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Cancer cell membrane targeting and red light-triggered carbon monoxide (CO) release for enhanced chemotherapy

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1. Materials and experimental procedure

1.1 Materials

Doxorubicin Hydrochloride (DOX) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China); Tetraethyl orthosilicate (TEOS, analytical grade) and cetyltrimethylammonium chloride (CTAC, 25 wt%) were purchased from Sinopharm Chemical Reagent Co., Ltd; 3-hydroxyflavone (3-HF) was obtained from Sigma-Aldrich LLC; Fetal bovine serum (FBS), RPMI-1640 cell culture medium, penicillin, and trypsin were bought from Gibco (USA); Annexin V-FITC/PI double staining cell apoptosis detection kit was got from Jiangsu KGI Biotechnology Co., Ltd (China); The bicinchoninic acid (BCA) protein detection kit, membrane protein extraction kit and phosphate buffer solution (PBS) were gained from Shanghai Shenggong Biotechnology Co., Ltd. (Shanghai, China); Thiazole Blue (MTT) and Hoechst 33324 were achieved from Gelest, Inc.

Human liver cancer cells (HepG2), human breast cancer cells (MCF-7), human lung cancer cells (A549), human normal hepatocytes (HL7702) and human cervical cancer cell (HeLa) cell lines were obtained from the cell bank of the Shanghai Institute of Cells (Chinese Academy of Sciences, China).

Balb/c mice (female, 18±2g), 4-5 weeks old, were got from Shanghai Lingchang Biological Technology Co., Ltd. (Shanghai, China), Animal Research Center is set up under standard conditions including a 25±2°C environmental temperature, a 60%±10% humidity, a light/dark cycle interval of 12 h, and an SPF barrier environment with IVC cages. The approval number of laboratory animal welfare ethics is IACUC-001-17. For all animal studies, mice were randomly assigned to each group.

1.2 Instruments and equipment

The instruments used here include as following: ZEN3600 dynamic light scattering analyzer (Malvern Instruments Limited, UK); UV-1750 ultraviolet spectrophotometer (Pekin Elmer, USA); FV1000MPE two-photon laser confocal microscope (Olympus Corporation, Japan); JEM-200CX perspective electron microscope (JEOL, Japan); Free-Zone freeze dryer (LABCONCO, USA); CR22GⅢ high-speed refrigerated centrifuge (Hitachi, Japan); H2050R-1 centrifuge (Hunan

Xiangyi Technology Co., Ltd., China); ELX800 microplate reader (Biotech, USA); Direct-Q 3UV ultrapure water preparation instrument (Shanghai Merck Chemical Technology Co., Ltd., China). ^{1}H NMR spectra were obtained on a Bruker AVANCE III 500 spectrometer (Germany) and all data was obtained as referenced to TMS (an internal standard). Mass spectrum of final product was achieved with a LCQ Fleet mass spectrometer (USA). The quantum yields of CO released from 3-HF were obtained from FLS980 steady-state/transient fluorescence spectrometer (Edinburgh, England). The excitation wavelength is 350 nm, and the scanning range is 200-600 nm. As the light source illuminates the sample, the sample emits fluorescence. According to the equation: $QY=\frac{1.2\times10^{8}(v\times K)}{I\times A\times\lambda}\times100\%$. Here, v represents reaction rate (mol·s^{-1}); K is number of reaction transferred electrons (K=2); I represents optical power density (W·m^{-2}); A is the area of incident light (m^2); λ is the wavelength of incident light.[1] The quantum yields were automatically achieved by the calculation via the software of the instrument.

A JEM-2010 transmission electron microscope (TEM) was used to measure the structure and particle size of the samples. A semiconductor laser with a wavelength of 980 nm was used as the excitation light source. The Hitachi F-4500 spectrophotometer was used to measure the up-conversion emission spectra of the samples.

Waters 1525 HPLC (Waters Company, USA) equipped with Vydac C18 was used to separation and analysis 3-HF and the products generated upon UCNP-induced CO release from 3-HF. The detailed conditions are as following. Eluant: A is methanol, B is water with 10% acetic acid and the ratio of A and B was changed from 3/7, 9/1 to 3/7; Velocity: mL/min; Detection wavelength: 245 nm; Column temperature: 25 ^{0}C; Injection volume: 100 μL.

1.3 Incubation and collection of cells

HepG2 cells were cultured in RPMI-1640 basal medium (RPMI-1640: FBS: penicillin=89:10:1), under the cultural environment of 37^{o}C, 5% CO_2. and full humidity. When the confluence of the cells reaches 80% or more, they are digested with 0.25% trypsin solution. The digested cells were precipitated by centrifugation at 1000 g for 3 min and washed with PBS solution for 3 times, then the precipitate was collected for further application.

1.4 Preparation of red blood cell membranes

3 mL blood was got from 3 Kunming mouse by using eyeball blood collection method. The

blood was collected in a 10 ml centrifuge tube containing 0.5 mL of sodium citrate solution with a mass fraction of 4% as an anticoagulant. The red blood cells were re-suspended in pre-cooled isotonic phosphate buffered saline (PBS, pH=7.4), and then centrifuged and washed for 3 times at 4°C to remove the serum. The washed red blood cells were put in a pre-cooled 0.2×PBS hypotonic buffer for hemolysis for 50 min, and then centrifuged at a high-speed centrifuge (4°C, 12000 r/min) for 10 min to precipitate the red blood cell membrane and remove hemoglobin. The centrifugal washing was repeated for 3 times. The pink red blood cell membrane on the lower layer was collected and selected with 1×PBS for later use.

1.5 Preparation of cancer cell membranes

The detailed preparation of cancer cell membranes was according to the instruction of the membrane protein extraction kit. HepG2 cells were cultivated and then collected by centrifugation. The cells were washed with a solution diluted by pre-cooled double-distilled water for three times. Centrifugation was performed at 800 g for 5 min and the precipitate was suspended in membrane protein extraction buffer to which 1 mL protease inhibitor, 1 mL dithiothreitol (DTT) and 1 mL extraction buffer were added. The cells were disrupted by ultrasonic for 30 s each time and 4 times in total. After each ultrasonic, the sample was placed on ice bath to cool for 1 min. According to the ultrasonic microscopy, the cell disruption rate is not less than 90%. The cells were treated at 4°C with 17500 g centrifugation for 10 min, and the supernatant was collected in a clean tube. The cells were put in 37°C water bath for 10 min, followed by centrifugation at room temperature with 17000 g for 10 min. The lower sediment was collected, then washed with pre-cooled sterile water. The sample was centrifuged and collected. The concentration of the extracted cell membrane was determined by using the bicinchoninic acid (BCA) protein detection kit. The obtained cell membrane was placed in the refrigerator at -80°C for later use.

1.6 Preparation of mesoporous silica nanoparticles

Mesoporous silica nanoparticles were prepared as follows: The SiO_2 template was synthesized in the first step. Specifically, 35.7 mL of ethanol, 5 mL of ultrapure water and 0.8 mL of ammonia were mixed and the resulting solution was stirred at 30°C for 10 min. 1 mL tetraethyl orthosilicate (TEOS) was added dropwise to above solution. The solution was stirred for 90 min to form milky white SiO_2. The SiO_2 was washed with water and re-suspended in 20 mL ultrapure water for later

use. SiO_2@MSN was synthesized in the second step. Specifically, 2 g cetyltrimethylammonium bromide (CTAB), 20 mg TEA, and 10 mL SiO_2 solution were dissolved in 20 mL ultrapure water. The solution was stirred at room temperature for 60 min. The solution was raised to 30°C by using a water bath. and then 0.15 mL of TEOS was added to stir for 60 min. In the third step, the temperature of the above-mentioned mixed solution was cooled to 50°C, and then 636 mg of Na_2CO_3 was added to react for 90 min. The precipitate was collected by centrifugation, and washed with 1 wt% HCl at room temperature to remove CTAB. The precipitate was re-suspended in water, washed with water for three times. Finally, the sample was dried in vacuum to obtain ordered mesoporous silica nanoparticles.

1.7 Preparation of DOX/3HF@MSN

10 mg nanoparticles were dispersed in 10 mL DOX aqueous solution (1 mg/mL) to stir at room temperature without exposure to light. After 24 h, the solution was centrifuged and washed with phosphate buffered solution (PBS, pH=7.4) for three times. The sample DOX@HMSNs was collected. The loading amount was calculated according to the UV-Vis absorption. 10 g dry DOX@HMSN and 10 mg 3-HF were dispersed in 10 mL ethanol solution to react for 24 h at room temperature without exposure to light. The nanoparticles were collected by centrifugation, and washed with ultrapure water for 3 times to obtain DOX/3HF@HMSN. According to the UV-Vis absorption of the sample, the loading capacity was calculated.

1.8 Preparation of bionic cell membrane nanoparticles

The preparation of drug-loaded bionic nanoparticles RBC@HepG2-M-DOX/3HF@MSN (termed as RHM) was according to the previous report.[2] In short, the collected erythrocyte membrane and HepG2 cell membrane were mixed with DOX/3HF@MSN under ultrasound. Subsequently, an Avanti micro extruder was used to extrude the mixture through a 400 nm × 200 nm polycarbonate porous membrane for 20 times. The solution was centrifuged to remove excess vesicles.

1.9 Characterization of nanoparticles

The nanoparticles before and after being wrapped with the cell membrane were characterized. The particle size and potential of the nanoparticles were measured by DLS, their morphology was observed by TEM, and the surface proteins of the nanoparticles were characterized by SDS-PAGE

electrophoresis.

