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Syntheses and biological evaluation of new fluoroquinolone antibacterials containing chiral oxiimino pyrrolidine

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Abstract—The design and syntheses of new fluoroquinolone antibacterial agents having pyrrolidine ring at C-7 position are described. The pyrrolidine ring is optically active and possesses methyloxime functional group. Two of them have excellent in vitro antibacterial activities and pharmacokinetic profiles.

Quinolone antibacterial agents are among the most attractive drugs in the anti-infective chemotherapy field. To date many quinolone antibacterials have been introduced into clinical use, and significant improvements in antibacterial spectrum and activity have been achieved. And since the discovery of Norfloxacin,[1] fluoroquinolones have changed the landscape of antimicrobial chemotherapy. Although the current fluoroquinolones are generally characterized by a broad antimicrobial spectrum, their activities against clinically important Gram-positive pathogens (including *Staphylococcus aureus*, *Streptococcus pyogens*, *Streptococcus pneumoniae* and *Enterococcus*) are relatively moderate.[2] In addition, extensive use of fluoroquinolones has brought increasing quinolone resistance to many pathogens and majority of *Methicillin-Resistant Staphylococcus aureus* (MRSA) have abtained resistance to Ciprofloxacin (CPFX),[3] a representative fluoroquinolone, as well as other antibacterials.

On the basis of these considerations, we have focused on development of compounds with good activity against both Gram-positive and resistant organisms. Inspired by three groups previous research results,[4–6] we planned to synthesize oxime-incorporated pyrrolidine rings as C-7 substituents. And methyl group was introduced into 3-position of pyrrolidine ring for the purpose of increasing Gram-positive antibacterial activity.[7] In the course of our research, Compound **8** and its enantiomer (**R**)-**8** were chosen as C-7 substituents and various quinolone and naphthyridone nuclei were introduced.

In this report, we describe the synthesis and antibacterial activity of a series of fluoroquinolones having optically active oxiimino pyrrolidine at C-7 position.

Initially, optically inactive pyrrolidine derivative (**8**) was prepared as depicted in Scheme 1. Methylation and subsequent ketalization with neopentylglycol of readily available compound **1**[8] gave properly protected compound **3**. But at this stage, we had to swich *N*-protecting group Cbz to Bn for the next steps harsh reaction condition. And then, the conversion of ester group of compound **4** to hydroxymethyl moiety was attempted by several methods using borohydride and aluminium hydride, thus lithium aluminium hydride and tetrahydrofuran turned out to be the best reaction condition. Amination of the alcohol (**5**) was carried out by successive mesylation, azidation and azide reduction to furnish compound **6**. The primary amine of **6** was protected with Boc and then selective *N*-deprotection of compound **7** was performed by catalytic hydrogenation to give compound **8**.

Condensation of **9a–e** with **8** was carried out according to the reactivities of **9a–e** (Scheme 2). In case of **9a**, a condition under acetonitrile and triethylamine at 45 °C was the most suitable, but for **9b–d**, additional heating was needed, and for **9e**, 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) was used instead of triethylamine. In the next step, **10a–e** were subjected to deprotection of ketal

