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A COLORIMETRIC CHEMOSENSOR BASED ON FLUORESCEIN FOR THE DETECTION OF Zn^{2+} IN AQUEOUS SOLUTION

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Abstract – A novel quinoline-functionalized fluorescein derivative **HL** was designed and used as a colorimetric chemosensor for the detection of Zn^{2+} over other commonly coexistent metal ions in aqueous solution at pH 7.2. Studies on its binding with different metal ions revealed a noticeable naked eye color change in the presence of Zn^{2+}. The mechanism has been supported by Job's plot evaluation, MS and ^1H NMR spectroscopic studies. The association constant and the detection limits of sensor **HL** to Zn^{2+} were determined as 1.17×10^5 M^{-1} and 5.7 nM. This excellent selectivity and sensitive of **HL** to Zn^{2+} exhibited its potential application value in the biological monitoring and tracking of Zn^{2+}.

INTRODUCTION

The development of the fluorescent probe with high selectivity, sensitivity for metal ions has attracted more and more attention because of their potential applications in medicinal and environmental research.[1-4] Zn^{2+} is the second most abundant transition metal ion in the human body after iron, and it plays a critical role in enzyme regulation structure and function, neural signal transmission and gene expression.[5-10] Furthermore, deficiency of Zn^{2+} may lead to many diseases ranging from Alzheimer's disease to prostate cancer and diabetes.[11,12] A variety of work has been focused on the design of chemosensors to determine the "free" zinc ions so as to understand their biological roles. Zn^{2+} does not give any spectroscopic or magnetic signals originating from its $3d^{10}4s^0$ electronic configuration, thus the fluorescence method stands out as an effective choice.[13] For detection of Zn^{2+}, most of the sensors are based on rhodamine compounds.[14,15] However, fluorescein-based probes have received comparatively little attention, there is still a demand for new indicators with improved properties, especially colorimetric probes that can make "naked eye" detection in the visible wavelength region easier due to their

requirement of less labor and expensive equipment.

Based on their unique photophysical properties, such as long absorption and emission wavelength, high fluorescence quantum yield, large extinction co-efficient and high stability to light, the fluorescein and rhodamine family dyes are usually introduced to construct optical sensor for many different target molecules especially metal ions, such as Zn^{2+}, Cu^{2+} and Fe^{3+}.[16-19] The sensing mechanism of these probes is based on the change in structure from spirocyclic to opencyclic form.

In this work, we report a novel colorimetric chemosensor **HL** based on fluorescein fluorophore for the quantification of Zn^{2+} in water. **HL** specifically binds to Zn^{2+} in the presence of other competing cations, and drastic fluorescence enhancement and obvious color change in aqueous solution are noticed. This response provides a convenient and practical way to detect both Zn^{2+} in environmental and biological samples.

RESULTS AND DISCUSSION

The fluorescein hydrazine (**2**) was synthesized by condensation reaction of fluorescein and hydrazine monohydrate in refluxing acetic acid for 8 h. The unique ligand, (*E*)-3', 6'-dihydroxy-2-((quinolin-2-ylmethylene)amino)spiro[isoindoline-1,9'-xanthen]-3-one (**HL**) was facilely synthesized by the condensation reaction of fluorescein hydrazine and 2-quinolinecarboxaldehyde. (Scheme 2). The molecular structure of **HL** was confirmed by NMR, mass spectra and elemental analysis (Figures S1-S3). The spectral data were in agreement with the desired structures.

In order to investigate the recognition abilities of the sensors **HL** in aqueous solution, we carried out a series of Host-Guest recognition experiments in DMSO/H_2O (3:7/v:v) HEPES buffered solution at pH 7.2. As shown in Figure 1, the free probe **HL** displayed a maximum absorption band at 335 nm. Upon addition of two equiv Fe^{3+}, the UV-vis spectrun of **HL** has absorption at longer wavelength region. When the same amounts of Zn^{2+} was added, a moderate enhancement of absorption intensity was observed. However, an obvious red shift of the maximum absorbance from 335 nm to 360 nm induced by addition of Zn^{2+}, the change in absorbance spectrum was accompanied by an obvious and characteristic color change from colorless to brown. Other metal ions including Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Ba^{2+}, Ag^{+}, Al^{3+}, Cr^{3+} and Cd^{2+} exhibited almost no change in absorption behaviour under above conditions. The results demonstrated that probe **HL** may be characteristic of selectivity to Zn^{2+} and Fe^{3+} over other competitive metal ions.

