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Supporting Information

Hydrofluorocarbons Nanoparticles for ^{19}F MRI-Fluorescence Dual Imaging and Chemo-Photodynamic Therapy

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1. General information

Phospholipid S75 was obtained from Lipoid GmbH. Pluronic F68 (average MW = 8350) and ICG were purchased from Adamas (Shanghai, China). Soybean oil of medicinal grade was acquired from Aladdin (Shanghai, China). SOSG and DCFH-DA were purchased from Thermo Fisher (United States). Calcein/PI Cell Viability/Cytotoxicity Assay Kit was purchased from Beyotime (Shanghai, China). Cell Counting Kit-8 (CCK-8) was purchased from Meilun (Suzhou, China). Human breast adenocarcinoma cell line MCF-7 and Human normal breast epithelial cells MCF-10A were supplied by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Beyotime (Shanghai, China) provided Human lung adenocarcinoma cell line A549. All reagents used in this study were either analytical or HPLC grades and were used without further treatment. Deionized water was used unless otherwise indicated. Column flash chromatography was performed on silica gel (200-300 mesh) with the eluent as indicated in the procedures.

$^{1}H/^{19}F/^{13}C$ NMR spectra of compounds were recorded on Bruker AVANCE III 400 MHz or 500 MHz or 600 MHz spectrometers. Chemical shifts are in ppm and coupling constants (*J*) were in Hertz (Hz). ^{1}H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using $CDCl_3$ as solvent. ^{13}C NMR spectra were referenced to solvent carbons (77.16 ppm for $CDCl_3$). ^{19}F NMR spectra were referenced to 2% perfluorobenzene (s, -164.90 ppm) in $CDCl_3$. The splitting patterns for ^{1}H NMR spectra are denoted as follows: s (singlet), d (doublet), q (quartet), p (quint), m (multiple).

UV-Vis spectra were recorded on a UV-2600 UV-Vis-NIR spectrophotometer

(Shimadzu, Japan). Fluorescence spectra were measured on an F-4700 spectrometer (Hitachi, Japan). Dynamic light scattering (DLS) was measured on a Nano ZS 90 particle size analyzer (Malvern, U.K). Transmission electron microscope (TEM) images of the emulsions were measured on the equipment of JEOL JEM-2100 (Jeol, Japan). Confocal microscope images were taken on Leica-LCS-SP8-STED (Lycra, Germany). Laser experiments used an LWIRL808-5W-F laser (Laserwave, Beijing). Temperatures were monitored by a Hikvision H13 thermal camera (Hikvision, Beijing).

2. Synthesis of compound 9

Under an argon atmosphere, to a solution of 2-pyridone (1.0 g, 10.5 mmol) in dry acetonitrile (15.0 mL) was added 3,5-bis(trifluoromethyl)benzyl bromide (6.4 g, 21.0 mmol) and K_2CO_3 (7.5 g, 54.3 mmol) at room temperature. The reaction mixture was refluxed for 8 hours. After cooling to room temperature, the reaction mixture was quenched with 50 mL of water and extracted with 20 mL of ethyl acetate (3 times). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by flash chromatography on silica gel (DCM/MeOH = 100/1) to give compound **9** as pale wax (2.3 g, yield 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91-7.71 (m, 3H), 7.46-7.35 (m, 2H), 6.63 (dd, *J* = 9.9, 1.4 Hz, 1H), 6.25 (td, *J* = 6.7, 1.4 Hz, 1H), 5.25 (s, 2H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -65.94. ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.5, 140.2, 139.1, 137.2, 132.2 (q, *J* = 34.0 Hz), 128.2, 128.1, 123.2 (q, *J* = 267.1 Hz), 121.7, 107.0, 51.8. HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{14}H_9F_6NO$, 322.0661, found 322.0625.

