Electronic Supplementary Information (ESI)

A Conjugated molecule based on tetra-fused thienoisoindigo ribbon for NIR-II photothermal cancer therapy

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Supporting Experimental Section

Materials: Chemical reagents were purchased from Energy Chemical, Aldrich, Laysan Bio or Alfa Aesar and used as received. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotin(polyethylene glycol)] (DSPE-MPEG$_{2000}$-Biotin) was purchased from Laysan Bio, Inc. (Arab, AL). The compound **1**, **2**, and **4ThIID** were prepared according to our previously reported literatures.[1]

Characterization: UV-vis-NIR absorption spectra were recorded on a Shimadzu UV-3600Plus UV-vis-NIR spectrometer. The size of 4ThIID NPs were measured by dynamic light scattering (DLS) carried with a particle size analyzer NanoBrook 90Plus instrument (Brookhaven Instruments Co. USA). Transmission electron microscopy (TEM) images were acquired from a TALOSF200X transmission electron microscope with an accelerating voltage of 200 kV.

Preparation of CP NPs*:* 4ThIID (3 mg) and amphiphilic lipid-PEG 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotin(polyethylene glycol)] (DSPE-MPEG$_{2000}$-Biotin, 12 mg) were dissolved in tetrahydrofuran (THF, 10 mL). The resulting tetrahydrofuran (THF) solution was added dropwise into ultra-purified water (40.0 mL), with sonication being used to aid in stirring at a constant rate for 24 hours. This was followed by a seven-day dialysis process at room temperature to eliminate any remaining THF. The NPs suspension were purified by ultrafiltration and filtered through a 0.2 μm syringe driven filter. Then the obtained NPs were

subsequently concentrated to 1.0 mg mL^{-1} and stored at 4 °C for further use.

***Photothermal Performance**:* NIR-II photothermal conversion properties of 4ThIID NPs in water were studied following our previously reported procedure.[2] Photothermal conversion experiments of 4ThIID NPs (25, 50, 75, or 100 μg/mL, 1.0 mL) were conducted in water under 1064 nm laser irradiation (1.0 W/cm^2) for 5 minutes, and then cooled down to room temperature. Water as the reference was also tested under the same condition. The photothermal conversion efficiency (η) values were calculated with the equation $\eta = (hA_{\triangle}T_{max}\text{-}Q_s)/[I(1\text{-}10^{-A_\lambda})]$ where h is the heat transfer coefficient, A is the surface area of the system, $_{\triangle}T_{max}$ is the temperature difference between the maximum steady-state temperature and ambient temperature, I is the laser power, A_λ is the absorbance of the solution at the irradiation wavelength, Q_s is the heat change of the pure solvent. The unknown hA value was calculated by the linear data of time versus –ln θ curve according to equation: $t = -(\sum_i m_i C_{p,i})\ln\theta/(hA)$.[2]

***Animals and Tumor Xenograft Model**:* All animal experiments were performed under the guidelines set by Nantong Committee of Use and Care of Laboratory Animals and the overall project protocols were approved by the Animal Ethics Committee of Nantong University. To established tumor-bearing mouse model, about 5-week-old female BALB/c mice were purchased from the Laboratory Animal Center of Nantong University (Nantong, China) for the experiment of photothermal therapy (PTT). We selected the murine 4T1 breast cancer cells in this study. To establish tumors in six-week-old BALB/c, 100 μL of cell culture medium containing 2-3 million murine 4T1 breast cancer cells were inoculated into the abdomen of mice of the BALB/c mouse. After about 7 days, the tumor-bearing mice were used for PTT.

