Supplementary Information

Design, Synthesis, Antibacterial Evaluation of Novel Azolylthioether Quinolones as MRSA DNA Intercalators

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1. Experimental Protocols

1.1 General Methods

Melting points were recorded on X-6 melting point apparatus and uncorrected. TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 cm^{-1} range. NMR spectra were recorded on a Bruker AV 300 and 600 spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t), as well as multiplet (m). The mass spectra were recorded on LCMS-2010A and the high-resolution mass spectra (HRMS) were recorded on an IonSpec FT-ICR mass spectrometer with ESI resource. All fluorescence spectra were recorded on F-7000 Spectrofluorimeter (Hitachi, Tokyo, Japan) equipped with 1.0 cm quartz cells, the widths of both the excitation and emission slit were set as 2.5 nm, and the excitation wavelength was 280 nm. Fluorescence spectra were recorded at 290 K in the range of 300–550 nm. The UV spectrum was recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells. HSA was obtained from Sigma–Aldrich (St. Louis, MO, USA). The DNA was isolated from MRSA bacteria. Tris and HCl were analytical purity. Sample masses were weighed on a microbalance with a resolution of 0.1 mg. All other chemicals and solvents were commercially available, and were used without further purification.

1.2 Biological Assay Procedures

Minimal inhibitory concentration (MIC, μg/mL) is defined as the lowest concentration of the new compounds that completely inhibit the growth of bacteria, by means of standard two folds serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS). The tested

microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Chloromycin, Norfloxacin, Ciprofloxacin, Clinafloxacin and Fluconazole were used as control drugs. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. All the bacteria and fungi growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimal inhibitory concentration (MIC).

1.2.1. Antibacterial Assays

The prepared compounds **3a–c**, **4a–f** and **5a–f** and their precursors were evaluated for their antibacterial activities against *Staphylococcus aureus* ATCC25923, *Methicillin-resistant Staphylococcus aureus* N315, *Bacillus subtilis* ATCC6633 and *Micrococcus luteus* ATCC4698 as Gram-positive, *Escherichia coli* JM109, *Pseudomonas aeruginosa* ATCC27853, *Bacillus proteus* ATCC13315 and *Eberthella typhosa* ATCC14028 as Gram-negative bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU/mL. The compounds were dissolved in DMSO to prepare the stock solutions. The compounds and reference drugs were prepared in Mueller–Hinton broth (Guangdong huaikai microbial sci.& tech co., Ltd, Guangzhou, Guangdong, China) by twofold serial dilution to obtain the required concentrations. These dilutions were inoculated and incubated at 37 ºC for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

1.2.2 Antifungal Assays

The synthesized compounds were also evaluated for their antifungal activities against *Candida albicans* ATCC76615, *Candida mycoderma* ATCC9888, *Candida utilis* ATCC9950, *Sacchromycese cervisea* ATCC9763 and *Aspergillus flavus* ATCC204304. A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was $1–5\times10^3$ spore/mL. From the stock solutions of the tested compounds and reference antifungal Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO., Ltd, Beijing, China) were made resulting in eleven wanted concentrations of each tested compounds. These dilutions were inoculated and incubated at 35 ºC for 24 h. The drug's MIC was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well.

1.3 Experimental Procedures and Represent Spectral Data for the Prepared Compound

*Ethyl-6-fluoro-7-(4-(oxiran-2-ylmethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2a**)*

A mixture of norfloxacin (0.96 g, 0.0027 mol) and sodium hydrogen carbonate (0.69 g, 0.0048 mol) in acetonitrile (100 mL) was stirred at 50 ℃ for 1 h. After the mixture was cooled to room temperature, 2-(chloromethyl)oxirane (1.00 g, 0.0027 mol) was added. The reaction mixture was then heated at 45 ℃ for 10 h. After the reaction was completed (monitored by TLC, chloroform/methanol (50/1, V/V)), the reaction was cooled to room temperature and treated with formic acid to adjust the pH value to 5.5–6.5. After the acetonitrile was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with chloroform/methanol (60/1, V/V) to give the pure target compound **2a** as white power (0.68 g). Yield: 67.4%; mp: 185–186 ℃; IR (KBr, cm^{-1}) ν: 3061 (Ar–H), 1804 (aromatic frame), 1718 (C=O), 1617, 1571 (aromatic frame); ^{1}H NMR (300 MHz, $CDCl_3$): δ 15.05 (s, 1H, COO*H*), 8.63 (s, 1H, quinolone 2-*H*), 7.99 (d, *J* = 13.0 Hz, 1H, quinolone 5-*H*), 6.80 (d, *J* = 6.8 Hz, 1H, quinolone 8-*H*), 4.53 (t, *J* = 8.3 Hz, 1H, CH_2), 4.30 (dd, 2H, quinolone N-CH_2), 3.96 (dd, *J* = 11.7, 6.0 Hz, 1H, O-C*H*), 3.59 (t, *J* = 5.0 Hz, 1H, CH_2), 3.32 (t, *J* = 14.3 Hz, 4H, piperazine N-CH_2), 2.81 (dd, *J* = 11.5, 6.7 Hz, 4H, piperazine N-CH_2), 2.69 (dd, *J* = 10.9, 5.5 Hz, 1H, CH_2), 2.58 (d, *J* = 6.6 Hz, 1H, CH_2), 1.56 (t, *J* = 7.2 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.3, 165.3, 153.7, 152.1, 142.8, 137.6, 121.2, 118.7, 112.4, 107.9, 55.8, 52.4, 49.6, 47.6, 44.9, 14.2 ppm; MS (m/z): 376 $[M+H]^+$; HRMS (TOF) calcd for $C_{19}H_{23}FN_3O_4$: $[M+H]^+$, 376.1673; found, 376.1674.

