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A Novel Naked-Eye and Dual-Channel Responsive Fluorescent Probe for Cu^{2+} Based on 3,4-Disubstituted-1,8-naphthalimide

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A new 3,4-disubstituted-1,8-naphthalimide derivative H1 was designed and synthesized as a selective fluorescent probe for Cu^{2+} over miscellaneous metal ions in aqueous media. Upon mixing with Cu^{2+} in CH_3OH:H_2O (1:1, volume ratio), the increase of fluorescence intensity and a bathochromic shift of absorbance of H1 could be observed with a notable color response (changing from yellow to pink). Furthermore, Cu^{2+} coordinates to the probe H1 and a 1:1 metal-ligand complex was formed.

Key words: 1,8-Naphthalimide, Copper(II), Fluorescence

I. INTRODUCTION

Cu^{2+} is not only a significant metallic pollutant, but also an essential element for living organisms [1, 2]. And it plays an important role in many fundamental physiological processes in organisms from bacteria to mammals [3]. But alterations in the cellular homeostasis of copper ions are related to many serious human afflictions including neurodegenerative diseases [4] such as Menkes and Wilson diseases [5], Alzheimer's disease [6], familial amyotropic lateral sclerosis [7], and prion diseases [8]. Thus, on-site and real-time detection and quantification of Cu^{2+} is important to take advantage of its beneficial aspects while avoiding its toxic effects [9].

In fact, a great number of techniques have been actualized to detect and analyze amounts of copper ions [10], including fluorescence, UV-Vis absorption, atomic absorption, and inductively coupled plasma atomic emission spectroscopy. Among these techniques, fluorescent molecular sensing, which can convert molecular recognition into highly sensitive and easily detected fluorescence signals [11], has received much attention in recent years because of their simplicity, high sensitivity, and real-time detection [12]. However, up to now, for most of the reported Cu^{2+} fluorescent sensors, the binding of the metal ion causes a quenching of the fluorescence emission [13], due to its paramagnetic nature [14]. And only a few sensors in which the binding of a Cu^{2+} ion causes an increase in the fluorescence have been reported [15]. So it is the most challengeable to develop simple-to-use, naked-eye and fluorescence enhancement probes for Cu^{2+}.

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The naphthalimide moiety has been widely used as a fluorophore for the design of functional supermolecules, due to its high absorption coefficient, high fluorescence quantum yield and high photostability [16]. Various derivatives of 4-amido-1,8-naphthalimide have been mainly studied [17]. And, disubstituted-1,8-naphthalimide [18] derivatives have been developed as a useful platform to construct various fluorescent probes for metal ions, because it possesses a relatively rigid and nice cation binding pocket composed of two nitrogen fragments [19], intrinsic cation-induced deprotonation of the N−H fragment [20] and the tunable selectivity through changing the type and amount of substituted amine [21]. Most of the reported disubstituted-1,8-naphthalimide fluorescent probes are basically 4,5-disamido-1,8-naphthalimide derivatives [22]. Due to the similar spatial structure of 3 and 4 positions of 1,8-naphthalimide to 4 and 5 positions of 1,8-naphthalimide, the performance of 3,4-disubstituted-1,8-naphthalimide is the same as that of 4,5-disubstituted. But there were only a few research and reports about 3,4-disubstituted-1,8-naphthalimide fluorescence probes [23]. In this work, probe H1 was designed and synthesized based on 3,4-diaminosubstituted-1,8-naphthalimide, which was expected to possess excellent performance.

II. SYNTHESIS

The synthesis route of H1, a 3,4-disubstituted-1,8-naphthalimide derivative, is shown in Scheme 1.

A. Compound 2: 3-nitro-4-[8′-(amino) quinolyl]-*N*-[2-(2′-hydroxyethoxy)ethyl]-1,8-naphthalimide

Compound **1** [24] (306 mg, 0.748 mmol) and 8-aminoquinoline (132 mg, 0.909 mmol) were dissolved