In Vivo PTT: The xenograft 4T1 tumor-bearing mice were randomly separated into 5 groups (5 mice per group) when the tumor volumes reached about 50-100 mm^3: "PBS", "PBS + laser", "4ThIID NPs", "4ThIID NPs + laser + chicken breast" and "4ThIID NPs + laser", respectively. The NPs (100 μL; 350 μg/mL based on 4ThIID NPs) or PBS (100 μL) were intravenously injected into the tumor-bearing mice, respectively. For the "PBS + laser", "4ThIID NPs + laser + chicken breast", and "4ThIID NPs + laser" groups, the tumor areas of mice were irradiated

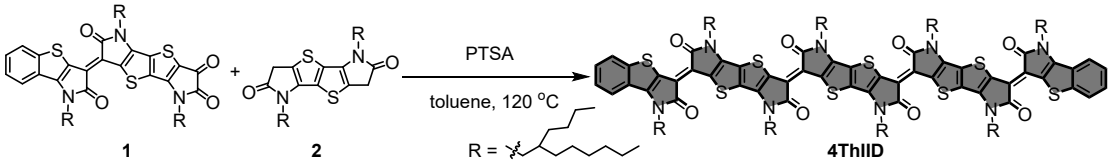
by a 1064 nm laser at 1.0 W cm^{-2} for 5 min at 6 h post-injection. After treatments, the mice weight and tumor volumes were measured every two days for 20 days. The tumor volume was calculated using the following formula: length × $width^2/2$ (length and width were the longest and shortest diameters of tumors, respectively). Relative tumor size was calculated as V/V_0 (V_0 was the tumor volume of day 0). The survival rates of mice in each group were also monitored.

Cell Culture and Cytotoxicity Assay: The murine 4T1 breast cancer cells were supplied by School of Pharmacy, Nantong University. The cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin−streptomycin (PS). The cells were maintained in an atmosphere of 5% CO_2 and 95% humidified air at 37 °C. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was conducted to evaluate the biocompatibility of 4ThIID NPs. First, 4T1 cancer cells were seeded in 96-well plates (Costar, IL, USA) at a density of 5 × 10^3 cells/well and incubated in complete DMEM (100 μL). After 24 h incubation, when the cell number of each well reached an appropriate density, both cells were added to a series of concentrations of NPs (0, 10, 20, 30,40,50,and 100 μg/mL based on CPs). After incubation for another 24 h, 20 μL of freshly prepared MTT solution (5 mg/mL) in PBS was added into each well. After 4 h, the MTT medium solution was carefully removed and then DMSO (150 μL) was added into each well and the plate was gently shaken for 5 min at room temperature to completely dissolve all the precipitates. The maximum absorbance of the precipitation mentioned above at 490 nm was then measured by the microplate Reader (INFINITE M NANO). The cell viability was expressed by the ratio of the absorbance of the cells incubated with 10, 20, 30,40,50,and 100 μg/mL of samples to that of the cells incubated with the sample concentration of 0 μg/mL.

Live/Dead Cell Staining: First, immerse the 14-mm circular cell climbing slices in 75% ethanol, and then soak them in complete medium to remove the residual alcohol. After that, place the cell climbing slices into a 24-well plate. After the digestion of 4T1 cells is completed, seed the cells into the wells of the plate at a density of 5×10^4 cells per well and incubate them for 24 hours. When observing under an optical microscope that the cells are completely attached to the wall and the density is appropriate, aspirate and discard the original medium, add the nanoparticle solution (5/15/25 μg/mL), incubate for 6 hours, then irradiate the cells with a 1064

nm laser at 1.00 W/cm^2 for 15 minutes, and continue the incubation for 4 hours. The blank group and the group without light irradiation serve as the corresponding control groups. Prepare the working solution of Calcein-AM (2 μM)/PI (4.5 μM), aspirate and discard the original medium, and carefully wash the wells three times with PBS. Add 500 μL of the working solution to each well, and continue the incubation in the incubator for 30 minutes. Drop the anti-fluorescence quenching mounting solution onto the glass slide. After preparing the slides, detect the live cells (yellow-green fluorescence) and dead cells (red fluorescence) under a fluorescence microscope using a 490 ± 10 nm laser filter.

***Histological Studies*:** After 22 days of mouse experiment, all groups of mice were sacrificed. The tumors and important normal organs (kidney, spleens and livers) were excised and fixed in 4% paraformaldehyde for two days before dehydrated overnight, and embedded in paraffin on the next day for H&E staining.



Scheme S1. Synthetic scheme for 4ThIID.

