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Research paper

A chromene pyrazoline derivatives fluorescent probe for Zn^{2+} detection in aqueous solution and living cells

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ABSTRACT

A new chromene pyrazoline derivatives fluorescent probe L was designed and synthesized. The probe L appears in a 55-fold fluorescence enhancement after 5 equiv. Zn^{2+} was added, and it also exhibits high sensitivity and selectivity for response to Zn^{2+} in ethanol-water (V:V = 1:1) solution through "OFF-ON" type process and a possible photoinduced electron transfer (PET). Notably, the probe L distinguishes between Zn^{2+} and Cd^{2+}. The association constant was considered as $2.38 \times 10^3\ M^{-1}$ via fluorescence titration experiments. The probe L-Zn^{2+} complex forms a 1:1 binding stoichiometry which was discussed by Job's plot. The probe is very highly sensitive with fluorometric detection limit of 1.603×10^{-10} M. It also shows good reversibility upon addition of EDTA. Furthermore, the viability of L to Zn^{2+} has practical application in live cell imaging.

1. Introduction

As we know, transition metal zinc is one of the most important in life system [1] and it is the second most rich transition metal element in the human body [2]. Zn^{2+} plays a crucial role in many biochemical processes such as cellular metabolism [3], muscle contraction [4], DNA-binding proteins [5], gene expression, apoptosis, enzyme regulation, immunity, metallo-enzyme function [6] and so forth. In pathology, Parkinson's disease [7], senile dementia [8], epilepsy disease [9], cerebral ischemia [10], diabetes [11], amyotrophic lateral sclerosis (ALS) [12], infantile diarrhea [13] and other diseases are related to the formation of Zn^{2+} and metabolic disorders. Therefore, it becomes very important to detect Zn^{2+} in both the environment and biological systems [14].

The development of fluorescent probes for Zn^{2+} detection has become a very active field in chemical biology. A lot of fluorescent probes for the detection and recognition of Zn^{2+} have been studied by various teams [15–19], but some of them can be applied only in organic solutions like acetonitrile toxic solvents, which restrict their potential applications, some of them have complex preparation process, inferior reversibility or selectivity [20–23]. In addition, some Zn^{2+} fluorescent probes display relatively low selectivity and suffer from the interferences from other metal ions, especially Cd^{2+} [24], which is the same group as Zn^{2+} in the periodic table and has similar binding properties with Zn^{2+} [25]. Therefore, similar fluorescence intensity changes and wavelength shifts are usually obtained when these two metal ions coordinate to the probe molecule respectively [26]. Thus, it is a great challenge to design and synthesize a fluorescent probe to sense and monitor Zn^{2+} with high selectivity and sensitivity in aqueous solutions [27].

In recent years, pyrazoline derivatives have drawn much attention because of their excellent blue fluorescence property, high fluorescence quantum yield, the rigid flat structure and high hole-transport efficiency [28–31].Chromene derivatives have been widely used as important intermediates in the synthesis of many natural products and medicinal agents. Many synthesized molecules based on the chromene ring system were found to be useful in antiproliferative activity [32,33]. Moreover chromone derivatives not only give fluorescence in the visible range but also cross the cell membrane very easily due to the lipophilic nature [34–38].

Chromene and pyrazoline have optical properties such as high fluorescence quantum yield, high light stability, large Stokes shift and non-toxicity. Taking all these into account, we have designed and synthesized a new compound L connecting pyrazoline ring and chromene ring. The L showed good selectivity and high sensitivity fluorescence response to Zn^{2+} over other metal ions,

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especially Cd^{2+} in ethanol-water (V:V = 1:1) solution. Hence, owing to the good selectivity, high sensitivity and complete reversibility for detection and recognition of Zn^{2+}, this probe L could be suitable for imaging in living cells. In contrast to previously reported Zn^{2+} fluorescent probes [39–42], the advantages of presenting new probe L are simple structure, easy synthesis, better fluorescence intensity enhancement, higher sensitivity and reversible.

