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A novel fluorescein derivative as a colorimetric chemosensor for detecting copper(II) ion

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ABSTRACT

A novel fluorescein derivative, synthesized by the reaction of fluorescein hydrazide and 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone, was evaluated as a chemoselective metal ion sensor. Addition of Cu^{2+} to an aqueous solution of the fluorescein derivative resulted in a rapid color change from colorless to deep yellow together with a distinctive change in UV–vis absorption spectrum. However, other common alkali-, alkaline earth-, transition- and rare earth metal ions induced no or minimal spectral changes. The stoichiometry of the reaction and association constant of the fluorescein derivative with Cu^{2+} are described. Experimental results indicate that the fluorescein derivative could provide a rapid, selective and sensitive response to Cu^{2+}, and could be used as a potential Cu^{2+} colorimetric chemosensor in aqueous solution.

1. Introduction

Metal ion sensors are employed in applications ranging from clinical toxicology, environmental bioinorganic chemistry, bioremediation, and waste management [1–3] and much attention has focussed to the development of sensing devices for copper(II). As the third most abundant divalent metal ion in the human body, after Fe^{2+} and Zn^{2+}, Cu^{2+} plays a pivotal role in a variety of fundamental physiological processes in organisms ranging from bacteria to mammals [4,5]. Copper compounds are also employed for plant diseases treatment, water treatment and as preservatives for wood and leather. Nonetheless, while a low-level background intake of copper is indispensable, high doses of copper can be harmful and even toxic to biological systems [4,6].

Many sensing methods for detecting Cu^{2+} have been described, such as colorimetric and fluorescent chemosensors, and electrochemical methods [7–9]. Colorimetric sensors are promising due to the simplicity of the assay. Furthermore, colorimetric assays have a significantly lower capital cost than closely related methods, such as fluorescent sensors, for which both spectrophotometric equipment and a UV light source are required [10–16].

The fluorescein and rhodamine family of dyes, with spirolactam structure (closed form) are non-fluorescent. As shown in Fig. 1, ring-opening of the spirolactam gives the open form and results in a strong fluorescence and obvious color change [17–20]. Due to large visible-range extinction coefficients and high fluorescence quantum yields for fluorescein and rhodamine, these compounds are excellent antenna chromophores [21–26].

In recent years, several rhodamine-based chemosensors and chemodosimeters for metal ions, such as Cu^{2+} [27–30], Hg^{2+} [20,31–34], Fe^{3+} [35], and Pb^{2+} [19] have been studied. The cation-sensing mechanism of these probes is based on the change in structure between spirocyclic and opencyclic forms. However, fluorescein-based probes have received comparatively little attention [36,37]. We synthesized a fluorescein-based colorimetric chemosensor, 1-phenyl-3-methyl-5-hydroxypyrazole-4-benzoyl (fluorescein)hydrazone (**1**), for rapid, selective and sensitive response to Cu^{2+} in aqueous media (Fig. 2). Solutions of **1** are colorless, but upon addition of micromolar Cu^{2+} a deep yellow color is obtained. Addition of other common alkali-, alkaline earth-, transition- and rare earth metal ions result in no or minimal spectral change. Compound **1** is a naked-eye chemosensor for

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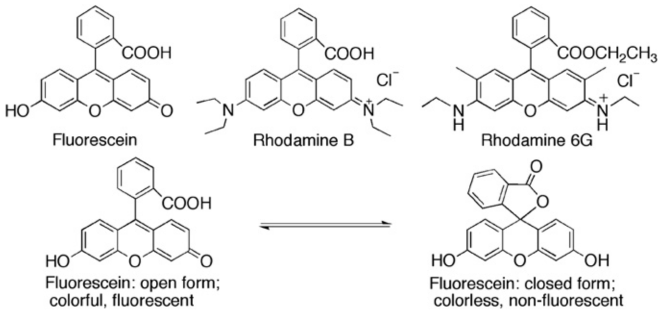

Fig. 1. Structure of fluorescein, rhodamine B and rhodamine 6G.

detection of Cu^{2+} that upon chelation of Cu^{2+} **1** will change to a strongly colored ring-opened form.

