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ORIGINAL PAPER

A Novel Colorimetric and Off–On Fluorescent Chemosensor for Cr^{3+} in Aqueous Solution and its Application in Live Cell Imaging

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Abstract A novel colorimetric and off–on fluorescent chemosensor **2** was designed and synthesized, which showed reversible and highly selective and sensitive recognition toward Cr^{3+} over other examined metal ions in aqueous solution. Upon addition of Cr^{3+}, the solution of chemosensor **2** resulted in a color change from colorless to obvious pink color, these significant changes in color could be used for naked-eye detection. Chemosensor **2** exhibited a stable response for Cr^{3+} in the range 0–10 μM with a detection limit of 1 ppm. Furthermore, fluorescence imaging experiments of Cr^{3+} ions in living MGC803 cells demonstrated its value of practical applications in biological systems.

Keywords Rhodamine B · Chemosensor · Cr^{3+} · Aqueous solution · Cell imaging

Introduction

Selective detection of transition metal ions has been of great interest because of their importance in biological and environmental processes [1–4]. Among transition metal ions, trivalent chromium(Cr^{3+}) is an essential element in human nutrition and plays an important role in the metabolism of carbohydrates, fats, proteins and nucleic acids as it can activate certain enzymes and stabilize proteins and nucleic acids [5]. The deficiency of Cr^{3+} would cause disturbances in the glucose levels and lipid metabolism, and lead to a variety of disease such as diabetes and cardiovascular disease [6]. On the other hand, chromium is an environmental pollutant and its build-up due to various industrial and agricultural activities is a matter of concern. Therefore, developing artificial receptors with high selectivity and sensitivity for chemical and biochemical agents for the efficient detection of Cr^{3+} is especially important for both the environment and human health.

In recent years, colorimetric and fluorimetric methods for detection of Cr^{3+} are very popular due to its operational simplicity, high selectivity, sensitivity, rapidity, low cost of equipment and direct visual perception [7–10]. Rhodamine spirolactam based chemosensors are especially attractive due to their excellent spectroscopic properties of large molar extinction coefficient, high emission quantum yields and long absorption and emission wavelength elongated to visible region [11–13]. As is well known, rhodamine derivatives with spirolactam structure are nonfluorescent and colorless, upon metal binding, their structures can undergo a change from the spirolactam to an open ring amide, gives rise to a strong fluorescence emission and a pink color [14]. Due to the quenching effect of the paramagnetic Cr^{3+}, fluorescent turn-on reagents suitable for monitoring intracellular Cr^{3+} remain underdeveloped. Only a few of fluorescent probes based on rhodamine derivatives for Cr^{3+} have been reported [15–18]. Additionally, there are few literatures available reporting rhodamine-based fluorescent turn-on sensors for Cr^{3+} either in vitro or in vivo systems [19].

Herein, we reported a new rhodamine B-based chemosensor(**2**) for Cr^{3+} detection in aqueous solution. As shown in Scheme 1, chemosensor **2** was obtained by a simple two-step reaction from rhodamine B. Chemosensor **2** exhibited highly selectivity and sensitivity signal behavior toward Cr^{3+} over other common interfering metal ions and anions. Its selectivity was excellent, and the detection limit was measured

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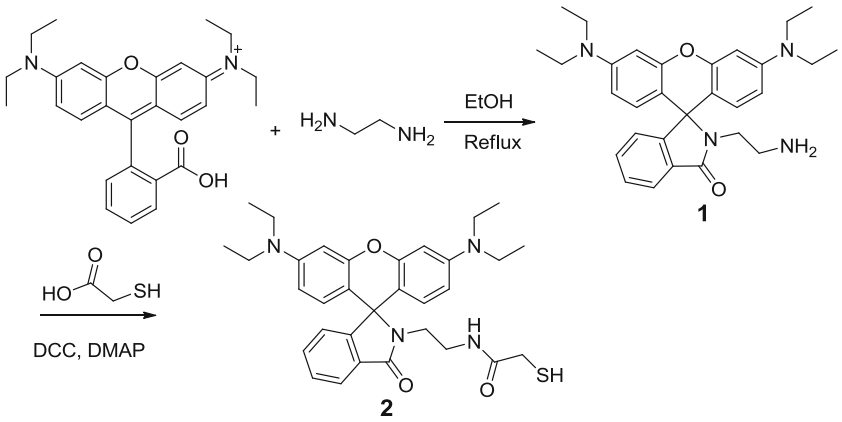
Scheme 1 Synthetic route of **2**



to be 1 ppm. Moreover, fluorescence imaging experiments of Cr^{3+} ions in living MGC803 cells demonstrated its value of practical applications in biological systems.

