Electronic Supplementary Material (ESI) for ChemComm.

A novel molecular scaffold resensitizes multidrug-resistant *S. aureus* to fluoroquinolones

Apurva Panjla,[a] Grace Kaul,[b] Manjulika Shukla,[b] Shubhandra Tripathi,[a] Nisanth N. Nair,[a*] Sidharth Chopra[b*] and Sandeep Verma[a*]

Abstract: Nosocomial infections arising from opportunistic pathogens, such as *Staphylococcus aureus*, are growing unabated, compounded by the rapid emergence of antimicrobial resistance. Herein, we demonstrate a new molecular design that exhibits excellent activity against multidrug-resistant *S. aureus* with no cytotoxicity and resensitizes fluoroquinolones (FQ) towards FQ-resistant methicillin-resistant *S. aureus* strains, with DNA gyrase B as the likely molecular target as determined by molecular dynamics simulations.

Table of contents

1. Experimental procedures

1.1 Materials and methods:

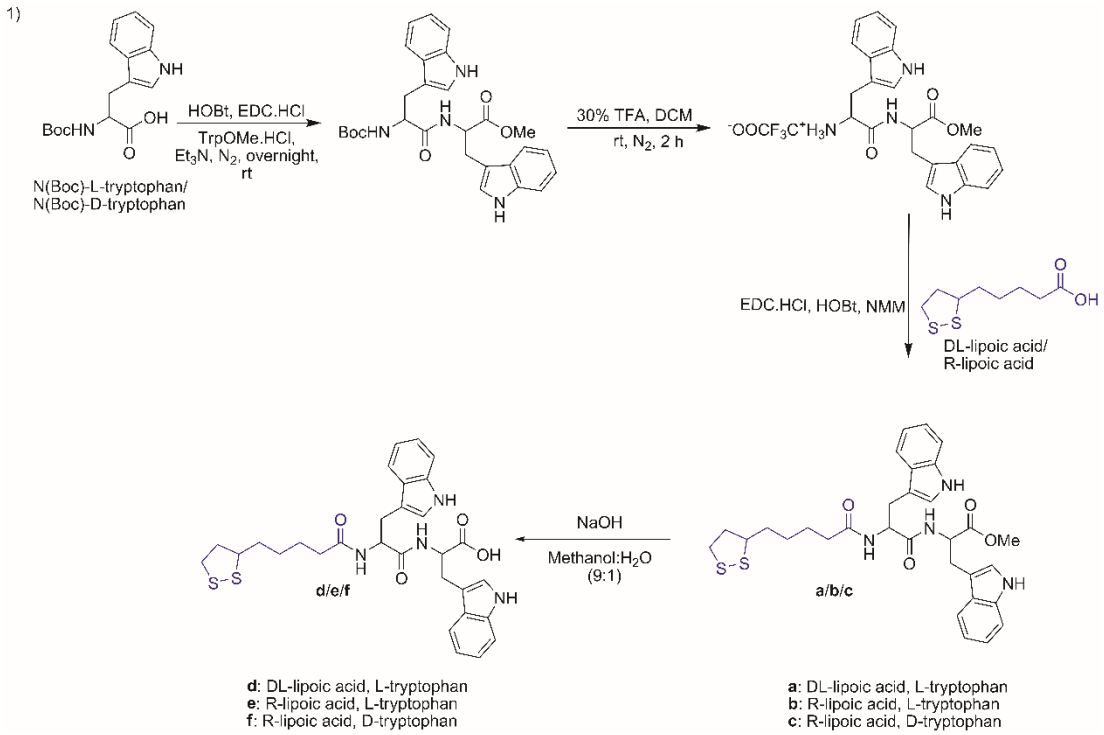
Acetonitrile (CH_3CN), dichloromethane (DCM), methanol (MeOH) were distilled following standard procedures before use. Boc anhydride, hexachloroethane, triphenylphosphine and thionyl chloride were obtained from Spectrochem Pvt. Ltd. (Mumbai, India). L-tryptophan, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, 1-hydroxybenzotriazole, trifluoroacetic acid, N-methylmorpholine were purchased from Avra synthesis Pvt. Ltd. (Hyderabad, India). DL-lipoic acid, rhodamine B and 1-boc-piperazine were purchased from Sisco Research Laboratories Pvt. Ltd. Sodium bicarbonate, HPLC water, and sodium chloride were purchased from Merck. Hydrochloric acid from Fischer scientific. R-lipoic acid was purchased from TCI Chemicals (India) Pvt. Ltd. Amberlite Resin, triethylamine were obtained from S. D. Fine Chem. Ltd. (Mumbai). ^1H and ^{13}C NMR spectra were recorded on JEOL-JNM ECS 400/500 model operating at 400/500 MHz and 100/125 MHz, respectively. HRMS spectra were recorded at IIT Kanpur, India, on Waters, Q-Tof Premier Micromass HAB 213 mass spectrometer using capillary voltage 2.6-3.2 kV.

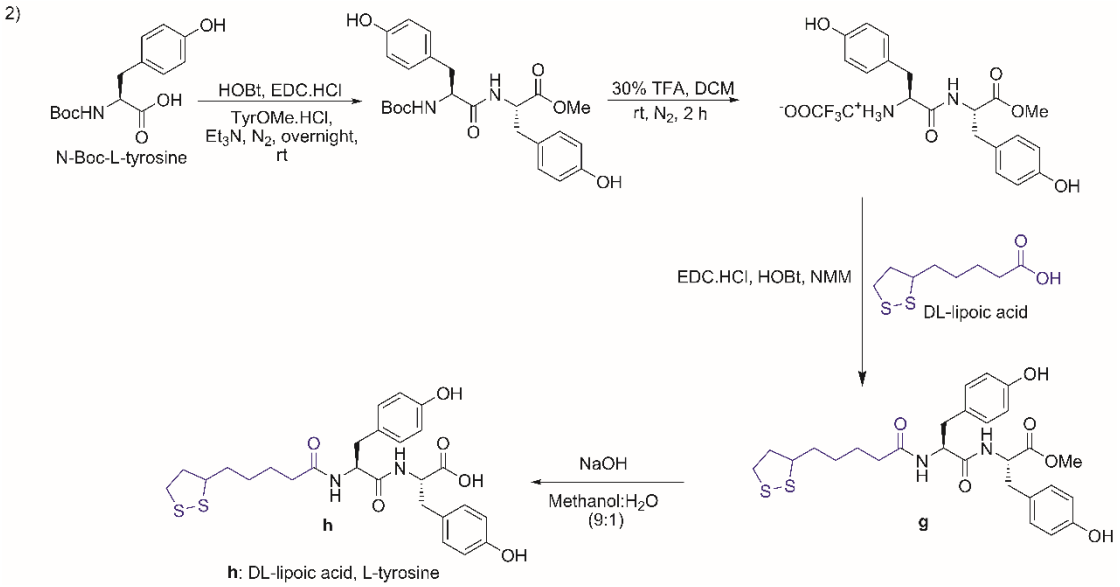
1.2 High-Performance Liquid Chromatography (HPLC)

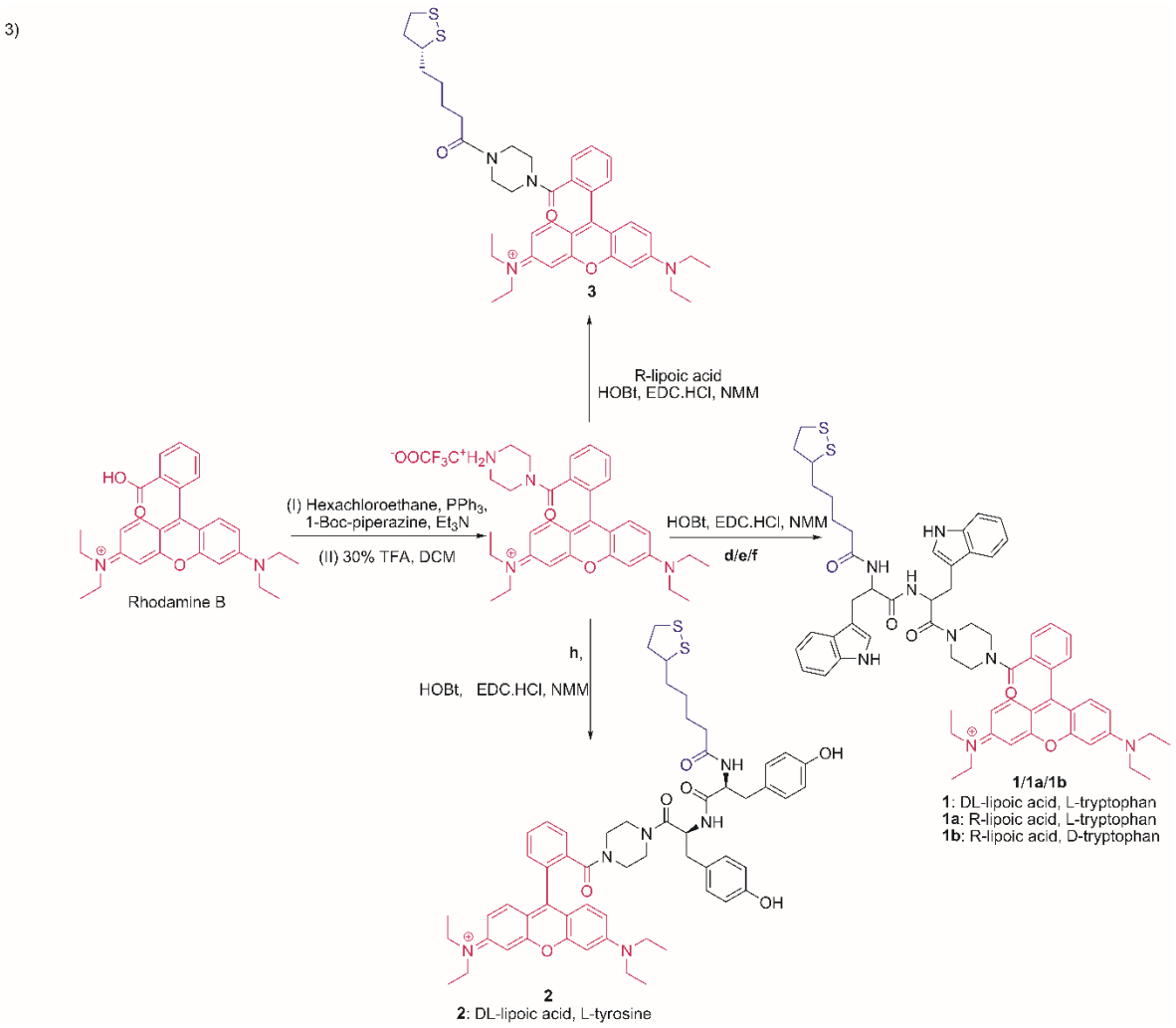
HPLC analysis was performed on HPLC system (Agilent Technologies 1260 infinity) equipped with a quaternary pump (G1311B), auto liquid sampler (G1329B), diode array detector (G1315D) and analytical scale-fraction collector (G1364C). Instrument control, data acquisition and data analysis were performed using ChemStation software (Agilent Technologies, Wokingham, UK). A ZORBAX Eclipse plus C18 (250 x 4.6 mm) column with 5 µm particle size at room temperature from Agilent technologies was used for compounds **1** and **1a**, and Lichrospher 100 RP-18 (5 µM) column from Merck was used for HPLC analysis of compounds **2** and **1b**. The mobile phase consisted of acetonitrile/water with 0.1% TFA, and the flow rate was 1.0 mL/min. Injection volume was 10 µL and the column effluent was monitored at 220 and 568 nm.

1.3 Synthesis and characterization of compounds

The designed peptide conjugates were synthesized using solution phase synthesis protocol (Scheme S1). Lipoic acid was conjugated to the amine end of the dipeptide via EDC coupling. Rhodamine B was functionalized with piperazine linker, which was then coupled to the acid end of lipoic acid-dipeptide conjugate via EDC coupling. The detailed scheme has been provided below.