2. Experimental

2.1. Materials and instruments

All the materials for synthesis were purchased from commercial suppliers and used without further purification. With the exception of $Al(NO_3)_3$ and $MgSO_4$, aqueous solutions of metal ions were prepared from their chloride salts.

NMR spectra were taken on a Bruker Avance DRX-200 or Varian Mercury Plus-300 BB spectrometer with TMS (tetramethylsilane) as internal standard and DMSO-d_6 as solvent. Mass spectra were obtained on a Bruker Esquire 6000 spectrometer. UV–vis absorption spectra were obtained with a Perkin Elmer Lambda 35 UV–vis spectrophotometer and recorded in quartz cells with 1 cm optical path length. Fluorescence spectra were recorded on a Hitachi RF-4500 fluorescence spectrophotometer.

2.2. General procedures of metal ion sensing

2.2.1. Job's plot analyses

For Job's plot analyses (in Section 3.3), a 1.0×10^{-3} M stock solution of Cu^{2+} in H_2O, and 1.0×10^{-3} M stock solution of **1** in DMSO (dimethylsulfoxide) were prepared. The Cu^{2+} stock solution and the stock solution of **1** were placed in a test tube to obtain a total volume of 20 μL, then diluted to 2 mL with DMSO/H_2O (4:6, v/v) to get the test solution (nine test solutions were got; for the first test solution, 2 μL stock solution of Cu^{2+} and 18 μL stock solution of **1** were used, then diluted to 2 mL with DMSO/H_2O (4:6, v/v) to get the first test solution; for the second test solution, 4 μL stock solution of Cu^{2+} and 16 μL stock solution of **1** were used; for the third test solution, 6 μL stock solution of Cu^{2+} and 14 μL stock solution of **1** were used; until 18 μL stock solution of Cu^{2+} and 2 μL stock solution of **1** were used to get the ninth test solution). All the analyses were performed with $[\mathbf{1}] + [Cu^{2+}] = 10.0$ μM. The absorbance at 495 nm of these test solutions was recorded.

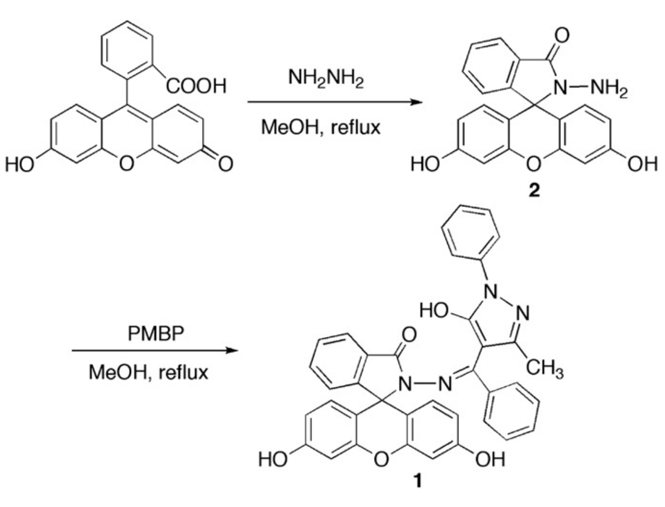

Fig. 2. Synthetic route of chemosensor **1**.

2.2.2. Spectral analyses

In other spectral analyses, a 1.0×10^{-3} M solution of **1** was prepared in DMSO, and then diluted by DMSO/H_2O (4:6, v/v) to give a 1.0×10^{-5} M stock solution of **1** for spectral analyses. Stock solutions of metal ions were prepared in H_2O (2.0×10^{-3} M of Cu^{2+} was used in Section 3.2; 2.0×10^{-2} M of metal ions were used in other sections, except Sections 3.2 and 3.3). Each time, a 2 ml stock solution of **1** (1.0×10^{-5} M) was added to the quartz cell, and the required quantity of stock solution of metal ions was added with a microsyringe.

