# **RSC Advances**



## **PAPER**

View Article Online



Cite this: RSC Adv., 2024, 14, 31861

# A rhodamine-based fluorescent probe bearing 8hydroxyquinoline group for the highly selective detection of Hg<sup>2+</sup> and its practical application in cell imaging†

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A highly selective and sensitive fluorescent probe, RHOQ, was designed for the detection of Hq<sup>2+</sup> by incorporating an 8-hydroxyguinoline moiety onto a rhodamine molecular platform with a suitable linker. In MeOH-Tris (20 mM, pH = 7.4, 1:9, v/v) buffer solution, RHOQ exhibited 550-fold fluorescence enhancement at 594 nm upon addition of  $Hg^{2+}$ , with a fast response and a low detection limit (9.67  $\times$ 10<sup>-8</sup> M). The 1:1 binding mode of RHOQ with Hq<sup>2+</sup> was established using Job's plot, UV-Vis, and fluorescence spectroscopic titration methods. Furthermore, RHOQ was successfully applied for the detection of Hg<sup>2+</sup> in living cells with good membrane permeability.

Received 23rd August 2024 Accepted 2nd October 2024

DOI: 10.1039/d4ra06115b

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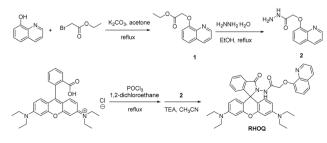
## Introduction

The mercury ion is a highly toxic heavy metal that poses severe health risks to humans and wildlife. It is a global environmental pollutant that can enter the food chain through various sources, including industrial activities, mining, and natural processes. Mercury ions can accumulate in the body and cause various health issues, such as neurological disorders, kidney damage, and cardiovascular diseases. When inorganic Hg2+ entered living organisms, it would transform to lipophilic and higher toxic methylmercury (CH<sub>3</sub>Hg<sup>+</sup>) and ethylmercury (CH<sub>3</sub>CH<sub>2</sub>-Hg<sup>+</sup>). Therefore, detecting and monitoring mercury ions in the environment and food chain is crucial to prevent their harmful effects on human health and the ecosystem.

However, traditional detection methods for mercury ions, such as atomic absorption spectroscopy (AAS), atomic emission spectrometry (AES), electrochemistry, and inductively coupled plasma-atomic emission spectroscopy (ICP-OES), require sophisticated and time-consuming pretreatment and equipment, making real-time onsite detection challenging.2-7 To overcome these limitations, fluorescent probes have emerged as a simple and cost-effective detection method with high sensitivity and selectivity, real-time monitoring capabilities, and easy

To date, many fluorescent probes based on various fluorophores have been developed for the detection of mercury ions in vitro and in vivo. 10-15 Among of them, rhodamine-based fluorophore has been widely used due to its commercial available, good photostability, relatively long absorption and emission wavelengths and simple structural derivatization. 16-30 Rhodamine derivatives exhibit very weak fluorescence in the spirocyclic form and strong fluorescence in the ring-opening form, triggered by specific analyte chelation. Hence, a new fluorescent probe, **RHOQ**, was developed for highly selective Hg<sup>2+</sup> detection based on the ring close/opening transformation of the rhodamine platform (Scheme 1). Compared with recently reported fluorescent probe for Hg2+ (Table S1†), probe RHOQ demonstrated faster response, lower detection limit and higher fluorescence enhancement ratio. Furthermore, RHOQ can successfully monitor Hg2+ in living HeLa cells with low cytotoxicity.

<sup>†</sup> Electronic supplementary information (ESI) available. DOI: https://doi.org/10.1039/d4ra06115b



Scheme 1 Synthetic route of probe RHOQ

procedures.8 Fluorescent probes have found applications in various fields, including environmental monitoring, food safety, live cell imaging, and medical diagnosis.9

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## 2 Experimental

#### 2.1 Reagents and instrumentation

High-purity solvents and chemicals were purchased from Sigma-Aldrich Inc. with analytical grade. Metal ions including Ag<sup>+</sup>, Li<sup>+</sup>, Ca<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup>, Hg<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, and Fe<sup>3+</sup> were purchased as perchlorates, K<sup>+</sup>, Na<sup>+</sup>, and Mg<sup>2+</sup> were purchased as chlorides.

