

A Schiff Base Fluorescent Probe for Mg^{2+} and Zn^{2+} Based on Coumarin

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(Received: 10 May 2012;

Accepted: 15 February 2013)

AJC-12999

A novel fluorescent Schiff base (**L**) based on coumarin was synthesized and characterized. Addition of Mg^{2+} and Zn^{2+} to fluorescent Schiff base in acetonitrile resulted in fluorescence enhancement and little influence was observed for other metal ions. It suggested that fluorescent Schiff base could be used as a potential Mg^{2+} and Zn^{2+} fluorescent probe in acetonitrile.

Key Words: Coumarin, Schiff base, Fluorescent probe, Mg^{2+} , Zn^{2+} .

INTRODUCTION

The design and synthesis of fluorescent probes with high selectivity and sensitivity is an active field of supramolecular chemistry, which plays fundamental role in medical, environmental and biological applications. Both Mg^{2+} and Zn^{2+} are biologically important metal ions and their selective detection is extremely important. Magnesium(II) is one of the most abundant divalent ions in the cell and plays a critical role in many biological activities, such as cell proliferation¹, cell death², signal transduction³, transporters⁴ and ion channels^{5,6}. Zinc(II), the second most abundant transition metal ion in the human body, plays vital role in many cellular processes, including gene expression⁷, apoptosis⁸, enzyme regulation⁹ and neurotransmission¹⁰. The lack of zinc ions will cause a reduction of the ability of the islet cell to produce and secrete insulin. However, Zn^{2+} is also a pollutant of the environment and toxic to soil microbes¹¹. Therefore, qualitative detection of Mg^{2+} and Zn^{2+} is needed in cell, industrial and environmental fields.

Because of their excellent photo physical properties, coumarin and its derivatives have been widely used to construct a variety of fluorescent probes and chemosensors¹². Hence, in this paper, we report a fluorescent probe Schiff base for Zn^{2+} and Mg^{2+} based on coumarin, which exhibited considerable fluorescence enhancement induced by Zn^{2+} and Mg^{2+} in acetonitrile.

EXPERIMENTAL

All UV-VIS spectra and fluorescence spectra were recorded on a TU-1901 double-beam UV-VIS spectrophotometer and RF-53010PC fluorescence spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker 400 spectrometer. Mass spectra were obtained on a Thermo-Finnigan LCQ

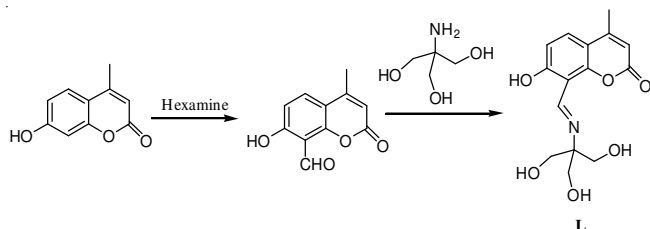
advantage mass spectrometer. Elemental analyses were taken with a Vario EL CHNS elemental analyzer.

7-Hydroxy-4-methylcoumarin and *tri*-(hydroxymethyl)-methylamine were purchased from Aldrich and used without further purification. Acetonitrile for spectral detection was HPLC reagent without fluorescent impurity. All solvents were analytical reagents.

8-Formyl-7-hydroxy-4-methylcoumarin: 8-Formyl-7-hydroxy-4-methylcoumarin was prepared by the known method¹³ with slight modification. 7-Hydroxy-4-methylcoumarin (5.00 g, 0.0284 mol) and hexamine (10.00 g, 0.071 mol) in acetic acid (37.5 mL) were stirred for 5.5 h at 95 °C. Thereafter, hydrochloric acid (75 mL, concd. HCl/H₂O = 84:100, v/v) was added and further heated for 45 min. After cooling, the reaction mixture was poured into ice-water (375 mL) and extracted with ethyl acetate three times. The combined organic layer was dried over sodium sulfate and the solvent removed. The residue was purified by column chromatography on silica gel using dichloromethane as eluent to provide the product as a light yellow solid. Yield 1.10 g (19 %).

8-((1,3-Dihydroxy-2-(hydroxymethyl)propan-2-ylimino)methyl)-7-hydroxy-4-methyl-2H-chromen-2-one (L**):** **L** was prepared in high yield by reacting 8-formyl-7-hydroxy-4-methylcoumarin with *tri*-(hydroxymethyl)-methylamine (**Scheme-I**). *Tri*-(hydroxymethyl)-methylamine (0.24 g, 0.2 mmol) was dissolved in ethanol (20 mL) and 8-formyl-7-hydroxy-4-methylcoumarin (0.40 g, 0.2 mmol) was added to the solution. The reaction mixture was refluxed for 8 h under nitrogen and then the mixture was cooled to room temperature. The precipitate was filtered off, washed with cold ethanol several times and dried to give the desired product as pale yellow solid. Yield 0.50 g (83 %). m.p. 228.1-229.8 °C.

