A Ratiometric Fluorescent Probe for Fast Detection and Bioimaging of Formaldehyde

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1. General imformation.

1.1 Chemical reagents

Malononitrile, isophorone, *p*-hydroxybenzaldehyde, trifluoroacetic acid (TFA), *n*-butylamine, formaldehyde (37% wt, in water) and other reagents were purchased from Innochem Technology Co., Ltd. All other routine reagents used in the experiments were analytical reagents.

1.2 Instruments

^{1}H and ^{13}C NMR spectra of **SWJT-10** were taken on a Bruker AVANCE OEO 400 spectrometer with TMS as internal standard and DMSO-d_6 as solvent. ESI-MS spectra of **SWJT-10** and compound 4 were recorded by using Bruker MicrO TOF spectrometer. The fluorescence spectra were obtained by Hitachi F-7000 spectrofluorometer with the slit widths at 10/10 nm. The absorption spectra were obtained by AOE A360 UV-Vis spectrophotometer. Confocal imaging of HeLa cells was performed by Nikon AR1+ confocal microscope. Zebrafish larvae images were obtained by Olympus BX61W1-FV1000 confocal scanning microscope.

1.3 Analysis experiments

SWJT-10 was weighed and dissolved in DMSO to prepare 1.0 mM stock solution. Formaldehyde, Acetaldehyde, Glyoxal, Methylglyoxal, Acrolein, Pyruvate, H_2O_2, ClO^- were taken in volume and diluted with distilled water to prepare 100.0 mM stock solutions. Cys, Hcy, GSH, glucose were weighed and dissolved in distilled water to prepare 100.0 mM stock solutions. Dilute 20.0 μL of **SWJT-10** stock solution to 1980 μL with DMSO/PBS, then add 20.0 μL of assay stock solution (2.0 mL final volume, DMSO/PBS, v/v, 1:1), and incubate the mixture at room temperature 15 minutes. For all fluorescence spectra, excitation was set to 435 nm and the excitation and emission gap was 10/10 nm.

The quantum yield was calculated by the following formula:

$$\Phi_u = \Phi_s (F_u/F_s)(A_s/A_u)$$

"F_u" and "A_u" represent the fluorescence emission peak integral value and absorbance value of **SWJT-10**, respectively. "F_s" and "A_s" were the fluorescence

emission peak integral value and absorbance value of Rhodamine B, respectively. "Φ_u" and "Φ_s" represent the fluorescence quantum yields of **SWJT-10** and Rhodamine B, respectively.

1.4 Biological experiments

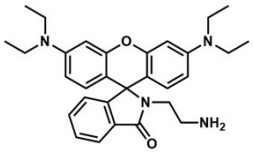
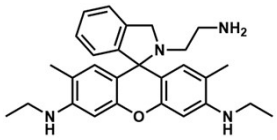
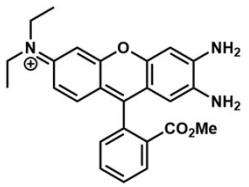
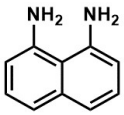
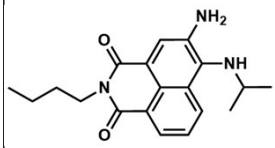
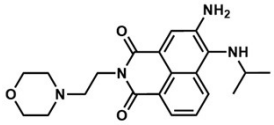
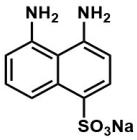
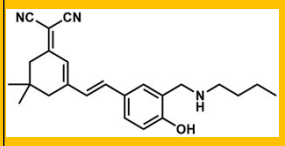
This study was approved by the Southwest Military Region General Hospital and Southwest Jiaotong University Animal Experiment Ethics Committee. The cytotoxicity of **SWJT-10** to HeLa cells was examined by CCK-8 assay method. HeLa cells were seeded at a 96-well culture plate. After growth at 37 ℃ in a 5% CO_2 for 24 h, treated with 0, 5.0, 10.0, 15.0, 20.0 μM **SWJT-10**. After incubation for 12 h, the CCK solution was added into each well for further incubation for 4 h. The absorbance at 540 nm was measured.

