A novel fluorescent probe of aluminium ions based on rhodamine derivatives and its application in biological imaging

Chun Kan*[a1]，Xing Wang [a1]，Xiaotao Shao [a] ,Linyun Wu [a], Siyan Qiu[b]and Jing Zhu[b]

[a] *College of Science, Department of Chemistry and Material Science, Nanjing Forestry University, 159 LongpanRoad, Nanjing 210037, China*
[b] *Department of Pharmacy, Jiangsu Key Laboratory for Pharmacology and Safety Evaluation of Chinese Materia Medica, Nanjing University of Chinese Medicine, 138 XianlinDadao, Nanjing 210023, China*

Contents
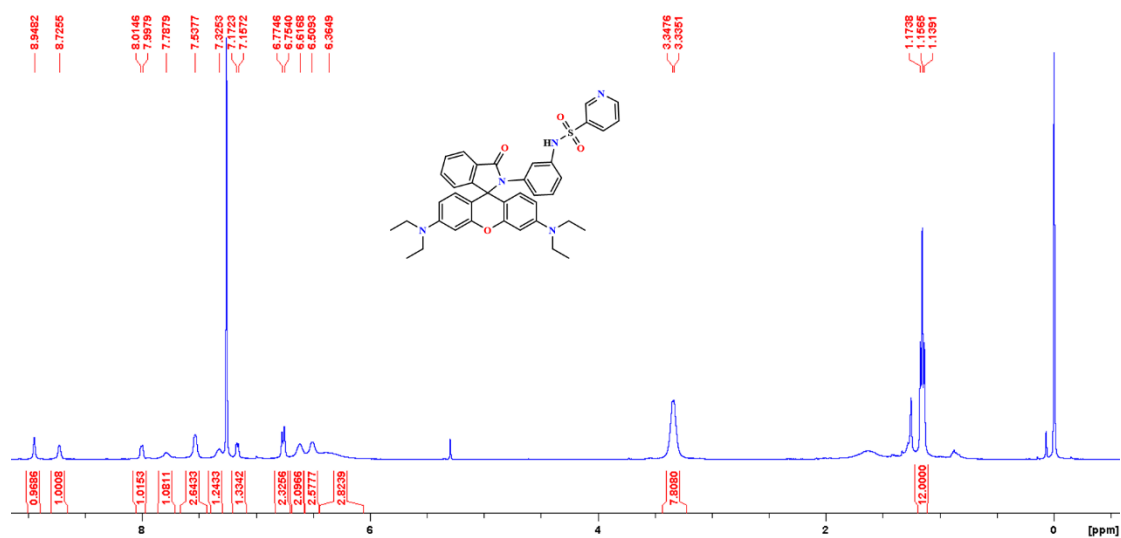
1. NMR and MS spectra

Fig. S1. ^{1}H NMR (CDCl$_3$, 400 MHz) spectra of compound **RBJF**.
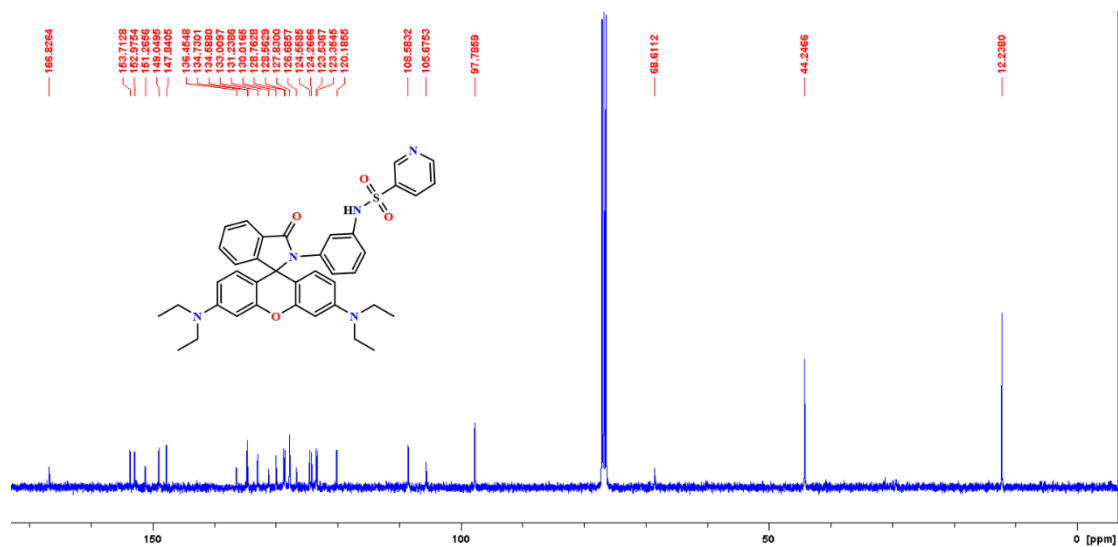

