Supporting Information for

A highly selective probe engineered to detect polarity and distinguish normal cells and tumor cells in tissue sections

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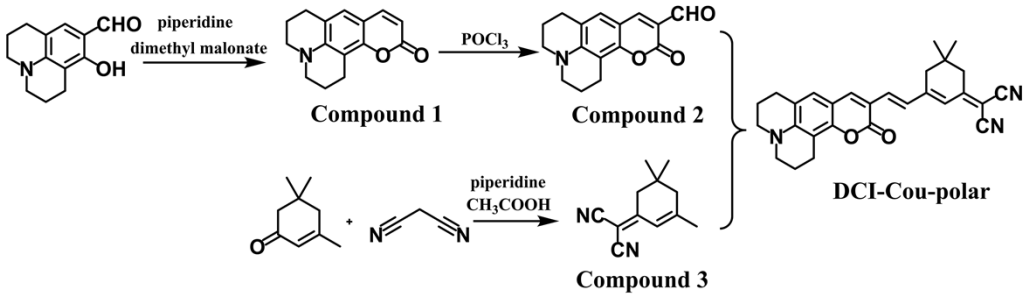
Table of contents

Page

Materials and instruments

Common reagents or materials were obtained from commercial suppliers without further purification except as otherwise noted. 8-Hydroxyjulolidine-9-Aldehyde, dimethyl malonate and isophorone are purchased from Bidepharm. Phosphorus oxychloride and malononitrile are purchased from Energy Chemical. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer (Japan), and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer (Japan). The fluorescence imaging of cells was performed with Leica TCS SP8 CARS confocal microscope (Germany). ^{1}H and ^{13}C NMR spectra were measured on a Bruker AVANCE III HD500 digital NMR spectrometer (Germany), using tetramethylsilane (TMS) as internal reference. High resolution mass spectrometric (HRMS) was acquired on Aglient 7250& JEOL-JMS-T100LP AccuTOF (Bruker Daltonics, Billerica, MA, USA).

Synthetic route of the fluorescent probe DCI-Cou-polar



Scheme S1. Synthetic route of the fluorescent probe **DCI-Cou-polar**.

Synthesis of compound 1

8-Hydroxyjulolidine-9-Aldehyde (100 mg, 0.46 mmol), dimethyl malonate (187 mg, 1.415 mmol) and piperidine (0.14 mL) were dissolved in 2 mL ethanol, and the reaction mixture was refluxed for 12 h under nitrogen protection. After the solvent was evaporated, 1 mL of concentrated hydrochloric acid and 1 mL of acetic acid were added, and the reaction mixture was refluxed for 25 h under the protection of nitrogen. After

cooling, 2 mL saturated NH_4Cl and 2 mL 40% NaOH were added to the reaction mixture to precipitate brown solid, and the precipitated solid was purified by silica gel column. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, *J* = 9.2 Hz, 1H), 6.86 (s, 1H), 6.01 (d, *J* = 9.2 Hz, 1H), 3.40 – 3.16 (m, 4H), 2.90 (t, *J* = 6.5 Hz, 2H), 2.77 (t, *J* = 6.3 Hz, 2H), 2.07 – 1.90 (m, 4H).

Synthesis of compound 2

Under nitrogen protection, 0.3 mL phosphorus oxychloride was added to 1 mL DMF and stirred at 0 ℃ for 0.5 h. Compound **1** (60 mg, 0.25 mmol) was dissolved in 1 mL anhydrous DMF, the dissolved solution was dropped into the reaction system, and then stirred at 70 ℃ for 12 h. After the reaction was completed, the reaction solution was poured into ice water to stop the reaction and the precipitated solid was filtered to obtain a purple solid (50 mg, 74% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.86 (s, 1H), 8.21 (s, 1H), 7.24 (s, 1H), 3.37 (dt, *J* = 9.3, 4.8 Hz, 4H), 2.71 (dt, *J* = 12.5, 6.3 Hz, 4H), 1.94 – 1.82 (m, 4H).

Synthesis of compound 3

Isophorone (1384 mg, 10 mmol) and malononitrile (782 mg, 12 mmol) were dissolved in 15 mL anhydrous ethanol. Two drops of acetic acid and two drops of piperidine were added to the reaction solution. Under nitrogen protection, the reaction system was refluxed for 6 h. After removing ethanol by rotary distillation under reduced pressure, water and dichloromethane were added for extraction. The resulting residue was purified by silica gel column to afford the compound **3** as white solid (541 mg, 29% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.63 (s, 1H), 2.53 (s, 2H), 2.19 (s, 2H), 2.05 (s, 3H), 1.03 (s, 6H).

Solution preparation

The probe solution (5 mM) was prepared by dissolving 2.2 mg probe **DCI-Cou-**

polar in 1 mL DMSO. The concentration of **DCI-Cou-polar** in the spectrum experiments were 10 μM. In the selectivity experiment, analytes with concentration of 100 mM or 10 mM were prepared in ultra-pure water as reserve solution. We chose 1,4 dioxane/DMSO as the polarity detection system in the spectrum experiment.

Culture and preparation of HepG2 cells and HL-7702 cells

HepG2 and HL-7702 cells cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Before the experiments, HepG2 and HL-7702 cells were inoculated in 35 mm glass-bottomed culture dishes and then cultured in 2 mL culture medium at 37℃ in an incubator containing 5% CO_2 and 95% air for 24 h. During this period, cells will adhere to the glass surface.

