Supplementary information

Fighting Metallodrug Resistance from Alternation of Drug Metabolism and Blockage of Autophagy Flux by Mitochondrial-Targeting AIEgens

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Experimental section

Materials and instrumentation

All the reagents for synthesis were purchased from J&K (China) and used as received without further purification. Solutions for all experiments were prepared with solvents of HPLC grade and doubly distilled water from Millipore (>18.2 MΩ). Deuterated solvents were obtained from Cambridge Isotope Lab. ROS assay kit, JC-1 assay kit, and DAPI were purchased from KeyGen Co. (China). Mito-tracker Red and Lyso-tracker Red were purchased from Beyotime (China). Antibodies, such as PINK1, Parkin, LC3B, p62, MRP2, CTR1, P-gp, GAPDH, Tubulin, for western blot or immunofluorescence, were purchased from Abcam Co. (China).

The ^{1}H and ^{13}C NMR data were recorded at 400 MHz on a Bruker DRX-400 NMR spectrometer. The ESI-MS data were determined using the LCQ electrospray mass spectrometer (Thermo Scientific). UV-vis absorption spectra and fluorescence emission spectra were recorded on a Lambda 365 UV-vis spectrophotometer and FS5 Spectrofluorometer. The confocal imaging was performed on a confocal laser scanning microscope (Nikon TI-E-A1, Japan). Flow cytometry analysis was performed with a flow cytometer (BD FACSverse, USA). The transmission electron microscopy (TEM) images were observed using a transmission electron microscope (H7650, Hitachi, Japan) at 80 kV in high contrast mode. Western blotting experiments were conducted on Tanon 5200 Multi and the signals were enhanced by Tanon High-sig ECL Western Blotting Substrate.

Synthesis and characterization

Synthesis of DP-OH: $Pd(OAc)_2$ (23 mg, 0.1 mmol), t-BuONa (0.58 g, 6.0 mmol), $(t\text{-}Bu)_3PHBF_4$ (88 mg, 0.3 mmol), N-O-Br (1.25 g, 5.0 mmol), 9,9-dimethyl-9,10-dihydroacridine (DMAC, 1.25 g, 6.0 mmol) and toluene (25 mL) were added into a 100 mL round-bottom flask. The mixture was refluxed under argon for 48 h. After cooling to room temperature, the mixture was poured into water, extracted with dichloromethane (DCM) (50 mL×3), and dried over anhydrous Na_2SO_4. After removal of the solvents, the crude product was purified via column chromatography on silica gel using DCM/ethyl acetate (20 : 1 v/v) as the eluent to give a yellow solid (1.00 g, yield 64%). ^{1}H NMR (400 MHz, Methanol-d_4) δ (ppm): 8.89-8.83 (m, 2H), 8.45-8.39 (m, 2H), 7.59 (t, J = 2.0 Hz, 1H), 7.51 (dd, J = 7.6, 1.7 Hz, 2H), 7.44 (t, J = 1.7 Hz, 1H), 7.05-6.91 (m, 5H), 6.36 (dd, J = 8.0, 1.4

Hz, 2H), 1.69 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 161.13, 154.57, 143.24, 140.36, 138.79, 130.04, 127.04, 126.04, 124.41, 121.33, 121.15, 121.07, 115.41, 114.10, 36.02, 31.98.

Synthesis of DP-PPh$_3$: DP-OH (37.8 mg, 0.1 mmol), TPP (62.2 mg, 0.13 mmol), K_2CO_3 (41.5 mg, 0.3 mmol), and acetone (15 mL) were added into a 50 mL round-bottom flask. The mixture was refluxed under argon for 12 h. After cooling to room temperature, the supernatant was obtained by centrifugation. Light yellow solid (61.3 mg, yield 79%) was obtained by recrystallization of dichloromethane and anhydrous ether. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm): 8.71-8.66 (m, 2H), 7.94-7.85 (m, 7H), 7.75 (dtd, J = 8.4, 6.9, 1.7 Hz, 4H), 7.67 (td, J = 7.5, 3.4 Hz, 5H), 7.62-7.57 (m, 2H), 7.49 (dd, J = 7.4, 1.9 Hz, 2H), 7.36 (t, J = 1.9 Hz, 1H), 7.23 (d, J = 1.7 Hz, 1H), 6.98 (dtd, J = 15.5, 7.3, 1.7 Hz, 4H), 6.35 (dd, J = 7.8, 1.7 Hz, 2H), 4.26 (t, J = 5.7 Hz, 2H), 4.03 (s, 2H), 2.37-2.25 (m, 2H), 1.91 (q, J = 7.7 Hz, 2H), 1.72 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 161.81, 150.76, 146.08, 143.03, 141.51, 140.43, 135.36, 134.17, 134.10, 134.08, 134.00, 130.79, 130.75, 130.66, 130.63, 130.00, 127.02, 126.08, 121.79, 121.05, 119.35, 118.50, 117.89, 114.07, 113.62, 67.17, 36.03, 32.15, 0.59. ESI-MS (CH_3OH): calcd for [**DP-PPh$_3$**-Br$^-$]$^+$ m/z = 774.24, found m/z = 695.40.

Synthesis of TPE-Br: Zinc dust (7.84 g, 120 mmol) was suspended in dry THF (150 mL) under argon in a flame-dried round-bottomed flask equipped with a reflux condenser. $TiCl_4$ (6.64 mL, 60 mmol) was added slowly and the resulting mixture was refluxed for 2 hours, then cooled down in an ice bath. Pyridine (1 mL) was added and after 10 min 4-bromobenzophenone (10.44 g, 40 mmol) dissolved in 20 mL THF was added drop-wise. After refluxing overnight, the mixture was quenched with 10% aqueous K_2CO_3 solution (100 mL) followed by extraction with CH_2Cl_2. The organic phase was washed with water and brine and dried over $MgSO_4$. After rotary evaporation, the crude product was obtained as a mixture of trans/cis isomers. Multiple recrystallizations from CH_2Cl_2/MeOH solution afforded the trans and cis compounds in their pure form. Afford 7 g TPE of yellow powder in a 67% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm): 7.28-7.22 (m, 4H), 7.17-7.11 (m, 6H), 7.01 (td, J = 7.0, 3.1 Hz, 4H), 6.90 (t, J = 8.5 Hz, 4H). To a solution of TPE (2.45 g, 5 mmol) in 40 mL, dry THF has added dropwise 2.4 mL (6 mmol) of n-BuLi (2.5 M in n-hexane) at -78 °C under stirring. The reaction mixture was stirred for 2 h to get a dark brown solution. The obtained solution was then added dry ice pieces in small portions under nitrogen. The solution was allowed to warm to

room temperature and stir for an additional 12 h. The solvent was evaporated under reduced pressure. The crude product was purified on a silica gel column using dichloromethane/methanol mixture as eluent. Yield 20%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.87 (s, 1H), 7.77-7.67 (m, 2H), 7.41-7.31 (m, 2H), 7.20-7.06 (m, 9H), 7.01-6.89 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 167.46, 147.96, 142.83, 142.79, 142.53, 142.45, 140.95, 140.83, 140.80, 133.24, 133.18, 131.41, 131.32, 131.28, 131.21, 131.12, 131.07, 131.02, 129.50, 129.33, 128.62, 128.53, 128.44, 128.32, 127.52, 127.44, 127.39, 127.25, 120.61, 120.51.

Synthesis of TPE-PPh$_3$

Synthesis of 1: Complex **1** was synthesized by a modified procedure from the literature.[1] Into a 500 mL two-neck flask equipped with a condenser were added 4-hydroxybenzophenone (1g, 5 mmol), 4-bromobenzophenone (2.60 g, 10 mmol), and zinc powder (2.95 g, 45 mmol) in 150 mL of THF in nitrogen. The reaction mixture was cooled to -78 °C, and $TiCl_4$ was then charged dropwise. The reaction was refluxed overnight with nitrogen protection. After cooling down to room temperature, this reaction mixture was acidified with hydrochlorid acid (1 M) to pH < 2. The organic phase was extracted with DCM and drying with anhydrous sodium sulfate. The crude product was purified using a silica column eluted with a solvent mixture (petroleum ether/ethyl acetate = 10:1) to give **1** as a white solid (1.4 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.885 (d, 1H), 6.53-6.60 (m, 2H), 6.86-6.91 (m, 4H), 6.98-7.20 (m, 10H), 7.25-7.35 (m, 2H).

Synthesis of 2: Complex **1** (855 mg, 2 mmol), 4-Methoxycarbonylphenylboronic acid (540 mg, 3 mmol), Na_2CO_3 (1.06 g, 10 mmol), 1, 4-dioxane (10 mL) and H_2O (4 mL) are mixed in a sealable tube. The mixture was argon degassed for 30 min, then Pd(dppf)Cl_2 (73 mg, 0.1 mmol) was added and the tube was sealed and heated at 100 °C overnight. The reaction mixture was then filtered and extracted with CH_2Cl_2. The organic phase was washed with water and brine and evaporated. The crude product was purified by column chromatography using petroleum ether and DCM mixture (1:1, v/v) as eluent to afford **2** (627 mg, 65 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.92(m, 3H), 4.95 (d, 1H), 6.53-6.60 (m, 2H), 6.86-6.91 (m, 4H), 6.98-7.20 (m, 10H), 7.30-7.45 (m, 2H).

Synthesis of 3: Into a two-neck flask equipped with a condenser was placed Complex **2** (2.7 g, 5.6 mmol) and K_2CO_3 (1.94 g, 14.0 mmol), DMF (50 mL), and (4-Bromobutyl) Triphenylphosphonium

Bromide (5.4 g, 11.3 mmol) was added, and the reaction was stirred overnight under nitrogen at 80 °C. After cooling down to room temperature, the mixture was extracted with DCM. The organic phase was washed with water three times and then dried with anhydrous $MgSO_4$. The crude product was purified using a silica gel column using petroleum ether and DCM mixture (5:1, v/v) as the eluent to give **3** (2.7 g, 55%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.60-1.75 (m, 2H), 1.78-1.91 (m, 2H), 3.55-3.70 (m, 2H), 3.82 (m, 3H), 3.91-3.99 (m, 2H), 6.61-6.70 (m, 2H), 6.85-7.20 (m, 14H), 7.49-7.61 (m, 2H), 7.68-7.99 (m, 19H).

Synthesis of TPE-PPh$_3$: To a MeOH (3.0 mL) solution of **3** (880 mg, 1 mmol), sodium hydroxide aqueous solution (10 eq) were added and stirred for 24h at room temperature. This reaction mixture was acidified with hydrochloric acid (1 M) to pH < 1. The organic phase was extracted with DCM and drying with anhydrous $MgSO_4$. The solvents were rotary evaporated to afford **TPE-PPh$_3$** (675 mg, 75%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.97 (d, J = 8.2 Hz, 2H), 7.80 (dddd, J = 38.0, 24.4, 12.7, 7.2 Hz, 17H), 7.54 (dd, J = 14.9, 8.4 Hz, 2H), 7.19-6.84 (m, 14H), 6.67 (dd, J = 11.0, 8.8 Hz, 2H), 4.02-3.89 (m, 2H), 3.66 (d, J = 6.2 Hz, 2H), 1.90-1.80 (m, 2H), 1.68 (d, J = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 167.54, 157.44, 143.89, 141.12, 139.50, 136.94, 135.84, 135.31, 134.08, 132.40, 131.91, 131.21, 130.75, 130.63, 130.38, 129.94, 128.34, 128.24, 127.18, 126.89, 126.64, 119.36, 118.51, 114.32, 114.17, 66.81, 34.45, 29.47, 20.27, 19.76, 18.86. ESI-MS (positive mode, m/z) found (calcd) for $[M-Br]^+$: 785.50 (785.30).

