Supporting Information

Mitochondria targeted small molecule-mediated chemo-photodynamic therapy induces apoptosis in cancer cells

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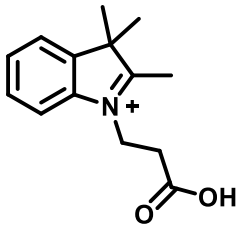
Materials: 3-bromopropanoic acid was purchased from GLR innovations, 2,3,3-Trimethylindolenine was purchased from Sigma, Dry solvents were purchased from Finar, Ethylene diamine, BOC anhydride, Aniline, HOBt, EDCI.HCl, DIPEA was purchased from Avra, Indomethacin was purchased from TCI chemicals, HOBt, Tetra methoxy propane and DPBF (1,3-diphenylisobenzofuran) was purchased from BLD Pharma. Glasswares were purchased from Borosil or Asco, TLC sheets of silica gel 60 F254 was used for monitoring the reaction completions, Heidolph Rotary Evaporator was used for solvent evaporation, silica gel of 60-120 mesh and 100-200 mesh were purchased from Avra, Solvents for column chromatography Hexane, Ethyl acetate, Dichloromethane, Methanol were obtained from Finar, UV was recorded using a V-750 JASCO spectrophotometer and Fluorescence using Fluorolog HORIBA JOBIN YVON instrument, NMR spectra was recorded using Ascend NMR-500 MHz (Bruker) spectrometer, using $CDCl_3$ or DMSO as solvent and spectra was analysed using solvent as a reference peak, FE-SEM was carried out using JeOL JSM-7600F (Japan), DLS was performed usingMulti-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, H2DCFDA was purchased from Sigma. BCl-2 (C-2) antibody was purchased from santa Cruz and COX-2, Cas-9, antibody was purchased from Thermo fischer scientific, Bax, PARP,Cas-3 was purchased from Cell Signaling. Imaging by confocal was performed by using Leica TCS SP8 microscope, Data was analysed and plotted by using Graph Pad prism, Origin, Confocal images was analysed and quantified by using Image j, western blot images were quantified by using Imaje Lab software.

Methods:

Synthesis of compound 3:



Procedure: In a 250mL round bottom flask, 3-bromopropanoic acid (1eq., 2 g), 2,3,3-Trimethylindolenine (1.2 eq., 2.5 g) and Potassium Iodide (1.2 eq., 2.6 g) was added to dry acetonitrile in the inert condition and refluxed at 80°C for 48h. The solvent was evaporated by rota evaporator, and the compound was purified by 60-120 mesh silica, product came between 10-12% of MeOH in DCM.

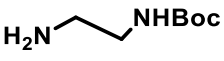
Yield: 85%

^{1}H NMR (500 MHz, DMSO): δ 8.00– 7.99 (m, 1H), 7.86 – 7.84 (m, 1H), 7.63-7.61 (m, 2H), 4.66 (t, *J* = 7.0 Hz, 2H), 2.99 (t, *J* = 7.0 Hz, 2H), 2.87 (s, 3H), 1.54 (s, 6H).

^{13}C NMR (126 MHz, DMSO): δ 198.39, 172.50, 171.99, 142.22, 141.28, 129.82, 129.39, 123.97, 116.04, 54.75, 49.05, 44.08, 31.59, 22.35, 21.53, 15.00.

Mass: m/z calculated for $C_{14}H_{18}NO_2$+, [M+]: 232.1332, Observed Mass: 232.1342

Synthesis of compound 5:



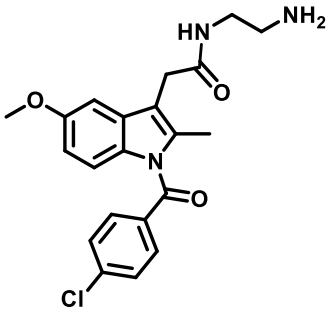
Procedure: In a 250 mL round bottom flask, Boc anhydride (1 eq.) was added drop wise to ethylenediamine (6 eq.) in dry DCM at 0°C in the inert condition and the reaction mixture was stirred overnight; the solvent was evaporated by rota evaporator, and the product was extracted three times with DCM/Water and finally dried over vacuum.

Yield: 80%

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.17 (s, 1H), 3.11 (d, *J* = 5.3 Hz, 2H), 2.73 (t, *J* = 5.9 Hz, 2H), 1.81 (s, 2H), 1.38 (s, 9H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 155.33, 78.16, 42.24, 40.81, 28.66, 27.40.

Synthesis of compound 6:



Procedure: In a 100 mL round bottom flask, Indomethacin (1 eq., 700 mg), compound 5 (1.5 eq., 470 mg), EDCI. HCl (1.6 eq., 600 mg), HOBt (1.6eq., 422.96) and DIPEA (1 eq., 340µL) were added into dry DMF at 0°C in the inert condition and the reaction mixture was stirred at room temperature for 30h. Completion of the reaction was monitored by TLC. The product was extracted with EtOAc/Water and purified by 100-200 mesh silica at 8-10% MeOH/DCM. The obtained product was dissolved in dry DCM in an inert condition, and 3-4 eq. of Trifluoroacetic acid (TFA) was added to it at 0°C, and the reaction was stirred for 2h; reaction completion was monitored by TLC, the solvent was evaporated to obtain compound 6 as the required product.

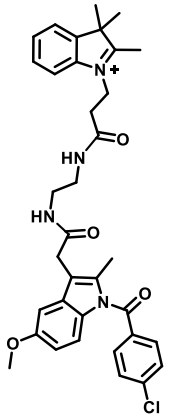
Yield: 70%

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.61 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 2.2 Hz, 1H), 6.81 (d, *J* = 9.0 Hz, 1H), 6.65-6.63 (m, 2H), 3.78 (s, 3H), 3.60 (s, 2H), 3.51 (s, 3H), 3.29 (d, *J* = 5.5 Hz, 2H), 2.81 (s, 2H), 2.34 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 170.25, 167.36, 155.19, 138.51, 135.36, 132.56, 130.17, 129.88, 129.43, 128.16, 114.03, 111.80, 110.94, 100.07, 54.72, 39.71, 39.38, 30.92, 30.56, 21.63, 12.17.

Mass: m/z calculated for $C_{21}H_{23}ClN_3O_3$ +, $[M + H]^+$: 400.1422, Observed Mass: 400.1437

Synthesis of compound 7:



Procedure: In a round bottle flask, compound 3 (1eq., 1.4g), compound 6 (1.2eq., 2.9 g), EDCI.HCl (1.2eq., 1.4 g), HOBt (1.2eq., 991 mg), and DIPEA (1eq., 1mL) were added to dry DMF in the inert condition at 0°C, and the reaction mixture was stirred at room temperature for 24h. Completion of the reaction was monitored through TLC. The solvent was evaporated, and the product was purified by 100-200 mesh silica in 9-11% MeOH/DCM.

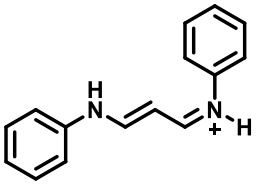
Yield: 55%

^{1}H NMR (500 MHz, DMSO): δ 7.99 (d, *J* = 8.4 Hz, 1H), 7.73-7.69 (m, 4H), 7.67-7.62 (m, 3H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.09 (d, *J* = 2.4 Hz, 1H), 6.95-6.92 (m, 1H), 6.71-6.68 (m, 1H), 3.88 (d, *J* = 27.3 Hz, 2H), 3.75 (s, 3H), 3.73 – 3.69 (m, 2H), 3.55 (s, 1H), 3.49 (s, 2H), 3.38 (s, 1H), 3.31 (d, *J* = 6.1 Hz, 1H), 3.07 (s, 4H), 2.22 (s, 3H), 1.26 (d, *J* = 5.8 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 170.86, 170.08, 168.32, 156.01, 138.11, 138.04, 135.69, 134.70, 131.62, 131.33, 130.76, 129.53, 129.49, 128.30, 127.78, 124.97, 119.57, 115.02, 114.58, 114.35, 111.69, 110.08, 102.28, 55.91, 55.86, 54.01, 39.61, 39.44, 37.11, 31.65, 18.50, 17.17, 13.82, 12.86

Mass: m/z calculated for $C_{35}H_{38}ClN_4O_4^+$: 613.2576, Observed Mass: 613.2584.

Synthesis of compound 8:



Procedure: A solution of aniline (2 eq., 5.5 mL) in dilute HCl was added dropwise by a dropping funnel over a period of 1h to a solution of 1,1,3,3-Tetramethoxy propane (1 eq., 5.2 mL) in dilute HCl taken in a 200 mL of round bottle flask. The colour of the solution changed to orange; the reaction mixture was further stirred for 4h at 50°C. Orange precipitate was formed which was filtered under vacuum filtration assembly, washed with water, and dried in a vacuum.

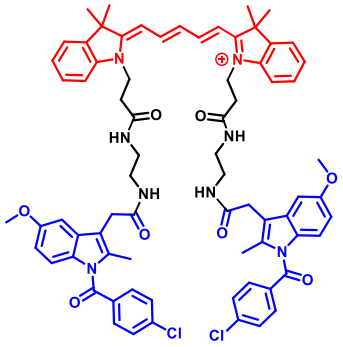
Yield: 92%

^{1}H NMR (500 MHz, DMSO): δ 12.70 (s, 2H), 8.91 (d, *J* = 11.3 Hz, 2H), 7.50 – 7.43 (m, 8H), 7.26 (t, *J* = 7.1 Hz, 2H), 6.51 (t, *J* = 11.5 Hz, 1H)

^{13}C NMR (126 MHz, DMSO): δ 198.39, 172.50, 171.99, 142.22, 141.28, 129.82, 129.39, 123.97, 116.04, 54.75, 49.05, 44.08, 31.59, 22.35, 21.53, 15.00

Mass: m/z calculated for $C_{15}H_{15}N_2^+$: 223.1230, Observed Mass:223.1221

Synthesis of compound Cy-(Indo)$_2$:



Procedure: In a round bottle flask of 50 mL, compound 8 (1eq., 130 mg), compound 7 (2 eq., 676.2 mg) and Sodium acetate (1.5 eq., 67.73 mg) were added in acetic anhydride and the reaction mixture was continued to stir at 70°C for 6h in the inert condition. Completion of the reaction was monitored through TLC. The solvent was evaporated by rota evaporator, followed by the purification of the desired molecule by 100-200 mesh silica in 8-10% MeOH/DCM. The molecule was purified three times by column chromatography and dried over vacuum to obtain the final blue solid.

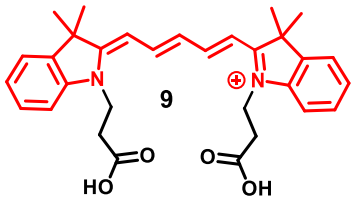
Yield: 60%

^{1}H NMR (500 MHz, DMSO): δ 8.32 (t, *J* = 13.0 Hz, 2H), 8.23 (s, 2H), 8.15 (s, 2H), 7.67 – 7.65 (m, 4H), 7.62 – 7.59 (m, 6H), 7.37 (t, *J* = 7.7 Hz, 2H), 7.32 (d, *J* = 7.9 Hz, 2H), 7.23 (t, *J* = 7.6 Hz, 2H), 7.09 (d, *J* = 2.5 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.66-6.63 (m, 2H), 6.48 (t, *J* = 12.3 Hz, 1H), 6.27 (d, *J* = 13.8 Hz, 2H), 4.23 (d, *J* = 6.5 Hz, 4H), 3.72 (s, 6H), 3.47 (s, 4H), 3.03 (s, 8H), 2.48-2.45 (m, 4H), 2.20 (s, 6H), 1.65 (s, 12H).

^{13}C NMR (126 MHz, DMSO): δ 173.13, 170.04, 169.82, 168.27, 155.98, 154.67, 142.20, 141.48, 138.02, 135.68, 134.68, 131.60, 131.33, 130.71, 129.47, 128.76, 125.14, 122.83, 114.97, 114.65, 111.68, 111.55, 103.89, 102.33, 55.87, 49.36, 38.97, 38.83, 33.67, 31.65, 27.56, 22.21, 14.38, 13.84.

Mass: m/z calculated for $C_{73}H_{75}Cl_2N_8O_8^+$: 1261.5079, Observed Mass: 1261.5085

Synthesis for compound 9:



Procedure: In a round bottle flask of 50 mL, compound 8 (1eq., 150 mg), compound 3 (2 eq., 295 mg) and Sodium acetate (1.5 eq., 78.16 mg) were added in acetic anhydride and the reaction mixture was continued to stir at 70°C for 3h in the inert condition. Completion of the reaction was monitored through TLC. The solvent was evaporated by a rota evaporator, followed by the purification of the desired molecule by 100-200 mesh silica in 15-20% MeOH/DCM with a few drops of acetic acid. The molecule was dried over a vacuum to obtain the final product as a blue solid.

^{1}H NMR (500 MHz, D_2O): δ 7.99 (t, *J* = 13.0 Hz, 2H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.27 (t, *J* = 7.5 Hz, 2H), 6.57 (t, *J* = 12.5 Hz, 1H), 6.30 (d, *J* = 13.6 Hz, 2H), 4.32 (t, *J* = 7.3 Hz, 4H), 2.66 (t, *J* = 7.3 Hz, 4H), 1.65 (s, 12H).

Mass: m/z calculated for $C_{31}H_{35}N_2O_4^+$: 499.2591, Observed Mass: 499.2592.

Reactive oxygen generation under 740nm:

The absorbance of a 30µM solution of DPBF was measured in the dark, followed by the addition of **Cy-(Indo)$_2$** in the same solution at a final concentration of 4µM. After mixing the solution well, the absorbance of the solution was recorded in the dark to obtain the baseline. Oxygen was purged into the solution and irradiated with 740 nm (0.9 W/cm^2) for 10 min., followed by the recording of absorbance at 30-second intervals for monitoring the reactive oxygen generation process under 740nm LED irradiation for a total of 1200 seconds. The interaction of DPBF and **Cy-(Indo)$_2$** under 740nm LED light and oxygen leads to the observed changes in the absorbance spectra.

ROS generation using H2DCFDA under 740nm:

In a beaker, the solution of 0.5 mL of 1 mM H2DCFDA and 2 mL of 10 mM NaOH in water was stirred for 30 minutes at room temperature. Thereafter, 10 mL of 1X PBS was added. The fluorescence intensity of the solution was recorded, followed by the addition of **Cy-(Indo)$_2$** in the same solution at a final concentration of 4μM and irradiation with 740 nm (0.9 W/cm^2) for 10 min. and the fluorescence intensity of DCF was monitored after every 10 seconds.

