Research article

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Received: 29 August 2014, Revised: 23 January 2015, Accepted: 28 January 2015 Published online in Wiley Online Library: 11 March 2015

(wileyonlinelibrary.com) DOI 10.1002/bio.2893

An aqueous fluorescent probe for Hg^{2+} detection with high selectivity and sensitivity

Qian Fang,[a] Qian Liu,[b] Xiangzhi Song[a,c,d]* and Jian Kang[e]*

ABSTRACT: An aqueous fluorescent probe, 1, was developed for the rapid detection of Hg^{2+} with high sensitivity and excellent selectivity. Upon the addition of Hg^{2+} in pure aqueous media, the Hg^{2+}-mediated hydrolysis of vinyl ether and subsequent cyclization reactions converted probe 1 into the corresponding iminocoumarin dye, which is strongly fluorescent when excited. The application of this probe for the detection of intracellular Hg^{2+} was successfully demonstrated in living cells. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: fluorescent probe; Hg^{2+}; hydrolysis reaction; vinyl ether; water-soluble

Introduction

Mercury is an extremely toxic element that is naturally occurring or introduced by human activities. Owing to the widespread distribution of mercury in water and soil, the mercury pollution has become a serious problem in the past several decades (1–3). Mercury is strongly thiophilic to enzymes and proteins, which can cause severe damage to the brain, kidney, nervous and digestive systems even at low-level exposure (4–9). Consequently, mercury pollution has become a serious threat to humans and wildlife. Generally, the mercury pollutant exists as Hg^{2+}. Therefore, it is necessary to develop effective methods for the qualitative and quantitative detection of Hg^{2+} in living species and the environment (10,11).

Fluorescence analysis has been widely used in molecular recognition owing to its great selectivity, high sensitivity, easy operation and non-destructiveness. Thus, a variety of fluorescent probes for Hg^{2+} detection has been developed in the past decade (12–15). Early on, most Hg^{2+} fluorescent probes were designed based on their coordinative property with heteroatom-based ligands. However, coordination-based fluorescent probes suffer from low selectivity to Hg^{2+} over other metal ions, particularly for intracellular detection in living cells. As a result, Hg^{2+}-promoted chemical reactions, including desulfurization, hydrolysis, cyclization and elimination have been employed in the design of fluorescent probes for Hg^{2+} detection to achieve good selectivity and high sensitivity (16–22). As Ando and Koide reported a turn-on Hg^{2+} fluorescent probe based on Hg^{2+}-mediated hydrolysis of vinyl ether, this strategy has gained great interest and has become a general approach in the design of Hg^{2+} fluorescent probes (23).

Iminocoumarin dyes are good fluorophores with relatively high fluorescent quantum yield, good water solubility and excellent photostability. Owing to the above-mentioned advantages of iminocoumarin dyes, recently they were used to construct fluorescent probes for the detection of F^-, Cu^{2+}, Zn^{2+} and thiophenol (24–26). In addition, Cho and Ahn developed a coumarin-based probe for the detection of Hg^{2+} with good selectivity and sensitivity (1). However, this probe can only function in organic co-solvent systems because of its poor water solubility and it is known that the introduction of cyano groups into organic compounds can effectively improve their water solubility (27). Herein, we set out to design and synthesize a new water-soluble fluorescent dye, probe **1**, for the rapid and selective detection of Hg^{2+}. The two cyano groups in probe **1** provide good water solubility, making this probe applicable in aqueous environments. When treated with Hg^{2+} in 100% aqueous solution (20 mm phosphate-buffered saline [PBS], pH 7.4), probe **1** rapidly generates the iminocoumarin, reference dye **2**, which is highly fluorescent upon excitation. The proposed mechanism of probe **1** with Hg^{2+} was presented in Scheme 1. Hg^{2+} induced the hydrolysis of the vinyl ether in probe **1** to generate a hydroxy intermediate, which was quickly transformed into reference dye **2** via an intramolecular nucleophilic reaction.

