Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic supplementary information

Design and application of a fluorescent probe based on ESIPT mechanism for the detection of Cys with o-hydroxyacetophenone structure

Experimental Section

Instrumentation and Reagents

Fluorescence spectra were obtained by a F-4600 fluorescence spectrometer (Hitachi, Japan). ^{1}H-NMR spectra were recorded on Avance III 500 NMR spectrometer (Bruker Daltonics Inc., Germany). Matrix-assisted laser desorption/ionization time of flight spectra (MALDI-TOF-MS) were performed on a Bruker Microflex TOF using DCTB as a matrix (Bruker Daltonics Inc., Germany). pH was measured on an INESA Scientific PHS-3C pH meter (INESA Scientific Instrument Co., Ltd., China). Cell imaging experiments were carried out on an LSM 710 laser scanning confocal microscope (Carl Zeiss, Oberkochen, Germany). All reagents were of analytical reagent grade and used without further purification or treatment. All aqueous solutions were prepared using ultrapure water obtained from a Milli-Q water purification system (18.2 MΩ cm).

Human serum samples and cells

Three human serum samples of volunteers were provided by the First Affiliated Hospital of Qiqihar Medical University (Qiqihar, China). All of the experiments were performed in compliance with the relevant laws and institutional guidelines of the People's Republic of China and the ethics committee of the First Affiliated Hospital of Qiqihar Medical University. The volunteer donors of the serum samples consented to the use of the samples for scientific research. The HepG2 cells were obtained from the

First Affiliated Hospital of Qiqihar Medical University.

Synthesis of DHOA

DHO was synthesized according to the method described in the literature.[1] ^{1}H NMR (500 MHz, Chloroform-*d*) δ 12.69 (s, 1H), 7.97 – 7.88 (m, 2H), 7.84 – 7.73 (m, 4H), 7.69 (d, *J* = 15.4 Hz, 1H), 7.59 – 7.50 (m, 1H), 7.08 (d, *J* = 8.7 Hz, 1H), 7.00 (t, *J* = 7.6 Hz, 1H), 3.22 (s, 6H). MALDI-TOF-MS: m/z 267.266.

To a solution of DHO (0.267 g, 1 mmol) in anhydrous CH_2Cl_2 (20 mL) was added triethylamine (0.5 mL) and acryloyl chloride (0.135 g, 1.5 mmol), respectively. The reaction mixture was stirred for 4 h at room temperature. The mixture was then poured into water and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified using silica-gel chromatography with petroleum ether:ethyl acetate (3:1 v/v) as the eluent to afford DHOA (0.27 g, 85% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.69 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.56 – 7.51 (m, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.37 (td, *J* = 7.6, 1.0 Hz, 1H), 7.24 (dd, *J* = 8.2, 1.0 Hz, 1H), 6.97 (d, *J* = 15.8 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 2H), 6.57 (dd, *J* = 17.2, 1.2 Hz, 1H), 6.28 (dd, *J* = 17.3, 10.5 Hz, 1H), 5.96 (dd, *J* = 10.6, 1.2 Hz, 1H), 3.07 (s, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 191.79, 164.33, 148.25, 146.55, 134.63, 133.22, 133.00, 131.73, 130.46, 129.75, 127.55, 126.01, 123.23, 120.99, 112.42, 40.53. MALDI-TOF-MS: m/z 321.265.

Fluorescence Measurements

The stock solution of DHO (1 mM) was prepared by dissolving an appropriate amount of probe in THF. HEPES buffer (1800 μL, 50 mM, pH = 7.4) was mixed with DHO stock solution (10 μL) in a 3-mL centrifuge tube. After that, Cys was added. The final volume of the solution was adjusted to 2 mL by ultra-pure water. The mixture was incubated at 37°C for 10 min to allow the reaction to take place.

Subsequently, it was subjected to measurement of fluorescence spectrum.

Theoretical calculation and analysis

The optimal geometrical structures of the DHO and DHOA were used of the DFT, B3LYP/6-31G(d,p) basis sets, by Gaussian 16 software. The effect of solvent was calculated by SMD model, and water was used as the solvent in the calculation.

Cytotoxicity test

In cytotoxicity tests, HepG2 cells were cultured with a culture density of 0.5×10^4 in a 96-well plate. Probe solutions with different concentrations ranging from 0 to 50 μM were added into the cells on the plate, and then the cells were cultured at 37°C for 24 h. Subsequently, MTT (5 mg/mL) was added, and the cells were then cultured for another 4 h. The cell survival rate was measured and calculated using an enzyme-labeled instrument.

