Supplementary Information

For

Zn^{2+}-Trigged Excited-State Intramolecular Proton Transfer: A Sensitive Probe with Near-infrared Emission from Bis(benzoxazole) Derivative

Yongqian Xu and Yi Pang*

Department of Chemistry & Maurice Morton Institute of Polymer Science, The University of Akron, Akron, OH, 44325
yp5@uakron.edu

1. Absorption Spectra

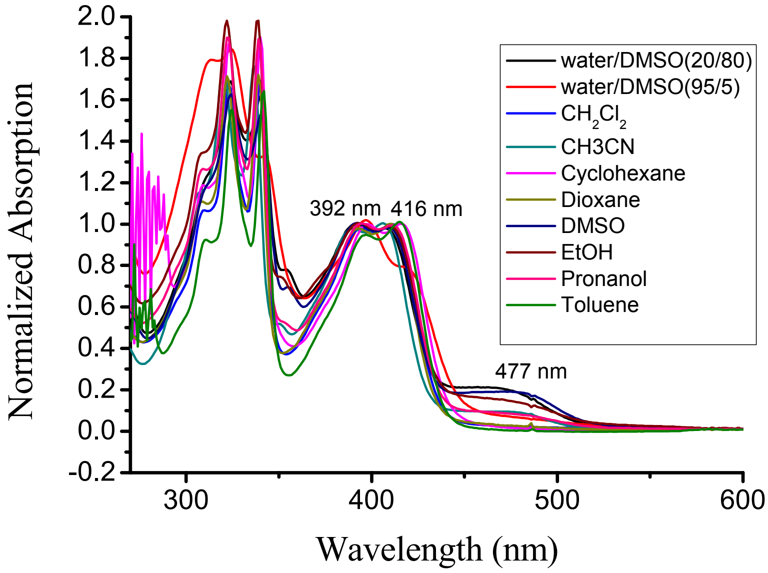


Fig. S1. UV-vis absorption spectra of **Zinhbo-1** in different solvents.

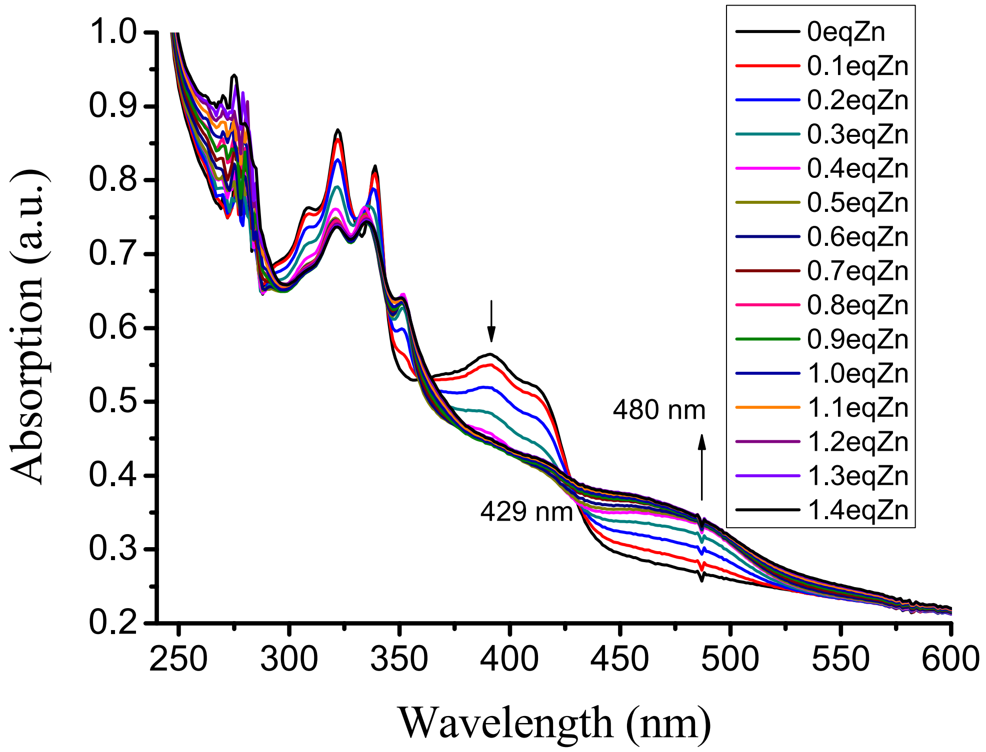


Fig. S2. Change in the UV-vis spectra for **Zinhbo-1**(1.0×10^{-5} M) in THF upon addition of zinc ions.