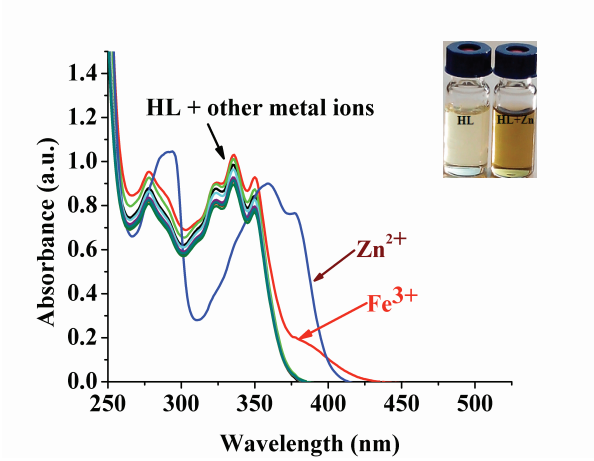


Figure 1. UV-vis spectra of **HL** (10 μM) before and after adding various cations (20 μM) in buffered DMSO/H_2O (v/v = 3:7) solution at pH 7.2

The fluorescence spectrum of **HL** with respective metal cations was shown in Figure 2, and the fluorescence excitation spectra was fixed as 397 nm (Figure S4). The sensor **HL** showed rather weak fluorescence emission at 450 nm. In fact, **HL** also did not give any observable response for many metal ions such as Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Fe^{3+}, Ba^{2+}, Ag^{+}, Al^{3+}, Cr^{3+} and Cd^{2+}. However, the addition of Zn^{2+} created a remarkable fluorescence enhancement at 509 nm occurred with a 59 nm bathochromic shift. The specific response of **HL** towards Zn^{2+} was assumed to be based on the opening function of the spirolactam ring.[24] Meanwhile, the reaction of Zn^{2+} with a chelating agent induced the rigidity in the resulting molecule and tended to produce a large chelation enhancement of the fluorescene (CHEF) which induced the large enhancement of fluorescence. These results demonstrated the high selectivity of **HL** toward Zn^{2+}.

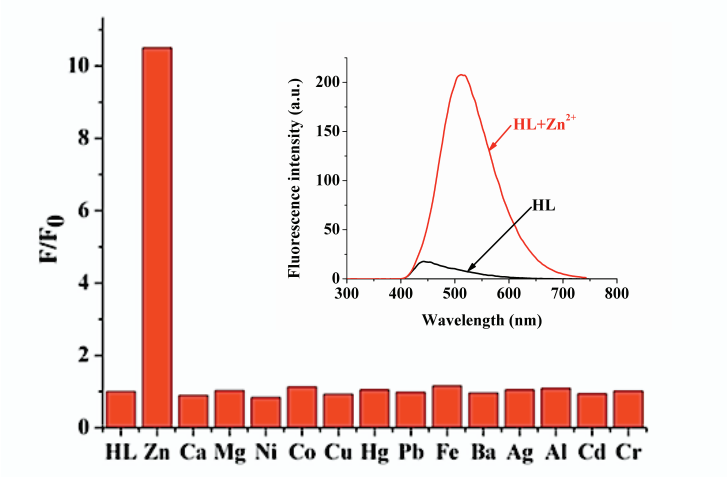


Figure 2. Fluorescence spectra of **HL** (10 μM) in the presence of different metal ions (20 μM). Inset: Photos of **HL** (10 μM) in the absence and presence of Zn^{2+} (20 μM) in DMSO/H_2O (3:7, v: v; HEPES buffered, pH 7.2)

