

Supporting Information

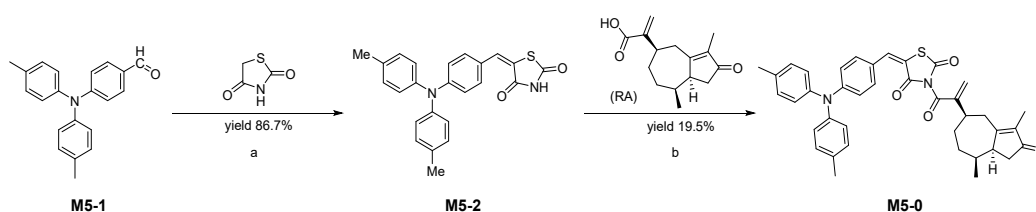
Synthesis of anti-tumor aggregation-induced emission-based fluorescent probe based on rupestonic acid

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1. Synthesis

1.1. Synthetic route of the probe



Compound M5-1 : 4-di-*p*-tolylaminobenzaldehyde ;

Compound M5-2 : 5-(4-(*p*-tolylamino)benzylidene)thiazolidine-2,4-dione ;

Compound RA : rupestonic acid;

The probe M5-0 : 5-(4-(di-*p*-tolylamino)benzylidene)-3-(artemisinin ketoacyl) thiazolidine-2,4-dione ;

1.2. Experimental methods

1.2.1. The Synthesis of M5-2

About 1 kg of *A. rupestris* L. (commercially available, purchased from Baokang Pharmaceutical Co., Ltd) were extracted three times with 75% ethanol (reflux 1h,2h,3h respectively). The extracts were combined and evaporated to dryness under reduced pressure, which yield 0.18 kg of extract. The extract was dissolved in 1L water, and extract three times with EA. Combine the organic phase and adjust pH to 8 by adding 5% NaHCO₃. After separating the aqueous phase, add dilute hydrochloric acid and adjust the pH value to 3. Aqueous phase extract three times with EA and the combined organic phase evaporated under reduced pressure. Obtain crude products purified by column chromatography (Petroleum ether: Ethyl acetate = 5:1), and recrystallization with ace-tone to give 0.8g pure rupestonic acid (purity >98%, mp: 132.7-134.2 °C).

1.2.2. The Synthesis of M5-2

A · To a stirred solution of M5-1(500 mg, 1.66 mmol), 2,4-Thiazolidinedione (214 mg,1.83 mmol), acetic acid (5 mg, 0.083 mmol), 3-picoline (7.7 mg, 0.083 mmol) in toluene (15 mL) was refluxed for 16 h. The reaction was concentrated under a vacuum pump and purified by chromatography on silica gel (EtOAc: PE =1:10) to afford M5-2(576 mg, red solid, mp: 185.4-186.7).

EtOAc: Ethyl acetate

PE: Petroleum ether

1.2.3. The Synthesis of M5-0

B · A mixture of M5-2 (268mg, 0.67mmol), rupestonic acid (150 mg, 0.61mmol), and DIEA (236mg, 1.83mmol) in dry DCM (10mL) was stirred at 0°C. Then HATU (346mg, 0.91mmol) was added and the reaction was slowly raised to room temperature for another 72 h. Concentrated under vacuum and purified by Prep-TLC (EtOAc : PE=1:10) to afford the probe M5-0 (75mg, orange solid, mp: 192.5-194.1).

DIEA: Diisopropylethylamine

HATU: N,N,N',N'-Tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate

DCM: Dichloromethane

2. Data Characterization

2.1. The NMR spectrogram and data of Rupestonic acid

^1H NMR (500 MHz, CDCl_3) δ 6.35 (s, 1H), 5.71 (s, 1H), 3.24–3.08 (m, 1H), 2.90–2.88 (m, 1H), 2.86–2.84 (m, 1H), 2.64–2.63 (m, 1H), 2.49–2.41 (m, 1H), 2.15 (s, 2H), 2.13–2.08 (m, 1H), 2.05–2.03 (m, 1H), 1.81 (s, 3H), 1.62–1.58 (m, 3H), 0.63 (d, $J = 7.1$ Hz, 3H).

^{13}C NMR (125 MHz, CDCl_3) δ 208.85, 175.20, 171.32, 145.75, 137.77, 125.33, 46.00, 41.30, 38.39, 37.69, 36.55, 35.27, 31.57, 12.11, 8.00.

