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Original article

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

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ABSTRACT

A series of novel 7-(4-alkoxyimino-3-amino-3-methylpiperidin-1-yl)fluoroquinolone derivatives were designed, synthesized and characterized by ^{1}H NMR, MS and HRMS. These fluoroquinolones were evaluated for *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains. All of the title compounds have considerable activity against the twelve strains, and exhibit exceptional potency in inhibiting the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae* (minimum inhibitory concentration (MIC): 0.06–8 μg/mL). The most active compound **17** is 4-fold more potent than levofloxacin against *S. aureus* and *S. epidermidis*, 32-fold more potent than levofloxacin against *Streptococcus pneumoniae*, and 16-fold more potent than IMB against *K. pneumoniae*.

1. Introduction

Quinolone antibacterial agents are among the most attractive drugs in the anti-infective chemotherapy field. These antibiotics exert their antimicrobial activity by binding to two type II bacterial topoisomerase enzymes, DNA gyrase (subunits encoded by gyrA and gyrB) and topoisomerase IV (subunits encoded by grlA and grlB for *Staphylococcus aureus*). This binding induces permanent double-stranded DNA breaks, and results in cell death [1,2].

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 *S. aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Enterococcus*) is relatively moderate, which has not only limited their use in infections caused by these organisms, but has also contributed to 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 and ofloxacin [3–6].

Structure–activity relationship (SAR) studies of quinolone antibacterial agents have demonstrated that the basic group at the C-7 position is the most adaptable site for chemical change and is an area that greatly influences potency, spectrum and safety [7–9]. In general, 5- and 6-membered nitrogen heterocycles including piperazinyl, pyrrolidinyl and piperidinyl type side chains have proven to be optimal substituents. Of the three, piperidinyl analogs are the least studied [9,10].

As part of an ongoing program to find potent new quinolones displaying strong Gram-positive activity, we have focused our attention on introducing new functional groups to the piperidine ring. Previous work on pyrrolidine analogs suggests that introduction of a methyl group in the 3-position of the pyrrolidine ring can increase Gram-positive activity [11]. 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 [12]. We applied this structural modification to IMB (Fig. 1), which shows good *in vitro* and *in vivo* antibacterial activity against Gram-negative and Gram-positive organisms, including MRSA and methicillin-resistant *S.taphylococcus epidermidis* (MRSE) [13]. 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 3-position and an alkoxyimino group at 4-position of the piperidine ring. Our primary objective was to optimize the potency of these compounds against Gram-positive and Gram-negative organisms.

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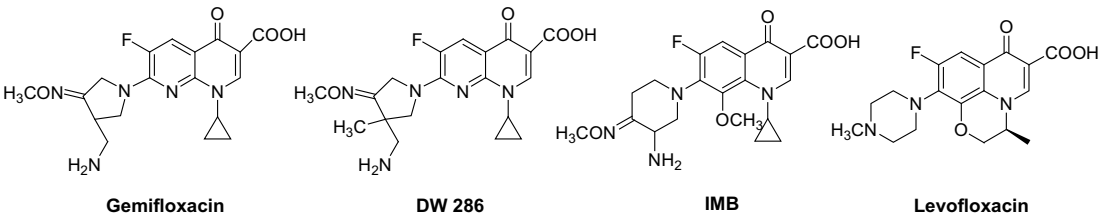


Fig. 1.

2. Results and discussion

2.1. Chemistry

Detailed synthetic pathways to new piperidine derivatives **9a**,**b** and novel fluoroquinolones **12**–**27** are depicted in Schemes 1 and 2, respectively. According to published procedures [14–17], *N-tert*-butoxycarbonyl-4-piperidone **1** reacted with dimethyl carbonate in the presence of sodium hydride in dry toluene to give the keto ester **2**, which was methylated with methyl iodide to provide methyl ketone **3**. Ketone **3** was smoothly converted to the oximes **4a**,**b** by reaction with *O*-alkylhydroxyamines in ethanol at 55–60 °C. However, direct ammonolysis of the ester moiety in oximes **4a**,**b** was unsuccessful.

Instead, the esters **4a**,**b** were first converted to acids **5a**,**b** by saponification and acidification. The acids **5a**,**b** were subsequently reacted with isobutyl chloroformate to give activated esters **6a**,**b**, which upon ammonolysis afforded amides **7a**,**b**, in an overall yield of 84% for the three steps.

Hoffmann degradation of amides **7a**,**b** used freshly prepared sodium hypobromite instead of commercial sodium hypochlorite. The *N-tert*-butoxycarbonyl protecting group on amines **8a**,**b** was removed with hydrogen chloride gas in methylene chloride to afford the new piperidine derivative dihydrochlorides **9a**,**b** in good yield.

