Supporting information for

Mitochondrial-targeted ratiometric fluorescent probe for the detection of sulfur dioxide in living cells

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Materials and instruments

The chemical reagents used in the experiments were all commercially available and could be used without further purification. The water in the experiment was double distilled water. Column chromatography used the silica gel (screen 200-300) purchased from Qingdao Ocean Chemicals Company. NMR spectra were obtained by an AVANCE III 400 MHz digital NMR spectrometer with tetramethylsilane (TMS) as a standard compound. High resolution mass spectra (HRMS) were recorded using a Bruker APEX IV-FTMS 7.0 T mass spectrometer. The UV absorption spectrum were measured with a Shimadzu UV-2600 spectrophotometer. The fluorescence emission spectrum were carried out on a Hitachi F4600 fluorescence spectrophotometer.

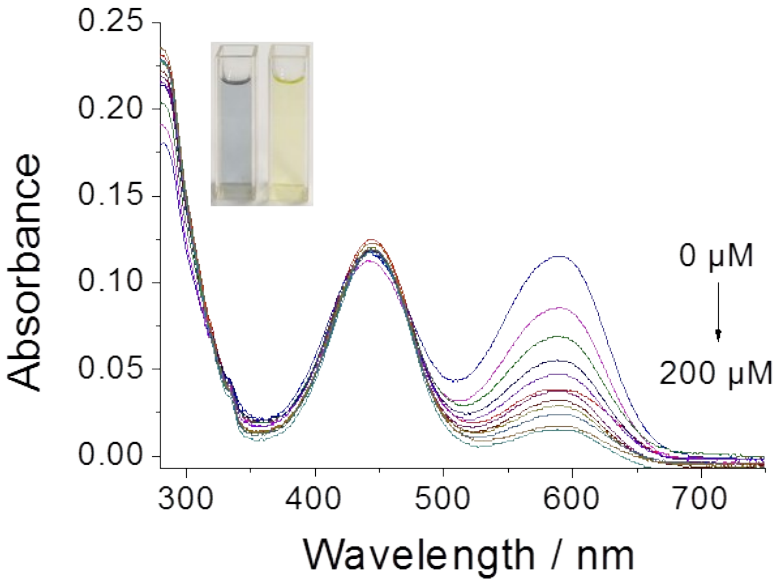

Fig. S1 UV-Vis spectra of 5 μM **MNP** in the absence and presence of Na_2SO_3 in PBS (pH = 7.4, 10% CH_3CN, 20 mM). Inset: Images of the probe in the absence (left) and presence (right) of 100 μM Na_2SO_3.

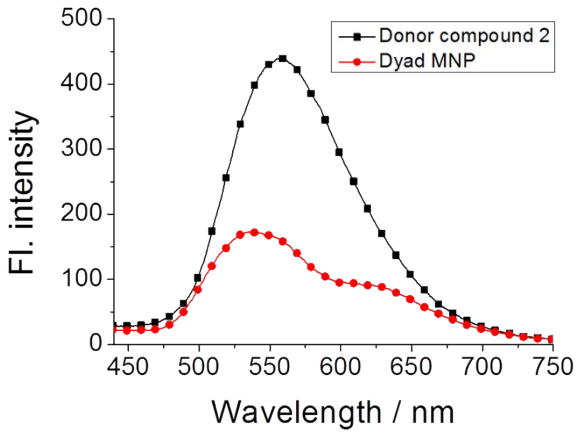

Fig. S2 Emission spectra of donor compound 2 (5 μM, red circle) and dyad **MNP** (5μM, blacks quare) in aqueous solution (pH = 7.4, 10% CH_3CN, 20 mM).