Experimental

Materials and instruments

Unless otherwise noted, all reagents were used as received from commercial suppliers without further purification. Silica gel (200–300 mesh) and thin-layer chromatography plates were purchased from Yantai Chemical Industry Research Institute

* Correspondence to: X. Song, College of Chemistry & Chemical Engineering, Central South University, Changsha, Hunan Province, Peoples Republic of China 410083. E-mail: xzsong@csu.edu.cn
* J. Kang, The Third Xiangya Hospital, Central South University, Changsha, Hunan Province, Peoples Republic of China, 410013. E-mail: kangjian@csu.edu.cn

[a] College of Chemistry & Chemical Engineering, Central South University, Changsha, Hunan Province, Peoples Republic of China

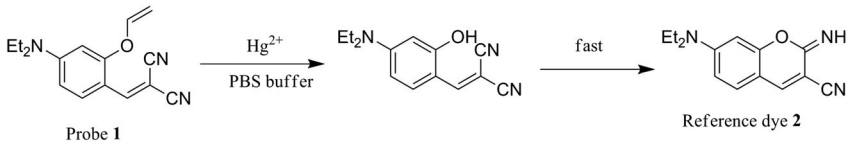
[b] College of Chemical Engineering and Modern Materials, Shangluo University, Shangluo, Shanxi Province, Peoples Republic of China

[c] State Key Laboratory for Powder Metallurgy, Central South University, Changsha, Hunan Province, Peoples Republic of China

[d] State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian, Liaoning Province, Peoples Republic of China

[e] The Third Xiangya Hospital, Central South University, Changsha, Hunan Province, Peoples Republic of China

LUMINESCENCE
The Journal of Biological and Chemical Luminescence



Scheme 1. Sensing mechanism of probe **1** with Hg^{2+}.

(China). All the ultraviolet-visible and fluorescence spectra were measured by Agilent Technologies (USA) and HITACHI F-4600 spectrometers (Japan), respectively. The pH was test by pHs-3B meter (Shanghai Precision & Scientific Instrument Co. Ltd, Shanghai, China). ^1H NMR spectra in $CDCl_3$ was measured by Bruker 400M NVANCE-III spectrometer (Switzerland) with chemical shifts reported as p.p.m. (tetramethylsilane as the internal standard). The accurate mass spectrometric experiment was performed on a micrOTOF-Q II mass spectrometer (BrukerDaltonik, Germany). Cell experiment were applied on an inverted fluorescence microscope (Olympus IX-70) connected with a digital camera (Olympus, c-5050).

Absorption and fluorescence analysis

The stock solution of probe **1** was prepared at 1 mm in dimethyl sulfoxide (DMSO). Stock solutions of various cations, including Na^+, Ca^{2+}, Cd^{2+}, Fe^{3+}, Ag^+, Co^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Mg^{2+}, Ba^{2+}, Al^{3+}, Mn^{2+} and Ni^{2+} were prepared in PBS buffer (20 mm, pH 7.4). The test samples for absorption and emission spectra measurements were prepared by placing appropriate amounts of stock solutions containing various cations into the corresponding solution of probe **1**. Unless otherwise noted, the excitation wavelength was 438 nm and the excitation and emission slit widths were set at 5 nm for all fluorescence measurements.

Synthesis of probe 1 and reference dye 2

The synthesis of reference dye **2** was obtained using the method in the literature (28). The synthetic route of probe **1** is shown in Scheme 2. Compound **b** was synthesized according to the method in the literature with 4-(diethylamino)salicylaldehyde as the starting material (1). Piperdine (0.3 mmol, 30.6 mg) in 2 mL of ethanol was added dropwise to a solution of compound **b** (1 mmol, 220 mg) and malononitrile (2 mmol, 130 mg) in 15 mL of anhydrous ethanol, while stirring at room temperature. The resulting mixture was kept at room temperature for 5 h. After removing the solvent, the residue was purified by silica gel column chromatography using petroleum ether/dichloromethane (v/v = 10: 1) as eluent to give pure probe **1** as a light yellow powder (180 mg, yield 71%). ^1H NMR (400 MHz, $CDCl_3$): δ1.25 (t, J = 7.6 Hz, ^6H), 3.47 (q, J = 8.8 Hz, ^4H), 4.65 (dd, J = 6.8, 1.6 Hz, ^1H), 4.92 (dd, J = 1.6, 1.6 Hz, ^1H), 6.22 (d, J = 2.4 Hz, ^1H), 6.66 (dd, J = 2.4, 2.4 Hz, ^1H), 6.95 (dd, J = 6.4, 5.6 Hz, ^1H), 7.09 (s, ^1H), 8.06 (d, J = 9.6 Hz, ^1H). MS (ESI): found: *m/z* = 268.18 $(M+1)^+$, calcd. for $C_{16}H_{17}N_3O$ = 268.14.