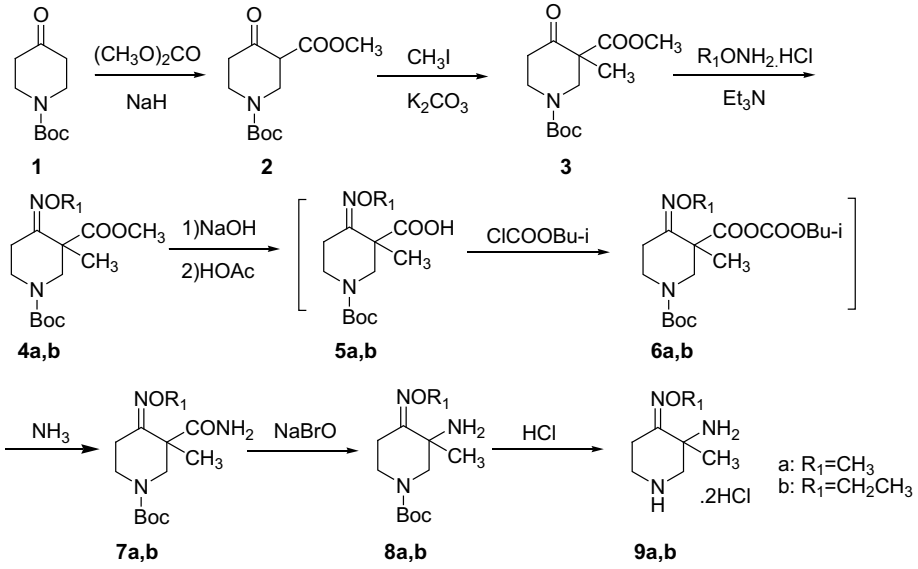
The coupling of the piperidine derivatives **9a**,**b** with various compounds containing quinolone and naphthyridine cores was carried out according to well-established literature procedures (Scheme 2) [18]. In the case of quinolones **12**–**23**, condensation of **9a**,**b** with **10a**–**f** was performed in the presence of triethylamine. However for **24**–**27**, boric chelates **11g**–**h** were required to increase reactivity. Table 1 shows the novel fluoroquinolone analogs, the yield of the final coupling step, and melting points of the purified compounds.

2.2. Pharmacology

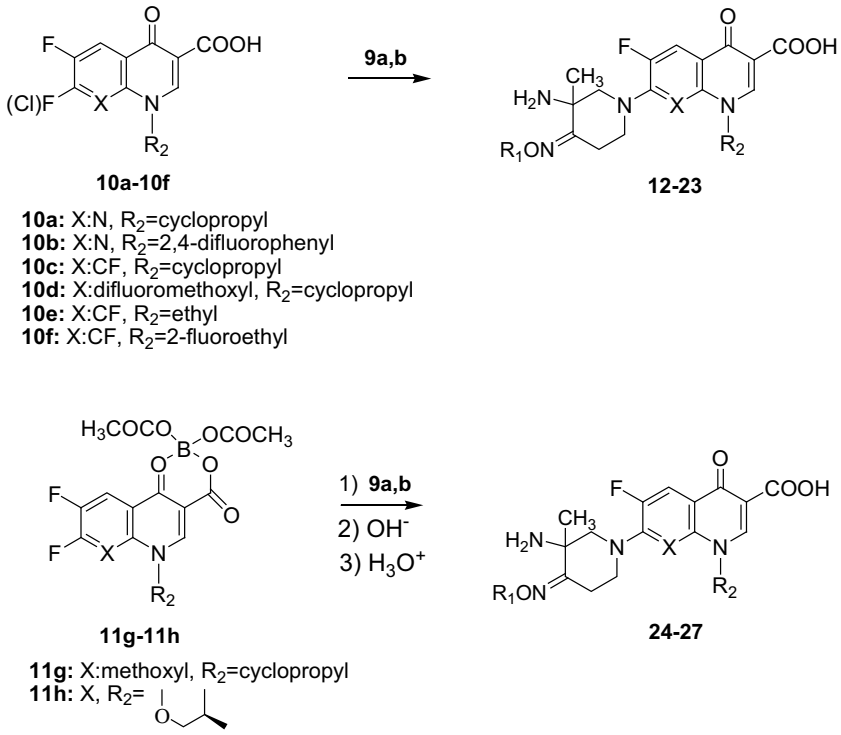
Fluoroquinolones **12**–**27** were evaluated for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques [19]. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the standard drugs – IMB, levofloxacin (LVFX) and GMFX for comparison are reported in Table 2.

The novel fluoroquinolones **12**–**27** have generally potent antibacterial activity against the twelve strains. They exhibit good potency in inhibiting the growth of *S. aureus*, *S. epidermidis* and *Klebsiella pneumoniae* (MIC: 0.06–8 μg/mL). The most active compound **17** is 4-fold more potent than LVFX against *S. aureus* and *S. epidermidis*, 32-fold more potent than LVFX against *S. pneumoniae*, and 16-fold more potent than IMB against *K. pneumoniae*. The compounds **16** and **26** are 4-fold more potent than LVFX against *Escherichia coli*, 32-fold more potent than IMB and 4-fold more potent than LVFX against *K. pneumoniae*.

Some compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line till 31.25 μg/mL concentration. After 72 h of exposure, viability was assessed on the basis of



Scheme 1. Synthesis of piperidine derivatives **9a**,**b**.



Scheme 2. Synthesis of novel fluoroquinolones **12**–**27**.

cellular conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan product and the results are reported in Table 1. Fifteen compounds when tested showed CC_{50} values ranging from 31.4 to 791.2 μM. A comparison of the substitution pattern at C-7 position demonstrated that ethyloxime-incorporated piperidino-substitutions were generally more cytotoxic than the analogs containing methyloxime.

3. Conclusion and discussion

In summary, a series of novel 7-(4-alkoxyimino-3-amino-3-methylpiperidin-1-yl) fluoroquinolone derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains. Generally, all of the novel fluoroquinolones demonstrated potent antibacterial activity. In particular, they exhibited good potency in inhibiting the growth of *S. aureus*, *S. epidermidis* and *K. pneumoniae*. However, overall the piperidinyl analogs were not superior to pyrrolidinyl analogs such as IMB and GMFX.

Novel fluoroquinolones **12**–**27** exhibited less activity than IMB against the tested Gram-positive, including resistant strains and Gram-negative strains, except *K. pneumoniae*. These results showed that introduction of another methyl group into 3-position of piperidine ring caused reduced antibacterial activity, which was contrary to the activity profiles of pyrrolidine-containing fluoroquinolones.

