

# New Application of a Known Molecule: Rhodamine B 8-hydroxy-2-quinolinecarboxaldehyde Schiff Base as a Colorimetric and Fluorescent “Off-On” Probe for Copper (II)

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Received: 13 January 2012 / Accepted: 27 June 2012 / Published online: 7 July 2012  
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**Abstract** Rhodamine B 8-hydroxy-2-quinolinecarboxaldehyde Schiff base (**1**) has been investigated as a colorimetric and fluorescent “off-on” probe for the recognition of  $\text{Cu}^{2+}$  in aqueous solution. Probe **1** was synthesized by condensation of rhodamine B hydrazide and 8-hydroxy-2-quinolinecarboxaldehyde, which exhibited good selectivity for  $\text{Cu}^{2+}$  among a range of biologically and environmentally important metal ions. The  $\text{Cu}^{2+}$  recognition event undergoes a  $\text{Cu}^{2+}$  promoted hydrolysis of probe **1** to release rhodamine B and the recognition process is barely interfered by other coexisting metal ions.

**Keywords** Fluorescence · Probe · Copper(II) recognition · Rhodamine

## Introduction

Highly selective and sensitive detection of transition and heavy metal ions by artificial chemoprobes is an

increasingly important research area [1–4]. Among the various detection methods available, UV–Vis absorption and fluorescence spectroscopy still remain the most frequently used modes due to their high sensitivity and easy operational use [5].

As an essential trace element for plants and animals, copper plays a crucial role in many fundamental physiological processes in organisms. It is well known that it serves as a catalytic cofactor for a variety of metalloenzymes such as superoxide dismutase, cytochrome c oxidase and tyrosinase. However, overloading of copper resulting toxicity associated with neurodegenerative diseases such as Alzheimer’s [6, 7] or Parkinson’s diseases [8, 9]. In addition, it has been suspected to cause infant liver damage in recent years [10, 11].

Rhodamines are the ideal platforms for development of chemoprobes for specific heavy and transition metal ions due to their excellent spectroscopic properties such as long-wavelength emission, high fluorescence quantum yield, and large molar extinction coefficient [12]. Recently, utilization of rhodamine spirolactam ring-opening process for the detection of metal ions has been well documented [13]. The rhodamine spirolactam form is basically colorless and non-fluorescent, while its metal induced ring-opened amide form has strong absorption within the visible range and usually displays strong fluorescence emission. As a consequence, much focus has been devoted to the development of rhodamine based probes for the recognition of  $\text{Cu}^{2+}$  [14–20].

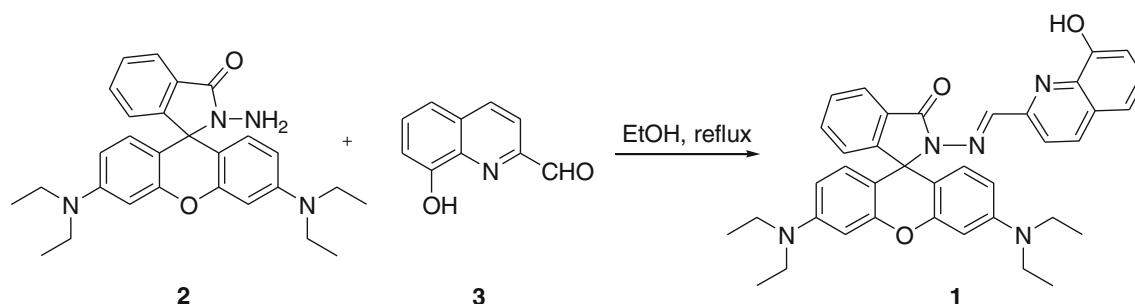
Herein, we report the  $\text{Cu}^{2+}$  recognition properties of a rhodamine B based probe, rhodamine B 8-hydroxy-2-

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**Electronic supplementary material** The online version of this article (doi:10.1007/s10895-012-1101-x) contains supplementary material, which is available to authorized users.

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**Scheme 1** Synthesis of probe **1**

quinolinecarboxaldehyde Schiff base (**1**) (Scheme 1). Probe **1** exhibits highly selective recognition of  $\text{Cu}^{2+}$  via colorimetric and fluorescent detection, respectively.

