Supporting Information for

A Dual-Response Near-Infrared Probe for Simultaneous Detection of Viscosity and pH in Nephritis Identification

Qiongdan Tan [a], Qian Zhang [a], Xugong Yuan [a], Wei Quan [a,b], Weiying Lin [a] *

[a] Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

[b] School of Chemistry and Materials Science, Hubei Engineering University, Xiaogan 432000, China

Email: weiyinglin2013@163.com

*Correspondence to: Weiying Lin, Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

Email: weiyinglin2013@163.com.

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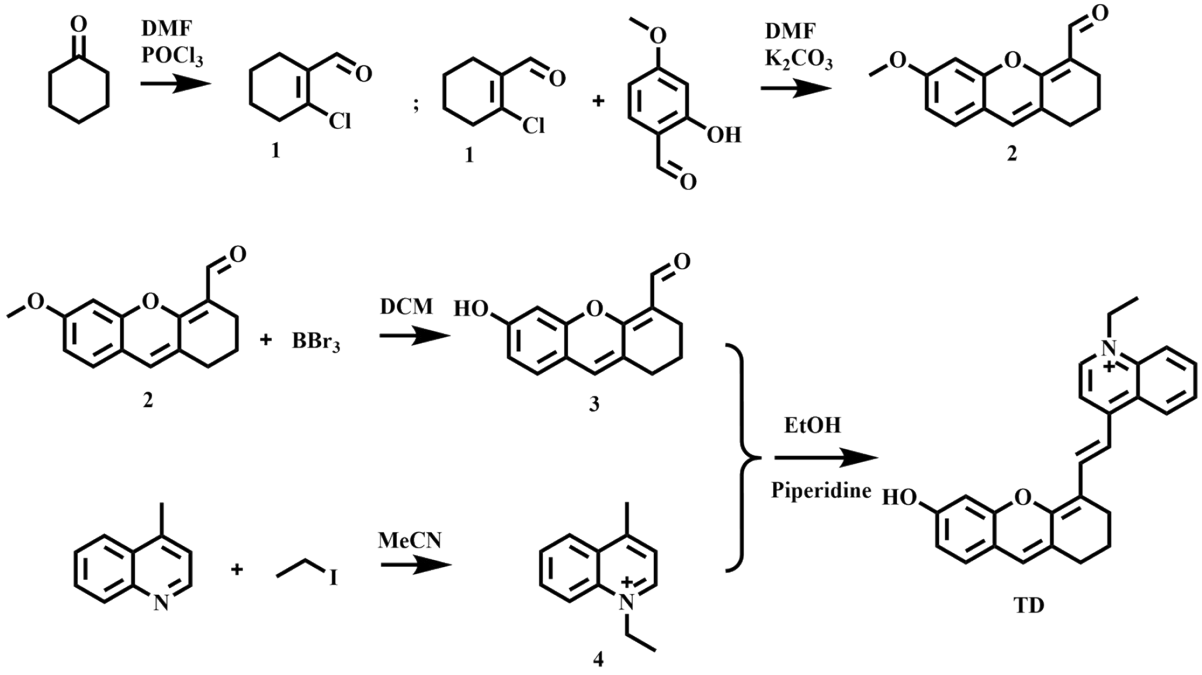
1. Materials and Instrumentations

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All experiments used ultra-pure water. Solvents were purified by standard methods prior. 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 Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance III HD 600 MHz NMR spectrometer (United States of America). High-resolution mass spectrometric (HRMS) analyses were measured on Brooke Solan X 70 FT-MS, Agilent 6540T. 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.

2 Synthesis



Scheme 1 The synthetic route of **TD**.

Synthesis of Compound 1: A mixture of phosphoryl chloride (5 mL) in dry Dimethylformamide (DMF) (5 mL) was stirred at 0 °C for 30 minutes. Cyclohexanone (5 mL) was then added dropwise, and the reaction mixture was stirred at room temperature for 30 minutes, followed by reflux at 70 °C for 6 hours. After completion, the reaction was carefully quenched with ice-water. The resulting mixture was extracted with ethyl acetate (EA). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the crude product, which was used directly in the next step without further purification, yield: 90%.

Synthesis of Compound 2: A mixture of 4-methoxysalicylaldehyde (2.00 g, 13.15 mmol), **compound 1** (2.28 g, 15.77 mmol), and potassium carbonate (5.45 g, 39.44 mmol) in DMF

(50.0 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was poured into water and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (eluent: petroleum ether/ethyl acetate = 10:2) to afford **compound 2**, yield: 78%. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 10.24 (s, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 6.96 (s, 1H), 6.88 (s, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 3.81 (s, 3H), 2.56 – 2.54 (m, 2H), 2.29 (t, *J* = 6.0 Hz, 2H), 1.63 (q, *J* = 6.1 Hz, 2H).

