A red-NIR fluorescence probe for rapid and visual detection of acrolein

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1. General Methods.

Malondicyanide, isophorone, vanillin and trifluoroacetic acid were purchased from Innochem. Hexamethylenetetramine (HMTA) and piperidine were purchased from Chronchem (Chengdu, China). Unless otherwise specified, all the chemicals were analytical reagent grade and can be used directly without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVB-400 spectrometer using TMS as the internal reference. Mass spectra (MS) were obtained by using Bruker Esquire 6000 spectrometer. High resolution mass spectrum (HRMS) was obtained by using Bruker MicrO TOF spectrometer and Bruker TI-00108 spectrometer. Fluorescence spectra were recorded by an F7000 spectrofluorimeter from Hitachi PharmaSpec with the excitation and emission slit widths at 10/10 nm.

SWJT-8 was weighed and dissolved in DMSO to prepare 1.0 mM stock solution. Formaldehyde, acetaldehyde, glyoxal, acetone aldehyde, n-propionaldehyde, pyruvic acid, acetone, allyl alcohol and acrolein were dissolved in distilled water prepare solution for standby. The test samples were prepared by dissolving 20.0 μL stock solution of **SWJT-8** and the appropriate analytical solution in the test tube. Before UV–vis absorption and fluorescence spectra were recorded, the mixture (final volume is 2.0 mL containing 50.0 % v/v DMSO) was incubated for 2 min at room temperature. **SWJT-8** stock solution was diluted to 10.0 μM with DMSO-PBS (1:1, v/v pH = 7.4). For all fluorescence spectra, the excitation was set at 500 nm, and the excitation and emission gaps were 10/10 nm. Fluorescence quantum yields were determined in solution, using Rhodamine B as a standard.

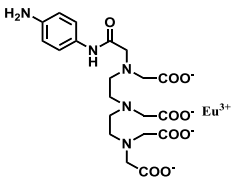
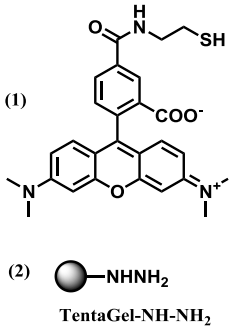
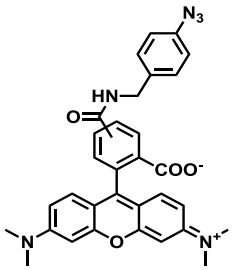
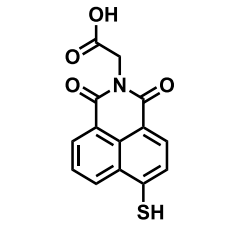
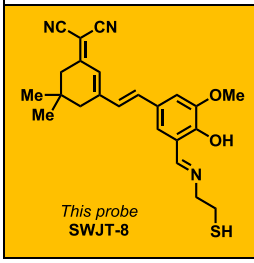
The B3LYP/6-31G(d,p) basis set was first used for optimizing the structure, and TD-DFT//B3LYP/6-31G(d,p) was then employed for studying the photophysical properties of **SWJT-8** and compound **M3**. (Gaussian 09 program)

The HepG2 cells were incubated in a glass-bottom petri dish (*Φ* 15 mm) and adhered at 37 ℃ for 24 hours. The cells were washed with phosphate buffered saline (PBS) and added 10.0 μM of **SWJT-8** at 37 ℃ for 30 minutes, then washed with PBS and imaged. After incubating with 100.0 μM acrolein for 30 min at 37 ℃ and then with **SWJT-8** (10.0 μM) for 30 min, HepG2 cells were washed with PBS and imaged

again. For oxidative stress tests, cells were treated with DMEM containing 200.0 μM H_2O_2 or 500.0 μM H_2O_2 for 2 hours, then added 10.0 μM of **SWJT-8** at 37 ℃ for 30 minutes before imaging. Fluorescence imaging of acrolein in HepG2 cells was recorded on a laser scanning confocal microscope. The excitation wavelength of the laser is 458 nm. The fluorescence signal was collected at the wavelength range of 650-690 nm.

2. Summary of fluorescent probes for acrolein.

Table S1

Probes	$\lambda_{ex}/\lambda_{em}$ nm	Linear range	Detection limit	Reaction temperature	Reaction time and pH	Ref.
	340/615	0-3 μM	0.96 μM	100 ℃	30 min pH = 3.5	Ref. 7a
	554/575	0-3 μM	0.54 μM	40 ℃	*Step 1*: 1 h, pH = 7.4 to 3.5; *Step 2*: 3 h, pH = 7.4	Ref. 7b
	554/575	N.A.	N.A.	rt	30 min	Ref. 8
	380/510	5-100 μM	0.02 μM (in CP buffer)	rt	90 min	Ref. 9
	490/672	0.5-5.0 μM	0.56 μM	rt	80 s, pH = 7.4	***This work***

3. Spectrum properties of M1 and M2.

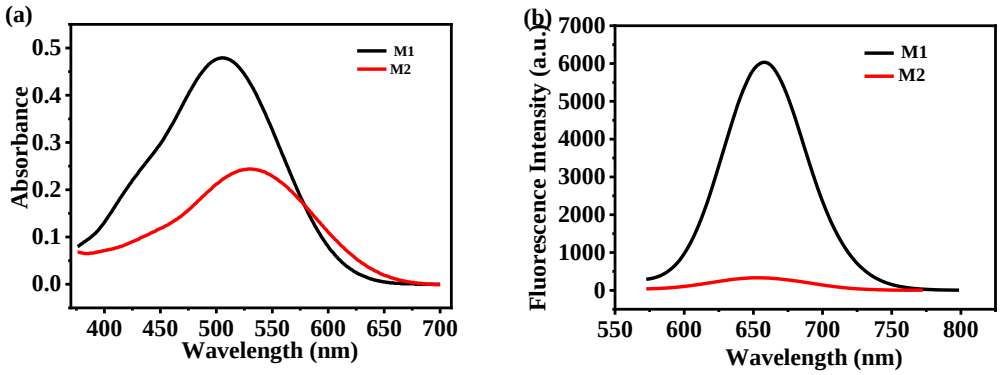


Figure S1. (a) Absorption and (b) fluorescence emission spectra of compound **M1** (10.0 μM) and compound **M2** (10.0 μM) in DMSO/PBS buffer (pH = 7.4, 1:1, V/V) solution. Compound **M1** : λ_{ex} = 504 nm; Compound **M2** : λ_{ex} = 530 nm.

