

# Synthesis and Analytical Application of a Novel Fluorescent Hg<sup>2+</sup> Probe 3', 6'-Bis(Diethylamino)-2-((2,4-Dimethoxybenzylidene) Amino)Spiro[Isoindoline-1,9'-Xanthene]-3-Thione

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**Abstract** A novel probe, 3',6' - bis(diethylamino) -2- ((2,4-dimethoxybenzylidene)amino) spiro [isoindoline-1,9'-xanthene]-3-thione (RBS), was designed and synthesized. Its structure was characterized with elemental analysis, IR spectra and <sup>1</sup>H NMR. The probe displayed highly selective and sensitive recognition of Hg<sup>2+</sup>. Reacting with mercury ions in aqueous solution, its fluorescence intensity was enhanced significantly, while its color was changed from colorless to pink. So, a new fluorescence method of detection of Hg<sup>2+</sup> was proposed. Its dynamic response concentration range and detection limit for Hg<sup>2+</sup> were 5.00 × 10<sup>-9</sup> M to 1.00 × 10<sup>-6</sup> M detected and 1.83 × 10<sup>-9</sup> M, respectively. Satisfying results were obtained when the probe was applied to detect spiked Hg<sup>2+</sup> in samples.

**Keywords** Fluorescent probe · Mercury ions · 3',6'-bis(diethylamino)-2-((2,4-dimethoxybenzylidene) amino)spiro[isoindoline-1,9'-xanthene]-3-thione · Fluorescent spectrometry

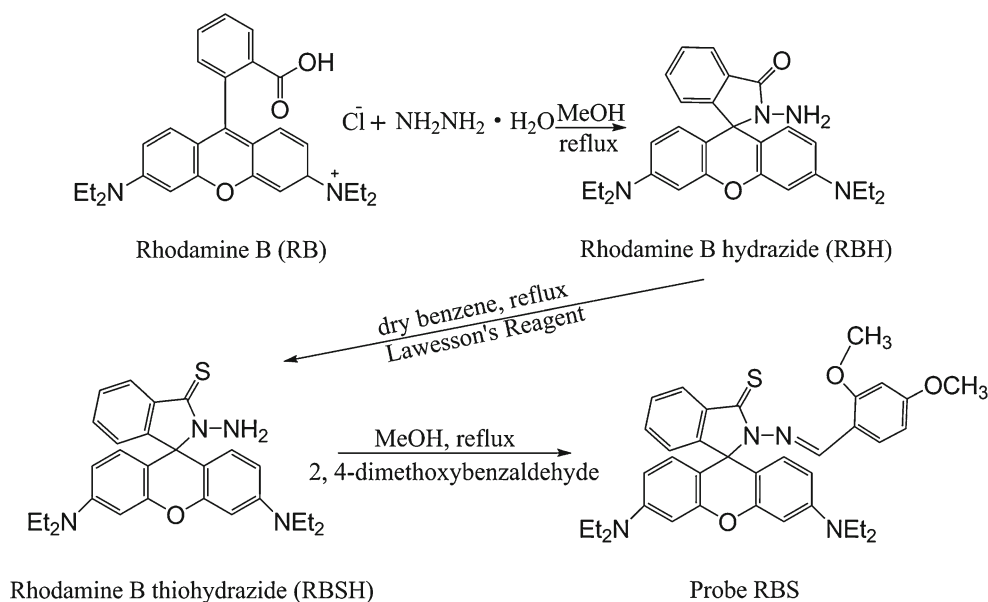
## Introduction

Mercury is a heavy metal element of strong biological toxicity [1] on earth. It can cause environmental and many human health problems, including neurological problems [2], myocardial infarction [3] and a possible involvement in the

development of some kinds of autism health problems [4]. Consequently, monitoring Hg<sup>2+</sup> in environment is of great significance. Many methods for detection of mercury have been reported including atomic absorption or emission spectroscopy [5, 6], voltammetry [7, 8], inductively coupled plasma mass spectrometry [9], gas chromatography method [10] and chemiluminescence method [11] etc. However, most of them need complex sample-preparation steps or require expensive equipment. Spectrofluorimetric method is an excellent approach because it is less labor-intensive and highly sensitive [12–18]. The focal point of the method is seeking highly selective and sensitive fluorogenic probe. Even though a number of Hg<sup>2+</sup> responsible fluorescent probes have been explored, higher selective and sensitive systems are still desired. Rhodamine derivative is an excellent fluorescent dye and widely used in the determination of metal ions and biological active substances [19, 20]. However, some disadvantage, such as interference from other metal ions, may seriously hamper their practicability in the detection of Hg<sup>2+</sup>. Some attractive fluorescent probes of rhodamine derivative have been designed and synthesized recently [21, 22]. A double bond of C=S was introduced to substitute for carbonyl group of rhodamine group in the probes. It increased the detection selectivity of probe because of the strong thiophilic affinity of Hg<sup>2+</sup>. We introduced 2, 4-dimethoxybenzaldehyde on the basis of rhodamine B thiohydrazide. With this modified moiety of the molecule, a new probe higher sensitive and selective was synthesized for determination of Hg<sup>2+</sup>. The new probe was nonfluorescent itself, however it exerts strong fluorescence when it reacts with Hg<sup>2+</sup> and the intensity of the fluorescence signal rises dramatically with increasing Hg<sup>2+</sup> concentration. It displayed an excellent selectivity and a high sensitivity toward the

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**Scheme 1** The structure and synthesis route of Compound 1



detection of  $\text{Hg}^{2+}$  in aqueous media. The probe was applied to determine  $\text{Hg}^{2+}$  in tap water, river water and soil samples. Satisfactory results were obtained.

