



Full Length Research Article

SYNTHESIS AND CHARACTERIZATION OF A NEW Zn²⁺-SELECTIVE PROBE BASED ON BENZOYL HYDRAZINE DERIVATIVE

¹Rijian Sun, ^{2,*}Yuxiang Ji, ²Na Li, ¹Jun Zhang and ^{1,*}Chunwei Yu

¹Laboratory of Environmental Monitoring, School of Tropical and Laboratory Medicine,
Hainan Medical University, Haikou, 571101, China

²Laboratory of Environmental Engineering Management, School of Tropical and Laboratory Medicine,
Hainan Medical University, Haikou, 571101, China

ARTICLE INFO

Article History:

Received 22nd September, 2014
Received in revised form
03rd October, 2014
Accepted 20th November, 2014
Published online 27th December, 2014

ABSTRACT

A benzoyl hydrazine derivative was successfully characterized as an “off-on” type fluorescent probe for Zn²⁺. A prominent fluorescence enhancement at 480 nm was observed in the presence of Zn²⁺, accompanied by the change in the absorption spectrum in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES). With the optimized experimental conditions, the probe exhibited a dynamic response range for Zn²⁺ from 0.5 μM to 4.0 μM with a detection limit of 0.16 μM.

Key words:

Benzoyl Hydrazine Derivative,
Zn²⁺,
Fluorescent Probe,
Schiff Base.

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INTRODUCTION

Some of the challenging areas of current research relevant to the receptor design and development are the detections of cations, anions and molecular species. Among them, Zn²⁺ is the second most abundant heavy metal ion after iron. It is an essential component of many enzymes and transcription factors (e.g., carbonic anhydrase, zinc finger proteins, etc.). (Takeda, 2001) However, over intake of Zn²⁺ can cause severe diseases. Therefore, the detection of Zn²⁺ by various synthetic receptors is an emerging area of current research. Due to the high selectivity, fast analysis with spatial resolution for providing in situ and real-time information, and especially the nondestructive sample preparation, the fluorescent probes method seems to be an ideal candidate for both sensing and bioimaging metal ions, anions and small molecules in various

samples (Quang and Kim, 2010; Guo *et al.*, 2014). Enhancement in the fluorescence intensity as a result of metal ion binding is more attractive than quenching (Yu *et al.*, 2014; Sargenti *et al.*, 2014). Thus, probes exhibit Zn²⁺-triggered fluorescent enhancement have attracted particular attention, and a number of the fluorescent probes that display fluorescence enhancements by the addition of the Zn²⁺ have been reported, (Quang and Kim 2010; Guo *et al.*, 2014; Huang *et al.*, 2011 and Cao *et al.*, 2012) and the design and synthesis of new Zn²⁺-selective “off-on” probes is still of great interest (Quang and Kim, 2010; Guo *et al.*, 2014). The selectivity was the key factor of probes, which was determined by the receptor sites of probes. Acylhydrazone receptors show good coordination property to heavy metals and have been successfully adopted for the construction of Mg²⁺- and Ag⁺-selective probes in our precious works (Yu *et al.*, 2014; Zhang *et al.*, 2012). Herein a Schiff base type Zn²⁺-selective probe was designed and characterized (Scheme 1).

Experimental Section

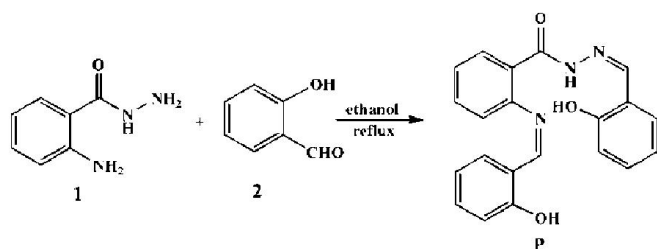
Reagents and Instruments: All of the materials were analytical reagent grade and used without further purification.

*Corresponding author: ¹Yuxiang Ji and ²Chunwei Yu,

¹Laboratory of Environmental Monitoring, School of Tropical and Laboratory Medicine, Hainan Medical University, Haikou, 571101, China

²Laboratory of Environmental Engineering Management, School of Tropical and Laboratory Medicine, Hainan Medical University, Haikou, 571101, China

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. Fluorescence emission spectra were conducted on a Hitachi 4600 spectrofluorimeter. UV-Vis spectra were obtained on a Hitachi U-2910 spectrophotometer.



