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SHORT COMMUNICATION

A Turn-on and Reversible Fluorescence Sensor for Zinc Ion Based on 4,5-Diazafluorene Schiff Base

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Abstract A new 4,5-diazafluorene-based fluorescent chemosensor has been synthesized by Schiff base condensation of 9,9-bis(3,5-dimethyl-4-aminophenyl)-4,5-diazafluorene with salicylaldehyde. The interaction of Schiff base with different metal ions has been studied over photofluorescent spectra. The results showed that Schiff base exhibited 194-fold enhancements in fluorescence at 465 nm after Zn^{2+} ions. Such fluorescent responses could be detected by naked eye under UV-lamp. The complex solution (**L**-Zn^{2+}) exhibited reversibility with EDTA.

Keywords Fluorescent sensor · Zn^{2+} ion · Schiff base · Turn-on

Introduction

In recent years, fluorescent chemosensors have attracted significant interest because of the simplicity and high sensitivity of fluorescence assays. Among various metal ions, fluorescent chemosensors for zinc ion have received wide attention in the past few decades due to the indispensable role of zinc ions to life [1–7]. Zinc is the second most abundant transition metal ion in the human body after iron. Zn^{2+} is now recognized as one of the most important cations in catalytic centers and structural cofactors of many Zn^{2+}-containing enzymes and DNA-binding proteins. Zinc is believed to be an essential factor in many biological processes such as brain function and pathology, gene transcription, immune function, and mammalian reproduction, as well as it is involved in the pathological processes in many diseases including Alzheimer's disease [8], epilepsy [9] and ischemic stroke [10]. Since there is a close association between Zn^{2+} and human health, developing fluorescent chemosensors with high selectivity and sensitivity for detecting trace amounts of Zn^{2+} has attracted increasing attention. In the past several years, considerable efforts have been made to develop fluorescent chemosensors for Zn^{2+} ions. However, most of them have disadvantages such as insufficient selectivity or sensitivity, or interference problems from other transition metal ions, especially Cd^{2+} which is in the same group of the periodic table and shows similar properties to Zn^{2+} [11, 12]. Furthermore, some of them often require laborious multistep organic synthesis [13, 14], which renders their discovery processes slow and caused prohibitively high cost. Therefore, for practical applications, it is still desirable to develop simple Zn^{2+} sensors with good selectivity and sensitivity. Schiff bases (imines) are known to be good ligands for metal ions [15–18]. In addition, Schiff base derivatives incorporating a fluorescent moiety are appealing tools for optical sensing of metal ions [19–21]. The 4,5-diazafluorene derivatives have been chosen as an ideal component of a fluorescent chemosensor due to its large π-system and bidentate coordination ability with various transition [22]. These compounds have similarities to 2,2′-bipyridyl and 1,10-phenanthroline derivatives. Furthermore, these compounds can form various complexes by coordination to metals such as Fe, Cu, Ag, Eu, and Ru. Only very few Schiff bases containing

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4,5-diazafluorene units have been synthesized. The present study is aimed to serve these purposes to develop the specific sensory materials for Zn^{2+} ion detections with optical turn-on responses.

Herein, we report the synthesis and characterization of a Schiff base ligand (**L**) as Zn^{2+} turn-on sensors. The interaction of Schiff base with different metal ions has been studied over photofluorescent spectra.

Experiment

Materials and Instrumentation

The reactants 4,5-diazafluoren-9-one (1) [23] was prepared according to the literature. 2,6-xylidine (Tianjin Guangfu) was purified by distillation prior to use. All organic solvents were of analytical reagent grade. Nitrate and chloride salt of all cations used were of high purity and used without any purification.

The ^1H NMR and ^{13}C NMR spectra were measured on a JEOL EX-400 spectrometer using Acetone-d_6 or DMSO-d_6 as solvent and tetramethylsilane as the internal reference. UV-vis spectra were examined on a Pgeneral TU-1810 spectrophotometer and fluorescent recorded on a F96PRO spectrofluorophotometer with excitation slit at 10.0 nm and emission at 20.0 nm.

