Supporting Information

Si-rhodamine fluorescent probe for monitoring of hypochlorous acid in the brains of mice afflicted with neuroinflammation

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Experimental Section

Materials and instruments

All raw materials are analytical grade and are used directly unless otherwise stated. All solvents such as tetrahydrofuran and ethyl ether in the synthesis step were further dried. Neuroinflammation stimulating drugs lipopolysaccharide (LPS) was purchased from Beijing InnoChem Science & Technology Co., Ltd. UV-Vis absorption and fluorescence spectra were obtained on HITACHI U-3900H and HITACHI F-4600 spectrophotometers, respectively. The biological reagents used in this experiment included cells and mice, of which HeLa cells and RAW264.7 cells were purchased from the Chinese Academy of Medical Sciences, C57BL/6 mice and feeds were purchased from Beijing Hfk Bioscience Co., Ltd. The MTT cytotoxicity test kit was purchased from Solarbio. *In vitro* imaging was carried out using a laser scanning confocal microscope (Leica SP8). *In vivo* imaging was conducted using an IVIS Spectrum Imaging System.

Preparation of HClO, other ROS and RNS

Commercial sodium hypochlorite solution was used as the source of HClO in this experiment, and its concentration was calibrated by absorbance at 292 nm (ε = 350 $M^{-1} \cdot cm^{-1}$). In the selectivity experiment, 19 substances were selected as interferents of HClO, among which ROS, RNS and active sulfur were prepared as follows: hydroxyl radical ·OH was prepared from the same concentration of hydrogen peroxide and ferrous sulfate, singlet oxygen $^1O_2$ was prepared from the same concentration of hydrogen peroxide and sodium hypochlorite, superoxide anion $O_2^{\bullet-}$ was prepared by dissolving potassium superoxide in DMSO, nitric oxide NO was prepared from copper and dilute nitric acid, hydrogen sulfide H_2S was prepared by dissolving sodium hydrosulfide in water, hydrogen peroxide H_2O_2 was prepared by diluting hydrogen peroxide solution, nitrite NO_2^- was prepared by directly dissolving

sodium nitrite in water, and hydrogen sulfite HSO_3^- was prepared by the corresponding concentration of Sodium Hydrogen Sulfite.

General procedure for fluorescence detection

Accurately weighed 10 mg of **SiR-FH** with an electronic balance, prepared it into a 1 mM stock solution using a solvent mixture with a volume ratio of DMSO/H_2O = 1/9, and the stock solution was diluted to 10 μM with a mixture of DMSO and H_2O. The scan range of UV-Vis absorption spectrum was set to 550 nm-700 nm, and tested the absorption curve of **SiR-FH** containing HClO (0-100 μM). When performing fluorescence spectroscopy tests, the excitation wavelength was adjusted to the appropriate wavelength according to the maximum absorption peak of the probe and the slit widths is 10.0 nm. In the experiment to explore the response time, the measurement mode of the fluorescence spectrometer was adjusted to "Time scan" mode, and tested the fluorescence change within 300 seconds.

Calculation formula for limit of detection (LOD) of HClO:

$$LOD = 3\sigma / s \quad \textbf{(1-1)}$$

where: σ is the standard deviation of **SiR-FH** fluorescence intensity in the absence of HClO, and s is the slope of the linear equation.

In vitro cytotoxicity assay

The cytotoxicity of the probe **SiR-FH** was detected by methyl thiazolyl tetrazolium (MTT) method, using HeLa cervical cancer cells, RAW264.7 macrophages and PC-12 neuronal cells. The cultured cells were digested and dispersed with trypsin, and counted under a microscope using a hemocytometer. The cells were then diluted into a suspension at a concentration of 10^5/mL and added to a 96-well plate. Replaced with cell culture medium containing different concentrations of **SiR-FH** (0-20 μM) after 24 h. Added MTT reagent after continued incubation for 24 h and switched to DMSO solution after 4 h. Cell viability was determined by measuring the UV-Vis absorption values in different well plates by the microplate reader.

Hemolysis experiment

Took 1 ml of human blood, added 1 ml of PBS and diluted to 2 mL. The sample was then centrifuged at 8000 rpm for 10 min. After removing the supernatant, added PBS again to 2 mL and centrifuged for 10 min (8000 rpm). Repeated this process 5 times to obtain blood cells from the serum. Dispersed the lower red blood cells in 10 mL of PBS as a blood cell standard solution. 200 μL of the standard solution was taken and transferred it to 800 μL of PBS solution containing different concentrations of probes (20-200 μM) as the test sample. 200 μL of standard solution was added to 800 μL of deionized water as a positive control, and 200 μL of standard solution was added to 800 μL of PBS as a negative control. The samples were left standing for 4 h and then centrifuged. The supernatant was placed in a 96-well plate, characterized by a microplate reader, and the hemolysis rate was calculated according to the following formula:

$$Hemolysis\ rate = (A_{sample} - A_{PBS}) / (A_{water} - A_{PBS}) * 100\% \quad \textbf{(1-2)}$$

Where: A_{sample}, A_{water} and A_{PBS} are the absorbances of the sample to be tested, water and PBS at 540 nm, respectively. The hemolysis of **SiR-FH** at different concentrations was characterized by the hemolysis rate.

Imaging of exogenous HClO in cells

HeLa cells were digested with trypsin and uniformly dispersed in 35 mm laser confocal culture dishes, each containing approximately 2×10^5 cells. The culture dishes were then placed in a carbon dioxide incubator for 24 h until the cells adhered. The adherent HeLa cells were divided into four groups. The first group of cells served as a control group and were incubated in medium containing **SiR-FH** (10 μM) for 60 min. The remaining three groups of cells were first incubated with **SiR-FH** (10 μM) for 60 min, and then switched to medium containing different concentrations of exogenous HClO (10, 30 and 50 μM). After 30 min of incubation, the medium was changed to normal medium to reduce cell damage from exogenous HClO.