2.0 Synthesis and characterization of up-conversion nanoparticles

Synthesis of $NaYF_4$: Tm, Yb Core UCNPs: The synthesis of up-conversion nanoparticles was according to relevant literature.[4] In short, $Y(AC)_3$ (0.695 mmol), $Yb(AC)_3$ (0.300 mmol), and $Tm(AC)_3$ (0.005 mmol) (the total mole of rare earth ions was fixed to 1 mmol), 6 mL of OA (oleic acid) and 15 mL ODE (octadecene) were separately added to a 50 mL double neck round bottom flask. Among these, OA is a stabilizer and ODE is a solvent. The mixed solution was heated to 150°C under vacuum and stirred for 30 min. When the solution was cooled to about 50 °C, 10 mL methanol solution containing 2.5 mmol NaOH and 4 mmol NH_4F was added and mixed for 10 min. The mixture was then degassed and heated up to 290°C. The reaction condition was maintained for 1.5 h. Subsequently, the solution was naturally cooled to room temperature. The same amount of ethanol was added. The solution was centrifuged at 7000 g for 10 min. The supernatant was discarded, and the precipitate was washed with a mixture of ethanol and cyclohexane (1:1) for three times. The solution was centrifuged at 6000 g for 10 min. The sample was dissolved in 2 mL of cyclohexane for later use.

Synthesis of $NaYF_4$: Tm, Yb@$NaYF_4$ Core/shell-UCNPs: 1 mmol $Y(AC)_3$ was added to a 50 mL double-necked round bottom flask, and then 6 mL of OA (oleic acid) and 15 mL of ODE were added. The resulting solution was heated to 150°C under vacuum, and stirred for 30 min, then cooled to room temperature. The UCNPs core prepared above in cyclohexane solution (2 mL) was added into a round bottom flask, and heated to 80°C to remove cyclohexane. After cooling to room temperature, 10 mL methanol solution containing 2.5 mmol NaOH and 4 mmol NH_4F was added to the flask. The solution was stirred and mixed for 30 min. The solution temperature is raised to 100°C, to remove the methanol. Under nitrogen atmosphere, the sample temperature was further increased to 290°C and kept for 1.5 h. After the temperature cooled to room temperature, the same amount of ethanol was added, and centrifuged at 7000 g for 10 min. The supernatant was discarded, and precipitate was washed with a mixture of ethanol and cyclohexane (1:1) for three times. The centrifugation was performed at 6000 g for 10 min to obtain $NaYF_4$: Tm, Yb@$NaYF_4$.

Characterization of up-conversion nanoparticles: The $NaYF_4$ shell layer was deposited on the core to obtain core/shell-featured UCNPs, which compensates for surface defects and improves

the optical properties of UCNPs. The particle diameter of synthetic UCNPs is about 25 nm (Figure S9B). Under 980 nm laser irradiation, the UCNPs with core-shell structure exhibited four emission bands at 345, 355, 455 and 475 nm. The intensity is about 3-fold that of core-UCNPs (Figure S9C).

Preparation of ligand-free nanoparticles: The oleic acid-encapsulated nanoparticles prepared above were dispersed in 2 mL of HCl solution (0.1 M), and sonicated for 15 min to remove surface ligands. The nanoparticles were then collected by centrifugation at 7500 g for 20 min. The further purified was performed by adding an acidic ethanol solution (pH 4) that was prepared by adding 0.1 M HCl aqueous solution to absolute ethanol. The resulting product was washed several times with ethanol and deionized water, and then dispersed in deionized water.

2.1 Determination of drug loading and encapsulation efficiency of RHM

A standard stock solution (100 μg/mL) of DOX was prepared in deionized water. The absorption values at 480 nm of the DOX solutions with concentrations of 0.06, 0.12, 0.6, 1.2, 1.8, and 2.4 μg/ml were carried out to obtain the linear fitting relationship between the absorption at 480 nm and DOX concentration. The standard stock solutions of 3-HF with the concentrations of 0.05, 0.1, 0.5, 1, 1.5, and 24 μg/mL in ethanol were prepared. The absorption values of these solutions at 344 nm were determined. The linear fitting curve between the absorption at 344 nm and 3-HF concentration was achieved for the determination of the concentration of 3-HF solution.

The drug loading (DL) and encapsulation efficiency (EE) of the drug-loaded nanoparticles were determined by the standard curve method. The drug-loaded nanoparticle RHM solution was centrifuged at 15,000 r/min for 10 min to collect unloaded 3-HF or DOX in the supernatant. The absorbance value of free 3-HF at 344 nm or free DOX at 480 nm was measured by UV-Vis spectrophotometer. According to the standard curve, the concentration of free 3-HF or DOX was calculated. The encapsulation efficiency and drug loading were calculated according to the following equation: Encapsulation efficiency (%) = (injection amount- free drug amount) /dosage ×100%, drug loading amount (%) = (Sample amount- free drug amount) /(Sample amount + material amount) ×100%.

2.2 Determination of the concentration of 3-HF in DOX/3HF@MSN

3-HF ethanol solutions with 0.05, 0.1, 0.5, 1, 1.5, 24 μg/mL were prepared and the absorbance of these solutions at 344 nm was measured. The standard curve of the concentration (X axis) of 3-

HF to the absorbance (Y axis) is obtained as Y=0.0803X-0.0014 and R^2=0.9997.

DOX@MSN were dispersed in ethanol solution, then 10 mg 3-HF was added to above solution. The resulting solution was stirred at room temperature under dark for 24 h, and then centrifuged at 1000 r/min for 5 min to remove un-encapsulated 3-HF. The precipitation (DOX/3HF@MSN) was weighted after freeze drying. The concentration and quantity of 3-HF in supernatant was achieved according to the standard curve and the volume of supernatant. The amount of 3-HF loaded in DOX/3HF@MSN can be calculated by subtracting the remaining amount of 3-HF in supernatant from total 10 mg 3-HF added. According to the weight percentage of 3-HF in DOX/3HF@MSN and the prepared concentration of DOX/3HF@MSN, the concentration of 3-HF in DOX/3HF@MSN is determined.

2.3 In vitro release detection

The dialysis bag-based method was used to evaluate the in vitro 3-HF or DOX release ability. The drug-loaded nanospheres RHM (corresponding to 2 mg DOX) prepared above were dispersed in 5 mL of deionized water. The solution was placed in a pre-treated dialysis bag. The molecular weight cut-off of the dialysis bag was 12,000. The whole dialysis bag was immersed in 20 mL PBS medium with pH 7.4 or 5.0. The in vitro release was investigated under constant shaking (100 r/min) at 37°C. The absorbance of the solution with the releasing 3-HF or DOX at each time point (0, 2, 4, 6, 8, 10, 12, and 24 h) was detected to calculate the release rate at different times, 3 samples in parallel were prepared for each group. The average value was used to get the release curve.

2.4 Investigation on the Cytotoxicity of Bionic Nanoparticles

HepG2 cells in logarithmic growth phase were used. 0.25% mass fraction of HepG2 cells were digested into a single cell suspension to seed in a 96-well plate at a cell density of 1×10^4 cells/mL and 100 μL/well. After the cells were cultured for 24 h, the medium was aspirated and the drugs with different concentration gradient were added. All cells were divided into 6 groups for control experiments, namely PBS, MSN, DOX, DOX@MSN, DOX/3HF@MSN, RHM, RHM+UCNPs. The final concentration of DOX was 2 μg/mL. The irradiation of RHM+UCNPs group was performed with laser (980 nm, 1.0 W/cm^2) for 5 min. After treatment for 24 h, 20 μL MTT (5 g/L) was added to each well to further culture for 4 h. The supernatant was removed, and then 150 μL

of dimethyl sulfoxide was added. After the solution was shaken for 10 min, the absorbance of the solution at 570 nm was measured to calculate the cell survival rate.

At the same time, the anti-tumor cells effect of nanoparticles was analyzed by laser confocal microscopes. HepG2 cells were incubated in a confocal culture dish for 24 h, then the medium was carefully aspirated. DOX, 3-HF, MSN, and DOX@MSN, DOX/3HF@MSN, RHM, RHM+UCNPs nanoparticles were separately added and kept for 4 h. The final concentrations of doxorubicin in DOX@MSN, DOX/3HF@MSN, RHM, RHM+UCNPs nanoparticles are 4 μmol/L. Subsequently, 1 μL Annexin V-FITC was added to mix well. 0.5 μL propidium iodide (PI) was added to incubate in the dark for 20 min, then the cells were washed with PBS buffer twice to remove excess dye. The cells were observed with a confocal laser microscope.

2.5 Uptake experiment of biomimetic nanoparticles

A qualitative analysis of the uptake of nanoparticles was carried out using a confocal laser microscope (CLSM). HepG2, HL7702, HeLa, MCF-7, A549 cells were incubated in a 35 mm Petri dish. The cells were cultured for 24 h to ensure them to be adhered to the wall. Then 2.5 μg/mL RHM was added to the culture solution to incubate at 37°C for 4 h. After the medium was removed, the cells were washed with PBS for three times. After the medium containing 10 μg/mL Hoechst 33342 was added to stain for 15 min, the cells were washed twice with PBS to remove excess dye. The uptake of nanoparticles was observed with CLSM.

Flow cytometry was used to quantitatively analyze the uptake of nanoparticles. The cells were cultured according to the above steps and then the drugs were added to incubate for 4 h. The cells were washed with PBS twice to remove the DOX that cannot enter into the cells. The cells were digested, collected, and filtered through a nylon mesh (300 mesh), and quantitatively analyzed with a flow cytometer (BD FACS Aria™ III, USA). Flow-jo software was used for data analysis.

2.6 Verification of CO release from biomimetic nanoparticles

The release of CO in vitro is detected by deoxy-myoglobin, because CO and deoxy-myoglobin have a strong binding ability. The binding process can be tracked and detected by a UV-Vis absorption. 10 mg myoglobin were dissolved in 10 mL of PBS solution, then 0.1 mg sodium dithionite was added to mix. Sodium dithionite can reduce myoglobin to deoxy-myoglobin. Nitrogen was added to remove air. 2 mL deoxy-myoglobin solution was pipetted into a cuvette,

then 20 μL nanoparticles (10 mmol/L) was added. The solution was filled with nitrogen gas, and detected by UV-Vis absorption.

