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Synthesis and *In Vitro* Antibacterial Activity of 7-(3-Alkoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-fluoroquinolone Derivatives

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A series of novel 7-(3-alkoxyimino-4-methyl-4-methylaminopiperidin-1-yl)fluoroquinolone derivatives were designed, synthesized, and characterized by ^{1}H-NMR, MS, and HRMS. These fluoroquinolones were evaluated for their *in-vitro* antibacterial activity against representative Gram-positive and Gram-negative strains. Generally, all of the target compounds have considerable antibacterial activity against the tested forty strains, and exhibit exceptional potency in inhibiting the growth of methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) ATCC33591 (MICs: 0.06 to 2 μg/mL). In particular, compounds **14**, **19**, **28**, and **29** are fourfold more potent than ciprofloxacin against MSSA 08-49. Compounds **23**, **26**, and **27** are twofold more potent than ciprofloxacin against MRSA ATCC33591 and MSSA ATCC29213. In addition, compound **14** exhibits excellent activity (MIC: 0.06 μg/mL) against *Acinetobacter calcoaceticus*, which is two- to 16-fold more potent than the reference drugs gemifloxacin, levofloxacin, and ciprofloxacin.

Keywords: Antibacterial activity / Fluoroquinolone / Synthesis

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Introduction

Quinolone antibacterial agents are among the most important drugs in the anti-infective chemotherapy field. These antibiotics exert their antimicrobial effect by inhibition of two type-II bacterial topoisomerase enzymes, DNA gyrase and topoisomerase IV, which are essential cellular enzymes that catalyze the double-strand breakage of DNA to allow strand passage and, thereby, control the topology and conformation of DNA [1–4].

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Abbreviations: ciprofloxacin (CPFX); gemifloxacin (GMFX); levofloxacin (LVFX); methicillin-resistant *S. aureus* (MRSA); methicillin-sensitive *S. aureus* (MSSA)

Although most of the quinolones currently on the market or under development are generally characterized by a broad antimicrobial spectrum, their activity against clinically important Gram-positive pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Enterococcus*, is relatively moderate, which has not only limited their use in infections caused by these organisms, but is also believed to be one of the reasons for the rapidly developing quinolone resistance. Thus, recent efforts have been directed toward the synthesis of new quinolones that can provide improved Gram-positive antibacterial activity, while retaining the good Gram-negative activity of early fluoroquinolones, such as ciprofloxacin (CPFX) and ofloxacin [5–8].

Structure-activity relationship (SAR) studies of quinolone antibacterial agents showed that the basic group at the C-7 position is the most adaptable site for chemical change and an area that greatly influences their potency, spectrum, and safety [9, 10]. In general, 5- and 6-mem-

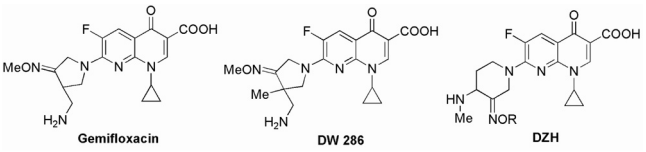
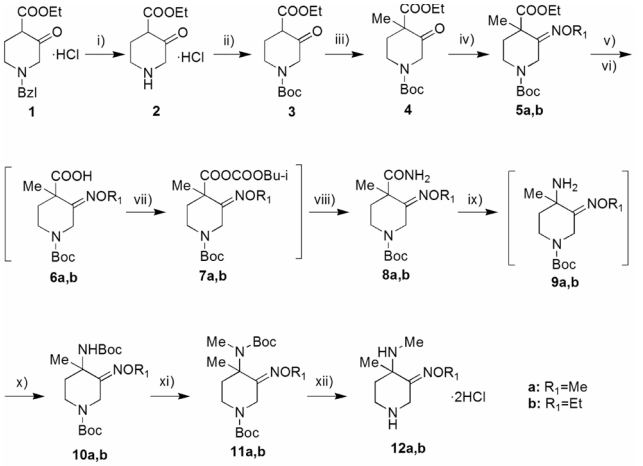


Figure 1. Structures of Gemifloxacin, DW 286, and DZH.

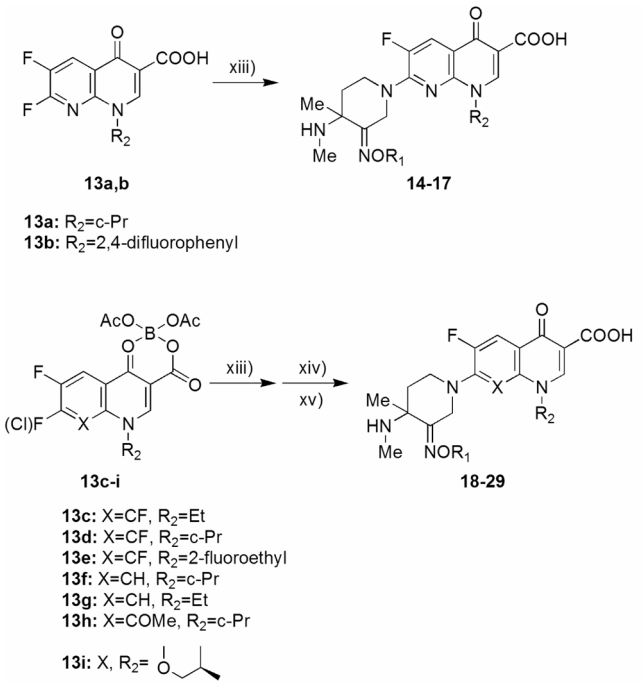
bered nitrogen heterocycles including piperazinyl-, pyrrolidinyl-, and piperidinyl-type side chains have been proven to be the optimal substituents [11]. However, relatively few quinolone antibacterial agents possessing piperidine substitution have been reported in the literature, as compared to that of piperazinyl- and pyrrolidinyl-based derivatives [12].

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 [13, 14]. Previous work on pyrrolidine analogs suggested that introduction of a methyl group in the 3-position of the pyrrolidine ring can increase Gram-positive activity [15]. For example, an analog of gemifloxacin (GMFX), DW 286 (Fig. 1), possessing an additional methyl group at the 3-position of the pyrrolidine ring, displays far more potent antibacterial activity than GMFX against important Gram-positive organisms, methicillin-resistant *S. aureus* (MRSA), and ofloxacin-resistant organisms, while maintaining an excellent pharmacokinetic profile [16]. 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* [12]. A series of fluoroquinolone compounds containing piperidinyl substitution at the C-7 position were designed and synthesized. These derivatives are structurally novel, having both a methylamino and a methyl group at the 4-position and an alkoxyimino group at the 3-position of the piperidine ring. Our primary objective was to optimize the potency of these compounds against Gram-positive and Gram-negative organisms.



