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Original article

Synthesis and *in vitro* antibacterial activity of 7-(3-Alkoxyimino-4-amino-4-methylpiperidin-1-yl) fluoroquinolone derivatives

Ju-Xian Wang [1], Yi-Bin Zhang [1], Ming-Liang Liu*, Bo Wang, Yun Chai, Su-Jie Li, Hui-Yuan Guo

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, PR China

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ABSTRACT

A series of novel 7-(3-alkoxyimino-4-amino-4-methylpiperidin-1-yl)fluoroquinolone derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activity and cytotoxicity. All of the target compounds have potent antibacterial activity against the tested Gram-positive and Gram-negative strains, and exhibit good potency in inhibiting the growth of *Staphylococcus aureus* including MRSA, *Staphylococcus epidermidis* including MRSE and *Streptococcus pneumoniae* (MICs: 0.125–4 μg/mL). Compound **22**, with the best activity against Gram-positive strains, is 4–16 fold more potent than gemifloxacin, gatifloxacin and levofloxacin against *Enterococcus faecalis*, and 16- and 4-fold more potent than levofloxacin against *S. epidermidis* 09-6 and *S. pneumoniae* 08-4, respectively.

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1. Introduction

Quinolone antibacterial agents are among the most attractive drugs in the anti-infective chemotherapy field. These antibiotics act against bacteria by selectively inhibiting two type II topoisomerase enzymes, DNA gyrase and topoisomerase IV, which both play a critical role in bacterial cell growth and division [1].

Because of increasing prevalence of bacterial infections, particularly those such as the clinically important pathogenic bacteria, *Staphylococci* (including methicillin-resistant *Staphylococcus aureus*, MRSA and methicillin-resistant *Staphylococcus epidermidis*, MRSE), *Streptococci* and *Enterococci*, drug-discovery efforts have been intensified in the past years to search for more effective antibacterial agents with a broad spectrum of activity, and activity against Gram-positive pathogens, particularly resistant pathogens [2,3]. The ideal strategy to such challenges is to find novel agents that inhibit new targets in bacteria. However, despite advances in drug development, finding new antibacterial agents with novel mechanisms of action remains extremely difficult. A more practical approach is to modify the structures of existing antibacterial agents to increase potency, and to overcome resistance.

From the chemical structural point of view, one of the most important features of the fluoroquinolone antibacterials is the presence of a 5- or 6-membered nitrogen heterocycle as side chain at the C-7 position, including piperazine, pyrrolidine and piperidine. Of the three, piperidinyl analogs are the least studied [4]. As part of an ongoing program to find potent new quinolones displaying strong Gram-positive antibacterial activity, we have focused our attention on introducing new functional groups to the piperidine ring [5–8]. Previous work on pyrrolidine analogs suggests that introduction of a methyl group in the 3-position of the pyrrolidine ring can increase Gram-positive antibacterial activity. For example, an analog of gemifloxacin (GMFX), DW 286 (Fig. 1), possessing an additional methyl group at 3-position of pyrrolidine ring, displays far more potent antibacterial activity than GMFX against important Gram-positive organisms, MRSA and ofloxacin resistant organisms, while maintaining an excellent pharmacokinetic profile [9]. We applied this structural modification to DZH (Fig. 1), which shows good *in vitro* activity against Gram-positive and Gram-negative organisms, including MRSA and *Pseudomonas aeruginosa* [4]. A series of fluoroquinolone compounds containing piperidinyl substitution at the C-7 position were designed and synthesized. These derivatives are structurally novel, having both an amino and a methyl group at the 4-position and an alkoxyimino group at 3-position of the piperidine ring. Our primary objective was to optimize the potency of these compounds against Gram-positive and Gram-negative organisms.

2. Chemistry

The new piperidine derivatives **10a,b** and novel fluoroquinolones **12**–**25** described herein were synthesized as shown in Schemes 1 and 2, respectively. Catalytic hydrogenation of ethyl *N*-benzyl-3-oxopiperidine-4- carboxylate hydrochloride **1** gave

* Corresponding author. Tel.: +86 10 63036965; fax: +86 10 63047865.
E-mail address: lmllyx@yahoo.com.cn (M.-L. Liu).
[1] These authors contributed equally to the work.

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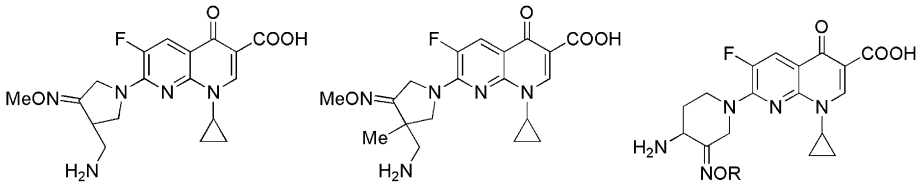
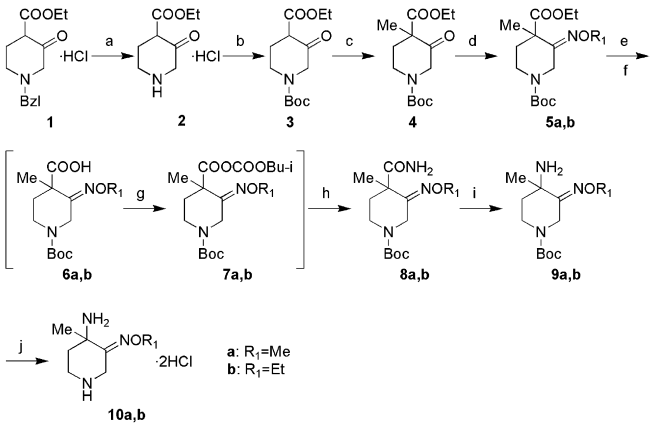


