Supporting Information

Rationally designed mitochondria-impairing small molecule enabled chemo-phototherapy to potentiate apoptosis and autophagy in cancer cells

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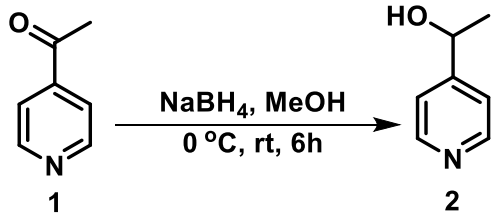
Materials: All the materials used for the experiments were purchased from various suppliers. The chemicals were purchase from BLD PHARMA , TCI, Avra, Finar and GLR innovation. Borosil or Asco glassware were used to set the reactions. Inert reactions were carried out under nitrogen or argon atmosphere. Analytical TLC was used were silica gel 60 F254 is coated on aluminium sheet (made in Germany). Heidolph Rotary evaporator was used to evaporate the solvent. Crude compounds were purified by column chromatography using silica 100-200 & 230-400 mesh size, as a stationary phase. UV spectra were recorded on a Shimadzu spectrophotometer. Fluorescence experiments were done on Fluorolog HORIBA JOBIN YVON. 1H and 13C spectra were recorded in DMSOd6 solvent and recorded on Ascend NMR-500MHz (Bruker) NMR spectrometer. Chemical shifts are indicated in parts per million (PPM). Residual protons in the deuterated solvents are considered as a reference ppm value. FE-SEM experiment was done using JeOL JSM-7600F.DLS was performed using a Multi-Angle DLS instrument from Malvern Panalytical. For cell biology experiments, cells were purchased from NCCS Pune and cultured using DMEM, 10 % FBS and Pen-Strep purchased from Gibco. MTT was used for measuring the cell viability and purchased from Sigma, ER Tracker green, Lyso Tracker Green DND-26, Golgi Tracker green (BODIPY FL C_5-Ceramide), Mito Tracker green FM, Annexin V, Propidium Iodide (PI), MitoSOX, TMRM, JC-1 was purchased from Thermo Fischer Scientific, and H2DCFDA was purchased from Sigma. BCl-2 (C-2), Beclin antibody was purchased from Santa Cruz and COX-2, Casp-9, Bax, PARP, Casp-3, and LC3 were purchased from Cell Signalling. Imaging was performed using a Leica TCS SP8 confocal microscope. Data was analysed and plotted by using GraphPad Prism and Origin. Confocal images were analysed and quantified by using ImageJ. Western blot images were quantified by using Image Lab software.

Methods:

Synthesis of compound 2.



In a clean, oven-dried 50 mL round-bottom flask equipped with a Teflon-coated magnetic stir bar, 4-acetylpyridine (600 mg, 1.0 eq, 4.12mmol) was dissolved in methanol (4 mL) under stirring. The reaction mixture was cooled to 0 °C using an ice-water bath. To the chilled solution, sodium borohydride (171.7mg, 1.1 eq, 4.53mmol) was added portion-wise over 5–10 minutes with constant stirring, maintaining the temperature at 0 °C. The reaction was stirred at 0 °C for an additional 30 minutes and then allowed to warm to room temperature and stirred for 12 hours. After completion, the methanol was evaporated under reduced pressure using a rotary evaporator and the residue was dissolved in ice-cold water to quench excess sodium borohydride using ammonium chloride. The resulting mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulphate and filtered, and concentrated under reduced pressure using a rotary evaporator. Pale yellow liquid was obtained. and which was used directly for further ester coupling reaction.

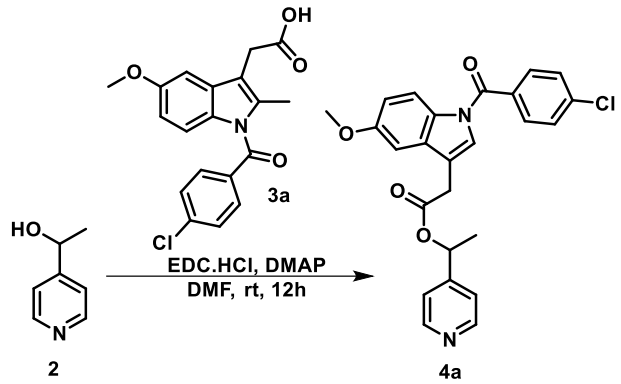
Yield: 95%

^{1}H NMR (500 MHz, CDCl3) δ 8.40 (d, J = 6.2 Hz, 1H), 7.31 (d, J = 5.3 Hz, 2H), 4.89 (q, J = 6.6 Hz, 1H), 1.47 (d, J = 6.6 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 156.18, 148.98, 120.70, 68.28, 25.01.

HRMS (ESI-TOF) (m/z): $[M+H]^+$ calculated for C_7H_9NO = 124.0762 Observed $[M+H]^+$ = 124.0769

Synthesis of compound 4a



In a clean, oven-dried 50 mL round-bottom flask equipped with a Teflon-coated magnetic stir bar, take 1-(pyridin-4-yl)ethan-1-ol (198mg, 1eq, 1.60mmol), Indomethacin (574mg, 1eq, 1.60mmol), EDC.HCl (338mg, 1.1eq, 1.76mmol) and 4-Dimethylaminopyridine (98mg, 0.5eq, 0.8mmol) were added under an inert nitrogen atmosphere. Anhydrous dimethylformamide (8 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 24 hours. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into ice and extracted thrice using ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulphate and filtered, and concentrated under reduced pressure using a rotary evaporator. The residue was further purified using silica gel column chromatography to obtain the pure product as a pale-yellow solid.

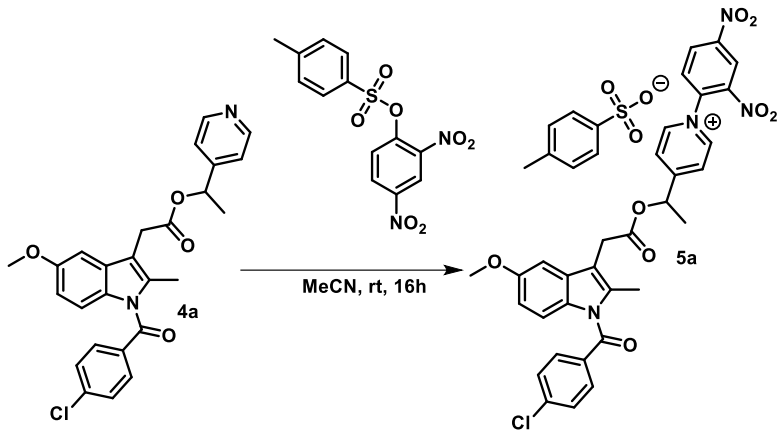
Yield: 85%

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 6.3 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.13 (d, *J* = 6.1 Hz, 2H), 6.92 (d, *J* = 2.5 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 6.68 (dd, *J* = 9.0, 2.5 Hz, 1H), 5.83 (q, *J* = 6.6 Hz, 1H), 3.78 (s, 3H), 3.72 (s, 2H), 2.38 (s, 3H), 1.52 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.82, 168.24, 156.12, 150.16, 150.03, 139.36, 135.97, 133.86, 131.15, 130.87, 130.48, 129.14, 120.53, 114.97, 112.22, 111.75, 101.40, 71.42, 55.67, 30.55, 21.86, 13.28.

HRMS (ESI-TOF) (m/z): $[M+H]^+$ calculated for $C_{26}H_{23}ClN_2O_4$ = 463.1425 Observed $[M+H]^+$ = 463.1425

Synthesis of compound 5a



In a clean oven-dried sealed tube with Teflon-coated magnetic stir bar, 2,4-dinitrophenyl p-toluenesulfonate (300mg, 1eq, 0.64mmol) and 1-(pyridin-4-yl)ethyl 2-(1-(4-chlorobenzoyl)-5-methoxy-1H-indol-3-yl)acetate (120mg, 1.1 eq, 0.70mmol) were dissolved in anhydrous acetonitrile (10 mL), and nitrogen was purged into the sealed tube. The reaction was refluxed for 2h and allowed to cool down to room temperature. The resulting precipitate was filtered, washed with diethyl ether and hexane thrice to remove trace impurities, and the solid product was further used for the synthesis of cyanine dye without any further purification.

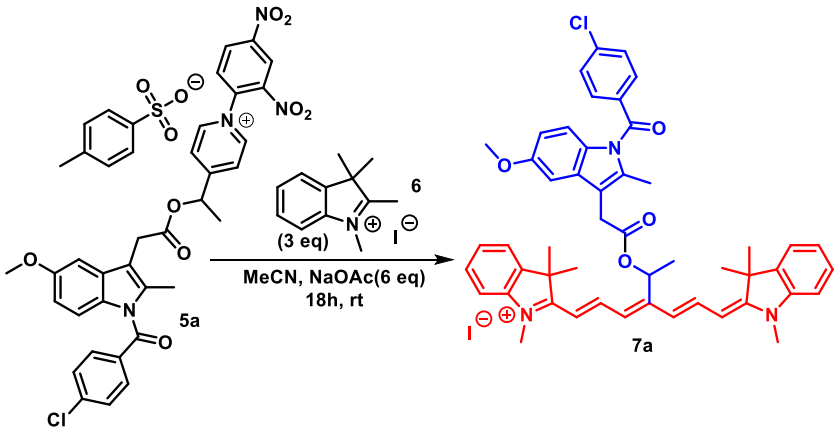
Yield: 75%

^{1}H NMR (500 MHz, DMSO) δ 9.37 (d, J = 6.9 Hz, 2H), 9.13 (d, J = 2.6 Hz, 1H), 8.97 (dd, J = 8.7, 2.6 Hz, 1H), 8.41 (t, J = 7.6 Hz, 3H), 7.71 – 7.63 (m, 4H), 7.47 (d, J = 8.2 Hz, 2H), 7.13 (d, J = 2.6 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 6.94 (d, J = 9.0 Hz, 1H), 6.74 (dd, J = 9.0, 2.6 Hz, 1H), 6.20 (q, J = 6.7 Hz, 1H), 4.04 (d, J = 2.7 Hz, 2H), 3.76 (s, 3H), 2.29 (s, 3H), 2.27 (s, 3H), 1.64 (d, J = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 170.32, 168.37, 164.37, 156.15, 149.67, 146.65, 146.45, 143.60, 138.95, 138.25, 137.92, 136.20, 134.52, 132.41, 131.64, 130.93, 130.78, 130.60, 129.57, 128.44, 125.97, 124.68, 121.90, 115.12, 112.70, 112.01, 102.27, 70.95, 56.00, 29.67, 21.76, 21.21, 13.68.

HRMS (ESI-TOF) (m/z): $[M]^+$ calculated for $C_{32}H_{26}ClN_4O_8^+$ = 629.1434 Observed $[M]^+$ = 629.1424

Synthesis of compound 7a



In a clean oven-dried 50ml round-bottom flask with Teflon-coated magnetic stir bar, take 4-(1-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetoxy)ethyl)-1-(2,4-dinitrophenyl)pyridin-1-ium 4-methylbenzenesulfonate (100mg, 1eq, 0.12mmol) and dissolve in

anhydrous acetonitrile (10 mL), in the presence of nitrogen atmosphere. Then 1,2,3,3-Tetramethyl-3H-indol-1-ium Iodide (112.7, 3eq, 0.36mmol) and Sodium acetate (298mg, 6eq, 0.72 mmol) were added, and the round-bottom flask was covered with aluminium foil to avoid light exposure. Further, the reaction was stirred for 16h at room temperature. After 16 hours, a green colour was observed and the solvent was evaporated under reduced pressure using a rotary evaporator. The resulting green residue was further purified using silica gel column chromatography with dichloromethane and methanol as the mobile phase, and the pure product was obtained as a dark green solid.

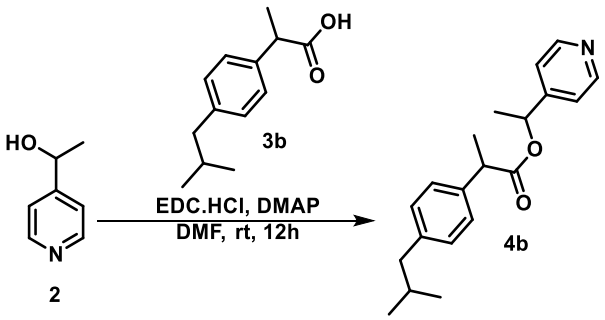
Yield: 80%

^{1}H NMR (500 MHz, DMSO) δ 8.05 (t, *J* = 13.4 Hz, 2H), 7.68 – 7.61 (m, 4H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.46 – 7.37 (m, 4H), 7.25 (td, *J* = 7.2, 1.5 Hz, 2H), 7.01 (d, *J* = 2.6 Hz, 1H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.71 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.63 (d, *J* = 13.3 Hz, 2H), 6.39 (d, *J* = 13.3 Hz, 2H), 6.12 (q, *J* = 6.7 Hz, 1H), 3.98 – 3.79 (m, 2H), 3.72 (s, 3H), 3.62 (s, 6H), 2.23 (s, 3H), 1.66 (s, 6H), 1.63 (s, 6H), 1.59 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 171.99, 170.24, 168.32, 159.08, 156.08, 143.42, 143.24, 141.38, 138.23, 134.48, 131.59, 130.88, 130.75, 129.53, 128.92, 125.19, 122.77, 121.06, 115.08, 112.93, 111.98, 111.53, 105.93, 102.10, 79.66, 69.63, 55.91, 49.14, 31.93, 29.98, 27.88, 27.80, 21.63, 13.68.

HRMS (ESI-TOF) (m/z): [M^+] calculated for $C_{50}H_{51}ClN_3O_4^+$ = 792.3563 Observed [M^+] = 792.3522

Synthesis of compound 4b



In a clean, oven-dried 50 mL round-bottom flask equipped with a Teflon-coated magnetic stir bar, take 1-(pyridin-4-yl)ethan-1-ol (900mg, 1eq, 7.31mmol), Ibuprofen (1500mg, 1eq, 7.31mmol), and EDC.HCl (1670mg, 1.1eq, 8.77mmol) and 4-Dimethylaminopyridine (446mg, 0.5eq, 3.65mmol) were added under an inert nitrogen atmosphere. Anhydrous dimethylformamide (8 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 24 hours. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into ice and extracted three times using ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulphate and filtered, and concentrated under reduced pressure using a rotary evaporator. The residue was further purified using silica gel column chromatography to obtain the pure product as a white solid.

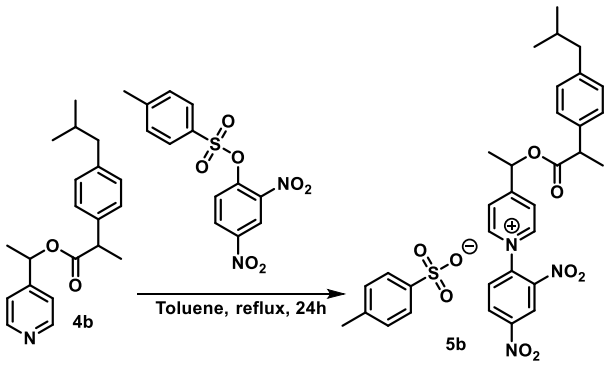
Yield: 70%

^{1}H NMR (500 MHz, CDCl3) δ 8.40 (d, *J* = 6.1 Hz, 2H), 7.15 (d, *J* = 8.1 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.88 (d, *J* = 6.1 Hz, 2H), 5.79 (q, *J* = 6.7 Hz, 1H), 3.76 (q, *J* = 7.1 Hz, 1H), 2.47 (d, *J*

= 7.2 Hz, 2H), 1.86 (dp, *J* = 13.5, 6.7 Hz, 1H), 1.50 (d, *J* = 7.2 Hz, 3H), 1.48 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 6H).

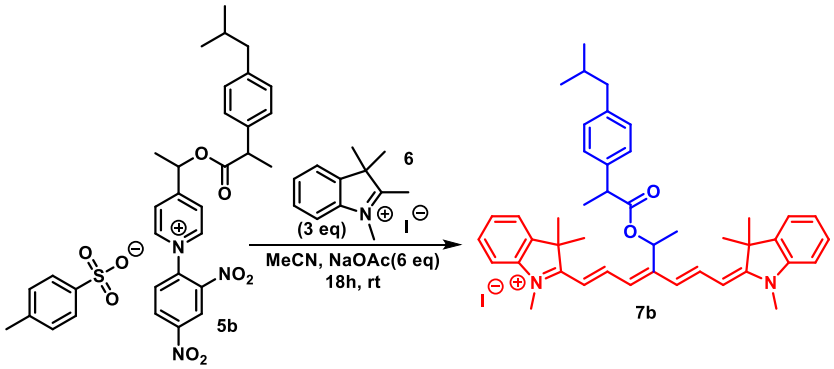
^{13}C NMR (126 MHz, CDCl3) δ 173.53, 150.64, 149.74, 140.78, 137.42, 129.38, 127.28, 120.23, 70.60, 45.19, 45.01, 30.20, 22.33, 22.31, 21.88, 17.97.

Synthesis of compound 5b



In a clean oven-dried sealed tube with a Teflon-coated magnetic stir bar, 2,4-dinitrophenyl p-toluenesulfonate (538mg, 1.1eq, 1.76mmol) and 1-(pyridin-4-yl)ethyl 2-(4-isobutylphenyl)propanoate (500mg, 1eq, 1.60mmol) were dissolved in anhydrous toluene (10 mL), and nitrogen was purged into the sealed tube. The reaction was refluxed for 24h, monitored by TLC, and cooled to room temperature once the starting materials were completely consumed. The resulting precipitate was dried under reduced pressure, and the solid product was used without further purification for the synthesis of a cyanine dye.

Synthesis of compound 7b



In a clean oven-dried 50ml round-bottom flask with Teflon-coated magnetic stir bar, take 1-(2,4-dinitrophenyl)-4-(1-((2-(4-isobutylphenyl)propanoyl)oxy)ethyl)pyridin-1-ium4-methylbenzenesulfonate (828mg, 1eq, 1.28 mmol) and dissolve in anhydrous acetonitrile (10 mL), in the presence of nitrogen atmosphere. Then 1,2,3,3-Tetramethyl-3H-indol-1-ium Iodide (1160mg, 3 eq, 3.85mmol) and Sodium acetate (756mg, 6 eq, 7.7mmol) were added, and the round-bottom flask was covered with aluminium foil to prevent light exposure. Further, the reaction was stirred for 16h at room temperature. After 16 hours, a green colour was observed, and the solvent was evaporated under reduced pressure using a rotary evaporator. The resulting green residue was further purified by silica gel column chromatography using dichloromethane and methanol as the mobile phase, yielding a dark green solid.

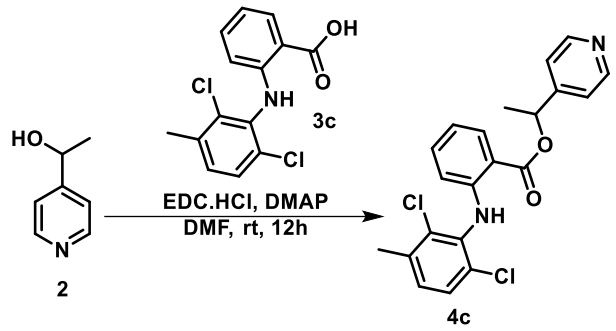
Yield: 72%

^{1}H NMR (500 MHz, CD_3CN) δ 8.14 (t, *J* = 13.4 Hz, 1H), 8.08 (t, *J* = 13.5 Hz, 1H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.27 (ddd, *J* = 10.8, 7.7, 2.5 Hz, 5H), 7.21 – 7.13 (m, 2H), 7.08 (d, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 13.4 Hz, 1H), 6.48 (d, *J* = 13.4 Hz, 1H), 6.35 (d, *J* = 13.4 Hz, 1H), 6.23 (d, *J* = 13.4 Hz, 1H), 6.12 (p, *J* = 7.0 Hz, 1H), 3.82 (dq, *J* = 11.1, 7.1 Hz, 1H), 3.59 (s, 3H), 3.56 (s, 3H), 2.48 (d, *J* = 7.2 Hz, 1H), 2.37 (d, *J* = 6.7 Hz, 5H), 1.89 – 1.79 (m, 1H), 1.75 (dq, *J* = 13.6, 6.7 Hz, 1H), 1.69 (s, 3H), 1.67 (s, 4H), 1.64 (s, 3H), 1.59 (s, 1H), 1.53 (d, *J* = 6.7 Hz, 1H), 1.45 (dd, *J* = 9.4, 7.1 Hz, 3H), 0.94 – 0.85 (m, 6H), 0.80 (dd, *J* = 10.8, 6.6 Hz, 3H).

^{13}C NMR (126 MHz, CD_3CN) δ 174.34, 174.31, 172.96, 172.88, 160.64, 160.53, 144.42, 143.87, 143.84, 141.81, 141.78, 141.30, 141.05, 138.50, 138.35, 129.94, 129.84, 129.19, 127.83, 127.78, 125.51, 125.49, 122.79, 121.61, 121.30, 117.86, 111.38, 111.35, 105.62, 105.49, 69.65, 69.47, 49.63, 49.62, 45.49, 45.29, 45.02, 44.99, 31.88, 31.86, 30.59, 30.52, 30.48, 27.82, 27.75, 27.70, 27.63, 22.16, 22.13, 22.09, 21.20, 21.08, 18.86, 18.63, 13.96, 13.91.

HRMS (ESI-TOF) (m/z): [M^+] calculated for $C_{44}H_{53}N_2O_2^+$ = 641.4102 Observed [M^+] = 641.4102

Synthesis of compound 4c



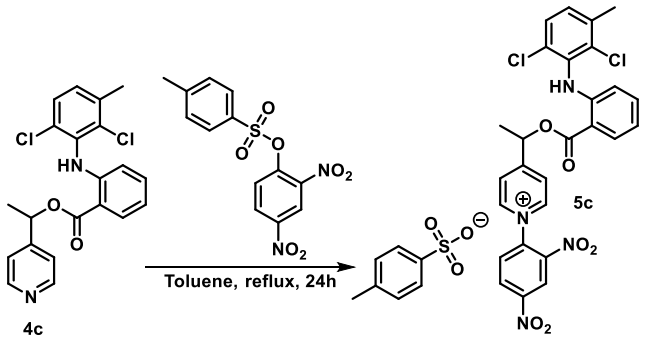
In a clean, oven-dried 50 mL round-bottom flask equipped with a Teflon-coated magnetic stir bar, take 1-(pyridin-4-yl)ethan-1-ol (480mg, 1eq, 3.8mmol), meclofenomic acid HCl salt (1295mg, 1eq, 3.8mmol), EDC.HCl (1490mg, 2.0eq, 7.6mmol) and 4-Dimethylaminopyridine (952mg, 0.5eq, 1.9mmol) were added under an inert nitrogen atmosphere. Anhydrous dimethylformamide (8 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 24 hours. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into ice and extracted thrice using ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulphate and filtered, and concentrated under reduced pressure using a rotary evaporator. The residue was further purified using silica gel column chromatography to obtain the pure product as a white solid.

Yield: 85%

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.62 (d, *J* = 5.2 Hz, 2H), 8.11 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.32 – 7.22 (m, 2H), 7.10 (d, *J* = 8.3 Hz, 1H), 6.80 (t, *J* = 7.6 Hz, 1H), 6.34 (d, *J* = 8.5 Hz, 1H), 6.12 (q, *J* = 6.6 Hz, 1H), 2.39 (s, 3H), 1.69 (d, *J* = 6.5 Hz, 3H).

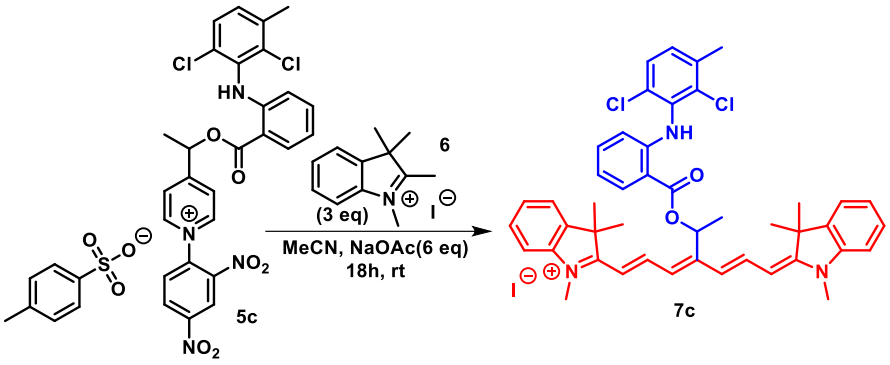
^{13}C NMR (126 MHz, $CDCl_3$) δ 167.41, 150.76, 150.14, 147.92, 136.51, 135.07, 134.33, 131.29, 128.62, 127.74, 120.62, 117.41, 113.94, 111.31, 70.97, 22.13, 20.56.

Synthesis of compound 5c



In a clean oven-dried sealed tube with a Teflon-coated magnetic stir bar, 2,4-dinitrophenyl p-toluenesulfonate (560mg, 1.1eq, 1.69mmol) and 1-(pyridin-4-yl)ethyl 2-((2,6-dichloro-3-methylphenyl)amino)benzoate (520mg, 1eq, 1.53mmol) were dissolved in anhydrous toluene (10 mL), and nitrogen was purged into the sealed tube. The reaction was refluxed for 24h, monitored by TLC, and cooled to room temperature once the starting materials were completely consumed. The resulting precipitate was dried under reduced pressure, and the solid product was used without further purification for the synthesis of a cyanine dye.

