Supporting Information for

Substituent effect on fluorophores instead of ionophores: its implication in highly selective fluorescent probe for Zn^{2+} over Cd^{2+}

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Materials. All chemicals and reagents were used directly as obtained commercially unless otherwise stated. All solvents were of reagent grade, The salt used in stock solutions of metal ions were $ZnCl_2$, $CaCl_2$, $MgSO_4$, KNO_3, $Ni(NO_3)_2\cdot 6H_2O$, $Co(OAc)_2\cdot 4H_2O$, $Mn(OAc)_2\cdot 4H_2O$, $Cu(NO_3)_2\cdot 3H_2O$, $Cd(OAc)_2\cdot 6H_2O$, $FeCl_2\cdot 4H_2O$, $AgNO_3$, $Hg(OAc)_2$, $Pb(OAc)_2\cdot 3H_2O$. Water used was ultra filter deionized.

Measurements

Absorption and emission spectra were collected by using a Shimadzu 1750 UV-visible spectrometer and a RF-5301 fluorescence spectrometer (Japan), respectively. NMR spectra were collected on a Bruker 500 avance III spectrometer. Chemical shifts (δ) were reported as ppm with TMS as the internal standard. Mass spectrometric (MS) data were obtained with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments.

Sample Preparation and Titration. Stock solutions of metal ions were prepared in deionized water. The concentration are fixed to 1.0×10^{-2} M. Stock solution of fluorescent sensors (5.0×10^{-4} M) were prepared in EtOH and then further diluted to 5.0×10^{-6} M for titration experiments. UV and fluorescence spectra were monitored within 15 seconds.

Calculation of quantum yield. The quantum yield of the sample was measured using quinine sulfate as the standard ($\Phi = 0.53$, 0.1 M H_2SO_4)[1] and calculated using eq 1:

$$\Phi_{\mathrm{unk}} = \Phi_{\mathrm{std}} \times \left(\frac{I_{\mathrm{unk}}}{I_{\mathrm{std}}}\right) \times \left(\frac{A_{\mathrm{std}}}{A_{\mathrm{unk}}}\right) \times \left(\frac{n_{\mathrm{unk}}}{n_{\mathrm{std}}}\right)^2 \quad (1)$$

Where Φ_{unk} is the fluorescence quantum yield of the sample, Φ_{std} is the fluorescence quantum yield of the standard, I_{unk} and I_{std} are the integrated emission intensities of the sample and the standard, respectively, A_{unk} and A_{std} are the absorbance of the sample and the standard at the excitation wavelength, respectively, and n_{unk} and n_{std} are the refractive indexes of the corresponding solution.

Calculation of association constants and detecting limit. The association constants were calculated employing Benesi-Hildebrand method[2] using equation (2), where K is the association constant, I is the fluorescence intensity of the free **H-3**, I_0 is the observed fluorescence intensity of the **H-3**·Zn^{2+} complex, and I_{fc} is the fluorescence intensity at the saturation. The plot of $1/(I\text{-}I_0)$ vs $1/[Zn^{2+}]$ gave a linear fitting, indicating a 1:1 stoichiometry between the **H-3** and Zn^{2+} ions. Detecting limit DL = $K \times S_{b1}/S$, where K=3, S_{b1} is the standard derivation of the blank solution and S is the slope of the calibration curve[3].

$$1/(I-I_0)=1/(I-I_{fc})+1/K(I-I_0)[\mathrm{Zn}^{2+}] \qquad (2)$$

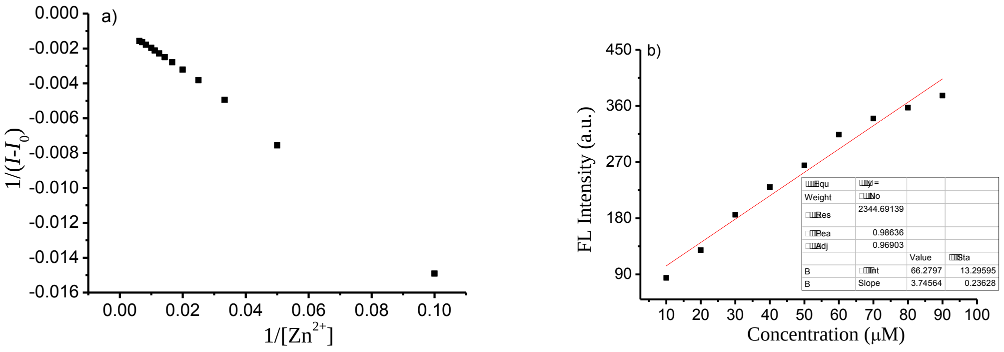


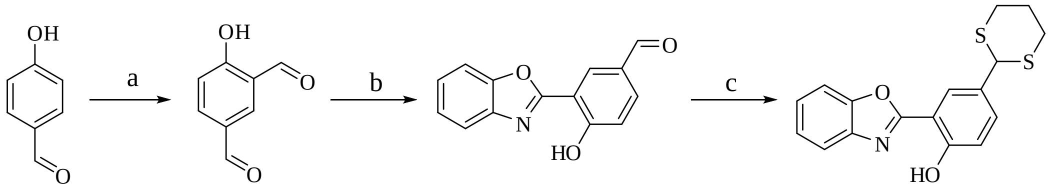
Fig S0. a) The plot of $1/(I\text{-}I_0)$ to $1/[Zn^{2+}]$; b) The plot of fluorescent intensity at 420 nm to the concentration of Zn^{2+}.

[1] J. N. Demasa and G. A. Crosby, *J. Phys. Chem.*, **1971**, *75*, 991-1024.

[2] (a) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **1949**, *71*, 2703. (b) L. L. Yannis, *J. Phys. Chem. B*, **1997**, *101*, 4863. (c) V. S. Jisha, A. J. Thomas and D. Ramaiah, *J. Org. Chem.* **2009**, *74*, 6667.

[3] A. Hakonen, Anal. Chem., **2009,** *81*, 4555-4559.

Synthesis.



H-2

H-3

2,4-dialdehydephenol

Hexamethylenetetramine (5.74 g, 41 mmol) was added to a solution of 4-hydroxybenzaldehyde (5.00 g, 41 mmol) in trifluoroacetic acid (45 mL) under N_2. Then the mixture was heated at 90°C for 24 hours. The reaction was quenched with 60 mL of 3 N HCl and cooled to room temperature. The mixture was extracted with CH_2Cl_2 (4×50 mL) and combined. After evaporation of the solvent, the solid residues were collected and purified on a silica gel column by using an eluant (petroleum ether: CH_2Cl_2 =3:1), the product was obtained as a white solid (2.35 g, 38%).

