



A highly sensitive and selective fluorescent probe for determination of Cu (II) and application in live cell imaging

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ABSTRACT

A highly sensitive fluorescent probe, 2 butyl 6 (2 ethylidenehydrazinyl) 1H benzo[de]isoquinoline 1,3(2H) dione (**P**) has been designed and synthesized to detect Cu²⁺ in CH₃CN-HEPES (4:1, v/v, pH = 7.4) solution. This probe functions via a distinctive hydrolysis mechanism through Cu²⁺-promoted accompany by extinction of the fluorescent and other competing metal ions did not showed any interference. The limit of detection toward Cu²⁺ was calculated of 320 nM. Probe **P** was not only successfully used for the determination of trace level Cu²⁺ in the CH₃CN-HEPES (4:1, v/v, pH = 7.4) solution, but also valid for fluorescence imaging of Cu²⁺ in lysosomes of 293T cells and it was applied in real water samples. This work indicated that **P** would be of great application prospect in environmental monitoring and medical diagnosis.

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1. Introduction

Copper (Cu²⁺) ranks the third abundant metal element in human bodies and is important in many fundamental physiological processes in organisms [1–3]. Deficiency of Cu²⁺ in humans will result in anemia, coronary heart diseases and bone abnormalities [4,5]. The World Health Organization (WHO) recommended the guidelines for drinking-water quality, stipulate the maximum of the acceptable range of oral intake in adults is most likely in the range of several milligrams per day (more than 2 or 3 mg/day) in adults but not much [6]. However, the excessive ingestion or uncontrolled increase of Cu²⁺ has bad effects on human health and leads to serious diseases such as amyotrophic lateral sclerosis, vomiting, liver, kidney damage or even death [7,8]. Cu²⁺ is widely released from the chemical industry and waste water, which may lead to pollution of the aquatic environment. And the bioaccumulation of Cu²⁺ in aquatic organisms can be detrimental to humans, leading to poisoning and even death in humans [9,10]. Therefore, it is key issue to monitor the concentration of Cu²⁺ in environmental systems to minimize the health risks and provide an effective guarantee of human health.

Recently, there are a lot of traditional techniques to detect the Cu²⁺ [11–20], such as atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry (ICP-AES), mass spectrometry (MS), flow injection analysis, surface plasmon resonance (SPR), and colorimetric methods based on coordination chemistry. However, most of

these methods are required high-cost, time-consuming, and operation difficultly.

Therefore, it is necessary to find a convenient and low cost test method to detect the Cu²⁺. Fluorescent probes are considered to be a promising detection tool, due to its high sensitivity, high selectivity, low cost, simplicity, easy detect ions and allow real-time imaging and useful applications in the environment [21]. In the past few years, there are a number of reports about fluorescent probes to detect Cu²⁺ ion [22–26].

Schiff bases [27–30] feature as good ligands for metal ions, and can be also used in analytical and pharmacological area (Scheme 1). However, most of them have been limited by its poor coordination ability, poor solubility and lack of spectroscopic characteristics. We have now designed and synthesized a Schiff base ligand **P** in excellent yields. The probe can detect Cu²⁺ applied in cell imaging and in real water.