Self-assembly & DLS Stability studies: A DMSO stock solution of Cy-(Indo)2 was prepared, and then samples of two different concentrations were prepared: 2 μM for measuring DLS & 10μM for measuring the Zeta potential of the Cy-(Indo)2 aggregates in water and cell culture complete media at room temperature and 4°C in water. Aggregation behaviour and zeta potential were recorded at different time points using a Malvern DLS instrument.

Field Emission Scanning Electron Microscopy (FE-SEM): FE-SEM experiments were performed on an Advance Analytical Field Emission Scanning Electron Microscope. 10 μL from 1 μM solution of compounds in water was placed on a commercially available silicon chip (1 × 1 cm), and it was dried under a high vacuum desiccator for 4 hours and kept under vacuum build a desiccator till analysis. The silicon chip was coated with gold for 60 seconds, and images were captured at different magnifications.

Atomic Force Microscopy (AFM): AFM experiments were performed on an Atomic Force Microscope. 10 μL from a 0.25 μM solution of compounds in water was placed on a commercially available glass slide attached, and it was dried under a high vacuum desiccator for 4 hours and kept under vacuum in a desiccator till analysis. The glass slide with the compound was used to capture the surfaces of aggregates at different magnifications.

Theoretical calculations:

The ORCA 5.0.3 software package was used for all quantum mechanical calculations [1]. The ground-state geometry of the molecule was optimized using the B3LYP functional in combination with the def2-SVP basis set. This optimized geometry was subsequently used to calculate the absorption spectrum *via* a single-point calculation. To analyze the nature of the frontier molecular orbitals (HOMO and LUMO) involved in the absorption process, a single-point excited-state calculation was performed using Gaussian 09 (G09) [2]. This provided insights into the electronic transitions during the excitation. Due to the molecule's large size and atomic complexity, triplet state lifetime calculations were carried out using the BP86 functional with the def2-SVP basis set. BP86, being a pure GGA functional, offers reduced computational cost compared to hybrid functionals such as B3LYP, making it more suitable for large systems.

Molecular dynamics (MD) simulations were conducted using the GROMACS software [3-4] to investigate the aggregation behavior of the molecule. The organic molecule was initially placed at the center of a simulation box and solvated with 1000 water molecules. The box was then replicated in three dimensions, resulting in a final system containing 8 organic molecules and 8000 water molecules. This configuration served as the starting point for MD simulations. The system was parameterized using the OPLS force field. Energy minimization was performed using the steepest descent algorithm to eliminate unfavorable contacts and ensure no atom overlaps. Subsequently, a 5.0 ns NPT ensemble simulation was conducted, employing the Verlet cutoff scheme and the Berendsen barostat for pressure coupling. After equilibration, the resulting morphologies were analyzed using Visual Molecular Dynamics (VMD) software [5] and radial distribution function (RDF) analysis.

Cell culture study

Cell culture: HeLa, HCT116, COS7 cells were cultured in complete media using DMEM, 1% Penstrep and 10% FBS in an incubator at 37°C, 95% humidity and 5% CO_2 atmosphere.

Cell Viability assay in the dark: In a 96-well plate, 5000 cells per well were seeded for HeLa, HCT116 and COS7, cells were left for attachment to the plate for 24h, and further cells were treated with **Cy-(Indo)$_2$** and compound 9 in a dose-dependent manner with 1% DMSO used as a control and incubated for next 24 h. Next day, media was aspirated and MTT reagent was added at the working concentration of 0.5 mg/mL in complete media, cells were

further incubated with MTT for 4h to allow the formation of formazan crystals, further 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 analyzed by Graph Pad prism to calculate the percentage cell viability.

Cell Viability assay in the light: In a 96-well plate, 5000 cells per well were seeded for HeLa, HCT116 and COS7, cells were left for attachment to the plate for 24h, and further cells were treated with **Cy-(Indo)$_2$** and compound 9 in a dose-dependent manner with 1% DMSO used as a control and incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then further incubated for next 4h to complete the 24h incubation time period. Next day, media was aspirated and MTT reagent was added at the working concentration of 0.5 mg/mL in complete media, cells were further incubated with MTT for 4h to allow the formation of formazan crystals, further 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 analyzed by Graph Pad prism to calculate the percentage cell viability.

Reactive oxygen generation: The absorbance of 30µM solution of DPBF was measured in dark, followed by the addition of **Cy-(Indo)$_2$** in the same solution at final concentration of 4µM. After mixing the solution well, the absorbance of the solution was recorded in the dark to obtain the baseline. Oxygen was purged into the solution and irradiated with light, followed by the recording of absorbance at 1 minute interval for monitoring the reaction progress. The interaction of DPBF and **Cy-(Indo)$_2$** in light and oxygen lead to the observed changes in the absorbance spectra.

Excitation and Emission range of Cy-(Indo)$_2$ in confocal studies:

Excitation: 633 nm, Emission: 650 nm-750 nm

Colocalisation study: Four live cell plates, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. Next day cells were treated with 3µM of **Cy-(Indo)$_2$** and incubated for 3 h time point for determining the colocalization in Mitochondria, Gogi apparatus and Lysosomes and endoplasmic reticulum. After the incubation period, media was aspirated, cells were washed with 1X PBS, followed by the addition of Mitotracker Green (100 nM), Golgi Tracker Green(1µg/mL), Lyso Tracker green (80nM) and ER tracker green (100 nM) to plate 1, 2, 3 and 4 respectively, and incubated further for 20 minutes. Further media was aspirated, cells were washed with 1X PBS and fresh DMEM was added. Samples were imaged by Leica confocal microscope and analysed by software Image J.

ROS generation by H2DCFDA: Four live cell plates, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. All the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 4µM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 4µM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, Media was aspirated, cells were washed with 1XPBS and H2DCFDA was added at the working concentration of 2µM in DMEM to all the four plates and incubated for 25 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, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. All the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 4µM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 4µM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, Media was aspirated, cells were washed with 1XPBS 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. While imaging cells were maintained at 37°C, 95% humidity and 5% CO_2 atmosphere.

TMRM Assay: Four live cell plates, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. All the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 4μM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 4μM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, Media was aspirated, cells were washed with 1XPBS and TMRM 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. While imaging cells were maintained at 37°C, 95% humidity and 5% CO_2 atmosphere.

JC1 Assay: Four live cell plates, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. All the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 4μM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 4μM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, Media was aspirated, cells were washed with 1XPBS and JC1 was added at the working concentration of 10 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.

MitoSOX Assay: Four live cell plates, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. All the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 4μM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 4μM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, Media was aspirated, cells were washed with 1XPBS and MitoSOX was added at the working concentration of 500 nM 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.

Annexin V Assay: Four live cell plates, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. All the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 5μM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 5μM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, Media was aspirated, cells were washed with 1XPBS and 5μL of FITC conjugated Annexin V was added to all the four plates and incubated for 25 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.

PI Assay: Four live cell plates, each containing 25000 HCT116 cells, were seeded in complete media and left for overnight attachment in an incubator at 37°C. All the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 5μM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 5μM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, Media was aspirated, cells were washed with 1XPBS and PI was added at the working concentration of 500 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. While imaging cells were maintained at 37°C, 95% humidity and 5% CO_2 atmosphere.

Western Blot: Four 3 cm tissue culture plates, each containing one lakh HCT116 cells, were seeded in complete media and left for 20 h attachment in an incubator at 37°C. Next day, all the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 7μM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 7μM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. After 24 h of incubation period, cells were washed with 1X PBS, trypsinised and whole cell lysate was formed by using lysis buffer containing protease phosphatase inhibitor in RIPA buffer, followed by quantification of protein by using Bradford reagent. Gel electrophoresis was carried out for separation of proteins by using SDS-page gel, equal amount of protein is loaded to each well followed by the running of gel and transferring of protein to nitrocellulose membrane. The membrane was cut and blocked by using 5X skimmed milk in 1X TBST, followed by overnight incubation at 4°C with primary antibody of BCl2, BAX, CAS-9, PARP, CAS-3 and COX2. The next day, the corresponding secondary antibody is added to the primary antibody and incubated at room temperature for 2h. By using enhanced chemiluminescence (ECL) reagent, blots were developed and imaged by Bio-Rad Gel documentation instrument and analysed by Image Lab software.

FACS: Four 3 cm tissue culture plates, each containing one lakh HCT116 cells, were seeded in complete media and left for 20 h attachment in an incubator at 37°C. Next day, all the four plates were treated in the following manner, Plate 1(cells + DMSO as a control) and Plate 2 (cells + **Cy-(Indo)$_2$** at 7μM) are incubated for 24 , Plate 3 (cells + DMSO as a control + light) and Plate 4 (cells + **Cy-(Indo)$_2$** at 7μM + Light) are incubated for 20 h followed by irradiation with 740 nm LED at 0.9 W/cm² for 10 minutes and then continued incubation for 4 h to complete the 24h time point. 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 by 1X Annexin V binding buffer 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 analysed by BD FACSAria Fusion machine.

Determination of ROS using Scavenger: 5000 HCT-116 cells were taken in a 96-well plate and incubated for 24 hours. Cells were incubated with various concentrations of **Cy-(Indo)$_2$**, 1% DMSO was used as a control, followed by the addition of scavengers like Mannitol (0.5mM) for hydroxy radical, Sodium Azide (0.01mM) for Singlet Oxygen, TEMPO (0.05mM) for Superoxide, and Sodium Pyruvate (1mM) for H_2O_2. After incubation for 20 hours, a 740 nm LED at 0.9W/cm^2 for 10 min was irradiated, and the sample was then incubated for another 4 hours to complete the 24 h incubation. Further steps involve removing the media and adding MTT reagent solution (0.5mg/ml in complete media with 10% PBS) to the plate. The plate containing the MTT reagent was incubated for 4 hours. After 4 hours, 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.

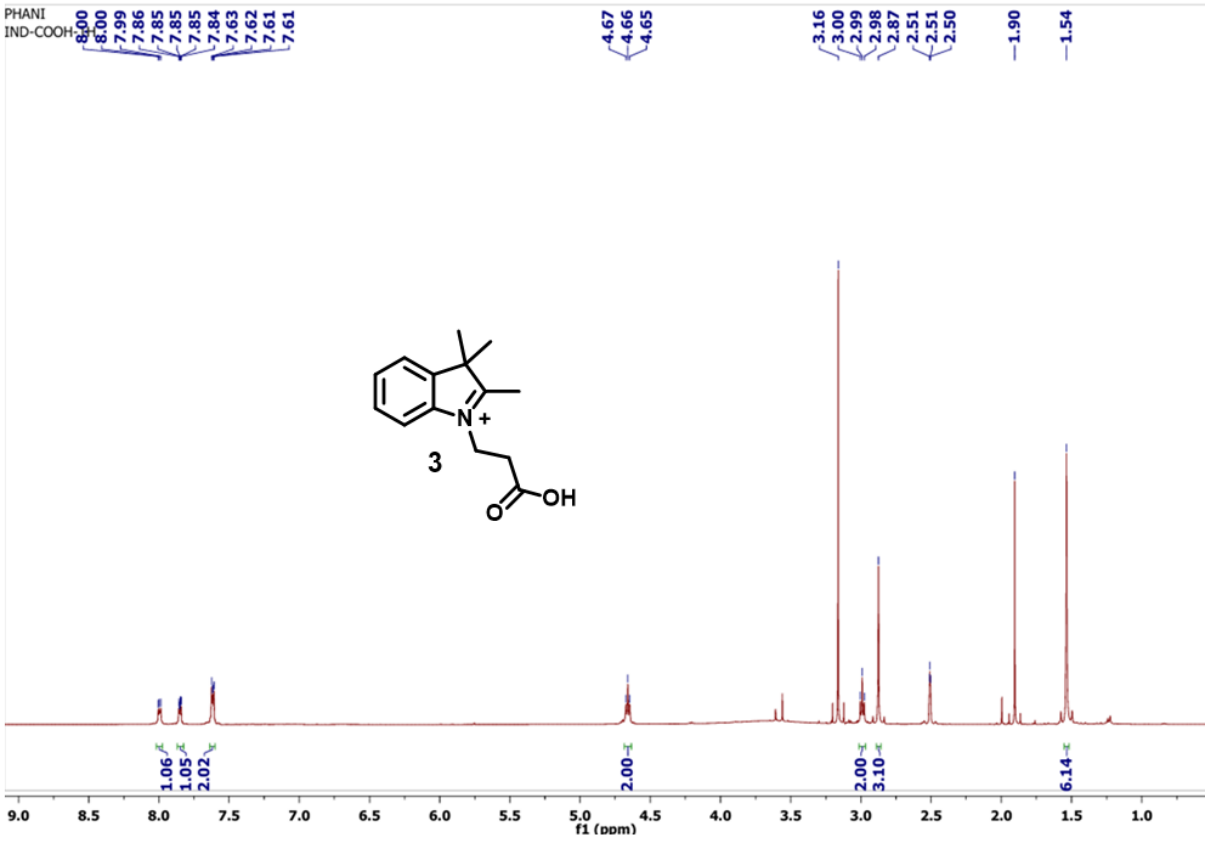


Fig. S1: ^{1}H NMR spectra of compound 3.