Reagents and conditions: i) H_2, Pd/C, EtOH; ii) Boc_2O, $NaHCO_3$, EtOH, H_2O; iii) MeI, K_2CO_3, Me_2CO; iv) R_1ONH_2 . HCl, Et_3N, EtOH, H_2O; v) aq. NaOH, EtOH; vi) HOAc, H_2O; vii) ClCOOBu-*i*, Et_3N, CH_2Cl_2; viii) NH_3, CH_2Cl_2; ix) aq. NaBrO, MeCN; x) Boc_2O, MeOH; xi) MeI, NaH, MeCN; xii) HCl, CH_2Cl_2.

Scheme 1. Synthesis of new piperidine derivatives **12a**, **b**.



Reagents and conditions: xiii) **12a**, **b**, Et_3N, MeCN; xiv) 2% NaOH; xv) aq. HCl.

Scheme 2. Synthesis of novel fluoroquinolones **14–29**.

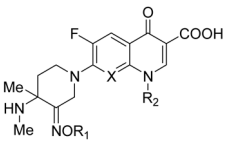
Results and discussion

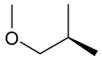
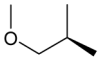
Chemistry

The new piperidine derivatives **12a**, **b** and novel fluoroquinolones **14–29** described herein were synthesized as shown in Schemes 1 and 2, respectively. According to published procedures [17–22], catalytic hydrogenation of ethyl *N*-benzyl-3-oxopiperidine-4-carboxylate hydrochloride **1** gave the secondary amine **2**. The amine **2** was subsequently treated with di-*tert*-butyl dicarbonate in ethanol to form the *tert*-butoxycarbonyl-protected compound **3**, which was methylated with methyl iodide to produce methyl ketone **4**. The ketone **4** was smoothly converted to oximes **5a**, **b** by reaction with alkoxylamines in ethanol at 60°C. However, direct ammonolysis of the ester moiety in **5a**, **b** was unsuccessful.

Therefore, the esters **5a**, **b** were first converted to the acids **6a**, **b** by saponification and acidification. The acids **6a**, **b** were subsequently reacted with isobutyl chlorofor-

Table 1. Structures and physical data of novel fluoroquinolones **14–29**.



Compound	R_1	R_2	X	Yield (%)	M.p. (°C)§	ESI-MS[M^+ + H]
14	Me		N	48	170–171	418
15	Et		N	47	199–201	432
16	Me	2,4-F_2-C_6H_3	N	37	115–118	490
17	Et	2,4-F_2-C_6H_3	N	32	161–164	504
18	Me	Et	CF	55	172–175	423
19	Et		CF	56	174–177	449
20	Me	2-F-C_2H_4	CF	41	171–174	441
21	Et	2-F-C_2H_4	CF	53	168–170	455
22	Me		CH	24	192–194	417
23	Et		CH	21	158–160	431
24	Me	Et	CH	17	168–169	405
25	Et	Et	CH	18	204–206	419
26	Me		COMe	36	82–84	447
27	Et		COMe	35	167–169	461
28	Me			40	169–170	433
29	Et			39	159–162	447

§ Melting points are uncorrected.

mate to give the activated esters **7a**, **b**, which upon ammonolysis afforded amides **8a**, **b** by pumping ammonia gas in methylene chloride. The three steps mentioned above were performed by one-pot method in an overall yield of 40%.

Hoffmann degradation of the amides **8a**, **b** used freshly prepared sodium hypobromite instead of commercially available sodium hypochlorite to give amines **9a**, **b**, which were subsequently protected by a *tert*-butoxycarbonyl group to form compounds **10a**, **b**. Methylation of **10a**, **b** was performed by reaction with methyl iodide in the presence of sodium hydride to produce compounds **11a**, **b**. Deprotection of the bis-*tert*-butoxycarbonyl groups on the amines **11a**, **b** was carried out by pumping dry hydrogen chloride gas in methylene chloride to afford the new piperidine derivative dihydrochlorides **12a**, **b** in a good yield.

The coupling reaction of the piperidine derivatives **12a**, **b** with various compounds containing quinolone and naphthyridine cores was performed according to well-established literature procedures (Scheme 2; [23]). In the case of quinolones **14–17**, condensation of **12a**, **b** with **13a**, **b** was carried out in the presence of triethylamine. However, for **18–29**, boric chelates **13c–i** were used to increase the reactivity. Table 1 shows the novel fluoroquinolone analogs, yields of the final coupling step, and the melting points of the purified compounds.

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 **14–29**. It was a pity that we were not successful in preparing X-ray-quality single crystals of compounds **14–29**. In the NOE spectra of **14–29**, there was no correlation between the oxime group and its neighbor group (methylamino 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 compound **8a** [24]. As expected, the geometry of the methyloxime group on the piperidine ring which adopts a boat conformation is the *E*-configuration (Fig. 2).

Table 2. *In-vitro* antibacterial activity of compounds **14–29**.

