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Supplementary Information

A heat shock protein-inhibiting molecular photothermal agent for mild-temperature photothermal therapy

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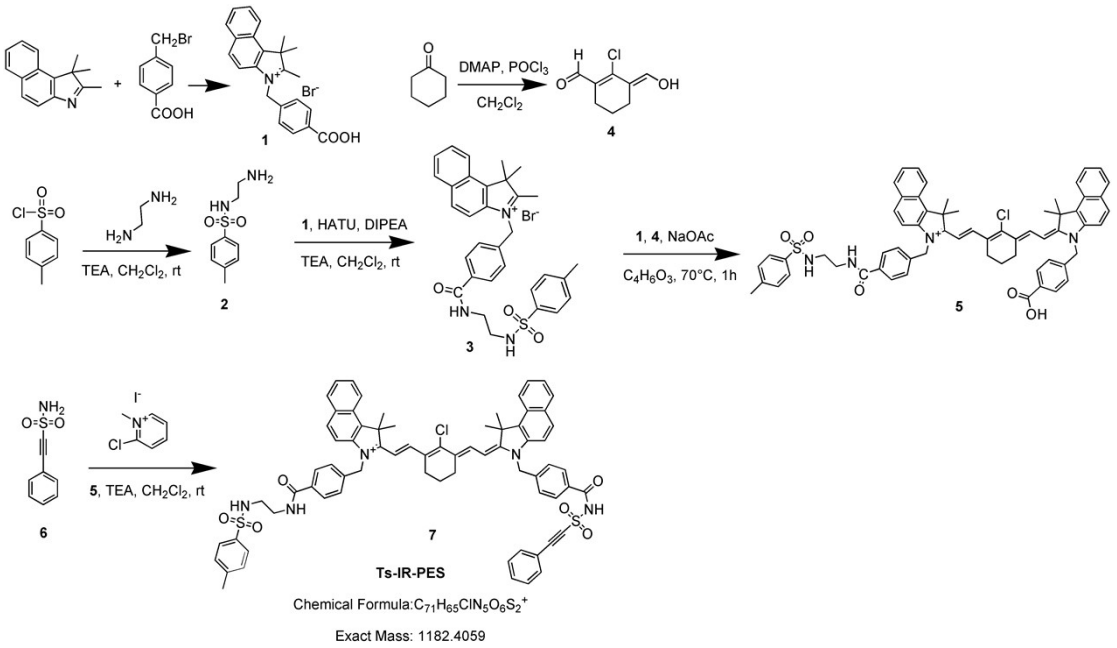
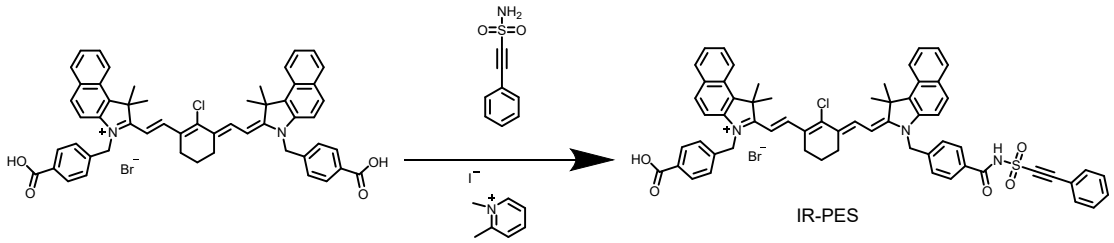
Experimental Procedures

Materials and Reagents. Alpha-bromo-*p*-toluic-acid，2,3,3-trimethyl-4,5-benzo-3H-indole were obtained from Tianjin Heowns Biochemical Technology Co., Ltd. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2h-tetrazoliubromide (MTT) and dialysis Membranes were obtained from Beijing Solarbio Science & Technology Co., Ltd. Annexin V-Alexa Fluor 488/PI Apoptosis Detection Kit was purchased from Yeasen (Shanghai, China). Antibody against HSP70 was purchased from Boster Biological Technology Co., Ltd. Acetonitrile, anhydrous diethyl ether, dichloromethane (DCM), dichlorobenzene, toluene, acetic anhydride dimethyl sulfoxide (DMSO) and triethylamine (TEA) were purchased from China National Pharmaceutical (Shanghai, China).

Instruments. High resolution transmission electron microscopy (HRTEM) was carried out on a JEM-2100 electron microscope. Fluorescence spectra were acquired with fluorescence spectrometer (FLS-980, Edinburgh, UK). UV-vis absorption spectra were measured on a pharmaspec UV-1700 UV-Visible spectrophotometer (Shimadzu, Japan). Confocal fluorescence imaging studies were performed with a TCS SP8 confocal laser scanning microscopy (Leica, Germany). Absorbance was measured in a microplate reader (Synergy 2, Biotek, USA) in the MTT assay. Success of each reaction step was confirmed by monitoring the changes in zeta potential with a Malvern Zeta Sizer Nano (Malvern Instruments). All the nuclear magnetic resonance (NMR) spectra were recorded on a Bruker NMR spectrometer. High resolution mass spectra (HRMS) were recorded on a Bruker Daltonics maXis UHR-TOF MS. HPLC was performed using a Shimadzu CBM-20A instrument.



Scheme S1. Synthesis of Ts-IR-PES.



Scheme S2. Synthesis of IR-PES.

Synthesis of Ts-IR-PES

Compound 1: The compound **1** was carefully prepared according to the references.[1] 2,3,3-trimethyl-4,5-benzo-3H-indole (TMBI, 10.0 g, 48 mmol) and alpha-bromo-*p*-toluic acid (BTA, 12.3 g, 57 mmol) were dissolved in 40 mL dichlorobenzene and stirred under 110 °C for 6 h. The solid crude product was then filtrated and recrystallized in methanol. The purified product as denoted intermediate **1** was dried under vacuum (80% yield).

Compound 2: The compound 2 was carefully prepared according to the references.[2] 4-toluene sulfonyl chloride (0.95 g, 5.0 mmol) was dissolved in 20 mL DCM under 0 °C. Ethylenediamine (0.15 g, 2.5 mmol) and triethylamine (70 μL, 0.50 mmol) were dissolved in 5 mL DCM. A mixture of 4-toluene sulfonyl chloride, ethylenediamine and triethylamine was stirred at 0 °C for 30 min. Then, the mixture was stirred at room temperature for another 2 h. The crude product was washed with NH_4Cl saturated solution and H_2O. The organic part was dried using $MgSO_4$, and removed

under vacuum. The purified product dried under high vacuum to afford **2** (60% yield**)**. ^{1}H NMR (400 MHz, d^6-DMSO): δ 7.71 – 7.69 (m, 2H), 7.40 – 7.38 (m, 2H), 3.60 (s, 2H), 2.75 – 2.71 (m, 2H), 2.54 – 2.51 (m, 2H), 2.37 (s, 3H). HRMS (ESI): *m/z* calcd for $C_9H_{15}N_2O_2S$ $[(M + H)]^+$: 215.0849, found: 215.0832.

Compound 3: A mixture of **1** (0.424 g, 1.0 mmol), *N*, *N*-diisopropylethylamine (200 μL) and HATU (1.14 g, 3 mmol) in DCM (20 mL) was stirred at 25 °C for 30 min. Then, **2** (0.428 g, 2 mmol) and triethylamine (417 μL, 3.0 mmol) were added to the mixture, and stirred at 25 °C for 24 h. After removal of the dichloromethane solvent under vacuum, the crude product was purified by column chromatography over silica gel using CH_2Cl_2/CH_3OH (v/v, 10:1) as the eluent (50% yield). ^{1}H NMR (400 MHz, d^6-DMSO): δ 7.76 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 8.0 Hz, 4H), 7.56 (d, J = 12.0 Hz, 2H), 7.49 (d, J = 12.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 4H), 6.96 (s, 1H), 6.90 (s, 1H), 4.73 (s, 3H), 3.76 (d, J = 4.0 Hz, 2H), 3.70 (s, 2H), 3.39 – 3.32 (m, 2H), 1.39 (s, 9H). HRMS (ESI): *m/z* calcd for $C_{32}H_{34}N_3O_3S^+$ $[(M\text{-}Br)]^+$: 540.2315, found: 540.2244.

Compound 4: The compound **4** was carefully prepared according to the references.[1] 80 mL DMF/dichloromethane mixture (1/1, v/v) was added dropwise into a solution of phosphoryl chloride (37 mL) and anhydrous dichloromethane (35 mL) under stirring in ice/water bath; afterwards, 10 g cyclohexanone was added dropwise. The ice/water bath was removed and the solution was then heated and refluxed for 3 h. The mixture was poured into ice, yielding a solid product which was collected by filtration and washed with iced diethyl ether. The resulting yellow product was denoted as intermediate **4** and used directly.

Compound 5 (Ts-IR): Under the protection of argon, **1** (0.424 g, 1.0 mmol), **3** (0.619 g, 1.0 mmol), **4** (0.172 g, 1.0 mmol), anhydrous sodium acetate (0.164 g, 2.0 mmol), and 10 mL acetic anhydride were added to a 50 ml reaction flask, the mixed solution was heated to 70 °C for 1 h. The mixture was cooled to room temperature, and ethanol was removed under reduced pressure to give a crude product. The crude product was purified by column chromatography over silica gel using CH_2Cl_2/CH_3OH (v/v, 10:1) as the eluent **(**40% yield**)**. ^{1}H NMR (400 MHz, d^4-CD_3OD): δ 8.50 (d, J = 20.0 Hz, 2H), 8.32 (d, J = 8.0 Hz, 2H), 8.05 – 7.98(m, 6H), 7.82 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 10.0 Hz, 4H), 7.59 – 7.49 (m, 4H), 7.42 (t, J = 12.0 Hz, 4H), 7.28 (d, J = 8.0 Hz, 2H), 6.30 (d, J = 8.0 Hz, 2H), 5.64 (s, 4H), 3.42 (t, J = 12.0 Hz, 2H), 3.23-3.18 (m, 2H), 3.04 (t, J = 12.0 Hz, 2H), 2.53 (s, 4H), 2.37 (s, 3H), 2.07 (s, 12H), 1.86 (s, 2H). HRMS (ESI): *m/z* calcd for $C_{63}H_{60}ClN_4O_5S^+$ $[(M\text{-}Br)]^+$: 1019.3967, found: 1019.4026.