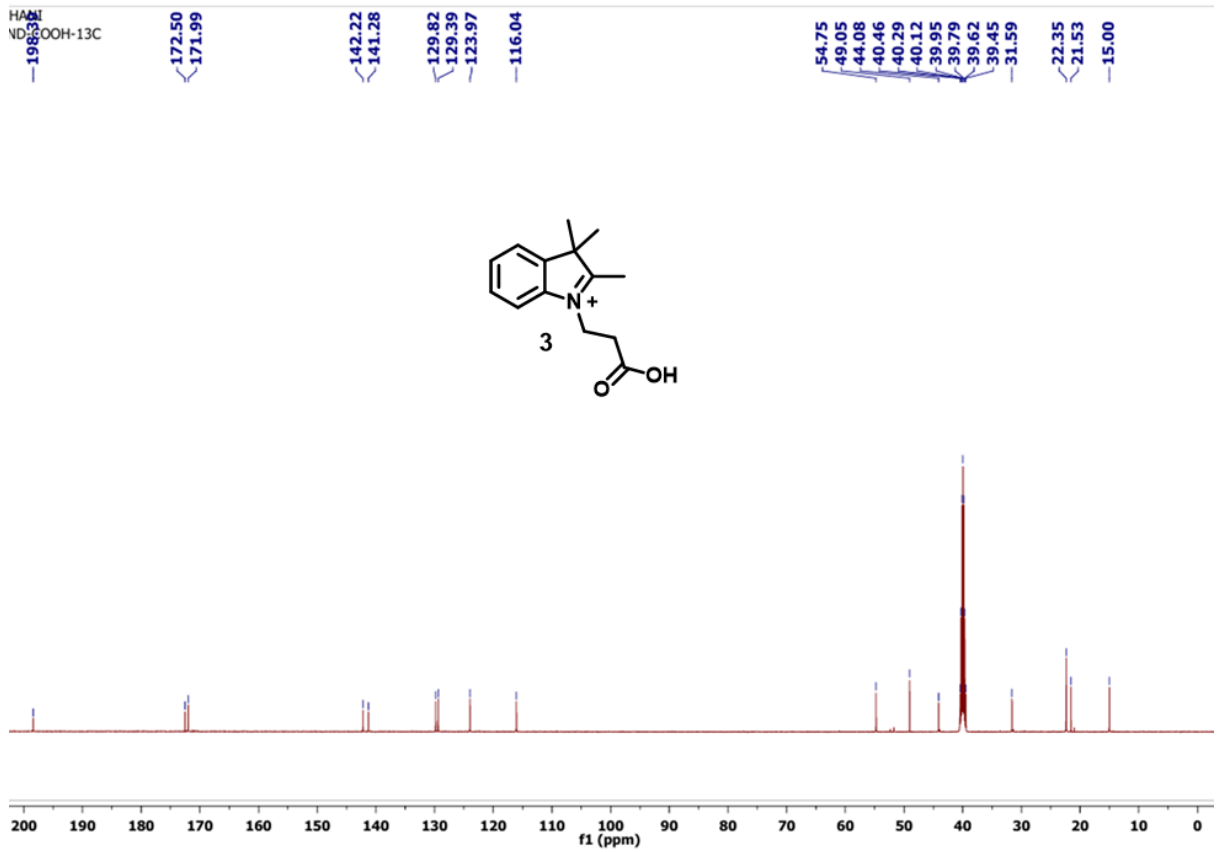


Fig. S2: ^{13}C NMR spectra of compound 3.

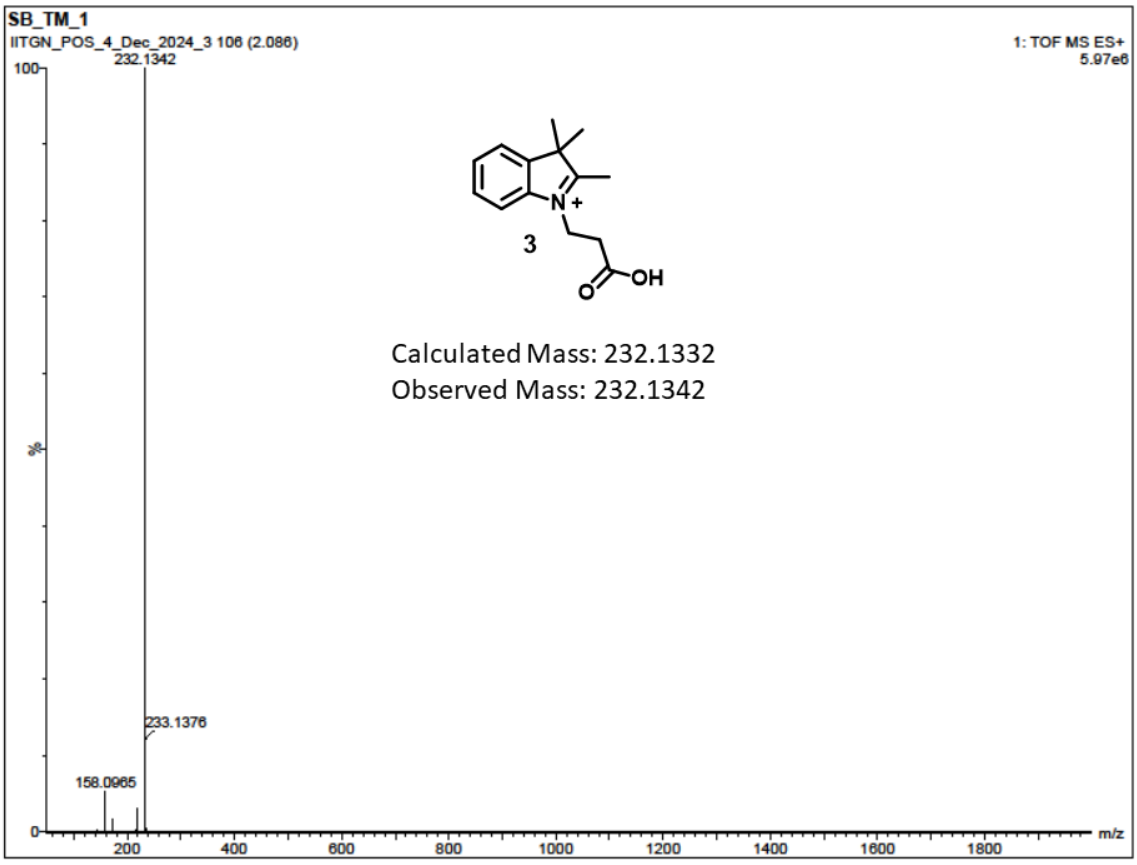


Fig. S3: HR-MS spectra of compound 3.

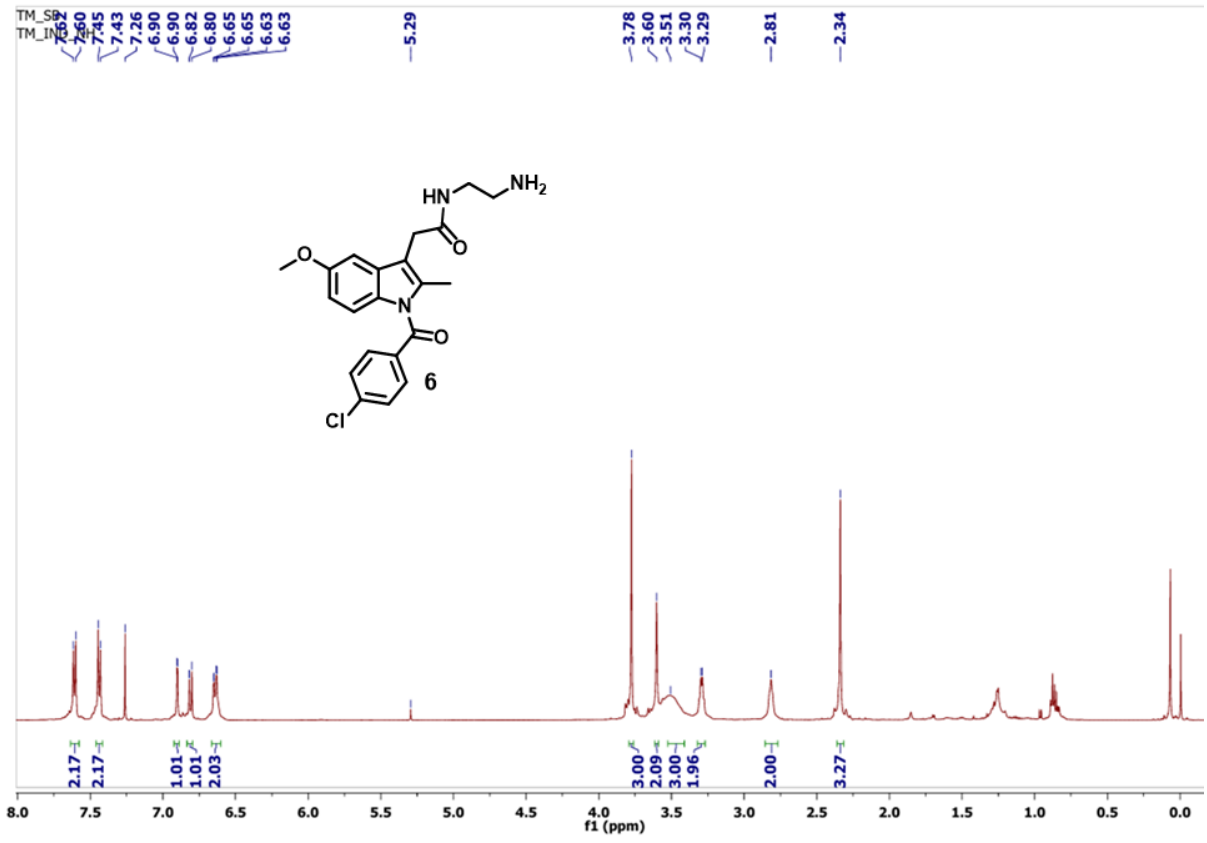


Fig. S4: ^{1}H NMR spectra of compound 6.

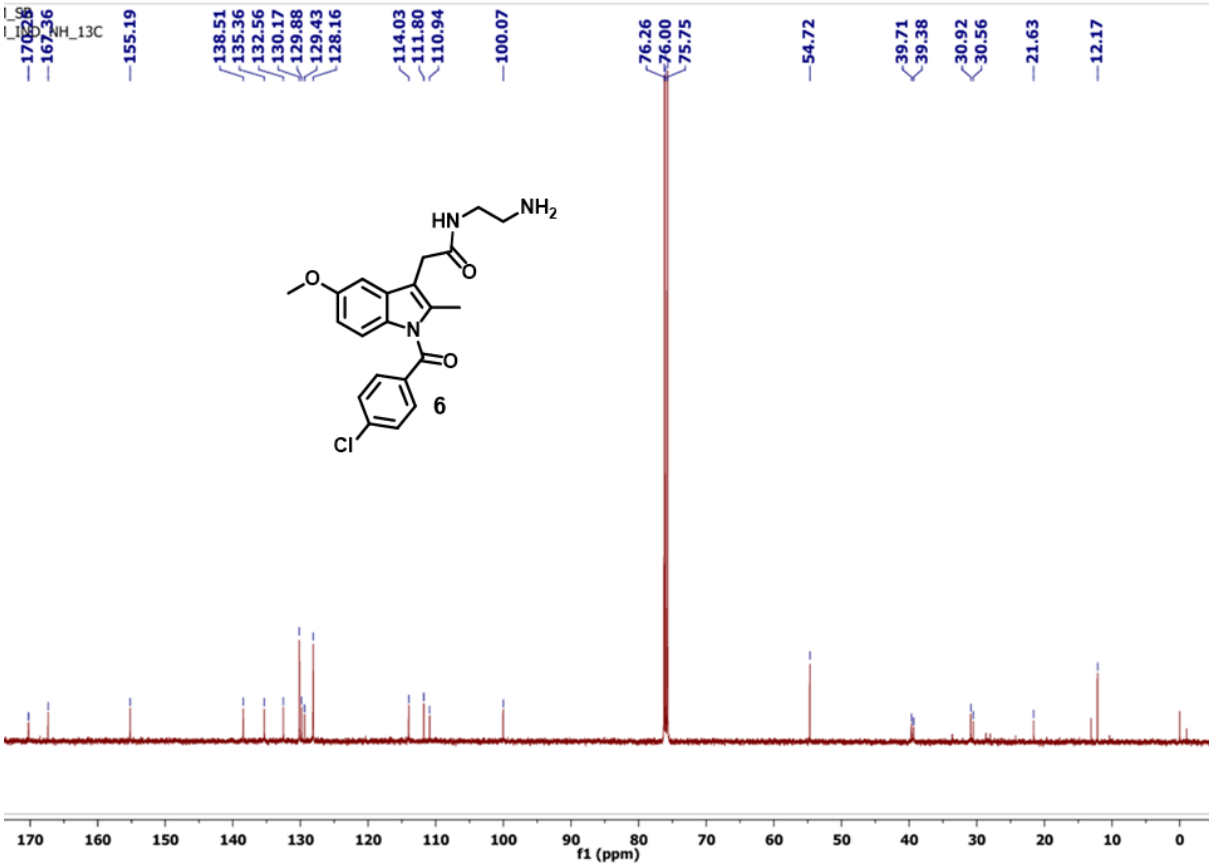


Fig. S5: ^{13}C NMR spectra of compound 6.

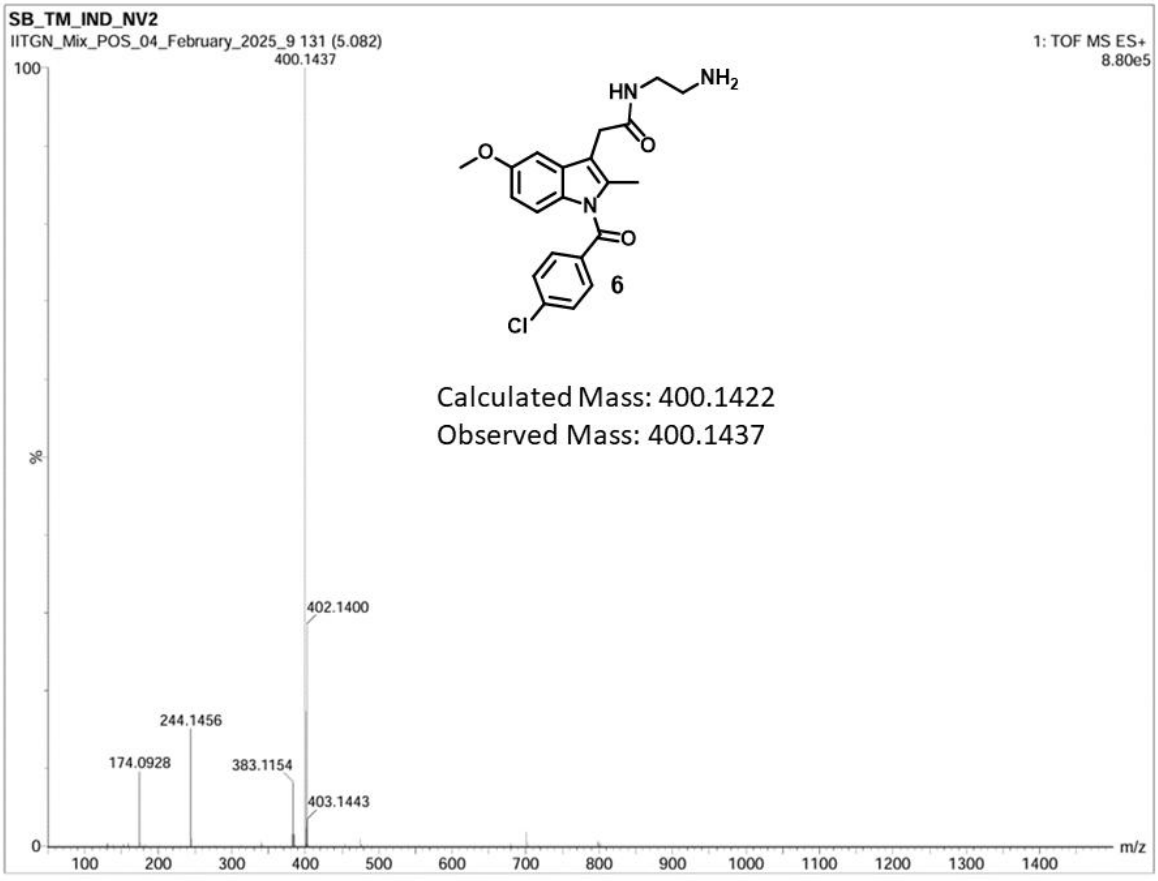


Fig. S6: HR-MS spectra of compound 6.

