Supplementary Information

One-step synthesis of a dual-functional AIE-active probe for ClO- detection and photodynamic therapy

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1 Experimental

1.1 Analytical instrument

^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded on a Bruker Avance-400 spectrometer (400 MHz, Bruker Co, Germany) in DMSO-d_6 [tetramethylsilane (TMS) as the internal reference. Electrospray ionization mass spectra (ESI–MS) were recorded on a Hybrid Quadrupole-TOF mass spectrometer. High-performance liquid chromatography (HPLC) was carried out using a C18 column (Titank. 4.6 mmID × 250 mmL). The mobile phase was methanol/H_2O (v/v=90:10), the injection volume was 10 μL, the flow rate was 0.8 mL min^{-1} and detection was performed at 380 nm and 25 ℃. The UV-Vis absorption spectrum of the probe was obtained by using a PerkinElmer Lambda 35 UV/Vis system using quartz cuvettes. The fluorescence data were recorded with a fluorescence spectrophotometer (FSP, model: C11367-11, Hamamatsu, Japan). The size and morphology of those as-prepared samples were characterized by transmission electron microscope (TEM) (Hitachi7650B microscope operating at 80 kV). The dynamic light scattering (DLS) was measured via Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK). X-ray photoelectron spectroscopy (XPS) was provided by the VGESCALAB 220-IXL spectrometer. Cell imaging was conducted on a confocal laser scanning microscope (CLSM, Zeiss 710 3-channel, Germany).

1.2 Materials and Solvents

Unless otherwise noted, all reagents were obtained from commercial suppliers and were used as received without further purification. 5-bromosalicylicaldehyde, 1,4-dimethylpyridine iodide, were purchased from Heowns Biochemical Technology Co., Ltd. (Tianjin, China). Analytical reagent grade solvents, such as tetrahydrofuran (THF), dimethylsulfoxide (DMSO), dichloromethane (DCM), absolute ethanol, anhydrous ether, piperidine, hydrochloric acid were bought from Damao Chemical Reagent Factory (DM). All the metal ions used are standard solutions of metal ions from Aladdin Industrial Co. Ltd. (Shanghai, China). Furthermore, monitoring of the reaction's progress was performed with F254 thin-layer chromatography (TLC) and visualization of the components was achieved via observation under UV light (365 nm).

1.3 Synthesis of probe BHSMP

First, 5-bromosalicylicaldehyde (200 mg, 1 mmol) and 1,4-dimethylpyridine iodide (282 mg, 1.2 mmol) were dissolved in 30 mL of absolute ethanol. Nitrogen is then flushed into the flask and the piperidine (0.5 mL) was added dropwise to the mixture. Finally, the mixture is refluxed overnight at 78

°C. After the reaction is completed and cooled to room temperature, the mixture is poured into anhydrous ether, and after a period of sedimentation, the precipitation is filtered and precipitated to obtain 285 mg of purple-black solid with a yield of 68%. The characterization results of ^{1}H NMR, ^{13}C NMR and ESI-MS were shown in **Fig. S1−4**, respectively. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.63 (d, J = 6.4 Hz, 2H), 8.07 – 7.91 (m, 3H), 7.66 – 7.56 (m, 2H), 7.16 (dd, J = 8.9, 2.6 Hz, 1H), 6.62 (d, J = 8.9 Hz, 1H), 4.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 164.96, 154.25, 144.80, 138.57, 134.44, 131.59, 124.76, 122.85, 121.74, 120.62, 105.55, 46.85. ESI-MS (ESI) m/z calculated for $C_{14}H_{13}BrNO^{+}$ $[M]^{+}$ 290.02, found $[M]^{+}$ 290.02.

1.4 Photophysical property

The photophysical properties of the 5-bromosalicylaldehyde was studied by Fluorescence spectrometer (FL). As shown in **Fig. S8**, the excitation and emission spectrum of 5-bromosalicylaldehyde in the mixture of dimethyl sulfoxide (DMSO) and water solution were located at 375 and 500 nm. There is almost no fluorescence signal in the pure DMSO solution, while a strong green fluorescence signal is emitted in the mixed solution of DMSO and H_2O, and the fluorescence intensity of 5-bromosalicylaldehyde reaches the maximum when the water ratio fraction is 50%, indicating that it has obvious AIE characteristics.