Compound	MIC (μg/mL)[§]																		
	14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	GMFX	LVFX	CPFX
S.a. 1[#]	0.5	0.5	0.5	0.5	2	0.5	2	2	0.5	0.25	1	2	0.25	0.25	1	0.5	≤0.03	0.125	0.5
S.a. 2	0.125	0.5	0.5	0.5	1	0.25	1	1	0.125	0.25	0.5	1	0.125	0.125	0.5	0.5	≤0.03	0.06	0.125
S.a. 3	0.5	0.5	0.5	0.5	2	0.5	2	2	0.5	0.25	1	2	0.25	0.25	1	0.5	≤0.03	0.125	0.5
S.a. 4	0.06	0.125	0.125	0.5	0.125	0.06	0.25	0.25	0.125	0.125	0.25	0.125	0.125	0.25	0.06	0.06	≤0.03	0.06	0.25
S.a. 5	64	64	>128	>128	>128	64	>128	>128	128	64	>128	64	32	64	128	64	0.5	1	4
S.e. 1	0.5	0.5	2	1	4	1	2	2	1	0.5	2	4	0.5	0.5	1	0.5	0.06	0.125	0.25
S.e. 2	2	2	4	8	16	2	8	16	2	2	16	32	1	1	2	2	0.06	1	2
S.e. 3	0.5	0.5	1	1	2	0.5	1	2	0.5	0.25	1	4	0.25	0.5	0.5	0.5	≤0.03	0.125	0.25
S.p. 1	2	1	1	2	16	1	8	16	0.5	2	8	64	0.5	1	2	2	≤0.03	0.5	1
S.p. 2	4	4	2	8	16	2	16	32	4	4	16	64	1	2	2	4	0.5	2	1
S.p. 3	128	128	>128	>128	>128	64	>128	>128	64	128	128	64	128	128	128	128	32	32	64
S.p. 4	1	1	1	2	16	1	16	16	0.5	2	8	16	0.5	1	2	2	≤0.03	0.5	1
S.py. 1	8	4	2	4	16	2	16	16	2	4	16	64	1	2	2	4	0.125	1	1
S.py. 2	8	4	4	8	16	4	32	32	4	8	16	64	2	2	4	8	1	2	1
S.py. 3	128	128	>128	>128	>128	128	>128	>128	128	128	128	64	128	128	128	128	4	32	32
E.fa. 1	1	1	4	16	8	0.5	4	4	2	1	4	8	1	1	2	2	≤0.03	0.5	0.5
E.fa. 2	1	1	2	4	4	0.5	4	4	2	1	2	16	1	1	2	2	0.06	0.25	0.5
E.fa. 3	1	1	4	4	8	1	8	8	2	1	4	16	1	2	2	2	0.06	0.5	1
E.fa. 4	2	4	8	16	8	2	8	8	2	2	4	32	1	2	2	4	0.5	2	1
E.fm. 1	128	64	>128	>128	>128	64	>128	128	64	64	128	64	128	64	128	128	32	64	>128
E.fm. 2	128	64	>128	>128	>128	128	>128	>128	64	64	128	128	128	>128	128	128	32	64	64
E.fm. 3	>128	64	>128	>128	>128	64	>128	>128	128	64	>128	128	64	64	>128	128	32	64	>128
E.co. 1	0.25	0.25	4	8	1	0.25	2	2	0.5	0.5	2	16	0.25	1	0.5	0.5	≤0.03	≤0.03	≤0.03
E.co. 2	0.25	0.5	4	16	2	0.25	2	2	0.5	1	2	32	0.25	1	0.5	1	≤0.03	≤0.03	≤0.03
E.co. 3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	32	128
K.p. 1	8	16	64	128	32	16	32	64	16	32	64	>128	16	32	16	32	0.5	0.5	0.5
K.p. 2	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	32	64
P.a. 1	>128	>128	>128	>128	>128	128	>128	>128	>128	>128	>128	>128	32	>128	>128	128	1	1	1
P.a. 2	1	2	8	32	8	2	8	8	4	8	16	64	2	2	2	8	0.125	0.25	0.25
P.a. 3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	>128	32	8	16
S.s.	0.06	0.5	4	8	2	0.25	4	2	0.5	1	2	4	0.25	1	0.5	2	≤0.03	≤0.03	≤0.03
B.t.	0.06	0.25	2	16	0.5	0.125	0.5	1	0.25	1	1	4	0.125	0.5	0.06	0.5	≤0.03	≤0.03	≤0.03
E.cl.	0.125	2	8	64	4	1	4	8	1	4	8	32	1	4	2	4	≤0.03	0.06	≤0.03
A.c.	0.06	0.5	2	8	2	0.25	4	4	1	1	4	16	0.25	1	2	8	0.125	0.25	1
E.a.	0.125	2	8	64	4	2	4	4	1	2	4	16	1	4	2	4	≤0.03	≤0.03	≤0.03
S.m.	1	4	8	64	4	2	4	4	2	4	4	16	8	8	2	4	0.125	0.25	0.25
M.m.	0.5	2	16	32	2	1	2	2	1	4	2	16	1	2	1	2	0.06	≤0.03	≤0.03
P.r.	1	4	8	32	8	2	4	8	2	4	8	32	2	4	2	8	0.06	0.25	≤0.03
P.v.	1	4	8	32	4	2	4	8	1	4	4	16	1	4	2	2	≤0.03	≤0.03	≤0.03
P.m.	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	128	128	8	1	2

§ The values were reproduced in three experiments; # Abbreviations: S.a. 1: *Staphylococcus aureus* ATCC29213; S.a. 2: *Staphylococcus aureus* ATCC13709; S.a. 3: *Staphylococcus aureus* ATCC33591; S.a. 4: methicillin-sensitive *Staphylococcus aureus* 08-49; S.a. 5: methicillin-resistant *Staphylococcus aureus* 08-52; S.e. 1: *Staphylococcus epidermidis* ATCC12228; S.e. 2: methicillin-sensitive *Staphylococcus epidermidis* 08-17; S.e. 3: methicillin-resistant *Staphylococcus epidermidis* 08-18; S.p. 1: *Streptococcus pneumoniae* ATCC49619; S.p. 2: *Streptococcus pneumoniae* ATCC6301; S.p. 3: *Streptococcus pneumoniae* ATCC10813; S.p. 4: penicillin-resistant *Streptococcus pneumoniae* 08-2; S.py. 1: *Streptococcus pyogenes* 556; S.py. 2: *Streptococcus pyogenes* 08-12; S.py. 3: *Streptococcus pyogenes* 08-13; E.fa. 1: *Enterococcus faecalis* ATCC29212; E.fa. 2: *Enterococcus faecalis* ATCC51299; E.fa. 3: *Enterococcus faecalis* HH22; E.fa. 4: vancomycin-resistant *Enterococcus faecalis* EFL1004; E.fm. 1: *Enterococcus faecium* ATCC700221; E.fm. 2: *Enterococcus faecium* 06-7; E.fm. 3: vancomycin-resistant *Enterococcus faecium* 05-7; E.co. 1: *Escherichia coli* ATCC25922; E.co. 2: *Escherichia coli* ATCC35218; E.co. 3: *Escherichia coli* 08-5 (ESBLs); K.p. 1: *Klebsiella pneumoniae* ATCC700603; K.p. 2: *Klebsiella pneumoniae* 08-2 (ESBLs); P.a. 1: *Pseudomonas aeruginosa* ATCC27853; P.a. 2: *Pseudomonas aeruginosa* 17; P.a. 3: *Pseudomonas aeruginosa* PA01; S.s.: *Shigella sonnei* 51592; B.t.: *Bacillus typhi* 50035; E.cl.: *Enterobacter cloacae* 45301; A.c.: *Acinetobacter calcoaceticus* 25001; E.a.: *Enterobacter aerogenes* 45102; S.m.: *Serratia marcescens* 41002; M.m.: *Morganella morganii* 49086; P.r.: *Proteus rettgeri* 49006; P.v.: *Proteus vuigaris* 56; P.m.: *Proteus mirabilis* 08-19.

