Electronic Supplementary Material (ESI) for Analytical Methods.

The D π A type ratiometric fluorescent probe to detect polarity changes and inhibition effect during 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 **Po-P** 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 **Po-P** 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 **Po-P**.

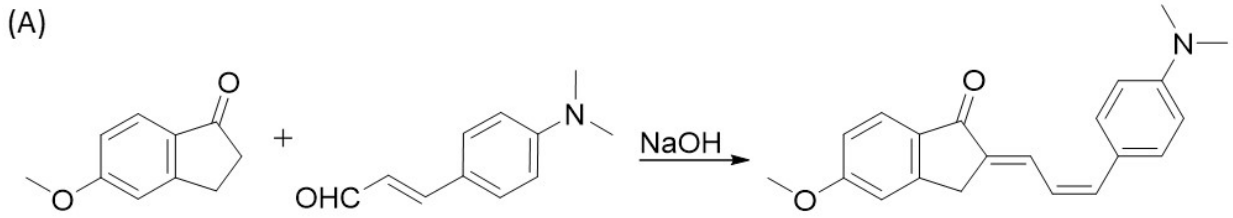

Figure **1.** Synthesis route of probe **Po-P**.

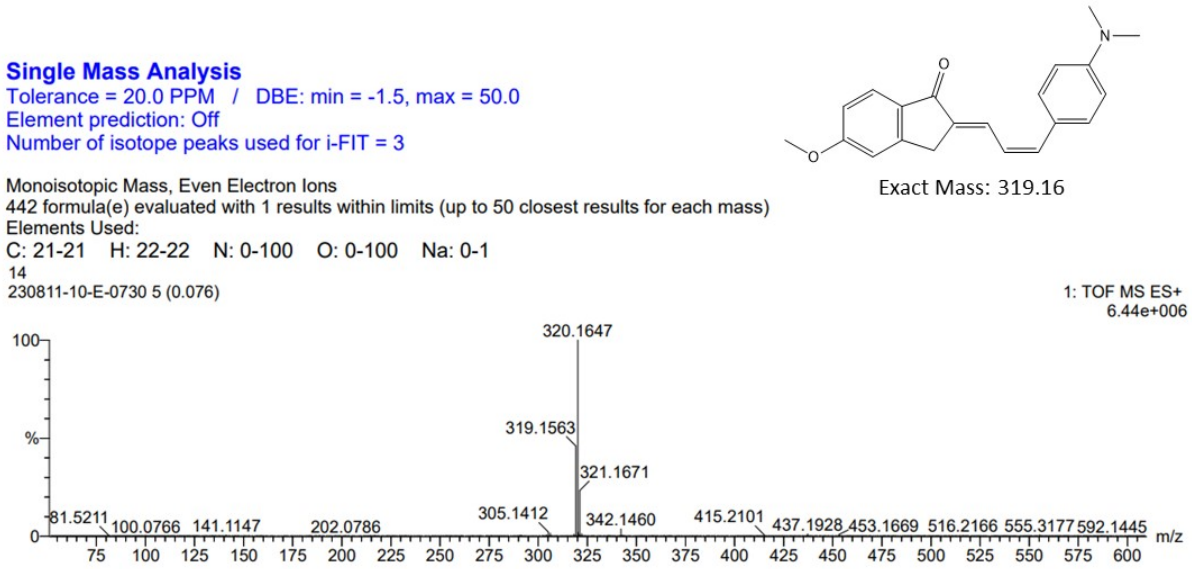

Figure **2**. Single mass analysis of the probe **Po-P**.