UV-vis and Photoluminescence spectroscopic study

The stock solution (10 mM) of complexes **DP-OH**, **DP-PPh$_3$**, **TPE-Br**, and **TPE-PPh$_3$** in DMSO were prepared before measurements and stored in a 4 °C refrigerator, and then diluted suitably with distilled water to the required concentration. The absorption spectra and fluorescence spectra were recorded at ambient temperature.

Cell culture

A549, A549R, A2780, MCF-7, HepG2, BEAS-2B, and HLF cells were obtained from the Experimental Animal Center of Sun Yat-Sen University (Guangzhou, China). Cisplatin-sensitive cells, A549, A2780, MCF-7, HepG2, BEAS-2B, and HLF, were routinely cultured in DMEM (Dulbecco's modified Eagle's medium, Gibco BRL) containing 10% FBS (fetal bovine serum, Gibco BRL), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL). Cisplatin-resistant cells,

A549R cells were cultured in RPMI 1640 with 1 μg/mL of cisplatin to maintain the drug resistance. Cells were cultured at 37 °C in a humidified incubator (BB 150 CO_2 incubator, Thermo Fisher Scientific, USA) containing 5% CO_2.

***In vitro* cytotoxicity assays**

The cytotoxicities of the complexes toward cancer cells were determined *via* MTT assays. Cells (100 μL) were seeded to a 96-well plate at a density of 5×10^4 /mL. After 24-hour incubation, solutions of the compounds **DP-OH**, **DP-PPh$_3$**, **TPE-Br**, and **TPE-PPh$_3$** and cisplatin (0-100 μM) were separately added into the wells and incubated for 48 h at 37 °C. Then an amount of 20 μL MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (5 mg mL^{-1}, PBS buffer) was added to each well and incubated for another 4 h. The medium was carefully removed and dissolved in DMSO (150 μL per well) was added and the absorbance at 570 nm of the purple formazan was collected on a microplate reader (LabServ K3, Thermo Scientific). The cell viabilities were calculated based on the data of three parallel tests. IC_{50} values quoted are mean ± SD. The maximum DMSO concentration was controlled at 1% (v/v) in all cases.

Cellular confocal images

A549R cells were seeded into 35 mm confocal dishes (JET BIOFIL, Canada) for confocal microscopy. After cultured overnight, the cells were pre-incubated with complex **DP-PPh$_3$** (10 μM) and **TPE-PPh$_3$** (20 μM) for 1 h at 37 °C. Subsequently, the medium was replaced with a staining medium containing Mito-Tracker Red (200 nM) or Lyso-Tracker Red (50 nM) and stained for 30 min at 37 °C. The staining medium was removed and the cells were washed with PBS three times and visualized immediately using confocal microscopy with a 63×oil-immersion objective lens under a confocal microscope (A1, Nikon, Japan). Complex **DP-PPh$_3$** and **TPE-PPh$_3$** were excited at 405 nm and emissions were collected at 510-560 nm. The excitation wavelength of Mito-Tracker Red and Lyso-Tracker Red was 561 nm and emission was collected at 575-625 nm, respectively.

Study on the intracellular generation of ROS

A549R cells were seeded into 35 mm confocal dishes (JET BIOFIL, Canada) for confocal microscopy. After cultured overnight, the cells were treated with complex **DP-OH** (2 μM), **DP-PPh$_3$** (2 μM), **TPE-Br** (4 μM) and **TPE-PPh$_3$** (4 μM) for 24 h at 37 °C. After stained with 10 μM DCFH-

DA probe and washed twice with serum-free medium, the cells were immediately observed by confocal microscopy (A1, Nikon, Japan) with excitation at 488 nm and emission at 530 ± 20 nm.

For the flow cytometry analysis of cellular ROS, A549R cells were seeded in 6-well plates for 24 h, incubated with complex **DP-OH** (2 μM), **DP-PPh$_3$** (2 μM), **TPE-Br** (4 μM), and **TPE-PPh$_3$** (4 μM) for 24 h at 37 °C. Then the cells were collected and washed with PBS twice. After that, the ROS probe (10 μM) DCFH-DA was added to the cell samples and incubated for 30 min. Then the cell samples were analyzed by flow cytometry after washing with culture medium without FBS and analyzed using a BD FACS Calibur flow cytometer with excitation at 488 nm and emission at 530 ± 30 nm. Data were analyzed using FlowJo 7.6.1 software. 10, 000 cells were acquired for each sample.

Mitochondrial membrane potential (MMP) detection

A549R cells were seeded into 35 mm confocal dishes (JET BIOFIL, Canada) for confocal microscopy. After cultured overnight, the cells were treated with complex **DP-OH** (2 μM), **DP-PPh$_3$** (2 μM), **TPE-Br** (4 μM) and **TPE-PPh$_3$** (4 μM) for 24 h at 37 °C. After stained with JC-1 working solution for 30 min, the cells were washed with 1× incubation buffer and then immediately observed by confocal microscopy (A1, Nikon, Japan).

For the flow cytometry analysis of MMP, A549R cells were seeded in 6-well plates for 24 h, incubated with **DP-OH** (2 μM), **DP-PPh$_3$** (2 μM), **TPE-Br** (4 μM) and **TPE-PPh$_3$** (4 μM) for 24 h. Then the cells were harvested with 0.25% trypsin, washed with PBS three times. After the addition of 0.5 mL JC-1 working solution, the cells were incubated at 37 °C for 30 min. Then the staining solution was removed, the cell samples were washed with 1× incubation buffer and analyzed by flow cytometry using a BD FACS Calibur flow cytometer.

Transmission electron microscopy assay

1×10^6 A549R Cells were cultured in 10 cm dishes and separately treated with 2 μM **DP-PPh$_3$** and 4 μM **TPE-PPh$_3$** for 24 h, and harvested in a 1.5 mL microcentrifuge tube for each sample. Then the cells were washed with cold PBS twice and fixed with cold fixation solution (2.5% glutaraldehyde in phosphate buffer) at 4 °C overnight. After that, the cell samples were dehydrated, fixed, embedded, and sliced, finally observed using transmission electron microscopy (Hitachi, Japan).

Mitochondrial Bioenergetics

The impact of **DP-PPh$_3$**, **TPE-PPh$_3$** on mitochondrial bioenergetics was investigated using a Seahorse Extracellular Flux analyser. A total of 2×10^4 cells/well were seeded in 24-well plates, followed by culturing at 37 °C for 18 h. Growth culture medium was replaced with medium supplemented with **DP-PPh$_3$**, **TPE-PPh$_3$** and incubated at 37 °C for 24 h. After that, XF assay medium (Seahorse Bioscience) containing 25 mM glucose, 1 mM pyruvate, or 2 mM glutamine for OCR and ECAR test, respectively, was added to the wells along with drugs to maintain a stimulating environment, and cells were equilibrated at 37 °C in a CO_2-free incubator for 1 h. OCR was measured using Seahorse XF24 extracellular flux analyser (Agilent Technologies), during which 1 μM oligomycin, 2 μM FCCP, and 1 μM rotenone + 1 μM antimycin A were injected consecutively every 24 min. ECAR was measured during which 10 mM glucose, 1 μM oligomycin, and 130 mM 2-DG were injected consecutively every 24 minutes. The average of four baseline rates and up to five test rates were used for data analysis. Data were recorded during the measurement. The OCR and ECAR data were normalized to per μg protein. The corresponding OCRs were calculated for basal respiration ($OCR_{initial}-OCR_{antimycin\ A/rotenone}$), ATP production ($OCR_{basal}-OCR_{oligomycin}$), max respiration ($OCR_{FCCP}$-$OCR_{basal}$), and spare respiration ($OCR_{FCCP}-OCR_{initial}$). The corresponding ECARs were calculated for glycolysis (before oligomycin injection), glycolytic capacity (after oligomycin injection and deduction of baseline), and glycolytic reserve (after oligomycin injection and deduction of glycolytic capacity).

RNA sequencing and analysis

A549R cells were seeded in a 10 cm culture dish and allowed to grow until the confluency reached 80%. Then the cells were treated with **DP-PPh$_3$** (2 μM), **TPE-PPh$_3$** (4 μM). After 24 h incubation, the cells were collected and the total RNA was extracted with TRIzol Reagent (Sigma-Aldrich). The samples were sent to Guangdong Magi Gene Technology Corporation for RNA sequencing (RNA-seq). RNA-seq was performed on Illumina HiSeq 2500 (Illumina, USA) with 150 bp paired-end reads. The differential expression analysis of digital gene expression data was analyzed with edge-R.[2] The enrichment analysis was performed by cluster Profilter using KEGG.[3]

Western Blot analysis

A549R Cells were cultured in 10 cm dishes and separately treated with **DP-OH**, **DP-PPh$_3$**, **TPE-Br**, **TPE-PPh$_3$** for 48 h, respectively. The cells were harvested in a 1.5 mL microcentrifuge tube for each sample. Then the proteins were extracted in ice-cold lysis buffer (KeyGen Biotech), the cell lysates were incubated on ice for 20 min and centrifuged at 12,000 g for 20 min at 4 °C. Protein concentrations were measured using protein assay reagents, and equal amounts of protein per lane were separated on SDS-PAGE gel and transferred to a PVDF membrane (Millipore). The membranes were incubated with specific antibodies at 4 °C overnight and then incubated with a horseradish peroxidase-coupled secondary antibody for 1 h at room temperature. After washing with PBST, the signal was visualized by Tanon High-sig ECL Western Blotting Substrate and Tanon 5200 Multi.

Immunofluorescence assay

A549R cells were seeded into 35 mm confocal dishes (JET BIOFIL, Canada) for confocal microscopy. After cultured overnight, the cells were treated with complex **DP-OH** (2 μM), **DP-PPh$_3$** (2 μM), **TPE-Br** (4 μM) and **TPE-PPh$_3$** (4 μM) for 48 h at 37 °C. Then A549R cells were fixed with 4% paraformaldehyde/PBS for 30 min. After washed with PBS three times, cells were then stained with anti-LC3B antibody (1: 100) for 1 h at room temperature, and detected with a secondary antibody (Fluorescein (FITC)-conjugated affinipure goat anti-rabbit lgG (H+L), 1: 50). The cells were counterstained with 300 nM DAPI for 10 min and immediately observed by confocal microscopy (A1, Nikon, Japan).

3D tumor spheroids viability assays

The spheroids were prepared with A549R cells by seeding 2,500 cells/well in an Ultra-Low Attachment 96-well plate (Corning).[4] The 4-day spheroids were incubated in a normal medium with **DP-OH**, **DP-PPh$_3$**, **TPE-Br**, **TPE-PPh$_3$** at the desired concentration. The spheroid growth was monitored using a live-cell phase-contrast microscope (Axio Observer, Zeiss). Then spheroids were washed twice with PBS, stained with Calcein AM/PI following the manufacturer's instructions (Beyotime, China), and fixed in 4% paraformaldehyde. Spheroids were placed in a glass-bottom dish and imaged at different depths (z-stacking) with a confocal scanning microscopy system (Calcein AM: λex = 488 nm and λem range 500-550 nm, PI: λex = 561 nm and λem range 570-620 nm).