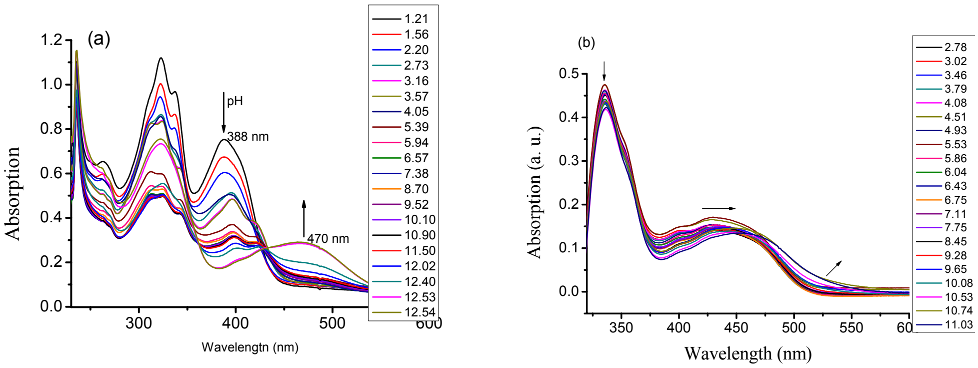


Fig. S3. Change in the UV-vis spectra for **Zinhbo-1** (2.0×10^{-6} M) in absence (a) and presence (b) of 1 equiv zinc ions in DMSO/H_2O(5:95) at different pH.

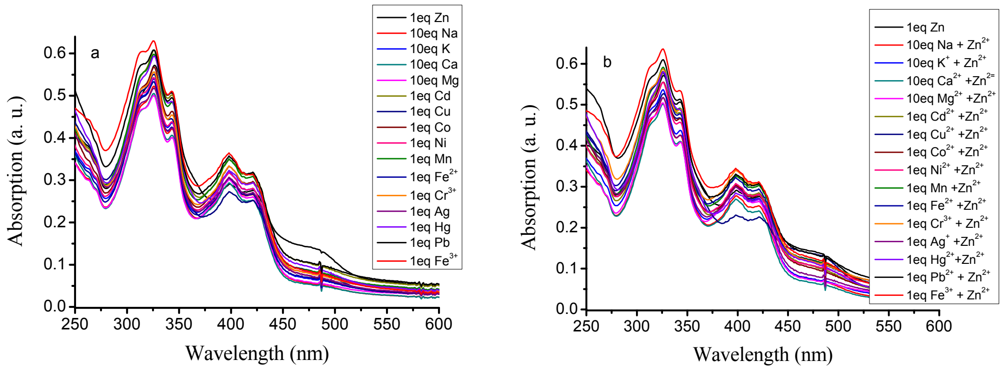
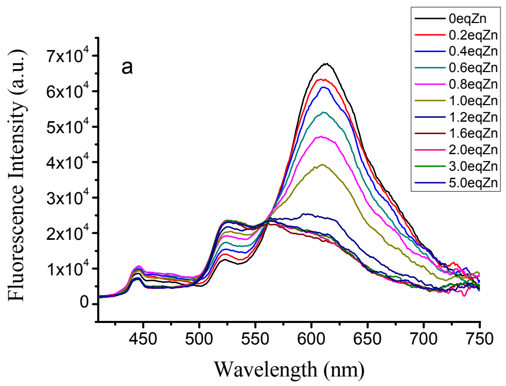
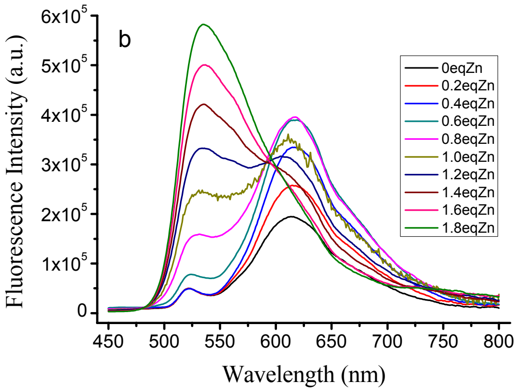


Fig. S4. Absorption spectra of **Zinhbo-1** (30 µ M) in absence (a) and presence (b) of 1 equiv zinc ions in HEPES buffer system containing 5% DMSO upon addition of different metal ions (30 µ M heavy metal or 0.3 mM Na^+, K^+, Ca^{2+}, Mg^{2+}).

