



## Full Length Research Article

### CHARACTERIZATION OF A Cu<sup>2+</sup>-SELECTIVE FLUORESCENT PROBE BASED ON BENZOYL HYDRAZINE DERIVATIVE

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#### ABSTRACT

A benzoyl hydrazine derivative was successfully characterized as Cu<sup>2+</sup>-selective fluorescent probe. Complexing with Cu<sup>2+</sup> triggers a prominent fluorescence enhancement at 408 nm, accompanied by the change in the absorption spectrum in ethanol phase. With the optimized experimental conditions, the probe exhibited a dynamic response range for Cu<sup>2+</sup> from 0.7 μM to 10 μM with a detection limit of 0.21 μM.

#### Key words:

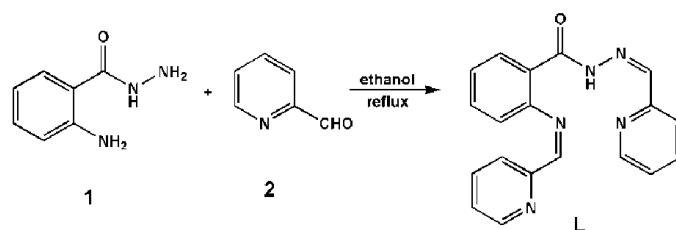
Cu<sup>2+</sup>;  
Fluorescent probe;  
Schiff base

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#### INTRODUCTION

Fluorescent probes are powerful tools for monitoring environmental species by virtue of their simplicity and high sensitivity (Duke *et al.*, 2010; Sun *et al.*, 2014 and Guo *et al.*, 2014). Among them, Cu<sup>2+</sup> is an essential element in living systems and has an extremely ecotoxicological impact on the human health (Yu *et al.*, 2014). However, Cu<sup>2+</sup> exhibits toxicity under overloading conditions in that it causes neurodegenerative diseases (Xie and Qin, 2011). Thus, it is necessary to trace the concentration of Cu<sup>2+</sup>, and many studies focus on the design of fluorescent probes and the analysis of Cu<sup>2+</sup> have been reported (Duke *et al.*, 2010; Guo *et al.*, 2014; Yu *et al.*, 2014; Xie and Qin, 2011; Shao *et al.*, 2005; Yu *et al.*, 2014; Zhang *et al.*, 2014 and Yu and Zhang, 2014). It is pitiful that only a few examples of “off-on” type probes available due to the fluorescence quenching nature of

paramagnetic Cu<sup>2+</sup> (Yu *et al.*, 2014; Yu *et al.*, 2014; Zhang *et al.*, 2014 and Yu and Zhang, 2014). Therefore, the development of highly sensitive and selective “off-on” chemosensor for Cu<sup>2+</sup> is necessary. With this intention, a Cu<sup>2+</sup>-specific fluorescent chemosensor **L** was synthesized and characterized (Scheme 1).



Scheme 1. Synthesis route of the proposed probe L.

#### Experimental Section

##### Reagents and Instruments

All of the materials were commercial available reagent and used without further purification. Fluorescence emission

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spectra were conducted on a Hitachi 4600 spectrofluometer. UV-Vis spectra were obtained on a Hitachi U-2910 spectrophotometer. NMR spectra were measured with TMS as an internal standard. MS spectra were recorded on a Thermo TSQ Quantum Access Agilent 1100. pH values were measured with a pH-meter PBS-3C.

### Synthesis

Under N<sub>2</sub> atmosphere, 1 mmol compound **1** (Fu *et al.*, 2012) and 2.2 mmol **2** were mixed in ethanol (30 mL). The reaction mixture was stirred at 80 °C for 4 h, and then cooled to room temperature. The yellow precipitate so obtained was filtered and used directly. Yields: 90%; m.p.: 201.2-203.0 °C; MS: *m/z* 329.06 (M<sup>+</sup>); IR (KBr tablet, cm<sup>-1</sup>): 3313.1 (N-H), 2912.0 (Ar-H), 1681.6 (C=N); <sup>1</sup>H NMR (δ: ppm, CDCl<sub>3</sub>): 9.04 (s, 1H, NH), 8.62-8.63 (d, 1H, By-H), 8.60-8.61 (d, 1H, By-H), 8.03-8.05 (d, 1H, By-H), 7.99-8.01 (d, 1H, By-H), 7.71-7.74 (t, 1H, Ar-H), 7.59-7.62 (t, 1H, Ar-H), 7.27-7.30 (m, 3H, Ar-H), 7.19-7.21 (m, 1H, Ar-H), 6.85-6.88 (t, 1H, Ar-H), 6.69-6.70 (d, 1H, Ar-H), 6.37 (s, 1H, N=CH), 5.83 (s, 1H, N=CH); <sup>13</sup>C NMR (δ: ppm, CDCl<sub>3</sub>): 161.33 (C=O), 157.64, 153.91 (N=C-C-By), 150.27, 149.97 (N=C-By), 149.53 (C=N), 145.24 (N=C-C-Ar), 137.01 (C=N), 136.45, 134.29, 129.13, 124.28, 123.45, 121.24, 120.74, 119.82, 116.15, 115.35 (ArC).