Fig. 1. Chemical structures of quinolone compounds.

secondary amine **2**. The amine **2** was subsequently treated with di-*tert*-butyl dicarbonate (Boc_2O) in ethanol to form Boc-protected compound **3**, which was methylated with methyl iodide at the presence of anhydrous potassium carbonate to produce methyl ketone **4**. The oximes **5a,b** were obtained from the reaction of **4** with alkoxylamines at 60 °C and converted to acids **6a,b** by saponification and acidification. The acids **6a,b** were subsequently reacted with isobutyl chloroformate to give activated esters **7a,b**, which upon ammonolysis afforded amides **8a,b** by pumping ammonia gas in methylene chloride. Hoffmann degradation of the amides **8a,b** used freshly prepared sodium hypobromite to give amines **9a,b**. Deprotection of Boc protective group on the amines **9a,b** was carried out by pumping dry hydrogen chloride gas in methylene chloride to afford new piperidine derivative dihydrochlorides **10a,b** in a good yield.

Finally, the target compounds **12**–**25** were obtained by coupling the new piperidine derivatives **10a,b** with various compounds containing quinolone or naphthyridine core according to the well-established literature procedures [10]. In the case of quinolones **12**–**21**, condensation of **10a,b** with **11a-f** was carried out in the presence of triethylamine. However for **22**–**25**, boric chelates **11g,h** were used to increase reactivity. Table 1 shows structures and cytotoxicity of the novel fluoroquinolones **12**–**25**.

Since the oxime group can exist in the *E* or *Z* configuration, it was necessary to determine the geometries of all the oxime target compounds **12**–**25**. It was a pity that we were not successful in preparing X-ray quality single crystals of compounds **12**–**25**. In NOE spectrums of **12**–**25**, there was no correlation between the oxime group and its neighbor group (amino group or methyl group). So it is quite probable that the geometry of the oxime group is the *E* configuration. Finally, we were able to obtain X-ray data for the compound **8a** [11]. As expected, the geometry of the methyloxime group on the piperidine ring which adopts a boat conformation is the *E* configuration (Fig. 2).



Scheme 1. Synthesis of new piperidine derivatives **10a,b**. Reagents and conditions: (a) Pd/C 5%, H_2 0.5 MPa, EtOH, rt, 4 h; (b) $NaHCO_3$, Boc_2O, EtOH 50%, rt, 1 h; (c) K_2CO_3, MeI, Me_2CO, 50 °C, 1.5 h; (d) $R_1ONH_2 \cdot HCl$, Et_3N, EtOH 80%, 60 °C, 2 h; (e) aq NaOH, EtOH, rt, 5 h; (f) HOAc, H_2O, rt, 0.5 h; (g) Et_3N, ClCOOBu-i, CH_2Cl_2, −15 °C, 2 h; (h) NH_3, CH_2Cl_2, 0 °C, 2 h; (i) aq NaBrO, MeCN, 5 °C, 10 h; (j) HCl gas, CH_2Cl_2, rt, 1 h.

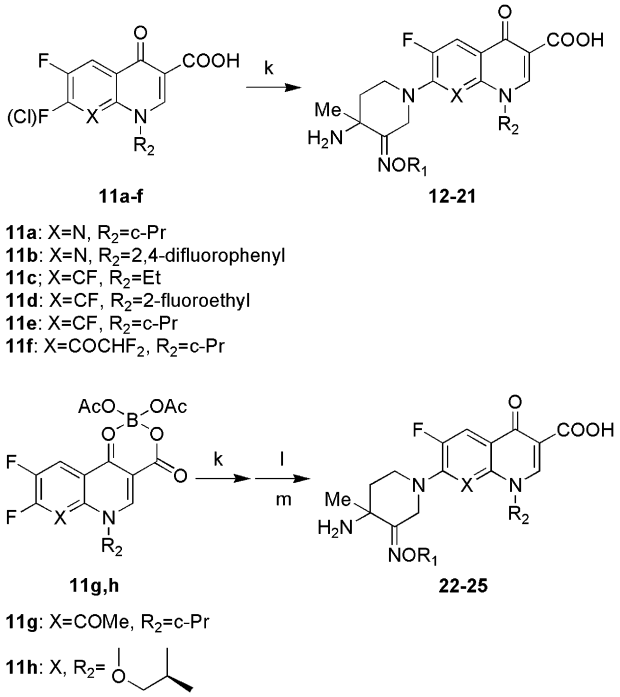
3. Results and discussion

3.1. Antibacterial activity

The novel fluoroquinolones **12**–**25** were evaluated for their *in vitro* antibacterial activity against ten Gram-positive and six Gram-negative strains using conventional agar-dilution method [12]. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the reference drugs GMFX, moxifloxacin (MXFX), gatifloxacin (GTFX) and levofloxacin (LVFX) for comparison are reported in Table 2.

