Supporting Information

An ESIPT based chromogenic and fluorescence ratiometric probe for Zn^{2+} with imaging in live-cells and tissues

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CONTENTS

1. UV-Vis Study of HBS:

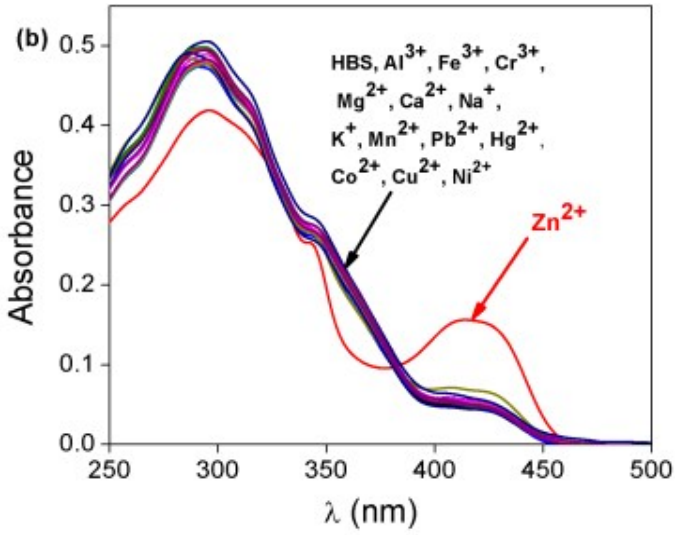


Fig. S1. UV-Vis spectra of chemosensor (HBS) (10 μM) upon addition of 2 equivalent of various metal ions i.e., Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}

2. UV-Vis Study of HBB:

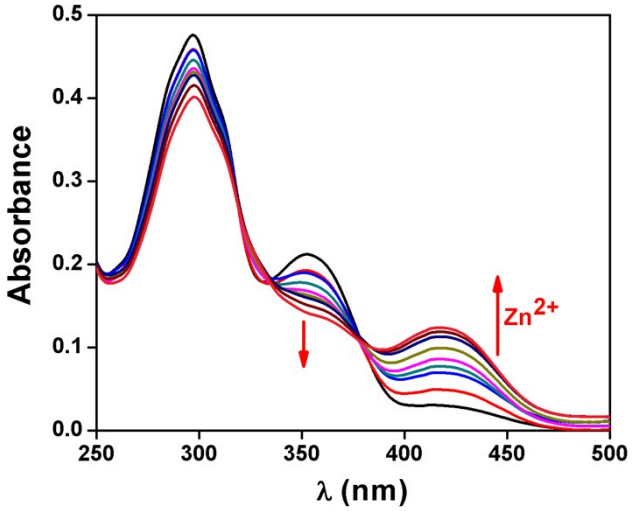


Fig. S2. Change of absorption spectra of HBB (10 μM) on gradual addition of Zn^{2+} (0 to 40 μM).

3. Emission study of HBB:

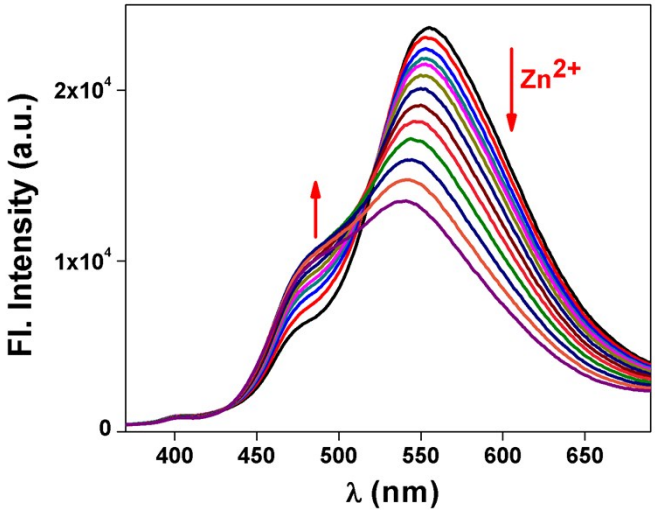


Fig. S3. Change of emission spectra of HBB (10 μM) upon gradual addition of Zn^{2+} (0 to 20 μM).