Synthesis of Compound 3: A solution of **compound 2** (1.00 g, 4.13 mmol) in dry dichloromethane (10.0 mL) was cooled to 0 °C. Boron tribromide (5.17 g, 20.64 mmol) was added dropwise. The reaction mixture was then warmed to room temperature and stirred overnight. The reaction was carefully quenched by the slow addition of water. The resulting precipitate was collected by filtration and used directly in the next step without further purification, yield: 80%. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 10.20 (s, 1H), 10.20 (s, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 6.93 (s, 1H), 6.63 (s, 1H), 6.61 (d, *J* = 8.3 Hz, 1H), 2.56 – 2.52 (m, 2H), 2.29 (t, *J* = 6.0 Hz, 2H), 1.62 (p, *J* = 6.1 Hz, 2H).

Synthesis of Compound 4: A mixture of 4-methylquinoline (2.00 g, 13.97 mmol) and iodoethane (2.61 g, 16.76 mmol) in dry acetonitrile (20.0 mL) was heated to reflux at 85 °C for 6 hours. After completion, the reaction mixture was cooled to room temperature. The resulting precipitate was collected by filtration, yielding **compound 4** as a yellow-green solid, which was used directly in the subsequent reaction, yield: 93%. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 9.43 (d, *J* = 6.1 Hz, 1H), 8.61 (d, *J* = 8.9 Hz, 1H), 8.56 (d, *J* = 7.1 Hz, 1H), 8.29 – 8.26 (m, 1H), 8.09 (d, *J* = 7.0 Hz, 1H), 8.06 (d, *J* = 7.2 Hz, 1H), 5.06 (q, *J* = 7.2 Hz, 2H), 3.01 (s, 3H), 1.59 (t, *J* = 7.2 Hz, 3H).

3 Optical studies and analysis

A stock solution (1 mM) of **TD** was initially prepared in dimethyl sulfoxide (DMSO). All spectrometric probes were used at a concentration of 10 μM. The adjunction of 20 μL of stock solution was added to 2.0 mL of different solvent systems to obtain **TD** diluent. The solutions of various interfering substances (cations, anions, amino acids and active small molecules) were prepared with twice-distilled water. The provided solutions were thoroughly mixed before the spectral test. Unless otherwise specified, the required fluorescence spectral measurement is generally an excitation wavelength of 650 nm, an excitation slit width of 5.0 nm, and an emission slit width of 5.0 nm.

4 Cell experiments

4.1 Culture and preparation of HeLa cells

HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Before the experiments, the Hela cells in 35-mm glass-bottomed dishes were cultured to a density of 2×105 cells per dish. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

4.2 Cytotoxicity assay

HeLa cells were seeded into 96-well plates, and 0, 1, 2, 5, 10, 20, 30, 40 and 50 μM (final concentration) of **TD** were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 h. Next, MTT (10 μL, 5 mg/mL) was injected into every well and incubated for 4 h. Then, violet formazan was dissolved with DMSO (100 μL). The absorbance of the solution was measured at 492 nm by way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **TD**.

Cell viability (%) = (OD_{sample} -OD_{blank}) / ($OD_{control}$ - OD_{blank}) × 100%.

4.3 Confocal imaging of intracellular viscosity

For cellular viscosity change, the HeLa cells were respectively incubated with LPS (10 μg/mL), Mon (10 μM), Nys (10 μM), for 30 minutes and then incubated with **TD** for 20 minutes. Afterward, the medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging. λ_{ex} = 650 nm, λ_{em} = 720-760 nm.

4.4 Confocal imaging of pH *in vitro*

HeLa cells were incubated with 10 μM **TD** for 20 minutes, after which DMEM medium was replaced with buffered solutions at pH= 5 to 9, and then fluorescence intensity was detected. λ_{ex} = 650 nm, λ_{em} = 720-760 nm.

4.5 Confocal imaging of intracellular pH

For monitoring cellular pH change, the HeLa cells were respectively incubated with CQ (100 μM) for 30 minutes, H_2O_2 (2.0 mM) for 6 h, NEM (20 μM) for 30 minutes and then incubated with **TD** for 20 minutes. Afterward, the medium was removed and the cells were rinsed three times with PBS (10 mM, pH= 7.4) for confocal imaging. λ_{ex} = 650 nm, λ_{em} = 720-760 nm.

5 Application of TD in a mouse model of nephritis

Four-week-old Kunming mice were randomly divided into two groups: normal control group and nephritis group. Mice in both groups were fed normally for three days. The nephritis groups received Intraperitoneal injection of cis-diamine dichloroplatinum (CDDP, 15 mg/kg) to establish the nephritis model, while the normal control groups received an intraperitoneal injection of PBS. Three days post-injection, three days later, kidneys were collected from both normal and nephritic mice for pathological analysis. Separately, the probe **TD** (10 μM) was administered intraperitoneally to another set of mice, and their kidneys were harvested 30 minutes later for fluorescence imaging.

