Chem Biol Drug Des 2014

Research Article

Design, Synthesis, Antibacterial Evaluation and Docking Study of Novel 2-Hydroxy-3-(nitroimidazolyl)-propyl-derived Quinolone

Qing Li[1], Junhao Xing[1], Haibo Cheng[2], Hui Wang[3], Jing Wang[2], Shuai Wang[2], Jinpei Zhou[2] and Huibin Zhang[1,*]

[1]Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China
[2]Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China
[3]School of Life Science and Technology, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China
**Corresponding author: Huibin Zhang, zhanghb80@cpu.edu.cn*

A novel series of 2-hydroxy-3-(nitroimidazolyl)-propyl-derived quinolones 6a–o were synthesized and evaluated for their in vitro antibacterial activity. Most of the target compounds exhibited potent activity against Gram-positive strains. Among them, moxifloxacin analog 6n displayed the most potent activity against Gram-positive strains including S. epidermidis (MIC = 0.06 μg/mL), MSSE (MIC = 0.125 μg/mL), MRSE (MIC = 0.03 μg/mL), S. aureus (MIC = 0.125 μg/mL), MSSA (MIC = 0.125 μg/mL), (MIC = 2 μg/mL). Its activity against MRSA was eightfold more potent than reference drug gatifloxacin. Finally, docking study of the target compound 6n revealed that the binding model of quinolone nucleus was similar to that of gatifloxacin and the 2-hydroxy-3-(nitroimidazolyl)-propyl group formed two additional hydrogen bonds.

Key words: antibacterial activity, docking study, nitroimidazole, quinolone

Abbreviations: 3D, three dimension; MDR, multidrug resistant; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MSSA, methicillin-sensitive *Staphylococcus aureus*; MSSE, methicillin-sensitive *Staphylococcus epidermidis*; MTB, *Mycobacterium tuberculosis*; PDB, protein data bank; VRE, vancomycin-resistant *Enterococcus faecalis*; VRSA, vancomycin-resistant *Staphylococcus aureus*.

Received 21 February 2014, revised 11 June 2014 and accepted for publication 3 July 2014

Increasing prevalence of bacterial resistance to antibiotics represents a serious medical and economical challenge (1–3). Infections caused by drug-resistant bacteria are often associated with considerable morbidity and mortality (4). Of particular concern are multidrug resistant (MDR) bacteria, such as methicillin-resistant *aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE), which are exhibiting increasing levels of resistance to commonly used antibiotics (5,6). Among them, MRSA is the leading cause of nosocomial and community-acquired infections worldwide, and it has been estimated that MRSA infection leads to 19 000 deaths annually in the United States (4). Thus, it is urgent for development of new antibacterial agents with the ability to exterminate these drug-resistant bacterial strains.

Quinolones, such as enoxacin (**1**), lomefloxacin (**2**) and ciprofloxacin (**3**) (Figure 1), which exert their antibacterial action by inhibition of DNA-gyrase and topoisomerase IV (7), are important antibacterial agents widely used in treatment of both Gram-positive and Gram-negative bacterial infections (8). Quinolones also possess exceptional antibacterial activity against *Mycobacterium tuberculosis* (MTB), gatifloxacin (**4**) and moxifloxacin (**5**) (Figure 1) are undergoing evaluation in phase III trial for drug-susceptible MTB (9). The SAR studies of quinolones have revealed that basic group at the C-7 position is the most adaptable site for regulating physicochemical property and an area that greatly influences their potency, spectrum and safety (10,11). Moreover, increasing bulk at C-7 position is tolerated (12). Thus, much effort has been made on the structural modification of C-7 position in quinolones by incorporating several bioactive moieties including macrolides (13,14), oxazolidinones (15), triazoles (16), 5-(nitroaryl)-1,3,4-thiadiazoles (17,18) and sulfonamides (19,20). This led to obtaining quinolone hybrids as a new class of antimicrobial agents (21). Several quinolone hybrids displayed enhanced antibacterial activity against both Gram-positive and Gram-negative organisms (13–20). Recently, chemometric studies coupled with 3D structure-based molecular modeling approaches were conducted to explore the possibilities for the optimization of quinolone compounds, which also revealed that several novel fragments attached at 1 and 7 positions of those quinolones

 doi: 10.1111/cbdd.12395

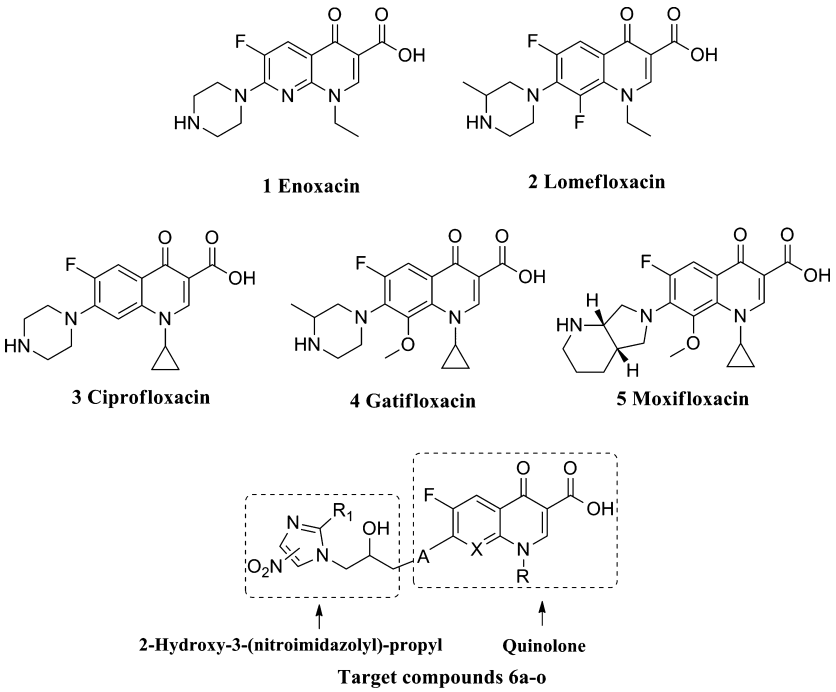


Figure 1: Structures of quinolones and design of target compounds **6a**–**o**.

could provide a good accommodation for binding of the active site (22,23). All these stimulated us with great interest to focus on further structural modification at the C-7 position of quinolone in order to find novel quinolone derivatives with potential activity against bacterial strains including drug-resistant microorganisms.

In this study, we introduced 2-hydroxy-3-(nitroimidazolyl)-propyl group which may severe as new hydrogen bond donors and receptors to increase binding affinity to the enzymes at C-7 position of quinolone. Thus, a series of novel compounds including 3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl-derived quinolones **6a**–**i** and 2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl-derived quinolones **6j**–**o** were designed and synthesized (Figure 1). All of the target compounds **6a**–**o** were evaluated for their *in vitro* activity against both Gram-positive and Gram-negative pathogens. Furthermore, the molecular docking was performed by inserting compound **6n** into the active site of the topoisomerase II DNA-gyrase to investigate binding model of the target compounds.

Methods and Materials

General chemistry

All reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China) and used without further purification. Melting points were measured on capillary tube and were uncorrected. IR spectra (in KBr pellets) were taken using Shimadzu FT-IR-8400S spectrophotometer (Nishinokyo-Kuwabara-cho, Nakagyo-ku, Kyoto, Japan). ^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6, $CDCl_3$) were recorded with a Bruker AV-300 or ACF 500 spectrometer (Beijing, China) in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (J) in Hz. High-resolution mass spectra were recorded using an Agilent QTOF 6520 (Beijing, China).

