Full Paper

Synthesis and *In-vitro* Antibacterial Activity of 7-(3-Aminopyrrolo[3,4-*c*]pyrazol-5(2*H*,4*H*,6*H*)-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid Derivatives

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A series of novel 7-(3-aminopyrrolo[3,4-*c*]pyrazol-5(2*H*,4*H*,6*H*)-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid derivatives was designed, synthesized and characterized by ^{1}H-NMR, MS and HRMS. These fluoroquinolones were evaluated for their *in-vitro* antibacterial activity against representative Gram-positive and Gram-negative strains. Generally, all of the target compounds display rather weak potency against the tested Gram-negative strains, but most of them exhibit good potency in inhibiting the growth of *S. aureus* including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* including methicillin-resistant *S. epidermidis* (MRSE) (MIC: 0.125–8 μg/mL). In particular, the compound **9g** is 2 to 32 fold more potent than gemifloxacin (GM), moxifloxacin (MX), gatifloxacin (GT), and levofloxacin (LV) against *S. pneumoniae* 08-3, *K. pneumoniae* 09-23, and *P. aeruginosa* ATCC27853, 4 to 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.

Keywords: Antibacterial activity / Fluoroquinolone / Structure–activity relationship

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Introduction

Since the discovery of nalidixic acid by Lesher *et al.* in 1962 [1], the quinolones have evolved into an important class of antibacterial agents used mainly for the treatment of respiratory tract infections (RTI), urinary tract infections (UTI), sexually transmitted diseases (STD), gastrointestinal and abdominal infections, skin and soft tissue infections, and infections of the bone and joints among many other uses [2]. These antibiotics act by binding to the quinolone-resistance-determining region (QRDR) in the catalytic domain of the topoisomerase II (DNA gyrase) or IV complex with DNA. Cell death is induced by trapping the topoisomerase protein–DNA complex thus disrupting normal DNA replication, inducing oxidative damage, and triggering cell-death mechanisms. DNA gyrase appears to be the primary target for quinolones in Gram-negative bacteria such as *Escherichia coli*, while topoisomerase IV is the primary target in Gram-positive bacteria such as *Staphylococcus aureus* [3, 4].

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 is also believed to be one of the reasons for 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 and ofloxacin [5].

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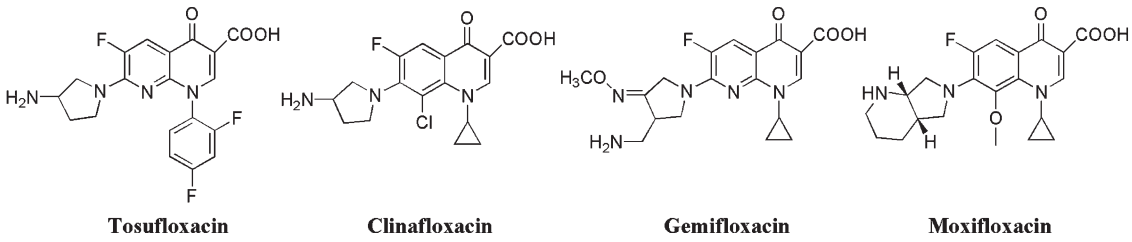


Figure 1. The structure of some quinolones containing an aminopyrrolidine residue at the C-7 position.

