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A Coumarin-based Fluorescent Sensor for Selective Detection of Copper (II)

Jian-Hong Wang,[†,*] Xin-Ling Guo,[†] Xu-Feng Hou,[†,‡] Hui-Jun Zhao,[†] Zhao-Yang Luo,[†] and Jin Zhao[†]

[†]*Key Laboratory of Natural Medicines and Immunotechnology of Henan Province, Henan University, Kaifeng 475004, Henan, China.* [*]*E-mail: jhworg@126.com*
[‡]*College of Chemistry and Chemical Engineering, Xuchang University, Xuchang 461000, Henan, China*
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Cu (II) detection is of great importance owing to its significant function in various biological processes. In this report, we developed a novel coumarin-based chemosensor bearing the salicylaldimine unit (**2**) for Cu^{2+} selective detection. The results from fluorescence spectra demonstrated that the sensor could selectively recognize Cu^{2+} over other metal cations and the detection limit is as low as 0.2 μM. Moreover, the confocal fluorescence imaging in HepG2 cells illustrated its potential for biological applications.

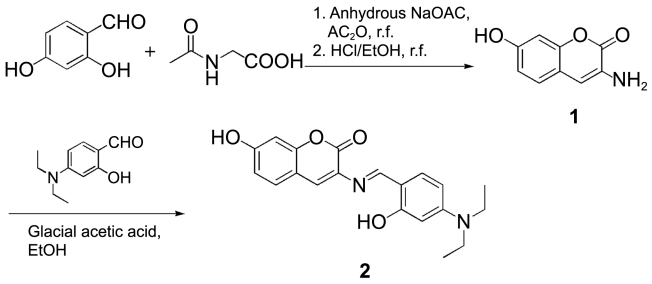
Key Words : Coumarin, Cu^{2+} detection, Confocal fluorescent, Biological imaging

Introduction

Copper is one of the transition metal abundant in the human body and actively participates in various biological processes.[1-4] It mainly binds to metallothionein in cytoplasm and involves in cellular respiration, antioxidant defence and neurotransmitter.[5,6] And the abnormal levels of copper were proposed to be related to certain diseases such as cardiovascular, diabetes, cancer and neurodegenerative diseases.[7-11] Therefore, it is of great importance in developing efficient methods for copper detection in physiological conditions.

Recently, Fluorescent probes have become a powerful tool for Cu^{2+} detection in living systems owing to its high sensitivity, selectivity and especially, non-destructive intracellular detection. To date, a variety of fluorescent Cu^{2+} sensors have been reported based on the mechanism of Cu^{2+} induced chemical reactions or Cu^{2+} coordination.[12-14] And most of these reported chemosensors decorate different fluorophores, including rhodamine,[15-17] fluoroscein,[18] naphthalimide[19] and BODIPY.[20] However, there are only a few sensors for Cu^{2+} detection based on the coumarin fluorophore so far. For example, Kim *et al.* reported a coumarin-based fluorogenic probe bearing the 2-picolyl unit as Cu^{2+} coordination site.[21] Yoon *et al.* constructed the coumarin-Cu^{2+} complex employing dipicolylamine as binding sites and demonstrated the application in pyrophosphate detection through a Cu-desorption procedure.[22] Leung and co-workers very recently developed a novel coumarin-DPA-Cu^{2+} chemosensing ensemble for selective detection of histidine in biological conditions.[23] In fact, the coumarin-based chemosensors are identified as a special class of sensitive fluorophores for their excellent fluorescence characteristics, favourable membrane permeability and good solubility.[24-26] Therefore, the development of new coumarin-chemosensors able to efficiently and selectively detect Cu^{2+} is of great importance.

Herein, we present a relatively simple chemosensor (**2**, Scheme 1), based on the coumarin scaffold bearing an aryl schiff base moiety in which the salicylaldimine framework will provide the coordination site for selective binding of Cu^{2+}.[27] Due to the intrinsic paramagnetic properties, Cu^{2+} has the propensity to quench the fluorescence of metal complex.[28] Accordingly, the potential of Cu^{2+} selective detection was examined both in organic aqueous solution and in living systems. The chemosensor **2** was synthesized as shown in Scheme 1.