Cytotoxicity assays

HL-7702 cells and HepG2 cells were inoculated into 96-well plates and the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) until 80-90% of the bottom area of the plates was covered by cells. Different concentrations of **DCI-Cou-polar** (0, 1, 2, 3, 5, 10, 20 μM) were added and cultured in the incubator for 24 h. After the culture medium with probe was sucked away, 90 μL DMEM medium and 10 μL MTT (5 mg/mL) were added to each well and incubated for 4 h. The excess MTT solution was then carefully removed from each well, and the formed formazan was dissolved in 100 μL of DMSO. The optical density of each well was measured at a wavelength of 490 nm using a microplate reader (Bio-Tek, USA). The viability of cells was determined by assuming that the viability of cells without **DCI-Cou-polar** was 100%.

Preparation of tumor and normal organ slices for imaging experiments

Five-week-old Female BALB/c mice (license number: SCXK 2019-0010) were purchased from SiPeiFu Biotechnology (Beijing, China), and were housed under aseptic conditions in small animal isolators with free access to food and water. All animal experiments were reviewed and approved by the Animal Care and Experiment Committee of Guangxi University. To establish 4T-1 tumor bearing BALB/c mice, 4T-1 cell suspensions (1 × 106 cells) were subcutaneously injected into the flank of BALB/c mice depilated. After 14 days, tumors and organs (heart, liver, and kidney) were dissociated from mice and washed 3 times with PBS. The organs and tumors were sliced and incubated with 50 μM **DCI-Cou-polar** in PBS for 30 min. After washing 3 times with PBS, the slices were observed under confocal microscope.

Calculation of fluorescence quantum yield of DCI-Cou-polar

The fluorescence quantum yields (Φ_F) were determined by using rhodamine B as the Φ_F reference according to the literature method. Quantum yields were corrected as follows:

$$\Phi_F = \Phi_r \left(\frac{n_s}{n_r}\right)^2 \left(\frac{A_r}{A_s}\right) \left(\frac{F_s}{F_r}\right)$$

Where the s and r indices designate the sample and reference samples respectively. A is the absorbance at λ_{ex}, n is the average refractive index of the appropriate solution, and F is the integrated area under the corrected emission spectrum.

Solvents	E_T (30)	λ_{abs}/nm	λ_{em}/nm	ε/L mol^{-1} cm^{-1}	Stokes shift/nm	Φ_F (%)
Toluene	33.9	536	654	36000	118	1.56%
Dioxane	36	530	660	35200	130	2%
THF	37.4	535	682	36700	147	3.34%
ACN	42.2	538	724	37700	186	2.13%
DMF	43.2	551	731	39000	180	2.02%
DMSO	45.1	561	742	36100	181	1.90%
PBS	63.1	529	766	17900	237	0.016%

Table S1. Photophysical properties of **DCI-Cou-polar** in various solvents.

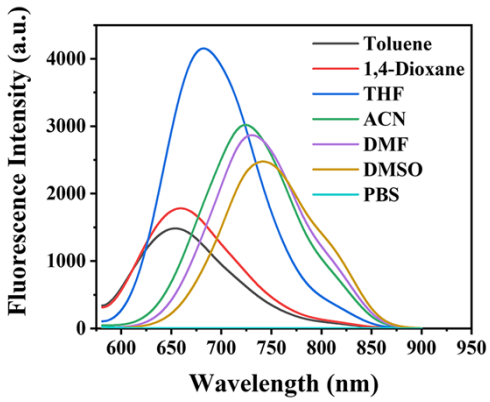


Fig. S1. The fluorescence spectra of **DCI-Cou-polar** in different polarity solvents.