Synthesis of compound 7c



In a clean oven-dried 50ml round-bottom flask with Teflon-coated magnetic stir bar, take 4-(1-((2-((2,6-dichloro-3-methylphenyl)amino)benzoyl)oxy)ethyl)-1-(2,4-dinitrophenyl)pyridin-1-ium4-methylbenzenesulfonate (485mg, 1eq, 0.65mmol) and dissolve in anhydrous acetonitrile (10 mL), in the presence of a nitrogen atmosphere. Then 1,2,3,3-Tetramethyl-3H-indol-1-ium Iodide (591mg, 3 eq, 1.96mmol) and Sodium acetate (385mg, 6 eq, 3.92mmol) were added, and the round-bottom flask was covered with aluminium foil to prevent light exposure. Further, the reaction was stirred for 16h at room temperature. After 16 hours, a green colour was observed, and the solvent was evaporated under reduced pressure using a rotary evaporator. The resulting green residue was further purified by silica gel column chromatography using dichloromethane and methanol as the mobile phase, yielding a dark green solid.

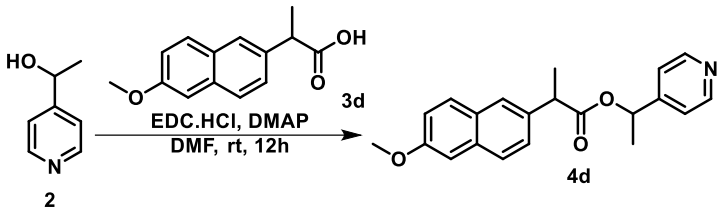
Yield: 75%

^{1}H NMR (500 MHz, CD_3CN) δ 9.15 (s, 1H), 8.27 – 8.19 (m, 3H), 7.62 (s, 1H), 7.50 (d, *J* = 7.4 Hz, 2H), 7.43 (td, *J* = 7.8, 1.2 Hz, 3H), 7.37 (ddd, *J* = 8.8, 7.2, 1.7 Hz, 1H), 7.30 – 7.22 (m, 5H), 6.89 (t, *J* = 7.6 Hz, 1H), 6.69 (d, *J* = 13.4 Hz, 2H), 6.36 (s, 1H), 6.35 – 6.27 (m, 3H), 3.58 (s, 6H), 2.39 (d, *J* = 14.4 Hz, 3H), 1.80 (d, *J* = 6.7 Hz, 3H), 1.72 (s, 6H), 1.65 (s, 6H).

^{13}C NMR (126 MHz, CD_3CN) δ 173.02, 168.22, 160.90, 148.53, 144.34, 143.86, 141.86, 137.58, 135.44, 135.39, 134.83, 132.14, 129.94, 129.19, 128.56, 125.53, 122.79, 121.55, 118.35, 114.31, 111.73, 111.38, 105.71, 78.80, 70.71, 49.68, 31.83, 27.74, 27.70, 21.34, 20.21.

HRMS (ESI-TOF) (m/z): $[M^+]$ calculated for $C_{45}H_{46}Cl_2N_3O_2^+$ = 730.2962 Observed $[M^+]$ = 730.3008

Synthesis of compound 4d



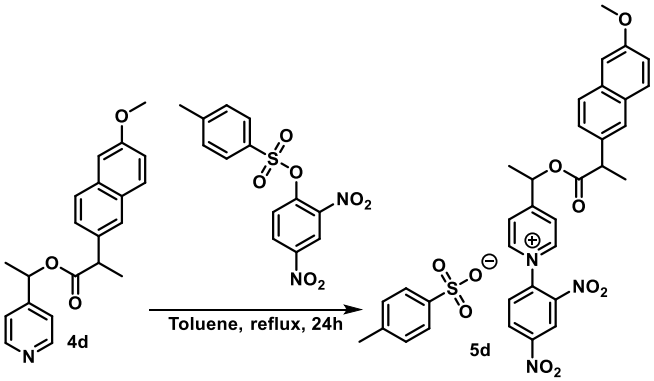
In a clean, oven-dried 50 mL round-bottom flask equipped with a Teflon-coated magnetic stir bar, take 1-(pyridin-4-yl)ethan-1-ol (800mg, 1eq, 6.45mmol), Naproxen (1495mg, 1eq, 6.45mmol), EDC.HCl (1490mg, 1.2eq, 7.09mmol) and 4-Dimethylaminopyridine (396mg, 0.5eq, 3.22mmol) were added under an inert nitrogen atmosphere. Anhydrous dimethylformamide (8 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 24 hours. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into ice and extracted thrice using ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulphate and filtered, and concentrated under reduced pressure using a rotary evaporator. The residue was further purified using silica gel column chromatography to obtain the pure product as a white solid.

Yield: 85%

^{1}H NMR (500 MHz, CDCl3) δ 8.55 – 8.50 (m, 1H), 8.35 – 8.30 (m, 1H), 7.74 – 7.63 (m, 2H), 7.59 (d, *J* = 1.9 Hz, 0.6H), 7.41 (dd, *J* = 8.5, 1.9 Hz, 0.4H), 7.32 (dd, *J* = 8.5, 1.9 Hz, 0.6H), 7.25 – 7.21 (m, 0.26H), 7.20 – 7.10 (m, 2H), 6.89 – 6.85 (m, 1H), 5.80 (p, *J* = 6.8 Hz, 1H), 3.91 (d, *J* = 3.2 Hz, 4H), 1.58 (dd, *J* = 8.6, 7.1 Hz, 3H), 1.47 (d, *J* = 6.7 Hz, 2H), 1.40 (d, *J* = 6.7 Hz, 1H).

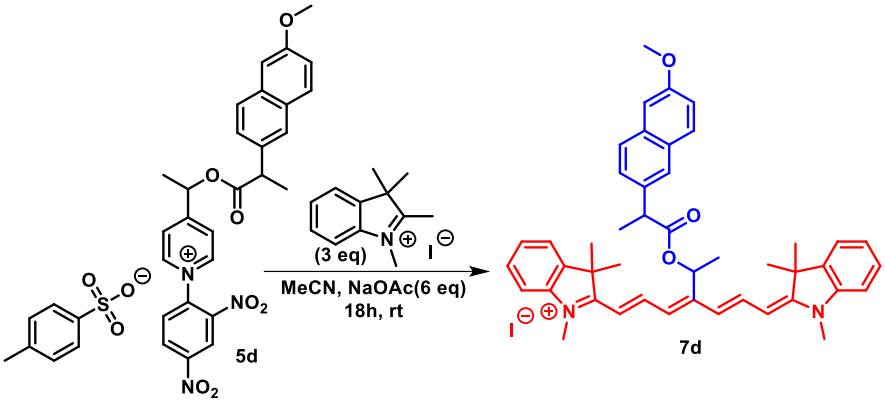
^{13}C NMR (126 MHz, CDCl3) δ 173.66, 173.51, 157.78, 157.76, 150.48, 150.45, 150.06, 150.01, 149.78, 135.29, 135.25, 133.78, 129.27, 129.22, 128.96, 128.92, 127.24, 127.20, 126.19, 126.15, 126.08, 125.97, 120.65, 120.59, 120.32, 119.14, 119.07, 105.68, 71.01, 70.89, 55.31, 45.54, 45.51, 21.87, 21.62, 18.27, 18.21.

Synthesis of compound 5d



In a clean oven-dried sealed tube with a Teflon-coated magnetic stir bar, 2,4-dinitrophenyl p-toluenesulfonate (1180mg, 1.1eq, 2.95mmol) and 1-(pyridin-4-yl)ethyl 2-(6-methoxynaphthalen-2-yl)propanoate (900mg, 1eq, 2.68mmol) were dissolved in anhydrous toluene (10 mL), and nitrogen was purged into the sealed tube. The reaction was refluxed for 24h, monitored by TLC, and cooled to room temperature once the starting materials were completely consumed. The resulting precipitate was dried under reduced pressure, and the solid product was used without further purification for the synthesis of a cyanine dye.

Synthesis of compound 7d



In a clean oven-dried 50ml round-bottom flask with Teflon-coated magnetic stir bar, take 1-(2,4-dinitrophenyl)-4-(1-((2-(6-methoxynaphthalen-2-yl)propanoyl)oxy)ethyl)pyridin-1-ium4-methylbenzenesulfonate (688mg, 1eq, 1.02mmol) and dissolve in anhydrous acetonitrile (10 mL), in the presence of nitrogen atmosphere. Then 1,2,3,3-Tetramethyl-3H-indol-1-ium Iodide (922mg, 3 eq, 3.06mmol) and Sodium acetate (601mg, 6 eq, 6.12mmol) were added, and the round-bottom flask was covered with aluminium foil to prevent light exposure. Further, the reaction was stirred for 16h at room temperature. After 16 hours, a green colour was observed, and the solvent was evaporated under reduced pressure using a rotary evaporator. The resulting green residue was further purified by silica gel column chromatography using dichloromethane and methanol as the mobile phase, yielding a dark green solid.

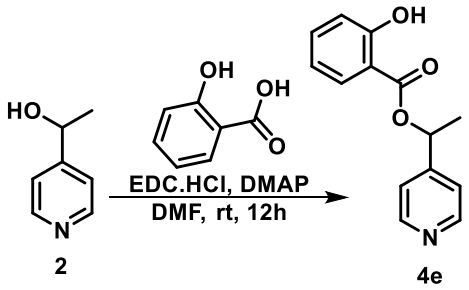
Yield: 68%

^{1}H NMR (500 MHz, CD_3CN) δ 8.10 (t, *J* = 13.4 Hz, 1H), 7.97 (t, *J* = 13.4 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.77 (d, *J* = 1.8 Hz, 0.5H), 7.70 (dd, *J* = 8.7, 5.3 Hz, 1H), 7.62 (d, *J* = 1.9 Hz, 0.5H), 7.49 (d, *J* = 7.4 Hz, 2H), 7.44 (qd, *J* = 7.3, 1.5 Hz, 2H), 7.36 (dd, *J* = 8.4, 1.9 Hz, 0.6H), 7.31 – 7.21 (m, 4H), 7.17 (dd, *J* = 9.0, 2.6 Hz, 0.6H), 7.12 (d, *J* = 2.6 Hz, 0.5H), 7.03 (dd, *J* = 8.9, 2.6 Hz, 0.5H), 6.54 (d, *J* = 13.4 Hz, 1H), 6.31 (d, *J* = 13.4 Hz, 1H), 6.24 (d, *J* = 13.4 Hz, 1H), 6.13 (dd, *J* = 6.8, 3.2 Hz, 1H), 5.91 (d, *J* = 13.4 Hz, 1H), 3.97 (dt, *J* = 10.3, 7.1 Hz, 1H), 3.87 (s, 1.4H), 3.70 (s, 1.4H), 3.55 (s, 3H), 3.41 (s, 3H), 1.68 – 1.59 (m, 12H), 1.58 – 1.49 (m, 6H).

^{13}C NMR (126 MHz, CD_3CN) δ 174.13, 174.11, 172.89, 172.65, 160.86, 160.53, 158.38, 158.19, 144.34, 144.07, 143.83, 143.74, 141.78, 141.74, 136.23, 136.21, 134.42, 134.27, 129.88, 129.80, 129.47, 129.17, 127.70, 126.96, 126.93, 126.45, 126.39, 125.48, 125.44, 122.76, 121.40, 120.72, 119.48, 119.23, 111.36, 111.27, 106.37, 106.18, 105.55, 105.32, 55.64, 55.51, 49.59, 49.53, 45.92, 45.54, 31.87, 31.72, 27.75, 27.69, 27.67, 27.61, 21.05, 18.66, 18.29.

HRMS (ESI-TOF) (m/z): [M^+] calculated for $C_{45}H_{49}N_2O_3^+$ = 665.3738 Observed [M^+] = 665.3723.

Synthesis of compound 4e



In a clean, oven-dried 50 mL round-bottom flask equipped with a Teflon-coated magnetic stir bar, take 1-(pyridin-4-yl)ethan-1-ol (395mg, 1eq, 3.2mmol), salicylic acid (443mg, 1eq, 3.2mmol), EDC.HCl (737mg, 1.1eq, 3.52mmol) and 4-Dimethylaminopyridine (195mg, 0.5eq, 1.6mmol) were added under an inert nitrogen atmosphere. Anhydrous dimethylformamide (8 mL) was added to the flask, and the reaction mixture was stirred at room temperature for 24 hours. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured into ice and extracted thrice using ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulphate and filtered, and concentrated under reduced pressure using a rotary evaporator. The residue was further purified using silica gel column chromatography to obtain the pure product as a white solid.

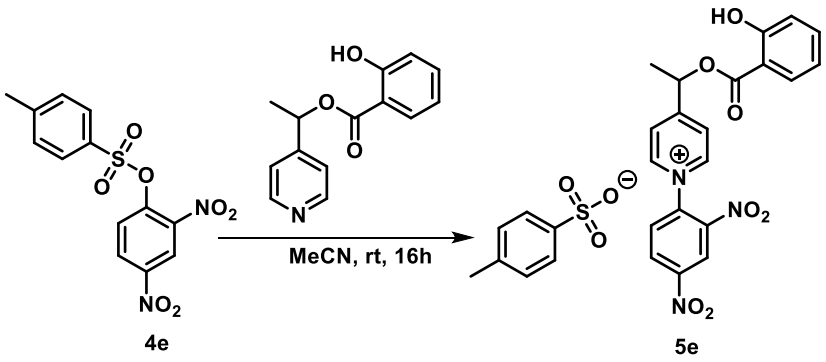
Yield: 85%

^{1}H NMR (500 MHz, CDCl3) δ 10.60 (s, 1H), 8.66 – 8.59 (m, 2H), 7.95 (dd, J = 8.0, 1.7 Hz, 1H), 7.49 (ddd, J = 8.7, 7.2, 1.7 Hz, 1H), 7.35 – 7.28 (m, 2H), 6.99 (dd, J = 8.4, 1.1 Hz, 1H), 6.93 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 6.10 (q, J = 6.7 Hz, 1H), 1.69 (d, J = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, CDCl3) δ 169.20, 161.93, 150.23, 149.92, 136.08, 129.76, 120.50, 119.29, 117.79, 112.19, 71.84, 21.92.

HRMS (ESI-TOF) (m/z): $[M + H]^+$ calculated for $C_{14}H_{13}NO_3$= 244.0968 Observed $[M + H]^+$ = 244.0858

Synthesis of compound 5e



In a clean oven-dried sealed tube with Teflon-coated magnetic stir bar, 2,4-dinitrophenyl p-toluenesulfonate (261.3mg, 1.1eq, 0.77mmol) and 1-(pyridin-4-yl)ethyl 2-hydroxybenzoate (171mg, 1eq, 0.70mmol) were dissolved in anhydrous acetonitrile (10 mL), and nitrogen was purged into the sealed tube. The reaction was refluxed for 2h and allowed to cool down to room temperature. The resulting precipitate was filtered, washed with diethyl ether and hexane thrice

to remove trace impurities, and the solid product was further used for the synthesis of cyanine dye without any further purification.

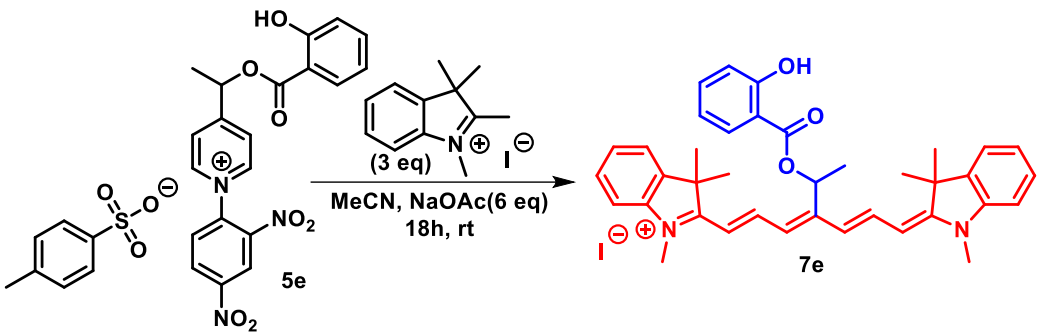
Yield: 80%

^{1}H NMR (500 MHz, DMSO) δ 10.35 (s, 1H), 9.39 (d, *J* = 6.9 Hz, 2H), 9.12 (d, *J* = 2.6 Hz, 1H), 8.97 (dd, *J* = 8.7, 2.5 Hz, 1H), 8.56 (d, *J* = 7.1 Hz, 2H), 8.43 (d, *J* = 8.7 Hz, 1H), 8.03 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.58 (ddd, *J* = 8.8, 7.2, 1.8 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.08 – 6.97 (m, 2H), 6.45 (q, *J* = 6.7 Hz, 1H), 2.29 (s, 3H), 1.78 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 167.61, 163.95, 160.36, 149.65, 146.81, 146.41, 143.60, 139.01, 137.94, 136.39, 132.42, 131.24, 130.62, 128.45, 125.96, 124.97, 121.90, 119.93, 118.01, 113.84, 71.54, 21.79, 21.22.

HRMS (ESI-TOF) (m/z): $[M]^+$ calculated for = 410.0983 Observed $[M]^+$ = 410.0977

Synthesis of compound 7e



In a clean oven-dried 50ml round-bottom flask with Teflon-coated magnetic stir bar, take 1-(2,4-dinitrophenyl)-4-(1-((2-hydroxybenzoyl)oxy)ethyl)pyridin-1-ium 4-methylbenzenesulfonate (200mg, 1eq, 0.34mmol) and dissolve in anhydrous acetonitrile (10 mL), in the presence of nitrogen atmosphere. Then 1,2,3,3-Tetramethyl-3H-indol-1-ium Iodide (310mg, 3eq, 1.02mmol) and Sodium acetate (202.5mg, 6eq, 2.04mmol) were added, and the round-bottom flask was covered with aluminium foil to avoid light exposure. Further, the reaction was stirred for 16h at room temperature. After 16 hours, a green colour was observed and the solvent was evaporated under reduced pressure using a rotary evaporator. The resulting green residue was further purified using silica gel column chromatography with dichloromethane and methanol as the mobile phase, and the pure product was obtained as a dark green solid.

Yield: 72%

^{1}H NMR (500 MHz, DMSO) δ 10.39 (s, 1H), 8.10 (t, *J* = 13.4 Hz, 2H), 7.91 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.58 – 7.51 (m, 1H), 7.45 – 7.36 (m, 4H), 7.25 (t, *J* = 7.2 Hz, 2H), 7.00 (t, *J* = 7.2 Hz, 2H), 6.70 (d, *J* = 13.4 Hz, 2H), 6.46 (d, *J* = 13.5 Hz, 2H), 6.39 – 6.30 (m, 1H), 3.63 (s, 6H), 1.73 (d, *J* = 6.7 Hz, 3H), 1.67 (s, 6H), 1.61 (s, 6H).

^{13}C NMR (126 MHz, DMSO) δ 172.02, 168.28, 160.44, 158.53, 143.42, 143.15, 141.46, 136.29, 130.72, 128.91, 125.20, 122.75, 121.58, 119.90, 117.97, 113.69, 111.54, 106.21, 70.84, 49.13, 31.91, 27.84, 27.79, 21.60.

HRMS (ESI-TOF) (m/z): $[M]^+$ calculated for $C_{38}H_{41}N_2O_3^+$ = 573.3112 Observed $[M]^+$ = 573.3090

Heat generation:

We add 10 and 20 μM of the compound in DMSO to a small beaker, then irradiate with the 808 nm laser at 0.8W/cm2 and measure heat generation every minute with an IR thermometer.

ROS Generation Study Using DPBF

ROS generation by compound 7a was further evaluated using DPBF as an absorbance-based probe. Initially, the absorbance of DPBF solution (30 μM) was recorded in the dark. Compound 7a was then added to the DPBF solution to obtain a final concentration of 10 μM, and the absorbance was recorded again before irradiation. The solution was then saturated with oxygen by bubbling oxygen through the solution, followed by irradiation with an 808 nm laser at a power density of 0.8 W/cm². The decrease in DPBF absorbance was monitored in a time-dependent manner at 30 s intervals.

ROS Generation Study Using H_2DCFDA

ROS generation by compound 7a was evaluated using H_2DCFDA as a fluorescent probe. Briefly, 0.5 mL of H_2DCFDA solution (1 mM) was mixed with 2 mL of NaOH solution (10 mM in water) in a beaker and stirred for 30 min at room temperature to allow deacetylation. After this, 10 mL of 1× PBS was added to the solution. The initial fluorescence intensity of the solution was recorded. Compound 7a was then added to the same solution to obtain a final concentration of 5 μM. The solution was irradiated with an 808 nm laser at a power density of 0.8 W/cm², and the fluorescence intensity of DCF was monitored at 5 s intervals.

Monitoring the Stability of Nanoparticles

The stability of nanoparticles formed from compound 7a was evaluated by monitoring changes in hydrodynamic diameter and zeta potential over time. Briefly, two separate samples of compound 7a were prepared in water at a final concentration of 0.1 μM. One sample was stored at 4 °C, while the other was stored at room temperature. The hydrodynamic diameter and zeta potential of each sample were measured using dynamic light scattering (DLS). Measurements were performed daily for up to 5 days to assess the colloidal stability of the nanoparticles under different storage conditions.

Indomethacin Release from Compound 7a by HPLC

The release of indomethacin from compound 7a was evaluated using HPLC analysis following near-infrared laser irradiation. Sodium acetate buffer was prepared by dissolving sodium acetate trihydrate (13.6 g) in 750 mL of HPLC-grade water, followed by the addition of triethylamine (1 mL). The solution was diluted to 1 L with HPLC-grade water, and the pH was adjusted to 5.0 using glacial acetic acid.

The HPLC mobile phase consisted of sodium acetate buffer and acetonitrile in a 40:60 (v/v) ratio. A 125 μM aqueous solution of compound 7a was prepared, and 20 μL of the solution was injected into the HPLC system. The chromatographic run time was set to 20 min.

To study photoinduced drug release, the sample was exposed to an 808 nm laser at a power density of 0.8 W/cm² for 5 min. After each irradiation period, the sample was analyzed by HPLC to determine the amount of indomethacin released. The irradiation–analysis cycle was repeated every 5 min, and the release profile was monitored up to 35 min of total irradiation time.

Cell Viability assay in the dark:

In a 96-well plate, 5000 cells per well were seeded for HeLa, HCT116 and MDA-MB231 and RPE1 cells were left for attachment to the plate for 24h, and further cells were treated with the compound in a dose-dependent manner, with 1% DMSO used as a control and incubated for the next 24 h. The next day, the media was aspirated, and MTT reagent was added at the working concentration of 0.5 mg/mL in complete media with 10% PBS. The cells were further incubated with MTT for 4h to allow the formation of formazan crystals. Further, the media was aspirated, and 100µL of DMSO was added to dissolve the formed crystals. Absorbance was recorded at 570nm using a Perkin Elmer multimode plate reader, and analysed in GraphPad Prism to calculate cell viability.

Cell Viability assay under laser irradiation:

In a 96-well plate, 5000 cells per well were seeded for HeLa, HCT116 and MDA-MB231 and RPE1, cells were left for attachment to the plate for 24h, and further cells were treated with compound in a dose-dependent manner with 1% DMSO used as a control and incubated for 20 h followed by irradiation with 808 nm Laser at 0.8 W/cm² for 10 minutes and then further incubated for next 4h to complete the 24h incubation time period. Then, the media was aspirated, and MTT reagent was added at the working concentration of 0.5 mg/mL in complete media with 10% PBS. The cells were further incubated with MTT for 4h to allow the formation of formazan crystals. Further, the media was aspirated, and 100µL of DMSO was added to dissolve the formed crystals. Absorbance was recorded at 570nm by using a Perkin Elmer multimode plate reader and analysed by Graph Pad Prism to calculate the percentage cell viability.

Colocalisation study:

Five Live cell plates were taken, and 25000 HCT-116 cells were added to each plate with complete media and kept in the incubator for cell attachment. The next day, compound 7a at a 0.5 µM concentration is prepared in complete media. Then the media from the plate is removed, and the prepared solution is added. For one sample, it is incubated for 30 min; for the other four, it is incubated for 3 hours. Then, the media from the plate is removed, and the plate is washed twice with PBS to remove the remaining molecules. Further, the addition of Mitotracker Green (100 nM), Golgi Tracker Green(1µg/mL), Lyso Tracker green (80nM) and ER tracker green (100 nM) to the incubation for 30 minutes. Further media was aspirated, cells were washed with 1x PBS, and fresh DMEM was added. Samples were imaged using a Leica confocal microscope and analysed using ImageJ.

ROS generation by H2DCFDA:

Four live-cell plates were seeded with 25000 HCT-116 cells and incubated in the incubator for 24 h to allow attachment. After that, all four plates were treated as follows: in Control Dark and in

Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.4 µM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm² for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After that, the media from the plate was removed, and we gave the PBS wash, and the H2DCFDA was added at the working concentration of 2µM in DMEM to all four plates and incubated for 25 minutes, after which the media was aspirated cells were washed with 1x PBS twice, fresh DMEM was added, and samples were imaged with a Leica confocal microscope. During imaging, cells were maintained at 37°C, 95% humidity, and 5% CO2.

JC1 Assay:

Four live-cell plates were seeded with 25000 HCT-116 cells and incubated in the incubator for 24 h to allow attachment. After that, all four plates were treated as follows: in Control Dark and in Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.4 µM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm² for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After that, the media from the plate was removed, and we performed a PBS wash, and JC1 was added at the working concentration of 5 µg/mL in PBS to all the four plates and incubated for 15 minutes, after which media was aspirated cells were washed with 1x PBS twice, fresh DMEM was added, and samples were imaged with Leica confocal microscope. While imaging cells were maintained at 37°C, 95% humidity and 5% CO_2 atmosphere.

