

## A Schiff Base Fluorescent Probe for Mg<sup>2+</sup> and Zn<sup>2+</sup> Based on Coumarin

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A novel fluorescent Schiff base (**L**) based on coumarin was synthesized and characterized. Addition of Mg<sup>2+</sup> and Zn<sup>2+</sup> 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<sup>2+</sup> and Zn<sup>2+</sup> fluorescent probe in acetonitrile.

**Key Words:** Coumarin, Schiff base, Fluorescent probe, Mg<sup>2+</sup>, Zn<sup>2+</sup>.

### 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<sup>2+</sup> and Zn<sup>2+</sup> 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 proliferation<sup>1</sup>, cell death<sup>2</sup>, signal transduction<sup>3</sup>, transporters<sup>4</sup> and ion channels<sup>5,6</sup>. Zinc(II), the second most abundant transition metal ion in the human body, plays vital role in many cellular processes, including gene expression<sup>7</sup>, apoptosis<sup>8</sup>, enzyme regulation<sup>9</sup> and neurotransmission<sup>10</sup>. The lack of zinc ions will cause a reduction of the ability of the islet cell to produce and secret insulin. However, Zn<sup>2+</sup> is also a pollutant of the environment and toxic to soil microbes<sup>11</sup>. Therefore, qualitative detection of Mg<sup>2+</sup> and Zn<sup>2+</sup> 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 chemosensors<sup>12</sup>. Hence, in this paper, we report a fluorescent probe Schiff base for Zn<sup>2+</sup> and Mg<sup>2+</sup> based on coumarin, which exhibited considerable fluorescence enhancement induced by Zn<sup>2+</sup> and Mg<sup>2+</sup> 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. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker 400 spectrometer. Mass spectra were obtained on a Thermo-Finnigan LCQ

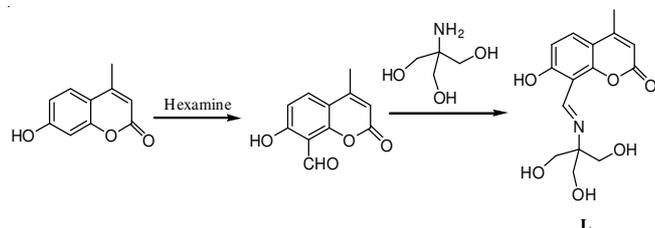
advantage mass spectrometer. Elemental analyses were taken with a Vario EL CHNS elemental analyzer.

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 method<sup>13</sup> 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/H<sub>2</sub>O = 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.

$^1\text{H}$  NMR ( $\text{CD}_3\text{SOCD}_3$ ,  $\delta$  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).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{SOCD}_3$ ,  $\delta$  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:  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Hg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ . As shown in Fig. 1, the fluorescence intensity of free **L** ( $10\ \mu\text{M}$ ) alone was weak at  $424\ \text{nm}$  when it was excited at  $341\ \text{nm}$ , 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 ion<sup>14</sup>. In the presence of  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ , **L** showed large fluorescence enhancement. In addition,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  resulted in an obvious red-shift of the  $\lambda_{\text{em}}$  of **L** from  $424$  to  $455\ \text{nm}$ . The sensor gave slight fluorescence enhancement with  $\text{Hg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Cr}^{3+}$ . Upon addition of  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Hg}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Pb}^{2+}$ , the emission intensity of **L** only slightly increased and the  $\lambda_{\text{em}}$  was red shifted to near  $455\ \text{nm}$ .

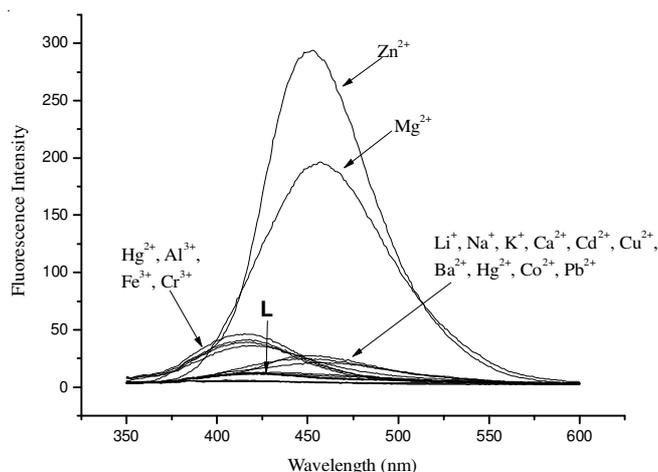


Fig. 1. Fluorescence spectra of **L** ( $10\ \mu\text{M}$ ,  $\lambda_{\text{ex}} = 341\ \text{nm}$ ) with addition of various metal ions (10 equiv, respectively) in acetonitrile

