Design, synthesis, and biological evaluation of pyrazole-ciprofloxacin hybrids as antibacterial and antibiofilm agents against *Staphylococcus aureus*

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Contents

1. Materials and methods

All the chemicals, reagents, and solvents were obtained from commercial providers and were used as such without any further purification. Reactions were monitored using TLC-MERCK pre-coated silica gel 60-F_{254} (0.5 mm) aluminum plates under UV light. Compounds were purified by column chromatography using silica of 60-120 mesh. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 500 MHz spectrometer using tetramethyl silane (TMS) as the internal standard and by making samples in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm and referenced to TMS (δ 0.00 for ^{1}H NMR and ^{13}C NMR) or solvents used for NMR recording $CDCl_3$ (δ 7.26 for ^{1}H NMR and 77.2 ^{13}C NMR) or DMSO-d_6 (δ 2.50 for ^{1}H NMR and 39.5 for ^{13}C NMR). Spin multiplicities for ^{1}H NMR are described as s (singlet), brs (broad singlet), d (doublet), dd (double doublet), t (triplet), and m (multiplet). Coupling constant values are reported in hertz (Hz). For fluorine-containing compounds, the carbon-fluorine coupling is denoted as J_{CF}. HRMS was recorded on an Agilent quadrupole-time-of-flight (QTOF) mass spectrometer 6540 series instrument and was performed in electrospray ionization (ESI) techniques at 70 eV. The melting point was taken using the Stuart R SMP30 apparatus. Microwave reactions were conducted in Monowave 300 single-mode microwave reactor (make-Anton Paar GmbH). Microwave reactions were performed in SiC10 Silicon Carbide reaction vessels (10 mL) or reusable Pyrex vials (30 mL). The strains *Staphylococcus aureus* ATCC 29213, *P. aeruginosa* PAO1 ATCC 15692, and Mtb H37Ra ATCC 25177 and the HepG2 cell line were procured from ATCC (American Type Culture Collection, USA). PUC19 plasmid was procured from Addgene, and E.Coli DH5 was procured from New England Biolabs, respectively.

1.1 General procedure A for the synthesis of intermediate 2-chloro-N-(4-(3-substitutedphenyl-1H-pyrazol-1-yl)phenyl)acetamides, 6a-q:

A mixture of appropriate pyrazole amine (**5a-q,** 1 equiv.) and triethylamine (1 equiv.) was stirred in adequate amount of dry methylene chloride at 0 °C for 30 min. To this 1.5 equiv. of chloroacetyl chloride was added dropwise. After stirring at 0 °C for 15 min and at room temperature for 2 h, the mixture was vacuum filtered and the resulting solids were washed with water and oven dried to furnish crude products **(6a-q)** which are used as such in the next step without any further purification.

1.2 General procedure B for the synthesis of final products, 7a-q:

A mixture of appropriate pyrazole acetamide **6a-q**, triethylamine (2 equiv.), and ciprofloxacin (1 equiv.) in acetonitrile (10 mL) was heated under reflux for 12-15 h. Upon completion, the reaction mixture was cooled to room temperature and crushed ice was added. The resultant solids were vacuum filtered washed with ice-cold water and oven dried. These crude solids were then purified by silica gel column chromatography using 10% methanol and DCM as the eluent.

1-Cyclopropyl-7-(4-(2-((4-(3-(3,4-dimethoxyphenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7a)

Light brown solid, yield 85%, mp: 200-202 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 9.95 (s, 1H), 8.67 (s, 1H), 8.47 (d, *J* = 2.5 Hz, 1H), 7.92 (d, *J* = 13.3 Hz, 1H), 7.86 (d, *J* = 9.1 Hz, 2H), 7.81 (d, *J* = 9.1 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 1.9 Hz, 1H), 7.45 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 2.5 Hz, 1H), 3.86 (s, 3H), 3.85 – 3.82 (m, 1H), 3.80 (s, 3H), 3.48 – 3.40 (m, 4H), 3.29 (s, 2H), 2.84 – 2.77 (m, 4H), 1.34 (q, *J* = 6.8 Hz, 2H), 1.23 – 1.17 (m, 2H);^{13}C NMR (125 MHz, DMSO-d_6) δ 176.33, 168.18, 165.93, 152.99 (d, *J* = 249.5 Hz), 151.69, 148.92, 148.87, 147.91, 145.15 (d, *J* = 10.0 Hz), 139.16, 136.65, 135.31, 128.87, 125.64, 120.29, 118.54 (d, *J* = 8.0 Hz), 118.47, 118.06, 111.89, 110.94 (d, *J* = 23.1 Hz), 109.03, 106.73, 106.26 (d, *J* = 3.2 Hz), 104.87, 61.44, 55.56, 55.52, 52.32, 49.33, 49.30, 35.84, 7.56; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{36}H_{36}FN_6O_6$ 667.2680; found 667.2649.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-((4-(3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-1-yl)phenyl)amino)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid(7b)

Beige solid, yield 85%, m.pt: 220-222 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 9.97 (s, 1H), 8.67 (s, 1H), 8.50 (d, *J* = 2.0 Hz, 1H), 7.95 – 7.90 (m, 1H), 7.87 (d, *J* = 8.9 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 6.4 Hz, 1H), 7.21 (s, 2H), 7.06 (d, *J* = 2.1 Hz, 1H), 3.88 (s, 6H), 3.85 (d, *J* = 7.0 Hz, 1H), 3.71 (s, 3H), 3.44 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.34 (q, *J* = 5.8 Hz, 2H), 1.23 – 1.17 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.78, 172.53, 168.69, 166.41, 153.66, 153.46 (d, *J* = 249.5 Hz), 152.12, 148.38, 145.63 (d, *J* = 9.8 Hz), 139.63, 138.00, 137.26, 135.70, 129.46, 128.91, 120.75, 119.06, 118.97, 111.41 (d, *J* = 23.4 Hz), 107.19, 106.72 (d, *J* = 3.4 Hz), 105.85, 103.32, 61.91, 60.57, 56.44, 52.80, 49.81, 49.77, 36.32, 8.03; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{37}H_{38}FN_6O_7$ 697.2786; found 697.2799.

1-Cyclopropyl-6-fluoro-7-(4-(2-((4-(3-(naphthalen-2-yl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7c)

Beige solid, yield 87%, m.pt: 225-227 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.22 (s, 1H), 10.01 (s, 1H), 8.66 (s, 1H), 8.56 (s, 1H), 8.45 (s, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 2H), 7.96 – 7.90 (m, 4H), 7.86 (s, 2H), 7.58 – 7.49 (m, 3H), 7.18 (s, 1H), 3.82 (s, 1H), 3.44 (s, 4H), 3.31 (s, 2H), 2.81 (s, 4H), 1.33 (s, 2H), 1.19 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.76, 168.72, 166.41, 153.44 (d, *J* = 250.4 Hz), 152.03, 148.29, 145.60 (d, *J* = 10.0 Hz), 139.59, 137.40, 135.72, 133.65, 133.16, 130.71, 129.72, 128.71, 128.50, 128.11, 126.94, 126.54, 124.37, 124.25, 120.79, 119.12, 111.39 (d, *J* = 22.9 Hz), 107.20, 106.67, 105.95, 61.91, 52.80, 49.80, 36.32, 8.03; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{38}H_{34}FN_6O_4$ 657.2626; found 657.2604

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-((4-(3-phenyl-1*H*-pyrazol-1-yl)phenyl)amino)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid(7d)

Grey solid, yield 94%, m.pt: 200-202 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.20 (s, 1H), 9.96 (s, 1H), 8.64 (s, 1H), 8.49 (s, 1H), 7.97 – 7.78 (m, 7H), 7.56 (s, 1H), 7.45 (t, *J* = 4.9 Hz, 2H), 7.39 – 7.31 (m, 1H), 7.01 (s, 1H), 3.81 (s, 1H), 3.42 (s, 4H), 3.28 (s, 2H), 2.79 (s, 4H), 1.32 (s, 2H), 1.18 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.32, 168.26, 165.97, 153.02 (d, *J* = 249.6 Hz), 151.61, 147.92, 145.19 (d, *J* = 9.8 Hz), 139.17, 136.86, 135.27, 132.76, 129.11, 128.75, 128.01, 125.43, 120.31, 118.61, 118.52, 110.95 (d, *J* = 23.5 Hz), 106.73, 106.28, 105.19, 61.48, 52.36, 49.36, 49.33, 48.64, 35.88, 7.59; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{34}H_{32}FN_6O_4$ 607.2469; found 607.2426.

1-Cyclopropyl-6-fluoro-7-(4-(2-((4-(3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7e)

White solid; yield 90%, m.pt: 207-209 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 9.95 (s, 1H), 8.67 (s, 1H), 8.46 (d, *J* = 2.5 Hz, 1H), 7.92 (d, *J* = 13.3 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 4H), 7.80 (d, *J* = 8.9 Hz, 2H), 7.59 (d, *J* = 7.1 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.94 (d, *J* = 2.5 Hz, 1H), 3.83 (s, 1H), 3.81 (s, 3H), 3.44 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.34 (d, *J* = 6.3 Hz, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.83, 168.78, 166.60, 159.65, 154.48, 153.49 (d, *J* = 249.5 Hz), 152.04, 148.44, 145.68 (d, *J* = 9.8 Hz), 139.67, 137.01, 135.83, 129.40, 127.24, 125.80, 120.88, 118.95, 114.60, 111.42 (d, *J* = 23.0 Hz) , 107.13, 106.74 (d, *J* = 3.0 Hz), 105.15, 61.85, 55.61, 52.76, 49.72, 36.33, 8.02; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{35}H_{34}FN_6O_5$ 637.2574; found 637.2518.

1-Cyclopropyl-6-fluoro-7-(4-(2-((4-(3-(3-methoxyphenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7f)

Light brown solid, yield 87%, m.pt: 213-215 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 9.98 (s, 1H), 8.67 (s, 1H), 8.51 (d, J = 2.2 Hz, 1H), 7.91 (d, J = 13.2 Hz, 1H), 7.87 (d, J = 9.0 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 7.59 (d, J = 6.7 Hz, 1H), 7.51 (d, J = 7.7 Hz, 1H), 7.47 (s, 1H), 7.37 (t, J = 7.9 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.93 (dd, J = 8.1, 2.2 Hz, 1H), 3.84 (s, 4H), 3.44 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.34 (q, J = 6.4 Hz, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.78, 168.69, 166.40, 160.09, 153.46 (d, J = 249.8 Hz), 151.94, 148.35, 145.62 (d, J = 9.9 Hz), 139.62, 137.33, 135.69, 134.59, 130.28, 129.51, 120.75, 119.09, 118.33, 114.05, 111.40 (d, J = 23.3 Hz), 111.21, 107.20, 106.71 (d, J = 2.2 Hz), 105.86, 61.92, 55.60, 52.80, 49.81, 49.78, 36.31, 8.03; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{35}H_{34}FN_6O_5$ 637.2574; found 637.2536.

1-Cyclopropyl-6-fluoro-7-(4-(2-((4-(3-(2-methoxyphenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7g)

Cream solid, yield 93%, m.pt: 223-225 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.22 (s, 1H), 9.95 (s, 1H), 8.65 (s, 1H), 8.46 (d, J = 2.2 Hz, 1H), 8.01 (dd, J = 7.6, 1.5 Hz, 1H), 7.89 (d, J = 13.6 Hz, 1H), 7.87 (d, J = 9.4 Hz, 2H), 7.81 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 6.6 Hz, 1H), 7.38 – 7.32 (m, 1H), 7.13 (d, J = 8.3 Hz, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 1H), 3.43 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.36 – 1.30 (m, 2H), 1.19 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.36, 168.23, 165.98, 156.68, 153.03 (d, J = 249.7 Hz), 148.68, 147.96, 145.23, 145.15, 139.20, 136.76, 135.32, 129.25, 127.97 (d, J = 8.2 Hz), 121.28, 120.55, 120.32, 118.59, 111.95, 110.97 (d, J = 23.3 Hz), 108.97, 106.74, 106.31, 61.46, 55.51, 52.35, 49.32, 35.88, 7.59; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{35}H_{34}FN_6O_5$ 637.2574; found 637.2543.

7-(4-(2-((4-(3-(4-Chlorophenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7h)

White solid, yield 92%, m.pt: 237-239 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 9.97 (s, 1H), 8.67 (s, 1H), 8.52 (s, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.91 (d, J = 13.3 Hz, 1H), 7.87 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 5.4 Hz, 1H), 7.52 (d, J = 8.3 Hz, 2H), 7.06 (d, J = 1.6 Hz, 1H), 3.84 (s, 1H), 3.44 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.33 (d, J = 5.0 Hz, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.75, 168.69, 166.38, 153.43 (d, J = 249.4 Hz), 150.92, 148.28, 145.59 (d, J = 10.1 Hz), 139.58, 137.42, 135.60,

132.92, 132.09, 129.76, 129.21, 127.54, 120.75, 119.13, 118.98 (d, *J* = 7.6 Hz),, 111.38 (d, *J* = 23.2 Hz), 107.20, 106.64 (d, *J* = 2.8 Hz), 105.77, 61.91, 52.80, 49.79, 49.76, 36.30, 8.03; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{34}H_{31}ClFN_6O_4$ 641.2079; found 641.2026.

7-(4-(2-((4-(3-(3-Chlorophenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7i)

Grey solid, yield 93%, m.pt: 223-225 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 10.00 (s, 1H), 8.67 (s, 1H), 8.54 (s, 1H), 7.98 (s, 1H), 7.92 (d, *J* = 6.4 Hz, 1H), 7.89 (d, *J* = 9.5 Hz, 3H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.59 (s, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.13 (s, 1H), 3.84 (s, 1H), 3.44 (s, 4H), 3.30 (s, 2H), 2.80 (s, 4H), 1.34 (d, *J* = 4.7 Hz, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.80, 168.70, 166.40, 153.46 (d, *J* = 249.6 Hz), 150.64, 148.36, 145.62 (d, *J* = 10.4 Hz), 139.63, 137.51, 135.56, 135.34, 134.10, 131.13, 129.85, 128.18, 125.39, 124.44, 120.76, 119.22, 119.01 (d, *J* = 8.2 Hz), 111.41 (d, *J* = 23.3 Hz), 107.20, 106.72, 106.04, 61.90, 52.79, 49.79, 49.77, 36.32, 8.04; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{34}H_{31}ClFN_6O_4$ 641.2079; found 641.2025.

7-(4-(2-((4-(3-(2-Chlorophenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7j)

Light brown solid, yield 92%, m.pt: 233-235 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.24 (s, 1H), 9.98 (s, 1H), 8.68 (s, 1H), 8.55 (s, 1H), 7.93 (d, *J* = 13.4 Hz, 1H), 7.91 – 7.80 (m, 5H), 7.63 – 7.54 (m, 2H), 7.49 – 7.39 (m, 2H), 6.99 (s, 1H), 3.84 (s, 1H), 3.44 (s, 4H), 3.30 (s, 2H), 2.80 (s, 4H), 1.34 (d, *J* = 5.0 Hz, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.81, 168.74, 166.42, 153.48 (d, *J* = 249.6 Hz), 149.78, 148.41, 145.64 (d, *J* = 10.2 Hz), 139.65, 137.55, 135.54, 132.05, 131.68, 131.13, 130.78, 130.06, 128.83, 127.83, 120.77, 119.33, 119.02 (d, *J* = 7.6 Hz), 111.42 (d, *J* = 23.0 Hz), 109.06, 107.20, 106.76, 61.92, 52.79, 49.78, 36.34, 8.04; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{34}H_{31}ClFN_6O_4$ 641.2079; found 641.2023.

1-Cyclopropyl-6-fluoro-7-(4-(2-((4-(3-(4-fluorophenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7k)

Beige solid, yield 93%, m.pt: 206-208 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.22 (s, 1H), 9.97 (s, 1H), 8.65 (s, 1H), 8.50 (s, 1H), 7.96 (s, 2H), 7.85 (dd, *J* = 26.4, 11.8 Hz, 5H), 7.57 (s, 1H), 7.33 – 7.24 (m, 2H), 7.02 (s, 1H), 3.82 (s, 1H), 3.43 (s, 4H), 3.30 (s, 2H), 2.81 (s, 4H), 1.34 (s, 2H), 1.19 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.72, 168.67, 166.38, 163.42, 161.48, 153.43 (d, *J* = 249.5 Hz), 151.17, 148.25, 145.56 (d, *J* = 15.9 Hz), 139.56, 137.32, 135.66, 129.79 (d, *J* = 2.0 Hz), 129.63, 127.86 (d, *J* = 8.0 Hz), 120.75, 119.05, 118.96 (d, *J* = 7.9 Hz), 116.14, 115.97, 111.36 (d, *J* = 22.6 Hz), 107.19, 106.61, 105.54, 61.91, 52.80, 49.77,

49.75, 36.28, 8.02; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{34}H_{31}F_2N_6O_4$ 625.2374; found 625.2370.

7-(4-(2-((4-(3-(4-Bromophenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7l)

Beige solid, yield 85%, m.pt: 220-223 °C; ^{1}H NMR (500 MHz, DMSO-*d*6) δ 15.24 (s, 1H), 9.97 (s, 1H), 8.68 (s, 1H), 8.53 (s, 1H), 7.93 (d, *J* = 13.2 Hz, 1H), 7.91 – 7.85 (m, 4H), 7.83 (s, 2H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.61 (s, 1H), 7.07 (s, 1H), 3.85 (s, 1H), 3.41 (d, *J* = 31.4 Hz, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.33 (s, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 176.82, 168.75, 166.46, 153.49 (d, *J* = 249.9 Hz), 150.99, 148.43, 145.61, 139.67, 137.42, 135.61, 132.45, 132.15, 129.83, 127.87, 121.52, 120.80, 119.18, 119.02 (d, *J* = 7.7 Hz), 111.44 (d, *J* = 23.2 Hz), 107.19, 106.77 (d, *J* = 2.5 Hz), 105.80, 61.90, 52.79, 49.81, 49.78, 36.35, 8.04; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{34}H_{31}BrFN_6O_4$ 685.1574; found 685.1544.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-((4-(3-(p-tolyl)-1*H*-pyrazol-1-yl)phenyl)amino)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid(7m)

White solid, yield 88%, m.pt: 223-225 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.28 (s, 1H), 10.01 (s, 1H), 8.72 (s, 1H), 8.54 (d, *J* = 2.5 Hz, 1H), 7.97 (d, *J* = 13.3 Hz, 1H), 7.92 (d, *J* = 9.1 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 4H), 7.65 (d, *J* = 7.2 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 2H), 7.03 (d, *J* = 2.5 Hz, 1H), 3.89 (s, 1H), 3.50 (s, 4H), 3.35 (s, 2H), 2.86 (s, 4H), 2.41 (s, 3H), 1.39 (d, *J* = 6.3 Hz, 2H), 1.26 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.76, 168.65, 166.40, 153.45 (d, *J* = 249.7 Hz), 152.11, 148.33, 145.61 (d, *J* = 10.1 Hz), 139.61, 137.74, 137.19, 135.77, 130.45, 129.74, 129.40, 125.79, 120.77, 118.99, 111.39 (d, *J* = 23.1 Hz), 107.20, 106.69 (d, *J* = 2.6 Hz), 105.39, 61.89, 52.79, 49.78, 49.75, 36.31, 21.32, 8.03; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{35}H_{34}FN_6O_4$ 621.2625; found 621.2576.

1-Cyclopropyl-7-(4-(2-((4-(3-(2,4-dichlorophenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7n)

White solid, yield 90%, m.pt: 217-219 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.24 (s, 1H), 9.99 (s, 1H), 8.67 (s, 1H), 8.57 (s, 1H), 7.97 – 7.92 (m, 1H), 7.91 (s, 1H), 7.85 (dd, *J* = 23.0, 8.2 Hz, 4H), 7.75 (d, *J* = 1.8 Hz, 1H), 7.59 (s, 1H), 7.54 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H), 3.84 (s, 1H), 3.44 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.33 (s, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.76, 168.73, 166.38, 153.44 (d, *J* = 249.7 Hz), 148.70, 148.31, 145.60 (d, *J* = 10.1 Hz), 139.59, 137.67, 135.43, 133.62, 132.48, 132.16, 130.98, 130.20, 129.04, 128.06, 120.76, 119.39, 119.02, 118.95, 118.99 (d, *J* = 7.7 Hz), 111.39 (d, *J*

= 23.0 Hz), 109.03, 107.21, 106.67, 61.92, 52.80, 49.80, 49.77, 36.31, 8.04; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{34}H_{30}Cl_2FN_6O_4$ 675.1689; found 675.1677.

1-Cyclopropyl-6-fluoro-7-(4-(2-((4-(3-(4-fluoro-3-methylphenyl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7o)

Cream solid, yield 93%, m.pt: 201-203 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.24 (s, 1H), 9.96 (s, 1H), 8.68 (s, 1H), 8.50 (d, *J* = 2.5 Hz, 1H), 7.93 (d, *J* = 13.3 Hz, 1H), 7.89 – 7.80 (m, 5H), 7.79 – 7.74 (m, 1H), 7.60 (d, *J* = 7.3 Hz, 1H), 7.21 (t, *J* = 9.1 Hz, 1H), 7.00 (d, *J* = 2.5 Hz, 1H), 3.84 (s, 1H), 3.44 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 2.32 (s, 3H), 1.34 (d, *J* = 6.3 Hz, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.84, 168.70, 166.43, 161.02 (d, *J* = 243.7 Hz), 153.50 (d, *J* = 249.7 Hz), 151.32, 148.46, 145.65 (d, *J* = 10.1 Hz), 139.68, 137.30, 135.69, 129.61, 129.47 (d, *J* = 3.3 Hz), 129.05 (d, *J* = 5.1 Hz), 125.24 (d, *J* = 8.2 Hz), 125.09, 124.95, 120.77, 119.07, 115.71 (d, *J* = 22.5 Hz), 111.45 (d, *J* = 23.0 Hz), 107.20, 106.80 (d, *J* = 2.9 Hz), 105.54, 61.91, 52.79, 49.82, 49.78, 36.34, 14.70, 14.68, 8.04; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{35}H_{33}F_2N_6O_4$ 639.2531; found 639.2508.