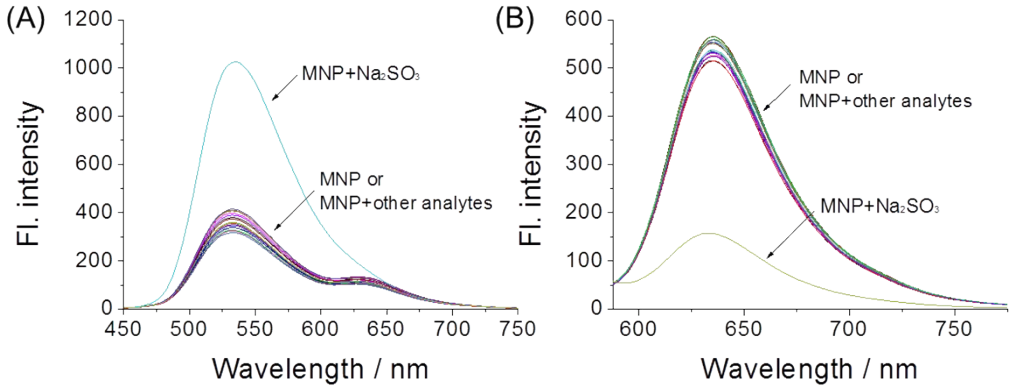

Fig. S3 Fluorescence spectra of 5 μM **MNP** in the presence of various species in PBS buffer (pH = 7.4, 10% CH_3CN, 20 mM) under excitation at 405 (A) and 561 nm (B). Concentration: GSH, 10 mM; Cys, 1 mM; other analytes, 100 μM.

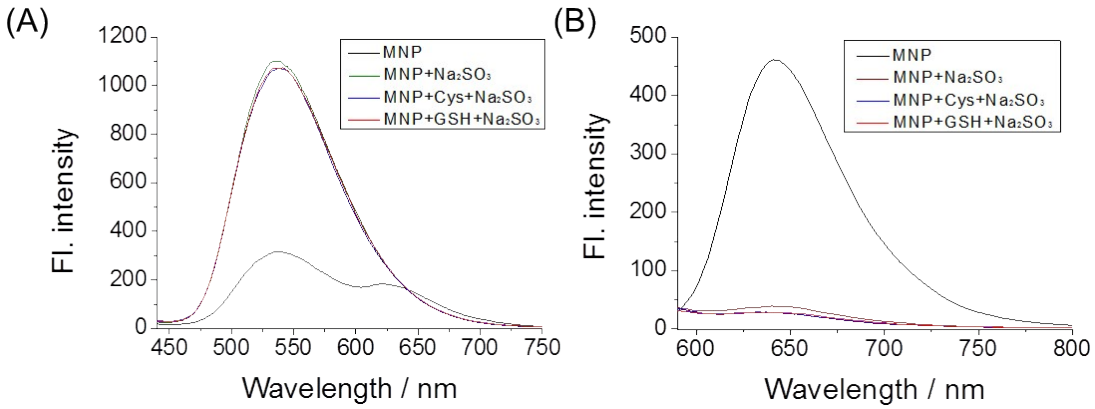

Fig. S4 Fluorescence spectra of 5 μM **MNP** in the absence and presence of higher concentration of Cys and GSH in PBS buffer (pH = 7.4, 10% CH_3CN, 20 mM)

under excitation at 405 (A) and 561 nm (B). Concentration: GSH, 10 mM; Cys, 1 mM; Na_2SO_3, 200 μM.

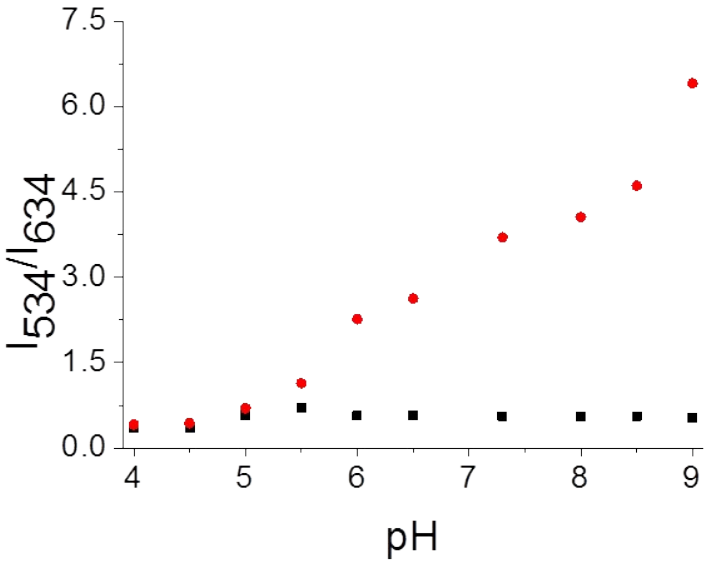

Fig. S5 Fluorescence intensity ratios (I_{534}/I_{634}) of **MNP** in the absence (black) and presence (red) of 100 μM Na_2SO_3 at various pH conditions. λ_{ex} = 405 and 561 nm.

