Supplementary Information

Orally Bioavailable STING Antagonists synthesized via Multi-component Povarov-Doebner Reaction

Kofi B. Owusu[a,b], Jyotrimayee Samal[a,b], Delmis E. Hernandez[a] and Herman O. Sintim*[a,b,c]

[a]Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, United States

[b]Institute for Drug Discovery, Purdue University, 720 Clinic Drive, West Lafayette, Indiana 47907, United States

[c]Purdue Institute of Inflammation, Immunology, and Infectious Disease, West Lafayette, IN, 47907, USA

*Corresponding author. Email: hsintim@purdue.edu

1. Chemistry

1.1. General synthesis of library compounds

General considerations. Solvents and reagents were obtained from commercial sources and utilized without further purification. ^{1}H and ^{13}C NMR spectra presented were obtained in methanol-d4 or DMSO-d6 using a Bruker AV500 (500 MHz) or (800 MHz) spectrometer with tetramethyl silane as an internal standard. ^{1}H NMR data were reported as shown: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Chemical shifts were reported in downfield order in parts per million (δ ppm). Multiplicities are reported as follows: s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, or combinations thereof. Electron spray ionization (ESI) technique and TOF mass analysis were used to record high-resolution mass spectra (HRMS). All the synthesized compounds were characterized using ^{1} H, ^{13}C and HRMS.

Synthesis of HSKB142 and analogues. The quinoline library was prepared through a previously established methodology, unless otherwise mentioned.[26] Briefly, in a 20 mL screw capped glass vial, the corresponding amine (1 mmol) and aldehyde (1 mmol) were refluxed in absolute ethanol (5 mL) for 2 hours. After that the reaction was cooled to room temperature followed by addition of corresponding ketone (2.5 mmol) and a catalytic amount of conc. hydrogen chloride. Further reaction was allowed to reflux for an additional 6 to 12 hours. Upon completion, the reaction mixture was concentrated and purified using silica gel column chromatography (hexanes: ethyl acetate 50 : 50 to 0 : 100) or ethyl acetate/methanol (99 : 01 to 80 : 20).

1.2. ^{1}H, ^{13}C and Mass characterization data of compounds

(3-fluoro-4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)phenyl)(4-(methylsulfonyl)piperazin-1-yl)methanone. (HSKB142) Light yellow solid (33 mg, 23%), ^{1}H NMR (500 MHz, DMSO) δ 8.59 (s, 1H), 7.89 (d, *J* = 9.1 Hz, 1H), 7.83 (d, *J* = 9.1 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.43 (dd, *J* = 9.8, 1.5 Hz, 1H), 7.39 (dd, *J* = 7.7, 1.5 Hz, 1H), 3.75 (s, 2H), 3.53 (s, 2H), 3.41 – 3.34 (m, 2H), 3.21 (s, 4H), 2.92 (s, 3H), 2.64 (t, *J* = 6.3 Hz, 2H), 2.00 (p, *J* = 5.5 Hz, 2H), 1.79 (d, *J* = 8.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 167.55, 167.53, 158.85 (d, *J* = 245.8 Hz), 151.16, 143.21, 142.01, 138.31, 137.90 (d, *J* = 103.3 Hz), 135.98, 131.70, 131.67, 129.71, 129.68, 129.28 (d, *J* = 74.9 Hz), 122.47 (d, *J* = 154.1 Hz), 115.91, 114.36 (d, *J* = 24.3 Hz), 114.26, 114.23, 46.66,

45.29, 34.15, 28.99, 26.91, 22.06, 21.63. HRMS (ESI), m/z calcd for $C_{26}H_{26}FN_5O_3S$ $[M+H]^+$ 508.1819, found 508.1817.

3-fluoro-*N*-(2-sulfamoylethyl)-4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)benzamide (HSKB143). Off white solid (38 mg, 29%). ^{1}H NMR (500 MHz, DMSO) δ 8.81 (t, *J* = 5.7 Hz, 1H), 8.59 (s, 1H), 7.88 (d, *J* = 9.0 Hz, 1H), 7.84 – 7.74 (m, 3H), 7.58 (t, *J* = 7.6 Hz, 1H), 6.96 (s, 2H), 3.68 (dt, *J* = 8.4, 5.8 Hz, 2H), 3.30 – 3.26 (m, 2H), 2.64 – 2.57 (m, 2H), 1.98 (p, *J* = 6.1 Hz, 2H), 1.81 – 1.74 (m, 2H), 1.25 – 1.18 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 165.3, δ 159.46 (d, *J* = 244.7 Hz), 151.6, 142.5, 136.6, 136.6, 136.5, 132.0, 132.0, 131.8, 131.6, 130.1, 129.5, 123.8, 122.4, 116.4, 114.9, 114.7, 54.1, 53.9, 40.4, 35.3, 29.5, 27.4, 22.5, 22.1. HRMS (ESI), m/z calcd for $C_{23}H_{22}FN_5O_3S$ $[M+H]^+$ 468.1506, found 468.1505.

1-(4-(4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)benzoyl)piperazin-1-yl)ethan-1-one. (1) Off white solid (70 mg, 62%), ^{1}H NMR (500 MHz, DMSO) δ 8.58 (s, 1H), 7.86 (q, *J* = 9.1 Hz, 2H), 7.66 – 7.64 (m, 2H), 7.54 – 7.52 (m, 2H), 3.52 (s, 10H), 2.80 (t, *J* = 6.2 Hz, 2H), 2.05 – 1.99 (m, 5H), 1.76 (q, *J* = 6.1 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 169.11, 168.49, 155.68, 143.19, 142.09, 142.04, 138.27, 135.95, 134.99, 129.15, 128.89, 126.73, 121.52, 115.98, 114.07, 45.58, 30.69, 29.21, 28.43, 22.11, 22.01, 21.26. HRMS (ESI), m/z calcd for $C_{28}H_{27}N_4O_2$ $[M+H]^+$ 454.2243, found 454.2247.

Morpholino(4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)phenyl)methanone. (2) Off white solid (106 mg, 89%), ^{1}H NMR (500 MHz, DMSO) δ 8.59 (s, 1H), 7.86 (m, *J* = 9.1 Hz, 2H), 7.66 – 7.63 (m, 2H), 7.54 – 7.51 (m, 2H), 3.64 (s, 8H), 3.39 – 3.35 (m, 2H), 2.81 (t, *J* = 6.1 Hz, 2H), 2.03 (q, *J* = 5.9 Hz, 2H), 1.78 (q, *J* = 5.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 168.96, 155.68, 143.18, 142.09, 142.00, 138.26, 135.95, 134.87, 129.13, 128.89, 126.74, 121.52, 115.98, 114.07, 66.13, 30.69, 29.20, 28.43, 22.11, 22.01. HRMS (ESI), m/z calcd for $C_{25}H_{24}N4O_2$ $[M+H]^+$ 413.1978, found 413.1988.

(4-hydroxy-4-methylpiperidin-1-yl)(4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)phenyl)methanone. (3) Off white solid (49 mg, 38%), ^{1}H NMR (500 MHz,

DMSO) δ 8.59 (s, 1H), 7.87 (t, *J* = 9.2 Hz, 2H), 7.64 – 7.62 (m, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 4.45 (s, 1H), 3.39 (d, *J* = 15.1 Hz, 4H), 3.25 (s, 1H), 2.81 (t, *J* = 6.1 Hz, 2H), 2.08 (d, *J* = 0.7 Hz, 1H), 2.03 (d, *J* = 7.0 Hz, 2H), 1.78 (d, *J* = 6.3 Hz, 2H), 1.48 (s, 4H), 1.18 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 168.68, 142.10, 141.65, 135.97, 129.07, 126.36, 114.09, 66.25, 30.69, 29.82, 29.22, 28.44, 22.12, 22.03. HRMS (ESI), m/z calcd for $C_{27}H_{28}N_4O_2$ $[M+H]^+$ 441.2291 found 441.2294.

(1,1-dioxidothiomorpholino)(4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)phenyl)methanone. (4) Off white solid (74 mg, 56%), ^{1}H NMR (500 MHz, DMSO) δ 8.59 (s, 1H), 7.87 (t, *J* = 8.8 Hz, 2H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.61 (d, *J* = 8.2 Hz, 2H), 4.03 (d, *J* = 7.1 Hz, 2H), 3.82 (s, 2H), 3.37 (s, 2H), 3.29 (d, *J* = 5.9 Hz, 2H), 3.17 (d, *J* = 5.2 Hz, 1H), 2.82 (t, *J* = 6.1 Hz, 3H), 2.08 (s, 1H), 2.03 (t, *J* = 6.0 Hz, 2H), 1.78 (q, *J* = 5.3 Hz, 2H).^{13}C NMR (126 MHz, DMSO) δ 169.40, 155.68, 143.19, 142.23, 142.11, 138.28, 135.97, 134.42, 129.18, 128.92, 126.62, 121.54, 115.98, 114.10, 50.82, 30.69, 29.22, 28.43, 22.12, 22.02. HRMS (ESI), m/z calcd for $C_{25}H_{24}N_4O_3S$ $[M+H]^+$ 461.1647 found 461.1651.

((2*S*,6*R*)-2,6-dimethylmorpholino)(4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)phenyl)methanone. (5) Off white solid (77 mg, 61%). ^{1}H NMR (500 MHz, DMSO) δ 8.59 (s, 1H), 7.87 (t, *J* = 9.7 Hz, 2H), 7.66 – 7.64 (m, 2H), 7.53 – 7.50 (m, 2H), 3.58 (dd, *J* = 12.9, 6.4 Hz, 4H), 3.39 – 3.34 (m, 2H), 3.24 (s, 1H), 2.89 (s, 1H), 2.81 (t, *J* = 6.1 Hz, 3H), 2.04 – 2.00 (m, 2H), 1.78 (d, *J* = 6.5 Hz, 2H), 1.10 (d, *J* = 59.5 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 168.48, 155.53, 143.01, 141.94, 141.79, 138.10, 135.79, 134.83, 128.97, 128.76, 126.62, 121.35, 115.81, 113.93, 71.04, 39.35, 29.07, 28.26, 21.95, 21.86, 18.27. HRMS (ESI), m/z calcd for $C_{27}H_{28}N_4O_2$ $[M+H]^+$ 441.2290, found 441.2292.

***N*-(1-(methylsulfonyl)piperidin-4-yl)-4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)benzamide. (6)** Off white solid (89 mg, 62%), ^{1}H NMR (500 MHz, DMSO) δ 8.59 (s, 1H), 7.86 (q, *J* = 9.4 Hz, 2H), 7.68 – 7.65 (m, 2H), 7.56 – 7.53 (m, 2H), 3.65 (d, *J* = 102.8 Hz, 5H), 3.21 (s, 4H), 2.93 (s, 3H), 2.81 (t, *J* = 6.1 Hz, 2H), 2.02 (dp, *J* = 9.8, 3.3 Hz, 2H), 1.77 (dq, *J* = 5.9, 3.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 169.06, 155.62, 143.17, 142.13,

138.28, 135.97, 134.78, 129.19, 128.91, 126.76, 121.57, 115.96, 114.13, 45.41, 34.13, 29.22, 28.42, 22.11, 22.01. HRMS (ESI), m/z calcd for $C_{26}H_{27}N_5O_3S$ $[M+H]^+$ 490.1913, found 490.1917.

(2-fluoro-4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)phenyl)(4-(methylsulfonyl)piperazin-1-yl)methanone. (7) Off white solid (172 mg, 62%), ^{1}H NMR (500 MHz, DMSO) δ 8.58 (s, 1H), 7.91 – 7.82 (m, 2H), 7.56 – 7.51 (m, 3H), 3.81 (t, *J* = 5.3 Hz, 2H), 3.45 (t, *J* = 5.1 Hz, 2H), 3.25 (t, *J* = 5.2 Hz, 2H), 3.16 (t, *J* = 5.0 Hz, 2H), 2.94 (s, 3H), 2.83 (t, *J* = 6.1 Hz, 2H), 2.01 (dp, *J* = 9.5, 3.0 Hz, 2H), 1.77 (ddt, *J* = 11.9, 8.2, 4.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 164.08, 157.23 (d, *J* = 246.1 Hz), 154.33, 144.35, 144.29, 143.13, 142.31, 138.33, 136.02, 129.13, 128.92, 128.60, 128.57, 125.77, 122.95, 122.81, 121.74, 116.63, 116.46, 115.93, 114.25, 46.26, 45.68, 45.25, 40.95, 34.25, 30.70, 29.22, 28.26, 22.07, 21.98. HRMS (ESI), m/z calcd for $C_{26}H_{26}FN_5O_3S$ $[M+H]^+$ 508.1819, found 508.1818.

(4-(methylsulfonyl)piperazin-1-yl)(5-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)pyridin-2-yl)methanone. (8) Off white solid (99 mg, 47%), ^{1}H NMR (500 MHz, DMSO) δ 8.82 (dd, *J* = 2.2, 0.9 Hz, 1H), 8.60 (s, 1H), 8.20 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.90 (t, *J* = 8.1 Hz, 2H), 7.75 (dd, *J* = 7.9, 0.8 Hz, 1H), 3.83 – 3.79 (m, 2H), 3.66 (t, *J* = 5.0 Hz, 2H), 3.39 – 3.35 (m, 2H), 3.26 (t, *J* = 5.2 Hz, 2H), 3.19 (t, *J* = 5.0 Hz, 2H), 2.93 (s, 3H), 2.85 (t, *J* = 6.1 Hz, 2H), 2.05 – 2.01 (m, 2H), 1.82 – 1.77 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 166.70, 152.82, 152.55, 148.45, 143.37, 142.44, 138.36, 137.85, 137.17, 136.08, 129.31, 129.15, 122.80, 121.83, 115.91, 114.36, 46.22, 45.76, 45.27, 41.20, 34.14, 29.22, 28.21, 22.03, 21.97. HRMS (ESI), m/z calcd for $C_{25}H_{26}N_6O_3S$ $[M+H]^+$ 491.1865, found 491.1863.

(4-(methylsulfonyl)piperazin-1-yl)(6-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)pyridin-3-yl)methanone. (9) Light yellow solid (52 mg, 11%), ^{1}H NMR (500 MHz, DMSO) δ 8.74 (d, *J* = 2.2 Hz, 1H), 8.59 (s, 1H), 8.04 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.88 (t, *J* = 7.8 Hz, 2H), 3.79 (s, 2H), 3.56 (s, 2H), 3.23 (s, 4H), 3.00 (t, *J* = 6.2 Hz, 2H), 2.93 (s, 3H), 2.11 – 1.94 (m, 4H), 1.79 – 1.74 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 167.02, 159.91, 153.45, 146.31, 142.86, 142.46, 138.43, 136.07, 135.60, 130.15, 129.63, 129.15,

124.14, 122.06, 115.94, 114.19, 45.22, 34.19, 30.67, 29.30, 27.76, 22.05, 21.86. HRMS (ESI), m/z calcd for $C_{25}H_{26}N_6O_3S$ $[M+H]^+$ 491.1865, found 491.1863.

(3-fluoro-4-(9-hydroxy-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-a]phenanthridin-7-yl)phenyl)(4-(methylsulfonyl)piperazin-1-yl)methanone. (10) Light orange solid (24 mg, 16%), ^{1}H NMR (500 MHz, DMSO) δ 8.60 (s, 1H), 7.89 (d, *J* = 9.0 Hz, 1H), 7.84 (s, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.45 (dd, *J* = 9.8, 1.5 Hz, 1H), 7.39 (dd, *J* = 7.7, 1.5 Hz, 1H), 4.90 (s, 1H), 4.00 (s, 1H), 3.75 (s, 1H), 3.52 (s, 4H), 3.21 (s, 4H), 2.91 (s, 3H), 2.82 (d, *J* = 16.0 Hz, 1H), 2.58 (dd, *J* = 16.8, 7.5 Hz, 2H), 2.16 (s, 1H), 1.91 (t, *J* = 6.2 Hz, 1H).

^{13}C NMR (126 MHz, DMSO) δ 168.01, 159.38 (d, *J* = 245.9 Hz), 151.85, 143.79, 141.91, 138.06, 136.51, 132.25, 130.13, 130.00, 129.41, 123.59, 121.88, 114.98, 114.79, 64.42, 45.80, 40.50, 40.33, 40.16, 40.00, 39.83, 39.66, 36.33, 34.63.
HRMS (ESI), m/z calcd for $C_{26}H_{26}FN_5O_4S$ $[M+H]^+$ 524.1767, found 524.1762.

(3-fluoro-4-(9-methyl-3H-pyrazolo[4,3-f]quinolin-7-yl)phenyl)(4-(methylsulfonyl)piperazin-1-yl)methanone. (11) Dark yellow (75 mg, 40%), ^{1}H NMR (500 MHz, DMSO) δ 8.62 (s, 1H), 8.14 (t, *J* = 7.9 Hz, 1H), 8.00 – 7.95 (m, 2H), 7.94 (s, 1H), 7.48 (dd, *J* = 11.0, 1.5 Hz, 1H), 7.44 (dd, *J* = 7.9, 1.6 Hz, 1H), 3.76 (s, 2H), 3.51 (s, 2H), 3.21 (s, 4H), 2.98 (s, 3H), 2.93 (s, 3H).^{13}C NMR (126 MHz, DMSO) δ 167.49, 159.61 (d, *J* = 250.2 Hz), 149.07, 143.79, 137.84, 137.78, 131.59, 129.16, 128.53, 128.44, 123.67, 123.41, 121.81, 115.30, 115.10, 45.31, 34.21, 22.41. HRMS (ESI), m/z calcd for $C_{23}H_{22}FN_5O_3S$ $[M+H]^+$ 468.1505, found 468.1503.

(4-(methylsulfonyl)piperazin-1-yl)(4-(3,8,10,11-tetrahydropyrazolo[4,3-f]thiopyrano[3,4-c]quinolin-7-yl)phenyl)methanone. (12) Off white (157 mg, 41%), ^{1}H NMR (500 MHz, DMSO) δ 8.66 (s, 1H), 7.94 (d, *J* = 9.1 Hz, 1H), 7.88 (s, 1H), 7.69 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 2H), 3.93 (s, 2H), 3.77 (s, 1H), 3.66 (s, 2H), 3.55 (s, 1H), 3.25 – 3.16 (m, 8H), 2.93 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 168.98, 154.16, 141.02, 135.24, 129.43, 129.22, 126.94, 121.97, 115.40, 45.41, 34.15, 30.62, 28.79, 25.56. HRMS (ESI), m/z calcd for $C_{25}H_{25}N_5O_3S_2$ $[M+H]^+$ 508.1477, found 508.1473.

(4-(9-hydroxy-8,9,10,11-tetrahydro-3H-pyrazolo[4,3-a]phenanthridin-7-yl)phenyl)(4-(methylsulfonyl)piperazin-1-yl)methanone. (13) Light purple (85 mg, 31%), ^{1}H NMR (500 MHz, DMSO) δ 8.65 – 8.58 (m, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.86 – 7.80 (m, 1H), 7.67 (d, *J* = 1.8 Hz, 1H), 7.66 (d, *J* = 1.9 Hz, 1H), 7.56 (d, *J* = 1.9 Hz, 1H), 7.55 (d, *J* = 1.8 Hz, 1H), 4.88 (d, *J* = 3.4 Hz, 1H), 3.98 (td, *J* = 8.3, 3.9 Hz, 1H), 3.76 (s, 2H), 3.49 (d, *J* = 17.9 Hz, 2H), 3.22 (s, 5H), 2.98 (dd, *J* = 16.4, 4.3 Hz, 2H), 2.93 (s, 3H), 2.78 (dd, *J* = 16.4, 7.5 Hz, 1H), 2.18 (dt, *J* = 11.5, 5.1 Hz, 1H), 1.94 (tt, *J* = 15.3, 7.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 169.04, 155.78, 143.31, 142.07, 141.54, 138.31, 135.98, 135.97, 134.81, 129.22, 129.08, 126.99, 126.76, 121.11, 115.96, 114.15, 64.20, 45.40, 37.23, 34.12, 30.01, 27.42. HRMS (ESI), m/z calcd for $C_{26}H_{27}N_5O_4S$ $[M+H]^+$ 506.1862, found 506.1850.