Bruker Avance-III 400 MHz spectrometer was used to collect <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra with TMS internal reference at 400 and 100 MHz, respectively. Bruker Autoflex mass spectrometer (MALDI-TOF) was used to record high-resolution mass spectrum (HRMS). PerkinElmer LS50B (Waltham, MA, USA) and Cary UV-300 spectrometer were used to collect fluorescence spectra and UV-Vis spectra, respectively. Orion 420A pH/mV/temperature benchtop meter was used for pH measurements.

#### 2.2 Synthesis of probe RHOQ

**Compound 1.** A mixture of 8-hydroxyquinoline (1.60 g, 11 mmol), ethyl bromoacetate (1.93 g, 15.5 mmol) and  $K_2CO_3$  (3.3 g, 24 mmol) in acetone (50 mL) was heated to reflux for 30 hours. After cooling, solid was filtered off and washed with small portions of ethyl acetate. The filtrates were combined and evaporated to dryness. The residue was purified by column chromatography on silica gel (PE:EA = 1:1) to give compound 1 (2.42 g, 95%) as a yellow oil.  $^1$ H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 8.96 (1H, dd, J = 4.1 Hz, J = 1.7 Hz), 8.15 (1H, dd, J = 8.3 Hz, J = 1.7 Hz), 7.47-7.41 (3H, m), 6.97 (1H, dd, J = 6.7 Hz, J = 2.2 Hz), 4.98 (2H, s), 4.28 (2H, q, J = 7.3 Hz), 1.27 (3H, t, J = 7.3 Hz).  $^{13}$ C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 168.83, 153.68, 149.57, 140.18, 135.93, 129.57, 126.36, 121.82, 120.90, 109.39, 66.19, 61.42, 14.18.

Compound 2. To a solution of compound 1 (1.5 g, 6.5 mmol) in EtOH (30 mL) was added hydrazine monohydrate (3 mL) in one portion and the reaction mixture was heated to reflux for 3 hours. After cooling, solvent was removed under reduced pressure, the solid was washed with small portions of water and EtOH and dried to give compound 2 (1.2 g, 85%) as a white solid.  $^1$ H-NMR (400 MHz, DMSO-d6)  $\delta$  (ppm): 9.55 (1H, s), 8.88 (1H, dd, J = 4.1 Hz, J = 1.6 Hz), 8.35 (1H, dd, J = 8.3 Hz, J = 1.4 Hz), 7.58–7.49 (3H, m), 7.23 (1H, dd, J = 7.7 Hz, J = 1.3 Hz), 4.74 (2H, s), 4.40 (2H, s).  $^{13}$ C-NMR (100 MHz, DMSO-d6)  $\delta$  (ppm): 166.79, 153.76, 149.29, 139.64, 136.07, 129.06, 126.75, 122.00, 120.84, 111.42, 68.07.

**Probe RHOQ.** To a suspension of rhodamine B (0.5 g, 1.0 mmol) in 1,2-dichloroethane (10 mL) was added POCl<sub>3</sub> (0.4 mL, 4.4 mmol) dropwise over 2 min under  $N_2$  atmosphere and the reaction mixture was refluxed for 4 hours. The mixture was then cooled and evaporated *in vacuo*, the residue was dissolved in CH<sub>3</sub>CN (80 mL) and this acid chloride solution was added dropwise to a solution of compound 2 (0.23 g, 1.0 mmol) and TEA (0.5 mL) in CH<sub>3</sub>CN (20 mL) over 1 hour at room temperature. The reaction mixture was then refluxed for 2 hours. After solvent was evaporated under reduced pressure, the crude product was purified by column chromatography on silica gel (PE:EA = 1:5) to give compound **RHOQ** (420 mg, 65%) as