Scheme S1: Synthetic route for compounds **1**, **1a**, **1b**, **2** and **3**.

Compound 1

Synthesis of **a**: A solution of lipoic acid (1.56 g, 7.56 mmol) in DCM (50 mL) was cooled to 0 °C and 1-hydroxybenzotriazole (1.33 g, 9.83 mmol) was added, followed by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.88 g, 9.83 mmol). After half an hour, a clear solution was obtained, to which TFA salt of tryptophan dipeptide methyl ester (4.31 g, 8.33 mmol) was added, followed by the addition of N-methylmorpholine (997 µL, 9.07 mmol). The reaction mixture was stirred for 12 h. The crude compound was acidified under ice cold condition with 1 N HCl to pH 2-3 and extracted with (3 x 15 mL) dichloromethane. The organic layer was washed with 10% $NaHCO_3$ solution (3 x 10 mL), and finally with saturated brine solution (2 x 10 mL), the combined organic layer was dried over anhydrous sodium sulphate. Dichloromethane was evaporated to get the crude compound which was further purified with a silica gel column chromatography (0-2% CH_3OH gradient in CH_2Cl_2) to give compound **a** as a light yellow solid (3.6 g, 80.32% yield), R_f value is 0.4 (5% methanol in dichloromethane). ^{1}H NMR (500 MHz, DMSO-d_6) = δ (ppm) 10.87 (s, 1H), 10.78 (s, 1H), 8.32 (d, J = 6.8 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.32 (dd, J = 11.0, 8.4 Hz, 2H), 7.15 (s, 1H), 7.12 – 7.01 (m, 3H), 6.97 (q, J = 7.3 Hz, 2H), 4.63 – 4.59 (m, 1H), 4.54 (q, J = 7.0 Hz, 1H), 3.55 (s, 3H), 3.51 – 3.43 (m, 1H), 3.20 – 3.02 (m, 5H), 2.88 (dd, J = 14.6, 9.3 Hz, 1H), 2.36 – 2.24 (m, 1H), 2.00 (d, J = 7.2 Hz, 2H), 1.81-1.72 (m,1H), 1.59 – 1.10 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) = δ (ppm) 172.68, 172.41, 136.57, 136.53, 127.88, 127.59, 124.25, 124.06, 121.50, 121.32, 119.02, 118.96, 118.65, 118.49, 111.96, 111.74, 110.66, 109.73, 56.54, 55.46, 53.64, 53.39, 52.35, 38.60, 38.58, 35.54, 34.58, 28.66, 28.24, 27.48, 25.41. HRMS-ESI: (m/z) Observed $[M+H]^+$ = 593.2250, Calculated $[M+H]^+$ = 593.2251.

Synthesis of **1**: Rhodamine B piperazine amide was synthesized using procedure reported by *Shuqi Wu and co-workers*.[1] Compound **a** (1 g, 1.6 mmol) was dissolved in 10 mL methanol. Then, a solution of NaOH (97.24 mg, 2.3 mmol) in distilled water (2 mL) was added. The mixture was stirred at room temperature for 2 h. The completion of reaction was monitored with TLC. The reaction mixture was passed over cation exchange resin. The solution obtained was evaporated under reduced pressure to obtain the crude product **d**. The crude product **d** (500 mg, 0.86 mmol) of lipoic acid ditryptophan conjugate was dissolved in dichloromethane (10 mL). To this solution, 1-hydroxybenzotriazole (151 mg, 1.12 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (215 mg, 1.12 mmol) were added at 0 °C under nitrogen atmosphere. After half an hour, N-methyl morpholine (113 µL, 1.04 mmol) was added followed by the addition of TFA salt of rhodamine piperazine amide (593 mg, 0.95 mmol) and stirring was continued for 12 h at room temperature. After completion of the reaction, the mixture was washed with 10% aqueous sodium bicarbonate solution twice and then twice with 1 N aqueous hydrochloric acid solution. The combined organic layer was washed with brine solution and dried over anhydrous sodium sulphate. The organic phase was subsequently evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-3% CH_3OH gradient in CH_2Cl_2) to give pure product as pink solid (600 mg, 62.28%). R_f value is 0.6 (10% methanol in dichloromethane). ^{1}H NMR (500 MHz, DMSO-d_6) = δ (ppm) 10.90 – 10.80 (m, 2H), 8.18 (s, 1H), 7.93 (s, 1H), 7.73 (s, 2H), 7.56 – 7.45 (m, 4H), 7.35 – 7.28 (m, 2H), 7.12 – 6.91 (m, 12H), 4.88 (s, 1H), 4.59 (dd, J = 13.3, 8.4 Hz, 1H), 3.65 – 3.48 (m, 8H), 3.22 – 2.84 (m, 12H), 2.36 – 2.25 (m, 1H), 2.04 (s, 2H), 1.81 – 1.72 (m, 1H), 1.57 – 1.38 (m, 4H), 1.22 – 1.16 (s, 17H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ (ppm) 173.28, 171.22, 170.44, 167.20, 157.73, 155.64, 136.51, 136.36, 130.39, 130.13, 127.76, 124.15, 121.55, 119.05, 118.37, 114.09, 113.98, 111.97, 111.95, 111.86, 108.87, 96.29, 77.48, 77.16, 76.84, 56.55, 56.49, 54.01, 46.11, 40.27, 38.53, 36.18, 34.59, 29.82, 28.81, 25.27, 12.61. HRMS-ESI: (m/z) Observed $[M]^+$ = 1071.4983, Calculated $[M]^+$ = 1071.4983.

Compound 1a

Synthesis of **b**: The same procedure was used as followed for compound **a** using R-lipoic acid. ^{1}H NMR (400 MHz, DMSO-d_6) = δ (ppm) 10.88 (s, 1H), 10.79 (s, 1H), 8.34 (d, J = 7.0 Hz, 1H), 8.03 – 7.82 (m, 1H), 7.64 – 7.55 (m, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.39 – 7.24 (m, 2H), 7.24 – 6.81 (m, 6H), 4.63 – 4.51 (m, 2H), 3.55 (s, 3H), 3.51 – 3.43 (m, 1H), 3.21 – 2.99 (m, 5H), 2.90 – 2.84 (m, 1H), 2.33 – 2.36 (m, 1H), 2.00 (d, J = 6.7 Hz, 2H), 1.78 – 1.71 (m, 1H), 1.54 – 1.13 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) = δ (ppm) 174.30, 172.06, 136.01, 127.36, 127.06, 123.66, 123.47, 120.92, 120.74, 118.40, 118.09, 117.92, 111.39, 111.21, 109.18, 109.10, 56.07, 55.99, 51.75, 38.07, 38.02, 35.02, 34.06, 34.01, 33.48, 29.76, 29.29, 28.17, 28.07, 24.83, 24.25. HRMS-ESI: (m/z) Observed $[M+H]^+$ = 593.2250, Calculated $[M+H]^+$ = 593.2251.

Synthesis of **1a**: The same procedure was used as followed for compound **1** using R-lipoic acid. ^{1}H NMR (400 MHz, DMSO-d_6) = δ 10.90 – 10.80 (m, 2H), 8.18 (s, 1H), 7.94 (s, 1H), 7.73 (s, 2H), 7.56 – 7.28 (m, 6H), 7.10 – 6.92 (m, 10H), 4.88 (s, 1H), 4.56 (s, 2H), 3.69 – 3.37 (m, merged with DMSO-d_6 residual water peak, 8H), 3.11 – 2.91 (m, 12H), 2.30 (s, 1H), 2.03 (s, 2H), 1.76

(d, 2H), 1.52 – 1.16 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) = δ 173.60, 171.54, 170.76, 167.51, 158.05, 155.96, 136.83, 136.68, 130.95, 130.70, 130.45, 128.08, 124.89, 124.46, 121.86, 119.37, 118.69, 114.29, 112.18, 109.19, 96.61, 56.81, 46.42, 40.59, 38.85, 36.50, 34.91, 30.14, 29.13, 25.59, 12.93. HRMS-ESI: (m/z): Observed $[M]^+$ = 1071.4988, Calculated $[M]^+$ = 1071.4983.

Compound 1b

Synthesis of **c**: The same procedure was used as followed for compound **a** using D-tryptophan and R-lipoic acid. ^{1}H NMR (400 MHz, DMSO-d_6) = δ (ppm) 10.88 (s, 1H), 10.79 (s, 1H), 8.34 (d, J = 7.3 Hz, 1H), 7.91 (d, J = 8.3 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.33 (t, J = 8.5 Hz, 2H), 7.18 – 6.86 (m, 6H), 4.66 – 4.51 (m, 2H), 3.61 – 3.50 (m, 3H), 3.51 – 3.43 (m, 1H), 3.24 – 2.97 (m, 5H), 2.89 (dd, J = 14.6, 9.2 Hz, 1H), 2.69 (s, 1H), 2.35 – 2.37 (m, 1H), 2.09 – 1.88 (m, 2H), 1.87 – 1.64 (m, 1H), 1.55 – 1.21 (m, 6 H). ^{13}C NMR (100 MHz, DMSO-d_6) = δ (ppm) δ 172.16, 171.95, 171.90, 136.06, 127.38, 127.09, 123.74, 123.54, 120.98, 120.81, 118.45, 118.14, 117.98, 111.45, 111.23, 110.15, 109.23, 56.03, 53.13, 52.89, 51.83, 38.07, 35.04, 34.07, 29.04, 28.14, 27.72, 26.97, 24.90. HRMS-ESI: (m/z) Observed $[M+H]^+$ = 593.2250, Calculated $[M+H]^+$= 593.2251.

Synthesis of **1b**: The same procedure was used as followed for compound **1** using using D-tryptophan and R-lipoic acid. ^{1}H NMR (400 MHz, DMSO-d_6) = δ (ppm) 10.90 – 10.70 (m, 2H), 8.18 (s, 1H), 7.94 (s, 1H), 7.73 (s, 1H), 7.56 – 7.46 (d, J = 39.1 Hz, 4H), 7.28 (s, 2H), 7.10 – 6.92 (m, 10H), 4.88 (s, 1H), 4.56 (s, 1H), 3.68 – 3.39 (m, merged with DMSO-d_6 residual water peak, 8H), 3.11 – 2.91 (m, 12H), 2.31 – 2.30 (m, 1H), 2.03 (s, 2H), 1.77 (s, 1H), 1.52 – 1.16 (m, 19H).^{13}C NMR (100 MHz, DMSO-d_6) = δ (ppm) 172.45, 171.70, 170.24, 166.87, 157.56, 155.59, 136.54, 135.55, 132.30, 130.94, 130.31, 127.83, 124.50, 124.05, 121.35, 121.30, 118.92, 118.82, 118.67, 118.60, 114.76, 113.52, 111.78, 110.65, 96.41, 56.52, 49.71, 45.90, 38.60, 35.58, 34.61, 28.62, 28.16, 25.44,12.93. HRMS-ESI: (m/z) Observed $[M]^+$ = 1071.4988, Calculated $[M]^+$ = 1071.4983.