The cellular level of CO is detected by the probe FL-CO-1 (Figure S2). The synthesis of the probe is based on previous literature[3] and verified by NMR spectrum. First, HepG2 cells and 5 μg/mL RHM+ up-conversion nanoparticles (10 μM) in PBS were co-cultured for 4 h, then the cells were irradiated with laser (980 nm, 1.0 W/cm^2) or without light treatment for 10 min. The living cells were incubated with a mixture of FL-CO-1 (1 μM) and $PdCl_2$ (1 μM) at 37^{o}C for 30 min. The PBS group was served as a control. The cells were washed with PBS for three times and imaged by CLSM.

2.7 ROS generation of biomimetic nanoparticles

The ROS sensitive probe 2', 7'-dichlorofluorescein diacetate (DCFH-DA) was used as an indicator of intracellular ROS production. HepG2 cells were incubated with 5 μg/mL RHM+ up-conversion nanoparticles (10 μM) in PBS for 4 h. Cells were irradiated with laser (980 nm, 1.0 W/cm^2) or without light treatment for 10 min. Then the cells were incubated with DCFH-DA (10 μM) for 30 min, and finally observed by CLSM.

2.8 Establishment of tumor-bearing mouse model

The human hepatocarcinoma HepG2 cells suspension was collected at a concentration of 1×10^7 cells/Ml. 0.1 mL cell solution was incubated subcutaneously into the right axillary of nude mice. The incubated white mice were placed in an independent ventilated cage system to closely observe the growth of the tumor at the incubated site. If the tumor cells are successfully implanted, there would be mass-like changes.

2.9 Measurement of outcome

The equation for the calculation of tumor volume is: $TV=1/2\times A\times B^2$. Where A represents the measurement of the long diameter of the tumor, and B represents the measurement of the short diameter of the tumor.

The equation for the calculation of relative tumor volume is: $RTV=V_t/V_0$. Wherein V_0 is the tumor volume measured in caged administration (i.e. d_0), and V_t is the tumor volume at each measurement.

Index for evaluation of anti-tumor activity is relative tumor proliferation rate T/C (%). The

equation for the calculation is: T/C (%) = (T_{RTV}/C_{RTV}) × 100%. Here T_{RTV} represents RTV in the treatment group, C_{RTV} represents RTV in the model group.

The tumor growth inhibition rate (%): tumor growth inhibition rate = (average tumor weight of the model group- average tumor weight of the administration group)/average tumor weight of the model group) × 100%.

2.10 In vivo experiments

In vivo imaging: A small animal live imaging system (IVIS® Spectrum, PerkinElmer) is used to verify the distribution of nanoparticles in animals. Fifteen mice were randomly divided into 3 groups, and 200 μL of DOX and RHM nanoparticles were injected into mice bearing tumor through the tail vein, and the DOX concentration was 5 mg/mL. The distribution of nanoparticles in tumor-bearing mice was tested at different time points after injection.

Drug distribution in vivo: PBS (100 μL), DOX (100 μL, 0.5 mg/mL) and RHM (100 μL, 5 mg/mL) were injected through the tail vein. After 5 h, the mice were euthanized, and the main organs and tumor tissues were dissected and separated. The near infrared fluorescence system was used to image them.

Evaluation after in vivo treatment: After the tumor grew up to 100 cm^3, they were randomly divided into 4 groups (7 in each group). The tail vein was injected with PBS (100 μL), DOX (100 μL, 0.5 mg/mL), RHM (100 μL, 5 mg/mL) and RHM (100 μL, 5 mg/mL) + UCNPs (20 μL). After 2 h of the injection, the mice were anesthetized and the tumor was exposed to a laser with a wavelength of 980 nm at 1 W/cm^2 for 10 min. All tumor-bearing mice were administered and weighed every two days. At the end of the experiment on the 16th day, the mice were euthanized and dissected to separate their main organs and tumor tissues.

Evaluation of animal survival time: For evaluating the impact of nanoparticles on the survival time of mice, we recorded the survival time of nude mice in different treatment groups. First, the mice were divided into four groups (6 mice in each group), and they were feed with PBS (100 μL), DOX (100 μL, 0.5 mg/mL), RHM (100 μL, 5 mg/mL), and RHM (100 μL, 5 mg/mL) + UCNPs (20 μL). The process was administered once every two days, and the treatment time was 30 days. The survival time of the mice was recorded.

Histopathological detection: After the treatment, the mice were euthanized, and the main

organs, heart, liver, spleen, lung, kidney, and tumor tissues were dissected and collected, and stained with hematoxylin and eosin (HE). Each tissue was sectioned and observed.

Statistical treatment: At least three independent sample replicates were performed for each experiment, the mean value was represented by X±SD, the analysis between groups was statistically processed by t-test, and the results were statistically analyzed by SPSS (Staffstical Package for the Social Science) 17.0.

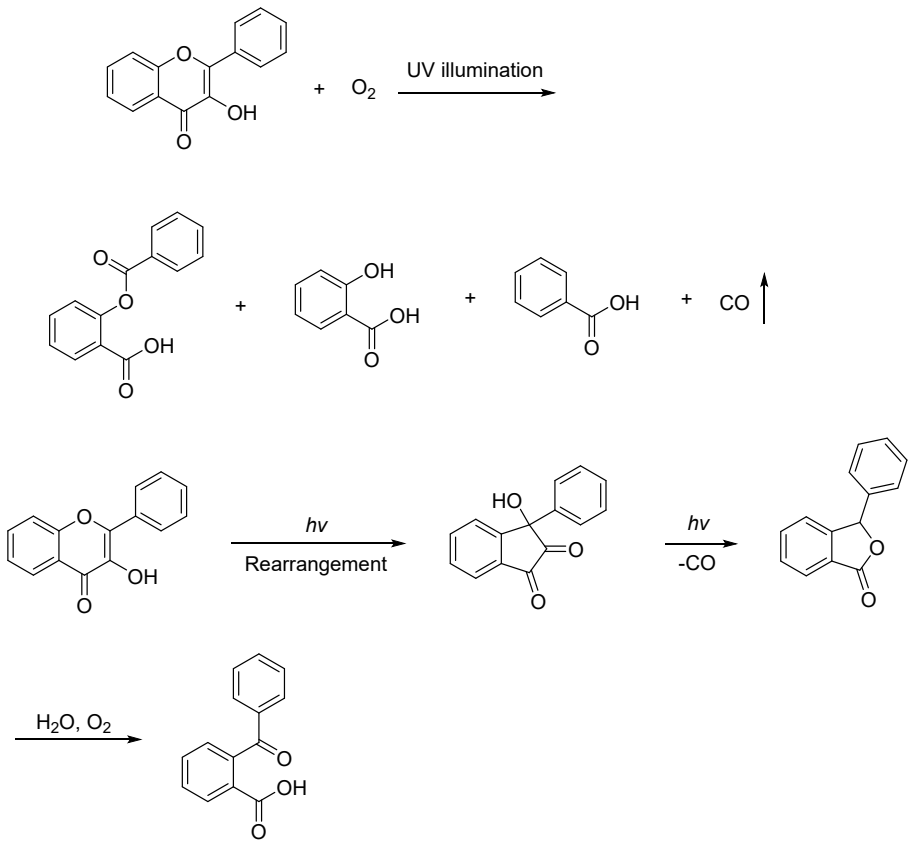
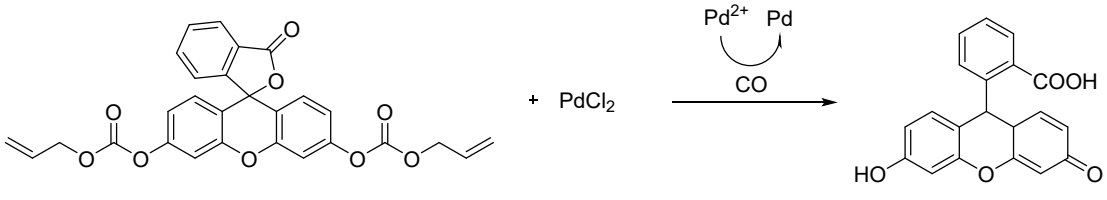


Figure S1. CO-release reactivity of 3-hydroxyflavone (3-HF) under UV-light-induced illumination.



FL-CO-1

Non fluorescent

Fluorescein

Highly fluorescent

Figure S2. The reaction of FL-CO-1 and $PdCl_2$ with CO for the detection of CO.

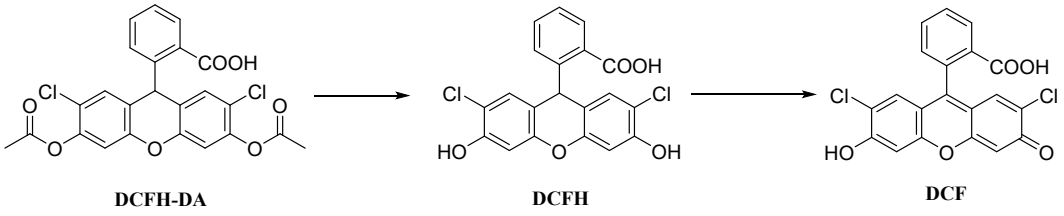


Figure S3. Reaction of DCFH-DA with ROS.

The synthetic MSN is regular and spherical structure, which can be seen from TEM spectrum (Fig. S4). DOX and 3-HF were co-loaded into the MSN through physical adsorption and hydrogen bond interactions. In FTIR spectra (Fig. S5), the absorption peak at 468 cm^{-1} is the bending vibration peak of the Si-O bond. As DOX was installed in MSN, two new absorption bands at 2854 and 2927 cm^{-1} that are attributed to the stretching vibration peaks of -OH in DOX occurred, meanwhile, the absorption peak intensity of MSN at 3000-3700 cm^{-1} weakened. After 3-HF was loaded in DOX@MSN, the skeleton vibration peak intensity of MSN weakened, and a new absorption peak at 1618 cm^{-1} assigned to the vibration peak of the carbonyl group in 3-HF appeared. These results indicated the successful construct of DOX/3-HF@MSN.

DOX has a characteristic absorption peak at 484 nm, while MSN and 3-HF have no absorption at this wavelength. As DOX was loaded into MSN, the UV absorption peak at 484 nm and fluorescence intensity of DOX significantly decreased, suggesting that DOX was successfully loaded into MSN. After the addition of 3-HF into DOX@MSN, the absorption intensity further decreased (Fig. S5). The spectra changes of DOX before and after the introduction of MSN and 3-HF supported the formation of DOX/3-HF@MSN.