a white solid.  $^1\mathrm{H}\text{-NMR}$  (400 MHz, CD<sub>3</sub>CN)  $\delta$  (ppm): 10.78 (1H, br. s), 8.23–8.14 (m, 2H), 7.87–7.85 (m, 1H), 7.61–7.48 (m, 4H), 7.28–7.20 (m, 2H), 7.04–7.02 (m, 1H), 6.59–6.58 (m, 2H), 6.32–6.24 (m, 4H), 4.65 (s, 2H), 3.33 (q, J=6.9 Hz, 8H), 1.11 (t, J=7.0 Hz, 12H).  $^{13}\mathrm{C}\text{-NMR}$  (100 MHz, CD<sub>3</sub>CN)  $\delta$  (ppm): 167.00, 163.52, 154.22, 153.34, 151.77, 148.60, 135.80, 132.85, 129.28, 128.95, 128.13, 126.55, 123.45, 122.40, 121.40, 107.37, 104.12, 96.78, 65.28, 43.62, 11.56. HRMS (ESI): m/z [M + H $^+$ ] calcd for C<sub>39</sub>H<sub>40</sub>N<sub>5</sub>O<sub>4</sub> $^+$ : 641.3002; found: 642.3053; m/z [M + Na $^+$ ] calcd for C<sub>39</sub>H<sub>39</sub>N<sub>5</sub>O<sub>4</sub>Na $^+$ : 664.2894; found: 664.1235; m/z [M + K $^+$ ] calcd for C<sub>39</sub>H<sub>39</sub>N<sub>5</sub>O<sub>4</sub>K $^+$ : 680.2634; found: 679.9705.

#### 2.3 Spectroscopic measurements

Various cation solutions including LiCl, NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> (100 mM), and AgClO<sub>4</sub>·H<sub>2</sub>O, Cd(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, Co(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, Cu(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, Fe(ClO<sub>4</sub>)<sub>2</sub>, Fe(ClO<sub>4</sub>)<sub>3</sub>, Hg(ClO<sub>4</sub>)<sub>2</sub>·3H<sub>2</sub>O, Ni(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, Pb(ClO<sub>4</sub>)<sub>2</sub>·3H<sub>2</sub>O, Zn(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (10 mM) were prepared in double-distilled water. Probe **RHOQ** was dissolved in MeOH to prepare 1.0 mM stock solution. All the absorption and fluorescence spectra measurements were conducted in MeOH–H<sub>2</sub>O solution (1:9, v/v, 40 mM HEPES, pH 7.4). In the fluorescence experiments, the excitation wavelength was set at 543 nm, and the excitation/emission slit widths were set at 5/5 nm, respectively.

#### 2.4 Cytotoxicity assay

The cellular toxicity of probe **RHOQ** was tested by MTT assay. HeLa cells were seeded into 96 well plates at a density of 4000 cells per well at 37 °C with 5%  $CO_2$  for 24 h, and then incubated with 0.1% DMSO and different concentration of probe solution (5, 10, 20, 30  $\mu$ M), respectively. Subsequently, 20  $\mu$ L of MTT solution was added to each well followed by incubation for an additional 4 h. The absorbance of each well was measured at 490 nm.

#### 2.5 Cell culture and imaging

Probe **RHOQ** (1.0 mM) was prepared in DMSO solution. HeLa cells were cultured in Minimum Essential Medium (MEM) with 10% fetal bovine serum (FBS) and 1% antibiotics at 37 °C in an atmosphere of 5% CO<sub>2</sub> and 95% humidity. The HeLa cells were plated on coverslips and washed 3 times with phosphate buffered saline (PBS) followed by incubating with the probe solution (10  $\mu$ M) in DMSO for 30 min at 37 °C, and then washed with PBS three times. After incubating with Hg(ClO<sub>4</sub>)<sub>2</sub> solution (10  $\mu$ M) in PBS for 30 min at 37 °C, the cells were washed with PBS three times again.

## 3 Results and discussion

#### 3.1 The synthesis of RHOQ

The synthetic route of probe **RHOQ** was outlined in Scheme 1. Intermediate 1 was obtained by reacting 8-hydroxyquinoline with ethyl bromoacetate with a high yield. Subsequently, the reaction of 1 with hydrazine under reflux conditions gave compound 2 with an easy work-up operation. Finally, the condensation of rhodamine B with compound 2 in CH<sub>3</sub>CN yielded the target probe **RHOQ**. All chemical structures were

confirmed by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, as well as high resolution mass spectroscopy (detailed in the Experimental section and ESI†).

