Design of a novel coumarin-based multifunctional fluorescent probe for Zn^{2+} / Cu^{2+} / S^{2-} in aqueous solution

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Abstract: A multifunctional fluorescent chemosensor 7-(diethylamino)-coumarin-3-carbaldehyde-(2'-methylquinoline-4'-formyl) hydrazone (HL) has been designed and synthesized. The sensor shows significant fluorescence enhancement in the presence of Zn^{2+} , which might be mainly due to the restricted -C=N isomerization process. In contrast, the fluorescence of the sensor is quenched by Cu^{2+} attributed to the inherent to the paramagnetic species. More interestingly, the 'in situ' prepared L-Cu exhibits a selective response to S^{2-} based on reversible formation–separation of complex L-Cu and CuS.

Keywords: Multifunctional; fluorescent sensor; Zinc; Copper; Sulfide

1. Introduction

As is well-known, zinc and copper, two of the most abundant transition metal ion, are widely dispersed and used in the environment around us [1-3]. They play some critical roles in a variety of biological process such as gene transcription, regulation of metalloenzymes, and neural signal transmission [4-5] superoxide dismutase,

cytochrome coxidase in human body [6-8]. Hence, a quantity of Zn^{2+} and Cu^{2+} is essential and beneficial for our good health. Whereas, overloading conditions, they exhibit toxicity in that they cause neurodegenerative diseases such as Alzheimers, Parkinsons [9-12], Menkes and familial amyotropic lateral sclerosis [13-15]. And in general, the normal Zn^{2+} content of blood plasma is 12 to 16 mM in the body [16], the maximum amount of Cu^{2+} in drinking water is limited to $20\mu\text{M}$ which is restricted by the Environmental Protection Agency (EPA) of the U.S regulations. [17-19]. Sulfide, as a traditional toxic anion, has many applicable utilities such as manufacture of sulfur and sulfuric acid, dyes and cosmetics [20-22]. On the other hand, it is to exposure to high levels of sulfide that can result in various physiological and biochemical problems including irritation in mucous membranes, unconsciousness, and respiratory paralysis [23]. For these reasons, in order to protect the environment and human health, it is crucial to develop a convenient analytical method for detection and recognition of excessive amounts of these ions.

Fluorescent probes with operational simplicity, high sensitivity, and inexpensive operational cost have attracted considerable attention [24-26]. However, the multifunctional fluorescent probe which is simultaneous detection and identification of multiple ions is still few in number. Compared to single-ion responsive molecular sensors, the multifunctional sensors have been actively developed because of the advantages such as analytical time reduction [27-29]. Coumarin is a kind of compounds with great fluorescent intensity, high quantum yield, very suitable to act as fluorescent chromophores [30-33]. In addition, Schiff bases are well known to be

good ligands which provide an electron-rich environment, which made it easy to bind with metal ions [34-36].

With this in mind, we have designed and synthesized a coumarin-based multifunctional fluorescent probe for Zn^{2+} and Cu^{2+} in aqueous solution (Scheme 1). On one hand, the sensor exhibits a large fluorescence enhancement in the presence of Zn^{2+} . On the other hand, the fluorescence of the sensor demonstrates obvious fluorescence quenching in the presence of Cu^{2+} . Even more surprising, the emission signals of ‘in situ’ prepared L-Cu can be restored with S^{2-} . Thus, the ‘in situ’ prepared L-Cu could be developed a platform as new sulfide sensor.

2. Experimental

2.1. Apparatus and reagents

Unless mentioned otherwise, all chemicals for synthesis were purchased from commercial suppliers and used without further purification. JNM-ECS 400MHz instruments using TMS as an internal standard, ESI-MS were determined on a Bruker esquire 6000 spectrometer. Perkin Elmer Lambda 35 UV–Vis spectrophotometer, Hitachi RF-5301 spectrophotometer equipped with quartz cuvettes of 1 cm path length, Beijing XT4-100x microscopic melting point apparatus.

