Supporting Information for

Real-time monitoring norepinephrine exocytosis by high K^+ via an endoplasmic reticulum-targeting fluorescent probe

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Experimental

1.Materials and Instrumentations

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. ^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker AM500 NMR spectrometer. Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). HRMS spectral data were recorded on a Bruke Daltonics Bio TOF mass spectrometer. Absorption spectra and fluorescent spectra were performed on a Shimadzu UV-2600 UV-Vis and a Hitachi F4700 fluorescence spectrophotometer, respectively. The cells imaging was conducted in Leica TCS SP8 confocal microscope with white light laser.

2.Synthesis and Characterization of Compound

2.1Synthesis of Compound 2.

N-Tosylethylenediamine (2.86g, 13.3 mmol) and compound 1 (3.08 g, 11.1 mmol) were dissolved in ethanol (30 mL). The mixture was stirred at 80 °C for 11 h under nitrogen protection and then poured into water (60 mL). After vacuum filtration, the residue was purified by column chromatography with CH_2Cl_2:CH_3OH = 20:1 as the eluent to obtain gray solid.

2.2Synthesis of Compound ER-OH.

Compound **2** (1 g, 2.11 mmol), 1-Hydroxysuccinimide (414 mg, 2.54 mmol) and K_2CO_3(438 mg, 3.17mmol) dissolved in DMSO (4 ml). The mixture was stirred at 120 °C for 10 h under nitrogen protection. After vacuum filtration, the residue was purified by column chromatography with CH_2Cl_2:CH_3OH = 10:1 as the eluent to obtain gray solid.

3. Calculation of Detection Limits.

Fluorescence titration was carried out in PBS (10 mM, pH 7.4) to determine the detection limit. The detection limit is calculated using the following equation:

$$LOD = 3\sigma/S$$

where σ is the standard deviation of blank measurements and S is the slope between the ration of fluorescent intensity (Fig.S4) and sample concentration.