(4-(methylsulfonyl)piperazin-1-yl)(4-(3,8,10,11-tetrahydropyrano[3,4-c]pyrazolo[4,3-f]quinolin-7-yl)phenyl)methanone. (14) Off white (110 mg, 28%), ^{1}H NMR (500 MHz, DMSO) δ 8.63 (s, 1H), 7.95 (d, *J* = 9.1 Hz, 1H), 7.88 (d, *J* = 9.2 Hz, 1H), 7.69 (d, *J* = 7.9 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 4.82 (s, 2H), 4.17 (t, *J* = 5.9 Hz, 2H), 3.76 (s, 2H), 3.53 (s, 2H), 3.43 (d, *J* = 6.0 Hz, 4H), 3.03 (s, 1H), 2.98 (s, 1H), 2.93 (s, 3H).^{13}C NMR (126 MHz, DMSO) δ 168.91, 152.30, 143.60, 140.30, 139.49, 135.41, 128.99, 128.78, 127.19, 126.98, 126.89, 121.09, 115.59, 66.35, 63.94, 45.41, 34.14, 28.25.
HRMS (ESI), m/z calcd for $C_{25}H_{25}N_5O_4S$ $[M+H]^+$ 492.1705, found 492.1695.

2-fluoro-*N*-(2-sulfamoylethyl)-4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)benzamide. (15) Light yellow solid (168 mg, 65%), ^{1}H NMR (500 MHz, DMSO) δ 8.59 (s, 1H), 8.51 (td, *J* = 5.7, 3.4 Hz, 1H), 7.94 – 7.83 (m, 2H), 7.80 (t, *J* = 7.7 Hz, 1H), 7.56 – 7.48 (m, 2H), 6.99 (s, 2H), 3.70 (dt, *J* = 7.9, 6.0 Hz, 2H), 3.35 (s, 2H), 3.28 (dd, *J* = 8.2, 6.2 Hz, 2H), 2.81 (t, *J* = 6.2 Hz, 2H), 2.02 (dq, *J* = 8.7, 4.5 Hz, 2H), 1.78 (ddt, *J* = 11.2, 8.3, 4.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 164.07, 157.22 (d, *J* = 246.0 Hz), 154.30, 144.35, 144.29, 143.13, 142.28, 138.32, 136.00, 129.11, 128.88, 128.59, 128.55, 125.75, 122.94, 122.80, 121.73, 116.62, 116.45, 115.91, 114.22, 46.25, 45.67, 45.24, 40.94, 34.25, 30.68, 29.19, 28.24, 22.05, 21.96. HRMS (ESI), m/z calcd for $C_{23}H_{22}FN_5O_3S$ $[M+H]^+$ 468.1506, found 468.1504.

***N*-(2-sulfamoylethyl)-5-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)picolinamide. (16)** Off white solid (69 mg, 15%), ^{1}H NMR (500 MHz, DMSO) δ 9.11 (t, *J* = 6.0 Hz, 1H), 8.87 (d, *J* = 2.1 Hz, 1H), 8.61 (s, 1H), 8.24 (dd, *J* = 8.0, 2.2 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 7.88 (q, *J* = 9.3 Hz, 2H), 7.00 (s, 2H), 3.78 (q, *J* = 6.7 Hz, 2H), 3.31 (d, *J* = 24.7 Hz, 4H), 2.82 (t, *J* = 6.1 Hz, 2H), 2.06 – 1.98 (m, 2H), 1.78 (ddt, *J* = 11.3, 8.4, 5.1 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 163.78, 152.66, 148.72, 148.62, 143.40, 142.50, 138.89, 138.19, 129.23, 129.10, 121.94, 121.31, 115.83, 114.41, 53.66, 34.37, 29.19, 28.18, 22.01, 21.95. HRMS (ESI), m/z calcd for $C_{22}H_{22}N_6O_3S$ $[M+H]^+$ 451.1552, found 451.1551.

***N*-(2-sulfamoylethyl)-6-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)nicotinamide. (17)** Off white solid (56 mg, 43%), ^{1}H NMR (500 MHz, DMSO) δ 9.07 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.91 (t, *J* = 5.7 Hz, 1H), 8.58 (s, 1H), 8.33 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.93 (dd, *J* = 8.1, 0.8 Hz, 1H), 7.91 – 7.79 (m, 2H), 6.97 (s, 2H), 3.75 – 3.68 (m, 2H), 3.33 (s, 4H), 2.95 (t, *J* = 6.2 Hz, 2H), 1.98 (qt, *J* = 6.4, 3.0 Hz, 2H), 1.75 (pd, *J* = 6.1, 4.1 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 164.89, 161.20, 153.48, 146.98, 142.86, 142.51, 138.45, 136.10, 135.64, 129.63, 129.16, 128.44, 124.13, 122.11, 115.95, 114.21, 53.49, 34.81, 30.69, 29.30, 27.76, 22.03, 21.87. HRMS (ESI), m/z calcd for $C_{22}H_{22}N_6O_3S$ $[M+H]^+$ 451.1552, found 451.1553.

N-(2-sulfamoylethyl)-4-(8,9,10,11-tetrahydro-3H-pyrazolo[4,3-a]phenanthridin-7-yl)benzamide. (18) White solid (90 mg, 26%), ^{1}H NMR (500 MHz, DMSO) δ 8.71 (t, *J* = 5.7 Hz, 1H), 8.59 (s, 1H), 7.98 – 7.93 (m, 2H), 7.87 (d, *J* = 5.9 Hz, 1H), 7.70 – 7.66 (m, 2H), 6.97 (s, 2H), 3.72 – 3.64 (m, 2H), 3.39 – 3.36 (m, 2H), 3.30 – 3.27 (m, 2H), 2.79 (t, *J* = 6.1 Hz, 2H), 2.06 – 1.97 (m, 2H), 1.77 (t, *J* = 6.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 166.15, 155.70, 143.69, 142.15, 138.21, 136.00, 133.33, 129.20, 129.09, 128.90, 126.90, 121.58, 116.00, 114.11, 53.61, 34.77, 29.22, 28.38, 22.10, 22.01. MS (ESI), m/z calcd for $C_{23}H_{23}N_5O_3$ $[M+H]^+$ 450.1600, found 450.1596.

***N*-(2-(*N*-methylsulfamoyl)ethyl)-4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)benzamide. (19)** Off white solid (57 mg, 42%), ^{1}H NMR (500 MHz, DMSO) δ 8.72 (t, *J* = 5.7 Hz, 1H), 8.59 (s, 1H), 7.95 (d, *J* = 8.1 Hz, 2H), 7.87 (q, *J* = 9.1 Hz, 2H), 7.71 – 7.65 (m, 2H), 7.05 (q, *J* = 4.9 Hz, 1H), 3.65 (dt, *J* = 7.9, 5.9 Hz, 2H), 3.37 (t, *J* = 7.1 Hz, 2H), 3.32 – 3.29 (m, 2H), 2.78 (t, *J* = 6.2 Hz, 2H), 2.62 (d, *J* = 4.9 Hz, 3H), 2.05 – 1.97 (m, 2H), 1.76 (tt, *J* = 8.3, 5.2

Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 166.16, 155.69, 143.73, 143.18, 142.15, 138.30, 135.99, 133.26, 129.20, 129.12, 128.91, 126.88, 121.57, 116.00, 114.11, 48.39, 34.43, 30.69, 29.23, 28.60, 28.38, 22.10, 22.02. HRMS (ESI), m/z calcd for $C_{24}H_{25}N_5O_3S$ $[M+H]^+$ 464.1756, found 464.1759.

***N*-methyl-*N*-(2-sulfamoylethyl)-4-(8,9,10,11-tetrahydro-3*H*-pyrazolo[4,3-*a*]phenanthridin-7-yl)benzamide. (20)** Off white solid (68 mg, 51%), ^{1}H NMR (500 MHz, DMSO) δ 8.59 (s, 1H), 7.87 (d, *J* = 4.9 Hz, 2H), 7.66 – 7.61 (m, 2H), 7.53 (d, *J* = 7.7 Hz, 2H), 6.96 (d, *J* = 26.7 Hz, 2H), 3.37 (q, *J* = 7.3 Hz, 6H), 3.02 (s, 3H), 2.80 (t, *J* = 6.1 Hz, 2H), 2.02 (tt, *J* = 8.6, 4.6 Hz, 2H), 1.81 – 1.73 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 170.27, 155.78, 143.19, 142.12, 141.89, 138.28, 135.97, 135.52, 129.19, 129.01, 126.64, 126.27, 121.53, 116.00, 114.08, 51.30, 42.66, 37.74, 29.24, 28.43, 22.13, 22.05. HRMS (ESI), m/z calcd for $C_{24}H_{25}N_5O_3S$ $[M+H]^+$ 464.1756, found 464.1761.

1.3. Purity analysis method for HSKB-142 and HSKB-143

HPLC Purity Chromatograms were performed on Agilent Eclipse instrument equipped with C18 column (3 μm, 4.6 × 100 mm^2). The eluent A was composed of 0.1% NH_4OH in H_2O and B was composed of MeOH. The UV detector was set at 260 nm wavelength in order to obtain the curve. Samples were injected at 25^{o}C. The curve for **HSKB-142** was obtained by running the flow with 50% B for 0 → 5 min, 50 to 100% B for 5 →10 min, 100% B for 10 →24 min followed by 50% B for 24→30 min. The curve for **HSKB-143** was obtained by running the flow with 50% B for 0 → 5 min, 50 to 100% B for 5 →8 min, 100% B for 8 →24 min followed by 50% B for 24→30 min.

2. Biology

2.1. Expression and affinity purification of hSTING

A cloned hSTING plasmid used in this study was gifted by from Prof. Pingwei Li, Texas A&M University. The hSTING plasmid (PET28a, SUMO) was introduced into Novagen's E. coli Rosetta™ 2(pLysS) cells employing kanamycin (50 μg mL^{-1}) and chloramphenicol (34 μg mL^{-1}) for resistance selection. Following transformation, a single colony was selected and cultured in 10 mL LB broth supplemented with kanamycin (50 μg mL^{-1}) and chloramphenicol (34 μg mL^{-1})

overnight at 37°C. Subsequently, the overnight culture was inoculated into expression media (Terrific Broth) facilitated with aforementioned antibiotics and was incubated at 37°C until attaining exponential phase (OD600 = 0.6) prior to induction for 18 hours at a lowered temperature of 16°C with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were subsequently harvested by centrifugation at 5000 rpm at 4°C for 30 minutes, and the resulting pellet was resuspended in 25 ml of lysis buffer comprising 20 mM imidazole, 5 mM mercaptoethanol, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 300Mm NaCl and 50mM Tris Base (pH 7.4). Lysis of the cells was achieved through sonication, followed by centrifugation at 20,000 rpm at 4°C for 30 minutes to collect the supernatant. The supernatant was subjected to purification via HisTrap-HP Nickel column (Cytiva), and the elute with pure hSTING protein was obtained using an elution buffer containing 50 mM Tris Base (pH = 7.4), 300 mM NaCl, 350 mM imidazole, and 10% glycerol. Subsequently, imidazole was removed by dialyzing the eluted hSTING protein overnight against a dialysis buffer (50 mM Tris Base (pH = 7.4) and 300 mM NaCl). Finally, the protein concentration was determined by measuring absorbance at 280 nm using the extinction coefficient of 47 955 M^{-1} cm^{-1}.

2.2. Cell culture methods and maintenance

THP1-Dual cells, KI-hSTING-S154 cells, RAW Dual and Jurkat cells were purchased from InvivoGen. THP1-Dual and KI-hSTING-S154 cells were cultured in RPMI1640 containing 10% heat inactivated FBS, 0.01% Penicillin streptomycin. RAW Dual cells were cultured in DMEM containing 10% heat inactivated FBS, 0.01% Penicillin streptomycin, Jurkat cells were cultured in RPMI1640 containing 10% FBS, 0.01% Penicillin streptomycin. Cells were maintained at 37°C. under 5% CO_2.

2.3. STING based fluorescence polarization assay.

50 nM of 2′-Fluo-AHC-c-diGMP (Biolog) was incubated with 10 μM hSTING and 20 μM of screening compounds in dimethyl sulfoxide (DMSO) for 5 minutes in 1× phosphate buffered saline at room temperature. Fluorescence polarization ($\lambda_{ex/em}$ = 485/528 nm) was then quantified via

Biotek Cytation 5 multi-mode reader, with anisotropy calculated using Gen5 microplate reader and imaging software. Anisotropy was normalized via equating measurements with 0 μM hSTING to zero. Experiments was performed in triplicates using 384 Greiner-Bio 384 fluorometric plate (flat plate). Anisotropy was converted to fraction bound by F-c-di-GMP using the following equation:

$$F_{bound} = \frac{r - r_{free}}{(r_{bound} - r)Q + (r - r_{free})}$$

where r refers to the anisotropy value at 10 μM hSTING concentration upon compound addition, r_{free} refers to the anisotropy value of unbound fluorophore, and Q refers to ratio of fluorescence intensities between bound and free fluorophore. Anisotropy changes with increasing concentration of HSD1077 was plotted, with a 4-parameter dose–response fit applied to obtain the reported IC_{50} via GraphPad Prism (San Diego, CA, USA).

2.4. Differential scanning fluorimetry (DSF) assay

STING buffer containing 100 mM Tris-HCl, pH=7.4, 150 mM NaCl was added to a solution of 150 μM of compounds in DMSO, 20 μM hSTING, and 1:500 (v/v) SYPRO Orange. The experiments were carried out in triplicates and denaturation performed using a real time PCR (RT-PCR) cycler. A gradient from 15 to 80 ºC, holding in increment of 1 ºC for 15 seconds was used for the denaturation of the ligand-protein complex. $\Delta Tm = Ty - Tx$. The ΔTm values for each compound were calculated by subtracting the average denaturing temperature (Tx) of untreated from the temperature (Ty) of the treated.

2.5. Cell viability of RAW ISG Blue reporter cells

RAW ISG Blue macrophage reporter cells (Invivogen) were cultured in DMEM containing 10% heat inactivated fetal bovine serum and 1X penicillin/streptomycin in 37 °C, 5% CO_2. 2×10^3 cells were seeded in 96 well plates and incubated for 24 hours to allow for adherence. Cells were

then treated with increasing concentrations of HSKB142 & HSKB143 for 24 hours. After which, CellTiter-Blue cell viability assay reagent (Promega) was added based on manufacturer recommendations and incubated for 3 hours. Fluorescence ($\lambda_{ex/em}$ = 560/590 nm) of each well was quantified via Biotek Cytation 5 multi-mode reader. Experiments were performed in biological triplicates, with data reported as the mean and standard deviation of 3 data points. Readings from cell samples treated with DMSO was normalized to 100%.

2.6. Cell viability of THP-1 dual reporter cells

THP-1 dual or THP-1 (STING N154S) dual reporter cells (Invivogen) were cultured in RPMI media containing 10% heat inactivated fetal bovine serum and 1× penicillin/streptomycin in 37 °C, 5% CO_2. 2×10^3 cells were seeded in 96 well plates and incubated for 24 hours to allow for stability. Cells were then treated with increasing concentrations of HSKB142 & HSKB143 for 24 hours. After which, CellTiter-Blue cell viability assay reagent (Promega) was added based on manufacturer recommendations and incubated for 3 hours. Fluorescence ($\lambda_{ex/em}$ = 560/590 nm) of each well was quantified via Biotek Cytation 5 multi-mode reader. Experiments were performed in biological triplicates, with data reported as the mean and standard deviation of 3 data points. Readings from cell samples treated with DMSO was normalized to 100%.

2.7. Cell viability of Jurkat cells

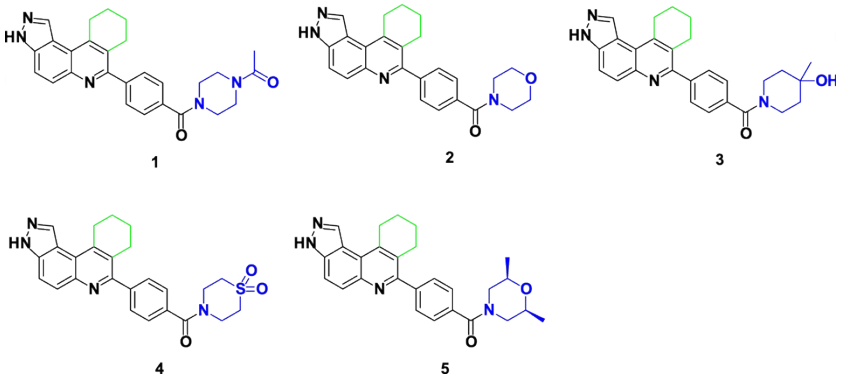
Jurkat cells (Invivogen) were cultured in RPMI media containing 10% fetal bovine serum and 1× penicillin/streptomycin in 37 °C, 5% CO_2. 2×10^3 cells were seeded in 96 well plates and incubated for 24 hours to allow for stability. Cells were then treated with increasing concentrations of HSKB142 & HSKB143 for 24 hours. After which, CellTiter-Blue cell viability assay reagent (Promega) was added based on manufacturer recommendations and incubated for 3 hours. Fluorescence ($\lambda_{ex/em}$ = 560/590 nm) of each well was quantified via Biotek Cytation 5 multi-mode reader. Experiments were performed in biological triplicates, with data reported as the mean and standard deviation of 3 data points. Readings from cell samples treated with DMSO was normalized to 100%.

2.8. IRF attenuation in THP1 cells

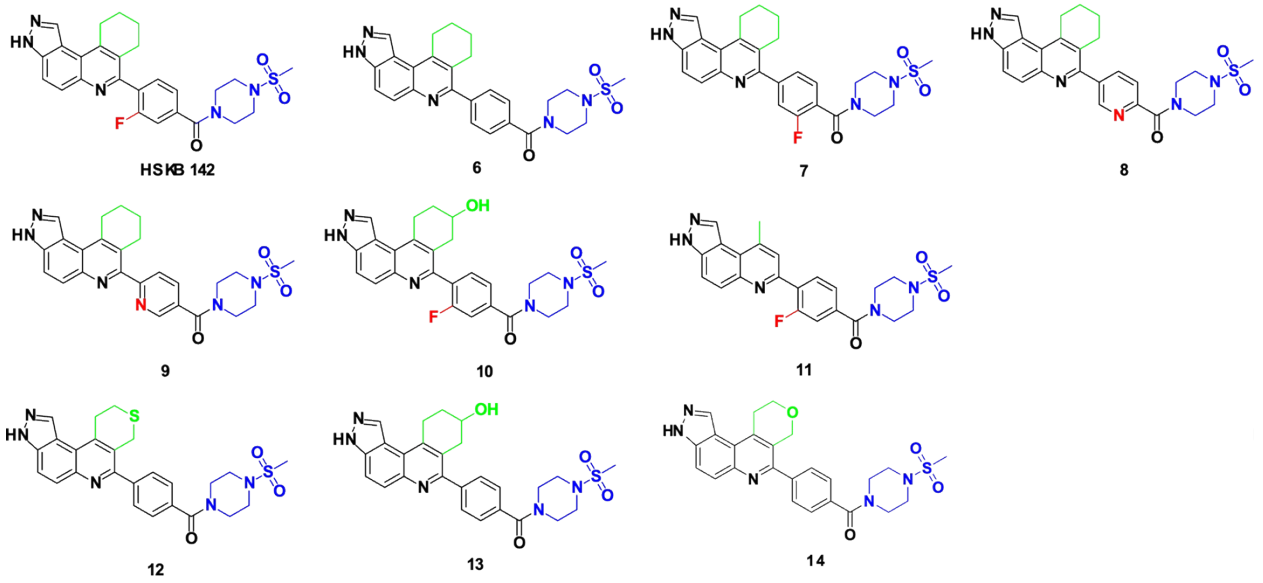
THP1 WT and THP1 (KI STING N154S) dual reporter cells (from invivogen) were cultured in RPMI media 10% heat inactivated fetal bovine serum and 1× penicillin/streptomycin in 37 °C, 5% CO2. 2 x 10^5 cells were seeded in 96 well plates and incubated for 24 hours to allow for stability. Cells were then treated in triplicates with compounds or DMSO as negative control and incubated for an hour, then 100 uM 2'3'cGAMP prior to incubation for 24 hours. 10 µL of media were collected and 50 µL solution of QUANTI-Luc™ reagent (Invivogen) was added and luminescence was determined via a Biotek Cytation 5 multi-mode reader.