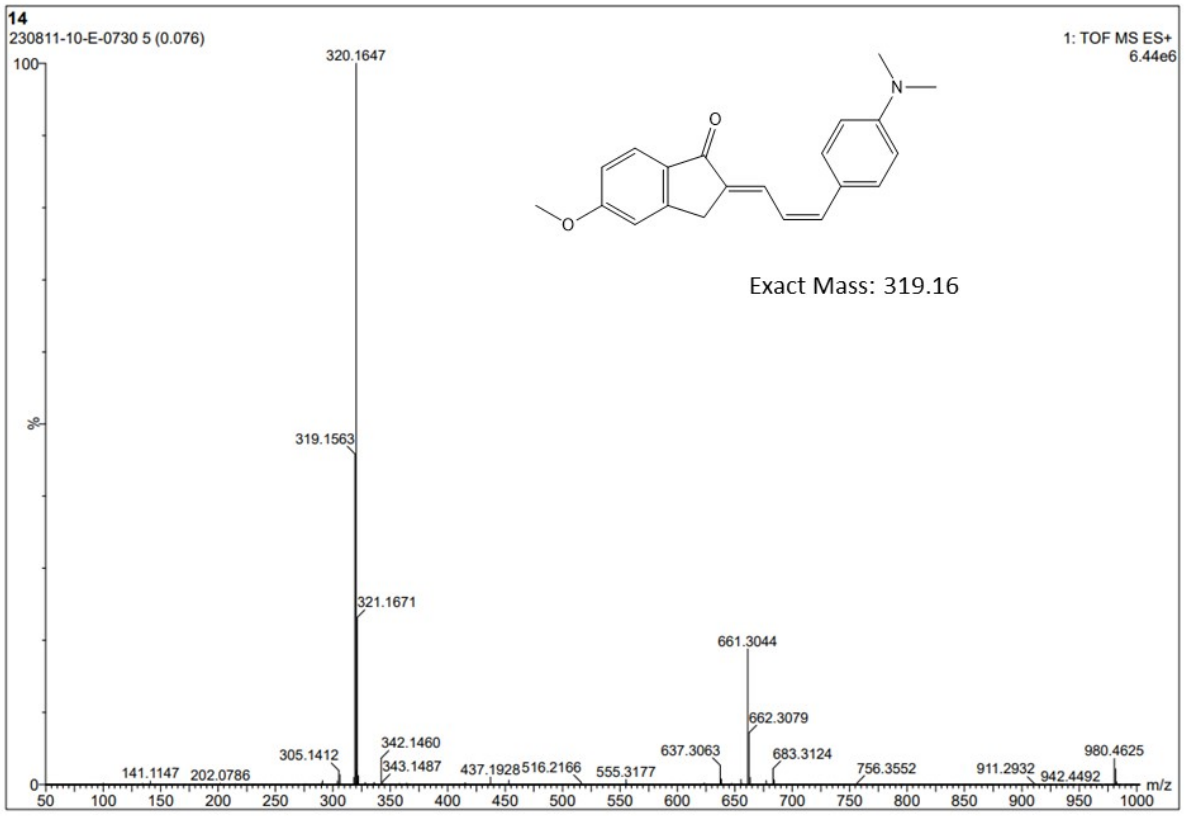

Figure **3**. HRMS of the probe **Po-P**.