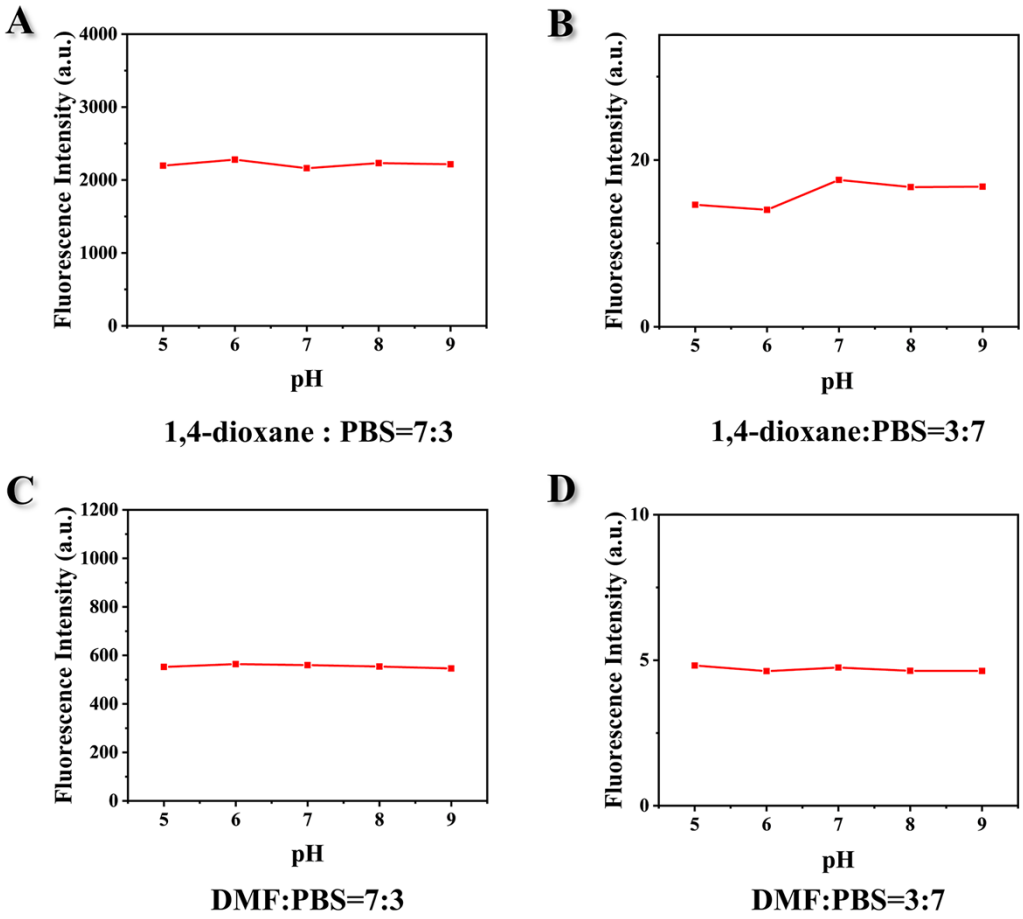


Fig. S2. (A) The fluorescence intensity of **DCI-Cou-polar** (10 μM) in solvents with ratio of 1,4-dioxane: PBS = 7: 3 under different pH, λ_{ex} = 561 nm, λ_{em} = 726 nm. (B) The fluorescence intensity of **DCI-Cou-polar** (10 μM) in solvents with ratio of 1,4-dioxane: PBS = 3: 7 under different pH, λ_{ex} = 561 nm, λ_{em} = 732 nm. (C) The

fluorescence intensity of **DCI-Cou-polar** (10 μM) in solvents with ratio of DMF: PBS = 7: 3 under different pH, λ_{ex} = 561 nm, λ_{em} = 747 nm. (D) The fluorescence intensity of **DCI-Cou-polar** (10 μM) in solvents with ratio of DMF: PBS = 3: 7 under different pH, λ_{ex} = 561 nm, λ_{em} = 717 nm.

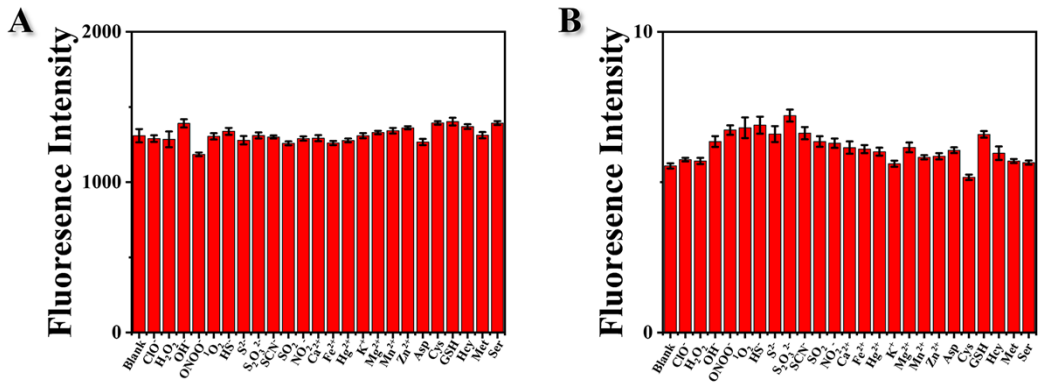


Fig. S3. (A) The fluorescence intensity of **DCI-Cou-polar** (10 μM) to various relevant analytes (100 μM，the concentration of Cys and GSH is 500 μM) in solvents with ratio of 1,4-dioxane: PBS = 7: 3, λ_{ex} = 561 nm, λ_{em} = 727 nm. (B) The fluorescence intensity of **DCI-Cou-polar** (10 μM) to various relevant analytes (100 μM，the concentration of Cys and GSH is 500 μM) in solvents with ratio of DMF: PBS = 3: 7, λ_{ex} = 561 nm, λ_{em} = 717 nm.

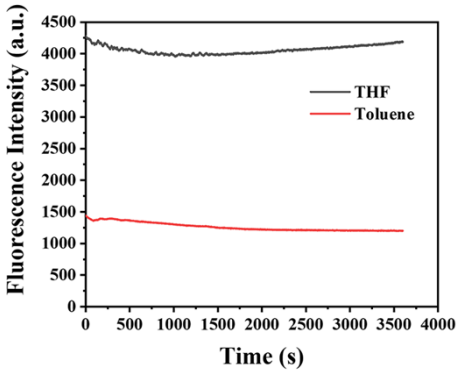


Fig. S4. Photostability of **DCI-Cou-polar** in THF and toluene with continuous irradiation for 60 min. λ_{ex} = 561 nm, λ_{em} = 654 nm.

