Supporting Information for

Evaluation of Cell Viability with Single Fluorescent Probe Based on Two Kinds of Fluorescence Signal Modes

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CONTENTS

Materials and methods

All reagents were obtained from commercial suppliers without further purification. All experiments used ultra-pure water. Solvents were purified by standard methods prior. Ultra-pure water is using by ULPURE. The pH measurements were performed with PHS-3E pH meter. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer. The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope. CCK-8 was purchased from Fluorescence imaging experiments were performed with TransGen Biotechnology. TLC analysis was carried out on silica gel plates, and column

chromatography was conducted over silica gel (mesh 200-300); both of them were purchased from the Qingdao Ocean Chemicals. Mito-TrackerTM Red was purchased from Thermo Fisher. ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity 600 spectrometer. High resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX 70 FT-MS, Agilent 6540T.

The synthesis of the probes ACD-E and CHD-V

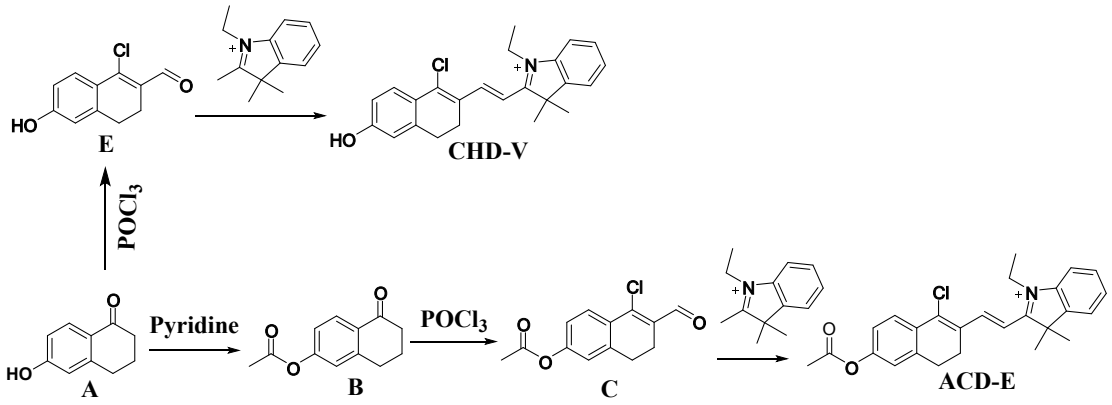

Figure S1. Synthesis routes of the probes **ACD-E** and **CHD-V**.

Characterization of compound E. Fresh N, N'-dimethylformamide (10 mL) was added dropwise to phosphorus oxychloride (2 mL) at 0 °C temperature, and the mixture was stirred for 30 min to yield an orange solution. Then 370 mg of 6-methoxytetralone (2.5 mmol, dissolved in 5 mL DMF) was added, and the solution was heated at 40 °C for 5 hours. The temperature was then raised to 70°C for 12 h. After cooled to 0 °C, the solution was poured onto 100 g of ice and neutralized by saturated sodium bicarbonate to adjust pH value. The crude product was purified by column chromatography on silica gel with petroleum ether and ethyl acetate as eluent to give the product as a yellow solid (137mg). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 10.32 (s, 1H), 10.19 (s, 1H), 7.66 (d, J = 8.6 Hz, 1H), 6.78 (dd, J = 8.6, 2.4 Hz, 1H), 6.71 (d, J = 2.3 Hz, 1H), 2.78 – 2.69 (m, 2H), 2.48 (dd, J = 9.0, 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-*d6*) δ 190.02, 161.35, 145.62, 141.91, 129.18, 128.66, 122.93, 115.36, 114.50, 27.07, 21.73.

Synthesis of compound B. Product A (2.3mmol,400 mg) was dissolved in anhydrous pyridine (10 mL), and then acetic anhydride added dropwise slowly at 0 ℃ under a nitrogen atmosphere. Mixture warm to room temperature, then the solution was refluxed stirred at 110 °C overnight (the reaction was monitored by a TLC plate), then cooled, pour the reactants into ice water. The solution extract 20 mL * 3 with ethyl acetate, and then add copper sulfate solution. Solvent was removed under reduced pressure and purified by column chromatography with a gradient elution (CH2Cl2) to give the product B as a white solid. (357 mg) ^{1}H NMR (500 MHz, DMSO-*d6*) δ 7.91 (d, J = 8.5 Hz, 1H), 7.32 – 6.84 (m, 2H), 2.95 (t, J = 6.0 Hz, 2H), 2.66 – 2.55 (m, 2H), 2.14 – 1.97 (m, 2H).

