Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Fluorescence imaging of cellular GSH to reveal the hindering influence of rutin on ferroptosis

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1. Materials and instruments

All solvents and reagents were commercially available and used without further purification. Doubly distilled water was used in all the experiments. Thin-layer chromatography (TLC) analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were purchased from the Qingdao Ocean Chemicals. Fluorescence spectra and relative fluorescence intensity were measured with a Hitachi F-4600 spectrofluorimeter with a 10 mm quartz cuvette. UV/vis spectra were obtained with a Shimadzu UV-2700 spectrophotometer. High-resolution mass spectra (HRMS) for the characterization of structures were collected using a Bruker apex-Ultra mass spectrometer (Bruker Daltonics Corp., USA) in electrospray ionization (ESI) mode. ^{1}H and ^{13}C NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using tetramethylsilane (TMS) as internal reference. LC-MS were collected using an Agilent 6510 Q-TOF LC/MS.

2. Cytotoxicity experiment

Exploited by standard MTT assay the cell viability of the probe **N-GSH** was judged. The HeLa cells inoculated in 96-well plates of density around 8000 cells/well. The HeLa cells were bred overnight down the medium of 100 μL along with consistency of various concentrations (0-50M) of the probe **N-GSH** for 24 hrs. 10 μL MTT was individually inserted to several wells for additional 3 hours incubation. Subsequently, 100 μL of DMSO was practiced for the dissolving of resulted-precipitate, thereafter the plate was shaken for 40 mints. The micro-plate reader (Thermo Fisher Scientific) was operated to determine the absorbance of resultant-solution and to estimate the cytotoxicity for the probe **N-GSH**.

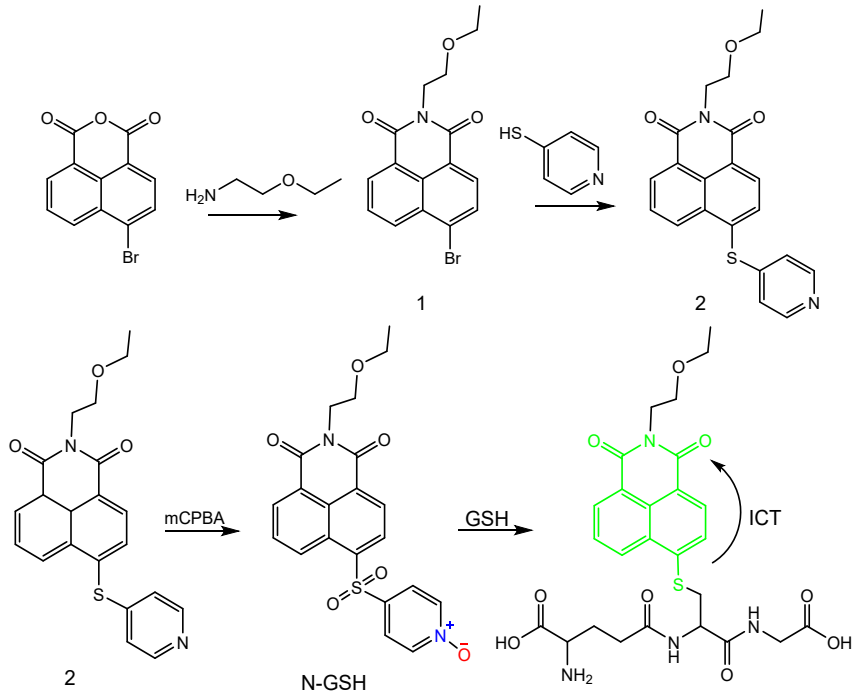


Figure S1. Synthesis route of probe N-GSH.

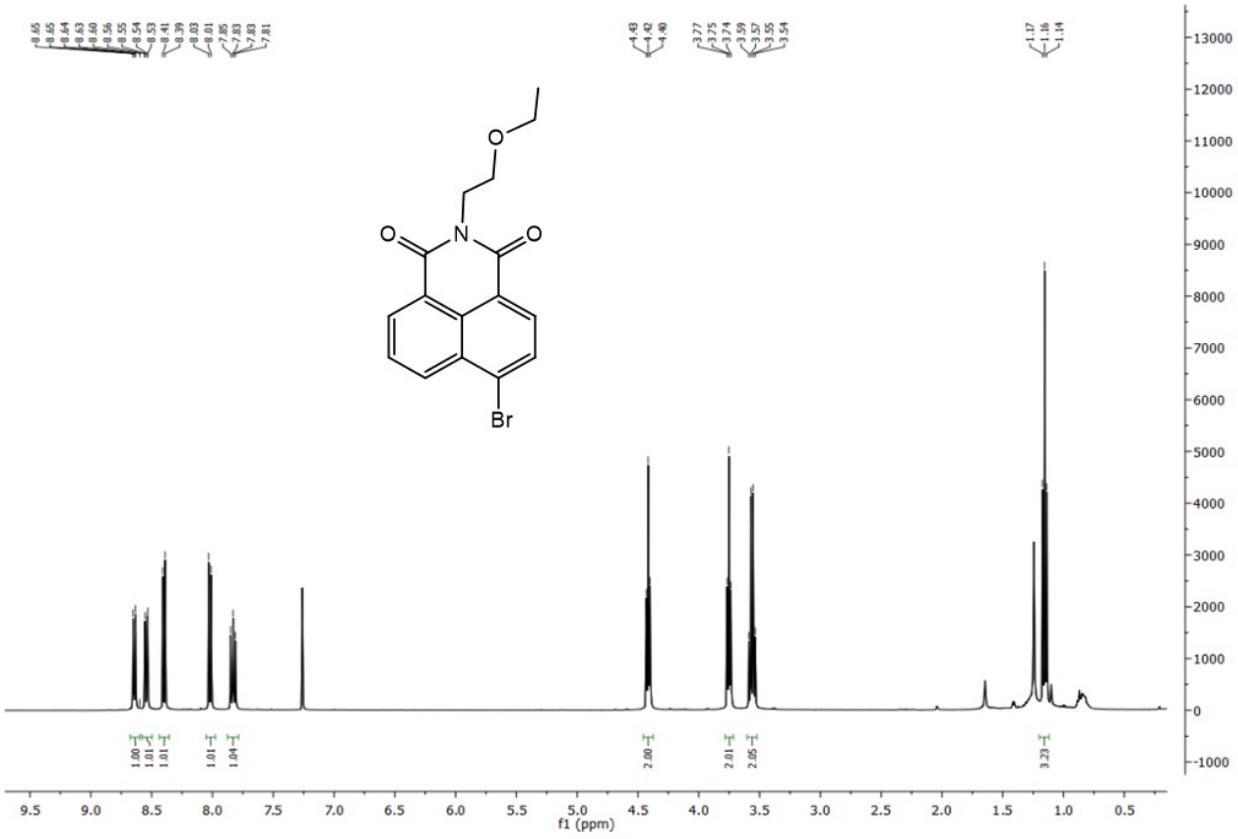


Figure S2. HNMR of compound 1.

