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Scientific paper

Benzothiazolylhydrazone-Based Turn-on Fluorescent Probe for Detecting Cu^{2+}: S-donor as a Cu^{2+}-induced Fluorescence Quenching Inhibitor

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Abstract

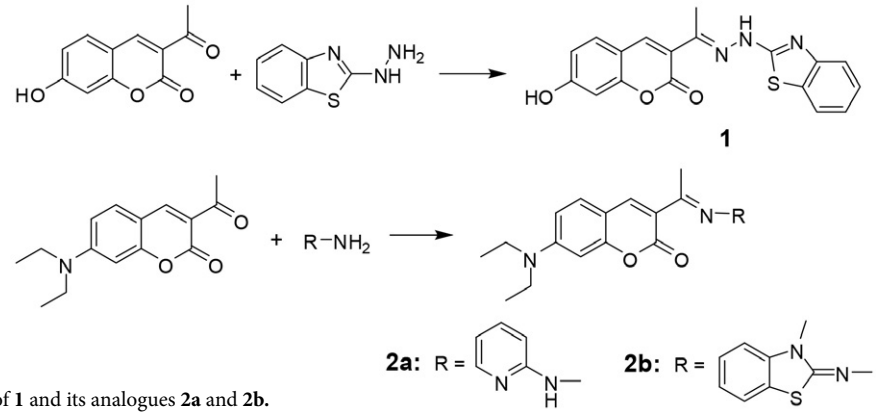
The fluorescent turn-on detection of metal ions is highly desirable for public health and environmental security. Herein, we report a rationally designed fluorescent probe (**1**) for the detection of Cu^{2+} synthesized by integrating 2-hydrazinylbenzothiazole with 3-acetyl-7-hydroxycoumarin. The probe alone is non-fluorescent due to the isomerization of C=N in the excited state. The addition of Cu^{2+} can cause a delayed fluorescence enhancement. A comparative study of **1** and its analogues indicated that the turn-on fluorescence response was thanks to the sulfur atom coordinating to Cu^{2+}. The response delay of **1** in sensing Cu^{2+} was ascribed to the gradual transition from N-coordination to S-coordination (N and S in thiazole moiety). The proposed new function of S-donor would provide a new approach for the turn-on fluorescence sensation of Cu^{2+}.

Keywords: Fluorescent probe; Coumarin; Benzothiazole; Cupric ion; S-donor

1. Introduction

Copper is an essential trace element important for the function of enzymes. It plays a pivotal role in cell physiology as a catalytic cofactor in the cellular redox reactions. Nevertheless, excess copper is implicated in various neurodegenerative disorders, such as Wilson's and Alzheimer's diseases.[1] In addition, Copper is an environmental pollutant having highly toxic effect on aquatic organisms, especially on algaes.[2] Thus, the convenient and fast methods for the detection of trace amounts of cupric ion are significant not only for public health, but also for environmental security. Fluorescent probes are a powerful tool for the detection of metal ions, especially for biomonitoring owing to their non-invasiveness, visualization and real-time.

The turn-on fluorescence probes allow detection with less false positives, providing a better opportunity to accurately monitor the target object than the turn-off flu-



Scheme 1. Synthesis of **1** and its analogues **2a** and **2b.**

orescence probe. However, it is difficult to achieve turn-on fluorescent sensing of cupric ion due to its paramagnetic nature which leads to vigorous fluorescence quenching.[3–8] During the course of our ongoing efforts to develop fluorescent probes for mental ions[9–11], we have firstly found that some probes containing S-donor show turn on fluorescence responses to Cu^{2+}, and so the S-donor may play an important role in protecting from Cu^{2+}-induced fluorescence quenching.[12–14] Following this idea, a rationally designed fluorescent probe containing S-donor (**1**) was synthesized by incorporating 3-acetyl-7-hydroxycoumarin and 2-hydrazinylbenzo[d]thiazole for the fluorescence turn-on detection of cupric ion in the present work (Scheme 1). The binding mode between **1** and Cu^{2+} was determined by ESI-MS. The function of S-donor in protecting from fluorescence quenching was affirmed by control experiments using the analogues of **1** (**2a** and **2b** in Scheme 1). Besides preventing from fluorescence quenching, S-donor should be helpful for the improvement of Cu^{2+}-selective binding.[15]