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

Experimental

All reagents and solvents were commercially available and used without further purification. Column chromatography was carried out using silica gel (300-400 mesh). Thin layer chromatography (TLC) was performed with silica gel 60 F254 indicator. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO on Bruker AV-400 spectrometer with TMS as internal standards. High resolution mass data were collected on Bruker ultrafleXtreme MALDI-TOF-TOF mass instrument. The fluorescence spectra were measured using a Varian Cary Eclipse Fluorescence spectrophotometer. The living cell imaging was performed using Zeiss LSM710 confocal fluorescence microscopy. All stock solutions of metal ions were prepared from analytical grade nitrate salts which were dissolved in acetonitrile. The HEPES buffer solutions were prepared as standard procedure.

Compound **1** was prepared following the method in the literature.[29]

Synthesis of (3-(4-(Diethylamino)-2-hydroxybenzylidene-amino)-7-hydroxy-coumarin (2). 3-Amino-7-hydroxy-coumarin **1** (1.77 g, 10.0 mmol) and 4-diethylamino-salicyl-aldehyde (1.93 g, 10.0 mmol) were added into anhydrous ethanol (60 mL), then a drop of glacial acetic acid was added. The reaction mixture was stirred at 78 °C for over-night under N_2 protection. After cooling to room temperature, the solvent was evaporated under reduced pressure, then the residue was recrystallized from ethanol to afford a yellow solid **2**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.54 (s, 1H), 8.90 (s, 1H), 7.83 (s, 1H), 7.46 (d, J = 8.6 Hz, 1H), 7.27 (d, J = 8.9 Hz, 1H), 6.75 (dd, J = 8.5, 1.8 Hz, 1H), 6.67 (s, 1H), 6.31 (dd, J = 8.9, 2.2 Hz, 1H), 6.05 (d, J = 2.1 Hz, 1H), 3.41-3.36 (m, 4H), 1.11 (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO) δ 163.6, 162.2, 161.5, 158.6, 153.5, 151.9, 134.1, 129.1, 128.9, 128.5, 114.3, 111.1, 108.8, 104.1, 102.1, 96.8, 44.1, 12.6. ESI-HRMS (*m/z*): Calcd. For $C_{20}H_{20}N_2O_4$ ($[M+H]^+$): 353.1496; Found 353.1495.

Results and Discussion

The selective response of **2** toward Cu^{2+} was evaluated by addition of 1 equiv. of various metal ions in HEPES buffer (0.1 M, pH 7.4, containing 0.2% CH_3CN) at 25 ºC. As shown in Figure 1, free **2** displayed strong fluorescence band centered at 518 nm. The addition of metal cations including Fe^{3+}, Cr^{3+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Mg^{2+}, K^+, Ag^+, and Na^+, respectively, did not alter the fluorescence intensity of **2**. In contrast, the Fe^{2+}, Co^{2+} induced fluorescence quenching to a certain degree. However, it is worth noting that the fluore-scence intensity (518 nm) of **2** was highly sensitive to Cu^{2+}, which was reduced by approximate 100% upon the addition of 1 equiv. of Cu^{2+}. The results obviously indicate sensor **2** has an excellent selectivity for Cu^{2+} over other metal cations. To further examine the sensitivity of **2** binding to Cu^{2+}, Cu^{2+} titration experiment was performed. As shown in Figure 2, upon addition of increasing amounts of Cu^{2+}, fluorescence intensity of **2** gradually decreases. When 1.0 equiv of Cu^{2+} was present, the total fluorescence intensity at 518 nm of **2** almost completely quenched. Using the fluorescence titration data, we then found that there exists a good linear correlation between the fluorescence intensity of **2** and Cu^{2+} in the range of 0-10 μM (Fig. 2, inset). Moreover, the detection limit is as low as 0.2 μM. The linear dependence of the intensity ratio within the equivalent range of Cu^{2+} demonstrated that **2** generate a 1:1 complex with Cu^{2+}. In addition, the Job's plot with fluorescence titrations further confirmed the 1:1 bind-ing between **2** and Cu^{2+} (data not shown). Considering the salicylaldimine unit existing in **2**, the recognition mechanism can be reasonably concluded to the formation of Cu^{2+} coordinating with salicylaldimine framework of **2**.

