Electronic Supplementary Material (ESI) for RSC Advances.


# Supporting Information

## Intra-Mitochondrial Reaction for Cancer Cell Imaging and Anti-Cancer Therapy by Aggregation-Induced Emission

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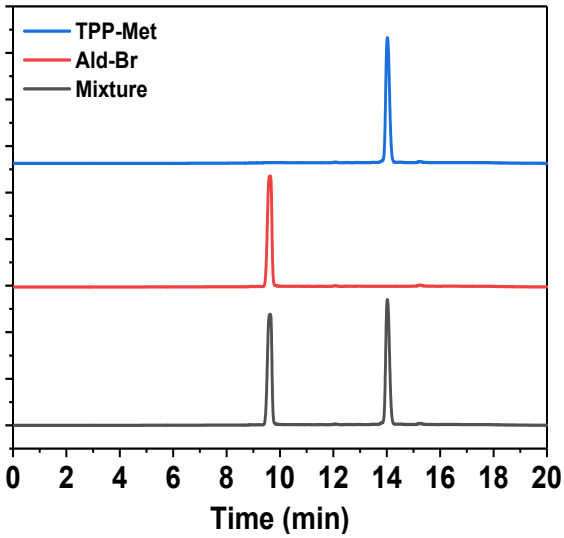



**Figure S1**. HPLC trace of 1mM TPP-Met, Ald-Br and the mixture of 1 mM TPP-Met and Ald-Br. The molecules were dissolved in pH 8.0 PBS and the intensity of the mixture was checked by using HPLC after stirring for 24h.

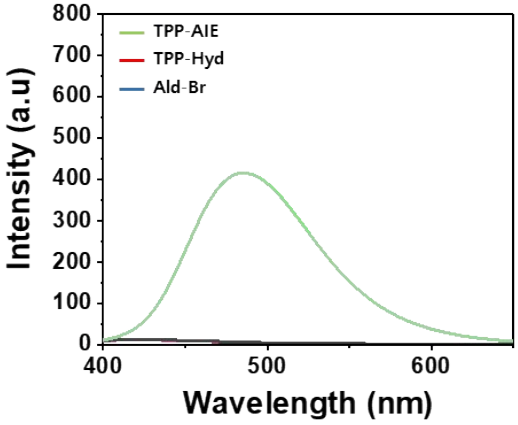



**Figure S2**. The fluorescence spectra of TPP-Hyd, Ald-Br and after stirring for 24 h in pH 8.0 PBS. The appearance of fluorescence was recorded in the mixture of 1 mM TPP-Hyd and Ald-Br, called TPP-AIE.

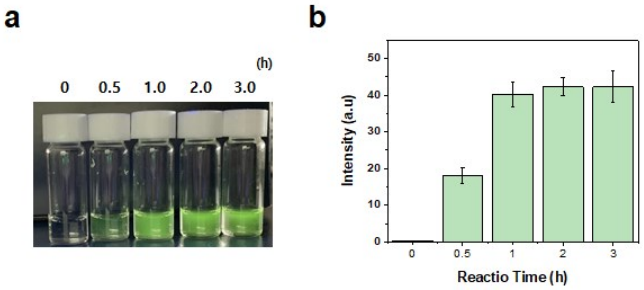



**Figure S3**. Time dependent carbonyl ligation was observed to form TPP-AIE. a) Optical image of the mixture of 0.5 mM TPP-Hyd and Ald-Br for showing appearance of fluorescence with 365 nm irradiation. b) Quantificatio of TPP-AIE in the mixture solution. The intensity was observed at 520 nm following 488 nm excitation.

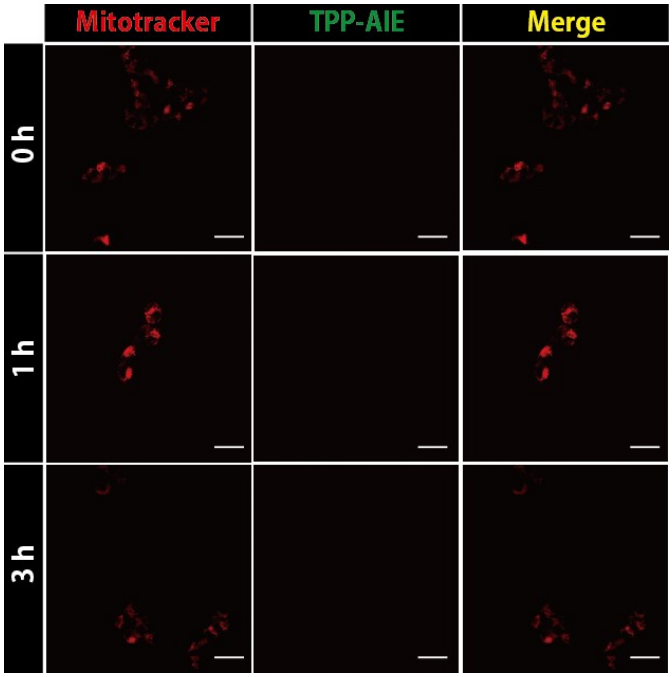



**Figure S4**. Flourescence image of intra-mitochondrial carbonyl ligation between TPP-Hyd and Ald-Br. The HEK293 cells were incubated with 20 μM TPP-Hyd and Ald-Br. Mitotracker Red was used to label mitochondira. The Scale bar for all images = 30 μm