Stock solutions of various metal ions (1 mM) were prepared using nitrate salts. Stock solutions of various anions (10 mM) were prepared using sodium salts. A stock

solution of HL (1 mM) was prepared. The excitation slit width was 3 nm, the emission slit width was 1.5 nm.

2.2. Synthesis of ethyl 2-methyl quinoline-4-carboxylate [37-38]

A mixture of isatin (6.8 mmol, 1.0 g) and 85% KOH (2.67g, 47.6 mmol) was heated to 50°C for 1 h. Then acetone (5 ml, 56.7 mmol) was added dropwise and stirred for 20 h. The reaction mixture was cooled to ambient temperature and acidified to pH 3 by 37% HCl to obtain heavy slurry which was filtered, washed and dried to afford **A**. Mp: 248-250°C. To an ethanol solution (75 ml) of **A** (3 mmol, 0.56g) was added H₂SO₄ (conc. 3.5 ml). After refluxed for 24 h, solvent was removed and the residue was partitioned between EtOAc and saturated NaHCO₃ solution. The organic layer was washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and condensed to give ethyl 2-methyl quinoline-4-carboxylate. Mp: 71-72°C, ¹H NMR (400 MHz, CDCl₃) (Fig. S1.): δ=1.47 (t, *J* = 7.2Hz, 3H), 2.82 (s, 3H), 4.51 (q, *J*₁= 7.2 Hz, *J*₂= 14.0 Hz, 2H), 7.61 (t, *J*= 8.0 Hz, 1H), 7.76 (t, *J*= 8.0 Hz, 1H), 7.82 (s, 1H), 8.10 (d, *J*= 8.4Hz, 1H), 8.69 (d, *J* = 8.4 Hz, 1H).

2.3. Synthesis of 2-methyl quinoline-4-carboxylic hydrazide

Hydrazine hydrate (80%, 4 ml) was added dropwise to an ethanol solution (40 ml) of ethyl 2-methyl quinoline-4-carboxylate (5 mmol, 1.015g) and refluxed under stirring for 20 h. Then the solvent was concentrated under reduced pressure and cooled to room temperature, a white needle crystal was observed. The final product was filtered, dried and recrystallized from ethanol. Mp: 178-179°C, ¹H NMR (400

MHz; CDCl₃) (Fig. S2.): δ = 2.71 (s, 3H_e), 4.25 (s, 2H_h), 7.29 (s, 1H_f), 7.54 (m, 1H_b), 7.59 (s, 1H_g), 7.73 (m, 1H_c), 8.05 (d, J =8.4 Hz, 1H_d), 8.14 (d, J =8.1 Hz, 1H_a).

2.4. Synthesis of 7-(diethylamino)- coumarin -3-carbaldehyde [39]

4-diethylaminosalicylaldehyde (1.93g, 10mmol), diethylmalonate (3.2g, 20 mmol), piperidine (1 ml) were stirred for 6 h at 80°C in 30 ml ethanol. Then ethanol was removed and 37% HCl (20 ml), glacial acetic acid (20 ml) were added with stirring for 6h, the mixture was poured into 100 ml ice water and was modulated pH to ~5 by 40% NaOH to afford **1**. Anhydrous DMF (2 ml) was added dropwise to POCl₃ (2 ml) stirred for 0.5 h at 30°C. This solution was combined with a portion of **1** (1.50 g, 6.91 mmol, dissolved in 10 mL DMF). After stirring for 12 h at 60°C, the mixture also was poured into 100 ml ice water, 20% NaOH was added to adjust the pH to yield large amount of precipitate. The crude product was filtered, thoroughly washed, dried and recrystallized from ethanol to give **2**, Mp: 163-164°C, ¹H NMR (400 MHz; CDCl₃) (Fig. S3.): δ = 1.23 (t, J =7.1 Hz, 6H₁), 3.45 (t, J =7.1 Hz, 4H₂), 6.45 (s, H₇), 6.62 (d, J =6.6 Hz, 6H₁), 1H_g), 7.39 (d, J =6.6 Hz, 1H₄), 8.23 (s, 1H₅), 10.1 (s, 1H₆).

