Supplementary Information

Direct readout protonophore induced selective uncoupling and dysfunction of individual mitochondria within the cancer cells

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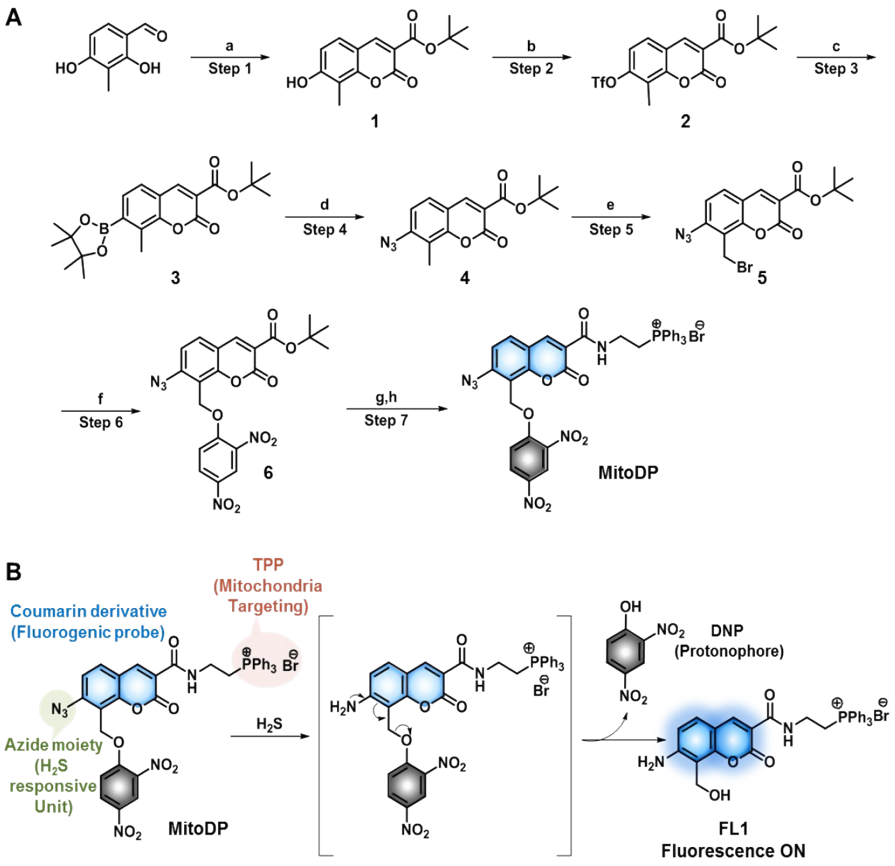
Materials, methods,and instrumentations

2-Methylresorcinol (Avra), phosphorous oxychloride (Avra), *t*-butyl ethyl malonate (Alfa aesar), piperidine (Avra), N-phenyl-bis(trifluoromethanesulfonimide)(Avra), sodium carbonate (Avra),bis(pinacolato)diboron(Avra),1,1'-Bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex(Avra),potassium acetate (Avra),sodium azide (Avra), copper acetate (Rankem), 2,4-dinitrophenol (Alfa aesar), potassium carbonate (Rankem), acetonitrile (Merck), *N, N*-dimethylformamide (Merck), trifluoroacetic acid (Avra),*N, N*-diisopropylethylamine (DIPEA) (Alfa-Aesar), diethyl ether (Loba chem., India), petroleum ether 60-80 (Avra), ethyl acetate (Avra) and DCM (Avra) were purchased commercially and used without further purification.Flash column chromatography was performed using silica gel (100-200 mesh) and analytical thin layer chromatography was performed using silica gel 60 (pre-coated sheets with 0.25 mm thickness). Mass spectra were recorded on anion SpecHiResESI mass spectrometer. NMR spectra were collected on a 400MHz spectrometer (Bruker, Germany).UPLC analyses were performed on a Waters Acquity UPLC (H class)/Q Daquadrupole MS analyzer or Acquity UPLC(H class)/Xevo TQD quadrupole MS/MS analyzer equipped with an Acquity UPLC BEH C18 column (Waters). Eluent A (0.1 % formic acid in Water) and eluent B (0.1 % in Acetonitrile) were used for UPLC analyses. HPLC analysis was performed on an InertsilODS-3V (150 × 4.6 mm × 5 μm) column (GL Sciences Inc.).Eluent A (0.1% TFA in H_2O), eluent B (Acetonitrile) with sample concentration 0.3 mg/mL. Gradient: T/%B:0/5, 20/90, 27/5, 30/5, Diluent = Water: Acetonitrile1:1), Flow rate: 1.0 mL/min.



Scheme S1. Synthetic scheme for **MitoDP** (A) and mode of activation in presence of H_2S followed by protonophore (DNP) release (B).Reagents and Reaction conditions: Scheme 1A a) 2, 4-dihydroxy-3-methylbenzaldehyde, *tert*-butyl ethyl malonate, piperidine, ethanol, RT, 6 h; b) N-Phenyl-bis(trifluoro-methanesulfonimide), Na_2CO_3, DMF, RT, 12 h; c) Bis(pinacolato)diboron, Pd(dppf)Cl_2-CH_2Cl_2, CH_3COOK, toluene, reflux, 2 h; d) NaN_3, $Cu(OAc)_2$, MeOH, 55 °C, 2 h; e) NBS, AIBN, CCl_4, reflux, 6 h; f) 2,4-dinitrophenol, K_2CO_3, CH_3CN, reflux, 6 h; g) TFA, DCM, 0 °C, 3 h; h) $NH_2CH_2CH_2PPh_3Br$.

Synthesis of 1: Piperidine (6.77 mL, 98.60 mmol) was added to a stirred solution of 2,4-dihydroxy-3-methylbenzaldehyde (3.0 g, 19.72 mmol), *tert*-butylethylmalonate (6.32 g, 39.43 mmol) in anhydrous ethanol (30 mL) and stirred for 6 h at room temperature (RT). After completion, the reaction mixture was concentrated under reduced pressure. The resulting mixture was dissolved in ethyl acetate (EA). The organic layer was washed with water and brine, dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated under vacuum. The crude compound was purified through the silica gel column chromatography using ethyl acetate: hexanes (50 %) as eluentto afford compound **1** (4.1 g, 75.36 %) as pale brown solid.^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.43 (s, 1H), 7.48 (d, *J* = 8.40 Hz, 1H), 6.80 (d, *J* = 2.80 Hz,1H), 2.09 (s, 3H,), 1.50 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 164.7, 162.88, 157.54, 155.52, 149.22, 129.22, 129.24, 114.24, 11.26, 110.80, 109.90, 81.23, 28.29, 21.78, 8.34. HRMS m/z (M^+ + Na): calcd. 299.0897, found 299.0899.

Synthesis of 2: Compound **1** (2.2 g, 7.97 mmol), *N*-phenyl bis-(trifluoromethanesulfonamide) (5.7 g, 15.94 mmol), and sodium carbonate (4.3 g, 39.85 mmol) in DMF (40 mL) were stirred under an inert atmosphere at RT for 12 h. The reaction mixture was then poured into ice water, precipitated solid was filtered, washed with water and dried in vacuo to afford **2** (3.0 g, 92.30%) as pale brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.26 (t, *J* = 1.2 Hz, 1H), 2.43 (s, 3H,), 1.67 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): 160.44, 154.72, 153.17, 149.95, 145.41, 126.65 119.59, 116.41, 82.31, 29.02, 8.50. ESI- HRMS m/z (M^+ + Na): calcd. 431.0491, found 431.0839.

Synthesis of 3: A mixture of compound **2** (2.52 g, 6.176 mmol), bis(pinacolato)diboron (2.04 g, 8.02 mmol), and potassium acetate (1.82 g, 18.52 mmol) in toluene (50 mL) was purged with argon gas for 15 min. Then, Pd(dppf)Cl_2-CH_2Cl_2 (1.52 g, 1.85 mmol) was added and mixture was additionally purged with argon gas for 5 min. The reaction mixture was heated at 110 °C for 4h. After cooling, the reaction mixture was concentrated in vacuo. The resulted crude compound was passed through a silica gel column using EA (50 %) in petroleum ether as the eluent to afford **3** (1.6 g, 67.22%) as off-white solid.^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.67 (d, *J* = 7.60 Hz, 1H), 7.39 (d, *J* = 7.60 Hz, 1H), 2.64 (s, 3H), 1.34 (s, 16H), 1.26 (s, 5H). ^{13}C-NMR (100 MHz, $CDCl_3$): 160.96, 156.20, 151.94, 146.65, 134.50, 131.76, 129.91, 118.56, 126.35, 124.36, 122.58, 118.91, 82.49, 81.71, 28.68, 27.18, 23.86, 13.19.ESI- HRMS m/z (M^+ + 1): calcd. 387.1901, found 387.2360.

Synthesis of 4: Compound **3** (1.7 g, 4.40 mmol), sodium azide (430.0 mg, 6.60 mmol), and copper acetate (172.0 mg, 0.88 mmol) in methanol (30 mL) was stirred at 55 °C for 4 h. After completion of the reaction, the reaction mixture was cooled to RT and then the reaction mixture was concentrated in vacuo. The crude compound was passed through the silica gel column using EA (50 %) in pet ether as the eluent to afford **4** (0.9 g,68.18 %) as pale-yellow solid.^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.46 (d, *J* = 8.40 Hz, 1H), 7.10 (d, *J* = 8.42 Hz, 1H), 2.29 (s, 3H), 1.60 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$): 160.93, 155.76, 153.29, 146.32, 143.31, 126.57, 116.61, 113.52, 81.65, 36.91, 29.01, 13.12.ESI- HRMS m/z (M^+ + Na): calcd. 324.0963, found 324.0968.

Synthesis of 5: AIBN (40.0 mg, 0.243 mmol), N-bromosuccinimide (288.0 mg, 1.61 mmol) were added successively to a stirred solution of compound **4** (400.0 mg, 1.33 mmol) in CCl_4 (20 mL) and mixture was refluxed for 6 h. After completion of the reaction, the reaction temperature was cooled to RT; and concentrated in vacuo. The resulted crude compound was passed through a silica gel column using EA (40 %) in petroleum ether as the eluent to afford **5** (300.0 mg, 56.0 %) as pale brown solid.^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 7.58 (d, *J* = 8.40 Hz, 1H), 7.14 (d, *J* = 7.89 Hz, 1H), 4.67 (s, 2H), 1.60 (s, 9H). ^{13}C-NMR (100 MHz, DMSO-d_6): 161.54, 155.44, 153.10, 131.48, 119.48, 117.32, 115.79, 115.33, 114.69, 114.47, 81.91, 27.69, 21.28. ESI- HRMS m/z (M^+ + Na): calcd. 402.0068, found 402.0063.

Synthesis of 6: 2, 4-Dinitrophenol (140.0 mg, 0.761 mmol), and potassium carbonate (158 mg, 1.142 mmol) were added to a solution of **5** (300.0 mg, 0.761 mmol) in acetonitrile (10 mL). The reaction mixture was refluxed for 6 h. The, reaction mixture was cooled to RT and concentrated in vacuo. The resulted crude compound was passed through a silica gel column using EA (40 %) in petroleum ether as the eluent to afford **6** (168.0 mg, 43.0 %) as yellow solid. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.76 (d, *J* = 2.64 Hz, 1H), 8.68 (s, 1H), 8.57 (dd, *J* = 9.24 Hz, 1H), 8.05 (d, *J* = 8.56 Hz, 1H), 7.91(q, *J* = 12.36 Hz, 1H), 7.50 (d, *J* = 8.48 Hz, 1H), 5.46 (s, 2H), 1.51 (s, 9H).^{13}C-NMR (100 MHz, DMSO-d_6): 161.72, 155.91, 154.20, 147.70, 145.24, 140.10, 138.92, 134.10, 129.34, 121.28, 117.35, 116.21, 115.86, 114.74, 81.90, 61.60, 29.41. ESI- HRMS m/z (M^++ 1): calcd. 484.1126, found 484.1555.

Synthesis of MitoDP: TFA (10%) in DCM (10 mL) was added to a stirred solution of compound **6** (168 mg, 0.347 mmol) in DCM (10 mL) at 0 °C and then, stirred for 1h. The reaction mixture was concentrated under reduced pressure and dried in vacuo. The crude intermediate was taken for

the next step without further purification. To a stirred solution of crude intermediate (150.0 mg, 0.351 mmol) in DMF (10 mL) at 0℃, DIPEA (0.32 mL, 1.75 mmol), (2-aminoethyl) triphenylphosphoniumbromide salt (197.0 mg, 0.42 mmol) and **1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate** (HATU) (200.0 mg, 0.526 mmol) were added. The reaction was continued to stir for 12h at RT. After completion, the reaction mixture was diluted with water and stirred for an additional15 min. The precipitated solid was filtered, washed with water and dried in vacuo. The crude product was purified by column chromatography over silica gel (100-200 mesh) using methanol in DCM (0.5: 9.5) as eluent to afford probe **MitoDP** as pale-yellow solid (125.0 mg; 45%).^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.82 (s, 1H), 8.77 (d, *J* = 3.20 Hz, 1H), 8.59 (d, *J* = 2.80 Hz, 1H), 8.14 (d, *J* = 8.14 Hz, 1H), 7.94-7.82 (m, 9H), 7.75-7.70 (m, 7H), 7.56 (d, *J* = 8.40 Hz, 1H), 5.52 (s, 2H), 4.01-3.89 (m, 2H), 3.88-3.82 (m, 2H).^{13}C-NMR (100 MHz, DMSO-d_6): 162.26, 161.30, 147.96, 145.43, 140.14, 134.87, 133.65, 130.11, 121.10, 118.61, 117.93, 116.39, 116.35, 116.30, 115.18, 112.08, 61.68, 35.75, 33.33. ESI- HRMS m/z (M^+): calcd. 715.1701, found 715.1742. HPLC purity: 98.11%.

Preparation of FL1: A mixture of **MitoDP** (50 mg, 0.069 mmol) and Na_2S (10 eq.) in acetonitrile: water (5 mL; 1:1) was stirred for 1 h at RT. The reaction mixture was concentrated under reduced pressure. Crude was purified by column chromatography using silica gel (100-200 mesh) using 10% methanol in DCM as eluent to afford compound **FL1** as brown solid (10 mg, 24 %).^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.53 (s, 1H), 7.89-7.74 (m, 15H), 7.58 (d, *J* = 8.52 Hz, 1H), 6.82 (s, 2H), 6.64 (d, J=MHz, 1H), 6.45 (s, 1H), 4.81(s, 1H), 3.85 (t, *J* = 6.48 Hz, 1H), 3.68 (bs, 2H),

Cell culture and treatment

HCT 116 (colon cancer), HeLa (cervical cancer), and 3T3-L1 (normal mouse fibroblast) cell lines were obtained from the ATCC through NCCS, India. HeLa and 3T3-L1 cells were grown in the DMEM high glucose media (Sigma) and HCT 116 cells were grown in the McCoy media (Sigma) using 10% FBS, 1%Penstrep and 0.2%of Amphotericin (Gibco Life Tech). All the cell lines were maintained at carbon dioxide (5%) at 37°C.

In vitro fluorescence imaging

Cells were seeded at a density of 0.3 × 10^5 cells per cm^2 with growth medium in 35 mm dishes and kept for overnight incubation. The cells were treated with indicated concentrations of **MitoDP**

and DNP for the required duration. DMSO was used as the vehicle. All the images for **MitoDP** fluorescence were measured at an excitation wavelength of 390 nm and emission wavelength being monitored over the 440–500 nm range in Zeiss A1 AxioVert Fluorescence Microscope at 40X magnification.

Inhibitor studies

Cancer cells (HCT 116 and HeLa cells) were pretreated with aminooxyacetic acid (AOAA, 2 mM) inhibitor for CBS, N-propargylglycine (PAG, 2 mM) inhibitor for CSE and co-treated with AOAA and PAG for 1 h before treating with 5μM**MitoDP**.

Co-localization experiments with Mitotracker red and Lysotracker red

Cells were incubated with **MitoDP** for 30 mins and then the dyes Mitotracker red (250 nM) and Lysotracker red (250 nM) were added to the cells in separate experiments. After incubation for 15 mins, cells were washed with PBS and fluorescence images were taken in Zeiss A1 AxioVert Fluorescence Microscope at 40X magnification. Colocalization 3D histogram was obtained using the Zen software.

Cells were grown on cover slips and incubated with Mito DP for 30 min and then Mitotracker red (250 nM) was added to the cells. After incubation for 15 min, cells were washed with PBS. Cover slips with cells were inverted on a concave microscopic glass slide and taken for imaging. Fluorescence images were taken in Olympus microscope (BX51) at 60X magnification. Zoomed images were cropped using Image J software

Estimation of mitochondrial membrane potential

For analyzing the change in mitochondrial membrane potential, cells were treated with **MitoDP** (10 μM) for indicated time points and tetramethylrhodamine, ethyl ester (TMRE) (100 nM) was added to the cells. After incubation for 15 mins, cells were washed with PBS and supplemented with fresh growth medium. The fluorescence images were taken at 540 nm/580 nm in Zeiss A1 AxioVert Fluorescence Microscope at 40X magnification.

Estimation of mitochondrial membrane potential through flow cytometry.

Cells were treated with Mito DP, FL1 and positive controlFCCP (mitochondrial uncoupler) for 12 and 24 h. Tetramethylrhodamine, ethyl ester (TMRE) (100 nM) was added to the cells. After incubation for 15 min cells were harvested, resuspended in 1X PBS and taken for FACS analysis.

Estimation of reactive oxygen species (ROS)

For measuring the levels of ROS generation, **MitoDP** treated cells were incubated with 2′,7′-dichlorofluorescein diacetate [DCFDA, (5 μM)] for 30 mins. Cells were washed with PBS and thereafter supplemented with fresh growth medium before taking fluorescence images at 495-510/517–540 nm in Zeiss A1 AxioVert Fluorescence Microscope at 40X magnification.

Measurement of Adenosine triphosphate production (ATP)

After the stipulated time of **MitoDP** exposure, cells were lysed in 70% perchloric acid and ATP in cell extract was measured using ATP Bioluminescent Assay Kit (Sigma) in Biotek Plate reader.

Cell viability assay

The cells were seeded in the 96 well plates and incubated overnight before treatment. Cells were treated with different concentration of **MitoDP** and DNP and incubated for 48 h and 72 h. After the stipulated time, MTT (5 mg/mL)was added and incubated for 2 h at 37 ^{o}C; the formazan crystals formed were dissolved in DMSO and absorbance was recorded at 590 nm usinga 620 nm reference filter.

Propidium iodide staining for apoptosis analysis

The HCT116 cells were treated with MitoDP (10 μm) and Staurosporine (1 μM) (positive control for apoptosis) for 48 h. After 48 h cells were harvested and fixed in ice cold 70% ethanol for 30 min. The fixed cells were incubated in DNA extraction buffer (0.2 M Na2HPO4 and 0.1% Triton X-100, pH 7.8) for 5 min and centrifuged at 400g for 5 min. The pellet was resuspended in DNA staining solution (20 μg/ml Propidium iodide and 200 mg/ml DNase free RNase), incubated for 30 mins and then taken for FACs analysis.

General methods for UV-Vis and Fluorescence Spectroscopy

All reagents and solvents used for fluorescence spectroscopy were commercial and used without further purification.Except under special case, the following methods provide all of measurements for fluorescence spectroscopy. Absorption spectra were recorded on an UV-1800 spectrophotometer (Shimadzu), and fluorescence spectra were recorded using an RF-6000 fluorescence spectrofluorometer (Shimadzu) with a 3000μL volume of 1 cm standard quartz cell. 500 μM Stock solution of the **MitoDP**probe was prepared by dissolving probe in DMSO.Stock solution of the Na_2S was prepared by dissolving in PBS buffer (pH=7.4). The fluorescence emission spectra were recorded at excitation wavelength of 395 nm and emission was monitored over wavelength the range

of 400-600 nm (λ_{em}= 450 nm) with slit width set at 3 nm. The solutions of biologically relevant species were prepared from cysteine (Cys), H_2O_2, $NaNO_2$, $Cu(OAc)_2$, $Zn(OAc)_2$, $FeSO_4$, $FeCl_3$, Na_2CO_3, GSH, ascorbic acid (AA), NO, Na_2S_2, K_2S_5in PBS buffer.