The activities of the quinolone nuclei in this study are in the order 1-cyclopropyl-8-fluoroquinolone > levofloxacin nuclei > 1-cyclopropyl-8-methoxylquinolone > 1-cyclopropyl-8-difluoromethoxyl quinolone > 1-ethyl-8-fluoroquinolone ≈ 1-(2-fluoroethyl)-8-fluoroquinolone ≈ naphthyridine. In addition, fluoroquinolones featuring methyloxime-incorporated piperidino-substitution at C-7 position are comparable to analogs containing ethyloxime.

4. Experimental protocols

4.1. Chemistry

Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR spectra were determined on a Varian Mercury-400 spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane as an internal standard. Electrospray ionization (ESI) mass spectra and high resolution mass spectra (HRMS) were obtained on a MDSSCIEX Q-Tap mass spectrometer. Fast Atom Bombardment (FAB) mass spectra and high resolution mass spectra (HRMS) were obtained on a MICROMASS AutoSpec Ultima-TOF mass spectrometer. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F_{254}).

4.2. Synthesis

4.2.1. *Methyl* N-tert-*butoxycarbonyl-4-oxopiperidine-3-carboxylate* ***2***

To a suspension of 70% sodium hydride (30.20 g, 0.88 mol) in dry toluene (500 mL) was added dropwise dimethyl carbonate (43.20 g, 0.48 mol) over 0.5 h at room temperature under an atmosphere of nitrogen. After addition of a few drops of methanol, a solution of *N-tert*-butoxycarbonyl-4-piperidone **1** (48.00 g, 0.24 mol) dissolved in dry toluene (200 mL) was added dropwise to the reaction mixture while stirring at 80 °C over 35 min. The reaction mixture was stirred for 3 h at the same temperature and then cooled to 0 °C (ice bath), adjusted to pH 6–6.5 with acetic acid. The resulting mixture was diluted with cold water (100 mL) and adjusted to pH 8 with 5% sodium hydroxide solution. The toluene layer was separated and the aqueous layer was extracted with toluene (3 × 20 mL). The combined toluenes were dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The solid obtained was dried *in vacuo* to give the title compound **2** (54.50 g,

Table 1
The structures, physical data and cytotoxicity of novel fluoroquinolones **12–27**.

Compd.	R_1	X	R_2	Yield (%)	mp (°C)	CC_{50} (μM)
12	CH_3	N		68	231–232	169.7
13	CH_2CH_3	N		70	187–188	92.8
14	CH_3	N	2,4-F_2-C_6H_3	66	215–216	258.7
15	CH_2CH_3	N	2,4-F_2-C_6H_3	64	210–211	31.4
16	CH_3	CF		67	212–213	575.3
17	CH_2CH_3	CF		65	194–195	146.9
18	CH_3	$COCHF_2$		59	216–217	791.2
19	CH_2CH_3	$COCHF_2$		59	166–169	721.9
20	CH_3	CF	CH_2CH_3	62	214–215	337.9
21	CH_2CH_3	CF	CH_2CH_3	65	236–239	219.2
22	CH_3	CF	CH_2CH_2F	55	216–217	626.1
23	CH_2CH_3	CF	CH_2CH_2F	50	192–194	243.2
24	CH_3	$COCH_3$		67	204–205	219.0
25	CH_2CH_3	$COCH_3$		70	155–157	342.6
26	CH_3			55	228–230	NT
27	CH_2CH_3			53	185–188	732.2
IMB	–	–	–	–	–	348.5
LVFX	–	–	–	–	–	>1000

CC_{50}: The 50% cytotoxic concentration; NT: not tested.

88.0%) as a off-white solid, mp: 32–34 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.47 (9H, s, Boc-9H), 2.35–2.38 (2H, t, *J* 6.0,C_5–2H), 3.54–3.57 (2H, t, *J* 6.0, C_6–2H), 3.76 (3H, s, OCH_3), 4.05 (2H, s, C_2–2H), 11.97 (1H, s, C_3-H). ESI-MS: *m*/*z* 258 $(M + H)^+$.

4.2.2. *Methyl* N-tert-*butoxycarbonyl-3-methyl-4-oxopiperidine-3-carboxylate* ***3***

To a suspension of methyl *N-tert*-butoxycarbonyl-4-oxopiperidine-3-carboxylate **2** (53.68 g, 0.21 mol) and anhydrous potassium carbonate (100.93 g, 0.73 mol) in dry acetone (500 mL) was added a solution of methyl iodide (23.38 mL, 0.38 mol) dissolved in dry acetone (250 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was heated to 40 °C and stirred for 6 h, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure. The residue was diluted with methylene chloride (300 mL) and washed with water then saturated saline, dried over anhydrous sodium sulfate, filtered and concentrated. The yellow oily residue was treated with petroleum ether (300 mL) and filtered. The solid obtained was washed twice with petroleum ether and dried *in vacuo* to give the title compound **3** (45.36 g, 80.1%) as a white solid, mp: 42–43 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.31 (3H, s, CH_3), 1.49 (9H, s, Boc-9H), 2.45–2.51 (1H, m), 2.76 (1H, s), 3.06–3.10 (1H, m), 3.30–3.37 (1H, m), 3.72 (3H, s, OCH_3), 4.12 (1H, s), 4.48–4.51 (1H, m). ESI-MS: *m*/*z* 272 $(M + H)^+$.