Compound 2

Synthesis of **g**: A solution of lipoic acid (1.63 g, 7.91 mmol) in DCM (50 mL) was cooled to 0 °C and 1-hydroxybenzotriazole (1.39 g, 10.29 mmol) was added, followed by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.96 g, 10.26 mmol). After half an hour, a clear solution was obtained, to which TFA salt of tyrosine dipeptide methyl ester (2.98 g, 8.72 mmol) was added, followed by the addition of N-methylmorpholine (1.13 mL, 9.5 mmol). The reaction mixture was stirred for 12 h. The crude compound was acidified under ice cold condition with 1 N HCl to pH 2-3 and extracted with (3 x 15 mL) dichloromethane. The organic layer was washed with 10% $NaHCO_3$ solution (3 x 10 mL), and finally with saturated brine solution (2 x 10 mL), and the combined organic layer was dried over anhydrous sodium sulphate. Dichloromethane was evaporated to get the crude compound which was further purified with a silica gel column chromatography (0-4% CH_3OH gradient in CH_2Cl_2) to give compound **g** as a light yellow solid (3.5 g, 81.04% yield), R_f value is 0.4 (6% methanol in dichloromethane). ^{1}H NMR (500 MHz, DMSO-d_6) = δ (ppm) 9.19-9.10 (m, 2H), 8.23 (d, J = 7.5 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.14 – 6.88 (m, 4 H), 6.66-6.61 (m, 4 H), 4.50 – 4.35 (m, 2H), 3.58 (d, J = 7.7 Hz, 3 H), 3.19-3.08 (m, 2 H), 3.00 – 2.71 (m, 4 H), 2.60-2.55 (m,1H), 2.43 – 2.32 (m, 1H), 2.06 – 1.96 (m, 2H), 1.86-1.79 (m, 5H), 1.59 – 1.19 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) = δ (ppm) 172.38, 172.29, 172.20, 172.17, 156.55, 156.22, 130.57, 130.52, 128.47, 127.48, 115.58, 115.41, 115.26, 56.59, 56.54, 54.44, 54.19, 52.28, 38.62, 38.60, 37.26, 36.47, 35.55, 35.51, 34.62, 28.61, 25.47. HRMS-ESI: (m/z) Observed $[M+H]^+$ = 547.1933, Calculated $[M+H]^+$ = 547.1931.

Synthesis of **2**: Compound **g** (1 g, 1.8 mmol) was dissolved in 10 mL methanol. Then, a solution of NaOH (97.24 mg, 2.3 mmol) in distilled water (2 mL) was added. The mixture was stirred at room temperature for 2 h. The completion of reaction was monitored with TLC. The reaction mixture was passed over cation exchange resin. The solution obtained was evaporated under reduced pressure to obtain the crude product **h**. The crude compound **h** (600 mg, 1.12 mmol) was dissolved in dichloromethane (10 mL) to obtain a clear solution, to which 1-hydroxybenzotriazole (198 mg, 1.47 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (280 mg, 1.46 mmol) at 0 °C under nitrogen atmosphere were added. After half an hour, N-methylmorpholine (148 µL, 1.46 mmol) was added followed by addition of TFA salt of rhodamine piperazine amide (774 mg, 1.23 mmol) and the stirring was continued for 12 h at room temperature. After completion of the reaction, the mixture was washed with 10% aqueous sodium bicarbonate solution twice and then with 1 N aqueous hydrochloric acid solution. The combined organic layer was washed with brine solution and dried over anhydrous sodium sulphate. The organic phase was subsequently evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-3% CH_3OH gradient in CH_2Cl_2) to give pure product as pink solid (700 mg, 62.25%). R_f value is 0.4 (10% methanol in dichloromethane). ^{1}H NMR (500 MHz, DMSO-d_6) = δ (ppm) 9.20 (s, 2H), 8.11 (s, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.75 (s, 2H), 7.67 (s, 1H), 7.51 (s, 1H), 7.13 (s, 5H), 7.01 – 6.82 (m, 7H), 6.61 (d, J = 8.0 Hz, 4H), 4.75 (d, J = 5.9 Hz, 1H), 4.39 (s, 1H), 3.89 – 3.47 (m, 10H), 3.37 (s, 2H), 3.21 – 3.06 (m, 6H), 2.78 (s, 2H), 2.68 (dd, J = 13.0, 6.0 Hz, 1H), 2.43 – 2.28 (m,

1H), 2.01 (s, 2H), 1.82 (dd, J = 12.7, 6.4 Hz, 2H), 1.64 – 1.51 (m, 1H), 1.47 (d, J = 7.9 Hz, 2H), 1.38 (s, 2H), 1.21 (s, 17H). ^{13}C NMR (125 MHz, DMSO-d_6) = δ (ppm) 172.36, 171.47, 157.59, 156.29, 155.66, 135.66, 132.25, 130.81, 130.49, 128.44, 115.48, 115.30, 114.81, 113.57, 96.46, 56.55, 53.31, 45.93, 38.61, 37.29, 37.15, 35.58, 34.65, 28.64, 25.47, 12.99. Observed mass $[M]^+$ = 1025.4663, Calculated $[M]^+$ = 1025.4664.

Compound 3:

Synthesis of **3**: A solution of lipoic acid (160 mg, 0.77 mmol) in DCM (20 mL) was cooled to 0 °C and 1-hydroxybenzotriazole (136 g, 1 mmol) was added, followed by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (193 mg, 1 mmol). After half an hour, a clear solution was obtained, to which TFA salt of rhodamine B piperazine amide (500 mg, 8.16 mmol) was added, followed by the addition of N-methylmorpholine (110 µL, 1 mmol). The reaction mixture was stirred for 12 h. The crude compound was acidified under ice cold condition with 1 N HCl to pH 2-3 and extracted with (3 x 15 mL) dichloromethane. The organic layer was washed with 10% $NaHCO_3$ solution (3 x 10 mL), and finally with saturated brine solution (2 x 10 mL), and the combined organic layer was dried over anhydrous sodium sulphate. The crude product was purified by silica gel column chromatography (0-3% CH_3OH gradient in CH_2Cl_2) to give pure product as pink solid (350 mg, 61%.) R_f value is 0.2 (10% methanol in dichloromethane).^{1}H NMR (400 MHz, DMSO-d_6) = δ 7.76 – 7.69 (m, 3H), 7.53 (s, 1H), 7.17 – 7.09 (m, 4H), 6.95 (s, 2H), 3.74 – 3.56 (m, 9H), 3.50 – 2.93 (m, 11H), 2.42 – 2.38 (m, 1H), 2.27 (t, *J* = 7.2 Hz, 2H), 1.89 – 1.81 (m, 1H), 1.74 – 1.29 (m, 7H), 1.20 (t, *J* = 6.8 Hz, 12 H).^{13}C NMR (125 MHz, DMSO-d_6) = δ (ppm) 172.36, 171.47, 157.59, 156.29, 155.66, 135.66, 132.25, 130.81, 130.49, 128.44, 115.48, 115.30, 114.81, 113.57, 96.46, 56.55, 53.31, 45.93, 38.61, 37.29, 37.15, 35.58, 34.65, 28.64, 25.47, 12.99. Observed mass $[M]^+$ = 699.3401, Calculated $[M]^+$ = 699.3397.

1.4 Biological assays

Bacterial strains and media

The bacterial panel consisting of ESKAPE pathogens namely *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (BAA-1705), *Acinetobacter baumannii* (BAA-1605), *Pseudomonas aeruginosa* (ATCC 27853) and, *Staphylococcus aureus* (ATCC 29213). This panel was further expanded to include drug-resistant clinical *S. aureus* strains, including resistant to vancomycin and other clinically utilized antibiotics. These strains were procured from BEI/NARSA/ATCC (Biodefense and Emerging Infections Research Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection, USA) and routinely cultivated on Mueller-Hinton Agar (MHA). Before starting the experiment, a single colony was picked from MHA plate, inoculated in Mueller-Hinton cation supplemented broth (CA-MHB II) and incubated overnight at 37 °C with shaking for 18-24 h to get the starter culture.

Minimal Inhibitory Concentration assays

Antibacterial susceptibility testing was carried out utilizing broth micro dilution assay according to CLSI guidelines. 10 mg/mL stock solutions of test and control compounds were prepared in DMSO and stored in -20 °C. Bacterial cultures were inoculated in appropriate media and OD_{600} of the cultures was measured, followed by dilution to achieve ~105 CFU/mL. The compounds were tested from 64-0.5 mg/L in two-fold serial diluted fashion with 2.5 µL of each concentration added per well of a 96-well round bottom microtiter plate. Later, 97.5 µL of bacterial suspension was added to each well containing the test compound along with appropriate controls. Presto blue (Thermo Fisher) resazurin-based dye used for visual identification of active drugs. The MIC plates were incubated at 37 °C for 18-24 h to obtain MIC.

Cell cytotoxicity assay

Cell toxicity was performed against Vero cells using the MTT assay. ~103 cells/well were seeded in 96 well plate and incubated at 37 °C with 5% CO_2 atmosphere. After 24 h, compound was added ranging from 100-12.5 mg/L and incubated for 72 h at 37 °C with 5% CO_2 atmosphere. After the incubation was over, MTT was added at 5 mg/L in each well, incubated at 37 °C for further 4 hours, residual medium was discarded, 0.1 mL of DMSO was added to solubilize the formazan crystals and OD was taken at 540 nm for the calculation of CC_{50}. CC_{50} is defined as the lowest concentration of compound which leads to a 50% reduction in cell viability. Doxorubicin was used as positive control and each experiment was repeated in triplicate.

Bacterial time kill kinetics

The presence or absence of bactericidal activity was assessed by the time-kill method. Briefly, *S. aureus* ATCC 29213 bacteria were diluted ~105 CFU/mL in MHB (Mueller-Hinton Broth) and treated with 1X and 10X of MIC of **1b** and vancomycin and incubated at 37 °C with shaking for 24 h. 100 µL samples were collected at the time intervals of 0 h, 1 h, 6 h and 24 h, serially diluted in PBS and plated on MHA followed by incubation at 37 °C for 18-20 h. Kill curves were constructed by counting the colonies from plates and plotting the CFU/mL of surviving bacteria at each time point in the presence and absence of compound. Each experiment was repeated three times in duplicate and mean data plotted.

Isolation of 1b resistant mutant in *S. aureus*

After the initial MIC experiment, serial passaging was initiated by harvesting bacterial cells growing at the highest concentration of the compound, the OD_{600} of the one-half the MIC well of the previous MIC assay and inoculating into fresh CA-MHBII. This inoculum was subjected to another MIC assay. After 24 h incubation period, cells growing in the highest concentration of the compound from the previous passage were once again harvested and assayed for the MIC. The process was repeated for 25 passages. The MIC value of the compound was plotted against the number of passages, and the fold increase in MIC was determined.

Synergism with FQ

Interaction of **1b** with FDA approved drugs was tested by the checkerboard method as reported earlier.[2] Serial two-fold dilutions of each drug were freshly prepared prior to testing. **1b** was diluted two-fold along the abscissa while the antibiotics were serially diluted along the ordinate. 95 µL of ~10^5 CFU/mL bacterial culture was added to each well and plates were incubated at 37 °C for 24 h. After the incubation was over, the ΣFICs (fractional inhibitory concentrations) were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone and FIC B is the MIC of drug B in the combination/MIC of drug B alone. The combination is considered synergistic when the ΣFIC is ≤0.5, indifferent when the ΣFIC is >0.5 to 4 and antagonistic when the ΣFIC is >4.

Time-kill analysis of NRS10193 and NRS10198 with Ciprofloxacin and 1b combination

To further validate the observed synergy by checkerboard method, time-kill analysis was performed using 1X MIC of ciprofloxacin and **1b** against FQ-resistant strains NRS10193 and NRS10198 along with appropriate controls. Briefly, FQ-resistant strains NRS10193 and NRS10198 were diluted ~10^5 CFU/mL in MHB and treated with 1X MIC of **1b** and Ciprofloxacin alone and in combination and incubated at 37 °C with shaking for 24 h. 100 µL samples were collected at the time intervals of 0 h, 1 h, 6 h and 24 h, serially diluted in PBS and plated on MHA followed by incubation at 37 °C for 18-20 h. Kill curves were constructed by counting the colonies from plates and plotting the CFU/mL of surviving bacteria at each time point in the presence and absence of compound. Each experiment was repeated three times in duplicate and the mean data is plotted.

1.5 Computational details

Molecular docking

Molecular docking was performed using AutoDock-4.2 Software[3] using Lamarckian Genetic Algorithm.[4] Grid covering the binding site with grid spacing of 0.375 was created. Molecular docking was performed following parameters: 150 randomly positioned poses; maximum number of 2.5 X 107 energy evaluations; maximum number of generations 2.5 X 107; mutation rate of 0.02; crossover rate 0.8 and elitism parameter 1.200 docked poses were generated and clustered with RMSD tolerance of 2 Å. On the basis of binding energy and clustering best docked conformation was selected and utilized to perform MD simulations.

