Electronic Supplementary Material (ESI) for Analytical Methods.

A fluorescent probe for rapid detection of low concentration mercury ions and its application in biological cells

Chun Kan*[a1], Xing Wang [a1], Linyun Wu [a], Xiaotao Shao [a], Haizhu Xing[b], Min You[b]and Jing Zhu[b]

College of Science, Department of Chemistry and Material Science, Nanjing Forestry University, 159 LongpanRoad, Nanjing 210037, China

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

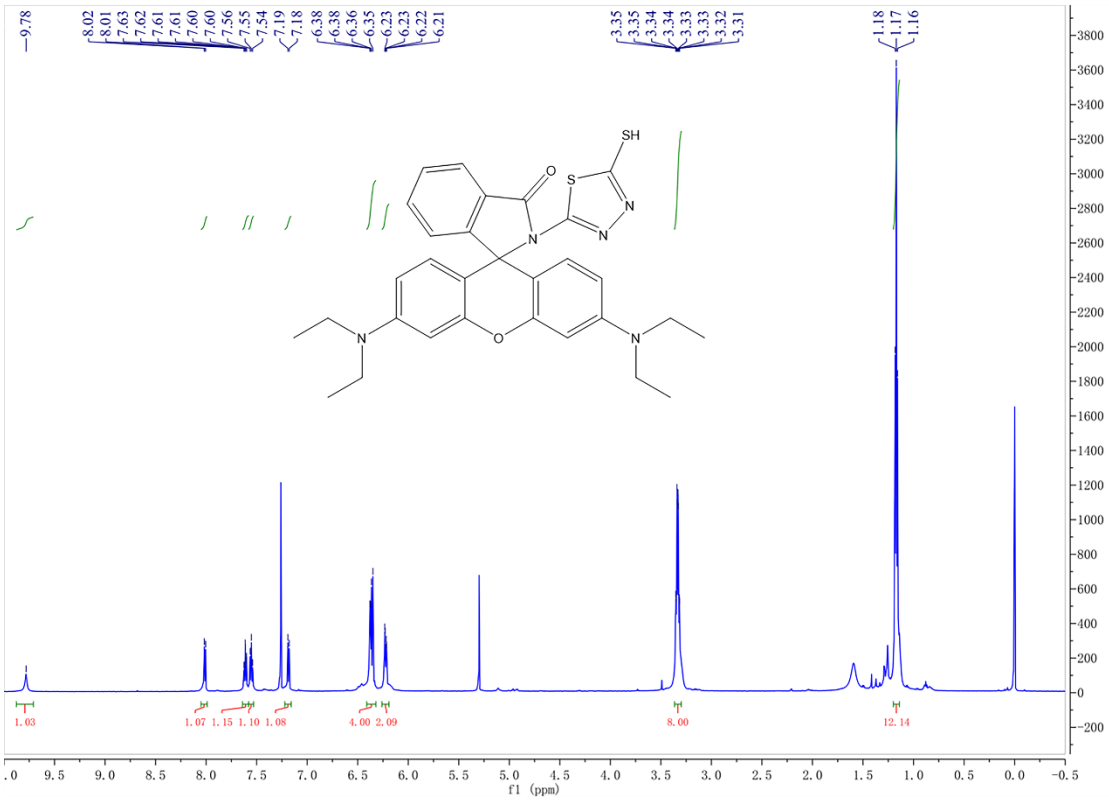


Fig. S1. ^{1}H NMR ($CDCl_3$, 600 MHz) spectra of compound RBSH.