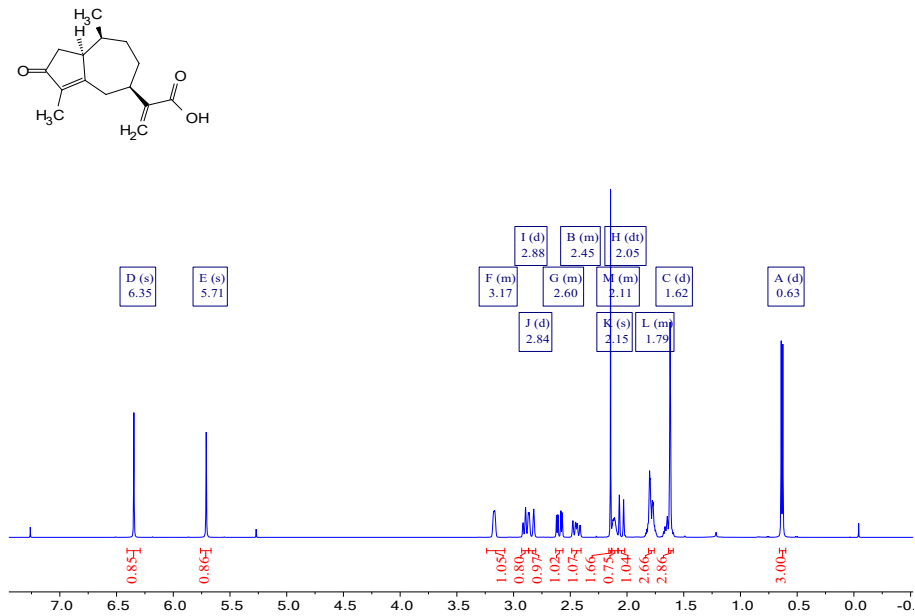


Figure SI-1. ^1H NMR spectrum of Rupestonic acid.

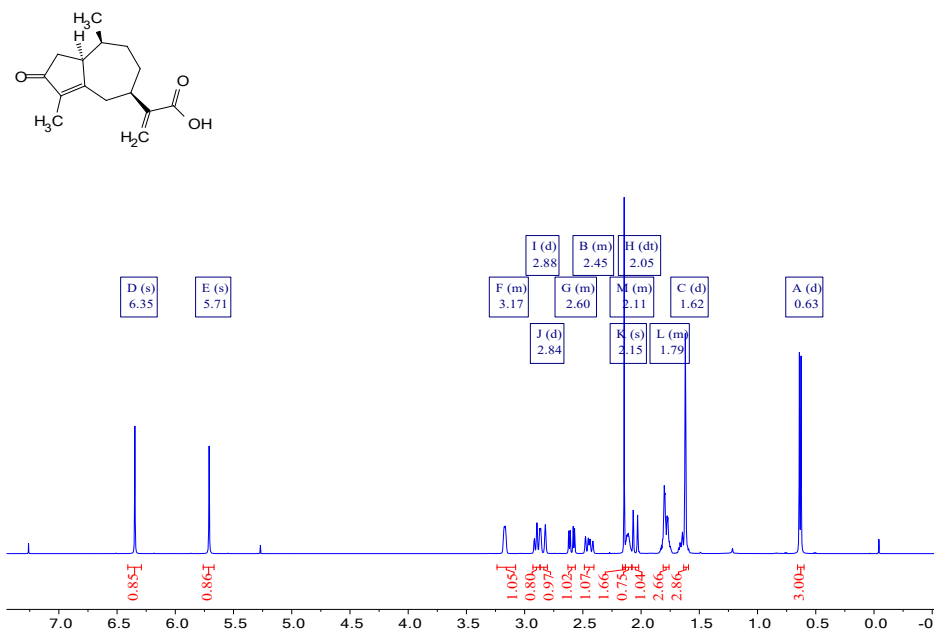


Figure SI-2. ^{13}C NMR spectrum of Rupestonic acid.

2.2. The NMR spectrogram and data of M5-2

^1H NMR (500 MHz, CDCl_3) δ 7.78 (s, 1H), 7.30 (d, $J = 9.0$ Hz, 2H), 7.13 (d, $J = 8.0$ Hz, 4H), 7.05 (d, $J = 8.4$ Hz, 4H), 6.97 (d, $J = 9.0$ Hz, 2H), 2.34 (s, 6H). ^{13}C NMR (125 MHz, CDCl_3) δ 172.34, 171.77, 167.99, 167.58, 150.51, 143.42, 134.59, 131.84, 130.15, 125.95, 123.99, 119.21, 117.21, 20.81.