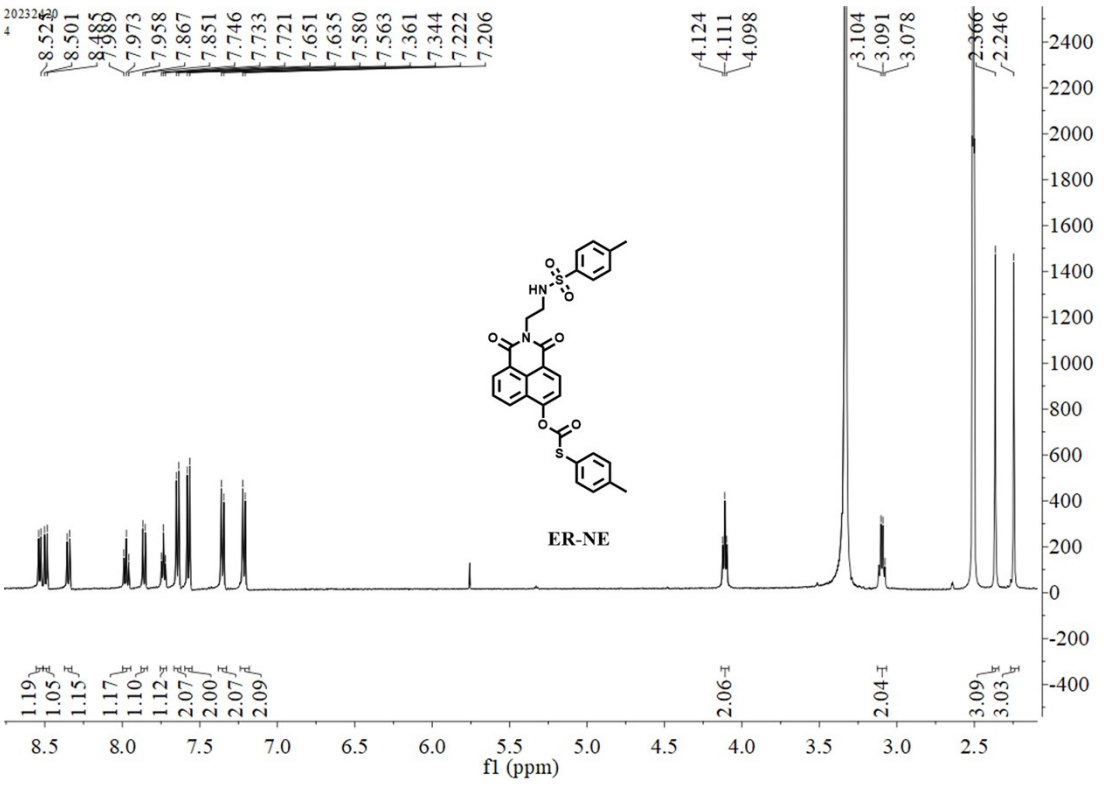

Fig. S1. [1] H spectra of probe **ER-NE** in DMSO-d_6.

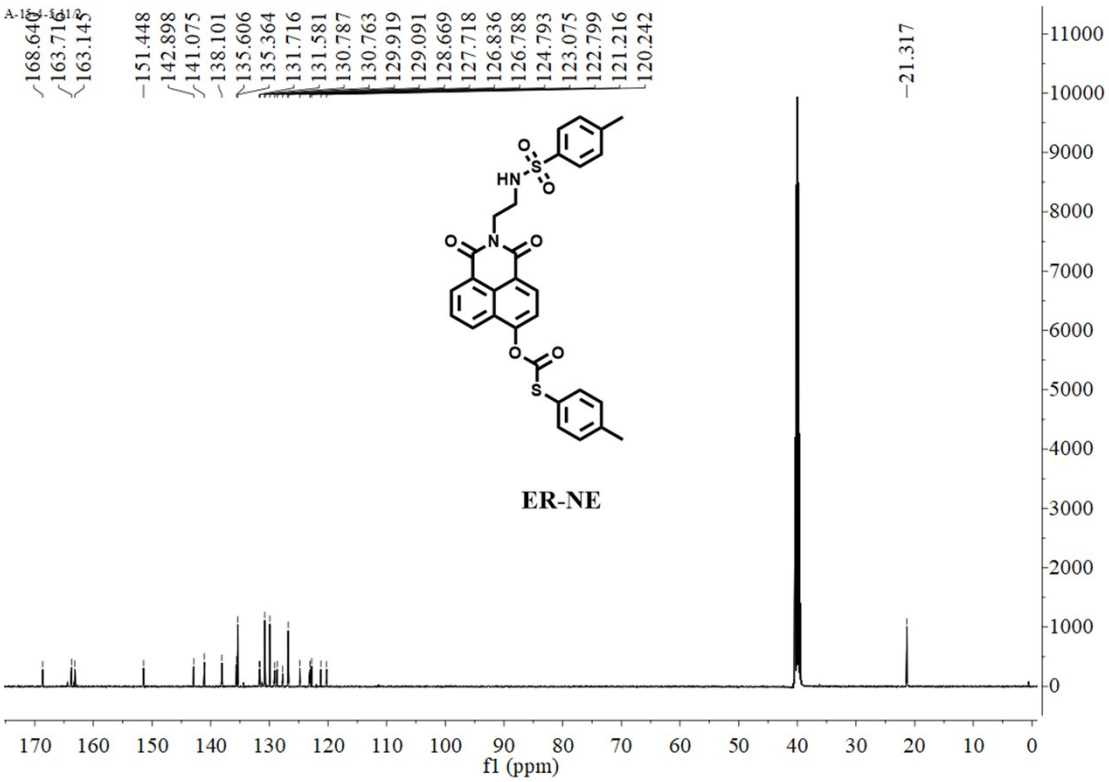

Fig. S2. [13]C NMR spectra of probe **ER-NE** in DMSO-d_6.

