

一种 Hg^{2+} 荧光探针的合成及其在活细胞成像中的应用张敏^a 肖慧丰^a 韩志湘^b 仰榴青^a 吴向阳^{*,b}^(a) 江苏大学化学化工学院 镇江 212013^(b) 江苏大学环境与安全工程学院 镇江 212013

摘要 设计合成了一个新的罗丹明席夫碱类荧光探针 **1**, 其结构经 ^1H NMR、 ^{13}C NMR、MS 和 X 射线单晶衍射的验证. 通过紫外-可见光谱法和荧光光谱法研究了探针 **1** 在乙醇-水(V/V=7/3, HEPES 10 mmol/L, pH=7.0)中对 Hg^{2+} 的识别性能. 探针 **1** 通过显著的荧光增强来识别 Hg^{2+} , 并具有良好的选择性和抗干扰能力. 通过 Job's plot 和 MS 证明了探针 **1** 和 Hg^{2+} 形成 1:1 的配合物. 探针 **1** 的荧光强度与 Hg^{2+} 浓度(0~50 $\mu\text{mol/L}$)呈良好的线性关系, 可以定量地检测该范围内的 Hg^{2+} . 在 MGC-803 活细胞中的荧光显微成像表明, 探针 **1** 可检测生物体内的 Hg^{2+} .

关键词 罗丹明 B; 合成; 荧光探针; Hg^{2+} ; 生物成像

A Hg^{2+} -Selective Fluorescence Probe: Synthesis and Application in Aqueous Solution and Living Cell ImagingZhang, Min^a Xiao, Huifeng^a Han, Zhixiang^b Yang, Liuqing^a Wu, Xiangyang^{*,b}^(a) School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013^(b) School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013

Abstract A novel rhodamine B Schiff-base fluorescence probe **1** was synthesized and characterized by ^1H NMR, ^{13}C NMR, MS and single-crystal X-ray analysis. The recognition properties of the probe **1** with Hg^{2+} ions were investigated in ethanol-water (V/V = 7/3, HEPES 10 mmol/L, pH = 7.0) by the UV-Vis spectrophotometry and the fluorescence spectrophotometry. The probe **1** showed remarkable "turn-on" fluorescent responses to Hg^{2+} with good selectivity over the other metal ions examined. The 1:1 binding model of probe **1** with Hg^{2+} was established by Job's plot and MS spectra. The probe can be applied to the quantitative analysis of Hg^{2+} with a linear range covering from 0 to 50 $\mu\text{mol/L}$. The fluorescence imaging experiments in MGC-803 living cells demonstrated that **1** could be used in biological systems to monitor Hg^{2+} .

Keywords rhodamine B; synthesis; fluorescent probe; Hg^{2+} ; bioimaging

1 Introduction

Mercury is highly toxic even at relatively low concentrations, and is widespread in air, water, and soil. What's worse, mercury can be accumulated through the food chain and atmospheric circulation.^[1,2] The exposure to mercury would cause a lot of severe environmental and health threats.^[3] For example, Hg^{2+} could block thiol group (SH) in enzymes and proteins, leading to the dysfunction of cells, and consequently, it will produce fatal damage to brain, nervous and endocrine systems, and even the kidneys.^[4,5]

Considerable efforts have been devoted to detecting Hg^{2+} via different analytical methods. In contrast to other

approaches, such as liquid chromatography,^[6] capillary electrophoresis,^[7] neutron activation analysis,^[8] and anodic stripping voltammetry,^[9] increasing attention has been paid to the development of colorimetric and fluorescent probes for the detection of Hg^{2+} owing to their high sensitivity and selectivity, easy operation, rapid measurement, low cost and real time monitoring.^[10-13] In the past decades, a great many fluorescent probes for Hg^{2+} have been explored,^[14-37] but many of them suffer the cross-sensitivity towards potential competitive metal ions, for example Cr^{3+} ,^[21,22,32] Cu^{2+} ,^[20,25,31,33,37] Fe^{3+} ,^[20,33,34] and Al^{3+} .^[23] Therefore, it is important to develop effective fluorescent probes, which could detect Hg^{2+} with excellent selectivity and sensitivity.