HeLa cells were incubated with 10.0 μM **SWJT-10** at 37°C, 5% CO_2 for 30 mins, and then washed three times with PBS to remove extracellular excess probe. Next, adding 1.0 mM FA to the experimental group, then incubate for another 30 mins, and imaging after washing three times. Fluorescence imaging was performed on a Nikon AR1+ confocal microscope. The excitation wavelength is 435 nm, the fluorescence at 575-625 nm is collected in the yellow channel, and the fluorescence at 655-705 nm is collected in the red channel.

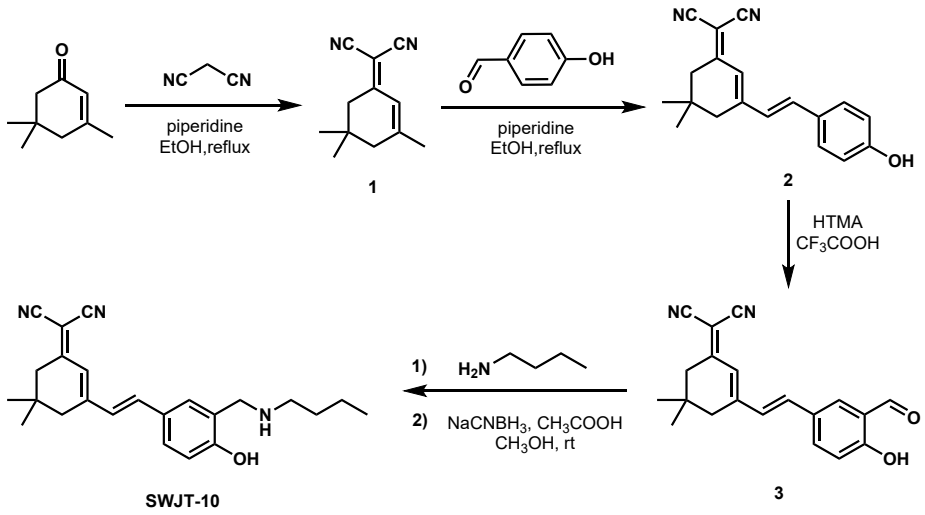
The zebrafish larvae were incubated with 10.0 μM **SWJT-10** in distilled water for 30 mins and then washed with distilled water. Next, 1.0 mM FA was added to the experimental group and incubated for another 30 mins. After that, zebrafish larvae were wished with distilled water three times. Finally, zebrafish larvae were immobilized with 1.5% agarose for confocal imaging using an Olympus BX61W1-FV1000 Confocal Scanning Microscope.

2. Summary of FA fluorescent probes based on cyclization mechanism.

Table S1

Name	Probes structure	$\lambda_{ex}/\lambda_{em}$ (nm)	Linear range	Detection limit	Response time	Referen-ces
dRB-EDA		560/590	-	-	1 h	Ref. 26
R6-FA		530/560	2-10 μM	0.77 μM	10 s	Ref. 27
L		520/620	0-3 mM	8.3 μM	5 min	Ref. 28
DAN		335/424	0-80 μM	0.95 μM	5 min	Ref. 29
Np2		380/444	0-250 μM	1.8 μM	2 h	Ref. 30
NP-lyso		380/444	0-200 μM	0.27 μM.	1 h	Ref. 31
DAS		345/490	0-100 μM	1 μM	40 min	Ref. 32
SWJT-10		435/600, 680	0-1 mM	4.5 μM	5 s	*This work*

3. Synthesis of SWJT-10.



Scheme S1. Synthesis of probe **SWJT-10**.

4. ^{1}H, ^{13}C NMR spectra and ESI-MS of SWJT-10.

