

A Simple Benzimidazole Based Fluorescent Sensor for Ratiometric Recognition of Zn²⁺ in Water

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A phenylbenzimidazole derivatized sensor (**L**) that behaves as a ratiometric fluorescent receptor for Zn²⁺ in water has been described. In HEPES buffer at pH 7.4, sensor **L** displays a weak fluorescence emission band at 367 nm. Upon addition of Zn²⁺, the emission intensity at 367 nm is decreased, concomitantly, a new emission band centered at 426 nm is developed, thus facilitates a ratiometric Zn²⁺ sensing behavior. Sensor **L** binds Zn²⁺ through a 1:1 binding stoichiometry with high selectivity over other metal cations. Sensor **L** displays a linear response to Zn²⁺ concentration from 0 to 6.0 × 10⁻⁵ M, sensor **L** also exhibits high sensitivity to Zn²⁺ with a detection limit of 3.31 × 10⁻⁷ M.

Key Words : Zinc ion, Fluorescence, Ratiometric, Benzimidazole

Introduction

The design and synthesis of metal ion selective fluorescent probes has been an important field in supramolecular chemistry and coordination chemistry in the past two decades.¹ As the second most-abundant transition metal ion in human body, Zn²⁺ plays vital roles in physiological and pathological processes such as brain activity, neural signal transmitters or modulators, regulators of gene transcription and immune function.² Both its deficiency and excess can cause some health problems, including acrodermatitis enteropathica, prostate cancer, diabetes, Alzheimers and Parkinson's diseases.^{2b,3} Although some analytical techniques including UV-vis spectroscopy,⁴ flame atomic absorption spectrometry⁵ and potentiometry⁶ have been applied to Zn²⁺ detection in various samples, considerable attention has been paid to the development of fluorescent Zn²⁺ sensors owing to the simplicity, low cost and high sensitivity of fluorescence techniques.^{1c,7}

To date, a large amount of Zn²⁺ selective fluorescent sensors have been reported. However, a majority of them could not perform in pure water. Thus, the design and synthesis of Zn²⁺ sensors that can be applied in absolute water solution is imperative. Ratiometric fluorescent sensors permit dual wavelength signals to detect target species by measuring the ratio of fluorescence intensities at two different wavelengths, which would increase the dynamic range of fluorescence measurements due to its built-in correction property.⁸ Consequently, it is of great significance to design ratiometric fluorescent sensors.⁹ To the best of our knowledge, only a few ratiometric fluorescent sensors that can detect Zn²⁺ in absolute water solution have been documented,¹⁰ however, these reported sensors requires intricate synthetic procedures, simple and water soluble fluorescent Zn²⁺ sensor based on benzimidazole fluorophore is still rare.

We herein report the synthesis and Zn²⁺ recognition pro-

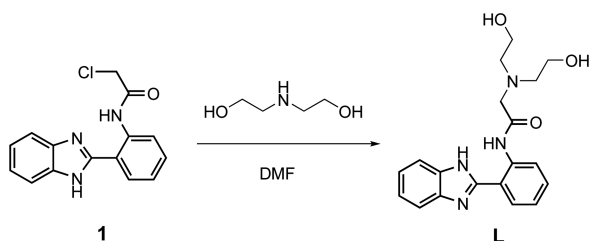
perty of a new 2-(2'-aminophenyl)benzimidazole derivatized fluorescent sensor **L** (Scheme 1). Sensor **L** exhibits ratiometric emission responses to Zn²⁺ in water solution (HEPES 10 mM, pH 7.4) with high selectivity and sensitivity.

Experimental

Instruments and Materials. Unless mentioned otherwise, all of the solvents and reagents used were purchased from commercial suppliers and used without further purification. Compound **1** was prepared according to the literature method.¹¹ Nuclear magnetic resonance (NMR) spectra were recorded on Agilent 400-MR NMR spectrometer. Chemical shifts (δ) were expressed in parts per million (ppm) and coupling constants (J) in Hertz. High resolution mass spectroscopy (HRMS) was measured on an Agilent 1200 time-of-flight mass spectrometer (Bruker, micrOTOF-Q). Fluorescence measurements were performed on a Sanco 970-CRT spectrofluorometer (Shanghai, China). The pH measurements were made with a Model PHS-25B meter (Shanghai, China).