4ThIID: To a solution of compound **1** (100 mg, 0.105 mmol, 2.2 e.q.), **2** (21.0 mg, 0.048 mmol, 1.0 e.q.) in toluene (3.5 mL), PTSA (2.8 mg, 0.017mmol, 0.35 e.q.) was added. The reaction mixture was stirred at 110 °C for 4 h, then cooled to room temperature. The mixture was poured into water, followed by extraction with $CHCl_3$. The combined organic layers were washed with water, brine and dried with $MgSO_4$ before filtered and concentrated in vacuum. The crude product was first purified by silica gel chromatography using PE/DCM (1/3, v/v) as eluent and then by gel permeation chromatography (GPC, porous styrene-divinylbenzene copolymer beads as filler) with toluene as eluent to afford **4ThIID** as a black solid (81.0 mg, yield 69%). HRMS (MALDI-TOF): Calcd for $[C_{146}H_{208}N_8O_8S_8]^+$: 2458.3914, found $[C_{146}H_{208}N_8O_8S_8+H]^+$: 2459.39883. Elemental Anal. Calcd for $C_{112}H_{158}N_6O_6S_6$ (%): C, 71.29; H, 8.52; N, 4.56, Found: C, 70.76; H, 8.455; N, 4.49. The ^{1}H NMR spectra of 4ThIID are shown in Fig. S2. Due to the open-shell characteristic of the 4ThIID

molecule,[1] no signals in the aromatic region were found in its ^{1}H NMR.

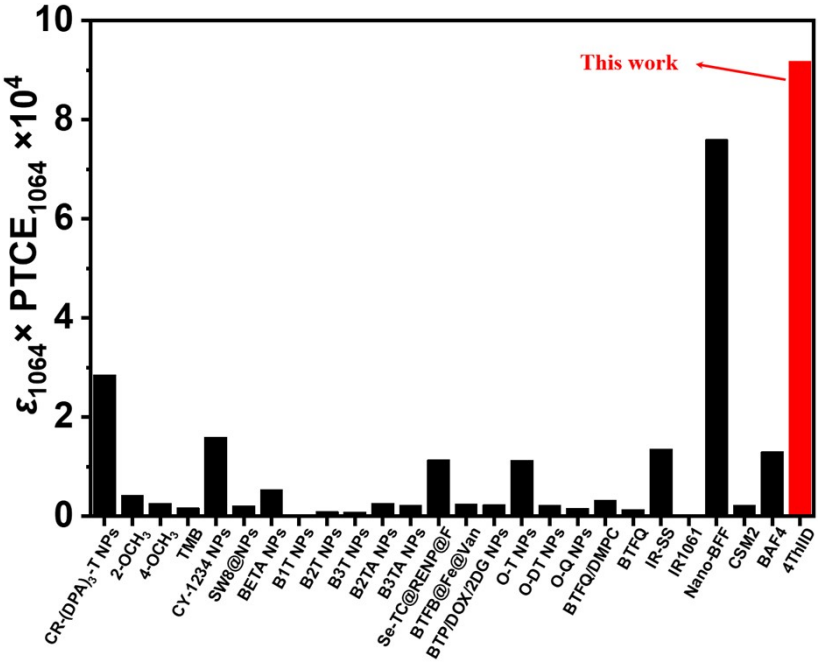


Fig. S1 The reported ε_{1064} × PTCE$_{1064}$ values of NIR-II small molecule photothermal reagents.

Table S1. Summary of NIR-II organinc small-molecule photothermal materials under 1064 nm laser irradiation.

