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Supplementary Information

A Triple-responsive Targeted Hybrid Liposomes with High MRI Performance for Tumor Diagnosis and Therapy

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1. Materials

Sodium carbonate (AR), anhydrous magnesium sulfate (AR), anhydrous sodium sulfate (AR), hydrobromic acid (HBr, 48%), dichloroethane (AR), hydrochloric acid (12 mol/L), N,N-dimethylformamide (AR), acetonitrile (AR), chloroform (AR), methanol (AR), dichloromethane (AR), ethyl acetate (AR) and triethylamine (AR) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. PBS solution (0.01mol/L) was prepared by sodium hydrogen phosphate, monobasic potassium phosphate, potassium chloride and sodium chloride according to a certain proportion. PB solution (0.02 mol/L) was prepared by sodium hydrogen phosphate, monobasic sodium phosphate according to a certain proportion. Sodium hydrogen phosphate (AR), monobasic potassium phosphate (AR), hydrogen peroxide (H_2O_2, 30%), potassium chloride (AR) and sodium chloride (AR) were purchased from Tianjin Bodi Chemical Co., Ltd. 6-nitro-3-benzodioxole-5-carboxaldehyde (97%), aluminum chloride (99%), Tris(2-carboxyethyl)phosphine (TCEP, 99%), n-bromodecane (98%), celecoxib (98%), succinic anhydride (99%) and sodium borohydride (99%) were purchased from J&K Chemical Ltd. Fetal bovine serum (FBS), trypsin–EDTA and penicillin/streptomycin (5000 U/mL) were purchased from Thermo Fisher Scientific. 3,3'-Dithiodipropionic acid (99%), oxalyl chloride (99%), 4-Dimethylaminopyridine (DMAP, 99%), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride (EDCI, 98%), methoxypolyethylene glycols (mPEG-2000),

polyethylene glycol (PEG-2000) and anhydrous pyridine (99%) were purchased from TCI. AA-PEG-OH, Gd-DTPA (99%) and 12,12-NB-DTPA-Gd (98%) were provided by our lab. Doxorubicin hydrochloride (DOX·HCl, 98%) was purchased from Dalian Meilun Biotech Co., Ltd. 4',6-diamidino-2-phenylindole (DAPI, 98%) was purchased from Sigma-Aldrich. MTT assays and Dulbecco's modified Eagle's medium (DMEM) were purchased from KGI Biotech. 96-well plates, 24-well plates and cell culture flask were purchased from Guangzhou Jet Bio-Filtration Co., Ltd.

2. Experimental methods

2.1. UV/ GSH and UV/ H_2O_2 responsiveness of molecules

0.1 mg/mL aqueous solution of each compound (10,10-NB-S-S-P-AA, 10,10-NB-OA-P-CE, 10,10-NB-S-S-P-OMe and 10,10-NB-OA-P-OMe) was put in quartz cell and irradiated by UV light for different time. The UV absorption curves of aqueous solution at different time points were recorded by UV/VIS/NIR Spectrometer (Lambda 750 S, PerkinElmer Co., Ltd.). 20 mg of each compound (10,10-NB-S-S-P-AA, 10,10-NB-OA-P-CE, 10,10-NB-S-S-P-OMe and 10,10-NB-OA-P-OMe) was dissolved in 2 mL CH_2Cl_2, respectively. The obtained solution was irradiated by UV light for 30 min, respectively. The changes in structure of every molecule (10,10-NB-S-S-P-AA, 10,10-NB-OA-P-CE, 10,10-NB-S-S-P-OMe and 10,10-NB-OA-P-OMe) before and after UV irradiation were recorded by MS.

TCEP is frequently used to replace GSH in the extracellular reduction-responsive experiment due to the stability and the high effectiveness of TCEP. 10,10-NB-S-S-P-AA or 10,10-NB-S-S-P-OMe was dissolved in 10 mmol/L TCEP aqueous solution for 24 h, and the degradation products were characterized by MS. The process of the extracellular H_2O_2-responsive experiment was similar with the extracellular reduction-responsive experiment. 10,10-NB-OA-P-CE or 10,10-NB-OA-P-OMe was dissolved in 10 mmol/L H_2O_2 aqueous solution for 24 h, and the degradation products were characterized by MS.

2.2. Hemolysis evaluation

To evaluate the hemolysis of each component (10,10-NB-S-S-P-AA, 10,10-NB-OA-P-CE, 10,10-NB-S-S-P-OMe and 10,10-NB-OA-P-OMe) in liposomes, the red blood cells (RBCs) was obtained by washing and centrifuging the pig blood sample. 2% (v/v) red blood cells

solution was made by using normal saline to dilute the red blood cells (RBCs). Different concentrations of 10,10-NB-S-S-P-AA, 10,10-NB-OA-P-CE, 10,10-NB-S-S-P-OMe and 10,10-NB-OA-P-OMe were co-incubated with 2% red blood cells solution in 24-well plate at 37 ° C for 4 h, respectively. The negative control group was 2% red blood cells solution treated with normal saline, while the positive control group was 2% red blood cells solution treated with 2% Triton X-100 (TX-100) solution. The absorbance values of different groups at 540 nm were measured by a microplate reader. The hemolytic rates of different samples were calculated by the equation below:

$$Hemolytic\ rate\ \% = \frac{A_{sample} - A_{negtive\ control}}{A_{positive\ control} - A_{negtive\ control}} \times 100$$

2.3. Fabrication of Targeted and Non-targeted liposomes (GNSOPAC and GNSOPM)

Targeted and Non-targeted liposomes (**GNSOPAC** and **GNSOPM**) were fabricated by the thin film dispersion method. In brief, 12,12-NB-DTPA-Gd, 10,10-NB-S-S-P-AA and 10,10-NB-OA-P-CE were mixed in a certain proportion and dissolved by 1 mL mixed solution of dichloromethane and methanol (1:1, v/v) in round-bottomed flask. The organic solvent of the mixed solution was removed by rotary evaporation to form a homogeneous thin film. Then, the homogeneous thin film was hydrated overnight by 4 mL of PBS solution. After hydration, the targeted liposomes (**GNSOPAC**) were fabricated by sonicating with ultrasound probe for 10 min (ultrasound 2 s, intermittent 5 s, 100 W). The preparation process of the non-targeted liposomes (**GNSOPM**) is similar with that of the targeted liposomes.

2.4. Preparation of drug-loaded liposomes

Drug-loading liposomes were prepared by dialysis method. 12,12-NB-DTPA-Gd, 10,10-NB-S-S-P-AA and 10,10-NB-OA-P-CE were mixed and made to a homogeneous thin film in round-bottomed flask. Then, the film was hydrated overnight by using 3 mL of PBS solution. After hydration, 1 mL of DOX•HCl solution (1 mg/mL) was added to the above round-bottomed flask. The obtained mixed solution was sonicated with an ultrasound probe for 10 min (ultrasound 2 s, intermittent 5 s, 100 W), and was put into a dialysis bag (MWCO 3500). The dialysis bag was immersed in 400 mL PBS solution (0.01 M; pH 7.4) with stirring at room temperature, and PBS solution was changed every half an hour until 4 h. The obtained targeted drug-loaded liposomes (**GNSOPACD**) in the dialysis bag were taken out for subsequent

experiments. The preparation process of the non-targeted drug-loaded liposomes (**GNSOPMD**) is similar with that of the targeted liposomes.

The drug entrapment efficiency (DEE) was calculated by the formula as follows. The mass of drug in liposomes was determined by the calibration curve (y=0.018x-0.001, R^2 = 0.999, where y is the UV absorption intensity at 486 nm, x is the concentration of DOX•HCl).

$$DEE\ (wt\%)\ = \frac{mass\ of\ drug\ in\ liposomes}{mass\ of\ drug\ in\ feed} \times 100\%$$

2.5. Stability of liposomes

The stability of liposomes is a prerequisite for clinical application of liposomes. To evaluate long-term stability of liposomes, the diameter and PDI of empty liposomes and drug-loaded liposomes were measured using a Zetasizer (Malvern Nano S90, Britain) at different time points (0, 1, 3, 5, and 7 d). To evaluate serum stability of liposomes, drug-loaded liposomes (GNSOPACD and GNSOPMD) were put into 10% serum solution and incubated at 37 °C for 72 h. The diameter and PDI of the obtained drug-loading liposomes were recorded using a Zetasizer (Malvern Nano S90, Britain) at different time points (0, 7, 24, 48, and 72 h).

2.6. UV/ GSH/ H_2O_2 responsiveness of liposomes

To evaluate UV/GSH/H_2O_2 responsiveness of liposomes, the diameter distributions of liposomes treated with TCEP, H_2O_2 and UV light were measured using a Malvern Zetasizer Nano S90. The morphology of liposomes after different treatments were observed by TEM (JOEL JEM-2000EX system). TEM samples of liposomes treated with different conditions were prepared by negative staining of 2% phosphotungstic acid solution.

2.7. *In vitro* drug release

To simulate the *in vivo* drug release of drug-loaded liposomes, *in vitro* drug release study was performed by putting drug-loaded liposomes in different conditions. The dialysis bags (MWCO 3500) containing drug-loading liposomes were treated with different conditions (pH 7.4, pH 6.5, pH 5.0, pH 5.0+10 mmol/L TCEP, pH 5.0+10 mmol/L H_2O_2 and pH 5.0+30 min UV) with stirring at 37 °C. The UV/ GSH responsive group or the UV/ H_2O_2 responsive group was performed by two steps. In the first step, the dialysis bag (MWCO 3500) containing drug-loading liposomes was immersed in PBS solution (pH 5.0+10 mmol/L TCEP or pH 5.0+10 mmol/L H_2O_2) for 4 h. In the second step, the dialysis bag (MWCO 3500) containing drug-

loading liposomes was re-immersed in PBS solution (pH 5.0+10 mmol/L TCEP or pH 5.0+10 mmol/L H_2O_2) after 30 min UV irradiation. 3 mL phosphate buffer solution was taken from beaker at predetermined time intervals and the fluorescence intensity at 590 nm was measured. Meanwhile, 3 mL fresh phosphate buffer solution needed to supplement into the beaker. The cumulative amount of DOX released was determined by the calibration curve (y = 133.8x-0.645, R^2 = 0.999, y is the fluorescence emission intensity at 590 nm, x is the concentration of DOX•HCl). The cumulative release curves of different groups were calculated by the equation below:

$$Cumulative\ release\ rate\ of\ DOX = \frac{M_t}{M_0} \times 100\%$$

Here, M_t is the cumulative release mass of DOX after t hour, and M_0 is the start mass of DOX encapsulated in the liposomes.

2.8. Longitudinal relaxation time measurement

The longitudinal relaxation time (T_1) of **GNSOPAC** and **GNSOPM** in different ratios at different Gd^{3+} concentration was measured by using a 0.54 T nuclear magnetic resonance imager (MesoQMR23-060h-1, Niumag Technology Co., Ltd.) at 37 °C. The Gd^{3+} concentration of **GNSOPAC** and **GNSOPM** liposomes was determined by ICP-AES. The longitudinal relaxivity (r_1) was obtained by the fitting linear slope of 1/relaxation time ($1/T_1$) *vs.* the Gd^{3+} concentration.

2.9. *In vitro* MR imaging

In vitro MR imaging results of **Gd-DTPA**, **GNSOPAC** and **GNSOPM** liposomes at various Gd^{3+} concentration (0.00376, 0.0376, 0.188, 0.376 and 0.94 mmol/L) were observed by 0.54 T nuclear magnetic resonance imager (MesoQMR23-060h-1, Niumag Technology Co., Ltd.) at 37 °C.

2.10. *In vitro* cytotoxicity study

In vitro cytotoxicity studies were performed with **GNSOPAC** and **GNSOPM** against MCF-7 cells by MTT assays. 1×10^5 cells/mL of MCF-7 cells in logarithmic growth stage was seeded in 96-well plates and cultured in a cell incubator (5% CO_2, 37 °C) overnight. When the cell confluence reached to 80%, the old cell culture medium was immediately replaced by 100 μL of fresh medium containing different concentration of **GNSOPAC** and **GNSOPM** (0.1, 1, 10,

25, 50, 100, 250, 400 and 500 μg/mL). After 24 h incubation, the culture medium in 96-well plates was discarded and used PBS solution to wash cells three times. 150 μL of MTT reagent was added in 96-well plates and incubated for 4 h. After discarding the residue MTT reagent, 150 μL of DMSO was added in 96-well plates to dissolve formazan. The absorbance of 96-well plates at 492 nm was measured by using a microplate reader. The cell viability was calculated to this formula:

$$Cell\ Viability\ (\%) = (A_{sample} - A_{control})/(A_{control} - A_0) \times 100$$

The method of *in vitro* photo-induced anticancer study of DOX·HCl, **GNSOPACD** and **GNSOPMD** liposomes (the concentrations of DOX·HCl are 0.1, 1, 5, 10, 15, 20, 25, 30 and 35 μg/mL) against MCF-7 and A549 cells are similar with *in vitro* cytotoxicity study.

2.11. *In vitro* cellular uptake

To evaluate the targeting activity and the cellular uptake level of drug-loaded liposomes, hydrophobic anticancer drug (DOX) was used as a fluorescent probe to encapsulate into empty liposomes. DOX was obtained by desalination of DOX·HCl. MCF-7 and A549 cells in logarithmic growth phase were seeded in glass bottom dishes in a humidified cell incubator (5% CO_2, 37 °C) overnight at 37 °C. Then, the culture medium was replaced with the fresh medium containing **GNSOPACD** and **GNSOPMD** liposomes (the concentration of DOX is 5 μg/mL) for 2 h, respectively. For the targeting-inhibition study, MCF-7 and A549 cells pre-treated with anisamide (5 μmol/L and 10 μmol/L) were incubated with **GNSOPACD** liposomes for 1 h. The cells of different treatments were washed three times with cold PBS and fixed with 4% formaldehyde for 15 min. The cells were further washed twice with PBS and the nucleus were stained with DAPI for 10 min. The excess DAPI was removed by washing with PBS three times. The cellular uptake and intracellular distribution of drug-loaded liposomes were observed by confocal laser scanning microscopy (CLSM, FV1000).