The rhodamine structure, which has excellent photo-

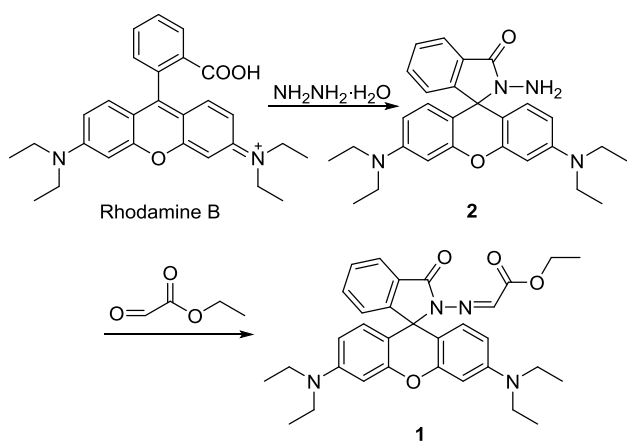
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physical properties, such as long absorption and emission wave-length, high fluorescence quantum yield, large absorption coefficients, has been extensively used as a signaling moiety in the construction of turn-on fluorescent probes for the detection of important cations and anions.^[14–17,1–29,31,32,34,35,38] The ester group in fluorescent probes can not only increase the chelating ability between the probes and metal ions^[39], but also improve the ability of application in the biological system.^[40] In view of the above-mentioned advantages of rhodamine and ester group, a new Schiff-base fluorescence probe **1** based rhodamine B and ethyl glyoxalate was designed and synthesized (Scheme 1). The probe shows a turn-on fluorescent response for Hg^{2+} in aqueous solution with remarkably high selectivity and sensitivity. Furthermore, it has been used for Hg^{2+} imaging in living cells with satisfying results.



Scheme 1 Synthesis of **1**

2 Results and discussion

2.1 Synthesis and characterization

The synthetic route of probe **1** was shown in Scheme 1. Rhodamine B hydrazide **2** was prepared according to the method described in our previous work.^[35] Condensation of **2** with ethyl glyoxalate yielded probe **1**. The structural characterization of **1** was characterized by ^1H NMR, ^{13}C NMR, and MS. Furthermore, the structure of probe **1** was confirmed through its single crystal X-ray analysis. Light pink single crystals were obtained by slow evaporation of the mixed CHCl_3 -petroleum ether (60–90 °C) solution at room temperature. A crystal (0.20 mm × 0.20 mm × 0.20 mm) was measured on a Rigaku Saturn 724 CCD area diffractometer equipped with a graphite-monochromatic Mo $\text{K}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). The crystal structure has been deposited at the Cambridge Crystallographic Data Centre (deposition number CCDC 1410668). The results reveal that probe **1** crystallizes with 0.64 CHCl_3 solvent molecule (Figure 1). The crystal belongs to monocline system with $P2_1/c$ space group.