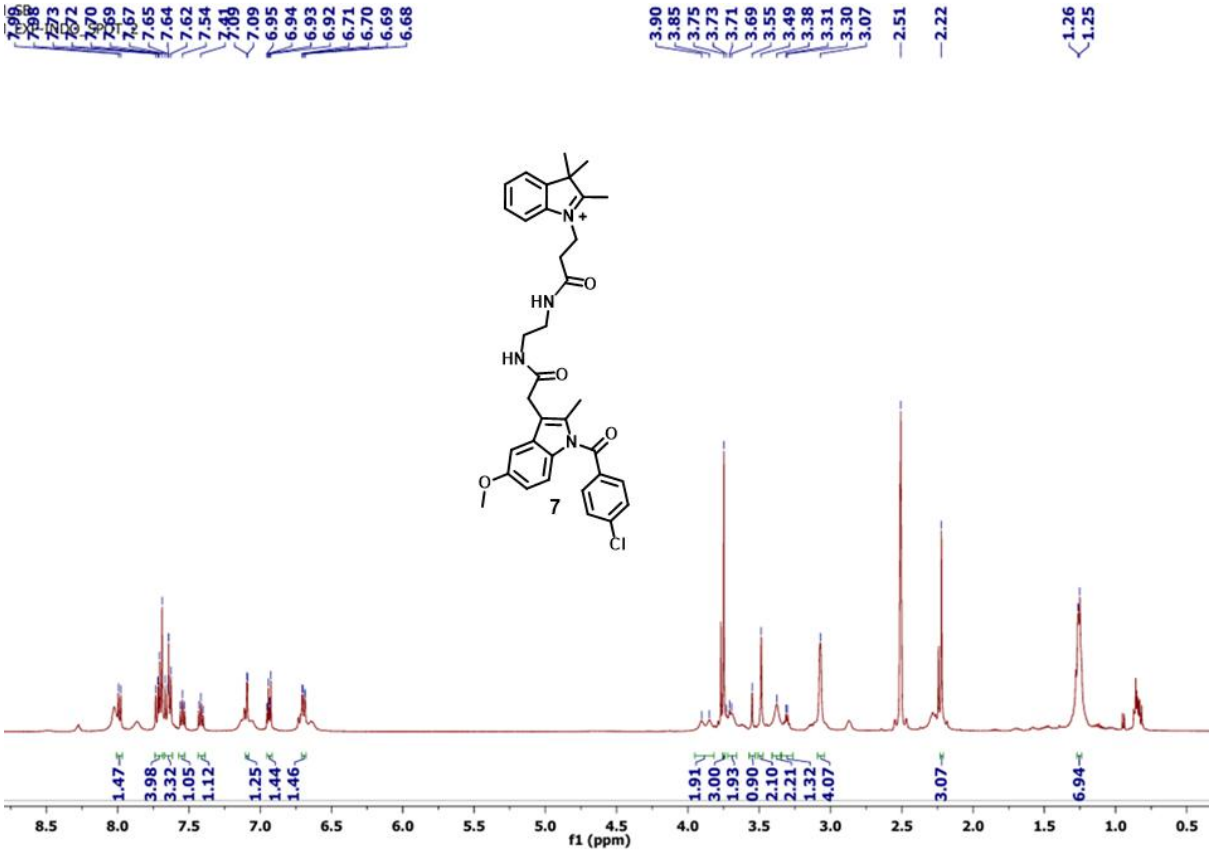


Fig. S7: ^{1}H NMR spectra of compound 7.

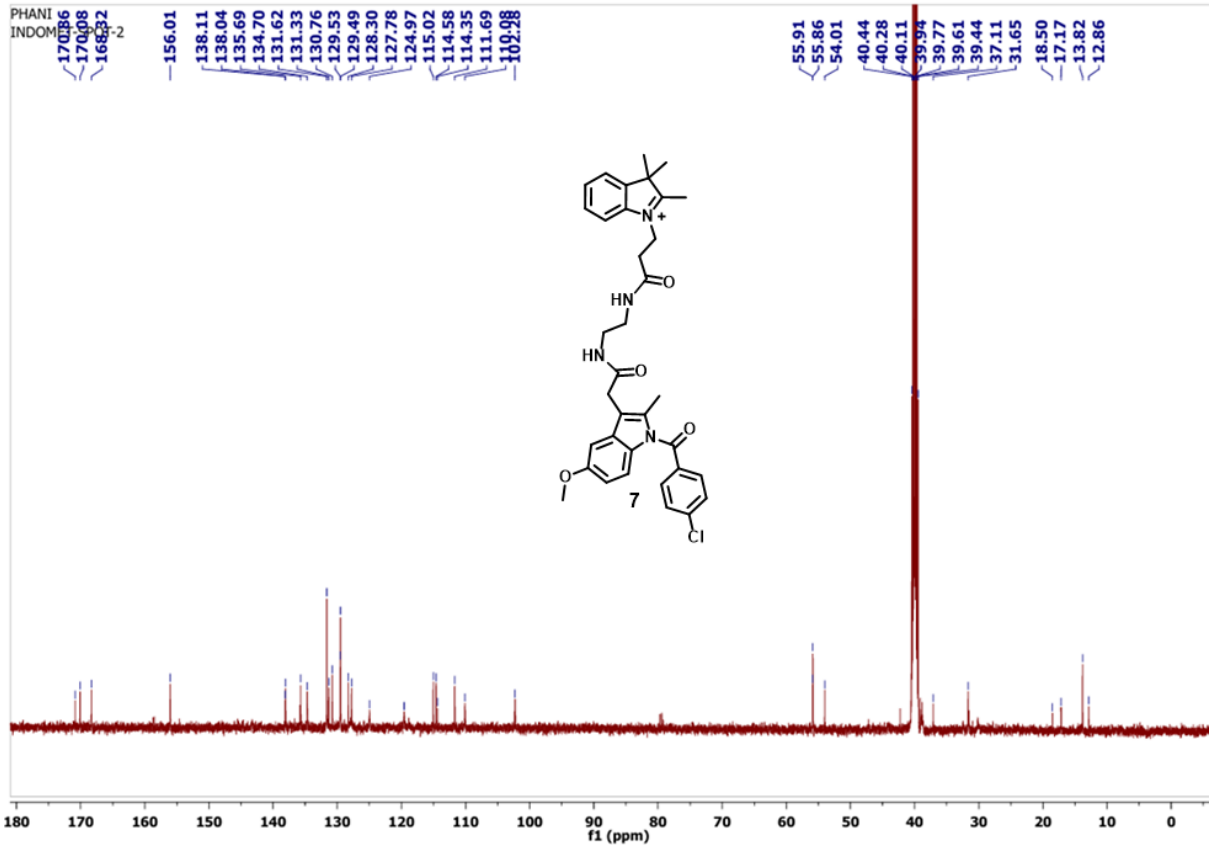


Fig. S8: ^{13}C NMR spectra of compound 7.

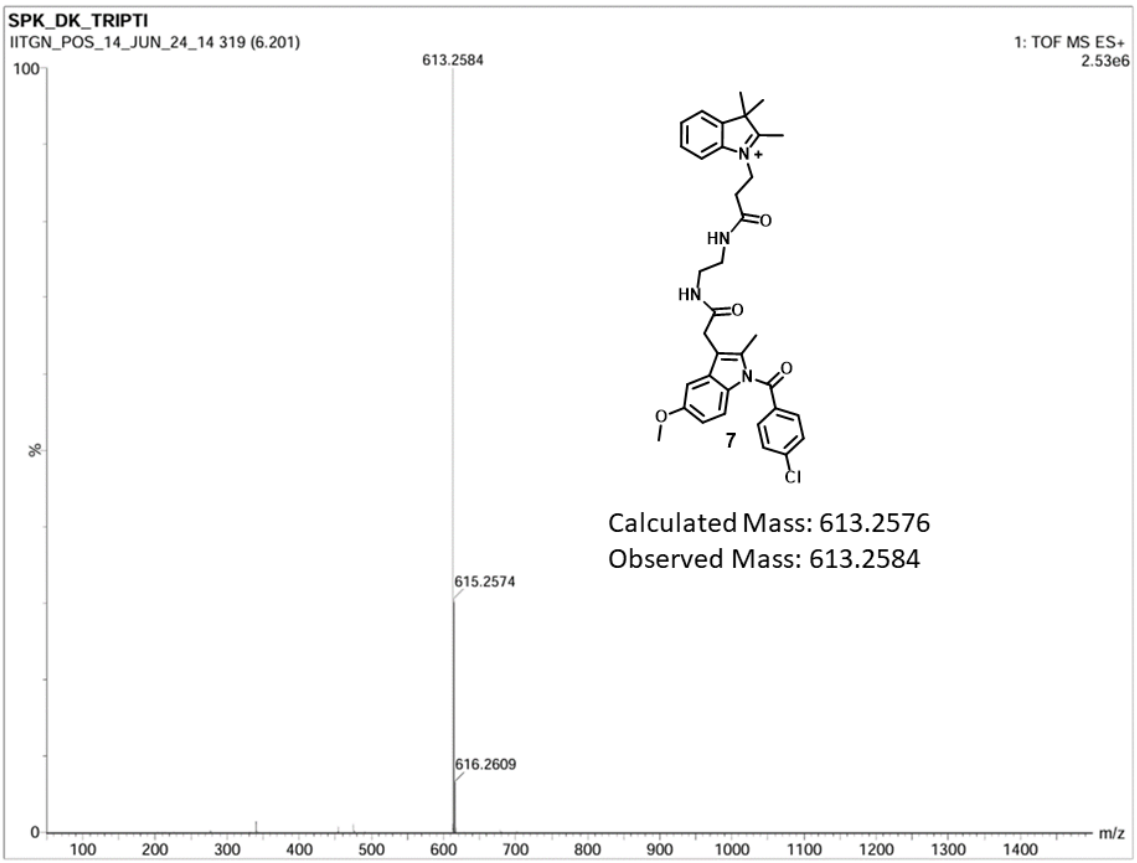


Fig. S9: HR-MS spectra of compound 7.

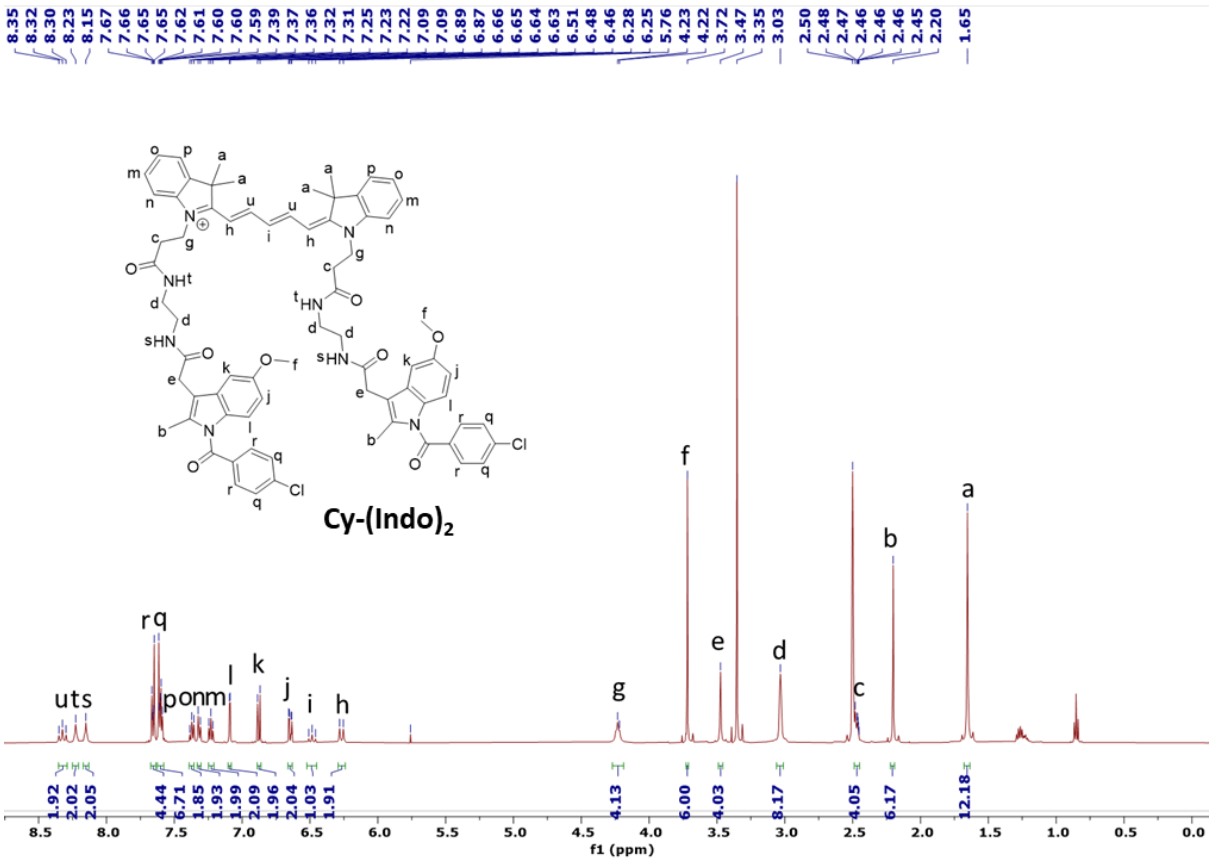


Fig. S10: ^{1}H NMR spectra of compound Cy-(Indo)$_2$.