Antibacterial activity

The novel fluoroquinolones **14–29** were evaluated for their *in-vitro* antibacterial activity against 22 Gram-positive and 18 Gram-negative strains using standard techniques [25]. 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 that of the standard drugs GMFX, CPFX, and levofloxacin (LVFX) for comparison are reported in Table 2.

All of the target compounds **14–29** generally have a potent antibacterial activity against the tested 40 strains. They exhibit good potency in inhibiting the growth of

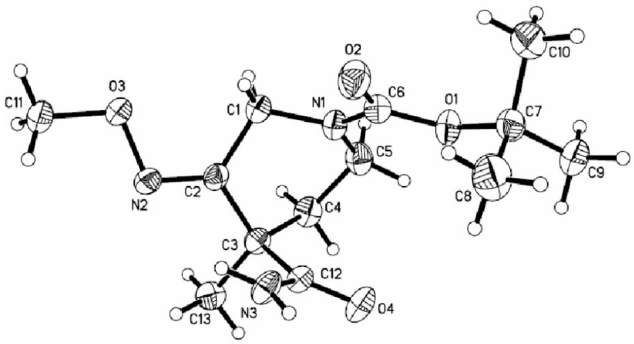


Figure 2. X-ray structure of compound **8a**.

methicillin-sensitive *S. aureus* (MSSA) and MRSA ATCC 33591 (MICs: 0.06–2 μg/mL). In particular, compounds **14**, **19**, **28**, and **29** are comparable to LVFX, and fourfold more potent than CPFX against MSSA 08-49. Compounds **23**, **26**, and **27** are twofold more potent than CPFX against MRSA ATCC33591 and MSSA ATCC29213. In addition, compound **14** exhibits excellent activity (MIC: 0.06 μg/mL) against *Acinetobacter calcoaceticus*, which is 2- to 16-fold more potent than GMFX, LVFX, and CPFX (MICs: 0.125 to 1 μg/mL). However, overall the piperidinyl analogs exhibit less activity than the reference drugs against Gram-positive and Gram-negative strains, except against MSSA.

Compound **14**, bearing the same quinolone nucleus as DZH, is much less active than GMFX against the tested strains, except against *A. calcoaceticus*. These results suggest that introduction of another methyl group into the 4-position of the piperidine ring causes reduced antibacterial activity, which is in contrary to the activity profiles of pyrrolidine-containing fluoroquinolones.

The activity of the quinolone nuclei in this study are in the order: 1-cyclopropyl-8-fluoroquinolone ≈ 1-cyclopropyl-8-methoxylquinolone > 1-cyclopropyl-8-hydrogenquinolone ≈ 1-cyclopropyl-1,8-naphthyridine > levofloxacin nuclei > 1-(2,4-difluorophenyl)-1,8-naphthyridine > 1-ethyl-8-hydrogen-quinolone ≈ 1-(2-fluoroethyl)-8-fluoroquinolone > 1-ethyl-8-fluoroquinolone against Gram-positive strains. In the case of Gram-negative strains, the activity is in the order: 1-cyclopropyl-8-fluoroquinolone > 1-cyclopropyl-1,8-naphthyridine > 1-cyclopropyl-8-methoxyl-quinolone ≈ 1-cyclopropyl-8-hydrogenquinolone ≈ levofloxacin nuclei > 1-ethyl-8-fluoroquinolone ≈ 1-(2-fluoroethyl)-8-fluoroquinolone > 1-ethyl-8-hydrogenquinolone > 1-(2,4-difluorophenyl)-1,8-naphthyridine. Generally, in both Gram-positive and Gram-negative strains, better results are obtained with a cyclopropyl group at the *N*-1 position, while analogs bearing an ethyl or 2-fluoroethyl group show relatively low potency. In addition, fluoroquinolones featuring methyloxime-incorporated piperidino-substitution at the C-7 position are at least as potent as the analogs containing ethyloxime.

Conclusion

In summary, a series of novel 7-(3-alkoxyimino-4-methyl-4-methylaminopiperidin-1-yl)fluoroquinolone derivatives were designed, synthesized, and evaluated for their *in-vitro* antibacterial activity against 40 Gram-positive and Gram-negative strains. Generally, all of the target compounds **14–29** demonstrate potent antibacterial activity, and they exhibit good potency in inhibiting the growth of MSSA and MRSA ATCC33591 (MICs: 0.06–2 μg/mL). In particular, compounds **14**, **19**, **28**, and **29** are fourfold more potent than CPFX against MSSA 08-49. Compounds **23**, **26**, and **27** are twofold more potent than CPFX against MRSA ATCC33591 and MSSA ATCC29213. In addition, compound **14** exhibits excellent activity (MIC: 0.06 μg/mL) against *A. calcoaceticus*, which is 2- to 16-fold more potent than GMFX, LVFX, and CPFX.

Experimental

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). 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 400 (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. Column chromatography was carried out on silica gel HG/T2354-92 made in Haiyang Chemical Company (Qingdao, China).

*Ethyl 3-oxopiperidine-4-carboxylate hydrochloride **2***

A mixture of **1** (18.0 g, 60.4 mmol), ethanol (200 mL) and 5% Pd/C (2.5 g) was stirred for 4 h at 0.5 MPa hydrogen pressure and room temperature and filtered. The filtrate was concentrated under reduced pressure. The resulting solid residue was recrystallized from methanol/ethyl acetate to afford compound **2** (11.8 g, 95%) as a white solid. M. p.: 200–202°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H: 1.29 (t, 3H, J = 6.5 Hz, OCH_2CH_3), 1.66–1.67 (m, 1H), 2.69–2.71 (m, 2H), 3.30–3.35 (m, 2H), 3.80–3.81 (m, 2H), 4.26 (q, 2H, J = 6.5 Hz, OCH_2CH_3), 10.24 (br, 2H, NH_2^+); ESI-MS m/z: 172 [M^+ + H].