Results and discussion

Proposed mechanism for the reaction of probe 1 with Hg^{2+}

First, we investigated the absorption and emission spectra of probe **1**, reference dye **2** and the reaction product of probe **1** with Hg^{2+}. The solution of probe **1** displays an absorption maximum at 447 nm and is essentially non-fluorescent (shown in Fig. 1). The reference dye **2** absorbs at 438 nm and was strongly fluorescent with a maximum at 486 nm (shown in Supplementary Fig. 2). Upon treatment with Hg^{2+} in aqueous PBS buffer, the solution of probe **1** showed a blue-shift to 438 nm in its absorption spectrum and exhibited a strong blue fluorescent signal with a maximum at 486 nm (Fig. 1). The optical spectra studies clearly suggested that Hg^{2+} induced the hydrolysis and generated reference dye **2**, which is highly fluorescent upon excitation.

To understand further the reaction mechanism of probe **1** with Hg^{2+}, ^1H NMR titration analysis was conducted for a mixture of probe **1** and Hg^{2+} in DMSO-d_6/D_2O (V/V, 4:1) solution (Fig. 2). Upon addition of Hg^{2+} to the solution of probe **1**, the protons at 4.68 p.p.m. (H_1), 4.96 p.p.m. (H_2), 6.98 p.p.m. (H_3) assigned to CH=CH_2 and 7.94 p.p.m. (H_4) assigned to CH=$(CN)_2$ disappeared and a new ^1H NMR spectrum was obtained, which is identical to that of reference dye **2**. Moreover, mass spectrum analysis on the reaction product of probe **1** with Hg^{2+} showed a peak at *m/z* = 242.1290 (Supplementary Fig. 9, Supporting Information), which is nearly

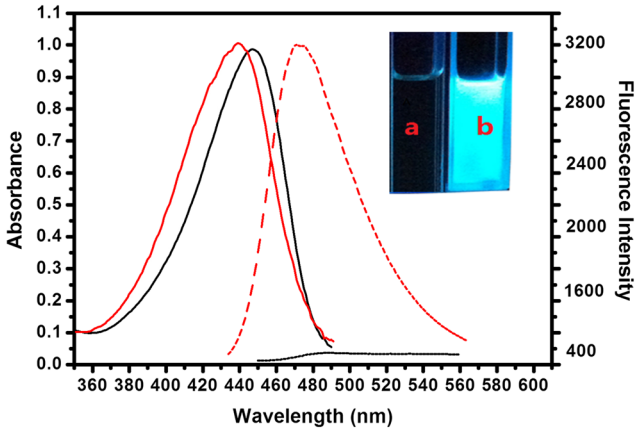
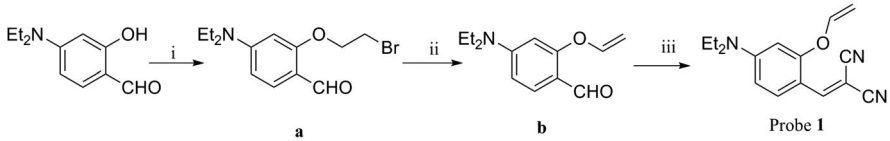


Figure 1. Ultraviolet-visible absorption (solid line) and emission (dot line) of probe **1** in the absence (black lines) and presence (red lines) of Hg^{2+} in phosphate-buffered saline (20 mm, pH 7.4). Inset: Emission images of probe **1** before (a) and after (b) addition of Hg^{2+} under a 365 nm ultraviolet lamp.



