Relocating NSAID into endoplasmic reticulum induces ER stress -mediated apoptosis in cancer cells

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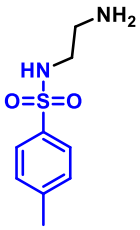
Materials:

Chemicals needed to synthesise the molecules were purchased from GLR Innovations, BLD Pharma, TCI or Avra. Solvents, 60-120 mesh, 100-200 mesh, and 230-400 mesh silica, needed for purification through column chromatography and dry solvents needed for setting up inert reactions were purchased from Finar. The nitrogen atmosphere is maintained for reactions in inert conditions. A Heidolph Rotary evaporator was used to evaporate the solvent. Required ^{1}H, ^{13}C, and ^{19}F NMR spectra are recorded in CDCl3 and DMSO purchased from Eurisotop, and the NMR spectrometer is Ascend NMR-500 MHz (Brucker). For cell biology experiments, DMEM, 10% FBS AND 1% Penicillin-streptomycin were purchased from Gibco and used for the complete media preparation for the culture of HeLa, HCT116, MCF7 and A549 cell lines. To determine the cell viability, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was purchased from Himedia. ER tracker Red needed for the colocalisation study was purchased from Invitrogen, Oil Red O was purchased from TCI chemicals, Alexa fluor 488 AnnexinV, Propidium Iodide, Pro-caspase 3 monoclonal antibody and COX-2 antibody were purchased from Invitrogen, LC3 was purchased from cell signalling and antibodies for PERK, CHOP, GRP94, Caspase-12, Beclin and IRE1 were purchased from Santa Cruz Biotechnology. A Perkin Elmer multimode Plate reader is used to record the absorbance, and Leica (TCS SP8) is used for confocal imaging; data was analysed by GraphPad prism and Image j software, respectively. For western blot experiments, data was quantified by using Image Lab software. Secondary anti-body Alexa Fluor 633 goat anti-mouse IgG2b(y2b) is purchased from Invitrogen.

Methods

Synthesis of compound 3a



3a

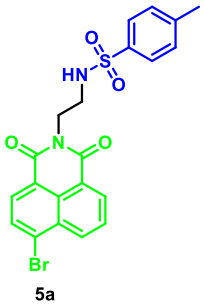
Procedure: In a round bottle flask, compound 1a (1 eq., 2000 mg) and an excess of compound 2, i.e. ethylene diamine (8 eq., 5.6 mL), were added to dry DCM at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for one hour at room temperature. Completion of the reaction was monitored through silica thin layer chromatography (TLC). The solvent was evaporated on the rota vapour, and the reaction mixture was purified with 60-120 mesh silica in 7% MeOH in DCM.

Yield: 60%

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 2H), 2.95 – 2.92 (m, 2H), 2.78-2.75 (m, 2H), 2.40 (s, 3H).

Mass: m/z: Calculated for $C_9H_{15}N_2O_2S+$, $[M + H]^+$: 215.0849, Observed Mass: 215.0813

Synthesis of compound 5a:



5a

Procedure: In a round bottle flask, compound 4 (1eq., 500mg) and compound 3a (1.5 eq., 579.48 mg) were added to ethanol and refluxed for 12h at 80°C. Completion of the reaction was monitored through TLC; the solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica in 1.5-2% MeOH in DCM.

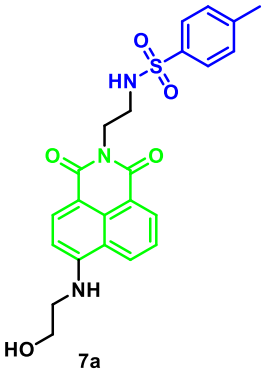
Yield: 70%

^{1}H NMR (500 MHz, DMSO) δ 8.50 – 8.47 (m, 2H), 8.23 (d, *J* = 7.9 Hz, 1H), 8.16 (d, *J* = 7.9 Hz, 1H), 7.97 – 7.93 (m, 1H), 7.75 (t, *J* = 6.3 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 2H), 7.18 (d, *J* = 8.0 Hz, 2H), 4.08 (t, *J* = 6.5 Hz, 2H), 3.08 (q, *J* = 6.5 Hz, 2H), 2.22 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 163.40, 163.34, 142.85, 138.17, 132.99, 131.93, 131.74, 131.31, 130.19, 129.91, 129.50, 129.21, 128.80, 126.76, 123.27, 122.49, 55.38, 21.31.

Mass: m/z: Calculated for $C_{21}H_{18}BrN_2O_4S+$, $[M + H]^+$: 473.0165, Observed: 473.0121

Synthesis of compound 7a



7a

Procedure: In a round bottle flask, compound 5a (1 eq., 100mg) and compound 6, i.e. ethanol amine (6 eq.,), was added in 2-methoxy ethanol and refluxed for 20h at 110°C. Completion of the reaction was monitored through TLC. The solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica at 2% MeOH in DCM.

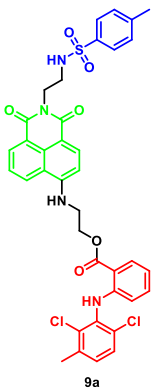
Yield: 80%

^{1}H NMR (500 MHz, DMSO) δ 8.66 (d, *J* = 8.4 Hz, 1H), 8.37 (d, *J* = 7.2 Hz, 1H), 8.19 (d, *J* = 8.5 Hz, 1H), 7.73 – 7.68 (m, 2H), 7.67 – 7.63 (m, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 1H), 4.90 (t, *J* = 5.6 Hz, 1H), 4.07 (t, *J* = 6.7 Hz, 2H), 3.70 (q, *J* = 5.8 Hz, 2H), 3.47 (dd, *J* = 11.5, 5.8 Hz, 2H), 3.05 – 3.00 (m, 2H), 2.26 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 164.30, 163.38, 151.29, 142.88, 138.06, 134.64, 131.06, 129.99, 129.91, 129.01, 126.85, 124.60, 122.32, 120.57, 108.09, 104.26, 59.28, 55.37, 46.03, 21.34.

Mass: m/z: Calculated for $C_{23}H_{24}N_3O_5S+$, $[M + H]^+$: 454.1431, Observed: 454.1449

Synthesis of compound 9a:



9a

Procedure: In a round bottle flask, compound 8 i.e., meclofenamic acid (1eq., 50mg), compound 7a (1.2 eq., 85.5mg), EDCI.HCl (1.6eq., 48.20mg) and DMAP (0.5 eq., 9.6mg) were added to dry THF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 40% EtOAc in DCM.

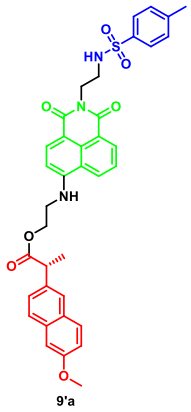
Yield: 30%

^{1}H NMR (500 MHz, DMSO) δ 9.05 (s, 1H), 8.65 (d, *J* = 8.5 Hz, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 8.19 (d, *J* = 8.5 Hz, 1H), 7.97 – 7.92 (m, 2H), 7.71 (s, 1H), 7.63 – 7.57 (m, 3H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.32 – 7.23 (m, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.75 (t, *J* = 7.5 Hz, 1H), 6.15 (d, *J* = 8.4 Hz, 1H), 4.62 (d, *J* = 4.9 Hz, 2H), 4.07 (t, *J* = 6.5 Hz, 2H), 3.86 (d, *J* = 4.9 Hz, 2H), 3.07 – 3.01 (m, 2H), 2.28 (s, 3H), 2.21 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 168.15, 164.25, 163.35, 150.90, 147.33, 142.82, 138.05, 136.86, 135.00, 134.75, 134.45, 134.06, 131.93, 131.02, 129.86, 128.87, 128.43, 126.82, 124.71, 122.30, 120.59, 117.92, 113.66, 111.58, 108.63, 63.24, 42.14, 40.79, 39.22, 21.29, 20.50.

Mass: m/z: Calculated for $C_{37}H_{33}Cl_2N_4O_6S$+, $[M + H]^+$: 731.1492, Observed: 731.1500

Synthesis of compound 9'a



9'a

Procedure: In a round bottle flask, compound 8' i.e., Naproxen (1eq., 50mg), compound 7a (1.2 eq., 118.17mg), EDCI.HCl (1.6eq., 66mg) and DMAP (0.6 eq., 15.91mg) were added to dry DCM in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 8% EtOAc in DCM.

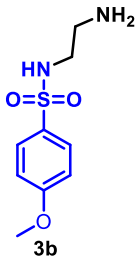
Yield: 38.2%

^{1}H NMR (500 MHz, DMSO) δ 8.49 (d, *J* = 8.4 Hz, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 8.11 (d, *J* = 8.5 Hz, 1H), 7.70 (dt, *J* = 10.5, 5.7 Hz, 2H), 7.61 – 7.58 (m, 5H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 1.9 Hz, 1H), 7.02 (m, 1H), 6.73 (d, *J* = 8.7 Hz, 1H), 4.34 (m, 2H), 4.07 (t, *J* = 6.6 Hz, 2H), 3.89 (d, *J* = 7.1 Hz, 1H), 3.81 (s, 3H), 3.64 – 3.60 (m, 2H), 3.04 (m, 2H), 2.21 (s, 3H), 1.43 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 174.51, 164.26, 163.32, 157.50, 150.81, 142.86, 138.05, 135.90, 134.39, 133.62, 130.97, 129.89, 129.81, 129.40, 128.70, 127.32, 126.84, 126.53, 125.95, 124.63, 122.28, 120.48, 118.99, 108.51, 106.00, 104.25, 62.82, 55.54, 44.93, 41.93, 40.75, 39.21, 21.29, 18.88.

Mass: m/z: Calculated for $C_{37}H_{36}N_3O_7S$+, $[M + H]^+$: 666.2268, Observed: 666.2285

Synthesis of compound 3b



3b

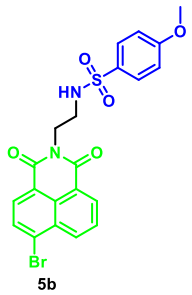
Procedure: In a round bottle flask, compound 1b (1 eq., 1700 mg) and an excess of compound 2, i.e. ethylene diamine (8 eq., 4.39 mL), were added to dry DCM at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for 10 minutes at 0°C. Completion of the reaction was monitored through (TLC). The solvent was evaporated on the rota vapour, and the reaction mixture was purified with 60-120 mesh silica in 3% MeOH in DCM.

Yield: 80%

^{1}H NMR (500 MHz, DMSO) δ 7.74 (d, *J* = 8.8 Hz, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 3.81 (s, 3H), 2.83 (t, *J* = 6.5 Hz, 2H), 2.67 (t, *J* = 6.5 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 162.64, 132.20, 129.22, 114.82, 56.12, 43.20.

Synthesis of compound 5b



5b

Procedure: In a round bottle flask, compound 3b (1eq., 2100mg) and compound 4 (1 eq., 2526 mg) were added to ethanol and refluxed for 12h at 80°C. Completion of the reaction was monitored through TLC; the solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica in 50 % EtOAc in Hexane.

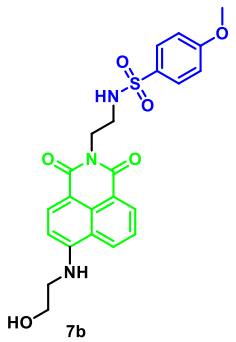
Yield: 59.2%

^{1}H NMR (500 MHz, DMSO) δ 8.49 (m, 2H), 8.24 (d, *J* = 7.9 Hz, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 7.96 (m, 1H), 7.68 (s, 1H), 7.60 – 7.57 (m, 2H), 6.89 (d, *J* = 8.9 Hz, 2H), 4.08 (t, *J* = 6.5 Hz, 2H), 3.71 (s, 3H), 3.07 (q, *J* = 6.5 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 163.43, 163.37, 162.33, 133.01, 132.64, 131.94, 131.73, 131.32, 130.21, 129.53, 129.20, 128.87, 128.82, 123.29, 122.49, 114.57, 55.94.

Mass: m/z: Calculated for $C_{21}H_{18}BrN_2O_5S+$, $[M + 2 + H]^+$: 491.0094, Observed: 490.92

Synthesis of compound 7b



7b

Procedure: In a round bottle flask, compound 5b (1 eq., 1100mg) and compound 6, i.e. ethanol amine (6 eq.,), was added in 2-methoxy ethanol and refluxed overnight at 110°C. Completion of the reaction was monitored through TLC. The solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica at 3-3.5 % MeOH in DCM.

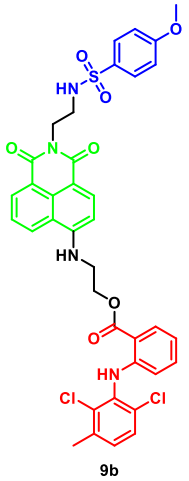
Yield: 48%

^{1}H NMR (500 MHz, DMSO) δ 8.67 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* = 7.2 Hz, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 7.73 (t, *J* = 5.5 Hz, 1H), 7.69 – 7.62 (m, 4H), 6.96 (d, *J* = 8.9 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 1H), 4.92 (t, *J* = 5.6 Hz, 1H), 4.08 (t, *J* = 6.7 Hz, 2H), 3.76 (s, 3H), 3.72 (q, *J* = 5.9 Hz, 2H), 3.48 (q, *J* = 5.8 Hz, 2H), 3.03 (dd, *J* = 13.2, 6.5 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 164.31, 163.38, 162.40, 151.29, 134.63, 132.55, 131.07, 129.97, 129.01, 128.97, 124.60, 122.31, 120.55, 114.60, 108.06, 104.25, 59.27, 55.94, 46.02, 40.74, 39.21.

Mass: m/z: Calculated for $C_{23}H_{24}N_3O_6S$+, $[M + H]^+$: 470.1380, Observed: 470.1398

Synthesis of compound 9b



9b

Procedure: In a round bottle flask, compound 8 i.e., meclofenamic acid (1eq., 150mg), compound 7b (1eq., 221.3mg), EDCI.HCl (1.6eq., 144.2mg) and DMAP (0.6 eq., 34.5 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 50% EtOAc in Hexane.

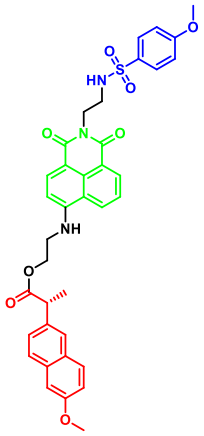
Yield: 42.2%

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.43 (d, *J* = 7.2 Hz, 1H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 8.3 Hz, 1H), 8.03 (m, 1H), 7.61 – 7.52 (m, 3H), 7.36 – 7.29 (m, 2H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.81 – 6.71 (m, 2H), 6.34 (d, *J* = 8.8 Hz, 4H), 5.36 (t, *J* = 4.7 Hz, 1H), 4.88 – 4.84 (m, 2H), 4.27 – 4.21 (m, 2H), 3.84 (d, *J* = 4.4 Hz, 2H), 3.49 – 3.44 (m, 2H), 3.43 (s, 3H), 2.42 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 168.80, 164.02, 163.52, 160.98, 148.52, 147.06, 135.67, 133.93, 133.75, 133.69, 133.28, 130.55, 130.38, 130.34, 130.22, 128.71, 127.91, 127.63, 126.84, 125.60, 123.99, 121.42, 119.22, 116.56, 113.00, 112.54, 109.42, 109.10, 103.02, 61.65, 53.96, 43.17, 42.00, 37.77, 28.68, 19.64, 13.10.

Mass: m/z: Calculated for $C_{37}H_{33}Cl_2N_4O_7S$+, $[M + H]^+$: 747.1442, Observed: 747.1429

Synthesis of compound 9'b



9'b

Procedure: In a round bottle flask, compound 8' i.e., Naproxen (1eq., 150mg), compound 7b (1 eq., 305.8 mg), EDCI.HCl (1.6eq., 199.2 mg) and DMAP (0.6 eq., 47.7 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 50% EtOAc in Hexane.

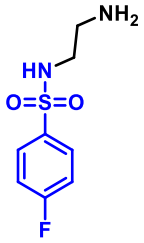
Yield: 29.8%

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (d, *J* = 7.2 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.59 – 7.54 (m, 3H), 7.51 – 7.44 (m, 3H), 7.31 (dd, *J* = 8.4, 1.5 Hz, 1H), 6.99 (m, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 6.38 (d, *J* = 8.9 Hz, 2H), 5.44 (d, *J* = 4.5 Hz, 1H), 5.35 (t, *J* = 4.9 Hz, 1H), 4.69 – 4.62 (m, 1H), 4.50 – 4.42 (m, 1H), 4.27 – 4.21 (m, 2H), 3.91 (d, *J* = 7.1 Hz, 1H), 3.85 (s, 3H), 3.59 (s, 2H), 3.46 (s, 3H), 3.46 – 3.40 (m, 2H), 1.58 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.70, 163.98, 163.41, 161.04, 156.64, 148.17, 134.04, 133.41, 132.57, 130.56, 130.10, 128.47, 127.92, 127.72, 126.33, 125.07, 124.69, 124.62, 123.66, 121.28, 118.95, 118.06, 112.60, 109.10, 104.41, 102.91, 76.25, 76.00, 75.74, 61.33, 54.29, 54.04, 44.44, 42.38, 41.92, 37.80, 28.68, 17.00.

Mass: m/z: Calculated for $C_{37}H_{35}N_3O_8S$+, $[M + H]^+$: 682.2218, Observed: 682.2217

Synthesis of compound 3c



3c

Procedure: In a round bottle flask, compound 1c (1 eq., 2000 mg) and an excess of compound 2, i.e. ethylene diamine (8 eq., 5.48 mL), were added to dry DCM at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for 40 minutes at 0°C. Completion of the reaction was monitored through (TLC). The solvent was evaporated on the rota vapour, and the reaction mixture was purified with 60-120 mesh silica in 7 % MeOH in DCM.

Yield: 39.5 %

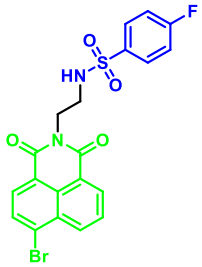
^{1}H NMR (500 MHz, DMSO) δ 7.88 – 7.85 (m, 2H), 7.41 (t, *J* = 8.8 Hz, 2H), 3.92 (s, 3H), 2.75 (t, *J* = 6.4 Hz, 2H), 2.54 (t, *J* = 6.4 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 165.52, 163.52, 137.45, 137.42, 129.95, 129.87, 116.80, 116.62, 46.35, 41.64.

^{19}F NMR (470 MHz, DMSO) δ -107.14.

Mass: m/z: Calculated for $C_8H_{12}FN_2O_2S$+, $[M + H]^+$: 219.0598, Observed: 219.0593

Synthesis of compound 5c



5c

Procedure: In a round bottle flask, compound 3c (1eq., 710 mg) and compound 4 (1 eq., 901.39 mg) were added to ethanol and refluxed for 1h at 80°C. Completion of the reaction was monitored through TLC; the solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica in 50 % EtOAc in Hexane.

Yield: 42.9%

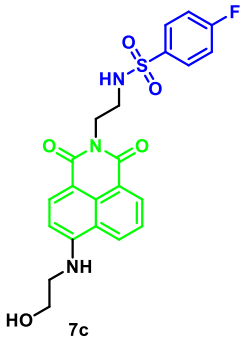
^{1}H NMR (500 MHz, DMSO) δ 8.56 – 8.52 (m, 2H), 8.29 (d, *J* = 7.9 Hz, 1H), 8.21 (d, *J* = 7.9 Hz, 1H), 7.99 (m, 1H), 7.92 (t, *J* = 6.3 Hz, 1H), 7.77 – 7.69 (m, 2H), 7.26 (t, *J* = 8.8 Hz, 2H), 4.09 (s, 2H), 3.11 (d, *J* = 6.5 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 163.43, 137.43, 133.10, 132.02, 131.81, 131.40, 129.78, 129.29, 123.34, 122.55, 116.77, 116.60.

^{19}F NMR (470 MHz, DMSO) δ -107.02.

Mass: m/z: Calculated for $C_{20}H_{15}BrFN_2O_4S+$, $[M + 2+H]^+$: 478.9894, Observed: 478.92

Synthesis of compound 7c



7c

Procedure: In a round bottle flask, compound 5c (1 eq., 1144 mg) and compound 6, i.e. ethanol amine (6 eq.,), was added in 2-methoxy ethanol and refluxed overnight at 110°C. Completion of the reaction was monitored through TLC. The solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica at 5 % MeOH in DCM.

Yield: 53.7%

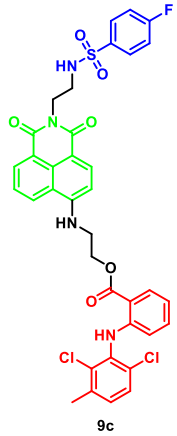
^{1}H NMR (500 MHz, DMSO) δ 8.67 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* = 7.3 Hz, 1H), 8.20 (d, *J* = 8.6 Hz, 1H), 7.89 – 7.84 (m, 1H), 7.77 (m, 2H), 7.72 (t, *J* = 5.4 Hz, 1H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.29 (t, *J* = 8.8 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 1H), 4.91 (t, *J* = 5.6 Hz, 1H), 4.07 (t, *J* = 6.7 Hz, 2H), 3.70 (q, *J* = 5.9 Hz, 2H), 3.47 (m, 2H), 3.06 (m, 2H).

^{13}C NMR (126 MHz, DMSO) δ 165.40, 164.30, 163.38, 151.33, 137.36, 134.68, 131.11, 129.99, 129.85, 129.77, 129.05, 124.63, 122.28, 120.56, 116.75, 116.57, 108.02, 104.27, 59.25, 46.01, 40.68, 39.19.

^{19}F NMR (470 MHz, DMSO) δ -107.04

Mass: m/z: Calculated for $C_{22}H_{21}FN_3O_5S+$, $[M + H]^+$: 458.1180, Observed: 458.17

Synthesis of compound 9c



9c

Procedure: In a round bottle flask, compound 8 i.e., meclofenamic acid (1eq., 100mg), compound 7c (1eq., 143.80 mg), EDCI.HCl (1.6eq., 96.14 mg) and DMAP (0.6 eq., 23.04 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 35 % EtOAc in Hexane.

Yield: 27.8%

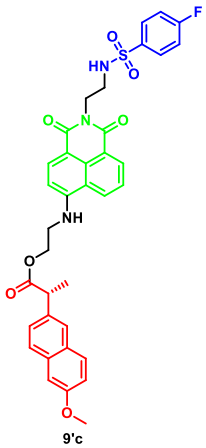
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.42 (d, *J* = 7.2 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 8.03 (d, *J* = 7.4 Hz, 1H), 7.62 (m, 2H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.31 (m, 3H), 7.15 (d, *J* = 8.3 Hz, 1H), 6.79 – 6.71 (m, 3H), 6.54 (t, *J* = 8.5 Hz, 2H), 6.34 (d, *J* = 8.1 Hz, 2H), 5.48 (t, *J* = 4.5 Hz, 1H), 4.87 – 4.83 (m, 2H), 4.27 – 4.23 (m, 2H), 3.84 (d, *J* = 4.5 Hz, 2H), 3.47 (d, *J* = 5.2 Hz, 2H), 2.42 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 168.70, 164.01, 163.49, 148.74, 147.03, 135.65, 133.85, 133.80, 133.27, 130.42, 130.22, 128.69, 128.25, 128.18, 127.87, 126.83, 125.83, 124.02, 121.21, 119.24, 116.54, 114.70, 114.52, 112.99, 109.53, 108.79, 103.10, 61.66, 43.05, 41.94, 37.66, 30.91, 28.68, 21.67, 19.63.

^{19}F NMR (470 MHz, $CDCl_3$) δ -105.86.

Mass: m/z: Calculated for $C_{36}H_{30}Cl_2FN_4O_6S^+$, $[M + H]^+$: 735.1242, Observed: 735.1242

Synthesis of compound 9'c



9'c

Procedure: In a round bottle flask, compound 8' i.e., Naproxen (1eq., 150mg), compound 7c (1 eq., 297.8 mg), EDCI.HCl (1.6eq., 199.2 mg) and DMAP (0.6 eq., 47.7 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 30% EtOAc in Hexane.

Yield: 24.8%

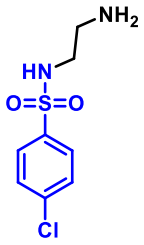
^{1}H NMR (500 MHz, DMSO) δ 8.53 (d, *J* = 8.3 Hz, 1H), 8.37 (d, *J* = 7.2 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 7.88 (s, 1H), 7.81 – 7.75 (m, 2H), 7.71 (s, 1H), 7.65 – 7.54 (m, 4H), 7.29 (t, *J* = 8.7 Hz, 3H), 7.13 (s, 1H), 7.03 (d, *J* = 8.9 Hz, 1H), 6.79 (d, *J* = 8.6 Hz, 1H), 4.35 (s, 2H), 4.08 (t, *J* = 6.4 Hz, 2H), 3.94 – 3.85 (m, 1H), 3.82 (s, 3H), 3.65 (d, *J* = 5.2 Hz, 2H), 3.07 (d, *J* = 6.2 Hz, 2H), 1.43 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 174.51, 164.27, 163.35, 157.50, 150.91, 135.91, 134.47, 133.61, 131.06, 129.84, 129.77, 129.40, 128.69, 127.31, 126.53, 125.94, 124.70, 122.30, 120.52, 118.99, 116.74, 116.56, 108.47, 106.02, 104.34, 62.82, 55.56, 44.92, 41.93, 39.19, 18.89.

^{19}F NMR (470 MHz, DMSO) δ -107.06.

Mass: m/z: Calculated for $C_{36}H_{33}FN_3O_7S+$, $[M + H]^+$: 670.2018, Observed: 670.2045

Synthesis of compound 3d



3d

Procedure: In a round bottle flask, compound 1d (1 eq., 2000 mg) and an excess of compound 2, i.e. ethylene diamine (8 eq., 5.00 mL), were added to dry DCM at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for 1h at 0°C. Completion of the reaction was monitored through (TLC). The solvent was evaporated on the rota vapour, and the reaction mixture was purified with 60-120 mesh silica in 10 % MeOH in DCM.

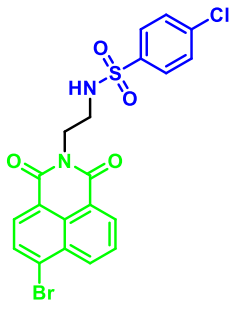
Yield: 85%

^{1}H NMR (500 MHz, DMSO) δ 7.79 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.6 Hz, 2H), 3.86 (s, 3H), 2.74 (t, *J* = 6.5 Hz, 2H), 2.52 (t, *J* = 6.6 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 139.95, 137.64, 129.81, 128.90, 46.37, 41.67.

Mass: m/z: Calculated for $C_8H_{12}ClN_2O_2S+$, $[M + H]^+$: 235.0303, Observed: 235.0328

Synthesis of compound 5d



5d

Procedure: In a round bottle flask, compound 3d (1eq., 200 mg) and compound 4 (1 eq., 236 mg) were added to ethanol and refluxed for 1h at 80°C. Completion of the reaction was monitored through TLC; the solvent was evaporated, and the reaction mixture was purified with 100-200 mesh silica in 60 % EtOAc in Hexane.

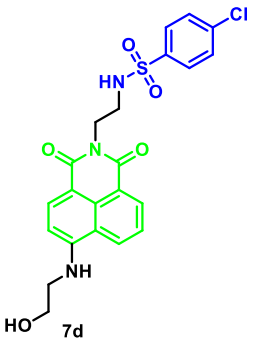
Yield: 41%

^{1}H NMR (500 MHz, DMSO) δ 8.43 (m, 2H), 8.14 (m, 2H), 7.97 (s, 1H), 7.93 – 7.88 (m, 1H), 7.69 – 7.63 (m, 2H), 7.45 – 7.41 (m, 2H), 4.06 (t, *J* = 6.4 Hz, 2H), 3.13 (q, *J* = 6.4 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 163.33, 163.27, 139.94, 137.52, 133.05, 131.91, 131.70, 131.28, 130.13, 129.65, 129.62, 129.12, 128.62, 123.03, 122.24, 60.24, 21.21, 14.53.

Mass: m/z: Calculated for $C_{20}H_{15}BrClN_2O_4S+$, $[M + 2+H]^+$: 494.9598, Observed: 494.92

Synthesis of compound 7d



7d

Procedure: In a round bottle flask, compound 5d (1 eq., 380 mg) and compound 6, i.e. ethanol amine (6 eq.,), was added in 2-methoxy ethanol and refluxed overnight at 110°C. Completion of the reaction was monitored through TLC. The solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica at 3 % MeOH in DCM.

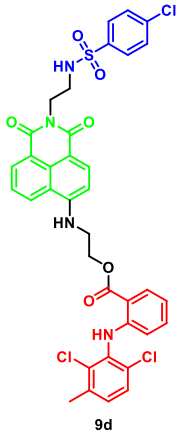
Yield: 51.6%

^{1}H NMR (500 MHz, DMSO) δ 8.66 (d, *J* = 6.2 Hz, 1H), 8.36 (s, 1H), 8.19 (d, *J* = 6.8 Hz, 1H), 7.92 (s, 1H), 7.67 (d, *J* = 28.4 Hz, 4H), 7.49 (d, *J* = 5.5 Hz, 2H), 6.78 (d, *J* = 6.7 Hz, 1H), 4.90 (s, 1H), 4.07 (s, 2H), 3.71 (s, 2H), 3.47 (s, 2H), 3.08 (s, 2H).

^{13}C NMR (126 MHz, DMSO) δ 164.31, 163.36, 151.34, 139.89, 137.55, 134.64, 131.07, 129.96, 129.64, 129.07, 128.72, 124.59, 122.24, 120.58, 108.01, 104.27, 59.28, 46.02, 40.74, 39.20.

Mass: m/z: Calculated for $C_{22}H_{21}ClN_3O_5S+$, $[M + H]^+$: 474.0885, Observed: 474.17

Synthesis of compound 9d



9d

Procedure: In a round bottle flask, compound 8 i.e., meclofenamic acid (1eq., 80mg), compound 7d (1eq., 143.01 mg), EDCI.HCl (1.6eq., 76.91 mg) and DMAP (0.6 eq., 18.43 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 30 % EtOAc in Hexane.

Yield: 26.9%

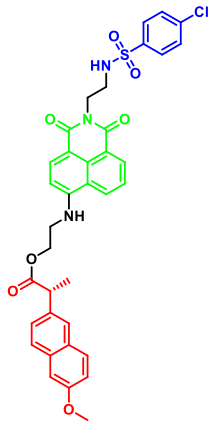
^{1}H NMR (500 MHz, DMSO) δ 9.05 (s, 1H), 8.69 (d, *J* = 8.4 Hz, 1H), 8.37 (d, *J* = 7.2 Hz, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 8.03 – 7.92 (m, 3H), 7.75 – 7.61 (m, 3H), 7.45 (m, 3H), 7.31 (m, 3H), 6.97 (d, *J* = 8.5 Hz, 1H), 6.77 (t, *J* = 7.5 Hz, 1H),

6.16 (d, *J* = 8.4 Hz, 1H), 4.63 (s, 2H), 4.08 (t, *J* = 6.2 Hz, 2H), 3.88 (d, *J* = 4.0 Hz, 2H), 3.11 (d, *J* = 6.1 Hz, 2H), 2.31 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 168.14, 164.29, 163.37, 151.02, 147.32, 139.90, 137.51, 136.89, 135.02, 134.79, 134.52, 134.08, 131.94, 131.03, 130.58, 129.89, 129.59, 129.01, 128.68, 128.47, 124.79, 122.29, 120.67, 117.94, 113.67, 111.62, 108.58, 104.50, 63.25, 42.15, 29.47, 20.51, 14.41.

Mass: m/z: Calculated for $C_{36}H_{30}Cl_3N_4O_6S+$, $[M + H]^+$: 751.0946, Observed: 751.0759

Synthesis of compound 9'd



9'd

Procedure: In a round bottle flask, compound 8' i.e., Naproxen (1eq., 50mg), compound 7d (1 eq., 123.49 mg), EDCI.HCl (1.6eq., 66.60 mg) and DMAP (0.6 eq., 15.91 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 25 % EtOAc in Hexane.

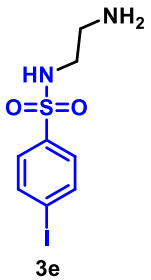
Yield: 31.3%

^{1}H NMR (500 MHz, DMSO) δ 8.52 (d, *J* = 8.3 Hz, 1H), 8.36 (d, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.95 (s, 1H), 7.72 (t, *J* = 9.1 Hz, 3H), 7.64 – 7.56 (m, 4H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.14 (s, 1H), 7.04 (d, *J* = 8.9 Hz, 1H), 6.76 (d, *J* = 8.6 Hz, 1H), 4.37 (s, 2H), 4.09 (t, *J* = 6.3 Hz, 2H), 3.90 (m, 1H), 3.83 (s, 3H), 3.71 – 3.61 (m, 2H), 3.11 (s, 2H), 1.45 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 174.51, 164.27, 163.32, 157.51, 150.89, 139.90, 137.55, 135.91, 134.42, 133.62, 131.00, 129.80, 129.62, 129.40, 128.84, 128.71, 127.32, 126.53, 125.95, 124.64, 122.24, 120.52, 118.99, 108.45, 106.02, 104.30, 62.83, 55.55, 44.93, 41.94, 40.73, 39.19, 18.89.

Mass: m/z: Calculated for $C_{36}H_{33}ClN_3O_7S+$, $[M + H]^+$: 686.1722, Observed: 686.1714

Synthesis of compound 3e



3e

Procedure: In a round bottle flask, compound 1e (1 eq., 1700 mg) and an excess of compound 2, i.e. ethylene diamine (8 eq., 3.00 mL), were added to dry DCM at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for 1h at 0°C. Completion of the reaction was monitored through (TLC). The solvent was evaporated on the rota vapour, and the reaction mixture was purified with 60-120 mesh silica in 9 % MeOH in DCM.

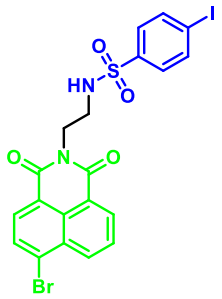
Yield: 61%

^{1}H NMR (500 MHz, DMSO) δ 7.98 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 4.77 (s, 3H), 2.78 (t, *J* = 6.5 Hz, 2H), 2.58 (t, *J* = 6.4 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 140.57, 138.57, 128.73, 100.84, 45.30, 41.17.

Mass: m/z: Calculated for $C_8H_{12}IN_2O_2S+$, $[M + H]^+$: 326.9659, Observed: 326.9653

Synthesis of compound 5e



5e

Procedure: In a round bottle flask, compound 3e (1eq., 650 mg) and compound 4 (1 eq., 553.4 mg) were added to ethanol and refluxed for 6h at 80°C. Completion of the reaction was monitored through TLC; the solvent was evaporated, and the reaction mixture was purified with 100-200 mesh silica in 70 % EtOAc in Hexane.

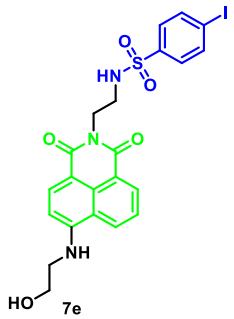
Yield: 38.3%

^{1}H NMR (500 MHz, DMSO) δ 8.48 – 8.45 (m, 2H), 8.21 (d, *J* = 7.9 Hz, 1H), 8.14 (d, *J* = 7.9 Hz, 1H), 7.97 – 7.91 (m, 2H), 7.79 – 7.76 (m, 2H), 7.45 – 7.40 (m, 2H), 4.07 (s, 2H), 3.11 (q, *J* = 6.5 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 163.38, 163.32, 140.68, 138.43, 133.11, 131.93, 131.76, 131.31, 130.18, 129.68, 129.16, 128.72, 128.45, 123.13, 122.36, 100.65, 31.61, 30.29, 29.47.

Mass: m/z: Calculated for $C_{20}H_{15}BrIN_2O_4S+$, $[M + 2 + H]^+$: 586.8955, Observed: 586.83

Synthesis of compound 7e



7e

Procedure: In a round bottle flask, compound 5e (1 eq., 600 mg) and compound 6, i.e. ethanol amine (6 eq.,), was added in 2-methoxy ethanol and refluxed overnight at 110°C. Completion of the reaction was monitored through TLC. The solvent was evaporated, and the reaction mixture was purified with 60-120 mesh silica at 2 % MeOH in DCM.

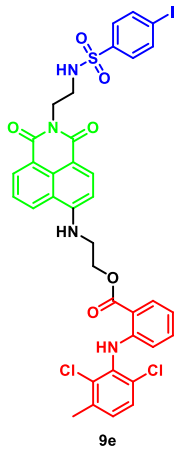
Yield: 41%

^{1}H NMR (500 MHz, DMSO) δ 8.68 (d, *J* = 8.4 Hz, 1H), 8.39 (d, *J* = 7.3 Hz, 1H), 8.21 (d, *J* = 8.6 Hz, 1H), 7.91 (m, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.74 (t, *J* = 5.3 Hz, 1H), 7.70 – 7.65 (m, 1H), 7.63 (d, *J* = 1.3 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 4.07 (t, *J* = 6.7 Hz, 2H), 3.70 (t, *J* = 6.0 Hz, 2H), 3.56 – 3.45 (m, 2H), 3.06 (m, 2H).

^{13}C NMR (126 MHz, DMSO) δ 164.36, 163.41, 151.39, 140.60, 138.42, 134.72, 131.15, 130.01, 128.53, 124.68, 122.28, 120.60, 108.01, 104.36, 100.61, 59.26, 46.01.

Mass: m/z: Calculated for $C_{22}H_{21}IN_3O_5S+$, $[M + H]^+$: 566.0241, Observed: 566.08

Synthesis of compound 9e



9e

Procedure: In a round bottle flask, compound 8 i.e., meclofenamic acid (1eq., 80mg), compound 7e (1eq., 142 mg), EDCI.HCl (1.6eq., 76.91 mg) and DMAP (0.6 eq., 18.43 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 35-50 % EtOAc in Hexane.

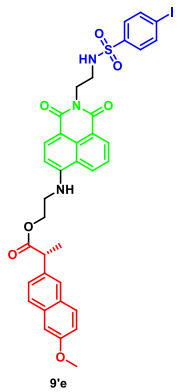
Yield: 21.5%

^{1}H NMR (500 MHz, DMSO) δ 9.05 (s, 1H), 8.68 (d, *J* = 8.1 Hz, 1H), 8.36 (d, *J* = 7.3 Hz, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 7.96 (d, *J* = 6.9 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.67 – 7.57 (m, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.35 – 7.25 (m, 2H), 6.95 (d, *J* = 8.7 Hz, 1H), 6.76 (t, *J* = 7.7 Hz, 1H), 6.16 (d, *J* = 8.4 Hz, 1H), 4.63 (t, *J* = 4.9 Hz, 2H), 4.08 (t, *J* = 6.3 Hz, 2H), 3.88 (d, *J* = 5.0 Hz, 2H), 3.15 – 3.01 (m, 2H), 2.30 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 168.14, 164.28, 163.36, 151.01, 147.31, 140.63, 138.40, 136.88, 135.02, 134.77, 134.51, 134.07, 132.53, 131.95, 131.07, 131.02, 129.87, 129.03, 128.78, 128.52, 128.45, 124.76, 122.27, 120.64, 117.94, 113.67, 111.60, 108.57, 104.49, 100.55, 63.28, 42.16, 31.75, 30.26, 29.50, 28.82, 20.51, 11.26.

Mass: m/z: Calculated for $C_{36}H_{30}Cl_2IN_4O_6S+$, $[M + H]^+$:843.0302, Observed: 843.0514

Synthesis of compound 9'e



9'e

Procedure: In a round bottle flask, compound 8' i.e., Naproxen (1eq., 80mg), compound 7e (1 eq., 196.4 mg), EDCI.HCl (1.6eq., 106.2 mg) and DMAP (0.6 eq., 25.46 mg) were added to dry DMF in the inert nitrogen condition at 0°C. The reaction was kept at stirring for 24h, and the progress of the reaction was monitored through TLC. The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 33 % EtOAc in Hexane.

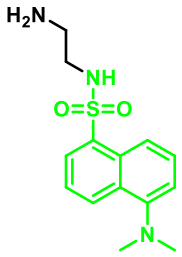
Yield: 23.3%

^{1}H NMR (500 MHz, DMSO) δ 8.52 (d, *J* = 8.4 Hz, 1H), 8.36 (d, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.97 (d, *J* = 6.0 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.71 (s, 1H), 7.66 (d, *J* = 5.0 Hz, 1H), 7.61 (dd, *J* = 12.7, 7.3 Hz, 4H), 7.51 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.15 (s, 1H), 7.05 (dd, *J* = 8.9, 1.7 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 4.38 (d, *J* = 3.0 Hz, 2H), 4.10 (t, *J* = 6.4 Hz, 2H), 3.92 (dd, *J* = 14.0, 6.9 Hz, 1H), 3.84 (s, 3H), 3.69 – 3.63 (m, 2H), 3.11 (d, *J* = 6.3 Hz, 2H), 1.51 – 1.40 (m, 3H).

^{13}C NMR (126 MHz, DMSO) δ 174.51, 164.26, 163.30, 157.50, 150.87, 140.63, 140.30, 138.42, 135.90, 134.40, 133.62, 132.57, 130.98, 129.78, 129.40, 128.83, 128.70, 128.55, 127.32, 126.53, 125.95, 124.61, 122.22, 120.49, 119.00, 108.45, 106.00, 104.29, 100.61, 62.84, 55.55, 44.94, 41.95, 40.73, 39.20, 18.91.

Mass: m/z: Calculated for $C_{36}H_{33}IN_3O_7S$+, $[M + H]^+$: 778.1078, Observed: 778.1046

Synthesis of compound 11



11

Procedure: In a round bottle flask, compound 10 (1 eq., 500 mg) and an excess of compound 2, i.e. ethylene diamine (8 eq.), were added to dry DCM at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for 6h at room temperature. Completion of the reaction was monitored through (TLC). The solvent was evaporated on the rota vapour, and the reaction mixture was purified with 60-120 mesh silica in 5 % MeOH in DCM.

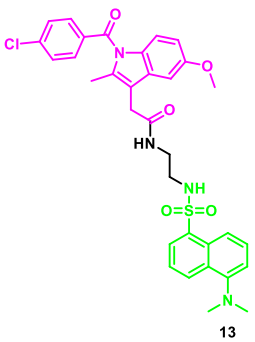
Yield: 65%

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, *J* = 8.5 Hz, 1H), 8.27 (m, 2H), 7.54 (m, 2H), 7.18 (d, *J* = 7.5 Hz, 1H), 2.93 – 2.90 (m, 2H), 2.88 (s, 6H), 2.74 – 2.71 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 152.02, 134.62, 130.45, 129.90, 129.68, 129.61, 128.44, 123.21, 118.73, 115.23, 45.43, 45.28, 40.77.

