Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 432

www.rsc.org/obc

PAPER

Highly sensitive and selective turn-on fluorescent and chromogenic probe for Cu^{2+} and ClO^- based on a *N*-picolinyl rhodamine B-hydrazide derivative†

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Received 10th July 2010, Accepted 17th September 2010
DOI: 10.1039/c0ob00411a

A new rhodamine B-based dual-function chromo- and fluorogenic probe for Cu^{2+} and ClO^- has been designed, synthesized, and characterized. The probe comprises a spectroscopic unit of rhodamine B and a Cu^{2+}-specific chelating unit of pyridinecarboxamide as well as a ClO^--specific reactive moiety of diacylhydrazine, and is a highly selective and extremely sensitive fluorescent and colorimetric sensor for Cu^{2+} and ClO^- in different pH conditions. Compared with the reported probes for Cu^{2+} or ClO^-, this is the first chemosensor based on a small molecule that can detect both Cu^{2+} and ClO^-, respectively, at 1 nM level.

Introduction

Development of selective and sensitive signalling systems for the determination and visualization of metal ions or anions with biological interest is a very attractive research topic in the chemosensing and molecular imaging fields.[1] Among the various transition metal ions, the copper ion plays a critical role as a catalytic co-factor for a variety of metalloenzymes, including superoxide dismutase, cytochrome *c* oxidase and tyrosinase.[2] However, under overloading conditions, copper exhibits toxicity in that it causes neurodegenerative diseases, probably by its involvement in the production of reactive oxygen species.[3] On the other hand, hypochlorite anion (ClO^-) is one of the biologically important reactive oxygen species (ROS),[4] and produced in organisms by the reaction of H_2O_2 with Cl^- ions under the catalysis of a heme enzyme, myeloperoxidase.[5] Endogenous ClO^- is essential to life and has important antibacterial properties. However, excessive or misplaced production of ClO^- can lead to tissue damage and diseases, such as atherosclerosis, arthritis, and cancers.[5,6] Therefore, highly selective and sensitive detection of Cu^{2+} or ClO^- in various samples is of toxicological and environmental concern.[7,8]

Sensors based on the chemical species induced changes in fluorescence appear to be particularly attractive due to the highly sensitive, quick, simple and real time monitoring of the fluorescence. In general, chemosensors exhibiting fluorescence enhancement (fluorescence "turn-on") are favored over those showing fluorescence quenching (fluorescence "turn-off"). Moreover, fluorescence "turn-off" sensors may report false positive results caused by other quenchers in practical samples and are undesirable for practical analytical applications. The rhodamine framework seems to be an ideal model to construct "turn-on" fluorescent chemosensors due to its particular structural property.[9] As is well-known, rhodamine derivatives with spirolactam structure are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam induced by metal ions gives rise to strong fluorescence emission and a pink color. However, very few research interests were drawn to such chemosensors until the first rhodamine-based fluorescent chemodosimeter for Cu^{2+} was reported by Czarnik's group.[10] Inspired by their successful work, a large number of rhodamine-based "turn-on" chemosensors and chemodosimeters for transition- or heavy-metal ions have been developed in recent years.[11] In these systems, the signal was transduced either through opening the spirolactam ring upon metal ion coordination or through irreversible chemical reactions induced by metal ion. Although anions play a fundamental role in a wide range of chemical and biological processes, a limited number of rhodamine-based fluorescent sensors have been made available for anions detection so far.[12] In view of the excellent structural and photophysical properties of rhodamine derivatives, the search for new rhodamine sensors with the characteristics of high selectivity and affinity, sensitive and reversible turn-on response to analytic species, as well as good water-solubility, and broad pH range has been the focus of extensive investigation.

In this paper, a new rhodamine B-based dual-function chemosensor for Cu^{2+} and ClO^- has been designed, synthesized, and characterized (Fig. 1). The probe comprises a spectroscopic unit of rhodamine B and a metal chelating unit of pyridinecarboxamide[13] as well as a ClO^- reactive moiety of diacylhydrazine.[14,12a] The probe itself is nearly nonfluorescent because of its spirolactam structure, however, it displays an excellent selectivity and an extremely high sensitivity (up to 1 nM) toward the detection of Cu^{2+} in MeCN/Tris-HCl buffer (pH 7.0)