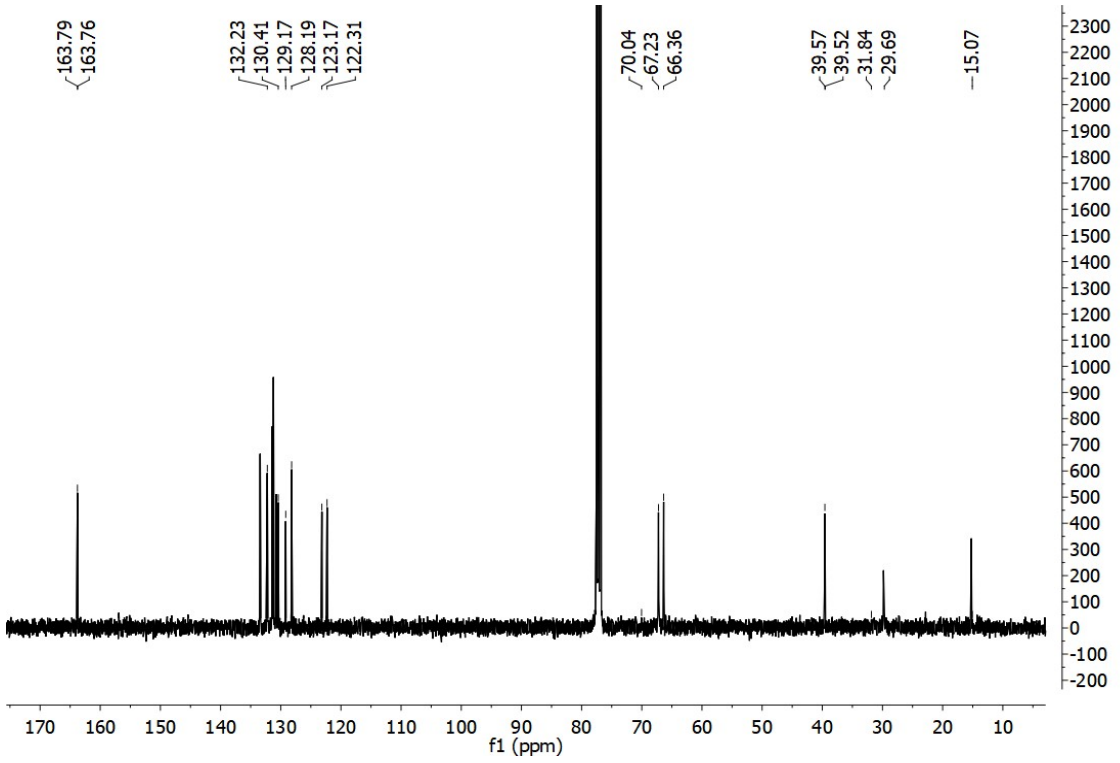
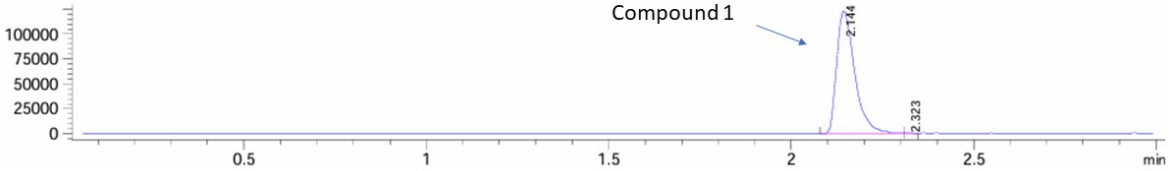


Figure S3. CNMR of compound 1.

A. HPLC data:



B. MS data:

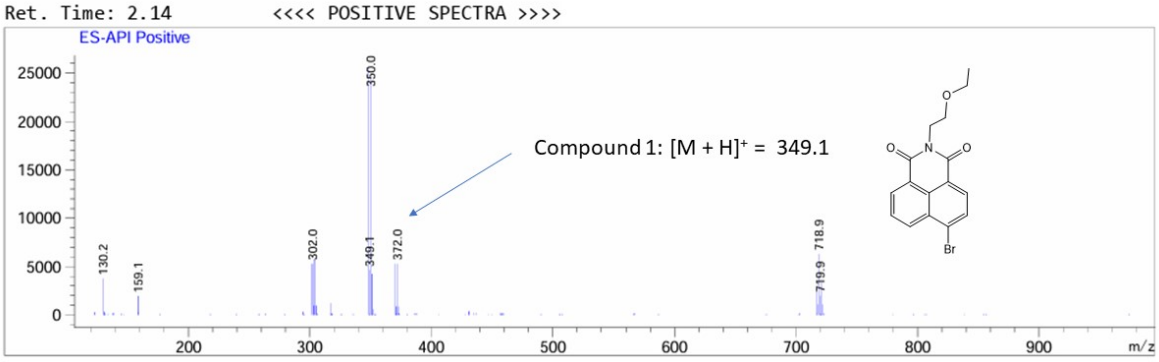


Figure S4. LCMS data of compound 1, Detection at 214 nm.