Imaging of endogenous HClO in cells

RAW264.7 cells were divided into three groups. The first group served as a control group and was co-incubated with **SiR-FH** (10 μM) for 60 min. The second group of cells were first co-incubated with **SiR-FH** (10 μM) for 60 min and then LPS (1 μg/mL) was added to the culture medium. The third group as a positive control group, RAW264.7 cells were first co-incubated with **SiR-FH** (10 μM) for 60 min, and then exogenous HClO (50 μM) was added.

Photostability test

The HeLa cells in confocal culture dishes were divided into two groups. The first group of cells was co-incubated with **SiR-FH** (10 μM) for 60 min, followed by the addition of exogenous HClO (50 μM) to turn on the fluorescence. The second group of cells were co-incubated with commercial mitochondrial dye Rhodamine 123 (5 μM) for 30 min. Photostability testing was performed by continuous laser irradiation for 300 s. Confocal fluorescence imaging was performed every 10 s during laser irradiation, and the fluorescence images were batch processed using ImageJ to quantify fluorescence intensity.

Construction of neuroinflammation mouse and fluorescence imaging

A model of neuroinflammation in the brain was constructed using C57BL/6 mice. 8-weeks-old female C57BL/6J mice (n = 6) were provided by Beijing Hfk Bioscience Co., Ltd, were randomly divided into two groups. The weight of each mouse was 17-20 g. The control group (n = 3) was intraperitoneally injected with normal saline (5 mL/Kg) every day, and the process lasted 7 days. The experimental group (n = 3) was intraperitoneally injected with LPS (0.25 mg/Kg) saline solution to induce neuroinflammation, and the process lasted 7 days. The injection time is between 15:00 and 16:00 every day. All mice were injected with **SiR-FH** (1.5 mg/Kg) saline solution through the tail vein. After 5 minutes of anesthesia, the mouse brain was imaged using

the IVIS Spectrum Imaging System and the fluorescence intensity was quantified by software ImageJ-Win64.

General procedure for detection of TNF-α and IL-1β concentration

Accurately weigh the weight of mice brains and add 9 times of the volume of homogenizing medium (0.86% or 0.9% normal saline) according to the proportion of weight (mg): volume (UL): 1: 9. Under the condition of ice water bath, mechanical homogenization is prepared into 10% homogenizing solution, 2500-3000 RPM, centrifugation for 10 minutes. After treatment, the supernatant was collected for detection of TNF-α concentration using TNF-α ELISA kit (EK282/4-96) and IL-1β ELISA kit (EK201B/3-96) according to the manufacturer's instructions.

Histological staining of the tissue slices

Killed the mice and the brains were collected for tissue analysis. Though a series of standard procedures, including fixation in 10% neutral buffered formalin, embedding into paraffin and sectioning at 3 μm thickness, the tissues were stained with hematoxylin-eosin (H&E). Thereafter, the prepared slices were observed with microscope inspection, image acquisition and analysis.

Synthesis of the Probe

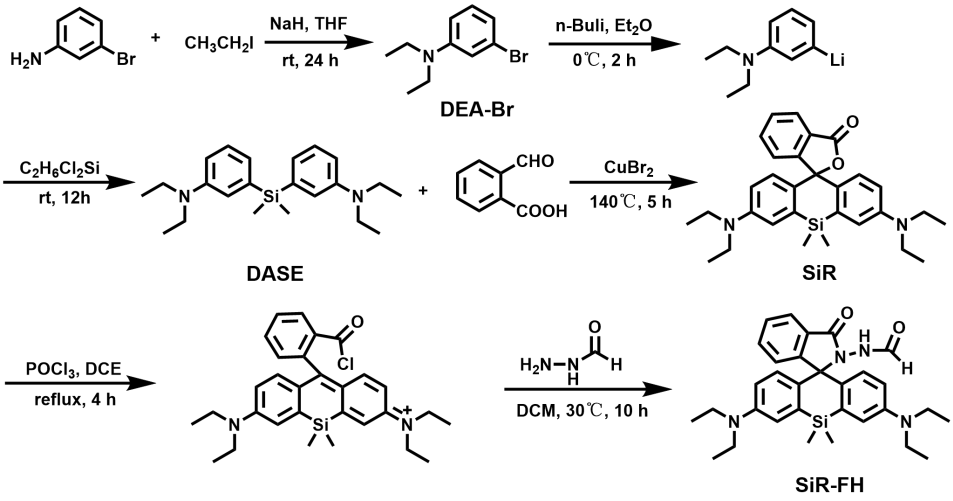


Fig. S1. Synthesis of probe **SiR-FH**.

Synthesis of DEA-Br

Pretreated the mineral oil dispersion of sodium hydride with cyclohexane. 2.50 g of sodium hydride mineral oil dispersion was added to a 250 mL double-necked flask and stirred with 30 mL of cyclohexane for 5 min, then stopped stirring and let stand. The upper layer of cyclohexane was removed and quenched with ethanol, repeated three times to increase the reaction contact area by wash away the mineral oil on the surface of sodium hydride. Tetrahydrofuran was added to the treated sodium hydride and placed in an ice-water bath at 0 °C, to which m-bromoaniline (4.27 g, 25 mmol) was added slowly dropwise and stirred continuously in the ice-water bath for 30 min. After the solution turned brown-black, ethyl iodide (9.82 g, 63 mmol) was added and stirred at room temperature for 24 h until the reaction solution turned brown and no longer changed color. After washing with water and drying, **DEA-Br** was obtained through purification with column chromatography (PE/ DCM = 80/1-60/1) as yellow oil (4.15 g, 73% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.06 (t, *J* = 8.1 Hz, 1H), 6.80 (s, 1H), 6.76 (d, *J* = 7.9 Hz, 1H), 6.59 (d, *J* = 6.2 Hz, 1H), 3.35 (q, *J* = 7.0 Hz, 4H), 1.18 (t, *J* = 7.0 Hz, 6H).