2. Fluorescence Spectra





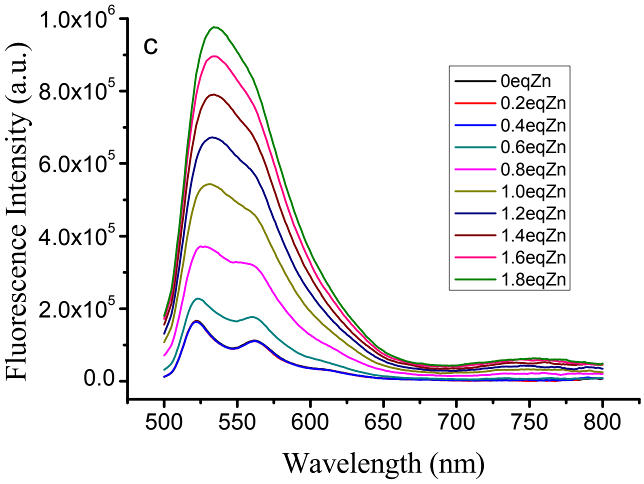


Fig. S5. Change in the fluorescence spectra for **Zinhbo-1**(1.0×10^{-5} M) in THF with addition of zinc ions upon excitation at (a) 390, (b) 429 and (c) 480 nm.

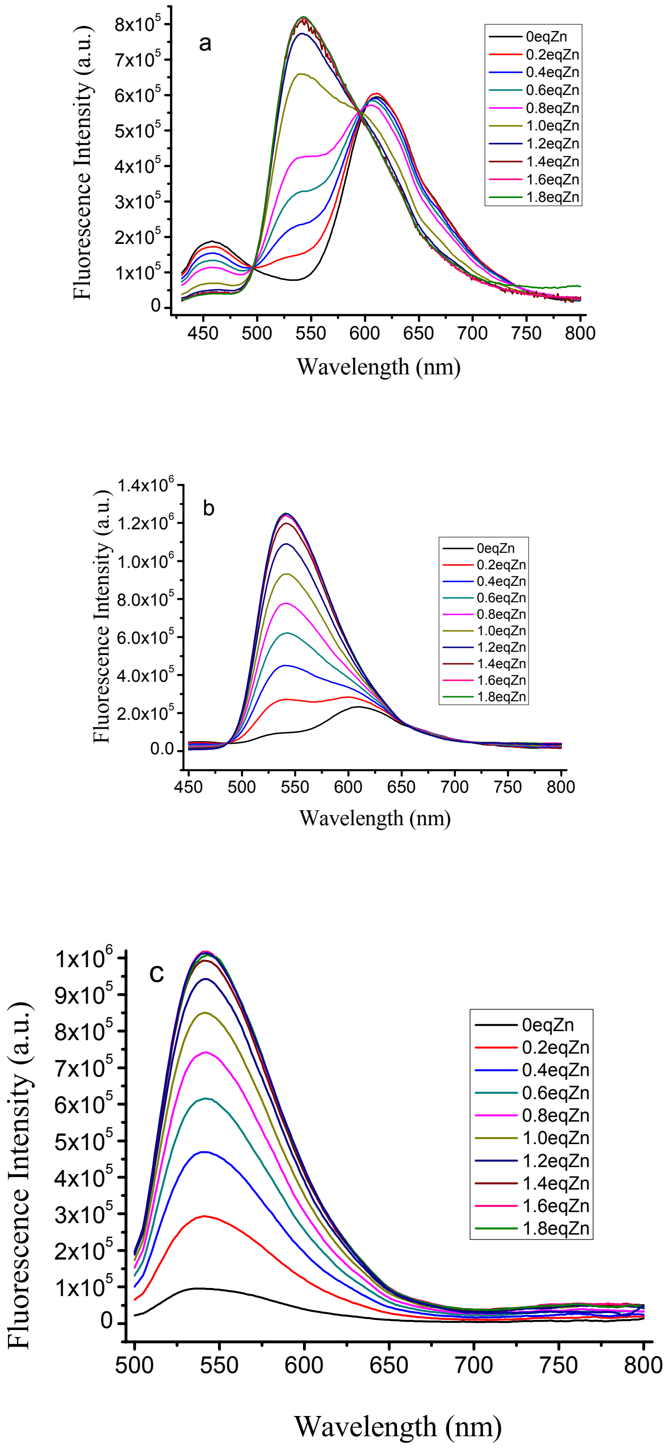


Fig. S6. Change in the fluorescence spectra for **Zinhbo-1**(1.0 × 10^{-5} M) in DMSO with addition of zinc ions upon excitation at (a) 410, (b) 427 and (c) 480 nm.

