Novel Fluorescence Probe toward Cu^{2+} Based on Fluorescein Derivatives and Its Bioimaging in Cell

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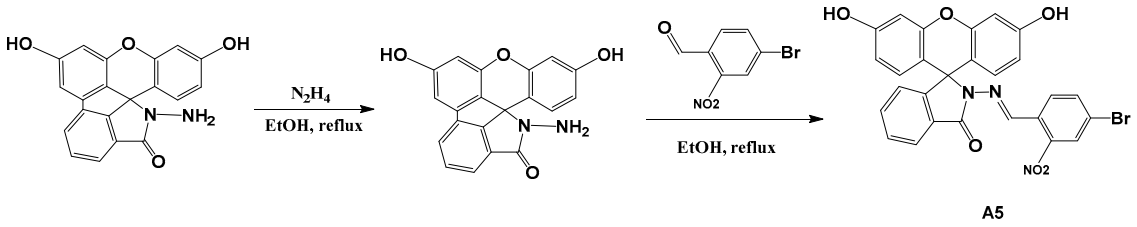
1. Synthesis of Probe A5



Scheme S1 Synthesis route of probe **A5**

Fluorescein hydrazine was synthesized from fluorescein and hydrazine according to the literature. Fluorescein hydrazine (3.39 g, 9.78 mmol) and 4-Bromo-2-nitrobenzaldehyde, (1.50 g, 6.52 mmol) were dissolved in 50 mL of ethanol, refluxed for 3 h, cooled to room temperature after the reaction. The precipitate was filtered out, washed several times with absolute ethanol, and the pale-yellow solid was obtained and placed in a dark place at 5 °C for use.