## Experimental

### Apparatus and Reagents

The Rhodamine B (RB), Lawesson's Reagent (2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide) were purchased from Aladdin (Shan Hai) and used without further purification. The 2,4-dimethoxybenzaldehyde was supplied by ACROS and used as received. Benzene was further treated by sodium chips and then distilled. All of other solvents and ion salt reagents used were of analytical reagent grade. Doubly distilled water was used throughout all experiments. Rhodamine B hydrazide and Rhodamine B thiohydrazide were prepared according to a minor modification of the literature [21, 23] and their spectroscopic and physical properties are consistent with published data, respectively.

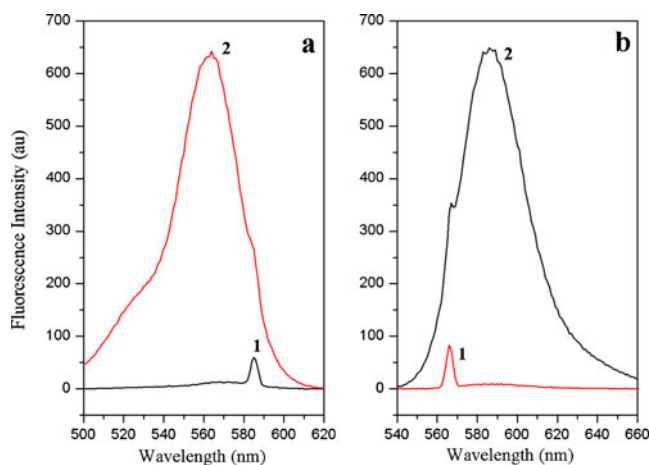
A RF-5301PC spectrofluorimeter (Shimadzu, Japan) equipped with a xenon lamp and a 1-cm quartz cell was used for fluorescence measurements. A pH-3CT digital pH meter (Shanghai Da Zhong Device Works, China) with a combined glass-calomel electrode was used for pH measurements. The  $^1\text{H}$  NMR was obtained in  $\text{CDCl}_3$  on Varian Mercury Plus 400 MHz NMR spectrometer using TMS as an internal standard. Elemental analysis and IR spectra were performed on a Vario EL III elementary analytical meter (Elementar, Germany) and Nicolet 380 IR spectrometer (Thermo, America), respectively. Melting point was measured with an Fb62 Digital Melting Point Apparatus

(Shanghai METTLER TOLEDO Instruments Corporation, China).

### Synthesis and Characterization of 3',6'-Bis(Diethylamino)-2-((2,4-Dimethoxybenzylidene) Amino)Spiro[Isosindoline-1,9'-Xanthene]-3-Thione

The fluorescent probe was synthesized according to Scheme 1.

Rhodamine B hydrazide (RBH) was synthesized by the literature [23]. Rhodamine B thiohydrazide (RBSH) was prepared according to a minor modification of the literature [21]. Rhodamine B hydrazide (2.28 g, 5.0 mmol) and Lawesson's Reagent (2.03 g, 5.0 mmol) were dissolved in



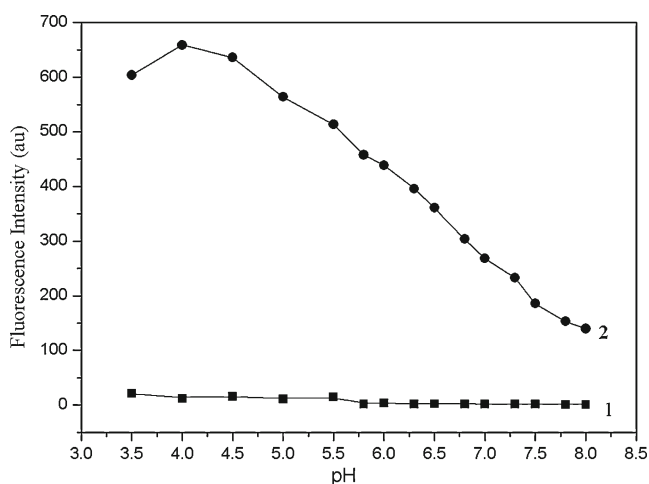
**Fig. 1** Excitation and emission spectra of the probe reagent. **a** excitation spectra ( $\lambda_{em}=585$  nm), **b** emission spectra ( $\lambda_{ex}=565$  nm), 1 buffer solution + RBS; 2 buffer solution + RBS +  $\text{Hg}^{2+}$ ; Buffer solution (HAc-NaAc), 0.20 M; pH, 4.00; slit width, 3 nm; t, 25 min; RBS,  $1.00 \times 10^{-6}$  M;  $\text{Hg}^{2+}$ ,  $1.00 \times 10^{-6}$  M

60 mL dry benzene, and the reaction mixture was refluxed for 24 h under  $N_2$  atmosphere and then concentrated by evaporation. The crude product was purified by silica gel column chromatography with  $CH_2Cl_2$ /petroleum ether (4/1, v/v), affording RBS as a light purple solid 0.54 g, yield 24.3 %.  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ (ppm): 8.09 (d, 1H, Ar-H), 7.46 (m, 2H, Ar-H), 7.10 (d, 1H, Ar-H), 6.43 (s, 2H, xanthene-H), 6.35 (d, 2H, xanthene-H), 6.27 (m, 2H, xanthene-H), 4.81 (s, 2H,  $NH_2$ ), 3.34 (q, 8H,  $NCH_2CH_3$ ), 1.16 (t, 12H,  $NCH_2CH_3$ ); elemental analysis (%) for  $C_{28}H_{32}N_4OS$ (calc.): C, 71.15 (71.24); H, 6.82 (6.86); N (11.77), 11.85. The data were in agreement with the reported values.