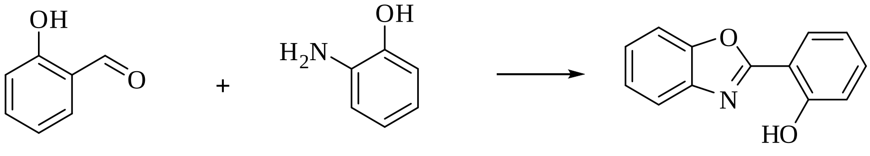
2-(2'-hydroxy-5'-formylphenyl)benzoxazole (H-2)

2,4-dialdehyde phenol (0.2 g, 1.32 mmol) was added to a solution of o-aminophenol (0.144 g, 1.32 mmol) in methanol (15 mL). After addition, the mixture was heated at 45 °C for 12 hours. Then the solvent was removed using rotary evaporator under reduced pressure. After that the solution of 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.3 g, 1.32 mmol) in 100 mL CH_2Cl_2 was added to the above residue. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was treated with 100 mL saturated Na_2CO_3, then washed with water, and dried over Na_2SO_4. After evaporation of the solvent, the solid residues were collected and purified on a silica gel column by using an eluant (petroleum ether: CH_2Cl_2 =2:1), the product **H-2** was obtained as a white solid (0.2 g, 66.7%). ^1H NMR (500 MHz, $CDCl_3$) δ=12.22 (s, 1H), 9.99 (s, 1H), 8.59 (s, 1H), 8.01 (d, 1H, *J*=10.75), 7.78 (d, 1H, *J*=8.10), 7.67 (d, 1H, *J*=8.35), 7.46 (m, 2H), 7.25 (s, 1H). ^{13}C NMR (100

MHz, $CDCl_3$) δ=110.91, 111.05, 118.37, 119.47, 125.41, 126.08, 129.01, 130.27, 134.11, 139.54, 149.19, 161.72, 163.48, 189.89. *m/z* (TOF-LD): Calcd. $[M+H]^+$ For $C_{14}H_9NO_3$: 240.0660, found: 240.0652.

2-[2'-hydroxy-5'-(1,3-dithiane)phenyl]benzoxazole (H-3)

1,3-dimercaptopropane (0.054 g, 0.6mmol) was added to a solution of compound **H-2** (0.11 g, 0.5 mmol) in $CHCl_3$. Then 0.013 g I_2 (0.05 mmol) was added to the above mixture. After addition, the mixture was stirred at room temperature for 8 hours. The reaction mixture was washed with 20 mL water. The organic layer was collected and the solvent was removed using rotary evaporator, the product **3** was obtained as a white solid (0.14 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 8.11 (d, 1H, *J*=2.95), 7.74 (m, 1H), 7.62 (m, 1H), 7.54 (dd, 1H, *J*=10.78, 2.92), 7.40 (m, 2H), 7.10 (d, 1H, *J*=11.05), 5.21 (s, 1H), 3.14 (m, 2H), 2.91 (m, 2H), 2.30 (m, 2H). δ= 25.01, 32.13, 36.88, 50.46, 110.68, 110.73, 117.79, 119.31, 125.06, 125.53, 126.51, 130.49, 133.09, 139.95, 149.17, 158.71, 162.48. *m/z* (TOF-LD): Calcd. $[M+H]^+$ For $C_{17}H_{15}NO_2S_2$: 330.0622, found: 330.0613.



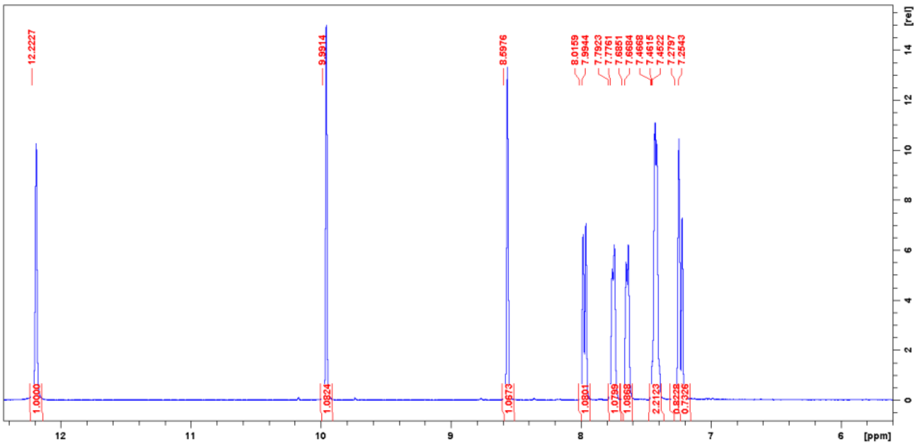
H-1

2-(2'-hydroxyphenyl)benzoxazole (H-1)

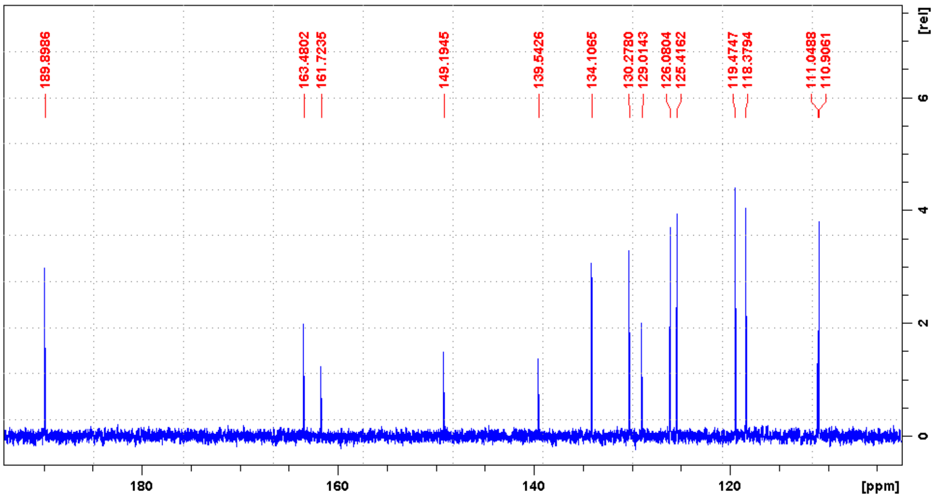
2-Hydroxybenzaldehyde (1.12 g, 9.2 mmol) was added to a solution of *o*-aminophenol (1.00 g, 9.2 mmol) in methanol (35 mL). After addition, the mixture was heated at 45°C for 12 hours. Then the solvent was removed using rotary evaporator. After that the solution of DDQ (2.0 g, 9.2 mmol) in 150 mL CH_2Cl_2 was added to the above residue. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was treated with 100 mL saturated Na_2CO_3, then washed with water, and dried over Na_2SO_4. After evaporation of the solvent, the solid residues were collected and purified on a silica gel column by using an eluant (petroleum ether: CH_2Cl_2 =2:1), the product **H-1** was obtained as a white solid (0.84 g, 45%). δ=11.51 (s, 1H), 8.83 (dd, 1H, *J*=2.05, 9.90), 7.76 (m, 1H), 7.64 (m, 1H), 7.47 (m, 1H), 7.42

(m, 2H), 7.15 (d, *J*=11.05, 1H), 7.05 (m, 1H). *m/z* (TOF-LD): Calcd. $[M+H]^+$ For $C_{13}H_9NO_2$: 212.0711, found: 212.0703.