4.2.3. *Methyl* N-tert-*butoxycarbonyl-4-methoxyimino-3-methylpiperidine-3-carboxylate* ***4a***

To a stirring solution of methyl *N-tert*-butoxycarbonyl-3-methyl-4-oxopiperidine-3-carboxylate **3** (15.00 g, 55.3 mmol) dissolved in ethanol (50 mL) was added dropwise a solution of methoxylamine hydrochloride (5.05 g, 60.5 mmol) and triethylamine (8.37 mL, 60.5 mmol) in 80% ethanol (25 mL) at 55–60 °C over 15 min. The reaction mixture was stirred at the same temperature for 1.5 h and concentrated under reduced pressure. The residue was diluted with distilled water (30 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with 1 N HCl then saturated brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was recrystallized from petroleum ether to give the title compound **4a** (14.71 g, 88.6%) as a white solid, mp: 75–76 °C. ^{1}H

Table 2
In vitro antibacterial activity of compounds **12–27** against selected strains.

Compd.	Strains MIC (μg/mL)											
	S. a.	*MRSA*	*S. e.*	*MRSE*	*S. p.*	*S. py.*	*E. f.*	*E. co.*	*K. p.*	*P. a.*	*S. s.*	*E. cl.*
12	4	128	4	32	32	64	32	4	4	32	16	4
13	2	128	2	64	16	128	16	2	4	32	8	4
14	1	>128	2	>128	8	>128	8	8	8	32	8	16
15	1	128	1	>128	16	128	4	8	8	64	16	16
16	0.125	16	0.125	16	2	2	0.5	0.25	0.25	4	0.25	0.5
17	0.06	16	0.125	16	1	4	0.5	0.5	0.5	8	0.5	1
18	0.25	64	1	64	8	8	2	2	2	32	4	8
19	0.5	64	2	64	8	>128	4	4	4	64	8	16
20	1	>128	1	>128	16	32	8	2	2	32	16	4
21	2	>128	2	>128	16	32	16	8	8	64	16	16
22	1	>128	1	>128	16	16	8	4	4	32	8	8
23	2	>128	2	>128	16	32	16	8	4	128	16	16
24	0.25	16	0.5	16	8	8	2	1	2	16	2	4
25	0.125	8	0.25	8	4	16	0.5	0.5	1	8	1	2
26	0.25	16	0.25	16	2	8	1	0.25	0.25	4	0.5	1
27	0.5	16	0.5	16	8	32	2	0.5	0.5	8	1	2
IMB	<0.03	1	<0.03	1	2	4	2	0.5	8	2	0.125	0.25
GMFX	<0.03	4	<0.03	2	2	2	0.06	0.06	0.5	0.125	0.06	0.06
LVFX	0.25	32	0.5	32	32	4	1	1	1	0.5	1	0.125

S. a., *Staphylococcus aureus* ATCC 29213; *MRSA*, Methicillin-resistant *Staphylococcus aureus* 05-3; *S. e.*, *Staphylococcus epidermidis* ATCC 12228; *MRSE*, *Methicillin-resistant Staphylococcus epidermidis* 05-1; *S. p.*, *Streptococcus pneumoniae* 97100; *S. py.*, *Streptococcus pyogenes* 9619; *E. f.*, *Enterococcus faecalis* ATCC 29212; *E. co.*, *Escherichia coli* 26; *K. p.*, *Klebsiella pneumoniae* 7; *P. a.*, *Pseudomonas aeruginosa* 17; *S. s.*, *Shigella sonnei* 51592; *E. cl.*, *Enterobacter cloacae* 45301.

NMR ($CDCl_3$, 400 MHz) δ_H 1.36 (3H, s, CH_3), 1.46 (9H, s, Boc-9H), 2.39–3.16 (4H, m), 3.70, 3.86 (6H, s, s, 2× OCH_3), 3.77–3.82 (1H, m), 4.32–4.36 (1H, m). ESI-MS: *m*/*z* 301 $(M+H)^+$.

4.2.3.1. Methyl N-tert-*butoxycarbonyl-4-ethoxyimino-3-methylpiperidine-3-carboxylate* ***4b***. The title compound was obtained in a similar manner as for the preparation of **4a** (90.3%). mp: 51–53 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.22–1.26 (3H, t, *J* 7.2, $OCH_2\underline{CH_3}$), 1.36 (3H, s, CH_3), 1.46 (9H, s, Boc-9H), 2.42–3.19 (4H, m), 3.70 (3H, s, OCH_3), 4.07–4.12 (2H, q, *J* 7.2, $O\underline{CH_2}CH_3$), 3.79 (1H, s), 4.30–4.33 (1H, m). ESI-MS: *m*/*z*: 315 $(M+H)^+$.

4.2.4. N-tert-*Butoxycarbonyl-3-carbamyl-4-methoxyimino-3-methylpiperidine* ***7a***

To a stirring solution of methyl *N-tert*-butoxycarbonyl-4-methoxyimino-3-methylpiperidine-3-carboxylate **4a** (17.00 g, 56.6 mmol) in methanol (100 mL) was added dropwise a solution of sodium hydroxide (4.53 g, 113.2 mmol) dissolved in distilled water (20 mL) at room temperature. The reaction mixture was heated to 50 °C and stirred for 2 h at the same temperature. After removal of the methanol under reduced pressure, the reaction mixture was diluted with distilled water (30 mL), adjusted to pH 6–6.5 with acetic acid and filtered to give the acid **5a** as a white solid. The solid **5a** was dissolved in methylene chloride (150 mL), and to this solution was added triethylamine (8.8 mL, 63.6 mmol). The reaction mixture was cooled to −12 to −14 °C using an ice-salt bath, isobutyl chloroformate (9.0 mL, 69.2 mmol) was added and stirred for 0.5 h at the same temperature to give the ester **6a**. To the reaction mixture containing the ester **6a** was pumped ammonia gas cautiously at 0–5 °C for 0.5 h. The mixture was then washed with 1 N HCl and saturated brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting yellow residue was recrystallized from ethyl acetate to give the title compound **7a** (13.50 g, 83.6%) as a white solid, mp: 126–127 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.37 (3H, s, CH_3), 1.47 (9H, s, Boc-9H), 2.14–3.86 (5H, m), 3.89 (3H, s, OCH_3), 4.35–4.37 (1H, m), 5.37 (1H, br, CONH), 6.19, 6.78 (1H, 2s, CONH). ESI-MS: *m*/*z* 286 $(M+H)^+$.

