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A new high selective and sensitive turn-on fluorescent and ratiometric absorption chemosensor for Cu^{2+} based on benzimidazole in aqueous solution and its application in live cell

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ABSTRACT

A new benzimidazole base turn-on fluorescent and ratiometric absorption chemosensor (L) bearing bidentate ligand for detection of Cu^{2+} was designed and synthesized. Fluorescence and UV–vis spectra studies demonstrated that L can detect Cu^{2+} ions in aqueous solution using fluorescence enhancement and ratiometric absorption sensing over a wide pH range. Both fluorescent and ratiometric absorption sensing of L for Cu^{2+} possessed high selectivity and sensitivity over other competitive metal ions and had low detection limit. Job's plot, mass spectra and DFT calculation indicated the sensing mechanism is the complex formation between L and Cu^{2+} with 1:2 stoichiometry. Fluorescence images of HepG2 in the absence and presence of Cu^{2+} displayed L had cell permeability and detection ability for Cu^{2+} in live cells.

1. Introduction

Fluorescent chemosensors for sensing and recognition of heavy and transition metal ions in environment and organisms have many advantages such as real-time monitoring with fast response time, intrinsic high selectivity and sensitivity, ease of handling and low-cost [1–6]. Among metal ions, Cu^{2+} ion plays a critical role in many biochemical process, such as enzyme catalysis, cellular metabolism and oxygen transportation [7]. However, overloading Cu^{2+} in living body, especially in neuronal cytoplasm can cause some serious diseases, such as prion disease, several neurodegenerative diseases including Menkes, Wilson, Alzheimer and Parkinson and others [8–11]. Thus monitoring the concentration of Cu^{2+} in environment and living body is necessary. In recent years, considerable efforts have been devoted to the development of high sensitive and selective fluorescent chemosensors for recognition of Cu^{2+} [2,12–17]. Most of the reported fluorescent chemosensors for Cu^{2+} is fluorescence “on-off” mode due to the paramagnetic property of Cu^{2+} ions after complex formation between chemosensor and metal ion [12–14,18,19]. Thus the recognition ability of fluorescent chemosensors for Cu^{2+} ions is easy affected by the other miscellaneous substances in practical applications. On the contrary, the “off-on” fluorescent chemosensors displayed more high sensitivity and can alleviate this influence by the significant fluorescence enhancement upon analyte recognition [15–17,20,21]. Although some “off-on” fluorescent chemosensors have been developed [22,23], some fluorescent chemosensor can only be applied in non-aqueous solvents which limit their application in biological systems. In addition, the fluorescence enhancement signal in most case is still too weak and often suffers from a high background signal. So it is still imperative to develop “off-on” fluorescent chemosensors with high selectivity and sensitivity for detection of Cu^{2+} ion in aqueous solution.

Furthermore, ratiometric chemosensors can be used to evaluate the analyte concentration via the measurement of the ration change of the two absorption/emission intensities and present some advantages. Ratiometric can provide a built-in correction for environmental effects such as photo-bleaching, sensor molecule concentration and the environmental factors around the sensor molecule (such as pH, polarity and temperature, etc.). Although some ratiometric fluorescent chemosensors for Cu^{2+} ions have been reported [24,25], ratiometric absorption chemosensors are still very rare. Therefore, development of more accurate and sensitive ratiometric absorption chemosensors for Cu^{2+} ions is also necessary.

Benzimidazole derivatives possess interesting optical and electronic properties, and often used as fluorescent emitting materials [26,27]. Many benzimidazole-containing compounds have been reported to be used as fluorescent chemosensors for selective detection of metal cations and anions [28,29].

