Supporting Information

Ferroptosis-inducing photosensitizers alleviate hypoxia tumor microenvironment for enhanced fluorescence imaging-guided photodynamic therapy

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Materials

The chemical reagents were procured from Energy Chemical Co. (China) or J&K Scientific Ltd. All solvents employed were of analytical grade. MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) was procured from Energy Chemical Co. Dulbecco's modified Eagle's medium (DMEM) and PBS buffer were obtained from KeyGEN BioTECH Ltd. Hoechst 33342, ER tracker Green, lysosome tracker blue, and MitoTracker Green were acquired from Life Technologies Co. (USA). Mouse breast cancer cells (4T1), Human liver carcinoma cells (HepG-2) and Human breast cancer cells (MCF-7) were procured from the Institute of Basic Medical Sciences (IBMS) at the Chinese Academy of Medical Sciences.

Methods

^{1}H-NMR and ^{13}C-NMR spectra were acquired using a Bruker Avance II 400 spectrometer. Mass spectrometric data (ESI-MS) was obtained using an LTQ Orbitrap XL and a TOF LC/MS (G6224A and G6230B). Absorption and emission spectra for **ICyHD NPs** and Hydrogen peroxide activation reactions were measured using a Lambda 35 UV-visible spectrophotometer from PerkinElmer and a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). The fluorescence response of **ICyHD NPs** with varying concentrations of H_2O_2 (Fig. 2b) were measured using SpectraMax M2/M2e Microplate Readers from Molecular Devices. Fluorescence images were captured using an FV3000 confocal laser scanning microscope from Olympus. Fluorescence imaging of small animals was performed

using a NightOWL II LB983 live imaging system.

Synthesis of Compound 1.

4-Iodoaniline (6.00 g, 27.4 mmol) was placed in a 250 mL round bottom flask, to which 4 mL H_2O and 20 mL concentrated hydrochloric acid were added for dissolution. Then $NaNO_2$ (2.08 g, 30.6 mmol) was dissolved in 10 mL H_2O, and the aqueous solution containing $NaNO_2$ was slowly dropped into a round bottom flask at 0℃, and stirred for 1 h until the solution turned brown and clear. Subsequently, $SnCl_2$ (10.42 g, 55.1 mmol) was dissolved in 8 mL H_2O and 7 mL concentrated hydrochloric acid, and then the solution was slowly added to the aforementioned reaction system. The mixture was stirred for 2 h until the solution turned dark yellow, and the brown precipitate was obtained after suction filtration and multiple washes with water. The brown precipitate was dissolved in NaOH solution, washed three times with CH_2Cl_2/saturated aqueous solution of NaCl, and then the organic layer was separated and dried over anhydrous Na_2SO_4. Under reduced pressure, the solvent was extracted by evaporation to yield **Compound 1** as brown solid (4.20 g, yield 70%). ^{1}H NMR (500 MHz, MeOD) δ 7.64 (d, J=8.4 Hz, 1H), 6.79 (d, J=8.4 Hz, 1H).

Synthesis of Compound 2.

Compound 1 (5.00 g, 21.4 mmol) and 3-methyl-2-butanone (3.68 g, 42.8 mmol) were dissolved in 50 mL acetic acid and then refluxed at 120℃ for ~5 h under N_2 atmosphere. The aforementioned reactions were monitored by TLC. After the reaction was completed, a saturated Na_2CO_3 solution was introduced to the system to eliminate

acetic acid, until no more bubbles formed. Subsequently, the system was washed three times with CH_2Cl_2/saturated aqueous solution of NaCl, the organic layer was separated and then dried over anhydrous Na_2SO_4, and the solvent was extracted by evaporation under reduced pressure. The crude product was purified by column chromatography with an eluent (Petroleum ether/EtOAc=1:1), obtaining **Compound 2** as brown liquid (3.78 g, yield 43.5%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73-7.43 (m, 1H), 5.30 (s, 1H), 2.26 (s, 2H), 1.49-1.11 (m, 4H). HRMS: m/z $[M+H]^+$ calculated value: 286.0014, test value: 286.0100.

Synthesis of Compound 3.

Compound 2 (5.00 g, 17.5 mmol) and 6-bromohexanoic acid (10.21 g, 52.6 mmol) were dissolved in 20 mL acetonitrile, and the reaction was refluxed under N_2 atmosphere for ~48 h to yield a dark purple solution. The reaction was monitored by TLC until the aromatic substrate was completely consumed. The reaction mixture was poured into cold diethyl ether (100 mL) after cooling to room temperature under stirring vigorously. The precipitate was then washed repeatedly with cold diethyl ether, ultimately yielding **Compound 3** as purple solid, which was used directly for the next step without further purification (7.12 g, yield 46.8%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.32 (s, 1H), 7.99 (d, J = 8.2 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 4.44 (t, J = 7.0 Hz, 2H), 2.85 (d, J = 9.8 Hz, 3H), 2.23 (t, J = 7.0 Hz, 2H), 1.82 (s, 2H), 1.55 (s, 2H), 1.54 (s, 6H), 1.42 (d, J = 6.5 Hz, 2H). HRMS: m/z $[M-Br]^+$ calculated value: 400.0768, test value: 400.0778.

Synthesis of Compound 4.

$POCl_3$ (15.91 g, 104.7 mmol) was slowly added dropwise to a 100 mL round-bottom flask containing 20 mL DMF at 0℃ under N_2 atmosphere. After stirring for 30 min, a white solid was produced. Subsequently, cyclohexanone (5.00 g, 51.0 mmol) was slowly added to the system which was heated to 80℃ and stirred for 4 h. The system was then placed in ice water (200 mL) and stirred for 1 h, resulting in the precipitation of **Compound 4** as yellow solid, which was used directly for the next step without further purification (3.12 g, yield 14.9%). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.87 (s, 1H), 10.26 (s, 1H), 7.67 (s, 1H), 2.48 (d, J = 6.2 Hz, 4H),1.60 (m, J = 7.1 Hz, 2H). HRMS: m/z $[M-H]^-$ calculated value: 171.0291, test value: 171.0223.

Synthesis of Compound 5.

Compound 3 (1.28 g, 2.6 mmol), **Compound 4** (0.24 g, 1.4 mmol), and anhydrous sodium acetate (0.52 g, 6.3 mmol) were dissolved in 10 mL acetic anhydride. The mixed solution was stirred at 90℃, under N_2 atmosphere and away from light, for 2 h. After the reaction, the mixture was cooled to room temperature, washed three times with CH_2Cl_2/saturated aqueous solution of NaCl, the organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was extracted by evaporation under reduced pressure, and the crude product was purified by column chromatography (CH_2Cl_2: CH_3OH=100:1), yielding **Compound 5** as green solid (0.58 g, yield 38%). ^{1}H NMR (400 MHz, MeOD) δ 8.43 (d, J = 14.1 Hz, 1H), 7.88 (s, 1H), 7.75 (t, J = 10.3 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 6.29 (d, J = 14.1 Hz, 1H), 4.14 (dd, J = 20.6,

13.4 Hz, 2H), 2.74 (s, 2H), 2.31 (t, J = 7.1 Hz, 2H), 1.99 (s, 2H), 1.88 – 1.81 (m, 2H), 1.71 (d, J = 7.1 Hz, 6H), 1.50 (d, J = 6.9 Hz, 2H), 1.28 (d, J = 2.3 Hz, 1H). HRMS: m/z $[M\text{-}Br]^+$ calculated value: 935.1543, test value: 935.1544.

Synthesis of Compound 6.

Nitrophenol (0.55 g, 3.9 mmol) and anhydrous K_2CO_3 (0.18 g, 3.9 mmol) were dissolved in a mixed solution of 10 mL acetonitrile and 5 mL DMF, the reaction was carried out under N_2 atmosphere at room temperature, and stirred for 10 min. Subsequently, a solution containing **Compound 5** (0.55 g, 0.5 mmol) in 5 mL acetonitrile was added dropwise to the aforementioned mixed solution, which was stirred at room temperature overnight and was monitored by TLC. After that the product was extracted and dried under reduced pressure to obtain green solid. The residue was dissolved in CH_2Cl_2, washed three times with an aqueous solution of CH_2Cl_2/saturated aqueous solution of NaCl, and then the organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was then extracted by evaporation under reduced pressure. The resulting solid was fully dissolved in 30 mL CH_3OH and used directly for the following step without purification.

$SnCl_2 \cdot 2H_2O$ (1.02 g, 5.3 mmol) was dissolved in 3 mL concentrated HCl and added dropwise to the above CH_3OH solution. The solution was stirred at 70℃ for 12 h under N_2 atmosphere. The reaction was monitored by TLC. After the reaction, the solvent was extracted under reduced pressure, the residue was dissolved in CH_2Cl_2, washed with CH_2Cl_2/saturated aqueous solution of NaCl three times, the organic layer

was separated and dried over anhydrous Na_2SO_4, then the solvent was extracted under reduced pressure, and the product was purified by column chromatography (CH_2Cl_2:CH_3OH=10:1), yielding **Compound 6** as blue solid (0.28 g, yield 25%). ^1H NMR (400 MHz, MeOD) δ 8.57 (d, J = 14.4 Hz, 1H), 7.87 (s, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.66 (s, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.7 Hz, 1H), 6.74 (s, 1H), 6.16 (d, J = 14.0 Hz, 1H), 5.41 (d, J = 61.5 Hz, 1H), 4.12 (t, J = 6.7 Hz, 2H), 2.79 (s, 2H), 2.30 (d, J = 6.9 Hz, 2H), 1.94 (s, 2H), 1.83 (d, J = 7.0 Hz, 2H), 1.76 (s, 6H), 1.68 (d, J = 7.1 Hz, 2H), 1.50 (d, J = 6.9 Hz, 2H), 1.36 (d, J = 9.9 Hz, 2H). HRMS: m/z $[M-Br]^+$ calculated value: 609.1608, test value: 609.1613.

Synthesis of ICy.

2-(4-nitrophenyl)-2-oxoacetic acid (0.047 g, 0.24 mmol), HATU (0.073 g, 0.19 mmol) and triethylamine (2 μL) were dissolved in 10 mL anhydrous CH_2Cl_2 and the mixture stirred under N_2 atmosphere at room temperature for 30 min. Then **Compound 6** (0.086 g, 0.12 mmol) was added to the mixture, which was stirred at room temperature for ~4 h. The reaction was monitored by TLC. After the reaction, the mixture was extracted by evaporation under reduced pressure, and the **ICy** was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH=50:1) as blue solid (0.069 g, yield 51%). ^1H NMR (400 MHz, MeOD) δ 8.74 (d, J = 14.6 Hz, 1H), 8.27 (d, J = 8.0 Hz, 2H), 8.07 (s, 1H), 7.98 (d, J = 9.3 Hz, 3H), 7.87 (d, J = 8.1 Hz, 1H), 7.58 (s, 1H), 7.50 (s, 1H), 7.44 – 7.30 (m, 3H), 6.51 (d, J = 13.0 Hz, 1H), 4.32 (s, 2H), 2.79 (s, 2H), 2.72 (s, 2H), 2.20 (s, 2H), 1.94 – 1.91 (m, 2H), 1.83 (s, 2H), 1.78 (s, 6H),

1.66 (d, J = 6.7 Hz, 3H), 1.51 – 1.47 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 187.28, 177.47, 173.67, 161.96, 160.93, 153.23, 150.96, 145.79, 144.74, 141.70, 141.22, 138.09, 138.02, 133.14, 132.25, 132.10, 129.51, 128.84, 124.26, 118.93, 118.07, 115.87, 115.17, 107.00, 105.43, 93.08, 51.67, 50.94, 40.90, 29.01, 27.68, 27.51, 25.88, 24.55, 24.01, 20.29. HRMS: m/z $[M-Br]^+$ calculated value: 786.1670, test value: 786.1673.

Synthesis of ICyHD.

ICy (0.061 g, 0.07 mmol), HATU (0.026 g, 0.06 mmol) and DIPEA (0.053 g, 0.4 mmol) were dissolved in 10 mL anhydrous CH_2Cl_2 and stirred under N_2 atmosphere at room temperature for 30 min. Then hydroxylamine hydrochloride (0.024 g, 0.34 mmol) was dissolved in 5 mL DMF, which was dropped into the above mixture. The mixture was stirred at room temperature for ~4 h. The reaction was monitored by TLC. After the reaction, the solvent was extracted by evaporation under reduced pressure, and **ICyHD** was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH=50:1) as blue solid (0.028 g, yield 32%). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.47 (s, 1H), 10.32 (s, 1H), 8.64 (s, 1H), 8.43 (d, J = 8.6 Hz, 2H), 8.35 (d, J = 8.7 Hz, 2H), 8.21 (s, 1H), 8.09 (s, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.76 (d, J = 8.9 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 8.9 Hz, 1H), 7.18 (s, 1H), 6.59 (d, J = 15.0 Hz, 1H), 5.32 (s, 1H), 4.38 (s, 2H), 2.76 (s, 2H), 2.70 (s, 2H), 2.30 (d, J = 19.1 Hz, 2H), 1.86 (s, 2H), 1.76 (s, 7H), 1.55 (s, 2H), 1.45 (s, 2H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 187.28, 177.47, 173.67, 161.96, 160.93, 153.23, 150.96,

145.79, 144.74, 141.70, 141.22, 138.09, 138.02, 133.14, 132.25, 132.10, 129.51, 128.84, 124.26, 118.93, 118.07, 115.87, 115.17, 107.00, 105.43, 93.08, 51.67, 50.94, 45.25, 33.49, 29.01, 27.68, 27.51, 25.88, 24.55, 20.29. HRMS: m/z $[M-Br]^+$ calculated value: 801.1779, test value: 801.1771.

The preparation of nanoparticles.

The amphiphilic polymer DSPE-PEG_{2000}-TAT was used to prepare **ICyHD NPs**. The procedure is as follows: First, 2 mg of **ICyHD** was dissolved in 300 μL of THF, 20 mg of DSPE-PEG_{2000}-TAT was dissolved in 3 mL ultrapure water, and then the THF solution containing **ICyHD** was slowly added dropwise to the ultrapure water containing DSPE-PEG_{2000}-TAT. The mixed solution was sonicated for 30 min at 180 W ultrasound power. Then, the resulting dispersion was poured into an aqueous dialysis bag with a molecular weight of 3500, and dialyzed in ultrapure water for 3 days, changing the water twice a day. After dialysis, the solution in the dialysis bag was filtered through a 0.1 μm water system filter membrane three times to obtain the **ICyHD NPs** solution. **ICy NPs** were prepared using the same preparation method as mentioned above.