2.5. Synthesis of HL

An ethanol solution (20 mL) of 2-methyl quinoline-4-carboxylic hydrazide (0.203g, 1 mmol) was added to another 20 ml ethanol containing 7-(diethylamino)-coumarin -3-carbaldehyde (1 mmol, 0.245 g), then the solution was refluxing for 12 h under stirring and some white precipitant appeared. The crude product was filtered, dried and recrystallization from DMF, Mp: 224-225°C, ¹H NMR (400MHz;

DMSO- d_6) (Fig. S4.): (the major) $\delta=1.11$ (t, $J=7.0$ Hz, $3H_{13}$, $3H_{14}$), 2.67 (s, $3H_5$), 3.43(q, $J=7.0$ Hz, $2H_{12}$, $2H_{15}$), 6.55 (d, $J=2.2$ Hz, H_{16}), 6.74 (dd, $J=2.2$ Hz, $J=9.0$ Hz, H_{11}), 7.55~7.57 (m, H_{10}), 7.59 (s, H_6), 7.64 (d, $J=9.0$ Hz, H_2), 7.72~7.77 (m, H_3), 7.97 (d, $J=8.3$ Hz, H_4), 8.07 (d, $J=8.1$ Hz H_1), 8.33 (s, H_9), 8.41 (s, H_8), 12.13 (s, H_7). ESI-MS (Fig. S5): $[M+1]^+$: 429.172. $[M+Na]^+$: 451.155.

3. Results and discussion

3.1. General information

The detection limit was calculated according to the following equation: detection limit $3\sigma/K$ based on the fluorescence titration, where σ was the standard deviation of blank measurements, and K was the slope between intensity versus sample concentration [40].

3.2. UV-vis titrations on Zn^{2+}

The mode of coordination of HL with Zn^{2+} was initially investigated by UV-vis titrations in ethanol and water (9:1, v/v). As shown in Fig. 1. The free receptor showed maximum absorption wavelength at 450 nm which might be assigned to the coumarin group [30-33]. Upon addition of Zn^{2+} , the absorption band at 450 nm exhibited a slightly bathochromic shift along with certain increasing intensity, the absorption band at 280 nm gradually decreased while a new absorption band appeared at 350 nm. In addition, two clear isosbestic points were observed at 312 nm, 365 nm which clearly indicated the presence of new complex in equilibrium with the receptor. All of these might be attributed to form a 1:1 stoichiometry of the binding mode of HL with Zn^{2+} , which were further confirmed by ESI-MS (Fig. S6) and Job's plot

analysis (Fig. S7).

3.3. Fluorescence response of HL to Zn^{2+}

The fluorometric behaviour of HL was evaluated upon addition of several metal ions such as Na^+ , K^+ , Li^+ , Cr^{3+} , Cu^{2+} , Hg^{2+} , Pb^{2+} , Ba^{2+} , Fe^{3+} , Fe^{2+} , Al^{3+} , Mn^{2+} , Co^{2+} , Ca^{2+} , Ag^+ , Cd^{2+} , Ni^{2+} , Mg^{2+} and Zn^{2+} in ethanol and water (9:1, v/v). As shown in Fig. 2, the free sensor revealed hypofluorescence at 505 nm, on the addition of various metal ions to aqueous solution of HL, the fluorescence intensity of HL showed significant fluorescence enhancement at 514 nm in the presence of Zn^{2+} , other relevant metal ions caused almost no fluorescence increase except Cd^{2+} which was in the same group with Zn^{2+} in the periodic table [41]. The phenomenon may be mainly explained by the restricted $-\text{C}=\text{N}$ isomerization, More specifically, before addition of Zn^{2+} , the $-\text{C}=\text{N}$ isomerization was the predominant decay process of the excited states for coumarin derivative with an unbridged $\text{C}=\text{N}$ structure so that the sensor showed weak fluorescence. however, up addition of Zn^{2+} , the binding of the nitrogen atom of the $-\text{C}=\text{N}$ with Zn^{2+} restricted $\text{C}=\text{N}$ isomerization in the excited states, as result, the fluorescence occurred remarkably at 514 nm as shown in Scheme 2 [39], [42-43]. In addition, part of the reason was attributed to the chelation of the nitrogen atom of the $-\text{C}=\text{N}$ group with Zn^{2+} which resulted in the efficient inhibition for the PET process of the $-\text{C}=\text{N}$ group [44]