Experimental

Apparatus

Fluorescence spectra measurements were performed on a HITACHI F-4500 fluorescence spectrophotometer, and the excitation and emission wavelength band passes were both set at 4.0 nm. Absorption spectra were measured on a Lambda 35. UV/VIS spectrometer, Perkin Elmer precisely. The melting points were determined by an X-4 microscopic melting point apparatus with a digital thermometer (Shanghai, China). The pH was measured with a Model pHs-3C meter (Shanghai, China). ^{1}H and ^{13}C NMR spectra were recorded using a Bruker DTX-400 spectrometer. Samples were dissolved in $CDCl_3$ and placed in 5 mm NMR tubes. TMS was used as internal reference. ESI mass spectra were carried out on an HPLC Q-Tof HR-MS spectromerer (Waters Micromass) by using methanol as mobile phase. IR spectra in KBr disks were conducted using a PE-1710 instrument. Fluorescence images experiments were carried out with a Nikon-80i inverted fluorescence microscope.

Materials

All chemicals and reagents were used as received from commercial sources without further purification. Solvents for chemical synthesis and analysis were purified according to standard procedures. Double distilled water was used throughout the experiment. Chloride salts of metal ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, and Hg^{2+}) and the nitrate salt of Ag^+ ions were used to evaluate the metal ion binding properties by synthesized compounds. The metal ions were prepared as 10.00 mmol/l in water solution. The stoichiometry of each compound and Cr^{3+} was determined by Job's method from the obtained absorption spectroscopic data. In the determination, the sum of concentration of Cr^{3+} and each compound was kept at 300 μM and the molar ratio of Cr^{3+} was changed from 0 to 1.0.

Synthesis

Compound **1** was synthesized by reported methods [20]. Compound **2** was synthesized by a similar way described in a reported method [21]. The concrete way was described as follows:

Thioglycolic acid (92 mg, 1 mmol), DCC (237 mg, 1 mmol), DMAP (10 mg, 0.06 mmol) were dissolved in 10 mL dry dichloromethane. After cooling to 0 °C in ice bath, to the solution was added dropwise a solution of the compound **1** (484 mg, 1 mmol) in 20 mL dichloromethane over 30 min. The resulting mixture was stirred for 24 h at room temperature. The reaction mixture was then evaporated and the crude product was purified by column chromatography (silica gel, petroleum ether: ethyl acetate = 1:2, v/v). The yield was 45.6 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm):

1.18(t, 12H, *J*=7.02 Hz), 1.97(t, 1H, *J*=8.72 Hz), 3.09(t, 4H, *J*=6.58 Hz), 3.34 (dd, 10H, *J*=7.08 Hz), 6.28 (dd, 2H, *J*=3.78 Hz), 6.38(d, 2H, *J*=2.44 Hz), 6.44(d, 2H, *J*=8.84 Hz), 7.09(dd, 1H, *J*=2.84 Hz), 7.47(dd, 2H, *J*=8.6 Hz), 7.92(dd, 1H, *J*=2.88Hz). ^{13}C NMR (100 MHz, $CDCl_3$ ppm)δ 12.60, 28.35, 39.91, 40.62, 44.36, 65.55, 97.72, 104.79, 108.22, 122.83, 123.92, 128.49, 130.52, 132.81, 148.91, 153.29, 153.72, 169.55, 169.72. HR-MS: Calcd for $[C_{32}H_{38}N_4O_3S]$: 558.2665. Found: 559.2734 $[M+H]^+$, M.p.: 155–157 °C.

Results and Analysis

Fluorescence and UV–vis studies were performed using a 10 μM solution of compound **2** in a CH_3OH-H_2O(2:8, v/v)

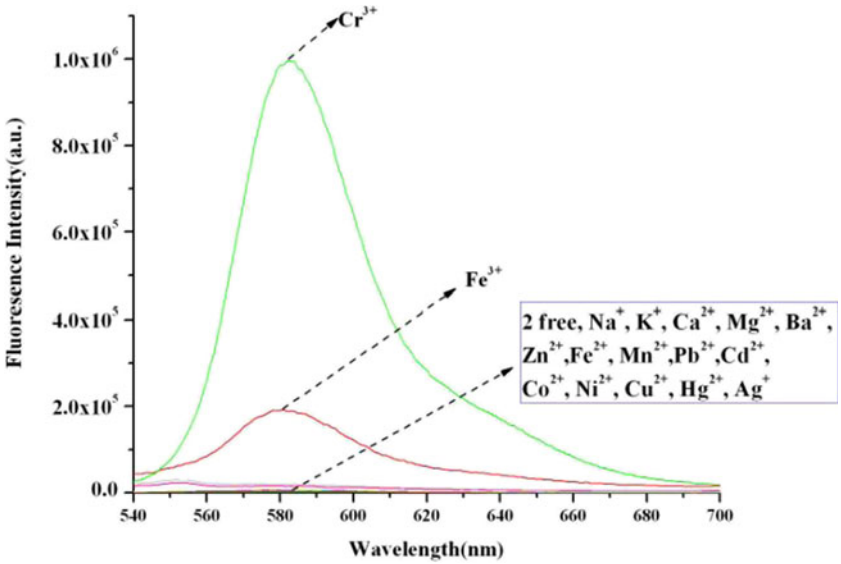


Fig. 1 Fluorescence spectra of **2**(10 μM)in CH_3OH-H_2O(2:8, v/v) with the presence of 10 eq. of various species (λ_{ex}=520 nm, slit=4 nm). Inset: fluorogenic response of **2** (10 μM) in H_2O to Cr^{3+} (10 eq.), λ_{ex}=520 nm

solution with appropriate amounts of metal ions. Compound **2** was colorless and found to be very stable in the above-mentioned solution system for more than two weeks. The absorption spectra of compound **2** in solutions did not show any peaks above 450 nm indicating the ring-closed spirolactone is predominant. In addition, a very weak fluorescence signal was observed at 580 nm (Fig. 1) upon excitation at 520 nm, confirming the presence of ring-closed spirolactone.