7-(4-(2-((4-(3-(Benzo[d][1,3]dioxol-5-yl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7p)

Beige solid, yield 88%, m.pt: 215-217 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 9.95 (s, 1H), 8.67 (s, 1H), 8.46 (d, *J* = 2.4 Hz, 1H), 7.92 (d, *J* = 13.3 Hz, 1H), 7.83 (dd, *J* = 23.2, 8.9 Hz, 4H), 7.60 (d, *J* = 7.2 Hz, 1H), 7.47 (d, *J* = 1.4 Hz, 1H), 7.44 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 6.07 (s, 2H), 3.84 (s, 1H), 3.44 (s, 4H), 3.29 (s, 2H), 2.80 (s, 4H), 1.34 (d, *J* = 6.4 Hz, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.74, 168.66, 166.39, 153.43 (d, *J* = 249.6 Hz), 151.87, 148.26, 148.18, 147.57, 145.59 (d, *J* = 10.0 Hz), 139.56, 137.15, 135.71, 129.37, 127.49, 120.74, 119.65, 118.92, 111.36 (d, *J* = 23.2 Hz), 108.97, 107.18, 106.63, 106.18, 105.34, 101.58, 61.92, 52.80, 49.78, 49.75, 36.28, 8.02; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{35}H_{32}FN_6O_6$ 651.2367; found 651.2313.

7-(4-(2-((4-(3-(Benzofuran-2-yl)-1*H*-pyrazol-1-yl)phenyl)amino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(7q)

Brown solid, yield 91%, m.pt: 221-223 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 10.01 (s, 1H), 8.66 (s, 1H), 8.60 (s, 1H), 7.92 (s, 1H), 7.89 (d, *J* = 6.3 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.68 (dd, *J* = 11.9, 8.0 Hz, 2H), 7.59 (s, 1H), 7.35 (t, *J* = 7.1 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 1H), 7.06 (d, *J* = 1.8 Hz, 1H), 3.86 (d, *J* = 22.9 Hz, 1H), 3.44 (s, 4H), 3.30 (s, 2H),

2.80 (s, 4H), 1.33 (s, 2H), 1.20 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.83, 168.77, 166.43, 154.58, 153.49 (d, J = 249.3 Hz), 150.59, 148.45, 145.65 (d, J = 10.2 Hz), 144.18, 139.67, 137.72, 135.40, 129.80, 128.88, 125.14, 123.77, 121.70, 120.78, 119.43, 119.04 (d, J = 7.7 Hz), 111.67, 111.44 (d, J = 23.0 Hz),107.20, 106.79 (d, J = 2.9 Hz), 106.70, 103.40, 61.92, 52.79, 49.79, 36.35, 8.05; HRMS-QTOF (ESI): m/z calcd. for $[M+H]^+$ $C_{36}H_{32}FN_6O_5$ 647.2418; found 647.2357.

1.3 *In vitro* determination of anti-bacterial activity

The in-vitro assay to determine the minimum inhibitory concentration (MIC) of the test molecules was performed in 96-well round bottom microtitre plates (Corning, USA). Briefly, two-fold serial dilutions of molecules were prepared in cation-adjusted Muller Hinton Broth at a volume of 100 µl per well. The suspensions of OD_{600}= 0.08-0.1 from overnight grown cultures of *Staphylococcus aureus* ATCC 29213, and *P. aeruginosa* PAO1 ATCC 15692 grown on Muller Hinton Agar (Difco Laboratories, Detroit, MI, USA) were made and diluted to hundred times. A 100 µl of diluted suspension was dispensed into each well of the 96-well round plate, and the assay plates were incubated at 37 °C for 18-24 hrs. After incubation, the plates were read visually, and the minimum concentration of the compound showing no turbidity was recorded as MIC[1].

1.4 Generation of one step ciprofloxacin-resistant mutants of *S. aureus*

The bacterial cells from log phase were harvested and the inoculum was adjusted to 10^{10} CFU/ml. The mutants were selected by plating 100 ul of the adjusted inoculum on ciprofloxacin containing plates of Mueller-Hinton agar medium. After incubation of 48 hrs at 37°C, the colonies were picked and passaged on ciprofloxacin containing plates. The mutants obtained were tested susceptibility for ciprofloxacin using liquid broth microdilution assay. Those mutants which showed more than eight fold increase in the MIC values for ciprofloxacin were selected for evaluation of proposed gyrase inhibitors.[2]

1.5 *In vitro* determination of anti-tubercular activity

The in-vitro assay to determine the MIC of the molecules was performed in 96-well round bottom microtiter plates (Corning, USA). The liquid broth micro-dilution method was used to determine the MIC of the molecules.[3] The mycobacterial suspension grown in 7H9 liquid broth medium supplemented with D-pantothenic acid (24 mg/L), L-leucine (50 mg/L), L-methionine (50 mg/L), L-arginine (200 mg/L), 0.2% glycerol, 0.05% Tween 80, and 10% Album-Dextrose-Saline (ADS) 7H9 liquid broth medium supplemented with D-pantothenic acid (24 mg/L), L-leucine (50 mg/L), L-methionine (50 mg/L), L-arginine (200 mg/L), 0.2% glycerol, 0.05% Tween 80, and 10% Album-Dextrose-Saline (ADS), after reaching the log

phase (i.e OD_{600} range of 0.4-0.6) which is equivalent to 1.0×10^8 CFU/ml was diluted OD_{600} of 0.005 (1.0×10^6 CFU/ml. The test compounds were two-fold serially diluted in 100 µl of 7H9 liquid broth media in each well. A volume of 100 µl of *Mycobacterium tuberculosis* (*Mtb)* H37Ra ATCC 25177 culture (OD600 0.005) was dispensed into each well of 96-well round plate and the assay plates were incubated at 37 °C for 10 days in 5% CO_2 . After incubation, the plates were read visually and the minimum concentration of the compound showing no turbidity was recorded as MIC.

1.6 Cytotoxicity

Potent compounds were examined for their cytotoxicity against Hep-G2 cell line (a human liver cancer cell line) using MTT assay. Metabolically active cells reduce MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) into insoluble formazan, which is measured as the level of absorbance denoting the viability of cells. Initially, cells were seeded in 96-well plates with the medium at a density of 1000-4000 per well in 100 µl and incubated overnight to grow and adhere. Then, the old media was replaced with fresh media, different concentrations of compounds ranging from 1000-0.78 µg/mL were added and incubated for 48 h. The treatment media was removed, and 100 µl of MTT (0.5 mg/mL) was added and incubated at 37 ^oC for 4 h. Then the supernatant was removed, and formazan crystals formed were solubilized by the addition of 100 µL of DMSO. The quantity of formazan was measured using a multimode plate reader (Perkin Elmer, USA) at 570 nM wavelength

1.7 Biofilm Inhibition

The active molecules were tested for biofilm inhibition activity against *Staphylococcus aureus* ATCC 29213. Briefly, the suspension of 0.015 at OD_{600} was prepared from overnight-grown cultures. Twelve twofold serial dilutions of test actives ranging from 128 to 0.06 µg/ml were prepared in 100 µl of Mueller Hinton Broth (MHB)supplemented with 2% sucrose in each well of 96-well flat-bottom polystyrene tissue culture plate (Tarsons, Mumbai, India), followed by the addition of 100µl of the above-mentioned suspension to each well of the plate. After incubation at 37°C in for 24 hrs, the supernatant from each well was decanted, and planktonic cells were removed by washing the wells with phosphate-buffered saline (PBS; pH 7.4). The biofilm was fixed with methanol for 15 min and then air-dried at room temperature. The wells of the dried plate were stained with 0.1% (wt/vol) crystal violet for 10 min and rinsed thoroughly with water until the negative control wells (without biofilms) appeared colourless. Biofilm formation was quantified by the addition of 200 µl of 95% ethanol to the crystal violet-stained wells, and the absorbance was recorded at

595 nm (A_{595}) using a microplate reader. The percentage of inhibition was calculated using the equation (1 – A_{595} of the test/A_{595} of non-treated control) × 100. Culture without the agents was used as the no-treatment control. The minimum biofilm inhibition concentration ($MBIC_{50}$) was defined as the lowest agent concentration that showed 50% or more inhibition on the formation of biofilm[4].

1.8 Characterization of the Supercoiling Activity of Gyrase

pUC19 containing DH5α strain of *E. coli* was grown to saturation by incubating at 37°C in LB media supplemented with 50 µg/mL of ampicillin. The overnight grown cells were pelleted down at 6000 rpm for 10 min and re-suspended in fresh LB media to an OD_{600} of 2.0. The adjusted suspensions were treated with DMSO, Ciprofloxacin (0.125 µg/ml i.e. MIC) and test compounds **(7a, 7d, & 7g)** at 0.25 µg/ml (2X MIC) and 1 µg/ml (8X MIC) followed by incubation at 37°C with 200 rpm for 90 minutes. For determining the supercoiling states of pUC19 DNA after treatment, cells were harvested and pUC19 was extracted from all the treated samples using a Qiagen QIAprep spin miniprep kit (Qiagen). The extracted plasmids were quantified using nanodrop, and 300 ng of plasmid from each sample was separated by electrophoresis in 0.8% agarose at 80 V for 30 min for determination of topological states.[5]

1.9 Molecular docking studies

Molecular docking studies were performed using the glide docking module of Schrödinger suite release 2021.[6] The structure coordinates of DNA gyrase in complex with ciprofloxacin (PDB: 2XCT) were obtained from the RCSB.[7] The protein was prepared using the Protein Preparation Wizard of the Schrödinger suite by adding hydrogens, bond orders were assigned, and water molecules were removed within 5 Å around the co-crystal. The protein structure was minimized using the OPLS-3e force field. The grid is generated by picking the active site where the co-crystal is located and by keeping the grid box of size 12 Å from the centroid to cover the entire vicinity of the active site. The generated grid is validated by re-docking of co-crystal to an RMSD of 0.92. Compound **7a, 7d, and 7g** were drawn and prepared using LigPrep, and was docked at the active site of gyrase using the GLIDE module[8].

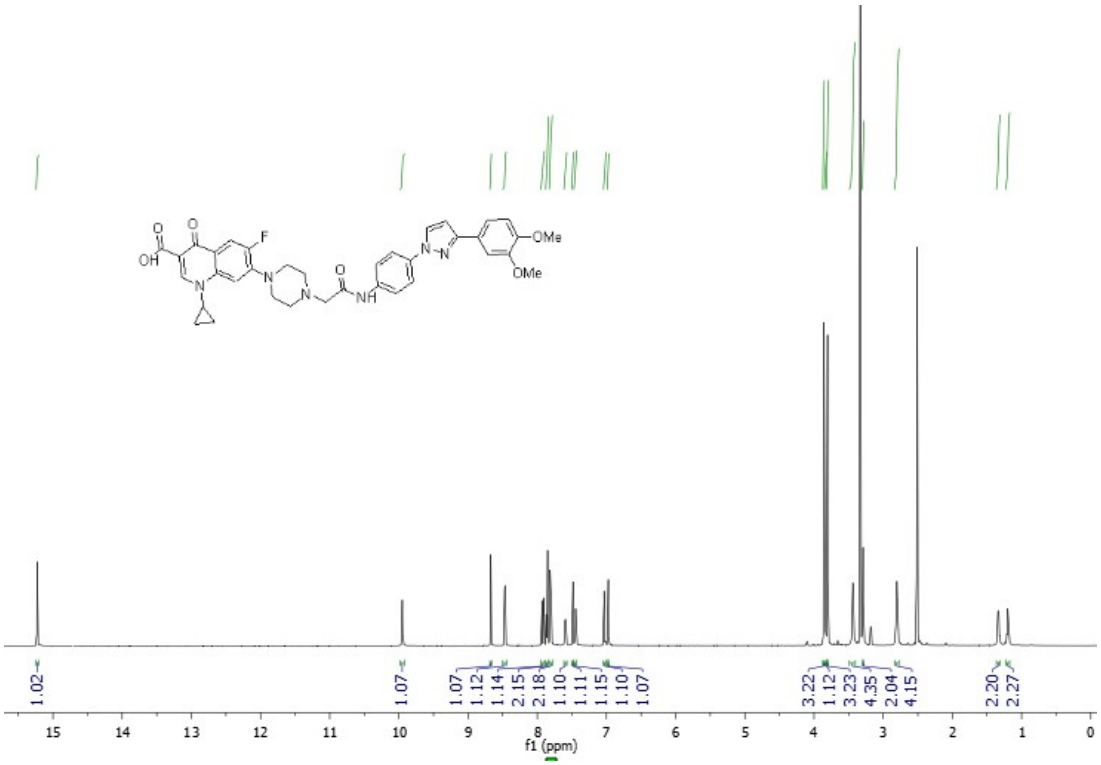
1.10 Time Kill Assay

The assay was performed for active molecules *i.e.*, **7a, 7d**, and **7g** against *Staphylococcus aureus* ATCC 29213. The log phase cultures of both bacteria were adjusted to an OD_{600} of 0.005 and incubated with respective molecules at concentrations equal to MIC, 4X MIC, and

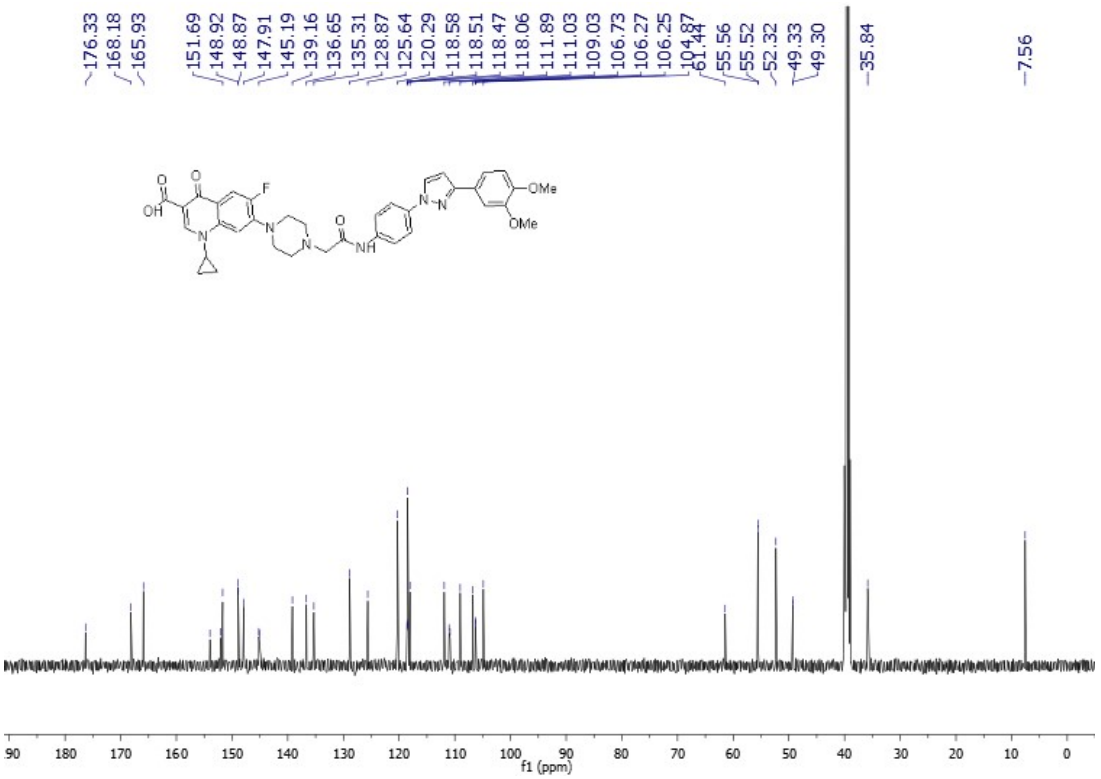
8X MIC at 37 °C. The viable bacterial count was determined in the form of CFU/ml by plating 10-fold serial dilutions for each sample on agar plates at regular intervals of time *i.e.,* 2, 4, 6, 8, 10, and 24 hrs. Killing curves were constructed by plotting the $\log_{10}$ CFU/ml versus time over 24 hrs[9].

2 ^{1}H, ^{13}C and HRMS spectra of final compounds, **7a-q**

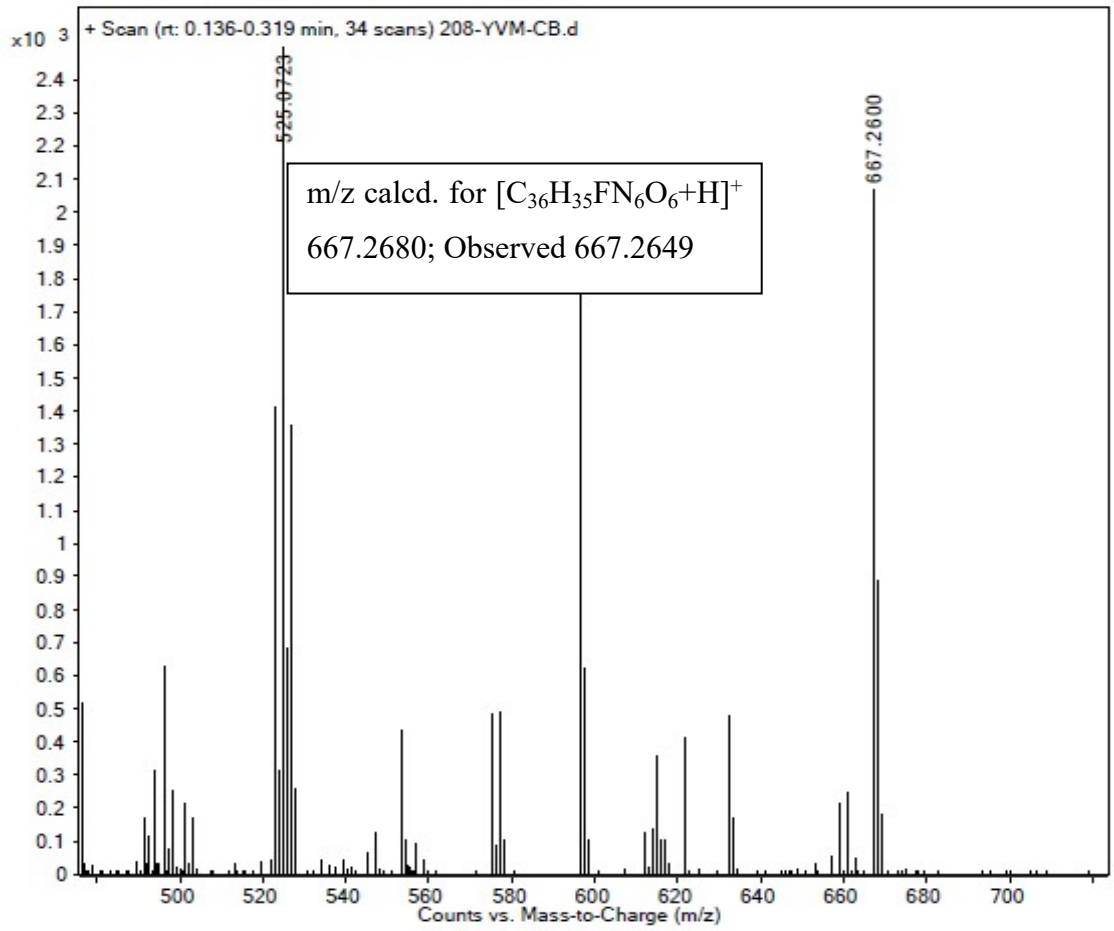
^{1}H, and ^{13}C spectra of final compounds, **7a-q**



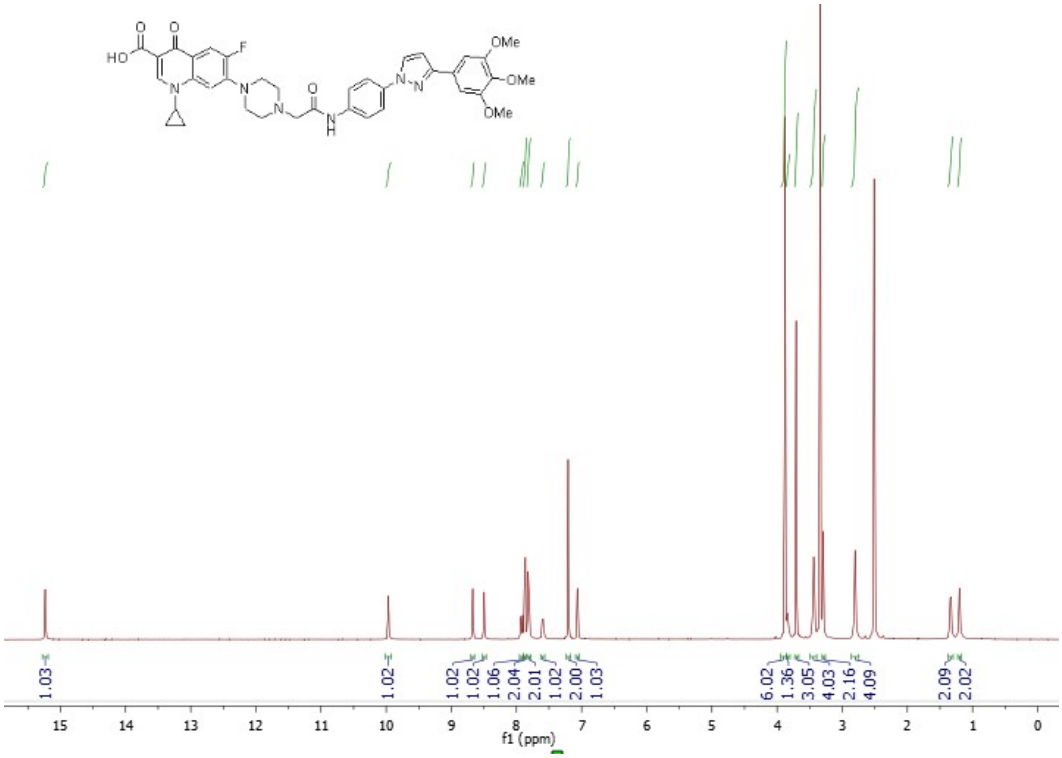
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7a**