Molecular dynamics

Molecular dynamics (MD) simulation was performed using GROMACS simulation package.[5] AMBER99SB force field[6] was used for protein, and GAFF force field[7] was used to define force field parameters for **1b**. The charges for **1b** were derived using the RESP method using the RED program package.[8] Water molecules were modelled using the rigid TIP3P force-field[9] and eight Na^+ ions were added to neutralize the system. After energy minimization, system was equilibrated in NPT ensemble for 4 ns for 1 atm and 300 K. For equilibrating in NPT ensemble, isotropic Berendsen barostat was employed with pressure coupling of 1.0 ps. At the equilibrated density of the system, NVT ensemble simulation was carried out for 50 ns. Velocity rescale thermostat with a coupling constant of 0.1 ps was used for thermostating the system at 300 K. Long-range electrostatics were handled by the Particle-Mesh-Ewald (PME) method.[10] A time step of 1 fs was used for integrating the equation of motion. The ligplot interaction image of **1b** and gyrase B was generated using the Ligplus program.[11] For other visualizations, VMD program package was employed.[12]

2. Results and discussion

Table S1: Synergism of **1b** with fluoroquinolones against *S. aureus* (ATCC 29213)

Drug	MIC (µg/mL)	MIC of **1b** in presence of drug (µg/mL) 'A'	MIC of drug in presence of **1b** (µg/mL) 'B'	FIC-A	FIC-B	FICI (FIC-A +FIC-B)	Inference
1b	4						
Levofloxacin	0.25	0.5	0.06	0.25	0.24	0.49	Synergic
Moxifloxacin	0.03	1	0.015	0.5	0.5	1	No interaction
Ciprofloxacin	0.5	1	0.125	0.5	0.25	0.75	No interaction

Table S2: Synergism of **1b** with fluoroquinolones against FQ-resistant NRS10193

Drug	MIC (µg/mL)	MIC of **1b** in presence of drug (µg/mL) 'A'	MIC of drug in presence of **1b** (µg/mL) 'B'	FIC-A	FIC-B	FICI----(FIC-A +FIC-B)	Inference
1b	4						
Levofloxacin	64	2	0.25	0.5	0.003	0.50	Synergic
Moxifloxacin	8	2	0.015	0.5	0.001	0.50	Synergic
Ciprofloxacin	128	1	0.2	0.25	0.001	0.25	Synergic

Table S3: Synergism of **1b** with fluoroquinolones against FQ-resistant NRS10198

Drug	MIC (µg/mL)	MIC of **1b** in presence of drug (µg/mL) 'A	MIC of drug in presence of **1b** (µg/mL) 'B'	FIC-A	FIC-B	FICI----- (FIC-A +FIC-B)	Inference
1b	4						
Levofloxacin	32	1	0.25	0.25	0.007813	0.25	Synergic
Moxifloxacin	4	1	2	0.25	0.5	0.75	No interaction
Ciprofloxacin	512	1	128	0.25	0.25	0.5	Synergic

Table S4: Binding energy of **1b** and known inhibitors with DNA Gyrase B in molecular docking

Molecules	Binding Energy (kcal/mol) against Gyrase B
Aminobenzimidazole	-8
Novobiocin	-9
1b	-11
2	-8
3	-8

Table S5: Table showing binding energy of **1b** and know inhibitor Novobiocin with ParE (Topoisomerase IV) in molecular docking

Ligand	Binding Energy (kcal/mol) with ParE
1b	-9
Novobiocin (Known Inhibitor)	-9

Table S6: Table showing the Hydrogen bond occupancy between **1b** and DNA Gyrase B during 50 ns MD simulation time period

H-bond Donor	Atom Name	H-bond Receptor	Atom Name	%Occupancy
Asn54	ND2	**1b**	O1	66
1b	N7	Asn54	OD1	12
1b	N6	Asp53	OD1	66
1b	N4	Glu50	OE1/OE2	68

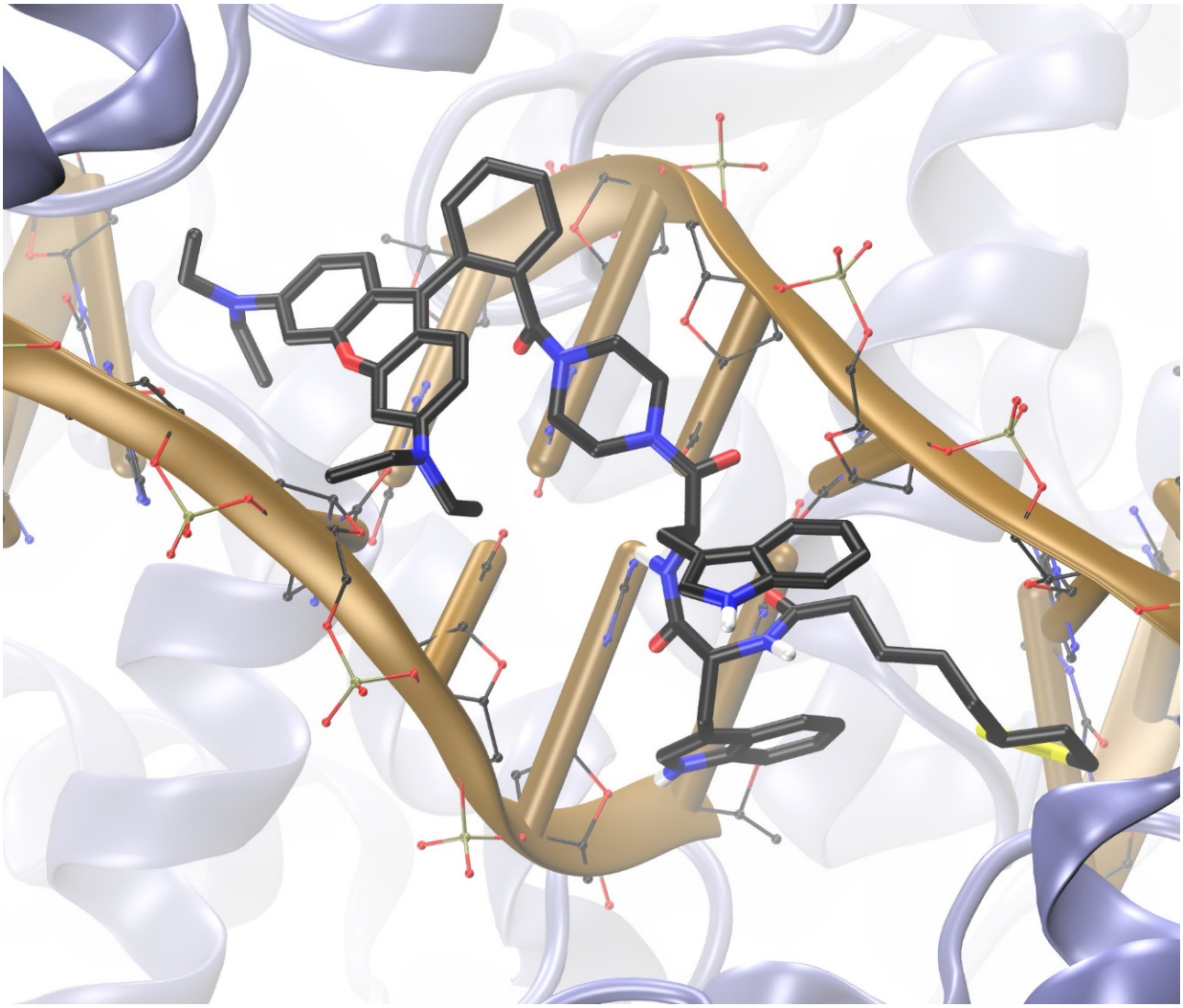


Figure S1: Figure showing **1b** is unable to intercalate between the nitrogen base pairs (like fluoroquinolones) of cleaved DNA in DNA gyrase A complex. Color code: DNA Gyrase A (violet); DNA (brown); DNA atoms shown in CPK representation and **1b** shown in licorice representation

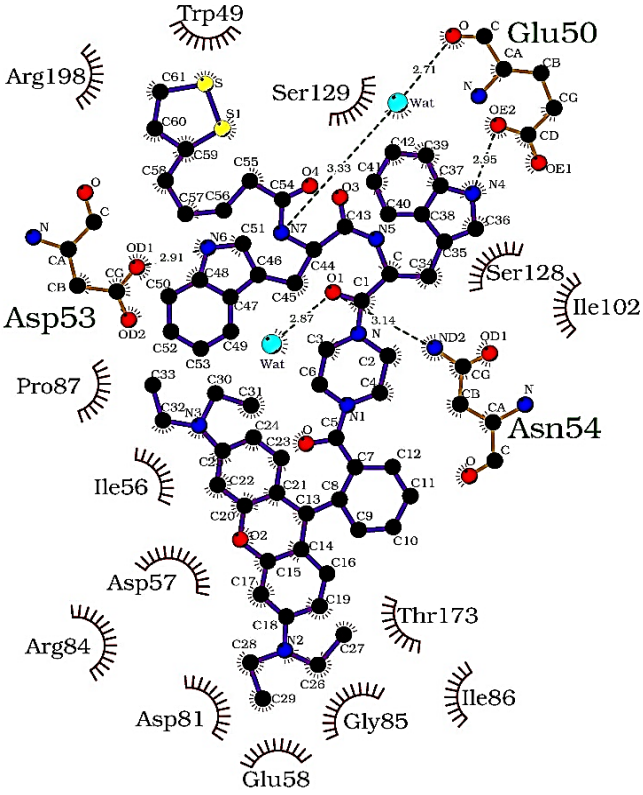


Figure S2: **1b** interactions with DNA gyrase B are shown using ligplot for the average structure of the top cluster computed from the canonical ensemble molecular dynamics trajectory. Potential hydrogen bonds are shown using green dashed lines and residues with hydrophobic contacts are indicated by arc with spokes pointing towards the ligand atoms, while the corresponding contact atoms of the ligands are shown with spokes pointing towards them

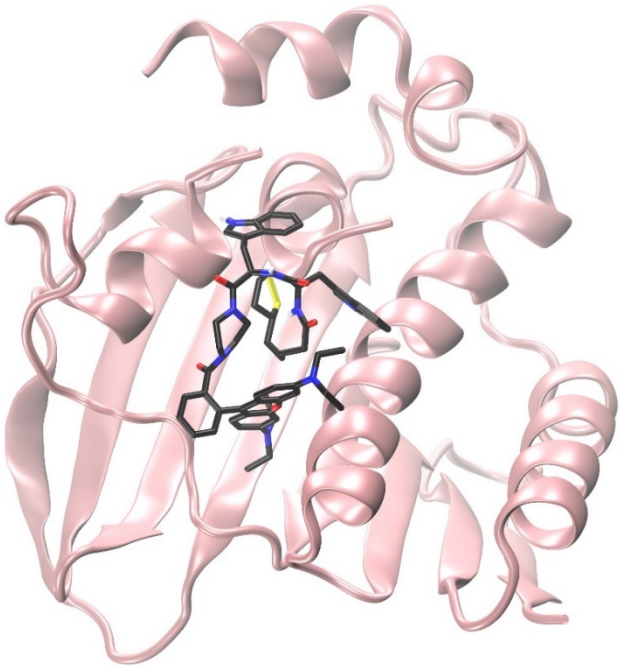


Figure S3: Binding conformation of **1b** in the molecular docking in the ATPase binding site of ParE (Topo IV). Color code: ParE (pink) and **1b** shown in licorice representation