The stock solution for **L** were prepared in DMSO (5 mM), stock solution for nitrate and chloride salt of different metals of Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} were prepared in water. All the metal ion solutions for the study were prepared in distilled water.

The binding constants of the inclusion complex were obtained from the fluorescence titration data. According to the Benesi–Hildebrand method, the equation for a 1: 2 host: guest complex is given below:

$$\log\frac{F-F_{\min}}{F_{\max}-F} = \log[M]^2-\log K_d$$

In this equation K_d is the dissociation constant and different fluorescence emission intensity $F_{\min}$, F and $F_{\max}$ are the find out at $\lambda_{\max} = 465$ nm, for the complex at initial, interval t, and the final state of complex. Concentrations of metal represent by M and association constant (K_a) was determined by $K_a = 1/K_d$.

The detection limits (DLs) were calculated using the following equation:

$$DL = \frac{3\sigma}{S}$$

where σ is the standard deviation of the blank measurement, which is 0.02 here, and S is the binding constant.

Monomer Synthesis

Synthesis of 9,9-Bis(3,5-Dimethyl-4-Aminophenyl)-4,5-Diazafluorene (2)

9,9-bis(3,5-dimethyl-4-aminophenyl)-4,5-diazafluorene was synthesized by accordingly to describe the method in Scheme 1. Trifluoromethanesulfonic acid (4.08 g, 27.2 mmol) was slowly added to the 2,6-xylidine (16.9 g, 139.49 mmol) was placed in a 100 ml three-necked flask with magnetic stirring and a nitrogen inlet. Then, 4,5-diazafluoren-9-one (3.62 g, 19.92 mmol) was added. The mixture was the heated to 165 °C for 24 h under an atmosphere of nitrogen with continuous stirring. The solution was cooled below 80 °C followed by neutralization with a solution of 5 % sodium hydroxide. The precipitate was filtered and washed with water. The crude product was purified by recrystallization to give a white powder (1.1 g, yield: 12 %), M.p.: 314–316 °C.

IR (KBr, cm^{-1}): 3472–3380 (N-H), 1519 cm^{-1} (C = N); ^1H NMR (400 MHz, Acetone-d_6, δ, ppm, TMS, Fig. 1): 2.02 (*s*, 12H, $-CH_3$), 4.18 (*s*, 4H, $-NH_2$), 6.71 (*s*, H^4, 4H), 7.31 (*dd*, H^9, 2H), 7.85 (*dd*, H^8, 2H), 8.61 (*dd*, H^{10}, 2H); ^{13}C NMR (100 MHz, Acetone-d_6, δ, ppm, TMS, Fig. 1): 18.0 (C^2), 60.8 (C^6), 121.6 (C^9), 123.9 (C^4), 128.2 (C^3), 133.0 (C^7), 134.5 (C^8), 143.8 (C^5), 148.5 (C^1), 150.0 (C^{10}), 158.4 (C^{11}).

Synthesis of 4,5-Diazafluorene Schiff Bases (L)

9,9-bis(3,5-dimethyl-4-aminophenyl)-4,5-diazafluorene (0.121 g, 0.3 mmol) was placed into a 100 ml round-bottom flask which was fitted with condenser, thermometer and magnetic stirrer. methanol (50 ml) was added into the flask and reaction mixture was heated up to 80 °C. A solution of salicylaldehyde (0.109 g, 1.2 mol) in 20 ml methanol was added into the flask. Reactions were maintained for 24 h under reflux. The precipitated monomers was filtered hot and dried in a vacuum desiccator. (0.14 g, yield: 95.4 %). ^1H NMR (DMSO-d_6, δ, ppm, TMS, Fig. 2): 8.72 (s, H^7, 1H), 8.59 (s, H^{17}, 1H), 8.09 (s, H^{15}, 1H), 7.58 (s, H^{16}, 1H), 7.41–7.46 (m, H^3, H^4, 2H), 6.92–6.97 (m, H^{11}, H^2, H^5, 4H), 3.35 (s, −OH), 2.04 (s, $-CH_3$). ^{13}C NMR (DMSO-d_6, δ, ppm, TMS, Fig. 2): 163.3 (C^1), 160.4 (C^7), 156.7 (C^{18}), 150.0 (C^8), 146.2 (C^{17}), 142.0 (C^{12}), 134.2 (C^{15}), 133.4 (C^3), 132.6 (C^{10}), 129.4 (C^5), 126.4 (C^{11}), 124.0 (C^4), 119.1 (C^{16}), 118.4 (C^6), 116.6 (C^2), 60.2 (C^{13}), 18.0 (C^9).

