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Synthesis and *in vitro* antibacterial activities of 7-(3-aminopyrrolo[3,4-c]pyrazol-5(2*H*,4*H*,6*H*)-yl)quinolone derivatives

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Abstract

A series of novel 7-(3-aminopyrrolo[3,4-c]pyrazol-5(2*H*,4*H*,6*H*)-yl)quinolone derivatives were designed, synthesized and evaluated for *in vitro* antibacterial activities. Compounds **6g**, **7g** and **7h** with the potencies similar to those of gemifloxacin, moxifloxacin, gatifloxacin and levofloxacin against Gram-positive organisms, worth further investigation.
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Keywords: Fluoroquinolone; Synthesis; Antibacterial activities

Since the discovery of norfloxacin, most of the quinolone antibacterial research has been focused on the basic group at the C-7 position to produce new potent quinolones, namely, ciprofloxacin, ofloxacin, lomefloxacin, fleroxacin, and sparfloxacin, all of which contain a piperazine derivative at the C-7 position [1–3]. Warner–Lambert reported that this piperazine structure has been successfully replaced with two appropriate mimics, 3-aminopyrrolidine and 3-(aminomethyl)pyrrolidine, tosufloxacin and clinafloxacin are two representative quinolones containing an aminopyrrolidine residue [4–6]. Moreover, gemifloxacin and moxifloxacin (Fig. 1), two important quinolones, also contain a pyrrolidine residue. Inspired by these previous research, we designed a series of 7-(3-aminopyrrolo[3,4-c]pyrazol-5(2*H*,4*H*,6*H*)-yl)quinolone derivatives. In this paper, we report the synthesis and *in vitro* antibacterial activities of these new quinolone derivatives.

Synthetic pathways toward side chains **2** and **4** and novel fluoroquinolone derivatives **6a–g** and **7a–h** are depicted in Scheme 1. 2,4,5,6-Tetrahydropyrrolo[3,4-c]pyrazol-3-amine hydrochloride **2** was prepared by *tert*-butyl 3-cyano-4-oxopyrrolidine-1-carboxylate **1** with hydrazine hydrochloride in ethanol. Another side chain was prepared by *tert*-butyl 3-cyano-4-oxopyrrolidine-1-carboxylate **1** with methylhydrazine in ethanol, and then compound **3** was subjected to deprotection by pumping hydrogen chloride gas, to provide 2,4,5,6-tetrahydro-2-methylpyrrolo[3,4-

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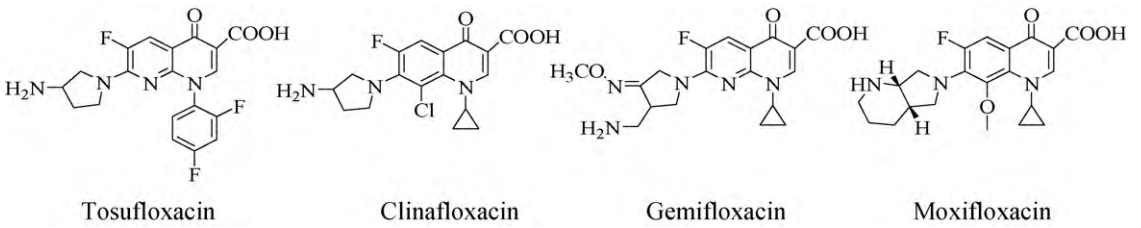
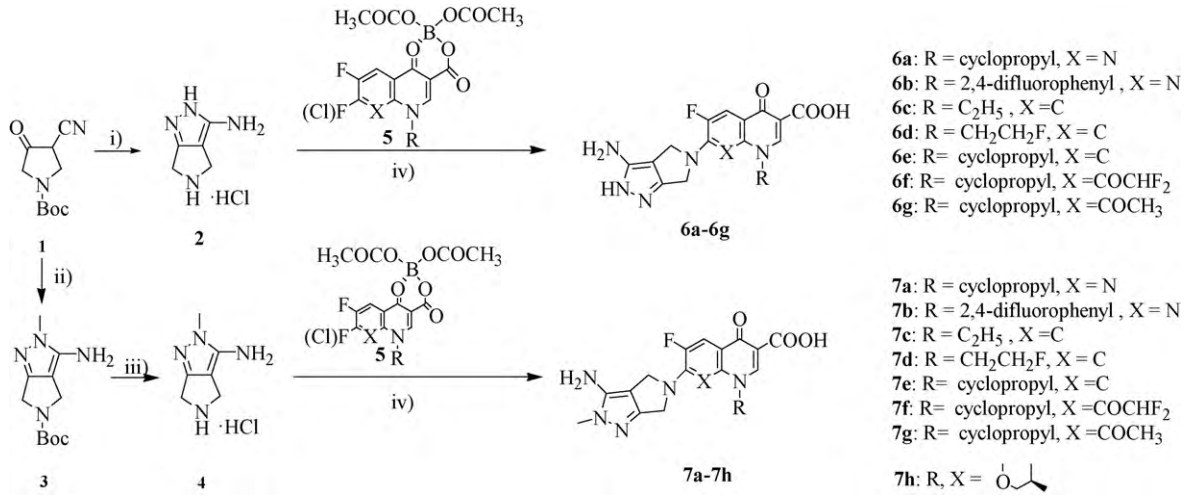