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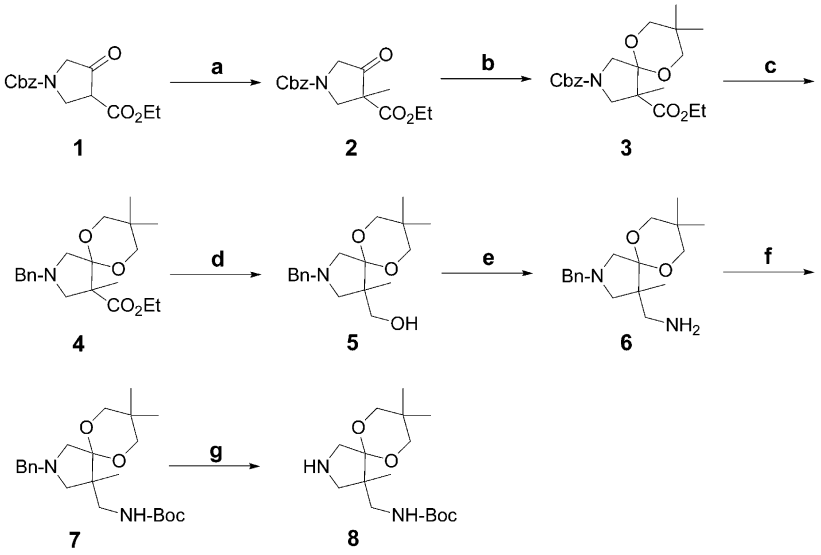
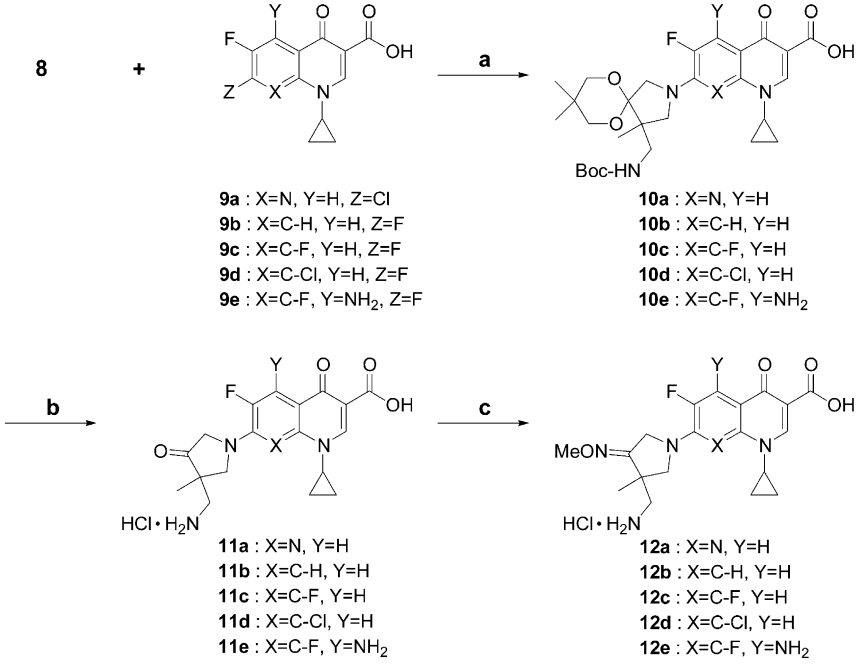
and Boc group simultaneously by means of concd. HCl to provide **11a–e**. Finally, Introducing methyloxime to ketone of **11a–e** gave **12a–e** as biologically applicable form. In addition, we also provided **14** (piperidine ring instead of pyrrolidine), **15** (replaced methyloxime with benzyloxime) and **16** (bulky ketal group in place of oxime) to prove the necessity of the functional groups in the C-7 substituent of **12a–e**.

The antibacterial activities of the synthesized compounds along with the reference agents CPFX and Gemifloxacin (GMFX) against general strains are listed in Table 1.

When we compared **12a** with **14–16**,[10] piperidine ring (**14**) showed significantly low Gram-negative activity and exchanging methyloxime to bulky ketal group (**16**)



Scheme 1. Reagents and conditions: (a) Methyl iodide, K_2CO_3, acetone, 45 °C, 3 h, 89%; (b) Neopentyl glycol, *p*-TSA, *n*-heptane, 120 °C, 6 h, 97%; (c) (i) H_2(g), 10% Pd/C, MeOH, rt, 3 h; (ii) Benzyl chloride, K_2CO_3, acetonitrile, reflux, overnight, 90% (two-step yield); (d) Lithium aluminium hydride, THF, −5 °C, 1.5 h, 93%; (e) (i) Methanesulfonyl chloride, triethylamine, MC, 0 °C–rt, 2 h; (ii) Sodium azide, DMF, reflux, overnight; (iii) Triphenylphospine, H_2O, THF, reflux, 5 h, 73% (three-step yield); (f) Di-*tert*-butyl dicarbonate, MC, 0 °C–rt, 1 h, 94%; (g) H_2(g), 10% Pd/C, MeOH, rt, 4 h, 91%.



Scheme 2. Reagents and conditons: (a) **9a**: Triethylamine, acetonitrile, 45 °C, 1.5 h, 89%; **9b–d**: Triethylamine, acetonitrile, reflux, overnight, 80–83%; **9e**; 1,8-diazabicyclo[5.4.0]-7-undecene, acetonitrile, reflux, overnight, 78%; (b) concd HCl, rt, overnight, 70–89%; (c) methoxylamine hydrochloride, pyridine, 45 °C, 4 h, 81–85%.