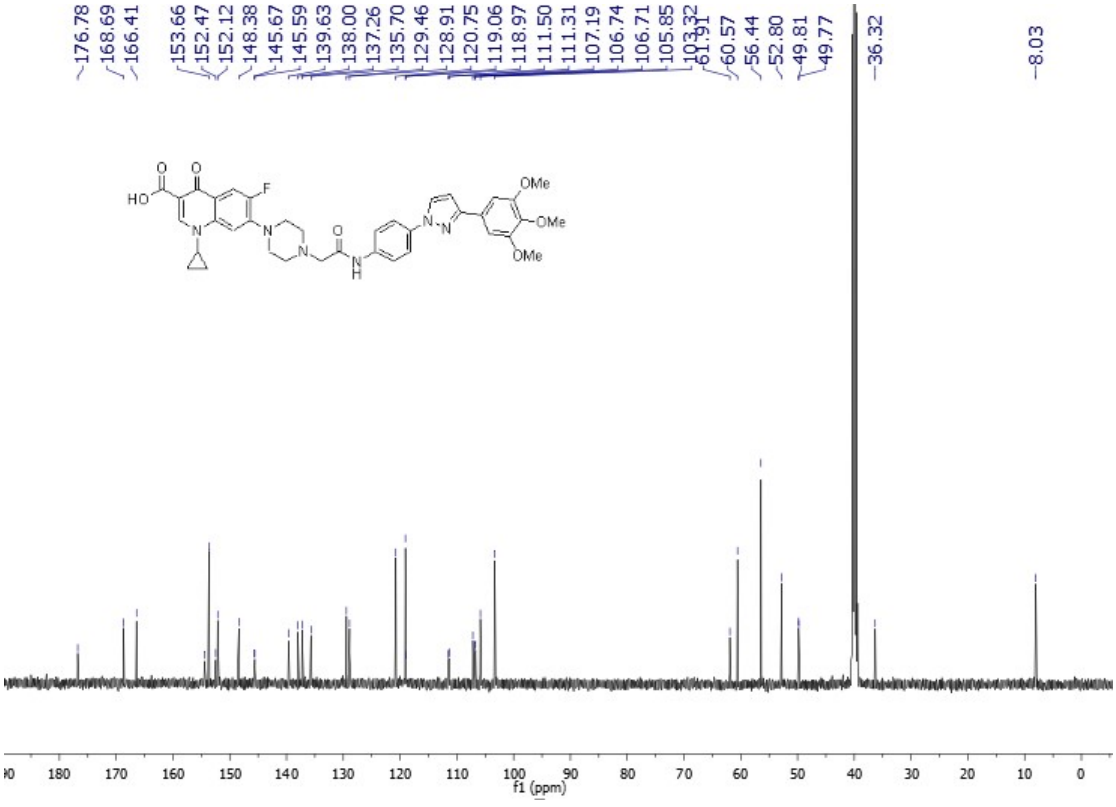
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7a**



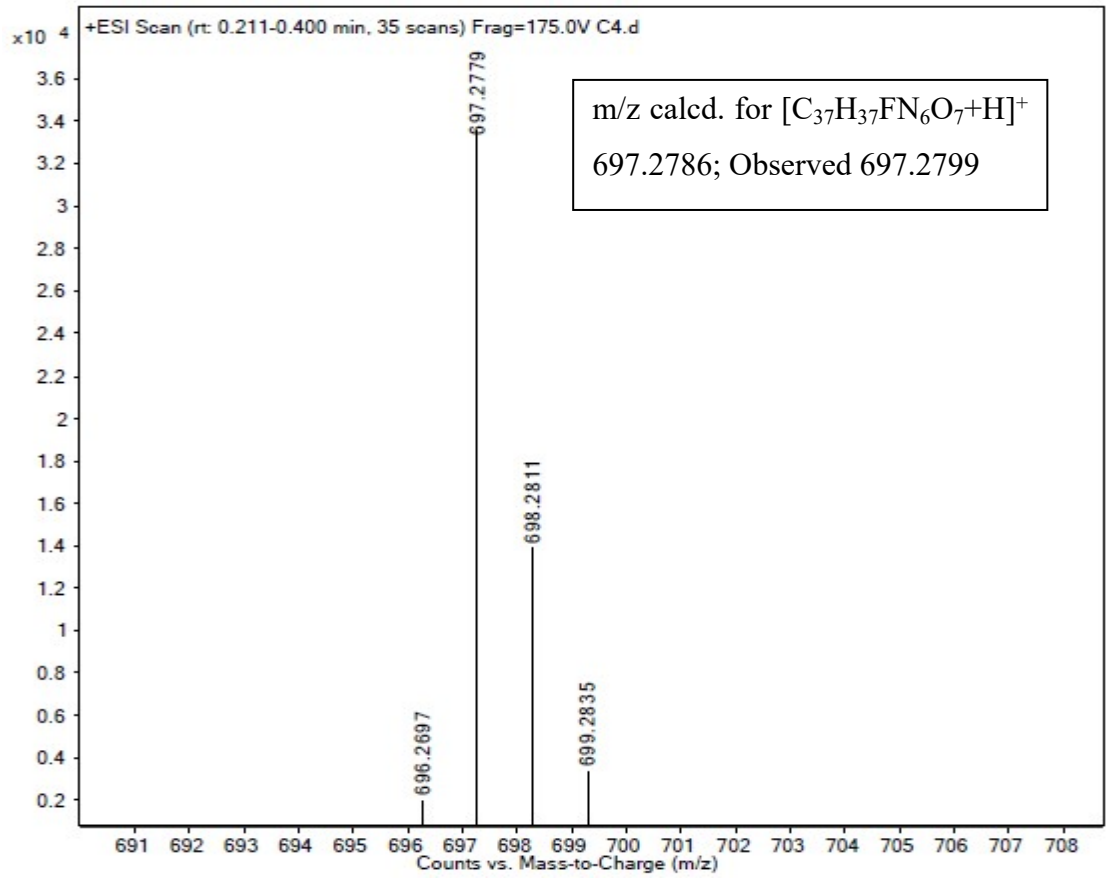
Mass spectrum of compound **7a**



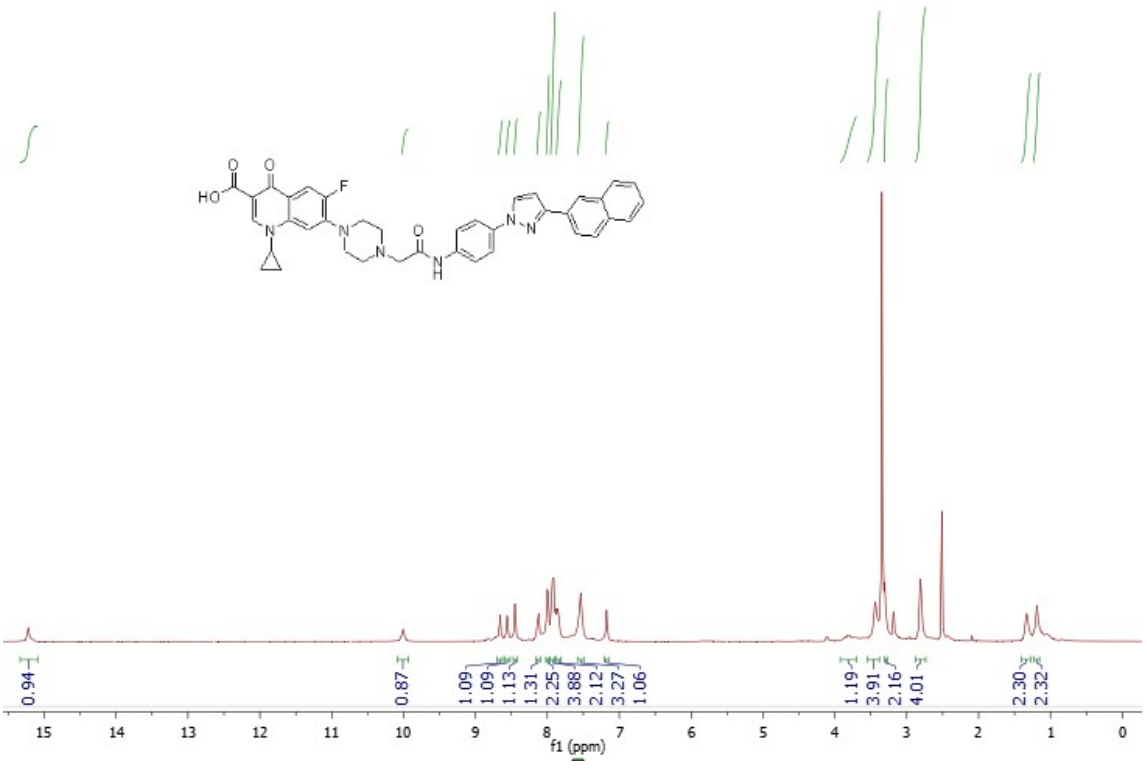
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7b**



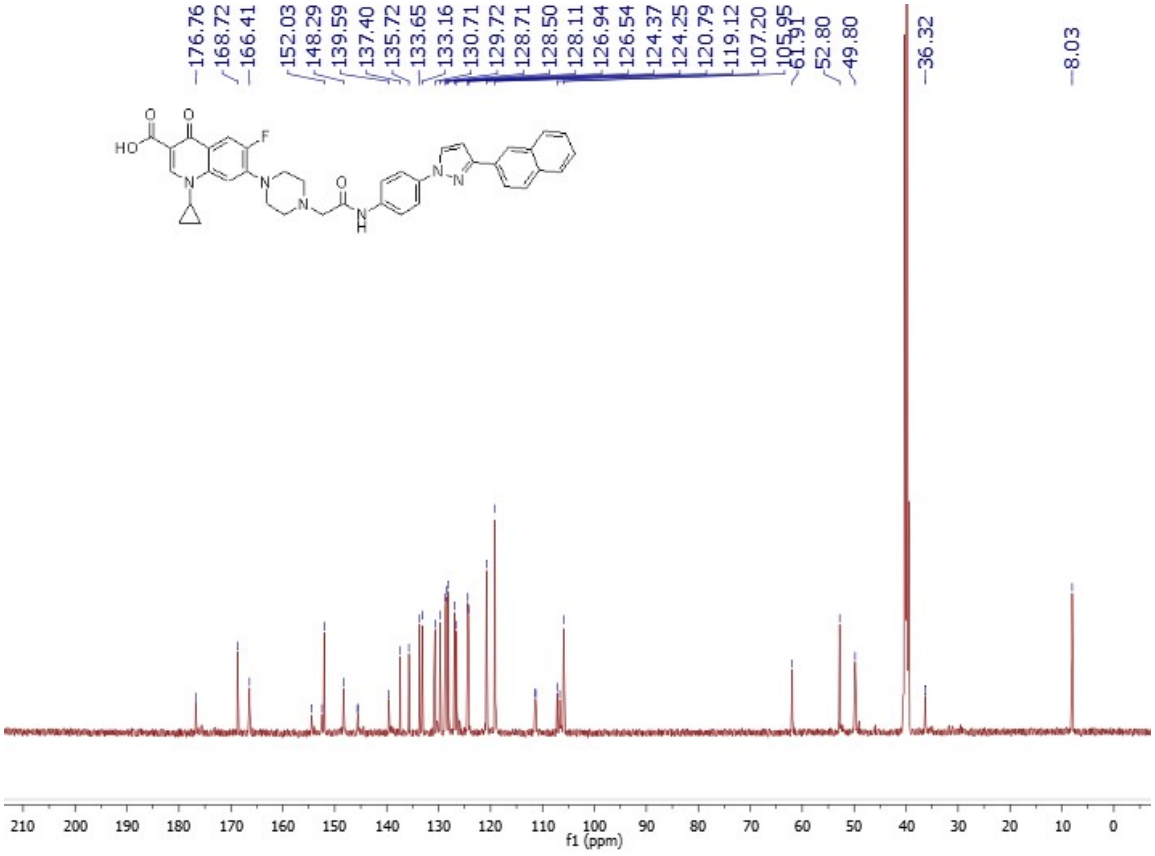
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7b**



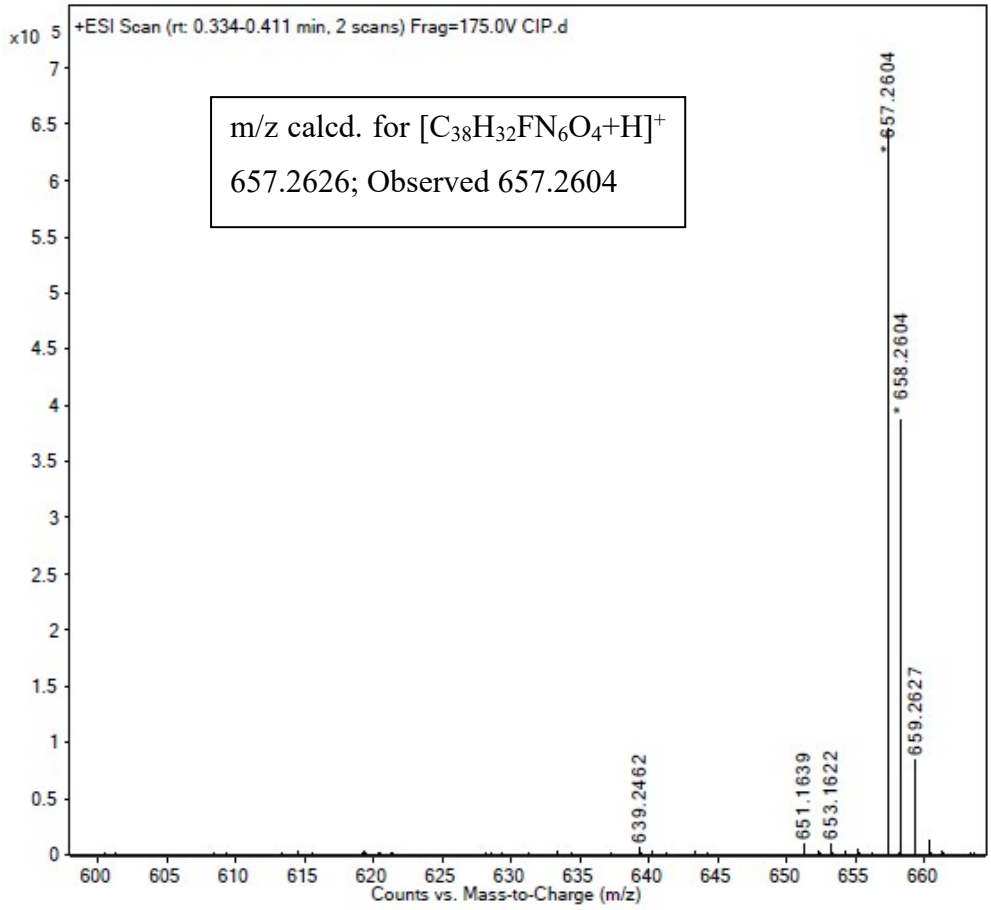
Mass spectrum of compound **7b**



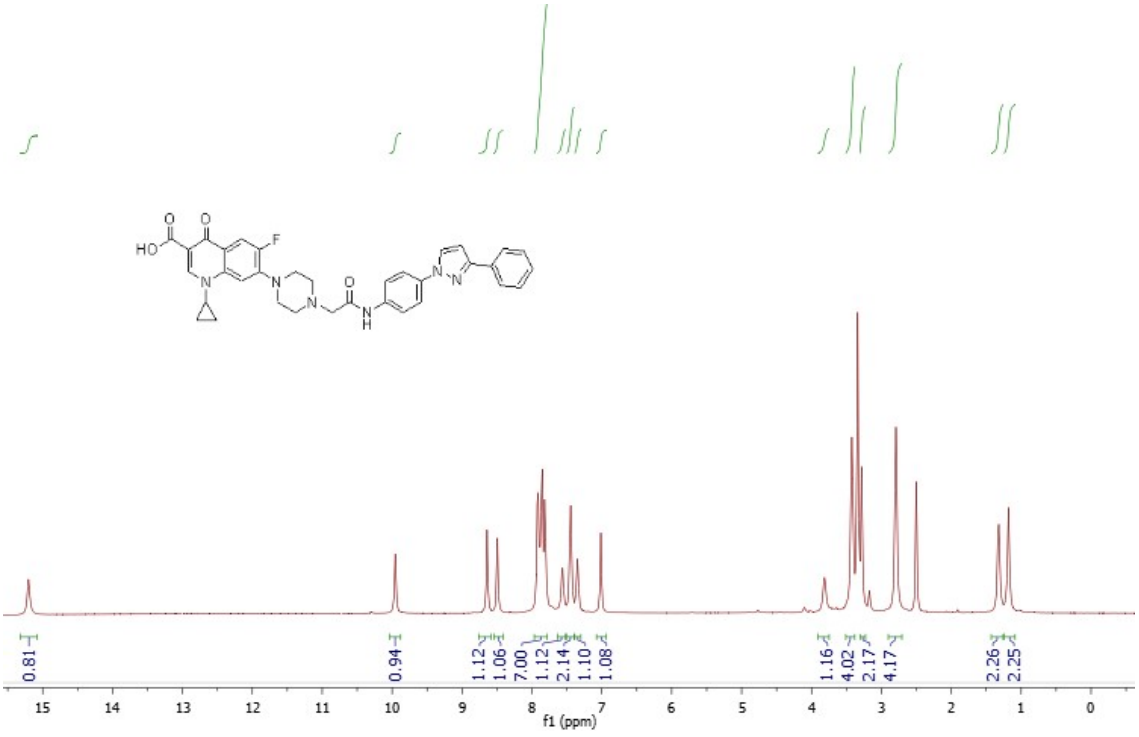
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7c**



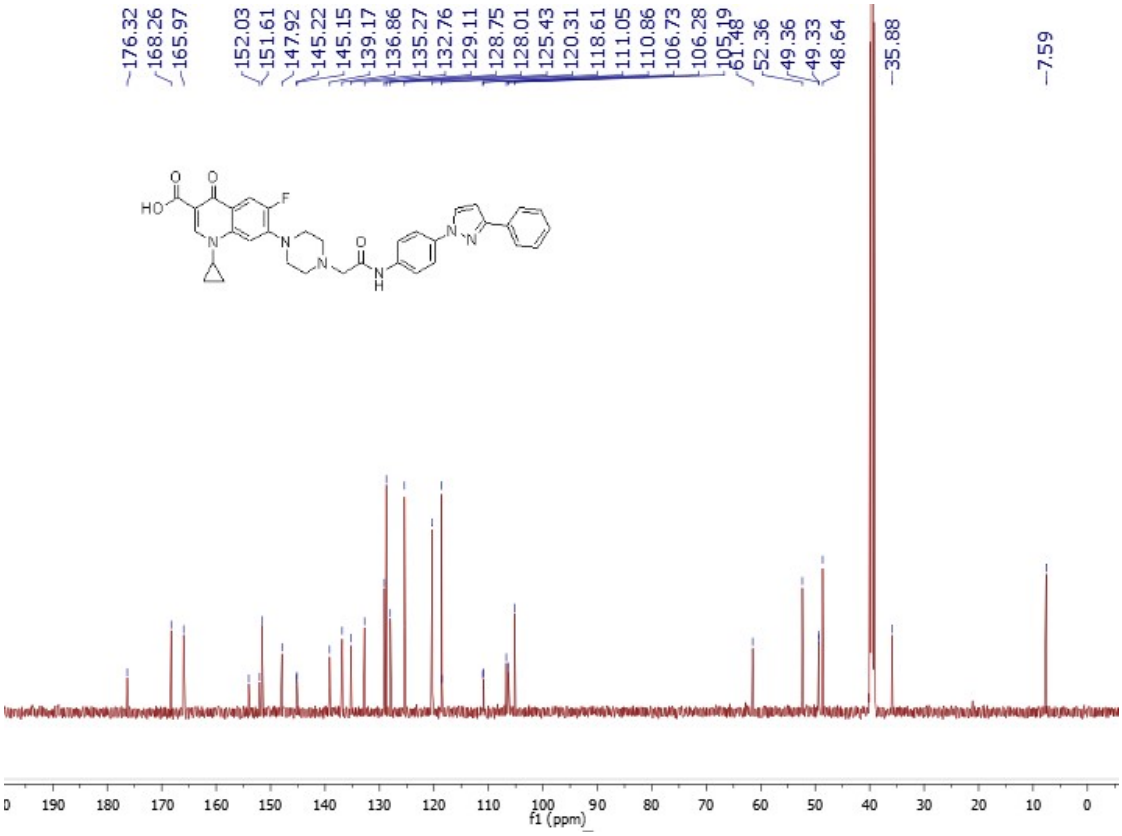
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7c**