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

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

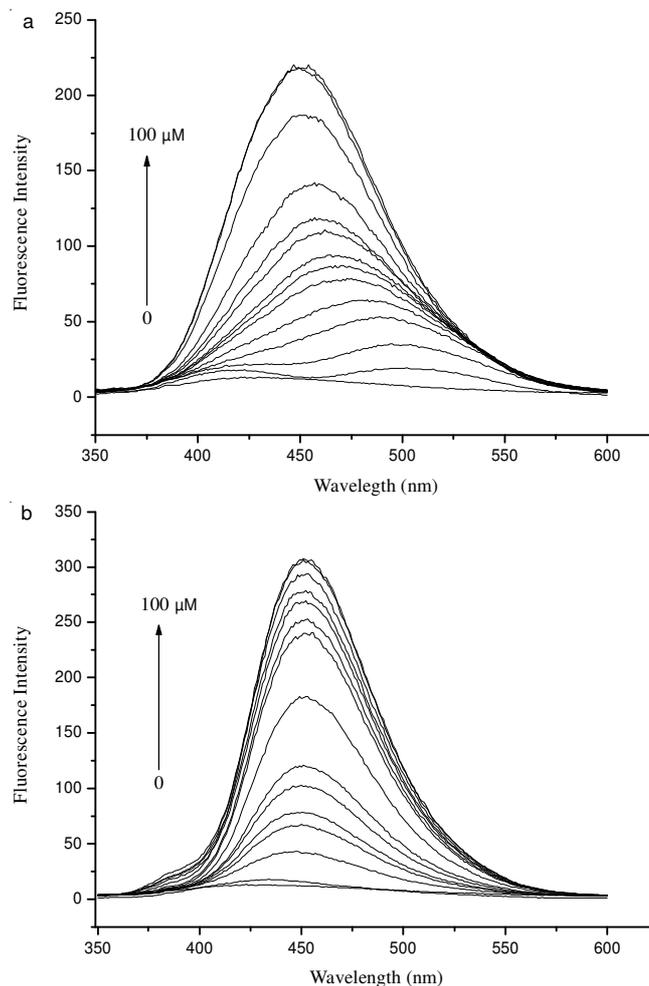


Fig. 2. Fluorescence spectra of **L** ( $10\ \mu\text{M}$ ,  $\lambda_{\text{ex}} = 341\ \text{nm}$ ) upon addition of (a)  $0$ – $100\ \mu\text{M}$  of  $\text{Mg}^{2+}$  and (b)  $0$ – $100\ \mu\text{M}$  of  $\text{Zn}^{2+}$  in acetonitrile

In order to further test the interference of other metal ions on the determination of  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ , competition experiments were performed in which **L** was added to a solution of  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  in the presence of other metal ions. As shown in Fig. 3a, addition of  $\text{Hg}^{2+}$  resulted in relative large increase of the fluorescence intensity of  $\text{Mg}^{2+}$  complex.  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$  and  $\text{Cu}^{2+}$  showed only slight interference on fluorescent enhancement upon the subsequent addition of  $\text{Mg}^{2+}$ . The other metal ions showed little influence on the determination of  $\text{Mg}^{2+}$ . As shown in Fig. 3b, the fluorescence intensity of  $\text{Zn}^{2+}$  complex increased with the addition of  $\text{Hg}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{K}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Li}^+$  and  $\text{Na}^+$ . With the addition of  $\text{Ba}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Pb}^{2+}$  and  $\text{Fe}^{3+}$ , the fluorescence intensity of  $\text{Zn}^{2+}$  complex decreased, but it still had strong fluorescence intensity.  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ , especially  $\text{Cu}^{2+}$  could quench fluorescence of **L**- $\text{Zn}^{2+}$  complex *via* energy or electron transfer<sup>15</sup>.

Detailed investigations were carried out to understand the coordinating behaviour of **L** with  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ . 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).