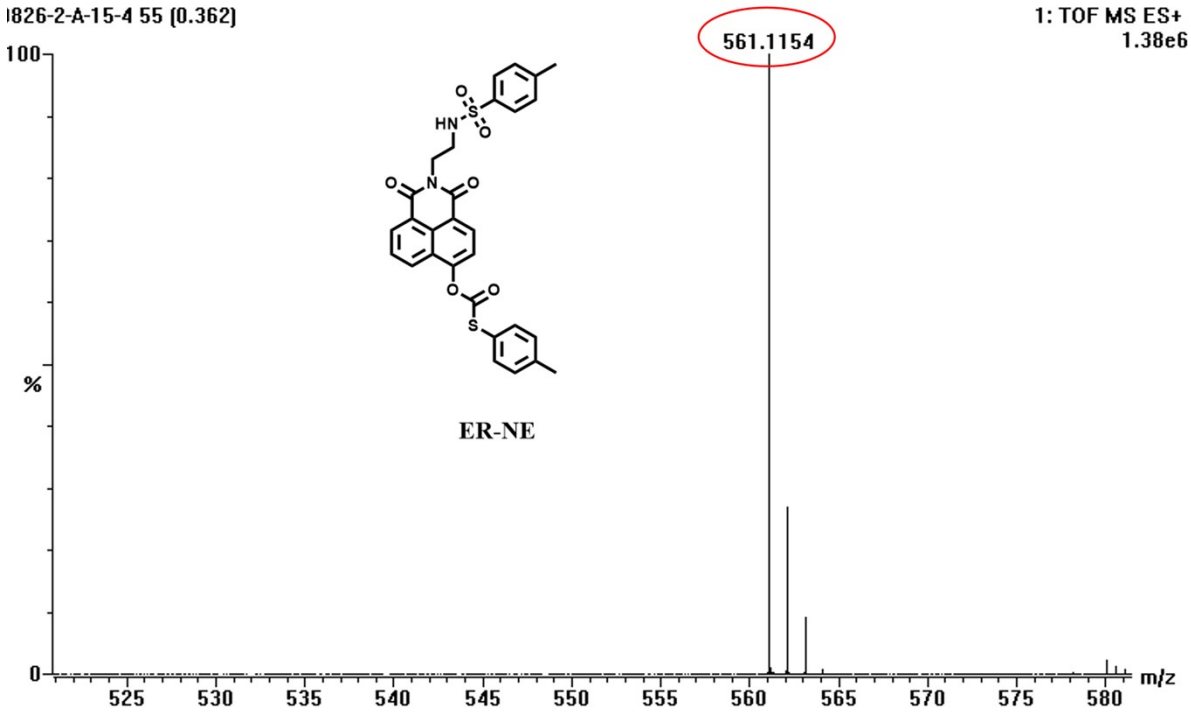

Fig. S3. HRMS spectral of compound **ER-NE**

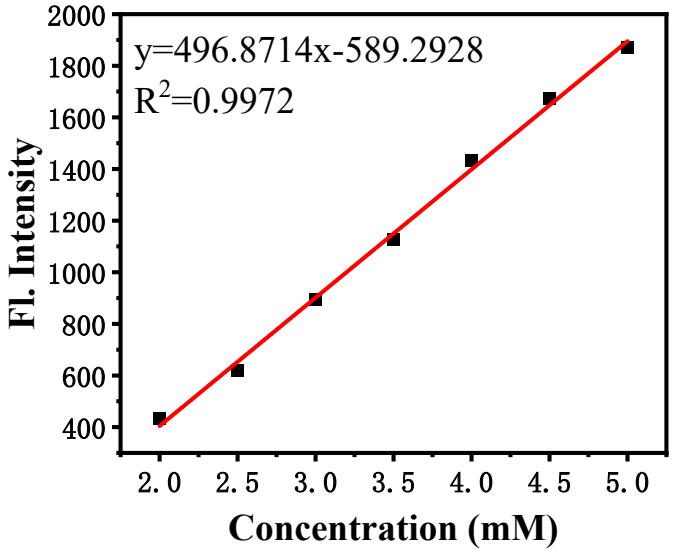

Fig. S4. Linear relationship between **ER-NE** and NE concentration. λ_{ex}:450 nm.