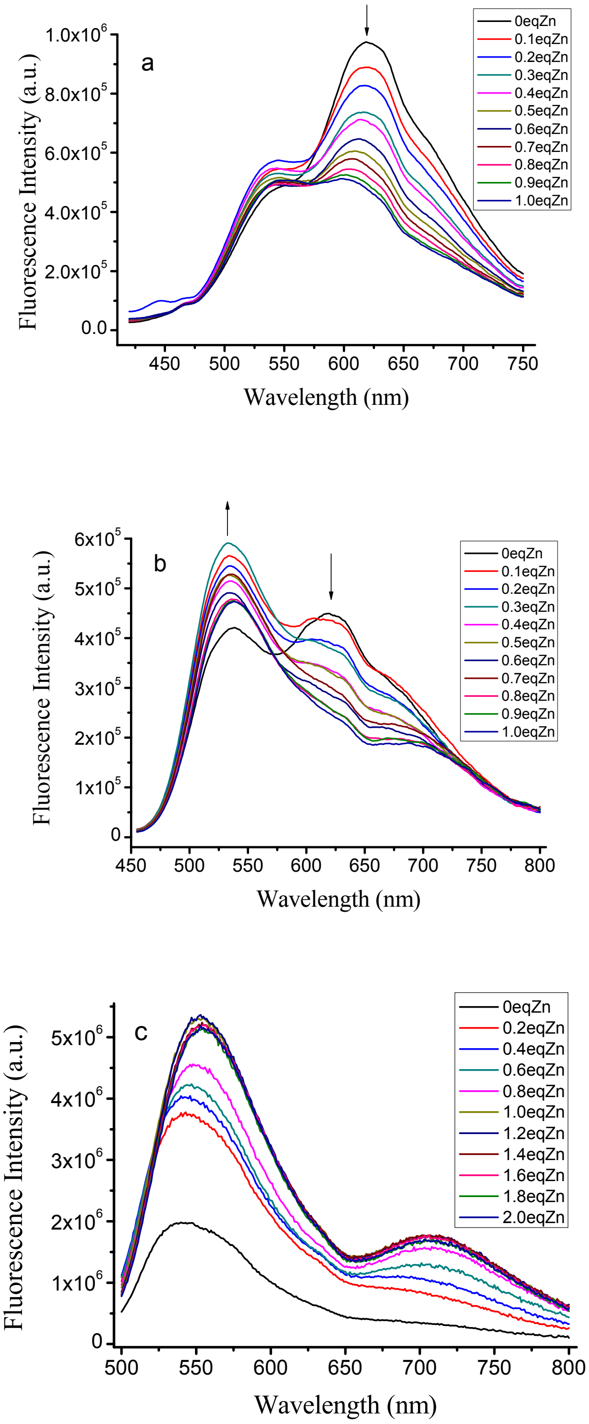


Fig. S7. Emission spectra of **Zinhbo-1**(10 μM) in HEPES buffer system containing 5% DMSO upon addition of zinc ions with the excitation at (a) 400, (b) 435 and (c) 480 nm.

