Bioorganic & Medicinal Chemistry Letters 20 (2010) 5195–5198

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and in vitro antibacterial activity of novel fluoroquinolone derivatives containing substituted piperidines

Yun Chai, Mingliang Liu *, Bo Wang, Xuefu You, Lianshun Feng, Yibin Zhang, Jue Cao, Huiyuan Guo

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

ARTICLE INFO

Article history:
Received 15 March 2010
Revised 1 July 2010
Accepted 2 July 2010
Available online 8 July 2010

Keywords:
Fluoroquinolone
Synthesis
Antibacterial activity

ABSTRACT

We report herein the synthesis of novel 7-(4-alkoxyimino-3-aminomethyl-3-methylpiperidin-1-yl) fluoroquinolone derivatives. The antibacterial activity of the newly synthesized compounds was evaluated and correlated with their physicochemical properties. Results reveal that all of the target compounds have good potency in inhibiting the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* including MRSE (MIC: 0.125–4 μg/mL). Compounds **12, 13** are more potent than or comparable to levofloxacin against MRSA, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella sonnei*. Compound **17** is more active than or comparable to levofloxacin against *S. aureus* including MRSA, *S. epidermidis* and *S. pyogenes*.

Quinolone antibacterial agents represent a fast growing group of antibiotics. 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]

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 cocci including *Staphylococci, Streptococci*, and *Enterococci* 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.[2]

The structure–activity relationship (SAR) study of fluoroquinolone 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.[3,4] In general, five- and six-membered nitrogen heterocycles including piperazinyl, pyrrolidinyl, and piperidinyl type side chains have proven to be the optimal substituents. However, piperidinyl analogs of the three are the least studied.[5,6]

Recently, as part of an ongoing program to find potent and broad-spectrum antibacterial agents that display strong Gram-positive activity, we also have focused on introducing new functional groups to the piperidine ring.[7–10] It was reported that DW286 (Fig. 1), possessing an additional methyl group at the 3-position of the pyrrolidine ring displays more antibacterial activity than Gemifloxacin against important Gram-positive organisms, methicillin-resistant *Staphylococcus aureus* (MRSA) and ofloxacin resistant organisms, while maintaining an excellent pharmacokinetic profile,[11,12] and IMB (Fig. 1), a 8-methoxyl fluoroquinolone with a methoxyimino group attached to the piperidine ring at C-7 position, shows good in vitro and in vivo antibacterial activity.[7] Inspired by those research results, a series of new fluoroquinolone derivatives were designed and synthesized. These derivatives are structurally novel, having both an aminomethyl 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.

The synthetic routes of new piperidine derivatives **9a**,**b** and novel fluoroquinolones **12–26** are shown in Schemes 1 and 2, respectively. Addition reaction of ethyl 3-aminopropionate hydrochloride **1** with acrylonitrile in the presence of sodium hydroxide gave the secondary amine **2**, which was subsequently treated with di-*tert*-butoxycarbonyl dicarbonate (Boc_2O) to produce Boc-protected cyano ester **3**. The compound **3** was cyclized to the ketone **4** by sodium hydride in refluxing toluene, with an overall yield of 83% for the three steps. The ketone **4** was methylated with methyl iodide to provide methyl ketone **5**. However, selective reduction of the cyano group to the primary amine, in the presence of the ketone moiety of cyano ketone **5** was unsuccessful.

Therefore, catalytic hydrogenation of both functional groups of the cyano ketone **5** and subsequent protection of the resulting

* Corresponding author. Tel.: +86 10 63036965; fax: +86 10 63047865.
E-mail address: lmllyx@yahoo.com.cn (M. Liu).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.006