*Cyclopropyl-6-fluoro-7-(4-(oxiran-2-ylmethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2b**)*

White solid; Yield: 64.0%; mp: 201–202 ℃; IR (KBr, cm^{-1}) ν: 3050 (Ar–H), 1805 (aromatic frame), 1716 (C=O), 1615, 1548, 1482 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.19 (s, 1H, COO*H*), 8.63 (s, 1H, quinolone 2-*H*), 7.85 (dd, *J* = 10.9, 7.6 Hz, 1H, quinolone 5-*H*), 7.54 (d, *J* = 6.1 Hz, 1H, quinolone 8-*H*), 5.13 (d, *J* = 4.5 Hz, 1H, quinolone N-C*H*), 3.91 (td, *J* = 9.9, 4.8 Hz, 1H, O-C*H*), 3.81 (s, 2H, O-CH_2), 3.71–3.58 (m, 4H, piperazine N-CH_2), 2.67 (td, *J* = 10.9, 6.3 Hz, 4H, piperazine N-CH_2), 2.43 (dd, *J* = 12.6, 6.1 Hz, 1H), 1.32 (d, *J* = 5.9 Hz, 2H, cyclopropyl-CH_2), 1.19 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 177.4 (quinolone 4-*C*), 166.4 (quinolone *C*OOH), 153.6, 151.1, 139.9, 136.4, 122.1, 116.8, 112.1, 105.1, 54.8, 52.3, 48.9, 46.8, 42.6, 8.1 ppm; MS (m/z): 388 $[M+H]^+$; HRMS (TOF) calcd for $C_{20}H_{22}FN_3O_4$: $[M+H]^+$, 388.1673; found, 388.1672.

*8-Chloro-1-cyclopropyl-6-fluoro-7-(3-(oxiran-2-ylmethylamino)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2c**)*

White solid; Yield: 62.4%; mp: 191–192 ℃; IR (KBr, cm^{-1}) ν: 3458 (NH), 3060 (Ar–H), 1805 (aromatic frame), 1717 (C=O), 1614, 1546, 1481 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 14.56 (s, 1H, COO*H*), 8.83

(s, 1H, quinolone 2-*H*), 7.93 (d, *J* = 11.9 Hz, 1H, quinolone 5-*H*), 4.42–4.37 (m, 1H, NH-CH_2), 3.92–3.86 (m, 1H, NH-CH_2), 3.70 (dd, *J* = 11.0, 4.1 Hz, 1H, cyclopropyl-C*H*), 3.60 (dd, *J* = 11.0, 5.5 Hz, 1H, O-C*H*), 3.34 (s, 4H, pyrrole N-CH_2), 2.63 (d, *J* = 17.1 Hz, 4H, pyrrole CH_2C*H*, N*H*), 2.49 (d, *J* = 6.2 Hz, 1H, O-CH_2), 2.42 (dd, *J* = 12.7, 6.3 Hz, 1H, O-CH_2), 1.22–1.16 (m, 2H, cyclopropyl-CH_2), 1.03–0.96 (m, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.2, 165.7, 165.5, 153.6, 152.0, 147.7, 145.0, 138.9, 118.3, 110.8, 110.6, 106.6, 106.1, 70.5, 69.8, 67.8, 60.7, 57.8, 52.9, 49.3, 48.6, 46.2, 35.6, 7.4 ppm; MS (m/z): 423 $[M+H]^+$; HRMS (TOF) calcd for $C_{20}H_{21}ClFN_3O_4$: $[M+H]^+$, 422.1283; found, 422.1282.

*Ethyl-6-fluoro-7-(4-(2-hydroxy-3-(1-methyl-1H-imidazol-2-ylthio)propyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4a**)*

A mixture of 2-thiol-1-methylimidazole (0.31 g, 0.0027 mol) and potassium carbonate (0.69 g, 0.0048 mol) in acetonitrile (20 mL) was stirred at 50 ℃ for 1 h. After the mixture was cooled to room temperature, compound **2a** (1.00 g, 0.0027 mol) was added. The reaction mixture was then heated at 80 ℃ for 20 h. After the reaction was completed (monitored by TLC, chloroform/methanol (25/1, V/V)), the reaction was cooled to room temperature and treated with formic acid to adjust the pH value to 5.5–6.5. After the ethanol was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with chloroform/methanol (30/1, V/V) to give the pure target compound **4a** as yellow power (0.35 g). Yield: 26.4%; mp: 109–110 ℃; IR (KBr, cm^{-1}) ν: 3441 (OH), 3030 (Ar–H), 2940 (CH_3), 1713 (C=O), 1628, 1540, 1497, 1457 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.35 (s, 1H, COO*H*), 8.93 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.22 (s, 1H, imidazole 3-*H*), 7.16 (d, *J* = 7.2 Hz, 1H, quinolone 8-*H*), 6.92 (s, 1H, imidazole 4-*H*), 4.59 (d, *J* = 14.5 Hz, 2H, CH_2-CH_3), 3.90 (s, 1H, O*H*), 3.59 (s, 3H, imidazole-CH_3), 3.30 (d, *J* = 4.7 Hz, 4H, piperazine N-CH_2), 3.27–3.22 (m, 2H, imidazole S-CH_2), 2.63 (td, *J* = 11.2, 6.0 Hz, 4H, piperazine N-CH_2), 2.48–2.40 (m, 2H, piperazine N-CH_2), 1.42 (t, *J* = 7.1 Hz, 3H, quinolone CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.5, 166.3, 150.7, 149.8, 147.3, 145.2, 138.1, 136.7, 120.3, 118.1, 110.2, 106.1, 67.0, 60.2, 53.5, 50.1, 49.7, 48.1, 13.4 ppm; MS (m/z): 490 $[M+H]^+$. HRMS (TOF) calcd for $C_{23}H_{28}FN_5O_4S$: $[M+H]^+$, 490.1924; found, 490.1927.

*Ethyl-6-fluoro-7-(4-(2-hydroxy-3-(3-mercapto-1H-1,2,4-triazol-1-yl)propyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3a**)*

Brown solid; Yield: 24.0%; mp: 219–220 ℃; IR (KBr, cm^{-1}) ν: 3451 (OH), 3081 (Ar–H), 2943 (CH_3), 1716 (C=O), 1614, 1541, 1523, 1420 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.13 (s, 1H, COO*H*), 14.05 (s, 1H, triazole S*H*), 8.91 (s, 1H, triazole 5-*H*), 8.37 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.3 Hz, 1H quinolone 5-*H*), 7.15 (d, *J* = 7.1 Hz, 1H, quinolone 8-*H*), 4.57 (dd, *J* = 14.0, 6.9 Hz, 2H,

CH_2-CH_3), 3.38 (s, 1H, O*H*), 3.13 (dd, *J* = 13.2, 7.0 Hz, 2H, triazole CH_2), 2.87–2.84 (m, 1H, HO-C*H*), 3.30–3.22 (m, 4H, piperazine N-CH_2), 2.65 (td, *J* = 11.2, 6.2 Hz, 4H, piperazine N-CH_2), 2.49 (dd, *J* = 13.5, 6.0 Hz, 2H, piperazine N-CH_2), 1.43 (t, *J* = 7.1 Hz, 3H, quinolone CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.6, 166.5, 154.1, 152.5, 151.4, 145.1, 111.5, 107.6, 106.2, 79.6, 67.0, 61.8, 53.9, 53.5, 50.0, 49.5, 14.8 ppm; MS (m/z): 477 $[M+H]^+$; HRMS (TOF) calcd for $C_{21}H_{25}FN_6O_4S$: $[M+H]^+$, 477.5323; found, 477.5324.