#### 3.2 Selectivity of RHOQ

After the synthesis of the probe, the selectivity of **RHOQ** (10  $\mu$ M) towards various metal ions, including Na<sup>+</sup>, K<sup>+</sup>, Li<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ag<sup>+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup>, Hg<sup>2+</sup>, Fe<sup>2+</sup> and Fe<sup>3+</sup>, was investigated in a MeOH-Tris buffer (20 mM, pH = 7.4, 1:9, v/v) solution using UV-Vis and fluorescence spectroscopy. The absorption spectrum of RHOQ exhibited negligible absorbance in the range from 400 to 700 nm because RHOQ mainly exists in spirolactam form. Upon the addition of Hg2+, a strong absorption peak at 566 nm with a characteristic broad shoulder band centered at about 521 nm appeared (Fig. 1), suggesting the conversion of the spirolactam form to the ring-opening amide form of RHOQ. As shown in the inset of Fig. 1, the addition of Hg<sup>2+</sup> to the solution of RHOQ induced a prominent colour changed from colourless to magenta. In addition, similar phenomenon was also observed upon the addition of Cu<sup>2+</sup>, while other metal ions did not trigger any changes. In the fluorescence experiments, the addition of Hg2+ induced a significant fluorescence enhancement (550-fold) at 594 nm, while Fe<sup>3+</sup> caused a relatively small fluorescence change (15fold) with a small hypsochromic shift (fluorescence maximum at 584 nm), and other metal ions induced negligible changes (Fig. 2). Consistent with the results of fluorescent experiments, only the addition of Hg2+ to the solution of RHOQ can emit distinct red fluorescence under the irradiation of UV lamp (inset of Fig. 2).

#### 3.3 Interference effect of RHOQ

After investigating the selectivity of **RHOQ** for various cations, the potential interference effect of other cations on the selectivity of **RHOQ** for Hg<sup>2+</sup> detection was examined. As shown in Fig. 3, the presence of high concentrations of various cations (40

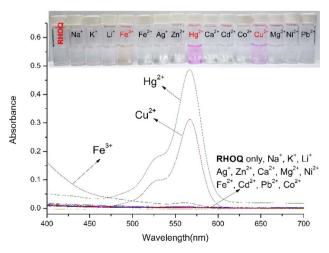


Fig. 1 Absorption spectrum of RHOQ (10  $\mu$ M) upon addition of different metal ions in MeOH–Tris buffer (20 mM, pH = 7.4, 1:9, v/v). Inset: visual color change of RHOQ upon addition of different metal ions.

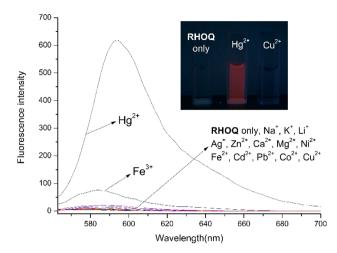


Fig. 2 Fluorescence spectrum of RHOQ (10  $\mu$ M) upon addition of different metal ions in MeOH–Tris buffer (20 mM, pH = 7.4, 1:9, v/v). Inset: visible emission of RHOQ observed under a UV lamp in the absence (left) and presence of Hq<sup>2+</sup> (middle) or Cu<sup>2+</sup> (right).

equiv.) did not interfere with Hg<sup>2+</sup> detection of **RHOQ**. Especially, the presence of Cu<sup>2+</sup>, known as a paramagnetic fluorescence quencher, did not affect the detection of Hg<sup>2+</sup> by the **RHOQ**. Similarly, absorbance measurements also showed no significant changes in the presence of various competing ions (Fig. S8†). These results confirmed that **RHOQ** has a highly potential application in the detection of Hg<sup>2+</sup> in living organisms. The selectivity and interference effect of **RHOQ** for Hg<sup>2+</sup> detection was confirmed, indicating its potential application in detecting Hg<sup>2+</sup> in living organisms.