To get an insight into the sensing properties of **HL** to Zn^{2+}, the fluorescence titration experiment was then

performed. As shown in Figure 3, upon the incremental addition of Zn^{2+} (0-2.0 equiv.) to **HL** solution, the fluorescence emission was gradually increased and reached the saturation state when 1.0 equiv. of Zn^{2+} ions was employed. According to linear Benesi-Hildebrand expression, the measured fluorescence intensity [$1/(F-F_0)$] at 509 nm varied as a function of $1/[Zn^{2+}]$ in a linear relationship (R^2=0.9911), which indicated the formation of 1:1 stoichiometry between Zn^{2+} and **HL** in the complex. The association constant of **HL** with Zn^{2+} in HEPES buffer was accordingly calculated to be 1.17×10^5 M^{-1} (Figure 4). The method of continuous variation (Job's plot) was applied to prove the complexation ratio between **HL** and Zn^{2+}. As shown in Figure S8, the maximum point at the mole fraction of 0.5 indicates the complexation ratio of **HL** and Zn^{2+} is 1:1. The detection limit for Zn^{2+} was estimated to be 5.7 nM based on a 3σ/slope analysis under these experimental conditions (Figure S9). The detection limit was sufficiently low to detect submicromolar concentration of the Zn^{2+}, which belonged to the range found in many chemical and biological systems, implying that probe **HL** holds great potential for the application in the development of sensor materials for Zn^{2+}.

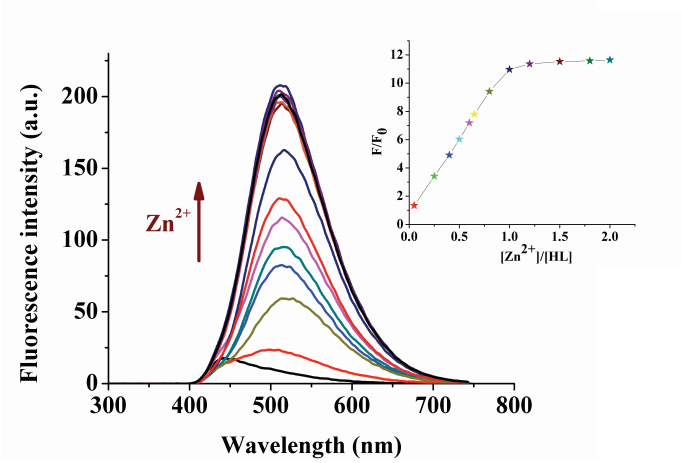


Figure 3. Enhancement in the fluorescence intensity of **HL** (10 μM) in DMSO/H_2O (3:7, v:v; HEPES buffered, pH 7.2) in presence of Zn^{2+} (0-2 equiv); Inset showed the change in the fluorescence intensity of the ligand with varying concentration of Zn^{2+} (λex = 397 nm).

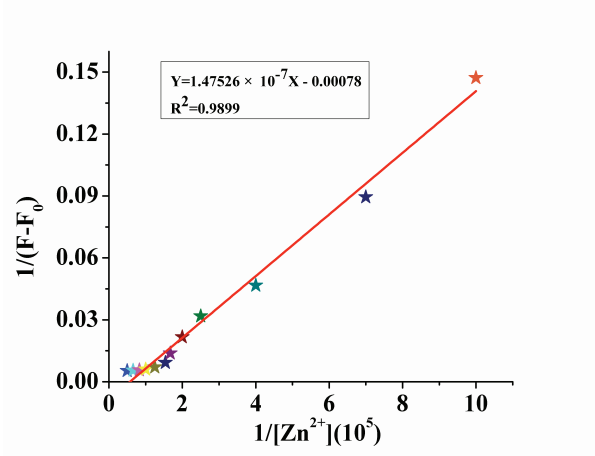


Figure 4. Benesi-Hildebrand plot of **HL** (10 μM) based on 1:1 binding stoichiometry (λex=397 nm)

Besides the high selectivity, a time course of the fluorescence response of **HL** upon the addition of Zn^{2+} was shown in Figure S7. The kinetics of fluorescence enhancement by **HL** was recorded, and results indicated that the recognizing event could complete in 20 min so that it could be used as a fluorescent probe for the fast detection of Zn^{2+}.

To check further the practical applicability of receptor **HL** as a Zn^{2+}-selective receptor, we carried out the competition experiments (Figure 5). For the competition tests, receptor **HL** was treated with 2 equiv. of Zn^{2+} and 2 equiv. of other coexistent metal ions such as Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Fe^{3+}, Ba^{2+}, Ag^{+}, Al^{3+}, Cr^{3+} and Cd^{2+}. As a result, no interference was observed for the detection of Zn^{2+} by **HL**. These results indicated that **HL** could be an excellent chemosensor for Zn^{2+} over competing relevant metal ions in aqueous solution.