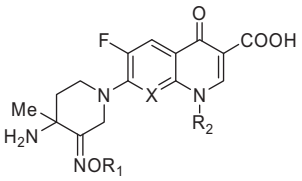
All of the target compounds **12**–**25** have generally potent antibacterial activity against the sixteen tested strains. They exhibit good potency in inhibiting the growth of *S. aureus* including MRSA, *S. epidermidis* including MRSE and *Streptococcus pneumoniae*, with MICs in the range of 0.125–4 μg/mL, which were comparable to that of the reference drugs. Compound **22**, with the best activity against Gram-positive strains, is found to be 4–16 fold more potent than GMFX, GTFX and LVFX against *Enterococcus faecalis*, and 16- and 4-fold more potent than LVFX against *S. epidermidis* 09-6 and *S. pneumoniae* 08-4, respectively.

As for Gram-negative strains, the novel fluoroquinolones **12**–**25** have poor or no activity against *Escherichia coli*, but show moderate activity against *P. aeruginosa*. For example, compounds **12**, **14**–**16**,



Scheme 2. Synthesis of novel fluoroquinolones **12**–**25**. Reagents and conditions: (k) **10a,b**, Et_3N, MeCN, rt, 3–10 h; (l) aq NaOH, rt, 1–4 h; (m) aq HCl, rt.

Table 1
The structures and cytotoxicity of compounds **12**–**25**.



Compd.	R_1	X	R_2	CC_{50} (μM)
12	Me	N		77.4
13	Et	N		300
14	Me	N	2,4-F_2-C_6H_3	93.0
15	Et	N	2,4-F_2-C_6H_3	128
16	Me	CF	Et	433
17	Et	CF	Et	592
18	Me	CF	2-F-C_2H_4	36.6
19	Et	CF		288
20	Me	$COCHF_2$		1068
21	Et	$COCHF_2$		518
22	Me	COMe		729
23	Et	COMe		280
24	Me			1506
25	Et			1157
GMFX				321
MXFX				161
GTFX				396

18, **20**, **21** and **23** are more active than or comparable to LVFX against these strains. In particular, compound **14** is 64-fold more potent than GTFX and LVFX, and comparable to GMFX and MXFX against *P. aeruginosa* 09-32. However, Compound **12**, possessing the same quinolone nucleus as DZH, is not superior to GMFX against the tested Gram-positive and Gram-negative strains, except *Klebsiella pneumoniae* 09-23. These results suggest that introduction of another methyl group into 4-position of piperidine ring does not cause increased antibacterial activity, which is not consistent with the activity profiles of the pyrrolidine-containing fluoroquinolones.

3.2. Cytotoxicity

Cytotoxicity of compounds **12**–**25** were also assessed in comparison with the reference drugs GMFX, MXFX and GTFX using CPE assay against a mammalian MDCK cell line. As listed in Table 1, 14 target compounds **12**–**25** show CC_{50} values ranging from 36.6 to 1506 μM. Among them, cytotoxicity of compounds **20**, **24** and **25** ($CC_{50} > 1000$ μM) is much less than the three reference drugs. The cytotoxicity of the substitution pattern at C-7 position depends on the quinolone nuclei. For example, fluoroquinolones fearturing ethyloxime-incorporated piperidino-substitution are less cytotoxic than the analogs containing methyloxime for naphthyridine nuclei, which is contrary to the cytotoxicity profiles for the quinolone nuclei of substitution linkage to the C-8 position though an oxygen atom.

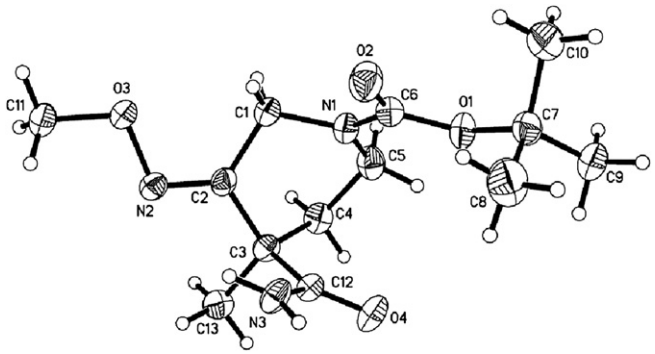


Fig. 2. X-ray structure of compound **8a**.

4. Conclusion

In summary, a series of novel 7-(3-alkoxyimino-4-amino-4-methylpiperidin-1-yl)fluoroquinolone derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activity and cytotoxicity. Generally, all of the target compounds **12**–**25** demonstrate potent antibacterial activity against the sixteen tested Gram-positive and Gram-negative strains. In particular, they exhibit good potency in inhibiting the growth of *S. aureus* including MRSA, *S. epidermidis* including MRSE and *S. pneumoniae* (MICs: 0.125–4 μg/mL), and some of them are much less cytotoxic than GMFX, MXFX and GTFX.