4. By fluorescence method:

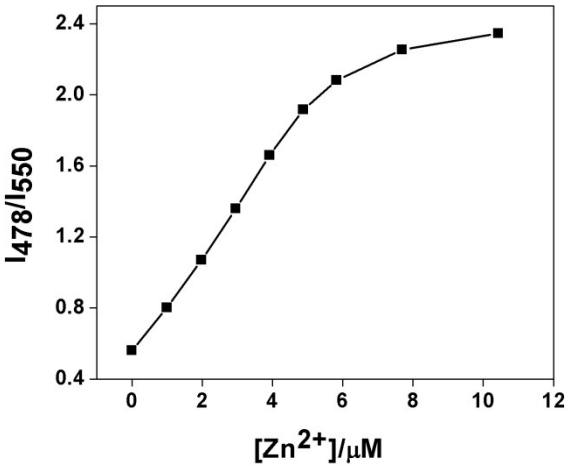


Fig. S4. Mole ratio plot of HBS depending on Zn^{2+} concentration

5. Job's plot of HBS for Zn^{2+}:

Stock solution of HBS and Zn^{2+} were prepared in the order of 10 μM in [MeOH/ H_2O, 1/1, v/v] (at 25 °C) at pH 7.2 in HEPES buffer in same concentration. The emission spectrum was recorded in each case with different *host-guest* ratio but the volume remains the same in each case. Job's plots were drawn by plotting $\Delta F.X_{host}$ *vs* X_{host} (ΔF = change of intensity of the emission spectrum at 478 nm during titration and X_{host} is the mole fraction of the host in each case respectively).

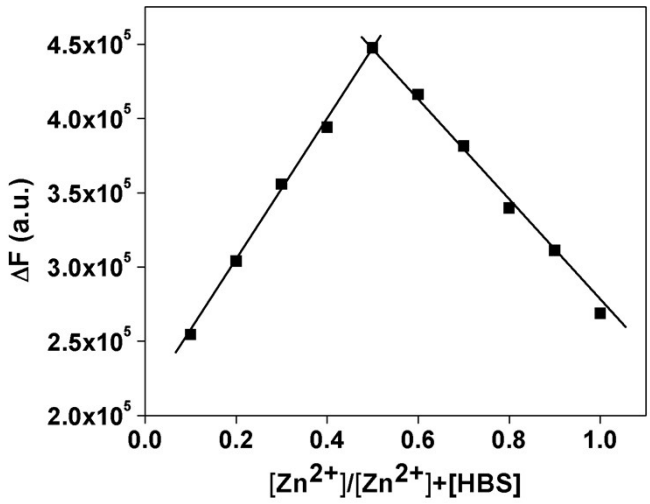


Fig. S5. Job's plot of HBS for Zn^{2+} (where ΔF indicates the change of emission intensity at 478 nm)

6. Determination of detection limit:

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of HBS without the ion (Zn^{2+}) was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit of HBS for Zn^{2+} was determined from the following equation[1]:

$DL = K \times Sb_1/S$

Where K = 2 or 3 (we take 3 in this case); Sb_1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

Thus using the formula, we get the Detection Limit = 1.6×10^{-7} M i.e., HBS can detect Zn^{2+} in this minimum concentration by fluorescence techniques.