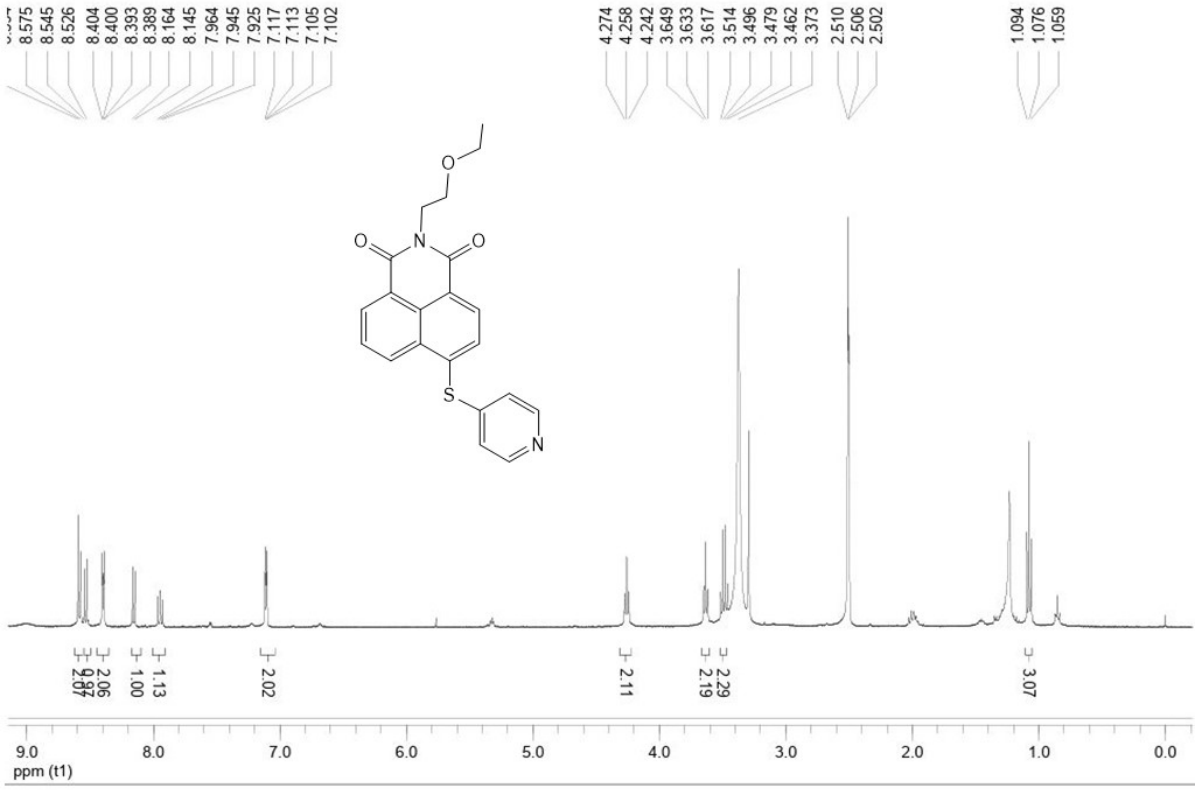


Figure S5. HNMR of compound 2.

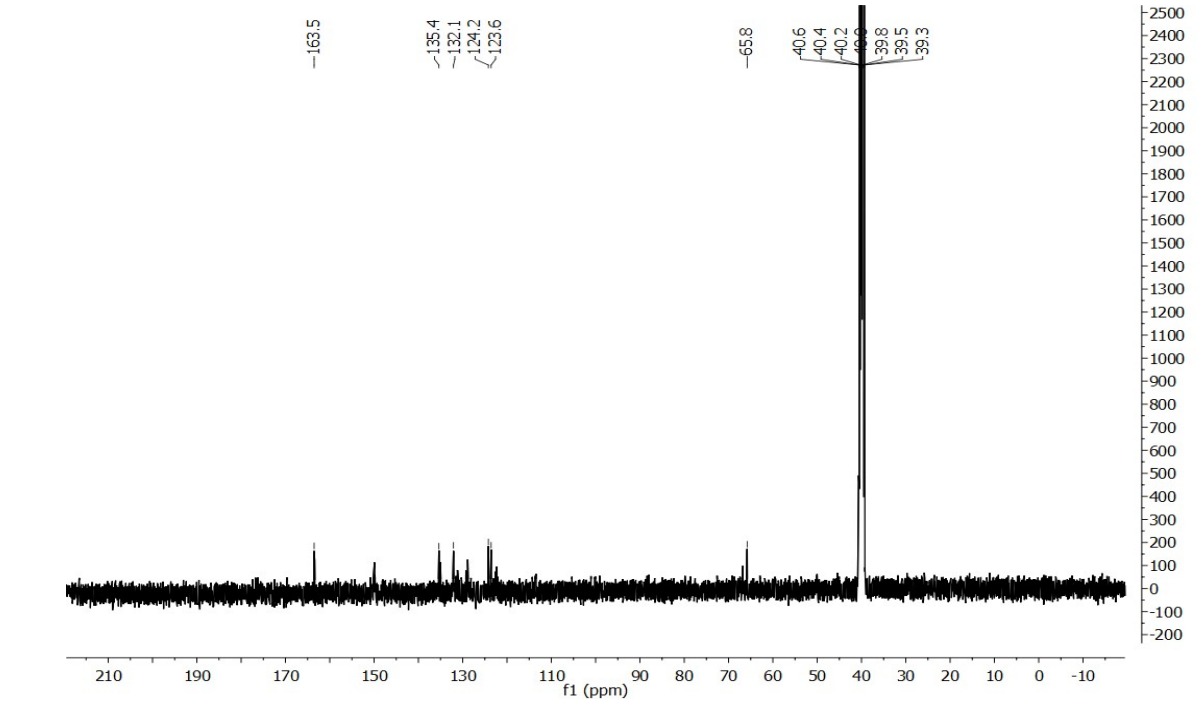


Figure S6. CNMR of compound 2.