### General spectroscopic methods

All of the UV-Vis and fluorescence titration data were recorded at room temperature. Test solutions were prepared by placing 50 μL of the **L** stock solution (1 mM) and an appropriate aliquot of individual ions stock solution into a test tube, and then diluting the solution to 5 mL with ethanol. For all fluorescent measurements, excitation and emission slit widths were 10 nm, respectively. Excitation wavelength was 260 nm.

## RESULTS AND DISCUSSION

### Uv-vis spectral response of **L**

The UV/vis of **L** (10 μM) in ethanol to various metal ions (K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, Ag<sup>+</sup>, Ni<sup>2+</sup>, Hg<sup>2+</sup>, Cr<sup>3+</sup> and Fe<sup>3+</sup>, 10 equiv.) and its selectivity for Cu<sup>2+</sup> were illustrated in Figure 1.

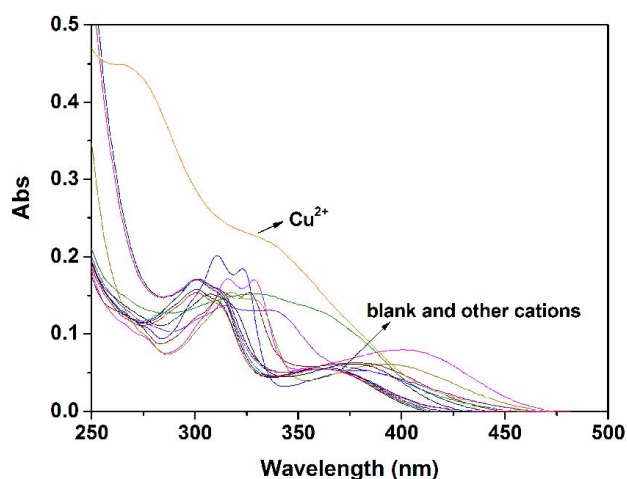


Figure 1. Spectra of **L** (10 μM) with various metal ions (100 μM) in ethanol

The results showed that the addition of Cu<sup>2+</sup> cause an obvious change of the absorption spectrum of probe **L**, which clearly suggested the binding of **L** with Cu<sup>2+</sup>. The titration experiment shows a significant increase of absorption band centered at 414 nm, and the probe exhibited a dynamic response range for Cu<sup>2+</sup> from 0.7 μM to 10 μM with a detection limit of 0.21 μM (Figure 2).

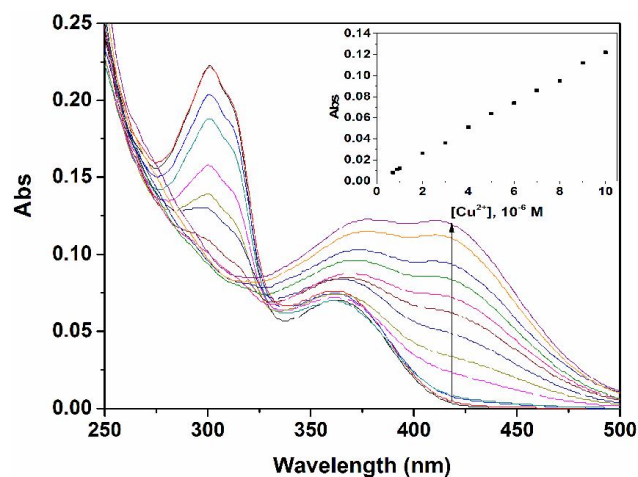


Figure 2. UV-Vis response of **L** (10 μM) with different concentrations of Cu<sup>2+</sup> in ethanol. Inset: the fluorescence of **L** (10 μM) as a function of Cu<sup>2+</sup> concentrations (0.5–10 μM)

### Fluorescence spectral response of **L**

To further evaluate the selectivity of **L**, the fluorescent spectra (ex=260 nm) of **L** (10 μM) in ethanol solution with the addition of respective metal ions (10 equiv.) as above mentioned was investigated (Figure 3). Compared to other tested ions, only Cu<sup>2+</sup> generated a significant “turn-on” fluorescent response at 408 nm with a prominent fluorescence enhancement. It suggested that **L** has a better selectivity toward Cu<sup>2+</sup> than to other metal ions.