4. Spectroscopic properties.

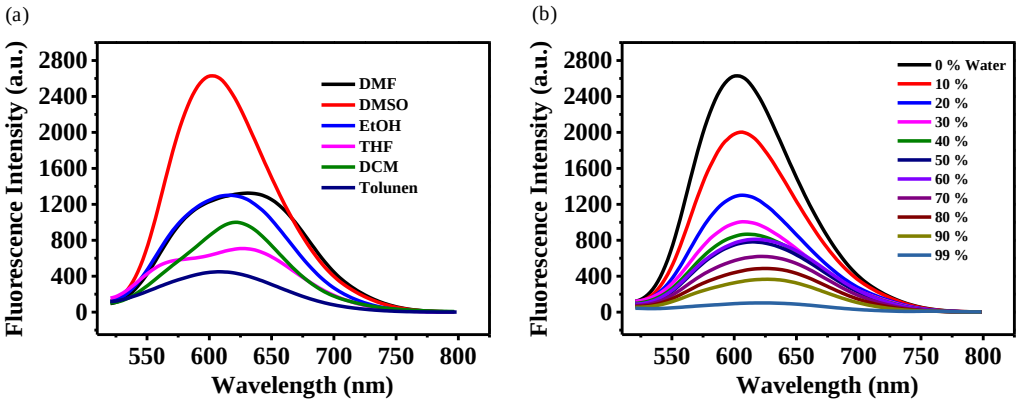
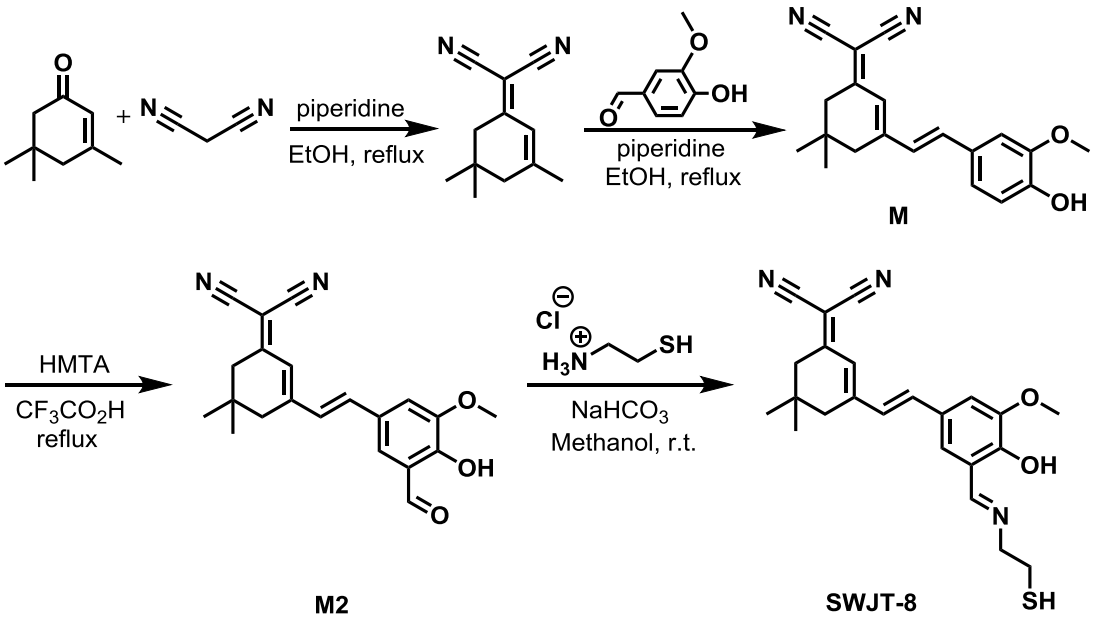


Figure S2. (a) Emission spectra of **SWJT-8** (10.0 μM) in N,N-Dimethylformamide (DMF), Dimethyl sulfoxide (DMSO), Ethanol (EtOH), Tetrahydrofuran (THF), Dichloromethane (DCM) and Toluene (λ_{ex} = 500 nm). (b) Emission spectra of **SWJT-8** (10.0 μM) in solvent with different DMSO-water fractions (fw).

5. Synthesis of probe SWJT-8.



Scheme S1. Synthesis of probe **SWJT-8**.

Probe **SWJT-8** was prepared according to the route shown in **Scheme S1**, and the synthesis of **M** followed the literature.[10a] Compound **M** (9.21 g, 28.75 mmol) and hexamethylenetetramine (6.91 g, 49.29 mmol) were dissolved in CF_3CO_2H (50 mL) and stirred at 90 ℃ for 6 h. After cooling to room temperature, HCl (40 mL) was added to the above mixture solution and stirred at room temperature for 20 min. Then the mixture solution was extracted with CH_2Cl_2 (3 × 150 mL) and dried with Na_2SO_4. Evaporation of the organic solvents gave the crude product, which was purified by flash column chromatography (petroleum ether : ethyl acetate = 4 : 1→1 : 1) on silica gel to afford compound **M2** (2.65 g, yield 26.5%) as a maroon solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.65 (s, 1H), 10.29 (s, 1H), 7.64 (d, J = 2.0 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 7.36 (d, J = 16.0 Hz, 1H), 7.28 (d, J = 16.0 Hz, 1H), 6.86 (s, 1H), 3.93 (s, 3H), 2.60 (s, 2H), 2.53 (s, 2H), 1.01 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 190.8, 170.4, 156.3, 152.3, 149.0, 137.4, 128.2, 127.6, 122.6, 122.2, 121.2, 114.6, 114.0, 113.3, 75.7, 56.4, 42.4, 38.3, 31.7, 27.5 (2C) ppm. HRMS (ESI): m/z calcd for $C_{21}H_{21}N_2O_3$ $[M+H]^+$: 349.1574, found: 349.1546, error 8.0 ppm.