Compound 6: The compound **6** was carefully prepared according to the references.[3] Phenylacetylene (0.54 mL, 4.9 mmol) was added to anhydrous THF (15 mL) and cooled to –78 °C using a mixture of liquid nitrogen and acetone. Once cooled, a 1 M solution of LiHMDS (5.4 mL, 5.4 mmol) was added dropwise and the mixture was left to stir for 10 min. HMPA (0.94 mL, 5.4 mmol) was then added before an additional 10 min of stirring. Freshly prepared sulfamoyl chloride (620 mg, 5.4 mmol) dissolved in anhydrous THF (5 mL) was subsequently added and the reaction mixture was left to stir for 1 h, maintaining the temperature at –78 °C. After stirring and returning to r.t., EtOAc (15 mL) was added and the mixture washed with aqueous NH_4Cl (15 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL), the organic layers combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting crude oil was subject to column chromatography eluting with 30% EtOAc/hexane to yield as a white solid **(**60% yield**)**.

Compound 7 (Ts-IR-PES): 2-Chloro-1-methylpyridin-1-ium iodide (1.010 g, 3.95 mmol) and **5** (3.613 g, 3.29 mmol) were dissolved in 10 mL CH_2C1_2. The mixture was stirred at room temperature for 10 min before adding **6** (1.788 g, 9.88 mmol). The mixture was stirred at room temperature for another 10 min. Then TEA (1.377 mL, 9.88 mmol) was slowly added. The reaction was stirred at room temperature for 1 h. The solvent was removed under vacuum. Then 1 equivalent of HC1 (3 mL) and 20 mL H_2O were added to the residue. The solution was extracted with ethyl acetate for three times. The ethyl acetate layers were combined, dried (using Na_2SO_4), filtered and concentrated under reduced pressure. Then the crude product was purified by column chromatography over silica gel using CH_2Cl_2/CH_3OH (v/v, 10:1) as the eluent **(**35% yield**)**. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.43 (d, J = 14.1 Hz, 1H), 8.35 (d, J = 13.7 Hz, 1H), 8.28 (s, 1H), 8.17 – 8.11 (m, 2H), 8.04 – 7.84 (m, 8H), 7.64 (dt, J = 14.3, 7.6 Hz, 5H), 7.44 (dd, J = 33.7, 8.2 Hz, 5H), 7.30 (d, J = 8.7 Hz, 2H), 7.23 (d, J = 7.6 Hz, 2H), 7.08 (t, J = 7.2 Hz, 2H), 6.16 (d, J = 14.4 Hz, 1H), 6.05 (t, J = 12.9 Hz, 1H), 5.42 (s, 3H), 5.14 (s, 2H), 3.87 (s, 2H), 3.45 (s, 2H), 3.12 (s, 2H), 2.44 (s, 4H), 2.22 (d, J = 5.4 Hz, 3H), 2.04 (d, J = 5.6 Hz, 12H), 1.78 (s, 2H), 1.26 (s, 2H). HRMS (ESI): m/z calcd for $C_{71}H_{65}N_5O_6S^{2+}$ $[(M\text{-}Br)]^+$: 1182.4059, found: 1182.4169.

IR-PES: 2-Chloro-1-methylpyridin-1-ium iodide (1.010 g, 3.95 mmol) and IR825[1] ($C_{54}H_{48}BrClN_2O_4$, 2.975 g, 3.29 mmol) were dissolved in 15 mL CH_2C1_2. The mixture was stirred at room temperature for 10 min before adding **6** (0.894 g, 4.94 mmol). The mixture was stirred at room temperature for another 10 min. Then TEA (0.689 mL, 4.94 mmol) was slowly added. The reaction was stirred at room

temperature for 1 h. The solvent was removed under vacuum. Then 1 equivalent of HC1 (3 mL) and 20 mL H_2O were added to the residue. The solution was extracted with ethyl acetate for three times. The ethyl acetate layers were combined, dried (using Na_2SO_4), filtered and concentrated under reduced pressure. Then the crude product was purified by column chromatography over silica gel using CH_2Cl_2/CH_3OH (v/v, 10:1) as the eluent (35% yield). ^{1}H NMR (400 MHz, d^6-DMSO): δ 13.05 (s, 1H), 8.42 (d, J = 12.0 Hz, 4H), 8.14 (t, J = 12.0 Hz, 4H), 8.04 (t, J = 16.0 Hz, 4H), 7.78 – 7.73 (m, 4H), 7.62 (t, J = 12.0 Hz, 4H), 7.52 – 7.43 (m, 5H), 6.48 (d, J = 16.0 Hz, 2H), 5.83 (s, J = 4.0 Hz, 4H), 3.89 (s, 1H), 2.64 (s, 4H), 2.10 (s, 12H), 1.83 (s, 2H), 1.40 (d, J = 12.0 Hz, 2H). HRMS (ESI): *m/z* calcd for $C_{62}H_{53}ClN_3O_5S^+$ $[(M-Br)]^+$: 986.3389, found: 1019.4026.

UV-vis spectra and fluorescent excitation spectra. The UV-vis absorption spectra of Ts-IR-PES were obtained using UV-visible spectrophotometer, and fluorescent spectra of Ts-IR-PES in H_2O and CH_3OH were acquired using fluorescence spectrometer (FLS-980, Edinburgh, UK).

Photostability Test. Aqueous soluntion of Ts-IR-PES in UV-quartz cuvette were exposed to the 808 nm laser (0.33 W/cm^2) for different time. UV-vis spectra of the solution before and after laser irradiation was recorded.

Photothermal effect and photothermal conversion efficiency. For the purpose of evaluating the photothermal ability, the Ts-IR-PES solution with different concentrations (0 mM, 0.05 mM, 0.10 mM, 0.20 mM, and 0.50 mM) were irradiated using 808 nm laser (0.33 W/cm^2). A thermometer submerged in the solution was used to monitor the temperature during the irradiated 600 s. To measure the photothermal conversion efficiency, Ts-IR-PES solution (0.50 mM) were exposed to 808 nm irradiation (0.33 W/cm^2) for 10 min, and then the irradiation was removed for cooling down to room temperature. The temperature of the solution was recorded with an interval of 20 s during this process. Ts-IR-PES solutions (0.50 mM) were imaged by Infrared Thermal Camera.

Calculation of the photothermal conversion efficiency. The photothermal conversion efficiencies (η) were measured according to a previously described method.[4]

$$\eta = \frac{hS(\Delta T_1 - \Delta T_2)}{I(1-10^{-A})} \qquad hS = \frac{mc}{\tau_s} \qquad t = -\tau_s \ln(\theta) \qquad \theta = \frac{T - T_{surr}}{T_{max} - T_{surr}}$$

Where ΔT_1 and ΔT_2 are the maximum temperature changes of sample and H_2O,

respectively. I represent the laser power. A is the absorbance of Ts-IR-PES (0.50 mM) at 816 nm. m and c are the mass and heat capacity of solvent, respectively. T is the temperature at moment t in the cooling process. T_{max} is the maximum temperature of sample. T_{surr} is the surrounding temperature.

Gel electrophoresis. HSP70 (10 μg/mL) was reacted with Ts-IR-PES (5 μg/mL) for 6 h, which donated as Ts-IR-PES + HSP70 group. The reaction mixture was dialyzed with a dialysis bag (MWCO 3500). HSP70 group and Ts-IR-PES group were used as controls. Then sample of three groups (HSP70; HSP70 + Ts-IR-PES; Ts-IR-PES) were separated by the 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), respectively. Then, the fluorescence bands of the three groups were displayed by a ChemiDoc™ Touch Imaging System (Bio-Rad). Then, gel was stained with coomassie blue for 1 h and stay in water for whole night. Finally, the coomassie blue bands of the three groups were displayed by a ChemiDoc™ Touch Imaging System (Bio-Rad).

Cell culture. 4T1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium. All cells were supplemented with 10% fetal bovine serum (BI) and 100 units/mL of 1% antibiotics penicillin/streptomycin (Gibco) to maintain at 37 ^{o}C in a 100% humidified atmosphere containing 5% CO_2.

Cytotoxicity Assays. 4T1 cells were seeded in 96-well plates with an amount of 5 × 10^3 for 24 h and incubated with Ts-IR-PES (0 μM, 5 μM, 10 μM, 20 μM and 30 μM) for another 24 h. During which, the cells were cultured with fresh complete medium, and with or without irradiation using 808 nm laser (0.33 W/cm^2, 10 min). After that, 150 μL MTT solution (0.5 mg/mL) was added to each well. The remaining MTT solution was removed 4 h later, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm with a RT 6000 microplate reader. The half-maximum inhibitory concentration (IC_{50}) value was calculated according to the MTT results.

For Calcein-AM and PI co-staining Assay, 4T1 cells (2.0 × 10^5 per dish) were seeded on 35 mm confocal dishes and allowed to stabilize for 24 h. Then, 4T1 cells incubated with 20 μM Ts-IR-PES were or not exposed to an 808 nm laser with the power density of 0.33 W/cm^2 for 10 min. After another 24 h of incubation, the cells were stained with Calcein-AM and PI for 30 min to evaluate the PTT efficacy using TCS SP8 confocal laser scanning microscopy.

The experiment of singlet oxygen generation detection. For detection of ROS experiment, 4T1 cells (2.0 × 10^5 per dish) were seeded on 35 mm confocal dishes and

allowed to stabilize for 24 h. Then, 4T1 cells incubated with 20 μM PBS/Ts-IR-PES/Ts-IR/IR-PES/methylene blue (MB) for 4h. And the cells were washed three times with PBS (10 mM, pH = 7.4) and stained with 5 μM dichlorodihydrofluorescein diacetate for 10 min. Cells were exposed to an 808 nm laser with the power density of 0.33 W/cm^2 for 10 min to evaluate the production of ROS using TCS SP8 confocal laser scanning microscopy.