All the measurements were taken at room temperature about 298 K. After 2 min of the mixing of metal ions with **1**, UV–vis absorption spectra or fluorescence emission spectra were measured, unless otherwise indicated. For all fluorescent tests, excitation wavelength was 287 nm, with excitation and emission slit widths both 5 nm. Addition of metal ions increased the volume by no more than 0.04 mL, so that dilution was insignificant.

2.3. Synthesis

2.3.1. Synthesis of fluorescein hydrazide (2)

In a 100 mL flask containing a suspension of fluorescein (6 g, 18.1 mmol) in 50 mL methanol, excess hydrazine hydrate (24 mL; hydrazine content >80 mass%) was added. The reaction mixture was heated to reflux for 7 h with stirring, during which time the suspended particles were consumed and a clear solution was obtained. The ensuing solution was allowed to cool and poured into 400 mL H_2O at which time, a yellow precipitated formed immediately, which was allowed to settle for 2 h. The aqueous suspension was filtered, washed with water until the filtrate was colorless, and washed 3 × 10 mL with cold absolute ethanol. The crude product was purified by recrystallization from ethanol to give 3.59 g of **2** as an off-white solid (57%). Melting point: 262–264 °C. ^1H NMR (200 MHz, DMSO-d_6), δ (ppm): 4.38 (s, 2H), 6.42 (m, 4H), 6.59 (s, 2H), 6.97 (m, 1H), 7.48 (m, 2H), 7.76 (m, 1H), 9.81 (s, 2H). ^{13}C NMR (50 MHz, DMSO-d_6), δ (ppm): 64.6, 102.3, 109.9, 111.9, 122.3, 127.9, 128.4, 129.3, 132.6, 151.5, 152.4, 158.1, 165.4.

2.3.2. Synthesis of 1-phenyl-3-methyl-5-hydroxypyrazole-4-benzoyl(fluorescein)hydrazone (1)

In a 25 mL flask, **2** (0.346 g, 1 mmol) and 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP; 0.278 g, 1 mmol) were suspended in 10 mL methanol. The mixture was refluxed for 8 h with stirring, during which time a clear solution formed. Following reaction, the mixture was allowed to cool to room temperature and a yellow precipitate formed. The precipitated was separated by filtration and washed with 3 × 10 mL methanol. After drying, 0.25 g of **1** (bright yellow solid) was prepared in 41% yield. Melting point >320 °C. ^1H NMR (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 3H), 5.96 (s, 2H), 6.35 (s, 2H), 6.61 (d, $J = 2.1$ Hz, 3H), 7.12 (m, 3H), 7.21 (s, 2H), 7.36 (m, 3H), 7.63 (m, 2H), 7.77 (d, $J = 8.1$ Hz, 2H), 7.89 (d, $J = 6.9$ Hz, 1H), 10.04 (s, 2H), 11.51 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6), δ (ppm): 15.1, 66.4, 100.7, 102.4, 107.5, 113.2, 118.2, 123.3, 124.4, 127.1, 127.7, 128.7, 129.5, 130.2, 134.7, 138.3, 147.4, 149.6, 152.8, 159.0, 164.7,

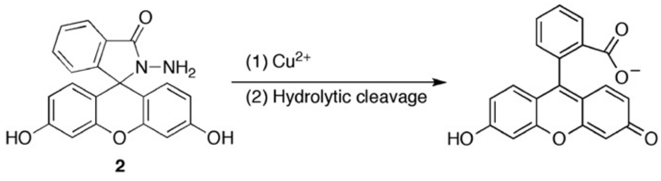

Fig. 3. According to literature, the reaction mechanism of **2** with Cu^{2+}.

166.7, 167.3. ESI mass spectrometry, *m*/*z*: 607.3 $(M + H)^+$, 629.3 $(M + Na)^+$.

3. Results and discussion

3.1. *Design and synthesis of sensor **1***

In past few years, some fluorescein-based metal ions sensors were reported. Lippard and co-workers installed a series of one or two binding units, such as 8-aminoquinoline, methylated dipicolylamine, or thioether on the xanthene moiety of fluorescein [38–42]. Similar work was also reported by Jun et al. [43] and Kim et al. [44]. Guo and co-workers attached some chelator groups to the benzene moiety of decarboxylated fluorescein [45,46].