Fig. 1. Structures of four quinolone derivatives.



Scheme 1. Reagents and conditions: (i) $NH_2NH_2{\cdot}HCl$, C_2H_5OH, r.t., 12 h, 50.6%; (ii) NH_2NHCH_3, C_2H_5OH, r.t., 12 h, 23.0%; (iii) MeOH, HCl (gas), r.t., 1 h; (iv) a: CH_3CN, Et_3N, 25–60 °C, 1–48 h; b: 5% NaOH/H_2O, 40 °C, 0.5–2 h; c: 2 mol/L HCl, r.t., 17.9–91.3%.

c]pyrazol-3-amine hydrochloride **4**. Finally, the target compounds **6a–g** and **7a–h** were obtained by S_N reaction of boric chelating compounds **5** with **2** or **4**, and then hydrolysis of chelating groups [7–9].

The novel fluoroquinolones **6a–g** and **7a-h** were evaluated for their *in vitro* antibacterial activity against representative Gram-negative and Gram-positive strains using standard techniques. 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 gemifloxacin (GM), moxifloxacin (MX), gatifloxacin (GT) and levofloxacin (LV) for comparison are reported in Table 1 [10].

All 15 compounds display generally rather weak potency against the tested Gram-negative strains, but most of them exhibit good potency in inhibiting the growth of *Staphylococcus aureus* including MRSA and *Staphylococcus epidermidis* including MRSE (MIC: 0.125–8 μg/mL). In particular, the most active compound **7g** is 2–32-fold more potent than MX, GM, GT and LV against *Streptococcus pneumoniae* 08-3, *Klebsiella pneumoniae* 09-23 and *Pseudomonas aeruginosa* ATCC27853, 4–32-fold more potent than MX, GM and LV against *K. pneumoniae* 09-21, and more active than or comparable to the four reference drugs against *P. aeruginosa* 09-32.

