Supporting Information

Cooperatively enhanced photothermal-chemotherapy via simultaneously downregulating HSPs and promoting DNA alkylation in cancer cells

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Materials and Instrumentation

All general chemicals for fluorescence detection and organic synthesis including chlorambucil, N, N-Diisopropylethylamine (DIEA), L-Glutathione, triphosgene and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased

from Energy Chemical Co. and all of the solvents were analytically pure. Singlet Oxygen Sensor Green (SOSG) was obtained from life technologies. Tris-HCl (1M), pBR322 plasmid DNA, Tris Acetate-EDTA (TAE) buffer, 1% agarose precast gel, DNA Damage Assay Kit, Annexin V-FITC apoptosis Detection Kit and Calcein AM/propidium iodide (PI) Detection Kit were purchased from Beyotime Biotechnology Co., Ltd (China). GSH and GSSG Content Assay Kit were purchased from Solarbio Science & Technology Co., Ltd (China). DNA Damage Detection Kit (SCGE) was purchased from Keygen Biotech Corp., Ltd (China). Human breast cancer MCF-7 cells, Human cervical cancer Hela cells, Human hepatoellular carcinomas HepG2 cells, Mouse breast cancer 4T1 cells and Mouse fibroblast 3T3 cells were purchased from Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences. ^{1}H-NMR and ^{13}C-NMR spectra of all compounds were performed with Bruker Avance III 400, 500 and 600 spectrometers. Mass spectrometric (ESI-HRMS) data were carried out using LTQ Orbit rap XL instruments. Absorption and emission spectra for Cy-NH_2 and Cy-S-S-Cbl were performed with a Lambda 35 UV-visible spectrophotometer (PerkinElmer) and a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018), respectively. Fluorescence quantum yield was obtained with the HAMAMATSU absolute fluorescence quantum yield spectrometer (Serial No. C11347). Confocal laser scanning microscope (CLSM) images were performed on Olympus FV3000 confocal laser scanning microscope. The gel scanner was obtained with the ProteinSimple (FluorChem). This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals

published by the US National Institutes of Health (8th edition, 2011). The animal protocol was approved by the local research ethics review board of the Animal Ethics Committee of Dalian University of Technology. Small animals' fluorescence imaging was carried out by NightOWL II LB983 living imaging system.

Synthesis

Synthesis of Cy-Cl

Cy-Cl was prepared according to the literature methods.[1]

Synthesis of Cy-NH_2

Cy-Cl (0.374 g, 0.5 mmol) and 4-aminothiophenol (0.25 g, 2 mmol) were dissolved in 10 mL anhydrous DMF under N_2 atmosphere and stirred at room temperature for 4 h. Then the mixture was poured into 150 mL of ether with stirring. The obtained solid was precipitated and purified by column chromatography (DCM/CH_3OH = 7: 1, v/v). The mainly blue-black solid was collected (Yield = 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, *J* = 14.1 Hz, 2H), 7.55 (d, *J* = 7.3 Hz, 2H), 7.46 – 7.36 (m, 4H), 7.23 (t, *J* = 7.2 Hz, 2H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.52 (d, *J* = 8.3 Hz, 2H), 6.34 (d, *J* = 14.2 Hz, 2H), 4.17 (d, *J* = 6.1 Hz, 4H), 2.72 (s, 4H), 1.83 – 1.67 (m, 10H), 1.53 (s, 12H), 1.45 (d, *J* = 7.7 Hz, 2H), 1.20 (d, *J* = 22.5 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.63, 152.26, 147.45, 145.53, 142.13, 141.03, 133.28, 128.48, 128.00, 124.74, 122.28, 120.91, 115.03, 111.34, 101.54, 50.68, 48.68, 43.58, 27.35, 26.04, 25.81, 22.50, 20.57. ESI-HRMS, calcd for ($C_{44}H_{52}N_3O_6S_3Na$) m/z: $[M- Na]^-$: 814.3024, found: 814.3027.

Synthesis of Cy-S-S-OH

Cy-NH_2 (0.2 g, 0.24 mmol) and N, N-Diisopropylethylamine (DIEA, 0.078 g, 0.6 mmol)

were dissolved in 10 mL anhydrous DCM under N_2 atmosphere and stirred at ice bath temperature for 10 min. Triphosgene (0.036 g, 0.12 mmol) in 5 mL anhydrous DCM was added dropwise to Cy-NH_2 solution and stirred at ice bath temperature for 2-3 h. After removal of unreacted phosgene gas by flushing argon gas, a solution of 2, 2'-dithiodiethanol (0.185 g, 1.2 mmol) in anhydrous DCM was added to the mixture and the reaction mixture was stirred overnight at room temperature. After removing the solvent under reduced pressure, the crude product was purified by silica gel chromatography (DCM/CH_3OH = 10: 1, v/v). The mainly blue-green solid was collected (Yield = 50%).^{1}H NMR (600 MHz, DMSO-d_6) δ 9.71 (s, 1H), 8.63 (d, J = 14.1 Hz, 2H), 7.53 (d, J = 7.4 Hz, 2H), 7.43 (t, J = 8.6 Hz, 4H), 7.38 (t, J = 7.6 Hz, 2H), 7.21 (dd, J = 19.9, 8.1 Hz, 4H), 6.37 (d, J = 14.2 Hz, 2H), 5.76 (s, 1H), 4.27 (t, J = 6.3 Hz, 2H), 4.17 (d, J = 6.9 Hz, 4H), 3.60 (dd, J = 12.0, 6.3 Hz, 2H), 2.97 (t, J = 6.3 Hz, 2H), 2.78 (dd, J = 14.3, 7.8 Hz, 4H), 1.81 – 1.68 (m, 10H), 1.45 (s, 12H), 1.34 (d, J = 9.1 Hz, 2H), 1.23 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.77, 153.10, 149.58, 145.09, 142.09, 141.06, 133.32, 129.67, 128.49, 126.46, 124.83, 122.30, 111.44, 101.75, 62.08, 59.35, 54.88, 50.68, 48.69, 45.46, 45.42, 41.03, 36.79, 31.25, 27.24, 26.08, 25.82, 22.50, 20.46. ESI-HRMS, calcd for ($C_{49}H_{60}N_3O_9S_5Na$) m/z: [M- Na]$^-$: 994.2939, found: 994.2946.

Synthesis of Cy-S-S-Cbl

Chlorambucil (152 mg, 0.5 mmol), DCC (309 mg, 1.5 mmol) and DMAP (24.5 mg, 0.2 mmol) were dissolved in dried DCM (5 mL) under N_2 atmosphere, and the mixture was stirred at 0 °C. After 4 h, Cy-S-S-OH (305 mg, 0.3 mmol) was added to the solution,

and the resulting solution was stirred for 48 h at room temperature in the dark. After removing the solvent under reduced pressure, the crude product was purified by silica gel chromatography (DCM/CH_3OH = 9: 1, v/v). The mainly dark green solid was collected (Yield = 50%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.60 (d, *J* = 14.0 Hz, 2H), 7.50 (d, *J* = 7.3 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 4H), 7.36 (t, *J* = 7.6 Hz, 2H), 7.19 (dd, *J* = 18.8, 7.8 Hz, 4H), 6.97 (d, *J* = 7.9 Hz, 2H), 6.62 (d, *J* = 7.9 Hz, 2H), 6.34 (d, *J* = 14.2 Hz, 2H), 4.24 (t, *J* = 6.2 Hz, 2H), 4.20 (t, *J* = 6.1 Hz, 2H), 4.15 (s, 4H), 3.68 – 3.61 (m, 8H), 2.94 (dd, *J* = 14.5, 6.6 Hz, 4H), 2.74 (s, 4H), 2.41 (t, *J* = 7.5 Hz, 2H), 2.23 (t, *J* = 7.2 Hz, 2H), 1.77 – 1.69 (m, 10H), 1.41 (s, 14H), 1.22 (d, *J* = 11.3 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.07, 172.29, 153.61, 150.13, 145.60, 144.99, 142.58, 141.57, 137.56, 133.84, 129.94, 129.80, 129.01, 126.97, 125.36, 122.81, 120.07, 112.42, 111.94, 102.26, 72.75, 62.50, 62.02, 60.71, 52.71, 51.17, 49.21, 44.17, 41.67, 37.27, 36.97, 33.69, 33.32, 27.76, 26.99, 26.58, 26.34, 22.99, 21.00. ESI-HRMS, calcd for ($C_{63}H_{77}Cl_2N_4O_{10}S_5Na$) m/z: $[M- Na]^-$: 1279.3626, found: 1279.3650.

UV-vis Spectra and Fluorescent Emission Spectra

The UV-vis spectrum of Cy-NH_2 and Cy-S-S-Cbl was obtained using UV-visible spectrophotometer and fluorescent emission spectrum of Cy-NH_2 and Cy-S-S-Cbl in PBS buffer (0.01 M PBS, PBS/DMSO = 9:1, v/v) was acquired with fluorescence spectrometer (FLS-980, Edinburgh, UK) under 690 nm excitation, while various amounts of GSH and other analytes were added for measurements.

Fluorescence Quantum Yield

The absolute fluorescence quantum yield apparatus was used to measure

fluorescence quantum yield of Cy-NH_2 and Cy-S-S-Cbl in CH_3OH, respectively. The excitation wavelength was 730 nm.