2. Experimental

All chemicals were purchased from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China) and used without further purification. Analytical grade acetonitrile and deionised water were used as solvents for all spectral measurements. The metal nitrates were used for the fluorescence sensing of metal ions. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Av400 NMR spectrometer (Bruker Co., Ltd., Karlsruhe, Germany). ESI-MS spectra were performed on a Bruker Esquire HCT mass spectrometer (Bruker Technologies, Bremen, Germany) equipped with an electrospray ion source. Fluorescence spectra were taken on a Hitachi F-7000 fluorescence spectrometer (Hitachi, Tokyo, Japan). The synthetic routes of **1**, **2a** and **2b** were illustrated in Scheme 1. ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of them were provided in the supporting information (Fig. S1-9).

3-(1-(2-(benzo[d]thiazol-2-yl)hydrazono)ethyl)-7-hydroxycoumarin (1):
3-acetyl-7-hydroxycoumarin (408 mg, 2.0 mmol), 2-hydrazinylbenzo[*d*]thiazole (330 mg, 2.0 mmol) and a catalytic amount of formic acid were added into 20 mL absolute ethanol and then refluxed for 3 h. A yellow solid precipitated out. The precipitate was collected by filtration and washed several times with ethanol to afford **1** (550 mg, 78 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.73 (s, 1H), 10.75 (s, 1H), 8.11 (s, 1H), 7.70 (s, 1H), 6.69 (d, 1H, J = 8.4 Hz), 7.35 (s, 1H), 7.29 (t, 1H, J = 7.6 Hz), 7.09 (t, 1H, J = 7.6 Hz), 6.84 (d, 1H, J = 8.4 Hz). 6.78 (s, 1H), 2.31 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 162.3, 160.1, 155.9, 141.6, 131.0, 126.5, 122.3, 122.2, 122.0, 114.1, 111.9, 102.3, 17.1. ESI-MS m/z calculated for $[M+H]^+$ 352.08, found 351.9; calculated for $[M-H]^-$ 350.06, found 349.8.

3-(1-(2-(pyridin-2-yl)hydrazono)ethyl)-7-diethylaminocoumarin (2a):
2a was synthesized according to the reported procedure[16]. Yield, 82%. ESI-MS m/z calculated for $[M+H]^+$ 351.18, found 351.0; calculated for $[M-H]^-$ 349.17, found 348.9.

3-(1-(3-methylbenzo[d]thiazol-2(3H)-ylidene)hydrazono)ethyl)-7-diethylaminocoumarin (2b):
3-acetyl-7-diethylaminocoumarin (519 mg, 2.0 mmol), 3-methyl-2-benzothiazolinone hydrazone hydrochloride (431 mg, 2.0 mmol) and triethylamine (274 μL, 2.0 mmol) were added into 20 mL absolute ethanol and then refluxed for 3 h. The resulting precipitation was collected by filtration to afford **2b** (603 mg, 72 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.56–7.60 (m, 2H), 7.27–7.36 (m, 2H), 7.08 (t, 1H, J = 7.2 Hz), 6.74 (d, 1H, J = 7.2 Hz), 6.57 (s, 1H), 3.59 (s, 3H), 3.46 (q, 4H, J = 6.8 Hz), 2.51 (s, 1H). 1.14 (t, 6H, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 160.6, 156.9, 156.4, 151.4, 141.9, 141.4, 130.7, 126.9, 124.1, 122.8, 122.1, 119.0, 110.4, 109.9, 108.3, 96.6, 44.6, 31.4, 17.4, 12.8. ESI-MS m/z calculated for $[M+H]^+$ 421.17, found 421.0.