Measurement of fluorescence intensity

Fluorescence intensity was assessed using Image J software.

Statistical analysis

Fluorescence intensity was measured for three different cells in images obtained from three different experiments (by Image J). Cells which did not take up the compound were not included (In HeLa cells). Averages of these readings were taken as representative values for tests done at each concentration. In HeLa cells, the values were differing by 4-8 units. The graph was prepared in Graph Pad keeping the values of HCT 116 and HeLa together. Comparative analysis was done using Dunnet's test comparing with their respective controls. All values are the mean ± S.E.M from three independent sets. The statistical comparisons were done using either one-way or two-way variance analysis using Dunnett's Multiple-Comparison Post-Test. The figures were also prepared using the GraphPad software.

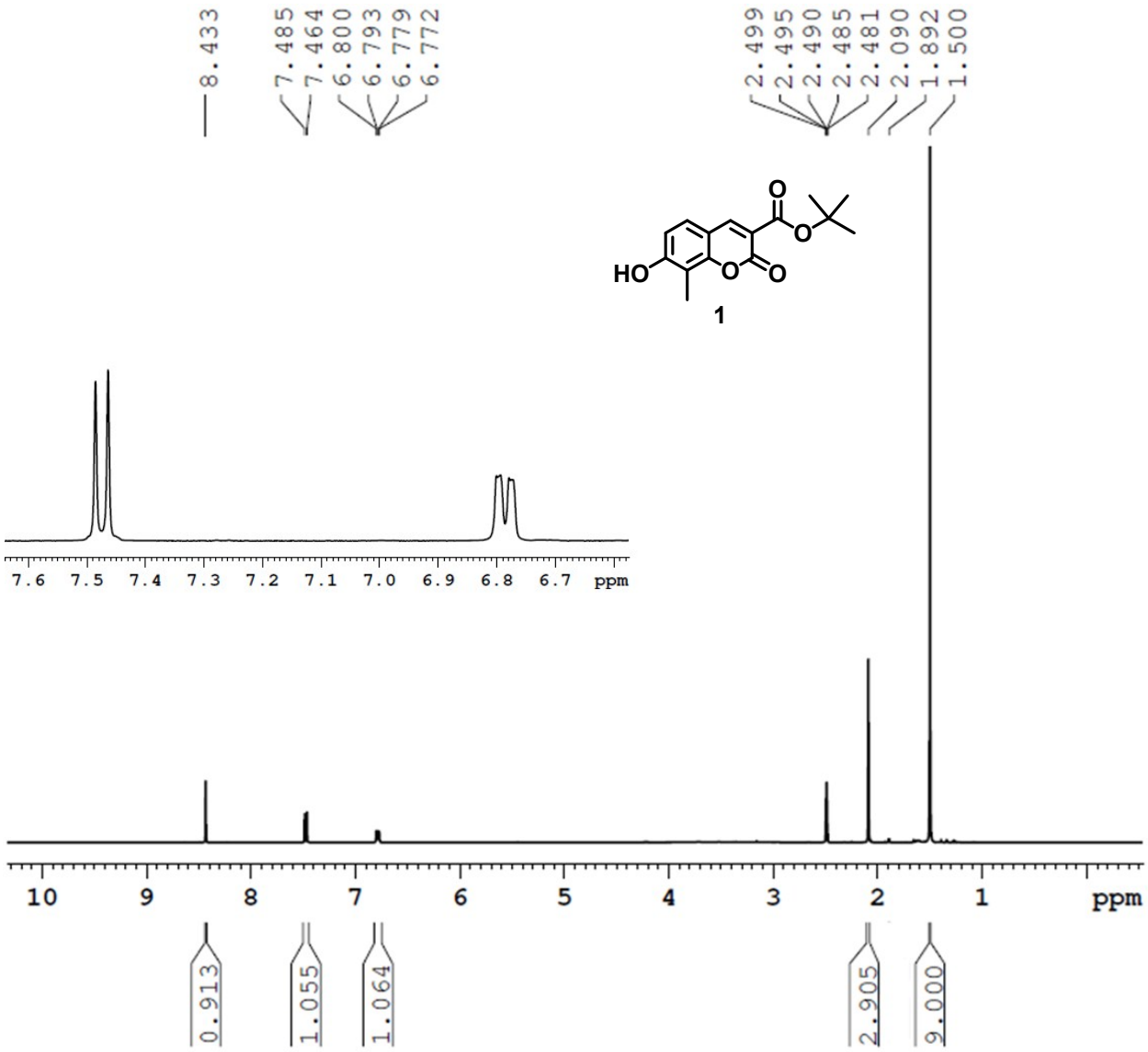


Fig. S1 ^{1}H-NMR of compound **1**in DMSO-$d_{6.}$

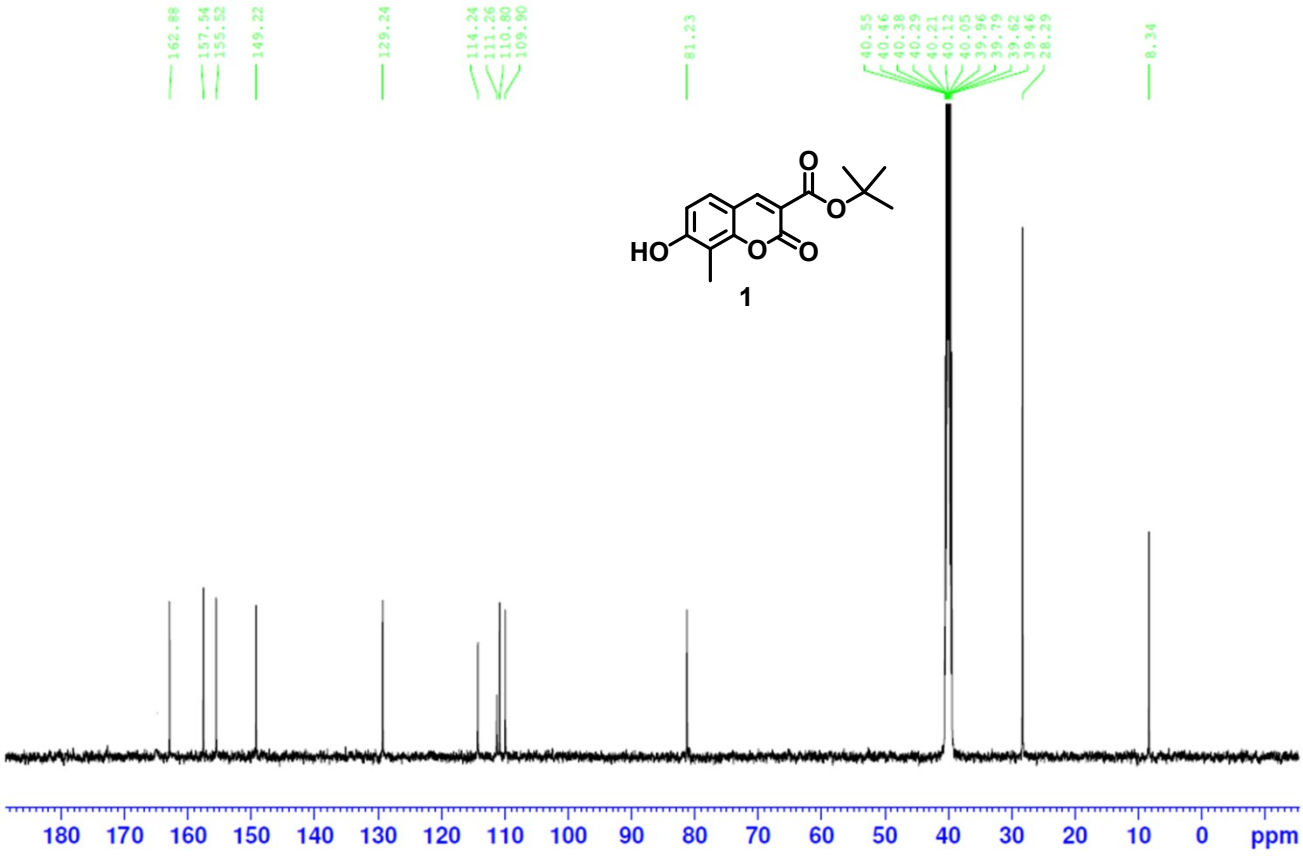


Fig.S2 ^{13}C-NMR of compound **1** in DMSO-d_6.

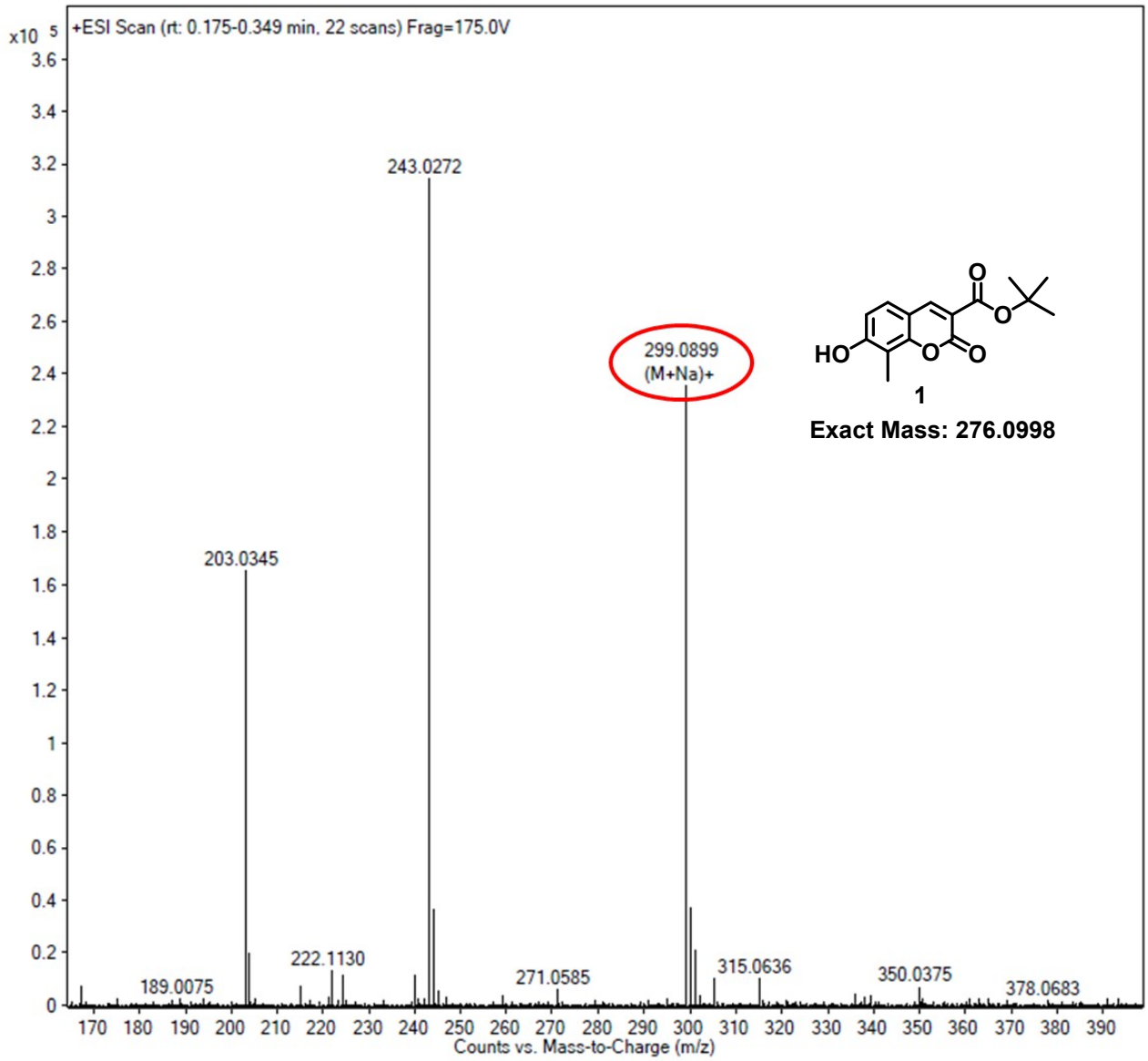


Fig.S3 HRMS of compound **1.**

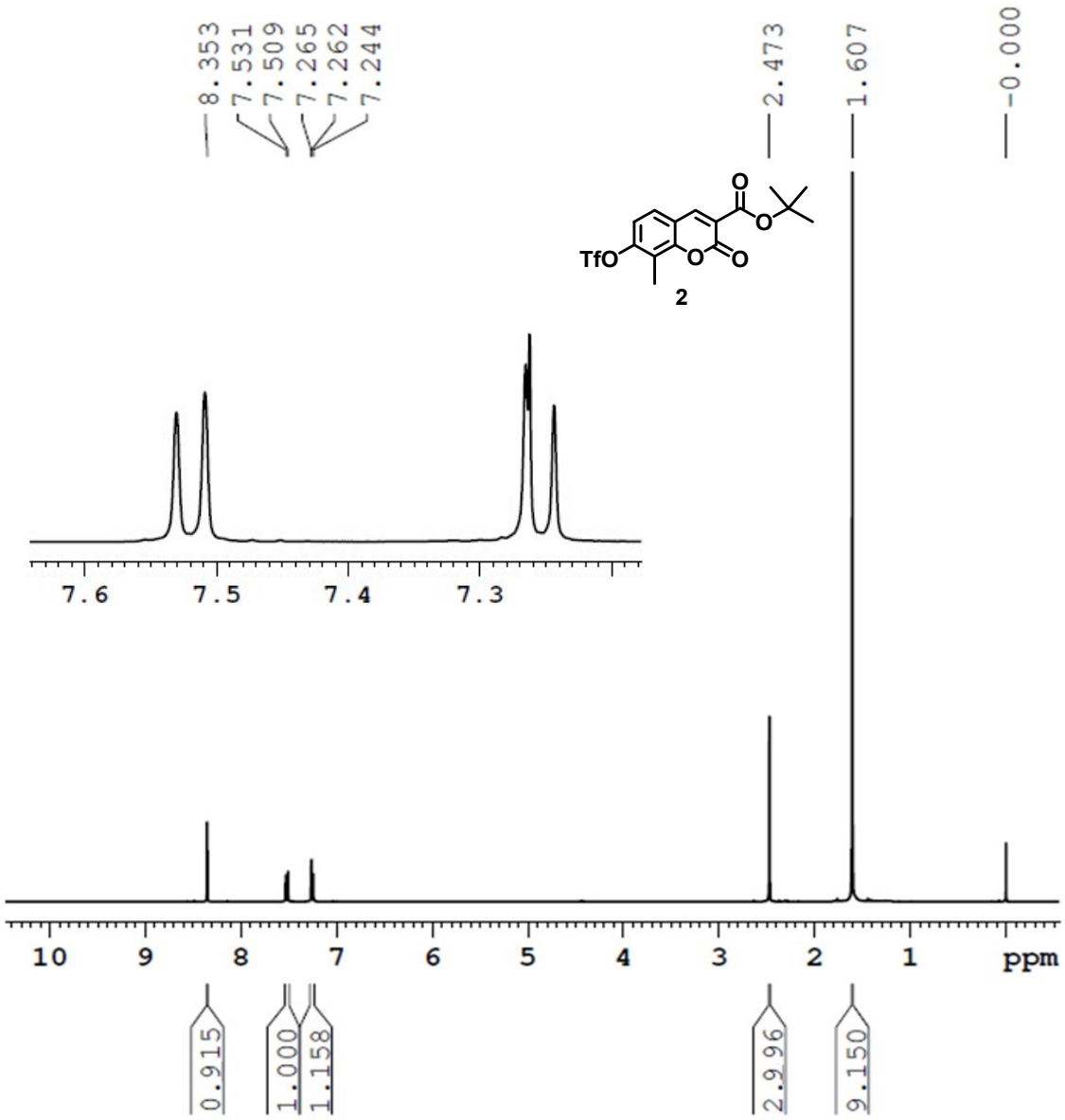


Fig. S4 ^{1}H-NMR of compound **2**in $CDCl_3$.

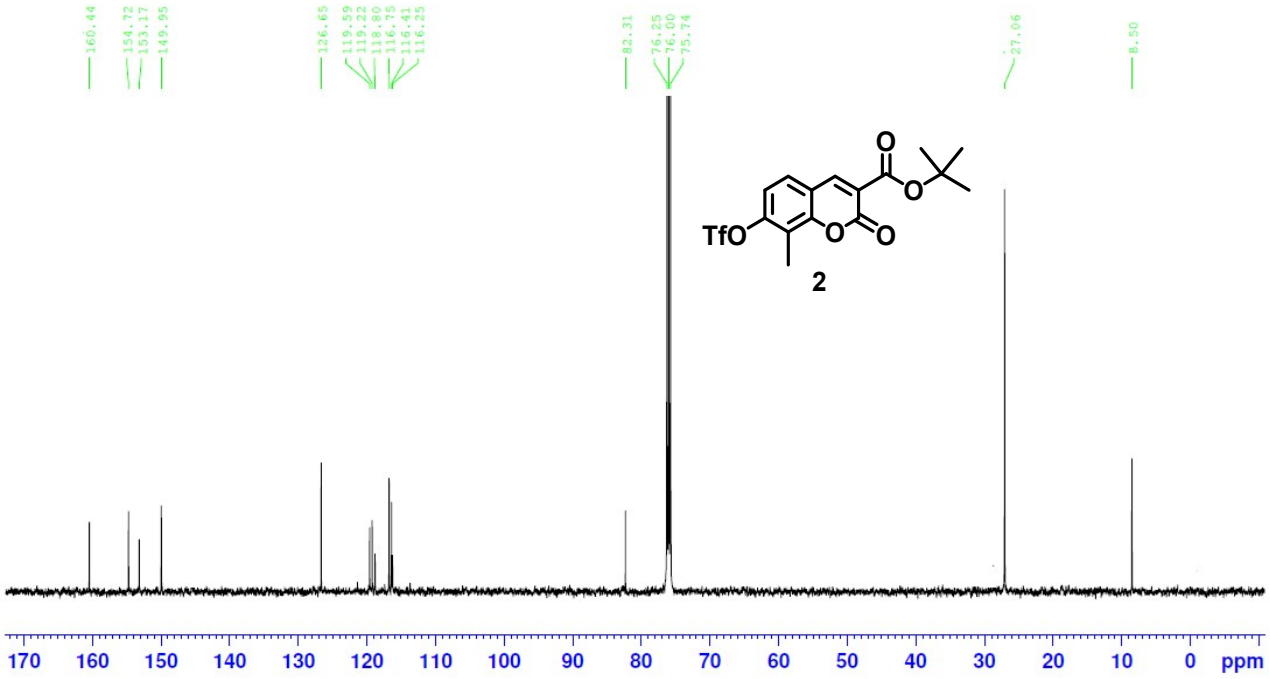


Fig.S5 ^{13}C-NMR of compound **2**in $CDCl_3$.