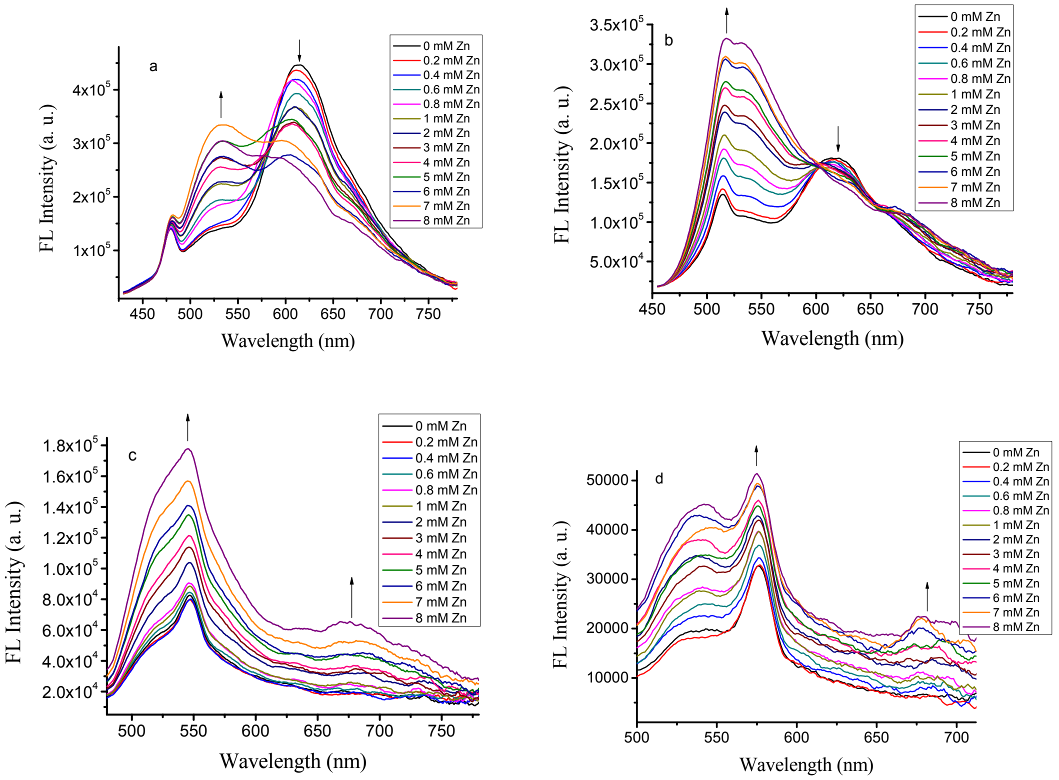


Fig. S8. Emission spectra of **Zinhbo-1**(1.5 μM) in HEPES buffer system containing 5% DMSO and 10 mM EGTA upon addition of zinc ions with the excitation at (a) 410, (b) 435, (c) 460 and 480 nm (d).

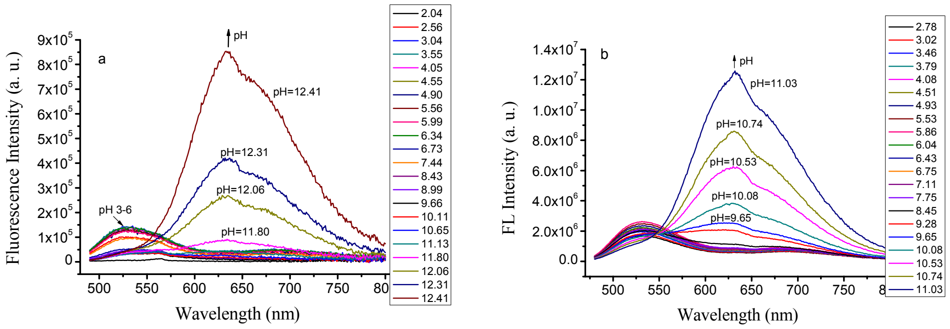


Fig. S9. Change in the fluorescence spectra for **Zinhbo-1**(1.0×10^{-6} M) in absence (a) and presence (b) of 1 equiv of zinc ion in DMSO/H_2O(5:95) with different pH (excitation at 470 nm). Left: In acidic solutions (pH=3-6), emission band was at ~530 nm, attributing to the *enol* form. In the basic solution (pH>12), the emisson was near ~635 nm, originating from the phenoxide anion.

6. Determination of binding constant.[S1]

A series of HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer solutions (50 mM, pH 7.20, 0.1 M KNO_3) containing various amounts of Zn^{2+} (0 ~ 9.5 mM) and 10 mM of EGTA (ethylenebis(oxyethylenenitrilo)tetraacetic acid) were prepared. The concentration of free Zn^{2+} was calculated with $[EGTA]_{total}$, $[Zn^{2+}]_{total}$, and $K'_{Zn\text{-}EGTA}$, the apparent binding constant at a given pH and ionic strength. $K'_{Zn\text{-}EGTA}$ value was calculated from Eq. 1 at pH = 7.20

$$K'_{Zn\text{-}EGTA} = K_{(ZnL)}(1+10^{(pK_{LMH}\text{-}pH)}/((1+10^{(pH\text{-}pK_{Zn})})(1+10^{(pK_1\text{-}pH)}+10(^{(pK_1+pK_2\text{-}2pH)})) \quad \text{(Eq. 1)}$$

using the following published pK and log K values for EGTA; pK_1 = 9.40, pK_2 = 8.79, pK_3 =2.70, pK_{LMH} = 9.40, log $K_{(ZnL)}$ = 12.6 (25 °C, μ = 0.1 M). All protonation constants were corrected upward by 0.11 when working in 0.1 M ionic strength. Thus, $K'_{Zn\text{-}EGTA}$ value at pH 7.20, 0.1 M ionic strength is 3.80×10^8 M^{-1}.