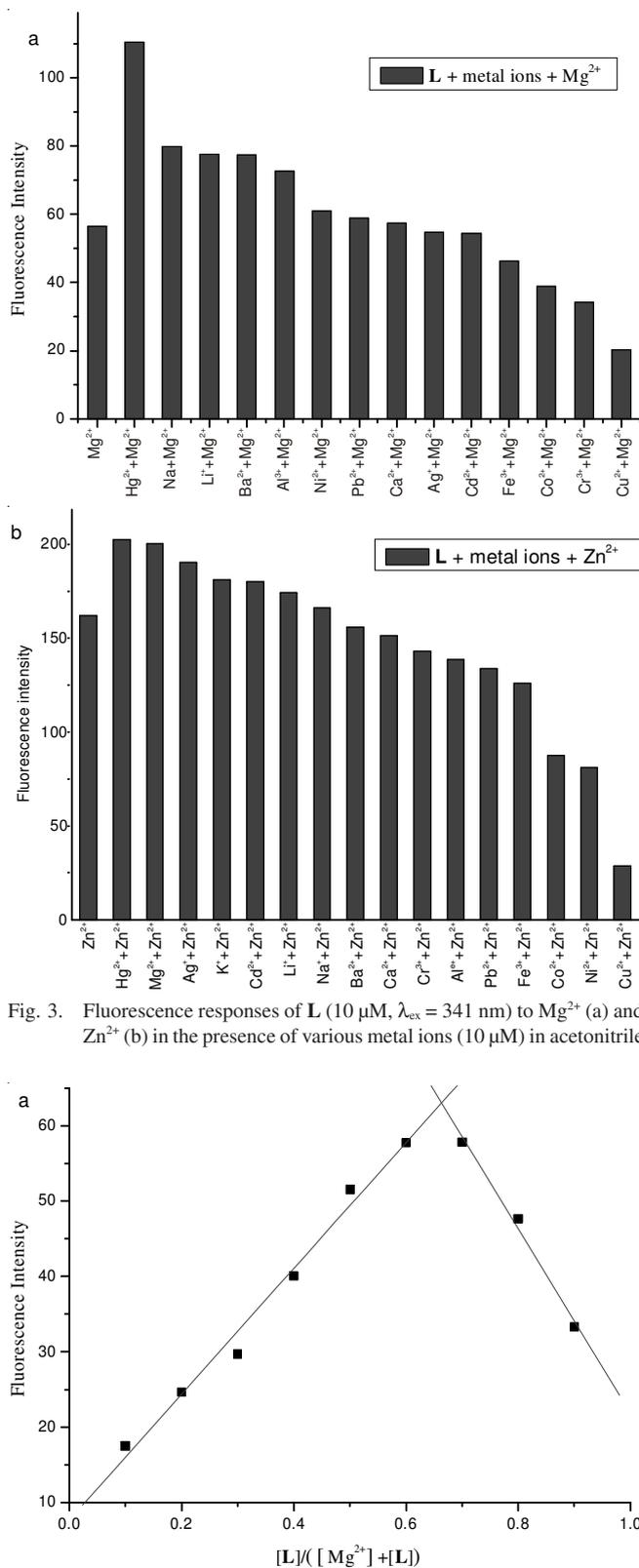


Fig. 3. Fluorescence responses of **L** (10 μM, λ<sub>ex</sub> = 341 nm) to  $Mg^{2+}$  (a) and  $Zn^{2+}$  (b) in the presence of various metal ions (10 μM) in acetonitrile

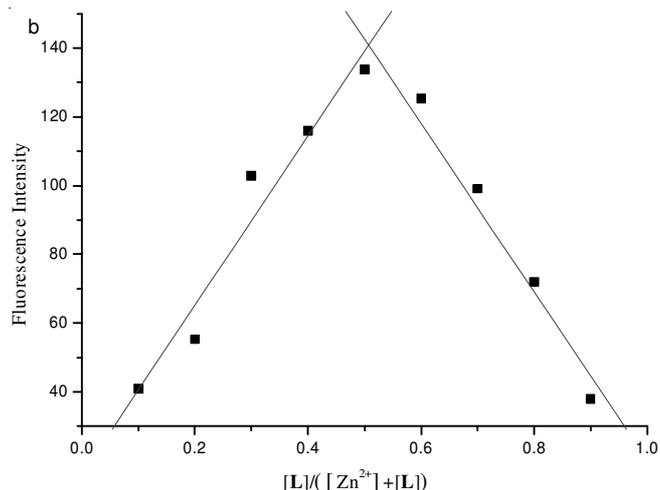


Fig. 4. Job's plot of **L** with  $Mg^{2+}$  (a) and  $Zn^{2+}$  (b) in acetonitrile. Total concentration of **L** and  $Mg^{2+}/Zn^{2+}$  is 20 μM (λ<sub>ex</sub> = 341 nm)

## 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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