Scheme 1. The synthesis route of the proposed probe P

Synthesis

Compound **1** 69.6 mg (0.5 mmol) and 120.23 μ L **2** (1.1 mmol) were mixed in ethanol (20 mL). The reaction mixture was stirred at 80 °C for 4 h, and then cooled to room temperature. The white precipitate so obtained was filtered and used directly.

Yields: 85.6 %. MS (ES+) *m/z*: 360.20 [M+H]⁺, 382.31 [M+Na]⁺. ¹H NMR (δ ppm, DMSO-*d*₆): 11.44 (s, 1H), 10.24 (s, 1H), 8.45 (s, 1H), 7.80 (d, 1H, *J* = 7.72), 7.48 (s, 1H), 7.39 (d, 1H, *J* = 7.40), 7.30 (d, 1H, *J* = 7.12), 7.27 (d, 1H, *J* = 7.20), 7.13 (t, 1H, *J* = 7.38), 6.97 (d, 1H, *J* = 7.60), 6.92 (d, 1H, *J* = 7.32), 6.89 (s, 1H), 6.87 (d, 1H, *J* = 7.64), 6.83 (d, 1H, *J* = 7.56), 6.76 (d, 1H, *J* = 7.36), 6.72 (d, 1H, *J* = 10.24), 6.68 (d, 1H, *J* = 7.48). ¹³C NMR (δ ppm, DMSO-*d*₆): 161.21, 158.51, 155.63, 150.64, 147.28, 135.14, 132.50, 131.38, 130.78, 129.00, 126.90, 125.08, 120.12, 119.93, 119.24, 118.54, 117.48, 116.75, 115.79, 114.11.

General spectroscopic methods

All of the UV-Vis and fluorescence titration data were recorded at 25 °C. Test solutions were prepared by placing 50 μ L of the P 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-water (9:1, v:v, pH 6.3, 20 mM HEPES). For all fluorescent measurements, excitation and emission slit widths were 10 nm, respectively. Excitation wavelength was 408 nm.

RESULTS AND DISCUSSION

pH effects on P and P with Zn²⁺

The titration experiment was firstly carried out to test the pH effect of probe P for Zn²⁺ sensing (Figure 1). The results showed that the emission intensities of the free probe P can be negligible in the range pH 4-10. After the addition of Zn²⁺, the emission intensity at 480 nm rapidly reached to a maximum in the range of pH 4-10. The pH-control emission measurements revealed that probe P showed the best respond to Zn²⁺ at pH 6.3. Therefore, further UV-vis and fluorescent studies were carried out in ethanol-water solution at pH 6.3 (9:1, v:v, 20 mM HEPES).

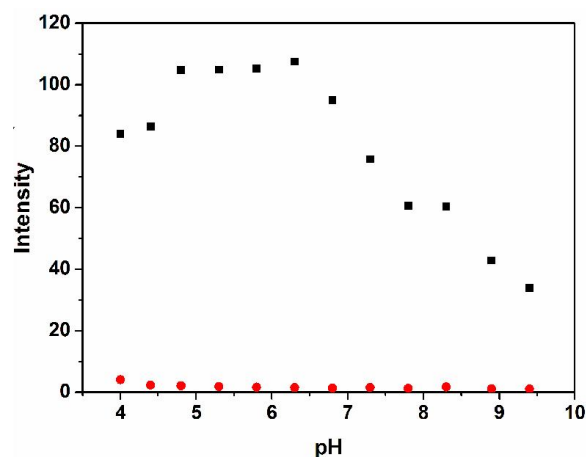


Figure 1. Influences of pH on the fluorescence spectra of P (●) and P (10 μ M) plus Zn²⁺ (10 μ M) (■) in ethanol-water solution (9:1, v:v). The pH was modulated by adding 1 M HCl or 1 M NaOH in HEPES buffers

UV-vis response of P

The absorption spectra of P in the absence and presence of Zn²⁺ were also recorded for the purpose of obtaining a better insight into the response mechanism of P with Zn²⁺ (Figure 2). The results showed that probe P (10 μ M) displays a very weak band over 420 nm in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES). However, electronic spectra of P (10 μ M) in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES) immediately resulted in a significant new absorption band at 420 nm upon the addition of Zn²⁺ (ϵ = 1.74 \times 10³), which clearly suggested the binding of P with Zn²⁺.