To quantitatively analyze cellular uptake of drug-loaded liposomes, the results of flow cytometry analysis were carried out by a flow cytometer (FACSCanto). COS-7, MCF-7 and A549 cells in logarithmic growth phase were seeded in 6-well plates at a density of 10^5 cells/well for overnight. When the cell confluence reached to 90%, MCF-7 and A549 cells were treated without or with anisamide (5 μmol/L and 10 μmol/L) in advance. The culture medium

was replaced with the fresh medium containing **GNSOPACD** liposomes (the concentration of DOX is 5 μg/mL), and cells were co-incubated with these liposomes for different time. After that, the cells of different treatments were washed three times with cold PBS and trypsinized, and then the culture medium containing 10% FBS was added in each well to stop trypsinization. The cells were collected in sample tubes by centrifugation and re-dispersed in PBS to prepare the single cell suspension. The DOX florescence intensity of cells with different treatments was measured by a flow cytometer.

2.12. Wound-Healing Assay

In order to study effect of liposomes on cell migration, the wound-healing assay was carried out. MCF-7 and COS-7 cells in logarithmic growth phase were seeded in 6-well plates at a density of 10^5 cells/well. When the cell density reached to 90% confluence, cell wounds were scraped using a sterile 200-μL pipette tip. The cells were carefully rinsed with PBS to remove cell debris and incubated with fresh FBS-free culture medium containing different samples (**GNSOPACD** and **GNSOPMD**) for 24 h. The wound-healing areas were visualized by using an inverted fluorescence microscope, and the distance of cell migration was counted by Image J.

2.13. *In vivo* antitumor activity

To evaluate the *in vivo* antitumor activity of different formulations, the subcutaneous xenograft model was established by subcutaneously injecting 4×10^7 cells/mL MCF-7 cells into the right axilla of BALB/c nude mice (6-7 weeks old). When the tumors were grown to about 80 mm^3 (V=L×W×W/2), the mice bearing breast tumor were randomly divided into five groups. Each group was treated with PBS, free DOX, **GNSOPMD**, **GNSOPACD** and **GNSOPACD** +UV, respectively. The drug formulation (the dosage of DOX•HCl is 3.5 mg/kg) of each group was injected through tail vein. The length and width of the tumors in different groups were measured with Vernier caliper and the body weight of mice were recorded by an electronic balance every three days.

2.14. *In vivo* nuclear magnetic imaging

In order to study the *in vivo* MR imaging and tumor targeting activity of liposomes, MCF-7 tumor bearing mice were treated with Gd-DTPA, **GNSOPM**, **GNSOPAC** and **GNSOPAC**+UV, respectively. Meanwhile, the formulation (the dosage of Gd^{3+} is 0.1

mmol/kg) of each group was injected through tail vein. Furthermore, *in vivo* T_1-weighted nuclear magnetic images of each group at 0.5 h, 1 h, 2 h and 3 h of post-injection were recorded by using a 0.5 T nuclear magnetic resonance imager (Niumag Technology Co., Ltd., MesoQMR23-060h-1, FOV Read = 100 mm, FOV Phase = 100 mm, TR = 500 ms, TE = 20 ms).

2.15. *In vivo* optical imaging and *ex vivo* biodistribution studies

A small-animal imager (LB983 NC100) was used to conduct the in vivo optical imaging and ex vivo biodistribution studies (Ex 480 nm, Em 590 nm). The nude mice bearing MCF-7 breast tumor were treated with DOX•HCl, **GNSOPMD, GNSOPACD** and **GNSOPACD**+UV (the dose of DOX•HCl is 3.5 mg/kg) via intravenous injection. The *in vivo* fluorescence distributions at 0.5 h, 1 h, 2 h and 3 h of post-injection of mice were photographed by using an imager. After 24 h of injection, mice were immediately sacrificed and the main organs (heart, liver, spleen, lung, kidney) and tumors were excised to observe the fluorescence distribution.

2.16. Pharmacokinetics

For the study of pharmacokinetics, SD rats (~200 g) abstained from fasting overnight. DOX•HCl and **GNSOPACD** (the dose of DOX•HCl is 2 mg/kg) were injected intravenously into SD rats. 0.5 mL of blood samples at different time points (10 min, 30 min, 60 min, 90 min, 2 h, 4 h, 8 h, 18 h, 20 h and 24 h) were collected in centrifuge tube containing EDTA-2Na via retro-orbital blood samplings. The blood cells in the blood samples were removed to get clear plasm by centrifugation at 3000 rpm. 200 μL of plasma was added to 1.8 mL of 6% hydrochloride ethanol solution, and insoluble subjects precipitated out. After centrifugation, the fluorescence intensity of the obtained clear solution was measured using a fluorescence spectrophotometer (Ex 480 nm, Em 590 nm). The pharmacokinetics profiles of DOX•HCl and **GNSOPACD** were determined by measuring the fluorescence intensity of the obtained clear solution at different time points.

3. Synthesis

Compound 1: 4,5-dihydroxy-2-nitrobenzaldehyde (2HO-NB-CHO)

1 g of 6-nitro-3-benzodioxole-5-carboxaldehyde (6-Nitropiperonal) was dissolved in 10 mL dichloroethane, the obtained solution was added dropwise in mixed slurry of aluminum chloride

(3.5 g) and dichloroethane with stirring for 2 h in ice bath. After the reaction, the reaction solution was poured into 25 mL hydrobromic acid (48%), and stirred at room temperature for 48 hours. The reaction mixture was diluted with a large amount of water, which was extracted with ethyl acetate for 3 times. The organic layer was dried by anhydrous magnesium sulfate and removed organic solvent to get crude product. The yellow product was obtained by recrystallization in water and the yield was 77.4%.

MS m/z $[M-H]^-$:191.96, $[2M-H]^-$:364.84

^{1}H NMR (500 MHz, DMSO-d_6): δ in ppm 7.10-7.60 (s, 2H, HO-C_6H_2-); 10.10-10.20 (s, 1H, -C_6H_3-*CHO*); 7.10-7.60 (s, 2H, 2*HO*-C_6H_2-)

^{13}C NMR (500MHz, DMSO-d_6): δ in ppm 111.60-150.90 (HO-C_6H_2-), 188.53 (-C_6H_3-*CHO*).

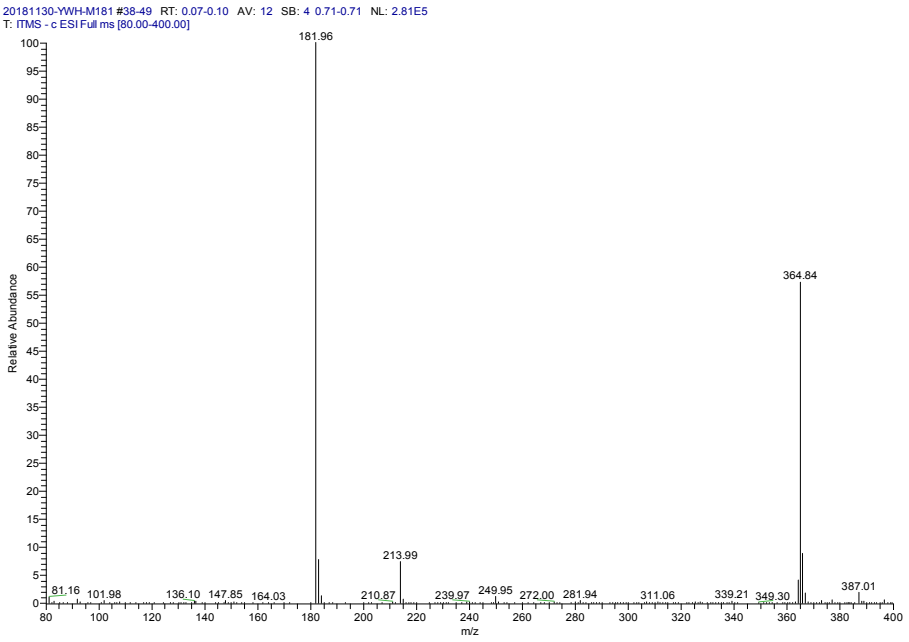

Fig. S1. MS of 2HO-NB-CHO

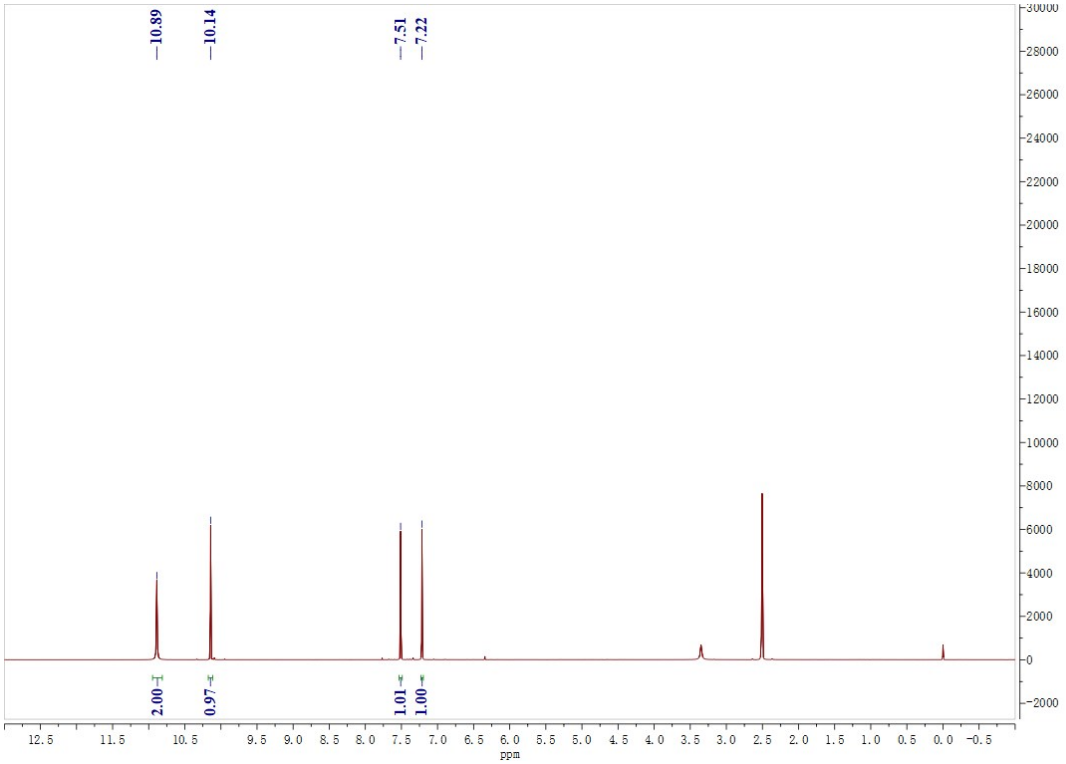

Fig. S2. ^{1}H NMR of 2HO-NB-CHO

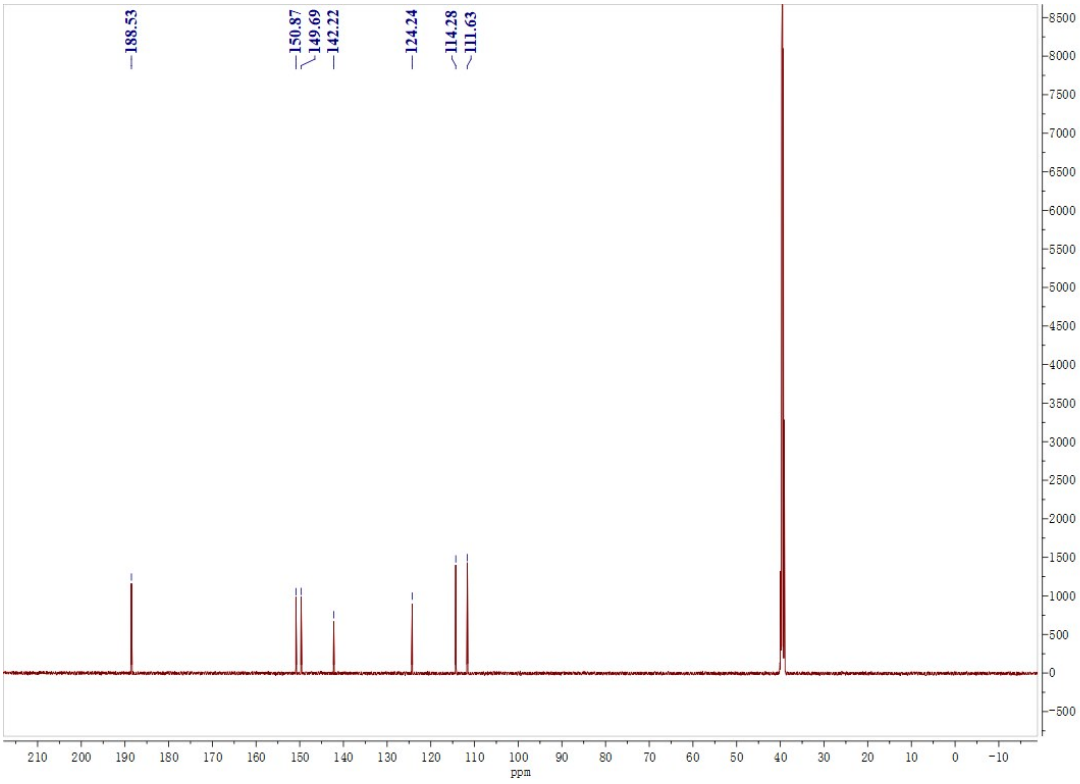

Fig. S3. ^{13}C NMR of 2HO-NB-CHO

Compound 2: 4,5-didecyloxy-2-nitrobenzaldehyde (10,10-NB-CHO)

10 mL N,N-Dimethylformamide solution containing 4,5-dihydroxy-2-nitrobenzaldehyde (1

g) was added into the mixture of anhydrous potassium carbonate (1.88 g) and n-bromodecane (3.58 g) with stirring and heated to 60 °C for 15 h in the N_2 stream. The resulting mixture was diluted with water, and extracted with 50 mL of diethyl ether for 3 times. The extract was dried with anhydrous magnesium sulfate and the solvent was evaporated by rotary evaporation. The yellow product was recrystallized from diethyl ether and the yield was 79.4%.