However, fluorescein-based metal ions sensors, which are based on the change in structure between spirocyclic and opencyclic forms of fluorescein moiety, have been seldom investigated up to now [36,37]. According to Ma and co-workers, the parent compound **2** can be used as a selective and sensitive probe for Cu^{2+} [36]. In aqueous solution, Cu^{2+} can be recognized and bound by compound **2**, and the subsequent complexation of Cu^{2+} promotes hydrolytic cleavage of the amide bond, causing the release of fluorophore (fluorescein) and thereby the retrievement of fluorescence (Fig. 3). A reaction time of 2 h at room temperature was used for their detecting system. Thus, compound **2** can not be used for real-time monitoring of Cu^{2+}.

Our considerations for synthesizing **1** are as follows: (a) PMBP is known to possess a high chelating ability with common metal ions [47–49]. Introducing PMBP moiety can generate the chelating ability of **1** toward some metal ions; (b) when coordination takes place, the spirolactam of fluorescein moiety can be opened, at the same time obvious color and optical change can be observed. Consequently, sensor **1** was synthesized via a simple two-step reaction, and confirmed by melting point, ^1H NMR, ^{13}C NMR, and mass spectrum.

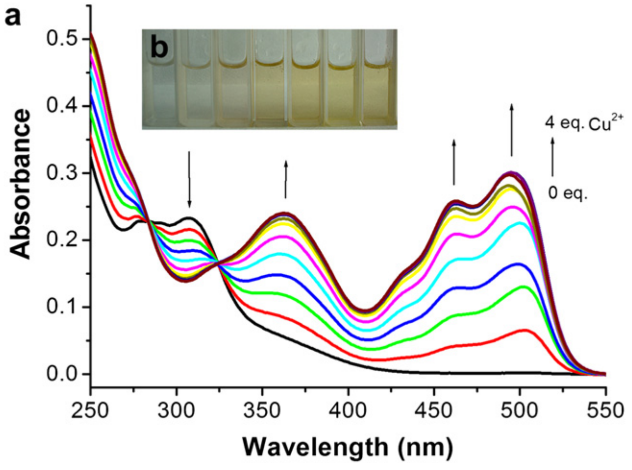

Fig. 4. UV–vis absorption spectra (a) and photographs of color changes (b) of **1** (10.0 μM) with gradual addition of Cu^{2+} [(a) 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 40 μM, respectively; (b) from left to right: 0, 2, 6, 10, 16, 20, and 40 μM, respectively] in aqueous solution ($DMSO/H_2O$ = 4:6, v/v).

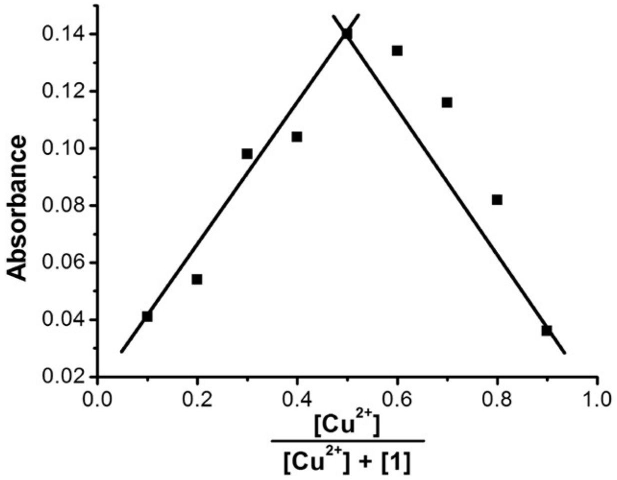

Fig. 5. Job's plot of **1** with Cu^{2+} in aqueous solution ($DMSO/H_2O$ = 4:6, v/v). Total concentration of **1** + Cu^{2+} was kept constant at 10.0 μM. The absorbance at 495 nm was used.