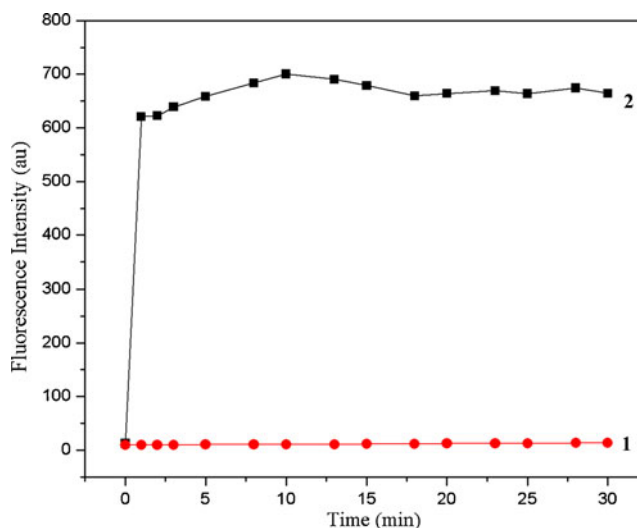
Synthesis of Compound RBS follows. 2,4-dimethoxybenzaldehyde (0.0664 g, 0.4 mmol) and Rhodamine B thiohydrazone (0.20 g, 0.4 mmol) were mixed in methanol and stirred for 10 min. Then the reaction mixture was heated to reflux, and 3 drops of acetic acid were added. After refluxing for 6 h, yellow precipitates obtained were filtered off, recrystallized by ethanol and dried under vacuum. Yield: 0.1207 g (48.67 %). Melting point: 265.1 °C;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$ (ppm): 8.92(s, 1H,  $CH=N$ ), 8.11 (d, 1H, Ar-H), 8.00 (m, 1H, Ar-H), 7.39(2H, Ar-H), 7.12 (d, 1H, Ar-H), 6.80 (d, 2H, Ar-H), 6.44 (d, 2H, xanthene-H), 6.30 (m, 4H, xanthene-H), 3.85 (s, 3H,  $OCH_3$ ), 3.82 (s, 3H,  $OCH_3$ ), 3.33 (q, 8H,  $NCH_2CH_3$ ), 1.15 (t, 12H,  $NCH_2CH_3$ ); elemental analysis (%) for  $C_{37}H_{40}N_4O_3S$ (calc.): C 71.52 (71.58), H, 6.49 (6.53), N (9.02) 9.11.

#### Preparation of Stock Solution

$2.00 \times 10^{-6}$  M stock solution of RBS was prepared in N,N-dimethylformamide (DMF).  $1.00 \times 10^{-3}$  M  $Hg^{2+}$  stock solution was prepared by dissolving 0.0318 g  $Hg(Ac)_2$  in 100 mL of 3 % nitric acid dilute solution. The standard



**Fig. 2** Effect of pH on the fluorescence intensity pH, 3.00–8.00; other conditions as in Fig. 1

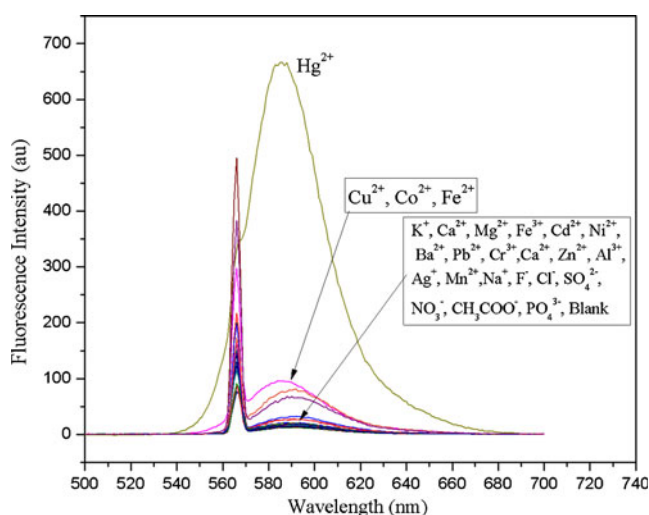


**Fig. 3** Effect of reaction time on the fluorescence intensity 1 buffer solution + RBS; 2 buffer solution + RBS +  $Hg^{2+}$ ; t, 0–30 min; other conditions as in Fig. 1

solution of  $Hg^{2+}$  was obtained by serial dilution of  $1.00 \times 10^{-3}$  M  $Hg(Ac)_2$  solution with doubly distilled water. A stock buffer solution of HAc-NaAc (pH 3.50–5.50, 0.20 M) and  $NaH_2PO_4$ - $Na_2HPO_4$  (pH 5.80–8.00, 0.02 M) were prepared for determining experiment. All stock solutions above were stored at 4 °C.