Synthesis of 2-chloro-4-nitroimdazole (10)

4-Nitroimidazole (22.6 g, 0.2 mol) was mixed with acetic anhydride (200 mL) in 500-mL three-neck round-bottomed flask. Fuming nitric acid (13.6 mL, 0.3 mol) was slowly added the well-stirred mixture at a rate such that the internal temperature is maintained at 0 °C. After addition was completed, the reaction mixture was warmed to 23–25 °C and stirred for 4 h. The mixture was poured into ice water (800 mL) and extracted with dichloromethane (200 mL × 3). The combined organic layers was washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo to afford 1,4-dinitro-1H-imidazole (**8**) as white solid (23.0 g, 73%).

The suspension of 1,4-dinitro-1H-imidazole (23.0 g, 0.15 mol) in toluene (200 mL) was refluxed for 16 h. After TLC analysis indicated the completed consumption of starting materials, the mixture was cooled to room temperature and a gradual formation of precipitate was observed. The precipitate was collected by filtration to afford 2,4-dinitro-1H-imidazole as yellowish solid (18.5 g, 80%).

2,4-dinitro-1H-imidazole (7.81 g, 0.05 mol) was mixed with concentrated hydrochloric acid (75 mL) and refluxed for 2 h. Then the mixture was cooled to room temperature,

addition of water (10 mL) afforded a white precipitate which was collected by filtration, dried in vacuo to give 2-chloro-4-nitro-1H-imidazole as white solid (3.61 g, 50%). m.p. 219–219.5 °C; ESI-MS m/z 148.3 $[M + H]^+$; ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 14 (brs, 1H), 8.45 (s, 1H).

Synthesis of 2-chloro-4-nitro-1-(oxiran-2-ylmethyl)-1H-imidazole (13)

To a solution of 2-chloro-4-nitro-1H-imidazole (3.7 g, 25 mmol) in anhydrous ethanol (100 mL) was added glycidol (2.5 mL, 38 mmol) and K_2CO_3 (0.7 g, 5 mmol), then the mixture was heated to reflux for 16 h. The solvent was removed and the residue was dissolved in water (20 mL) and extracted with ethyl acetate/methanol (20/1, 30 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give yellow oil, The resulting crude oil was purified by silica gel column chromatography (dichloromethane/methanol, 20:1) to afford 3-(2-chloro-4-nitro-1H -imidazol-1-yl)propane-1,2-diol (**11**) as a yellow solid (3.2 g, 63%). ESI-MS m/z 222.1 $[M + H]^+$; ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.40 (s, 1H, H_5-imidazole), 5.26 (d, J = 5.4 Hz, 1 H, OH), 4.91 (t, J = 5.4 Hz, 1H, CH_2O), 4.19 (dd, 1H, J = 3.3, 14.1 Hz, NCH_2), 3.97 (dd, 1H, J = 8.4, 14.1 Hz, NCH_2), 3.76–3.82 (m, 1H, CHO), 3.42–3.45 (m, 1H, CH_2O), 3.27–3.31 (m, 1H, CH_2O).

To a solution of resulting compound **11** (5.52 mg, 25 mmol) in pyridine (25 mL) maintained at 10 °C, methanesulfonyl chloride (3.4 mg, 30 mmol) was slowly added. After addition was completed, DMAP (0.28 mg, 2.5 mmol) was added and stirred for 2 h. The reaction mixture was poured into water (100 mL) and pH value was adjusted to 4–5 and extracted with ethyl acetate (30 mL × 3). The combined organic layers was wash with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford compound **12** as yellow oil. The resulting crude oil was directly used in the next step without purification. ESI-MS m/z 300.0 $[M + H]^+$, 322.0 $[M + Na]^+$; ^1H NMR (DMSO-d_6, 500 MHz, ppm) δ: 8.48 (s, 1H, H_5-imidazole), 5.76 (br s, 1H, CHOH), 4.09–4.25 (m, 5H, propyl), 3.23 (s, 3 H, CH_3).

The obtained crude compound **12** was dissolved in ethyl acetate (100 mL), DBU (40 mmol) was added and stirred at room temperature for 2 h. After TLC analysis indicated the completed consumption of starting materials, the reaction mixture was washed with brine and dried over anhydrous Na_2SO_4. After evaporation of solvent, the crude product was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 1:1) to yield 2-chloro-4-nitro-1-(oxiran-2-ylmethyl)-1H-imidazole (**13**) as brown oil, two steps yield 36%. ESI-MS m/z 242.2 $[M + K]^+$; ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.51 (1H, s, H_5-imidazole), 4.48 (dd, 1H, J = 3.5, 14.5 Hz, NCH_2), 4.19 (dd, 1H, J = 5.5, 14.5 Hz, NCH_2), 3.39–3.41 (m, 1H, CHO), 2.86–2.88(m, 1H, CH_2O), 2.62–2.63(m, 1H, CH_2O).

General procedure for the preparation of compounds 6a–i

A mixture of 2-chloro-4-nitro-1-(oxiran-2-ylmethyl)-1H-imidazole **13** (3.7 mmol) and quinolones (2.7 mmol) in ethanol (25 mL) was refluxed for 16 h. The solution was cooled to room temperature and evaporated under reduced pressure. The residues were purified by silica gel column chromatography (dichloromethane/methanol, 100:1) to afford compounds **6a**–**i** as yellow solid.

7-(4-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a)

Gatifloxacin(1 g, 2.7 mmol), yield 50%; m.p. 210–212 °C; ESI-MS m/z 579.3$[M + H]^+$, 601.3 $[M + Na]^+$; IR (ν_{max}/cm): 3410 (COOH), 1727 and 1618 (C=O), 1443 and 1316 (N=O); ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 14.92 (br s, 1H, COOH), 8.70 (s, 1H, H_2-quinolone), 8.53 (s, 1H, H_5-imidazole), 7.75 (d, $J_{H,F}$ = 12 Hz, H_5-quinolone), 5.28–5.18 (m, 1H, HO-propyl), 4.32–3.93 (m, 5H, propyl, and CH-cyclopropyl), 3.74 (s, 3H, CH_3O), 3.40–3.33 (m, 3H, piperazine), 3.04–2.96 (m, 2H, piperazine), 2.75–2.61 (m, 2H, piperazine), 2.46–2.30 (m, 1H, propyl), 1.03–1.12 (m, 5H, cyclopropyl, and CH_3-piperazine), 0.81–0.85 (m, 2H, cyclopropyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.74, 166.07, 157.57, 154.30, 150.91, 146.18, 144.84, 139.50, 134.57, 132.54, 124.89, 121.22, 106.98, 67.49, 66.75, 63.40, 58.08, 57.46, 57.14, 56.97, 55.88, 55.50, 52.64, 52.44, 52.26, 51.65, 50.88, 41.19, 15.71, 14.83, 9.45, 9.29; HR-MS (ESI) m/z: calculated for $C_{25}H_{29}ClFN_6O_7$ $[M + H]^+$: 579.1765, found: 579.1762.

7-((4aS,7aS)-1-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)hexahydro-1H-pyrrolo[3,4-b]pyridin-6(2H)-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6b)

Moxifloxacin hydrochloride (1.18 g, 2.7 mmol), yield 42%; m.p. 203–206 °C; ESI-MS m/z 605.2 $[M + H]^+$; IR (ν_{max}/cm): 3411 (COOH), 1727 and 1620 (C=O), 1436 and 1343 (N=O); ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 15.18 (br s, 1H, COOH), 8.64 (s, 1H, H_2-quinolone), 8.39 (s, 1H, H_5-imidazole), 7.64 (d, 1H, $J_{H,F}$ = 14 Hz, H_5-quinolone), 5.21 (m, 1H, HO-propyl), 4.19–4.11 (m, 2H, propyl), 3.97–3.96 (m, 1H, cyclopropyl), 3.82–3.75 (m, 2H, propyl), 3.71(br s, 1H, pyrrolo[3,4-b]pyridine), 3.56 (s, 3H, CH_3O), 3.49–3.42 (m, 1H, pyrrolo[3,4-b]pyridine), 3.10 (s, 1H, pyrrolo[3,4-b] pyridine), 3.50–3.47 (m, 1H, pyrrolo[3,4-b]pyridine), 2.54–2.53 (m, 1H, pyrrolo[3,4-b]pyridine), 2.92–2.90 (m, 1H, pyrrolo[3,4-b]pyridine), 2.39–2.25 (m, 3H, propyl, and pyrrolo[3,4-b]pyridine), 1.77–1.53 (m, 4H, pyrrolo[3,4-b] pyridine), 1.17–0.93 (m, 4H, cyclopropyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.33, 166.30, 155.50, 151.71, 150.51, 144.76, 141.08, 137.50, 134.96, 132.24, 124.74, 117.30, 106.95, 67.07, 62.05, 61.64, 59.05, 53.80, 53.40, 51.42, 50.34, 41.12, 37.05, 23.42, 22.21,

9.76, 9.00; HR-MS (ESI) m/z: calculated for $C_{27}H_{31}ClFN_6O_7$ $[M + H]^+$: 605.1921, found: 605.1930.