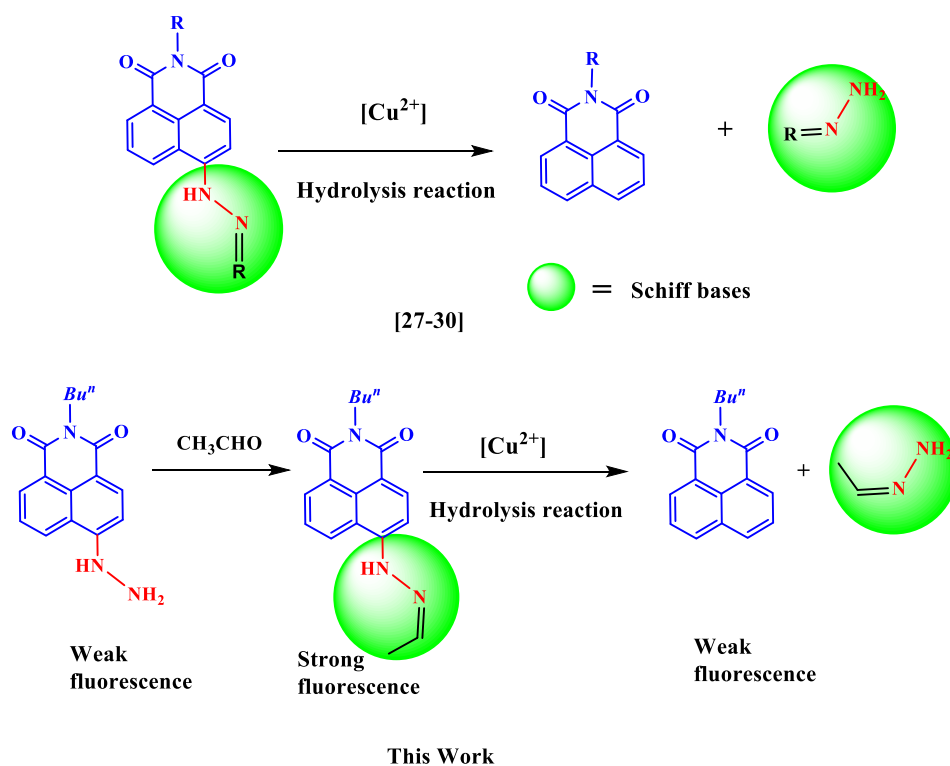
2. Experimental

2.1. Material Methods

All of the materials were commercially available, they were used without purification. The stock solution of probe **P** (10⁻⁴ M) was prepared in CH₃CN-HEPES (4:1, v/v). HEPES buffer solution (100 mM) was prepared by dissolving HEPES in ultrapure water, and the pH was adjusted to 7.4 through NaOH solution (1.0 M). In the experiment of pH effect of **P** toward Cu²⁺, pH adjustment was made with dilute hydrochloric acid and sodium hydroxide. The metal ions were prepared with deionized water from KCl, NaCl, MgCl₂, ZnCl₂, FeCl₃, SnCl₂, CaCl₂, Al

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Scheme 1. Design of the novel 1,8 naphthalimide-based chemosensor.

(NO₃)₃, CuCl₂, NiCl₂, MnCl₂, CuCl, CrCl₃, Hg(OAc)₂, PbCl₂, BaCl₂, AgNO₃, CoCl₂, FeCl₂, AuCl₃.

The FT-IR spectra were recorded on the Bruker ALPHA-T spectrometer (Bruker, Germany). The melting points were determined on a Beijing Taikemelting point apparatus (X-4). The NMR spectra were recorded on a Bruker AVANVE 400 MHz spectrometer (Bruker, Germany). The high-resolution mass spectrometry (HRMS) was recorded on a FTMS Ultra Apex MS spectrometer (Bruker Daltonics Inc., USA). Absorption spectra were collected using a UV-2550 ultraviolet spectrophotometer (Shimadzu, Japan). Fluorescence measurements were obtained using a PerkinElmer LS55 fluorescence spectrometer (PerkinElmer, UK). The pH values were measured on a PHS-3C pH meter (Inesa, China). The cell images were recorded by LEICA TCS SP2 confocal laser scanning microscopy (Leica, Germany). All experiments were carried out in the temperature of 25 °C and within a 10 mm quartz cell.

2.2. Synthesis of Compound P

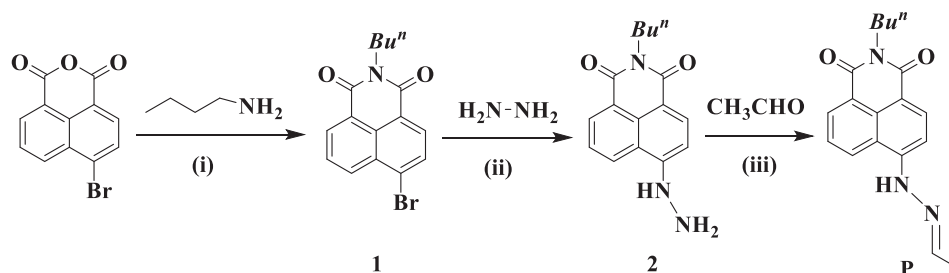
*N*n butyl 4 bromo 1,8 naphthalimide (**1**) was prepared by modifying a previously reported procedure. *N*n butyl 4 hydrazine hydrate 1,8 naphthalic anhydride (**2**) was synthesized according to the literature [31]. The synthesis of compound **P** was that compound **2**

(283.0 mg, 1 mmol) was dissolved in 20 mL EtOH, then 3.0 mL acetaldehyde (40%, w/w) was added to the solution. The mixture was stirred for 2 h at room temperature. The precipitate was filtered and washed with EtOH to get the orange solid (217 mg, yield: 66%) (**Scheme 2**). *m. p.* 184.5–185.1 °C; FT-IR ν (cm⁻¹): 3291, 3265, 2933, 2849, 2837, 1671, 1625, 1603, 1561, 1417, 1380, 1350, 1232, 1127, 1093, 1069, 958, 928, 870, 836, 769, 750, 577, 493. ¹H NMR, (400 MHz, DMSO *d*₆) δ (ppm): 11.01 (s, NH, 1H), 8.70–7.70 (m, Ar-H, 5H), 7.49 (d, *J* = 8.5 Hz, C-CH, 1H), 4.01 (t, *J* = 7.3 Hz, C-CH₂-C 2H), 2.04 (s, C-CH₃, 3H), 1.47–1.76 (m, C-CH₂-C, 2H), 1.34 (q, *J* = 7.4 Hz, C-CH₂-C, 2H), 0.92 (t, *J* = 7.3 Hz, C-CH₃, 3H). ¹³C NMR (100 MHz, DMSO *d*₆) δ (ppm): 164.12, 163.39, 147.46, 145.65, 134.13, 131.21, 129.65, 128.74, 125.09, 122.38, 118.58, 110.45, 106.46, 30.26, 20.30, 19.01, 14.22. HRMS (ESI): *m/z* calcd for C₁₈H₁₉N₃O₂ ([M + H]⁺): 310.1556; found 310.1550.

3. Results and Discussion

3.1. Synthesis and Spectral Characterization

The synthesis of probe **P** was displayed in **Scheme 2**. Compound **1** and **2** were synthesized according to the previously reported literature



Scheme 2. Synthesis route to compound **P**. Reagents and conditions: (i) EtOH, 20 h, reflux (ii) 2 methoxyethanol, 4 h, reflux (iii) EtOH, 2 h.

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