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm 0.80-0.90 (m, 6H, -CH_2-CH_3); 1.26-1.49 (m, 24H, -$(CH_2)_6$-CH_3); 1.80-1.95 (m, 8H, -O-CH_2-$(CH_2)_2$-); 4.10-4.18 (m, 4H, -O-CH_2-CH_2-); 7.3-7.4 (s, 1H, -C_6H_2-NO_2); 7.5-7.6 (d, 1H, -C_6H_2-CHO);10.39-10.46 (s, 1H, -C_6H_2-CHO).

^{13}C NMR (500MHz, $CDCl_3$): δ in ppm 14.1-32.0 (-$(CH_2)_8$-CH_3); 69.70-69.90 (-O-CH_2-$(CH_2)_8$-); 108.10-153.30 (-C_6H_2-NO_2);187.92 (-C_6H_2-CHO).

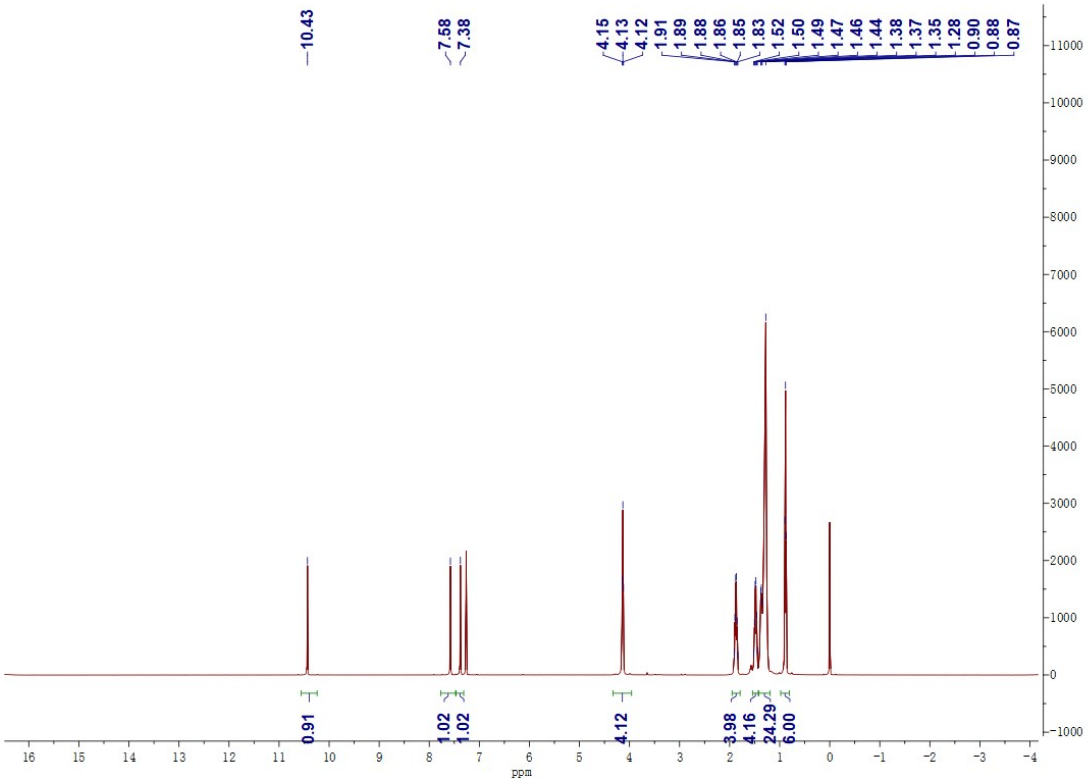

Fig. S4. ^{1}H NMR of 10,10-NB-CHO

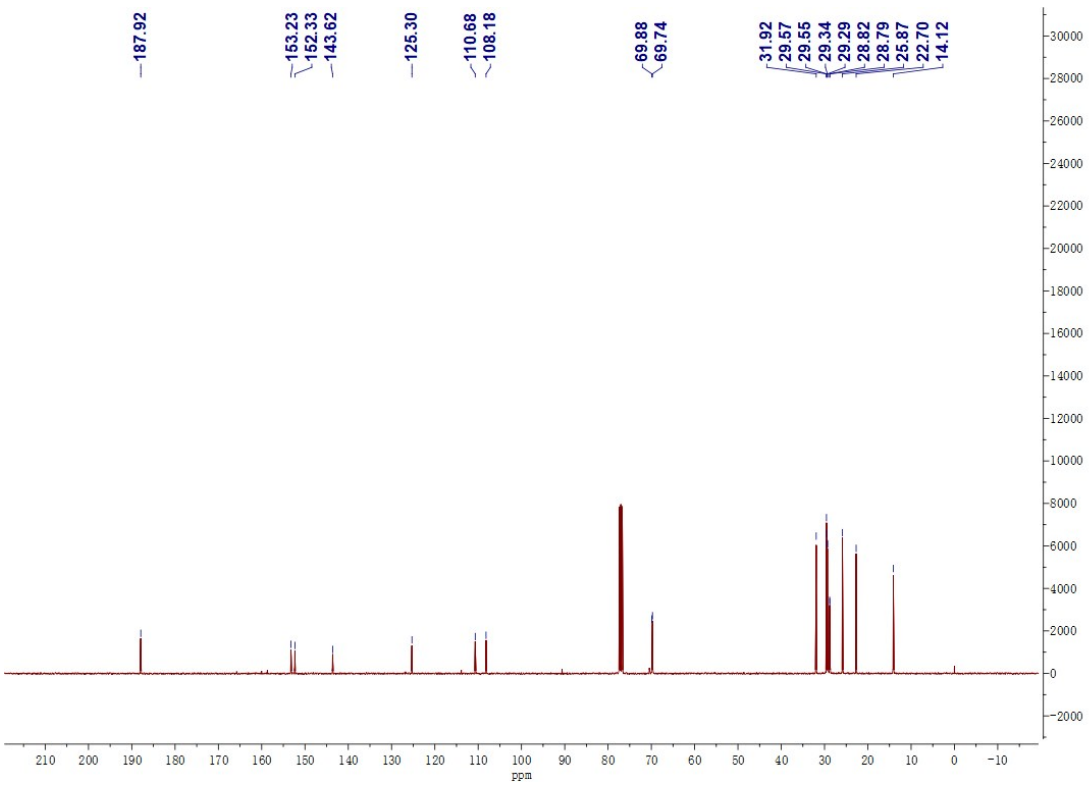

Fig. S5. ^{13}C NMR of 10,10-NB-CHO

Compound 3: 4,5-Didecyloxy-2-nitrobenzyl alcohol (10,10-NB-CH_2OH)

0.4 g of sodium borohydride was slowly added to the mixed solution of methanol and tetrahydrofuran (*v*:*v*=1:1) containing 4,5-didecyloxy-2-nitrobenzaldehyde (2 g) with stirring for 30min in ice bath, and continue to react at room temperature for 3 h. The residue was obtained by removing solvent of the mixed reaction solution, which was dissolved in chloroform and washed with hydrochloric acid (0.1 mol/L) and deionized water for 3 times. The organic solvent was dried with anhydrous sodium sulfate and the yellow product was obtained by removing the solvent. The yield was 95.1%.

MS m/z $[M+H]^+$: 478.43

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm 0.83-0.93 (m, 6H, -CH_2-CH_3); 1.26-1.40 (m, 24H, -$(CH_2)_6$-CH_3); 1.40-1.90 (m, 8H, -O-CH_2-$(CH_2)_2$-); 4.01-4.18 (m, 4H, -O-CH_2-CH_2-); 4.82-4.99 (s, 2H, -CH_2-OH); 7.05-7.13 (s, 1H, -C_6H_2-CH_2OH); 7.60-7.72 (s, 1H, -C_6H_2-NO_2).

^{13}C NMR (500MHz, $CDCl_3$): δ in ppm 14.09-31.91(-$(CH_2)_6$-CH_3); 62.95 (-CH_2-OH); 69.47-69.64 (-O-CH_2-$(CH_2)_8$-); 109.93-154.21 (-C_6H_2-CH_2OH).

