Rapid sensing and imaging of methylglyoxal in living cell enabled by a near-infrared fluorescent probe

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1. General Methods

Hexamethylenetetramine (HMTA), isophorone, malondicyanide, sodium cyanoborohydride ($NaCNBH_3$), o-phenylenediamine, p-hydroxybenzaldehyde, trifluoroacetic acid and other analytical reagents were all purchased from Innochem.

^1H 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 MicrO TOF spectrometer and Bruker TI-00108 spectrometer. Fluorescence spectra were recorded by an F4600 spectrofluorimeter from Hitachi PharmaSpec with the excitation and emission slit widths at 20/20 nm. Fluorescence imaging of MGO in HeLa cells was recorded on a Carl Zeiss LSM700 laser scanning confocal microscope.

SWJT-2 was weighed and dissolved in DMF to prepare 1.0 mM stock solution. Formaldehyde, acetaldehyde, glyoxal, pyruvic acid, *n*-propionaldehyde, Acrolein, H_2O_2, OCl^-, $ONOO^-$, sodium nitroprusside and FBS were dissolved in distilled water or DMF prepare solution for standby. The test samples were prepared by dissolving 20 μL stock solution of **SWJT-2** 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 DMF) was incubated for 15 min at room temperature. **SWJT-2** stock solution was diluted to 10.0 μM with DMF-PBS (1:1, v/v pH = 7.4). For all fluorescence spectra, the excitation was set at 440 nm, and the excitation and emission gaps were 20/20 nm.

The quantum yield was calculated through the following formula:

$$\Phi u = \Phi s\,(Fu/Fs)(As/Au)(\eta_u^{\,2}/\eta_s^{\,2})$$

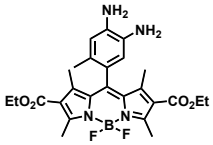
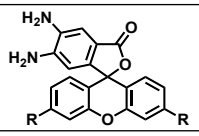
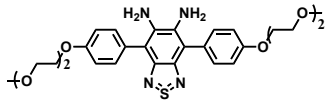
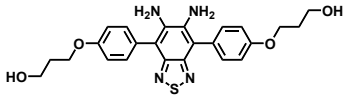
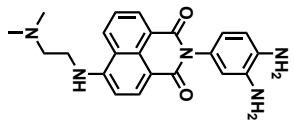
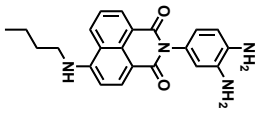
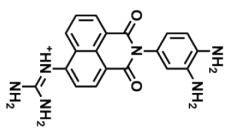
"*Fu*" and "*Au*" represent the integral of the fluorescence emission and absorbance spectra of **SWJT-2**, respectively. "*Fs*" and "*As*" were the integral of the fluorescence emission and absorbance spectra of fluorescein, respectively. "Φu" and "Φs" represent fluorescence quantum yield of **SWJT-2** and fluorescein, respectively. "η_u" and "η_s" are the refractive index of the solvent of **SWJT-2** and fluorescein, respectively.

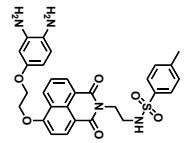
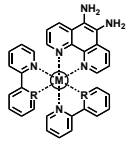
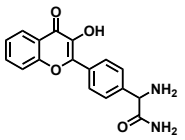
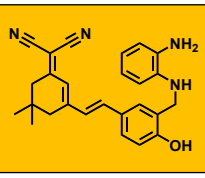
The B3LYP/6-31G(d,p) basis set was first used for optimizing the structure, and TD-DFT//B3LYP/6-31G(d,p) was then employed for studying the photophysical properties of **SWJT-2** and compound **M2**. (Gaussian 09 program)

The HeLa cells were incubated in a glass-bottom petri dish (Φ 15 mm) and adhered at 37 °C for 24 hours. The cells were washed with phosphate buffered saline (PBS) and added 10.0 μM of **SWJT-2** at 37 °C for 30 minutes, then washed with PBS and imaged. After incubating with 50.0 μM MGO for 30 min at 37 °C, HeLa cells were washed with PBS and imaged again. For inhibition tests, cells were treated with DMEM containing 1 mM N-acetylcysteine (NAC) for 6 hours, then added 10.0 μM of **SWJT-2** at 37 °C for 30 minutes before imaging. To confirm the result, the test cells were further incubated with MGO (50.0 μM) for imaging. Fluorescence imaging of intracellular MGO in HeLa cells was recorded on a laser scanning confocal microscope. The excitation wavelength of the laser is 488 nm. All cells were dyed with DAPI (4',6-diamidino-2-phenylindole).

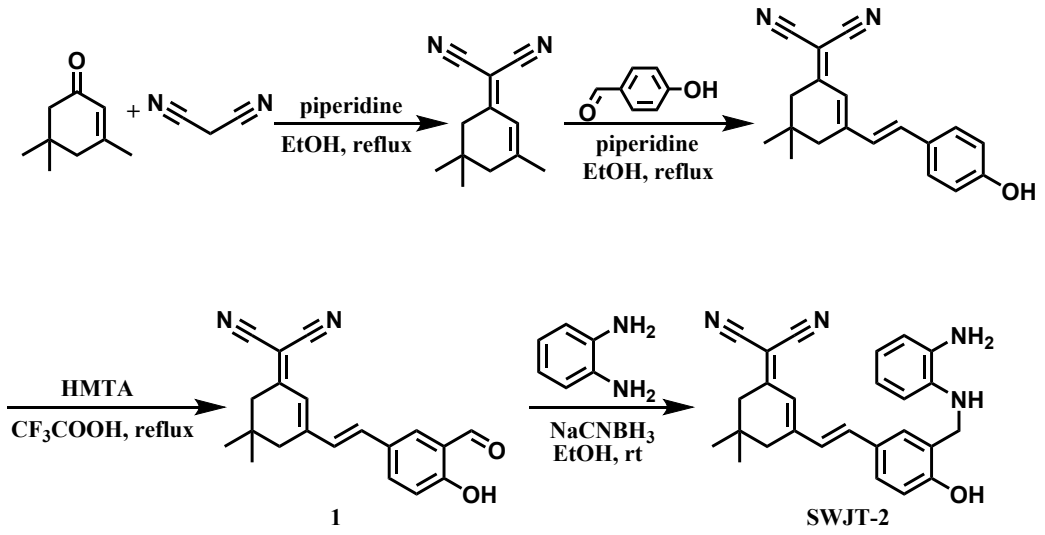
2. Summary of all fluorescent probes for methylglyoxal