3.2. *UV–vis titration of **1** with Cu^{2+}*

Fig. 4a shows the UV–vis absorption spectra of **1** with various concentrations of Cu^{2+} in aqueous solution at room temperature. In aqueous solution ($DMSO/H_2O$ = 4:6, v/v), **1** (10.0 μM) had nearly no absorption in the visible region, which was ascribed to the closed spirolactam form of **1**. The characteristic peak of 66.4 ppm (9-carbon) in ^{13}C NMR spectrum of **1** also supported this dentification.

Each time, 2 μL of Cu^{2+} stock solution (2.0×10^{-3} M) was added to the stock solution of **1** (10.0 μM). For the spectra of **1**, upon addition of Cu^{2+}, the band at 307 nm decreased and three new bands at 363 nm, 460 nm, and 495 nm appeared, respectively. Correspondingly, the solution of **1** changed from colorless to deep yellow (Fig. 4b). The significant color change indicates that **1** is a sensitive naked-eye indicator for Cu^{2+}.

According to linear Benesi–Hildebrand expression, the measured absorbance $[1/(A - A_0)]$ at 370 nm varied as a function of $1/[Cu^{2+}]$ in a linear relationship ($R = 0.9932$), indicating formation of 1:1 stoichiometry between Cu^{2+} and **1** [13,50,51]. The association constant of **1** with Cu^{2+} in the solution of $DMSO/H_2O$ (4:6, v/v) was calculated to be 3.3×10^4 M^{-1}.

Reactions of **1** with $Cu(AcO)_2$, $CuSO_4$, or $Cu(NO_3)_2$ gave the same changes as the cases of **1**-$CuCl_2$ on the UV–vis absorption spectra, demonstrating that counter-anion effect is negligible.

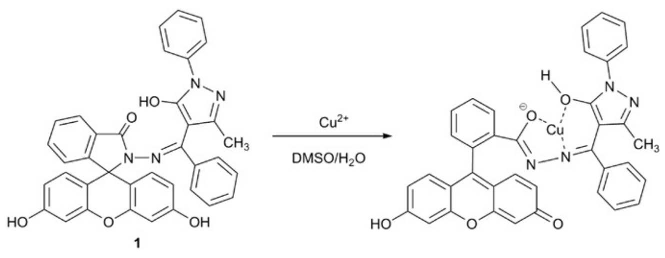

Fig. 6. Proposed binding mechanism of **1** with Cu^{2+}.

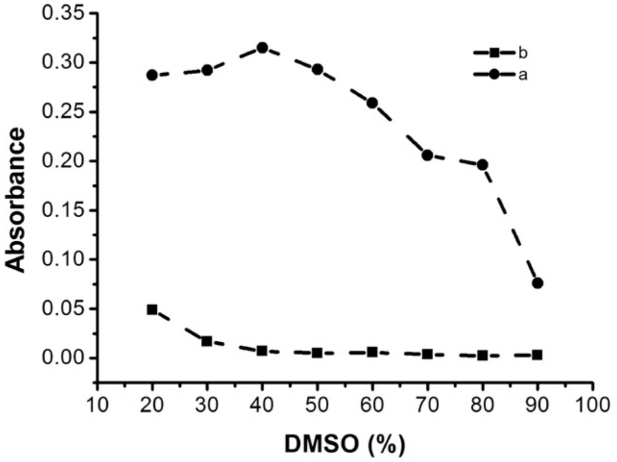

Fig. 7. Effect of DMSO content on the absorbance of **1** (10.0 μM) at 495 nm in the presence (a) and absence (b) of Cu^{2+} (20.0 μM) in DMSO aqueous solution.

3.3. *Job's plot analyses*

For further determination the stoichiometry between **1** and Cu^{2+}, Job's plot analyses were also used. Total concentration of **1** + Cu^{2+} was kept constant at 10.0 μM. When molar fraction of Cu^{2+} was 0.5, the absorbance at 495 nm got to maximum (Fig. 5), indicating that forming a 1:1 complex between **1** and Cu^{2+}.