To understand the recognition abilities of HL toward Zn^{2+} , competition experiments (Fig. 3) and fluorescent titrations (Fig. 4) were carried out. From the fluorescence titration profiles, the stability constant was determined as 1.19×10^5

based on Benesi–Hildebrand equation (Fig. S8.)[45-46] and the detection limit reached at 5.76×10^{-7} M which may find potential applications for detection of micromolar concentrations of Zn^{2+} (Fig. S9.). According to competition experiments, L-Zn complex mixing with the other metal ions showed no significant variation in the case of Cu^{2+} , Fe^{3+} , Fe^{2+} , although the fluorescence intensity was low but detectable. Thus, it meant that the sensor can be used as a selective fluorescent sensor for Zn^{2+} in the presence of most competing metal ions.

3.4 ^1H NMR titration

For the formation of 1:1 complex, it was necessary to ^1H NMR titration except for the above experiments, Zn^{2+} (as its nitrate salt) was added to the DMSO-d_6 solution of HL, significant spectral changes were observed as shown in Fig. 5, the peaks at 12.12 ppm should be corresponding to the labile protons of -C-NH. Upon addition of Zn^{2+} , the proton peak of the -CO-NH at 12.12 ppm disappeared, This was possible because addition of Zn^{2+} to HL caused the enolization of -CO-NH ($\text{COH}=\text{N}$) [47] and the loss of the -OH proton with subsequent binding to electron deficient Zn^{2+} , the signals (8,9) were shifted to low-field compared to that in the ^1H NMR spectrum of the parent ligand HL which were attributed to the binding mode of Zn^{2+} and the oxygen of -COO and the nitrogen atom of the $-\text{C}=\text{N}$, in addition, the aryl protons also have different changes in chemical shift, Therefore, the large differences of the ^1H NMR spectra of HL in the absence and presence of Zn^{2+} , couple with ESI-MS and Job's plot analysis, suggested that HL chelated Zn^{2+} through interactions with the oxygen of -COO, the nitrogen atom of the $-\text{C}=\text{N}$ and oxygen of the $\text{COH}=\text{N}$ and forms 1:1

complex.

3.5. UV-vis titrations on $\text{Cu}^{2+}/\text{S}^{2-}$

To investigate the binding property of sensor HL toward $\text{Cu}^{2+}/\text{S}^{2-}$, we measured the UV-vis spectra of HL and $\text{Cu}^{2+}/\text{S}^{2-}$ in ethanol and water (9:1, v/v). As shown in Fig. 6a. Upon addition of Cu^{2+} , the absorption band at 452 nm shifted to 484 nm along with intensity increasing and a new absorption peak appeared at 342 nm. Moreover, two clear isosbestic points were observed at 317 nm, 372 nm which indicated that the complex L-Cu was formed. In contrast, as shown in Fig. 6b, upon addition of S^{2-} , the absorption band at 450 nm exhibited a blue shifted and appeared certain decrease. Compared to Fig. 5a, the spectra of reversible changes was because that Cu^{2+} was released from complex L-Cu and the inorganic small molecular compound (CuS) was formed, which was further confirmed by ESI-MS (Fig. S10, S11). [48].

3.6. Fluorescence response of HL to Cu^{2+}

As shown in Fig. 2, the fluorescence of HL was almost completely quenched in the presence of only Cu^{2+} . This was because that Cu^{2+} with open shell d-orbitals often quench, the fluorescence of fluorophores due to the electron or energy transfer between Cu^{2+} and fluorophores, providing a very fast and efficient nonradiative decay of the excited states [48], [49]. As for others transition metal ions which did not quench fluorescence, the reason could be explained by the competition between the fluorescence enhancement due to the inhibition of C=N isomerization and quenching of fluorescence from the metal cation-induced electron or energy transfer processes

[39].