4.2.4.1. N-tert-*Butoxycarbonyl-3-carbamyl-4-ethoxyimino-3-methylpiperidine* ***7b***. The title compound was obtained in a similar manner as for the preparation of **7a** (78.58%). mp: 108–109 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.24–1.27 (3H, t, *J* 7.2, $OCH_2\underline{CH_3}$), 1.38 (3H, s, CH_3), 1.52 (9H, s, Boc-9H), 2.20–3.78 (5H, m), 4.11–4.16 (2H, q, *J* 7.2, $O\underline{CH_2}CH_3$), 4.33–4.35(1H, m), 5.46 (1H, br, CONH), 6.19, 6.66 (1H, 2s, CONH). ESI-MS: *m*/*z* 300 $(M+H)^+$.

4.2.5. N-tert-*Butoxycarbonyl-3-amino-4-methoxyimino-3-methylpiperidine* ***8a***

To a solution of *N-tert*-butoxycarbonyl-3-carbamyl-4-methoxyimino-3-methylpiperidine **7a** (2.00 g, 7.0 mmol) in acetonitrile (80 mL) was added dropwise a freshly prepared sodium hypobromite solution (14 mL) at 5 °C for 0.5 h. The reaction mixture was stirred at the same temperature for 3 h, and then concentrated under reduced pressure. The residue was dissolved in distilled water (30 mL), then the solution was adjusted to pH 2.5 with 2 N HCl, washed with ethyl acetate (3 × 30 mL). The aqueous layer was adjusted to pH 12 with 5% sodium hydroxide solution, and then extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with saturated brine, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give the title compound **8a** (0.91 g, 50.6%) as a colorless liquid. ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.27 (3H, s, CH_3), 1.43 (9H, s, Boc-9H), 1.75 (2H, br, NH_2), 2.30–3.33 (1H, m), 2.90–3.00 (3H, m), 3.67–3.70 (1H, m), 3.83 (3H, s, OCH_3), 3.84–3.87 (1H, m). ESI-MS: *m*/*z* 258 $(M+H)^+$.

4.2.5.1. N-tert-*Butoxycarbonyl-3-amino-4-ethoxyimino-3-methylpiperidine* ***8b***. The title compound was obtained in a similar manner as for the preparation of **8a** (66.0%). ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.22–1.26 (3H, t, *J* 7.2, $OCH_2\underline{CH_3}$), 1.28 (3H, s, CH_3), 1.46 (9H, s, Boc-9H), 1.79 (2H, br, NH_2), 2.28–3.93 (6H, m), 4.05–4.10 (2H, q, *J* 7.2, $O\underline{CH_2}CH_3$). ESI-MS: *m*/*z* 272 $(M+H)^+$.

4.2.6. 3-Amino-4-methoxyimino-3-methylpiperidine dihydrochloride ***9a***

To a stirring solution of *N-tert*-butoxycarbonyl-3-amino-4-methoxyimino-3-methylpiperidine **8a** (2.58 g, 10 mmol) dissolved in methylene chloride (50 mL) was pumped dried hydrochloride gas at 0–5 °C for 0.5 h. The reaction mixture was allowed to stir for another 0.5 h at room temperature, the resulting solid was collected by suction, and the solid was dried *in vacuo* to give the title compound **9a** (2.27 g, 98.7%) as a white solid, mp: 121–122 °C. ^1H NMR (DMSO-d_6 + D_2O, 400 MHz) δ_H 1.65 (3H, s, CH_3), 2.54–2.62 (1H, m), 2.90–2.98 (1H, m), 3.11–3.17 (1H, m), 3.26–3.32 (2H, m), 3.57–3.60 (1H, m), 3.88 (3H, s, OCH_3). ESI-MS: *m*/*z* 158 $(M+H)^+$.

4.2.6.1. 4-Amino-3-ethoxyimino-4-methylpiperidine dihydrochloride ***9b***. The title compound was obtained in a similar manner as for the preparation of **9a** (98.6%). mp: 135–137 °C. ^1H NMR (DMSO-d_6 + D_2O, 400 MHz) δ_H 1.24 (3H, t, *J* 7.2, $OCH_2\underline{CH_3}$), 1.62 (3H, s, CH_3), 2.45–2.53 (1H, m), 2.89–3.16 (1H, m), 3.17–3.53 (3H, m), 3.57–3.60 (1H, m), 4.12 (2H, q, *J* 7.2, $O\underline{CH_2}CH_3$). ESI-MS: *m*/*z* 172 $(M+H)^+$.

4.2.7. General procedure for the synthesis of 7-(4-alkoxyimino-3-amino-3- methylpiperidin-1-yl) fluoroquinolone derivatives ***12–23***

A mixture of **10a–f** (1 mmol), **9a**,**b** (1.3 mmol), triethylamine (5 mmol) and dry acetonitrile (10 mL) was stirred at 30–60 °C for 1–6 h. The resulting solid was collected by suction, and dried *in vacuo* to give the title compounds **12–23**.