Compound **M2** (63.2 mg, 181.4 μmol), cysteamine hydrochloride (20.6 mg, 181.4 μmol) and $NaHCO_3$ (15.2 mg, 181.4 μmol) were dissolved in methanol (6 mL), and the resulting mixture was stirred for 3 h at room temperature. The reaction mixture was then filtered, and the crude product was recrystallized from methanol to give **SWJT-8** (40.2 mg, 54.4% yield) as a black solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.30 (d, J = 1.8 Hz, 1H), 7.27 (d, J = 1.8 Hz, 1H), 7.22 (s, 2H), 6.79 (s, 1H), 5.71 (s, 1H), 3.85 (s, 3H), 3.38 (brs, 1H), 3.09 (brs, 1H), 2.92 (t, J = 6.0 Hz, 2H), 2.59 (s, 2H), 2.53 (s, 2H), 1.01 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.2, 156.8, 147.9, 139.1, 127.9, 126.7, 126.4, 121.3, 120.7, 114.3, 113.5, 109.2, 74.6, 67.1, 56.0, 52.5, 42.4, 38.3, 35.2, 31.7, 27.5 (2C) ppm. ESI-MS: m/z 408.18 $[M + H]^+$.

6. ^{1}H, ^{13}C NMR and HRMS copies of M2.

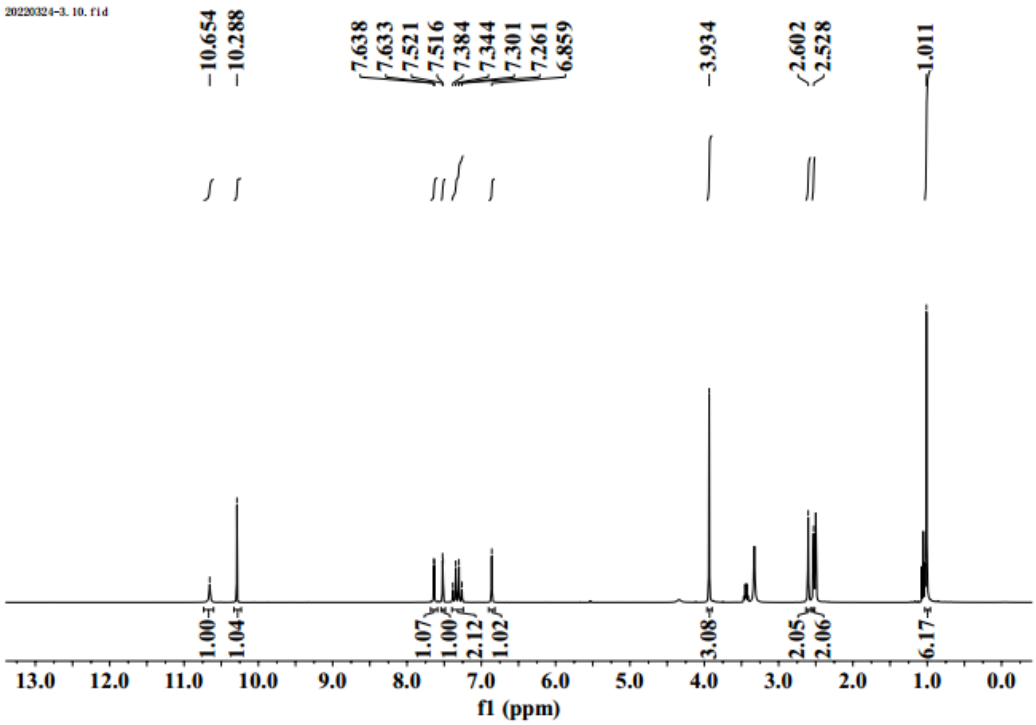


Figure S3. ^{1}H NMR spectrum of compound **M2** (400 MHz, DMSO-d_6).

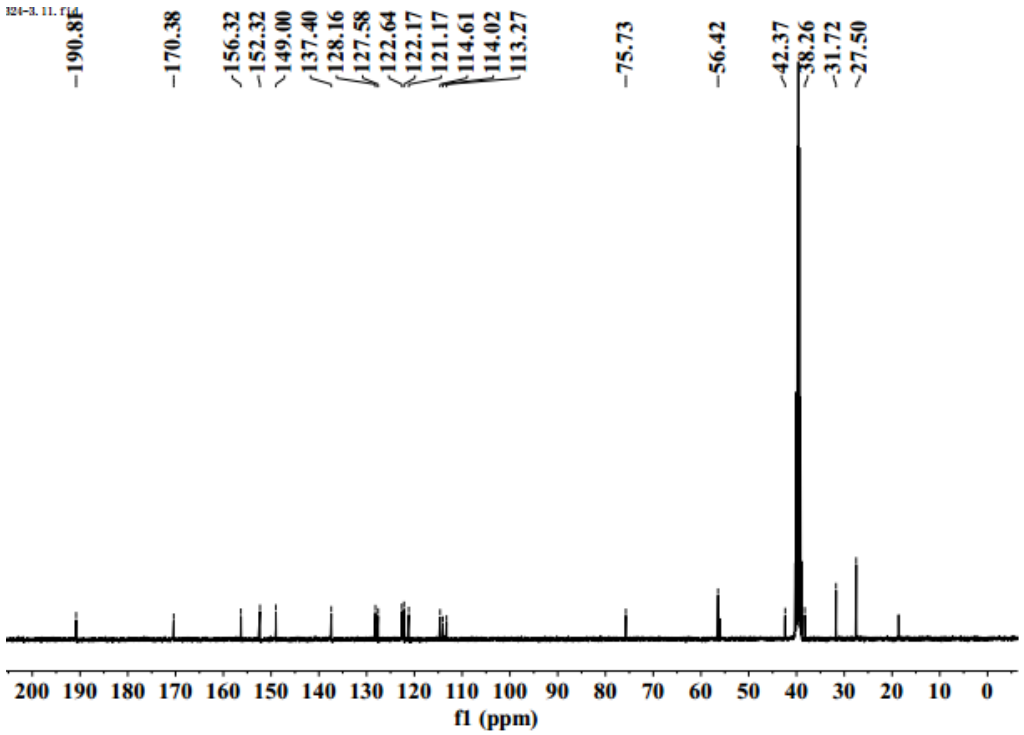


Figure S4. ^{13}C NMR spectrum of compound **M2** (100 MHz, DMSO-d_6).