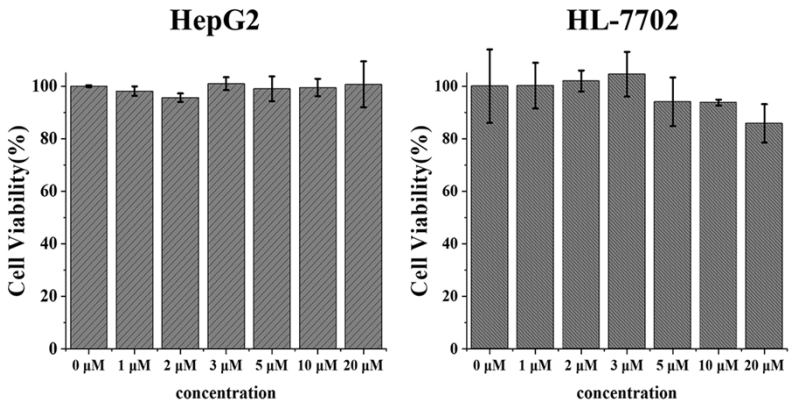


Fig. S5. The cytotoxicity of **DCI-Cou-polar** in HL-7702 cells and HepG2 cells.

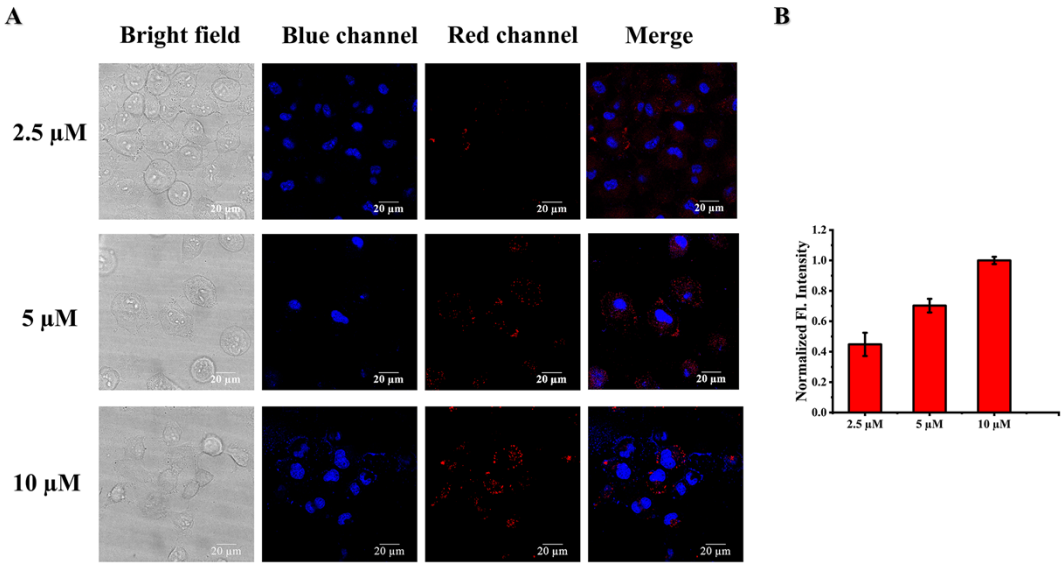


Fig. S6. (A) Confocal imaging of HepG2 cells treated with different concentrations of **DCI-Cou-polar**. (The blue channel is the nuclear commercial dye **Hoechst 33342**. The Red channel is **DCI-Cou-polar**). (B) Normalized fluorescence intensity of HepG2 cells treated with different concentrations of **DCI-Cou-polar**. Blue channel: λ_{ex} = 405 nm, λ_{em} = 425-510 nm; Red channel: λ_{ex} = 561 nm, λ_{em} = 620-786 nm. Scale bar: 20 μm.

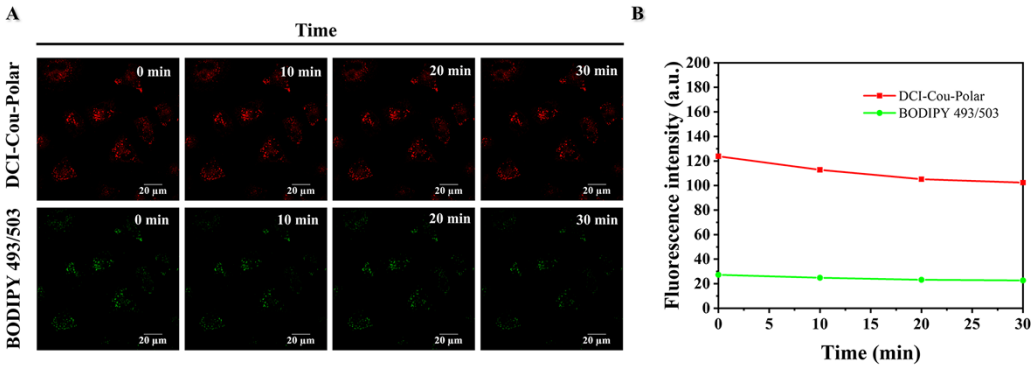


Fig. S7. Fluorescence intensity of **DCI-Cou-polar** and commercial dye **BODIPY 493/503** at different time. Green channel: λ_{ex} = 493 nm, λ_{em} = 522-539 nm; Red channel: λ_{ex} = 561 nm, λ_{em} = 620-786 nm. Scale bar: 20 μm.