***In vivo* antitumor study**

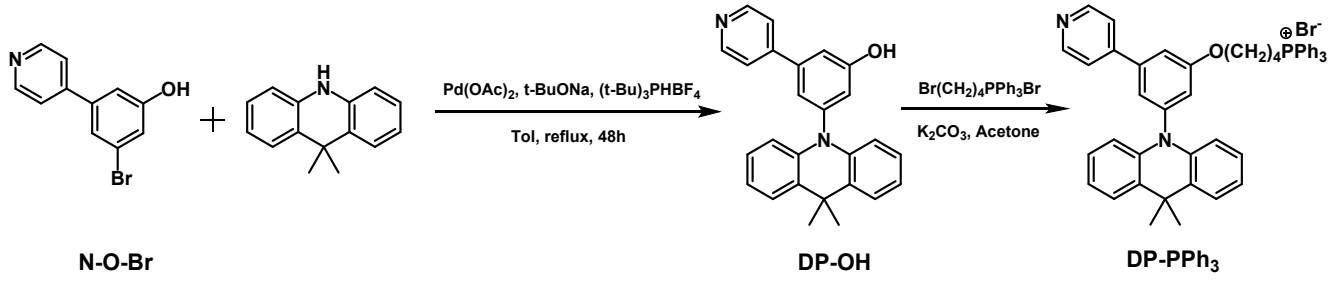
The *in vivo* anticancer activity studies were carried out in A549 tumor-bearing nude mice models. Balb/c nude mice were purchased from SPF (Beijing) Biotechnology Co., Ltd (ages 6 to 8 weeks, 18-20 g). All of the animal experiments were approved by the university animal care and use committee of Nanjing Normal University. To build the subcutaneous tumor model, the mice were inoculated by injecting 2×10^6 A549 cells in the back of each mice. The mice were randomly divided into 4 groups (6 mice/group) when the tumors grew to 80-100 mm^3. The groups were injected with various compounds of **DP-PPh$_3$**, **TPE-PPh$_3$**, saline and cisplatin (5 mg kg^{-1} body weight, dissolved in containing 0.9% saline solution) once every second day for 14 days, respectively. The growth of the subtaneous tumor was monitored, and the body weight of mice was recorded every 2 days as well. The volume of tumor was measured and calculated according to the following formula: Volume=$width^2\times length\times0.5$. At the end of experiments, the mice was sacrificed, the tumors and the organisms such as heart, liver, lung, spleen and kidney were collected for hematoxylin and eosin (H&E), and the TUNEL apoptosis staining.

Statistical analysis

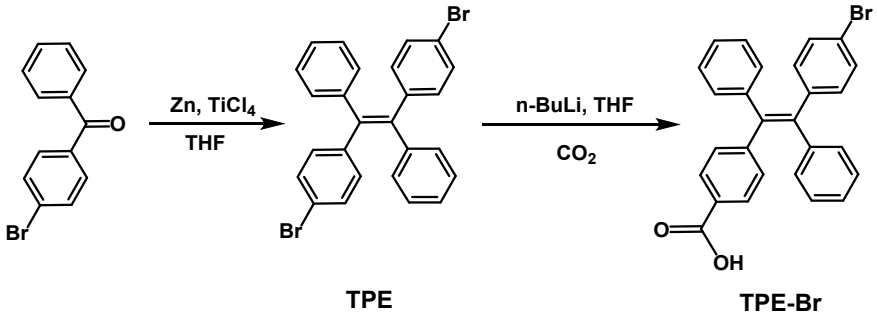
Data are given as Mean ± SD. Statistical significance was performed using a two-tailed Student's *t*-test. Statistical significance was set at $^{*}P < 0.05$, and extreme significance was set at $^{**}P < 0.01$, and $^{***}P < 0.001$.

Table S1. IC_{50} values (μM, 48 h) towards variable cancer cells. Data are shown as mean ± standard deviation (SD, n=3).

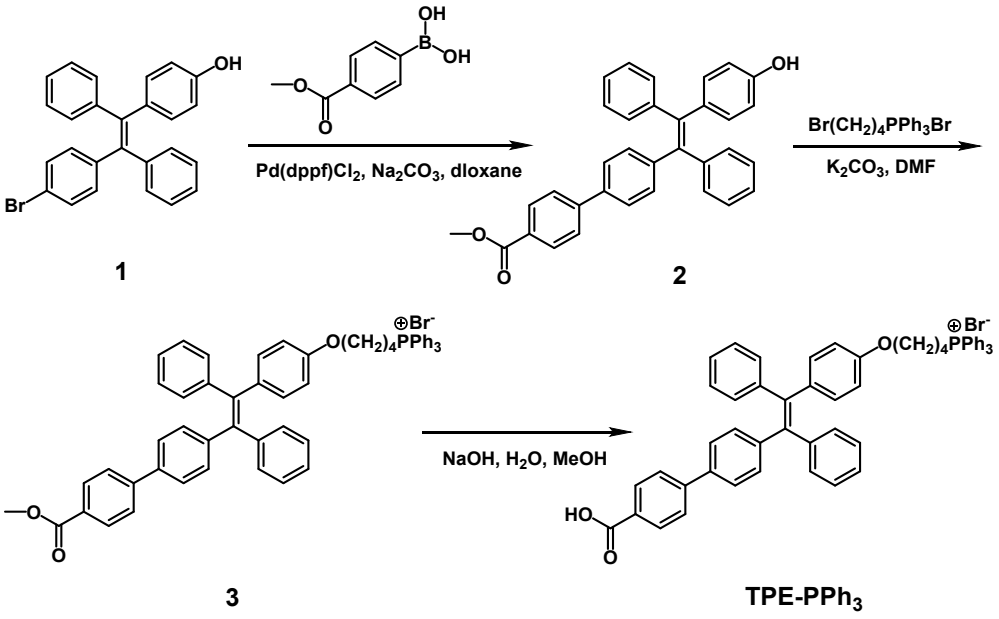
Complexes	A2780	MCF-7	HepG2	BEAS-2B	HLF
DP-OH	42.89±0.28	42.95±1.59	35.56±0.51	49.84±0.96	32.57±0.49
DP-PPh$_3$	1.44±0.05	1.34±0.03	3.12±0.10	1.77±0.09	2.72±0.09
TPE-Br	84.65±4.06	115.07±4.97	81.27±1.90	153.61±0.86	126.12±3.61
TPE-PPh$_3$	2.55±0.03	1.88±0.08	6.40±0.14	3.44±0.01	2.16±0.07
cis-Pt	5.71±0.32	3.88±0.50	3.42±0.23	8.45±0.37	6.43±0.56



Scheme S1: Synthesis route of **DP-PPh$_3$**.



Scheme S2: Synthesis route of **TPE-Br**.



Scheme S3: Synthesis route of **TPE-PPh$_3$**.

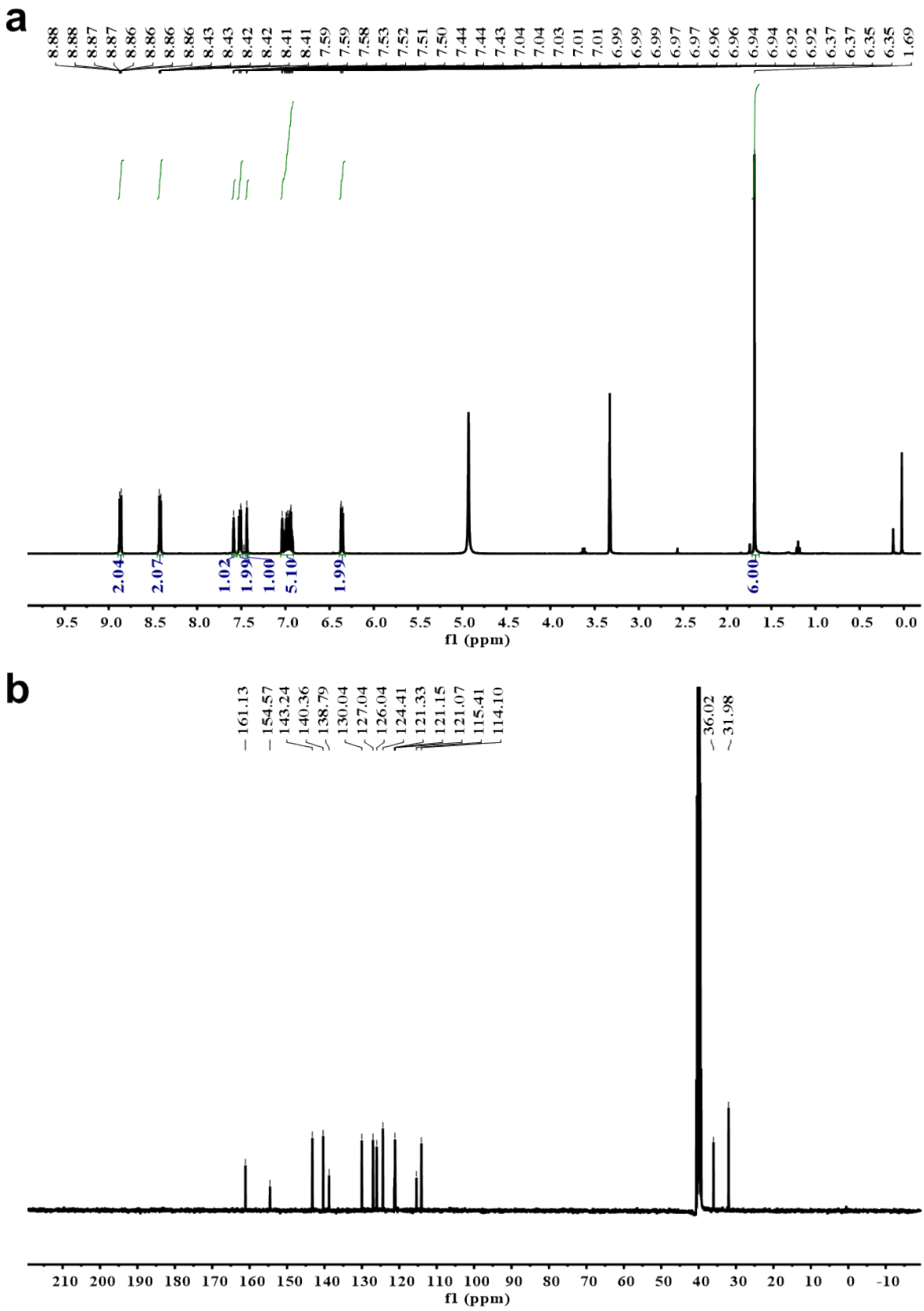


Figure S1. (a) ^{1}H NMR spectrum of (400 MHz, Methanol-d_4) and (b) ^{13}C NMR spectrum of (101 MHz, DMSO-d_6) of **DP-OH**.