Synthesis of compound C. The compound C was synthesized according to produce E procedure. 1H NMR (500 MHz, DMSO-d6) δ 10.27 (s, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.19 (dd, J = 8.4, 2.4 Hz, 1H), 7.17 (s, 1H), 2.85 (s, 3H), 2.56 (d, J = 8.3 Hz, 2H), 2.30 (s, 3H). 13C NMR (126 MHz, DMSO-d6) δ 190.52, 169.37, 153.14, 143.89, 141.18, 132.25, 129.33, 127.75, 121.88, 121.07, 26.53, 21.58, 21.33

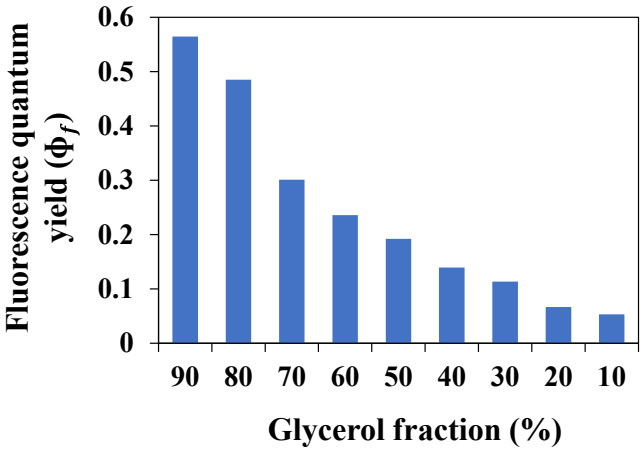

Figure S2 The fluorescence quantum yield (Φ_f) of the probe **CHD-V** in different ratio of ethanol and glycerol.

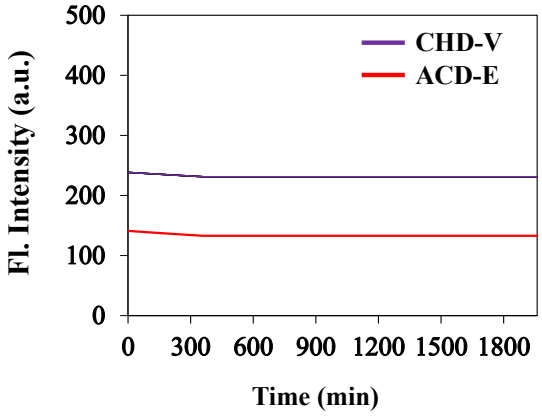

Figure S3 The intensity of fluorescence spectra of probe **CHD-V** (10 μM) at 698 nm in EtOH and **ACD-E** (10 μM) at 609 nm in EtOH/PBS (1:20, v/v) in different time at room temperature.

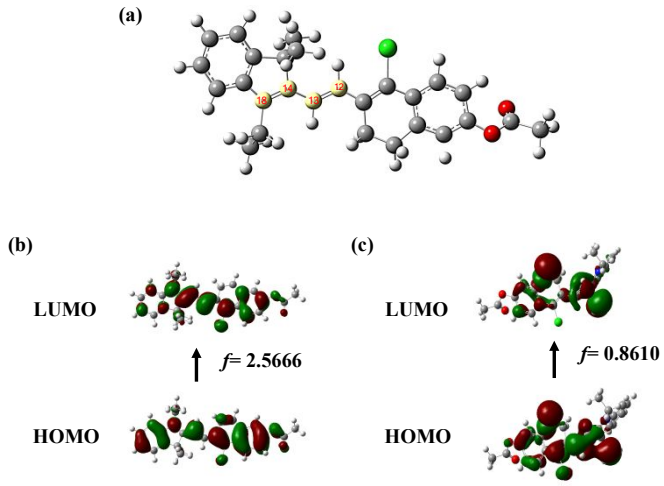

Figure S4 (a) The optimized geometries of **ACD-E** in the excited states; (b) the frontier molecular orbital of **ACD-E** in the excited states with a dihedral angle of 0° at around C12–C13–C14–N18; (c) the frontier molecular orbital of **ACD-E** in the excited states with a dihedral angle of 90° at around C12–C13–C14–N18. Calculations were performed by the DFT method (PCM model) with a B3LYP/6-31G (d, f) basis set using Gaussian 09.