Mass: m/z: Calculated for $C_{14}H_{20}N_3O_2S$+, $[M + H]^+$: 294.1271, Observed: 294.1265

Synthesis of compound 13



13

Procedure: In a round bottle flask, compound 12, i.e. Indomethacin (1 eq., 30mg), compound 11 (2 eq., 49.1 mg), triethylamine (1.5 eq., 16.5µL), DMAP (0.2 eq., 2.048 mg), HOBt (1.2 eq., 38mg) and EDCI.HCl(1.2 eq., 19.28mg) was added to dry DMF at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for 24h at room temperature. Completion of the reaction was monitored through (TLC). The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 40-50 % EtOAc in Hexane.

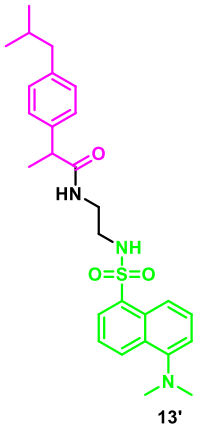
Yield: 30%

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 1H), 8.02 (m, 1H), 7.72 – 7.67 (m, 2H), 7.56 – 7.51 (m, 1H), 7.50 – 7.42 (m, 3H), 7.17 (d, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 9.0 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 6.71 (m, 1H), 6.23 (t, *J* = 5.5 Hz, 1H), 5.30 (t, *J* = 5.7 Hz, 1H), 3.81 (s, 3H), 3.59 (s, 2H), 3.27 (m, 2H), 2.95 – 2.91 (m, 2H), 2.87 (s, 6H), 2.33 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.23, 168.46, 156.28, 152.13, 139.46, 136.67, 134.21, 133.65, 131.33, 131.05, 130.66, 130.37, 129.94, 129.60, 129.43, 129.19, 128.57, 123.15, 118.46, 115.29, 112.36, 112.19, 100.77, 55.80, 45.40, 43.16, 39.67, 32.12, 29.71, 13.28.

Mass: m/z: Calculated for $C_{33}H_{34}ClN_4O_5S+$, $[M+H]^+$: 633.1933, Observed: 633.1941

Synthesis of compound 13'



13'

Procedure: In a round bottle flask, compound 12', i.e. Ibuprofen (1 eq., 60mg), compound 11 (1.5 eq., 128 mg), triethylamine (1.5 eq., 60µL), DMAP (0.6 eq., 21.32 mg), HOBt (1.4 eq., 154.43 mg) and EDCI.HCl(1.4 eq., 78.066 mg) was added to dry DMF at 0°C in the inert atmosphere of nitrogen, and the reaction was stirred for 24h at room temperature. Completion of the reaction was monitored through (TLC). The compound was extracted with ethyl acetate and purified with 100-200 mesh silica at 35-45 % EtOAc in Hexane.

Yield: 32%

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.5 Hz, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 8.18 (m, 1H), 7.61 – 7.55 (m, 1H), 7.50 (m, 1H), 7.20 (d, *J* = 7.5 Hz, 1H), 7.11 – 7.00 (m, 4H), 5.78 (t, *J* = 5.7 Hz, 1H), 5.55 (t, *J* = 5.8 Hz, 1H), 3.35 – 3.23 (m, 2H), 3.14 (m, 1H), 3.01 – 2.92 (m, 2H), 2.89 (s, 6H), 2.43 (d, *J* = 7.2 Hz, 2H), 1.83 (m, 1H), 1.38 (d, *J* = 7.2 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.60, 151.05, 139.68, 137.20, 133.42, 129.52, 128.91, 128.58, 128.55, 128.48, 127.51, 126.21, 122.15, 117.69, 114.26, 45.40, 44.40, 43.97, 42.33, 38.31, 29.12, 21.37, 17.31.

Mass: m/z: Calculated for $C_{27}H_{36}N_3O_3S+$, $[M+H]^+$:482.2472, Observed:482.2493

Biological studies:

Cell Viability assay: Cells were seeded in the 96 well plates with 5000 cells per well and left for attachment for 24 h, at 37°C in an incubator; the next day, cells were treated with the compounds 9a-9e, 9'a-9'e, 13 and 13' at different concentrations as well as in combination with bafilomycin A, for combinatorial treatment bafilomycin A was used at the final concentration of 25nM, 1% of DMSO was taken as a control, followed by the 24h incubation period time. MTT was added at the final concentration of 0.5 mg/mL and incubated for 3h; MTT was aspirated, and 100µL of DMSO was added per well to dissolve the formed frozman crystals and absorbance was recorded with envision microplate reader, the data obtained was analysed by Graph Pad Prism software.

Colocalisation Study: 25,000 HCT116 cells were seeded in a live cell plate and left for overnight attachment at 37°C in an incubator. Next day, compound 9e is incubated for 6h at the final concentration of 3µM and 13' at the final concentration of 5µM for 3h and 6h. After incubation, media was aspirated, cells were washed with 1mL 1X PBS twice, and ER tracker Red was added at the final concentration of 100nM in DMEM and incubated for 20 minutes. Next, DMEM was aspirated, cells were washed with 1mL 1X PBS twice, and fresh DMEM was added to the plate, sample was imaged by Leica confocal microscopy and analysed by Image j software.

AV staining: 25,000 HCT116 cells were seeded in a live cell plate and left for overnight attachment at 37°C in an incubator. The next day, compound 13' is incubated at the final concentration of 15µM for 24h and another plate is treated with an equivalent amount of DMSO as a control. After incubation, media was aspirated, cells were washed with 1mL 1X PBS twice, and 5µL of AV was added to 2mL DMEM and incubated for 20 minutes. Next, DMEM was aspirated, cells were washed with 1mL 1X PBS twice, and fresh DMEM was added to the plate; the sample was imaged by Leica confocal microscopy and analysed by Image j software.

PI staining: 25,000 HCT116 cells were seeded in a live cell plate and left for overnight attachment at 37°C in an incubator. The next day, compound 13' is incubated at the final concentration of 15µM for 24h and another plate is treated with an equivalent amount of DMSO as a control. After incubation, media was aspirated, cells were washed with 1mL 1X PBS twice, and PI was added to the final working concentration of 500nM and incubated for 20 minutes. Next, DMEM was aspirated, cells were washed with 1mL 1X PBS twice, and fresh DMEM was added to the plate; the sample was imaged by Leica confocal microscopy and analysed by Image j software.

Oil Red O assay: 25,000 HCT116 cells were seeded on the coverslip and left for overnight attachment at 37°C in an incubator. The next day, compound 13' is incubated at the final concentration of 15µM for 24h and another plate is treated with an equivalent amount of DMSO as a control. After incubation, media was aspirated, cells were washed with 1mL 1X PBS twice, and then the cells were fixed with 10 % Formalin for 15 minutes, followed by the washing and then the filtered Oil Red O solution was added at the dilution of 1:2 in ddH_2O and incubated for 20 minutes. Next, the solution was aspirated, cells were washed with 1mL 1X PBS thrice to remove any traces of dye present, and fresh DMEM was added to the plate; the sample was imaged by Leica confocal microscopy and analysed by Image j software.

Immunofluorescence Assay: In a six-well plate, 50,000 HCT116 cells were seeded on the coverslip in each well and left for overnight attachment at 37°C. Cells were treated with molecule 13' around IC_{50} value with an equivalent amount of DMSO in the control and incubated for 24h. Further, cells were washed with 1X PBS twice and incubated with 4% paraformaldehyde for 12 minutes for fixation, followed by incubation with PBST (0.2% Tween 20 in PBS) for 10 minutes at room temperature for permeabilisation. The next step involves blocking with 1% BSA for 30 minutes at 37°C in an incubator. After washing the cells with 1X PBS, the primary antibody for IRE1α, PERK and Caspase-12 was added to the cells after diluting in 1% BSA and incubated at 37°C for 4h. The next step involves washing cells with PBST and adding a secondary antibody conjugated to Alexa Fluor 633 with incubation at room temperature in dark at 37°C for 1 h. Finally, cells were washed with 1X PBS thrice, and with the help of mounting media with DAPI coverslip was mounted on the glass slide; the sample was imaged by a Leica confocal microscope and analysed by image J software.

Western Blot: In two 6cm plates, ten lakh HCT116 cells were seeded per plate and incubated for 24h at 37°C. The next day, cells were treated with 13' at 12µM in one plate, and an equivalent amount of DMSO was added to the other plate. The cells were washed with 1X PBS, trypsinised, and cell lysate was formed by lysis buffer, followed by protein quantification using the Bradford reagent. The protein of interest was separated by running the SDS page gel and transferred to the nitrocellulose membrane; the membrane was washed with 1X TBS, followed by blocking the membrane with 5% skimmed milk in 1X TBST. The membranes were washed with 1X TBST, and the primary antibodies for PERK, CHOP, IRE1α, LC3, Caspase-12, COX-2, Beclin, and Caspase-3 were added to the membrane and incubated overnight at 4°C. After washing with 1X TBST the next day, secondary antibodies for respective primary antibodies were added and incubated for 2h at room temperature. Blots were imaged and analysed for the protein of interest. For control loading, an HRP-conjugated beta-actin antibody was added to the blots and incubated for 2h at room temperature, followed by the imaging and analysis of the sample. The reference ladder

varies from 250kD-10Kd.Blots were developed using clarity-enhanced chemiluminescence (ECL) reagent and imaged by Bio-Rad Gel documentation instrument, followed by the analysis by Image Lab software.

FACS: $1*10^6$ HCT116 cells seeded in tissue culture plates and left for attachment for 24h. The next day, cells were treated with the 13' at 15µM in one plate, and an equivalent amount of DMSO was added as a control to the other plate and incubated for 24h. The cells were then washed with 1X PBS, detached with trypsin and then transferred to the microcentrifuge tube and centrifuged for 5 minutes at 10,000 rpm. The supernatant was removed, and the pellet was washed with 1X PBS twice. 5µL of Alexa Fluor 488 annexin V and 1µL of 100µg/mL PI working solution were added to the pellet dissolved in 100µL of 1X AV binding buffer and incubated for 20 minutes at room temperature. Now, after the incubation period, 400µL of 1X AV binding buffer was added, mixed and analysed using a BD FACSAria Fusion machine using a 488nm laser.

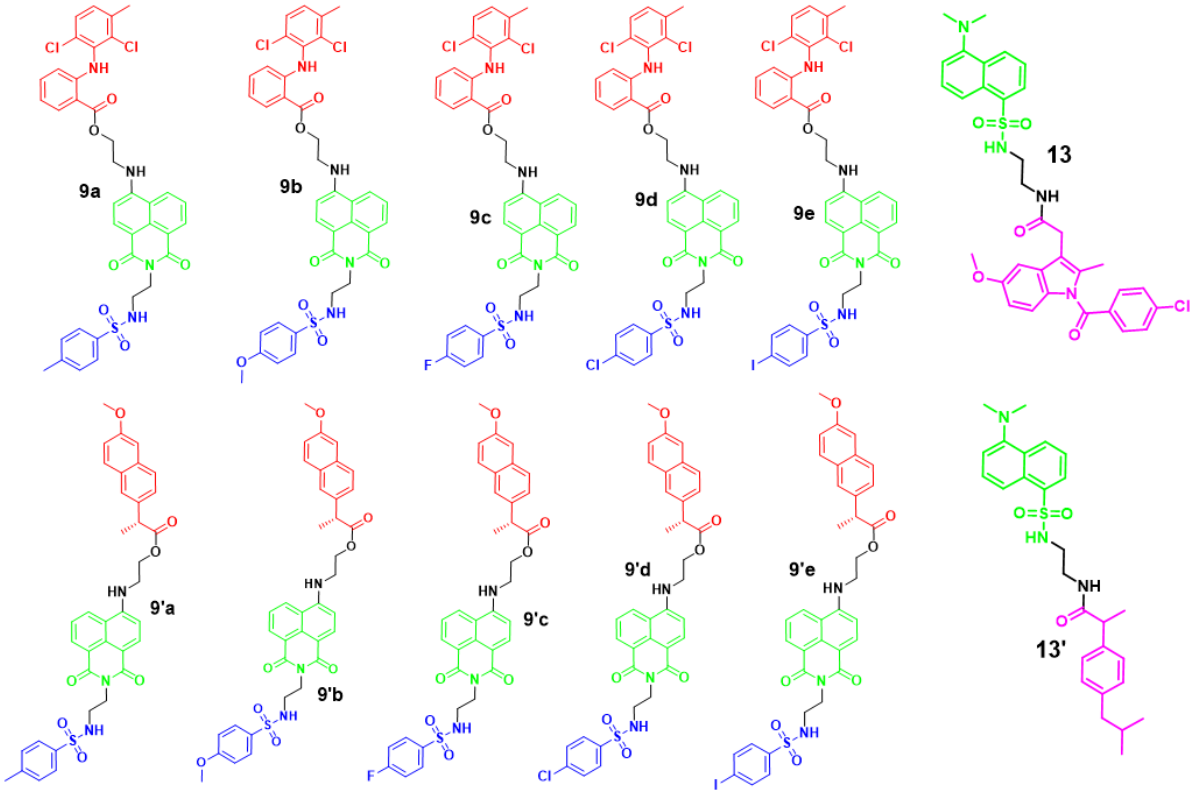


Fig. S1: Chemical structures of the library members.

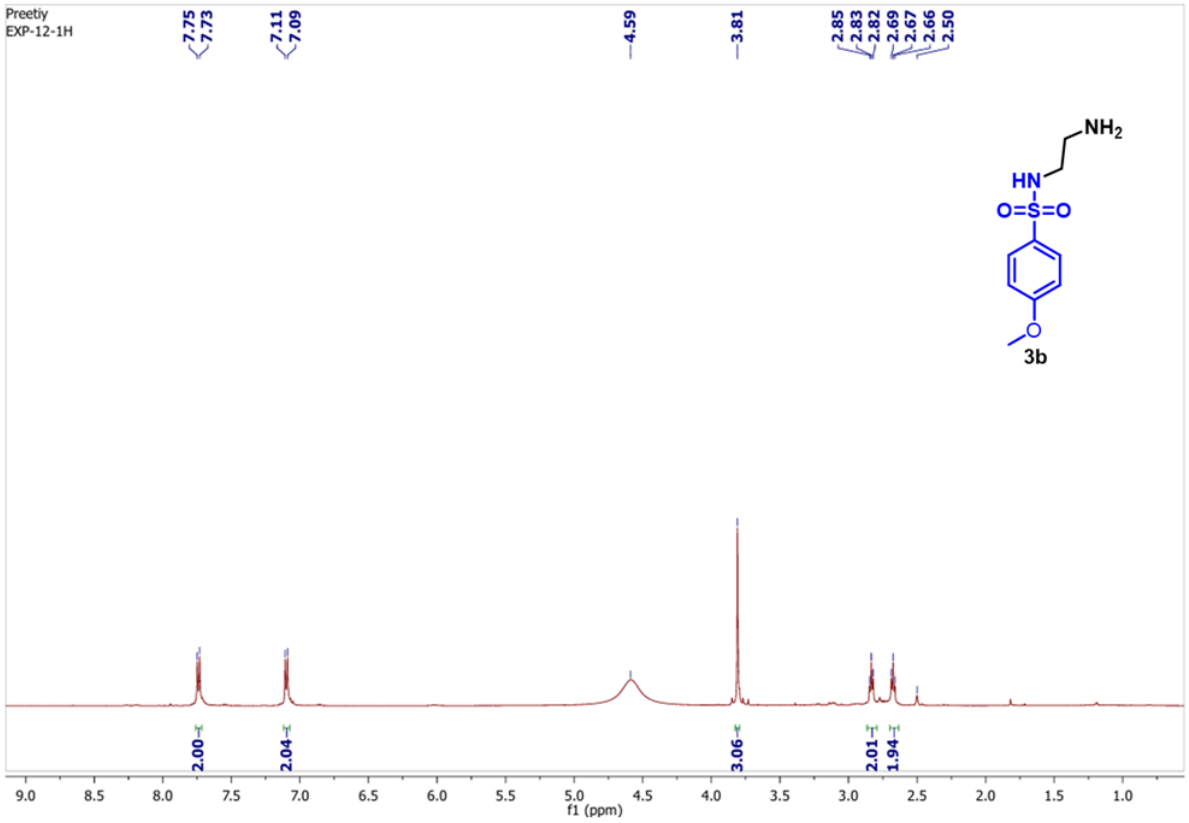


Fig. S2: ^{1}H NMR spectra of 3b.

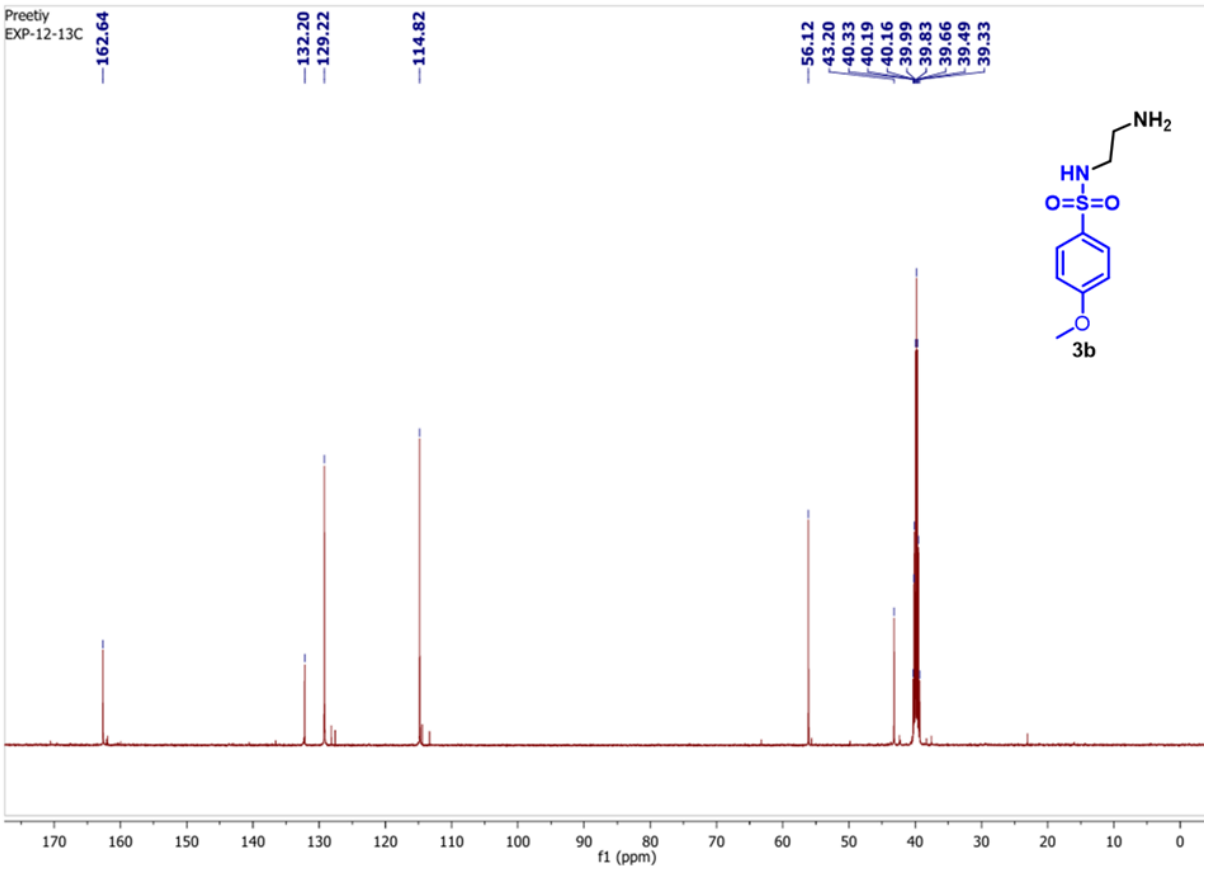


Fig. S3: ^{13}C NMR spectra of 3b.

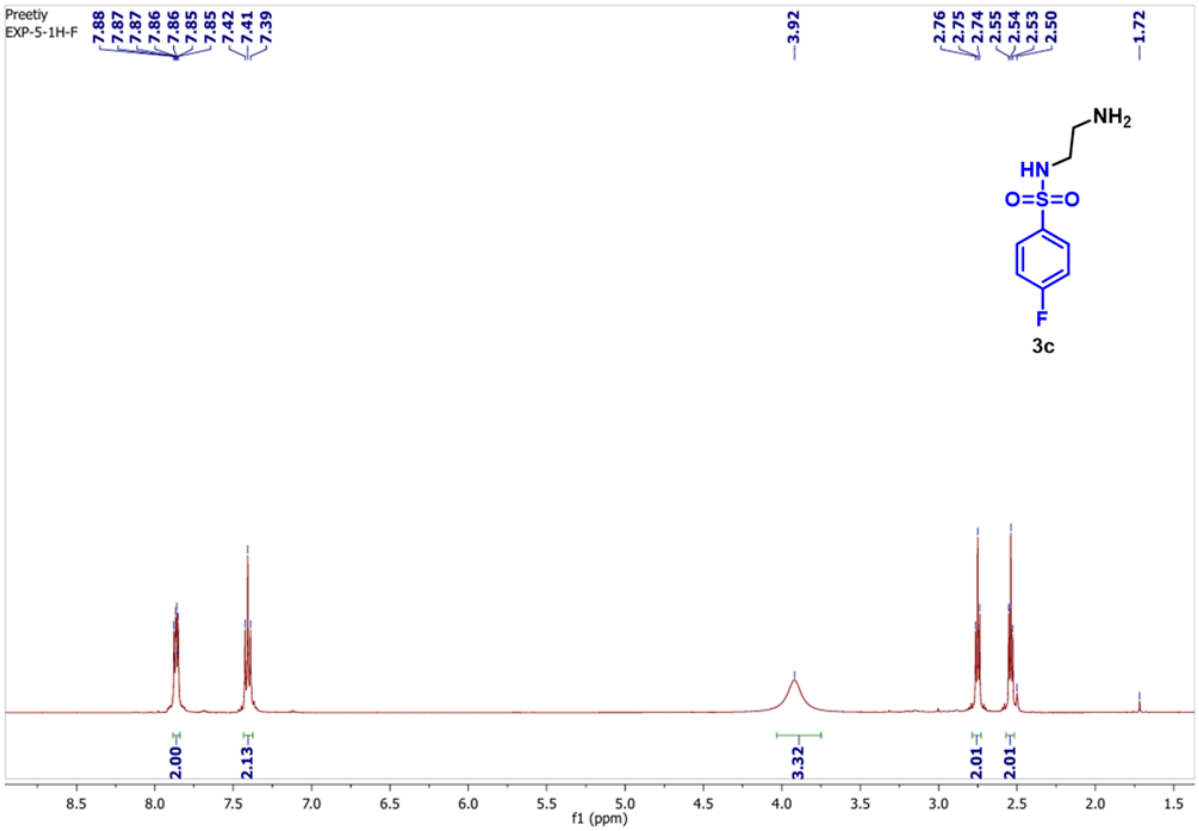


Fig. S4: ^{1}H NMR spectra of 3c.

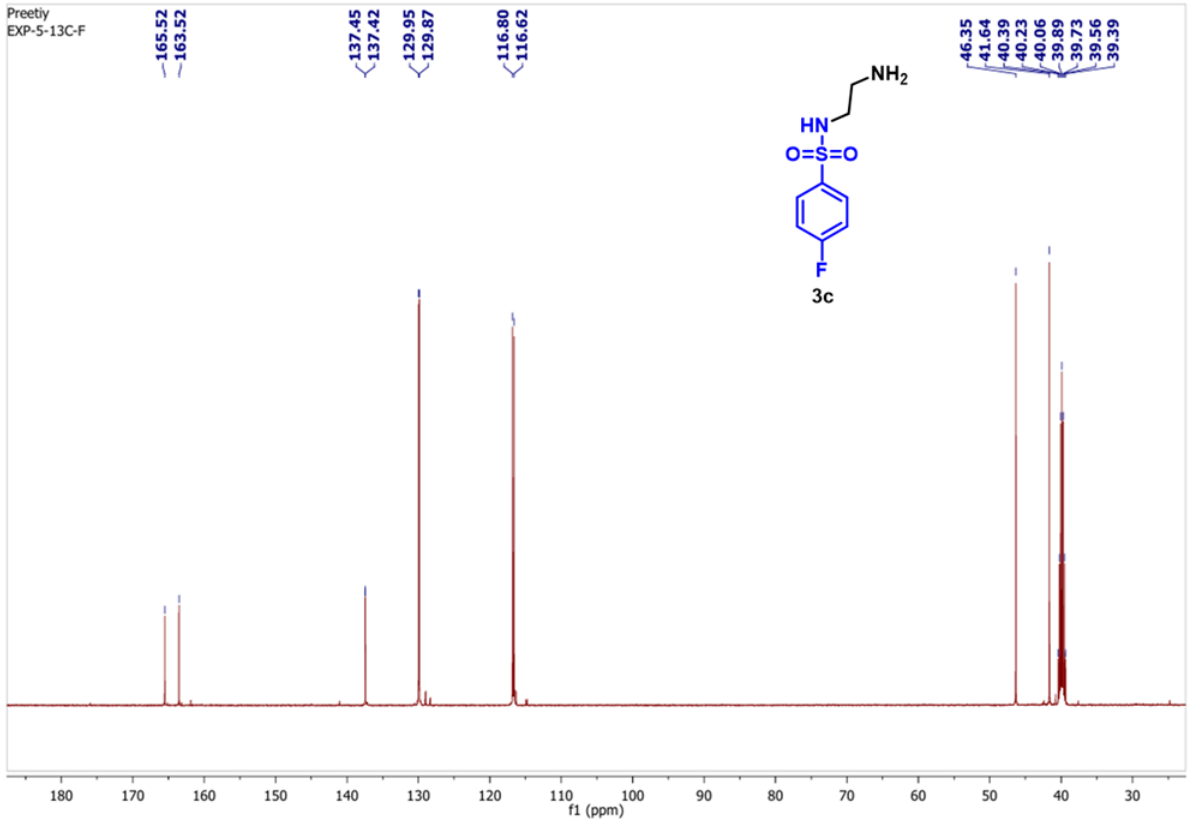


Fig. S5: ^{13}C NMR spectra of 3c.

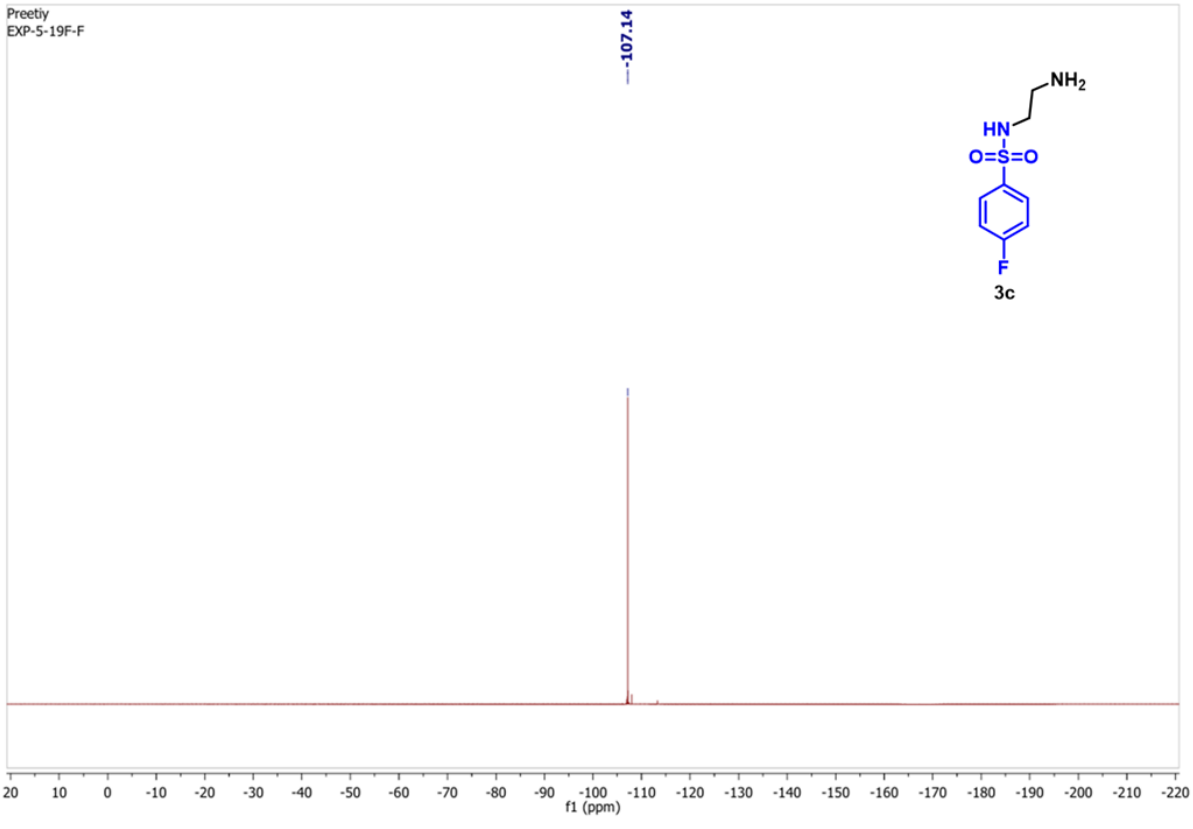


Fig. S6: ^{19}F NMR spectra of 3c.

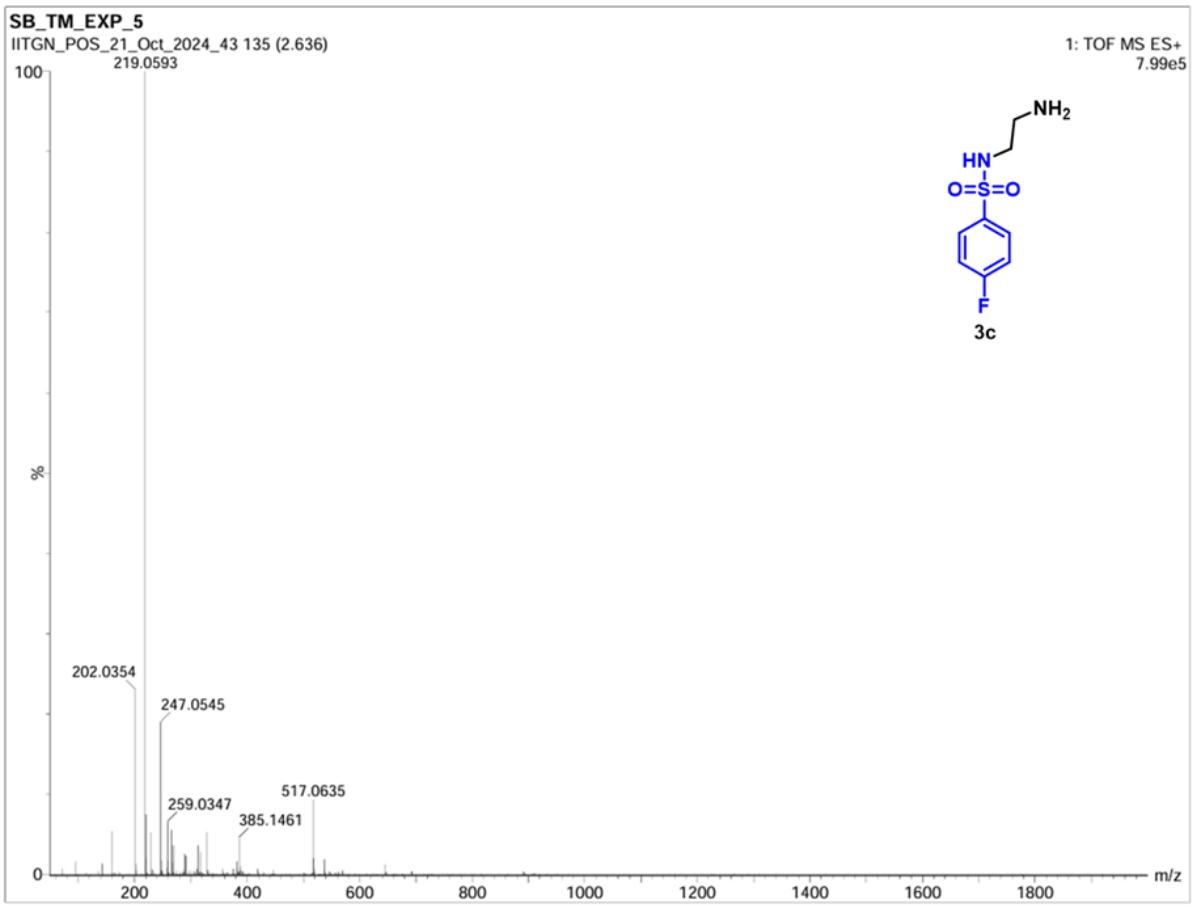


Fig. S7: HR-MS spectra of 3c.

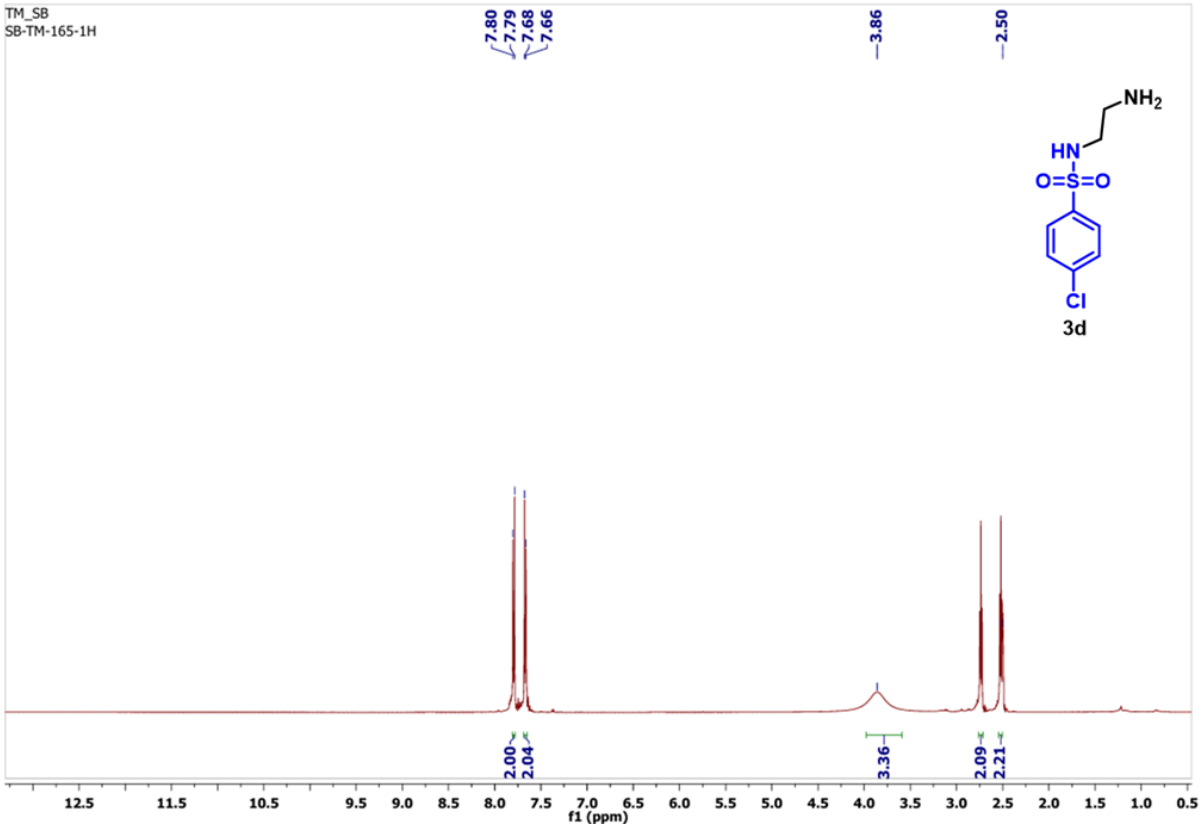


Fig. S8: ^{1}H NMR spectra of 3d.

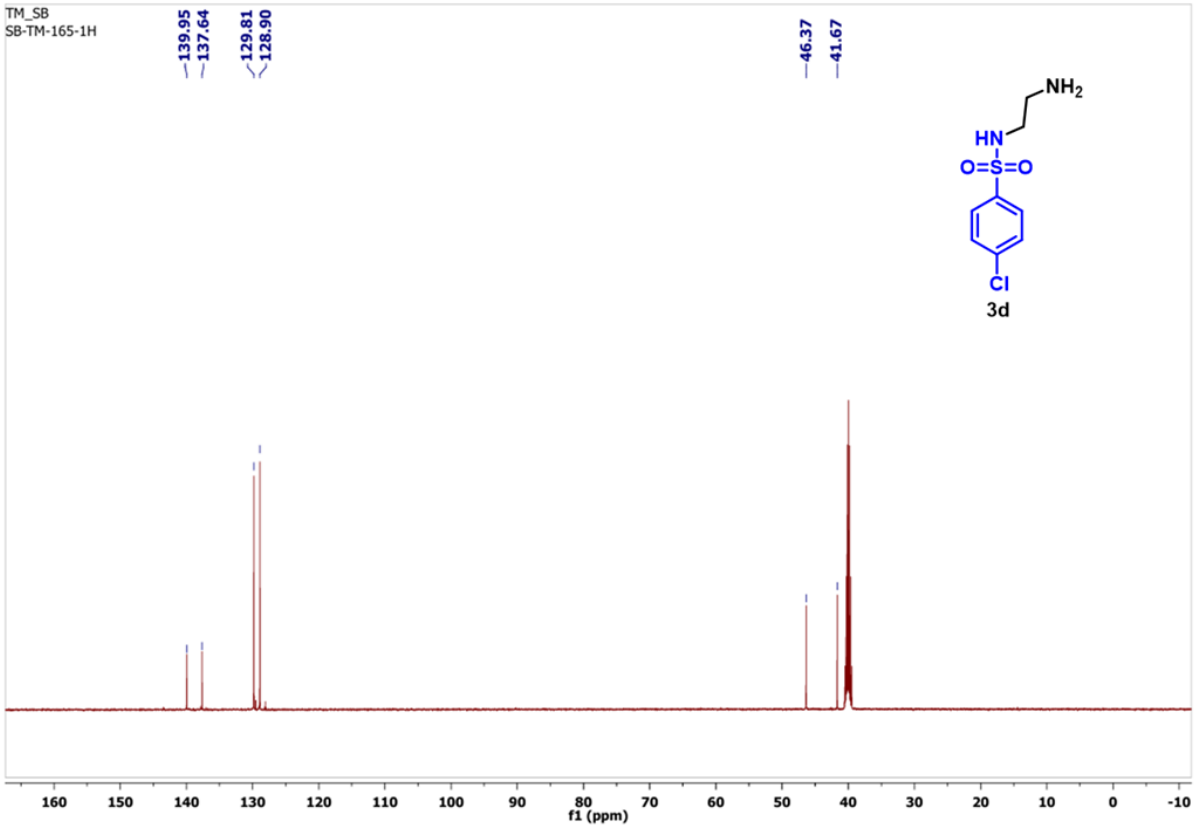


Fig. S9: ^{13}C NMR spectra of 3d.

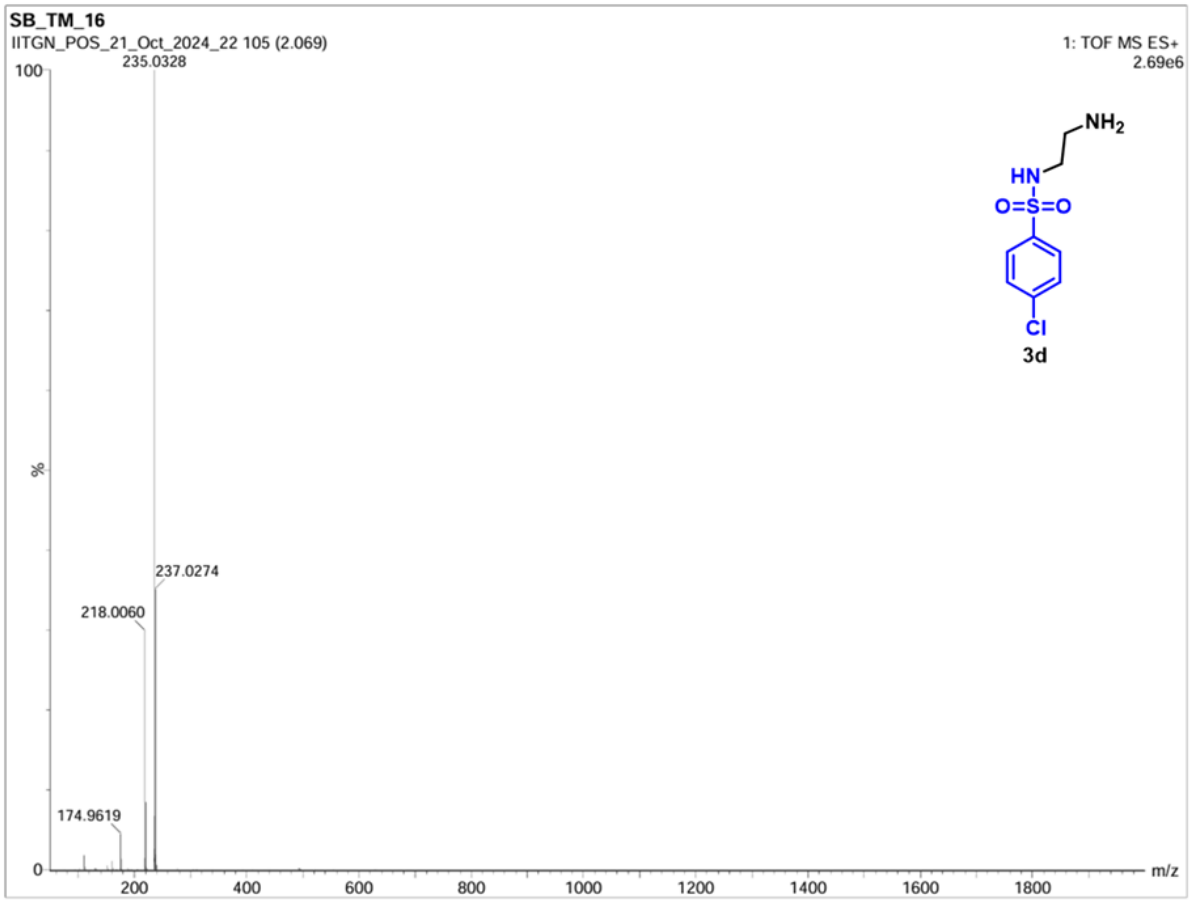


Fig. S10: HR-MS spectra of 3d.