General Methods. All titration experiments were carried out at (298.2 ± 0.1) K. Fluorescence spectra were measured with 10 μ M solution of sensor **L** in buffered water solution (HEPES 10 mM, pH = 7.4) and the solutions of metal salts were prepared in distilled water. All of the metal salts were used in water-soluble sulfate, nitrate or chloride salts. These solutions were used for all spectroscopic studies after appropriate dilution. Double-distilled water was used throughout the experiments. Moreover, the fluorescence spectra were measured 3 minutes after metal ion addition.

Synthesis of Sensor L. At room temperature, diethanolamine (498 mg, 4.74 mmol) was added to a stirred solution of *N*-[2-(1*H*-benzimidazol-2-yl) phenyl]-2-chloroacetamide (**1**) (450 mg, 1.58 mmol) in 10 mL of *N,N*-dimethylformamide (DMF). The reaction mixture was stirred overnight at room temperature, then poured into 300 mL of distilled water and



Scheme 1. Synthesis of sensor **L**.

led up to pH = 7 by diluted HCl solution. The mixture was extracted with CH_2Cl_2 (3×100 mL). The combined organic phase was dried over anhydrous MgSO_4 , followed by filtration and rotary evaporation. The residue was purified by a silica gel column chromatography with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (6:1, v/v) as eluent to give 442 mg of **L** as brown solid. Yield: 79%. mp 179.5–180 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 13.10 (s, 2H), 8.85 (d, $J = 8.8$ Hz, 1H), 8.05 (d, $J = 7.2$ Hz, 1H), 7.73 (s, 1H), 7.59 (s, 1H), 7.49 (t, $J = 7.2$ Hz, 1H), 7.29–7.25 (m, 3H), 4.58 (s, 2H), 3.57 (d, $J = 4.4$ Hz, 4H), 3.43 (s, 2H), 2.79 (t, $J = 4.4$ Hz, 4H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 172.08, 150.88, 138.18, 131.06, 128.35, 123.34, 120.24, 116.62, 60.95, 59.59, 58.27. HRMS(+). Calcd for $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_3\text{Na}$ [$\text{M}+\text{Na}^+$] $^+$: m/z 377.1590. Found: m/z 377.1582.

Results and Discussion

Metal Ion Recognition in Water. Sensor **L** was facilely synthesized by the reaction of **1** with diethanolamine (Scheme 1) and was characterized by ^1H NMR, ^{13}C NMR and HRMS. The good water solubility of **L** makes it has a potential application for metal ion detection in water solution. Based on this, we select the HEPES buffered water solution at pH 7.4 (HEPES 10 mM) as the working moiety. The fluorescence spectra were obtained with the excitation at 310 nm. The fluorescence properties of sensor **L** (10 μM) were investigated with various metal ions. As shown in Figure 1, sensor **L** exhibits a very weak fluorescence at 367 nm, which is

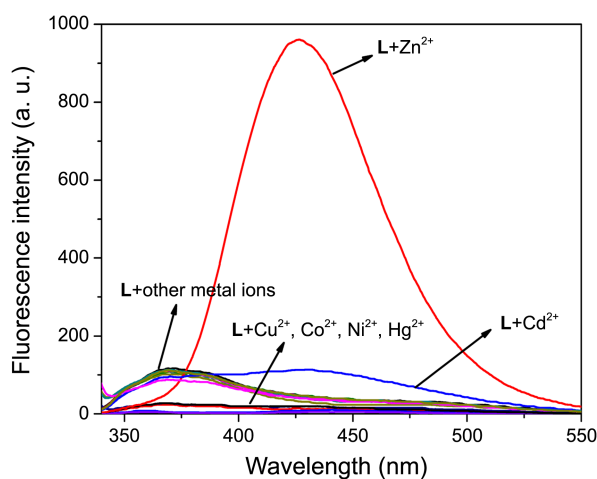


Figure 1. Fluorescence spectra changes of **L** (10 μM) in H_2O (HEPES 10 mM, pH = 7.4) in the presence of different metal ions (80 μM of each, $\lambda_{\text{ex}} = 310$ nm).