*Cyclopropyl-6-fluoro-7-(4-(2-hydroxy-3-(3-mercapto-1H-1,2,4-triazol-1-yl)propyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3b**)*

White solid; Yield: 20.5%; mp: 214–215 ℃; IR (KBr, cm^{-1}) ν: 3443 (OH), 3010 (Ar–H), 1718 (C=O), 1625, 1545, 1498, 1455 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.21 (s, 1H, COO*H*), 13.99 (s, 1H, triazole S*H*), 8.65 (d, *J* = 5.1 Hz, 1H, triazole 5-*H*), 8.36 (d, *J* = 57.3 Hz, 1H, quinolone 2-*H*), 7.87 (t, *J* = 13.0 Hz, 1H, quinolone 5-*H*), 7.55 (d, *J* = 5.8 Hz, 1H, quinolone 8-*H*), 5.05 (d, *J* = 24.6 Hz, 1H, cyclopropyl-C*H*), 3.98–3.92 (m, 1H, HO-C*H*), 3.82 (s, 1H, O*H*), 3.71–3.63 (m, 1H, triazole CH_2), 3.38 (dd, *J* = 12.9, 4.5 Hz, 4H, piperazine N-CH_2), 3.09 (ddd, *J* = 20.2, 12.8, 6.9 Hz, 1H, triazole CH_2), 2.67 (d, *J* = 23.4 Hz, 4H, piperazine N-CH_2), 2.50–2.44 (m, 2H, piperazine N-CH_2), 1.32 (d, *J* = 6.8 Hz, 2H, cyclopropyl-CH_2), 1.19 (d, *J* = 3.3 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.8, 166.4, 154.3, 152.6, 148.3, 145.6, 139.7 118.9, 111.4, 106.7, 79.6, 71.0, 67.6, 64.9, 63.2, 53.5, 50.0, 36.2, 8.0 ppm; MS (m/z): 489 $[M+H]^+$; HRMS (TOF) calcd for $C_{22}H_{25}FN_6O_4S$: $[M+H]^+$, 489.1720; found, 489.1723.

*8-Chloro-1-cyclopropyl-6-fluoro-7-(3-(2-hydroxy-3-(3-mercapto-1H-1,2,4-triazol-1-yl)propylamino)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3c**)*

White solid; Yield: 25.6%; mp: 222–223 ℃; IR (KBr, cm^{-1}) ν: 3443 (OH), 3021 (Ar–H), 1717 (C=O), 1625, 1541, 1494, 1451 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 14.57 (s, 1H, COO*H*), 14.00 (s, 1H, triazole S*H*), 8.83 (s, 1H, triazole 5-*H*), 8.32 (s, 1H, quinolone 2-*H*), 7.93 (d, *J* = 11.7 Hz, 1H, quinolone 5-*H*), 5.04 (s, 1H, O*H*), 4.41–4.37 (m, 1H, cyclopropyl-C*H*), 3.96–3.92 (m, 1H, HO-C*H*), 3.39 (s, 1H, triazole CH_2), 3.38–3.33 (m, 4H, pyrrole N-CH_2), 3.12 (dd, *J* = 12.4, 6.9 Hz, 1H, triazole CH_2), 2.61 (d, *J* = 24.7 Hz, 4H, pyrrole CH_2C*H*, N*H*), 2.49–2.44 (m, 2H, NH-CH_2), 1.19 (p, *J* = 6.9 Hz, 2H, cyclopropyl-CH_2), 1.00–0.96 (m, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.8, 165.5, 156.8, 153.3, 144.3, 138.6, 123.1, 119.7, 110.9, 108.1, 79.6, 67.8, 63.6, 54.5, 51.6, 11.2, 8.1 ppm; MS (m/z): 523 $[M+H]^+$; HRMS (TOF) calcd for $C_{22}H_{24}ClFN_6O_4S$: $[M+H]^+$, 523.1331; found, 523.1327.

Ethyl-6-fluoro-7-(4-(2-hydroxy-3-(1-methyl-1H-tetrazol-5-ylthio)propyl)piperazin-1-yl)-4-oxo-1,4-

*dihydroquinoline-3-carboxylic acid (**4b**)*

Brown solid; Yield: 22.1%; mp: 213–214 ˚C; IR (KBr, cm^{-1}) ν: 3440 (OH), 3025 (Ar–H), 2940 (CH_3), 1715 (C=O), 1625, 1541, 1498, 1455 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.21 (s, 1H, COO*H*), 8.80 (s, 1H, quinolone 2-*H*), 7.91–7.87 (m, 1H, quinolone 5-*H*), 7.56 (d, *J* = 7.3 Hz, 1H, quinolone 8-*H*), 4.59 (dd, *J* = 5.1 Hz, 2H, quinolone N-CH_2), 4.05 (dt, *J* = 11.3, 5.7 Hz, 1H, HO-C*H*), 3.95 (s, 3H, tetrazole-CH_3), 3.81 (s, 1H, O*H*), 3.65–3.60 (m, 1H, tetrazole S-CH_2), 3.59–3.56 (m, 1H, tetrazole S-CH_2), 3.34 (d, *J* = 17.6 Hz, 4H, piperazine N-CH_2), 3.31 (d, *J* = 7.5 Hz, 2H, quinolone N-CH_2), 2.73–2.61 (m, 4H, piperazine N-CH_2), 1.61 (m, *J* = 7.2 Hz, 3H, quinolone CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.9, 165.6, 154.9, 148.3, 145.2, 139.6, 118.6, 111.1, 107.4, 106.8, 67.2, 62.8, 54.0, 50.6, 46.5, 36.1, 34.0, 8.0 ppm; MS (m/z): 492 $[M+H]^+$; HRMS (TOF) calcd for $C_{21}H_{26}FN_7O_4S$: $[M+H]^+$, 492.1829; found, 492.1831.