Characterization of nanoparticles.

The hydrodynamic diameters (DLS) of **ICyHD NPs** and **ICy NPs** were characterized using Zetasizer Nano-ZS90, and their micromorphology were examined using transmission electron microscopy (TEM). Subsequently, the concentrations of the prepared **ICyHD NPs** and **ICy NPs** were calibrated using a UV-vis spectrometer.

Characterization of H_2O_2 response.

To a 300 μL PBS incubation system containing 1% methanol, 30 μL of 10 mM H_2O_2 (with a final concentration of 100 μM) and 6 μL of 5 mM stock solutions of **ICyHD NPs** and **ICy NPs** were added, bringing their total concentration to 10 μM. Subsequently, 261 μL of PBS and 3 μL of methanol were mixed in, and the solution was incubated at 37℃ for 30 min in a constant temperature shaker. Upon completion of the incubation, the solution was transferred to a 96-well plate and its fluorescence intensity was measured using a Varioskan LUX Multimode Microplate Reader with an enzyme-linked immunosorbent assay (ELISA) protocol (λ_{ex}=680 nm; λ_{em}=690-850 nm).

Fluorescence titration experiment with H_2O_2 in vitro.

To incubate the PBS (with 1% methanol) containing 10 μM **ICyHD NPs** nanoparticles with varying concentrations of H_2O_2, the concentrations were set in sequence to 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM. After waiting for the H_2O_2 reaction to reach equilibrium (30 min), the fluorescence emission spectra of each group were collected using an enzyme-linked immunosorbent assay (ELISA) reader and analyzed.

Minimum detection limit.

The co-incubation test system containing 10 μM **ICyHD NPs** and various concentrations of H_2O_2 was tested three times in parallel, followed by 11 parallel tests

on blank samples without H_2O_2. The test data were then summarized, and the minimum detection limit of **ICyHD NPs** was calculated according to the provided formula. The formula for calculating the minimum detection limit is as follows[1]:

$$\text{Detection Limit (DL)} = 3\sigma/k$$

DL represents the minimum detection limit of **ICyHD NPs**; σ represents the standard deviation of the blank sample; k represents the slope of the linear relationship between the fluorescence intensity of **ICyHD-NH_2 NPs** at 720 nm and the concentration of H_2O_2.

Singlet oxygen detection in vitro.

In this study, DPBF (1,3-diphenlisobenzofuran) was used as the probe to detect the generation of singlet oxygen. DPBF (maximum absorption peak about 1.0) was dissolved in dichloromethane and ICy-NH_2 and ICyHD-NH_2 (both maximum absorption peak about 0.3) was added to the above mixed solution, respectively. The mixed solution was irradiated every 30s with a 700 nm laser (5.0 mW/cm^2) and the UV-vis spectrum of the mixture was measured immediately after each irradiation.

Cell culture.

Mouse breast cancer cells (4T1) and human breast cancer cells (MCF-7) were cultured in Dulbecco's modified Eagle's medium (DMEM/High) containing 4.5 g/L glucose, 4.0 mM L-glutamine, and 110 mg/L sodium pyruvate. Fetal bovine serum supplemented with 10% and 1% penicillin was used as a supplement. 5 μM **ICyHD NPs** and **ICy NPs** were added to the confocal Petri dish and co-cultured with the cells

while they were fully adhered to the wall in the incubator (37°C, 5% CO_2). All kinds of adherent cells were digested with trypsin, gently washed, transferred to 35 mm confocal dishes and 96-well plates, and incubated for 24 h under the above conditions to achieve complete adhesion, followed by subsequent cell experiments.

Cell uptake.

To the culture dish with a MCF-7 cell density of about 60%, 1 mL of 5 μM **ICyHD NPs** or **ICy NPs** was added. The solution was then incubated in a 37℃ incubator with 5% CO_2. A confocal microscope （λ_{ex}=640 nm, λ_{em}=700-790 nm） was used to collect images of intracellular fluorescence changes over time and conduct quantitative analysis.

Subcellular location.

MCF-7 cells were seeded into culture dishes, cultured until the cell density was about 60%, and then incubated with **ICyHD** (5 μM) for 2 h. After that, the culture dish was treated with four commercial dyes: Hoechst 33342 (nuclear dye), ER-Tracker Green (endoplasmic reticulum dye), Mito-Tracker Green (mitochondrial dye), and Lyso-Tracker Green (lysosome dye). The mixture was then incubated for an additional 0.5 h in a constant temperature incubator. Subsequently, the culture medium was discarded and the dish was gently washed twice with PBS. Fresh culture medium was then added, and the cells were examined under a confocal microscope for fluorescence localization.

MCF-7 cells were seeded into culture dishes, cultured until the cell density reached

approximately 60%, and then incubated with fresh medium containing **ICyHD** or **ICyHD NPs** (5 μM) for 10 h. Afterward, Hoechst 33342 (a nuclear dye) was added to culture dishes, mixed thoroughly, and incubated for an additional 0.5 h in a constant temperature incubator. Subsequently, the culture medium was discarded and the culture dishes gently washed twice with PBS. Fresh medium was then added, and confocal microscopy was used to capture intracellular fluorescence images and perform co-localization analysis.

Intracellular ROS detection.

MCF-7 cells were seeded in culture dishes and cultured until the cell density was about 60%. Then the cells were divided into six groups: Control group, **ICyHD NPs** or **ICy NPs** (5 μM) without light group, **ICyHD NPs** or **ICy NPs** (5 μM) with light group and SAHA (5 μM) Group. Fresh culture medium containing the above components was added and incubated for 18 h, and then the cells were exposed to light (40 mW/cm^2; 700 nm; 5 min). After the light exposure, 2 μM DCFH-DA was added for an additional 30 min incubation (DCFH-DA is a commonly used high sensitivity probe for detecting intracellular ROS)[2]. Subsequently, the culture medium was discarded and the cells gently washed twice with PBS, followed by the addition of fresh culture medium. Finally, confocal microscopy was used to capture intracellular fluorescence images for quantitative analysis.

Calcein AM/PI staining assay.

Calcein-AM and Propidium Iodide (PI) staining assay was used to study the effect of

combined therapy. In this study, MCF-7 cells were incubated with 5 μM **ICyHD NPs** for 18 h, followed by 700 nm light irradiation for 10 min (40 mW/cm^2). After washing the cells with PBS for three times, the cells were stained with AM (0.1 μL)/PI (0.1 μL) for 15 min. Finally, the fluorescence images were imaged by a confocal laser scanning microscope (green channel: λ_{ex}=488 nm and λ_{em}=50-580 nm; red channel: λ_{ex}=561 nm and λ_{em}=600-700 nm).

In vitro cytotoxicity evaluation.

On one hand, the cytotoxicity of photosensitizers is a critical factor in evaluating their biocompatibility, and on the other hand, it is also an important indicator for assessing the efficacy of photodynamic therapy[3]. The MTT assay was employed to evaluate the cytotoxicity of **ICyHD NPs** and **ICy NPs**. The complete culture medium, containing cells (10%FBS, 100 μL), was seeded into 96-well plates at a density of 5×10^4 cells/mL. After a cell attachment period of either 24 or 48 h, each well was washed with 100 μL PBS. Subsequently, the cells attached to the plate were cultured for 12 hours in complete culture medium with different concentrations of **ICyHD NPs** and **ICy NPs** (0, 1, 2, 3, 4, 5, 6, and 7 μM). To ensure accurate statistical analysis in the experimental group, four replicates were performed for each group. After 24 h of incubation, 10 μL of MTT (5 mg/mL) in PBS was introduced into each well of the 96-well plate to assess cell viability. The 96-well plates were then incubated in a constant temperature moist environment with a CO_2 concentration of 5% and a temperature of 37°C. After an incubation period of 4 h, the medium was carefully aspirated, and the

purple crystals were dissolved in 100 μL of DMSO per well. Since cell viability corresponds to the absorption of methylthioninium chloride, it can be quantitatively analyzed using a microplate reader. Therefore, the methylthioninium chloride optical density in the experimental group was compared with that in the control group (0 μM) to calculate the percentage of vitality in the experimental group.

Lipid peroxidation detection in MCF-7 cells.

C11-BODIPY 581/591 is a lipid peroxide detection probe, capable of being specifically oxidized by lipid peroxides and emitting a strong green fluorescence. It is used to detect the level of LPO in cells. First, the C11-BODIPY 581/591 probe was stained, and then the LPO level was evaluated by confocal imaging. MCF-7 cells were incubated at 37°C for 24 h in a glass plate, and then exposed to the following different treatments: 1) untreated MCF-7 cells (control), 2) MCF-7 cells incubated with **ICyHD NPs** (5 μM) for 18 h at 37 °C. 3) MCF-7 cells incubated with **ICyHD NPs** (5 μM) for 18 h at 37 °C, followed by irradiation with 700 nm light for 5 min at a power density of 40 mW/cm^2. 4) MCF-7 cells incubated with **ICy NPs** (5 μM) for 18 h at 37 °C. 5) MCF-7 cells incubated with **ICy NPs** (5 μM) for 18 h at 37°C, followed by irradiation with 700 nm light for 5 min at a power density of 40 mW/cm^2. The cells were then co-incubated with a solution containing C11-BODDIPY 581/591 (10 μM) in the culture medium for 30 min, followed by confocal fluorescence imaging (λ_{ex}=488 nm, λ_{em}=500-540 nm).

GSH determination in 4T1 cells.

4T1 cells were cultured on 6-well plates at 37°C and incubated until the cell confluence reached 70%. There are five groups including 1) Control, 2) **ICy NPs** (5μM), 3) **ICy NPs** (5μM) irradiated with 700 nm red LED light (40 mW/cm^2, 5 min), 4) **ICyHD NPs** (5μM), 5) **ICyHD NPs** (5μM) irradiated with 700 nm red LED light (40 mW/cm^2, 5 min). After 4 h of incubation, GSH and GSSG Assay Kit was used according to the manufacture instruction and 96-well plates were determined by a microplate reader (SpectraMax M2e, Molecular Devices Inc. USA).

Western blot analysis.

4T1 cells were seeded in 10 cm cell culture dish (Nest) and cultured with medium containing **ICyHD NPs** (5 μM) respectively. After 18 h of incubation at 37°C in the dark, the cells were irradiated with 700 nm red LED light for 5 min at a power density of 40 mW/cm^2. After 4 h of incubation, cells were lysed by RIPA lysis buffer with protease and phosphatase inhibitor in ice for 30 min. After measurement of protein concentration by BCA assay, equal amounts of protein were added to each lane of SDSPAGE gel for electrophoresis, and then transferred onto polyvinylidene fluoride (PVDF) membranes. After blocking by western blocking buffer and specific primary antibodies incubation at 4°C overnight, membranes were incubated by peroxidase-labeled goatanti-rabbit HRP secondary antibodies for 1 h at room temperature. The immunoblots were visualized by FluorChem E (ProteinSimple Co, Ltd., USA) with BeyoECL Plus (Beyotime Biotechnology Co., Ltd., Shanghai).

Confocal imaging of nucleus morphology of MCF-7 cells.

MCF-7 cells were cultured on 35 mm glass-bottom culture dishes for 24 h at 37°C, then exposed to different following treatments: 1) untreated (Control), 2) erastin (10 μM), 3) cisplatin (50 μM), 4) **ICyHD NPs** (5 μM), 5) **ICyHD NPs** (5 μM) with irradiation (700 nm, 5 min, 40 mW/cm^2). After different treatments, cells were stained by Hoechst 33342 (100 nM) for 10 min. The excitation wavelength for Hoechst 33342 was 405 nm and the emission wavelength was collected from 440 to 480 nm.

In vivo antitumor evaluation.

The Balb/c mice were purchased from Dalian Medical University. The animal experiments involved in this work were approved by the Dalian University of Technology Animal Care and Use Committee (DUT20210902). When the mice tumor reached ~100 mm^3, these mice were treated via the tail vein injection of: (1) PBS (100 μL) (PBS group); (2) **ICy NPs** (100 μL, 100 μM in PBS) (**ICy NPs** group); and (3) **ICy NPs** (100 μL, 100 μM in PBS) with light irradiation (700 nm, 100 mW/cm^2, 15 min) after 18 h injection (**ICy NPs+Light** group); (4) **ICyHD NPs** (100 μL, 100 μM in PBS) (**ICyHD NPs** group); and (5) **ICyHD NPs** (100 μL, 100 μM in PBS) with light irradiation (700 nm, 100 mW/cm^2, 15 min) after 18 h injection (**ICyHD NPs+Light** group). Each group contained five mice. The body weight of the mice and tumor volume were measured every other day.

In vivo antitumor evaluation.

All the experimental data were performed at least three independent measurements ($n \geq 3$). In vivo therapeutic experiments were performed five independent measurements. The data in this paper were expressed as the mean±standard deviations (SD). The statistical analysis and data processing were performed by Origin 2019 Software. Excel software was used for one-way ANOVA analysis and statistical analysis of Student's t-test. Significant difference was defined as $*p<0.05$, $**p<0.01$, and $***p<0.001$.