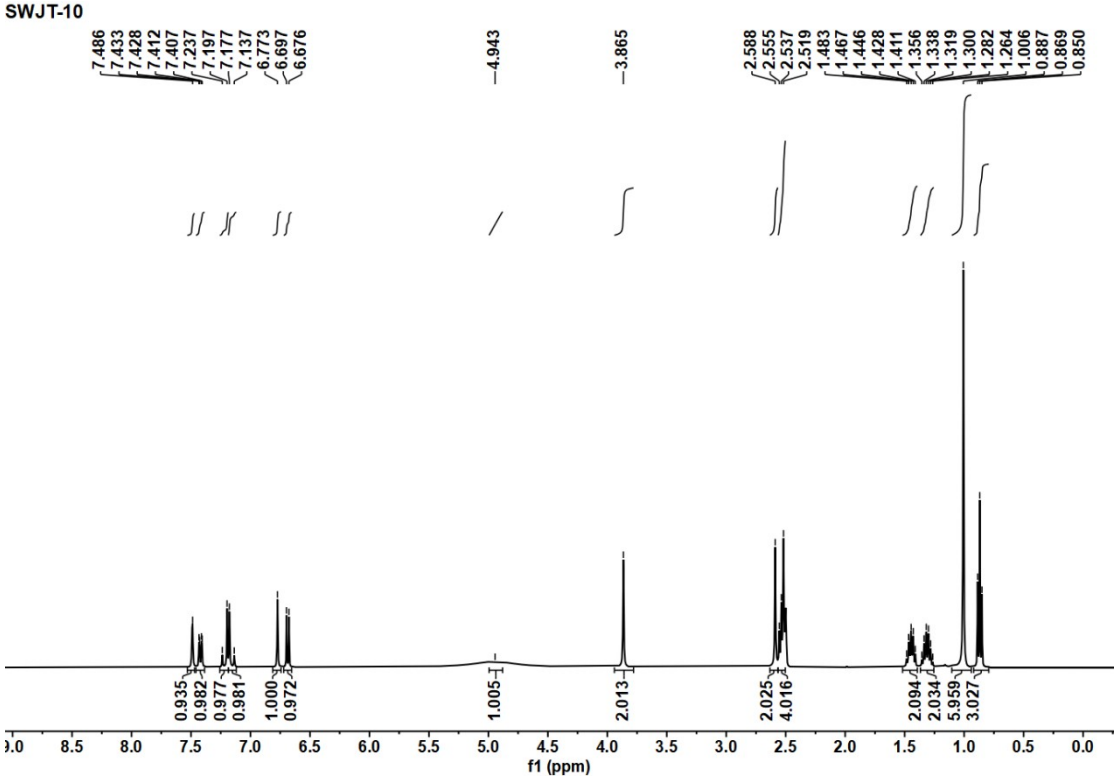


Fig. S1. ^{1}H NMR spectrum of **SWJT-10** in DMSO-d_6(400MHz).

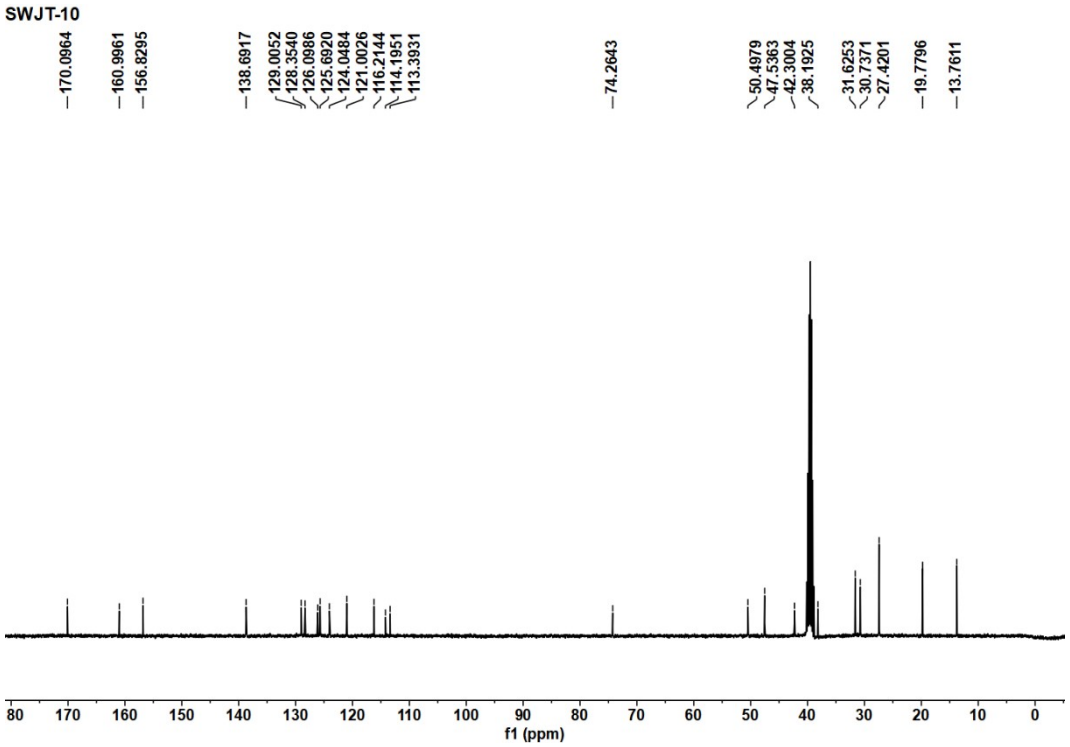


Fig. S2. ^{13}C NMR spectrum of **SWJT-10** in DMSO-d_6(100MHz).