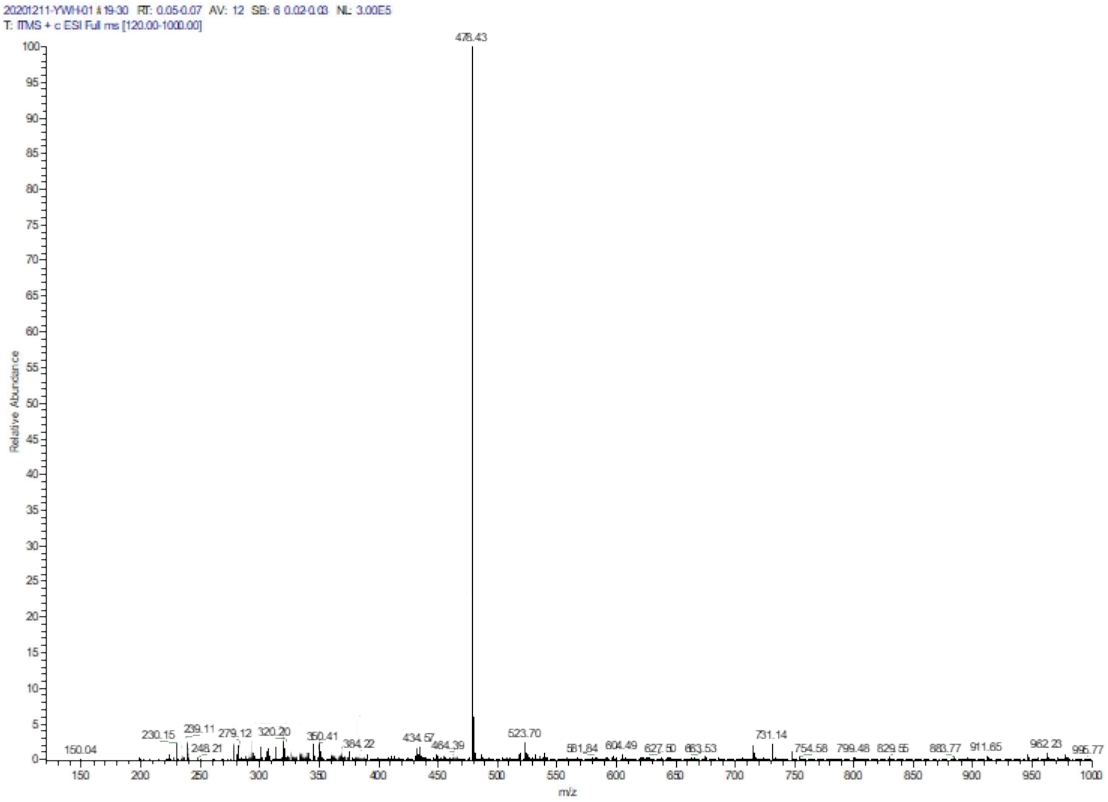

Fig. S6. MS of 10,10-NB-CH_2OH

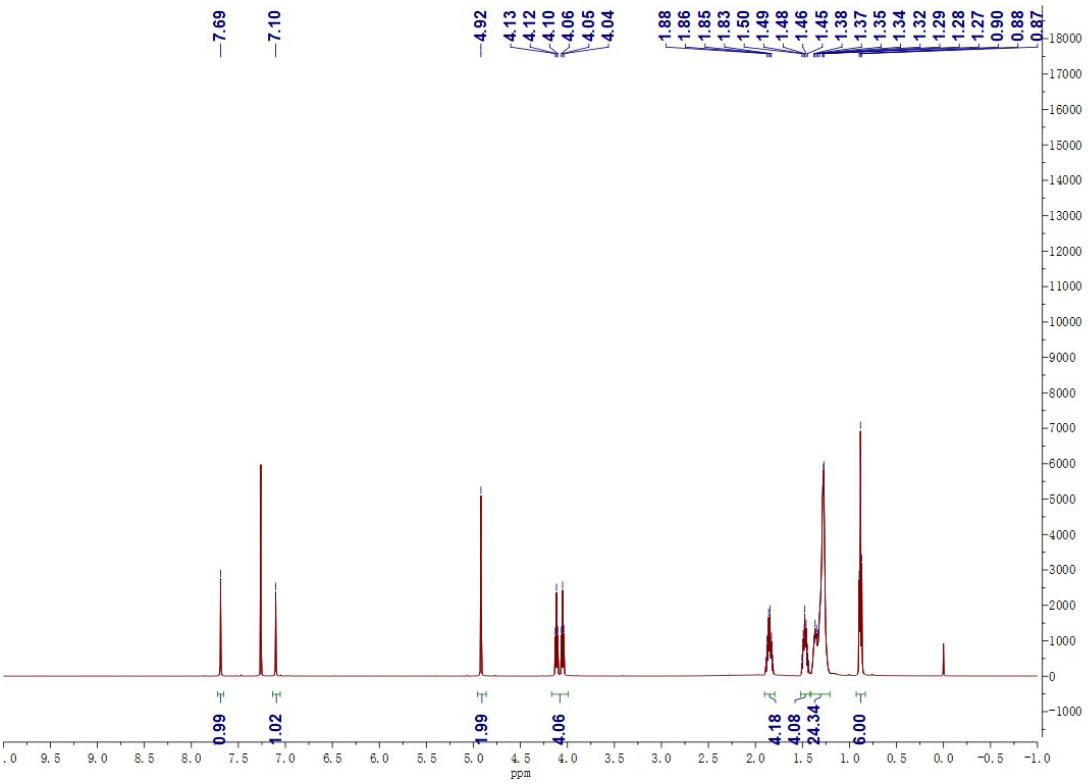

Fig. S7. ^1H NMR of 10,10-NB-CH_2OH

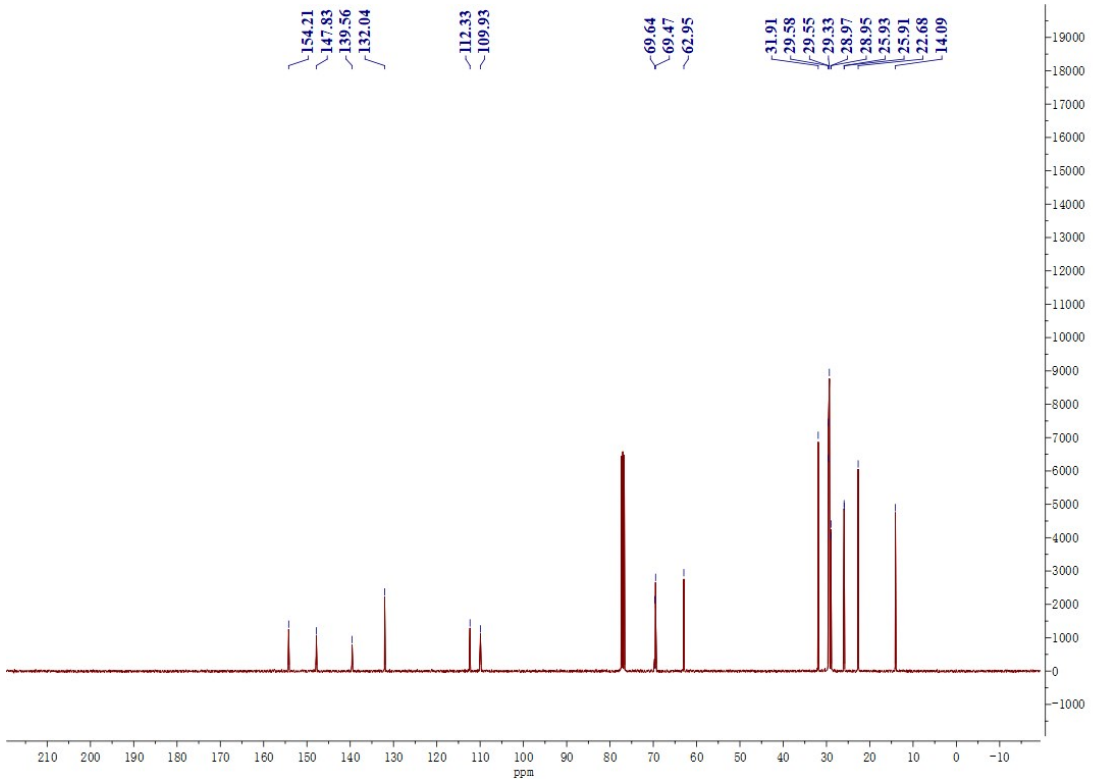

Fig. S8. ^{13}C NMR of 10,10-NB-CH_2OH

Compound 4: 10,10-NB-S-S-COOH

First, 2 g of 3,3'-dithiodipropionic acid was added to 10 mL of acetyl chloride and refluxed at 65 °C for 2 h. The solvent was removed by rotary evaporation, and the solid was washed with cold diethyl ether repeatedly and placed in vacuum drier for 12 h to obtain DTDPA. 1 g of 4,5-Didecyloxy-2-nitrobenzyl alcohol, 0.8 g of DTDPA and 2 mL of pyridine were dissolved in 20 mL of anhydrous dichloromethane and stirred for 24 h. The obtained mixture was washed with hydrochloric acid (0.1 mmol/L) and saturated NaCl solution and subsequently dried with anhydrous sodium sulfate. After filtration, the solvent was removed by rotary evaporation in vacuum. Finally, the pure product was obtained by silica gel column chromatography (dichloromethane: methanol=100:1) and the yield was 72.1%.

MS m/z $[M-H]^-$:656.41, $[M+Cl]^-$:692.28

^1H NMR (500 MHz, $CDCl_3$): δ in ppm 0.83-0.93 (m, 6H, -CH_2-CH_3); 1.26-1.40 (m, 24H, -$(CH_2)_6$-CH_3); 1.40-1.90 (m, 8H, -O-CH_2-$(CH_2)_2$-); 2.70-3.10 (-$(CH_2)_2$-S-S-$(CH_2)_2$-); 4.01-4.18 (m, 4H, -O-CH_2-CH_2-); 5.48-5.56 (s, 2H, -C_6H_2-CH_2-OC=O); 6.90-7.0 (s, 1H, -C_6H_2-CH_2-); 7.65-7.75 (s, 1H, -C_6H_2-NO_2).

^{13}C NMR (500MHz, $CDCl_3$): δ in ppm 14.09-31.92(-*$(CH_2)_6$-CH_3*); 32.59-34.00 (-*$(CH_2)_2$*-S-S-*$(CH_2)_2$*-); 63.82 (-C_6H_2-*CH_2*-OC=O); 69.47-69.64 (-O-*CH_2*-$(CH_2)_8$-); 109.85-153.74 (-*C_6H_2*-CH_2OH);171.15 (-C_6H_2-*CH_2*-O*C=O*);177.39 (-*CH_2-COOH*).

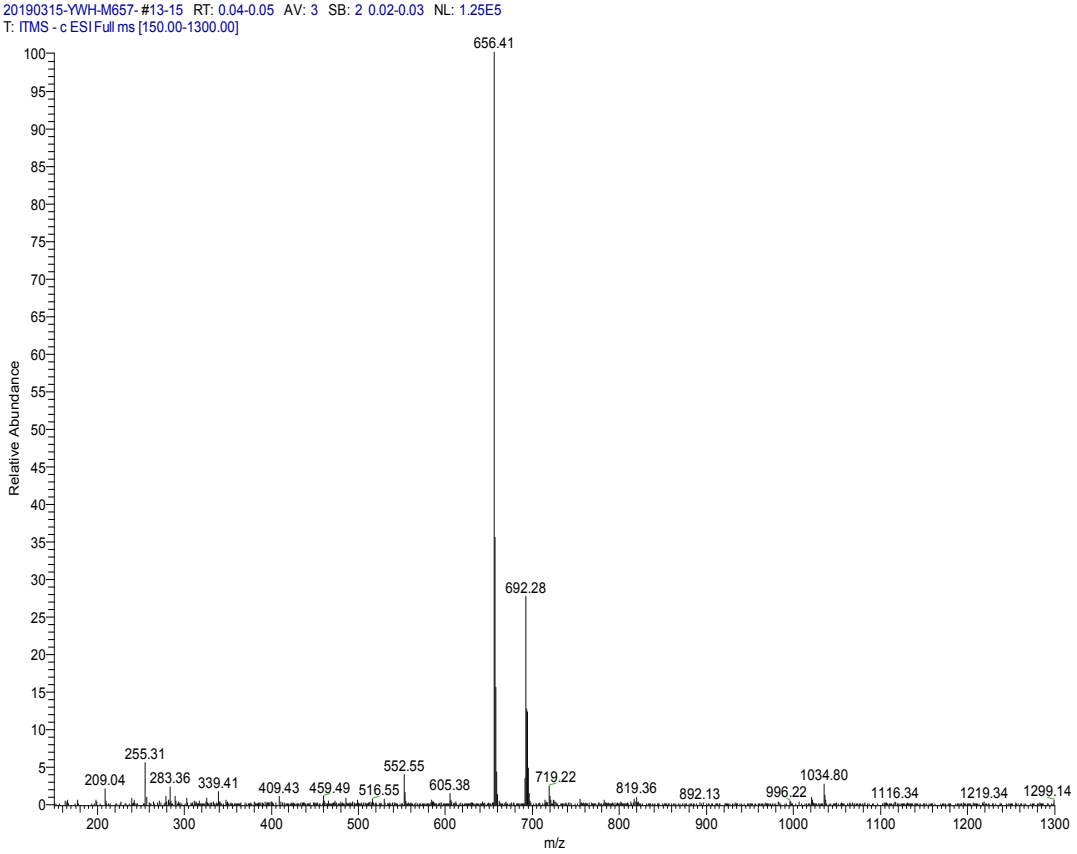

Fig. S9. MS of 10,10-NB-S-S-COOH

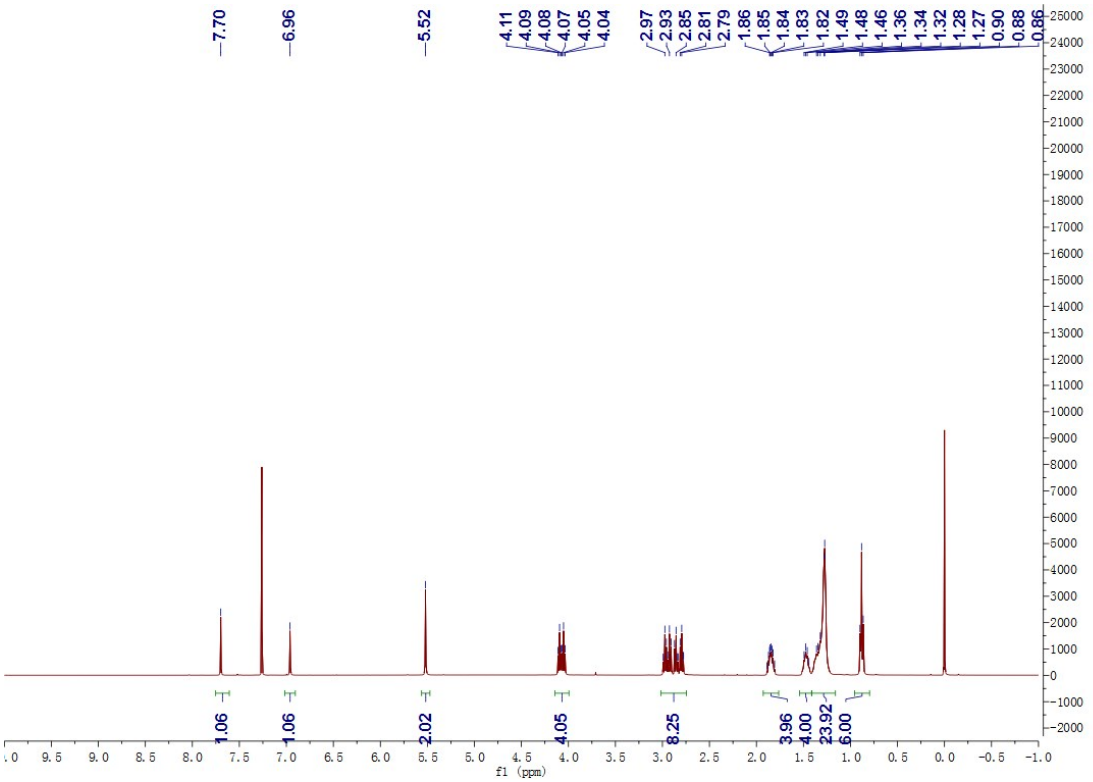

Fig. S10. ^{1}H NMR of 10,10-NB-S-S-COOH

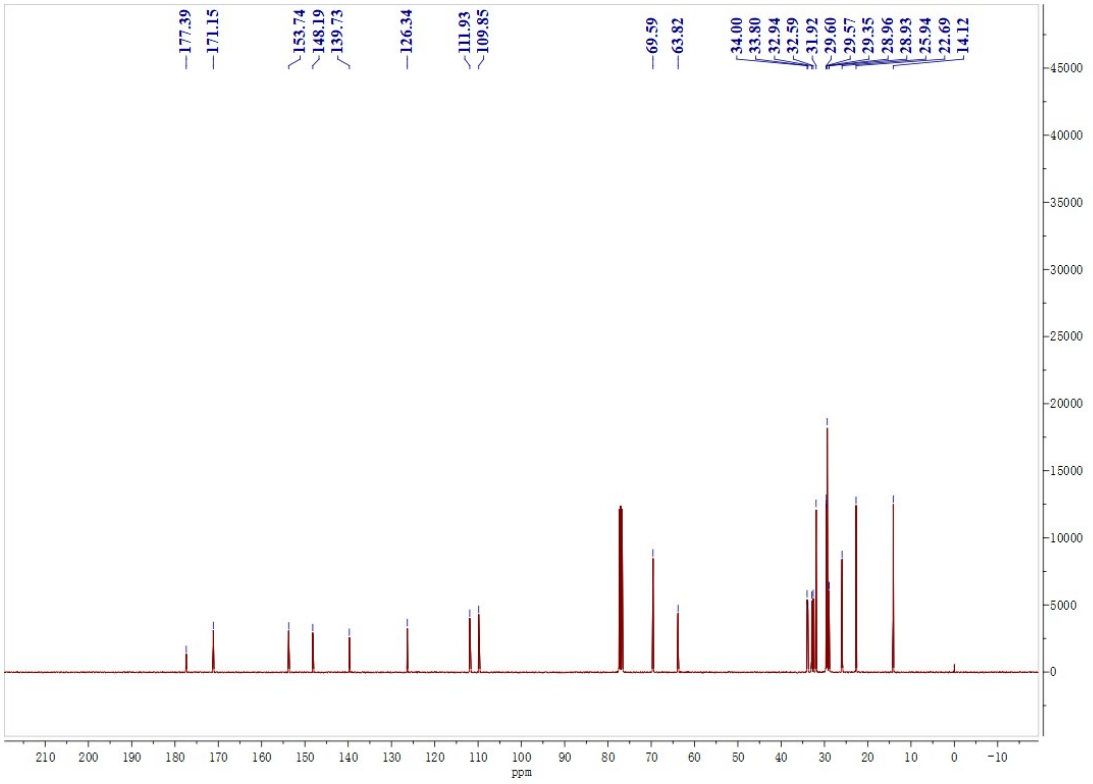

Fig. S11. ^{13}C NMR of 10,10-NB-S-S-COOH

Compound 5: Celecoxib-sussinamidic acid

0.3 g of succinic anhydride and TEA (0.5 mL) were dissolved in 20 mL of anhydrous CH_3CN

by stirring 0.5 h. 1 g of celecoxib was added to the above solution and stirred at 55 °C for 4 h. The solvent of reaction solution was removed by rotary evaporation in vacuum, and the residue was dissolved in dichloromethane. The obtained solution was washed sequentially with hydrochloric acid (1 mol/L) and saturated NaCl solution three time, and subsequently dried with anhydrous sodium sulfate. After filtration, the solvent was removed by rotary evaporation in vacuum. Finally, the pure product was obtained by silica gel column chromatography (dichloromethane: methanol=100:1) and the yield was 56.2%.