Scheme 2. The synthetic route of probe **1**. (i) $BrCH_2CH_2Br$, K_2CO_3, acetone; (ii) *tert*-BuOK, dimethyl sulfoxide; (iii) malononitrile, piperidine, EtOH.

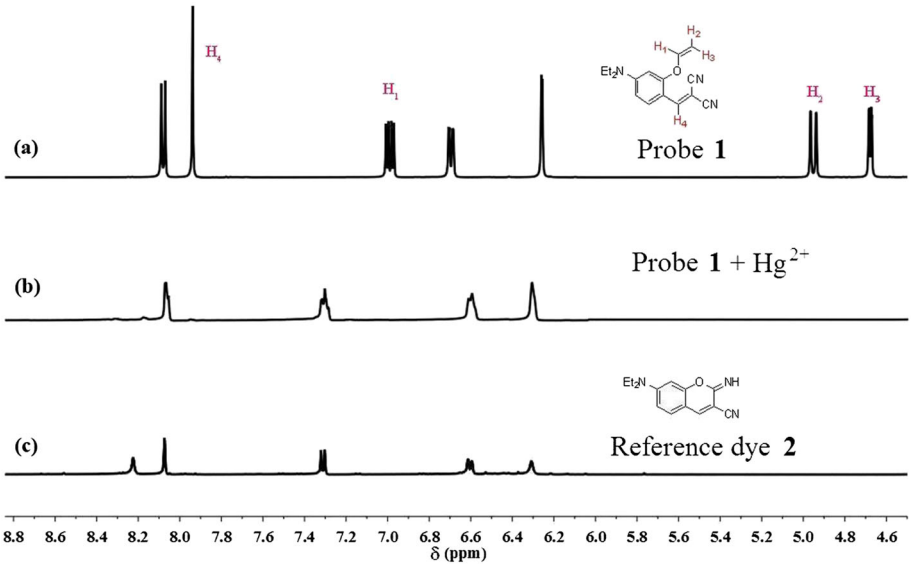


Figure 2. Partial ^{1}H NMR spectra of (a) probe **1** (20 mm) in DMSO-d_6, (b) probe **1** (20 mm) with Hg^{2+} (60 mm) in DMSO-d_6/D_2O (v/v=4:1) and (c) reference dye **2** in DMSO-d_6. DMSO, dimethyl sulfoxide.

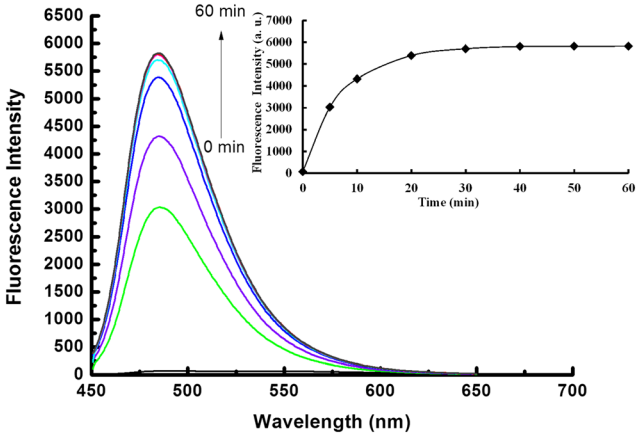


Figure 3. The time-dependent fluorescence of probe **1** (10 μm) with Hg^{2+} (20 μm) in phosphate-buffered saline (20 mm, pH 7.4) within 30 min.