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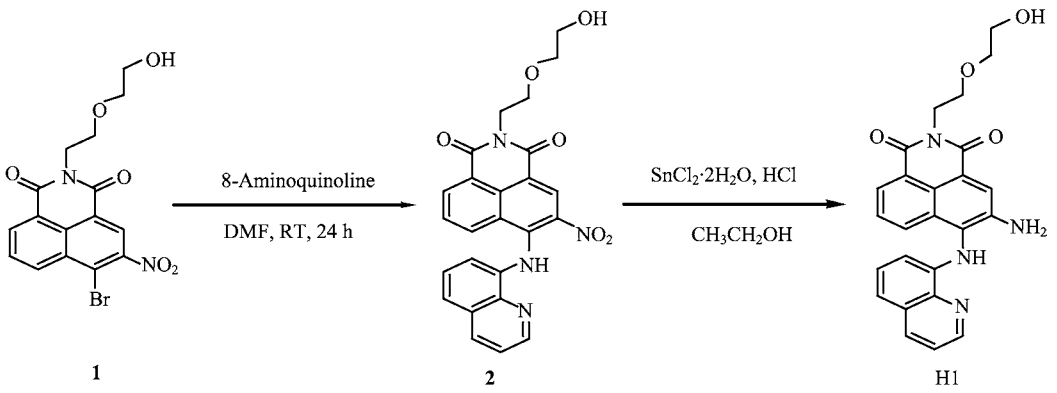

1 2 H1

Scheme-1 Design of the fluorescence probe H1.

in 5 mL dry DMF, then stirred for 24 h at room temperature under nitrogen and monitored by thin layer chromatograph. After the reaction was completed, the reaction mixture was poured into the ice water, filtered, and dried to get the red-brown solid. The crude product was purified by flash chromatography on silica (CH_3Cl:CH_3OH, 20:1, volume ratio), which afforded compound **2** as a red-brown solid (300 mg, 85.0%). mp: 272.3−273.3 °C. IR(KBr) ν: 3452.4, 2962.1, 2880.4, 1695.4, 1658.6, 1593.2, 1572.8, 1511.5, 1474.7, 1458.3, 1392.9, 1352.1, 1335.8, 1298.9, 1213.2, 1057.89, 820.9, 784.1 cm^{-1}. ^1H NMR (400 MHz, DMSO) δ(ppm): 10.61 (s, 1H), 8.90 (d, J=5.6 Hz, 1H), 8.61 (s, 1H), 8.60 (m, 2H), 8.44 (t, J=7.2 Hz, 1H), 7.78 (t, J=8.0 Hz, 1H), 7.65 (m, 2H), 7.42 (t, J=8.0 Hz, 1H), 7.09 (d, J=7.6 Hz, 1H), 4.85 (t, J=6.0 Hz, 1H), 4.17 (t, J=6.4 Hz, 2H), 3.64 (m, 2H).

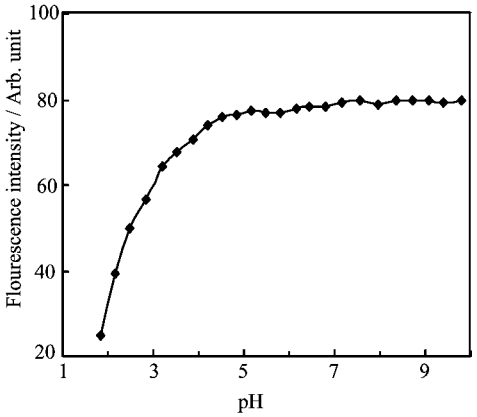

FIG. 1 Influence of pH on the fluorescence of H1 in the methanol-water (1:1, volume ratio, 10 mmol/L HEPES buffer solution). Excitation wavelength was 350 nm. Emission wavelength was 460 nm, [H1]=5.0 μmol/L.