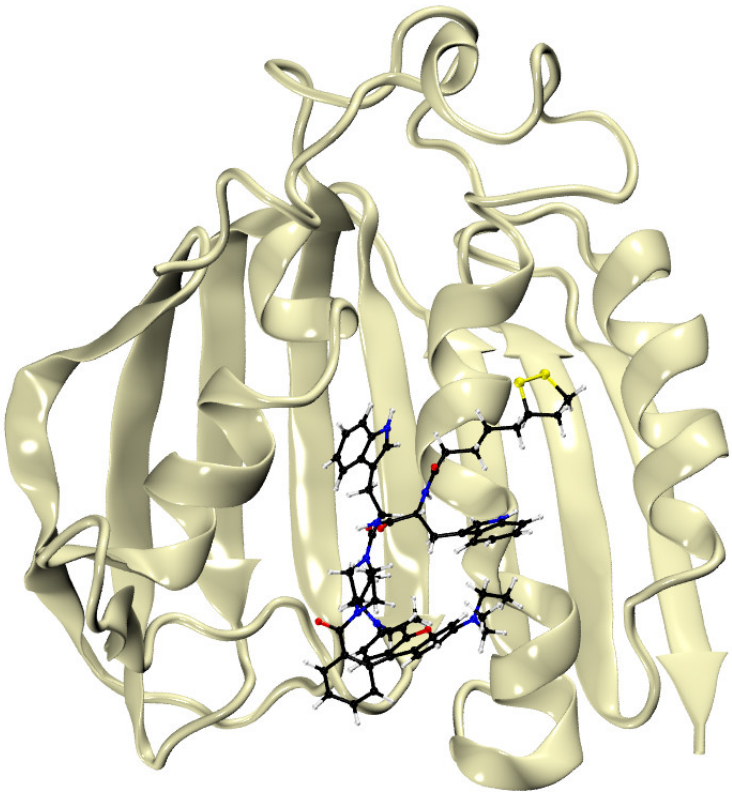
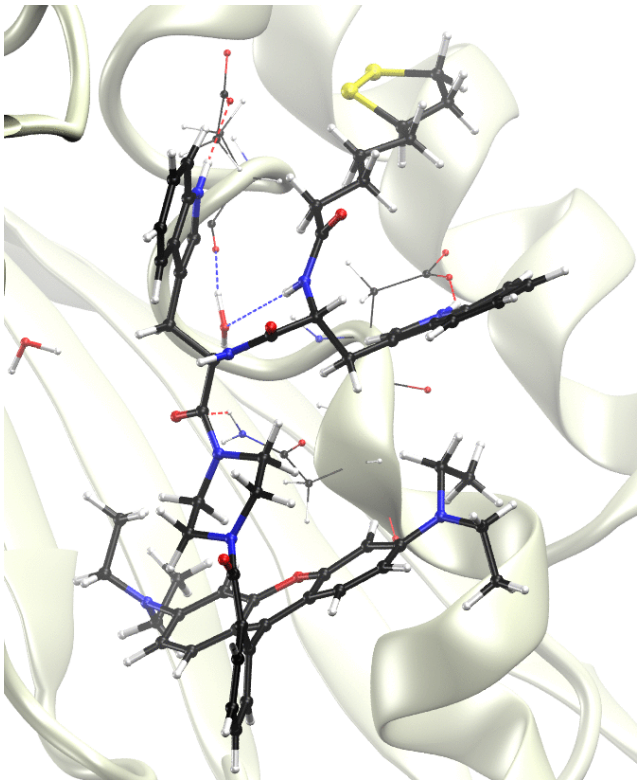


Figure S4: Binding conformation of **1b** in the MD equilibrated structure in the ATPase binding site of Gyrase B. Solvent molecules are not shown for clarity



Movie S1: Binding interaction of **1b** in the ATPase binding site of gyrase B from the MD simulation trajectory at 300 K. Solvent molecules are not shown for clarity

3. HPLC Chromatograms

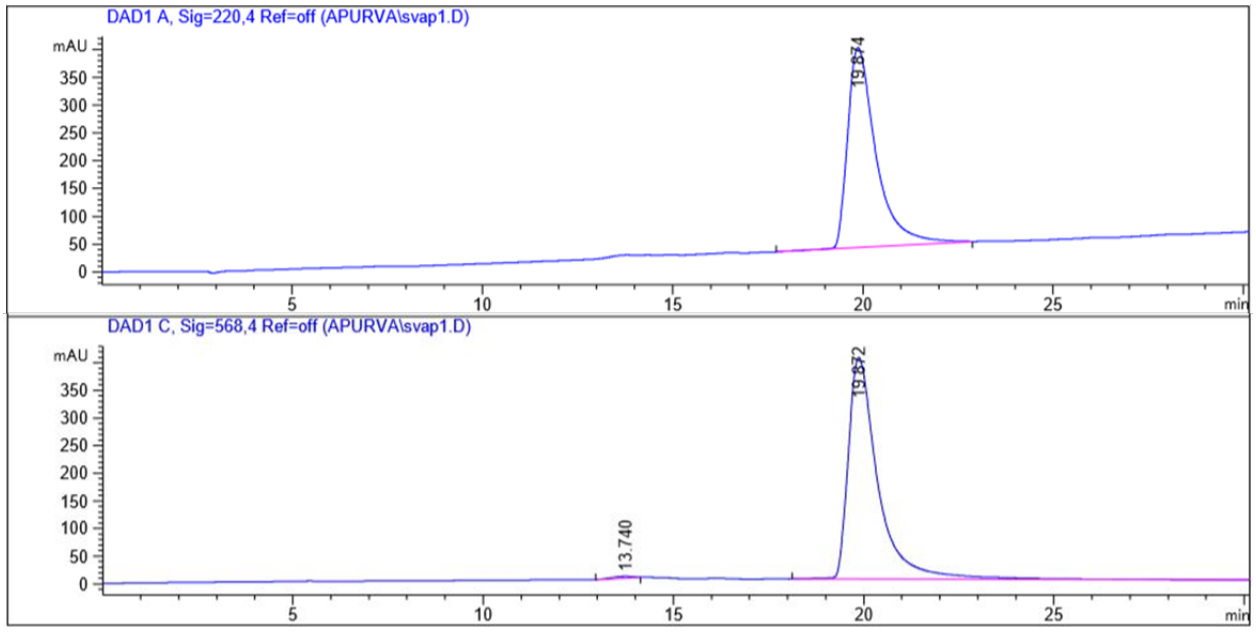


Figure S5: HPLC chromatograms of **1** at 220 nm and 568 nm in 0.1% TFA-CH_3CN/H_2O gradient system

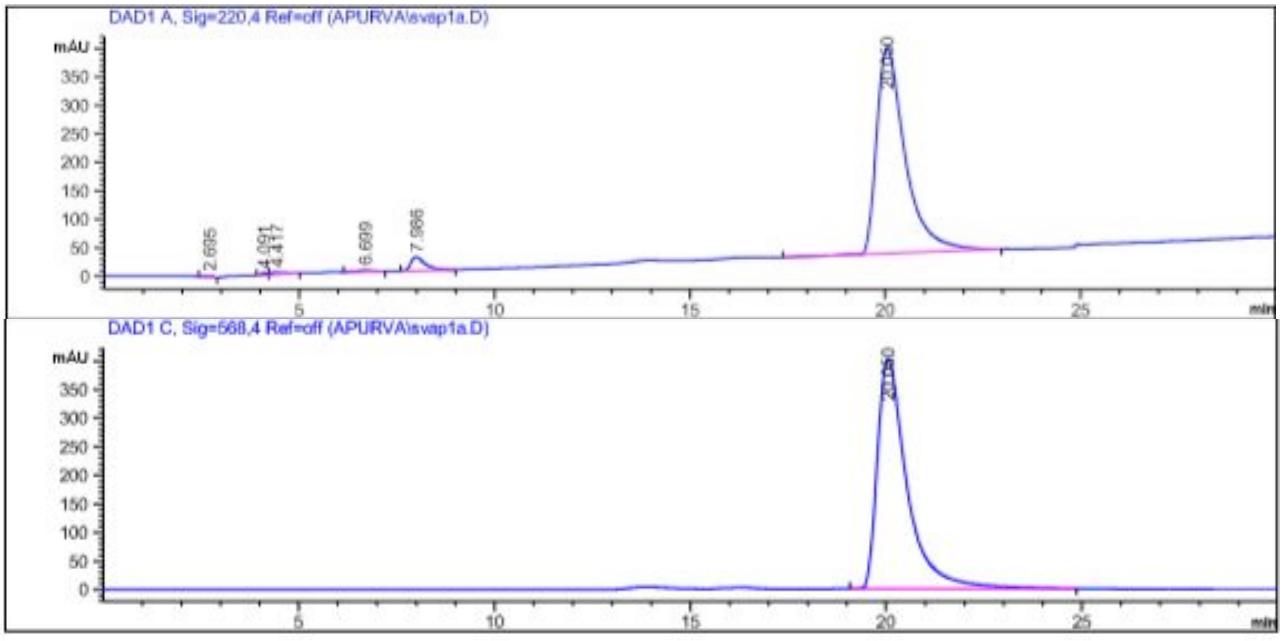


Figure S6: HPLC chromatograms of **1a** at 220 nm and 568 nm in 0.1% TFA- CH_3CN /H_2O gradient system

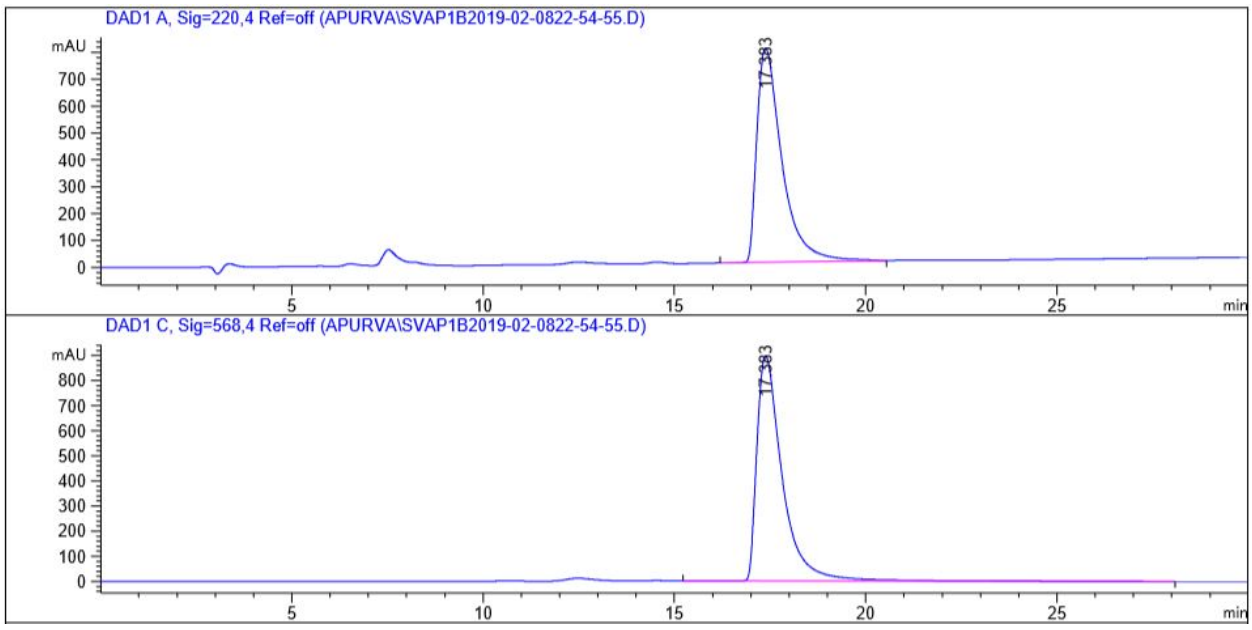


Figure S7: HPLC chromatograms of **1b** at 220 nm and 568 nm in 0.1% TFA- CH_3CN /H_2O gradient system