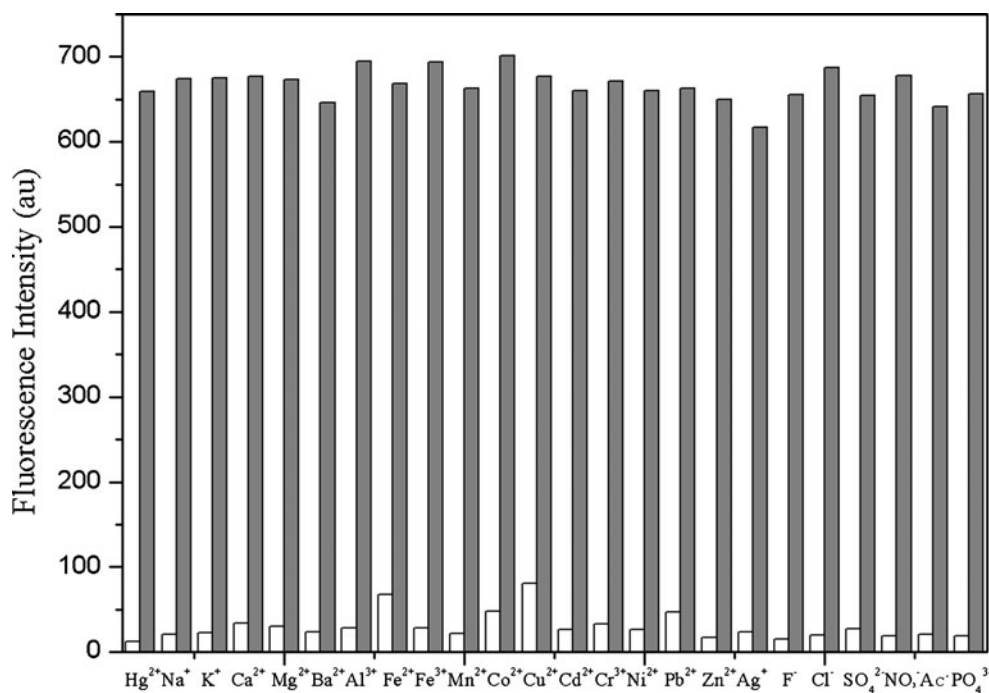
#### Preparation of Tap Water Sample, River Water Sample and Soil Sample

Tap water sample and river water sample were collected from laboratory and the Nai River in Taian city, filtered with 45  $\mu m$  apertures filter membrane and then spiked with



**Fig. 4** Fluorescence spectra of RBS in DMF- $H_2O$ (1:1, v/v) with the presence of various ions background ions,  $4.00 \times 10^{-5}$  M and  $1.00 \times 10^{-4}$  M; other conditions as in Fig. 1

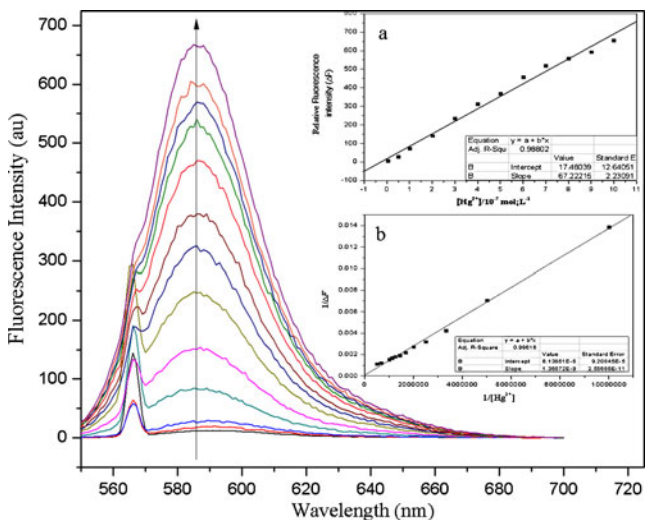
**Fig. 5** Fluorescence intensity of RBS upon the addition of  $1.00 \times 10^{-6}$  M  $\text{Hg}^{2+}$  in the presence of background ions in DMF- $\text{H}_2\text{O}$ (1:1, v/v) background ions,  $4.00 \times 10^{-5}$  M and  $1.00 \times 10^{-4}$  M; other conditions as in Fig. 1



different concentration of  $\text{Hg}^{2+}$  solution to the final concentration  $3.00 \times 10^{-7}$  M,  $6.00 \times 10^{-7}$  M and  $8.00 \times 10^{-7}$  M. The soil used was collected from flower bed of Shandong Agricultural University (China). It was dried for 4 h in a programmable hot air oven at  $110^\circ\text{C}$  to make the water content less than 5 %, milled into a powder and then sifted with sieve of 100–200 mesh. Three 10.0 g samples were mixed 1 h with 3.0 mL, 6 mL and 8 mL of  $1.0 \times 10^{-5}$  M  $\text{Hg}^{2+}$  standard solution, respectively. Then 30 mL aqua regia

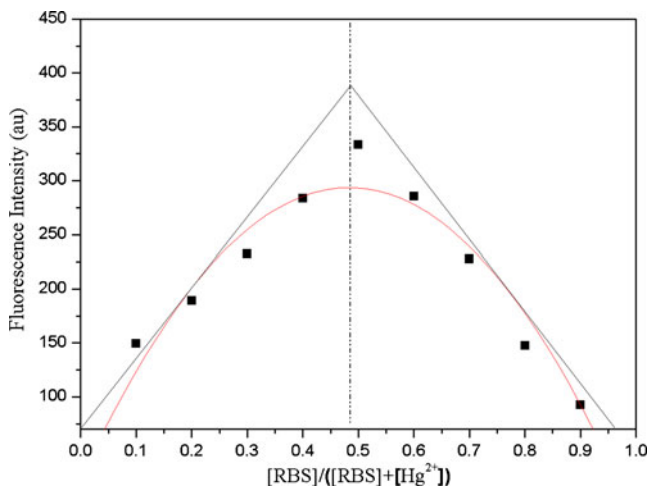
was added slowly. The mixtures were boiled for 2 h and stirred overnight. Subsequently they were filtered through a  $45\ \mu\text{m}$  filter. The filtrates were diluted to 100 mL with double distilled water. The test samples were obtained.