2.2 UV-Vis spectral responses

The UV-Vis absorption spectra of **1** with adding different

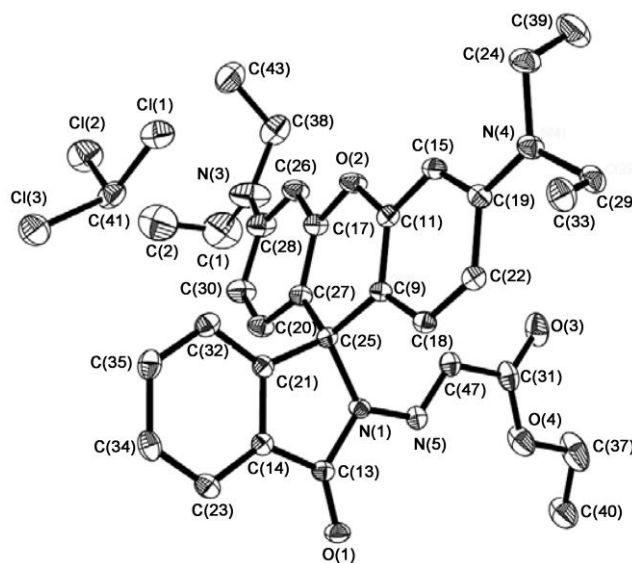


Figure 1 Single crystal structure of **1**

concentrations of Hg^{2+} was carried out in ethanol-water ($V/V=7/3$) buffer (HEPES 10 mmol/L, $\text{pH}=7.0$), and the results were shown in Figure 2. The solution of **1** was colorless and exhibited no absorption peak in the visible wavelength range (450–600 nm). After addition of Hg^{2+} , characteristic absorption peak at 560 nm appeared and gradually increased with addition of increasing concentration of Hg^{2+} , which demonstrated the formation of the ring-opened form of **1** upon Hg^{2+} binding. Meanwhile, an obvious color change from colorless to pink occurred, suggesting that **1** can be used as a “naked-eye” probe for Hg^{2+} in test systems (Figure 2, inset). Job’s method for the absorbance was used to define the stoichiometry of the **1**- Hg^{2+} complex by keeping total concentration of Hg^{2+} and **1** at 10 $\mu\text{mol/L}$ and changing the molar ratio of **1** from 0 to 1. As shown in Figure 3a, a maximum absorption occurred when the molar fraction of [**1**] vs. ([**1**] + [Hg^{2+}]) was 0.50, which revealed that **1** and Hg^{2+} formed a complex with the molar ratio of 1 : 1. Moreover, ESI-MS analysis also confirmed the 1 : 1 binding model. **1** has a peak at m/z 541.46 ($\text{M} + \text{H}^+$), whereas a peak at m/z 803.91 shows up upon the addition of Hg^{2+} , which corresponds to $[\text{M} + \text{Hg}^{2+} + \text{NO}_3^-]$ (calcd 804.23). Thus, the proposed binding model of **1** with Hg^{2+} was given in Figure 3b.

2.3 Fluorescence spectral responses

Fluorescence titration of **1** with Hg^{2+} was investigated in ethanol-water ($7/3=V/V$) buffer (HEPES 10 mmol/L, pH 7.0) (Figure 4). The free **1** showed almost no fluorescent emission. Upon addition of Hg^{2+} to the solution of **1**, an emission peak value at 583 nm was observed and fluorescence intensity gradually increased with the increase of Hg^{2+} concentration. The fluorescence intensity reached a peak after addition of 8.0 equiv. of Hg^{2+} with a 49-fold fluorescence intensity enhancement. The fluorescent response of probe **1** towards Hg^{2+} was calculated to cover a linear range from 0 to 50 $\mu\text{mol/L}$ ($R^2=0.9955$) (Figure 4

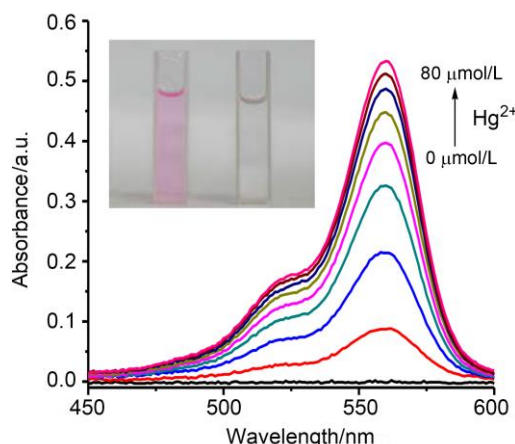


Figure 2 UV-Vis absorption titration spectra of **1** (10 $\mu\text{mol/L}$) with Hg^{2+} (0~8.0 equiv.) in ethanol-water (V/V=7/3) buffer (HEPES 10 mmol/L, pH 7.0)