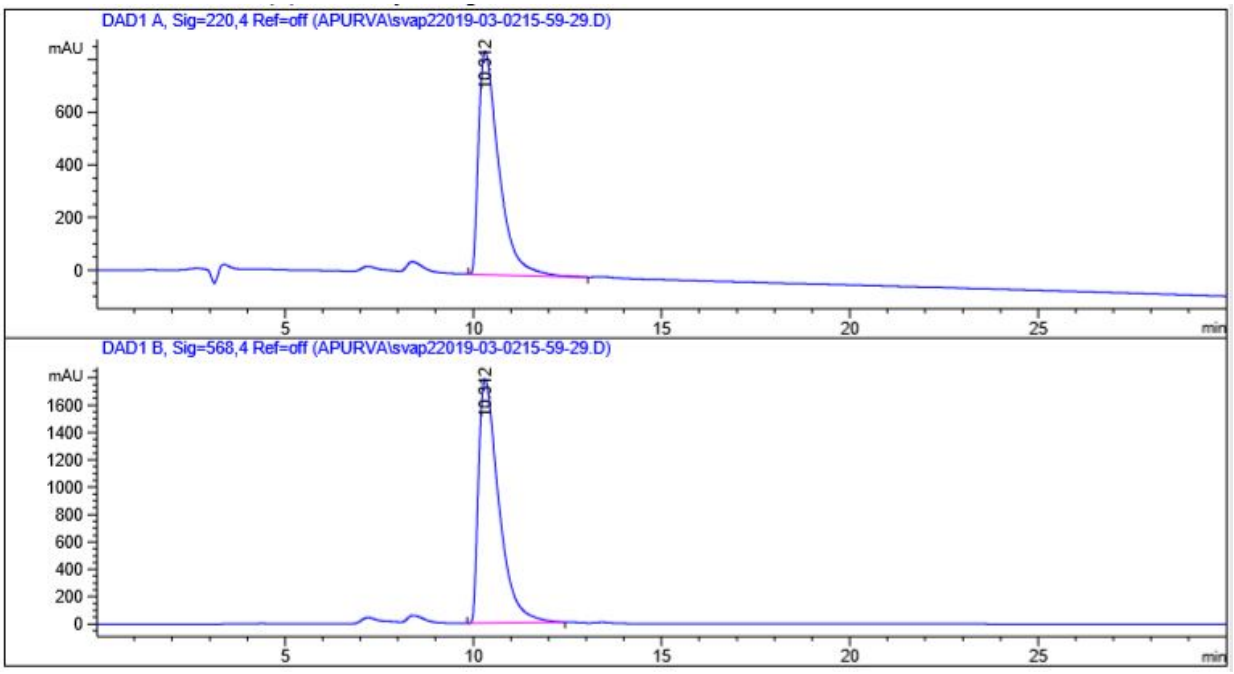


Figure S8: HPLC chromatograms of **2** at 220 nm and 568 nm in 0.1% TFA- CH_3CN /H_2O gradient system