Synthesis of DASE

Added **DEA-Br** (3.41 g, 15 mmol) and 40 mL of anhydrous ether to a dry 250 mL two-necked flask. Cooled the mixture with liquid nitrogen to solidify and used the Schlenk line to repeatedly pump nitrogen to ensure that the mixture was in the anhydrous and anaerobic state. Then transferred to an ice-water bath at 0 °C, carefully took 2.40 mol/L of n-butyllithium (6.55 mL, 15.72 mmol), slowly added it to the reaction system and continued stirring for 2 h. Dichlorodimethylsilane (1.10 mL, 9 mmol) was added, and the mixture was stirred at room temperature for 12 h. The solution changed from pale yellow to pure white and solid was precipitated. **DASE** was obtained through purification with column chromatography (PE/EA = 50/1-40/1) as yellow oil (2.18 g, 38% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.27 – 7.20

(m, 2H), 6.89 (d, *J* = 2.8 Hz, 2H), 6.87 (d, *J* = 7.1 Hz, 2H), 6.73 (dd, *J* = 8.4, 2.8 Hz, 2H), 3.35 (q, *J* = 7.0 Hz, 8H), 1.16 (t, *J* = 7.0 Hz, 12H), 0.55 (s, 6H).

Synthesis of SiR

DASE (387 mg, 1.09 mmol), Carboxybenzaldehyde (819 mg, 5.46 mmol) and cupric bromide (38 mg, 0.17 mmol) were added to a 100 mL glass thick wall pressure tube and heated to 140 °C and continue stirring for 5 h. After cooling, the reactant was transferred to dichloromethane with an ultrasonic machine, washed with water and dried to obtain a dark green solution. Purify using column chromatography (with a small amount of triethylamine added to prevent tailing) (PE/EA/TEA = 80/2/1). **SiR** will appear blue on a weakly acidic silica gel column, and emit red fluorescence under fluorescent light, which is easy to distinguish from impurities. **SiR** (247 mg, 47% yield) was obtained after chromatographic separation. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 (d, *J* = 7.7 Hz, 1H), 7.68 (t, *J* = 7.5 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 6.94 (s, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 6.50 (d, *J* = 9.1 Hz, 2H), 3.38 (q, *J* = 7.1 Hz, 8H), 1.17 (t, *J* = 7.1 Hz, 12H), 0.64 (d, *J* = 7.8 Hz, 6H).

Synthesis of SiR-FH

Phosphorus oxychloride (460 mg, 3 mmol) was slowly added to 10 mL of 1, 2-dichloroethane solution containing **SiR** (484 mg, 1 mmol), and refluxed at 80 °C for 4 h, the reaction solution turned dark blue. After cooling, the reaction solvent was distilled under reduced pressure, and the obtained product was dissolved in anhydrous dichloromethane. Formohydrazide (180 mg, 3 mmol) and 200 μL of N, N-diisopropylethylamine were slowly added to the reaction system and stirring was continued at 30 °C for 10 h. The reaction solution changed from dark blue liquid to pale yellow transparent liquid. **SiR-FH** was obtained through purification with column chromatography (DCM/MeOH = 50/1) as pale-yellow oil (130 mg, 27% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 (d, *J* = 7.4 Hz, 1H), 7.59 – 7.44 (m, 3H), 7.09 (d, *J* = 7.2 Hz, 1H), 6.80 (s, 2H), 6.56 (s, 4H), 3.37 (q, *J* = 7.1 Hz, 8H), 1.19 (t, *J* = 7.0 Hz, 12H), 0.53 (d, *J* = 18.6 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ

167.67, 165.62, 153.33, 146.34, 136.78, 133.82, 129.43, 128.59, 128.04, 127.78, 124.20, 123.85, 114.65, 114.04, 73.49, 44.16, 12.59. HR-MS (MALDI-TOF): calcd for 527.283679, found 527.283774.

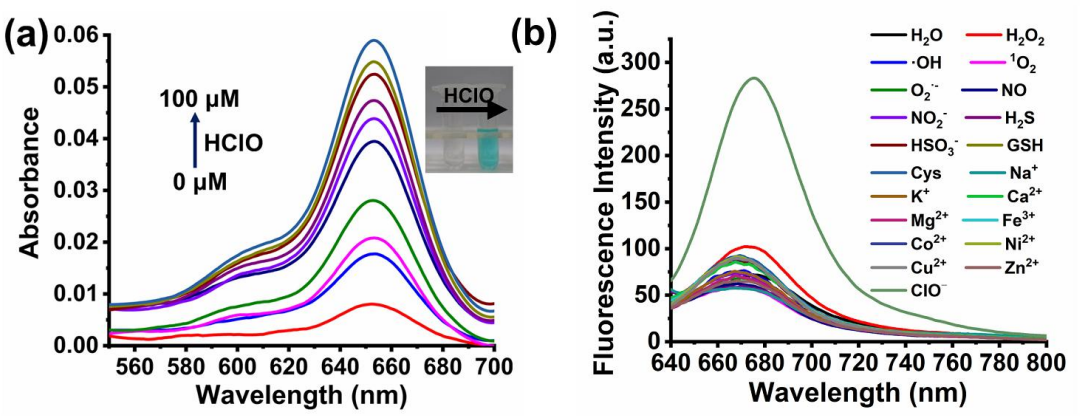


Fig. S2. (a) UV-Vis absorption spectra of **SiR-FH** (10 μM) with HClO (0-100 μM) in aqueous solution, the inset image showed the colour change before and after the addition of HClO. (b) Fluorescence intensity change of **SiR-FH** (10 μM) in aqueous solution under the interference (50 μM) of H_2O_2, ·OH, $^1O_2$, $O_2^{\cdot-}$, NO, NO_2^-, H_2S, HSO_3^-, Cys, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, except GSH (1 mM), λ_{ex} = 610 nm.