7-(4-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6c)

Norfloxacin (0.86 g, 2.7 mmol), yield 46%; m.p. 210–212 °C; ESI-MS m/z 523.3 $[M + H]^+$; IR (ν_{max}/cm): 3426 (COOH), 1719 and 1628 (C=O), 1474 and 1340 (N=O); ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 15.34 (br s, 1H, COOH), 8.95 (s, 1H, H_2-quinolone), 8.51 (s, 1H, H_5-imidazole), 7.91(d, 1H, $J_{H,F}$ = 13.2 Hz, H_5-quinolone), 7.16 (d, 1H, $J_{H,F}$ = 7.2 Hz, H_8-quinolone), 5.29 (d, 1H, J = 5.1 Hz, OH), 4.59 (q, 2H, J = 7.2 Hz, CH_2-ethyl), 4.23–4.26 (d, 1H, propyl), 4.09–3.97 (m, 2H, propyl), 3.30 (s, 4H, piperazine), 2.60–2.72(m, 4H, piperazine), 2.43 (d, 2H, J = 5.7 Hz, propyl), 1.42 (t, 3H, J = 7.2 Hz, CH_3-ethyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.10, 166.07, 154.47, 151.17, 148.41, 145.30, 144.39, 137.15, 132.19, 124.40, 119.08, 111. 05, 106.78, 66.10, 61.22, 52.97, 51.96, 49.10, 48.88, 14.27; HR-MS (ESI) m/z: calculated for $C_{22}H_{25}ClFN_6O_6$ $[M + H]^+$: 523.1503, found: 523.1509.

7-(4-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (6d)

Enoxacin (0.86 g, 2.7 mmol), yield 15%; m.p. 220–222 °C; ESI-MS m/z 524.3 $[M + H]^+$; IR (ν_{max}/cm): 3447 (COOH), 1724 and 1628 (C=O), 1472 and 1371 (N=O); ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 15.30 (br s, 1H, COOH), 8.97 (s, 1H, H_2-quinolone), 8.52 (s, 1H, H_5-imidazole), 8.06 (d, 1H, $J_{H,F}$ = 13.8 Hz, H_5-quinolone), 5.29 (d, 1H, J = 4.8 Hz, OH), 4.49 (q, 2H, J = 7.2 Hz, CH_2-ethyl), 4.24 (d, 1H, propyl), 4.04–3.97 (m, 2H, propyl), 3.82–3.79 (m, 4H, piperazine), 2.56–2.69 (m, 4H, piperazine), 2.43 (d, 2H, J = 5.7 Hz, propyl), 1.39 (t, 3H, J = 7.2 Hz, CH_3-ethyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.26, 165.78, 149.80, 147.63, 145.10, 144.77, 144.39, 132.25, 124.37, 119.20, 112.56, 108.02, 66.09, 61.07, 53.02, 51.92, 47.13, 46.65, 14.61; HR-MS (ESI) m/z: calculated for $C_{21}H_{24}ClFN_7O_6$ $[M + H]^+$: 524.1455, found: 524.1462.

7-(4-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6e)

Lomefloxacin (1.05 g, 2.7 mmol), yield 42%; m.p. 215–216 °C; ESI-MS m/z 555.1 $[M + H]^+$; IR (ν_{max}/cm): 3411 (COOH), 1722 and 1624 (C=O), 1470 and 1329 (N=O); ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 14.91 (br s, 1H, COOH), 8.93 (s, 1H, H_2-quinolone), 8.52 (s, 1H, H_5-imidazole), 7.76 (d, 1H, $J_{H,F}$ = 12 Hz, H_5-quinolone), 5.23 (d, 1H, J = 20.1 Hz, HO-propyl), 4.59 (q, 2H, J = 6 Hz, CH_2-ethyl), 4.32–4.20 (m, 1H, propyl), 4.07–3.89 (m, 2H, propyl), 3.42–3.36- (m, 3H, piperazine), 3.08–2.91 (m, 2H, piperazine), 2.73–2.55- (m, 2H, piperazine), 2.45–2.31 (m, 2H, propyl), 1.44 (t, 3H, J = 6.6 Hz, CH_3-piperazine), 1.06 (t, 3H, J = 6 Hz, CH_3-ethyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 175.49, 165.51, 156.16, 152.87, 151.10, 147.50, 144.30, 133.62, 132.08, 127.25, 124.36, 120.35, 106.96, 68.48, 67.09, 66.36, 63.50 57.54, 57.06, 56.80, 56.43, 55.25, 54.86, 53.78, 53.57, 52.12, 51.95, 51.45, 50.67, 46.60, 15.91, 14.76, 13.81; HR-MS (ESI) m/z: calculated for $C_{23}H_{26}ClF_2N_6O_6$ $[M + H]^+$: 555.1565, found: 555.1575.

7-(4-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)piperazin-1-yl)-6,8-difluoro-1-(2-fluoroethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6f)

Fleroxacin (1 g, 2.7 mmol), yield 42%; m.p. 191–192 °C; ESI-MS m/z 559.3 $[M + H]^+$; IR (ν_{max}/cm): 3415 (COOH), 1720 and 1626 (C=O), 1474 and 1335 (N=O); ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 14.76 (br s, 1H, COOH), 8.86 (s, 1H, H_2-quinolone), 8.51 (s, 1H, H_5-imidazole), 7.87 (d, 1H, $J_{H,F}$ = 6.9 Hz, H_5-quinolone), 5.25 (d, 1H, J = 2.7 Hz, HO-propyl), 4.98–4.84 (m, 4H, fluoroethyl), 4.25–4.22 (m, 1H, propyl), 4.06–3.99 (m, 2H, propyl), 3.33–3.32(m, 4H, piperazine), 2.63–2.52 (m, 4H, piperazine), 2.43 (d, 2H, J = 3 Hz, propyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 175.66, 165.37, 156.20, 152.88, 152.33, 144.38, 133.75, 132.19, 127.35, 124.42, 120.15, 107.10, 106.78, 83.00, 80.84, 65.98, 61.49, 57.83, 53.79, 51.98, 50.35; HR-MS (ESI) m/z: calculated for $C_{22}H_{23}ClF_3N_6O_6$ $[M + H]^+$: 559.1314, found: 559.1319.