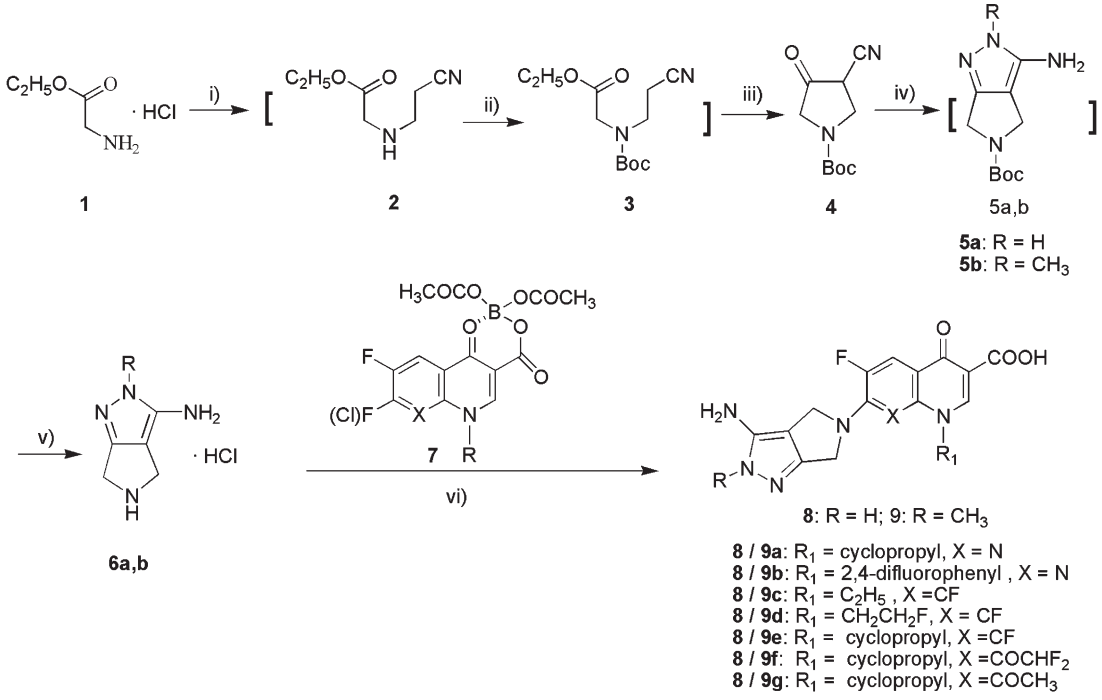
Structure–activity relationship (SAR) studies of quinolone antibacterial agents showed that the basic group at the C-7 position is the most adaptable site for chemical change and an area that greatly influences their potency, spectrum, and safety [6]. In general, 5- and 6-membered nitrogen heterocycles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

The introduction of the pyrrolidine derivatives to the quinolones resulted in a dramatic improvement of Gram-positive activity compared to piperazinyl analogs. Many new fluoroquinolones used currently in the clinic, including tosufloxacin, sitafloxacin, trovafloxacin, gemifloxacin (GM), and moxifloxacin (MX), contain an aminopyrrolidine residue at the C-7 position [8–12] (Fig. 1). In this study, additional attempts have been made to modify the pyrrolidinyl moiety. The 3-aminopyrazole function group at the 3-positions of some fourth generation cephalosporins, such as cefoselis [13], was fused with a pyrrolidine to offer the 3-amino-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole which can be readily obtained from the corresponding 3-cyano-4-oxopyrrolidine in a single operation. A series of novel fluoroquinolone compounds containing these bicyclic amines at the 7-position were designed and synthesized. Our primary objective was to optimize the potency of these compounds against Gram-positive and Gram-negative organisms.

Results and discussion

Chemistry

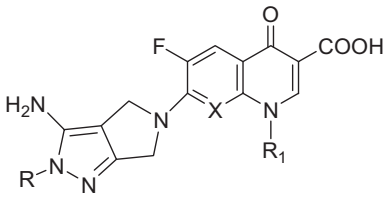
The synthesis of the novel fluoroquinolone derivatives **8a–g** and **9a–g** is outlined in Scheme 1. Addition reaction of ethylglycinate hydrochloride **1** with acrylonitrile in the presence of



Reagents and conditions: (i) MeOH, NaOH, Acrylonitrile; (ii) Boc_2O; (iii)NaH, Toluene; (iv) $NH_2NH_2{\cdot}HCl$ / NH_2NHCH_3, C_2H_5OH; (v) MeOH, HCl (gas); (iv) a: CH_3CN, Et_3N; b: 5% $NaOH/H_2O$; c: 2 mol/L HCl.

Scheme 1. Synthesis route of compounds **8a–g** and **9a–g**.

Table 1. Structures and physical data of novel fluoroquinolones **8a–g** and **9a–g**.