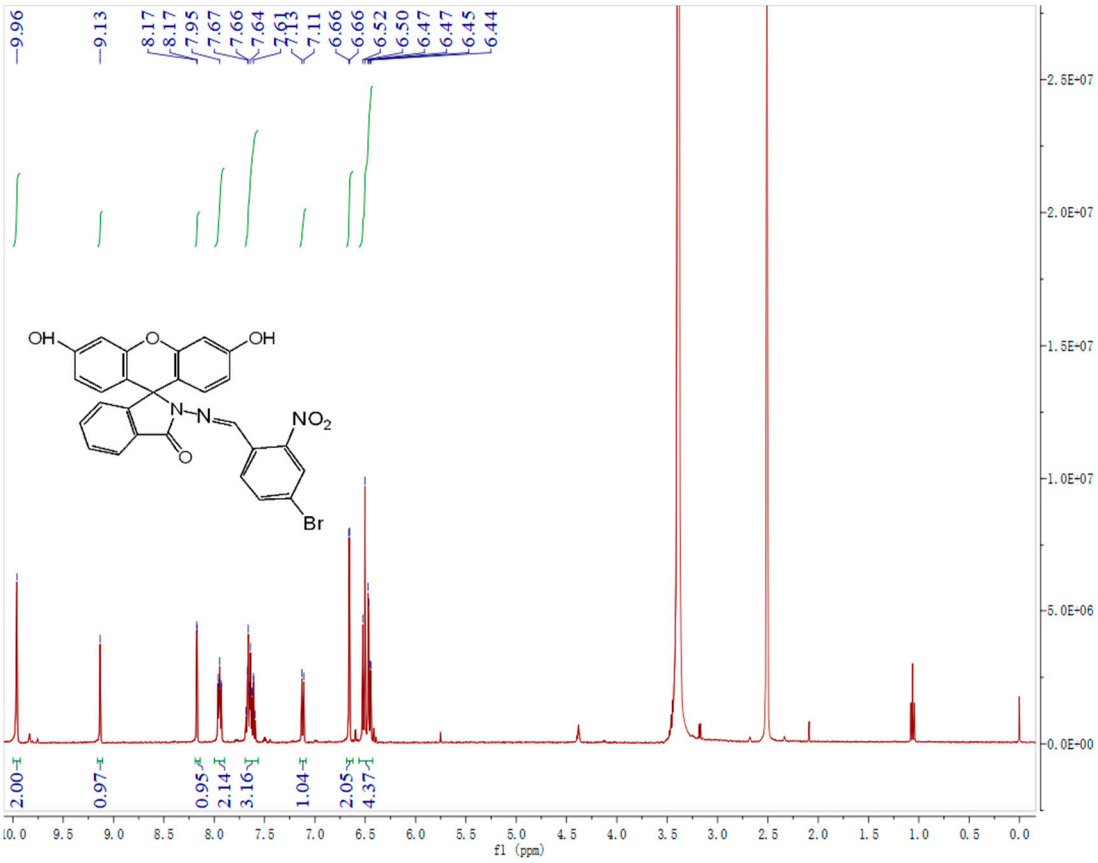


Figure S1 ^{1}H NMR spectrum of compound A5 in d_6-DMSO.

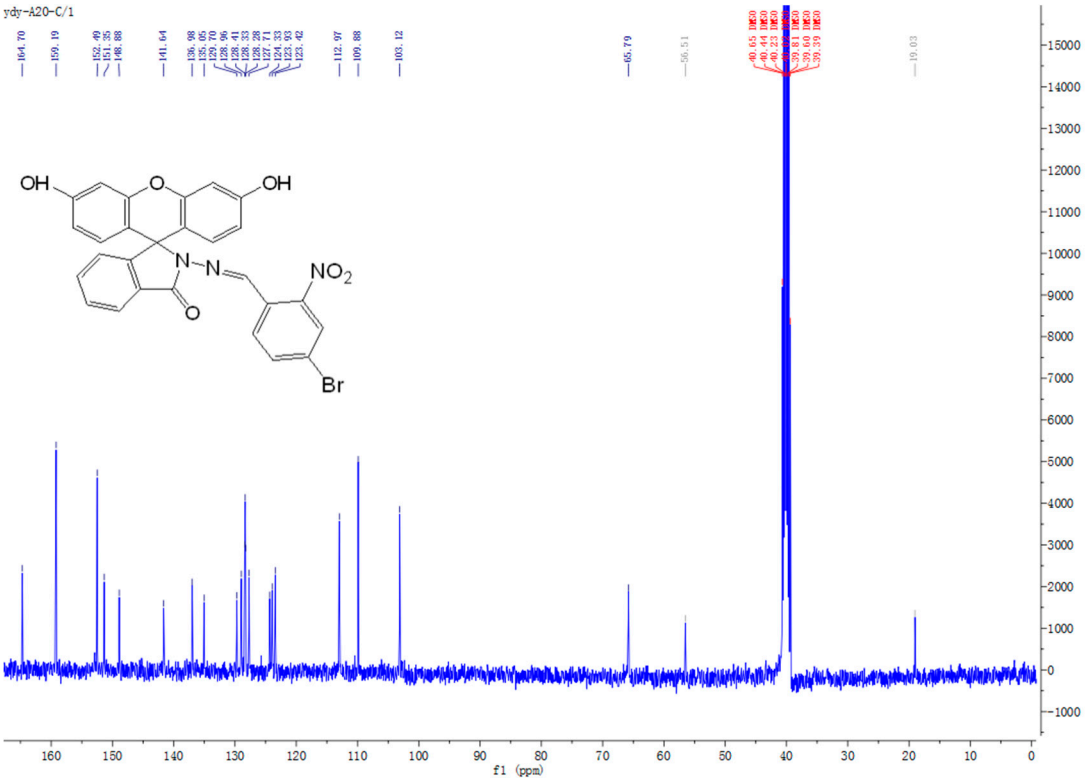


Figure S2 ^{13}C NMR spectrum of compound A5 in d_6-DMSO.