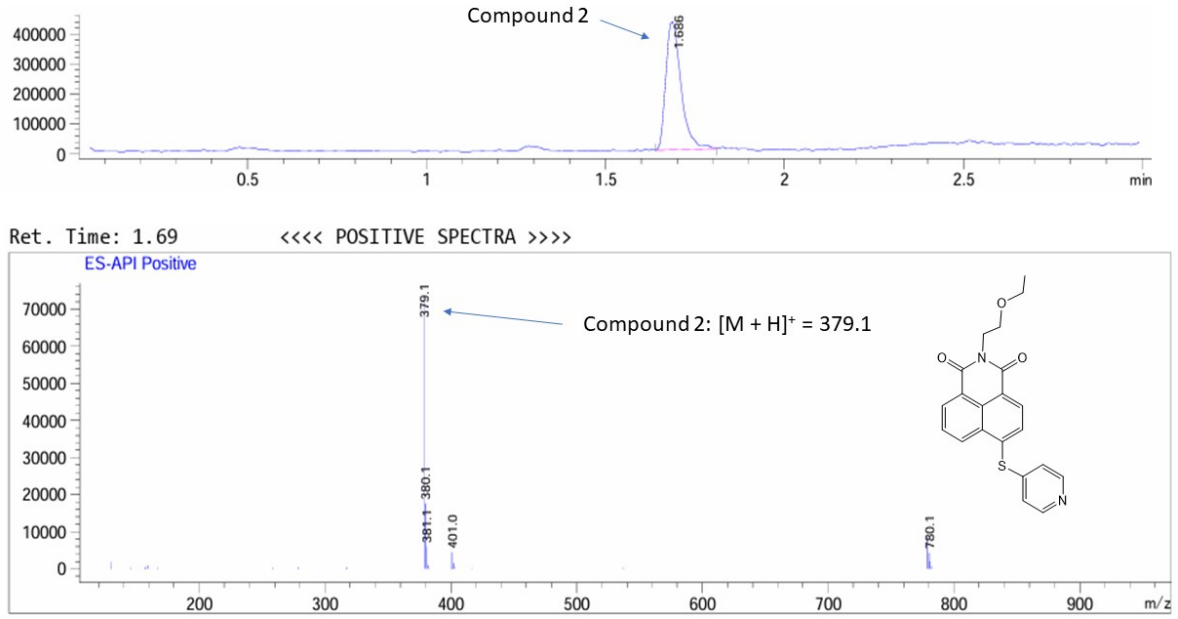


Figure S7. LCMS data of compound 2.

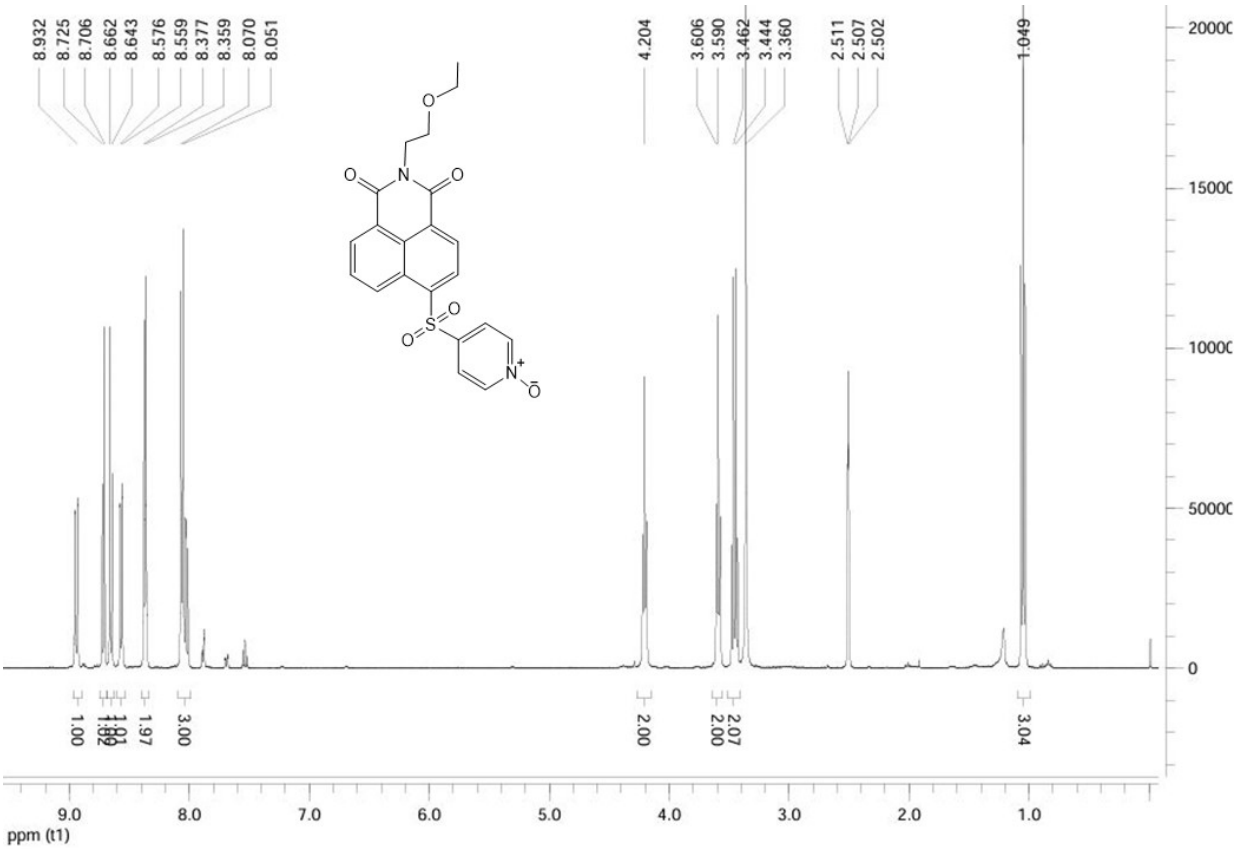


Figure S8. HNMR of probe N-GSH.