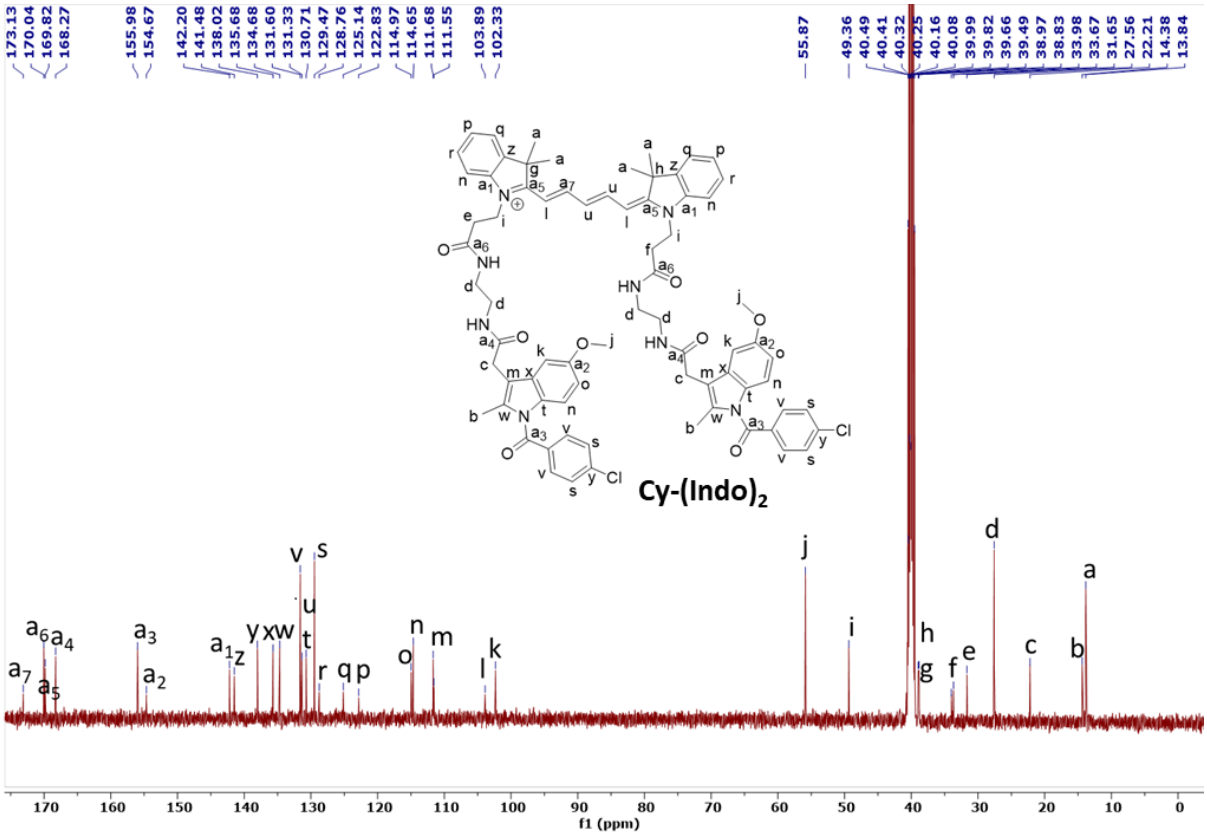


Fig. S11: ^{13}C NMR spectra of compound Cy-(Indo)$_2$.

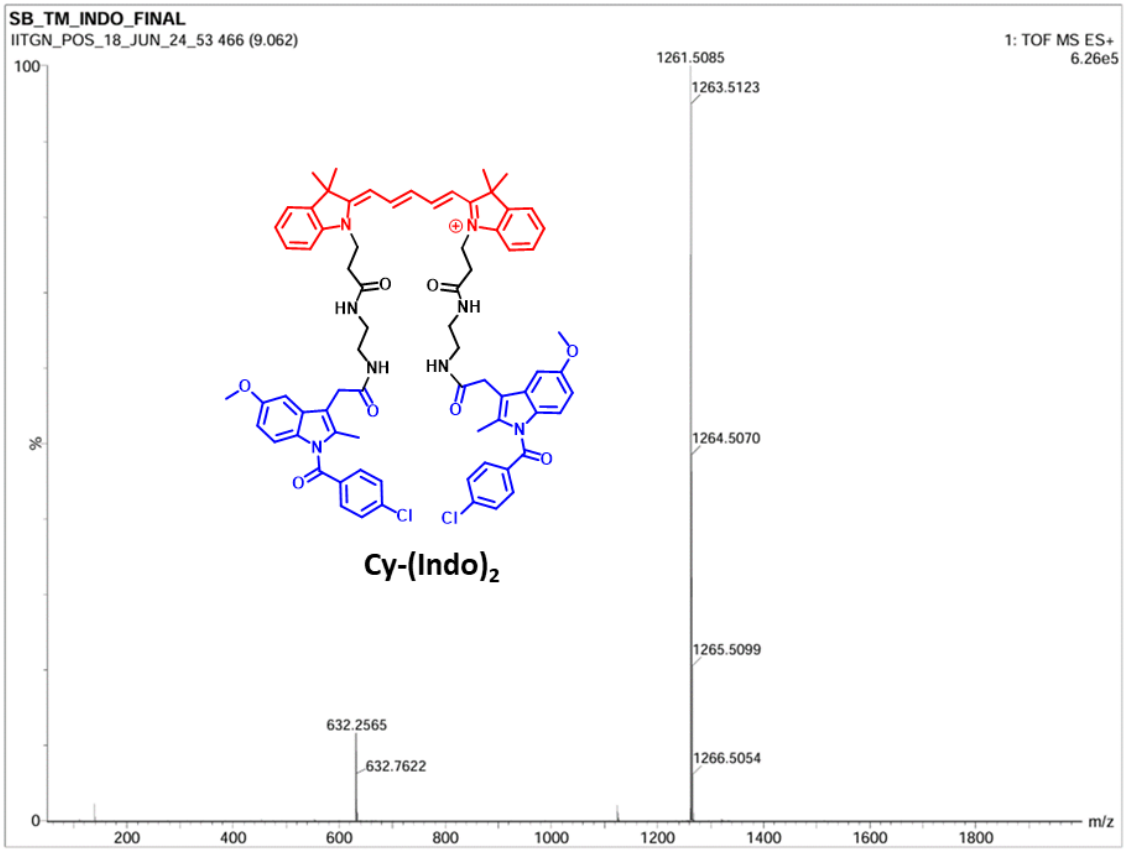


Fig. S12: HR-MS spectra of compound Cy-(Indo)$_2$.

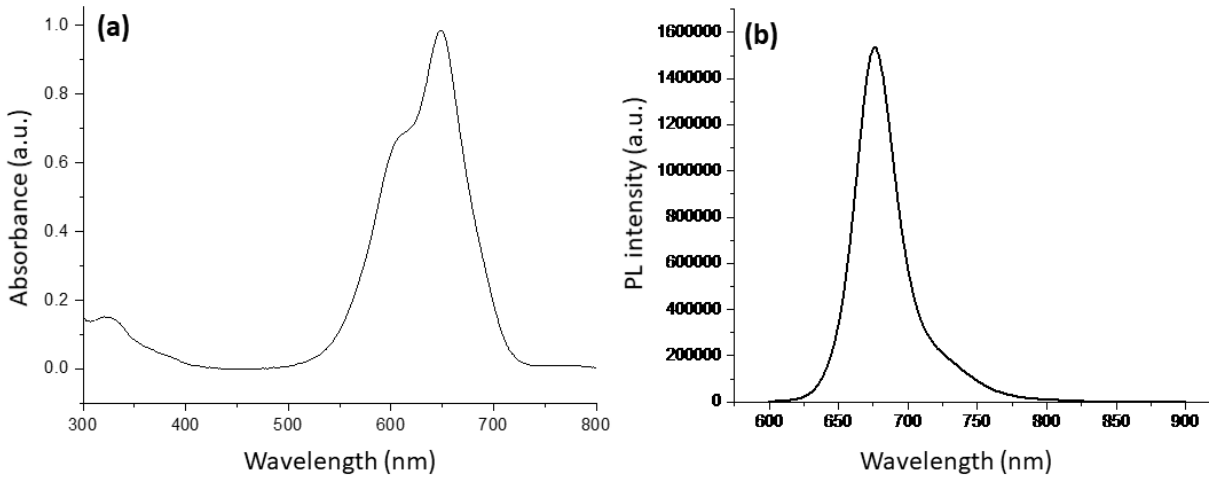


Fig. S13: (a) UV-Vis and (b) fluorescence emission spectra of Cy-$(Indo)_2$.

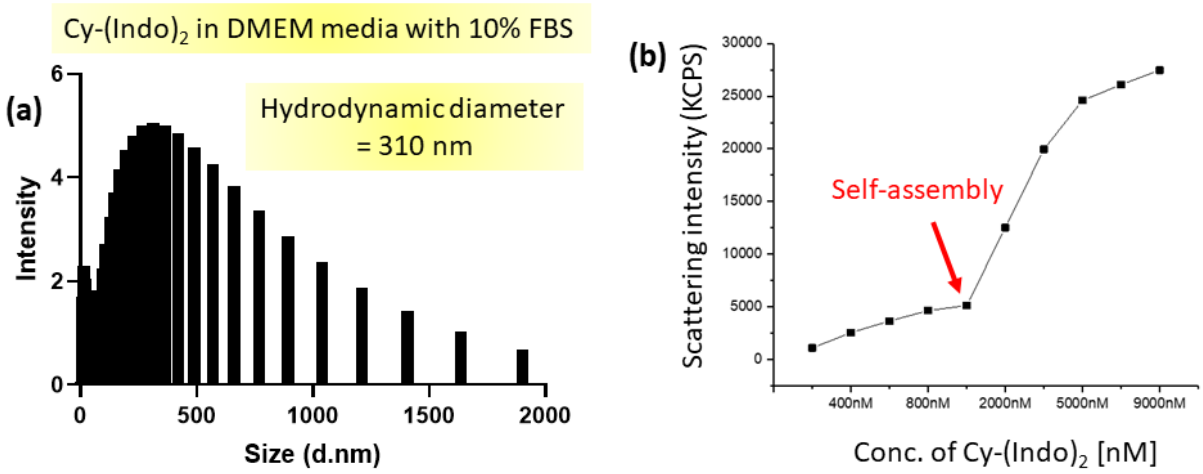


Fig. S14: (a) Size of the self-assembled Cy-$(Indo)_2$ nanoparticles in DMEM cell culture media with 10% FBS measured by DLS. (b) Concentration dependent change in scattering intensity of self-assembled Cy-$(Indo)_2$ in water.

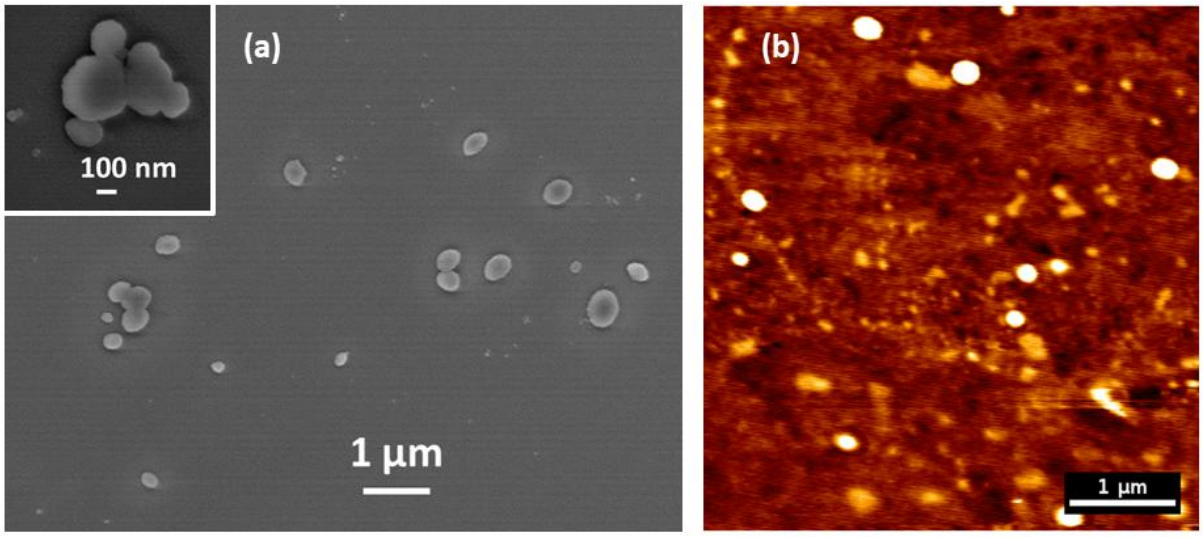


Fig. S15: (a) FESEM and (b) AFM images of self-assembled Cy-$(Indo)_2$ in water.

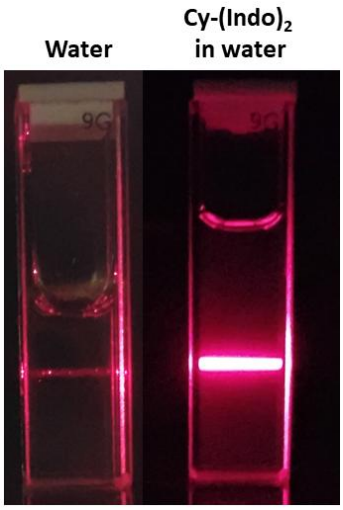


Fig. S16: Tyndall effect of Cy-(Indo)$_2$ in water.