Compound	R	R_1	X	Yield (%)	Mp (°C)[a)]	ESI-MS $(M + H)^+$
8a	H		N	67	>300	371
8b	H	2,4-F_2-C_6H_3	N	72	283–285 (dec)	443
8c	H	C_2H_5	CF	56	260–263 (dec)	376
8d	H	2-F-C_2H_4	CF	84	256–258 (dec)	394
8e	H		CF	39	273–275 (dec)	388
8f	H		$COCHF_2$	43	239–241 (dec)	436
8g	H		$COCH_3$	18	244–245 (dec)	400
9a	CH_3		N	83	214–216 (dec)	385
9b	CH_3	2,4-F_2-C_6H_3	N	91	236–237 (dec)	457
9c	CH_3	C_2H_5	CF	77	223–225 (dec)	390
9d	CH_3	2-F-C_2H_4	CF	68	243–245 (dec)	408
9e	CH_3		CF	69	267–268 (dec)	402
9f	CH_3		$COCHF_2$	47	220–223 (dec)	450
9g	CH_3		$COCH_3$	26	231–232 (dec)	414

[a)] Melting points are uncorrected.

sodium hydroxide gave the secondary amine **2**, which was subsequently treated with di-*tert*-butoxycarbonyl dicarbonate (Boc_2O) to produce the Boc-protected cyano ester **3**. The compound **3** was cyclized to the cyano ketone **4** by sodium hydride in refluxing toluene, with an overall yield of 66% for the three steps. The cyano ketone **4** was treated with hydrazine or methylhydrazine in ethanol to give the Boc-protected bicyclic amines **5a,b** which upon deprotection afforded the key intermediates **6a,b** by pumping hydrogen chloride gas [14–16].

Finally, the target compounds **8a–g** and **9a–g** were obtained by the coupling reaction of the intermediates **6a,b** with various boric chelates containing quinolone and naphthyridone cores **7**, and then hydrolysis of chelating groups according to well-established literature procedures [17–19]. Table 1 shows the novel fluoroquinolone analogs, yields of the final coupling step, and melting points of the purified compounds.

Antibacterial activity

The novel fluoroquinolones **8a–g** and **9a–g** were evaluated for their *in-vitro* antibacterial activity against representative Gram-negative and Gram-positive strains using standard techniques [20]. 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 GM, MX, gatifloxacin (GT), and levofloxacin (LV) for comparison are reported in Table 2.

The novel fluoroquinolones **8a–g** and **9a–g** display generally rather weak potency against the tested Gram-negative strains, but most of them exhibit good potency in inhibiting the growth of *S. aureus* including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* including methicillin-resistant *S. epidermidis* (MRSE) (MIC: 0.125–8 μg/mL). In particular, the most active compound **9g** is 2 to 32 fold

Table 2. *In-vitro* antibacterial activity of compounds **8a–g** and **9a–g**.