3. Results and Discussion

3.1. Fluorescence Sensing of Cu^{2+}

With the compound **1** in hand, its fluorescence responds to various metal ions, including Ba^{2+}, Ca^{2+}, Mg^{2+}, K^+, Al^{3+}, Na^+, Zn^{2+}, Ag^+, Fe^{3+}, Mn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Co^{2+}, Cr^{3+}, Hg^{2+} and Cu^+, were examined. As shown in Fig. 1, Probe **1** alone in CH_3CN/H_2O (1/1) is nearly non-fluorescent due to the C=N isomerization which is a radiationless decay process of the excited states.[16,17] When adding Cu^{2+} into the solution and allowing it to sit for

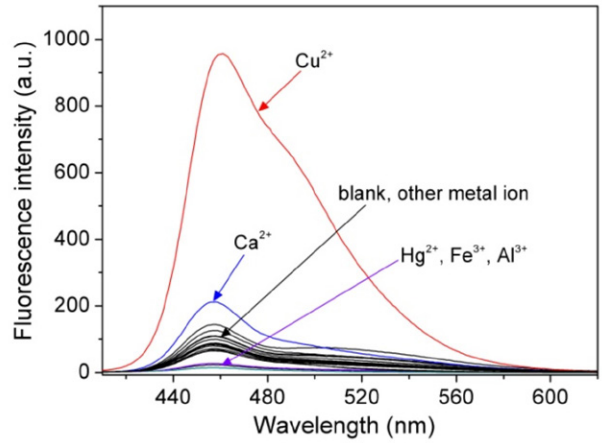


Figure 1. Fluorescence spectra of **1** (10 μM) in CH_3CN/H_2O (1/1) upon adding different metal ions (10 μM) and then allowing to sit for 2 hour at 30 °C when excited at 390 nm.

some time, an enhancement of fluorescence at 460 nm was observed under ultraviolet excitation at 360nm, which gives bright cyan luminescence. Ca^{2+} can also cause an slight enhancement of fluorescence at about 460 nm, but which is too negligible to cause visible fluorescence. Besides, Hg^{2+}, Fe^{3+} and Al^{3+} can induce a degree of fluorescence quenching. Other ions did not cause obvious fluorescence changes.

It is uncommon that the probe can not give an immediate fluorescence turn-on response to Cu^{2+}. For the determination of the optimum incubation time, time-dependent fluorescence spectra were carried out (Fig. 2). It was found that the rate of the fluorescence enhancement correlates with temperature. The fluorescence intensities of the mixture of **1** and Cu^{2+} reached a plateau in 100 minutes at 30 °C. When the temperature is 40 °C, the fluorescence emission maximum was found in 40 minutes.

The fluorescence titration of **1** with Cu^{2+} shows that the fluorescence emission maximum was observed when the Cu^{2+} reached 10 μmol/L (1 equiv), which suggested a high-affinity binding of Cu^{2+} to **1** with 1:1 stoichiometry (Fig. 3). The "turn-on" fluorescence response of **1** to Cu^{2+} should be ascribed to the coordination between them and the consequent restriction of the C=N isomerisation.[16] For demonstrating the binding between **1** and Cu^{2+}, ESI-MS spectrum of **1** in the presence of Cu^{2+} were scanned. The positive ion mode ESI-MS spectrum of the mixture of **1** and $Cu(NO_3)_2$ (1/1) in CH_3CN exhibits the base peak at m/z 412.8, which was assigned to [**1**-H+Cu]$^+$. The observed and calculated isotopic patterns (calcd 413.0) agree well with each other (Fig. 4). This indicated the deprotonation of the secondary amino group (NH) upon coordination with Cu^{2+}.