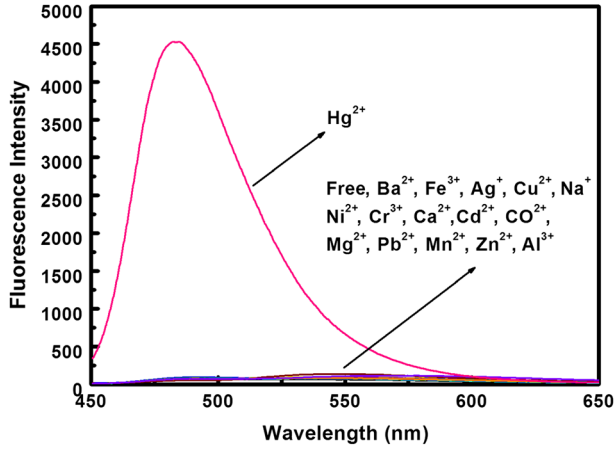


Figure 5. Fluorescence responses of probe **1** (10 μm) with various cations (10 μm) in phosphate-buffered saline (20 mm, pH 7.4) measured after 30 min mixing.

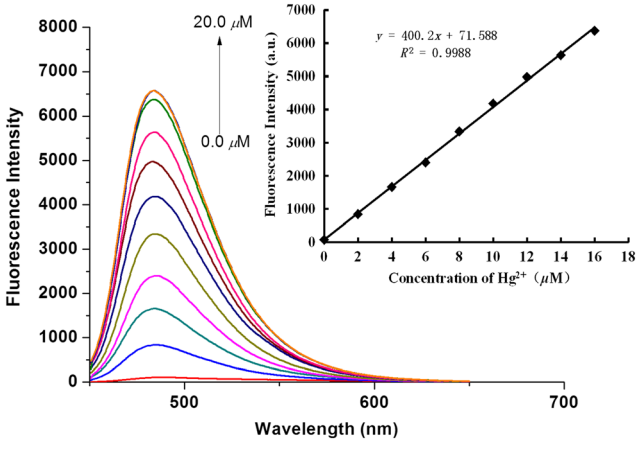


Figure 4. The changes of fluorescence intensity at 486 nm of probe **1** (10 μm) upon the addition of Hg^{2+} (0.0–20 equiv.) in phosphate-buffered saline (20 mm, pH 7.4). Inset: (top) the dependence of the fluorescence intensity (at 486 nm) on the concentrations of Hg^{2+}; (bottom) fluorescence images of probe **1** (10 μm) in the absence (a) and presence (b) of Hg^{2+} in phosphate-buffered saline (20 mm, pH 7.4).

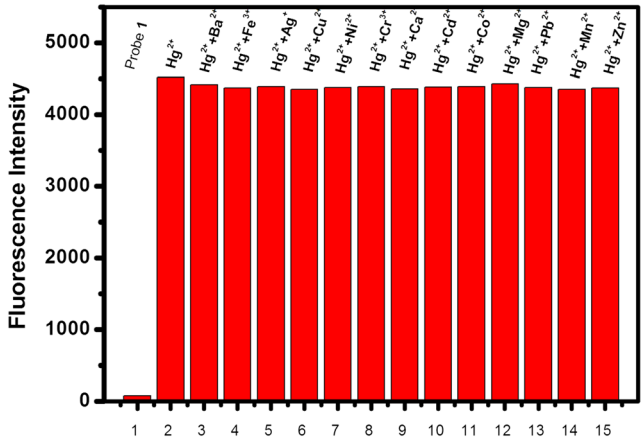


Figure 6. Interfering effect of various analytes on the fluorescence intensity at 486 nm of probe **1** (10 μm) with Hg^{2+} (20 μm) in phosphate-buffered saline (20 mm, pH 7.4) after incubated for 30 min.

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

identical to the theoretical molecular mass of reference dye **2** ([M + 1] = 242.2884). These data provide strong support for our proposed mechanism for the manner in which Hg^{2+} reacts with probe **1** to generate the strong fluorescent reference dye **2**.

Fluorescence studies

To obtain an appropriate reaction time, the time-dependent fluorescence studies of probe **1** with Hg^{2+} were conducted. It can be seen in Fig. 3 that a pronounced fluorescence enhancement was observed within 5 min and the fluorescence intensity became saturated after 30 min, indicating probe **1** could make a rapid detection of Hg^{2+}.