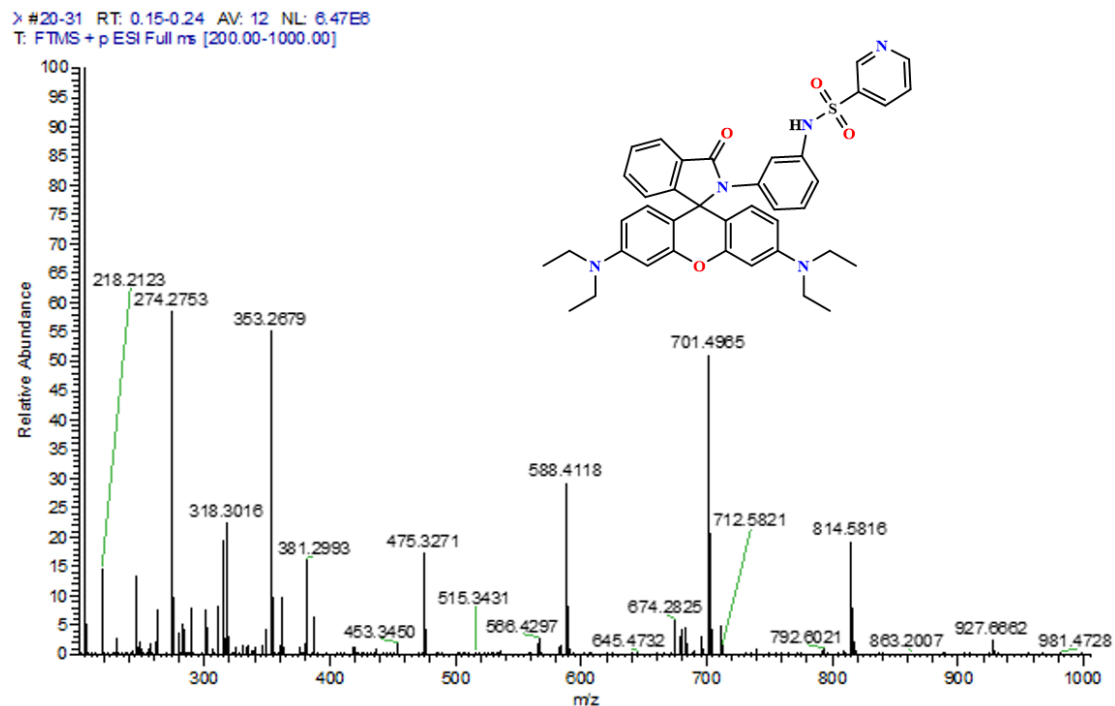Fig. S2. ^{13}C NMR (CDCl$_3$, 600 MHz) spectra of compound **RBJF**.

Fig. S3. ESI-MS spectrum of compound **RBJF**.