Calculation of singlet oxygen generation and quantum Yield. The $^1O_2$ quantum yield was detected according to the literature method.[5] The singlet oxygen ($^1O_2$) generation efficiency of Ts-IR-PES was evaluated by singlet oxygen capture agent, DPBF. Briefly, the absorbance of DPBF at 415 nm was adjusted to about 1.0, and then Ts-IR-PES or methylene blue (MB) was added to the cuvette and the absorbance was adjusted to 0.3 at *ca.* 800 or 660 nm, respectively. The relative quantum yields were calculated with reference to MB in DMSO for which the quantum yield is 0.52.[6] The mixture was then placed in a cuvette and irradiated with a 808 nm light source for different time (0, 2, 4, 6, 8, 10 min), and the corresponding absorption spectra was measured immediately. The slopes of absorbance of DPBF at 415 nm versus irradiation time were measured and used to compare the $^1O_2$ generation ability. The emission maxima of DPBF with different irradiation times were obtained, and the singlet oxygen quantum yields were determined using the following equation:

$$\Phi_{\Delta sam} = \Phi_{\Delta std}\left(\frac{m_{sam}}{m_{std}}\right)\left(\frac{F_{std}}{F_{sam}}\right)$$

where “sam” and “std” designate the “Ts-IR-PES” and “MB”, respectively. “m” is the slope of absorbance attenuation curve of DPBF at 415 nm, and “F” is the absorbance correction factor, which is obtained by $F = 1-10^{-O.D.}$. (O.D. is the absorbance of the solution at 808 nm).

Immunofluorescence imaging experiment. For fluorescence imaging, 4T1 cells (4×10^3/well) were passed on confocal dishes and incubated for 24h. Cells were washed three times with PBS (10 mM, pH = 7.4), and incubated with different materials for 4 h at 37 °C (PBS, Ts-IR, IR-PES and Ts-IR-PES) (20 μM). Then cells were treated with 4% (w/v) paraformaldehyde for 20 min at 4 °C. Cells were washed three times with PBS (10 mM, pH = 7.4) and permeabilized with 0.25% (v/v) Triton X-100 for 5 min at 25 °C. Next, cells were treated with 5% bovine serum albumin (BSA) for 60 min at 25 °C. Then cells incubated with antibody against HSP70 for 60 min at 25 °C. Cells were washed three times with PBS (10 mM, pH = 7.4), and

incubated with FITC-labeled secondary antibody for 60 min at 25 °C. Cells were washed three times with PBS (10 mM, pH = 7.4) and taken under a confocal microscope.

Co-localization analysis of Ts-IR-PES in 4T1 cells by CLSM. 4T1 cells were cultured in RPMI-1640 containing 10% fetal bovine serum and antibiotics penicillin/streptomycin at 37 °C in a 5% CO_2/95% air incubator. For fluorescence imaging, cells (4×10^3/well) were passed on confocal dishes and incubated for 24 h. Before the staining experiment, cells were washed three times with PBS (10 mM, pH = 7.4), and then incubated with 20 μM Ts-IR-PES or 20 μM IR-PES for 4 h at 37 °C. Then the petri dish was washed with PBS (10 mM, pH = 7.4) for another three times, and incubating with ER-Tracker Red (a commercial ER dye, 20 nM) for 15 min at 37 °C. Finally, wash each dish with PBS (10 mM, pH = 7.4) for three times, and analyzed with a TCS SP8 confocal laser scanning microscopy.

Animal tumor xenograft models. Animal experiments were reviewed and approved by the Ethics Committee of Shandong Normal University, Jinan, P. R. China (approval number AEECSDNU 2021045). All animal experiments were conducted and obeyed the Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China. All animal experiments were carried out in accordance with the principles of laboratory animal care (ROC). Balb/c mice (4 – 6 weeks old, female, weighing about 18 g) were fed under normal conditions with an average of 12 h of light and dark cycles per day, and given enough food and water.

The establishment of mouse tumor model: 4T1 cells were trypsin zed and redispersed in 50 μL RPMI 1640 serum-free medium (about 2×10^5 cells), and then subcutaneously inoculated into the right armpit of the mouse. By measuring the length (L) and width (W) of mouse tumors, the tumor volume (V) of tumor-bearing mice were calculated ($V = L \times W^2/2$). The calculation method of the relative tumor volume of mice is: V/V_0 (V_0 is the tumor volume of mice before treatment). When the mouse tumor volume reached about 20 – 40 mm^3, the Balb/c mice were treated.

Living tumor treatment experiment. The tumor-bearing mice were randomly divided into 6 groups (5 in each group): (i) 50 μL PBS buffer solution, (ii) 50 μL PBS buffer solution with laser irradiated, (iii) 50 μL Ts-IR-PES solution (0.20 mM), (iv) 50 μL Ts-IR solution (0.20 mM) with laser irradiated, (v) 50 μL IR-PES solution (0.20 mM) with laser irradiated, and (vi) 50 μL Ts-IR-PES solution (0.20 mM) with laser irradiated. The mice with laser groups were treated with 808 nm laser irradiation

(0.33 W/cm^2) for 10 min. During the treatment, every other day, the mouse weight was measured with a scale and the change of tumor volume was measured with a vernier caliper, and proceeded to the 20th day.

***In vivo* biological safety experiment.** The mice were divided into 6 groups and treated with different materials, and five major organs (liver, lung, spleen, kidney, and heart) were dissected after 7 days for H&E staining.

The mice were subjected to eyeball blood collection and divided into two batches. One aliquot was immediately stored at 4 °C for routine blood testing. The other was stored at room temperature for 1 h, centrifuged at 4500 rpm at 4 °C, and the supernatant was stored at –80 °C for biochemical analysis.

Supplementary Figures

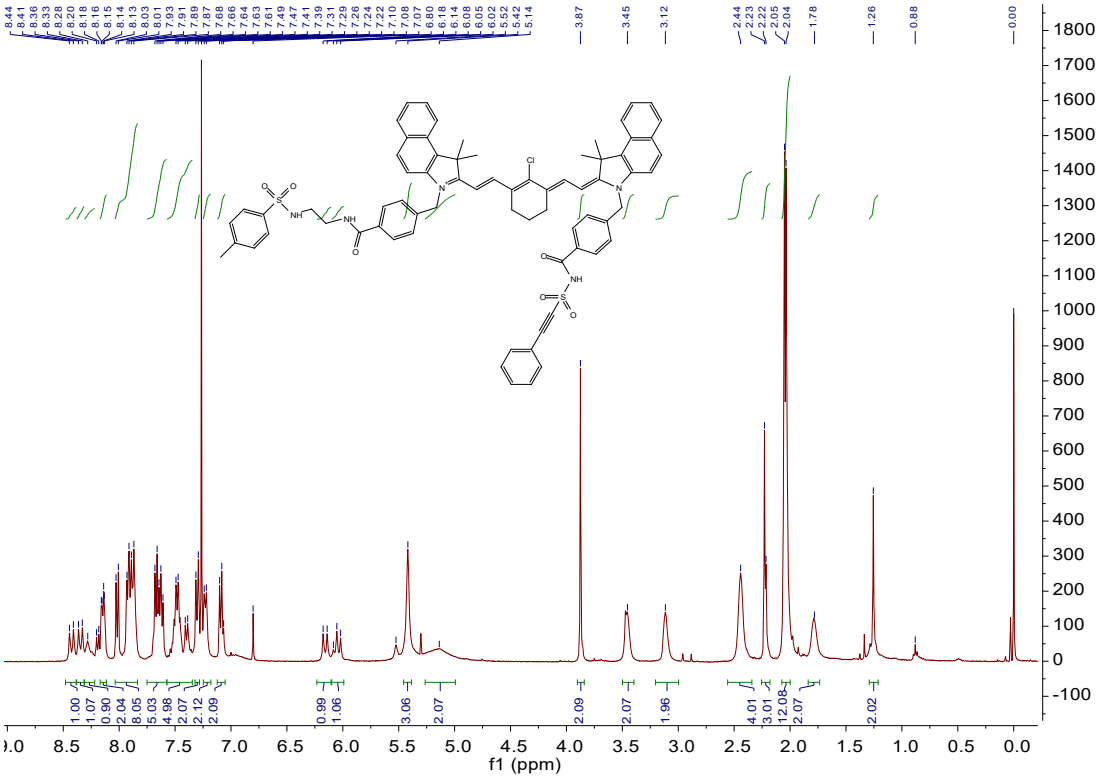


Fig. S1 ^{1}H NMR spectrum of Ts-IR-PES (Chloroform-*d*).

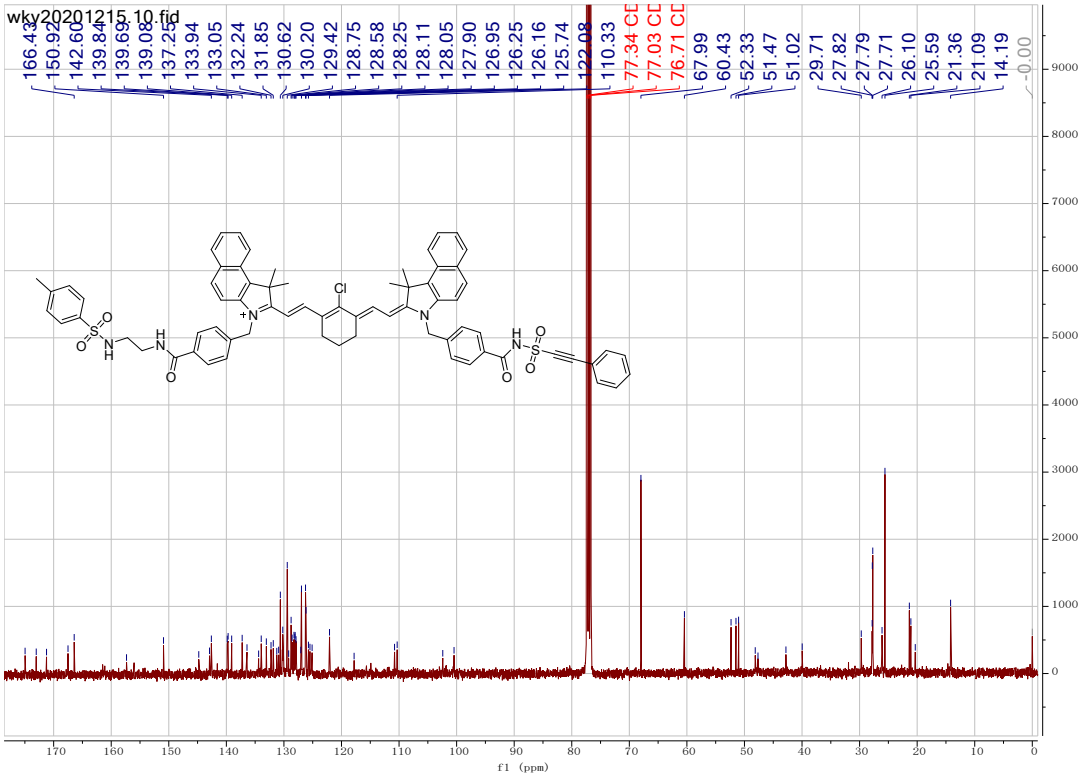


Fig. S2 ^{13}C NMR spectrum of Ts-IR-PES (Chloroform-*d*).