4.2.7.1. 1-Cyclopropyl-6-fluoro-7-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ***12***. The title compound was obtained as off-white solid. ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.09–1.10 (2H, m, cyclopropyl CH_2), 1.30–1.32 (2H, m, cyclopropyl CH_2), 1.41 (3H, s, CH_3), 2.59–2.66 (1H,m), 3.09–3.12 (1H, m), 3.68–3.73 (3H, m), 3.89 (3H, s, OCH_3), 4.22–4.25 (2H, m), 8.11 (1H, d, *J* 13.2, C_5–H), 8.73(1H, s, C_2–H). ESI-MS: *m*/*z* 404 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{19}H_{23}FN_5O_4$ $(M+H)^+$: 404.17341; Found 404.17398.

4.2.7.2. 1-Cyclopropyl-6-fluoro-7-(3-amino-4-ethoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ***13***. The title compound was obtained as off-white solid. ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.08–1.12 (2H, m, cyclopropyl CH_2), 1.26–1.30 (5H, m, cyclopropyl CH_2, $OCH_2\underline{CH_3}$), 1.35 (3H, s, CH_3), 2.62–2.70 (1H,m), 3.08–3.14 (1H, m), 3.59–3.77 (3H, m), 4.09–4.24 (4H, m), 8.10 (1H, d, *J* 13.2, C_5–H), 8.74 (1H, s, C_2-H). FAB-MS: *m*/*z* 418 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{20}H_{25}FN_5O_4$ $(M+H)^+$: 418.1891; Found 418.1874.

4.2.7.3. 1-(2,4-Difluorophenyl)-6-fluoro-7-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid ***14***. The title compound was obtained as off-white solid. ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.20 (3H, s, CH_3), 2.36–2.48 (1H, m), 2.78–2.88 (1H, m), 3.33–3.51 (2H, m), 3.67–3.82 (2H, m), 3.85 (3H, s, OCH_3), 7.08–7.14 (2H, m, ph-2H), 7.38–7.44 (1H, m, ph-H), 8.14 (1H, d, *J* 13.2, C_5–H), 8.68 (1H, s, C_2–H). ESI-MS: *m*/*z* 476 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{22}H_{21}F_3N_5O_4$ $(M+H)^+$: 476.15456; Found 476.15598.

*4.2.7.4. 1-(2,4-Difluorophenyl)-6-fluoro-7-(3-amino-4-ethoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid **15***. The title compound was obtained as off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.23–1.27 (6H, m, $OCH_2\underline{CH_3}$, CH_3), 2.37–2.49 (1H, m), 2.82–2.91 (1H, m), 3.34–3.52 (2H, m), 3.70–3.89 (2H, m), 4.07–4.12 (2H, m, $O\underline{CH_2}CH_3$), 7.09–7.14 (2H, m, ph-2H), 7.39–7.45 (1H, m, ph-H), 8.14 (1H, d, *J* 13.2, C_5–H), 8.68 (1H, s, C_2–H). FAB-MS: *m*/*z* 490 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{23}H_{23}F_3N_5O_4$ $(M+H)^+$: 490.1702; Found 490.1713.

*4.2.7.5. 1-Cyclopropyl-6,8-difluoro-7-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **16***. The title compound was obtained as off-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 1.15–1.22 (4H, m, 2× cyclopropyl CH_2), 1.58 (3H, s, CH_3), 2.53–2.59 (1H, m), 3.08–3.14 (1H, m), 3.22–3.28 (1H, m), 3.49–3.55 (2H, m), 3.60–3.63 (1H, m), 3.90 (3H, s, OCH_3), 4.11–4.13 (1H, m), 7.90 (1H, d, *J* 10.8, C_5–H), 8.69 (1H, s, C_2–H). ESI-MS: *m*/*z* 421 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{20}H_{23}F_2N_4O_4$ $(M+H)^+$: 421.16874; Found 421.17060.

*4.2.7.6. 1-Cyclopropyl-6,8-difluoro-7-(3-amino-4-ethoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **17***. The title compound was obtained as off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.18 (2H, m, cyclopropyl CH_2), 1.24–1.34 (5H, m, $OCH_2\underline{CH_3}$, cyclopropyl CH_2), 1.40 (3H, s, CH_3), 2.73–2.79 (1H, m), 2.99–3.05 (1H, m), 3.25–3.46 (4H, m), 3.99–4.01 (1H, m), 4.10–4.15 (2H, m, $O\underline{CH_2}CH_3$), 7.95 (1H, d, *J* 11.2, C_5–H), 8.80 (1H, s, C_2–H). FAB-MS: *m*/*z* 435 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{21}H_{25}F_2N_4O_4$ $(M+H)^+$: 435.1844; Found 435.1843.

*4.2.7.7. 1-Cyclopropyl-6-fluoro-7-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-8-difluoromethoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **18***. The title compound was obtained as off-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 0.967 (2H, m, cyclopropyl CH_2), 1.13–1.15 (2H, m, cyclopropyl CH_2), 1.26 (3H, s, CH_3), 2.63–2.68 (1H, m), 2.84–2.88 (1H, m), 3.20–3.32 (4H, m), 3.78 (3H, s, OCH_3), 4.03 (1H, m), 6.98 (1H, t, *J* 73.2, $OCHF_2$), 7.91 (1H, d, *J* 11.6, C_5–H), 8.75 (1H, s, C_2–H), ESI-MS: *m*/*z* 469 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{21}H_{24}F_3N_4O_5$ $(M+H)^+$: 469.16988; Found 469.16722.