Femtosecond Transient Absorption Spectra

The femtosecond pump-probe TA measurements were performed using a regenerative amplified Ti:sapphire laser system (Coherent; 800 nm, 70 fs, 6 mJ/pulse, and 1 kHz repetition rate) as the laser source and a Femto-100 spectrometer (Time-Tech LLC). Briefly, the 800 nm output pulse from the regenerative amplifier was split in two parts with a 50% beam splitter. The transmitted part was used to pump a TOPAS Optical Parametric Amplifier (OPA) which generated a wavelength-tunable laser pulse from 250 nm to 2.5 μm as pump beam. The reflected 800 nm beam was split again into two parts. One part with less than 10% was attenuated with a neutral density filter and focused into a crystal to generate a white light continuum (WLC) used for probe beam. The probe beam was focused with an Al parabolic reflector onto the sample. After the sample, the probe beam was collimated and then focused into a fiber-coupled spectrometer with CMOS sensors and detected at a frequency of 1 KHz. The intensity of the pump pulse used in the experiment was controlled by a variable neutral-density filter wheel. The delay between the pump and probe pulses was controlled by a motorized delay stage. The pump pulses were chopped by a synchronized chopper at 500 Hz and the absorbance change was calculated with two adjacent probe pulses (pump-blocked and pump-unblocked). The linear polarization angle difference between the pump and probe light keeps magic angle (54.7°) to record the isotropic response.

The GSH-activable Cy-S-S-Cbl

The fluorescence changes of Cy-S-S-Cbl (10 μM) were detected over time with different concentrations of GSH (0 mM, 0.02 mM, 0.04 mM, 0.08 mM, 0.16 mM, 0.32 mM, 0.64 mM, 1.28 mM, 2.56 mM) in PBS buffer (PBS/DMSO = 8/2, v/v). Moreover, the fluorescence changes of Cy-S-S-Cbl mixed GSH (1.5 mM) were detected every 15 min.

Selectivity of Cy-S-S-Cbl

The selectively activated performance of Cy-S-S-Cbl was investigated with the treatment of others common metal ions and amino acids, such as K^+, Ca^{2+}, Na^+, Mg^{2+}, Ala, Arg, Glu, Ser, Thr, Trp, Tyr, DTT, Cys, Hcy and GSH (2.5 mM).

Photothermal Performance Detection

Cy-NH_2 was irradiated by 808 nm light at different power densities (0 W/cm^2, 0.1 W/cm^2, 0.2 W/cm^2, 0.4 W/cm^2 and 0.6 W/cm^2) to discuss the effect of power density on conversion efficiency, respectively. Next, different concentrations of Cy-NH_2 were prepared including 0 μM, 10 μM, 20 μM and 40 μM and then irradiated by 808 nm light (0.5 W cm^{-2}), respectively. A thermometer submerged in the solution was used to monitor the temperature during 300 s. Besides, Cy-NH_2 and Cy-S-S-Cbl solutions were imaged by infrared thermal camera. Next, the same concentration of Cy-NH_2, Cy-S-S-Cbl or ICG was irradiated by 808 nm light (0.5 W cm^{-2}), respectively. To measure the conversion efficiency, 40 μM Cy-NH_2 or Cy-S-S-Cbl was exposed to 808 nm light (0.5 W cm^{-2}) irradiation for 5 min, and then the irradiation was removed for cooling down to room temperature. The temperature of the solution was recorded at an interval of 30 s during this process. The photothermal conversion efficiencies (η) were measured

according to a previously described method:[2]

$$\eta = \frac{hs(T_{Max}-T_{Surr})-Q_{Dis}}{I(1-10^{-A_{808}})}$$ Equation S1

h is the heat transfer coefficient, *s* is the surface area of the container, and the value of *hs* is determined from the equation (2). Q_{Dis} represents heat dissipated from the light mediated by the solvent and container. *I* is the light power and *A* is the absorbance at 808 nm.

$$\mathrm{hs} = \frac{mC}{\tau_s}$$ Equation S2

m is the mass of the solution containing the photoactive material, *C* is the specific heat capacity of the solution, and τ_s is the associated time constant, which can be determined from equation (3).

$$\mathrm{t} = -\tau_s In(\theta)$$ Equation S3

θ is a dimensionless parameter, known as the driving force temperature, as calculated using equation (4).

$$\theta = \frac{T-T_{Surr}}{T_{Max}-T_{Surr}}$$ Equation S4

T_{max} and T_{Surr} are the maximum steady state temperature and the environmental temperature, respectively.

Singlet Oxygen Detection

The singlet oxygen ($^1O_2$) generated was measured using 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) and singlet oxygen sensor green (SOSG). For ABDA assay, Cy-NH_2 and Cy-S-S-Cbl was mixed ABDA were prepared in PBS buffer (0.01 M), respectively. Then the cuvette was exposed to 808 nm light (0.1 W cm^{-2}), and the absorption spectrum was observed immediately after each irradiation. For SOSG assay, the $^1O_2$ generation efficiency of blank, Cy-NH_2 and Cy-S-S-Cbl were evaluated

by singlet oxygen capture agent SOSG in CH_3OH, respectively. Briefly, the fluorescence intensity of SOSG at 525 nm was adjusted to about 100, and then Cy-NH_2 or Cy-S-S-Cbl was added to the cuvette, respectively. The mixture was then placed in a cuvette and irradiated with 808 nm light source (0.1 W cm^{-2}) for different time 0-300 s, and the corresponding emission spectra was measured immediately.

Cell Culture Conditions and Cellular Uptake

Human cervical cancer Hela cells, Human breast cancer MCF-7 cells, Human hepatoellular carcinomas HepG2 cells, Mouse breast cancer 4T1 cells and Mouse fibroblast 3T3 cells were maintained in DMEM medium. All of them were supplemented with 1% penicillin-streptomycin and 15% FBS, and the atmosphere was of 5% CO_2 and 95% air at 37 ºC. Cancer cells were incubated with Cy-S-S-Cbl or Cy-S-S-Cbl + NEM at 37 °C for 3 h, respectively. After cells were washed with PBS for twice, the confocal fluorescence imaging was performed and images were collected (excited at 640 nm, monitored at 740-800 nm).

***In Vitro* GSH Assays**

The Hela, MCF-7, HepG2, 4T1 or 3T3 cells was collected into multiple PE tubes (every tube about 10 mg) to detect the content of GSH according to the Reduced Glutathione (GSH) Content Assay Kit, respectively. After incubated with PBS, Cy-NH_2 or Cy-S-S-Cbl for 4 h, the content of GSH and GSSG of Hela and 4T1 cells were detected according to GSH and GSSG Content Assay Kit.

Dead/Live Cell Co-staining

Firstly, Hela cells were incubated in DMEM medium for 24 h, then exposed to

different following treatments: group 1, untreated (Control); group 2, irradiated with 808 nm light (0.5 W cm^{-2}) for 5 min; group 3, incubated with 40 μM Cy-S-S-Cbl for 4 h at 37 ºC; group 4, incubated with 40 μM Cy-S-S-Cbl for 4 h at 37 ºC and irradiated with 808 nm light (0.5 W cm^{-2}) for 5 min; group 5, incubated with 2.5 mM NEM for 8 h and then incubated with 40 μM Cy-S-S-Cbl for 4 h at 37 ºC; group 6, incubated with 2.5 mM NEM for 8 h and then incubated with 40 μM Cy-S-S-Cbl for 4 h at 37 ºC and irradiated with 808 nm light (0.5 W cm^{-2}) for 5 min. After different treatments, Calcein AM and Propidium Iodide co-staining was performed. The excitation wavelength of Calcein AM and Propidium Iodide were 488 nm, and emission signals were collected from 505 to 545 nm with green fluorescence and from 600 to 700 nm with red fluorescence, respectively. The same method was applied to other four kinds of cells treated with Cy-S-S-Cbl to compare the difference killing effect on each group.

Flow Cytometric Assay

For apoptosis analysis, Hela cells (1×10^6 cells per well) were seeded in 6-well plates and cultured overnight. Then cells were incubated with different conditions and divided into six groups: group 1, untreated; group 2, irradiated with 808 nm light (0.5 W cm^{-2}) for 5 min; group 3, incubated with 30 μM Cy-NH_2 at 37 ºC for 4 h; group 4, incubated with 30 μM Cy-NH_2 for 4 h at 37ºC and irradiated with 808 nm light (0.5 W cm^{-2}) for 5 min; group 5, incubated with 30 μM Cy-S-S-Cbl for 4 h at 37ºC; group 6, incubated with 30 μM Cy-S-S-Cbl for 4 h at 37ºC and irradiated with 808 nm light (0.5 W cm^{-2}) for 5 min. After incubation for another 24 h, the cells were centrifuged at 1300 rpm for 5 min and resuspended in Annexin V binding buffer (10 mM HEPES, 150 mM NaCl, 5

mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$). Then cells were stained with Annexin V-FITC/propidium iodide (PI) Apoptosis Detection Kit according to the manual. Finally, cells were analyzed on a flow cytometer.