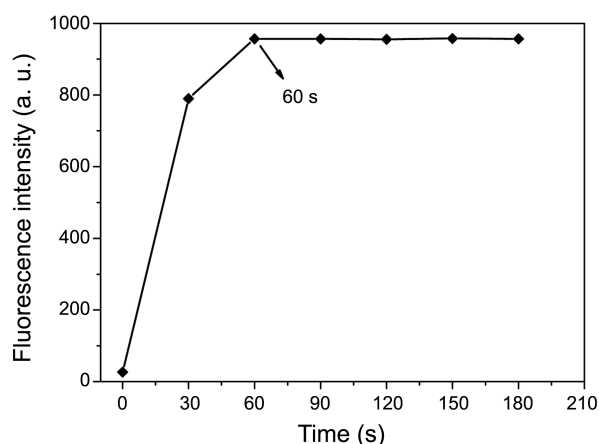


Figure 2. Fluorescence intensity changes of **L** (10 μM) in H_2O (HEPES 10 mM, pH = 7.4) in the presence of Zn^{2+} (80 μM) against time. $\lambda_{\text{ex}} = 310$ nm, $\lambda_{\text{em}} = 426$ nm.

attributed to the photoinduced electron transfer (PET) from diethanolamine nitrogen atom to the photoexcited phenylbenzimidazole moiety.^{9f} However, a significant fluorescence enhancement at 426 nm with a remarkable 59 nm red-shift was observed upon addition of 80 μM of Zn^{2+} . A fluorescence quenching was detected on addition of Cu^{2+} , Co^{2+} , Ni^{2+} and Hg^{2+} , implying that these metal ions could also bind with **L** but lead to fluorescence quenching due to their paramagnetic nature. The addition of Cd^{2+} elicits a slight fluorescence enhancement at 426 nm. By contrast, other metal ions such as Ag^+ , Pb^{2+} , Sr^{2+} , Ba^{2+} , Fe^{2+} , Mn^{2+} , Fe^{3+} , Al^{3+} , Cr^{3+} , Mg^{2+} , K^+ , Ca^{2+} and Na^+ do not cause any significant fluorescence spectrum changes. These results indicate that sensor **L** has a specific selectivity to Zn^{2+} through emission enhancement and red-shift. In addition, a time course study reveals that the Zn^{2+} -induced fluorescence enhancement can complete within 1 minute (Figure 2), indicating the rapid response of sensor **L** to Zn^{2+} .

Fluorescence Titration Studies. To further understand the sensing properties of **L** to Zn^{2+} , the fluorescence titration

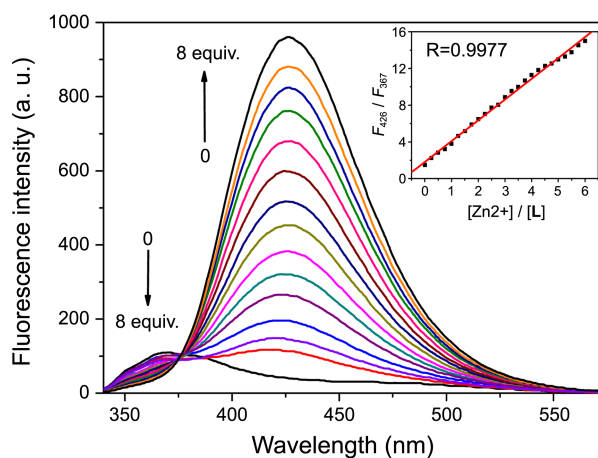


Figure 3. Fluorescence spectra of sensor **L** solution (10 μM) in water (HEPES 10 mM, pH = 7.4) on addition of different amounts of Zn^{2+} (0 to 8 equiv.). Inset: Fluorescence ratio (F_{426}/F_{367}) changes on addition of Zn^{2+} (0 to 6 equiv.) in water.