Fluorescence Spectral Responses of **2**

As shown in Fig. 1, the solution of **2**(10 μM) showed a very weak fluorescence in the absence of metal ions. When 10 eq. metal ions of Zn^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Hg^{2+}, Ba^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Ag^{+}, Co^{2+}, Cu^{2+} and Na^{+} were added, no obvious changes on fluorescence intensity and color could be observed (Fig. 1). However, under the same condition of Cr^{3+} (10 eq.) resulted in a remarkably enhancement of fluorescence at 580 nm. Moreover, the competitive experiments also confirmed that the background metal ions showed small interference with the detection of Cr^{3+} in CH_3OH-H_2O (2:8, v/v) (Fig. S4). Also, it was investigated that the fluorescence response of compound **2** toward Cr^{3+} in the presence response of various coexistent anion such as Cl^{-}, Br^{-}, NO^{3-}, SO_4^{2-} and ClO_4^{-}. It is gratifying to note that all the tested anions have no interference (Fig. 2).

In order to investigate the influence of the different acid concentration on the spectra of compound **2** and find a suitable pH span in which compound **2** can selectively detect Cr^{3+}

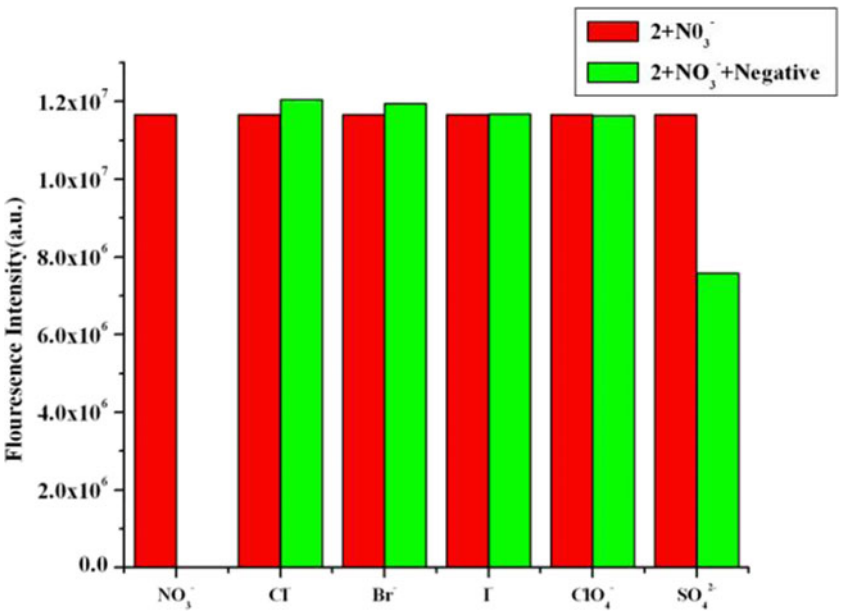


Fig. 2 Fluorescence intensity (at 580 nm) of **2**(10 μM) upon the addition of 10 eq. Cr^{3+} in the presence of 100 μM background negative ions in CH_3OH-H_2O (2/8, v/v), λ_{ex}=520 nm