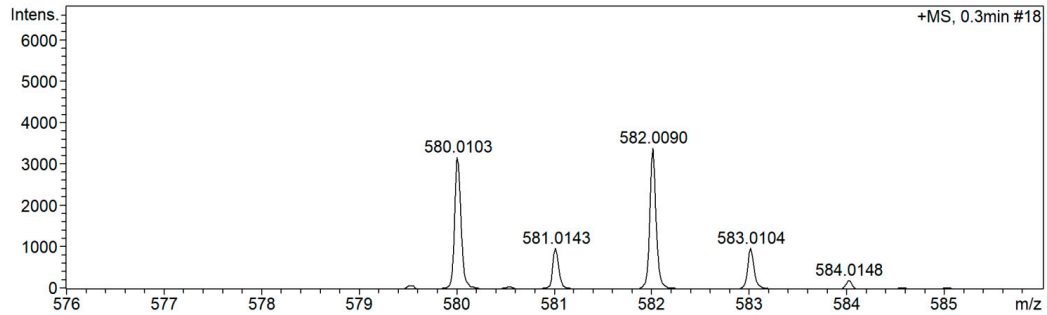
Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	2018/4/27 15:51:19
Analysis Name	C:\Users\ÀîÐËÐË\Desktop\ÐÂ½ÎÄ¼þ¼Ð\ÁõÁÖ\ÁõÁÖ\Ó«ºâËØëÂÓëÈ©ÀàµÄ·Ó¦\Ó«ºâËØëÂÓë4-äá-2-Ïõ»ù±½¼×È©·Ó¦		
Method	tune_low_2010-608.m	Operator	service
Sample Name	Id016-003	Instrument / Ser#	micrOTOF-Q II 10280
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	110.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¡¥ Conf	N-Rule
580.0103	1	C 27 H 16 Br N 3 Na O 6	100.00	580.0115	1.1	2.0	14.5	20.5	even	ok