7-(4-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6g)

Ciprofloxacin (0.89 g, 2.7 mmol), yield 31%; m.p. 193–194 °C; ESI-MS m/z 535.1 $[M + H]^+$; IR (ν_{max}/cm): 3415 (COOH), 1721 and 1629 (C=O), 1472 and 1339 (N=O); ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 14.94 (br s, 1H, COOH), 8.66 (s, 1H, H_2-quinolone), 8.53 (s, 1H, H_5-imidazole), 7.90 (d, 1H, $J_{H,F}$ = 13.2 Hz, H_5-quinolone), 7.55 (d, 1H, $J_{H,F}$ = 7.8 Hz, H_8-quinolone), 5.30 (d, 1H, J = 4.5 Hz, HO-propyl), 4.27–4.23 (m, 1H, propyl), 4.05–3.98 (m, 2H, propyl), 3.84–3.82 (m, 1H, cyclopropyl), 3.32–3.30 (m, 4H, piperazine), 2.73–2.61 (m, 4H, piperazine), 2.48–2.46 (m, 2H, propyl), 1.33–1.19 (m, 4H, cyclopropyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.74, 166.36, 155.07, 151.77, 148.40, 145.60, 144.85, 139.58, 132.66, 124.50, 119.95, 111.19, 107.16, 66.53, 61.72, 53.40, 52.44, 49.83, 36.24, 7.97; HR-MS (ESI) m/z: calculated for $C_{23}H_{25}ClFN_6O_6$ $[M + H]^+$: 535.1503, found: 535.1508.

7-(4-(3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (6h)

1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (0.89 g, 2.7 mmol), yield 46%; m.p. 211–212 °C; ESI-MS m/z 536.1 $[M + H]^+$; IR (ν_{max}/cm): 3404 (COOH), 1724 and 1628 (C=O), 1472 and 1342 (N=O); ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 14.98 (br s, 1H, COOH), 8.53 (s, 1H, H_2-quinolone), 8.52 (s, 1H, H_5-imidazole), 8.00 (d, 1H, $J_{H,F}$ = 13.8 Hz, H_5-quinolone), 5.30 (m, 1H, HO-propyl), 4.27–4.23 (m, 1H, propyl), 3.98–4.05 (m, 2H, propyl), 3.79 (s, 4H, piperazine), 3.61–3.53 (m, 1H, cyclopropyl), 2.67–2.54 (m, 4H, piperazine), 2.43–2.41 (m, 2H, propyl), 1.17–1.01 (m, 4H, cyclopropyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.35, 165.66, 149.66, 148.58, 147.05, 146.60, 145.14, 144.40, 132.24, 124.36, 119.20, 112.18, 107.56, 66.09, 61.15, 53.16, 53.93, 46.65, 34.85, 6.79; HR-MS (ESI) m/z: calculated for $C_{22}H_{24}ClFN_7O_6$ $[M + H]^+$: 536.1455, found: 536.1453.

7-(3-((3-(2-chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxypropyl)(methyl)amino)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6i)

Balofloxacin (1.05 g, 2.7 mmol), yield 44%; m.p. 202–203 °C; ESI-MS m/z 593.3 $[M + H]^+$; IR (ν_{max}/cm): 3411 (COOH), 1727 and 1618 (C=O), 1434 and 1315 (N=O); ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 14.98 (br s, 1H, COOH), 8.70 (s, 1H, H_2-quinolone), 8.48 (d, 1H, J = 2.1 Hz, H_5-imidazole), 7.74 (d, 1H, $J_{H,F}$ = 12 Hz, H_5-quinolone), 5.17 (d, 1H, J = 3.6 Hz, HO-propyl), 4.25–4.15 (m, 2H, propyl, and cyclopropyl), 3.94–3.88 (m, 2H, propyl), 3.74(s, 3H, CH_3O), 3.57–3.37 (m, 2H, piperidine), 3.12–2.99 (m, 2H, piperidine), 2.72–2.61 (m, 1H, piperidine), 2.56–2.52 (m, 2H, piperidine), 2.34 (d, 3H, J = 3 Hz, CH_3N-piperidine), 1.98–1.77(m, 2H, propyl), 1.71–1.41 (m, 2H, piperazine), 1.17–0.97(m, 4H, cyclopropyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.30, 165.64, 157.30, 153.39, 150.40, 145.68, 144.34, 139.60, 134.11, 132.00, 124.40, 120.50, 106.48, 67.15, 62.70 60.48, 57.53, 53.10, 51.93, 50.79, 40.77, 26.47, 25.98, 25.39, 9.02, 8.77; HR-MS (ESI) m/z: calculated for $C_{26}H_{31}ClFN_6O_7$ $[M + H]^+$: 593.1921, found: 593.1926.

General procedure for the preparation of compounds 6j–l

A mixture of quinolone (2.67 mmol), ornidazole (2.67 mmol) and KOH (6.9 mmol) in ethanol (20 mL) and H_2O (20 mL) was refluxed for 12 h. After TLC analysis indicated the completed consumption of starting materials, the solvent was evaporated in vacuo, and the resulting residues were dissolved in water (20 mL) and the pH value was adjusted to 4–5 with aqueous 50% HCl, a yellow precipitate was formed and collected by filtration to give the crude **6j**–**l**, then purification was achieved by silica gel column chromatography (dichloromethane/methanol, 50:1) to afford compounds **6j**–**l** as yellow solid.

1-cyclopropyl-6-fluoro-7-(4-(2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6j)

Gatifloxacin (1 g, 2.67 mmol), yield 93%; m.p. 225–229 °C; ESI-MS m/z 559.3$[M + H]^+$; IR(ν_{max}/cm): 3497 (COOH), 1700 and 1620 (C=O), 1433 and 1377 (N=O); ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 14.55 (br s, 1H, COOH), 8.69 (s, 1H, H_2-quinolone), 8.02 (s, 1H, H_4-imidazole), 7.72 (d, 1H, $J_{H,F}$ = 14.5 Hz, H_5-quinolone), 5.07 (d, 0.5H, J = 5.0 Hz, HO-propyl), 5.03 (d, 0.5H, J = 5.0 Hz, HO-propyl), 4.73 (d, 0.5H, J = 14 Hz, propyl), 4.59 (d, 0.5H, J = 14 Hz, propyl), 4.17–3.92 (m, 3H, propyl, and cyclopropyl), 3.76 (s, 3H, CH_3O), 3.43–3.34 (m, 3H, piperazine), 3.05–2.96 (m, 2H, piperazine), 2.81–2.61 (m, 2H, piperazine), 2.49 (s, 3H, CH_3-imidazole), 2.44–2.34 (m, 2H, propyl), 1.09–1.06 (m, 3H, CH_3-piperazine), 1.12–0.99 (m, 4H, cyclopropyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.78, 166.12, 157.64, 154.32, 152.74, 150.96, 146.28, 139.50, 138.94, 134.63, 133.45, 121.20, 107.02, 68.33, 67.65, 63.44, 58.30, 57.63, 57.43, 55.73, 55.57, 52.54, 51.91, 51.25 51.08, 50.03, 41.17, 16.00, 15.04, 14.97, 9.50, 9.30; HR-MS (ESI) m/z: calculated for $C_{26}H_{31}N_6O_7FNa$ $[M + Na]^+$: 581.2136, found: 581.2143.

1-cyclopropyl-6-fluoro-7-((4aS,7aS)-1-(2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl) hexahydro-1H-pyrrolo[3,4-b]pyridin-6(2H)-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6k)

Moxifloxacin hydrochloride (1.16 g, 2.67 mmol), yield 87%, m.p. 128–131 °C; ESI-MS m/z 585.0 $[M + H]^+$; IR(ν_{max}/cm): 3428 (COOH), 1717 and 1616 (C=O), 1437 and 1362 (N=O); ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 14.97 (br s, 1H, COOH), 8.76 (s, 1H, H_2-quinolone), 7.93 (s, 1H, H_4-imidazole), 7.75 (d, 1H, $J_{H,F}$ = 14 Hz, H_5-quinolone), 4.55 (dd, 1H, J = 12.0, 21.5 Hz, HO-propyl), 4.05–3.99 (m, 4H, propyl, pyrrolo[3,4-b]pyridine, and cyclopropyl), 3.82–3.80 (m, 2H, propyl), 3.56 (d, 3H, J = 11.5 Hz, CH_3O), 3.50–3.42 (m, 2H, pyrrolo[3,4-b]pyridine), 3.16–2.70 (m, 3H, pyrrolo[3,4-b]pyridine), 2.50 (d, 3H, J = 32.5 Hz, CH_3-imidazole), 2.60–2.39 (m, 2H, propyl), 1.82–1.57 (m, 5H, pyrrolo[3,4-b]pyridine), 1.25–0.93 (m, 4H, cyclopropyl); ^{13}C NMR ($CDCl_3$, 500 MHz, ppm): δ 176.89, 167.00, 154.60, 152.51, 152.00, 149.60, 140.80, 138.36, 137.37, 134.45, 133.22, 118.42, 107.77, 67.79, 66.99, 62.79, 61.81, 61.06, 58.96, 58.07, 55.30, 53.89, 53.60, 51.19, 50.37, 50.02, 49.36, 48.17, 40.40, 37. 41, 36.94, 24.19, 23.37, 23.22, 22.00, 14.69, 9.88, 9.02; HR-MS (ESI) m/z: calculated for $C_{28}H_{33}FN_6O_7Na$ $[M + Na]^+$: 607.2292, found: 607.2300.