Inset: the photograph shows the color change from free **1** (right) to **1**+ Hg^{2+} (left) under normal light

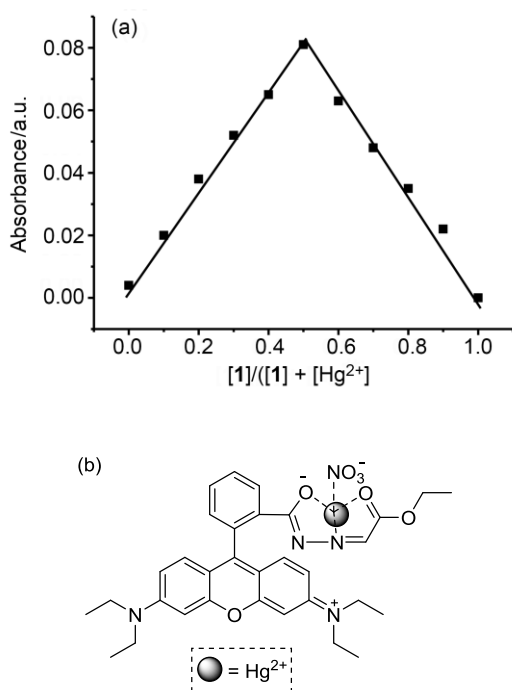


Figure 3 (a) Job's plot of **1** and Hg^{2+} and (b) the proposed binding model of **1** with Hg^{2+}

inset), which can be applied to the quantitative analysis of Hg^{2+} in this range.

The selectivity of probe **1** to various competing ions was also performed in ethanol-water (V/V=7/3) buffer (HEPES 10 mmol/L, pH=7.0). The addition of 1.0 equiv. of other metal ions (Fe^{3+} , Co^{2+} , Mg^{2+} , Zn^{2+} , Al^{3+} , Ni^{2+} , Cr^{3+} , Mn^{2+} , Ca^{2+} , Li^{+} , Cd^{2+} and Cu^{2+}) to the solution of **1** (10 $\mu\text{mol/L}$) showed no color change and no fluorescence. Upon the addition of Hg^{2+} , a visible pink color appeared and orange fluorescence was observed. As shown in Figure 5, only Hg^{2+} could induce a fluorescence increase, whereas the other metal ions did not show this behavior, suggesting that

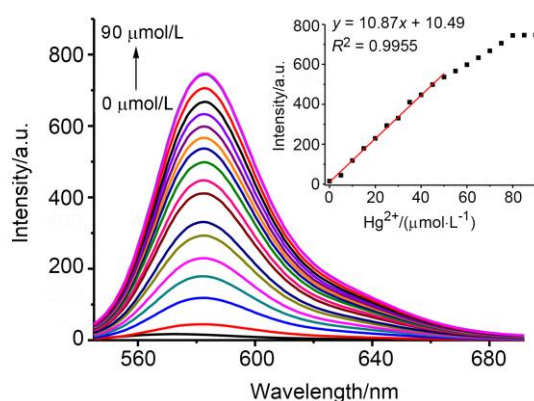


Figure 4 Fluorescence emission spectra of **1** (10 $\mu\text{mol/L}$) upon titration of Hg^{2+} (0~9.0 equiv.) in ethanol-water (V/V=7/3) buffer (HEPES 10 mmol/L, pH 7.0) (λ_{ex} =530 nm, λ_{em} =583 nm) Inset: the linear relationship between $[\text{Hg}^{2+}]$ and fluorescence intensity of **1**