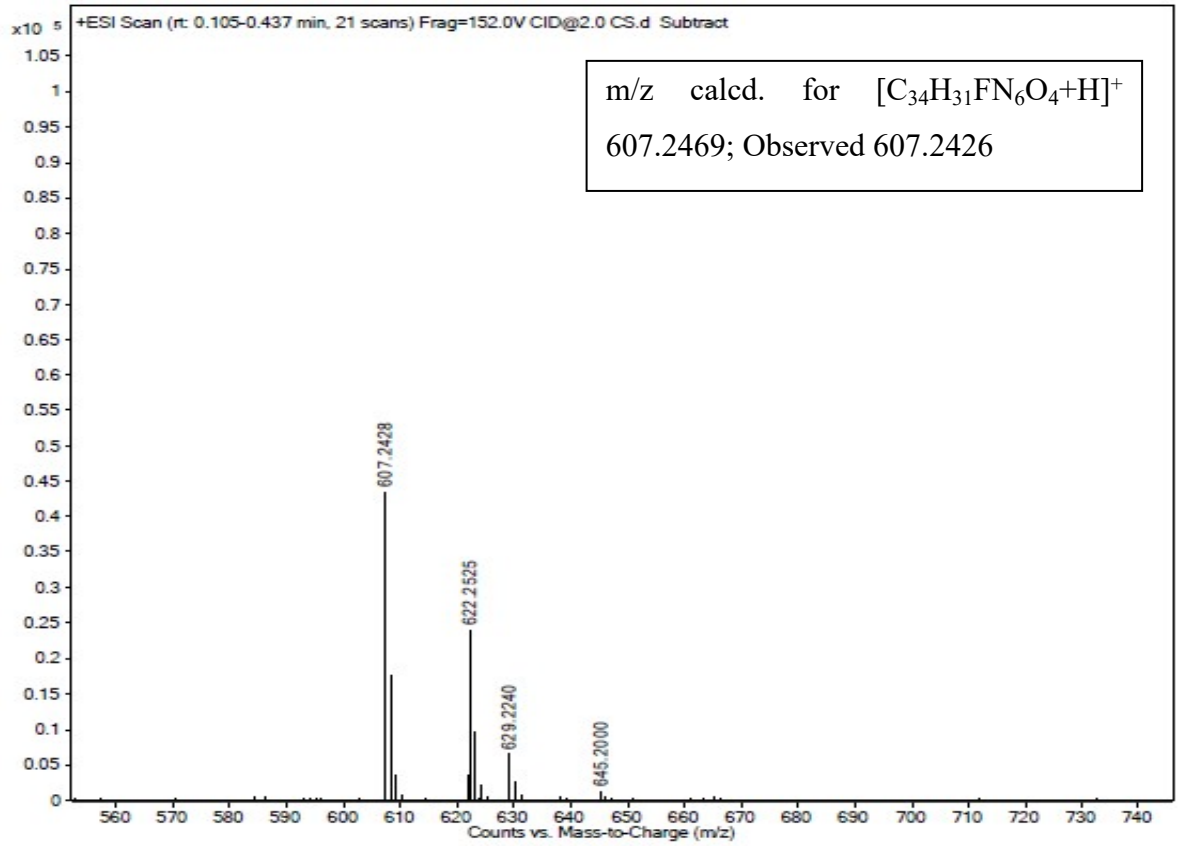
Mass spectrum of compound **7c**



^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7d**

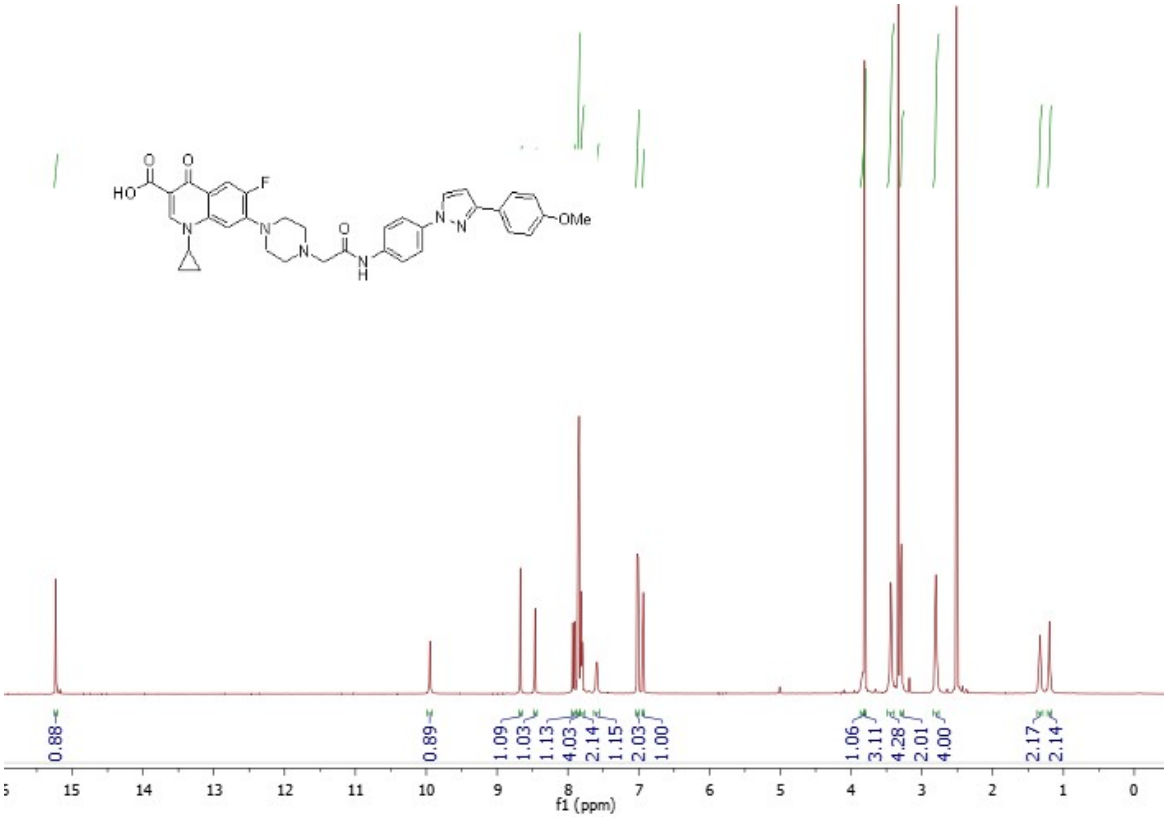


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7d**

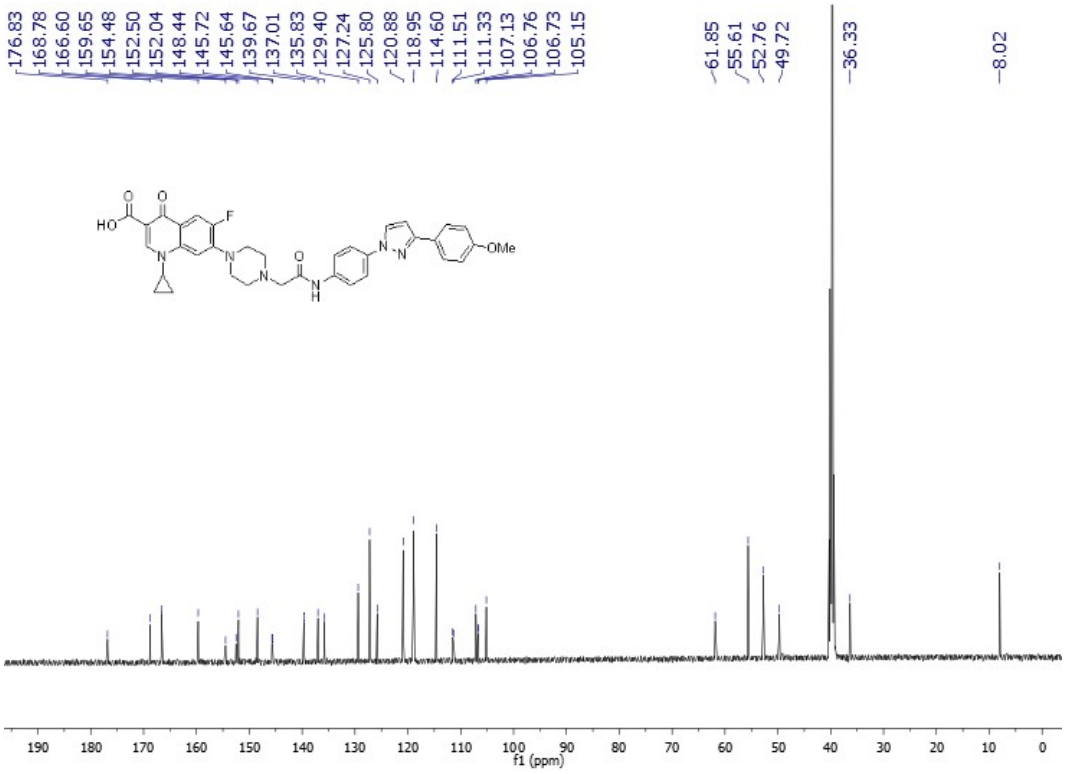


m/z calcd. for $[C_{34}H_{31}FN_6O_4+H]^+$ 607.2469; Observed 607.2426

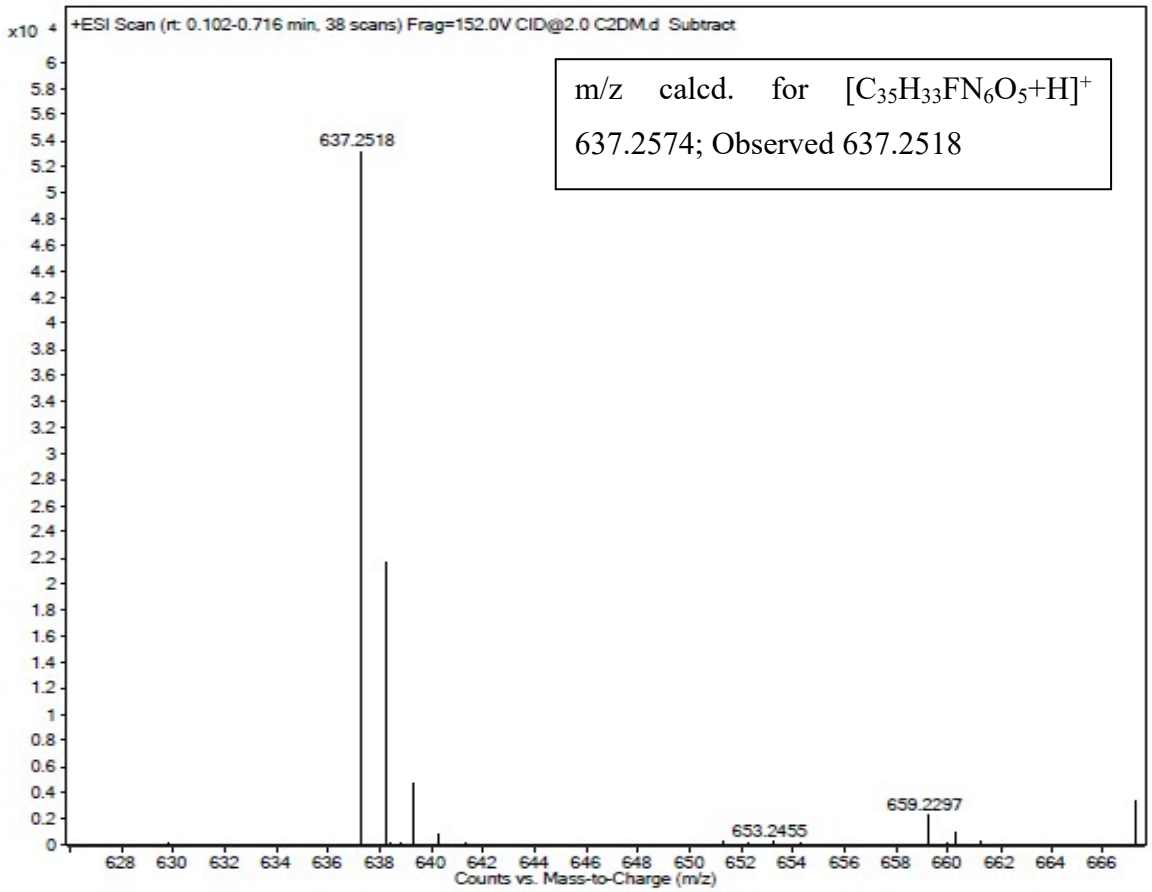
Mass spectrum of compound **7d**



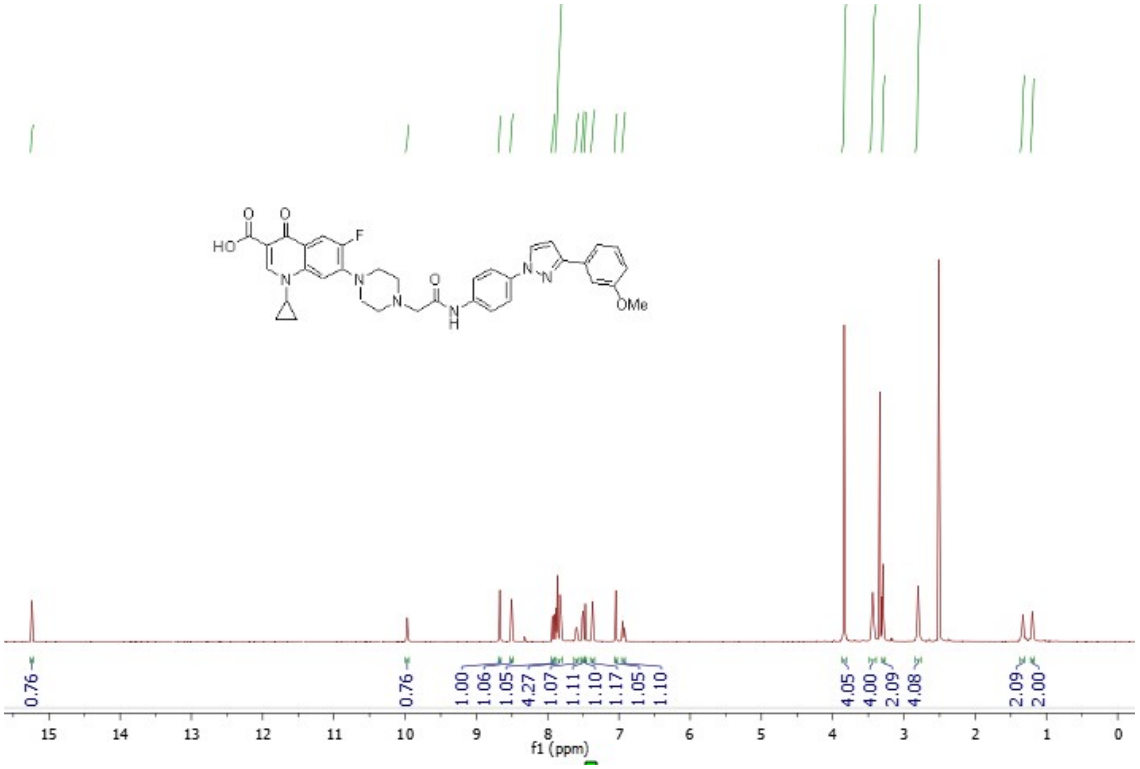
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7e**



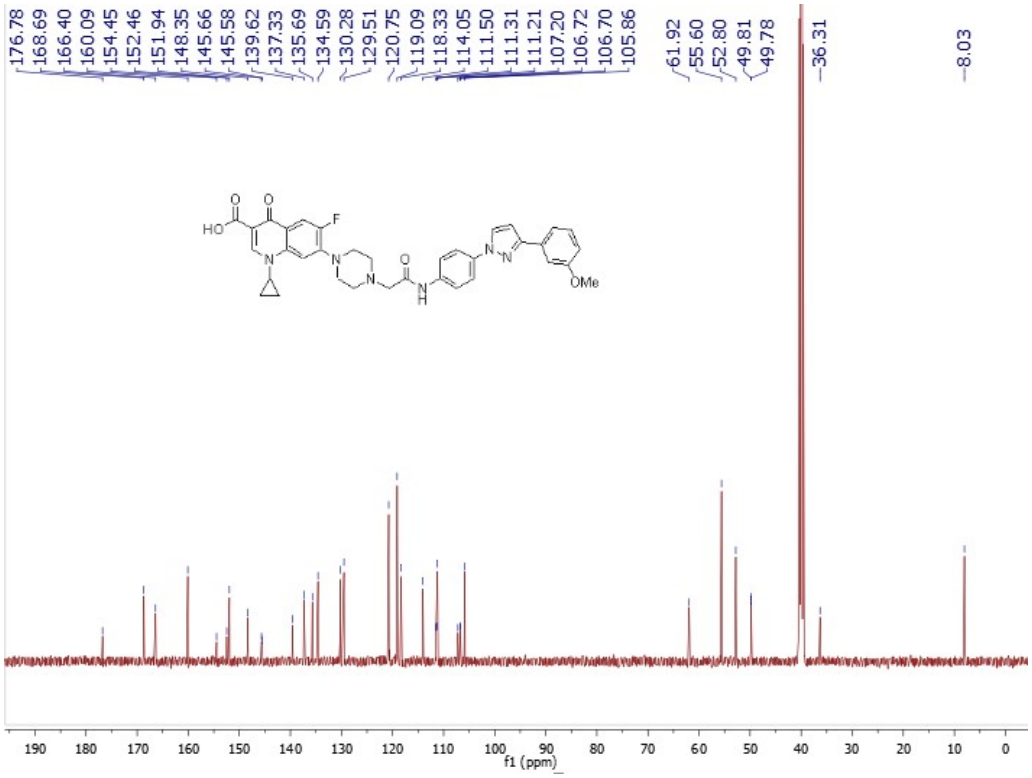
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7e**



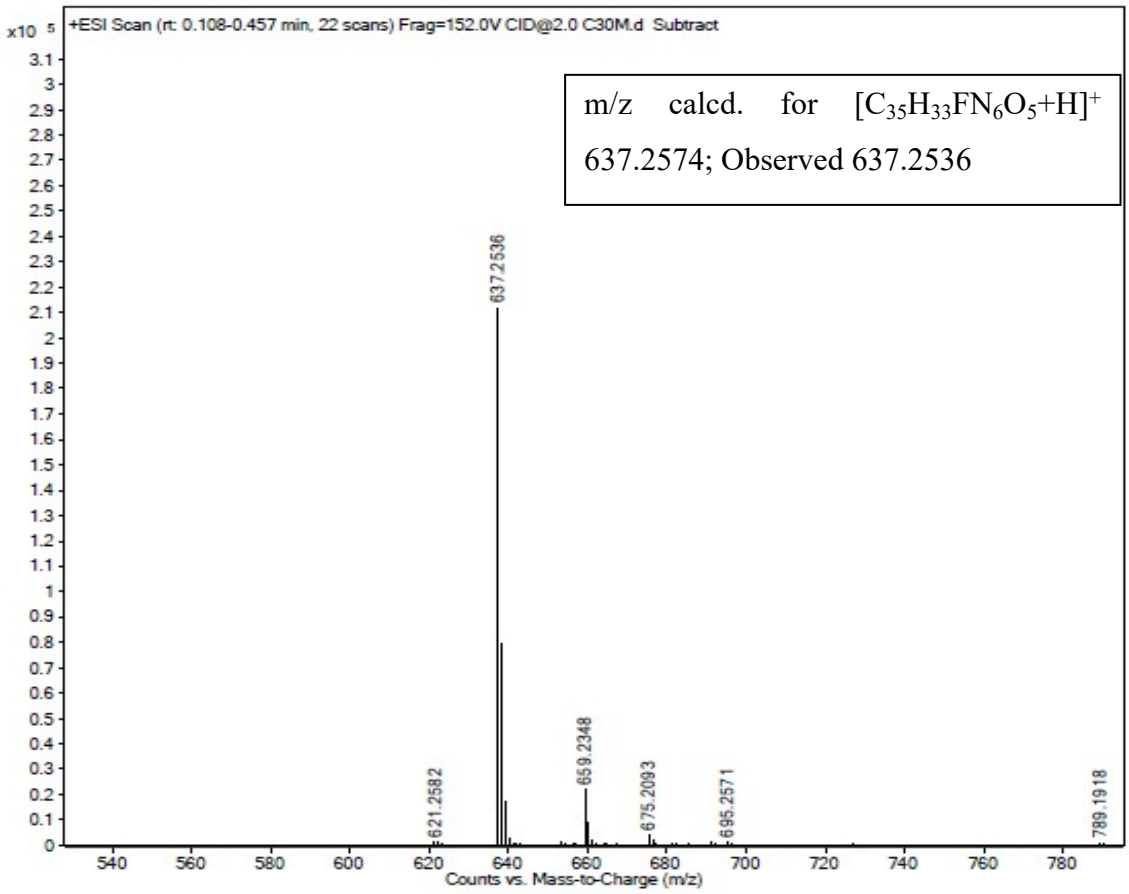
Mass spectrum of compound **7e**



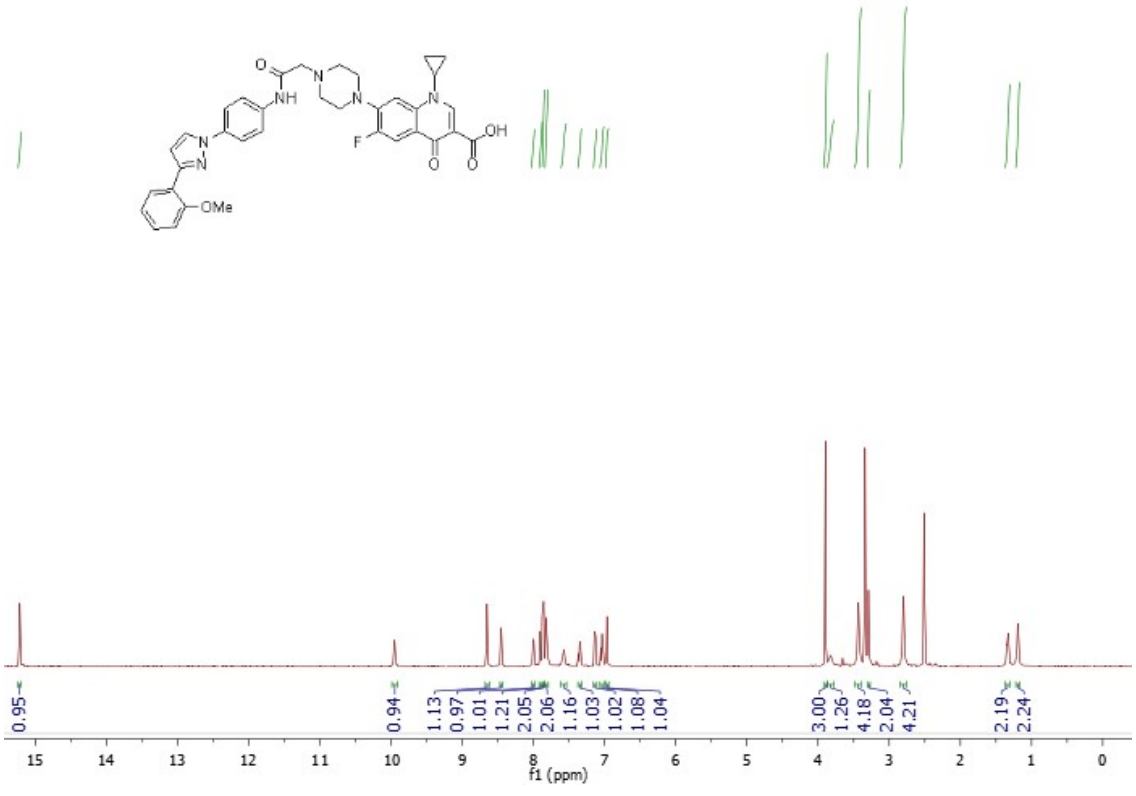
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7f**



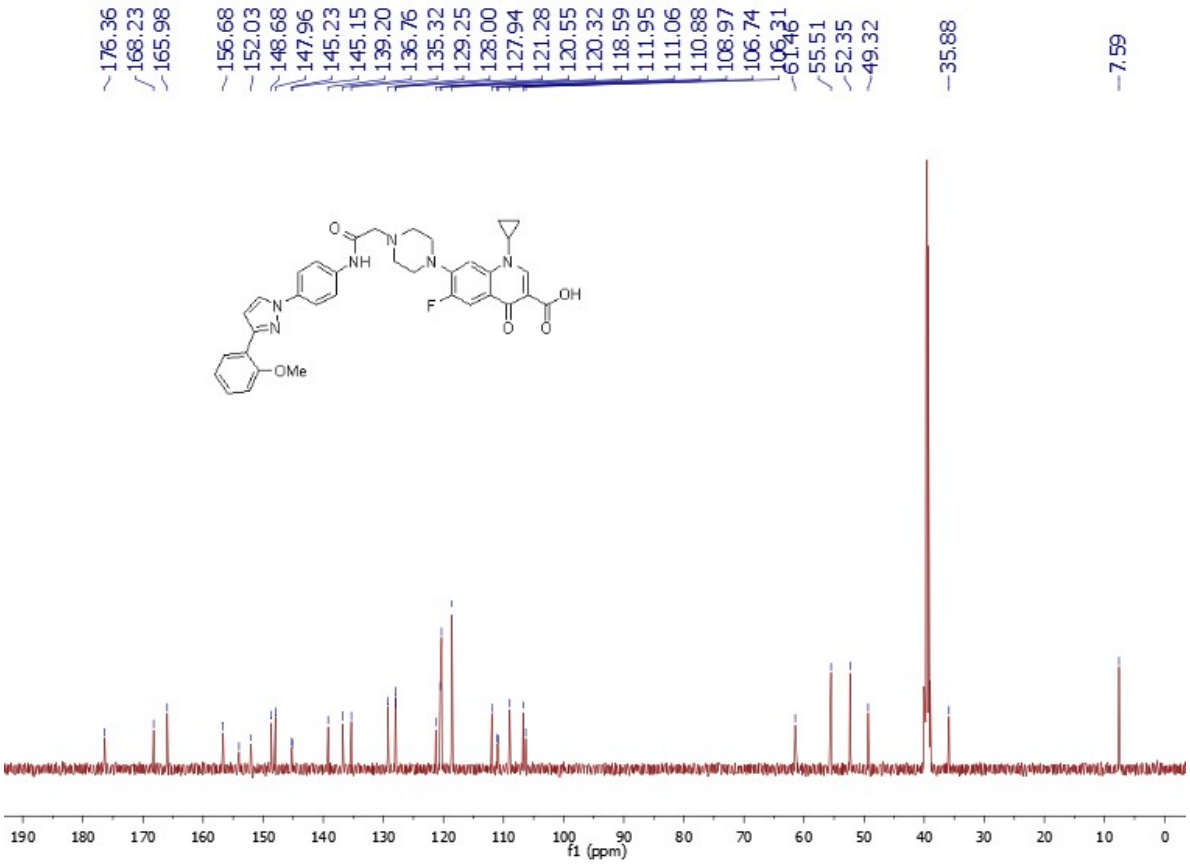
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7f**