In addition, according to fluorescence titration profiles, the association constant was determined as 1.45×10^5 (Fig. S12.) and the detection limit reached at 3.1×10^{-7} M (Fig. S13.) which was lower than the limitation by the Environmental Protection Agency (EPA) of the U.S. To check the practical applicability of HL as a selective fluorescent sensor for Cu^{2+} , we also carried out competition experiment in ethanol and water (9:1, v/v). As shown in Fig. S14, addition of transition metal ions also induced some fluorescence quenching, and then addition of Cu^{2+} , the fluorescence exhibited total quenching, which suggested that the probe was not interfered by others metal ions.

3.7. Fluorescence response of L-Cu to S^{2-}

On the other hand, the ‘in situ’ prepared L-Cu was evaluated as a multi-ion sensor for recognition of S^{2-} . As shown in Fig. 7, the fluorescence of HL could be regenerated only by addition of S^{2-} to the solution containing L-Cu. Other anions including F^- , Cl^- , Br^- , I^- , CO_3^{2-} , NO_3^- , SO_4^{2-} , H_2PO_4^- , SCN^- , N_3^- , ClO_4^- , BF_4^- , PF_6^- , AcO^- did not generate the noteworthy results. We presumed the ‘in situ’ prepared L-Cu exhibited a selective response to S^{2-} based on simple inorganic-reaction. And, in short, firstly, the fluorescence of HL was quenched by Cu^{2+} , and then Cu^{2+} would be dragged out from the complex by S^{2-} which was known to react with Cu^{2+} to make a very stable CuS ($K_{\text{sp}} = 6.3 \times 10^{-36}$), as result, the quenched fluorescence recurred remarkably [50-55]. It was further confirmed by the UV-vis spectra of reversible change. [56-58].

Moreover, the selectivity of L-Cu for S^{2-} was not affected by the presence of some other anions (Fig. 8) and the detection limit for S^{2-} was measured to be 1.9×10^{-5} M (Fig. S15). Hence, the 'in situ' prepared L-Cu was highly selective and sensitive for S^{2-} detection, which made it feasible for biological and environmental applications.

4. Conclusion

In summary, we have successfully developed a multi-ion responsive molecular sensor 7-(diethylamino)-coumarin-3-carbaldehyde-(2-methylquinoline-4-formyl) hydrazone (HL) for Zn^{2+} , Cu^{2+} , S^{2-} in aqueous solution. More importantly, because of these lower detection limit, the multi-functional fluorescent probe may find potential applications for detect micromolar concentrations of $Zn^{2+}/Cu^{2+}/S^{2-}$ and molecular recognition in the environment.

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Figure captions

Fig.1. Absorption spectra of HL (10.0 μM) in ethanol and water (9:1, v/v) upon the addition of Zn^{2+} (0, 2, 4, 6, 8, 9, 10, 11, 12 μM).

Fig.2. Fluorescence responses of HL (10.0 μM) in ethanol and water (9:1, v/v) with 10.0 μM of Na^+ , K^+ , Li^+ , Cr^{3+} , Cu^{2+} , Hg^{2+} , Pb^{2+} , Ba^{2+} , Fe^{3+} , Fe^{2+} , Al^{3+} , Mn^{2+} , Co^{2+} , Ca^{2+} , Ag^+ , Cd^{2+} , Ni^{2+} , Mg^{2+} , and 10.0 μM of Zn^{2+} . Excitation wavelength was 469 nm.

Fig.3. Fluorescence intensity of HL and its complexation with Zn^{2+} in the presence of various metal ions. Black bar: HL (10.0 μM) and HL with 1.0 equiv. of Mg^{2+} , Mn^{2+} , Ca^{2+} , Ni^{2+} , Ag^+ , K^+ , Li^+ , Co^{2+} , Cr^{3+} , Ba^{2+} , Pb^{2+} , Na^+ , Cd^{2+} , Hg^{2+} , Cu^{2+} , Fe^{2+} , Al^{3+} and Fe^{3+} stated. Red bar: 10.0 μM of HL and 1.0 equiv. of Zn^{2+} and 10.0 μM of HL and 1.0 equiv. of Zn^{2+} with 1.0 equiv. of metal ions stated ($\lambda_{\text{ex}}=469$ nm).