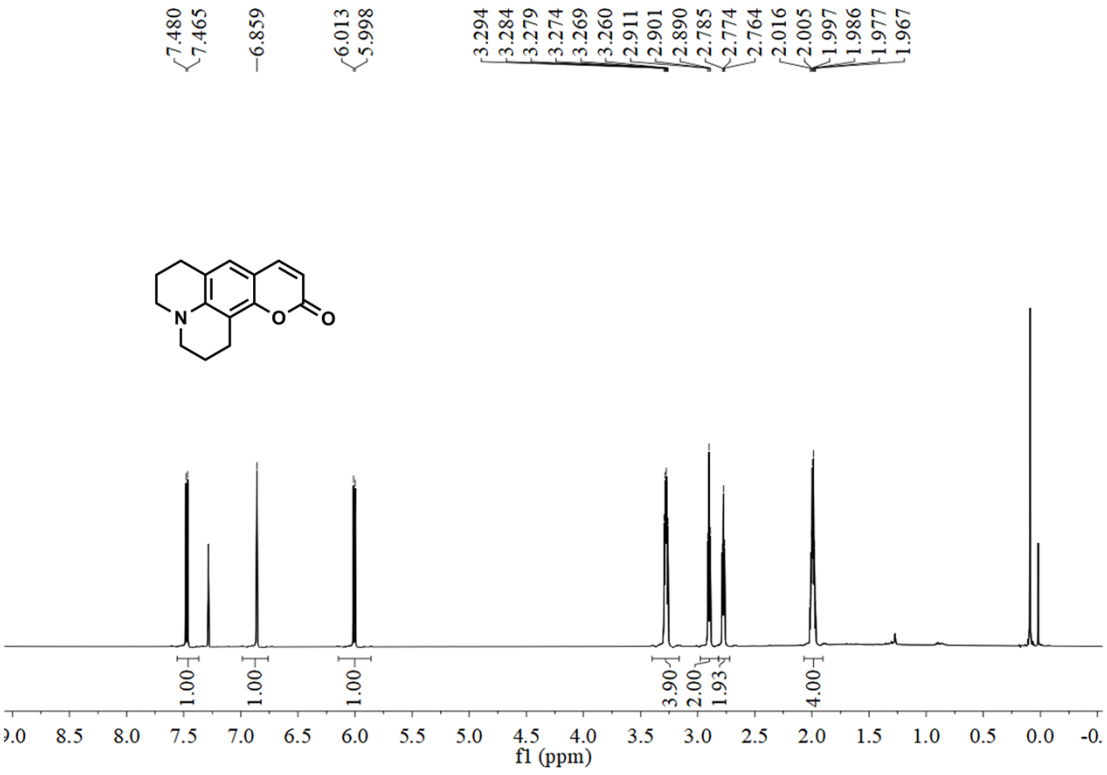


Fig. S8. ^{1}H NMR spectrum of **compound 1** in $CDCl_3$.

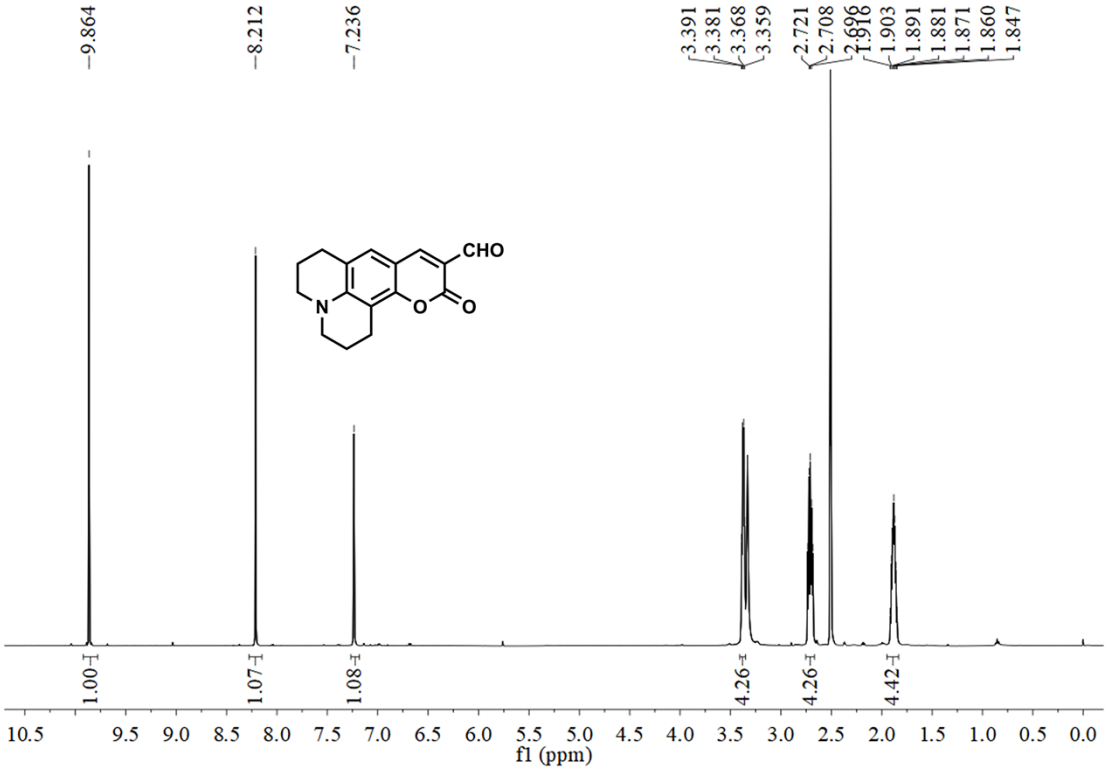


Fig. S9. ^{1}H NMR spectrum of **compound 2** in DMSO-d_6.