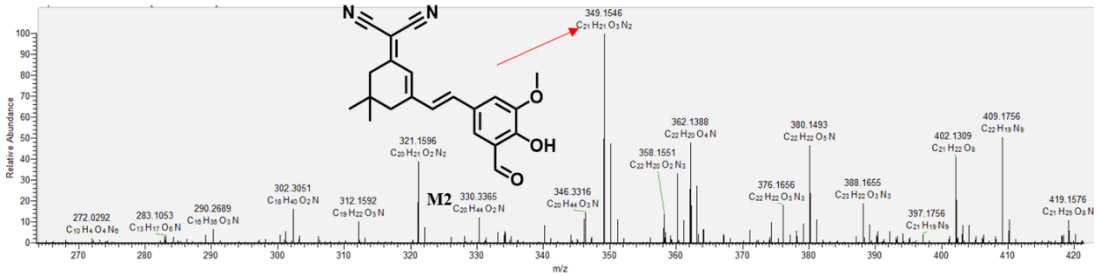


Figure S5. HRMS of compound **M2**.

7. ^{1}H, ^{13}C NMR and ESI-MS copies of SWJT-8.

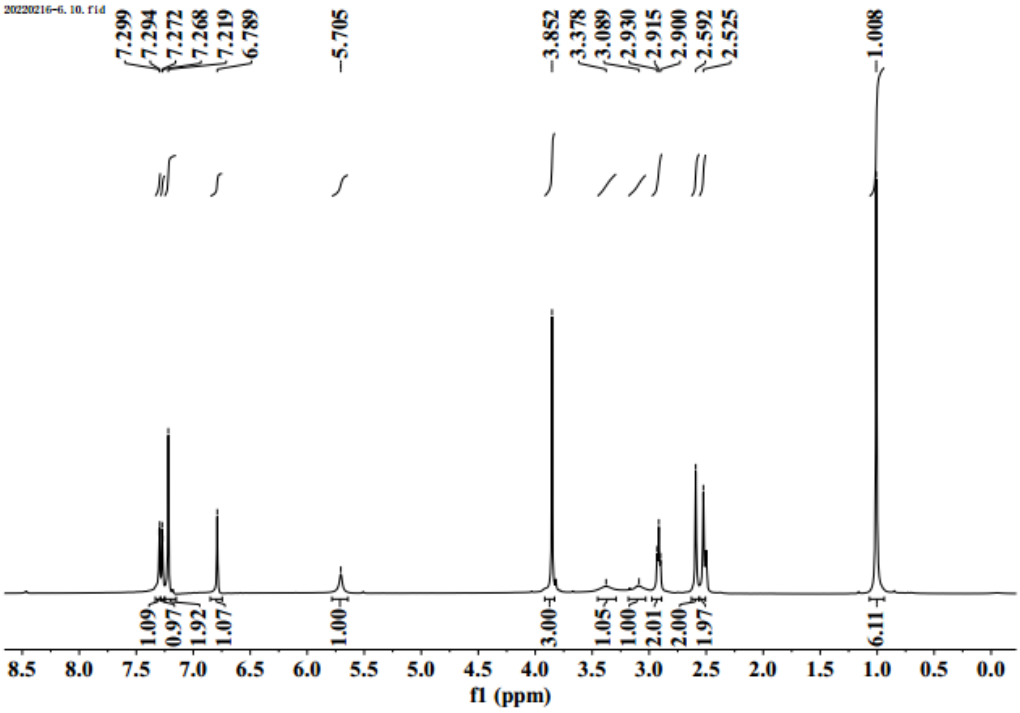


Figure S6. ^{1}H NMR spectrum of probe **SWJT-8** (400 MHz, DMSO-d_6).