Table 1. In vitro antibacterial activities against general strains[9]

Compd	Minimum inhibitory concentration (μg/mL)									
	S.p. 308A	*S.p.* 77A	*S.a.* SG511	*S.a.* 285	*E.c.* 078	*E.c.* DC 2	*P.a.* 1592E	*P.a.* 1771M	*S.t.*	*E.cl.* 1321 E
12a	0.098	0.025	0.004	0.007	0.004	0.049	0.781	0.391	0.007	0.004
12b	0.098	0.013	0.007	0.013	0.013	0.025	0.781	0.391	0.013	0.013
12c	0.098	0.025	0.013	0.013	0.025	0.049	1.563	0.781	0.025	0.013
12d	0.195	0.025	0.007	0.007	0.007	0.025	0.781	0.195	0.025	0.004
12e	0.013	<0.002	<0.002	<0.002	<0.002	0.007	0.391	0.195	<0.002	<0.002
14	6.250	6.250	0.781	1.563	1.563	12.500	12.500	6.250	0.781	0.781
15	0.195	0.025	<0.002	<0.002	0.049	0.098	6.250	1.563	0.098	0.049
16	0.391	0.098	0.013	0.025	0.195	0.195	6.250	3.125	0.195	0.098
(R)-12a	0.098	0.013	0.004	0.007	0.025	0.049	1.563	0.781	0.013	0.007
(S)-12a	0.195	0.098	0.049	0.049	0.025	0.098	0.781	0.391	0.013	0.007
(R)-12e	0.004	<0.002	<0.002	<0.002	<0.002	<0.002	0.391	0.195	<0.002	<0.002
(S)-12e	0.049	0.013	<0.002	0.004	0.004	0.013	0.391	0.195	<0.002	<0.002
CPFX	3.125	0.391	0.195	0.781	0.004	0.098	0.195	0.098	0.013	<0.002
GMFX	0.195	0.025	0.013	0.025	0.007	0.025	0.391	0.195	0.004	0.004

S.p.: *Streptococcus pyogenes*, *S.a.*: *Staphylococcus aureus*, *E.c.*: *Escherichia coli*, *P.a.*: *Pseudomonas aeruginosa*, *S.t.*: *Salmonella typhimurium*, *E.cl.*: *Enterobacter cloacae*.

could not maintain activity against both Gram-positive and negative. And benzyloxime group (**15**) also did not improve Gram-negative activity. Compared with GMFX, **12a** demonstrated improved Gram-positive antibacterial activity as we expected. When it comes to quinolone nuclei, all members of the series (**12a–e**) had more potent activities than CPFX and GMFX, and 8-halogenated quinolone nuclei (**12c** and **12d**) showed slightly better antibacterial efficacy than **12b**.

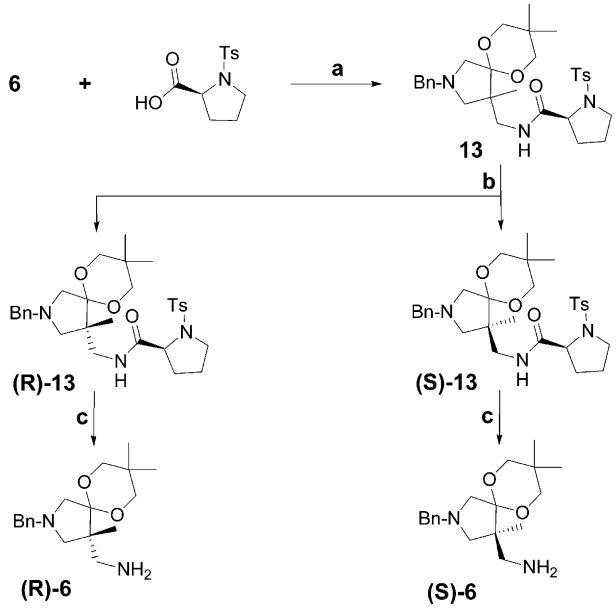
Especially, 5-amino-8-fluoro derivative (**12e**) demonstrated extremely high activity and naphthyridone nucleus derivative (**12a**) also gave strong and broad antibacterial spectrum. So at this point, we selected **12a** and **12e** for further study and tried to get chiral substituent for biological evaluation. Simply, we attatched *N*-tosyl protected L-proline to the primary amine of **6** and provided diastereomer **13** as separable form by column chromatography (Scheme 3). Through column chromatography and base-catalyzed hydrolysis, optically active (**R**)-**6** and (**S**)-**6** were obtained and we assigned their absolute configuration by X-ray crystallography.[11] Following the each chemical modification described in Scheme 1 and 2, we could also easily provide (**R**)-**12a**(**DW-286a**), (**S**)-**12a**, (**R**)-**12e** and (**S**)-**12e** from (**R**)-**6** and (**S**)-**6**.[12]