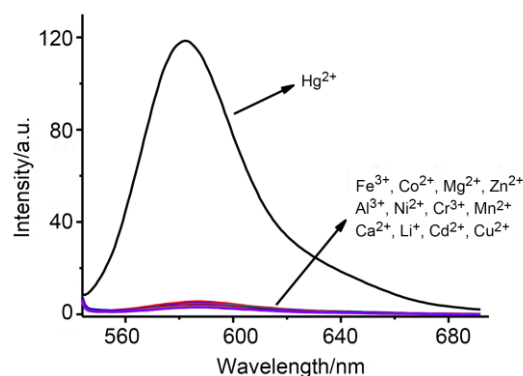


Figure 5 Fluorescence spectrum of probe **1** (10 $\mu\text{mol/L}$) upon addition of 1.0 equiv. Fe^{3+} , Co^{2+} , Mg^{2+} , Zn^{2+} , Al^{3+} , Ni^{2+} , Cr^{3+} , Mn^{2+} , Ca^{2+} , Li^{+} , Cd^{2+} , Cu^{2+} , Hg^{2+} in ethanol-water (V/V=7/3) buffer (HEPES 10 mmol/L, pH 7.0) (λ_{ex} =530 nm)

probe **1** is highly selective for Hg^{2+} over the other metal ions. To investigate the practical applicability of **1** for Hg^{2+} , the interference experiments were carried out with the excitation fixed at 530 nm and the emission at 583 nm (Figure 6). No obvious fluorescence intensity changes of probe **1** were observed upon addition of common interferences such as Fe^{3+} , Al^{3+} , Cr^{3+} , Al^{3+} , and so on. According to Figure 3b, the ester group of probe **1** may contribute to the high selectivity.

Furthermore, the effect of pH on the fluorescence intensity of **1** in the absence and presence of Hg^{2+} was explored at a pH range from 3.0 to 9.0. The fluorescence intensity at 583 nm of free probe **1** was not affected by a pH range of 5.0~8.5, and that of **1**- Hg^{2+} did not vary with pH in the range of 6.5~8.5. The results suggested that probe **1** can be employed to monitor Hg^{2+} under physiological pH conditions.

2.4 Cell cytotoxicity and fluorescence microscopy imaging

The *in vitro* cytotoxicity of probe **1** was evaluated using a MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay by measuring anti-proliferation

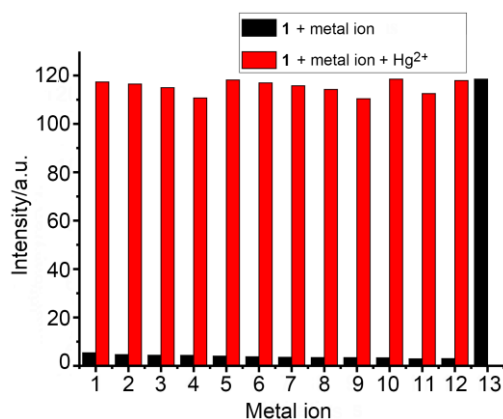


Figure 6 Fluorescence intensity of **1** (10 $\mu\text{mol/L}$) after addition of Hg^{2+} (10 $\mu\text{mol/L}$) in the presence of other metal ions (10 $\mu\text{mol/L}$) in ethanol-water ($V/V=7/3$, HEPES 10 mmol/L, pH 7.0) $\lambda_{\text{ex}}=530$ nm, from left to right (1) Fe^{3+} , (2) Co^{2+} , (3) Mg^{2+} , (4) Zn^{2+} , (5) Al^{3+} , (6) Ni^{2+} , (7) Cr^{3+} , (8) Mn^{2+} , (9) Ca^{2+} , (10) Li^{+} , (11) Cd^{2+} , (12) Cu^{2+} , (13) Hg^{2+}