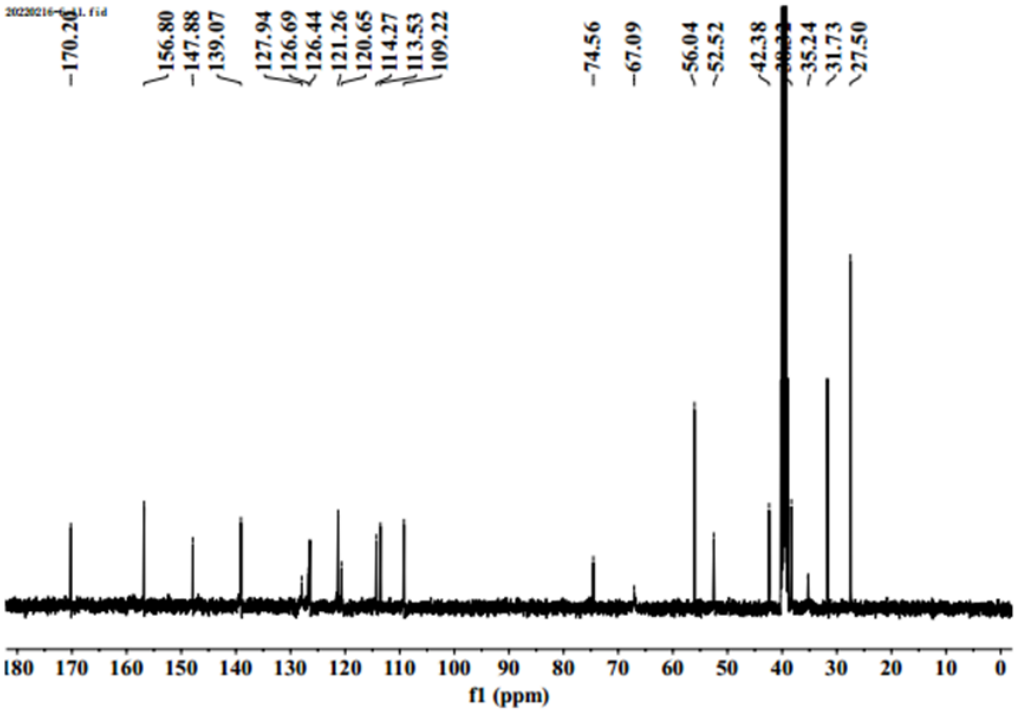


Figure S7. ^{13}C NMR spectrum of probe **SWJT-8** (100 MHz, DMSO-d_6).

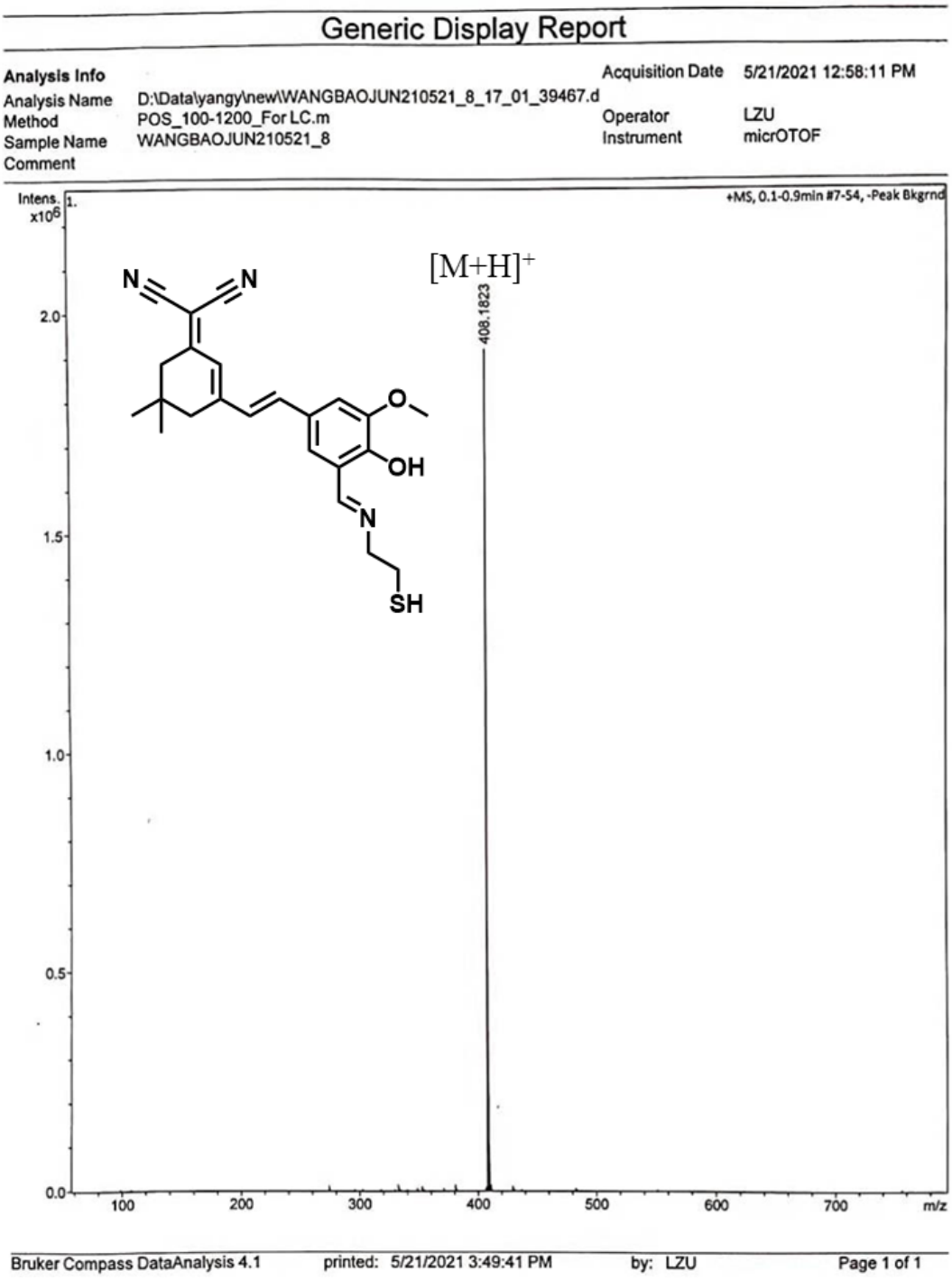


Figure S8. ESI-MS spectrum of probe **SWJT-8**.

8. pH Effects.

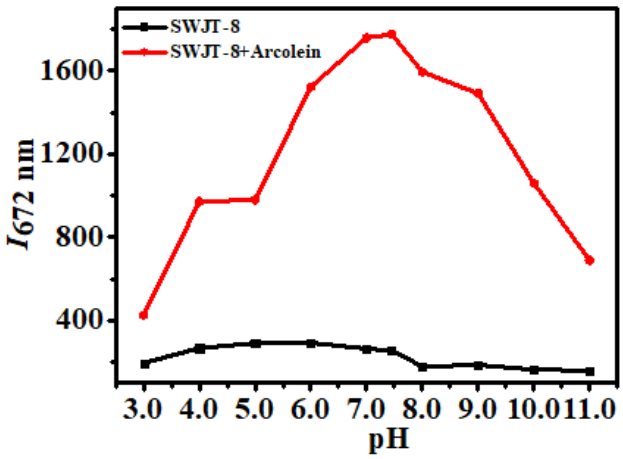


Figure S9. Effects of pH on the reaction of **SWJT-8** (10.0 μM) with acrolein (100.0 μM) (λ_{ex} = 500 nm).