5. Experimental protocols

5.1. Chemistry

All chemical reagents and solvents used in this study were purchased from Beihua Fine Chemicals Company (Beijing, China). The starting material **1** was purchased from Bayoupharm Company (Chengdu, China). The compounds **8a,b** were prepared from **1** according to the literature [5]. Melting points were determined in open glass capillaries and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Mercury-400 or an INOVA-500 spectrometer (both: Varian Inc., Palo Alto, CA, USA) using tetramethylsilane as internal standard. Electron spray ionization (ESI) mass spectra and high-resolution mass spectra (HRMS) were recorded on a MDSSCIEX Q-Tap mass spectrometer (Applied Biosystems, USA). Merck silica gel ART5554 $60F_{254}$ plates (Merck, Germany) were used for analytical TLC.

5.1.1. *N-tert-Butoxycarbonyl-4-amino-3-methoxyimino-4-methylpiperidine (**9a**)*

To a stirring solution of **8a** (2.8 g, 10.0 mmol) in acetonitrile (150 mL) was added dropwise a freshly prepared sodium hypobromite solution (25 mL) at 5 °C. The reaction mixture was stirred for 10 h at the same temperature. After removal of the acetonitrile under reduced pressure, the reaction mixture was diluted with water (30 mL), adjusted to pH 2.5–3 with 2 N hydrochloric acid and washed with methylene chloride (3 × 30 mL). Then the water layer was adjusted to pH 12 with 6 N sodium hydroxide solution and extracted with methylene chloride (3 × 50 mL). The combined extracts were washed with saturated brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford compound **9a** (2.1 g). Yield: 83%, colorless liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.33 (3H, s, CH_3), 1.46 (9H, s, Boc-9H), 1.68–1.80 (4H, m), 3.32–3.38 (1H, m), 3.67–3.72 (1H, m), 3.85 (3H, s, OCH_3), 4.02 (1H, d, $J = 17.2$ Hz), 4.61 (1H, d, $J = 17.2$ Hz). ESI-MS m/z: 258 $(M + H)^+$.

5.1.2. *N-tert-Butoxycarbonyl-4-amino-3-ethoxyimino-4-methylpiperidine (**9b**)*

The title compound was obtained in a similar manner as for the preparation of **9a**. Yield: 58%, colorless liquid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.22 (3H, t, $OCH_2\underline{CH_3}$, $J = 7.2$ Hz), 1.31 (3H, s, CH_3), 1.45 (9H, s, Boc-9H), 1.64–1.78 (4H, m), 3.31–3.38 (1H, m), 3.62–3.66 (1H, m), 4.01–4.09 (3H, m), 4.61 (1H, d, $J = 17.2$ Hz). ESI-MS m/z: 272 $(M + H)^+$.

Table 2
In *vitro* antibacterial activity of compounds **12**–**25**.

	MIC (μg/mL)																	
Compd.	12	13	14	15	16	17	18	19	20	21	22	23	24	25	GMFX	MXFX	GTFX	LVFX
S.a.1[a]	0.25	0.125	0.25	0.25	0.5	0.25	0.125	0.5	0.25	0.125	0.125	0.25	0.25	0.25	0.125	0.125	0.06	0.25
S.a.2	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.06	0.125	0.06	0.125
S.a.3	0.25	0.25	0.25	2	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.125
S.e.1	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.125	0.25	4
S.e.2	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.125	0.25	0.25	0.25
S.p.	4	0.25	1	0.25	0.25	0.25	2	4	0.25	0.25	0.5	0.25	0.25	2	0.25	0.125	0.25	2
E.fa.1	16	>128	64	8	32	4	128	4	8	8	1	1	16	32	16	1	8	8
E.fa.2	16	>128	64	32	64	>128	32	>128	>128	32	1	128	>128	64	16	2	4	8
E.fm.1	64	>128	>128	64	>128	>128	32	>128	>128	128	>128	64	>128	32	2	2	2	4
E.fm.2	16	>128	>128	>128	>128	>128	128	>128	>128	128	>128	128	>128	>128	16	2	4	16
E.c.1	8	128	16	16	128	>128	32	128	128	16	>128	128	128	32	4	2	4	2
E.c.2	16	>128	>128	32	>128	>128	32	>128	>128	128	>128	>128	>128	64	16	2	8	16
K.p.1	16	>128	64	32	32	>128	16	4	2	16	>128	1	2	64	8	1	0.25	16
K.p.2	2	>128	64	32	64	>128	32	>128	>128	8	>128	>128	>128	2	8	1	2	16
P.a.1	4	8	4	4	4	4	2	8	4	4	16	4	8	4	2	2	1	4
P.a.2	16	2	0.25	0.5	4	64	0.5	4	2	16	8	0.5	128	64	0.25	0.25	16	16