MS m/z $[M-H]^-$:480.20

^{1}H NMR (500 MHz, DMSO-d_6): δ in ppm 2.23 (s, 3H, -C_6H_4-CH_3); 2.34-2.50 (t, 4H, -CH_2-CH_2-COOH); 7.15-7.25 (m, 5H, -C_6H_4-CH_3 and -C_3N_2H-CF_3); 12.13 (s, 1H, -CH_2-*COOH*); 12.27 (s, 1H, -SO_2-*NH*-).

^{13}C NMR (500MHz, DMSO-d_6): δ in ppm 20.76 (-C_6H_4-CH_3); 27.78-30.41 (-CH_2-CH_2-COOH); 125.15 (-C_3N_2H-CF_3); 106.20-145.36 (CH_3-C_6H_4-, -C_6H_4-SO_2, -C_3N_2H-CF_3); 170.81 (-CH_2-*COOH*); 173.12 (-CH_2-*CO*-NH-)

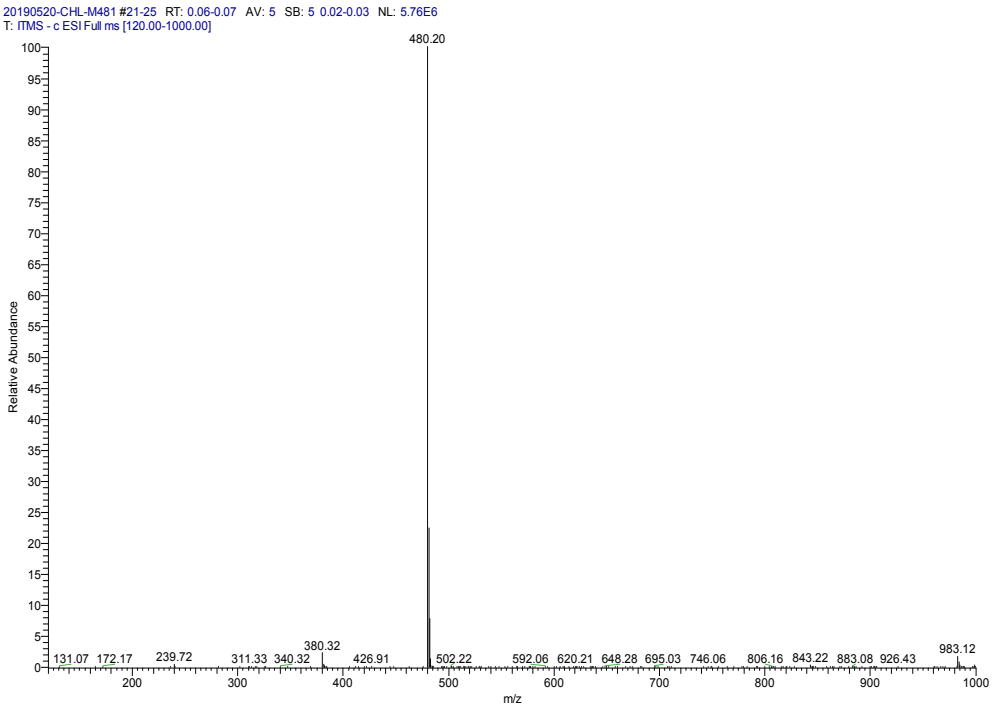

Fig. S12. MS of celecoxib-sussinamidic acid

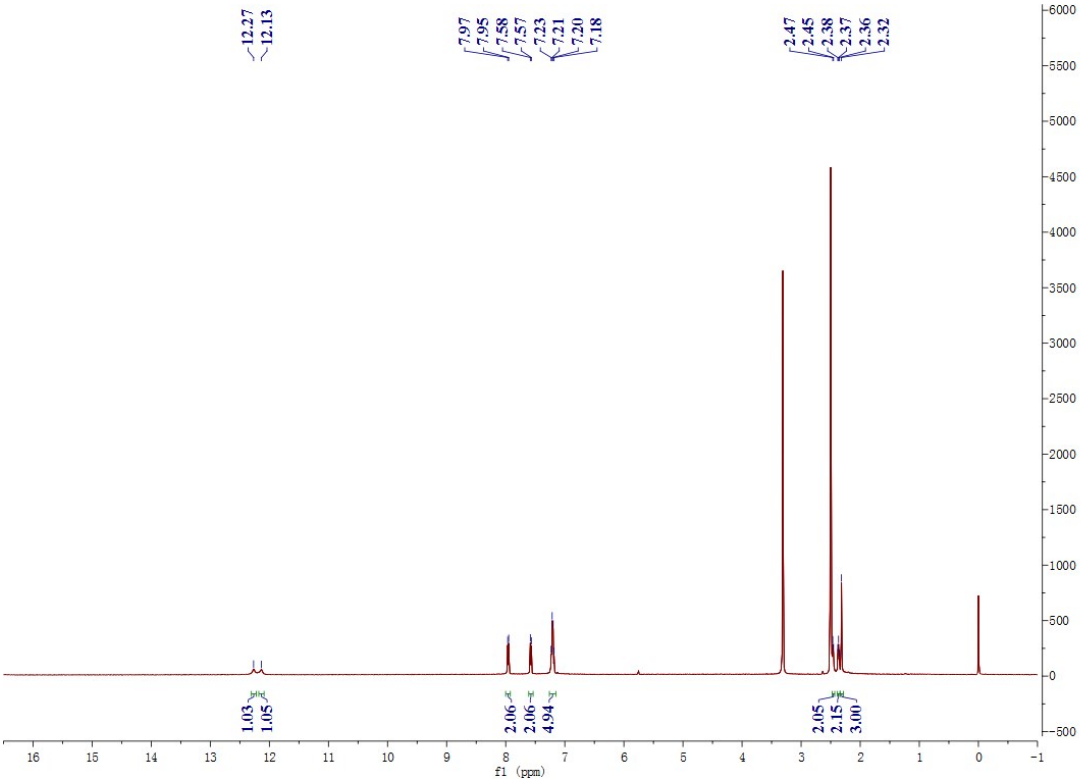

Fig. S13. [1]H NMR of celecoxib-sussinamidic acid

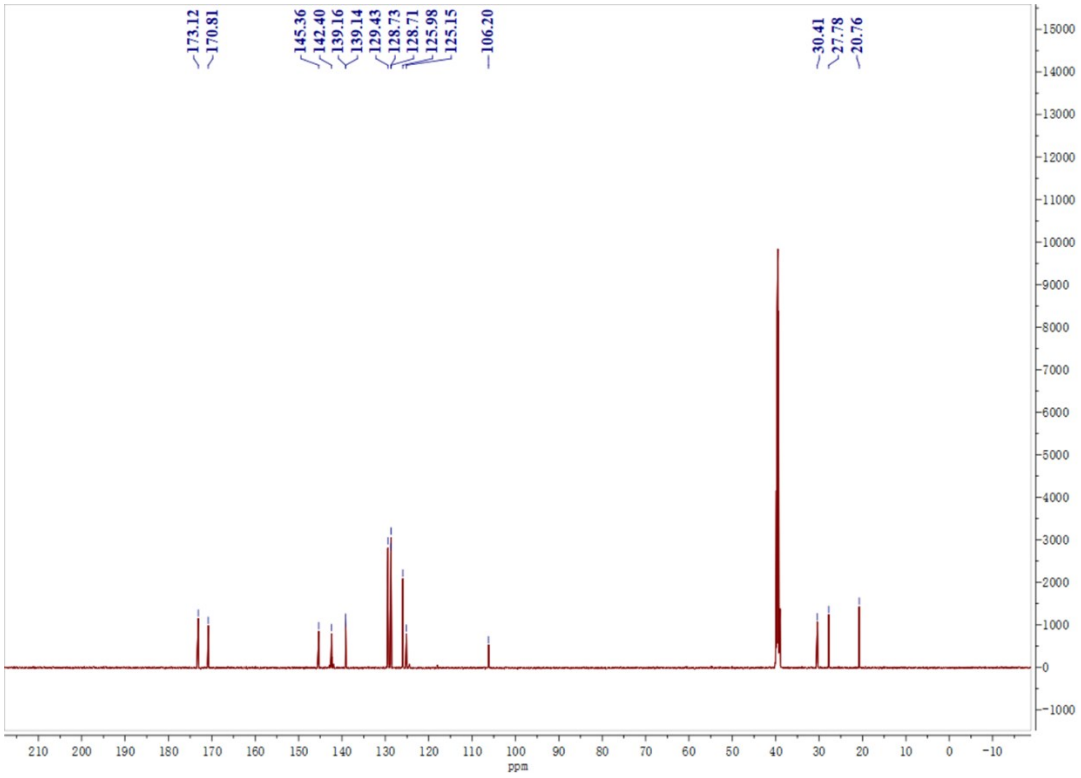

Fig. S14. [13]C NMR of celecoxib-sussinamidic acid

Compound 6: CE-PEG-OH

0.55 g of celecoxib-sussinamidic acid, 0.33g of EDCI and 27 mg of DMAP were dissolved in 30 mL anhydrous chloroform under vigorous magnetic stirring for 4 h. 3 g of HO-PEG-OH was added to the above reaction solution and stirred for 24 h. Then, the obtained mixture was washed with hydrochloric acid (0.1 mol/L) and saturated NaCl solution three times, and subsequently dried with anhydrous sodium sulfate. After filtration, the solvent was removed by rotary evaporation in vacuum. Finally, the pure product was obtained by silica gel column chromatography (dichloromethane: methanol=20:1) and the yield was 59.2%.^{1}H NMR of **CE-PEG-OH** was verified in Fig. S15.

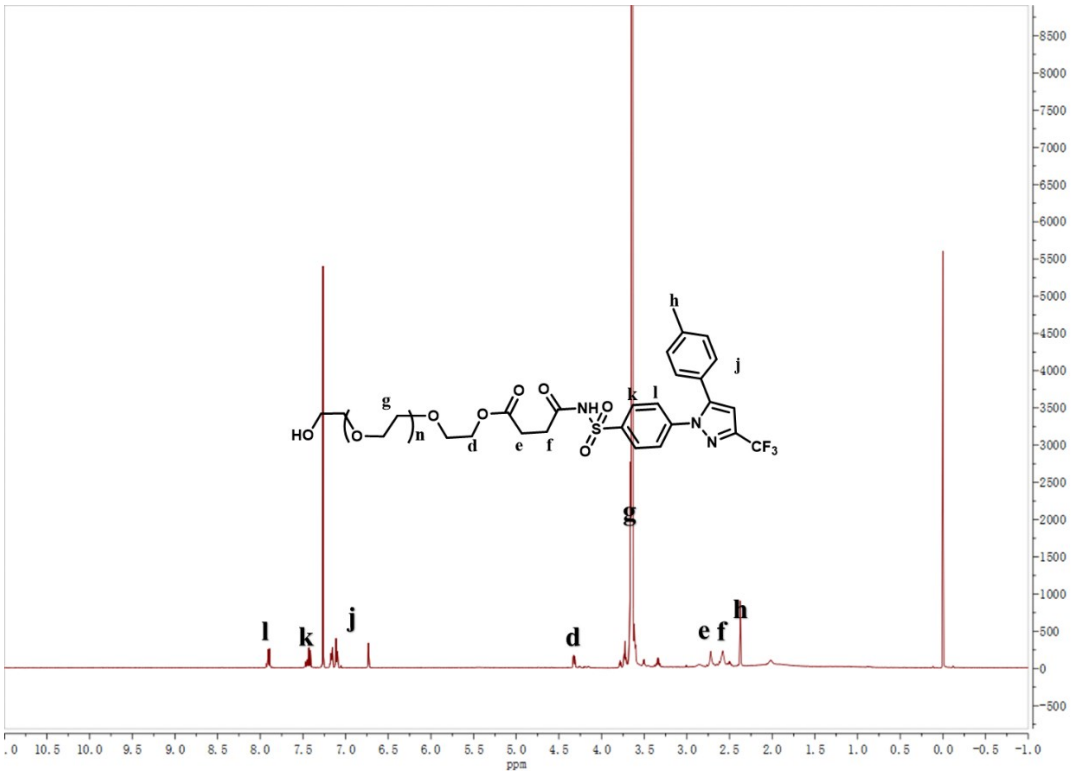

Fig. S15. ^{1}H NMR of CE-PEG-OH

Compound 7: 10,10-NB-OA-P-OMe and 10,10-NB-OA-P-CE

0.5 mL of oxalyl chloride was added to 10 mL of anhydrous dichloromethane in ice bath, 10 mL of dichloromethane solution containing 0.45 g of 10,10-NB-CH_2OH was gradually added to the above solution and stirred for 2 h. The excess oxalyl chloride in reaction solution was removed by rotary evaporation in vacuum to get an oil liquid. The oil liquid was redissolved in 10 mL of anhydrous dichloromethane. The obtained dichloromethane was added dropwise to 10 mL of dichloromethane solution containing 1.5 g of HO-PEG-OMe and 1 mL of pyridine

under vigorous magnetic stirring for 24 h. The solvent in mixture was removed by rotary evaporation in vacuum to get a yellow solid. The yellow solid was dissolved in deionized water and the insoluble matter was removed by filtration. The aqueous solution was dialyzed against deionized water (12 times, 1 L) by using a dialysis membrane (MWCO 500) for 5d to remove the excess reactants and salt. Finally, the pure product 10,10-NB-OA-P-OMe was obtained by lyophilization and the yield was 67.6%. ^{1}H NMR of 10,10-NB-OA-P-OMe was shown in Fig. S16. 10,10-NB-OA-P-CE was synthesized by the same method and the yield was 69.1%. ^{1}H NMR of 10,10-NB-OA-P-CE was shown in Fig. S17.