The calculated $[Zn^{2+}]_{free}$ concentration of each solution is:

$[Zn^{2+}]_{total}$ (mM)	*0.2*	*0.4*	*0.6*	*0.8*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	**8**
$[Zn^{2+}]_{free}$ (nM)	0.054	0.11	0.168	0.229	0.29	0.66	1.1	1.8	2.6	4.0	6.1	11

The intensities at 710 nm in the emission spectrum of each solution was measured with the excitation wavelength at 480 nm and was fitted to the following equation (Eq. 2).

$$F_0/(F\text{-}F_0) = \alpha/(K_s[M])+\alpha \qquad \text{(Eq. 2)}$$

Where $\alpha = a/(b\text{-}a)$, $[M] \approx C_M$ is Zn^{2+} ion concentration, a and b are proportional to the molar absorption coefficients (at the excitation wavelength) and the fluorescent quantum yields of the ligand and complex, respectively. K_s can be determined by a fit curve analysis of F versus C_M.

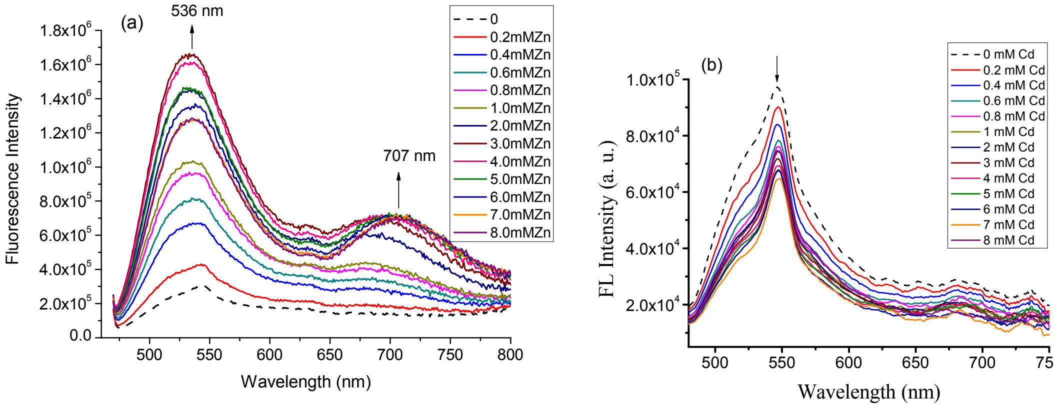


Fig.S10. Emission spectra of **Zinhbo-1**(1.5 μ M) in HEPES buffer system containing 5% DMSO and 10 mM EGTA upon addition of zinc ions (a) and Cd^{2+} (b) with the excitation at 460 nm. Low concentration of Cd^{2+} cation induced slight decrease in fluorescence, in contrast to the addition of Zn^{2+} cation under the same concentration range.

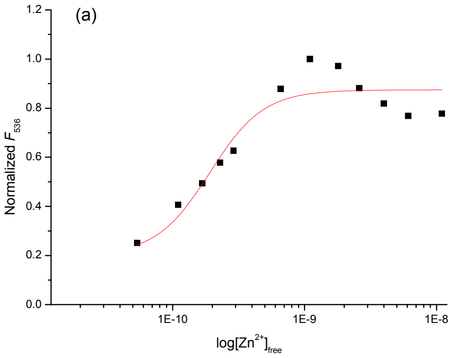


Figure 11. Plots of fluorescence of **Zinhbo-1** (1.5 μ M) in HEPES buffer system containing 5% DMSO and 10 mM EGTA upon addition of zinc ions. The fluorescence intensity at 536 was used to calculate dissociation constant. $K_d = 1.91 \pm 0.56 \times 10^{-10}$ M (detection at 536 nm)