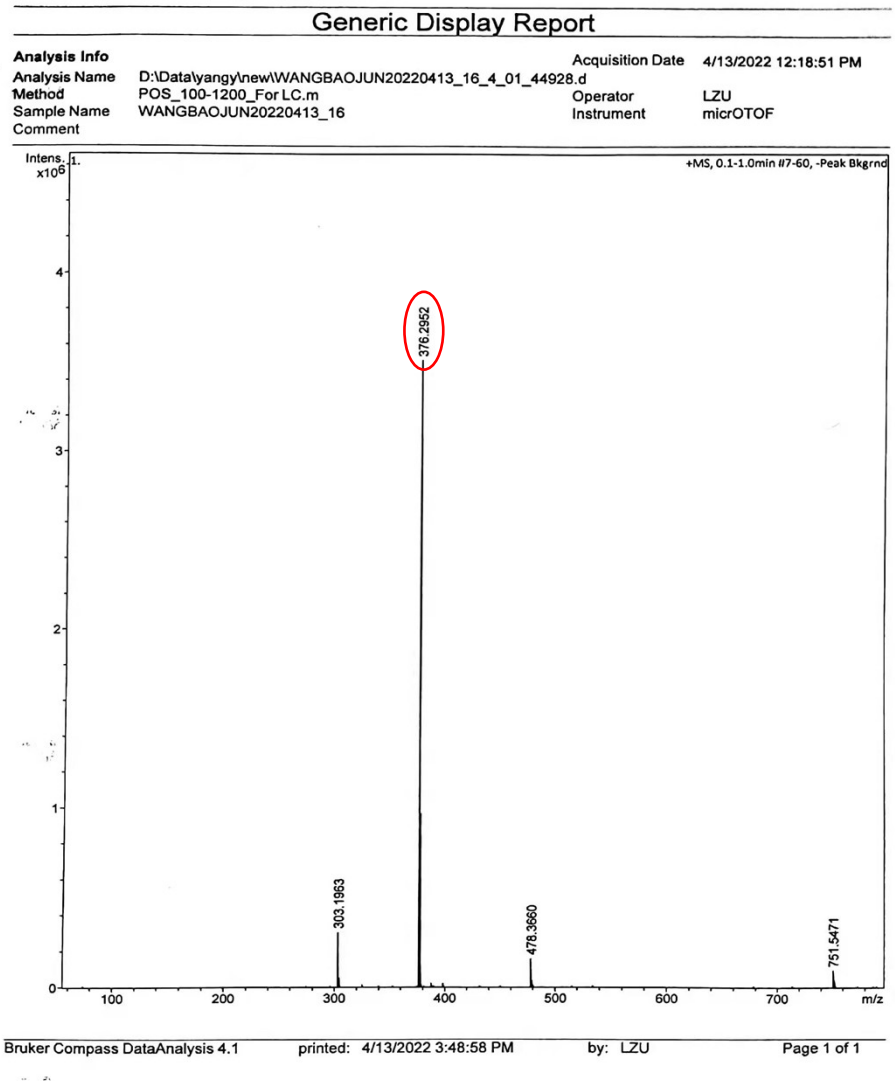


Fig. S3. ESI-MS spectrum of **SWJT-10**.