***In Vitro* Cytotoxicity Studies**

Hela cells were seeded onto 96-well plates at 10000 cells per well and incubated at 37 ºC for 24 h. Different concentrations of Cy-NH_2, Cy-S-S-Cbl and Cy-S-S-Cbl + NEM in DMEM medium were added to the wells, respectively. Then, the cells were further incubated for 4 h and were subjected with 808 nm light (0.5 W cm^{-2}) for 5 min. After 24 h, MTT solution (5 mg mL^{-1}) was added in DMEM medium to each well. The solution in each well was removed and then added 100 μL DMSO to each well after incubating the cells for 4 h. The absorbance at 490 nm was measured with a Bio-Rad microplate reader. The cell viability was obtained by the following equation:

$$\text{cell viability (\%)} = \left(OD_{ps} - \frac{OD_{black\ control}}{OD_{control}} - OD_{black\ control}\right) \times 100\%$$

For dark toxicity of Cy-NH_2, Cy-S-S-Cbl and Cy-S-S-Cbl + NEM, no light irradiation was applied to this experiment, and all other steps were the same. The same method was used to analysis the cell cytotoxicity on MCF-7 cells, HepG2 cells, 4T1 cells and 3T3 cells after treated with Cy-S-S-Cbl, respectively.

Interactions with Plasmid DNA

A solution containing 100 ng/μL of pBR322 DNA in PBS was first incubated with 10 mM GSH at 37 °C for 8 h and then incubated with Cy-S-S-Cbl at different concentrations (25, 50 and 100 μM) or Cy-NH_2 (100 μM) at 37°C for 4 h. An untreated sample used as a reference and Cbl (100 μM) were also incubated at 37°C for 4 h. After

incubation, the mobility of the DNA-3 samples was analyzed with no further treatment by gel electrophoresis on a 1% agarose precast gel (Beyotime, China) at 150 V at 25°C for 30 min in Tric-acetate/EDTA buffer. The bands were analyzed with a ProteinSimple gel scanner. The same method was used to analysis other groups treated with Cy-S-S-Cbl or Cy-NH_2 with/without 808 nm light irradiation.

γ-H2AX Immunofluorescence Assay

Hela cells were incubated with PBS, Cy-NH_2 or Cy-S-S-Cbl for 4 h, respectively. After cells were washed with PBS, the cells were treated according to the DNA Damage Assay Kit (Beyotime). The confocal fluorescence imaging was performed and images were collected. The excitation wavelength of DAPI and γ-H2AX were 405 nm and 488 nm, and emission signals were collected from 415 to 485 nm with blue fluorescence and from 500 to 545 nm with red fluorescence, respectively.

Alkaline Comet Assay

Hela cells were incubated with PBS or different concentrations of Cy-S-S-Cbl (10 μM, 20 μM and 40 μM) for 4 h, respectively. After cells were washed with PBS, the cells were treated according to the DNA Damage Detection Kit (Keygen). Finally, images were analyzed using a confocal laser scanning microscope. The excitation wavelength of PI was 488 nm and the emission signal was collected from 600 to 680 nm with red fluorescence.

Intracellular HSP70 Expression

Hela cells were seeded in 10 cm cell culture dish (Nest) and cultured with medium containing Cy-S-S-Cbl or Cy-NH_2 (20 μM), respectively. After 4 h of incubation at

37°C in the dark, the cells were irradiated with 808 nm light for 5 min (0.5 W/cm^2). After 12 h of incubation, cells were lysed by RIPA lysis buffer with protease and phosphatase inhibitor on ice for 20 min. After measurement of protein concentration by BCA assay, equal amounts of protein were added to each lane of SDS-PAGE gel for electrophoresis and then transferred onto polyvinylidene fluoride (PVDF) membranes. The membrane was incubated with HSP70 primary antibodies incubation at 4°C overnight and then incubated by peroxidase-labeled goat anti-rabbit HRP secondary antibodies for 2 h at room temperature. The immunoblots were visualized by FluorChem E (ProteinSimple Co, Ltd., United States) with BeyoECL Plus (Beyotime Biotechnology Co., Ltd., Shanghai).

***In Vivo* Fluorescence Imaging**

The female SCID/BALB/c-nude mice, 5-6 weeks of age, were obtained from Liaoning Chang Sheng Biotechnology Co., Ltd. Then, 1×10^6 Hela cells were injected subcutaneously into the selected armpit positions to establish the solid tumor model of mice. After about 18 days, tumors grew to about 130 mm^3 in volume before used for *in vivo* imaging and phototherapy. For *in vivo* tumor imaging, Hela tumor-bearing SCID/BALB/c-nude mice were divided into two groups: 1) Injecting Cy-S-S-Cbl (300 μM/L, 100 μL) into the mice; 2) Preinjecting NEM (2 mM/L, 50 μL) into the mice for 6 h and then Cy-S-S-Cbl (300 μM/L, 100 μL) was injected intratumorally. The fluorescence signals were monitored at different post-injection time (0.5 h, 1 h, 2 h, 4 h and 6 h). The excitation wavelength was 740 nm, and the collected emission wavelength was 810-830 nm.

In Vivo Photothermal Imaging

For *in vivo* photothermal imaging (IR), Hela tumor-bearing SCID/BALB/c-nude mice were divided three groups: 1) Injecting PBS (100 μL) into the mice; 2) Preinjecting NEM (2 mM/L, 50 μL) into the mice for 6 h and then Cy-S-S-Cbl (300 μM/L, 100 μL) was injected. 3) Injecting Cy-S-S-Cbl (300 μM/L, 100 μL) into the mice. Then, the tumor sites were irradiated with 808 nm light (0.5 W cm^{-2}) for 8 min after 4 h postinjection and the temperature changes of the tumor site were recorded by IR thermal camera every 60 s.

In Vivo Tumor Therapy

Hela tumor-bearing SCID/BALB/c-nude mice were randomly divided into five groups (n = 5 per group), including group 1, “PBS”; group 2, “PBS + light”; group 3, “Cbl”; group 4, “Cy-S-S-Cbl”; group 5, “Cy-S-S-Cbl + light”, respectively. On day 0, PBS (100 μL) were injected intratumorally in group “PBS” and “PBS + light”. At the same time, mice of group “Cbl”, “Cy-S-S-Cbl”, and “Cy-S-S-Cbl + light” were injected with each of the corresponding drugs (300 μM/L, 100 μL) in the same way, respectively. After injection for 4 h, group “PBS + light”, and “Cy-S-S-Cbl + light” were continuously irradiated with 808 nm light (0.5 W cm^{-2}) for 8 min and group “PBS”, “Cbl” and “Cy-S-S-Cbl” without subsequent light irradiation. In the following 21 days, the tumor volume of all mice was measured every three days using a vernier caliper. Then, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were used to calculate the tumor volume, as follows: tumor volume = width × width × length/2. Relative tumor volume was calculated as V/V_0 (V_0 was the initial

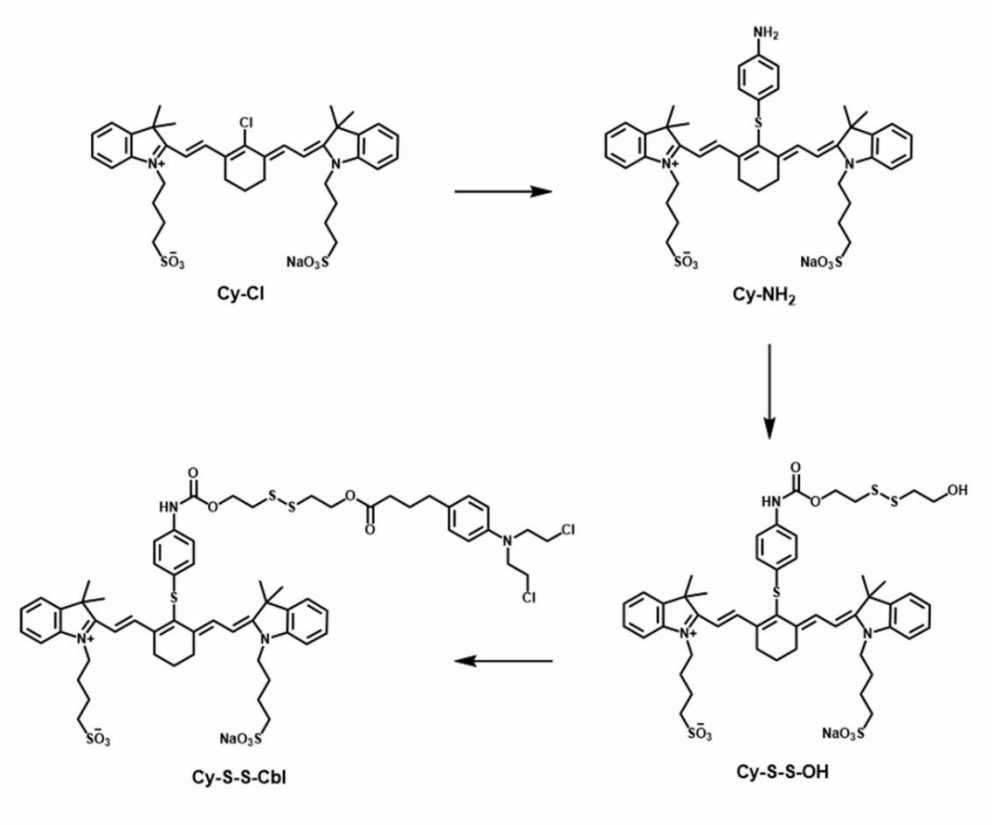
tumor volume).

In Vivo Biosafety Assay

The *in vivo* biosafety was evaluated by the body weight of all mice and H&E slice histological analysis major organs. After 21 d post-treatment, the mice were euthanized, and main organs including heart, liver, spleen, lung, kidneys and tumor were harvested for histological analysis by means of hematoxylin-eosin (H&E) staining.