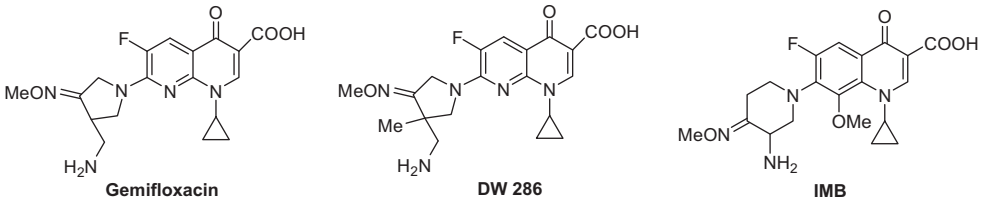
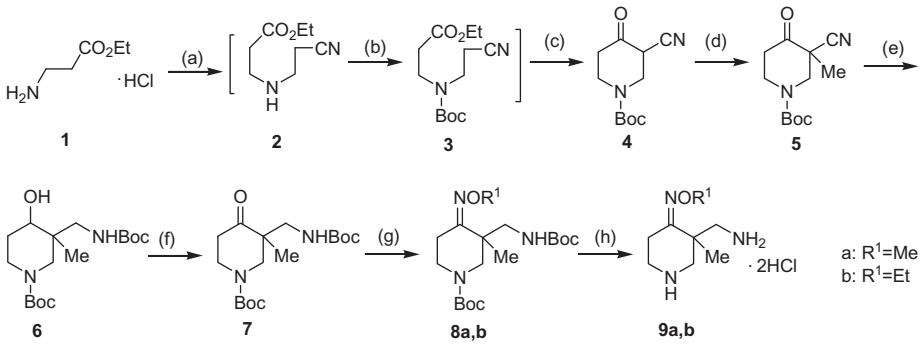
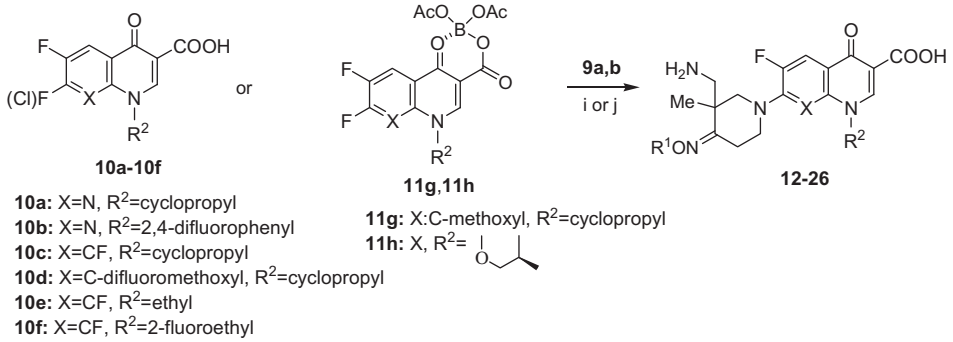


Figure 1. Structures of Gemifloxacin, DW286 and IMB.



Scheme 1. Reagents and conditions: (a) acrylonitrile, NaOH, EtOH, 50 °C, 5 h; (b) Boc_2O, MeOH, 50 °C, 3 h; (c) 60% NaH, toluene, reflux, 2 h, 83% (three steps); (d) MeI, K_2CO_3, acetone, 40 °C, 6 h, 82%; (e) H_2(g), 5% Pd/C, Boc_2O, MeOH, rt, 8 h, 65%; (f) Jone's reagent, acetone, 0 °C, 1 h, 86%; (g) R^1ONH_2·HCl, Et_3N, MeOH, 55–60 °C, 2 h, 88–91%; (h) HCl(g), CH_2Cl_2, rt, 1 h, 98%.



Scheme 2. Reagents and conditions: (i) Et_3N, MeCN, 25–50 °C, 2–10 h, 50–70%; (j) a—Et_3N, MeCN, 25–50 °C, 2–24 h; b—5% NaOH/H_2O, 40 °C, 0.5–2 h; c—2 N HCl, rt, 50–60%.

amine by Boc group yielded the bis-Boc-protected amino alcohol **6**. Oxidation of compound **6** by Jone's reagent afforded the corresponding ketone **7**, on which the oxime function group was introduced via condensation with methoxylamine/ethoxylamine to yield amines **8a,b**. The bis-Boc-protecting groups of amines **8a,b** were removed by pumping hydrogen chloride gas in methylene chloride to afford the new piperidine derivative dihydrochlorides **9a,b** in good yield.