*Cyclopropyl-6-fluoro-7-(4-(2-hydroxy-3-(1-methyl-1H-imidazol-2-ylthio)propyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4c**)*

White solid; Yield: 32.2%; mp: 111–112 ˚C; IR (KBr, cm^{-1}) ν: 3439 (OH), 3031 (Ar–H), 2940 (CH_3), 1713 (C=O), 1628, 1540, 1497, 1457 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.35 (s, 1H, COO*H*), 8.88 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.0 Hz, 1H, quinolone 5-*H*), 7.18 (s, 1H, imidazole 3-*H*), 7.14 (d, *J* = 6.5 Hz, 1H, quinolone 8-*H*), 6.87 (s, 1H, imidazole 4-*H*), 5.23 (d, *J* = 4.2 Hz, 1H, cyclopropyl-C*H*), 3.91 (s, 1H, O*H*), 3.55 (s, 3H, imidazole-CH_3), 3.32 (d, *J* = 4.7 Hz, 4H, piperazine N-CH_2), 3.28–3.21 (m, 2H, imidazole S-CH_2), 2.62 (td, *J* = 11.1, 6.3 Hz, 4H, piperazine N-CH_2), 2.47–2.41 (m, 2H, piperazine N-CH_2), 1.31 (d, *J* = 6.1 Hz, 2H, cyclopropyl-CH_2), 1.22 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.4, 166.2, 150.6, 149.5, 147.2, 145.8, 138.2, 136.5, 120.2, 118.2, 110.6, 106.1, 67.2, 60.7, 53.4, 50.8, 49.2, 48.1, 13.2, 8.1 ppm; MS (m/z): 502 $[M+H]^+$. HRMS (TOF) calcd for $C_{24}H_{28}FN_5O_4S$: $[M+H]^+$, 502.1924; found, 502.1927.

*Cyclopropyl-6-fluoro-7-(4-(2-hydroxy-3-(1-methyl-1H-tetrazol-5-ylthio)propyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4d**)*

White solid; Yield: 28.4%; mp: 212–213 ˚C; IR (KBr, cm^{-1}) ν: 3443 (OH), 3091 (Ar–H), 2947 (CH_3), 1718 (C=O), 1628, 1540, 1500, 1451 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.22 (s, 1H, COO*H*), 8.66 (d, *J* = 4.0 Hz, 1H, quinolone 2-*H*), 7.91–7.87 (m, 1H, quinolone 5-*H*), 7.56 (d, *J* = 7.3 Hz, 1H, quinolone 8-*H*), 5.22 (d, *J* = 5.1 Hz, 1H, cyclopropyl-C*H*), 4.02 (dt, *J* = 11.3, 5.7 Hz, 1H, HO-C*H*), 3.95 (s, 3H, tetrazole-CH_3), 3.83 (s, 1H, O*H*), 3.68–3.60 (m, 1H, tetrazole S-CH_2), 3.60–3.56 (m, 1H, tetrazole S-CH_2), 3.34 (d, *J* = 17.6 Hz, 4H, piperazine N-CH_2), 3.31 (d, *J* = 7.5 Hz, 2H, quinolone N-CH_2), 2.73–2.61 (m, 4H, piperazine N-CH_2), 1.33–

1.30 (m, 2H, cyclopropyl-CH_2), 1.20–1.17 (m, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.9, 165.8, 154.9, 148.4, 145.2, 139.6, 118.6, 111.1, 107.5, 106.8, 67.2, 62.9, 54.0, 50.6, 46.5, 36.0, 34.0, 8.0 ppm; MS (m/z): 504 $[M+H]^+$. HRMS (TOF) calcd for $C_{22}H_{26}FN_7O_4S$: $[M+H]^+$, 504.1829; found, 504.1828.

*8-Chloro-1-cyclopropyl-6-fluoro-7-(3-(2-hydroxy-3-(1-methyl-1H-imidazol-2-ylthio)propylamino)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4e**)*

White solid; Yield: 31.8%; mp: 241–242 ℃; IR (KBr, cm^{-1}) ν: 3445 (OH), 3090 (Ar–H), 2951 (CH_3), 1727 (C=O), 1625, 1520, 1467, 1476, 1451 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 14.56 (s, 1H, COO*H*), 8.83 (s, 1H, quinolone 2-*H*), 7.91 (d, *J* = 11.9 Hz, 1H, quinolone 5-*H*), 7.22 (d, *J* = 0.8 Hz, 1H, imidazole 3-*H*), 6.92 (d, *J* = 0.9 Hz, 1H, imidazole 4-*H*), 5.29 (s, 1H, O*H*), 4.41–4.36 (m, 1H, cyclopropyl-C*H*), 3.90 (dt, *J* = 12.1, 6.2 Hz, 1H, HO-C*H*), 3.60 (s, 3H, imidazole-CH_3), 3.33 (s, 4H, pyrrole N-CH_2), 3.26 (dd, *J* = 13.2, 4.4 Hz, 1H, imidazole-CH_2), 3.07–3.00 (m, 1H, imidazole-CH_2), 2.60 (d, *J* = 20.9 Hz, 4H, pyrrole CH_2C*H*, N*H*), 2.48–2.41 (m, 2H, NH-CH_2), 1.19 (q, *J* = 5.8 Hz, 2H, cyclopropyl-CH_2), 0.98 (q, *J* = 6.9 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.8, 144.4, 138.7, 128.8, 123.4, 111.1, 67.8, 63.5, 54.3, 51.4, 42.0, 33.3, 11.2 ppm; MS (m/z): 536 $[M+H]^+$; HRMS (TOF) calcd for $C_{24}H_{27}ClFN_5O_4S$: $[M+H]^+$, 536.1535; found, 536.1535.