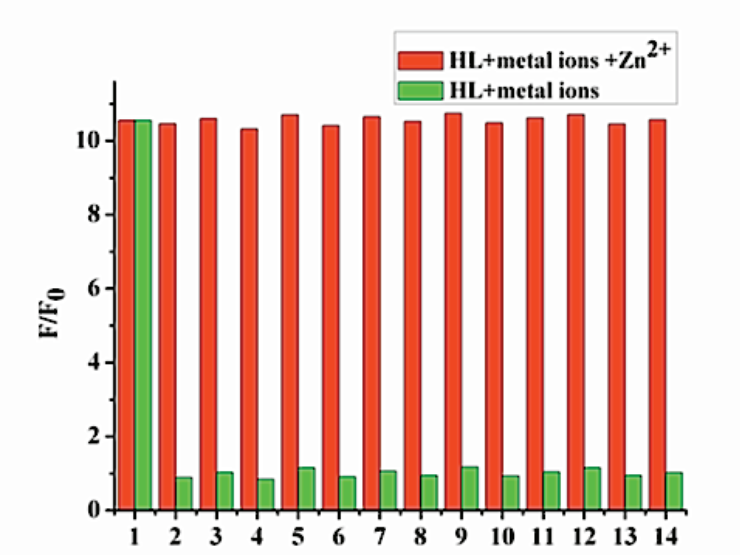


Figure 5. Relative fluorescence intensity of **HL** (10 μM) to various cations in DMSO/H_2O (3:7, v:v; HEPES buffered, pH 7.0). The green bars represented the enhancement degree of **HL** in the presence of cations of interest (all were 20 μM). The red bars represented the enhancement degree of the emission that occured subsequent upon the addition of 30 μM of Zn^{2+} to the above solution (λex=397 nm). (1) Zn^{2+}; (2) Ca^{2+}; (3) Mg^{2+}; (4) Ni^{2+}; (5) Co^{2+}; (6) Cu^{2+}; (7) Hg^{2+}; (8) Pb^{2+}; (9) Fe^{3+}; (10) Ba^{2+}; (11) Ag^{+}; (12) Al^{3+}; (13) Cd^{2+}; (14) Cr^{3+}

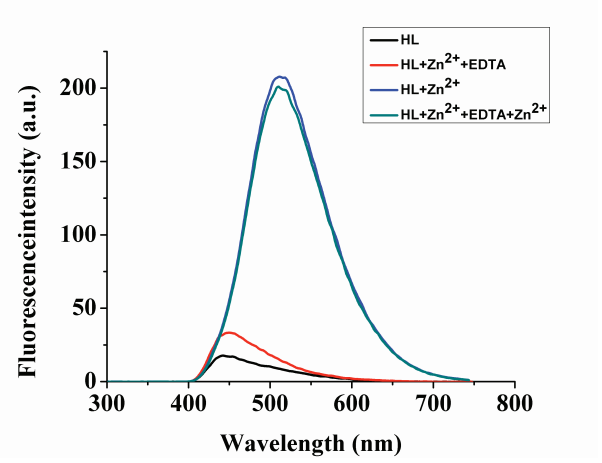


Figure 6. Fluorescence responses of **HL** (10 μM) after the sequential addition of Zn^{2+} and EDTA

The reversibility of the recognition process of **HL** was performed by adding a bonding agent, Na_2EDTA. As shown in Figure 6, the addition of Na_2EDTA to a mixture of **HL** and Zn^{2+} resulted in the diminution of the fluorescence intensity, which indicated the regeneration of the free sensor **HL**. Therefore, it meant that the receptor **HL** could be used as a selective fluorescent sensor for detection and recognition of Zn^{2+} in such fields of environmental analysis.

For the biological application of **HL**, the sensing should operate in the physiological range. As shown in Figure 7, the fluorescence intensity of **HL** is stable at pH 5-8 without obvious florescence responses. When the pH was more than 9, the deprotonation of the hydroxyl group led to the fluorescence enhancement due to the ring opening of the spirolactam at alkaline conditions. Meanwhile, it can be seen that the fluorescence intensity of **HL**-Zn^{2+} was evidently enhanced when the pH was more than 7.5 and reached its maximum at pH 9.5. The finding was attributed to the deprotonation of the hydroxyl group being favorite to the chelation of **HL** to Zn^{2+}. However, Fe^{3+} can be bound with OH^- in concentrated alkaline solution, which resulted in the fluorescence intensity of **HL**-Zn^{2+} became much weaker. The results indicated that the **HL** can be used as a chemosensor for Zn^{2+} detection under physiological conditions.