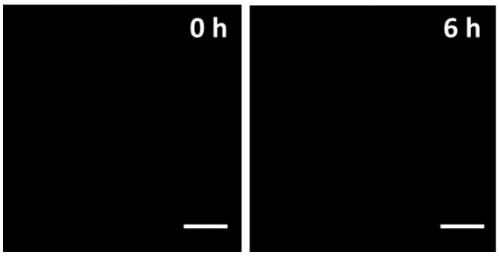



**Figure S5.** CLSM image in HeLa cells treated with 20 μM TPP-Met and Ald-Br for 4h.

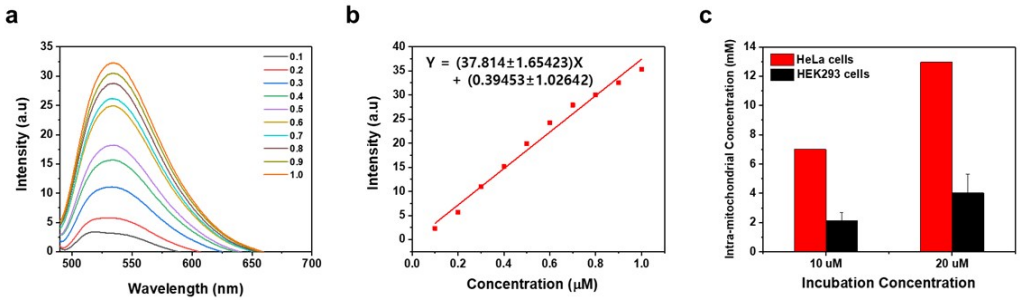



**Figure S6.** a) Emission spectra of TPP-AIE in pH 8.0 PBS solution monitored for calibration plot. b) Calibration plot of TPP-AIE from the emission spectra. c) Intra-mitochondrial concentration of TPP-AIE after 6 h treatment with TPP-Hyd and Ald-br against HeLa cells and HEK293 cells. Data represent mean ± s.d from three independent experiments.

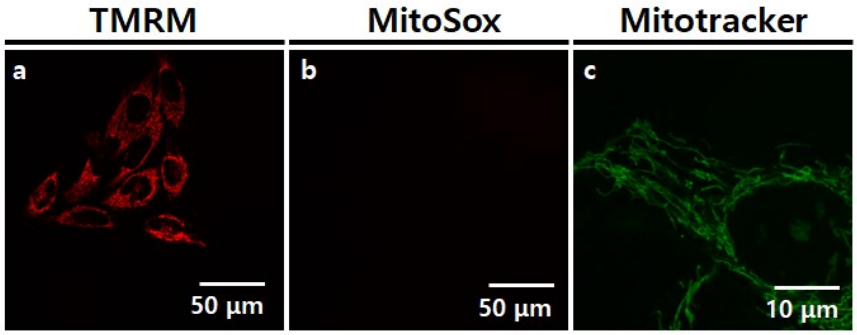



**Figure S7.** Confocal microscopy image of showing healthy mitochondrial condition in HeLa cells labeled a) TMRM , b) MitoSOX, and c) Mitotracker. The HeLa cells were incubated with 20 μM TPP-Met and Ald-Br. The Scale bar for all images = 30 μm

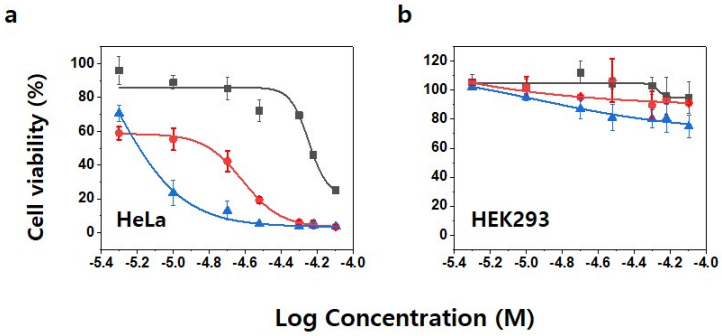



**Figure S8.** Cellular viability of a) HeLa cells and b) HEK293 cells treated with different concentrations of TPP-Hyd and Ald-Br. (Black line : 12h incubation, Red line : 24 h incubation, Blue line : 48 h incubation)

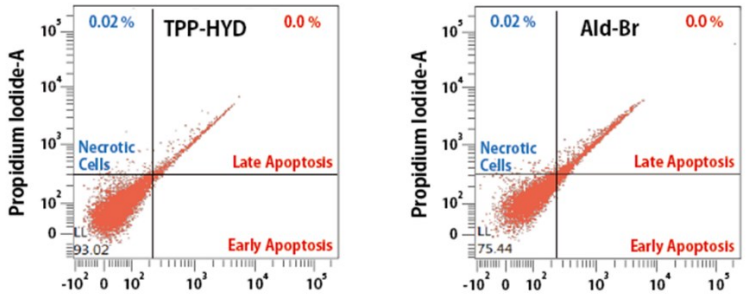



**Figure S9.** FACS analysis against HeLa cells treated with 20 μM TPP-HYD and Ald-Br individually for 12h. Annexin V-FITC and PI were used to monitor apoptosis of the cells.