on MGC-803 cells. A cytotoxicity screen was conducted in the concentration of 0~100 $\mu\text{mol/L}$. The cellular viability estimated was *ca.* 97% and 54% in 24 h after treatment with 10 and 80 $\mu\text{mol/L}$ **1**, respectively, and the IC_{50} value was calculated as 83.44 $\mu\text{mol/L}$. Due to the above-mentioned characteristics of probe **1**, bioimaging applications of **1** for detecting of Hg^{2+} in living cells were then explored. After MGC-803 cells were cultured with a solution of 10 $\mu\text{mol/L}$ probe **1** for 15 min at 37 $^{\circ}\text{C}$ and then washed with phosphate-buffered saline (PBS) three times, no evidence of fluorescence appeared (Figure 7b). After further incubation with 80 $\mu\text{mol/L}$ $\text{Hg}(\text{NO}_3)_2$ for another 15 min at 37 $^{\circ}\text{C}$, the red fluorescence was observed (Figure 7d). The bright field image of MGC-803 cells proved that probe **1** was practical on cell imaging experiments (Figures 7a and 7c). It was proven that probe **1** is cell-permeable and may be used for monitoring Hg^{2+} in living cells.

3 Conclusions

In summary, we have developed an effective rhodamine B-ethyl glyoxalate based turn-on fluorescent probe for Hg^{2+} in aqueous media and living cells with high sensitivity and selectivity. The 1 : 1 binding stoichiometry between probe **1** and Hg^{2+} was established by Job's plot and MS spectra. Furthermore, it can be applied to the quantitative analysis of Hg^{2+} in the range of 0~50 $\mu\text{mol/L}$. Most importantly, the probe was effectively used in cell imaging, indicating its promising value in the practical application in biological systems.

4 Experimental section

4.1 Materials and instruments

All analytical grade chemicals were used as received from commercial suppliers. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE II 400 MHz instrument in CDCl_3 with TMS as an internal standard. Mass

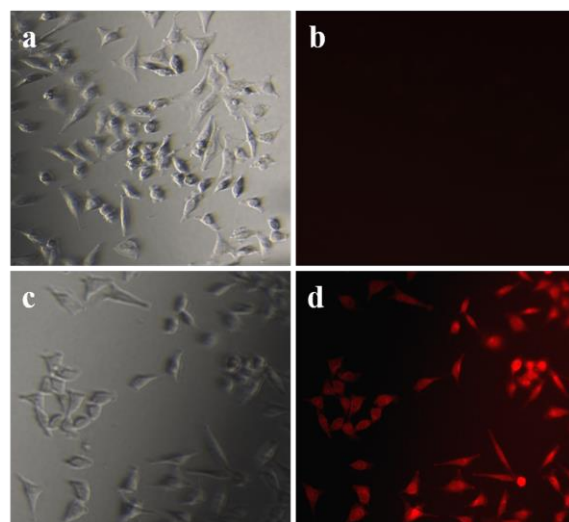


Figure 7 Fluorescence images of Hg^{2+} in MGC-803 cells with 10 $\mu\text{mol/L}$ probe **1** for 15 min

Bright-field transmission images (a and c) and fluorescence images (b and d) of MGC-803 cells incubated with 0, 80 $\mu\text{mol/L}$ Hg^{2+} for 15 min, respectively

spectra were obtained from a Thermo LXQ Liquid Chromatography-Ion Trap Mass Spectrometry. The single-crystal structure was determined on a Rigaku Saturn 724 CCD area-detector diffractometer. The UV-vis absorption spectra were obtained on a Shimadzu UV-2450 scanning spectrophotometer. All fluorescence spectra were measured with a Varian Cary Eclipse spectrophotometer. Fluorescence images of MGC-803 cells were carried out with an inverted fluorescence microscope (Carl Zeiss, Axio Observer A1).

4.2 Synthesis

4.2.1 Synthesis of rhodamine B hydrazide **2**

As showed in Scheme 1, rhodamine B hydrazide **2** was synthesized following the procedure described in our previous work.^[35] m.p. 175~176 $^{\circ}\text{C}$ (Lit.^[35] 175~176 $^{\circ}\text{C}$); ^1H NMR (400 MHz, CDCl_3) δ : 7.95~7.92 (m, 1H), 7.48~7.42 (m, 2H), 7.13~7.08 (m, 1H), 6.50~6.40 (m, 4H), 6.36~6.24 (m, 2H), 3.61 (s, 2H), 3.34 (q, $J=7.2$ Hz, 8H), 1.16 (t, $J=7.2$ Hz, 12H). ESI-MS m/z : 457.49 (20, $[\text{M}+\text{H}^+]$), 935.41 (100, $[2\text{M}+\text{Na}^+]$).