[a] Abbreviations: S.a.1, *Staphylococcus aureus* ATCC25923; S.a.2, methicillin-sensitive *Staphylococcus aureus* 08-1; S.a.3, methicillin-resistant *Staphylococcus aureus* 08-1; S.e.1, methicillin-sensitive *Staphylococcus epidermidis* 09-6; S.e.2, methicillin-resistant *Staphylococcus epidermidis* 09-2; S.p., *Streptococcus pneumoniae* 08-4; E.fa.1, *Enterococcus faecalis* 08-10; E.fa.2, *Enterococcus faecalis* 08-12; E.fm.1, *Enterococcus faecium* 08-2; E.fm.2, *Enterococcus faecium* 06-7; E.c.1, *Escherichia coli* ATCC25922; E.c.2, *Escherichia coli* 08-21; K.p.1, *Klebsiella pneumoniae* 09-22; K.p.2, *Klebsiella pneumoniae* 09-23; P.a.1, *Pseudomonas aeruginosa* ATCC27853; P.a.2, *Pseudomonas aeruginosa* 09-32.

*5.1.3. 4-Amino-3-methoxyimino-4-methylpiperidine dihydrochloride (**10a**)*

To a stirring solution of **9a** (2.6 g, 10.0 mmol) dissolved in methylene chloride (50 mL) was pumped dry hydrogen chloride gas at room temperature for 0.5 h. The reaction mixture was allowed to stir for another 0.5 h at the same temperature. The resulting solid was collected by suction, and dried *in vacuo* to give the title compound **10a** (2.3 g). Yield: 98%, white solid, mp: 178–180 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.54 (3H, s, CH_3), 2.18–2.21 (2H, m), 3.23–3.25 (1H, m), 3.39–3.42 (1H, m), 3.87–3.96 (4H, m), 4.34–4.38 (1H, m), 8.80 (3H, s, NH_3^+), 9.70 (2H, s, NH_2^+). ESI-MS *m*/*z*: 158 $(M+H)^+$.

*5.1.4. 4-Amino-3-ethoxyimino-4-methylpiperidine dihydrochloride (**10b**)*

The title compound was obtained in a similar manner as for the preparation of **10a**. Yield: 95%, white solid, mp: 152–154 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.24 (3H, t, $OCH_2\underline{CH_3}$, $J = 7.2$ Hz), 1.54 (3H, s, CH_3), 2.14–2.24 (2H, m), 3.21–3.23 (1H, m), 3.29–3.42 (1H, m), 3.89 (1H, d, $J = 17.2$ Hz), 4.15 (2H, q, $O\underline{CH_2}CH_3$, $J = 7.2$ Hz), 4.36 (1H, d, $J = 17.2$ Hz), 8.80 (3H, s, NH_3^+), 9.68 (2H, d, NH_2^+, $J = 46.0$ Hz). ESI-MS *m*/*z*: 172 $(M+H)^+$.

*5.1.5. General procedure for the synthesis of **12**–**21***

A mixture of **11a-f** (1.0 mmol), **10a,b** (1.2 mmol), dry triethylamine (8.0 mmol) and dry acetonitrile (20 mL) was stirred at room temperature under an atmosphere of nitrogen for 3–8 h. The resulting solid was collected by suction, and dried *in vacuo* to give **12**–**21**.

*5.1.5.1. 1-Cyclopropyl-6-fluoro-7-(4-amino-3-methoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8- naphthyridine-3-carboxylic acid (**12**).* Yield: 67%, off-white solid, mp: 168–170 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.05–1.10 (2H, m, cyclopropyl CH_2), 1.26–1.31 (2H, m, cyclopropyl CH_2), 1.50 (3H, s, CH_3), 2.04–2.05 (2H, m), 3.62–3.65 (1H, m), 3.84–3.92 (4H, m), 4.19–4.23 (1H, m), 4.57 (1H, d, $J = 16.8$ Hz), 5.12 (1H, d, $J = 16.8$ Hz), 8.08 (1H, d, C_5-H, $J = 13.2$ Hz), 8.72 (1H, s, C_2-H). ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 6.85 (2C, s, cyclopropyl CH_2), 26.94 (1C, s), 34.94 (1C, s), 37.90 (1C, s), 40.32 (1C, s), 42.74 (1C, s), 51.67 (1C, s), 61.41 (1C, s, $NOCH_3$), 107.62 (1C, s), 112.30 (1C, s), 119.44 (1C, d, $J = 88.0$ Hz), 146.53 (1C, s), 146.72 (1C, d, $J = 1032.4$ Hz, C-6), 147.22 (1C, s), 149.33 (1C, d, $J = 34.4$ Hz), 157.70 (1C, s, C=N), 165.66 (1C, s, COOH), 176.40 (1C, s, C=O). HRMS-ESI *m*/*z*: Calcd for $C_{19}H_{23}FN_5O_4$: 404.17341. Found: 404.17228 $(M+H)^+$.