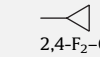
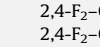
Finally, the target compounds **12–26** were obtained by coupling the new piperidine derivatives **9a**,**b** with various compounds containing quinolone and naphthyridone cores according to well-established literature procedures (Scheme 2).[13] In the case of quinolones **12–23**, condensation of **9a**,**b** with **10a–f** was performed in the presence of triethylamine. However for **24–26**, boric chelates **11g–h** were required to increase reactivity. All of the synthetic compounds were well characterized through the spectral characteristics.[14]

It is obvious that the target compounds **12–26** and intermediates **9a,b** are all racemes. Since the oxime group can exist in the *E*- or *Z*-configuration, it was necessary to determine the geometries of all the oxime target compounds **12–26**. Although we were not successful in preparing X-ray quality single crystals of compounds **12**–**26**, we were able to obtain X-ray data for the intermediate **9a**. As expected, the six-membered piperidine ring adopts a chair conformation and the methyloxime geometry of **9a** was confirmed to have the *E*-configuration (Fig. 2).[15]

Fluoroquinolones **12–26** were evaluated for their in vitro antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques. The minimum inhibitory concentration values (MICs) were compared with those of levofloxacin (LVFX) (Table 1).

The novel fluoroquinolones **12–26** have potent antibacterial activity against the tested strains. They exhibit good potency in inhibiting the growth of *S. aureus* and *Staphylococcus epidermidis* including MRSE (MIC: 0.125–4 μg/mL). Compounds **12, 13** are more potent than or comparable to LVFX against MRSA, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella sonnei*. Compound **17** is more active than or comparable to LVFX against *S. aureus* including MRSA, *S. epidermidis* and *S. pyogenes*. However, most of the target compounds exhibit less activity than LVFX against the tested Gram-positive and Gram-negative strains. These results show that introduction of a methyl group into 3-po-

Table 1
In vitro antibacterial activity and the cytotoxicity of the target compounds **12–26**

Strains	MIC (μg/mL)														CC_{50} (μg/mL)
Compound	R^1	X	R^2	*S.a.*	MRSA	*S.e.*	MRSE	*S.p.*	*S.py.*	*E.f.*	*E.co.*	*K.p.*	*P.a.*	*S.s.*	*E.cl.*	
12	Me	N		0.5	8	1	0.25	32	8	32	0.5	0.5	8	1	4	176.78
13	Et	N		0.25	8	0.5	0.25	4	16	4	1	1	16	1	8	44.19
14	Me	N	2,4-F_2–C_6H_3	1	128	2	2	64	64	>128	8	128	32	8	32	88.39
15	Et	N	2,4-F_2–C_6H_3	2	128	4	2	128	64	>128	8	64	128	16	32	78.75
16	Me	CF		0. 25	16	0.25	0.25	32	32	64	0. 5	64	16	1	4	88.39
17	Et	CF		0.125	8	0.25	0.25	8	16	2	2	2	16	2	16	62.5
18	Me	$COCHF_2$		0.25	32	1	1	128	64	128	4	4	32	4	8	88.39
19	Et	$COCHF_2$		1	16	1	2	16	128	2	8	8	64	16	16	44.19
20	Me	CF	CH_2CH_3	1	32	2	2	128	64	128	8	64	64	8	32	314.98
21	Et	CF	CH_2CH_3	2	16	4	2	64	64	128	16	16	128	32	128	78.75
22	Me	CF	CH_2CH_2F	2	64	4	2	128	128	>128	8	64	64	16	64	314.98
23	Et	CF	CH_2CH_2F	2	64	4	4	128	128	128	16	64	128	64	64	157.49
24	Me	$COCH_3$		0.5	16	4	1	64	64	32	8	8	64	8	32	157.49
25	Et	$COCH_3$		2	128	4	2	16	128	8	16	32	16	2	128	19.69
26	Me			1	64	2	1	128	64	128	4	128	32	8	16	176.78
LVFX				0.125	16	0.25	0.125	2	16	0.5	1	1	0.25	1	0.125	>1000