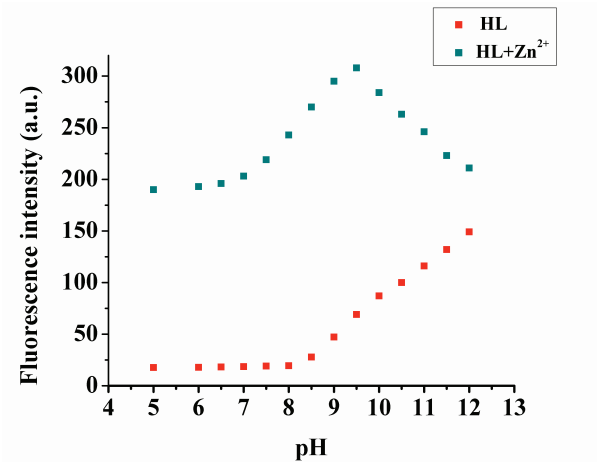


Figure 7. Variations of fluorescence intensity of **HL** (10 μM) with 2.0 equiv. Zn^{2+} in DMSO/H_2O as a function of pH

In order to show the feasibility of the hybrid material as a chemosensor and absorbent for Zn^{2+} in potential practical applications, we studied the performance of **HL** in natural water samples. The addition of 5 μM (red bars) and 10 μM (green bars) of Zn^{2+} to the **HL** suspension dispersed in laboratory pure water (LW) afforded about 5.3-fold and 10.5-fold enhancement of fluorescence intensity, respectively. Then the responding ability of **HL** to Zn^{2+} dispersed in natural water was tested. Water samples were collected from two significantly different sources: drinking water (DW) from Anshan City (Liaoning Province, China) and seawater (SW) from Dalian (Liaoning Province, China). The Zn^{2+}-induced fluorescence enhancement was not significantly affected in the presence of different water source (Figure

8). The preliminary investigations in natural water samples including seawater and drinking water indicated that **HL** featured an excellent Zn^{2+} chemosensor and adsorbent in complex natural samples.

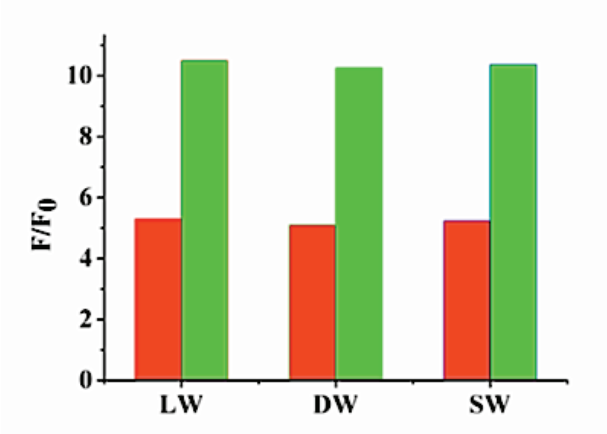


Figure 8. The sensitivity of **HL** to Zn^{2+} in different water samples: laboratory pure water (LW), drinking water (DW) and seawater (SW). The red and green bars represented the emission intensities of **HL** in the presence of 5 μM (red bars) and 10 μM (green bars) of Zn^{2+}, respectively.

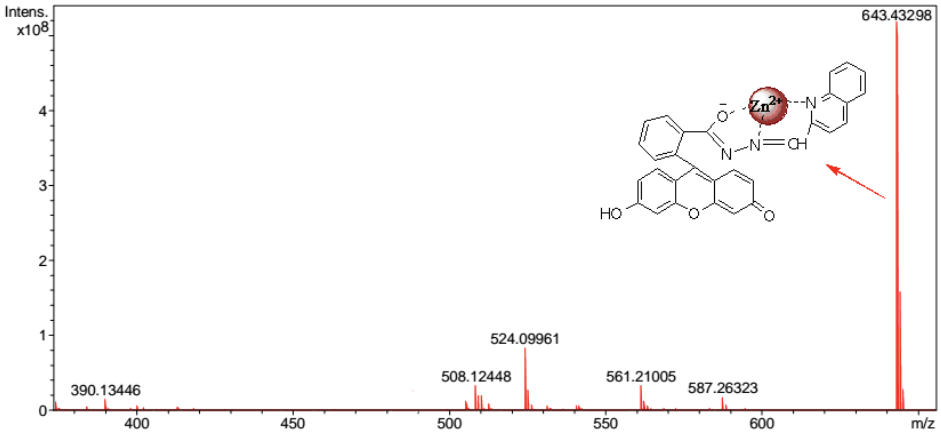
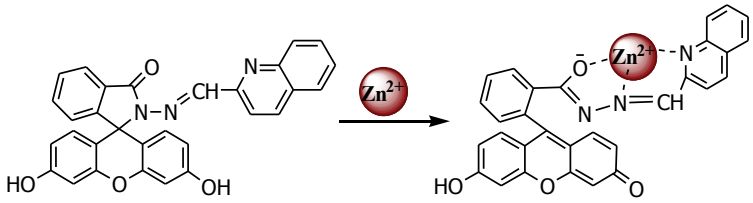