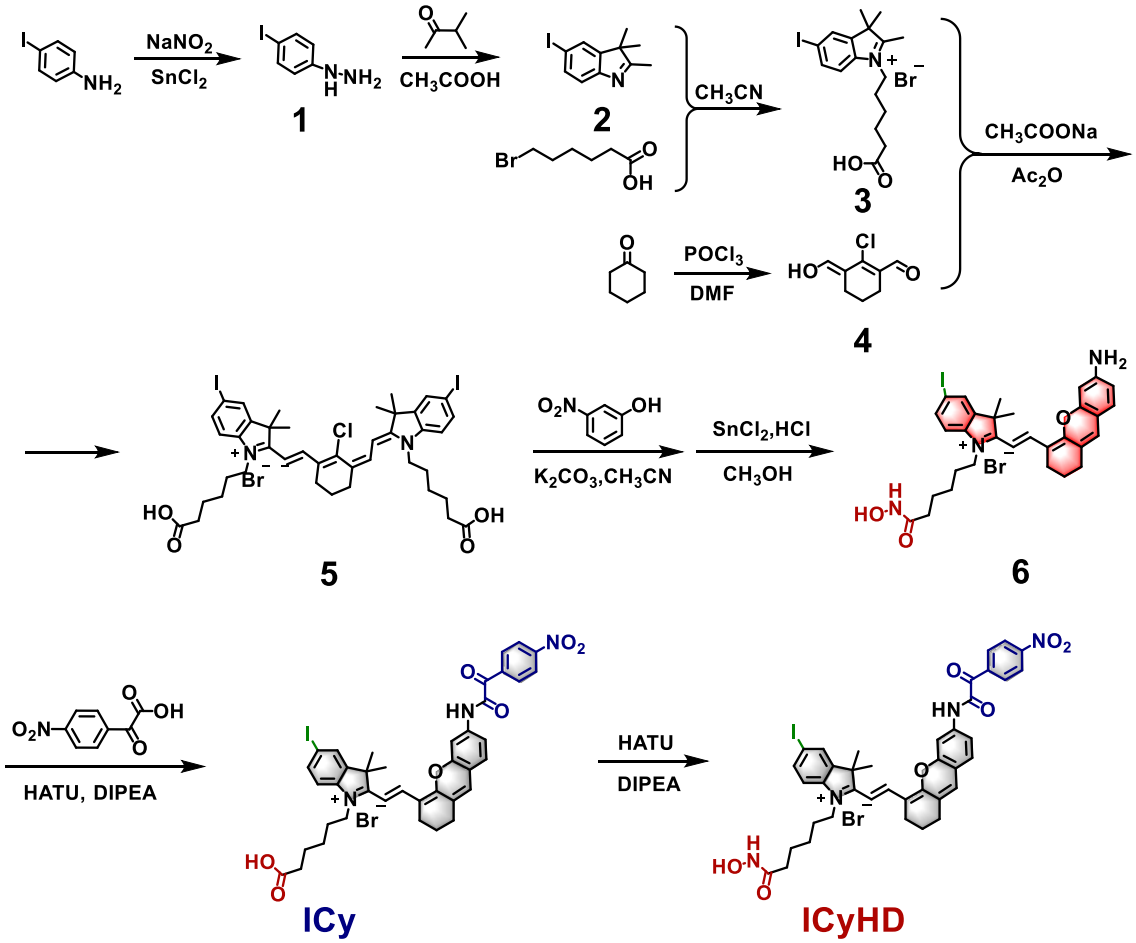

Fig. S1. Synthetic route of **ICyHD**.

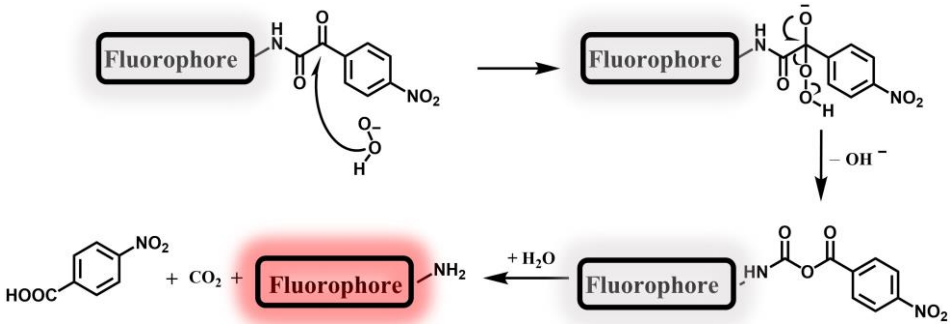

Fig. S2. Proposed Mechanism for Recognizing H_2O_2 Based on a Fluorophore Accommodating α-Ketoamide.

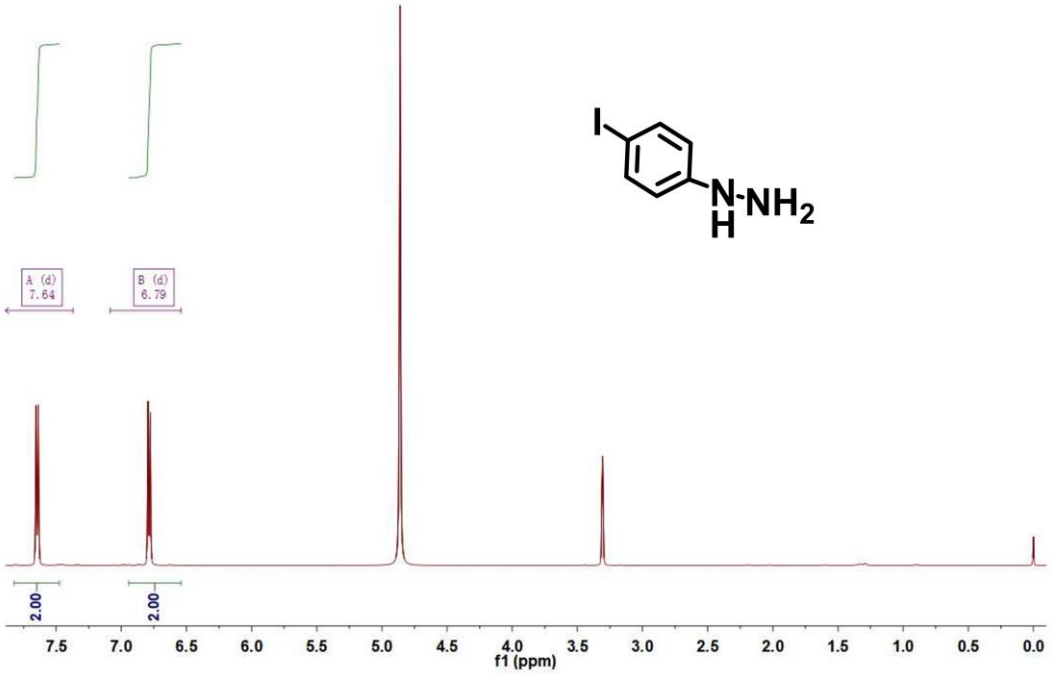

Fig. S3. [1]H NMR spectrum of **Compound 1** (500 MHz, MeOD).

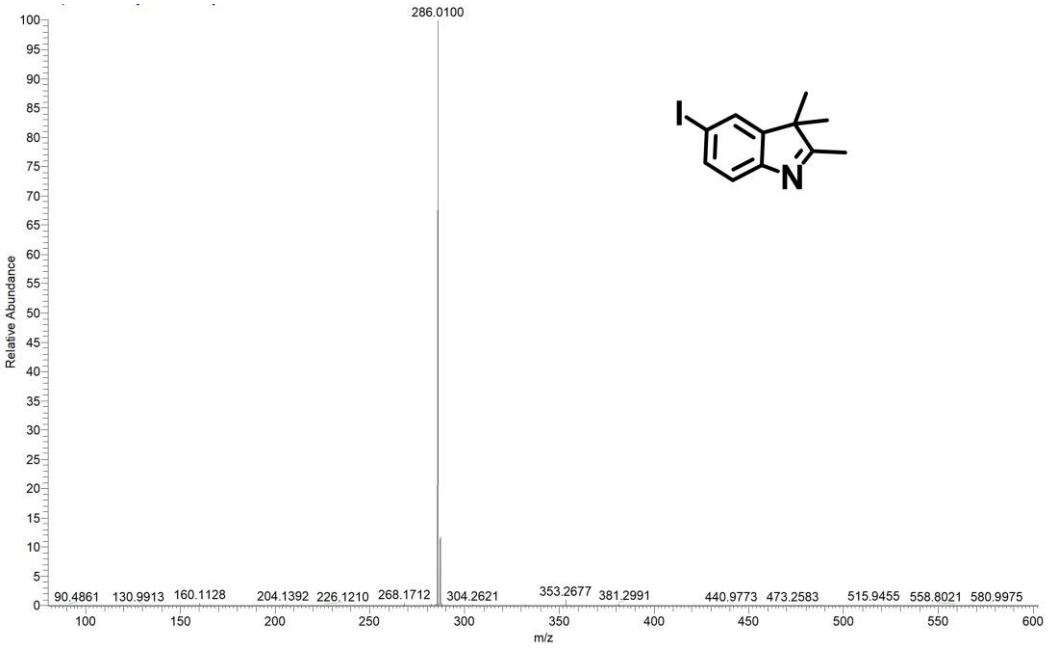

Fig. S4. Mass spectrum of **Compound 2**.

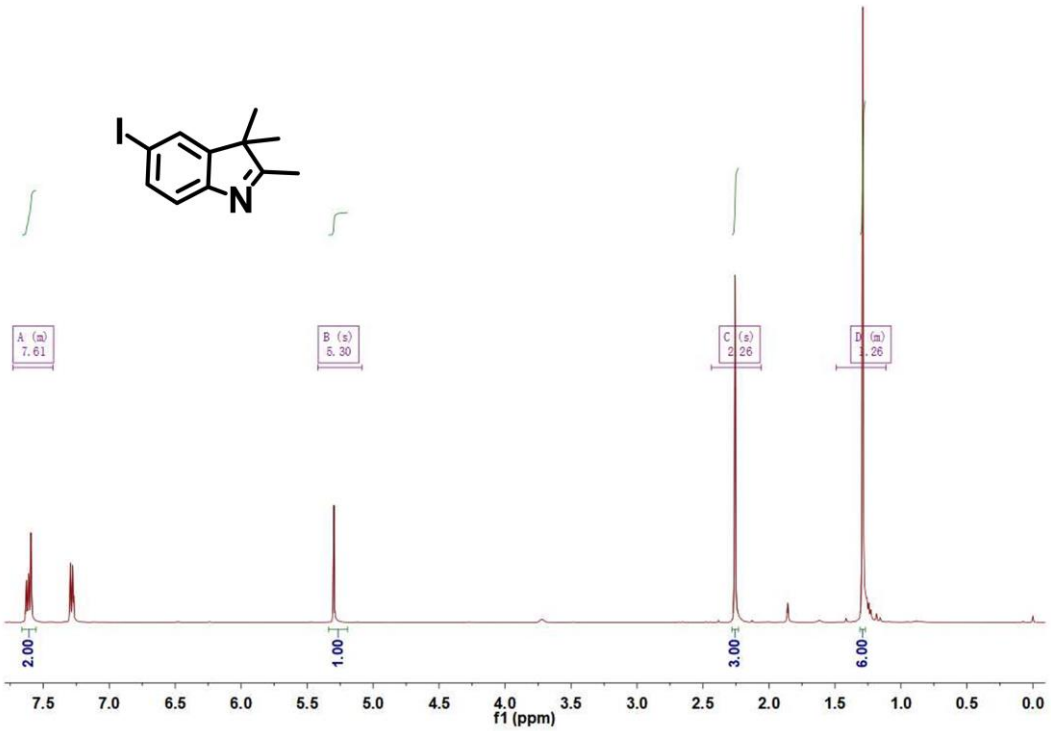

Fig. S5. ^{1}H NMR spectrum of **Compound 2** (500 MHz, $CDCl_3$).

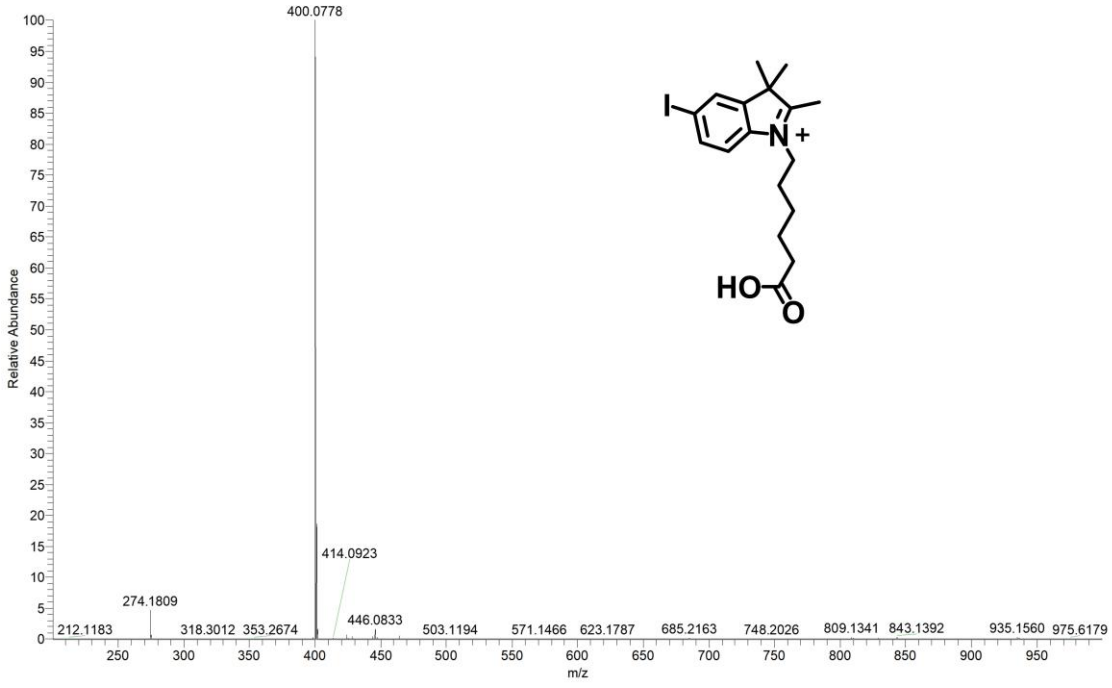

Fig. S6. Mass spectrum of **Compound 3**.