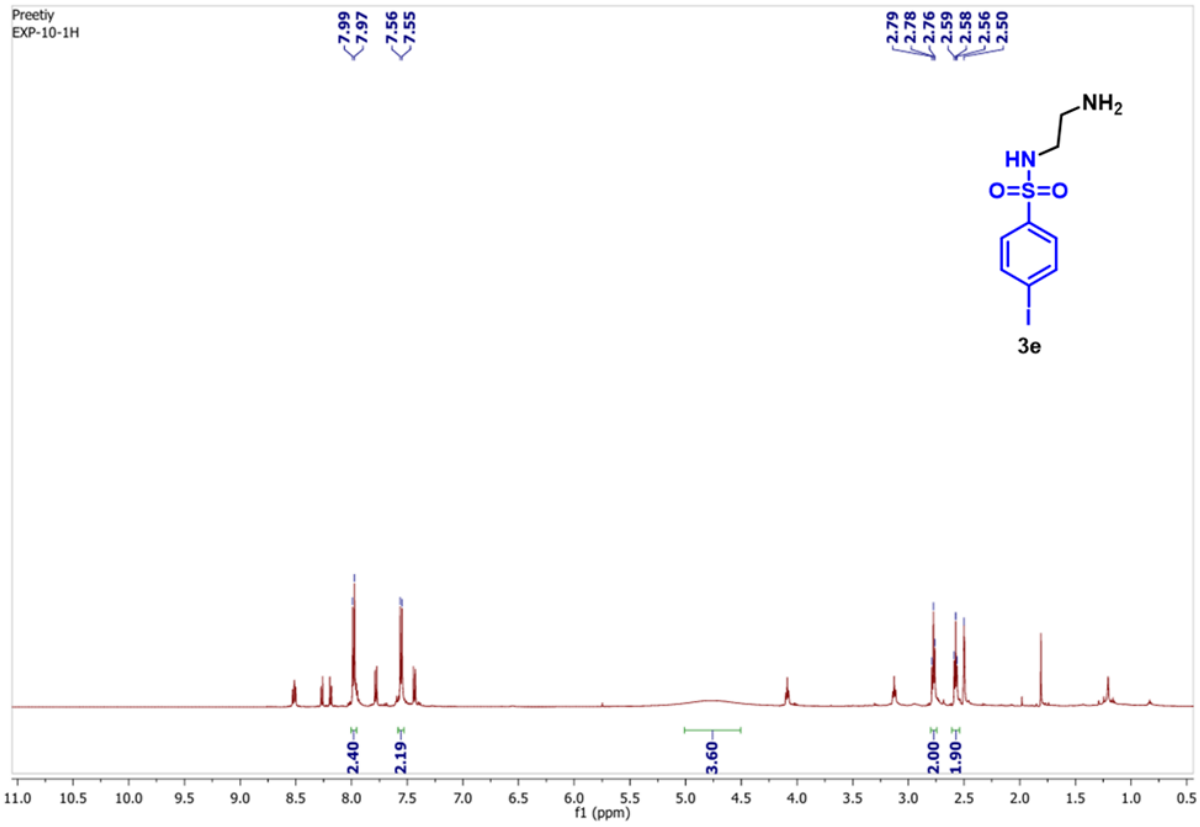


Fig. S11: ^{1}H NMR spectra of 3e.

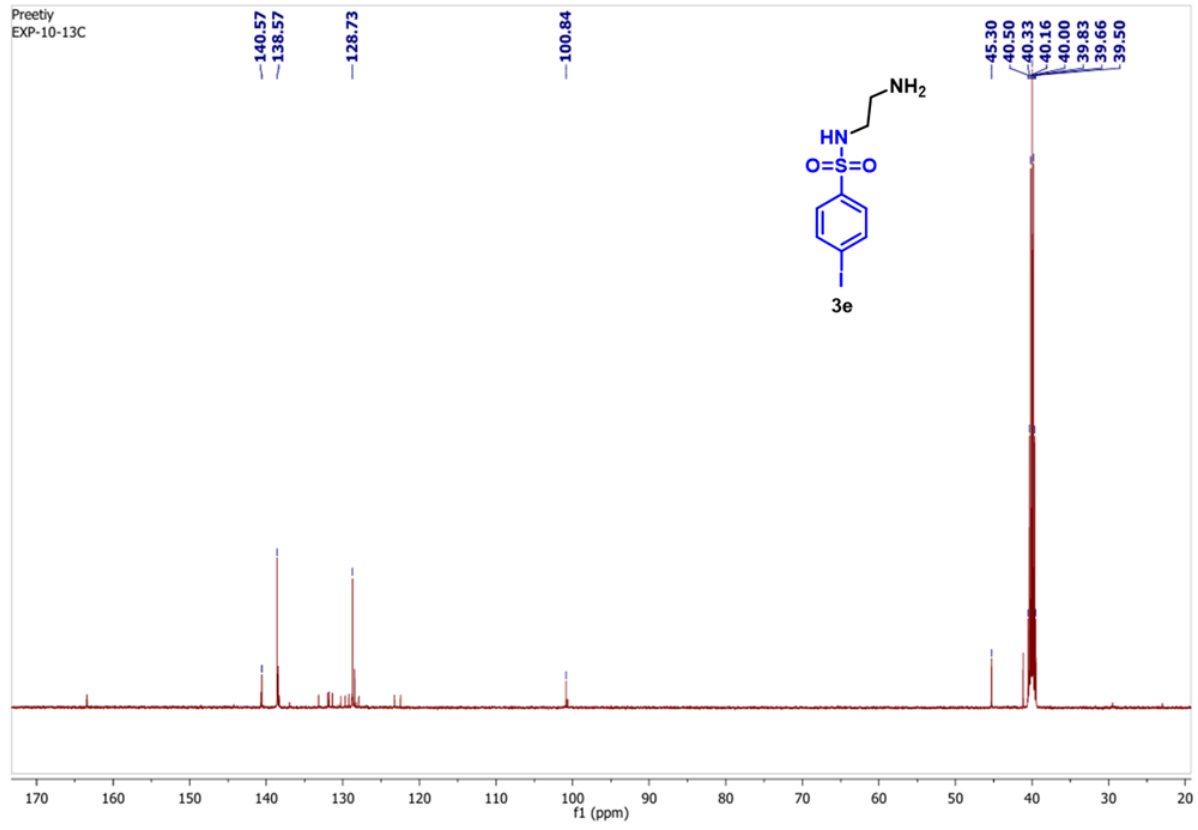


Fig. S12: ^{13}C NMR spectra of 3e.

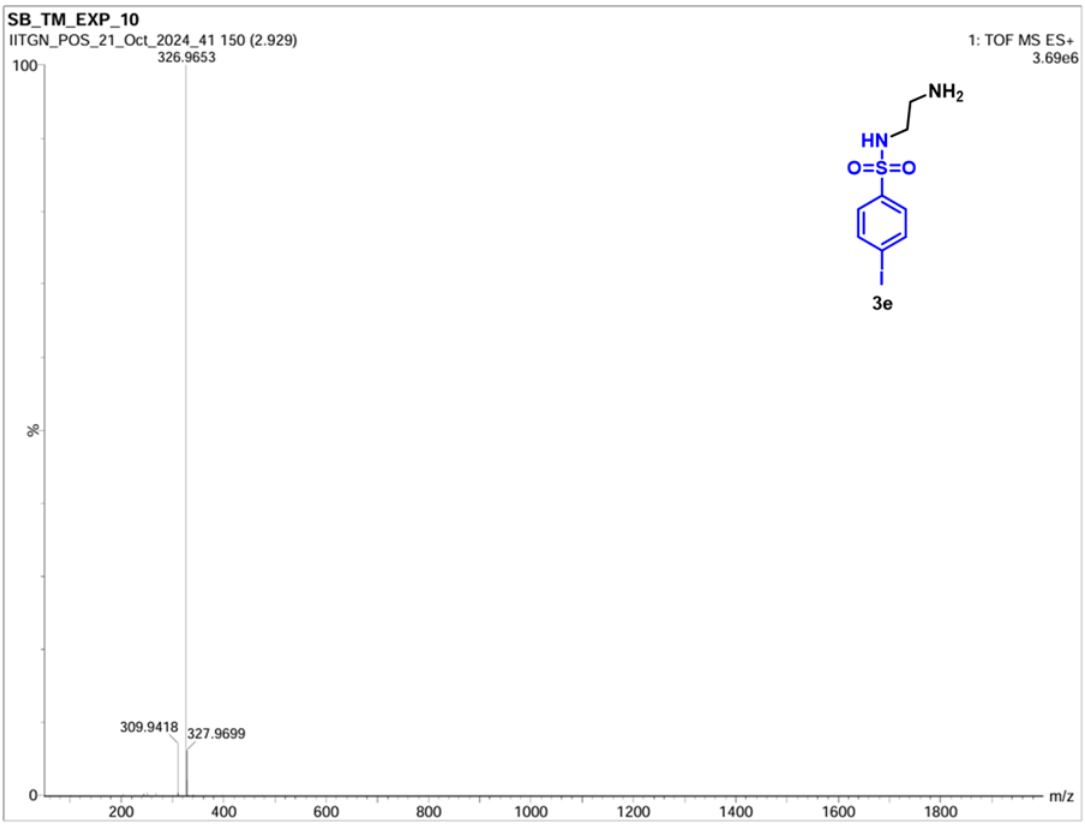


Fig. S13: HR-MS spectra of 3e.

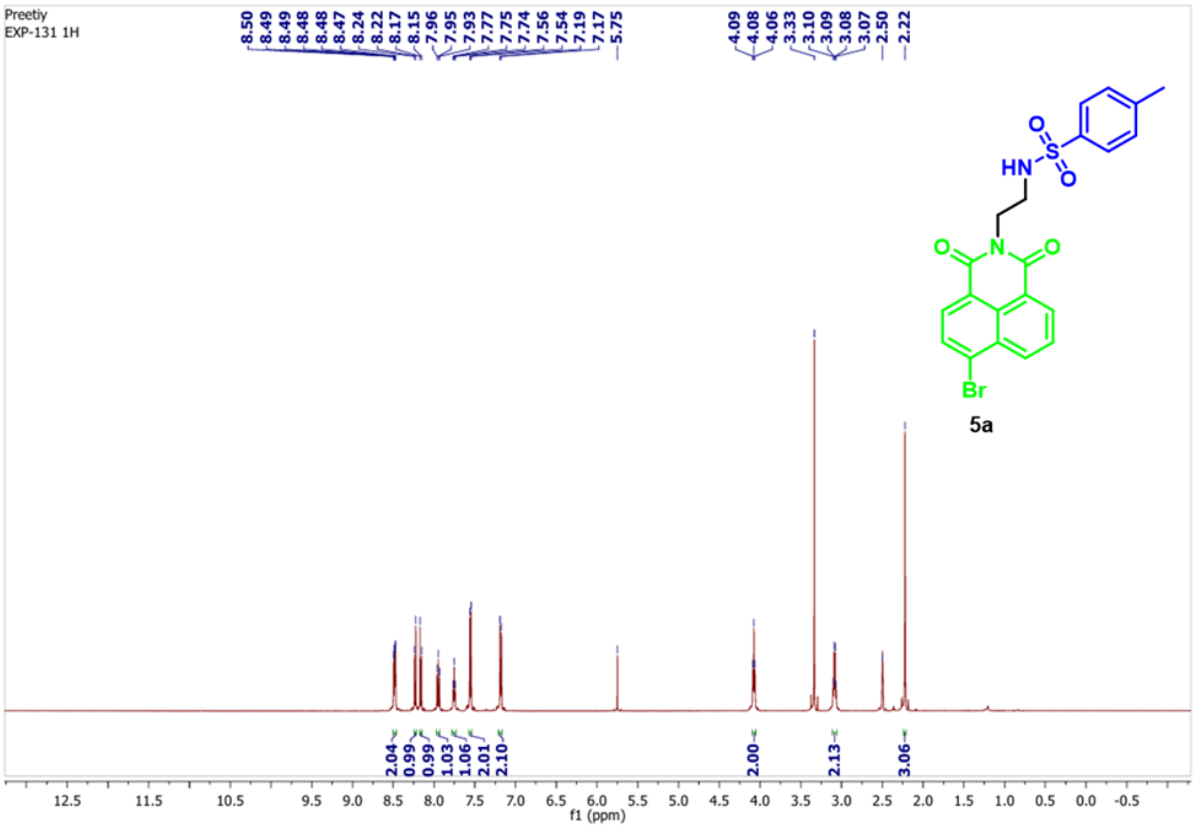


Fig. S14: ^{1}H NMR spectra of 5a.

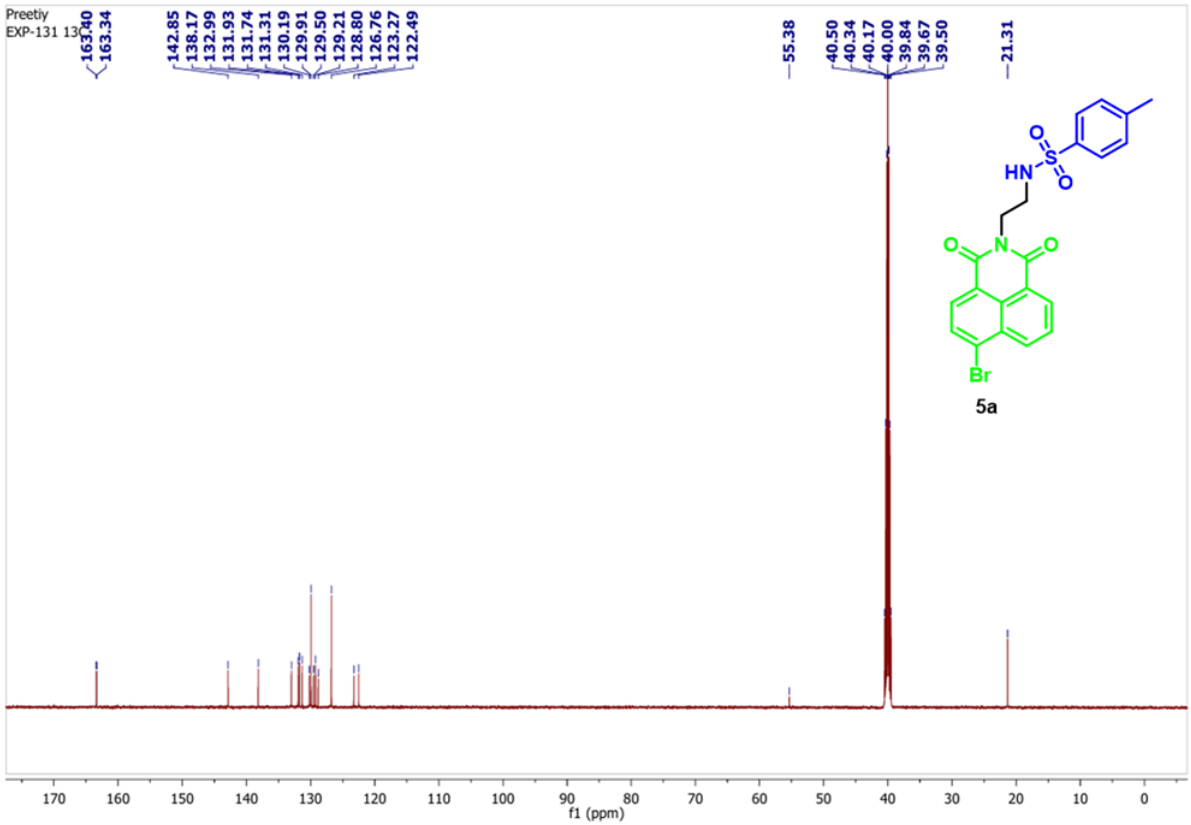


Fig. S15: ^{13}C NMR spectra of 5a.

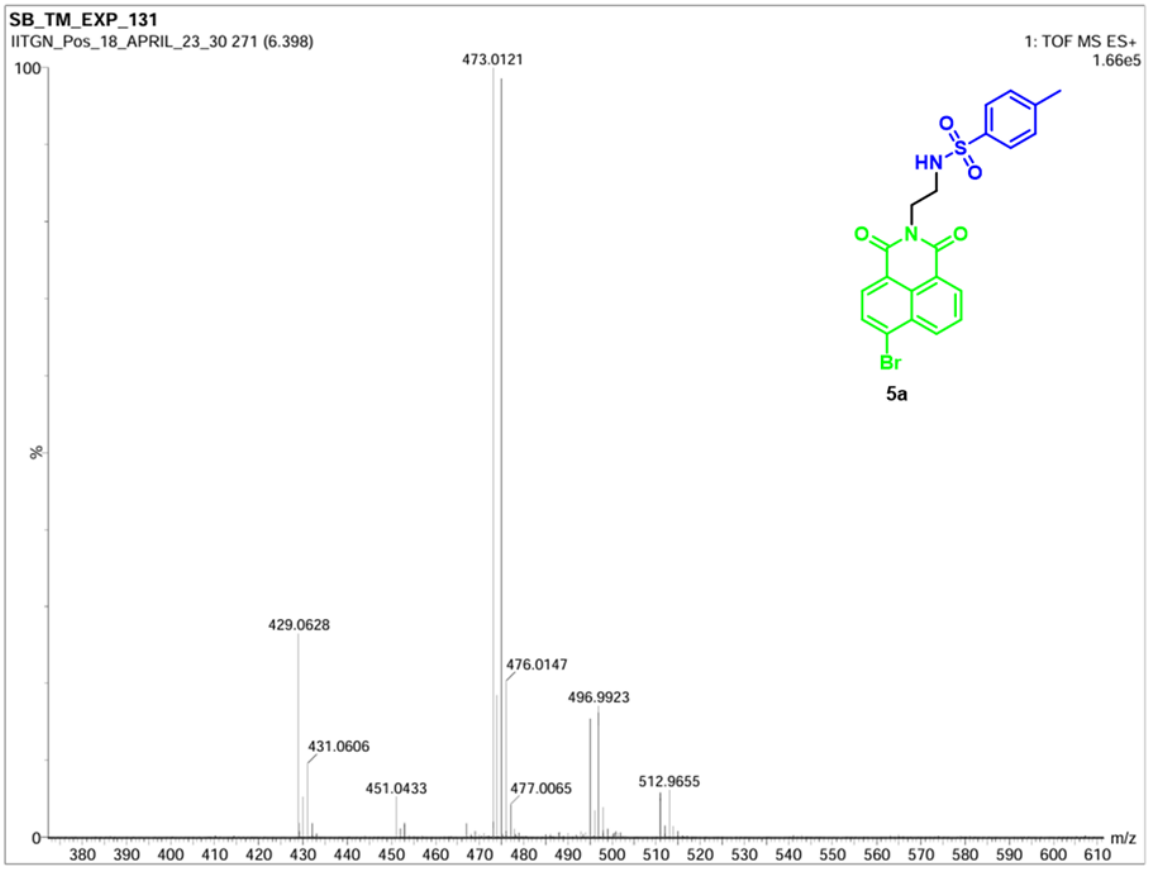


Fig. S16: HR-MS spectra of 5a.

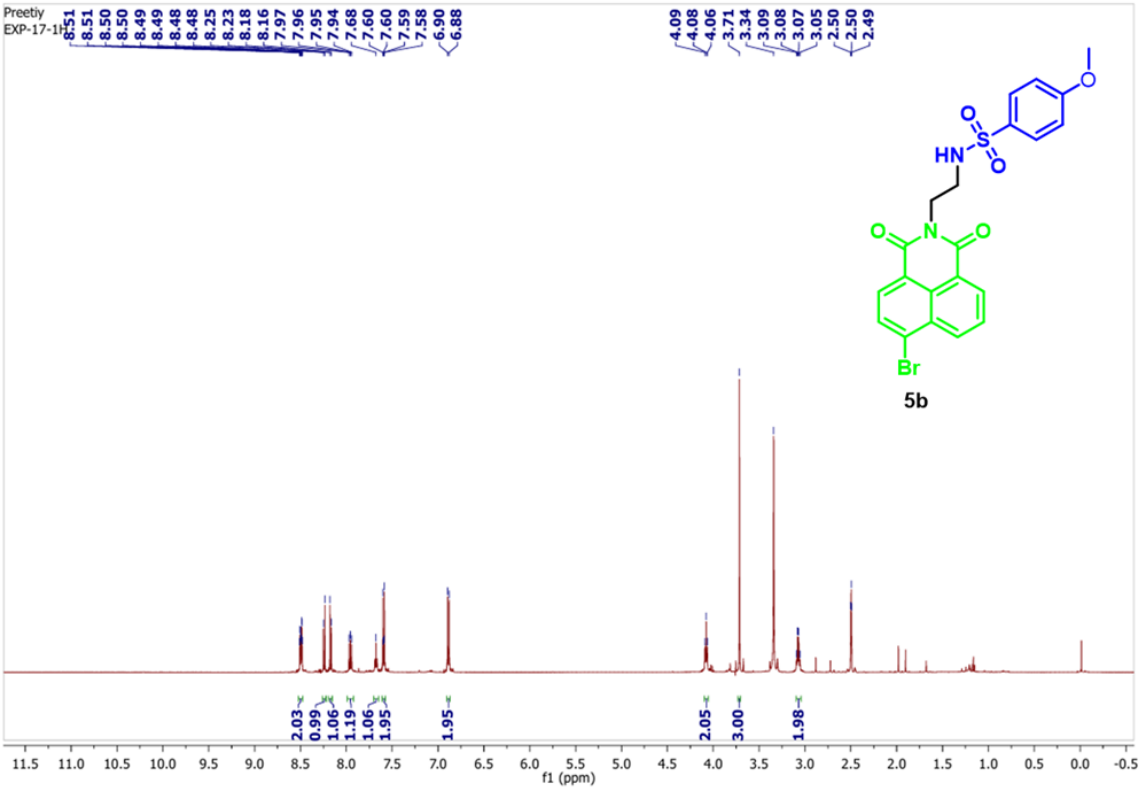


Fig. S17: ^{1}H NMR spectra of 5b.

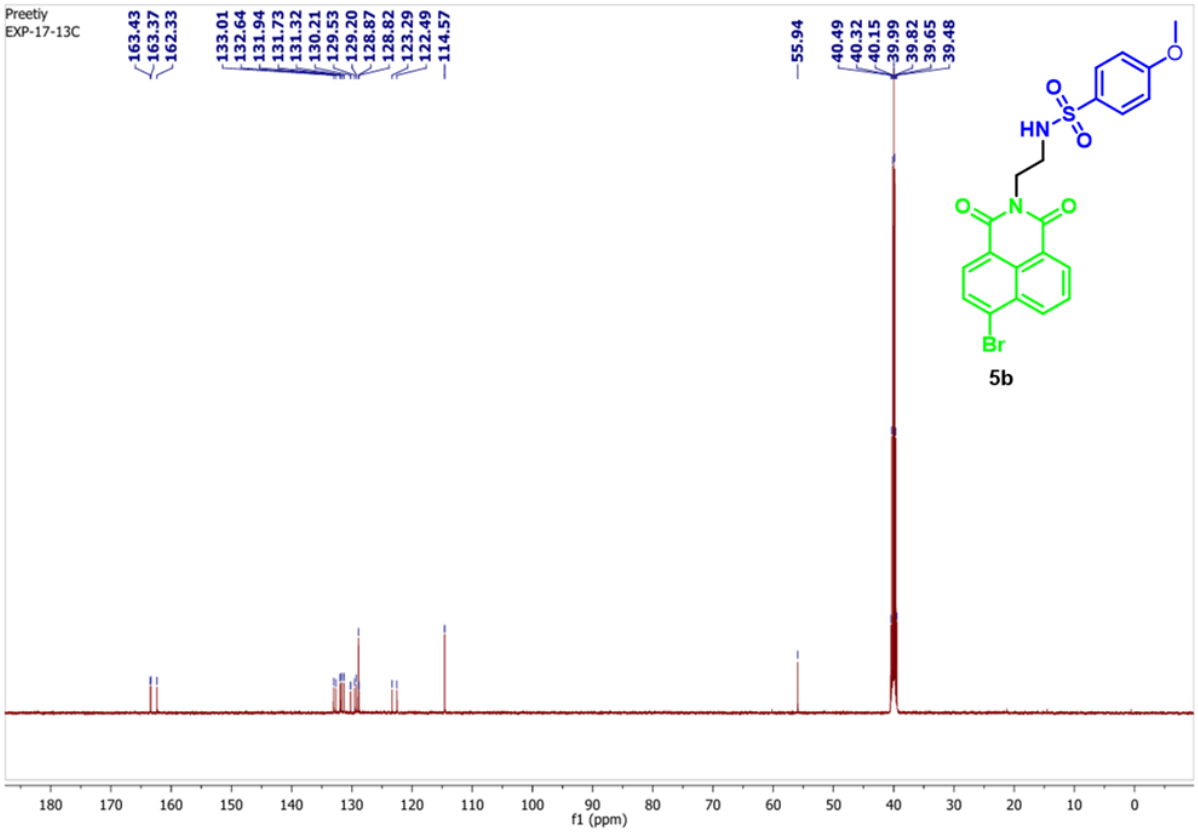


Fig. S18: ^{13}C NMR spectra of 5b.

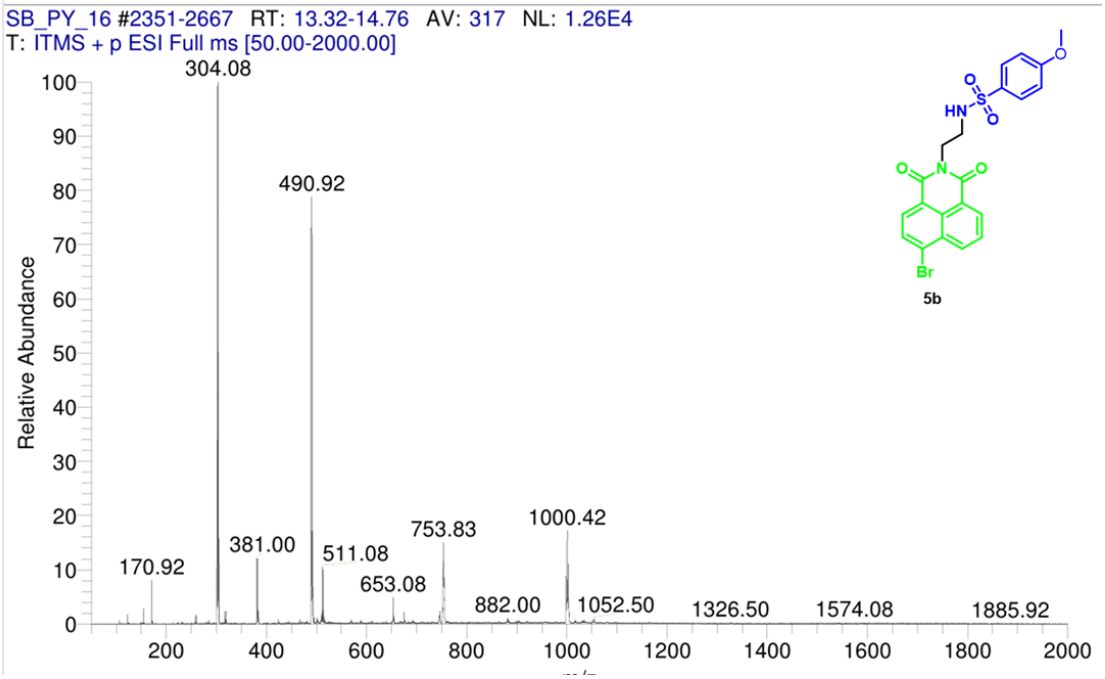


Fig. S19: HR-MS spectra of 5b.

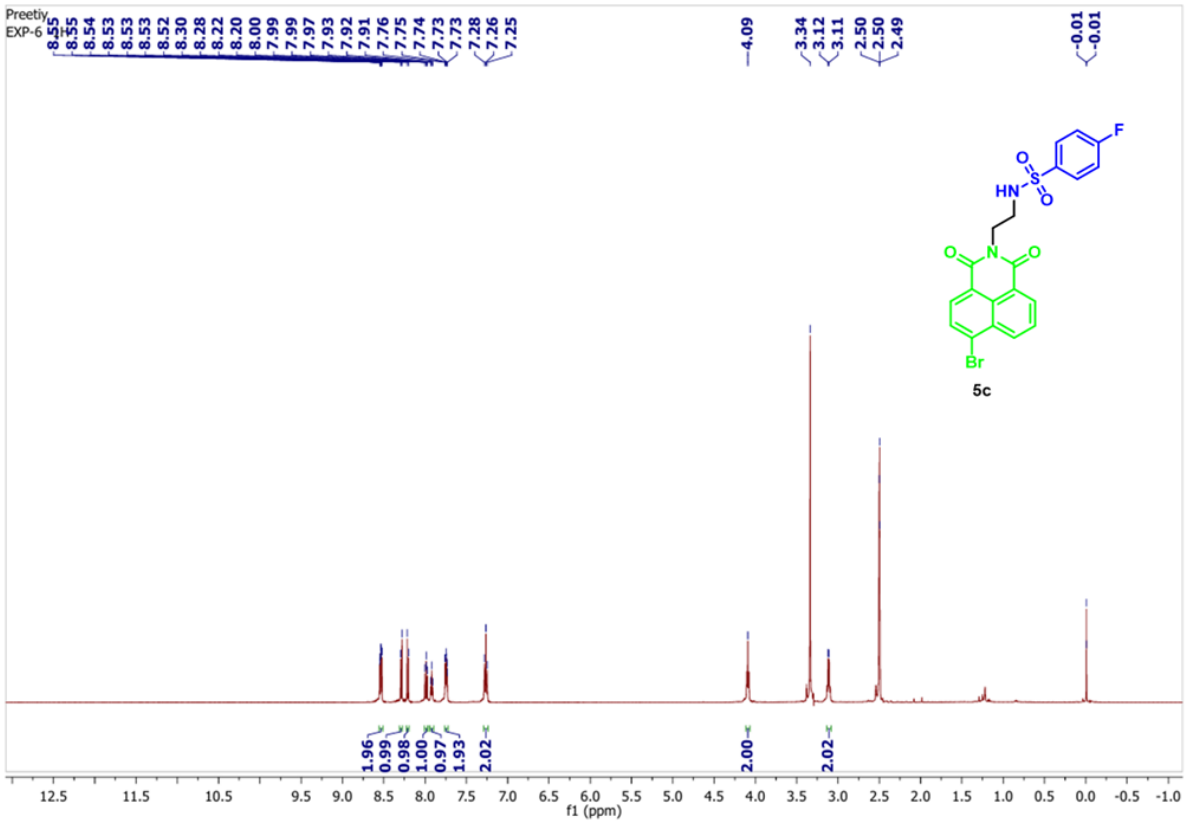


Fig. S20: ^{1}H NMR spectra of 5c.

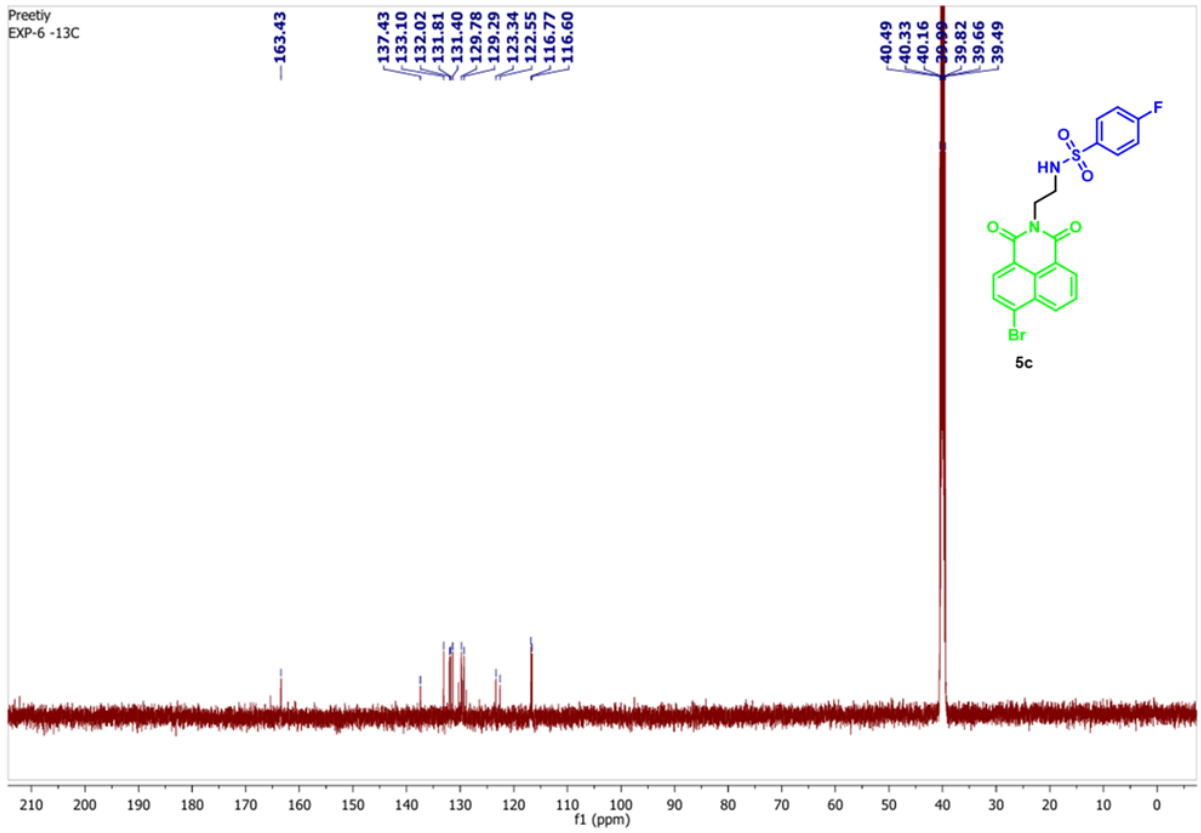


Fig. S21: 13C NMR spectra of 5c.

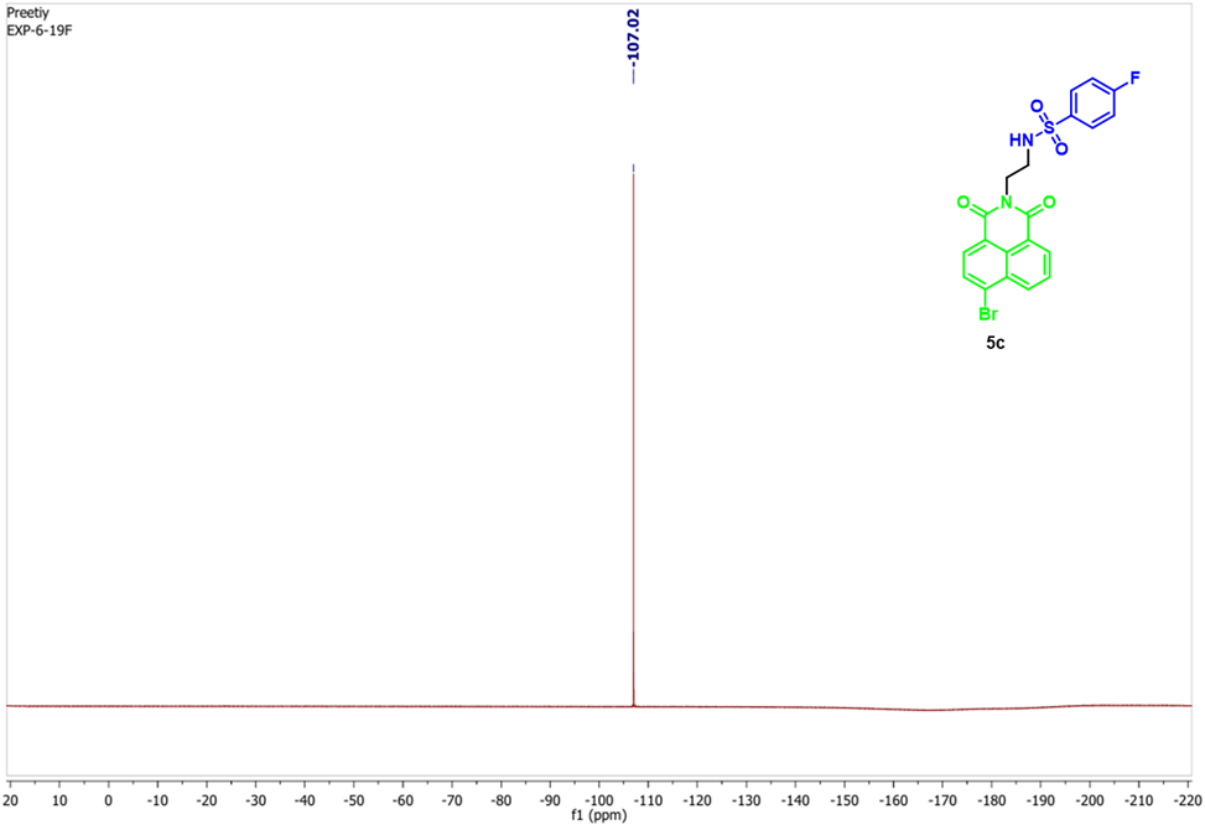


Fig. S22: ^{19}F NMR spectra of 5c.

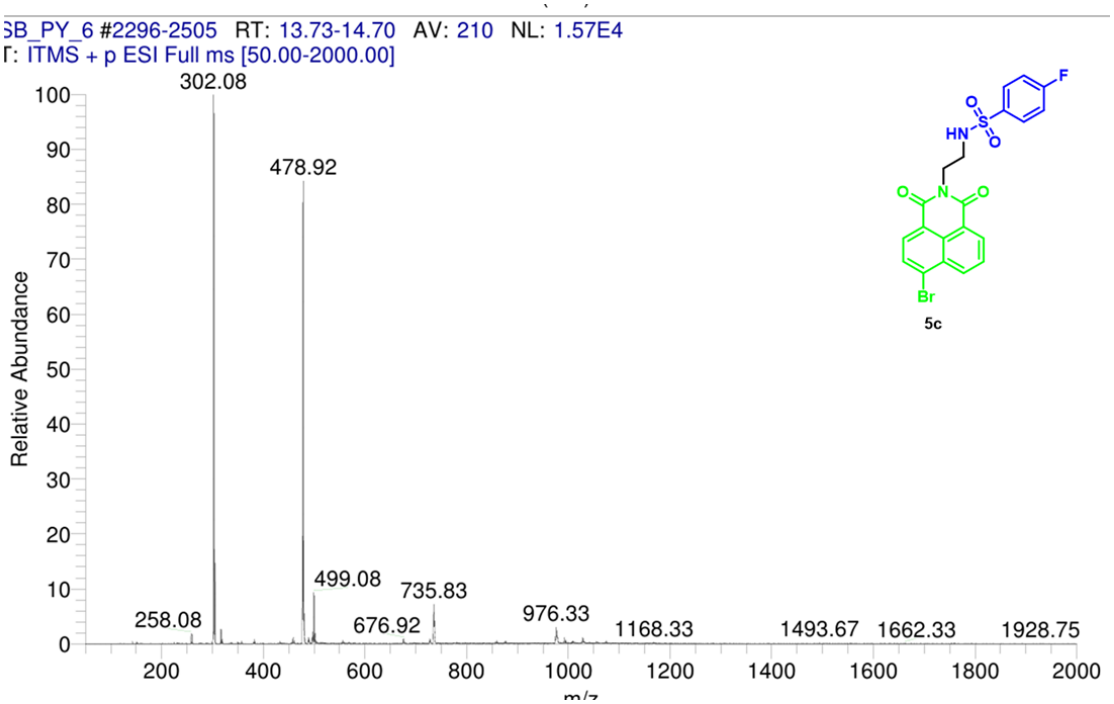


Fig. S23: HR-MS spectra of 5c.

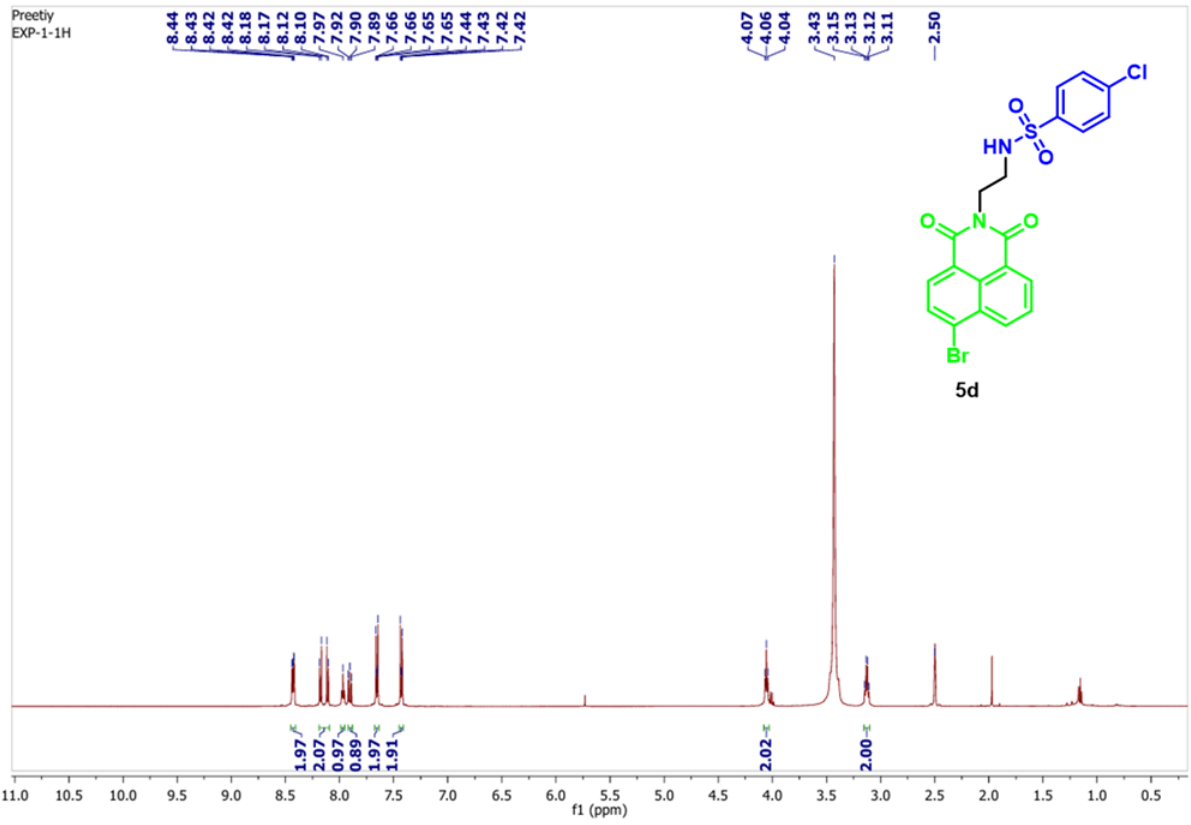


Fig. S24: ^{1}H NMR spectra of 5d.