Preparation of National Environmental Standard Sample (Water-Mercury (Numbers: GSBZ 50016–90, Batch Number: 202030))

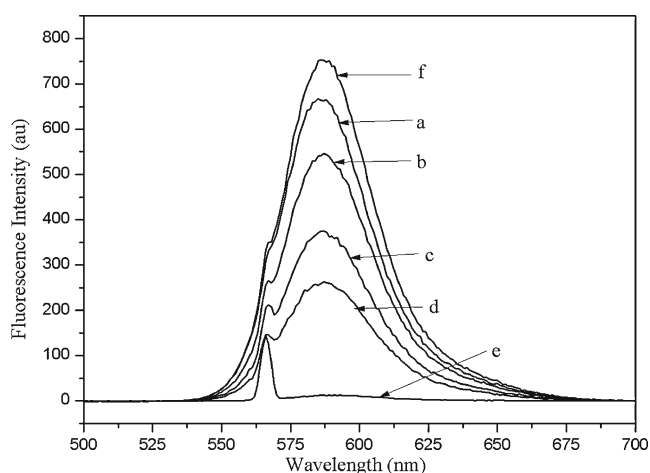


**Fig. 6** Fluorescence spectra of RBS upon the addition of  $\text{Hg}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $5.00 \times 10^{-9}$  M and  $1.00 \times 10^{-6}$  M; other conditions as in Fig. 1. Inset: **a** the plot of fluorescent emission intensity at 585 nm as a function of  $\text{Hg}^{2+}$  concentration; **b** the Benesi-Hildebrand plot of RBS upon the addition of  $\text{Hg}^{2+}$

The ampoule was opened just before use, and 10.00 mL solution was transferred to a 250 mL volumetric flask and diluted to 250 mL with 3 % nitric acid solution.



**Fig. 7** Job's plot of RBS and  $\text{Hg}^{2+}$ . The total concentration of RBS and  $\text{Hg}^{2+}$  was kept at a fixed  $1.00 \times 10^{-6}$  M; other conditions as in Fig. 1



**Fig. 8** Reversible titration response of RBS to  $\text{Hg}^{2+}$  in the DMF/ $\text{H}_2\text{O}$  (V/V, 1/1) solution. a: RBS ( $1.00 \times 10^{-6}$  M) with  $\text{Hg}^{2+}$  ( $1.00 \times 10^{-6}$  M); b: RBS ( $1.00 \times 10^{-6}$  M) with  $\text{Hg}^{2+}$  ( $1.00 \times 10^{-6}$  M) and then addition of KI ( $2.00 \times 10^{-6}$  M); c: RBS ( $1.00 \times 10^{-6}$  M) with  $\text{Hg}^{2+}$  ( $1.00 \times 10^{-6}$  M) and then addition of KI ( $4.00 \times 10^{-6}$  M); d: RBS ( $1.00 \times 10^{-6}$  M) with  $\text{Hg}^{2+}$  ( $1.00 \times 10^{-6}$  M) and then addition of KI ( $6.00 \times 10^{-6}$  M); e: RBS ( $1.00 \times 10^{-6}$  M); f: RBS ( $1.00 \times 10^{-6}$  M) with  $\text{Hg}^{2+}$  ( $1.00 \times 10^{-6}$  M), then addition of KI ( $2.00 \times 10^{-6}$  M) and then addition of  $\text{Hg}^{2+}$  ( $1.00 \times 10^{-6}$  M) once again; other conditions as in Fig. 1

## Experimental Procedure

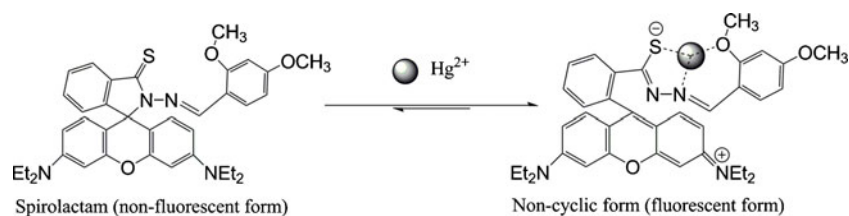
To 10 mL colorimetric cylinder containing 5.00 mL  $2.00 \times 10^{-6}$  M DMF solution of RBS, appropriate volumes of the standard solution of  $\text{Hg}^{2+}$  was added directly with a transfer pipette. The solutions were diluted with buffer solution to 10 mL and then equilibrated within 25 min. And then the fluorescence spectra were measured immediately with 1.00 cm quartz cell. Excitation and emission slits were set to 3 nm.

## Results and Discussion

### Design and Synthesis of Compound 1

Probe RBS is a schiff base including a rhodamine B thiohydrazide group and an acylhydrazone block. Initial considerations for RBS acting as an  $\text{Hg}^{2+}$ -selective fluorescent chemosensor are as follows: (1) the rhodamine framework is an ideal mode for constructing OFF-ON fluorescent and chromophoric chemosensors because of its particular structural

**Scheme 2** The proposed binding mechanism of RBS with  $\text{Hg}^{2+}$



property; (2) a sulfur-based functional group should be considered and introduced because of sulphophile of  $\text{Hg}^{2+}$ ; (3) the structure of schiff base gives a strong coordination affinity for some specific metal ions; (4)  $-\text{NH}_2$  replaced by schiff base containing benzaldehyde derivative group which include two electron-donating group ( $-\text{OCH}_3$ ) affording N, S, and 2,4-dimethoxybenzaldehyde  $-\text{O}$  atoms as the  $\text{Hg}^{2+}$  binding site that might reduce the response time and change the sensing behavior.

### Excitation and Emission Spectra

RBS, as a turn-on fluorescent probe, was almost non-fluorescence in DMF- $\text{H}_2\text{O}$  (1:1, v/v) solution at pH 4.00. On treatment with  $\text{Hg}^{2+}$  ions, a strong yellow fluorescence emerged. It can be ascribed to the delocalized xantheno moiety of rhodamine. As shown in Fig. 1, the optimum excitation and emission wavelengths were 565 and 585 nm, respectively. In addition, the solution changes from colorless to pink-red during the process. Its intensity increased with increasing of the  $\text{Hg}^{2+}$  concentration. The phenomenon indicated that the probe can also be used for visual technique to detect  $\text{Hg}^{2+}$ .