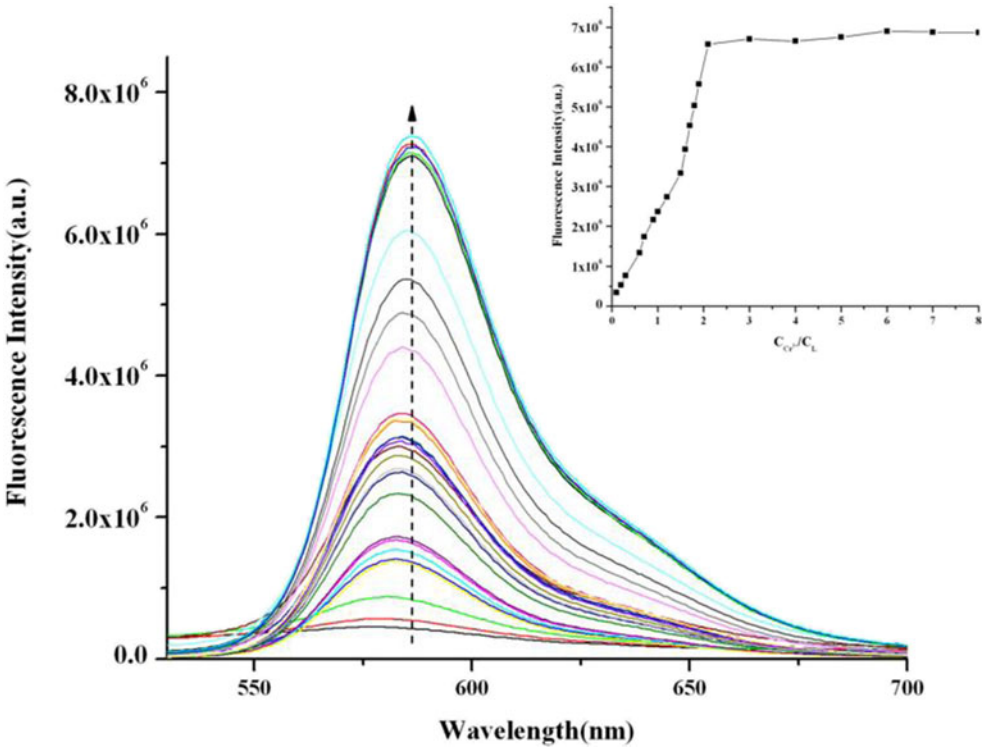


Fig. 3 Fluorescence spectra of **2** (100 μM)in CH_3OH-H_2O(2:8, v/v) upon addition of different amounts of Cr^{3+} ions. Inset: Changes in the emission intensity at 580 nm, λ_{ex}=520 nm

efficiently, the acid titration experiments were performed (Fig. S5). The results showed that free compound **2** did not have obvious absorption between pH 1.0 and 14.0, indicating that the spirocyclic form of compound **2** was not sensitive to pH changes in this range. However, the addition of Cr^{3+} led to the fluorescence enhancement over a comparatively wide pH range (4.0–8.0), which is attributed to opening of the rhodamine ring. Consequently, **2** may be used to detect Cr^{3+} in a comparatively wide pH.

To further investigate the binding stoichiometry of **2** and Cr^{3+} ion, a fluorescence titration experiment was carried out. An increase of fluorescence intensity of **2** could be observed

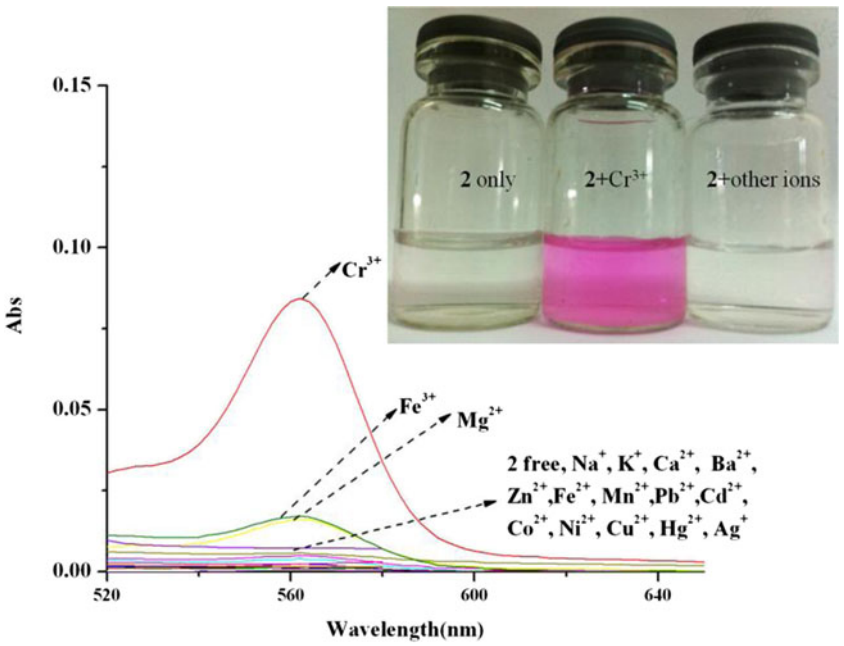


Fig. 4 Absorption spectra of **2** (10 μM) in CH_3OH-H_2O(2/8, v/v) with the presence of 10 eq. of various species (10 eq. of Cr^{3+}). Insert shows the photo of **2** with different metal ions in CH_3OH-H_2O(2/8, v/v)