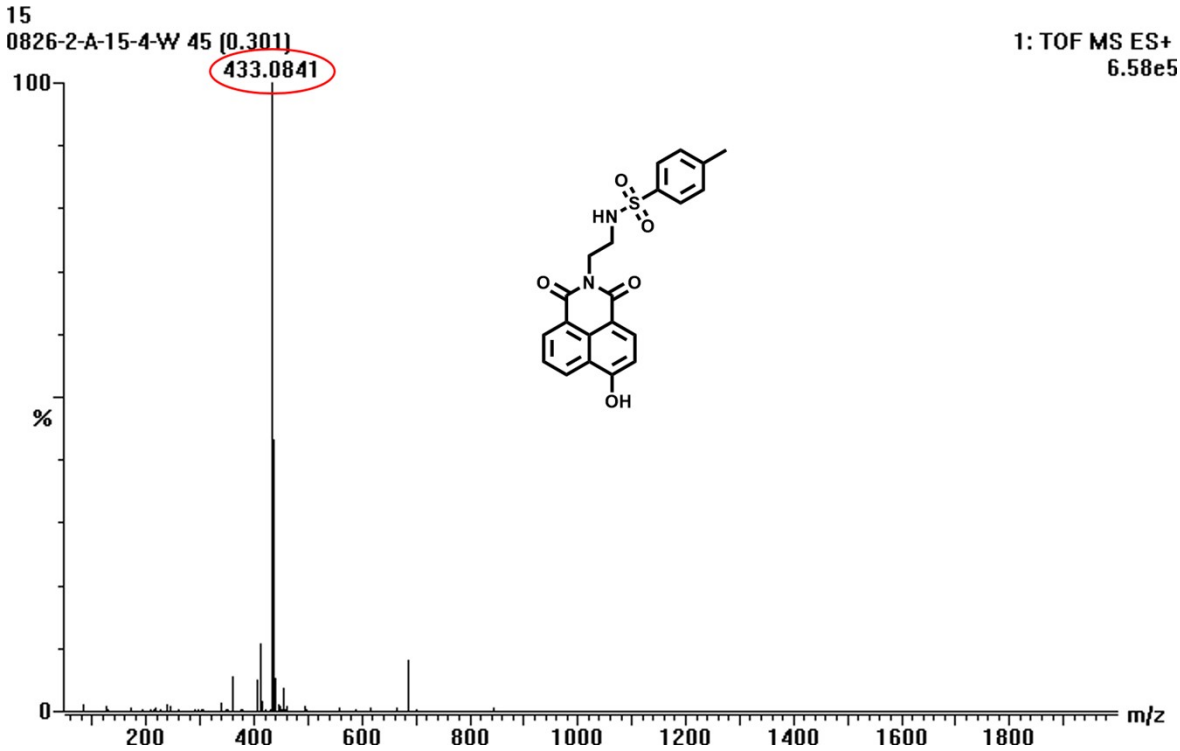

Fig. S5. The HRMS of the product after the reaction between **ER-NE** and NE. HR-ESIMS calcd for $C_{21}H_{18}N_2NaO_5S^+$: 433.0829 $[M+Na]^+$; found, 433.0841 $[M+Na]^+$.