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† Electronic supplementary information (ESI) available: ^{1}H NMR, ^{13}C NMR, ESI-MS and additional spectroscopic data of **1**. See DOI: 10.1039/c0ob00411a

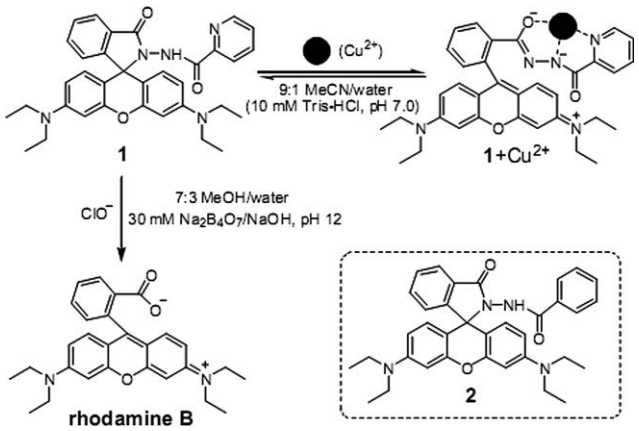

Fig. 1 The proposed sensing mechanisms of **1** for Cu^{2+} and ClO^-.

and ClO^- in MeOH/$Na_2B_4O_7$–NaOH buffer (pH 12.0) over a wide range of tested metal ions and anions with remarkably enhanced fluorescent intensities and also clear color changes from colorless to pink. Moreover, most of the background metal ions and anions show small or no interference with the detection of Cu^{2+} or ClO^-. Compared with the reported probes for Cu^{2+} or ClO^-, this is the first chemosensor based on a small molecule that can detect both Cu^{2+} and ClO^- at 1 nM level.

Results and discussion

The probe **1** was readily prepared by treating rhodamine B base with $POCl_3$, which was followed without purification by pyridine-2-carboxylic acid hydrazide. The structure of **1** was confirmed by ^1H NMR, ^{13}C NMR, MS, and elemental analysis.

An MeCN–water solution of **1** is colorless and nonfluorescent, indicating that the spirolactam form of **1** exists only. The characteristic peak of the 9-carbon of **1** at 66.3 ppm in the ^{13}C NMR also supports this consideration.[15] As we expected, addition of Cu^{2+} to the MeCN–water solution of **1** caused an obvious pink color and bright orange fluorescence as a result of the Cu^{2+}-induced ring opening of the spirolactam form. The other ions of our interest, such as Na^+, K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+} and Ag^+, showed little interference (Fig. S1, ESI†). The above results suggested that **1** can serve as a selective "naked-eye" and fluorescent sensor for Cu^{2+}.

To apply **1** in more complicated systems, the spectra response of **1** in the absence and presence of Cu^{2+} in different pH values were evaluated (Fig. S2, ESI†). Without Cu^{2+}, no obvious characteristic absorption of rhodamine could be observed for **1** between pH 4.0 and 10.0. Upon addition of Cu^{2+}, **1** responds stably to Cu^{2+} in the same region without any interference by protons. When the pH value is lower than 4.0, the spectra response also occurs upon the coordination of Cu^{2+}, but the intensity of the free **1** increases slowly with the decreasing pH values. These results indicate that **1** successfully reacts with Cu^{2+} and allows Cu^{2+} detection in a wide pH range.

A 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0) was selected as a testing system to investigate the chemical response of **1** to Cu^{2+} at room temperature (about 20 °C). A time course of the absorption response of **1** upon addition of Cu^{2+} revealed the recognizing event could complete in less than 5 s (Fig. S3, ESI†), and remains quite stable from 5 s to 2 min, suggesting that **1** has rapid detection ability for Cu^{2+}. The feature of real-time response to Cu^{2+} is particularly important in practical application. The UV-vis spectrum of **1** (20 μM) showed no absorbance above 500 nm, which is ascribed to its spirolactam form in solution. Upon addition of Cu^{2+} to a solution of **1**, the solution turned from colorless to pink rapidly, and the absorbance was significantly enhanced with a new peak appearing at around 565 nm (Fig. 2a), clearly suggesting the formation of the ring-opened amide form of **1** as a result of Cu^{2+} binding. The association constant for Cu^{2+} was estimated to be 3.9×10^5 M^{-1} ($R^2 = 0.990$) on the basis of nonlinear fitting of the titration curve assuming 1 : 1 stoichiometry (Fig. 2a inset). This 1 : 1 binding mode was also supported by a Job plot (Fig. S4, ESI†). The solid evidence of the 1 : 1 binding mode comes from the ESI-MS spectra of the complex of **1** with Cu^{2+}, in which the peaks at m/z 623.1958 (calcd = 623.1952) corresponding to $[1+Cu–H]^+$ was clearly observed (Fig. S5, ESI†).