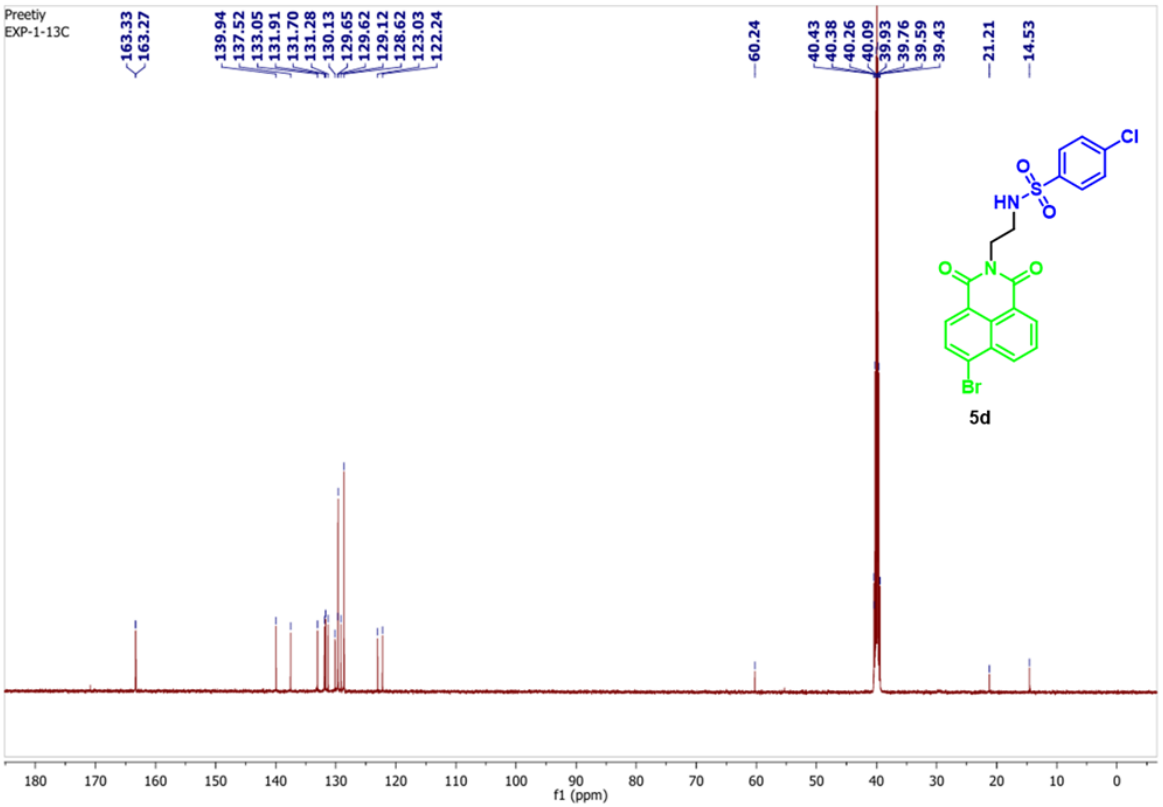


Fig. S25: ^{13}C NMR spectra of 5d.

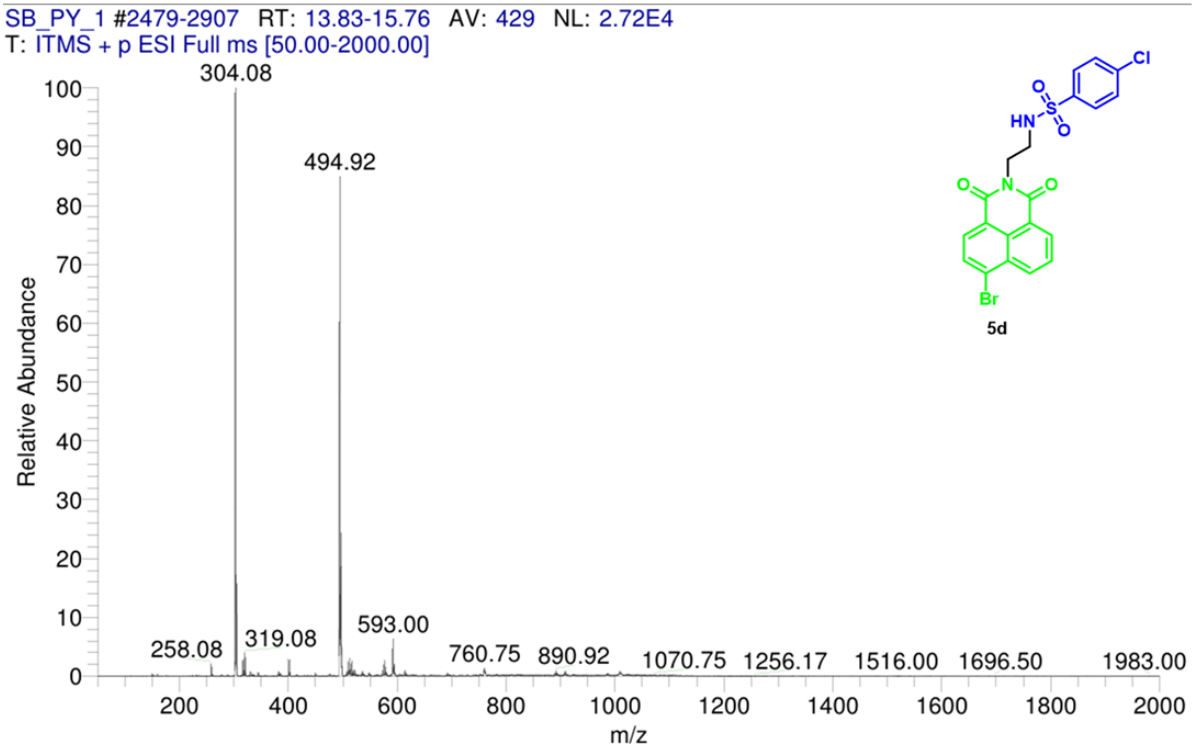


Fig. S26: HR-MS spectra of 5d.

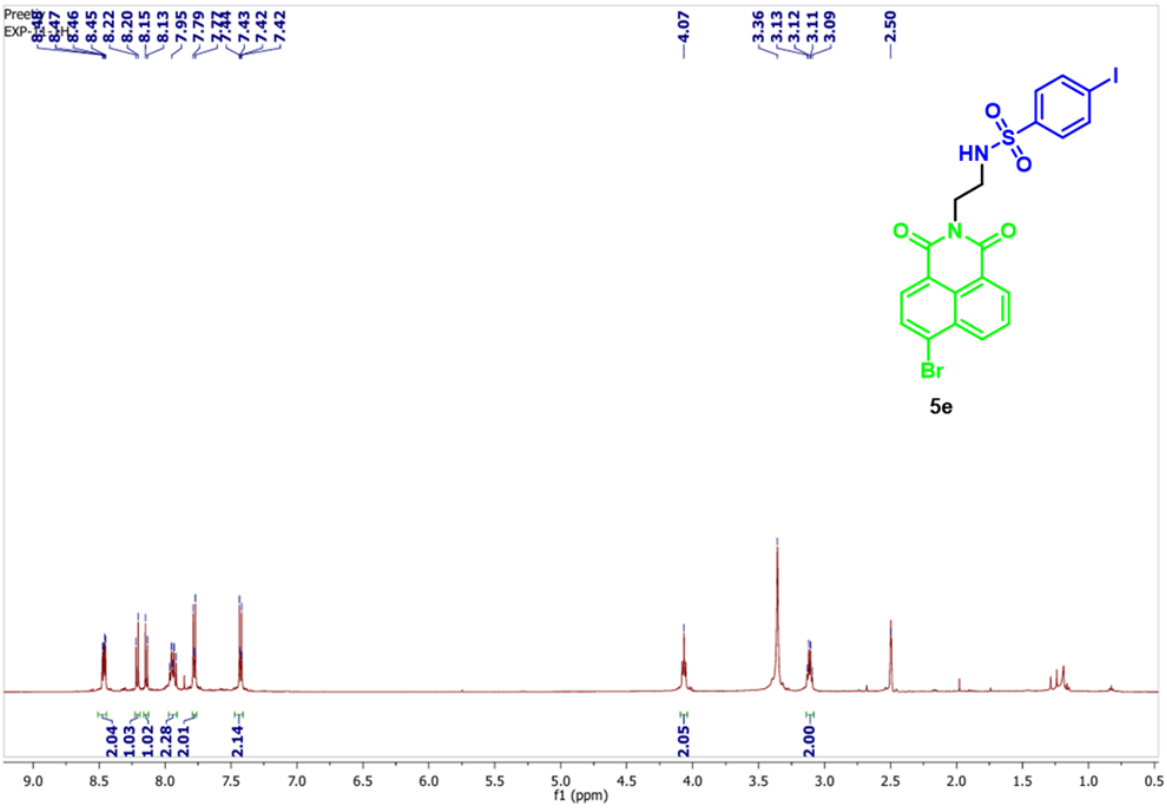


Fig. S27: ^{1}H NMR spectra of 5e.

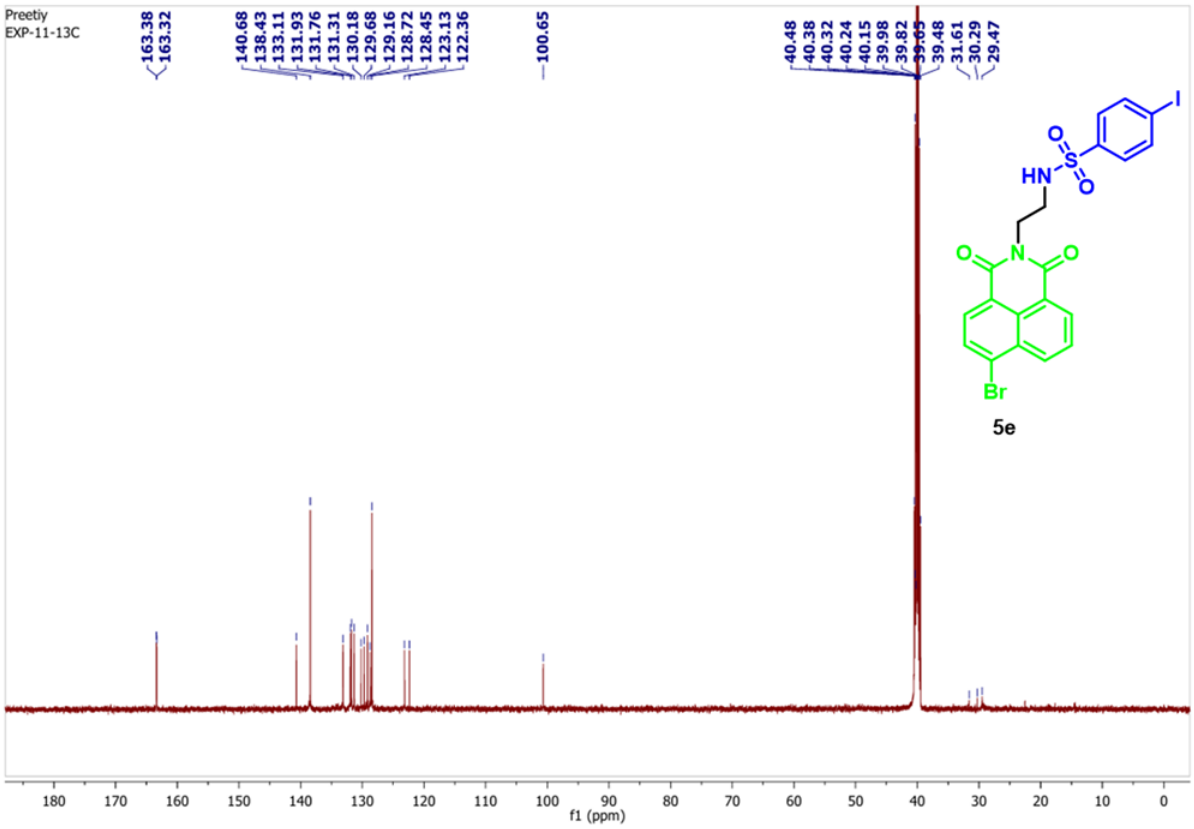


Fig. S28: ^{13}C NMR spectra of 5e.

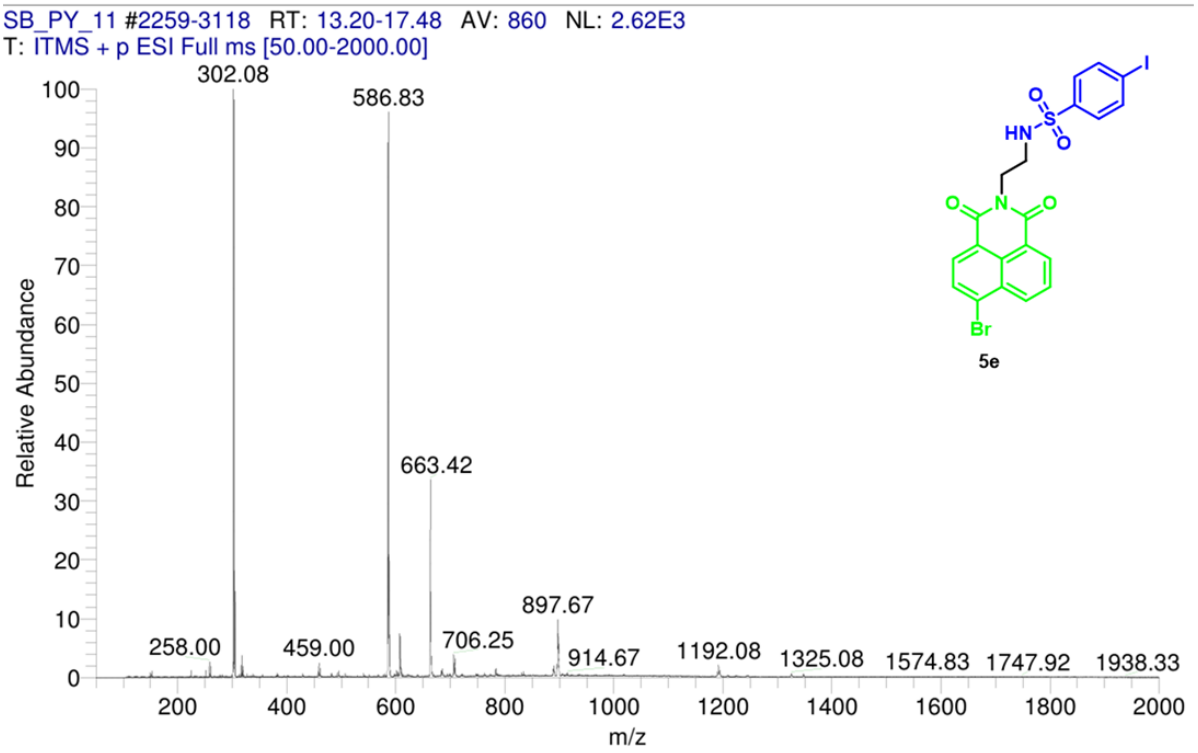


Fig. S29: HR-MS spectra of 5e.

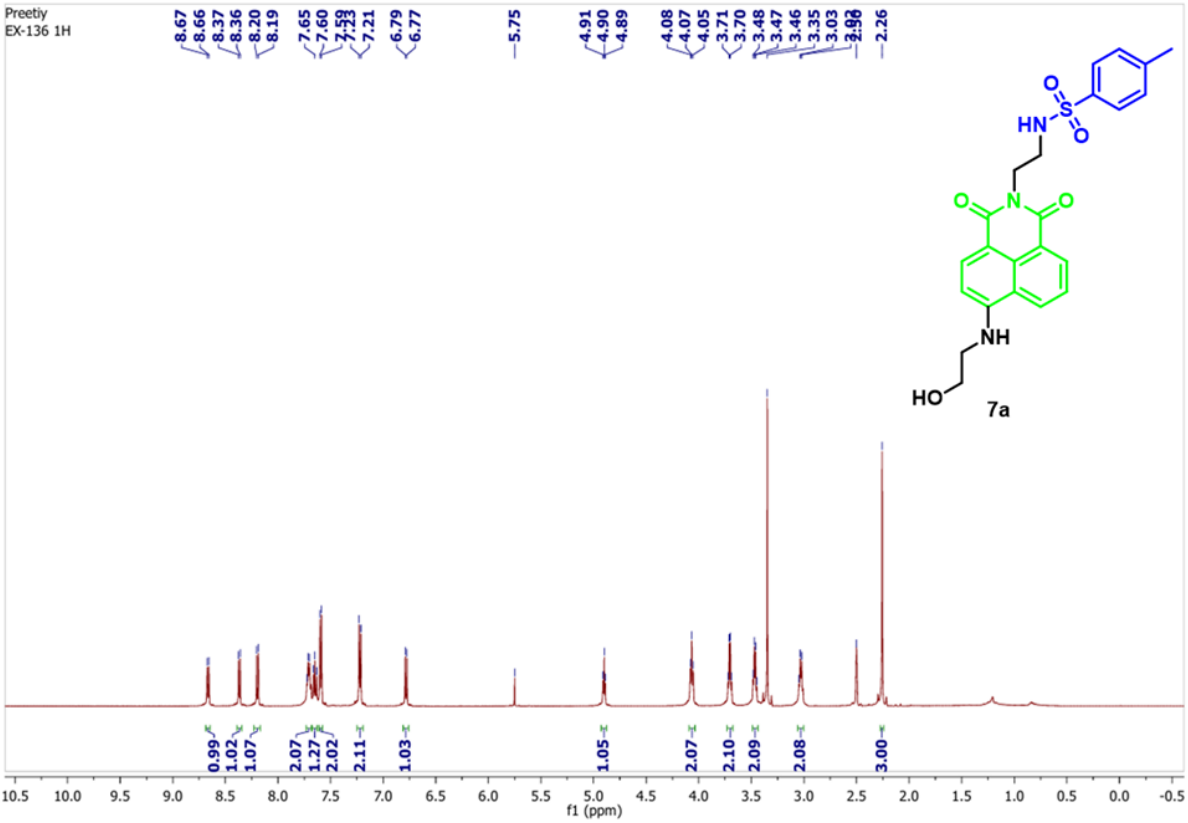


Fig. S30: ^{1}H NMR spectra of 7a.

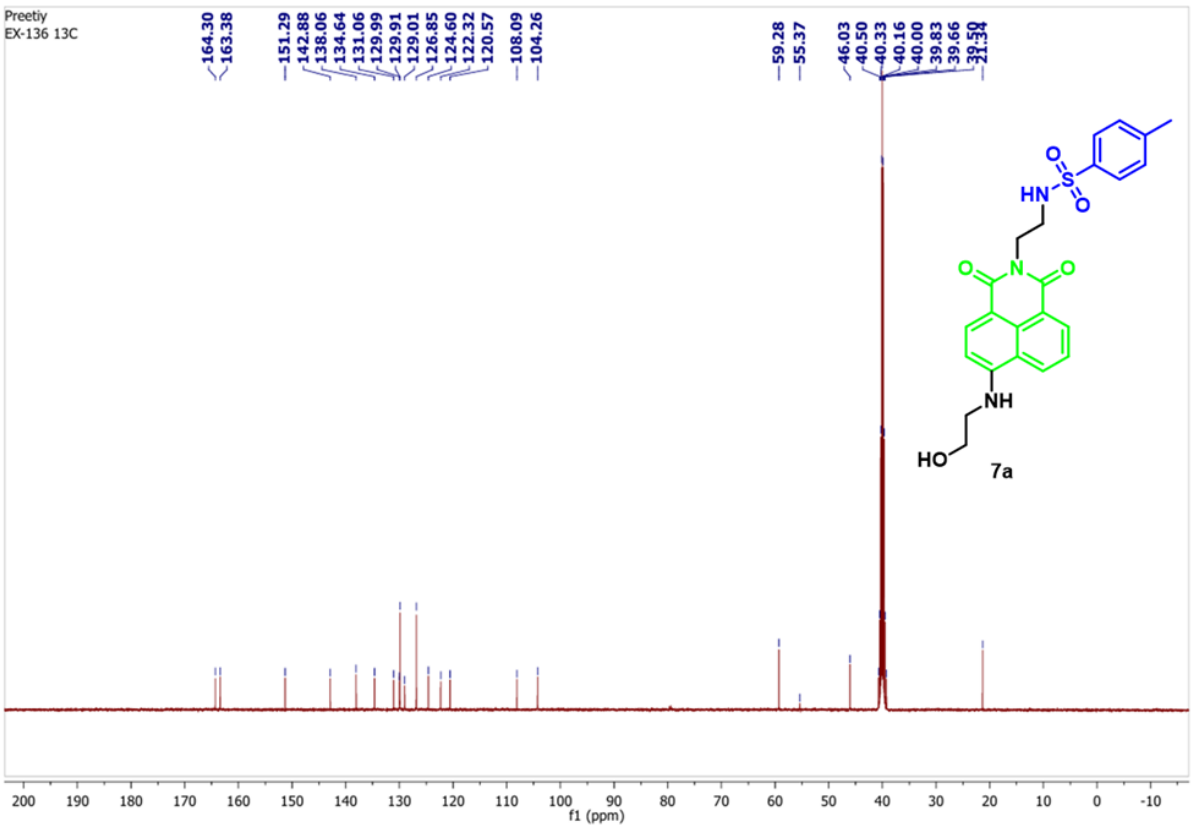


Fig. S31: ^{13}C NMR spectra of 7a.

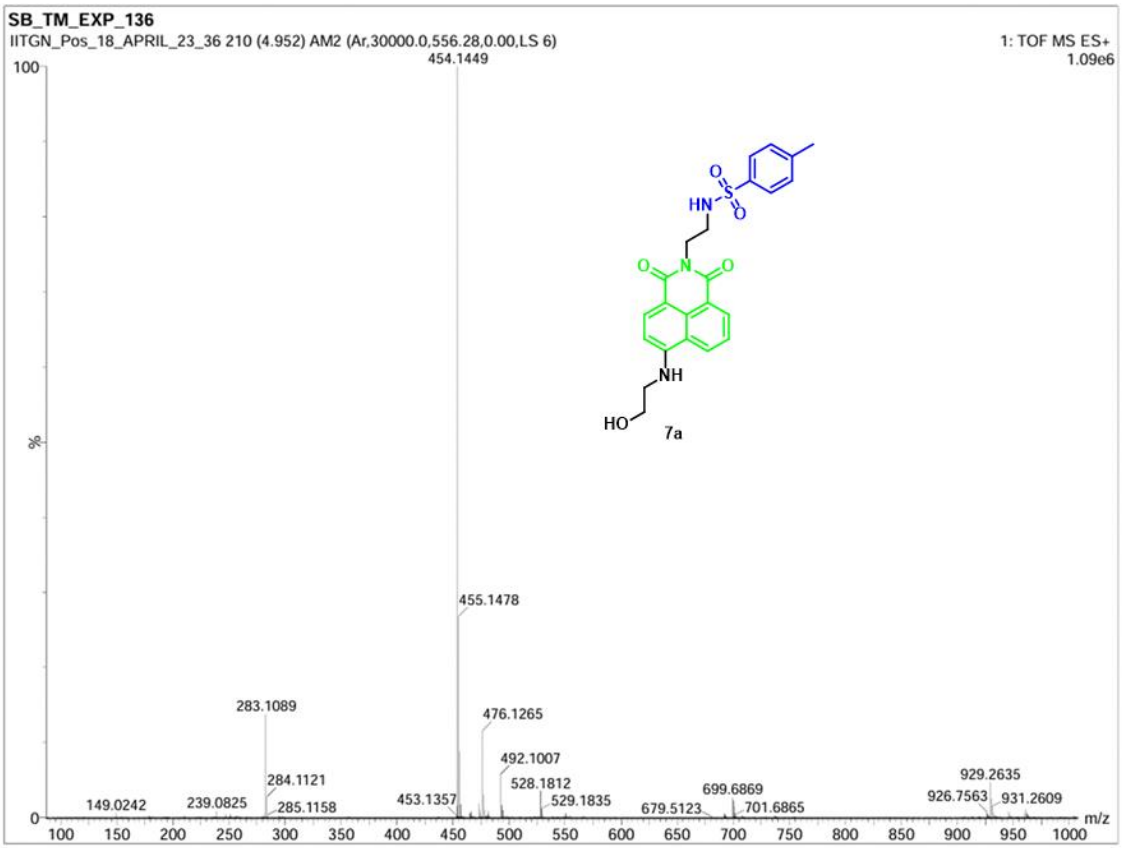


Fig. S32: HR-MS spectra of 7a.

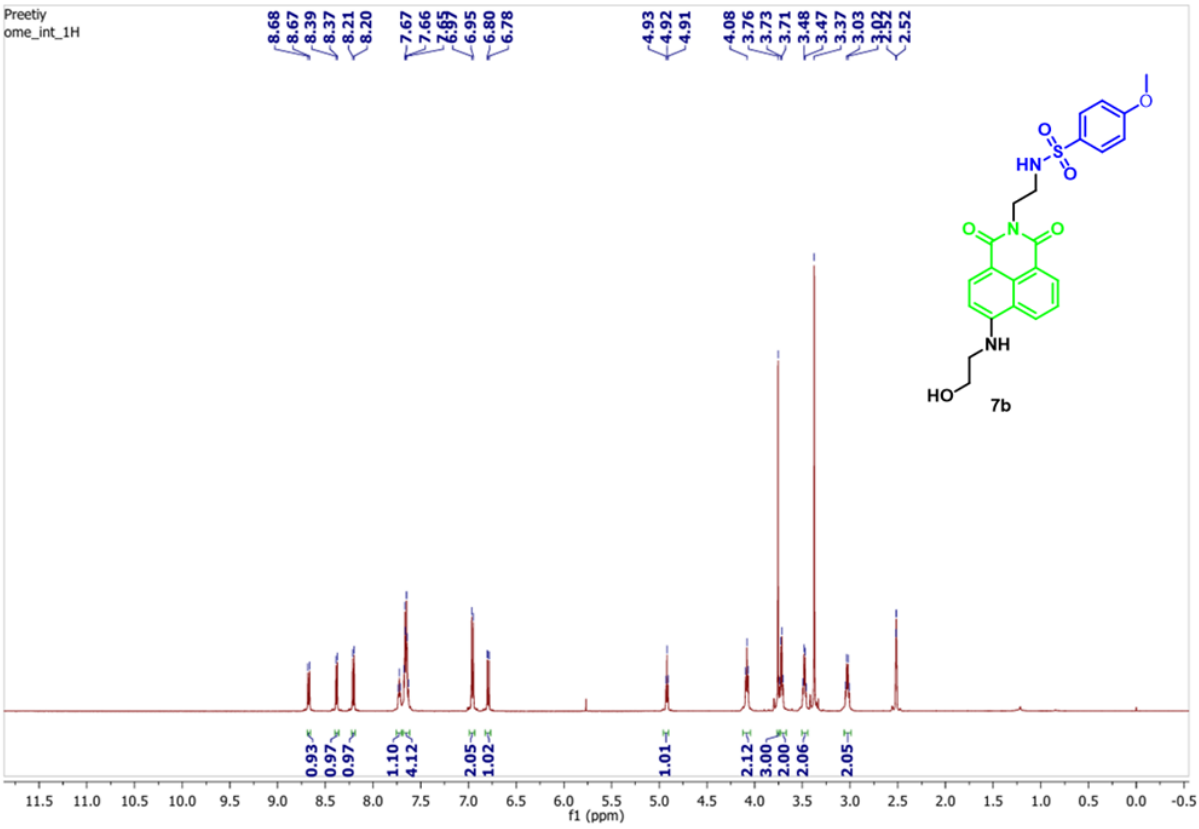


Fig. S33: ^{1}H NMR spectra of 7b.

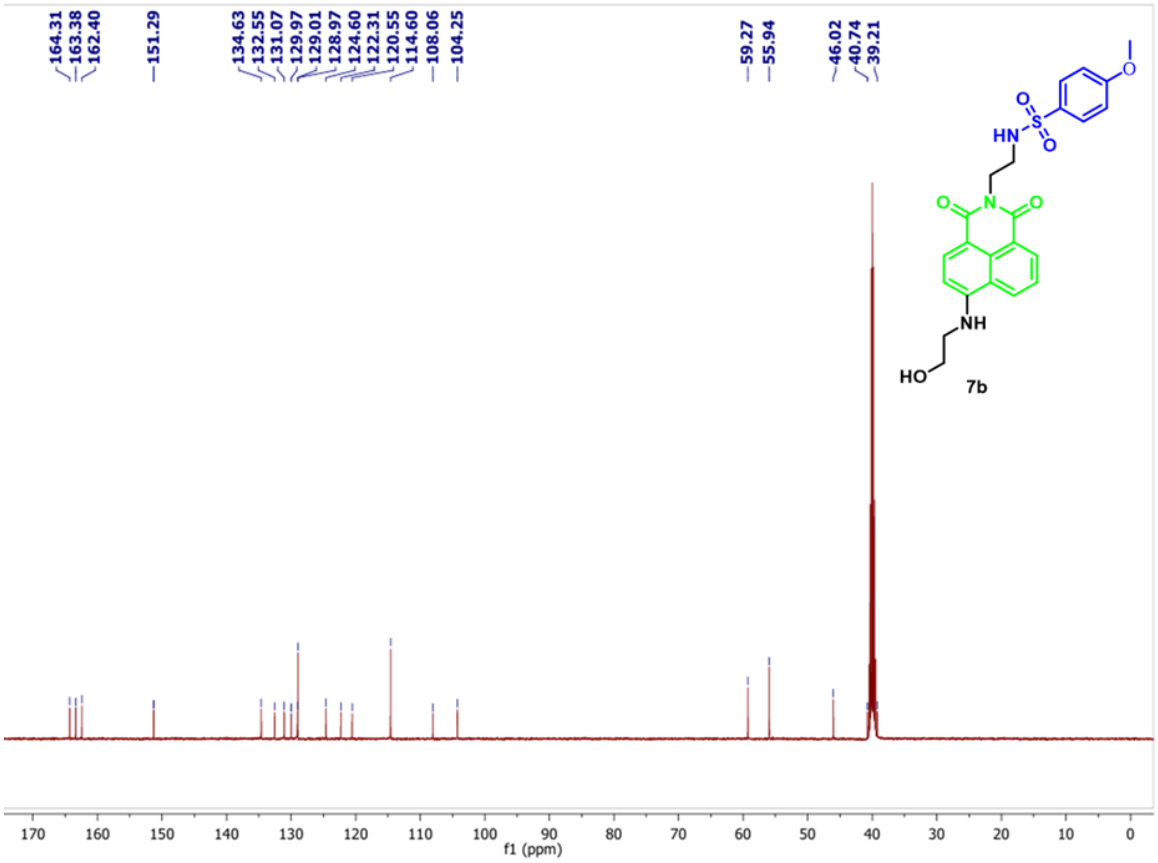


Fig. S34: ^{13}C NMR spectra of 7b.

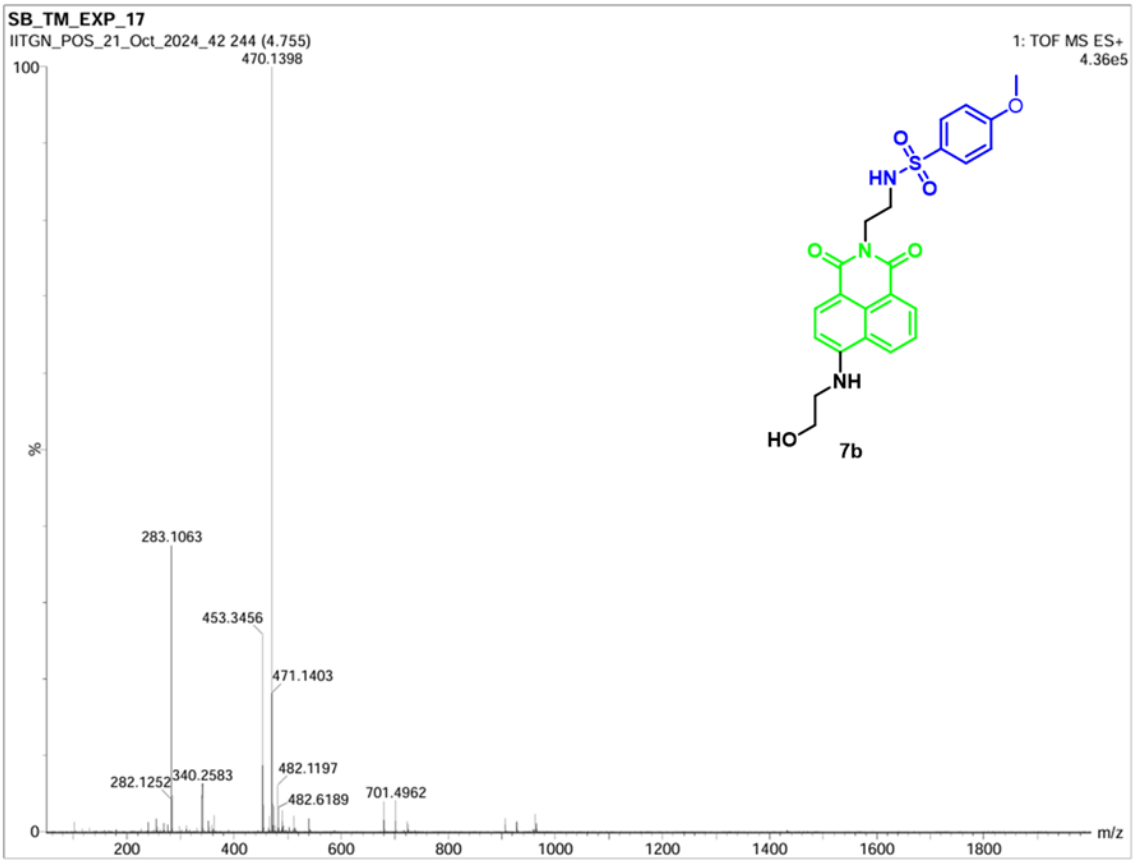


Fig. S35: HR-MS spectra if 7b.

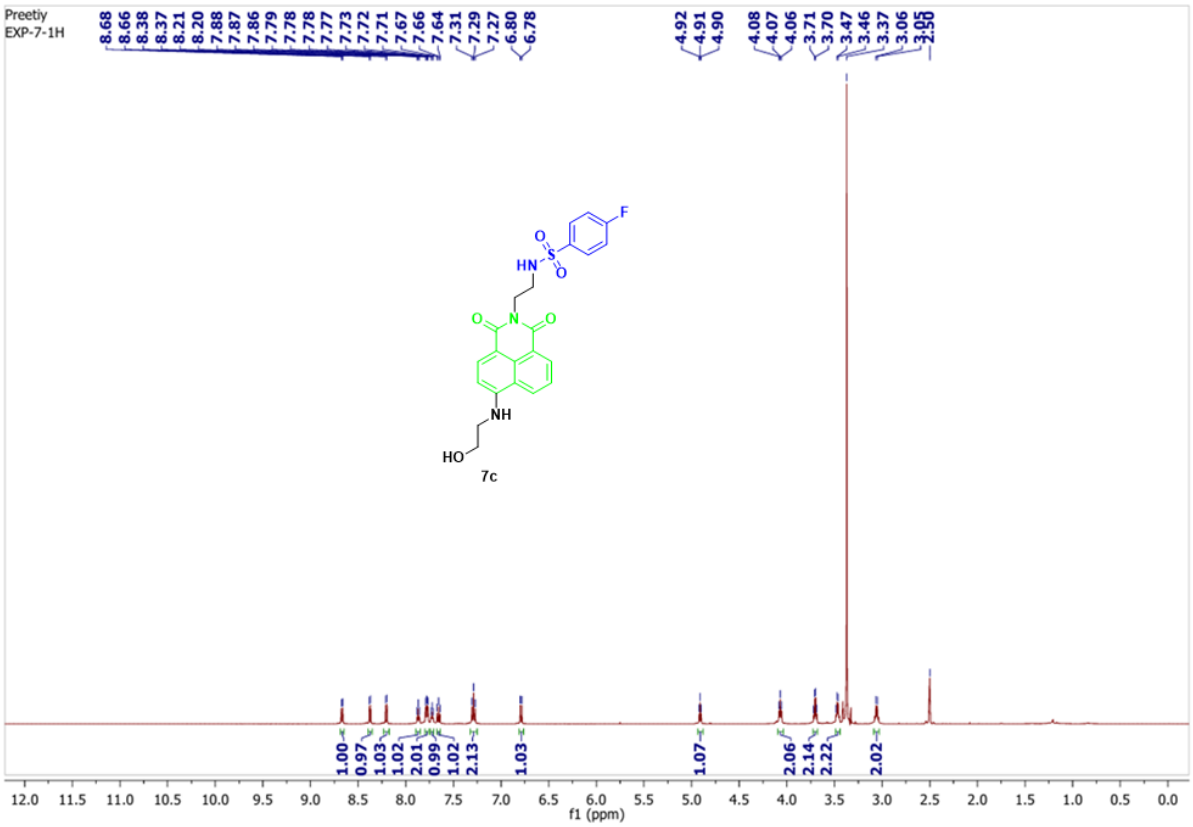


Fig. S36: ^{1}H NMR spectra of 7c.

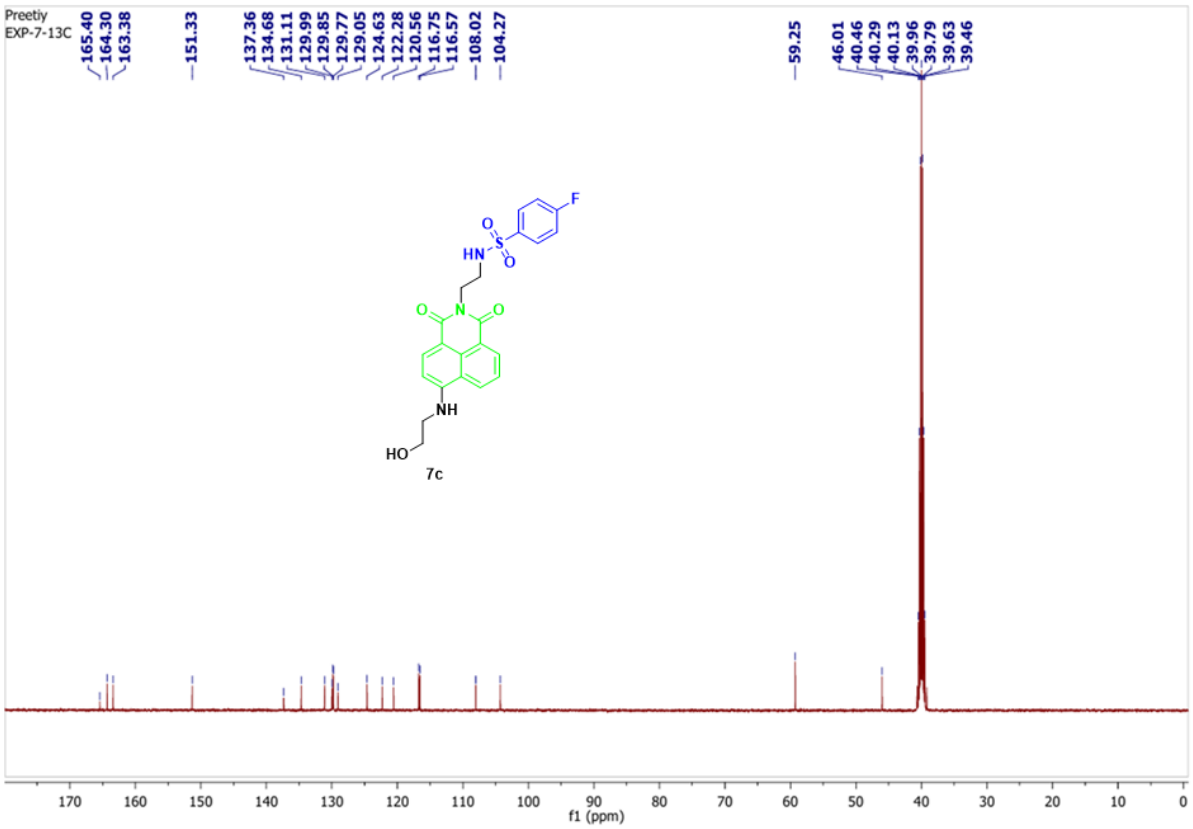


Fig. S37: ^{13}C NMR spectra of 7c.

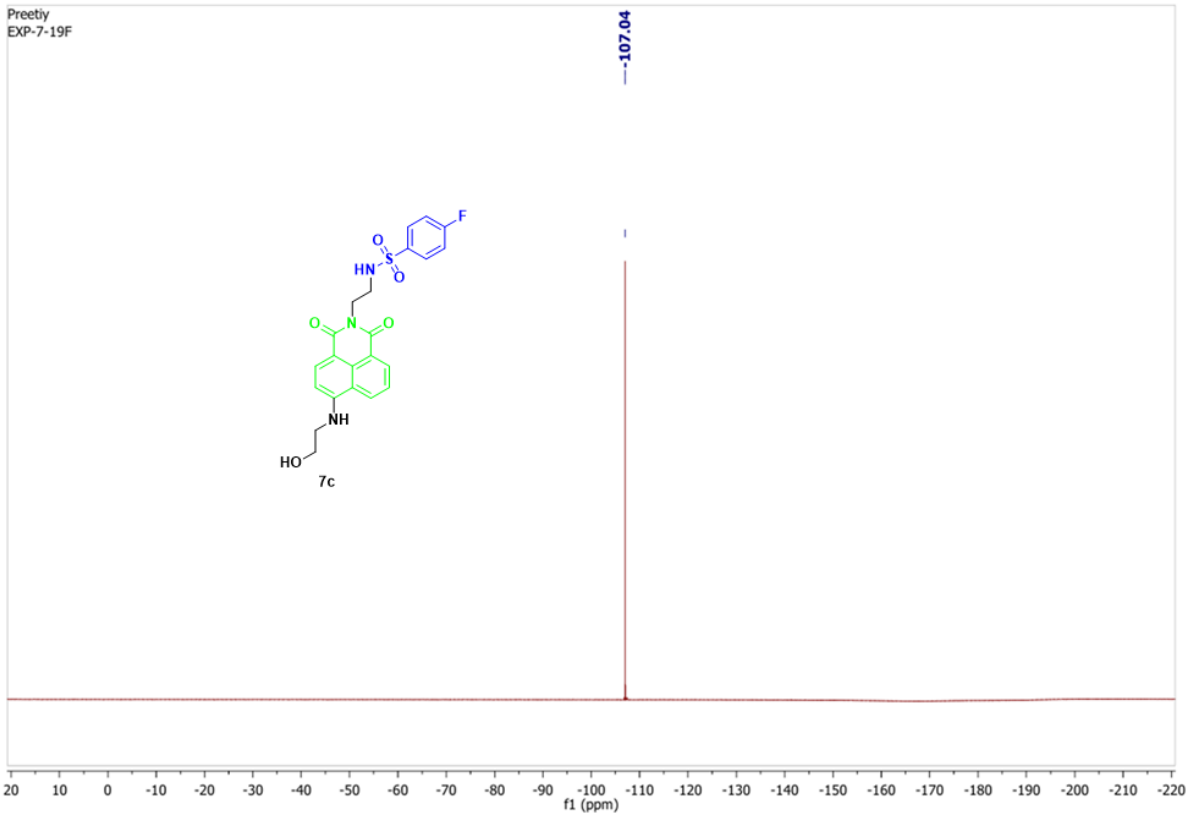


Fig. S38: ^{19}F NMR spectra of 7c.