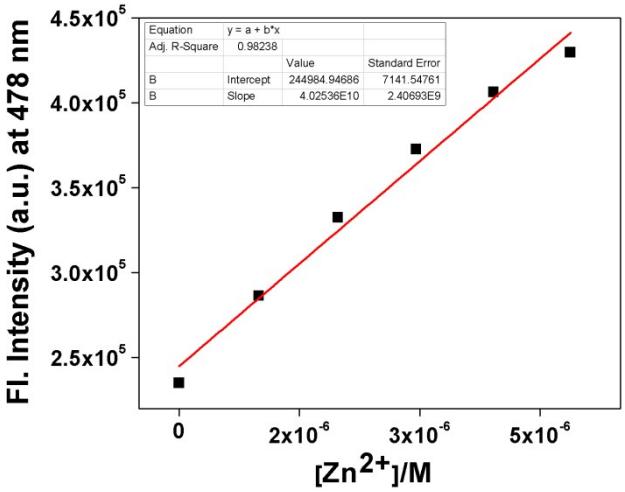


Fig. S6: Linear response curve of HBS at 478 nm depending on the Zn^{2+} concentration

7. Determination of association constant:

Binding constant was calculated according to the Benesi-Hildebrand equation. K_a was calculated following the equation stated below.

$$1/(F\text{-}F_o) = 1/\{K_a(F_{max}–F_o)\ [M^{n+}]^x\} + 1/[F_{max}\text{-}F_o]$$

Here F_0, F and F_{max} indicate the emission in absence of, at intermediate and at infinite concentration of metal ion respectively.

Plot of $1/\ [F\text{-}F_0]$ vs. $1/[Zn^{2+}]$ gives a straight line indicating 1:1 complexation between HBS and Zn^{2+} where K_a is found to be $1.6\times10^5\ M^{-1}$ for HBS.

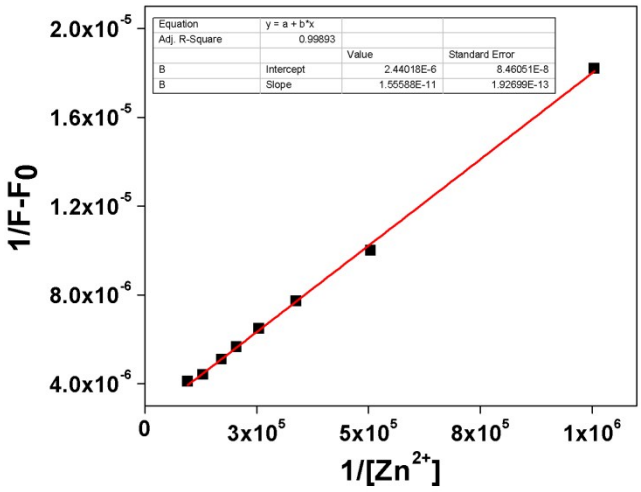


Fig. S7: Determination of association constant of HBS at 478 nm depending on the Zn^{2+} concentration using Benesi-Hildebrand equation

8. Lifetime study:

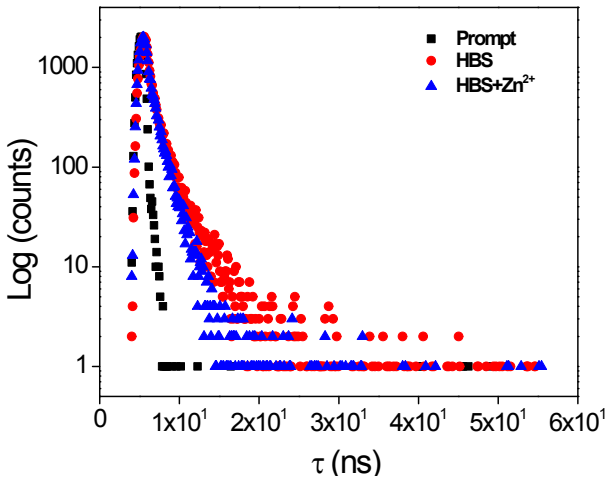


Fig. S8. Lifetime decay plot of HBS and the HBS+Zn^{2+} complex

Table S1: Fluorescence lifetime data[a]