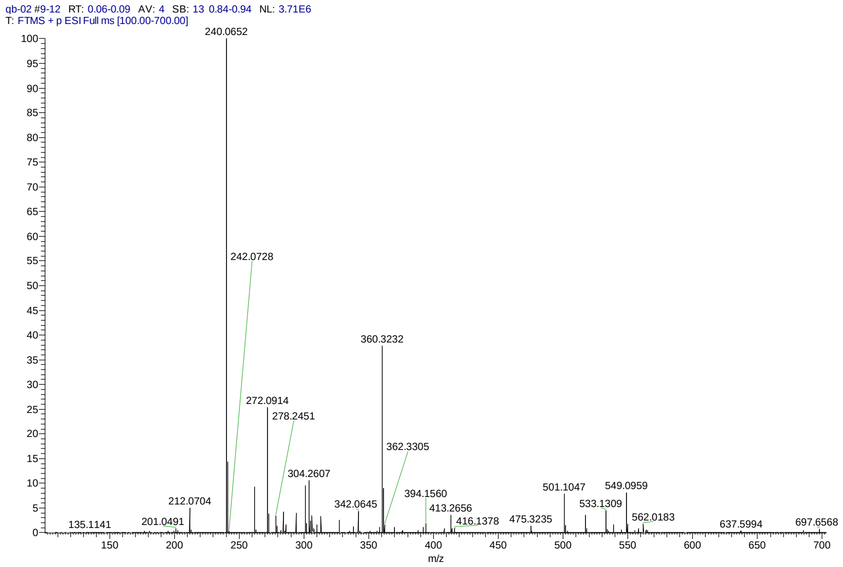
^{1}H NMR spectrum of **H-2**



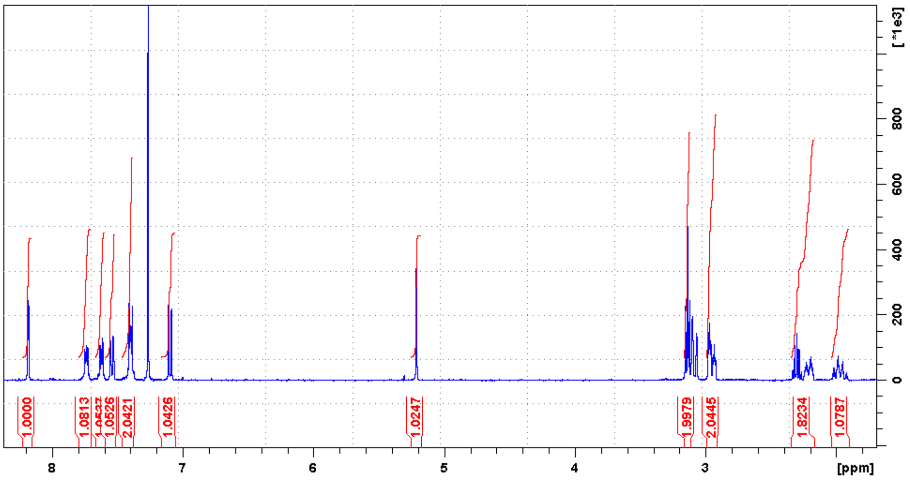
^{13}C NMR spectrum of **H-2**



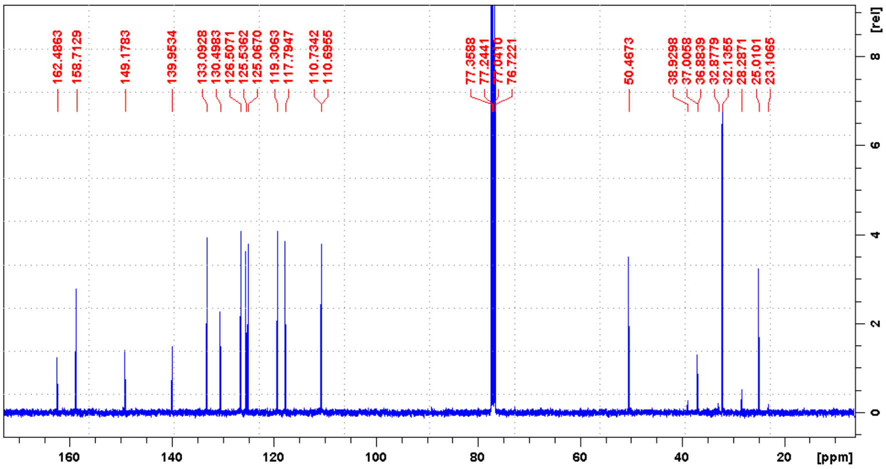
HRMS spectrum of **H-2**



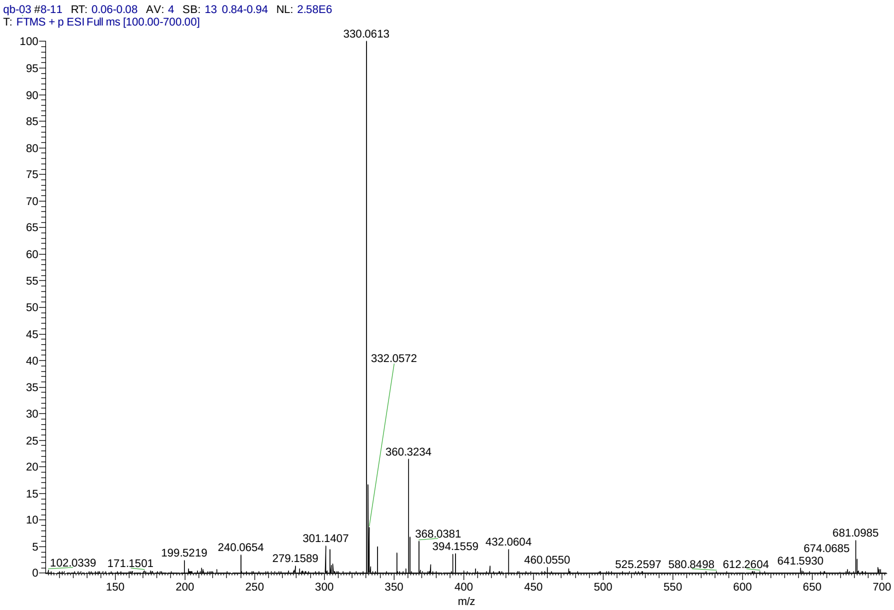
^{1}H NMR spectrum of **H-3**



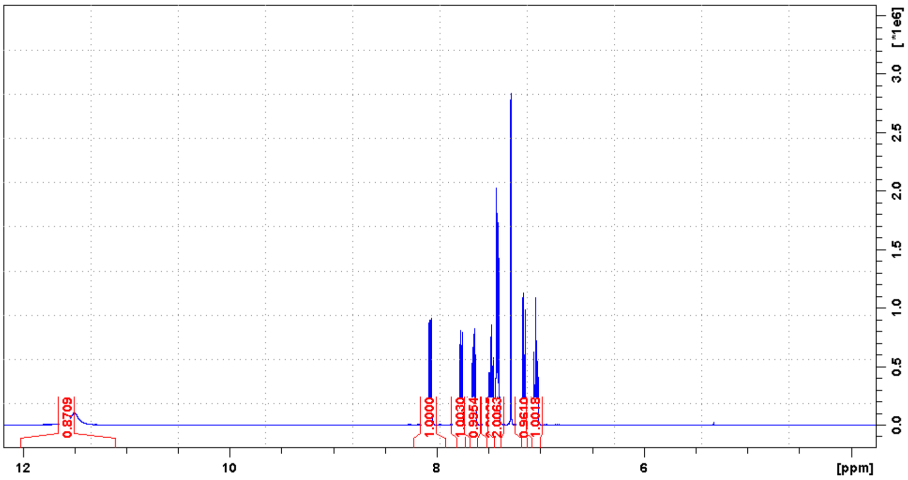
^{13}C NMR spectrum of **H-3**



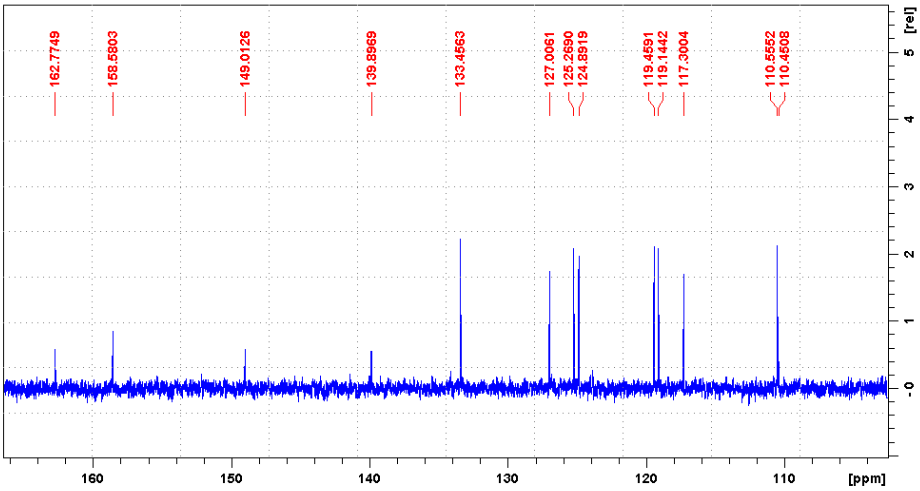
HRMS spectrum of **H-3**



^{1}H NMR spectrum of **H-1**



^{13}C NMR spectrum of **H-1**



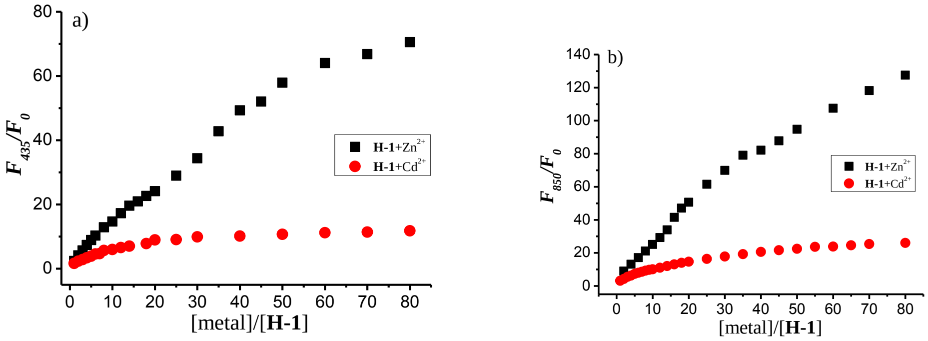