6. Supplementary Figures

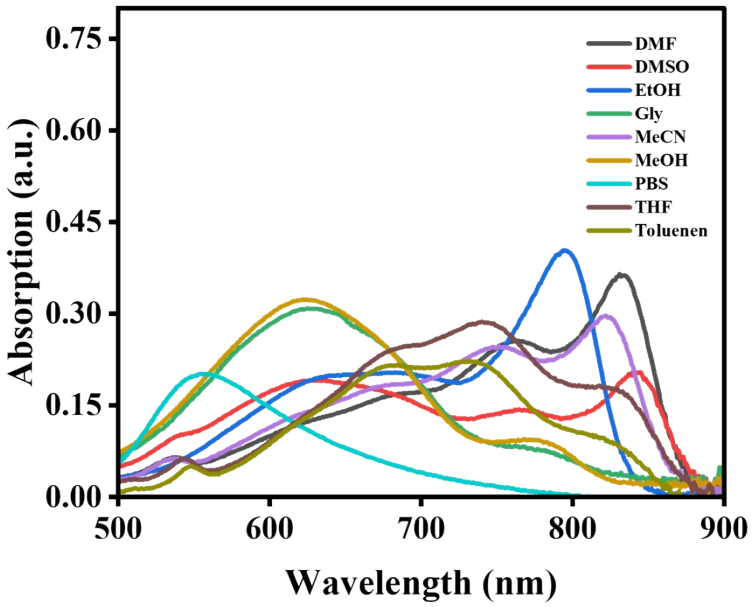


Fig. S1 UV absorption spectra of **TD** (10 μM) in different solvents.

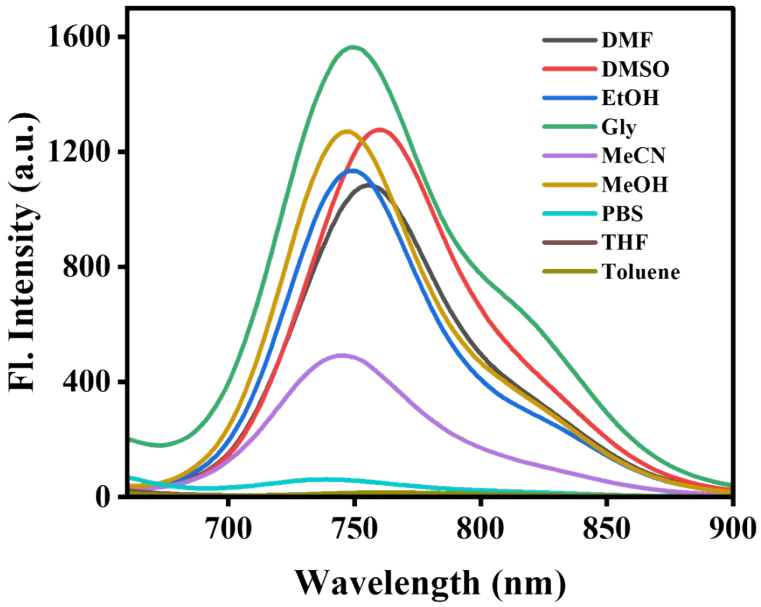


Fig. S2 The fluorescence spectra of **TD** (10 μM) in different solvents. λ_{ex} = 650 nm, slit = 5/5 nm.