The detailed concentration of DOX (1.46 mg/mL) and 3-HF (0.91 mg/mL) in MSN was determined by their characteristic absorption intensity change (Fig. S6). According to the standard curve, the amounts of DOX and 3-HF encapsulated in MSN are calculated to be about 12.0% and 21.4%, respectively (Fig. S7-8).

The protein components of the RBC membranes, HepG2 membranes, RBC-H hybrid membrane vesicles and RBC-H membrane encapsulated on MSNs were further analyzed by SDS-

PAGE experiments (Fig. S9A).

2. Spectral analysis

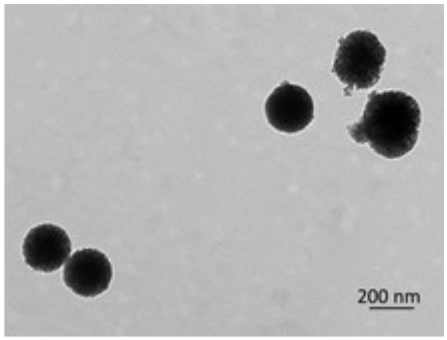


Figure S4. TEM of MSN.

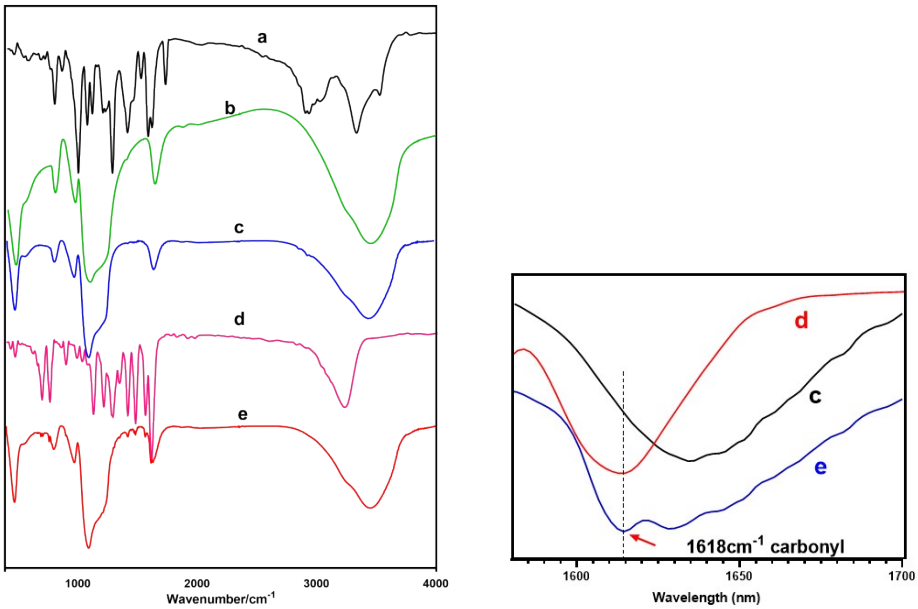


Figure S5. (Left) Representative FTIR spectra, a) DOX; b) MSN; c) DOX@MSN; d) 3-HF; e) DOX/3HF@MSN. (Right) Enlarged view of partial area.

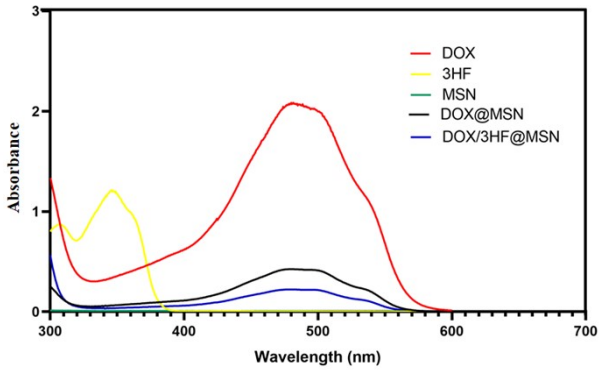


Figure S6. UV absorption spectra of various molecules or assemblies. The detailed concentrations are as following. MSN: 4 mg/mL; 3-HF: 2 mg/mL; DOX: 2 mg/mL; DOX@MSN: 16.67 mg/mL (12% DOX, w/w); DOX/3HF@MSN: 12.17 mg/mL (12% DOX and 30% 3-HF, w/w). The concentrations of DOX and 3-HF in DOX/3-HF@MSN are 1.46 and 0.91 mg/mL, respectively.

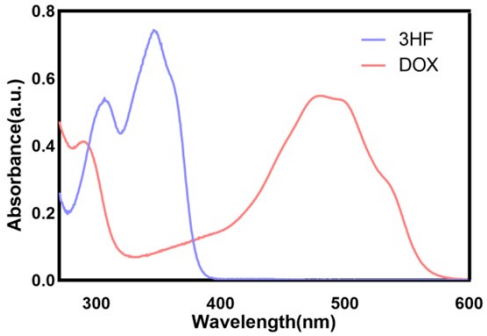


Figure S7. The UV-Vis absorption spectra of DOX and 3-HF.

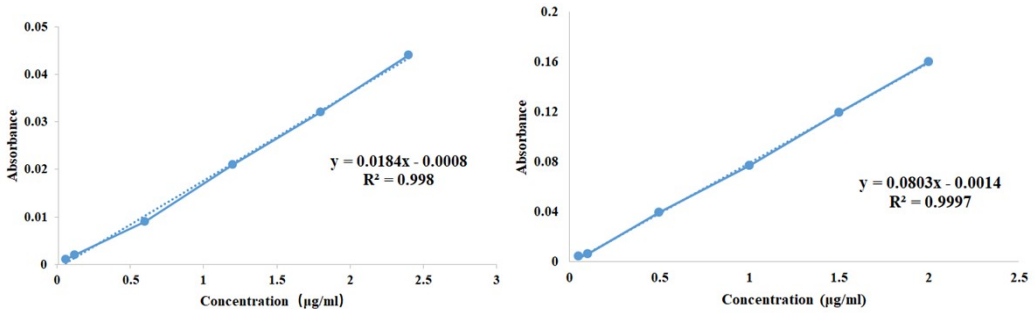


Figure S8. The standard curves of DOX (left) and 3-HF (right) between absorbance and different concentrations. The absorption wavelengths for DOX and 3-HF are at 480 and 344 nm, respectively.

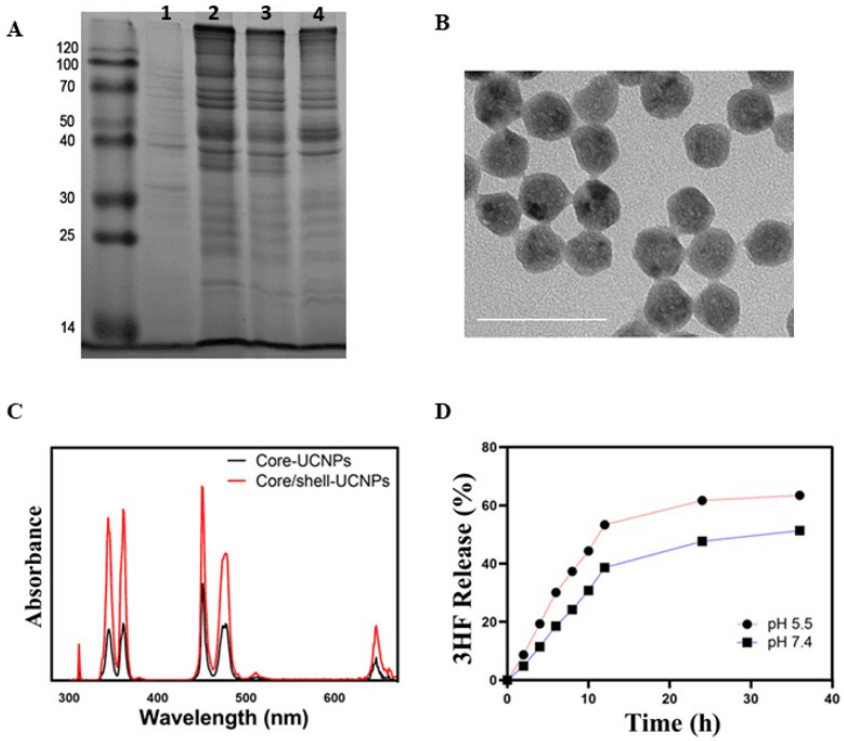


Figure S9. A) SDS-PAGE protein analysis of RBC membrane (1), HepG2 membrane (2), RBC-H hybrid membrane capsule (3) and RHM (4); B) TEM image of $NaYF_4$: Tm, Yb@$NaYF_4$, Scale bar: 50 nm; C) Luminescence spectra of core-UCNPs and core/shell-UCNPs; D) The cumulative release of 3-HF at pH 5.5 and 7.4 with different time duration.

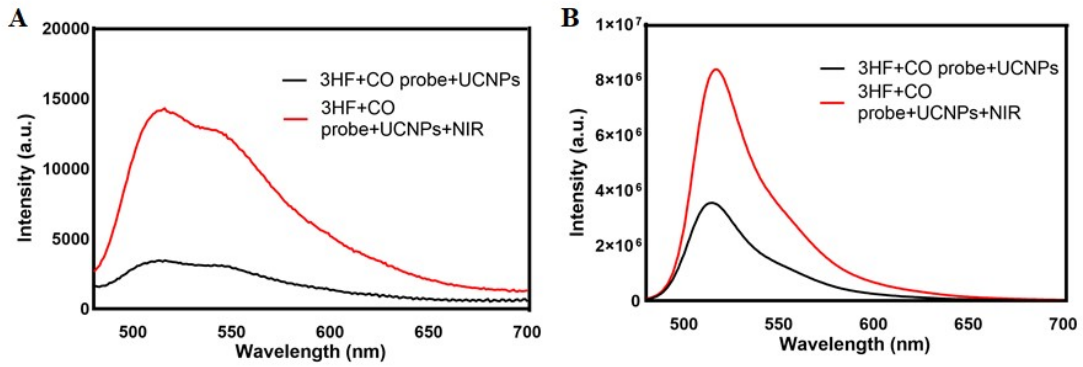


Figure S10. Fluorescence spectra of FL-CO-1+ $PdCl_2$ (1 μM) in the solution with pH 5.4 (A) and 7.4 (B) before and after illumination (980 nm, 1.0 W/cm^2, 15 min). The solutions include 0.5 mg/mL of 3-HF and 10 μM of UCNPs.