9. ESI-MS copies of compound M3.

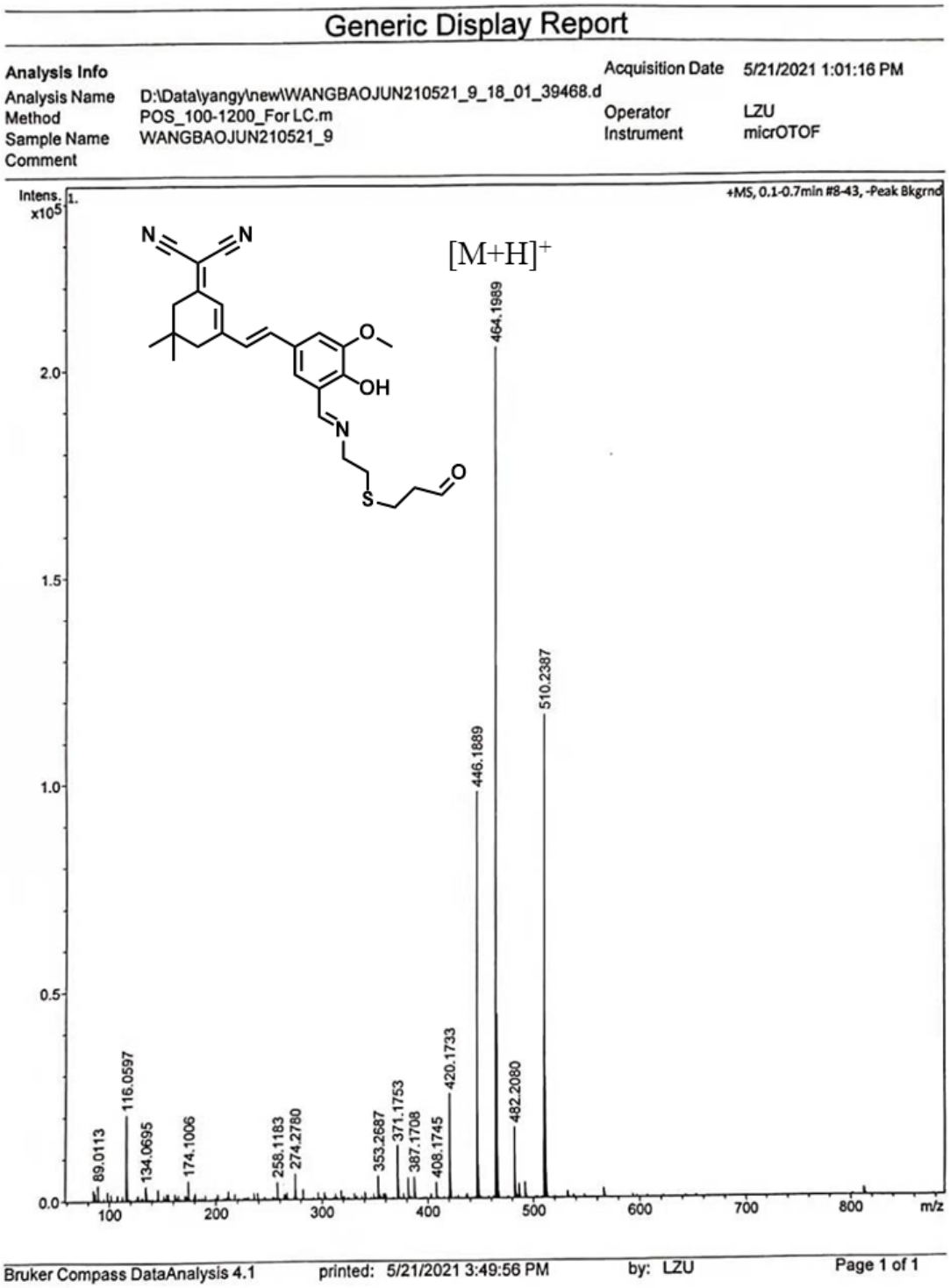


Figure S10. ESI-MS spectrum of compound **M3**.

10. ^{1}H NMR titration.

