Chinese Chemical Letters 27 (2016) 813–816

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

A rhodamine derivative for Hg^{2+}-selective colorimetric and fluorescent sensing and its application to *in vivo* imaging

Xue-Mei Li [a], Rui-Rui Zhao [b], Yu-Ling Wei [a], Dan Yang [b], Zhang-Jian Zhou [c], Jun-Feng Zhang [c,*], Ying Zhou [b,*]

[a] Department of Safety Evaluation, Technology Center of China Tobacco Yunnan Industrial Co., Ltd., Kunming 650106, China
[b] College of Chemical Science and Technology, Yunnan University, Kunming 650091, China
[c] College of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming 650500, China

ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form 15 March 2016
Accepted 21 March 2016
Available online 11 April 2016

Keywords:
Chemsensor
Mercury
Rhodamine
Colorimetric
Fluorescence
In vivo imaging

ABSTRACT

A rhodamine-based sensor has been developed for the detection of mercuric ions. The colorimetric and fluorescence responses, allowing naked-eye detections, are based on Hg^{2+}-induced opening of the rhodamine spirocycle. Among all the testes ions, only Hg^{2+} generated a significant fluorescence enhancement of up to 300-fold, with a bright yellow–green emission. This sensor was a low toxic compound, and was successfully applied in the *in vivo* imaging of Hg^{2+} in Spill 2 cells and *C. elegans*. This approach provides a sensitive and accurate method for the estimation of Hg^{2+} in environmental, tobacco and biological applications.

1. Introduction

Mercury is one of the most toxic heavy metals found in aquatic systems, and it has become a worldwide issue in recent years due to its severe risk to human health and to the environment. Compared with many of the current techniques for mercury detection, such as atomic absorption spectrometry, inductively coupled plasma mass spectroscopy, spectrophotometry, neutron activation analysis, anodic stripping voltammetry and X-ray fluorescence spectrometry, fluorescence sensing provide simple, safe, effective and rapid detection of Hg^{2+}, paving the way for its applications [1]. Even a number of research groups have reported their recent achievements on important ion-targeted or anion-targeted fluorescent sensors [2], the development of organic probes for sensing of environmentally hazardous Hg^{2+} ions is still of great importance due to their implications in broad areas.

Herein, we have designed and synthesized a simple and easy to prepare fluorescent chemosensor (**1**) for the Hg^{2+} ion sensing. In molecule **1**, rhodamine group acts as a fluorophore group, with a hydroxyquinoline group as a recognition group, allowing the coordination capacity required to chelate mercury ions. Sensor **1** showed colorimetric and fluorescent selectivity for Hg^{2+} in DMSO–HEPES buffer (0.02 mol/L, pH 7.4; v/v = 6:4) solution over other common physiologically important metal ions. It showed that sensor **1** was a low toxic compound, and was successfully applied in the *in vivo* imaging of Hg^{2+} in Spill 2 cells and *C. elegans*.

2. Experimental

2.1. Reagents and chemicals

All reagents were purchased from commercial sources and were used without further purification. Flash chromatography was carried out on silica gel (230–400 mesh). ^{1}H NMR spectra (DMSO-d_6) were recorded using Ascend ^{TM} 400 spectrometer; ^{13}C NMR spectra (DMSO-d_6) were recorded using Avance ^{III} 400 spectrometer; mass spectrometry was recorded with Xevo TQ-S mass spectrometer. The UV–vis spectrum was obtained using UV-240IPC spectrophotometer. The fluorescence spectra were obtained with F-4500 FL spectrometer with a 1 cm standard quartz cell. The cells were imaged using an Olympus IX71 inverted fluorescence microscopy. The mounted nematodes were imaged using an Olympus BX51 inverted fluorescence microscopyll reagents were of analytical grade or the best grade commercially

* Corresponding authors.
E-mail addresses: junfengzhang78@aliyun.com (J.-F. Zhang), yingzhou@ynu.edu.cn (Y. Zhou).

http://dx.doi.org/10.1016/j.cclet.2016.04.001

available, and were put into use without further purification. Deionized water was used throughout. HEPES buffer solutions (0.02 mol/L, pH 7.4) were prepared in deionized water. Analyte solutions of the perchlorate of Al^{3+}, Na^{+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ag^{+}, Fe^{3+}, Hg^{2+} and Cr^{3+} were prepared by dissolving the salts in distilled water to final concentrations of 0.1 mol/L.