Name	ε_{1064}(Lmol^{-1} cm^{-1})	PTCE$_{1064}$(%)	ε_{1064}× PTCE$_{1064}$	Reference
CR-(DPA)$_3$-T NPs	4.722×10^4	60.4	2.85×10^4	3
2-OCH$_3$	1.48×10^4	28.8	4.262×10^3	
4-OCH$_3$	1.59×10^4	16.8	2.671×10^3	4
TMB	6.06×10^3	27.5	1.667×10^3	
CY-1234 NPs	2.1×10^4	76.01	1.5962×10^4	5
SW8@NPs	2.867×10^3	75	2.15×10^3	6
BETA NPs	1.13×10^4	47.6	5.379×10^3	
B1T NPs	0.59×10^3	12.5	7.4×10^1	
B2T NPs	3.18×10^3	29.5	9.38×10^2	*7*
B3T NPs	3.13×10^3	28.2	8.83×10^2	
B2TA NPs	7.38×10^3	36.2	2.672×10^3	
B3TA NPs	6.18×10^3	35.8	2.212×10^3	
Se-TC@RENP@F	3.0793×10^4	36.9	1.1363×10^4	8
BTFB@Fe@Van	8.862×10^3	28.4	2.517×10^3	9
BTP/DOX/2DG NPs	7.837×10^3	29.8	2.335×10^3	10
O-T NPs	1.55×10^4	73	1.1315×10^4	
O-DT NPs	0.36×10^4	62	2.232×10^3	11
O-Q NPs	0.32×10^4	51	1.632×10^3	
BTFQ/DMPC	1.03×10^4	30.8	3.172×10^3	12
BTFQ	0.36×10^4	37.2	1.339×10^3	
IR-SS	1.77×10^4	77	1.36×10^4	13
IR1061	NA	45.25		14
Nano-BFF	2.22×10^5	34.3	7.6×10^4	15
CSM2	7.17×10^3	31.6	2.27×10^3	16
BAF4	1.63×10^4	80	1×10^4	17
4ThIID	**1.25×10^5**	**73.4**	**9.18×10^4**	**This wok**

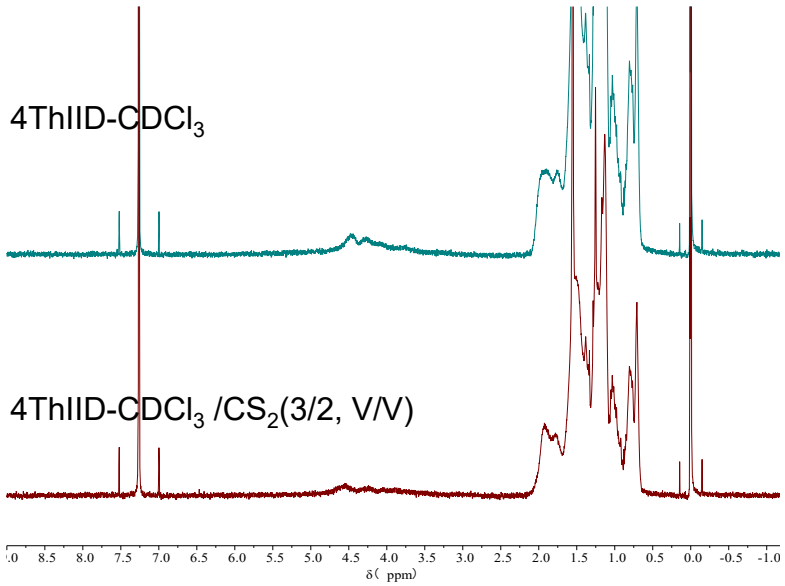


Fig. S2 ^{1}H NMR spectra of 4ThIID in $CDCl_3$ (top, 25 °C) and in the $CDCl_3/CS_2$ mixed solvent (bottom, 25 °C).