1-cyclopropyl-6-fluoro-7-(3-((2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)(methyl)amino)piperidin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6l)

Balofloxacin (1.0 g, 2.67 mmol), yield 91%, m.p. 112–116 °C; ESI-MS m/z 573.2 $[M + H]^+$; IR (ν_{max}/cm): 3411 (COOH), 1727 and 1618 (C=O), 1434 and 1345 (N=O); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 14.77 (br s, 1H, COOH), 8.79 (s, 1H, H_2-quinolone), 7.94 (s, 1H, H_4-imidazole), 7.80 (d, 1H, $J_{H,F}$ = 12 Hz, H_5-quinolone), 4.58 (d, 1H, J = 13.2 Hz, HO-propyl), 4.09–3.99 (m, 3H, cyclopropyl, propyl, and piperidine), 3.76 (d, 3H, J = 1.8 Hz, CH_3O), 3.67–3.43 (m, 3H, propyl, and piperidine), 3.12–3.01 (m, 2H, piperidine), 2.80–2.75 (m, 2H, piperidine), 2.56–2.48 (m, 1H, piperidine), 2.56 (s, 3H, CH_3-imidazole), 2.40 (d, 3H, J = 4.8 Hz, CH_3N-piperidine), 2.09–1.49 (m, 4H, piperidine, and propyl), 1.24–0.84 (m, 4H, cyclopropyl); ^{13}C NMR ($CDCl_3$, 300 MHz, ppm): δ 176.02, 165.74, 157.00, 153.66, 151.15, 148.83, 144.47 138.80, 137.43, 132.95, 132.30, 120.80, 106.90, 66.35, 61.49, 60.12, 58.85, 56.46, 56.01, 52.67, 52.33, 50.38, 49.30, 39.60, 37.28, 36.84, 26.58, 25.95, 24.80, 13.80, 8.66, 8.38; HR-MS (ESI) m/z: calculated for $C_{27}H_{34}FN_6O_7$ $[M + H]^+$: 573.2468, found: 573.2488.

General procedure for the preparation of compounds 6 m-o

A mixture of quinolone (2.3 mmol), (*S*)-ornidazole (2.53 mmol) and KOH (6.9 mmol) in ethanol (20 mL) and H_2O (20 mL) was refluxed for 12 h. After TLC analysis indicated the completed consumption of starting materials, the solvent was evaporated in vacuo, and the resulting residues were purified as in method for preparation of compounds **6j**–**l**.

1-cyclopropyl-6-fluoro-7-(4-((R)-2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6m)

Gatifloxacin (1 g, 2.67 mmol), yield 87%; m.p. 224–226 °C; ESI-MS m/z 559.3 $[M + H]^+$; IR(ν_{max}/cm): 3408 (COOH), 1733 and 1598 (C=O), 1433 and 1314 (N=O); ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 14.55 (br s, 1H, COOH), 8.69 (s, 1H, H_2-quinolone), 8.02(s, 1H, H_4-imidazole), 7.72 (d, $J_{H,F}$ = 14.5 Hz, H_5-quinolone), 5.07 (d, 1H, J = 5 Hz, HO-propyl), 4.67 (d, 1H, J = 14 Hz, propyl), 4.17–4.01 (m, 3H, propyl, and cyclopropyl), 3.77 (s, 3H, CH_3O), 3.41–3.34 (m, 3H, piperazine), 3.05–2.96 (m, 2H, piperazine), 2.82–2.63 (m, 2H, piperazine), 2.49 (s, 3H, CH_3-imidazole), 2.49–2.35 (m, 2H, propyl) 1.09–1.06 (m, 3H, CH_3-piperazine), 1.14–1.03 (m, 4H, cyclopropyl); ^{13}C NMR (DMSO-d_6, 300 MHz, ppm): δ 176.78, 166.12, 157.64, 154.32, 152.74, 150.96, 146.28, 139.50, 138.94, 134.63, 133.45, 121.20, 107.02, 67.65, 63.38, 58.30, 57.36, 57.63, 55.57, 52.52, 51.25 50.03, 41.25, 16.00, 14.97, 14.92, 9.50, 9.30; HR-MS (ESI) m/z: calculated for $C_{26}H_{31}N_6O_7FNa$ $[M + Na]^+$: 581.2136, found: 581.2142.

1-cyclopropyl-6-fluoro-7-((4aS,7aS)-1-((R)-2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)hexahydro-1H-pyrrolo[3,4-b]pyridin-6(2H)-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6n)

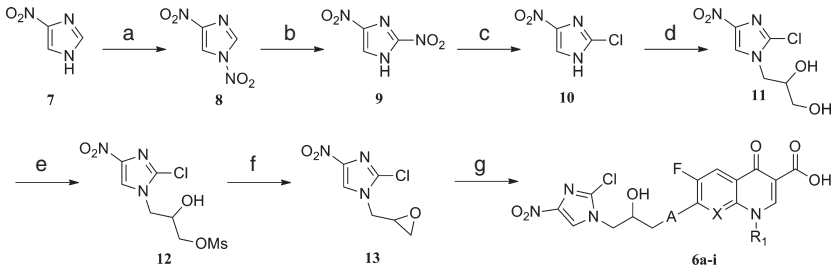
Moxifloxacin hydrochloride (1.16 g, 2.67 mmol), yield 88%; m.p. 128–130 °C; ESI-MS m/z 585.0 $[M + H]^+$; IR(ν_{max}/cm): 3361 (COOH), 1708 and 1619 (C=O), 1443 and 1259 (N=O); ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 14.95 (br s, 1H, COOH), 8.76 (s, 1H, H_2-quinolone), 7.93 (s, 1H, H_4-imidazole), 7.79 (d, 1H $J_{H,F}$ = 14 Hz, H_5-quinolone), 4.57 (d, 1H, J = 12.0 Hz, HO-propyl), 3.99–4.07 (m, 4H, propyl, pyrrolo[3,4-b]pyridine, and cyclopropyl), 3.84 (s, 1H, propyl), 3.58 (s, 3H, CH_3O), 3.49–3.44 (m, 3H, pyrrolo[3,4-b]pyridine), 2.87–2.76 (m, 3H, pyrrolo[3,4-b]pyridine), 2.54 (s, 3H, CH_3-imidazole), 2.58–2.46 (m, 2H, pyrrolo[3,4-b]pyridine), 1.84–1.57 (m, 5H, pyrrolo[3,4-b]pyridine, and propyl), 1.27–0.90 (m, 4H, cyclopropyl); ^{13}C NMR ($CDCl_3$, 500 MHz, ppm): δ 176.66, 166.00, 154.69, 152.69, 152.00, 149.62, 140.92, 138.40, 137.30, 134.44, 133.28, 118.63, 107.87, 67.78, 62.85, 61.11, 58.97, 55.35, 50.34, 49.25, 48.04, 40.40, 36.94, 24.24, 23.41, 14.73, 9.85, 9.07; HR-MS (ESI) m/z: calculated for $C_{28}H_{33}FN_6O_7Na$ $[M + Na]^+$: 607.2292, found: 607.2301.