experiments were performed. As shown in Figure 3, the emission intensity at 367 nm of **L** solution progressively decreased on incremental increasing the added Zn²⁺ concentration (0 to 80 μM), meanwhile, the emission intensity at 426 nm increased impressively. The observed two wavelength variations behave ratiometric feature with an isoemissive point at 376 nm. Under the present conditions, the fluorescence ratio at 426 nm and 367 nm (F_{426}/F_{367}) increased linearly against Zn²⁺ concentration (0 to 60 μM, $R = 0.9977$) (Figure 3, inset), which allowed the detection of Zn²⁺ by the ratiometric fluorescence method. The detection limit of sensor **L** to Zn²⁺ was determined to be 3.31×10^{-7} M.¹²

Sensing Mechanism. In order to explore the recognition mechanism of the sensor **L**, the ¹H NMR spectra of **L** before and after addition of Zn²⁺ was compared (Figure 4). On addition of Zn²⁺, the intensity of NHs signal in free **L** at 13.10 ppm (Figure 4(a)) decreased to one NH signal intensity and became broadened (Figure 4(b)). Addition of Zn²⁺ led to down-field shift of tertiary N neighboring CH₂ proton signals (H_a and H_b). However, the proton signals in CH₂OH moiety (H_c and OH) did not show significant shifts, indicating the absence of interaction of OH with Zn²⁺. The signal at 8.85 ppm in free **L** could be assigned to H_d due to the possible hydrogen bonding between H_d and amide O atom, Zn²⁺ binding destroyed the hydrogen bonding and led to its up-field shift.¹³ These results indicate that sensor **L** may bind Zn²⁺ with an imidic acid form through tertiary N and imidic acid O atoms. This binding mode of sensor **L** with Zn²⁺ suppressed the PET process from diethanolamine nitrogen to phenylbenzimidazole moiety,¹⁴ which is responsible for the fluorescence enhancement. In addition, a chelation promoted amide NH deprotonation process (it was supported by HRMS analysis) exerted an internal charge transfer (ICT) from phenylbenzimidazole to Zn²⁺ ion (Figure 5), thus led to the observed emission red-shift.¹⁵

Binding Stoichiometry. Job's plot analysis shows that the fluorescence intensity of the tested solutions reach maximum when the mole fraction of Zn²⁺ is 0.5 (Figure 6), which advocates the formation of 1:1 complex between **L** and Zn²⁺. The 1:1 interaction of **L** and Zn²⁺ was further supported by

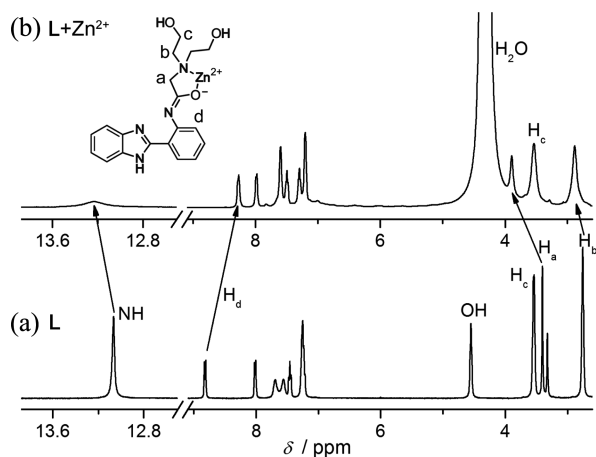


Figure 4. ¹H NMR spectrum (in DMSO-*d*₆) of **L** before (a) and after (b) addition of Zn²⁺.

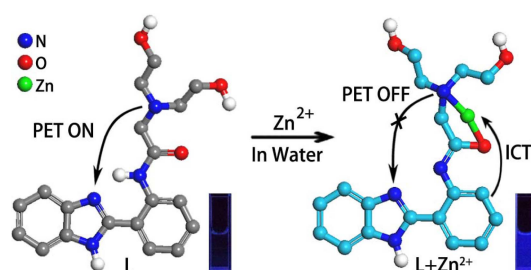


Figure 5. Proposed sensing mechanism of **L** to Zn²⁺.