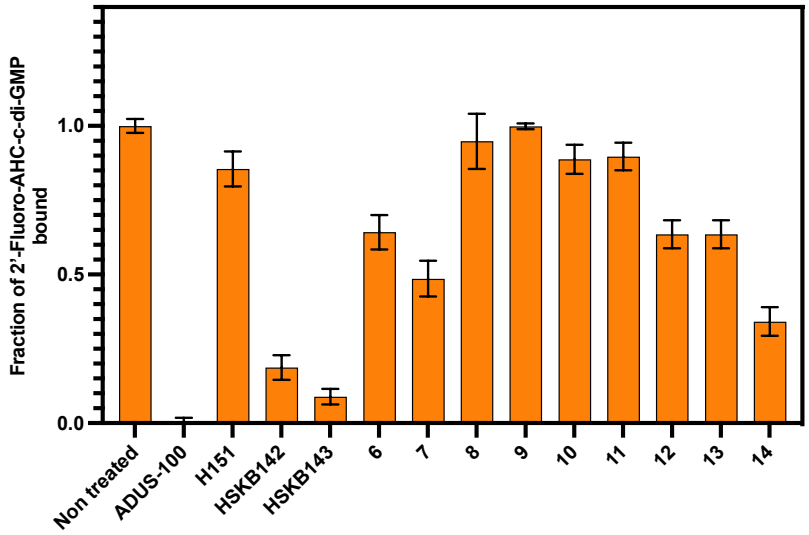
2.8. Biological characterization figures

Series A

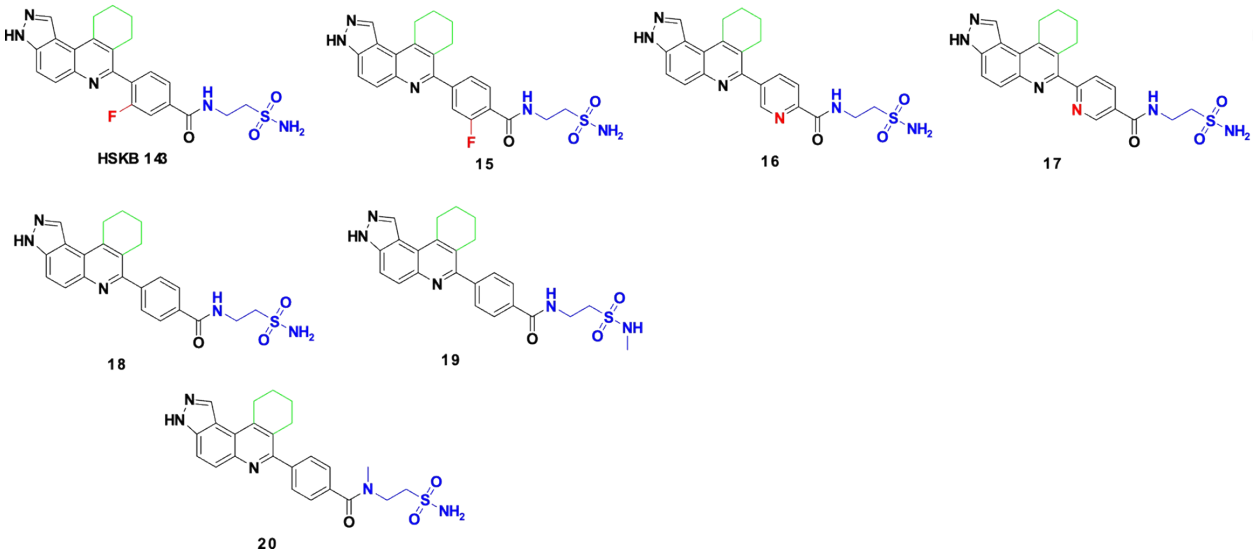


Series B


HSKB 142
6
7
8
9
10
11
12
13
14


Fraction of 2'-Fluoro-AHC-c-di-GMP bound
1.0
0.5
0.0
Non treated
ADUS-100
H151
HSKB142
HSKB143
6
7
8
9
10
11
12
13
14

Series C



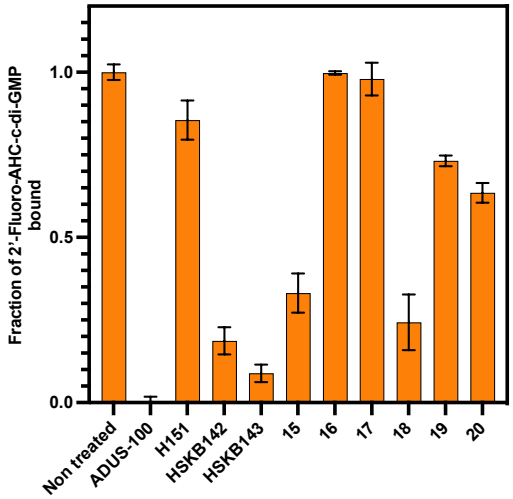
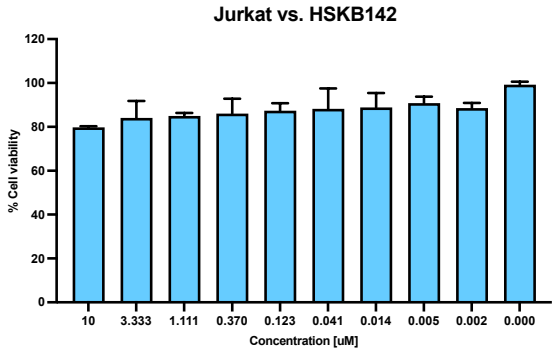
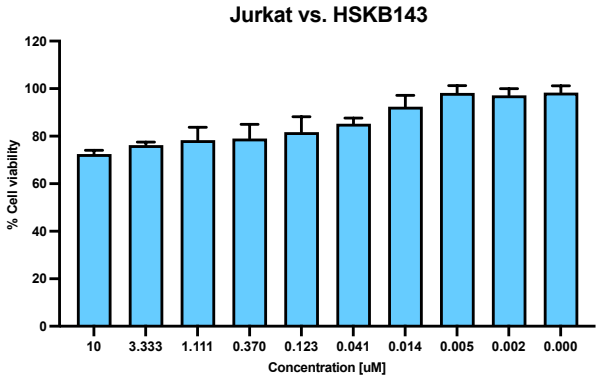


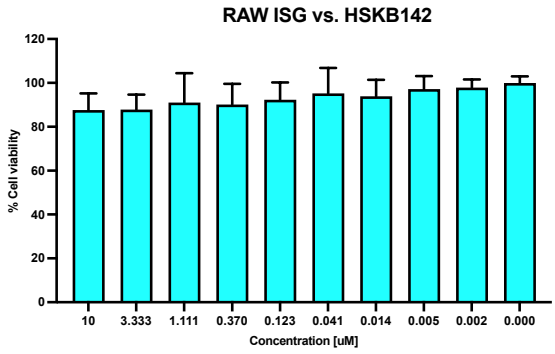
Fig. S1: Compounds tested for STING binding through STING-FP Assay

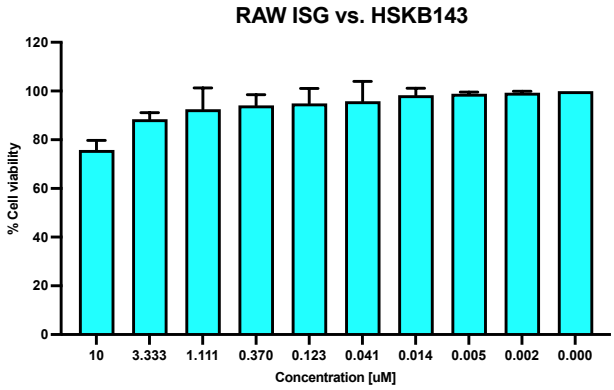
A)


Jurkat vs. HSKB142
% Cell viability
120
100
80
60
40
20
0
10
3.333
1.111
0.370
0.123
0.041
0.014
0.005
0.002
0.000
Concentration [uM]


Jurkat vs. HSKB143
% Cell viability
120
100
80
60
40
20
0
10
3.333
1.111
0.370
0.123
0.041
0.014
0.005
0.002
0.000
Concentration [uM]

B)


RAW ISG vs. HSKB142
% Cell viability
120
100
80
60
40
20
0
10
3.333
1.111
0.370
0.123
0.041
0.014
0.005
0.002
0.000
Concentration [uM]


RAW ISG vs. HSKB143
% Cell viability
120
100
80
60
40
20
0
10
3.333
1.111
0.370
0.123
0.041
0.014
0.005
0.002
0.000
Concentration [uM]

C)

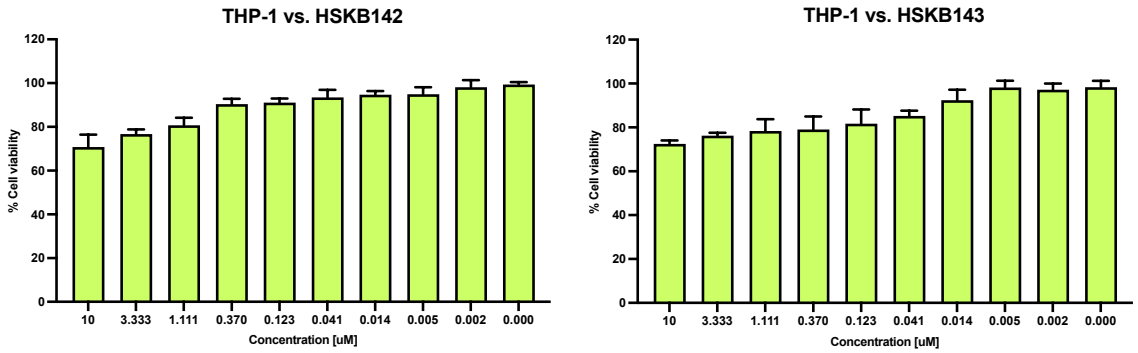


Fig. S2: Cell viability of compounds against immune cells.

3.Pharmacokinetic (PK) parameters

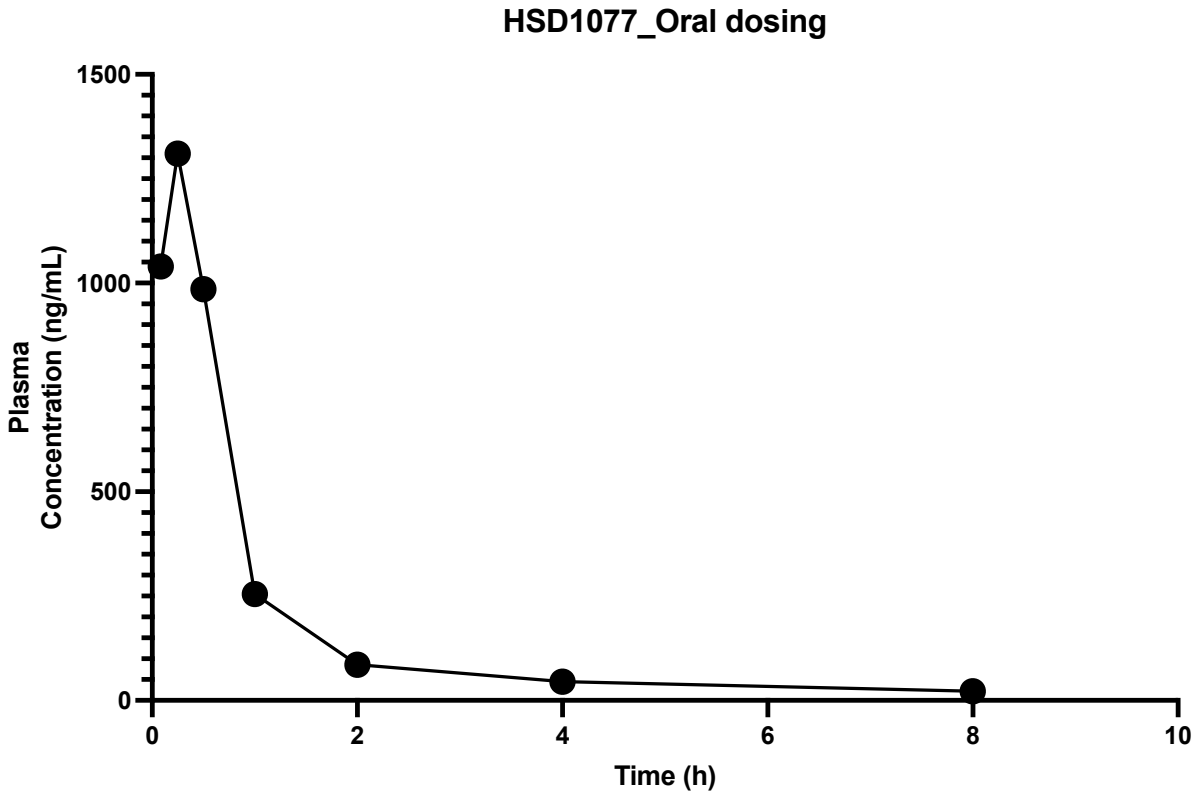


Fig. S3: Total plasma concentration vs time profile for HSD1077 after 20 mg/kg PO in Male CD1 Mice (n = 3). The three samples were pooled together, and drug concentration analyzed via LC-MS (as contract research at Pharmaron).

Table S1; Summary of HSD1077 pharmacokinetic parameters after PO dose of 20 mg/kg

PK parameters	Unit	Mean
$T_{1/2}$	h	3.18
T_{max}	h	0.250
C_{max}	ng/mL	1310
AUC_{last}	h*ng/mL	1272
AUC_{Inf}	h*ng/mL	1375
$AUC\_{}_{\%Extrap}\_obs$	%	7.45
MRT_{Inf}_obs	h	2.11
AUC_{last}/D	h*mg/mL	63.6
F	%	91.8

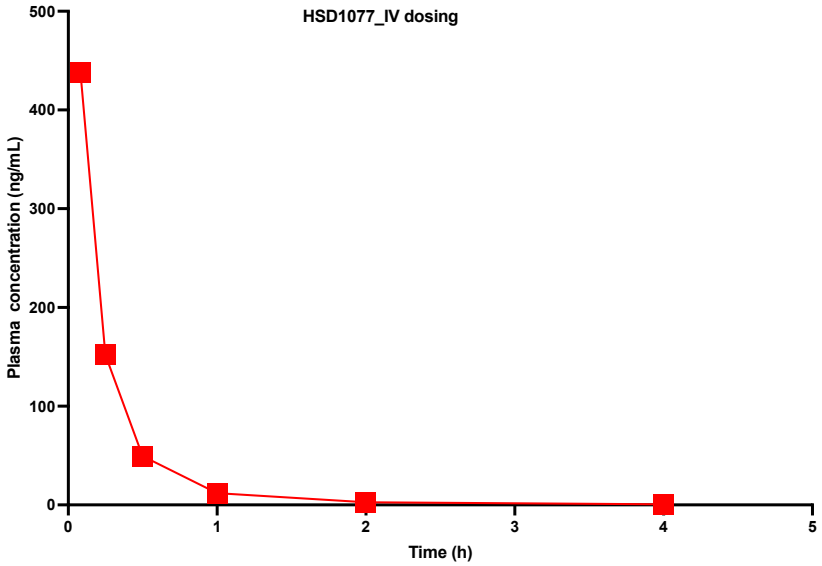


Fig. S4: Total plasma concentration vs time profile for HSD1077 after 2 mg/kg dosing (IV) in Male CD1 Mice (n = 3). The three samples were pooled together, and drug concentration analyzed via LC-MS (as contract research at Pharmaron).

Table S2; Summary of HSD1077 pharmacokinetic parameters after IV dose of 2 mg/kg

PK parameters	Unit	Mean
Cl_obs	mL/min/kg	222
$T_{1/2}$	h	0.700
C_0	ng/mL	743
AUC_{last}	h*ng/mL	149
AUC_{Inf}	h*ng/mL	150
$AUC\_{}_{\%Extrap}\_obs$	%	0.371
MRT_{Inf}_obs	h	0.290
AUC_{last}/D	h*mg/mL	74.6
V_{ss}_obs	L/kg	3.87

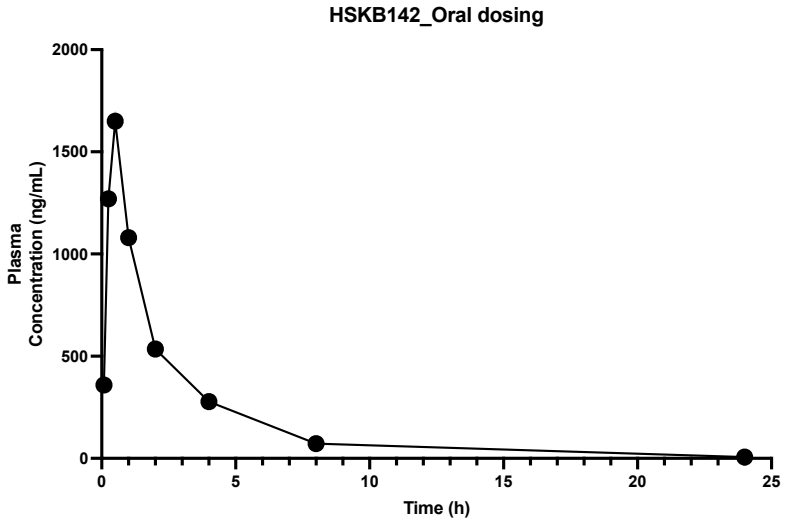


Fig. S5: Total plasma concentration vs time profile for HSKB142 after 20 mg/kg PO in Male CD1 Mice (n = 3). The three samples were pooled together, and drug concentration analyzed via LC-MS (as contract research at Pharmaron).

Table S3; Summary of HSKB142 pharmacokinetic parameters after PO dose of 20 mg/kg

PK parameters	Unit	Mean
$T_{1/2}$	h	3.96
T_{max}	h	0.5
C_{max}	ng/mL	1650
AUC_{last}	h*ng/mL	4147.57
AUC_{Inf}	h*ng/mL	4185.98
$AUC\_{}_{\%Extrap}\_obs$	%	0.92
MRT_{Inf}_obs	h	3.42
AUC_{last}/D	h*mg/mL	207.38
F	%	41.08

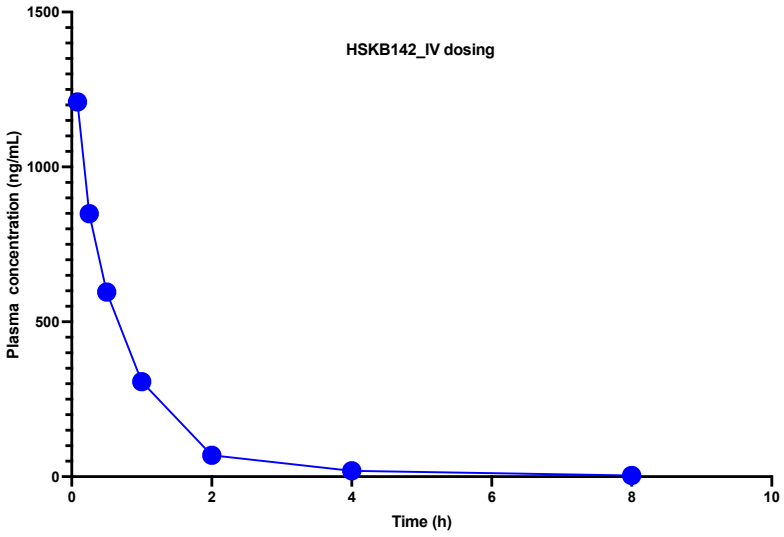


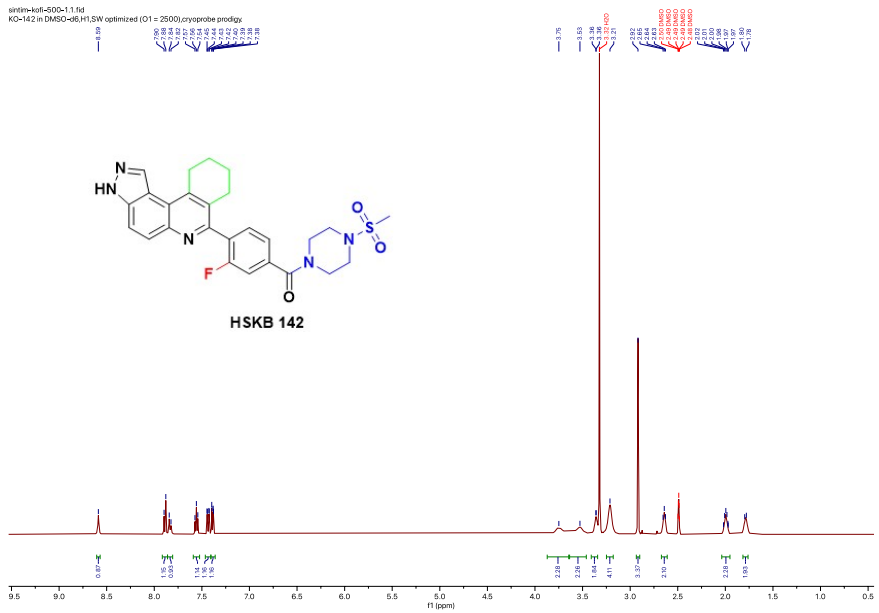
Fig. S6: Total plasma concentration vs time profile for HSKB142 after 2 mg/kg dosing (IV) in Male CD1 Mice (n = 3). The three samples were pooled together, and drug concentration analyzed via LC-MS (as contract research at Pharmaron).