^1H NMR (CD_3SOCD_3 , δ ppm): 2.33 (s, 3H); 3.65 (s, 6H); 5.26 (s, 3H); 5.96 (s, 1H); 6.44 (d, $J = 9.6$ Hz, 1H); 7.56 (d, $J = 9.6$ Hz, 1H); 8.79 (d, $J = 12.4$ Hz, 1H). ^{13}C NMR (CD_3SOCD_3 , δ ppm): 18.53, 60.38, 65.89, 103.15, 104.92, 106.41, 120.39, 131.16, 154.82, 156.46, 158.18, 159.76, 178.27. MS m/z : 306.2 $[\text{M}-\text{H}]^+$. Found, %: C, 58.68; H, 5.50; N, 4.59. Calculated for $\text{C}_{15}\text{H}_{17}\text{NO}_6$: C, 58.63; H, 5.58; N, 4.56.



Scheme-I: Synthetic route of compound **L**

RESULTS AND DISCUSSION

We examined the chemosensing behaviour of the fluorescent sensor **L** by fluorescence measurement in the presence of various metal ions in acetonitrile by comparing the fluorescence intensities of the solutions before and after addition of 10 equiv. of the following metal ions as: Li^+ , Na^+ , K^+ , Ca^{2+} , Ba^{2+} , Cu^{2+} , Fe^{3+} , Ag^+ , Hg^{2+} , Al^{3+} , Co^{2+} , Cd^{2+} , Ni^{2+} , Pb^{2+} , Mg^{2+} and Zn^{2+} . As shown in Fig. 1, the fluorescence intensity of free **L** (10 μM) alone was weak at 424 nm when it was excited at 341 nm, due to isomerization of the $\text{C}=\text{N}$ double bond in Schiff base. Compound with an unbridged $\text{C}=\text{N}$ structure is often nonfluorescent due to the $\text{C}=\text{N}$ isomerization, but it may be inhibited by complexation with special ion¹⁴. In the presence of Mg^{2+} and Zn^{2+} , **L** showed large fluorescence enhancement. In addition, Mg^{2+} and Zn^{2+} resulted in an obvious red-shift of the λ_{em} of **L** from 424 to 455 nm. The sensor gave slight fluorescence enhancement with Hg^{2+} , Al^{3+} , Fe^{3+} and Cr^{3+} . Upon addition of Li^+ , Na^+ , K^+ , Ca^{2+} , Ba^{2+} , Cu^{2+} , Ag^+ , Hg^{2+} , Cd^{2+} , Ni^{2+} and Pb^{2+} , the emission intensity of **L** only slightly increased and the λ_{em} was red shifted to near 455 nm.

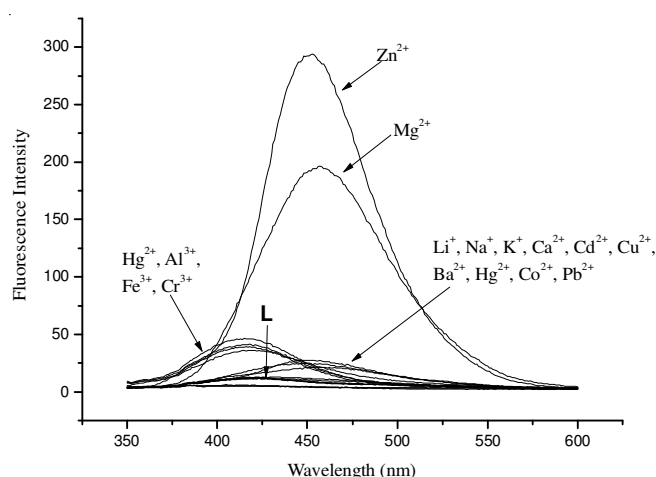


Fig. 1. Fluorescence spectra of **L** (10 μM , $\lambda_{\text{ex}} = 341$ nm) with addition of various metal ions (10 equiv, respectively) in acetonitrile

To further investigate the selectivity of **L** to Mg^{2+} and Zn^{2+} , we performed fluorescence titrations of **L** in acetonitrile upon excitation at 341 nm. As shown in Fig. 2, upon gradual addition

of Mg^{2+} and Zn^{2+} into the acetonitrile solution of sensor **L**, a new emission peak at about 450 nm appeared and the intensity was dramatically increased. When the concentration of Mg^{2+} and Zn^{2+} arrived at 100 μM , the fluorescence intensity reached the maximum and showed 17.6-fold and 24-fold enhancement, respectively.