Schiff bases, condensation of reactive aldehydes and amines, are important compounds with various potential applications. Some of Schiff base derivatives had been used as coordination unit in fluorescent chemosensors [30–33]. The bridged $C{=}N$ structure of Schiff bases easily

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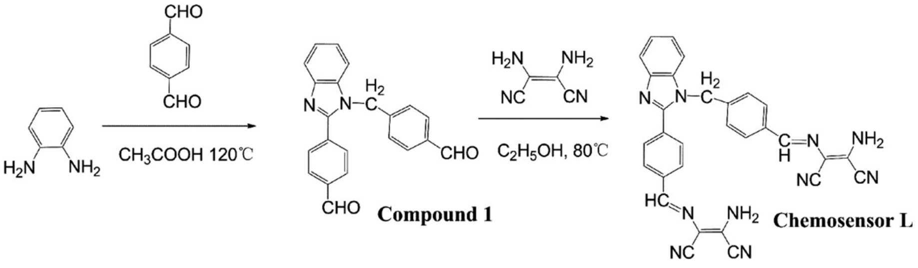

Scheme 1. Synthesis of chemosensor L.

isomerize in the excited state and tend to exhibit very weak fluorescence. But when they coordinate with some special metal ions, the C═N isomerization is inhibited, and then Schiff bases show strong fluorescence [34,35]. Therefore, the fluorescent chemosensors containing Schiff base unit often display fluorescence "off-on" performance upon the complex effect of metal ions.

In this work, we describe the synthesis and ion recognition properties of a new benzimidazole derivative with bidentate diaminomaleonitrile ligands (sensor L), the connect units are Schiff bases between benzimidazole and diaminomaleonitrile (Scheme 1). Sensor L not only exhibited fluorescent and ratiometric absorption recognition ability for Cu^{2+} in aqueous solution with high selectivity and sensitivity but also displayed the detection ability for Cu^{2+} ions in living cells.

2. Experimental

2.1. Instruments and Materials

All the solvents and reagents were of analytical grade from commercial suppliers and used without further purification. All chromatographic examinations were performed using silica gel, and TLC was performed on silica plates (made in China). ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Unity Inova Spectrometer (500 MHz and 600 MHz) at room temperature using DMSO d_6 as solvent. ESI-MS mass spectra were obtained on an agilent 1200 HPLC/Micro TOF II mass spectrometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. The absorbance spectra were examined using a Varian Cary 500 UV–vis spectrophotometer. Fluorescence microscope images were acquired on a Olympus FV-1000 Confocal Laser Scanning Microscope. All the pH values were measured with a METTLER TOLEDO FE20 pH meter. Deionized water was used throughout all experiments. The salts used in stock solutions of metal ions were $Cu(NO_3)_2\cdot3H_2O$, $Ni(NO_3)_2\cdot6H_2O$, $Al(NO_3)_3\cdot9H_2O$, $Zn(NO_3)_2\cdot6H_2O$, $Cr(NO_3)_3\cdot9H_2O$, $Cd(NO_3)_2\cdot4H_2O$, $Fe(NO_3)_3\cdot9H_2O$, $Co(NO_3)_2\cdot6H_2O$, $Mg(NO_3)_2\cdot6H_2O$, KNO_3, $AgNO_3$, $BaCl_2\cdot2H_2O$, $MnCl_2$, $HgCl_2$, $PbCl_2$, $CaCl_2$, $SrSO_4$ and $FeSO_4\cdot7H_2O$.

2.2. Synthesis

2.2.1. Synthesis of 4-(1-(4-Formylbenzyl)-1H-benzo[d]imidazol-2-yl) benzaldehyde (Compound 1)

Compound 1 was prepared by the reported method [36] with a slight modification. *o*-Phenylenediamine (1.08 g, 10 mmol), terephthalaldehyde (3.35 g, 25 mmol) and glacial acetic acid (100 mL) were mixed in a 250 mL flask and refluxed for 1 h. Then the reacting solution was cooled to room temperature and then adjusted to pH 5.0 with $NaHCO_3$ aqueous solution. The resulting yellow solid was collected by filtration and washed with water. This yellow solid was further dried and purified by silica gel column chromatography using dichloromethane/ethyl acetate (15:1, v/v) as eluent to afford the pure product (1.21 g, 35.6%). ^1H NMR (500 MHz, DMSO) δ 10.1 (s, 1H), 9.93 (s, 1H), 8.03 (d, $J = 8.4$ Hz, 2H), 7.96 (d, $J = 8.2$ Hz, 2H), 7.83 (d, $J = 8.2$ Hz, 2H), 7.78 (s, 1H), 7.55 (s, 1H), 7.33–7.28 (m, 2H), 7.20 (d, $J = 8.0$ Hz, 2H), 5.77 (s, 2H).