strains	Compd. MIC (μg/mL) [a]																
	8a	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**MX**	**GM**	**GT**	**LV**
*S.a.*1	2	2	4	0.25	0.25	0.25	0.125	0.25	0.25	0.125	0.5	0.5	0.125	0.125	0.125	0.125	0.06	0.125
*S.a.*2	16	8	32	0.25	0.25	0.25	0.25	0.25	0.25	0.25	1	0.5	0.25	0.25	0.25	0.25	0.125	0.125
*S.a.*3	8	4	8	0.25	0.25	0.25	0.25	0.25	0.25	0.25	1	2	0.25	0.25	0.125	0.06	0.06	0.125
*S.a.*4	8	8	8	>128	0.25	0.25	0.25	0.25	0.25	0.125	4	2	0.125	0.125	0.06	0.125	0.06	0.125
*S.e.*1	8	8	8	0.25	0.25	0.25	0.25	0.25	0.25	0.25	1	2	0.125	0.25	0.25	0.125	0.25	0.25
*S.e.*2	8	4	8	0.25	0.25	0.25	0.25	0.25	0.25	0.25	1	2	0.125	0.25	0.125	0.06	0.25	0.25
*S.e.*3	8	4	8	0.25	2	0.25	0.25	8	4	0.125	1	2	0.25	0.25	0.06	0.03	0.125	0.25
*S.e.*4	8	32	8	0.25	0.25	0.25	32	0.25	0.25	16	1	2	0.5	0.5	0.06	0.125	0.25	0.125
*S.e.*5	8	4	32	0.25	0.25	0.25	0.25	0.25	0.25	0.125	1	2	0.5	0.25	0.125	0.25	0.25	4
*S.p.*1	>128	32	32	0.25	>128	>128	32	>128	>128	>128	>128	64	128	64	1	16	8	3
*S.p.*2	16	32	32	0.25	0.25	16	2	0.25	4	16	8	0.25	64	0.5	1	8	4	2
*S.p.*3	16	32	>128	0.25	2	0.25	2	8	4	16	>128	0.25	4	0.25	0.125	0.25	0.25	2
*E.fa.*1	>128	>128	>128	128	>128	>128	64	>128	>128	>128	>128	128	64	8	1	16	8	8
*E.fa.*2	>128	>128	>128	128	>128	>128	64	>128	>128	>128	>128	128	128	>128	2	16	4	8
*E.fm.*1	>128	>128	>128	>128	>128	>128	64	>128	>128	>128	>128	128	64	>128	2	2	2	4
*E.fm.*2	>128	>128	32	>128	>128	>128	64	>128	>128	>128	>128	128	128	>128	2	16	4	16
*E.c.*1	>128	>128	>128	>128	128	128	128	>128	128	128	>128	4	32	128	2	4	2	2
*E.c.*2	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	8	64	>128	2	16	8	16
*E.c.*3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	2	16	8	8
*E.c.*4	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	2	16	2	16
*E.c.*5	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	2	32	1	16
*K.p.*1	>128	>128	>128	>128	>128	>128	64	>128	>128	>128	128	128	128	0.5	2	2	0.125	16
*K.p.*2	>128	>128	>128	128	16	64	128	>128	>128	>128	128	4	4	>128	1	8	0.06	16
*K.p.*3	>128	>128	8	>128	>128	>128	64	>128	>128	>128	128	64	64	0.5	1	8	2	16
*P.a.*1	>128	>128	>128	128	16	8	8	128	128	>128	128	128	8	1	2	2	2	4
*P.a.*2	>128	>128	>128	128	16	0.25	8	>128	16	>128	4	4	4	0.25	0.25	0.5	16	16
*P.a.*3	>128	>128	>128	128	>128	>128	128	>128	>128	>128	128	32	32	64	2	2	4	4
*P.a.*4	>128	>128	>128	128	>128	>128	64	>128	>128	>128	32	32	32	0.5	0.25	0.5	1	2
*P.a.*5	>128	>128	>128	>128	32	64	16	>128	16	>128	8	8	8	2	0.5	0.5	0.5	0.5

[a] The values were reproduced in three experiments. [b] Abbreviations: *S.a.*1, *Staphylococcus aureus* ATCC259223; *S.a.*2, methicillin-resistant *Staphylococcus aureus* 08-1; *S.a.*3, methicillin-sensitive *Staphylococcus aureus* 08-1; *S.a.*4, 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.

more potent than the reference drugs MX, GM, GT, and LV against *S. pneumoniae* 08-3, *K. pneumoniae* 09-23 and *P. aeruginosa* ATCC27853, 4 to 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 of 1-(2-fluoroethyl)-8-fluoroquinolone > 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.

Conclusion

In summary, we report herein the synthesis of a series of novel 7-(3-aminopyrrolo[3,4-*c*]pyrazol-5(2*H*,4*H*,6*H*)-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid derivatives. The

antibacterial activity of the newly synthesized compounds was evaluated. 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. The reduced activity might be due to the conjugated effect between the introduced new double bond and the amino group possessing a lone pair of electrons on the nitrogen, hindering the amino group from participating in hydrogen bonding with the drug target, which in case of quinolone is DNA gyrase.