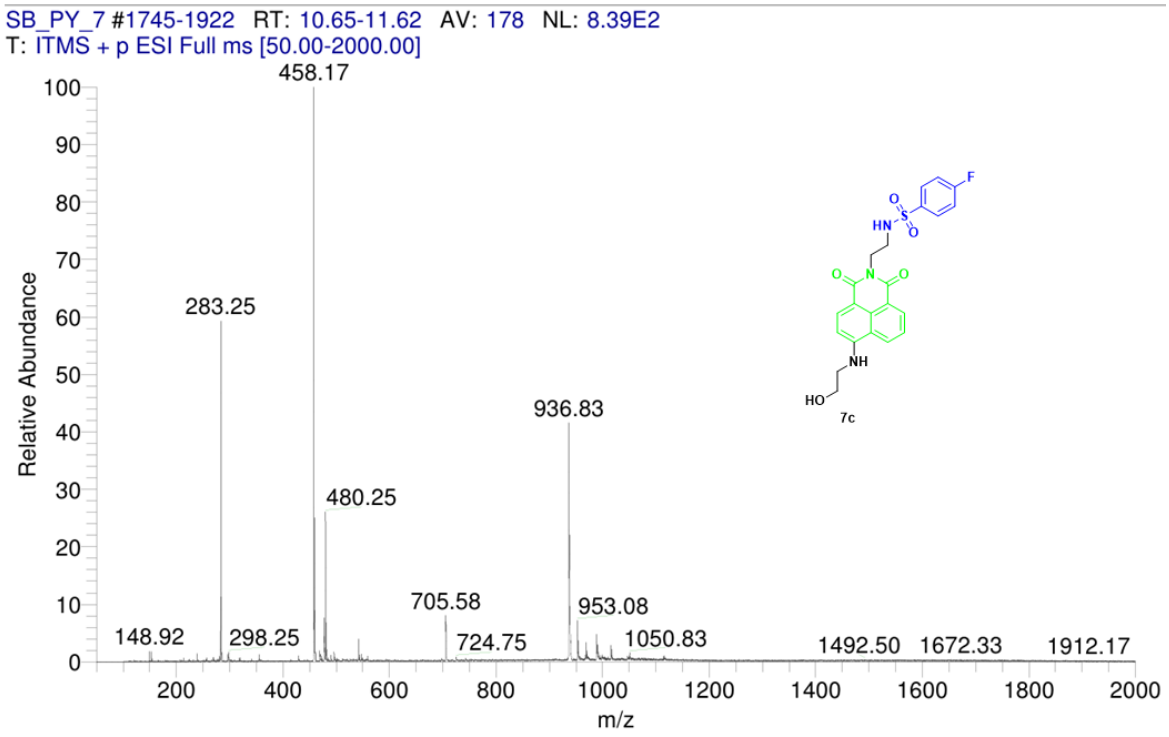


Fig. S39: HR-MS spectra of 7c.

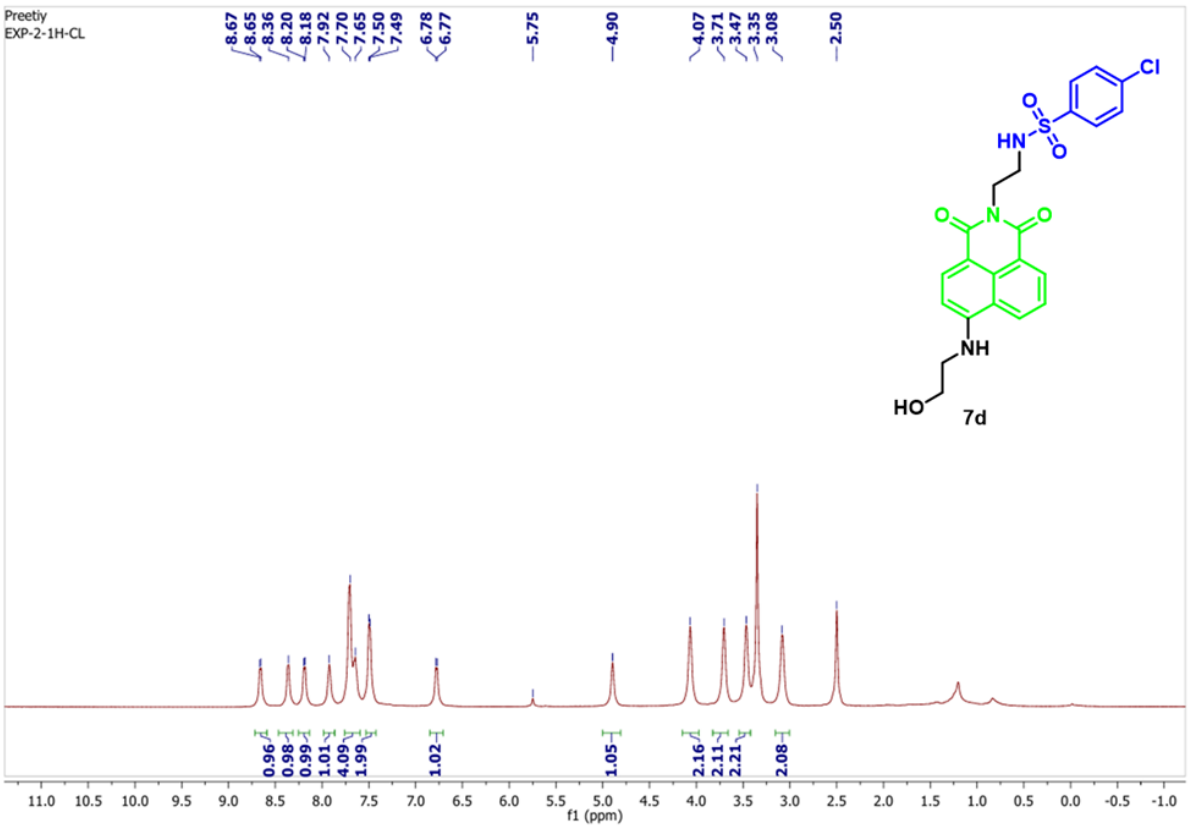


Fig. S40: ^{1}H NMR spectra of 7d.

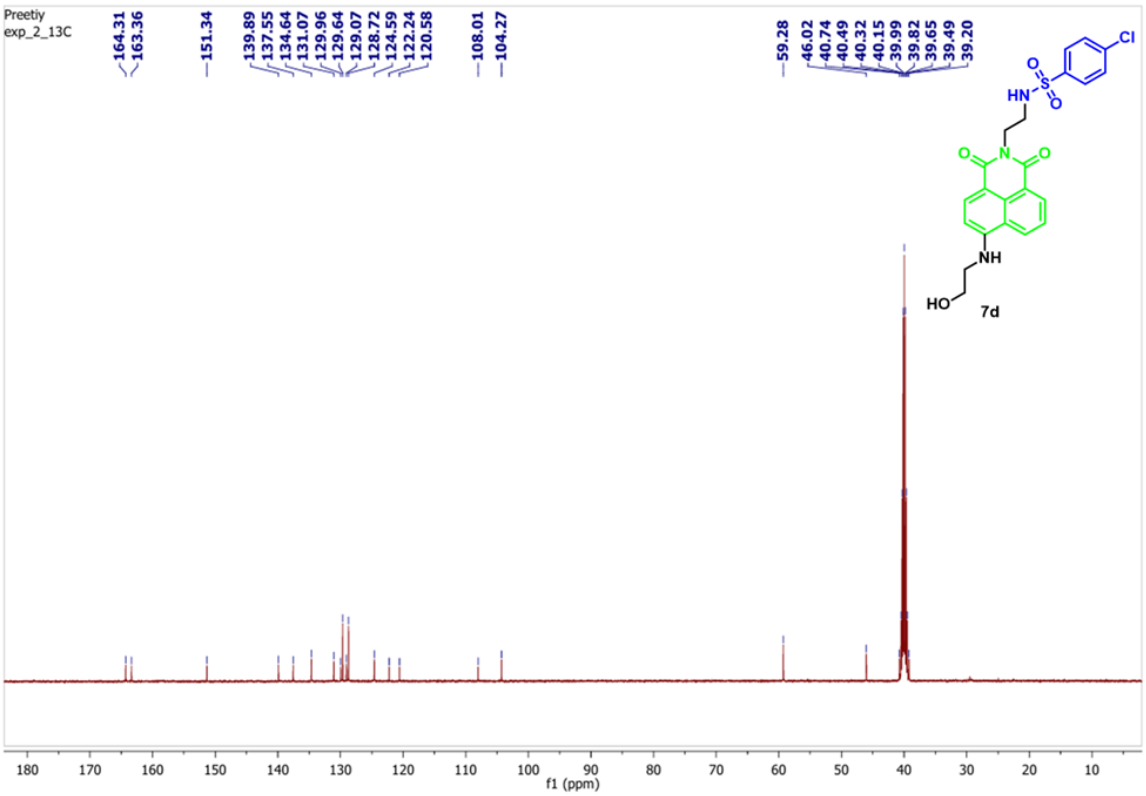


Fig. S41: ^{13}C NMR spectra of 7d.

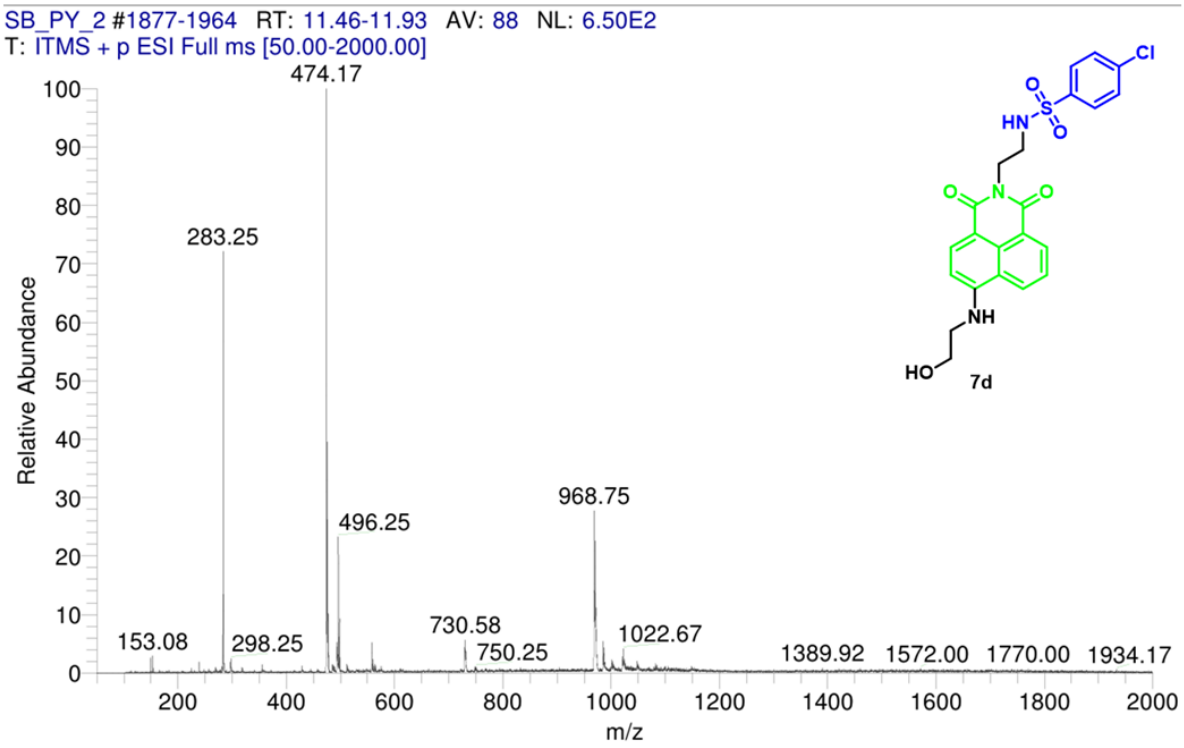


Fig. S42: HR-MS spectra of 7d.

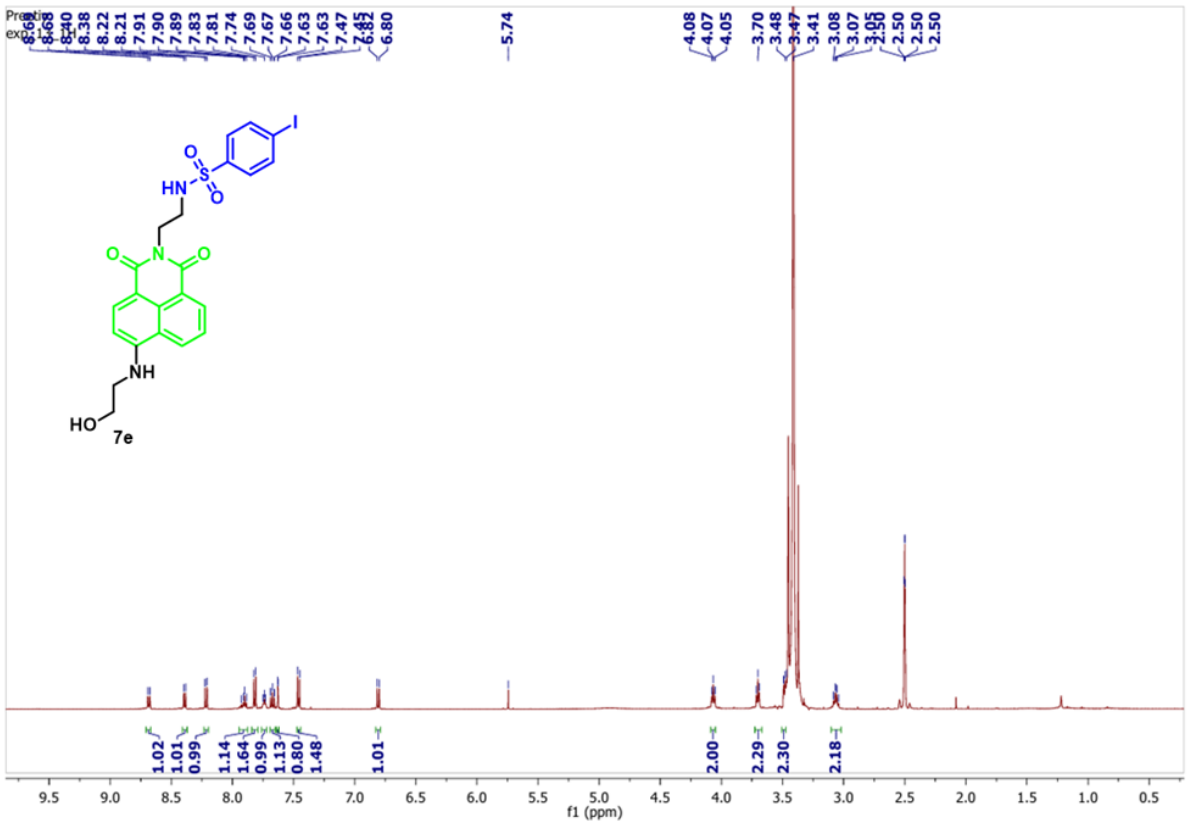


Fig. S43: ^{1}H NMR spectra of 7e.

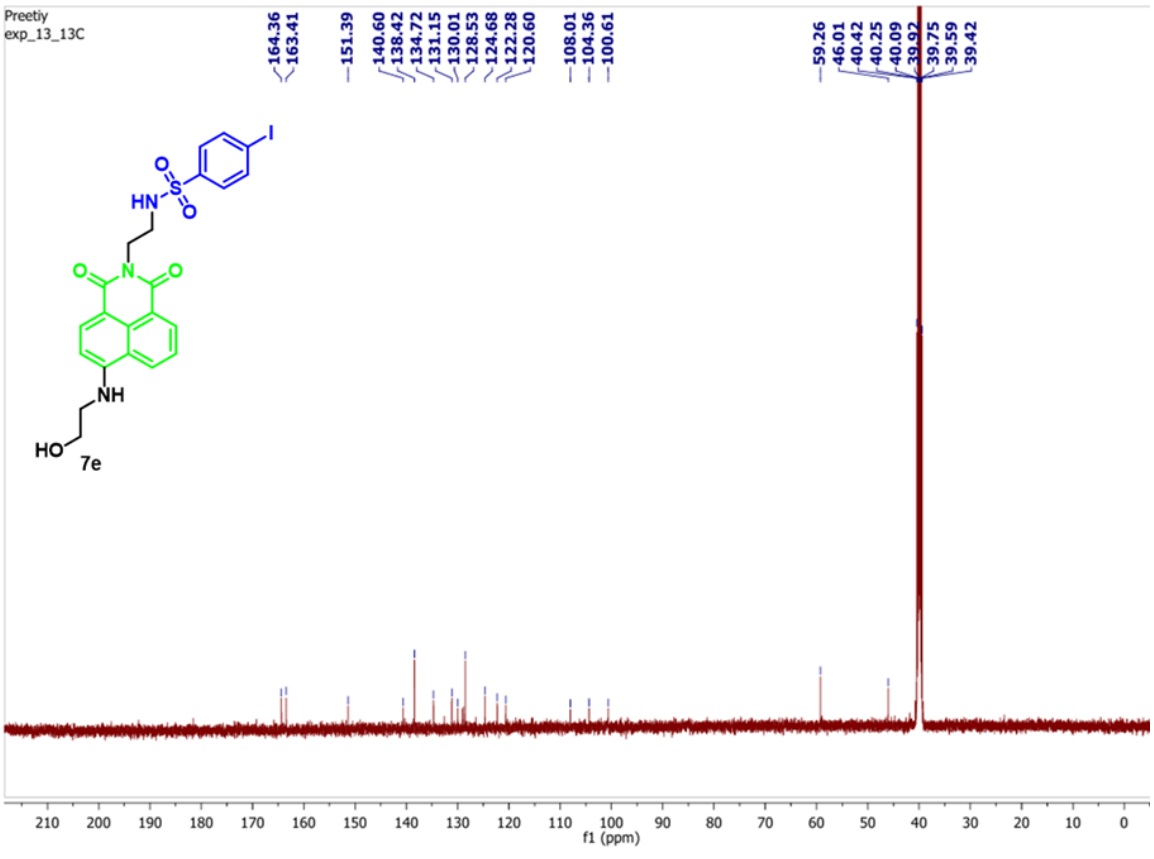


Fig. S44: ^{13}C NMR spectra of 7e.

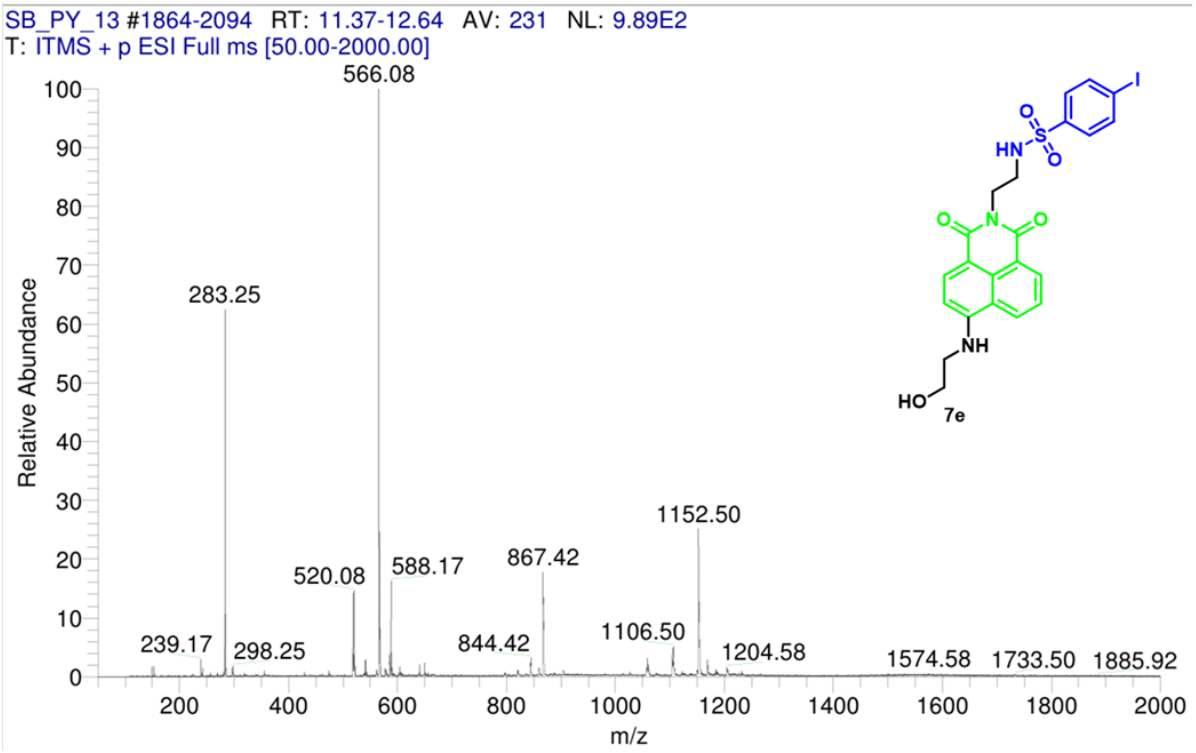


Fig. S45: HR-MS spectra of 7e.

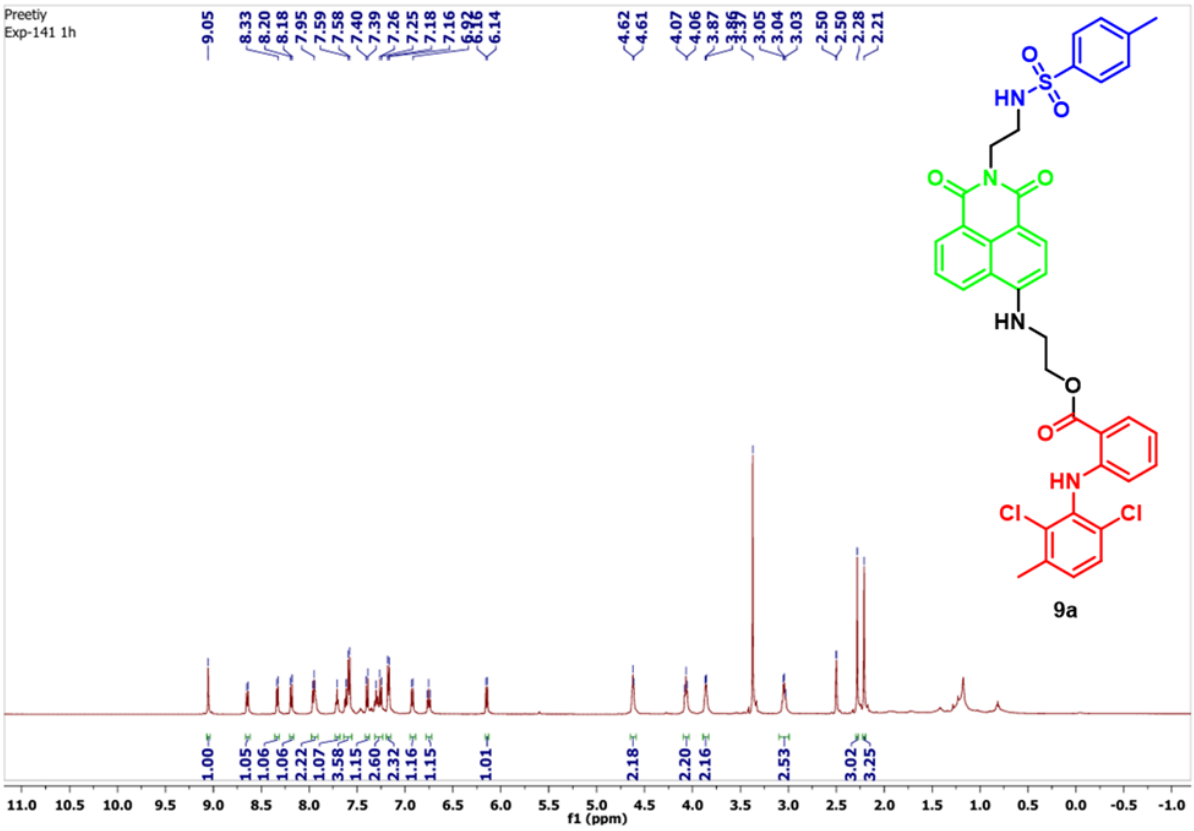


Fig. S46: ^{1}H NMR spectra of 9a.

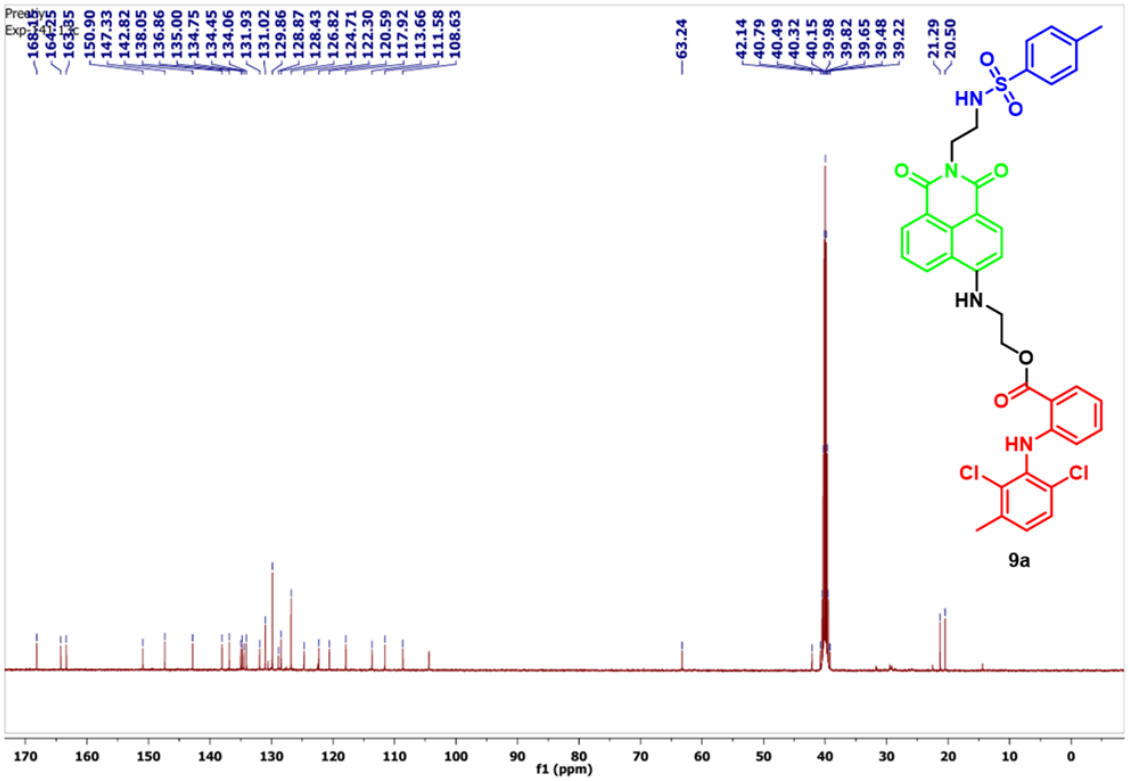


Fig. S47: ^{13}C NMR spectra of 9a.

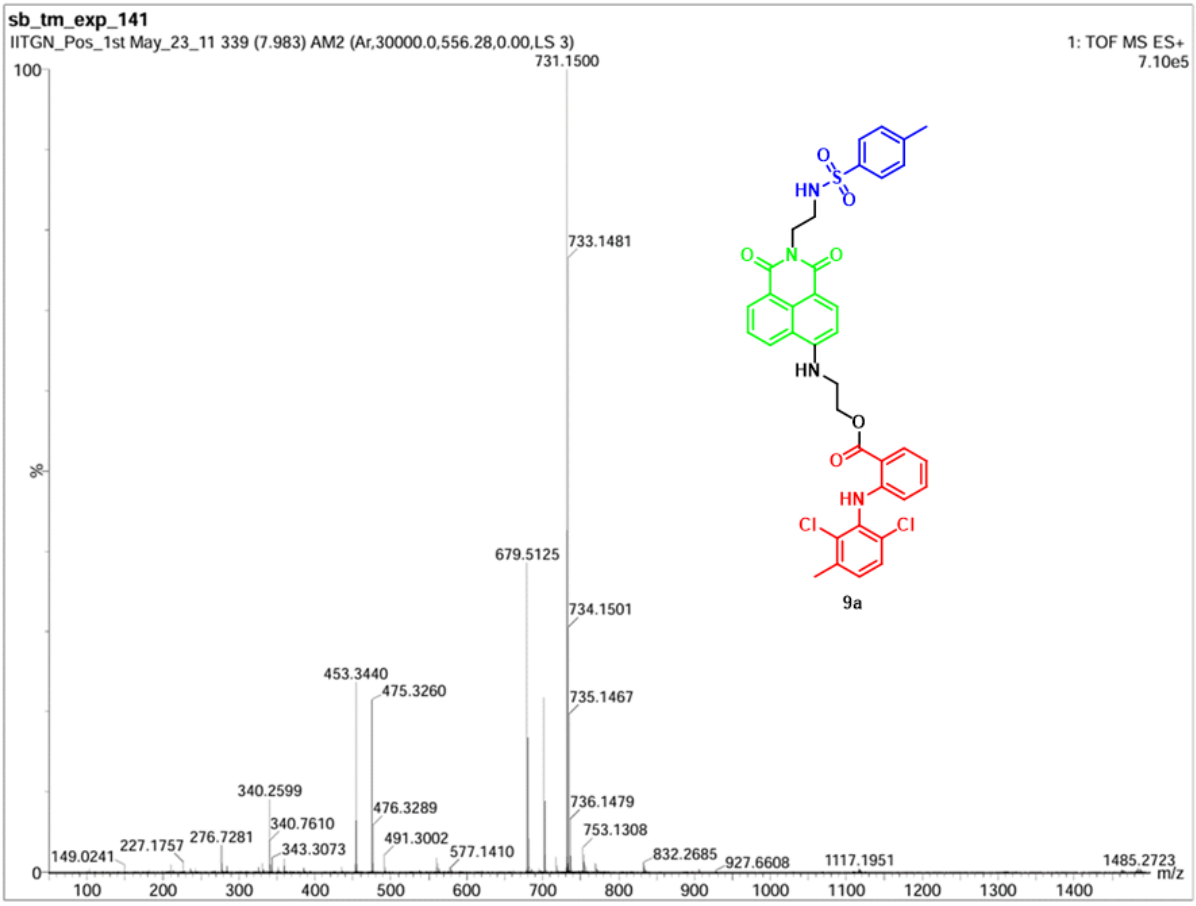


Fig. S48: HR-MS spectra of 9a.

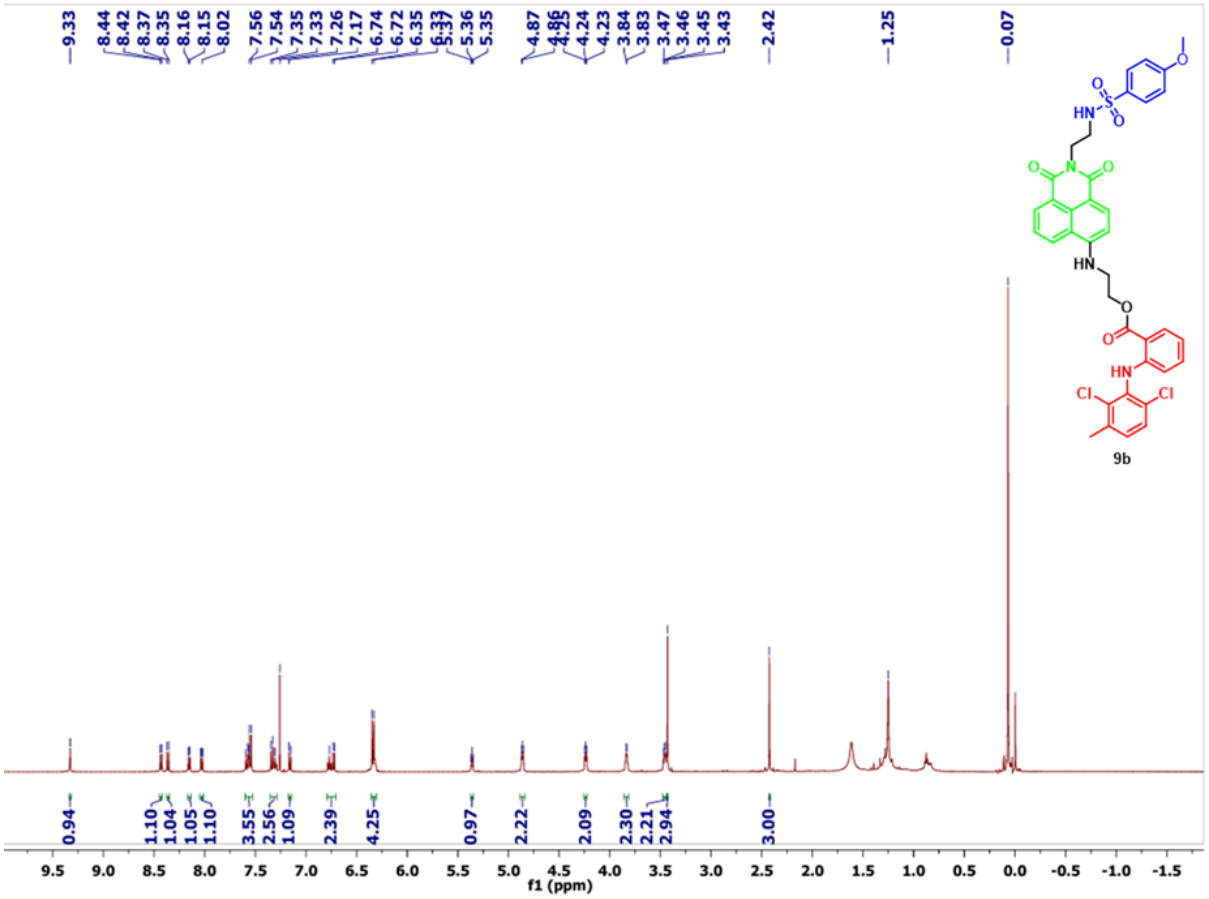


Fig. S49: ^{1}H NMR spectra of 9b.

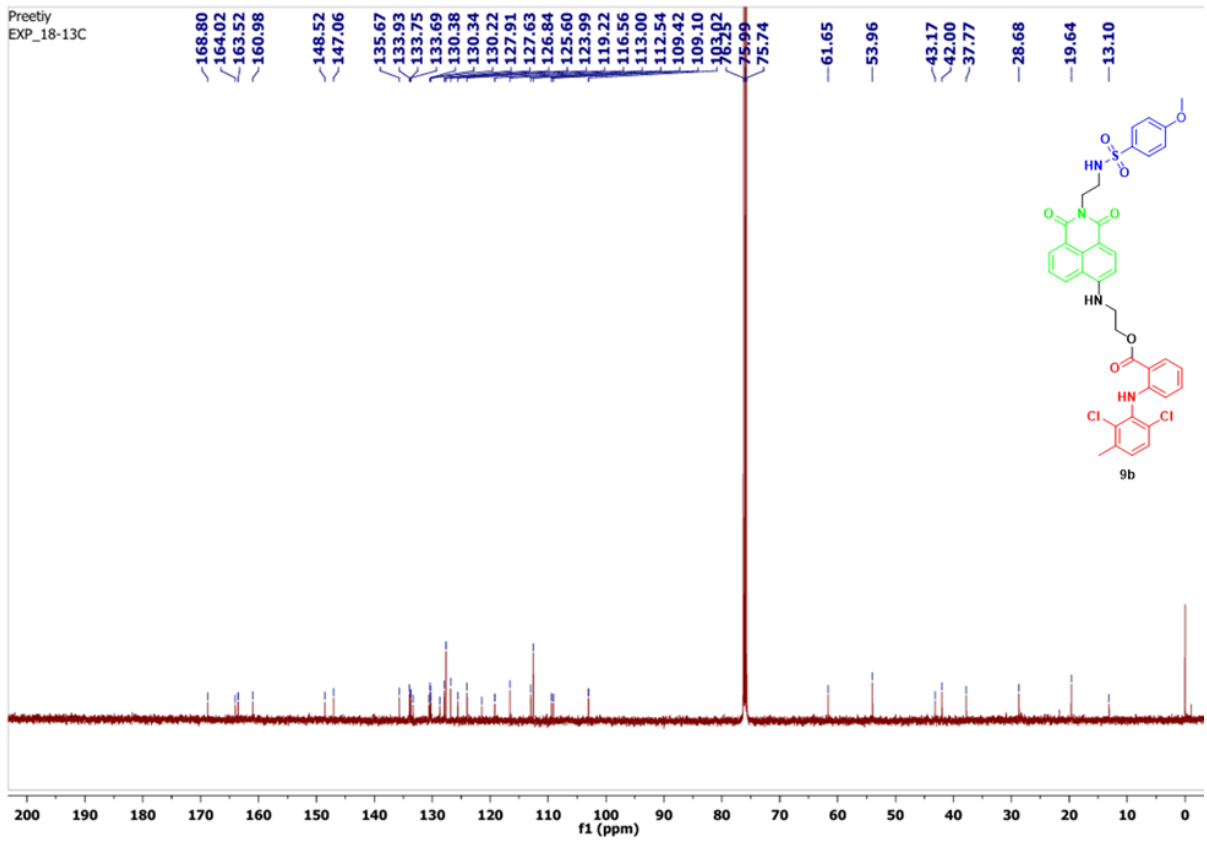


Fig. S50: ^{13}C NMR spectra of 9b.

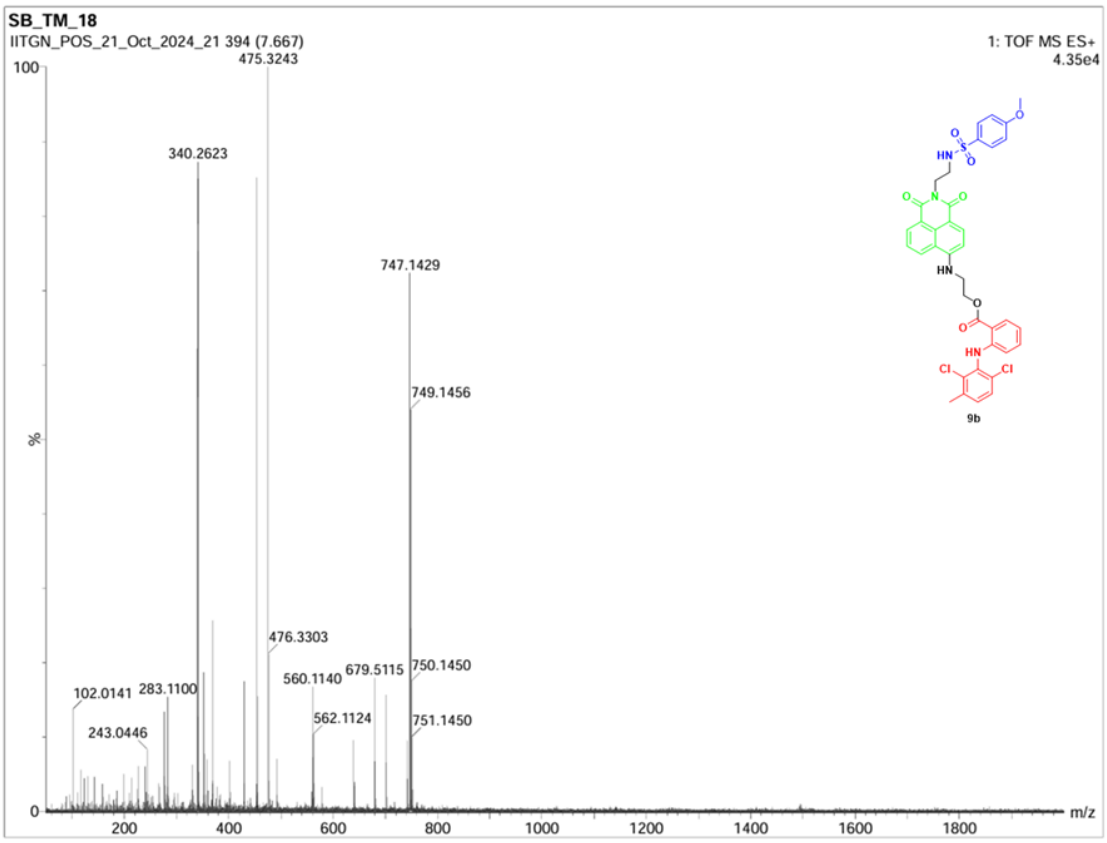


Fig. S51: HR-MS spectra of 9b.

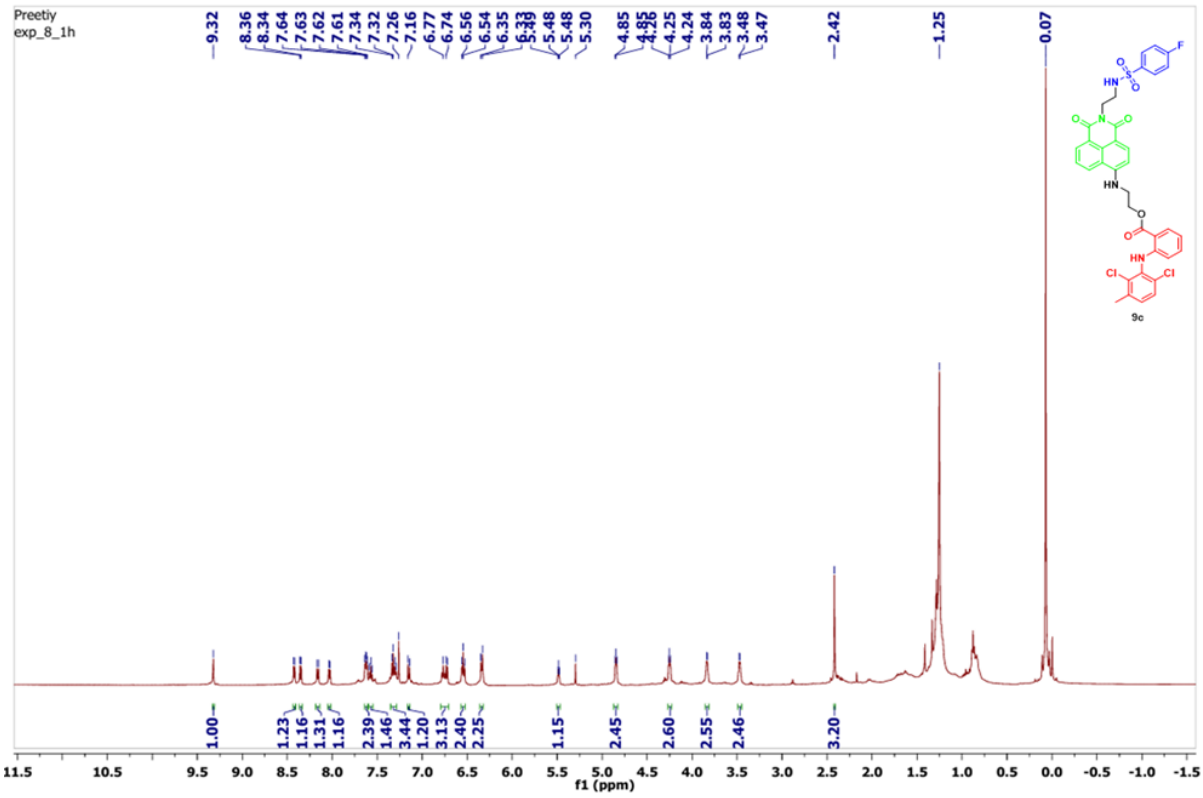


Fig. S52: ^{1}H NMR spectra of 9c.