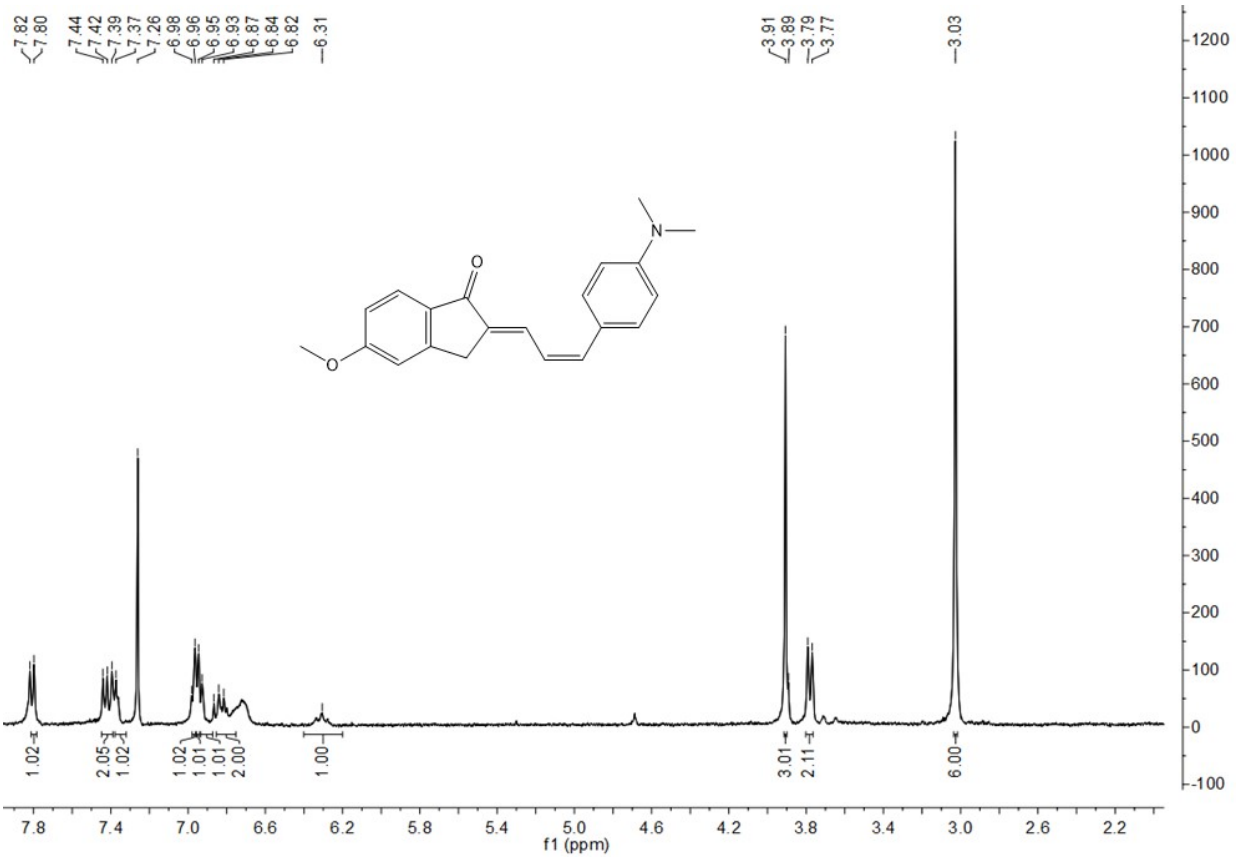

Figure **4**. ^{1}H NMR of the probe **Po-P**.

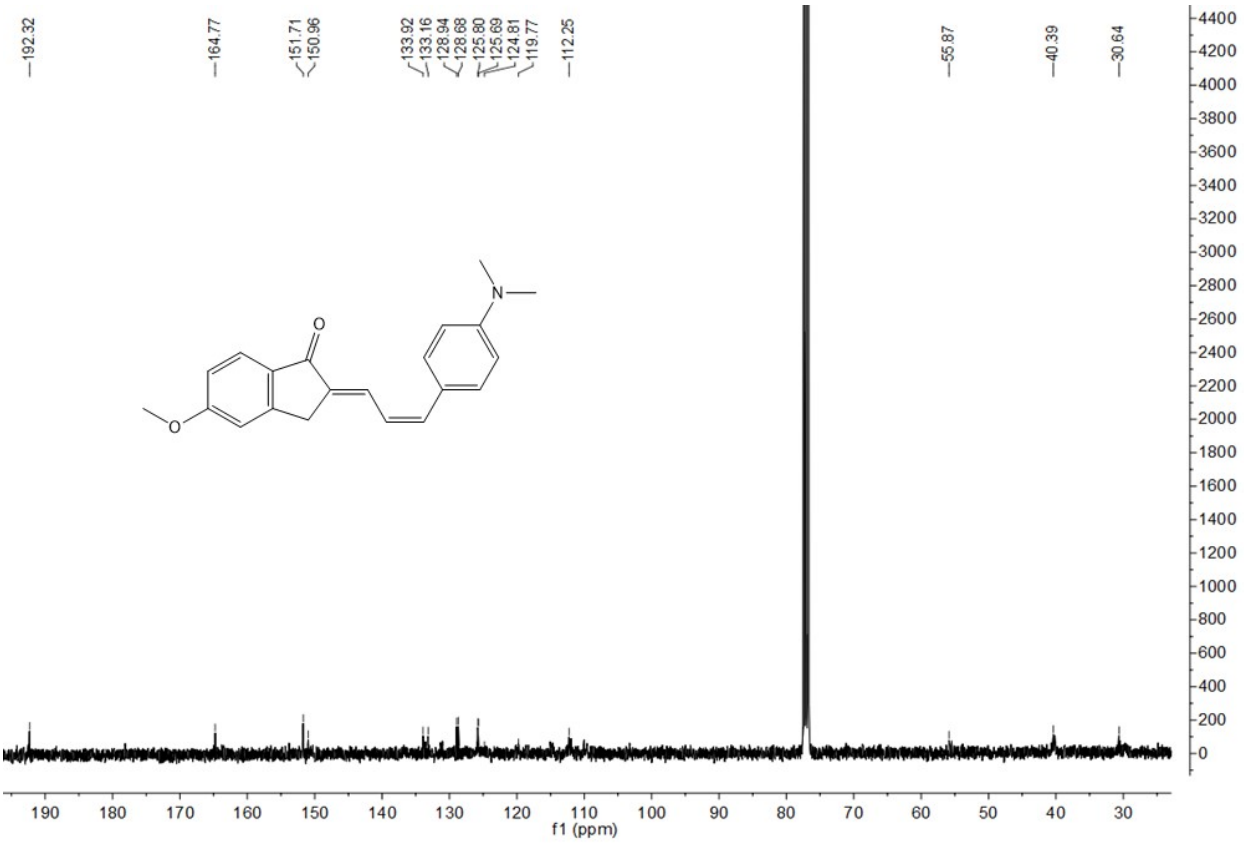

Figure **5**. ^{13}C NMR of the probe **Po-P**.