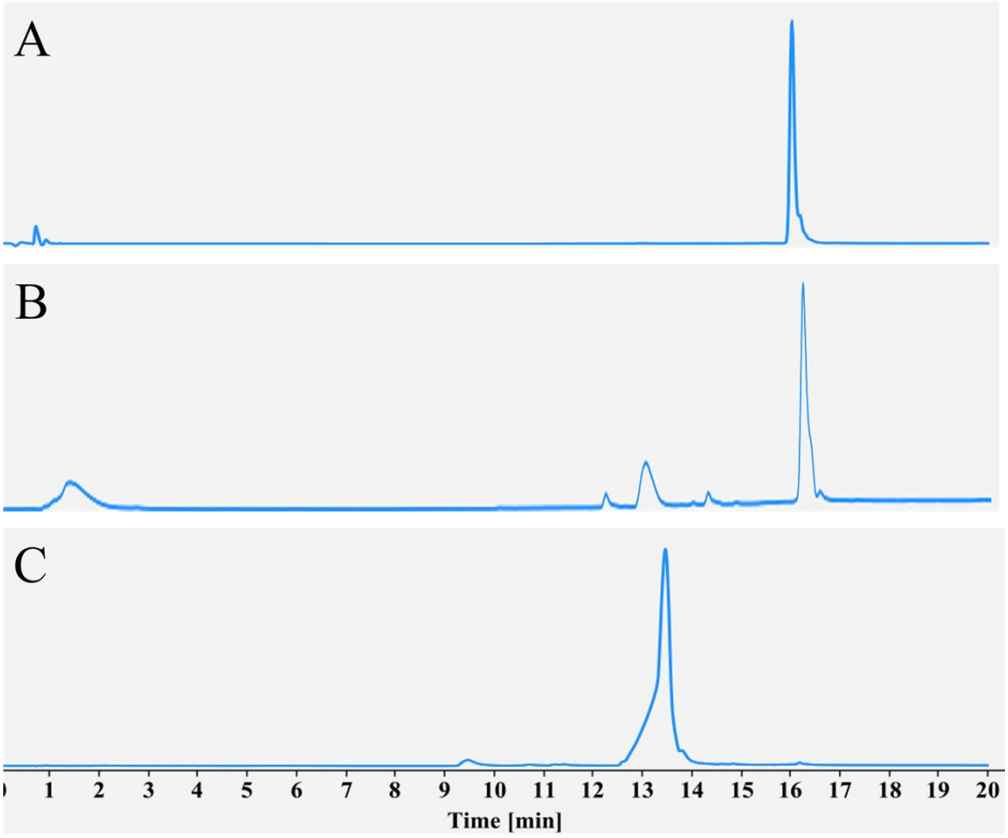

Fig.S6 The HPLC of (A)ER-NE; (B) ER-NE in the presence of NE; (C) ER-OH.

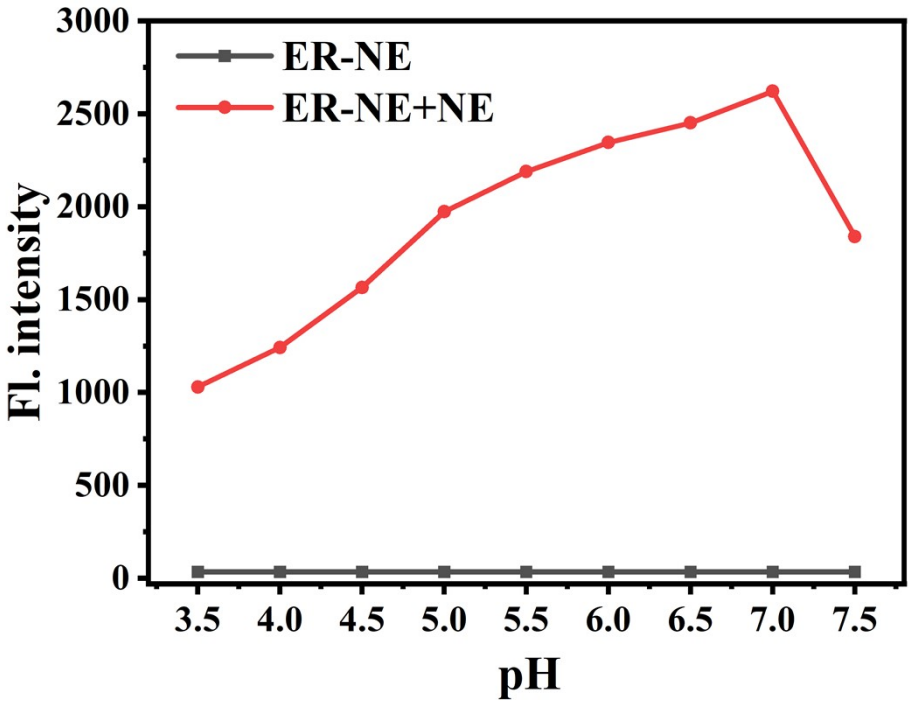

Fig. S7. Emission intensity changes of **ER-NE** (10 μM) at 575 nm with NE (5 mM) at indicated pH values in phosphate buffer (PBS) (10 mM, pH 7.4, containing 50% MeCN). λ_{ex}:450 nm.