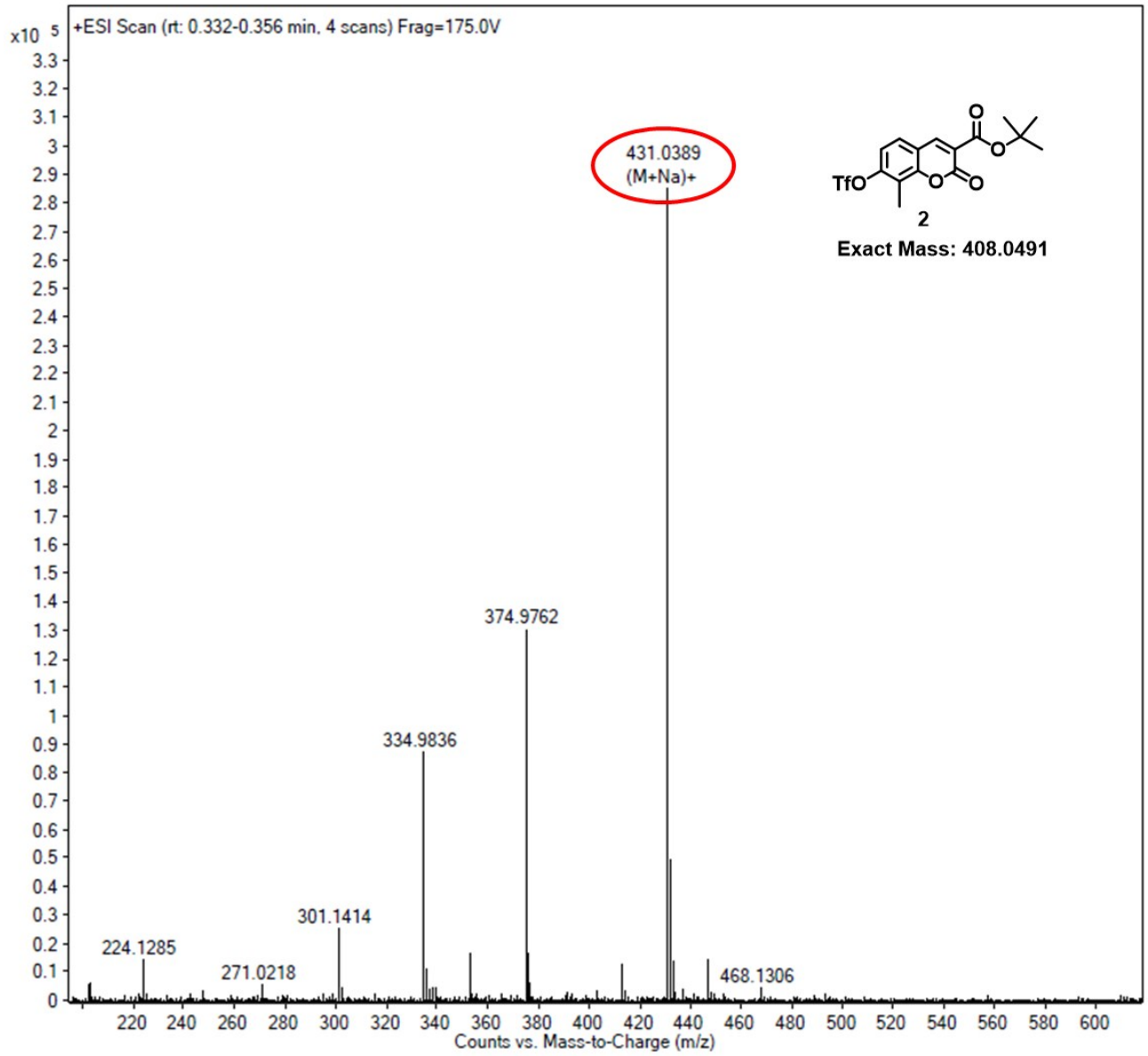


Fig. S6HRMS of compound **2.**

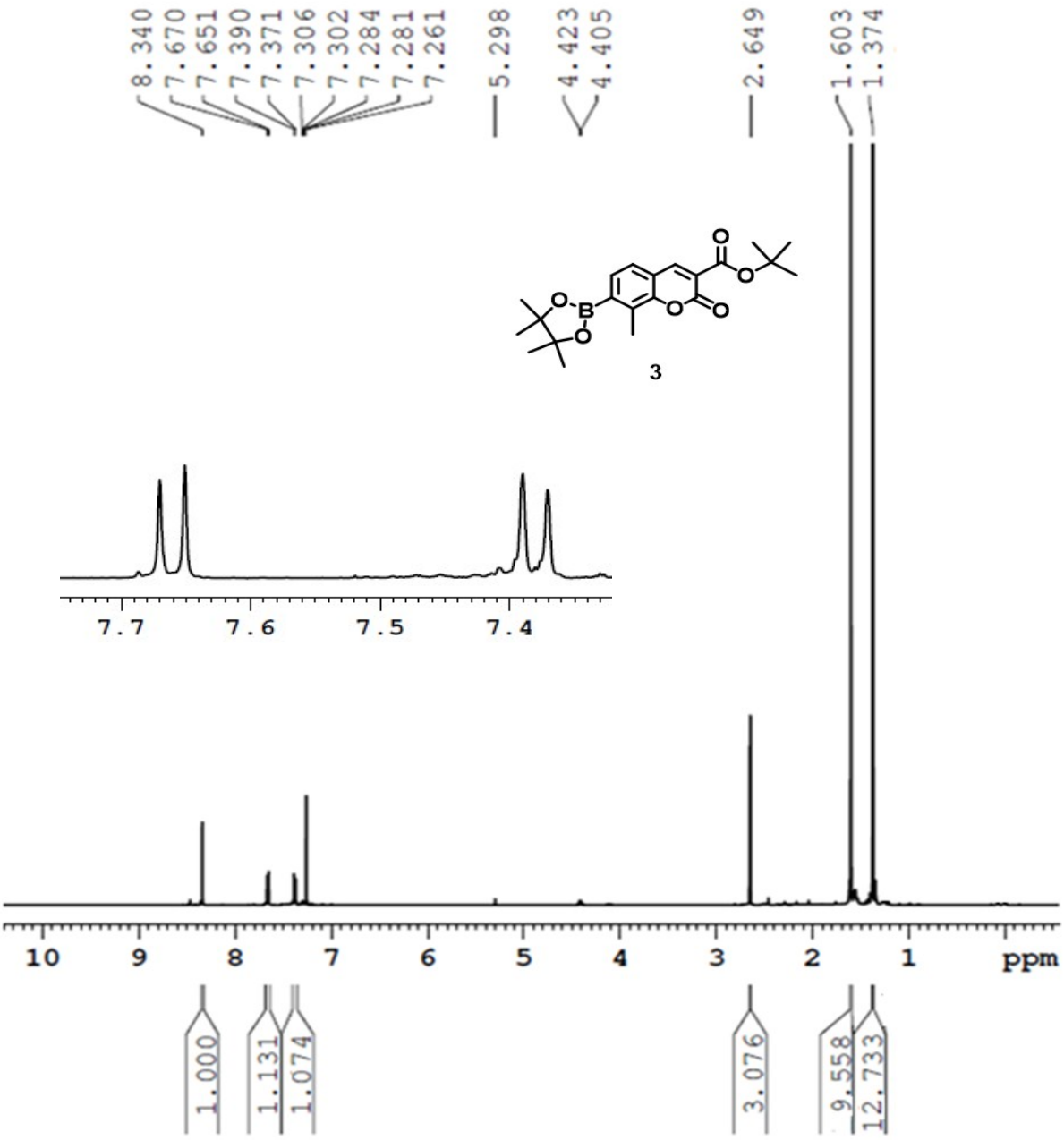


Fig. S7 ^{1}H-NMR of compound **3**in $CDCl_3$.

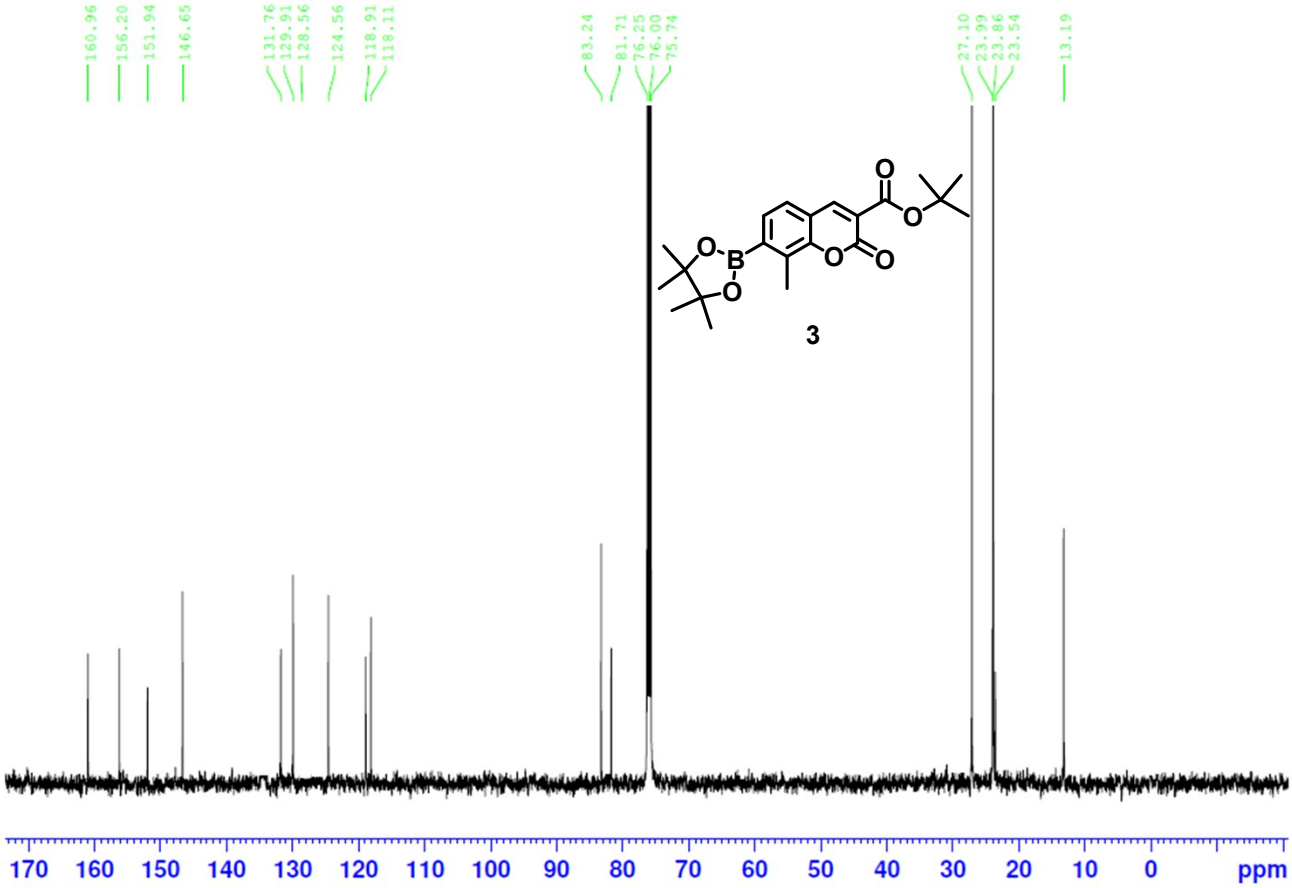


Fig. S8 ^{13}C-NMR of compound **3**in $CDCl_3$.

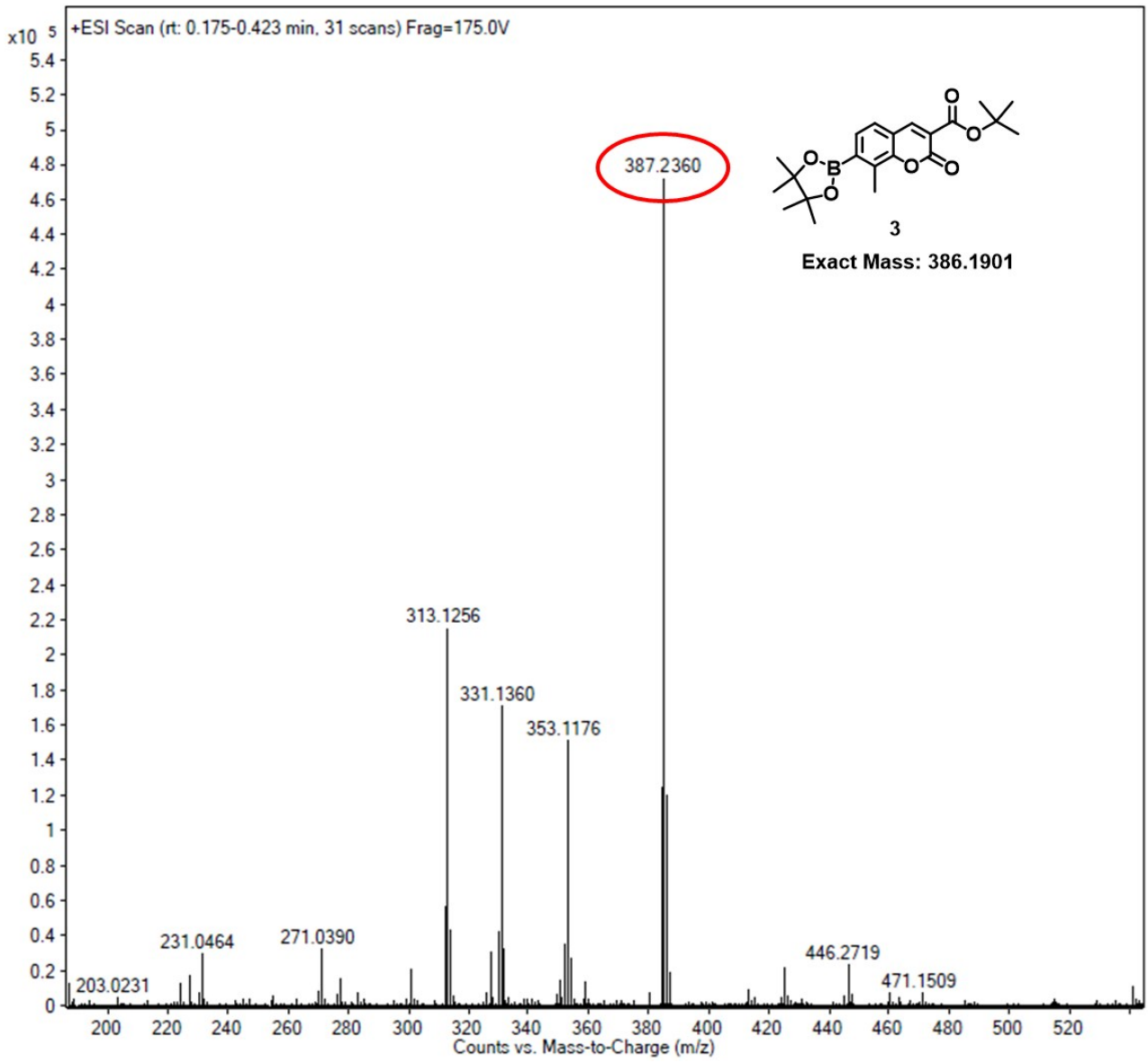


Fig. S9 HRMS of compound **3.**

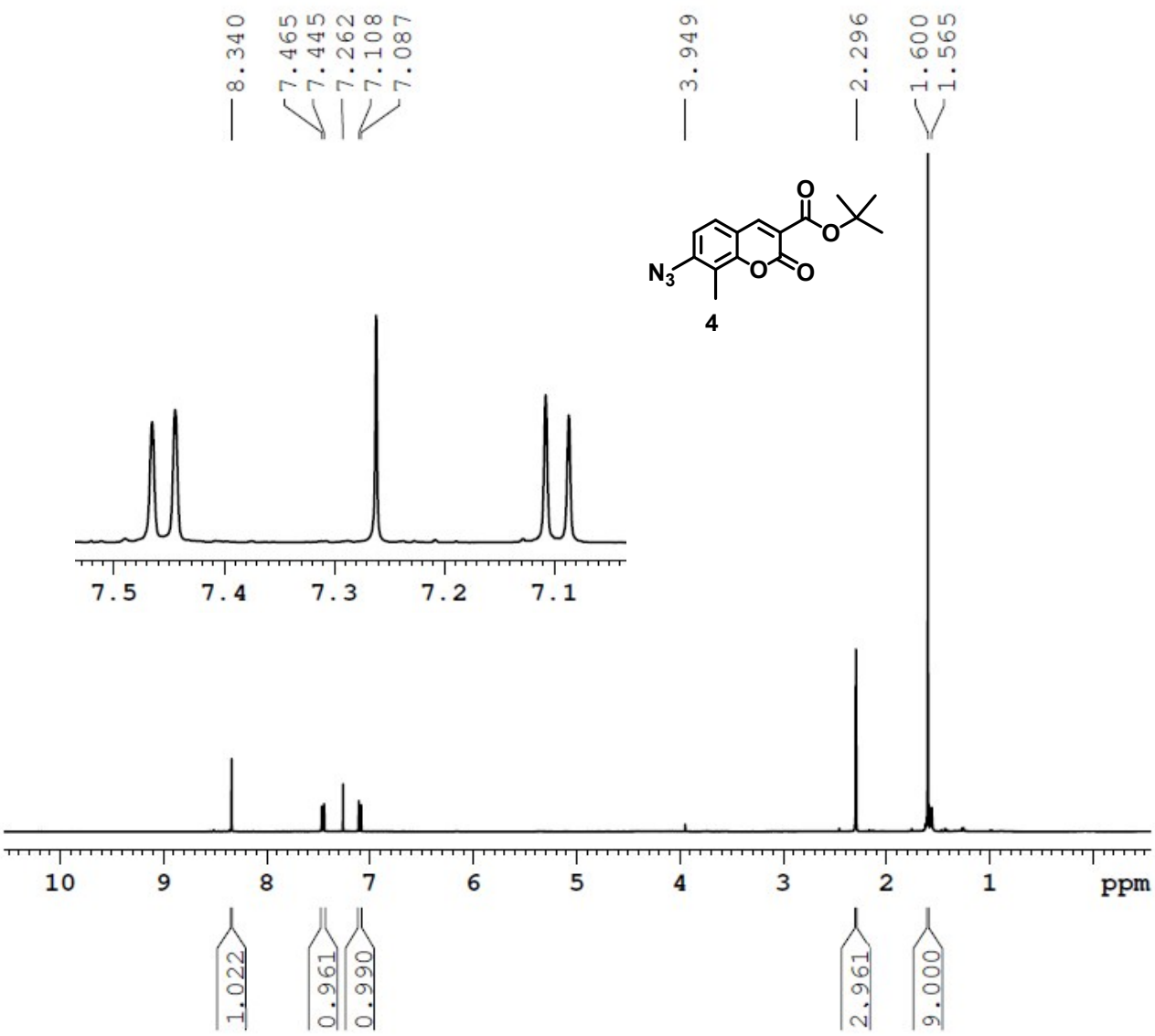


Fig.S10 ^{1}H-NMR of compound **4**in $CDCl_3$.