5. Screening conditions for the detection of FA by SWJT-10.

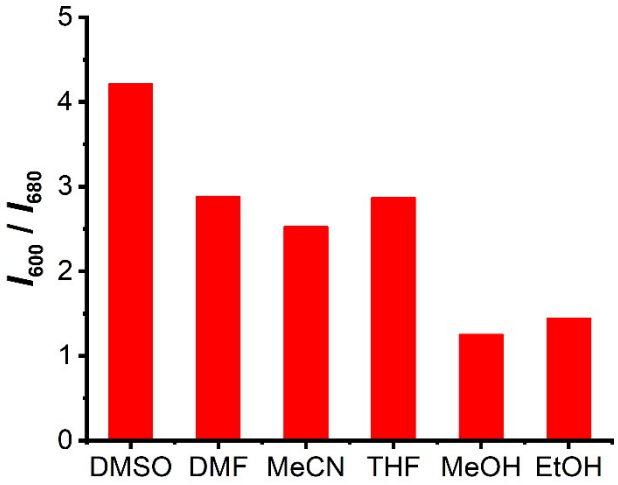


Fig. S4. The increase of the fluorescence ratio (I_{600}/I_{680}) of **SWJT-10** in different organic solvents, after adding 1.0 mM FA (λ_{ex} = 435 nm).

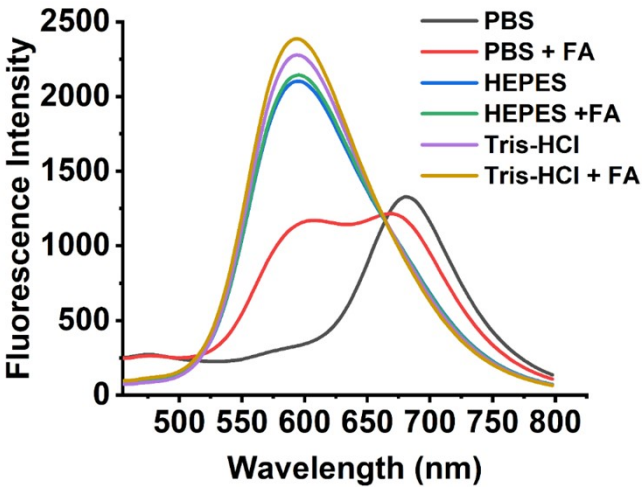


Fig. S5. Fluorescence spectral changes in different buffer solution (PBS, HEPES, Tris-HCl) (λ_{ex} = 435 nm).

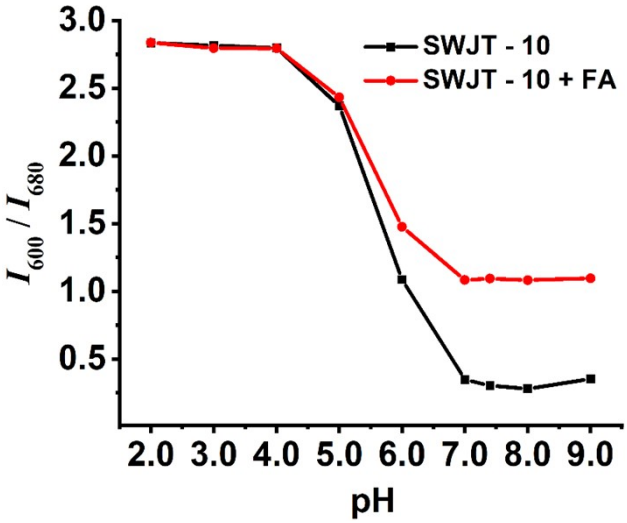


Fig. S6. pH effect of **SWJT-10** (10.0 μM) to FA (1.0 mM) (λ_{ex} = 435 nm).

6. The stability of SWJT-10 and SWJT-10 +FA

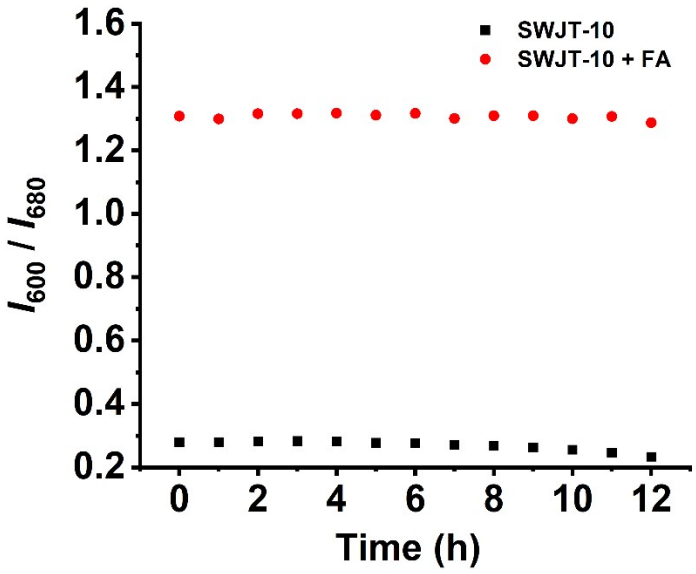


Fig. S7. The stability of SWJT-10 and SWJT-10 +FA (λ_{ex} = 435 nm).

