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# A Novel Chromone Schiff-Base Fluorescent Chemosensor for Cd(II) Based on C=N Isomerization

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Abstract A new chromone Schiff-base fluorescent probe 7'methoxychromone-3'-methylidene-1,2,4-triazole-3-imine (L) was designed and synthesized for selective recognition  $Cd^{2+}$ . With the fluorescence titration and the ESI-MS data, we reach the conclusion that the binding mode of the ligand-metal (L- $Cd^{2+}$ ) complex is 1:1. The sensor showed a strong fluorescence enhancement in ethanol system of  $Cd^{2+}$  (excitation 409 nm and emission 462 nm) and the sensing mechanism based on the fact that C=N isomerization can be used to explain this phenomenon.

Keywords Fluorescent sensor  $\cdot$  C=N isomerization  $\cdot$  Chromone  $\cdot$  Cd^{2+}

### Introduction

In recent years, with the increasingly deterioration of the environment, the toxic heavy metal ions are becoming more and more threatening to human health, which seriously affects people's quality of life [1–5]. So it is of great significance to recognize heavy metal ions quickly and efficiently. Cadmium (Cd), classified as a human carcinogen in 1993, is one of the

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Zheng-yin Yang yangzy@lzu.edu.cn most harmful pollutants [6–10]. Excessive Cd ingestion can result in plant growth inhibition or even death [11]. Worse is that it can enter the human body with the food and accumulate in liver or kidney threatening health, especially for children and pregnant women [12]. Nevertheless, we cannot stop using cadmium for its wide application in industry such as battery manufacturing and electroplating [13, 14]. Hence it is very important to develop highly selective and efficient chemosensors for the detection of Cd in production and life.

Nowadays fluorescent chemosensors have been paid much attention to detect metal ions because of their highly sensitive and selective character when mixed with these ions [15-18]. And many excellent chemosensors for detecting transition and heavy metal ions have been designed. However, there are only a few fluorescent chemosensors that have been reported for recognition of  $Cd^{2+}$  [19–22]. It is urgent to develop some fluorescent chemosensors that can detect cadmium efficiently both in the natural environment and biological systems. In our study, 7-methoxychromone-3-carbaldehyde and 3-amino-1,2, 4-triazole were selected as reactants to get a Schiff-base in that Schiff-base compounds and their metal complexes have been widely used in medicine, analytical chemistry, and the field of photochromic effect. What's more, as good ligands, Schiffbases can be used to identify the content of metal ions and carry out the quantitative analysis of metal ions [23–25]. The triazoles and their derivatives have also been widely used in fields of analytic approach and biology [26-28]. For these reasons, that Schiff-base was considered as a potential fluorescent probe for the determination of metal cations.

Herein, we designed a new chromone Schiff-base chemosensor L that allows the  $Cd^{2+}$  to be differentiated on the basis of distinct fluorescence responses in ethanol. The free L emitted weak fluorescence due to the C=N

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Scheme 1 Synthesis of 7'-methoxychromone-3'-methylidene-1,2,4-triazole-3-imine (L)



Fig. 1 Absorption spectra of L in EtOH (50.0  $\mu$ M) upon the addition of Cd(NO<sub>3</sub>)<sub>2</sub> (0–1.1 equiv.)





isomerization phenomenon; while when it was bound with  $Cd^{2+}$ , the C=N isomerization was inhibited and the molecular structure changed from flexible to rigid, which dramatically increased the fluorescence intensity [29, 30].

# Experimental

#### Materials and Instrumentation

All chemicals were obtained from commercial suppliers and used without further purification. ESI-MS were determined on a Bruker esquire 6000 spectrometer. Fluorescence spectra were recorded on a Hitachi RF-4500 spectrophotometer equipped with quartz cuvettes of 1 cm path length. <sup>1</sup>H NMR spectra were measured on the Bruker 400 MHz instruments using TMS as an internal standard. UV-Vis absorption spectra were determined on a Shimadzu UV-240 spectrophotometer.

## Synthesis

7-methoxychromone-3-carbaldehyde was obtained according to the literature procedures [31]. Synthesis of L was based on the following method (Scheme 1): an ethanol solution (12 mL) of 3-amino-1,2,4-triazole (0.042 g, 0.5 mmol) was added dropwise to a solution (25 mL) of 7-methoxychromone-3-carbaldehyde (0.102 g, 0.5 mmol) in ethanol. Then the solution was reflux for 6 h under stirring and some white precipitant appeared [32]. The mixture was filtered and dried under vacuum. Recrystallization from ethanol gave 7'-methoxychromone-3'methylidene-1,2,4-triazole-3-imine (L) which was dried under vacuum. Yield, 69.3 %, m.p: 223-224 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz): δ 11.28 (s, 1H, -NH-), 9.64 (s, 1H,  $H^{5}$ ), 9.03 (s, 1H,  $H^{6}$ ), 8.77 (s, 1H,  $H^{1}$ ), 7.62 (d, J = 8.0 Hz, 1H,  $H^{2}$ ), 6.54 (dd, J = 4.0 Hz and J = 8.0 Hz, 1H,  $H^{3}$ ), 6.53 (d,  $J = 4.0 \text{ Hz}, 1\text{H}, \text{H}^4$ , 3.81 (s, 3H, -CH<sub>3</sub>) (Figure S1). MS (ESI)  $m/z 271.1 [M + H]^+$ , (Figure S2).