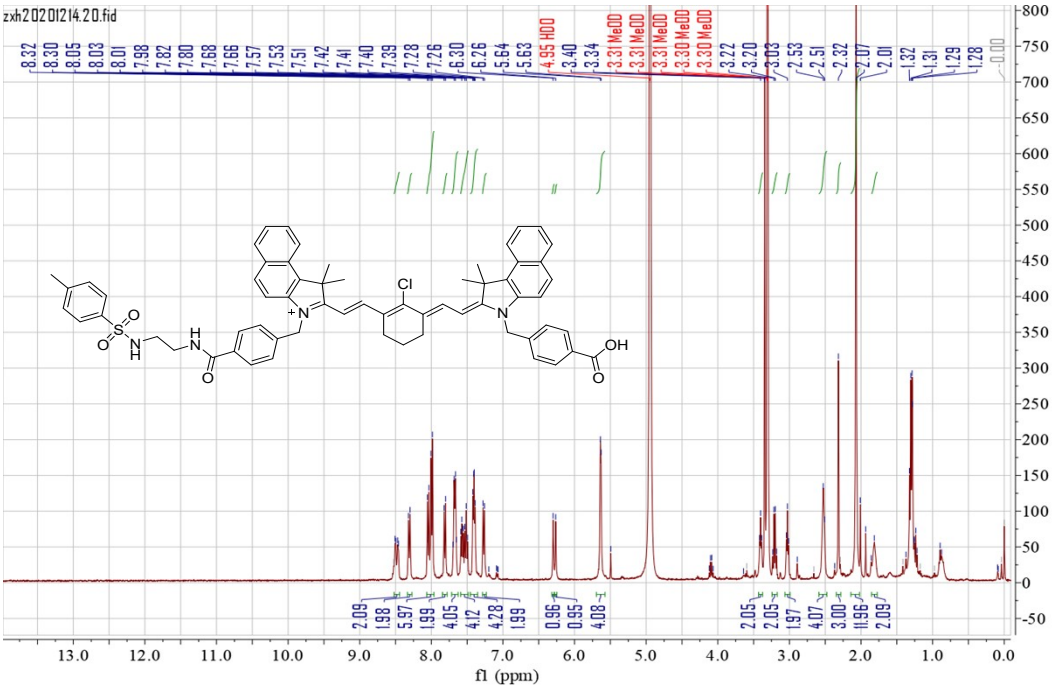


Fig. S3 ^{1}H NMR spectrum of Ts-IR (d^4-CD_3OD).

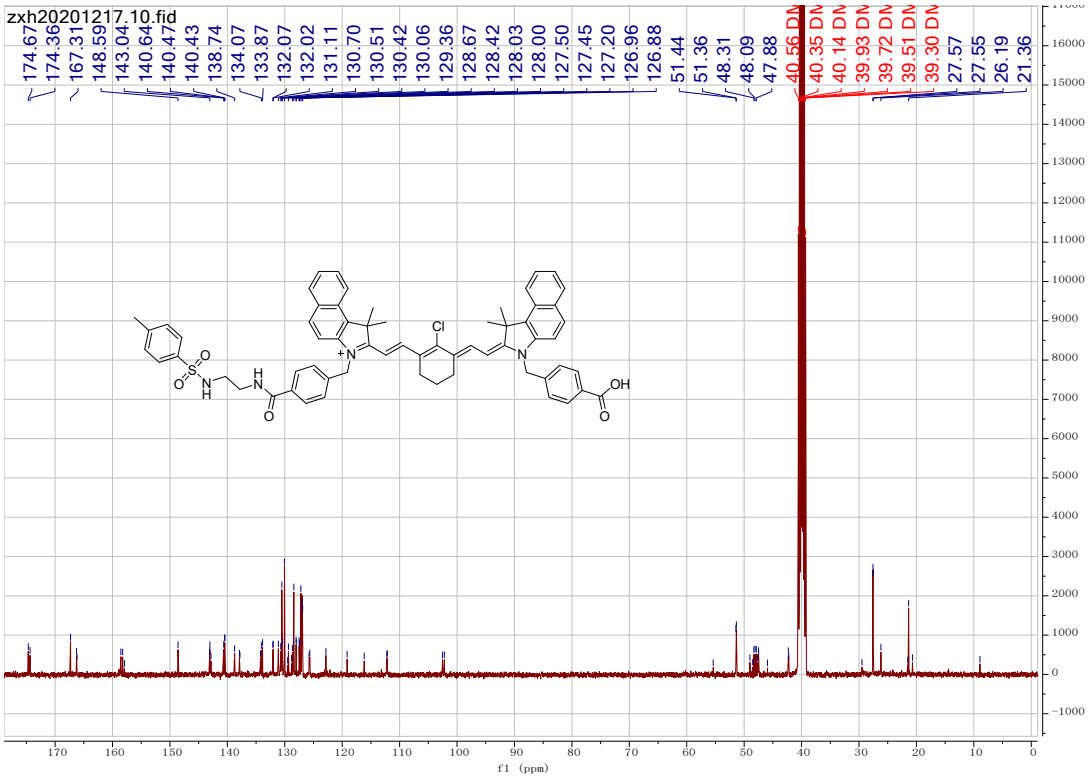


Fig. S4 ^{13}C NMR spectrum of Ts-IR (d^6-DMSO).

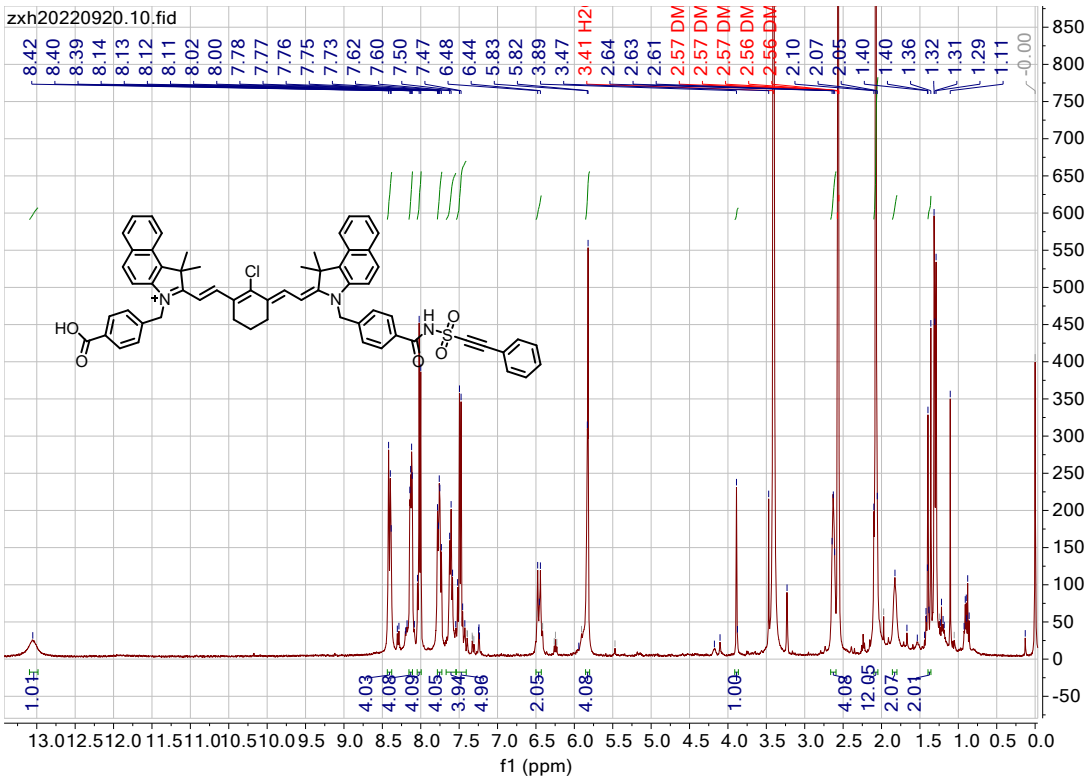


Fig. S5 ^{1}H NMR spectrum of IR-PES (d^{6}-DMSO).

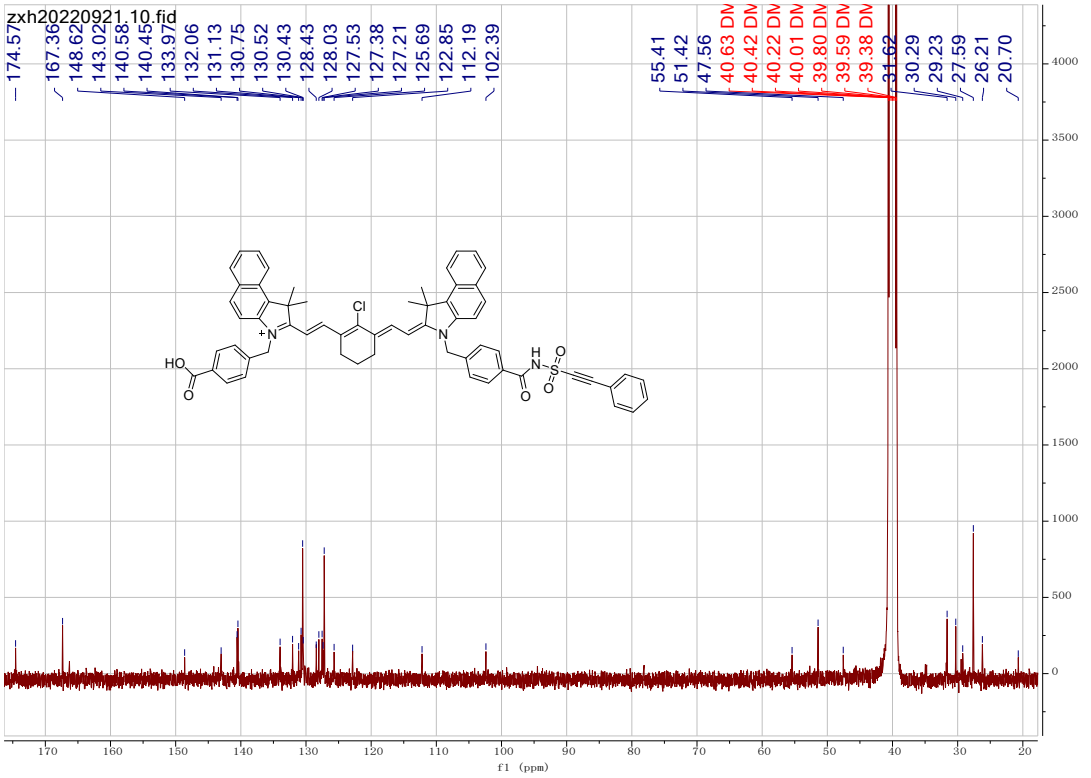


Fig. S6 ^{13}C NMR spectrum of IR-PES (d^{6}-DMSO).