Table S1

Probes	$\lambda_{ex}/\lambda_{em}$ nm	Linear range	Detection limit	Reaction time	Solution	References
	505/532	50-100 nM	50 nM	1 h (8 h)	PBS	Ref. 7a
	435/509 545/566	50-100 nM N. A.	700 nM N. A.	N. A.	PBS	Ref. 7b
	497/650	1-10 μM	18 nM	3 h	DMAC:PBS= 4:6	Ref. 7c
	500/650	4-16 μM	262 nM	1.5 h	DMAC:PBS= 4:6	Ref. 7d
	438/528	0-10 μM	77 nM	12 h	DMSO:PBS= 1:9	Ref. 7e
	440/555	0-6 μM	1.47 μM	40 min (1 h, Imaging)	DMSO:PBS= 1:19	Ref. 7f
	425/559	0-80 μM	2.2 μM	1.5 h	PBS	Ref. 7g

	380/460	0-5 μM	56 nM	2 h	DMF:PBS=1:9	Ref. 7h
	448/612	2-300 μM/2-1000 μM	0.78/1.15 μM	2 h	EtOH/PBS=2:1	Ref. 7i
	350/440, 525	0-600 μM	0.24/0.5 μM	1.5 h	DMSO/PBS=1:9	Ref. 7j
	440/674	0.0-10.0 μM	0.32 μM	15 min	DMF:PBS=1:1	***This work***

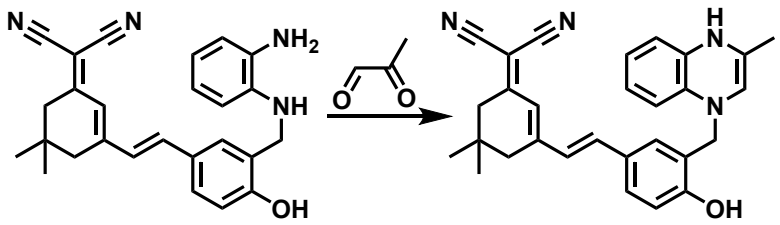
3. Synthesis of the probe SWJT-2 and compound M2



Scheme. S1 Synthesis route of probe **SWJT-2.**

The synthesis reference of compound **1** was synthesized according to the literature. [1] Compound **1** (123.1 mg, 0.39 mmol) and *o*-phenylenediamine (43.2 mg, 0.40 mmol) in 10 mL methanol and the mixture solution was stirred at room temperature for 20 min. Then, $NaCNBH_3$ (76.1 mg, 1.21 mmol) and CH_3COOH (90.0 μL) were added into the mixture. After stirring at room temperature for 3.5 h. The solvent was evaporated, the crude product was purified by silica gel column chromatography (petroleum ether : ethyl acetate = 2 : 1) to afford probe **SWJT-2** as a black solid (115.3 mg, 72.6 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.20 (s, 1H), 7.58 (d, J = 2.3 Hz, 1H), 7.47 (dd, J = 8.4, 2.2 Hz, 1H), 7.26 – 7.06 (m, 2H), 6.87 (d, J = 8.5 Hz, 1H), 6.75 (s, 1H), 6.62 – 6.53 (m, 1H), 6.52 – 6.29 (m, 3H), 4.86 (s, 1H), 4.55 (s, 2H),

4.21 (s, 2H), 2.57 (s, 2H), 2.50 (s, 2H), 0.99 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 170.30, 157.3, 156.8, 138.7, 135.9, 135.6, 129.0, 127.9, 127.1, 126.7, 126.3, 121.4, 117.7, 117.3, 115.6, 114.4, 114.2, 113.4, 110.4, 74.8, 42.4, 42.2, 38.2, 31.7, 27.5 (2C) ppm. HRMS (ESI): calcd for $C_{26}H_{27}N_4O$ $[M+H]^+$ 411.2179, found 411.2186, error 1.7 ppm.



SWJT-2 **M2**

Scheme. S2 Synthesis of **M2.**

Probe **SWJT-2** (100.1 mg, 0.24 mmol) and MGO (351.4 mg, 1.46 mmol, 30 wt. % in H_2O) were added in THF (10 mL), and the mixture solution was stirred at room temperature for 1 h. The solvent was evaporated, and the crude product was purified by column chromatography (DCM : MeOH = 20 : 1) on silica gel to afford compound **M2** (46.7 mg, 41 %) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.98 (s, 1H, OH), 9.08 (s, 1H, NH), 8.02 (dd, J = 6.3, 3.2 Hz, 2H), 7.90 (d, J = 2.2 Hz, 1H), 7.63 (dd, J = 6.3, 3.2 Hz, 2H), 7.60–7.56 (m, 1H), 7.20 (s, 2H), 7.01 (d, J = 8.5 Hz, 1H), 6.78 (s, 1H), 6.69 (d, J = 8.3 Hz, 1H), 5.68 (s, 2H, CH_2), 2.60 (s, 2H), 2.52 (s, 2H), 1.33 (s, 3H, CH_3), 1.01 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6)**:** δ = 170.2, 157.4, 156.6, 155.7, 138.5, 136.4, 129.2, 127.9, 127.1, 126.6, 126.3, 121.4, 117.7, 115.7, 114.2, 113.3, 110.4, 74.8, 42.4, 40.1, 38.3, 31.7, 30.8, 27.5 (2C) ppm.