3. Preparation of nanoemulsions E1-E10

Using the formulation of nanoemulsion E9 as a typical procedure: A homogeneous solution of **9** (10 mg), **3** (1 mg), **5** (2 mg) and **2** (60 mg) was obtained by 1 minute of ultrasound treatment, which was then mixed with a solution of S75 (10 mg/mL) and ICG (1 mg) in 2 ml of ethanol in a round-bottomed flask. Then 50 mg of soybean oil was added and the resulting mixture was put on a rotary evaporator under vacuum at 45 ^{o}C for 30 minutes to completely remove the ethanol. To the flask was added 2 mL aqueous solution of F68 (10 mg/mL) and the mixture was treated with ultrasound in an ice bath for 20 min to give the colostrum.

A solution of ferric chloride (20 μL, 12.35 mM) was added to 980 μL of the above colostrum, which was stirred for 10 h at room temperature in the dark. Then a solution of DSPE-PEG_{2000}-RGDyC (S75/DSPE-PEG_{2000}-RGDyC = 20/1) was added and the resulting mixture was shaken at 200 r/min at room temperature for 2 h. The resulting emulsion was placed in a liposome extruder with a filtration membrane of 100 nm in pore size. After repeated extruding 20 times, a green translucent nanoemulsion **E9** was obtained.

Other nanoemulsions **E1**-**E8** and **E10** were obtained with the corresponding ingredients through the same procedure as **E9**.

4. Profile of ICG encapsulating percentage and loading efficacy

The ICG encapsulating percentage (EE_{ICG}) and loading efficacy (LC_{ICG}) in **E8** was determined as 37.4±0.5 % and 0.38±0.01 % on an HPLC (Shimadzu, Japan). The chromatographic conditions of HPLC were as follows: Column: RP C18 column, 5 μm,

4.6 × 150 mm; mobile phase: A H_2O, B MeOH; gradient: B 30% - 100% in 30 min; wavelength: 254 nm; flow rate: 0.7 mL/min.

Then, encapsulating and loading of the ICG were calculated by generating standard curves (Figure S1). The specific calculation formula is as follows:

EE_{ICG} = [(the weight of ICG added – the weight of ICG in the supernatant)/ the weight of ICG added]×100%;

LC_{ICG} = [(the weight of ICG added – the weight of ICG in the supernatant)/the total weight of nanoemulsions]×100%.

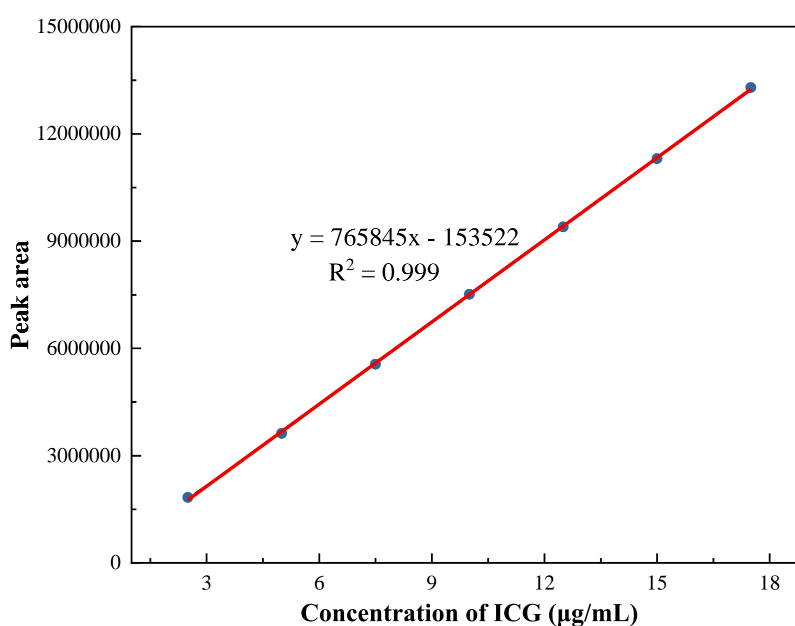


Figure S1. Standard curve of ICG concentration measured by HPLC.

5. ^{19}F MRI phantom experiments

All ^{19}F MRI phantom experiments were performed on a 400 MHz Bruker BioSpec MRI system at 24 °C. Nanoemulsions **E7** and **E8** were serially diluted with water to give a series of ^{19}F concentrations: 160 mM, 80 mM, 40 mM, 20 mM, 10 mM, 5 mM, and 2.5 mM, respectively.