2.2.2. Synthesis of Chemosensor L

Compound 1 (1.21 g, 3.6 mmol) and diaminomaleonitrile (0.78 g, 7.2 mmol) were dissolved and refluxed in ethanol (25 mL) for 12 h. Then the reacting solution was cooled and filtrated to get yellow solid. This yellow solid was further purified by silica gel column chromatography, using dichloromethane/ethanol (54:1, v/v) as eluent to afford the desired product (0.58 g, 31%). ^1H NMR (600 MHz, DMSO) δ 8.33 (s, 1H), 8.22–8.16 (m, 3H), 8.08 (s, 2H), 7.98–7.89 (m, 4H), 7.82 (d, $J = 8.4$ Hz, 2H), 7.80–7.75 (m, 1H), 7.56 (dd, $J = 5.8$, 3.3 Hz, 1H), 7.33–7.26 (m, 2H), 7.09 (d, $J = 8.3$ Hz, 2H), 5.71 (s, 2H). ^{13}C NMR (151 MHz, DMSO) δ (ppm):154.76, 154.30, 152.97, 143.17, 140.85, 137.10, 136.63, 135.35, 132.83, 129.89, 129.68, 128.00, 127.51, 126.93, 123.61, 123.02, 119.94, 114.77, 114.13, 111.56, 102.96, 48.01. HRMS: *m*/*z*: found [M + H]. 521.1951; molecular formula $C_{30}H_{21}N_{10}$, calcd. [M + H] 521.5680.

2.3. Solution Preparation Process for Fluorescence and Ultraviolet Spectra

Stock solutions of the salts were prepared in distilled water. Chemosensor L was dissolved in CH_3CN-Tris (20 mM, pH = 7.2, 1:1 v/v) solution to prepare the stock solution. Test solutions were prepared by adding 50 μL of each metal stock solution (5 mM) into 5 mL of the L stock solution for selective and competitive experiments. In titration experiment, the Cu^{2+} stock solutions with different concentrations were used, the whole added volume of the Cu^{2+} stock solution was less than 2% volume of L solution in case that the concentration of L was obviously influenced. The test solutions for Job's plot were prepared by mixing different volumes of L stock solution (100 μM) with different volumes of Cu^{2+} stock solution (100 μM) in CH_3CN-Tris (20 mM, pH = 7.2, 1:1, v/v) and fixing the mixed solution volume to 10 mL. pH of the test solutions were

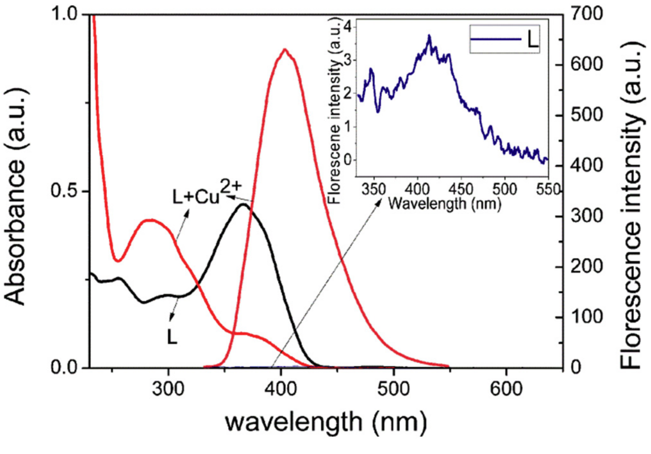

Fig. 1. Absorbance and fluorescence spectra of L (10 μM) in CH_3CN-Tris (20 mM, pH = 7.2, 1:1, v/v) solutions before and after addition of 5 equiv. Cu^{2+}. For fluorescence spectra, the excitation wavelength was 310 nm.