*4.2.7.8. 1-Cyclopropyl-6-fluoro-7-(3-amino-4-ethoxyimino-3-methylpiperidin-1-yl)-8-difluoromethoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **19***. The title compound was obtained as off-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 0.98 (2H, m, cyclopropyl CH_2), 1.14–1.15 (2H, m, cyclopropyl CH_2), 1.19–1.23 (3H, t, $OCH_2\underline{CH_3}$), 1.37 (3H, s, CH_3), 2.58–2.63 (1H, m), 2.95–2.98 (1H, m), 3.23–3.43 (4H, m), 4.04–4.11 (3H, m), 7.01 (1H, t, *J* 73.2, $OCHF_2$), 7.94 (1H, d, *J* 11.6, C_5–H), 8.77 (1H, s, C_2–H), FAB-MS: *m*/*z* 483 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{22}H_{26}F_3N_4O_5$ $(M+H)^+$: 483.1855; Found 483.1864.

*4.2.7.9. 1-Ethyl-6,8-fluoro-7-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **20***. The title compound was obtained as off-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 1.40–1.44 (3H, t, $NCH_2\underline{CH_3}$,), 1.54 (3H, s, CH_3), 2.53–2.58 (1H, m), 3.04–3.08 (1H, m), 3.20–3.25 (1H, m), 3.42–3.53 (3H, m), 3.88 (3H, s, OCH_3), 4.53–4.56 (2H, m, NCH_2), 7.91 (1H, d, *J* 10.8, C_5–H), 8.87 (1H, s, C_2–H), ESI-MS: *m*/*z* 409 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{19}H_{23}F_2N_4O_4$ $(M+H)^+$: 409.16874; Found 409.16956.

*4.2.7.10. 1-Ethyl-6,8-difluoro-7-(3-amino-4-ethoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **21***. The title compound was obtained as off-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 1.25 (3H, t, *J* 6.8, $OCH_2\underline{CH_3}$), 1.44 (3H, t, *J* 7.2, $NCH_2\underline{CH_3}$), 1.55 (3H, s, CCH_3), 2.53–2.61 (1H, m), 3.07–3.11 (1H, m), 3.22–3.28 (1H, m), 3.46–3.58 (3H, m), 4.12–4.17 (2H, m, $O\underline{CH_2}CH_3$), 4.59–4.60 (2H, m, $N\underline{CH_2}CH_3$), 7.95 (1H, d, *J* 11.6, C_5–H), 8.97 (1H, s, C_2–H). FAB-MS: *m*/*z* 423 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{20}H_{25}F_2N_4O_4$ $(M+H)^+$: 423.1844; Found 423.1809.

*4.2.7.11. 1-(2-Fluoroethyl)-6,8-difluoro-7-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **22***. The title compound was obtained as off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.40 (3H, s, CH_3), 2.73–2.78 (1H, m), 2.95–2.99 (1H, m), 3.22–3.41 (4H, m), 3.88 (3H, s, OCH_3), 4.70–4.89 (4H, m, NCH_2CH_2F), 8.02 (1H, d, *J* 11.2, C_5–H), 8.61 (1H, s, C_2–H). ESI-MS: *m*/*z* 427 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{19}H_{22}F_3N_4O_4$ $(M+H)^+$: 427.15931; Found 427.15950.

*4.2.7.12. 1-(2-Fluoroethyl)-6,8-difluoro-7-(3-amino-4-ethoxyimino-3-methylpiperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **23***. The title compound was obtained as off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.27 (3H, t, $OCH_2\underline{CH_3}$), 1.39 (3H, s, CH_3), 2.74–2.79 (1H, m), 2.95–3.02 (1H, m), 3.21–3.44 (4H, m), 4.09–4.15 (2H, m, $O\underline{CH_2}CH_3$), 4.69–4.88 (4H, m, NCH_2CH_2F), 8.01 (1H, d, *J* 11.2, C_5–H), 8.60 (1H, s, C_2–H). FAB-MS: *m*/*z* 441 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{20}H_{24}F_3N_4O_4$ $(M+H)^+$: 441.1750; Found 441.1752.

*4.2.8. General procedure for the synthesis of 7-(4-alkoxyimino-3-amino-3- methylpiperidin-1-yl) fluoroquinolone derivatives **24**–**27***

A mixture of **11g**–**h** (1 mmol), **9a**,**b** (1.3 mmol), triethylamine (5 mmol) and dry acetonitrile (10 mL) was stirred at 30–60 °C for 1–6 h. After completion of the condensation, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in 5% sodium hydroxide solution (6.0 mL), heated to 40–55 °C and stirred for 2–5 h at the same temperature. The reaction mixture was cooled to room temperature and adjusted to pH 7–7.5 with 2 N HCl. The solid product was collected by suction, and dried *in vacuo* to give the title compounds **24**–**27**.

*4.2.8.1. 1-Cyclopropyl-6-fluoro-7-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-8-methoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **24***. The title compound was obtained as pale yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 0.93–1.18 (4H, m, 2× cyclopropyl CH_2), 1.53 (3H, s, CH_3), 2.49–2.57 (1H, m), 3.09–3.13 (1H, m), 3.17–3.23 (1H, m), 3.41–3.55 (3H, m), 3.71 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 4.13–4.15 (1H, m), 7.81 (1H, d, *J* 11.6, C_5–H), 8.73 (1H, s, C_2–H), ESI-MS: *m*/*z* 433 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{21}H_{26}FN_4O_5$ $(M+H)^+$: 433.18872; Found 433.18866.