Table S4; Summary of HSKB142 pharmacokinetic parameters after IV dose of 2 mg/kg

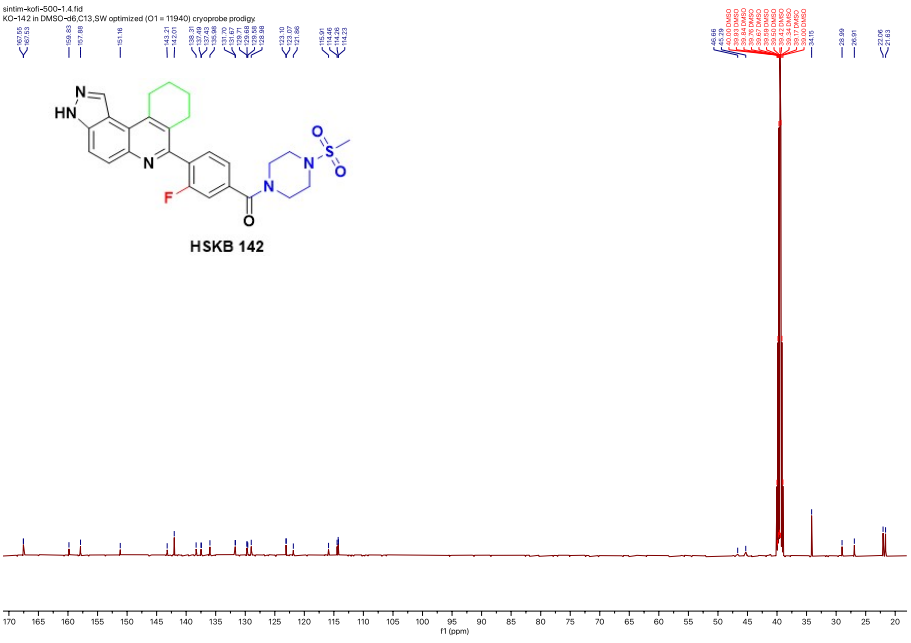
PK parameters	Unit	Mean
Cl_obs	mL/min/kg	32.7
$T_{1/2}$	h	1.50
C_0	ng/mL	1443
AUC_{last}	h*ng/mL	1010
AUC_{Inf}	h*ng/mL	1019
$AUC\_{\%Extrap}\_obs$	%	0.843
MRT_{Inf}_obs	h	0.965
AUC_{last}/D	h*mg/mL	505
V_{ss}_obs	L/kg	1.89