Fig. S1 The relative fluorescence intensity changes at 435 (a) and 850 nm (b) of **H-1** (10 μM) in aqueous solution upon addition of Zn^{2+} and Cd^{2+} (λ_{ex}=350 nm).

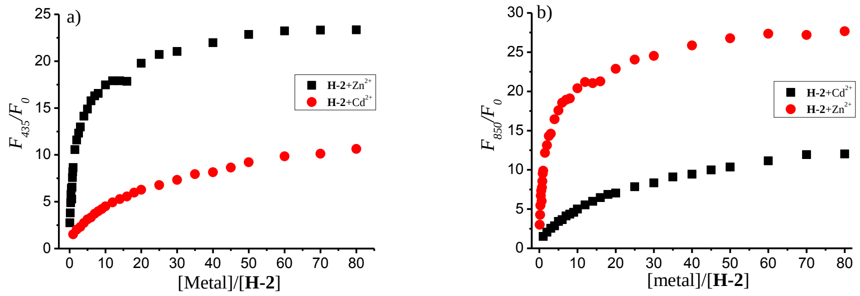


Fig. S2 The relative fluorescence intensity changes at 435 (a) and 850 nm (b) of **H-2** (10 μM) in aqueous solution upon addition of Zn^{2+} and Cd^{2+} (λ_{ex}=350 nm).

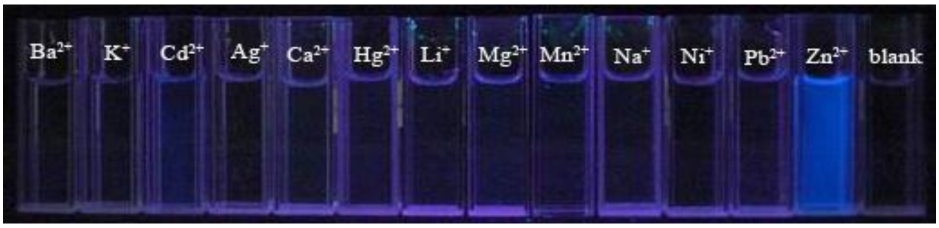


Fig. S3 Fluorescence change of **H-3** (5.0×10^{-5} M) with various metal ions in aqueous solution, where [metal ions] = 1.5×10^{-3} M, excitation by hand-hold UV lamp.