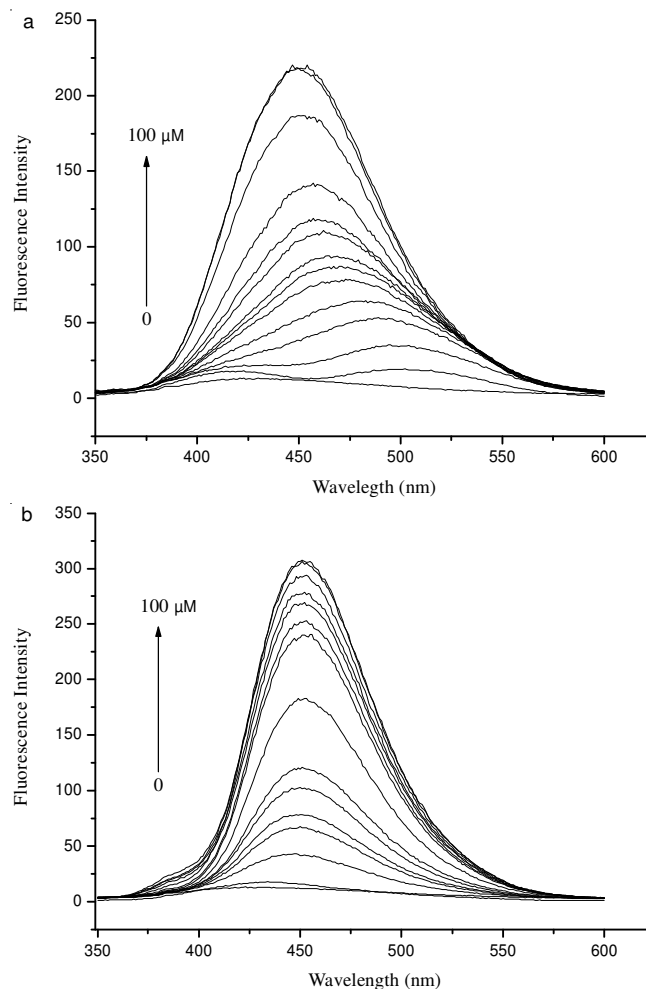


Fig. 2. Fluorescence spectra of **L** (10 μM , $\lambda_{\text{ex}} = 341$ nm) upon addition of (a) 0-100 μM of Mg^{2+} and (b) 0-100 μM of Zn^{2+} in acetonitrile

In order to further test the interference of other metal ions on the determination of Mg^{2+} and Zn^{2+} , competition experiments were performed in which **L** was added to a solution of Mg^{2+} and Zn^{2+} in the presence of other metal ions. As shown in Fig. 3a, addition of Hg^{2+} resulted in relative large increase of the fluorescence intensity of Mg^{2+} complex. Co^{2+} , Cr^{3+} and Cu^{2+} showed only slight interference on fluorescent enhancement upon the subsequent addition of Mg^{2+} . The other metal ions showed little influence on the determination of Mg^{2+} . As shown in Fig. 3b, the fluorescence intensity of Zn^{2+} complex increased with the addition of Hg^{2+} , Mg^{2+} , Ag^+ , K^+ , Cd^{2+} , Li^+ and Na^+ . With the addition of Ba^{2+} , Ca^{2+} , Cr^{3+} , Al^{3+} , Pb^{2+} and Fe^{3+} , the fluorescence intensity of Zn^{2+} complex decreased, but it still had strong fluorescence intensity. Co^{2+} , Ni^{2+} , especially Cu^{2+} could quench fluorescence of **L**- Zn^{2+} complex *via* energy or electron transfer¹⁵.

Detailed investigations were carried out to understand the coordinating behaviour of **L** with Mg^{2+} and Zn^{2+} . The stoichi-

ometry of the coordinating species was examined by the method of continuous variation (Job's plot) and found to be 1:2 and 1:1 with respect to **L** to Mg^{2+} and Zn^{2+} , respectively (Fig. 4).