Statistical Analysis

Data were expressed as mean ± standard deviation. Student's t test was used to evaluate the statistical significance. P values < 0.05 were regarded statistically significant (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).



Scheme S1. Synthesis routes of Cy-S-S-Cbl.

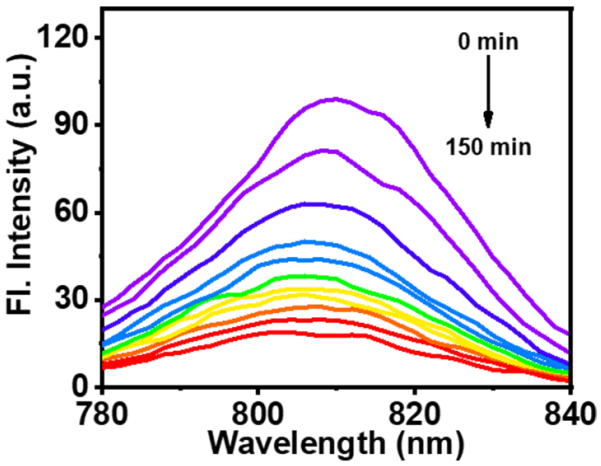


Figure S1. The emission changes of Cy-S-S-Cbl (10 μmol/L) in the presence of GSH (2.5 mmol/L, 250 equiv.) in PBS buffer solutions (0.01 M PBS, PBS/DMSO = 8:2 v/v) over time.

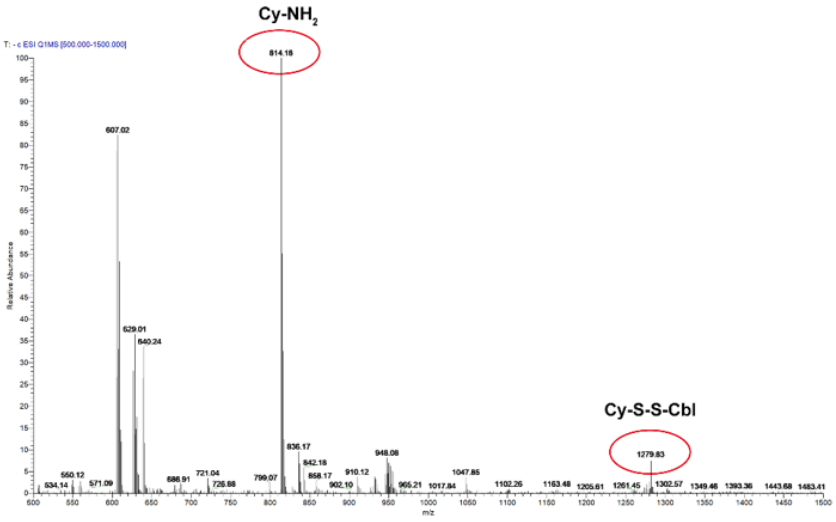


Figure S2. ESI-MS of the reaction solution of Cy-S-S-Cbl (10 μM) with GSH (2.5 mM).

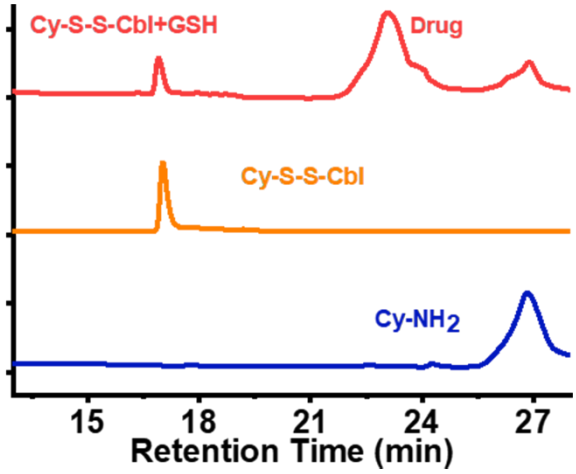


Figure S3. The HPLC analysis of Cy-NH_2, Cy-S-S-Cbl and Cy-S-S-Cbl with GSH for 1 h at 37 °C. The retention time at 16.9 min corresponds to Cy-S-S-Cbl and the retention time at 26.8 min corresponds to Cy-NH_2. The signals were monitored at 254 nm.

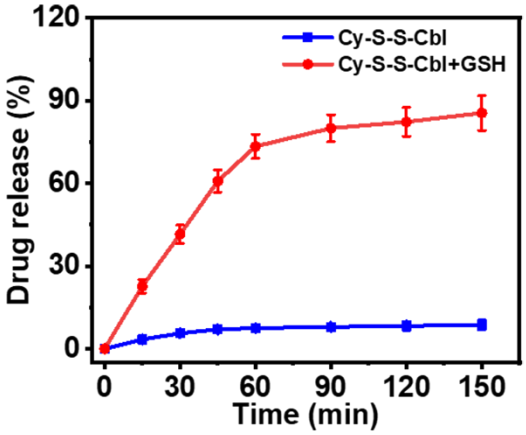


Figure S4. Drug release rate of Cy-S-S-Cbl with or without GSH in 150 min.

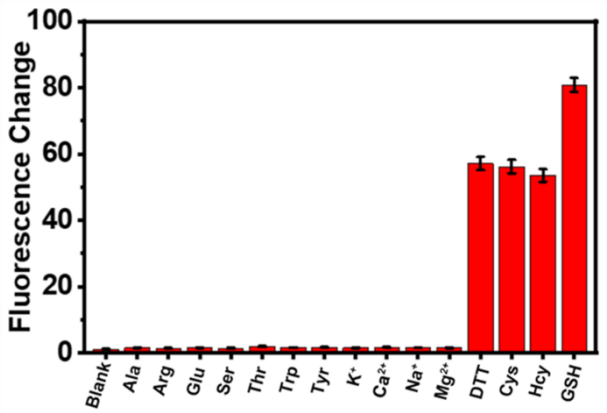


Figure S5. Fluorescence responses of Cy-S-S-Cbl (10 μM) to diverse analytes: Control (blank), Ala (1 mM), Arg (1 mM), Glu (1 mM), Ser (1 mM)), Thr (1 mM), Trp (1 mM), Tyr (1 mM), KCl (1 mM), $CaCl_2$ (1 mM), NaCl (1 mM), $MgCl_2$ (1 mM), DTT (1 mM), Cys (1 mM), Hcy (1 mM) and GSH (1 mM). The results are the mean ± standard deviation of three separate measurements. λ_{ex}=680 nm.

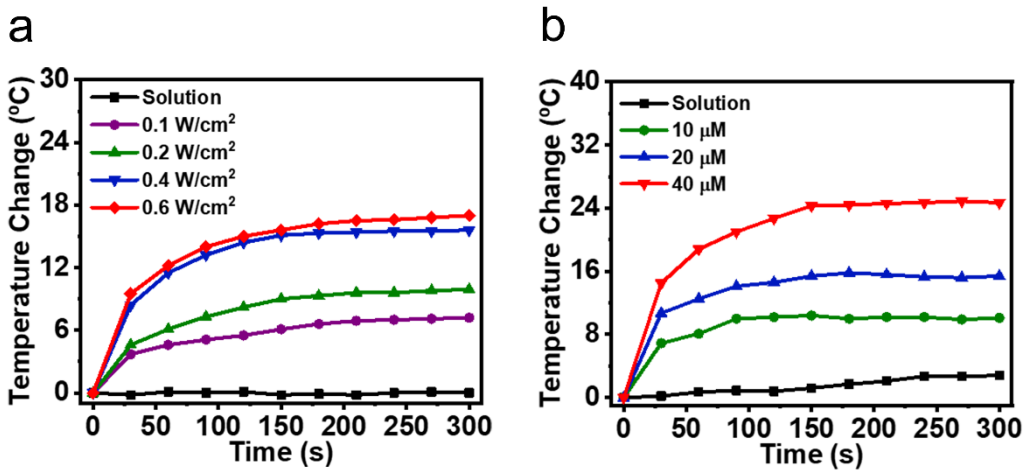


Figure S6. (a) Photothermal heating curves for the change of temperature with time when Cy-NH_2 (40 μM) was irradiated by 808 nm light at different power densities (0 W/cm^2, 0.1 W/cm^2, 0.2 W/cm^2, 0.4 W/cm^2 and 0.6 W/cm^2). (b) Photothermal temperature change curves of Cy-NH_2 in the PBS buffer solutions (0.01 M PBS, PBS/DMSO = 7:3 v/v) at various concentrations (0 μM, 10 μM, 20 μM and 40 μM)

under 808 nm light irradiation (0.5 W cm^{-2}).

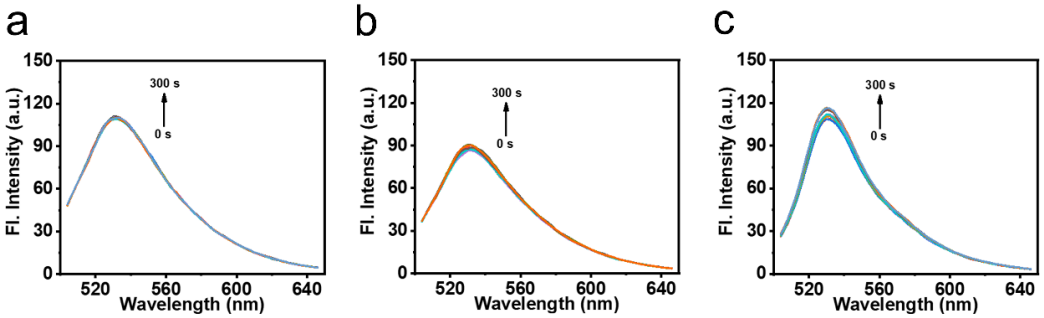


Figure S7. SOSG fluorescence change in the presence of (a) blank, (b) Cy-NH_2 or (c) Cy-S-S-Cbl under 808 nm light (0.1 W cm^{-2}) irradiation in CH_3OH.