*Ethyl N-tert-butoxycarbonyl-3-oxopiperidine-4-carboxylate **3***

To a stirred solution of **2** (2.1 g, 10.1 mmol) in ethanol (12 mL) were added sodium bicarbonate (1.0 g, 10.1 mmol) dissolved in water (12 mL) and di-*tert*-butyl dicarbonate (2.2 g, 10.1 mmol), respectively. The reaction mixture was stirred for 1 h at the room temperature and concentrated under reduced pressure. The residue was diluted with water (20 mL) and extracted with methylene chloride (3 × 15 mL). The combined extracts were washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate) to afford compound **3** (2.7 g, 100%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H: 1.32 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.47 (s, 9H, Boc-9H), 1.52–1.53 (m, 1H), 2.32–2.34 (m, 2H), 3.47–3.49 (m, 2H), 4.02–4.03 (m, 2H), 4.23 (q, 2H, J = 7.0 Hz, OCH_2CH_3); ESI-MS m/z: 272 [M^+ + H].

*Ethyl N-tert-butoxycarbonyl-4-methyl-3-oxopiperidine-4-carboxylate **4***

A solution of methyl iodide (20.0 mL, 0.32 mol) in dry acetone (90 mL) was added to a suspension of **3** (43.6 g, 0.16 mol) and

anhydrous potassium carbonate (88.8 g, 0.32 mol) in dry acetone (300 mL) while stirring at 50°C under an atmosphere of nitrogen. The reaction mixture was stirred for 1.5 h at the same temperature, and then cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure. The residue was diluted with ethyl acetate (100 mL) and washed with water and saturated saline, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate) to afford compound **4** (32.0 g, 70%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.23 (t, 3H, J = 7.2 Hz, OCH_2CH_3), 1.32 (s, 3H, CH_3), 1.44 (s, 9H, Boc-9H), 1.66–1.71 (m, 1H), 2.52–2.57 (m, 1H), 3.60–3.61 (m, 3H), 4.13–4.19 (m, 3H); ESI-MS m/z: 284 [M^+ + H].

Ethyl N-tert-butoxycarbonyl-3-methoxyimino-4-methylpiperidine-4-carboxylate ***5a***

A solution of methoxylamine hydrochloride (21.0 g, 0.25 mol) and triethylamine (35.0 mL, 0.25 mol) in 80% ethanol (85 mL) was added dropwise to a solution of **4** (65.5 g, 0.23 mol) in ethanol (250 mL) while stirring at 60°C. The reaction mixture was stirred for 2 h at the same temperature and concentrated under reduced pressure. The residue was diluted with water (100 mL) 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. The crude product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate) to afford compound **5a** (64.2 g, 89%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.22–1.25 (m, 3H), 1.39 (s, 3H, CH_3), 1.45 (s, 9H, Boc-9H), 1.55–1.62 (m, 1H), 2.38–2.44 (m, 1H), 3.36–3.49 (m, 2H), 3.88 (s, 3H, OCH_3), 4.03–4.06 (m, 1H), 4.17–4.20 (m, 2H), 4.53–4.57 (m, 1H); ESI-MS m/z: 315 [M^+ + H].

Ethyl N-tert-butoxycarbonyl-3-ethoxyimino-4-methylpiperidine-4-carboxylate ***5b***

The title compound was obtained in a similar manner as for the preparation of **5a** (80%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H: 1.23–1.26 (m, 6H), 1.40 (s, 3H, CH_3), 1.45 (s, 9H, Boc-9H), 1.52–1.62 (m, 1H), 2.38–2.42 (m, 1H), 3.41–3.51 (m, 2H), 4.07–4.20 (m, 5H), 4.57–4.63 (m, 1H); ESI-MS m/z: 329 [M^+ + H].

N-tert-Butoxycarbonyl-4-carbamyl-3-methoxyimino-4-methylpiperidine ***8a***

To a stirring solution of **5a** (64.2 g, 0.20 mol) in ethanol (60 mL) was added dropwise a solution of sodium hydroxide (13.9 g, 0.35 mol) dissolved in water (30 mL) at room temperature. The reaction mixture was stirred for 5 h at the same temperature. After removal of the ethanol under reduced pressure, the reaction mixture was diluted with water (30 mL), adjusted to pH = 6–6.5 with acetic acid (24.0 mL, 0.42 mol), and then extracted with methylene chloride (3 × 50 mL). The combined extracts were washed with saturated brine, dried over anhydrous sodium sulfate, and filtered. To the filtrate containing **6a** was added triethylamine (70 mL, 0.50 mol). The reaction mixture was cooled to −15 to −20°C using an ice-salt bath, isobutyl chloroformate (65 mL, 0.50 mol) was added dropwise and stirred for 2 h at the same temperature to give the activated ester **7a**. To the reaction mixture containing **7a** ammonia gas was cautiously pumped at 0–5°C for 1 h. The reaction mixture was then washed with 10% acetic acid solution, water, and saturated brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate) to afford compound **8a** (22.6 g, 40%) as a white solid. M. p.: 140–142°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.37–1.46 (m, 12H), 1.50–1.57 (m, 1H), 2.43–2.49 (m, 1H), 3.38–3.53 (m, 2H), 3.89 (s, 3H, OCH_3), 4.17–4.45 (m, 2H), 5.57 (br, 1H), 6.00 (br, 1H); ESI-MS m/z: 286 [M^+ + H].

N-tert-Butoxycarbonyl-4-carbamyl-3-ethoxyimino-4-methylpiperidine ***8b***

The title compound was obtained in a similar manner as for the preparation of **8a** (41%) as a white solid. M.p.: 88–90°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.27 (t, 3H, J = 6.8 Hz, OCH_2CH_3), 1.42–1.45 (m, 12H), 1.50–1.58 (m, 2H), 2.45–2.51 (m, 1H), 3.32–3.51 (m, 2H), 3.88 (q, 2H, J = 6.8 Hz, OCH_2CH_3), 4.20–4.33 (m, 1H), 5.23 (br, 1H), 6.01 (br, 1H); ESI-MS m/z: 300 [M^+ + H].