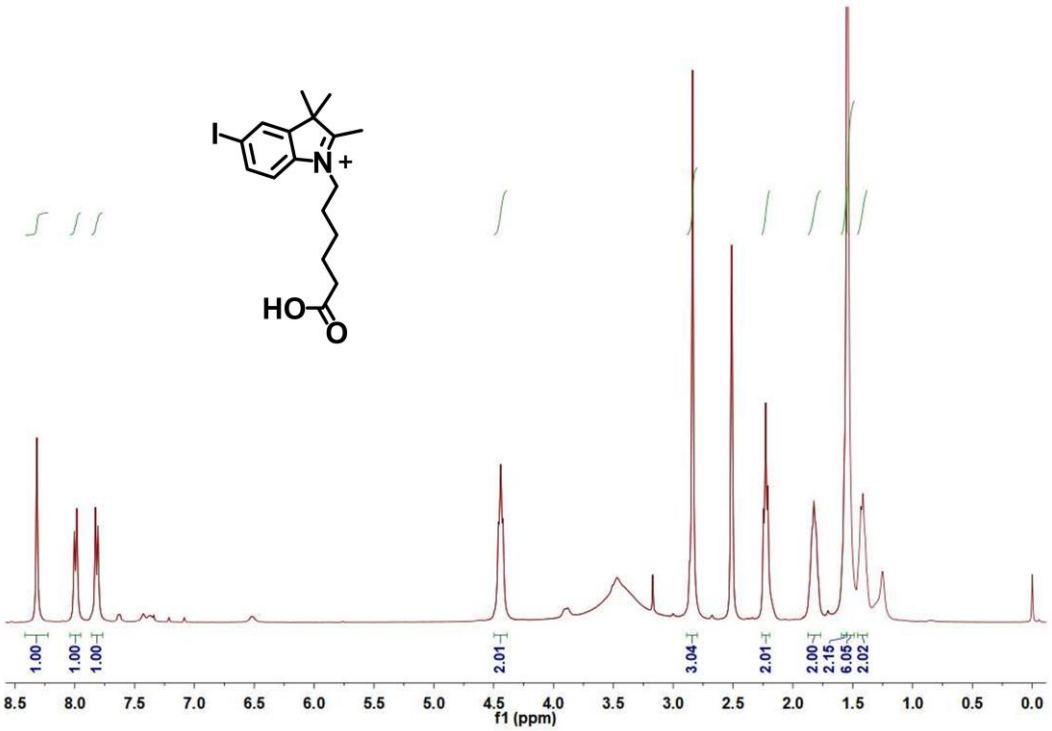

Fig. S7. ^{1}H NMR spectrum of **Compound 3** (400 MHz, DMSO-d_6).

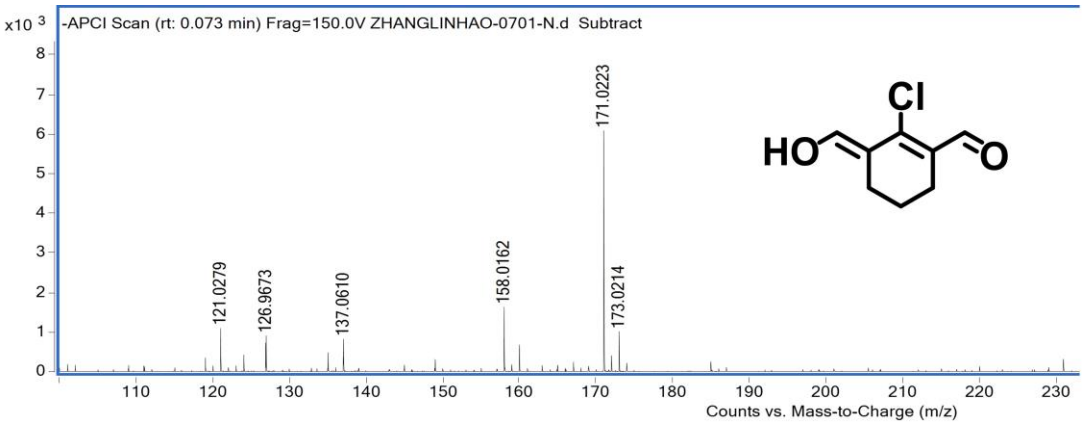

Fig. S8. Mass spectrum of **Compound 4**.

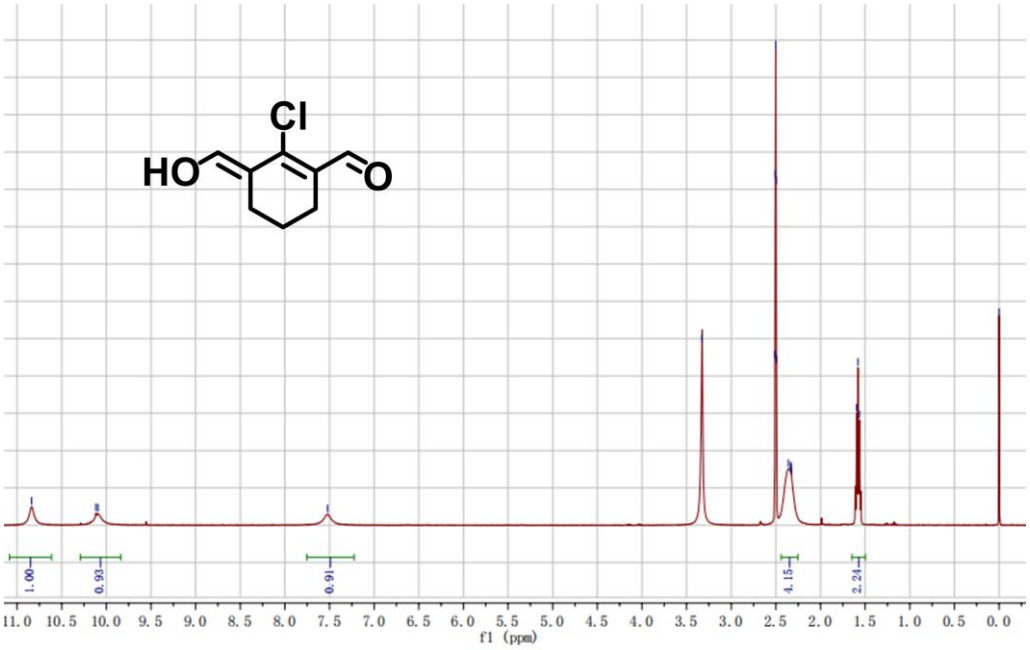

Fig. S9. ^{1}H NMR spectrum of **Compound 4** (400 MHz, DMSO-d_6).

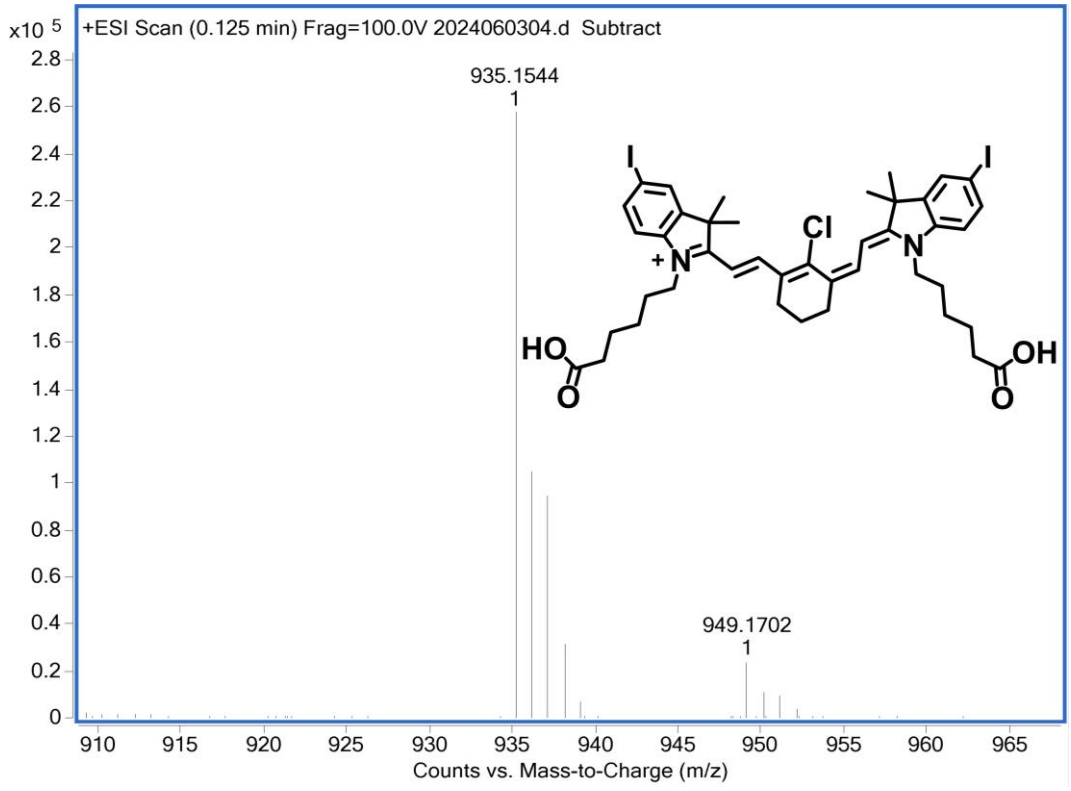

Fig. S10. Mass spectrum of **Compound 5**.

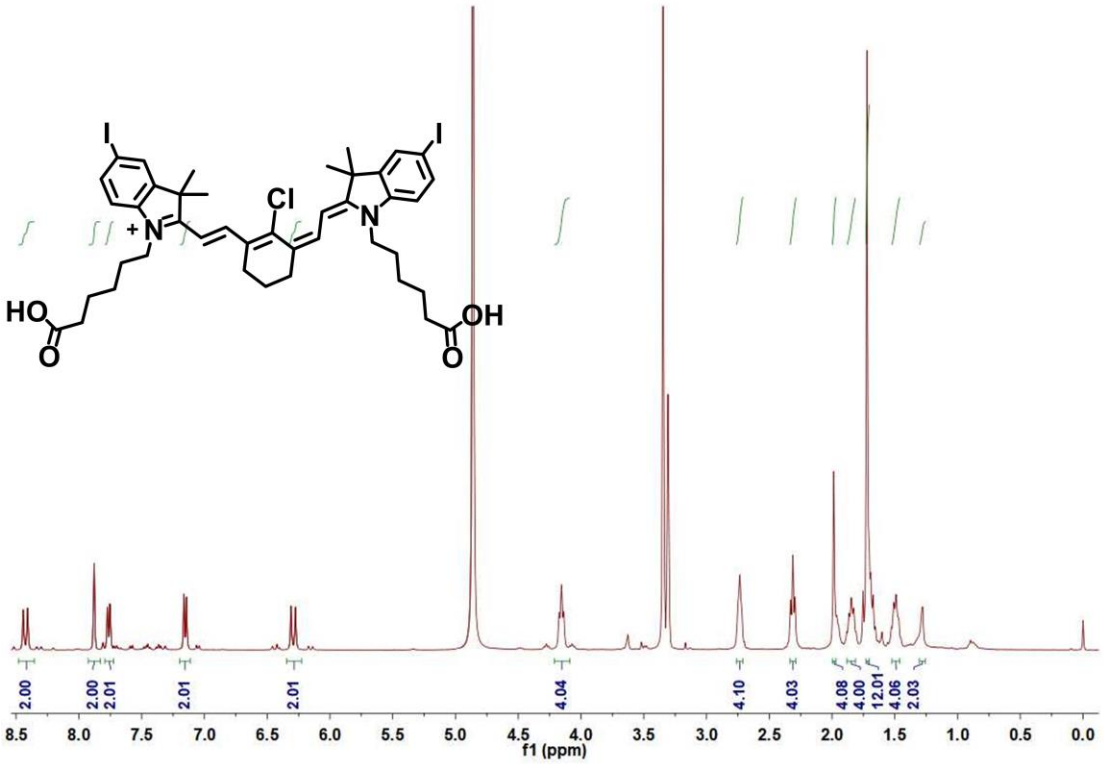

Fig. S11. ^{1}H NMR spectrum of **Compound 5** (400 MHz, MeOD).

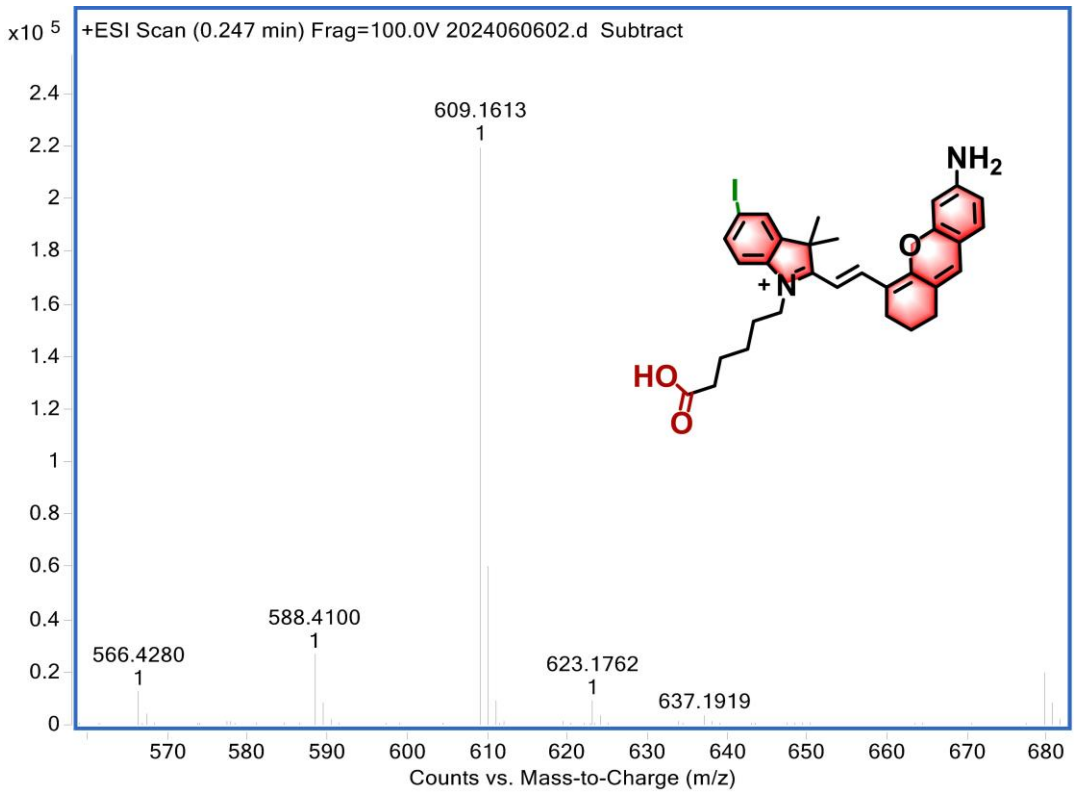

Fig. S12. Mass spectrum of **Compound 6**.

