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Synthesis and in vitro antibacterial activity of 7-(3-alkoxyimino-5-amino/methylaminopiperidin-1-yl)fluoroquinolone derivatives

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ABSTRACT

We report herein the design and synthesis of novel 7-(3-alkoxyimino-5-amino/methylaminopiperidin-1-yl)fluoroquinolone derivatives based on the structures of new fluoroquinolones IMB and DZH. The antibacterial activity of these newly synthesized compounds was also evaluated and compared with gemifloxacin, ciprofloxacin, and levofloxacin. Results revealed that all of the target compounds **10–27** have good potency in inhibiting the growth of *Staphylococcus aureus* including MSSA (MIC: 0.125–8 μg/mL), *Staphylococcus epidermidis* including MRSE (MIC: 0.25–16 μg/mL), *Streptococcus pneumoniae* (MIC: 0.125–4 μg/mL), and *Escherichia coli* (MIC: 0.25–0.5 μg/mL). In particular, some compounds showed useful activity against several fluoroquinolone-resistant strains, and the most active compound **15** was found to be 16–128, 2–32, and 4–8-fold more potent than the three reference drugs against fluoroquinolone-resistant MSSA, MRSA, and MRSE.

Quinolones represent an extremely successful family of antibiotics that have potent, a broad spectrum of antibacterial activity, and relatively few side effects.[1] They inhibit two type-II topoisomerases, DNA gyrase and topoisomerase IV, by binding to the intermediate catalytic enzyme–DNA complex. The stabilization of the resulting quinolone-enzyme–DNA complex leads to the generation of double-strand DNA breaks that trigger a cascade of events leading to cell death.[2,3]

Most of the quinolones currently on the market or under development are generally characterized by a broad antibacterial spectrum, but their activity against clinically important Gram-positive cocci, including *Staphylococci*, *Streptococci*, and *Enterococci*, is relatively moderate. This insufficient activity has not only limited their use in infections caused by these organisms, but has also contributed to 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.[4]

The general chemical structure of the quinolone antibacterial agents consists of a 4-quinolone/naphthyridone-3-carboxylic acid nucleus and a secondary amino group attached to the C-7 position of the heterocyclic core, which has a great impact of modulating potency, spectrum and pharmacokinetics.[5] In general, the optimal substituents have proven to be 5- and 6-membered nitrogen heterocycles that contain peripheral nitrogens, such as piperazinyl, pyrrolidinyl, and piperidinyl groups. However, so far the piperidinyl-based quinolone analogs of the three are the least reported in the literature.[6]

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 and found IMB (Fig. 1), a 8-methoxyl fluoroquinolone containing an 3-amino-4-methoxyiminopiperidin-1-yl group at the C-7 position, have good in vitro and in vivo antibacterial activity which was comparable to moxifloxacin and gemifloxacin (GMFX).[7–13] It was also reported that DZH, a fluoronaphthyridone with a 3-methoxyimino-4-methylaminopiperidin-1-yl group at the C-7 position, showed excellent in vitro antibacterial activity against Gram-positive and Gram-negative organisms, including methicillin-sensitive *Staphylococcus aureus* (MSSA), *Streptococcus pneumoniae*, *E. faecalis*, and *P. aeruginosa*, which was better than vancomycin and GMFX.[6]

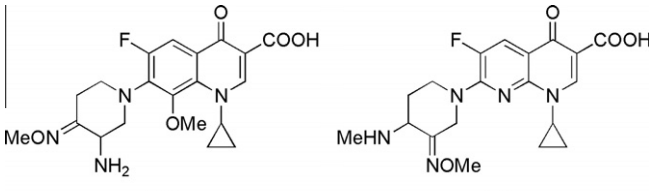

Figure 1. Structures of IMB and DZH.