N-tert-Butoxycarbonyl-4-(N-tert-butoxycarbonyl)amino-3-methoxyimino-4-methylpiperidine ***10a***

To a solution of **8a** (11.5 g, 40.4 mmol) in acetonitrile (300 mL) a freshly prepared sodium hypobromite solution (95 mL) was added dropwise at 5°C. The reaction mixture was stirred for 10 h at the same temperature and then concentrated under reduced pressure. The residue containing **9a** was dissolved in methanol (150 mL), and to this solution di-*tert*-butyl dicarbonate (4.4 g, 20.2 mmol) was added portionwise and stirred for 2 h at room temperature. After removal of the methanol under reduced pressure, the residue was diluted with water (200 mL) and extracted with methylene chloride (3 × 100 mL). The combined extracts were washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, petroleum ether / ethyl acetate) to afford compound **10a** (6.4 g, 44%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.41–1.47 (m, 18H, 2 Boc-9H), 1.54 (s, 3H, CH_3), 1.97–2.04 (m, 1H), 2.44–2.45 (m, 1H), 3.22–3.29 (m, 1H), 3.68–3.74 (m, 1H), 3.85–3.95 (m, 4H), 4.72–4.73 (m, 1H), 5.64 (br, 1H); ESI-MS m/z: 358 [M^+ + H].

N-tert-Butoxycarbonyl-4-(N-tert-butoxycarbonyl)amino-3-ethoxyimino-4-methylpiperidine ***10b***

The title compound was obtained in a similar manner as for the preparation of **10a** (50%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.26 (t, 3H, J = 6.8 Hz, OCH_2CH_3), 1.42–1.47 (m, 18H, 2 Boc-9H), 1.54 (s, 3H, CH_3), 1.99–2.05 (m, 1H), 2.44–2.45 (m, 1H), 3.22–3.29 (m, 1H), 3.70–3.73 (m, 1H), 3.94–3.95 (m, 1H), 4.13 (q, 2H, J = 6.8 Hz, OCH_2CH_3), 4.75–4.76 (m, 1H), 5.66 (br, 1H); ESI-MS m/z: 372 [M^+ + H].

N-tert-Butoxycarbonyl-3-methoxyimino-4-methyl-4-(N-tert-butoxycarbonyl)methylaminopiperidine ***11a***

To a stirring solution of **10a** (5.0 g, 14.4 mmol) in dry acetonitrile (80 mL) 60% sodium hydride (1.7 g, 42.5 mmol) was added at 5°C under an atmosphere of nitrogen and then stirred for 0.5 h at the same temperature. A solution of methyl iodide (2.6 mL, 42.0 mmol) in dry acetonitrile (20 mL) was added to the suspension and stirred for 3 h at 5°C. To this mixture enough water was added dropwise to consume the excess sodium hydride, and then concentrated under reduced pressure. The residue was diluted with water (100 mL) and extracted with methylene chlor-

ide (3 × 50 mL). The combined extracts were washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate) to afford compound **11a** (4.2 g, 81%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.42–1.46 (m, 18H, 2 Boc-9H), 1.58 (s, 3H, CH_3), 2.80–2.86 (m, 4H), 3.41–3.52 (m, 2H), 3.78–3.88 (m, 4H), 4.24–4.66 (m, 2H); ESI-MS *m/z*: 394 [M^+ + Na].

N-tert-Butoxycarbonyl-3-ethoxyimino-4-methyl-4-(N-tert-butoxycarbonyl)methylaminopiperidine ***11b***

The title compound was obtained in a similar manner as for the preparation of **11a** (92%) as a colorless liquid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.24 (t, 3H, *J* = 6.8 Hz, OCH_2CH_3), 1.43–1.49 (m, 18H, 2 Boc-9H), 1.55 (s, 3H, CH_3), 2.79–2.88 (m, 4H), 3.35–3.56 (m, 2H), 3.87–3.88 (m, 1H), 4.10 (q, 2H, *J* = 6.8 Hz, OCH_2CH_3), 4.33–4.70 (m, 2H); ESI-MS *m/z*: 386 [M^+ + H].

3-Methoxyimino-4-methyl-4-methylaminopiperidine dihydrochloride ***12a***

To a stirring solution of **11a** (5.1 g, 13.6 mmol) dissolved in methylene chloride (100 mL) dry hydrogen chloride gas was pumped 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 **12a** (3.1 g, 94%) as a white solid. ^{1}H-NMR (D_2O, 400 MHz) δ_H: 1.72 (s, 3H, CH_3), 2.27–2.28 (m, 1H), 2.44–2.45 (m, 1H), 2.79 (s, 3H, NCH_3), 3.48–3.50 (m, 1H), 3.70–3.71 (m, 1H), 3.83–3.85 (m, 1H), 4.02 (s, 3H, OCH_3), 4.80–4.83 (m, 1H); ESI-MS *m/z*: 172 [M^+ + H].

3-Ethoxyimino-4-methyl-4-methylaminopiperidine dihydrochloride ***12b***

The title compound was obtained in a similar manner as for the preparation of **12a** (99%) as a white solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H: 1.24 (t, 3H, *J* = 6.8 Hz, OCH_2CH_3), 1.55 (s, 3H, CH_3), 2.11–2.17 (m, 1H), 2.24–2.33 (m, 1H), 2.51 (s, 3H, NCH_3), 3.20–3.26 (m, 1H), 3.44–3.50 (m, 1H), 3.90–3.91 (m, 1H), 4.17 (q, 2H, *J* = 6.8 Hz, OCH_2CH_3), 4.35–4.55 (m, 1H), 9.75–9.94 (m, 4H, 2 NH_2^+); ESI-MS *m/z*: 186 [M^+ + H].

General procedure for the synthesis of 7-(3-alkoxyimino-4-methyl-4-methylaminopiperidin-1-yl)fluoroquinolone derivatives ***14–17***

A mixture of **13a**, **b** (1.0 mmol), **12a**, **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 **14–17** as a yellow solid.