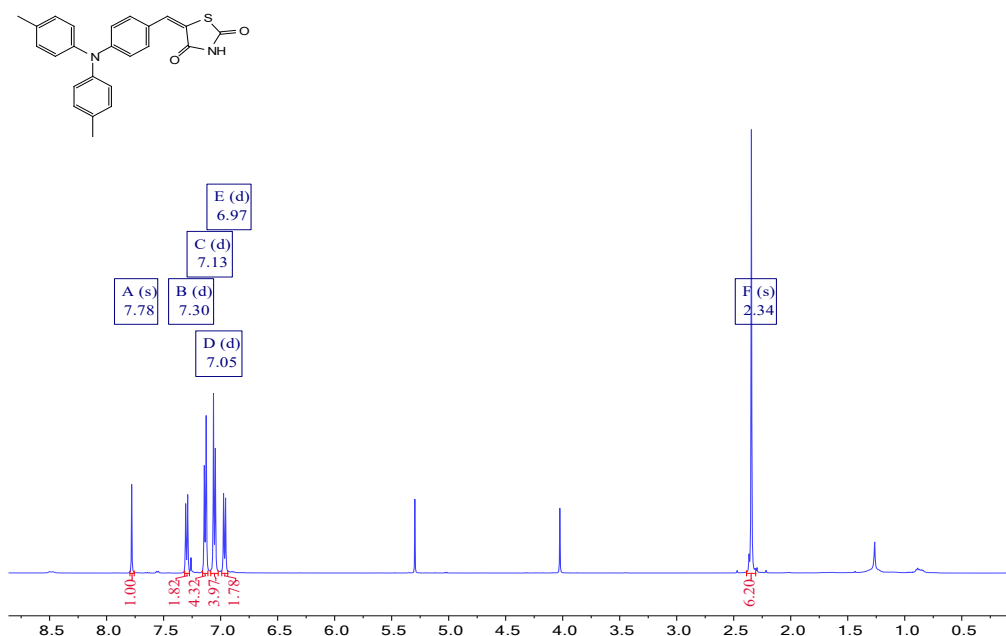


Figure SI-3. ^1H NMR spectrum of the compound M5-2.

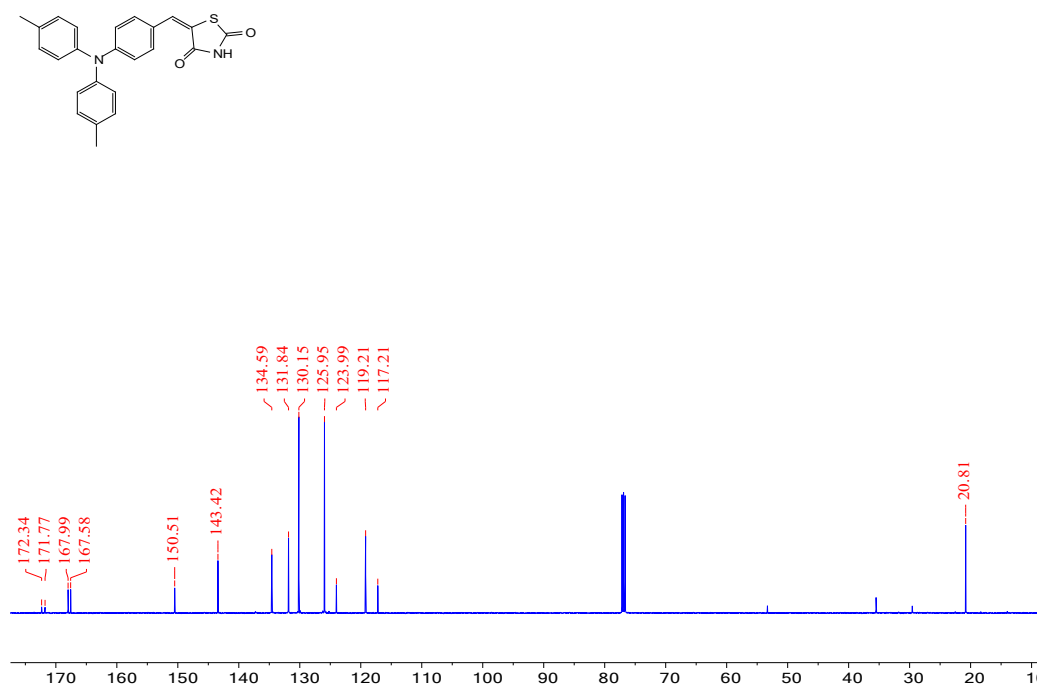


Figure SI-4. ^{13}C NMR spectrum of the compound M5-2.