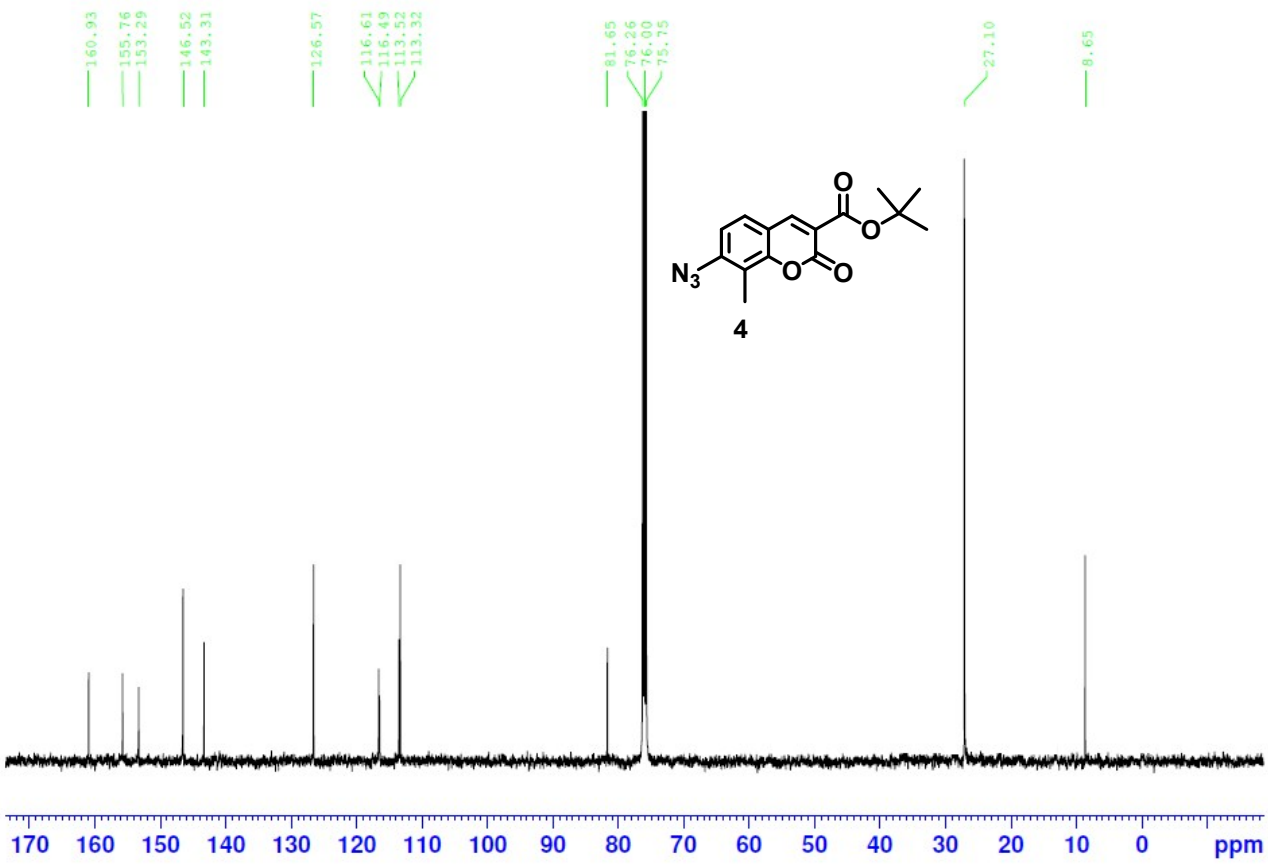


Fig. S11 ^{13}C-NMR of compound **4**in $CDCl_3$.

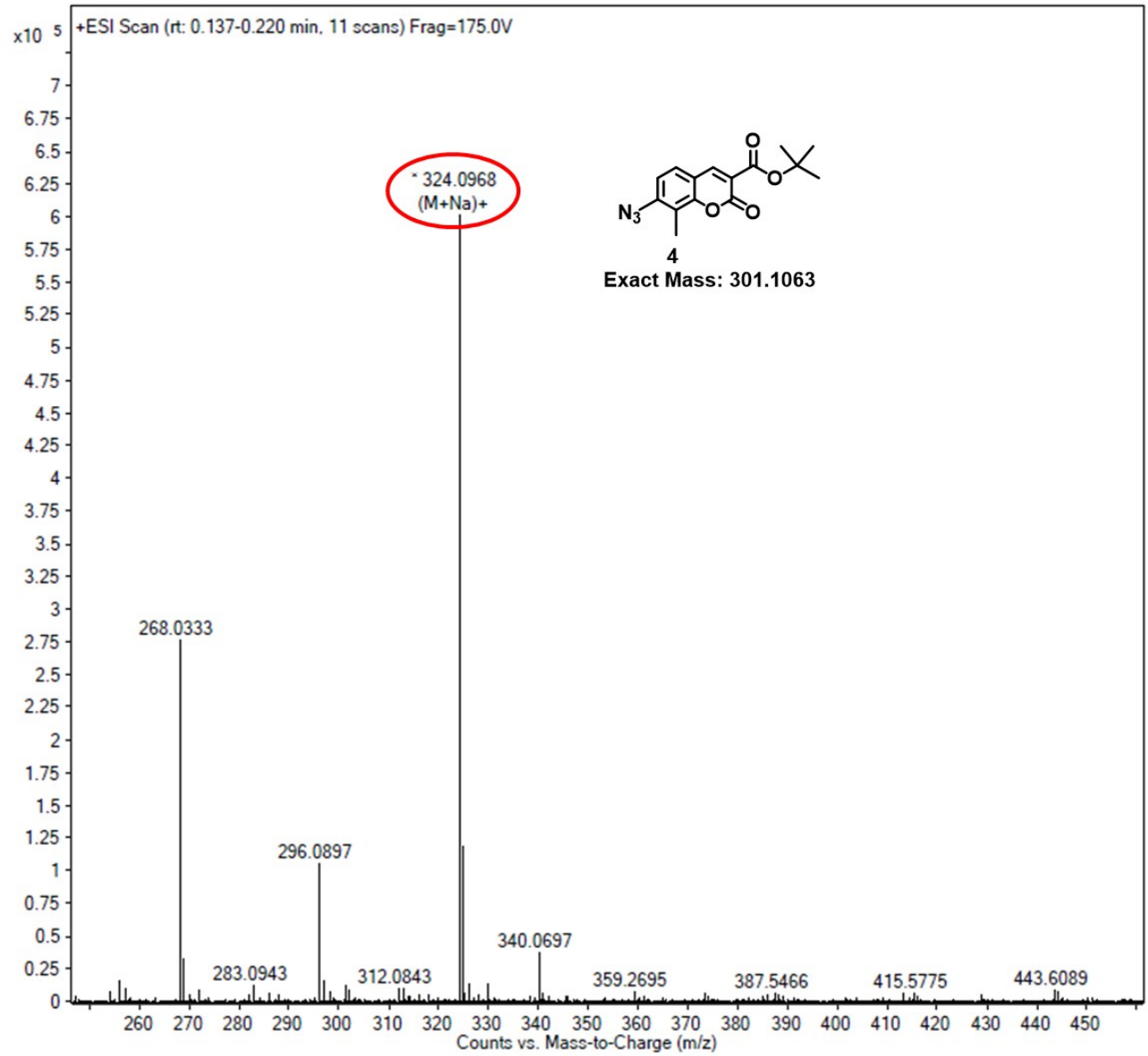


Fig. S12 HRMS of compound **4.**

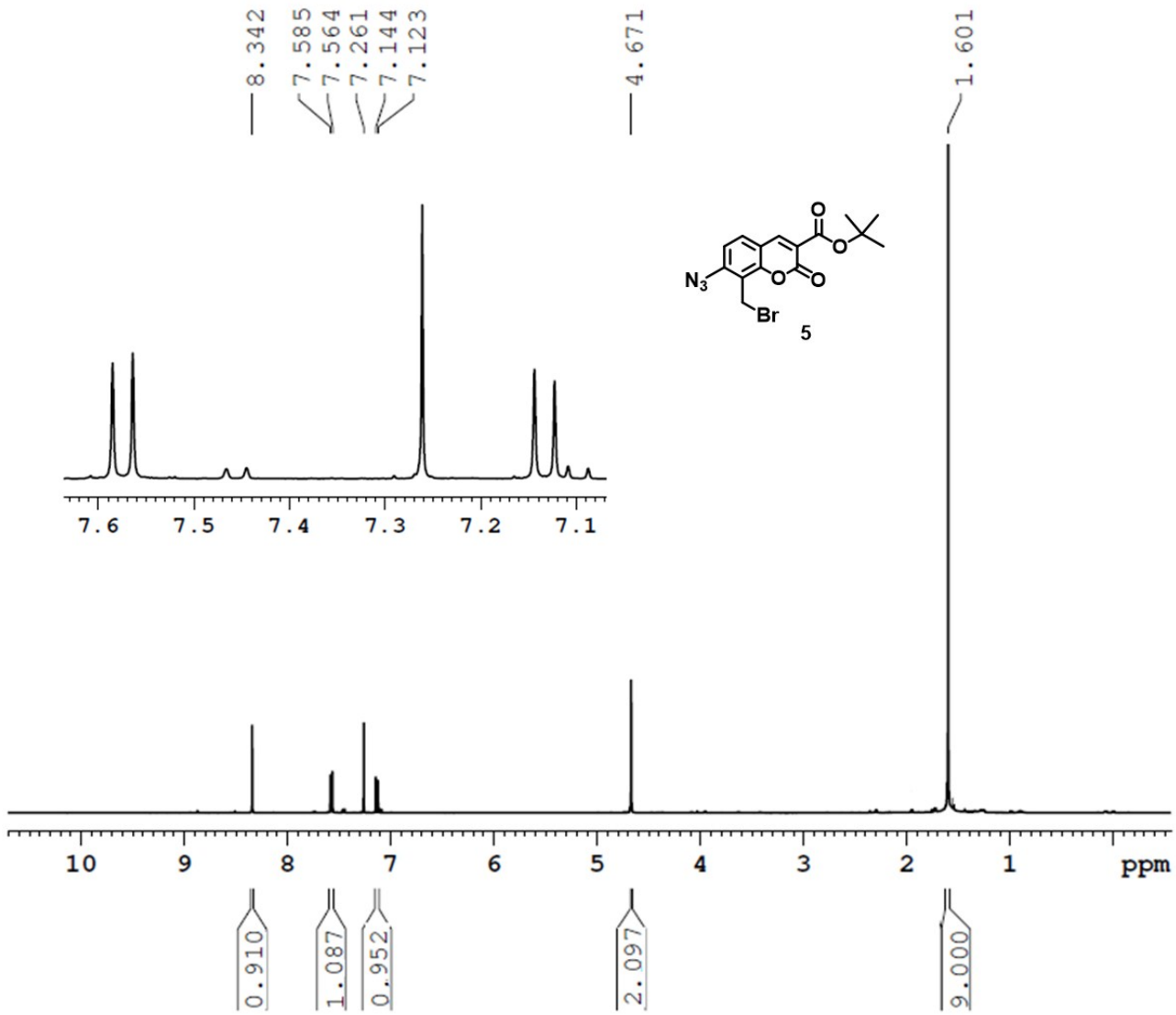


Fig. S13 ^{1}H-NMR of compound **5**in $CDCl_3$.

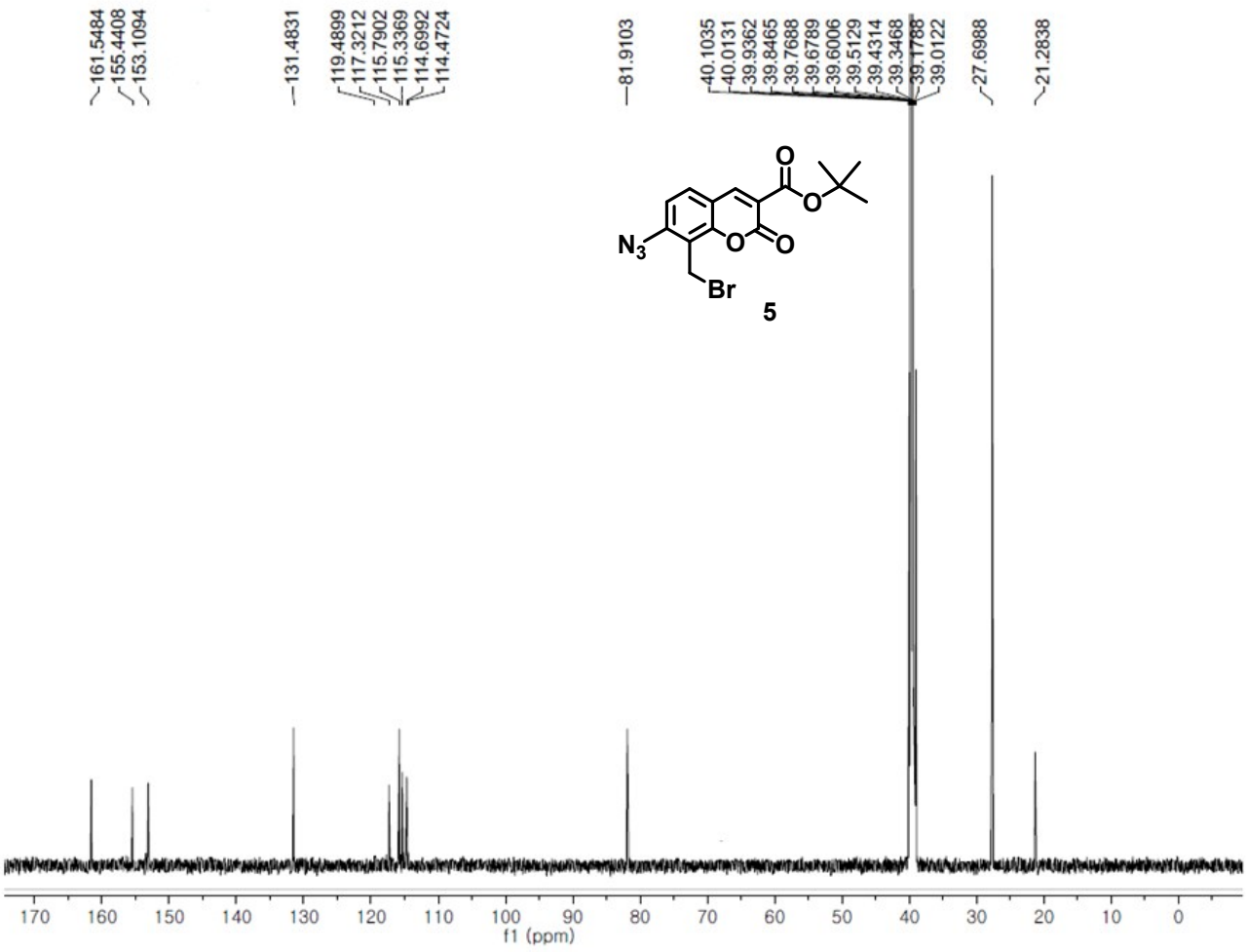


Fig. S14 ^{13}C-NMR of compound **5**in DMSO-d_6.