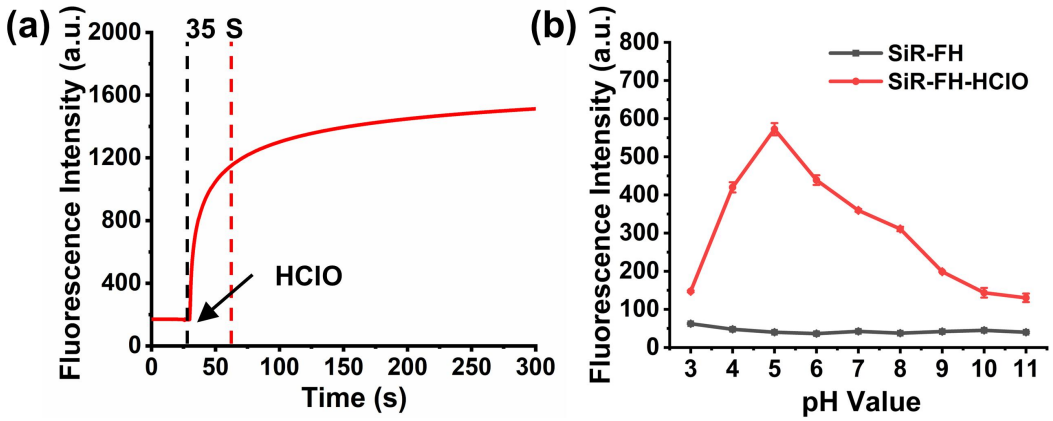


Fig. S3. (a) Time-dependent change in the fluorescence intensity of **SiR-FH** at 669 nm, λ_{ex} = 610 nm. (b) Change in fluorescence intensity of **SiR-FH** (10 μM) and **SiR-FH-HClO** (10 μM **SiR-FH** with 50 μM HClO) in buffer solutions of different pHs, λ_{ex} = 610 nm.

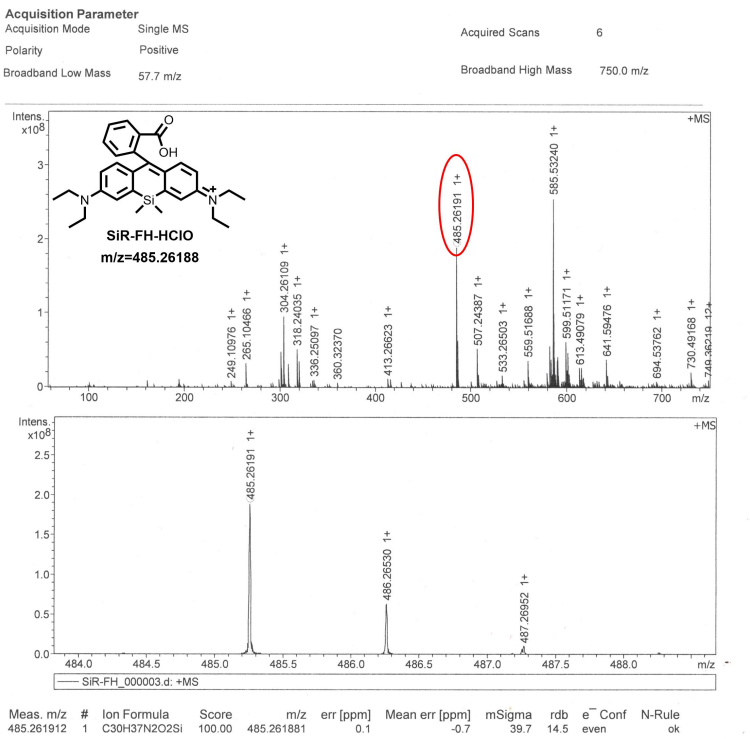


Fig. S4. ESI-FTICR-MS of the reaction product of **SiR-FH** with HClO.