B. Compound H1: 3-amido-4-[8′-(amino)quinolyl]-*N*-[2-(2′-hydroxyethoxy)ethyl]-1,8-naphthalimide

Compound **2** (271 mg, 0.574 mmol) was dissolved in 8 mL hydrochloric acid and 20 mL anhydrous alcohol, after which $SnCl_2{\cdot}H_2O$ (520 mg, 2.305 mmol) was added within half an hour. The reaction mixture was stirred at 40−50 °C water-bath for 2 h under nitrogen and monitored by TLC. After the reaction was completed, the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography on silica (CH_3Cl:CH_3OH, 30:1, volume ratio) to give H1 as a yellowish-brown solid in 33.4% yield (100 mg). mp: 279.9−280.4 °C. IR(KBr) ν: 3456.5, 3362.5, 3064.3, 2953.9, 2864, 1695.4, 1646.3, 1617.7, 1572.8, 1519.6, 1478.8, 1413.4, 1384.8, 1335.8, 1294.9, 1221.3, 780, 747.3 cm^{-1}. ^1H NMR (400 MHz, DMSO) δ(ppm): 8.94 (s, 1H), 8.43 (s, 1H), 8.34 (d, J=8.0 Hz, 1H), 8.25 (s, 1H), 8.12 (d, J=7.6 Hz, 1H), 7.92 (d, J=7.6 Hz, 1H), 7.62 (m, 1H), 7.57 (t, J=7.2 Hz, 1H), 7.24 (m, 2H), 6.11 (t, J=4.4 Hz, 1H), 5.79 (s, 2H), 4.84 (s, 1H), 4.16 (t, J=6.4 Hz, 2H), 2.62 (t, J=6.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ(ppm): 164.37, 163.91, 148.08, 144.68, 142.52, 138.23, 136.61, 131.05, 129.09, 128.17, 127.87, 127.33, 125.99, 123.08, 122.87, 122.50, 122.35, 122.17, 120.92, 116.06, 107.77, 58.33, 42.33.

III. RESULTS AND DISCUSSION

A. pH-titration and spectral responses

Fluoroionophores are usually disturbed by a proton in the detection of metal ions. Thus, the influence of pH on the fluorescence of H1 was first determined by fluorescence titration in methanol-water (1:1, volume ratio) solutions. The fluorescence of H1 at 460 nm remains unaffected between pH=9.8 and 4.5 and then gradually decreases from pH 4.5 to 1.8. The fluorescence quenching was most likely caused by the protonation of 3-NH_2 of the 3,4-diamine-1,8-naphthalimide chromophore and the photoinduced electron transfer (PET) from the fluorophore to protonated quinoline [25]. de Silva found the similar phenomenon in the design of an "off-on-off"

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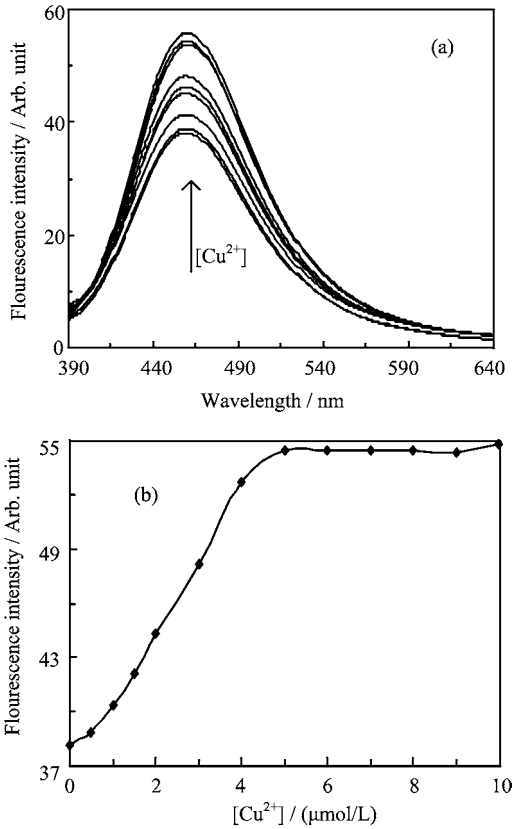

FIG. 2 (a) Cu^{2+}-titration induced the fluorescence spectra (λ_{ex}=350 nm) change of H1 (5 μmol/L) in HEPES (10 mmol/L, pH=7.07) buffer. (b) Fluorescence intensity of H1 at 460 nm as a function of Cu^{2+} ion concentration. The arrow shows the increase of $[Cu^{2+}]$.