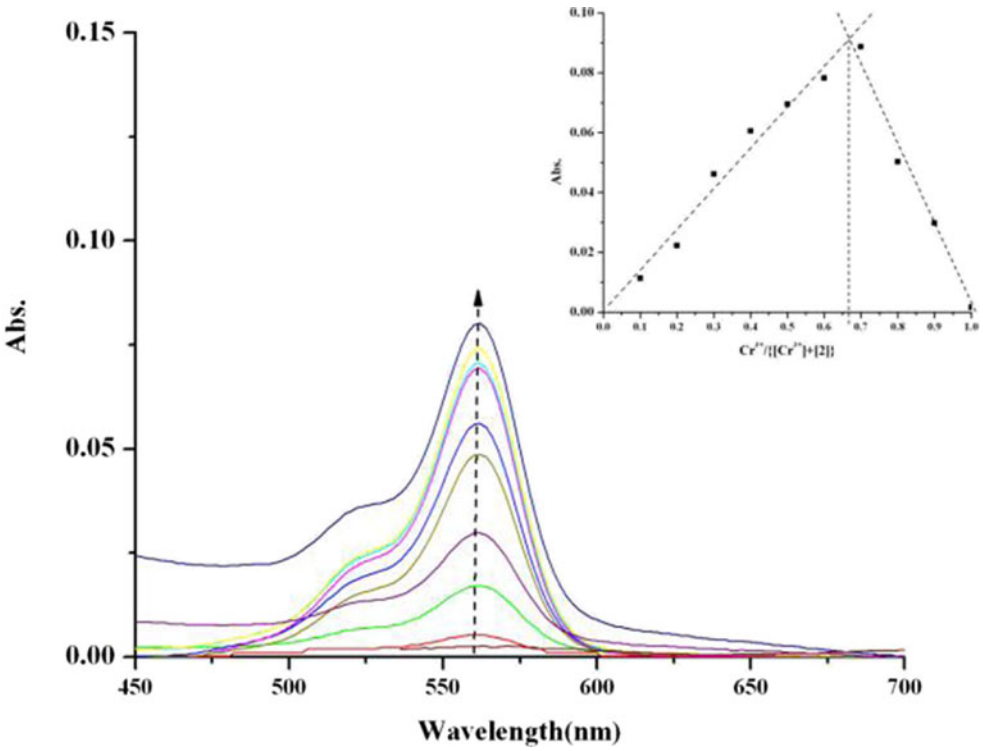


Fig. 5 UV–vis absorption spectra of Job's plots of the complexation between **2** and Cr^{3+}. Insert: Job's plot for the complex of **2** and Cr^{3+} at 562 nm. Total concentration of [**2**]+[Cr^{3+}] was kept constant at 300 μM

with gradual addition of Cr^{3+} ion (Fig. 3). The increment saturated after adding 2.0 equiv of Cr^{3+} (Fig. 3, inset), this also indicating a 2:1 stoichiometry of the Cr^{3+} to **2** in the complex.

UV–vis Spectral Responses of **2**

As shown in Fig. 4. UV–vis spectrum of **2** (10 μM) exhibited only very weak bands over 450 nm. Addition of 10 eq. Cr^{3+}

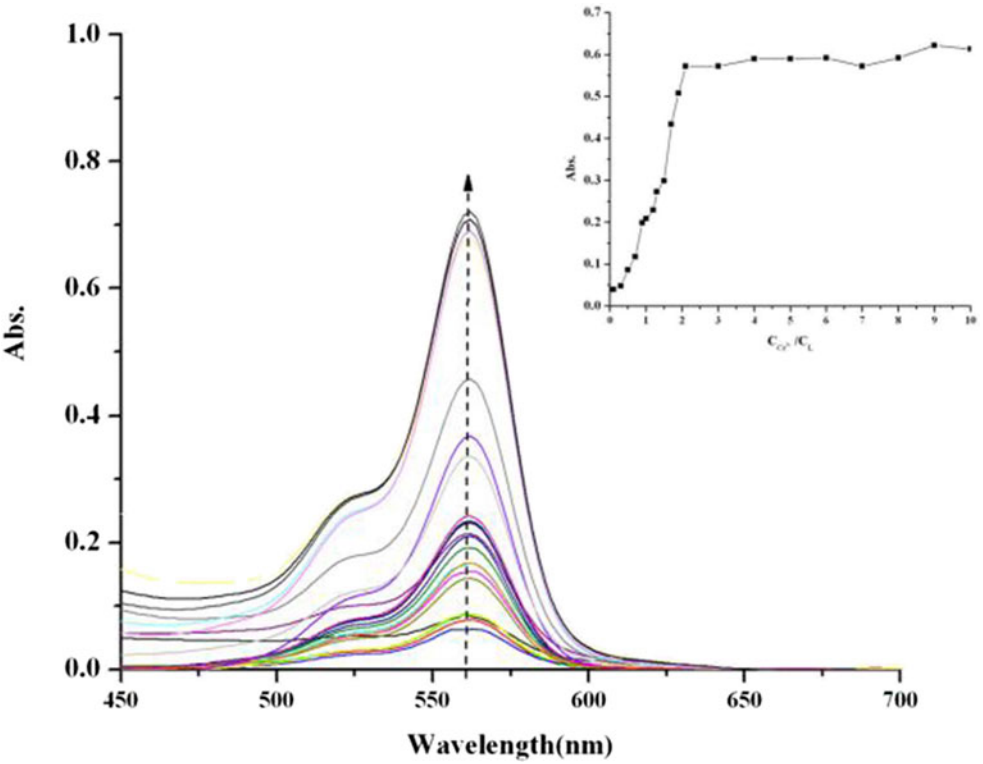


Fig. 6 UV–vis absorption spectra of **2** in CH_3OH-H_2O(2:8, v/v) upon addition of different amounts of Cr^{3+} ions. Inset: Changes in the absorption intensity at 562 nm