The MIC data against general strains were also listed in Table 1 and there were slight improvements in Gram-positive antibacterial activities for (**R**)-**12a** and (**R**)-**12e**. But for resistant strains including MRSA and Ofloxacin[13] resistant strains, (**R**)-**12a** and (**R**)-**12e** exhibited far superior antibacterial activities to CPFX and GMFX (Table 2). Generally, R-form enantiomers [(**R**)-**12a** and (**R**)-**12e**] were more potent than the S-form counterparts [(**S**)-**12a** and (**S**)-**12e**] by 10-fold and their racemates (**12a** and **12e**) had intermediate activities in MIC.

The phamacokinetic profiles of (**R**)-**12a** and (**R**)-**12e** were examined to know whether they could be applied as useful drugs to the body (Table 3). In rats, both of them have excellent advantages in maximal concentration in blood, half-life period and area under curve over GMFX and CPFX used as control.

It was reported that introduction of methyl group into 3-position of pyrrolidine could cause increment of cytotoxicity against mammalian cell as well as Gram-positive activity.[7] (**R**)-**12a** showed similar cytotoxity with GMFX, but, (**R**)-**12e** was more cytotoxic than GMFX (Table 4).

In summary, new optically active quinolone antibacterial agent (**R**)-**12a** and (**R**)-**12e** were designed and synthesized. They displayed far more potent antibacterial activity than CPFX and GMFX on important



Scheme 3. Reagents and conditions: (a) Ethyl chloroformate, triethylamine, MC, 0 °C–rt, 2 h, 86%; (b) column chromatography (eluent: ethyl acetate/*n*-Hexane = 2/3); (c) Potassium hydroxide, *iso*-propyl alcohol, reflux, overnight, quant.

Table 2. In vitro antibacterial activities against drug-resistant strains[8]

Strain		Minimum inhibitory concentration (μg/mL)							
		12a	**(R)12a**	**(S)12a**	**12e**	**(R)12e**	**(S)12e**	**CPFX**	**GMFX**
MRSA	690 E	0.004	<0.002	0.025	<0.002	<0.002	0.004	0.391	0.098
	692 E	<0.002	<0.002	0.025	<0.002	<0.002	0.004	0.391	0.049
	697 E	0.007	<0.002	0.025	<0.002	<0.002	0.004	0.391	0.049
	701 E	0.007	<0.002	0.049	<0.002	<0.002	0.007	0.391	0.098
	705 E	0.013	0.004	0.049	0.004	<0.002	0.007	0.391	0.098
	707 E	<0.002	<0.002	0.013	<0.002	<0.002	<0.002	0.195	0.025
Ofloxacin Resistant	*S.a.* 179	0.195	0.098	1.563	0.025	0.013	0.195	12.500	0.781
	S.a. 293	0.195	0.098	1.563	0.025	0.013	0.195	12.500	0.781
	S.a. 303	0.195	0.098	0.781	0.025	0.013	0.195	12.500	0.781
	S.e. 291	0.391	0.195	3.125	0.025	0.049	0.391	50.000	1.563
	S.e. 319	0.391	0.391	6.250	0.049	0.025	0.781	100.00	3.125
	E.k. 101	0.098	0.098	0.391	0.025	0.013	0.195	0.781	0.098
	P.a. 279II	6.250	6.250	6.250	6.250	3.125	3.125	6.250	6.250
	K.s. 30-92	1.563	1.563	3.125	0.781	0.781	1.563	1.563	0.781

S.a.: *Staphylococcus aureus*, *S.e.*: *Staphylococcus epidermidis*, *E.k.*: *Enterococcus knothe*, *P.a.*: *Pseudomonas aeruginosa*, *K.s.*: *Klebsiella species*.

Table 3. Pharmacokinetics of **(R)-12a** and **(R)-12e** in rats

Parameter	**(R)-12a**	**(R)-12e**	**GMFX**	**CPFX**
C_{max} (μg/mL)	9.06±2.040	6.67±3.327	6.63	4.39±1.220
T_{max} (h)	2.00	1.00	1.00	0.50
$T_{1/2}$ (h)	4.50	6.94	2.12	2.07
AUC (μg·h/mL)	90.82	68.77	25.79	12.72

SD rat, administered at a dose of 50mg/kg

Table 4. Cytotoxicity of **(R)-12a** and **(R)-12e** (IC_{50}, μg/mL)

	(R)-12a	**(R)-12e**	**GMFX**	**Trovafloxacin**	**CPFX**
V79-4	5.49	0.21	10.08	3.77	58.55
HepG2	37.26	11.59	13.49	11.16	37.47

V79-4 and HepG2 were incubated in the presence of each sample for 72 h. Cell density was determined by MTT assay or by staining the cells with crystal violet.