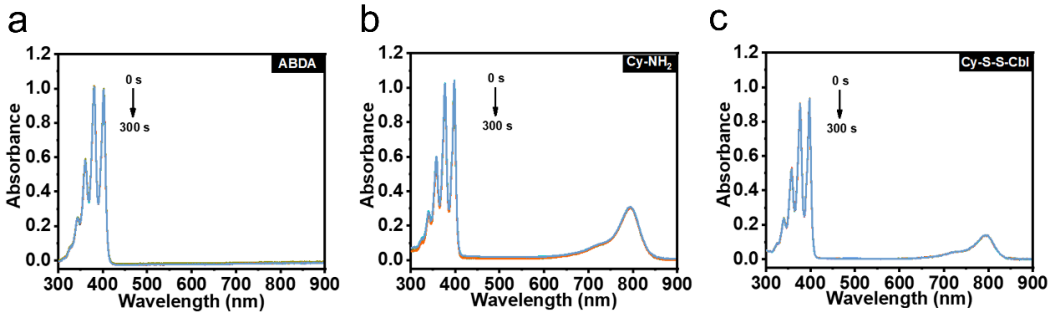


Figure S8. Photo-oxidation of ABDA as a function of irradiation time in the presence of (a) blank, (b) Cy-NH_2 or (c) Cy-S-S-Cbl under 808 nm light (0.1 W cm^{-2}) irradiation in PBS buffer (0.01 M).

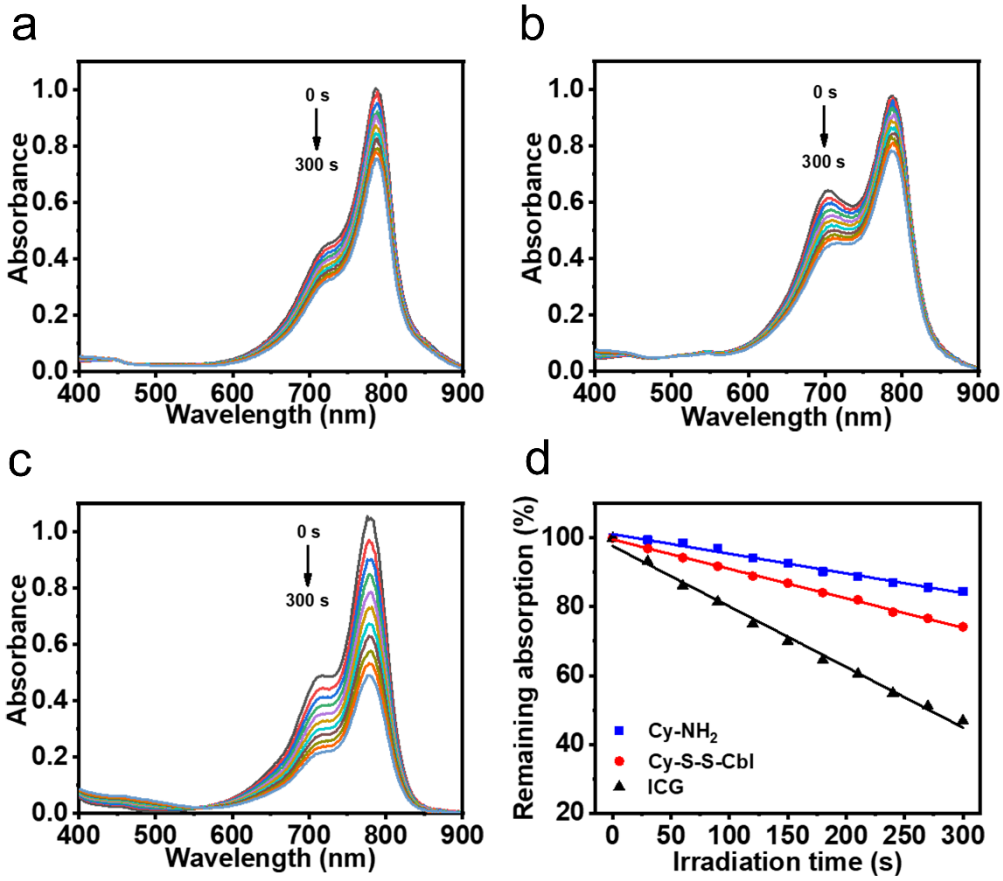


Figure S9. Absorption spectra of Cy-S-S-Cbl (a), Cy-NH_2 (b) or ICG (c) under 808 nm light irradiation (0.5 W cm^{-2}, 300 s) in PBS buffer solutions (0.01 M PBS, PBS/DMSO = 7:3 v/v). (d) The absorbance change at 790 nm after the Cy-S-S-Cbl, Cy-NH_2 or ICG was treated with 808 nm irradiation, respectively.

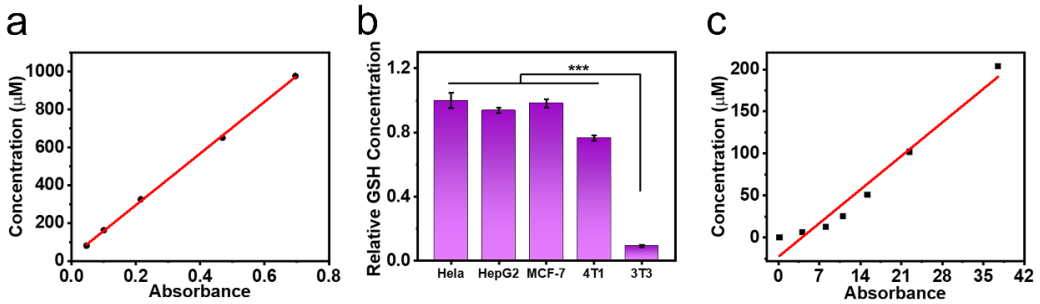


Figure S10. (a) The standard curve of GSH content was obtained by the Reduced Glutathione (GSH) Content Assay Kit. (b) Relative GSH concentration of different

kinds of cells. (c) The standard curve of GSSG content was obtained by the GSSG Content Assay Kit. Data represent the mean ± SD, n = 3, ***P < 0.001.

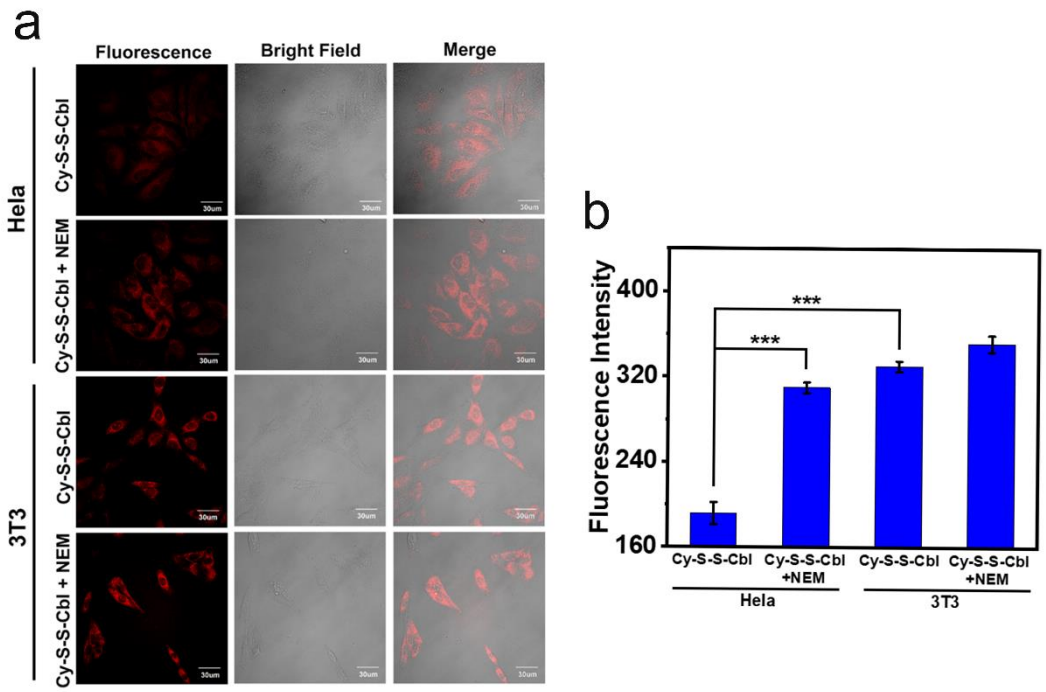


Figure S11. (a) Cellular uptake of Cy-S-S-Cbl or Cy-S-S-Cbl + NEM in Hela and 3T3 cells. Scale bar: 30 μm. (b) Fluorescence intensity of Cy-S-S-Cbl or Cy-S-S-Cbl + NEM in Hela and 3T3 cells, which was the quantitative data of (a). Statistical significance: ***P < 0.001.