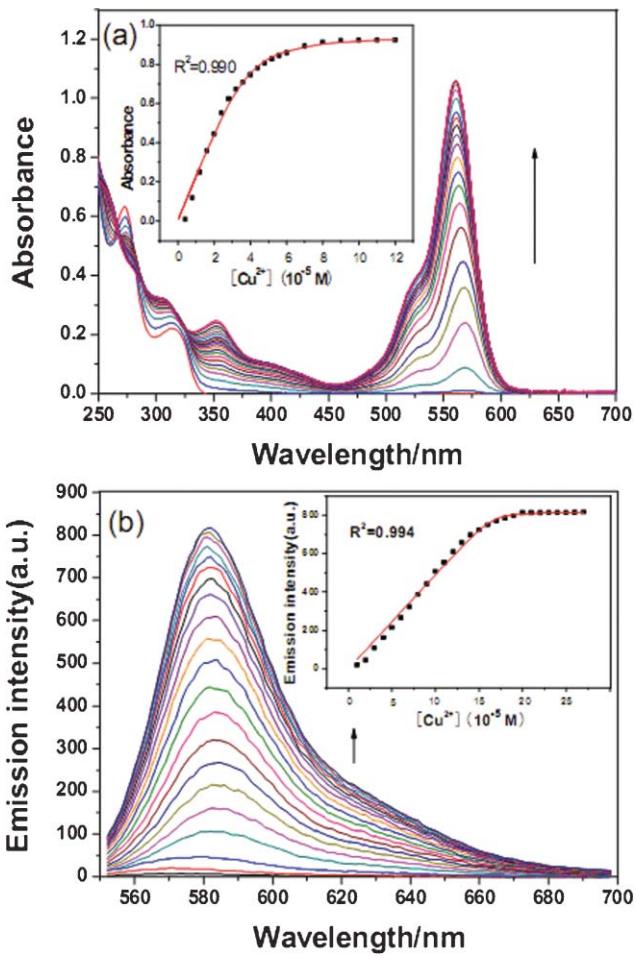

Fig. 2 Changes in absorption (a) and fluorescence emission (b) spectra of **1** (20 μM) in 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0) with various amounts of Cu^{2+} ions (λ_{ex} = 540 nm). Inset: absorption titration profile (a) and fluorescence titration profile (b) *vs.* concentration of Cu^{2+} in solution for **1**.

To achieve this 1 : 1 stoichiometry, carbonyl O atom, deprotonated amide N atom, and pyridine N atom of **1** are the most possible binding sites for Cu^{2+} (Fig. 1). IR spectra of **1** and **1**-Cu^{2+} were taken in KBr disks, respectively (Fig. S6, ESI†). The

peak at 1731.9 cm^{-1}, which corresponds to the characteristic amide carbonyl absorption of **1**, was shifted to 1631.7 cm^{-1} upon chelating with Cu^{2+}, indicating that rhodamine carbonyl group is involved in Cu^{2+} coordination. The deprotonation reaction of amide NH during the Cu^{2+} binding could be demonstrated by the ESI-MS spectra of **1**-Cu^{2+} complex (Fig. S5, ESI†), and ^1H NMR titration experiments (Fig. S7, ESI†), in which the amide NH proton (at around δ 9.1 ppm) disappeared upon Cu^{2+} addition. In addition, the pyridine N atom of **1** plays an indispensable role in the affinity of **1** to Cu^{2+} because the control compound **2** (Fig. 1) displayed no response to Cu^{2+} in absorption spectra when treated with excess Cu^{2+}.