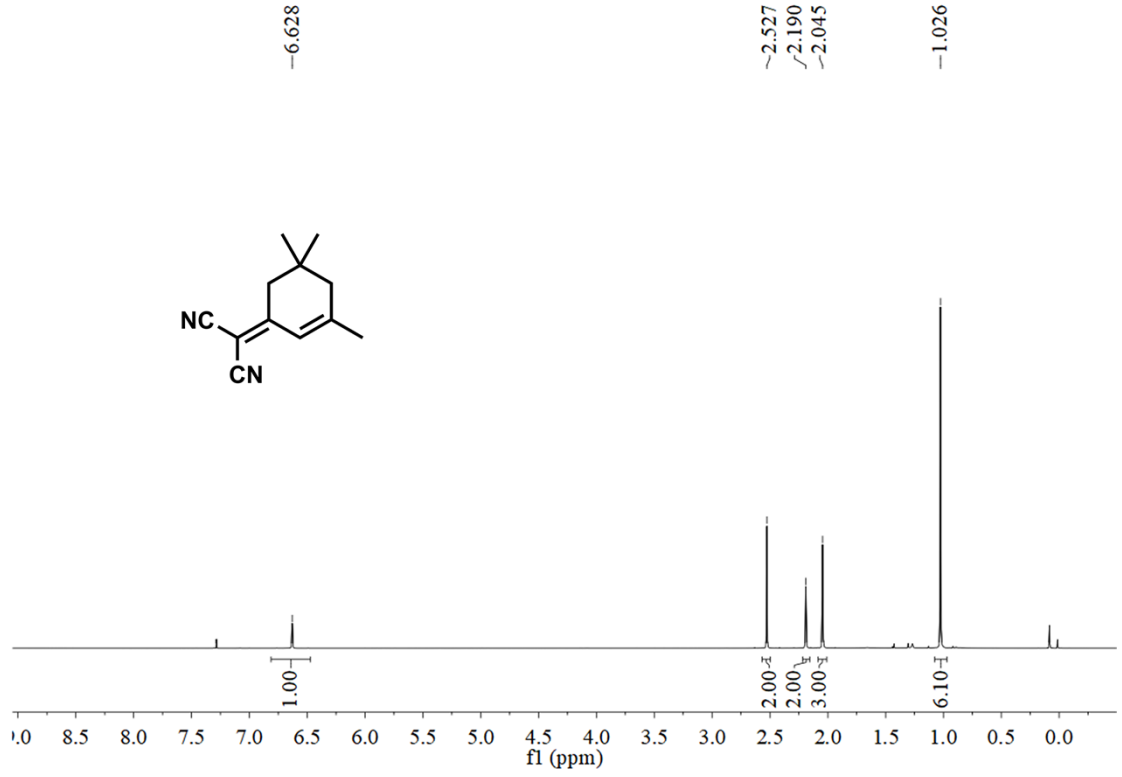


Fig. S10. ^{1}H NMR spectrum of **compound 3** in $CDCl_3$.