1-Cyclopropyl-6-fluoro-7-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ***14***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.05–1.10 (m, 2H), 1.25–1.27 (m, 2H), 1.37 (s, 3H, CH_3), 1.94–2.01 (m, 2H), 2.34 (s, 3H, NCH_3), 3.63–3.66 (m, 1H), 3.89 (s, 3H, OCH_3), 4.00–4.04 (m, 1H), 4.06–4.11 (m, 1H), 4.40–4.41 (m, 1H), 5.24–5.25 (m, 1H), 8.07 (d, 1H, *J* = 13.2 Hz, C_5-H), 8.73 (s, 1H, C_2-H). HRMS-ESI *m/z* calcd. for $C_{20}H_{25}FN_5O_4$ [M^+ + H]: 418.18905. Found: 418.18978.

1-Cyclopropyl-6-fluoro-7-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridin-3-carboxylic acid ***15***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.06–1.10 (m, 2H), 1.23–1.32 (m, 5H), 1.35 (s, 3H, CH_3), 1.90–2.02 (m, 3H), 2.32 (s, 3H, NCH_3), 3.62–3.66 (m, 1H), 3.97–4.02 (m, 1H), 4.06–4.16 (m, 3H), 4.40 (d, 1H, *J* = 16.4 Hz, C_2-H), 5.24 (d, 1H, *J* = 16.4 Hz, C_2-H), 8.07 (d, 1H, *J* = 13.2 Hz, C_5-H), 8.73 (s, 1H, C_2-H). HRMS-ESI *m/z* calcd. for $C_{21}H_{27}FN_5O_4$ [M^+ + H]: 432.20471. Found: 432.20362.

1-(2,4-Difluorophenyl)-6-fluoro-7-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ***16***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.27 (s, 1H, CH_3), 1.72–1.88 (m, 3H), 2.22–2.25 (m, 3H), 3.78–3.85 (m, 5H), 4.05–4.12 (m, 1H), 4.65–4.76 (m, 1H), 7.05–7.11 (m, 2H, ph-2H), 7.36–7.39 (m, 1H, ph-1H), 8.11 (d, 1H, *J* = 13.2 Hz, C_5-H), 8.68 (s, 1H, C_2-H). HRMS-ESI *m/z* calcd. for $C_{23}H_{23}F_3N_5O_4$ [M^+ + H]: 490.17021. Found: 490.17115.

1-(2,4-Difluorophenyl)-6-fluoro-7-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ***17***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.20–1.28 (m, 6H, OCH_2CH_3, CH_3), 1.56 (br, 1H, NH), 1.73–1.80 (m, 1H), 1.83–1.87 (m, 1H), 2.23–2.26 (m, 3H), 3.76–3.77 (m, 2H), 4.06–4.13 (m, 3H), 4.67–4.78 (m, 1H), 7.05–7.11 (m, 2H, ph-2H), 7.36–7.42 (m, 1H, ph-1H), 8.11 (d, 1H, *J* = 13.2 Hz, C_5-H), 8.68 (s, 1H, C_2-H). HRMS-ESI *m/z* calcd. for $C_{24}H_{25}F_3N_5O_4$ [M^+ + H]: 504.18586. Found: 504.18675.

General procedure for the synthesis of 7-(3-alkoxyimino-4-methyl-4-methylaminopiperidin-1-yl)fluoroquinolone derivatives ***18–29***

A mixture of **13c–i** (1.0 mmol), **12a**, **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 **18–29** as a yellow solid.

1-Ethyl 6,8-difluoro-7-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***18***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.36 (s, 3H, CH_3), 1.57 (t, 3H, *J* = 7.2 Hz, NCH_2CH_3), 1.93–1.96 (m, 2H), 2.33 (s, 3H, NCH_3), 3.32–3.35 (m, 1H), 3.63–3.66 (m, 1H), 3.87 (s, 3H, OCH_3), 4.11–4.12 (m, 1H), 4.40–4.42 (m, 1H), 4.44–4.49 (m, 2H), 7.98 (d, 1H, *J* = 11.6 Hz, C_5-H), 8.61 (s, 1H, C_2-H). HRMS-ESI *m/z* calcd. for $C_{20}H_{25}F_2N_4O_4$ [M^+ + H]: 423.18439. Found: 423.18571.

1-Cyclopropyl-6,8-difluoro-7-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***19***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.18–1.35 (m, 10H), 1.93–1.97 (m, 2H), 2.33 (s, 3H, NCH_3), 3.35–3.39 (m, 1H), 3.62–3.67 (m, 1H), 3.99–4.02 (m, 1H), 4.09–4.15 (m, 3H), 4.42–4.43 (m, 1H), 7.91–7.93

(d, 1H, J = 11.6 Hz, C_5-H), 8.79 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{22}H_{27}F_2N_4O_4$ [M^+ + H]: 449.20004. Found: 449.20110.

1-(2-Fluoroethyl)-6,8-difluoro-7-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***20***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.35 (s, 3H, CH_3), 1.92–1.95 (m, 2H), 2.32 (s, 3H, NCH_3), 3.31–3.34 (m, 1H), 3.63–3.66 (m, 1H), 3.87 (s, 3H, OCH_3), 4.09–4.11 (m, 1H), 4.39–4.40 (m, 1H), 4.68–4.88 (m, 4H), 8.00 (d, 1H, J = 11.6 Hz, C_5-H), 8.59 (s, 1H, C_2-H). HRMS-ESI: m/z calcd. for $C_{20}H_{24}F_3N_4O_4$ [M^+ + H]: 441.17497. Found: 441.17585.

1-(2-Fluoroethyl)-6,8-difluoro-7-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***21***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.24 (t, 3H, J = 7.2 Hz, OCH_2CH_3), 1.35 (s, 3H, CH_3), 1.63 (br, 1H, NH), 1.92–1.94 (m, 2H), 2.33 (s, 3H, NCH_3), 3.32–3.35 (m, 1H), 3.62–3.68 (m, 1H), 4.08–4.15 (m, 3H), 4.40–4.41 (m, 1H), 4.68–4.88 (m, 4H), 8.00 (d, 1H, J = 11.2 Hz, C_5-H), 8.60 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{21}H_{26}F_3N_4O_4$ [M^+ + H]: 455.19061. Found: 455.19287.