Subsequently, the complexation of Cu^{2+} by **1** was investigated by means of fluorescence titration in 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0). However, a time-course study revealed that the fluorescence intensity increased with the increase of reaction time at room temperature (about 20 °C), and 30 min later a plateau of fluorescence enhancement was achieved (Fig. S8, ESI†). As a result, a time of 30 min at room temperature was chosen for the present fluorescence studies. Upon addition of Cu^{2+}, a new emission band of **1** showing a maximum at 582 nm appeared (Fig. 2b). In the presence of 8 equiv of Cu^{2+}, the mixture showed an intense red fluorescence (Fig. S1, ESI†), and a big enhancement in the fluorescence intensity at 582 nm was observed. Fluorescence titration profile at 582 nm *versus* concentration of Cu^{2+} is shown in Fig. 2b inset. Based on nonlinear fitting of the titration curve assuming 1 : 1 stoichiometry, the association constant for Cu^{2+} was estimated to be 1.79×10^6 M^{-1} ($R^2 = 0.994$). The high enhancement of fluorescence of **1** upon the binding of Cu^{2+} and the large association constant ($K_a > 10^6$) of the **1**-Cu^{2+} complex suggested the possibility of Cu^{2+} sensing at a very low concentration level by **1**. When **1** was employed at 1 μM in 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0), the fluorescent intensity of **1** was proportional to the concentration of Cu^{2+} added (Fig. 3). This clearly demonstrates that **1** can respond up to ~1.0 nM level of the aqueous Cu^{2+} ion.

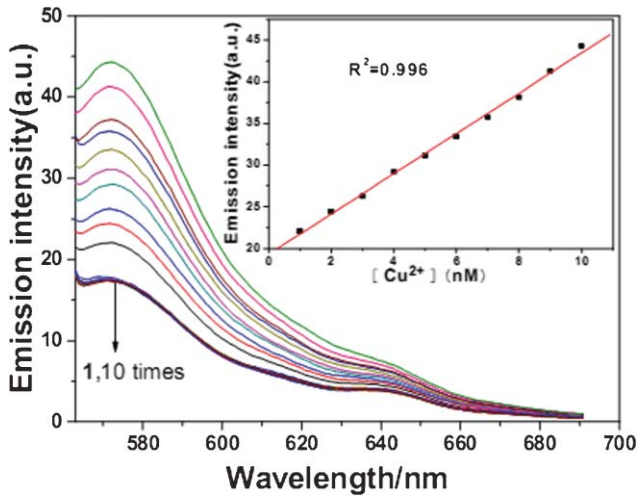

Fig. 3 Fluorescence response of **1** (1 μM) to Cu^{2+} in 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0). The excitation wavelength was 540 nm, and the splits were 10 nm. Inset: titration curve of $I_{586\,nm}$ *vs.* Cu^{2+} concentration.

An important feature of **1** is its high selectivity toward the Cu^{2+} over the other competitive species. Changes of fluorescence and UV/Vis spectra of **1** (20 μM) caused by Cu^{2+} and miscellaneous metal ions including Na^+, K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+} and Ag^+ in 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0) are recorded in Fig. 4 and Fig. S9 (see ESI†), respectively. The miscellaneous competitive cations did not lead to any significant fluorescence and absorption changes in the visible region. Moreover, in the presence of miscellaneous competitive metal ions, the Cu^{2+} ion still resulted in the similar fluorescence and absorption changes. In addition, we also investigated the spectra change of **1** (20 μM) for Cu^{2+} in the presence of various anions, such as F^-, Cl^-, Br^-, I^-, CN^-, NO_3^-, SiO_3^{2-}, SO_4^{2-}, AcO^-, $H_2PO_4^-$, ClO^-, ClO_3^-, MnO_4^- (Fig. S10, ESI†). Due to the much higher stability constant of CN^- with Cu^{2+}[12c] as well as the redox reaction between I^- and Cu^{2+},[16] the added CN^- or I^- could snatch the copper ions from the complex of **1**-Cu^{2+}, resulting in a sharply decreased absorption intensity accompanied by a color change. However, all the other selected anions have no interference. These results indicated that probe **1** exhibited the remarkable selectivity for Cu^{2+}, and the sensing could be a reversible process.