2.Experiment methods and graphs

Calculation of association constant

The association constant (K_a) of RBJF-Al^{3+} complex was determined by Benesi-Hildebrand Formula (1):

$$\triangle F = F - F_0 = \Delta F = \Delta F = [Al^{3+}](F_{max} - F_0)/(1/K_a + [Al^{3+}])$$

Where F is the fluorescence intensity at 582 nm upon addition of different concentration of Al^{3+}, F_0 is the fluorescence intensity at 582 nm in the absence of Al^{3+} and F_{max} is the saturated intensity at 582 nm in the presence of Al^{3+}.The association constant (Ka) was evaluated graphically by plotting $1/[F-F_0]$ against $1/[Al^{3+}]$. Linear fit to the data according to the formula **(1)**, through the slope and intercept, the binding constant of **RBJF** was calculated as $0.455* 10^4 M^{-1}$.

Determination of detection limit

According fluorescence titration experiments, we can also calculate the detection limit of **RBJF** for Al^{3+}. The fluorescence intensity of the blank samples was measured for 10 times, calculate the standard deviation of the fluorescence intensity at 582 nm. Then, make a curve based on the fluorescence intensity of **RBJF** at 582 nm and the concentration of $Al^{3+}$to obtain the slope. The detection limit was calculated according to the following formula:

$$\text{Detection limit} = \frac{3\text{SD}}{\text{S}} \qquad \text{SD} = \sqrt{\frac{1}{\text{N-1}}\sum_{i=1}^{N}(X_i - \bar{X})^2} \qquad \textbf{(2)}$$

Where SD is the standard deviation of the blank solution detected for 10 times; S is the slope of the calibration curve. Finally, the detection limit of **RBJF** is calculated to be 0.314μM.
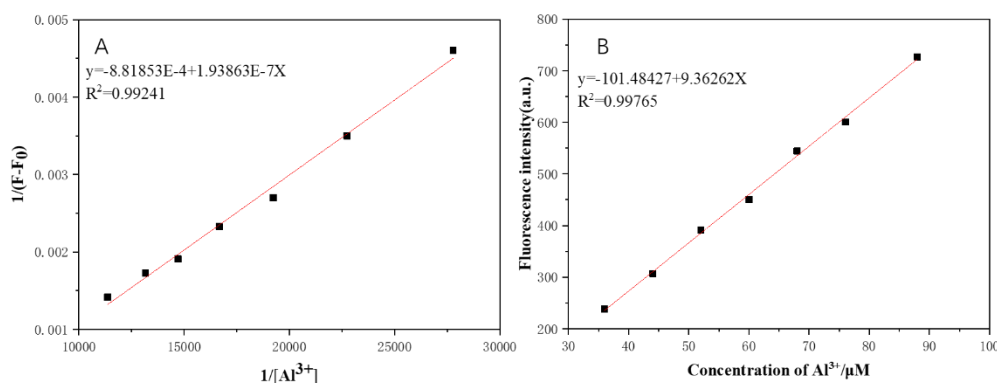
Fig. S4. (A) Benesi-Hildebrand plot (λ_{em} = 582 nm) of $1/(F-F_0)$ vs $1/[Al^{3+}]$. (B) Fluorescent intensity at 582 nm of **RBJF** (10 μM) in EtOH/H_2O solution (1:2, v/v) with different amounts of Al^{3+}. The excitation wavelength is 520 nm.