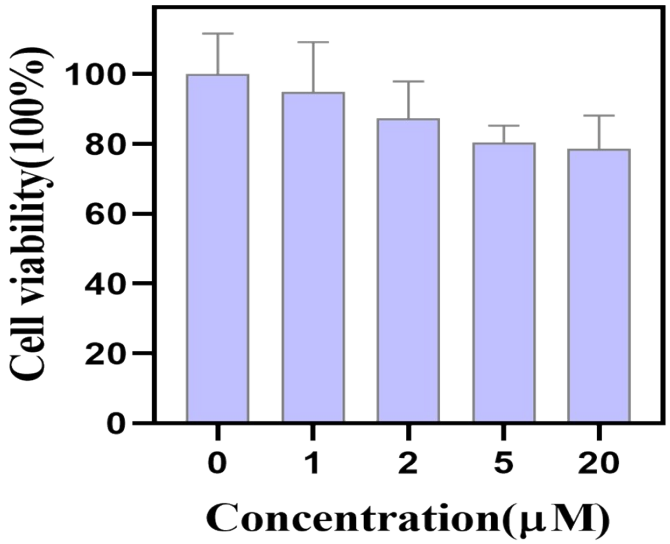

Fig. S8. Cytotoxicity of probe **ER-NE** at different concentrations for PC12 cells.

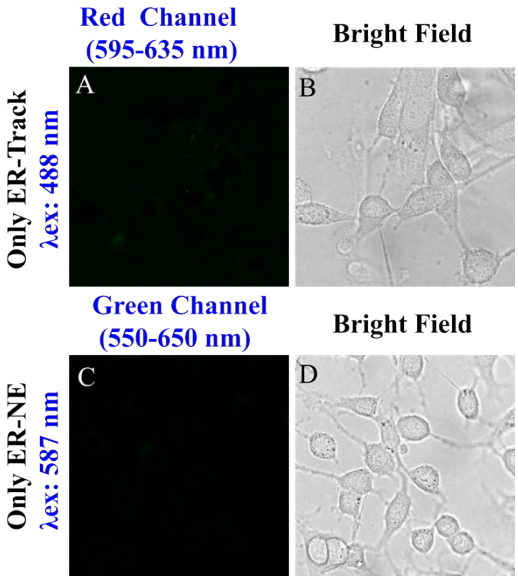

Fig.S9 PC12 cells image treated with only ER-NE/ER Track. A: PC12 cells only treated with ER Track (excitation at 488 nm, and collection the Red channel (595-635 nm)); B: Bright Field; C: PC12 cells only treated with ER-NE (excitation at 587 nm, and collection the Green channel (550-650 nm)); D: Bright Field.