The fluorescence enhancement times of CO probe (FL-CO-1+ $PdCl_2$) at pH 7.4 and 5.4 are 4.1- and 2.3-fold, respectively (Figure S10). According to the absorption change of 3-HF at 350 nm, the percentage of CO from 3-HF at pH 7.4 and 5.4 are 51.9% and 93.4%, respectively. The ratio of the CO release from 3-HF at pH 5.4 and 7.4 calculated from probe (FL-CO-1+ $PdCl_2$) is 1.78 (4.1/2.3), which is consistent with that achieved from absorption (1.79, 93.4%/51.9%), implying that tumor microenvironment (TME) is beneficial for the UCNP-induced CO release from 3-HF.

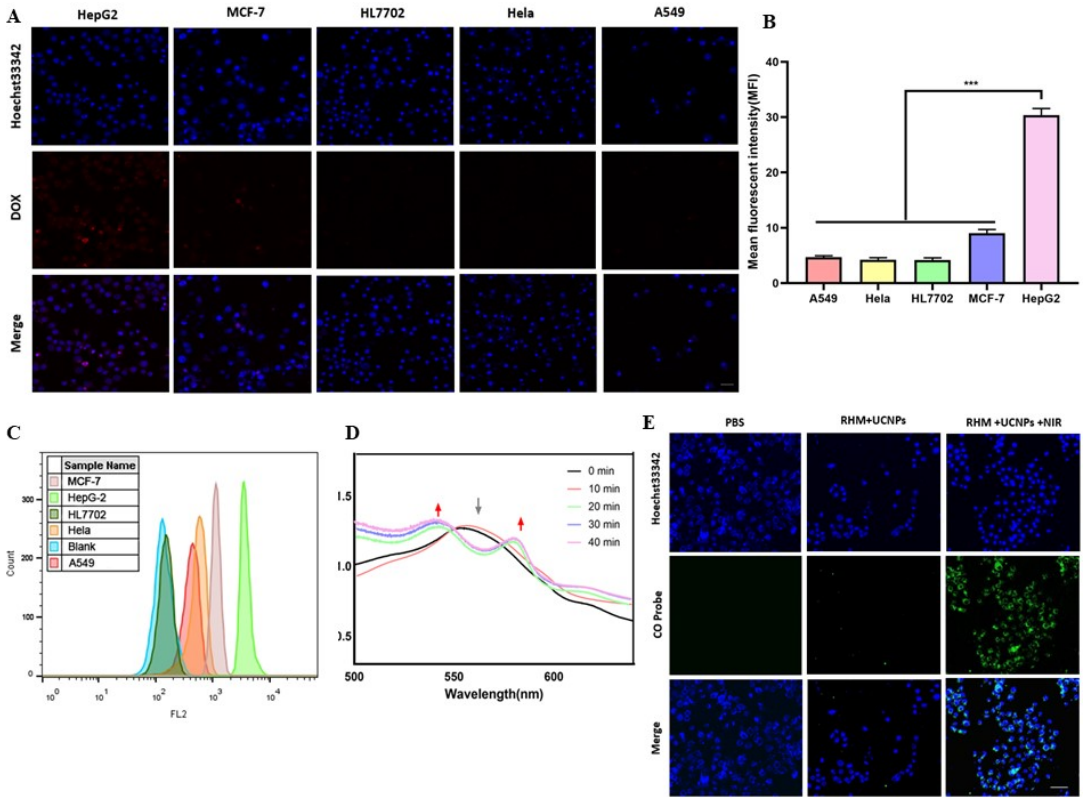


Figure S11. CLSM images (A) and fluorescence intensity (B) of different cells incubated with RHM nanoparticles, scale bar = 50 nm; C) Flow cytometric analysis of RHM phagocytosed by different cells; D) The CO release from RHM measured by using a deoxy-myoglobin assay; E) Fluorescent imaging of CO in HepG2 cells through the probe system (FL-CO-1+ $PdCl_2$, 1 μM), the cells were treated with UCNPs under excitation at 980 nm with1.0 W/cm^2 (scale bar =50 nm).

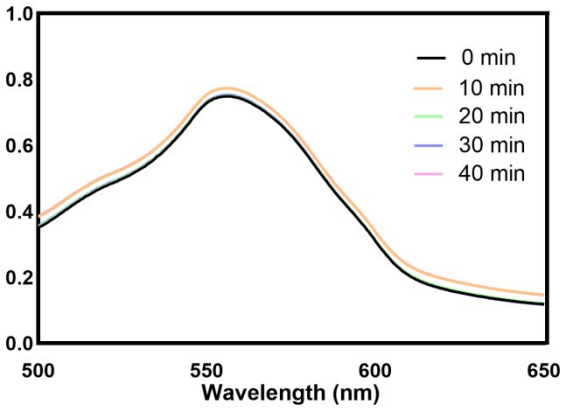


Figure S12. Absorption spectra of deoxy-myoglobin treated with RHM+UCNPs group without laser treatment. The spectra were recorded after different duration.

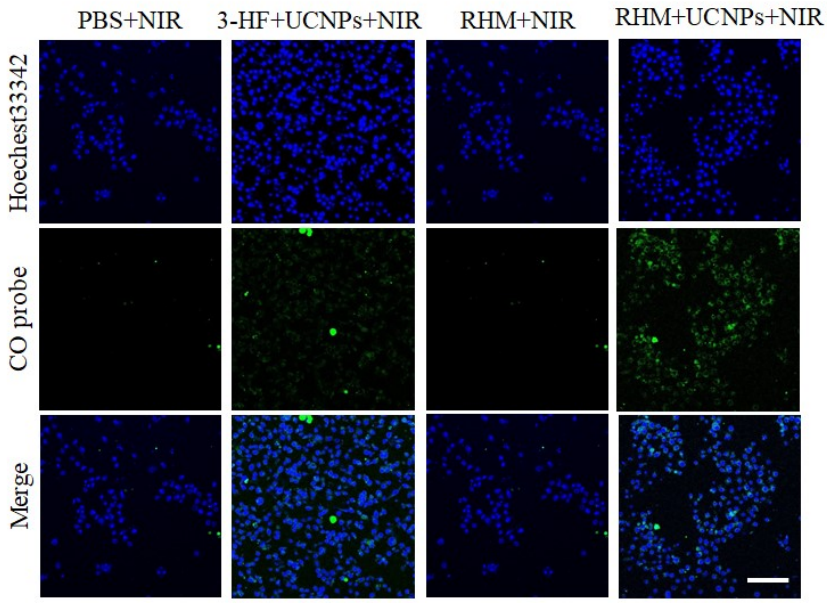


Figure S13. Images of CO released from 3-HF in the absence and presence of UCNPs in HepG2 cells. The cells were irradiated by 980 nm light with 1.0 W/cm^2 and CO was detected by the probe of FL-CO-1+ $PdCl_2$ (1 μM). Scale bar =50 nm. To increase the cell penetration of 3-HF, herein 3-HF was loaded in MSNs and then covered with mixed membrane of HepG2 cells and erythrocyte (RBC).

The dominant absorption band of 3-HF is in the UV region, implying that 3-HF can only be excited by UV light. The importance characteristic of UCNP is that it can convert red-light to UV light which can be employed to excite 3-HF. In the absence of UCNP, almost no CO can be produced as 3-HF was irradiated by red-light. In contrast, obvious CO signal monitored by CO probe was observed in the presence of UCNP.

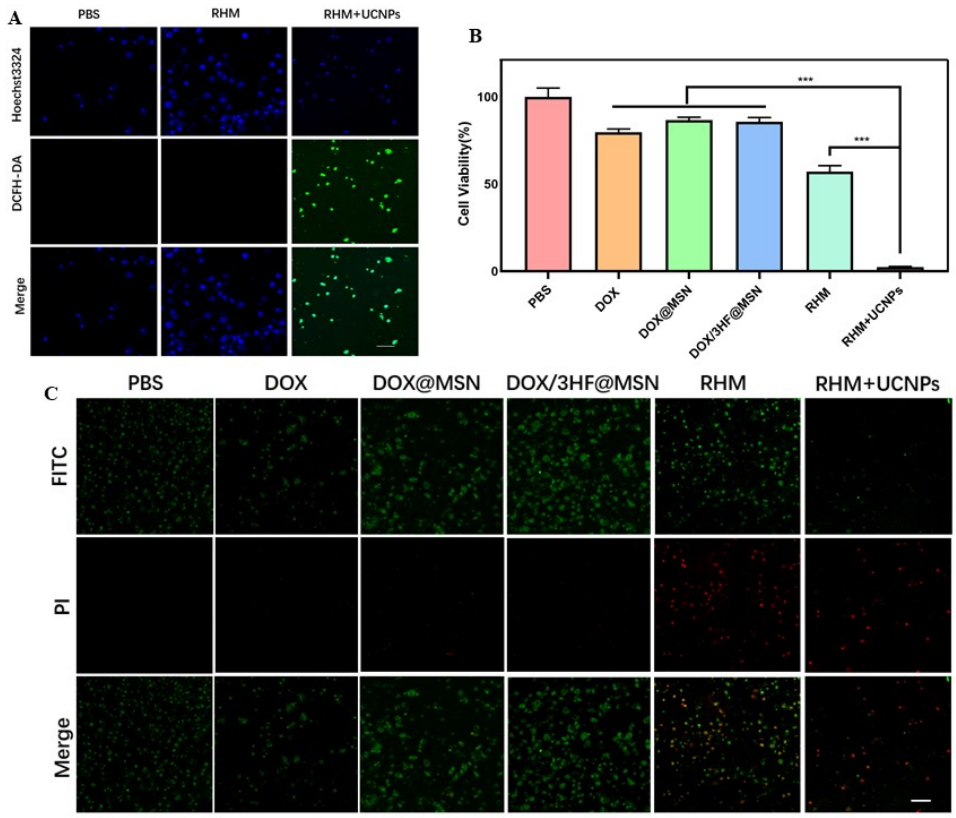


Figure S14. A) The ROS levels induced by the CO release in HepG2 cells; The viability of different tumor cells after treatment with PBS, MSN, DOX, DOX@MSN, DOX/3-HF@MSN, RHM, RHM + UCNPs, which is evaluated by MTT analysis (B) and CLSM analysis (C).