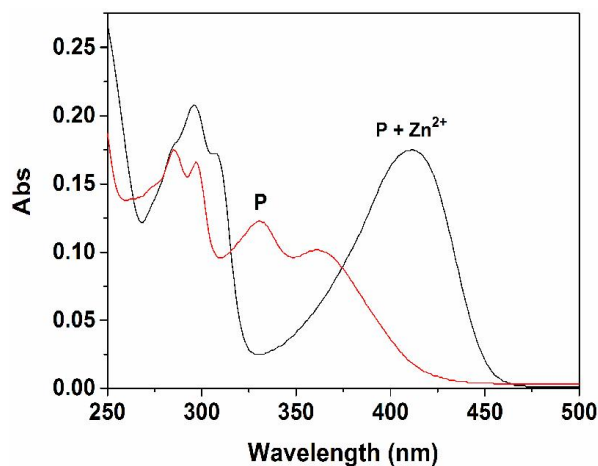


Figure 2. UV-vis spectra of P (10 μ M) with Zn²⁺ (100 μ M) in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES)

Fluorescence spectral response of P

The fluorescent spectra (ex=408 nm) of P (10 μ M) in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES) with the addition of respective metal ions (K⁺, Na⁺, Ca²⁺, Mg²⁺, Pb²⁺, Co²⁺, Cd²⁺, Ag⁺, Zn²⁺, Ni²⁺, Hg²⁺, Cr³⁺ and Fe³⁺, 10 equiv.) was investigated to evaluate the selectivity of probe P (Figure 3). Compared to other examined ions, only Zn²⁺ generated a significant “turn-on” fluorescent response at 480 nm with a fluorescence enhancement up to 35-fold. It suggested that P has a higher selectivity toward Zn²⁺ than other metal ions.

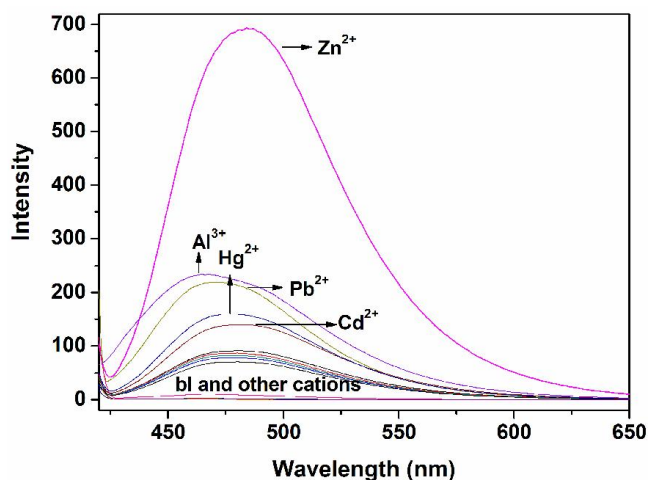


Figure 3. Fluorescence response of P (10 μM) with different metal ions (100 μM) in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES)

To further investigate the interaction of Zn^{2+} with the proposed probe P, the fluorescent titration experiment was carried out. Upon titration with Zn^{2+} , the fluorescence intensity of the monomer peak at 480 nm increased gradually (Figure 4), and the fluorescent intensity of P was proportional to the concentration of Zn^{2+} in the range of 0.5 μM to 4 μM ($R^2=0.993$) with a detection limit of 0.16 μM Zn^{2+} . This clearly demonstrated that probe P could sensitively detect environmentally relevant levels of Zn^{2+} .

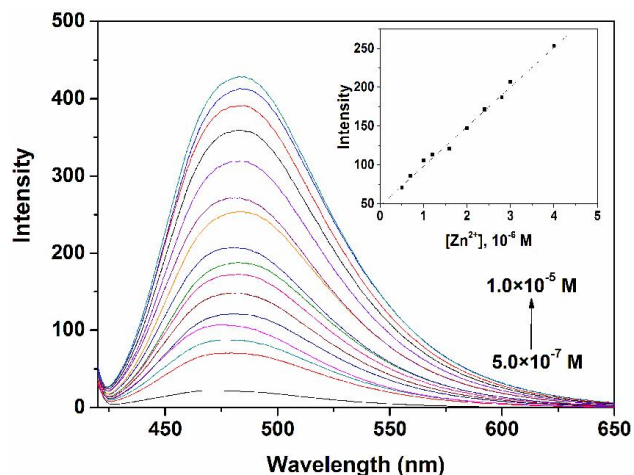


Figure 4. Fluorescence response of P (10 μM) with different concentrations of Zn^{2+} in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES). Inset: the fluorescence of P (10 μM) as a function of Zn^{2+} concentrations (0.5–4 μM)

The proposed binding mode of P with Zn^{2+}

The linear dependence of the intensity at 480 nm within the equivalent range of the Zn^{2+} showed that a 1:1 complex was formed between P and Zn^{2+} . Moreover, binding analysis using the method of continuous variations (Job's plot) was measured (Figure 5), and a maximum fluorescent intensity at 480 nm was observed when the molecular fraction of P was close to 0.5, which established the 1:1 complex formation between P and Zn^{2+} . Thus, according to the obtained results and reported work,¹⁴ the binding mode of P and Zn^{2+} was proposed as shown in Scheme 2.