2.3. The NMR spectrogram and data of M5-0

^1H NMR (500 MHz, CDCl_3) δ 8.09 (s, 1H), 7.58 (d, $J = 5.5$ Hz, 1H), 7.44 (d, $J = 7.9$ Hz, 4H), 7.35 (d, $J = 8.0$ Hz, 4H), 7.25 (d, $J = 8.4$ Hz, 2H), 4.65–4.04 (m, 2H), 3.39–3.32 (m, 1H), 3.27–3.14 (m, 2H), 3.03–2.91 (m, 1H), 2.92–2.83 (m, 1H), 2.82–2.72 (m, 1H), 2.65 (s, 6H), 2.43–2.34 (m, 2H), 2.22–2.08 (m, 1H), 1.24–1.12 (m, 1H), 0.91 (d, $J = 7.0$ Hz, 3H). ^{13}C NMR

(125 MHz, CDCl₃) δ 209.01, 175.20, 174.99, 168.75, 168.71, 167.03, 167.00, 151.01, 143.85, 138.39, 138.26, 135.14, 135.01, 132.30, 130.66, 126.45, 124.48, 119.63, 116.02, 116.01, 51.03, 46.13, 41.67, 41.28, 37.40, 36.77, 35.59, 30.05, 21.30, 12.47, 8.44.

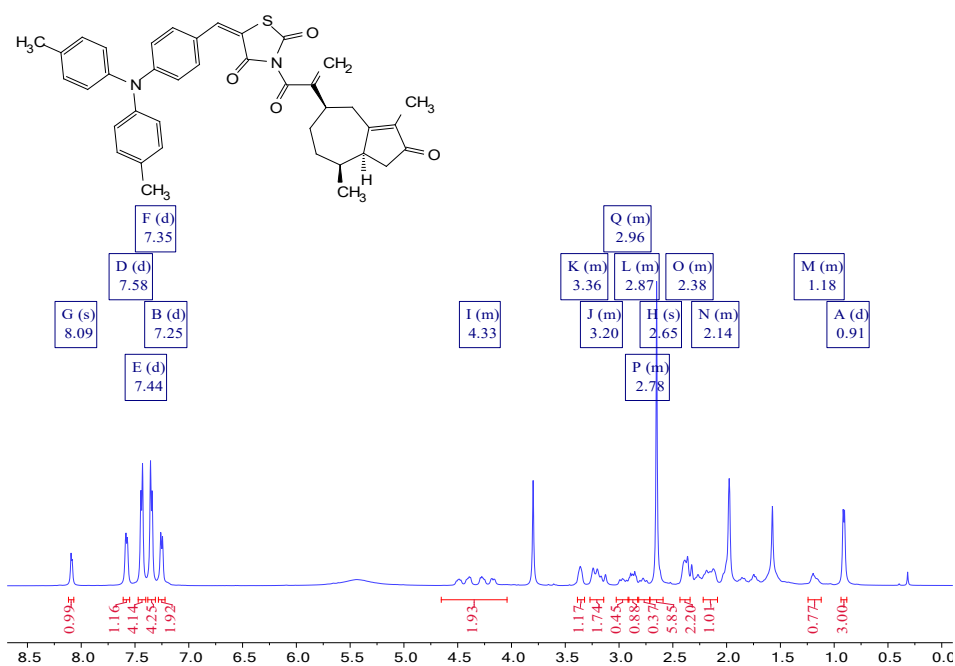


Figure SI-5. ¹H NMR spectrum of fluorophore M5-0.