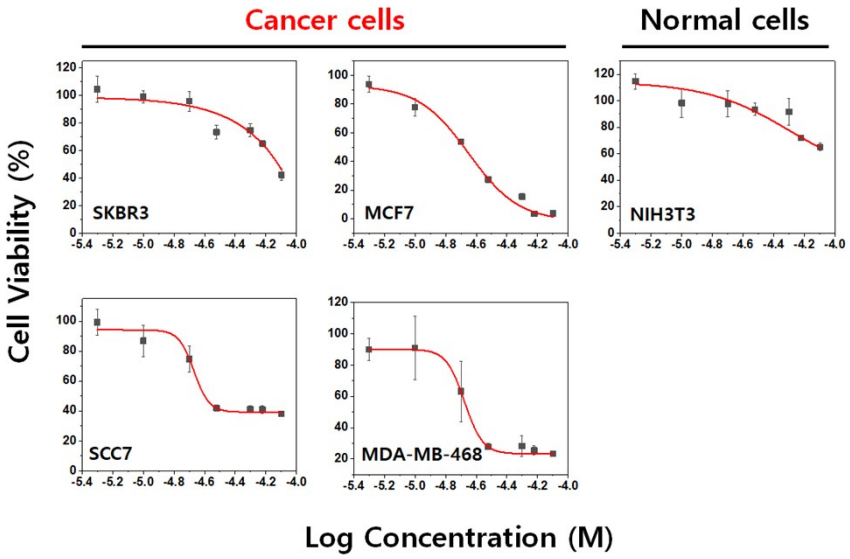
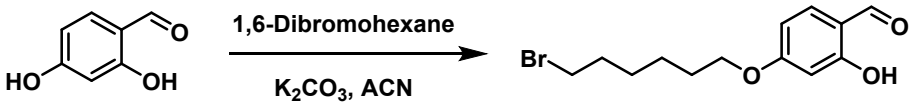



**Figure S10.** Cellular viability of cancer cell lines (SKBR3, MCF7, SCC7, MDA-MB-468) and normal cell line (NIH3T3) treated with different concentrations of TPP-Hyd and Ald-Br after 24 h incubation.

**Synthesis of materials**

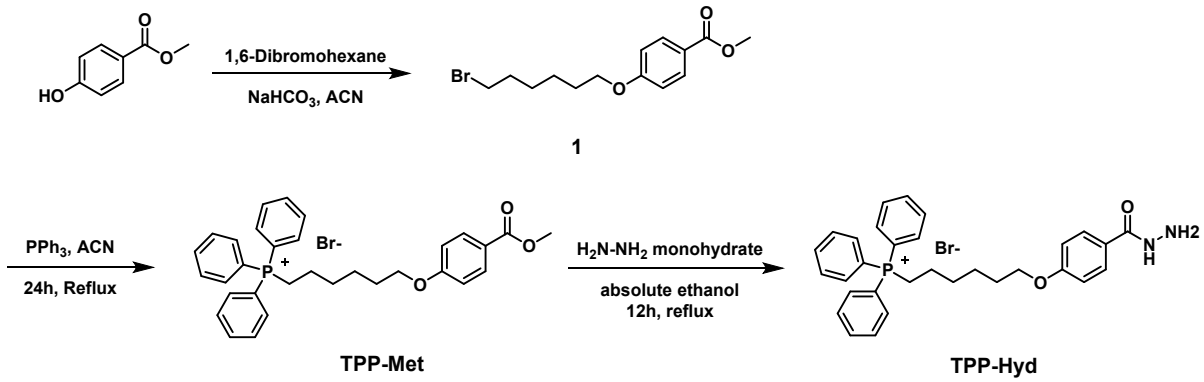



**Ald-Br**

**Ald-Br** 2,4-dihydroxy benzaldehyde (14.5 mmol, 2 g) and potassium carbonate (14.5 mmol, 1.22 g) were suspended in Acetonitrile 15 mL after that, 1,6-dibromohexane (14.5 mmol, 2.23 mL) were added. The reaction mixture was stirred for 12h at 60°C with TLC monitoring. After completion of the reaction, the Acetonitrile was distilled in vacuum. Then the mixture was extracted in $CH_2Cl_2/H_2O$ system for 3 times. The organic phase was dried with anhydrous $MgSO_4$ and then distilled in vacuum for crude product. The crude product was purified through

column chromatography (Hex/EA = 4:1 as the eluent) to obtain 1.12 g light yellow liquid compound **1** (3.7187 mmol, 25.6 % yield)