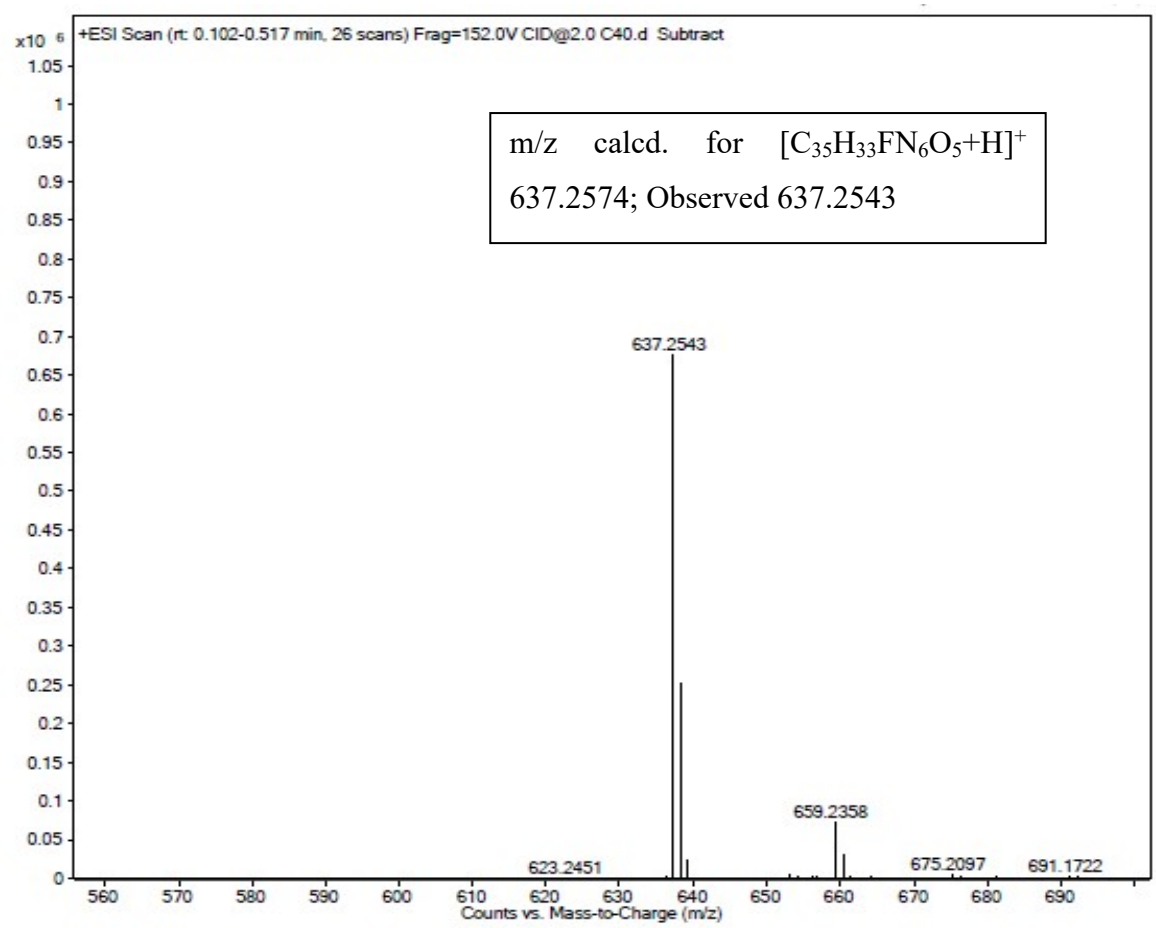
Mass spectrum of compound **7f**



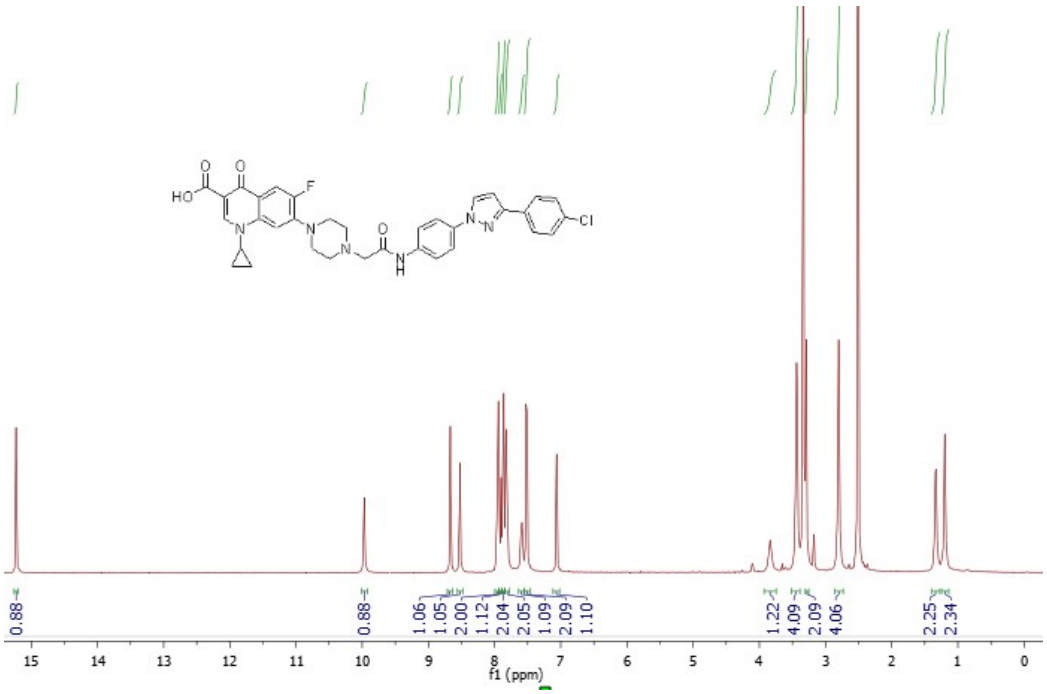
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7g**



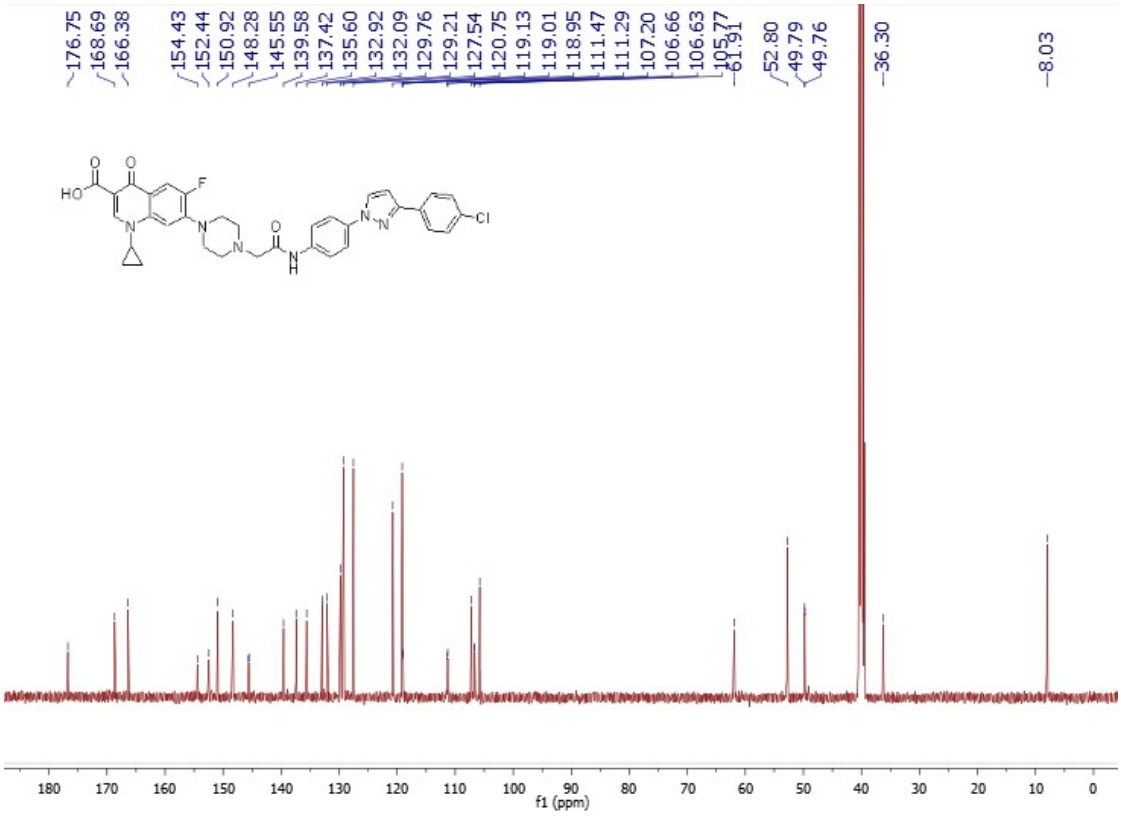
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7g**



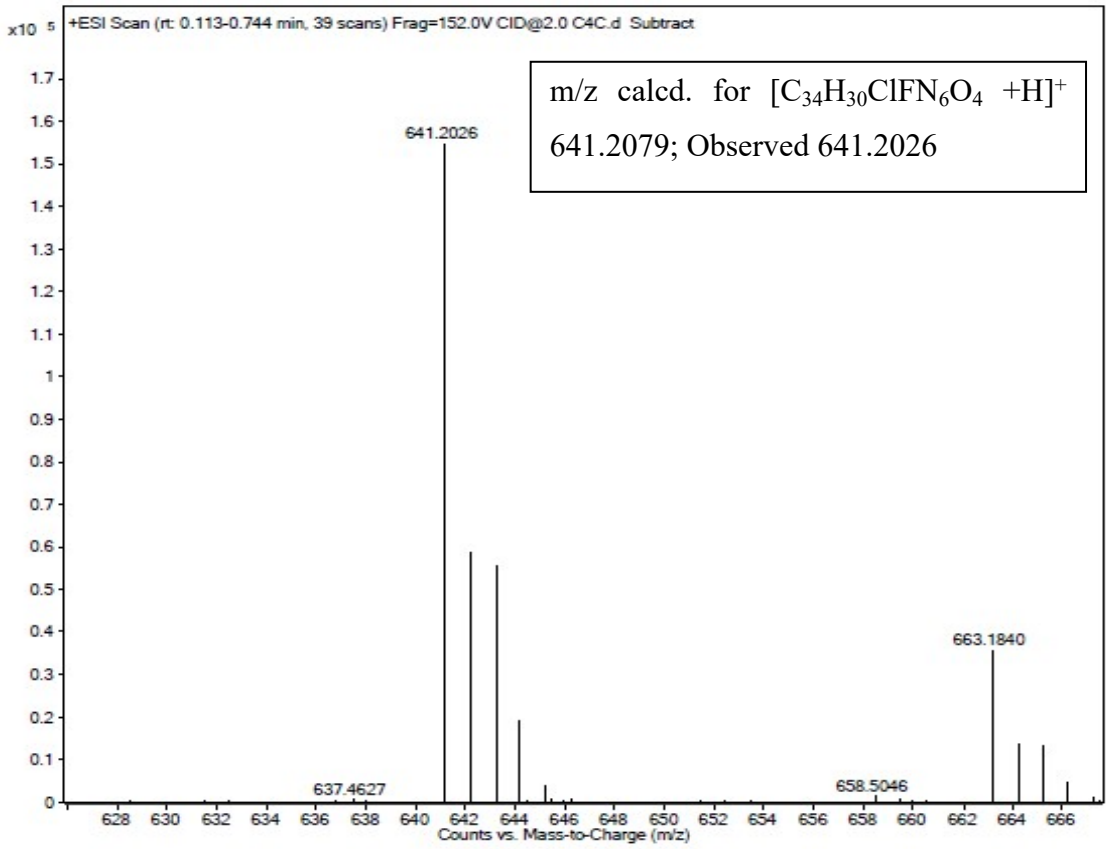
Mass spectrum of compound **7g**



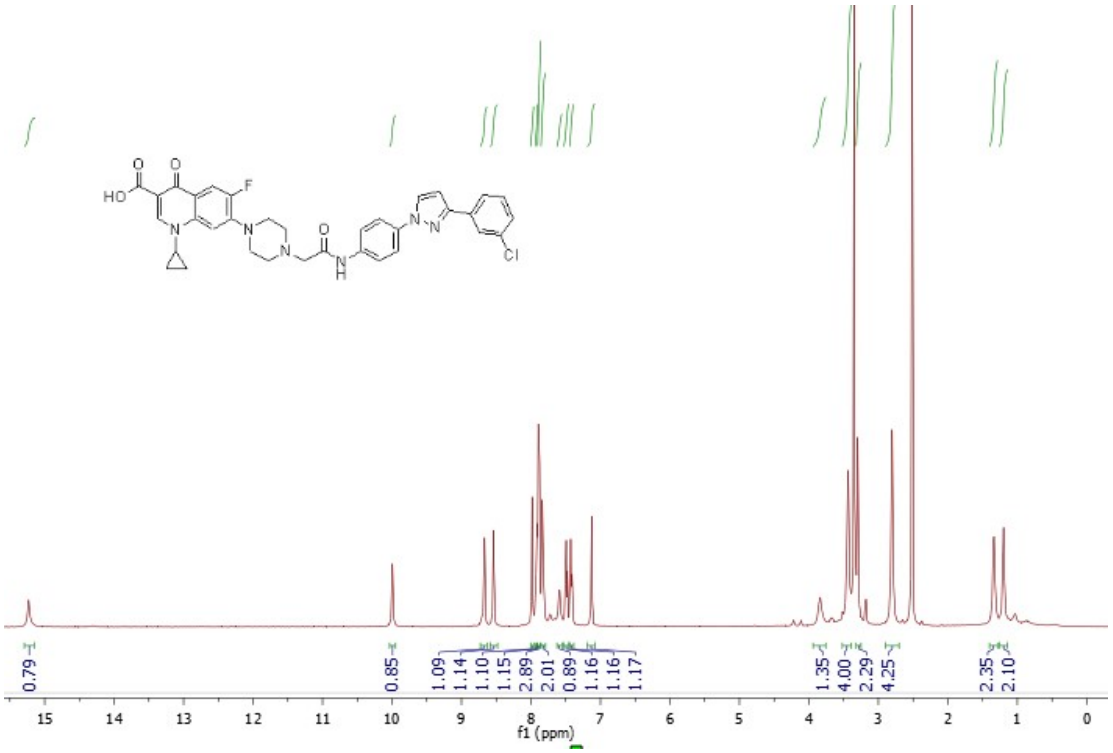
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7h**



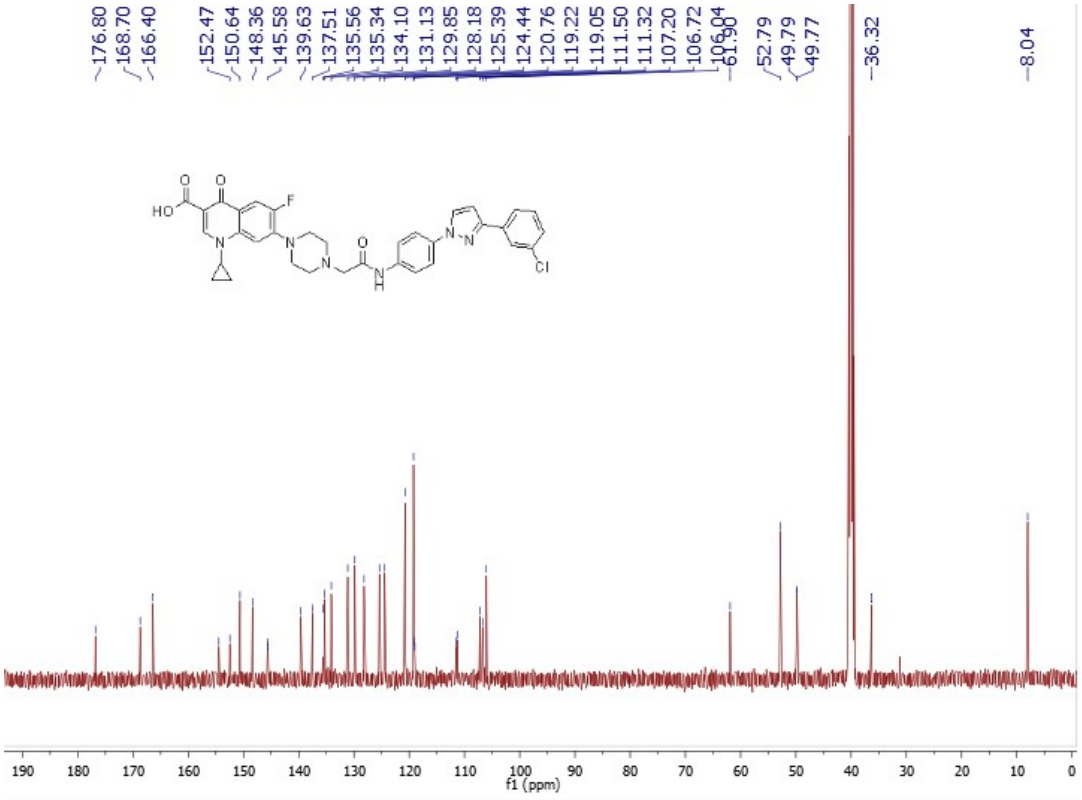
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7h**



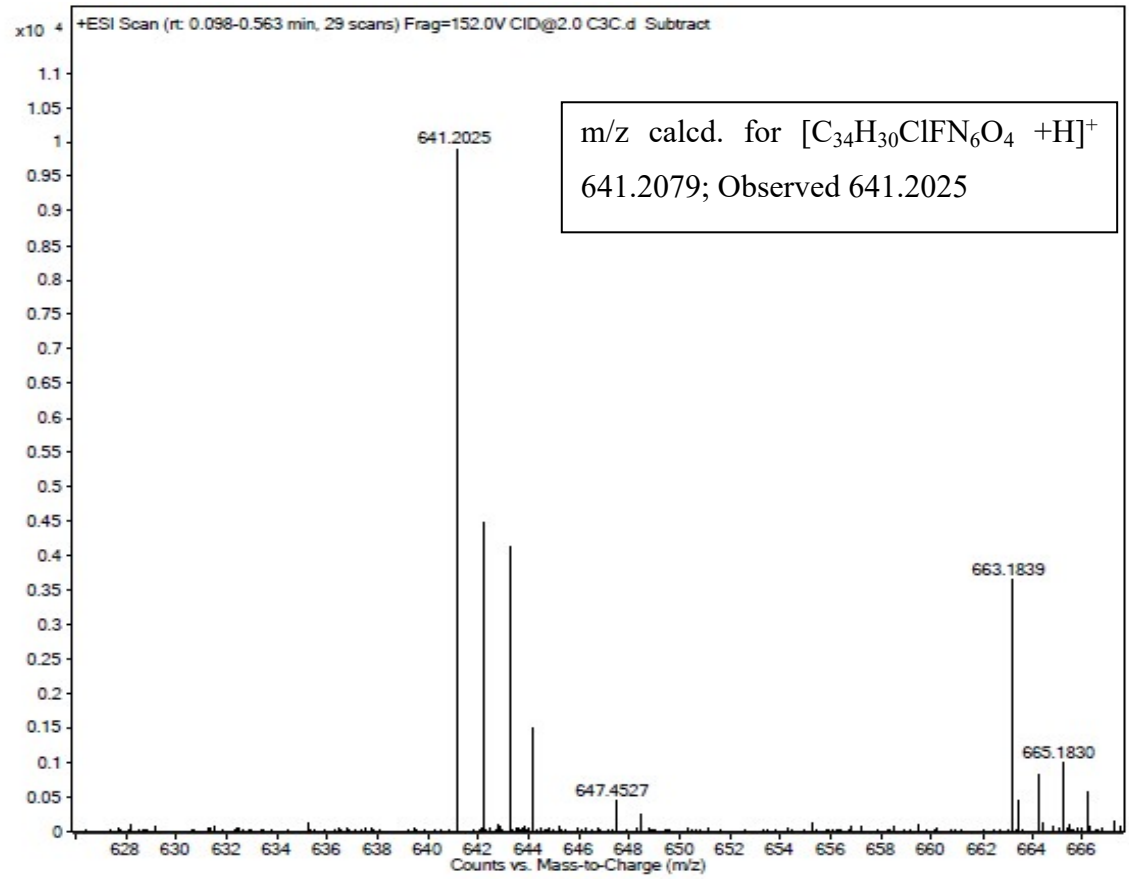
Mass spectrum of compound **7h**



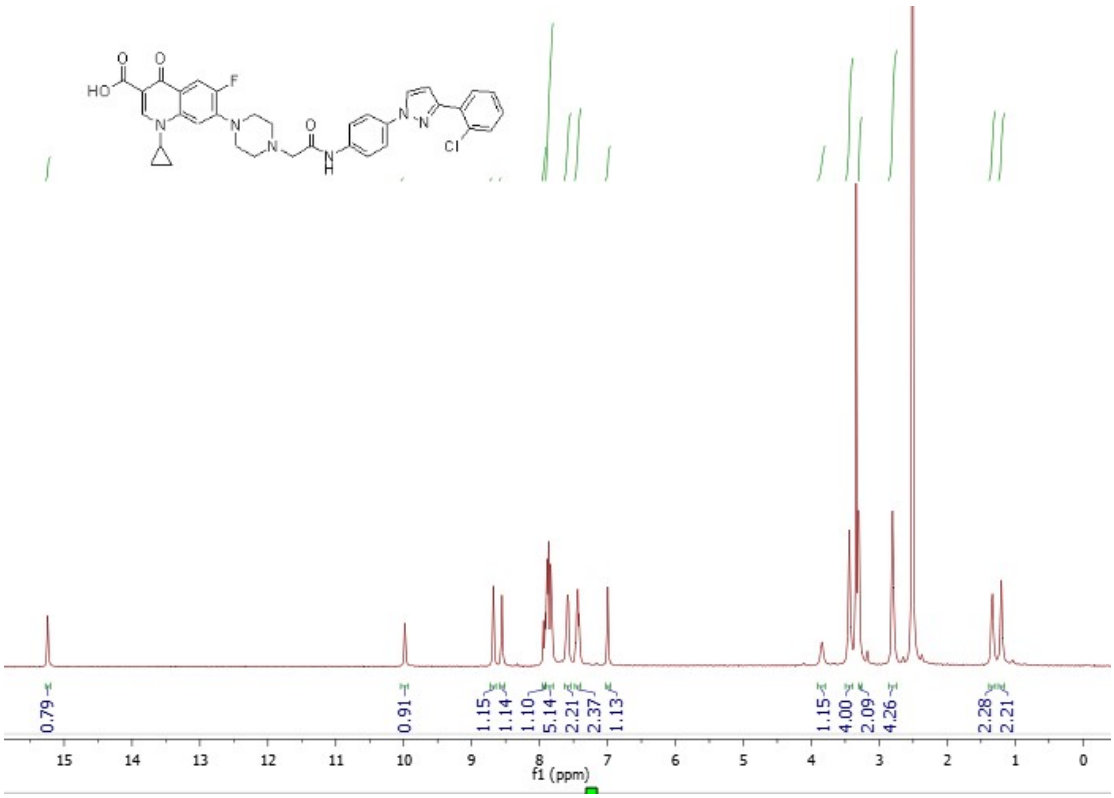
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7i**