Result and Discussion

In order to perform the fluorescence characterization of the **L**, the emission spectra of **L** in the presence of 1.0 equiv. of Zn^{2+} were recorded in different solvents. As shown in Fig. 3, on the addition of 1.0 equiv. of Zn^{2+}, the fluorescence intensity of L increased in EtOH and MeOH, there was no obvious change in THF, CH_3CN

Scheme 1 Synthesis of the Schiff base **L**

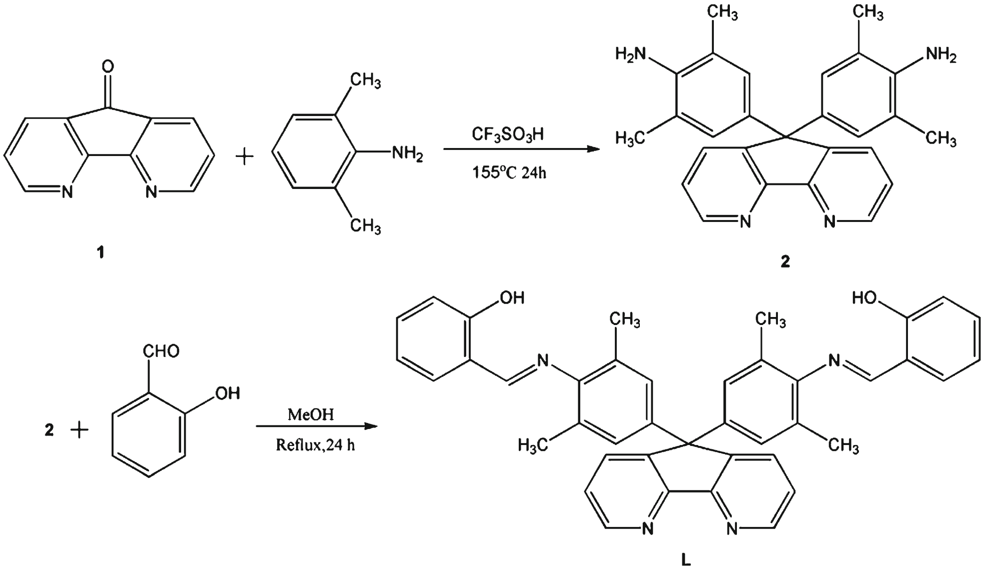


Fig. 1 ^{1}H NMR and ^{13}C NMR spectra of compound **2**

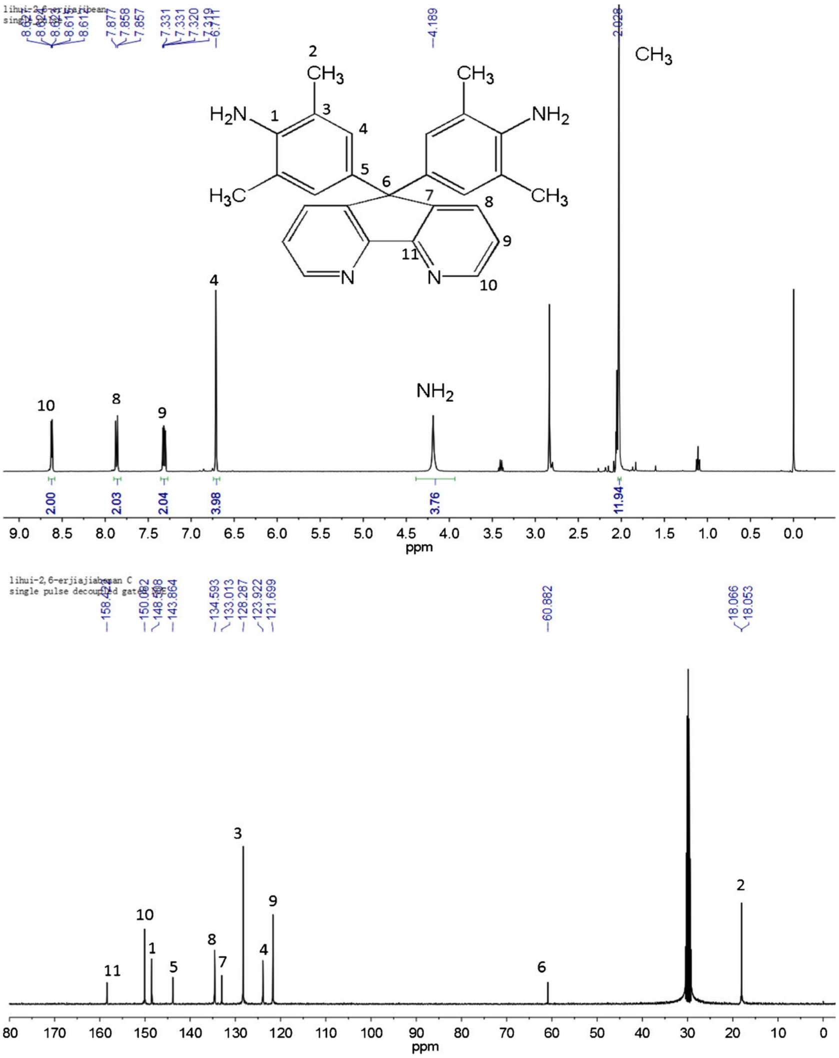
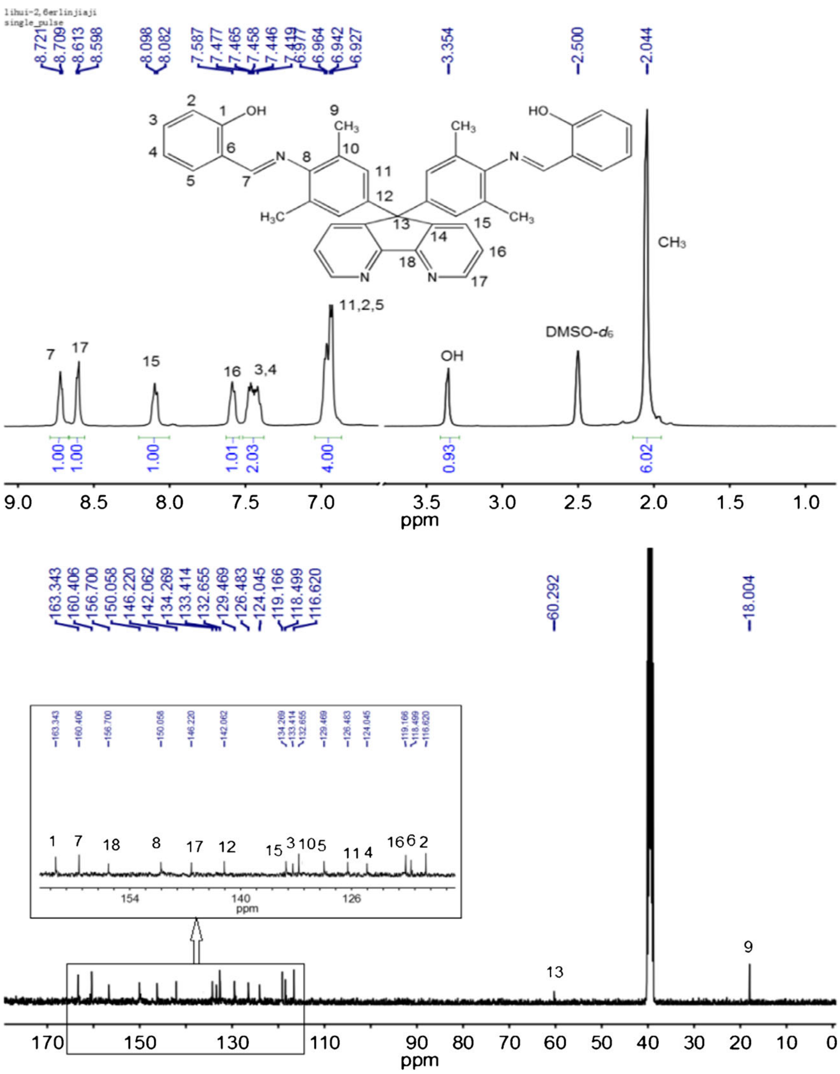