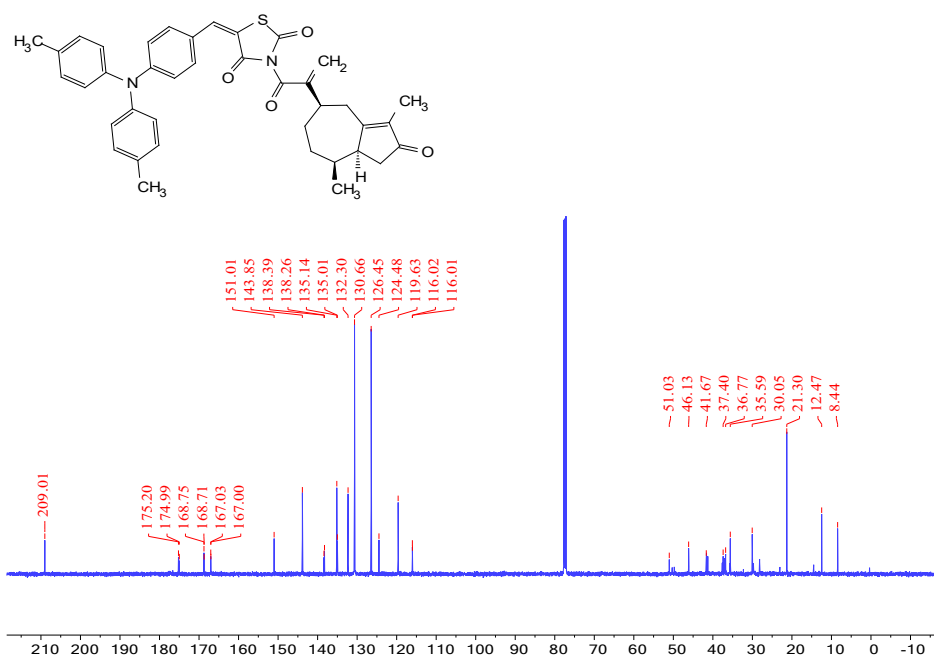


Figure SI-6. ¹³C NMR spectrum of fluorophore M5-0.

Data File: \\Deep-20160624\df\data1\XUXINFANG\ABDL\M05.lcd

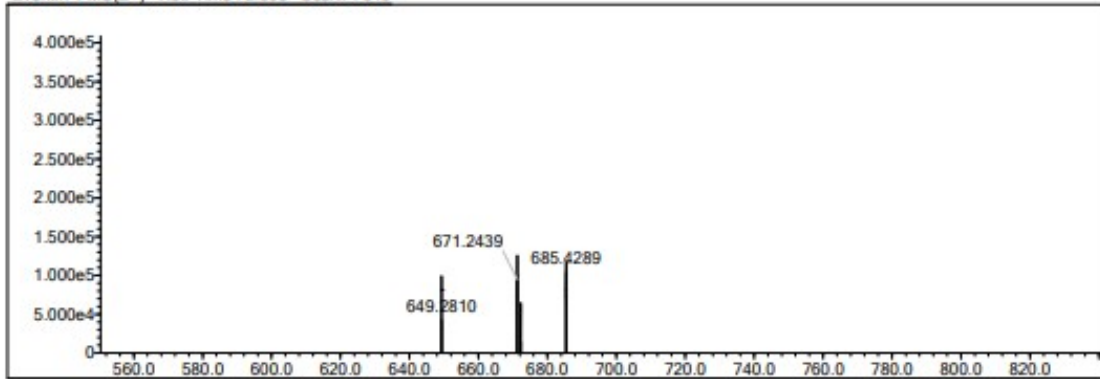
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B	3	0	0	O	2	4	4	S	2	1	1	I	3	0	0	Na
C	4	39	39	F	1	0	0	Cl	1	0	0					NH4

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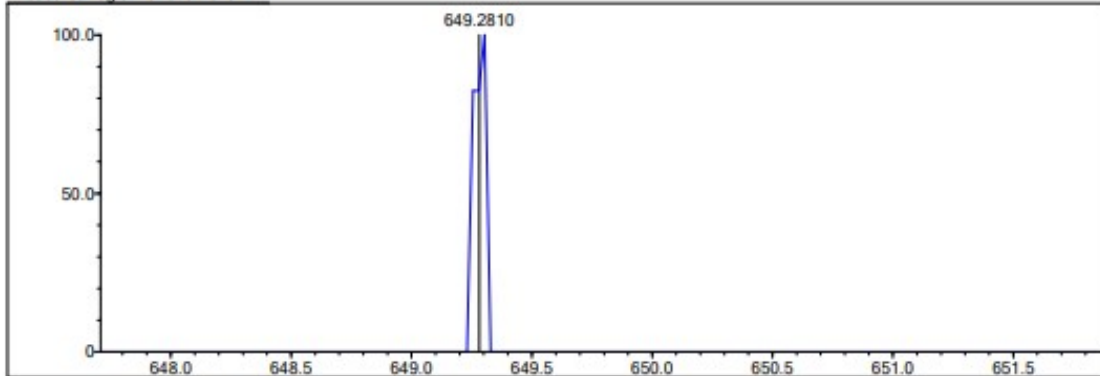
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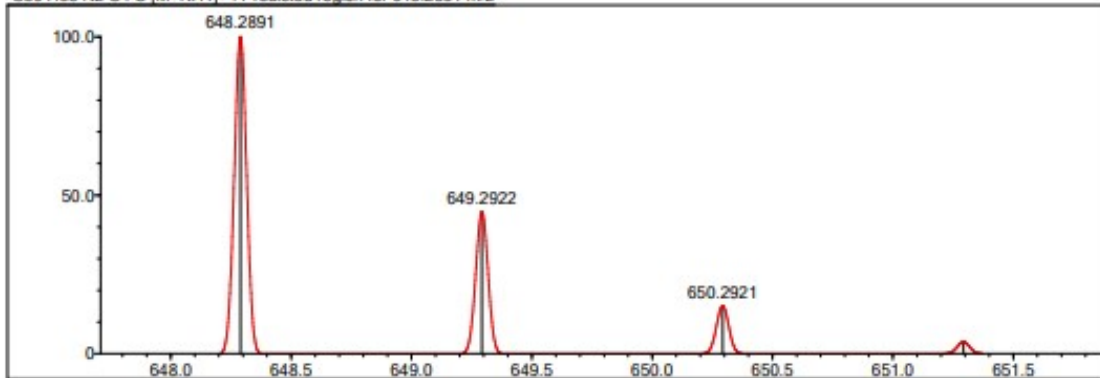
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Measured region for 648.2810 m/z