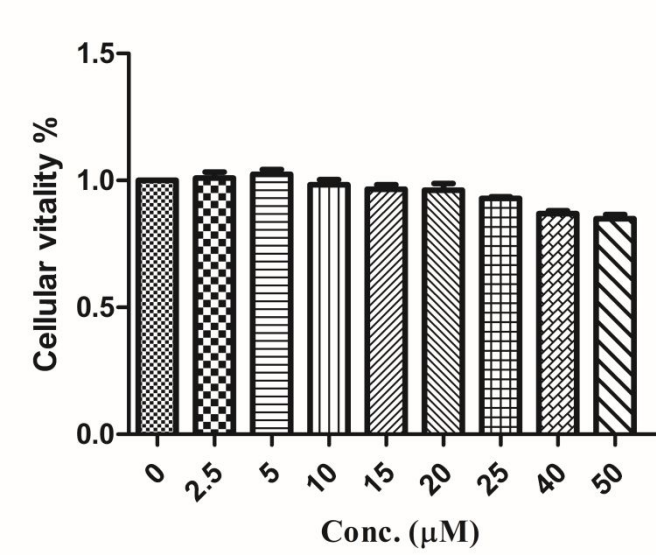

Figure S5. Effects of the probe **ACD-E** with varied concentrations (0-50 μM) on the viability of the Hela cells. The probe with varied concentrations was incubated with the cells for 24 h. The viability of the cells in the absence of the probe is defined as 100 %, and the data are the mean standard deviation of five separate measurements.

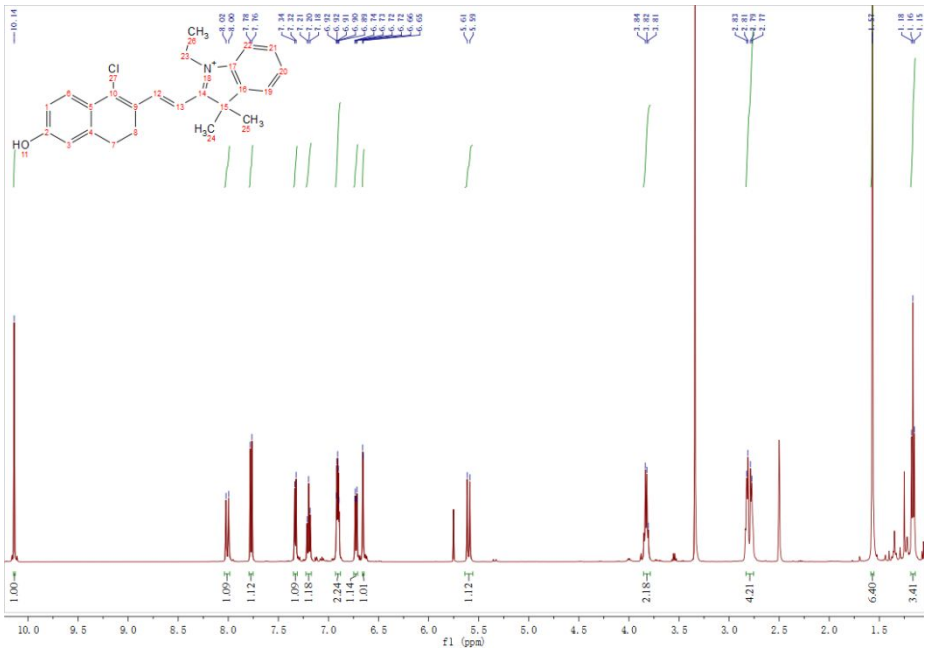

Figure S6 ^{1}H NMR spectra of probe **CHD-V** in DMSO-*d6*.