HRMS (ESI): calcd for $C_{29}H_{29}N_4O$ $[M+H]^+$ 449.2236, found 449.2237, error 0.22 ppm.

4. ^1H, ^{13}C NMR and ESI-MS copies of SWJT-2

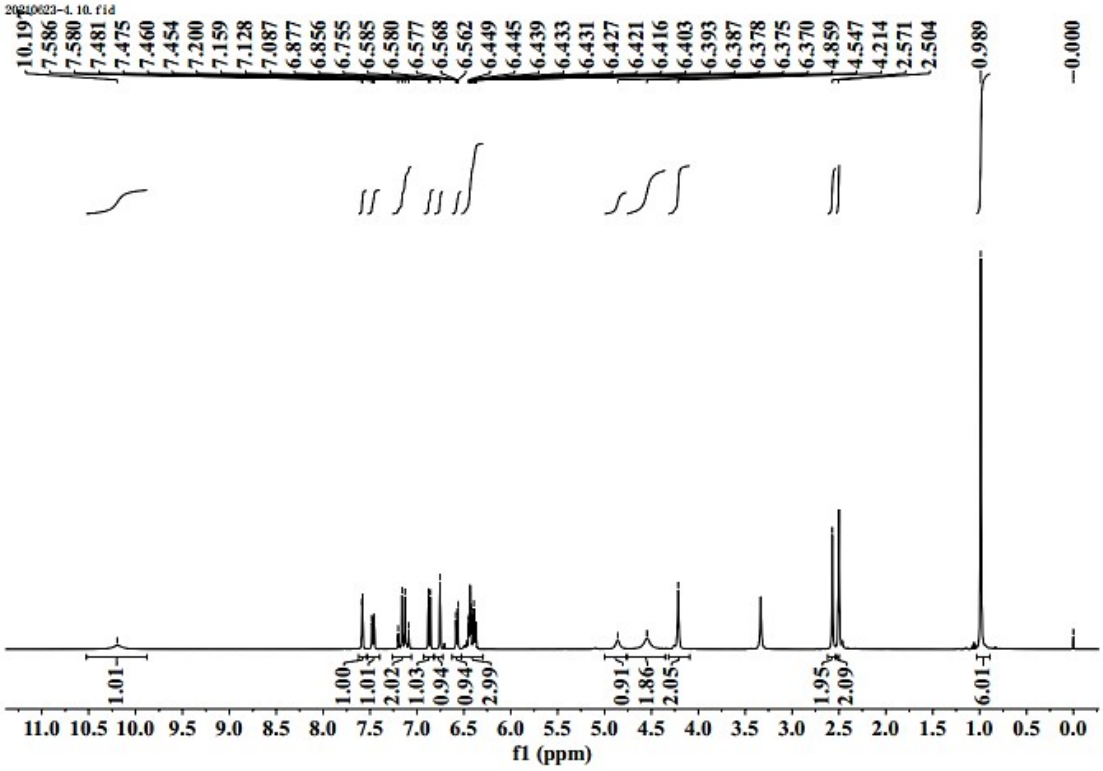


Fig. S1. ^1H NMR spectrum of probe **SWJT-2** (400 MHz, DMSO-d_6).