4.2.2 Synthesis of compound **1**

The mixture of rhodamine B hydrazide **2** (0.46 g, 1.0 mmol), ethyl glyoxalate (*ca.* 50% solution in toluene, 2 mL), anhydrous sodium sulfate (1.0 g) and methanol (10 mL) was stirred overnight at room temperature. The solvent was removed under reduced pressure to obtain the crude product. After recrystallization from ethyl acetate, 0.33 g of **1** was collected as a pale pink solid (yield 61%), m.p. 142~143 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ : 8.04 (d, $J=7.2$ Hz, 1H), 7.54~7.45 (m, 2H), 7.16 (s, 1H), 7.07 (d, $J=7.2$ Hz, 1H), 6.52~6.36 (m, 4H), 6.28~6.24 (m, 2H), 4.22 (q, $J=7.2$ Hz, 2H), 3.34 (q, $J=7.2$ Hz, 8H), 1.31 (t, $J=7.2$ Hz, 3H), 1.18 (t, $J=7.2$ Hz, 12H); ^{13}C NMR (100 MHz, CDCl_3) δ : 165.7, 163.7, 153.2, 152.5, 149.2, 134.6, 133.4, 128.4, 127.5, 126.4, 124.0, 123.8, 108.2, 104.0, 98.2, 65.5, 61.4,

44.3, 14.1, 12.6. ESI-MS m/z : 541.46 $[M+H]^+$.

4.3 Preparation of titration

Solutions of different metal salts were obtained by dissolving corresponding nitrate salts in deionized water. Deionized water was used throughout the work. HEPES buffer solutions (10 mmol/L, pH=7.0) were prepared in water. 1 mmol/L stock solution of **1** was performed by dissolving **1** in ethanol.

4.4 Cytotoxicity assay and cell imaging

The MGC-803 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). 24 h before imaging, the cells were seeded into 24 well plates. The cells were incubated with 10 $\mu\text{mol/L}$ **1** for 15 min at 37 $^{\circ}\text{C}$, washed three times with PBS and imaged. Then the cells were supplemented with 80 $\mu\text{mol/L}$ Hg^{2+} for 15 min at 37 $^{\circ}\text{C}$, washed three times with PBS and imaged, which was observed by an inverted fluorescence microscope.

Cytotoxicity of **1** in cells was assessed by MTT assay. MGC-803 cells were seeded into 96-well cell culture plate, and cultured at 37 $^{\circ}\text{C}$ with 5% CO_2 for 24 h. After removing DMEM with 10% FBS, new DMEM and different concentrations of **1** were added into plates. The cells were incubated at 37 $^{\circ}\text{C}$ with 5% CO_2 for another 24 h. After that, 100 μL of MTT (5 mg/mL) was added to each well and incubated for additional 4 h at 37 $^{\circ}\text{C}$ with 5% CO_2 . Then cells were dissolved in 600 μL of dimethyl sulfoxide (DMSO) per well, and MTT formazan was recorded by a microplate reader. The cell viability was calculated as the equation: cell viability(%) = $\text{OD}_{\text{sample}}/\text{OD}_{\text{control}} \times 100\%$.

IC_{50} value of **1** against MGC-803 was calculated with SPSS 16.0 version (SPSS Inc., Chicago, USA).

Supporting Information ^1H NMR and MS spectra of **2**, ^1H NMR, ^{13}C NMR and MS spectra of probe **1**, MS spectra of 1-Hg^{2+} , the colorimetric and fluorometric changes of **1**, effect of pH on the fluorescence intensity of **1** and cell viability of **1**. The Supporting Information is available free of charge via the Internet at <http://sioc-journal.cn/>.

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