Fig. 2 ^1H NMR and ^{13}C NMR spectra of Schiff base **L**



and DMSO. Since the highest fluorescence intensity of **L** was observed in EtOH, so the EtOH was choose as the test solvent.

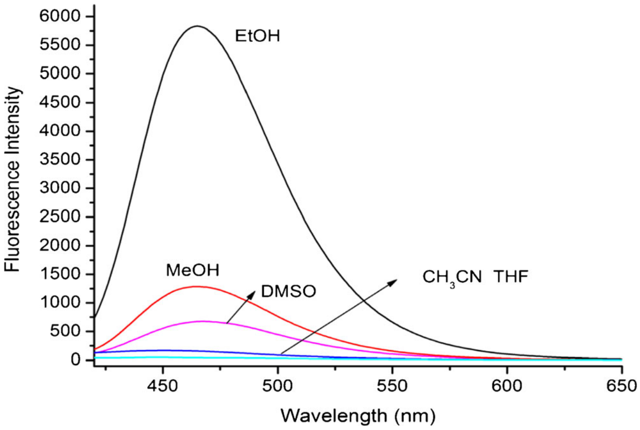


Fig. 3 The fluorescence spectra of the **L** (30 μM) in the presence of 1.0 equiv. of Zn^{2+} in different solvents. The inset depicts the changes of PL intensity upon the addition of Zn^{2+}. Excitation wavelength (nm): 394

The chemosensor behavior of receptor **L** with the following 16 metal ions: Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} in EtOH was investigated by flourescence measurements. As seen in Fig. 4a, receptor **L** alone and other cations all displayed a very weak single flourescence emission band at 465 nm when it was excitied at 394 nm except for Zn^{2+}. Adding 1.0 equivalents of Zn^{2+} ions, the fluorescence intensity of **L** (30 μM) increased rapidly. The fluorescence enhancement efficiency observed at 465 nm was 194-fold greater than the control in the absence of Zn^{2+}. Based on the use of a UV lamp, the solution of receptor **L** showed a dramatic color change from colorless to fluorescent blue in the presence of Zn^{2+} ion, which could easily be detected by the naked-eye (Fig. 4b). Fluorescence emission enhancement of **L** upon binding with Zn^{2+} is considered to be due to the formation of **L**-Zn^{2+} coordination complex. In addition, receptor **L** has an intramolecular hydrogen bond between the phenolic O-H and the nitrogen of the imine that undergoes excited state intramolecular proton transfer (ESIPT) [24, 25]. The receptor **L**