3. NMR Spectra

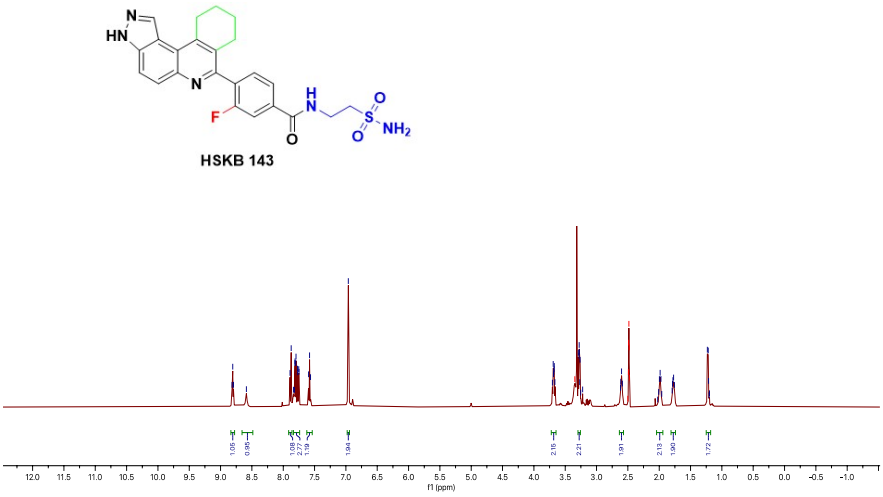
^{1}H NMR (500 MHz, DMSO) : **142**



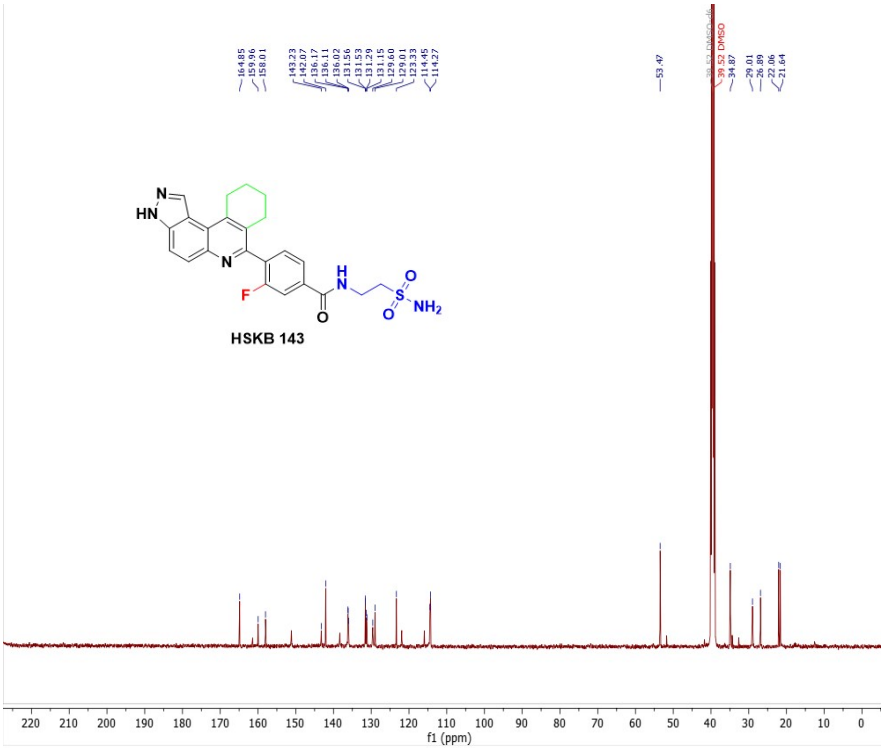
^{13}C NMR (126 MHz, DMSO) : **142**



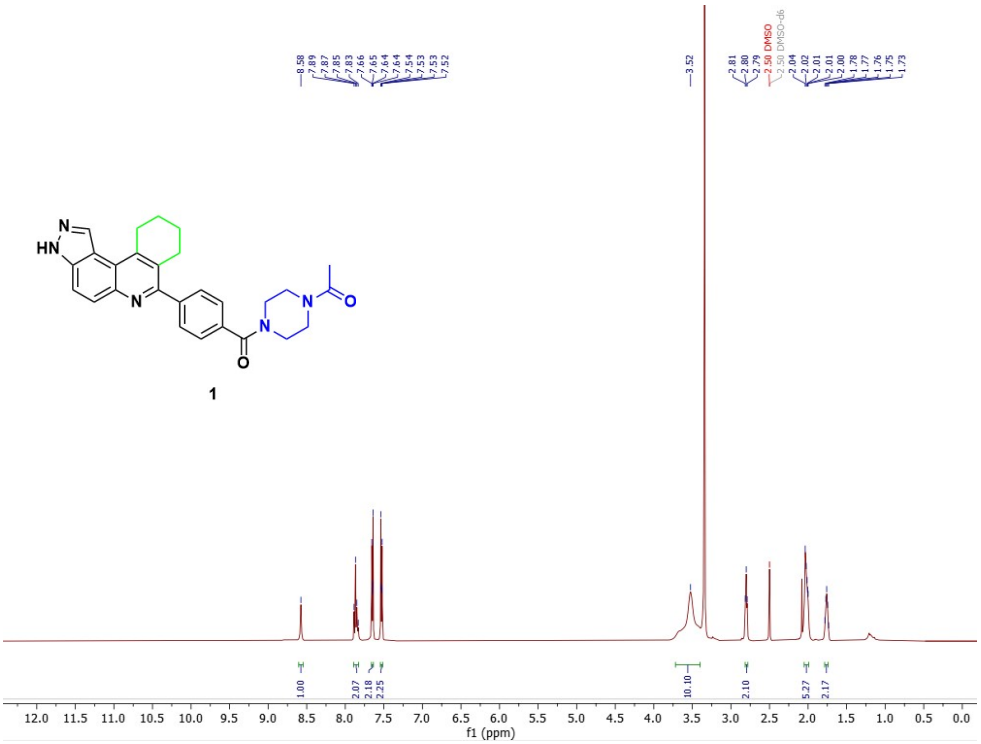
^{1}H NMR (500 MHz, DMSO) : **143**



^{13}C NMR (126 MHz, DMSO) : **143**

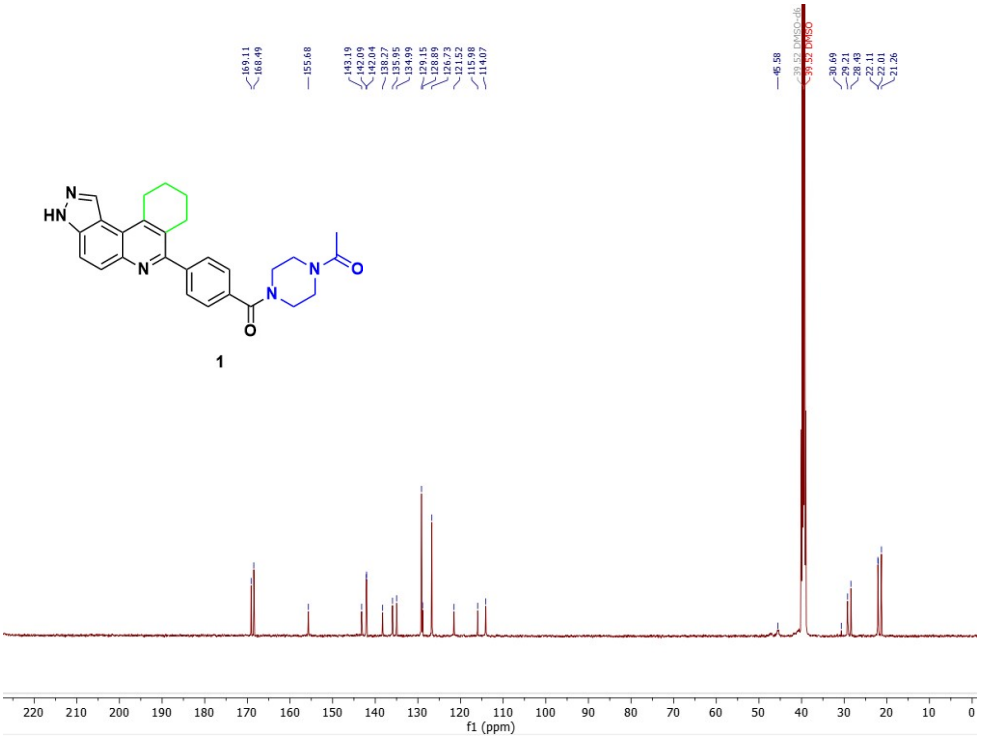


^{1}H NMR (500 MHz, DMSO) : **1**



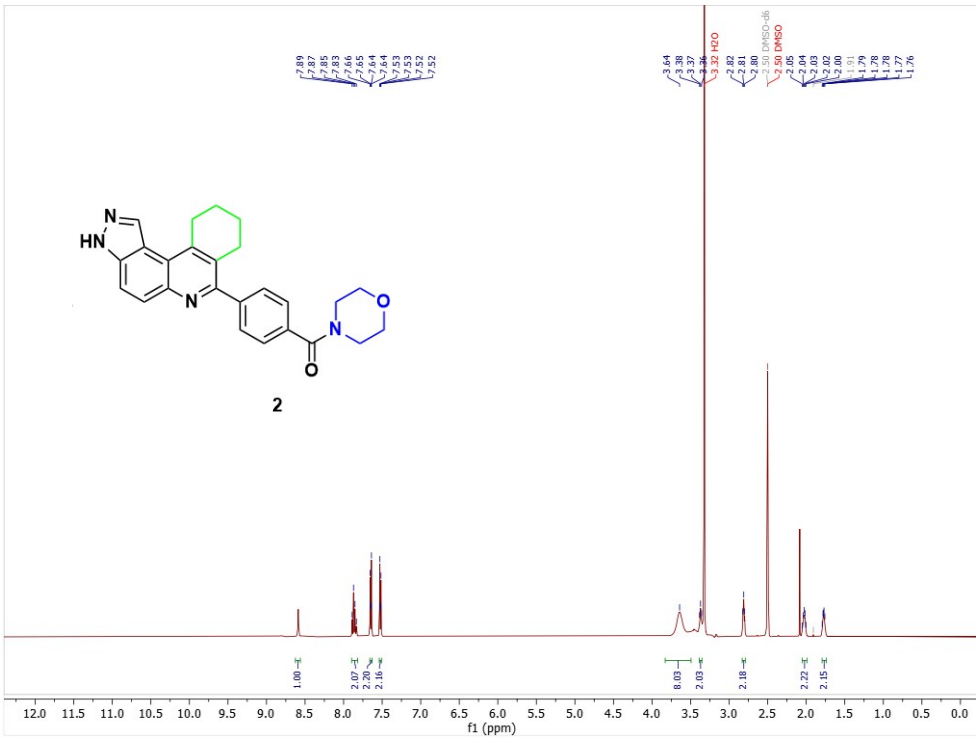
1

^{13}C NMR (126 MHz, DMSO) : **1**

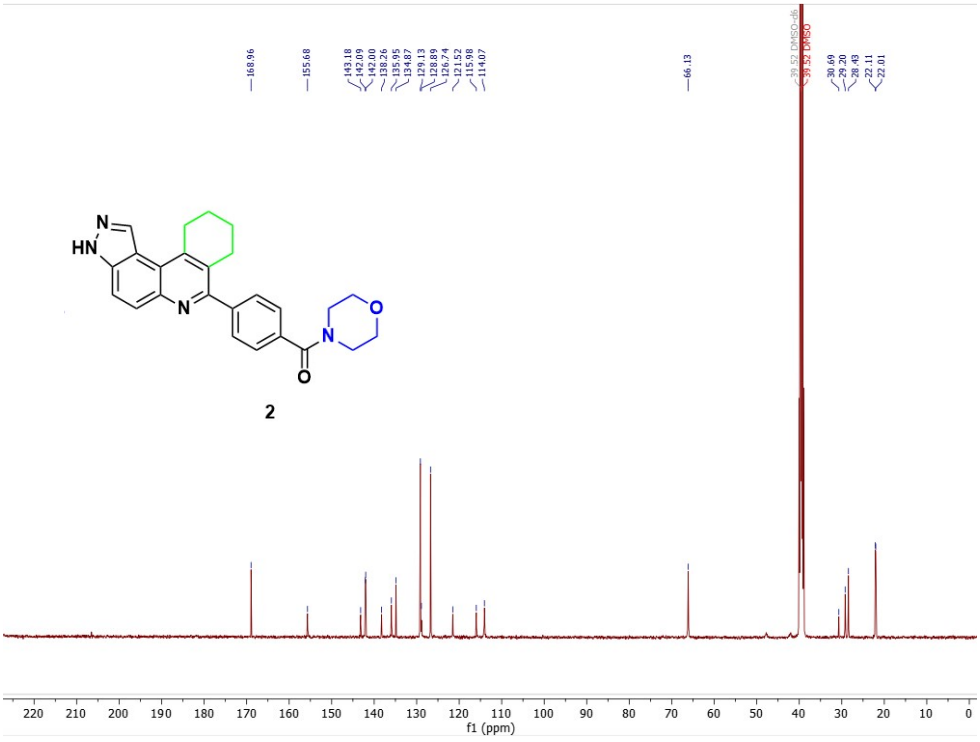


1

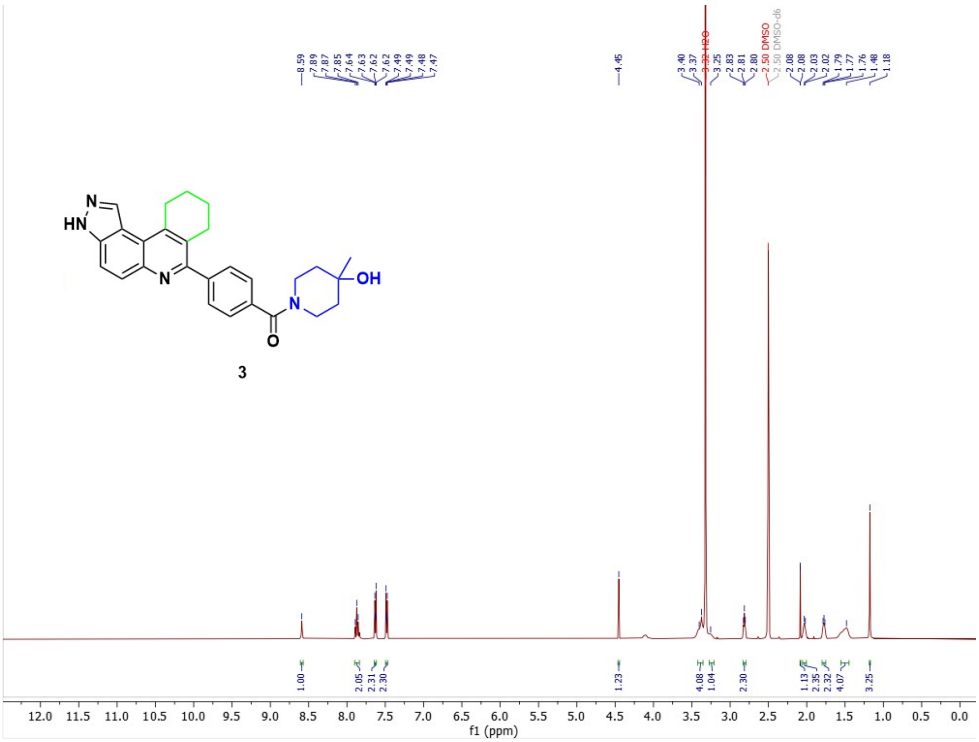
^{1}H NMR (500 MHz, DMSO) : **2**



^{13}C NMR (126 MHz, DMSO) : **2**

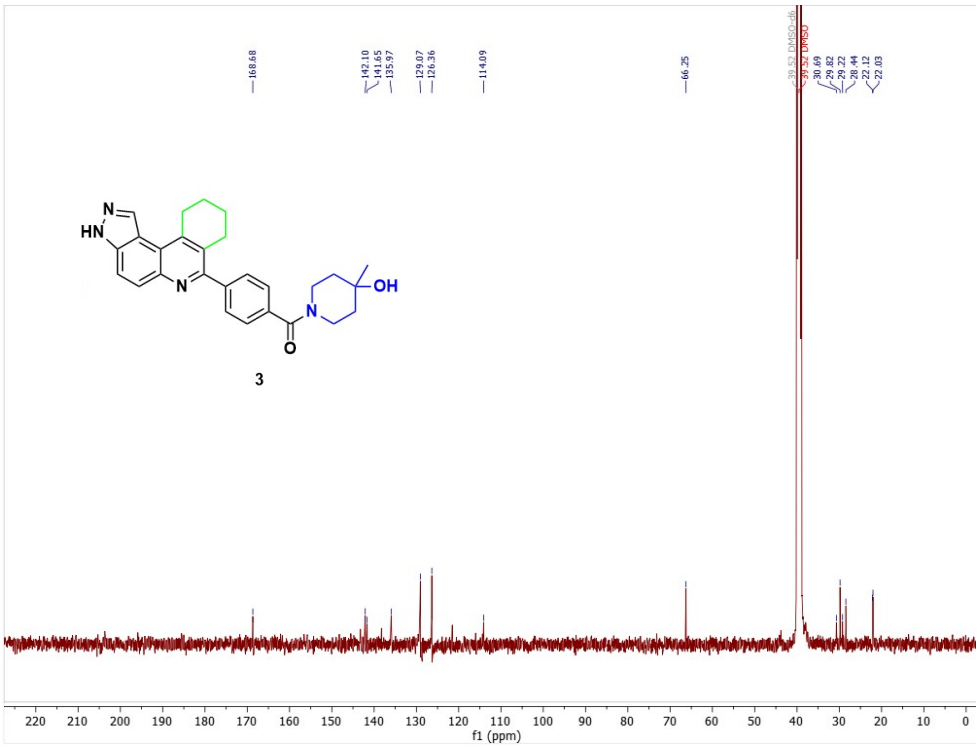


^{1}H NMR (500 MHz, DMSO) : **3**



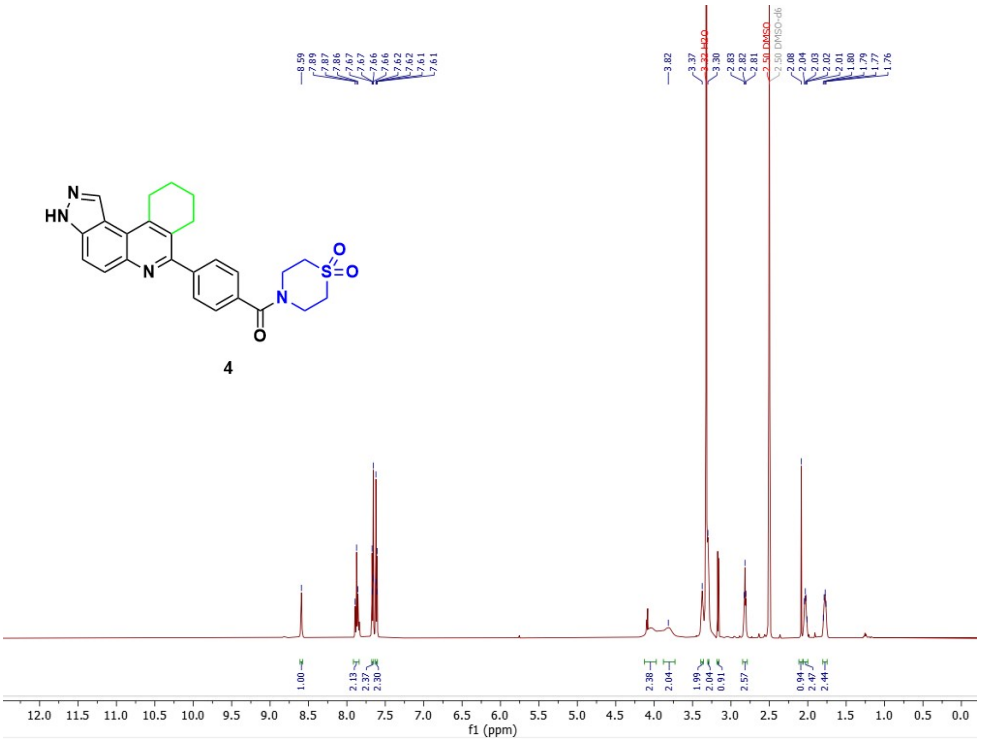
3

^{13}C NMR (126 MHz, DMSO) : **3**



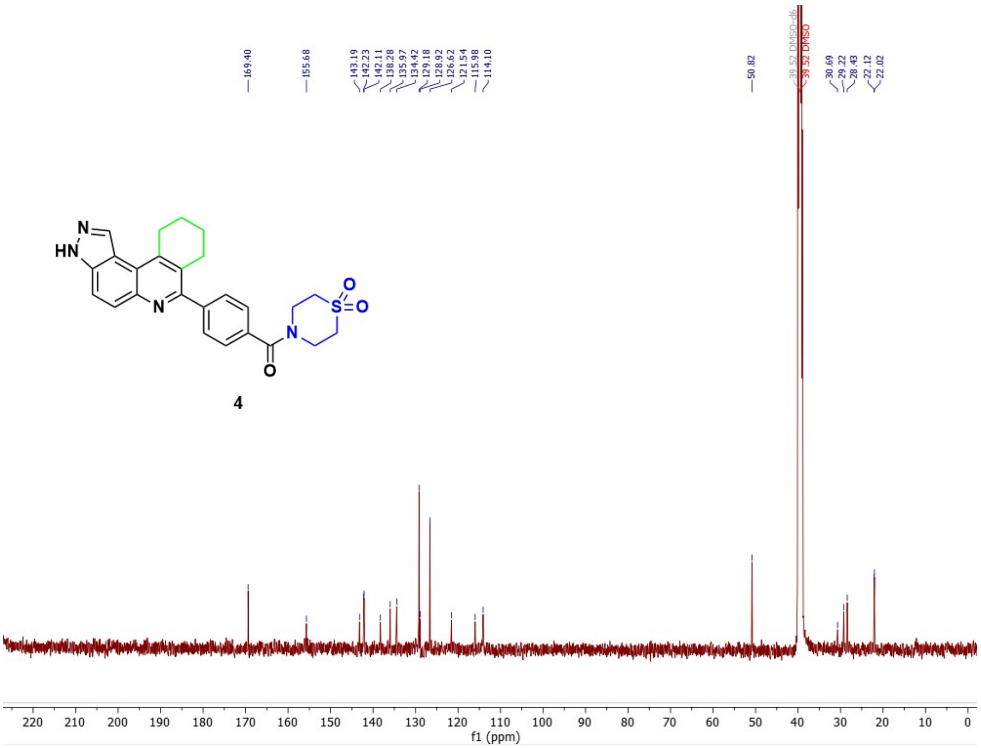
3

^{1}H NMR (500 MHz, DMSO) : **4**



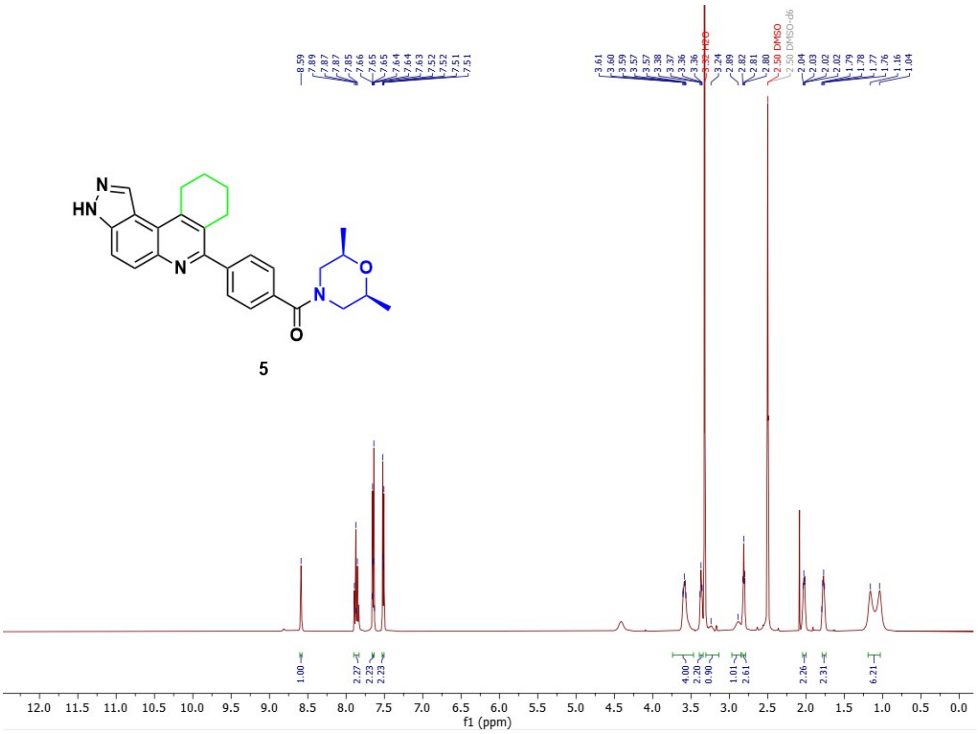
4

^{13}C NMR (126 MHz, DMSO) : **4**