Figure 9. FT-MS of **HL**-Zn^{2+} ensemble

The recognition mechanism of the sensor **HL** with Zn^{2+} was investigated by FT-MS spectra and ^1H NMR titration. FT-MS spectra showed a molecular-ion peak [**HL**+Na^+]$^+$ at *m/z* 508.12607. When the Zn^{2+} ion was added into the solution of **HL**, the peak at *m/z* 643.43298 was assignable to [**HL**-H^++Zn^{2+}+2Cl^-+Na^+]$^+$ species (Figure 9). This result confirmed that 1:1 stoichiometry complex between **HL** and Zn^{2+}. ^1H NMR titration experiments were carried out independently with **HL** and **HL**-Zn^{2+} (Figures S5, S6). Upon complexation of **HL** with Zn^{2+} caused the imino proton signals (H_b) underwent a down field shift from 8.723 to 8.670 ppm with Zn^{2+} addition, which indicated that the nitrogen atom of CH=N may participate in binding with Zn^{2+}. The two proton signals (H_a) of O-H decreased to one and underwent a downfield shift from 9.990 to 10.098 ppm, which astritted to the opencycle mechanism and the phenol O-H appeared tautomerism to C=O. Therefore, the obvious

differences of the ^{1}H NMR spectra of **HL** in the absence and presence of Zn^{2+}, coupling with ESI-MS and Job's plot analysis, suggested that **HL** could chelate Zn^{2+} through the interactions with carboxylate oxygen, imine nitrogen and nitrogen of quinoline group and formed 1:1 complex (Scheme 1).



HL **HL-Zn^{2+}**

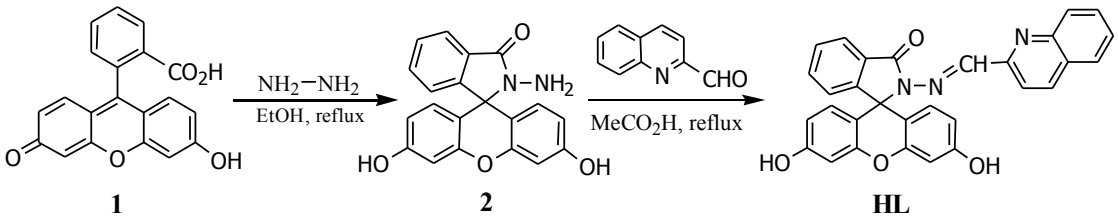
Scheme 1

CONCLUSIONS

In summary, we have developed a new fluorescein-based fluorescent chemosensor **HL** for Zn^{2+} detection in mixed aqueous medium (DMSO/H_2O = 3:7, HEPES buffered, pH =7.2). The experimental results clearly indicated that compound **HL** was a highly sensitive and selective chemosensor for Zn^{2+}. Furthermore, receptor selectivity and sensitivity were not affected in the presence of other competing metal ions. Moreover, according to Job's plot, 1:1 stoichiometry complex between **HL** and Zn^{2+} was formed. This excellent selectivity of chemosensor for Zn^{2+} in aqueous media exhibited its potential application value in the biological monitoring and tracking of Zn^{2+}.