4. ^{1}H NMR, ^{13}C NMR, and HRMS-ESI spectra

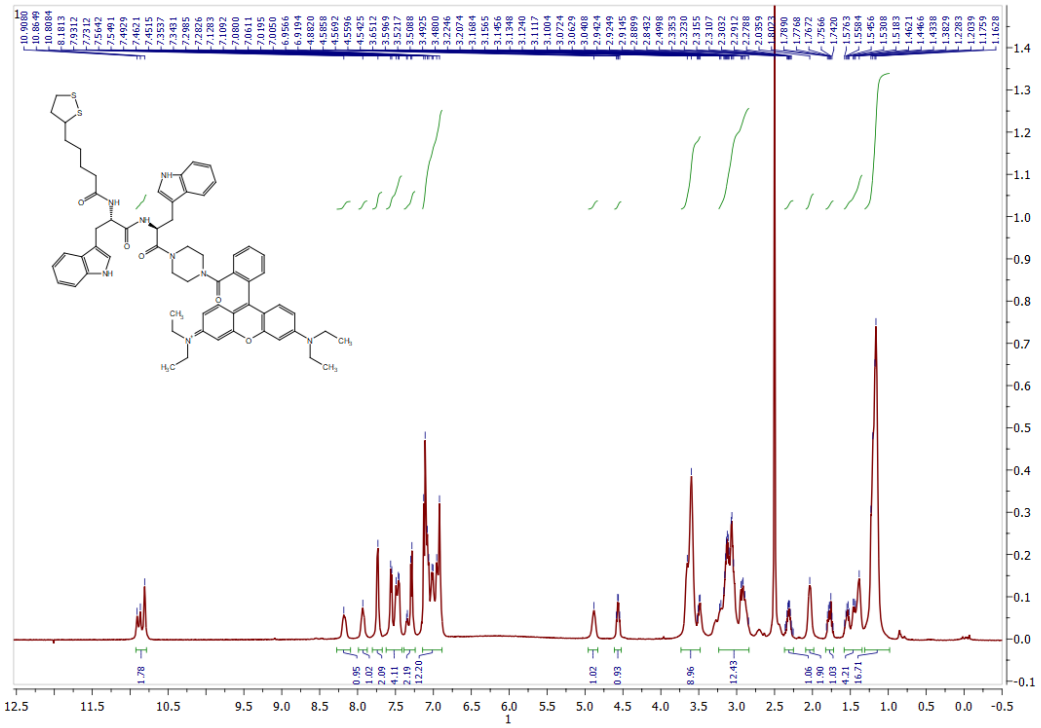


Figure S9: ^{1}H NMR spectrum of **1** in DMSO-d_6

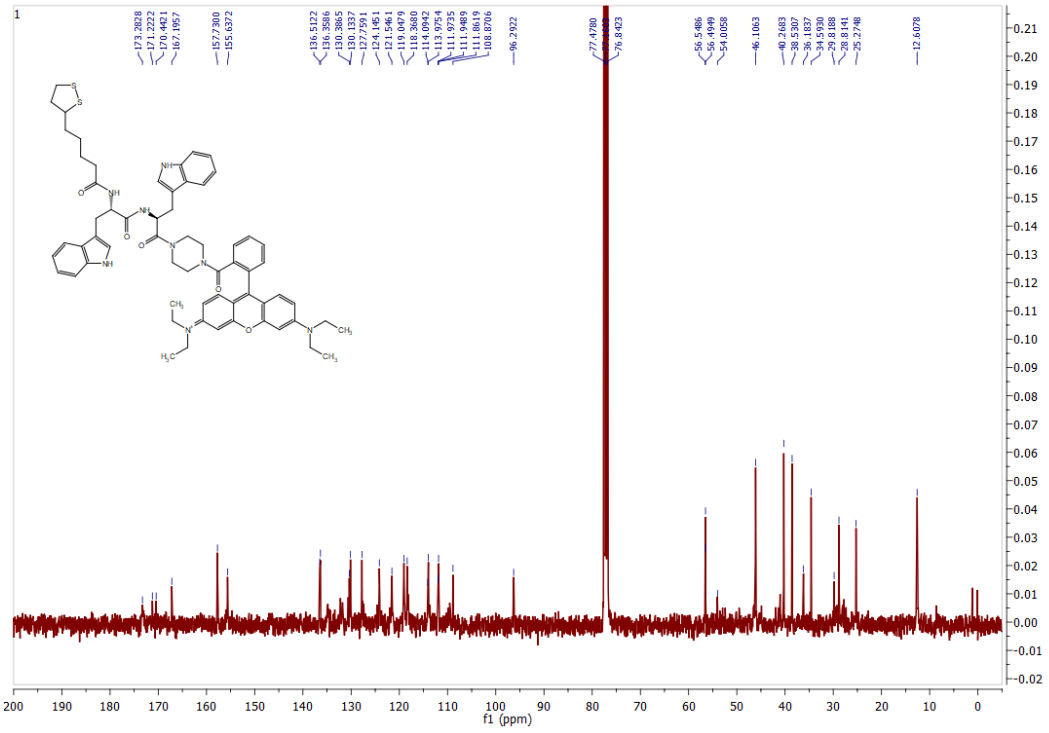


Figure S10: ^{13}C NMR spectrum of **1** in $CDCl_3$

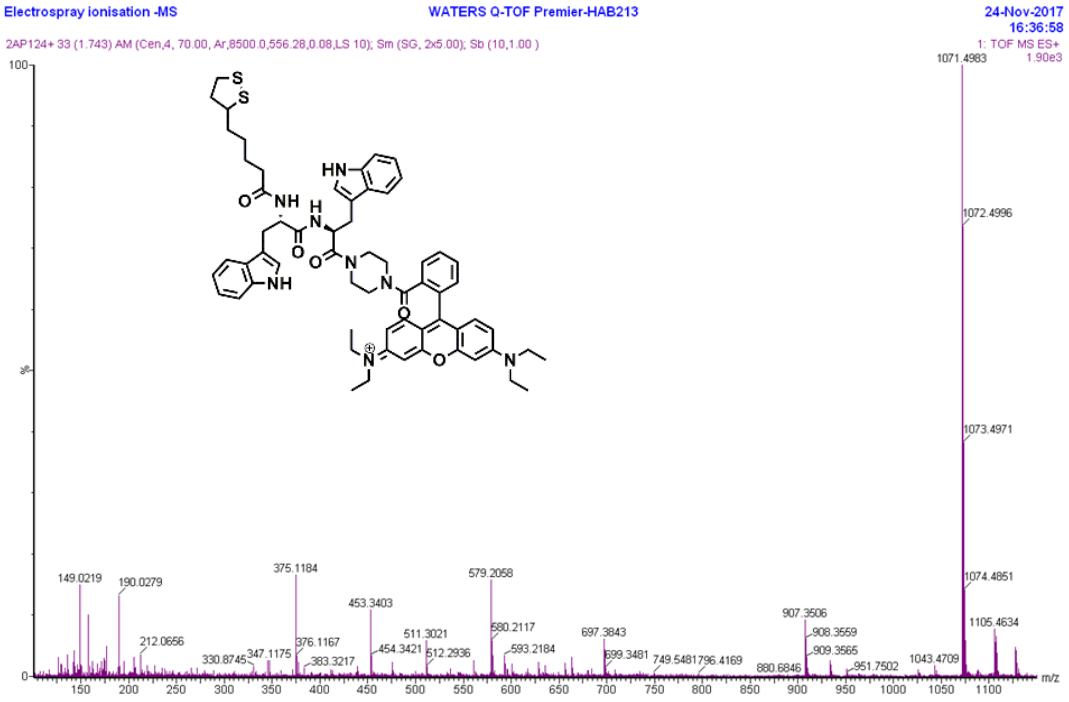


Figure S11: HRMS-ESI spectrum of **1**

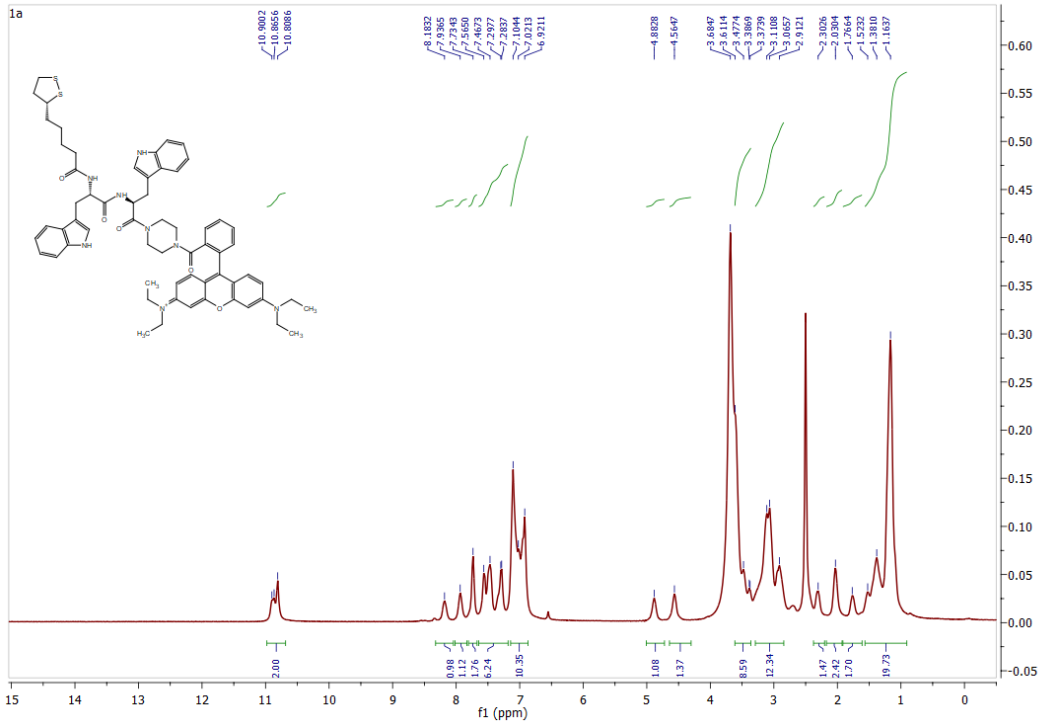


Figure S12: ^{1}H NMR spectrum of **1a** in DMSO-d_6

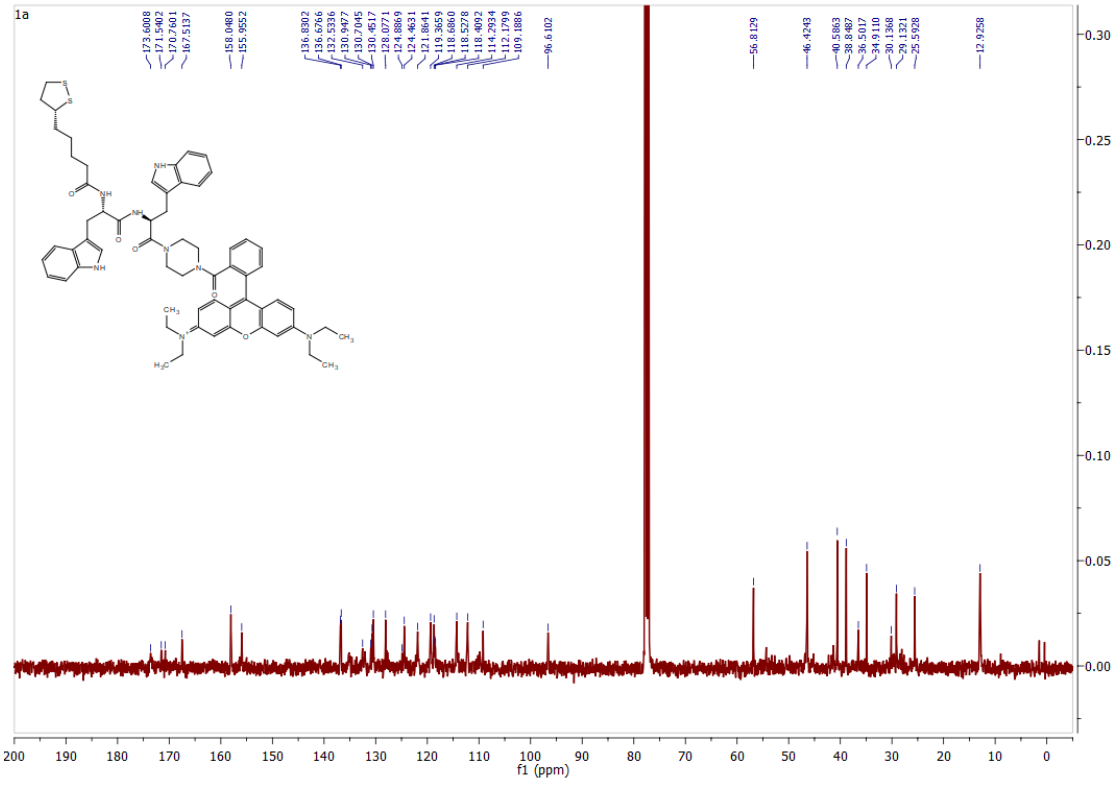


Figure S13: ^{13}C NMR spectra of **1a** in $CDCl_3$

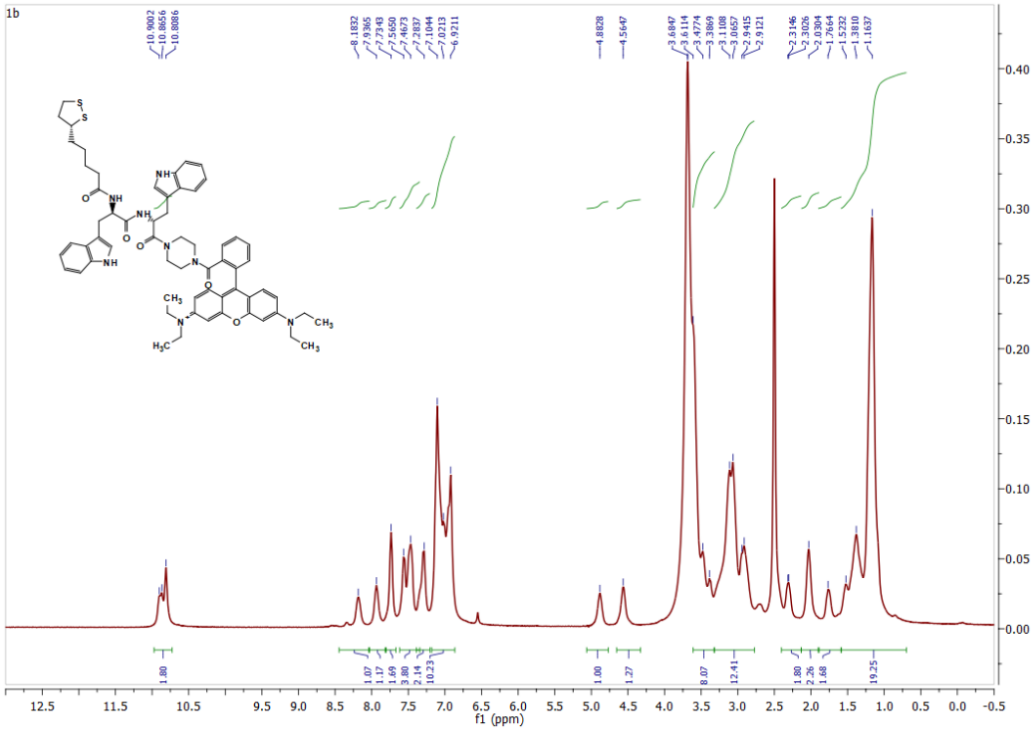


Figure S14: ^{1}H NMR spectra of **1b** in DMSO-d_6

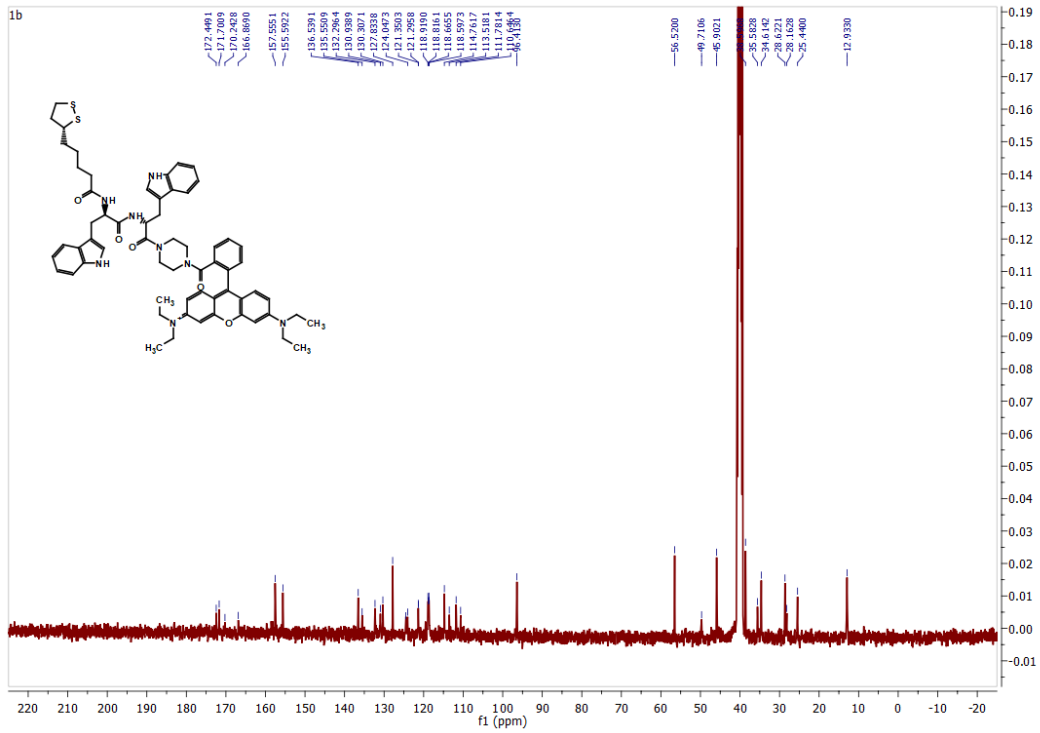


Figure S15: ^{13}C NMR spectra of **1b** in DMSO-d_6

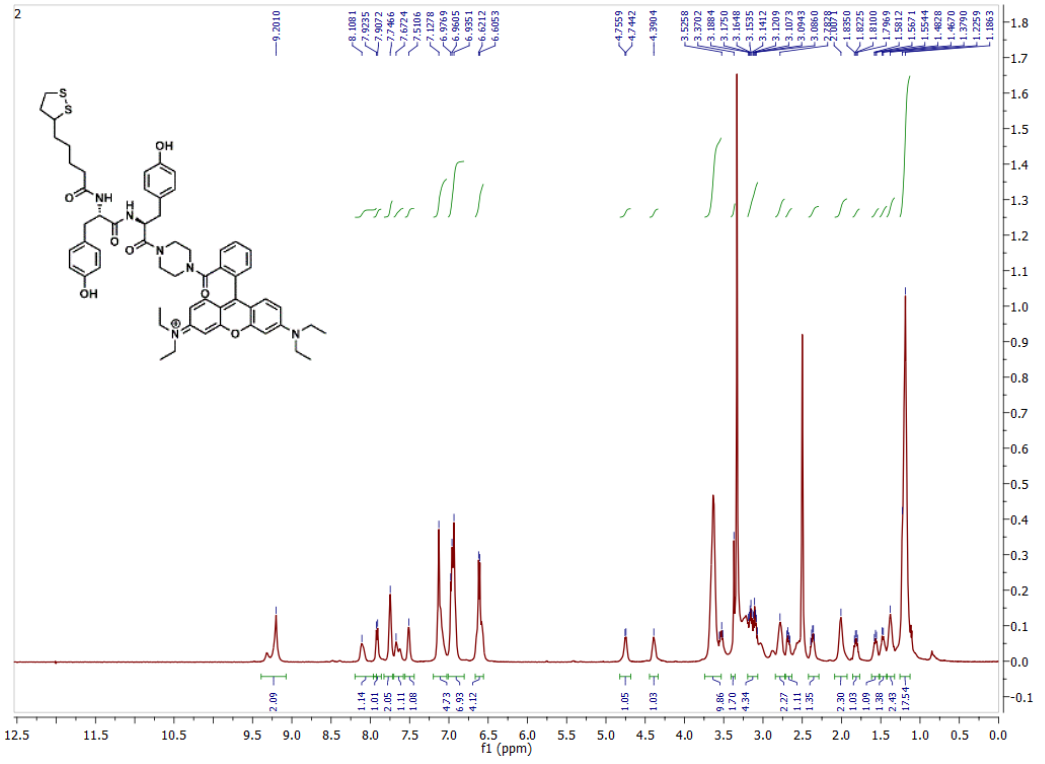


Figure S16: ^{1}H NMR spectra of **2** in DMSO-d_6

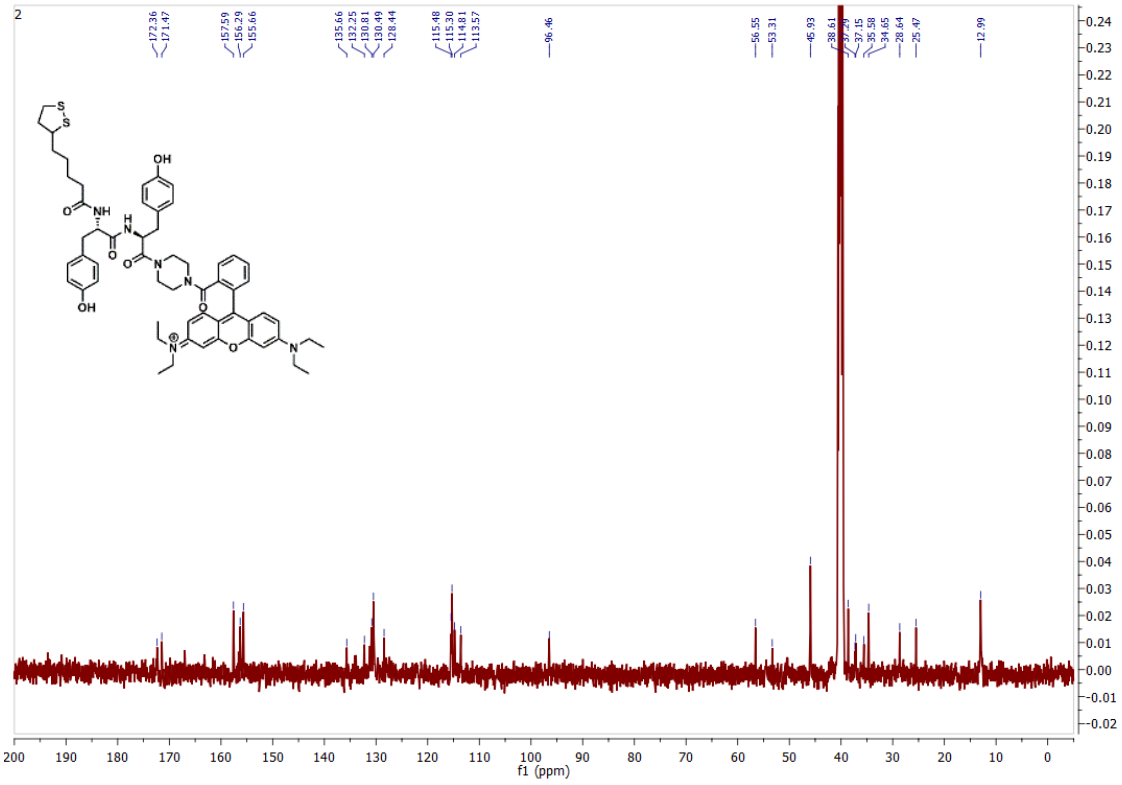


Figure S17: ^{13}C NMR spectrum of **2** in DMSO-d_6

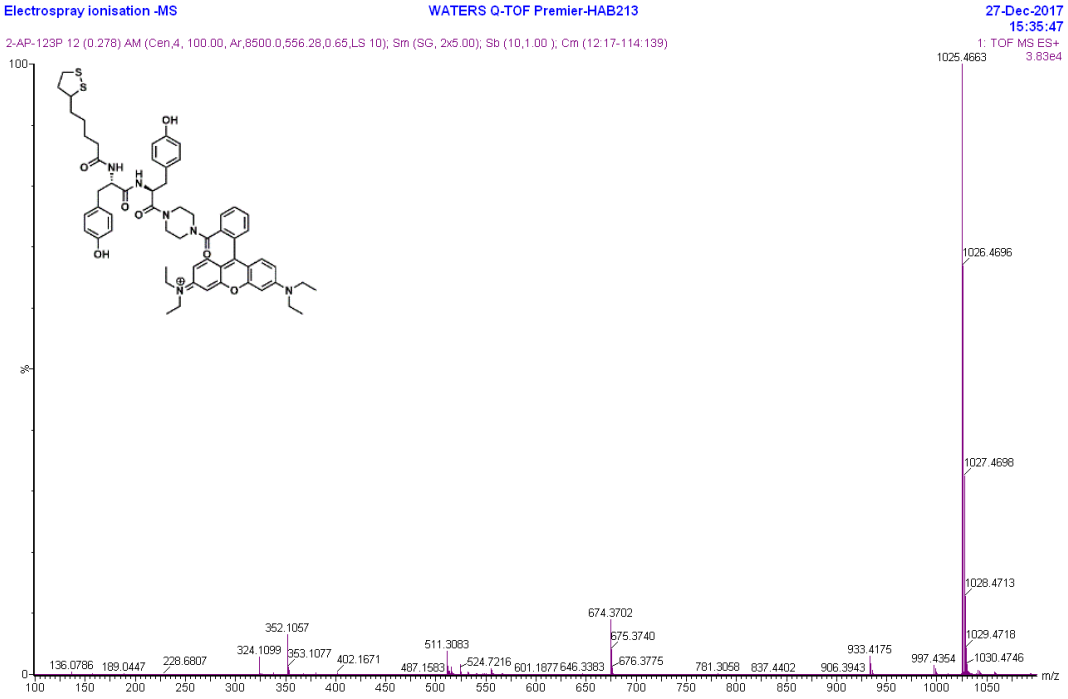


Figure S18: HRMS-ESI spectrum of **2**

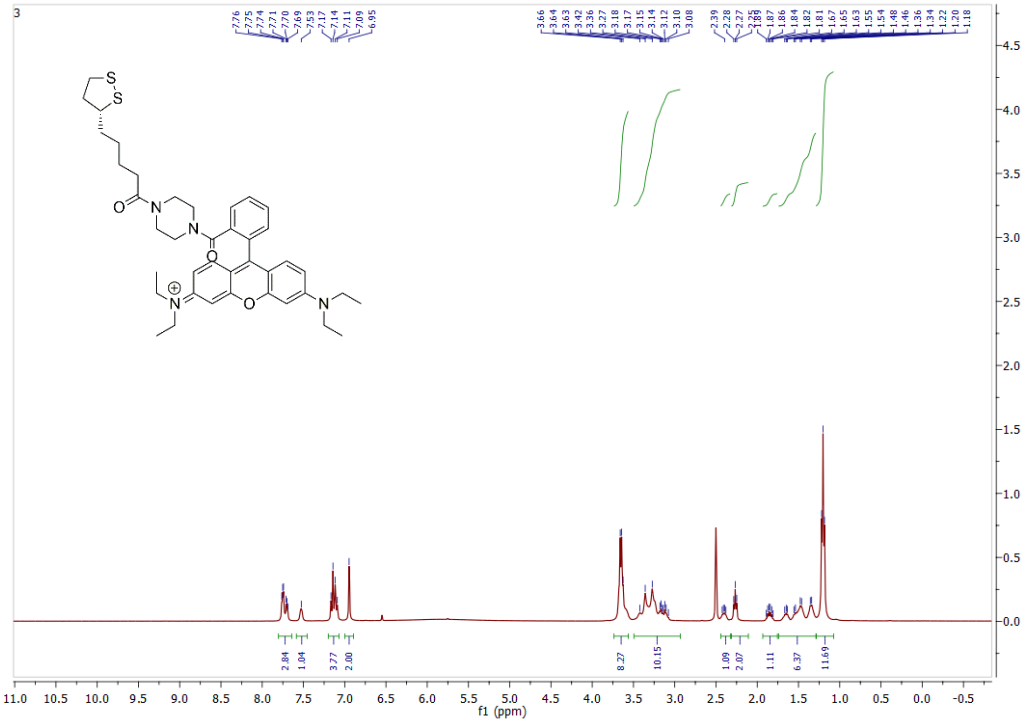


Figure S19: ^{1}H NMR spectrum of **3** in DMSO-d_6

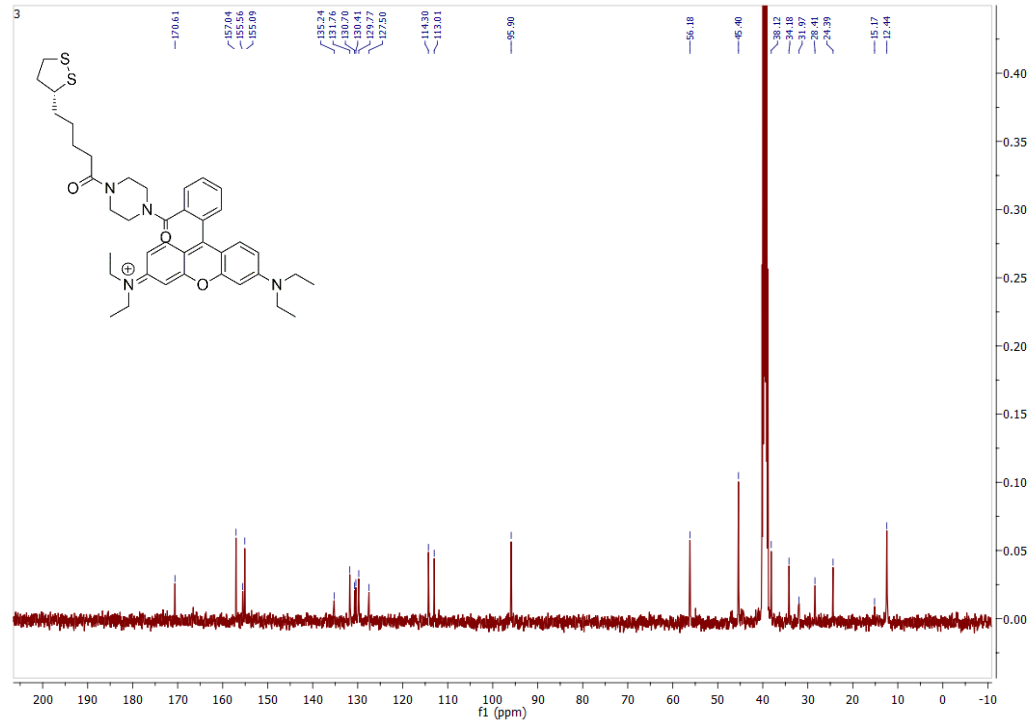


Figure S20: ^{13}C NMR spectrum of **3** in DMSO-d_6

5. References

1. S. Wu, Y. Song, Z. Li, Z. Wu, J. Han and S. Han, *Analytical Methods* 2012, **4**, 1699-1703.