### 3.4 Titration experiments of RHOQ

Additionally, UV/Vis and fluorescence titration of RHOQ in MeOH-Tris buffer (20 mM, pH = 7.4, 1:9, v/v) upon the

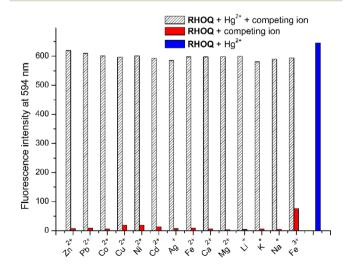


Fig. 3 Change of fluorescence intensity at 594 nm of RHOQ ( $10~\mu$ M) in MeOH–Tris buffer (20~mM, pH = 7.4, 1:9, v/v) upon addition of competing metal ions (40~equiv.) in the absence (black) and presence (red) of Hg<sup>2+</sup> (40~equiv.).

addition of  $\mathrm{Hg}^{2^+}$  were conducted. After the addition of  $\mathrm{Hg}^{2^+}$  (40 equiv.), the binding of **RHOQ** with  $\mathrm{Hg}^{2^+}$  reached equilibrium, as evidenced by both absorbance and fluorescence measurement. Nonlinear fitting of the titration curves using the Benesi–Hildebrand equation (eqn (1))<sup>31,32</sup> assuming a 1:1 stoichiometry for the **RHOQ**– $\mathrm{Hg}^{2^+}$  complex yielded association constant  $K_{\mathrm{ass}}$  of about 1.05  $\times$  10<sup>5</sup> L mol<sup>-1</sup> ( $R^2=0.990$ ) and 9.18  $\times$  10<sup>3</sup> L mol<sup>-1</sup> ( $R^2=0.990$ ) from UV/Vis (Fig. 4) and fluorescence (Fig. 5) titration, respectively.

$$\frac{1}{F - F_0} = \frac{1}{K(F_{\text{max}} - F_0)[Hg^{2+}]} + \frac{1}{F_{\text{max}} - F_0}$$
(1)

where  $F_0$  is the absorbance or fluorescence intensity of **RHOQ**, F is the absorbance or fluorescence intensity of **RHOQ**–Hg<sup>2+</sup> complex,  $F_{\rm max}$  is the absorbance or fluorescence intensity of **RHOQ** with excess amount of Hg<sup>2+</sup>, K is the association constant (L mol<sup>-1</sup>), and [Hg<sup>2+</sup>] is the concentration of Hg<sup>2+</sup> (mol L<sup>-1</sup>). According to the fluorescence titration experiment, a linear fluorescence intensity enhancement was observed in the range of 0–120  $\mu$ M of Hg<sup>2+</sup> with a calculated limit of detection (LOD =  $3\sigma/K$ ) of 9.67  $\times$  10<sup>-8</sup> M (Fig. S9†). <sup>33–35</sup> Therefore, probe **RHOQ** could be utilized as a promising method for the sensitive and quantitative detection of Hg<sup>2+</sup> in an aqueous solution.

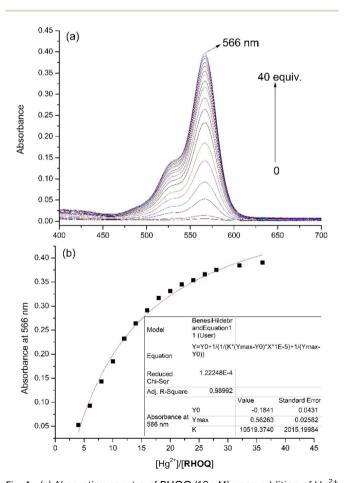
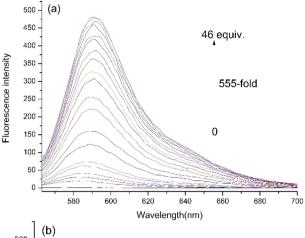


Fig. 4 (a) Absorption spectra of RHOQ (10  $\mu$ M) upon addition of Hg<sup>2+</sup> in MeOH–Tris buffer (20 mM, pH = 7.4, 1:9, v/v). (b) Absorbance at 566 nm of RHOQ as a function of Hg<sup>2+</sup> concentration (0–40 equiv.).