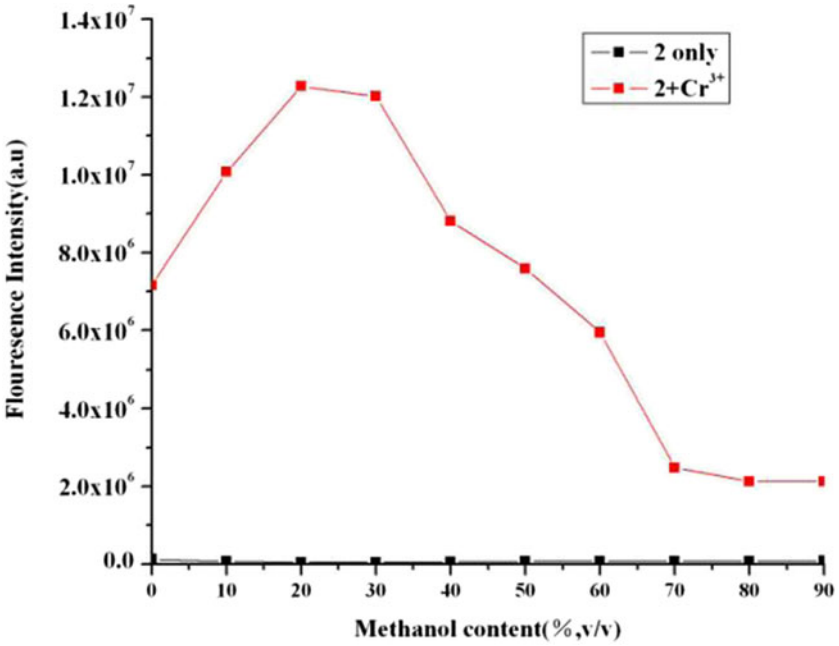


Fig. 7 Effect of the methanol content on the fluorescence intensity (at 580 nm) of **2** (10 μM) in the absence and presence of Cr^{3+} (100 μM), λ_{ex}=520 nm

into solution immediately resulted in a significant enhancement of absorbance at about 562 nm simultaneously the color change into red(Fig. 4. inset). This strongly suggested that compound **2** can serve as a "naked eye" probe and a high sensitivity for Cr^{3+}. Under the identical condition, no obvious response could be observed upon the addition of other ions including Zn^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Hg^{2+}, Ba^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Ag^{+}, Co^{2+}, Cu^{2+} and Na^{+} (Fig. 4). The results demonstrated that compound **2** was characteristic of high selectivity to toward Cr^{3+} over other competitive metal ions.

To determine the stoichiometry of the chromium-ligand complex, Job's method for absorbance measurement was applied [22]. A plot of $[Cr^{3+}]/\{[Cr^{3+}]+[2]\}$ versus the molarfraction of Cr^{3+} was provided in Fig. 5. The absorbance reached a maximum when the ratio was about 0.667, indicating a 2:1 stoichiometry of the Cr^{3+} to **2** in the complex. The ultraviolet titration experiment was also carried out to determine the stoichiometry of the chromium-ligand complex. As shown in Fig. 6, upon addition of increasing concentrations of Cr^{3+} ions to the solution, a new absorption band centered at

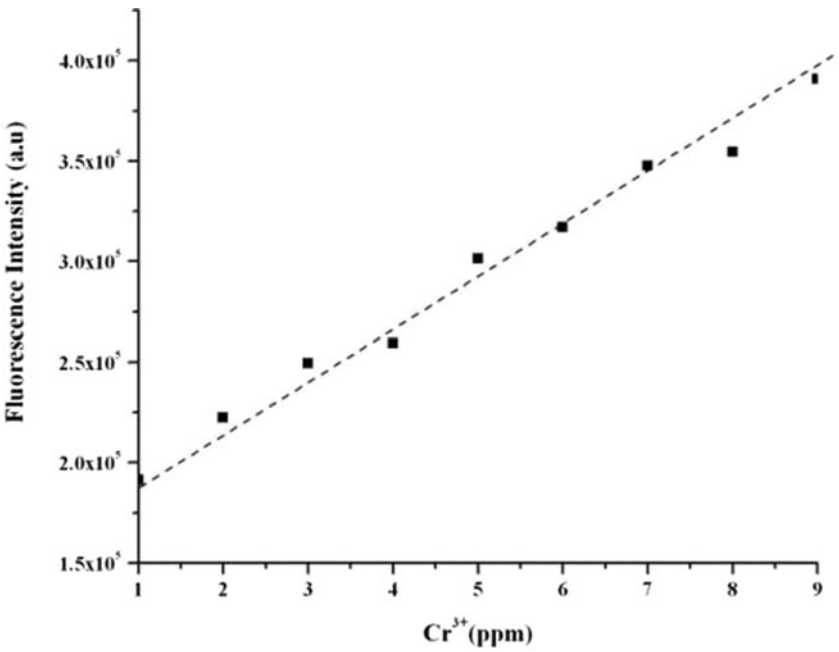


Fig. 8 The fluorescence at 580 nm of compound **2** (10 μM) as a function of the Cr^{3+} concentration, λ_{ex}=520 nm