C39 H38 N2 O4 S [M+NH4]⁺ : Predicted region for 648.2891 m/z



Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	0.00	C39 H38 N2 O4 S	[M+NH4] ⁺	648.2810	648.2891	-8.1	-12.49	0.00	22.0

Figure SI-7. High-resolution mass spectrometry of M5-0. (Calculated: 630.2552, Observed: [M+NH₄]⁺ = 648.2810.)

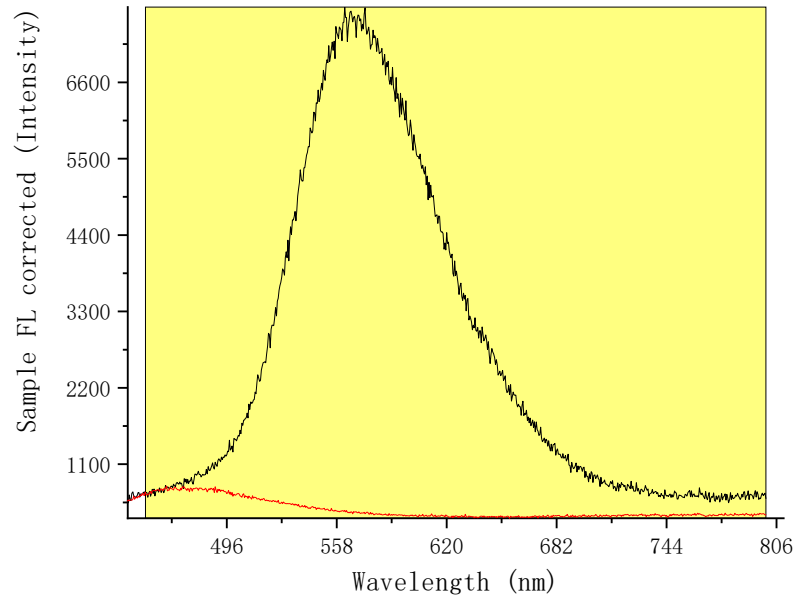


Figure SI-8. Emission fluorescence of **M5-0** in water (QYs =20.34)

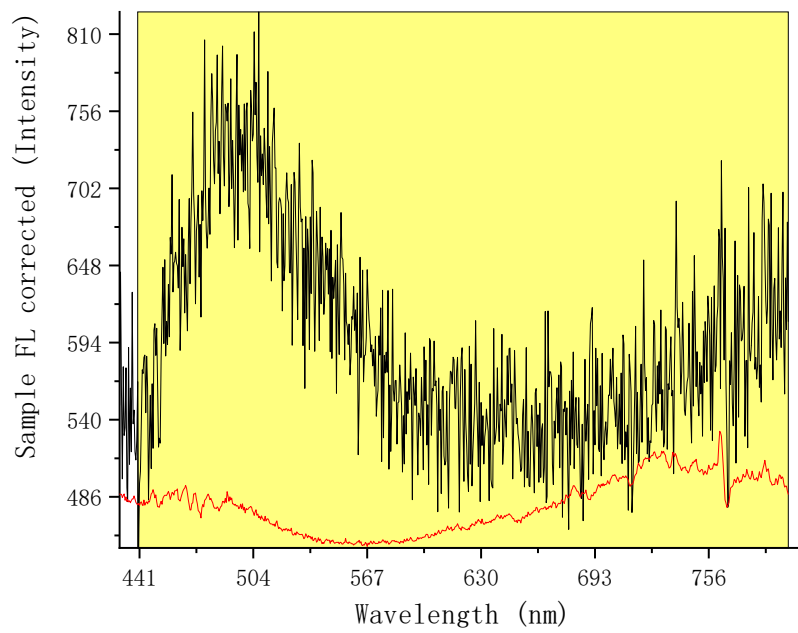


Figure SI-9. Emission fluorescence of **M5-0** in MeOH (QYs =0.92)