Mitochondria morphology:

Four live-cell plates were seeded with 25000 HCT-116 cells and incubated in the incubator for 24 h to allow attachment. After that, all four plates were treated as follows: in Control Dark and in Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.4 µM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm² for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After that, the media from the plate was removed, and we gave the PBS wash, and Mito Tracker green was added at the working concentration of 100 nM in DMEM to all the four plates and incubated for 15 minutes, after which media was aspirated cells were washed with 1x PBS twice, fresh DMEM was added, and samples were imaged with Leica confocal microscope. During imaging, cells were maintained at 37°C, 95% humidity, and 5% CO2.

TMRM Assay:

Four live-cell plates were seeded with 25000 HCT-116 cells and incubated in the incubator for 24 h to allow attachment. After that, all four plates were treated as follows: in Control Dark and in Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.4 µM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm² for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After that, the media from the plate was removed, and we performed a PBS wash, then added TMRM at a working concentration of 100 nM in DMEM to all four plates and incubated for 15 minutes. Afterwards, the media was aspirated,

cells were washed twice with 1x PBS, fresh DMEM was added, and samples were imaged with a Leica confocal microscope. During imaging, cells were maintained at 37°C, 95% humidity, and 5% CO2.

MitoSOX Assay:

Four live-cell plates were seeded with 25000 HCT-116 cells and incubated in the incubator for 24 h to allow attachment. After that, all four plates were treated as follows: in Control Dark and in Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.4 μM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm^2 for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After that, the media was removed from the plate, and we performed a PBS wash. MitoSOX was added at the working concentration of 500 nM in PBS to all four plates and incubated for 15 minutes. After which, the media was aspirated, cells were washed twice with 1x PBS, fresh DMEM was added, and samples were imaged with a Leica confocal microscope. While imaging cells were maintained at 37°C, 95% humidity and 5% CO_2 atmosphere.

Determination of ROS using Scavenger.

5000 HCT-116 cells were seeded in a 96-well plate and incubated for 24 hours. Cells were incubated with various concentrations of 7a, and 1% DMSO was used as a control, followed by the addition of scavengers such as Mannitol (0.5 mM) for hydroxyl radical, Sodium Azide (0.01 mM) for singlet oxygen, TEMPO (0.05 mM) for superoxide, and Sodium Pyruvate (1 mM) for H_2O_2. After incubating for 20 hours, the sample was irradiated with an 808 nm laser at 0.8 W/cm^2 for 10 min and then incubated for another 4 hours to complete the 24 h incubation. Further steps involve removing the media and adding MTT reagent solution (0.5 mg/ml in complete media with 10% 1xPBS) to the plate. The plate containing the MTT reagent was incubated for 4 h. After 4 h, the media was removed, DMSO (100 μL) was added to each well, and absorbance was measured with a Perkin Elmer multimode plate reader. The absorbance data were analysed and plotted using the software GraphPad Prism.

Annexin V Assay:

Four live-cell plates were seeded with 25000 HCT-116 cells and incubated in the incubator for 24 h to allow attachment. After that, all four plates were treated as follows: in Control Dark and in Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.4 μM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm^2 for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After that, the media was removed from the plate, and we performed a PBS wash. 5 μL of FITC-conjugated Annexin V was added to all four plates, and the plates were incubated for 25 minutes. After which, the media was aspirated, cells were washed twice with 1x PBS, fresh DMEM was added, and samples were imaged with a Leica confocal microscope. While imaging cells were maintained at 37°C, 95% humidity and 5% CO_2 atmosphere.

PI Assay:

Four live-cell plates were seeded with 25000 HCT-116 cells and incubated in the incubator for 24 h to allow attachment. After that, all four plates were treated as follows: in Control Dark and in Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.4 µM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm^2 for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After that, the media was removed from the plate, and cells were washed with PBS, and PI was added at the working concentration of 500 nM in DMEM to all four plates and incubated for 15 minutes, after which media was aspirated, cells were washed with 1x PBS twice, fresh DMEM was added, and samples were imaged with a Leica confocal microscope. While imaging cells were maintained at 37°C, 95% humidity and 5% CO_2 atmosphere.

FACS:

Four 6 cm tissue culture plates, each containing one lakh HCT116 cells, were seeded in complete media and incubated in the incubator for 24 h to allow the cells to attach. After that, all four plates are treated as follows: in Control Dark and Control Laser, only DMSO was added; in Treatment Dark and Treatment Laser, 0.6 µM of compound 7a was added. And incubated for 20 hr. After 20 h, the control and treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm^2 for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After 24 h of incubation period, cells were washed with 1x PBS, trypsinised, and centrifuged at 5000 rpm for 5 minutes, supernatant was discarded and cells were washed with PBS and again centrifuged for 5 minutes, cells were dissolved in 100µL of AV binding buffer and 5µL of FITC conjugated Annexin V and 1µL of 100µg/mL was added to the samples and incubated for 20 minutes in dark, followed by 400µL of addition of 1X AV binding buffer, samples were mixed properly and analyzed by BD FACSAria Fusion machine.

Western Blot:

Six 6 cm tissue culture plates, each containing one lakh HCT116 cells, were seeded in complete media and incubated in the incubator for 24 h to allow the cells to attach. After that, two sets of three plates were treated as follows: the control dark group received only DMSO, while the treatment dark and treatment laser groups received compound 7a at a final concentration of 0.6 µM. And incubated for 20 hr. After 20 h, the two treatment laser plates were irradiated with an 808 nm laser of power 0.8 W/cm^2 for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After a 24 h incubation period, cells were washed with 1X PBS, trypsinised, and a whole-cell lysate was prepared using lysis buffer containing protease and phosphatase inhibitors in RIPA buffer, followed by protein quantification using Bradford reagent. Gel electrophoresis was performed using SDS-PAGE to separate proteins; an equal amount of protein was loaded into each well, followed by electrophoresis and transfer to a nitrocellulose membrane. The membrane was cut and blocked with 5% skimmed milk in 1X TBST, followed by overnight incubation at 4°C with primary antibodies against Bcl-2, Bax, Casp-9, PARP, Casp-3, LC-3, Beclin, and COX-2. The next day, the corresponding secondary antibody was added to the primary antibody and incubated at room temperature for 2h. Using an enhanced chemiluminescence (ECL) reagent, blots were developed, imaged with the Bio-Rad Gel documentation instrument, and analysed with Image Lab software.

Autophagy flux by MTT

5000 HCT-116 cells were seeded in a 96-well plate and incubated for 24 hours. Cells were incubated with various concentrations of 7a, and 1% DMSO was used as a control, followed by the addition of Bafilomycin (25 nM), Chloroquin (12.5 μM) for H_2O_2. After incubating for 20 hours, the sample was irradiated with an 808 nm laser at 0.8 W/cm² for 10 min and then incubated for another 4 hours to complete the 24 h incubation. Further steps involve removing the media and adding MTT reagent solution (0.5 mg/ml in complete media with 10% 1xPBS) to the plate. The plate containing the MTT reagent was incubated for 4 h. After 4 h, the media was removed, DMSO (100 μL) was added to each well, and absorbance was measured with a Perkin Elmer multimode plate reader. The absorbance data were analysed and plotted using the software GraphPad Prism.

Autophagy flux by Western blot

Ten 6 cm tissue culture plates, each containing one lakh HCT116 cells, were seeded in complete media and incubated in the incubator for 24 h to allow the cells to attach. After that, two sets of five plates were treated as follows: the control dark group received only DMSO, while the treatment dark, treatment laser, and treatment laser bafilomycin groups received compound 7a at a final concentration of 0.6 μM. And treatment laser bafilomycin and bafilomycin is received at 25 nM, and incubated for 20 hr. After 20 h, the two treatment laser and two treatment laser bafilomycin plates were irradiated with an 808 nm laser of power 0.8 W/cm² for 10 minutes, then further incubated for 4 hours to complete the 24 h incubation. After a 24 h incubation period, cells were washed with 1X PBS, trypsinised, and a whole-cell lysate was prepared using lysis buffer containing protease and phosphatase inhibitors in RIPA buffer, followed by protein quantification using Bradford reagent. Gel electrophoresis was performed using SDS-PAGE to separate proteins; an equal amount of protein was loaded into each well, followed by electrophoresis and transfer to a nitrocellulose membrane. The membrane was cut and blocked with 5% skimmed milk in 1X TBST, followed by overnight incubation at 4°C with primary antibodies against LC-3 The next day, the corresponding secondary antibody was added to the primary antibody and incubated at room temperature for 2h. Using an enhanced chemiluminescence (ECL) reagent, blots were developed, imaged with the Bio-Rad Gel documentation instrument, and analysed with Image Lab software.

Uptake assay for HeLa 3D-tumor spheroids.

To form the HeLa spheroids, 5,000 cells were seeded in the U-bottom ultra-low attachment plates (Nunclon Sphera 96 well plate, Thermo Scientific, Cat. No. 174925). The plate is then centrifuged at 2,000 rpm for 10 mins to form uniform aggregates in each well. These are then cultured for 3 days in incubator with 5% CO_2 supply and 95% humidity at 37˚C temperature. They were observed each day till they were compact. These spheroids were then treated with 7a at 0.8μM and 1.2 μM working concentrations. Post 20 hours of incubation they were irradiated with 808 nm laser for 10 min. Post treatment spheroids were washed with 1X PBS. Later, these spheroids were exposed to 4% paraformaldehyde for fixation followed by conterstaining with 0.1X TritonX + Phallodidin (Thermo Scientific Cat. No. A12379**)** and DAPI (Merck, Cat. No. 10236276001), each for 30 mins at 37˚C. The spheroids were then mounted using Mowiol®. These were then imaged using Laser Scanning Confocal Microscope (Leica TCS SP8) with 405nM, 488nM and 633nM lasers. They were imaged at 20X with 1μM step size. These were then analysed with Fiji ImageJ software. For calculating the Fluorescence intensity, ImageJ software was used.

Live Dead Assay for HeLa 3D-tumor spheroids.

Once these spheroids are formed and treated with the 7a, spheroids were then exposed to 5 µg/mL Calcein AM (Cayman Chemical, Item No. 14948) and 20µg/mL Propidium iodide (Cayman Chemical, Item No. 14289) in complete media for 3 hours. Live imaging was carried out using Laser Scanning Confocal Microscope (Leica TCS SP8) with 488nM and 561nM lasers at 10X magnification. These were then analysed with Fiji ImageJ software. For calculating the Fluorescence intensity, ImageJ software was used.

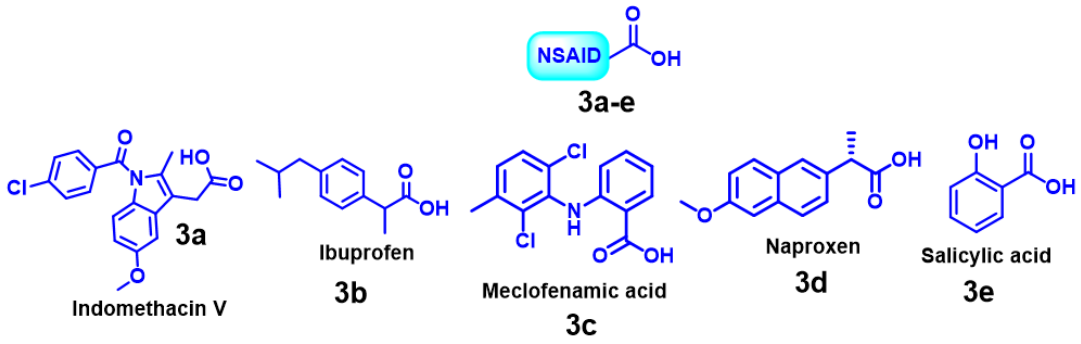


Fig. S1: Chemical structures of the NSAIDs conjugated.

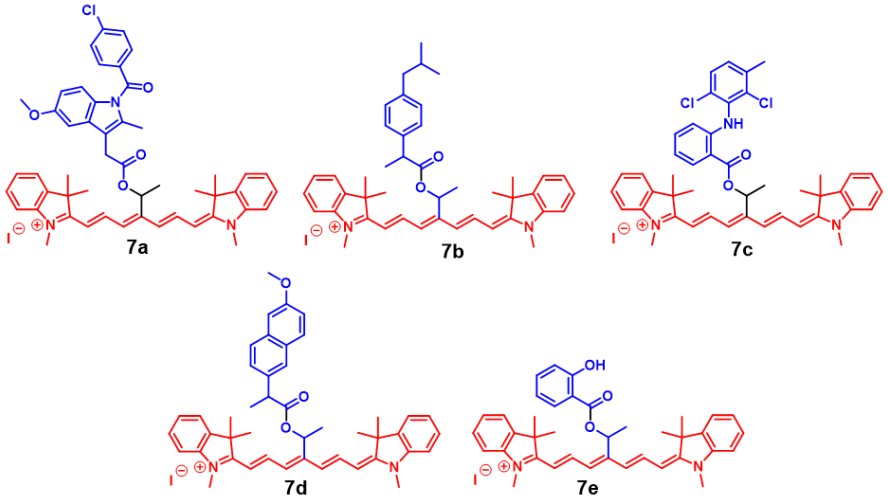


Fig. S2: Chemical structures of the heptamethine cyanine-NSAID conjugates.

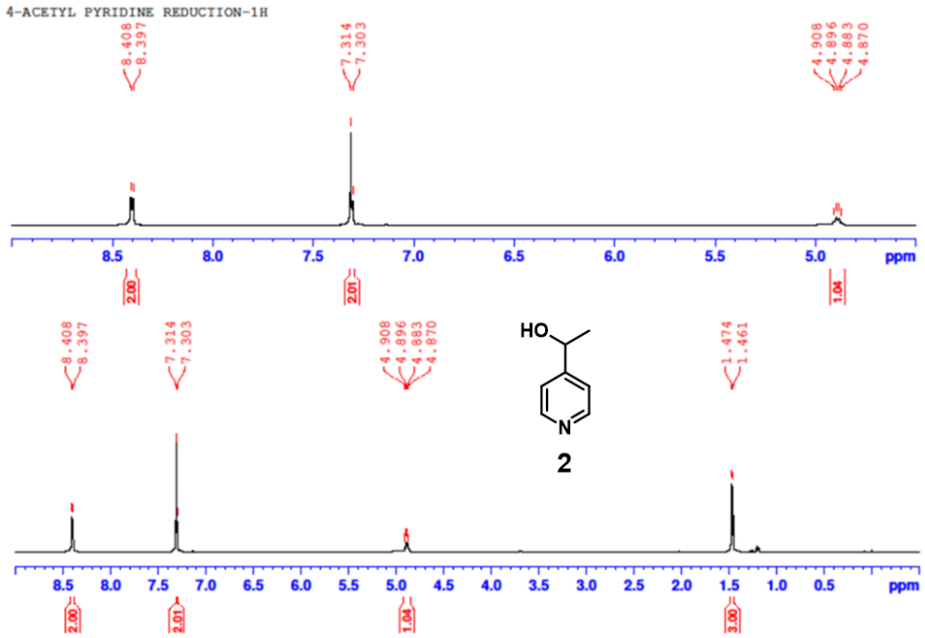


Fig. S3: ^{1}H NMR spectra of 2.

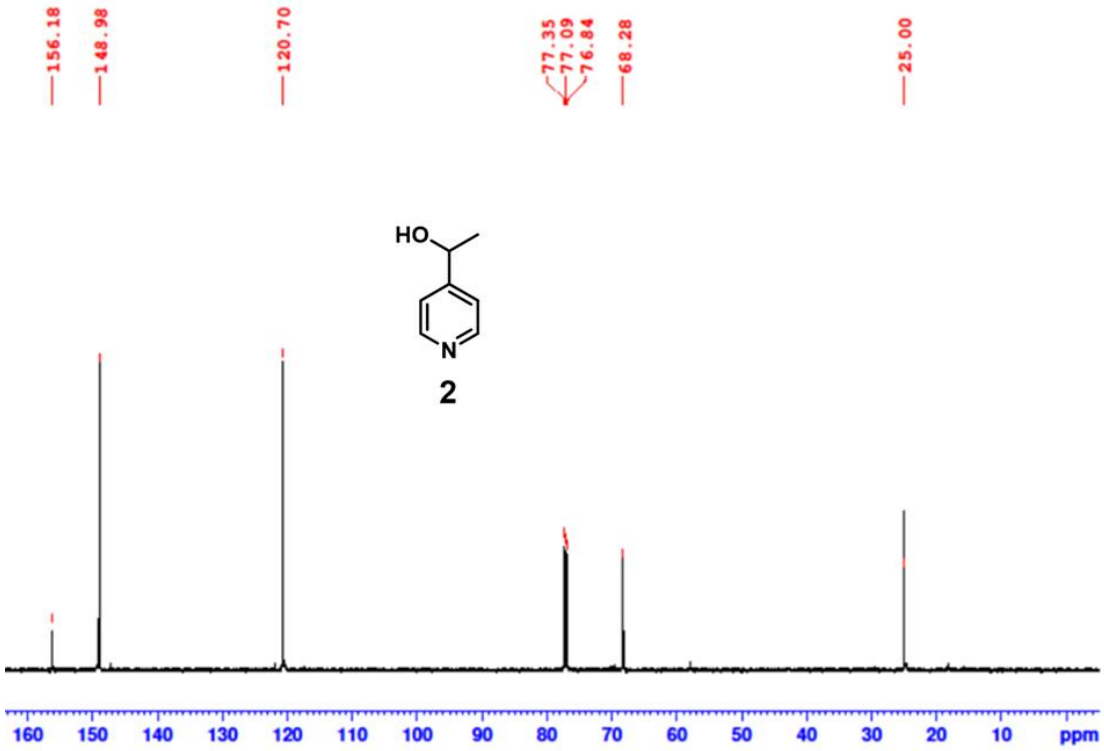


Fig. S4: ^{13}C NMR spectra of 2.

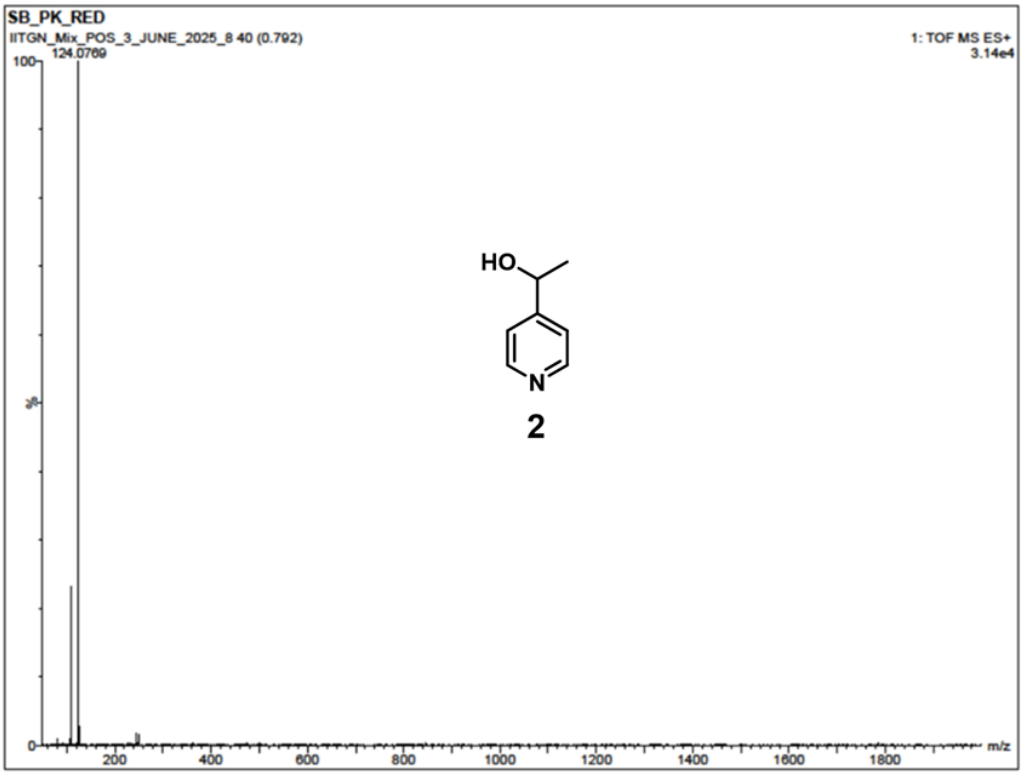


Fig. S5: HR-MS spectra of 2.

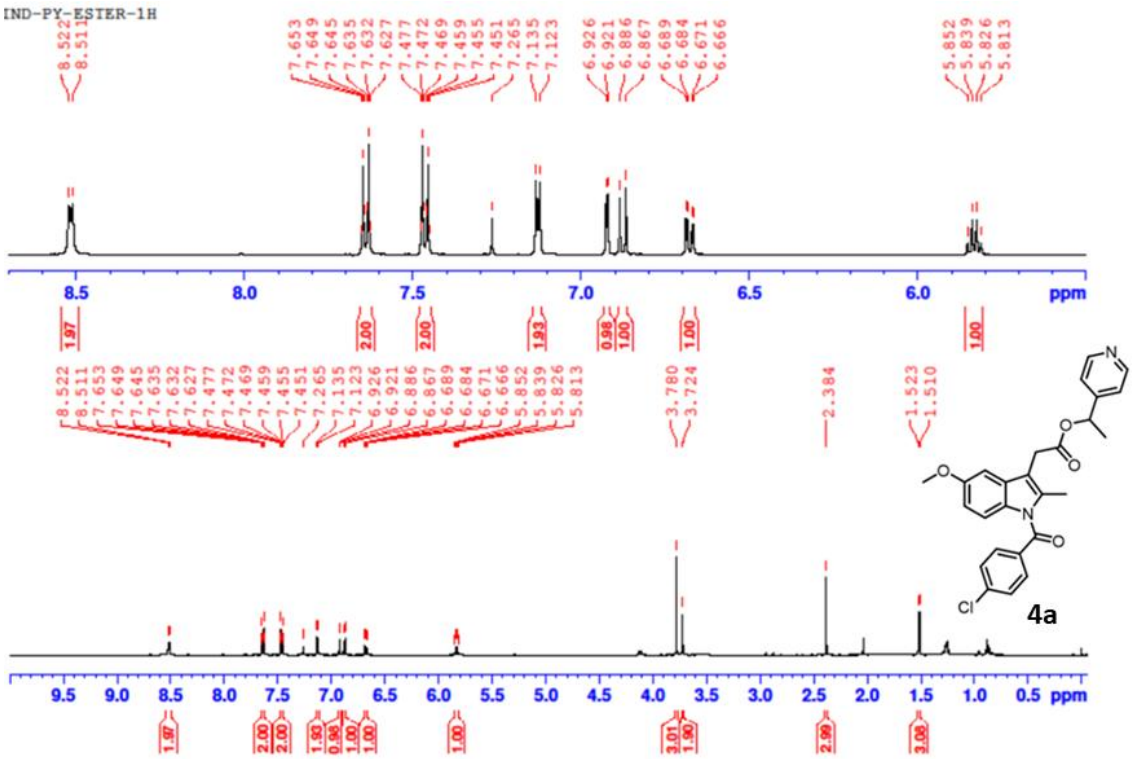


Fig. S6: ^{1}H NMR spectra of 4a.

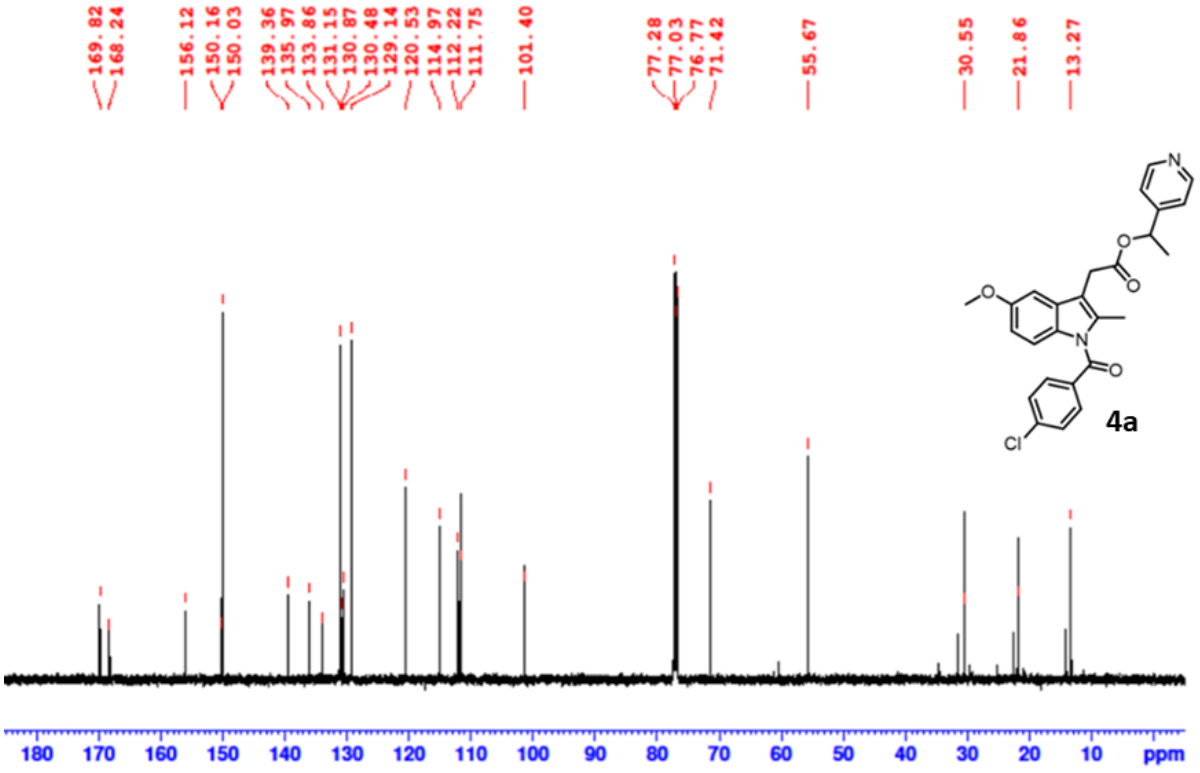


Fig. S7: ^{13}C NMR spectra of 4a.