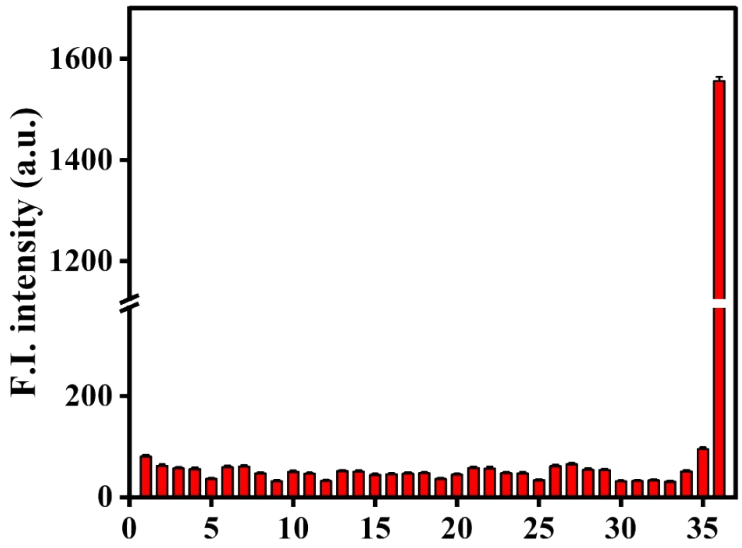


Fig. S3 The fluorescence spectra of **TD** (10 μM) in PBS after introducing 100 μM of various analytes. (1. **TD**, 2. $CaCl_2$, 3. $CdCl_2$, 4. $CoCl_2$, 5. $CuSO_4$•$5H_2O$, 6. $FeCl_3$, 7. $FeSO_4$, 8. Glucose, 9. H_2O_2, 10. Hcy, 11. Hg+, 12. KF, 13. L-Leucine, 14. Tryptophan, 15. L-Serine, 16. L-Aspartic acid, 17. L-Isoleucine, 18. $MgCl_2$, 19. $MnCl_2$, 20. Na_2CO_3, 21. $Na_2S_2O_3$•$5H_2O$, 22. Na_2SO_4, 23. Na_3PO_4, 24. NaBr, 25. NaCl, 26. NaClO, 27. NaH_2PO_4, 28. $NaHCO_3$, 29. $NaHSO_3$, 30. $NaNO_2$, 31. NaOAc,32. NaSCN, 33. $NiCl_2$,34. $ZnCl_2$,35. NaHS, 36. Gly.) λ_{ex} = 650 nm, λ_{em} = 750 nm；slit = 5/5 nm.

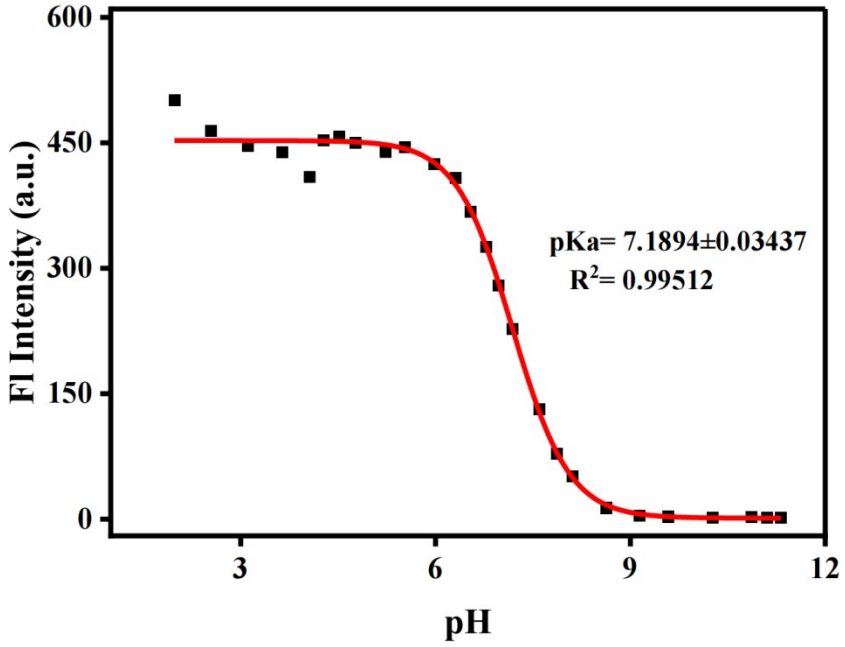


Fig. S4 Non-linear fitting curves of fluorescence spectra of **TD** in different pH buffer solutions. λ_{ex} = 650 nm, λ_{em}= 730 nm. slit= 5/5 nm.

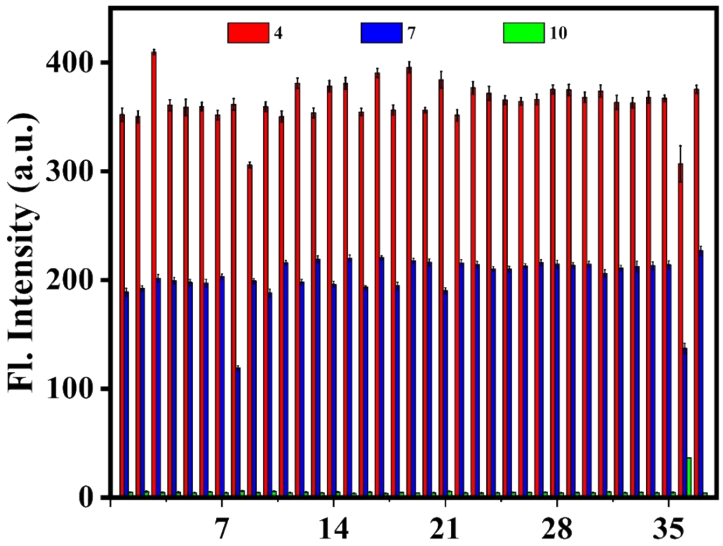


Fig. S5 Fluorescence intensities of **TD** (10 μM) with different interfering substances: 1.$CaCl_2$, 2.$CdCl_2$, 3.NaClO, 4.$CoCl_2$, 5.CuCl, 6.$FeCl_2$, 7.$FeCl_3$, 8.GSH, 9.H_2O_2, 10.KCl, 11.KI, 12.$MgCl_2$, 13.$MnCl_2$, 14.Na_2SO_4, 15.NaBr, 16.NaCl,17.NaClO,18.NaF,19.$NaHSO_3$,20.$NaNO_2$,21.NaSCN,22.NiF_2,23.Cys,24.Phenylalanine,25.CH_3COONa,26.Glycine,27.Arginine,28.L-Lysine,29.L-leucine, 30.Proline, 31. Glucose, 32.Tryptophan Serine, 33.Asparticacid, 34.Valine, 35.Isoleucine, 36.NaHS, 37.**TD** at pH = 4, 7 and 10 respectively. λ_{ex} = 650 nm, λ_{em} = 730 nm； slit = 5/5 nm.