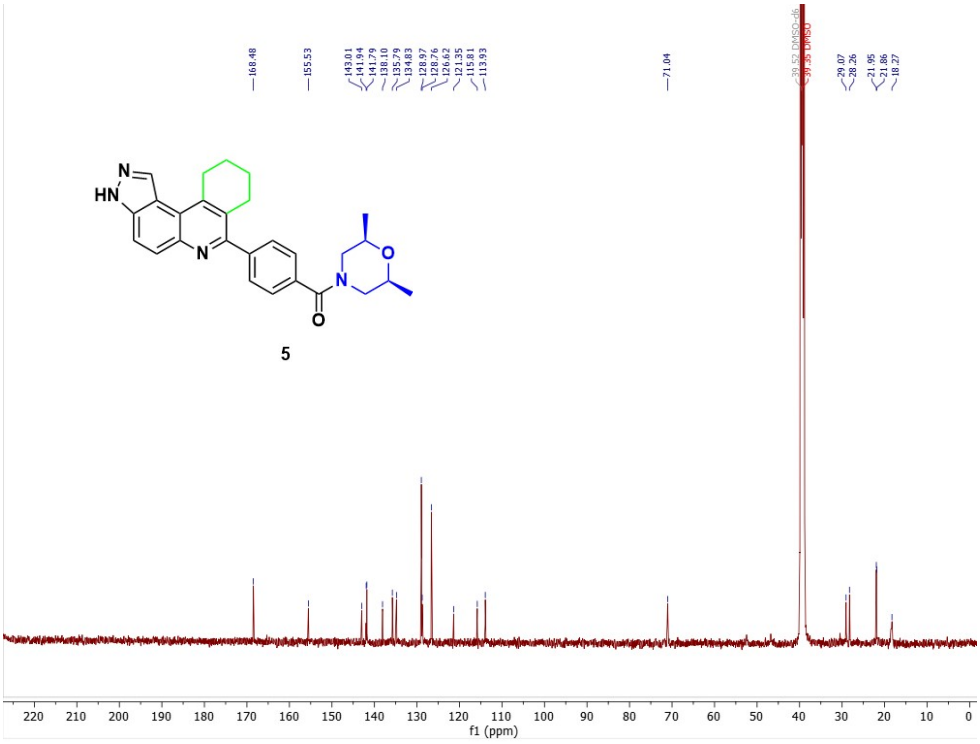


4

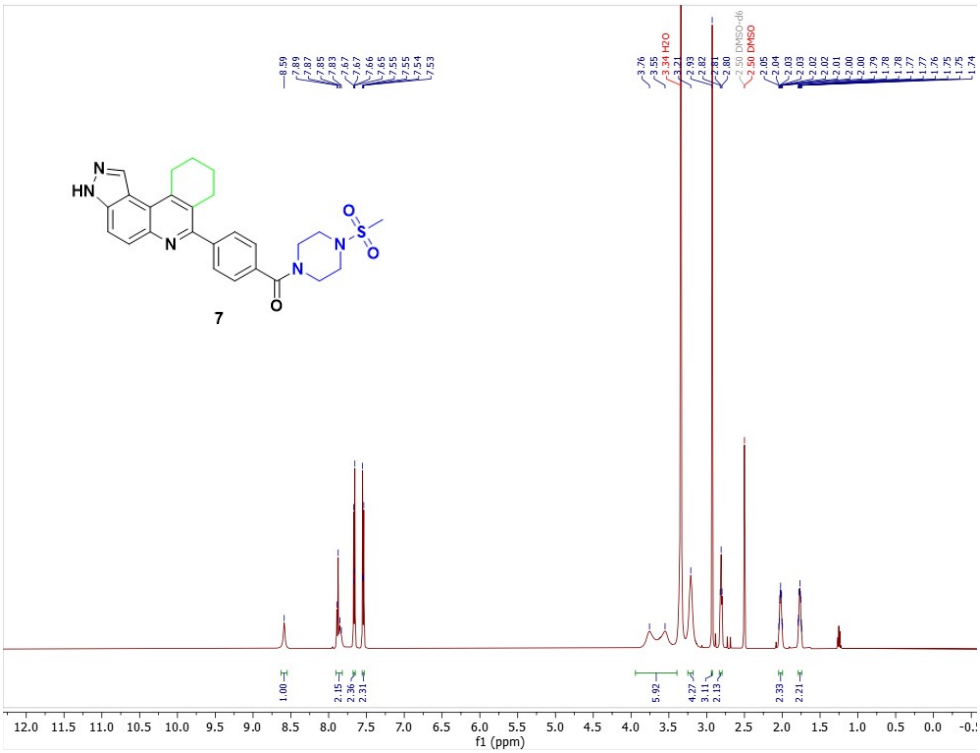
^{13}C NMR (126 MHz, DMSO) : **5**



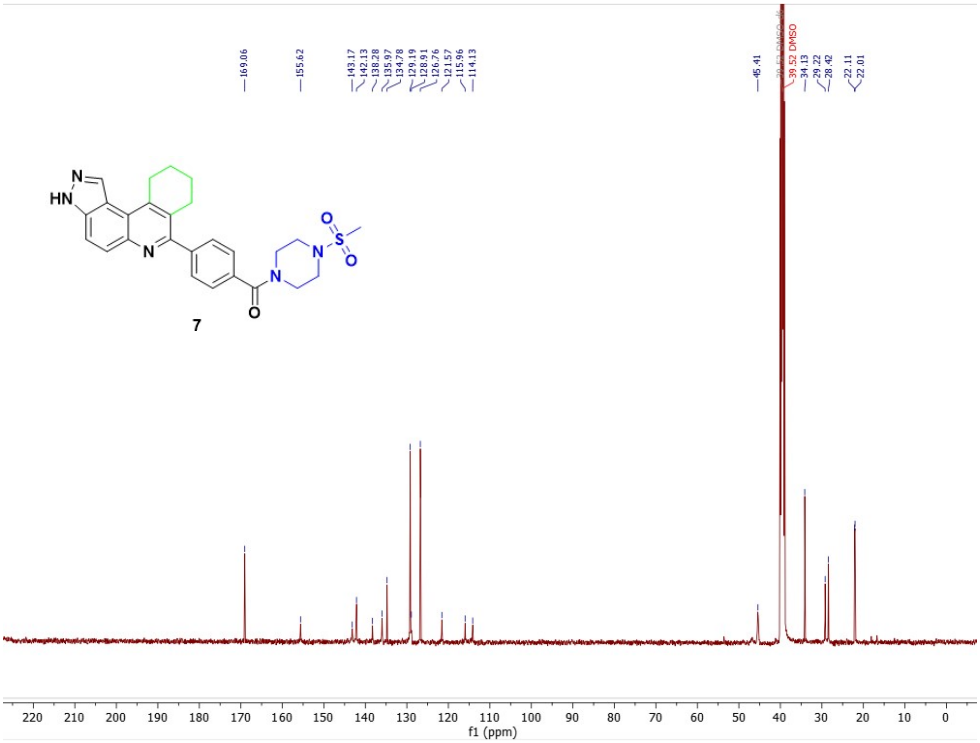
^{1}H NMR (500 MHz, DMSO) : **5**



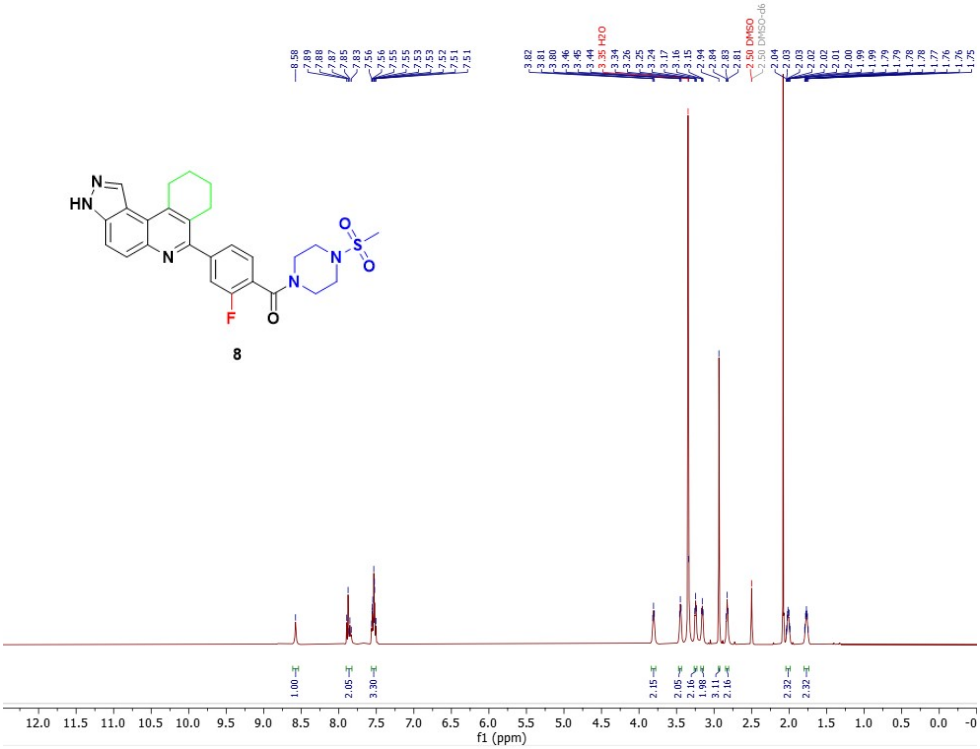
^{1}H NMR (500 MHz, DMSO) : **6**



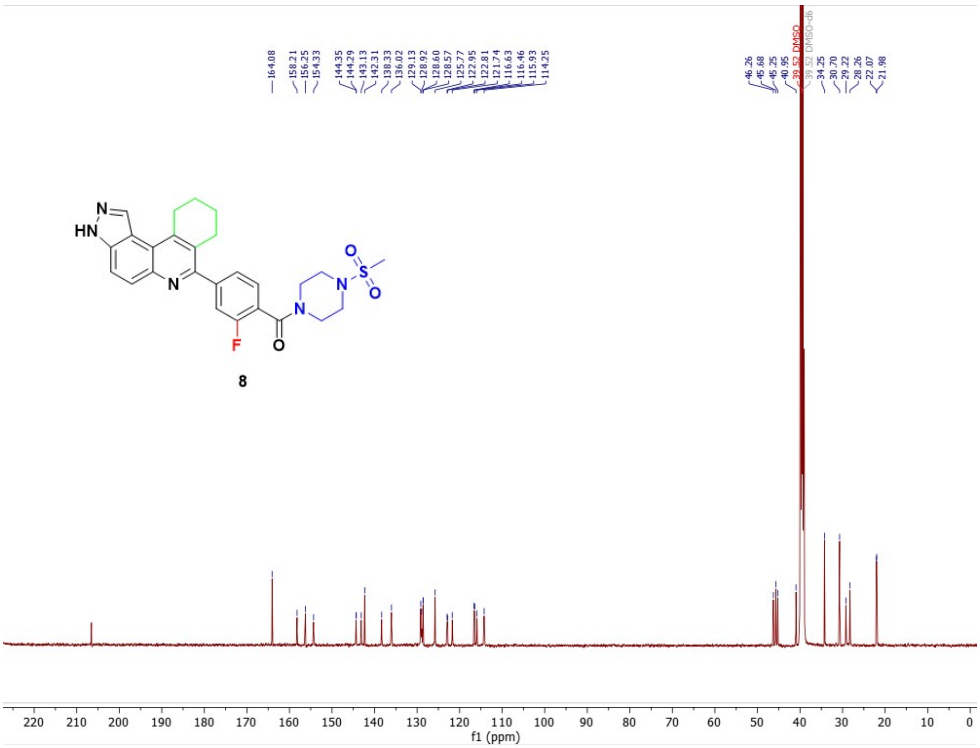
^{13}C NMR (126 MHz, DMSO) : **6**



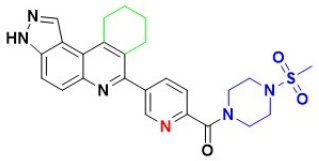
^{1}H NMR (500 MHz, DMSO) : **7**



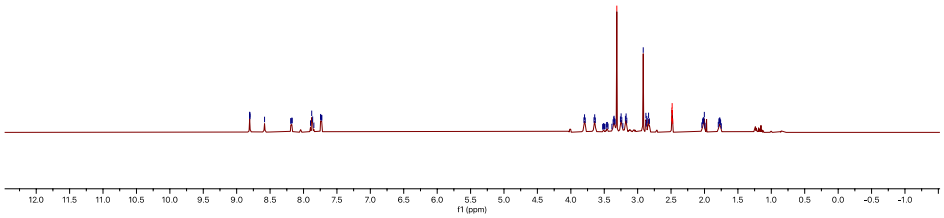
^{13}C NMR (126 MHz, DMSO) : **7**



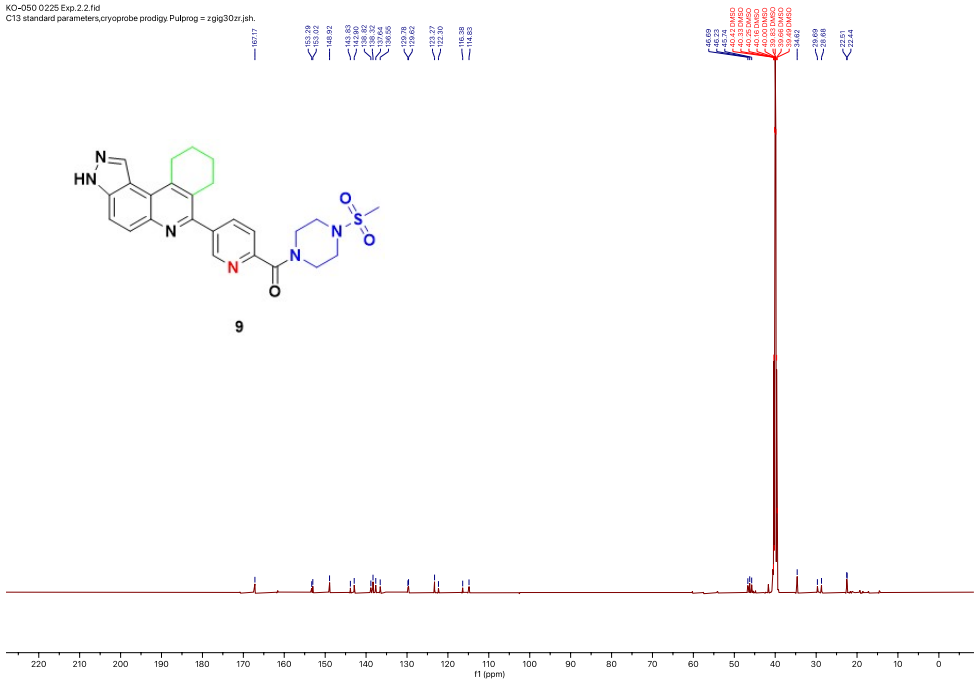
^{1}H NMR (500 MHz, DMSO) : **8**



9

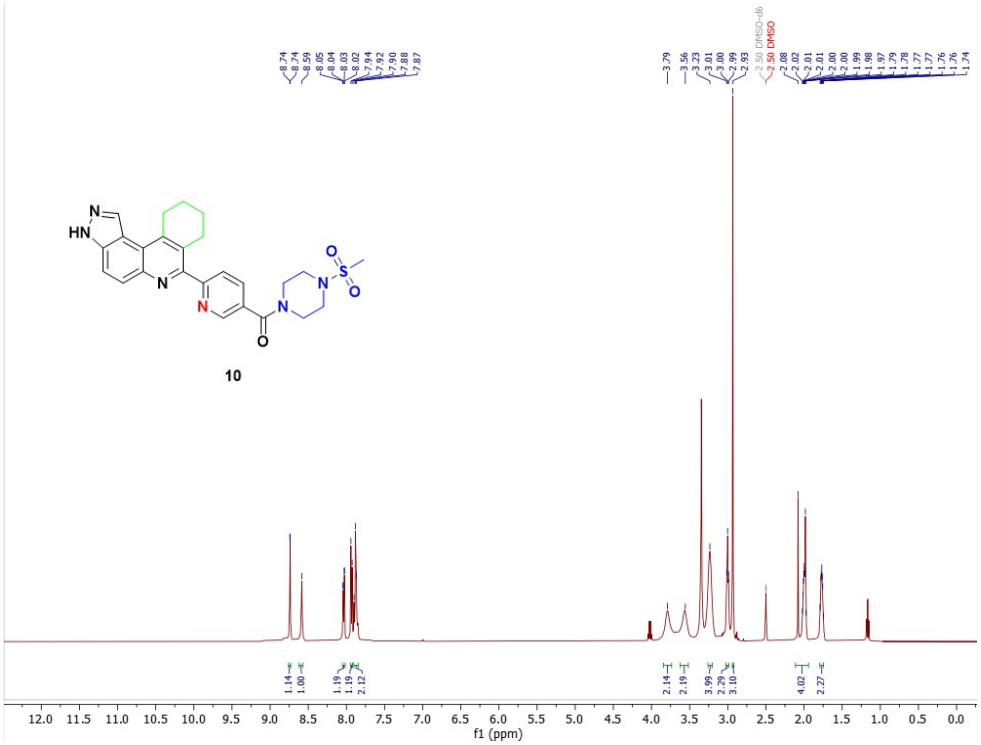


^{13}C NMR (126 MHz, DMSO) : **8**

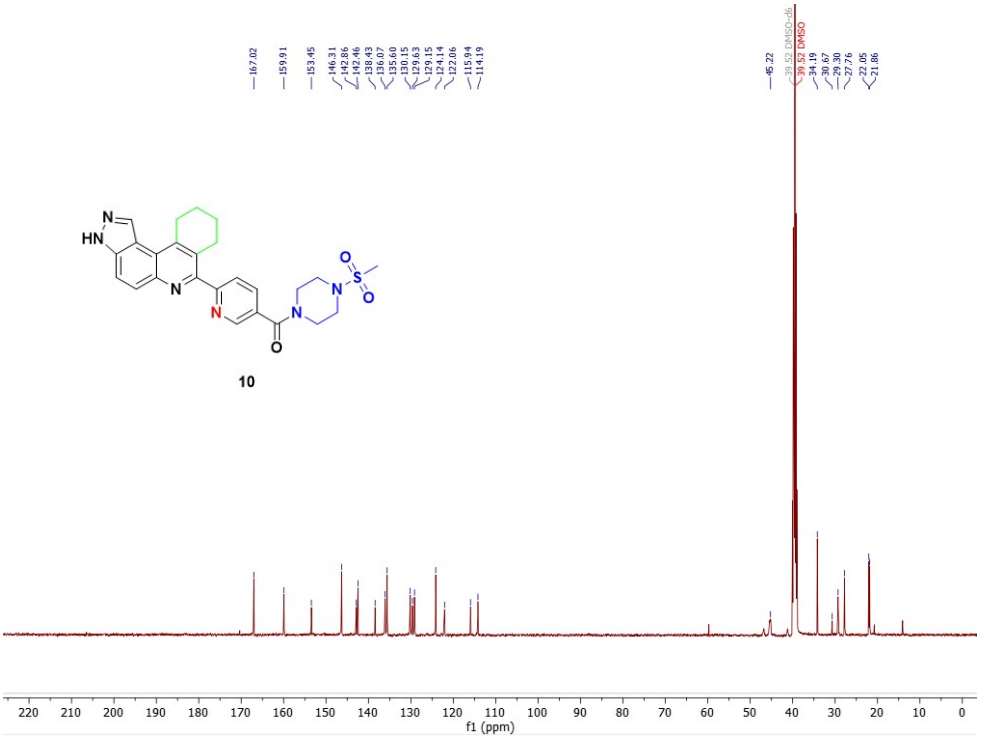


9

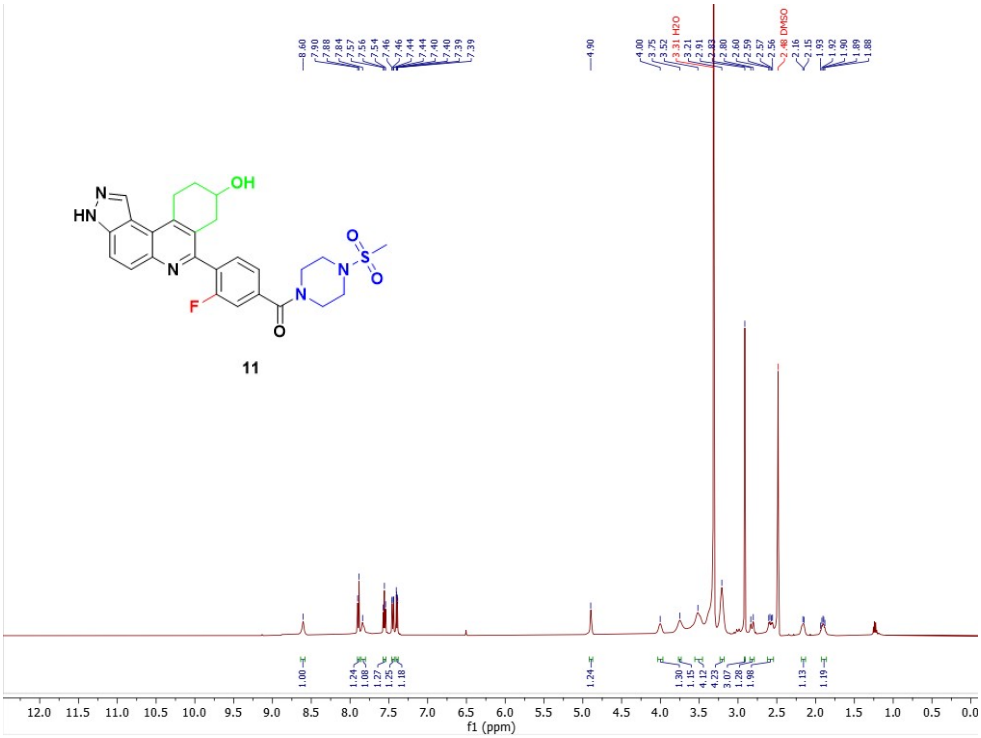
^{1}H NMR (500 MHz, DMSO) : **9**



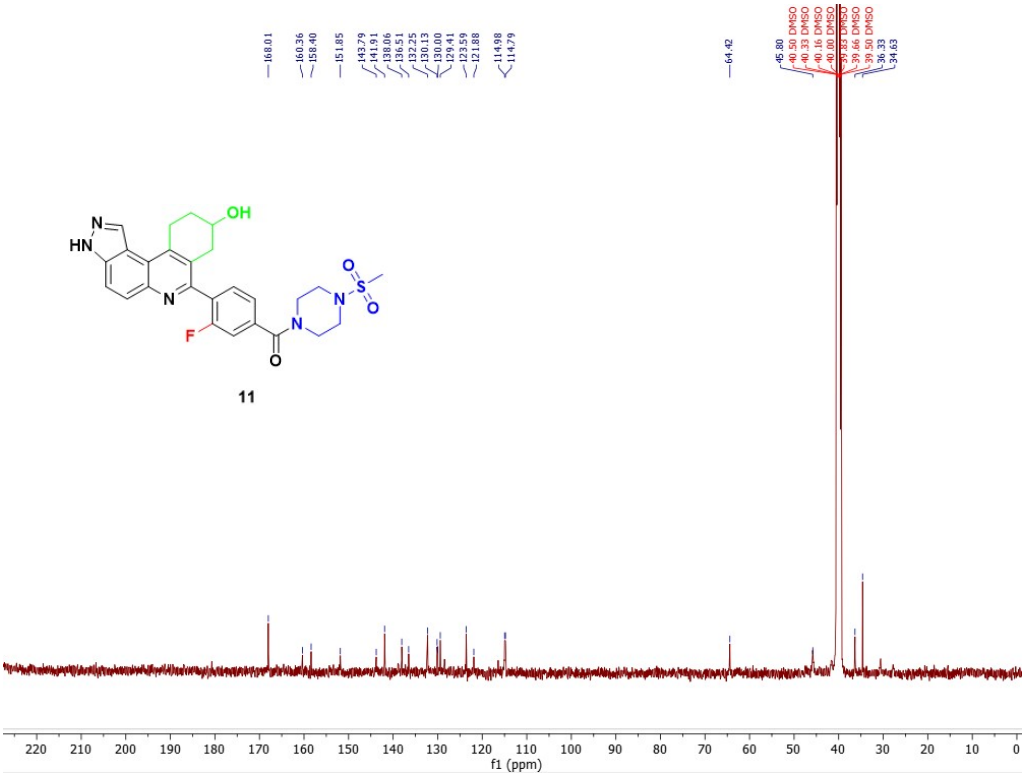
^{13}C NMR (126 MHz, DMSO) : **9**



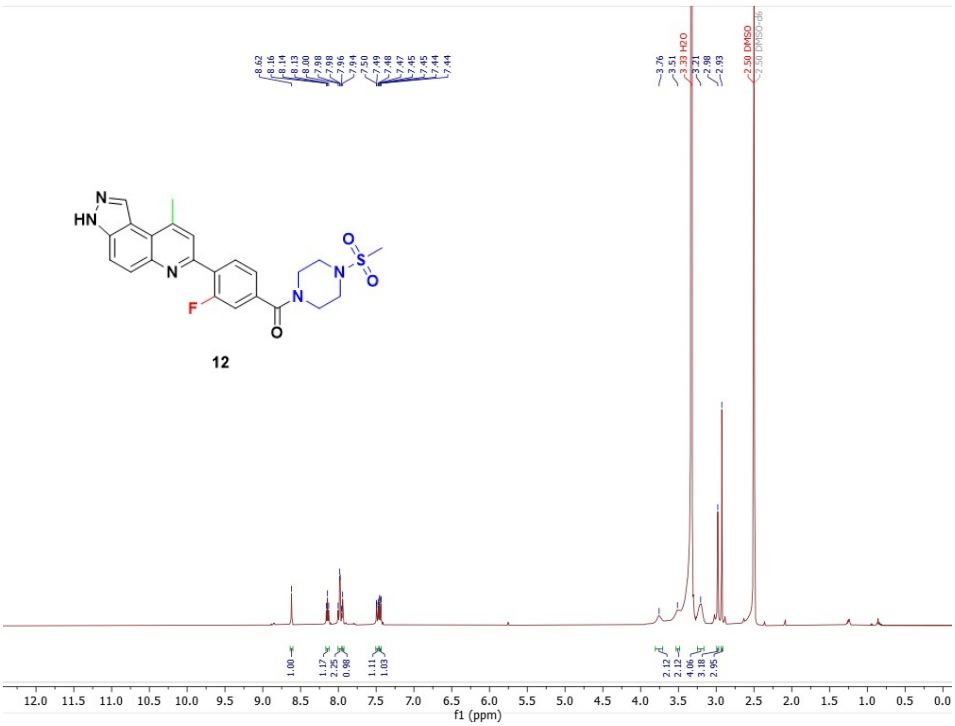