*8-Chloro-1-cyclopropyl-6-fluoro-7-(3-(2-hydroxy-3-(1-methyl-1H-tetrazol-5-ylthio)propylamino)pyrrolidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4f**)*

White solid; Yield: 35.4%; mp: 213–214 ℃; IR (KBr, cm^{-1}) ν: 3442 (OH), 3091 (Ar–H), 2941 (CH_3), 1727 (C=O), 1626, 1520, 1497, 1471, 1450 (aromatic frame); ^{1}H NMR (300 MHz, DMSO-d_6): δ 14.57 (s, 1H, COO*H*), 8.83 (s, 1H, quinolone 2-*H*), 7.94 (d, *J* = 11.8 Hz, 1H, quinolone 5-*H*), 5.19 (dd, *J* = 11.6, 5.1 Hz, 1H, cyclopropyl-C*H*), 4.42–4.37 (m, 1H, HO-C*H*), 4.01 (dd, *J* = 9.6, 5.0 Hz, 1H, O*H*), 3.95 (s, 3H, tetrazole-CH_3), 3.93 (s, 1H, tetrazole S-CH_2), 3.58 (dd, *J* = 13.0, 3.9 Hz, 1H, tetrazole S-CH_2), 3.46–3.41 (m, 1H, NH-CH_2), 3.34 (s, 4H, pyrrole N-CH_2), 3.24 (dd, *J* = 12.9, 7.7 Hz, 1H, NH-CH_2), 2.62 (d, *J* = 37.5 Hz, 4H, pyrrole CH_2C*H*, N*H*), 1.19 (d, *J* = 6.7 Hz, 2H, cyclopropyl-CH_2), 0.98 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.9, 165.2, 157.3, 152.7, 144.5, 137.9, 119.2, 108.1, 79.3, 66.8, 64.9, 54.3, 51.4, 42.0, 37.9, 34.1, 11.2, 7.2 ppm; MS (m/z): 538 $[M+H]^+$; HRMS (TOF) calcd for $C_{22}H_{25}ClFN_7O_4S$: $[M+H]^+$, 538.1440; found, 538.1442.

*7-(4-(3-(1-(3,4-dichlorobenzyl)-1H-1,2,4-triazol-3-ylthio)-2-hydroxypropyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5b**)*

Yellow solid; Yield: 24.8%; mp: 217–219 ℃; IR (KBr, cm^{-1}) ν: 3429 (OH), 3042 (Ar–H), 2939 (CH_3), 1726

(C=O), 1611, 1548, 1525, 1493, 1448 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): δ 15.36 (s, 1H, COO*H*), 8.96 (s, 1H, quinolone 2-*H*), 8.47 (s, 1H, triazole 5-*H*), 7.93 (d, *J* = 12.9 Hz, 1H, quinolone 5-*H*), 7.63 (s, 1H, quinolone 8-*H*), 7.51 (d, *J* = 7.5 Hz, 1H, Ph 5-*H*), 7.35 (d, *J* = 7.3 Hz, 1H, Ph 2-*H*), 7.17 (s, 1H, Ph 6-*H*), 4.59 (d, *J* = 4.9 Hz, 2H, Ph-CH_2), 4.38 (s, 2H, CH_2-CH_3), 4.26 (d, *J* = 12.7 Hz, 1H, HO-C*H*), 4.10 (s, 4H, piperazine N-CH_2), 4.00 (s, 1H, O*H*), 3.17 (s, 2H, triazole S-CH_2), 2.63 (s, 4H, piperazine N-CH_2), 2.38 (s, 2H, piperazine N-CH_2), 1.42 (s, 3H, quinolone CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ 176.6, 165.4, 154.3, 152.8, 152.4, 151.4, 148.5, 145.6, 144.5, 137.6, 121.7, 119.6, 111.3, 108.2, 106.6, 106.0, 70.1, 66.7, 64.1, 63.1, 56.4, 55.8, 53.5, 52.3, 42.2, 11.3 ppm; MS (m/z): 636 $[M+H]^+$; HRMS (TOF) calcd for $C_{28}H_{29}Cl_2FN_6O_4S$: $[M+H]^+$, 635.1410; found, 635.1413.

2. Figures of Molecular Modeling

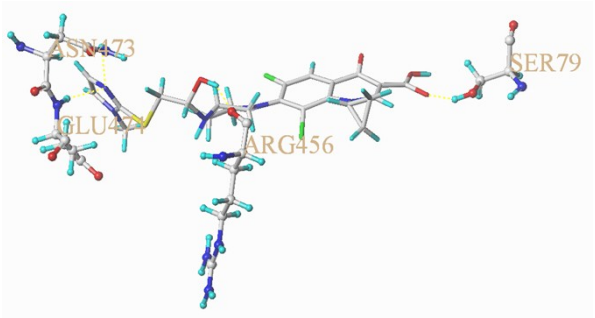


Figure S1. Three-dimensional conformation of compound **4e** docked in topoisomerase IV-DNA complex (a)

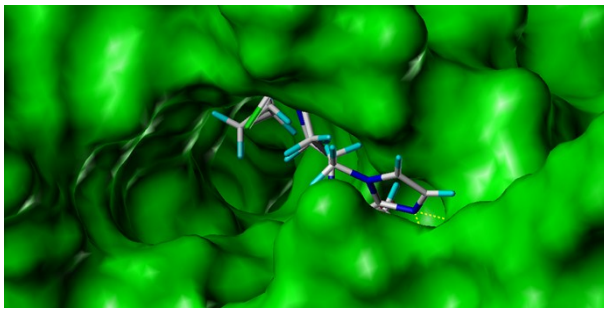


Figure S2. Three-dimensional conformation of compound **4e** docked in topoisomerase IV-DNA complex (b)

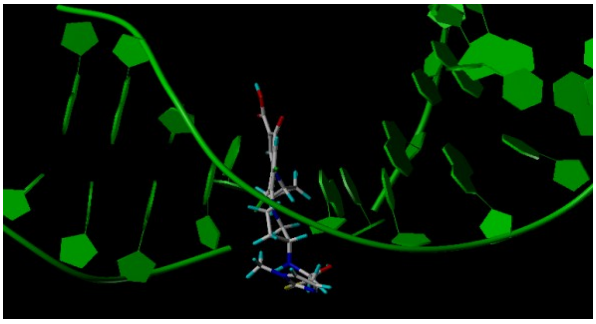


Figure S3. Three-dimensional conformation of compound **4e** with DNA of the topoisomerase IV-DNA complex (c)

3. Procedures for Isolating Genomic DNA from MRSA Bacteria

(1) An overnight culture (1 mL) was added to a microcentrifuge tube (1.5 mL), and then centrifuged at 13,000–16,000 × *g* for 2 minutes to pellet the cells. The supernatant was removed and the cells were resuspend thoroughly in EDTA (480 μL, 50 mM). The lytic enzyme (120 μL) was added appropriately to the resuspended

cell pellet, and mixed gently. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lysis can take place. After the sample was incubated at 37 ℃ for 30–60 minutes, it was centrifuged for 2 minutes at 13,000–16,000 × *g* and the supernatant was removed. Nuclei Lysis solution (600 μL) was added to the sample, it was gently pipeted until the cells are resuspended. It was incubated at 80 ℃ for 5 minutes to lyse the cells, then it was cooled to room temperature. RNase solution (3 μL) was added to the cell lysate, the tube was inverted to mix for 2–5 times. After the sample was incubated at 37 ℃ for 15–60 minutes, then it was cooled to room temperature.