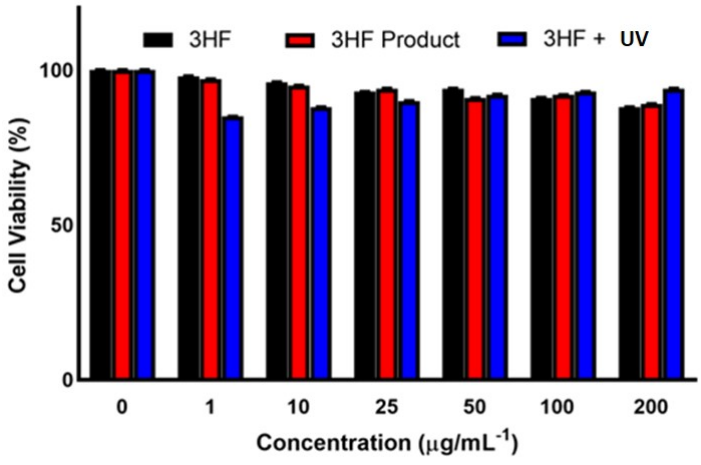


Figure S15. The cell viabilities of HepG2 cells incubated with different concentrations of 3-HF or its byproducts obtained after the CO release or 3-HF upon UV-light illumination (365 nm, 0.5 W/cm^2, 15 min) to produce CO. The cells were incubated for 24 h.

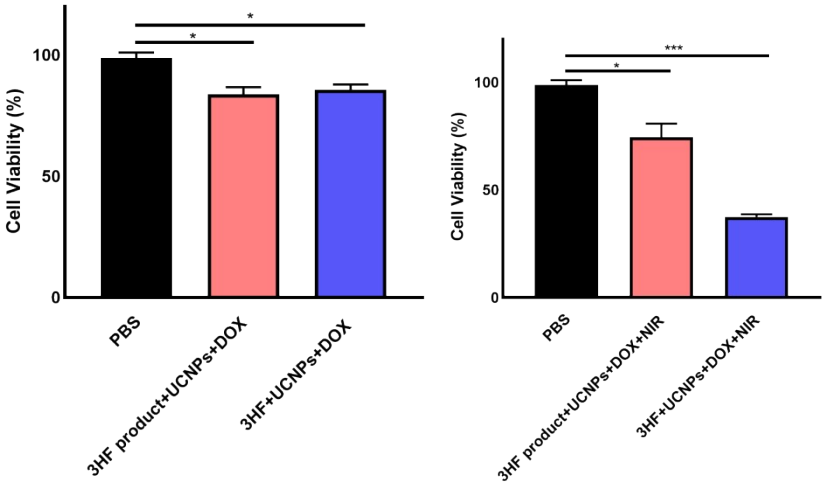


Figure S16. The cell viabilities of HepG2 cells incubated with different combinations for 12 h. The detailed conditions: 2 μg/mL DOX, 5 μg/mL 3-HF or 3-HF's product, and UCNPs (10 μM) with illumination (980 nm, 1.0 W/cm^2, 30 min) (right) or without illumination (left).

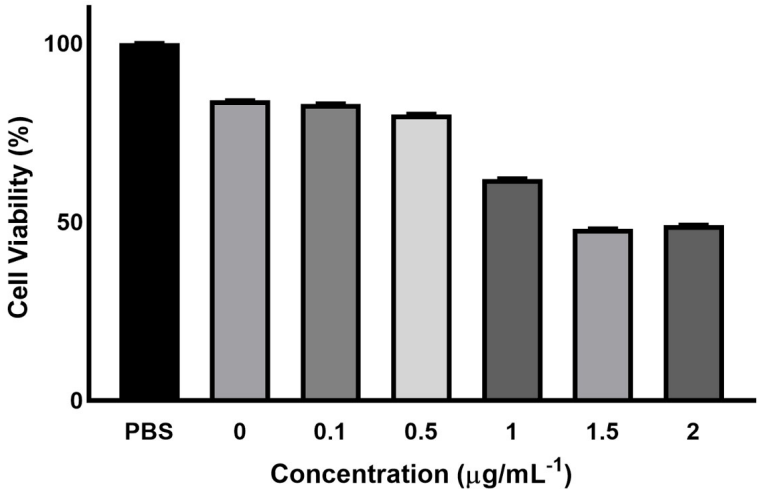


Figure S17. The cell viabilities of HepG2 cells in the presence of DOX (2 μg/mL) and UCNPs (10 μM) after incubation with different concentration of 3-HF and then illumination (980 nm, 1.0 W/cm^2, 30 min) for 12 h.

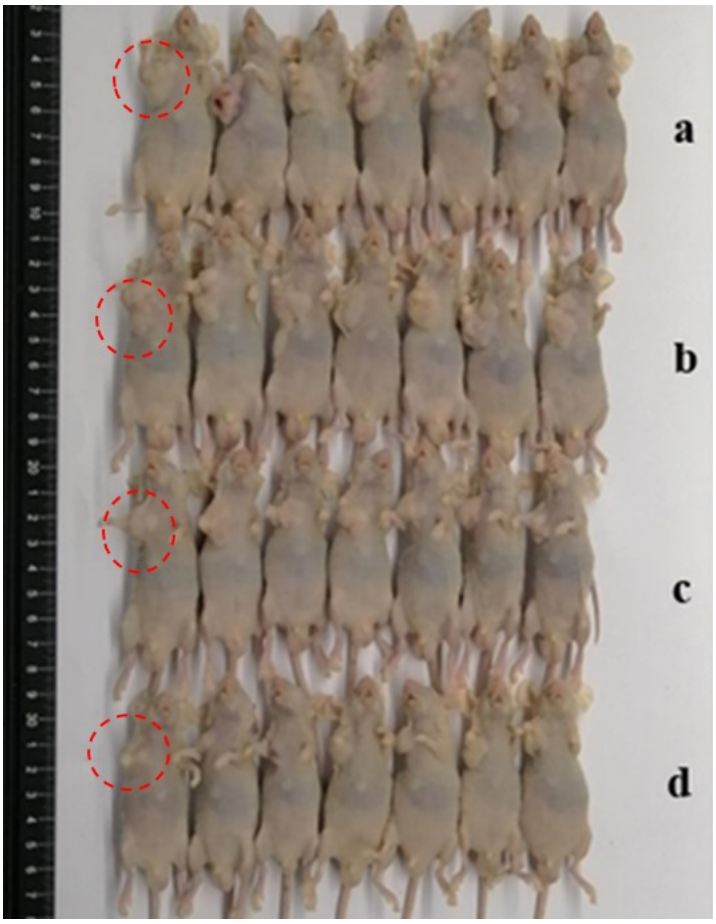


Figure S18. Photos of animals after the indicated treatments (a) PBS; b) DOX; c) RHM; d) RHM+UCNPs).

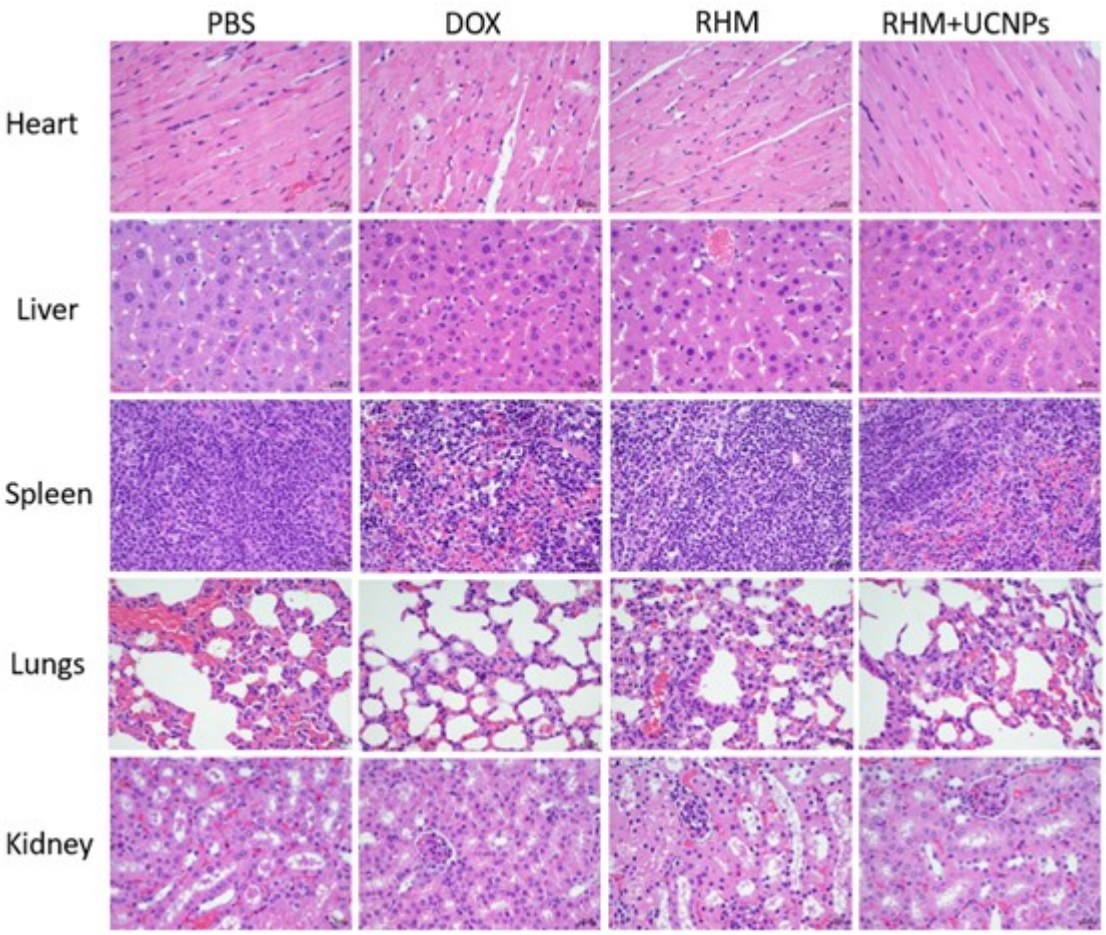


Figure S19. H&E staining images of major organs dissected from various groups with different treatments. Scale bar: 20 μm.

