Supplementary information

Rhodamine based near-infrared fluorescent probe for Selective Detection of Cu^{2+} Ions and its applications in bioimaging

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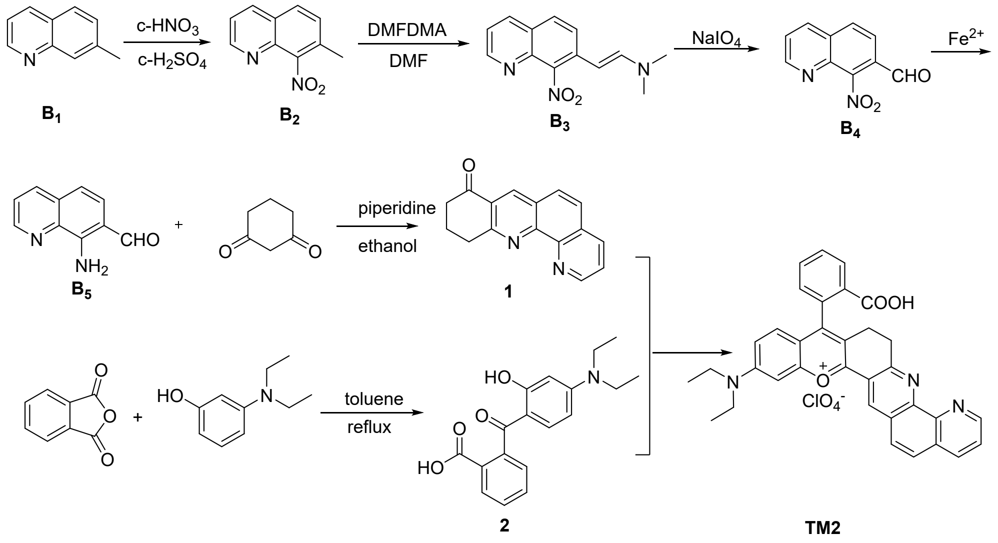
Synthesis and Characterization of Fluorescent Probe TM2



Scheme S1 Synthesis pathway of compound **TM2**

1 and **2** were synthesized according to known procedure.[S1a,b,c,d]

Synthesis of compound 2

Synthesis of Compound **2**. Under Ar atmosphere, 3-diethylaminophenol (10 g, 60.52 mmol) and Phthalic anhydride (8.96 g, 60.52 mmol) were added to toluene (51 ml), refluxed at 110 °C for 4 hours, and the mixture was cooled to 55 °C. 50 ml of 35% NaOH (w/w) aqueous solution was added, then heated to 90 °C for 6 hours. The reaction mixture was poured into 200 g of ice, adjusted the pH to 6 with concentrated hydrochloric acid, and stirred for 2 hours. The suspension was filtered and the crude product was recrystallized with ethanol to obtain a light purple solid **2** (11.7 g, yield 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.57 (s, 1H), 7.97 (dd, J = 7.8, 1.3 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.62 (t, J = 7.0 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 6.80 (d, J= 9.1 Hz, 1H), 6.19 (dd, J = 9.2, 2.5 Hz, 1H), 6.08 (d, J = 2.4 Hz, 1H), 3.40 (t, J = 7.0 Hz, 4H), 1.10 (t, J = 7.0 Hz, 6H).

Synthesis of compound B2-B5, 1

Synthesis of Compound **B2**.

Under an ice bath, concentrated sulfuric acid (15.4 ml, 0.286 mol) was added to a 50 ml flask. After 10 minutes, 7-methylquinoline (6.46 g, 0.04534 mol) was added and stirred for dissolution. Concentrated nitric acid (3.6 ml, 0.0546mol) was added dropwise and stirred at room temperature for 1 hour. The reaction solution was poured into 68 ml of ice water dropwise while stirring. The resulting mixture was filtered and the precipitate was washed with water. White product **B2** was obtained (6.96 g, yield 82%), which was directly used for the next step without further purification.

Synthesis of Compound **B3**.

Under Ar atmosphere, **B2** (3.0 g, 16.0 mmol) and N,N-Dimethylformamide dimethyl acetal (5.7 g, 6.4 ml, 48 mmol) were dissolved in 25 ml of anhydrous DMF. The solution was stirred at room temperature for 30 min and then heated to 145 °C for 18 h. The resulting mixture was diluted with water (50 mL) and extracted with DCM (25 mL x 3). The combine organic phases were washed with brine, dried over sodium sulfate and concentrated to give **B3** (4.05 g, yield 90%) as a red solid. The crude product was used for the next step without further purification.

Synthesis of Compound **B4**.

B3 (4.05g, 16.34 mmol), ethanol aqueous solution (1:1, v/v, 244 ml) and $NaIO_4$ (10.62 g, 49.49 mmol) was added to a 500 ml three necked flask. The mixture was stirred at r.t. for 4 hours. Insoluble substances were filtered out. The mixture was extract with EA, washed with saturated $NaHCO_3$ aqueous solution and then washed with water. The organic phases were dried over sodium sulfate and concentrated to obtain pure **B4** (3.7559 g, yield 80%). The crude product was used for the next step without further purification.