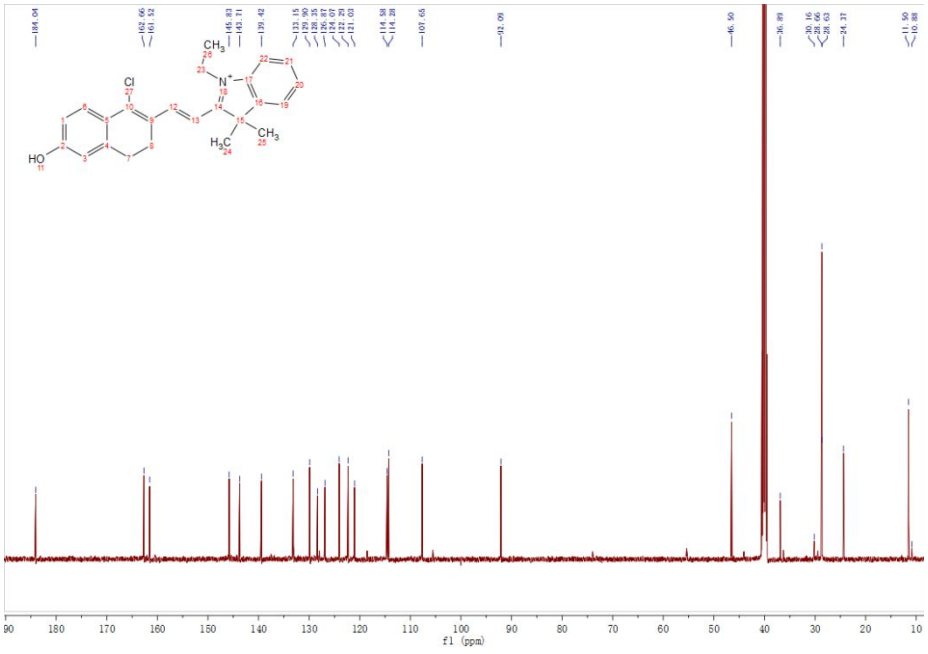

Figure S7 ^{13}C NMR spectra of probe **CHD-V** in DMSO-*d6*.