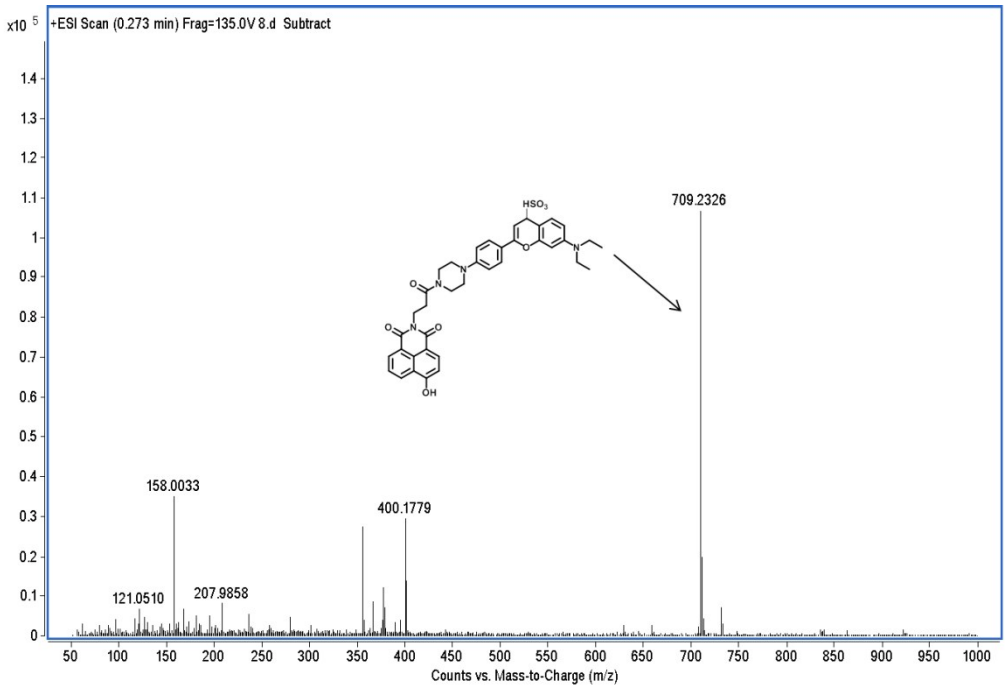

Fig. S6 HRMS data of the reaction product of the probe **MNP** with Na_2SO_3.

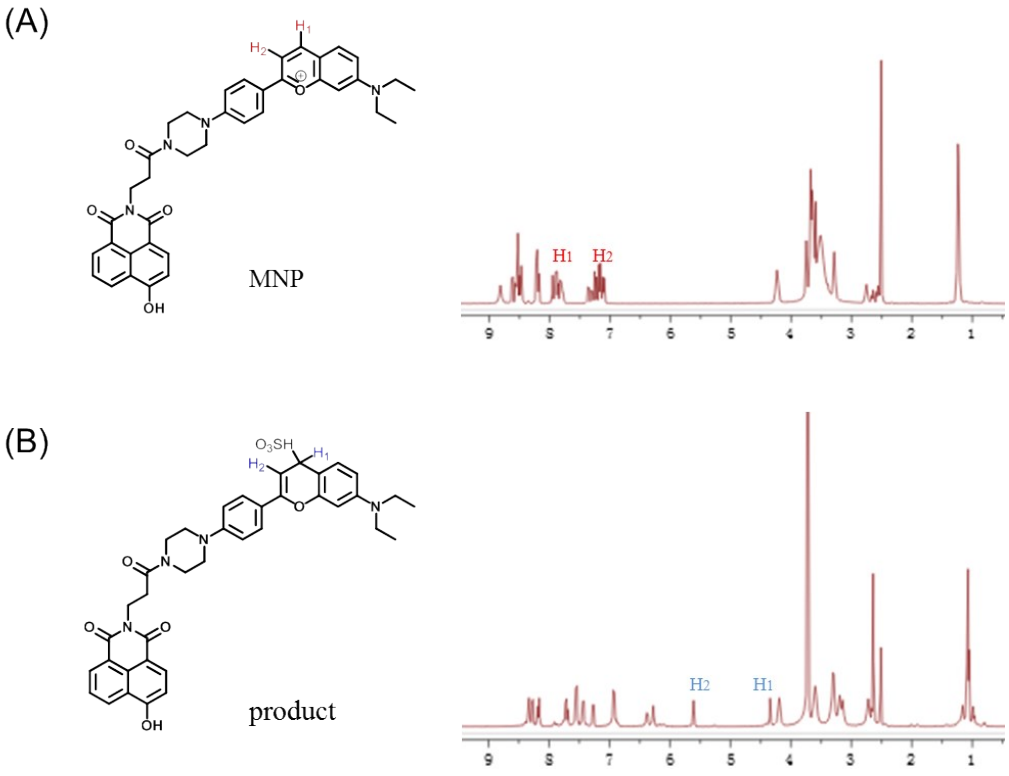

Fig. S7 ^{1}H NMR spectral changes of probe **MNP** and the product of MNP with SO_3^{2-} in a DMSO-d_6/D_2O (v/v =4:1) solution.