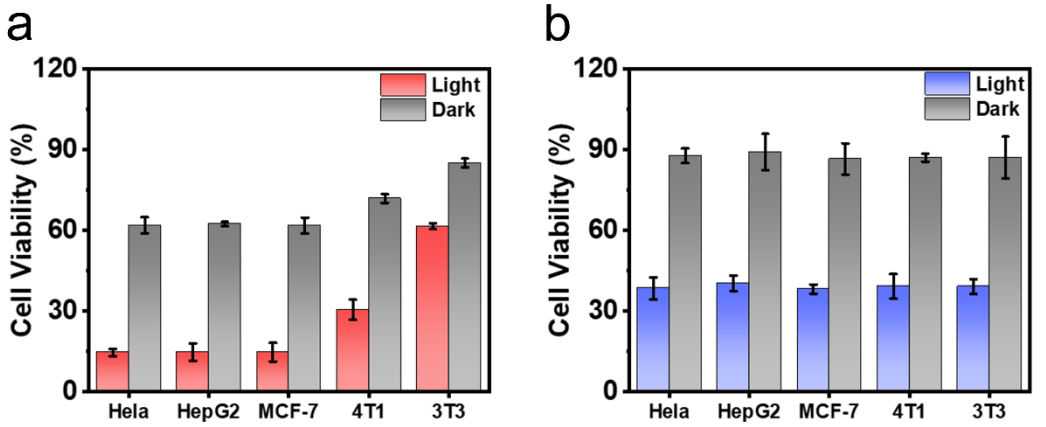


Figure S12. Phototoxicity and dark toxicity effects for Hela, HepG2, MCF-7, 4T1 and 3T3 cells treated with Cy-S-S-Cbl (a) or Cy-NH_2 (b).

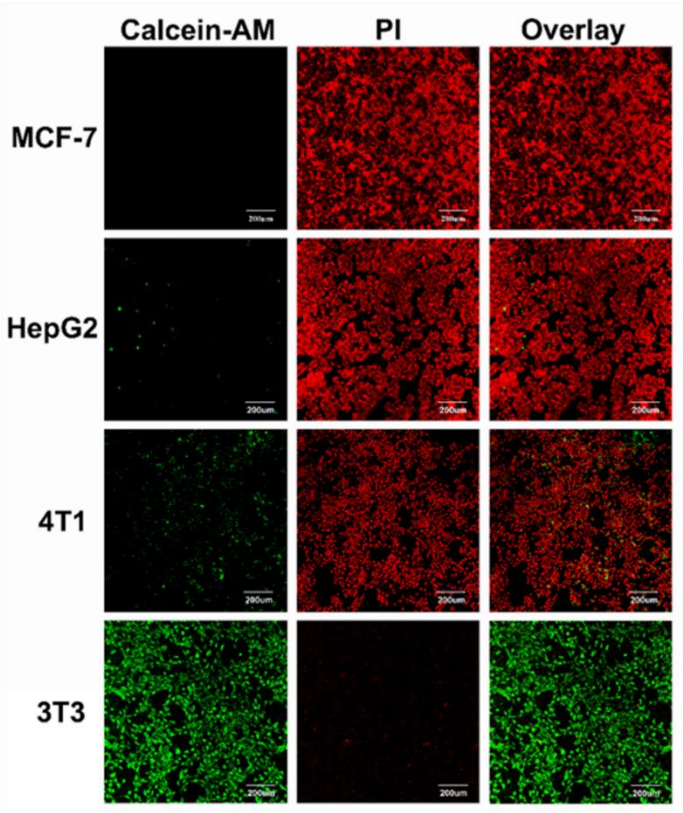


Figure S13. Calcein AM and propidium iodide co-staining fluorescence imaging after treated with Cy-S-S-Cbl under 808 nm light irradiation (0.5 W cm^{-2}) in MCF-7, HepG2 4T1 and 3T3 cells. Scale bar: 200 μm.

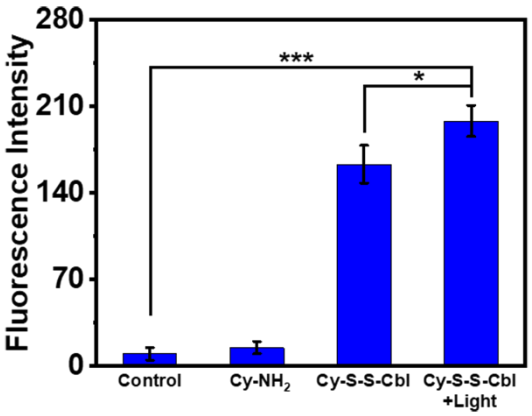


Figure S14. Fluorescence intensity of γ-H2AX in HeLa cells with different treatments, including Cy-NH_2, Cy-S-S-Cbl, Cy-S-S-Cbl + Light., which was the quantitative data of Fig. 3c. Statistical significance: *P < 0.05, **P < 0.01 and ***P < 0.001.

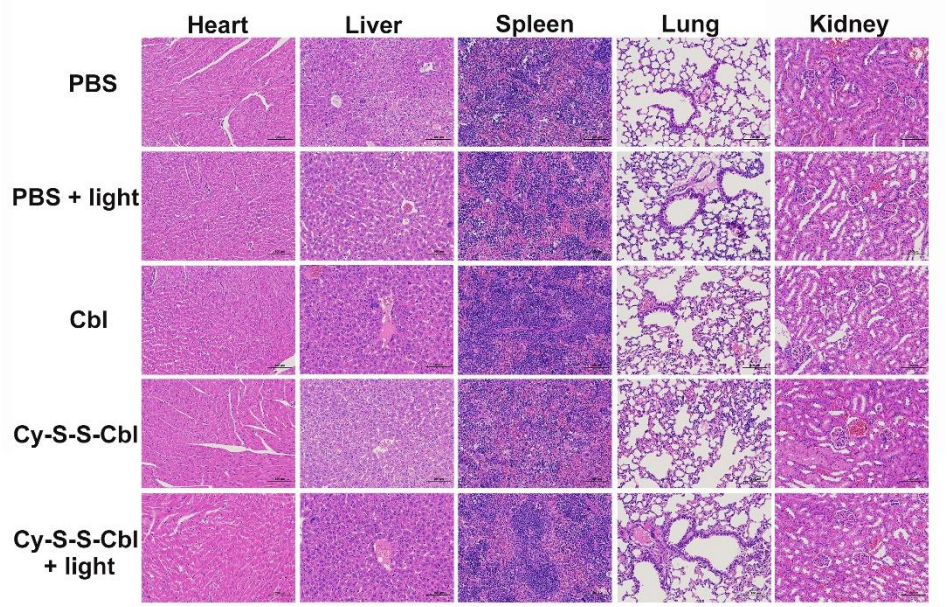


Figure S15. *In vivo* biosafety assay. H&E staining of heart, liver, spleen, lung, and kidney of mice from different treatment groups after 21 days of treatment by intratumoral injection. Scale bar = 100 μm.

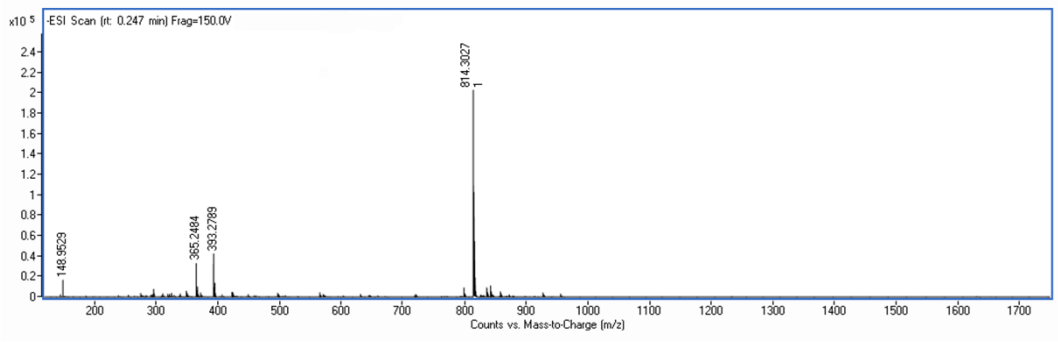


Figure S16. ESI-HRMS spectrum of compound Cy-NH_2.