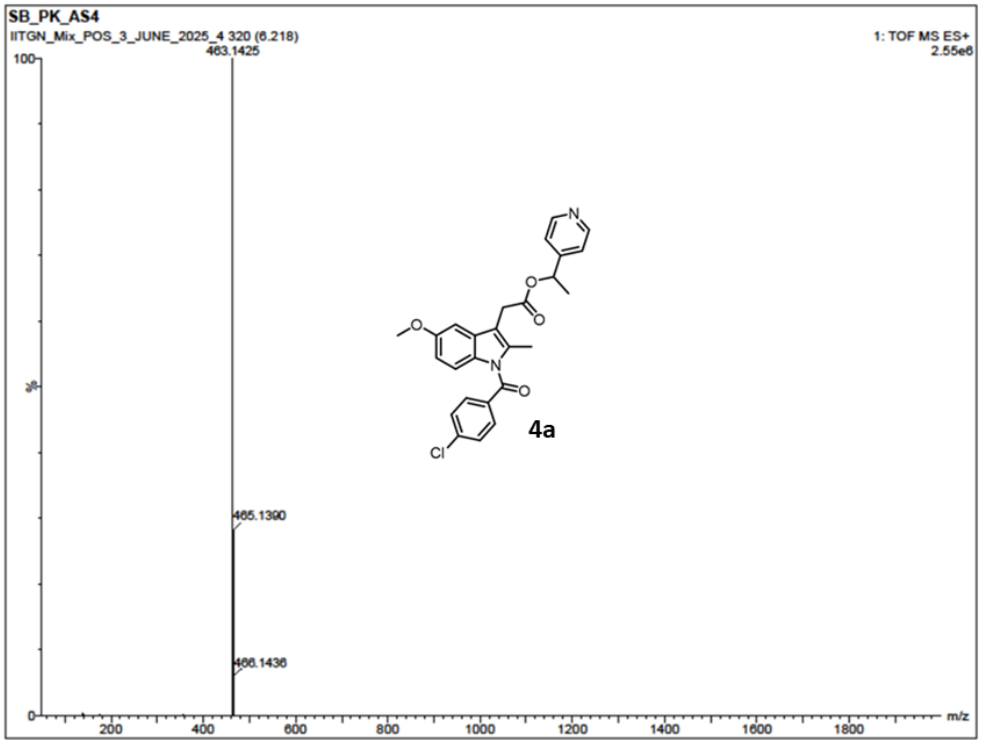


Fig. S8: HR-MS spectra of 4a.

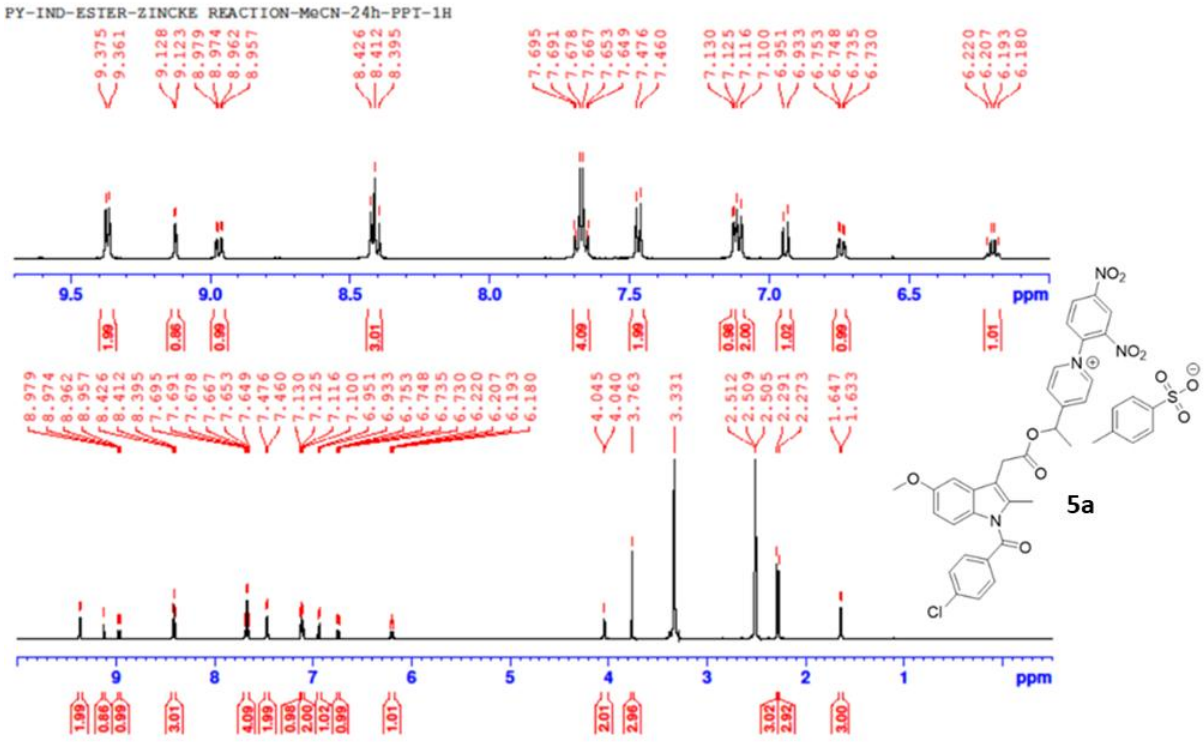


Fig. S9: ^{1}H NMR spectra of 5a.

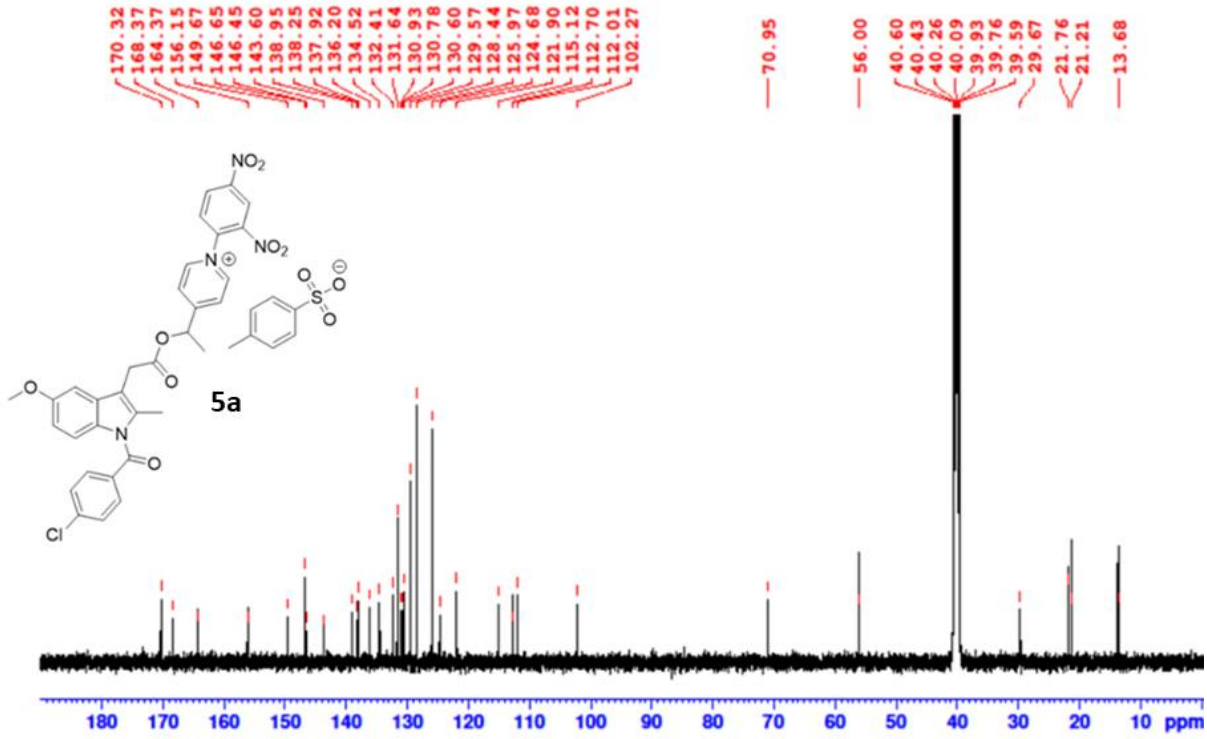


Fig. S10: ^{13}C NMR spectra of 5a.

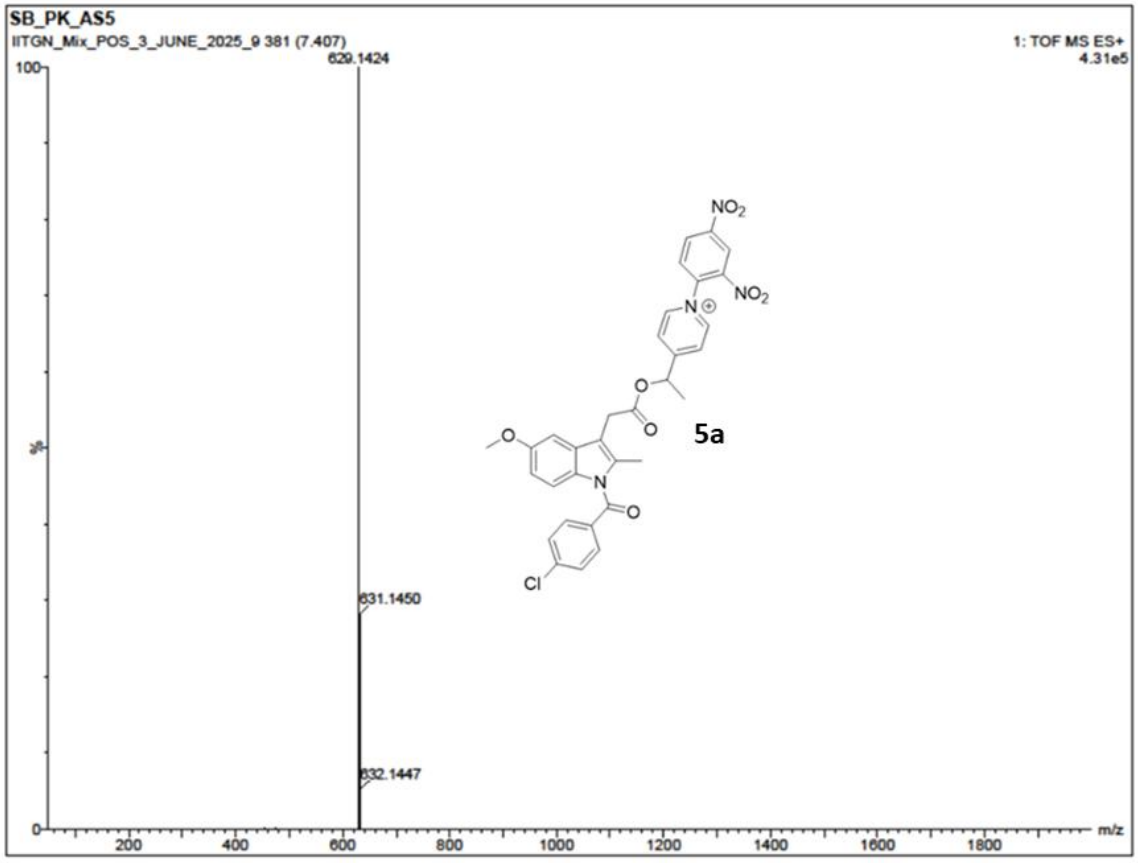


Fig. S11: HR-MS spectra of 5a.

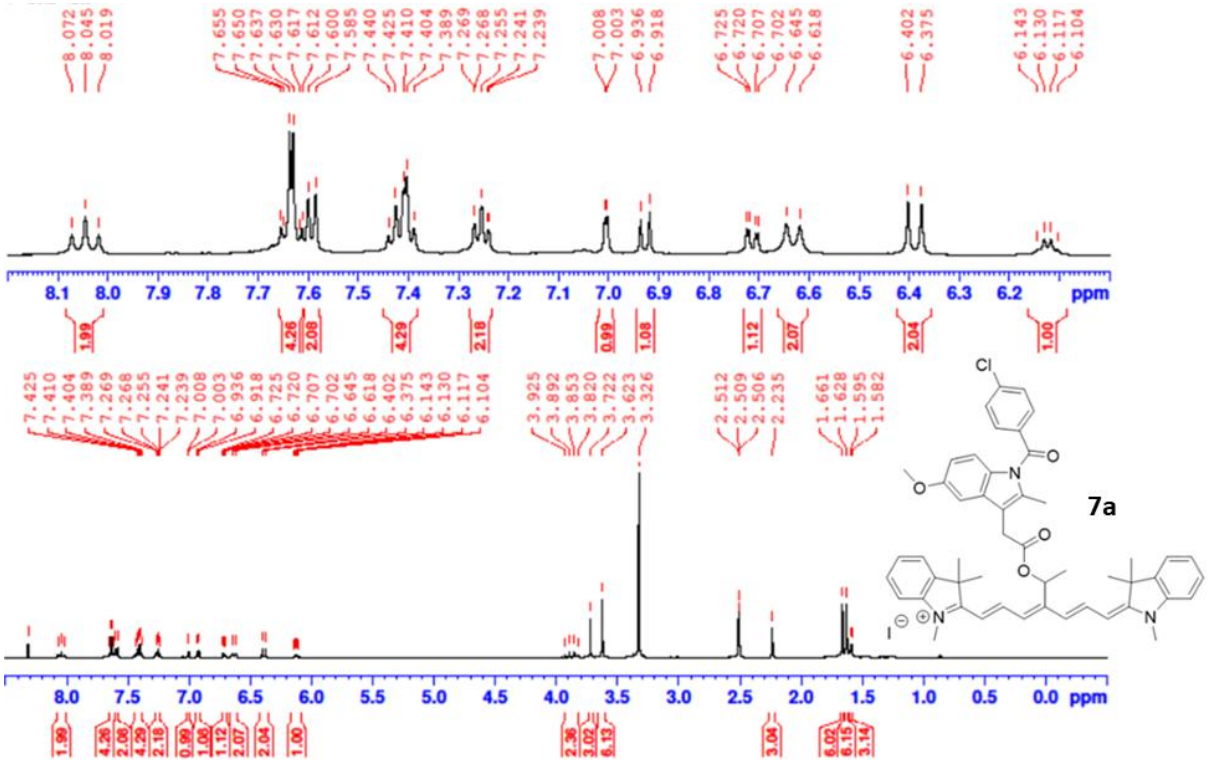


Fig. S12: ^{1}H NMR spectra of 7a.

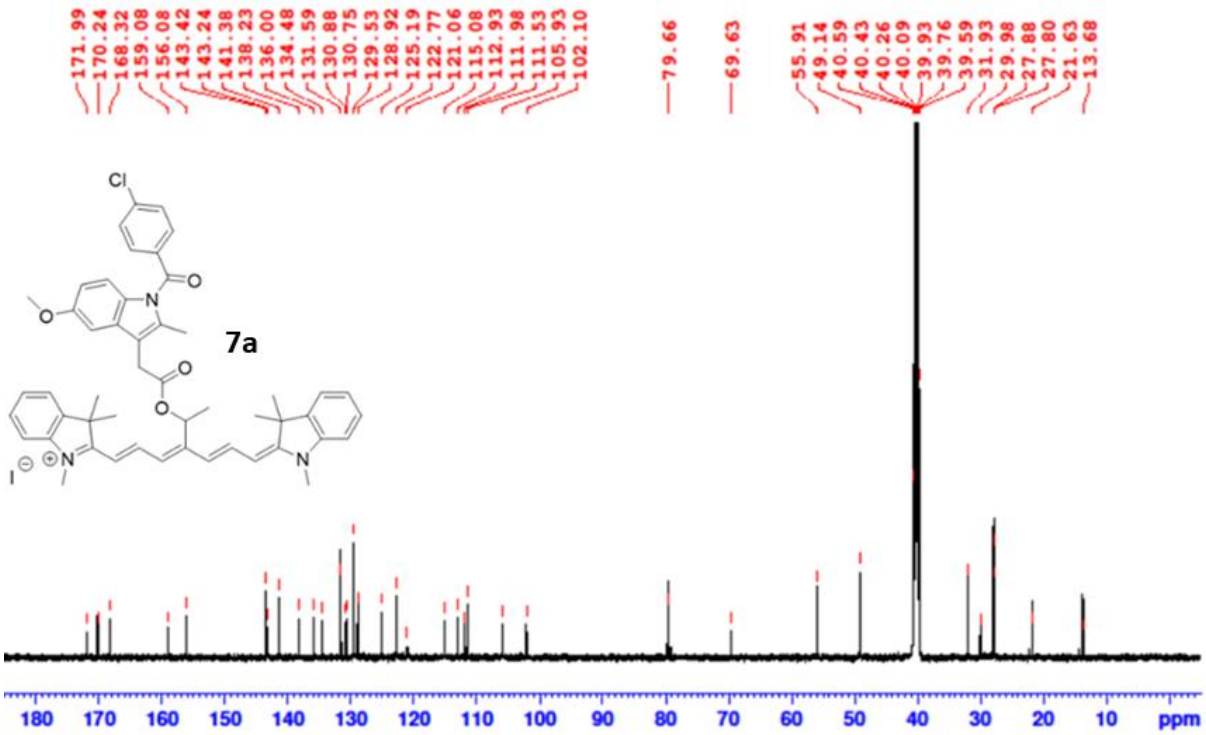


Fig. S13: ^{13}C NMR spectra of 7a.

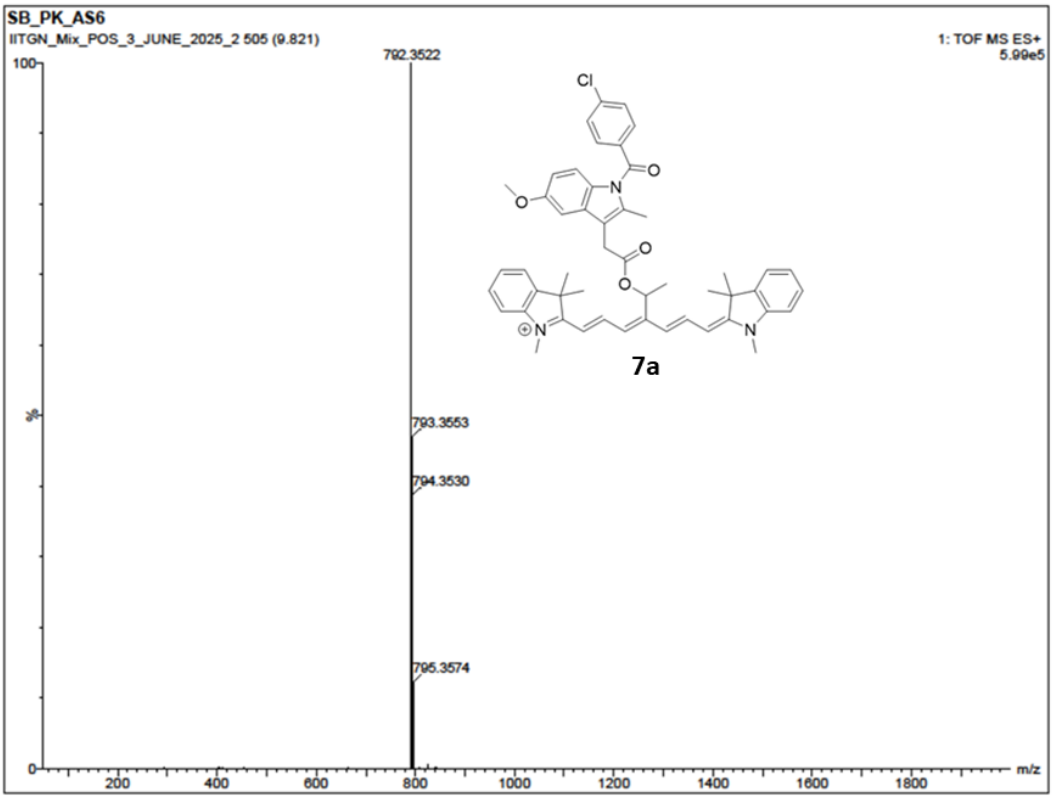


Fig. S14: HR-MS spectra of 7a.

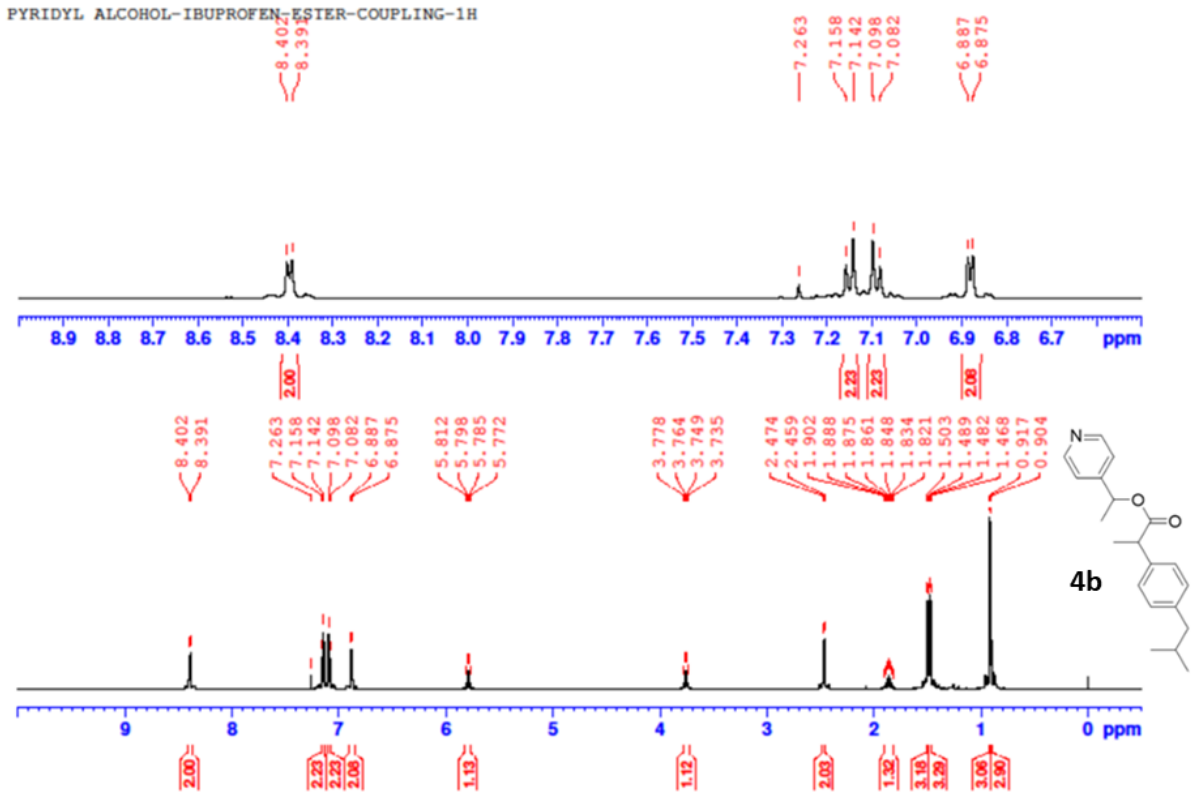


Fig. S15: ^{1}H NMR spectra of 4b.

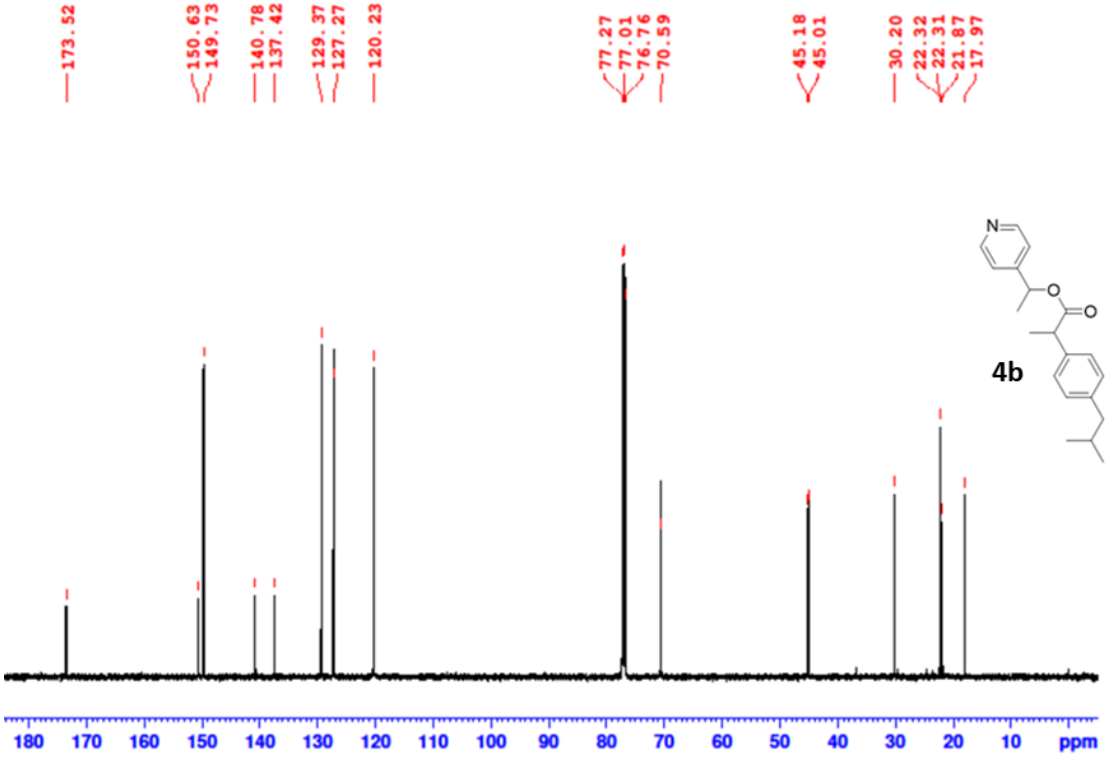


Fig. S16: ^{13}C NMR spectra of 4b.