$^1$H NMR (400 MHz, $CDCl_3$): δ 11.43 (s, 1H), 9.65 (s, 1H), 7.37 (d, 1H), 6.48 (d, 1H), 6.35 (s, 1H), 3.96 (t, 2H), 3.37(s, 3H), 1.81 (m, 4H), 144 (m, 4H). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 194.28, 166.30, 164.48, 135.21, 115.05, 108.69, 101.05, 76.90, 68.29, 33.68, 32.49, 27.28, 25.17. ESI (M/Z) : 301.24




**Compound 1.** Methyl-4-hydroxybenzoate (6 mmol, 912.9 mg), 1,6-dibromohexane (6 mmol, 0.923 ml) and sodium bicarbonate (6 mmol, 504.06 mg) were dissolved in acetonitrile 10 mL. after that, the reaction mixture were stirred at 80$^o$C for 24h. after completion of the reaction, the acetonitrile was distilled in vacuum, and then the mixture was extracted in $CH_2Cl_2/H_2O$ system for 3 times. The organic phase was dried with anhydrous $MgSO_4$ and then distilled in vacuum for crude product. The crude product was purified through column chromatography (Hex/EA = 4:1 as the eluent) to obtain 663.7 mg white solid compound **3** (3.1 mmol, 35 % yield)

$^1$H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, 2H), 6.89 (d, 2H), 4.00 (t, 2H), 3.87 (s, 3H), 3.41(td 2H), 1.85 (m, 4H), 1.49 (m, 4H).

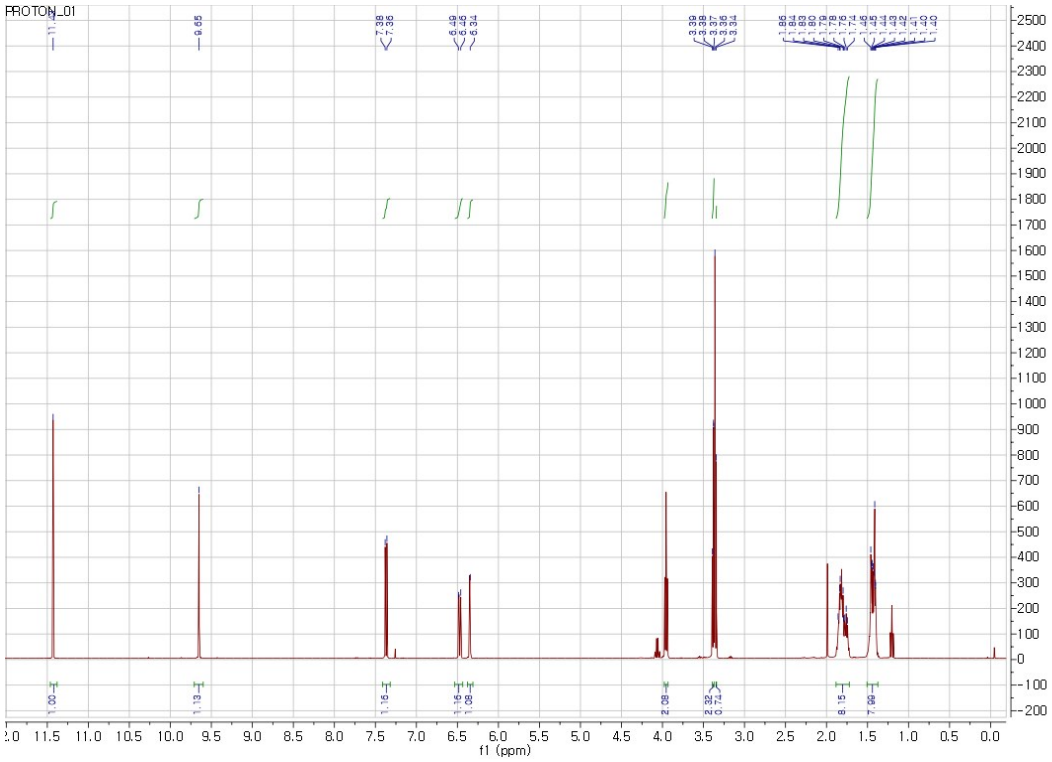
**TPP-Met.** Triphenylphosphine (4.755 mmol, 1.247 g) was added in **compound 3** (3.17 mmol, 1 g) in Acetonitrile 40 mL. after that, reaction mixture was stirred for 24h under the reflux condition with TLC monitoring. After completion of the reaction, the acetonitrile was distilled in vacuum. Then the mixture was purified through column chromatography ($CH_2Cl_2$/EA/MeOH = 5:5:1 as the eluent) to obtain 535.3 mg clear sticky liquid compound **4** (1.07 mmol, 34 % yield)

$^1$H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, 2H), 7.63-7.87 (m, 15H), 6.89 (d, 2H), 4.00 (t, 2H), 3.87 (s, 3H), 3.41(td 2H), 1.85 (m, 4H), 1.49 (m, 4H). ESI (M/Z) : 497.10

**TPP-Hyd.** Hydrazine monohydrate excess (8 mmol, 0.388 mL) was added in **compound 4** (0.4 mmol, 200 mg) in methanol 10 ml. after that, reaction mixture was stirred for 12h under the reflux condition with TLC monitoring. After completion of the reaction, the methanol was distilled in vacuum. Then the mixture was purified through column chromatography ($CH_2Cl_2$/EA/MeOH = 5:5:1 as the eluent) to obtain mg clear sticky liquid compound **5** (mmol, yield)