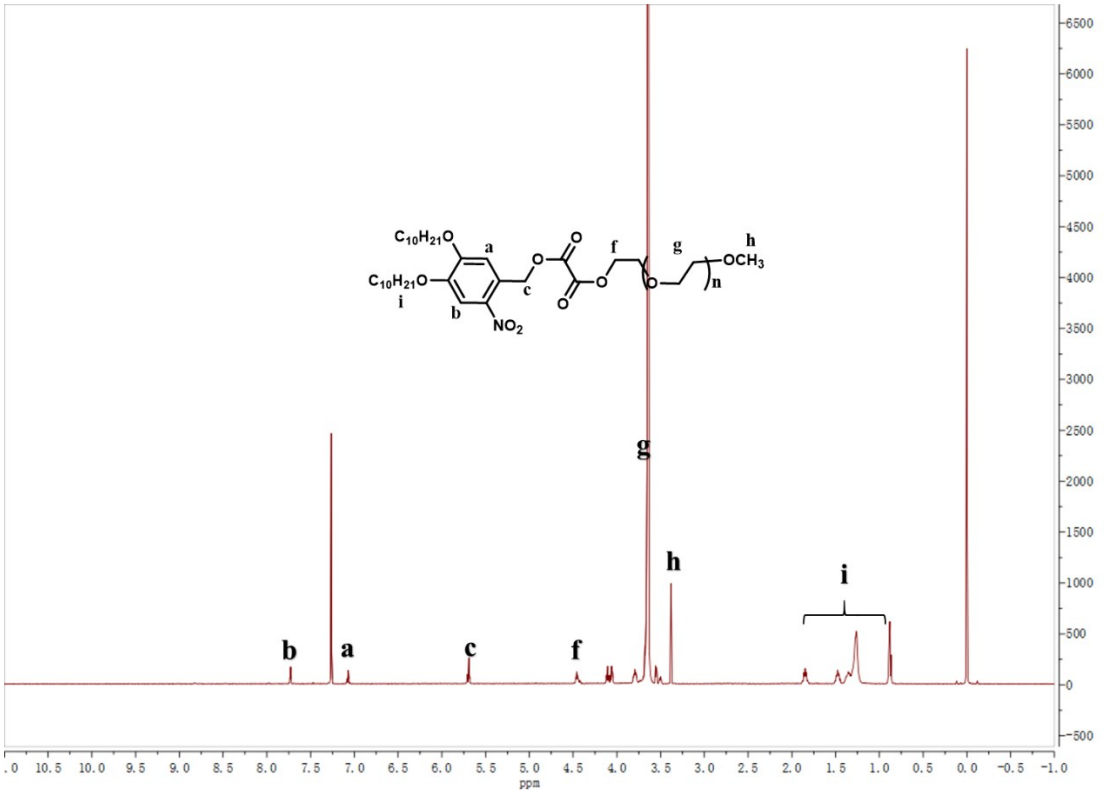

Fig. S16. ^{1}H NMR of 10,10-NB-OA-P-OMe

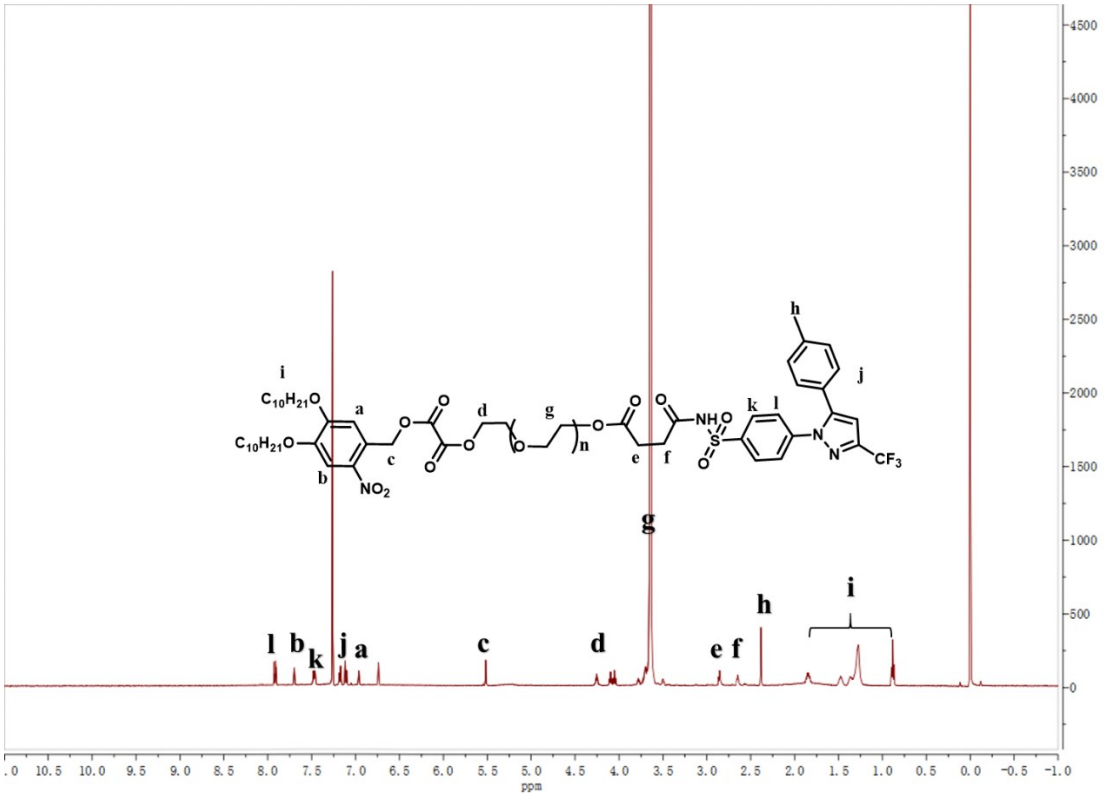

Fig. S17. ^{1}H NMR of 10,10-NB-OA-P-CE

Compound 8: 10,10-NB-S-S-P-OMe and 10,10-NB-S-S-P-AA

1 g of 10,10-NB-S-S-COOH, 0.3 g of EDCI and 3 mg of DMAP were dissolved in 30 mL of anhydrous chloroform and stirred for 4 h. 3 g of HO-PEG-OMe was added to the above reaction solution and stirred for 24 h. Then, the obtained mixture was washed with hydrochloric acid (0.1M) and saturated NaCl solution three times, and subsequently dried with anhydrous sodium sulfate. After filtration, the solvent was removed by rotary evaporation in vacuum. Finally, the pure product was obtained by silica gel column chromatography (dichloromethane: methanol=20:1) and the yield was 45.2%.^{1}H NMR of 10,10-NB-S-S-P-OMe was presented in Fig. S18. 10,10-NB-S-S-P-AA was synthesized by the same method and the yield was 61.2%. ^{1}H NMR of 10,10-NB-S-S-P-AA was shown in Fig. S19.