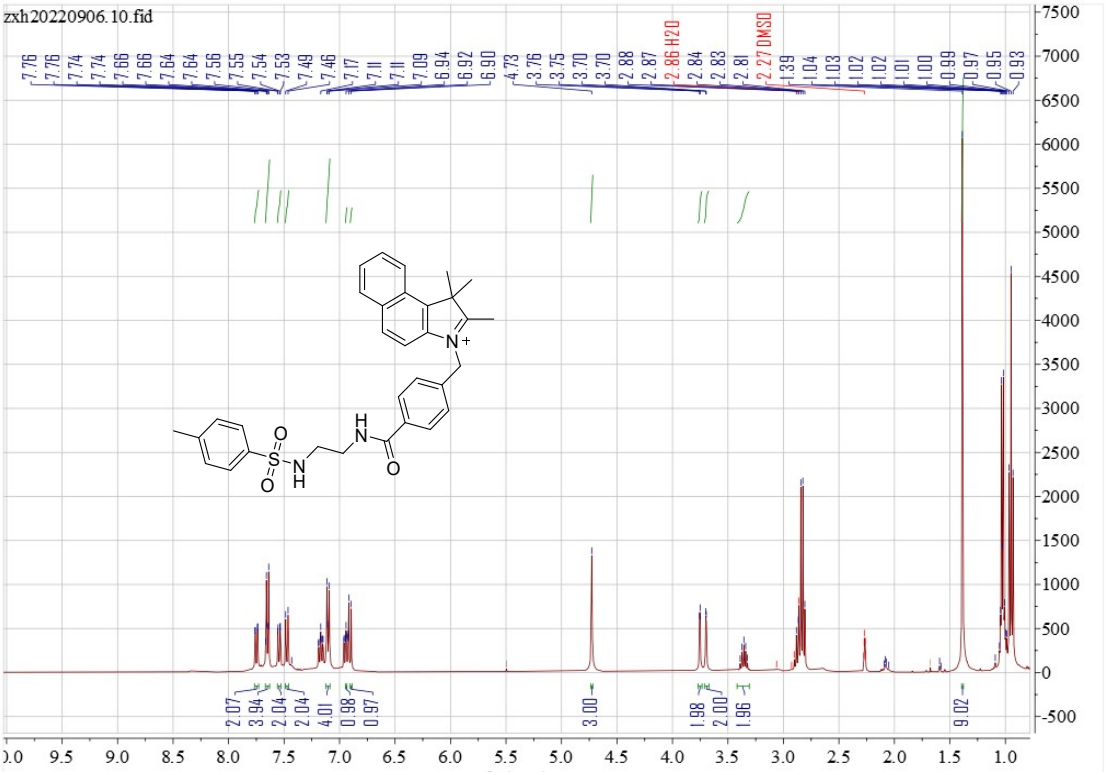


Fig. S7 ^{1}H NMR spectrum of Compound **3** (d^{6}-DMSO).

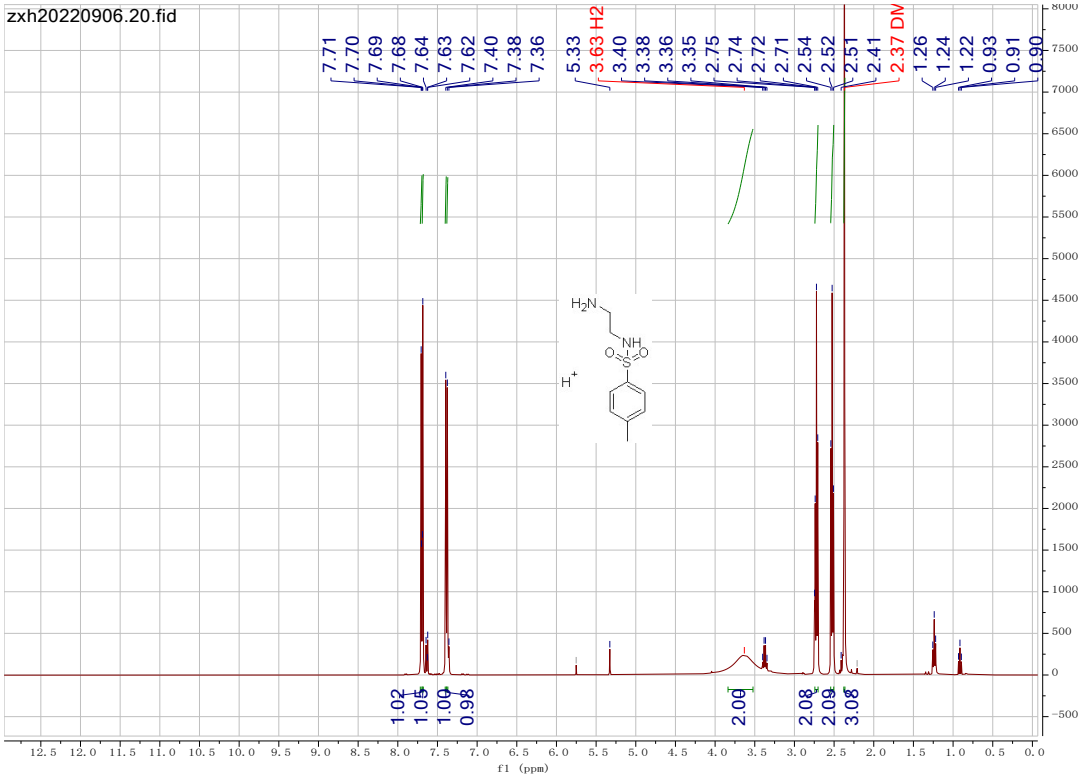


Fig. S8 ^{1}H NMR spectrum of Compound **2** (d^{6}-DMSO).

HPLC Analysis Report

Sample ID： TP-CH-2238

Sequeece: Ts-IR-PES

Columan ..... :4.6*150mm,kromasil C18-5

Solvent A ..... :0.1%Trifluoroacetic in 100% Acetonirile

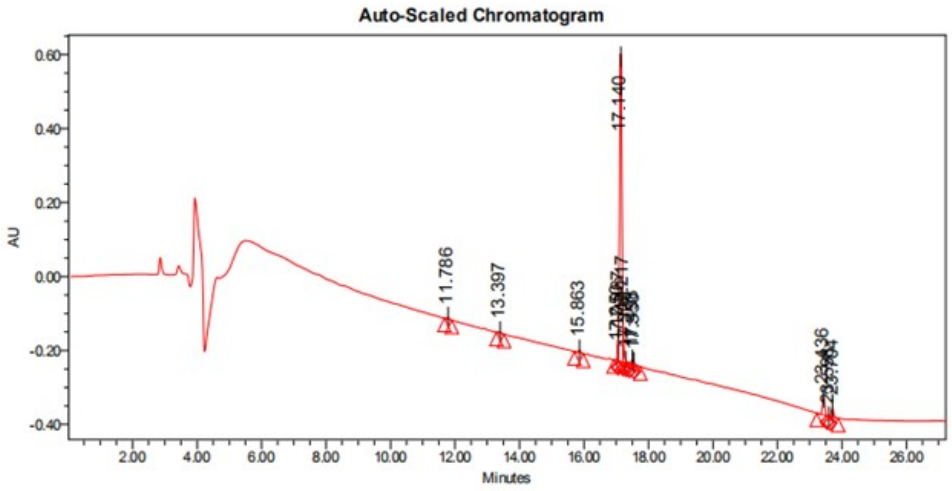
Solvent B ..... :0.1%Trifluoroacetic in 100% Water

Gradient	:	A	B
	0.01min	5%	95%
	25.0min	70%	30%
	30min	95%	10%

Flow rate ..... :1.0ml/min

Wavelength ..... :214nm

Volume ..... :20ul



Peak Results

	RT	Area	Height	% Area
1	11.786	23983	5330	0.49
2	13.397	19920	4106	0.41
3	15.863	39481	7968	0.81
4	17.050	25573	16255	0.52
5	17.067	36036	55816	0.74
6	17.140	4125108	831986	84.46

Fig. S9 HPLC spectrum of Ts-IR-PES. The product percentage was calculated to be 84.46%, with the retention time of 17.14 min under our condition.

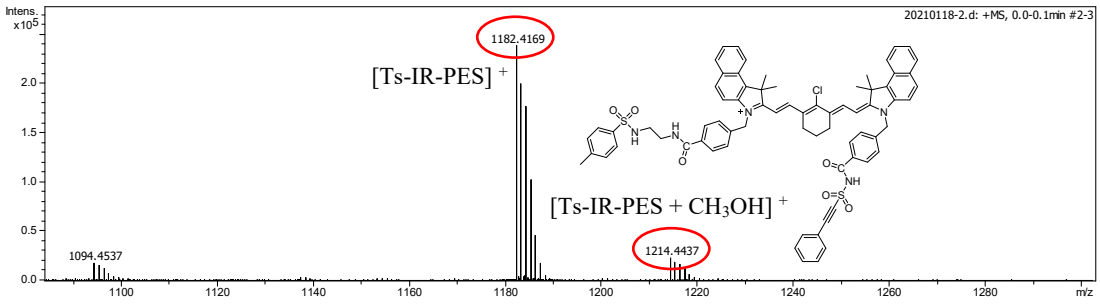


Fig. S10 HRMS spectrum of Ts-IR-PES. The *m*/*z* values of 1182.4169 and 1214.4437 are for $[Ts\text{-}IR\text{-}PES]^+$ and $[Ts\text{-}IR\text{-}PES + CH_3OH]^+$ (calculated 1182.4059 and 1214.4321), respectively.