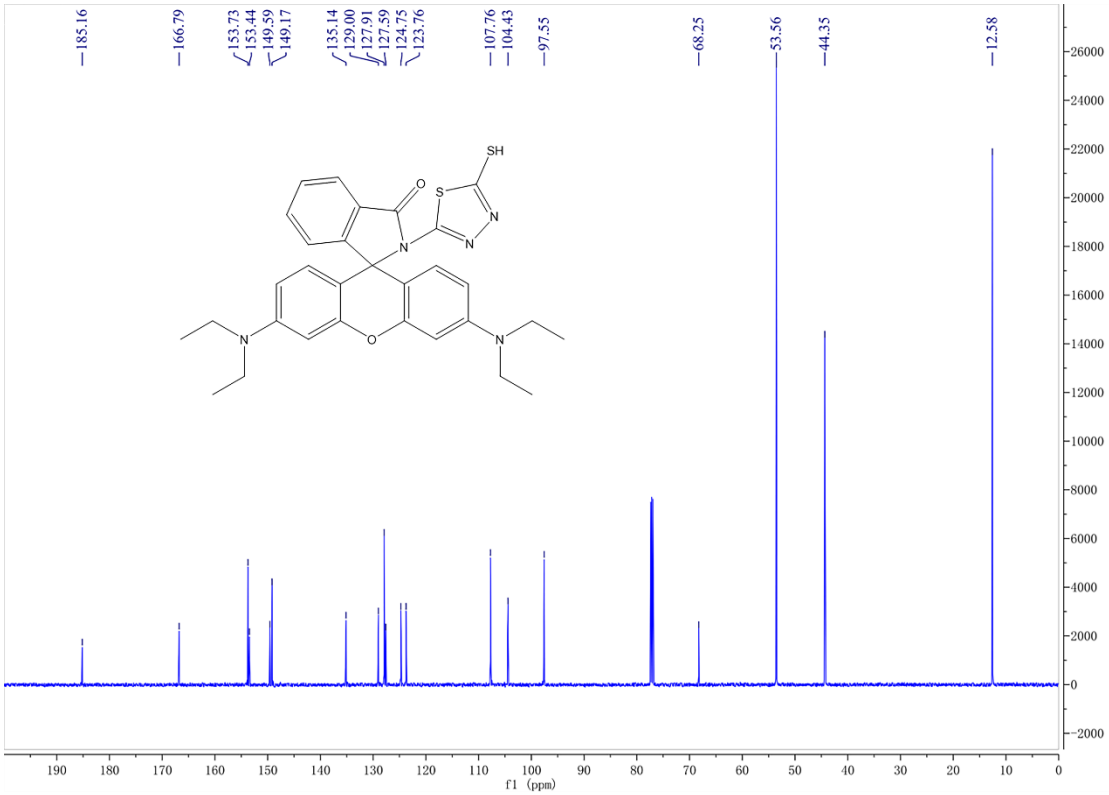


Fig. S2. ^{13}C NMR ($CDCl_3$, 600 MHz) spectra of compound RBSH.

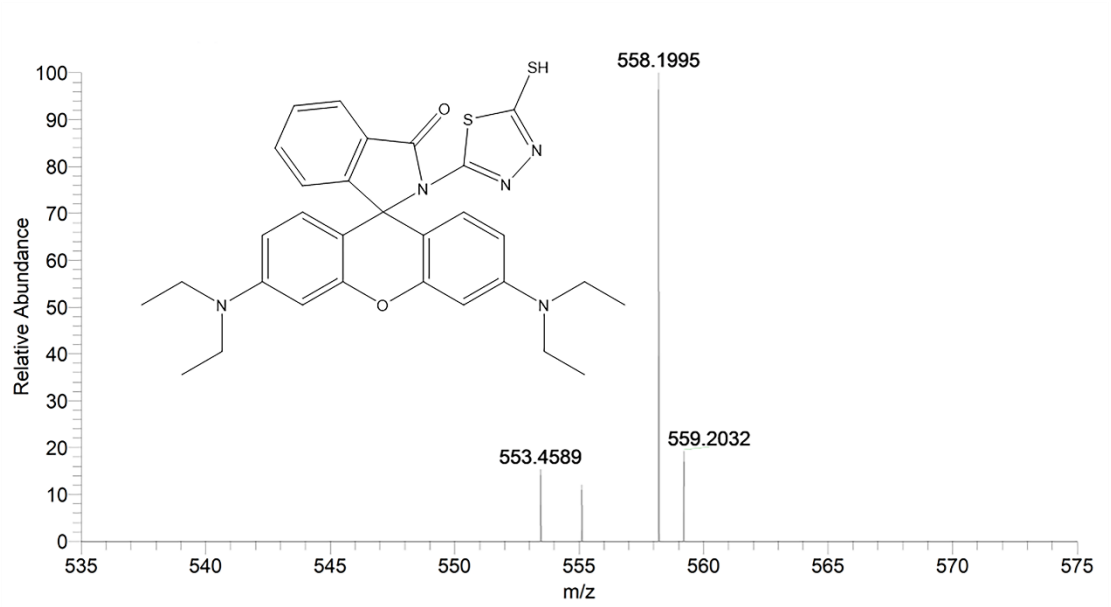


Fig. S3. ESI-MS spectrum of compound RBSH.