3.4. *Binding mode studies*

Usually, for most of rhodamine-based probes and a few fluorescein-based probes, the cation-sensing mechanism is based on the change in structure between spirocyclic and opencyclic forms. In the absence of metal ions, these probes exist in the spirocyclic forms which are colorless and non-fluorescent. Addition of metal ions makes the spirocycle open via coordination or irreversible chemical reaction, resulting in color change and generation of strong fluorescence [27–37].

For sensor **1**, obvious color and electronic absorption spectrum changes appeared with the addition of Cu^{2+}. The possible reason is that sensor **1** coordinates to Cu^{2+}, and meanwhile spirocyclic opening in fluorescein backbone happens (Fig. 6). As a result of the coordination, the absorption bands at 307 nm decrease and the absorption bands at 363 nm increase simultaneously. Isosbestic points at 284 nm and 324 nm also indicate the presence of a new metal complex in equilibrium with the free ligand **1** [16]. Meanwhile, the spirocyclic opening in fluorescein backbone gives the characteristic absorption of fluorescein at 460 nm and 495 nm in the visible region [52]. They are responsible for the color change from colorless to deep yellow.

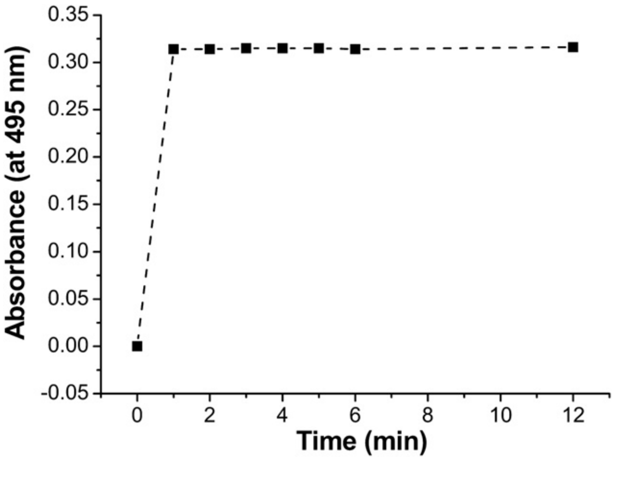

Fig. 8. Time course of the response of **1** (10.0 μM) to 2 equiv. of Cu^{2+} in aqueous solution (DMSO/H_2O = 4:6, v/v).

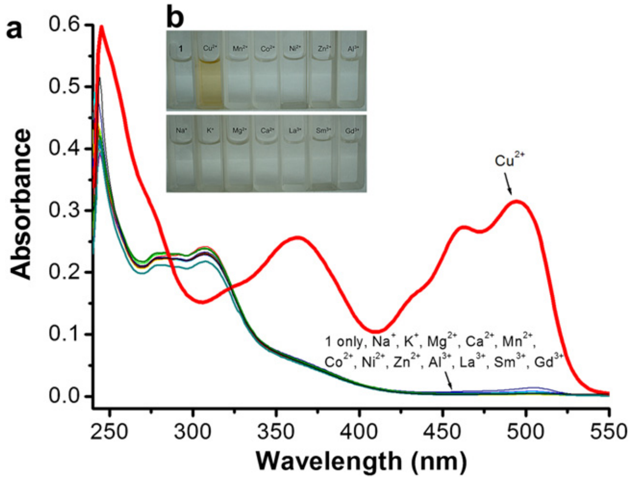

Fig. 9. UV–vis absorption spectra (a) and photographs of color changes (b) of **1** (10.0 μM) upon addition of various metal ions (20.0 μM) in aqueous solution (DMSO/H_2O = 4:6, v/v).

On addition of $EDTANa_2$ (Ethylenediaminetetraacetic acid disodium salt) to the solution of **1** containing Cu^{2+}, the solution changed from deep yellow to colorless, indicating a reversible coordination between **1** and Cu^{2+}. The reversible response toward Cu^{2+} implies that **1** is a chemosensor not a chemodosimeter of Cu^{2+}.