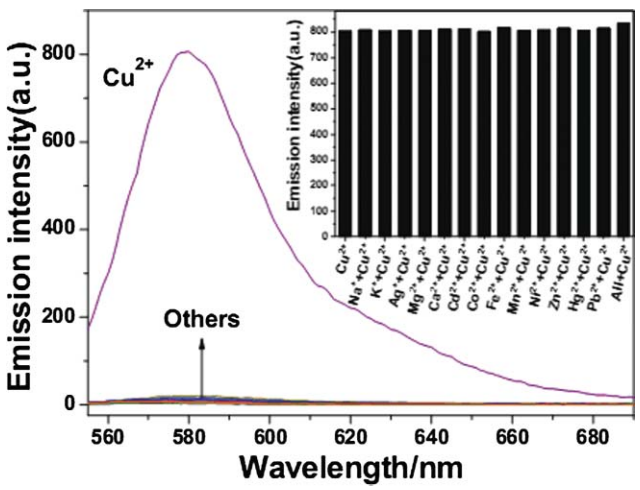

Fig. 4 The fluorescence spectra of **1** (20 μM) upon addition of 8 equiv of Cu^{2+} and various other metal ions including of Na^+, K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+}, Ni^{2+} and Ag^+ (8 equiv) in 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0). Inset: Fluorescence response of **1** (20 μM) to 8 equiv of Cu^{2+} in 9 : 1 (v/v) MeCN–water solution (10 mM Tris-HCl, pH 7.0) containing 8 equiv of various metal ions. $\lambda_{ex}/\lambda_{em}$ = 540/582 nm.

Recently, Ma *et al.* reported a *N*-benzoyl rhodamine B-hydrazide derivative (**2**) as a fluorescent probe for ClO^- by an oxidation-hydrolysis mechanism.[12a] Although high selectivity for ClO^- has been obtained, the probe has delayed response and need take 30 min to complete the ring-opening reaction. Inspired by this work, we decided to test the potential of **1** for ClO^-. Due to the poor water solubility of **1**, the use of 70% (v/v) MeOH as a co-solvent is required in this system. In fact, the choice of co-solvent is considerably important in this study. In the solution of MeCN–H_2O or THF–H_2O, the addition of ClO^- did not lead to any significant fluorescence and absorption changes of **1**. In contrast, in the solution of MeOH–H_2O, **1** showed rapid and

sensitive response to ClO^-. Firstly, the responses of **1** in the absence and presence of ClO^- in different pH values were evaluated by absorption spectra (Fig. S11, ESI†). As can be seen, pH 12.0 can be used for the present system. Although milder pH conditions, such as pH 4–10, can also be used, the sensitivity is relatively low. It is noteworthy that a time course study of the absorption and fluorescence responses revealed the ClO^--induced oxidation of **1** takes place rapidly at room temperature, and could complete within 10 s (Fig. S12, ESI†). As a result, a reaction medium of 30 mM $Na_2B_4O_7$/NaOH buffer (pH 12.0) that contained 70% (v/v) MeOH, and a reaction time of 10 s at room temperature were chosen for the present studies.

The UV/visible spectra of **1** (20 μM) shows no absorbance above 500 nm. Upon addition of ClO^- to a solution of **1**, the solution turned from colorless to pink immediately (Fig. S1, ESI†), and the absorbance was significantly enhanced with a new peak appearing at around 558 nm (Fig. 5a), indicating that probe **1** can serve as a "naked-eye" indicator for ClO^- concentration. The absorption intensity at 558 nm increased 220-fold and was saturated at 15 equiv of ClO^-. The obvious spectra and color changes are ascribed to the selective oxidation of diacylhydrazine group of **1** by ClO^-, which promotes the opening of the closed spirolactam ring.[12a] In addition, the response of ClO^- by **1** was also investigated by means of fluorescence titration in 7 : 3 (v/v) MeOH–$Na_2B_4O_7$/NaOH buffer (30 mM, pH 12.0). Upon addition of ClO^-, a new emission band showing a maximum at 580 nm that is characteristic of rhodamine B appeared immediately (Fig. 5b). In the presence of 20 equiv of ClO^-, this mixture showed an intense red fluorescence, and a >420-fold enhancement in the fluorescence intensity at 580 nm was estimated.