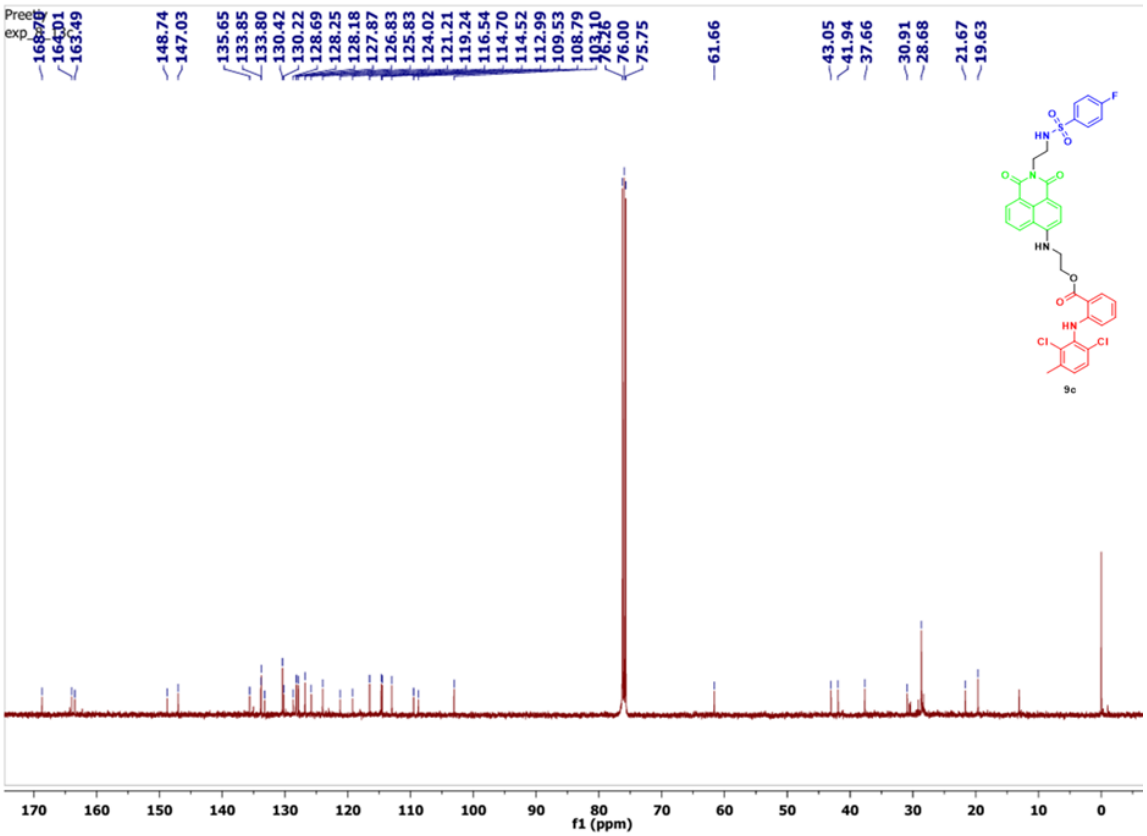


Fig. S53: ^{13}C NMR spectra of 9c.

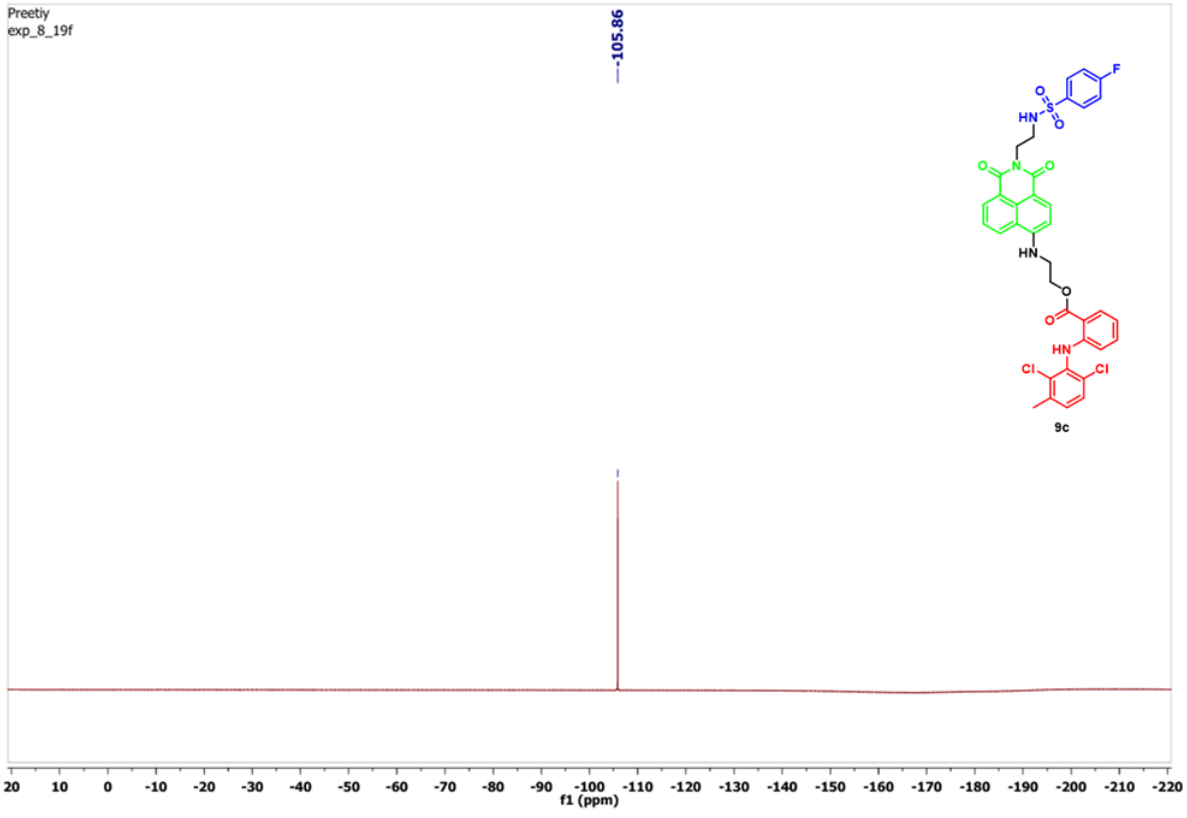


Fig. S54: ^{19}F NMR spectra of 9c.

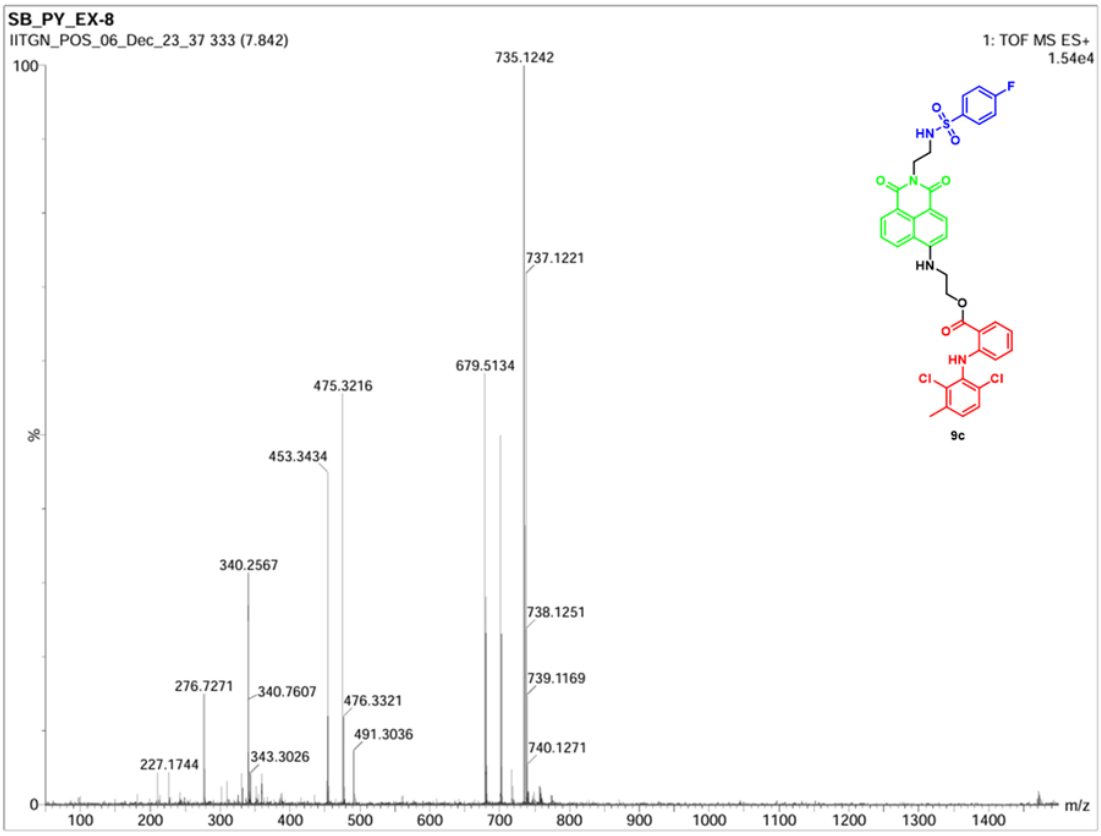


Fig. S55: HR-MS spectra of 9c.

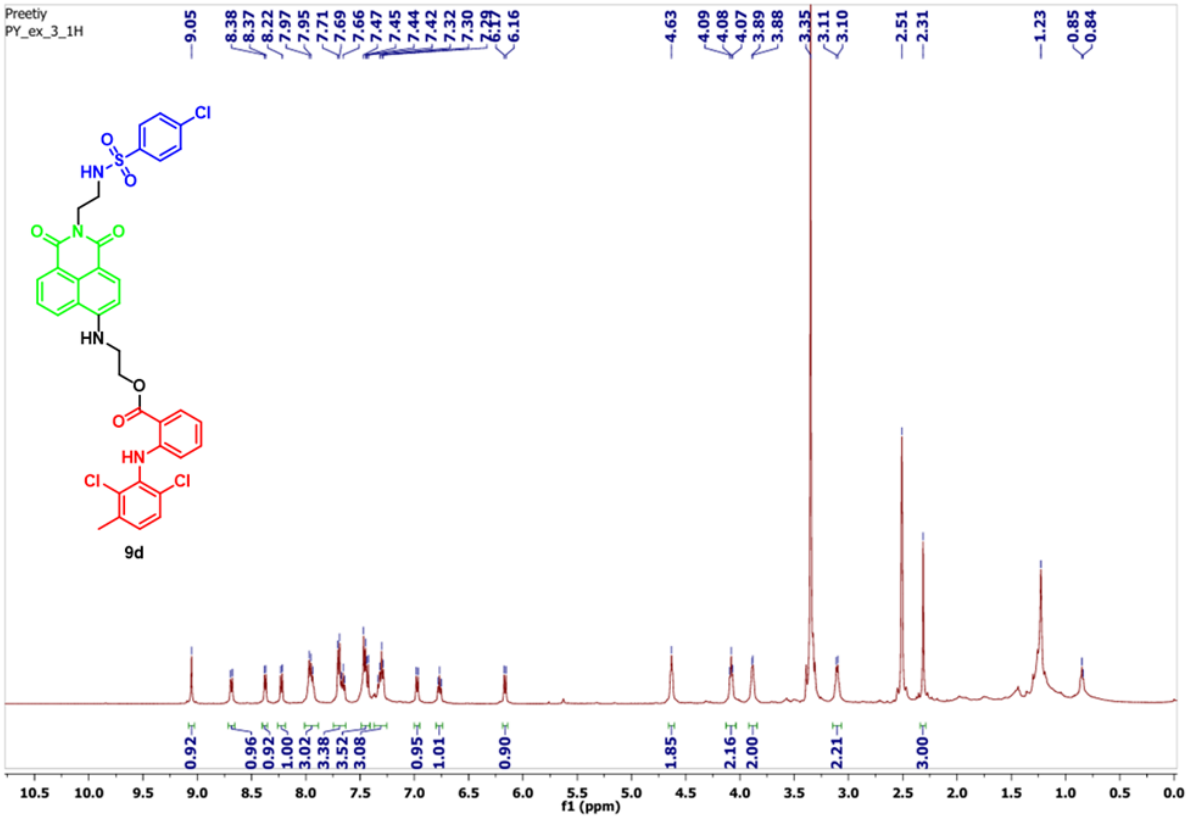


Fig. S56: ^{1}H NMR spectra of 9d.

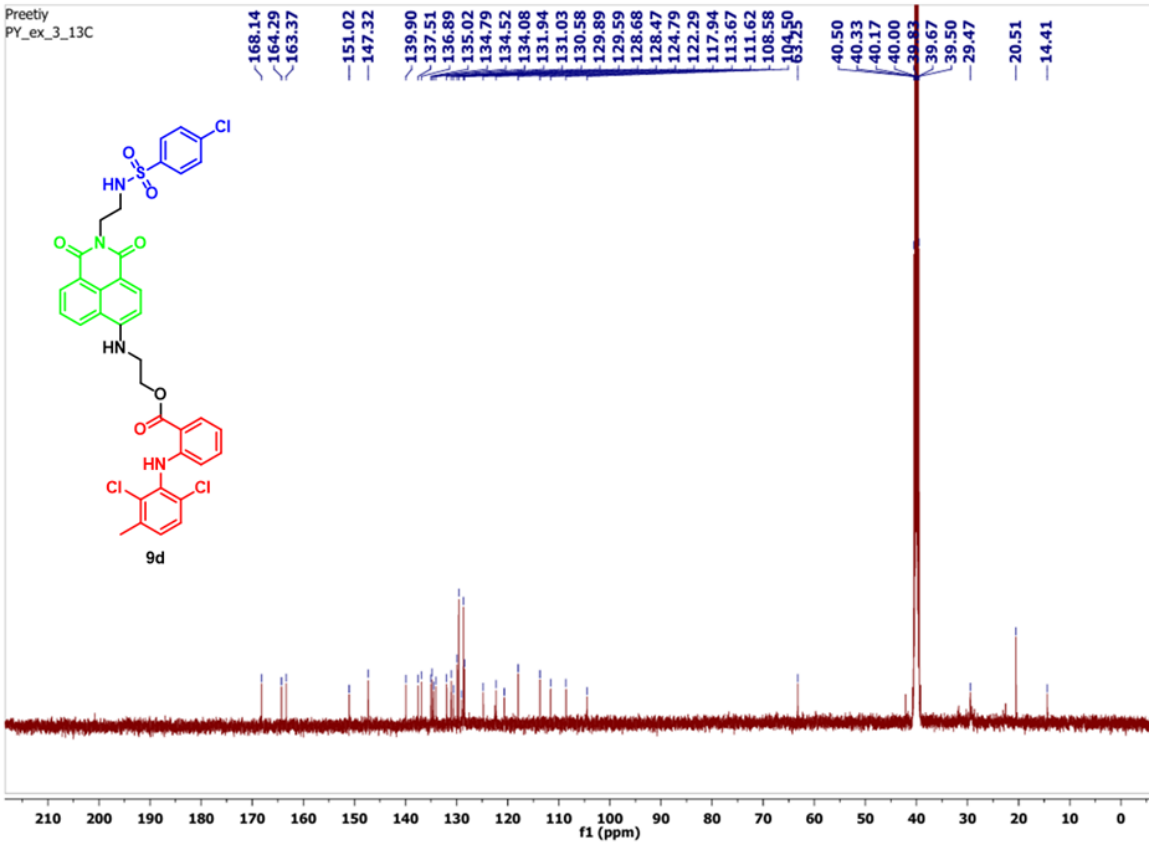


Fig. S57: ^{13}C NMR spectra of 9d.

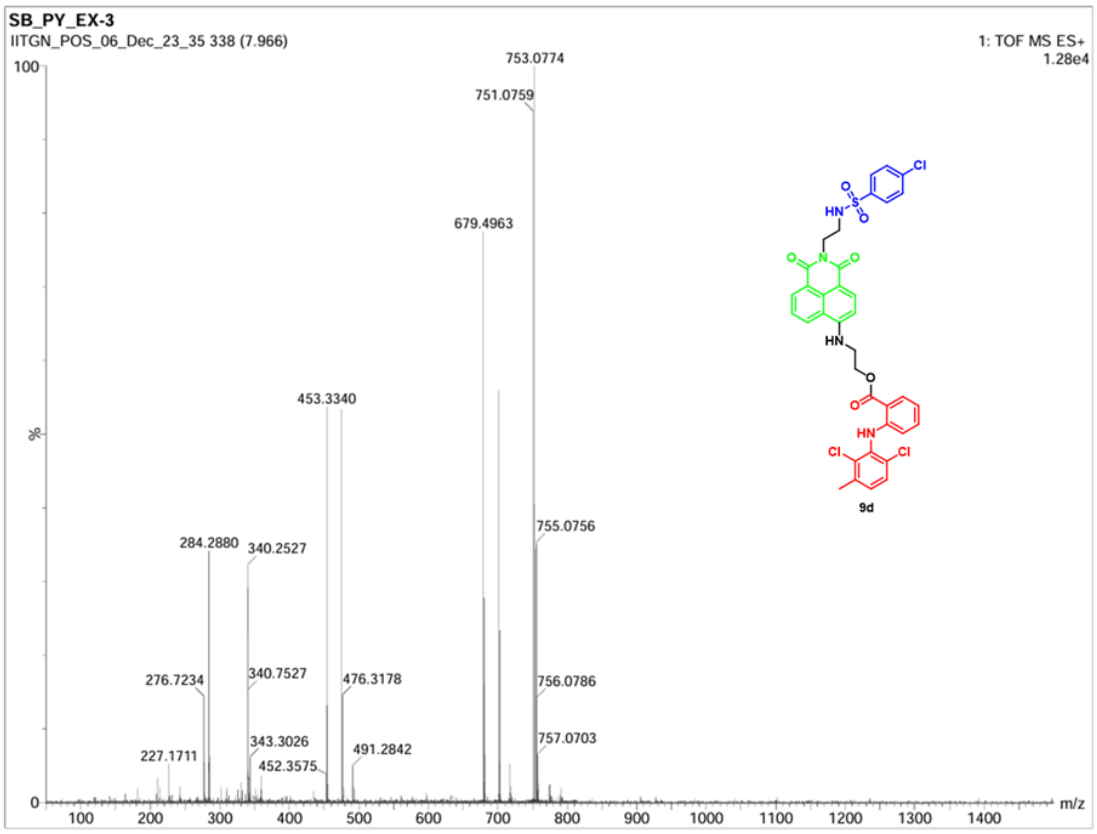


Fig. S58: HR-MS spectra of 9d.

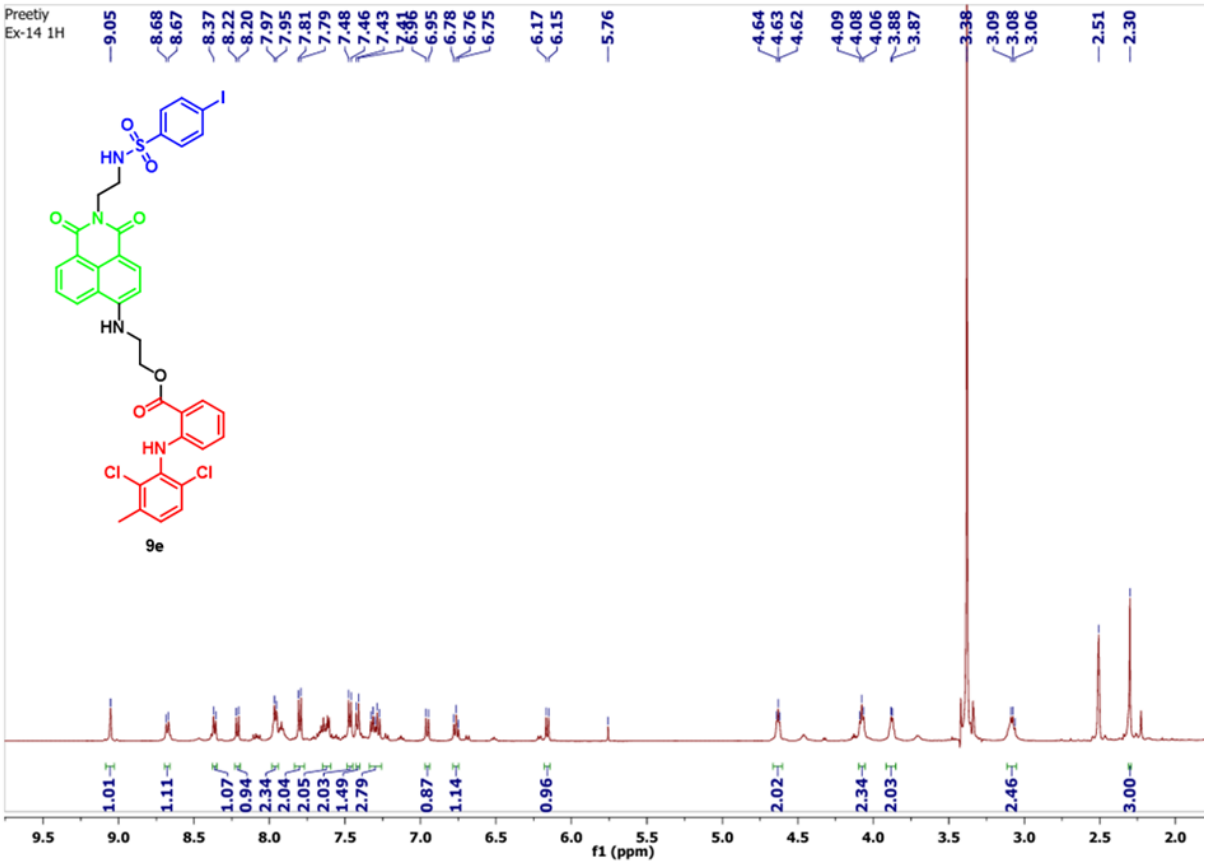


Fig. S59: ^{1}H NMR spectra of 9e.

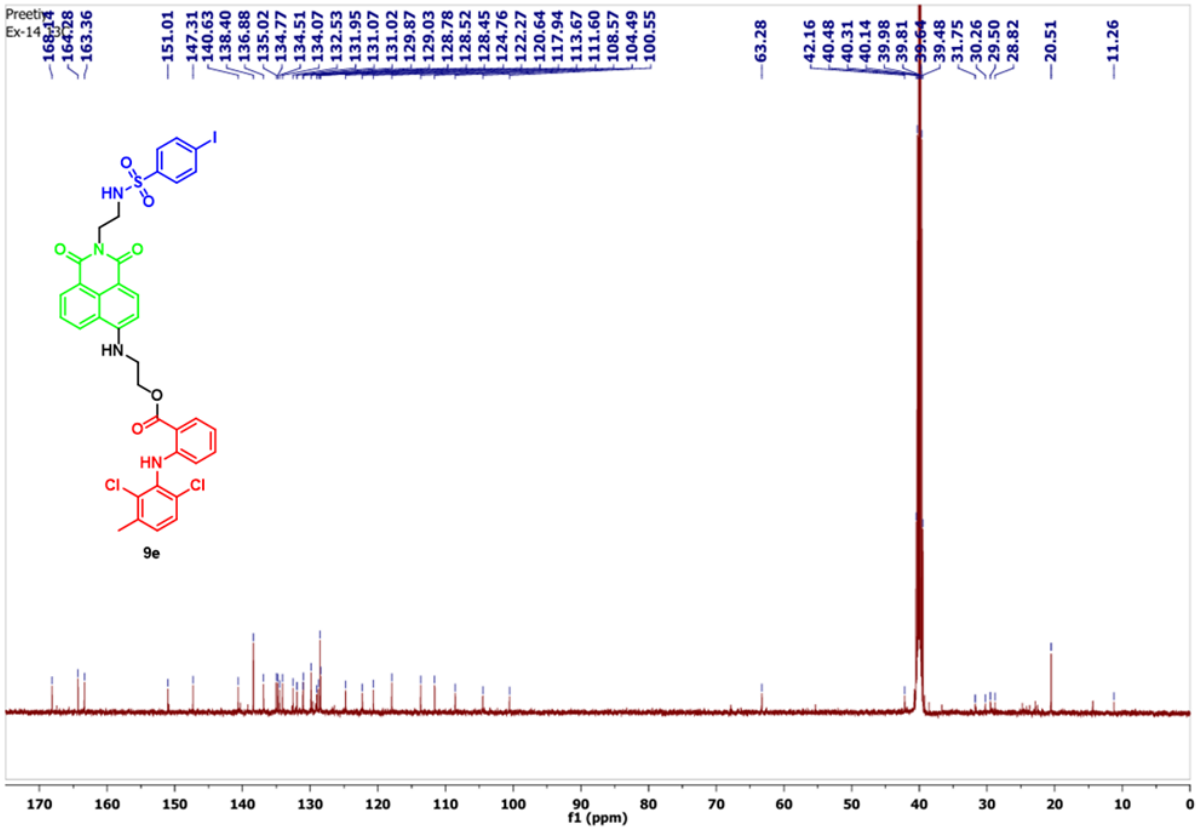


Fig. S60: ^{13}C NMR spectra of 9e.

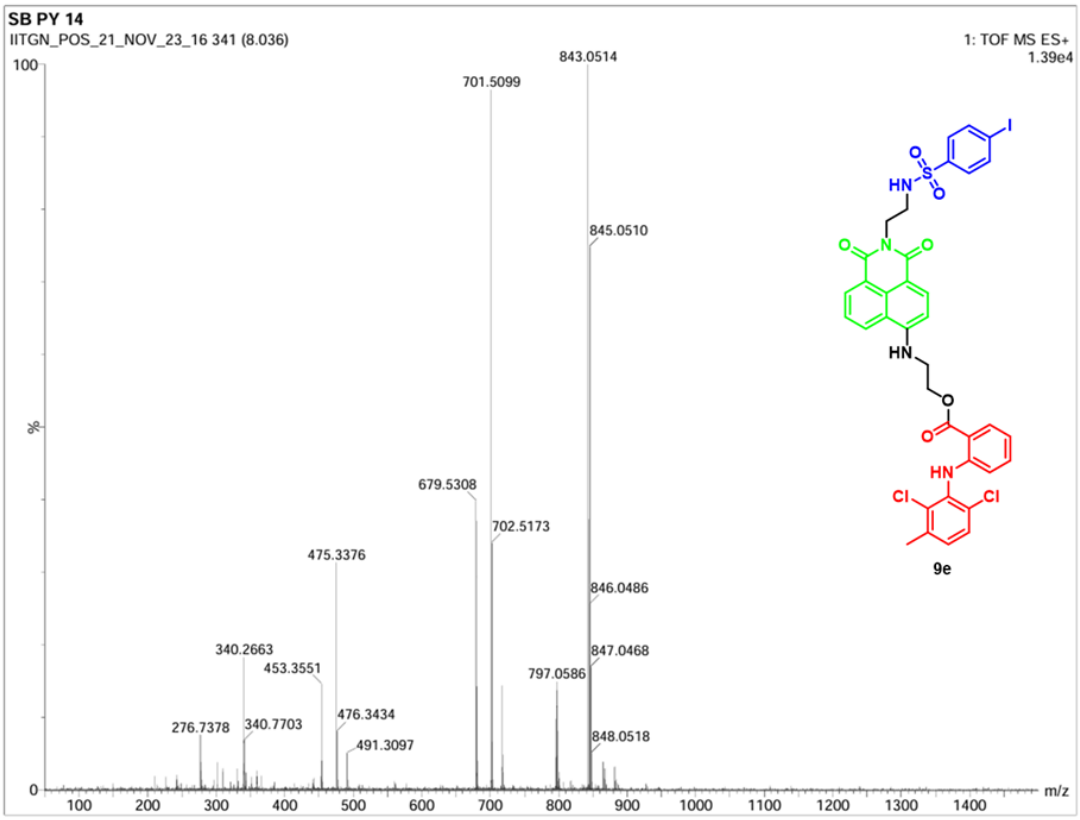


Fig. S61: HR-MS spectra of 9e.

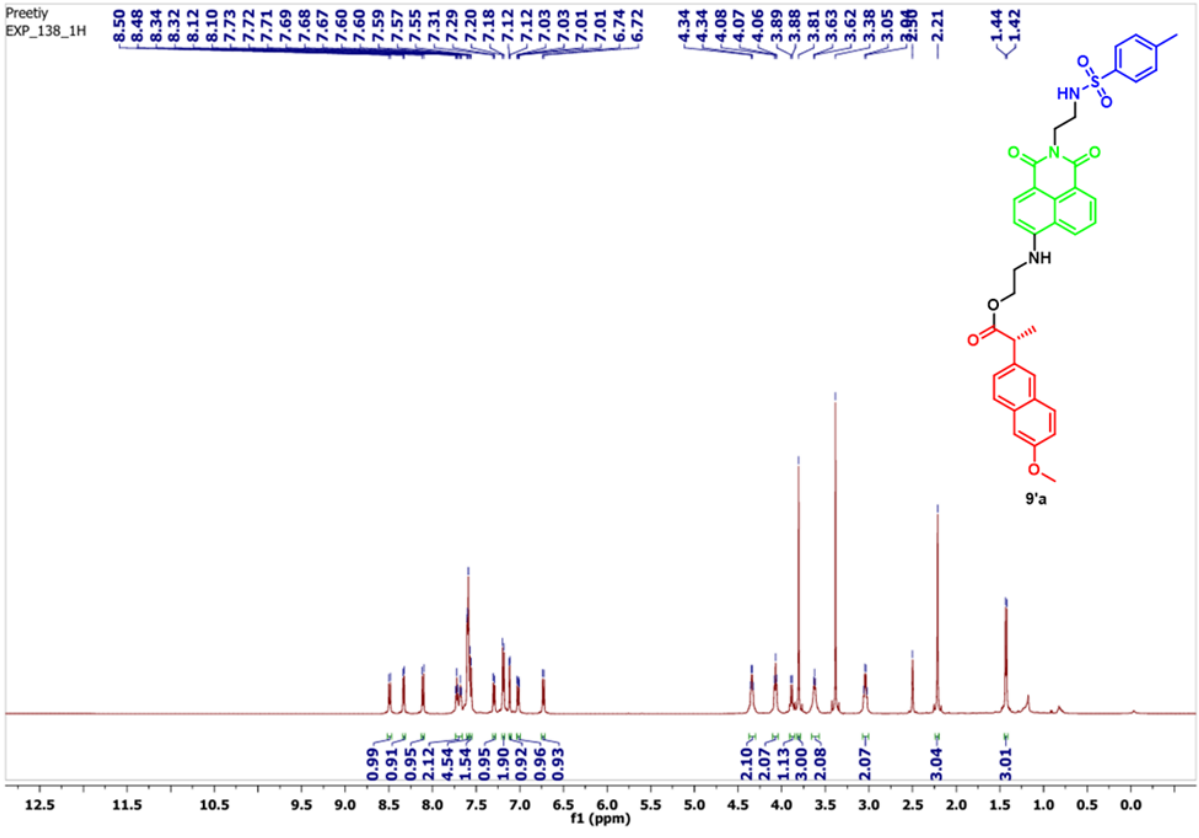


Fig. S62: ^{1}H NMR spectra of 9'a.

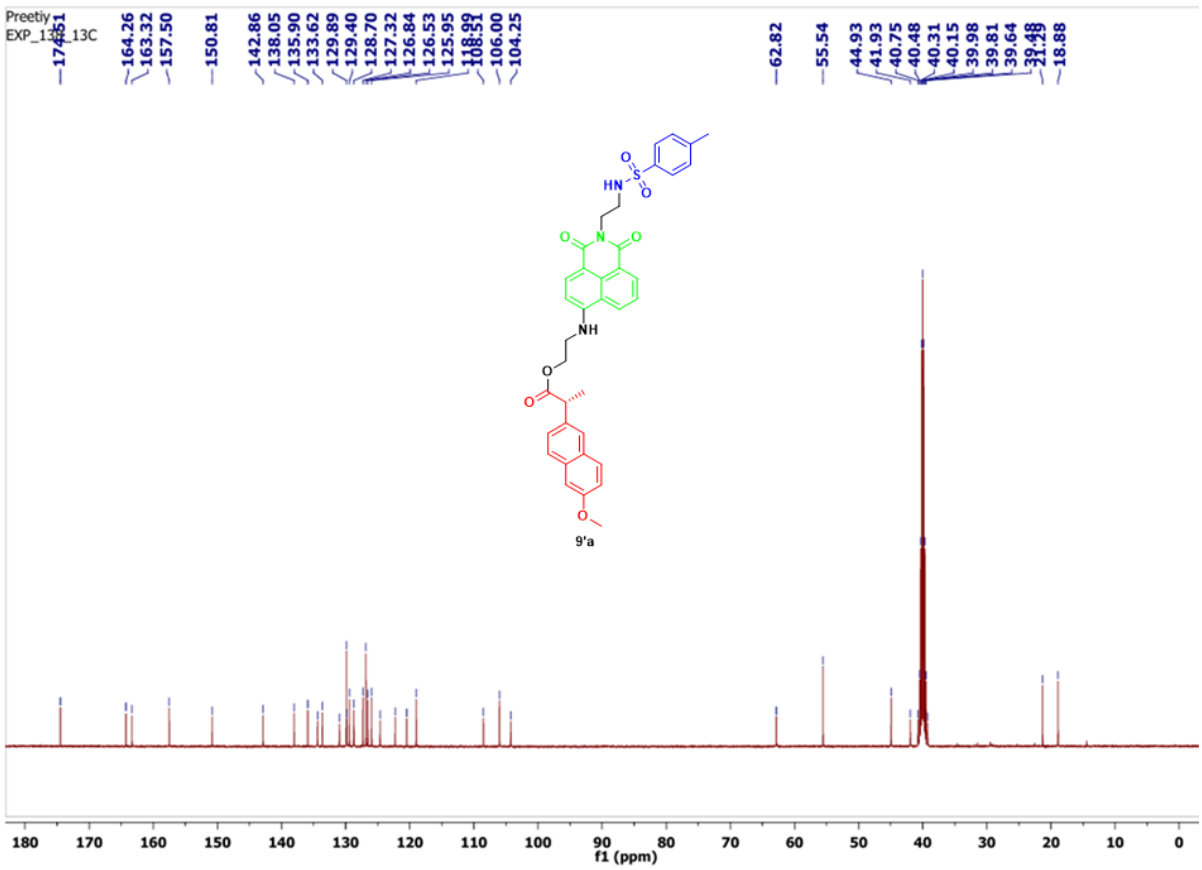


Fig. S63: ^{13}C NMR spectra of 9'a.

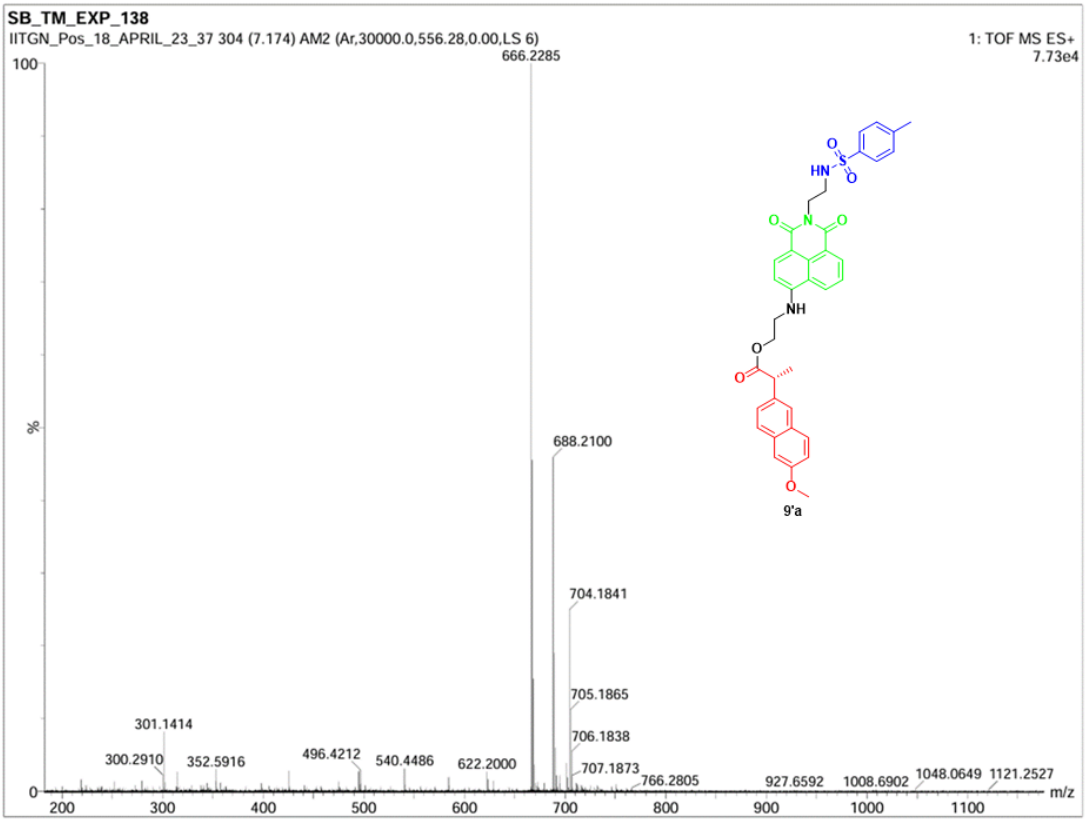


Fig. S64: HR-MS spectra of 9'a.

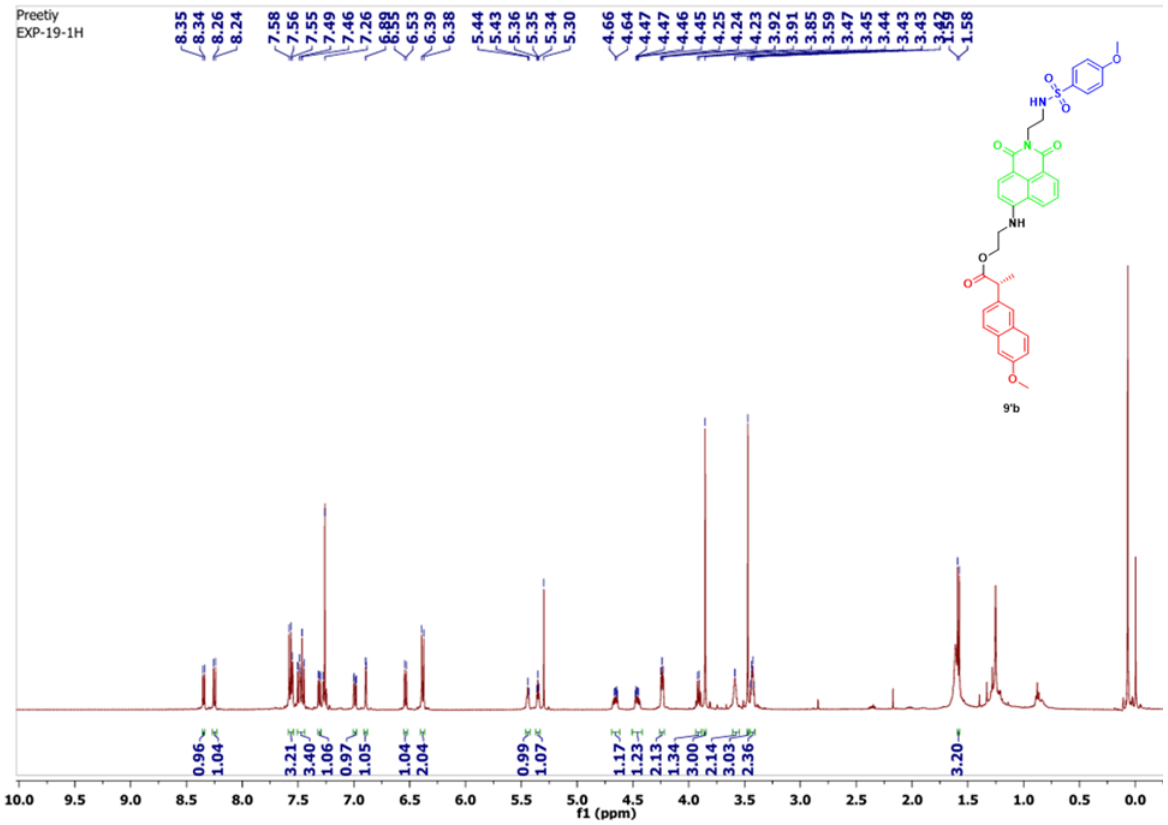


Fig. S65: ^{1}H NMR spectra of 9'b.

