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Novel selective fluorescent probes for sensing Zn^{2+} ions based on a coumarin Schiff-base

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ABSTRACT

Two Zn^{2+} ion fluorescent probes based on a coumarin Schiff-base were designed and synthesized, which have highly selective sensing of Zn^{2+} ions over other metal ions in aqueous solution based on a C=N isomerization mechanism. Both the X-ray crystal structure of the zinc complex and the Job's plots showed a 1:1 probe-Zn^{2+} ion identification with high selectivity and sensitivity. The **Cp1** probe was also used to image intracellular Zn^{2+} ions in MCF-7 cells with a good performance.

Zinc is the second most abundant transition metal in the human body, and it plays a myriad of roles in various biochemical processes, such as enzyme regulation, structure and function, neural signal transmission, and gene expression.[1] Although the majority of biological zinc ions are tightly sequestered by proteins, the presence of 'free zinc' in certain cells may still be possible and is closely related to severe physiological diseases such as Alzheimer's, amyotrophic lateral sclerosis (ALS), Parkinson's, ischemia, and epilepsy.[2] Thus, considerable interest has focused on the development of specific methods of detection for Zn^{2+} ions.[3] Up to now, significant advances have been made in the design of fluorescent probes for Zn^{2+} ions.[4] However, most of these probes display interference from cadmium because zinc and cadmium are located in the same group of the periodic table, resulting in very similar photophysical changes of the probes.[3] The design and synthesis of fluorescent probes to clearly discriminate Zn^{2+} ions from Cd^{2+} ions have therefore aroused considerable interest.

Since coumarin derivatives exhibit several advantages such as large Stokes shifts, good photostability, and high fluorescence quantum yields, they could be used as efficient fluorophores. Consequently, in recent years, the design and synthesis of many coumarin-based fluorescent probes for the detection of anions[5], cations,[6] and neutral molecules[7] have been reported.

The C=N isomerization mechanism, a new fluorescent probe functioning mechanism, has been developed recently.[8] Specifically, the imine with an unfixed C=N structure is nonfluorescent because C=N isomerization is the predominant decay process in excited states. In contrast, complexation with metal ions restricts the rotation of the C=N bond and results in the suppression of C=N isomerization so that its fluorescence increases drastically. Moreover, studies have shown that probes for Zn^{2+} ions based on such a mechanism could achieve selective sensing of Zn^{2+} over Cd^{2+} ions.[9] Following this mechanism, to develop a simple, facile, and reliable Zn^{2+} ion probe with better sensitivity and selectivity, in the present Letter, we elaborately designed and synthesized two Zn^{2+} ions fluorescent probes, **Cp1** and **Cp2**, based on a coumarin Schiff-base (Scheme 1). Differing from **Cp1**, as the improver of **Cp1**, there is a methyl group adjacent to C=N in **Cp2**, which makes **Cp2**-Zn^{2+} have a better fluorescent property due to the electron-donating effect of the methyl group (Supplementary Fig. S1). Compared with other probes in literatures based on such a mechanism, the two probes have the advantages of easily available raw materials, simple synthetic route, good water solubility, strong affinity to Zn^{2+} ions, little cytotoxicity in cell culture experiment, especially highly selective sensing of Zn^{2+} ions over Cd^{2+} ions in aqueous solution.

Ethyl 7-hydroxycoumarin-3-carboxylate was formylated to obtain ethyl 7-hydroxy-8-formylcoumarin-3-carboxylate (**Cp3**). Then a condensation reaction between **Cp3** and aminomethyl benzimidazole was conducted to produce (*E*)-ethyl 8-(((1*H*-benzo-[*d*]imidazol-2-yl) methylimino)methyl)-7-hydroxycoumarin-3-carboxylate (**Cp1**)

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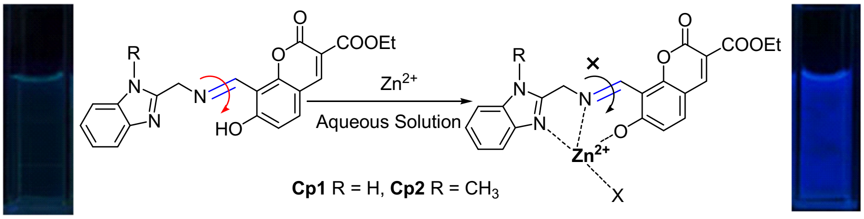
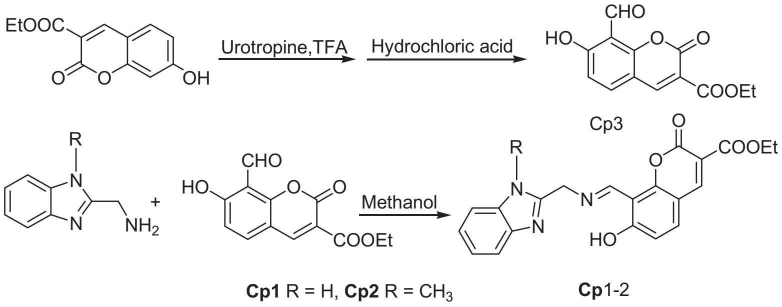


Scheme 1. Proposed sensing mechanism.



Scheme 2. Synthesis of **Cp1** and **Cp2**.