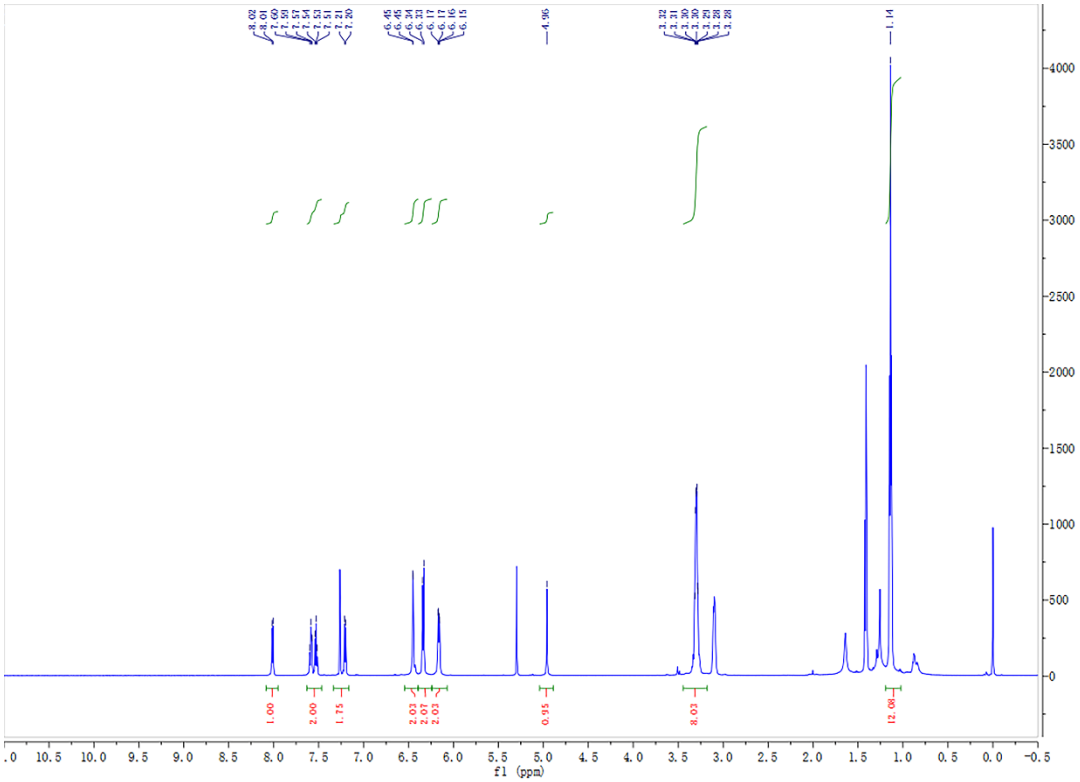


Fig. S4. ^{1}H NMR ($CDCl_3$, 600 MHz) spectra of compound RBSH+Hg^{2+}.

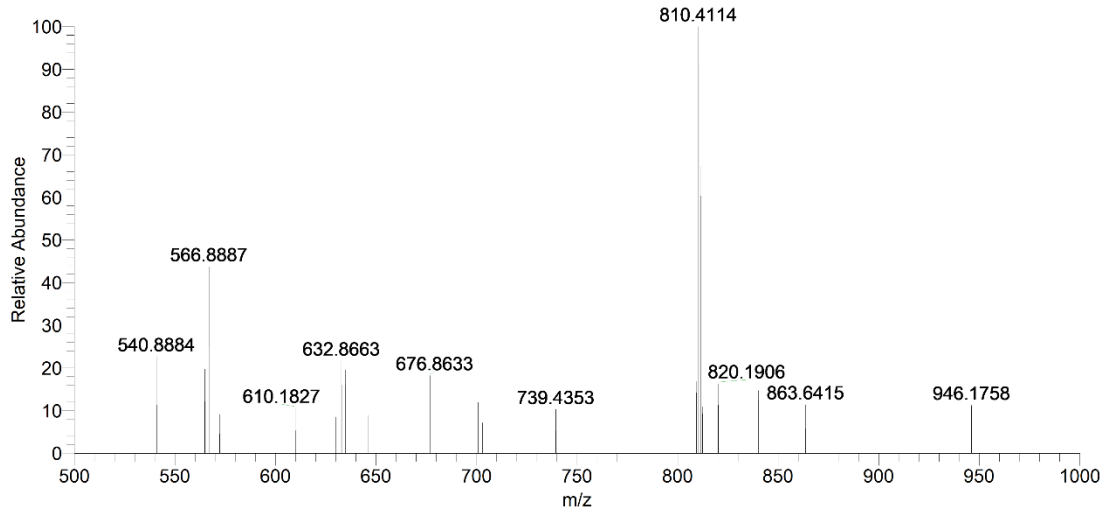


Fig. S5. ESI-MS spectrum of compound [L + CH_3COOHg^++H^+].