Synthesis of Compound **B5**.

B4 (3.755 g, 18.6 mmol) and Fe powder (7.651 g, 136.4 mmol) were added to a mixed solution (EtOH: HOAc: H_2O, 2:2:1, 143 ml) under Ar atmosphere. Then seven drops of concentrated hydrochloric acid was added. The mixture was heated at 90 °C for 40 minutes until the solution turns orange. After stirred at room temperature for 30 minutes, the insoluble substances in the solution were filtered and removed. The solvent was removed by rotary evaporation. The resulting mixture was diluted with water (200 mL) and extracted with DCM (100 mL x 3). The combine organic phases were washed with brine, dried over sodium sulfate and concentrated to give **B5** (2.9 g, 90%). The crude product was used for the next step without further purification.

Synthesis of Compound **1**.

B5 (1.9 g, 11.05 mmol), 1,3-cyclohexanedione (1.24 g, 11.05 mmol) and piperidine (11.1 ml) were refluxed in absolute ethanol (221 ml) at 90 °C overnight. TLC was used to monitor the reaction process. After the reaction was completed, the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (eluting with DCM/methanol) to give **1** (2.57 g, yield 94%) as a yellow solid. ^1H NMR (500 MHz, Chloroform-d) δ 9.26 (s, 1H), 8.89 (s, 1H), 8.27 (d, J=8.0 Hz, 1H), 7.87 (d, J=8.8 Hz, 1H), 7.78 (d, J=8.8 Hz, 1H), 7.69 (dd, J=8.0, 4.3 Hz, 1H), 3.59 (t, J=6.2 Hz, 2H), 2.92 – 2.77 (m, 2H), 2.41 – 2.28 (m, 2H).

Synthesis of compound TM2

Synthesis of Compound **TM2**.

At 0 °C, 2 (0.3 g, 1 mmol) and **1** (248 mg, 1 mmol) were added to 5 ml of concentrated H_2SO_4. The mixture was stirred at 90 °C for 7.5 h. After the reaction was completed, it was naturally cooled to room temperature. The reaction solution was slowly dropped into ice water with stirring. Then perchloric acid (70%, 1 ml) was added dropwise. After the mixture was left standing for 0.5 h, the suspended solids were filtered and washed with cold water for three times. The residue was purified by silica gel column chromatography (eluting with DCM/methanol) to give **TM2** (0.563g, yield 90%) as a purple solid. ^1H NMR (500 MHz, DMSO-d6) δ 9.09 (s, 1H), 8.84 (s, 1H), 8.49 (d, J=9.3 Hz, 1H), 8.14 (d, J=8.8 Hz, 1H), 8.06 – 7.95 (m, 2H), 7.87 – 7.69 (m, 3H), 7.43 (d, J=7.6 Hz, 1H), 6.66 (s, 1H), 6.58 – 6.48 (m, 2H), 3.40 (q, J=6.9 Hz, 4H), 3.27 – 3.15 (m, 2H), 2.30 – 2.19 (m, 1H), 2.08 – 1.92 (m, 1H), 1.14 (t, J=7.0 Hz, 5H). ^{13}C NMR (125 MHz, DMSO) δ 169.15, 157.09, 151.87, 151.45, 150.08, 149.16, 145.12, 144.77, 136.29, 135.60, 130.26, 129.01, 128.93, 128.64, 127.40, 126.91, 126.77, 126.65, 124.82, 124.01, 123.94, 123.36, 109.56, 107.34, 104.38, 97.07, 45.88, 43.93, 30.91, 20.46, 12.54, 8.76. ESI-MS: m/z calcd for $C_{34}H_{28}N_3O_3^+$ (M^+): 526.21, found $[M]^+$=526.13.

References:

S1: (a) Tamura M, Ogata H, Ishida Y, Takahashi Y. Design and synthesis of chiral 1,10-phenanthroline ligand, and application in palladium catalyzed asymmetric 1,4-addition reactions. Tetrahedron Lett 2017;58:3808-13. https://doi.org/10.1016/j.tetlet.2017.08.041.

(b) Zibaseresht R, Karimi P, Mohit-Azar S, Amirloo M, Azimi M. An improved synthesis of 2-(pyrid-2′-yl)-1,10-phenanthroline tridentate terpyridyl ligand. Int J Chem Res 2013;5:153-8. http://dx.doi.org/10.9735/0975-3699.5.1.153-158.

(c) Hu Y, Xiang Q, Thummel R. Bi-1,10-phenanthrolines and Their Mononuclear Ru(II) Complexes. Inorg Chem 2002;41:3423. https://doi.org/10.1021/ic011126f.

(d) Wang H, Guan J, Han X, Chen S, Li T, Zhang Y, Yuan M, Wang J. Benzothiazole modified rhodol as chemodosimeter for the detection of sulfur mustard simulant. Talanta 2018;189:39-44. https://doi.org/10.1016/j.talanta.2018.06.066.