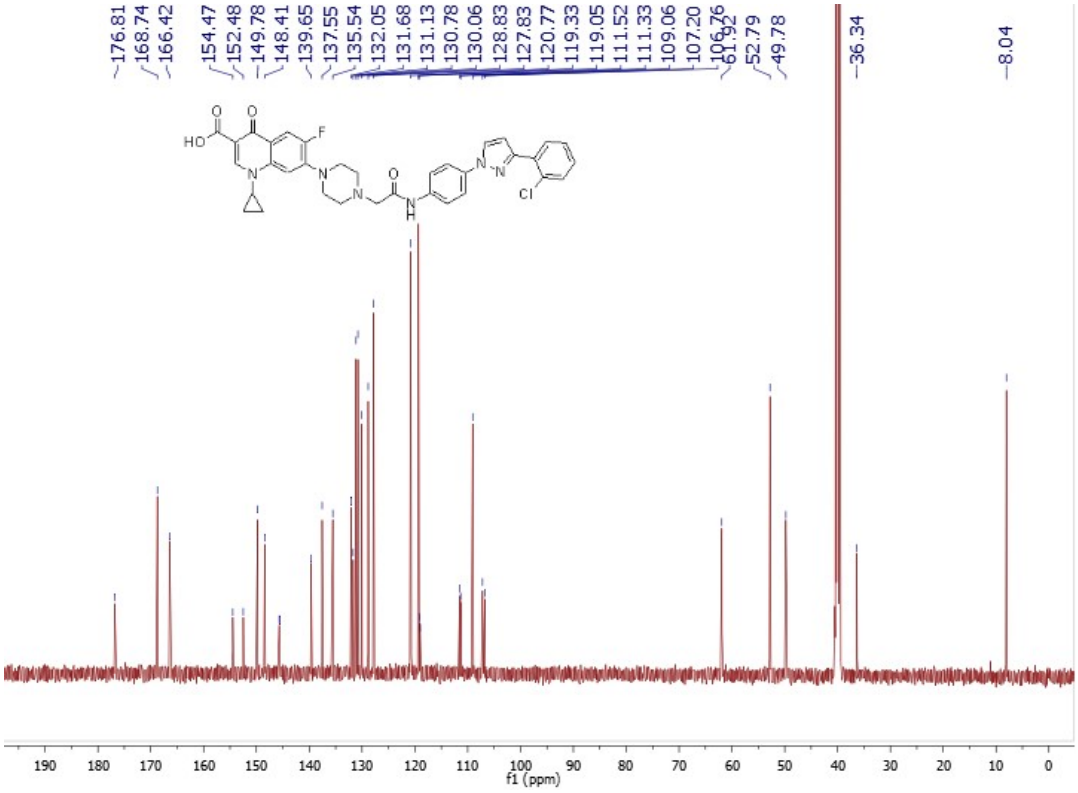
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7i**



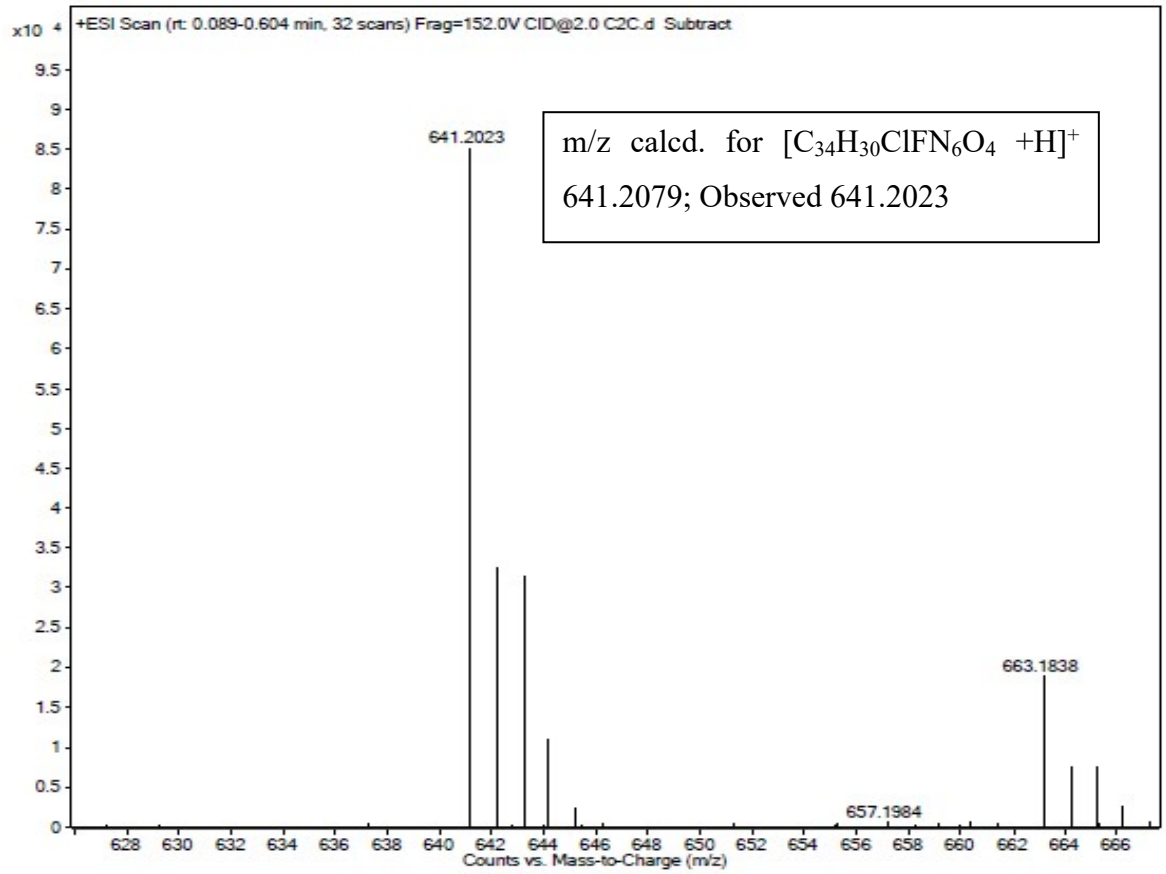
Mass spectrum of compound **7i**



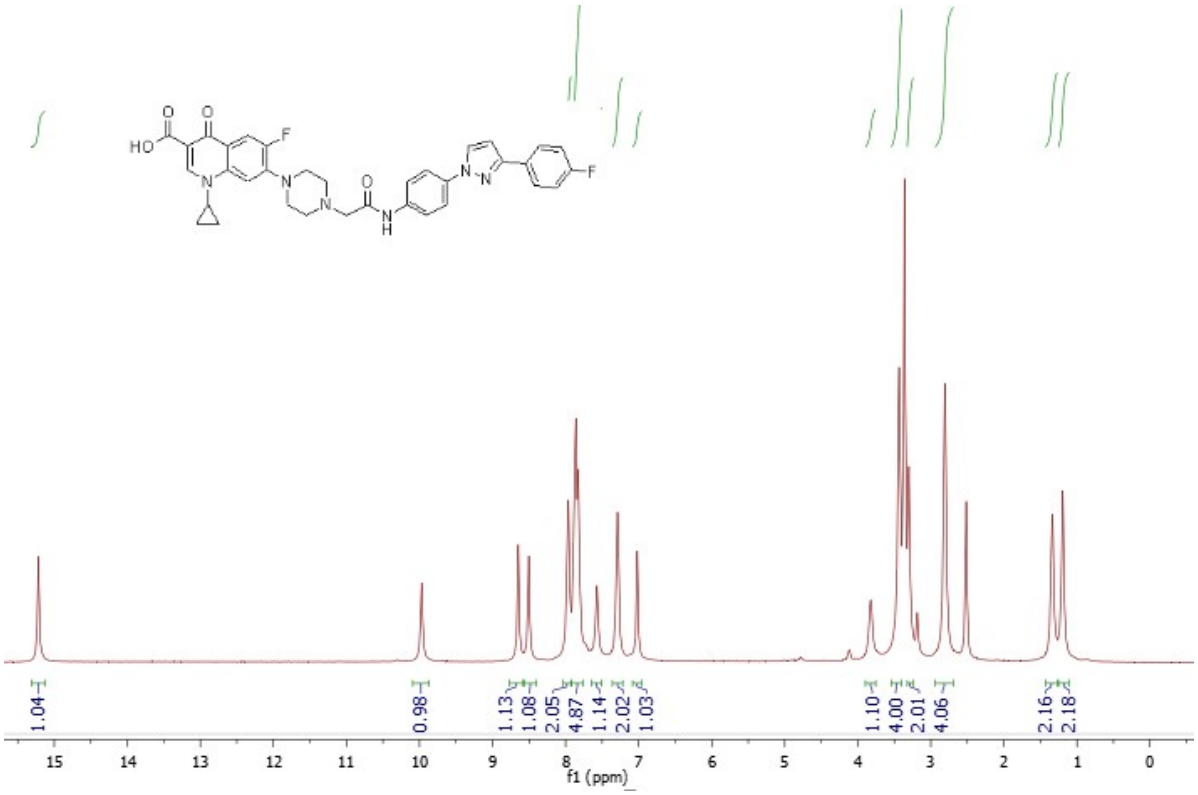
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7j**



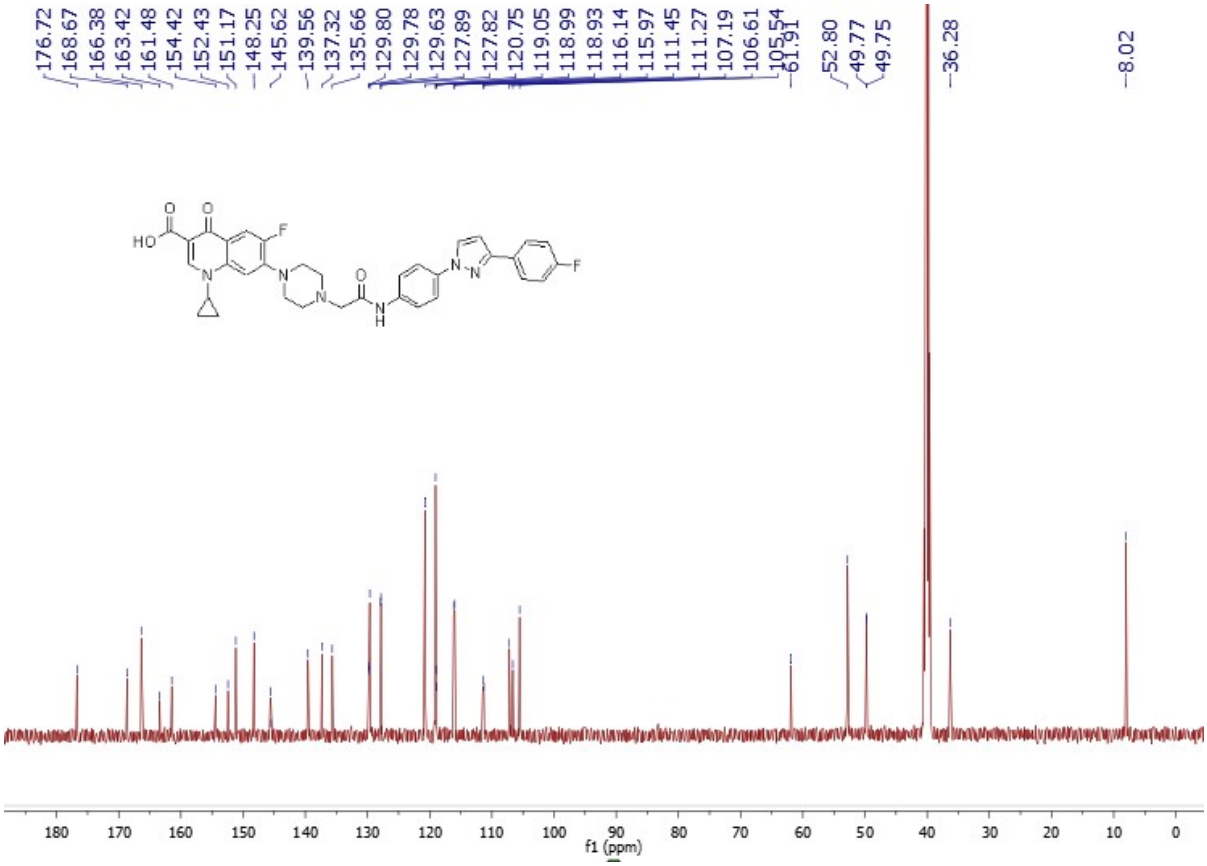
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7j**



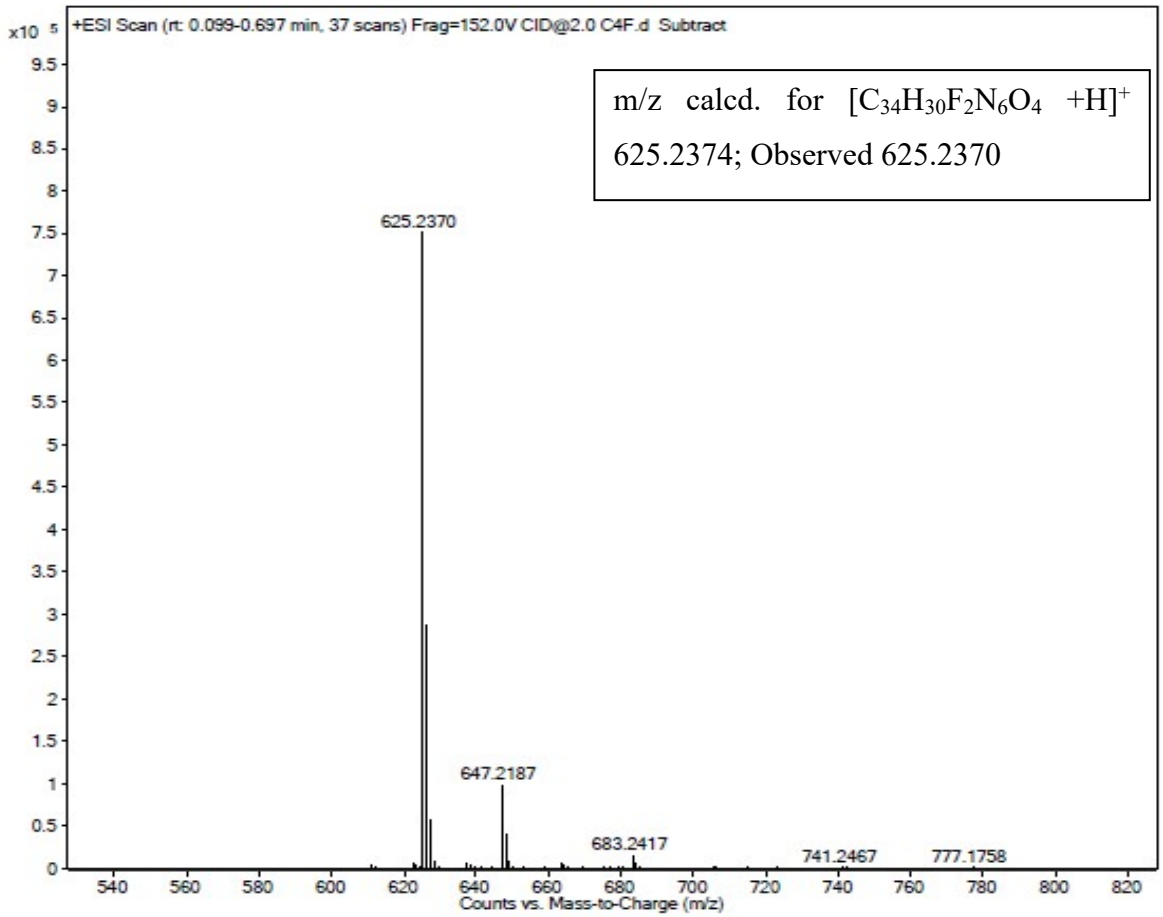
Mass spectrum of compound **7j**



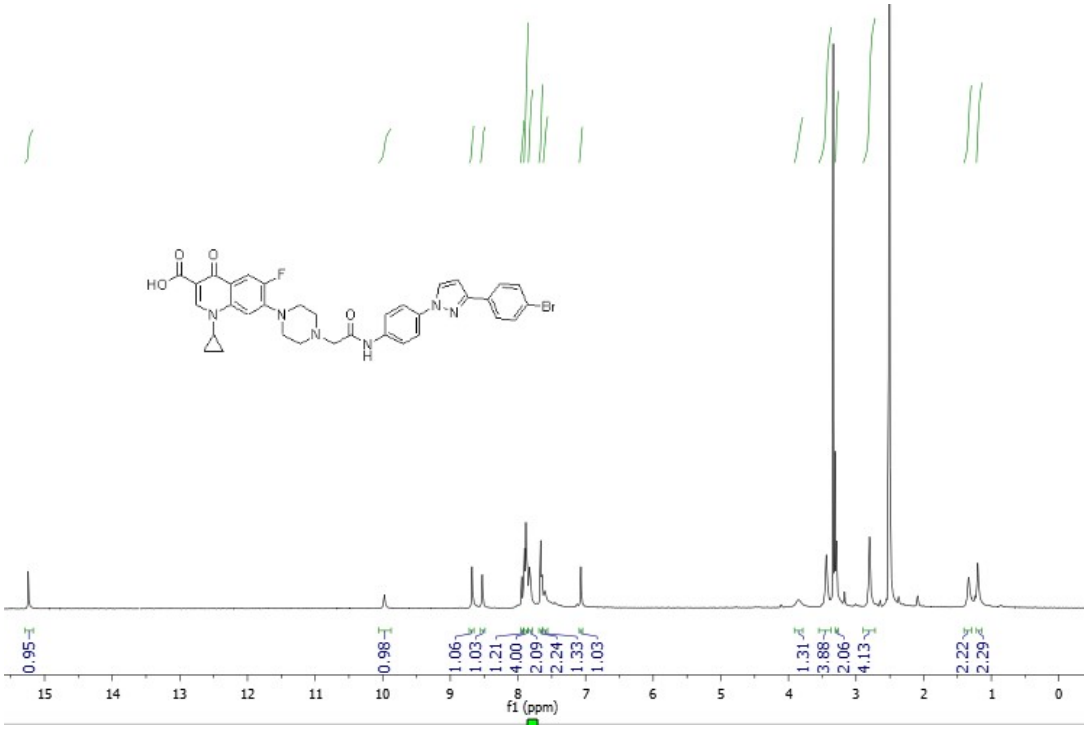
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7k**



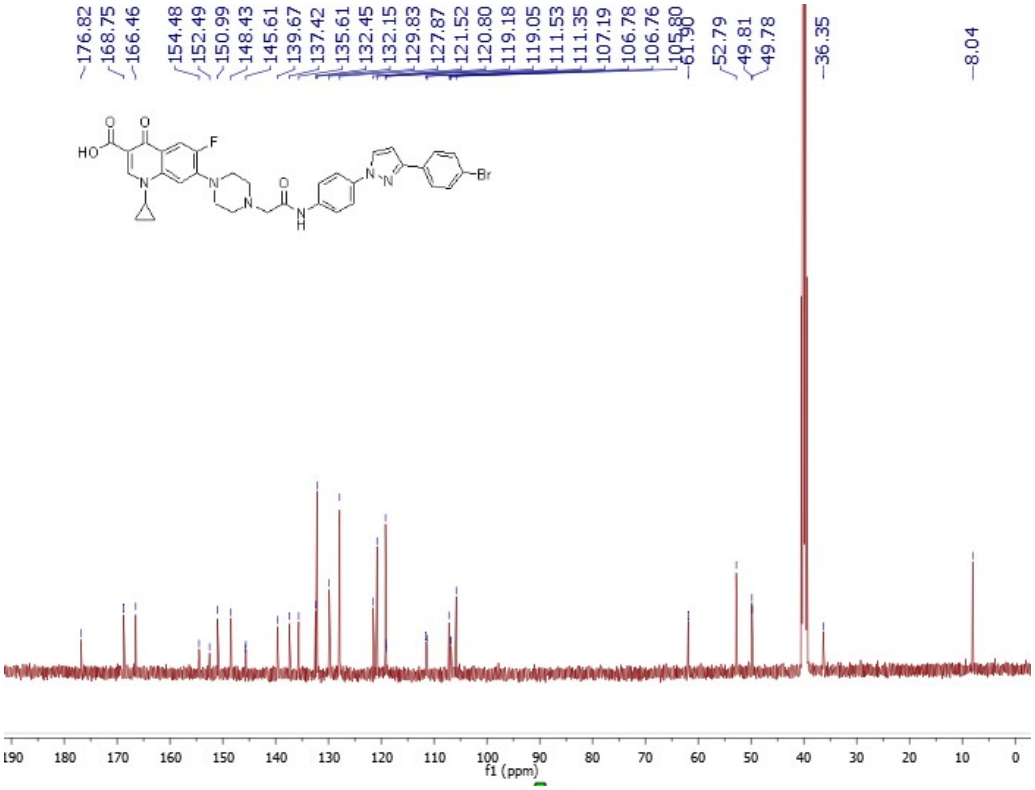
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7k**