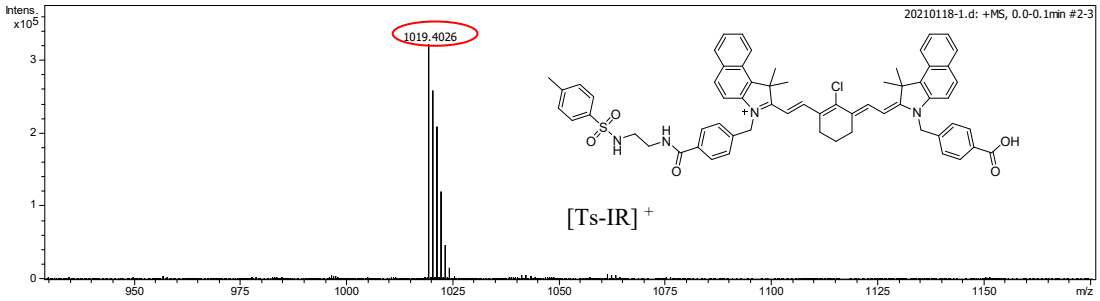


Fig. S11 HRMS spectrum of Ts-IR. The *m*/*z* values of 1019.4026 is for $[Ts\text{-}IR]^+$ (calculated 1019.3967).

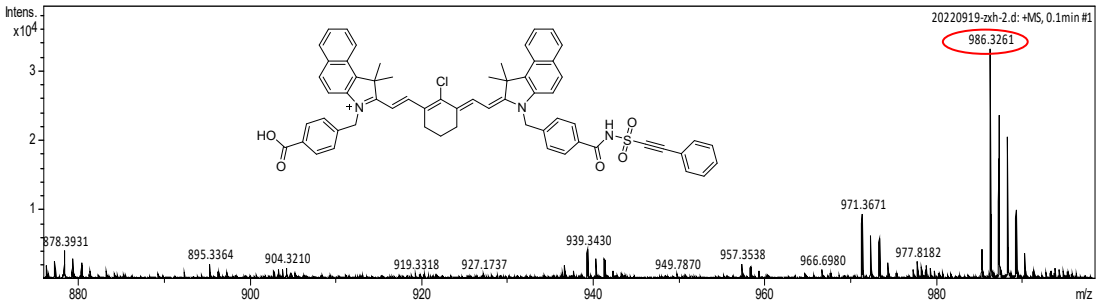


Fig. S12 HRMS spectrum of IR-PES. The *m*/*z* values of 986.3261 is for $[IR\text{-}PES]^+$ (calculated 986.3389).

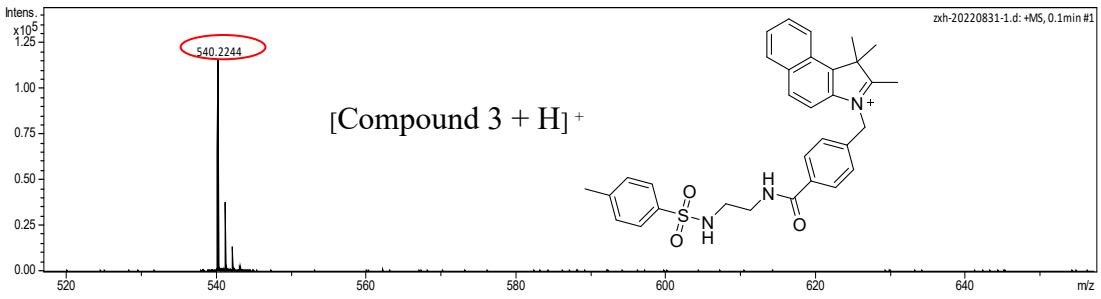


Fig. S13 HRMS spectrum of compound **3**. The *m*/*z* values of 540.2244 is for $[Compound\ 3 + H]^+$ (calculated 540.2315).

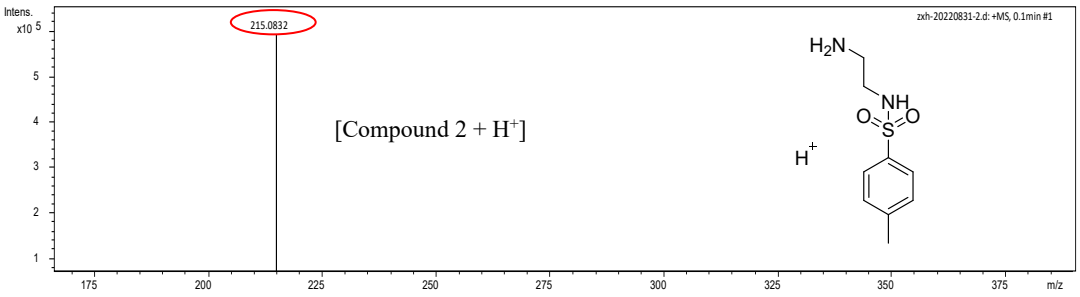


Fig. S14 HRMS spectrum of compound **2**. The *m/z* value of 215.0832 is for [Compound **2** + H]$^+$**(**calculated 215.0849**)**.

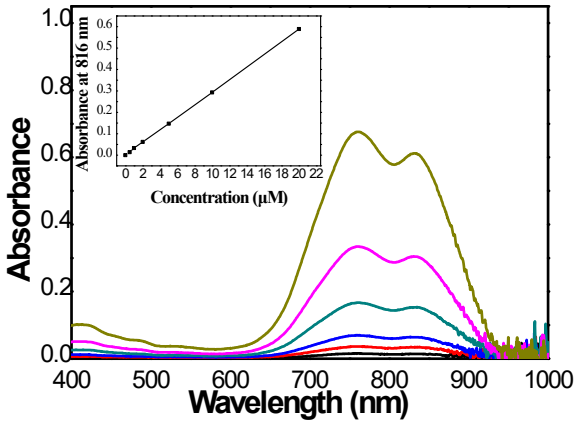


Fig. S15 UV-vis absorption spectra of Ts-IR-PES in different concentrations (Bottom to top: 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μM) in H_2O and methanol (v/v = 1:1). The inset shows concentration versus absorbance at 816 nm.

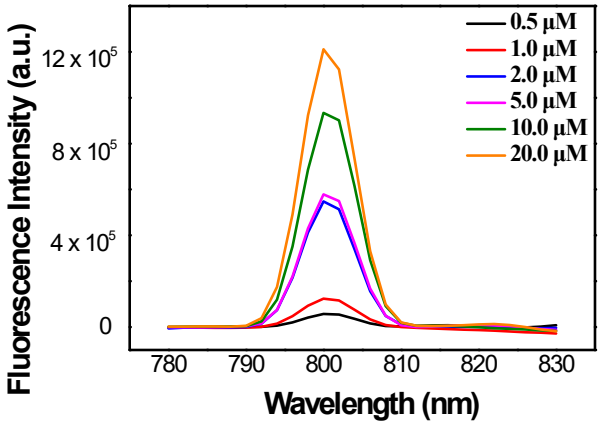


Fig. S16 Fluorescence spectra of Ts-IR-PES in different concentrations (0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μM) excited at the wavelength of 800 nm in H_2O and methanol (v/v = 1:1).

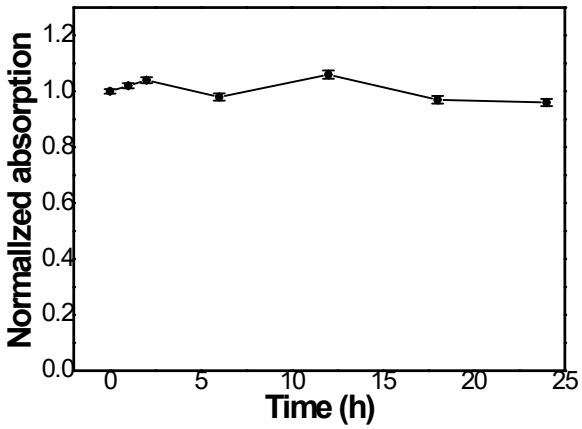


Fig. S17 Photostability of Ts-IR-PES (20.0 μM) in water.

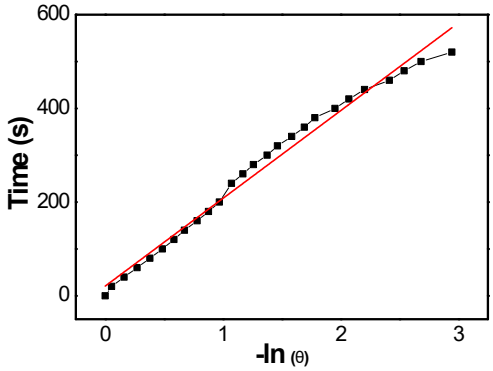


Fig. S18 Plot of time versus –ln (θ). (τ_s=181.13483) [θ is the driving-force temperature] from the data recorded during the cooling period of the experiment outlined in Fig. 2c.

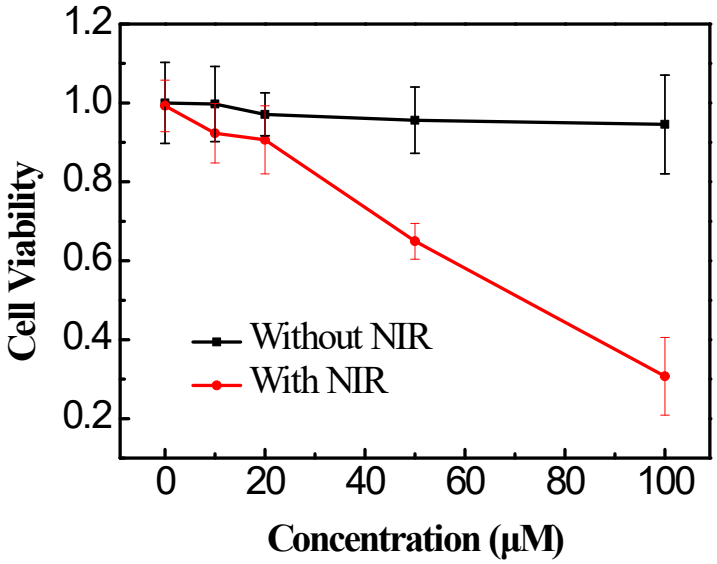


Fig. S19 Cell viabilities of 4T1 cells treated with different concentrations of ICG without or with 808 nm laser irradiation (0.33 W/cm^2, 808 nm, 600 s).