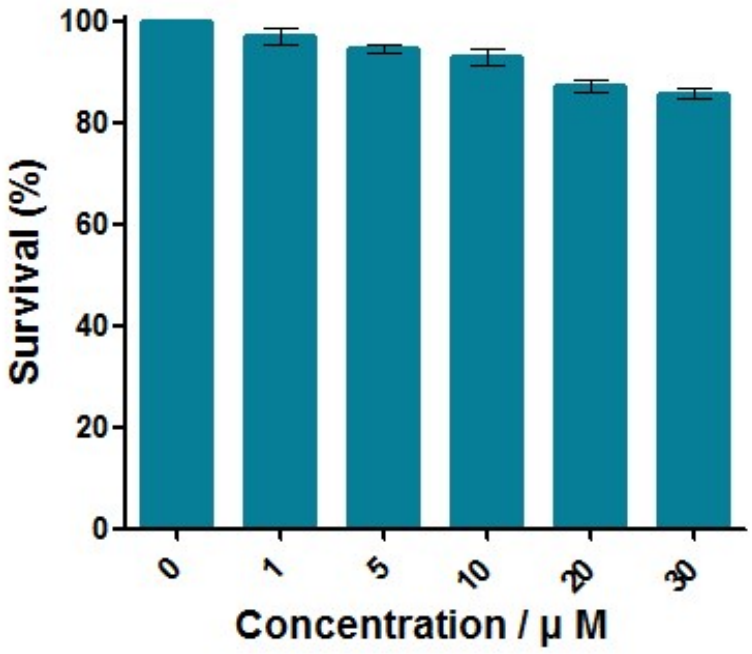

Fig. S8 Survial of HeLa cells in the presence of **MNP** at various concentrations measured using MTT assay.

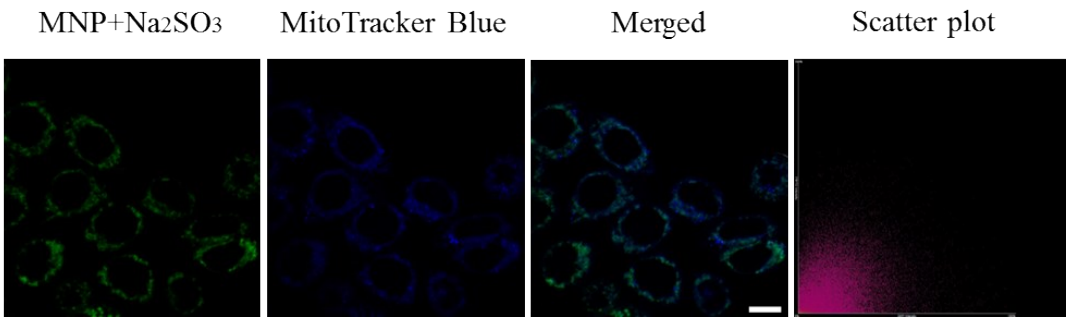

Fig. S9 Images of HeLa cells costained with 5 μM **MNP** for 30 min and then treated with 100 μM Na_2SO_3 for another 30 min, next treated with 1 μM MitoTracker Blue for 5 min, and the co-localization coefficient of **MNP** and Mito-Tracker Blue. Scale bar = 10 μm.