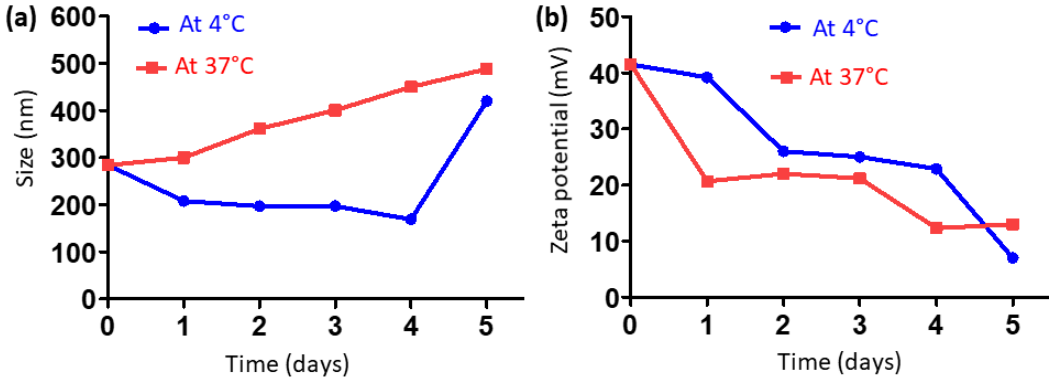


Fig. S17: Change in the (a) size and (b) zeta potential of Cy-(Indo)$_2$-NPs at 4°C and 37°C for 5 days measured by DLS.

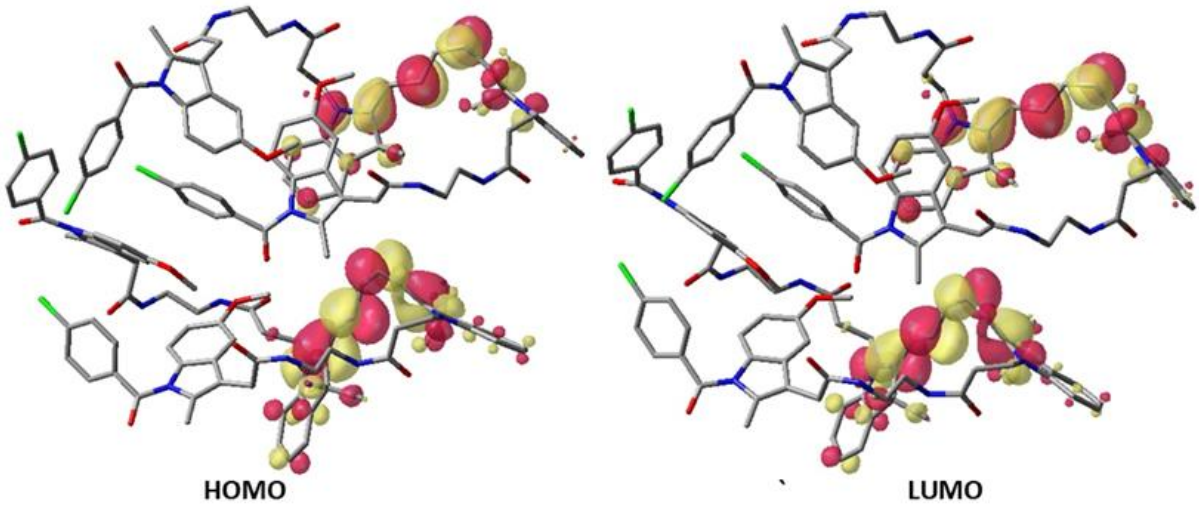


Fig. S18: Ground-state HOMO and LUMO orbitals of a representative dimer structure extracted from the MD trajectory. The orbitals are predominantly localized on the conjugated cores of the monomer units, indicating that key electronic interactions remain confined within the π-systems even in the aggregated state. Isovalue for orbital visualization: 0.02 a.u.

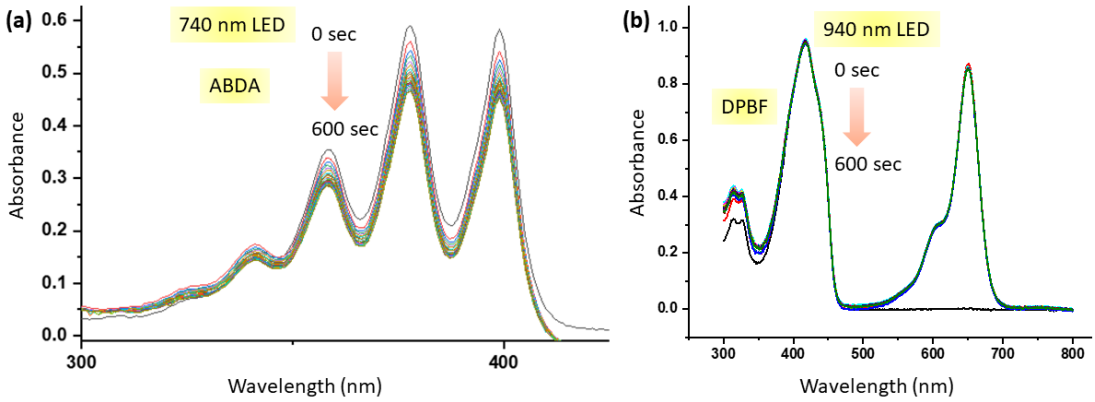


Fig. S19: (a) Time dependent UV-Vis spectra of Cy-$(Indo)_2$ along with ABDA after irradiation with 740 nm LED for 10 min (b) Time dependent UV-Vis spectra of Cy-$(Indo)_2$ along with DPBF after irradiation with 940 nm LED for 10 min.

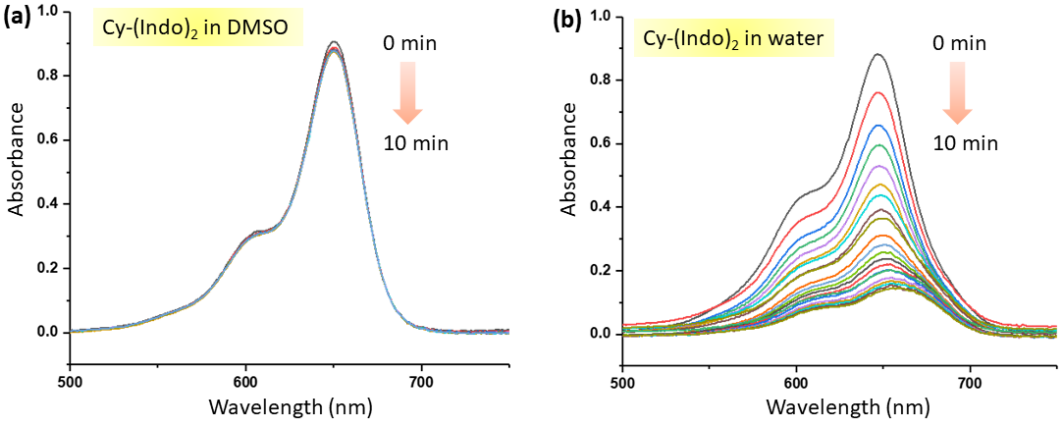


Fig. S20: (a, b) Photostability of Cy-$(Indo)_2$ in DMSO and water respectively under 740 nm LED for 10 min.

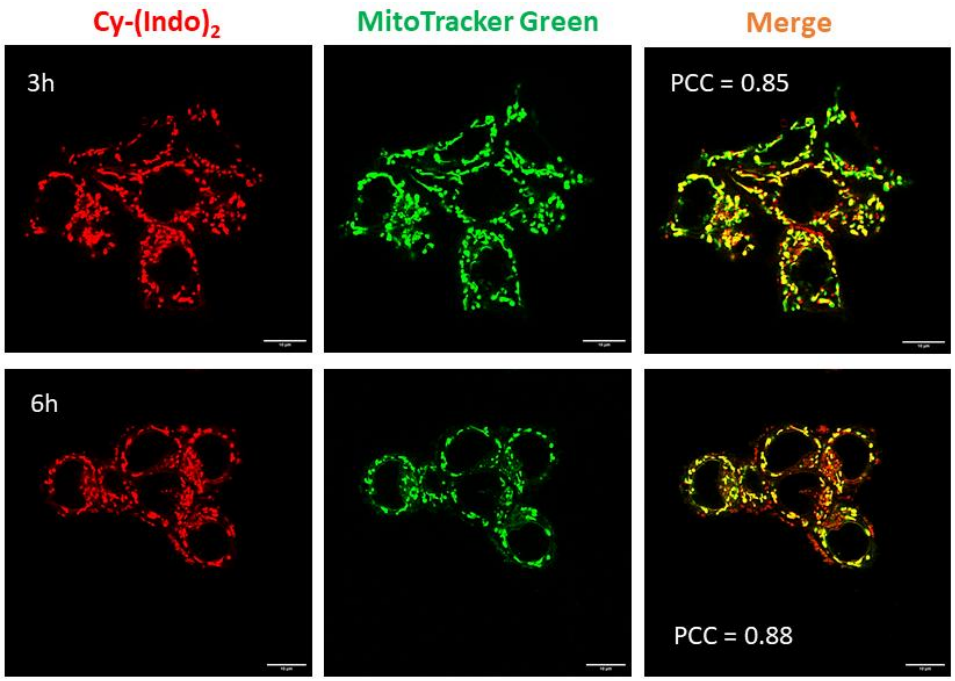


Fig. S21: Confocal laser scanning microscopy images of HCT-116 cells incubated with Cy-$(Indo)_2$ for 3h and 6h followed by staining the mitochondria with MitoTracker Green dye. Scale bar = 10 µm.

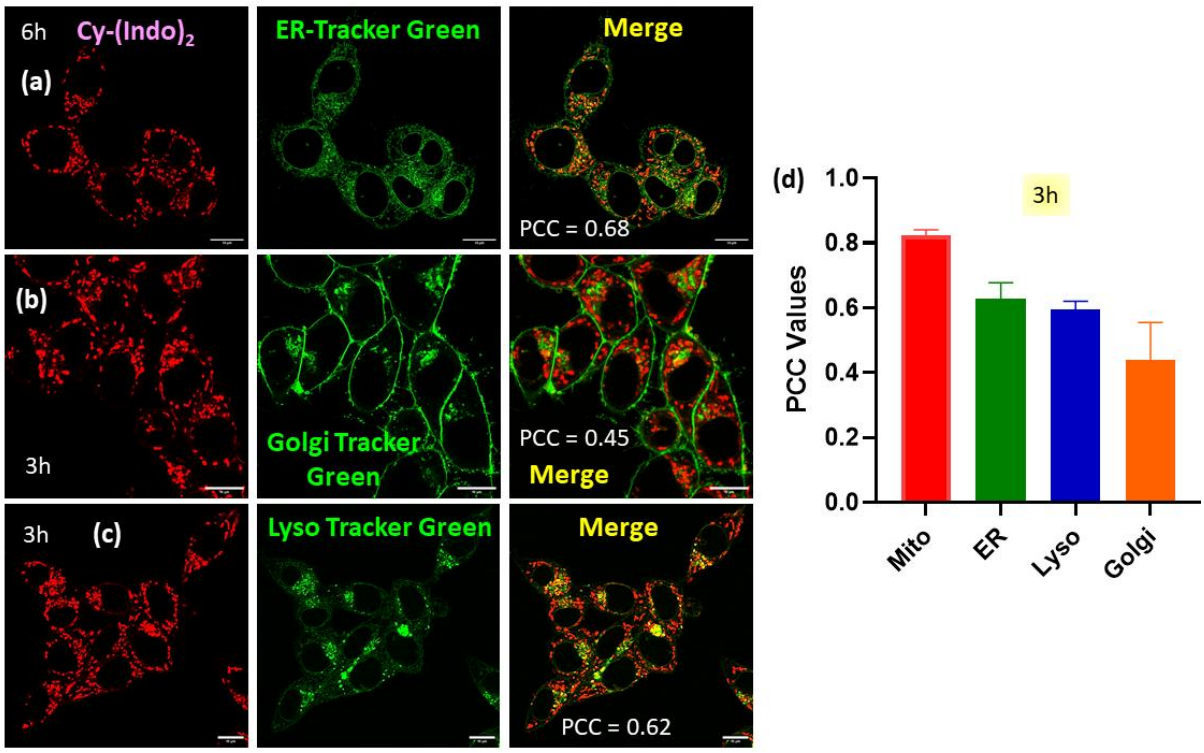


Fig. S22: (a) Confocal laser scanning microscopy images of HCT-116 cells after treatment with Cy-(Indo)$_2$ for 6h and co-stained the ER with ER-Tracker Green dye. (b, c) Confocal laser scanning microscopy images of HCT-116 cells treated with Cy-(Indo)$_2$ for 3h followed by staining the Golgi apparatus and lysosomes by Golgi Tracker Green and LysoTracker Green dyes. Scale bar = 10 µm. (d) Quantification of the organelle homing efficiency in different organelles by calculating PCC values at 3h.

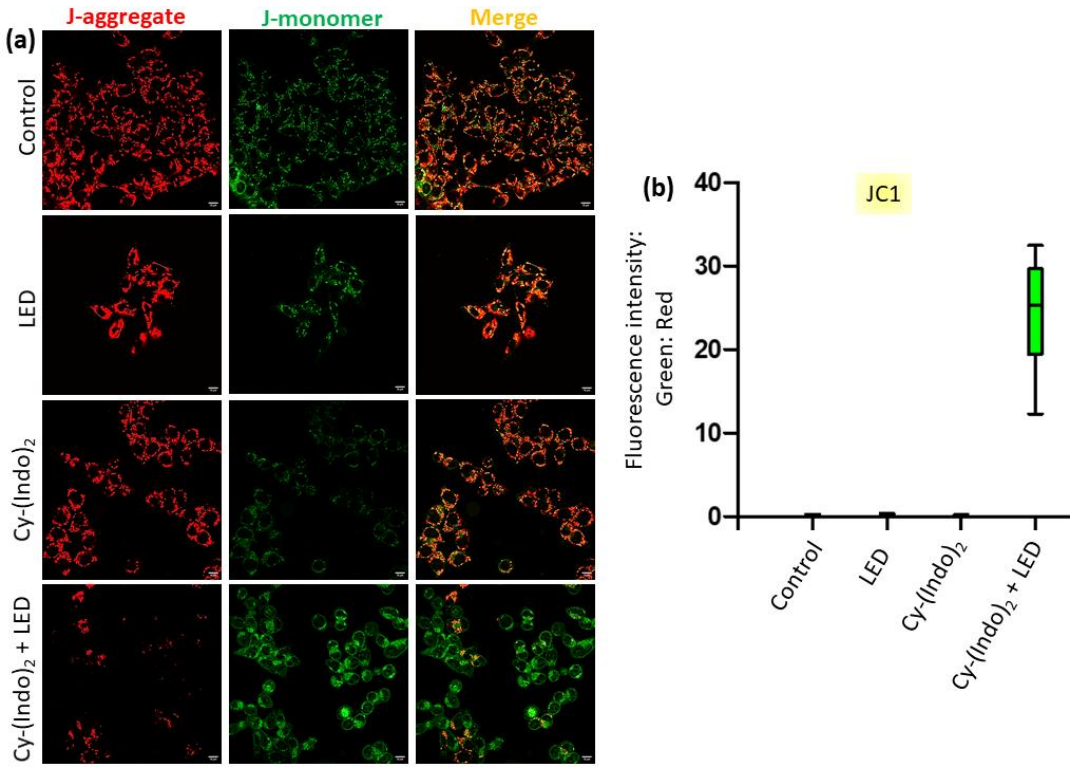


Fig. S23: (a) Confocal laser scanning microscopy images of the HCT-116 cells after incubating with Cy-(Indo)$_2$ for 24h followed by irradiating with or without 740 nm LED for 10 min. The cells were stained with JC1 dye. Scale bar = 10 µm. (b) Quantification of green: red fluorescence intensity from JC1 assay from confocal microscopy.