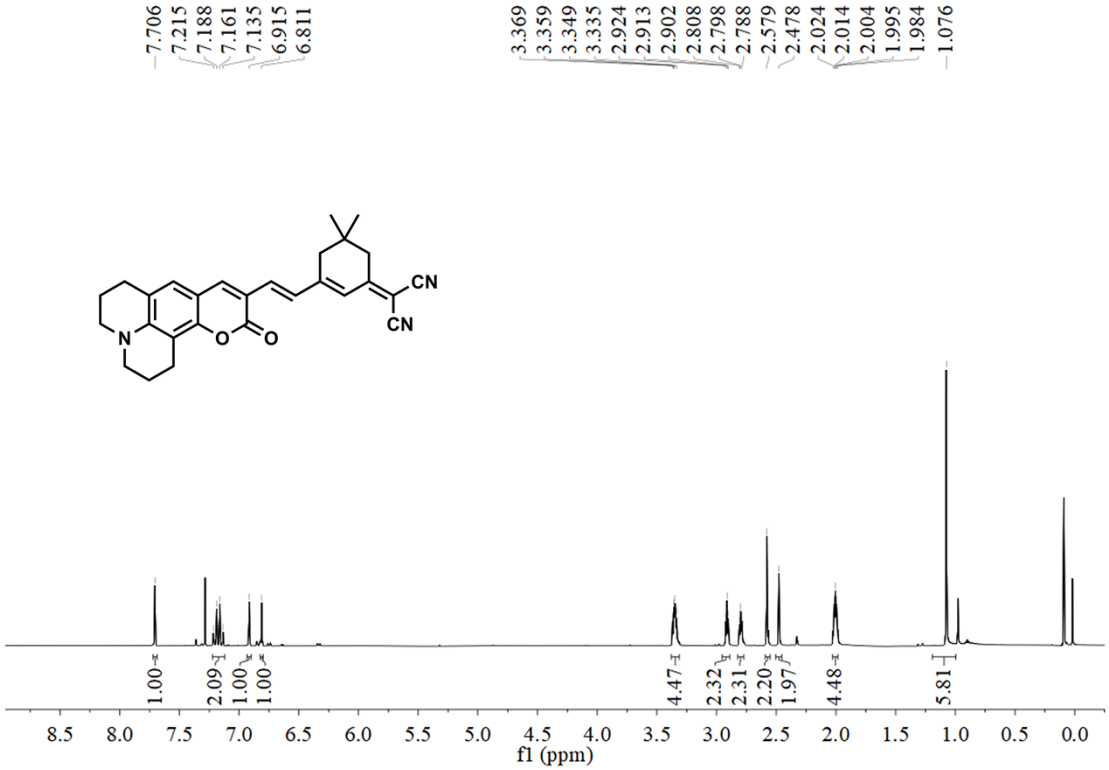


Fig. S11. ^{1}H NMR spectrum of **DCI-Cou-polar** in $CDCl_3$.

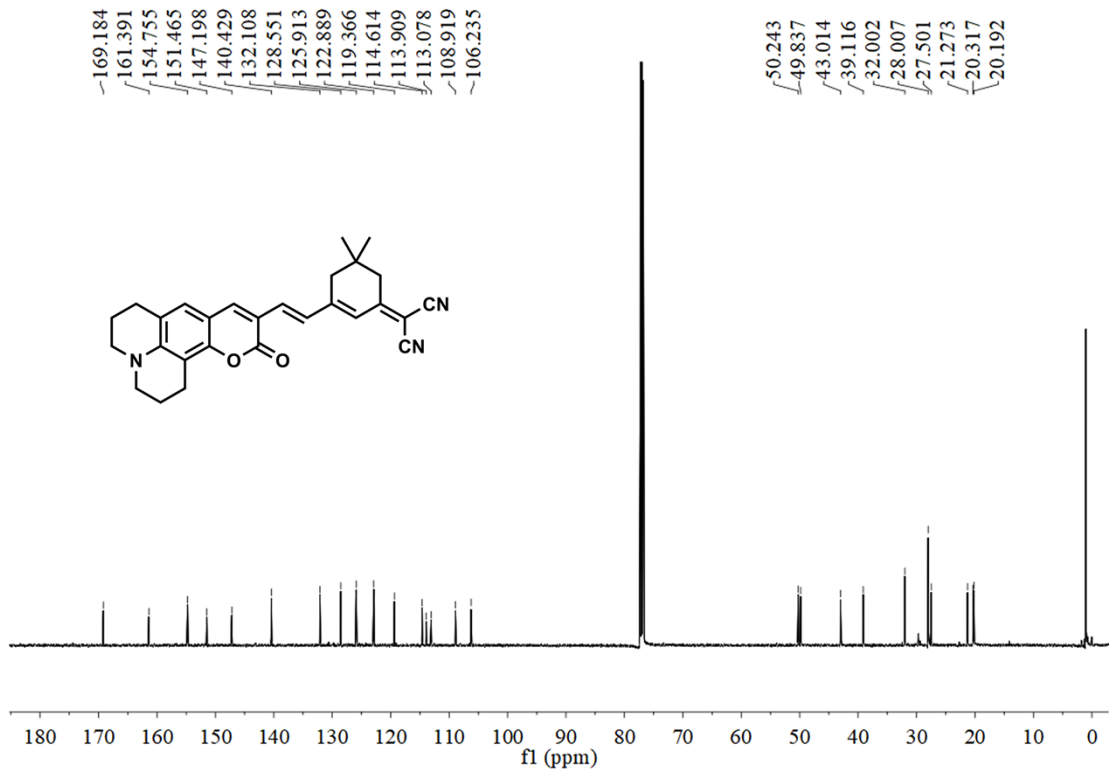


Fig. S12. ^{13}C NMR spectrum of **DCI-Cou-polar** in $CDCl_3$.