2. Experiment methods and graphs

Calculation of association constant

The association constant (K_a) of RBSH- Hg^{2+} complex was determined by Benesi-Hildebrand Formula (1):

$$\Delta F = F - F_0 = \Delta F = [Hg^{2+}](F_{max} - F_0)/\left(\frac{1}{K_a} + [Hg^{2+}]\right)$$

Where F is the fluorescence intensity at 592 nm upon addition of different concentration of Hg^{2+}, F_0 is the fluorescence intensity at 592 nm in the absence of Hg^{2+} and F_{max} is the saturated intensity at 582 nm in the presence of Hg^{2++}.The association constant (Ka) was evaluated graphically by plotting 1/[F-F_0] against 1/ [Hg^{2+}]. Linear fit to the data according to the formula **(1)**, through the slope and intercept, the binding constant of **RBSH** was calculated as 1.658*10^5 M^{-1}.

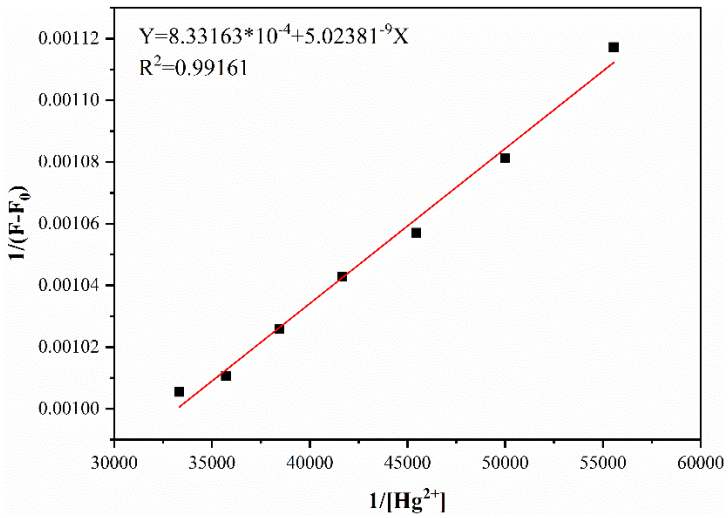


Fig. S6. Binding constant.

Determination of detection limit

According fluorescence titration experiments, we can also calculate the detection limit of **RBSH** for Hg^{2+}. The fluorescence intensity of the blank samples was measured for 10 times, calculate the standard deviation of the fluorescence intensity at 592 nm. Then, make a curve based on the fluorescence intensity of **RBSH** at 592 nm and the concentration of $Hg^{2+}$to obtain the slope. The detection limit was calculated according to the following formula:

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

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 **RBSH** is calculated to be 5.9 nM.

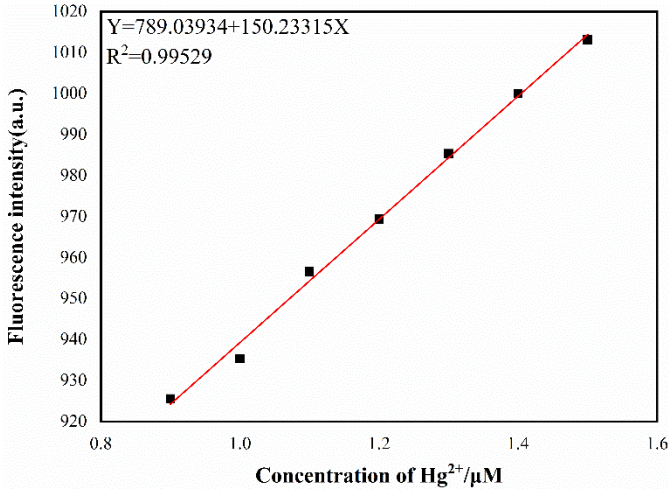


Fig. S7. Minimum detection limit

MTT method

MTT method was used to perform cytotoxicity test to detect cell survival and growth. We used Hela cells as the experimental cells. Hela cells were placed in a 96-well plate and placed in atmosphere at 37 °C 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 150μL 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 Hela cells as the cells used for imaging experiments. Hela 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 37°C, and the culture environment was in an air atmosphere of 5% CO_2. Hela cells incubated in the incubator were seeded in 24-well plates at 1.5×10^5 cells per well. Six hours after the probe **RBSH** was added, the cells were washed three times with PBS and live-cell imaging was performed. In addition, pretreatment with 10 μM Hg^{2+} for 1h, and then adding 10 μM probe **RBSH** for 5h as a control group. Fluorescence images of cells were recorded under an inverted fluorescent microscope.