^{1}H NMR (500 MHz, DMSO) : **10**



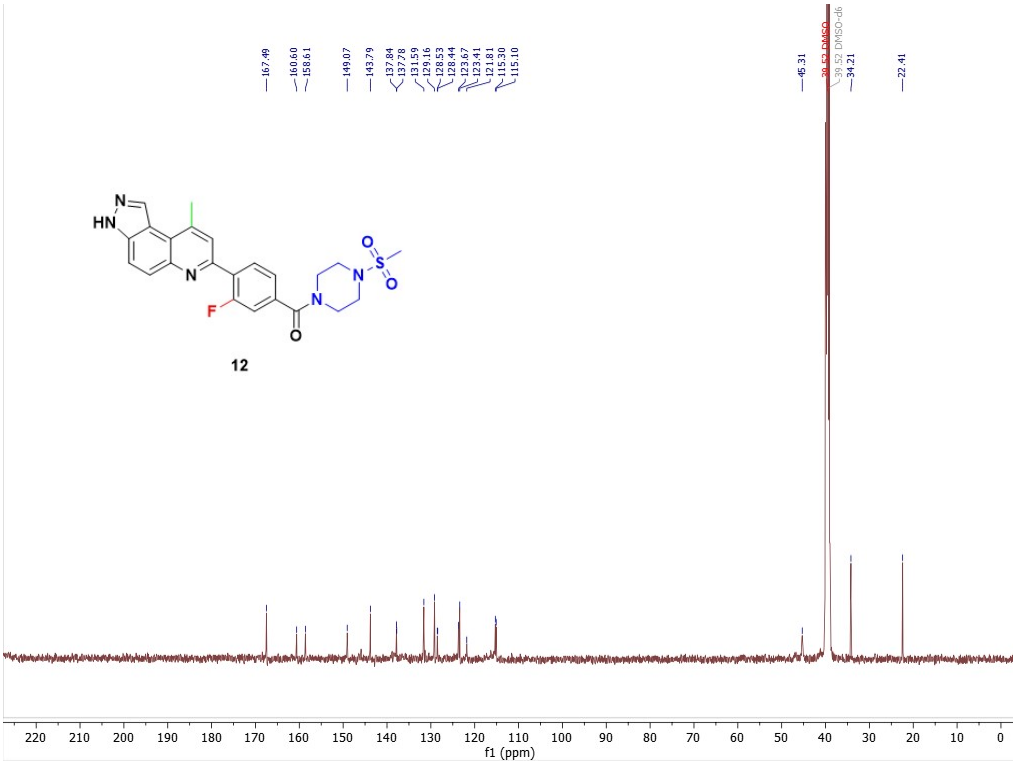
^{13}C NMR (126 MHz, DMSO) : **10**



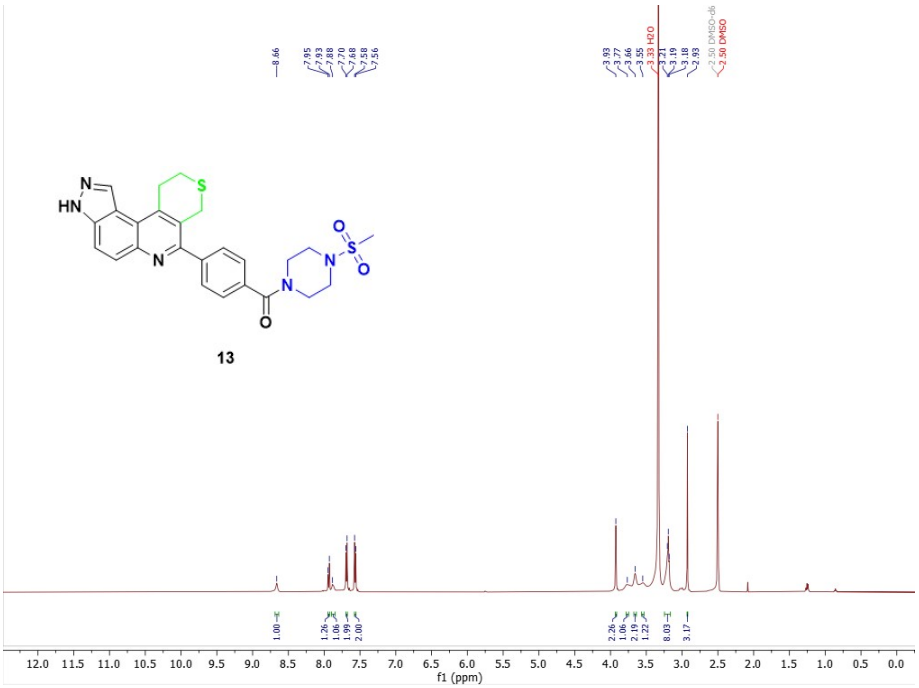
^{1}H NMR (500 MHz, DMSO) : **11**



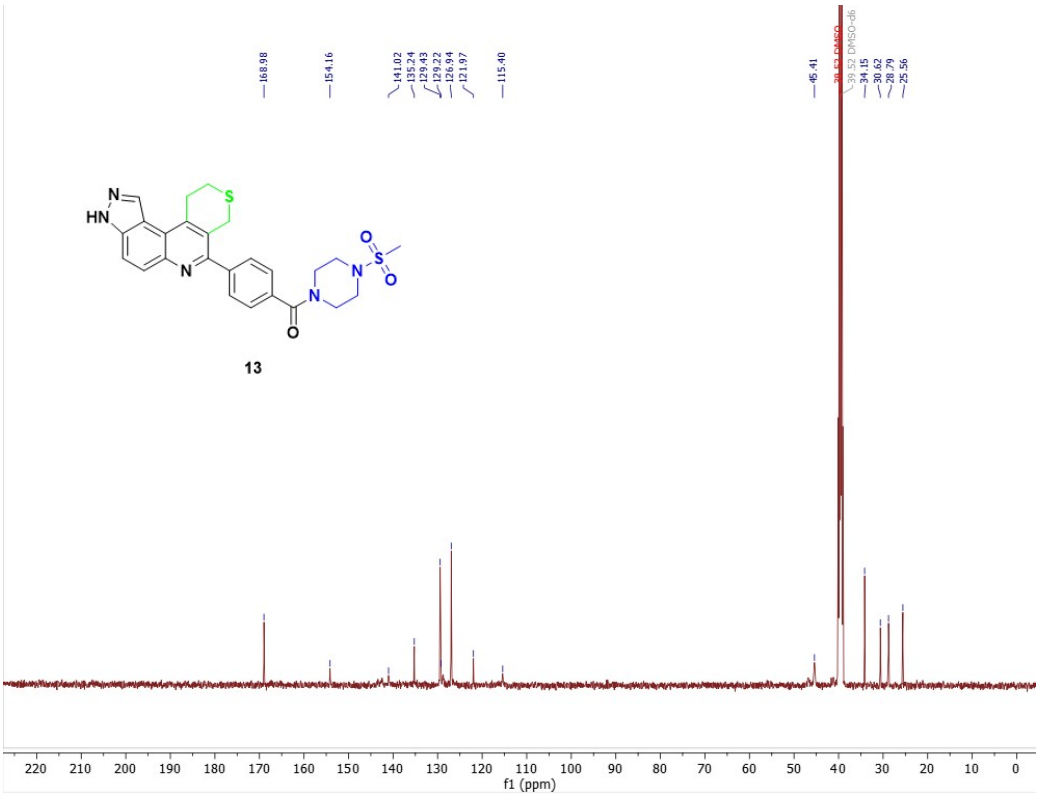
^{13}C NMR (126 MHz, DMSO) : **11**



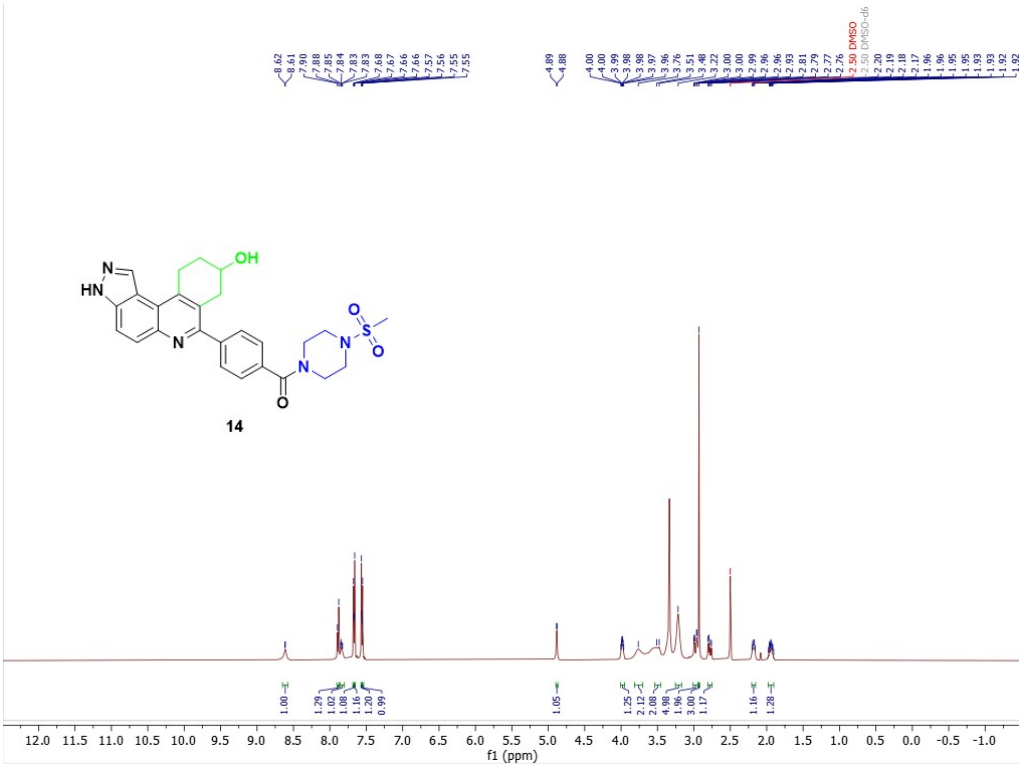
^{1}H NMR (500 MHz, DMSO) : **12**



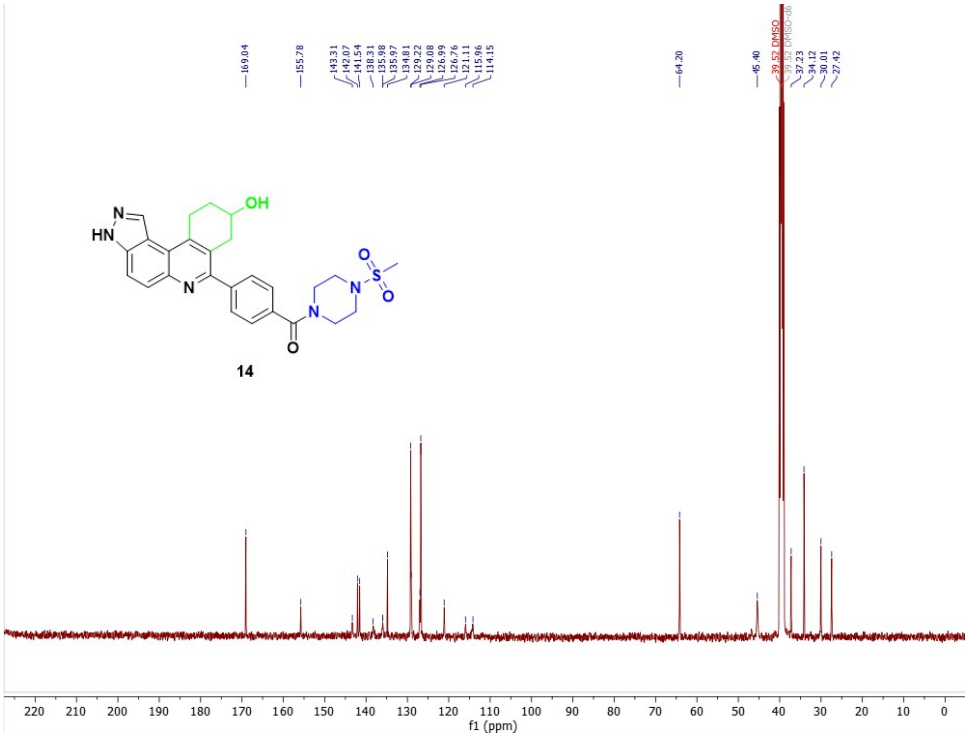
^{13}C NMR (126 MHz, DMSO) : **12**



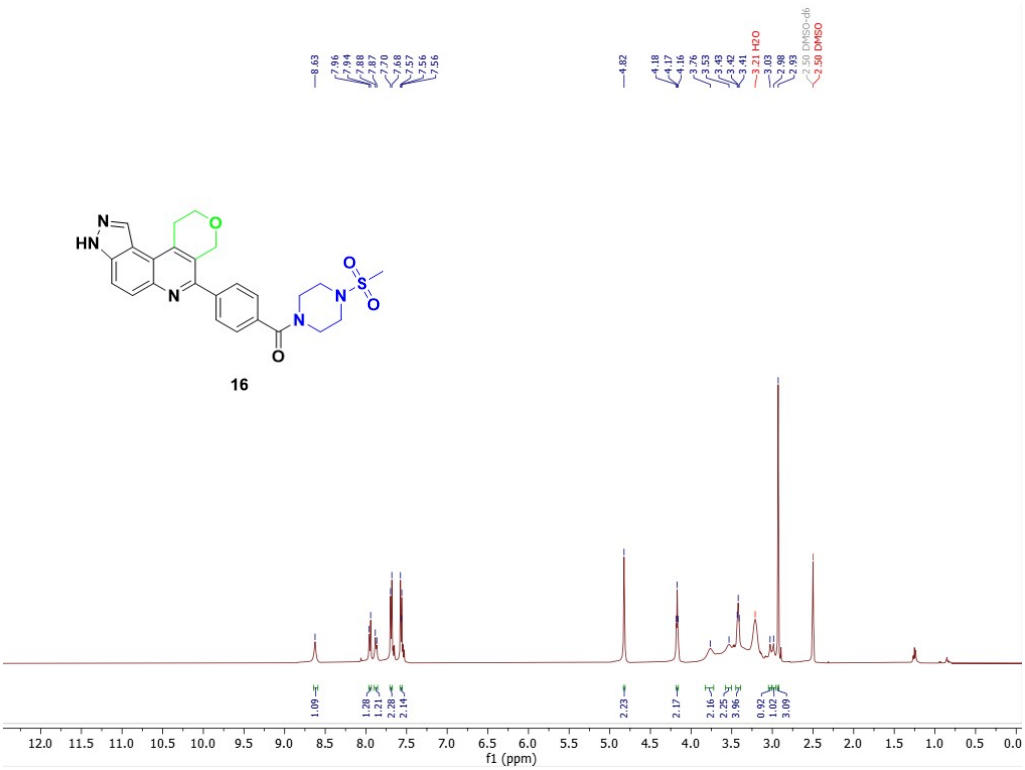
^{1}H NMR (500 MHz, DMSO) : **13**



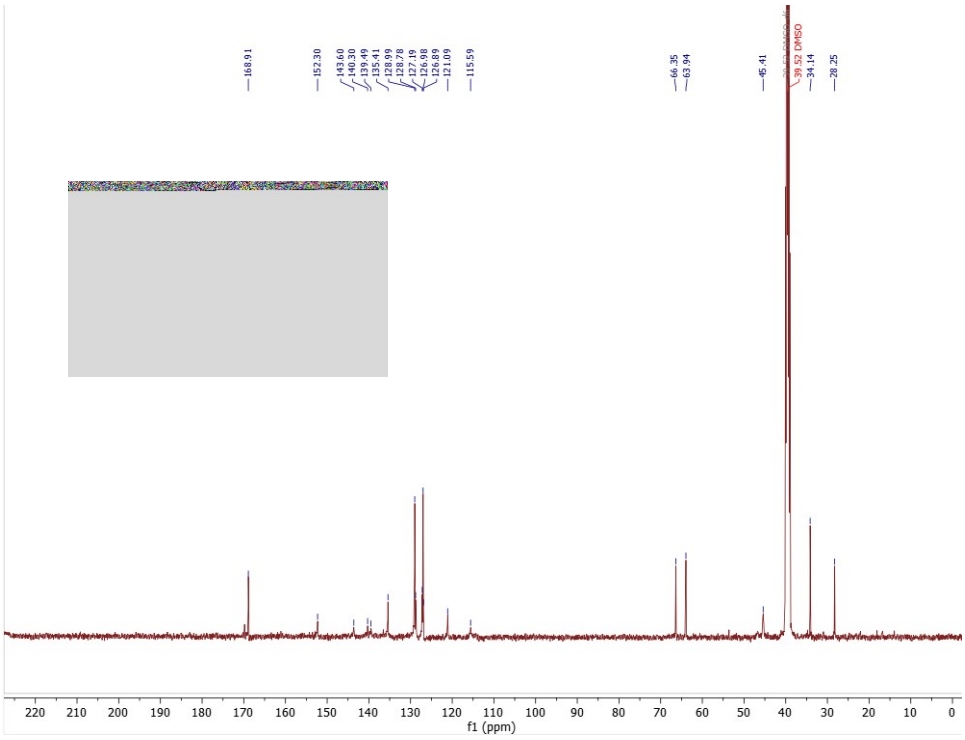
^{13}C NMR (126 MHz, DMSO) : **13**



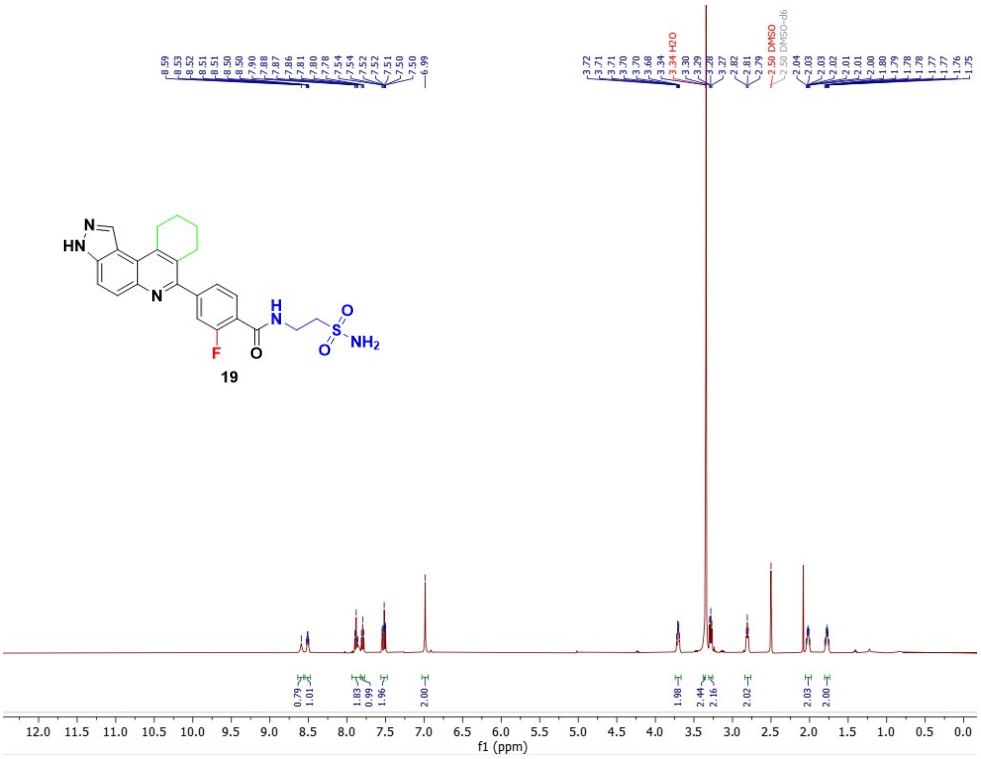
^{1}H NMR (500 MHz, DMSO) : **14**



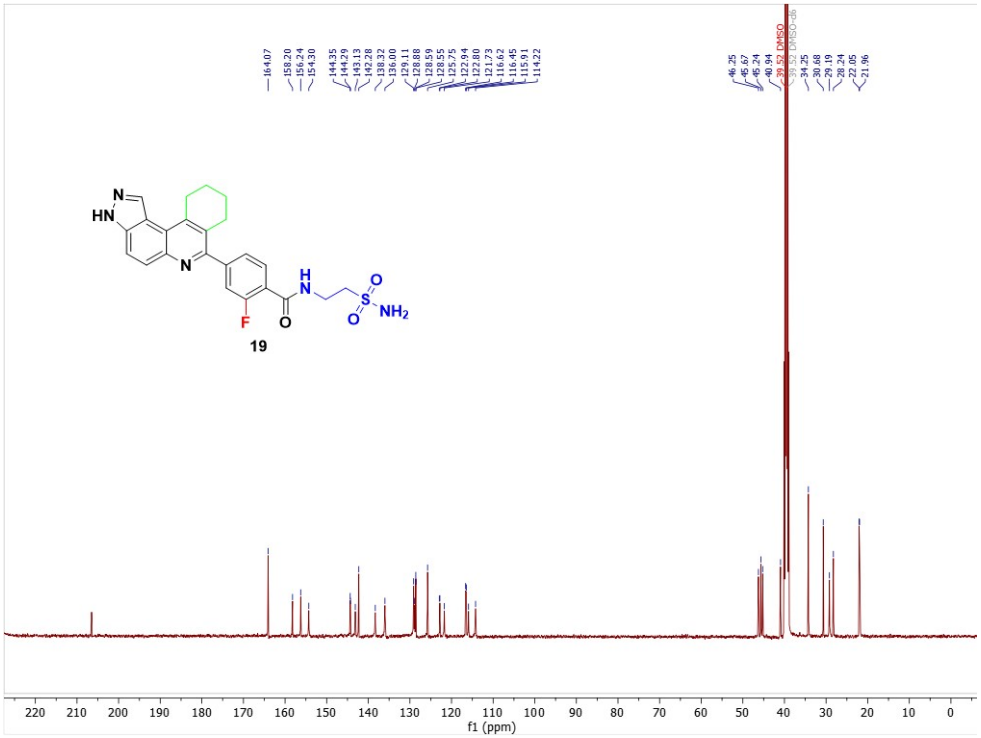
^{13}C NMR (126 MHz, DMSO): **14**


168.91
152.30
143.60
140.30
139.49
135.41
128.99
128.78
127.19
127.08
126.89
121.09
115.59
66.35
63.94
45.41
39.52 DMSO
34.14
28.25
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