Experimental

All chemical reagents and solvents used in this study were purchased from Beihua Fine Chemicals Company (Beijing, China). Melting points were determined in open glass capillaries and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Mercury-400 or an INOVA-500 spectrometer using tetramethylsilane as internal standard. Electron spray ionization (ESI) mass spectra and high resolution mass spectra (HRMS) were recorded on a MDSSCIEX Q-Tap mass spectrometer. Merck silica gel ART5554 60F254 plates were used for analytical TLC. Column chromatography was carried out on silica gel HG/T2354-92 made in Haiyang Chemical Company (Qingdao, China).

*tert-Butyl 3-cyano-4-oxopyrrolidine-1-carboxylate **4***

A mixture of ethylglycinate hydrochloride **1** (167.4 g, 1.2 mol), sodium hydroxide (48.0 g, 1.2 mol), and methanol (600 mL) was stirred for 0.5 h, and then acrylonitrile (79.5 g, 1.5 mol) was added dropwise over a period of 40 min at room temperature. The reaction mixture was heated to 65°C and stirred for 3 h to give the secondary amine **2**, which was pure enough to be used for the next step without further purification.

To the reaction mixture containing the amine **2** was added $(Boc)_2O$ (218.0 g, 1.0 mol) at room temperature, stirred at 55–60°C for 1 h and filtered. The filtrate was concentrated under reduced pressure. The residue was diluted with ethyl acetate (400 mL), washed with water and then saturated saline, dried over anhydrous sodium sulfate and filtered. The filter was concentrated under reduced pressure to afford the cyano ester **3** as a colorless oil.

To a refluxing suspension of sodium hydride (70%, 41.1 g, 1.2 mol) in dry toluene (600 mL) was added dropwise a solution of the cyano ester **3** dissolved in dry toluene (200 mL) under over a period of 0.5 h. The reaction mixture was stirred for 1 h at the same temperature, cooled to room temperature and then water (400 mL) was added slowly. The aqueous layer was separated and adjusted to pH 7 with 10% glacial acetic acid. The solid obtained was filtered, washed twice with water and dried *in vacuo* to give the title compound **4** (190.0 g, 75%) as a white solid, mp: 160–161°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 1.47 (9H, s, Boc-9H), 3.64–4.06 (4H, m, pyrrolidine), 4.34–4.38 (1H, m, pyrrolidine). ESI-MS: *m/z* 209 $(M - H)^-$.

*3-Amino-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole hydrochloride **6a***

A solution of *tert*-butyl 3-cyano-4-oxopyrrolidine-1-carboxylate **4** (2.1 g, 10.0 mmol) and hydrazine hydrochloride (1.1 g, 10.0 mol) dissolved in ethanol (50 mL) was stirred for 12 h at room temperature to give the Boc-protected bicyclic compound **5a**. To the reaction mixture containing compound **5a** was pumped dried hydrochloride gas at room temperature for 0.5 h, and then stirred for another 0.5 h at the same temperature. The resulting solid was collected by suction, and dried *in vacuo* to give the title compound **6a** (1.0 g, 51%) as a white solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 4.13 (2H, s, CH_2), 4.28 (2H, s, CH_2). ESI-MS: *m/z* 125 $(M + H)^+$.

*3-Amino-2-methyl-2,4,5,6-tetrahydro-pyrrolo[3,4-c]-pyrazole hydrochloride **6b***

The title compound was obtained in a similar manner as for the preparation **6a** (23%) as a white solid. ^{1}H-NMR (DMSO-d_6, 400MHz) δ_H 3.69 (3H, s, CH_3), 4.20 (2H, s, CH_2),4.41 (2H, s, CH_2). ESI-MS: *m/z* 139 $(M + H)^+$.

*General procedure for the synthesis of 7-(3-aminopyrrolo-[3,4-c]pyrazol-5(2H,4H,6H)-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid derivatives **8a–g** and **9a–g***

A mixture of **7** (1.0 mmol), **6a,b** (1.5 mmol), triethylamine (8.0 mmol), and dry acetonitrile (20 mL) was stirred at 25∼60°C under an atmosphere of nitrogen for 1∼48 h and detected with TLC (ethyl acetate as the mobile phase). The resulting solid was collected by suction, and dried *in vacuo* to give the title compounds **8a–g** and **9a–g**.