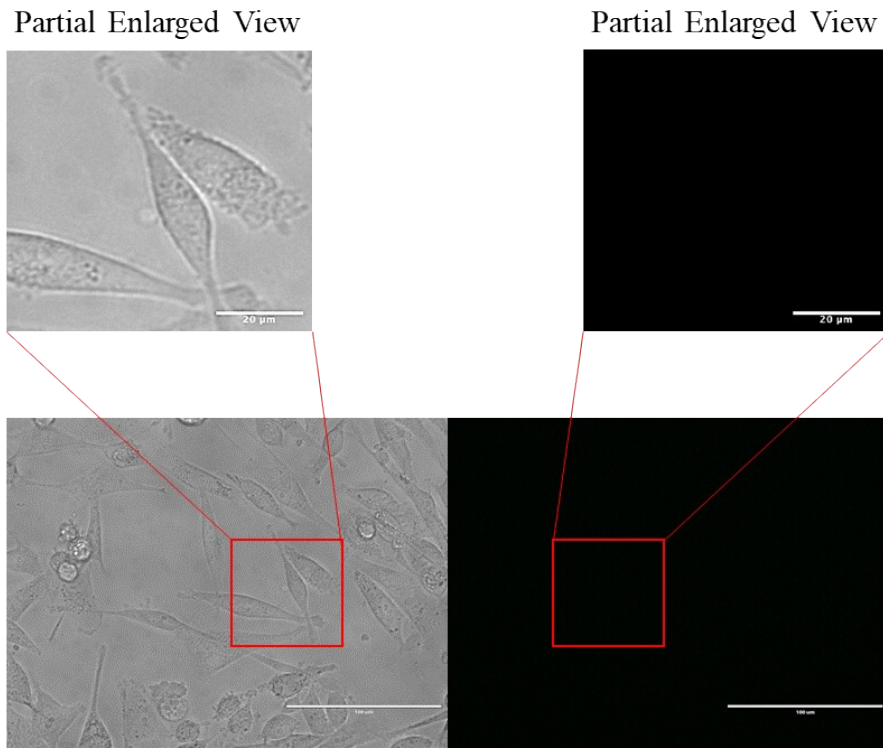


Figure SI-10. Original and partially enlarged views of fluorescence image of the probe on MDA-MB-231 (ctrl)

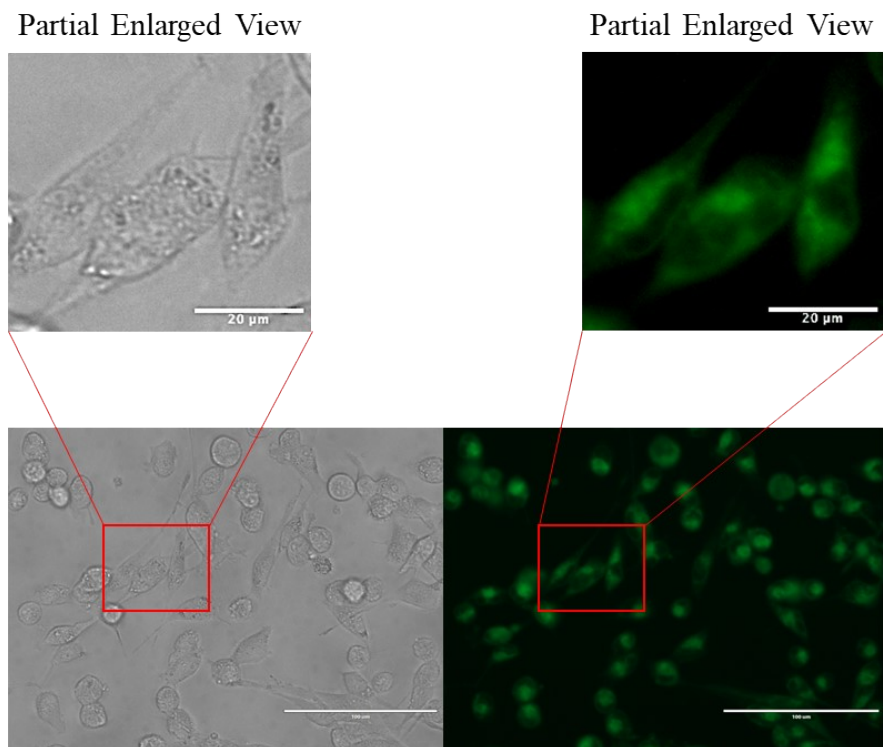


Figure SI-11. Original and partially enlarged views of fluorescence image of the probe on MDA-MB-231 (10 μM)