2. Materials and methods

The materials used for this study were obtained from commercial suppliers and used without further purification. ^1H NMR and ^{13}C NMR spectrum were measured on the Bruker Avance 400 (400 MHz) spectrometer. Chemical shifts are reported in ppm using TMS as an internal standard. HR-ESI-MS were determined on a Bruker esquire 6000 spectrometer. UV–vis absorption spectrum were monitored with a UV-2700 spectrophotometer. Fluorescence spectrum were determined on a Hitachi F-7000 spectrophotometer equipped with quartz cuvettes of 1 cm path length. The melting point was determined on an XRC-1u Melting Point Apparatus.

Stock solution of L (1×10^{-2} M) was prepared in N, N-Dimethylformamide. Stock solutions of various metal ions (1×10^{-2} M) and EDTA (1×10^{-2} M) in distilled water were also prepared. All absorption and fluorescence emission spectrum were measured in a 1 cm optical path length quartz optical cell at room temperature. All fluorescence measurements were carried out upon excitation at 382 nm. Excitation and emission slit widths were 5.0 nm and 10.0 nm respectively.

BHK-21 cells were maintained in DMEM supplemented with 10% FBS at 37 °C under a humidified atmosphere containing 5% CO_2. Cells were plated on 18 mm glass coverslips and allowed to adhere for 24 h, treated with L (20 μM in cell culture medium), and incubated for 30 min. Subsequently, the cells were treated with Zn^{2+} (100 μM in cell culture medium). Cells were incubated for 30 min and rinsed with PBS three times to remove free compound and ions before analysis. Cells incubated with only 20 μM L for 30 min acted as a control. The cytotoxic activity experiment of the complex against BHK-21 cells was tested according to MTS assay procedures: BHK-21 cells were seeded into 96-well plates for 24 h. The different volume concentration of probe L was dissolved in DMSO make the final concentration, and diluted in culture medium at concentrations of 5, 10, 25, 50, 100 μM as working-solution and each concentration in quintuplicate, DMSO as a negative. After incubation for 24 h, the cells were added 10 μL solution of MTS in incubator for 4 h. After sufficient reaction with cells, the OD of each well was measured at the wavelength of 490 nm using a microplate spectrophotometer.

3. Experimental

The synthetic route of L (1-(3-phenyl-5-(2-phenyl-2H-chromen-3-yl)-4,5-dihydr o-1H-Pyrazol-1-yl)ethanone) was shown in Scheme 1. The probe is easy to synthesize in three steps. According to the literature [37], compound **3** readily prepared from compound **1** and **2** in 79% yield, A mixture of compound **3** (0.3384 g, 1.0 mmol) and 80% hydrazine hydrate (0.3065 g, 5.0 mmol) were taken in a 100 mL reaction flask in the presence of glacial acetic acid (15 mL) and refluxed at 120 °C for 6 h. After completion of reaction, it was cooled and poured into crushed ice. The resulting precipitate was filtered and recrystallized from ethanol to yield probe L. Pale yellow solid; Yield: 71%; mp: 216–219 °C. ^1H NMR (400 MHz, $CDCl_3$, TMS) (Fig. S1): δ_H ppm 9.98 (s, 1H), 7.40–7.27 (m, 5H), 7.17 (d, J = 5.2 Hz, 1H), 7.10–7.00 (m, 3H), 6.90 (m, 2H), 6.70–6.56 (m, 2H), 5.79 (s, 1H), 5.02 (dd, J = 7.8, 4.2 Hz, 1H), 3.45 (dd, J = 10.8, 7.8 Hz, 1H), 3.20 (dd, J = 10.8, 4.2 Hz, 1H), 1.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS) (Fig. S2): δ_c ppm 167.60, 157.52, 156.20, 151.66, 138.26, 133.27, 132.28, 129.77, 129.03, 128.59, 128.24, 127.64, 126.87, 121.80, 121.21, 120.80, 119.72, 117.05, 115.96, 114.73, 77.86, 56.81, 39.60, 21.32. HR-ESI-MS (Fig. S3) calculated for $[M-H]^+$ 409.1630, found 409.2733.