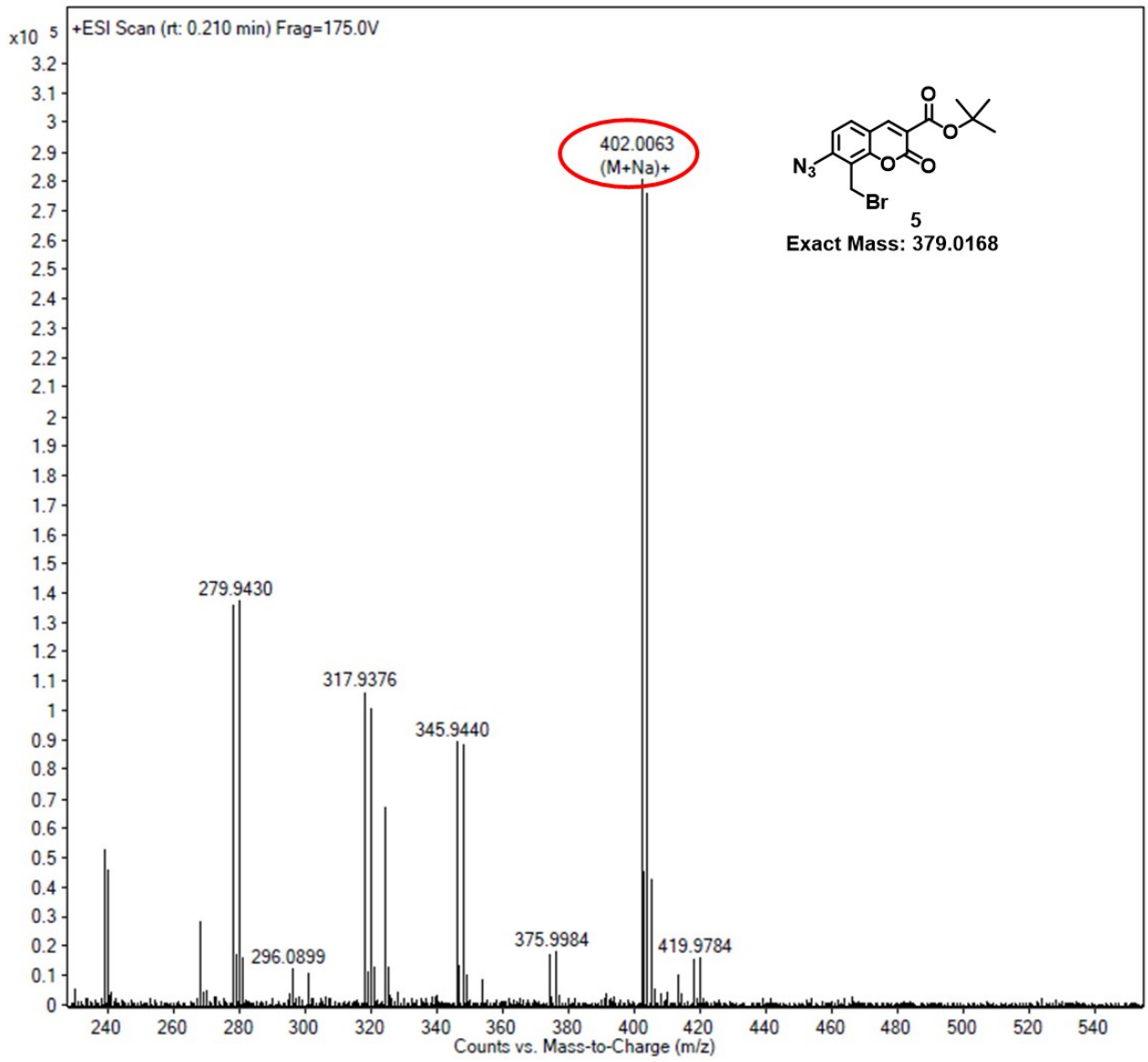


Fig.S15 HRMS of compound **5.**

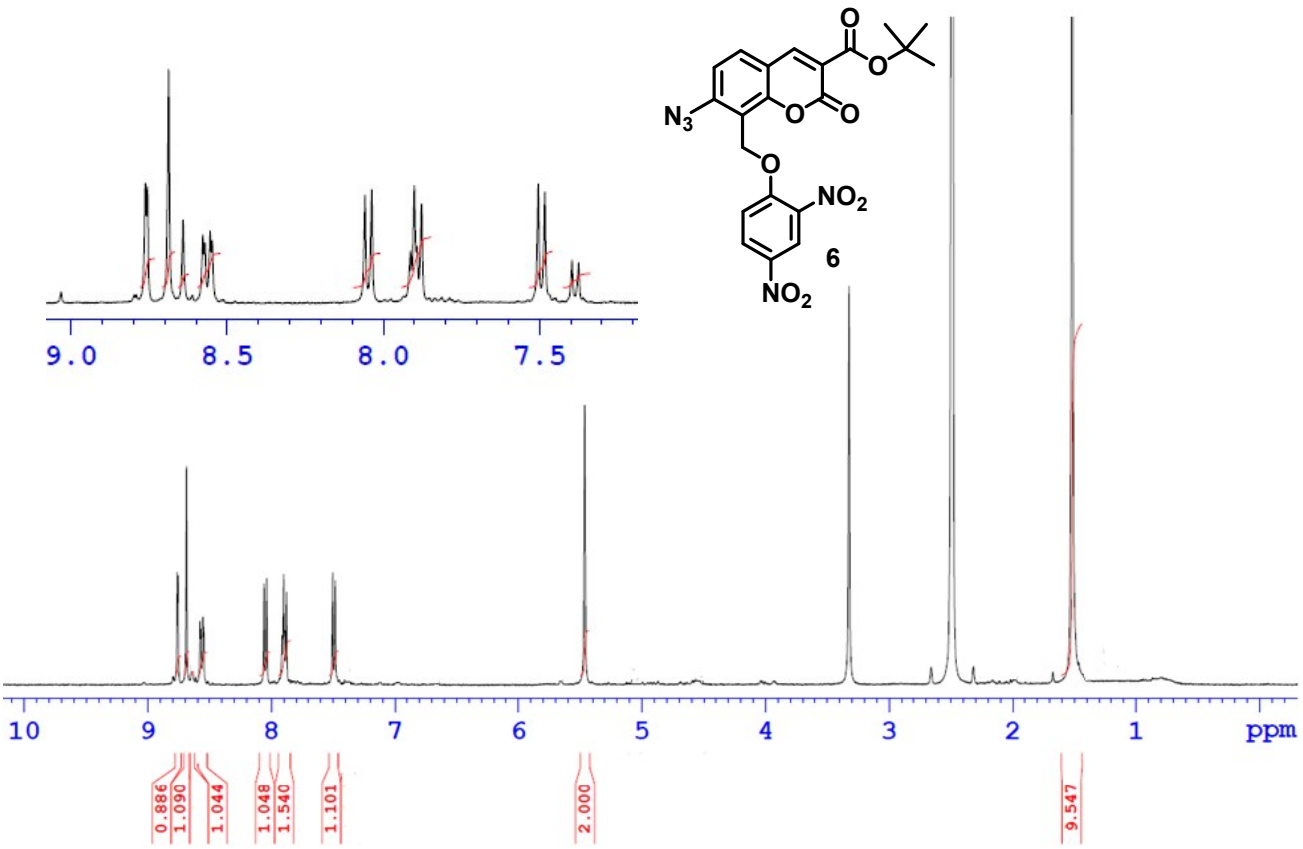


Fig. S16 ^{1}H-NMR of compound **6**in DMSO-$d_{6.}$

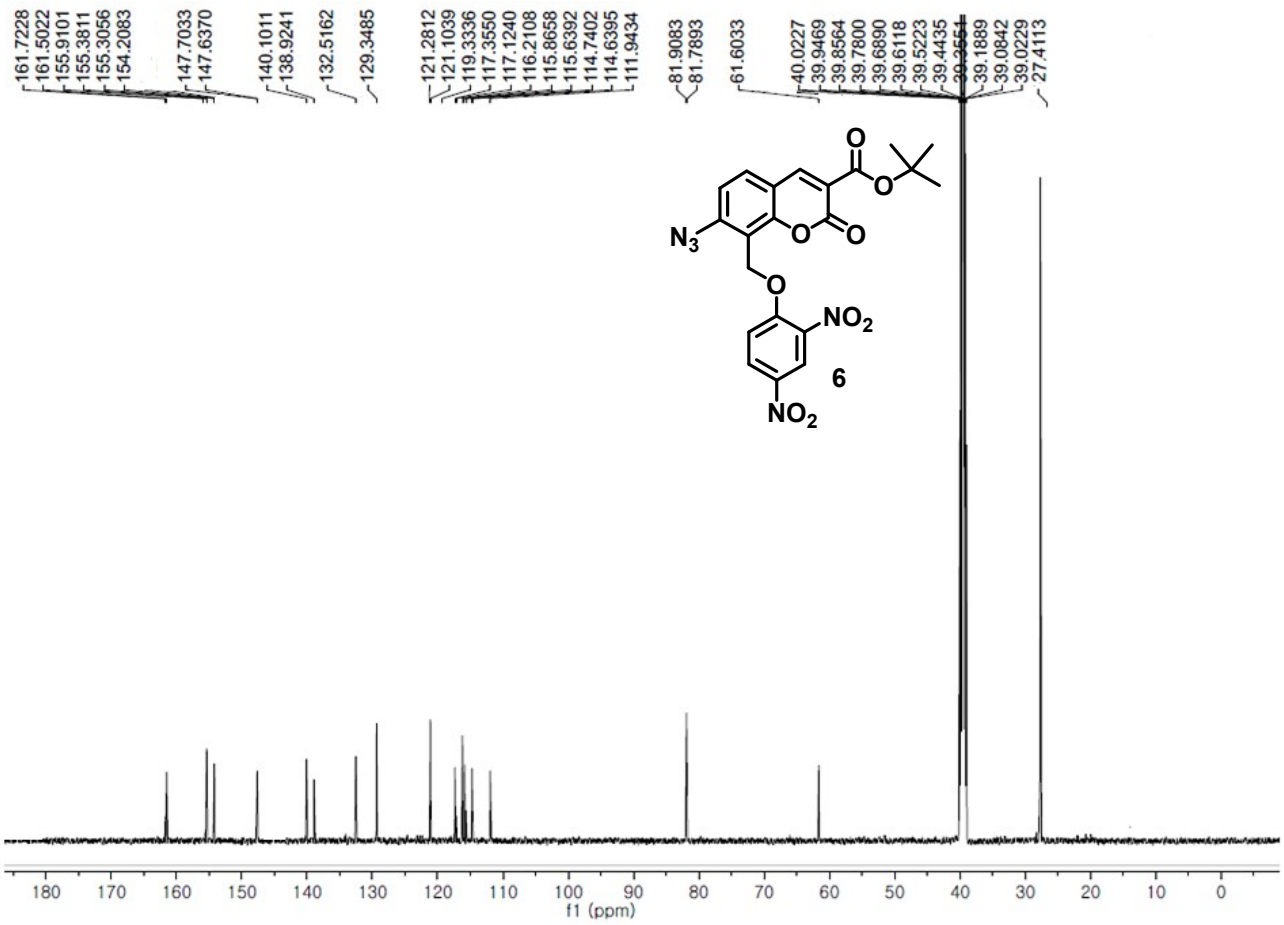


Fig. S17 ^{13}C-NMR of compound **6**in DMSO-d_6.

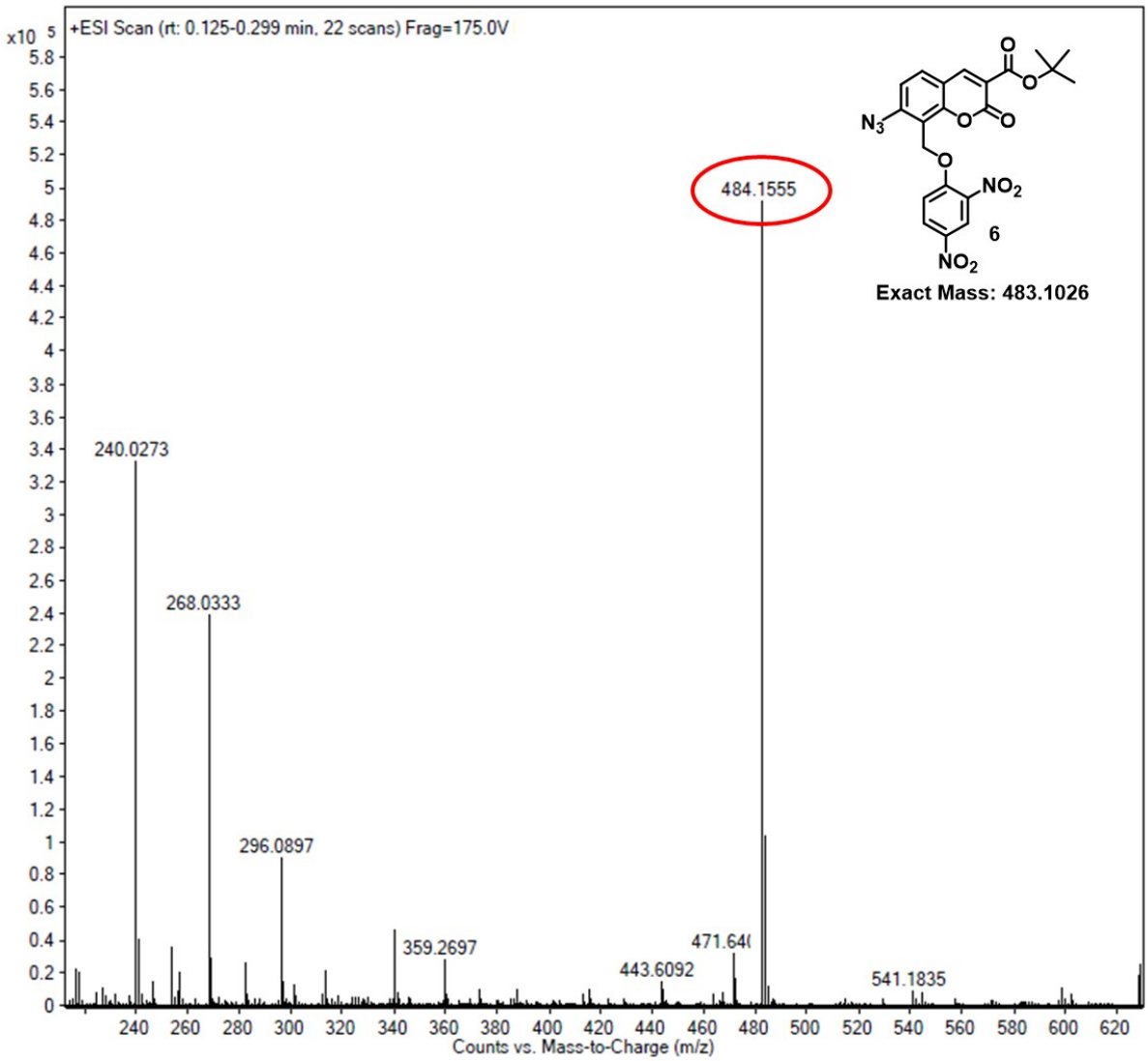


Fig. S18 HRMS of compound **6.**

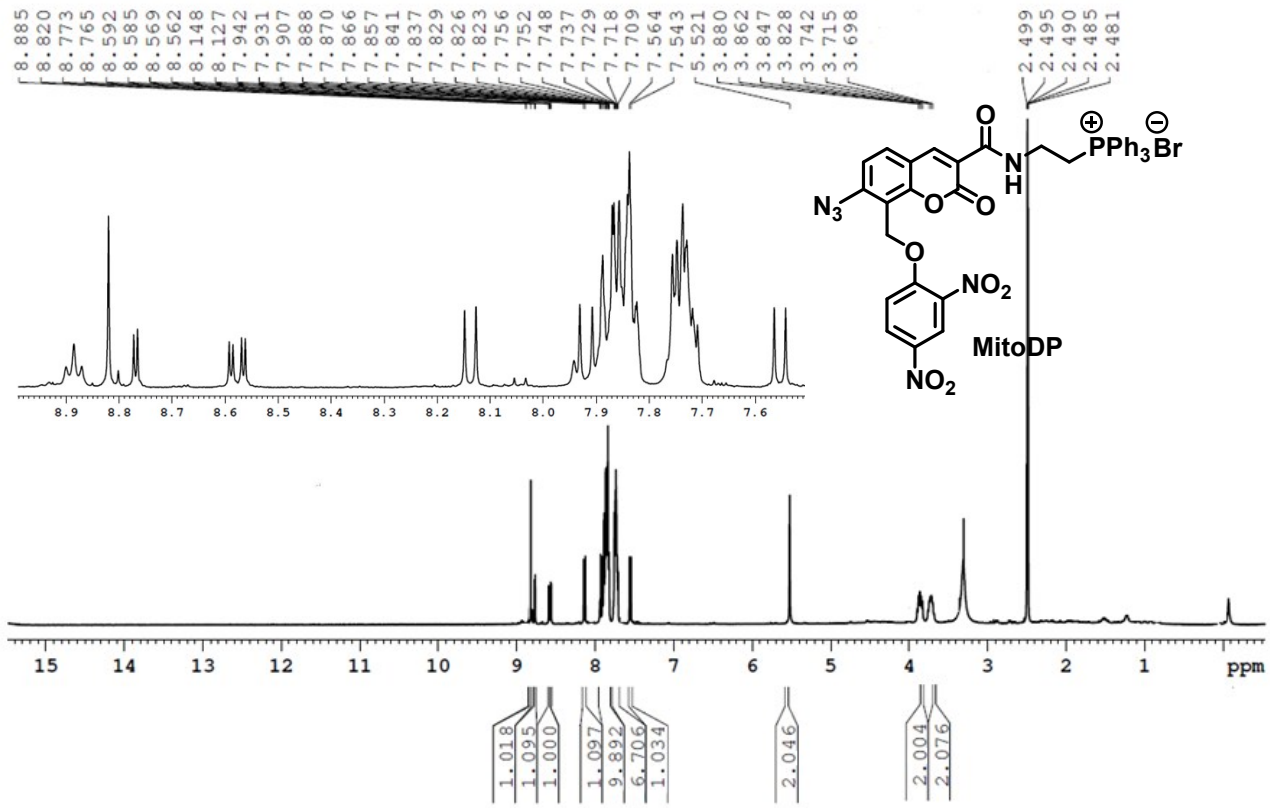


Fig. S19 [1]H-NMR of **MitoDP**in DMSO-d_6.

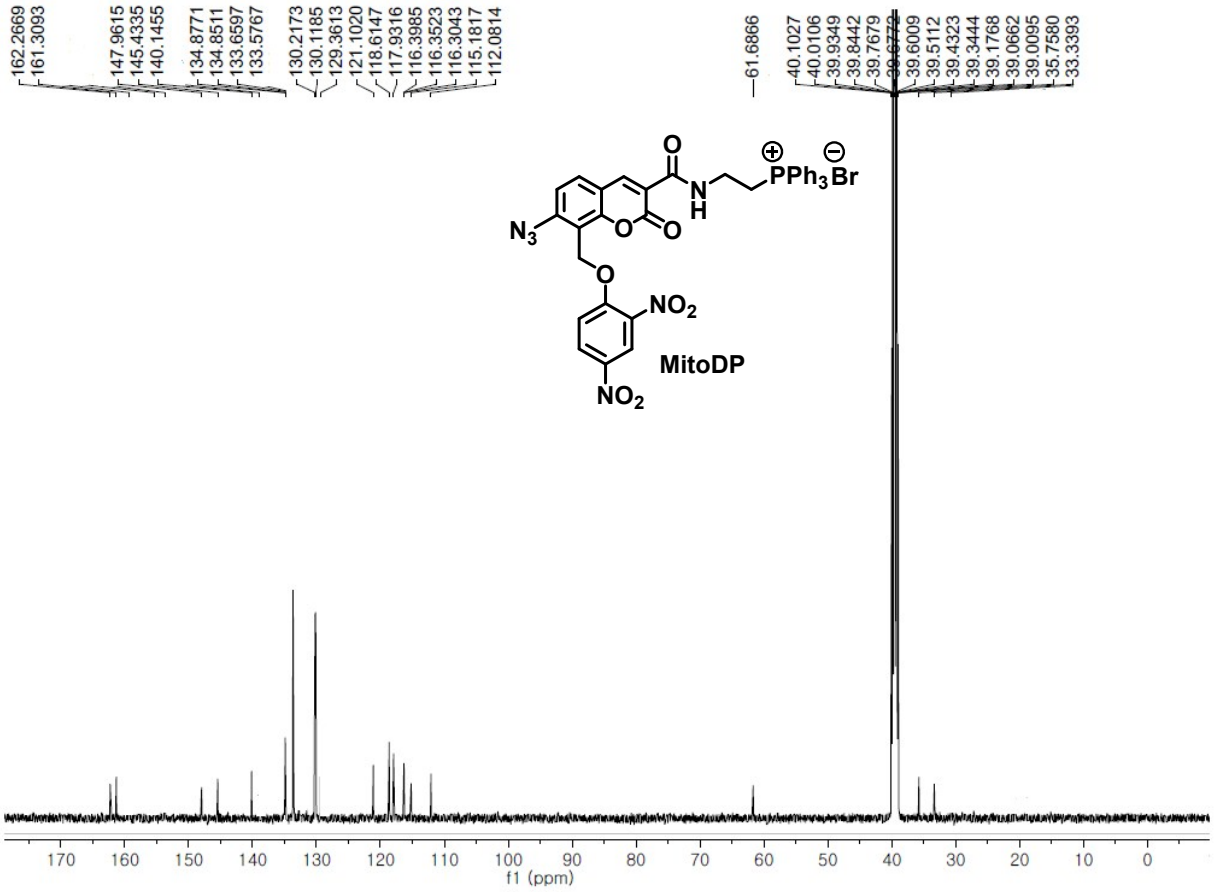


Fig. S20 ^{13}C-NMR of **MitoDP**in DMSO-d_6.

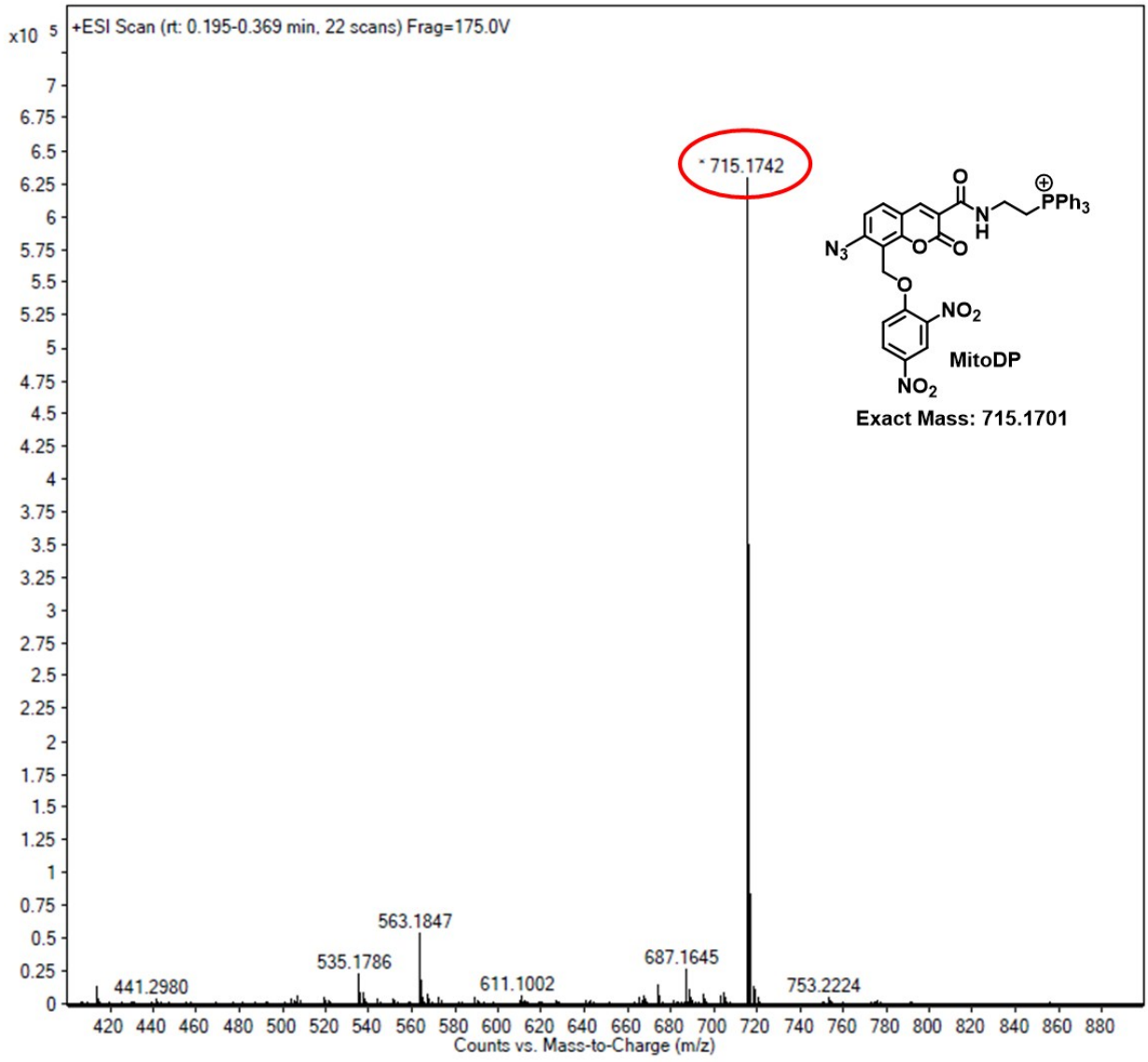


Fig. S21 HRMS of **MitoDP**.