### Effect of pH

As shown in Fig. 2, the pH titration experiment was exhibited. The fluorescence intensity of RBS displayed weak and steady signals in the pH range from 3.00 to 8.00. But the fluorescence intensity of RBS-mercury complex displayed a maximum signal toward the  $\text{Hg}^{2+}$  under pH 4.00. As the pH value increasing continued, the fluorescence intensity decreased. It may be the reason that the hydrolysis results in the actual concentration of  $\text{Hg}^{2+}$  in the sample solution reduction. By comprehensive consideration of the influence of every factor, further fluorescent studies were performed in HAc-NaAc buffer solution (pH 4.00, 0.20 M).

### Effect of Reaction Time

As the data shown in Fig. 3, effect of reaction time was investigated. The recognition interaction was almost complete within 5 min and the color of RBS solution changed into pink immediately after adding appropriate concentration of  $\text{Hg}^{2+}$ . The fluorescence intensity got the maximum

**Table 1** Recovery study of spiked  $\text{Hg}^{2+}$  in practical samples

Sample	$\text{Hg}^{2+}$ added ( $\text{mol}\cdot\text{L}^{-1}$ )	$\text{Hg}^{2+}$ founded ( $\text{mol}\cdot\text{L}^{-1}$ )	Recovery (%)	RSD (% , $n=10$ )
Tap water	0	Not detected	–	–
	$3.00\times 10^{-7}$	$3.18\times 10^{-7}$	105.8	1.91
	$6.00\times 10^{-7}$	$6.39\times 10^{-7}$	106.6	
	$8.00\times 10^{-7}$	$8.17\times 10^{-7}$	102.1	
River water	0	Not detected	–	–
	$3.00\times 10^{-7}$	$3.09\times 10^{-7}$	103.1	1.43
	$6.00\times 10^{-7}$	$6.58\times 10^{-7}$	109.7	
	$8.00\times 10^{-7}$	$8.05\times 10^{-7}$	100.6	
Soil sample	0	Not detected	–	–
	$3.00\times 10^{-7}$	$2.99\times 10^{-7}$	99.9	2.94
	$6.00\times 10^{-7}$	$6.47\times 10^{-7}$	107.9	
	$8.00\times 10^{-7}$	$7.98\times 10^{-7}$	99.8	

value at 10 min, remained constant after that. So the reaction time was selected as 25 min.

### The Selectivity of RBS

High selectivity is an important factor of probe towards analyte. The result of the selectivity test was shown in Fig. 4. When some other ions, such as alkali or alkaline-earth metal ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Ba}^{2+}$ ) ( $1.00\times 10^{-4}$  M), heavy and transition metal ions ( $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Mn}^{2+}$ ) ( $4.00\times 10^{-5}$  M), other metal ion ( $\text{Al}^{3+}$ ) ( $1.00\times 10^{-4}$  M) and anions ( $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{PO}_4^{3-}$ ) ( $1.00\times 10^{-4}$  M) were added to the solution of RBS under the same conditions, they cannot induce any obvious color change and fluorescent enhancement, only  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Fe}^{2+}$  caused the ignored fluorescence increase. From the above experimental results, it demonstrated that RBS was a selective probe for  $\text{Hg}^{2+}$  in aqueous condition.

### The Competition Experiments of RBS for $\text{Hg}^{2+}$

The result of the competition experiments of RBS for  $\text{Hg}^{2+}$  was shown in Fig. 5. The competition experiments were also carried by adding  $\text{Hg}^{2+}$  to the solution of RBS in the presence of other ions, such as alkali or alkaline-earth metal ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Ba}^{2+}$ ) ( $1.00\times 10^{-4}$  M), heavy and transition metal ions ( $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Mn}^{2+}$ ) ( $4.00\times 10^{-5}$  M), other metal ion ( $\text{Al}^{3+}$ ) ( $1.00\times 10^{-4}$  M) and anions ( $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{PO}_4^{3-}$ ) ( $1.00\times 10^{-4}$  M). From the above experimental results, it revealed that  $\text{Hg}^{2+}$ -induced fluorescence enhancement was not significantly interfered by these metal ions and anions mentioned above. The effect of  $\text{Hg}_2^{2+}$  on the detection of  $\text{Hg}^{2+}$  has been studied, too. Unfortunately,  $\text{Hg}_2^{2+}$  has a significant influence on the measurement results of  $\text{Hg}^{2+}$ . The existence of  $\text{Hg}_2^{2+}$  leads

to fluorescence increase obviously. This interference may be due to the decomposition of  $\text{Hg}_2^{2+}$  into liquid Hg and  $\text{Hg}^{2+}$  compounds on exposure to water.

### Linear Range and Association Constant

The fluorescence spectra of RBS in the presence of different concentrations of  $\text{Hg}^{2+}$  in DMF- $\text{H}_2\text{O}$  (V/V, 1/1) solution were recorded as shown in Fig. 6. When excited at 565 nm, free RBS exhibits very weak fluorescence due to its spirocyclic structure. The addition of different concentrations  $\text{Hg}^{2+}$  led to significant fluorescence emission enhancement (up to 80-fold enhancement) peaking at 585 nm, due to the delocalized xanthene moiety of RBS. There was a good linear relationship with a correlation coefficient 0.994 between the fluorescence intensity and the concentration of  $\text{Hg}^{2+}$  in the range of  $5.00\times 10^{-9}$  M to  $1.00\times 10^{-6}$  M as shown in the inset (a) of Fig. 6. The Linear equation was  $\Delta F = 6.72 \times 10^8 c + 17.48$  where  $\Delta F$  is the change in the fluorescence intensity at 585 nm and  $c$  is the