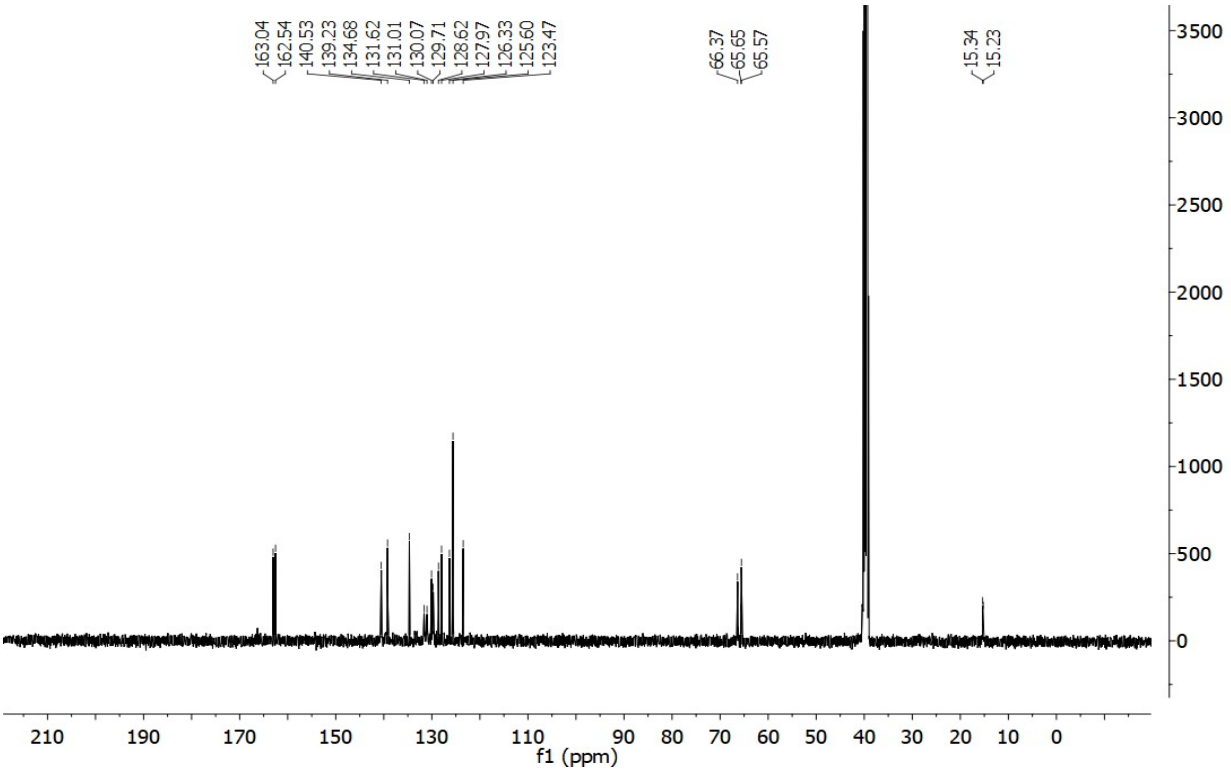


Figure S9. CNMR of probe N-GSH.

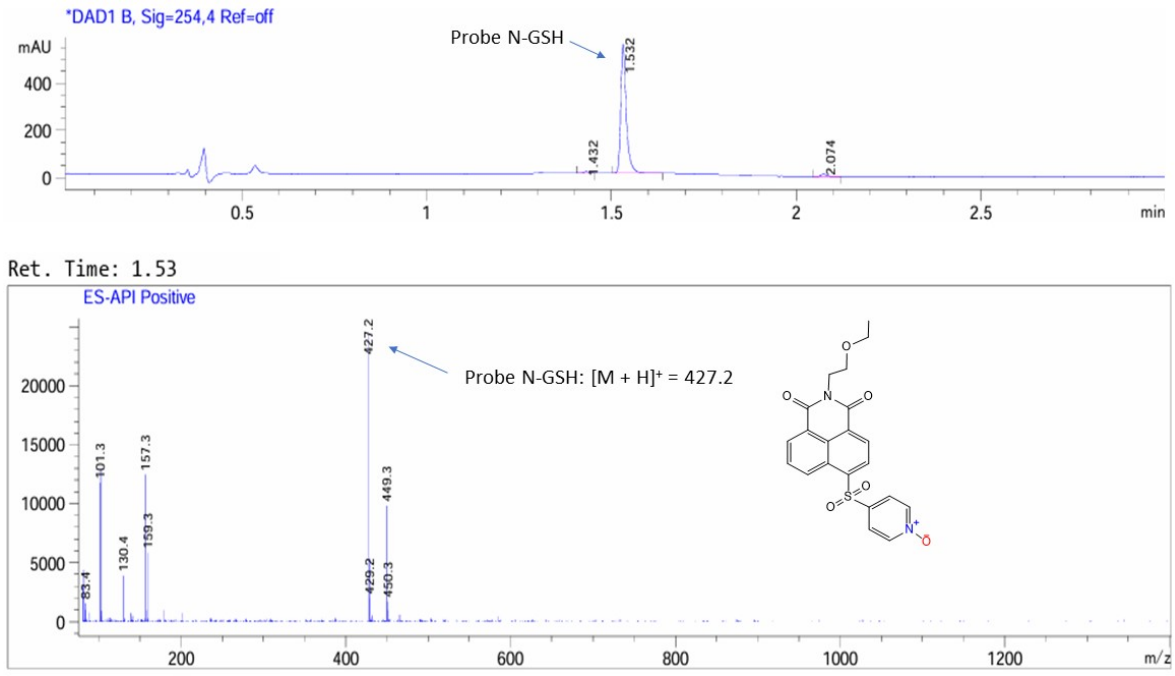


Figure S10. LC-MS data of probe N-GSH.