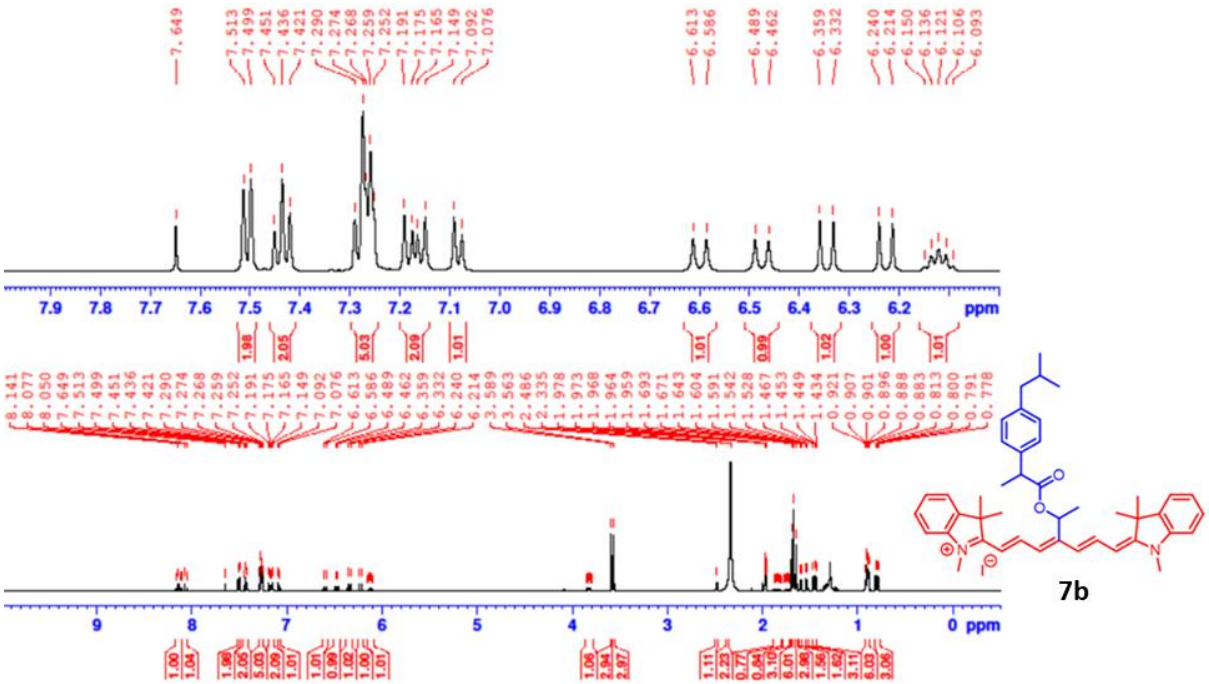


Fig. S17: ^{1}H NMR spectra of 7b.

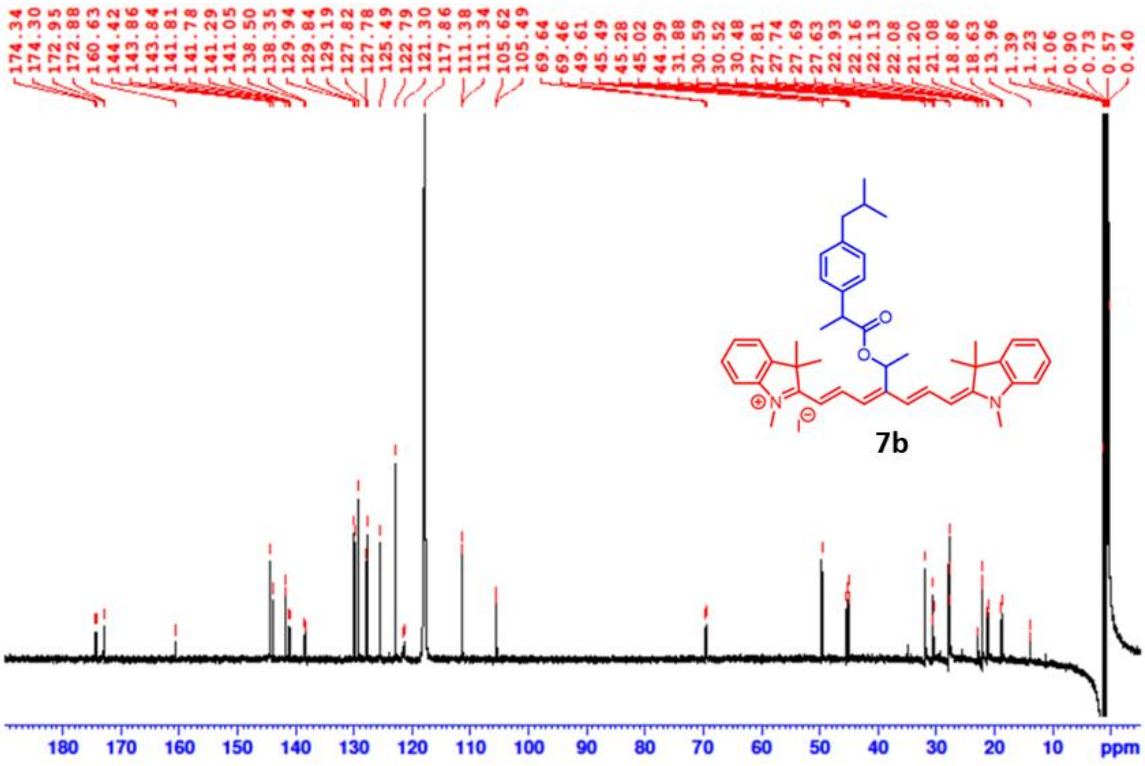


Fig. S18: ^{1}H NMR spectra of 7b.

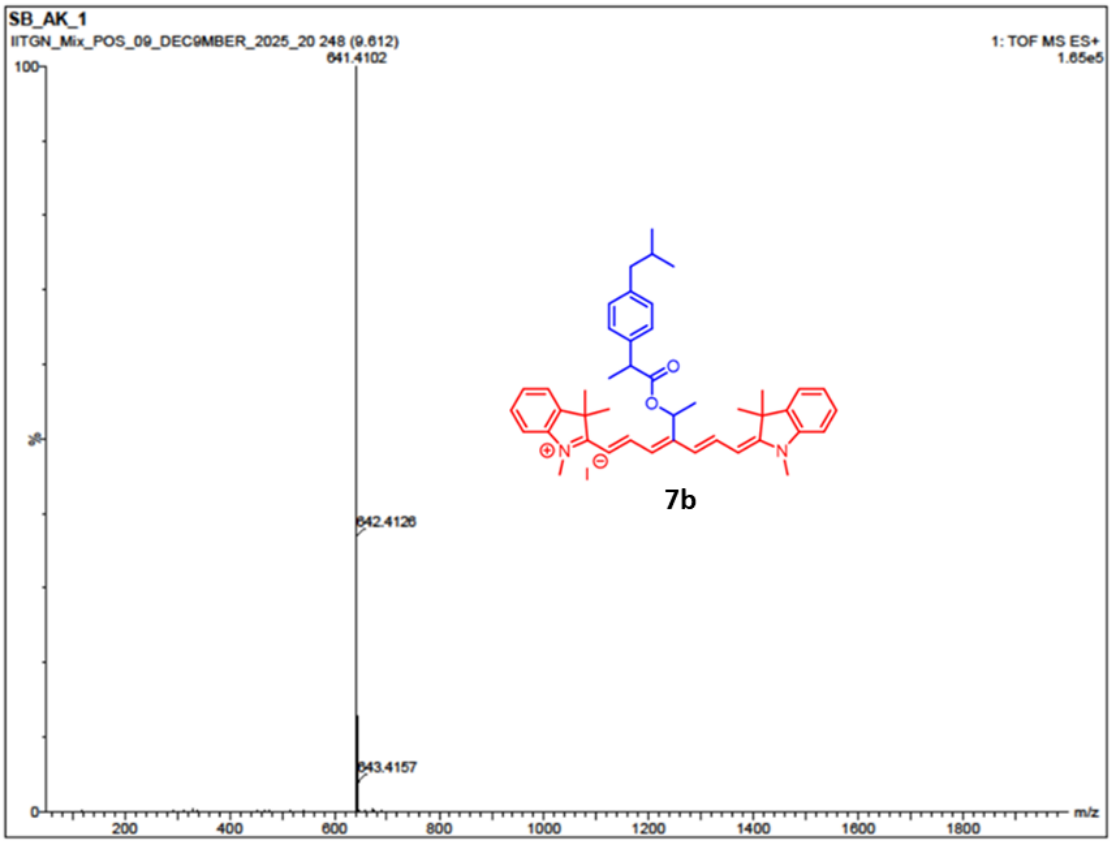


Fig. S19: HR-MS of 7b.

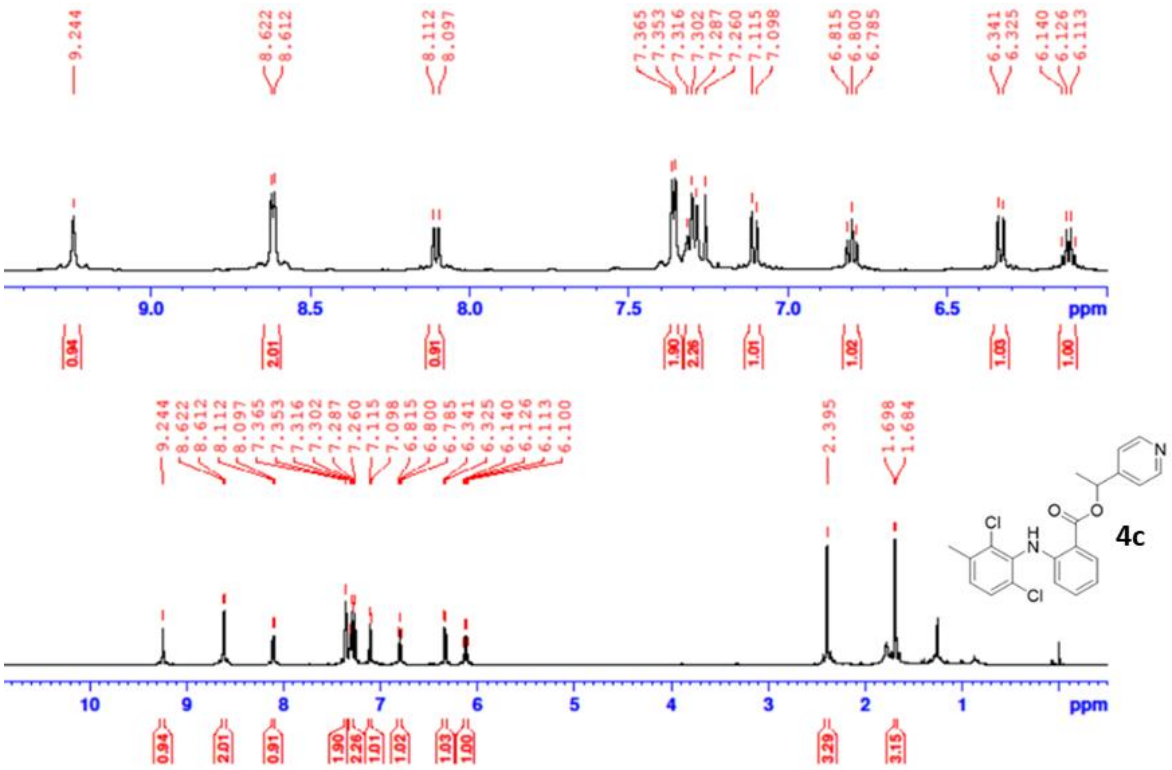


Fig. S20: ^{1}H NMR spectra of 4c.

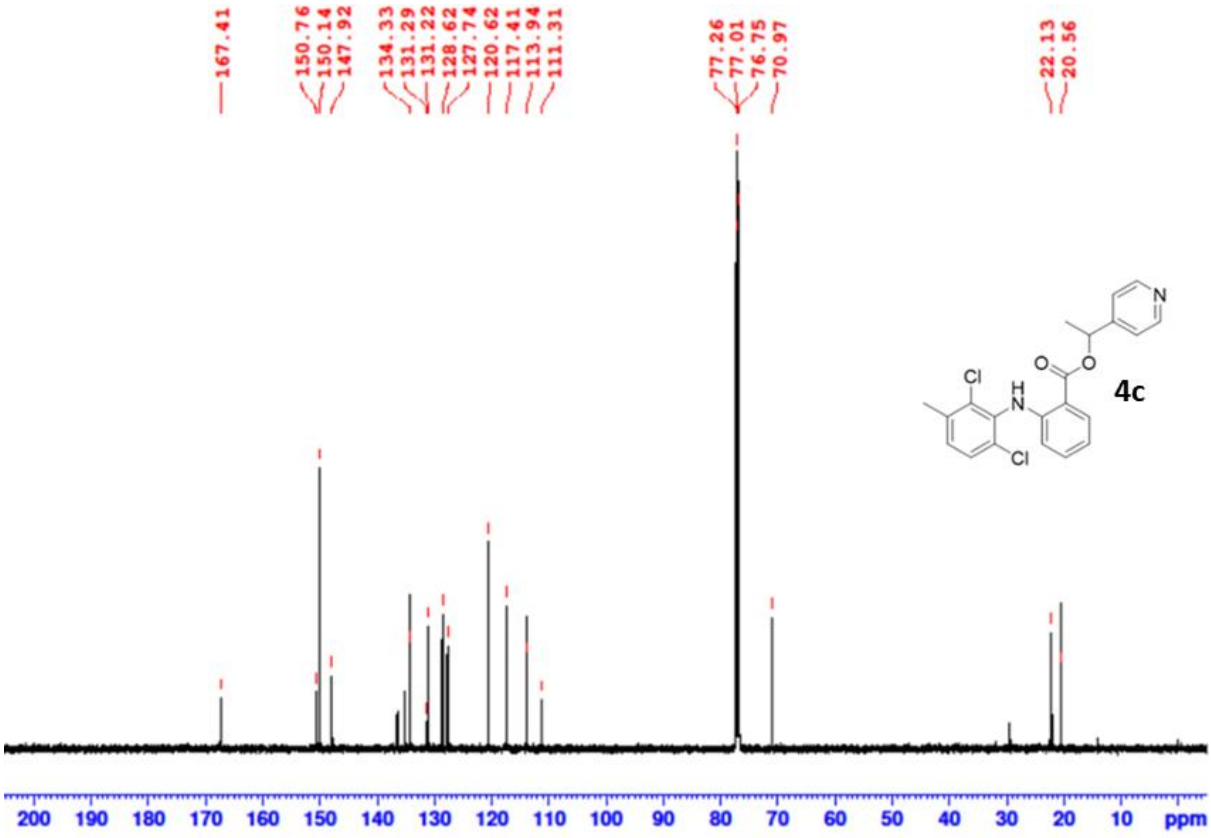


Fig. S21: ^{13}C NMR spectra of 4c.

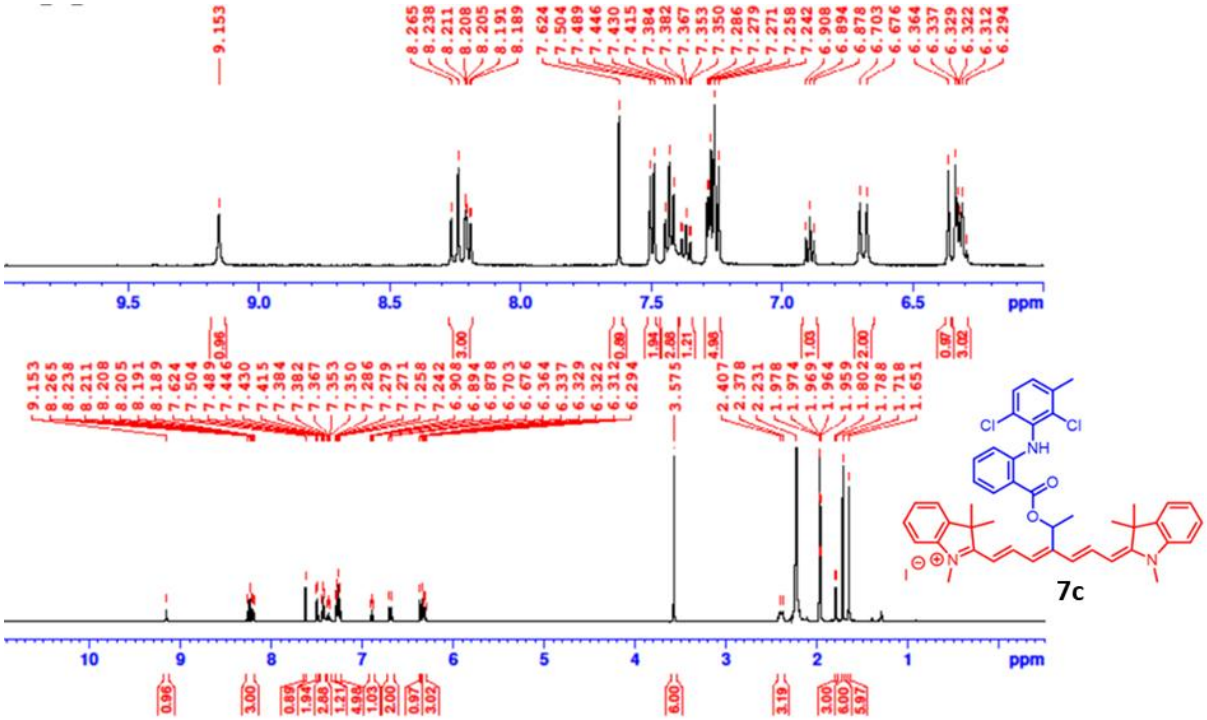


Fig. S22: ^{1}H NMR spectra of 7c.

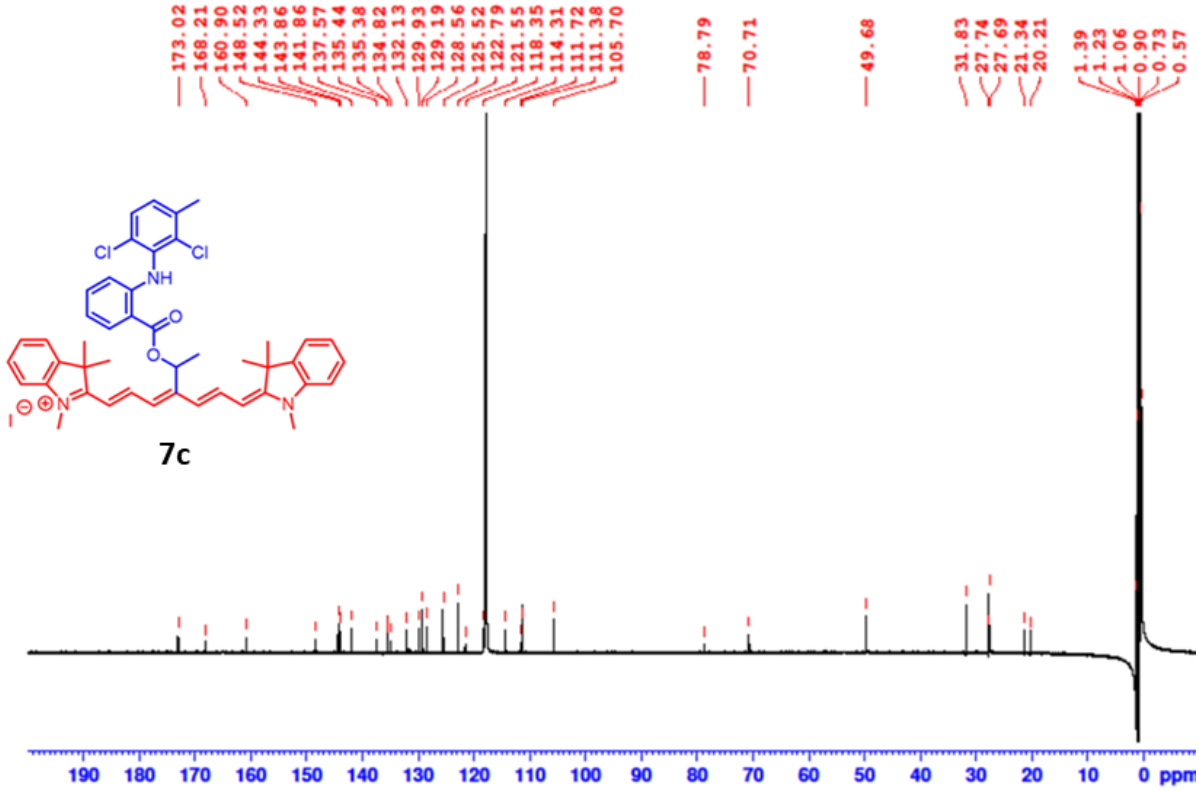


Fig. S23: ^{13}C NMR spectra of 7c.

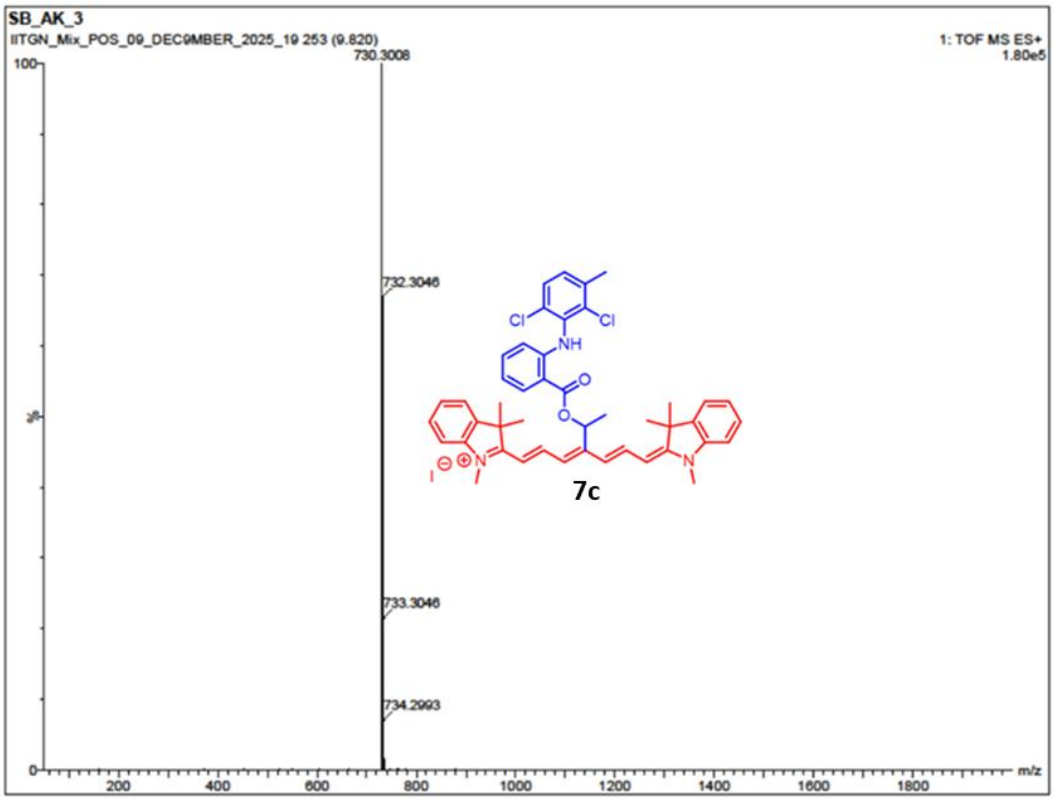


Fig. S24: HR-MS spectra of 7c.