$^{1}$H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, 2H), 7.64-7.82 (m, 15H), 6.8 (d, 2H), 4.00 (t, 2H), 1.89 (t, 2H), 1.87 (t, 2H), 1.85 (t, 2H), 1.43 (t, 2H). $^{13}$C NMR (400 MHz, $CDCl_3$): δ 144.09, 129.91, 127.78, 125.77, 35.45, 28.32. ESI (M/Z) : 497.42

All materials were checked by $^{1}$H NMR spectroscopy analysis (UNIST Center Research Facilities, Agilent 400 MR-DD2 NMR spectroscopy, Republic of Korea).



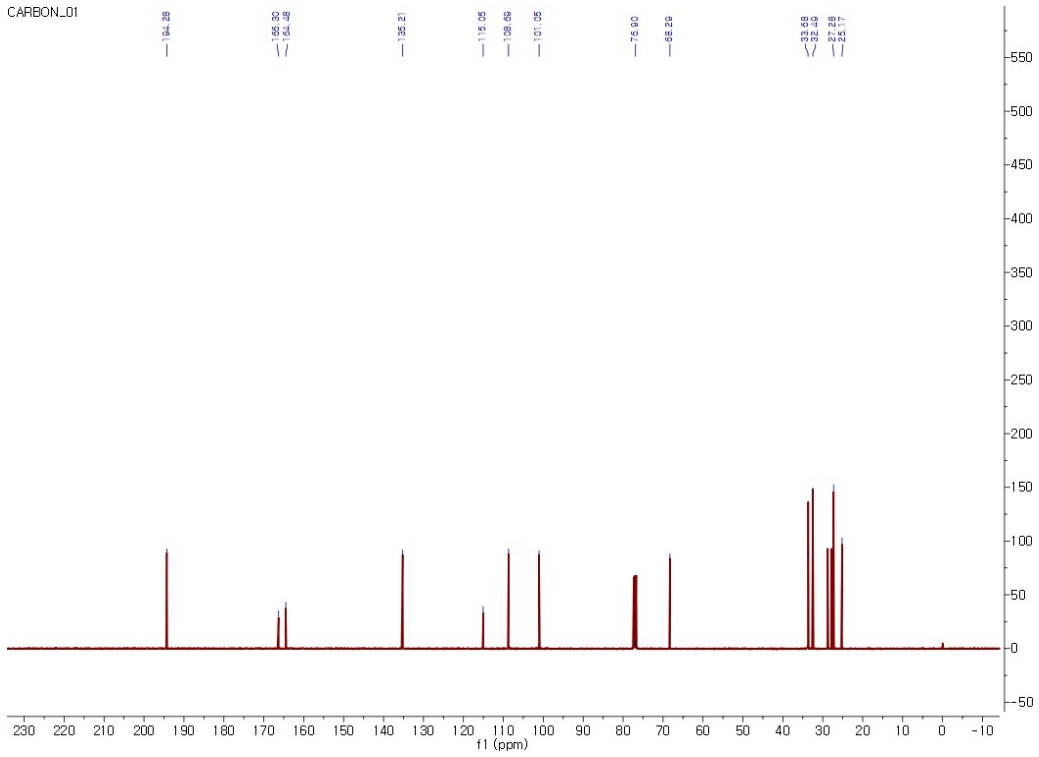



**Figure S11**. $^{1}H$ NMR and $^{13}C$ NMR spectra of Br-ALD

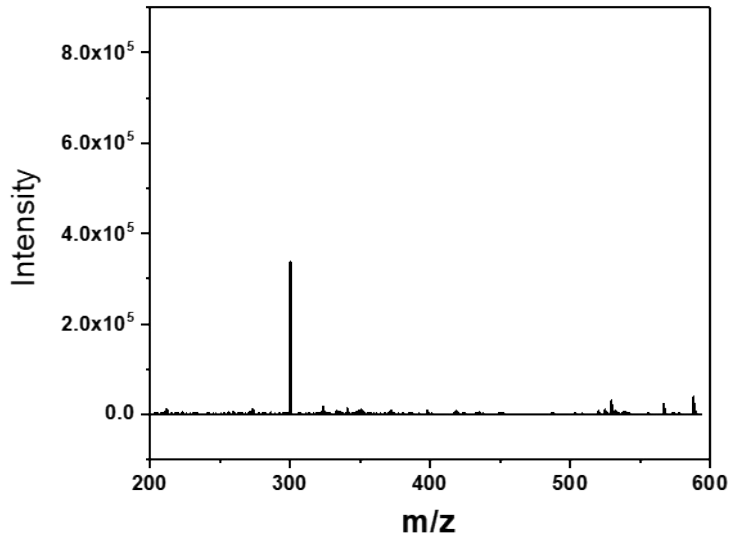



**Figure S12.** ESI analysis of Br-ALD

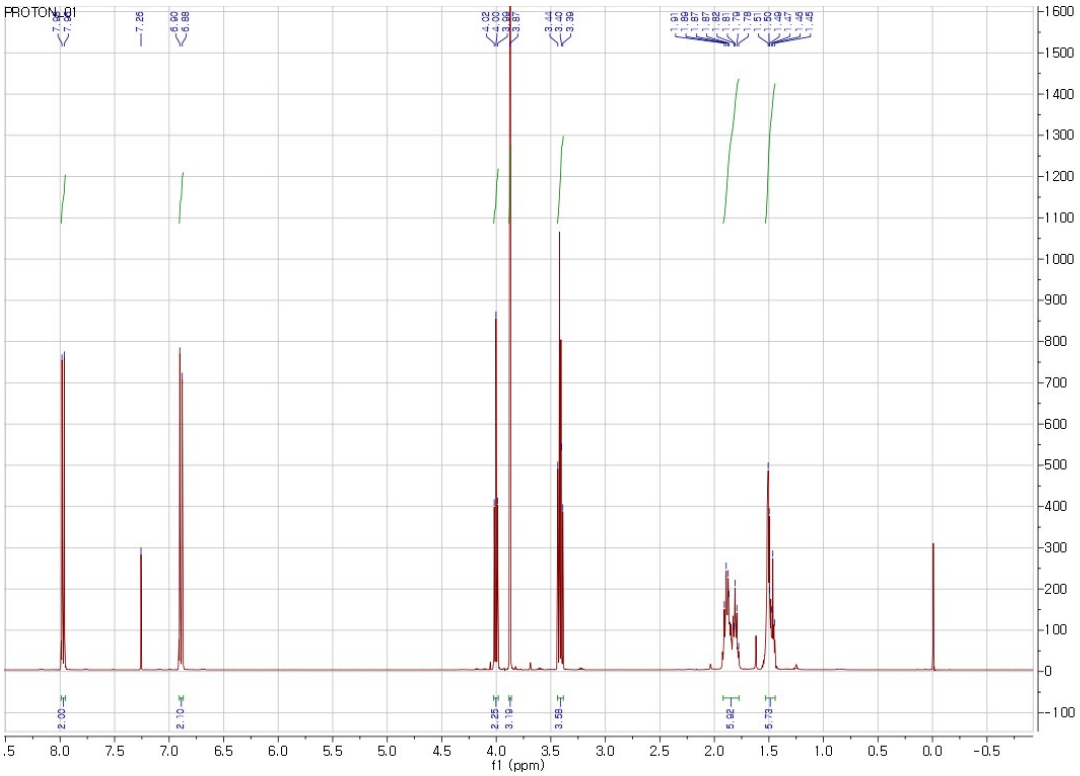



**Figure S13.** $^{1}H$ NMR spectra of Compound 1

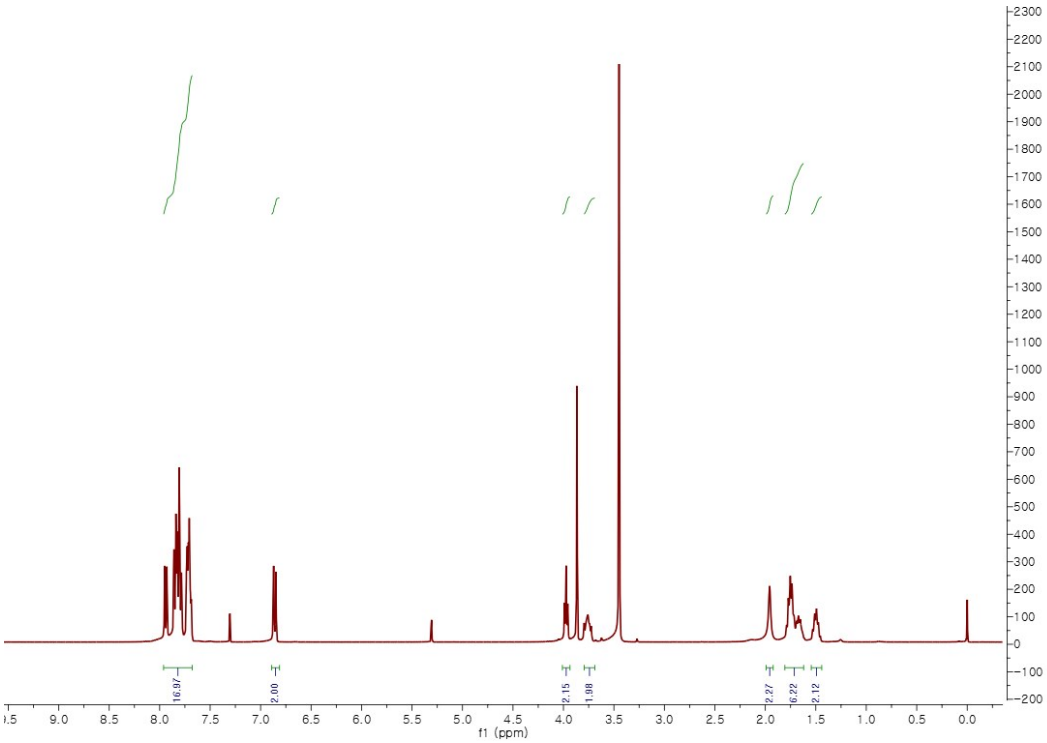



**Figure S14.** $^{1}H$ NMR and $^{13}C$ NMR spectra of TPP-Met

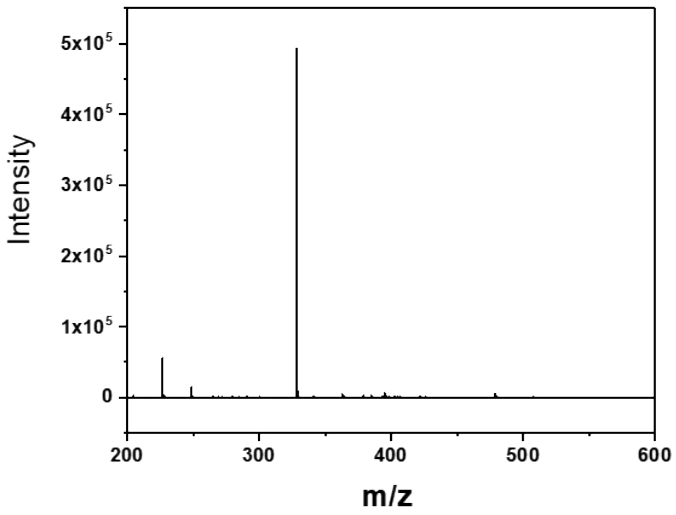



**Figure S15.** ESI analysis of **TPP-Met**

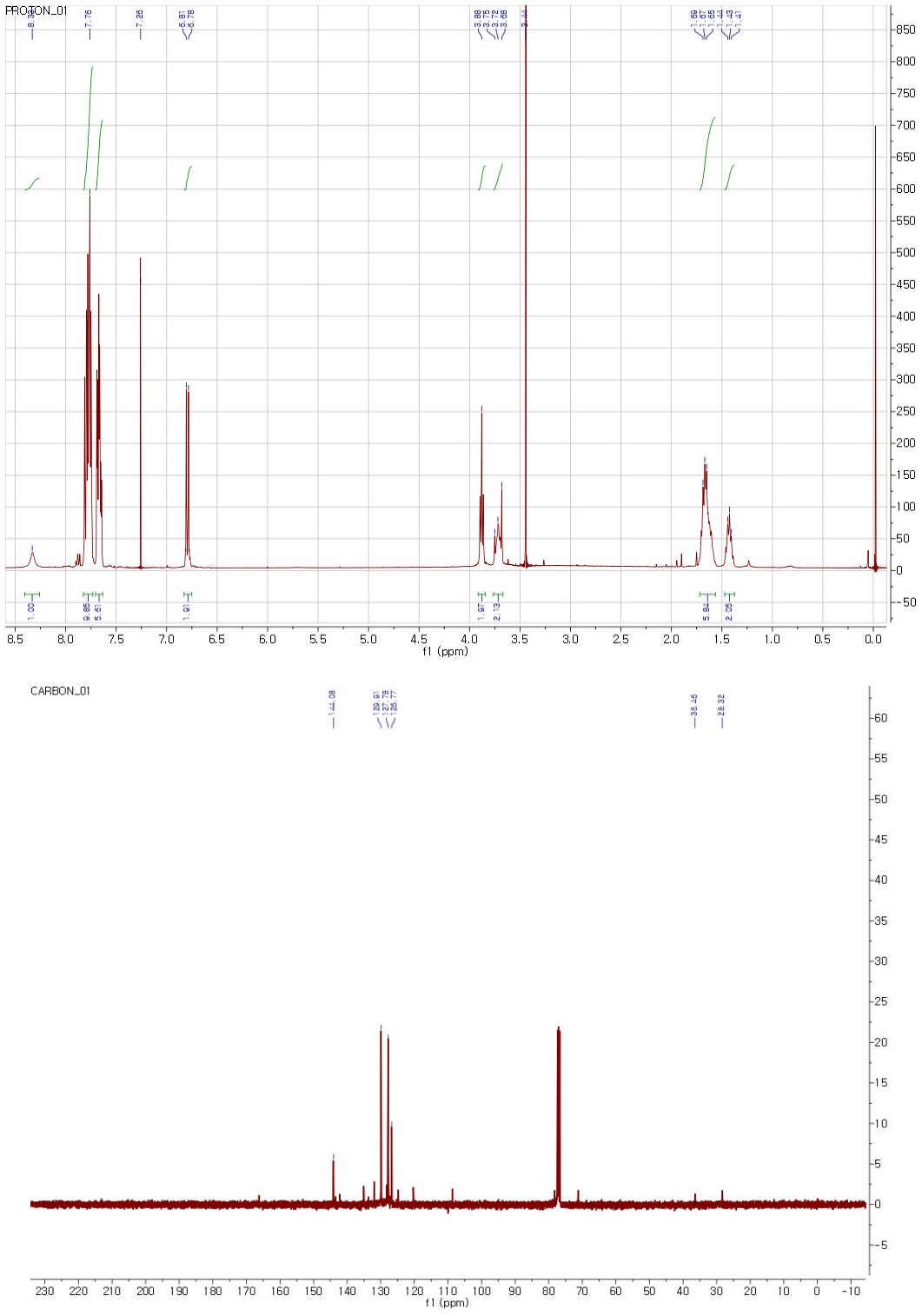



**Figure S16.** $^{1}H$ NMR and $^{13}C$ NMR spectra of **TPP-Hyd**.

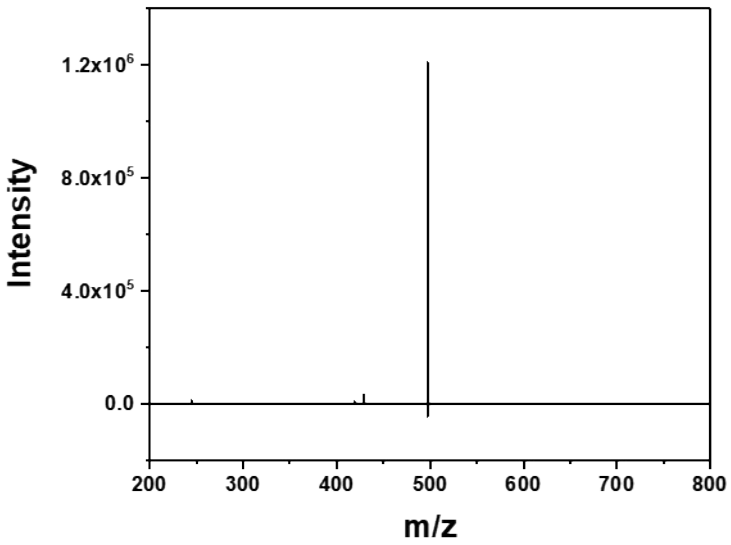