*5.1.5.2. 1-Cyclopropyl-6-fluoro-7-(4-amino-3-ethoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8- naphthyridine-3-carboxylic acid (**13**).* Yield: 80%, off-white solid, mp: 209–211 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.05–1.10 (2H, m, cyclopropyl CH_2), 1.23–1.32 (5H, m, cyclopropyl CH_2, $OCH_2\underline{CH_3}$), 1.44 (3H, s, CH_3), 1.88–2.03 (2H, m), 3.61–3.66 (1H, m), 3.86–3.92 (1H, m), 4.09–4.22 (3H, m), 4.63 (1H, d, $J = 17.2$ Hz), 5.07 (1H, d, $J = 17.2$ Hz), 8.08 (1H, d, C_5-H, $J = 13.2$ Hz), 8.74 (1H, s, C_2-H). ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 6.83 (2C, s, cyclopropyl CH_2), 14.34 (1C, s, $NOCH_2\underline{CH_3}$), 27.73 (1C, s), 34.92 (1C, s), 38.43 (1C, s), 40.40 (1C, s), 42.77 (1C, s), 51.25 (1C, s), 68.71 (1C, s, $NOCH_2$), 107.60 (1C, s), 112.16 (1C, s), 119.32 (1C, d, $J = 88.4$ Hz), 146.57 (1C, s), 146.74 (1C, d, $J = 1030.8$ Hz, C-6), 147.21 (1C, s), 149.37 (1C, d, $J = 34.4$ Hz), 158.15 (1C, s, C=N), 165.69 (1C, s, COOH), 176.40 (1C, s, C=O). HRMS-ESI *m*/*z*: Calcd for $C_{20}H_{25}FN_5O_4$: 418.18906. Found: 418.19072 $(M+H)^+$.

*5.1.5.3. 1-(2,4-Difluorophenyl)-6-fluoro-7-(4-amino-3-methoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**14**).* Yield: 60%, light yellow solid, mp: 168–171 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35 (3H, s, CH_3), 1.43–1.74 (2H, m), 3.64–3.64 (1H, m), 3.82 (3H, s, OCH_3), 3.82–3.93 (1H, m), 4.16–4.26 (1H, m), 4.59–4.69 (1H, m), 7.07–7.09 (2H, m, ph-2H), 7.36–7.39 (1H, m, ph-H), 8.12 (1H, d, C_5-H, $J = 13.2$ Hz), 8.68 (1H, s, C_2-H). HRMS-ESI *m*/*z*: Calcd for $C_{22}H_{21}F_3N_5O_4$: 476.15456. Found: 476.15426 $(M+H)^+$.

*5.1.5.4. 1-(2,4-Difluorophenyl)-6-fluoro-7-(4-amino-3-ethoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8- naphthyridine-3-carboxylic acid (**15**).* Yield: 60%, light yellow solid, mp: 169–171 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.20–1.27 (3H, m, $OCH_2\underline{CH_3}$), 1.41 (3H, s, CH_3), 1.90 (2H, brs), 3.61–3.63 (1H, m), 3.90–3.97 (1H, m), 4.05–4.10 (2H, m), 4.15–4.23 (1H, m), 4.65–4.72 (1H, m), 7.05–7.39 (3H, m, ph-3H), 8.13 (1H, d, C_5-H, $J = 12.8$ Hz), 8.68 (1H, s, C_2-H). HRMS-ESI *m*/*z*: Calcd for $C_{23}H_{23}F_3N_5O_4$: 490.17021. Found: 490.16883 $(M+H)^+$.

*5.1.5.5. 1-Ethyl-6,8-fluoro-7-(4-amino-3-methoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3- carboxylic acid (**16**).* Yield: 65%, off-white solid, mp: 199–201 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.29–1.59 (8H, m), 2.01–2.12 (2H, m), 3.54–3.85 (2H, m), 3.87–4.11

(5H, m), 7.90 (1H, d, C_5-H, J = 11.5 Hz), 8.94 (1H, s, C_2-H). HRMS-ESI m/z: Calcd for $C_{19}H_{23}F_2N_4O_4$: 409.16874. Found: 409.17062 $(M+H)^+$.

*5.1.5.6. 1-Ethyl-6,8-difluoro-7-(4-amino-3-ethoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3- carboxylic acid (**17**).* Yield: 55%, off-white solid, mp: 182–184 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.25 (3H, t, $OCH_2\underline{CH_3}$, J = 6.8 Hz), 1.43 (3H, s, CH_3), 1.59 (3H, t, $NCH_2\underline{CH_3}$, J = 7.2 Hz), 1.91–1.99 (2H, m), 3.46–3.56 (2H, m), 4.08–4.14 (3H, m), 4.47–4.51 (3H, m), 7.99 (1H, d, C_5-H, J = 10.8 Hz), 8.62 (1H, s, C_2-H). HRMS-ESI m/z: Calcd for $C_{20}H_{25}F_2N_4O_4$: 423.18439. Found: 423.18460 $(M+H)^+$.

*5.1.5.7. 1-(2-Fluoroethyl)-6,8-difluoro-7-(4-amino-3-methoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**18**).* Yield: 64%, light yellow solid, mp: 205–207 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.10–1.41 (7H, m), 1.79–1.90 (2H, m), 3.55–3.81 (5H, m), 4.14–4.35 (2H, m), 7.89 (1H, d, C_5-H, J = 10.5 Hz), 8.87 (1H, s, C_2-H). HRMS-ESI m/z: Calcd for $C_{19}H_{22}F_3N_4O_4$: 427.15931. Found: 427.15679 $(M+H)^+$.