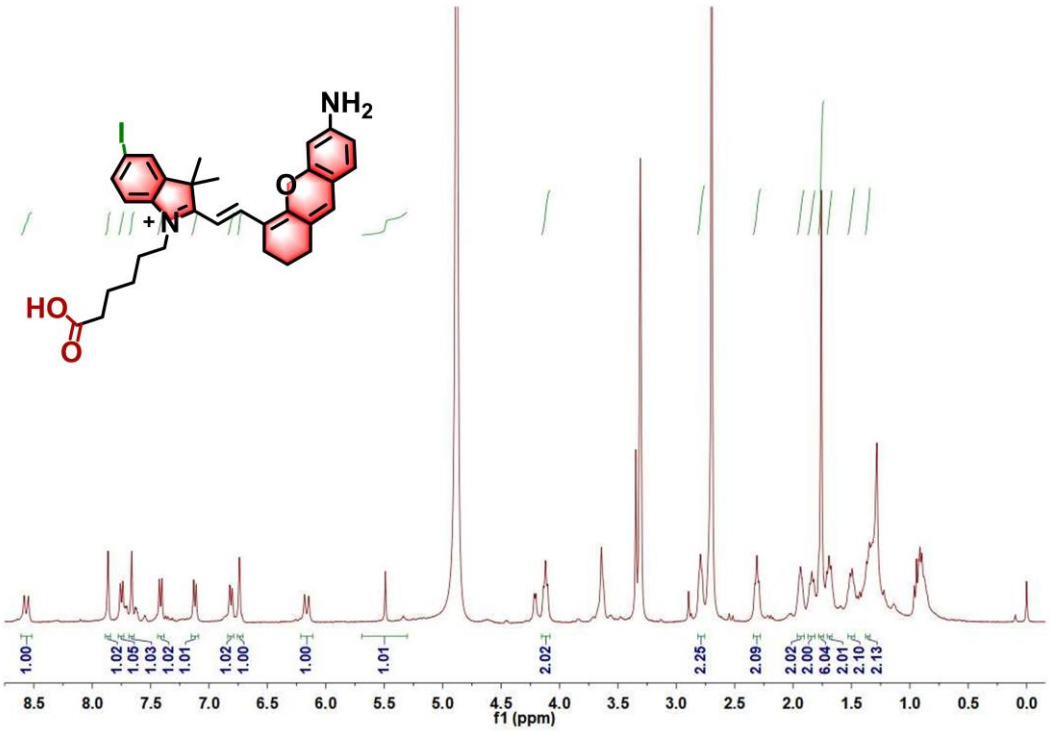

Fig. S13. ^{1}H NMR spectrum of **Compound 6** (400 MHz, MeOD).

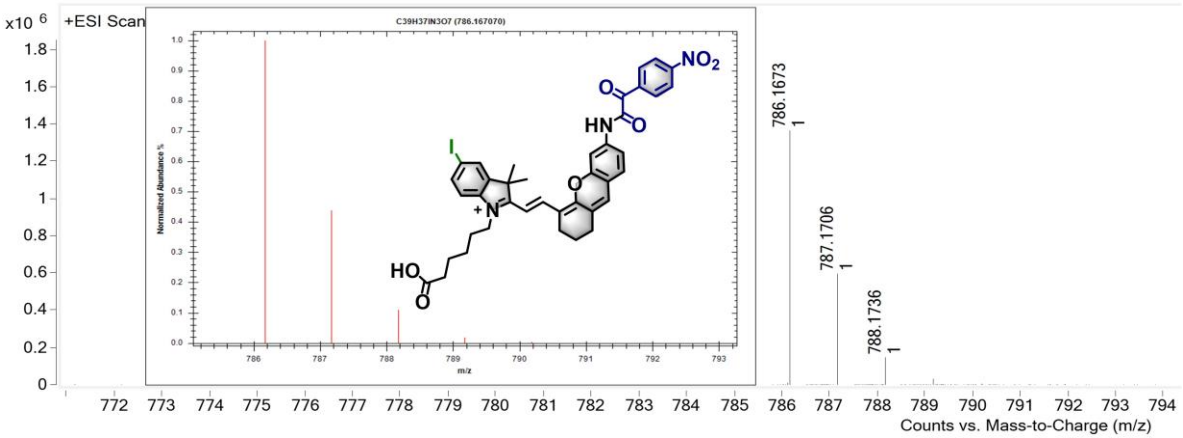

Fig. S14. Mass spectrum of **ICy**.

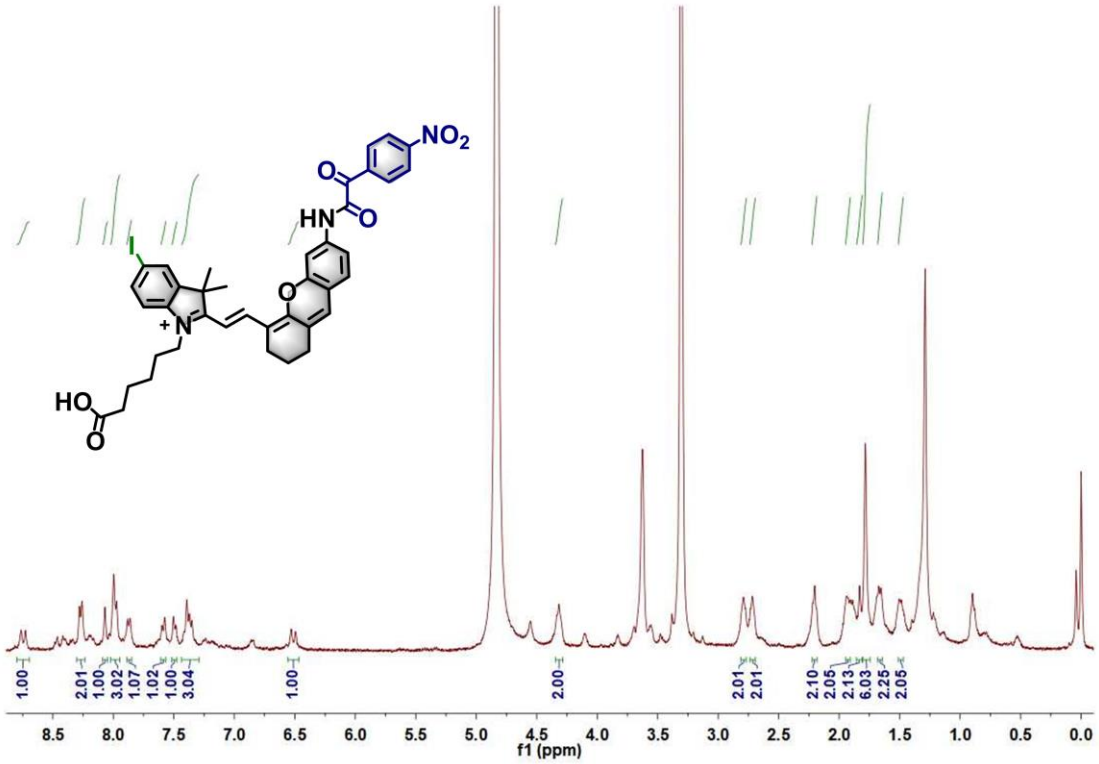

Fig. S15. ^{1}H NMR spectrum of **ICy** (400 MHz, MeOD).

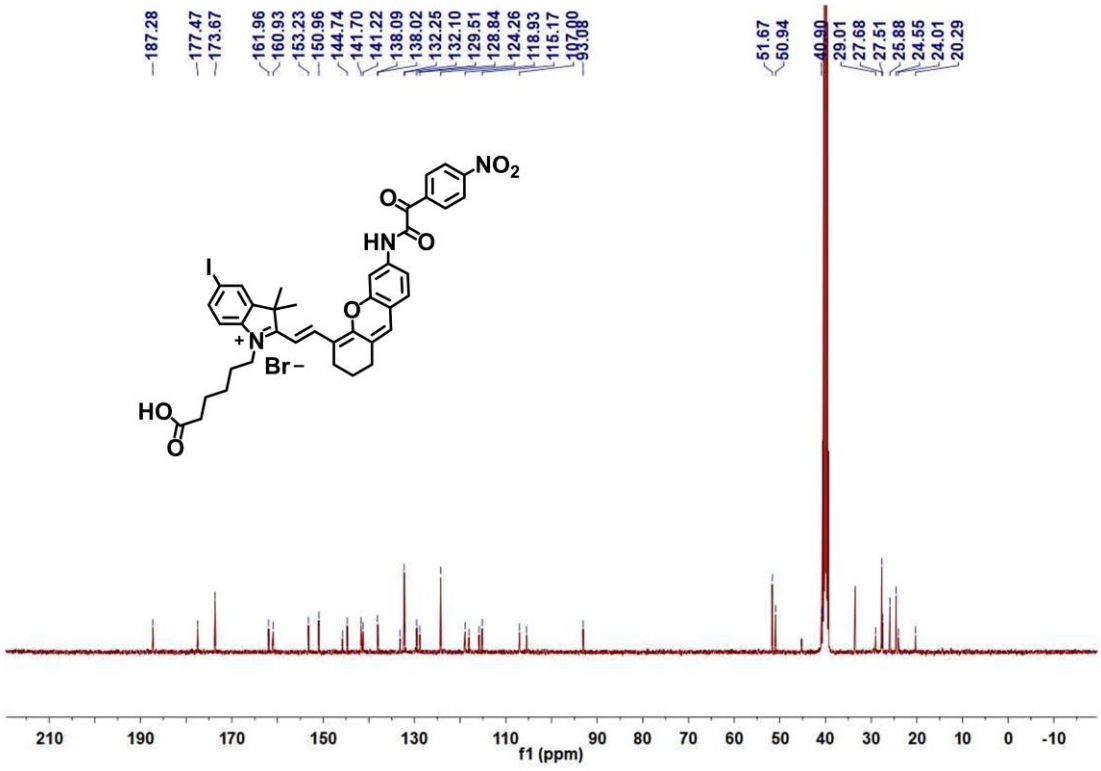

Fig. S16. ^{13}C NMR spectrum of **ICy** (101 MHz, DMSO-d_6).

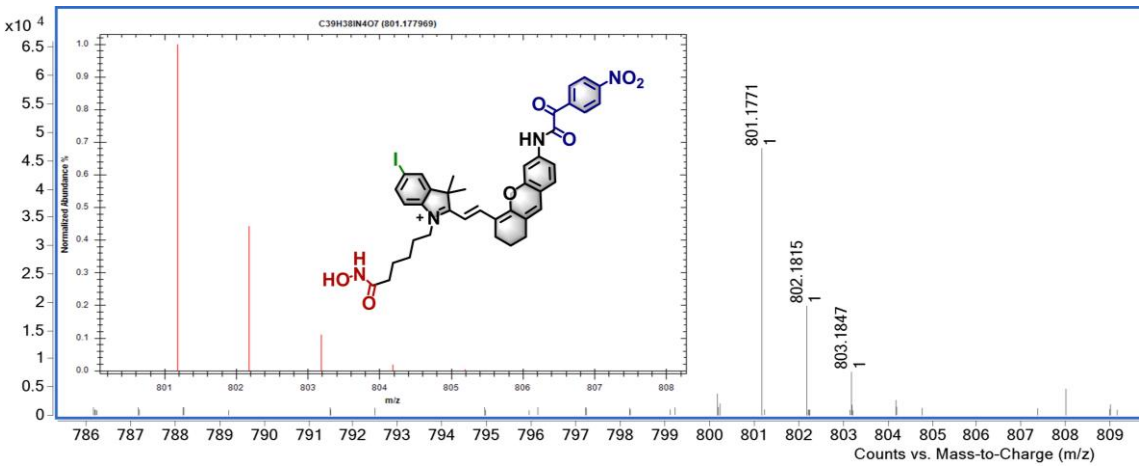

Fig. S17. Mass spectrum of **ICyHD**.

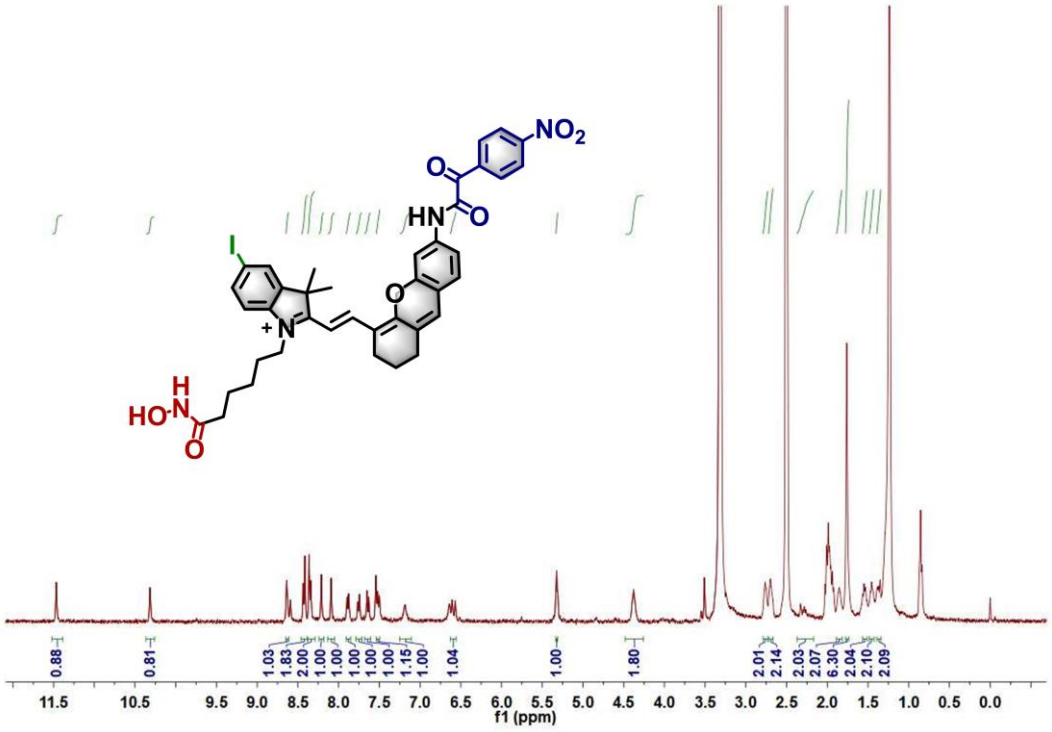

Fig. S18. ^{1}H NMR spectrum of **ICyHD** (400 MHz, DMSO-d6).