3.5. *Effect of reaction media and reaction time*

For practical applicability, the Cu^{2+} detection were carried out in aqueous solution (DMSO mixed with water). In order to get optimal experimental phenomenon, the effect of DMSO content on the absorbance of **1** was studied.

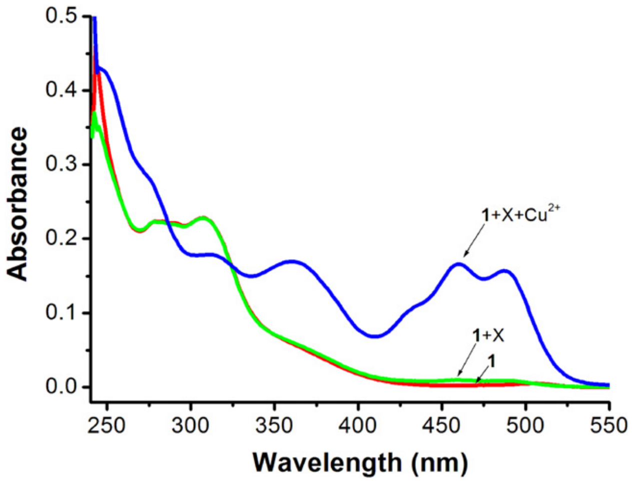

Fig. 10. UV–vis absorption spectra of **1** (10.0 μM) in aqueous solution (DMSO/H_2O = 4:6, v/v) in the presence of Cu^{2+} (20.0 μM) and miscellaneous cations (X) including Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, La^{3+}, Sm^{3+}, and Gd^{3+} (20.0 μM, respectively).

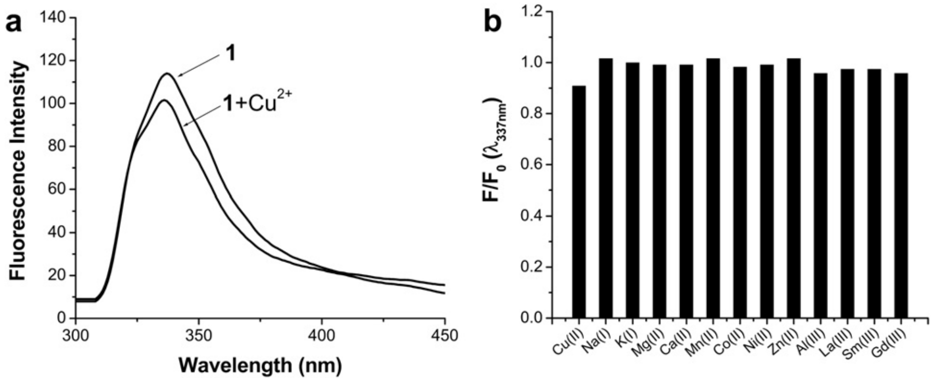

Fig. 11. (a) Fluorescence emission spectra of **1** (10.0 μM) and that after addition of Cu^{2+} (20.0 μM) in aqueous solution ($DMSO/H_2O = 4:6$, v/v). (b) Changes in fluorescence emission intensity ratio (F/F_0) at 337 nm of **1** (10.0 μM) in the presence of various metal ions in aqueous solution ($DMSO/H_2O = 4:6$, v/v). F_0 is the intensity of free **1**, and F is the intensity of **1** after addition of metal ion. Excitation wavelength was 287 nm.

As shown in Fig. 7, for the free **1**, when DMSO concentration is at or above 40%, **1** has very weak absorbance at 495 nm, therefore, gives lower background signal. In the presence of 2 equiv. of Cu^{2+}, when DMSO concentration is above 40%, the absorbance of **1** (at 495 nm) dramatically decreased. It means that when DMSO concentration is at 40%, the detection system can give lower background signal and maximum detection signal. As a result,$DMSO/H_2O = 4:6$ (volume ratio) as the solvent was employed in all spectral analysis experiments.