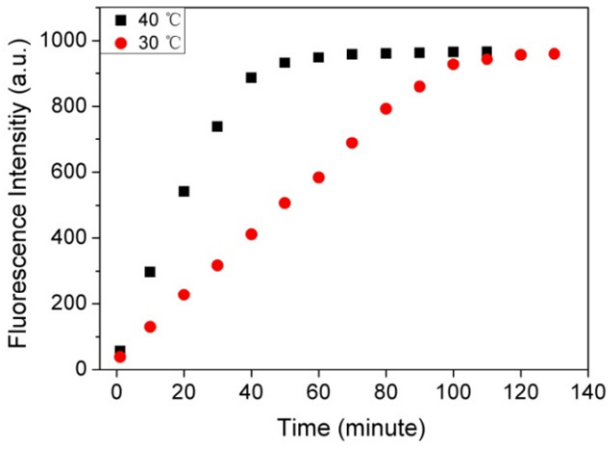


Figure 2. *Time-dependent* fluorescence responses of **1** (10 μM) to Cu^{2+} (10 μM).

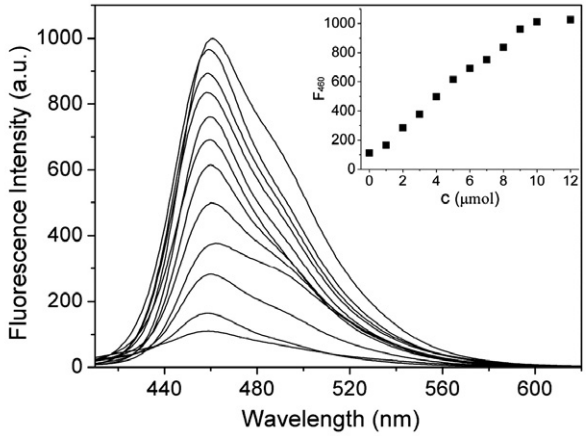


Figure 3. Fluorescence spectra of **1** (10 μM) in CH_3CN /H_2O (1/1) upon adding different concentrations of Cu^{2+} and then allowing to sit for 2 hour. The inset shows the emission intensities of **1** (10 μM) at 460 nm as a function of Cu^{2+} concentration.

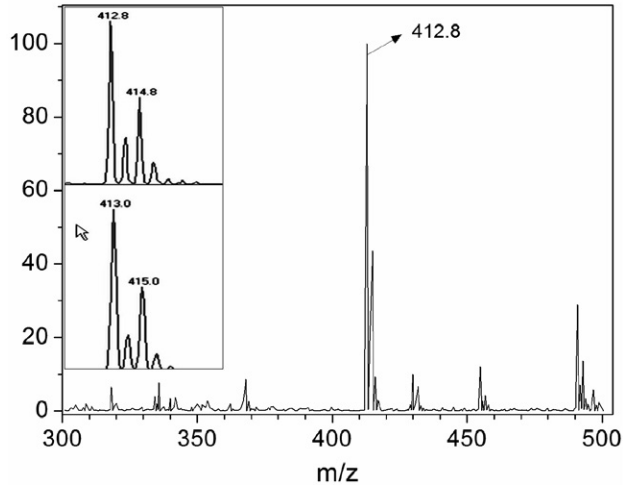


Figure 4. ESI-MS spectra of **1** in the presence of Cu^{2+}. The inset shows the observed (upper) and calculated (under) isotopic patterns.

For further evaluating the effects of common metal ions on the selectivity of **1** for Cu^{2+}, competition experiments were carried out by measuring the fluorescence res-

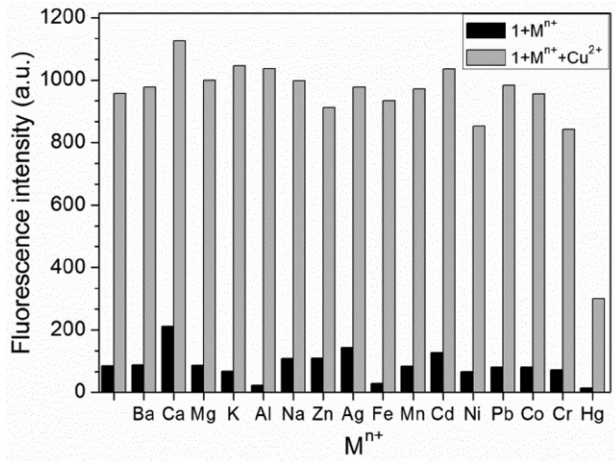


Figure 5. Selective fluorescence responses of **1** (10 μM) to Cu^{2+} (10 μM) in the presence of various foreign ions (10 μM).