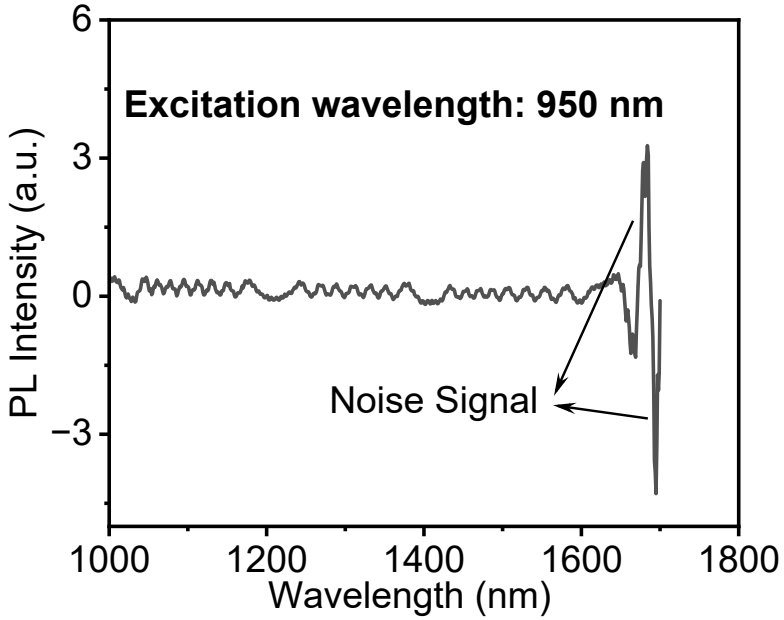


Fig. S3. Emission spectrum of 4ThIID in deoxygenated $CHCl_3$ solution at room temperature with excitation wavelength at 950 nm.

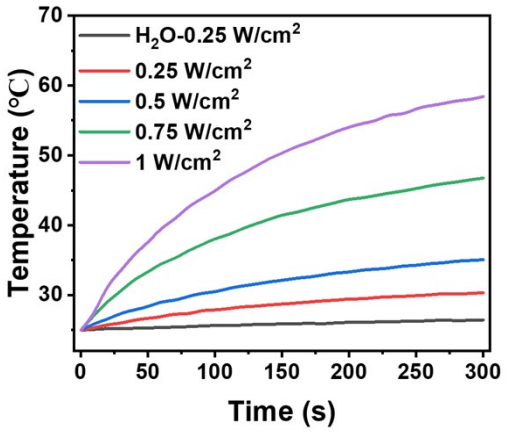


Fig. S4 The temperature rising curves of the 4ThIID NPs aqueous solution (50 μg mL^{-1}) under 1064 nm laser irradiation at different laser intensities.

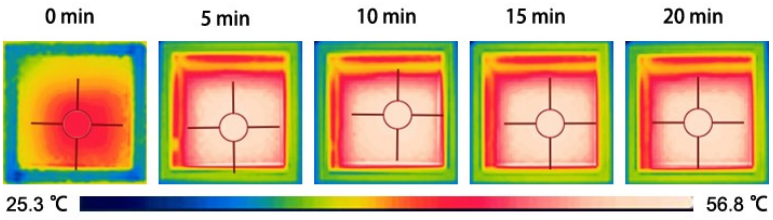


Fig. S5 The photothermal stability of the 4ThIID NPs aqueous solution (50 μg mL^{-1}) under continuous laser irradiation (1.0 W cm^{-2}).