Gram-positive organisms, MRSA and Ofloxacin resistant organisms. And with excellent pharmacokinetic profiles, both **(R)-12a** and **(R)-12e** are expected to be the best drug candidates near future. Now, we make efforts to optimize the synthetic scheme of them and develop better C-7 substituents.

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8. Coupling glycine ethylester hydrochloride with ethyl acrylate in the presence of triethylamine, Cbz protection and potassium *tert*-butoxide assisted cyclization can give the indicated starting material **1** (1-benzyl 3-ethyl 4-oxo-1,3-pyrrolidinedicarboxylate).
9. All cultures were obtained from Hoechst and MICs were determined by the agar dilution method as recommended by the National Committee for Clinical Laboratory Standards.
10. **14**: 7-(3-Aminomethyl-4-methoxyimino-3-methyl-piperidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid hydrochloride, **15**: 7-(3-Aminomethyl-4-benzyloxyimino-3-methyl-pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid hydrochloride, **16**: 7-(4-Aminomethyl-4,8,8-trimethyl-7,9-dioxa-2-aza-spiro[4.5]dec-2-yl)-1-cyclo-

propyl-6-fluoro-4oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid hydrochloride.

11. We could obtain single crystal of Cbz substituted (**S**)-**13** derivative [(*S*)-4,8,8-trimethyl-4-({[1-(toluene-4-sulfonyl)-pyrrolidine-2-carbonyl]-amino}-methyl)-6,10-dioxa-2-aza-spiro[4.5]decane-2-carboxylic acid benzyl ester, the chiral centre of this compound has same absolute configuration to that of (**S**)-**13**] to assign absolute configuration of the chiral centre of (**R**)-**13** and (**S**)-**13**. Crystal data: $C_{31}H_{14}O_7N_3S$ from Ethanol and Butanol solution, F.W. = 599.75, triclinic, $a = 10.1966$; $b = 12.2398$; $c = 13.6633$; $\alpha = 69.007$; $\beta = 88.535$; $\gamma = 86.991$; $V = 1589.82^3$, $d_{calc} = 1.253 g/cm^3$, Radiation = Mo Kα($\lambda = 0.71069$), Space group = P1, $Z = 2$, Final $R = 0.0793$.
12. (**R**)-**12a**: (*R*)-(+)-7-(3-Aminomethyl-4-methoxyimino-3-methyl-pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid hydrochloride, ^{1}H NMR(DMSO-d_6 + CF_3OOD, ppm) 1.05 (2H, bs), 1.20 (2H, d, $J = 7.3$ Hz), 1.34 (3H, s), 3.08 (1H, d, $J = 13.2$ Hz), 3.14 (1H, d, $J = 13.2$ Hz), 3.15 (2H, m), 3.66 (1H, bs), 3.86 (4H, bs), 4.08 (1H, d, $J = 12.7$ Hz), 4.61 (2H, s), 8.99 (1H, d, $J = 12.4$ Hz), 8.56 (1H, s), $[\alpha]_D = +5.77$ ($c = 2.33$, H_2O, 25 °C), (**S**)-**12a**: $[\alpha]_D = -3.74$ ($c = 1.32$, H_2O, 25 °C), (**R**)-**12e**: (*R*)-(−)-5-Amino-7-(3-aminomethyl-4-methoxy-imino-3-methyl-pyrrolidin-1-yl)-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid hydrochloride, ^{1}H NMR (DMSO-d_6 + CF_3COOD, ppm) 0.98 (2H, bs), 1.03 (2H, d, $J = 6.8$ Hz), 1.28 (3H, s), 3.00 (1H, d, $J = 13.2$ Hz), 3.05 (1H, d, $J = 13.2$ Hz), 3.59 (1H, d, $J = 10.8$ Hz), 3.79 (4H, bs), 3.91 (1H, bs), 4.25 (1H, d, $J = 17.3$ Hz), 4.41 (1H, d, $J = 17.3$ Hz), 8.45 (1H, s), $[\alpha]_D = -15.89$ ($c = 0.54$, H_2O, 25 °C), (**S**)-**12e**: $[\alpha]_D = +17.12$ ($c = 1.10$, H_2O, 25 °C).
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