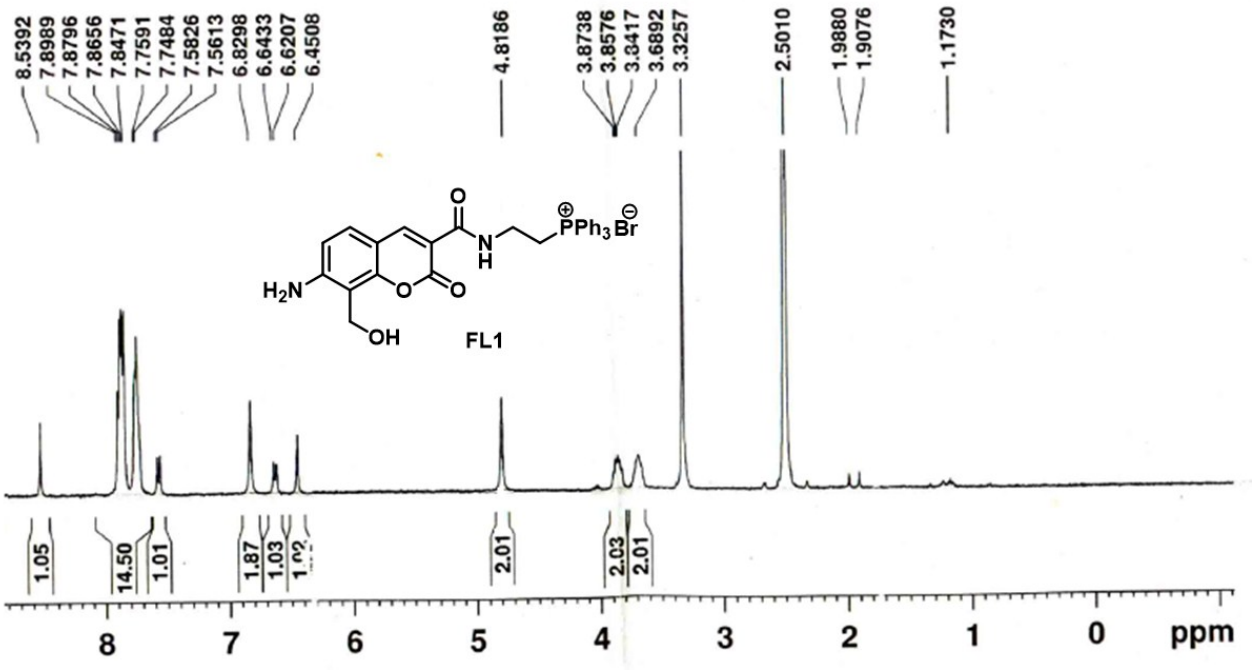


Fig. S22 ^{1}H-NMR of **FL1**after reaction of **MitoDP** with H_2S.

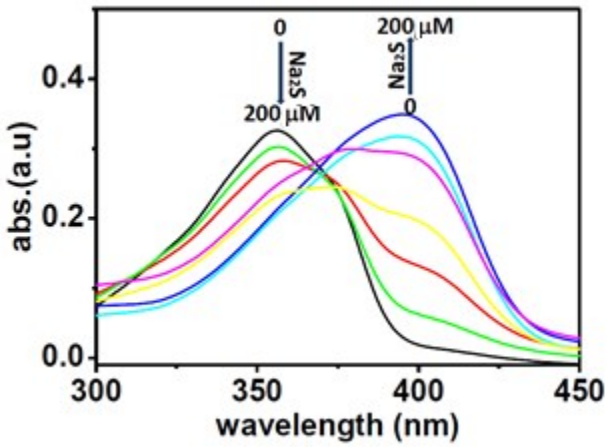


Fig. S23 UV-Vis. response of probe **MitoDP**.Change is absorption spectra of the probe **MitoDP** (5.0 µM) in PBS buffer (pH =7.4; 0.5% DMSO) recorded in the presence of Na_2S (0-200 µM). The spectra were recorded after incubation of the probe with Na_2S for 1 h.

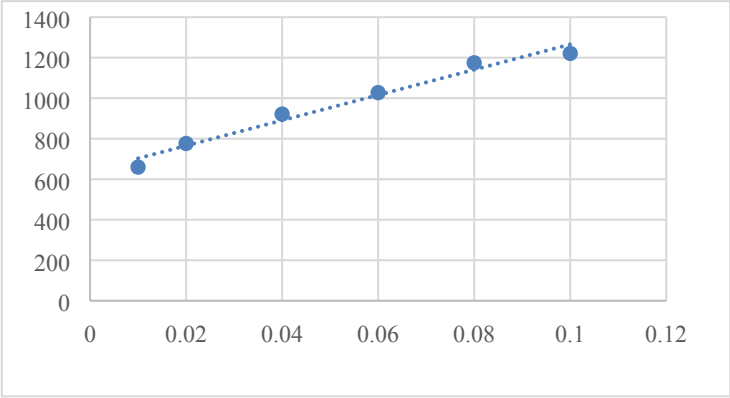


Fig. S24 Changes in fluorescence intensity of **MitoDP** (5.0 µM) at 450 nm against varied concentrations of $Na_2S$from 0 to 2 X 10^{-8} M in PBS buffer (pH =7.4; 0.5% DMSO) with the slit width 3/3 nm.

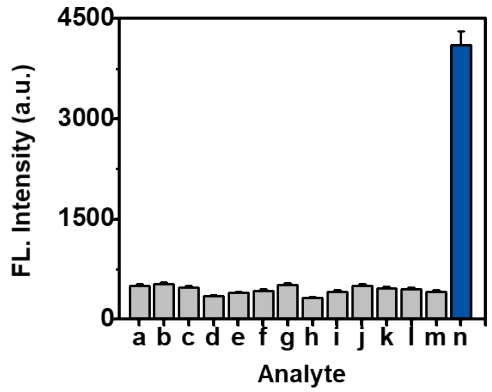


Fig.S25 The fluorescence responses of **MitoDP** (5.0 μM) to various relevant species (each analyte 200 μM); (a) cysteine (Cys), (b) H_2O_2, (c) $NaNO_2$, (d) $Cu(OAc)_2$, (e) $Zn(OAc)_2$, (f) $FeSO_4$, (g) $FeCl_3$, (h) Na_2CO_3, (i) glutathione (GSH) (5 mM), (j) ascorbic acid (AA), (k) NO, (l) Na_2S_2, (m) K_2S_5 and (n) Na_2S (200 μM) in PBS buffer (pH 7.4). All data were obtained after incubation for 30 min at 37 °C. Excitation effected at 395 nm and emission was collected at 450 nm with slit width set at 3 nm. Each bar represents the mean ± S.E. (0.03) of triplicate determinations from three independent experiments.

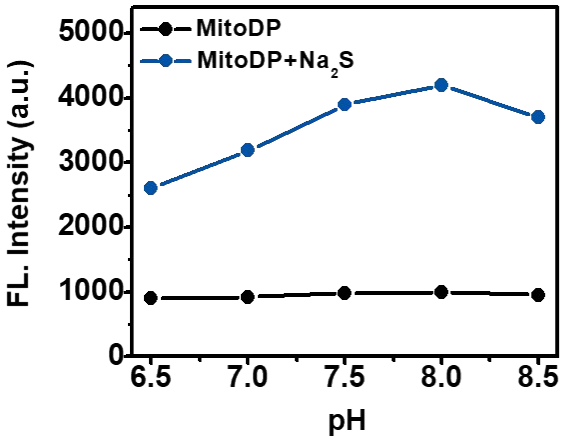


Fig. S26 The fluorescence changes of probe **MitoDP** (5.0 μM) in the absence and presence of Na_2S (200 μM) in a pH (6.5-8.5) ranges. Excitation effected at 395 nm and emission was collected at 450 nm with slit width set at 3 nm.

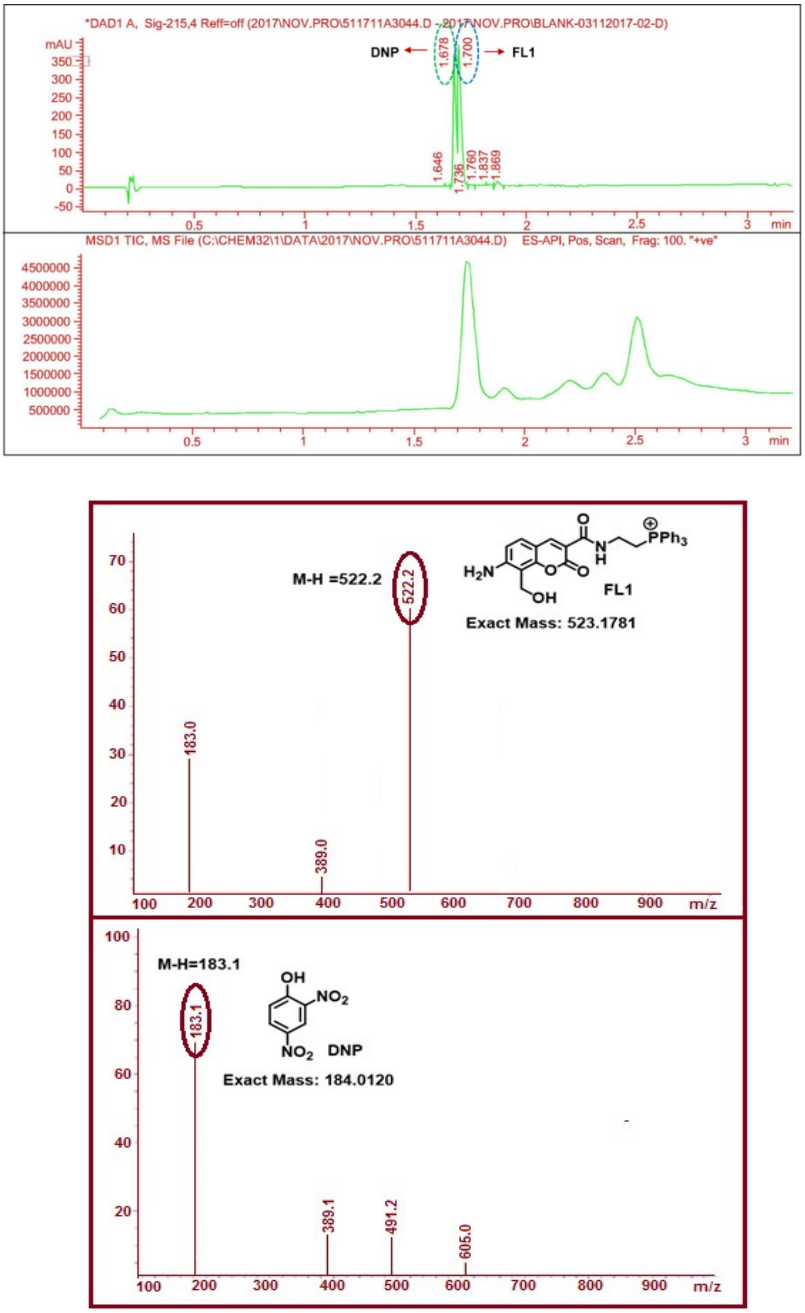


Fig. S27 LC-MS of **MitoDP** after treatment with H_2S (Na_2S).

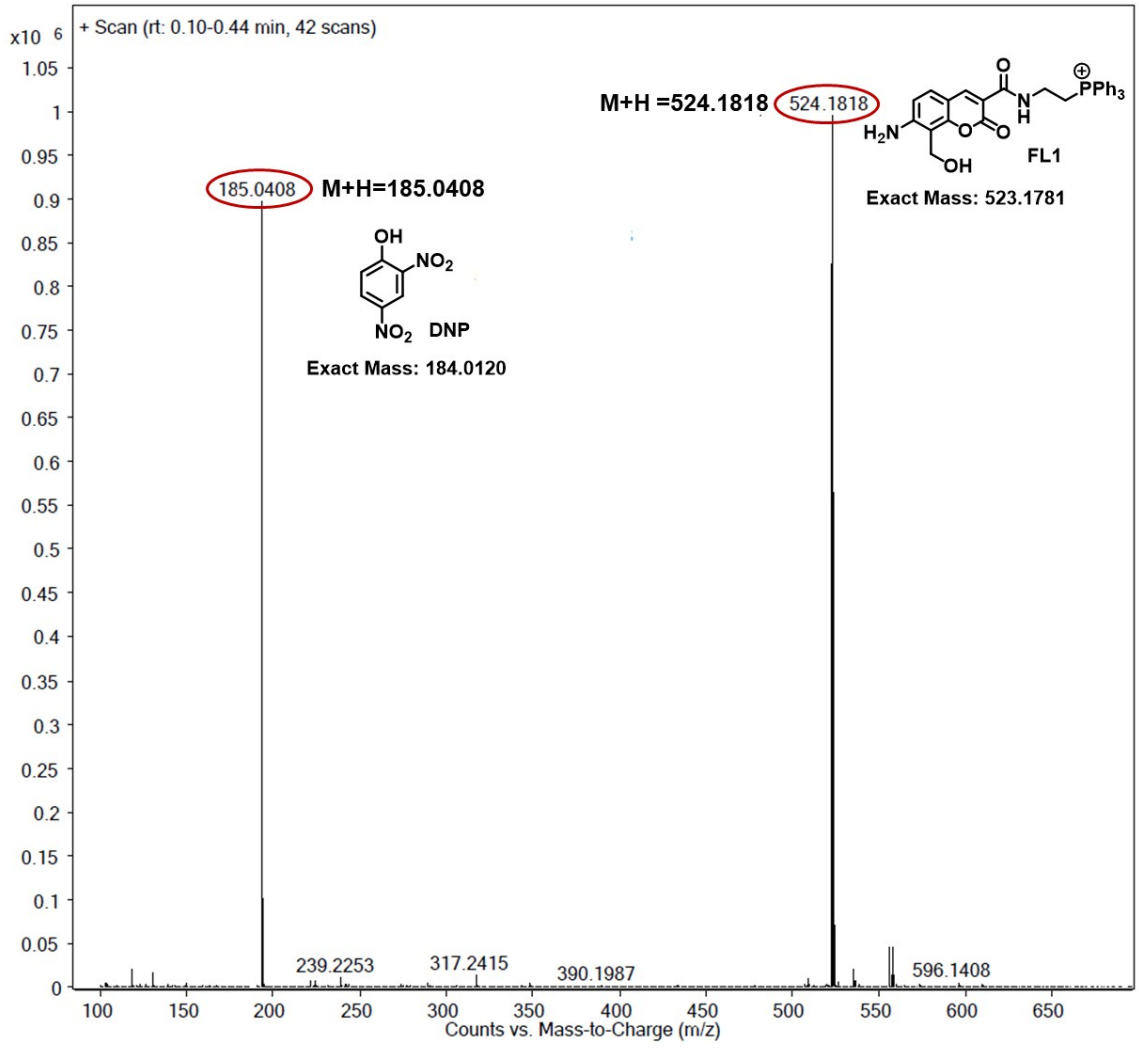
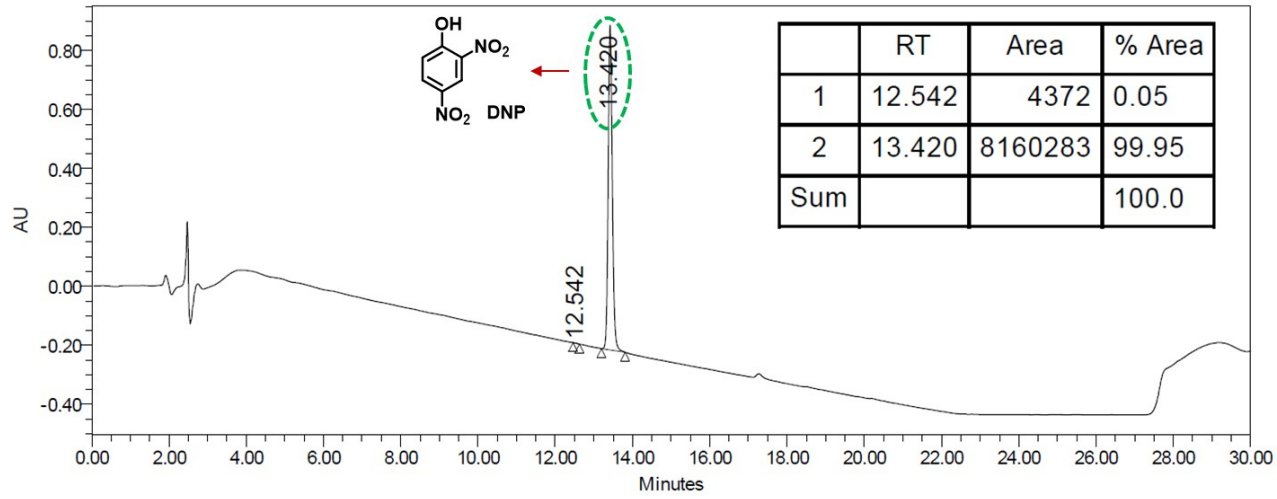
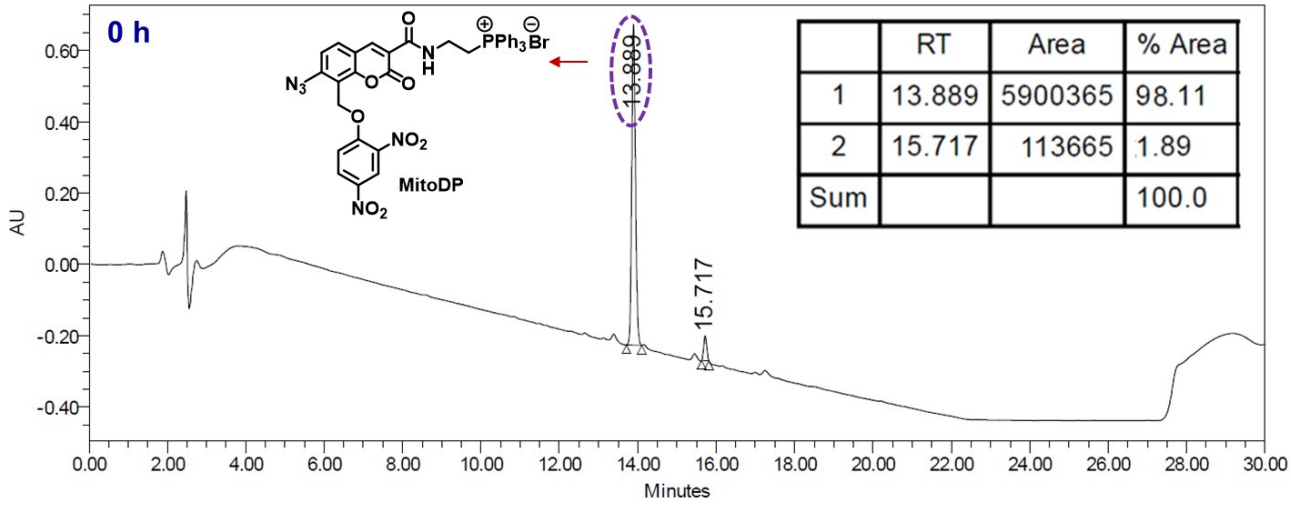


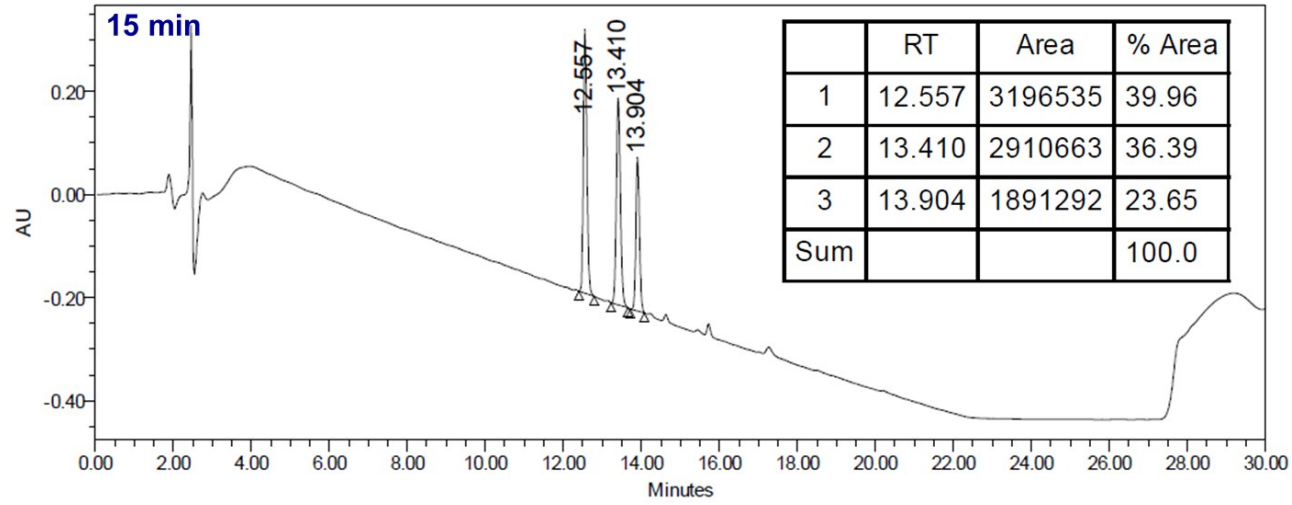
Fig. S28 HRMS of **MitoDP** after treatment with H_2S (Na_2S).