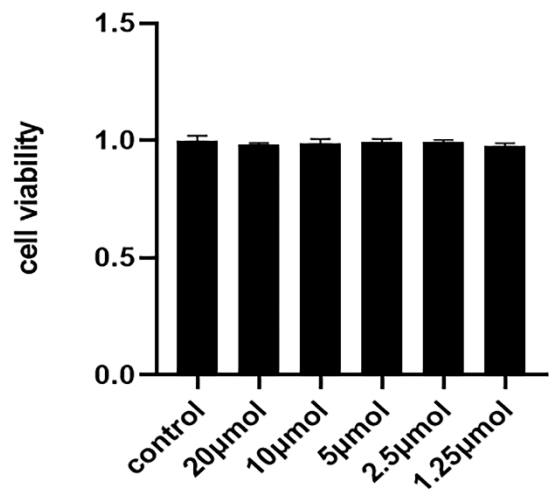
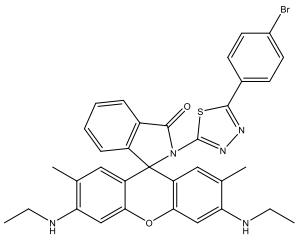
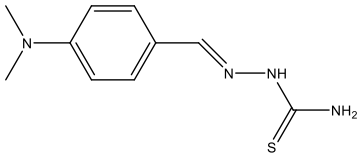
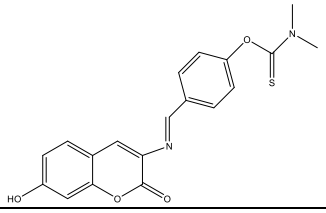
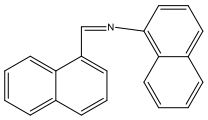
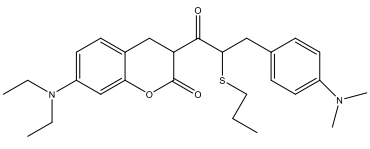


Fig. S8. Cytotoxicity assay of probe RBSH for Hela cells by the MTT test. Hela cells were respectively cultured in the presence of different concentrations of RBSH (1.25, 2.5, 5, 10, and 20μM) at 37°C for 24h. For the control group, HELA cells were incubated under the same conditions but without the probe RBSH.

Mercury ion probe comparison

Probe structures	Reagents	Detection Limit / nM	References
	THF: H_2O (8:2, v/v)	30.37	B. D. Vanjare, P. G. Mahajan, H.-I. Ryoo, N. C. Dige, N. G. Choi, Y. Han, S. J. Kim, C.-H. Kim and K. H. Lee, Sensors and Actuators B: Chemical, 2021, 330.
	0.01 M acetic acid/ sodium acetate buffer	0.77*10^-3	Y. Yu, L. R. Lin, K. B. Yang, X. Zhong, R. B. Huang and L. S. Zheng, Talanta, 2006, 69, 103-106.
	DMSO/H_2O (1:99, v/v)	4.5*10^-3	O. García-Beltrán, A. Rodríguez, A. Trujillo, A. Cañete, P. Aguirre, S. Gallego-Quintero, M. T. Nuñez and M. E. Aliaga, Tetrahedron Letters, 2015, 56, 5761-5766.
	DMSO	55.95	T.-b. Wei, G.-y. Gao, W.-j. Qu, B.-b. Shi, Q. Lin, H. Yao and Y.-m. Zhang, Sensors and Actuators B: Chemical, 2014, 199, 142-147.
	EtOH	0.5	W. Sheng, Y. Yu, N. Gao, M. Jin, L. Wang, N. Li, C. Li, H. Zhang, Y. Zhang and K. Liu, Anal Methods, 2021, 13, 1043-1048.

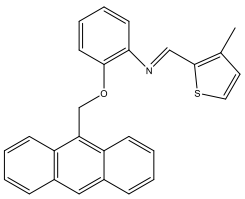
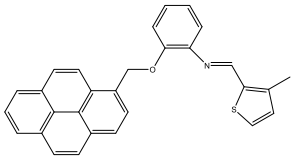
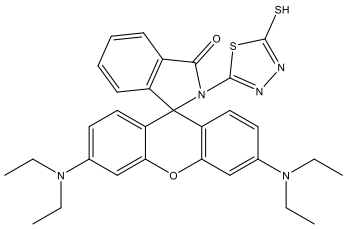
	H_2O: DMSO (1:2, v/v)	12.22	S. O. Tümay and S. Yeşilot, Journal of Photochemistry and Photobiology A: Chemistry, 2021, 407.
	H_2O: DMSO (1:2, v/v)	8.32	S. O. Tümay and S. Yeşilot, Journal of Photochemistry and Photobiology A: Chemistry, 2021, 407.
	EtOH/H_2O (1:3, v/v)	5.9	This work

Table S1 LOD comparison