1.5 Light-triggered ROS generation

To demonstrate the potential photodynamic therapeutic role of BHSMP upon photoexcitation, their ROS-generating capacity was studied. The commonly used ROS indicator 2′,7′-dichlorodihydro-fluorescein diacetate (DCFH-DA) could be used to measure the efficiency of their production. DCFH-DA ethanol stock solution with a concentration of 1.0 mM was prepared, 1 mL of mother liquor was mixed with 4 mL of 0.01 mM sodium hydroxide solution, stirred at room temperature in the dark for 30 min, and then diluted to 1 μM with PBS (pH 7.4) for later use. The fluorescence spectra of DCFH blank and DCFH+BHSMP were measured at 1 min intervals under a light of (450 nm, 60 mW cm^{-2}). The fluorescence intensity was significantly enhanced after the addition of BHSMP, indicating that BHSMP had ROS production efficiency.

2 Cellular study

2.1 Cell viability

The cytotoxicity of BHSMP NPs was evaluated by a standard cell counting kit-8 (CCK-8) assay. U-2OS cancer cells were inoculated in 96-well plates with a density of 5 $\times 10^{3}$ cells/well, and Dulbeccos

Modified Eagle Medium (DMEM) containing 10% fetal bovine serum. The cells were cultured in cell culture box for another 24 h until the cell fusion rate reached over 80 % and completely adhered to the wall. Different concentrations of BHSMP NPs (0, 5, 10, 20, 40, 60, 80 and 160 μg/mL) were incubated with U-2OS cancer cells for 24 h, and CCK-8 solution (5 mg/mL) was added to each well. After 3 h, the absorbance of each well was determined at a wavelength of 490 nm. The experiment was repeated three times and the cell viability and standard deviation were calculated by absorbance. The cells without incubation with BHSMP NPs served as the control group.

2.2 Cell imaging

Fluorescence imaging of cells was taken under a confocal scanning microscope (CLSM) using a 450 nm laser. U-2OS cancer cells were incubated with DMEM supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum under a humidified incubator at 37 ℃, in 5% CO_2/95% air. To evaluate the detection of ClO^- in living cells, the U-2OS cancer cells were incubated for BHSMP NPs (50 μg/mL) for 3 h. Afterward, cells were washed three times with PBS to remove BHSMP then ClO^- (50 μM) was added and fluorescence imaging was recorded at 0, 10, 30, 60 min after the addition of ClO^-. The CLSM images were obtained by excited with a 450 nm laser to evaluate the response of the probe toward ClO^-.

2.3 General ROS detection *in vitro*

The production of reactive oxygen species in cells under light conditions was detected with DCFH-DA as an indicator. U-2OS cancer cells are first seeded in 96-well plates with a cell density of 5,000 cells and incubated for 24 h. The probe (concentration 20 μg/mL) was added into the cell culture dish and incubated in the dark for 3 h, then the culture medium was removed and washed three times with PBS buffer solution to remove the remaining culture medium. The DCFH-DA was added and continued incubation with cells for 30 min. After that the cells were carefully washed again with PBS buffer two to three times, The cells were irradiated under a laser of (450 nm 25 mW cm^{-2}) for 5 min. Fluorescence images are captured by fluorescence microscopy. The DCF-DA signal is excited by a 488 nm laser that collects the emitted light in the range of 500-530 nm.