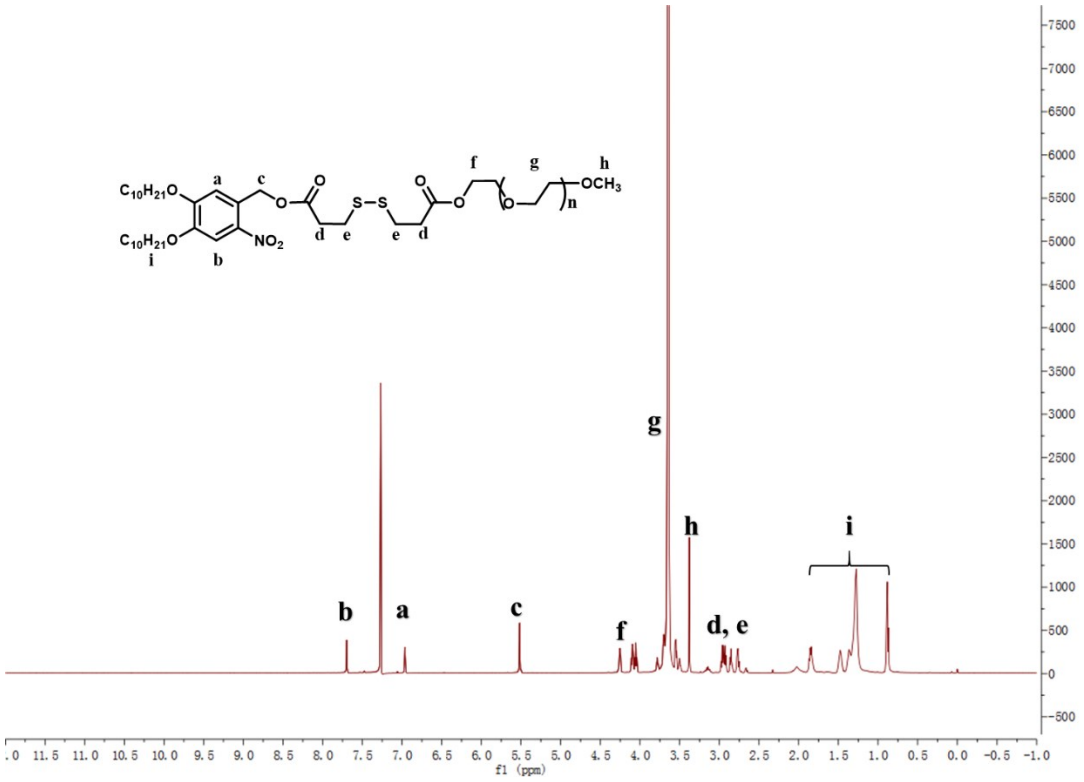

Fig. S18. ^{1}H NMR of 10,10-NB-S-S-P-OMe

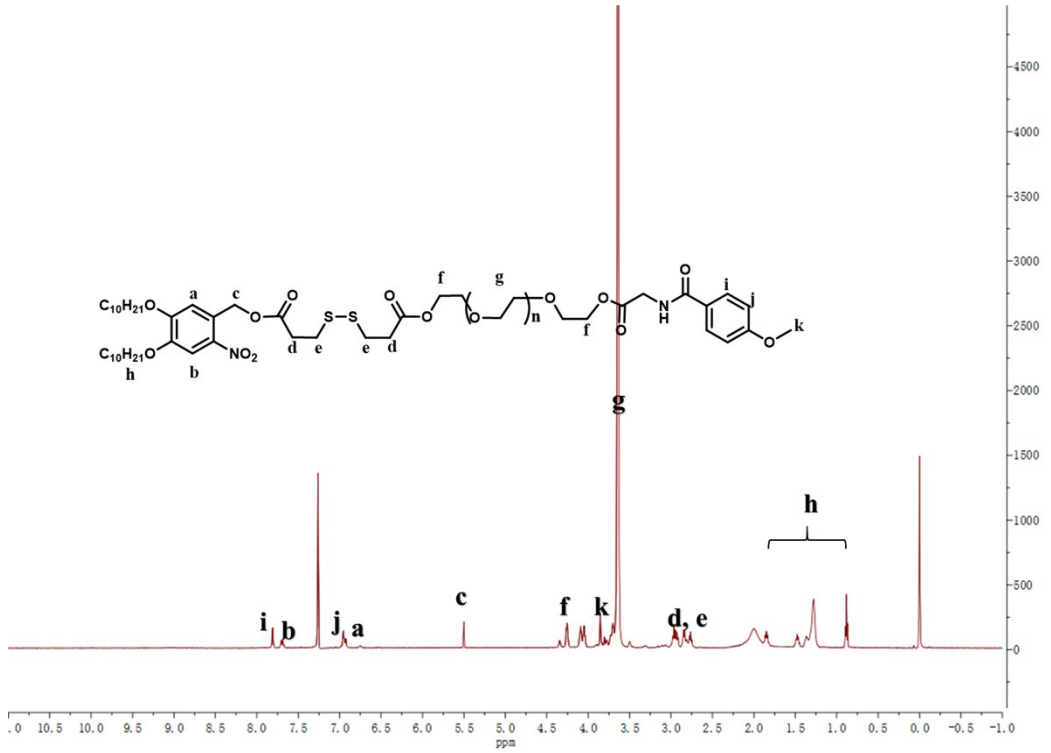

Fig. S19. ^{1}H NMR of 10,10-NB-S-S-P-AA

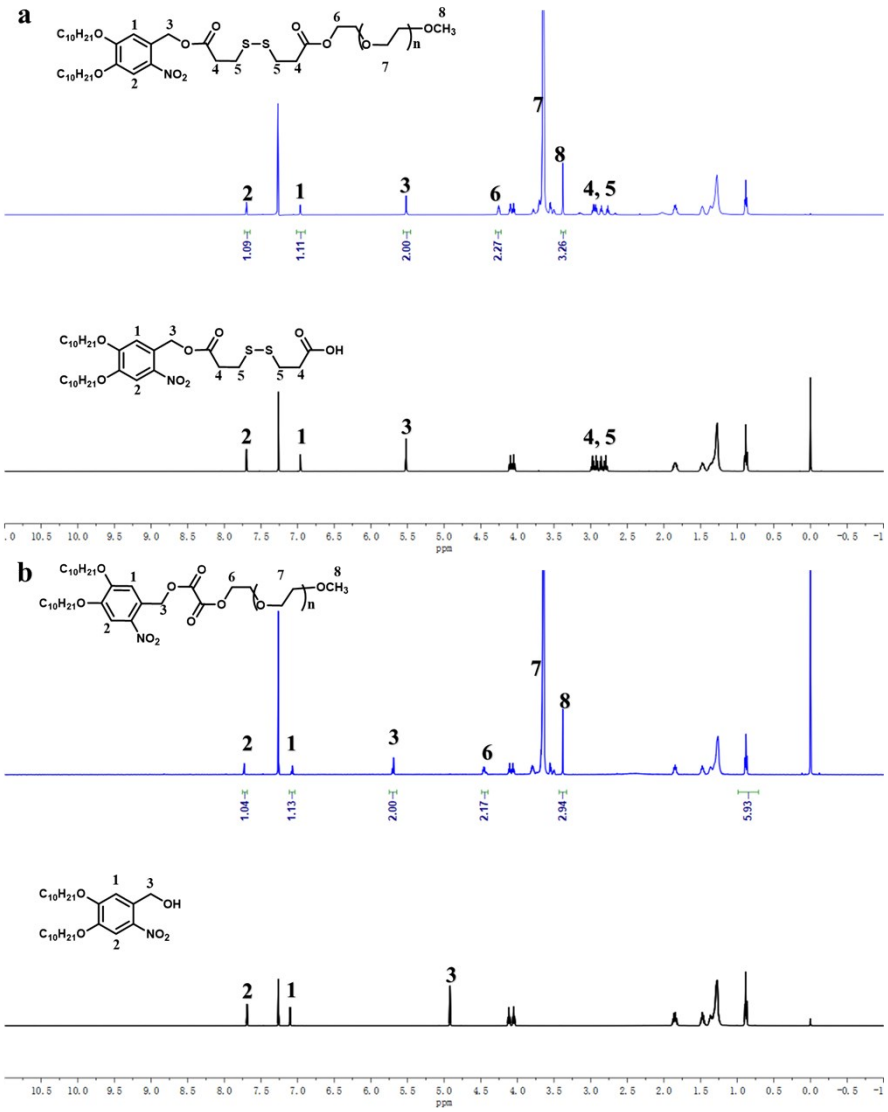

Fig. S20. ^{1}H NMR spectra of (a) 10,10-NB-S-S-P-OMe and (b) 10,10-NB-OA-P-OMe

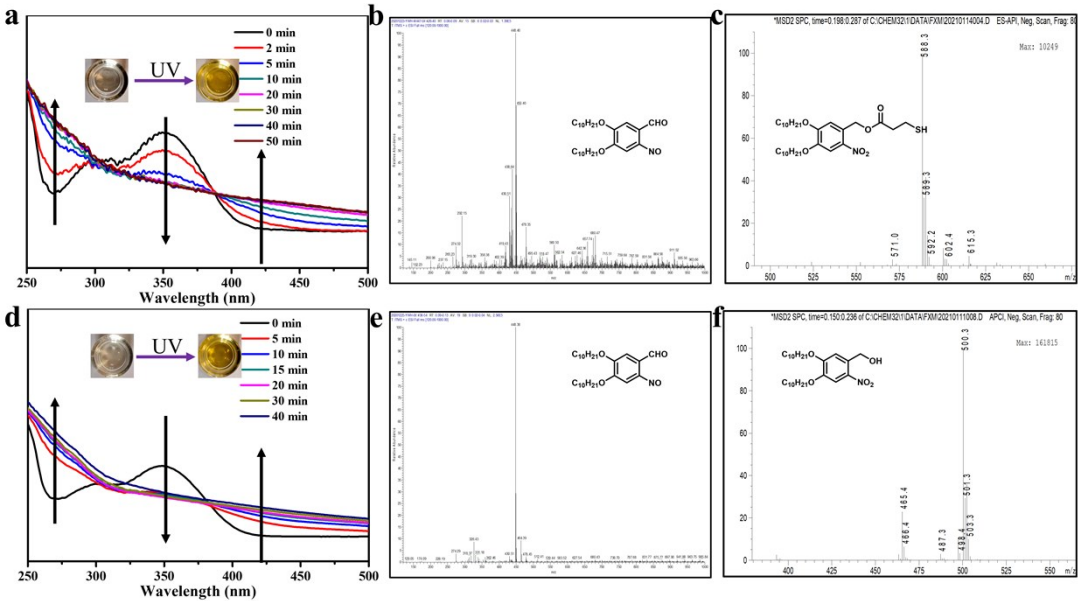

Fig. S21. Changes in UV-Vis spectra of (a) 10,10-NB-S-S-P-OMe and (c) 10,10-NB-OA-P-OMe

with different UV light irradiation time, MS of (b) 10,10-NB-S-S-P-OMe and (e) 10,10-NB-OA-P-OMe after UV light irradiation, MS of (c) 10,10-NB-S-S-P-OMe treated with TCEP and (f) 10,10-NB-OA-P-OMe treated with H_2O_2

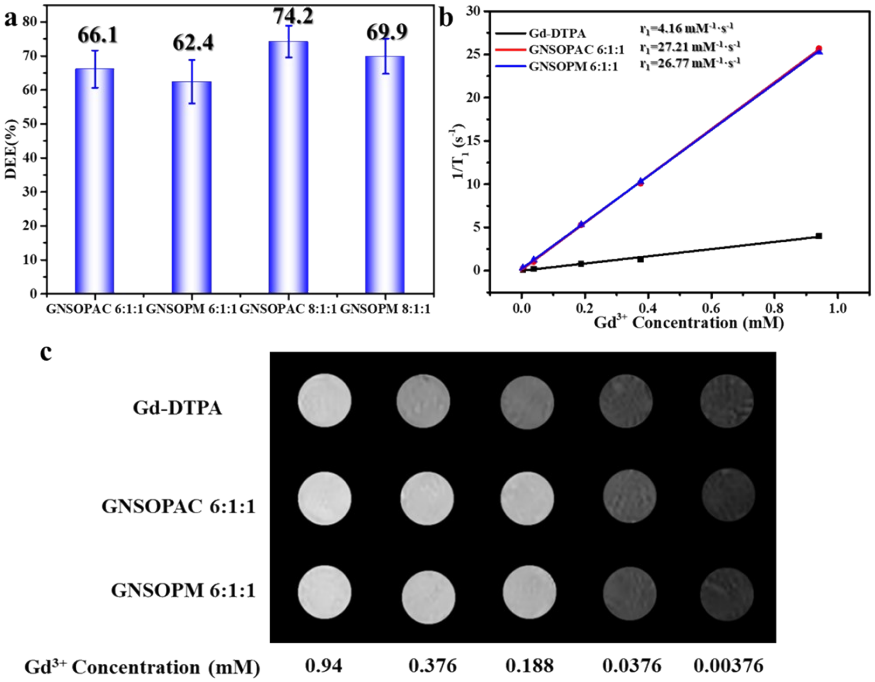

Fig. S22. (a) DEE of GNSOPAC and GNSOPM liposomes with different ratios (12,12-NB-DTPA-Gd: 10,10-NB-S-S-P-LA or 10,10-NB-S-S-P-OMe), (b) Longitudinal relaxation rate ($1/T_1$) as a function of Gd^{3+} concentration in different samples, (c) T_1-weighted MR images of different samples at different Gd^{3+} concentrations

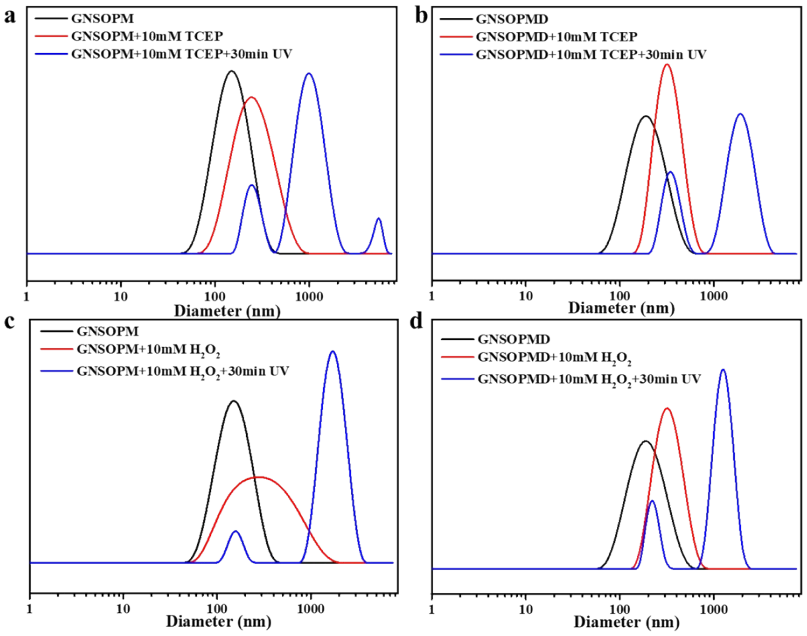

Fig. S23. Changes in diameter of (a, b) GNSOPM and (c, d) GNSOPMD liposomes after different treatments

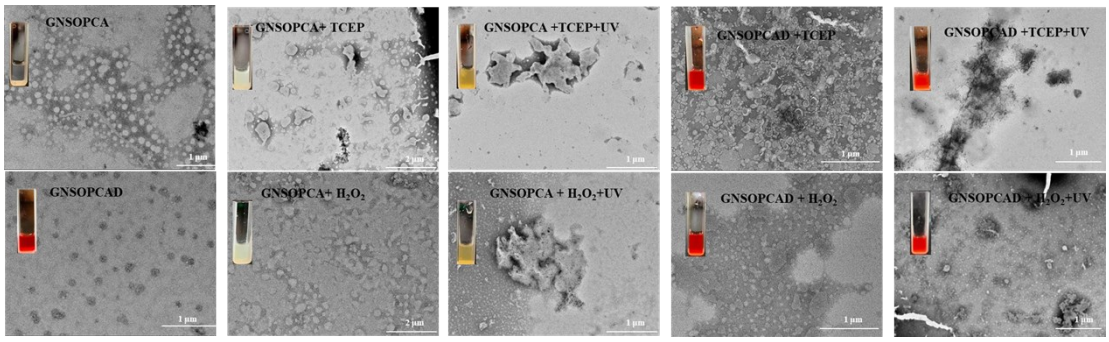

Fig. S24. Changes in TEM of GNSOPAC and GNSOPACD liposomes after different treatments

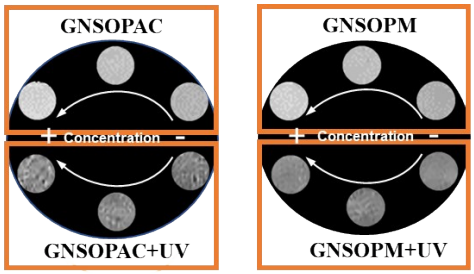

Fig. S25. Changes in T_1-weighted MR images of GNSOPAC and GNSOPM after UV irradiation

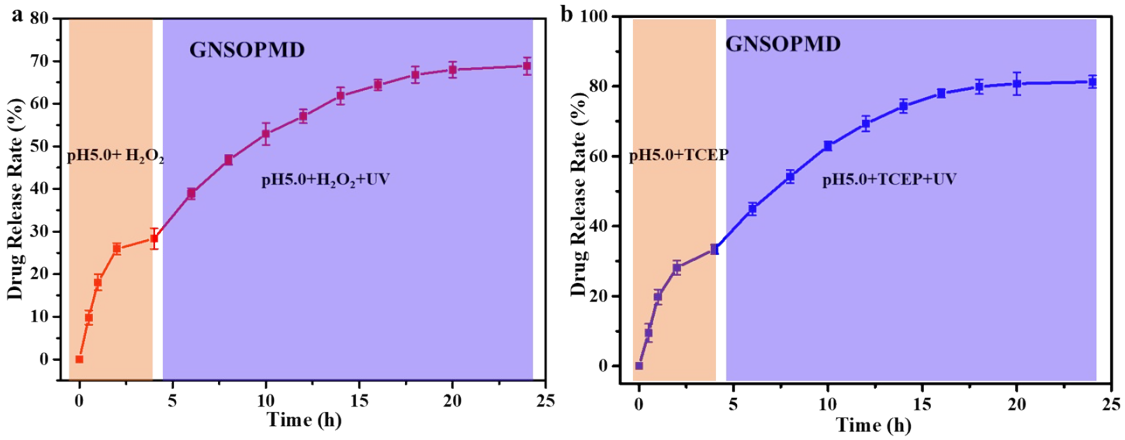

Fig. S26. Cumulative release profiles of GNSOPMD liposomes under stage-dependent treatments