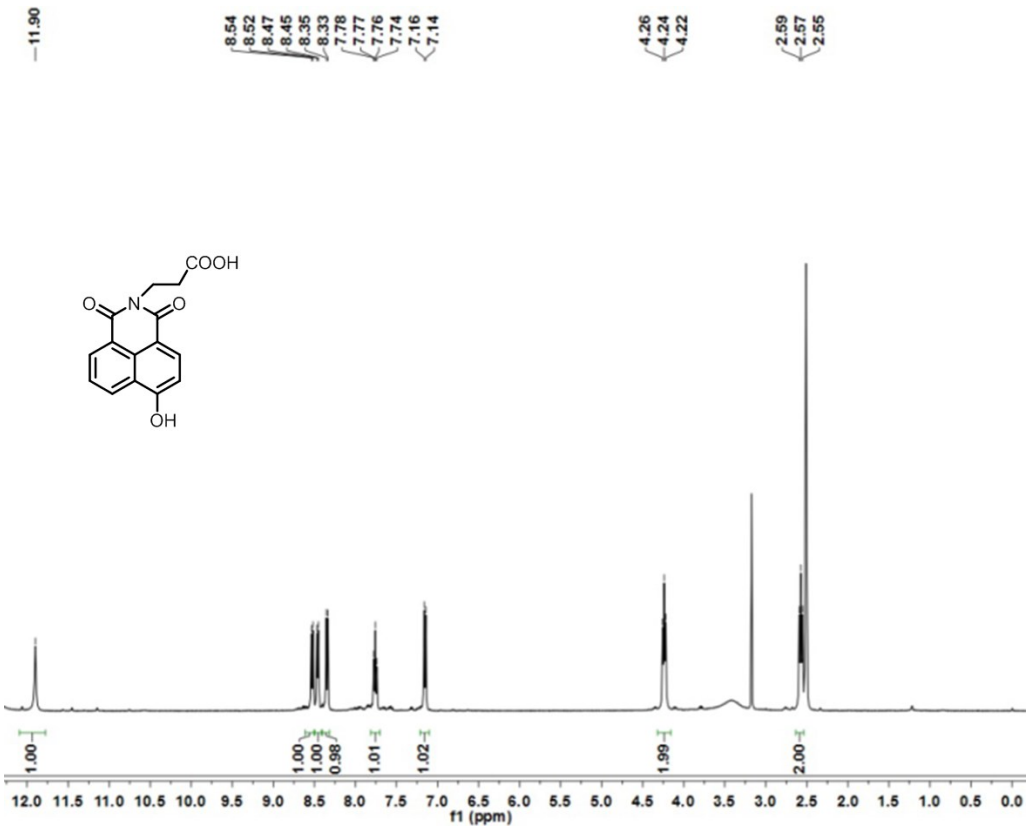

Fig. S10 ^{1}H NMR data of the Compound 2 (400 MHz, DMSO-d_6).

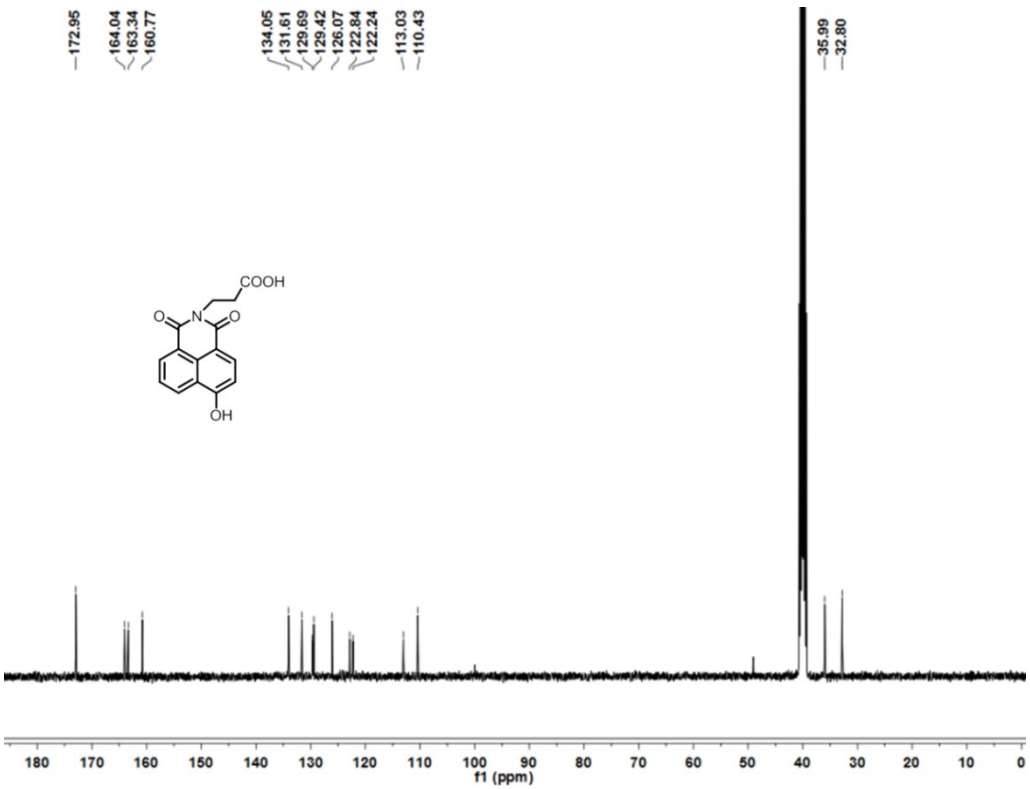

Fig. S11 ^{13}C NMR data of the Compound 2 (100 MHz, DMSO-d_6).