Figures

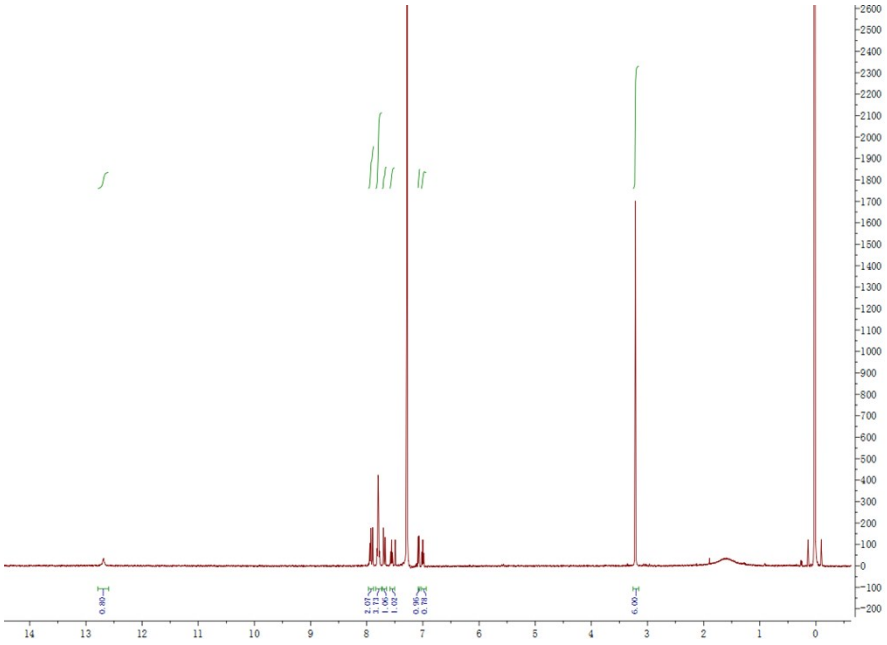

Fig. S1 ^{1}H NMR (500 MHz, Chloroform-*d*) of DHO.

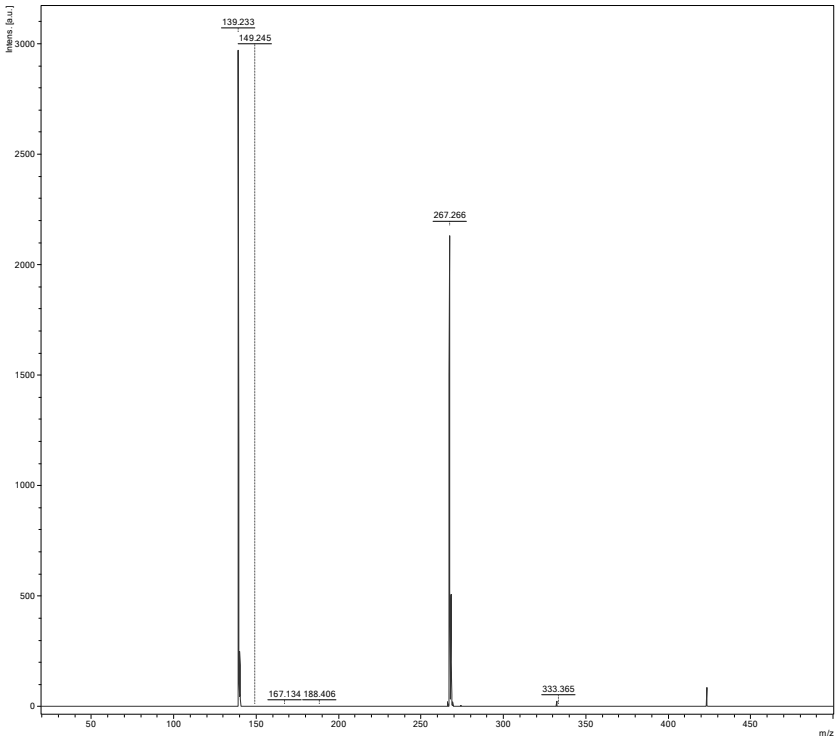

Fig. S2 MALDI-TOF-MS spectrum of DHO.

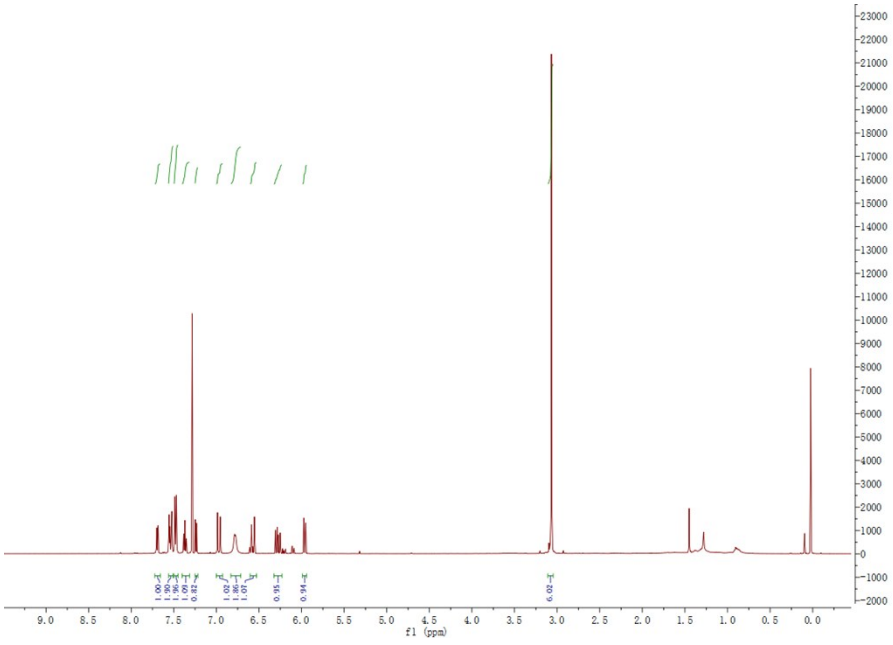

Fig. S3 ^{1}H NMR (500 MHz, DMSO-*d6*) of DHOA.