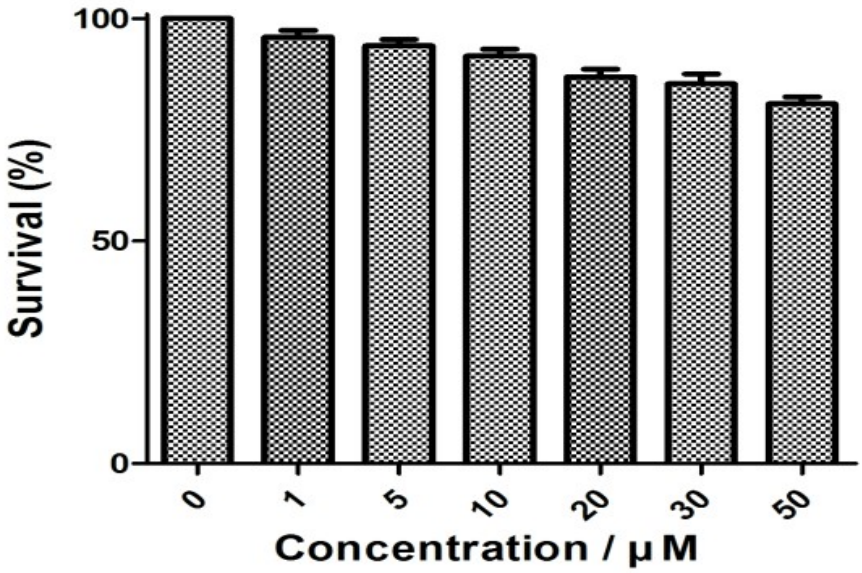

Figure **6**. Viability of HeLa cells treated with various concentrations of the probe **Po-P**.

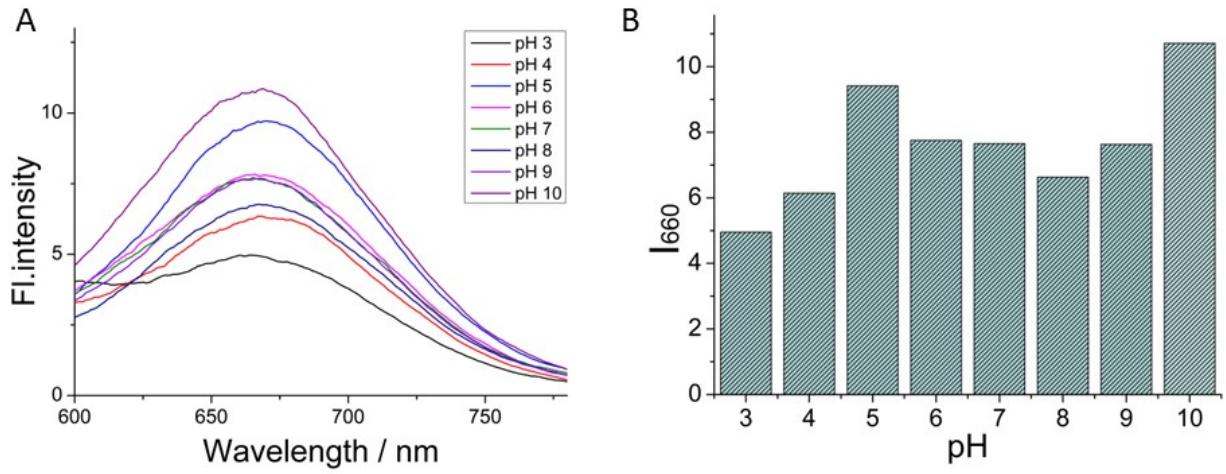

Figure **7**. **A.** Fluorescence spectra of 5 μM Po-P at various pH. **B.** Fluorescence spectra of 5 μM **Po-P** at 660 nm.