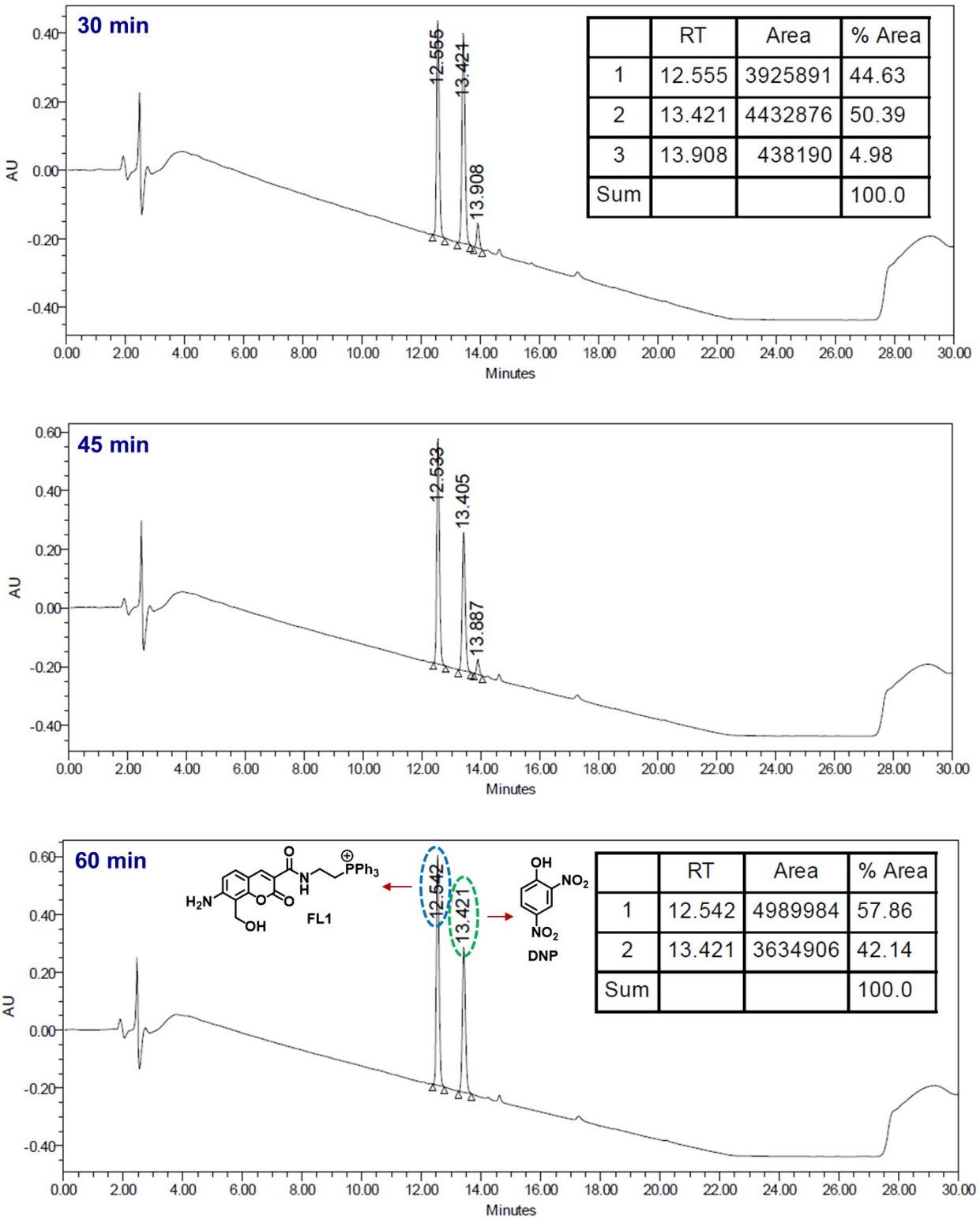
	RT	Area	% Area
1	12.542	4372	0.05
2	13.420	8160283	99.95
Sum			100.0



	RT	Area	% Area
1	13.889	5900365	98.11
2	15.717	113665	1.89
Sum			100.0



	RT	Area	% Area
1	12.557	3196535	39.96
2	13.410	2910663	36.39
3	13.904	1891292	23.65
Sum			100.0



	RT	Area	% Area
1	12.555	3925891	44.63
2	13.421	4432876	50.39
3	13.908	438190	4.98
Sum			100.0

	RT	Area	% Area
1	12.542	4989984	57.86
2	13.421	3634906	42.14
Sum			100.0

Fig. S29 HPLC of **MitoDP** after treatment with H_2S at different time intervals(0, 15, 30, 45 and 60 min).

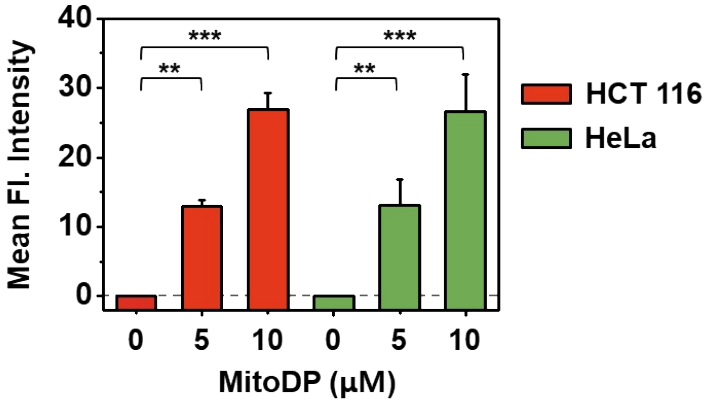


Fig.S30 Dose-dependent fluorescence intensity changes with **MitoDP** (0-10 μM) were calculated using ImageJ software. Each bar represents the mean ± S.E. of triplicate determinations from three independent experiments. ***P < 0.001, **P < 0.01(Two-way analysis of variance with Dunnett's multiple comparison post-test).

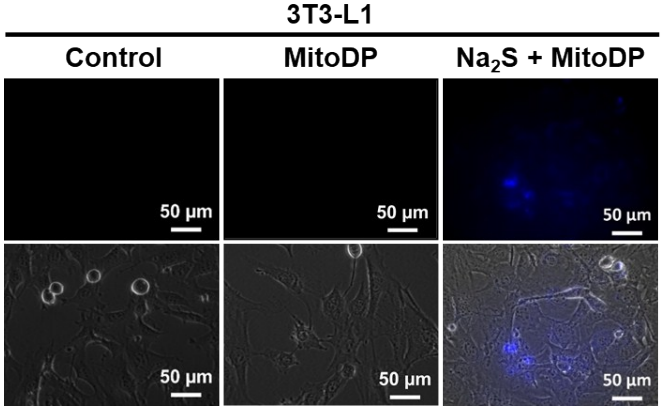


Fig. S31 Fluorescence images of **MitoDP** (0/10 μM) treated 3T3-L1 in the absence and presence of exogenous H_2S (Na_2S, 500 μM). Images were recorded using an excitation wavelength of 390 nm, and the emission monitored in the 440–500 nm range.

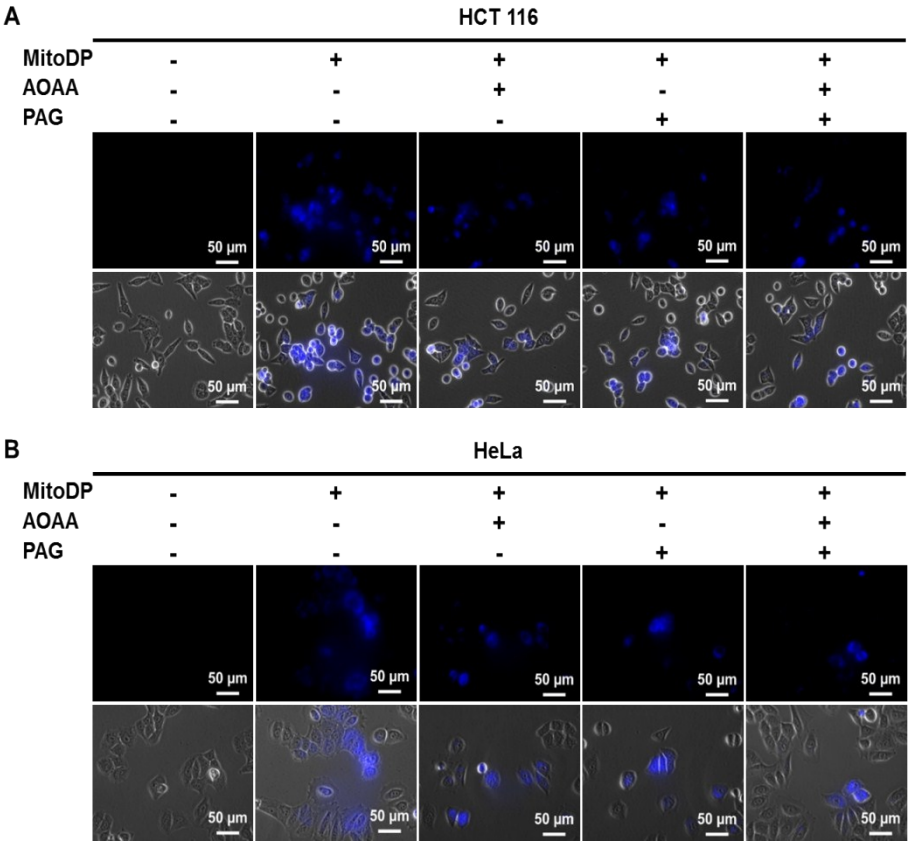


Fig. S32 Fluorescence images of HCT 116 cells (A) and HeLa cells (B) pretreated with AOAA (2 mM) and PAG (2 mM) for 1 h followed by incubation with **MitoDP** (5 µM) for 30 mins Upper panel is fluorescence images and lower panel is overlay of DIC images and corresponding fluorescence images. Images were recorded using an excitation wavelength of 390 nm, and the emission monitored in the 440–500 nm range. Scale bar, 50 µm

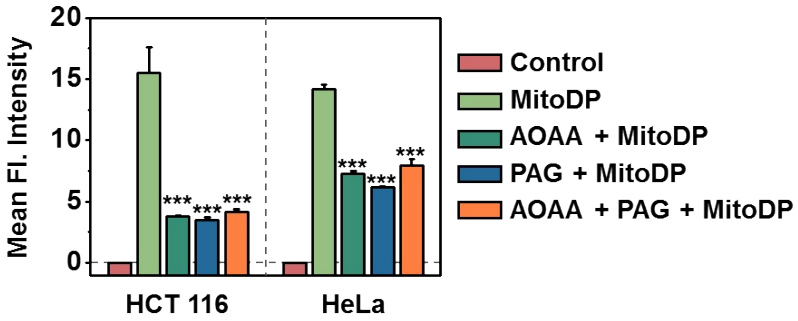


Fig.S33 Fluorescence intensity (recorded at λ_{em} 450 nm) in **MitoDP**(10 μM) pretreated cancer cells upon incubation without/with CBS/CSE inhibitors (AOAA/PAG) (2mM).Each bar represents the mean ± S.E. of triplicate determinations from three independent experiments. ***P < 0.001. (Two-way analysis of variance with Dunnett's Multiple Comparison Post-Test).

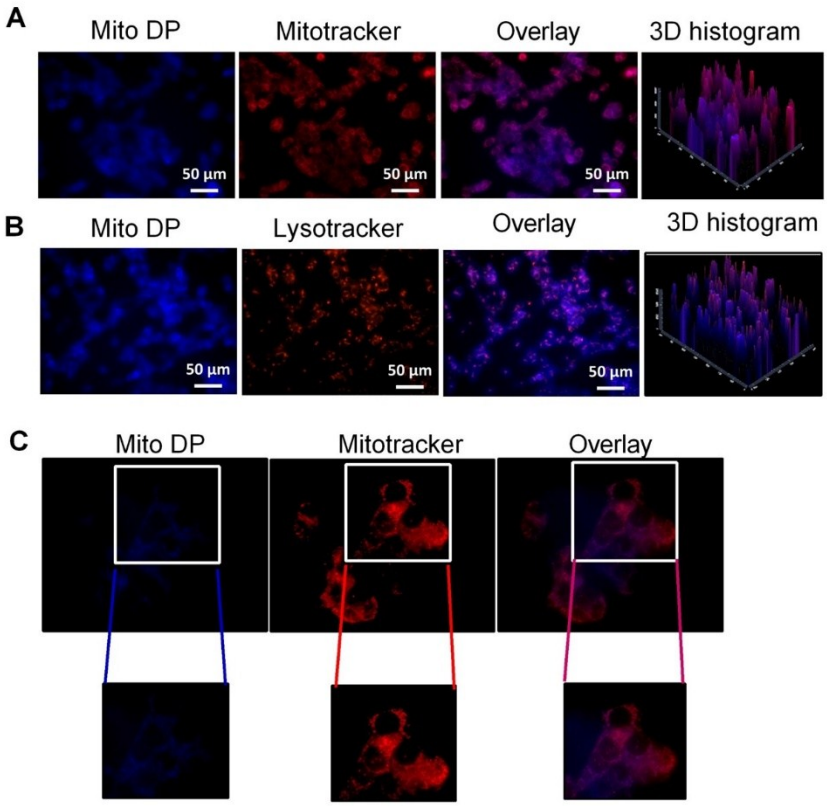


Fig. S34 Fluorescence and co-localization images of **MitoDP** with Mitotracker red (A) and Lysotracker red (B). HCT116 cells were treated with **MitoDP** (10 µM) and then stained with Mitotracker red (250 nM) and Lysotracker red (100nM). (C) High resolution images of **MitoDP** /Mitotrackertreated HCT116 cells. Images were collected at ex.390 nm/em 440–500 nm for blue channel and ex. 510 nm/em. 580 nm for the red channel.

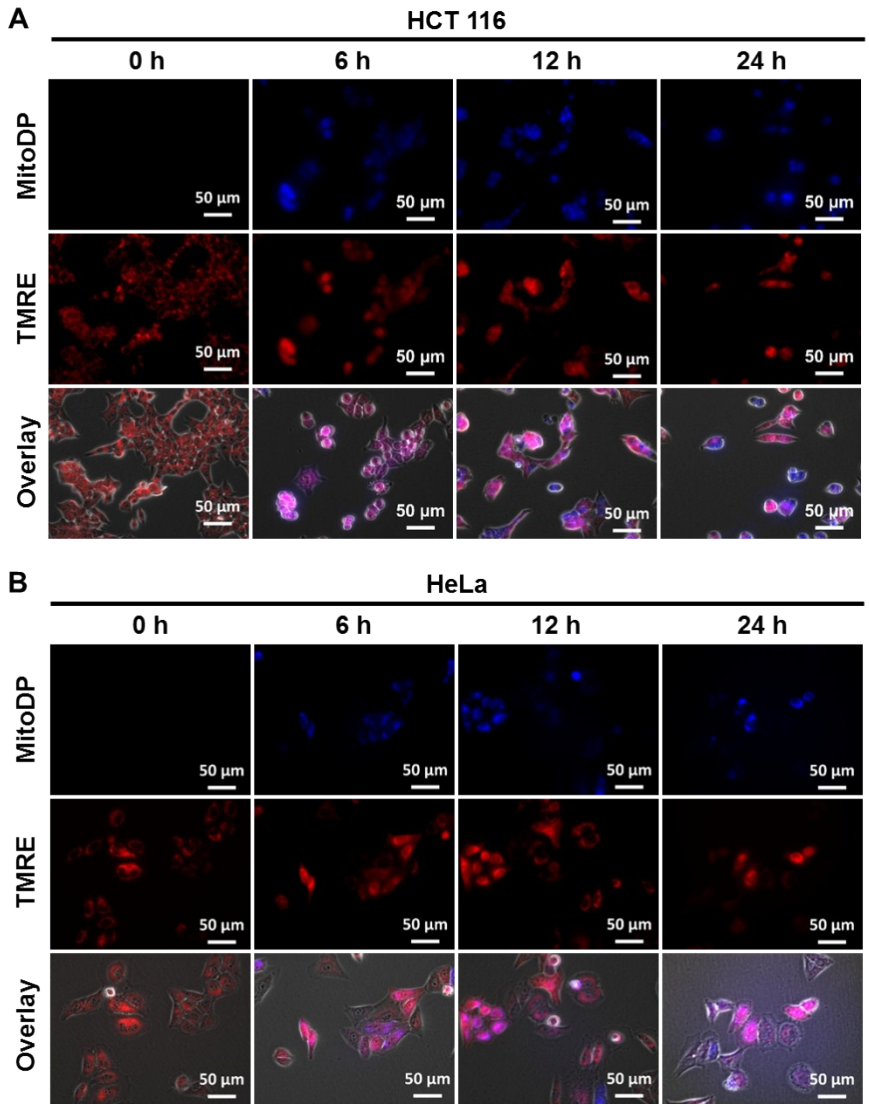


Fig. S35 Time-dependent measurement of mitochondrial membrane potential changes through fluorescence imaging of TMRE in **MitoDP** pretreated HCT116 cells (A), HeLa cells (B) with time (0- 24h)

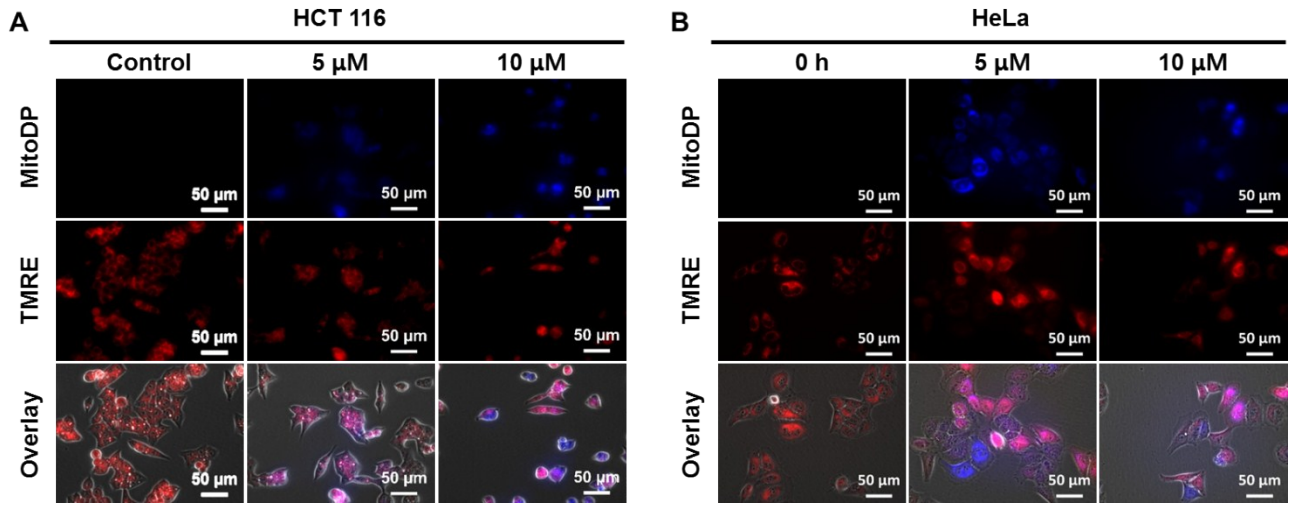


Fig. S36 Dose-dependent measurement of mitochondrial membrane potential changes in **MitoDP**(10 μM)treated HCT116 cells (A), HeLa cells (B) using TMRE(100 nM). The images were collected at ex.390 nm/em. 440–500 nm for blue channel and ex. 540 nm/em. 580 nm for red channel (TMRE).