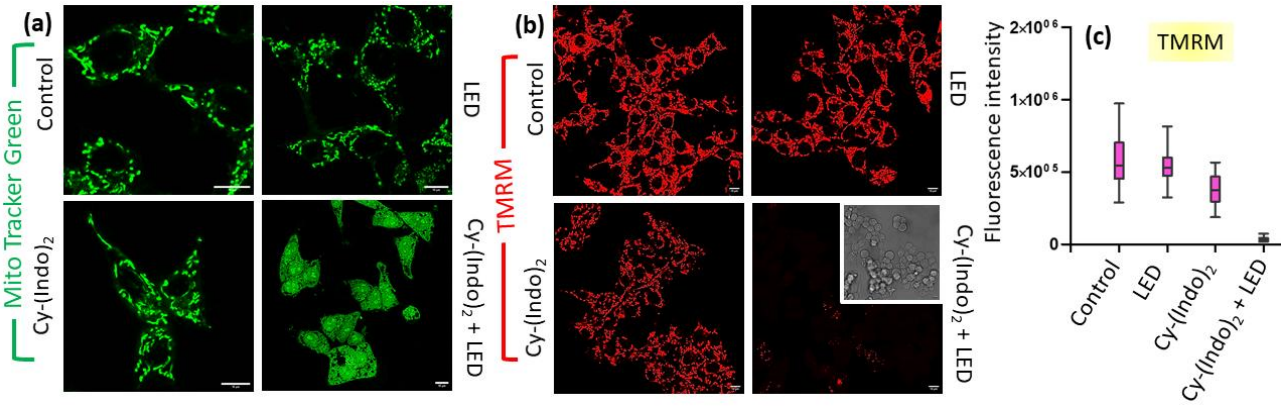


Fig. S24: Confocal laser scanning microscopy images of the HCT-116 cells after treatment with Cy-$(Indo)_2$ for 24h followed by irradiating with or without 740 nm LED for 10 min. (a) The mitochondria were stained with MitoTracker Green dye to visualize the mitochondrial morphology damage. (b) The cells were stained with TMRM (Red) dye. Scale bar = 10 µm. (c) Quantification of red fluorescence intensity of TMRM assay from the confocal microscopy.

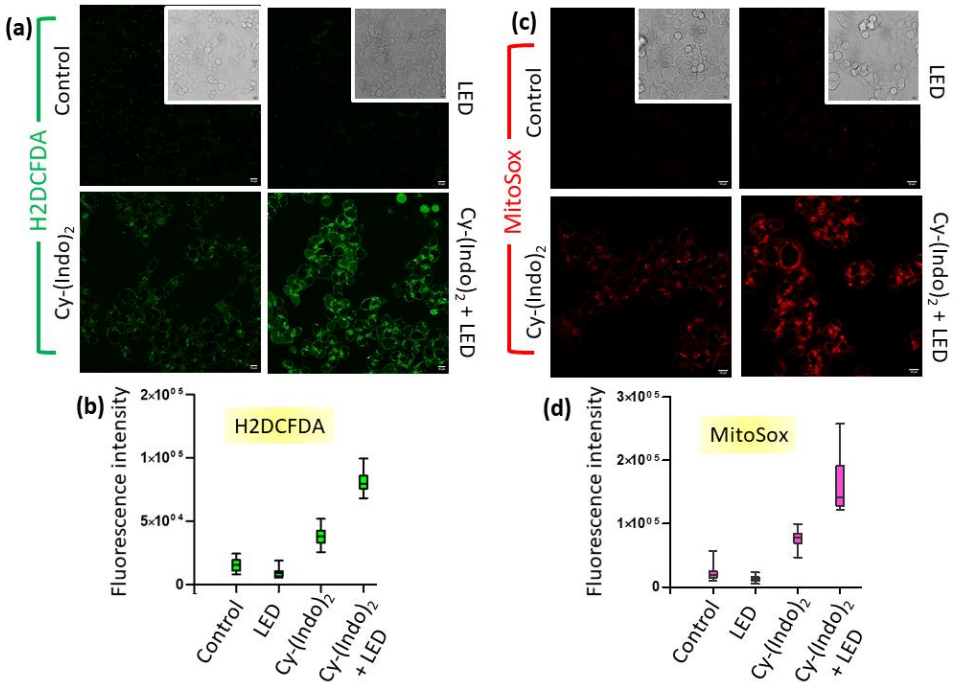


Fig. S25: Confocal laser scanning microscopy of HCT-116 cells treated with Cy-$(Indo)_2$ for 24h followed by irradiation with/without 740 nm LED for 10 min. (a) The cells were then incubated with the H2DCFDA dye to visualize ROS generation. (c) Cells were stained with MitoSox (Red) dye to visualize the superoxide generated into mitochondria. Scale bar = 10 µm. (b, d) Quantification of green and red fluorescence intensity of H2DCFDA and MitoSox from the confocal images respectively.

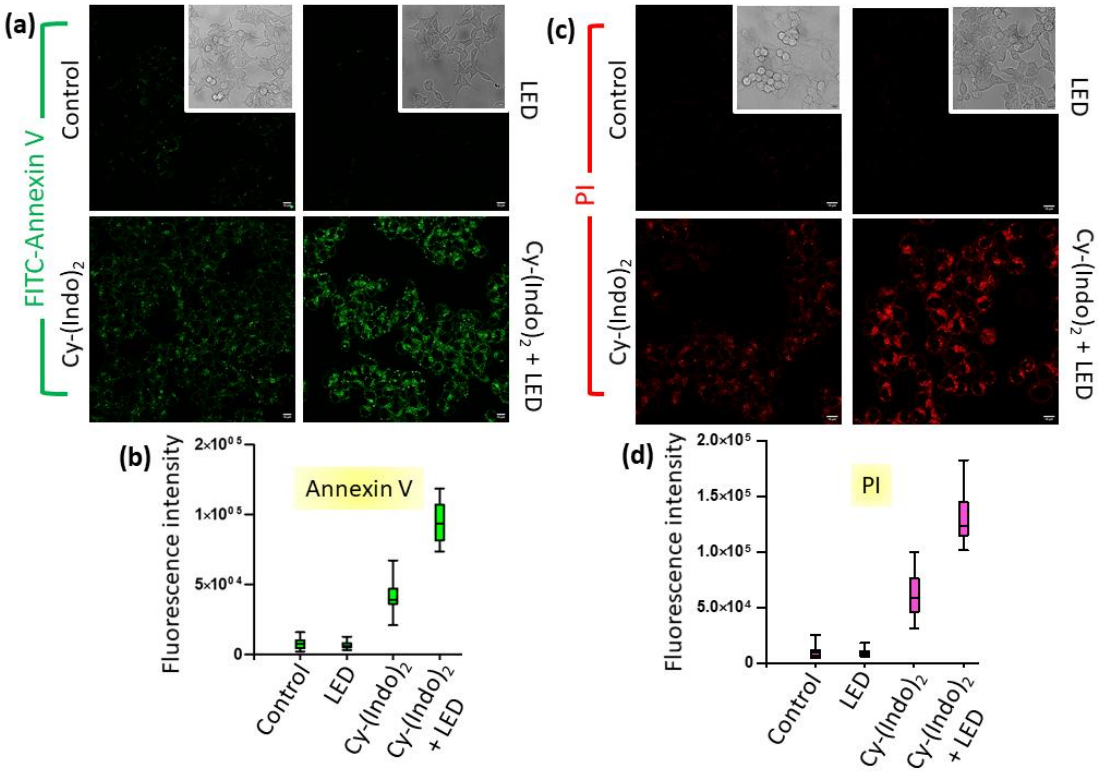


Fig. S26: (a, c) Confocal laser scanning microscopy images of the HCT-116 cells after treatment with Cy-$(Indo)_2$ for 24h followed by irradiating with or without 740 nm LED for 10 min. The cells were then stained with FITC-Annexin V (green) and PI (red) respectively. Scale bar = 10 µm. (b, d) Quantification of green and red fluorescence intensity of FITC-Annexin V and PI from the confocal images respectively.

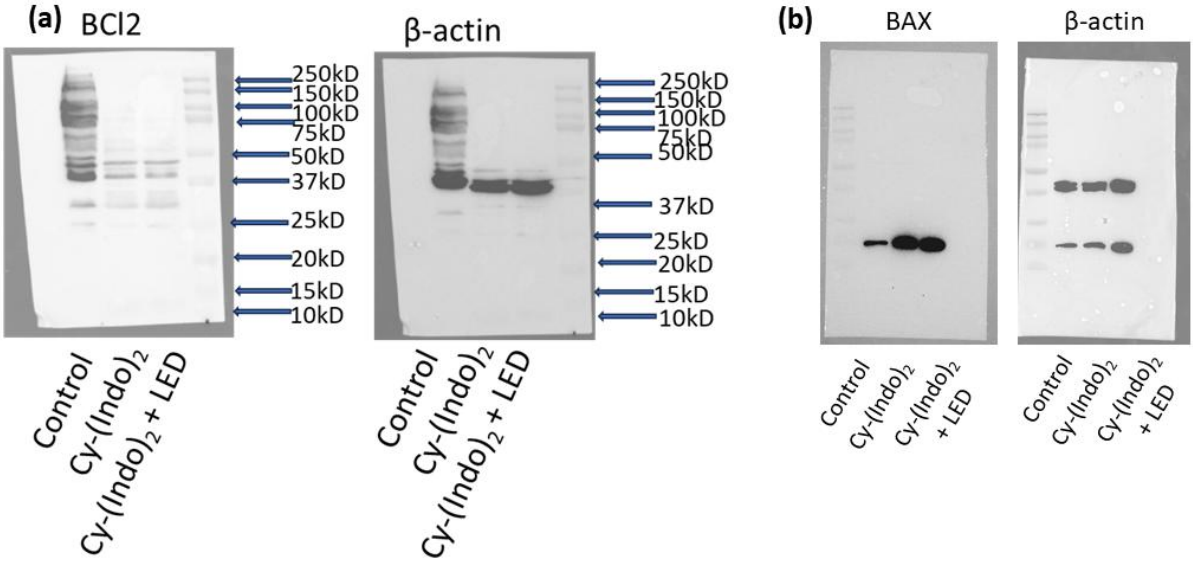


Fig. S27: Western blot images of (a) Bcl-2 and (b) BAX in HCT-116 cells after treatment with Cy-$(Indo)_2$ for 24h followed by irradiating with or without 740 nm LED for 10 min.

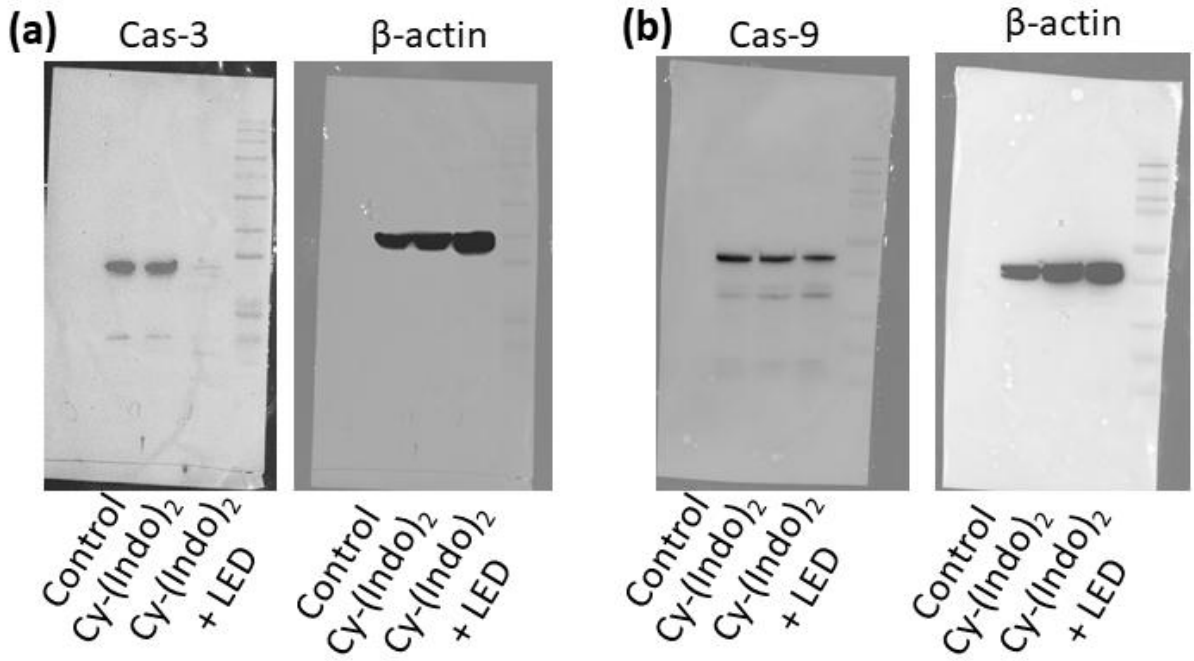


Fig. S28: Western blot images of (a) Cas-3 and (b) Cas-9 in HCT-116 cells after treatment with Cy-$(Indo)_2$ for 24h followed by irradiating with or without 740 nm LED for 10 min.