3 Results

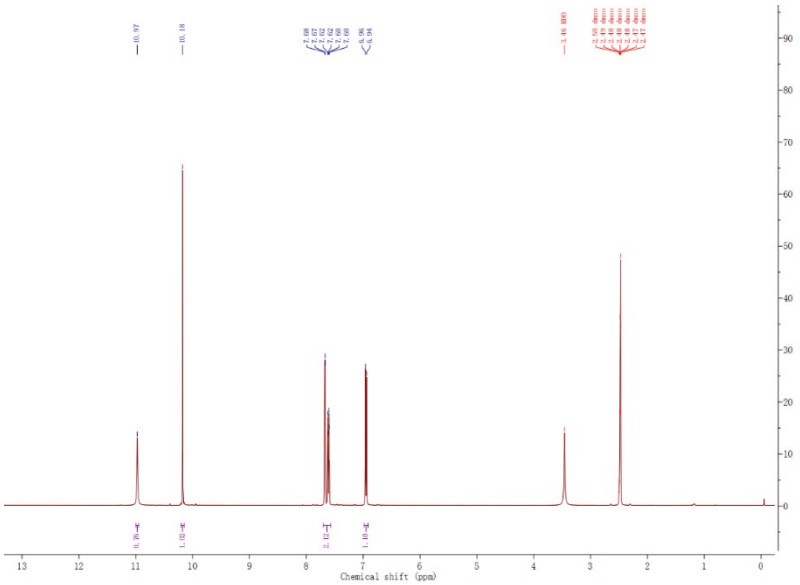

Fig. S1 [1]H-NMR spectrum of 5-bromosalicylaldehyde in DMSO-d_6.

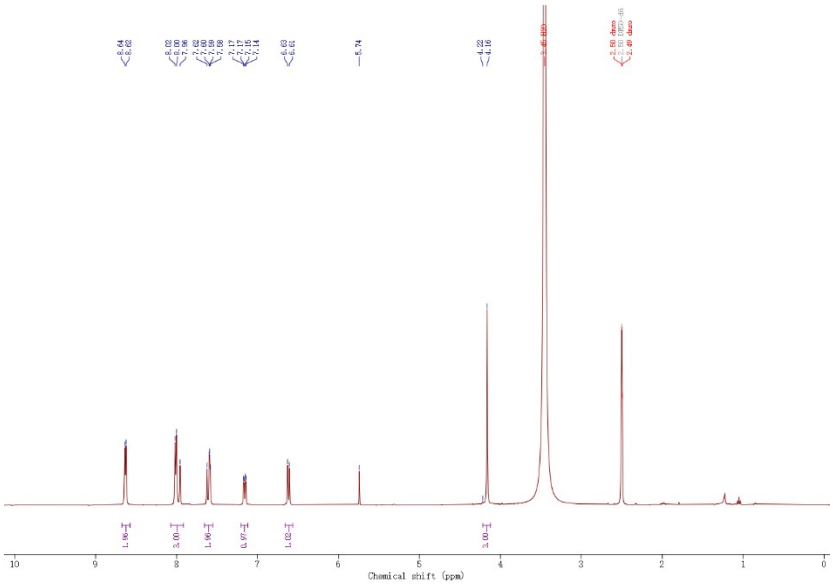

Fig. S2 [1]H-NMR spectrum of BHSMP in DMSO-d_6.

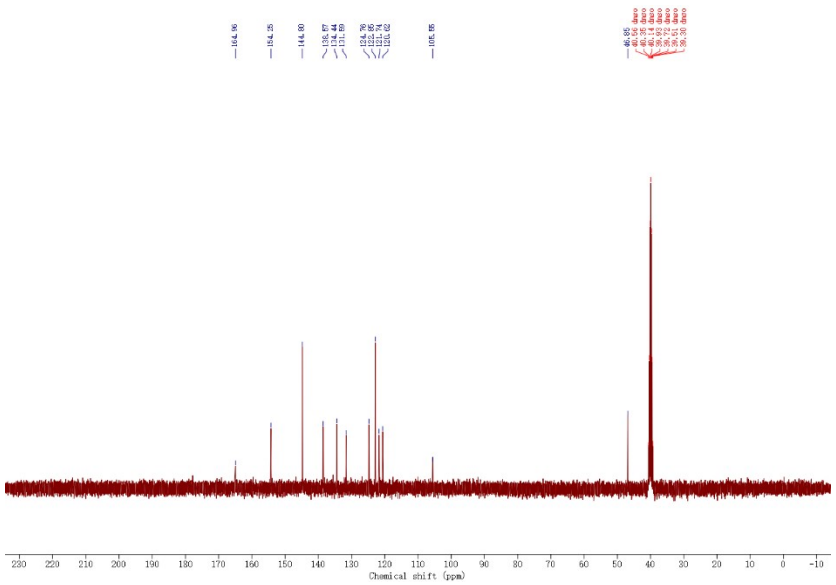

Fig. S3 ^{13}C-NMR spectrum of BHSMP in DMSO-d_6.