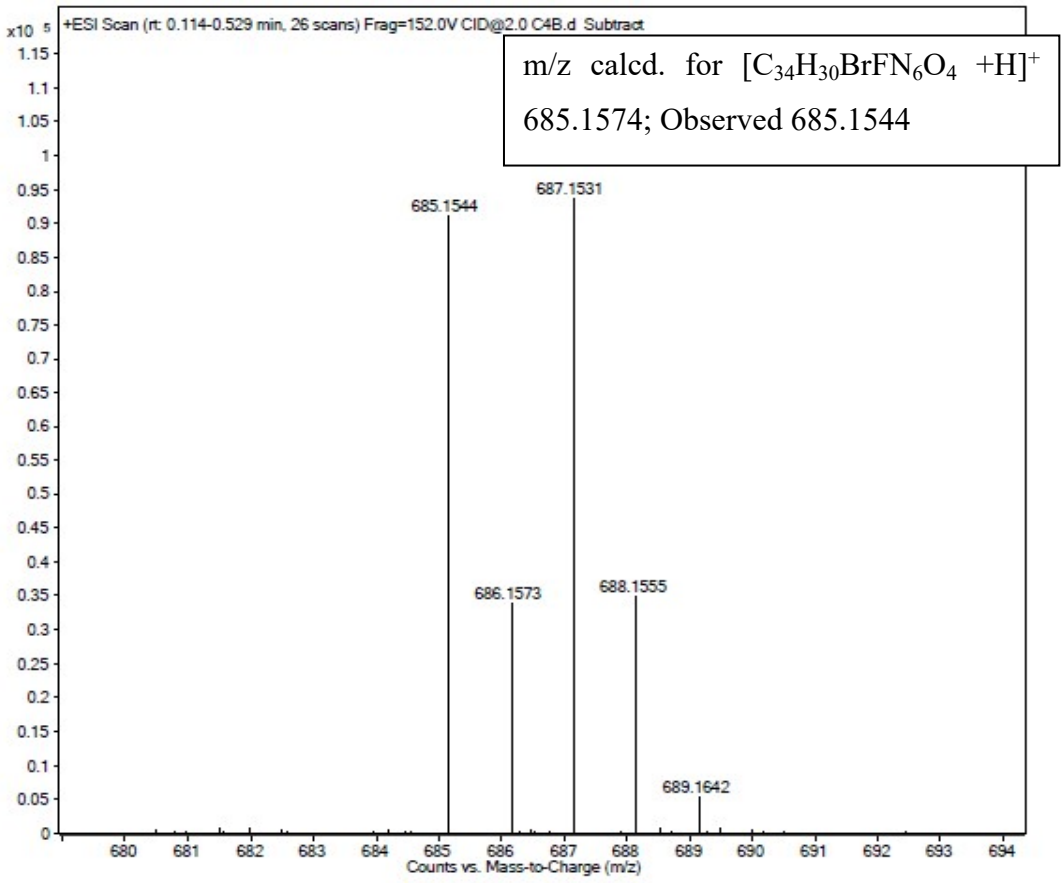
Mass spectrum of compound **7k**



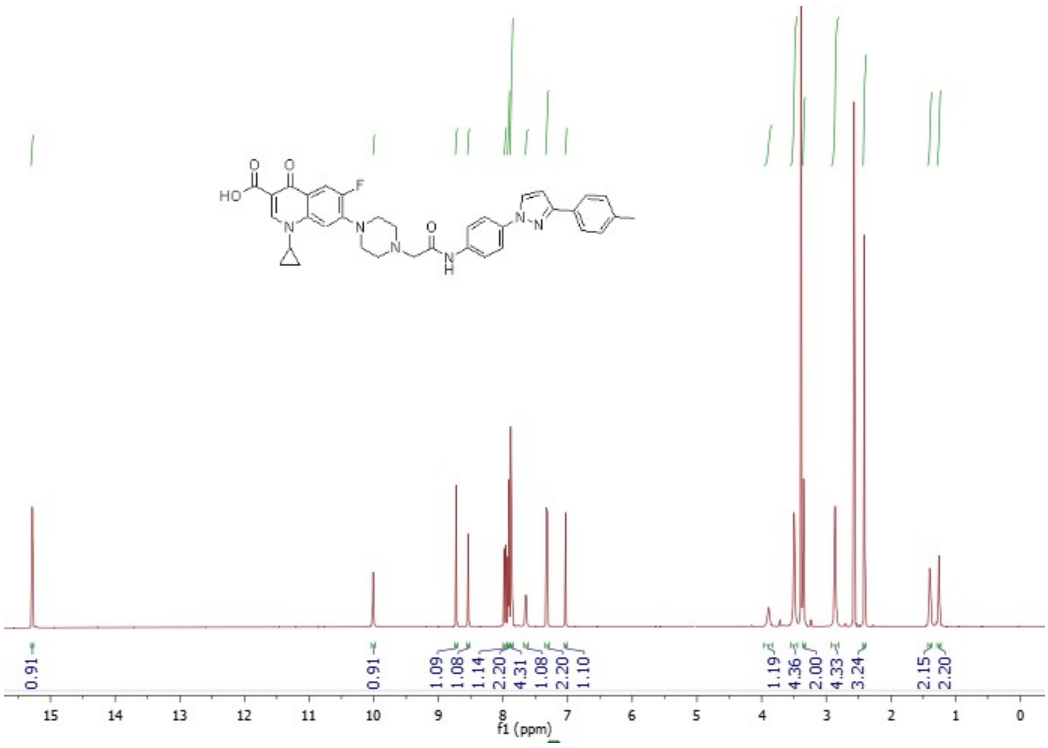
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7l**



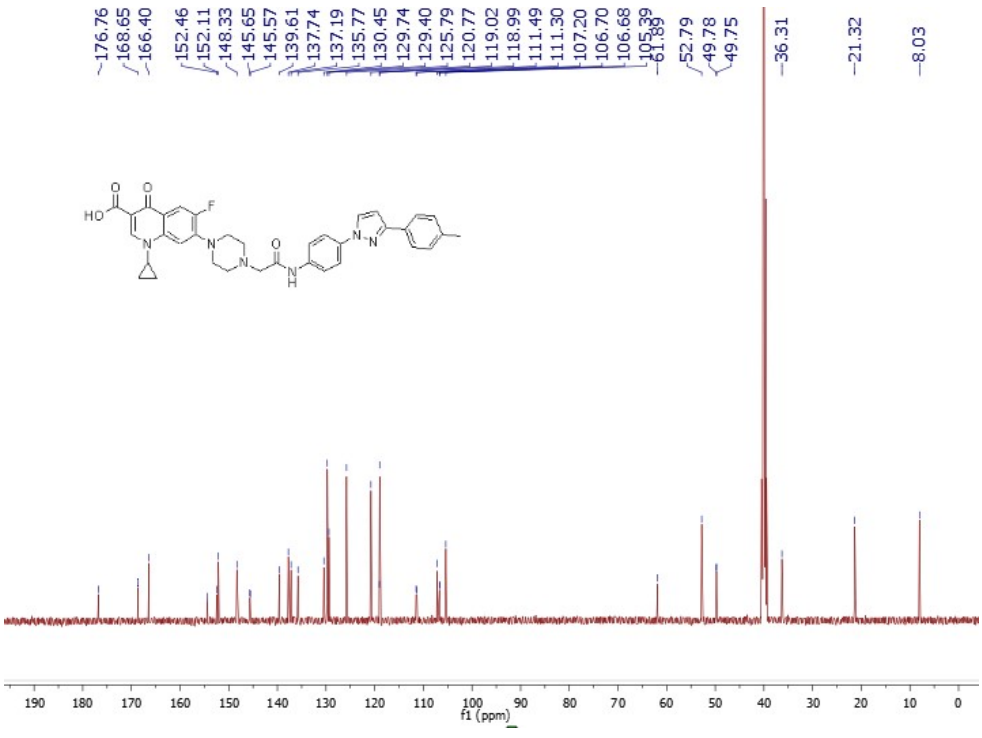
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7l**



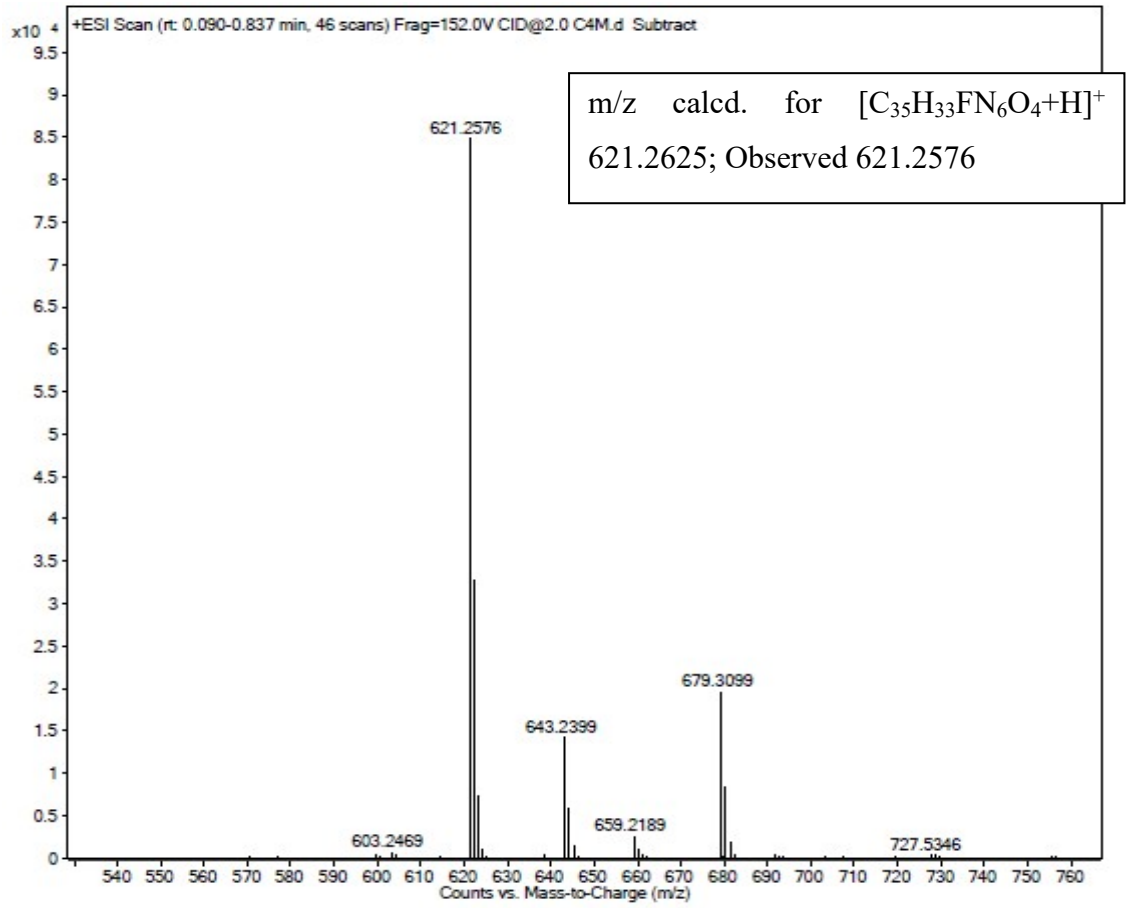
Mass spectrum of compound **7l**



^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7m**

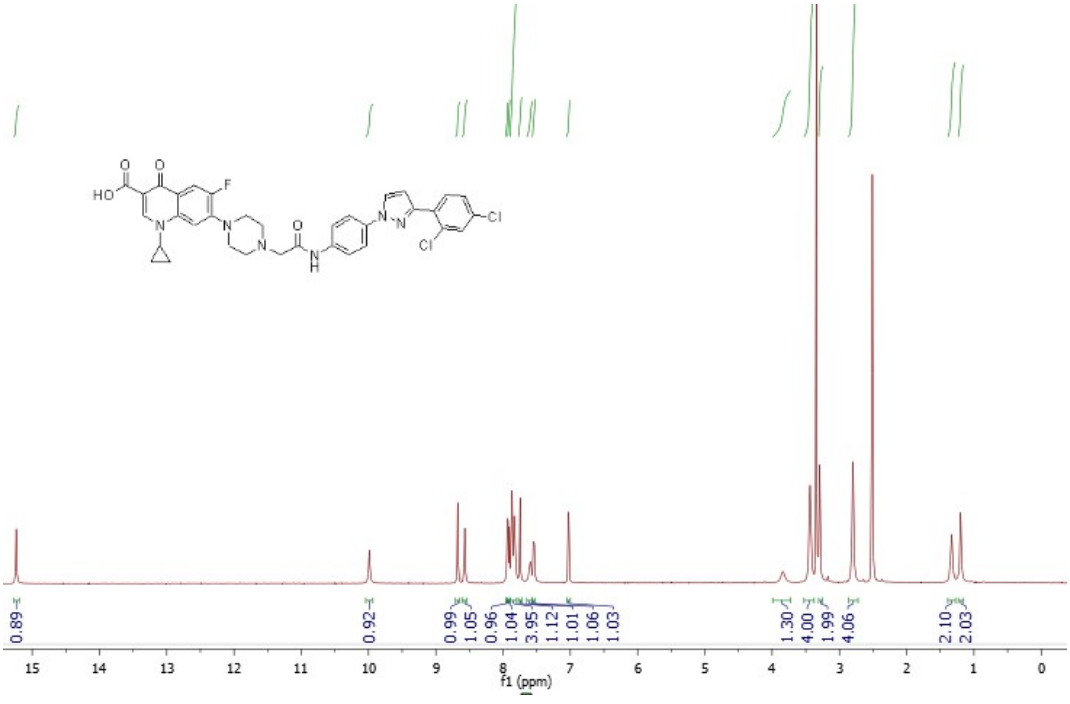


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7m**

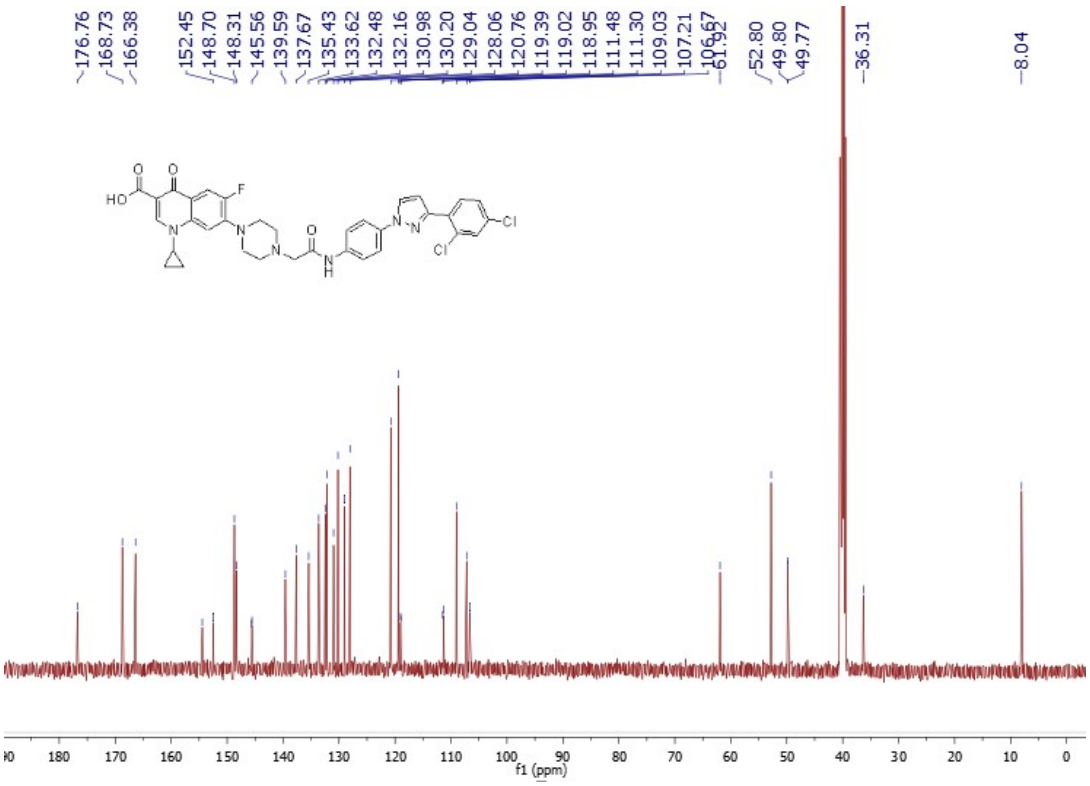


m/z calcd. for $[C_{35}H_{33}FN_6O_4+H]^+$ 621.2625; Observed 621.2576

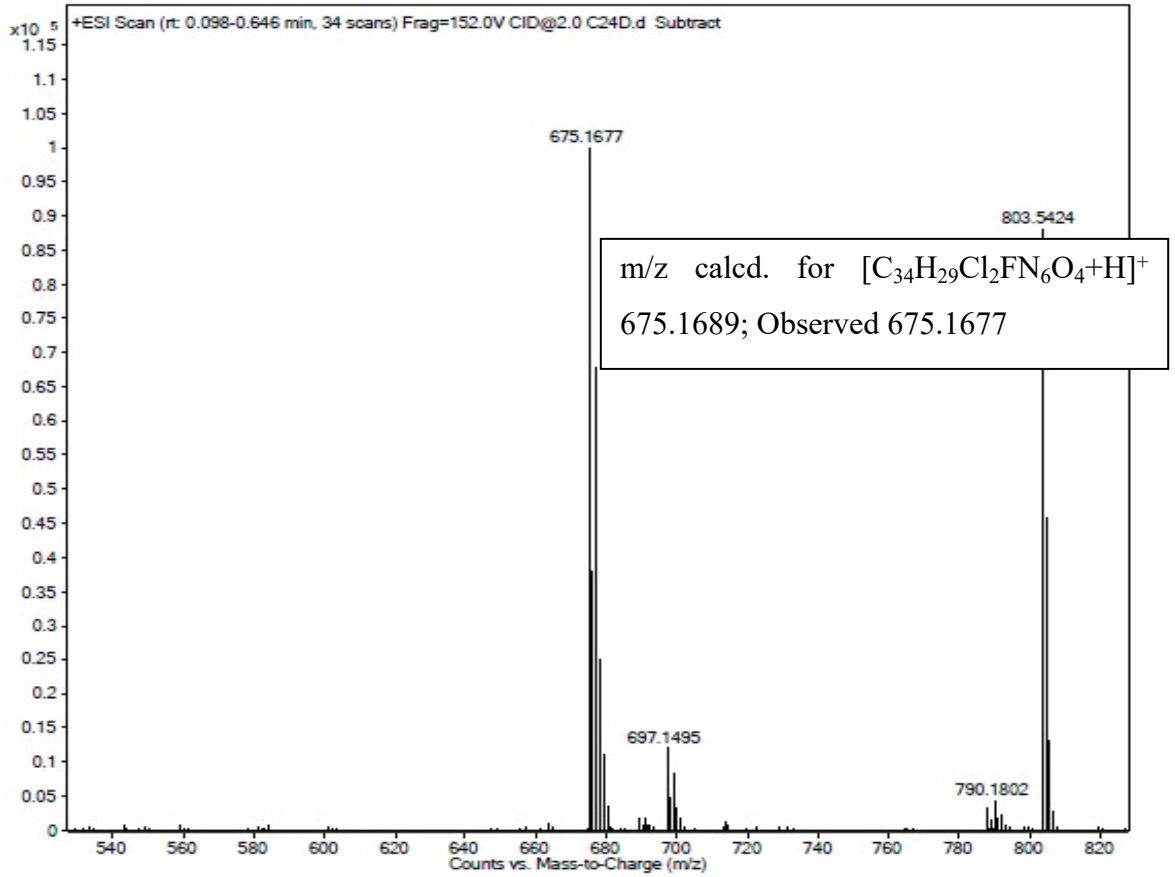
Mass spectrum of compound **7m**



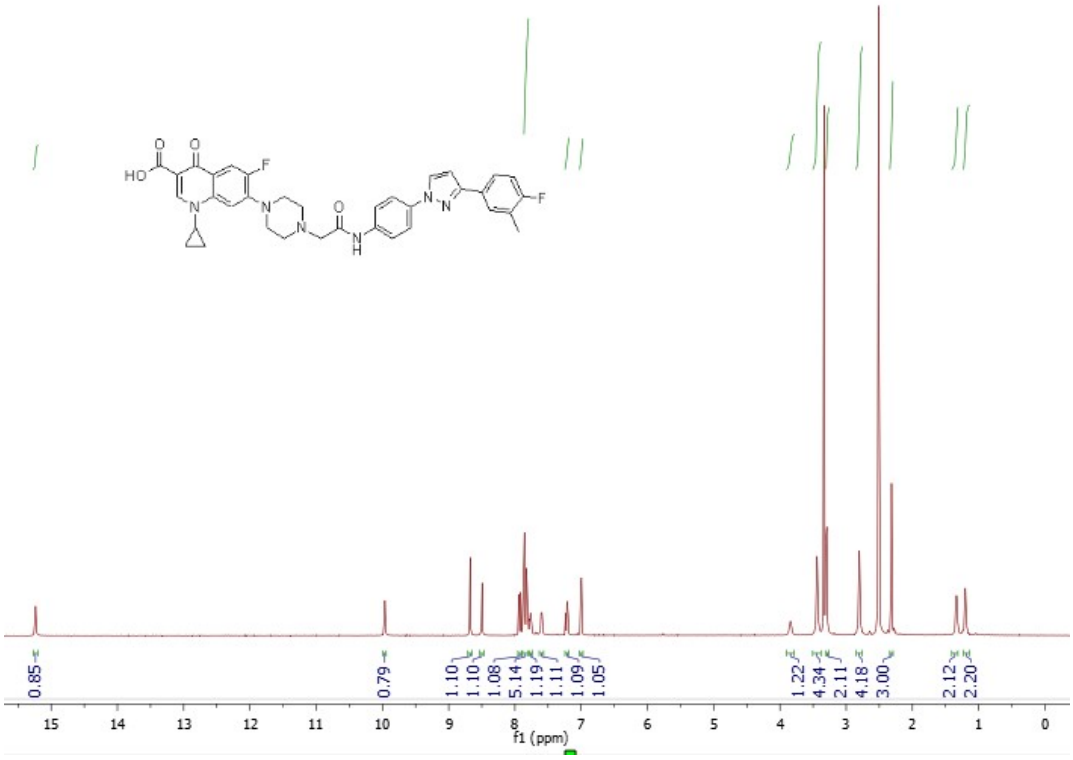
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7n**