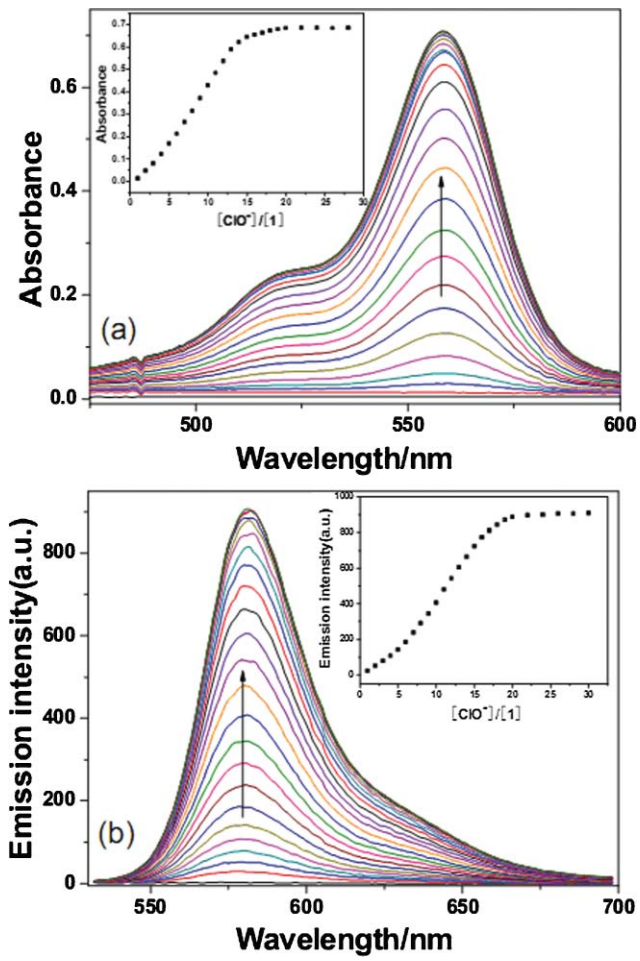

Fig. 5 Changes in absorption spectra of **1** (20 μM) (a) and fluorescence emission spectra of **1** (10 μM) (b) in 7 : 3 (v/v) MeOH–$Na_2B_4O_7$/NaOH buffer (30 mM, pH 12.0) with various amounts of ClO^- (λ_{ex} = 515 nm). Inset: absorption titration profile (a) and fluorescence titration profile (b) *vs.* concentration of ClO^- in solution for **1**.

We then evaluated the analytical performance of **1** for the determination of ClO^-. Under the present conditions, when **1** was employed at 1 μM level, the fluorescent intensity of **1** was proportional to the concentration of ClO^- added in the range of 1×10^{-9} M to 1×10^{-8} M ($R^2 = 0.994$), as shown in Fig. 6. This clearly demonstrates that **1** can respond up to ~ 1.0 nM level of the aqueous ClO^-, which is much lower than recently reported fluorescent methods for ClO^- determination.[12a,12b]

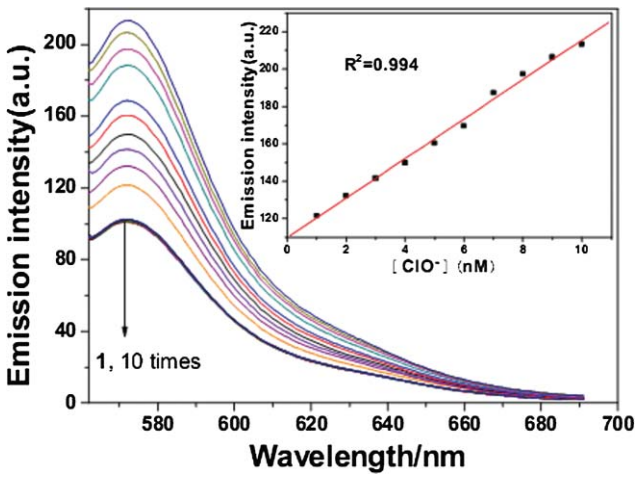

Fig. 6 Fluorescence response of **1** (1 μM) to ClO^- in 7 : 3 (v/v) MeOH–$Na_2B_4O_7$/NaOH buffer (30 mM, pH 12.0). The excitation wavelength was 515 nm, and the splits were 10 nm. Inset: titration curve of $I_{580\,nm}$ *vs.* ClO^- concentration.

Noteworthy is that probe **1** shows high selectivity toward the ClO^- over the other competitive species involving of a wide range of common cations, anions, and some oxidants. Changes of fluorescence and UV/Vis spectra of **1** caused by ClO^- and these competitive species in 7 : 3 (v/v) MeOH–$Na_2B_4O_7$/NaOH buffer (30 mM, pH 12.0) are recorded in Fig. 7 and Fig. S13 (see ESI†), respectively. The miscellaneous competitive species did not lead to any significant fluorescence and absorption changes in the visible region. Moreover, in the presence of miscellaneous competitive species, the ClO^- anion still resulted in the similar fluorescence and absorption changes. The results confirmed the remarkable selectivity of the probe **1** for ClO^-.