Generally, the activity of the quinolone nuclei in this study is in the order 1-(2-fluoroethyl)-8-fluoro-quinolone > 1-cyclopropyl-8-methoxylquinolone > 1-cyclopropyl-8-fluoroquinolone ≈ 1-cyclopropyl-8-difluoromethoxylquinolone > 1-cyclopropyl-1,8-naphthyridone ≈ 1-(2,4-difluorophenyl)-1,8-naphthyridine ≈ 1-ethyl-8-fluoroquinolone for fluoroquinolones containing 3-aminopyrrolo[3,4-c]pyrazol-5(2*H*,4*H*,6*H*)-yl substitution at C-7 position; 1-cyclopropyl-8-fluoroquinolone > 1-cyclopropyl-8-difluoromethoxylquinolone > 1-cyclopropyl-1,8-naphthyridone ≈ 1-(2,4-difluorophenyl)-1,8-naphthyridine ≈ 1-ethyl-8-fluoroquinolone > 1-(2-fluoroethyl)-8-fluoroquinolone ≈ 1-cyclopropyl-8-fluoroquinolone for fluoroquinolones containing 3-amino-2-methyl-pyrrolo[3,4-c]pyrazol-5(2*H*,4*H*,6*H*)-yl substitution at C-7 position. In addition, fluoroquinolones containing 3-aminopyrrolo[3,4-c]pyrazol-5(2*H*,4*H*,6*H*)-yl substitution at C-7 position appear to be less than corresponding 2-methyl analogs.

Table 1
In vitro antibacterial activities of target compounds **6a–g** and **7a–h** (MIC: μg/mL).

Compd.	Organism														
	*S.a.*1	*S.a.*2	*S.a.*3	*S.a.*4	*S.a.*5	*S.e.*1	*S.e.*2	*S.e.*3	*S.e.*4	*S.e.*5	*S.p.*1	*S.p.*2	*S.p.*3	*E.fa.*1	*E.fa.*2
6a	2	16	>128	8	8	8	8	8	8	8	>128	16	16	>128	>128
6b	2	8	32	4	8	8	4	4	32	4	32	32	32	>128	>128
6c	4	32	128	8	8	8	8	8	8	32	32	32	>128	>128	>128
6d	0.25	0.25	>128	0.25	>128	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	128	128
6e	0.25	0.25	>128	0.25	0.25	0.25	0.25	2	0.25	0.25	>128	0.25	2	>128	>128
6f	0.25	0.25	32	0.25	0.25	0.25	0.25	0.25	0.25	0.25	>128	16	0.25	>128	>128
6g	0.125	0.25	1	0.25	0.25	0.25	0.25	0.25	32	0.25	32	2	2	64	64
7a	0.25	0.25	32	0.25	0.25	0.25	0.25	8	0.25	0.25	>128	0.25	8	>128	>128
7b	0.25	0.25	>128	0.25	0.25	0.25	0.25	4	0.25	0.25	>128	4	4	>128	>128
7c	0.125	0.25	8	0.25	0.125	0.25	0.25	0.125	16	0.125	>128	16	16	>128	>128
7d	0.5	1	>128	1	4	1	1	1	1	1	>128	8	>128	>128	>128
7e	0.5	0.5	2	2	2	2	2	2	2	2	64	0.25	0.25	128	128
7f	0.125	0.25	0.25	0.25	0.125	0.125	0.125	0.25	0.5	0.5	128	64	4	64	128
7g	0.125	0.25	0.125	0.25	0.125	0.25	0.25	0.25	0.5	0.25	64	0.5	0.25	8	>128
7h	0.25	0.25	0.5	0.125	0.25	0.25	0.5	0.125	0.25	0.5	64	0.25	0.25	>128	>128
MX	0.125	0.25	0.125	0.125	0.06	0.25	0.125	0.06	0.06	0.125	1	1	0.125	1	2
GM	0.125	0.25	0.25	0.06	0.125	0.125	0.06	0.03	0.125	0.25	16	8	0.25	16	16
GT	0.06	0.125	0.25	0.06	0.06	0.25	0.25	0.125	0.25	0.25	8	4	0.25	8	4
LV	0.125	0.125	0.25	0.125	0.125	0.25	0.25	0.25	0.125	4	3	2	2	8	8