*5.1.5.8. 1-Cyclopropyl-6,8-difluoro-7-(4-amino-3-ethoxyimino-4-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**19**).* Yield: 76%, off-white solid, mp: 164–166 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.18–1.20 (2H, m, cyclopropyl CH_2), 1.25 (3H, t, $OCH_2\underline{CH_3}$, J = 6.8 Hz), 1.28–1.33 (2H, m, cyclopropyl CH_2), 1.48 (3H, s, CH_3), 1.99 (2H, brs), 3.47–3.58 (2H, m), 3.99–4.01 (1H, m), 4.07–4.14 (3H, m), 4.53 (1H, d, J = 15.6 Hz), 7.94 (1H, d, C_5-H, J = 11.2 Hz), 8.80 (1H, s, C_2-H). HRMS-ESI m/z: Calcd for $C_{21}H_{25}F_2N_4O_4$: 435.18439. Found: 435.18382 $(M+H)^+$.

*5.1.5.9. 1-Cyclopropyl-6-fluoro-7-(4-amino-3-methoxyimino-4-methylpiperidin-1-yl)-8-difluoromethoxyl-1,4- dihydro-4-oxo-quinoline-3-carboxylic acid (**20**).* Yield: 54%, off-white solid, mp: 164–166 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.98–1.28 (4H, m, 2× cyclopropyl CH_2), 1.84 (3H, s, CH_3), 2.42–2.57 (2H, m), 3.54–3.64 (3H, m), 3.94 (3H, s, OCH_3), 4.10 (1H, s), 4.71 (1H, d, J = 15.2 Hz), 6.56 (1H, $OCHF_2$, t, J = 68.4 Hz), 8.05 (1H, d, C_5–H, J = 11.6 Hz), 8.86 (1H, s, C_2–H), 14.27 (1H, brs, COOH). HRMS-ESI m/z: Calcd for $C_{21}H_{24}F_3N_4O_5$: 469.16988. Found: 469.17033 $(M+H)^+$.

*5.1.5.10. 1-Cyclopropyl-6-fluoro-7-(4-amino-3-ethoxyimino-4-methylpiperidin-1-yl)-8-difluoromethoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**21**).* Yield: 64%, off-white solid, mp: 235–238 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.99–1.28 (7H, m, 2× cyclopropyl CH_2, $OCH_2\underline{CH_3}$), 1.69 (3H, s, CH_3), 2.27 (2H, brs), 3.53–3.64 (2H, m), 3.96 (1H, d, J = 15.2 Hz), 4.11–4.19 (3H, m), 4.67 (1H, d, J = 15.2 Hz), 6.52 (1H, t, $OCHF_2$, J = 74.8 Hz), 8.01 (1H, d, C_5-H, J = 11.6 Hz), 8.84 (1H, s, C_2-H). HRMS-ESI m/z: Calcd for $C_{22}H_{26}F_3N_4O_5$: 483.18553. Found: 483.18588 $(M+H)^+$.

*5.1.6. General procedure for the synthesis of **22**–**25***

A mixture of **11g,h** (1.0 mmol), **10a,b** (1.2 mmol), dry triethylamine (8.0 mmol) and dry acetonitrile (20 mL) was stirred for 6–10 h at room temperature under an atmosphere of nitrogen. After completion of the condensation, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in 2% sodium hydroxide solution (20 mL) and stirred for 1–4 h at room temperature. The reaction mixture was adjusted to pH 7 with 6 N hydrochloric acid. The resulting solid was collected by suction, and dried *in vacuo* to give **22**–**25**.

*5.1.6.1. 1-Cyclopropyl-6-fluoro-7-(4-amino-3-methoxyimino-4-methylpiperidin-1-yl)-8-methoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (**22**).* Yield: 69%, light yellow solid, mp: 229–231 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.02–1.13 (4H, m, 2× cyclopropyl CH_2), 1.61 (3H, s, CH_3), 2.10–2.21 (2H, m), 3.54 (2H, brs), 3.75 (3H, s, OCH_3), 3.87 (3H, s, $NOCH_3$), 3.94 (1H, d, J = 15.2 Hz), 4.15–4.19 (1H, m), 4.65 (1H, d, J = 15.2 Hz), 7.76 (1H, d, C_5-H, J = 12.0 Hz), 8.69 (1H, s, C_2-H), 14.85 (1H, brs, COOH). HRMS-ESI m/z: Calcd for $C_{21}H_{26}FN_4O_5$: 433.18872. Found: 433.18958 $(M+H)^+$.

*5.1.6.2. 1-Cyclopropyl-6-fluoro-7-(4-amino-3-ethoxyimino-4-methylpiperidin-1-yl)-8-methoxyl-1,4-dihydro-4-oxo- quinoline-3-carboxylic acid (**23**).* Yield: 51%, light yellow solid, mp: 79–81 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.01–1.25 (7H, m, 2× cyclopropyl CH_2, $OCH_2\underline{CH_3}$), 1.44 (3H, s, CH_3), 1.88–1.97 (2H, m), 3.48–3.59 (2H, m), 3.76 (3H, s, OCH_3), 4.02–4.13 (4H, m), 4.53 (1H, d, J = 15.2 Hz), 7.89 (1H, d, C_5–H, J = 12.0 Hz), 8.82 (1H, s, C_2-H). HRMS-ESI m/z: Calcd for $C_{22}H_{28}FN_4O_5$: 447.20437. Found: 447.20451 $(M+H)^+$.