1-cyclopropyl-6-fluoro-7-(3-(((R)-2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl)(methyl)amino)piperidin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6o)

Balofloxacin (1.0 g, 2.67 mmol), yield 68%, m.p. 112–115 °C; ESI-MS m/z 573.2 $[M + H]^+$; IR (ν_{max}/cm): 3410 (COOH), 1727 and 1617 (C=O), 1435 and 1342 (N=O); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 14.73 (br s, 1H, COOH), 8.74(s, 1H, H_2-quinolone), 7.92 (s, 1H, H_4-imidazole), 7.81 (d, 1H, J = 12 Hz, H_5-quinolone), 4.60 (s, 1H, HO-propyl), 4.12–3.98 (m, 3H, cyclopropyl, propyl, and piperidine), 3.76 (s, 3H, CH_3O), 3.68–3.42 (m, 3H, propyl, and piperidine), 3.14–3.02 (m, 2H, piperidine), 2.81–2.74 (m, 2H, piperidine), 2.56 (s, 3H, CH_3-imidazole), 2.41 (d, 3H, J = 4.8 Hz, CH_3N-piperidine), 2.10–1.49 (m, 4H, piperidine, and propyl), 1.25–1.17(m, 3H, piperidine, and cyclopropyl), 1.01–0.94 (m, 2H, cyclopropyl); ^{13}C NMR ($CDCl_3$, 300 MHz, ppm): δ 175.98, 165.64, 156.99, 153.69, 151.15, 148.83, 144.52, 138.90, 137.45, 132.95, 132.28, 120.75, 107.12, 66.33, 61.45, 58.90, 56.42, 52.36, 50.33, 49.25, 39.59, 37.25, 36.80, 26.60, 25.97, 24.82, 13.80, 8.65, 8.36; HR-MS (ESI) m/z: calculated for $C_{27}H_{34}FN_6O_7$ $[M + H]^+$: 573.2468, found: 573.2486.

Minimum inhibitory concentration measurement

The minimum inhibitory concentration (MIC) was performed by agar dilution method according to Clinical and Labora-



Scheme 1: Synthesis of the target compounds **6a**–**i**. Reagents and conditions: (a) Fuming nitric acid, Ac_2O, 0 °C, 4 h; (b) toluene, reflux, 16 h; (c) con.HCl, reflux, 2 h; (d) Gycidol, K_2CO_3, EtOH, reflux, 16 h; (e) pyridine, MsCl, DMAP, 10 °C, 2 h; (f) EtOAc, DBU, r.t., 2 h; (g) quinolones, EtOH, reflux, 16 h.

tory Standards Institute guidelines (24). A series of twofold dilutions of the test compounds **6a**–**o** and gatifloxacin were prepared in DMSO (1 mL). Each dilute was added to molten Mueller-Hinton Agar (MHA) media at 56 °C to obtain the desired final concentrations ranging from 1280 to 0.031 μg/mL. The turbidity of bacterial suspensions were adjusted to approximately 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and further diluted to give 10^8 CFU/mL. Plates were inoculated with 1 μL of the appropriate bacterial suspension and incubated at 35 °C for 16 h. The MICs were the lowest concentration of the test compound at which no growth was observed on the plate. Media without any drugs was made as control.

Docking study

The automated docking studies were carried out using AutoDock version 4.2 (25). The 3.5 Å crystal structure of topoisomerase II DNA-gyrase (PDB ID: 2XCT) (26) was derived from PDB. The Discovery Studio 2.5 (Accelrys, Inc., San Diego, CA, USA) package was used to prepare the docking files. The protein targets were prepared by removing water molecules and bound ligands. Polar hydrogens were added. The manganese ion at the active site was retained and the charge was set to +2 *e* (26). For the ligands, the protonation state was adjusted to physiological pH, specifically, carboxylic acid moieties were deprotonated and amino groups were protonated. Conjugate gradient minimizations with CHARMm forcefield were performed.

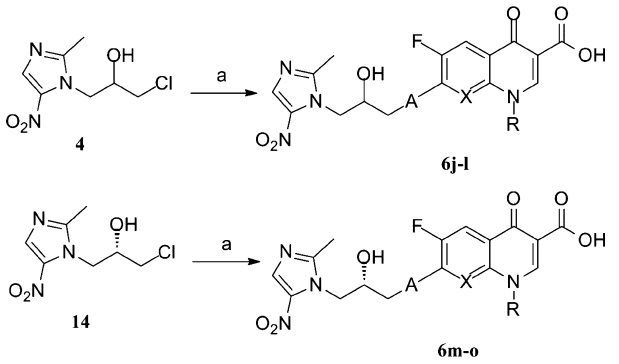
The grid size was set to 40 × 50 × 60 points in the x, y and z dimensions, with a grid spacing of 0.375 Å centered on the original ligand in crystal structure complex. Then automated docking studies were carried out to evaluate the binding free energy of the three selected compounds within the macromolecules. The Lamarckian genetic algorithm was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) (La Jolla, CA, USA). Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 7.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Simulations were ranked according to the docked energy between the protein and the ligand and clustered together by <2 Å in root-mean-square deviation (RMSD). The results of the most favorable free energy of binding were selected as the resultant complex structures.

Results and Discussion

Chemistry

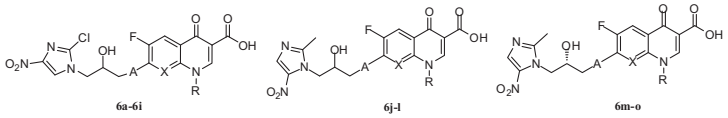
The synthetic routes adopted to obtain the target compounds **6a**–**o** were diagramed in Schemes 1 and 2. The structures of target compounds were confirmed by IR, NMR and MS spectral data. As depicted in Scheme 1, 2-chloro-4-nitroimdazole **10** was synthesized using the reported procedure (27). Nitration of commercially available 4-nitiroimidazole **7** with fuming nitric acid in acetic anhydride to give 1,4-dinitroimidazole **8**, which was subsequently undergone thermal rearrangement in toluene to obtain 2,4-dinitroimidazole **9**. Compound **9** was then halogenated with concentrated hydrochloric acid to afford 2-chloro-4-nitroimdazole **10**. According to the reported procedure (28,29), ring-opening reaction of compound **10** with glycidol gave compound **11**, then sulfonating of compound **11** with Msyl chloride/pyridine and cyclization with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at room temperature gave key intermediate 2-chloro-4-nitro-1-(oxiran-2-ylmethyl)-1H-imidazole **13**. Condensation of compounds **13** with commercially available quinolones provided target compounds **6a**–**i** (Table 1).

The synthesis of compounds **6j**–**o** (Table 1) was depicted in Scheme 2. Compounds **6j**–**l** were synthesized by the condensation of quinolones with ornidazole (**4**) in



Scheme 2: Synthesis of the target compounds **6j**–**o**. Reagents and conditions: (a), quinolones, KOH, H_2O/EtOH, reflux, 12 h.

Table 1: Structures and calculated physicochemical parameters of the target compounds **6a**–**o**



Compound	A[a]	X	R	*clogP*[b]	solubility[b]	TPSA Å[2b]
6a		C-OCH_3	Cyclopropyl	−0.70	−4.13	157.1
6b		C-OCH_3	Cyclopropyl	−0.39	−4.65	157.1
6c		CH	Ethyl	−1.08	−3.27	147.9
6d		N	Ethyl	−1.55	−3.52	160.8
6e		CF	Ethyl	−0.66	−3.96	147.9
6f		CF	FCH_2CH_2-	−1.21	−3.66	147.9
6g		CH	Cyclopropyl	−0.96	−3.73	147.9
6h		N	Cyclopropyl	−1.43	−3.99	160.8
6i		C-OCH_3	Cyclopropyl	−0.79	−4.37	157.1
6j, **6m**		C-OCH_3	Cyclopropyl	−1.15	−3.25	157.1
6k, **6n**		C-OCH_3	Cyclopropyl	−0.83	−3.77	157.1
6l, **6o**		C-OCH_3	Cyclopropyl	−1.24	−3.49	157.1
Gatifloxacin	–	–	–	−1.27	−3.72	82.11

[a]q is quinolone nucleus; n is 2-hydroxy-3-(nitroimidazolyl)-propyl.
[b]*clogP*, solubility and TPSA (topological polar surface area) were calculated by OSIRIS Property Explorer Version 2.