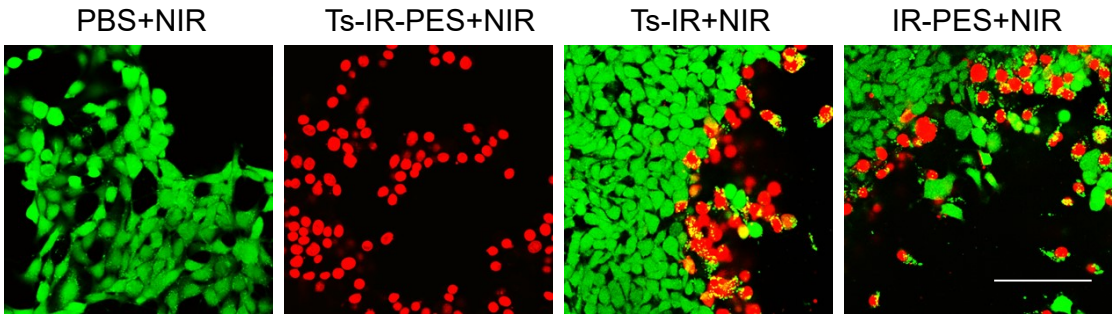


Fig. S20 Fluorescence confocal imaging of 4T1 cells contained with calcein AM (live cells, green fluorescence) and PI (dead cells, red fluorescence) after different treatments (concentration of the PS: 20 μM). Scar bar = 100 μm.

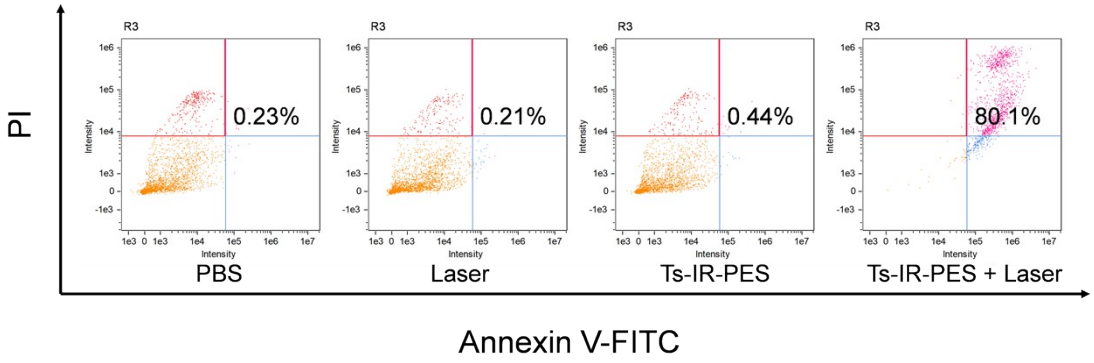


Fig. S21 Flow cytometry analysis of apoptosis of 4T1 cells after different treatments (NIR Laser, 808 nm, 0.33 W/cm^2).

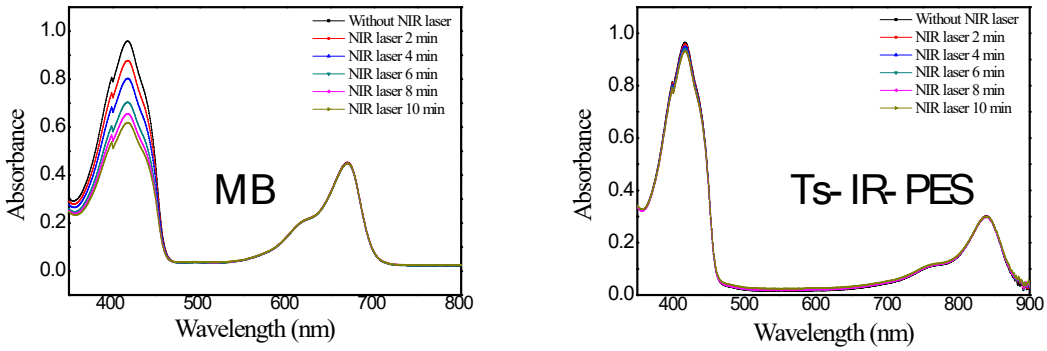


Fig. S22 The absorbance of DPBF and MB/Ts-IR-PES in DMSO. Then solution irradiated with 808 nm laser (0.33 W/cm^2) for different time.

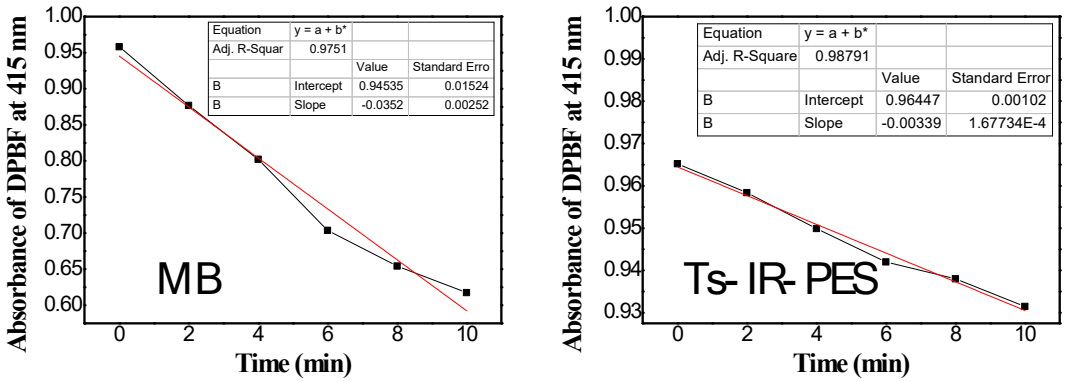


Fig. S23 The slope of absorbance attenuation curve of DPBF at 415 nm.

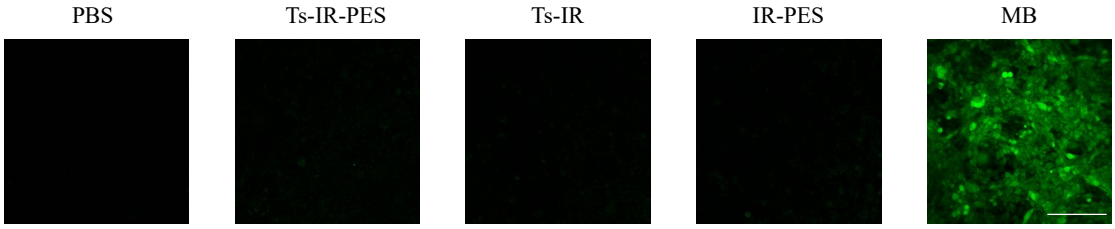


Fig. S24 The CLSM images. 4T1 cells incubated with different materials (concentration of the PS: 20 μM) and then stained with ROS sensitive probe DCFH-DA. And then, cells were treated with NIR laser (808 nm, 0.33 W/cm^2) for 10 min. MB: methylene blue. Scale bars, 100 μm.

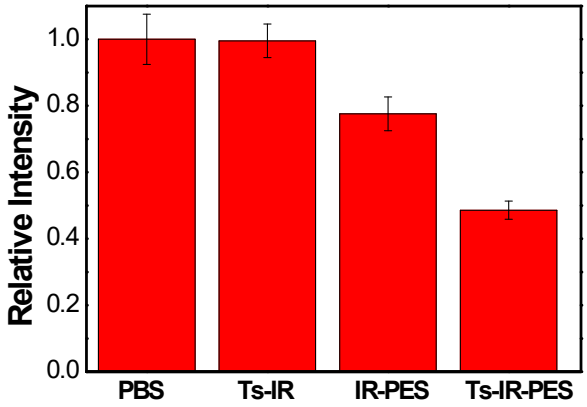


Fig. S25 Quantified HSP expression for corresponding (Fig. 3e) immunofluorescent images.

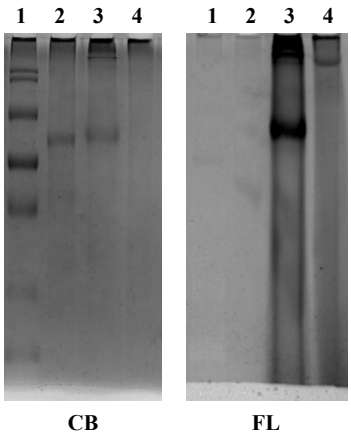


Fig. S26 Gel electrophoresis experiment of Ts-IR-PES reacting with HSP 70. The protein bands in the figure represent from right to left: 1: Marker; 2: HSP70; 3: Ts-IR-PES + HSP70; 4: Ts-IR-PES. CB: Fluorescence staining of gel electrophoresis samples with stained with coomassie blue; FL: Fluorescence imaging of gel electrophoresis samples.

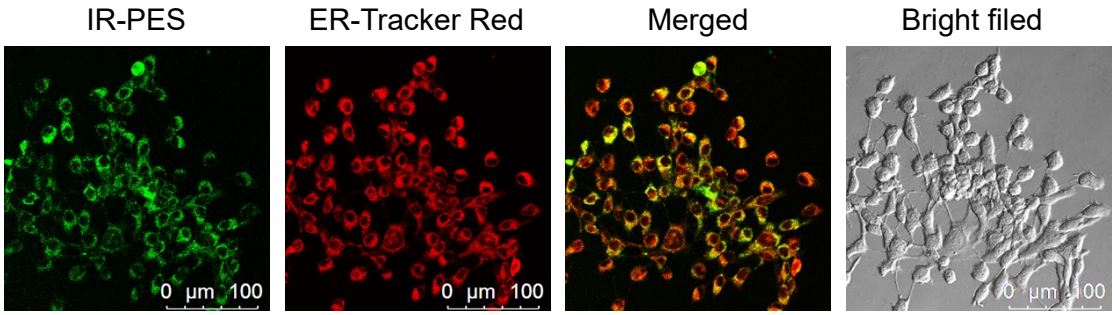


Fig. S27 Determination of ER localization of IR-PES by confocal microscopy.