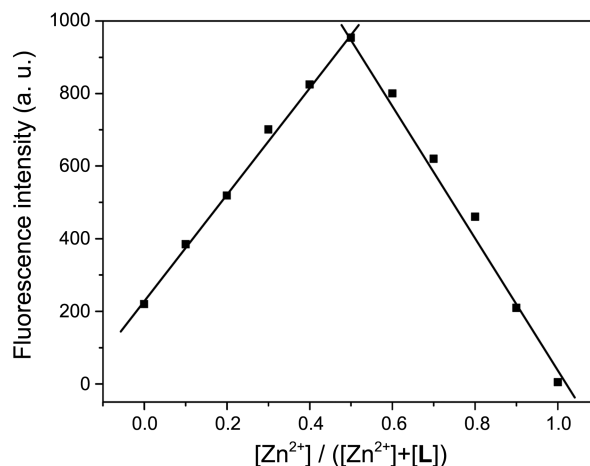


Figure 6. Job's plot for **L**-Zn²⁺ complex in HEPES-buffered H₂O solution (pH = 7.4). The total concentration of Zn²⁺ and **L** was 20 μM.

mass spectrometry analyses, in which the most prominent peak appeared at m/z 417.0896 is assignable to $[\text{L}+\text{Zn}^{2+}-\text{H}^+]^+$ (calcd = 417.0894). Moreover, from the fluorescence titration profile, the association constant (K_a) of **L** and Zn²⁺ was calculated to be 1.4×10^5 M⁻¹ by nonlinear least-squares fitting ($R^2 = 0.9988$) based on the 1:1 binding equation (Figure 7).¹⁶

Reversibility and pH Effect. As a sensor, the recognition process should be reversible. Upon addition 100 μM of ethylenediamine tetracetic acid disodium salts (EDTA_NA₂)

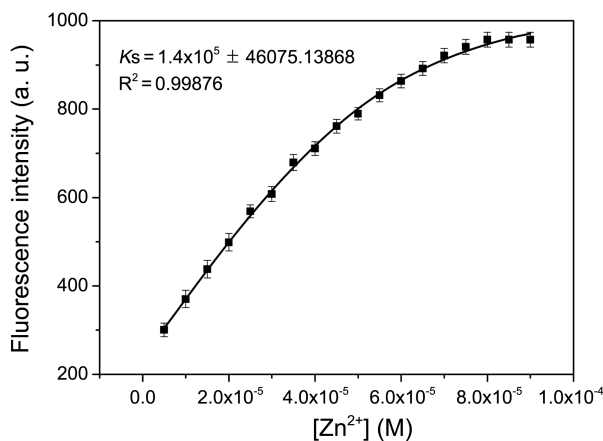


Figure 7. Nonlinear fitting of fluorescence intensity against Zn²⁺ concentration ($\lambda_{\text{em}} = 426$ nm).

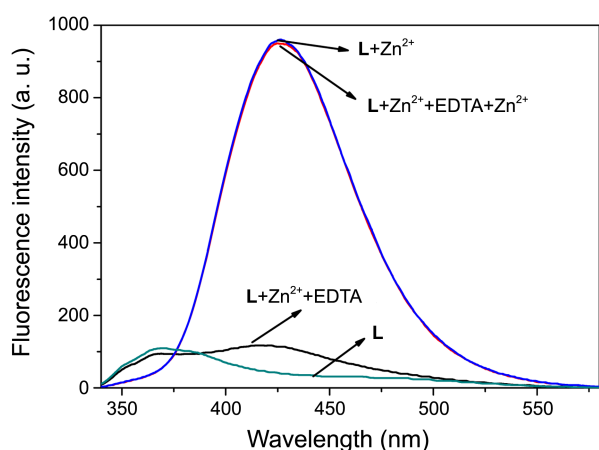


Figure 8. Fluorescence changes of **L** solution by alternative addition of Zn^{2+} and EDTA ($\lambda_{\text{ex}} = 310 \text{ nm}$).