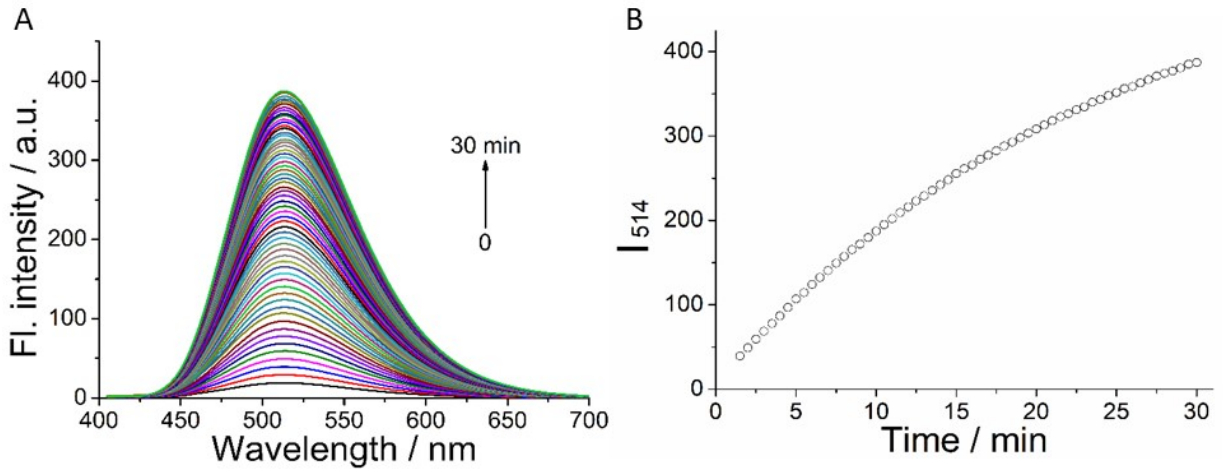


Figure S11. Time-dependent fluorescence spectra **A.** and fluorescence intensity (I514) **B.** of 5 μM N-GSH treated with 3 mM GSH in PBS (20 mM, pH 7.4, 5% MeOH).

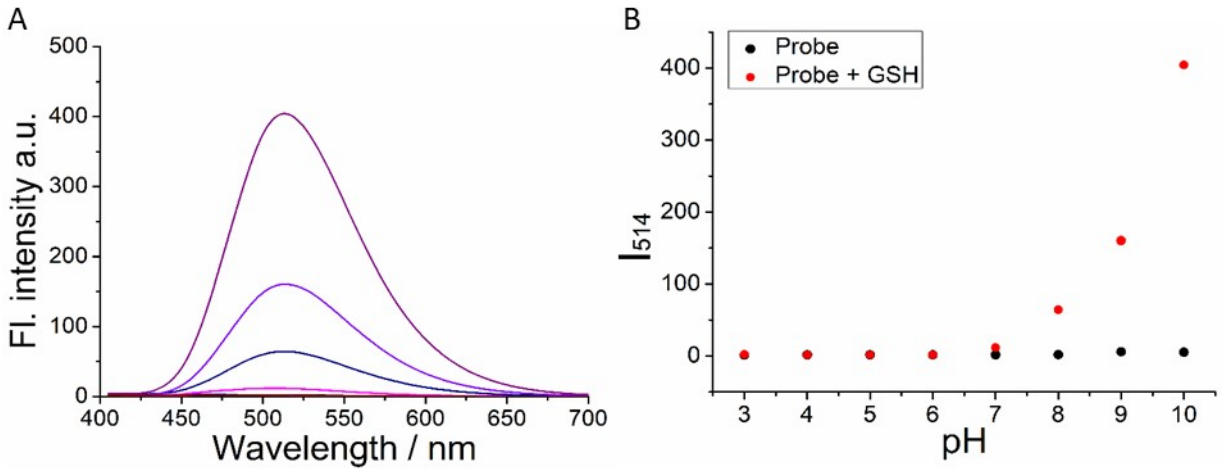


Figure S12. **A.** Fluorescence spectra of 5 μM N-GSH at various pH. **B.** Fluorescence spectra of 5 μM **N-GSH** treated with 100 μM GSH at various pH. (C) Quantified fluorescence intensity (I514) for (A) and (B). λ_{ex} = 405 nm.

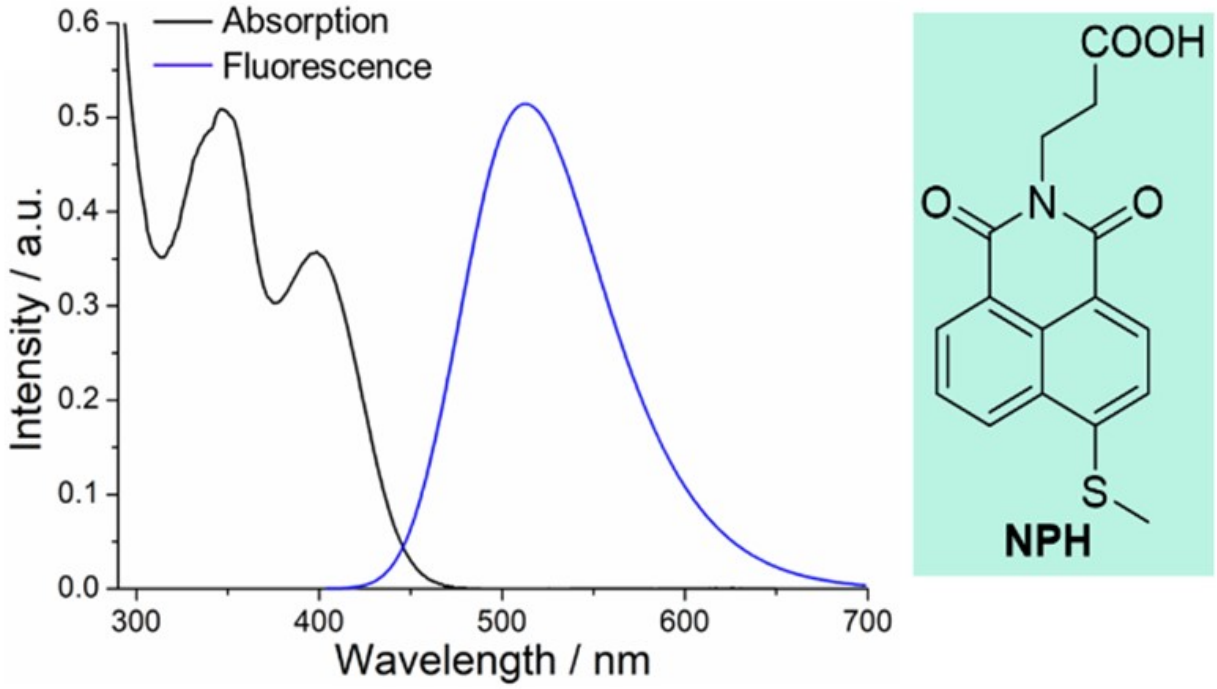


Figure S13. Normalized absorption and fluorescence spectrum of NPH.