**Table 2** The measurement results of environment standard sample

The $\text{Hg}^{2+}$ content of standard sample ( $\mu\text{g/L}$ )	Test No.	Measured value ( $\mu\text{g/L}$ )	Average value ( $\mu\text{g/L}$ )	RSD (% , $n=10$ )
Water – mercury (6.14)	1	6.23	6.15	3.25
	2	6.18		
	3	5.91		
	4	6.28		
	5	6.54		
	6	5.96		
	7	6.14		
	8	5.86		
	9	6.23		
	10	6.18		

concentration of  $\text{Hg}^{2+}$ . The detection limit of RBS for  $\text{Hg}^{2+}$  was  $1.83 \times 10^{-9}$  M (based on  $S/N=3$ ). Thus, RBS was capable of detecting both qualitatively and quantitatively of  $\text{Hg}^{2+}$  utilizing fluorospectrophotometry.

A series of solutions containing RBS and  $\text{Hg}^{2+}$  were prepared as the sum of the total concentration remained constant at  $1.00 \times 10^{-6}$  M and then the 1:1 stoichiometry between RBS and  $\text{Hg}^{2+}$  was confirmed by the Job's plot (the method of continuous variations) shown in Fig. 7. The association constant of the complex was estimated on the basis of 1:1 stoichiometry by the linear Benesi–Hildebrand expression [24]:

$$\frac{1}{\Delta F} = \frac{1}{[\text{RBS}]\Delta\Phi} + \frac{1}{K_a[\text{RBS}]\Delta\Phi} \frac{1}{[\text{Hg}^{2+}]}$$

Where  $\Delta F$  is the change in the fluorescence intensity at 585 nm;  $K_a$  is the association constant;  $\Delta\Phi$  is the difference of fluorescence quantum yields between the complex and RBS; and  $[\text{RBS}]$  and  $[\text{Hg}^{2+}]$  are the concentrations of RBS and  $\text{Hg}^{2+}$ , respectively. On the basis of the plot of  $1/\Delta F$  and  $1/[\text{Hg}^{2+}]$  as shown in the inset (b) of Fig. 6, the association constant was calculated to be  $5.96 \times 10^4 \text{ M}^{-1}$  from the ratio of the slope and intercept.

#### Reversibility and the Proposed Binding Mechanism

The reversibility and regeneration is an important factor for a fluorescence chemosensor to detect its specific analyte in practical application. In light of strong binding ability of the iodide anion ( $\text{I}^-$ ) toward  $\text{Hg}^{2+}$ , it was imagined that addition of  $\text{I}^-$  will take away  $\text{Hg}^{2+}$  from the RBS- $\text{Hg}^{2+}$  complex, liberating the spirolactone RBS and decreasing the fluorescence intensity at 585 nm. To colorless solution of RBS, 1 equiv of  $\text{Hg}^{2+}$  was added, then the resulting pink solution was immediately treated with KI (2, 4 and 6 equiv of  $\text{Hg}^{2+}$ ), which led to light-color solution and decreased fluorescence intensity as shown in Fig. 8. Further addition of  $\text{Hg}^{2+}$  to colourless solution of  $\text{I}^-$  mediated  $\text{Hg}^{2+}$ -decomplexed RBS led to reappearance of pink colour and fluorescence intensity enhancement at 585 nm which exceeded the extent of those upon first  $\text{Hg}^{2+}$  addition to RBS. Therefore the response of RBS to  $\text{Hg}^{2+}$  is reversible rather than a cation-catalyzed reaction.

Due to the 1:1 stoichiometry and the result of reversibility experiment, it could be concluded to the  $\text{Hg}^{2+}$ -binding complexation species RBS- $\text{Hg}^{2+}$ . The binding event was proposed to involve the thione-S atom, the imino-N atom and the methoxy-O atom, forming a stable metal complex which required to open the spiro ring to establish the delocalized xanthene moiety. The proposed binding mechanism of RBS with  $\text{Hg}^{2+}$  was shown in Scheme 2.

#### Recovery Study and Determination of Environment Standard Sample

To develop practical applicability of RBS toward  $\text{Hg}^{2+}$ , it was applied to the determination of  $\text{Hg}^{2+}$  in tap water, river water and soil samples. Tap water, river water and solid samples were obtained from laboratory tap water, Nai River and campus soil respectively. As results shown in Table 1, it can be confirmed that the results for recoveries study of spiked  $\text{Hg}^{2+}$  detected by the RBS probe were satisfying and the relative standard deviation (RSD) was 1.91 %, 1.43 % and 2.94 % with 10 determinations, respectively. To prove the reliability of the method, it was applied to the determination of  $\text{Hg}^{2+}$  in the environment standard sample (Water-mercury (Numbers: GSBZ 50016–90, batch number: 202030)). As results shown in Table 2, the relative standard deviation (RSD) was 3.25 % and the average value was 6.15  $\mu\text{g/L}$  consistent with the standard value 6.14  $\mu\text{g/L}$ . So the method was high reliability.

#### Conclusions

In summary, a new fluorogenic probe for detection of  $\text{Hg}^{2+}$  was synthesized. As a turn-on fluorescent probe, it responds to mercury ions rapidly with an excellent selectivity and competitiveness. The linear response range and the detection limit of this method were  $5.00 \times 10^{-9}$  to  $1.00 \times 10^{-6}$  M and  $1.83 \times 10^{-9}$  M, respectively. In addition, the fluorescence can be switched on and off by the addition of  $\text{Hg}^{2+}$  and KI alternately. It was successfully applied to detect  $\text{Hg}^{2+}$  levels in tap water, river water and soil samples. We anticipate that the probe would be more useful for detection of  $\text{Hg}^{2+}$  in future application.