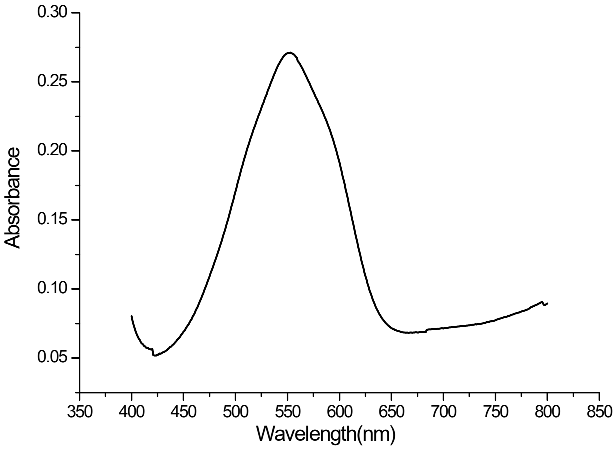


Figure S1. (a) Absorbance spectra of compound **TM2** (10 μM) in ethanol/H_2O (5:5, v/v) media.

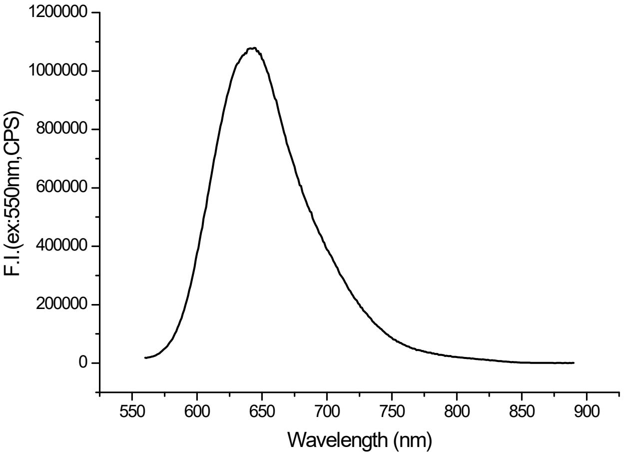


Figure S1. (b) Fluorescence spectra of compound **TM2** (10 μM) in ethanol/H_2O (5:5, v/v) media. (λ_{ex}: 550 nm).

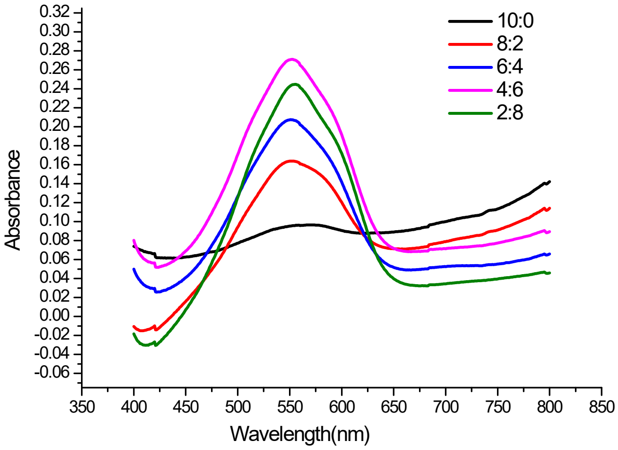


Figure S1. (c) Absorbance spectra of **TM2** (10 μM) in different ration of ethanol/H_2O mixtures.

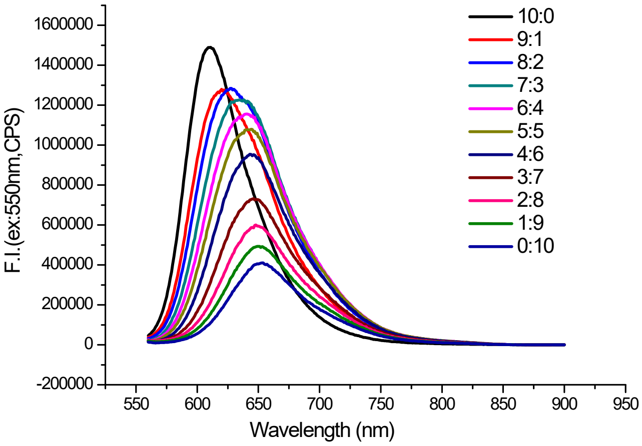


Figure S1. (d) Fluorescence spectra of **TM2** (10 μM) in different ration of ethanol/H_2O mixtures (λ_{ex}: 550 nm).

Determination of Detection Limit

The detection limit was calculated based on the fluorescence titration. Before adding Cu^{2+}, the emission intensity of compound **TM2** was measured by 10 times and the standard deviation of blank measurements was determined. Then a good linear relationship between the fluorescence intensity and Cu^{2+} concentration could be obtained in the 0-8 μM ($R^2 = 0.98$). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 2.3 μM.