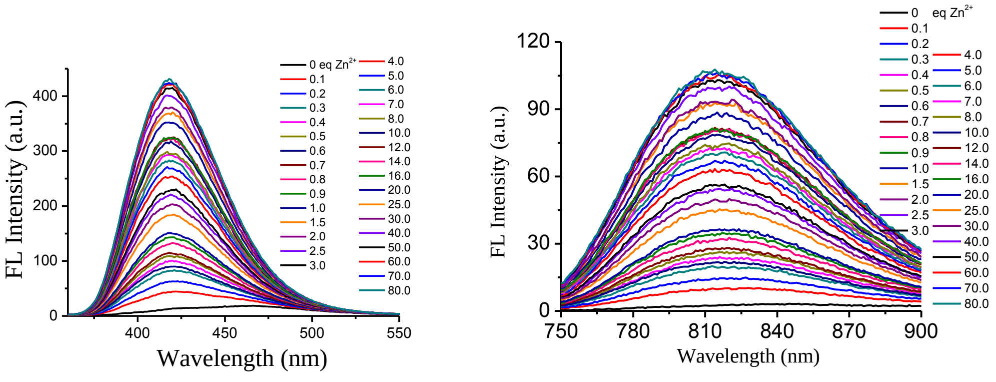


Fig. S4 The fluorescent spectra change of **H-2** (10 μM) at short and long wavelength region upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v), λ_{ex}=350 nm.

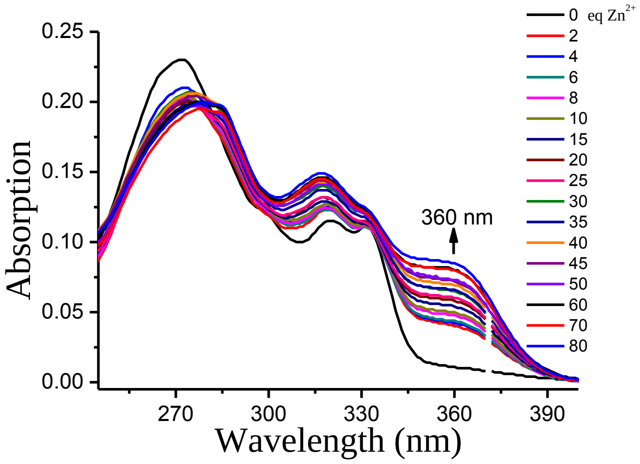


Fig. S5 The absorption spectra change of **H-2** (5 μM) upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v).

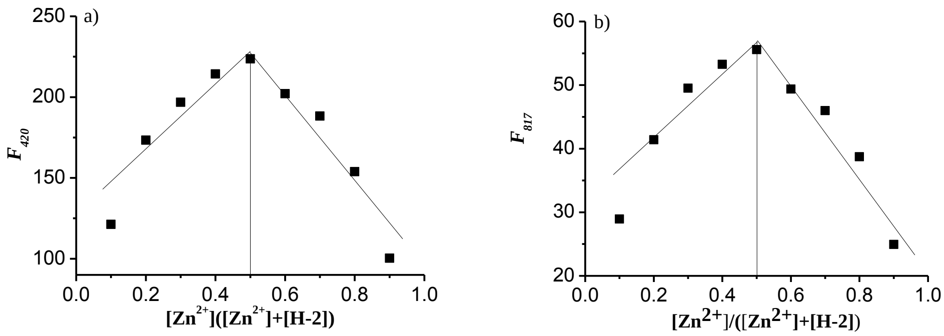


Fig. S6 The Job's plot of fluorescence intensity response of **H-2** at 420 (a) and 817 nm (b) to mole fraction of Zn^{2+} in EtOH-H_2O (1:1, V/V), λ_{ex}=350 nm.

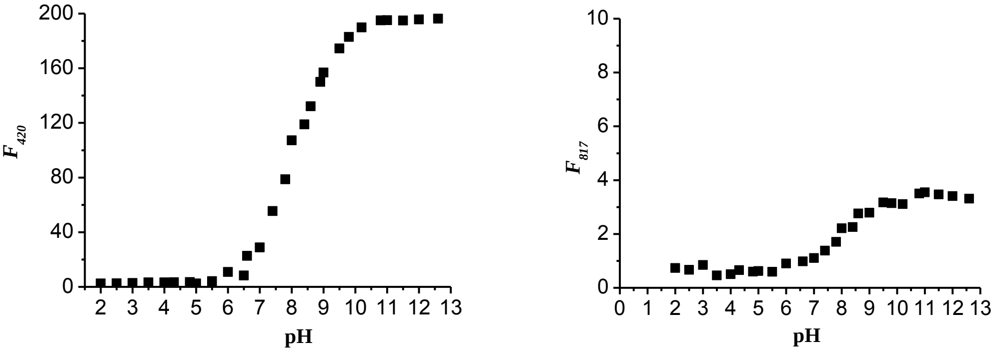


Fig. S7 The fluorescence intensity response of **H-2** (5 μM) at 420 and 817 nm to pH in EtOH-H_2O (1:1, V/V), λ_{ex}=350 nm.

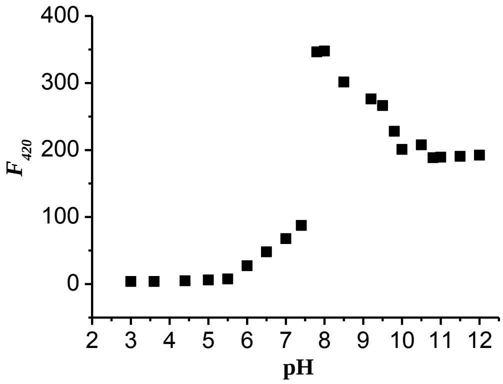


Fig. S8 The fluorescence intensity response of **H-2** (10 μM) at 420 nm to pH in EtOH-H_2O (1:1, V/V) in the presence of 1 equivalent of Zn^{2+}, λ_{ex}=350 nm.