(2) Protein precipitation solution (200 μL) was added to the RNase-treated cell lysate, and vortex vigorously at high speed for 20 seconds to mix the protein precipitation solution with the cell lysate. The sample was incubated on ice for 5 minutes, and centrifuged at 13,000–16,000 × *g* for 3 minutes.

(3) The supernatant containing the DNA was transfered to a clean microcentrifuge tube (1.5 mL) containing room temperature isopropanol (600 μL). It was gently mixed by inversion until the thread-like strands of DNA form a visible mass, and was centrifuged at 13,000–16,000 × *g* for 2 minutes. The supernatant was carefully poured off and the tube was drained on a clean absorbent paper. The room temperature ethanol (70%, 600 μL) was added to the tube and it was gently inverted several times to wash the DNA pellet. After it was centrifuged at 13,000–16,000 × *g* for 2 minutes, and then the ethanol was carefully aspirated. The tube was drained on a clean

absorbent paper to allow the pellet to air-dry for 10–15 minutes.

(4) DNA rehydration solution (100 μL) was added to the tube and the DNA was rehydrated by incubating at 65 ℃ for 1 hour. The solution was mixed periodically by gently tapping the tube. Alternatively, the DNA was rehydrated by incubating the solution overnight at room temperature or at 4 ℃. The obtained DNA was stored at 2–8 ℃.

4. Interactive Mode Discussion

Interactions with MRSA DNA

The application of absorption spectroscopy is one of the most useful techniques in DNA–binding studies. In absorption spectroscopy, hypochromism and hyperchromism are very important spectral features to distinguish the change of DNA double-helical structure. Due to the strong interaction between the electronic states of intercalating chromophore and that of the DNA base, the observed large hypochromism strongly suggests a close proximity of the aromatic chromophore to the DNA bases.

The interaction between compound **4e** and Cu^{2+} was investigated (Figure S4) and it showed that compound

4e had very intense intrinsic fluorescence in aqueous solution at $\lambda_{ex}/\lambda_{em}$ = 280/430 nm. The fluorescence intensity of 6.0×10^{-6} mol/L compound **4e** solution at 430 nm dropped regularly with the increasing concentration of copper(II) ion. This may be due to a strong interaction between compound **4e** and copper(II) ion. The composition of the binary complex can be deduced from the following equations.

$$M + nL = ML_n \quad (1)$$

$$\log[(F_0/F)-1] = \log K_a + n\log[M] \quad (2)$$

Where M is the quencher, L is the drug molecule with a fluorophore, ML_n is binary complex whose resultant constant is K_a. A plot of $\log[(F_0/F)-1]$ versus log[M] will give straight line with a slope of n and y-axis intercept logKa.

Figure S5 was obtained by keeping the compound **4e** concentration (6.0×10^{-6} mol/L) constant and changing the concentration of Cu^{2+} ion. The data obtained was well fitted to Equation 2 and the slope was 1.23, the correlation coefficient was 3.52, respectively. The result indicated that compound **4e** can form a stable 1:1 complex with Cu^{2+} ion.

The data (Figure S6) were well fitted to Equation 2 and the slope was 1.13, the correlation coefficient was 8.0×10^{4}, respectively. The result indicated that compound **4e** in the presence of Cu^{2+} ion can form a stable 1:1 complex with DNA.

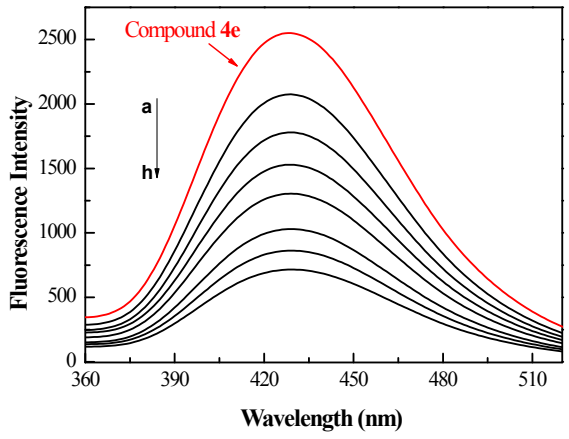


Figure S4. Emission spectra of compound **4e** in the presence of various concentrations of Cu^{2+} ions. *c*(compound **4e**) = 6.0×10^{-6} mol/L; *c*(Cu^{2+})/(1.0×10^{-5} mol/L), *a*–*h*: from 0.0 to 2.10 at increments of 0.30; red line shows the emission spectrum of compound **4e** only; T = 290 K, λ_{ex} = 280 nm.

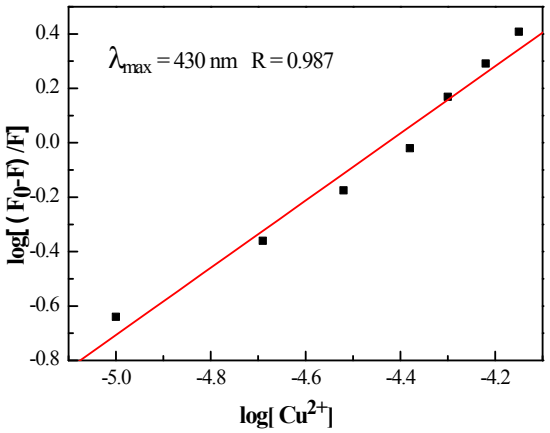


Figure S5. Estimation of the composition of the compound **4e**–Cu^{2+} complex, λ_{ex} = 280 nm, *c*(compound **4e**) = 6.0×10^{-6} mol/L.