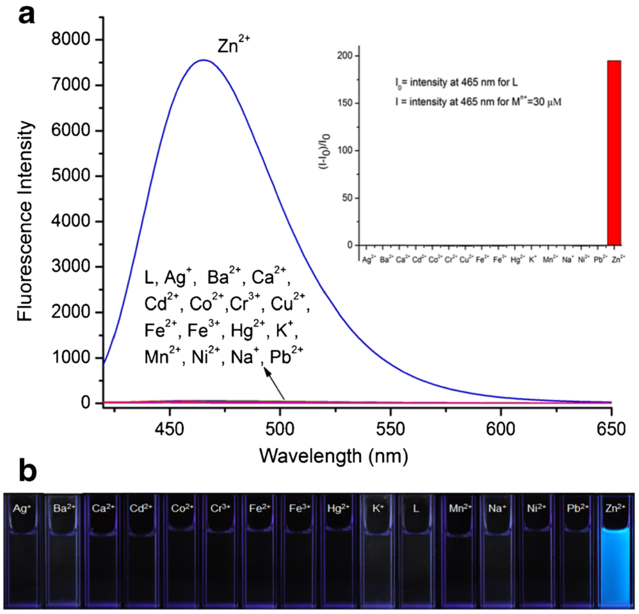


Fig. 4 **a** The fluorescence spectra of the **L** (30 μM) in the presence of 1.0 equiv. of various metal ions in ethanol.Excitation wavelength (nm): 394. Inset: fluorescence emission response profiles for **L** (30 μM) by adding various metal ions (30 μM) in ethanol. **b** visual fluorescence emissions of sensor **L** after addition of Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} (1 equiv.) in ethanol on excitation at 365 nm using UV lamp at room temperature

toward various metal cations, Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+} and Zn^{2+} are also investigated by UV-vis spectroscopy in ethanol. Interaction of receptor **L** with 1.0 equiv. of various metal ions don't result any significant changes in the absorption spectrum except Cr^{3+}, Cu^{2+} and Fe^{3+} (Fig. S1).

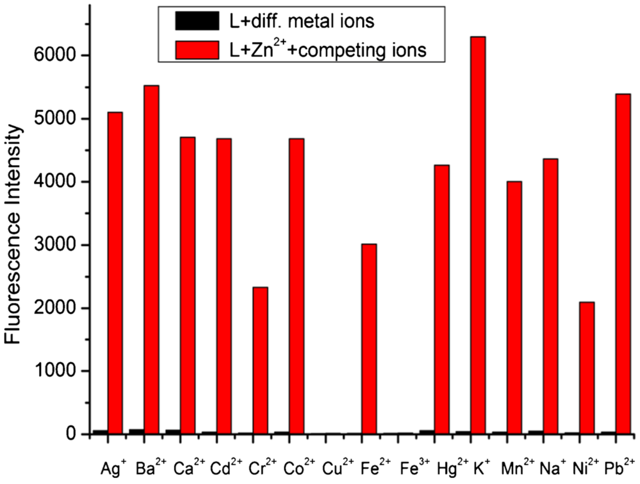


Fig. 5 Fluorescence response of receptor **L** (30 μM). The black bar represents emission intensity after adding 30 μM selected metal ions (Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, , Mn^{2+}, Na^+, Ni^{2+}, and Pb^{2+}) in ethanol solution and red bar represent emission intensity after adding 30 μM of Zn^{2+} ions in each of the above samples. Excitation wavelength (nm): 394

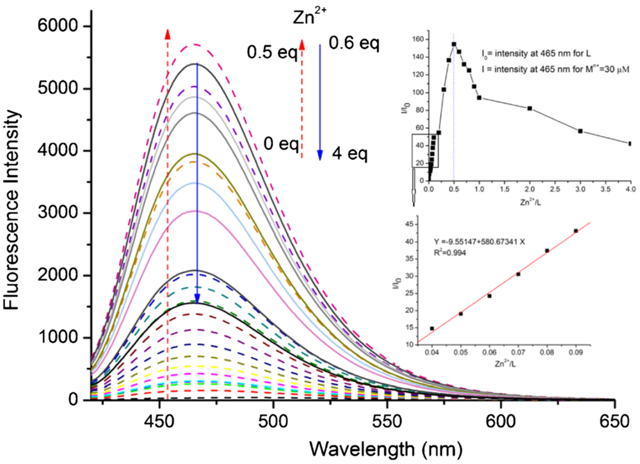


Fig. 6 Fluorescence emission spectra of receptor **L** (30 μM) in ethanol solution upon addition of increasing concentration of Zn^{2+}. Inset: fluorescence intensity at 465 nm versus the number of equivalents of Zn^{2+} added. Excitation wavelength (nm): 394

In order to establish the specific selectivity of **L** to Zn^{2+}, we performed the single and dual metal competitive analysis, as shown in Fig. 5. In a single metal system (black bars), all the metal ions (Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mn^{2+}, Na^+, Ni^{2+} and Pb^{2+}) concentrations were kept as 30 μM towards **L**. From the bar diagram (Fig. 5), the PL intensity of the **L** shows no obvious changes except for Cu^{2+} and Fe^{3+}. It is worth noting that the receptor **L** for Zn^{2+} did not have any interference from Cd^{2+} ion, due to Cd^{2+} and Zn^{2+} have similar properties and generally cause a strong interference when they are placed in solution together, which makes it difficult to distinguish each other [24]. Thus, the receptor **L** has displayed a considerable ability to distinguish Zn^{2+} and Cd^{2+} in a common solution.