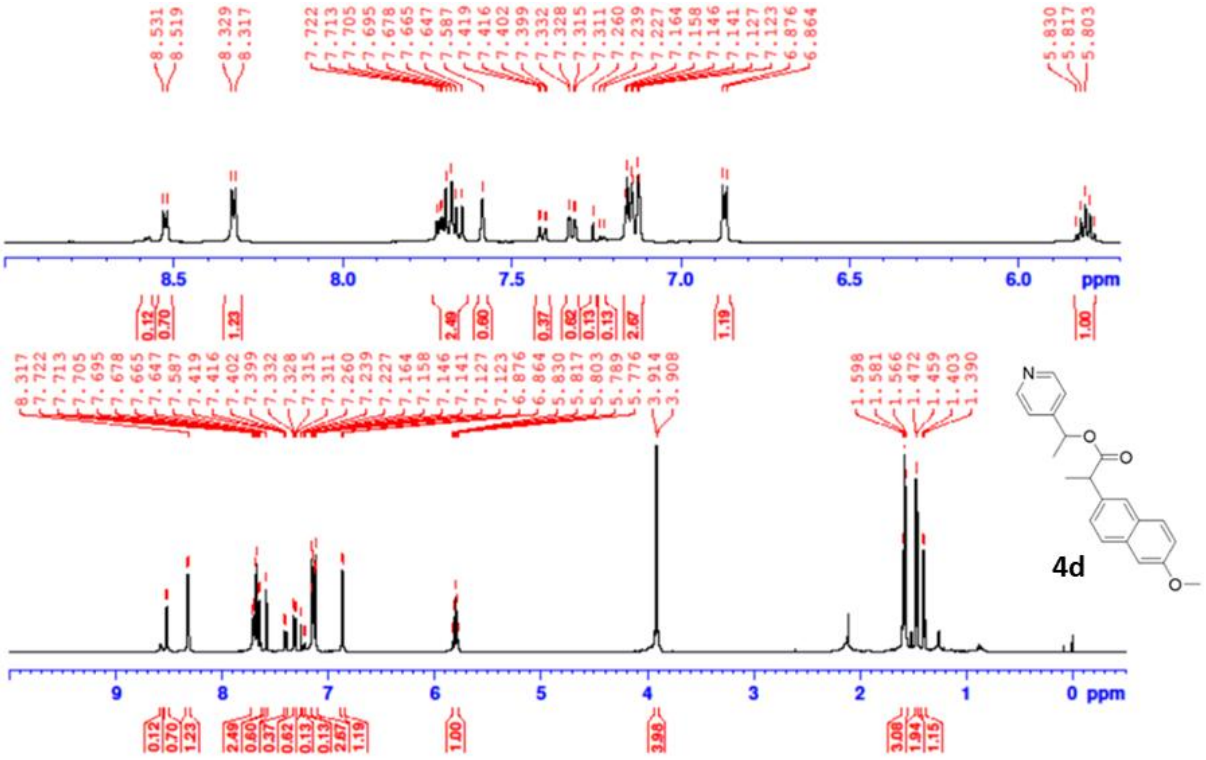


Fig. S25: ^{1}H NMR spectra of 4d.

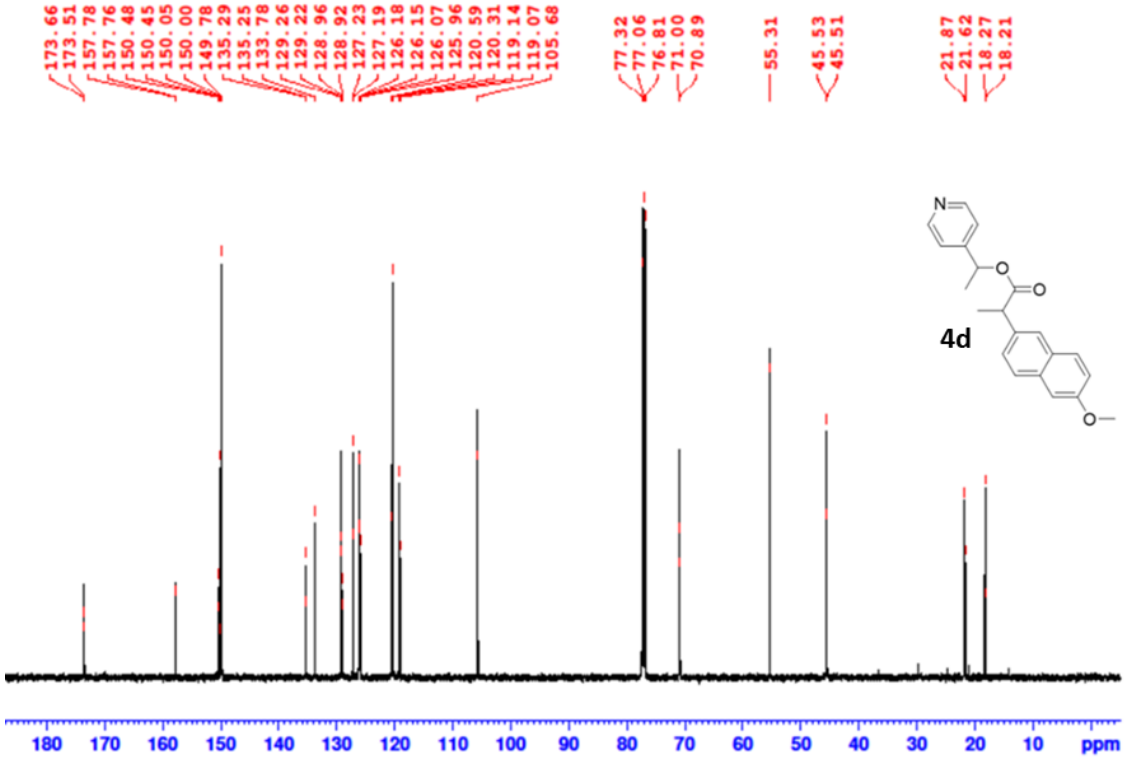


Fig. S26: ^{13}C NMR spectra of 4d.

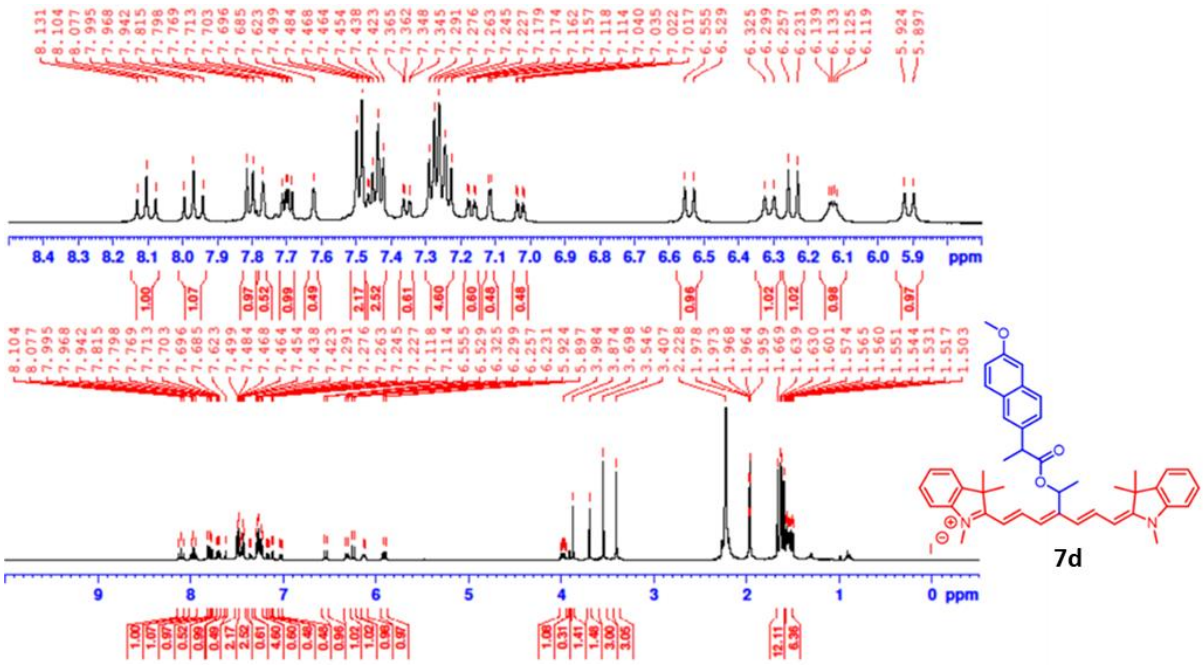


Fig. S27: ^{1}H NMR spectra of 7d.

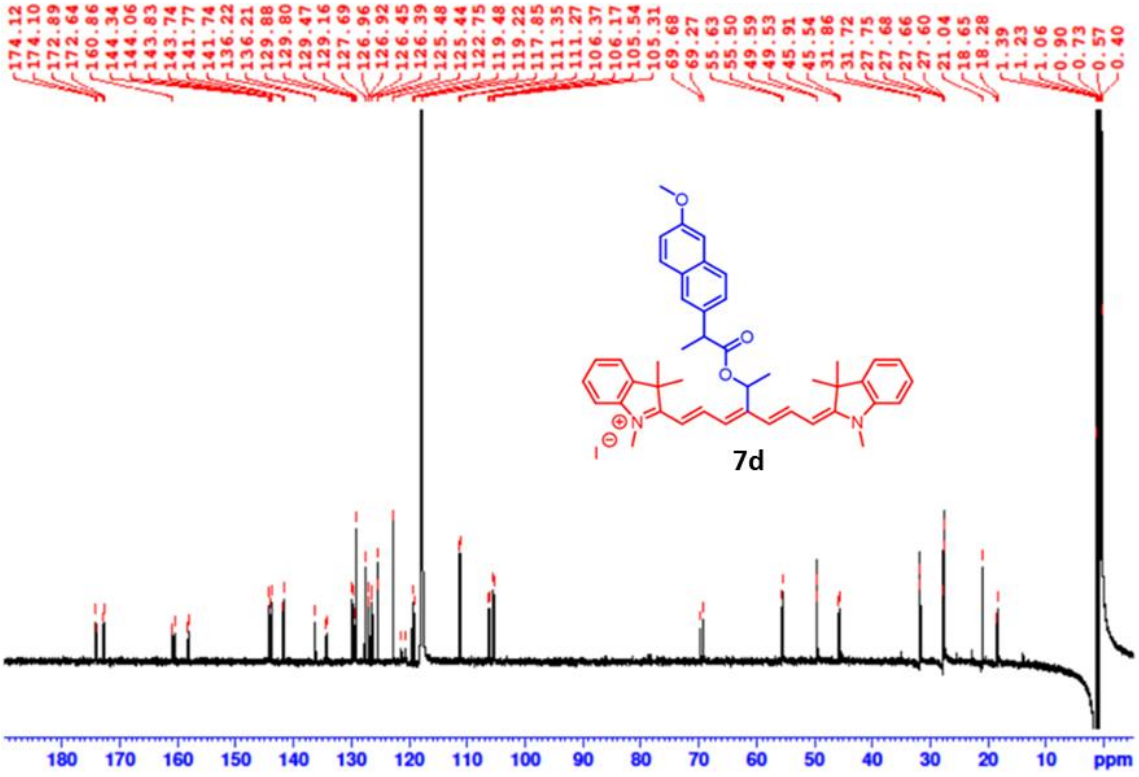


Fig. S28: ^{13}C NMR spectra of 7d.

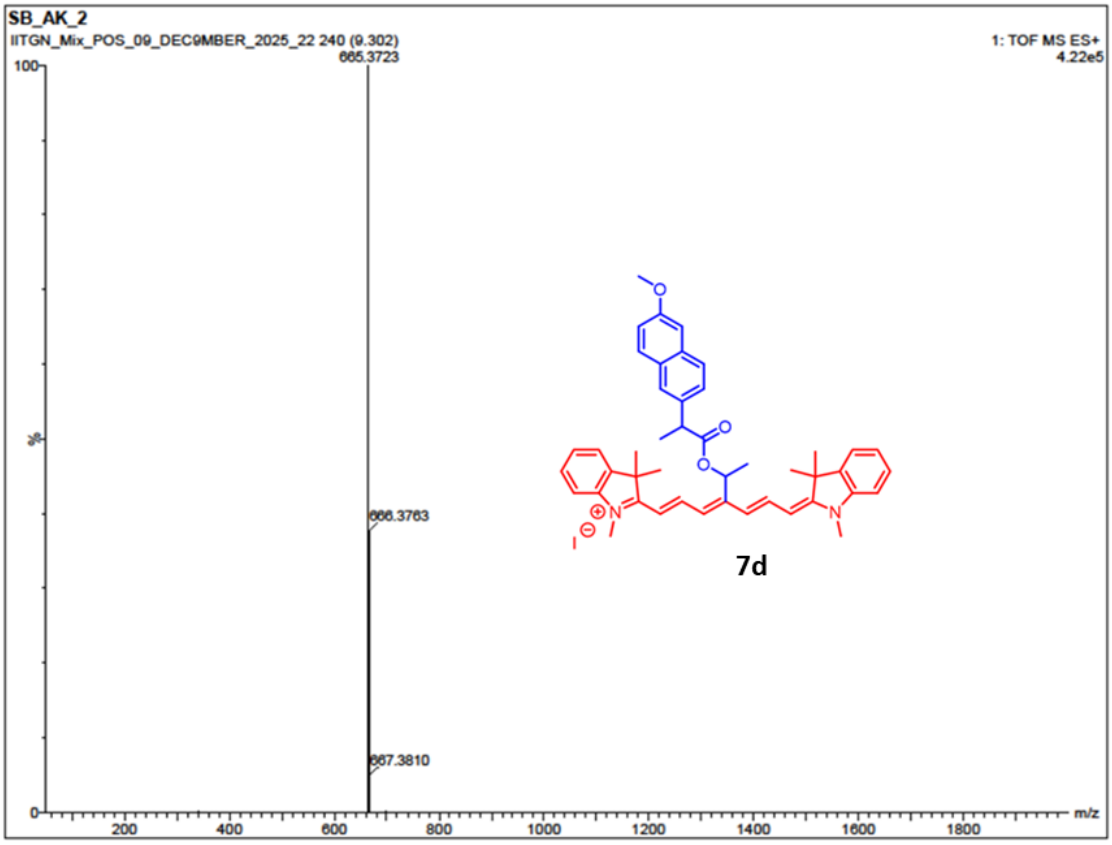


Fig. S29: HR-MS spectra of 7d.

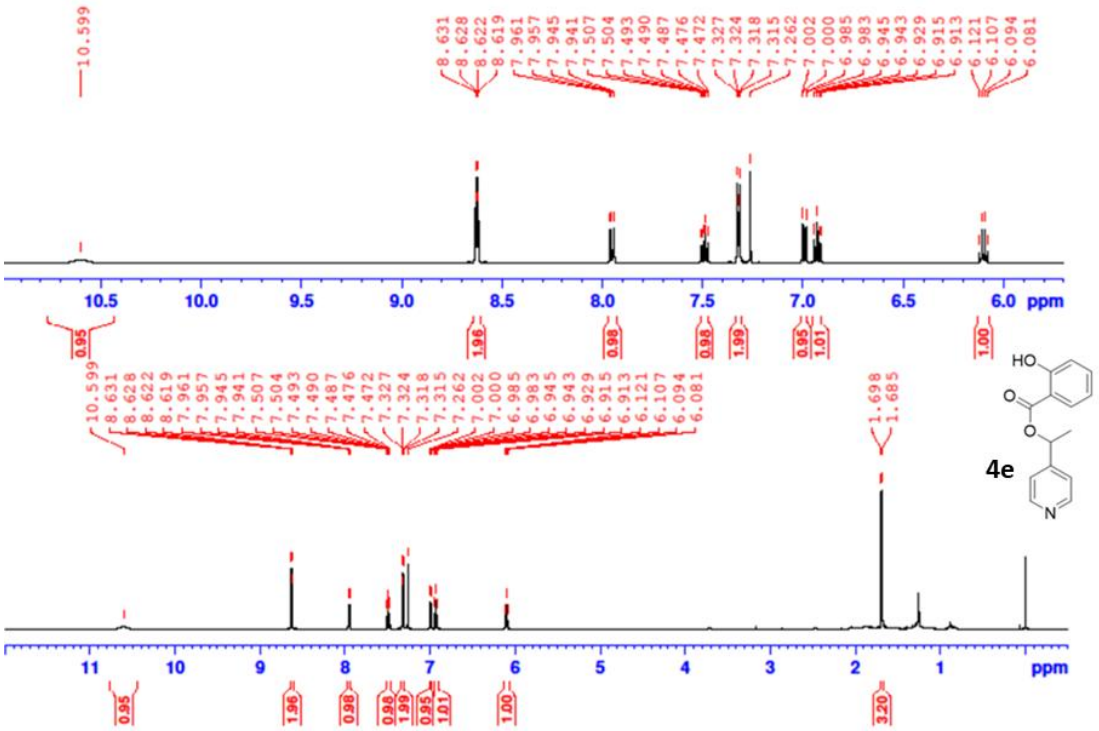


Fig. S30: [1]H NMR spectra of 4e.

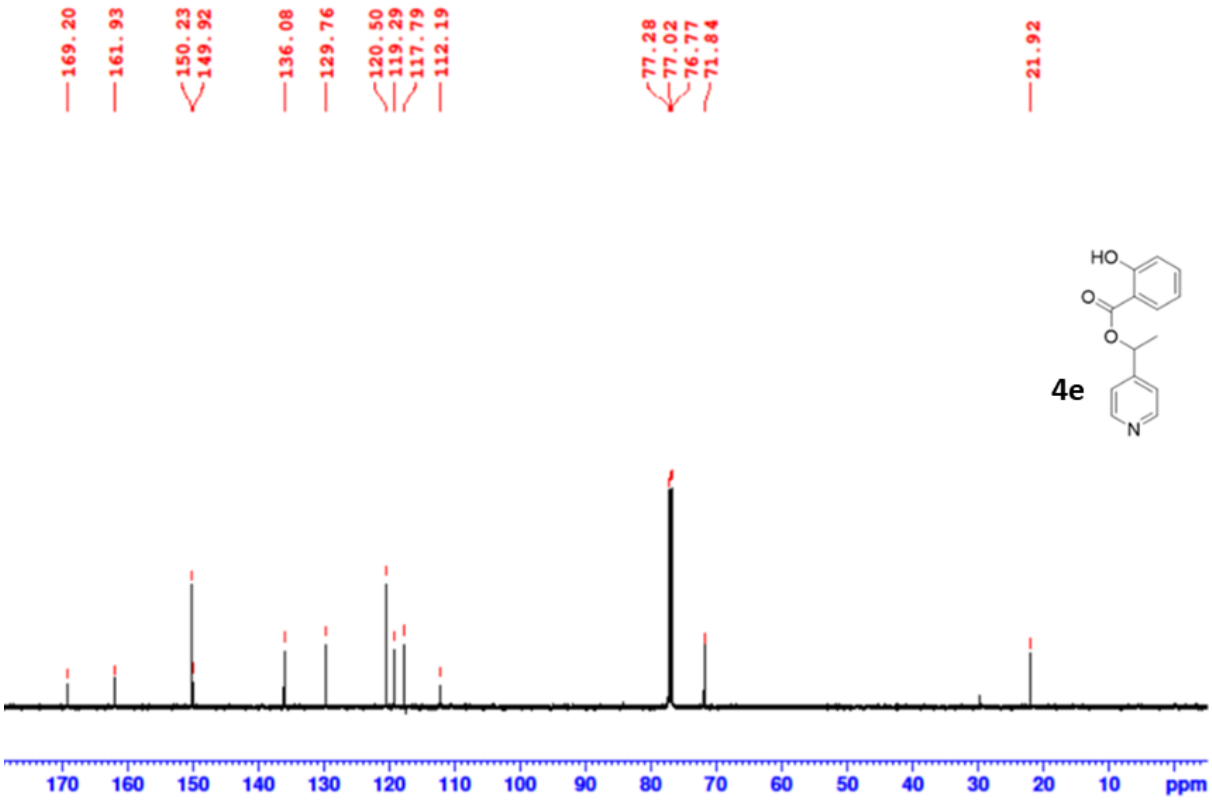


Fig. S31: [13]C NMR spectra of 4e.

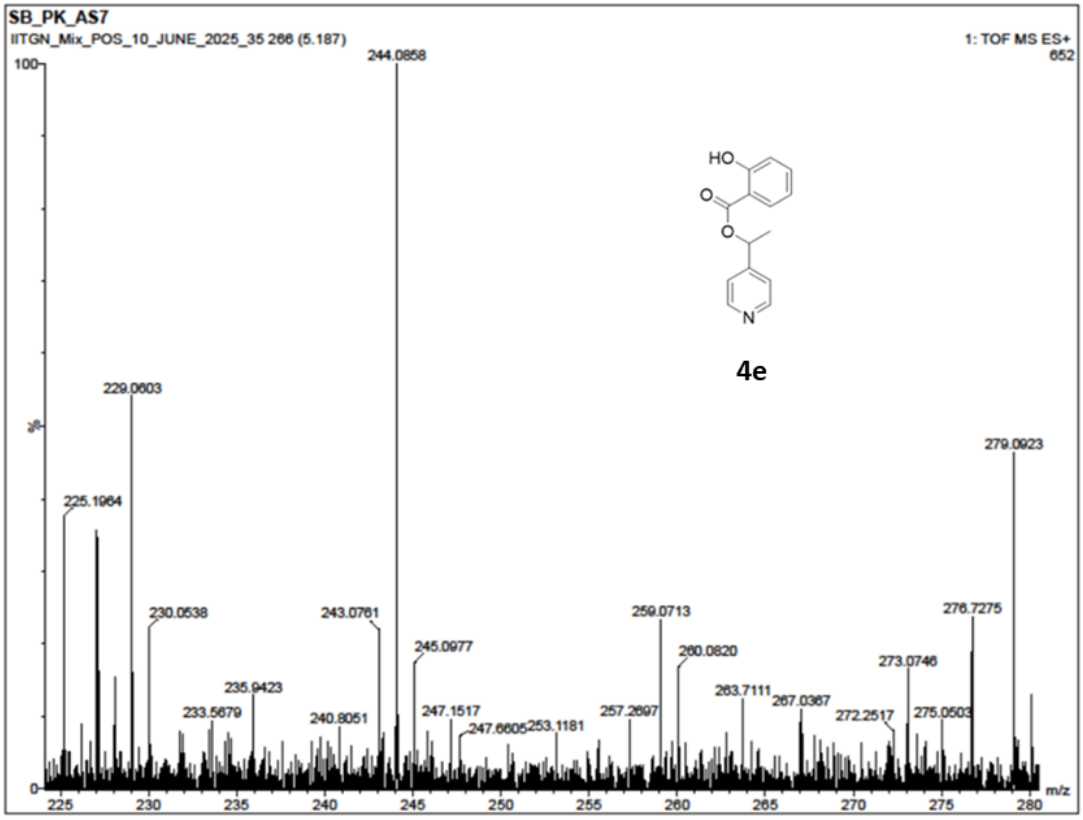


Fig. S32: HR-MS spectra of 4e.

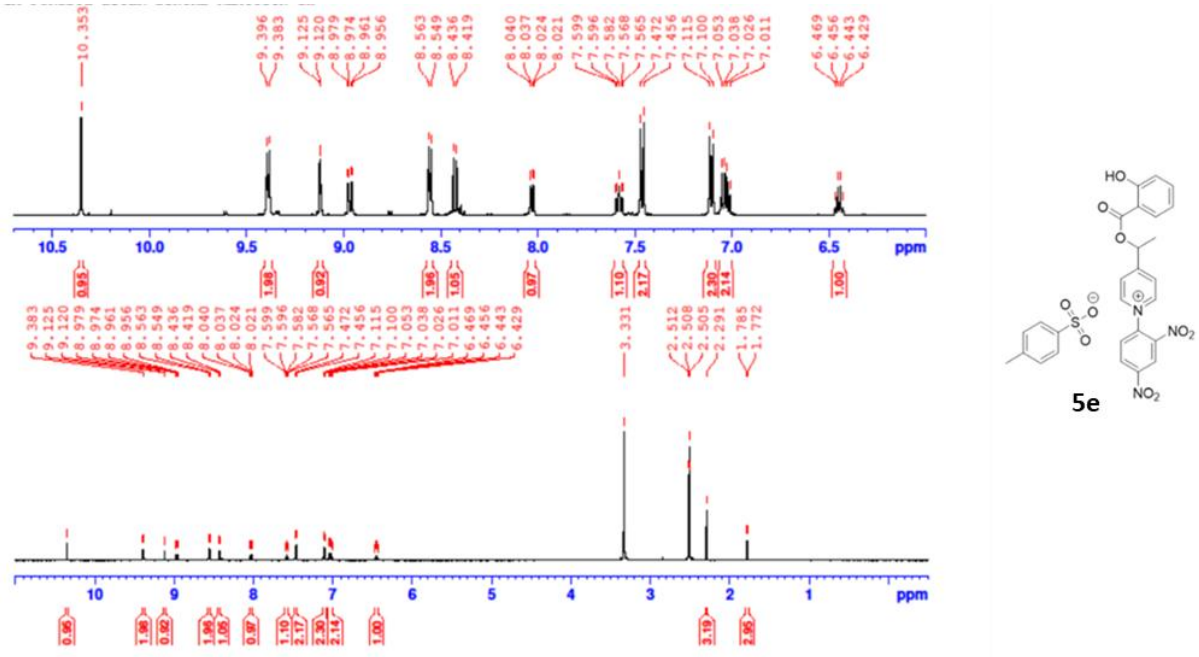


Fig. S33: ^{1}H NMR spectra of 5e.

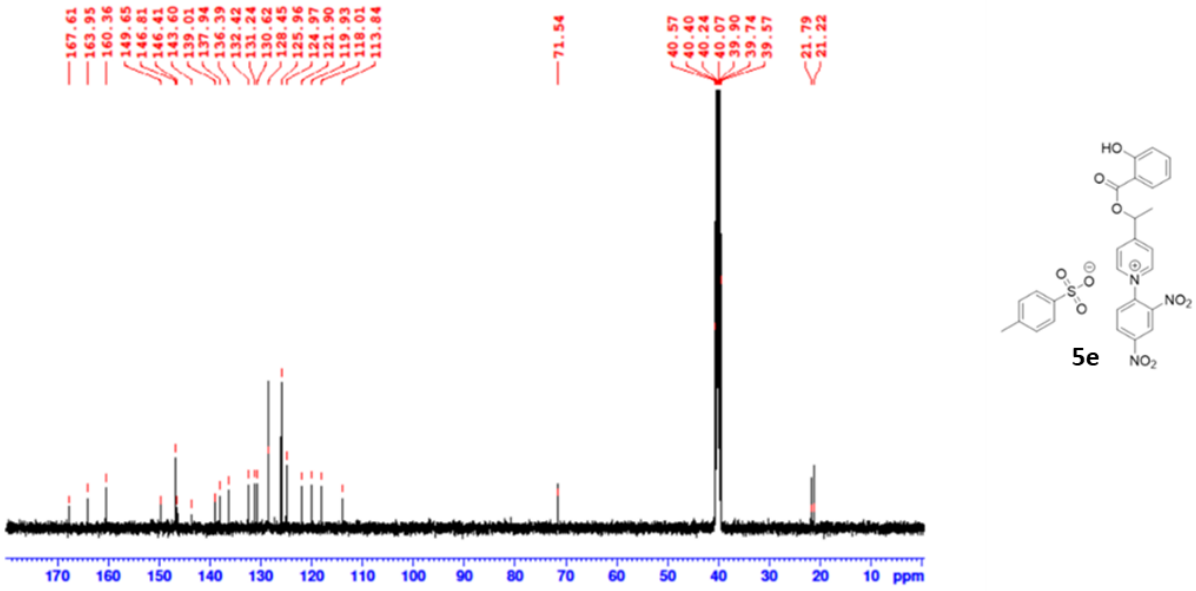


Fig. S34: ^{13}C NMR spectra of 5e.