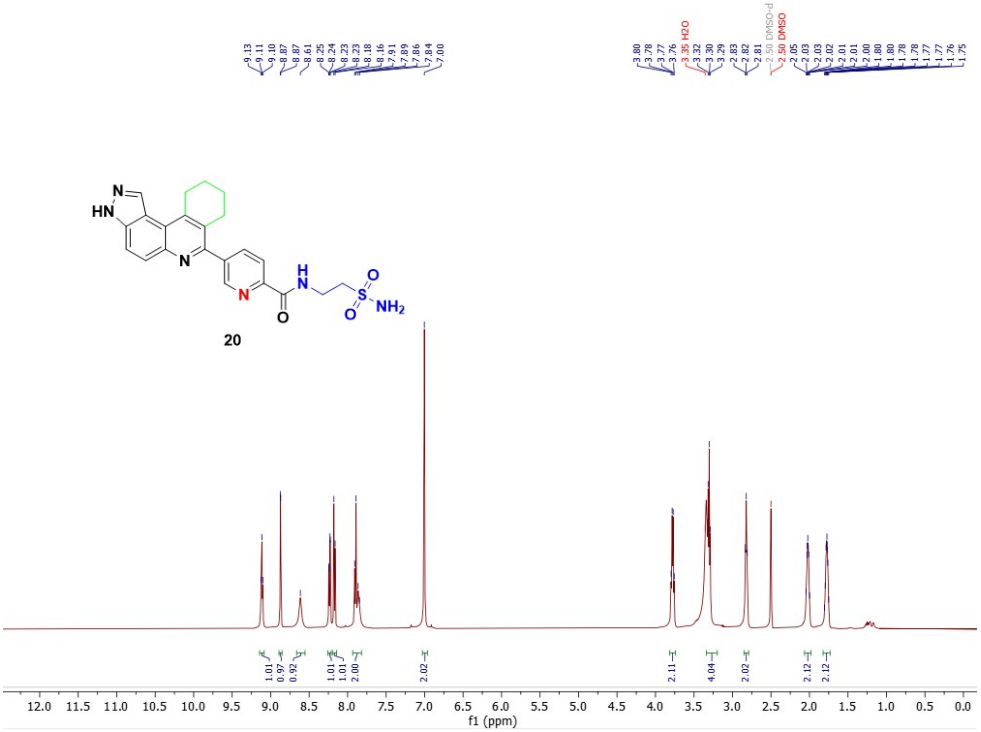
^{1}H NMR (500 MHz, DMSO) : **15**



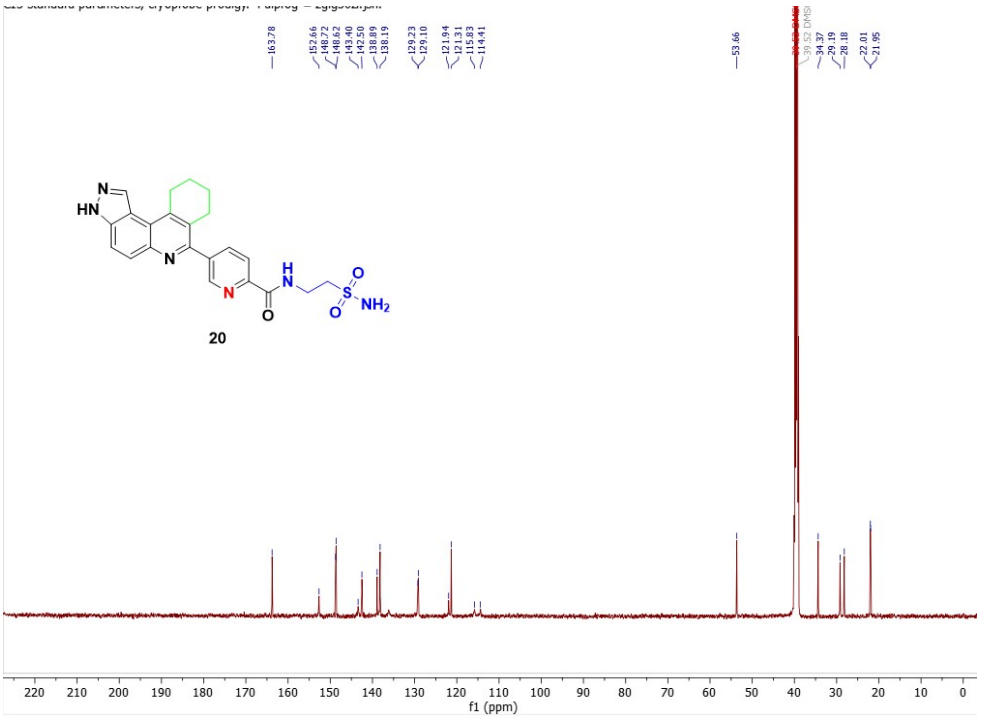
^{13}C NMR (126 MHz, DMSO) : **15**



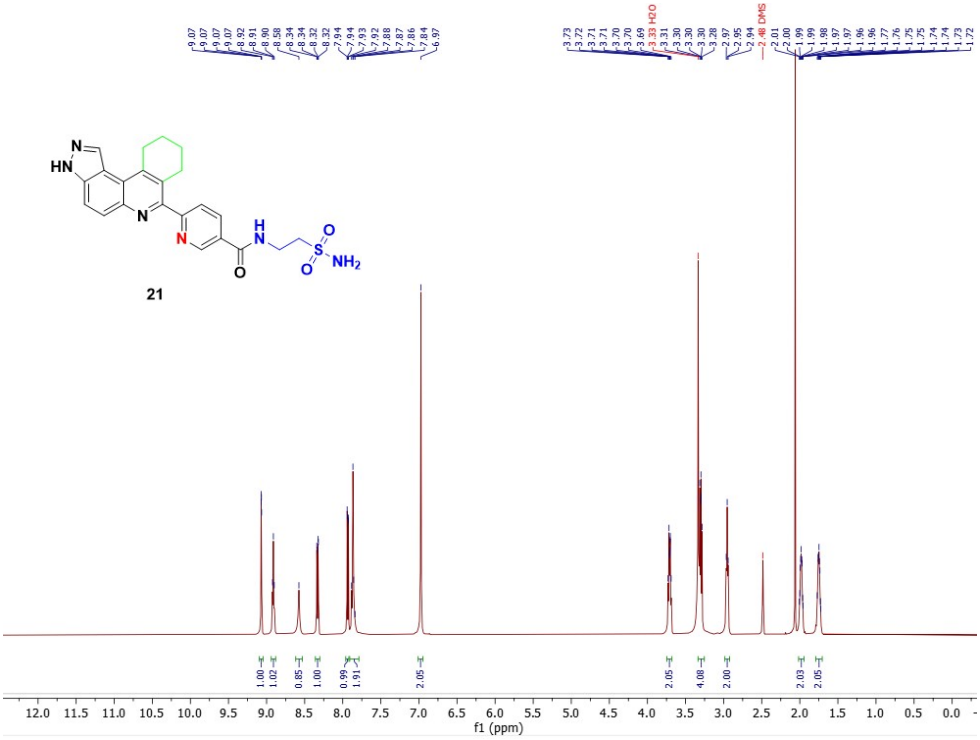
^{1}H NMR (500 MHz, DMSO) : **16**



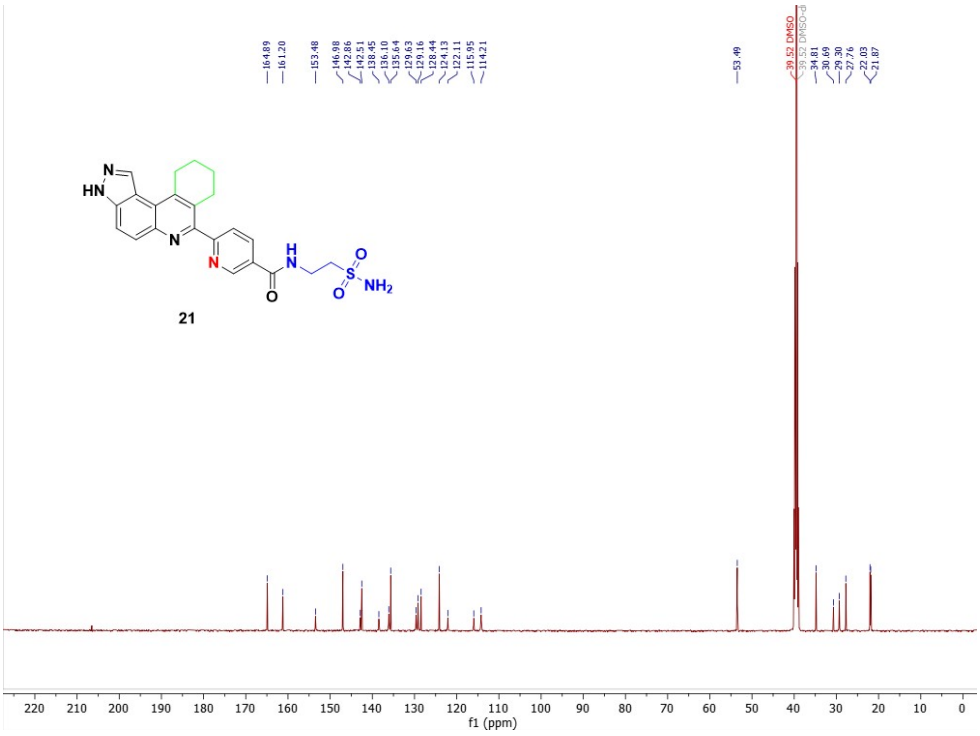
^{13}C NMR (126 MHz, DMSO) : **16**



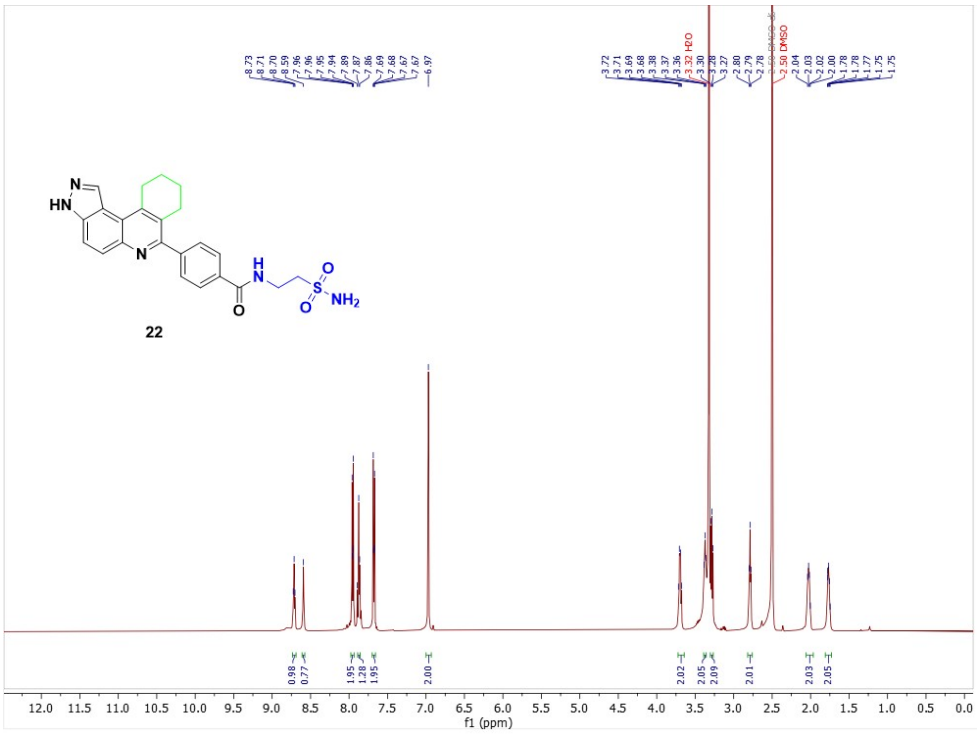
^{1}H NMR (500 MHz, DMSO) : **17**



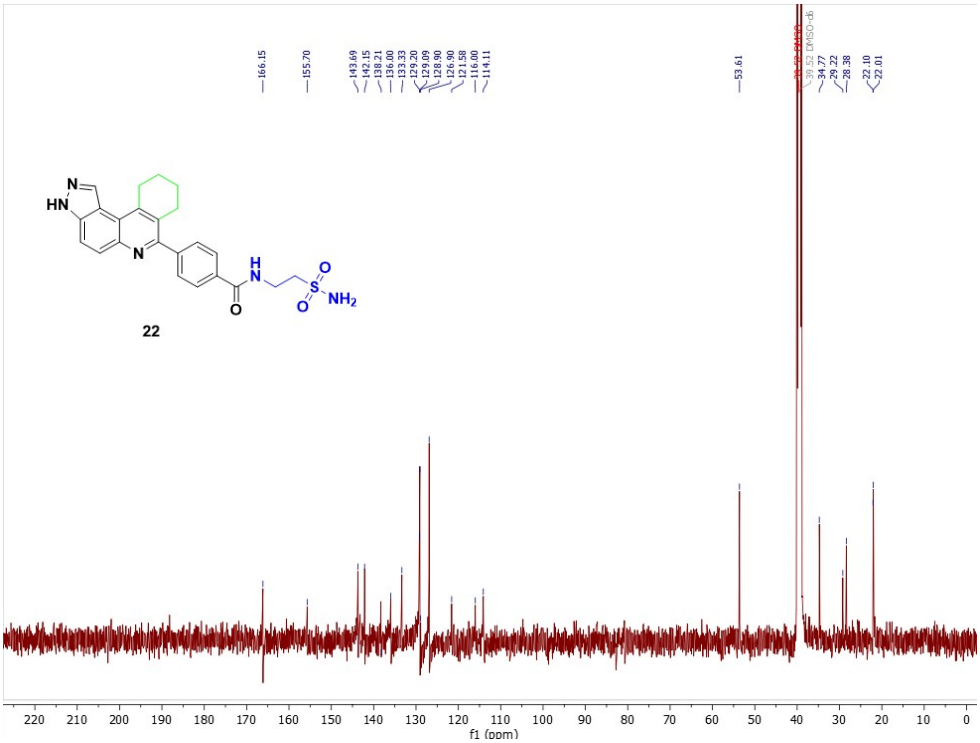
^{13}C NMR (126 MHz, DMSO) : **17**



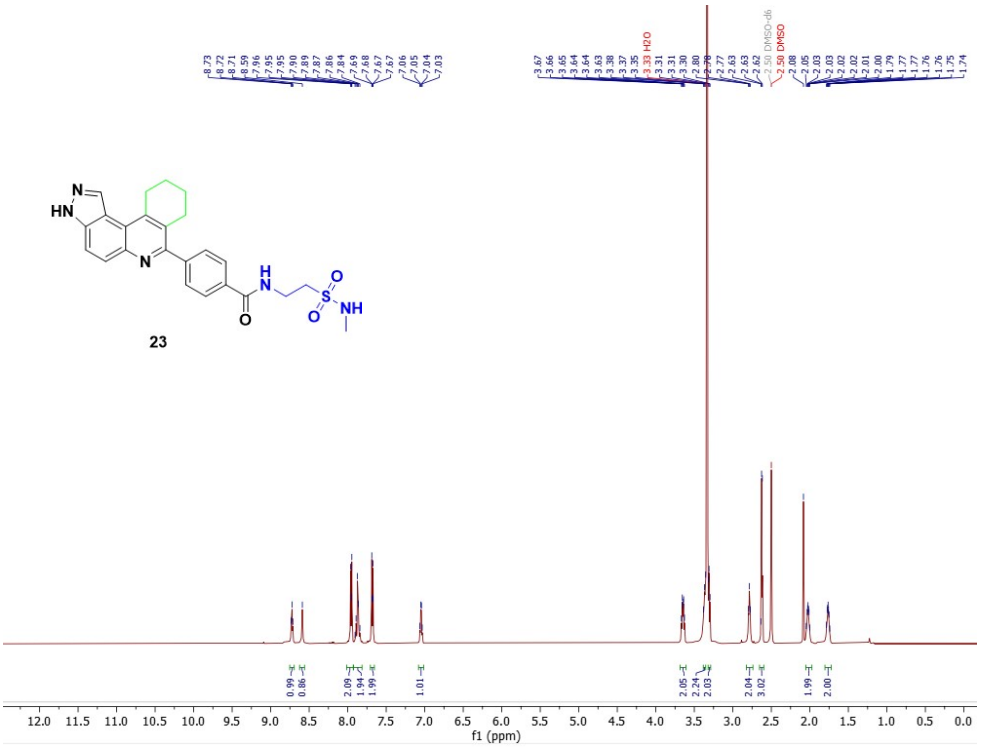
^{1}H NMR (500 MHz, DMSO) : **18**



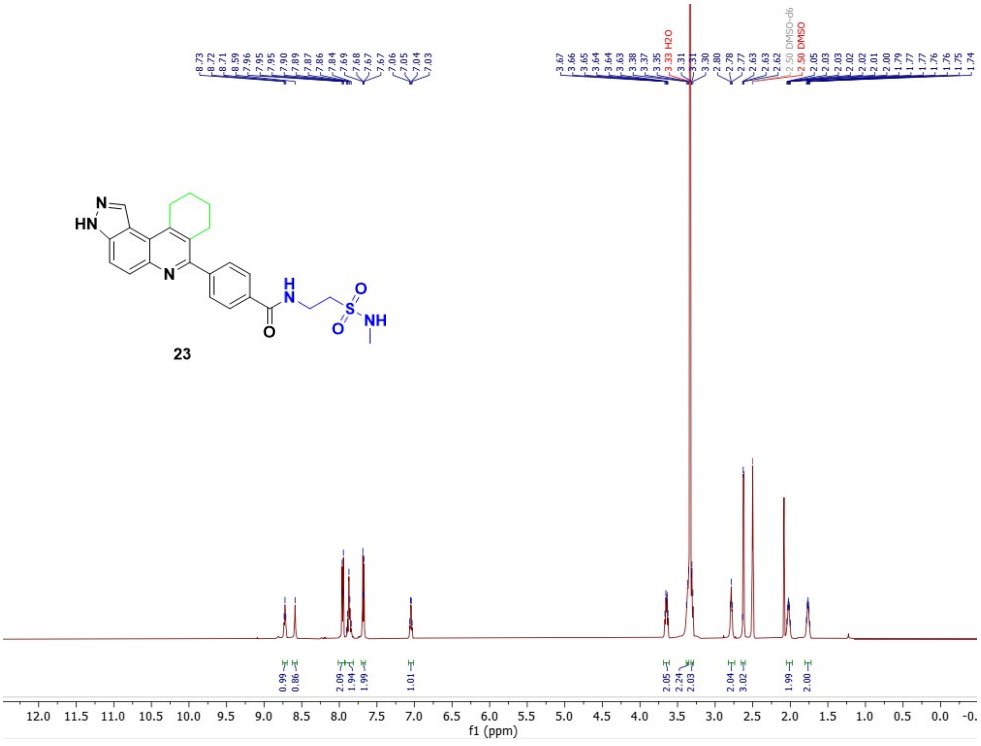
^{13}C NMR (126 MHz, DMSO) : **18**



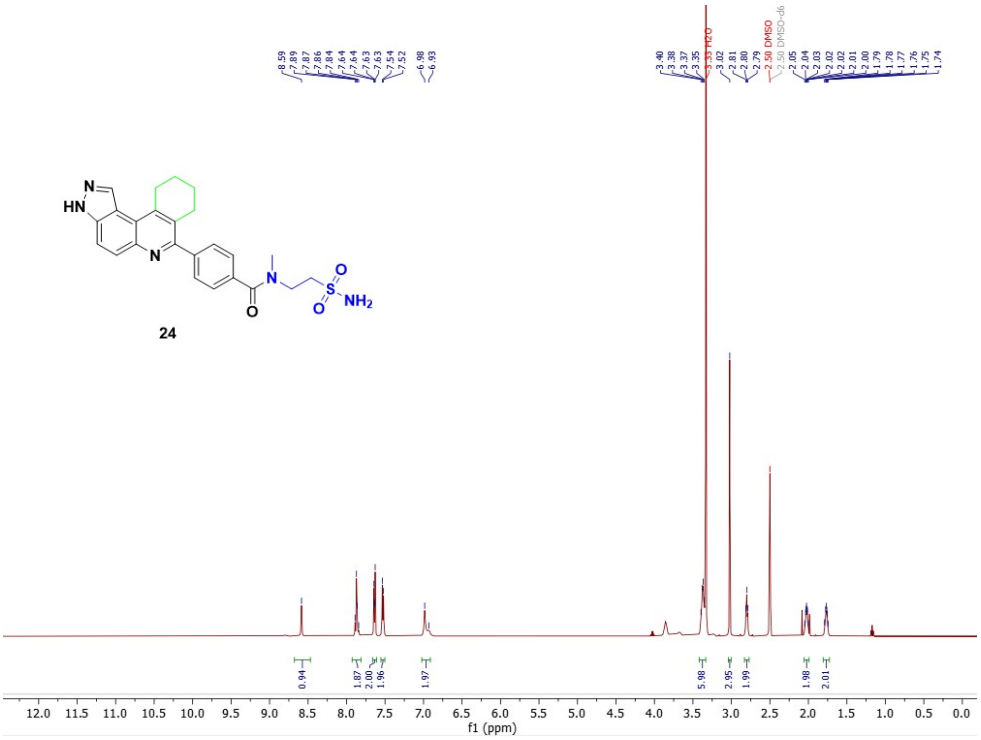
^{1}H NMR (500 MHz, DMSO) : **19**



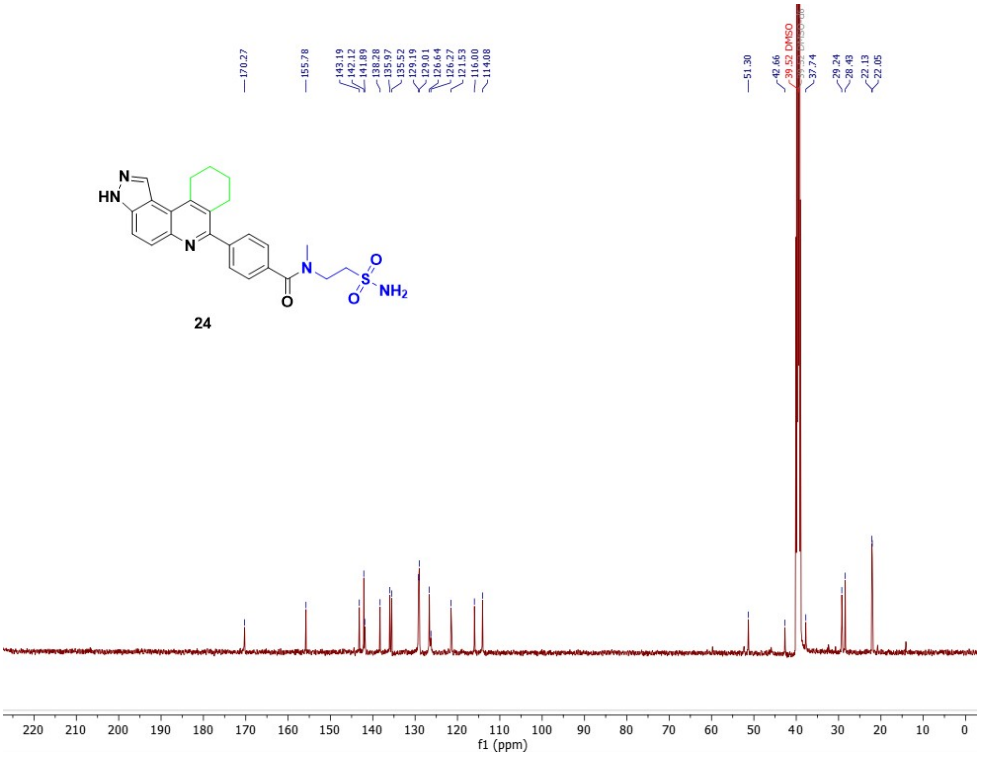
^{13}C NMR (126 MHz, DMSO) : **19**



^{1}H NMR (500 MHz, DMSO) : **20**

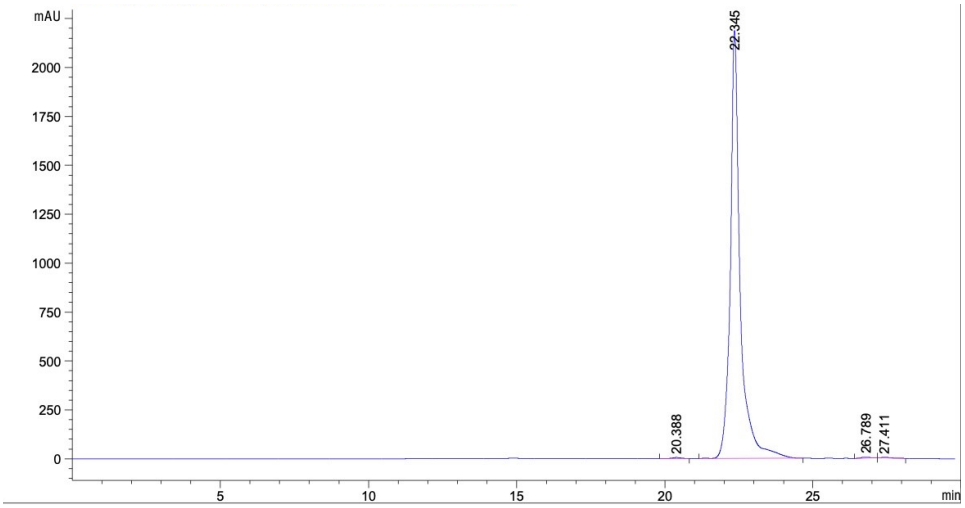


^{13}C NMR (126 MHz, DMSO) : **20**



4. HPLC Purity trace for HSKB-142 and HSKB-143

A. HSKB-142



B. HSKB-143