S.a., *Staphylococcus aureus* ATCC 29213; MRSA, methicillin-resistant *Staphylococcus aureus* 08-52; *S.e.*, *Staphylococcus epidermidis* ATCC 12228; MRSE, methicillin-resistant *Staphylococcus epidermidis* 08-18; *S.p.*, *Streptococcus pneumoniae* ATCC 49619; *S.py.*, *Streptococcus pyogenes* 06-1; *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.

sition of piperidine ring possessing an aminomethyl group in place of the amino group of IMB reduces antibacterial activity, which is contrary to our expected.

Generally, the activity of the quinolone nuclei in this study are in the order 1-cyclopropyl-1,8-naphthyridone ≈ 1-cyclopropyl-8-fluoroquinolone > 1-cyclopropyl-8-methoxylquinolone ≈ 1-cyclopropyl-8-difluoromethoxylquinolone > levofloxacin nuclei ≈ 1-(2,4-difluorophenyl)-1,8-naphthyridone ≈ 1-ethyl-8-fluoroquinolone > 1-(2-fluoroethyl)-8-fluoroquinolone. In addition, fluoroquinolones featuring methyloxime- incorporated piperidino-substitution at C-7 position are comparable to analogs containing ethyloxime.

Some compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line from 1000 to 7.81 μg/mL concentrations. After 48 h of exposure, viability was assessed and the results are reported in Table 1. Fifteen compounds when tested showed CC_{50} values ranging from 314.98 to 19.69 μg/mL. 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.

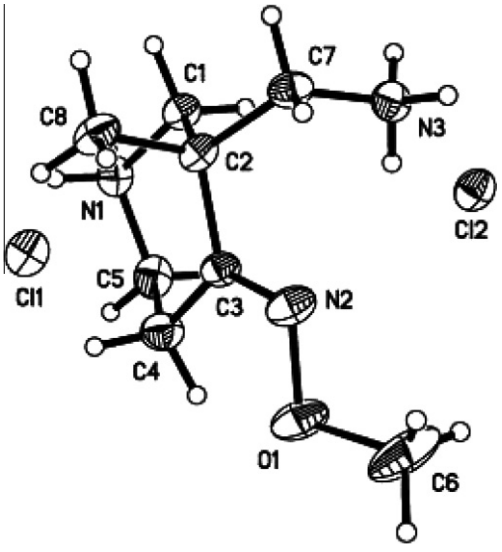


Figure 2. X-ray structure of **9a**.

In summary, we report herein the synthesis of some novel 7-(4-alkoxyimino-3-aminomethyl-3-methylpiperidin-1-yl) fluoroquinolone derivatives. The antibacterial activities of the newly synthesized compounds were evaluated and correlated with their physicochemical properties. Results reveal that all of the target compounds have good activity against *S. aureus* and *S. epidermidis* including MRSE, but most of them exhibit less activity than LVFX against the remaining Gram-positive and Gram-negative strains.

Acknowledgment

The work was supported by the National S&T Major Special Project on Major New Drug Innovation. Item No. 2009ZX09301-003.