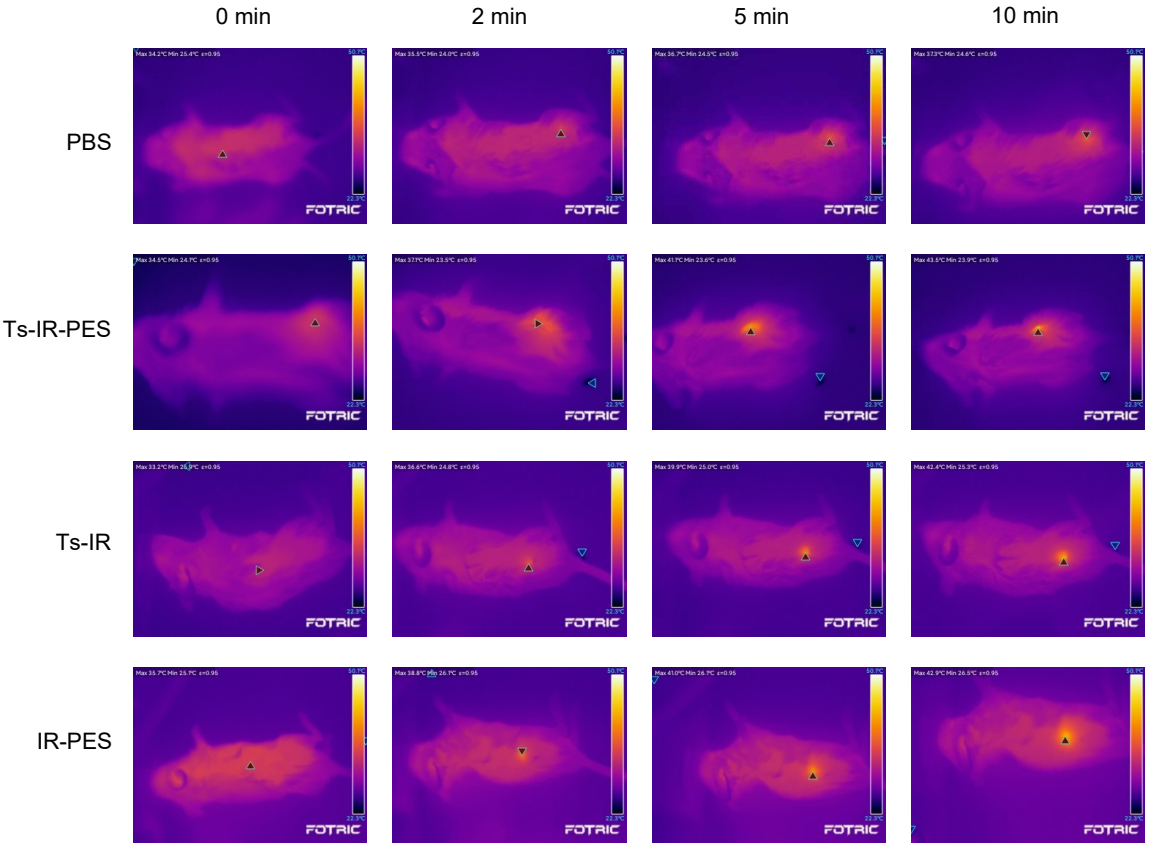


Fig. S28 IR thermal images of 4T1 tumor-bear mice under NIR irradiation (0.33 W/cm^2) with different treatments.

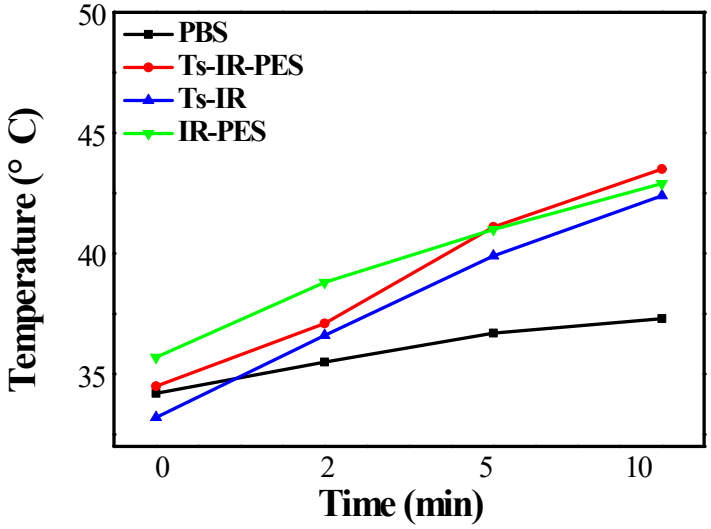


Fig. S29 Quantitative treatment of IR thermal images of 4T1 tumor-bear mice under NIR irradiation (0.33 W/cm^2) with different treatments.

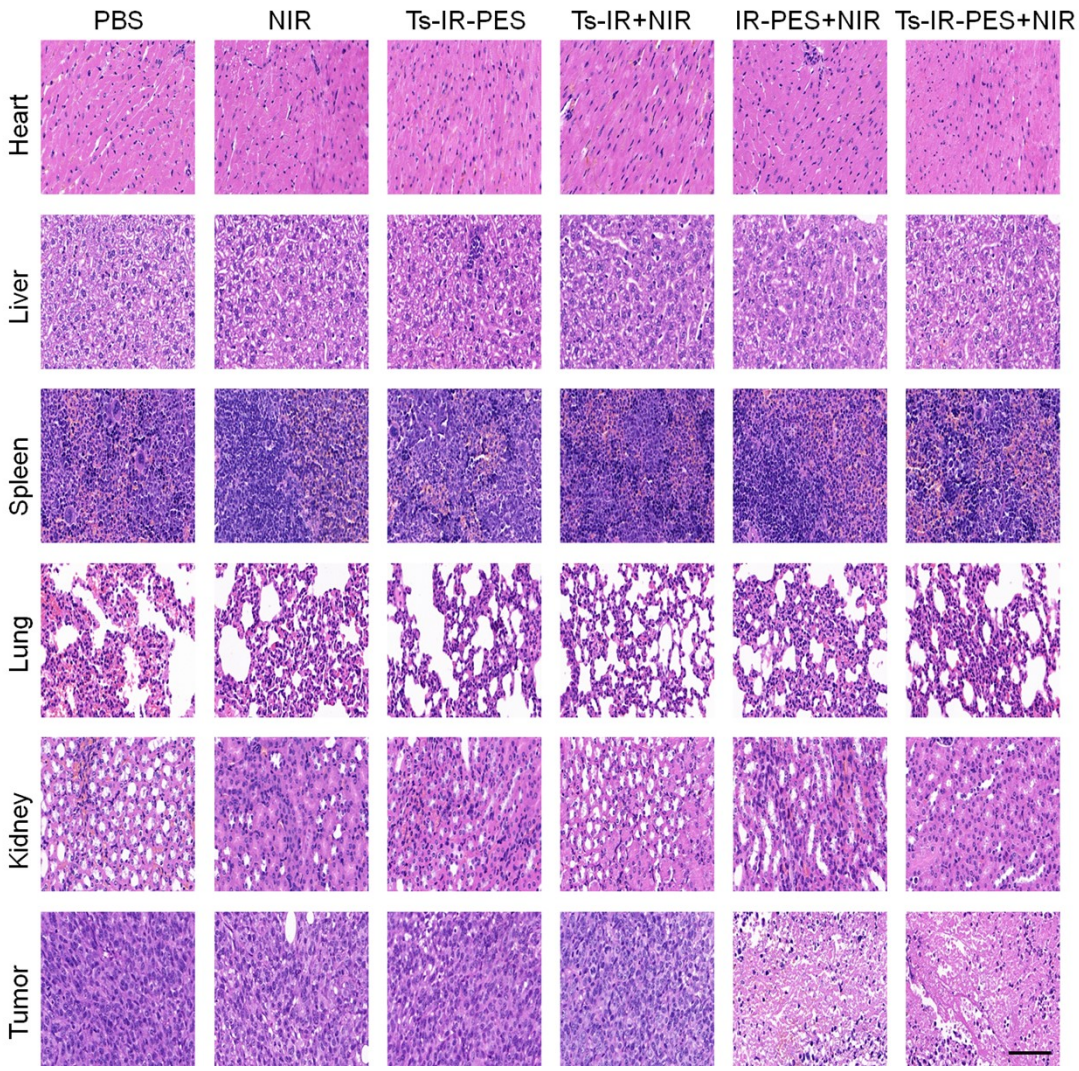


Fig. S30 Hematoxylin and eosin (H&E) staining images of tumor and major organs (heart, liver, spleen, lung, and kidney) of mice in different treatment groups. Scale bar = 200 μm.

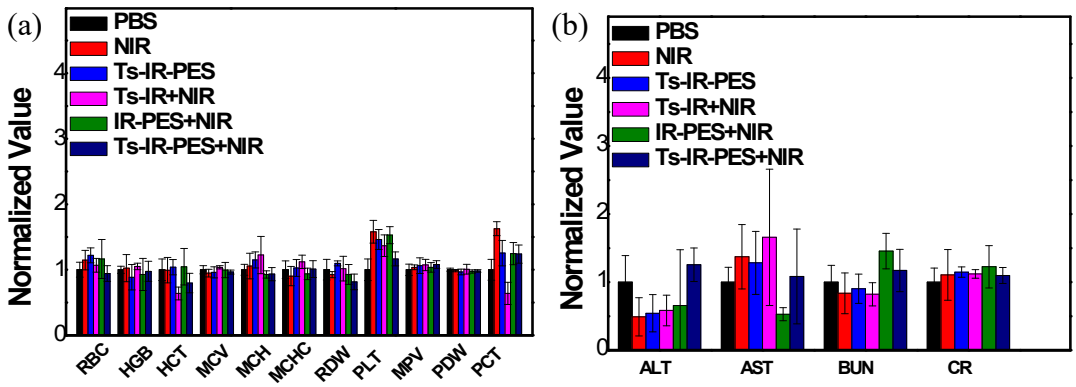


Fig. S31 Blood routine (a) and blood biochemistry (b) of analysis with different groups. RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concerntration; RDW: red cell distribution width; PLT: platelets; MPV: mean platelet volume; PDW: platelet distribution width; PCT: platelet cubic thrombocytocrit; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen and CR: creatinine.

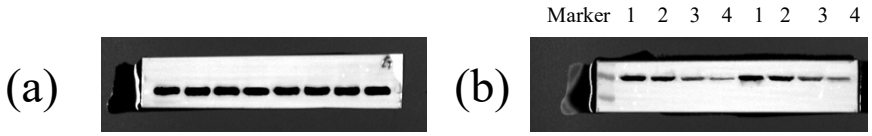


Fig. S32. The original pictures of the western blot after different treatments. (a) GAPDH and (b) HSP70. (1: PBS; 2: Ts-IR; 3: IR-PES; 4: Ts-IR-PES).

References

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