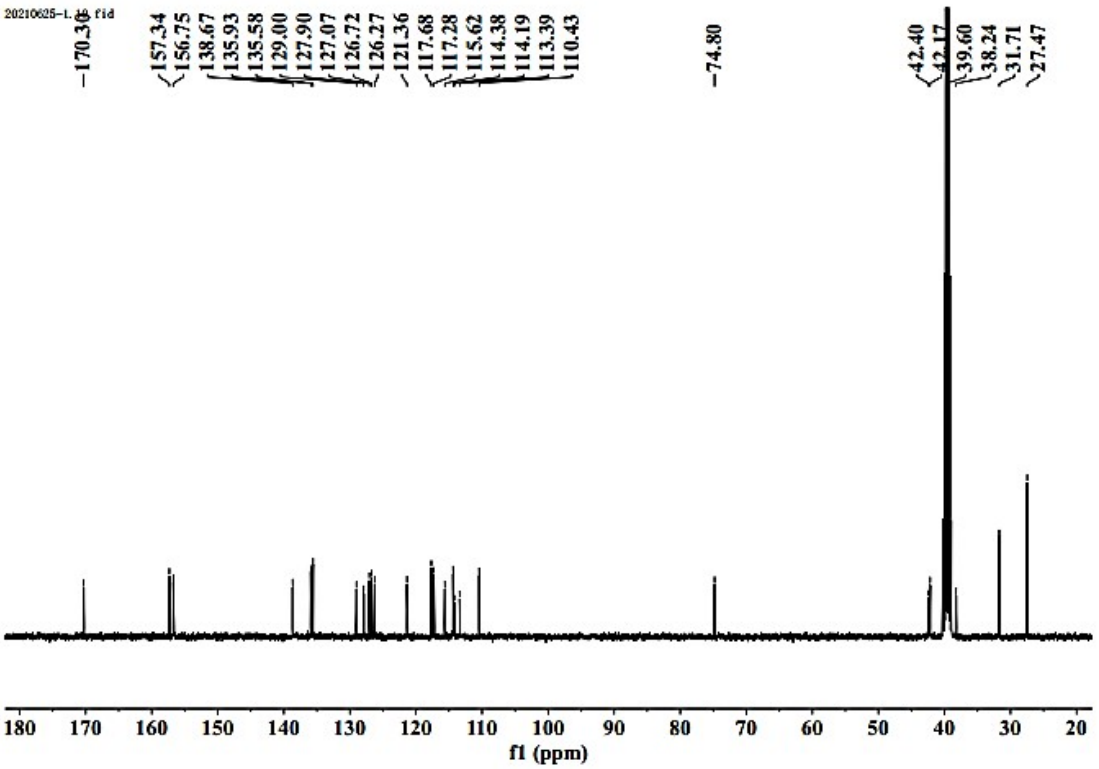


Fig. S2. ^{13}C NMR spectrum of probe **SWJT-2** (100 MHz, DMSO-d_6).

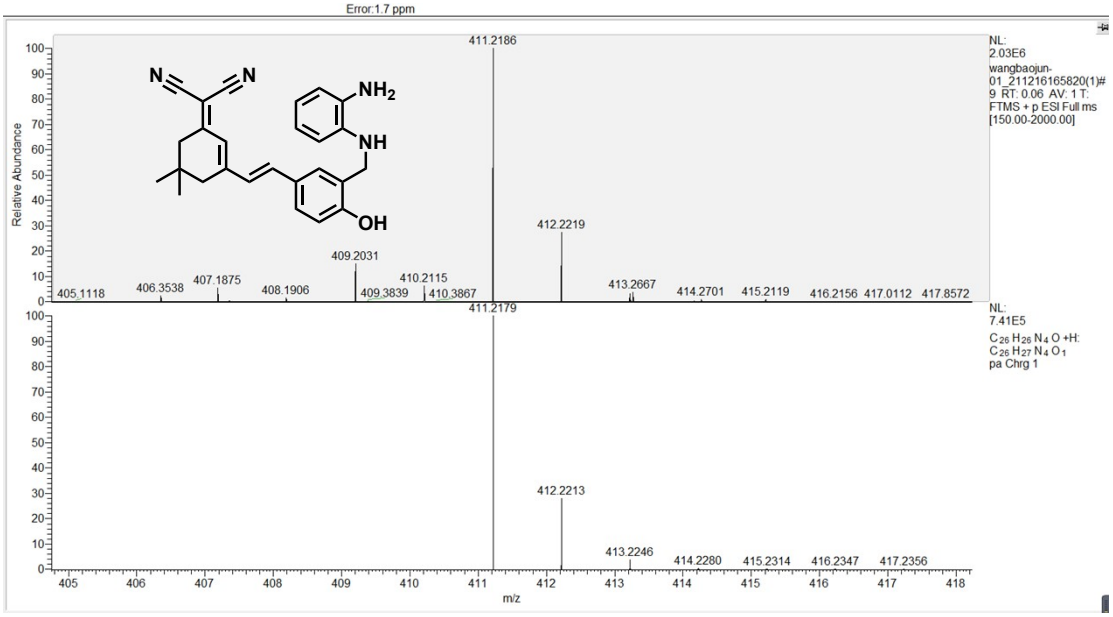


Fig. S3. HRMS of probe **SWJT-2**.

5. ^{1}H, ^{13}C NMR and HRMS copies of compound M2

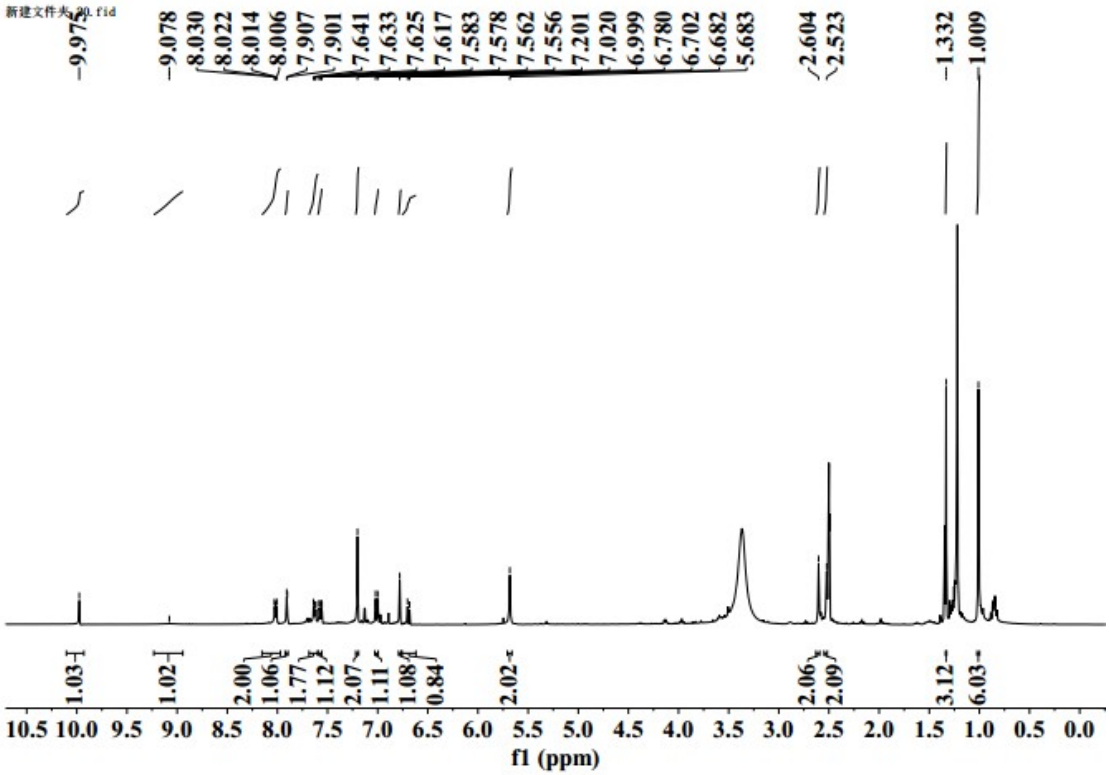


Fig. S4. ^{1}H NMR spectrum of compound **M2** (400 MHz, DMSO-d_6).