For T_1-weighted MRI, a spin-echo (SE) pulse sequence was used with the following parameters (method = RARE, matrix size = 32 × 32, SI = 20 mm, FOV = 3.0 cm, TR = 100 ms, TE = 3 ms, NS=256, scan time = 819 s).

6. Singlet oxygen detection in nanoemulsion solutions

Singlet Oxygen Sensor Green reagent (SOSG, Thermo Fisher) was used as a probe to study the singlet oxygen production of the nanoemulsions. The working solution was prepared by adding SOSG (100 μL of 20 μM solution) to the **E8** solution (900 μL, 2.5 μg/mL ICG). The working solutions were radiated with 808nm laser at the preset time (0, 1, 2, 3, 4 min) and power density (0, 0.5, 1, 2, 3 W/cm^2), respectively. After the irradiation, the solution was subjected to a fluorescence spectrophotometer to measure the fluorescence emission of SOSG ($\lambda_{excitation}/\lambda_{emission}$=504 nm/525 nm). Water was used as a control.

7. Photothermal effect and photostability of nanoemulsions

All photothermal experiments were carried out at 25 ºC. A series of nanoemulsion **E8** solutions with ICG concentrations at 0, 2.5, 5, 10, 20, 40 μg/mL were irradiated with an 808 nm laser at the preset power intensities (range 0 to 3 W/cm^2) and times (0, 1, 2, 3, 4, 5 min). During the irradiation, a thermal imaging camera (Hikvision) was used to monitor the temperature changes of **E8** every 10 seconds.

During the photothermal stability experiments, a solution of **E8** (with 40 μg/mL ICG) was irradiated with an 808 nm laser for 2 minutes and cooled in the dark to ambient temperature over 5 minutes. The process was repeated 5 times and a thermal imaging camera was used to monitor the temperature changes. Water was used as a control.

8. Cell culture and assessment of cell viability

A549 cells, MCF-10A cells, and MCF-7 cells were cultured in DMEM-high glucose medium with fetal bovine serum and 1% penicillin and/streptomycin. All cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

To study the cell uptake, A549 cells (1×10^5 cells) were incubated with nanoemulsion **E8** (10 μg/mL ICG) for 2, 6, 12 hours, respectively, which were then washed with PBS three times and fixed with 1 mL of 4% paraformaldehyde. After staining with 200 μL DAPI for 10 minutes, the cell images were obtained by a confocal laser scanning microscope.

In vitro cytotoxicity was evaluated by a CCK-8 assay. The cells (A549, MCF-7 and MCF-10A cells) were inoculated at about 1×10^4 cells per well in 96-well plates (n = 6) and cultured for 24 hours. The cells were then incubated with nanoemulsions **E1**, **E5**, **E8**, or salinomycin **5** for 12 hours, respectively. After washing 3 times with PBS (pH = 7.4), each well was added with 100 μL of CCK-8 (10% v/v) solution and incubated for another 3 hours. A microplate reader was employed to measure the absorbance at 450 nm.

9. *In vitro* anticancer efficacy

A549 cells were pre-seeded in 96-well plates and incubated with a series of nanoemulsions **E8** or salinomycin **5** solution at a salinomycin concentration range of 1.25 μg/mL to 20 μg/mL for 12 hours. One group of **E8**-treated A549 cells was washed with PBS three times and then exposed to an 808 nm laser for 5 minutes. Finally, the

CCK-8 solution was added to all groups to measure the cell viability using the above methods.

MCF-10A cells were pre-seeded in 96-well plates and incubated with a series of nanoemulsions **E8** at a salinomycin concentration range of 1.25 μg/mL to 20 μg/mL for 12 hours, then exposed to an 808 nm laser for 5 minutes. Finally, the CCK-8 solution was added to measure the cell viability (Figure S2).