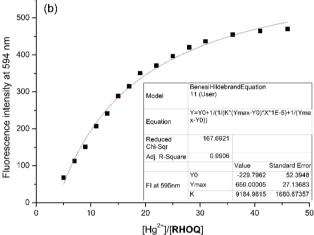


Fig. 5 (a) Fluorescence spectra of RHOQ (10  $\mu$ M) upon addition of Hg<sup>2+</sup> in MeOH–Tris buffer (20 mM, pH = 7.4, 1:9, v/v). (b) Fluorescence intensity at 594 nm of RHOQ as a function of Hg<sup>2+</sup> concentration (0–40 equiv.).

#### 3.5 pH effect of RHOQ

The pH-dependent emission measurements revealed that **RHOQ** exhibited high stability and non-fluorescence in the pH range from 4.0–10.0 (Fig. 6). A slight fluorescence enhancement of **RHOQ** below pH 4.0 may be attributed to the well-known spirolactam ring opening in strong acidic condition. On the other hand, the addition of  $Hg^{2+}$  to the solution of **RHOQ** induced a significant fluorescence enhancement in acidic or weak alkaline conditions (pH < 8.0), while a dramatic decrease in fluorescence intensity was observed at pH higher than 8.2. This result may be ascribed to the formation of insoluble  $Hg(OH)_2$  in alkaline conditions, leading to the conversion of the ring-opening **RHOQ**- $Hg^{2+}$  complex back to the original spirolactam compound. These findings support the suitability of **RHOQ** for the detection of  $Hg^{2+}$  in living cells.

#### 3.6 Reversibility and time-dependent response of RHOQ

The reversibility of probe for metal ion detection is a crucial characteristic for practical applications. To test the reversibility of RHOQ, an aqueous solution of Na<sub>2</sub>EDTA (disodium ethylenediaminetetraacetate) was introduced to the solution of

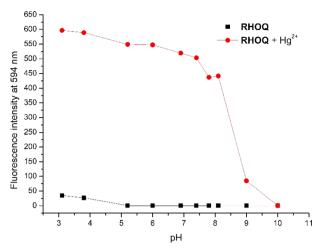


Fig. 6 Plot of fluorescence intensity at 594 nm of RHOQ (10  $\mu$ M) in MeOH-Tris buffer (20 mM, 1:9, v/v) in the presence (red dot) and absence (black square) of Hg<sup>2+</sup> (40 equiv.) as a function of pH.

RHOQ (10  $\mu$ M) and Hg<sup>2+</sup> (Fig. 7). The fluorescence intensity of RHOQ-Hg<sup>2+</sup> complex decreased significantly and approached the original intensity of RHOQ. These outcomes can also be attributed to the spirolactam ring opening/closing interconversion, which aligns with the findings obtained from the pH-dependent experiments.

We also investigated the time-dependent responses of **RHOQ** to Hg<sup>2+</sup> (Fig. 8). Upon the addition of Hg<sup>2+</sup>, the solution of **RHOQ** showed an instantaneous color change to red, with stable maximum fluorescence intensity for at least 30 minutes, demonstrating the suitability of **RHOQ** for real-time Hg<sup>2+</sup> detection.

#### 3.7 Binding mode of RHOQ and Hg<sup>2+</sup>

To elucidate the possible binding mode between **RHOQ** and  $Hg^{2+}$ , Job's plot experiments were conducted using fluorescent method. The result clearly indicated that the maximum

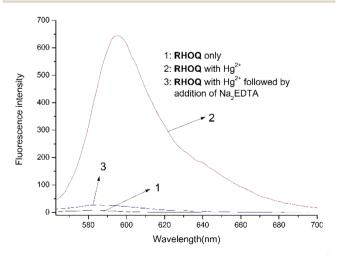


Fig. 7 Fluorescence spectra of RHOQ (10  $\mu$ M) upon addition of Hg<sup>2+</sup> (40 equiv.) followed by the addition of Na<sub>2</sub>EDTA in MeOH–Tris buffer (20 mM, pH = 7.4, 1:9, v/v).