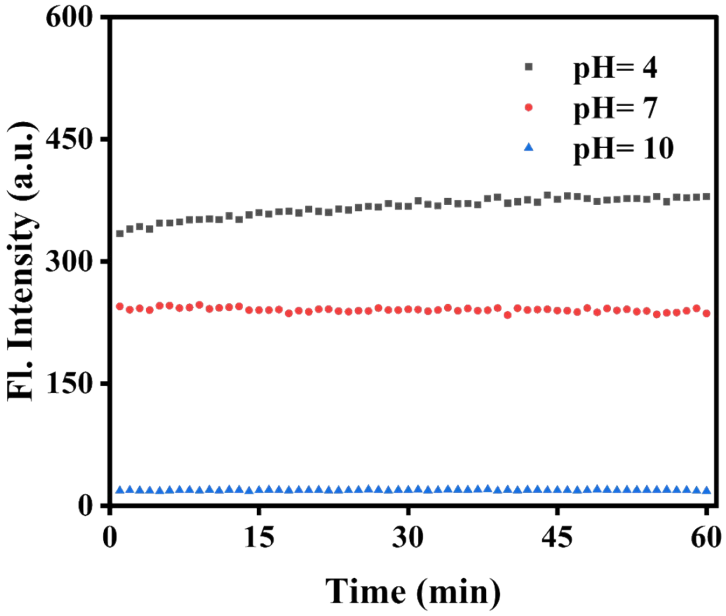


Fig. S6 Fluorescence stability of **TD** (10 μM) in buffer solutions of pH = 4, 7 and 10. λ_{ex} = 650 nm, λ_{em} = 730 nm； slit = 5/5 nm.

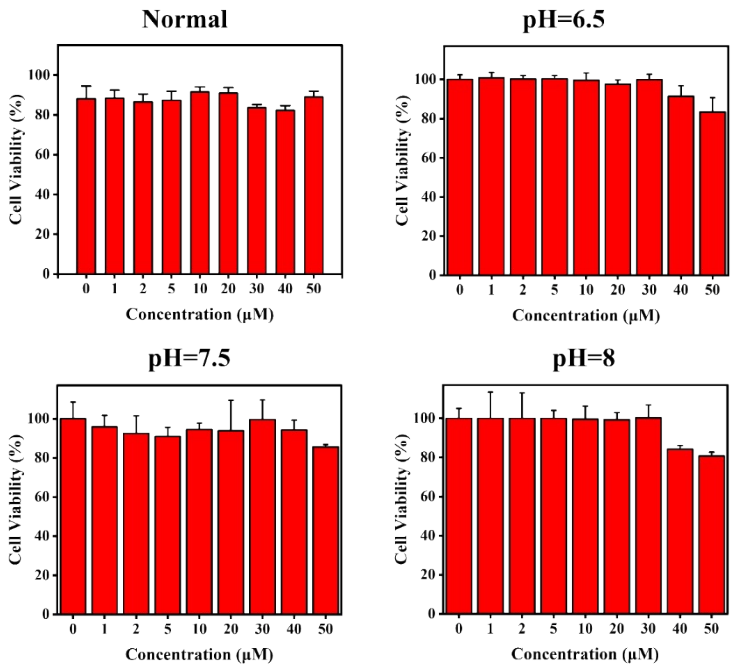


Fig. S7 Cytotoxicity assays of **TD** at different pH conditions for HeLa cells.

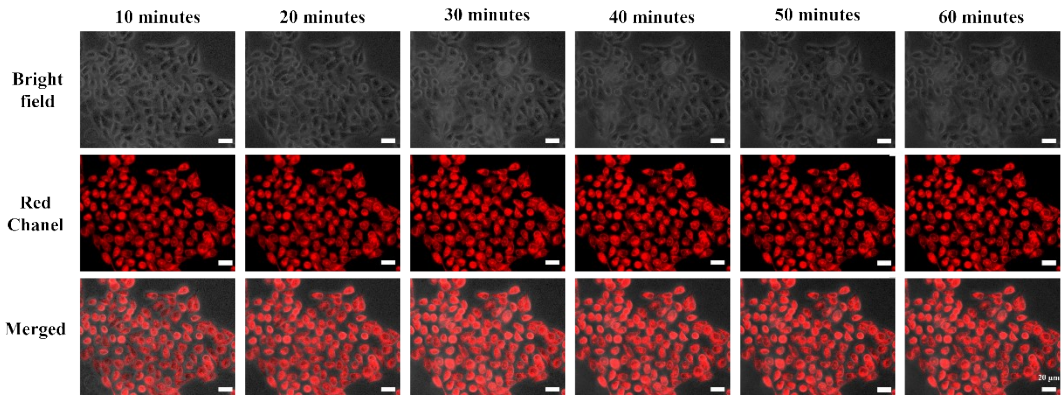
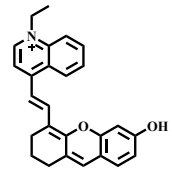
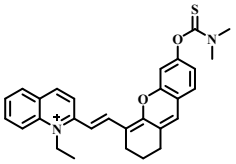
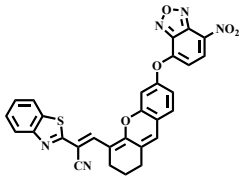
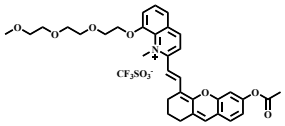
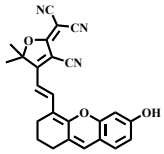
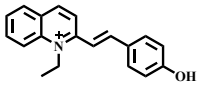
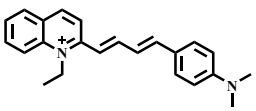
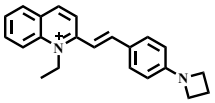