Fig.4. Fluorescence spectra of HL (10 μM) in ethanol and water (9:1, v/v) upon the addition of Zn^{2+} (0-11 μM) with an excitation of 469 nm.

Fig.5. ^1H NMR spectra of HL with metal ions in DMSO-d_6 : (I) HL; (II) HL with Zn^{2+}

Fig.6. (a) Changes in the absorption spectra of HL (10 μM) in ethanol and water (9:1, v/v) at room temperature as a function of added Cu^{2+} (0, 2, 4, 6, 7, 8, 9, 10, 11 μM).

(b) Changes in the absorption spectra of 10.0 μM of L-Cu in ethanol and water (9:1, v/v) at room temperature as a function of added S^{2-} (0–5 equiv.).

Fig.7. Fluorescence spectra of L-Cu (10 μM) upon the addition of anions (1.0 equiv.) of F^- , Cl^- , Br^- , I^- , SCN^- , CO_3^{2-} , SO_4^{2-} , NO_3^- , AcO^- , N_3^- , ClO_4^- , BF_4^- , PF_6^- , H_2PO_4^- and S^{2-} in ethanol and water (9:1, v/v). Excitation wavelength was 469 nm.

Fig.8. Fluorescence intensity of L-Cu with S^{2-} in the presence of various anions Black bar: HL (10.0 μ M) and Cu^{2+} ; HL and Cu^{2+} with 10 equiv. of various anions: 1. $H_2PO_4^-$, 2. SCN^- , 3. BF_4^- , 4. ClO_4^- , 5. PF_6^- , 6. Ac^- , 7. NO_3^- , 8. SO_4^{2-} , 9. CO_3^{2-} , 10. Cl^- , 11. Br^- , 12. F^- , 13. I^- , 14. N_3^- ; 15. L-Cu stated. Red bar: 10.0 μ M of HL and Cu^{2+} and S^{2-} ; 10.0 μ M of HL and Cu^{2+} and S^{2-} with 10 equiv. of anions: 1. $H_2PO_4^-$, 2. SCN^- , 3. BF_4^- , 4. ClO_4^- , 5. PF_6^- , 6. Ac^- , 7. NO_3^- , 8. SO_4^{2-} , 9. CO_3^{2-} , 10. Cl^- , 11. Br^- , 12. F^- , 13. I^- , 14. N_3^- , 15. L-Cu+S²⁻ stated (λ_{ex} =469 nm).

Scheme 1. Reagents and conditions: (a) (i) acetone, aq KOH, reflux, 15 h; (ii) conc HCl; (b) conc H_2SO_4 , EtOH, reflux, 18 h; (c) EtOH, $N_2H_4 \cdot H_2O$, 80 °C, 20 h ; (d) EtOH, diethylmalonate, piperidine , reflux, 10-12 h. (e) HCl (37%,) and glacial acetic acid ; (f) Anhydrous DMF, $POCl_3$

Scheme2. Proposed mechanism for detection of Zn^{2+} by HL.

Scheme3. Proposed mechanism for detection of Cu^{2+}/S^{2-} by HL.

Fig.1. Absorption spectra of HL (10.0 μM) in ethanol and water (9:1, v/v) upon the addition of $\text{Zn}(\text{NO}_3)_2$ (0, 2, 4, 6, 8, 9, 10, 11, 12 μM).

Fig.2. Fluorescence responses of HL (10.0 μM) in ethanol and water (9:1, v/v) with 10.0 μM of Na^+ , K^+ , Cr^{3+} , Cu^{2+} , Hg^{2+} , Pb^{2+} , Ba^{2+} , Fe^{3+} , Mn^{2+} , Co^{2+} , Ca^{2+} , Ag^+ , Cd^{2+} , Ni^{2+} , Mg^{2+} , and 10.0 μM of Zn^{2+} . Excitation wavelength was 469 nm.