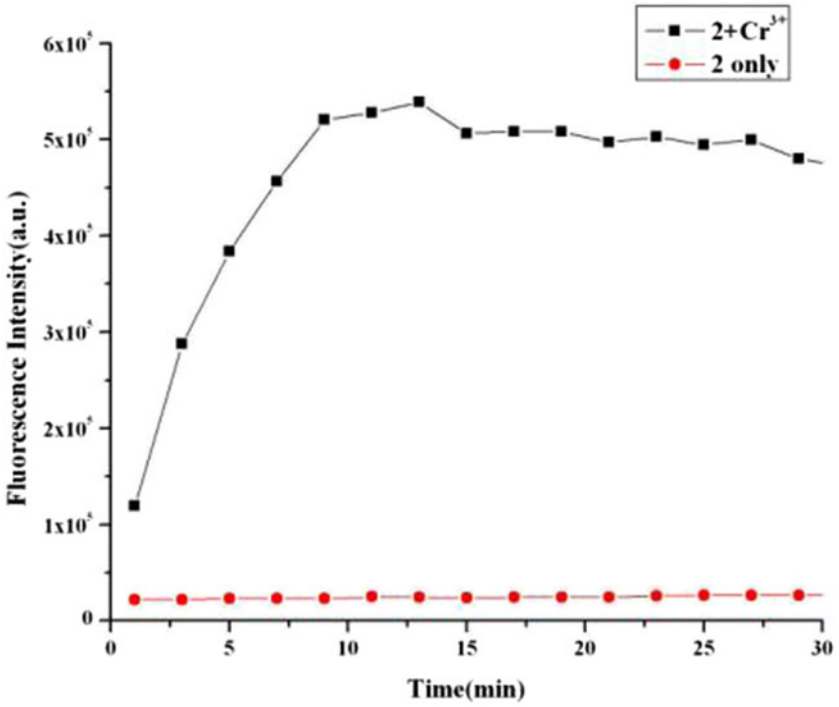


Fig. 9 Effect of reaction time on the fluorescence intensity (at 580 nm) of **2** (10 μM) in the absence and presence of 10 eq. Cr^{3+} in CH_3OH-H_2O (2/8, v/v), λ_{ex}=520 nm

562 nm appeared with increasing intensity, which can be ascribed to the formation of the ring-opened amide form of **2** upon Cr^{3+} ions binding. The increment saturated after adding 2.0 equiv of Cr^{3+} (Fig. 6, inset), this also indicating a 2:1 stoichiometry of the Cr^{3+} to **2** in the complex. These results were in accordance with the fluorescence titration experiment.

Effect of Media

The effect of methanol content on the fluorescent measurement of Cr^{3+} was investigated and results were shown in Fig. 7, it can be observed that the fluorescence signal reached its maximum value at 20 % aqueous methanol. Hence, 20 % aqueous methanol media was selected for the fluorimetric and colorimetric method.

The Detection of Cr^{3+}

Generally, one of the most important and useful application for a fluorescent chemosensor is the detection of metal ions. When the compound **2** was employed at 10 μM in a MeOH-H_2O solution (2/8, v/v), the fluorescent intensity of compound

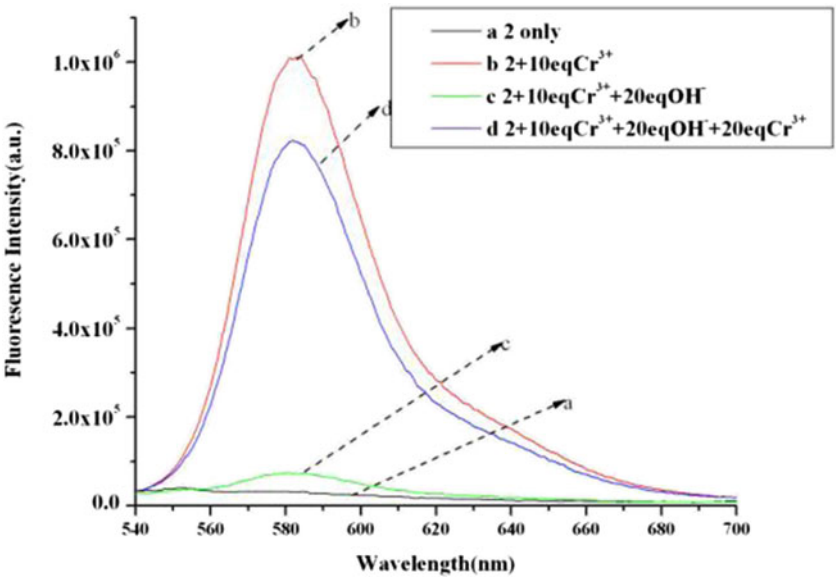


Fig. 10 Fluorescence intensity of **2** (10 μM) to Cr^{3+} in CH_3OH-H_2O (2/8, v/v) solution, λ_{ex}=520 nm