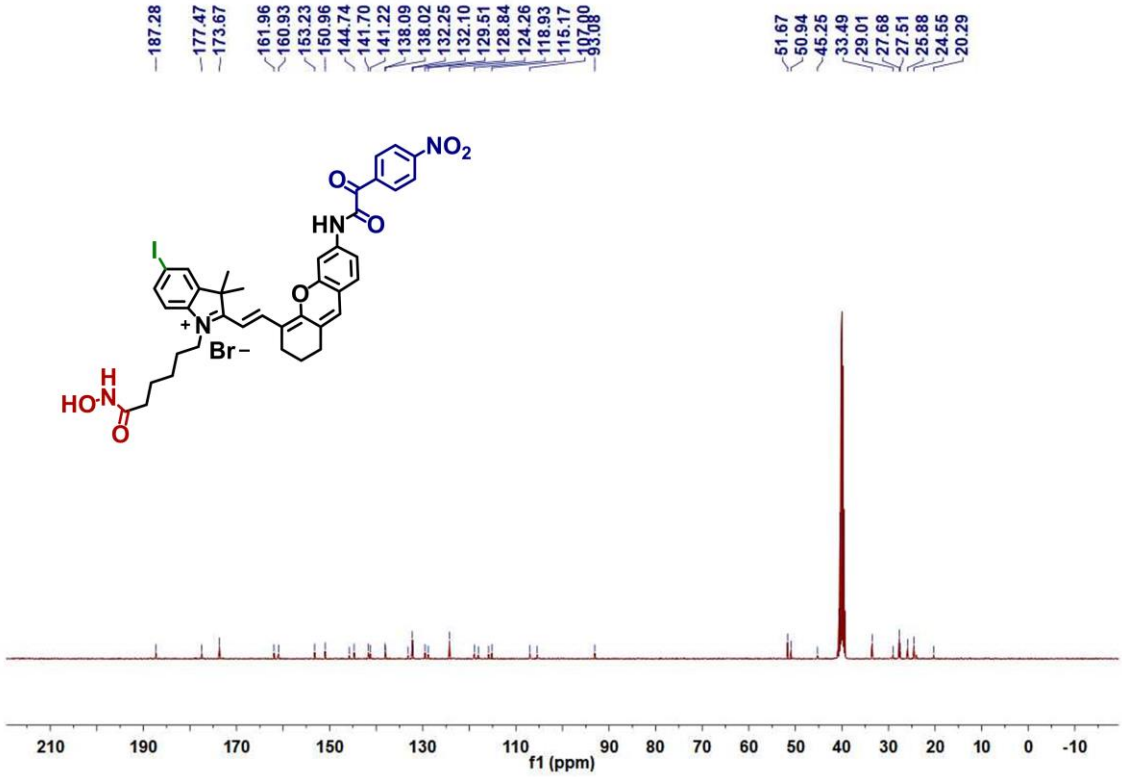

Fig. S19. ^{13}C NMR spectrum of **ICyHD** (101 MHz, DMSO-d_6).

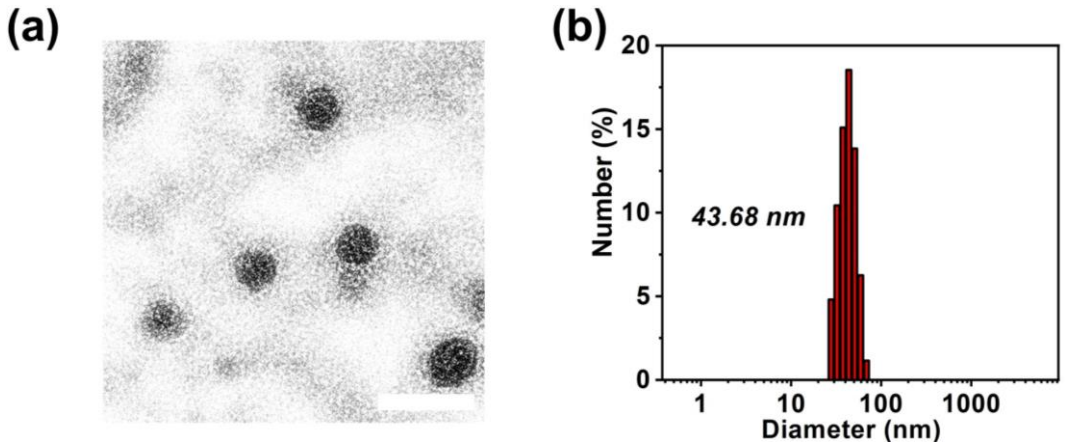

Fig. S20. (a) TEM images and (b) DLS images of **ICyHD NPs**. Scale bar: 100 nm.

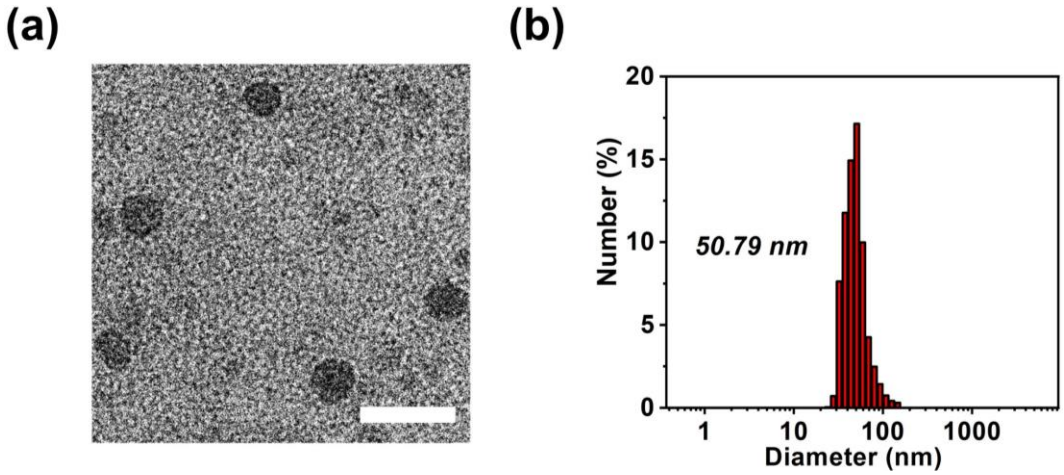

Fig. S21. (a) TEM images and (b) DLS images of **ICy NPs**. Scale bar: 100 nm.

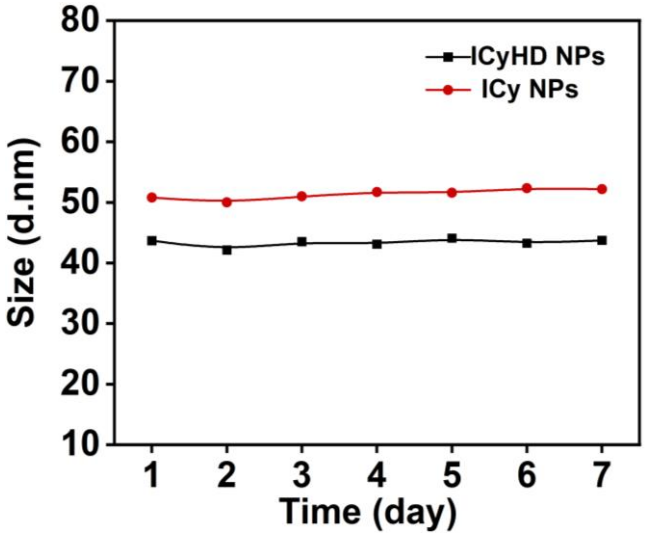

Fig. S22. Stability of **ICyHD NPs** and **ICy NPs**.

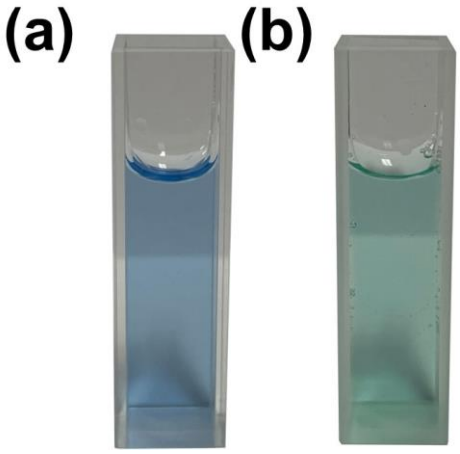

Fig. S23. Images of **ICyHD NPs** and H_2O_2 Systems Before (a) and After (b) Responses.

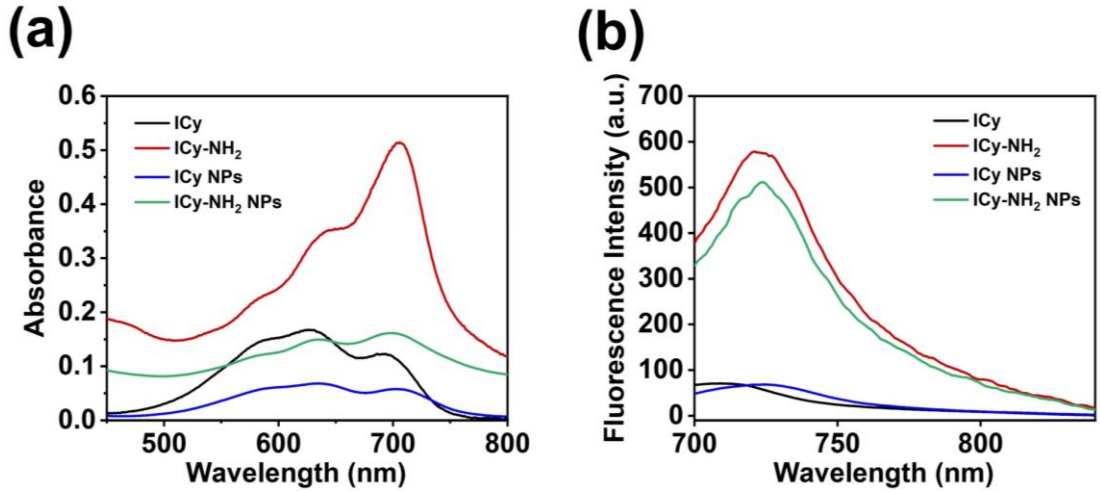

Fig. S24. (a) Absorption spectrum changes before and after 30 min reaction of 10 μM **ICy** or **ICy NPs** with 100 μM H_2O_2 in PBS (pH = 7.4) (containing 1% methanol); (b) Fluorescence emission spectrum changes before and after 30 min reaction of 10 μM **ICy** or **ICy NPs** with 100 μM H_2O_2 in PBS (pH = 7.4) (containing 1% methanol), λ_{ex} = 700 nm.

Tab. S1 Fluorescence Quantum Yields of ICy and ICyHD NPs.

Compound	ICy NPs	ICyHD NPs	ICy-NH_2 NPs	ICyHD-NH_2 NPs
Fluorescence* Quantum Yield	0.007	0.005	0.024	0.027

*The Fluorescence Quantum Yield is evaluated in PBS (pH=7.4).

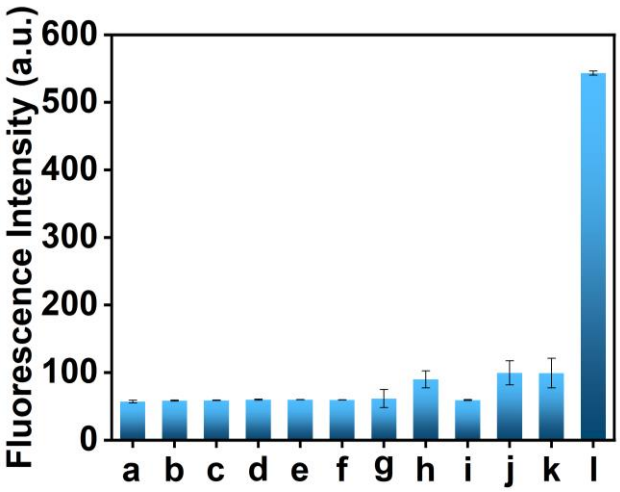

Fig. S25. Selectivity of **ICyHD NPs** toward different analytes of PBS. Unless mentioned otherwise, all concentrations were 100 μM. Insert a: blank; b: K^+; c: Ca^{2+}; d: Zn^{2+}; e: Cu^{2+}; f: Mg^{2+}; g: Fe^{2+}; h: NO_3^-; i: NO_2^-; j: $S_2O_3^{2-}$; k: vitamin C; and l: H_2O_2.

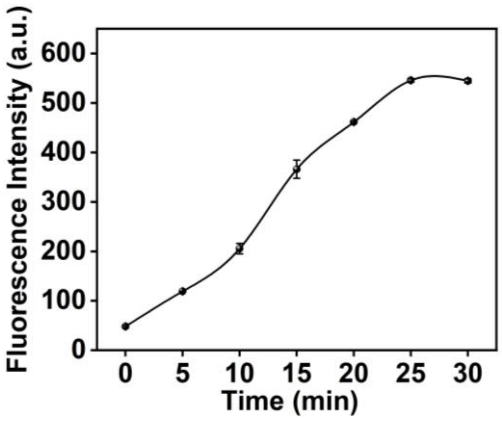

Fig. S26. Time-dependent changes in the fluorescence emission of **ICyHD NPs** in the presence of H_2O_2 (100 μM).

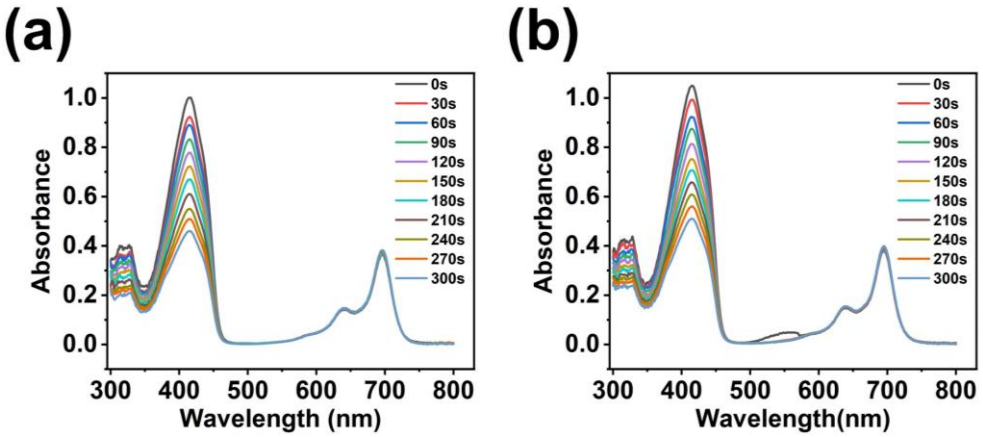

Fig. S27. The absorbance of DPBF in the presence of (a) **ICy-NH2** and (b) **ICyHD-NH2** under light irradiation (700 nm, 5 mW/cm^2) in DCM solution.