Figure S3 ESI-MS spectrum of A5

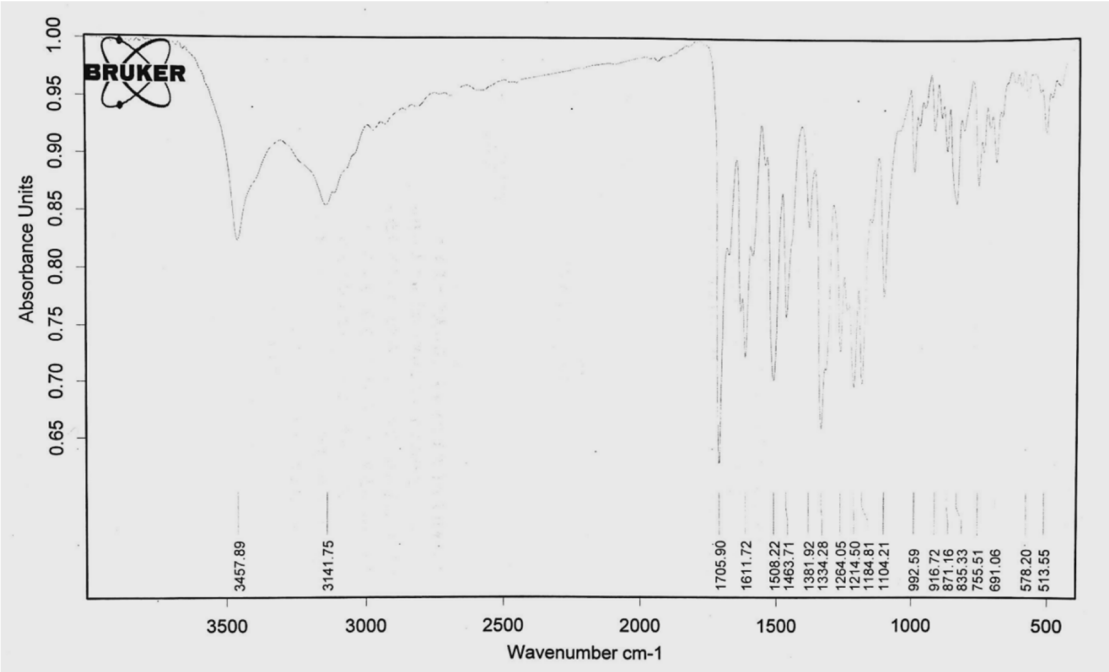


Figure S4 IR spectrum of A5

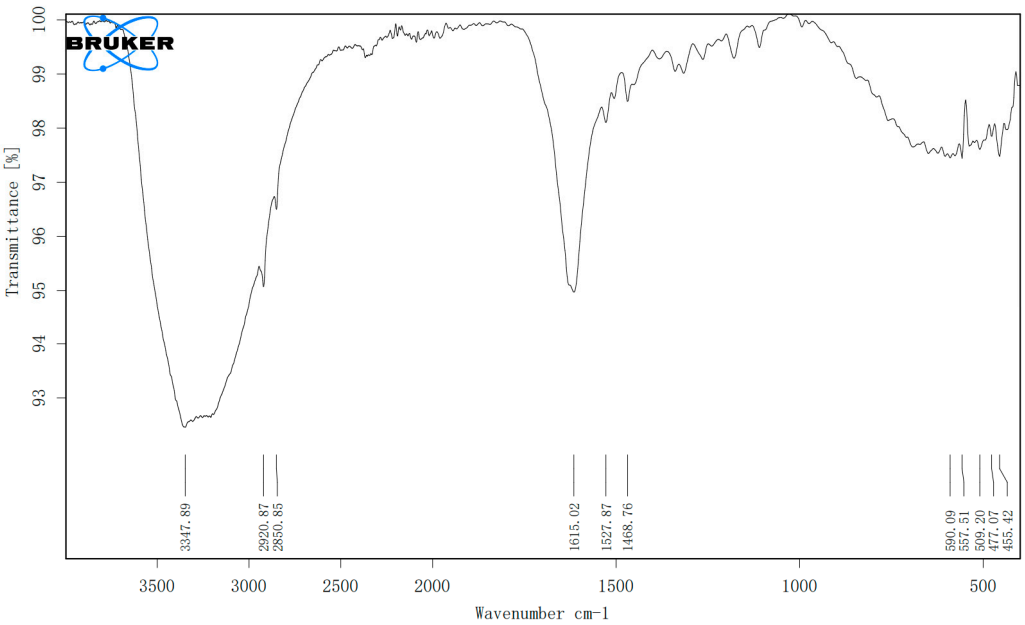


Figure S5 IR spectrum of A5 and Cu^{2+}

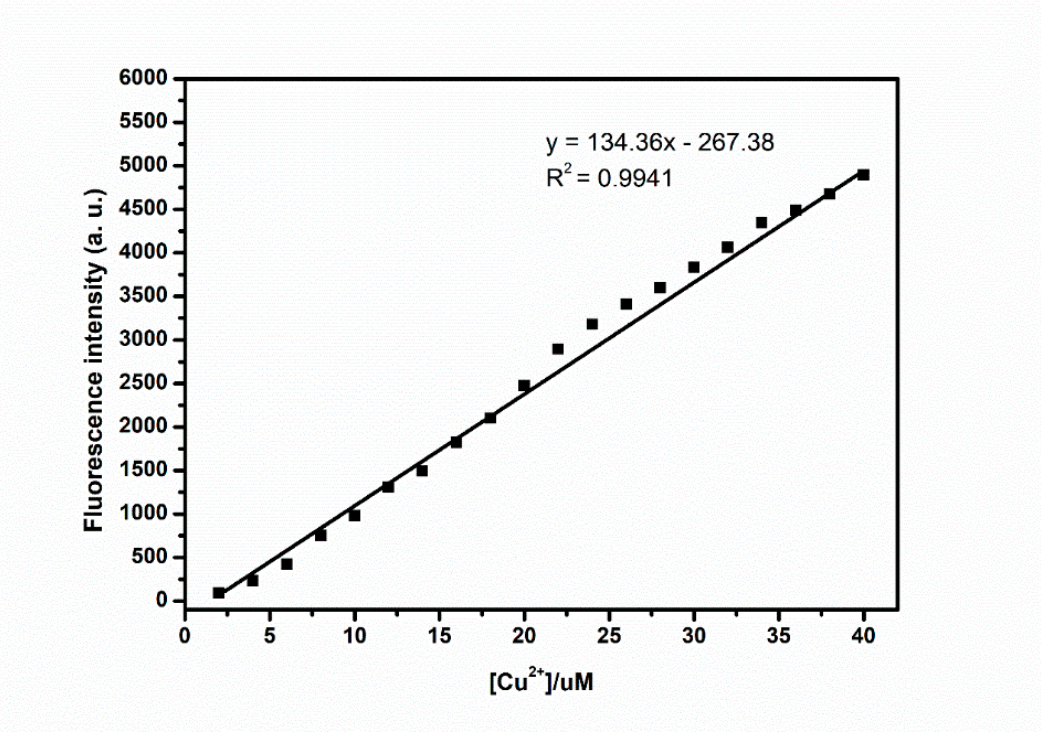


Figure S6 The linear correlation between the maximum fluorescence intensity (525 nm) and the concentration of Cu^{2+}; λex = 440 nm.