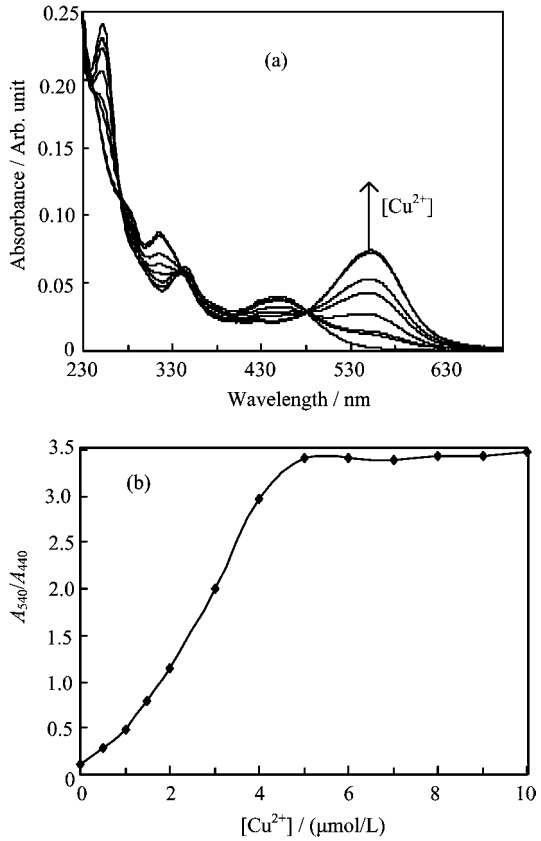

FIG. 3 (a) UV-Vis absorption spectra of 5 μmol/L H1 in the presence of different concentrations of Cu^{2+} in HEPES buffer solutions (10 mmol/L, pH=7.07). The arrow shows the increase of $[Cu^{2+}]$. (b) Ratiometric calibration curve A_{540}/A_{440} as a function of Cu^{2+} concentration.

fluorescent PET sensor [26]. Therefore, further fluorescence studies were carried out at pH=7.07 maintained with 10 mmol/L of HEPES buffer.

B. Cu^{2+}-titration and spectral responses

The emission spectra of H1 and its fluorescence titration with Cu^{2+} were recorded in HEPES buffer solutions (0.01 mol/L, pH=7.07) (Fig.2). When excited at 350 nm, the fluorescence intensity at 460 nm increased gradually with the sequential addition of Cu^{2+}. However, there was no wavelength change in the emission spectra. The fluorescence intensity changed at 460 nm as a function of the amount of Cu^{2+} (Fig.2(b)), and it could be estimated that the stoichiometry of H1 with Cu^{2+} is 1:1 in buffer solution. The quantum yield of H1-Cu^{2+} complex is 0.025 at 460 nm (λ_{ex}=350 nm) when combining the solution of probe H1 with 1 equiv. Cu^{2+} in pH=7.07 buffered solution.

Free H1 showed a broad absorption with a maximum at 430 nm (Fig.3). Upon addition of Cu^{2+}, the intensity of absorption band at 440 nm decreased and two new stronger absorption bands at 300 and 540 nm were formed and developed, which led to a large 100 nm red shift in absorption from 440 nm to 540 nm with two clear isosbestic points at 470 and 330 nm, and a color change from yellow to pink which was clearly evident to the naked eye. Figure 3(b) exhibits the dependence of the intensity ratios of absorption at 540 nm to that at 440 nm (A_{540}/A_{440}) on Cu^{2+}, which indicates the formation of a H1/Cu^{2+} adduct of 1:1 stoichiometry, with an association constant of K_a=2.56×10^5 mol/L.

C. Influence of pH on H1/Cu^{2+} adduct and spectral responses

To further evaluate the effect of pH on the H1/Cu^{2+} complex, the pH-titration was performed in the presence of H1 (5.0 μmol/L) and Cu^{2+} ion (5.0 μmol/L), as shown in Fig.4. The curve shape of its pH-titration was very similar to the pH-titration of free H1. With the increase of pH from 1.6 to 4.0, the fluorescence of the H1/Cu^{2+} complex at 460 nm gradually enhanced. When the pH was in the range of 4.0 to 10.0, the fluorescence intensity of H1/Cu^{2+} adduct at 460 nm was

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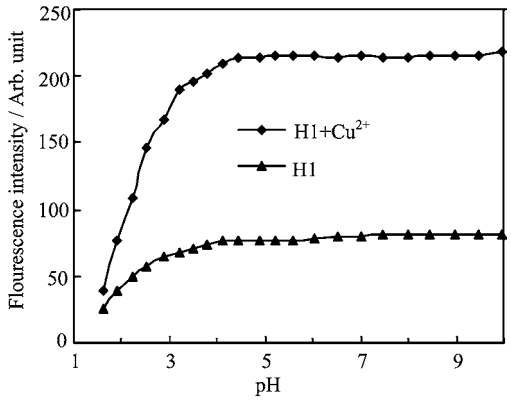

FIG. 4 The pH-titration profiles of H1 (5.0 μmol/L) in the presence of Cu^{2+} (5.0 μmol/L) in methanol/water (1/1, volume ratio) solution. Emission wavelength was 460 nm.

hardly changed, which demonstrated that the probe H1 could detect Cu^{2+} during such a wide pH range from 4 to 10.