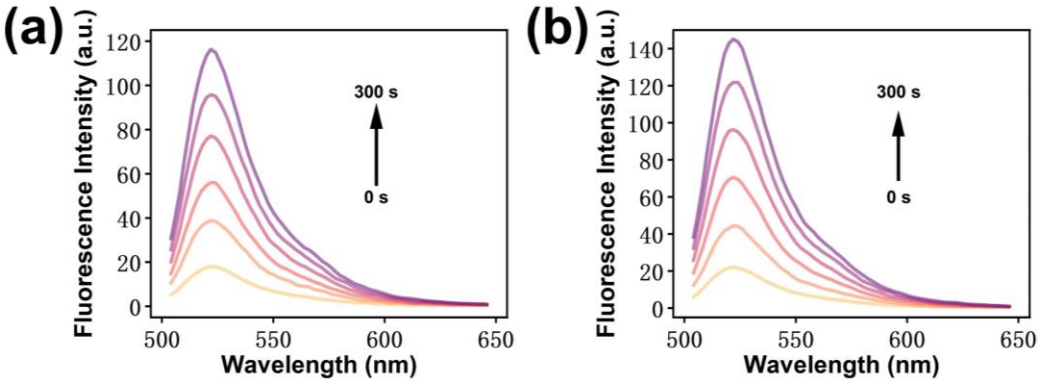

Fig. S28. The fluorescence emission spectrum of DCFH in the presence of (a) **ICyHD-NH2** or (b) **ICyHD-NH2 NPs** under light irradiation for different times (700 nm, 5 mW/cm^2, 0-300 s) in H_2O solution.

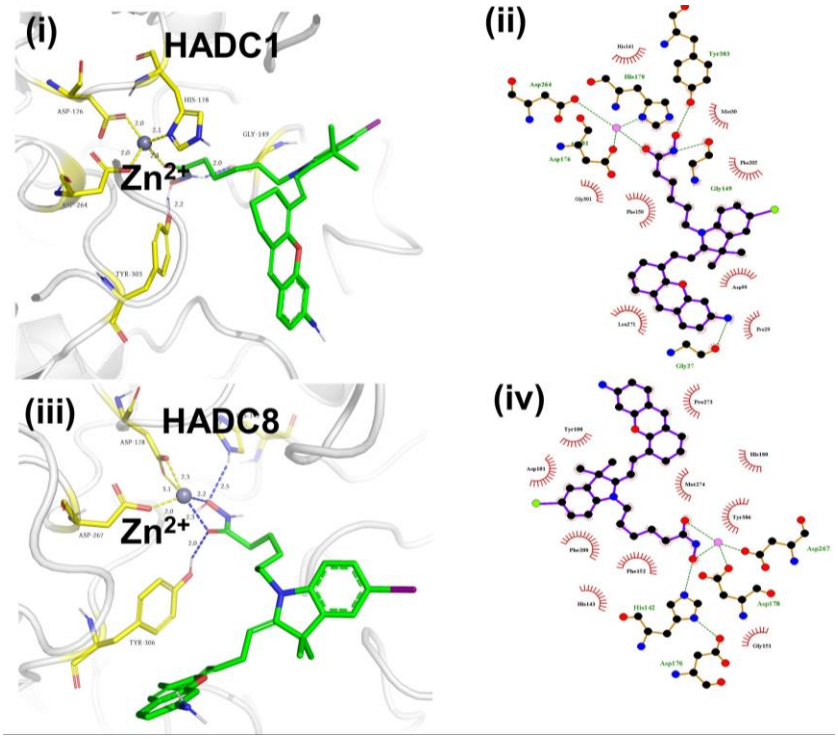

Fig. S29. Proposed binding study of **ICyHD-NH2** with Zn^{2+} in both HDAC1 and HDAC8. (i) Model structure of HDAC1 and (ii) its intricate relationship with **ICyHD-NH2**. (iii) Model structure of HDAC8 and (iv) its intricate relationship with **ICyHD-NH2**.

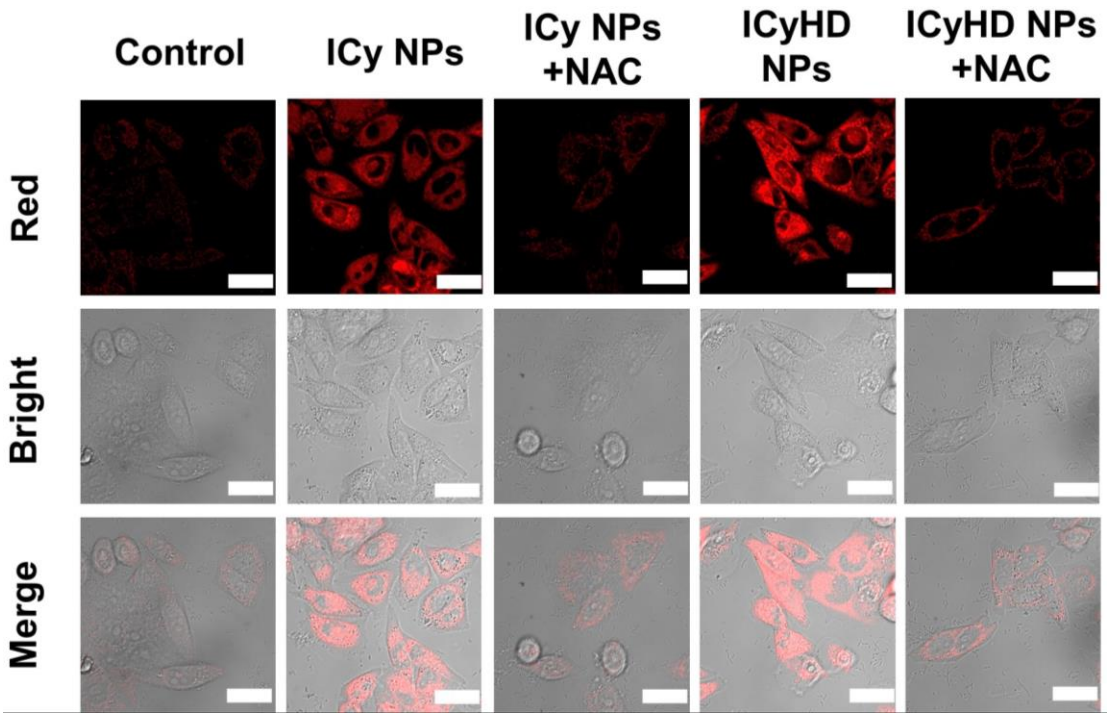

Fig. S30. Chemical inhibition test of NAC (20 mM) pretreated cells for 1 h. Scale bar: 10 μm.

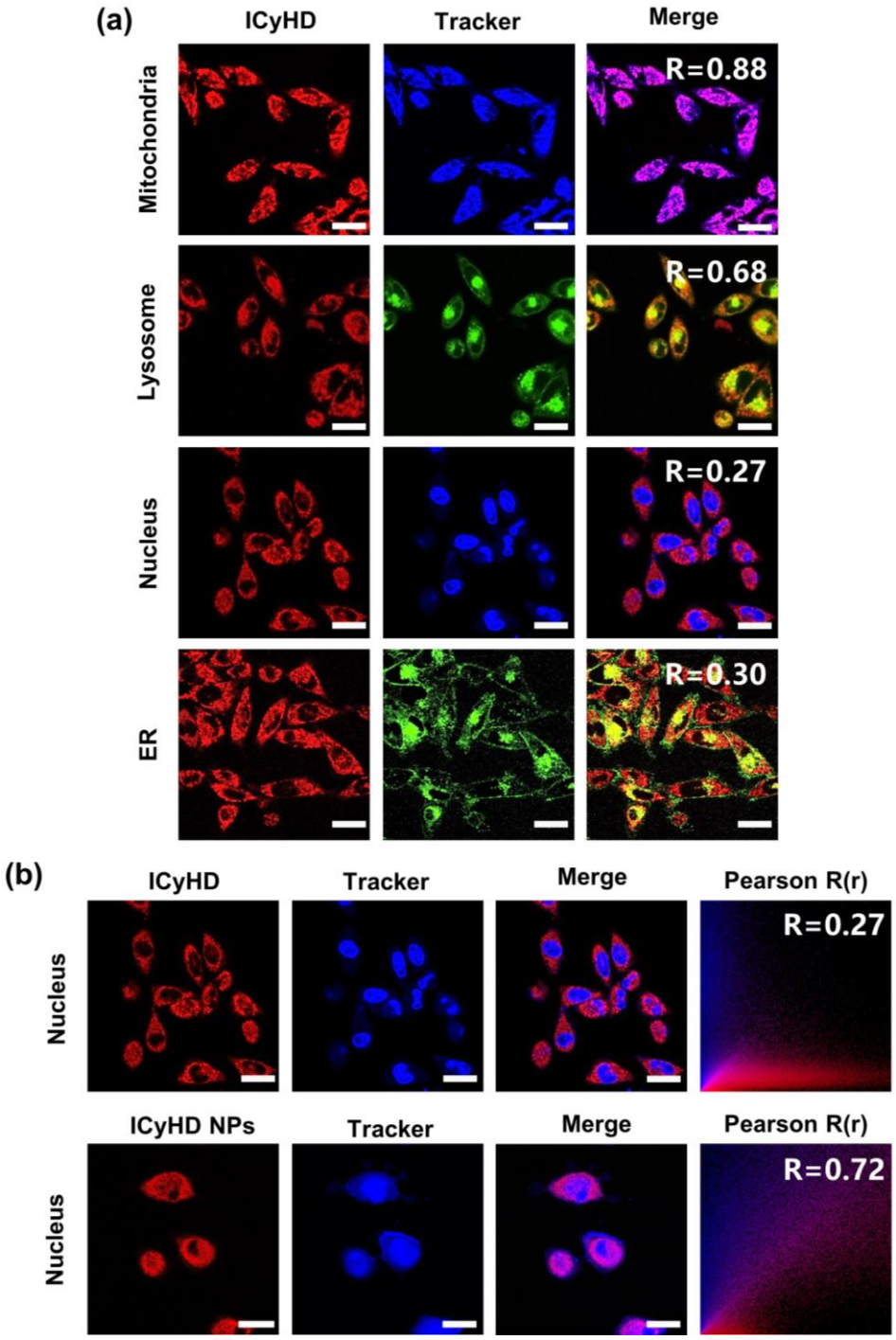

Fig. S31. (a) Organelle co-localization of **ICyHD**. Mitochondria stained with Mito-Tracker Green (λ_{ex}=488 nm, λ_{em}=500-550 nm); Lysosomes stained with Lyso-Tracker Green (λ_{ex}=488 nm, λ_{em}=500-550 nm); Nuclei stained with Hoechst 33342 (λ_{ex}=405 nm, λ_{em}=430-470 nm); endoplasmic reticulum stained with ER-Tracker Green (λ_{ex}=488 nm, λ_{em}=500-550 nm); (b) Organelle co-localization of **ICyHD** with **ICyHD NPs**. Nuclei stained with Hoechst 33342 (λ_{ex}=405 nm, λ_{em}=430-470 nm). Scale bar: 10 μm.

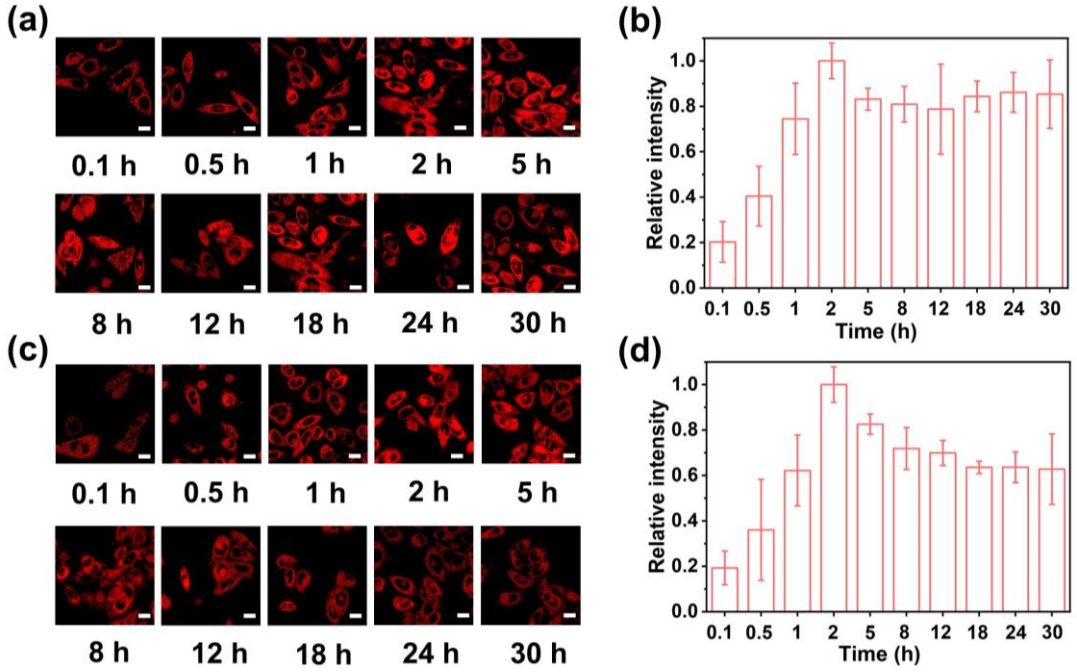

Fig. S32. (a) Retention analysis of the intracellular fluorescence imaging of MCF-7 cells after incubation with **ICyHD NPs** (λ_{ex} =700 nm; λ_{em} =710-800 nm; Scale bar: 20 μm); (b) Relative fluorescence intensity of the corresponding images in panel a; the intensity of image "a 2 h" was considered as 1.0; (c) Retention analysis of the intracellular fluorescence imaging of MCF-7 cells after incubation with **ICy NPs** (λ_{ex} =700 nm; λ_{em} =710-800 nm; Scale bar: 20 μm); (d) Relative fluorescence intensity of the corresponding images in panel a; the intensity of image "c 2 h" was considered as 1.0.