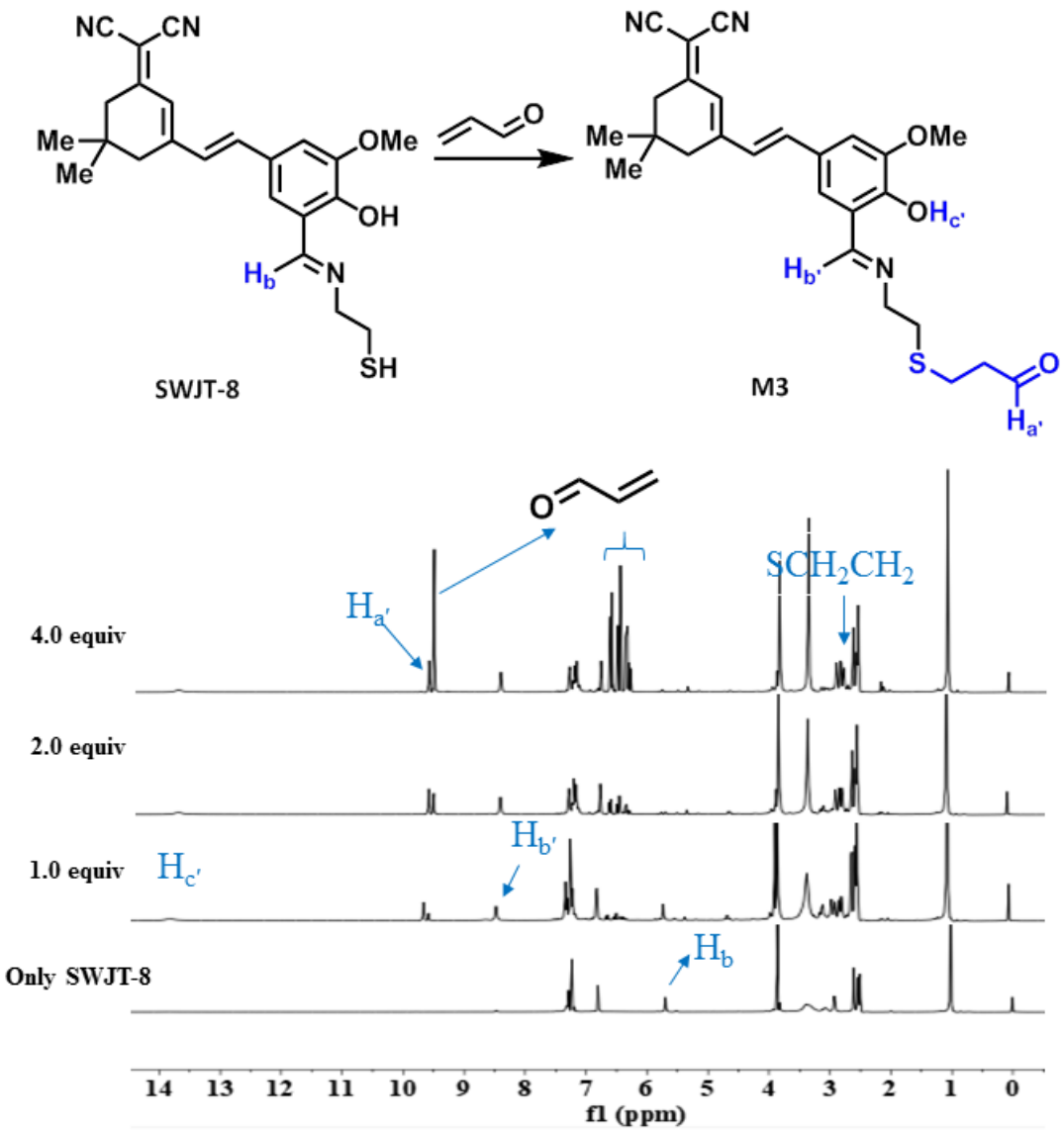


Figure S11. ^{1}H NMR spectra of **SWJT-8**, **SWJT-8** + 1.0 equiv acrolein, **SWJT-8** + 2.0 equiv acrolein, **SWJT-8** + 4.0 equiv acrolein in DMSO-d_6 (400 MHz).

10. Theoretical calculation.

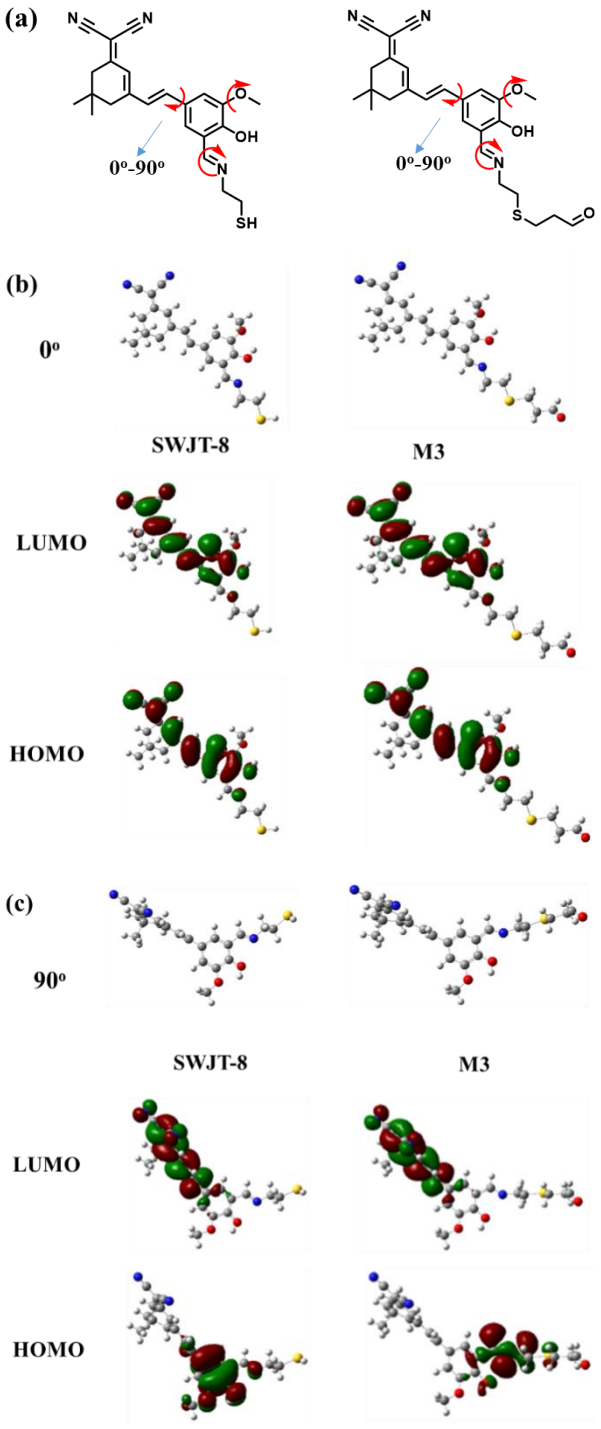


Figure S12. Molecular structures of **SWJT-8** and **M3**. Molecular orbitals of (a) **SWJT-8**, (b) **M3** molecule with different dihedral angles (0 ° and 90 °) along the rotation of the phenyl ring.