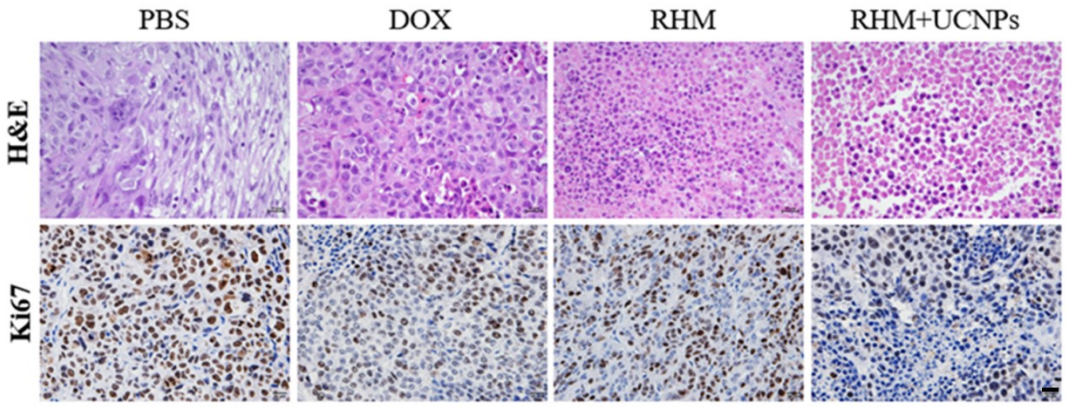


Figure S20. H&E staining and Ki-67 staining tumor sections of different treatment groups. The scale bar is 200 μm.

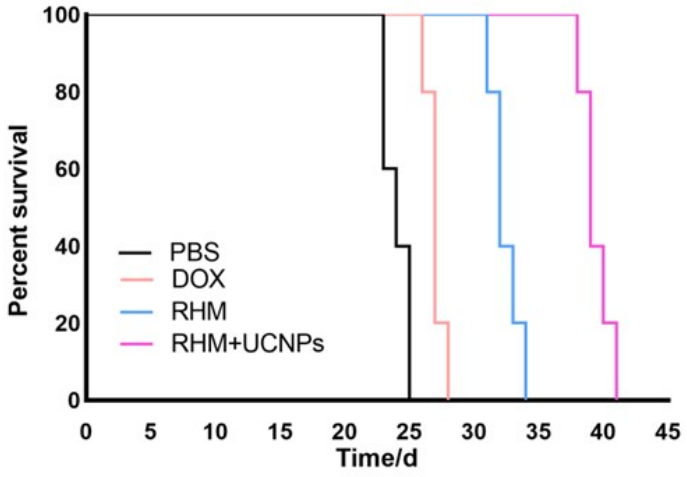


Figure S21. Survival curves of different treatment groups.

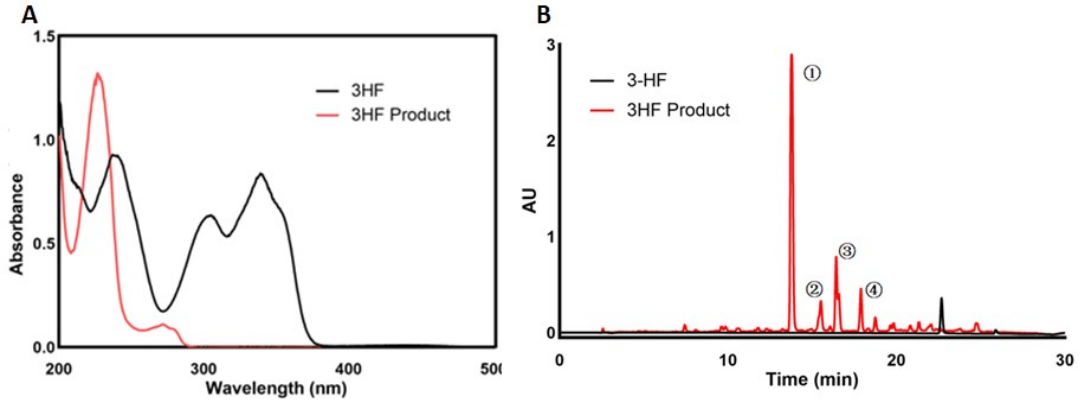


Figure S22. Absorption spectra (A) and HPLC spectra (B) of 3-HF and the organic reaction products generated upon UCNP-induced CO release from 3-HF.

3-HF has a main absorption band at 350 nm, but the absorption peak completely disappears after the CO release, indicating that there is no 3-HF in the reaction products. There are four main peaks marked as component 1-4 in HPLC, which suggests there are four main products generated upon UCNP-induced CO release from 3-HF. The percentages of the amounts of the products are 47.42%, 10.83%, 23.27%, 14.53%, respectively. Due to that the amounts of component 2 and 4 are small, no enough compound can be obtained for NMR analysis. According to the analysis of NMR and MS spectra (Fig. S23-28), components 1 and 3 are salicylic acid and 2-(benzoyloxy)benzoic acid, respectively. Based on the MS data, components 2

and 4 were inferred to be probably 2-benzoylbenzoic acid and benzoic acid, respectively. Although the products are diverse, the main product 2-(benzoyloxy)benzoic acid is unambiguous, because the other three products are the hydrolysis products or derivatives of 2-(benzoyloxy)benzoic acid.

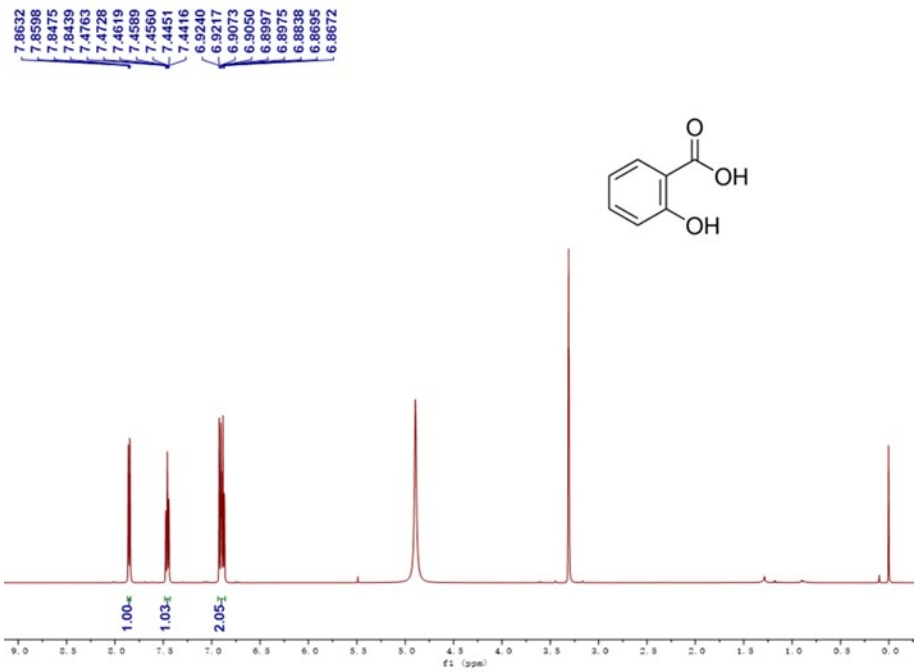


Figure S23. The ^{1}H NMR spectrum of component 1 in HPLC spectrum. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.85 (dd, J = 1.7, J = 7.9 Hz, 1H), 7.46 (dd, J = 1.8, J = 5.2 Hz, 1H), 6.90 (dd, J = 1.7, J = 7.9 Hz, 2H).

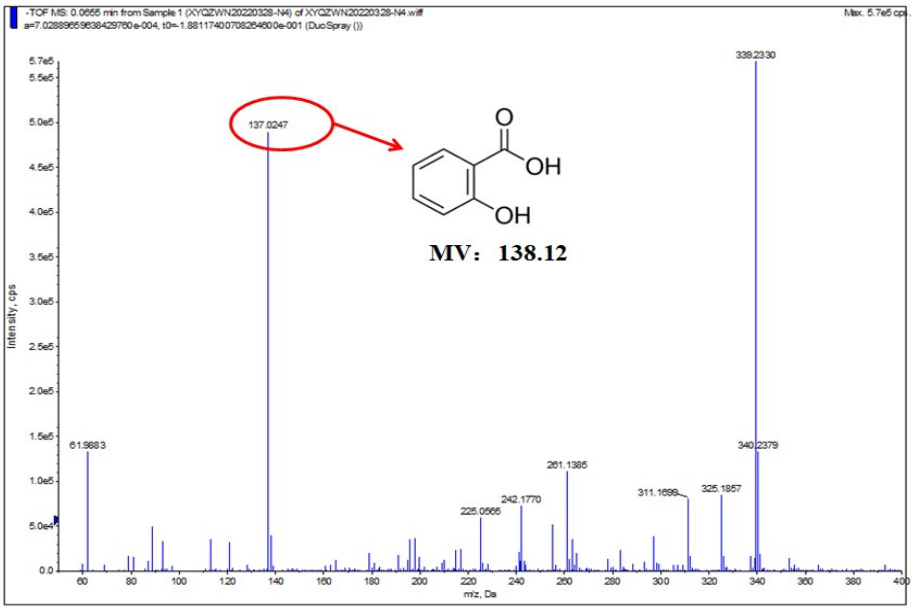


Figure S24. The MS spectrum of component 1 in HPLC spectrum. *m/z* 137.02; Calcd. $(M-H)^-$ 137.02.