To gain insight into the analytic performance of probe **1** for Hg^{2+} detection, the fluorescence behavior of probe **1** (10 μm) in response to varying concentrations of Hg^{2+} was investigated. In Fig. 4, a good linear relationship was observed between the fluorescence intensity and the concentration of Hg^{2+}. The detection limit of probe **1** for the detection of Hg^{2+} is calculated to be 20 nm. In the presence of 2.0 equivalents of Hg^{2+}, probe **1** (10 μm) produced a strong fluorescence enhancement (68-fold) (Fig. 4). These results evidently indicated that probe **1** could effectively detect Hg^{2+} qualitatively and quantitatively.

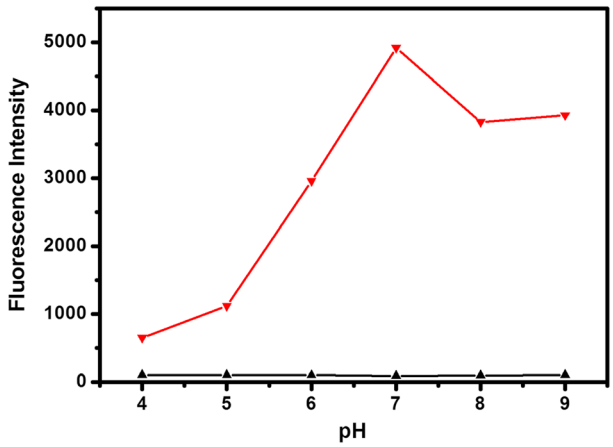


Figure 7. pH effect on the fluorescence intensity at 486 nm of probe **1** (10 μm) only (black line) and probe **1** (10 μm) with Hg^{2+} (10 μm) (red line).

To evaluate the selectivity of probe **1**, the fluorescence experiments were carried out on probe **1** in response to other common metal ions, including Fe^{3+}, Cr^{3+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Ba^{2+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Al^{3+} and Na^{+} in PBS buffer (20 mm, pH 7.4). As seen in Fig. 5, Hg^{2+} induced a prominent fluorescence enhancement whereas all other metal ions only trigger a faint fluorescence signal. Moreover, the coexistence of other interfering metal ions showed little effect on the detection of Hg^{2+} for probe **1** (Fig. 6). The above results implied that probe **1** exhibited high selectivity for Hg^{2+} detection.

It is known that Hg^{2+}-mediated hydrolysis is pH dependent. Thus, we also investigated the pH effect on the fluorescence behavior of probe **1** in response to Hg^{2+}. As shown in Fig. 7, the fluorescence of probe **1** is negligible under pH values from 4.0 to 9.0, indicating probe **1** has a good stability within a wide pH range. The solution of probe **1** with Hg^{2+} produced a strong fluorescence signal between pH 6.0 and 8.0, suggesting that this probe is suitable for the detection of Hg^{2+} in physiological conditions.

Cell imaging

Encouraged by the good selectivity, high sensitivity and good water solubility of probe **1**, we set out to evaluate the capability of this probe for the detection of Hg^{2+} in living cells. When HeLa cells were incubated with the 10 μm probe **1** for 30 min at 37°C and then washed with PBS buffer three times, only little fluorescence was produced, as shown in Fig. 8. In contrast, a strong green fluorescence signal was observed inside HeLa cells