Compound **6** was prepared in using the same method with probe 4. White solid; yield: 81%; mp: 136–138 °C. ^1H NMR (400 MHz, $CDCl_3$, TMS) (Fig. S4): δ_H ppm 7.67 (d, J = 5.2 Hz, 2H), 7.58–7.20 (m, 8H), 7.14–6.98 (m, 2H), 6.85 (d, J = 4.4 Hz, 1H), 6.66 (d, J = 4.8 Hz, 1H), 6.57 (s, 1H), 5.87 (s, 1H), 5.00 (d, J = 5.2 Hz, 1H), 3.32 (dd, J = 8, 11.6 Hz, 1H), 3.06 (d, J = 11.6 Hz, 1H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS) (Fig. S5): 168.80, 153.92, 151.78, 138.59, 134.57, 131.10, 130.35, 129.55, 128.90, 128.73, 128.54, 127.80, 126.79, 126.45, 121.15, 121.11, 120.87, 115.93, 78.25, 58.07, 39.64, 21.36. HR-ESI-MS (Fig. S6) calculated for $[M+H]^+$ 395.1681, found 395.2558.

4. Results and discussion

4.1. Uv–vis studies of L to Zn^{2+}

The absorption spectral property of L toward different metal ions (Ag^+, Al^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Cu^{2+}, Cd^{2+}, K^+, Mg^{2+}, Na^+, Hg^{2+}, Zn^{2+}, Pb^{2+}, Li^+, Mn^{2+} all the metal ions solution was 5 equiv. of L got by dissolving their corresponding nitrate salts in H_2O) was measured in ethanol-water (V:V = 1:1). As shown in Fig. S7. L alone (10 μM) presents a broadband center at 280 nm and 320 nm. We also found that Ag^+, Al^{3+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, K^+, Mg^{2+}, Na^+, Hg^{2+}, Zn^{2+}, Pb^{2+}, Li^+, Mn^{2+} did not cause significant changes in absorption spectrums. In contrast, Cu^{2+} caused a new band at 350–430 nm and Fe^{3+} had considerable changes in absorption bands.

4.2. Fluorescence studies of L to Zn^{2+}

The fluorescence change of L with respective metal ions was monitored in ethanol-water (V:V = 1:1) solution. Among various metal ions (Ag^+, Al^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Cu^{2+}, Cd^{2+}, K^+, Mg^{2+}, Na^+, Hg^{2+}, Zn^{2+}, Pb^{2+}, Li^+ and Mn^{2+} all the metal ions solution was 5 equiv. of L), Zn^{2+} created almost 55-fold fluorescence enhancement at 471 nm(Fig. 1). And a small red shift with fluorescence enhancement was observed. The change in spectral wavelength from 441 nm to 471 nm is caused by restricted C=N isomerization mechanism and an inhibition of photo-induced electron transfer (PET) process [43,44].

Furthermore, competition experiments for other metal ions in the L-Zn^{2+} were conducted in the same condition. As displayed in Fig. 2. Hg^{2+} and Pb^{2+} can partly quench fluorescence of L-Zn^{2+}, whereas Al^{3+}, Fe^{3+} and Cu^{2+} completely quenched fluorescence of L-Zn^{2+}. This may be attributed to the paramagnetic properties of these three metal ions and fluorescence quenching was observed when complex with some paramagnetic metal ions, such as Fe^{3+} and Cu^{2+}, are always encountered in other metal ion probes [45–47]. Thus, when they were bound to probes, the emission would be strongly quenched by a photoinduced metal into fluorophore electron or energy transfer mechanism [48–51]. Most of metal ions, including Ag^+, Co^{2+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, K^+, Mg^{2+}, Na^+, Hg^{2+}, Pb^{2+}, Li^+ and Mn^{2+} show a very negligible effect, and Al^{3+}, Cu^{2+} and Fe^{3+}could quench the fluorescence, which was often encountered in other probes. This is limited to the application of probe L in complicated environment samples. However, it is surprising that L-Zn^{2+} complex eliminated the influence of Cd^{2+} by blocking PET and restricting mechanism of C=N isomerization. These results show that L strongly coordinates with Zn^{2+} which