**Figure S17.** ESI analysis of **TPP-Hyd**

**Cell culture.** Human cancer cells originating from the cervix (HeLa) and noncancerous fibroblast cells (HEK293) were cultured in Dulbecco's Modified Eagle Medium (DMEM) and RPMI (Life Technologies) containing 10% fetal bovine serum (FBS) (Life Technologies) and 1% penicillin/streptomycin (Life Technologies) at 37 °C in a humidified atmosphere of 10% $CO_2$. For the cytotoxicity analysis, the cells were cultured in the presence of different concentrations of the TPP-Hyd, TPP-Met, Ald-Br (0, 10, 20, 30, 50, 60, 80 μM). The cell viability was measured after 24 h using the MTT assay. All data points were measured in quadruplicate. Fluorescence measurements were conducted by observing the absorbance at 595 nm emission using a microplate reader (SpectraMAX 384). 96-well Nunc (Thermo Fisher Scientific, USA) plates were seeded with cells at a density of $4 \times 10^3$ cells/well. The cells were allowed to settle by incubation at 37 °C under 5% $CO_2$ in the respective growth medium for 24 h.

**Imaging of mitochondrial depolarization.** HeLa and HEK293 cells were seeded on a Lab Tek II slide chamber at 80% confluence in DMEM (Life Technologies) supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were incubated at 37 °C under 5% $CO_2$ for 24 hours.. Following incubation in the presence of 20 μM of both TPP-Hyd and Ald-Br according to the manufacturer's protocol (Thermo Fisher Scientific, I34361), the cell culture medium was replaced with a 2 μM TMRM working solution to cover the adherent cells. The

cells were subsequently incubated for 15–30 min at 37 °C in the dark. The cells were gently washed with PBS prior to analysis using FV1000 CLSM.

**Imaging of ROS generation in cells.** The HeLa and HEK293 cells were seeded on a Lab Tek II slide chamber at 80% confluence in DMEM (Life Technologies) supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were incubated at 37 °C under 5% $CO_2$ for 24 hours. Following incubation in the presence of 20 μM of the molecule according to the manufacturer's protocol (MitoSox, M36008), the cell culture medium was replaced with a 10 μM MitoSox working solution to cover the adherent cells. The cells were subsequently incubated for 15 min at 37 °C in the dark. The cells were gently washed with PBS prior to the analysis using FV1000 CLSM.

**Annexin V/PI assay by flow cytometry analysis:**

HeLa cells ($2\times10^5$ cells/well) in DMEM (Life Technologies) supplemented with 10% FBS and 1% penicillin/streptomycin were cultured in 6-well plates overnight at 37 °C under 5% $CO_2$. Then the cells are treated with 20 μM TPP-Hyd and Ald-Br for 12h. After that the cells were washed, trypsinized and collected by centrifugation. After washing with 1X annexin-binding buffer, the cells were incubated with 100 μL solution of 1X annexin-binding buffer containing 5 μL of Propidium iodide (PI) (stock concentration was 50 μg/mL) and 5 μL of annexin V (stock concentration was 200 μg/mL) for 15 min. After that another 400 μL of 1X annexin-binding buffer was added to the cells and analyzed by BD FACSVerse flow cytometer using emission filters at 530 and 610 nm.