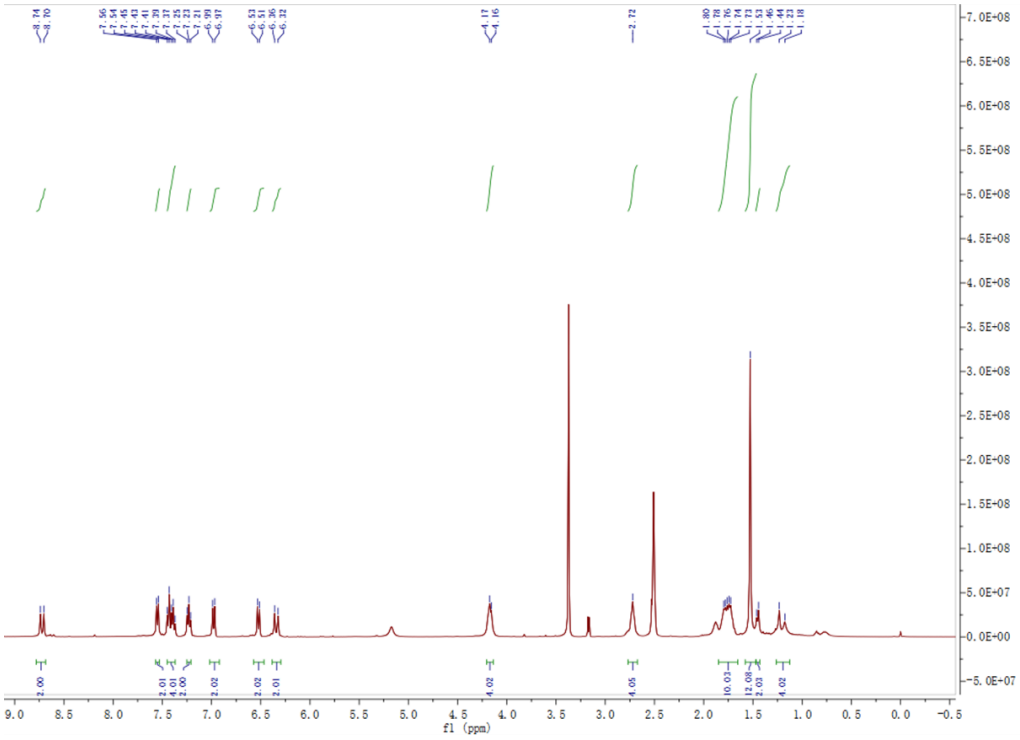


Figure S17. ^{1}H NMR spectrum of compound Cy-NH_2 in DMSO-d_6.

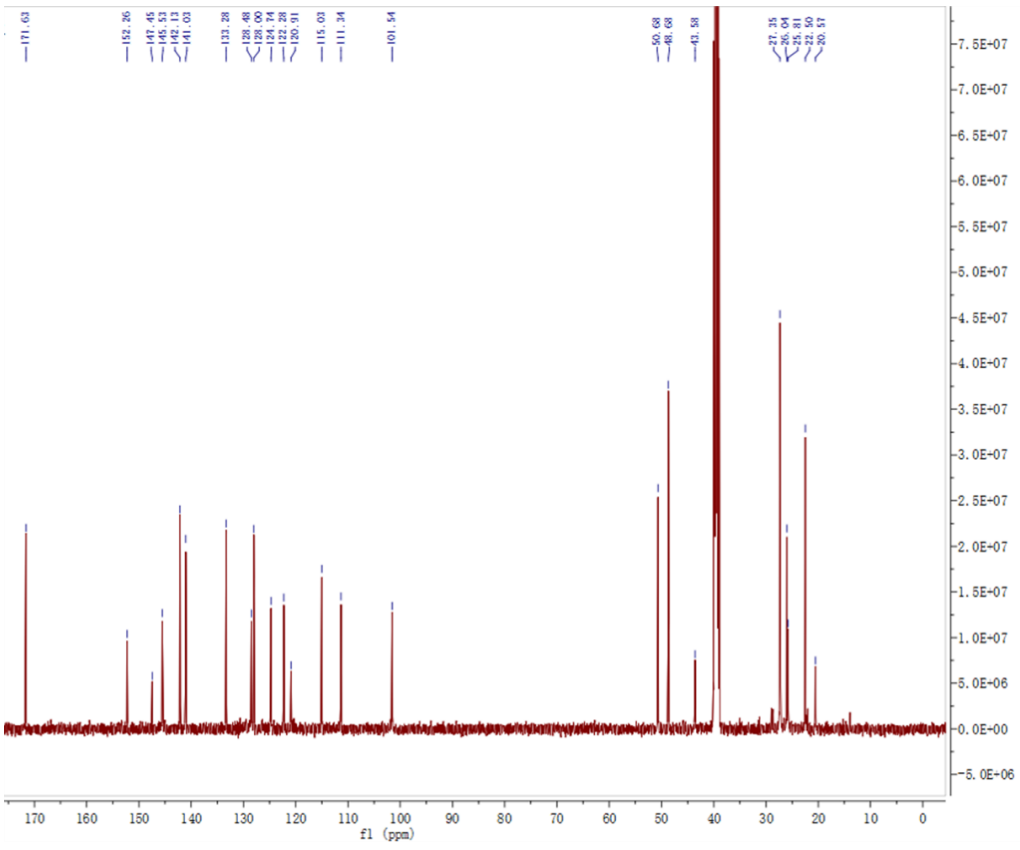


Figure S18. ^{13}C NMR spectrum of compound Cy-NH_2 in DMSO-d_6.

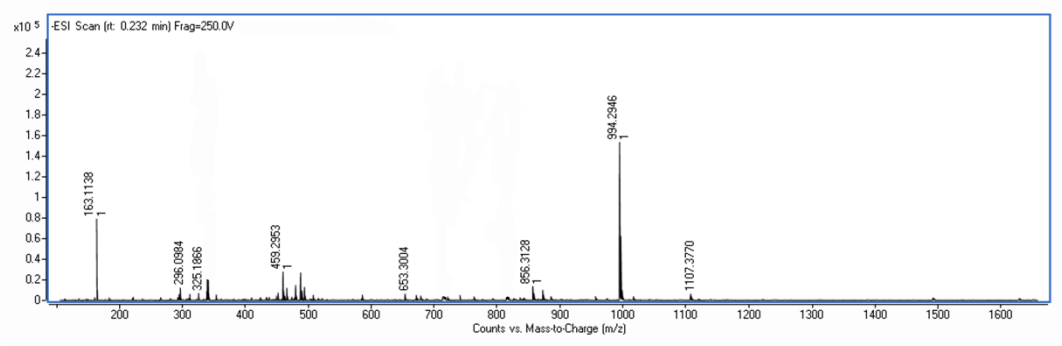


Figure S19. ESI-HRMS spectrum of compound Cy-S-S-OH.

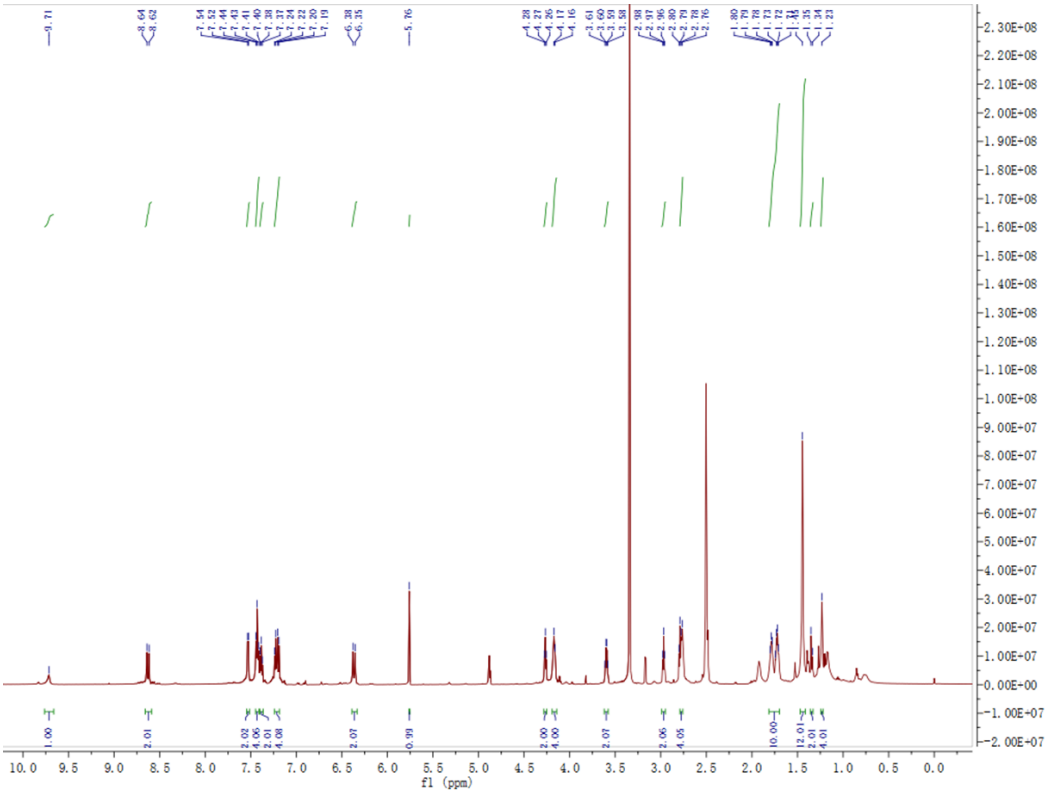


Figure S20. ^{1}H NMR spectrum of compound Cy-S-S-OH in DMSO-d_6.