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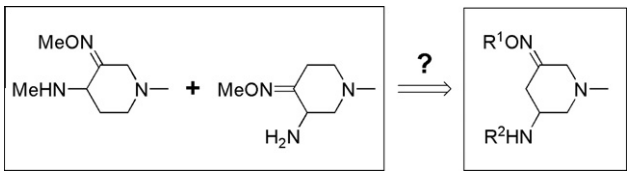


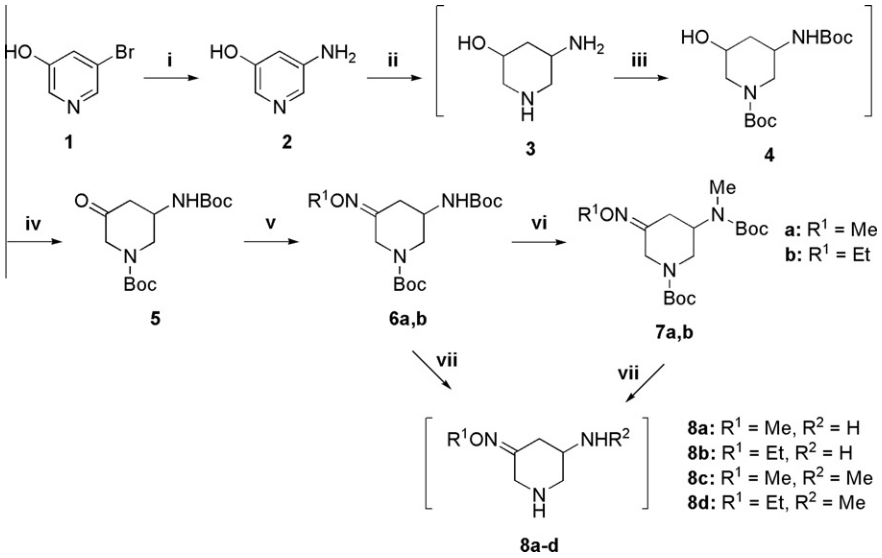
Figure 2. Design of the new piperidinyl side chain.

Based on these research results, a series of new fluoroquinolone compounds containing substituted piperidines were designed and synthesized. These derivatives are structurally novel, having an alkoxyimino group at the 3-position and an amino/methylamino group at the 5-position of the piperidine ring (Fig. 2), which are the position isomers of the side chains at the C-7 position of IMB and DZH. Our primary objective was to optimize the potency of these compounds against Gram-positive and Gram-negative organisms.

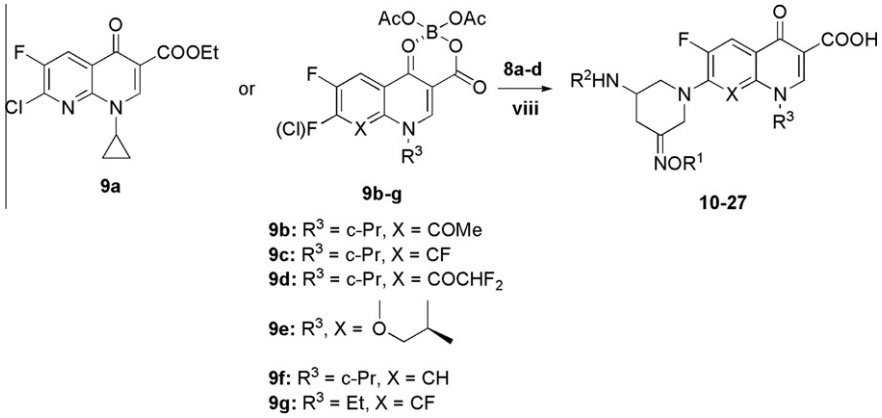
The new piperidine derivatives and novel fluoroquinolones described herein were synthesized as shown in Schemes 1 and 2, respectively. Using copper sulfate as the catalyst, amination of 5-bromopyridin-3-ol **1** performed smoothly in ammonia water at 100 °C gave 5-aminopyridin-3-ol **2**. The aminopyridine **2** was converted into aminopiperidine **3** by Rh/C-catalyzed hydrogenation in water at 80 °C, during which two equivalents of phosphonic acid was added to avoid the catalyst poisoning caused by the amino group. The amine **3** was subsequently treated with di-*tert*-butyl dicarbonate (Boc_2O) in NaOH–H_2O–EtOH solution to form bis-Boc-protected amino alcohol **4**, which was oxidated to the corresponding piperidone **5** by Jones reagent, with an overall yield of 26% for the three steps. The alkoxyimino group was introduced into the ring by coupling **5** with methoxylamine or ethoxylamine at 70 °C to afford amines **6a,b**. Methylation of the amines **6a,b** by reaction with methyl iodide in the presence of sodium hydride produced methylamines **7a,b**.