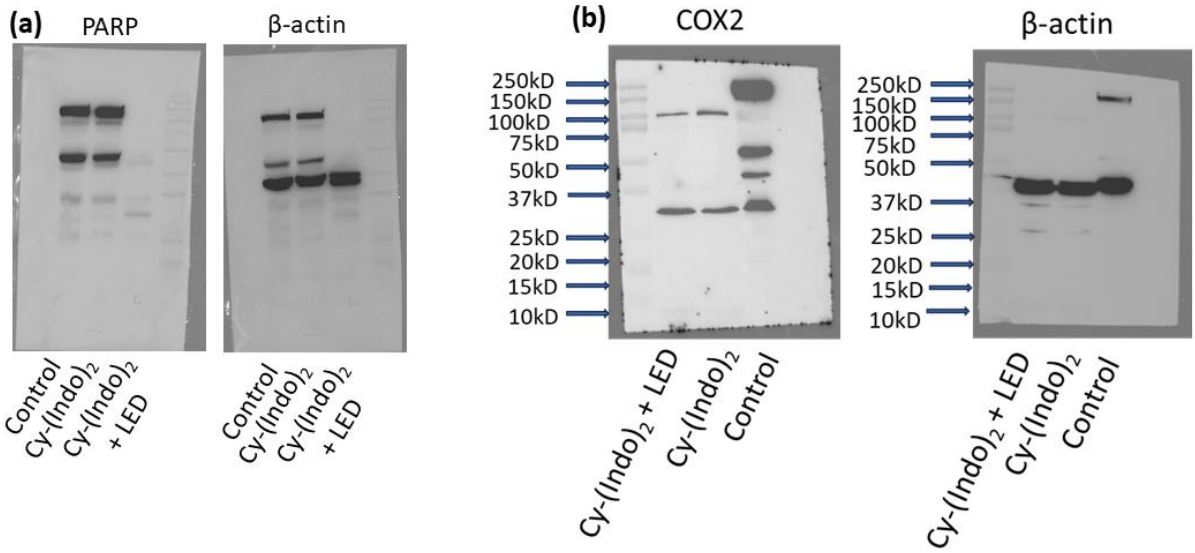


Fig. S29: Western blot images of (a) PARP and (b) Cox-2 in HCT-116 cells after treatment with Cy-$(Indo)_2$ for 24h followed by irradiating with or without 740 nm LED for 10 min.

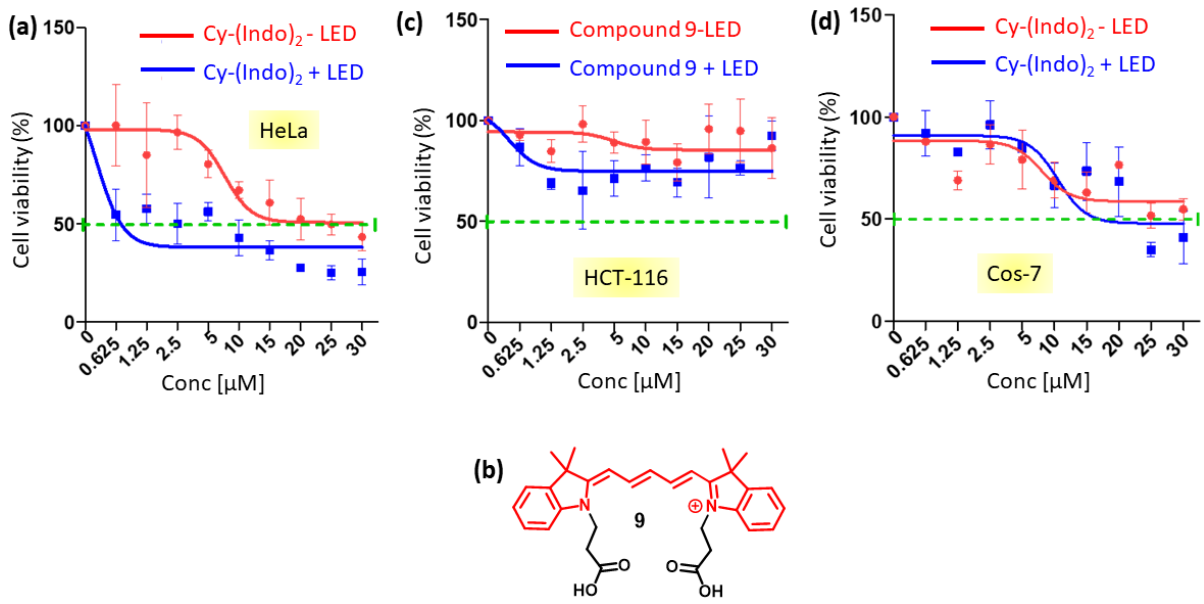


Fig. S30: (a, d) Viability of the HeLa and Cos-7 cells after dose dependent treatment with Cy-$(Indo)_2$ for 24h followed by irradiating them with or without 740 nm LED respectively. (b) Chemical structure of compound 9. (c) Viability of the HCT-116 cells after dose dependent treatment with compound 9 for 24h followed by irradiating them with or without 740 nm LED respectively.

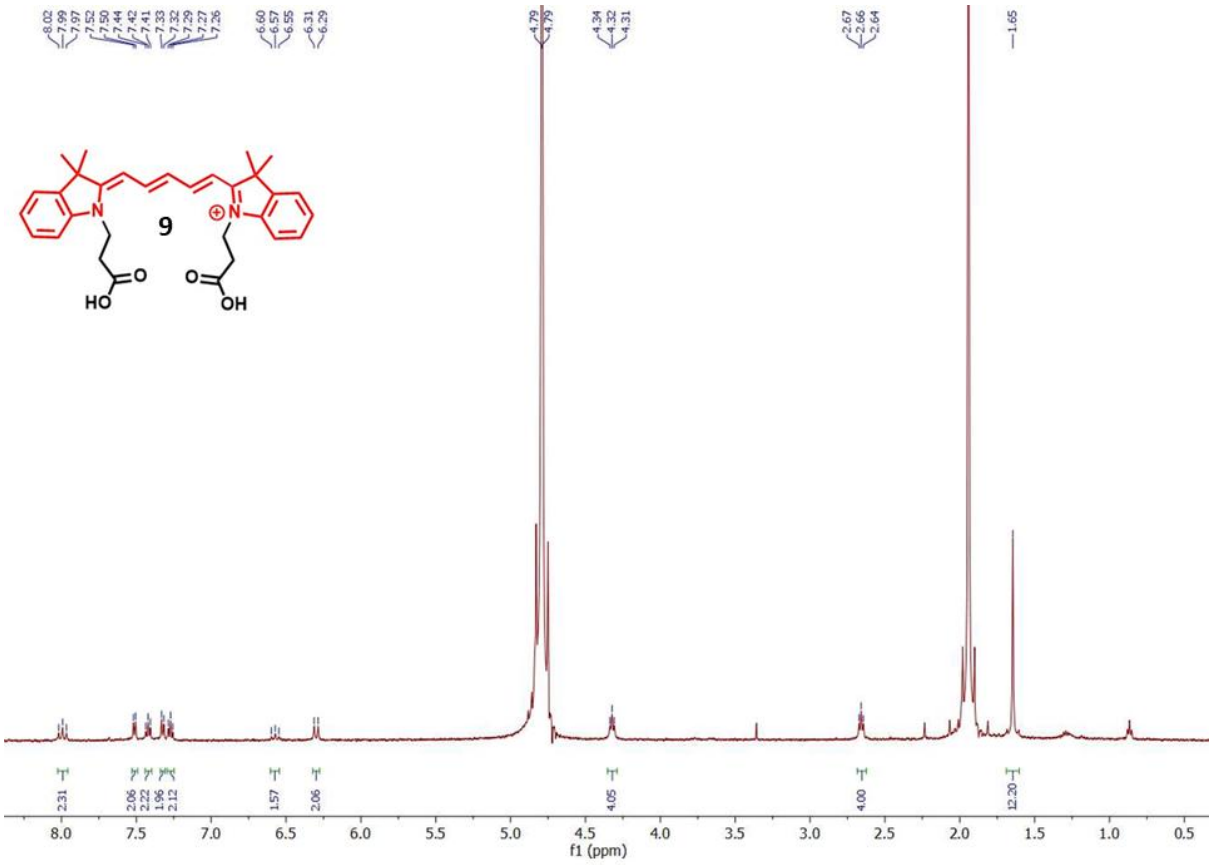


Fig. S31: ^{1}H NMR spectra of compound 9.

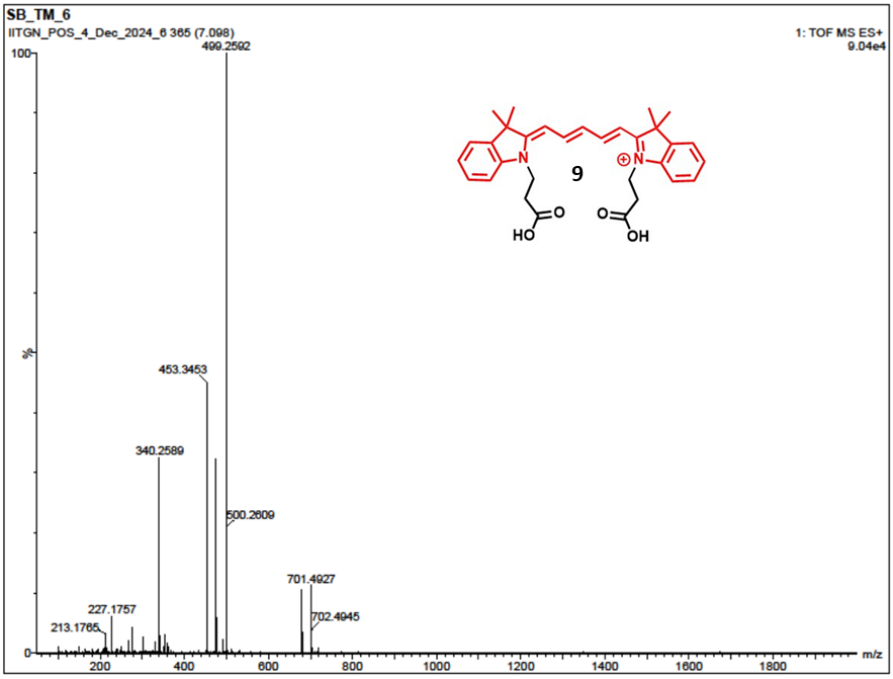


Fig. S32: HR-MS spectra of compound 9.

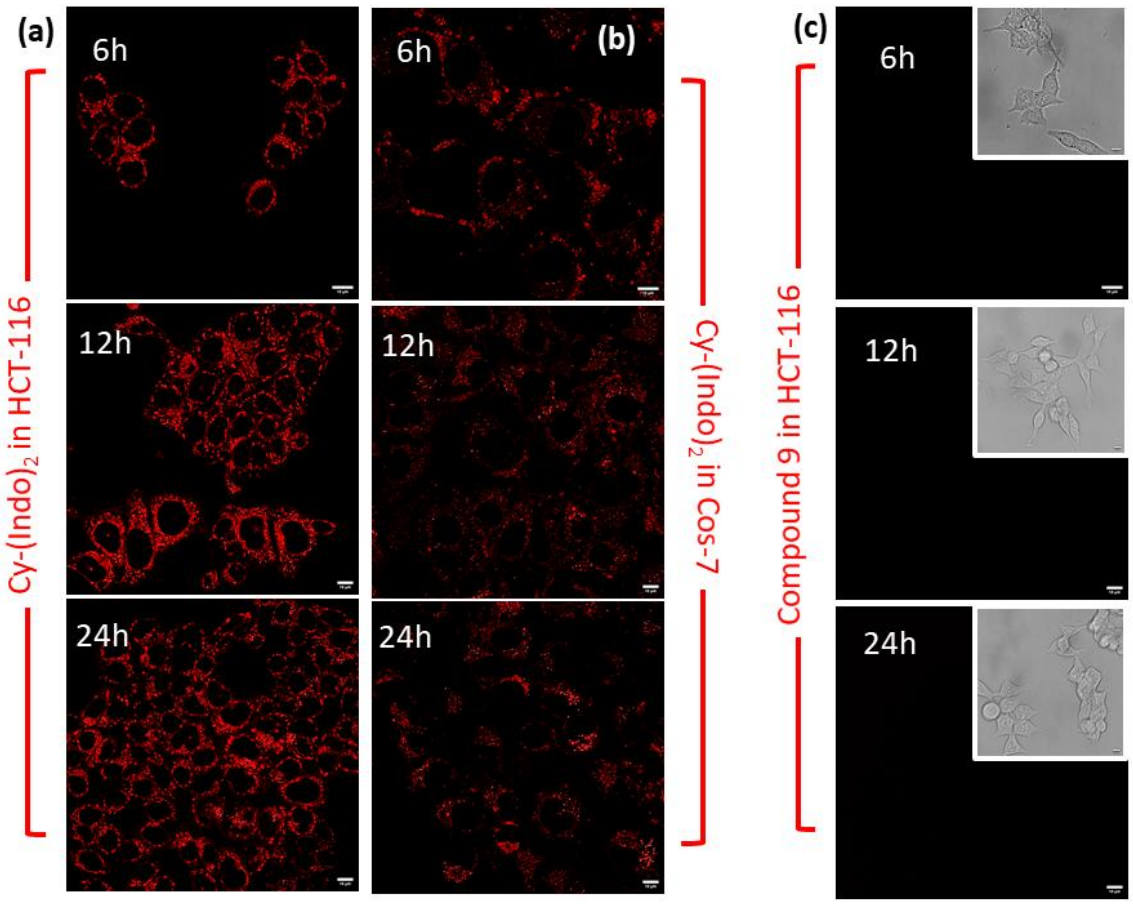


Fig. S33: (a,b) Confocal laser scanning microscopy images of HCT-116 and Cos-7 cells after incubating with Cy-$(Indo)_2$ at 3 µM in a time dependent manner for 6h, 12h and 24h. (c) Confocal laser scanning microscopy images of HCT-116 cells after incubating with compound 9 at 3 µM in a time dependent manner for 6h, 12h and 24h.

References: ORCA, Gaussian, GROMACS, VMD.

1) Neese, F. The ORCA program system. WIREs Comput. Mol. Sci. 2012, 2, 73–78.

2) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A. et al. Gaussian 09 Revision E.01. Gaussian Inc. Wallingford CT 2009.

3) B. Hess, C. Kutzner, D. Van Der Spoel, and E. Lindahl, "GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation," *J. Chem. Theory Comput.* 4, 435–447 (2008).

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