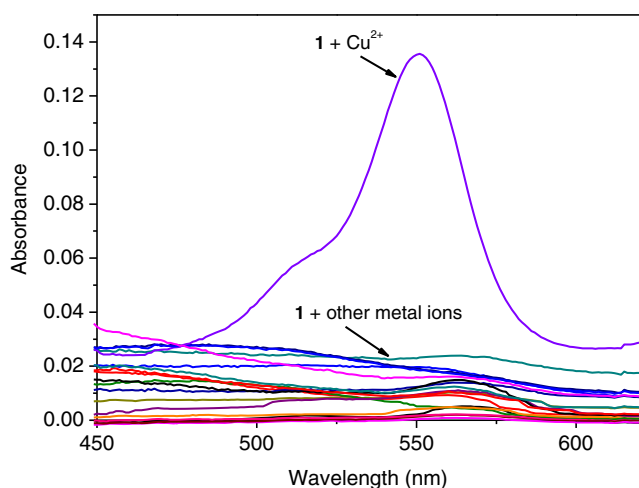
## Experimental

**General Methods and Materials** All the solvents were of analytical grade from commercial sources and used without further purification. Column chromatography was performed on silica gel (200–300 mesh). UV–vis absorbance spectra were measured on a SP-1900 spectrophotometer. Fluorescence measurements were performed on a 970 CRT spectrofluorometer (Shanghai Sanco, China). High resolution mass spectrum was measured on an Agilent 1200 time-of-flight mass spectrometer (Bruker, micrOTOF-Q). The pH measurements

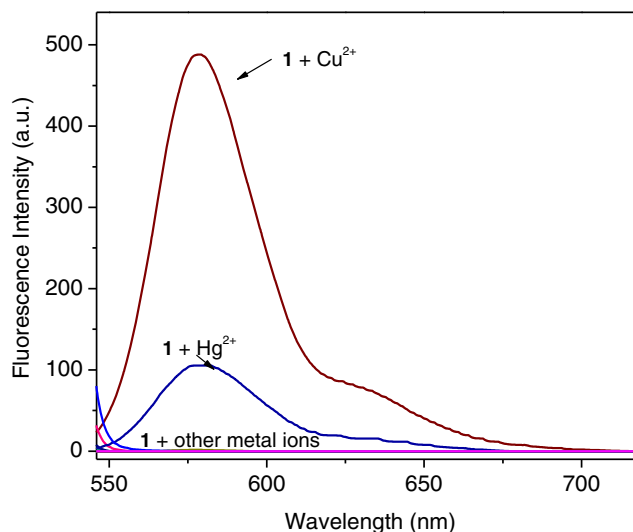
were made with a Model phs-25B meter. Compound **1** was prepared according to the literature method [21].

## Results and Discussion

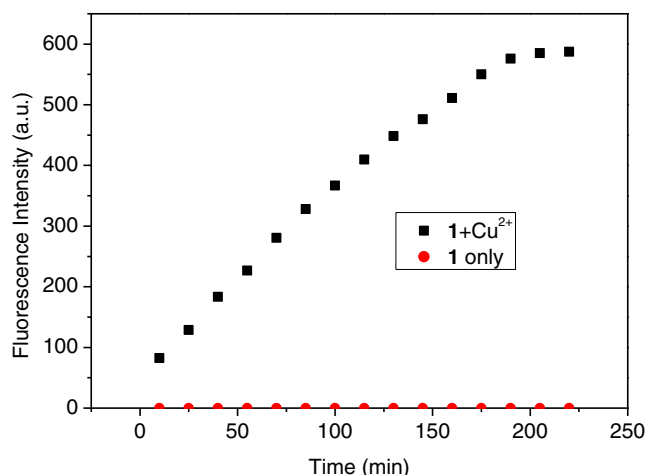
Compound **1** [21] has been synthesized as an intermediate for the preparation of some fluorescence resonance energy transfer (FRET) fluorescence probes. It is worth noting that **1** itself is also a promising probe for the sensing of certain metal ions because 8-hydroxyquinoline moiety has been frequently employed as a platform for the construction of functional ionophores for the recognition of metal ions [22, 23] and the structural similarity of **1** to some known rhodamine type metal ion sensors [24–26]. Thus, compound **1** was pre-



**Fig. 1** Absorbance changes of **1** ( $1.0 \times 10^{-5}$  M) solution ( $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ , 1:1, v/v, HEPES 10 mM, pH=7.0) upon addition of various metal ions ( $2.5 \times 10^{-4}$  M of each)



**Fig. 2** Fluorescence changes of **1** ( $1.0 \times 10^{-5}$  M) solution ( $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ , 1:1, v/v, HEPES 10 mM, pH=7.0) in the presence of various metal ions ( $2.5 \times 10^{-4}$  M of each, excited at 530 nm)



**Fig. 3** Time dependent of fluorescence intensity changes of **1** and **1** +  $\text{Cu}^{2+}$

pared by condensation of rhodamine B hydrazide (**2**) and 8-hydroxyquinoline-2-aldehyde (**3**) (Scheme 1) according to the previously method [21], its potential metal ion recognition behavior was subsequently explored.