Removal of the bis-Boc-protecting groups on the amines **6a,b** and methylamines **7a,b** turned out to be a challenging task, mainly due to the instability of the alkoxyimino groups to hydrochloric acid and trifluoroacetic acid, which are commonly used in the Boc deprotection reaction. After a number of attempts, we successfully removed the Boc groups in neutral condition by reaction with trimethyl silane iodine (TMSI) to afford the new piperidine derivatives **8a–d**.

Finally, the target compounds **10–27** were obtained by coupling the new piperidine derivatives **8a–d** with various compounds containing quinolone and naphthyridone cores and then hydrolysis



Scheme 1. Reagents and conditions: (i) $NH_3{\cdot}H_2O$, $CuSO_4{\cdot}5H_2O$, 100 °C, 12 h, 75%; (ii) H_3PO_4, H_2, 5% Rh/C, H_2O, 80 °C, 40 h; (iii) NaOH, Boc_2O, EtOH–H_2O, rt, 2 h; (iv) Jones reagent, Me_2CO, 0 °C, 0.5 h, 26% (three-step yield); (v) $R^1ONH_2{\cdot}HCl$, Et_3N, EtOH–H_2O, 70 °C, 2 h, 72%; (vi) NaH, MeI, MeCN, 0 °C, 3 h, 81%; (vii) TMSI, CH_2Cl_2, rt, 0.5 h.



Scheme 2. Reagents and conditions: (viii) (a) Et_3N, MeCN, rt to 50 °C, 10–70 h; (b) 2% NaOH/H_2O, rt, 2 h; (c) 6 N HCl, H_2O, rt, 9–42% (from **9a** to **9g**).

according to the well-established literature procedures (Scheme 2).[14] In the case of quinolones **10–13**, condensation of ester **9a** with **8a–d** was performed in the presence of triethylamine. However for **14–27**, boric chelates **9b–g** were required to increase reactivity. All of the synthetic compounds were well characterized through the spectral characteristics.[15]

Fluoroquinolones **10–27** were evaluated for their in vitro antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques and the minimum inhibitory concentration values (MICs) along with those of the reference drugs GMFX, CPFX, and levofloxacin (LVFX) for comparison are shown in Table 1.[16]

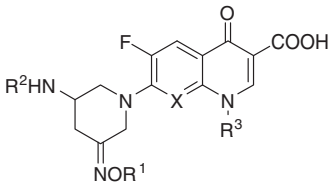
All of the target compounds **10–27** have potent antibacterial activity against the twelve tested strains. They generally exhibited good potency in inhibiting the growth of most tested Gram-positive strains, such as *S. aureus* including MSSA (MIC: 0.125–8 μg/mL), *Staphylococcus epidermidis* including methicillin-resistant *S. epidermidis* (MRSE) (MIC: 0.25–16 μg/mL) and *S. pneumoniae* (MIC: 0.125–4 μg/mL), as well as some of the tested Gram-negative strains such as *Escherichia coli* (MIC: 0.25–0.5 μg/mL). It is worth noting that some compounds showed useful activity against several fluoroquinolone-resistant strains. For example, compounds **14**–**19**, **21**, **23**, **25**, and **27** (MICs: 0.125–0.5 μg/mL) were 4–128-fold more potent than the three reference drugs (MICs: 2–16 μg/mL) against MSSA. Compounds **14–17**, **19–21**, **23**, and **25** (MICs: 1–4 μg/mL) showed 2–16-fold more potent activity than the references (MICs: 8–16 μg/mL) against MRSE. The most active compound **15** was found to be 16–128, 2–32, and 4–8-fold more potent than the references against fluoroquinolone-resistant MSSA, MRSA, and MRSE. In addition, compound **14** was 2–32-fold more potent than the references against fluoroquinolone-resistant *S. hemolyticus*, and the same for compound **16** against MRSA.