#### Analysis

Stock solutions (5.0 mM) of the nitrate salts of  $Al^{3+}$ ,  $Ba^{2+}$ ,  $Ca^{2+}$ ,  $Cu^{2+}$ ,  $Hg^{2+}$ ,  $Fe^{3+}$ ,  $K^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Na^+$ ,  $Li^+$ ,  $Pb^{2+}$ ,  $Zn^{2+}$  and  $Cd^{2+}$  in ethanol were prepared. Stock solutions of L (5.0 mM) were prepared in ethanol. Test solutions were

Fig. 3 Fluorescence spectra of L (50.0  $\mu$ M) with addition of increasing amount of Cd<sup>2+</sup> (0–1.4 equiv.) in EtOH ( $\lambda$ ex =409 nm, slit: 3 nm/3 nm). Inset: Fluorescence emission intensity of L at 462 nm as a function of (0–1.4 equiv.) Cd<sup>2+</sup> concentration



**Fig. 4** Job's plot of **L** in EtOH showing 1:1 stoichiometry of the complex between **L** and Cd<sup>2+</sup>. The total concentration of **L** and Cd<sup>2+</sup> was 50.0  $\mu$ M. Fluorescence emission intensity was recorded at 462 nm

prepared by placing 20  $\mu$ L of the probe stock solution into cuvettes, adding an appropriate aliquot of each ions stock, and diluting the solution to 2 mL with ethanol solutions. Both the excitation and emission slit widths were 3.0 nm.

## **Results and Discussion**

#### **Absorption Studies**

The binding properties of **L** with  $Cd^{2+}$  were studied by UV-Vis titration in the above solution at room temperature (Fig. 1). Upon addition of  $Cd^{2+}$  ions (0–1.1 equiv.), the absorbance bands at 262 nm and 409 nm enhanced gradually. Moreover,



there were clearly two isosbestic points at 278 and 354 nm, and the presence of these two different isosbestic points for the sensor suggested the formation of a complex [33].

## **Emission Studies**

A fluorescence study was further used to determine the selectivity and sensitivity of L in the presence of various metal ions, such as  $Al^{3+}$ ,  $Ba^{2+}$ ,  $Ca^{2+}$ ,  $Cu^{2+}$ ,  $Hg^{2+}$ ,  $Fe^{3+}$ ,  $K^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Na^+$ ,  $Li^+$ ,  $Pb^{2+}$ ,  $Zn^{2+}$  and  $Cd^{2+}$ . Fascinatingly, the treatment of **L** (50.0  $\mu$ M) with  $Cd^{2+}$  (50.0  $\mu$ M) resulted in a strong increase in the fluorescence intensity in the emission spectrum at 462 nm upon excitation at 409 nm (Fig. 2). Interestingly, no such effect was observed when **L** was treated with other cations (50.0  $\mu$ M). The probe exhibited high selectivity for  $Cd^{2+}$  over other metal ions.



To explore the possibility of using L as a practical ion selective fluorescent chemosensor for  $Cd^{2+}$ , competition experiments were carried out. For this purpose, L was treated with  $Cd^{2+}$  (50.0  $\mu$ M) in the presence of other metal ions (50.0  $\mu$ M) (Figure S3). The presence of some cations such as Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, or Ca<sup>2+</sup> had little interference with the fluorescence of Cd<sup>2+</sup>. And the responding for Cd<sup>2+</sup> in the presence of Ba<sup>2+</sup>, Hg<sup>2+</sup>, Pb<sup>2+</sup> or Zn<sup>2+</sup> were relatively low but clearly detectable. When Cd<sup>2+</sup> was added to the solution of L in the presence of Al<sup>3+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Mg<sup>2+</sup> or Mn<sup>2+</sup>, however, the fluorescence of the ligand was quenched or had a little enhancement that may be due to an energy or electron transfer [34]. As reported in the literature [35, 36], it is probably because the formed complex between these cations and L was too stable to be replaced by Cd<sup>2+</sup>.