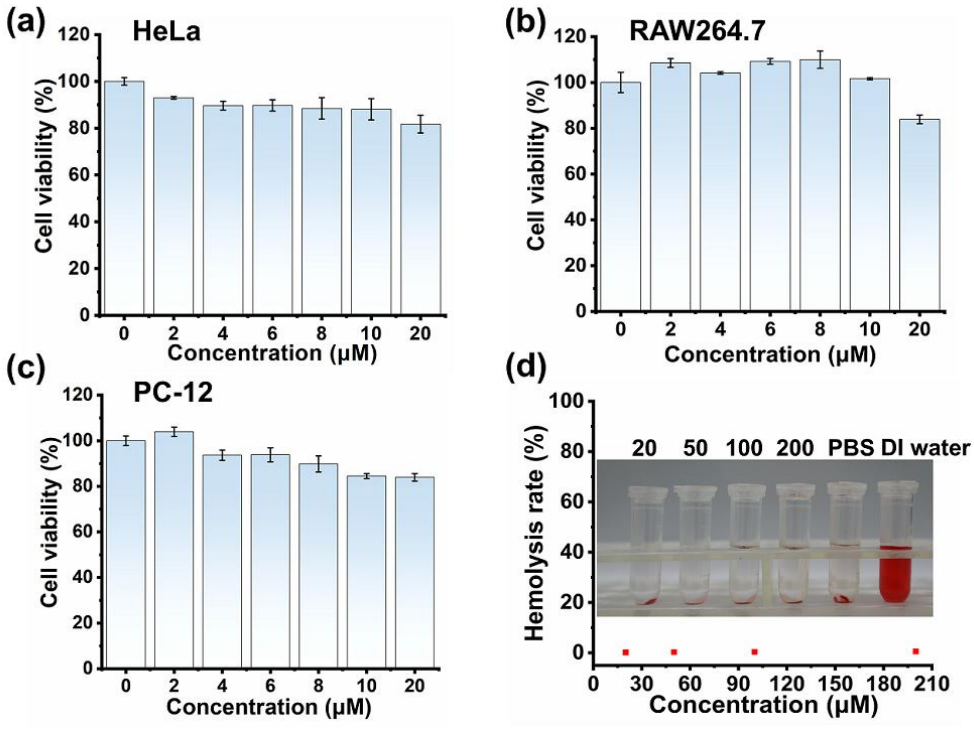


Fig. S5. Cell viability of (a) HeLa cells, (b) RAW264.7 cells and (c) PC-12 cells in different concentrations of **SiR-FH** (0-20 μM). (d) Hemolysis analysis of red blood cells treated with different concentration of **SiR-FH** (0-200 μM). Inserted picture represented the hemolysis result.

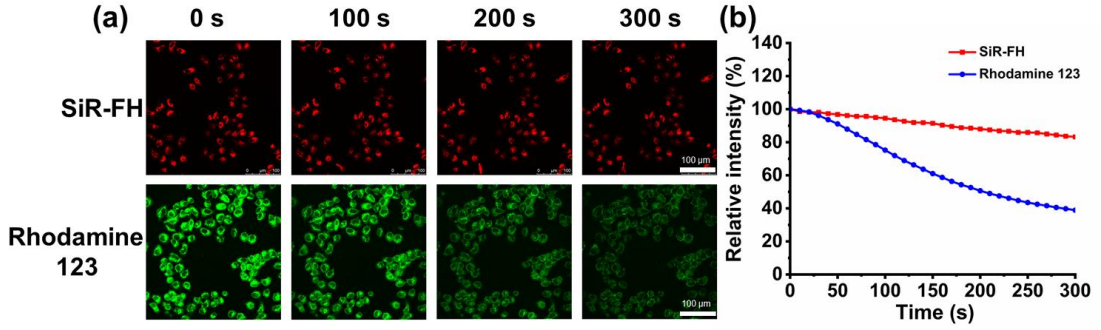


Fig. S6. (a) Confocal fluorescence images of **SiR-FH** (10 μM) and Rhodamine 123 (5 μM) in HeLa cells under continuous laser irradiation for 300 s, the excitation wavelengths of the red/green channels are 633/488 nm, scale bar: 100 μm. (b) Variation curve of fluorescence intensity of two probes with laser irradiation time.

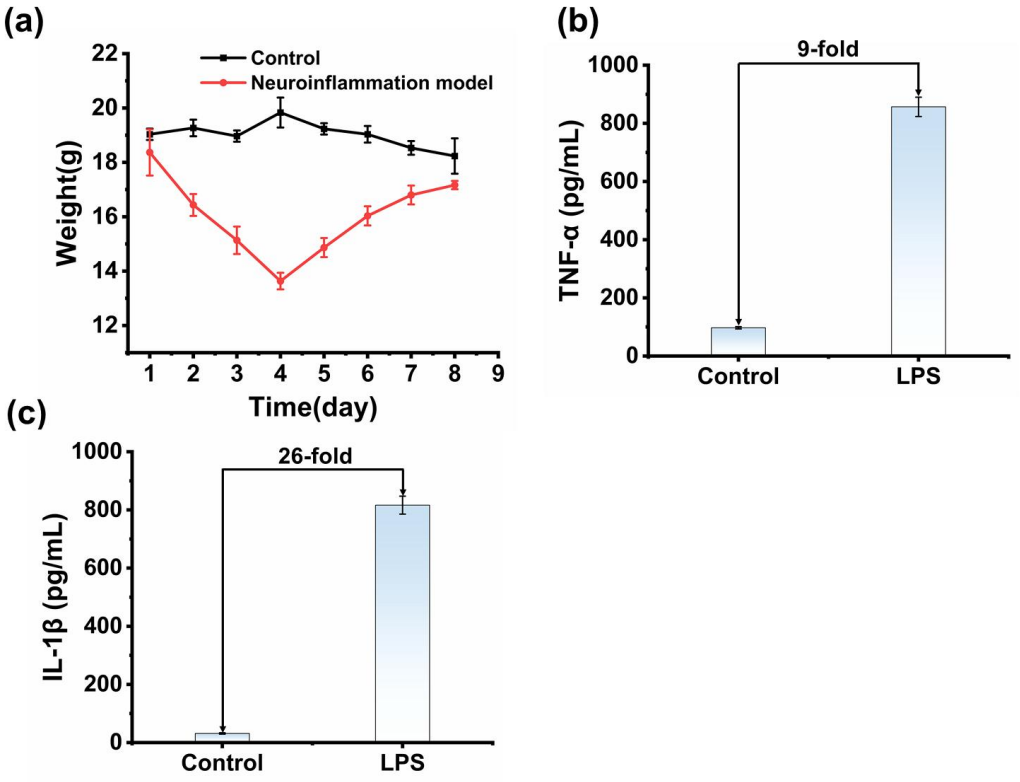


Fig. S7. (a) Body weight change curves of mice. Proinflammatory cytokine (e.g., TNF-α (b), IL-1β (c)) expression analysis of mice after normal saline or LPS injection for 7 consecutive days.