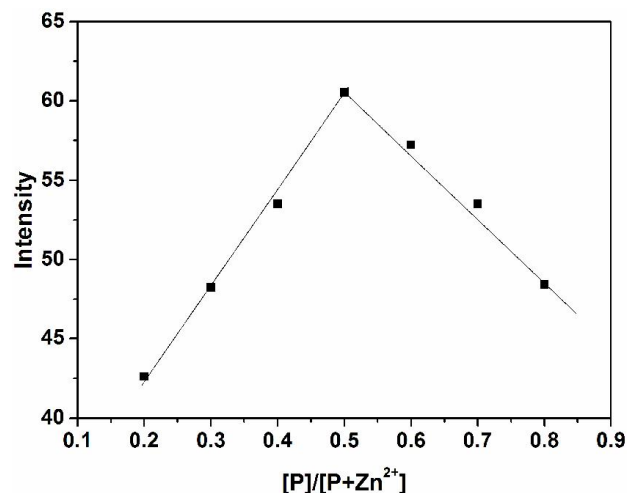
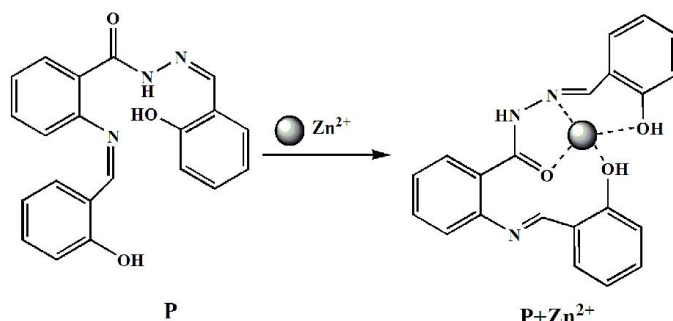


Figure 5. Job's plot curve of P with Zn^{2+} in ethanol-water solution (9:1, v:v, pH 6.3, 20 mM HEPES). The total concentration of P and Zn^{2+} was kept 10 μM .



Scheme 2. Proposed binding mode of P with Zn^{2+}

The ^1H NMR experiment was performed to understand the nature of P– Zn^{2+} interactions. The comparison of ^1H NMR spectra of P and P mixed with 1.0 equiv. of Zn^{2+} is shown in Figure 6. Apparently, the addition of Zn^{2+} into the solution of P led to an apparent downfield shift of the signals of –OH and –NH to certain degrees.

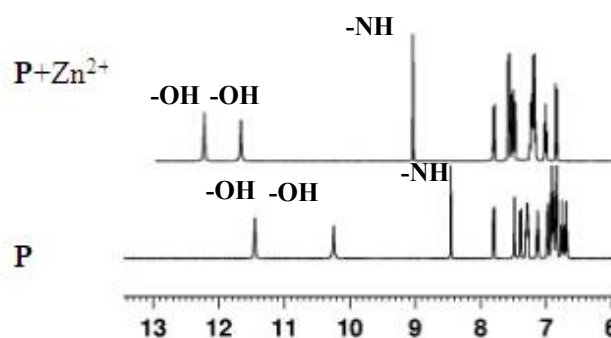


Figure 6. Partial ^1H NMR spectra of P and P– Zn^{2+}

Conclusions

In summary, a simple “off-on” type probe P for Zn^{2+} was presented. The conception may expand a promising approach to develop selective detection method for Zn^{2+} and lead to the development “off-on” type probes for other metal ions.

Acknowledgments: This work was financially supported by the National Natural Science Foundation of China (No. 81260268, 81360266), the Natural Science Foundation of

Hainan Province (No. 812188, 413131), the Colleges and Universities Scientific Research Projects of the Education Department of Hainan Province (Hjkj2013-29), the Research and Training Fundation of Hainan Medical University (HYCX201209), the National Training Programs of Innovation and Entrepreneurship for Undergraduates (201311810042).

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