Generally, the activity of the quinolone nuclei against Gram-positive strains was in the order: 1-cyclopropyl-8-methoxylquinolone > 1-cyclopropyl-8-fluoroquinolone > 1-ethyl-8-fluoroquinolone > levofloxacin nuclei > 1-cyclopropylquinolone ≈ 1-cyclopropyl-8-difluoromethoxylquinolone > 1-cyclopropyl-1,8-naphthyridine. In addition, fluoroquinolones featuring ethyloxime-incorporated piperidino-substitution at the C-7 position were at least as potent as the analogs containing methyloxime, and introduction of a methyl group to the amino group at the 5-position of the piperidine ring did not change the Gram-positive antibacterial activity remarkably.

As for Gram-negative strains, the novel fluoroquinolones **10–27** did not provided satisfactory potency. All of them were generally less active than the reference drugs GMFX, CPFX, and LVFX, although they demonstrated good activity against *E. coli* (MIC: 0.25–0.5 μg/mL).

Fluoroquinolones **10–27** were further examined for cytotoxicity (CC_{50}) in a mammalian Vero cell line from 1024 to 4 μg/mL concentrations. After 24 h of exposure, viability was assessed and the results are reported in Table 1. The eighteen compounds when tested showed CC_{50} values ranging from 593 to 1524 μM. A comparison of the substitution pattern at the C-7 position demonstrated that ethyloxime-incorporated piperidino-substitutions were generally less

Table 1
In vitro antibacterial activity and cytotoxicity of novel fluoroquinolones **10**–**27**



Strains	R^1	R^2	R^3	X	MIC (μg/mL)												CC_{50} (μM)
Compound					S.a.1	S.a.2	S.a.3	S.e.1	S.e.2	S.p	S.h	E.c.1	E.c.2	K.p	P.a.1	P.a.2	
10	Me	H	*c*-Pr	N	4	8	128	2	16	4	128	0.25	0.25	128	128	128	822
11	Et	H	*c*-Pr	N	4	2	16	4	8	4	128	0.25	0.5	128	128	128	967
12	Me	Me	*c*-Pr	N	0.5	4	128	2	16	4	>128	0.25	0.25	>128	128	>128	645
13	Et	Me	*c*-Pr	N	2	4	8	8	16	4	>128	0.25	0.25	>128	128	>128	731
14	Me	H	*c*-Pr	COMe	0.25	0.5	4	2	4	0.25	4	0.25	0.25	64	4	4	885
15	Et	H	*c*-Pr	COMe	0.25	0.125	2	1	2	0.125	8	0.25	0.25	128	8	>128	879
16	Me	Me	*c*-Pr	COMe	0.5	0.5	2	1	4	0.5	8	0.25	0.25	128	64	128	1088
17	Et	Me	*c*-Pr	COMe	0.5	0.5	8	0.25	4	1	128	0.25	0.25	128	128	128	1524
18	Me	H	*c*-Pr	CF	0.25	0.5	8	4	8	0.25	128	0.25	0.25	128	128	128	739
19	Et	H	*c*-Pr	CF	0.25	0.5	4	0.5	4	0.25	64	0.25	0.25	64	8	>128	1323
20	Me	Me	*c*-Pr	CF	0.25	2	8	2	2	1	8	0.25	0.25	128	8	128	1023
21	Et	Me	*c*-Pr	CF	0.5	0.25	4	0.5	2	1	128	0.25	0.5	>128	128	>128	1013
22	Me	H	*c*-Pr	$COCHF_2$	2	8	128	2	8	4	>128	0.25	0.25	>128	128	>128	667
23	Et	H	*c*-Pr	$COCHF_2$	0.5	0.25	4	0.25	1	1	128	0.25	0.25	>128	128	>128	1523
24	Me	H			0.5	2	128	4	8	0.5	64	0.25	0.5	64	8	64	1002
25	Et	H			0.25	0.5	4	0.5	2	0.25	8	0.25	0.25	64	8	32	1244
26	Me	H	*c*-Pr	CH	0.5	4	64	8	16	4	128	0.25	0.25	64	2	>128	593
27	Me	H	Et	CF	0.25	0.25	32	16	16	0.5	128	0.25	0.25	>128	8	>128	913
GMFX					0.008	2	4	0.25	8	0.008	8	0.015	0.06	32	2	1	
LVFX					0.125	8	32	2	8	0.015	32	0.015	0.5	32	4	2	
CPFX					0.25	16	64	1	16	0.03	64	0.008	0.25	32	32	1	