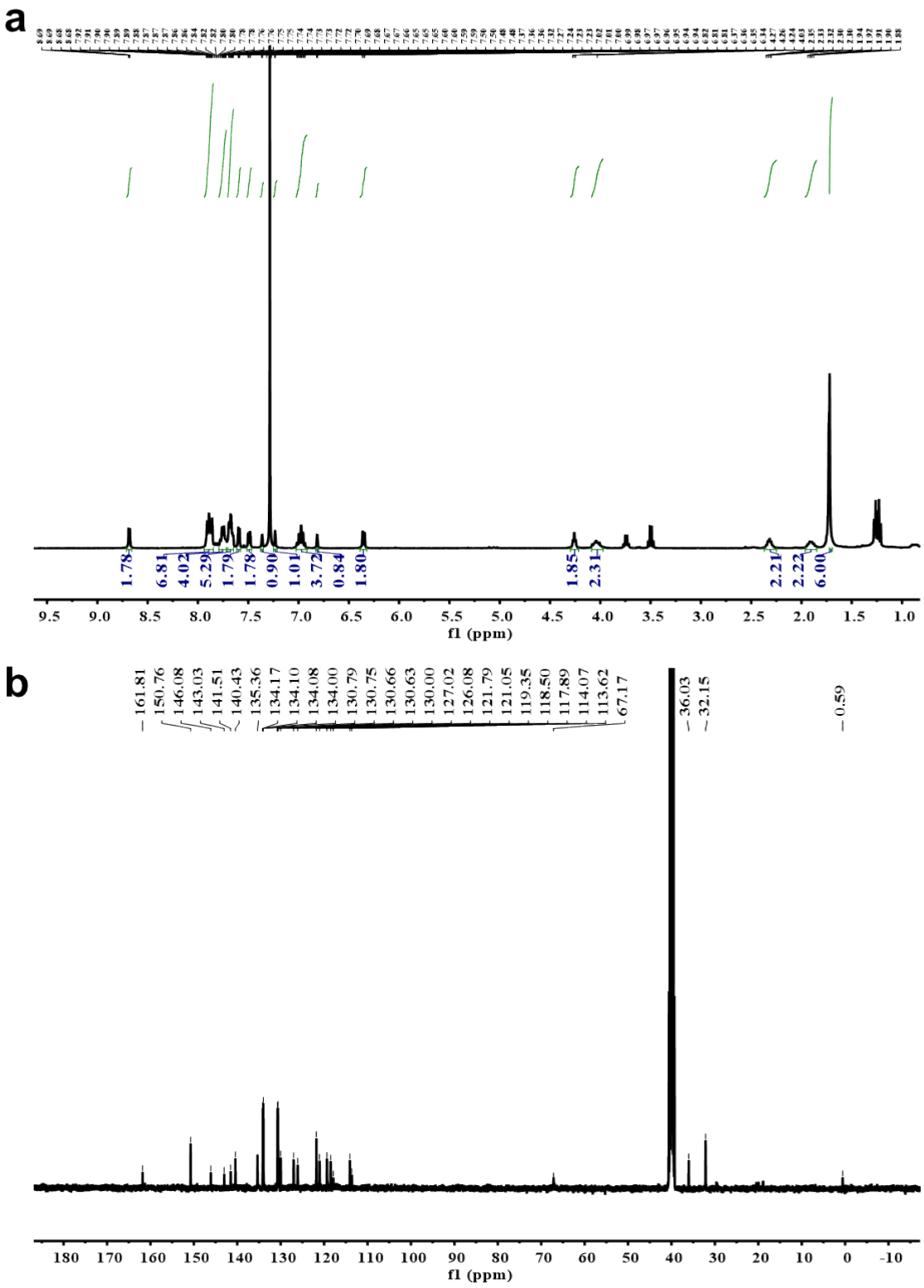


Figure S2. (a) ^{1}H NMR spectrum of (400 MHz, Methanol-d_4) and (b) ^{13}C NMR spectrum of (101 MHz, DMSO-d_6) of **DP-PPh$_3$**.

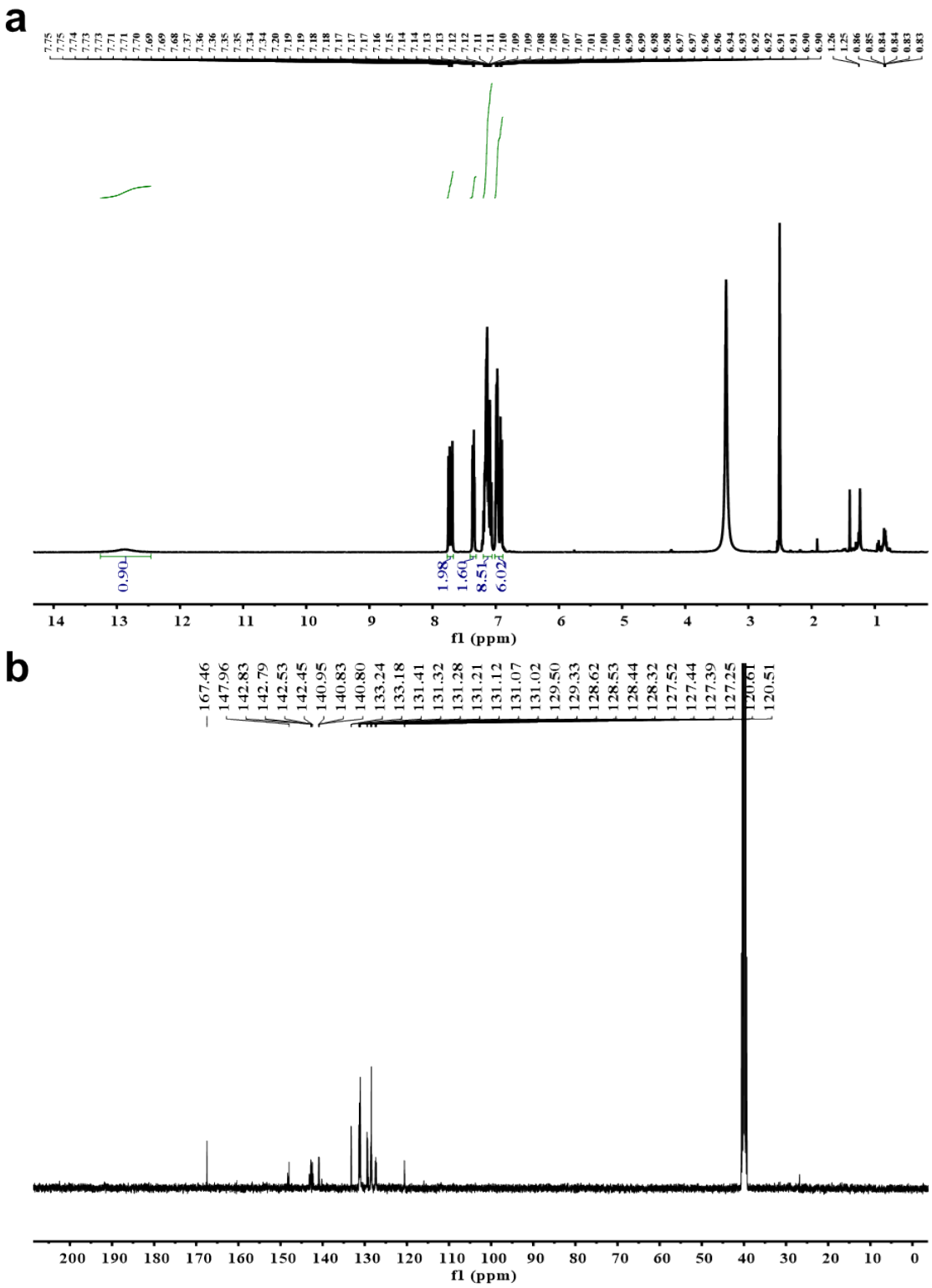


Figure S3. (a) ^{1}H NMR spectrum of (400 MHz, DMSO-d_6) and (b) ^{13}C NMR spectrum of (101 MHz, DMSO-d_6) of **TPE-Br**.

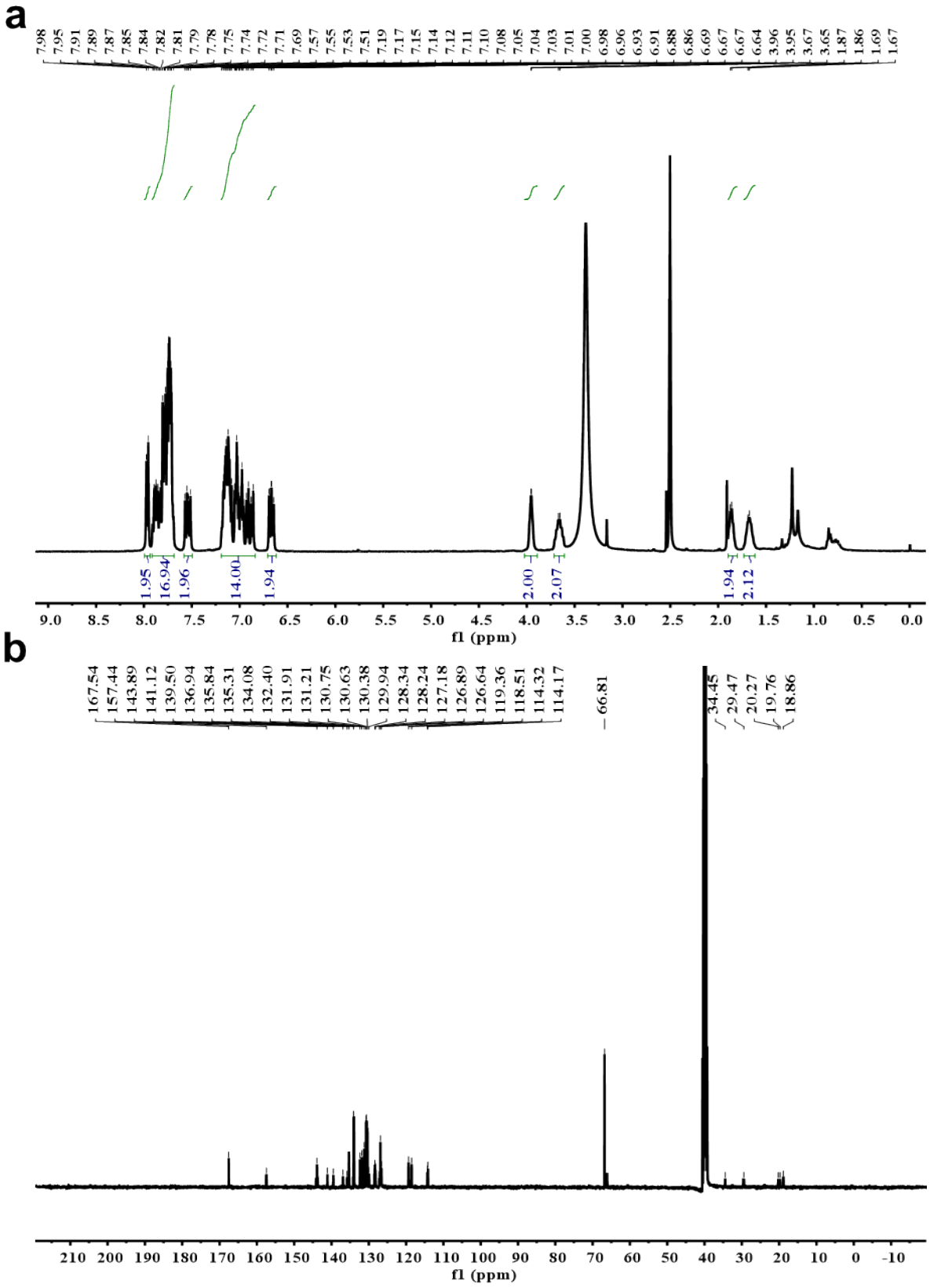


Figure S4. (a) ^{1}H NMR spectrum of (400 MHz, DMSO-d_6) and (b) ^{13}C NMR spectrum of (101 MHz, DMSO-d_6) of **TPE-PPh$_3$**.

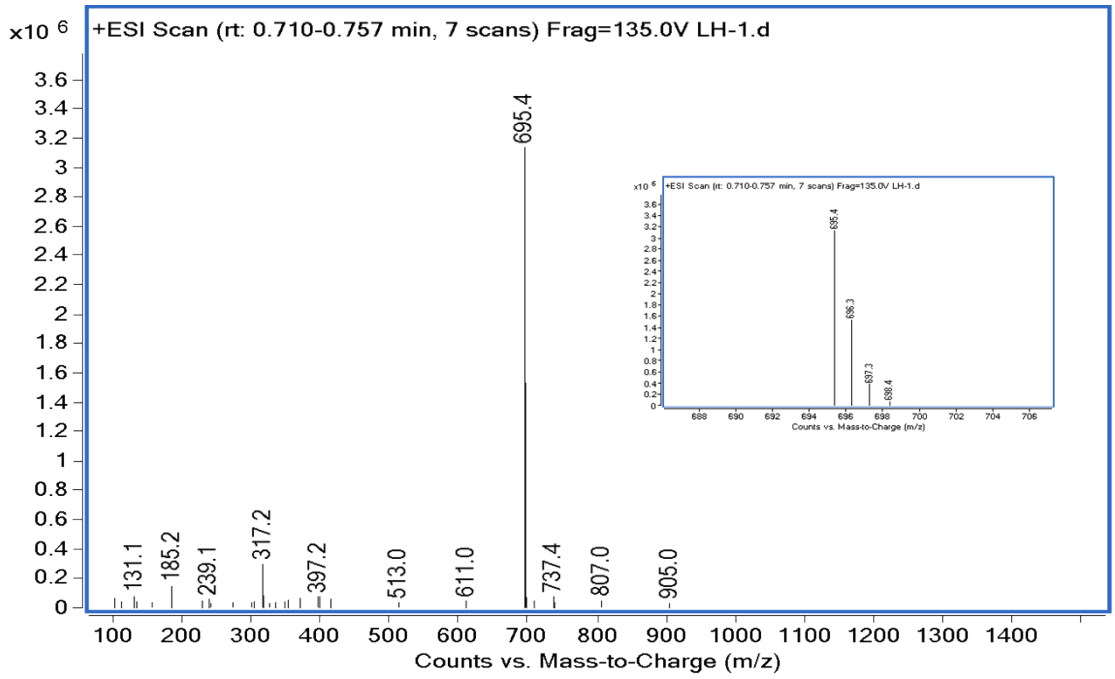


Figure S5. ESI-MS spectrum (CH_3OH) of **DP-PPh$_3$**.