As shown in Fig. 3, the free L (50.0  $\mu$ M,  $\lambda ex = 409$  nm) emitted weak fluorescence at 490 nm. Upon addition of 1.4 equiv. Cd<sup>2+</sup>, a strong fluorescence enhancement was observed, and the emission peak at 490 nm gradually blue shifted to 462 nm. And the stoichiometry of L-Cd<sup>2+</sup> complex was determined by changes in the fluorogenic response in the presence of varying Cd<sup>2+</sup> concentrations, and the results indicated the formation of a 1:1 complex.

#### **Binding Studies**

In order to determine the stoichiometry between L and Cd<sup>2+</sup>, the method of continuous variation (Job's plot) was used [37]. The total concentration of the L and Cd<sup>2+</sup> was constant (50.0  $\mu$ M), with a continuous variable molar fraction of guest ([L]/[L] + [Cd<sup>2+</sup>]). Figure 4 showed the Job plot of L with Cd<sup>2+</sup> (at 462 nm) and revealed that the L-Cd<sup>2+</sup> complex concentration approached a maximum when the molar fraction of Cd<sup>2+</sup> was 0.5, which meant L and Cd<sup>2+</sup> formed a 1:1 complex. In addition, the formation of a 1:1 complex between L and Cd<sup>2+</sup> was further conformed by the appearance of a peak at *m*/*z* 429.2, assignable to [L + Cd<sup>2+</sup> + C<sub>2</sub>H<sub>5</sub>OH - H]<sup>+</sup> in the ESI-MS spectra (Figure S4).

NMR studies provide additional evidence of the interaction between L and Cd<sup>2+</sup>. <sup>1</sup>H NMR spectra of L were recorded in DMSO- $d_6$  upon the addition of Cd<sup>2+</sup> or D<sub>2</sub>O. Significant



Fig. 7 Benesi-Hildebrand analysis of the emission changes for the complexation between L and  $Cd^{2\scriptscriptstyle +}$ 

spectral changes were observed as shown in Fig. 5. There were a number of small signals coming into being upon the addition of  $Cd^{2+}$ , such as the signal peaks of  $\delta$  9.32,  $\delta$  8.86,  $\delta$  8.12 and so on. And when a drop of D<sub>2</sub>O was added, the signal of the -NH- disappeared. These phenomena indicated that L had a proper structure to coordinate with  $Cd^{2+}$  by taking advantage of the C=N isomerization (Fig. 6) [38].

As shown in Fig. 7, the association constant  $K_a$  of the complex was then calculated to be  $8.9 \times 10^4 \text{ M}^{-1}$  with a linear relationship by Benesi-Hildebrand equation (Eq. (1)) [39], where  $F_{\text{max}}$ , F and  $F_{\text{min}}$  are fluorescence intensities of L in the presence of Cd<sup>2+</sup> at saturation, any intermediate Cd<sup>2+</sup> concentration, and free L.

$$\frac{1}{F - F_{min}} = \frac{1}{K(F_{max} - F_{min}) \left[ \text{Cd}^{2+} \right]} + \frac{1}{F_{max} - F_{min}} \tag{1}$$

The detection limit of **L** as a fluorescent sensor for the analysis of  $Cd^{2+}$  was determined from a plot of normalized fluorescence intensity as a function of the concentration of the added metal ions (Figure S5). According to the equation: detection limit =  $3\sigma/k$  [40], it was discovered that **L** had a detection limit of  $1.1 \times 10^{-6}$  M for  $Cd^{2+}$ .

Reversibility is a prerequisite in developing novel chemosensors for practical application. The reversibility of the recognition process of  $\mathbf{L}$  was performed by adding a



Fig. 6 Proposed binding mode for interaction of Cd<sup>2+</sup> with L

 $Cd^{2^+}$  bonding agent, Na<sub>2</sub>EDTA. The addition of Na<sub>2</sub>EDTA to a mixture of L and  $Cd^{2^+}$  resulted in diminution of the fluorescence intensity, which indicated the regeneration of the free chemosensor L (Figure S6). Such reversibility and regeneration are vital for the fabrication of devices to sense the  $Cd^{2^+}$ ions.

## Conclusion

In summary, we have developed a novel chemosensor L based on C=N isomerization mechanism. The probe exhibited high selectivity for  $Cd^{2+}$  over other metal ions with a strong fluorescence enhancement and high sensitivity with the detection limit reaching at  $10^{-6}$  M level in ethanol. This discovery of the dramatically enhanced fluorescence is probably due to the formation of a 1:1 complex L-Cd<sup>2+</sup> in which the rotation of acyclic C=N is frozen. Thus, we believe L has the ability to serve as a practical sensor for Cd<sup>2+</sup> detection in biological systems and environment. This strategy may provide a general way for designing new C=N isomerization sensors to detect other environmentally and biologically relevant species.

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