Abbreviations: S.a.1: *Staphylococcus aureus* ATCC25923; S.a.2: methicillin-sensitive *Staphylococcus aureus* 10-04; S.a.3: methicillin-resistant *Staphylococcus aureus* 10-05; S.e.1: methicillin-sensitive *Staphylococcus epidermidis* 10-4; S.e.2: methicillin-resistant *Staphylococcus epidermidis* 10-5; S.p: *Streptococcus pneumoniae* 1001; S.h: *Streptococcus hemolyticus* 1002; E.c.1: *Escherichia coli* ATCC25922; E.c.2: *Escherichia coli* 10-02; K.p: *Klebsiella pneumoniae* 10-1; P.a.1: *Pseudomonas aeruginosa* ATCC27853; P.a.2: *Pseudomonas aeruginosa* 10-1.

cytotoxic than the analogs containing methyloxime. In addition, a methylamino group in place of the amino group at the 5-position of the piperidine ring increased cytotoxicity of naphthyridones (**10–13**), but contrary for quinolones (**14–21**).

In summary, we report herein the design and synthesis of some novel 7-(3-alkoxyimino-5-amino/methylaminopiperidin-1-yl)fluoroquinolone derivatives based on the structures of new fluoroquinolones IMB and DZH. The antibacterial activity of these newly synthesized compounds was also evaluated and compared with GMFX, CPFX, and LVFX. Results reveal that all of the synthesized fluoroquinolones **10–27** have good potency in inhibiting the growth of *S. aureus* including MSSA, *S. epidermidis* including MRSE, *S. pneumoniae* and *E. coli*. In particular, some compounds showed useful activity against several fluoroquinolone-resistant strains, and the most active compound **15** was found to be 16–128, 2–32, and 4–8-fold more potent than the three reference drugs against fluoroquinolone-resistant MSSA, MRSA, and MRSE.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.073.

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15. To a stirring solution of **7b** (1.0 g, 2.7 mmol) in dry dichloromethane (20 mL) was added TMSI (0.8 mL, 5.7 mmol) at room temperature under an atmosphere of nitrogen and stirred for 0.5 h at the same temperature. Enough methanol was added to quench the reaction and then the mixture was concentrated under reduced pressure. The residue containing **8d** was dissolved in dry acetonitrile (20 mL), and to this solution dry triethylamine (1.1 mL, 8.1 mmol) and **9c** (0.9 g, 2.2 mmol) were added. The mixture was stirred at room temperature under an atmosphere of nitrogen for 20 h and then was concentrated under reduced pressure. The residue was dissolved in 2% sodium hydroxide solution (15 mL) and stirred for 2 h at the same temperature. The reaction mixture was adjusted to pH 7 with 6 N hydrochloric acid. The resulting solid was collected by suction to afford **21** [0.4 g, 42% (from **9c**), purity by HPLC: 54% (X) + 45% (Y) = 99%] as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17–1.32 (7H, m, 2 × cyclopropyl CH_2, $NOCH_2CH_3$), 2.50–2.60 (4H, m, NCH_3, Y-$C_{4'}$-H), 2.80–2.83 (1H, m, X-$C_{4'}$-H), 3.10–3.21 (2H, m, $C_{5'}$-H, $C_{4'}$-H), 3.37 (1H, br, cyclopropyl CH), 3.71 (1H, br, $C_{6'}$-H), 3.94–4.14 (6H, m, $NOCH_2CH_3$, X-$C_{2'}$-2H, Y-$C_{2'}$-H, $C_{6'}$-H), 4.37 (1H, d, J = 15.6 Hz, Y-$C_{2'}$-H), 7.89 (1H, d, J = 11.6 Hz, C_5-H), 8.77 (1H, s, C_2-H). MS (ESI, m/z): 435 $(M+H)^+$. HRMS (ESI, m/z): $C_{21}H_{25}F_2N_4O_4$ $(M+H)^+$. Calcd: 435.18439, found: 435.18042.
16. Compounds **10–27** 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, 0.03, 0.015 and 0.008 μg/mL. Petri dishes were incubated with 10^4 colony forming units (cfu) and incubated at 35 °C for 18–24 h. The MIC was the lowest concentration of the test compound, which resulted in no visible growth on the plate.