#### Metal ion Selectivity

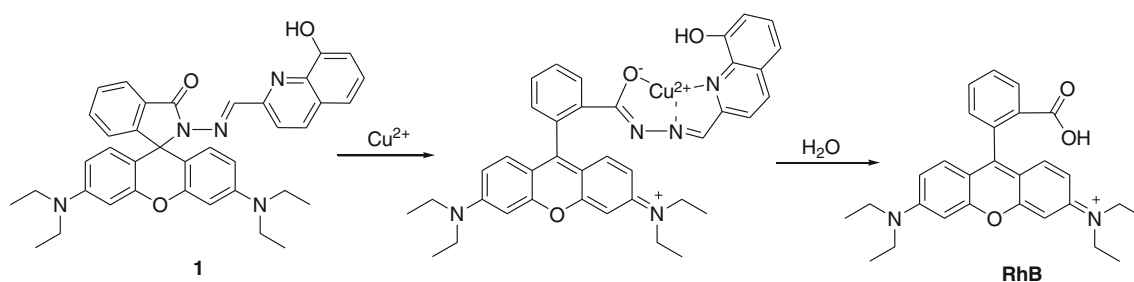
After screening of a series of solvent systems,  $\text{CH}_3\text{CN}-\text{H}_2\text{O}$  (1:1, v/v, HEPES 10 mM, pH=7.0) co-solvent was selected for the investigation of optical properties. Firstly, the metal ion selectivity of **1** was conducted. **1** itself in solution ( $1.0 \times 10^{-5}$  M) showed no absorption band above 500 nm and has no fluorescence emission because the ring closed form of rhodamine spirolactam is prevail. Upon addition of 25 equiv. of  $\text{Cu}^{2+}$  into **1** solution, a strong absorption band centered at 551 nm appeared (Fig. 1) accompanied with a color change from colorless to pink (Left part of Abstract Fig.). The colored solution also presents strong fluorescence emission intensity centered at 578 nm (Fig. 2). Addition of other tested metal ions such as  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ag}^+$ ,

$\text{Ba}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  can not cause any apparent color and fluorescence emission changes (Fig. 2). It is noteworthy that addition of  $\text{Hg}^{2+}$  induced a slight fluorescence enhancement, however, the  $\text{Hg}^{2+}$  induced fluorescence intensity enhancement is far below that caused by  $\text{Cu}^{2+}$  ( $F_{\text{Cu}}/F_{\text{Hg}}=4.6$ ) under the same conditions, which indicating the lower affinity of  $\text{Hg}^{2+}$  to **1**.

The  $\text{Cu}^{2+}$  induced gradually fluorescence intensity changes of the solution urged us to study the effect of time on response of probe **1** to  $\text{Cu}^{2+}$ . The time course study revealed that the recognition event could complete in 3 h (Fig. 3). Thus, in our work, each sample was checked after 3 h after the addition of metal ions.