1-Cyclopropyl-6-fluoro-7-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***22***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.18–1.19 (m, 2H), 1.38–1.54 (m, 6H), 1.90–1.94 (m, 2H), 2.36 (s, 3H, NCH_3), 3.54–3.61 (m, 2H), 3.72–3.74 (m, 1H), 3.91 (s, 3H, OCH_3), 4.07–4.09 (m, 1H), 4.51–4.52 (m, 1H), 7.42 (d, 1H, J = 7.2 Hz, C_8-H), 8.02 (d, 1H, J = 13.2 Hz, C_5-H), 8.76 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{21}H_{26}FN_4O_4$ [M^+ + H]: 455.19380. Found: 417.19537.

1-Cyclopropyl-6-fluoro-7-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***23***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.16–1.18 (m, 2H), 1.28 (t, 3H, J = 7.2 Hz, OCH_2CH_3), 1.37–1.40 (m, 5H), 1.94–1.96 (m, 3H), 2.34 (s, 3H, NCH_3), 3.56–3.60 (m, 2H), 3.70–3.72 (m, 1H), 4.03–4.18 (m, 3H), 4.54–4.55 (m, 1H), 7.42 (d, 1H, J = 6.4 Hz, C_8-H), 8.01 (d, 1H, J = 13.2 Hz, C_5-H), 8.76 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{22}H_{28}FN_4O_4$ [M^+ + H]: 431.20946. Found: 431.20919.

1-Ethyl-6-fluoro-7-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***24***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.31 (s, 3H, CH_3), 1.57 (t, 3H, J = 7.2 Hz, NCH_2CH_3), 1.76–1.79 (m, 2H), 2.31 (s, 3H, NCH_3), 3.67–3.71 (m, 1H), 3.77–3.82 (m, 1H), 3.80 (s, 3H, OCH_3), 4.05–4.06 (m, 1H), 4.31 (q, 2H, J = 7.2 Hz, NCH_2CH_3), 4.50–4.52 (m, 1H), 7.03 (d, 1H, J = 7.2 Hz, C_8-H), 8.05 (d, 1H, J = 13.2 Hz, C_5-H), 8.67 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{20}H_{26}FN_4O_4$ [M^+ + H]: 405.19381. Found: 405.19377.

1-Ethyl-6-fluoro-7-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***25***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.30–1.34 (m, 6H), 1.56 (t, 3H, J = 7.2 Hz, NCH_2CH_3), 1.82 (br, 3H), 2.32 (s, 3H, NCH_3), 3.65–3.68 (m, 1H), 3.76–3.81 (m, 1H), 4.05–4.06 (m, 1H), 4.18–4.26 (m, 2H), 4.30 (q, 2H, J = 7.2 Hz, NCH_2CH_3), 4.52–4.54 (m, 1H), 7.00 (d, 1H, J = 6.8 Hz, C_8-H), 8.05 (d, 1H, J = 13.2 Hz, C_5-H), 8.66 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{21}H_{28}FN_4O_4$ [M^+ + H]: 419.20946. Found: 419.20961.

1-Cyclopropyl-6-fluoro-7-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-8-methoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***26***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.00–1.06 (m, 2H), 1.21–1.26 (m, 2H), 1.39 (s, 3H, CH_3), 1.59 (br, 1H, NH), 1.96–1.99 (m, 2H), 2.37 (s, 3H, NCH_3), 3.38–3.41 (m, 1H), 3.65–3.67 (m, 1H), 3.76 (s, 3H, OCH_3), 3.87 (s, 3H, $NOCH_3$), 4.02–4.06 (m, 1H), 4.13–4.14 (m, 1H), 4.40–4.42 (m, 1H), 7.89 (d, 1H, J = 12.0 Hz, C_5-H), 8.82 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{22}H_{28}FN_4O_5$ [M^+ + H]: 447.20437. Found: 447.20548.

1-Cyclopropyl-6-fluoro-7-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-8-methoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid ***27***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 0.99–1.05 (m, 2H), 1.19–1.27 (m, 5H), 1.40 (s, 3H, CH_3), 1.96–2.01 (m, 2H), 2.38 (s, 3H, NCH_3), 3.39–3.42 (m, 1H), 3.67–3.72 (m, 1H), 3.77 (s, 3H, OCH_3), 4.02–4.06 (m, 1H), 4.10–4.17 (m, 3H), 4.42–4.43 (m, 1H), 7.88 (d, 1H, J = 12.0 Hz, C_5-H), 8.82 (s, 1H, C_2-H). HRMS-ESI m/z calcd. for $C_{23}H_{30}FN_4O_5$ [M^+ + H]: 461.22002. Found: 461.21986.

9-Fluoro-3(S)-methyl-10-(3-methoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-7-oxo-2,3-dihydro-7H-pyrrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ***28***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.36 (s, 3H, C_4-CH_3), 1.62 (d, 3H, J = 6.4 Hz, C_3-CH_3), 1.90–2.04 (m, 3H), 2.35 (s, 3H, NCH_3), 3.31–3.35 (m, 1H), 3.55–3.58 (m, 1H), 3.87 (s, 3H, OCH_3), 4.06–4.14 (m, 1H), 4.29–4.38 (m, 2H), 4.44–4.49 (m, 2H), 7.75 (d, 1H, J = 12.0 Hz, C_8-H), 8.62 (s, 1H, C_5-H). HRMS-ESI m/z calcd. for $C_{21}H_{26}FN_4O_5$ [M^+ + H]: 433.18872. Found: 433.19204.

9-Fluoro-3(S)-methyl-10-(3-ethoxyimino-4-methyl-4-methylaminopiperidin-1-yl)-7-oxo-2,3-dihydro-7H-pyrrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ***29***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.24 (t, 3H, J = 7.2 Hz, OCH_2CH_3), 1.34 (s, 3H, C_4-CH_3), 1.62 (d, 3H, J = 6.8 Hz, C_3-CH_3), 1.88–2.04 (m, 2H), 2.34 (s, 3H, NCH_3), 3.31–3.36 (m, 1H), 3.54–3.59 (m, 1H), 4.07–4.14 (m, 3H), 4.31–4.52 (m, 4H), 7.73 (d, 1H, J = 12.0 Hz, C_8-H), 8.63 (s, 1H, C_5-H). HRMS-ESI m/z calcd. for $C_{22}H_{28}FN_4O_5$ [M^+ + H]: 447.20437. Found: 447.20392.

Antibacterial activity

Compounds **14–29** were evaluated for their *in-vitro* antibacterial activity using conventional agar-dilution method in comparison to the reference drugs. 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. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate.

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