Compd.	Organism														
	*E.fm.*1	*E.fm.*2	*E.c.*1	*E.c.*2	*E.c.*3	*E.c.*4	*E.c.*5	*K.p.*1	*K.p.*2	*K.p.*3	*P.a.*1	*P.a.*2	*P.a.*3	*P.a.*4	*P.a.*5
6a	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
6b	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
6c	>128	32	>128	>128	>128	>128	>128	>128	>128	8	>128	>128	>128	>128	>128
6d	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	128	128	128	128	>128
6e	>128	>128	128	>128	>128	>128	>128	>128	16	>128	16	16	>128	>128	32
6f	>128	>128	128	>128	>128	>128	>128	>128	64	>128	8	0.25	>128	>128	64
6g	64	64	128	>128	>128	>128	>128	64	128	64	8	8	128	64	16
7a	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	>128	>128
7b	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	128	16	>128	>128	16
7c	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
7d	>128	>128	>128	>128	>128	>128	>128	128	128	128	128	4	128	32	8
7e	128	128	4	8	>128	>128	>128	128	4	64	128	4	32	32	8
7f	64	128	32	64	>128	>128	>128	128	4	64	8	4	32	32	8
7g	>128	>128	128	>128	>128	>128	>128	0.5	>128	0.5	1	0.25	64	0.5	2
7h	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	4	4	>128	128	4
MX	2	2	2	2	2	2	2	2	1	1	2	0.25	2	0.25	0.5
GM	2	16	4	16	16	16	32	2	8	8	2	0.5	2	0.5	0.5
GT	2	4	2	8	8	2	1	0.125	0.06	2	2	16	4	1	0.5
LV	4	16	2	16	8	16	16	16	16	16	4	16	4	2	0.5

*S.a.*1, *Staphylococcus aureus* ATCC259223; *S.a.*2, methicillin-resistant *Staphylococcus aureus* 08-1; *S.a.*3, methicillin-resistant *Staphylococcus aureus* 08-2; *S.a.*4, methicillin-sensitive *Staphylococcus aureus* 08-1; *S.a.*5, methicillin-sensitive *Staphylococcus aureus* 08-2; *S.e.*1, methicillin-resistant *Staphylococcus epidermidis* 09-2; *S.e.*2, methicillin-resistant *Staphylococcus epidermidis* 09-3; *S.e.*3, methicillin-resistant *Staphylococcus epidermidis* 09-4; *S.e.*4, methicillin-sensitive *Staphylococcus epidermidis* 09-3; *S.e.*5, methicillin-sensitive *Staphylococcus epidermidis* 09-6; *S.p.*1, *Streptococcus pneumoniae* 08-2; *S.p.*2, *Streptococcus pneumoniae* 08-3; *S.p.*3, *Streptococcus pneumoniae* 08-4; *E.fa.*1, *Enterococcus faecalis* 08-10; *E.fa.*2, *Enterococcus faecalis* 08-12; *E.fm.*1, *Enterococcus faecium* 08-2; *E.fm.*2, *Enterococcus faecium* 08-7; *E.c.*1, *Escherichia coli* ATCC 25922; *E.c.*2, *Escherichia coli* 08-21; *E.c.*3, *Escherichia coli* 08-22; *E.c.*4, *Escherichia coli* 08-23; *E.c.*5, *Escherichia coli* 08-24; *K.p.*1, *Klebsiella pneumoniae* 09-21; *K.p.*2, *Klebsiella pneumoniae* 09-22; *K.p.*3, *Klebsiella pneumoniae* 09-23; *P.a.*1, *Pseudomonas aeruginosa* ATCC27853; *P.a.*2, *Pseudomonas aeruginosa* 09-32; *P.a.*3, *Pseudomonas aeruginosa* 09-33; *P.a.*4, *Pseudomonas aeruginosa* 09-34; *P.a.*5, *Pseudomonas aeruginosa* 09-35.

In summary, we report herein the synthesis of a series of novel 7-(3-amino-(2-methyl-)pyrrolo[3,4-c]-pyrazol-5(2*H*,4*H*,6*H*)-yl)fluoroquinolone derivatives. The antibacterial activity of the newly synthesized compounds were evaluated and correlated with their physicochemical properties. Results reveal that most of the target compounds have good activity *S. aureus* including MRSA and *S. epidermidis* including MRSE. However, all of them display generally rather weak potency against the tested Gram-negative strains.