*5.1.6.3. 9-Fluoro-3(S)-methyl-10-(4-amino-3-methoxyimino-4-methylpiperidin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**24**).* Yield: 47%, light yellow solid, mp: 166–168 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.45 (3H, s, $C_{4'}$-CH_3), 1.62 (3H, d, C_3-CH_3, J = 6.8 Hz), 1.93–1.96 (2H, m), 3.40–3.46 (2H, m), 3.85 (3H, s, OCH_3), 4.01–4.08 (1H, m), 4.38–4.51 (4H, m), 7.73 (1H, d, C_8-H, J = 12.0 Hz), 8.63 (1H, s, C_5-H). HRMS-ESI m/z: Calcd for $C_{20}H_{24}FN_4O_5$: 419.17307. Found: 419.17502 $(M+H)^+$.

*5.1.6.4. 9-Fluoro-3(S)-methyl-10-(4-amino-3-ethoxyimino-4-methylpiperidin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (**25**).* Yield: 46%, light yellow solid, mp: 122–124 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.24 (3H, t, $OCH_2\underline{CH_3}$), 1.41 (3H, s, $C_{4'}$-CH_3), 1.62 (3H, d, C_3-CH_3, J = 4.4 Hz), 1.90 (2H, t, J = 5.6 Hz), 3.42–3.48 (2H, m), 4.03–4.12 (3H, m), 4.38–4.49 (4H, m), 7.74 (1H, d, C_8-H, J = 11.6 Hz), 8.62 (1H, s, C_5-H). HRMS-ESI m/z: Calcd for $C_{21}H_{26}FN_4O_5$: 433.18872. Found: 433.18662 $(M+H)^+$.

5.2. Antibacterial activity

Compounds **12**–**25** were evaluated for their *in vitro* antibacterial activity using standard techniques in comparison to the reference drugs GMFX, MXFX, GTFX and LVFX. Drugs (10.0 mg) were dissolved in 0.1 N sodium hydroxide solution and water (10 mL). Further progressive twofold serial dilution with melted Mueller-Hinton agar was performed to obtain the required concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, and 0.03 μg/mL. Petri dishes were incubated with 10^4 colony-forming units (cfu) and incubated at 35 °C for 18 h. MIC was the lowest concentration of the test compound, which resulted in no visible growth on the plate.

5.3. Cytotoxicity

Compounds **12**–**25** were examined for their toxicity (CC_{50}) in a mammalian MDCK cell line till 3.9 μg/mL concentrations. The MDCK cells were maintained in culture medium (Minimum Essential Medium with Earle's salt, supplemented with 10% fetal bovine serum) at 37 °C under 5% CO_2. Cells were seeded in 96-well plate at the plating density of 2.5×10^4 cells per well and allowed to recover for 24 h. Culture medium was replaced by assay medium containing the compound to be tested or drug-free. After 48 h of exposure, cells were harvested and cell viability was assessed by CPE assay. The CC_{50} values were calculated by Reed–Muench analyses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.03.026.

References

[1] T.V. Andriole, Clin. Infect. Dis 41 (2005) S113–S119.
[2] H.G. Talbot, J. Bradley, E.J. Edwards, D. Gilbert, M. Scheld, G.J. Bartlett, Clin. Infect. Dis 42 (2006) 657–668.
[3] U. Theuretzbacher, H.J. Toney, Curr. Opin. Investig. Drugs 7 (2006) 158–166.
[4] Z. Dang, Y.S. Yang, R.Y. Ji, S.H. Zhang, Bioorg. Med. Chem. Lett. 17 (2007) 4523–4526.
[5] Y.B. Zhang, L.S. Feng, X.F. You, Q. Guo, H.Y. Guo, M.L. Liu, Arch. Pharm. Chem. Life. Sci. 343 (2010) 143–151.
[6] J.X. Wang, Q. Guo, Y. Chai, L.S. Feng, H.Y. Guo, M.L. Liu, Chin. Chem. Lett. 21 (2009) 55–58.
[7] Y. Chai, Z.L. Wan, B. Wang, H.Y. Guo, M.L. Liu, Eur. J. Med. Chem. 44 (2009) 4063–4069.
[8] X.Y. Wang, Q. Guo, Y.C. Wang, B.Q. Liu, M.L. Liu, L.Y. Sun, H.Y. Guo, Acta. Pharmaceutica. Sinica 43 (2008) 819–827.
[9] 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.
[10] N. Takagi, H. Fubasami, H. Matsukubo, Chem. Abstr. 116 (1992) 152003e EP 464823.
[11] J.X. Wang, M.L. Liu, J. Cao, Y.C. Wang, Acta. Cryst. E64 (2008) o2294.
[12] Performance Standards for Antimicrobial Susceptibility Testing: 17th Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA, 2007, M100-S17.