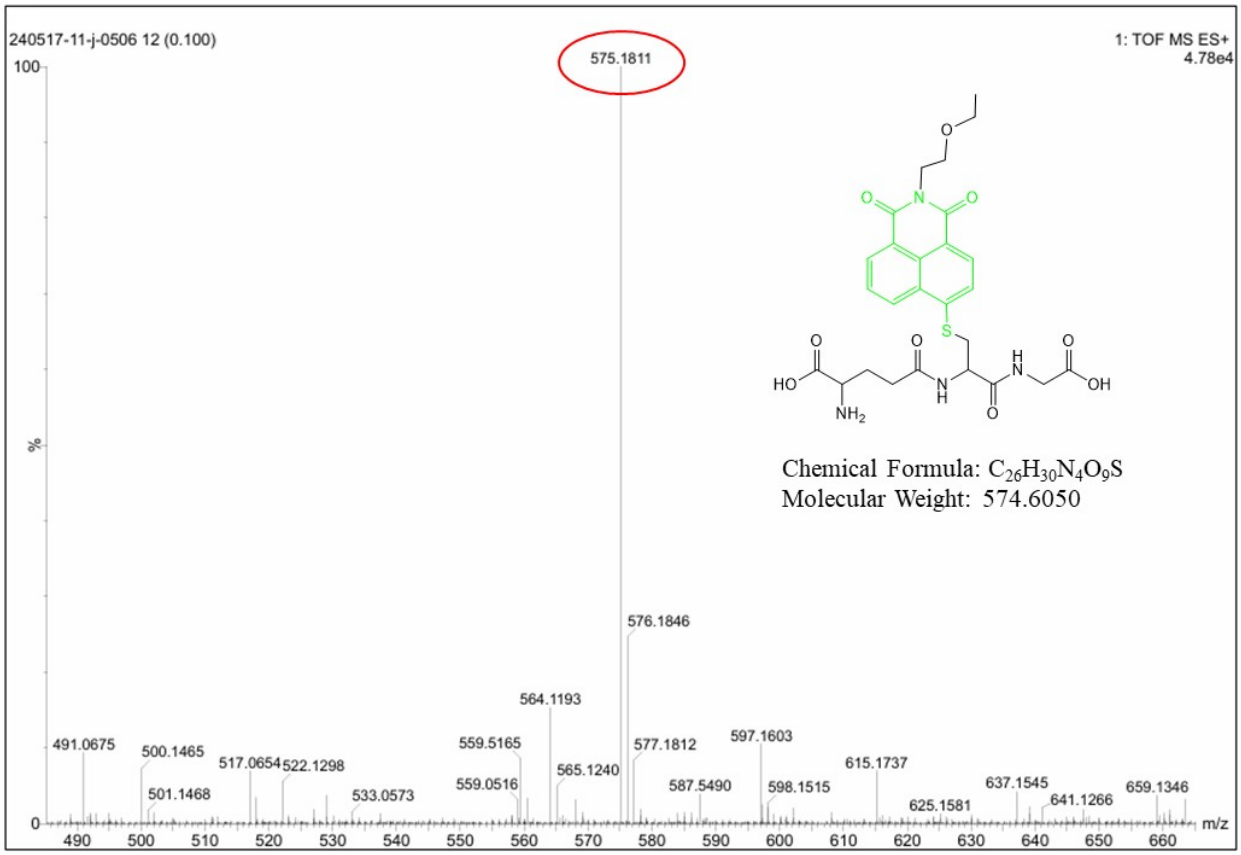


Figure S14. HRMS data of the probe N-GSH to GSH.

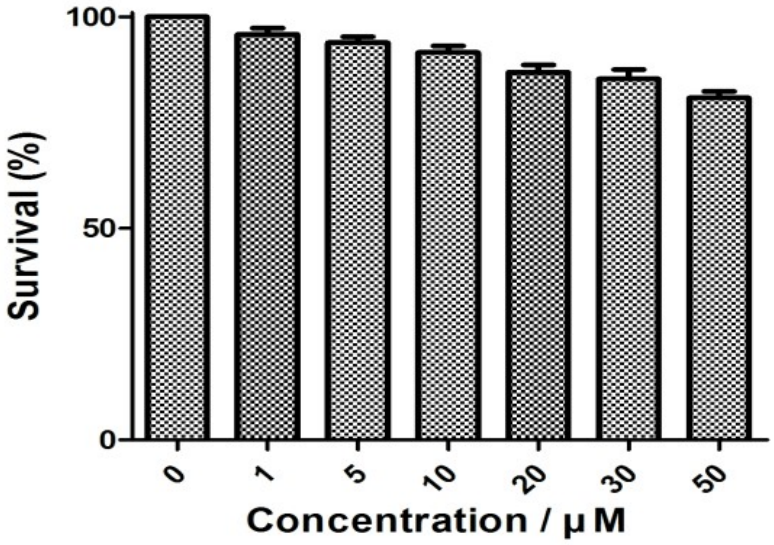


Figure S15. Viability of HeLa cells treated with various concentrations of the probe N-GSH.