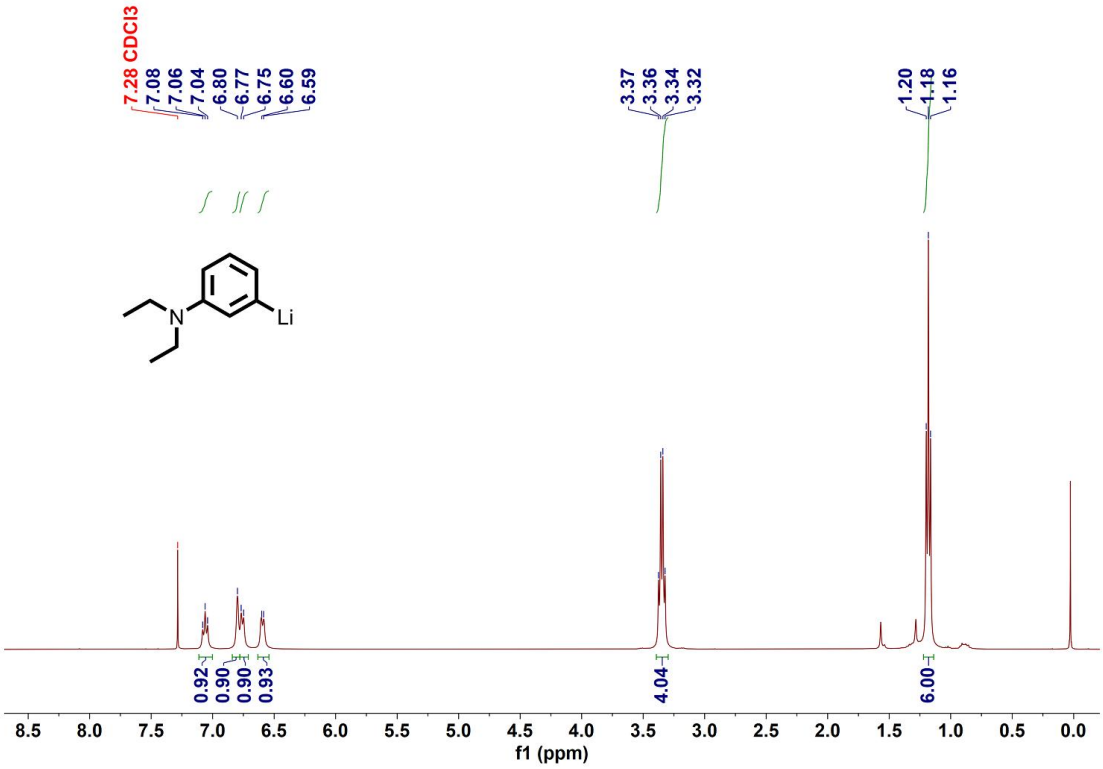


Fig. S8. ^{1}H NMR spectrum of **DEA-Br** in $CDCl_3$.

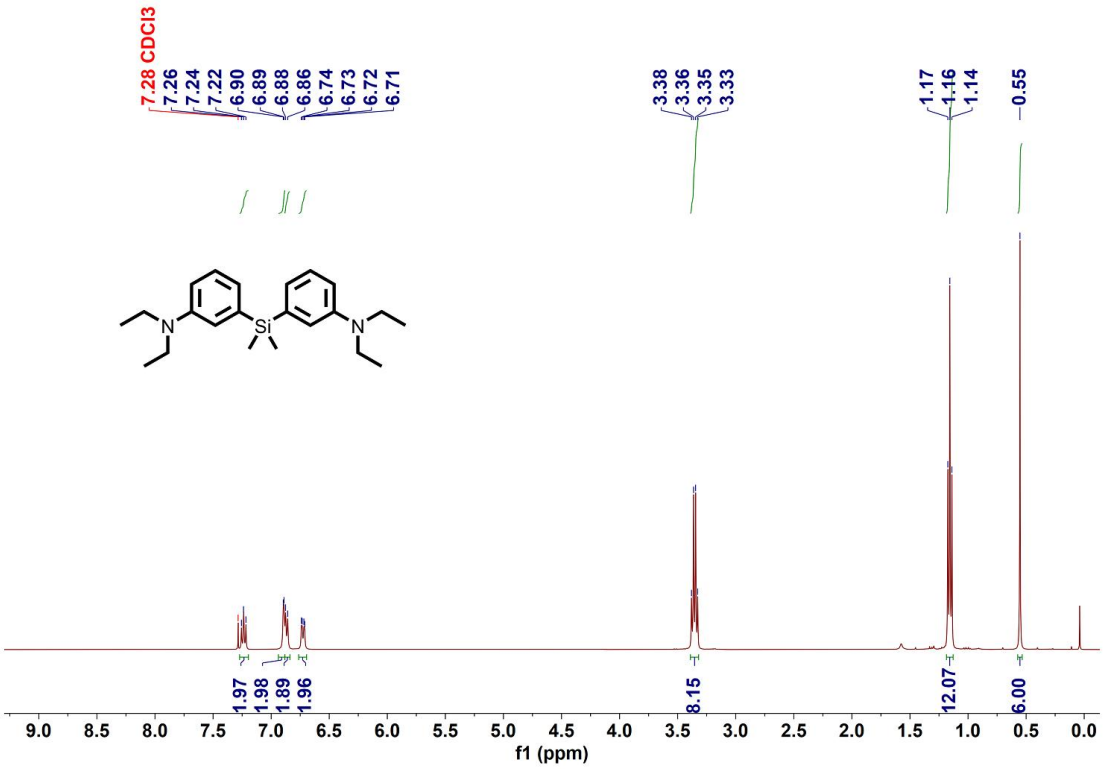


Fig. S9. ^{1}H NMR spectrum of **DASE** in $CDCl_3$.