D. The responses of H1 to various metal ions

The fluorescence titration of H1 with various metal ions was conducted to examine the selectivity, as shown in Fig.5 and Fig.6. Only Cu^{2+} induced a notable color response (changing from yellow to pink) in buffer solution, while other cations did not give rise to any response (Fig.6). As shown in Fig.5(a), there was no response of H1 to other heavy metal ions and alkaline ions except Cu^{2+} ions. The control experiments were conducted in the presence of 1.0 equiv. H1 mixed with 5.0 equiv. various metal ions. As shown in Fig.5(b), only Cd^{2+} slightly disturbed the intensity ratios (I/I_0 at 460 nm) compared with other metal ions including Cd^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Fe^{3+}, Cr^{3+}, Ag^{+}, Hg^{2+} except Cu^{2+}, but fortunately it did not affect the color change. This indicates that H1 is a highly selective and sensitive allochroic fluorescence probe for Cu^{2+} in aqueous buffer solution.

IV. CONCLUSION

We have described a simple and easy-to-prepare fluorescent probe H1 for Cu^{2+} based on 3,4-disubstituted-1,8-naphthalimide. Probe H1 displays high selectivity and sensitivity for Cu^{2+} with fluorescence enhancement, a large red-shift (100 nm) in UV/Vis and a color change from yellow to pink which was clearly evident to the naked eye in neutral aqueous buffer solution, attributed to the Cu^{2+}-induced deprotonation of the amines directly conjugating with the 3,4-diamine-1,8-naphthalimide chromophore. These results made H1 serve as a naked-eye, dual-channel responsive fluorescent probe for Cu^{2+}. Moreover, it could work over a

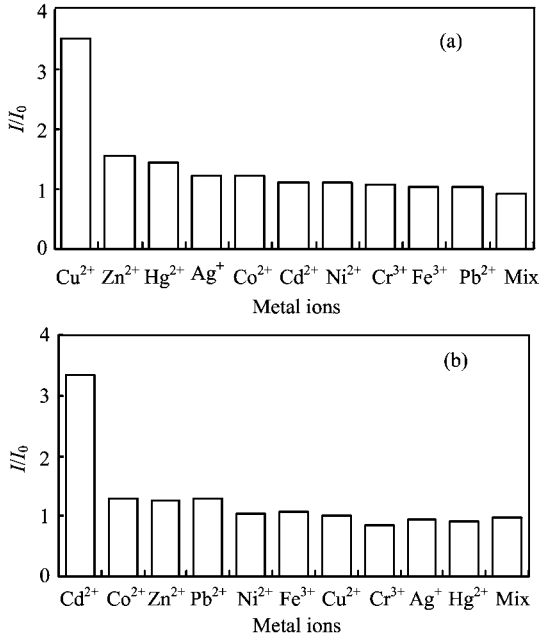

FIG. 5 (a) Fluorescent response of H1 (5.0 μmol/L) at 460 nm in HEPES (10 mmol/L, pH=7.07) buffer solution after the addition of 25.0 μmol/L of various metal ions. (b) Fluorescent response of H1 (5.0 μmol/L) to Cu^{2+} (25.0 μmol/L) in the presence of other metal ions (25.0 μmol/L) at 460 nm in HEPES (10 mmol/L, pH=7.07) buffer solution. Note: Excitation wavelength was set at 350 nm with excitation and emission slit at 2.0/2.5 nm. Mix represented the alkali and alkaline earth metals.

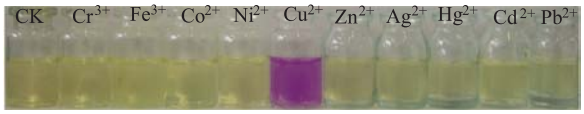

FIG. 6 Color changes of H1 (5.0 μmol/L) upon addition of various metal ions (25.0 μmol/L) in HEPES (10 mmol/L, pH=7.07) buffer solution, CK indicated free H1.

wide pH range from 4.0 to 10.0, which was important to use in practical view. We anticipate that the design strategy and remarkable photophysical properties of the probe would help to extend the development of 3,4-disubstituted-1,8-naphthalimide fluorescent probes.

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