Conclusions

In summary, a *N*-picolinyl rhodamine B-Hydrazide derivative has been characterized as a new dual-function chromo- and fluorogenic probe for Cu^{2+} and ClO^-, respectively, in different pH conditions. The probe displays high selectivity and extremely high sensitivity for Cu^{2+} in 9 : 1 (v/v) MeCN–water (10 mM

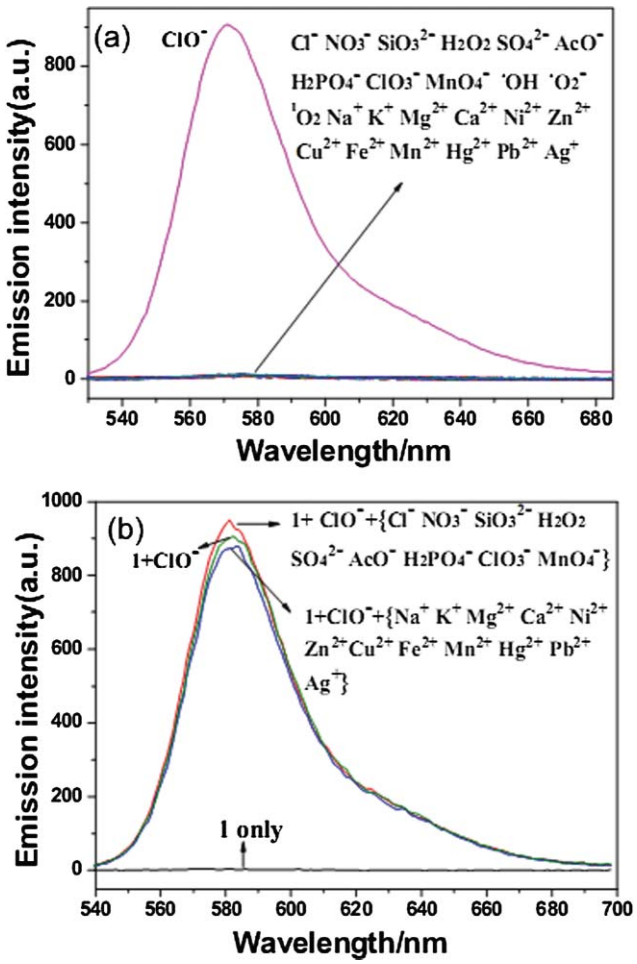

Fig. 7 (a) The fluorescence spectra of **1** (10 μM) in 7 : 3 (v/v) MeOH–$Na_2B_4O_7$/NaOH buffer (30 mM, pH 12.0) upon addition of 20 equiv of ClO^- and various species. (b) Fluorescence response of **1** (10 μM) to 20 equiv of ClO^- in 7 : 3 (v/v) MeOH–$Na_2B_4O_7$/NaOH buffer (30 mM, pH 12.0) containing various species. Species: Cl^-, NO_3^-, SiO_3^{2-}, H_2O_2, SO_4^{2-}, AcO^-, $H_2PO_4^-$ (1 mM); ClO_3^- (0.1 mM); MnO_4^- (20 μM); ·OH (100 μM Fe^{2+} + 1 mM H_2O_2); $\cdot O_2^-$ (1 mM KO_2); $^1O_2$ (1 mM Na_2MoO_4 + 2 mM H_2O_2); Na^+, K^+ (1 mM); Mg^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+} (0.1 mM); Cu^{2+}, Fe^{2+}, Mn^{2+}, Hg^{2+}, Pb^{2+}, Ag^+ (20 μM). λ_{ex} = 515 nm, λ_{em} = 580 nm.

Tris-HCl, pH 7.0), and for ClO^- in 7 : 3 (v/v) MeOH–water (30 mM $Na_2B_4O_7$/NaOH, pH 12.0). We expect that these methods will serve as practical tool for environmental samples analysis and biological studies.