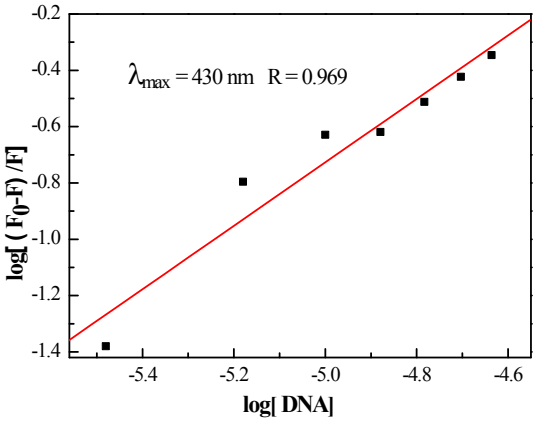


Figure S6. Estimation of the composition of the compound **4e**–Cu^{2+}–DNA complex, *c*(compound **4e**) = 6.0 × 10^{-6} mol/L; T = 290 K, λ_{ex} = 280 nm.

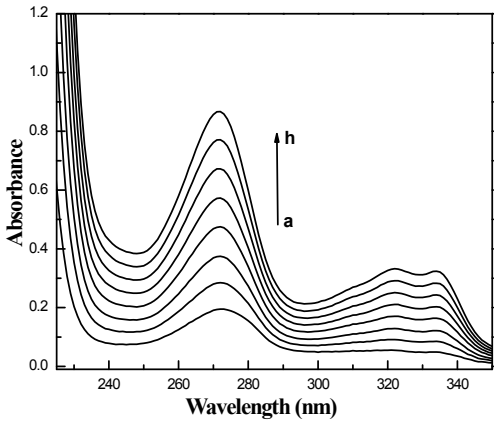
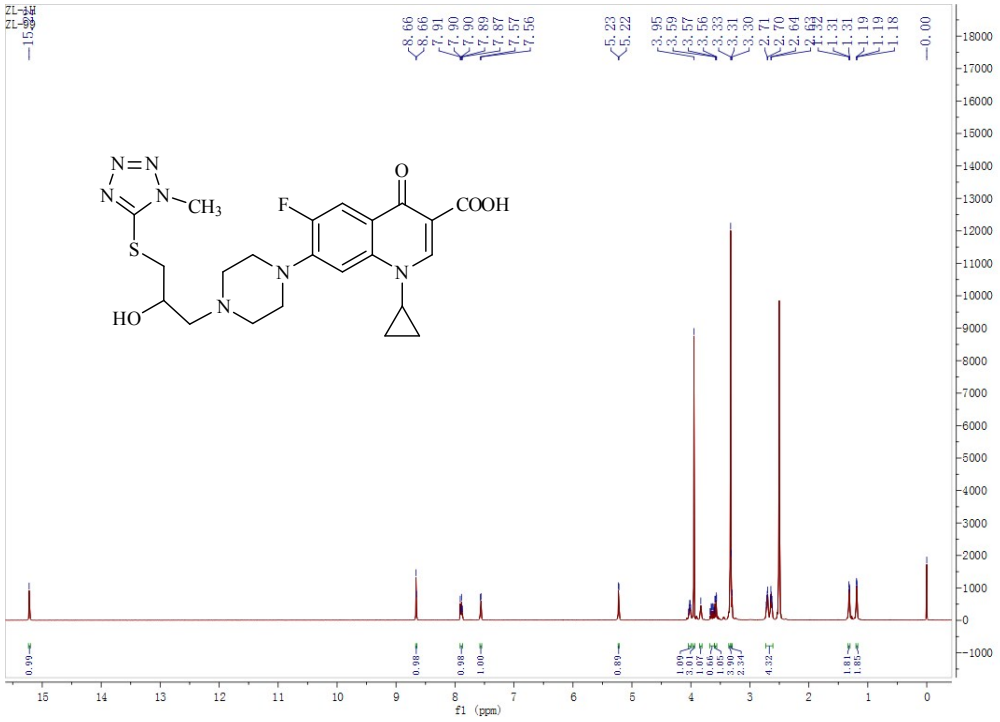


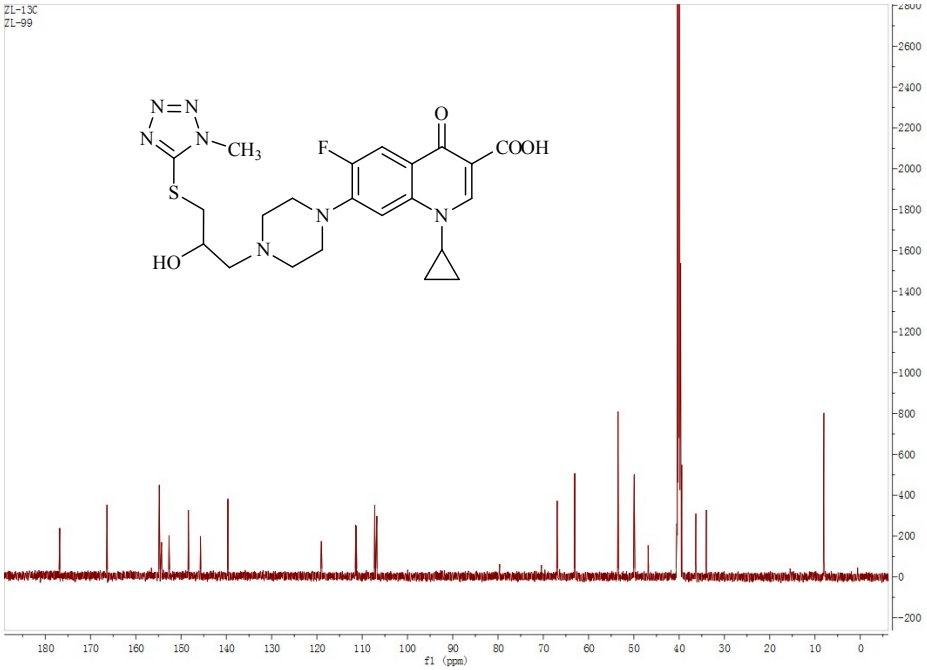
Figure S7. UV absorption spectra of different concentrations of compound **4e** (pH = 7.4, T = 290 K). *c*(compound **4e**) = 0–1.2 × 10^{-5} mol/L for curves *a*-*h* respectively at increment 0.15 × 10^{-5}.