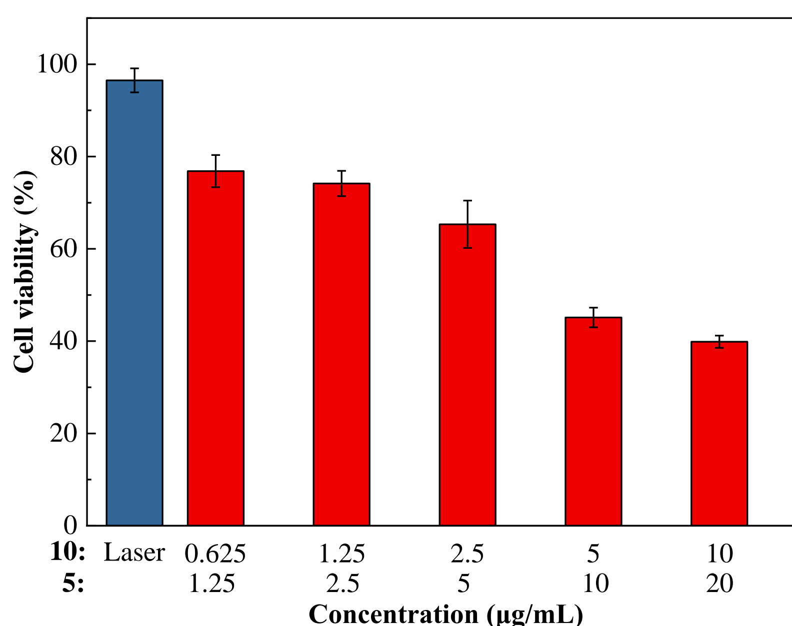


Figure S2. CCK-8 cytotoxicity assay of **E8** with laser irradiation in MCF-10A cells.

A549 cells were incubated with **E8** (at 10 μg/mL ICG) or PBS for 12 hours and then stained with Calcein-AM and PI in a buffer solution for 30 minutes. After washing with PBS 3 times, the live and dead cells were observed with a confocal laser scanning microscope.

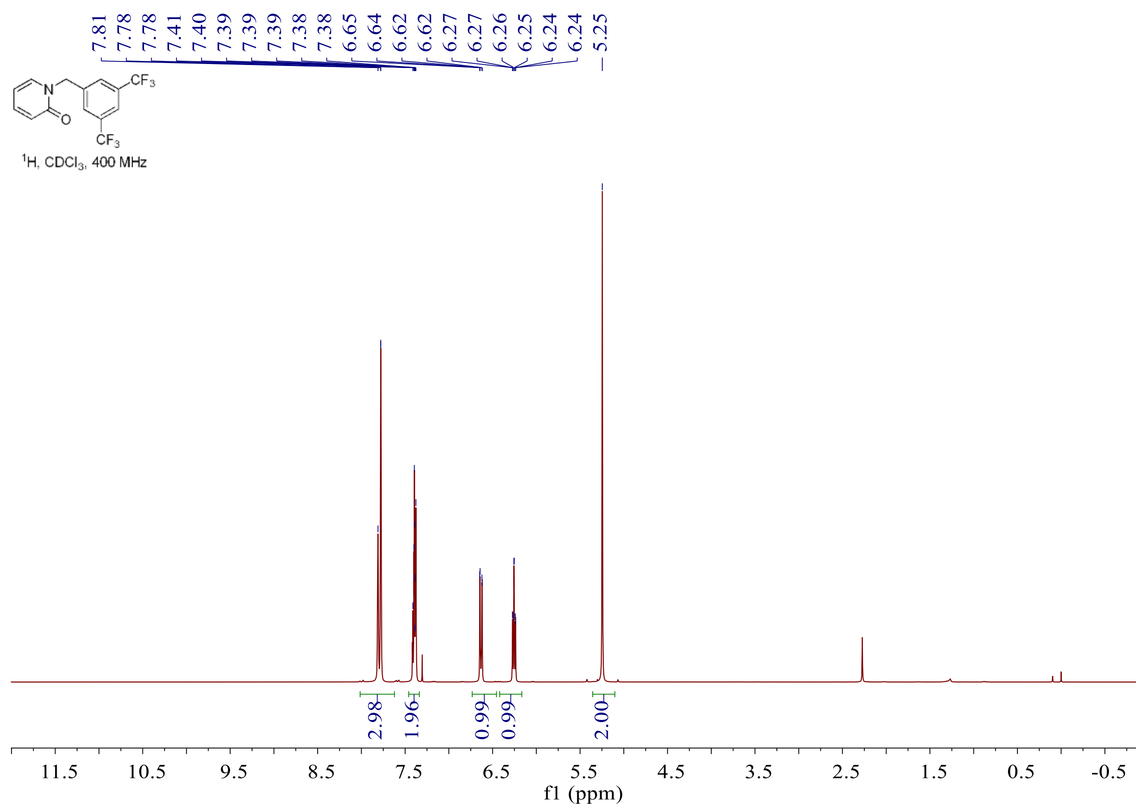
10. Intracellular ROS detection.