References and notes

1. Hoshino, K.; Kitamura, A.; Morrissey, I.; Sato, K.; Kato, J.; Ikeda, H. *Antimicrob. Agents Chemother.* **1994**, *38*, 2623.
2. Srivastava, B. K.; Solanki, M.; Mishra, B.; Soni, R.; Jayadev, S.; Valani, D.; Jain, M.; Patel, P. R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1924.
3. Bryskier, A.; Chantot, J. F. *Drugs* **1995**, *49*, 16.
4. Forroumadi, A.; Emami, S.; Mehni, M.; Moshafi, M. H.; Shafiee, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4536.
5. Domagala, J. M. *J. Antimicrob. Chemother.* **1994**, *33*, 685.
6. Dang, Z.; Yang, Y. S.; Ji, R. Y.; Zhang, S. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4523.
7. Wang, X. Y.; Guo, Q.; Wang, Y. C.; Liu, B. Q.; Liu, M. L.; Sun, L. Y.; Guo, H. Y. *Acta Pharm Sin* **2008**, *43*, 819.
8. Wang, J. X.; Guo, Q.; Chai, Y.; Feng, L. S.; Guo, H. Y.; Liu, M. L. *Chin. Chem. Lett.* **2010**, *21*, 55.
9. Chai, Y.; Wan, Z. L.; Wang, B.; Liu, M. L.; Guo, H. Y. *Eur. J. Med. Chem.* **2009**, *44*, 4063.
10. Zhang, Y. B.; Feng, L. S.; You, X. F.; Guo, Q.; Guo, H. Y.; Liu, M. L. *Arch. Pharm. Chem. Life Sci.* **2010**, *343*, 143.
11. Suto, M. J.; Domagala, J. M.; Roland, G. E.; Mailloux, G. B.; Cohen, M. A. *J. Med. Chem.* **1992**, *35*, 4745.
12. Yun, H. J.; Min, Y. H.; Lim, J. A.; Kang, J. W.; Kim, S. Y.; Kim, M. J.; Jeong, J. H.; Choi, Y. J.; Kwon, H. J.; Jung, Y. H.; Shim, M. J.; Choi, E. C. *Antimicrob. Agents Chemother.* **2002**, *46*, 3071.
13. MICs were determined by the agar dilution method as recommended by the National Committee for Clinical Laboratory Standards. 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. The cytotoxicity was tested in a mammalian Vero cell line and was assessed by CPE assay.

14. A mixture of **10a** (1 mmol), **9a** (1.3 mmol), triethylamine (5 mmol), and dry acetonitrile (10 mL) was stirred at 30 °C for 3 h. After the reaction was completed, the reaction mixture was filtered. The resulting solid was purified via silica gel column chromatography (chloroform/methanol, 10:1, v/v) to give the title compound **12** as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.09–1.10 (2H, m, cyclopropylCH_2), 1.17 (3H, s, CH_3), 1.27–1.29 (2H, m, cyclopropylCH_2), 2.78–2.94 (4H, m), 3.62–3.64 (1H, m), 3.86–3.87 (2H, m), 3.89 (3H, s, OCH_3), 3.99–4.10 (2H, m), 8.10 (1H, d, J = 13.2, C_5-H), 8.74 (1H, s, C_2–H). ESI-MS: m/z 418 $(M+H)^+$. HRMS-ESI: m/z Calcd for $C_{20}H_{25}FN_5O_4$ $(M+H)^+$: 418.18906; found 418.18798. A mixture of **11g** (1 mmol), **9a** (1.3 mmol), triethylamine (5 mmol) and dry acetonitrile (10 mL) was stirred at 60 °C for 6 h. After the reaction was completed, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in 5% sodium hydroxide solution (6.0 mL), heated to 45 °C and stirred for 2 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, purified via silica gel column chromatography (chloroform/methanol, 10:1, v/v) to give the title compound **24** as pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 0.99–1.04 (2H, m, cyclopropylCH_2), 1.19–1.22 (2H, m, cyclopropylCH_2), 1.27 (3H, s, CH_3), 2.48–2.54 (1H, m), 2.71–2.78 (1H, m), 2.83–2.91 (2H, m), 2.95–3.06 (2H, m), 3.61–3.66 (1H, m), 3.72 (3H, s, OCH_3), 3.76–3.81 (1H, m), 3.91 (3H, s, OCH_3), 3.95–3.98 (1H, m), 7.85 (1H, d, J = 12.4, C_5–H), 8.75 (1H, s, C_2–H), ESI-MS: m/z 447 $(M+H)^+$. HRMS-ESI: m/z Calcd for $C_{22}H_{28}FN_4O_5$ $(M+H)^+$: 447.19858; found 447.20148.
15. The crystallographic data of **9a** has been deposited at the Cambridge Crystallographic Data Center (CCDC No. 767681).