3. MTT Assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MCF-7 cells were seeded in 96-well plates at a density of 5000 cells/well. Plates were maintained at 37 ºC in a 5% CO_2/ 95% air incubator for 24 hours. Then, the culture medium in each well was replaced by fresh medium containing different concentrations of probe **A5**. After 24 hours treatment, into each well, 15μL MTT solutions (5 mg/mL in phosphate buffer solution) were added. After 4 hours incubation at 37 ºC, the absorbance of each wells at 490 nm was recorded by the Elx800 Absorbance Microplate Reader.

Table S1. The cell viability of different concentration of probe **A5**

Sample	2.5 μM/L	5 μM/L	10 μM/L	20 μM/L	40 μM/L
A5	98.7	91.0	90.7	82.0	73.3

4. Visual Detection

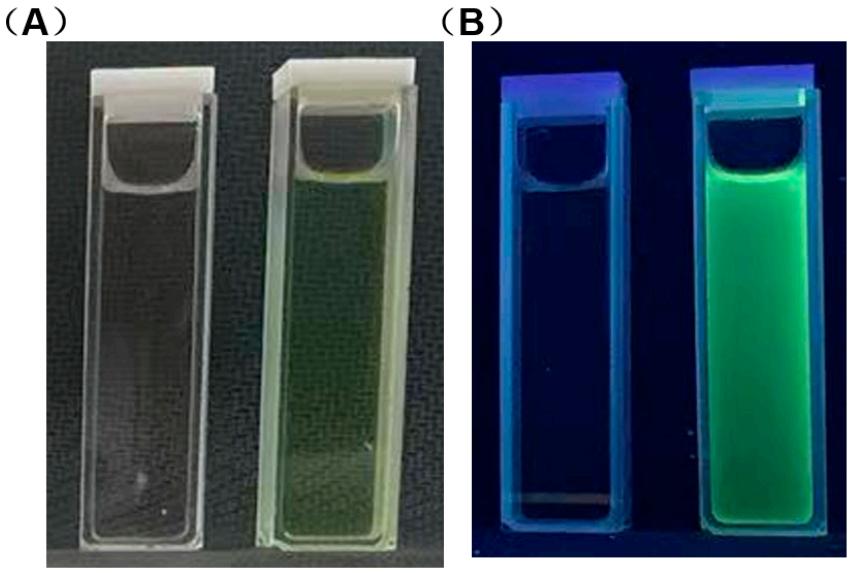


Figure S7 (A) Pictures under ambient light. (B) Pictures under 365 UV lamp.

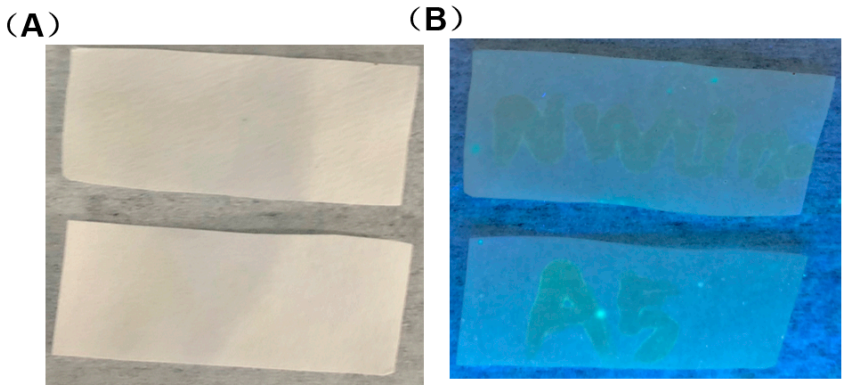


Figure S8 Photographs of A5 and after Cu^{2+} was written on a test paper, (A) Pictures under ambient light. (B) Pictures under 365 UV lamp.