Experimental section

Materials and general methods

Rhodamine B base,[17] pyridine-2-carboxylic acid hydrazide[18] and control compound **2**[12a] were prepared by literature procedures. All other reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel 60 (230–400 mesh). Absorption spectra were taken on an Agilent 8453 spectrophotometer. Fluorescence spectra were taken on Varian Cary Eclipse fluorescence spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. Mass spectra were obtained using electron ionization (EI) mass spectrometer.

Synthesis of probe 1

A solution of rhodamine B base (0.44 g, 1 mmol) in 1,2-dichloromethane (15 mL) was stirred, and phosphorus oxychloride (0.4 mL) was added dropwise over 10 min. The solution was refluxed for 4 h. The reaction mixture was cooled and the solvent was evaporated *in vacuo* to give rhodamine B acid chloride, which was directly used for next step without further purification.

The crude acid chloride was dissolved in acetonitrile (50 mL) and added dropwise over 2 h to a solution of pyridine-2-carboxylic acid hydrazide (0.14 g, 1 mmol) containing TEA (0.8 mL) in acetonitrile (20 mL) at room temperature. The reaction mixture was then stirred at room temperature for 4 h. The solvent was evaporated *in vacuo* to give crude product, which was purified by column chromatography (PE : EtOAc, 5 : 1, v/v) to provide pure **1**. Yield: 0.34 g, 60%. ^{1}H NMR (300 MHz, CD_3CN): δ 9.12 (s, 1H), 8.44 (d, 1H), 7.84–7.93 (m, 3H), 7. 47–7.59 (m, 3H), 7.02 (d, J = 7.2, 1H), 6.68 (d, J = 8.7, 2H), 6.35–6.39 (m, 2H), 6.30 (d, J = 2.4, 2H), 3.32 (q, J = 7.2, 8H), 1.08 (t, J = 7.2, 12H).^{13}C NMR (75 MHz, CD_3CN): 184.5, 175.92, 174.8, 171.3, 170.93, 160.30, 155.82, 151.82, 150.94, 149.67, 146.1, 145.3, 144.67, 130.23, 126.42, 119.46, 66.3, 34.2. IR (KBr) cm^{-1}: 3419, 1732, 1633, 1614, 1515, 1427, 1118. HRMS (ESI) calcd. for $(M + Na)^+$ 584.2632, found 584.2638. Anal. Calcd for $C_{34}H_{35}N_5O_3$: C, 72.70; H, 6.28; N, 12.47. Found: C, 72.73; H, 6.30; N, 12.43.

Procedures of ions sensing

Deionized water was used throughout all experiments. Solutions of Fe^{2+} and Ca^{2+} were prepared from their chloride salts; solutions of Hg^{2+}, Na^+, K^+, Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+} and Ag^+ were prepared from their nitrate salts; all anions except for MnO_4^- (potassium salt) were prepared from their sodium salts; Superoxide solution ($\cdot O_2^-$) was prepared by adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min;[19] Hydroxyl radical (·OH) was generated through the Fenton reaction of ferrous ammonium sulfate and hydrogen peroxide;[20] Singlet oxygen was produced from the H_2O_2/MoO_4^{2-} system in alkaline media.[21] Stock solutions of Cu^{2+} and ClO^- (50 mM, 5 mM, 0.5 mM) and the other ions (5 mM) were prepared in deionized water. A stock solution of **1** (25 mM) was prepared in DMF. The stock solution of **1** was then diluted to the corresponding concentration (20 μM, 10 μM and 1 μM) with the corresponding buffer solution [for Cu^{2+} sensing: MeCN–water (9 : 1, v/v) buffer solution (10 mM Tris-HCl, pH 7.0; for ClO^- sensing: MeOH–water (7 : 3, v/v) buffer solution (30 mM, $Na_2B_4O_7$/NaOH, pH 12.0)]. In the titration experiments, a 2.5 mL solution of **1** (20 μM, 10 μM and 1 μM) was poured into a quartz optical cell of 1 cm optical path length each time, and Cu^{2+} or ClO^- stock solution was added into the quartz optical cell gradually by using a micro-pipette. Spectra data were recorded in an indicated time after the addition. In selectivity experiments, the test samples

were prepared by placing the appropriate amounts of ions stock solution into 2.5 mL the corresponding buffer solution, and then adding stock solution of **1**.

Acknowledgements

We would like to thank the Natural Science Foundation of China (NSFC No. 20772073) and the Natural Science Foundation of Shanxi Province (2008011015) for financial support.

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