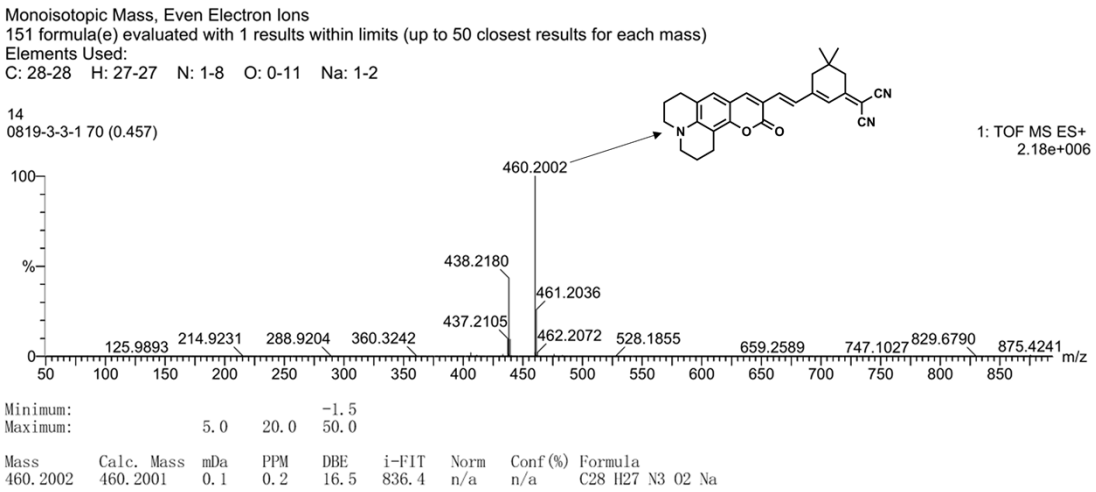
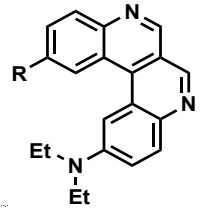
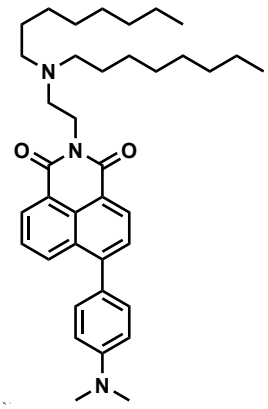
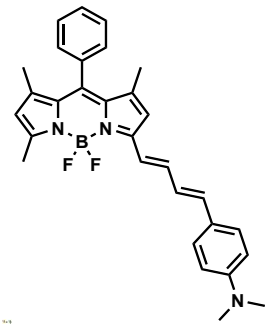
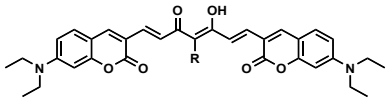
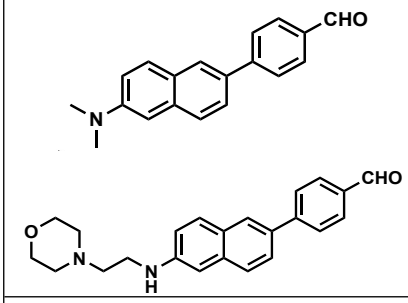
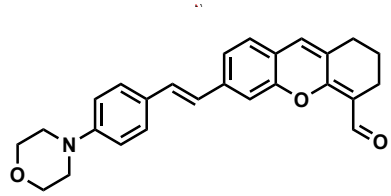
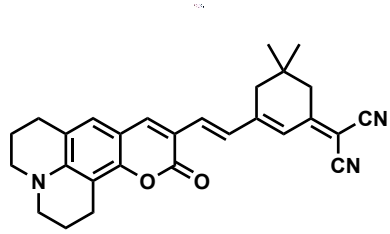


Fig. S13. HRMS (ESI) spectrum of **DCI-Cou-polar**.

Table S2. Comparison of this work with reported fluorescent probes.

The structure of the probes	Maximum Stokes shift/nm	Number of ROS and RSS in selective experiments	Bioimaging application	Ref.
	146	3（in non-lipid and lipid environment）	Cells	[1]
	No mentioned	7（in lipid environment）	Cells Tissues	[2]

	84	3（in non-lipid environment）	Cells Tissues Mice	[3]
	94	3（in non-lipid environment）	Cells	[4]
	227	5（in non-lipid environment）	Cells	[5]
	223	3（in non-lipid and lipid environment）	Cells	[6]
	237	11（in non-lipid and lipid environment）	Cells Tissues	This work

[1] M.-N. Li, J.-Y. Zhao, G. Zhang, Y.-J. Xu, J.-F. Ge, R. Sun, Naphthyridine-based neutral fluorescent probes for dynamic monitoring lipid droplet polarity, Dyes Pigment. 220 (2023) 111731.
[2] S. Biswas, M. Baruah, A. Shil, S. Sarkar, M. Ali, A. Samanta, S. Bhuniya, Polarity-Driven Two-Photon Fluorescent Probe for Monitoring the Perturbation in Lipid Droplet Levels during Mitochondrial Dysfunction and Acute Pancreatitis, ACS Sensors 8(10) (2023) 3793-3803.
[3] J. Zhang, W. Han, X. Zhou, X. Zhang, H. Zhang, T. Li, J. Wang, Y. Yuan, Y. He, J. Zhou, A Lipid Droplet-Specific NIR Fluorescent Probe with a Large Stokes Shift for

In Vivo Visualization of Polarity in Contrast-Induced Acute Kidney Injury, Anal. Chem. 95(31) (2023) 11785-11792.
[4] L. Shan, X. Li, X. Zheng, J. Wu, H. Ren, W. Liu, P. Wang, Two Polarity-Sensitive Fluorescent Probes Based on Curcumin Analogs for Visualizing Polarity Changes in Lipid Droplets, Molecules 28(18) (2023) 6626.
[5] Y. Dai, Z. Zhan, Q. Li, R. Liu, Y. Lv, Simultaneous monitoring of polarity changes of lipid droplets and lysosomes with two-photon fluorescent probes, Anal. Chim. Acta 1136 (2020) 34-41.
[6] Q. Hu, H. Zhang, P. Ye, S. Ma, X. Zhu, Y. Bai, Xanthene-based polarity-sensitive fluorescent probe with large Stokes shifts for simultaneous two-color visualizing of lipid droplets and lysosomes, Dyes Pigment. 208 (2023) 110874.