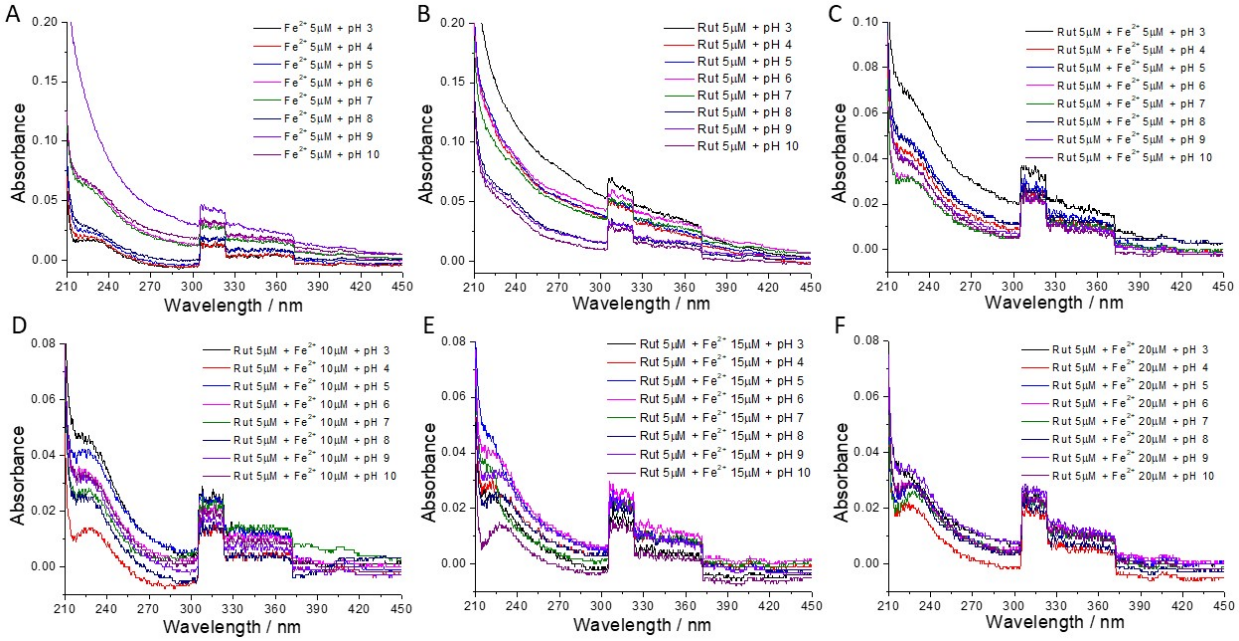


Figure S16. Absorbance spectrum of 5 uM Rutin/Fe^{2+} with various concentrations and pH 3-10.

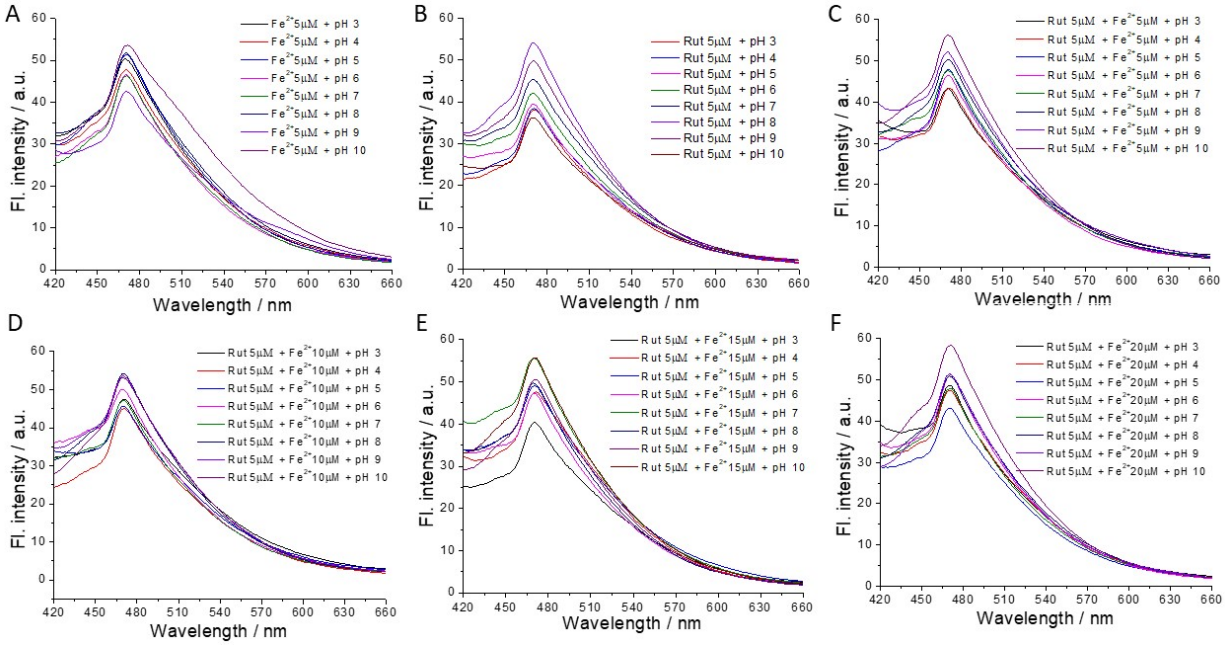


Figure S17. Fluorescence spectrum of 5 uM Rutin/Fe^{2+} with various concentrations and pH 3-10.

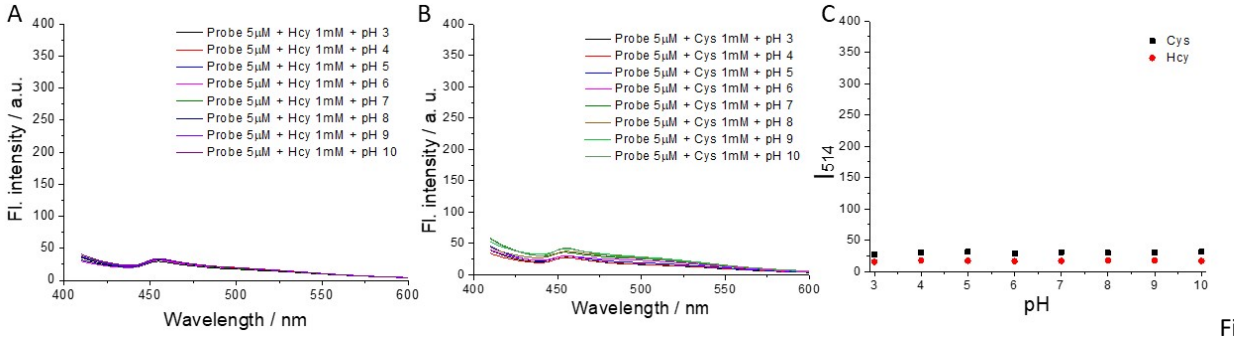


Figure S18. (A) Fluorescence spectra of 5 μM probe N-GSH treated with 1mM Hcy at various pH (3-10). (B) Fluorescence spectra of 5 μM probe N-GSH treated with 1mM Cys at various pH. (C) Quantified fluorescence intensity (I514) of N-GSH with Hcy and Cys at various pH (3-10).