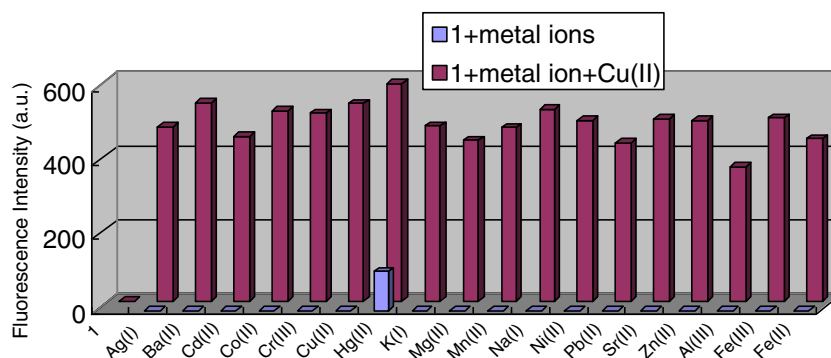
#### Recognition Mechanism

To examine the reversibility of the so-produced spectroscopic signals, excess ethylenediamine tetraacetic acid disodium salt ( $\text{EDTANa}_2$ ) as  $\text{Cu}^{2+}$  chelating agent was added to the colored solution of **1**- $\text{Cu}^{2+}$ . However, both the color and fluorescence intensity of the solution were scarcely changed. These results indicate the irreversible feature of the recognition process. A control experiment using anhydrous  $\text{CH}_3\text{CN}$  instead of  $\text{CH}_3\text{CN}-\text{H}_2\text{O}$  (1:1, v/v, HEPES 10 mM, pH=7.0) buffered solution was conducted. Without water, the previously described color change and fluorescence turn on processes could not occur. These contrary results in the presence and absence of water suggest that the  $\text{Cu}^{2+}$  recognition event is associated with probe hydrolysis. The reaction product of a high concentrated detection solution (0.1 mM of **1** + 25 equiv. of  $\text{Cu}^{2+}$ ) was proved to be rhodamine B by thin layer chromatography (TLC) and high resolution ESI-MS analysis. The main ion peak detected at  $m/z$  443.2321 ( $[\text{M}+\text{H}]^+$ ) presented a solid evidence for the generation of rhodamine B as a final product (Fig. S1). Similar to some reported rhodamine B based chemodosimeters [27, 28], a  $\text{Cu}^{2+}$  promoted hydrolysis of **1** to release rhodamine B is responsible for its dual recog-



**Scheme 2** Proposed mechanism for  $\text{Cu}^{2+}$  induced hydrolysis of **1**

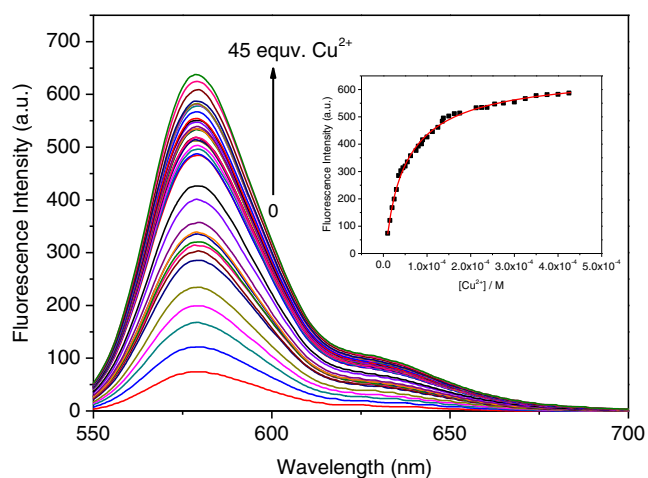
**Fig. 4** Fluorescence emission changes of **1** ( $1.0 \times 10^{-5}$  M) solution to various metal ions in  $\text{CH}_3\text{CN-H}_2\text{O}$  (1:1, v/v, HEPES 10 mM, pH=7.0). The blue bars represent the fluorescence of **1** in the presence of 25 equiv. of different metal ions; the red bars represent the fluorescence of the above solution upon the addition of 25 equiv. of  $\text{Cu}^{2+}$



nitration behaviors to  $\text{Cu}^{2+}$ . The proposed mechanism for  $\text{Cu}^{2+}$  induced hydrolysis of **1** is depicted in Scheme 2.

### Competitive Experiments

As a probe, achieving highly selective response to the target analyte over a wide range of potentially competing ions is an important requirement. Then, the fluorescence competition experiments in the presence of potentially competitive metal ions, such as alkali or alkaline-earth metals ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$ ), heavy and transition metal ions ( $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Ag}^+$ ) as well as  $\text{Al}^{3+}$  were conducted. As shown in Fig. 4, all other metal ions (25 equiv. to **1**) did not induce significant fluorescence emission changes. However, upon addition of  $\text{Cu}^{2+}$  (25 equiv.) to the solution containing **1** and a certain competitive metal ion, a dramatic increase in fluorescence intensity was occurred. These results revealed that the recognition of  $\text{Cu}^{2+}$  by **1** is not significantly perturbed by other coexisting metal ion, therefore, **1** exhibits a high selectivity toward  $\text{Cu}^{2+}$ .