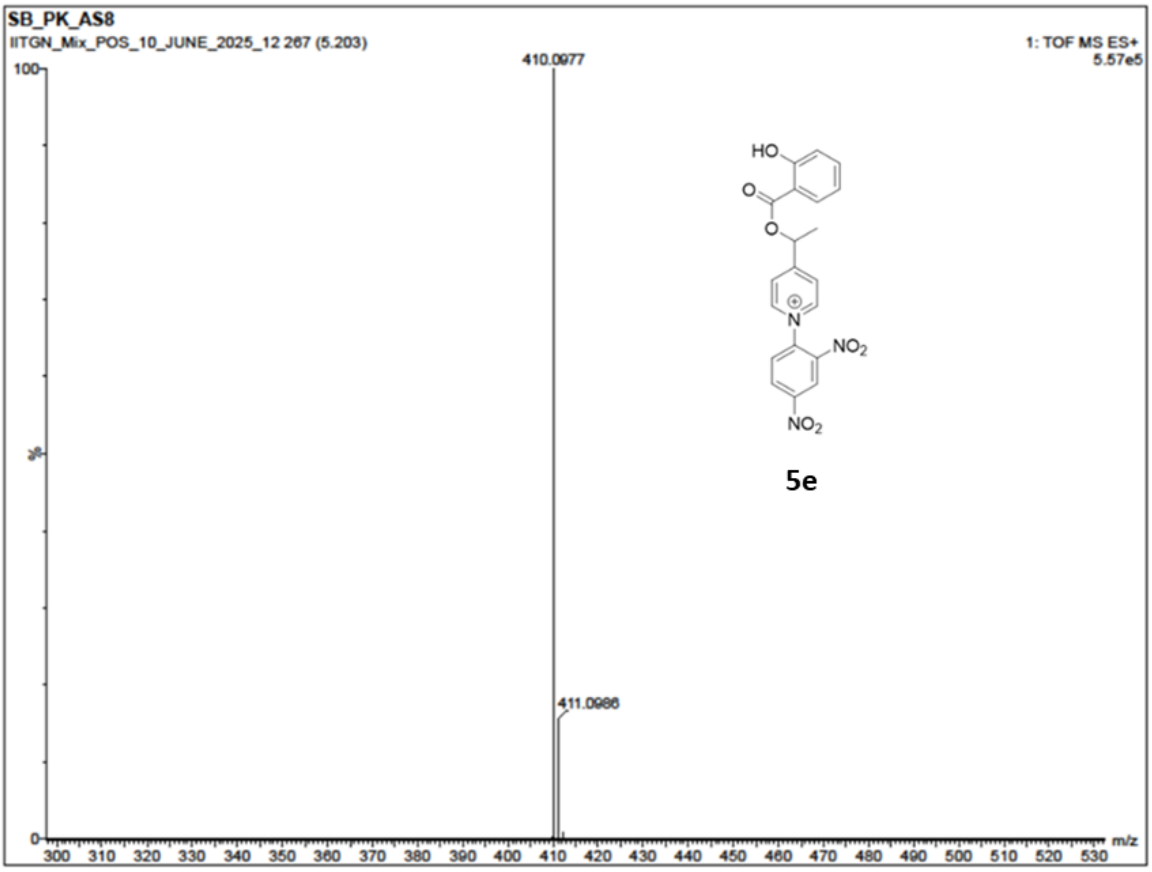


Fig. S35: HR-MS spectra if 5e.

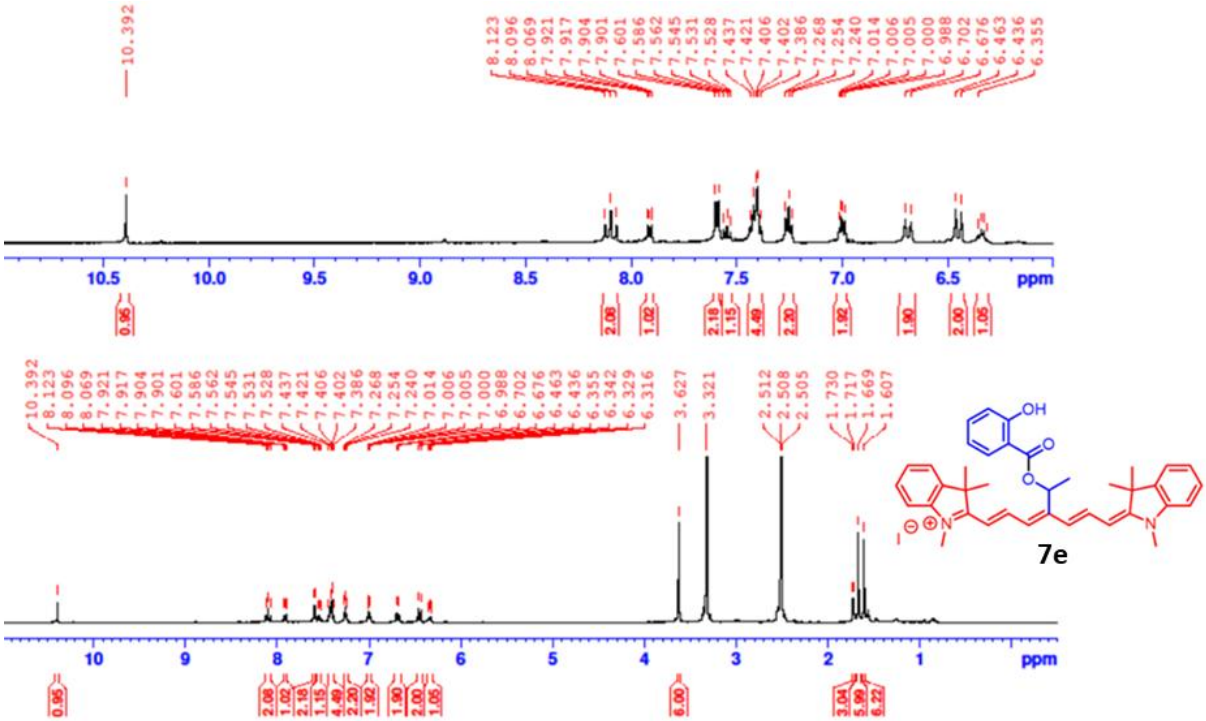


Fig. S36: [1]H NMR spectra of 7e.

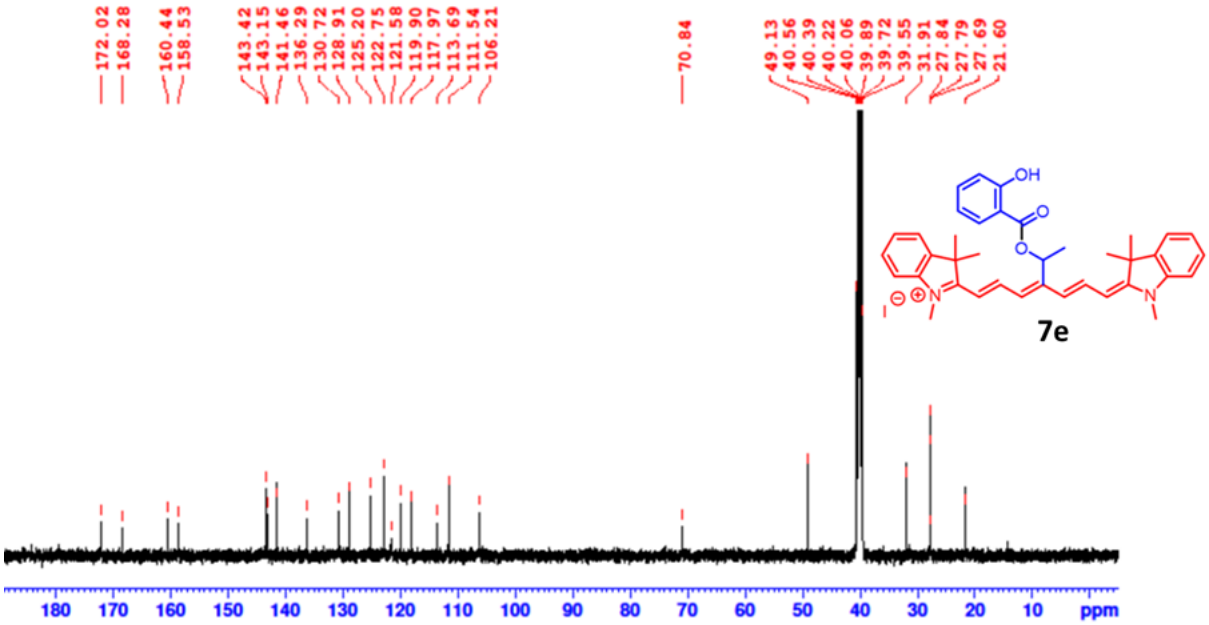


Fig. S37: [13]C NMR spectra of 7e.

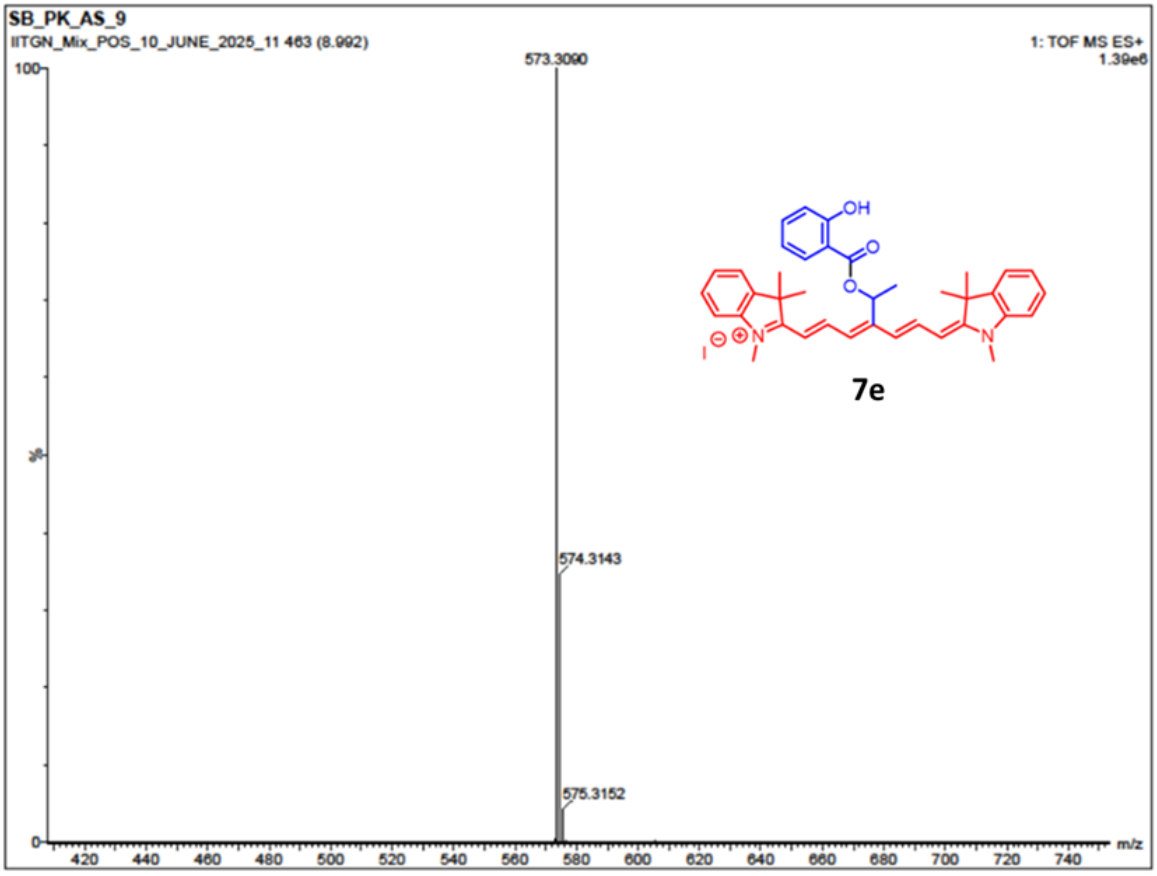


Fig. S38: HR-MS spectra of 7e.

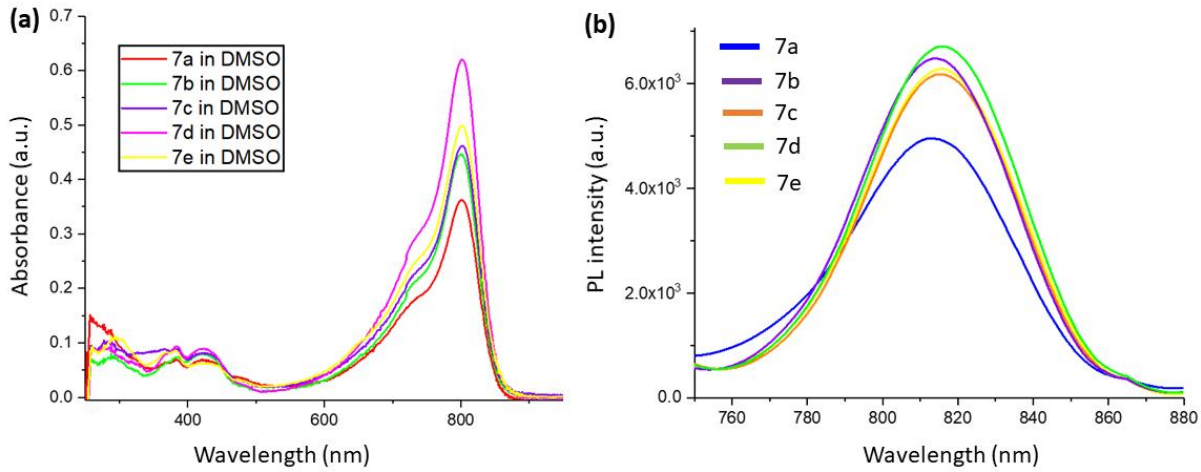


Fig. S39: UV-Vis and fluorescence spectra of 7a-7e in DMSO.

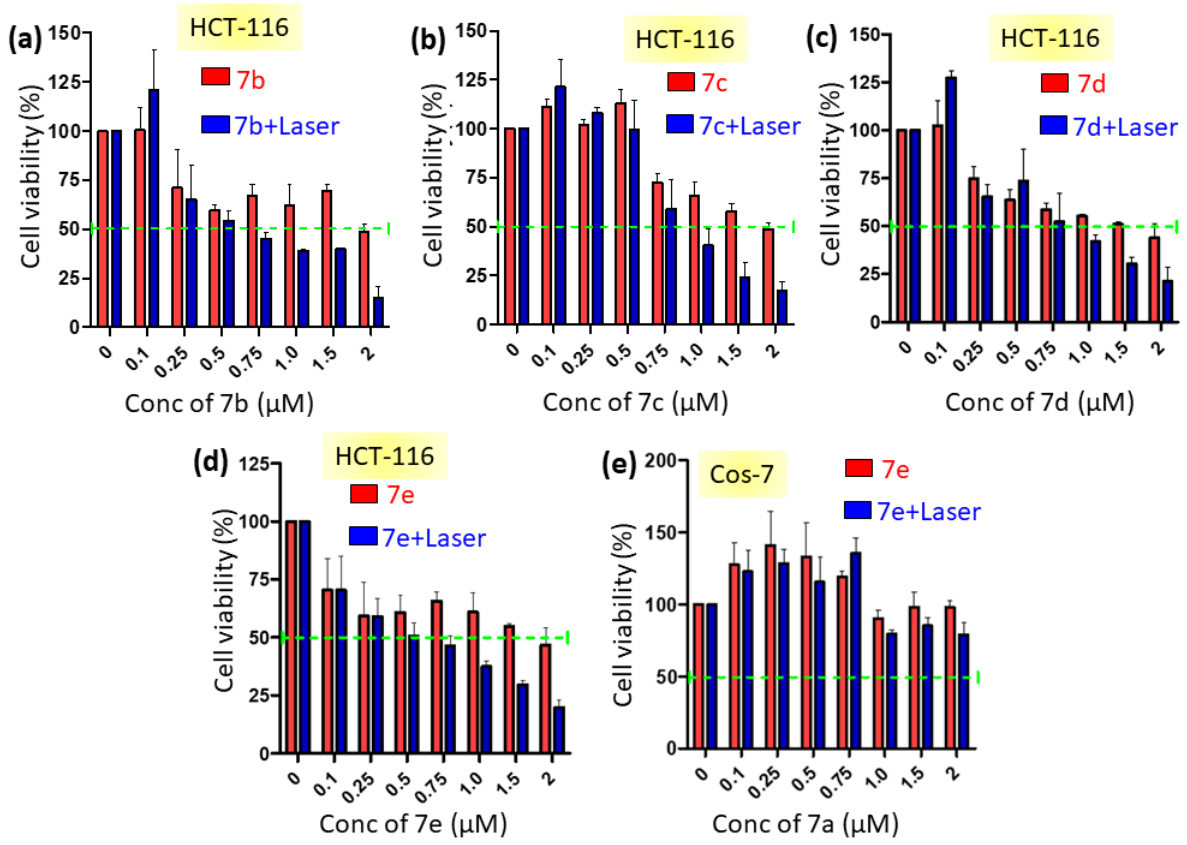


Fig. S40: (a-d) Dose dependent cell viability assay of 7b-e in HCT-116 cells at 24h with or without irradiation under 808 nm NIR laser for 10 min. (e) Dose dependent cell viability assay of 7a in Cos-7 cells at 24h with or without irradiation under 808 nm NIR laser for 10 min.

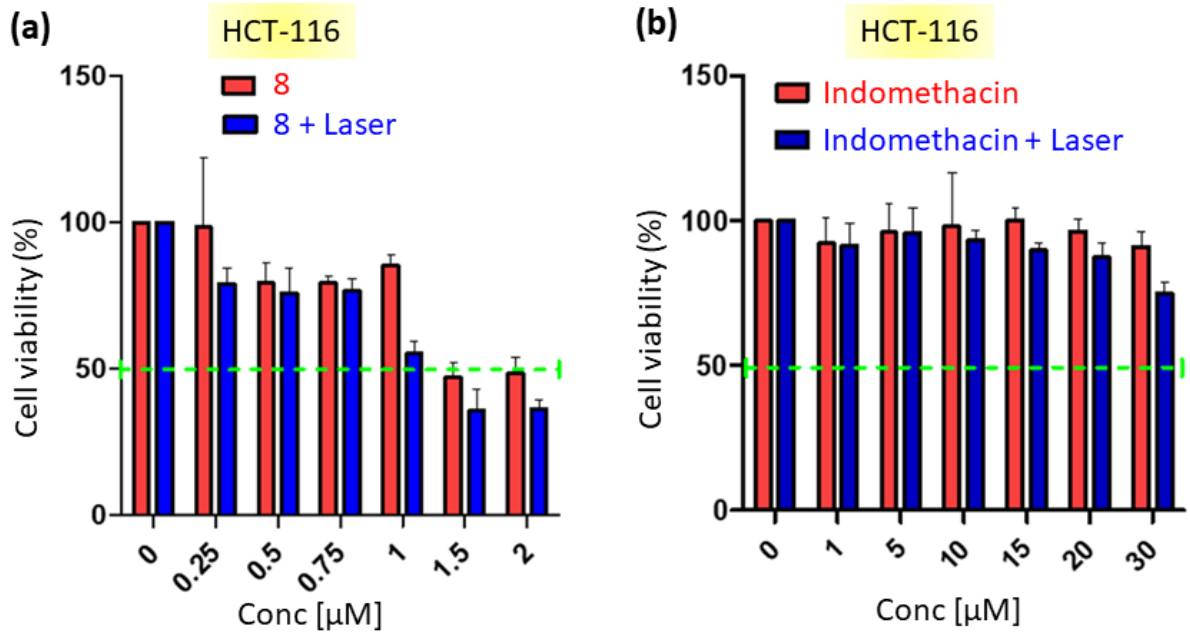


Fig. S41: (a,b) Dose dependent cell viability assay of 8 and indomethacin in HCT-116 cells at 24h with or without irradiation under 808 nm NIR laser for 10 min.

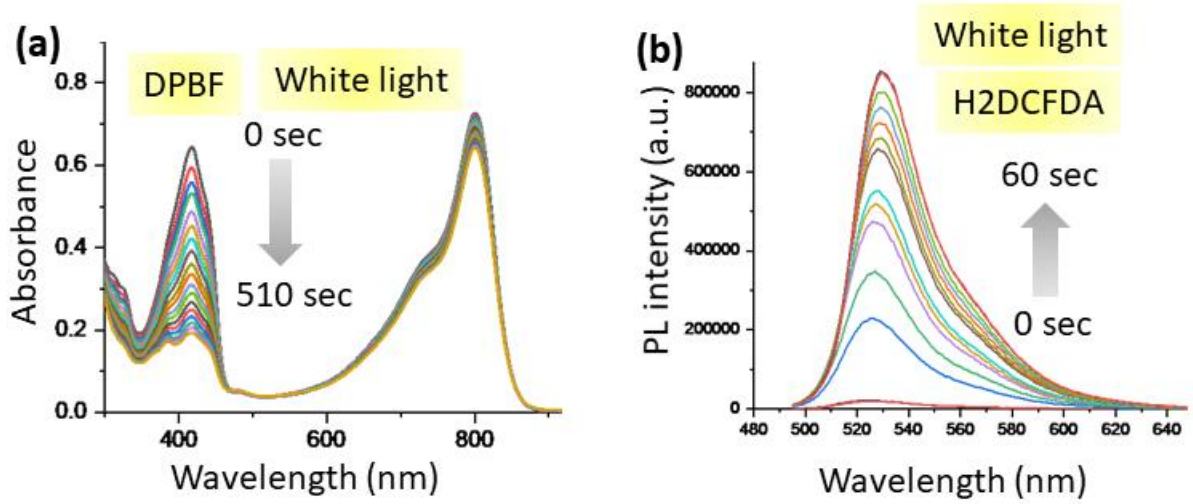


Fig. S42: (a) DPBF study of compound 7a in a time-dependent manner monitored by UV-visible spectra after irradiation with white light. (b) H2DCFDA study of compound 7a in a time-dependent manner by fluorescence spectra under irradiation with white light.

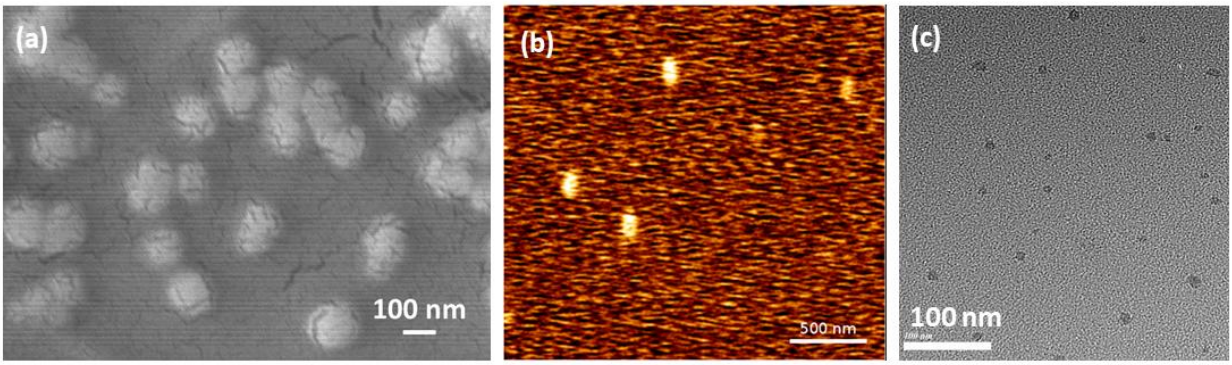


Fig. S43: (a) FESEM, (b) AFM and (c) TEM images of the self-assembled nanostructure of compound 7a in water.