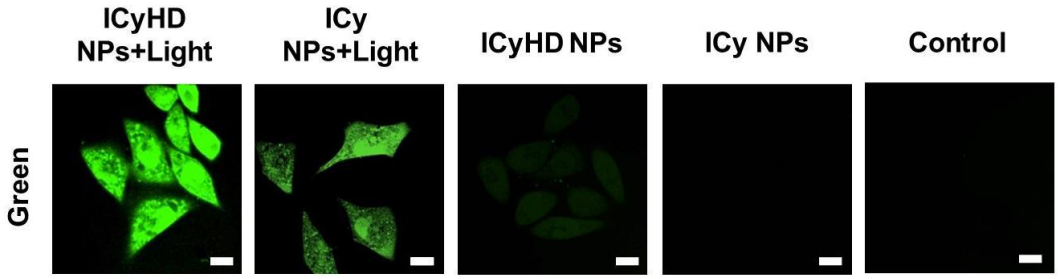

Fig. S33. Analysis of intracellular ROS production (λ_{ex}=488 nm, λ_{em}=500-600 nm). Scale bar: 10 μm.

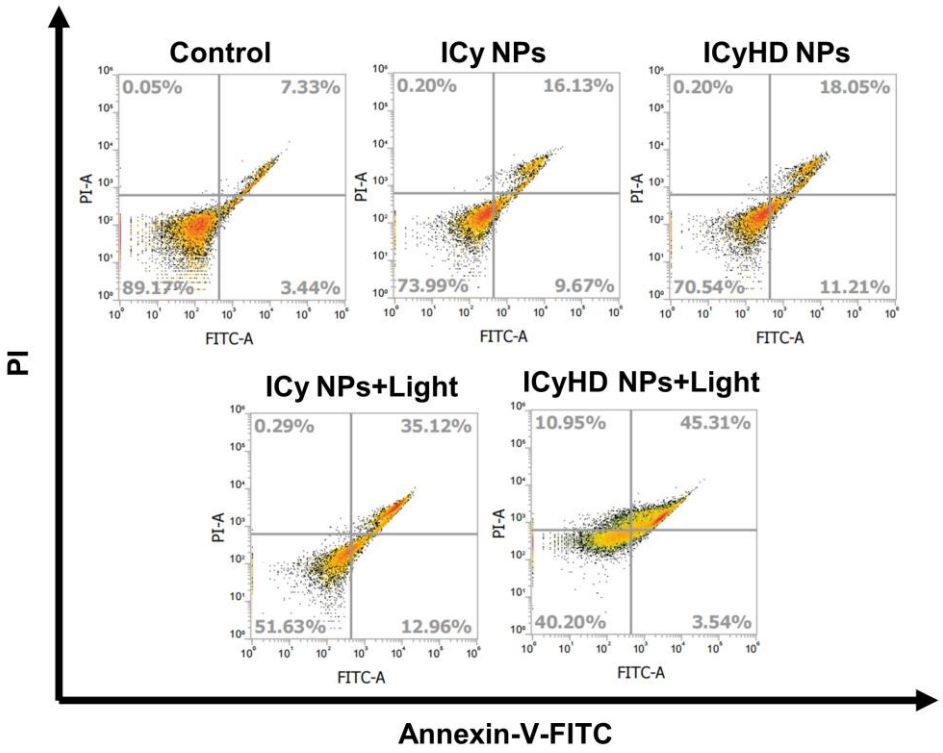

Fig. S34. Cell apoptosis of MCF-7 cancer cells with different treatments as determined by flow cytometry.

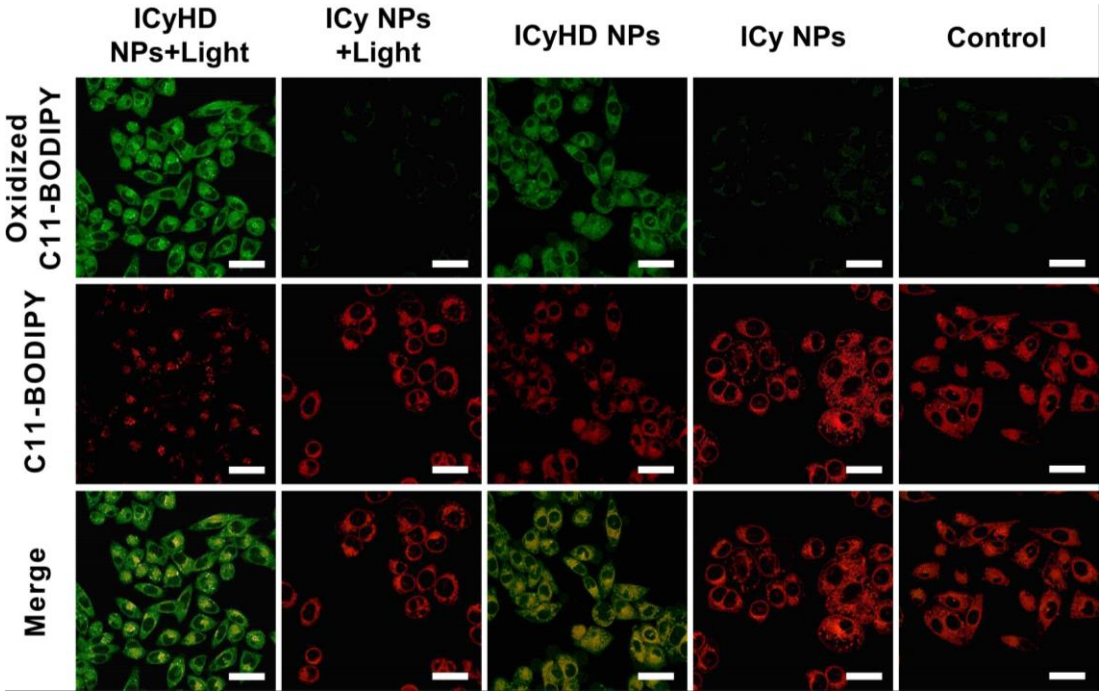

Fig. S35. CLSM images of C11-BODIPY 581/591 dye-stained MCF-7 cells in different treatment groups. Scale bars, 20 μm.

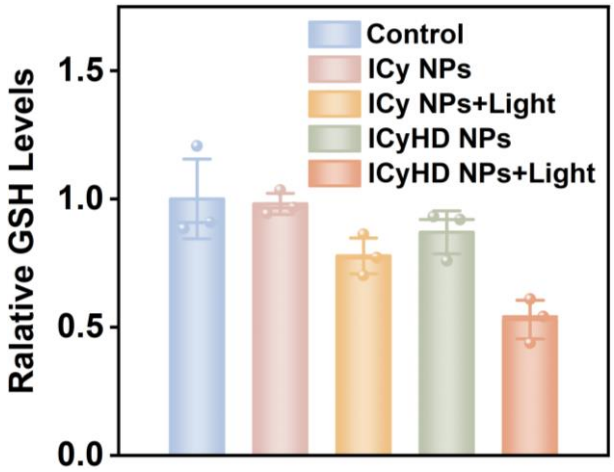

Fig. S36. Intracellular relative GSH levels in 4T1 cells under different treatment conditions.

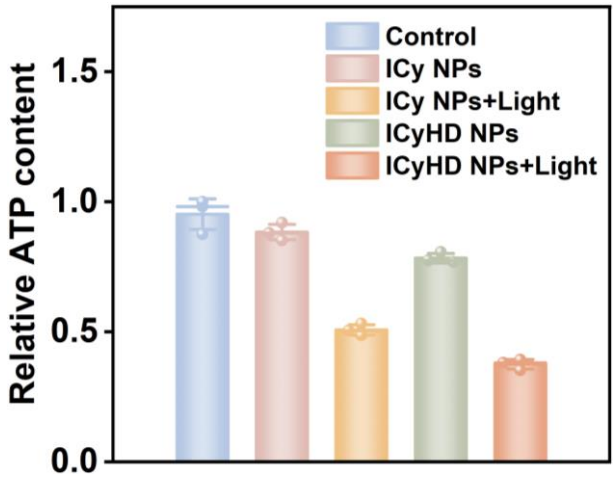

Fig. S37. Intracellular relative ATP levels in 4T1 cells under different treatment conditions.

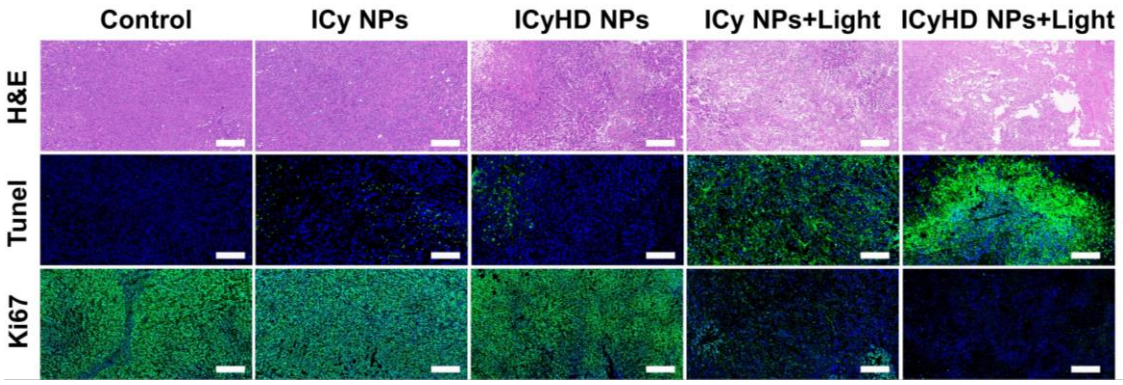

Fig. S38. H&E, TUNEL and Ki67 assay analysis of tumor tissues in the different treatments. Scale bar: 100 μm for H&E and 50 μm for TUNEL and Ki67.

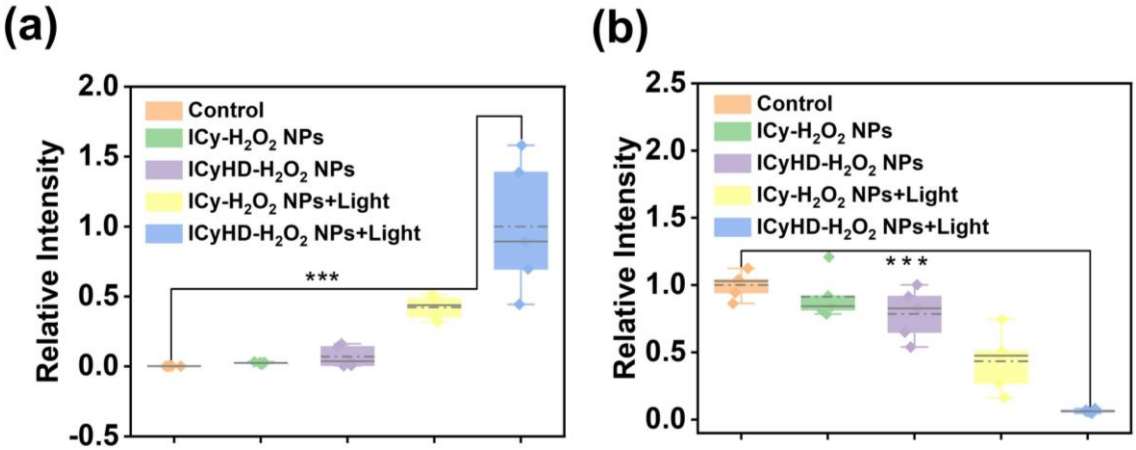

Fig. S39. (a) The normalized mean intensity of TUNEL assay analysis of tumor tissues in the different treatments. (b) The normalized mean intensity of Ki67 assay analysis of tumor tissues in the different treatments.. The statistical data are presented as mean ± standard deviation (n=5; ***P < 0.001).

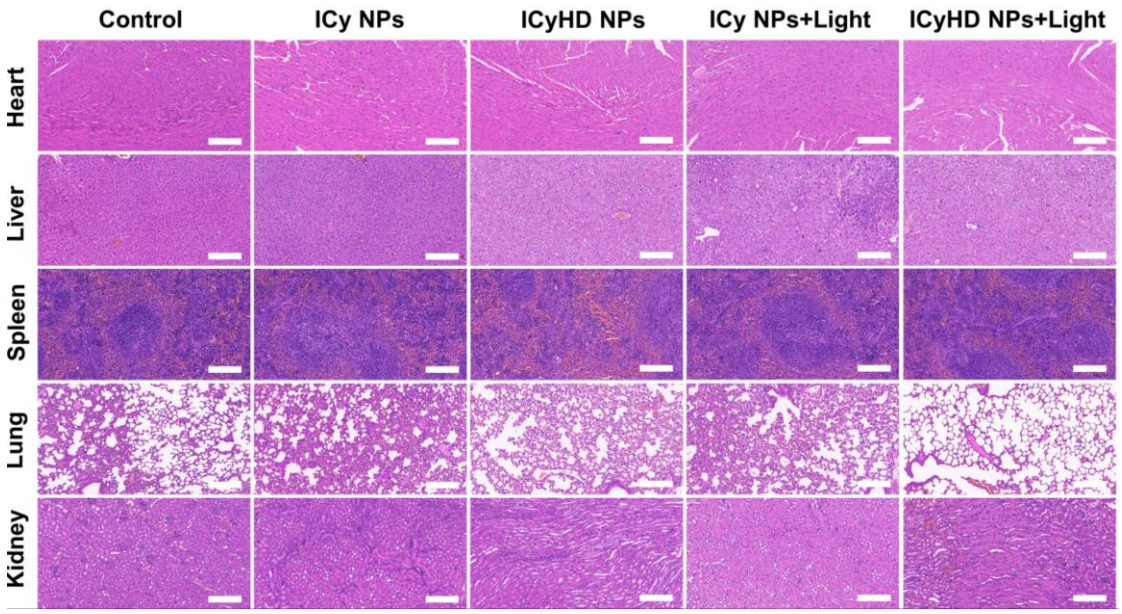

Fig. S40. In vivo biosafety assay. H&E staining of major organs of mice from different treatment groups after 14 d of treatment. Scale bars: 100 μm.

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2 M. Nahorniak, V. Oleksa, T. Vasylyshyn, O. Pop-Georgievski, E. Rydvalová, M. Filipová and D. Horák, *Nanomaterials*, 2023, **13**, 1535.

3 Rajneesh, J. Pathak, A. Chatterjee, S. P. Singh and R. P. Sinha, *Bio-Protoc.*, 2017, **7**, e2545.