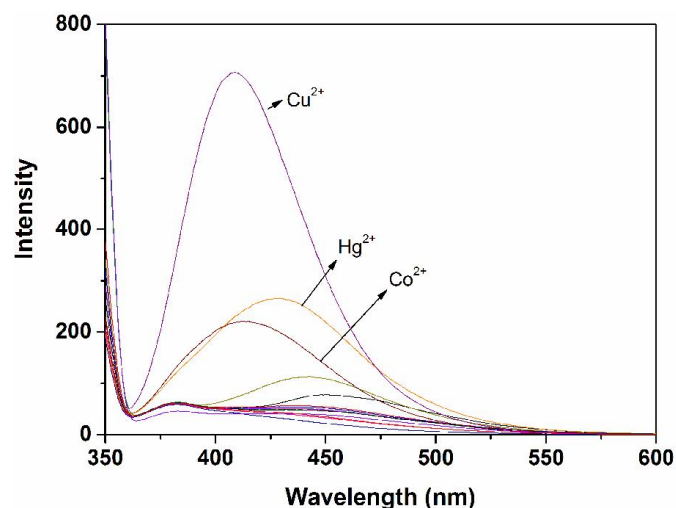


Figure 3. Fluorescence response of **L** (10 μM) with different metal ions (100 μM) in ethanol

For the Cu<sup>2+</sup> probe, cross-sensitivity to the other metal ions was also a challenge. Therefore, competition experiments were conducted in the presence of 10 equiv of Cu<sup>2+</sup> mixed with 10

equiv of other metal ions mentioned above. No significant variation in fluorescence intensity was found by comparison with that the same amounts of  $\text{Cu}^{2+}$  solution without other metal ions (Figure 4). It is gratifying to note that all the tested metal ions have no interference.

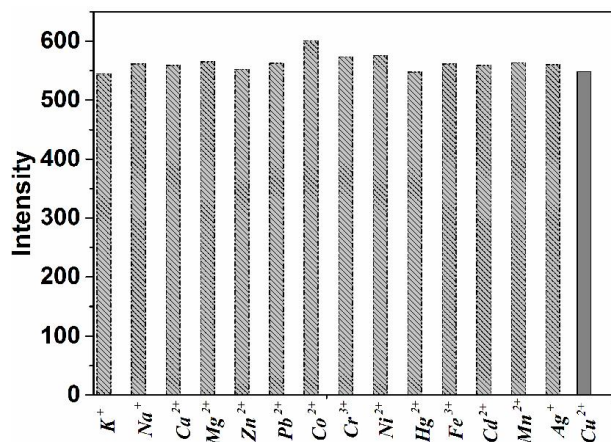


Figure 4. Fluorescence response of L (1.0  $\mu\text{M}$ ) to 10  $\mu\text{M}$  of  $\text{Cu}^{2+}$  and to the mixture of 10  $\mu\text{M}$  individual other metal ions with 10  $\mu\text{M}$  of  $\text{Cu}^{2+}$

#### Proposed binding mode of L with $\text{Cu}^{2+}$

Binding analysis using the method of continuous variations (Job's plot) was measured (Figure 5), and a maximum absorption at 414 nm was observed when the molecular fraction of L was close to 0.5, which established the 1:1 complex formation between L and  $\text{Cu}^{2+}$ .

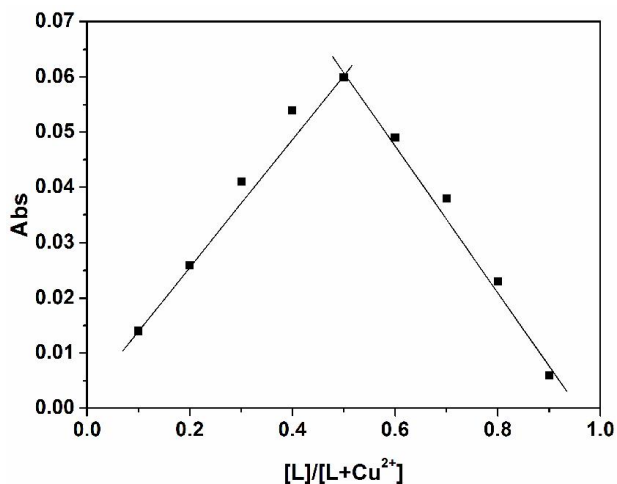
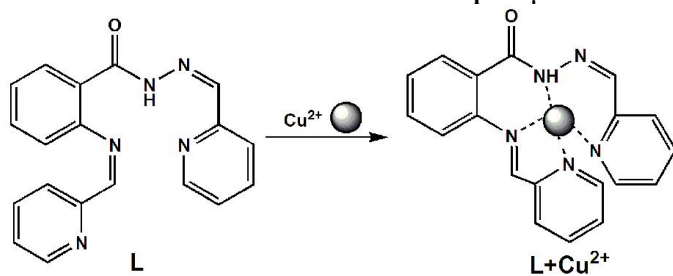


Figure 5. Job's plot curve of L with  $\text{Cu}^{2+}$  in ethanol. The total concentration of L and  $\text{Cu}^{2+}$  was kept 10  $\mu\text{M}$



Scheme 2. Proposed binding mode of L with  $\text{Cu}^{2+}$

Thus, according to the obtained results and reported work, the binding mode of L and  $\text{Cu}^{2+}$  was proposed as shown in Scheme 2.

#### Conclusions

In summary, a simple "off-on" type probe L for  $\text{Cu}^{2+}$  was presented. The conception may expand a promising approach to develop selective detection method for  $\text{Cu}^{2+}$  and lead to the development "off-on" type probes for other metal ions.

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