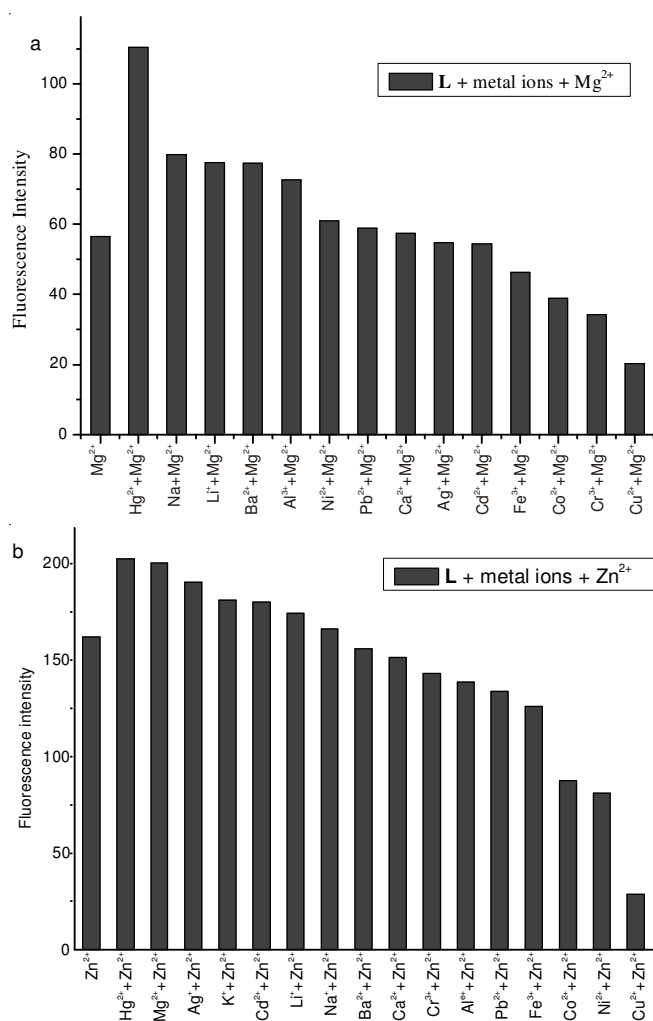


Fig. 3. Fluorescence responses of **L** (10 μM, $\lambda_{\text{ex}} = 341$ nm) to Mg^{2+} (a) and Zn^{2+} (b) in the presence of various metal ions (10 μM) in acetonitrile

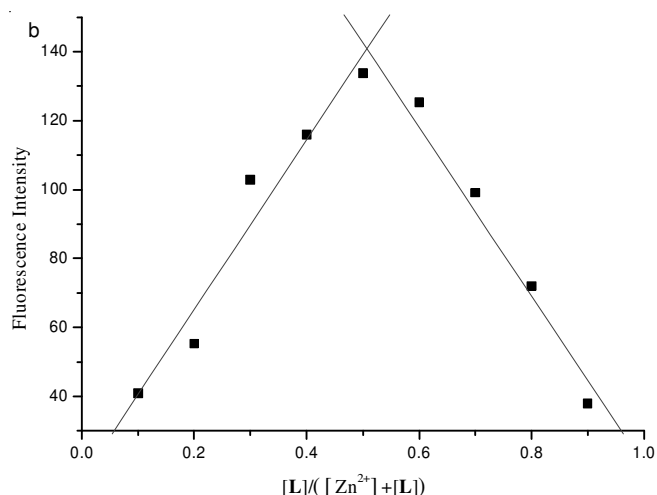
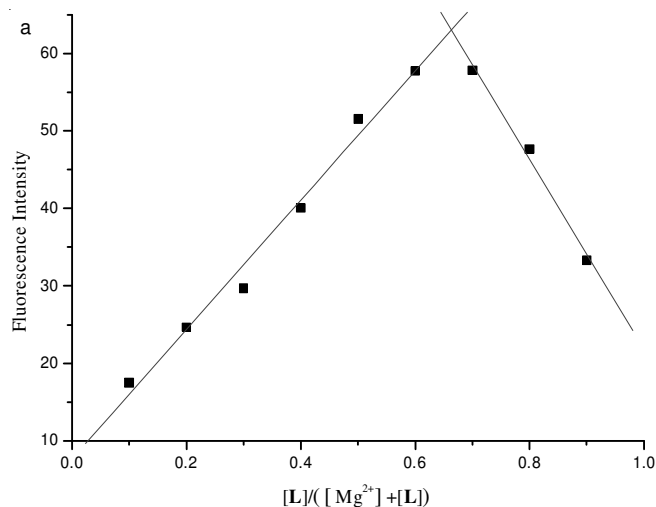


Fig. 4. Job's plot of **L** with Mg^{2+} (a) and Zn^{2+} (b) in acetonitrile. Total concentration of **L** and $\text{Mg}^{2+}/\text{Zn}^{2+}$ is 20 μM ($\lambda_{\text{ex}} = 341$ nm)

Conclusion

In conclusion, a Schiff base probe **L** based on coumarin was designed and synthesized. Upon treatment with Mg^{2+} and Zn^{2+} in acetonitrile, **L** showed large fluorescence enhancement and formed 1:2, 1:1 complexes with Mg^{2+} , Zn^{2+} . Probe **L** can be prepared from low cost starting materials with easy preparation, which is important for practical application.

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