2.2. Synthesis of probe 1

Compound **2** was synthesized from 2-methyloxine by the procedure published in literature [3]. Compound **3**, as a known rhodamine 6G derivative, was synthesized as described previously [4]. Compounds **2** (210 mg, 1.2 mmol) and **3** (0.307 mg, 0.8 mmol) were mixed in boiling ethanol with three drops of acetic acid (Scheme 1). After 8 h of stirring, the pink precipitate formed was removed by filtration, washed with ethanol/diethyl ether (1:1), and purified by silica gel column chromatography to afford orange–yellow solid product **1** (230 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.87 (s, 1H), 8.69 (s, 1H), 8.24–8.22 (d, 1H, J = 8.8 Hz), 7.98–7.96 (d, 1H, J = 7.2 Hz), 7.87–7.85 (d, 1H, J = 8.8 Hz), 7.62–7.57 (m, 2H), 7.40–7.38 (d, 2H, J = 7.6 Hz), 7.33–7.31 (d, 1H, J = 8.0 Hz), 7.09–7.05 (t, 2H, J = 7.2 Hz), 6.40 (s, 2H), 6.27 (s, 2H), 5.10 (s, 2H), 3.17–3.12 (m, 4H), 1.85 (s, 6H), 1.23–1.19 (t, 6H, J = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.87, 153.90, 152.51, 152.39, 151.26, 148.35, 145.98, 138.54, 137.00, 134.80, 129.29, 129.24, 128.71, 127.96, 126.93, 124.16, 123.82, 118.86, 118.26, 117.46, 112.79, 105.10, 96.58, 66.02, 32.00, 17.44, 14.64. HRMS (ESI, m/z): calcd. for $C_{36}H_{33}N_5O_3$ 584.2583 $[M+H]^+$, 606.2476 $[M+Na]^+$, found 584.2650 $[M+H]^+$; 606.2477 $[M+Na]^+$.

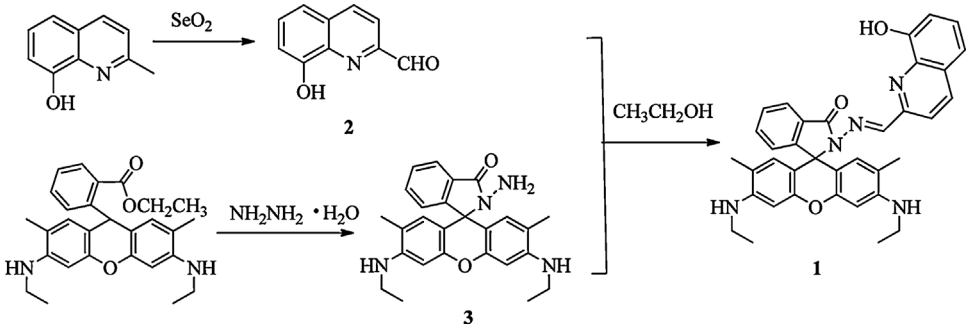
3. Results and discussion

In order to clarify the interaction of **1** with metal ions, the UV–vis absorption spectra of **1** were first studied in DMSO–HEPES buffer (0.02 mol/L, pH 7.4, v/v = 6:4) solutions. Cd^{2+}, Ni^{2+}, Zn^{2+}, Fe^{3+}, Cr^{3+}, Al^{3+}, Ag^{+}, Co^{2+}, Cu^{2+}, and Hg^{2+} were used to measure the selectivity of probe **1**. All spectra were recorded after three minutes upon addition of 25 equiv. of each of these ions. As shown in Fig. 1, compound **1** exhibits no major absorption band. Upon addition of different metal ions, only the presence of Hg^{2+} could lead to an obvious absorption increase at 533 and 662 nm (Fig. 1). It revealed that **1** had a good selectivity toward Hg^{2+} in the absorption among the tested ions. In the titration tests, with the addition of Hg^{2+}, the absorbance at 533 and 662 nm increased sharply, which induced a color change from colorless to pink (Fig. 2c).