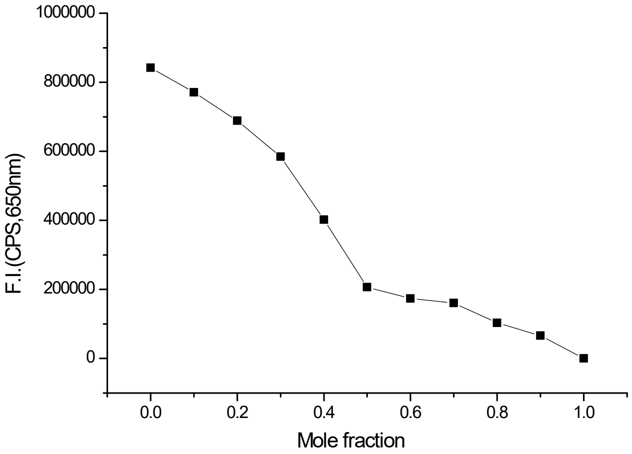


Figure S2. Job's plot of compound **TM2** with Cu^{2+} obtained by fluorescence measurements (λex: 550 nm, λem: 650 nm) measured in ethanol/H_2O (5:5, v/v) medium.The total concentration of **TM2** and Cu^{2+} is 10 μM.

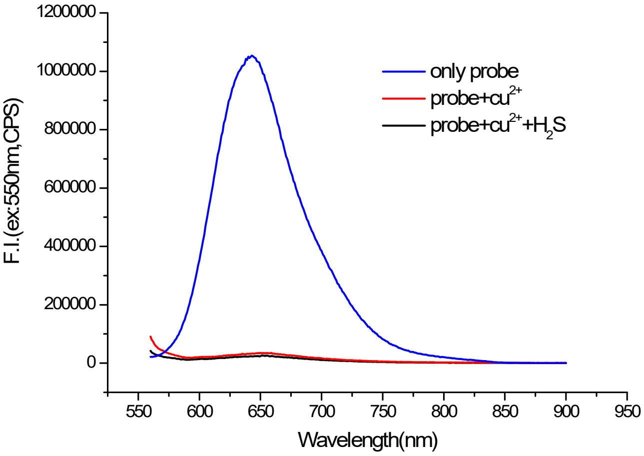


Figure S3. Fluorescence spectra of **TM2**(10 μM), **TM2**(10 μM)+Cu^{2+} (200 μM, 20 equiv.), and **TM2**(10 μM)+Cu^{2+}(200 μM, 20 equiv.)+H_2S(800 μM, 80 equiv.) in ethanol/water (5:5, v/v) mixtures. (λex: 550 nm)

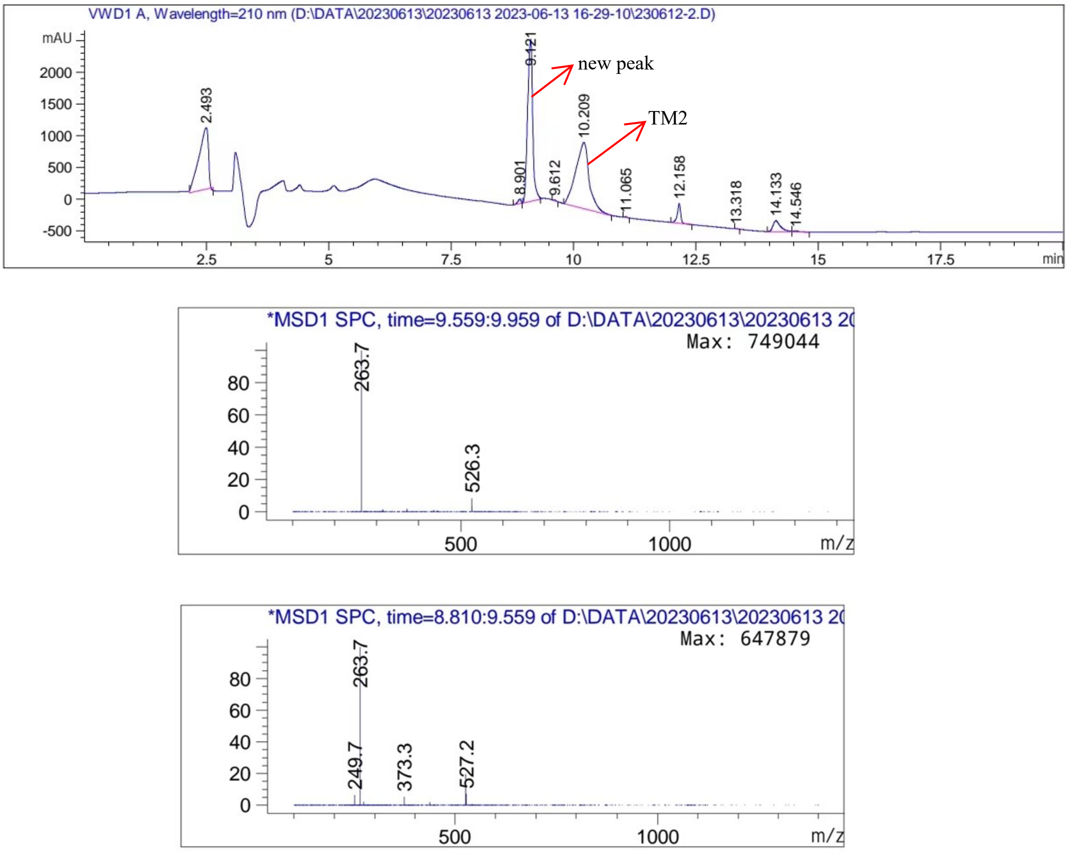
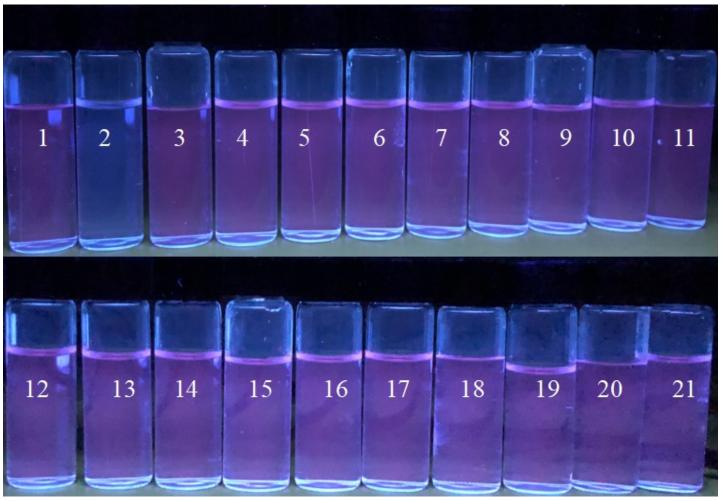