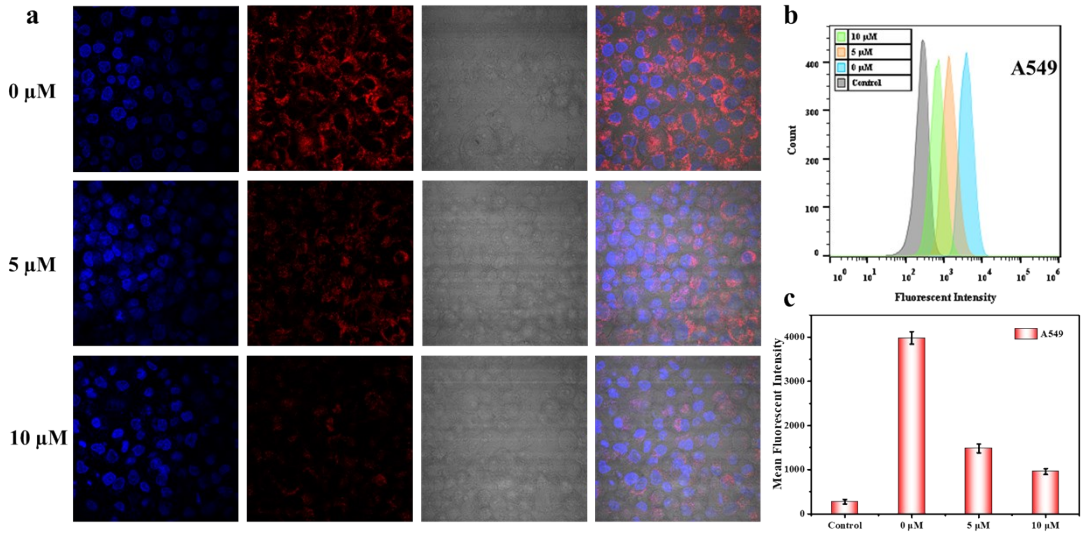

Fig. S27. (a) CLSM images, (b) flow cytometric profiles and (c) mean fluorescence intensity of A549 cells pretreated with or without free anisamide after 1 h incubation with GNSOPACD liposomes

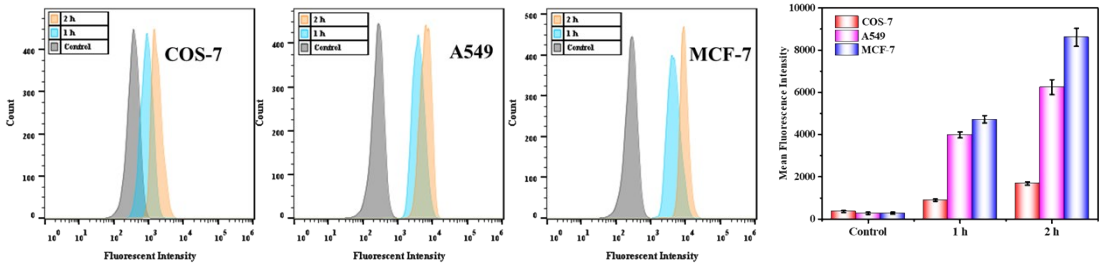

Fig. S28. Flow cytometric profiles and mean fluorescence intensity of COS-7, A549 and MCF-7 cells after 1 h incubation with GNSOPACD liposomes

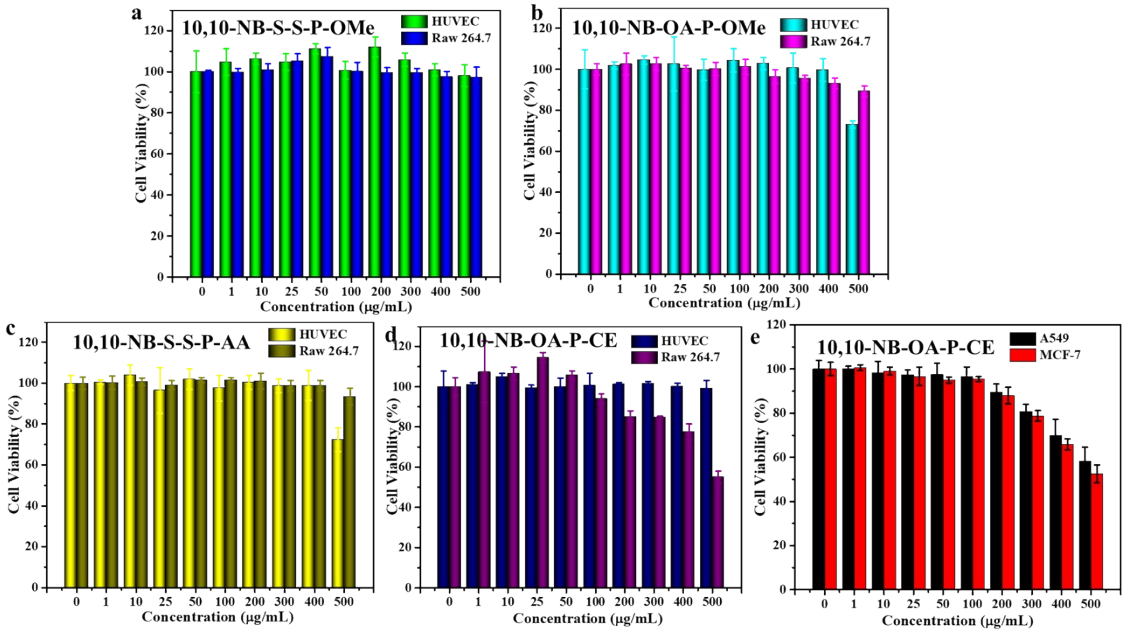

Fig. S29. The viability of HUVEC and Raw 264.7 cells incubated with (a) 10,10-NB-S-S-P-OMe, (b)10,10-NB-OA-P-OMe, (c) 10,10-NB-S-S-P-AA and (d) 10,10-NB-OA-P-CE at different concentration for 24 h. (e) The viability of A549 and MCF-7 cells incubated with10,10-NB-OA-P-CE at different concentration for 24 h

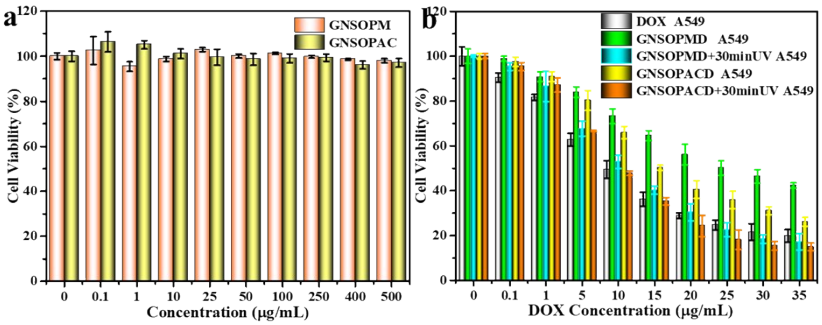

Fig. S30. The cell viability of A549 cells treated with (a) empty liposomes and (b) drug-loaded liposomes at various DOX concentrations for 24 h

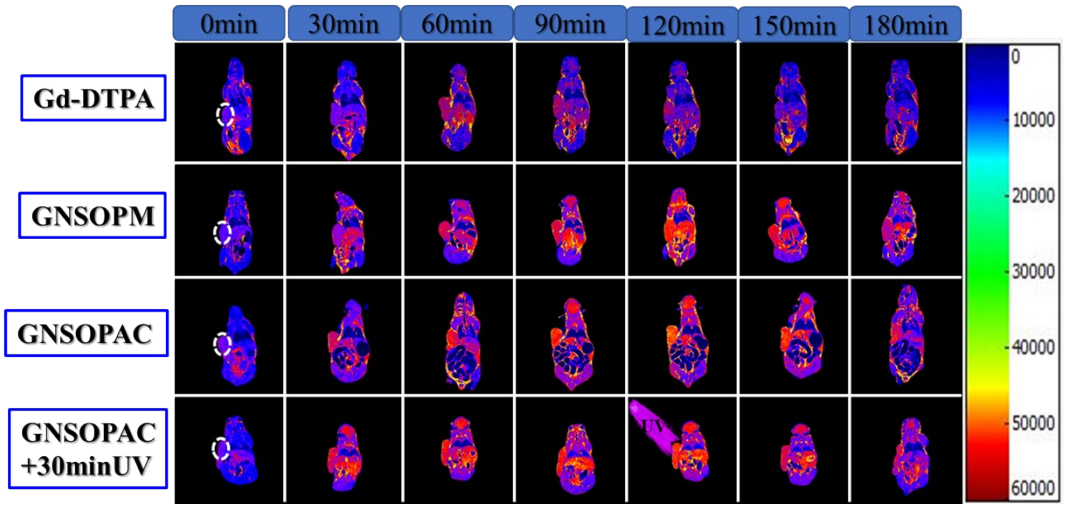

Fig. S31. *In vivo* MRI signal in a pseudo-color rainbow scale of nude mice bearing MCF-7 tumors at 0, 30, 60, 90, 120,150 and 180 min after intravenous injection of Gd-DTPA, GNSOPM and GNSOPAC liposomes (the dose of Gd^{3+} is 0.1 mmol/kg)

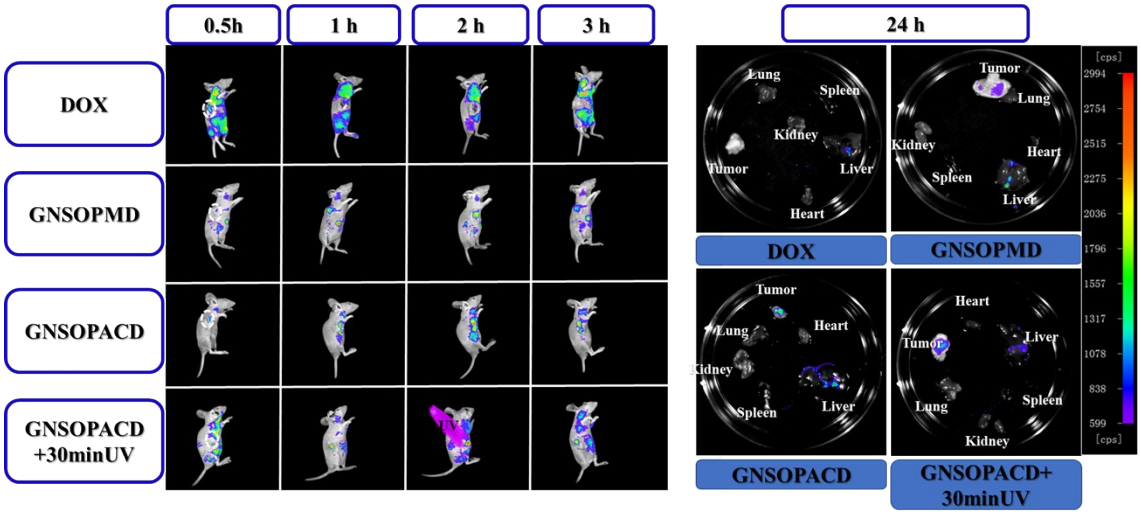

Fig. S32. *In vivo* biodistribution of nude mice after treatment with DOX, GNSOPMD, GNSOPACD and GNSOPACD+UV, and images of the major organs and tumors harvested at 24 h post-injection

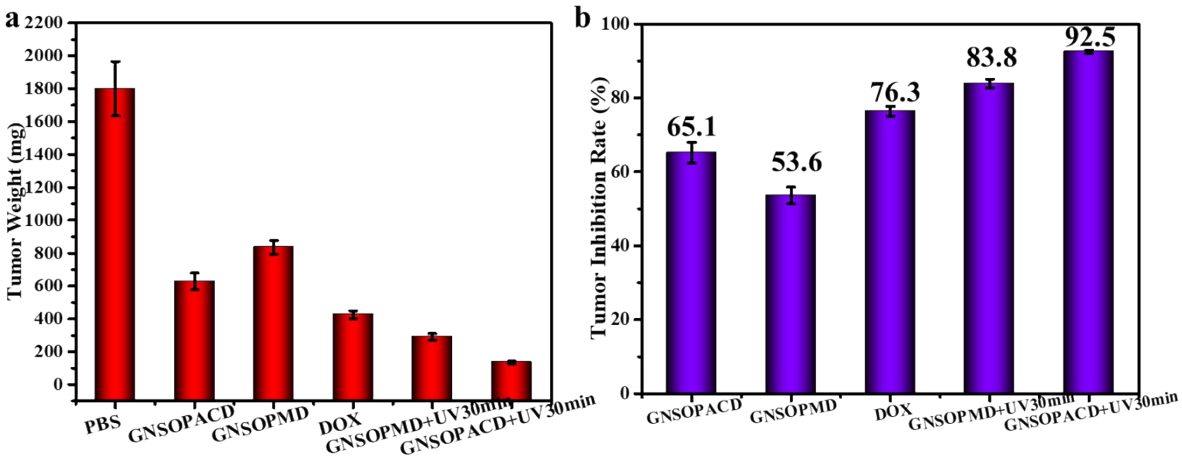

Fig. S33. (a) The weight of tumor tissue excised from nude mice treated with PBS, GNSOPMD, DOX, GNSOPACD, GNSOPMD +30 min UV and GNSOPACD +30 min UV after 30 days, (b) the tumor inhibition rate of different treatments after 30 days