ponse of **1** to Cu^{2+} in the presence of various foreign metal ions including Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Fe^{3+}, Hg^{2+} and so on. As illustrated in Fig. 5, when adding the mixtures of Cu^{2+} and various foreign metal ions to the solution of **1** and then allowing it to sit for 2 h, the fluorescence intensity at 460 nm is similar to that upon the addition of only Cu^{2+} except for Hg^{2+}, which can partly quench the Cu^{2+}-induced fluorescence. These results suggested the high selectivity of **1** as an efficient probe for the detection of Cu^{2+}.

3. 2. Sensing Mechanism

It is well-known that Cu^{2+} is the most vigorous quencher among transition metal ions, which has been found for some reported probes with similar structures.[16,17] To explore the reason for the unusual fluorescence turn-on response of **1** to Cu^{2+}, the sensing reversibility was checked by the addition of a competing ligand (EDTA) to the fluorescence solution. As seen in Fig. 6, the Cu^{2+}-induced fluorescence was suppressed by the addition of EDTA, and then recovered by further addition of Cu^{2+}, which suggested the reversible coordination interaction between **1** and Cu^{2+}. The complex between **1** and Cu^{2+} has been further confirmed by ESI-MS (Fig. 4). The thiazole moiety of the probe molecule has two potential coordination atoms (S and N atom), which results in two possible binding models in which N or S of thiazole moiety serves as donor atom respectively. In order to determine the binding model, two analogues of **1** (**2a** and **2b)** were synthesized for the control

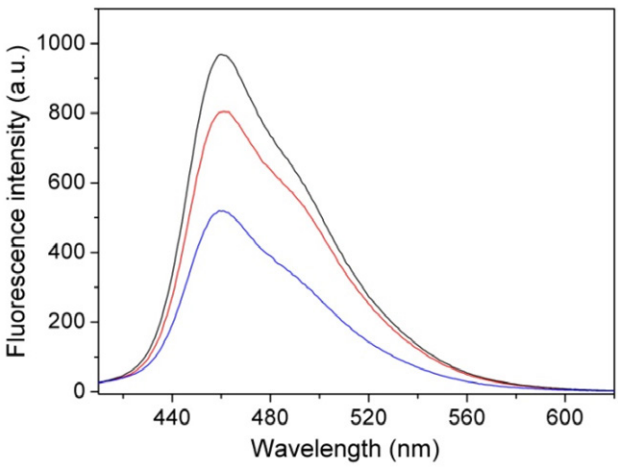


Figure 6. Fluorescence spectra of **1** (10 μM) upon successive addition of Cu^{2+} (blank), EDTA (blue) and then Cu^{2+} (red).