Figure S4. LC-MS of **TM2** (1 mM) in d6-DMSO upon addition of 0.5 equiv. of Cu^{2+}.

(a) Visible light



(b) UV light (365 nm lamp)

Figure S5. Observed visual color and fluorescence changes of compound **TM2** (10 μmol/L) upon addition of 20 equiv. of different ions in ethanol/H_2O (5:5, v/v) media: 1, no ions；2, Cu^{2+}；3, Fe^{2+}；4, Mn^{2+}；5, Co^{2+}；6, Ba^{2+}；7, Ag^{+}；8, Cr^{3+}；9, Na^{+}；10, Fe^{3+}；11, H_2O_2；12, Al^{3+}；13, Pb^{2+}；14, Ni^{2+}；15, Mg^{2+}；16, Cd^{2+}；17, Hg^{2+}；18, K^{+}；19, Zn^{2+}；20, H_2S；21, NaHS.

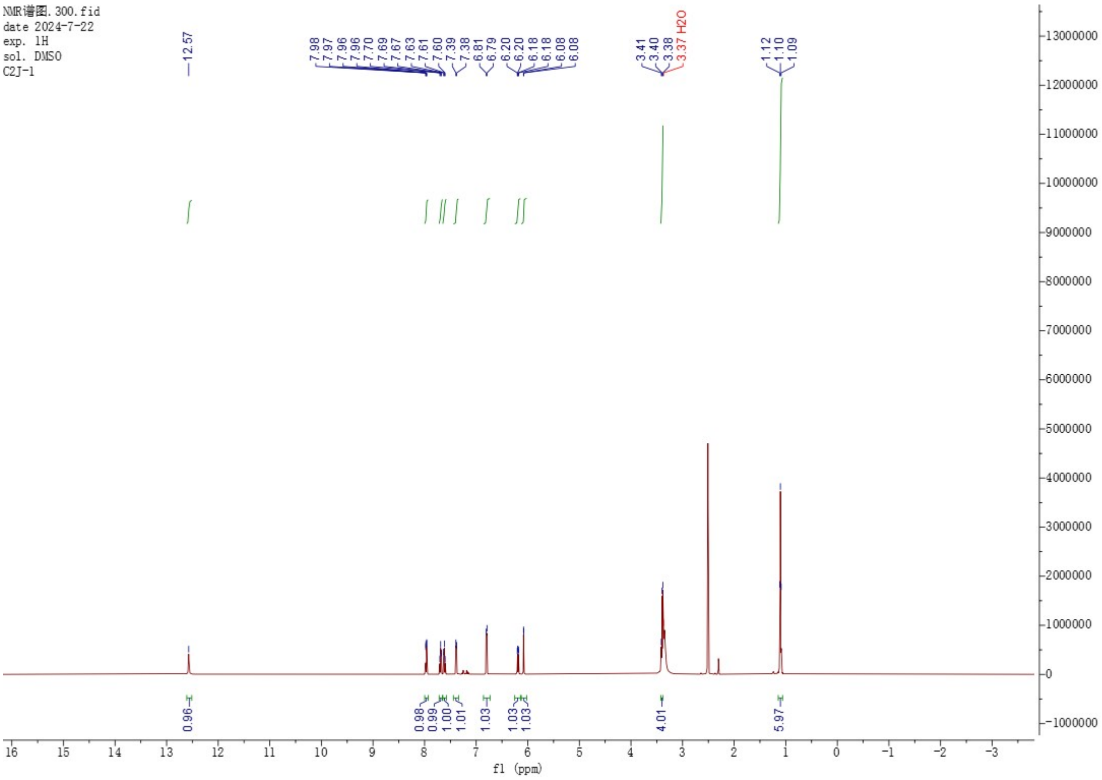


Figure S6. ^1H NMR spectrum of compound **2** in d6-DMSO

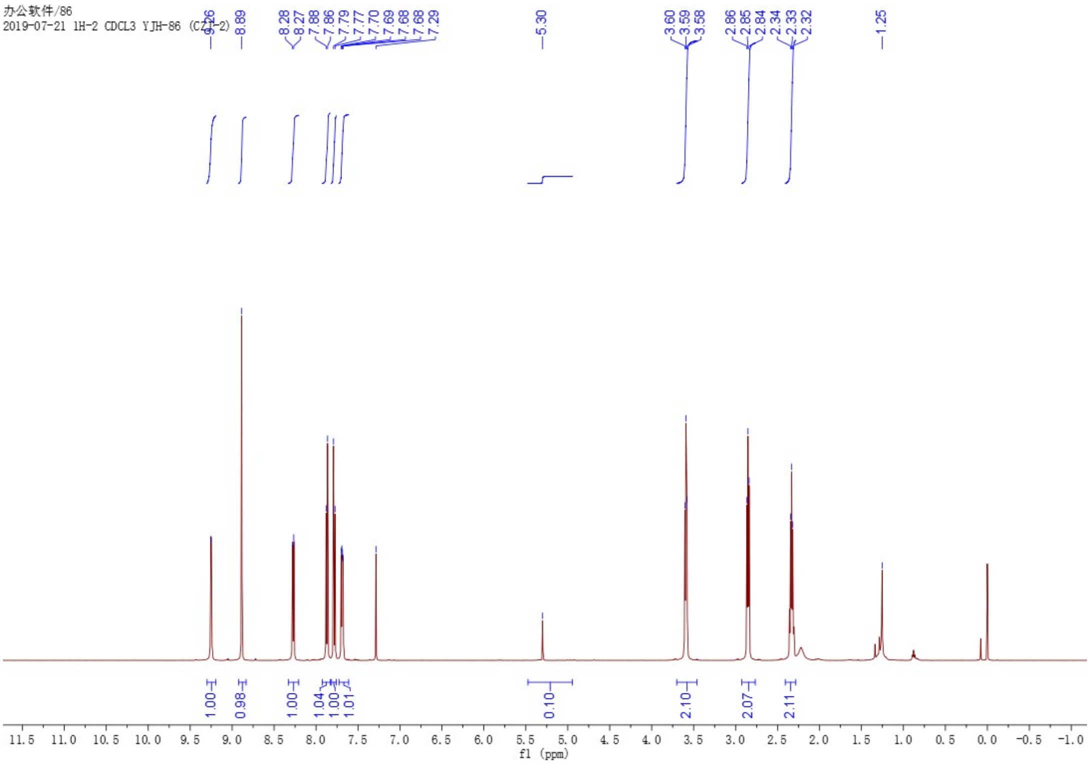


Figure S7. ^{1}H NMR spectrum of compound **1** in Chloroform-d