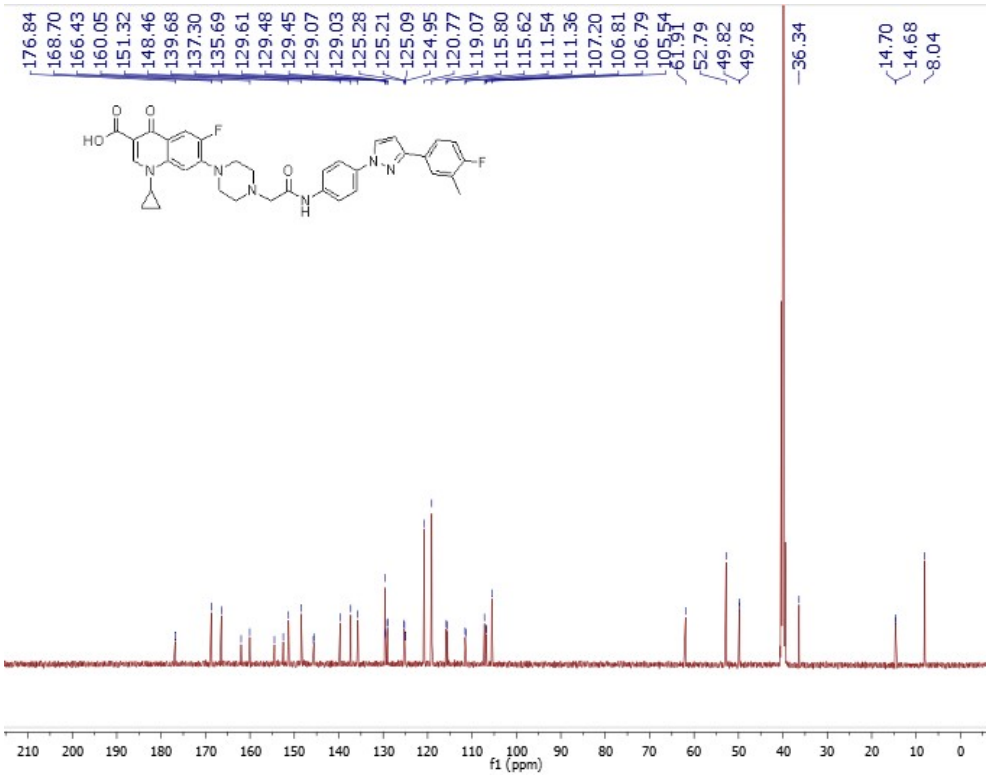
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7n**



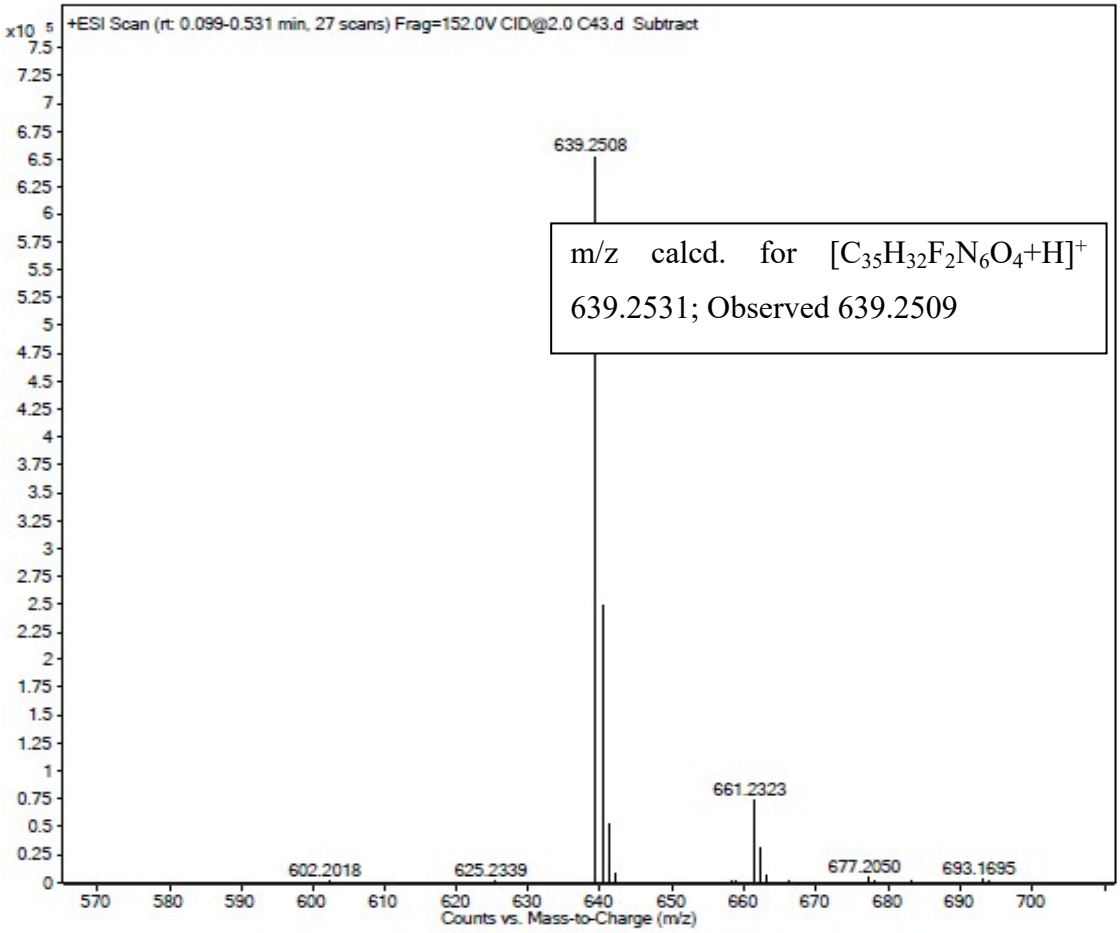
Mass spectrum of compound **7n**



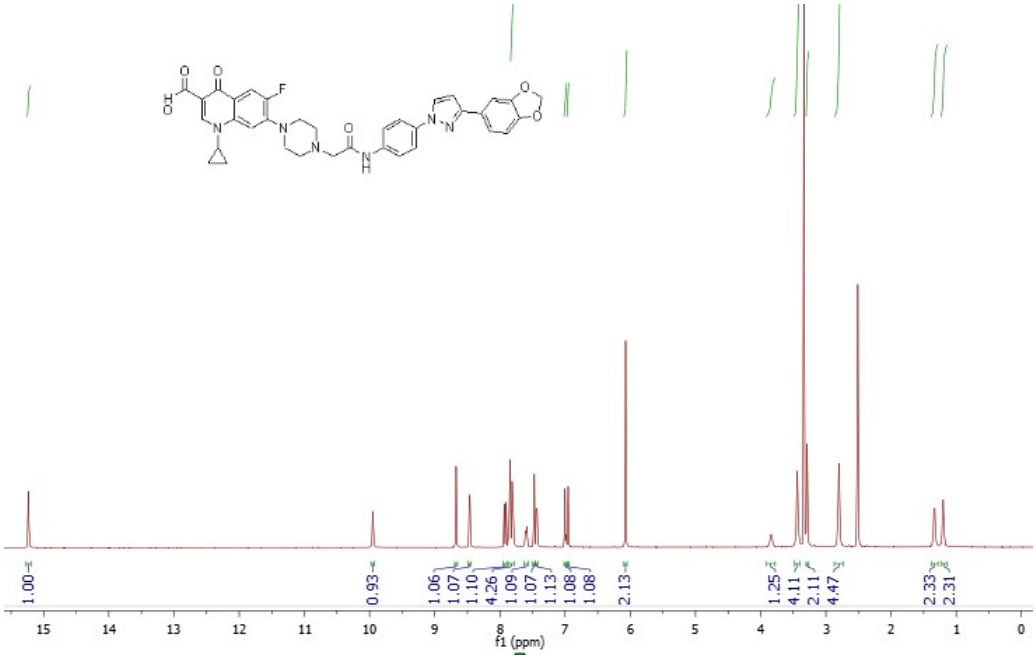
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7o**



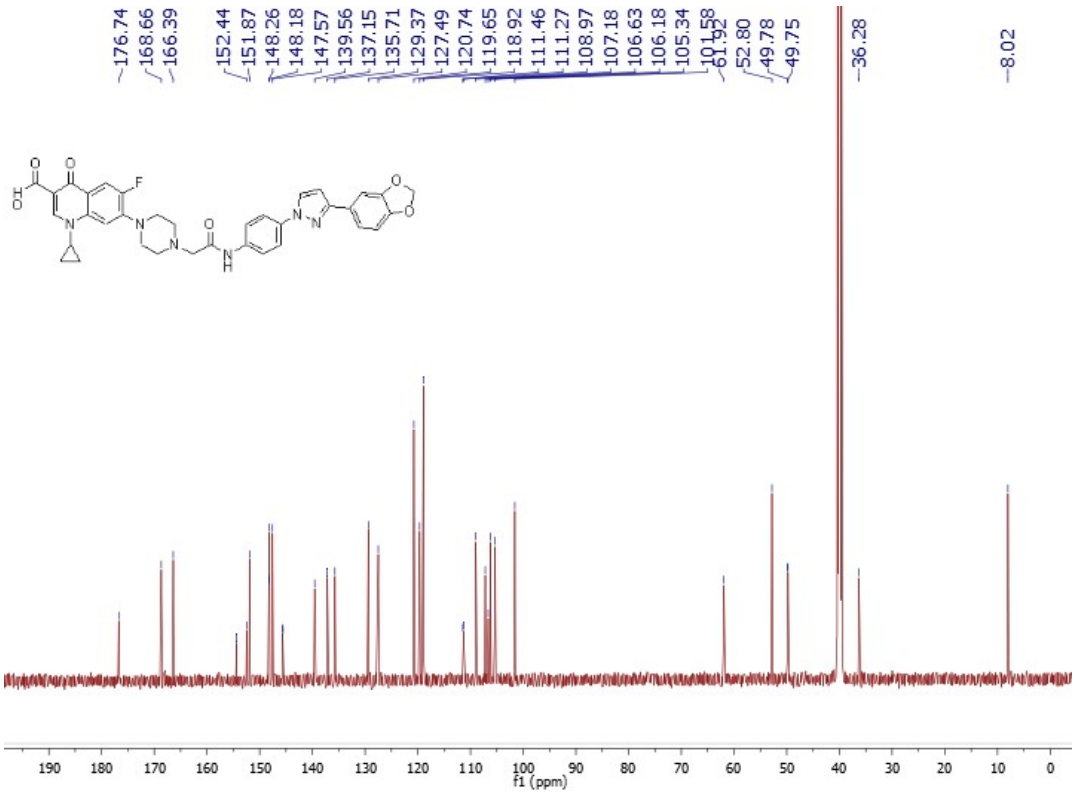
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7o**



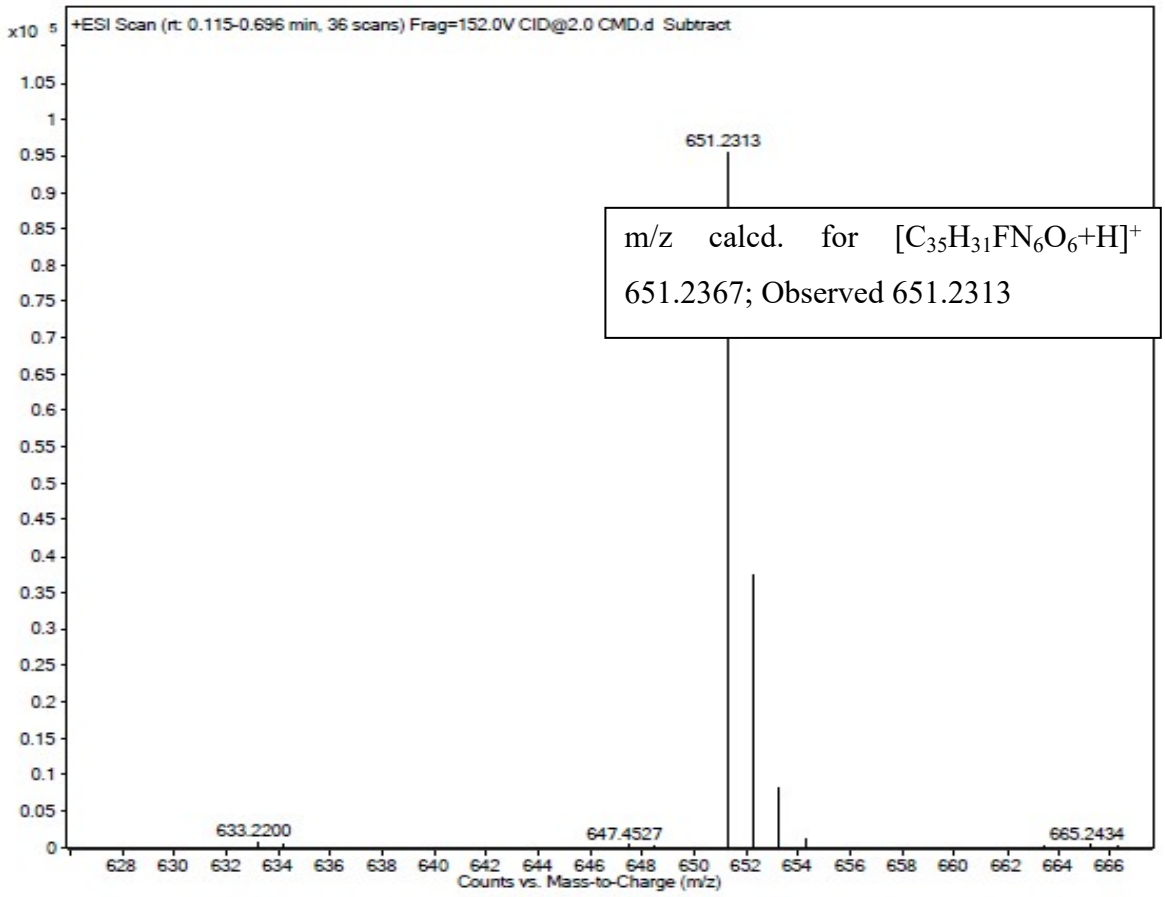
Mass spectrum of compound **7o**



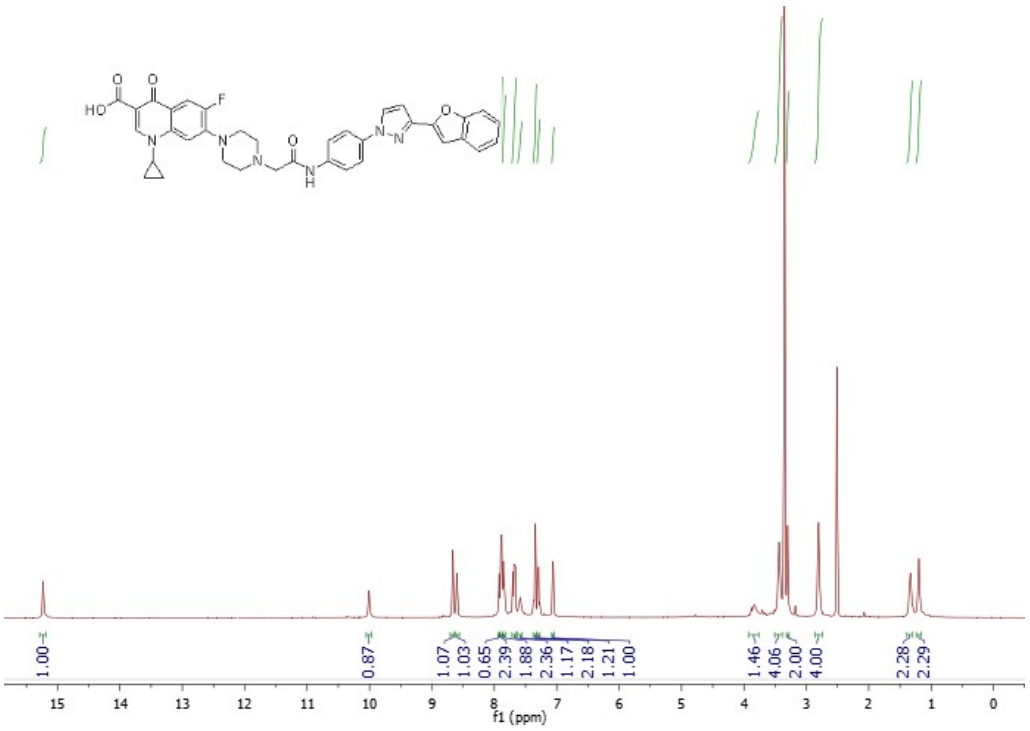
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7p**



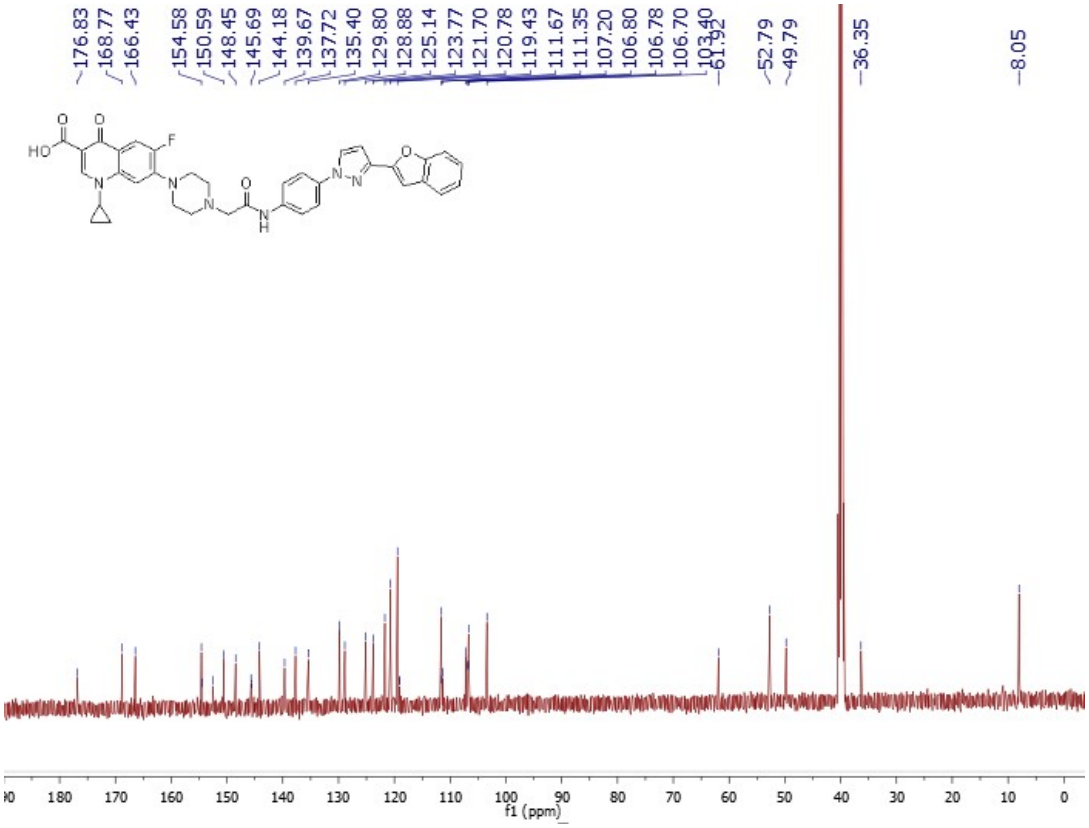
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7p**



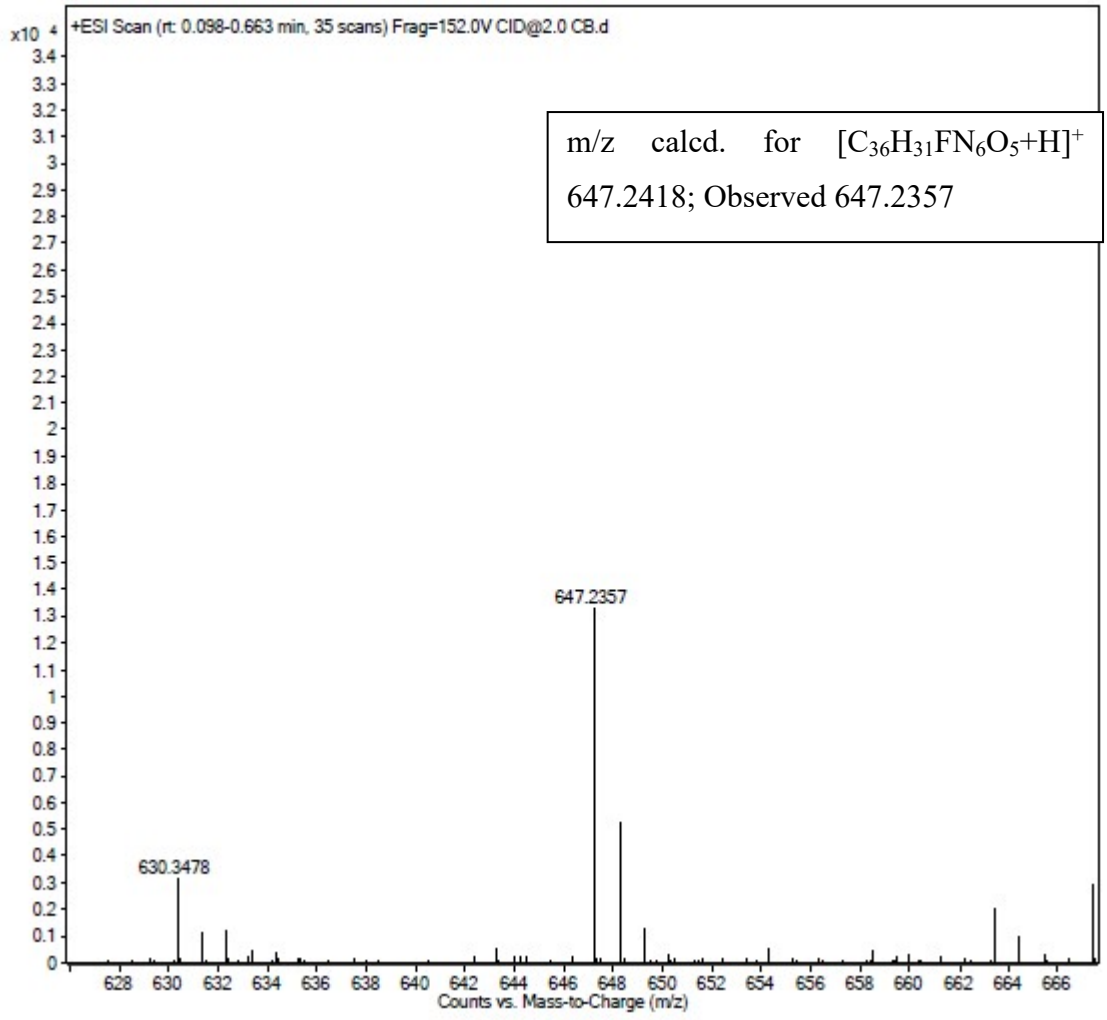
Mass spectrum of compound **7p**



^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **7q**



^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **7q**



Mass spectrum of compound **7q**

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