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[8] Synthesis of 1-cyclopropyl-6-fluoro-7-(3-aminopyrrolo[3,4-c]pyrazol-5(2*H*,4*H*,6*H*)-yl)-1,4-dihydro-4-oxo-1,8-naphthy-ridine-3-carboxylic acid (**6a**): a mixture of **2** (0.38 g, 1.9 mmol), **5** (0.53 g, 1.3 mmol), triethylamine (1.0 mL) and anhydrous acetonitrile (10 mL) was stirred at room temperature for 0.5 h, and then concentrated *in vacuo*. To the residue was added 5% NaOH solution (6.0 mL), the reaction mixture was stirred at 40 °C for 0.5 h and then adjusted to pH 5 with 2 mol/L HCl. The precipitate was collected by suction to give off-white amorphous product **6a** (0.32 g, 66.5%).
[9] Typical data of the synthetic compounds: **6f** ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00–1.18 (m, 4H), 4.05–4.09 (m, 1H), 4.57–4.60 (m, 4H), 6.88 (t, 1H), 8.63 (s, 1H), 7.87 (d, 1H), 8.75 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 9.3, 40.8, 49.5, 49.8, 107.2, 108.8, 109.1, 114.7, 117.3, 118.2, 119.9, 130.7, 135.4, 142.2, 151.2, 152.4, 154.9, 165.3, 175.7; HRMS-ESI *m/z*: 458.10521 (calcd. for $C_{19}H_{16}F_3N_5O_4Na$ [M+Na]$^+$). **7f** ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00–1.18 (m, 4H), 3.52 (s, 3H), 4.05–4.09 (m, 1H), 4.55–4.73 (m, 4H), 6.90 (t, 1H), 7.89 (d, 1H), 8.75 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 9.3, 36.4, 40.8, 48.8, 50.6, 105.6, 107.3, 108.9, 114.7, 117.3, 118.6, 119.9, 131.0, 135.3, 137.7, 144.0, 147.4, 151.3, 165.3, 175.7; HRMS-ESI *m/z*: 450.13891 (calcd. for $C_{20}H_{19}F_3N_5O_4$ [M+H]$^+$). **7g** ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02–1.25 (m, 4H), 3.66 (s, 3H), 3.72 (s, 3H), 4.03–4.07 (m, 1H), 4.63–4.79 (m, 4H), 7.88 (d, 1H), 8.81 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 8.9, 40.9, 48.5, 50.1, 61.4, 106.0, 106.1, 106.4, 119.0, 134.2, 136.0, 136.1, 143.7, 144.5, 147.4, 150.3, 153.1, 155.6, 165.6, 176.0; HRMS-ESI *m/z*: 414.15776 (calcd. for $C_{20}H_{21}FN_5O_4$ [M+H]$^+$). **7h** ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45–1.47 (m, 3H), 3.50 (s, 3H), 4.32–4.35 (m, 1H), 4.52–4.91 (m, 6H), 7.59 (d, 1H), 8.93 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 17.8, 36.3, 49.0, 50.6, 54.8, 67.9, 103.5, 106.2, 117.5, 124.9, 130.0, 137.6, 144.6, 146.1, 147.3, 152.5, 154.9, 166.1, 176.0; HRMS-ESI *m/z*: 400.14211 (calcd. for $C_{19}H_{19}FN_5O_4$ [M+H]$^+$).
[10] MICs were determined as described by the NCCLS (see National Committee for Clinical Laboratory Standards (2001). Performance standards for antimicrobial susceptibility testing: 11th informational supplement, vol. 21, M100-S11. National Committee for Clinical Laboratory Standards, Wayne, PA). The MIC was defined as the lowest concentration of each compound resulting in inhibition of visible growth of bacteria after incubation at 37 °C for 18–24 h.