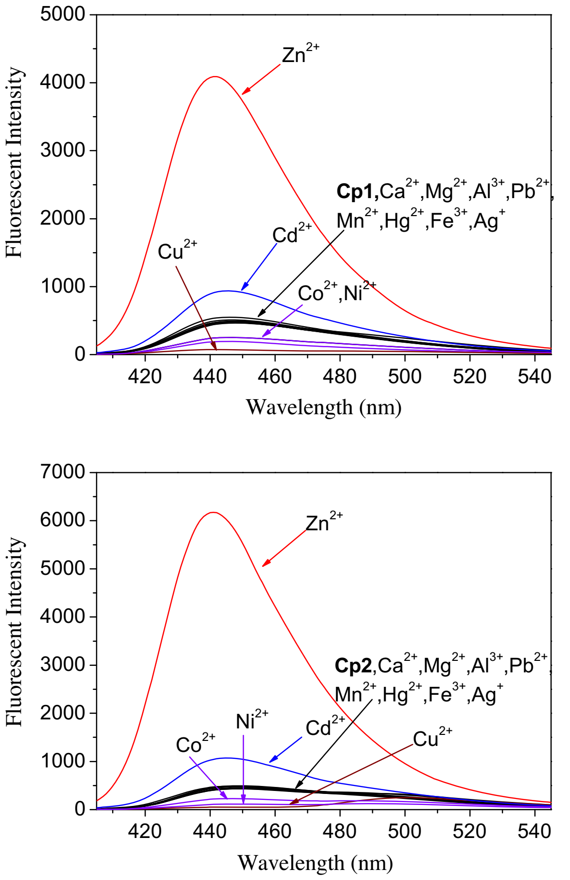


Figure 1. Fluorescence emission spectra of **Cp1** and **Cp2** (20 μM) in the presence of different ions such as Ca^{2+}, Mg^{2+}, Al^{3+}, Pb^{2+}, Mn^{2+}, Hg^{2+}, Fe^{3+}, Ag^{+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, and Zn^{2+} ion in Tris–HCl buffer (10 mM, pH 7.2). (The concentration of Zn^{2+} ions was 20 μM; that of all the other metal ions was 40 μM). The excitation was at 390 nm.

and (*E*)-ethyl 8-(((1methyl-1*H*-benzo-[*d*]imidazol-2-yl)methylimino)methyl)-7-hydroxycoumarin-3-carboxylate (**Cp2**) (Scheme 2).

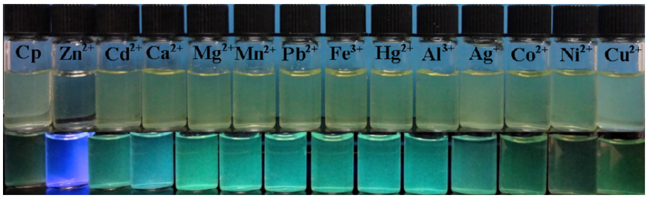


Figure 2. Photograph of **Cp1** (20 μM) upon the addition of metal ions (40 μM) in Tris–HCl buffer (10 mM, pH 7.2). The top image is with the naked eye, the bottom one is under irradiation at 365 nm.

Structure identification of **Cp1** and **Cp2** was confirmed by ^{1}H NMR, ^{13}C NMR, IR, HRMS spectroscopy, and X-ray crystal analysis (see Supplementary data).

To obtain insight into the fluorescent properties of **Cp1** and **Cp2** toward metal ions, the emission changes were investigated with different ions such as Ca^{2+}, Mg^{2+}, Al^{3+}, Pb^{2+}, Mn^{2+}, Hg^{2+}, Fe^{3+}, Ag^{+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} ion in Tris–HCl buffer (10 mM, pH 7.2). The emission spectra were investigated by exciting **Cp1** or **Cp2** at 390 nm (Fig. 1). From the emission spectra, it can be seen that there is a great enhancement in the fluorescence intensity of Zn^{2+} ions with both **Cp1** and **Cp2**, which could be clearly distinguished from the other metal ions. The emission spectra of the added Zn^{2+} ions showed a 5 nm blue shift: concomitantly, the maximum emissive wavelength shifted from 448 nm to 443 nm (Fig. 1). The prominent fluorescence changes of **Cp1** were also observable by a handgun UV lamp (Fig. 2). The weak yellow-green color that came from the solution of **Cp1** faded away only by the addition of Zn^{2+} ions. The bright blue fluorescence was only observed with **Cp1**-Zn^{2+}. The combined information of fluorescence and visible changes will be useful to discriminate Zn^{2+} ions from other metal ions.

In order to validate the high sensitivity of **Cp1** and **Cp2** to Zn^{2+} ions in practice, competition experiments were carried out by adding Zn^{2+} ions to the solution of **Cp1** or **Cp2** in the presence of Cd^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Pb^{2+}, Fe^{3+}, Hg^{2+}, Al^{3+}, Ag^{+}, Co^{2+}, Ni^{2+}, and Cu^{2+} ion (Fig. 3). It was observed that the presence of metal ions such as Cd^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Pb^{2+}, Fe^{3+}, Hg^{2+}, Al^{3+}, and Ag^{+} did not interfere with the Zn^{2+}-**Cp** enhanced fluorescence. However, the presence of Co^{2+}, Ni^{2+}, and Cu^{2+} ions totally quench the fluorescence,