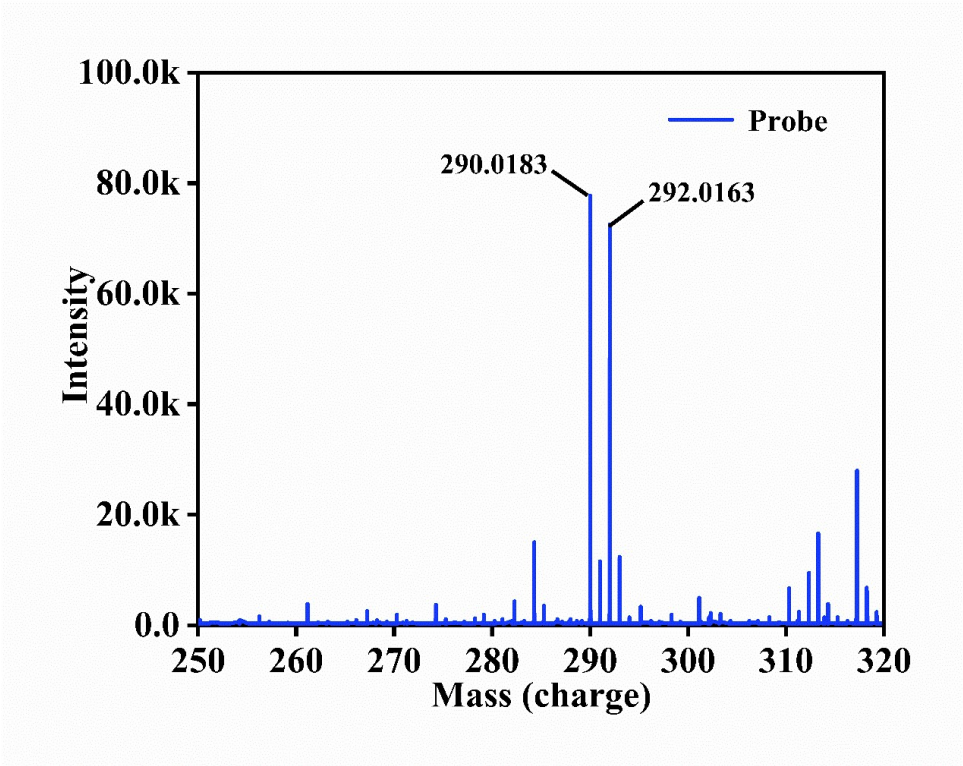

Fig. S4 ESI-MS data of BHSMP.