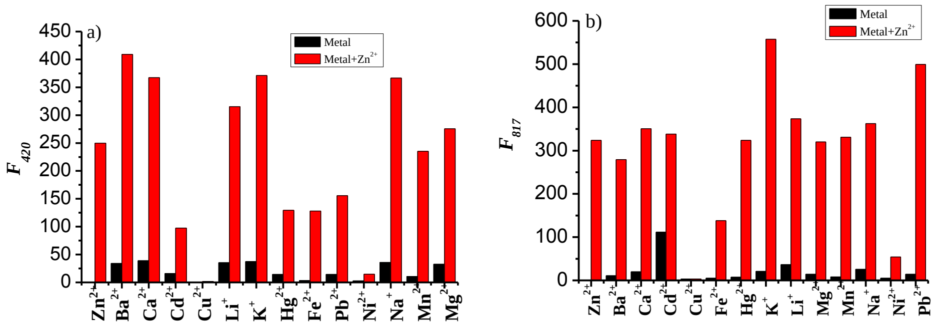


Fig. S9 Emission intensity of **H-2** (5 μM) at 420 (a) and 817 nm (b) in EtOH-H_2O (1:1, V/V) in the presence of different metal ions (150 μM) with the excitation at 350 nm (black bar). Red bars represent the intensity with subsequent addition of Zn^{2+} ions (150 μM).

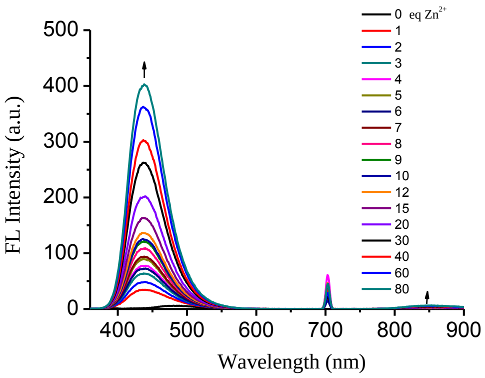


Fig. S10 The fluorescent spectra change of **H-3** (10 μM) upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v), λ_{ex}=350 nm.

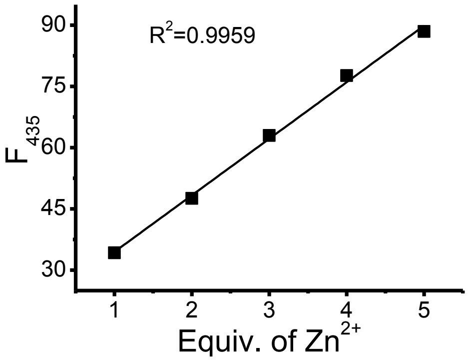


Fig. S11 The fluorescence intensity change of **H-3** (5 uM) at 435 nm upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v), indicative of good linear relationship.

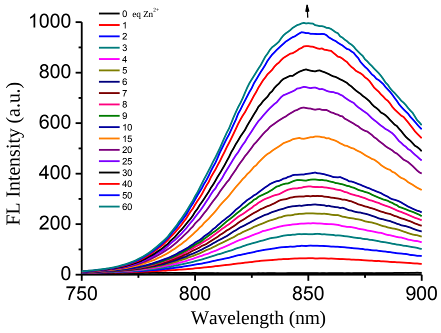


Fig. S12 The fluorescent spectra change of **H-3** (10 μM) in near infrared region upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v), λ_{ex}=350 nm.

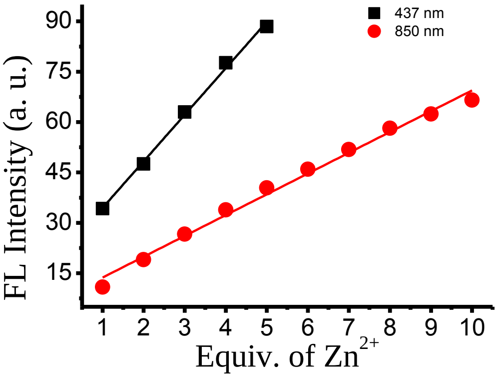


Fig. S13 The fluorescence intensity change of **H-3** (5 uM) at 437 and 850 nm upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v), indicative of good linear relationship.

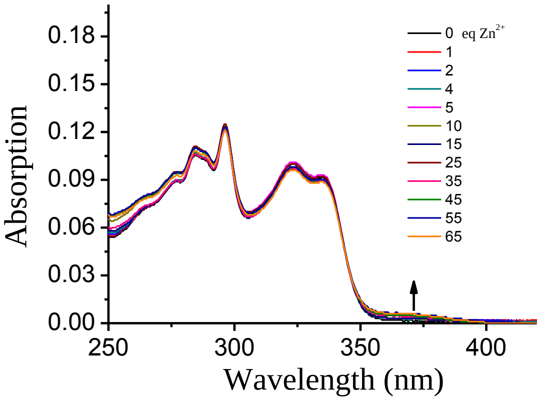


Fig. S14 The absorption spectra change of **H-3** (5 μM) upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v).

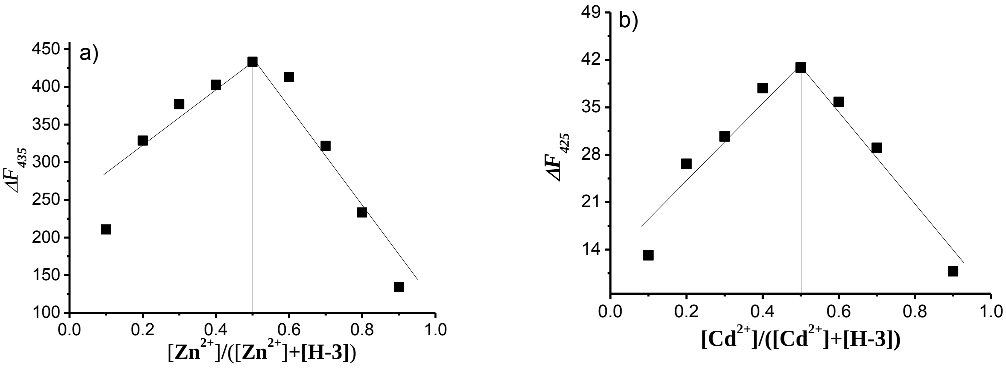


Fig. S15 The Job's plot of fluorescence intensity response of **H-3** to mole fraction of Zn^{2+} at 435 nm (a) and mole fraction of Cd^{2+} at 425 in EtOH-H_2O (1:1, V/V), λ_{ex}=350 nm.