The effect of reaction time on the UV–vis absorption spectra of the detecting system was studied and the results are shown in Fig. 8. Adding 2 equiv. of Cu^{2+} to aqueous solution of **1**, 1 min later the absorbance at 495 nm of the system gets to maximum and does not increase with more reaction time. It means the spirocycle of **1** can be opened in very short time, and **1** could be used as a real-time Cu^{2+} chemosensor. 2 min was selected as the detection time in this paper.

3.6. Interference from other metal ions

Selectivity is an important characteristic of an ion-selective chemosensor. To examine the selectivity of **1**, the affinity of **1** for other surveyed metal ions, such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, La^{3+}, Sm^{3+} and Gd^{3+}, was investigated. As shown in Fig. 9, addition 2 equiv. of these alkali-, alkaline earth-, transition- and rare earth metal ions do not cause significant UV–vis absorption spectral and color changes of **1**. However, under the same conditions, addition of Cu^{2+} results in an obvious change, which indicates that chemosensor **1** has higher binding affinity toward Cu^{2+} than other surveyed metal ions.

Furthermore, to explore the utility of **1** as a cation-selective chromogenic chemosensor, competition experiment was carried out by adding 20.0 μM Cu^{2+} to solution of **1** (10.0 μM) in the presence of miscellaneous cations including Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, La^{3+}, Sm^{3+}, and Gd^{3+} (20.0 μM, respectively; Fig. 10). These miscellaneous competitive cations did not lead to any significant absorption changes of **1**. However, upon addition of 2 equiv. of Cu^{2+} to the solution, the unique spectral and color changes were still displayed. These results indicate that **1** has a good selectivity toward Cu^{2+} over other competitive cations, and more, the detection of Cu^{2+} by **1** is hardly affected by these common coexistent metal ions.

3.7. Influence of metal ions on the fluorescence intensity of 1

The fluorescence titration of **1** (10.0 μM) with 2 equiv. of metal ions was conducted in aqueous solution ($DMSO/H_2O = 4:6$, v/v). Excitation wavelength was 287 nm, and fluorescence emission intensity was recorded at 337 nm.

In aqueous solution, the fluorescence intensity of **1** was extremely weak. It was also due to the closed spirolactam form of **1**. As shown in Fig. 11, upon addition of 2 equiv. of Cu^{2+}, emission intensity of **1** was slightly quenched. Addition of 2 equiv. of other surveyed metal ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, La^{3+}, Sm^{3+} and Gd^{3+}, respectively) almost did not produce a change in the fluorescence spectra of **1**, in terms of maximum emission intensity and general peak shape. Fluorescence intensity ratios of **1** in the presence and absence of these surveyed metal ions at 337 nm are almost equal to one (Fig. 11b).

After coordination to Cu^{2+}, **1** only gave the characteristic color change, and did not generate the strong fluorescence emission of fluorescein moiety. We attribute this to intramolecular fluorescence quenching by paramagnetic Cu^{2+}. Similar phenomena was also observed in other experiments [44,53].

Quenching of the fluorescence of luminescent ligand by coordination to a paramagnetic Cu^{2+} center can be restored by nitric oxide-induced reduction to a diamagnetic Cu^+ species. It is a strategy for metal-based nitric oxide sensing [54]. Can **1**-Cu^{2+} complex be used as a nitric oxide sensor? It deserves further research.

4. Conclusions

In conclusion, a novel fluorescein derivative (**1**) has been synthesized by the reaction of fluorescein hydrazide and 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone. Addition of Cu^{2+} to an aqueous solution ($DMSO/H_2O = 4:6$, v/v) of **1** can result in an obvious color change (from colorless to deep yellow) and UV–vis absorption spectral change in a very short time. This makes the real-time, simple-to-use and naked-eye detection of Cu^{2+} possible. As a result of coordination of **1** with Cu^{2+}, the spirocyclic opening of fluorescein moiety in **1** is proposed. Moreover, **1** possesses a good selectivity toward Cu^{2+} over other competitive cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, La^{3+}, Sm^{3+} and Gd^{3+}). These features indicate that **1** is a good candidate, for rapid, selective and sensitive detecting Cu^{2+} in aqueous media.

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