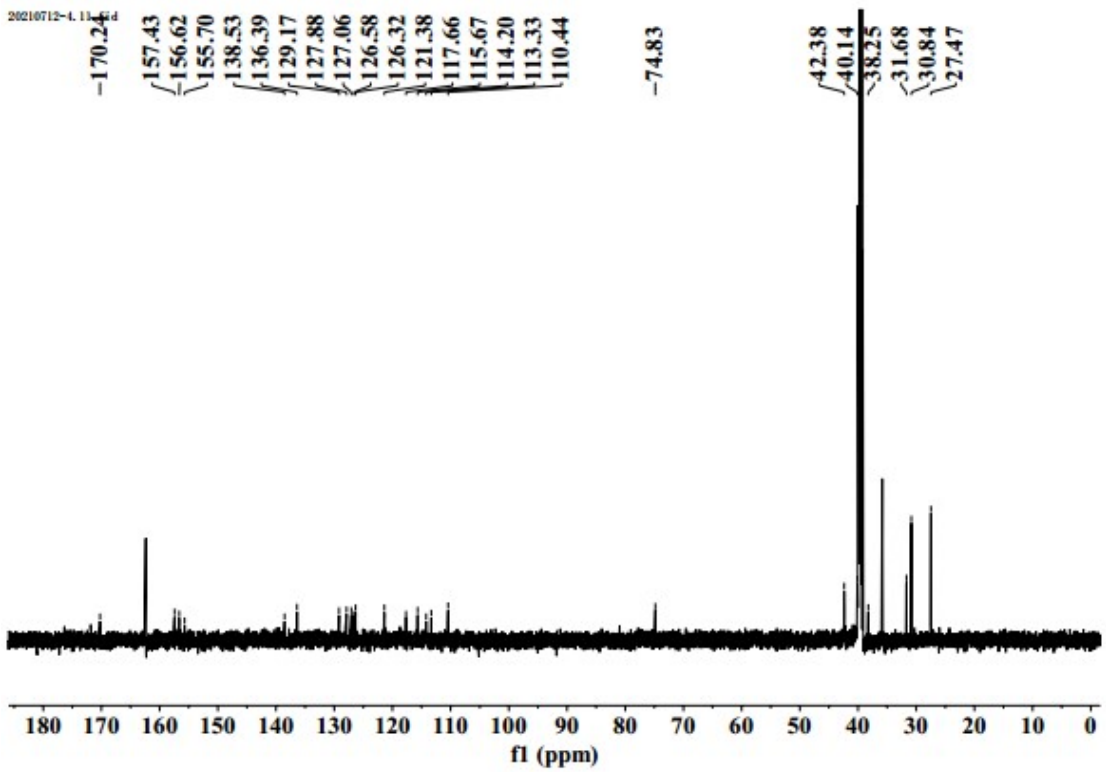


Fig. S5. ^{13}C NMR spectrum of probe **M2** (100 MHz, DMSO-d_6).

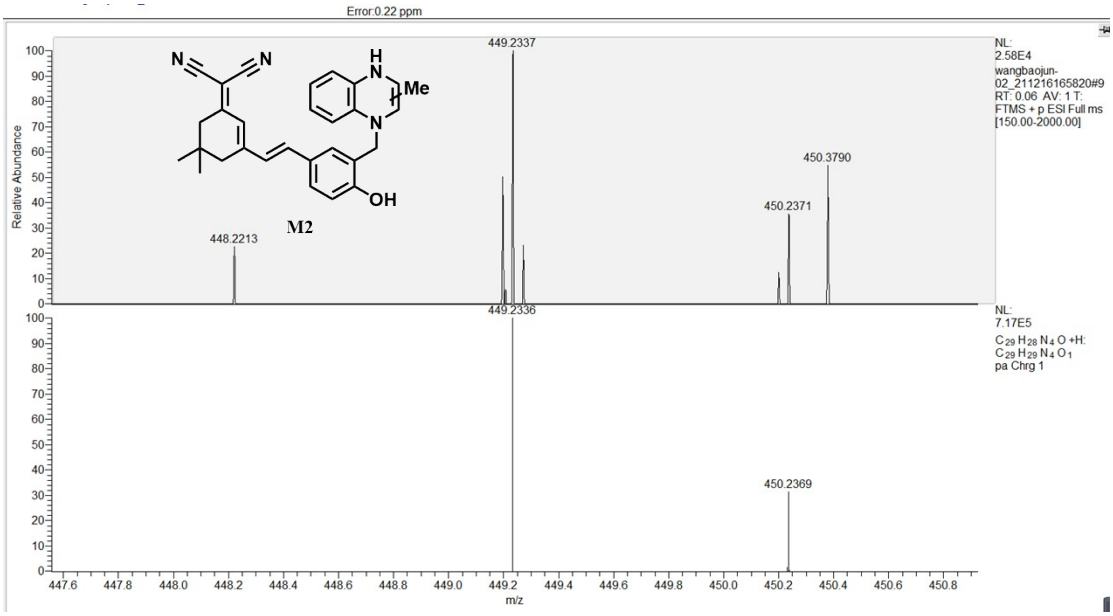


Fig. S6. HRMS of probe **M2**.