H_2O/ethanol (1/1) in the presence of KOH at reflux temperature. (*R*)-2-hydroxy-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propyl-derived quinolones **6m**–**o** were synthesized in the same condition by using enantiomerically pure (*S*)-ornidazole (**14**) as starting material to determine the influence of the geometries for activity.

The presence of a 2-hydroxy-3-(nitroimidazolyl)-propyl group in C-7 position of quinolones could influence the physicochemical properties of quinolone compounds. Their physicochemical parameters were calculated and presented in Table 1. From the parameters, it was observed that all the target compounds exhibited high TPSA values

Table 2: *In vitro* antibacterial activity of the target compounds **6a**–**o**

	MIC (μg/mL)							
	Gram-positive						Gram-negative	
Compound	S.e.[a]	MSSE[b]	MRSE[c]	S. a.[d]	MSSA[e]	MRSA[f]	E. c.[g]	K. p.[h]
6a	0.03	0.25	0.03	1	0.5	8	4	>32
6b	0.125	0.25	0.125	0.5	0.5	16	2	>32
6c	32	>32	>32	>32	>32	>32	>32	>32
6d	4	2	4	>32	16	32	>32	>32
6e	0.25	2	0.125	16	4	32	4	32
6f	16	16	2	>32	16	32	>32	>32
6g	2	2	2	8	4	32	16	>32
6h	2	2	0.125	8	4	16	16	>32
6i	0.5	0.5	0.25	0.5	0.5	8	4	>32
6j	1	1	1	32	1	32	8	>32
6k	0.125	0.25	0.125	0.25	0.5	8	4	8
6l	0.25	0.5	0.125	0.5	0.5	8	4	2
6m	16	32	32	32	32	16	32	32
6n	0.03	0.125	0.03	0.125	0.125	2	2	1
6o	0.25	1	0.25	1	1	32	8	16
Gatifloxacin	0.06	0.06	0.06	0.125	0.125	16	0.06	1

[a]*Staphylococcus epidermidis* ATCC 12228.
[b]Methicillin-sensitive *Staphylococcus epidermidis* clinical isolates.
[c]Methicillin-resistant *Staphylococcus epidermidis* clinical isolates.
[d]S. a., *Staphylococcus aureus* ATCC 29213.
[e]Methicillin-sensitive *Staphylococcus aureus* clinical isolates.
[f]Methicillin-resistant *Staphylococcus aureus* clinical isolates.
[g]E. c., *Escherichia coli* ATCC 700603.
[h]K. p., *Klebsiella pneumoniae* ATCC 25922.

when compared to gatifloxacin and similar lipophilicity (clog*P* around −1) and solubility (solubility around −3.5) to reference drug gatifloxacin.

Antibacterial evaluation

The target compounds **6a**–**o** were tested for their *in vitro* antibacterial activity against eight bacterial strains including *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 700603, *Klebsiella pneumonia* ATCC 25922, methicillin-sensitive *Staphylococcus epidermidis* (MSSE), methicillin-resistant *Staphylococcus epidermidis* (MRSE), methicillin-sensitive *Staphylococcus aureus* (MSSA) and MRSA. The minimum inhibitory concentration values (MICs) were determined by a standard agar dilution method (24) using gatifloxacin as reference drug. The results of MIC values were listed in Table 2.

The MIC values of compounds **6a**–**o** against *S. epidermidis* strains indicated that most compounds displayed potent activity against both standard strains and clinical isolates with respect to gatifloxacin. Gatifloxacin analog **6a** and moxifloxacin analog **6n** exhibited the most potent inhibitory activity against *S. epidermidis* and MRSE with the same MIC value of 0.03 mg/mL, which were twofold more potent than reference drug gatifloxacin. The MIC values of compounds **6a**–**o** against *S. aureus* indicated some compounds possessed a comparable or better activity in comparison with reference drug. Moxifloxacin analog **6n** showed the most potent activity (MIC = 2 mg/mL) against MRSA, and its activity was eightfold more than that of gatifloxacin. In addition, compounds **6a**, **i**, **k** and **l** exhibited potent activity (MIC = 8 mg/mL) against MRSA, which were twofold more potent than gatifloxacin. For Gram-negative pathogens, compounds **6a**–**b**, **6e**, **6i**–**6l** and **6n**–**o** had respectable *in vitro* activity against *E. coli* (MIC = 2–8 μg/mL), but were less active than reference drug (MIC = 0.06 μg/mL). All synthesized compounds showed poor or no activity (MIC > 32 μg/mL) against *K. pneumoniae* except compounds **6l** and **6n**, with MIC of 2 and 1 μg/mL, were equipotent to the standard gatifloxacin (MIC = 1 μg/mL).

Among synthesized compounds, moxifloxacin analog **6n** displayed the most potent activity against Gram-positive strains including *S. epidermidis* (MIC = 0.06 μg/mL), MSSE (MIC = 0.125 μg/mL), MRSE (MIC = 0.03 μg/mL), *S. aureus* (MIC = 0.125 μg/mL), MSSA (MIC = 0.125 μg/mL), MRSA (MIC = 2 μg/mL), compared with other synthesized compounds and reference drug gatifloxacin. Its inhibitory activity against *K. pneumoniae* (MIC = 1 mg/mL)