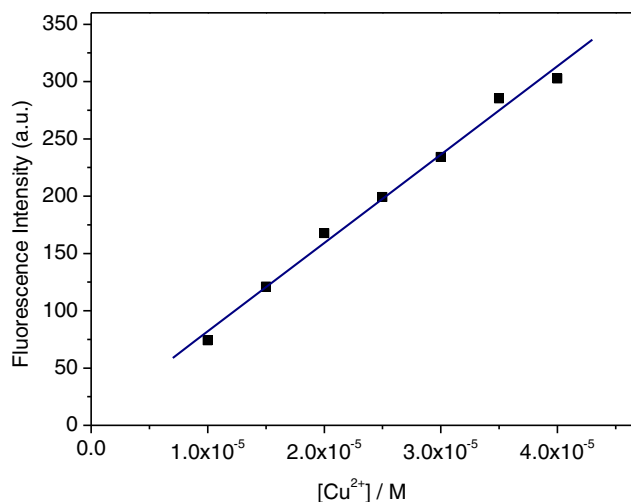
**Fig. 5** Fluorescence spectrum changes of **1** ( $1.0 \times 10^{-5}$  M) solution ( $\text{CH}_3\text{CN-H}_2\text{O}$ , 1:1, v/v, HEPES 10 mM, pH=7.0) upon addition of different amounts of  $\text{Cu}^{2+}$  (0–45 equiv, excited at 530 nm). Inset: The plot of fluorescence intensity changes against the  $\text{Cu}^{2+}$  concentration

### Fluorescence Titrations and Detection Limit

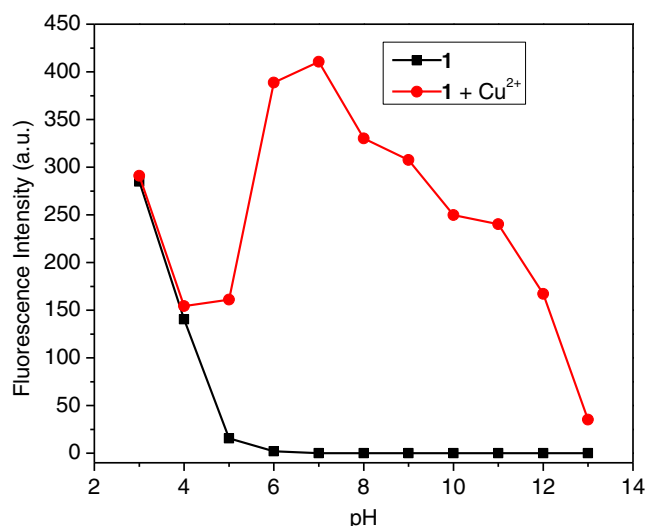
Subsequently, titration of **1** solution with different amounts of  $\text{Cu}^{2+}$  was carried out. Upon incremental addition of  $\text{Cu}^{2+}$  (0 to 45 equiv) to **1** solution ( $1.0 \times 10^{-5}$  M), the fluorescence emission band centered at 578 nm gradually increased and reached the saturation when 45 equiv. of  $\text{Cu}^{2+}$  was added (Fig. 5). Probe **1** showed a linear fluorescence response within the  $\text{Cu}^{2+}$  concentration range of  $1.0 \times 10^{-5}$  M to  $4.0 \times 10^{-5}$  M and the detection limit was calculated to be  $5.3 \times 10^{-6}$   $\text{M}^{-1}$  (Fig. 6).

### Effect of pH

To get further insight into the practicability of the probe, the effect of pH on the fluorescence of **1** was explored. As shown in Fig. 7, probe **1** alone has no effective fluorescence between pH 5 and 13, but its fluorescence increased distinctly when the pH value is smaller than 5.5. In the presence of  $\text{Cu}^{2+}$ , the fluorescence intensity of **1** solution increased remarkably between pH 6 and 8. These results strongly advocate that **1** is suitable for detection of  $\text{Cu}^{2+}$  at near neutral pH conditions.



**Fig. 6** Fluorescence intensity changes of **1** versus the concentration of  $\text{Cu}^{2+}$  at low concentration range



**Fig. 7** Effect of pH on fluorescence intensity of **1** ( $1.0 \times 10^{-5}$  M) solution ( $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ , 1:1, v/v) in the absence and presence of  $\text{Cu}^{2+}$

## Conclusions

In summary, we have developed a new application of rhodamine B 8-hydroxy-2-quinolinecarboxaldehyde Schiff base (**1**) as a dual mode probe for recognition of  $\text{Cu}^{2+}$ . Probe **1** exhibits highly selective colorimetric and fluorescent recognition of  $\text{Cu}^{2+}$  in  $\text{CH}_3\text{CN}-\text{H}_2\text{O}$  (1:1, v/v, HEPES 10 mM, pH=7.0) solution. The  $\text{Cu}^{2+}$  recognition event undergoes a  $\text{Cu}^{2+}$  promoted hydrolysis of probe **1** to release rhodamine B and the recognition process is barely interfered by other coexisting metal ions.

**Acknowledgements** We are grateful to the NSFC (21176029), the Natural Science Foundation of Liaoning Province (20102004) for financial support. The project is also sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

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