12. Absorbance titration.

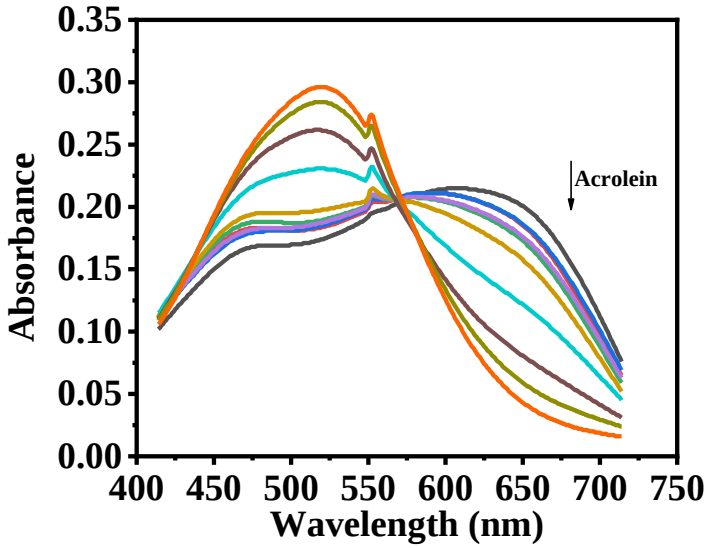


Figure S13. Fluorescence titration of 10.0 μM **SWJT-8** upon the addition of acrolein (0.0-60.0 μM) in DMSO/PBS buffer (pH = 7.4, 1:1, V/V) solution.

13. Linear concentration range of SWJT-8 with acrolein.

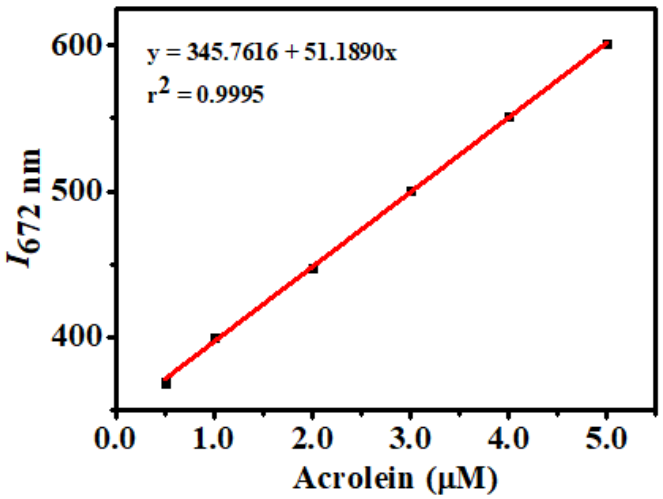


Figure S14. Fluorescence intensity ($I_{672\ nm}$) of **SWJT-8** (10.0 μM) change as a function of acrolein (0.5-5.0 μM) in DMSO/PBS buffer solution (pH = 7.4, 1:1, v/v) (λ_{ex} = 500 nm).

The result of the analysis as follows:
noise ratio (S/N) = 3:1
LOD = 3σ/slope=0.56 μM
LOQ=3LOD=1.68 μM

14. Stability of SWJT-8 in solution.

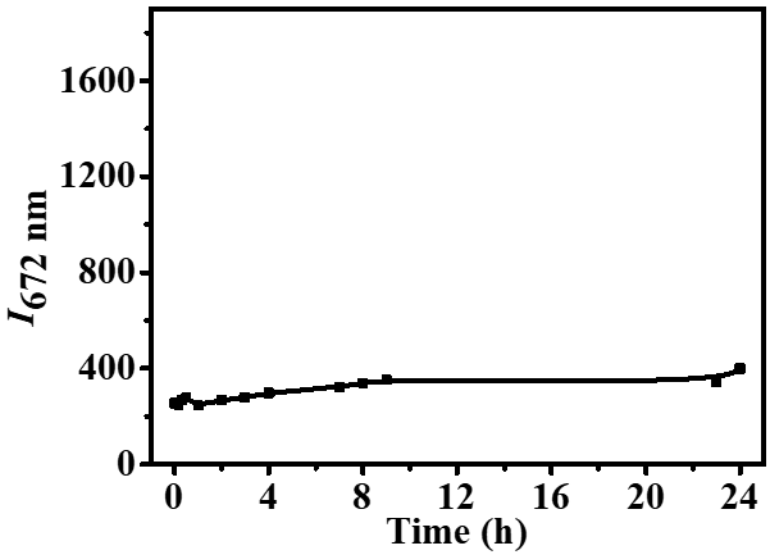


Figure 15. Spot chart of fluorescent intensity changes at 672 nm (**SWJT-8**, 10.0 μM) over time in in DMSO/PBS buffer solution (pH = 7.4, 1:1, v/v) (λ_{ex} = 500 nm).

15. Investigation of UV interference.

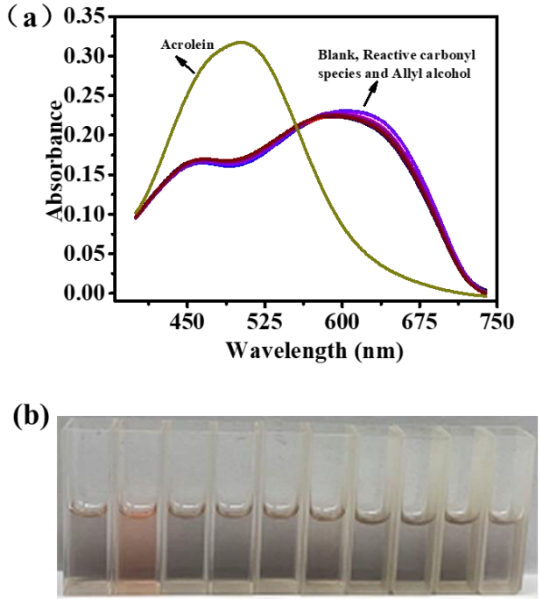


Figure 16. (a) UV spectra of **SWJT-8** (10.0 μM) with various analytes (100.0 μM). (b) The color changes of **SWJT-8** (10.0 μM) with various analytes (100.0 μM). The pictures were recorded after the addition of the analytes (from left to right: blank, acrolein, formaldehyde, acetaldehyde, glyoxal, acetone aldehyde, n-propionaldehyde, pyruvic acid, acetone and allyl alcohol, respectively) in in DMSO/PBS buffer solution (pH = 7.4, 1:1, v/v) (λ_{ex} = 500 nm).