6. pH Effects

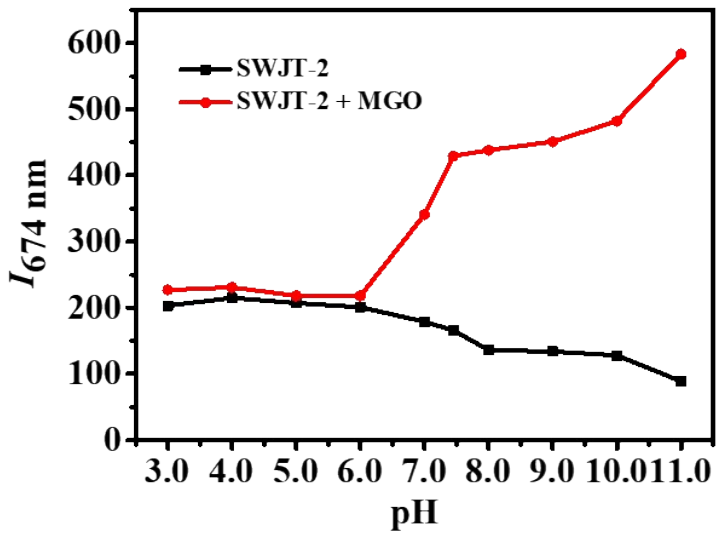


Fig. S7. Fluorescence responses of **SWJT-2** and **SWJT-2** + MGO (50.0 μM) under different pH conditions.

7. DFT calculations.

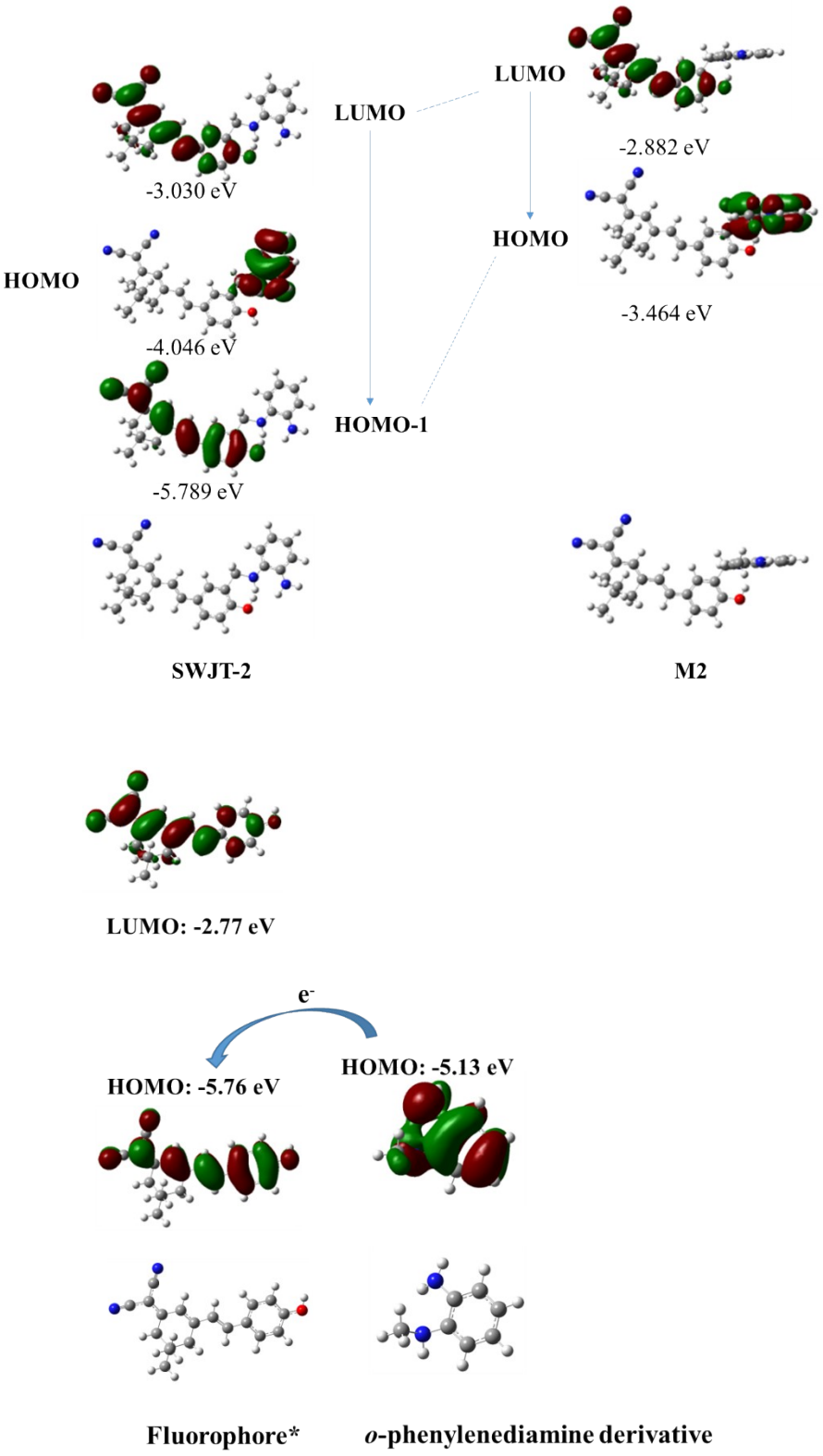


Fig. S8. Optimized structures, energy levels, and molecular orbital plots of **SWJT-2**, **M2**, Fluorophore, and *o*-phenylenediamine derivative.