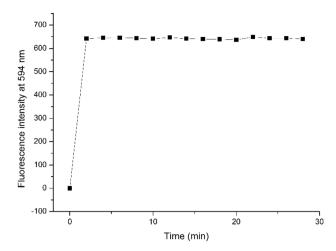


Fig. 8 Time-dependent fluorescence intensity at 594 nm of RHOQ (10  $\mu$ M) in MeOH-Tris buffer (20 mM, pH = 7.4, 1:9, v/v) upon the addition of Hg<sup>2+</sup> (40 equiv.).

fluorescence intensity was located at a mole fraction around 0.5 for Hg<sup>2+</sup> (Fig. 9), confirming a 1:1 binding stoichiometry between RHOQ and Hg2+. This result is consistent with the nonlinear fitting of titration curve using the Benesi-Hildebrand equation. In order to investigate the binding mode of RHOQ and Hg<sup>2+</sup>, <sup>1</sup>H NMR titration experiments were conducted. Upon addition of solution of Hg(ClO<sub>4</sub>)<sub>2</sub> in D<sub>2</sub>O to the solution of **RHOQ** in CD<sub>3</sub>CN, the proton peaks corresponding to  $H_a$ ,  $H_b$  and H<sub>c</sub> shifted to lower field (Fig. 10). This observation implied that Hg<sup>2+</sup> coordinated with N atom on quinoline group and O atom on carbonyl group (-COCH<sub>2</sub>O-). According to these results, we proposed a possible fluorescence OFF-ON mechanism of RHOQ upon addition of Hg<sup>2+</sup>, Fe<sup>3+</sup> and Cu<sup>2+</sup>, respectively (Scheme 2). As we know, rhodamine B or its derivatives generally exist in five-membered spirolactam form with non-fluorescent behaviour. While in the presence of specific metal cations, the coordination of metal cation with oxygen atom on the spirolactam

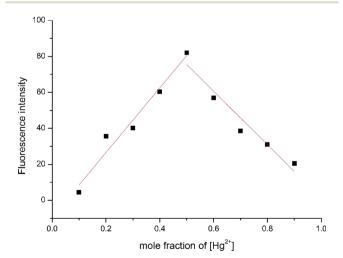


Fig. 9 Job's plot by fluorescence method of the complex between RHOQ and  $Hg^{2+}$  in MeOH-Tris buffer (20 mM, pH = 7.4, 1:9, v/v). Total concentration of RHOQ and  $Hg^{2+}$  is 50  $\mu$ M.

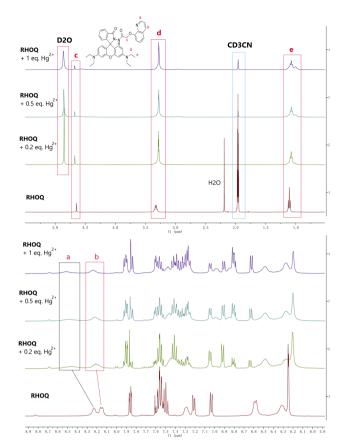
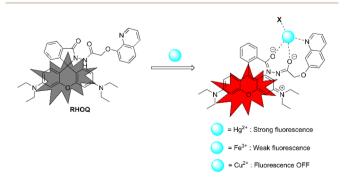


Fig. 10  $^{1}$ H NMR spectra corresponding to the titration of RHOQ in CD<sub>3</sub>CN with Hg(ClO<sub>4</sub>)<sub>2</sub> in D<sub>2</sub>O.

moiety of rhodamine B derivatives triggers the opening of spirolactam form to give a strong red fluorescence. To the probe **RHOQ**, the introduction of another amide group and 8-hydroxyquinoline moiety provides specific coordination sites for Hg<sup>2+</sup>, thus the selectivity of probe **RHOQ** to Hg<sup>2+</sup> was enhanced.

Additionally, when Cu<sup>2+</sup> was added to **RHOQ** solution, we observed an absorption spectrum similar to that of Hg<sup>2+</sup>, but no fluorescence was produced (Fig. 1 and 2). This phenomenon can be attributed to the relatively high binding affinity of Cu<sup>2+</sup> for **RHOQ** and its well-known paramagnetic nature.<sup>36,37</sup> Fortunately, the high concentration of Cu<sup>2+</sup> did not interfere with the



Scheme 2 Proposed binding mechanism of RHOQ upon addition of  $Hg^{2+}$ ,  $Fe^{3+}$  and  $Cu^{2+}$ , respectively. (X = coordinating anion or solvent).