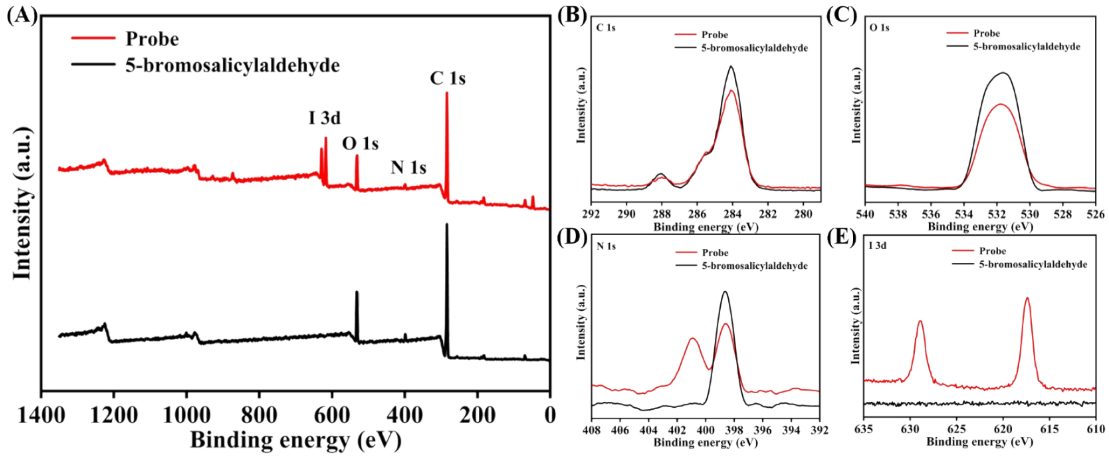

Fig. S5 (**A**) XPS spectra of BHSMP; (**B**) C 1s spectrum; (**C**) O 1s spectrum; (**D**) N 1s spectrum; (**E**) I 3d spectrum.

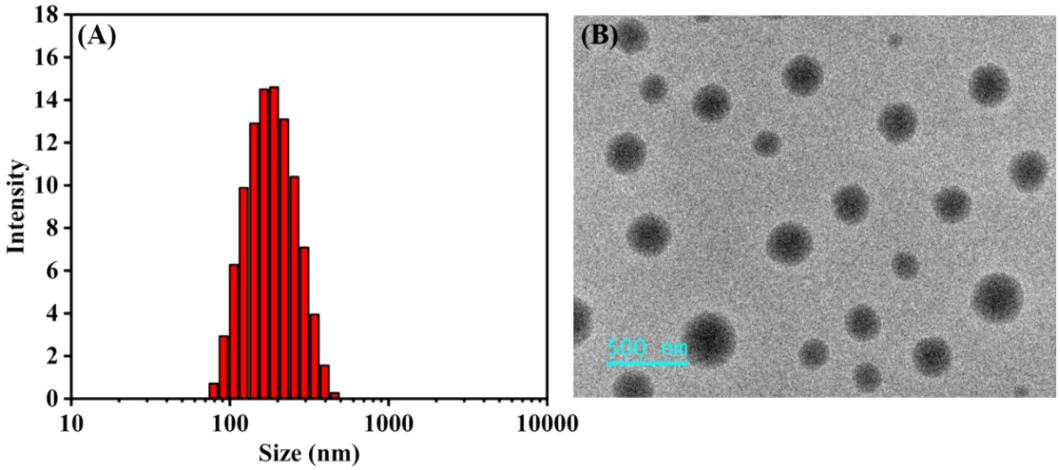

Fig. S6 (**A**) The DLS measurement of BHSMP in water, shows that the size distribution of the nanoparticles was about 200 nm with a polydispersity index of 0.22. (**B**) The TEM image of self-assembled nanoparticles, BHSMP NPs, the scale bar is 500 nm.

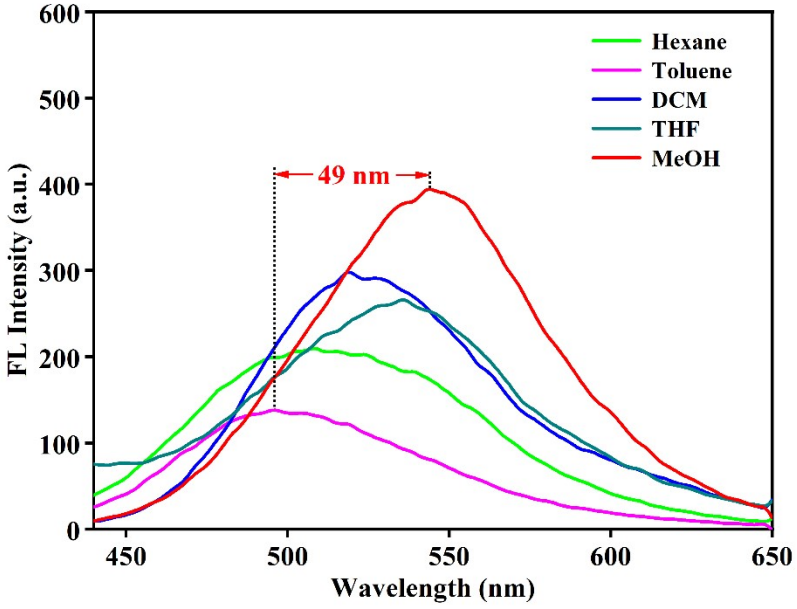

Fig. S7 Fluorescent emission spectra of BHSMP in various solvents. (50 μM; Ex=410 nm).