8. Linear concentration range.

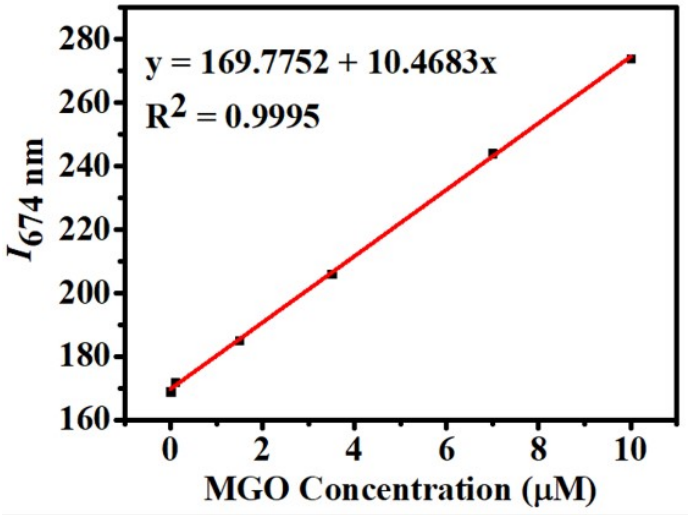


Fig. S9. Linear relationship between the fluorescence intensity at 674 nm and MGO concentration from 0.0 to 10.0 μM.

9. The kinetic study of the response of SWJT-2 to MGO.

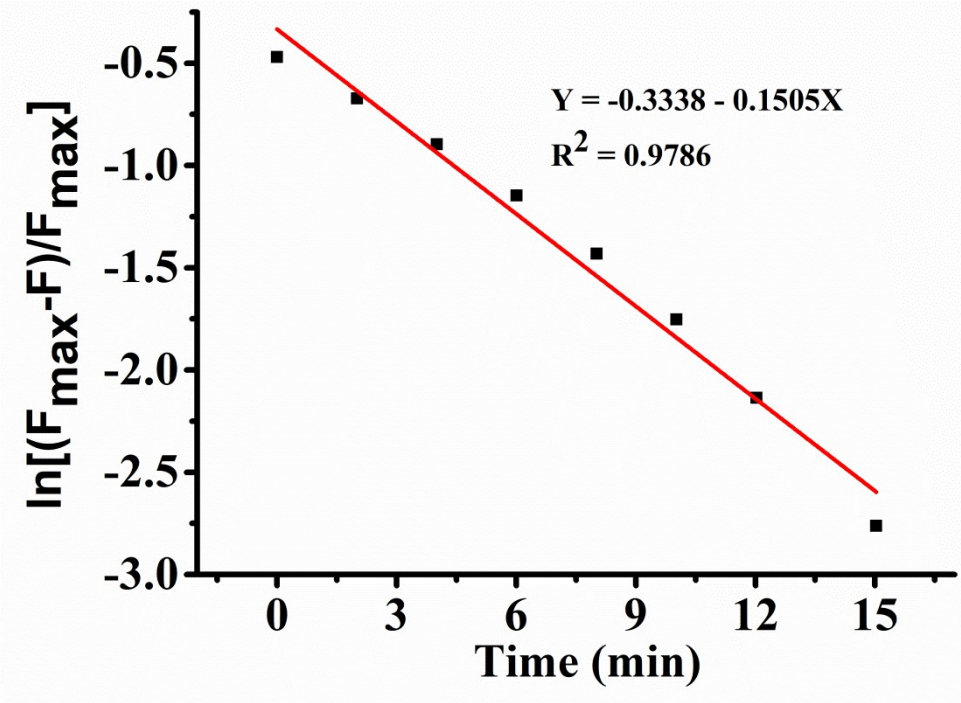


Fig. S10. Pseudo first-order kinetic plots of **SWJT-2** (10.0 μM) with the addition of MGO. (λ_{ex} = 450 nm)

The result of the analysis as follows：

$$\ln [(F_{max}-F_t) / (F_{max})] = -k_{obs}t$$

$$t_{1/2} = \ln 2/k_{obs}$$

Where F_{max} and F_t are the fluorescent intensity at maximum emission wavelength and time t. k_{obs} is the pseudo-first-order rate constant.