*4.2.8.2. 1-Cyclopropyl-6-fluoro-7-(3-amino-4-ethoxyimino-3-methylpiperidin-1-yl)-8-methoxyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **25***. The title compound was obtained as pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 0.93–1.04 (2H, m, cyclopropyl CH_2), 1.18–1.31 (5H, m, cyclopropyl CH_2, $OCH_2\underline{CH_3}$), 1.59 (3H, s, CH_3), 2.68–2.70 (1H, m), 3.12–3.16 (1H, m), 3.34–3.60 (4H, m), 3.71 (3H, s, OCH_3), 3.99–4.00 (1H, m), 4.10–4.16 (2H, m, $O\underline{CH_2}CH_3$), 7.87 (1H, d, *J* 11.6, C_5–H), 8.83 (1H, s, C_2–H), FAB-MS: *m*/*z* 447 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{22}H_{28}FN_4O_5$ $(M+H)^+$: 447.2044; Found 447.2026.

*4.2.8.3. 9-Fluoro-3(S)-methyl-10-(3-amino-4-methoxyimino-3-methylpiperidin-1-yl)-7-oxo-2,3-dihydro-7H-pyrrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid **26***. The title compound was obtained as pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.46 (3H, s, CH_3), 1.62 (3H, d, *J* 6.8, $CH\underline{CH_3}$), 2.75–2.82 (1H, m), 2.88–2.95 (1H, m), 3.25–3.44 (4H, m), 3.88 (3H, s, OCH_3), 4.36–4.52 (3H, m), 7.75 (1H, d, *J* 11.6, C_8–H), 8.63 (1H, s, C_5–H). ESI-MS: *m*/*z* 419 $(M+H)^+$. HRMS-ESI: *m*/*z* Calcd. for $C_{20}H_{24}FN_4O_5$ $(M+H)^+$: 419.17307; Found 419.17072.

4.2.8.4. 9-Fluoro-3(S)-methyl-10-(3-amino-4-ethoxyimino-3-methyl-piperidin-1-yl)-7-oxo-2,3-dihydro-7H-pyrrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid ***27****.* The title compound was obtained as pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.22 (3H, t, $OCH_2\underline{CH_3}$), 1.37 (3H, s, CH_3), 1.45 (3H, d, *J* 6.4,$CH\underline{CH_3}$), 2.63–2.66 (1H, m), 2.90–2.91 (1H, m), 3.27–3.33 (4H, m), 4.05–4.07 (2H, m, $O\underline{CH_2}CH_3$), 4.37–4.41 (1H, m), 4.59–4.63 (1H, m), 4.92–4.94 (1H, m), 7.62 (1H, d, *J* 11.6, C_8–H), 8.99 (1H, s, C_5–H). FAB-MS: *m*/*z* 433 $(M+H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{21}H_{26}FN_4O_5$ $(M+H)^+$: 433.1887; Found 433.1871.

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 [19]. 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.

4.4. Cytotoxicity

Some compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line till 31.25 μg/mL concentrations. The Vero 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 1×10^4 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 72 h of exposure, cells were harvested and cell viability was assessed by MTT assay. The CC_{50} values were calculated by Bliss analyses.

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References

[1] J.M. Domagala, C.L. Heifetz, T.F. Mich, J.B. Nichos, J. Med. Chem. 29 (1986) 445–448.
[2] K. Hoshino, A. Kitamura, I. Morrissey, K. Sato, J. Kato, H. Ikeda, Antimicrob. Agents Chemother. 38 (1994) 2623–2627.
[3] C.Y. Hong, Y.K. Kim, Y.H. Lee, J.H. Kwak, Bioorg. Med. Chem. Lett. 8 (1998) 221–226.
[4] L.J.V. Piddock, Antimicrob. Agents Chemother. 38 (1994) 163–169.
[5] V. Cecchetti, A. Fravolini, M.C. Lorenzini, O. Tabarrini, P. Terni, T. Xin, J. Med. Chem. 39 (1996) 436–445.
[6] D.R. Choi, J.H. Shin, J. Yang, S.Y. Yoon, Y.H. Jung, Bioorg. Med. Chem. Lett. 14 (2004) 1273–1277.
[7] A. Bryskier, J.F. Chantot, Drugs 49 (Suppl. 2) (1995) 16–28.
[8] H. Koga, A. Itoh, S. Murayama, S. Suzue, T. Irikura, J. Med. Chem. 23 (1980) 1358–1363.
[9] J.M. Domagala, J. Antimicrob. Chemother. 33 (1994) 685–706.
[10] Z. Dang, Y.S. Yang, R.Y. Ji, S.H. Zhang, Bioorg. Med. Chem. Lett. 17 (2007) 4523–4526.
[11] M.J. Suto, J.M. Domagala, G.E. Roland, G.B. Mailloux, M.A. Cohen, J. Med. Chem. 35 (1992) 4745–4750.
[12] 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.
[13] 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.
[14] J. Bonjoch, A. Inares, M. Guardià, J. Bosch, Heterocycles 26 (1987) 2165–2174.
[15] M.R. Almond, J.B. Stimmel, E.A. Thompson, G.M. Loudon, Org. Synth. 8 (1993) 132–137.
[16] 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.
[17] S. Huang, R.G. Xie, B.Z. Tian, Organic Synthesis Reagent Preparation Manual [M], Sichuan University Press, 1988, p. 443.
[18] N. Takagi, H. Fubasami, H. Matukubo, EP 464823, 1992; Chem. Abstr. 116 (1992) 152003e.
[19] MICs 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, National Committee for Clinical Laboratory Standards, Wayne, PA, 2001, M100-S11. The MIC was defined as the lowest concentration of each compound resulting in inhibition of visible growth of bacteria after incubation at 35 °C for 18–24 h.