5. Some Representative Spectra

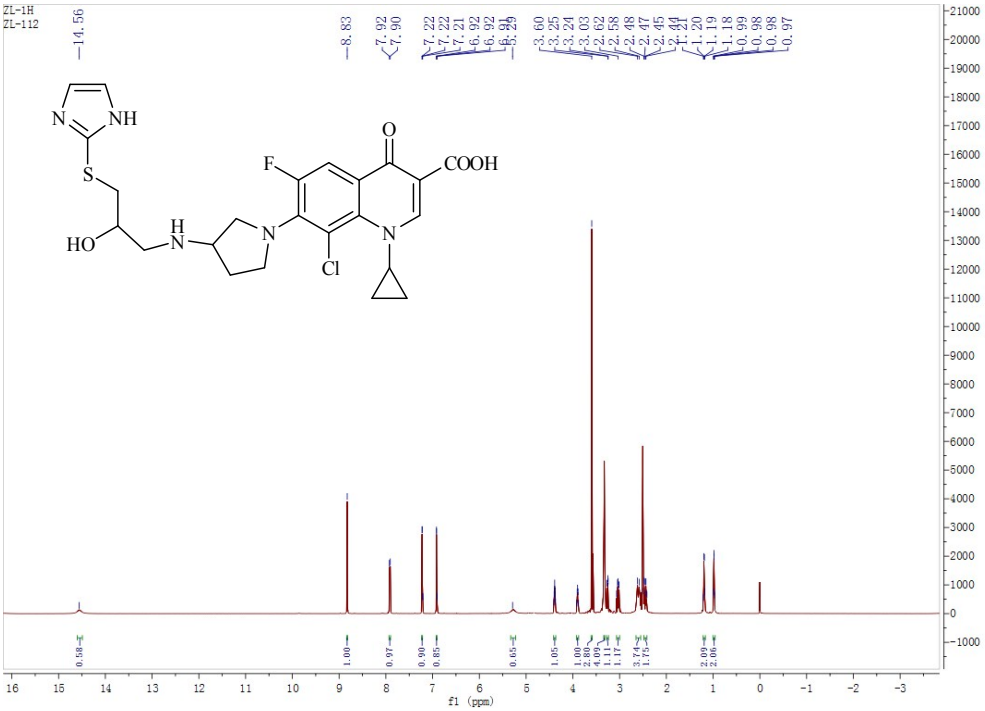
^{1}H NMR Spectrum of Compound 4d



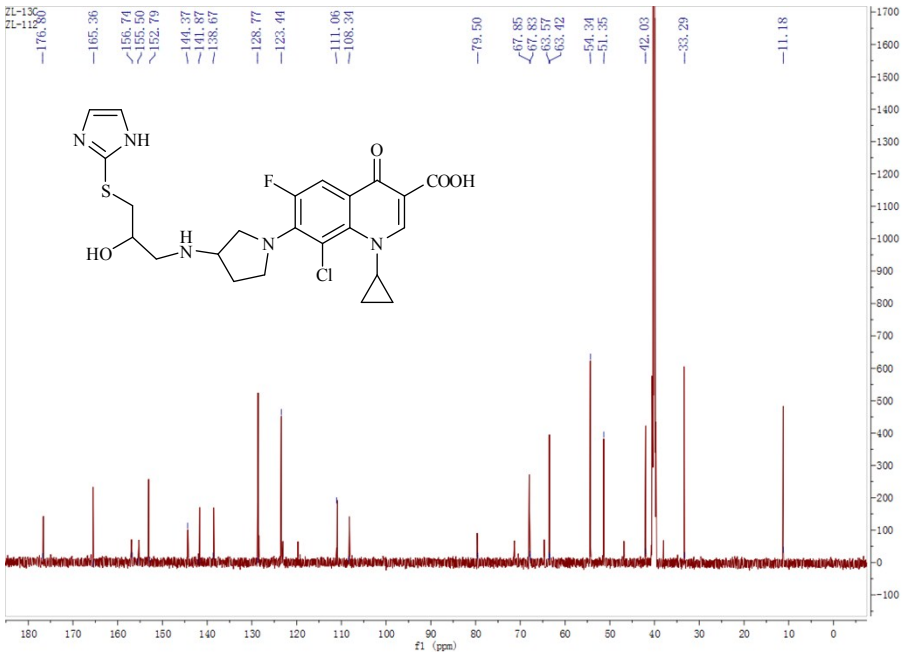
^{13}C NMR Spectrum of Compound 4d



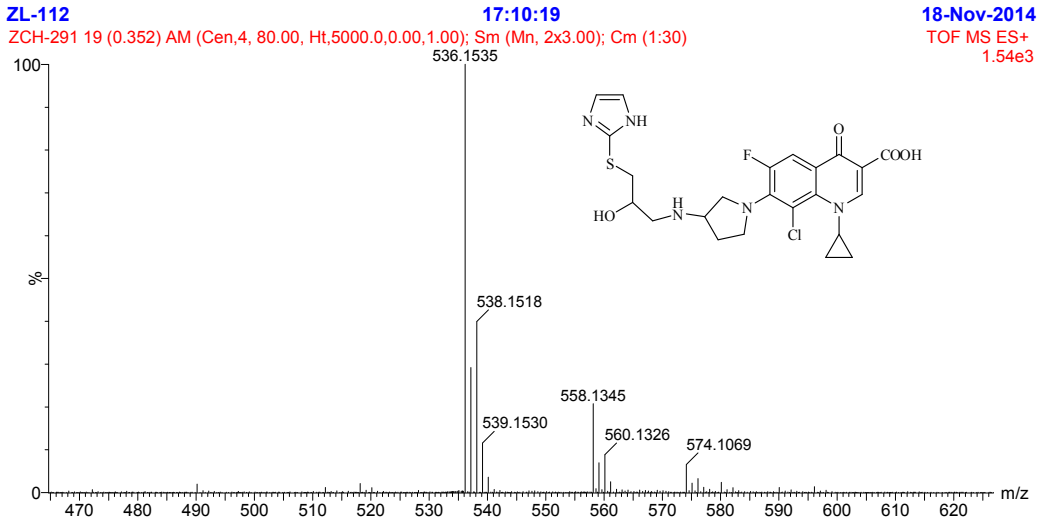
[1]HNMR Spectrum of Compound 4e



[13]C NMR Spectrum of Compound 4e



HRMS Spectrum of Compound 4e



HRMS (TOF) calcd. for $C_{24}H_{27}ClFN_5O_4S$: $[M+H]^+$, 536.1535; found, 536.1535