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#### References

1. Wolfe MF, Schwarzbach S, Sulaiman RA (1998) Effects of mercury on wildlife: a comprehensive review. *Environ Toxicol Chem* 17(2):146–160
2. Weiss B, Clarkson TW, Simon W (2002) Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environ Health Perspect* 110(Suppl 5):851–854
3. Guallar E, Sanz-Gallardo MI, Veer P, Bode P, Aro A, Gómez-Aracena J, Kark JD, Riemersma RA, Martín-Moreno JM, Kok FJ (2002) Mercury, fish oils, and the risk of myocardial infarction. *N Engl J Med* 347(22):1747–1754
4. George GN, Prince RC, Gailer J, Buttigieg GA, Denton MB, Harris HH, Pickering IJ (2004) Mercury binding to the chelation therapy agents DMSA and DMPS and the rational design of custom chelators for mercury. *Chem Res Toxicol* 17(8):999–1006

5. Huang RJ, Zhuang ZX, Tai Y, Huang RF, Wang XR, Lee FSC (2006) Direct analysis of mercury in Traditional Chinese Medicines using thermolysis coupled with on-line atomic absorption spectrometry. *Talanta* 68(3):728–734
6. Lobet JM, Falco G, Casas C, Teixido A, Domingo JL (2003) Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *J Agric Food Chem* 51(3):838–842
7. Dias Filho NL, do Carmo DR (2006) Study of an organically modified clay: selective adsorption of heavy metal ions and voltammetric determination of mercury (II). *Talanta* 68(3):919–927
8. Zejli H, Sharrock P, Hidalgo-Hidalgo de Cisneros JL, Naranjo-Rodriguez I, Tamsamani KR (2005) Voltammetric determination of trace mercury at a sonogel-carbon electrode modified with poly-3-methylthiophene. *Talanta* 68(1):79–85
9. Wang M, Zhang Y, Feng WY, Guan M, Wang B, Shi JW, Zhu MT, Li B, Zhao YL, Chai ZF (2007) Determination of mercury in fish by isotope dilution inductively coupled plasma-mass spectrometry. *Chin J Anal Chem* 35(7):945–948
10. Fitzgerald WF, Gill GA (1979) Subnanogram determination of mercury by two-stage gold amalgamation and gas phase detection applied to atmospheric analysis. *Anal Chem* 51(11):1714–1720
11. Yoon S, Albers AE, Wong AP, Chang CJ (2005) Screening mercury levels in fish with a selective fluorescent chemosensor. *J Am Chem Soc* 127(46):16030–16031
12. Wanichacheva N, Sethakarn K, Prapawattanapol N, Hanmeng O, Sanghiran Lee V, Grudpan K (2012) Rhodamine B-based “turn-on” fluorescent and colorimetric chemosensors for highly sensitive and selective detection of mercury (II) ions. *J Lumin* 132(1):35–40
13. Ko SK, Yang YK, Tae J, Shin I (2006) In vivo monitoring of mercury ions using a rhodamine-based molecular probe. *J Am Chem Soc* 128(43):14150–14155
14. Wang L, Yan JX, Qin W, Liu W, Wang R (2012) A new rhodamine-based single molecule multianalyte ( $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ ) sensor and its application in the biological system. *Dye Pigment* 92(3):1083–1090
15. Yoon S, Miller EW, He Q, Do PH, Chang CJ (2007) A bright and specific fluorescent sensor for mercury in water, cells, and tissue. *J Angew Chem Int Ed* 46(35):6658–6661
16. Yang H, Zhou Z, Huang K, Yu M, Li F, Yi T, Huang C (2007) Multisignaling optical-electrochemical sensor for  $\text{Hg}^{2+}$  based on a rhodamine derivative with a ferrocene unit. *Org Lett* 9(23):4729–4732
17. Nolan EM, Lippard SJ (2007) Turn-on and ratiometric mercury sensing in water with a red-emitting probe. *J Am Chem Soc* 129(18):5910–5918
18. Liu J, Lu Y (2007) Rational design of “turn-on” allosteric DNzyme catalytic beacons for aqueous mercury ions with ultrahigh sensitivity and selectivity. *Angew Chem* 46(40):7587–7590
19. Zhang D, Li M, Wang M, Wang J, Yang X, Ye Y, Zhao Y (2013) A rhodamine-phosphonate off-on fluorescent sensor for  $\text{Hg}^{2+}$  in natural water and its application in live cell imaging. *Sensors Actuator B Chem* 177:997–1002
20. Yapici NB, Jockusch S, Moscatelli A, Mandalapu SR, Itagaki Y, Bates DK, Wiseman S, Gibson KM, Turro NJ (2011) New rhodamine nitroxide based fluorescent probes for intracellular hydroxyl radical identification in living cells. *Org Lett* 14(1):50–53
21. Zheng H, Qian ZH, Xu L, Yuan FF, Lan LD, Xu JG (2006) Switching the recognition preference of rhodamine B spirolactam by replacing one atom: design of rhodamine B thiohydrazide for recognition of Hg (II) in aqueous solution. *Org Lett* 8(5):859–861
22. Lin WY, Cao XW, Ding YD, Yuan L, Yu QX (2010) A reversible fluorescent  $\text{Hg}^{2+}$  chemosensor based on a receptor composed of a thiol atom and an alkene moiety for living cell fluorescence imaging. *Org Biomol Chem* 8(16):3618–3620
23. Yang XF, Guo XQ, Zhao YB (2002) Development of a novel rhodamine-type fluorescent probe to determine peroxynitrite. *Talanta* 57(5):883–890
24. Benesi HA, Hildebrand JH (1949) A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. *J Am Chem Soc* 71(8):2703–2707