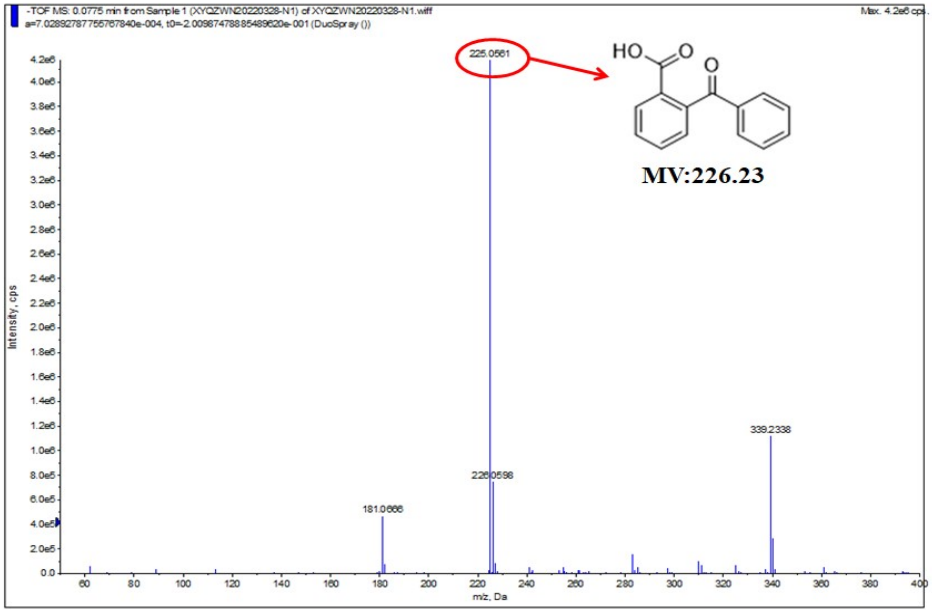


Figure S25. The MS spectrum of component 2 in HPLC spectrum. *m/z* 225.06; Calcd. $(M-H)^-$ 225.06.

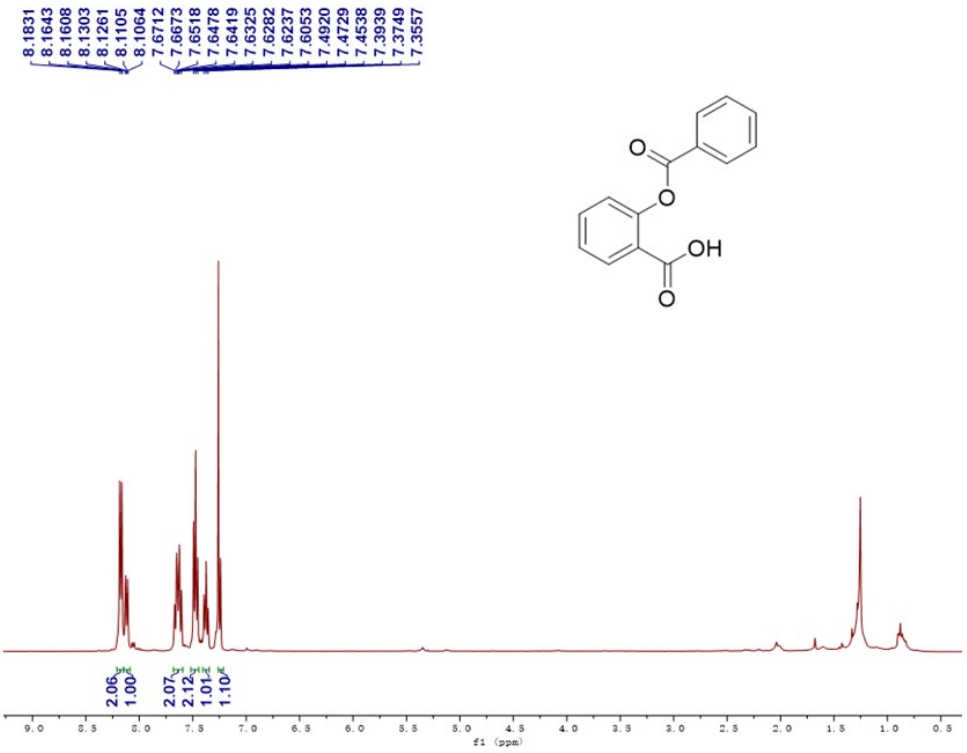


Figure S26. The ^{1}H NMR spectrum of component 3 in HPLC spectrum. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.16 (dd, J = 1.8, J = 9.4 Hz, 2H), 8.12 (dd, J = 2.1, J = 7.8 Hz, 1H), 7.77 (m, 2H), 7.64 (m, 2H), 7.47 (m, 1H), 7.36 (d, J = 9.6 Hz, 1H).

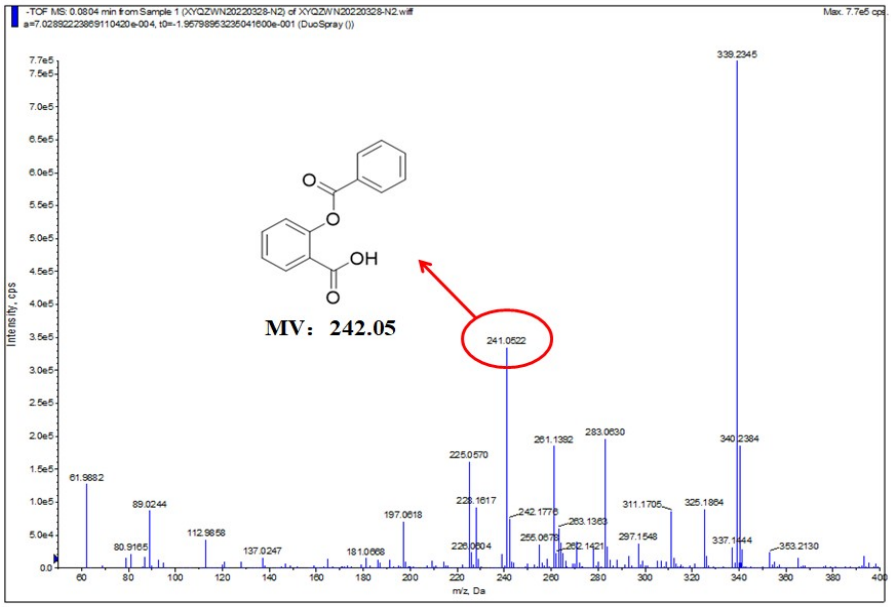


Figure S27. The MS spectrum of component 3 in HPLC spectrum. *m/z* 241.05; Calcd. $(M-H)^-$ 241.05.

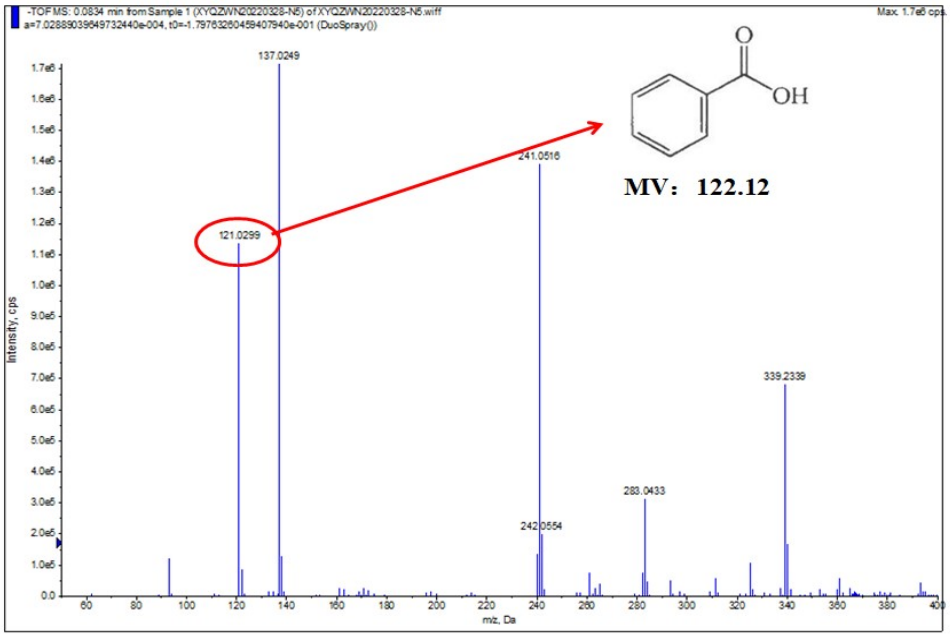


Figure S28. The MS spectrum of component 4 in HPLC spectrum. *m/z* 121.03; Calcd. $(M-H)^-$ 121.03.

3. References

1. H. Lin, K. Zhang, G. Yang, Y. Li, X. Liu, K. Chang, Y. Xuan, J. Ye, *Applied Catalysis B: Environmental*, 2020, **279**, 119387.
2. C. M. J. Hu, L. Zhang, S. Aryal, C. Cheung, R. H. Fang and L. F. Zhang, *P. Natl. Acad. Sci. USA.,* 2011, **108**, 10980-10985.
3. Y. Zhang, K. W. Ren, X. B. Zhang, Z. C. Chao, Y. Q. Yang, D. J. Ye, Z. H. Dai, Y. Liu and H. X. Ju, *Biomater.*, 2018, **163**, 55-66.
4. W. Y. Feng, D. D. Liu, S. M. Feng and G. Q. Feng, *Anal. Chem.*, 2016, **88**, 10648-10653.