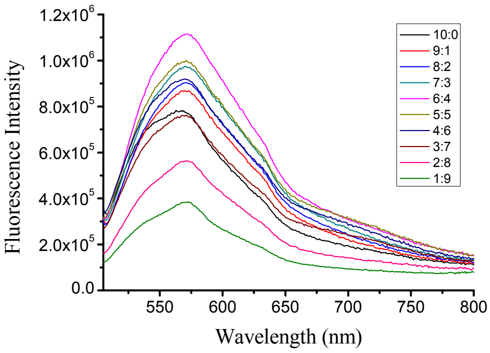


Fig. S12. Emission spectra of **Zinhbo-1**(1.5 μM) and zinc ions in HEPES buffer system containing 5% DMSO with the excitation at 460 nm.

3. Determination of quantum yield.[S2]

The quantum yield can be determined according to the literature.

$$\mathrm{QY_u} = \frac{(\mathrm{QY_s})(\mathrm{FA_u})(\mathrm{A_s})(\lambda_{\mathrm{exs}})(\eta_u^2)}{(\mathrm{FA_s})(\mathrm{A_u})(\lambda_{\mathrm{exu}})(\eta_s^2)}$$

where QY = quantum yield; FA = integrated area under the emission spectrum; A = absorbance at the excitation wavelength; λ_{ex} = the excitation wavelength; η = the refractive index of the solution; and the subscripts *u* and *s* refer to the unknown and the standard, respectively.

4. Fluorescence spectra with various metal ions

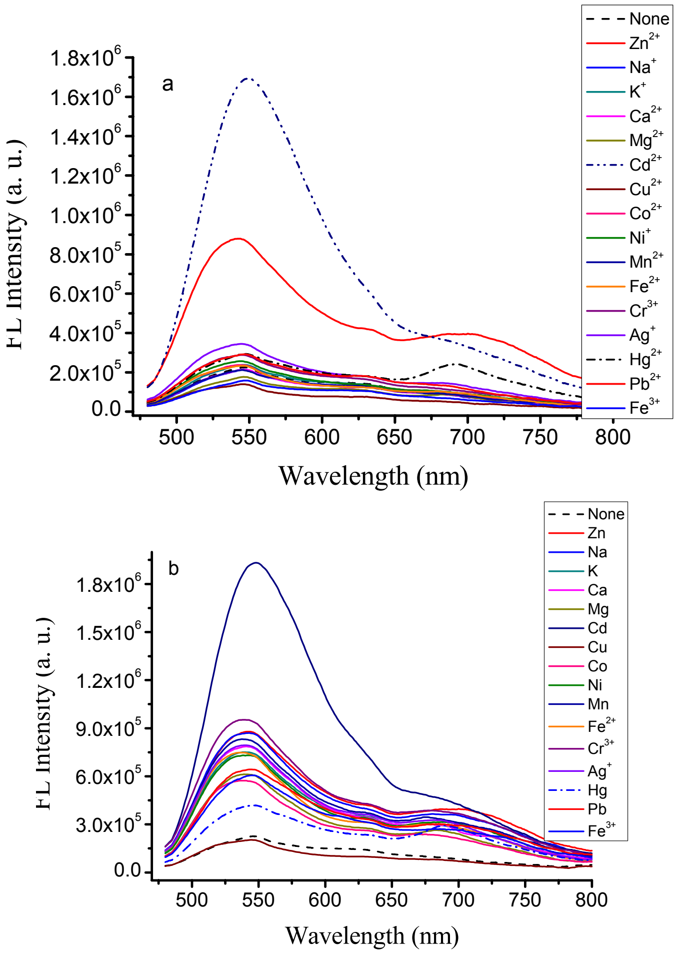


Fig. S13. Emission spectra of **Zinhbo-1**(30 μ M) in absence (a) and presence (b) of zinc ion (30 μ M) in HEPES buffer system(1:1) containing 5% DMSO upon addition of different metal ions (30 μ M heavy metal or 0.3 mM Na^+, K^+, Ca^{2+}, Mg^{2+}) with the excitation at 460 nm.

5. References

S1. (a) Valeur, B. Molecular *Fluorescence: Principles and Application*, Wiley-VCH,Weinheim, 2002

(b) Taki, M.; Wolford, J. L.; O'halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712-713

S2. (a) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; 2nd.; Kluwer Academic/Plenum: New York, 1999

(b) Velapoldi R. A.; Tønnesen H. H. *J. Fluorescence*, **2004**, *14*, 465-472.