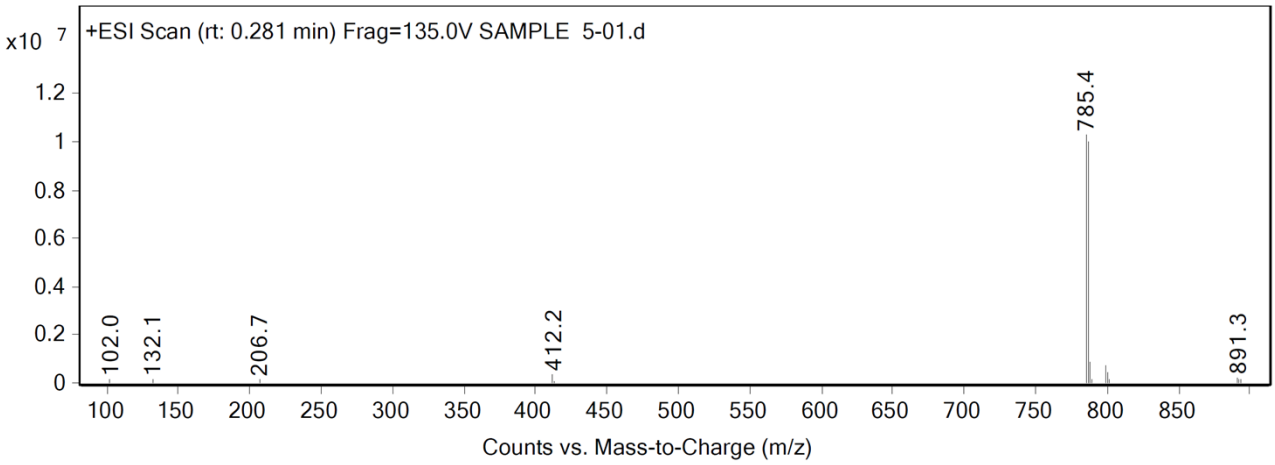


Figure S6. ESI-MS spectrum (CH_3OH) of **TPE-PPh$_3$**.

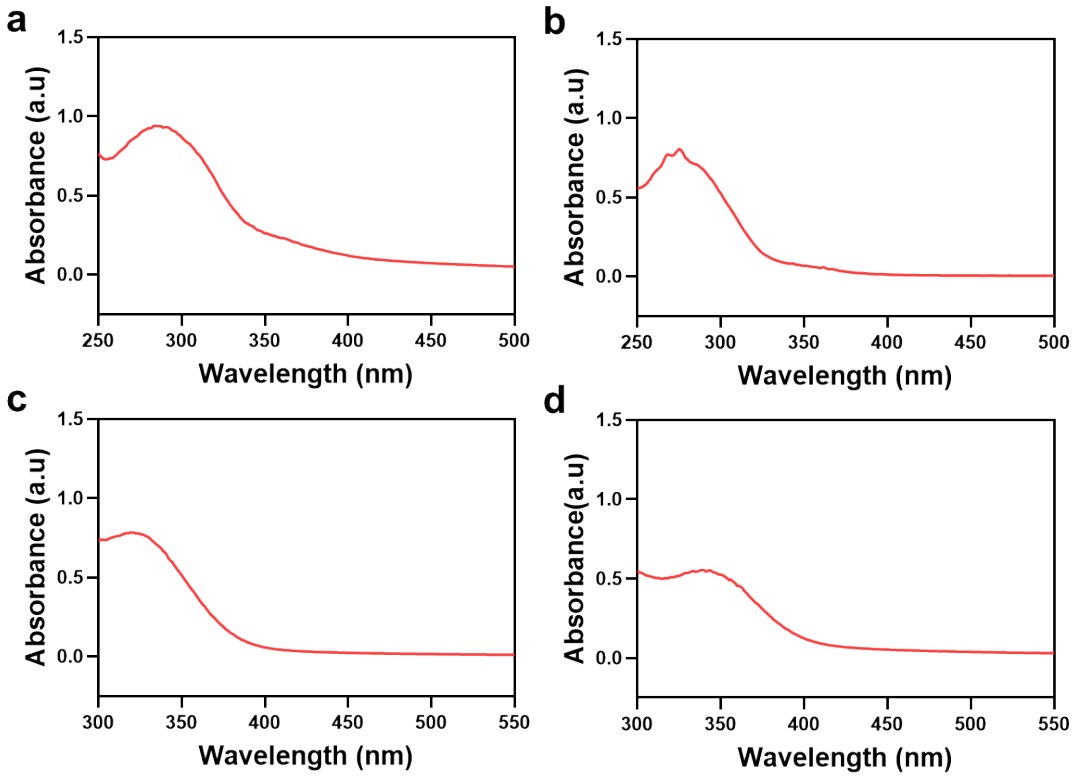


Figure S7. UV-vis spectra for 50 μM solution of complexes (a) **DP-OH**, (b) **DP-PPh$_3$**, (c) **TPE-Br** and (d) **TPE-PPh$_3$** in DMSO/water (v/v, 1:99) at room temperature.

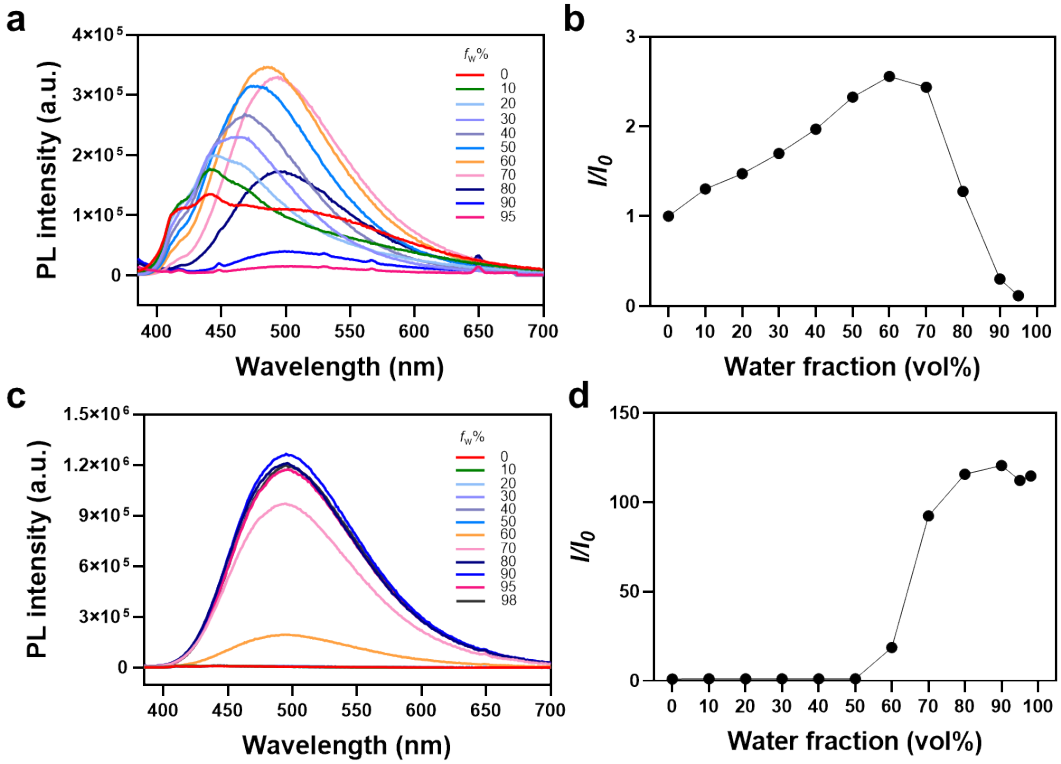


Figure S8. Photoluminescence (PL) spectra and relative maximum intensity ratio I/I_0 of **DP-OH** (a-b, 50 μM) and **TPE-Br** (c-d, 50 μM) in the DMSO/water mixture with different water volume fractions (*fw*). I_0 and I were the PL intensity in pure DMSO or in different composition of DMSO/water. Ex = 405 nm.

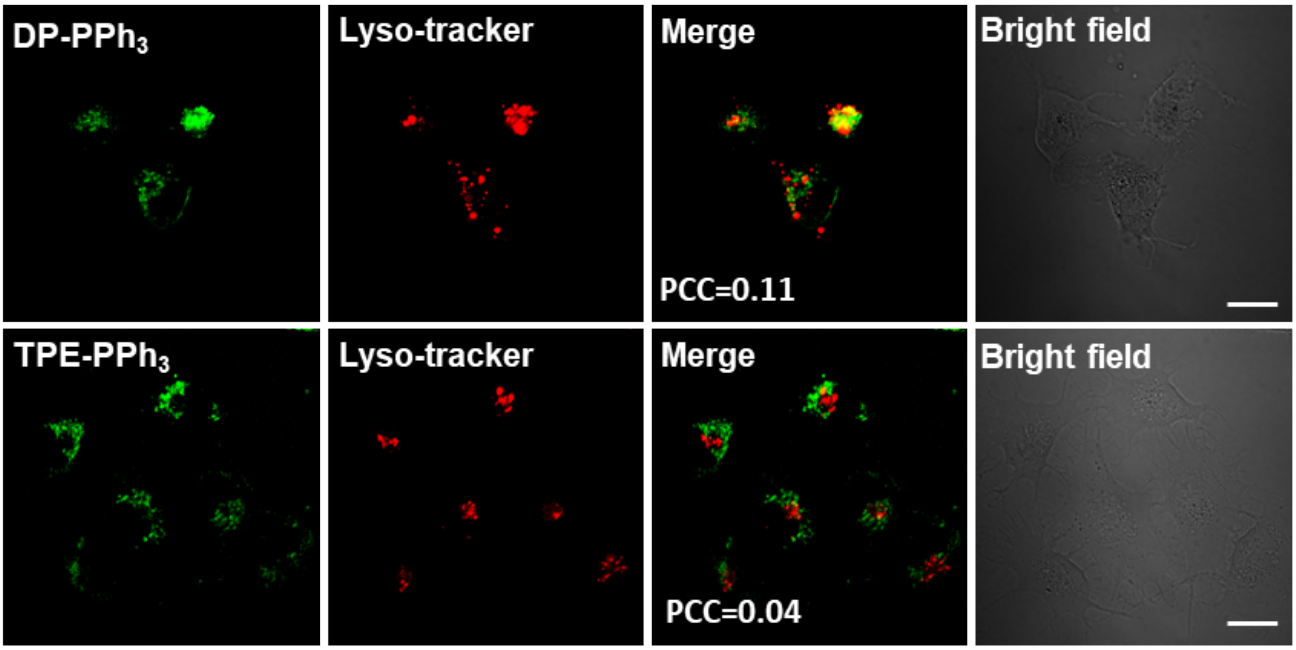


Figure S9. Confocal images of A549R cells treated with **DP-PPh$_3$** (10 μM) or **TPE-PPh$_3$** (20 μM). The cells were co-stained with Lyso-tracker-Red (50 nM). For the AIE probe, an excitation wavelength λex=405 nm and a band-pass filter of λ=510-560 nm were used. For Lyso-tracker-Red: λex=561 nm, band-pass filter λ=575-625 nm. Scale bar: 20 μm.