EXPERIMENTAL

Fluorescein hydrazine[20] was prepared by previously reported procedures. All other chemicals used in this study were commercially available, and the organic solvents were dried over appropriate drying agents and distilled prior to use. Double-distilled water was used throughout the experiment. Nitrate salts of metal ions (Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Fe^{3+}, Ba^{2+}, Ag^{+}, Al^{3+}, Cr^{3+} and Cd^{2+}) were used to evaluate the metal ion binding properties. The infrared spectra were performed on a Perkin Elmer spectrophotometer. ^{1}H NMR spectra were recorded using a Bruker AV 500-MHz spectrometer using DMSO-d_6 as a solvents and TMS as an internal standard. The ^{13}C NMR spectra were determined using TMS as an internal reference with a Bruker AV 500-MHz spectrometer operating at 150MHz. Molecular weight was recorded on a Bruker Solarix 70 high-resolution mass spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 microanalyser. The UV-vis and fluorescence experiments were performed on a Lambda-900 spectrometer and a Perkin-Elmer LS-55 fluorescence spectrophotometer respectively.



Scheme 2

General Procedure for the Preparation of Products Synthesis of (*E*)-3',6'-dihydroxy-2-((quinolin-2-ylmethylene)amino)spiro[isoindoline-1,9'-xanthen]-3-one (HL)

A mixture of 2-quinolinecarboxaldehyde (0.471 g, 5 mmol), fluorescein hydrazine (5 mmol) were magnetically stirred in 30 mL of acetic acid at refluxing until the reactions were completed (monitored by TLC). A white solid appeared which was then filtered from solution. The crude product was recrystallized from EtOH to give the target product as a yellow powder in 68% yields. IR (KBr): 3326, 3031, 2968, 2872, 1689, 1640, 1605, 1492, 1441, 1267, 882, 742cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.990 (s, 2H, OH), 8.723 (s, 1H, CH=N), 8.339 (d, *J*=8.5 Hz, 1H, Ar), 8.008 (m, 2H, Ar), 7.933 (d, *J*=8.0 Hz, 1H, Ar), 7.831 (d, *J*=8.5 Hz, 1H, Ar), 7.747 (t, *J*=7.0 Hz, 1H, Ar), 7.689 (t, *J*=7.0 Hz, 1H, Ar), 7.633 (m, 2H, Ar), 7.168 (d, *J*=7.0 Hz, 1H, Ar), 6.750 (s, 2H, Ar), 6.608 (d, *J*=8.5 Hz, 2H, Ar), 6.500 (d, *J*=8.0 Hz, 2H, Ar). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 164.71, 159.30, 154.16, 152.46, 151.54, 147.72, 147.05, 137.30, 135.02, 130.52, 129.68, 129.36, 128.38, 128.31, 127.86, 124.22, 124.00, 117.05, 113.07, 110.10, 103.24. MS, *m/z*: 508.12607 (**HL**+Na$^+$); Anal. Calcd for $C_{30}H_{19}N_3O_4$: C, 74.22; H, 3.94; N, 8.66. Found: C, 74.28; H, 3.92; N, 8.63.

Recognition Studies

The metal binding studies were performed in 10-mL volumetric flasks with the fixed concentration of particular metal ions (0.2 mM) along with compound **HL** (0.1 mM) in Tris–HCl buffer solution (50 mM, pH 7.2) system. The titration experiments were conducted manually stepwise addition of Zn^{2+} in the buffer solution of compound **HL** (10 mL). To guarantee the uniformity of solution, enough time was given before recording any spectrum. The association constant was calculated according to linear Benesi-Hildebrand.[21] The detection limit was calculated by the using the Stern-Volmer equation.[22] The stoichiometry of compound **HL** and Zn^{2+} was determined by Job's method from the obtained fluorescent spectroscopic data.[23] For fluorescence intensity measurements, the excitation wavelength was fixed at 397 nm. The slit widths were 10 nm/10 nm.

Association constant calculation

Generally, for the formation of 1:1 complexation species formed by the chemosensor compound and the guest cations, the Benesi-Hildebrand equation used is as follow:

$$\frac{1}{F\text{-}F_0} = \frac{1}{K_a(F_{max}\text{-}F_0)[Zn^{2+}]} + \frac{1}{F_{max}\text{-}F_0}$$

where F and F_0 represent the fluorescence emission of **HL** in the presence and absence of Zn^{2+}, respectively, F_{max} is the saturated emission of **HL** in the presence of excess amount of Zn^{2+}; $[Zn^{2+}]$ is the concentration of Zn^{2+} ion added, and Ka is the binding constant.

ACKNOWLEDGEMENTS

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