*7-(3-Aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **8a***

The title compound was obtained as a white solid (23%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.11–1.22 (4H, m, 2 cyclopropyl CH_2), 3.74–3.76 (1H, m, cyclopropyl CH), 4.43–4.81 (6H, m, 2 CH_2 and NH_2), 5.14 (1H, br, NH), 8.03 (1H, d, $J = 12.8$Hz, C_5-H), 8.59 (1H, s, C_2-H), 15.36 (1H, br, COOH); HRMS-ESI: *m/z* calcd. for $C_{17}H_{16}FN_6O_3$ $(M + H)^+$: 371.12679. Found 371.12655.

*7-(3-Aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **8b***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 3.87–4.05 (4H, m, 2 CH_2), 4.75 (2H, br, NH_2), 5.09 (1H, br, NH), 7.33–7.37 (1H, m, ph-1H), 7.60–7.63 (1H, m, ph-1H), 7.79–7.82 (1H, m, ph-1H), 8.09 (1H, d, $J = 12.8$ Hz, C_5-H), 8.80 (1H, s, C_2-H), 15.27 (1H, br, COOH); HRMS-ESI: *m/z* calcd. for $C_{20}H_{14}F_3N_6O_3$ $(M + H)^+$: 443.10795. Found 443.10891.

*7-(3-Aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **8c***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.22 (3H, t, $J = 6.8$Hz, NCH_2CH_3), 4.56 (2H, q, $J = 6.8$ Hz, NCH_2CH_3), 4.70–5.12 (7H, m, 2 CH_2 and NH_2 and NH), 7.81 (1H, d, $J = 14.8$ Hz, C_5-H), 8.86 (1H, s, C_2-H), 15.13 (1H, br, COOH); HRMS-ESI: *m/z* calcd. for $C_{17}H_{16}F_2N_5O_3$ $(M + H)^+$: 376.12212. Found 376.12131.

7-(3-Aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-(2-fluoroethyl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***8d***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 4.69–4.88 (4H, m, 2 CH_2), 4.95–5.07 (7H, m, CH_2 CH_2F and NH_2 and NH), 7.82 (1H, d, J = 14.4 Hz, C_5-H), 8.80 (1H, s, C_2-H), 15.21 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{17}H_{15}F_3N_5O_3$ $(M + H)^+$: 394.11270. Found 394.11090.

7-(3-Aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***8e***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.13–1.17 (4H, m, CH_2CH_2), 3.89–3.94 (1H, m, CH), 4.69–4.73 (4H, m, 2 CH_2), 4.79 (2H, br, NH_2), 5.07 (1H, br, NH),7.76 (1H, d, J = 14.4 Hz, C_5-H), 8.63 (1H, s, C_2-H), 15.24 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{18}H_{16}F_2N_5O_3$ $(M + H)^+$: 388.12212. Found 388.11966.

7-(3-Aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6-fluoro-8-difluoromethoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***8f***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.00–1.18 (4H, m, CH_2CH_2), 4.05–4.09 (1H, m, CH), 4.57–4.60 (6H, m, 2 CH_2 and NH_2), 5.07 (1H, br, NH), 6.88 (1H, t, J = 73.2 Hz, $OCHF_2$), 7.87 (1H, d, J = 13.6 Hz, C_5-H), 8.75 (1H, s, C_2-H), 15.24 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{19}H_{16}F_3N_5O_4Na$ $(M + Na)^+$: 458.10521. Found 458.10554.

7-(3-Aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6-fluoro-8-methoxyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***8g***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.00–1.14 (4H, m, CH_2CH_2), 3.59 (3H, s, OCH_3), 4.15–4.20 (1H, m, CH), 4.53–4.56 (6H, m, 2 CH_2 and NH_2), 5.05 (1H, br, NH), 7.72 (1H, d, J = 13.6 Hz, C_5-H), 8.67 (1H, s, C_2-H), 15.07 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{19}H_{18}FN_5O_4Na$ $(M + Na)^+$: 422.12405. Found 422.12722.