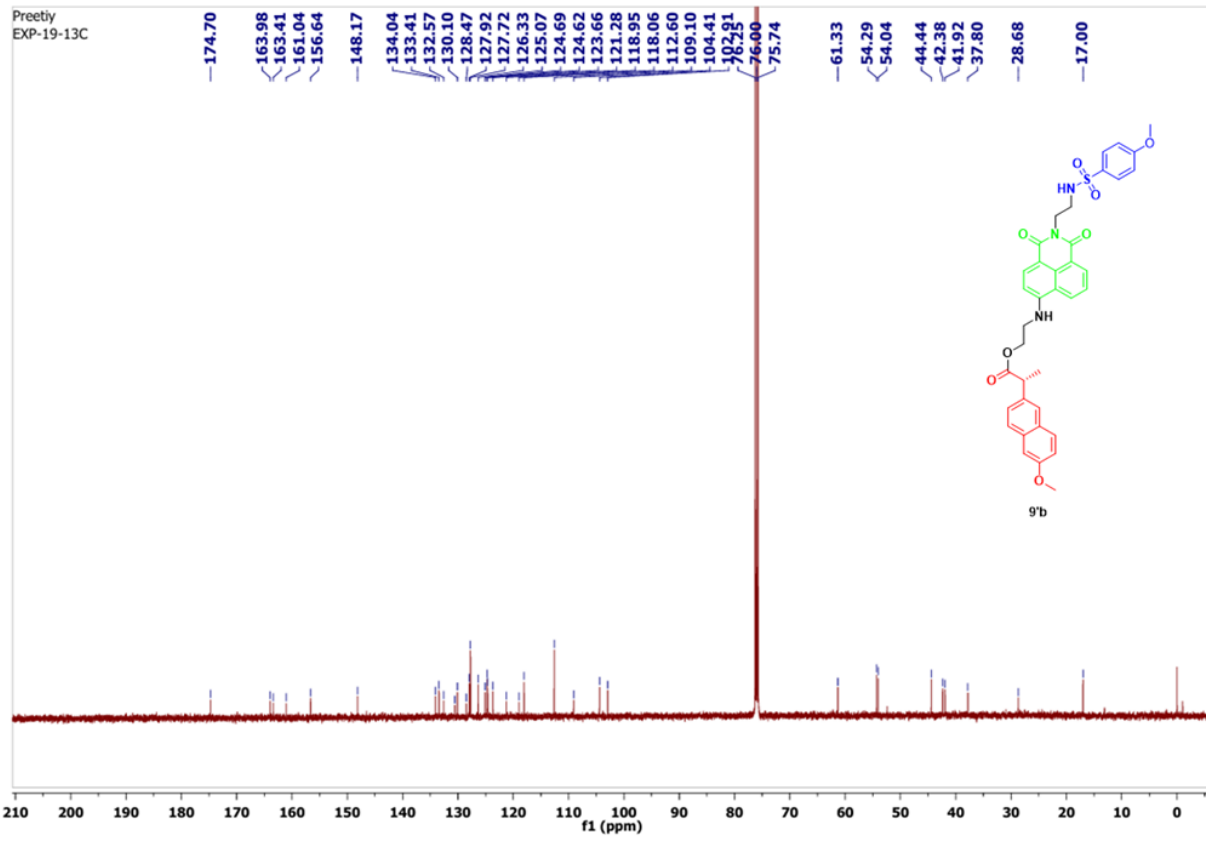


Fig. S66: ^{13}C NMR spectra of 9'b.

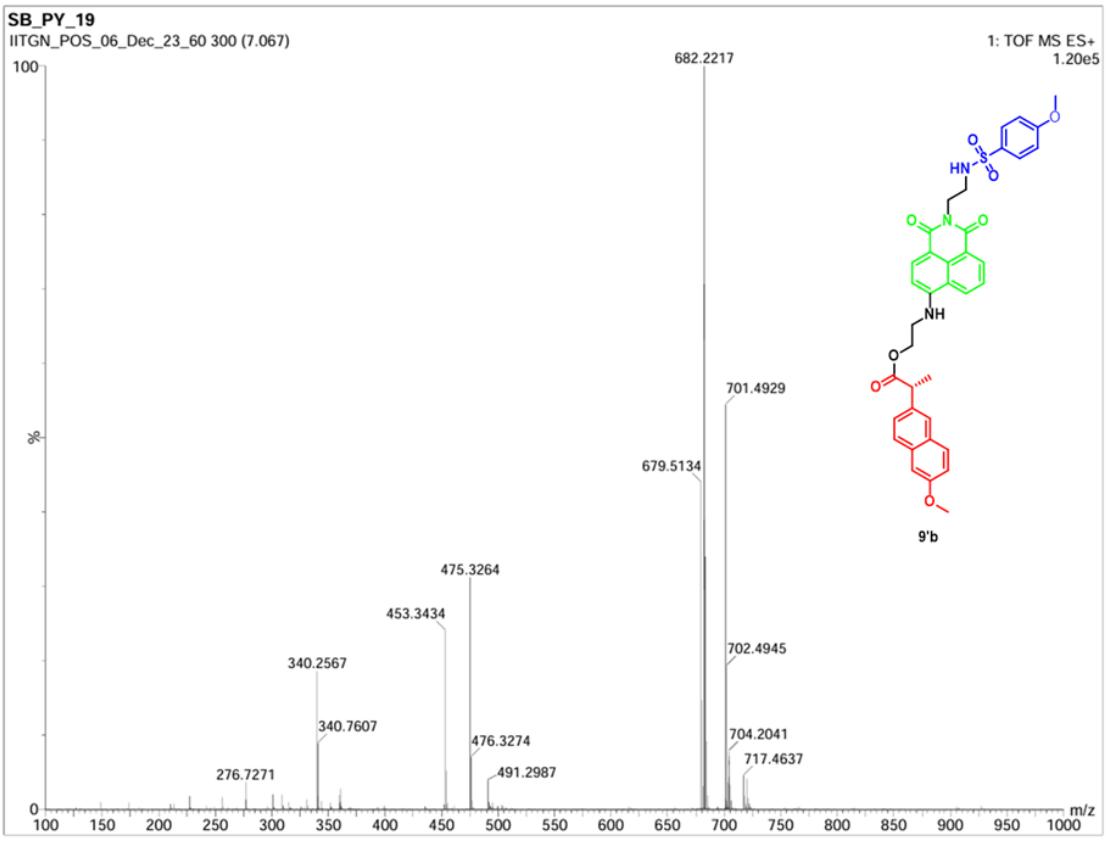


Fig. S67: HR-MS spectra of 9'b.

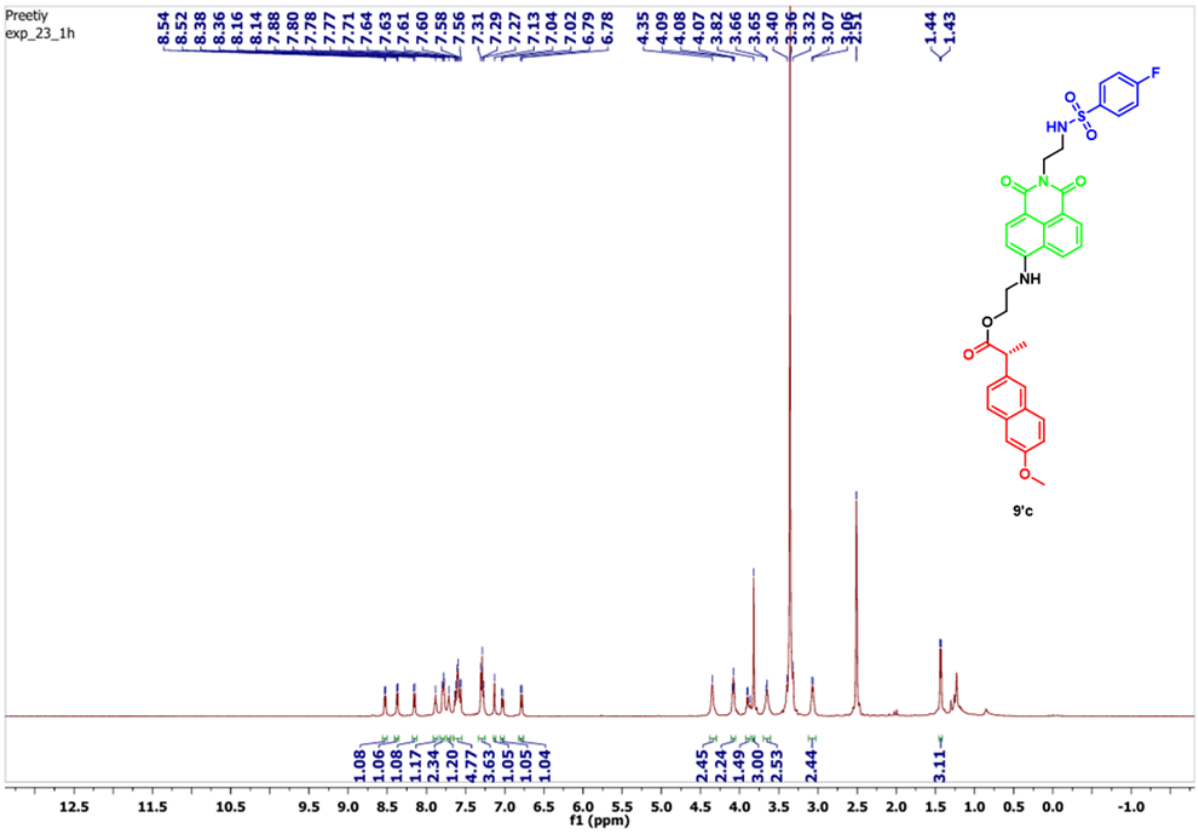


Fig. S68: ^{1}H NMR spectra of 9'c.

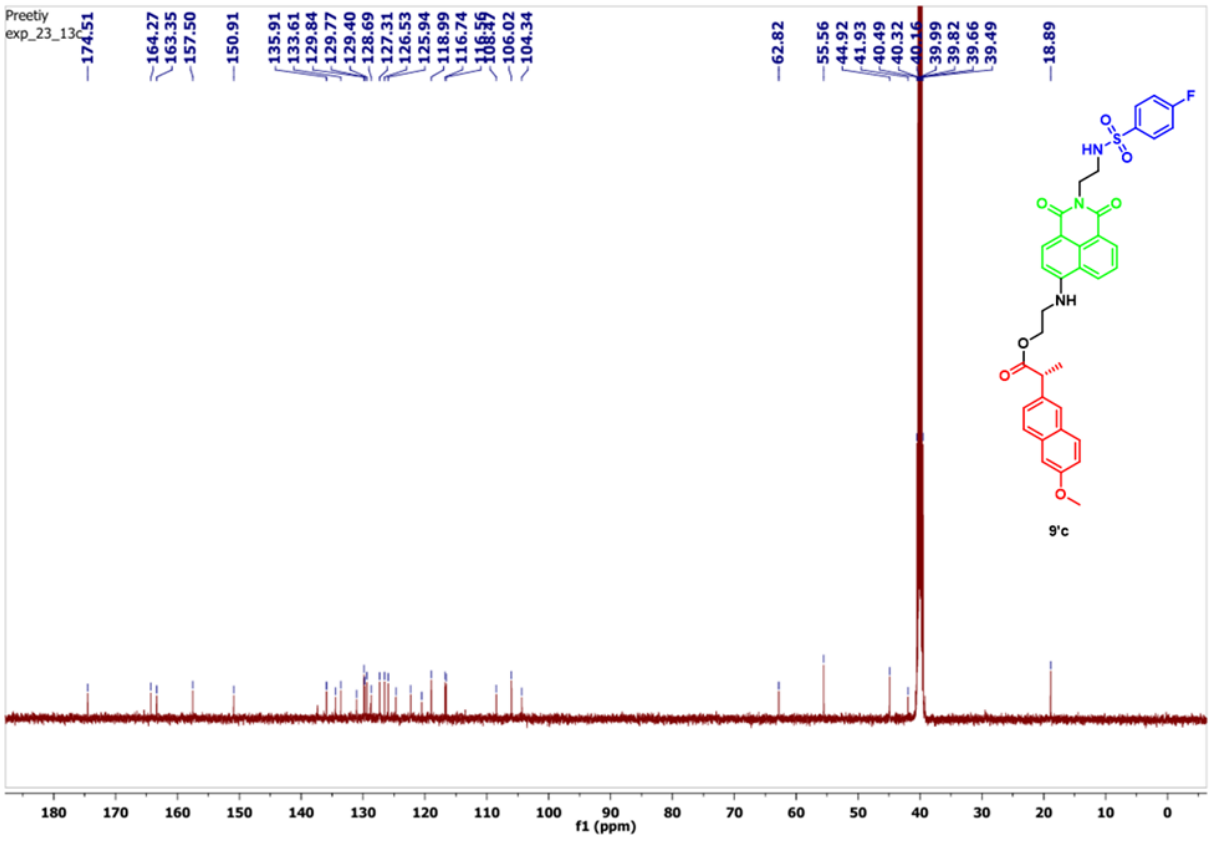


Fig. S69: ^{13}C NMR spectra of 9'c.

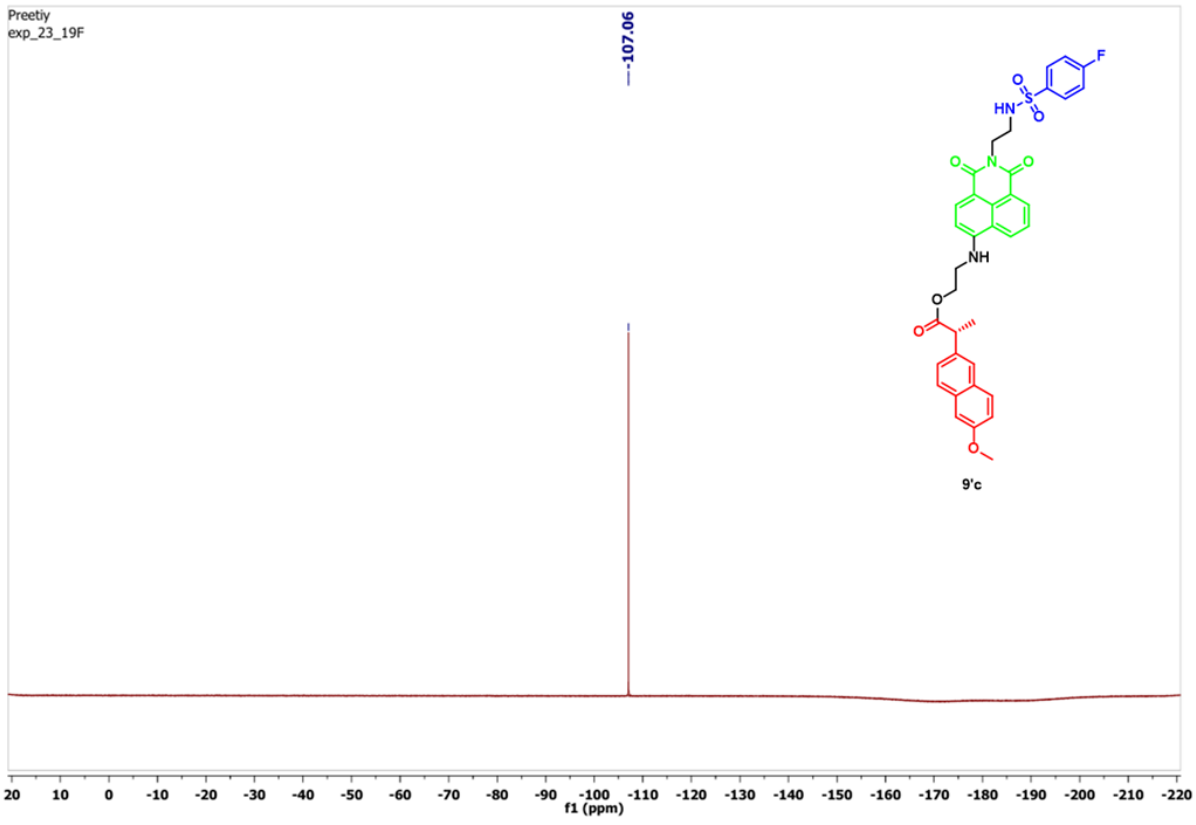


Fig. S70: ^{19}F NMR spectra of 9'c.

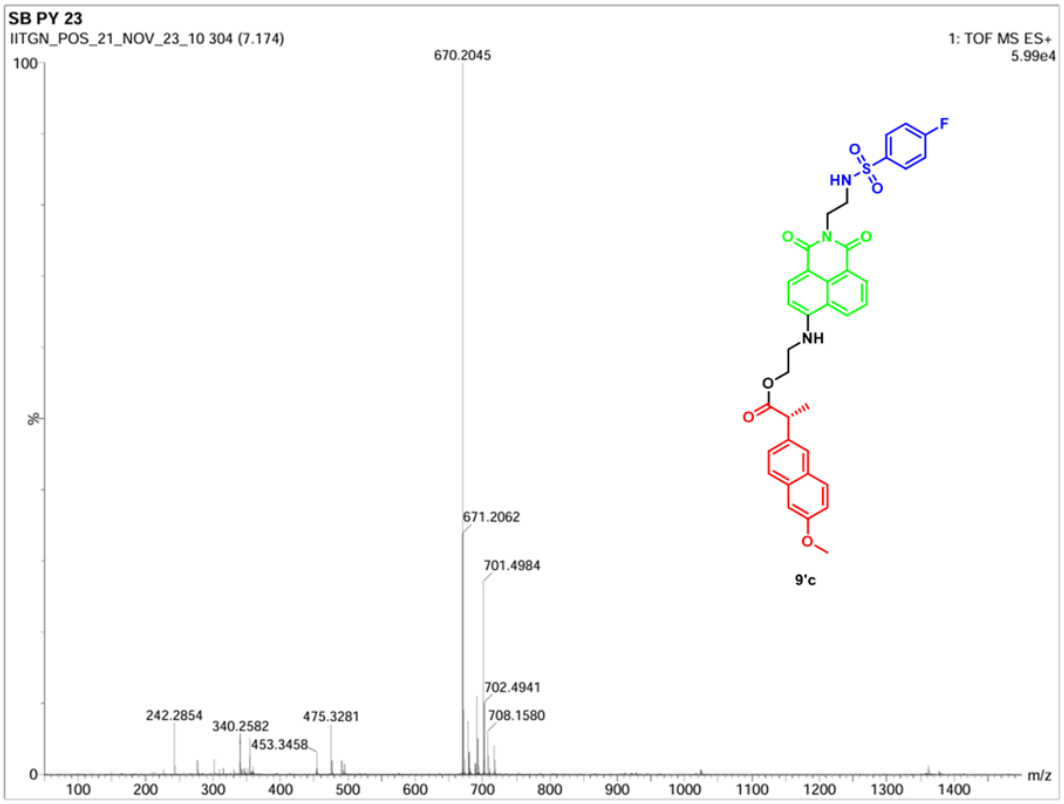


Fig. S71: HR-MS spectra of 9'c.

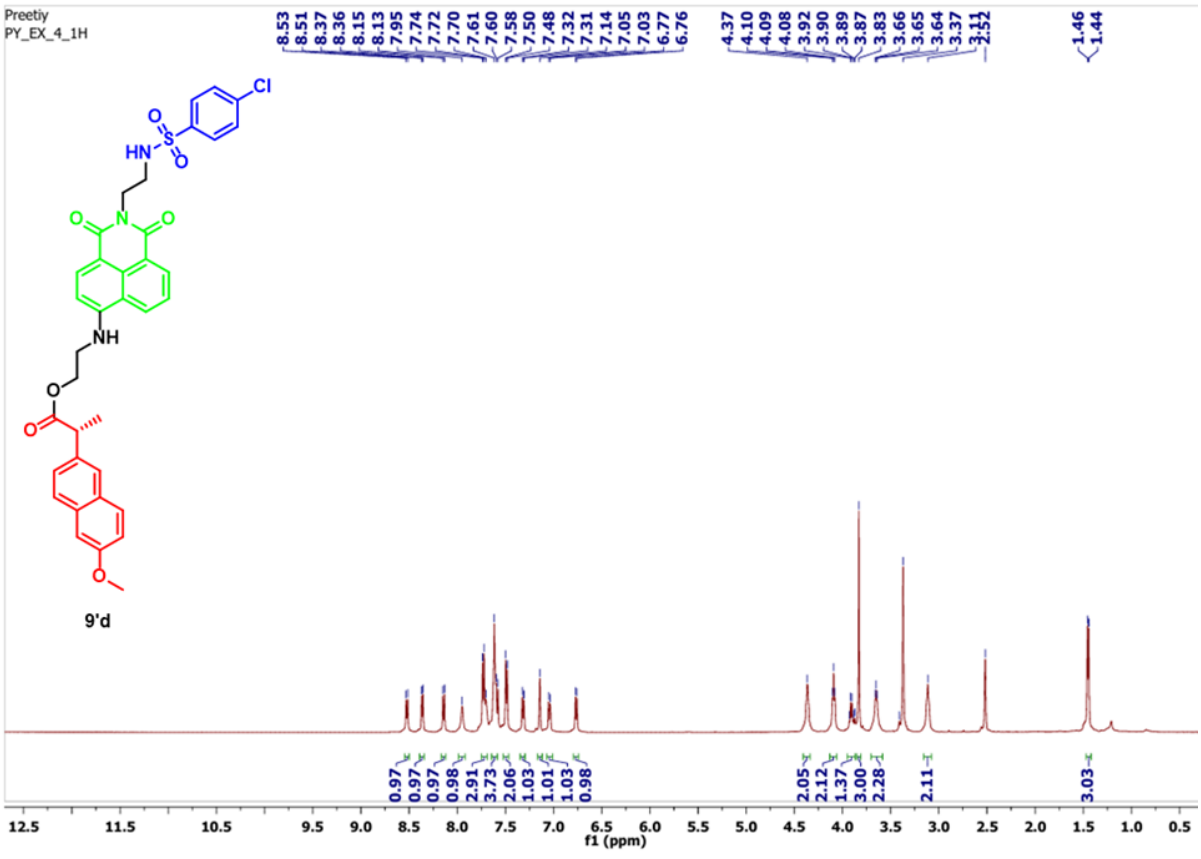


Fig. S72: ^{1}H NMR spectra of 9'd.

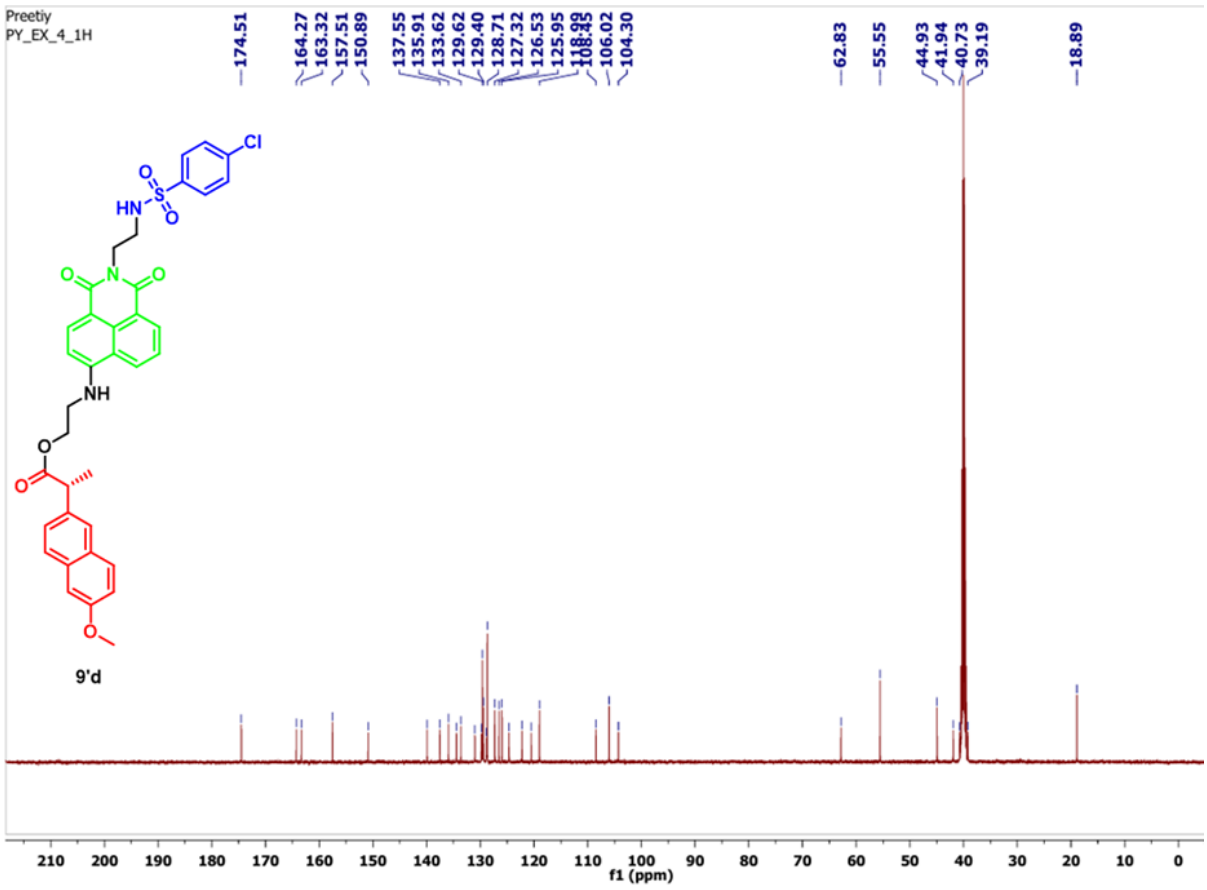


Fig. S73: ^{13}C NMR spectra of 9'd.

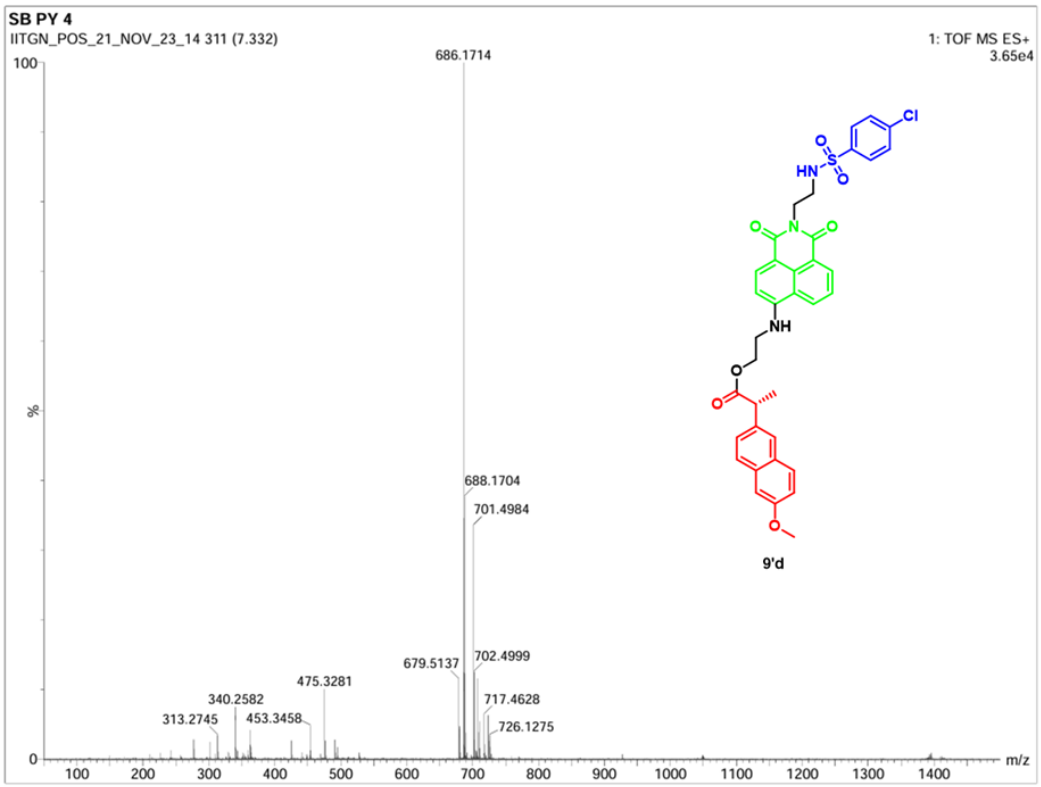


Fig. S74: HR-MS spectra of 9'd.

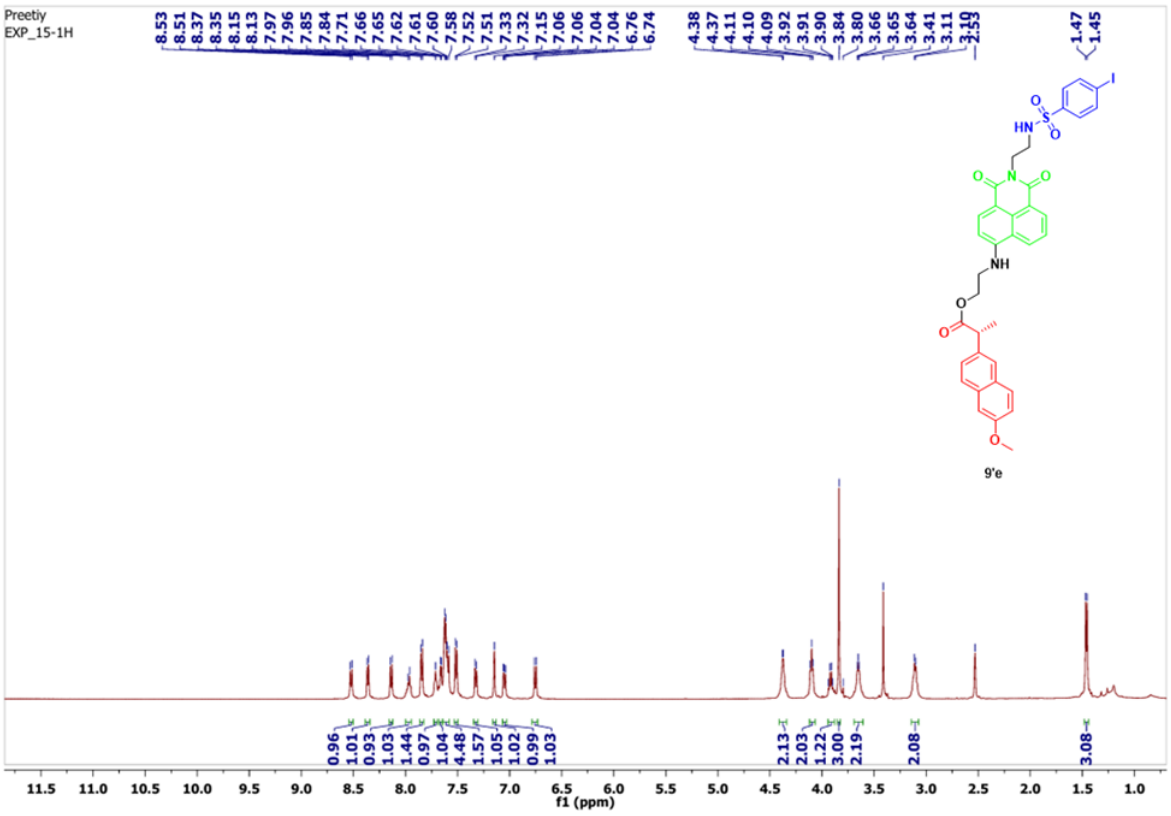


Fig. S75: ^{1}H NMR spectra of 9'e.

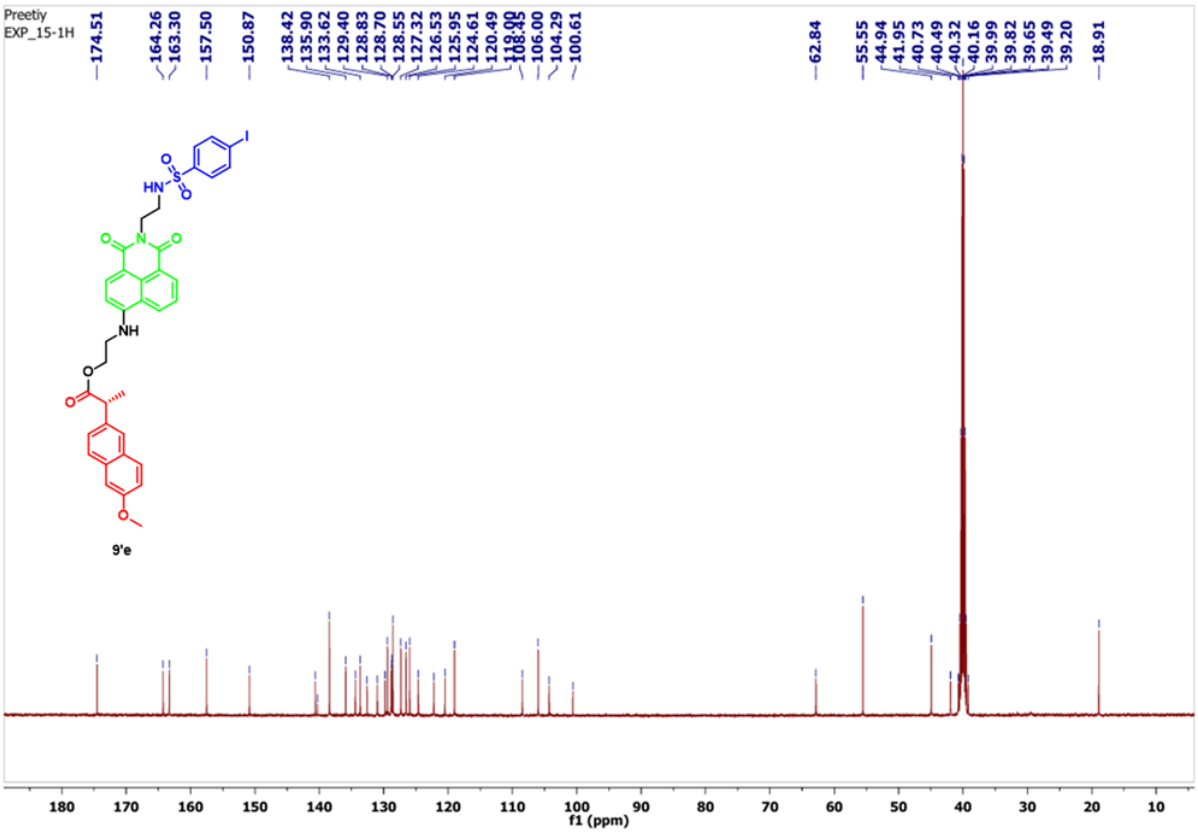


Fig. S76: ^{13}C NMR spectra of 9'e.

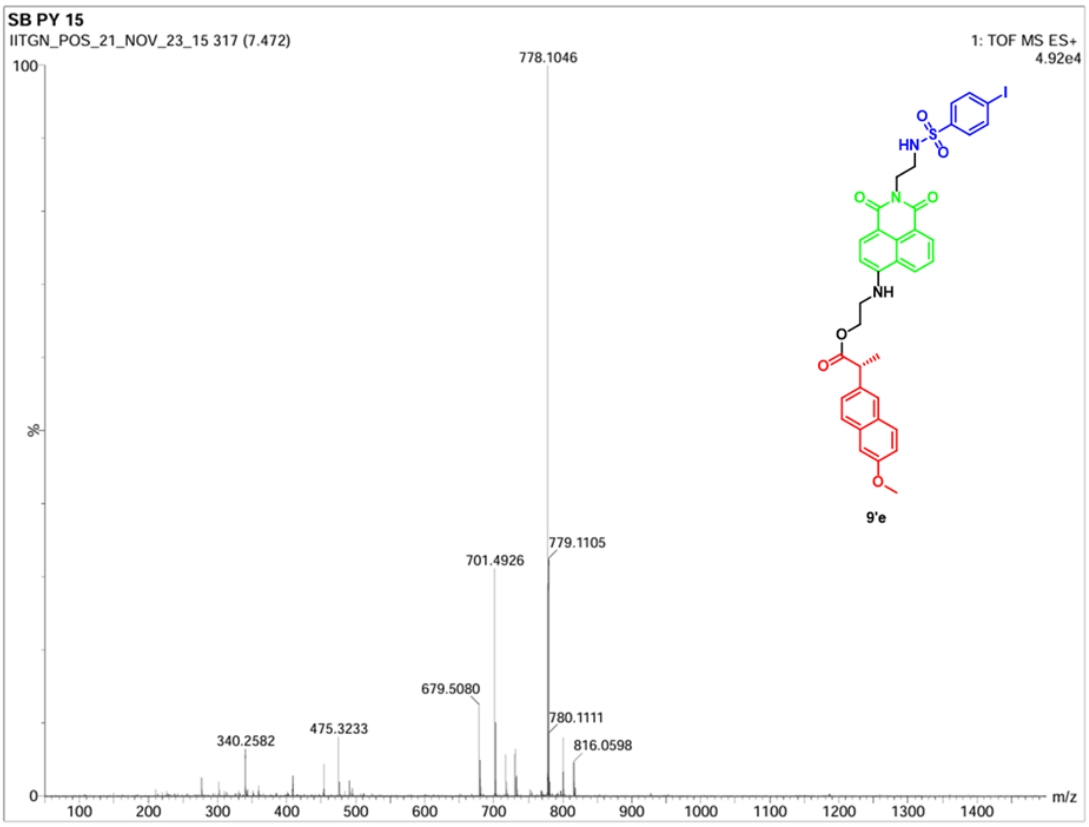


Fig. S77: HR-MS spectra of 9'e.

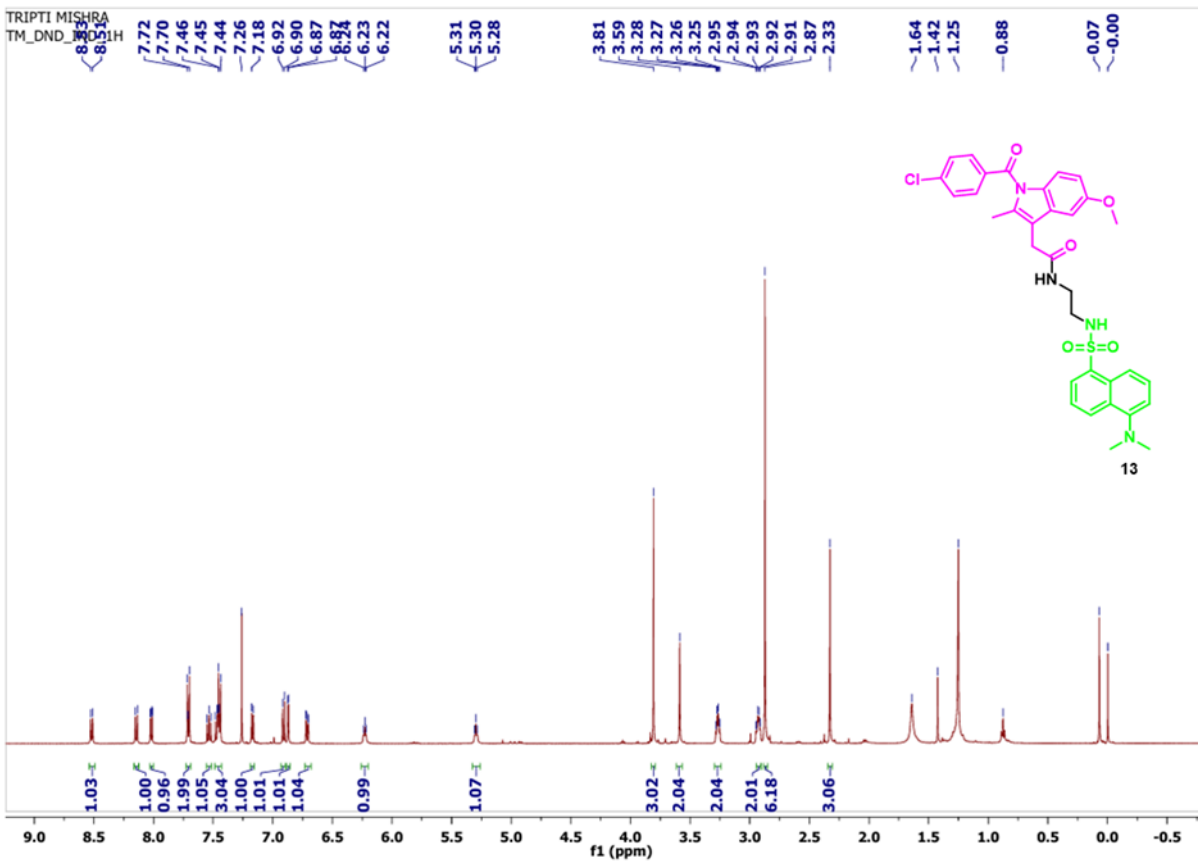


Fig. S78: ^{1}H NMR spectra of 13.

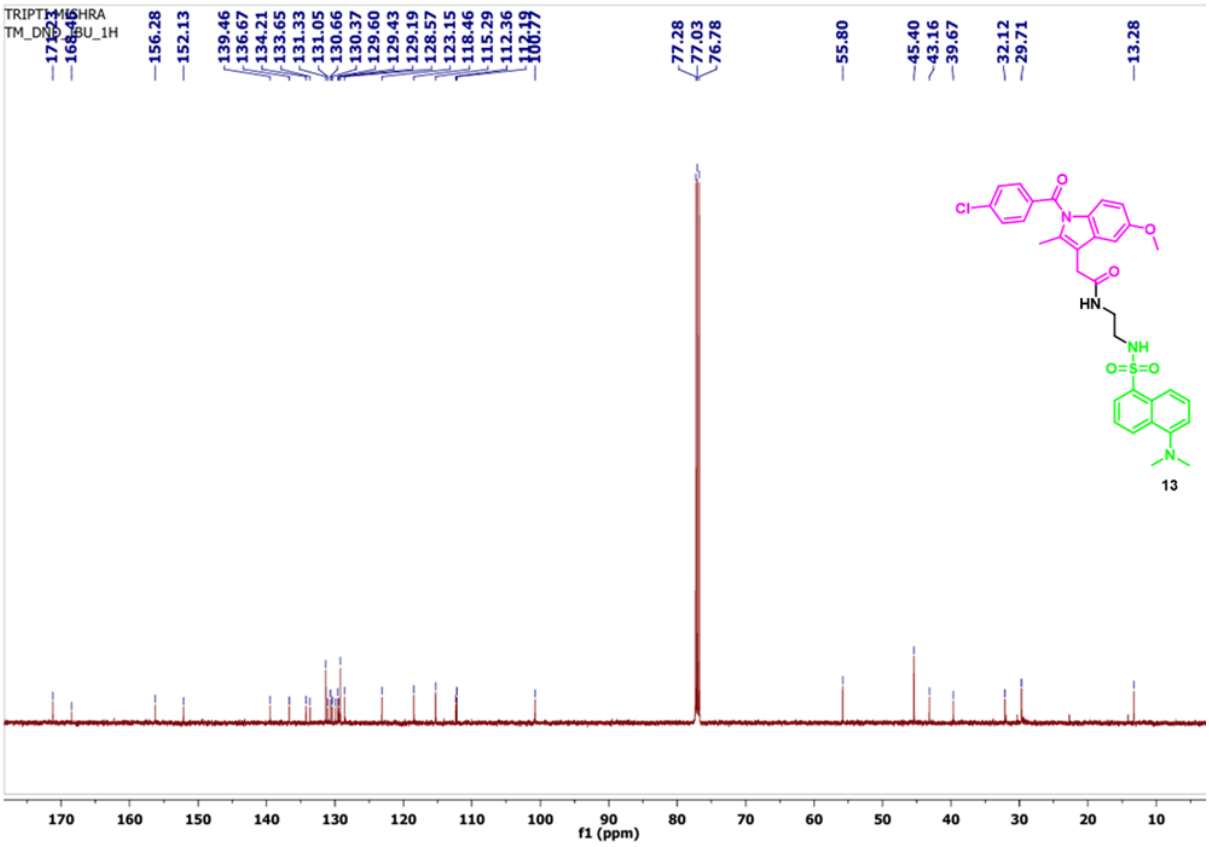


Fig. S79: ^{13}C NMR spectra of 13.