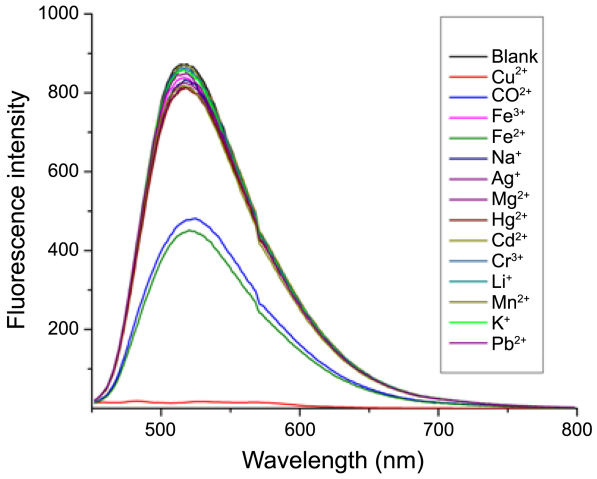


Figure 1. Fluorescence emission spectra of **2** (10 μM) in HEPES buffer (0.1 M, pH 7.4, 0.2% CH_3CN) in the presence of different metal cations (100 μM). Slite 5, λ_{ex} = 420 nm.

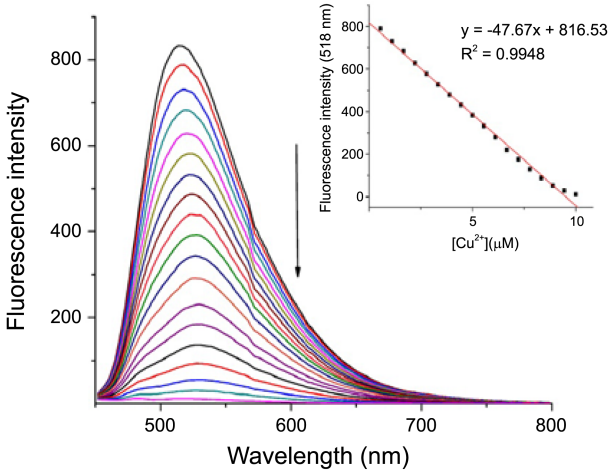


Figure 2. Emission spectra of **2** (10 μM) in HEPES buffer (0.1 M, pH 7.4, 0.2% CH_3CN) upon addition of various concentrations of Cu^{2+} (0-2 equiv.). Excitation wavelength was 420 nm. Inset: the plot of emission intensity at 518 nm as a function of Cu^{2+} concentration.

Based on the highly selective and sensitive Cu^{2+} detection

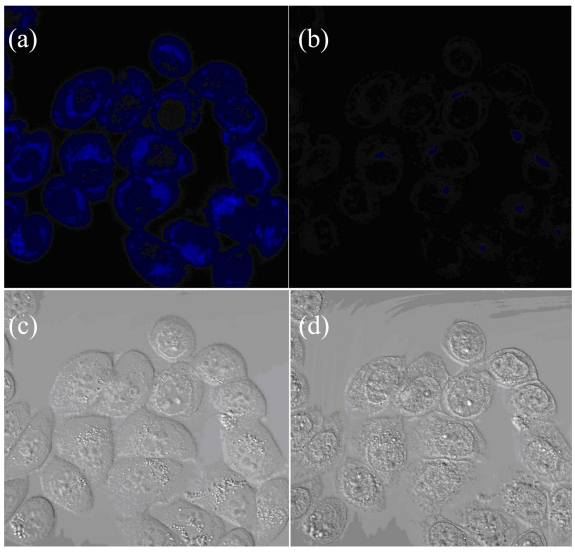


Figure 3. (a) Confocal fluorescence images of HepG2 cells treated with **2** (10 μM) for 30 min. (b) Fluorescence images of HepG2 cells treated with Cu^{2+} (10 mM). (c) Bright field images of (a). (d) Bright field images of (b).

profiles of chemosensor **2** in HEPES buffer, we further test the ability of **2** for intracellular Cu^{2+} imaging by using confocal fluorescence microscopy. HepG2 cells, incubated with probe **2** (10 μM) in culture medium for 30 min at 37 °C, exhibited strong fluorescence (Fig. 3(a)). Upon addition of Cu^{2+} (10 μM), the fluorescence intensity of **2** was immediately quenched (Fig. 3(b)), indicating the potential application of sensor **2** in visualizing intracellular Cu^{2+} levels in HepG2 cells.

Conclusion

In summary, a novel coumarin-based chemosensor **2** for Cu^{2+} selective detection was developed. The sensor exhibits highly sensitive and selective "turn-off" fluorescence detection towards Cu^{2+} over other metal cations. Moreover, the intracellular imaging in HepG2 cells demonstrates its potential for biological applications. Thus, the present results would broaden design strategies for Cu^{2+} detection.

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