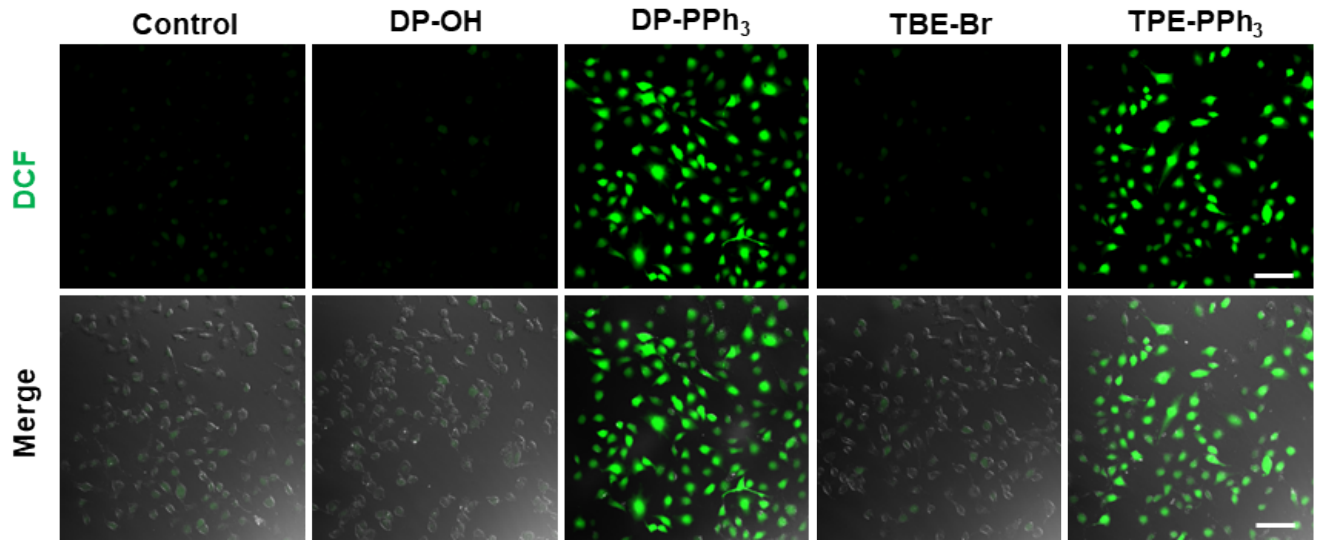


Figure S10. Confocal images of the ROS generation measured by DCF in A549R cells after treatment with **DP-OH** (2 μM), **DP-PPh$_3$** (2 μM), **TPE-Br** (4 μM) or **TPE-PPh$_3$** (4 μM) for 24 h. For DCF: λex=488 nm, emission was detected at λ=510-560 nm. Scale bar =100 μm.

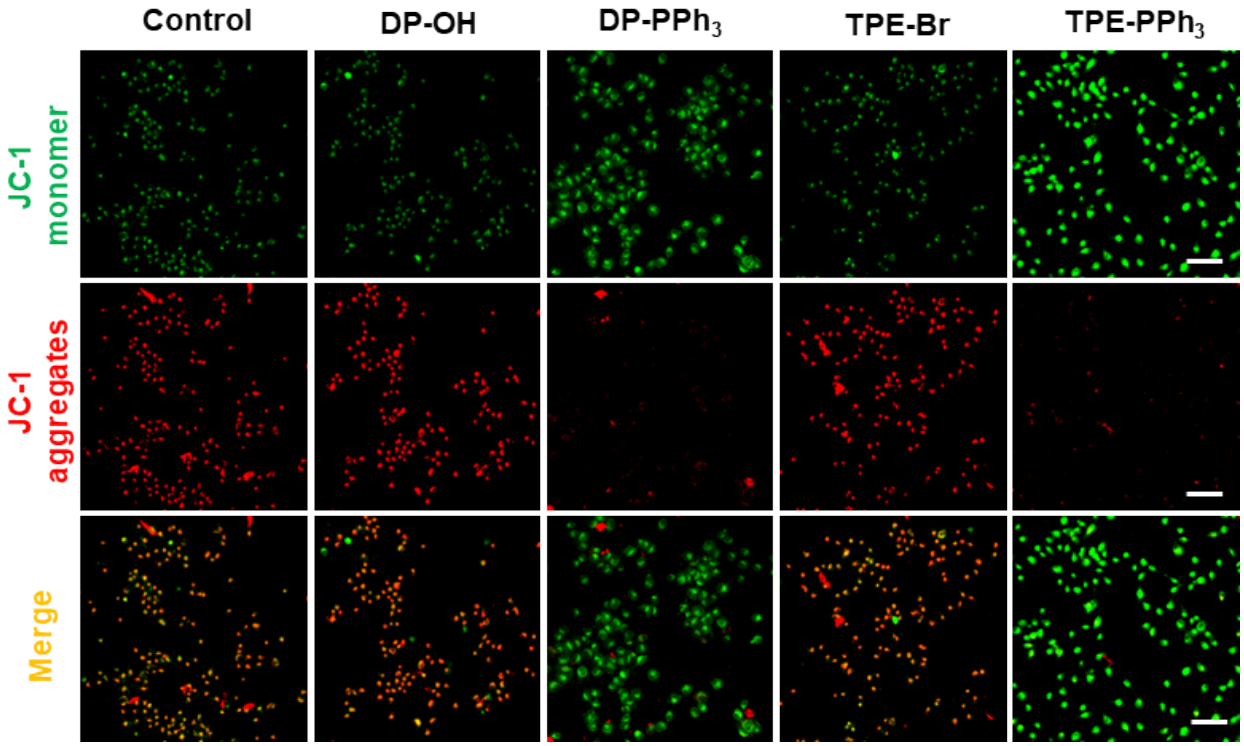


Figure S11. Confocal images of JC-1-labeled A549R cells treated with **DP-OH** (2 μM), **DP-PPh$_3$** (2 μM), **TPE-Br** (4 μM) or **TPE-PPh$_3$** (4 μM) at 37 °C for 24 h. For JC-1: λex = 488 nm, λem = 530 nm (green) and 590 nm (red). Scale bar =100 μm.

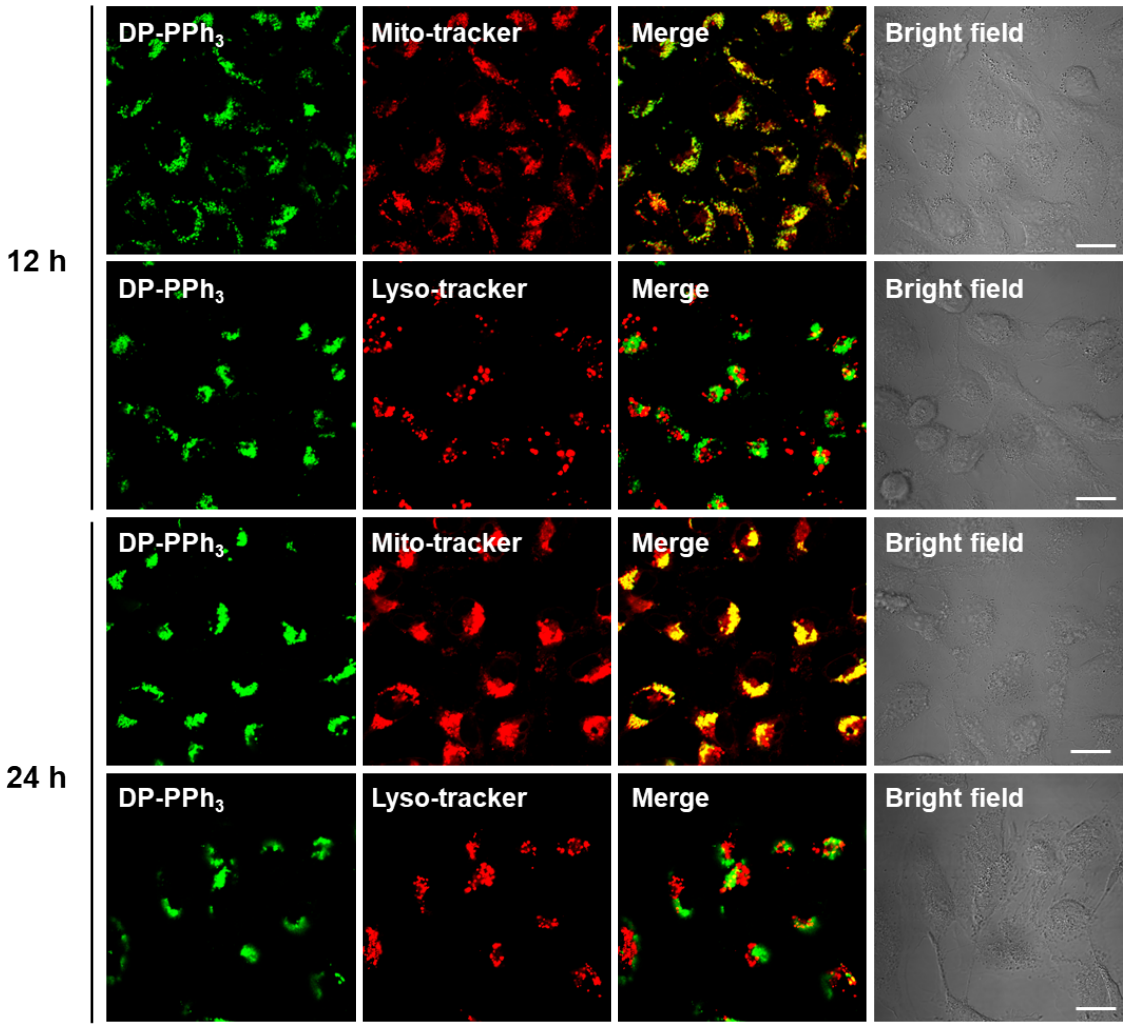


Figure S12. Time-dependent confocal images of A549R cells treated with **DP-PPh$_3$** (2 μM) for 12 h or 24 h, respectively. The cells were co-stained with Mito-tracker-Red (200 nM) or Lyso-tracker-Red (50 nM). For the **DP-PPh$_3$** probe, an excitation wavelength λex=405 nm and a band-pass filter of λ=510-560 nm were used. For Mito-tracker-Red or Lyso-tracker-Red: λex=561 nm, band-pass filter λ=575-625 nm. Scale bar: 20 μm.