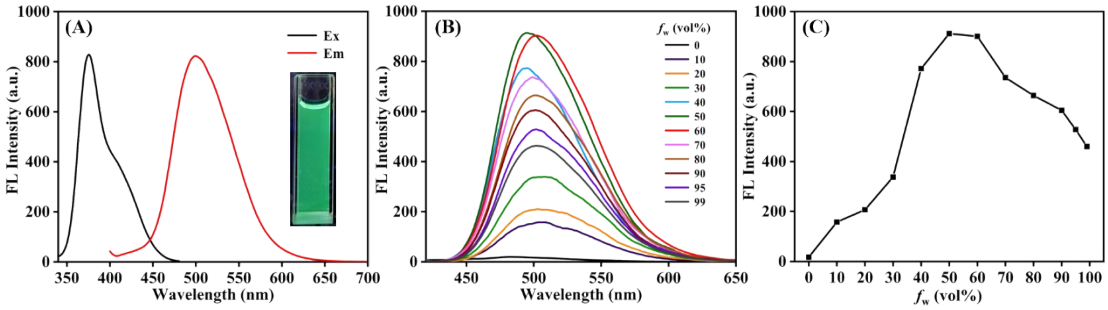

Fig. S8 (**A**) The excitation and emission spectrum of 5-bromosalicylaldehyde in DMSO/H_2O mixture. (Ex/Em 375 nm/500 nm) The inset picture in Figure is the solution of 5-bromosalicylaldehyde under irradiation with UV lamp at 365 nm. (**B**) Emission spectrum of 5-bromosalicylaldehyde (30 μM) in DMSO/H_2O mixtures with different water volume fractions (f_w). (**C**) The relationship of the fluorescence spectrum of 5-bromosalicyl-aldehyde versus water fraction in mixed solution.

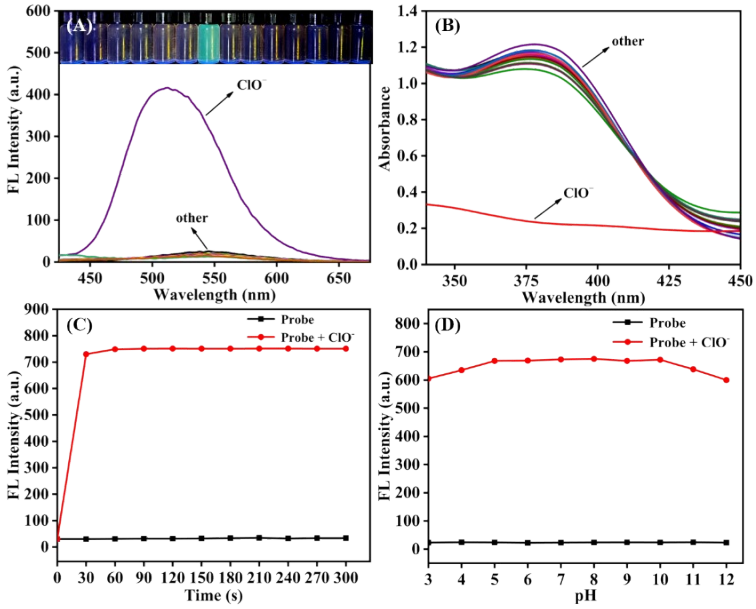

Fig. S9 The fluorescence spectrum (**A**) and the UV-Vis absorption spectrum (**B**) of probe (10 μM) after adding 60 μM ClO^- and other cations (H_2O_2, CO_3^{2-}, HCO_3^-, HSO_3^-, NO_3^-, AcO^-, H_2PO^{4-}, S^{2-}, F^-, Cl^-, Br^-, I^- and ClO^-) in PBS (60 μM, pH 7.4). (**C**) the fluorescence intensity of the probe as a function of reaction time. (**D**) Fluorescence intensity of BHSMP at various pH values.