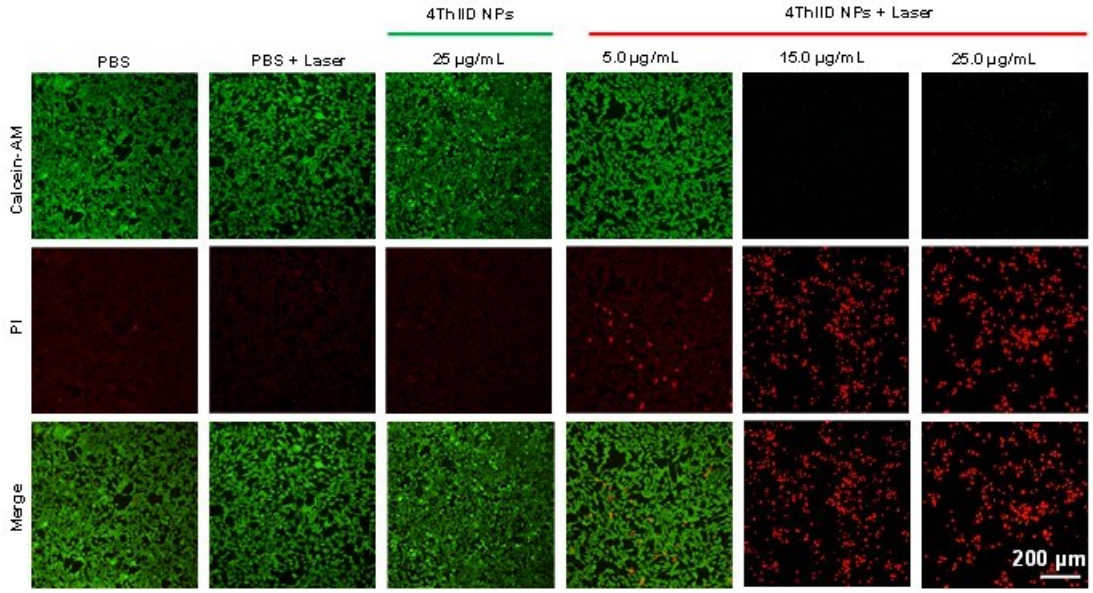


Fig. S6 Fluorescence photographs of 4T1 cells stained with Calcein-AM and PI after being treated in different ways; live cells exhibit green fluorescence, dead cells show red fluorescence, and the scale bar is 200 μm.

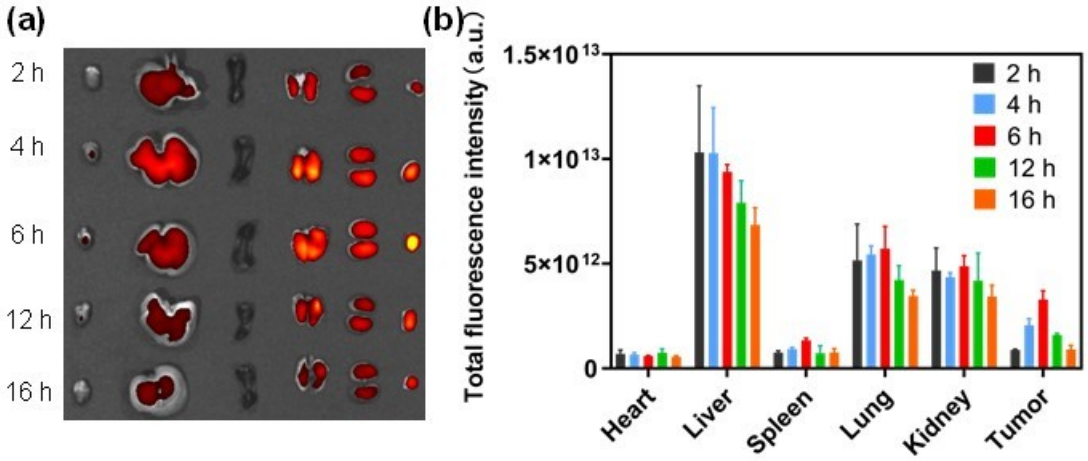


Fig. S7 (a) Ex vivo NIR fluorescence images and (b) total fluorescence intensity of heart, liver, spleen, lung, kidney and tumor for different time.

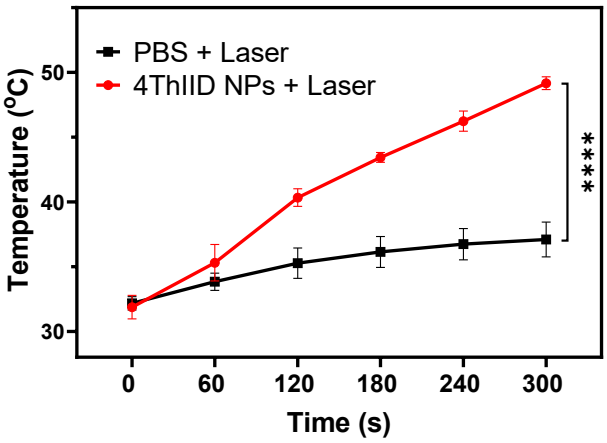


Fig. S8 The curves of the temperature changes in the tumor areas of mice in different groups with the laser irradiation time. ****$p < 0.0001$, two-way ANOVA analysis method.

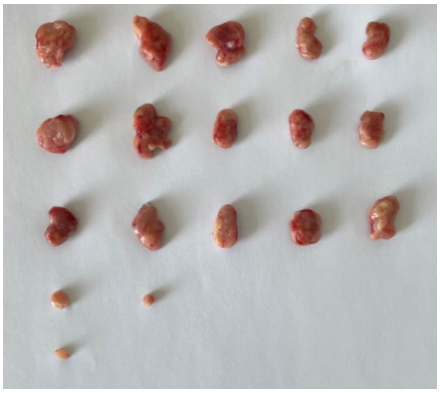


Fig. S9 Photos of tumors excised from tumor-bearing mice at 22 days of the treatment.