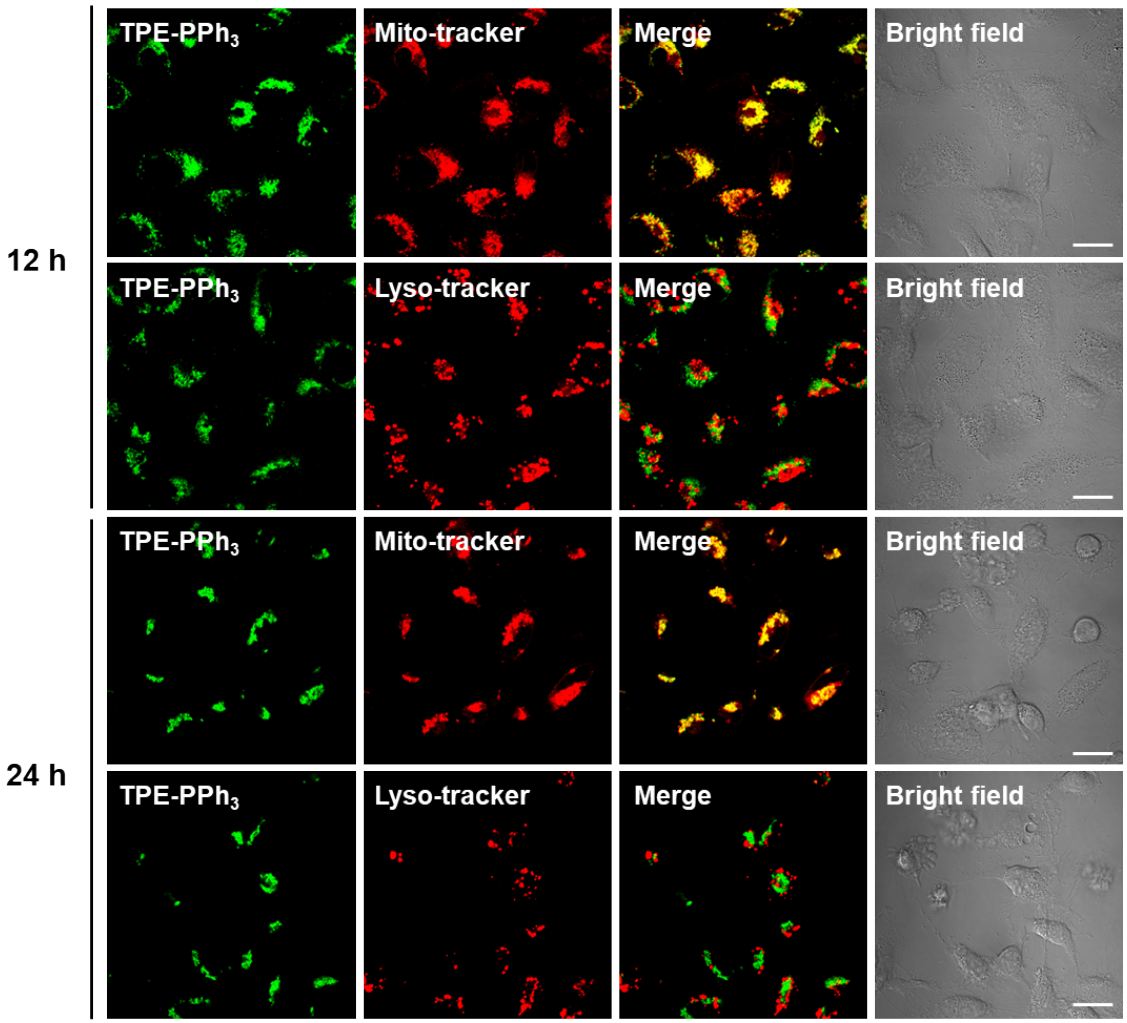


Figure S13. Time-dependent confocal images of A549R cells treated with **TPE-PPh$_3$** (4 μM) for 12 h or 24 h, respectively. The cells were co-stained with Mito-tracker-Red (200 nM) or Lyso-tracker-Red (50 nM). For the **TPE-PPh$_3$** probe, an excitation wavelength λex=405 nm and a band-pass filter of λ=510-560 nm were used. For Mito-tracker-Red or Lyso-tracker-Red: λex=561 nm, band-pass filter λ=575-625 nm. Scale bar: 20 μm.

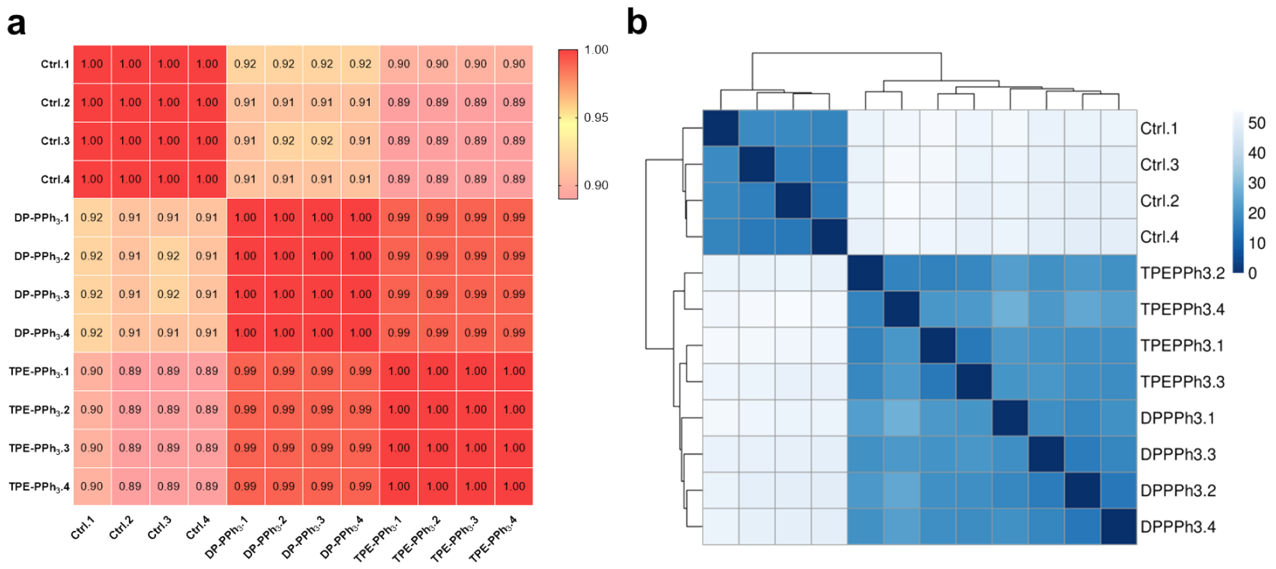


Figure S14. (a) Heatmap diagram of Pearson correlation between samples. (b) Cluster analysis displays the relationship between the samples.

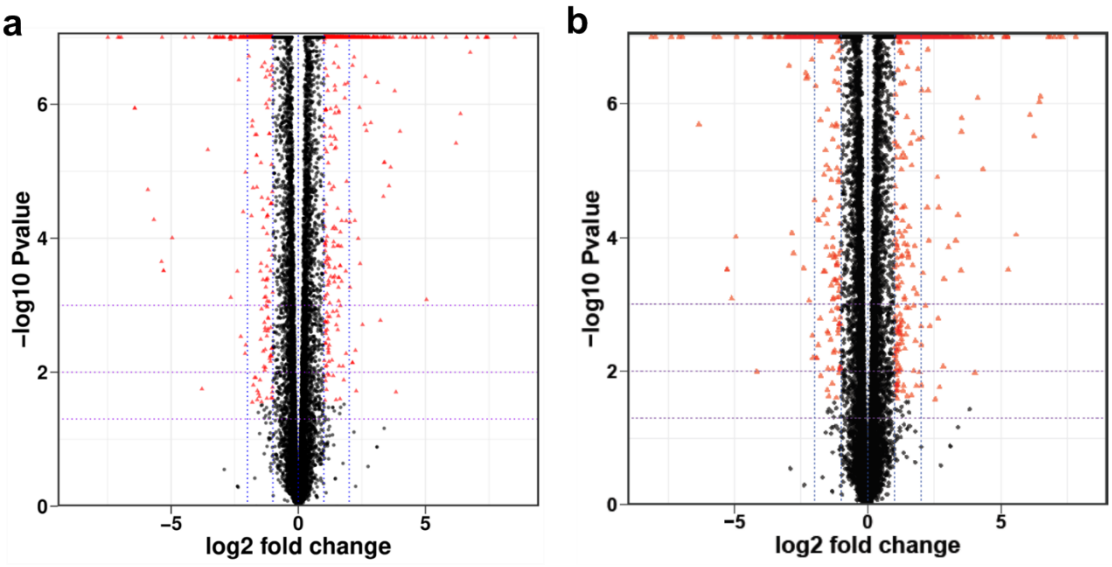


Figure S15. Volcano plot showing the differentially expressed genes between (a) **DP-PPh$_3$**-treated (2 μM), (b) **TPE-PPh$_3$**-treated (4 μM) A549R cells, and untreated A549R cells. The x-axis represents the log 2-fold changes (FC) of genes and the y-axis represents the –log10 P values for the two groups. Each dot represents a gene. The red-colored fields represent the up-regulated and down-regulated genes that met the selection threshold of FC > 2 and $p < 0.05$, respectively.

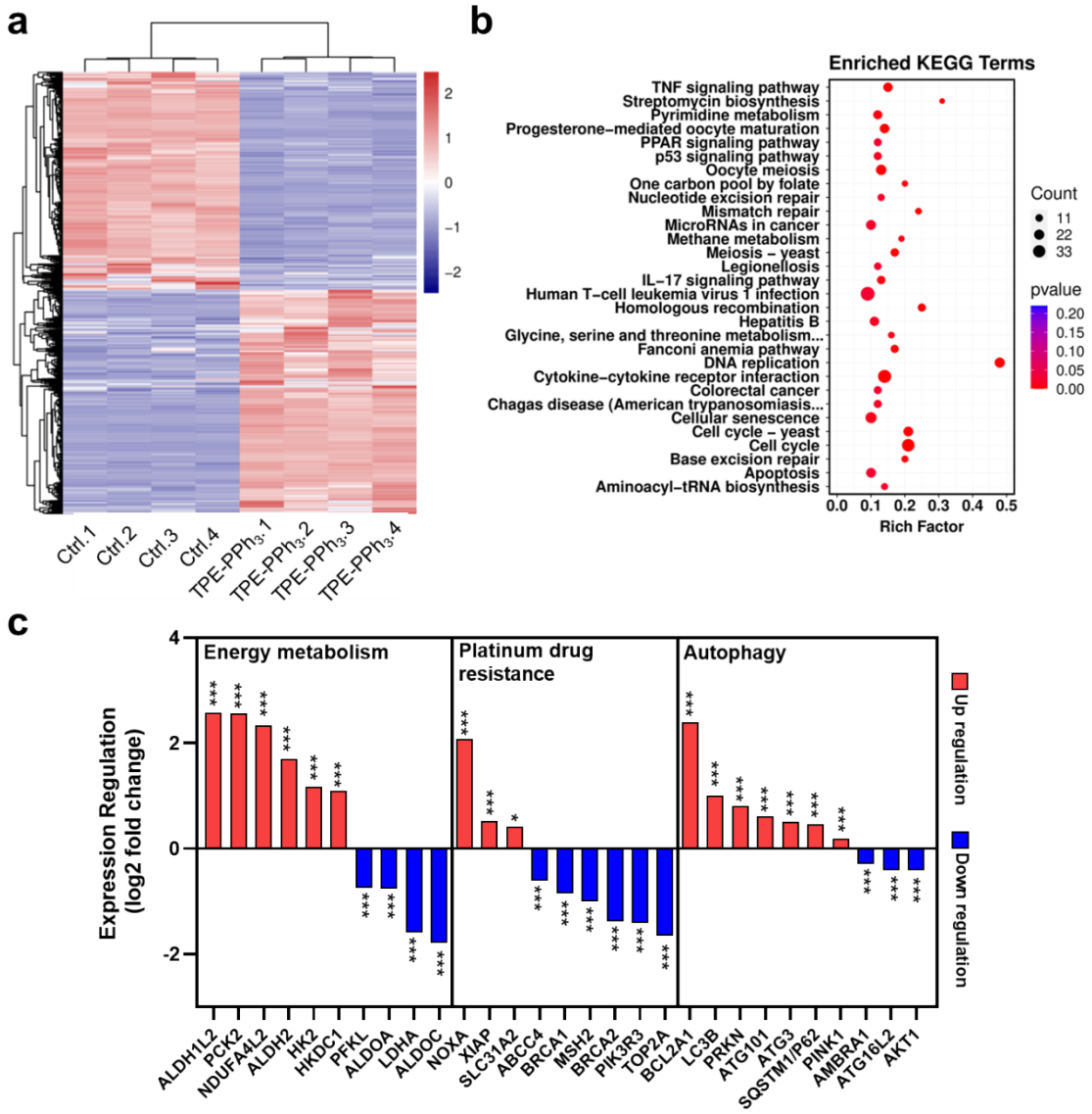


Figure S16. RNA sequence analysis of differential gene expression (a), KEGG enrichment analysis to identify pathways (b) and expression regulations (c) in A549R cells after **TPE-PPh$_3$** treatment (2 μM). *p < 0.05, **p < 0.01, ***p < 0.001.