Fig. S8 Photostability of the probe **TD** (10 μM) in living HeLa cells, Scale bar: 20 μm.

Table S1 The similar structure of the probe detection and imaging in some relevant works

Molecular structure	detection	Imaging	Reference
	Viscosity, pH	cells; mice	This work
	HClO	cells; zebrafish	*Dyes and Pigments* 245 (2026) 113200
	H_2S	cells; mice	*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 340 (2025) 126377
	N_2H_4	cells	*Journal of Molecular Structure* 1351 (2026) 144268
	Viscosity	cells, mice	*Journal of Luminescence* 289 (2026) 121600
	Viscosity	cells; elegans	*Microchemical Journal* 219 (2025) 116257
	Viscosity	cells	*Microchemical Journal* 218 (2025) 115572
	Viscosity	cells, zebrafish	*New J. Chem.* 2025, 49, 10166

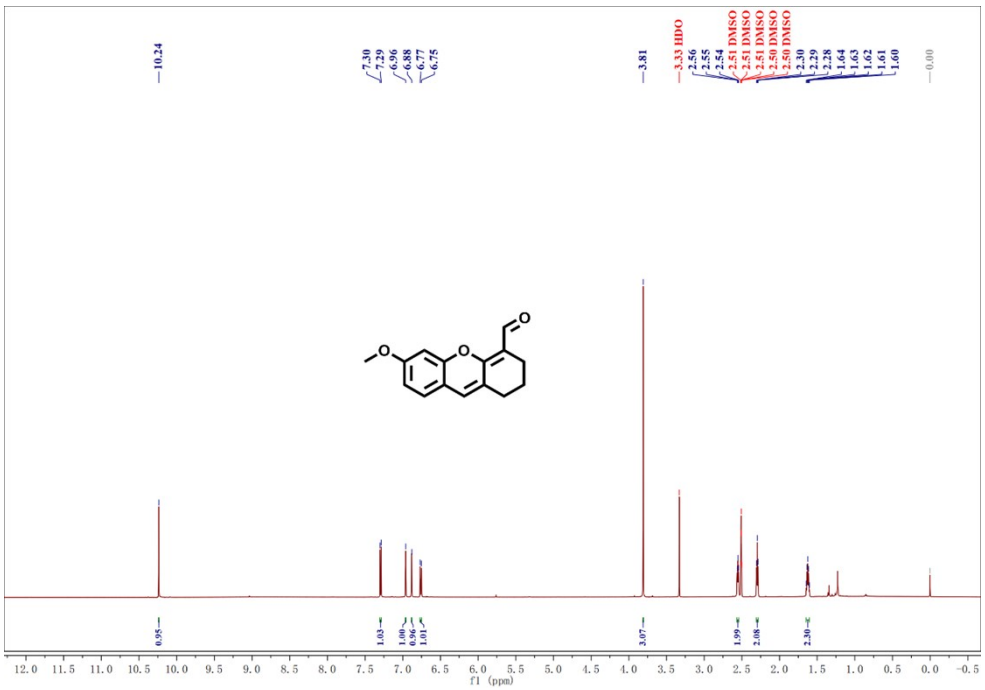


Fig. S9 ^{1}H NMR spectrum of **Compound 2** in DMSO-*d6*.