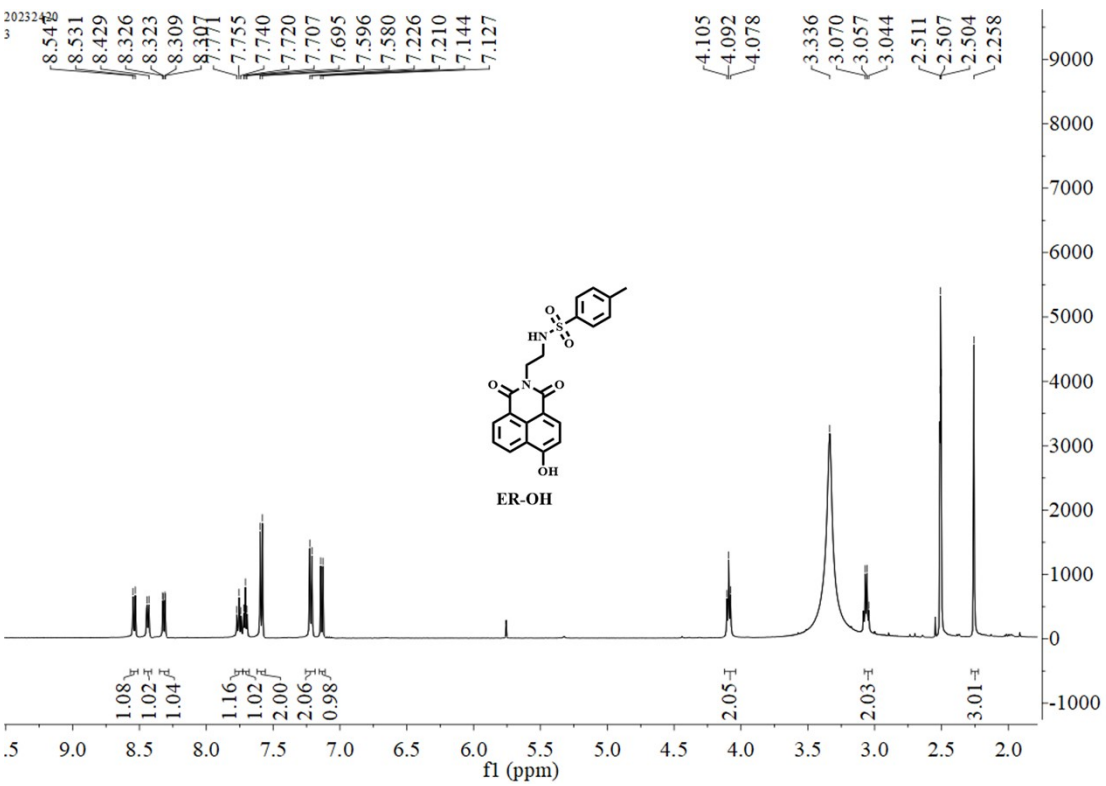

Fig. S10. ^{1}H NMR spectra of probe **ER-OH** in DMSO-d_6.

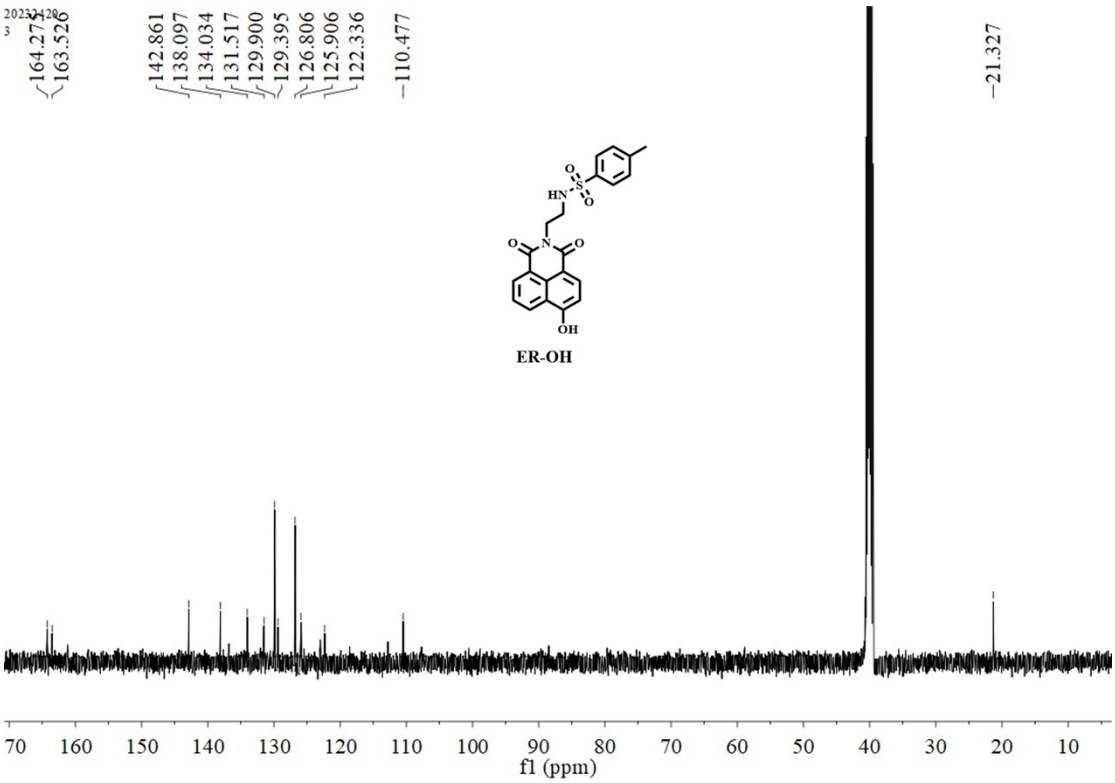

Fig. S11. ^{13}C NMR spectra of probe **ER-OH** in DMSO-d_6.