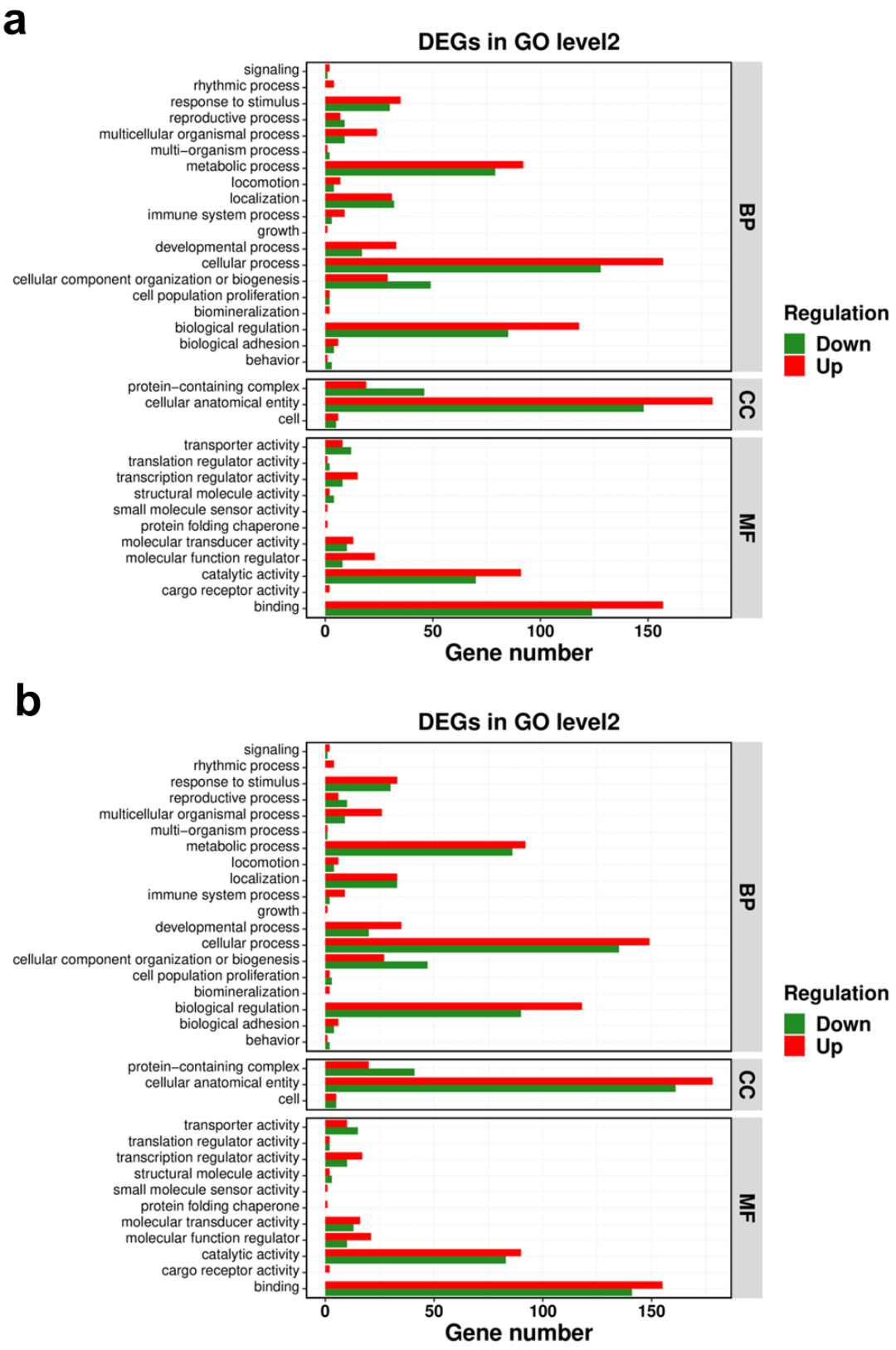


Figure S17. Differential expression genes (DEGs) in Gene Ontology (GO) categorization of biological process (BP), cellular component (CC), and molecular function (MF) in level 2 for the transcriptome induced by (a) **DP-PPh$_3$**-treatment or (b) **TPE-PPh$_3$**-treatment.

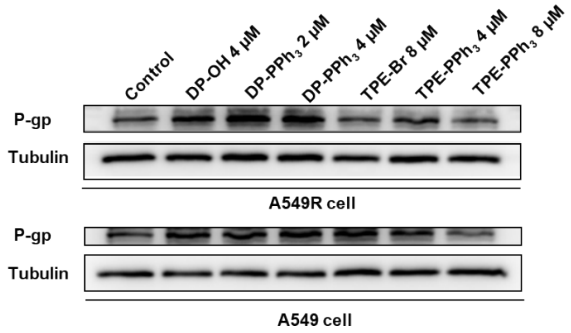


Figure S18. Immunoblotting of P-gp in A549R and A549 cells treated with **DP-OH**, **DP-PPh$_3$**, **TPE-Br**, or **TPE-PPh$_3$** for 48 h.

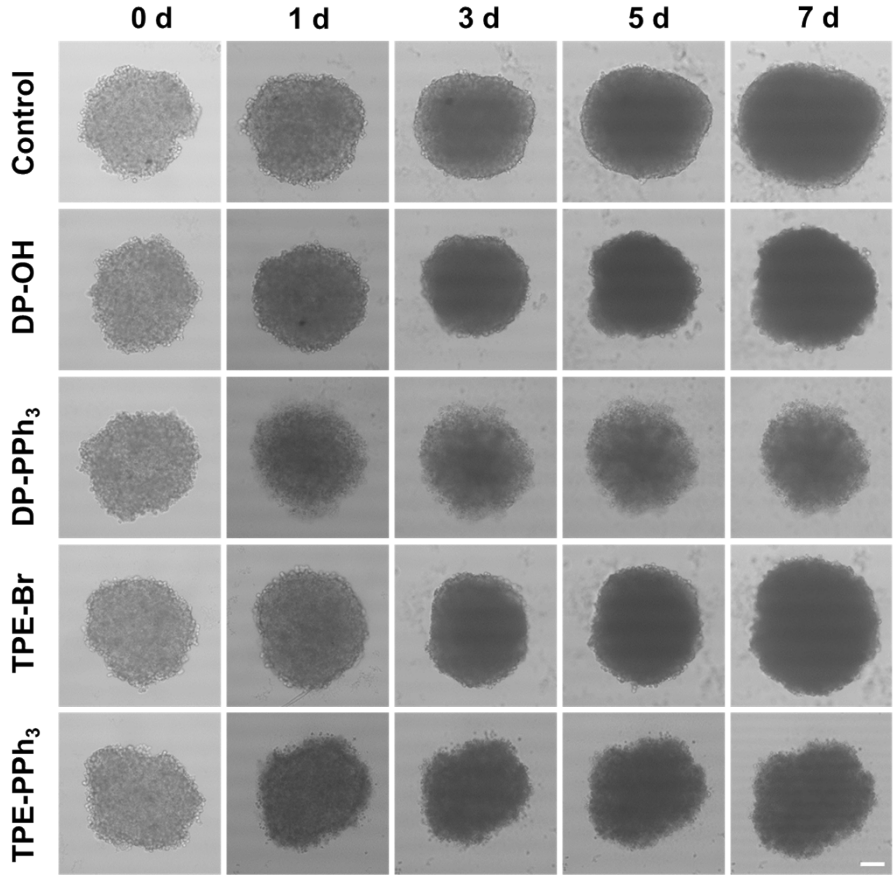


Figure S19. Representative pictures of 3D A549R tumor spheroids treated with **DP-OH** (10 μM), **DP-PPh$_3$** (10 μM), **TPE-Br** (20 μM) or **TPE-PPh$_3$** (20 μM) at different times. Scale bar: 100 μm.

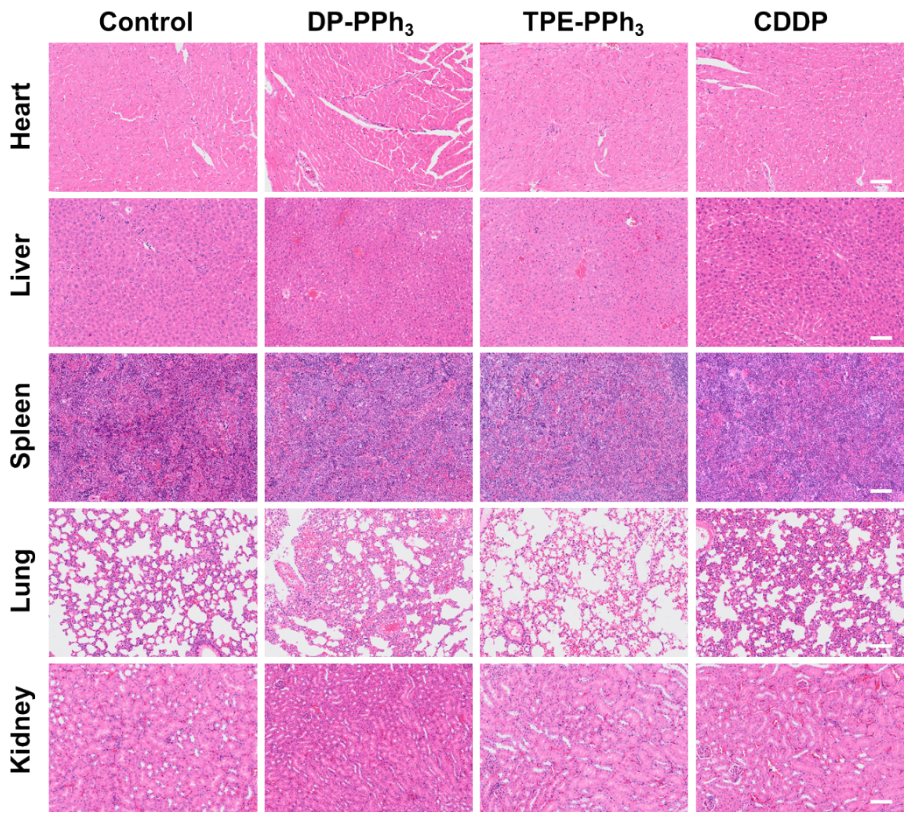


Figure S20. H&E-stained tissues sections of heart, liver, spleen, lung, and kidney collected from mice after treatment with saline, **DP-PPh$_3$**, **TPE-PPh$_3$**, and CDDP, respectively. Scale bar = 100 μm.

Reference

1. W. Zhang, Y. Huang, Y. Chen, E. Zhao, Y. Hong, S. Chen, J. W. Y. Lam, Y. Chen, J. Hou and B. Z. Tang, *ACS Appl. Mater. Interfaces*, 2019, **11**, 10567-10577.
2. M. D. Robinson, D. J. McCarthy and G. K. Smyth, *Bioinformatics*, 2010, **26**, 139-140.
3. G. Yu, L.-G. Wang, Y. Han and Q.-Y. He, *OMICS: A Journal of Integrative Biology*, 2012, **16**, 284-287.
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