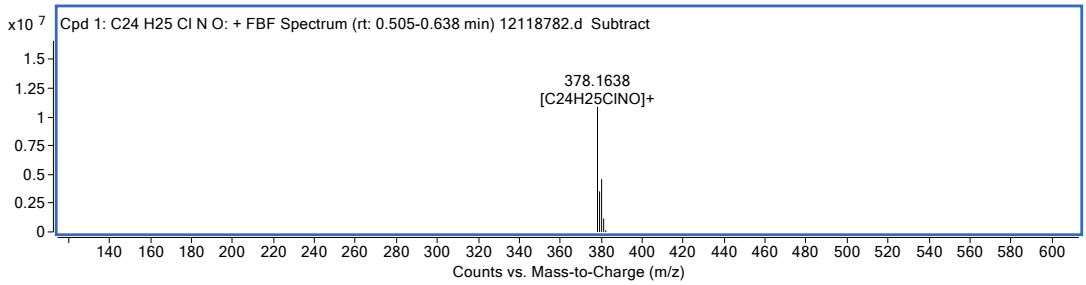

Figure S8 HRMS spectrum of **CHD-V**

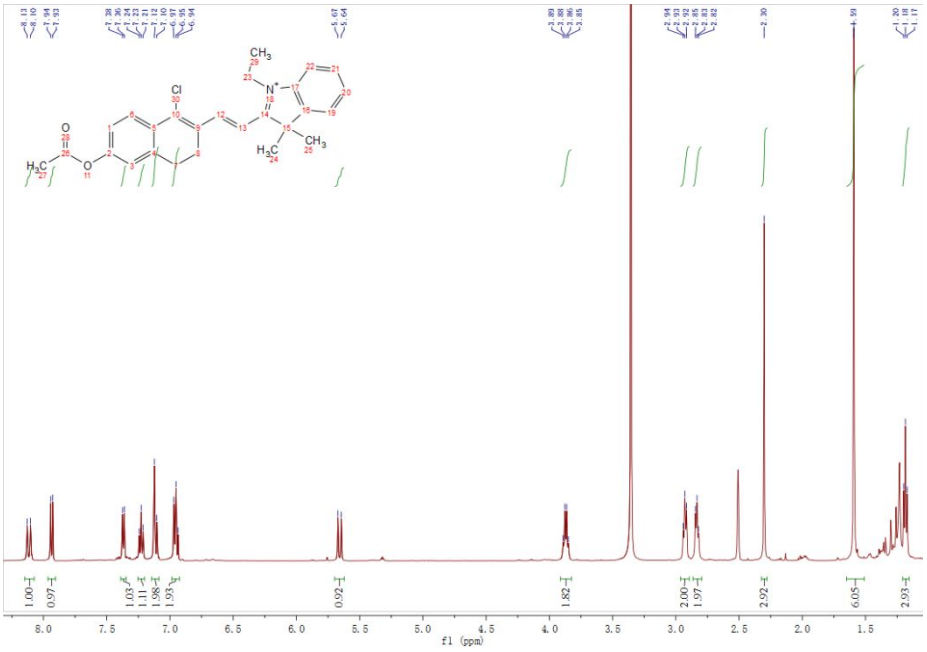

Figure S9 ^{1}H NMR spectra of probe **ACD-E** in DMSO-*d6*.

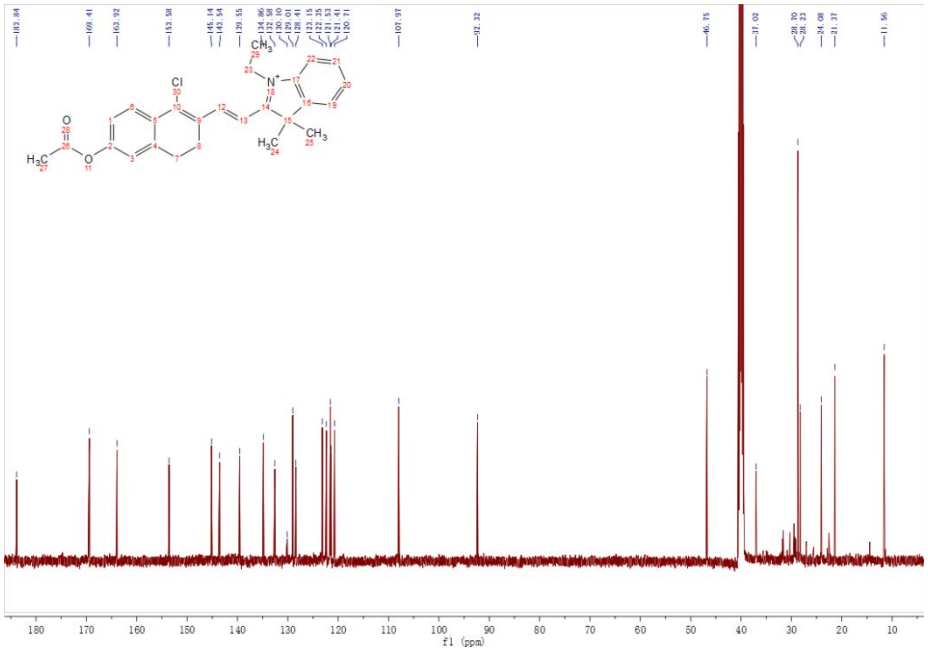

Figure S10 ^{13}C NMR spectra of **ACD-E** in DMSO-*d6*.

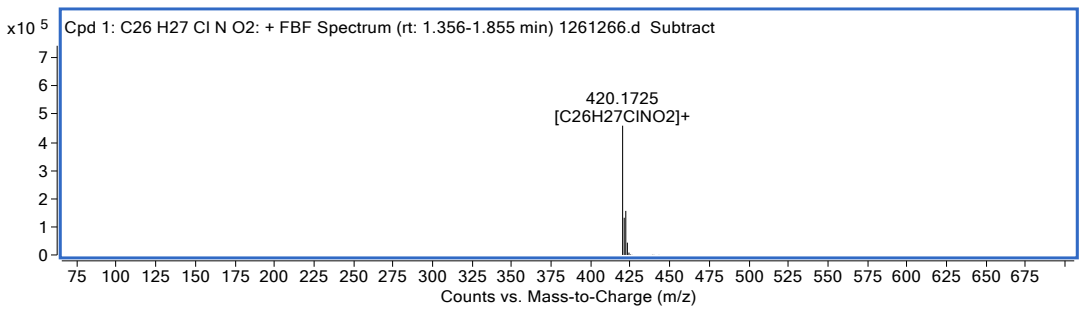

Figure S11 HRMS spectrum of **ACD-E**