7. The detection limit of SWJT-10 to FA.

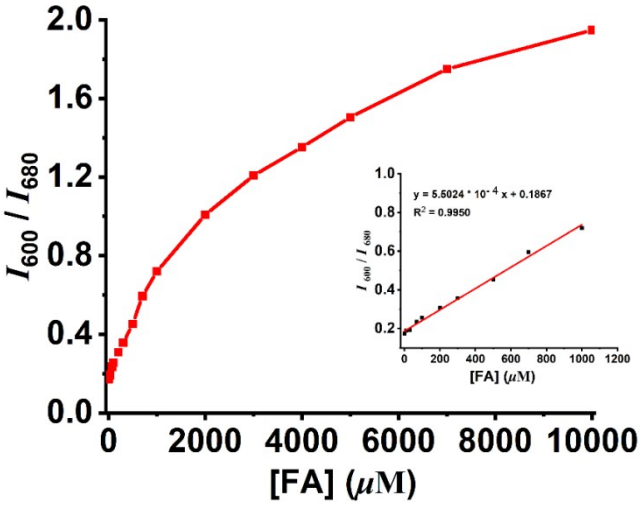


Fig. S8. Variation of I_{600} / I_{680} with increasing concentration (0.0 – 10.0 mM) of FA in PBS/DMSO (1: 1, v/v, pH=7.4) buffer solution (λ_{ex} = 435 nm). Linear relationship between I_{600} / I_{680} and FA concentration (inset).

The detection limit is calculated according to the formula:

$$LOD = K \times \delta / S$$

Linear Equation: $Y = 5.5024 \times 10^{-4} \times X + 0.1867$

$$S = 5.5024 \times 10^{-4}$$

$$\delta = 8.2476 \times 10^{-4}$$

$$K = 3$$

$$LOD = K \times \delta / S = 4.4967\ \mu M$$

8. Selectivity and anti-interference studies.

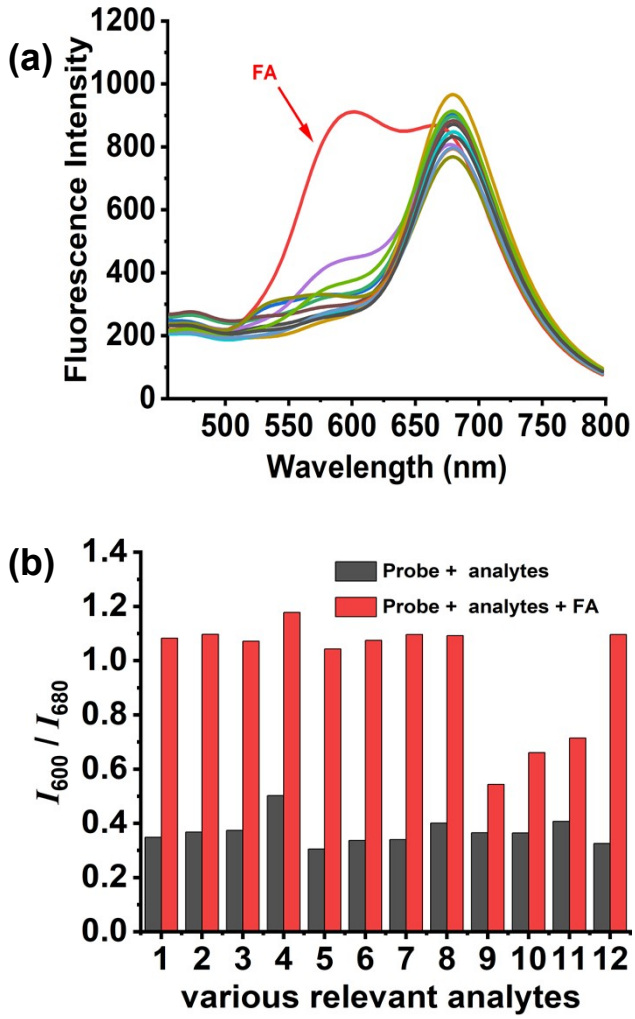


Fig. S9. (a) Fluorescence response of **SWJT-10** to FA and other analytes in PBS/DMSO (1: 1, v/v, pH=7.4) buffer solution (λ_{ex} = 435 nm). (b) Fluorescence response of **SWJT-10** (10.0 μM) to FA (1.0 mM) in the presence of various analytes (1.0 mM). (1) blank; (2) acetaldehyde; (3) glyoxal; (4) methylglyoxal; (5) acrolein; (6) pyruvate; (7) H_2O_2; (8) HClO; (9) Cys; (10) Hcy; (11) GSH; (12) Glucose

9. ^{1}H NMR titration spectrum of SWJT-10 with FA.

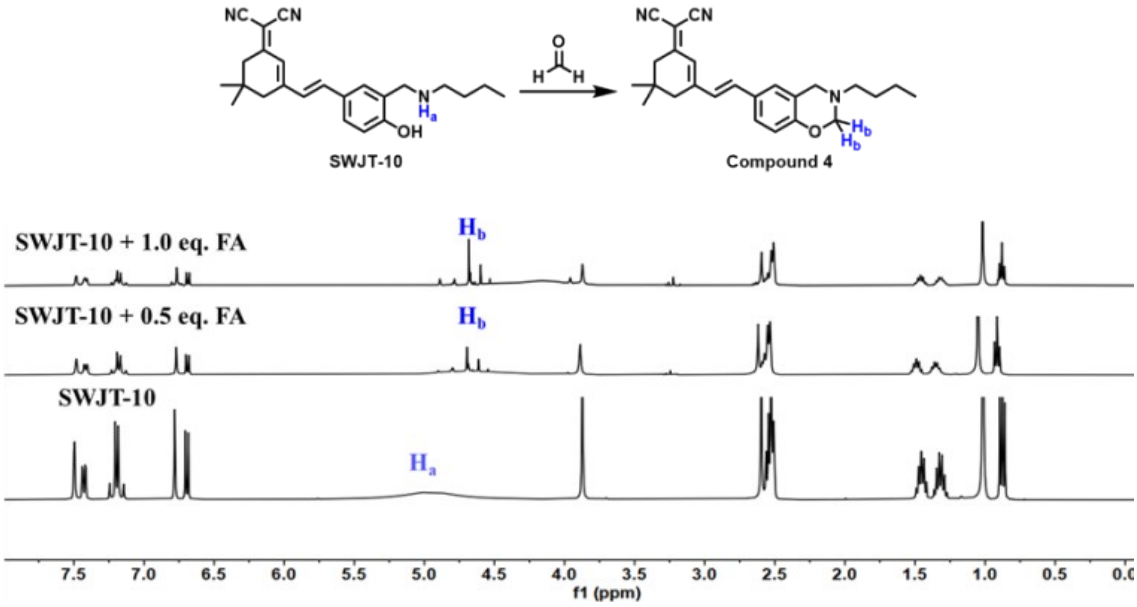


Fig. S10. ^{1}H NMR spectra of **SWJT-10** and **SWJT-10** + FA in DMSO-d_6 (400 MHz).