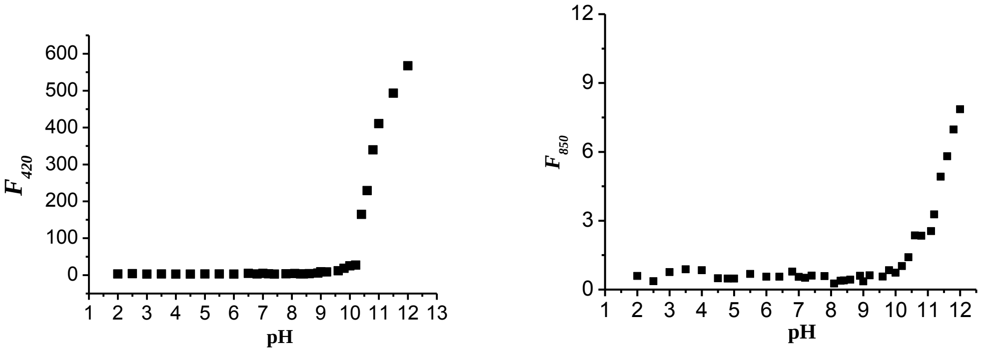


Fig. S16 The fluorescence intensity response of **H-3** (5 μM) at 420 and 850 nm to pH in EtOH-H_2O (1:1, V/V), λ_{ex}=350 nm.

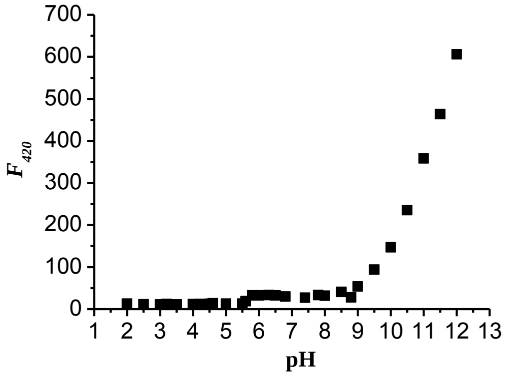


Fig. S17 The fluorescence intensity response of **H-3** (5 μM) at 420 nm to pH in EtOH-H_2O (1:1, V/V) in the presence of 1 equivalent of Zn^{2+}, λ_{ex}=350 nm.

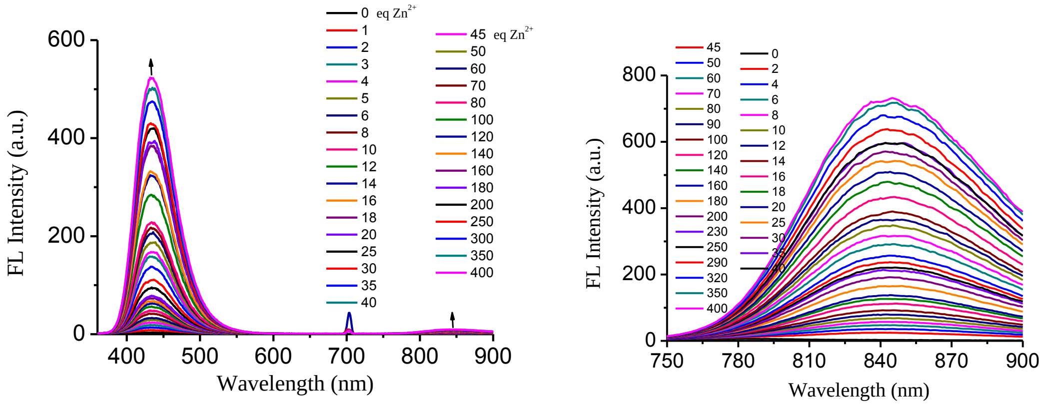


Fig. S18 The fluorescent spectra change of **H-1** (5 μM) at short and long wavelength region upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v), λ_{ex}=350 nm.

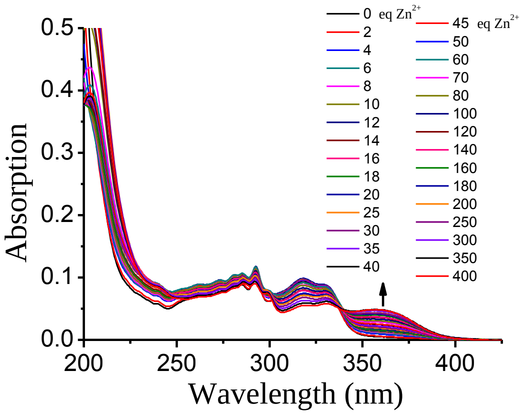


Fig. S19 The absorption spectra change of **H-1** (5 μM) upon addition of Zn^{2+} in EtOH-H_2O (1:1, v/v).

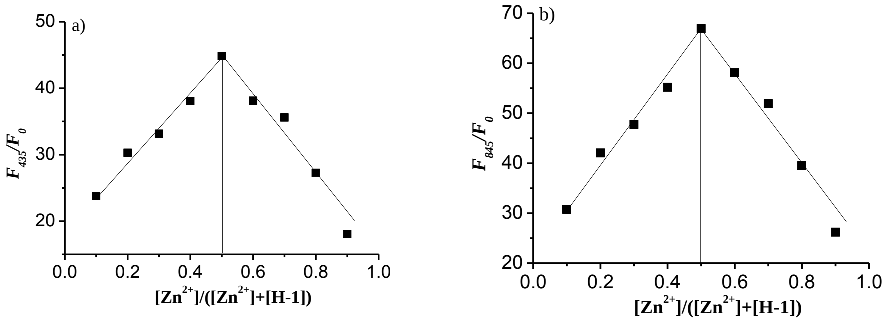


Fig. S20 The Job's plot of fluorescence intensity response of **H-1** at 435 (a) and 845 nm (b) to mole fraction of Zn^{2+} in EtOH-H_2O (1:1, V/V), λ_{ex}=350 nm.

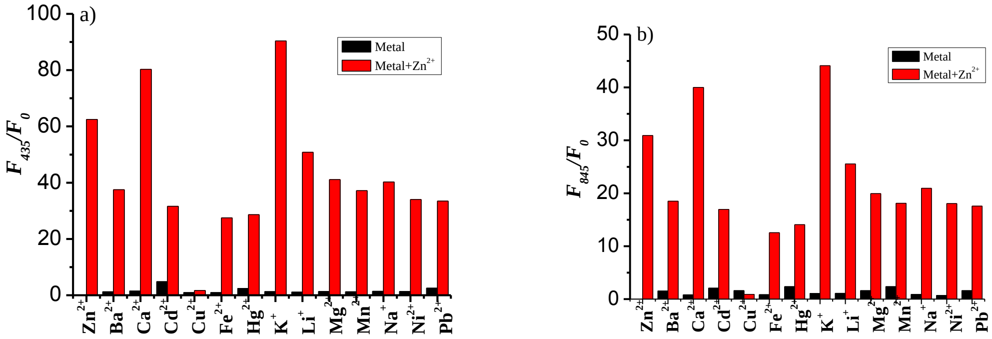


Fig. S21 Emission intensity of **H-1** (5 μM) at 420 (a) and 845 nm (b) in EtOH-H_2O (1:1, V/V) in the presence of different metal ions (150 μM) with the excitation at 350 nm (black bar). Red bars represent the intensity with subsequent addition of Zn^{2+} ions (150 μM).