A549 cells were incubated with nanoemulsion **E8** or PBS in a confocal culture dish for 12 hours, respectively, which was then added 2,7-dichlorodihydrofluorescein

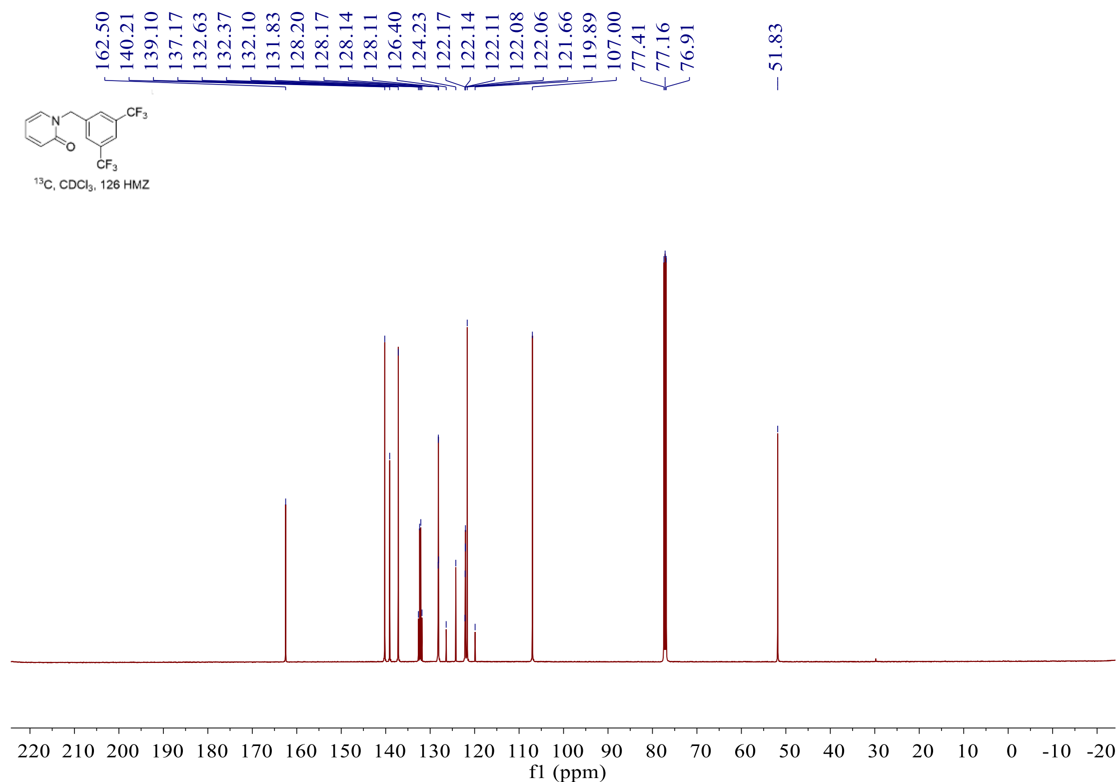
diacetate (DCFH-DA, 10 μmol) and incubated for 30 minutes. After irradiating them with a laser (808 nm, 1 W/cm^2) for 5 minutes, their fluorescence images were recorded on a confocal laser scanning microscope. Samples incubated with DCFH-DA were excited with a 488 nm laser, and fluorescence is collected from 500 to 600 nm.