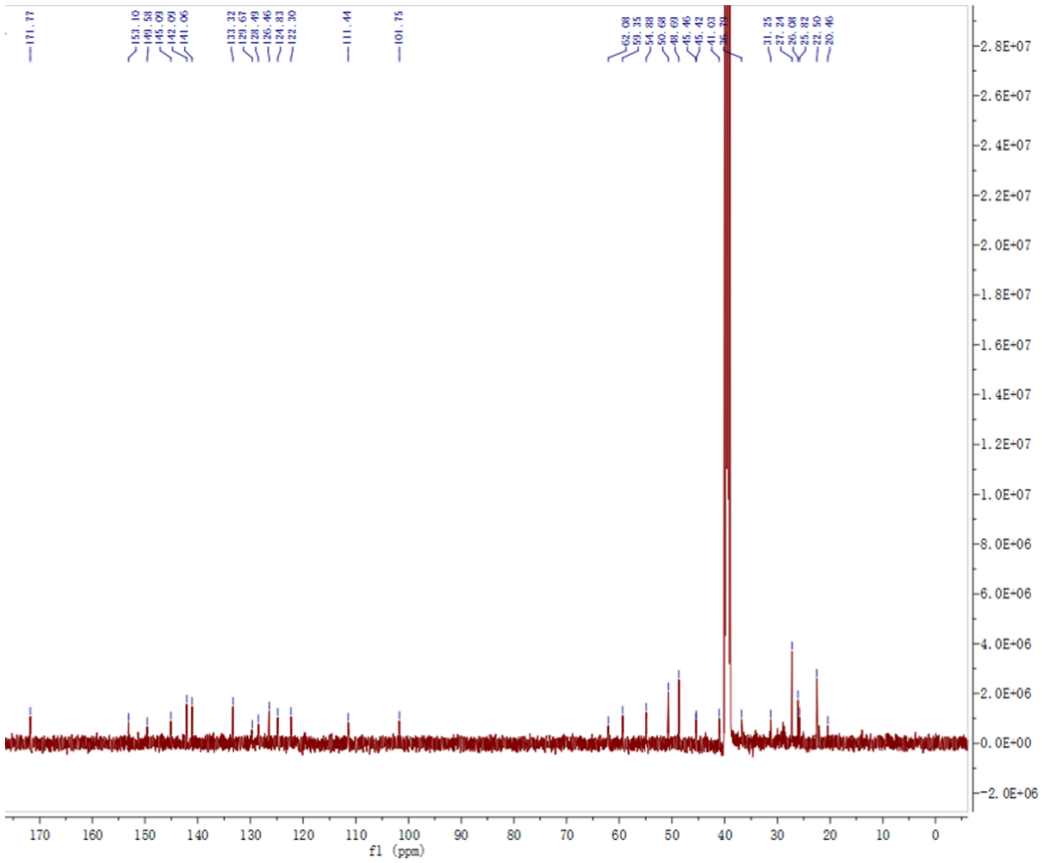


Figure S21. ^{13}C NMR spectrum of compound Cy-S-S-OH in DMSO-d_6.

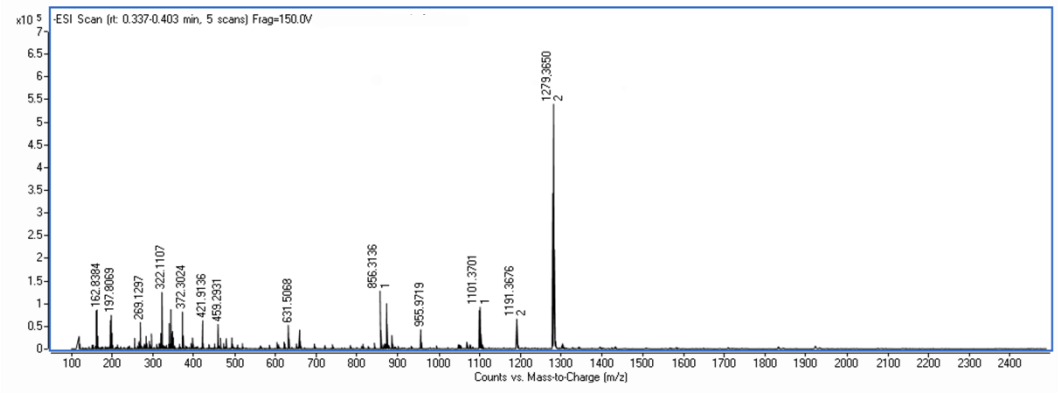


Figure S22. ESI-HRMS spectrum of compound Cy-S-S-Cbl.

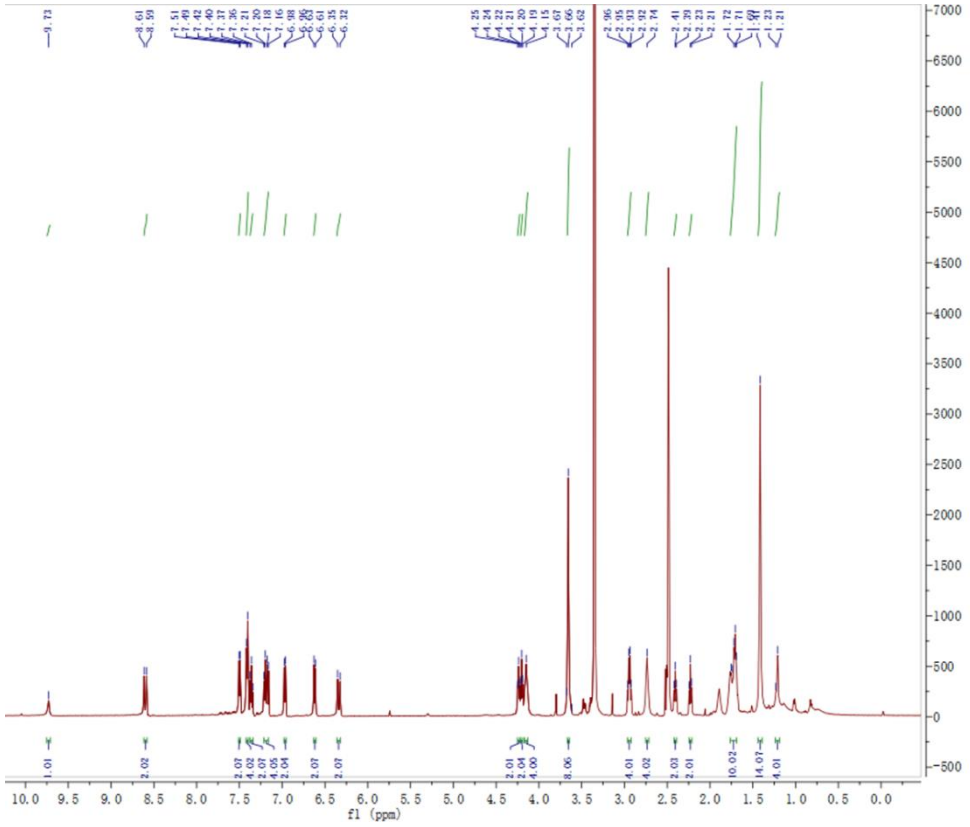


Figure S23. ^{1}H NMR spectrum of compound Cy-S-S-Cbl in DMSO-d_6.

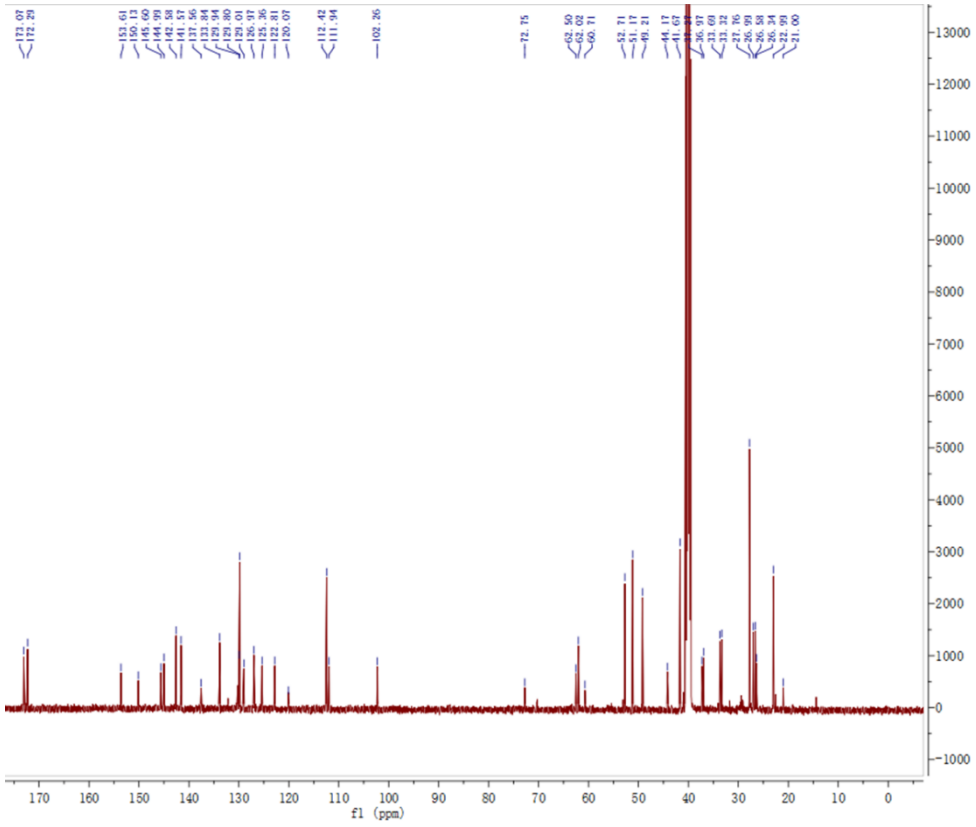


Figure S24. ^{13}C NMR spectrum of compound Cy-S-S-Cbl in DMSO-d_6.

Supporting References

[1] N. Narayanan, G. Patonay, *J. Org. Chem.* **1995**, *60*, 2391.

[2] Q. Tian, F. Jiang, R. Zou, Q. Liu, Z. Chen, M. Zhu, S. Yang, J. Wang, J. Wang, J. Hu, *ACS. Nano.* **2011**, *5*, 9761.