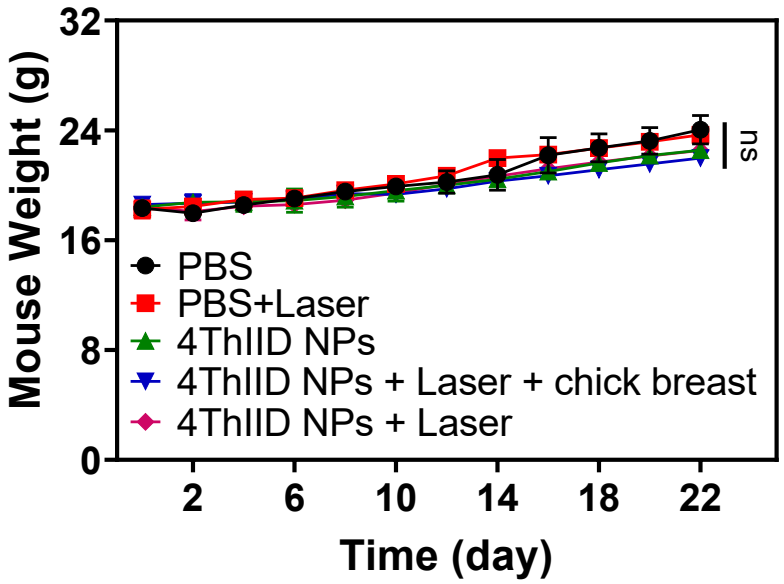


Fig. S10 During the entire experimental period, the curves of the weight changes of mice in different treatment groups over time.

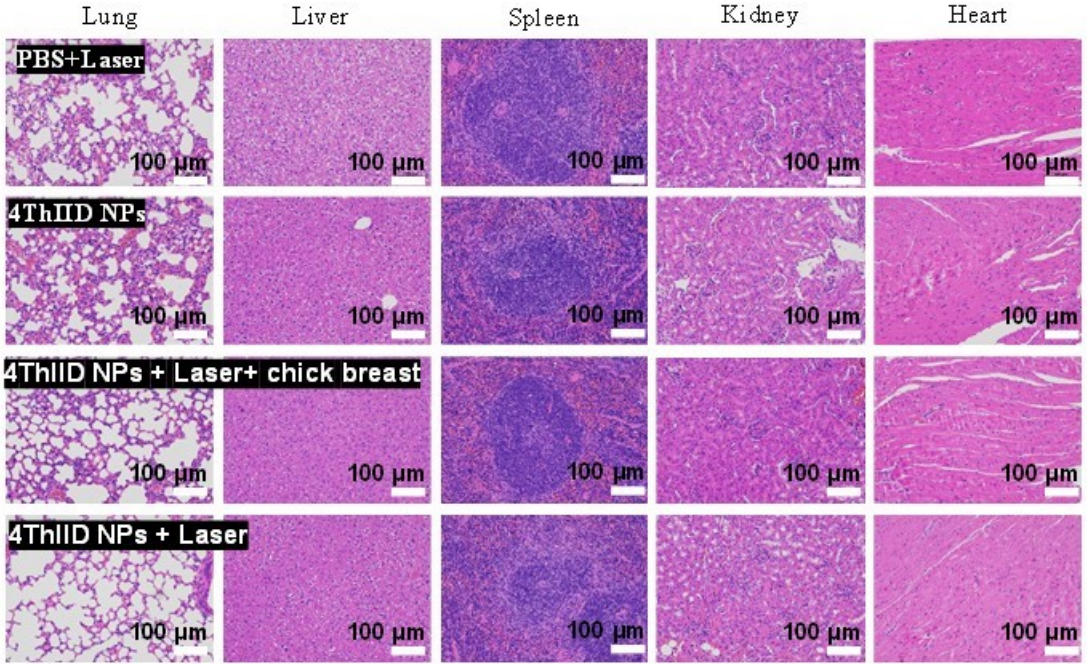


Fig. S11 Histological H&E staining of lung, livers, spleens, kidney and heart on day 22 after different treatments indicated.

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