10. ESI-MS spectra of Compound 4.

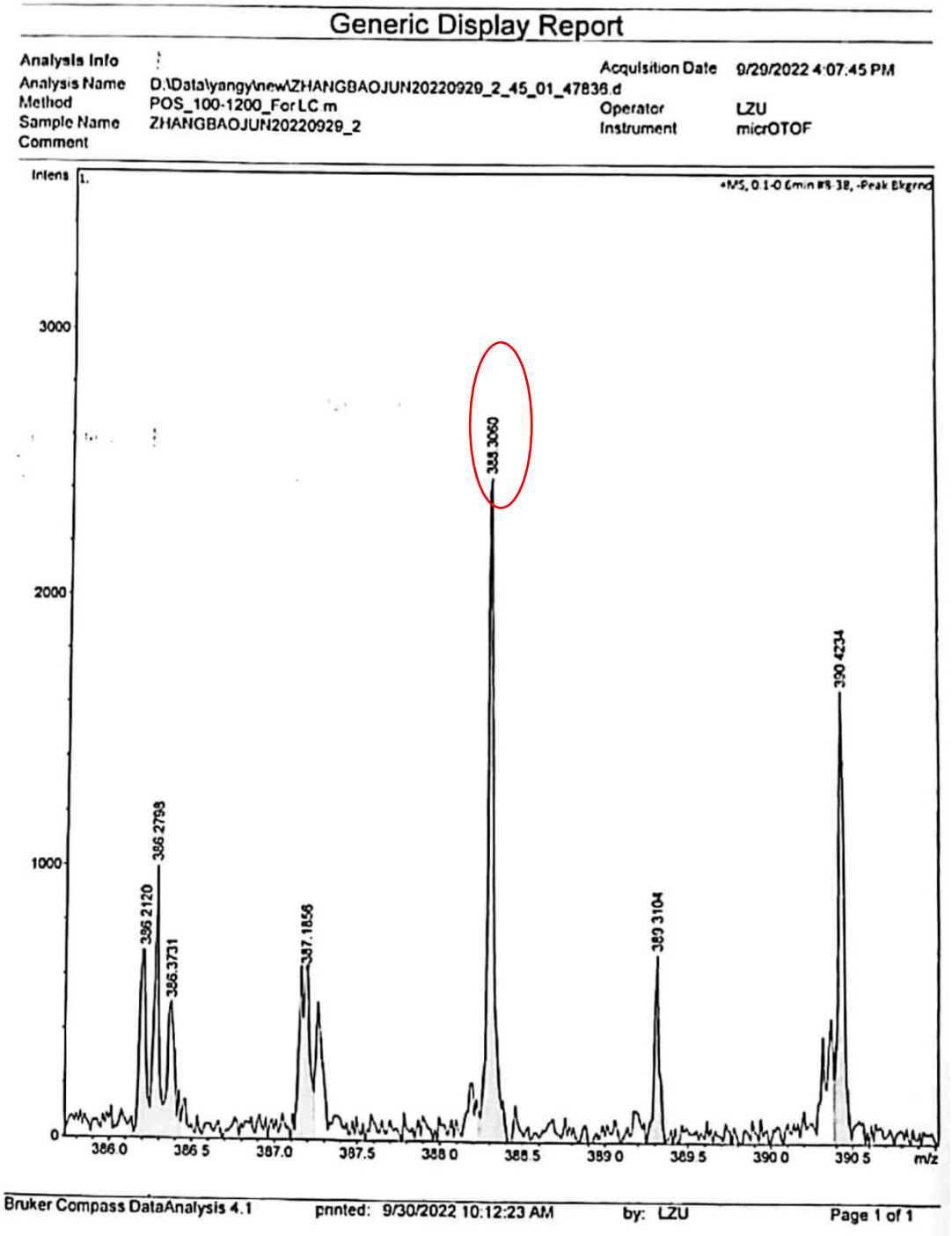


Fig. S11. ESI-MS spectrum of **SWJT-10 + FA.**

11. Cell viability of SWJT-10.

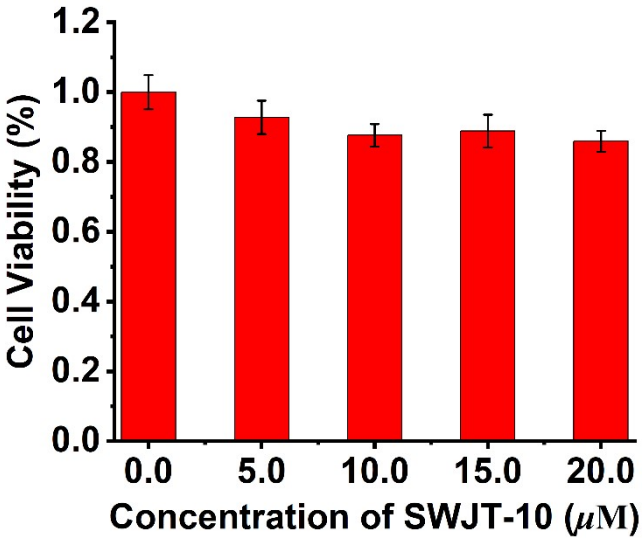


Fig. S12. Cytotoxicity of **SWJT-10** at different concentrations (0.0, 5.0, 10.0, 15.0, 20.0 μM).