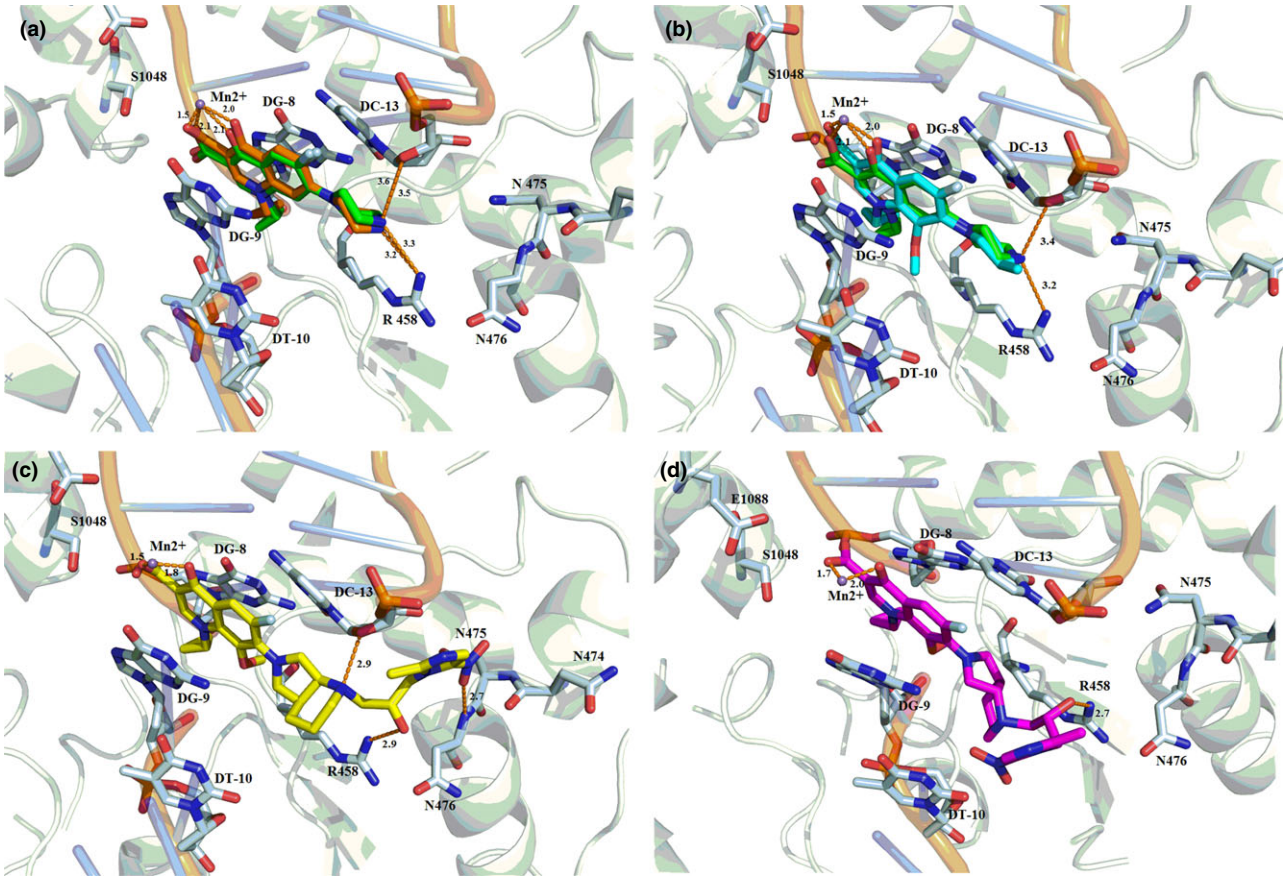


Figure 2: The binding model of docked compounds with topoisomerase II DNA-gyrase. The enzyme is shown as cartoon, while residues (white) in the active site and docked compounds are shown as sticks. H-bonding interactions are shown in orange dotted lines. (A) ciprofloxacin (orange) and original X-ray structure of ciprofloxacin (green), (B) gatifloxacin (cyan) and original X-ray structure ciprofloxacin (green), (C) compound **6n** (yellow), (D) compound *S*-**6n** (magenta). This figure was made using PyMol.

was equal to reference drug. The results were in accordance with the reported finding that one of the promising hits originated from moxifloxacin chemical class (22,23).

Structurally, the C-8 methoxy group substituted compounds except **6m** were statistically better than the remaining tested compounds in antibacterial activity, demonstrating that introduction of the methoxy group at the C-8 position of quinolone seems to contribute to the antibacterial activity. The naphthyridine derivatives **6d** and **6h** were more potent than its corresponding quinolone congeners **6c** and **6g**, Thus, replacement of CH at 8-position with N increased the antibacterial activity. The comparison of the MIC values of 2-chloro-4-nitroimidazole derivatives **6a**–**b**, **6i** and corresponding 2-methyl-5-nitroimidazole derivatives **6l**–**o** revealed that the substitution position of nitro group at imidazole has no prominent effect on activity. (*R*)-isomers **6m**–**o** displayed dramatically dissimilar antibacterial activity with their racemate **6j**–**l**, which suggested that the stereochemistry of 2-hydroxy at the propyl group may play an important role for the binding of the target compounds with DNA-gyrase.

Docking analysis

With the aim to investigate binding model and understand the potent activity observed, compound **6n** with the most potent antibacterial activity was selected for docking study by using AutoDock 4.2 software (25). Gatifloxacin and (*S*)-isomer of compound 6n (*S*-6n) were also docked into the active site for comparison. To evaluate the docking accuracy of AutoDock 4.2, the cocrystallized ligand ciprofloxacin was redocked within the active site of topoisomerase II DNA-gyrase (26). As shown in Figure 2, Autodock was successful in reproducing the binding position for ciprofloxacin, showing a RMSD of 0.62 Å for all atoms in comparison with original poses of X-ray structure complexes (30). The binding models of gatifloxacin, compound **6n** and *S*-**6n** with DNA-gyrase were depicted in Figure 2.

As shown in Figure 2, gatifloxacin in binding model nearly took the same pose with that observed in the X-ray structure of DNA-gyrase ciprofloxacin complexes (Figure 2B). The docked gatifloxacin formed two coordination bonds with Mn^{2+} ion by the carbonyl and carboxylate groups of quinolone scaffold with **1.5 Å** and **2.0 Å** distance, respec-

tively, and two π-π stacks between quinolone ring and pyrimidine rings in DG-8 and DG-9. The nitrogen atom of piperazine ring formed two hydrogen bonds with Arg 456 (**2.3 Å**) and DC-13 (**2.4 Å**). The docking binding model of compound **6n** indicated that compound **6n** was well filled in the active site (Figure 2C). The binding model of quinolone nucleus of compound **6n** was similar with that of ciprofloxacin and gatifloxacin. Two strong π–π stacks were formed between the quinolone moiety of compound **6n** and the purine rings of DG-8 and DG-9. The carbonyl and carboxylate groups of quinolone scaffold formed two coordinate bonds with Mn^{2+} ion (1.5 and 1.8 Å, respectively). The protonated N of pyrrolo[3,4-b]pyridine was engaged in a hydrogen bond with oxygen atom of DC-13. The 2-hydroxy-3-(nitroimidazolyl)-propyl group was oriented toward the hydrophilic site composed of Arg 458, Asn 474, Asn 475 and Asn 476, and formed two additional hydrogen bonds that 2-hydroxy made a hydrogen bond with guanidinium ion hydrogen atom of Arg 458 (2.9 Å) and nitro oxygen connected with NH group of Asn 476 (2.7 Å). This result revealed that the introduced 2-hydroxy-3-(nitroimidazolyl)-propyl group could serve as new hydrogen bond receptors and donors. Enhanced antibacterial activity of compound **6n** may be attributed to additional affinity to the enzyme by two new hydrogen bonding interactions in 2-hydroxy-3-(nitroimidazolyl)-propyl group. On the other hand, the binding model of quinolone moiety in compound *S*-**6n** (Figure 2D) was similar with that of compound **6n**. However, the 2-hydroxy-3-(nitroimidazolyl)-propyl group of *S*-**6n** was oriented toward DT-10, 2-hydroxy made a hydrogen bond with guanidinium ion hydrogen atom of Arg 458 (2.7 Å) and no hydrogen bond was formed with nitro group. This may give an explanation for the decreased antibacterial activity of racemate **6k** (**6n** versus **6k**).

Conclusion

In this paper, we have synthesized a new series of 2-hydroxy-3-(nitroimidazolyl)-propyl-derived quinolones and evaluated for their *in vitro* antibacterial activity against eight bacterial strains. Most of the target compounds exhibited potent activity against *S. epidermidis* and *S. aureus*. Among them, moxifloxacin analog **6n** displayed the most potent activity against Gram-positive strains including *S. epidermidis* (MIC = 0.06 μg/mL), MSSE (MIC = 0.125 μg/mL), MRSE (MIC = 0.03 μg/mL), *S. aureus* (MIC = 0.125 μg/mL), MSSA (MIC = 0.125 μg/mL), (MIC = 2 μg/mL). Its activity against MRSA was eightfold more potent than reference drug gatifloxacin. Docking study of the target compound **6n** revealed that the binding model of quinolone nucleus was similar with that of gatifloxacin and the 2-hydroxy-3-(nitroimidazolyl)-propyl group formed two additional hydrogen bonds. Given the potent *in vitro* antibacterial activity of **6n**, it might represent a promising lead for developing new antibacterial therapeutics.

Acknowledgments

Authors are really grateful to Prof. Wenbin Shen, China pharmaceutical University for providing ^{1}H NMR & ^{13}C NMR spectra. This study was supported by the China National Key Hi-Tech Innovation Project for the R&D of Novel Drugs (No. 2013ZX09301303-002).

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