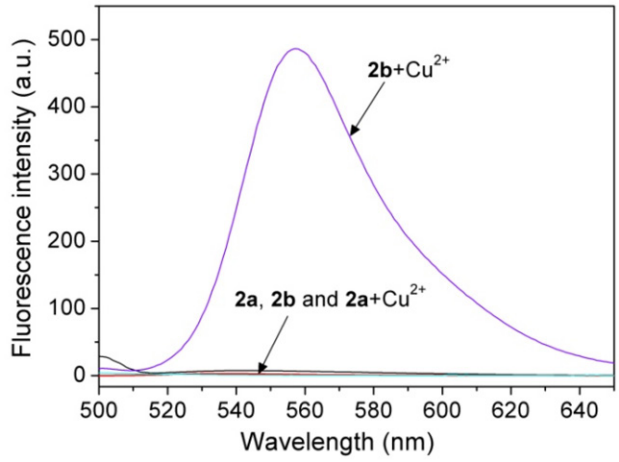
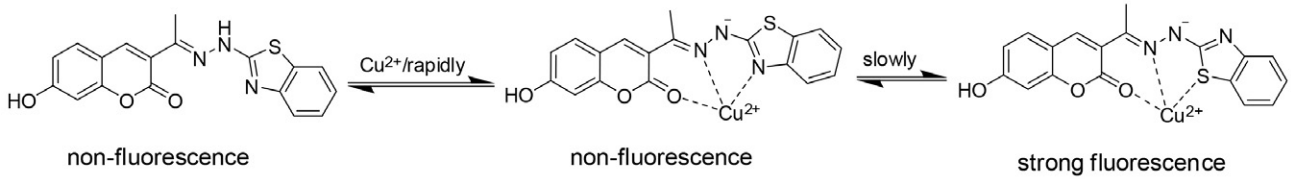


Figure 7. Fluorescence spectra of **2a** and **2b** (10 μM) in the absence and presence of Cu^{2+} (10 μM)

experiments. Sulfur-free **2a** provides a NNO donor set, but **2b** can only provides a NOS donor set, which correspond to the two possible binding models between **1** and Cu^{2+}. The fluorescence properties of **2a** and **2b** were illustrated in Fig 7. **2a** is non-fluorescent both in the presence and absence of Cu^{2+}. In contrast, **2b** gives an immediate turn-on fluorescence response to Cu^{2+}, which should be thanks to the S-donor. The similar phenomenon can been found in the other S-containing probe developed by Lee.[15] With this in mind, we can reasonably expect that the S-donor should be responsible for the fluorescence turn-on response of **1** to Cu^{2+}. The delayed fluorescence of **1** in sensing Cu^{2+} might be ascribed to the gradual formation of the **1**-Cu^{2+} complex with S-donor, that is, Cu(II) complex with NNO donor sites formed first, then gradually changed to the complex with NOS donor sites. On the basis of above discussion and the MS analysis (Fig. 4), the schematic diagram was proposed for illustrating the delayed fluorescence response of **1** to Cu^{2+} and the possible interaction between them as shown in Scheme 2.

4. Conclusion

A rationally designed turn-on fluorescent probe has been developed for the detection of Cu^{2+} which is the most vigorous fluorescence quencher among transition metal ions. The S-donor in the **1**-Cu^{2+}complex plays a crucial



Scheme 2. Delayed fluorescence response of 1 to Cu^{2+} resulting from slowly transition from the NNO coordination to the NOS coordination

role for the turn-on fluorescence. The delay of the fluorescence response should be ascribed to the transition from the complex with NNO donor sites to the complex with NOS donor sites. We believe that the new proposed fluorescence turn-on mechanism would show great potential in fluorescence sensing of Cu^{2+}.

5. References

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Povzetek

Detekcija kovinskih ionov z uporabo fluorescence je zelo zaželena za zagotavljanje javnega zdravja in varnosti okolja. V tem članku poročamo o racionalno zasnovani fluorescenčni sondi (**1**) za detekcijo Cu^{2+}, ki smo jo sintetizirali z uporabo 2-hidrazinilbenzotiazola s 3-acetil-7-hidroksikumarinom. Zaradi izomerizacije C = N v vzbujenem stanju sama sonda ne fluorescira. Dodatek Cu^{2+} lahko povzroči zakasnitev povečanje fluorescence. Primerjalna študija sonde **1** in njenih analogov je pokazala, da je odziv fluorescence na vklop zaradi koordinacije atoma žvepla in Cu^{2+}. Zakasnitev odziva sonde **1** pri zaznavanju Cu^{2+} je bila pripisana postopnemu prehodu iz N-koordinacije v S-koordinacijo (N in S v tiazolnem delu). Predlagana nova funkcija S-donorja omogoča nov pristop k detekciji Cu^{2+} z vklapljanjem fluorescence.