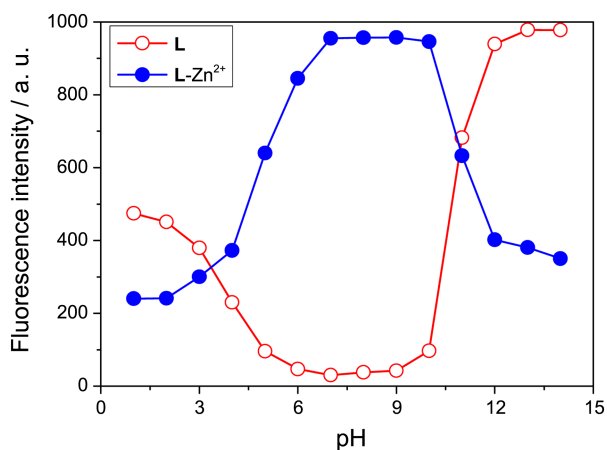


Figure 9. The fluorescence changes of **L** ($\lambda_{\text{em}} = 367 \text{ nm}$) and **L-Zn²⁺** ($\lambda_{\text{em}} = 426 \text{ nm}$) solution at different pH values ($\lambda_{\text{ex}} = 310 \text{ nm}$).

to **L-Zn²⁺** solution, the fluorescence emission restored to the similar state of free **L** (Figure 8), indicative of the reversibility of Zn^{2+} recognition.

Furthermore, the fluorescence changes of sensor **L** with and without Zn^{2+} at different pH values were also investigated (Figure 9). Acid-base titration results show that free sensor **L** displays weak emission at near neutral pH conditions, acidic ($\text{pH} < 5$) and basic ($\text{pH} > 10$) conditions can induce fluorescence enhancement. Therefore, sensor **L** can successfully detect Zn^{2+} in water in a wide pH range from 5 to 10. Moreover, the fluorescence intensity of **L-Zn²⁺** complex is stable between pH 7 to 10. These results indicate that sensor **L** has a potential practical applicability for Zn^{2+} detection in physiological and environmental systems.

Competition Experiment Studies. The fluorescence changes of **L** solution to Zn^{2+} in the presence of potential competitive metal ions were investigated to evaluate the anti-interference of Zn^{2+} recognition (Figure 10). Significant fluorescence quenching of **L-Zn²⁺** solution was observed in the presence of paramagnetic metal ions of Cu^{2+} and Hg^{2+} , this phenomenon was usual in some previously reported metal ion sensors.¹⁷ However, other metal ions do not induce

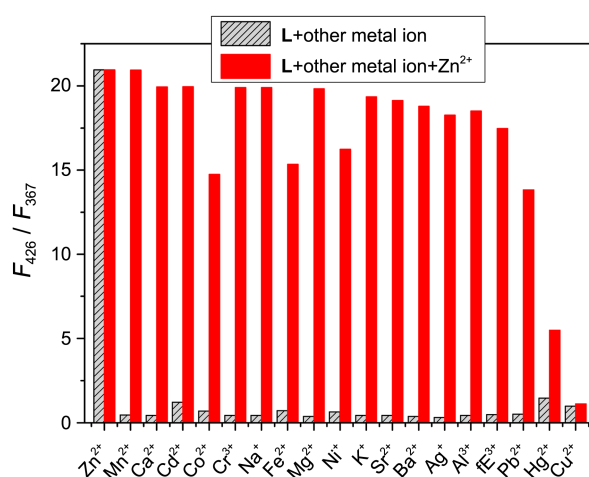


Figure 10. Fluorescence responses of **L** solution ($10 \mu\text{M}$) in the presence of $80 \mu\text{M}$ of miscellaneous metal ions (black bars) and upon further addition of $80 \mu\text{M}$ of Zn^{2+} (red bars) ($\lambda_{\text{ex}} = 310 \text{ nm}$).

such dramatic fluorescence quenching effect. Thus, sensor **L** displays a good selectivity to Zn^{2+} over other metal ions except Cu^{2+} and Hg^{2+} .

Conclusion

In summary, we have developed a new ratiometric fluorescent sensor **L** for Zn^{2+} recognition in absolute water solution at pH 7.4. Free sensor **L** displays weak fluorescence emission, however, chelating with Zn^{2+} results in fluorescence enhancement with a remarkable emission red-shift, which leads to ratiometric output signal. Sensor **L** shows good selectivity and sensitivity for Zn^{2+} over other metal ions. Job's plot and HRMS analyses indicate the formation of a 1:1 complex.

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