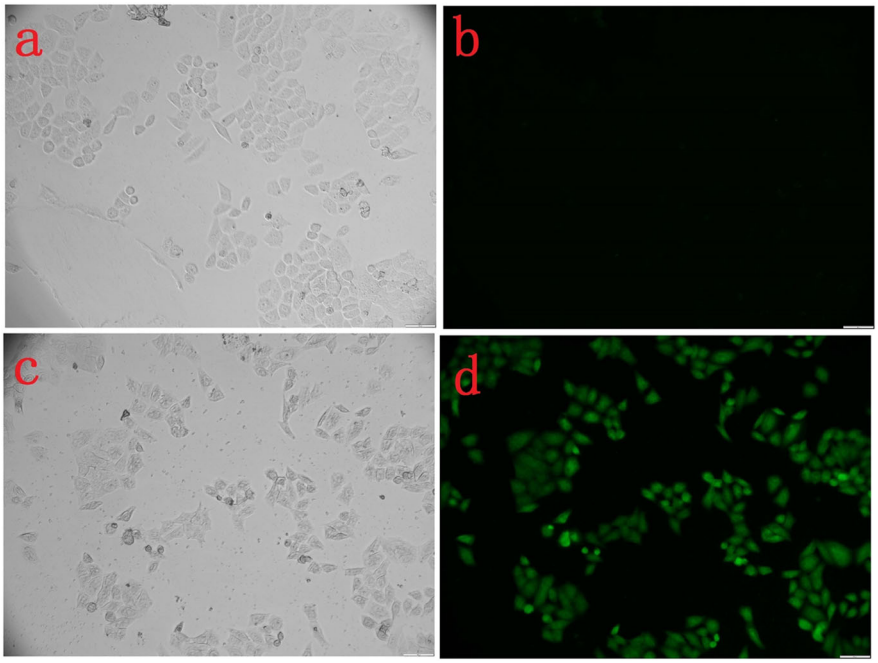


Figure 8. Images of HeLa cells: (a) bright-field and (b) fluorescent images of HeLa cells incubated with probe **1** (10 μm); (c) bright-field and (d) fluorescent images of HeLa cells treated with probe **1** (10 μm) for 30 min and then incubated with 30 μm Hg^{2+} for another 30 min.

when the cells were pretreated with probe **1** and further incubated with 30 μm Hg^{2+} for another 30 min. This result demonstrated that probe **1** had the potential for the detection of intracellular Hg^{2+} in biological samples.

Conclusions

We have developed a fluorescent probe for the detection of Hg^{2+} with high sensitivity and good selectivity. In the presence of Hg^{2+}, this probe produced a 90-fold fluorescence enhancement. Owing to its good water solubility, this probe can detect Hg^{2+} in pure aqueous solution with a fast response time. Importantly, the successful imaging of Hg^{2+} in living cells was demonstrated.

Acknowledgements

The research was supported by State Key Laboratory of Fine Chemicals (KF1202) and Hunan Nonferrous Metals Holding Group Co. Ltd.