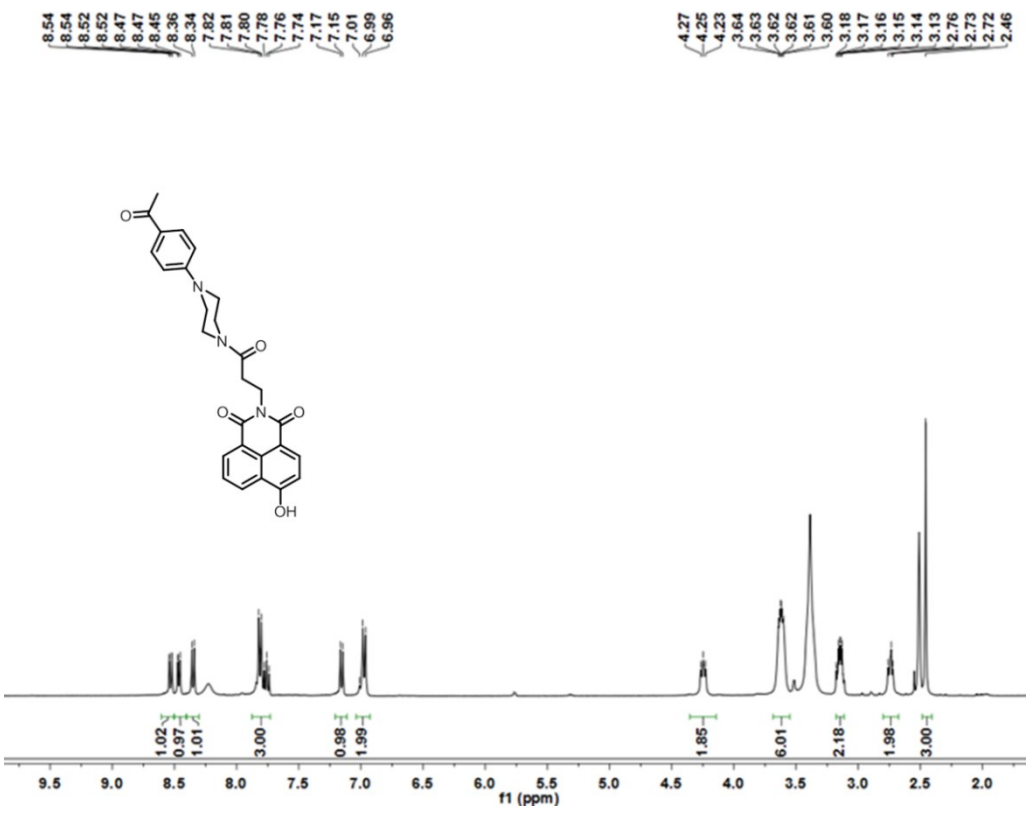

Fig. S12 ^{1}H NMR data of the Compound 3 (400 MHz, DMSO-d_6).