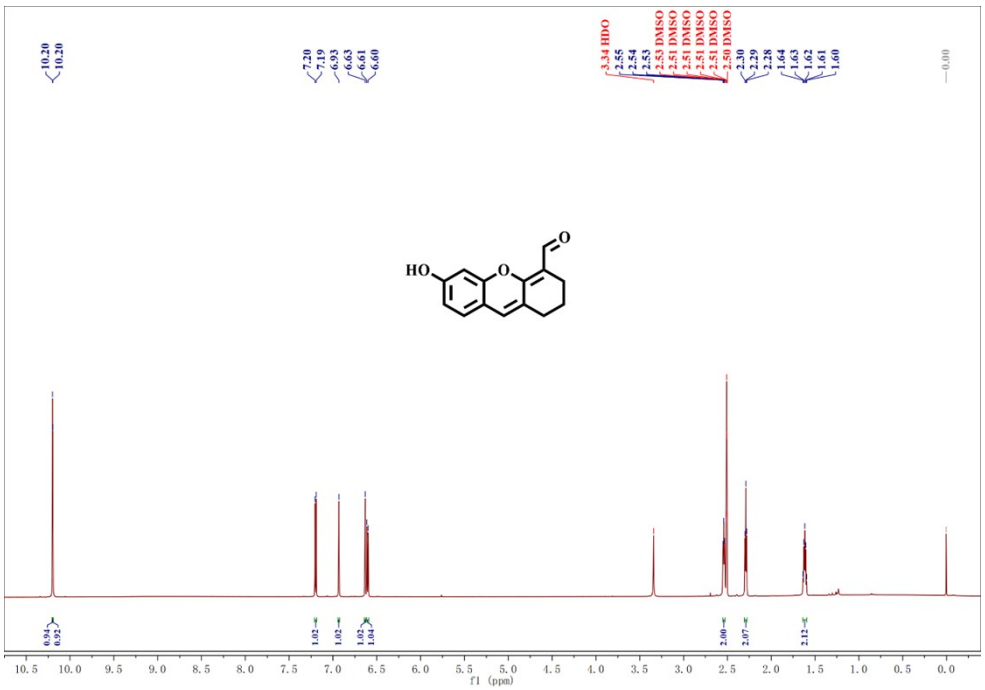


Fig. S10 ^{1}H NMR spectrum of **Compound 3** in DMSO-*d6*.

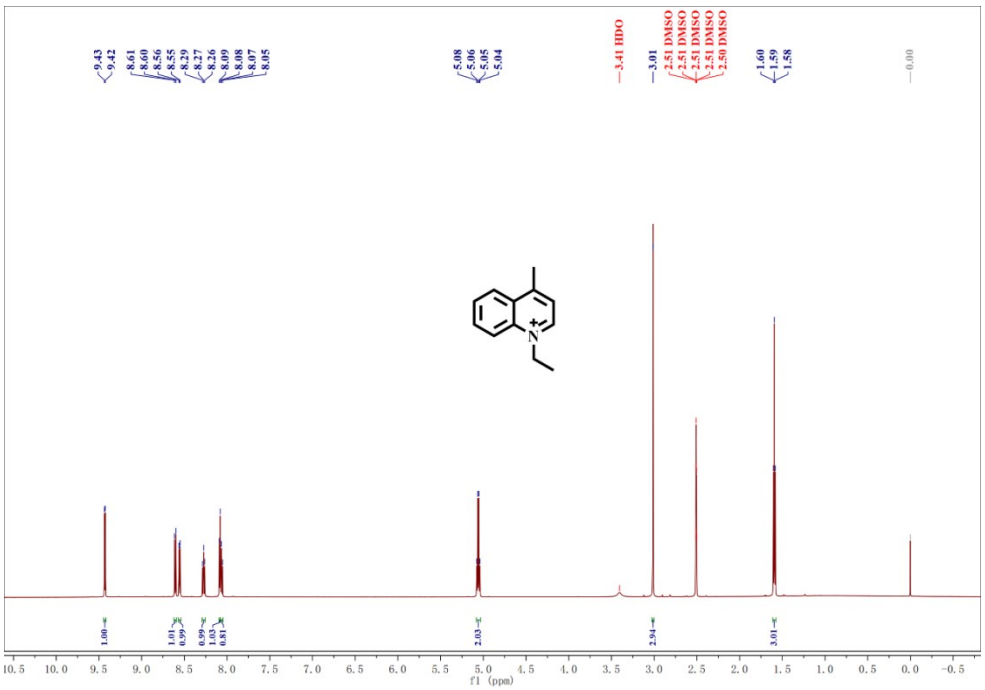


Fig. S11 ^{1}H NMR spectrum of **Compound 4** in DMSO-*d6*.