$$k_{obs} = 5.56 \times 10^{-3}\ s^{-1}$$

10. The investigation of probe 1 for MGO.

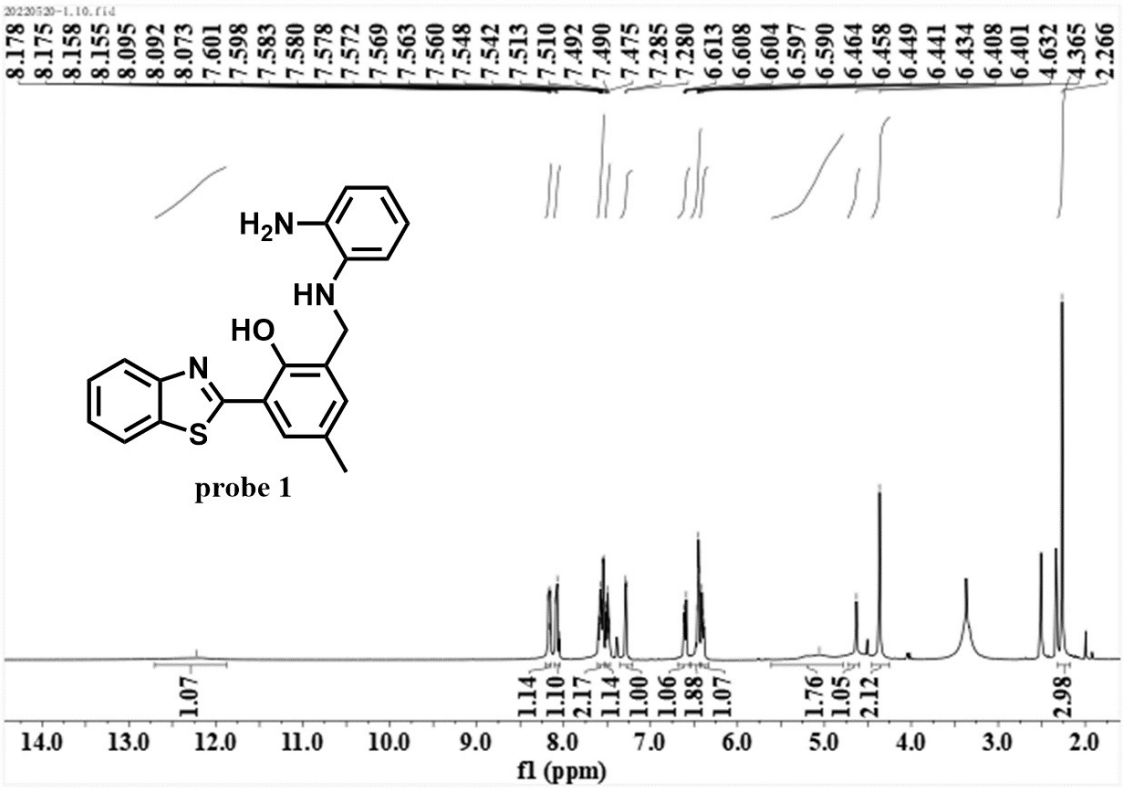


Fig. S11 ^{1}H NMR spectrum of compound probe **1** (400 MHz, DMSO-d_6).

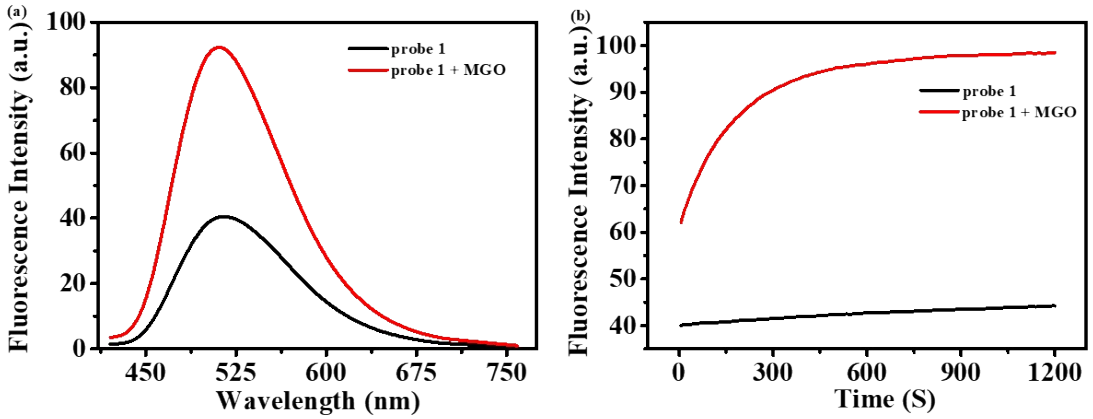


Fig. 12 (a) Fluorescence emission spectra of probe **1** (10.0 μM) in DMF/PBS buffer solution (1:1, v/v, pH = 7.4) in the absence (black) or presence (red) of MGO (50.0 μM). (b) Time-dependent fluorescence intensity ($I_{510\ nm}$) changes of probe **1** (10.0 μM) in the absence (black) and presence (red) of 50.0 μM MGO in DMF-PBS solution (1:1, V/V, pH = 7.4). (λ_{ex} = 400 nm)

11. Selectivity of SWJT-2 toward MGO.

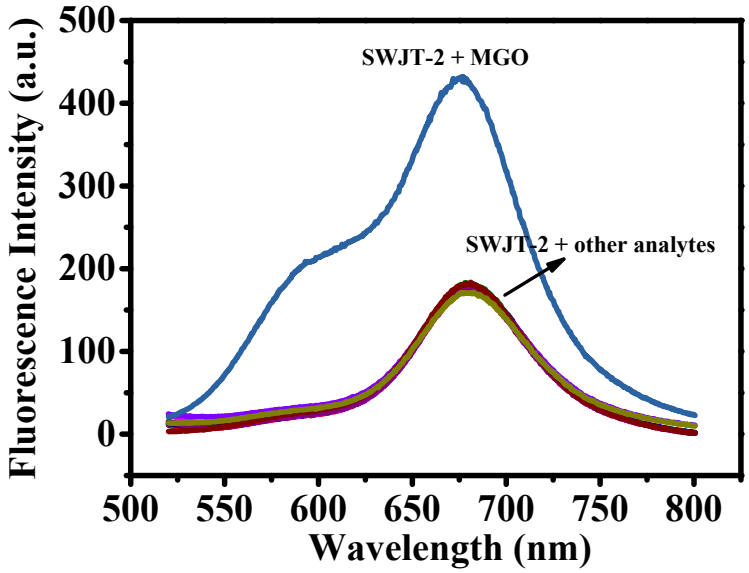


Fig. S13. Fluorescence spectra of **SWJT-2** (10.0 μM) in the presence of other analytes (50.0 μM) and MGO (50.0 μM).

Notes and references

[1] F. J. Huo, Y. Q. Zhang, J. B. Chao, Y. B. Zhang, C. X. Yin, *Dyes Pigm*, 2017, **143**, 270-275.