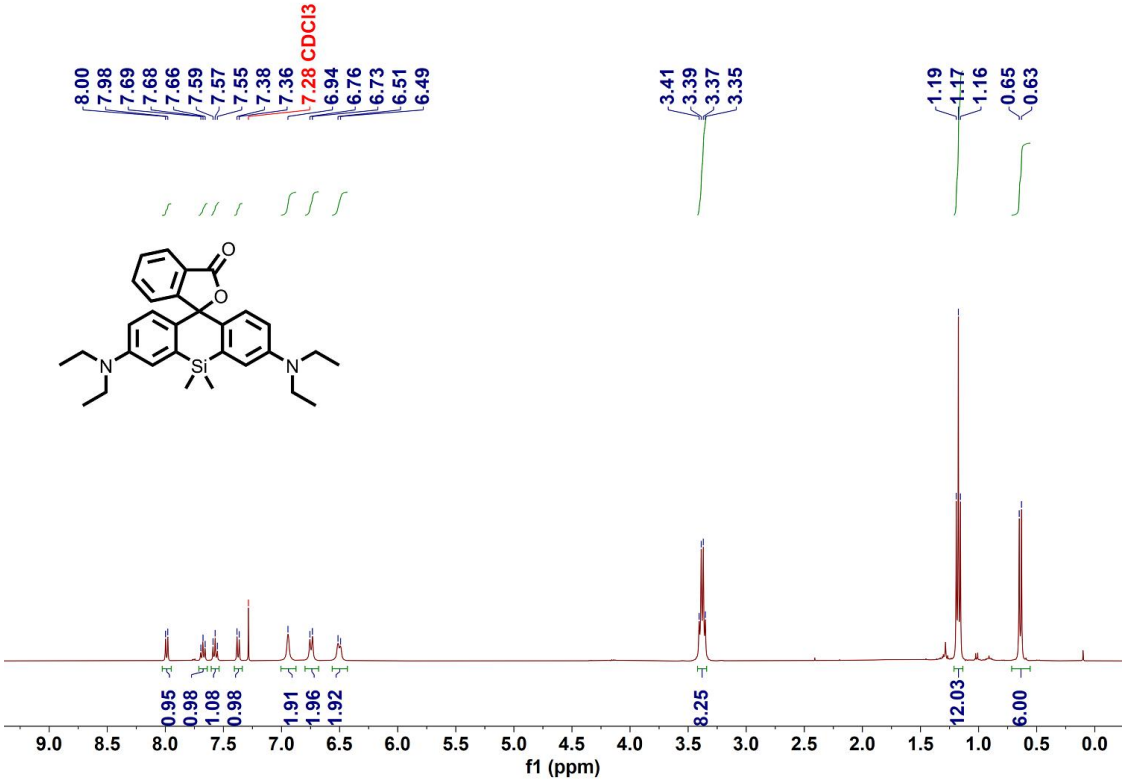
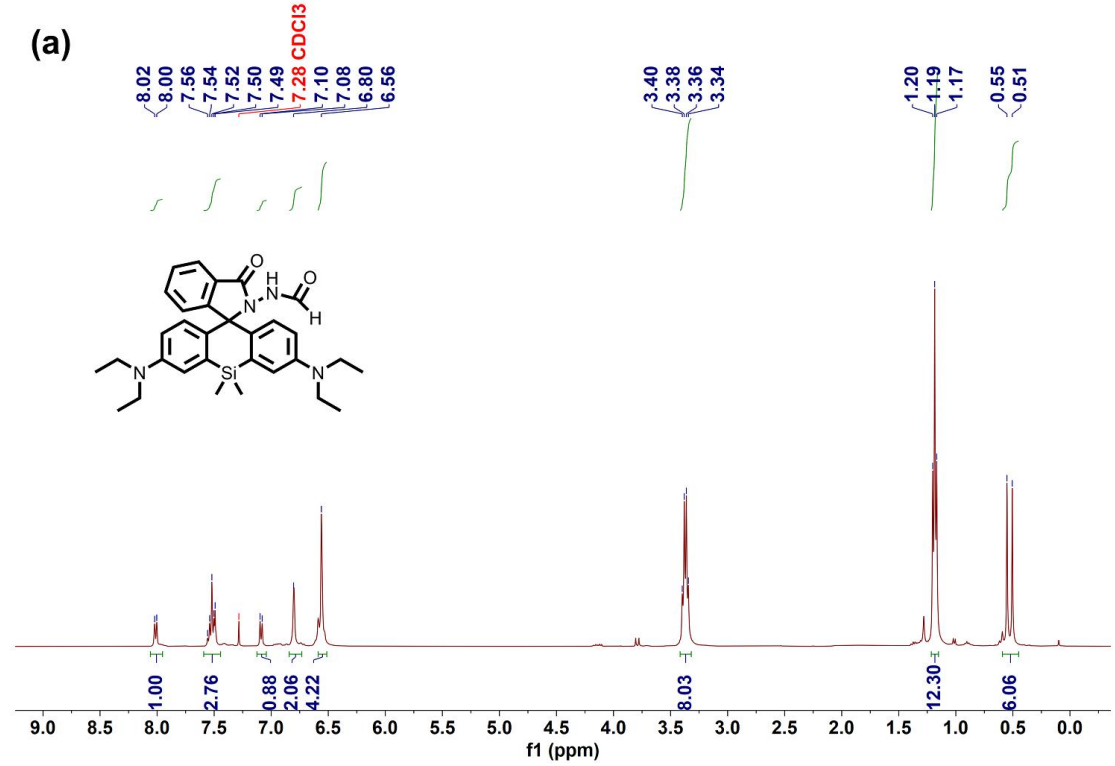


Fig. S10. ^{1}H NMR spectrum of **SiR** in $CDCl_3$.