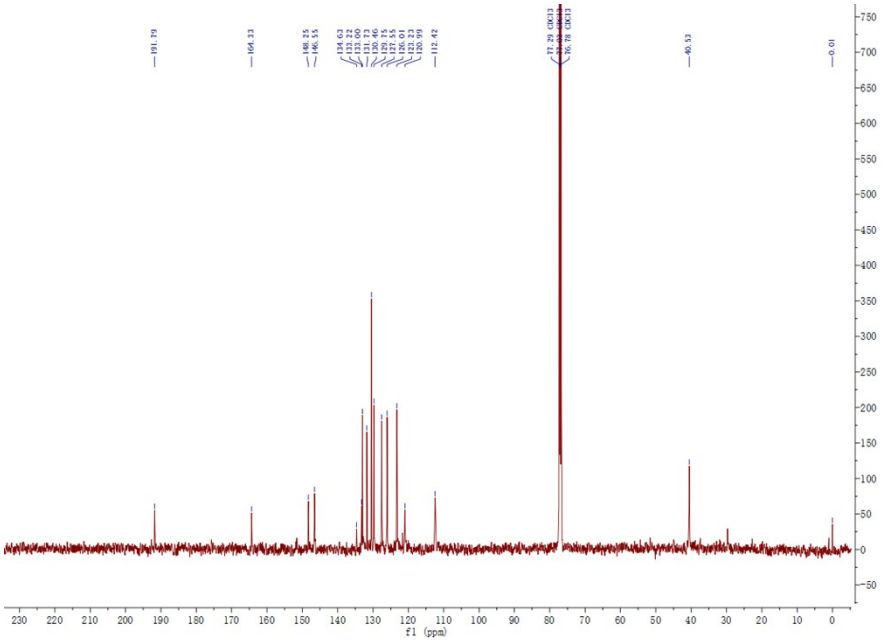

Fig. S4 ^{13}C NMR (126 MHz, Chloroform-*d*) of DHOA.

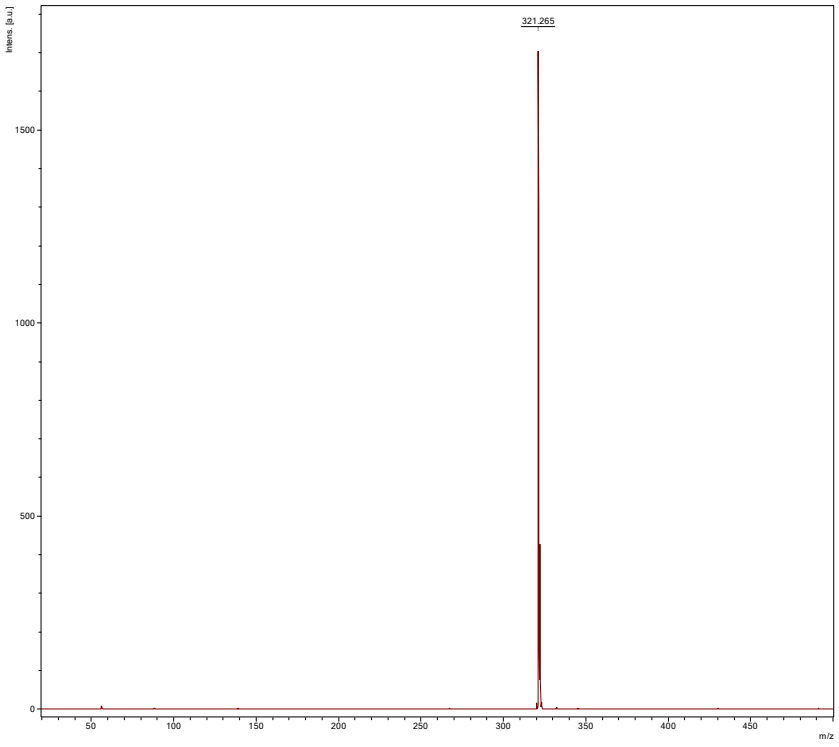

Fig. S5 MALDI-TOF-MS spectrum of DHOA.

References

1. Z. Song, R. T. Kwok, E. Zhao, Z. He, Y. Hong, J. W. Lam, B. Liu and B. Z. Tang, *ACS Appl Mater Interfaces*, 2014, **6**, 17245-17254.