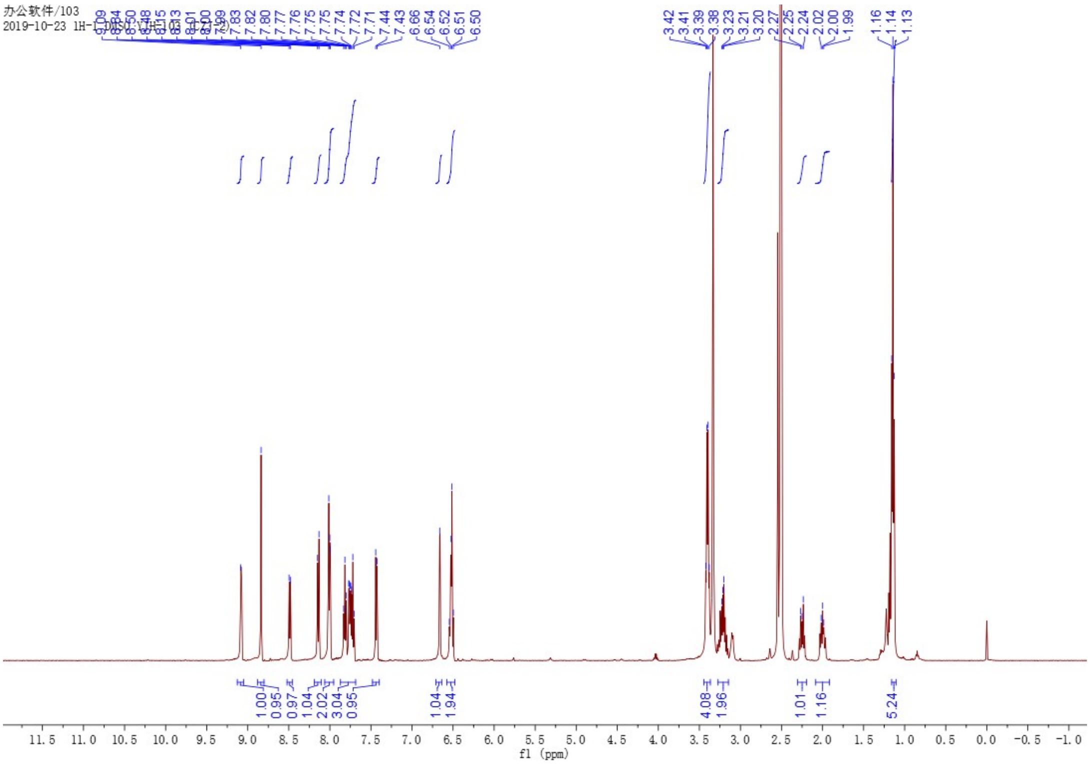


Figure S8. ^{1}H NMR spectrum of compound **TM2** in d6-DMSO

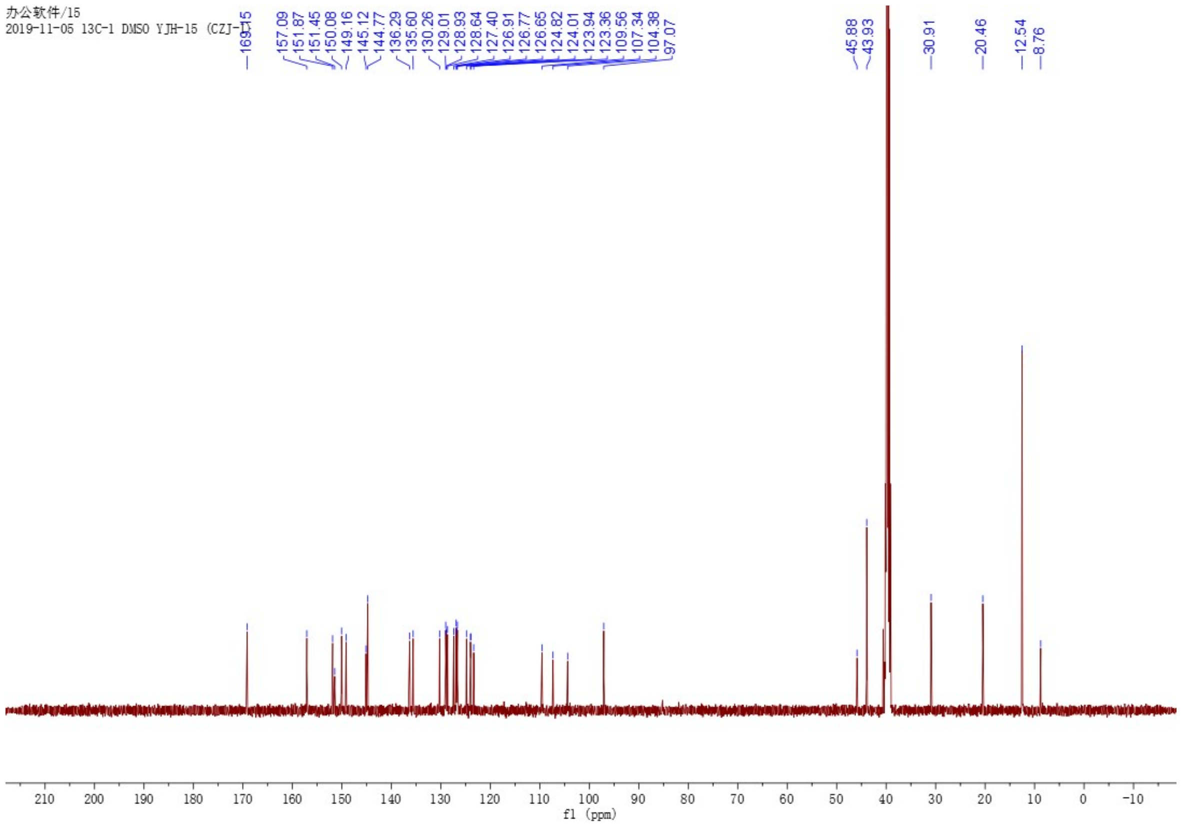


Figure S9. ^{13}C NMR spectrum of compound **TM2** in d6-DMSO

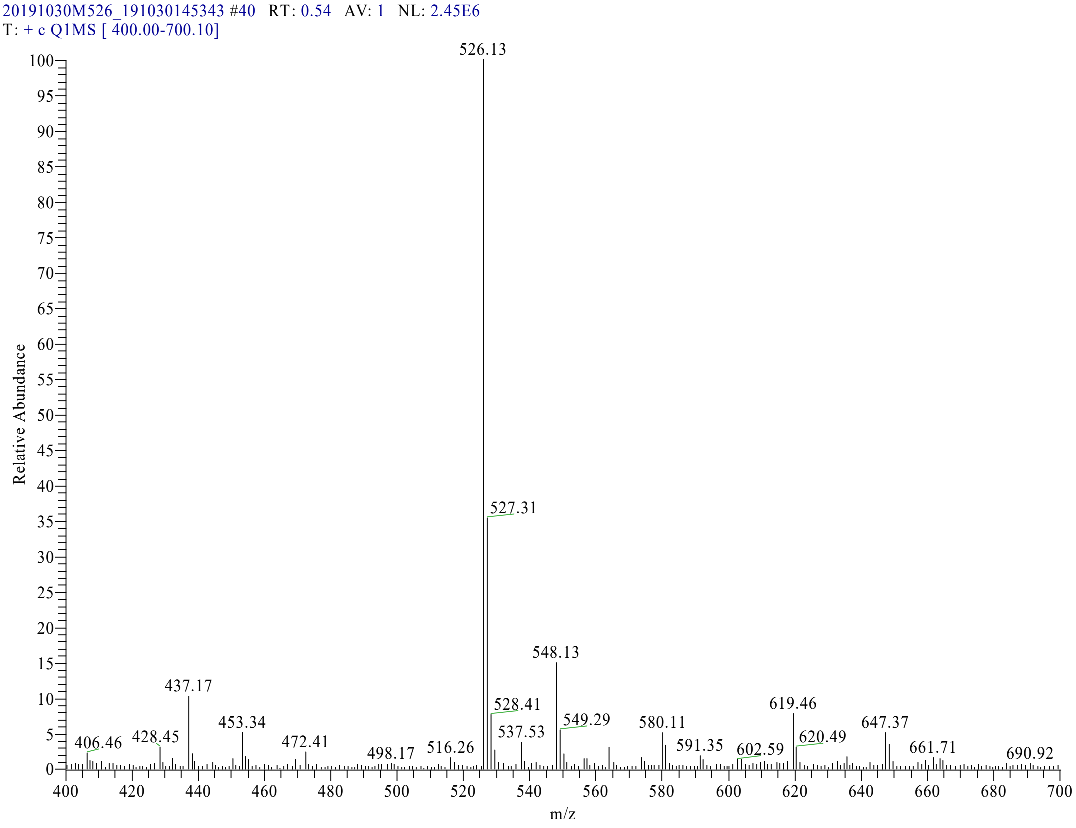
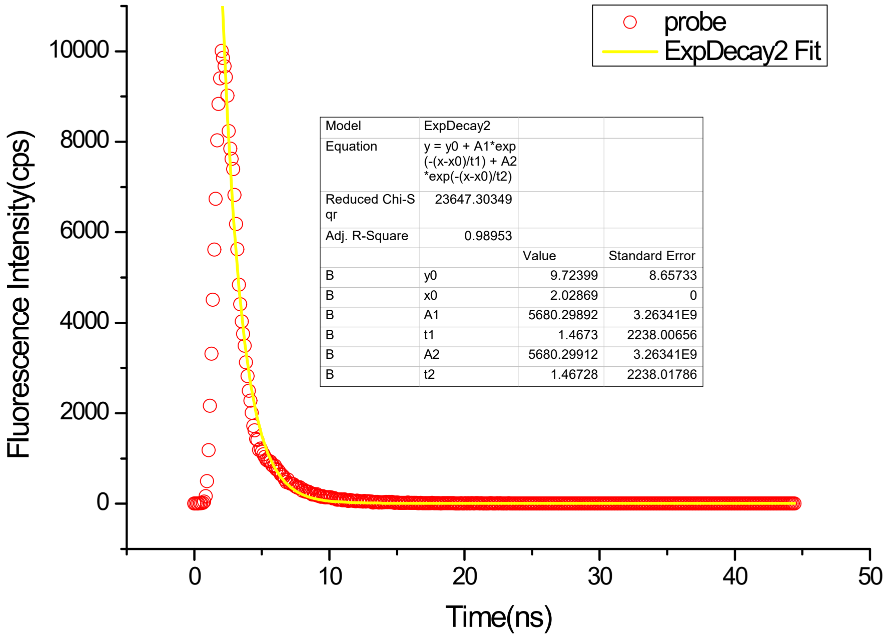


Figure S10. MS spectrum of compound **TM2**



Model	ExpDecay2		
Equation	y = y0 + A1*exp(-(x-x0)/t1) + A2*exp(-(x-x0)/t2)		
Reduced Chi-Sqr	23647.30349		
Adj. R-Square	0.98953		
		Value	Standard Error
B	y0	9.72399	8.65733
B	x0	2.02869	0
B	A1	5680.29892	3.26341E9
B	t1	1.4673	2238.00656
B	A2	5680.29912	3.26341E9
B	t2	1.46728	2238.01786

Figure S11. Fluorescence lifetime decay trace of probe **TM2** in ethanol/water (5:5, v/v) mixtures (10 μM, λ_{ex} = 550 nm).