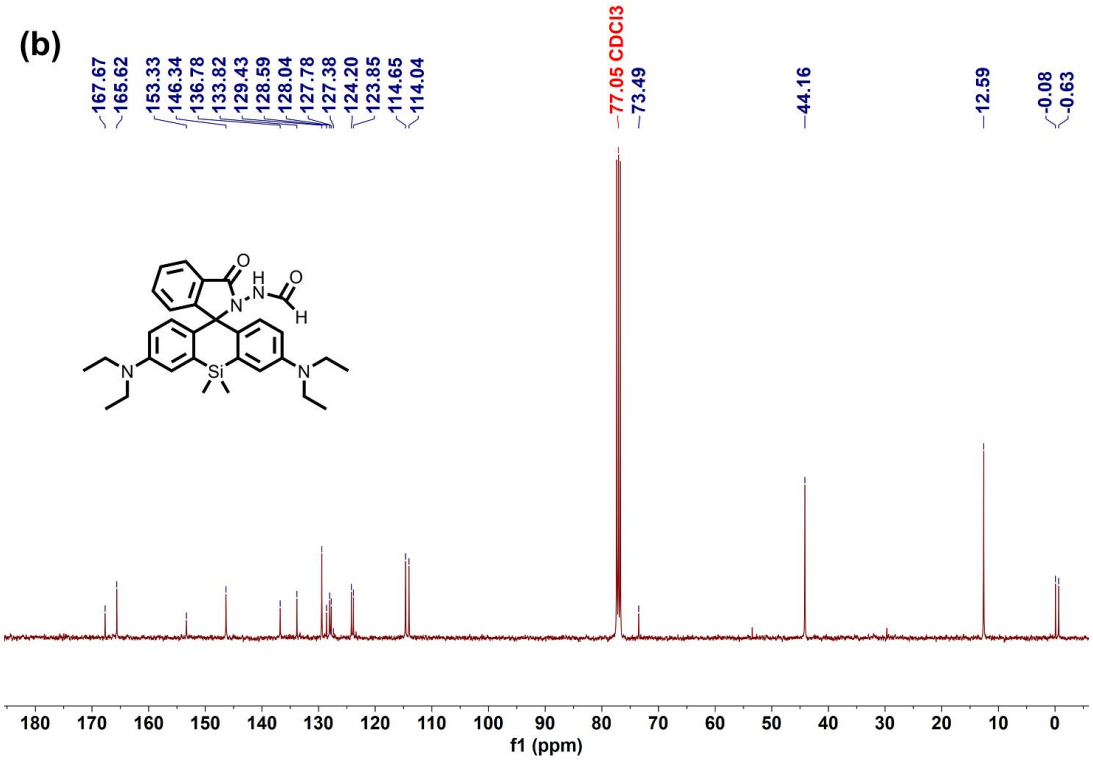


Fig. S11. ^{1}H NMR spectrum (a) and ^{13}C NMR spectrum (b) of **SiR-FH** in $CDCl_3$.

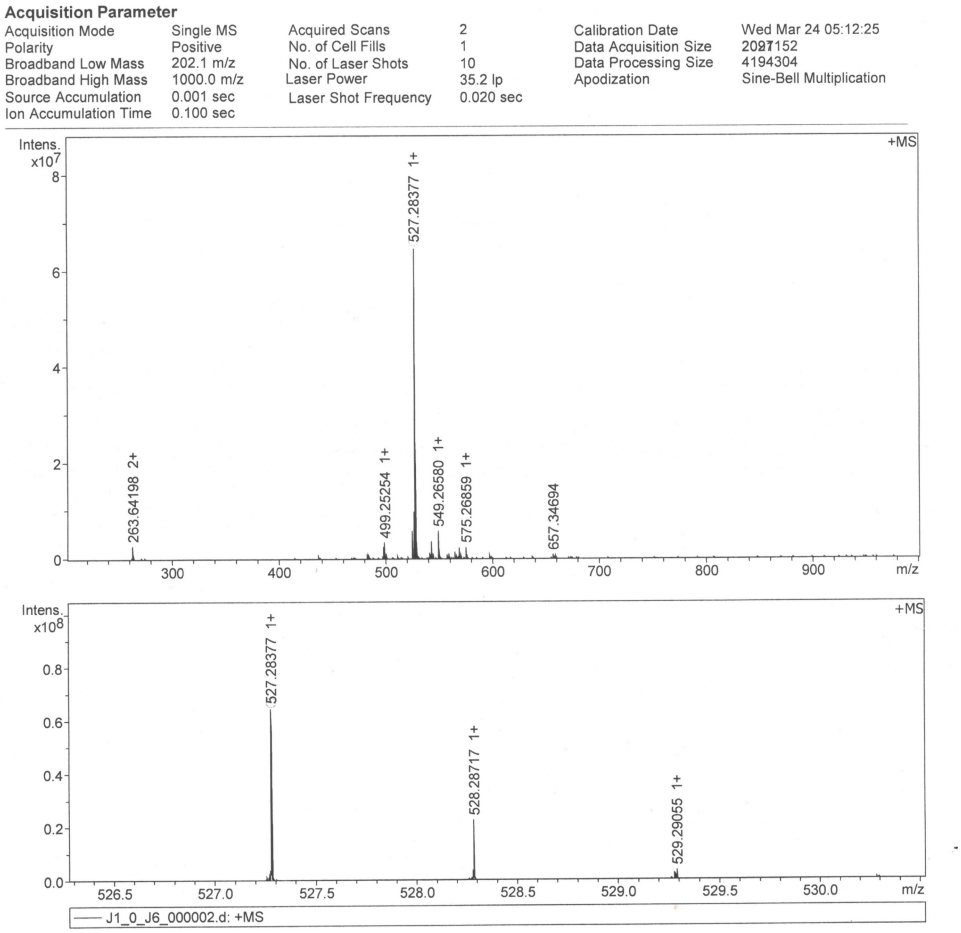


Fig. S12. HR-MS spectrum of **SiR-FH**.