Then, Cd^{2+}, Ni^{2+}, Zn^{2+}, Na^{+}, Fe^{3+}, Cr^{3+}, Al^{3+}, Pb^{2+}, Ag^{+}, Co^{2+}, Cu^{2+} and Hg^{2+} were used to evaluate fluorescent selectivity of compound **1** in DMSO–HEPES buffer (0.02 mol/L, pH 7.4, v/v = 6:4) solutions. From the fluorescence experiments (Fig. 3a), clear "off-on" fluorescence changes of **1** to Hg^{2+} were observed. Among the tested metal ions (30 equiv.), **1** showed a selective fluorescence enhancement only with Hg^{2+}, indicating that **1** displayed a high Hg^{2+} selectivity (Fig. 3b).

Compound **1** displays almost no fluorescence. When Hg^{2+} was added to the solution, a significant increase of the fluorescence intensity of 556 nm, which was attributed to the Hg^{2+}-induced ring opening of the spirolactam moiety, was observed. Hg^{2+} generated a significant fluorescence enhancement of up to 300-fold, with a bright yellow–green emission (Fig. 4). These results suggested that **1** has high fluorescence selectivity for Hg^{2+} compared to the other ions.

The proposed binding mechanism of compound **1** with Hg^{2+} is shown in the Scheme 2. The spirolactam moiety of the rhodamine group acts as a signal switcher, when **1** binds Hg^{2+}, the fluorescence-off state of **1** converts to the Hg^{2+}-promoted ring-opened amide form with a fluorescence-on state. Moreover, **1** is most likely to bind Hg^{2+} *via* the imide N and quinoline O atoms like other reported researches [5]. The detection limit was calculated



Scheme 1. Synthetic route of probe **1**.

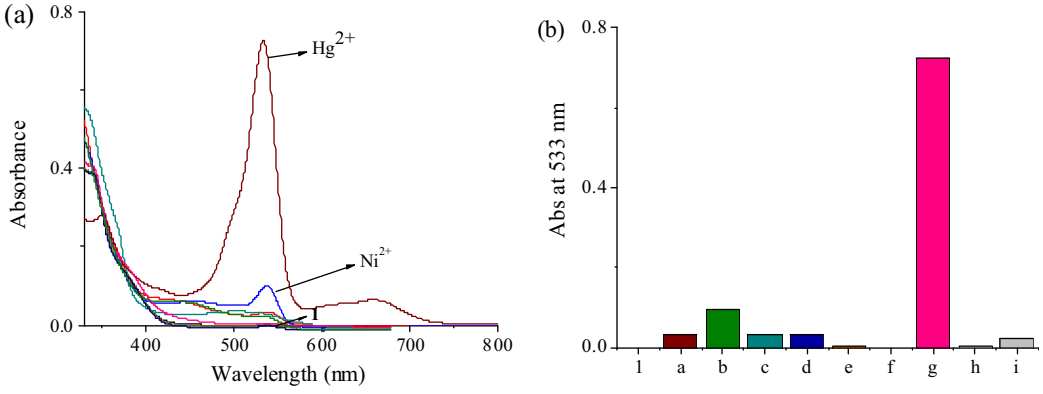


Fig. 1. (a) Absorption spectra of **1** (2.0×10^{-5} mol/L) in DMSO–HEPES buffer (0.02 mol/L, pH 7.4, v/v = 6:4) with 25 equiv. of Fe^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Na^{+}, Al^{3+}, Cd^{2+}, Zn^{2+}, Hg^{2+}; (b) Absorbances of **1** (2.0×10^{-5} mol/L) at 533 nm after addition of 25 equiv. selected ions (1: blank, a: Zn^{2+}, b: Ni^{2+}, c: Cd^{2+}, d: Fe^{3+}, e: Al^{3+}, f: Na^{+}, g: Hg^{2+}, h: Cr^{3+}, i: Co^{2+}).