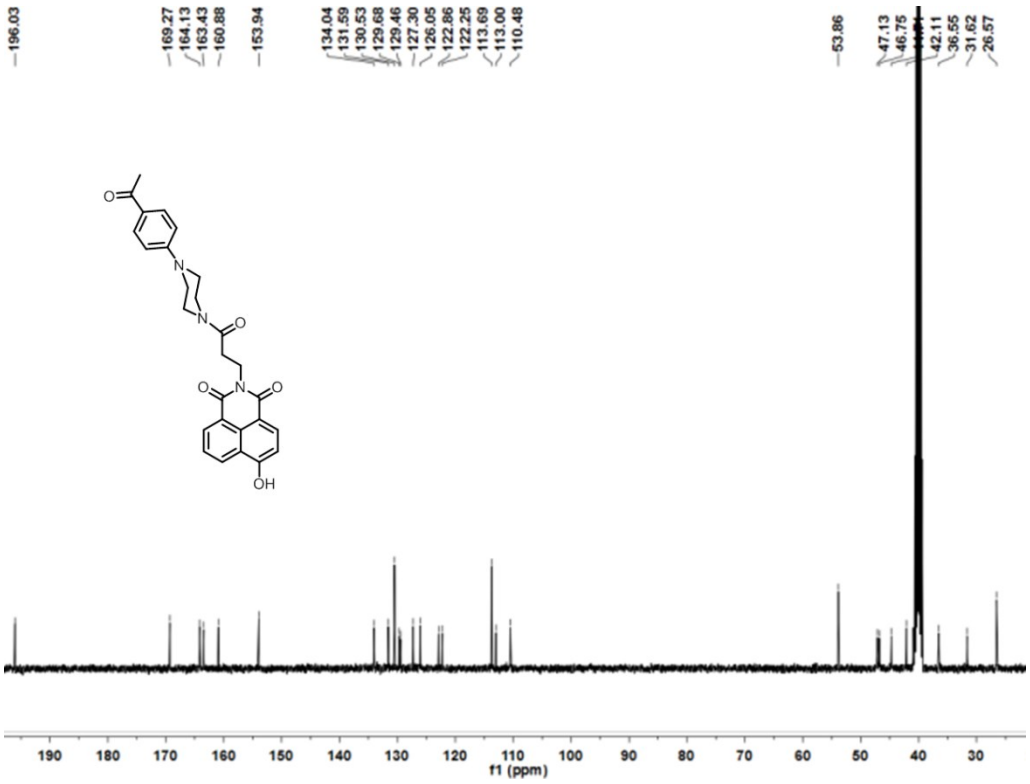

Fig. S13 ^{13}C NMR data of the Compound 3 (100 MHz, DMSO-d_6).

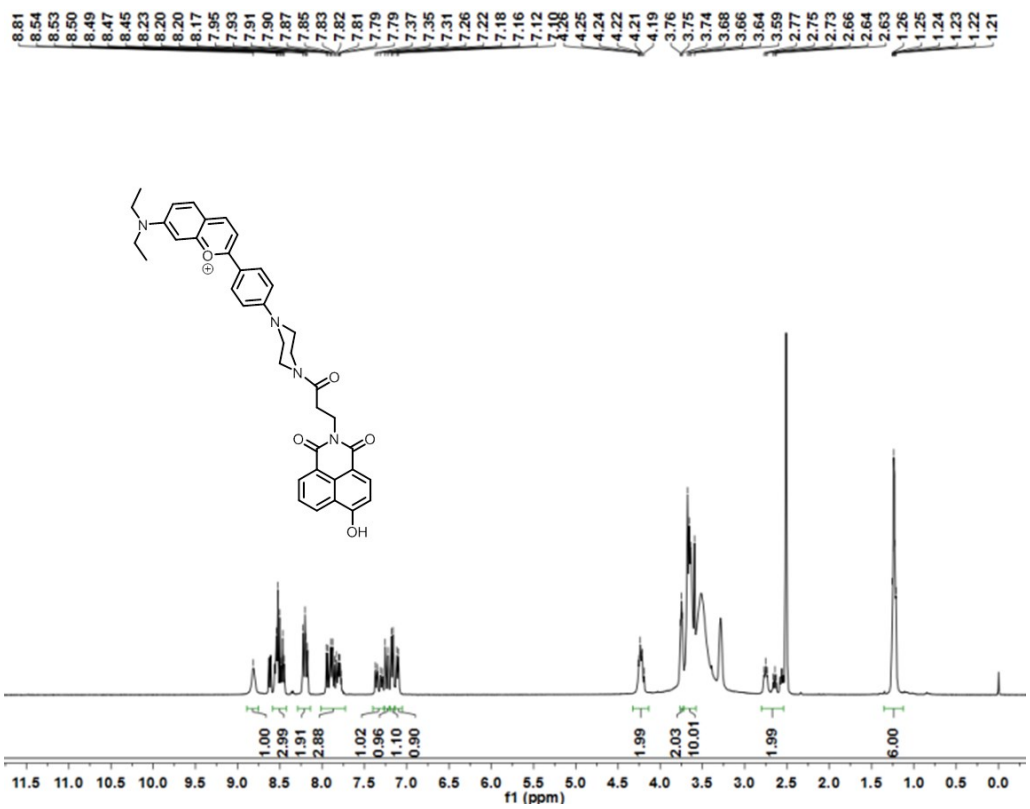

Fig. S14 ^{1}H NMR data of the probe **MNP** (400 MHz, DMSO-d_6).