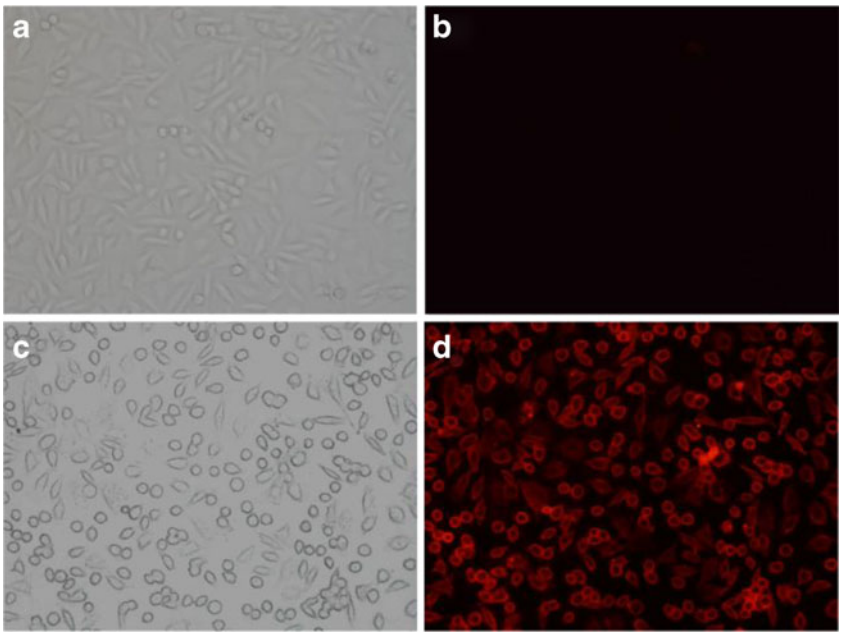


Fig. 11 Fluorescence images of Cr^{3+} in MGC-803 cells with 10 μM solution of **2** in CH_3OH-H_2O (2/8, v/v) for 30 min at 37 °C, Bright-field transmission images (**a**, **c**) and fluorescence images (**b**, **d**) of MGC-803 cells incubated with 0 μM, 40 μM of Cr^{3+} for 30 min, respectively

2 was proportional to be 1.0 ppm(Fig. 10), establishing that **2** was capable of distinguishing safe and toxic levels of Cr^{3+} in drinking water according to the China SA standard [23] (Fig. 8).

The Time Dependence of the Response

As shown in Fig. 9, the time dependence of the response of **2** to Cr^{3+} ions was investigated. It can be seen that the fluorescence signal of the compound **2** with Cr^{3+} ion increased for a few minutes, and leveled off as the time continues, while the fluorescence intensity of blank solution (only **2**, 10 μM) showed almost unchanged at the same conditions. The fluorescence intensity of **2** with Cr^{3+} reached its high value at about 10 min, after which the fluorescence intensity remained almost constant. There, a 10 min reaction time was selected in subsequent experiments in order to make the metal ions chelate with the sensors sufficiently.

Mechanism

As is well known, the reversibility is an important matter to obtain an excellent chemical sensor. Thus, the OH^--adding experiments were conducted to introduction of OH^- into the system containing **2** (10 μM) and Cr^{3+}(100 μM). The experiment showed that the introduction of OH^-(2 eq. to Cr^{3+}) could immediately restore the fluorescence intensities of **2**. When Cr^{3+} was added to the system again, the fluorescence intensity of **2** was enhance (Fig. 10). The above results also further elicited that the spectral response of **2** to Cr^{3+} is likely due to the chelation-induced ring opening of rhodamine spirolactam. Considering the behaviors of absorption and fluorescence spectra, the turn-on response of **2** may be explained by the spirocycle open-close mechanism.

Bioimaging Applications of Compound 2 in MGC-803 Cells [24, 25]

To further assess the potential applications of the probe as Cr^{3+} probe in living cells, fluorescent imaging inside MGC-803 cells was monitored by fluorescence microscopy. Incubation of MGC-803 cells with 10 μM of the probe **2** in CH_3OH-H_2O (2/8, v/v) for about 30 min at 37 °C gave almost no intracellular fluorescence. After washing with water two times, 40 μM of Cr^{3+} were then supplemented to the cells, and all the reaction mixtures were incubated at 37 °C for another 30 min. After that, the fluorescence from the intracellular area was observed (Fig. 7d), providing visual evidence of the probe **2** entering cells and information on the intracellular existence of Cr^{3+}. Furthermore, a bright-field transmission image of cells treated with **2** and Cr^{3+} confirmed that the cells were viable throughout the imaging experiments(Fig. 11a and c). These preliminary experimental results demonstrated that **2** could be used for detecting Cr^{3+} in biological samples.

Conclusions

In conclusion, we synthesized and reported an easily available fluorescent chemosensor **2** based on rhodamine B. The compound **2** exhibited a strong fluorescence enhancement upon addition of Cr^{3+} while showing almost no response to other cations. In addition, the limit of detection for Cr^{3+} in CH_3OH/

H_2O (2/8, v/v) was found to be 1 ppm. Furthermore, fluorescence imaging experiments of Cr^{3+} ions in living MGC803 cells demonstrated its value of practical applications in biological systems.

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