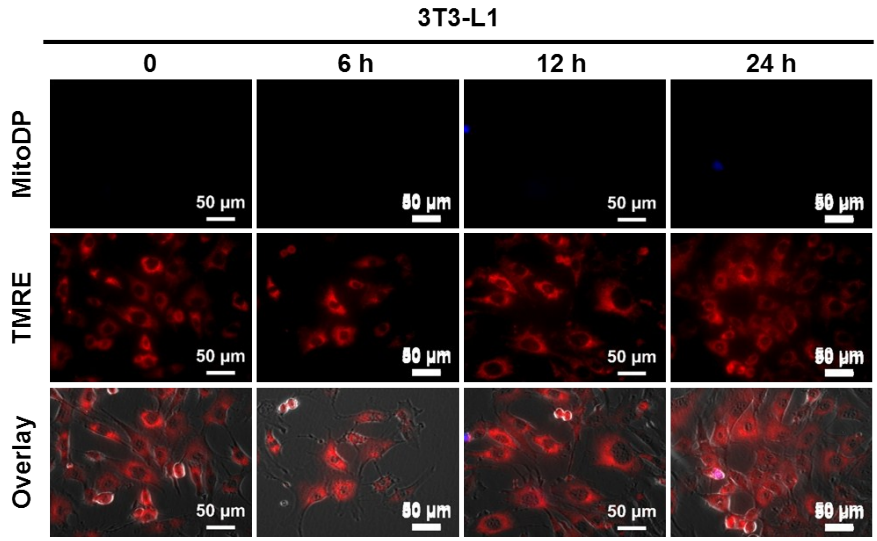


Fig. S37 Time-dependent measurement of mitochondrial membrane potential changes through fluorescence imaging with TMRE(100 nM) in **MitoDP**(10 μM)pretreated 3T3-L1 cells. The images were collected at ex.390 nm/em. 440–500 nm for blue channel and ex. 540 nm/em. 580 nm for red channel (TMRE).

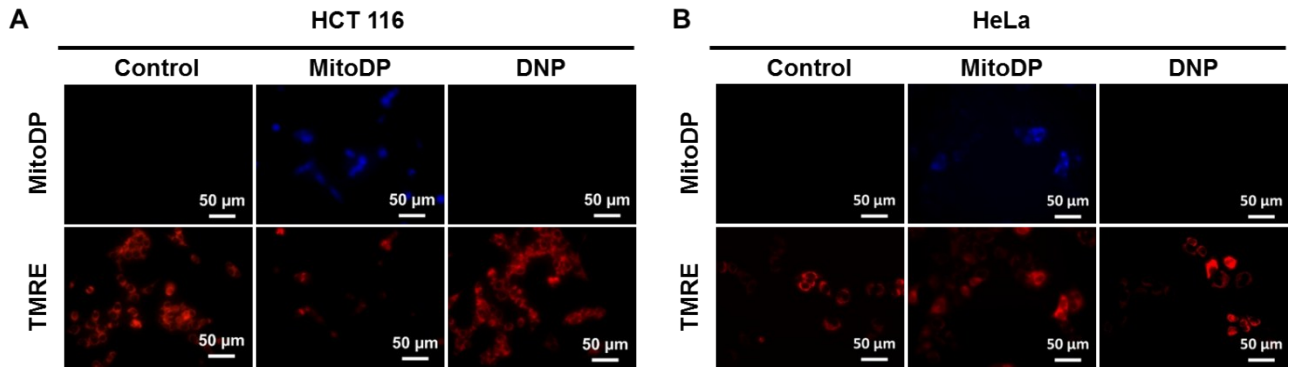


Fig. S38 The extent of depolarization of mitochondria as measured by CLSM images of TMRE (100 nM)in **MitoDP**(10 µM)/DNP (10 µM)treated HCT116 (A) and HeLa cells (B) after 24h.The images were collected at ex.390 nm/em. 440–500 nm for blue channel and ex. 540 nm/em. 580 nm for red channel (TMRE).

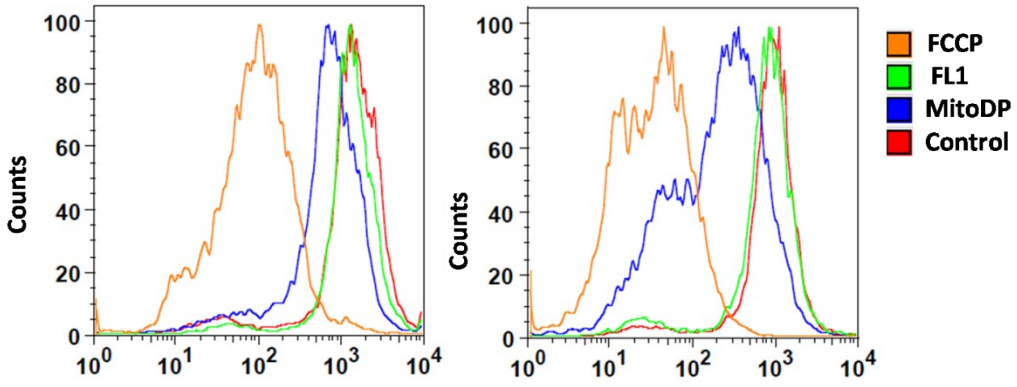


Fig. S39. Flow cytometryic analysis of TMRE stained HTCT116 cellstreated with FCCP, **MitoDP** and **FL1**separately at 12h (left) and 24 h (right).

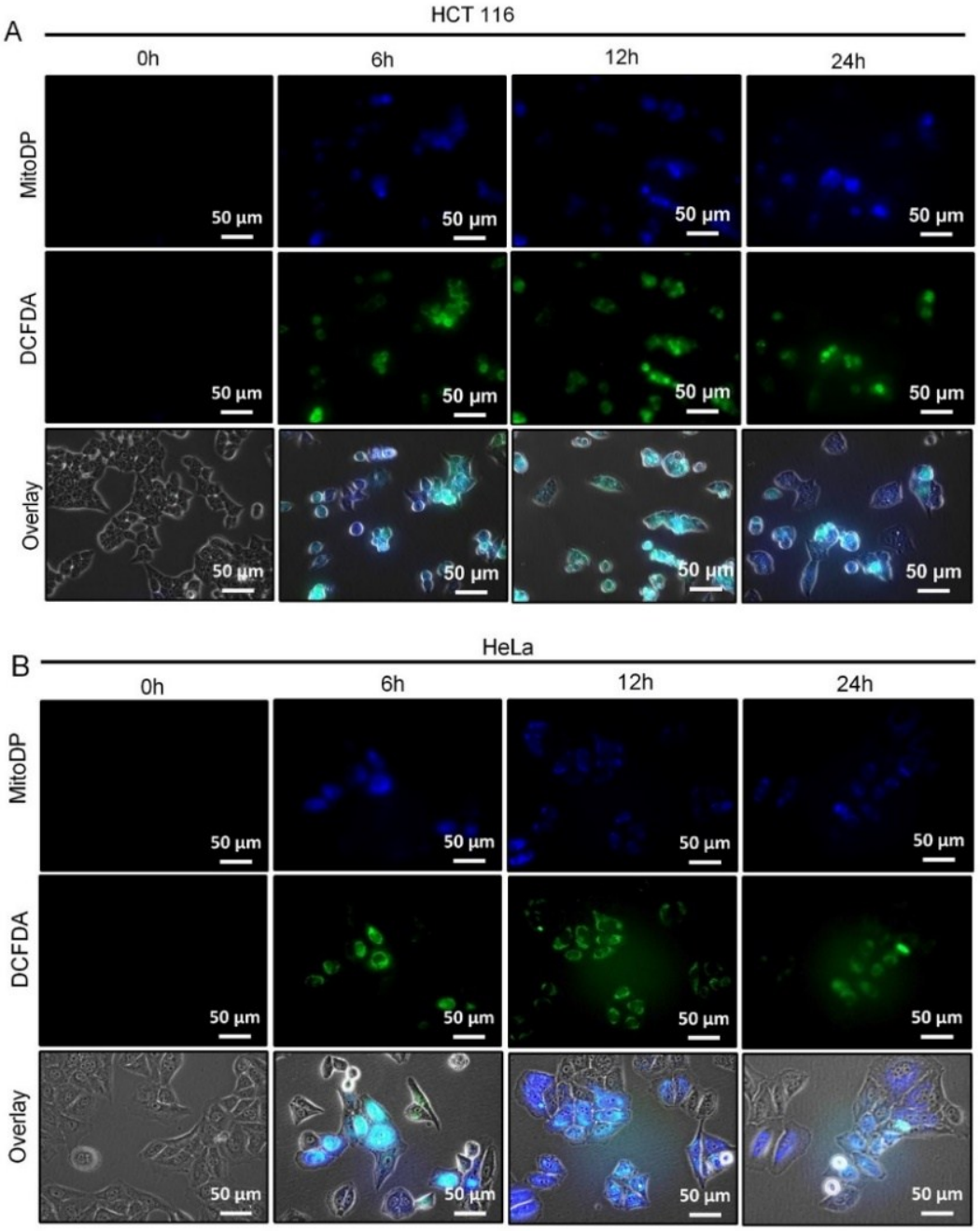


Fig. S40 Time-dependent measurement of ROS formation in mitochondria through fluorescence imaging of DCFDA in **MitoDP** pretreated HCT116 cells (A)and HeLa cells (B) with time (0- 24 h).

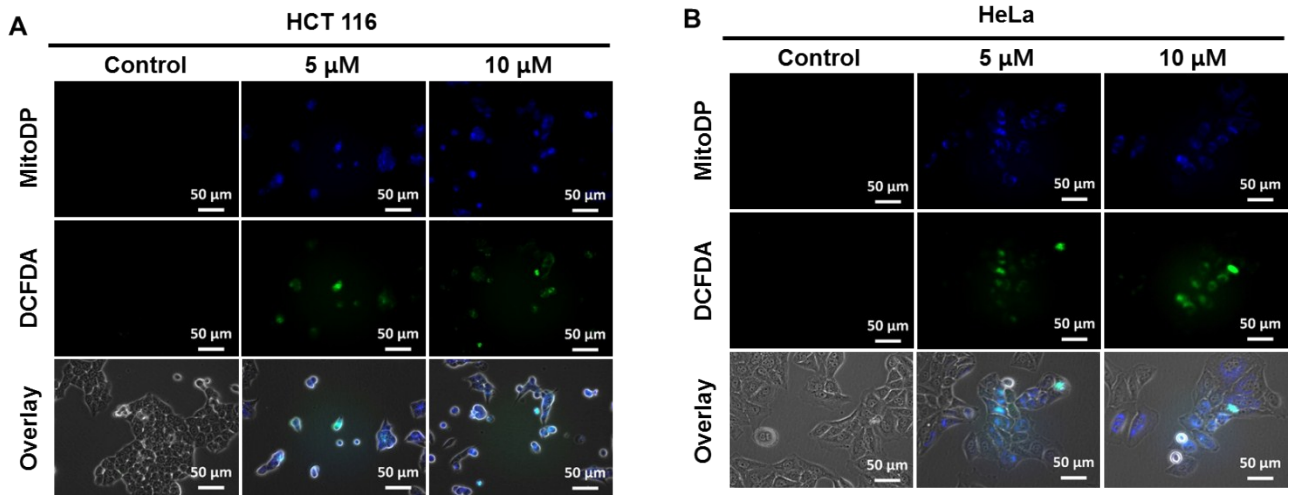


Fig. S41 MitoDP(0-10 µM)dose-dependent measurement of ROS production in treated HCT116 cells (A), HeLa cells (B) using DCDFA (5 µM) stain. The images were collected at ex.390 nm/em. 440–500 nm for blue channel and ex. 510 nm/em. 540 nm for green channel (DCFDA).

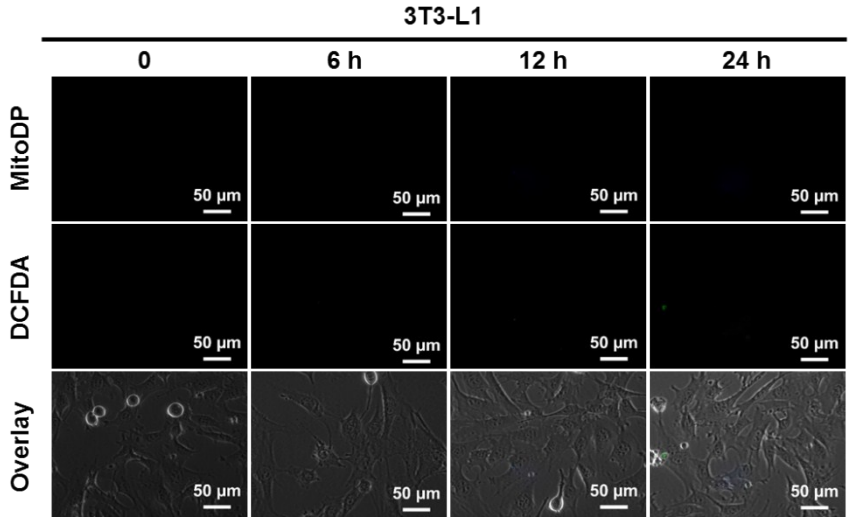


Fig. S42 Time-dependent measurement of ROS formation through fluorescence imaging with DCFDA (5 µM) in **MitoDP**(10 µM)pretreated 3T3-L1 cells. The images were collected at ex.390 nm/em. 440–500 nm for blue channel and ex. 510 nm/em. 540 nm for green channel (DCFDA).

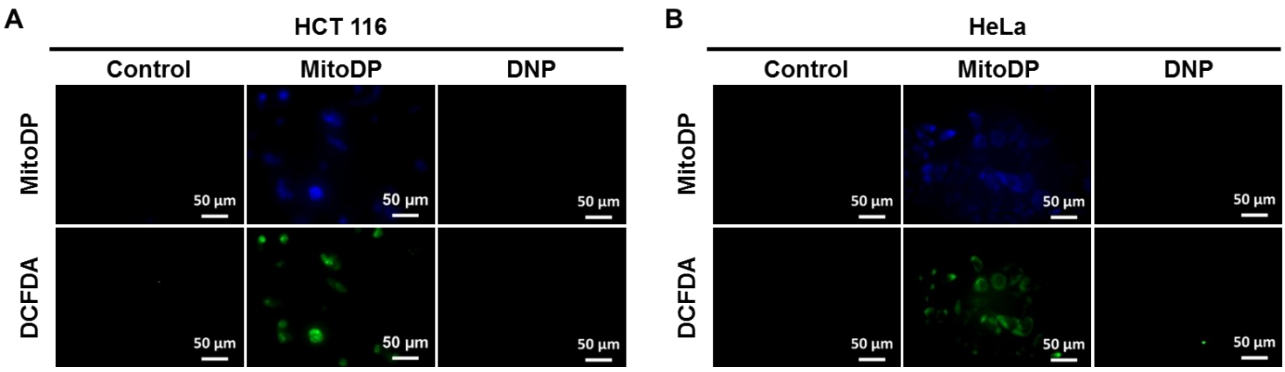


Fig.S43 The extent of ROS formation in **MitoDP**/DNP treated HCT116 (A) and HeLa cells (B) after 24h. The images were collected at ex.390 nm/em. 440–500 nm for blue channel and ex. 510 nm/em. 540 nm for green channel.

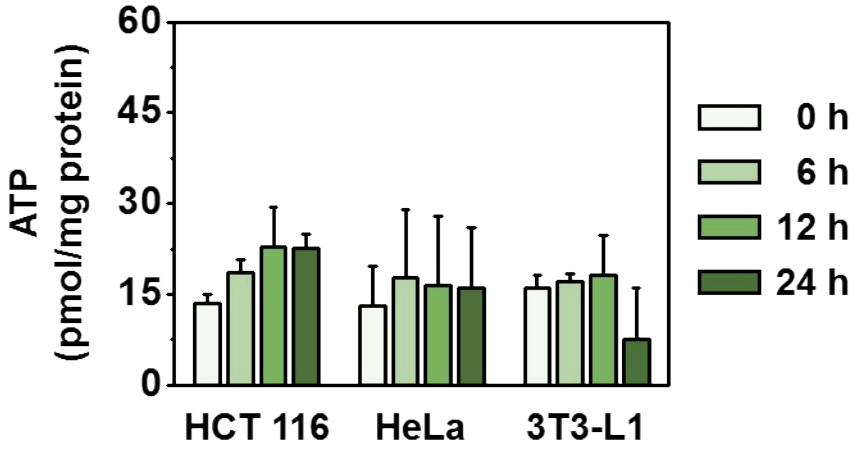


Fig. S44 Time-dependentATP levels measurement in DNP treated HCT116, HeLa, and 3T3-L1 cells (0-24 h).

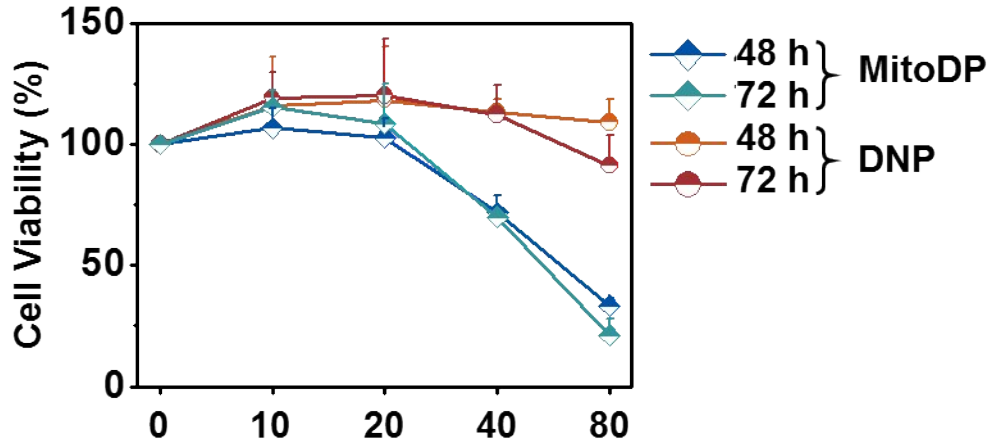


Fig. S45 Time and dose-dependent MTT Assay of HeLa cells treated with **MitoDP** and DNP for 48 and 72 h.

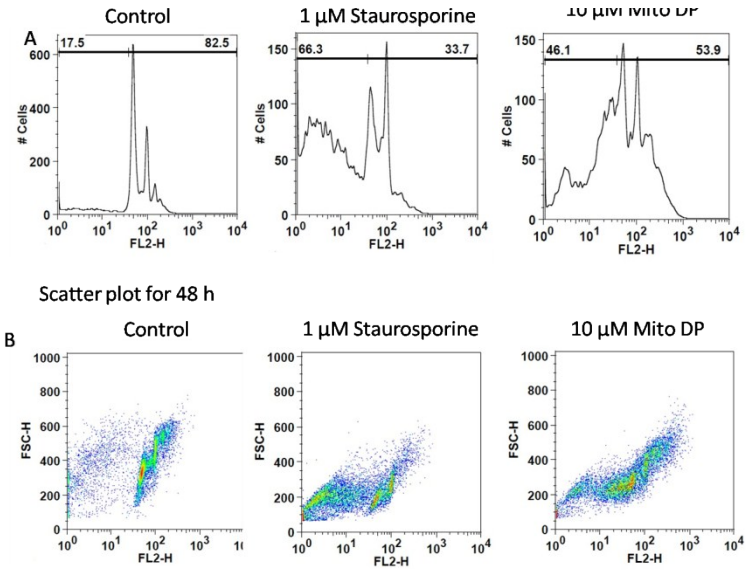


Fig S46. Analysis of apoptosis by Propidium staining and flow cytometry in HCT116. A) Histogram plot for the DNA analyzis of cells treated with 10 μM **MitoDP** and 1 μM Staurosporine for 48 h. B) Dot plot analysis of the same.