Fig.3. Fluorescence intensity of HL and its complexation with Zn²⁺ in the presence of various metal ions. Black bar: HL (10.0 μ M) and HL with 1.0 equiv. of Mg²⁺, Mn²⁺, Ca²⁺, Ni²⁺, Ag⁺, K⁺, Li⁺, Co²⁺, Cr³⁺, Ba²⁺, Pb²⁺, Na⁺, Cd²⁺, Hg²⁺, Cu²⁺, Fe²⁺, Al³⁺ and Fe³⁺ stated. Red bar: 10.0 μ M of HL and 1.0 equiv. of Zn²⁺ and 10.0 μ M of HL and 1.0 equiv. of Zn²⁺ with 1.0 equiv. of metal ions stated (λ_{ex} =469 nm).

Fig.4. Fluorescence spectra of HL (10 μM) in ethanol and water (9:1, v/v) upon the addition of Zn²⁺ (0-11 μM) with an excitation of 469 nm.

Fig. 5. ¹H NMR spectra of HL with metal ions in DMSO-d₆: (I) HL; (II) HL with Zn²⁺

Fig.6. (a) Changes in the absorption spectra of HL (10 μM) in ethanol and water (9:1, v/v) at room temperature as a function of added Cu^{2+} (0, 2, 4, 6, 7, 8, 9, 10, 11 μM).

(b) Changes in the absorption spectra of 10.0 μM of L-Cu in ethanol and water (9:1, v/v) at room temperature as a function of added S^{2-} (0–5 equiv.).

Fig.7. Fluorescence spectra of L-Cu ($10\ \mu\text{M}$) upon the addition of anions (1.0 equiv.) of F^- , Cl^- , Br^- , I^- , SCN^- , CO_3^{2-} , SO_4^{2-} , NO_3^- , Ac^- , N_3^- , ClO_4^- , BF_4^- , PF_6^- , $H_2PO_4^-$ and S^{2-} in ethanol and water (9:1, v/v). Excitation wavelength was 469 nm.

Fig.8. Fluorescence intensity of L-Cu with S²⁻ in the presence of various anions Black bar: HL (10.0 μ M) and Cu²⁺; HL and Cu²⁺ with 10 equiv. of various anions: 1. H₂PO₄⁻, 2. SCN⁻, 3. BF₄⁻, 4. ClO₄⁻, 5. PF₆⁻, 6. Ac⁻, 7. NO₃⁻, 8. SO₄²⁻, 9. CO₃²⁻, 10. Cl⁻, 11. Br⁻, 12. F⁻, 13. I⁻, 14. N₃⁻; 15. L-Cu stated. Red bar: 10.0 μ M of HL and Cu²⁺ and S²⁻; 10.0 μ M of HL and Cu²⁺ and S²⁻ with 10 equiv. of anions: 1. H₂PO₄⁻, 2. SCN⁻, 3. BF₄⁻, 4. ClO₄⁻, 5. PF₆⁻, 6. Ac⁻, 7. NO₃⁻, 8. SO₄²⁻, 9. CO₃²⁻, 10. Cl⁻, 11. Br⁻, 12. F⁻, 13. I⁻, 14. N₃⁻; 15. L-Cu+S²⁻ stated (λ_{ex} =469 nm).

Scheme 1. Reagents and conditions: (a) (i) acetone, aq KOH, reflux, 15 h; (ii) conc HCl; (b) conc H₂SO₄, EtOH, reflux, 18 h; (c) EtOH, N₂H₄·H₂O, 80°C, 20 h ; (d) EtOH, diethylmalonate, piperidine , reflux, 10-12 h. (e) HCl (37%,) and glacial acetic acid ; (f) Anhydrous DMF, POCl₃; (g) EtOH, reflux, 10 h.

Scheme 2. Proposed mechanism for detection of Zn^{2+} by HL.

Scheme 3. Proposed mechanism for detection of $\text{Cu}^{2+}/\text{S}^{2-}$ by HL.

Graphical abstract