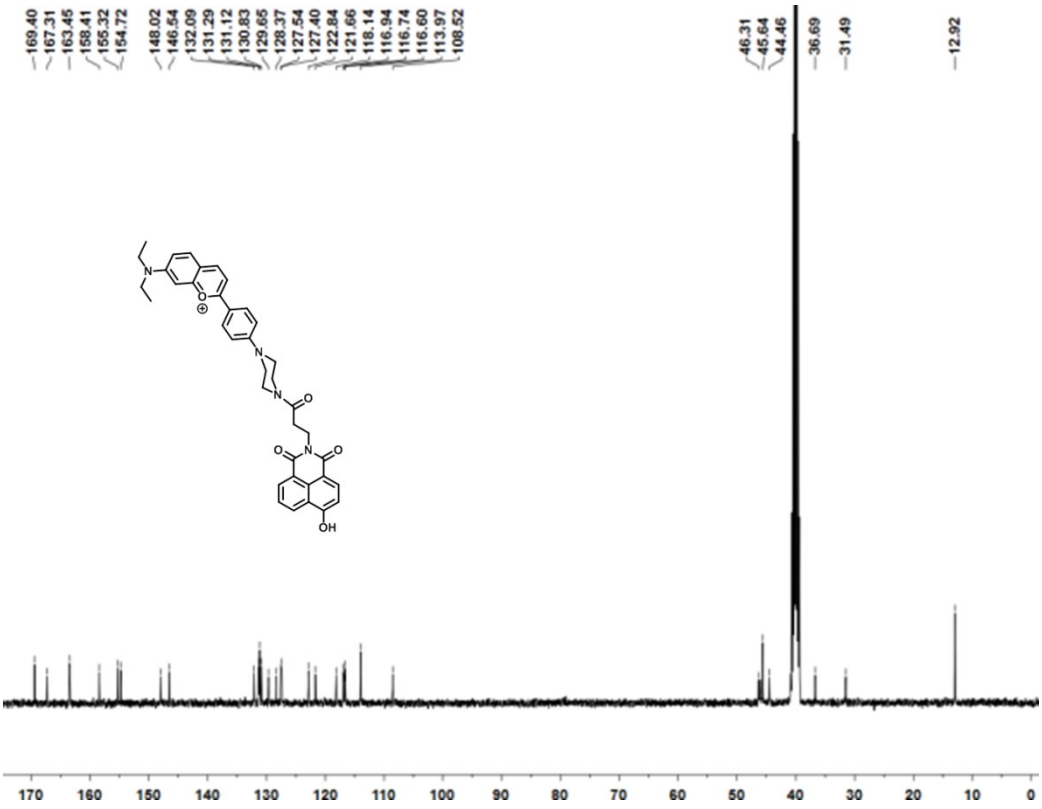

Fig. S15 ^{13}C NMR data of the probe **MNP** (100 MHz, DMSO-d_6).

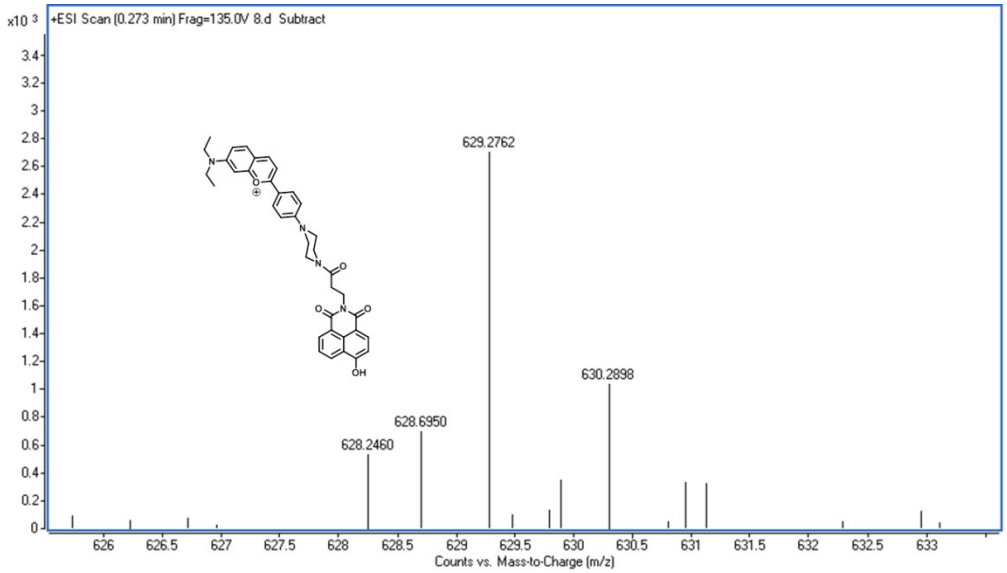

Fig. S16 HRMS data of the probe **MNP**.

Reference:

1. Dong, B.; Lu, Ya.; Zhang, N.; Song, W.; Lin, W. Ratiometric imaging of cysteine level changes in endoplasmic reticulum during H_2O_2-induced redox imbalance. *Anal. Chem.* **2019**, 91, 5513−5516.