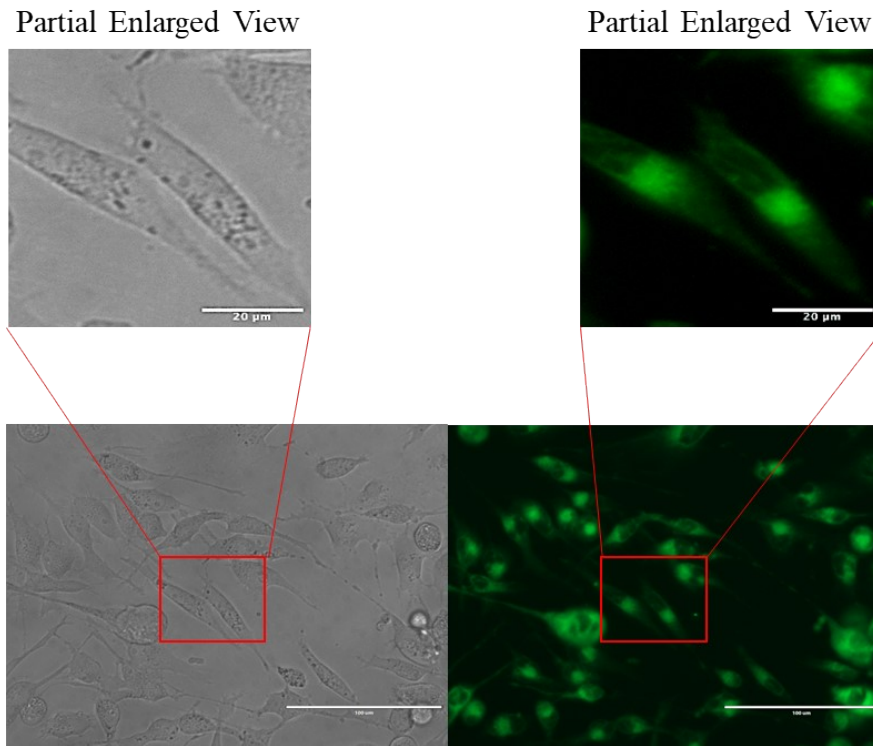


Figure SI-12. Original and partially enlarged views of fluorescence image of the probe on MDA-MB-231 (20µM)

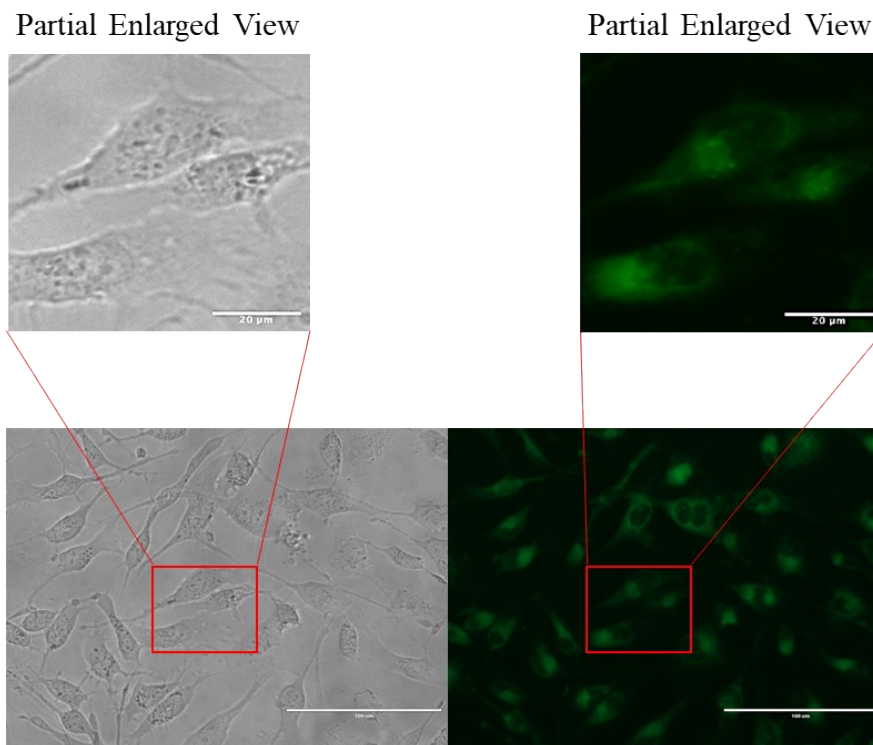


Figure SI-13. Original and partially enlarged views of fluorescence image of the probe on MDA-MB-231 (40µM)