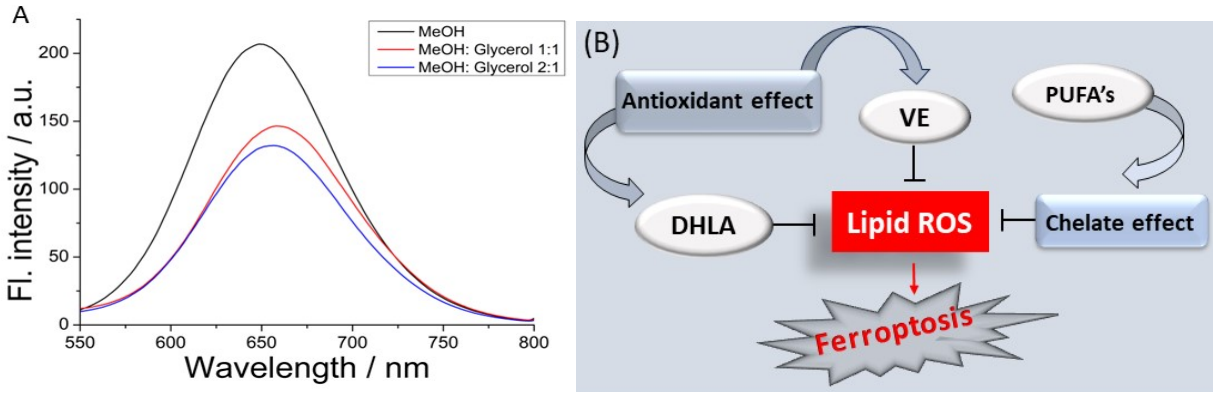

Figure **8**. **A.** Fluorescence spectra of 5 μM **Po-P** in MeOH-glycerol system. λ_{ex} = 410 nm. B**.** (B) Inhibition procedure of Vit E, and DHLA during ferroptosis.

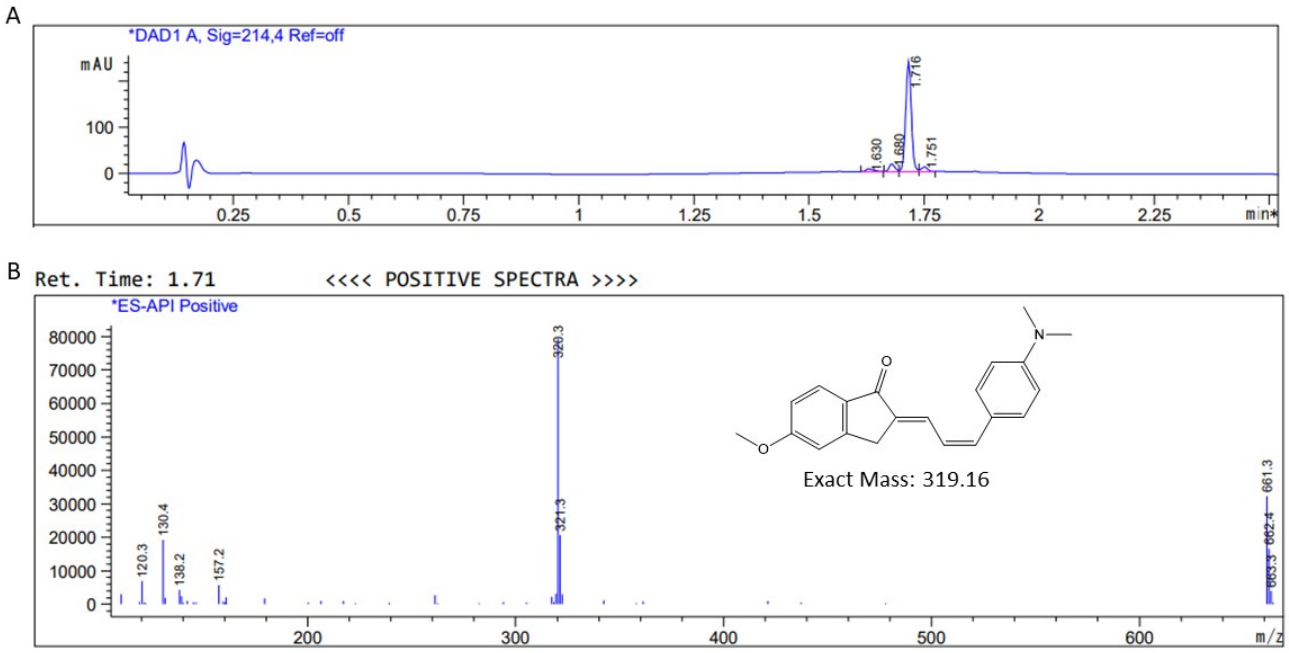

Figure **9**. (A-B) LCMS data of the probe **Po-P**.

Graphical Abstract