To further investigate the chemosensing properties of receptor **L**, fluorescence titration of receptor **L** with Zn^{2+} was performed. Upon increasing the concentration of Zn^{2+} ions (0–0.5

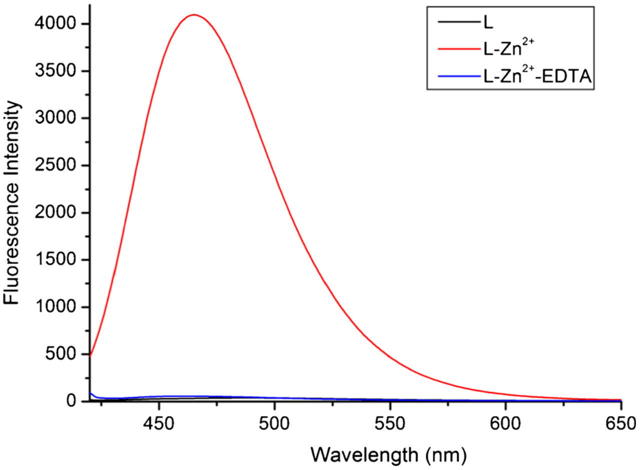


Fig. 7 Fluorescence emission spectra of receptor **L** (30 μM) in the presence of Zn^{2+} ions (30 μM) or EDTA (30 μM) in ethanol. Excitation wavelength (nm): 394

equiv.) a remarkable increase in emission intensity was observed at 465 nm (Fig. 6), further additions of Zn^{2+} ions (0.6–4.0 equiv.) result in the emission at 465 nm undergoes significant fluorescence decrease (Fig. 6). These results indicate that there is a resonance energy transfer mechanism operating between the 4,5-diazafluorene moiety and Schiff base moiety on addition of Zn^{2+} ions in ethanol. The formation of a rigid structure between imine and Zn^{2+} ion inhibits the C = N isomerization, leading to fluorescence enhancement. As the concentration of Zn^{2+} ions increased, the quenching mechanism of Zn^{2+} should be an intramolecular charge transfer (ICT) effect. A Job plot indicated a 1:2 stoichiometric complexation of receptor **L** with Zn^{2+} (Fig. S2). From the fluorescent titration data, the dissociation constants of **L**-Zn^{2+} was calculated to be 2.5×10^3 M^{-1} (Fig. S3). Moreover, the detection limits was also measured to be 5 ppm.

Furthermore, the reversibility nature of chemosensor **L** was measured by the titration of EDTA with fluorescent probes (**L** + Zn^{2+}), results are shown (Fig. 7) that after addition of EDTA, fluorescence emission intensity due to **L**-Zn^{2+} probe returened to lower level for chemosensor **L** indicate regeneration of free chemosensors **L**, quenching in fluorescent intensity stop after adding 10 equiv. EDTA.

Conclusion

In conclusion, a 4,5-diazafluorene based Schiff-base as metal binding site has been synthesized. The receptor **L** have turn on fluorescent behavior with Zn^{2+} ions in EtOH. More importantly, Fluorescence titration studies of Schiff base with Zn^{2+} in ethanol showed a remarkable enhancement in emission intensity was observed Zn^{2+} ions (0–0.5 equiv.) at 465 nm, further additions of Zn^{2+} ions (0.6–4.0 equiv.) resulted in the emission at 465 nm undergoes significant fluorescence decrease. Such fluorescent responses could be detected by naked eye under UV-lamp. The complex solution (**L**-Zn^{2+}) exhibited reversibility with EDTA.

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