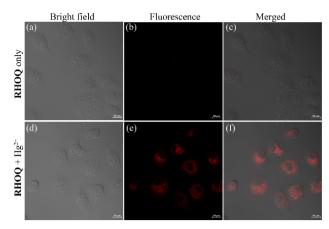


Fig. 11 Confocal microscopy images of HeLa cell staining with RHOQ (10  $\mu$ M) (a–c) followed by the incubation of Hg<sup>2+</sup> (10  $\mu$ M) (d–f). (a) and (d) bright field images; (b) and (e) fluorescence images collected in the range of 580–640 nm; (c) overlay of (a) and (b); (f) overlay of (d) and (e).

sensing of Hg<sup>2+</sup> by **RHOQ**. Due to the higher binding affinity of Hg<sup>2+</sup> for **RHOQ** compared to Cu<sup>2+</sup>, Hg<sup>2+</sup> would replace Cu<sup>2+</sup> in the **RHOQ**-Cu<sup>2+</sup> complex to form a new **RHOQ**-Hg<sup>2+</sup> complex, thereby regenerating the fluorescence. Similarly, the much lower binding affinity of Fe<sup>3+</sup> for **RHOQ** resulted in significantly low absorbance and fluorescence intensity.

#### 3.8 Cytotoxicity and bioimaging application of RHOQ

Building on the above experimental results, further studies were conducted in biological environments using HeLa cells. The cytotoxicity of RHOQ was evaluated using MTT assays before cell imaging (Fig. S10†). Living HeLa cells were incubated with different concentrations of probe RHOQ (0–30  $\mu M$ ) for 24 h at 37 °C, and the results suggested that the cell viability had negligible change, which indicated that RHOQ was low cytotoxicity to living cells.

To test the capability of **RHOQ** to detection  $Hg^{2^+}$  in living cells, HeLa cells were incubated with probe **RHOQ** (10  $\mu$ M) at 37 °C for 30 min. Negligible fluorescence was found in the range of 580–640 nm (Fig. 11a–c). Upon further incubation of  $Hg(ClO_4)_2$  for 30 min, a strong red fluorescence was observed (Fig. 11d–f), indicating that the intracellular uptake of  $Hg^{2^+}$  resulted the spirolactam ring opening of **RHOQ** and fluorescence enhancement. These results showed that **RHOQ** has good cell membrane permeability and can be utilized for the detection of  $Hg^{2^+}$  in living cells.

## 4 Conclusions

In summary, a highly Hg<sup>2+</sup>-selective rhodamine-based turn-on fluorescent probe **RHOQ**, was designed and investigated for Hg<sup>2+</sup> detection. The probe exhibited a visual color change to Hg<sup>2+</sup> and Cu<sup>2+</sup>, while only the presence of Hg<sup>2+</sup> triggers intense fluorescence enhancement of **RHOQ** due to the formation of spirolactam ring-opening form of **RHOQ**. Common alkali, alkali earth and transition metal ions did not cause any interference to the fluorescence detection of Hg<sup>2+</sup>. With fast response, a wide

applicable pH range, and a low detection limit  $(9.67 \times 10^{-8} \text{ M})$ , **RHOQ** holds promise for the sensitive and quantitative detection of  $\text{Hg}^{2^+}$  in aqueous solutions. Additionally, MTT assays confirmed that **RHOQ** has low cytotoxicity toward living cells and can be utilized for the detection of  $\text{Hg}^{2^+}$  in living cells. These results manifest that **RHOQ** has a good application prospect in the detection of  $\text{Hg}^{2^+}$ .

## Data availability

The data supporting this article have been included as part of the ESI.†

### **Author contributions**

Lei Zhang: investigation, formal analysis, resources, writingoriginal draft, funding acquisition. Jun Guo: investigation, formal analysis, resources. Qihua You: conceptualization, methodology, writing-review & editing, supervision.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This work was supported by the Key R&D Program of Xinzhou Science and Technology Project (No. 20220301), and Scientific Research Project of Xinzhou Normal University (Grant No. 00001022).

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