7-(2-Methyl-3-aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9a***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.11–1.27 (4H, m, CH_2CH_2), 3.52 (3H, s, CH_3), 3.65–3.76 (1H, m, CH), 4.66–5.00 (6H, m, 2 CH_2 and NH_2), 8.03 (1H, d, J = 12.8 Hz, C_5-H), 8.59 (1H, s, C_2-H), 15.35 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{18}H_{18}FN_6O_3$ $(M + H)^+$: 385.14244. Found 385.14036.

7-(2-Methyl-3-aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9b***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 3.46 (3H, s, CH_3), 3.82–4.18 (4H, m, 2 CH_2), 4.94 (2H, br, NH_2), 7.32–7.36 (1H, m, ph-1H), 7.58 (1H, s, ph-1H), 7.79–7.85 (1H, m, ph-1H), 8.01 (1H, d, J = 12.8 Hz, C_5-H), 8.80 (1H, s, C_2-H), 15.10 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{21}H_{16}F_3N_6O_3$ $(M + H)^+$: 457.12360. Found 457.11910.

7-(2-Methyl-3-aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9c***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.43 (3H, t, J = 6.4 Hz, NCH_2CH_3), 3.51 (3H, s, CH_3), 4.55 (2H, q, J = 6.8 Hz, NCH_2CH_3), 4.70–4.86 (6H, m, 2 CH_2 and NH_2), 7.79 (1H, d, J = 14.4 Hz, C_5-H), 8.86 (1H, s, C_2-H), 15.04 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{18}H_{18}F_2N_5O_3$ $(M + H)^+$: 390.13777. Found 390.13416.

7-(2-Methyl-3-aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-(2-fluoroethyl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9d***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 3.50 (3H, s, CH_3), 4.69–4.83 (4H, m, 2 CH_2H), 4.86–4.94 (6H, m, CH_2 CH_2F and NH_2), 7.82 (1H, d, J = 14.4 Hz, C_5-H), 8.81 (1H, s, C_2-H), 14.94 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{18}H_{16}F_3N_5O_3Na$ $(M + Na)^+$: 430.11029. Found 430.10827.

7-(2-Methyl-3-aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9e***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.16–1.17 (4H, m, CH_2CH_2), 3.51 (3H, s, CH_3), 4.10–4.11 (1H, m, CH), 4.70–4.87 (6H, m, 2 CH_2 and NH_2), 7.77 (1H, d, J = 14.0 Hz, C_5-H), 8.64 (1H, s, C_2-H), 14.90 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{19}H_{17}F_2N_5O_3Na$ $(M + Na)^+$: 424.11971. Found 424.11852.

7-(2-Methyl-3-aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6-fluoro-8-difluoromethoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9f***

^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 1.00–1.18 (4H, m, CH_2CH_2), 3.52 (3H, s, CH_3), 4.05–4.09 (1H, m, CH), 4.55–4.73 (6H, m, 2 CH_2 and NH_2), 6.90 (1H, t, J = 73.6 Hz, $OCHF_2$), 7.89 (1H, d, J = 13.6 Hz, C_5-H), 8.75 (1H, s, C_2-H), 14.81 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{20}H_{19}F_3N_5O_4$ $(M + H)^+$: 450.13891. Found 450.13566.

7-(2-Methyl-3-aminopyrrolo[3,4-c]pyrazol-5(2H,4H,6H)-yl)-1-cyclopropyl-6-fluoro-8-methoxyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9g***

^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H 1.02–1.25 (4H, m, CH_2CH_2), 3.66 (s, 3H), 3.72 (3H, s, CH_3), 4.03–4.07 (1H, m, CH), 4.63–4.79 (6H, m, 2 CH_2 and NH_2), 7.88 (1H, d, J = 13.6 Hz, C_5-H), 8.81 (1H, s, C_2-H), 14.81 (1H, br, COOH); HRMS-ESI: m/z calcd. for $C_{20}H_{21}FN_5O_4$ $(M + H)^+$: 414.15776. Found 414.16054.

Antibacterial activity

Compounds **8a–g** and **9a–g** 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, and 0.03 μg/mL. Petri dishes were incubated with 104 colony forming units (cfu) and incubated at 35°C for 18 h. The MIC was the lowest concentration of the test compound, which resulted in no visible growth on the plate.

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