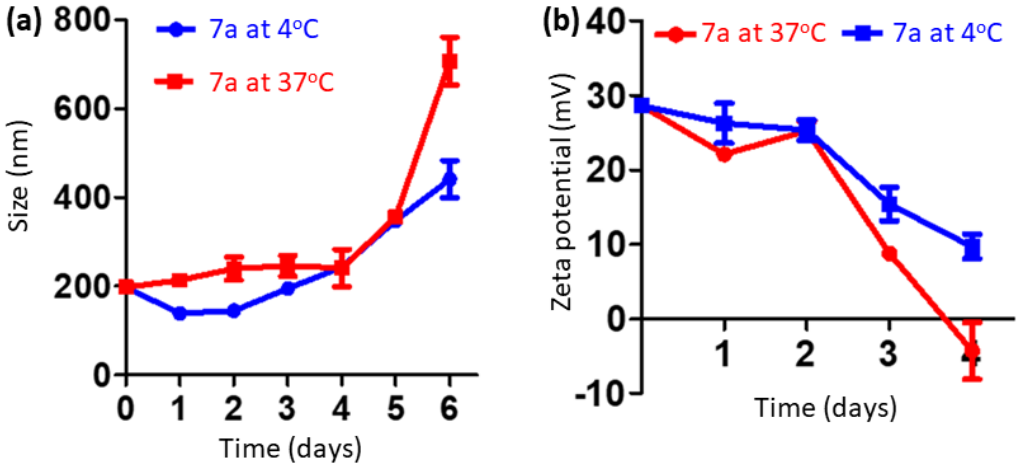


Fig. S44: (a, b) Change of size and zeta potentials of self-assembled nanoparticle of compound 7a in water at 4°C and 37°C in a time dependent manner.

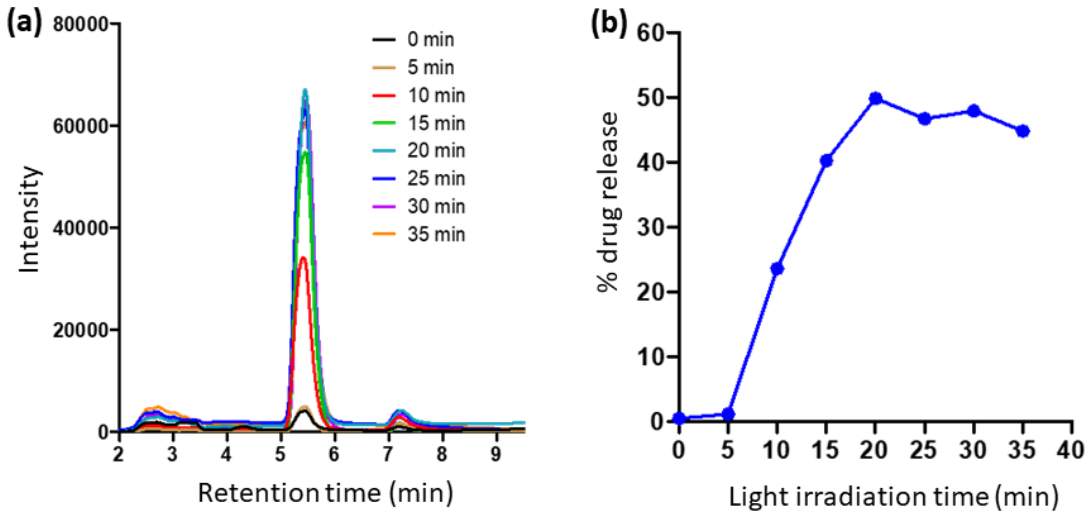


Fig. S45: Release of indomethacin from compound 7a under irradiation with 808 nm laser for 40 min determined by (a) HPLC and (b) quantification of % of indomethacin released in different time points.

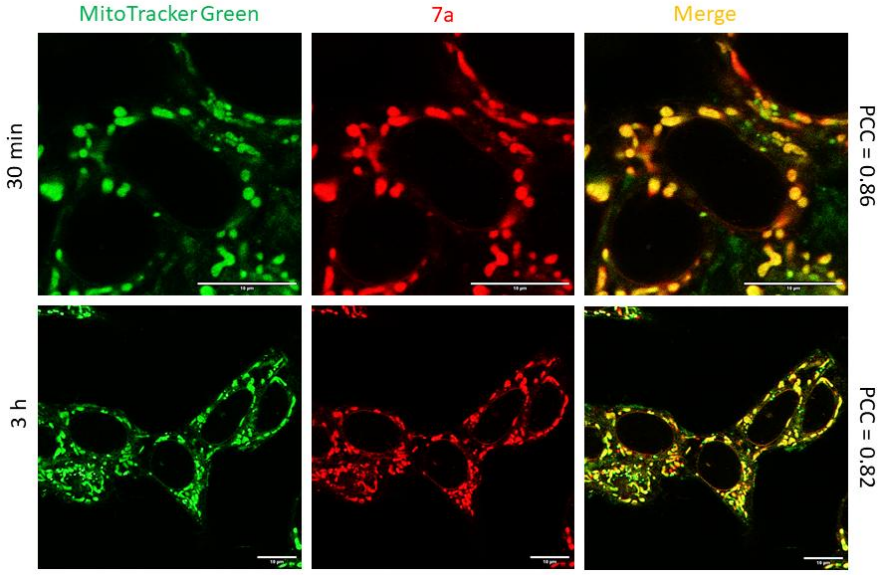


Fig. S46: Confocal laser scanning microscopy images of HCT-116 cells treated with compound 7a for 30 min and 3h followed by staining the mitochondria with MitoTracker Green dye. Scale bar = 10 μm.

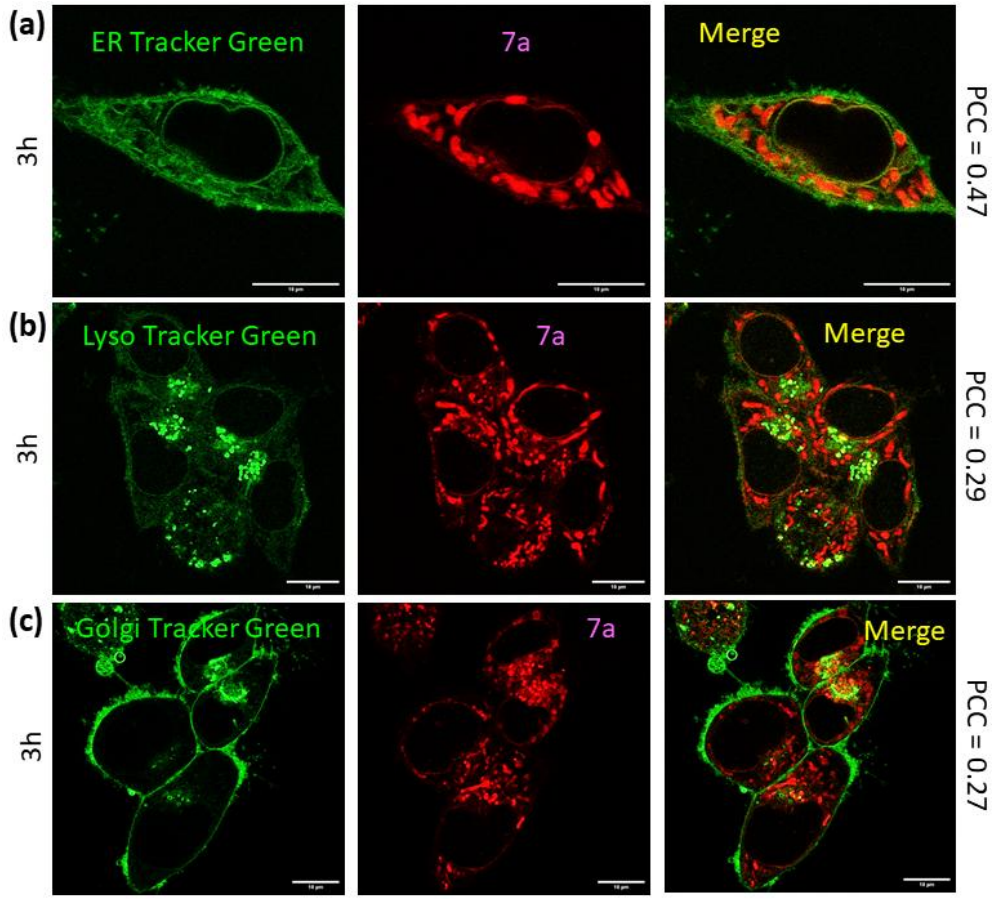


Fig. S47: Confocal laser scanning microscopy images of HCT-116 cells treated with compound 7a for 3h followed by staining with (a) ER Tracker Green, (b) LysoTracker Green and (c) Golgi Tracker Green dyes. Scale bar = 10 µm.

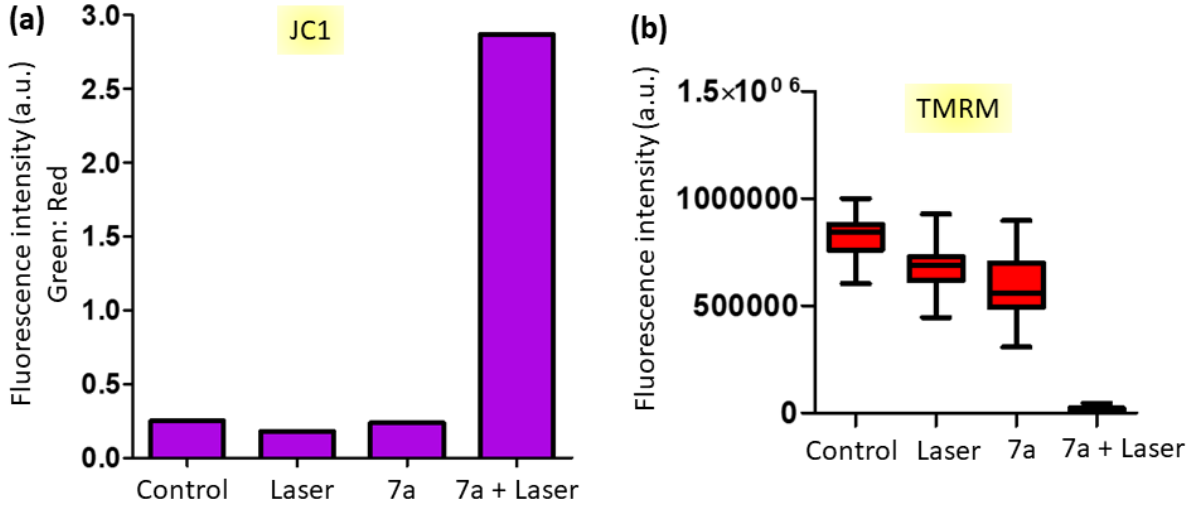


Fig. S48: Quantification of the fluorescence intensity from the confocal images of HCT-116 cells treated with compound 7a for 24h followed by irradiation with or without 808 nm laser for 10 min and stained the cells with (a) JC-1 and (b) TMRM to determine the mitochondrial outer membrane depolarization and damage respectively.

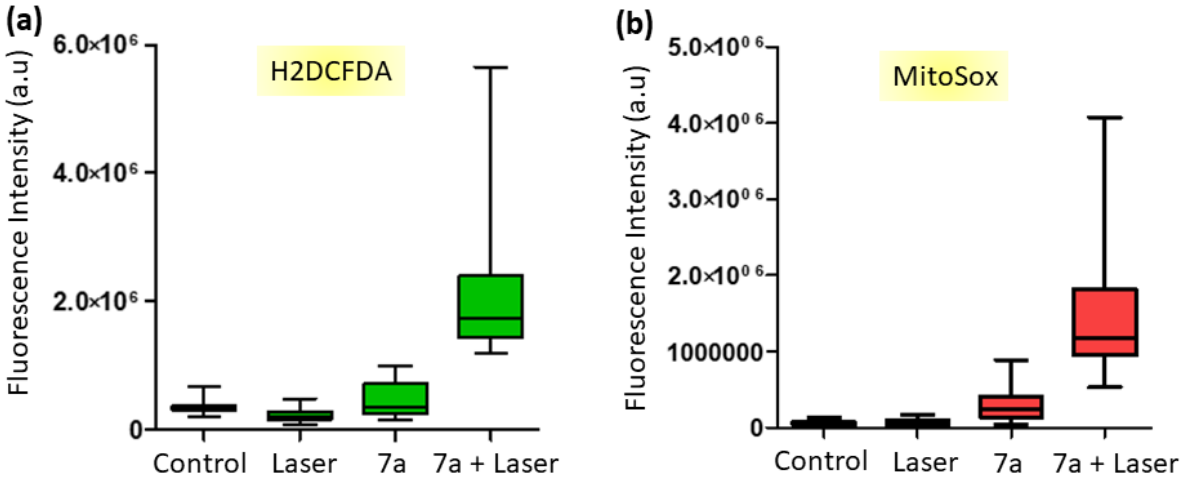


Fig. S49: Quantification of the fluorescence intensity from the confocal images of HCT-116 cells treated with compound 7a for 24h followed by irradiation with or without 808 nm laser for 10 min and stained the cells with (a) H2DCFDA and (b) MitoSox to determine the ROS and superoxide generation respectively.

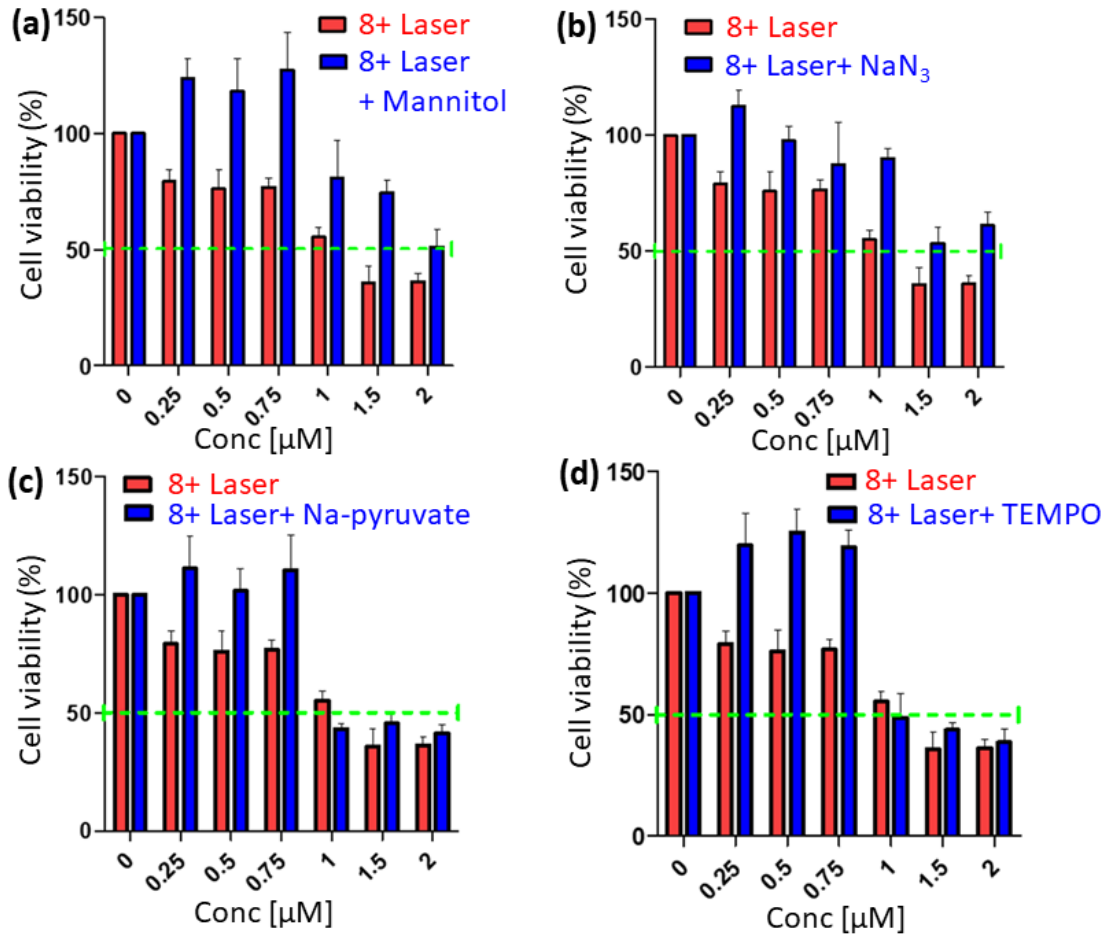


Fig. S50: (a-d) Viability of HCT-116 cells treated with a combination of compound 8 and mannitol, sodium azide, sodium pyruvate and TEMPO, respectively, for 24 h followed by irradiation under an 808 nm (0.8 W/cm2) laser for 10 min, measured by MTT assay.

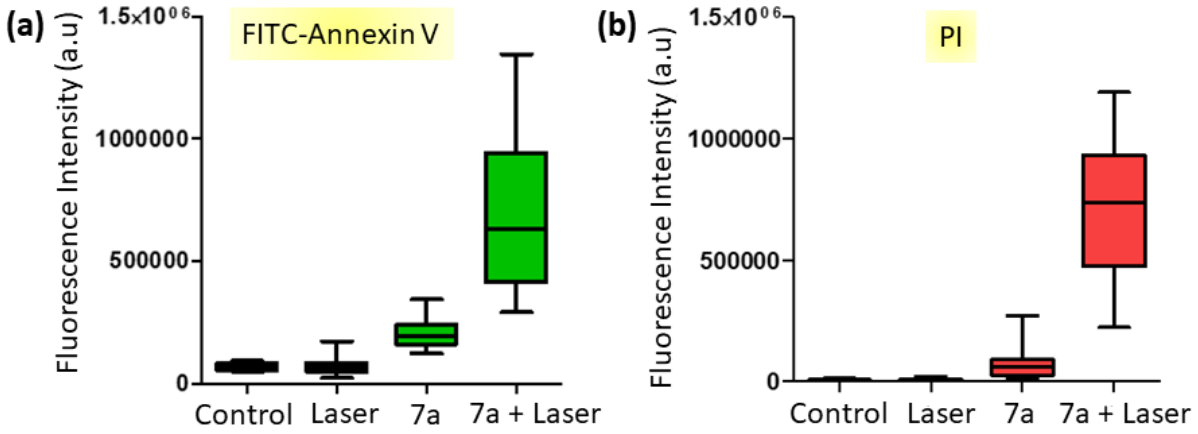


Fig. S51: Quantification of the fluorescence intensity from the confocal images of HCT-116 cells treated with compound 7a for 24h followed by irradiation with or without 808 nm laser for 10 min and stained the cells with (a) FITC-Annexin V and (b) PI to determine the early/late apoptosis and late apoptosis/necrosis respectively.

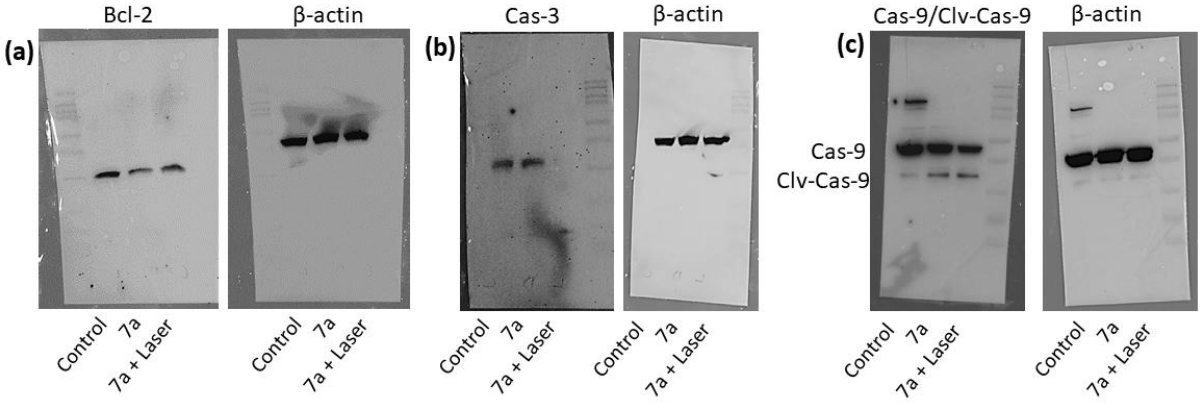


Fig. S52: Western blot images of (a) Bcl-2, (b) Cas-3 and (c) Cas-9/cleaved Cas-9 in HCT-116 cells treated with compound 7a for 24h followed by with or without irradiation under 808 nm laser for 10 min.

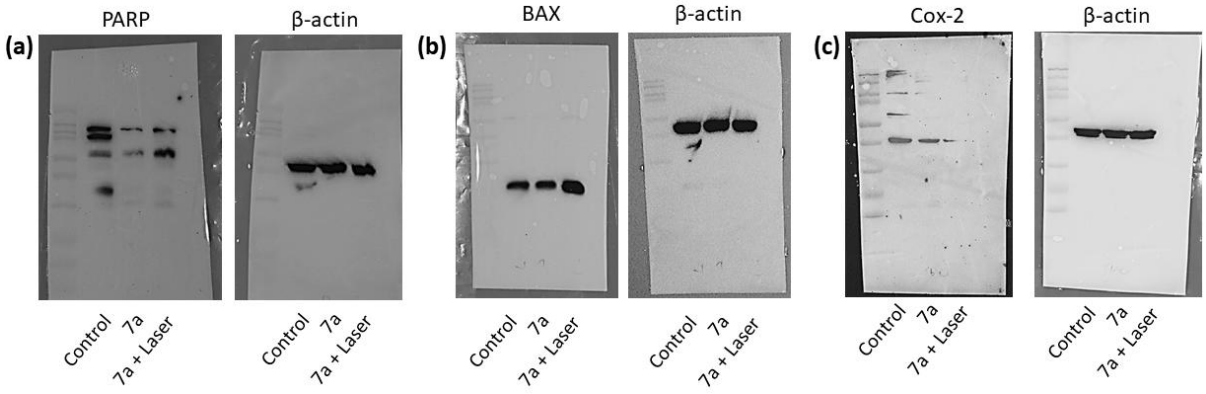


Fig. S53: Western blot images of (a) PARP, (b) BAX and (c) Cox-2 in HCT-116 cells treated with compound 7a for 24h followed by with or without irradiation under 808 nm laser for 10 min.

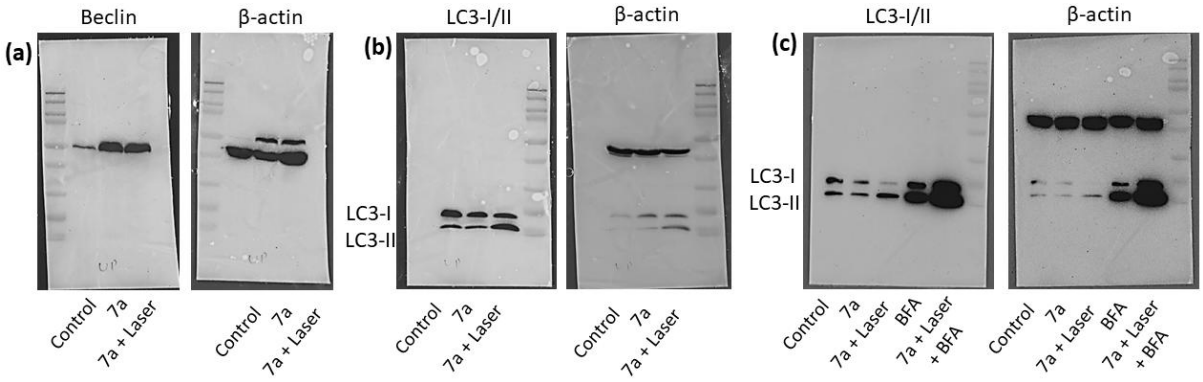


Fig. S54: Western blot images of (a) Beclin and (b) LC3-I/II in HCT-116 cells treated with compound 7a for 24h followed by with or without irradiation under 808 nm laser for 10 min. (c) Western blot images of LC3-I/II in HCT-116 cells treated with compound 7a for 24h followed by with or without irradiation under 808 nm laser for 10 min also in combination with autophagy inhibitor bafilomycin A.

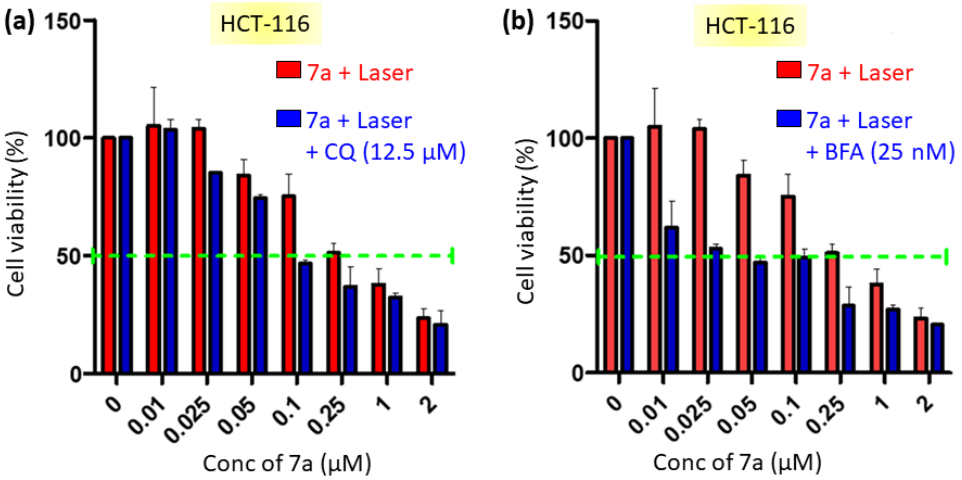


Fig. S55: Dose dependent cell viability assay of 7a in HCT-116 cells at 24h with or without irradiation under 808 nm NIR laser for 10 min in combination with autophagy inhibitors (a) chloroquine and (b) bafilomycin A.

IC50 (μM) under 808 nm laser irradiation					
Compound	**HCT-116**	**MDA-MB-2321**	**HeLa**	**RPE-1**	**Cos-7**
7a	0.08-0.12	0.4-0.5	0.17-0.2	> 2	> 2
7b	0.6-0.7	-	-	-	-
7c	0.85-0.95	-	-	-	-
7d	0.8-0.9	-	-	-	-
7e	0.45-0.55	-	-	-	-

Table S1: IC_{50} values of 7a-e in different cell lines at 24h incubation followed by irradiation under 808 nm (0.8 W/cm^2) NIR laser for 10 min.