References

1. Cho YS, Ahn KH. A "turn-on" fluorescent probe that selectively responds to inorganic mercury species. Tetrahedron Lett 2010; 51: 3852–4.
2. Zhang JF, Lim CS, Cho BR Kim JS. A two-photon excited luminescence of water-soluble rhodamine–platinum (II) complex: fluorescent probe specific for Hg^{2+} detection in live cell. Talanta 2010; 83: 658–62.
3. Kumar M, Kumar N, Bhalla V, Singh H, Sharma PR Kaur T. Naphthalimide appended rhodamine derivative: through bond energy transfer for sensing of Hg^{2+} ions. Org Lett 2011; 13: 1422–5.
4. Mutter J, Naumann J Guethlin C. Comments on the article 'the toxicology of mercury and its chemical compounds' by clarkson and magos. Crit Rev Toxicol 2006; 37: 537–49.
5. Nolan EM, Lippard SJ. Tools and tactics for the optical detection of mercuric ion. Chem Rev 2008; 108: 3443–80.
6. Yuan M, Li Y, Li J, Li C, Liu X, Lv J, et al. A colorimetric and fluorometric dual-modal assay for mercury ion by a molecule. Org Lett 2007; 9: 2313–16.
7. Wang J, Qian X. A series of polyamide receptor based PET fluorescent sensor molecules: positively cooperative Hg^{2+} ion binding with high sensitivity. Org Lett 2006; 8: 3721–4.
8. Tchounwon PB, Ayensu WK, Ninashvili N Sutton D. Review: environmental exposure to mercury and its toxicopathologic implications for public health. Environ Toxicol 2006; 18: 149–75.
9. De Silva AP, Gunaratne HQN, Gunnlaugsson T, Huxley A, Mccoy CP, Rademacher JT, et al. Signaling recognition events with fluorescent sensors and switches. Chem Rev 1997; 97: 1515–66.
10. Zhu M, Yuan M, Liu X, Lv J, Huang C, Liu H, et al. Visible near-infrared chemosensor for mercury ion. Org Lett 2008; 10: 1481–4.
11. Zeng D, Chen Y. A selective, fluorescence probe for Hg^{2+} detection in aqueous solution. Sci Direct 2007; 186: 121–4.
12. Yoon S, Albers AE, Wong AP Chang CJ. Screening mercury levels in fish with a selective fluorescent chemosensor. J Am Chem Soc 2005; 127: 16030–1.
13. Yuan M, Zhou W, Liu X, Zhu M, Li J, Yin X, et al. A multianalyte chemosensor on a single molecule: promising structure for an integrated logic gate. J Org Com 2008; 73: 5008–14.
14. Martínez-Máñez R, Sancenón F. Fluorogenic and chromogenic chemosensors and reagents for anions. Chem Rev 2003; 103: 4419–76.
15. Kim JS, Quang DT. Calixarene-derived fluorescent probes. Chem Rev 2007; 107: 3780–99.
16. Zheng H, Qian ZH, Xu L, Yuan FF, Lan L Xu J. 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 2006; 8: 859–61.
17. Nolan EM, Lippard SJ. A 'turn-on' fluorescent sensor for the selective detection of mercuric ion in aqueous media. J Am Chem Soc 2003; 125: 14270–1.
18. Chae MY, Czarnik AW. Fluorometric chemodosimetry mercury (II) and silver (I) indication in water via enhanced fluorescence signaling. J Am Chem Soc 1992; 114: 9704–5.
19. Yang YK, Yook KJ Tae J. A rhodamine-based fluorescent and colorimetric chemodosimeter for the rapid detection of Hg^{2+} ions in aqueous media. J Am Chem Soc 2005; 127: 16760–1.
20. Zhang G, Zhang D, Yin X, Yang Z, Shuai Z Zhu D. 1,3-Dithiole-2-thione derivatives featuring an anthracene unit: new selective chemodosimeters for Hg(II) ion. Chem Commun 2005; 16: 2161–3.
21. Liu B, Tian H. A selective fluorescent ratiometric chemodosimeter for mercury ion. Chem Commun 2005; 25: 3156–8.
22. Ko SK, Yang YK, Tae J Shin I. In vivo monitoring of mercury ions using a rhodamine-based molecular probe. J Am Chem Soc 2006; 128: 14150–5.
23. Ando S, Koide K. Development and applications of fluorogenic probes for mercury (II) based on vinyl ether oxymercuration. J Am Chem Soc 2011; 133: 2556–66.
24. Komatsu K, Urano Y, Kojima H Nagano T. Development of an iminocoumarin-based zinc sensor suitable for ratiometric fluorescence imaging of neuronalzinc. J Am Chem Soc 2007; 129: 13447–54.
25. Kand D, Mandal PS, Datar A Talukdar P. Iminocoumarin based fluorophores: indispensable scaffolds for rapid, selective and sensitive detection of thiophenol. Dyes Pigments 2014; 106: 25–31.
26. Peng Y, Dong YM, Dong M Wang YW. A Selective, sensitive, colorimetric, and fluorescence probe for relay recognition of fluoride and Cu (II) ions with "off–on–off" switching in ethanol–water solution. J Org Chem 2012; 77: 9072–80.
27. Hunt J, Ding C Batorsky R. Discovery of (R)-7-cyano-2, 3, 4, 5-tetrahydro-1-(1H-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-H-1, 4-benzodiazepine (BMS-214662), A farnesyltransferase inhibitor with potentpreclinical antitumor activity. J Med Chem 2000; 43: 3588–95.
28. Volmajer J, Toplak R, Leban I Majcen A. Synthesis of new iminocoumarins and their transformations into N-chloro and hydrazono compounds. Tetrahedron 2005; 61: 7012–21.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web site.