2. F. C. Odds, *J. Antimicrob. Chemother.*, 2003, **52**, 1-1.

3. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, *J. Comput. Chem.*, 2009, **30**, 2785-2791.
4. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *Journal of computational chemistry* 1998, **19**, 1639-1662.
5. B. Hess, C. Kutzner, D. Van Der Spoel and E. Lindahl, *Journal of chemical theory computation*2008, **4**, 435-447.
6. V. Hornak, R. Abel, A. Okur, B. Strockbine, A. Roitberg and C. J. P. S. Simmerling, Function,, *Proteins: Structure, Function, Bioinformatics*, 2006, **65**, 712-725.
7. J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, *Journal of computational chemistry* 2004, **25**, 1157-1174.
8. F.-Y. Dupradeau, A. Pigache, T. Zaffran, C. Savineau, R. Lelong, N. Grivel, D. Lelong, W. Rosanski and P. Cieplak, *Physical Chemistry Chemical Physics*, 2010, **12**, 7821-7839.
9. P. Mark and L. Nilsson, *The Journal of Physical Chemistry A* 2001, **105**, 9954-9960.
10. T. Darden, D. York and L. Pedersen, *The Journal of chemical physics* 1993, **98**, 10089-10092.
11. R. A. Laskowski and M. B. Swindells, *J. Chem. Inf. Model*, 2011.
12. B. Israelewitz, J. Baudry, J. Gullingsrud, D. Kosztin and K. Schulten, *Journal of Molecular Graphics Modelling* 2001, **19**, 13-25.