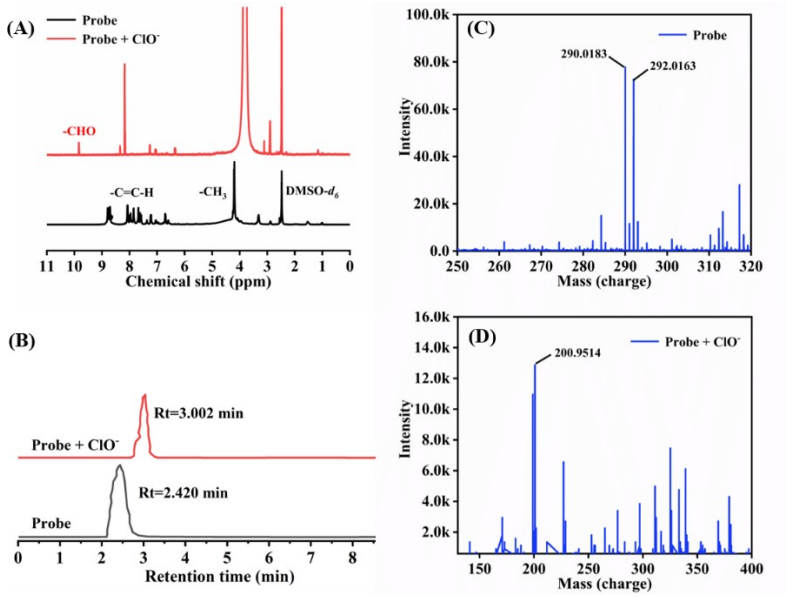

Fig. S10 (**A**) ^1H NMR spectrum of BHSMP in the absence and presence of ClO^-. (**B**) HPLC results of probe BHSMP (50 μg/mL) in the absence (black curve) and presence (red curve) of ClO^- (100 μM). ESI-MS data of (**C**) BHSMP and (**D**) BHSMP + ClO^-.

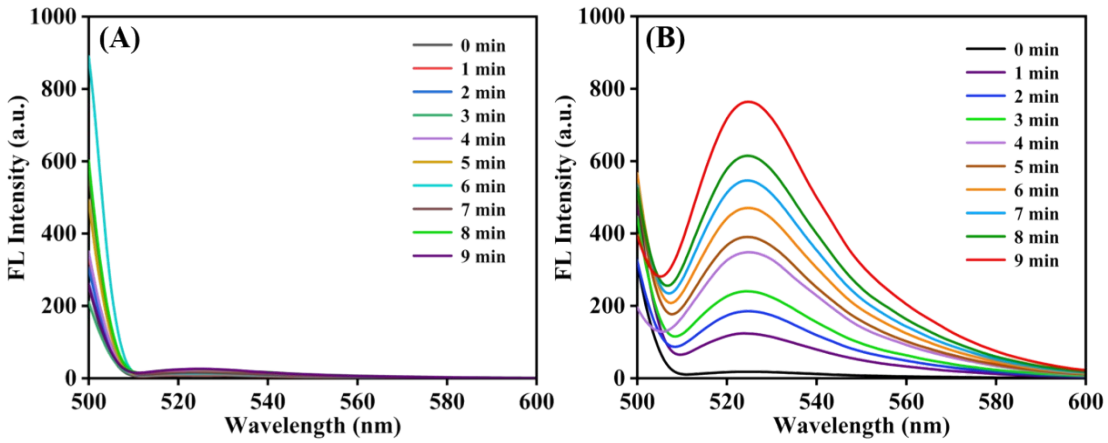

Fig. S11 Fluorescence spectra of the DCFH-DA (1 μM) in the presence of 10 μL BHSMP (50 μg/mL) under irradiation with different time points (**A**) Blank, (**B**) BHSMP.