MTT method

MTT method was used to perform cytotoxicity test to detect cell survival and growth. We used human breast cancer cells (MCF-7) as the experimental cells. MCF-7 cells were placed in a 96-well plate and placed in atmosphere at 310.15K and 5% CO_2 for 12 hours. Take the supernatant and add probe culture solution of different concentrations, then take the supernatant and add 5mg/mL MTT stock solution and leave it for 4h. Finally, take the supernatant again, add 150 μL DMSO to each well plate. The absorbance of each well was measured at 550 nm using a microplate reader (Bio-Rad, Model 550). The experiment was repeated three times to get the average value. We selected human breast cancer cell (MCF-7) cells as the cells used for imaging experiments. MCF-7 cells were placed in high glucose medium supplemented with 10% FBS (fetal bovine serum), 100 units/mL penicillin and 100 units/mL streptomycin temperature streptomycin in high glucose medium. The temperature was 310.15K, and the culture environment was in an air atmosphere of 5% CO_2. MCF-7 cells incubated in the incubator were seeded in 24-well plates at 1.5×10^5 cells per well. Six hours after the probe **RBJF** was added, the cells were washed three times with PBS and live-cell imaging was performed. In addition, pretreatment with 20 μM Al^{3+} for 1h, and then adding 10 μM probe **RBJF** for 5h as a control group. Fluorescence images of cells were recorded under an inverted fluorescent microscope.
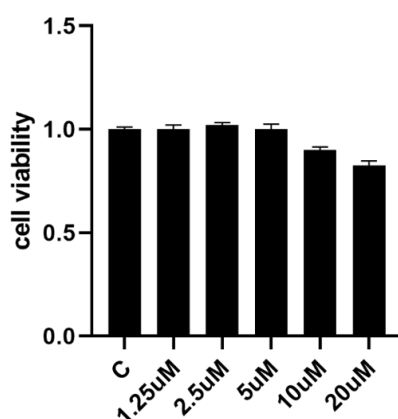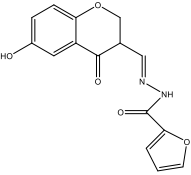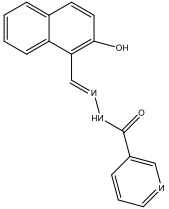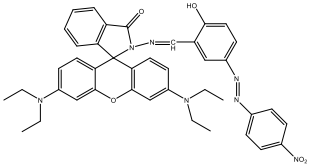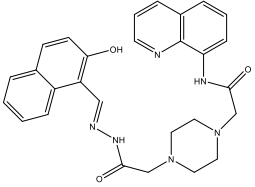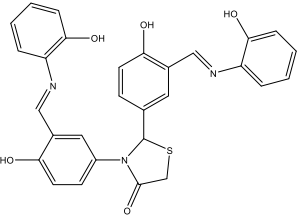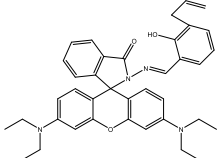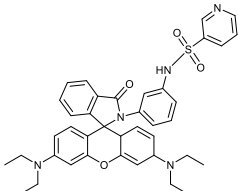

Fig. S5. Cytotoxicity assay of probe RBJF for MCF-7 cells by the MTT test. MCF-7 cells were respectively cultured in the presence of different concentrations of RBJF (1.25, 2.5, 5, 10, and 20 μM) at 310.15K for 24h. For the control group, MCF-7 cells were incubated under the same conditions but without the probe RBJF.

Compared the LOD

Probe structures	Reagents	Detection Limit / μM	References
	EtOH	0.0387	C. R. Li, J. C. Qin, B. D. Wang, L. Fan, J. Yan and Z. Y. Yang, J Fluoresc, 2016, 26, 345-353.
	near 100% aqueous media (Tris–HCl, pH 7.2)	0.4	J.-c. Qin, Z.-y. Yang and P. Yang, Inorganica Chimica Acta, 2015, 432, 136-141.
	EtOH-H$_2$O (4:1, v/v)	0.11	S. Mabhai, M. Dolai, S. Dey, A. Dhara, B. Das and A. Jana, New Journal of Chemistry, 2018, 42, 10191-10201.
	DMF/H$_2$O (1/9, v/v)	0.0367	S. Zeng, S. J. Li, X. J. Sun, M. Q. Li, Y. Q. Ma, Z. Y. Xing and J. L. Li, Spectrochim Acta A Mol Biomol Spectrosc, 2018, 205, 276-286.
	ACN/H$_2$O (1/1, v/v)	4.4	D. Aydin, E. Karakilic, S. Karakurt and A. Baran, Spectrochim Acta A Mol Biomol Spectrosc, 2020, 238, 118431.
	H$_2$O: CH$_3$CN (3:7, v/v)	0.572	A. Rai, A. K. Singh, K. Tripathi, A. K. Sonkar, B. S. Chauhan, S. Srikrishna, T. D. James and L. Mishra, Sensors and Actuators B: Chemical, 2018, 266, 95-105.
	MeOH/H$_2$O (1:1, v/v)	0.314	This work