11. Copies of $^{1}H/^{13}C/^{19}F$ NMR, and HRMS spectra of compound 9

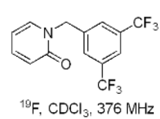
^{1}H NMR spectrium of compound **9** (400 MHz, $CDCl_3$)

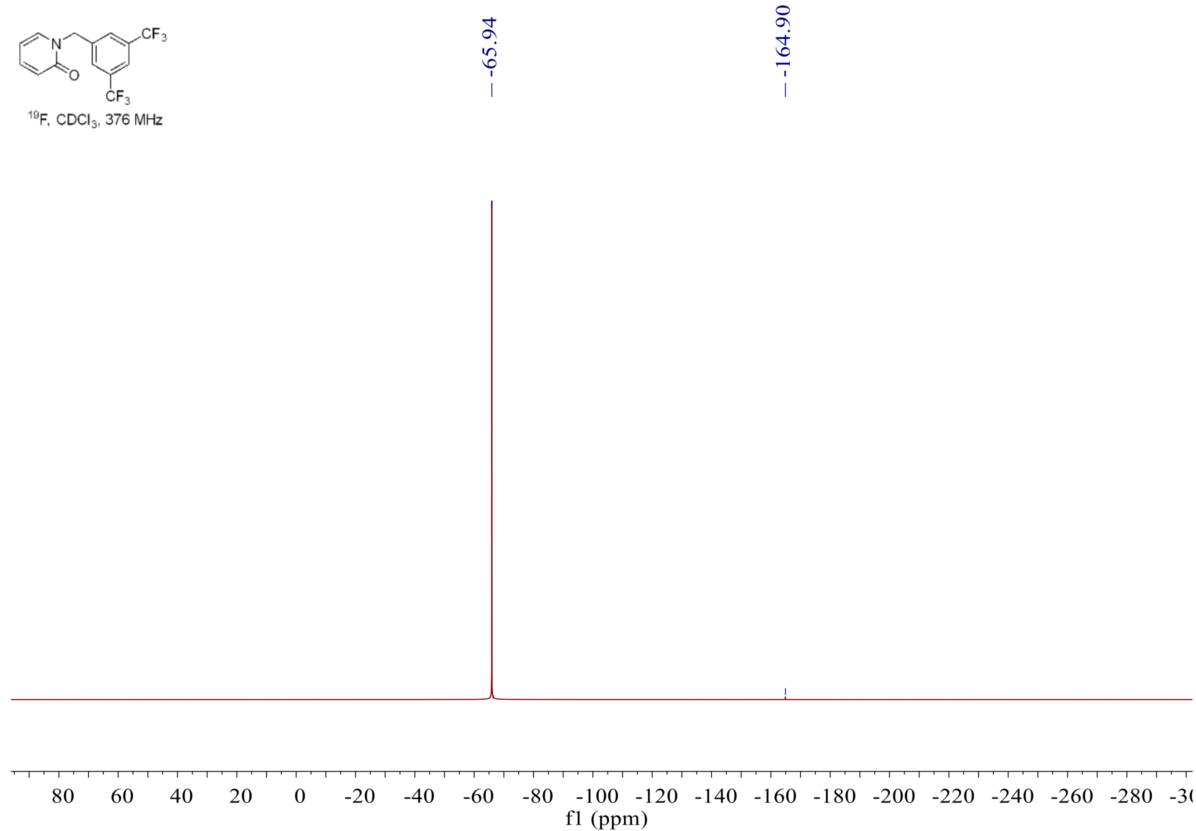


^{13}C NMR spectrium of compound **9** (126 MHz, $CDCl_3$)



^{19}F NMR spectrum of compound **9** (376 MHz, $CDCl_3$)





HRMS (ESI) spectrum of compound **9**