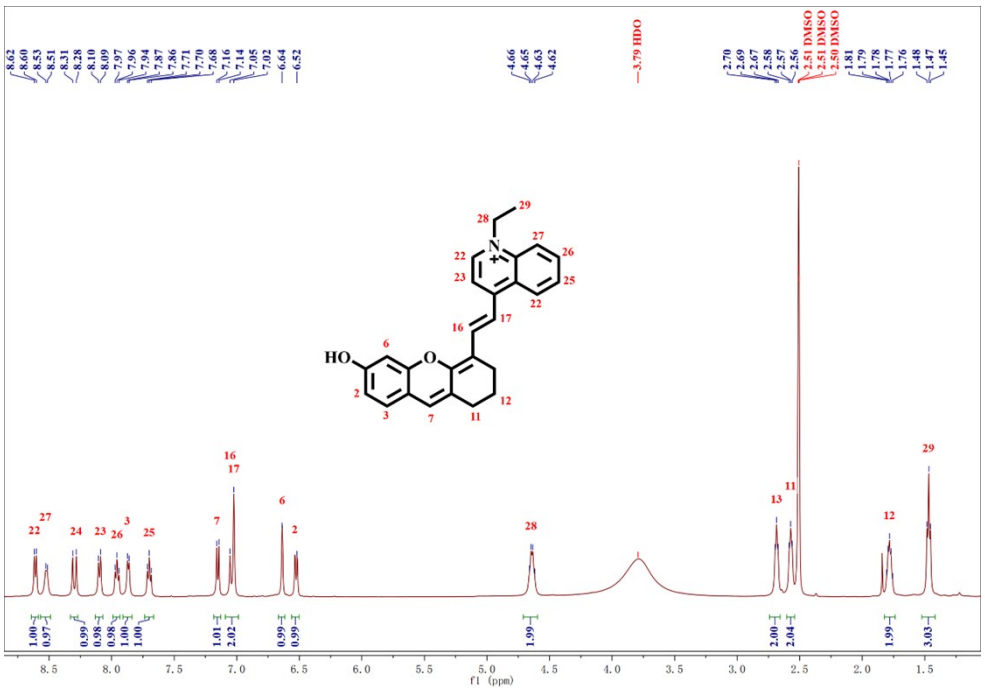


Fig. S12 ^{1}H NMR spectrum of **TD** in DMSO-*d6*.

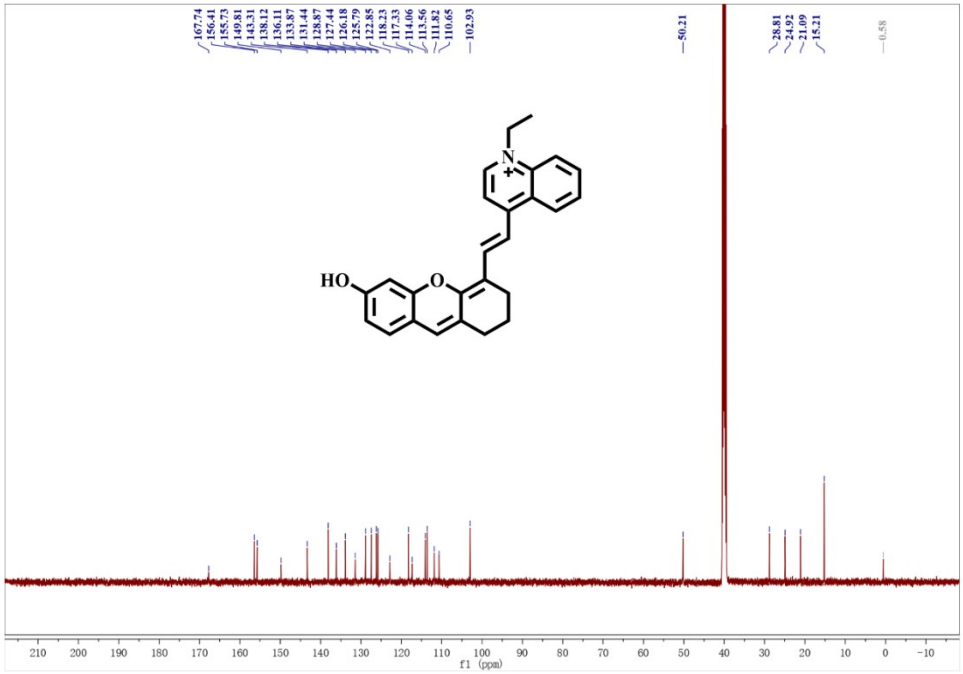


Fig. S13 ^{13}C NMR spectrum of **TD** in DMSO-*d6*.

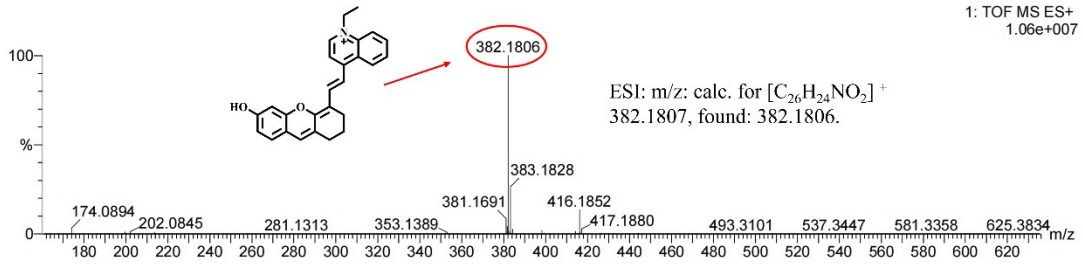


Fig. S14 The ESI-MS spectrum of **TD**.