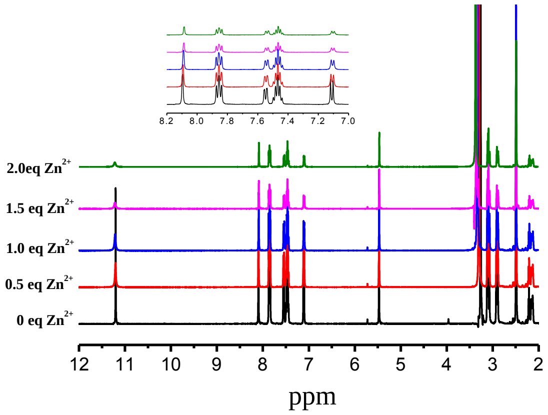


Fig. S22 ^{1}H NMR titration of **H-3** in DMSO-d_6 upon addition of Zn^{2+} (Inset: the enlarged spectra in the region of 7.0-8.2).

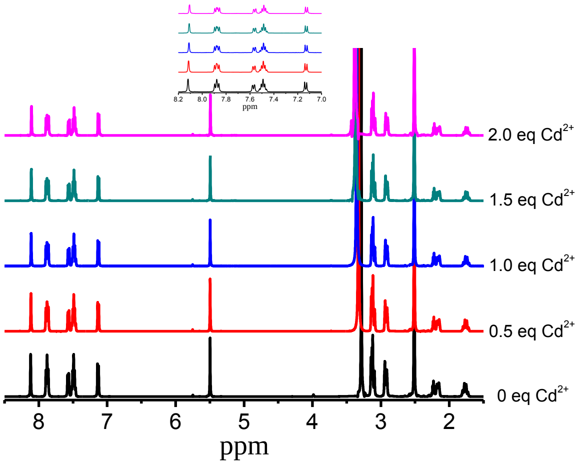


Fig. S23 ^{1}H NMR titration of **H-3** in DMSO-d_6 upon addition of Cd^{2+} (Inset: the enlarged spectra in the region of 7.0-8.2).

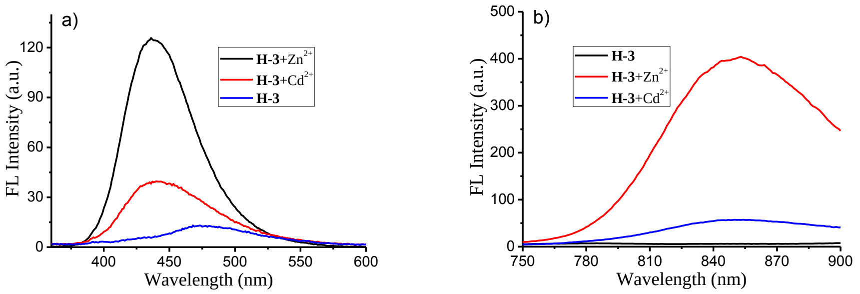


Fig. S24 Fluorescence spectra change of **H-3** (10 μM) in EtOH-H_2O (1:1, v/v) in the absence and presence of Zn^{2+} or Cd^{2+} (100 μM), λ_{ex}=350 nm.

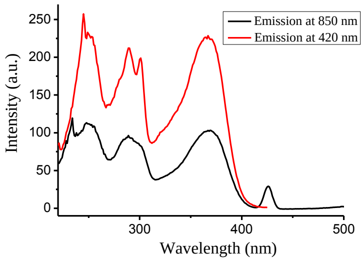


Fig. S25 Excitation spectra of probe **H-3** (10 μM) with 10 equivalent of Zn^{2+} in EtOH/H_2O (1:1, v/v).

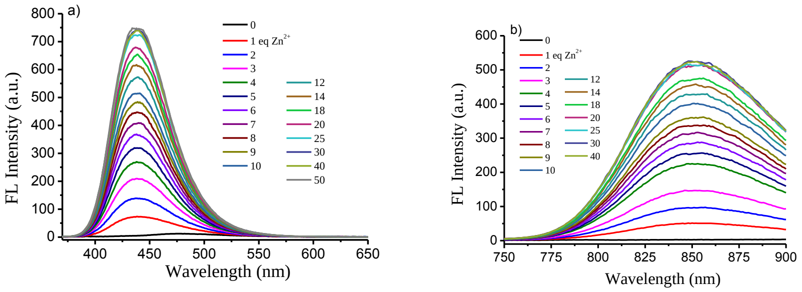


Fig. S26 Emission spectra change of probe H-3 (10 μM) at 420 (a) and 850 nm (b) with increasing concentration of Zn^{2+} in EtOH/HEPES buffer (pH 7.4, 1:1, v/v), λ_{ex}=350 nm.

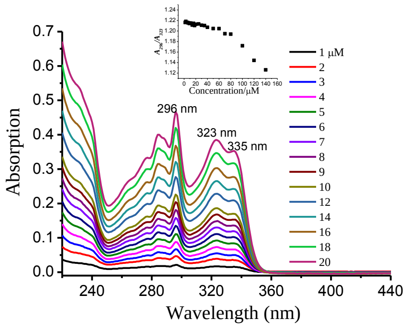


Fig. S27 The absorption spectra change of **H-3** with increasing concentration in EtOH-H_2O (1:1, v/v). Inset: the ratio change of A_{296}/A_{323} of **H-3** with increasing concentration.

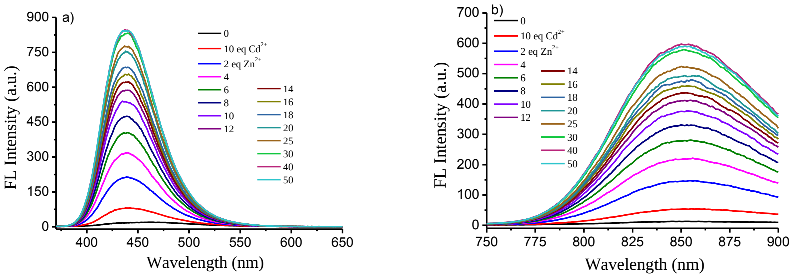


Fig. S28 Emission spectra change of **H-3** (10 μM) at 420 (a) and 850 nm (b) with increasing concentration of Zn^{2+} in the presence of 10 equivalent of Cd^{2+} in EtOH/HEPES Buffer (pH 7.4,1:1, v/v), λ_{ex}=350 nm.