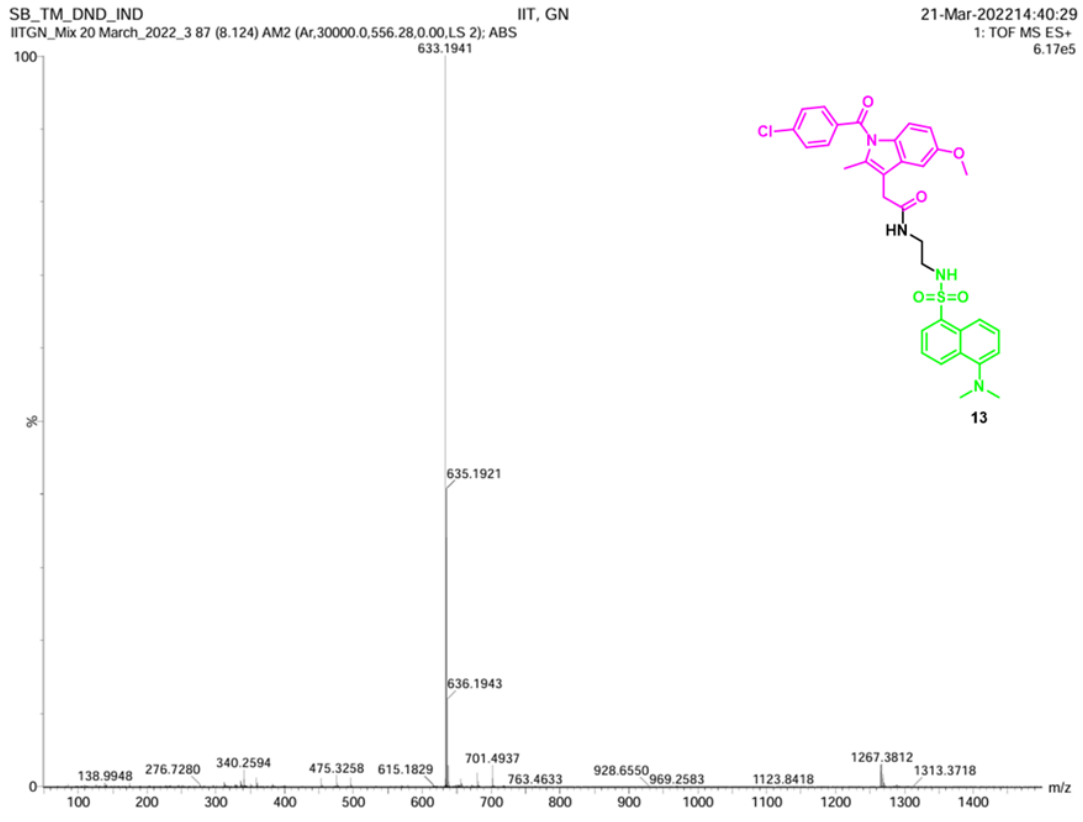


Fig. S80: HR-MS spectra of 13.

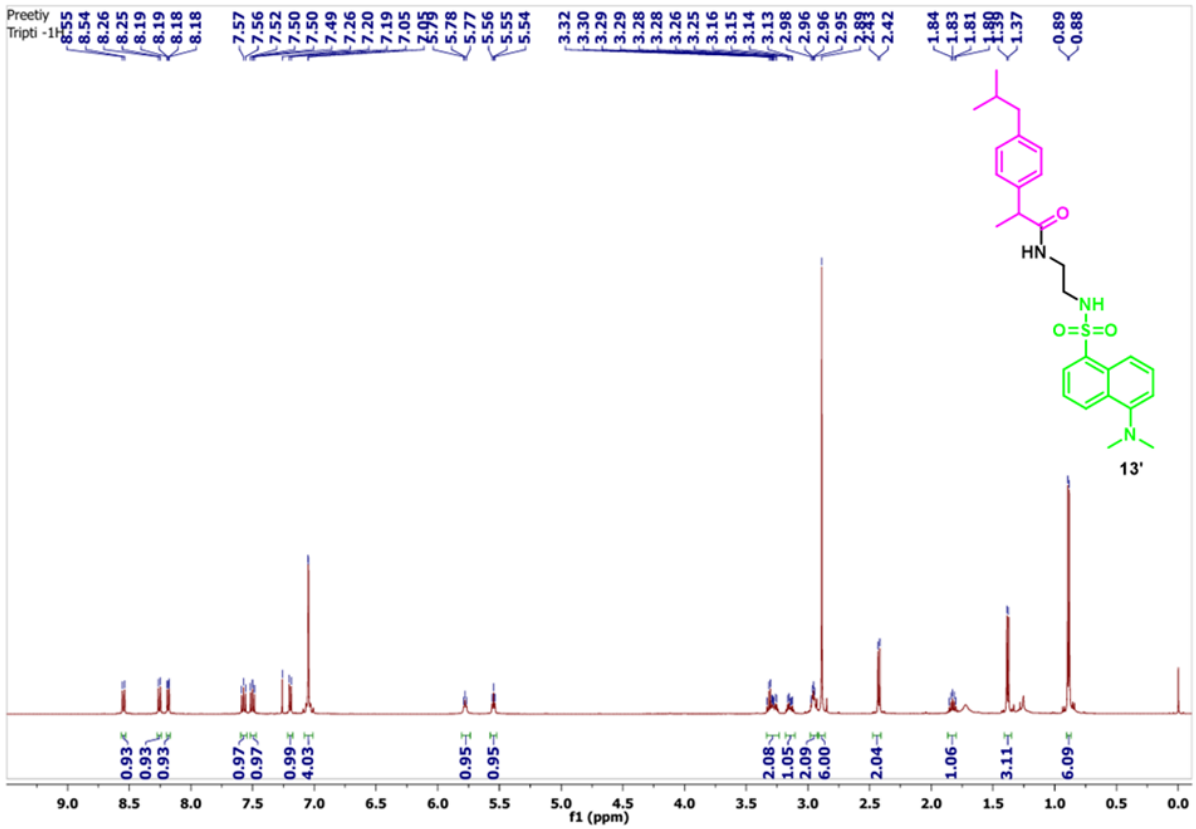


Fig. S81: ^{1}H NMR spectra of 13'.

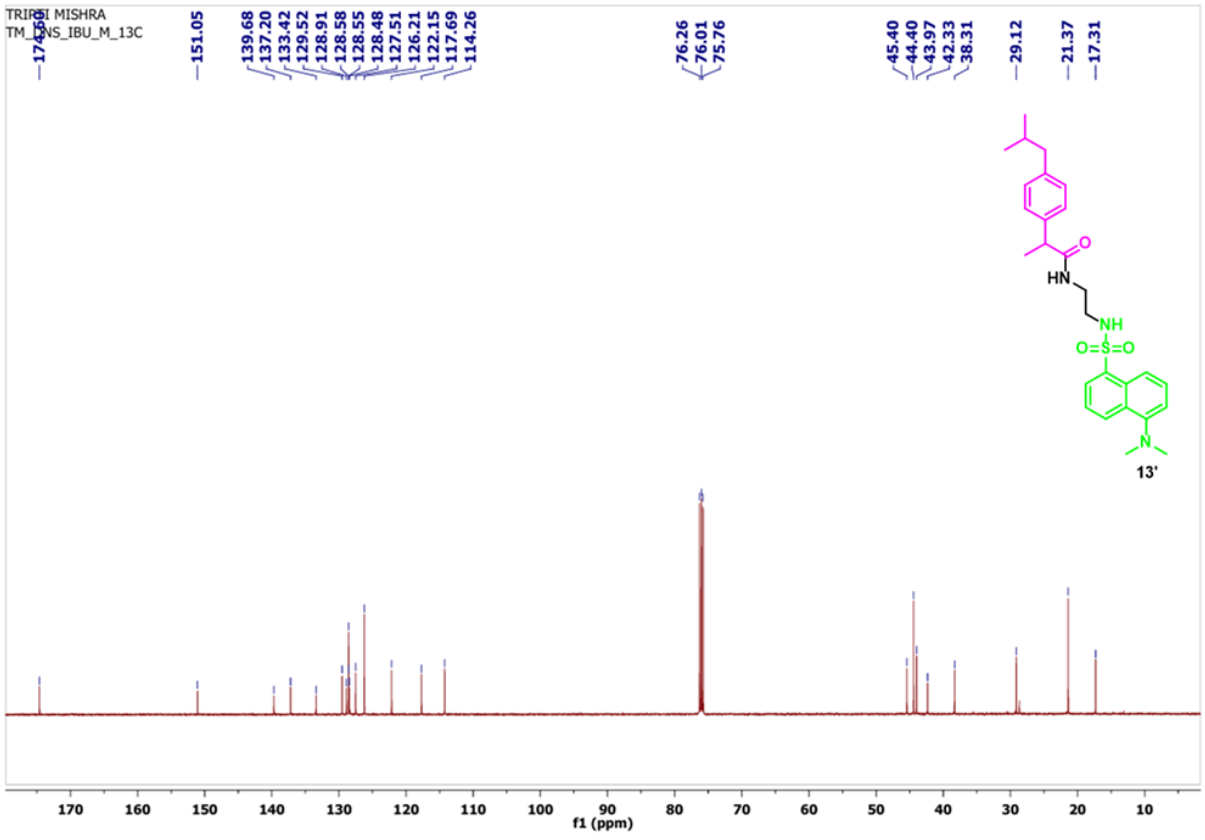


Fig. S82: ^{13}C NMR spectra of 13'.

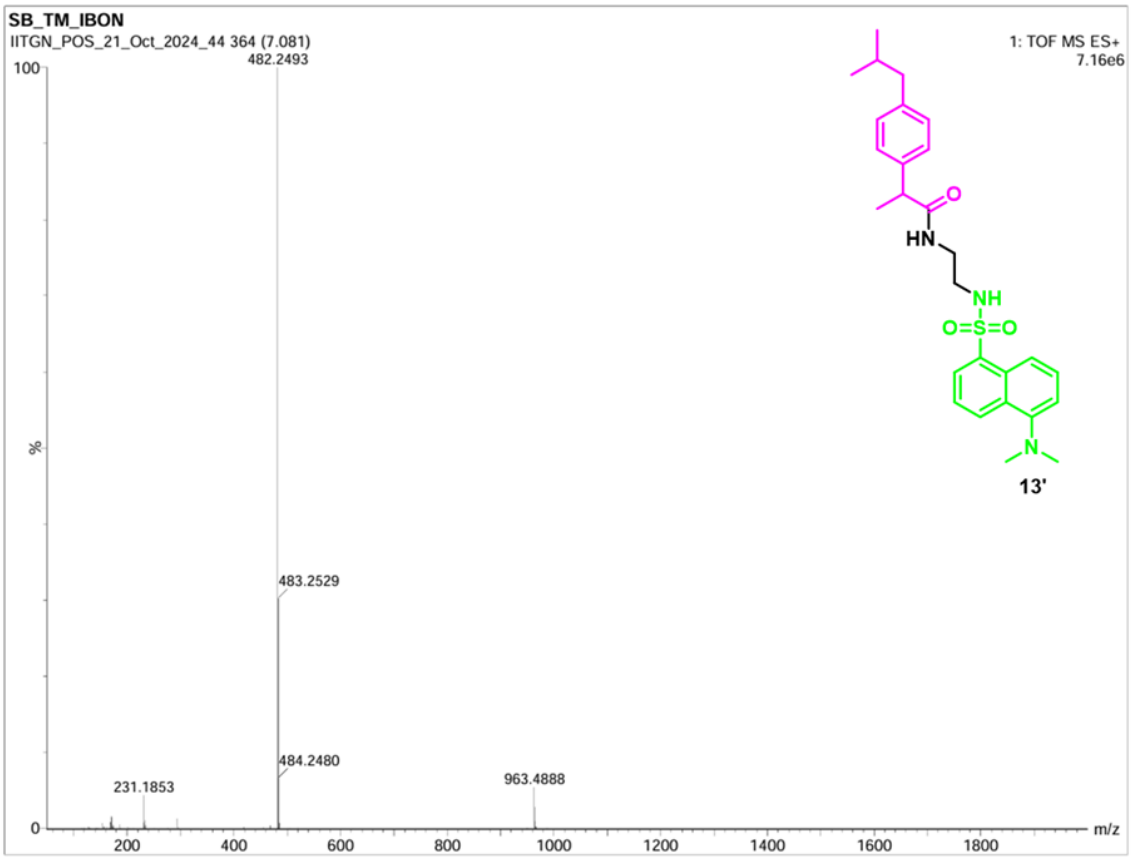


Fig. S83: HR-MS spectra of 13'.

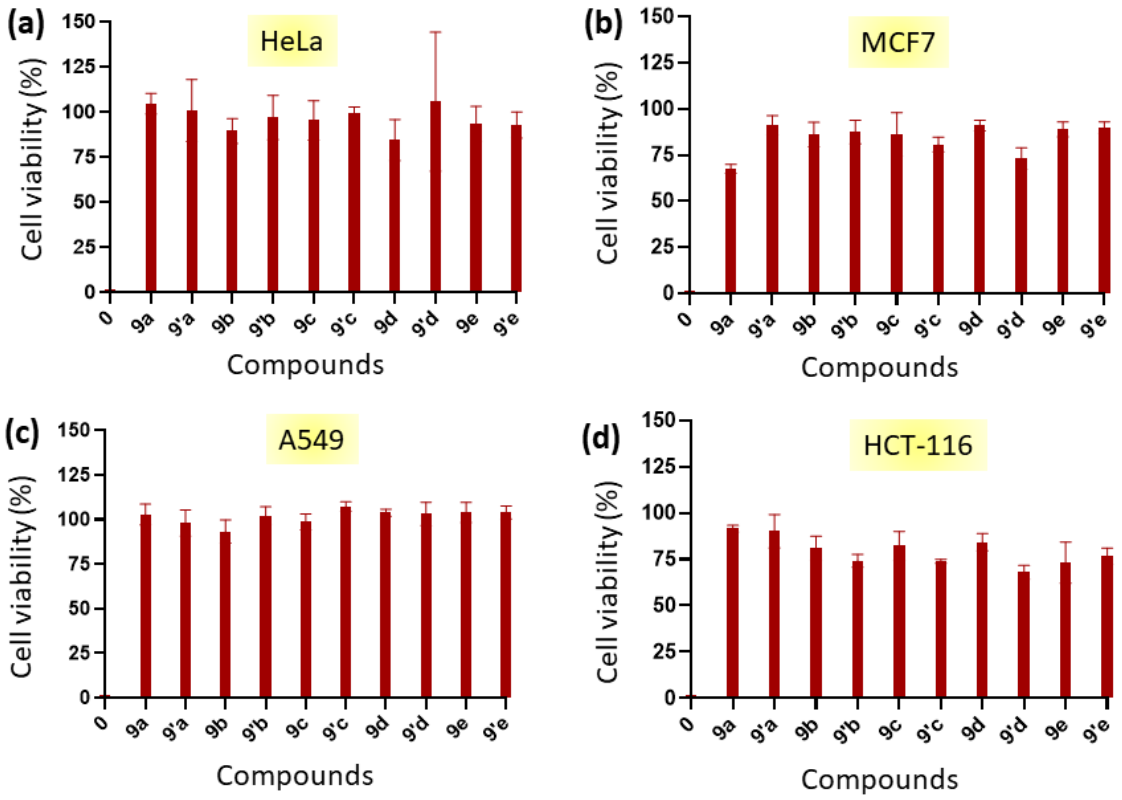


Fig. S84: (a-d) Cell viability assay of 9a-9e and 9'a-9'e at 15 µM concentration at 24h post-incubation in HeLa, MCF7, A549 and HCT-116 cells respectively.

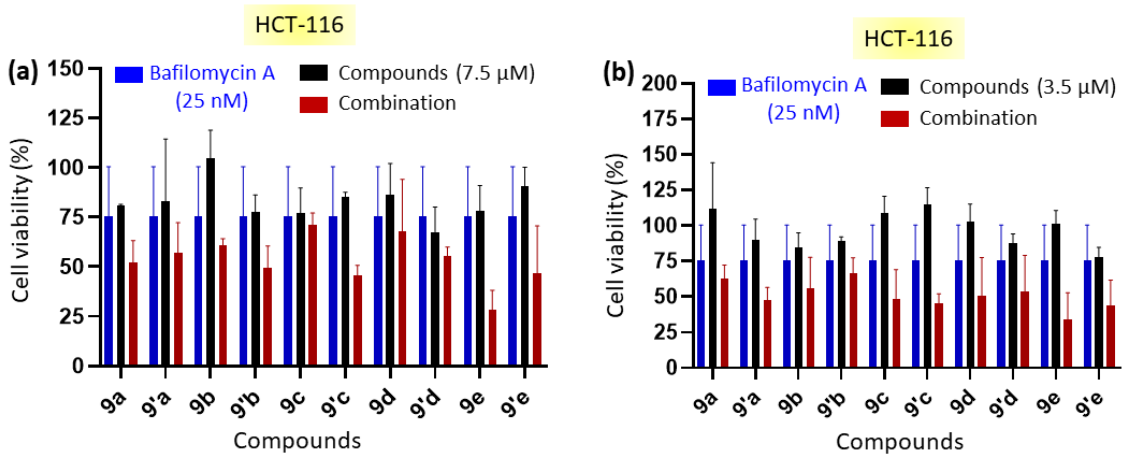


Fig. S85: (a, b) Cell viability assay of 9a-9e and 9'a-9'e at 7.5 and 3.5 µM concentrations in combination with Bafilomycin A (25 nM) at 24h post-incubation in HCT-116 cells respectively.

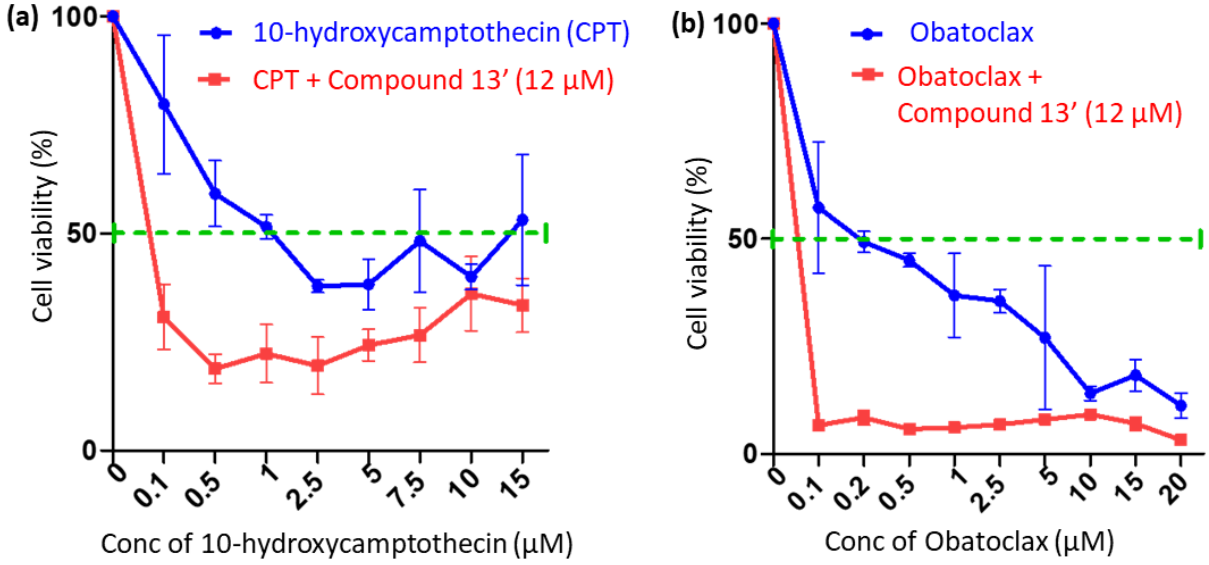


Fig. S86: (a,b) MTT assay of compound 13' in combination with 10-hydroxycamptothecin and obatoclax in HCT-116 cells at 24h post incubation.

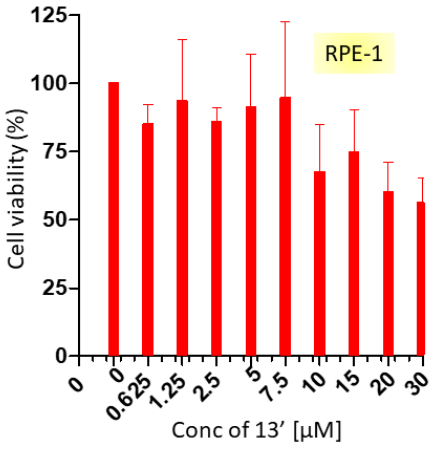


Fig. S87: Dose dependent cell viability of compound 13' in RPE-1 cells at 24h post-incubation.

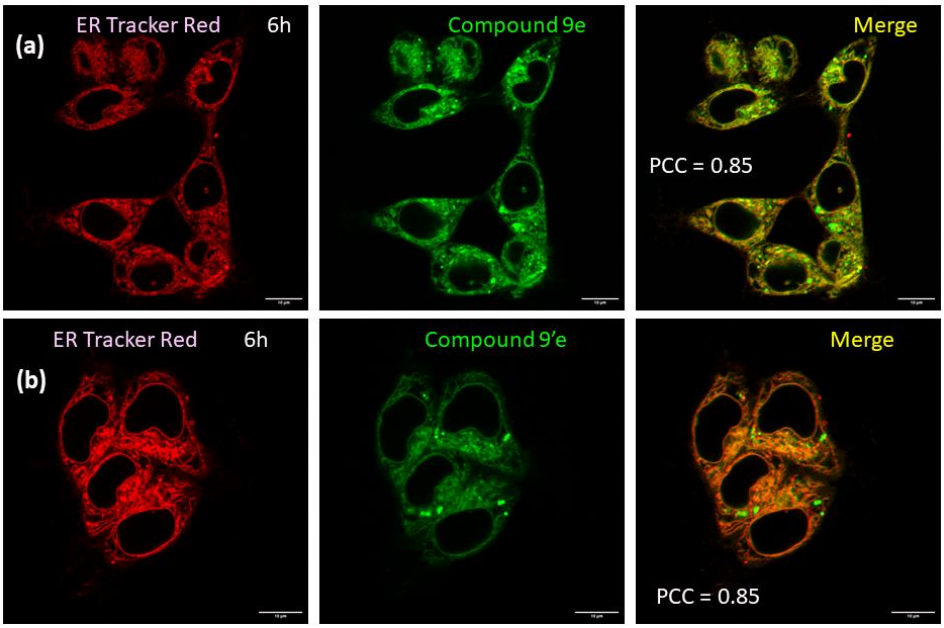


Fig. S88: (a,b) Confocal laser scanning microscopy images of HCT-116 cells after incubating with 9e and 9'e for 6h followed by staining the ER with ER Tracker Red dye. Scale bar = 10 µm.

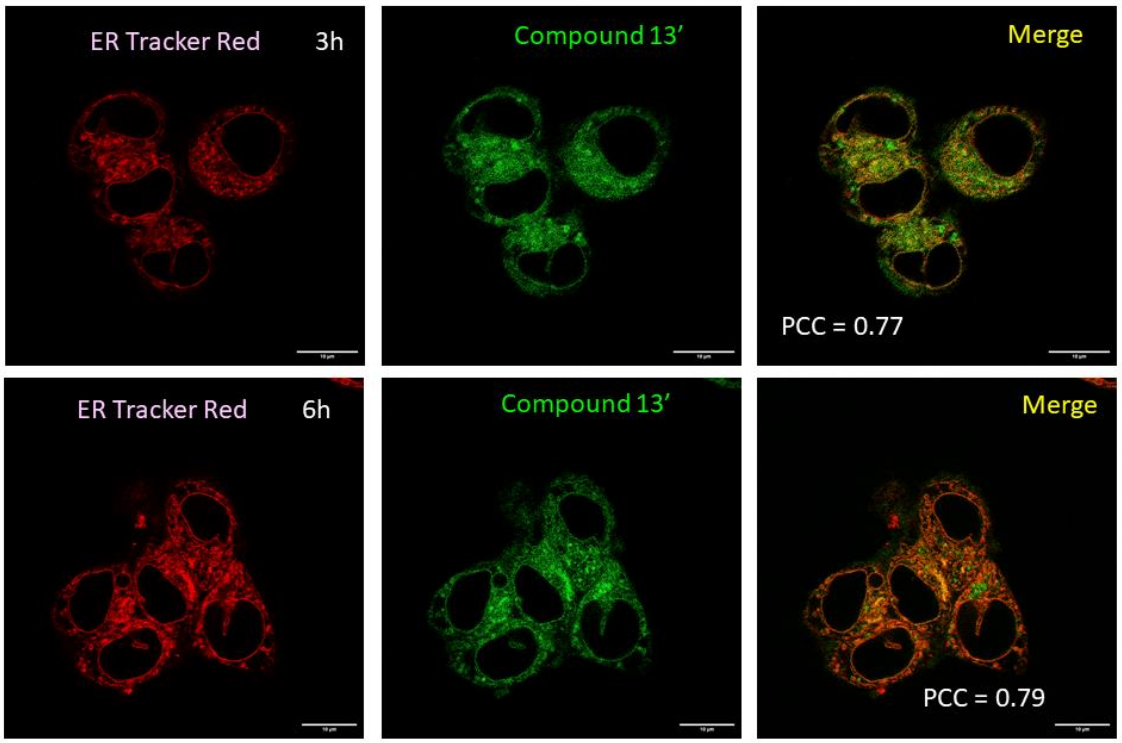


Fig. S89: Confocal laser scanning microscopy images of HCT-116 cells after incubating with 13' for 3h and 6h followed by staining the ER with ER Tracker Red dye. Scale bar = 10 µm.

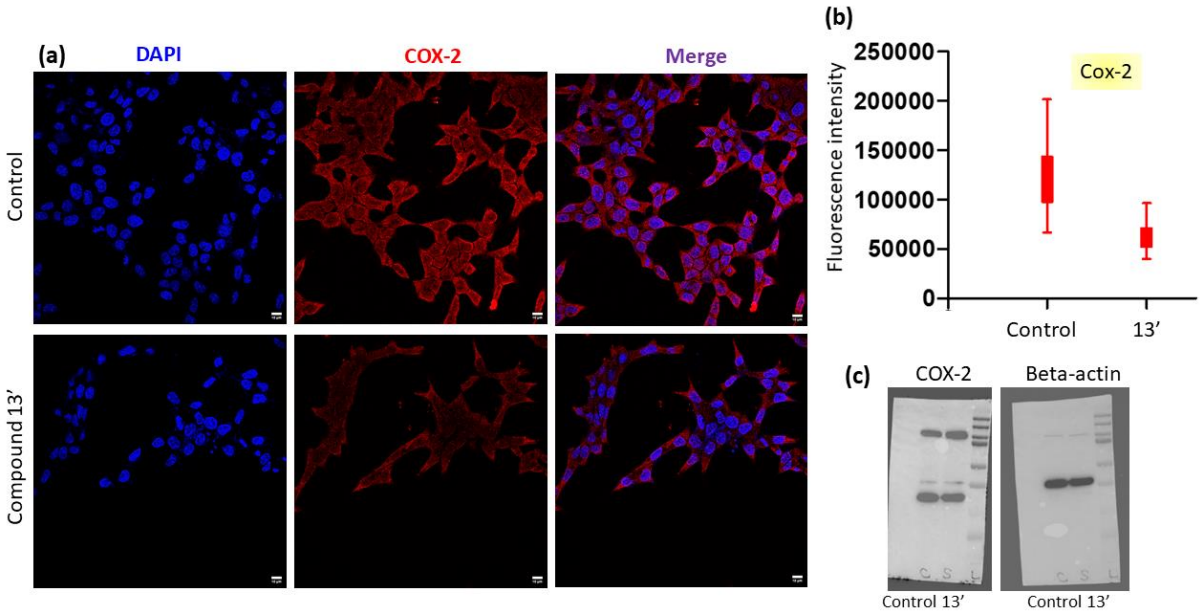


Fig. S90: (a) Confocal laser scanning microscopy images and (b) quantification from the confocal imaging of HCT-116 cells after treatment with compound 13' for 24h, followed by staining with primary antibodies specific for Cox-2. The cells were further stained with Alexa Fluor 633-labelled secondary antibody (Red). Nuclei were stained with DAPI (blue). Scale bar = 10 µm. (c) Expression of Cox-2 in HCT-116 cells after treatment with compound 13' for 24h followed by Western blot analysis.

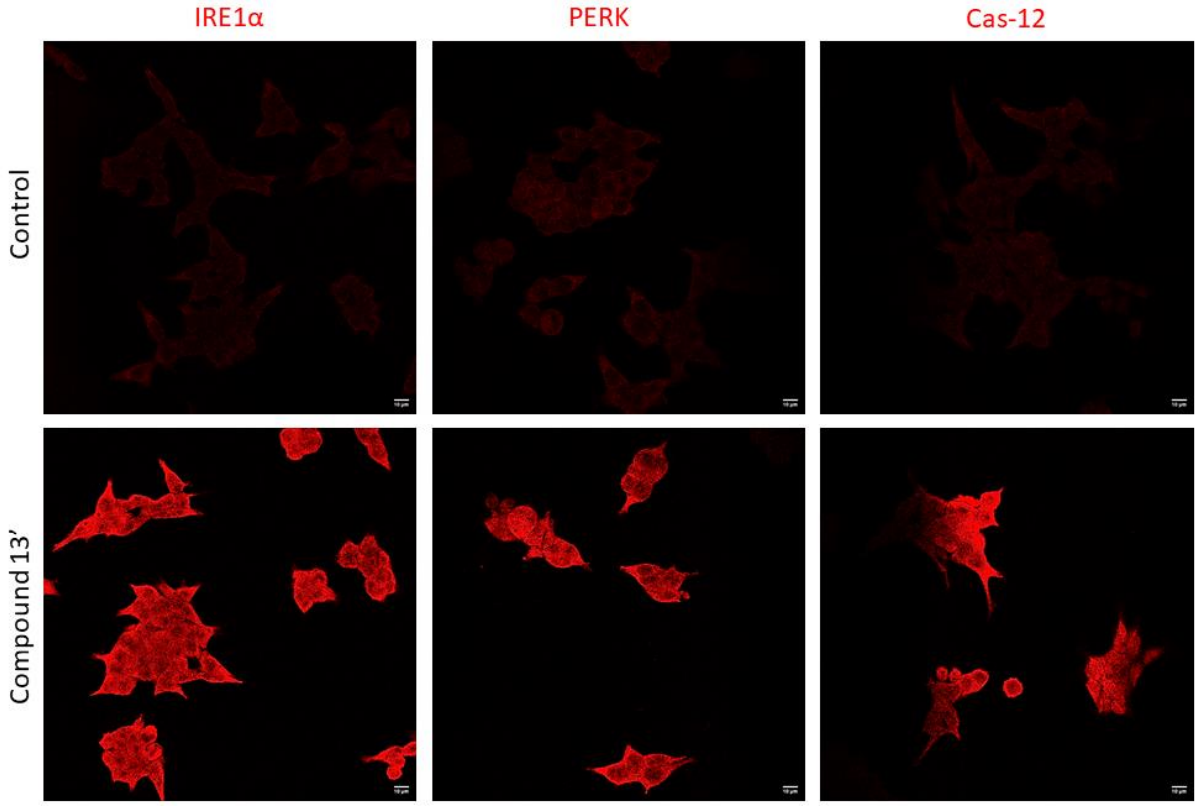


Fig. S91: Confocal laser scanning microscopy images of HCT-116 cells after treatment with compound 13' for 24h, followed by staining with primary antibodies specific for IRE1α, PERK and Cas-12. The cells were further stained with Alexa Fluor 633-labelled secondary antibody (Red). Scale bar = 10 µm.

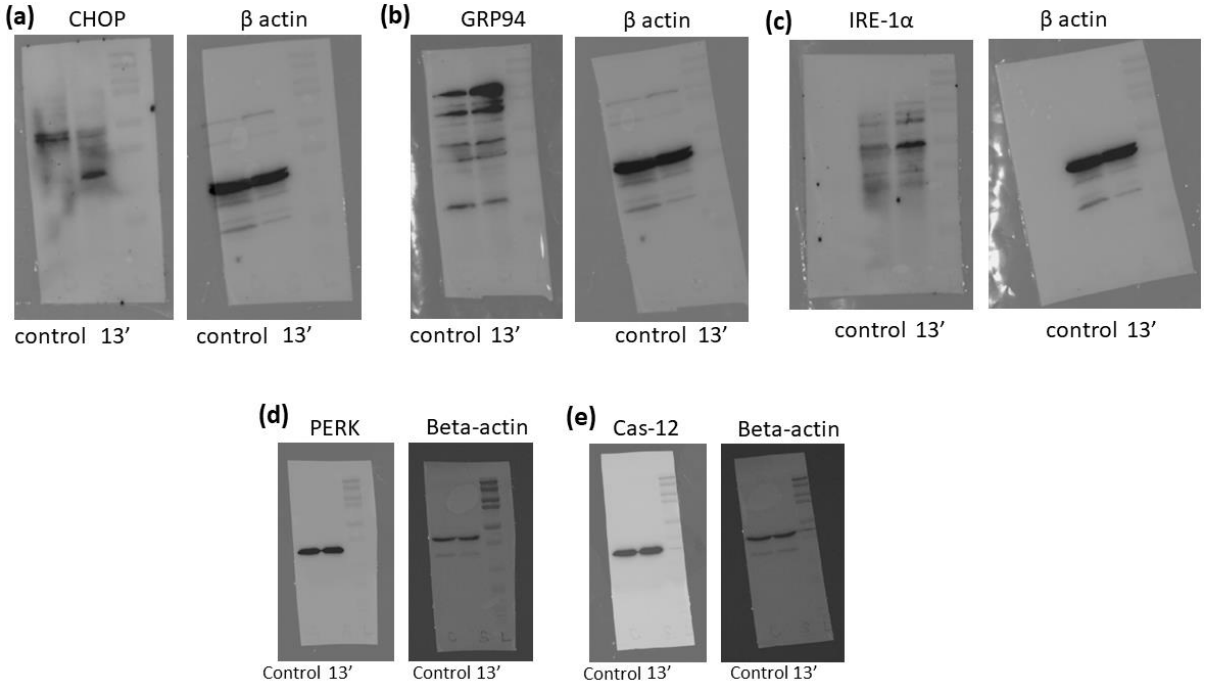


Fig. S92: Western blot images of (a-e) CHOP, GRP94, IRE-1α, PERK and Cas-12 expressions respectively after treating the HCT-116 cells with compound 13' for 24h.

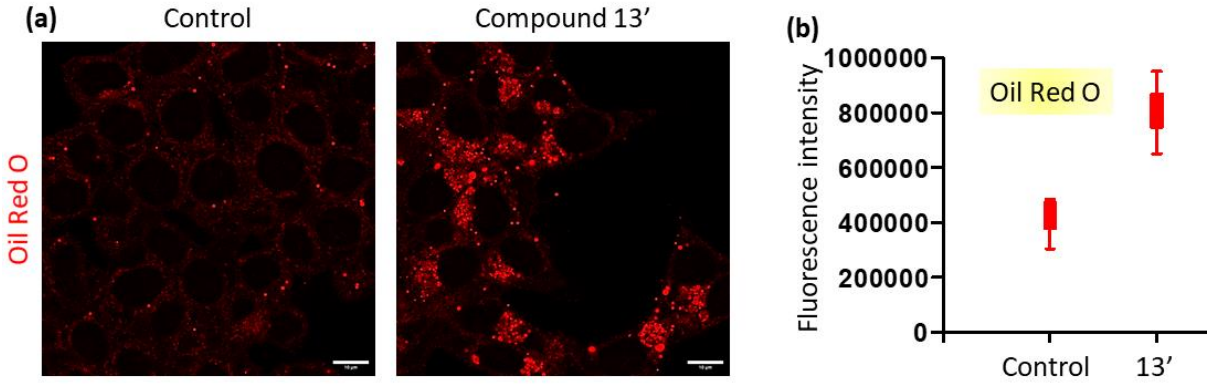


Fig. S93: (a) The confocal microscopy images and (b) quantification from the confocal imaging of HCT-116 cells after incubation with compound 13' for 24 h followed by staining with Oil Red O dye (red). Scale bar = 10 µm.

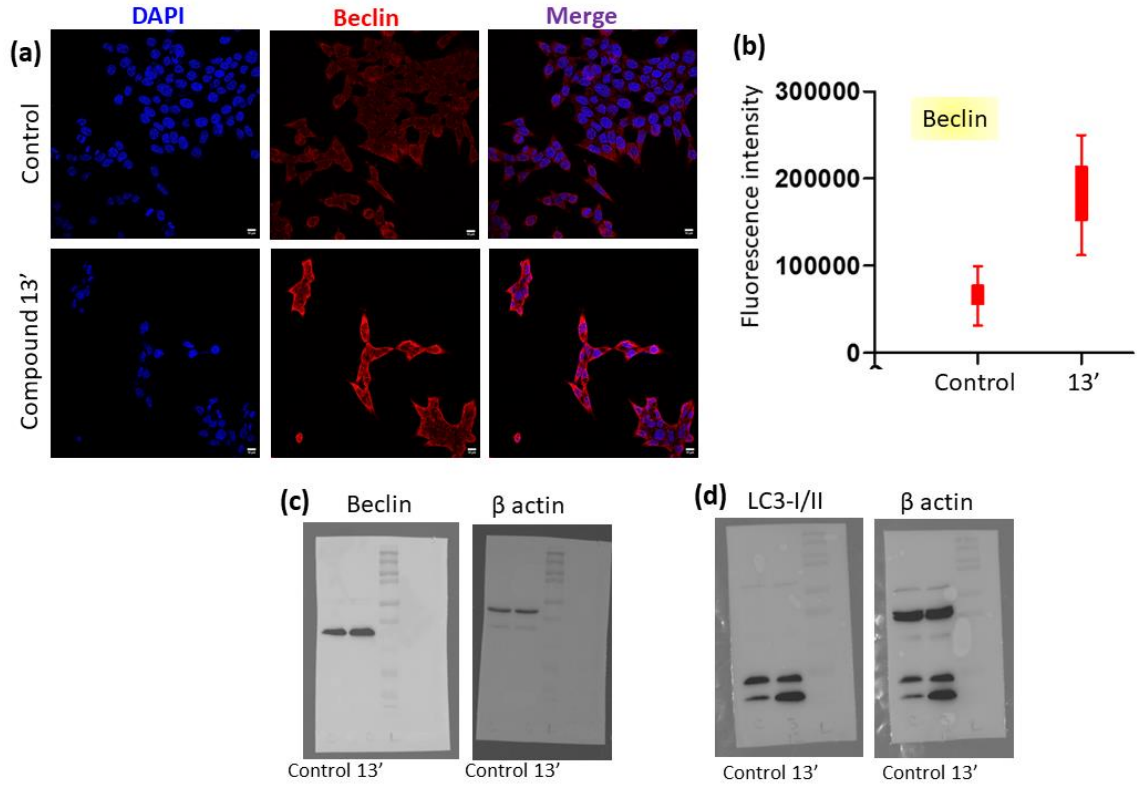


Fig. S94: (a) Confocal laser scanning microscopy images and (b) quantification form the confocal imaging of HCT-116 cells after treatment with compound 13' for 24h, followed by staining with primary antibodies specific for Beclin. The cells were further stained with Alexa Fluor 633-labelled secondary antibody (Red). Nuclei were stained with DAPI (blue). Scale bar = 10 µm. (c, d) Expression of Beclin and LC3-I/II in HCT-116 cells after treatment with compound 13' for 24h followed by Western blot analysis.

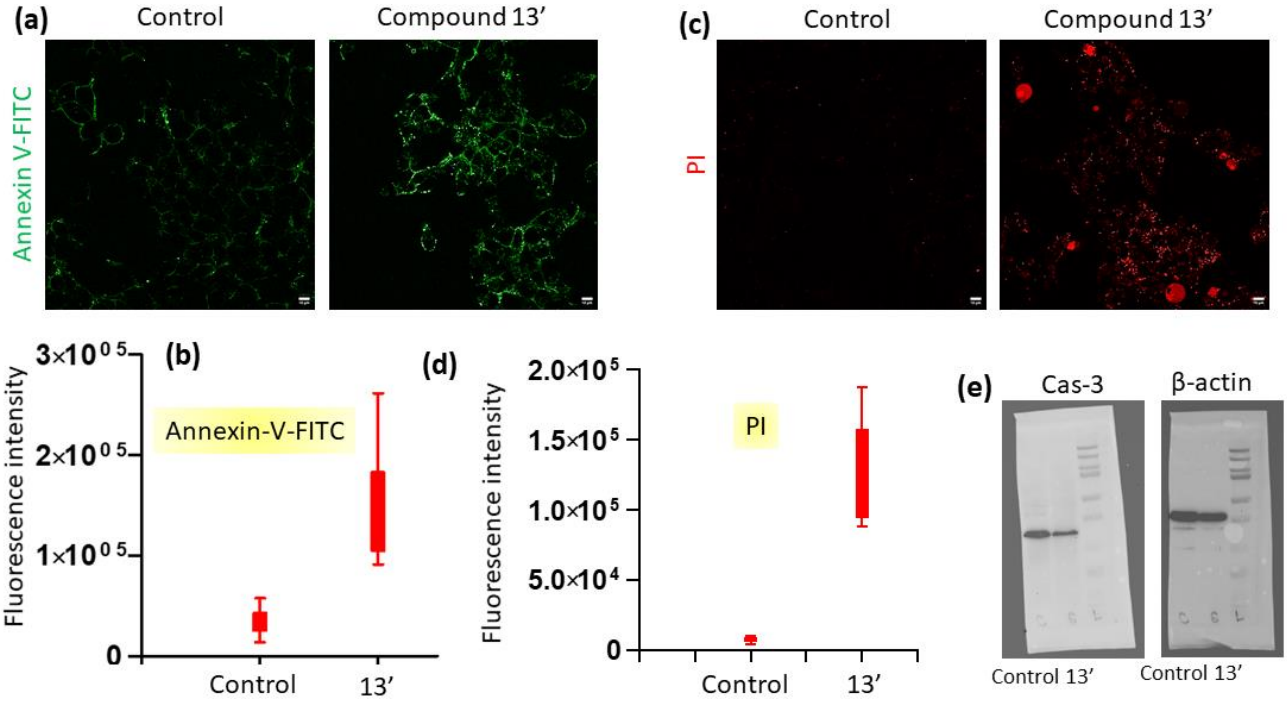


Fig. S95: (a-d) Confocal microscopy images and quantification from the confocal imaging of HCT-116 cells after treatment with compound 13' for 24h followed by staining the cells with Annexin-V-FITC (green) and PI (red) to visualize apoptosis. Scale bar = 10 µm. (e) Expression of Cas-3 in HCT-116 cells after treatment with compound 13' for 24h followed by Western blot analysis.