MeOH (solvent)	Quantum yield *(φ)*	τ (ns)	k_r ($10^8 \times s^{-1}$)	k_{nr} ($10^8 \times s^{-1}$)
HBS	0.155	1.73	0.90	4.88
HBS-Zn^{2+}	0.167	0.82	2.04	10.16

[a] Radiative rate constant K_r and total non radiative rate constant K_{nr} have been calculated using the equation $\tau^{-1} = K_r + K_{nr}$ and $K_r = \varphi_f / \tau$

9. Fluorescence study:

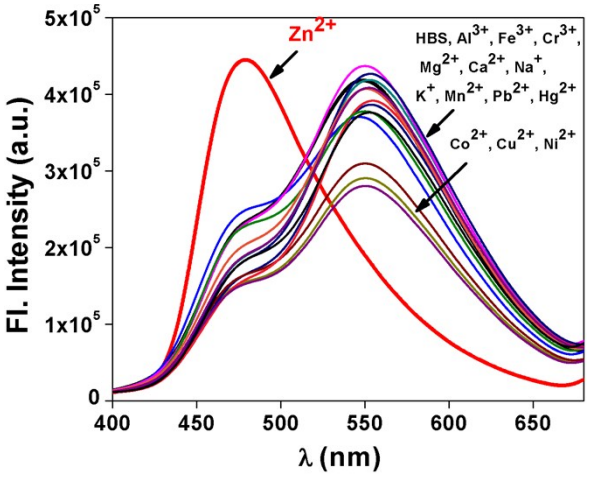


Fig. S9. Change in emission spectrum of HBS (10 μM) upon addition of Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, Al^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+} (10 μM) in MeOH:H_2O (1:1, v/v, pH=7.2)

10. ^{1}H NMR spectrum of HBS:

Fig. S10: ^{1}H NMR (300 MHz) spectra of HBS in DMSO-d_6

11. ^{13}C NMR spectrum of HBS:

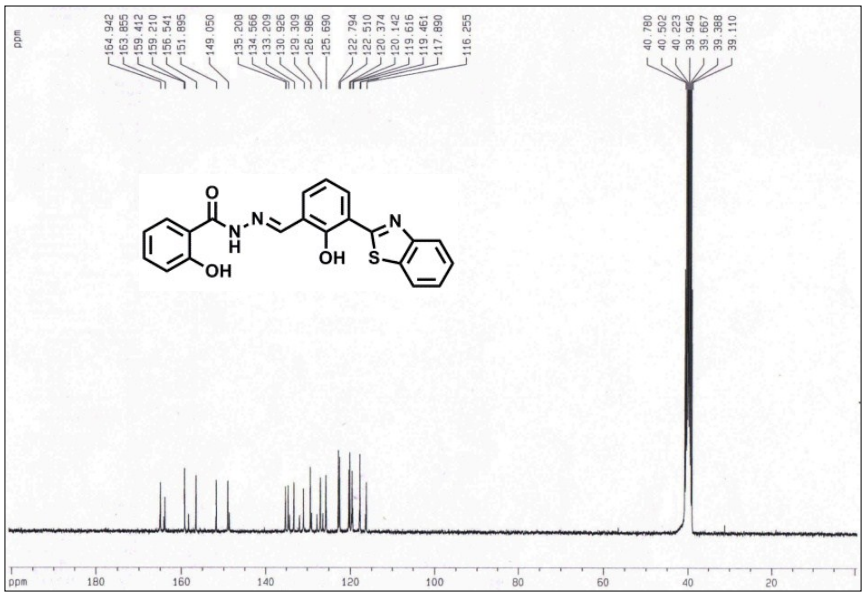


Fig. S11: ^{13}C NMR (150 MHz) spectra of HBS in DMSO-d_6

12. Mass spectrum (HRMS) of HBS:

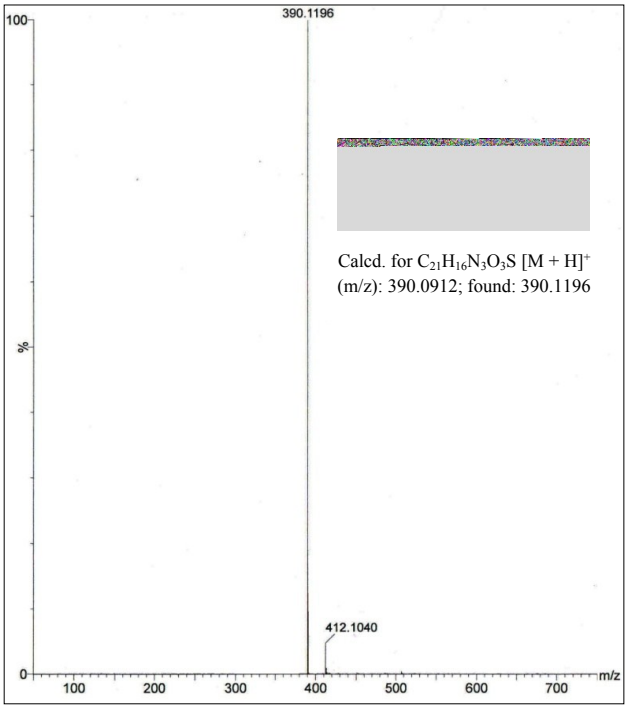


Fig. S12: HRMS of the probe (HBS)

13. ^{1}H NMR spectrum of HBB:

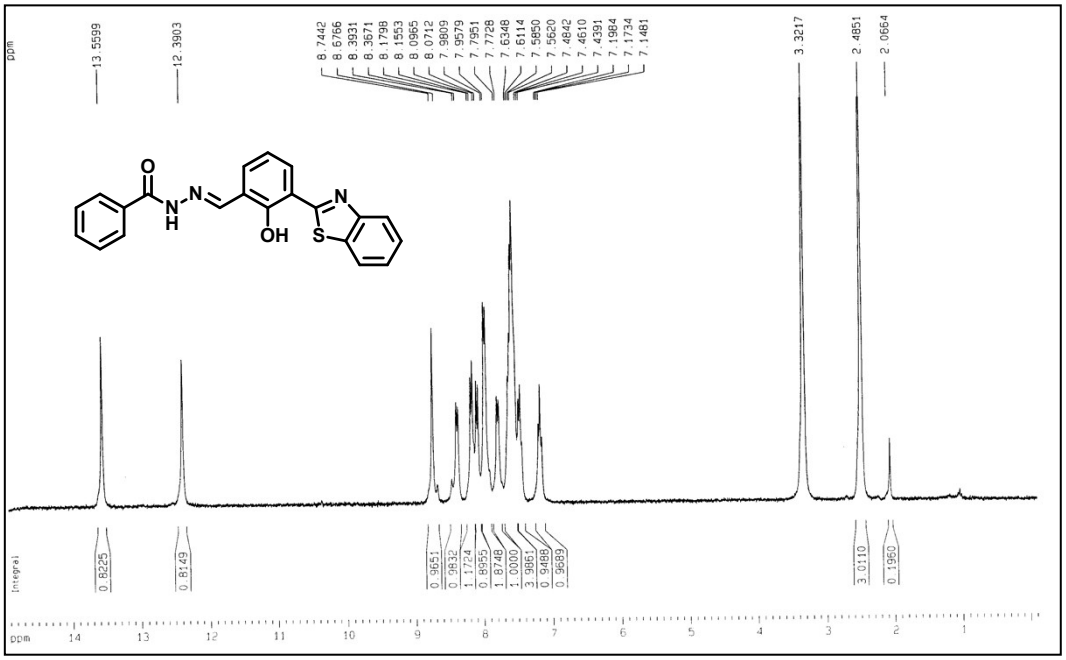


Fig. S13: ^{1}H NMR (300 MHz) spectra of HBB in DMSO-d_6

14. ^{13}C NMR spectrum of HBB:

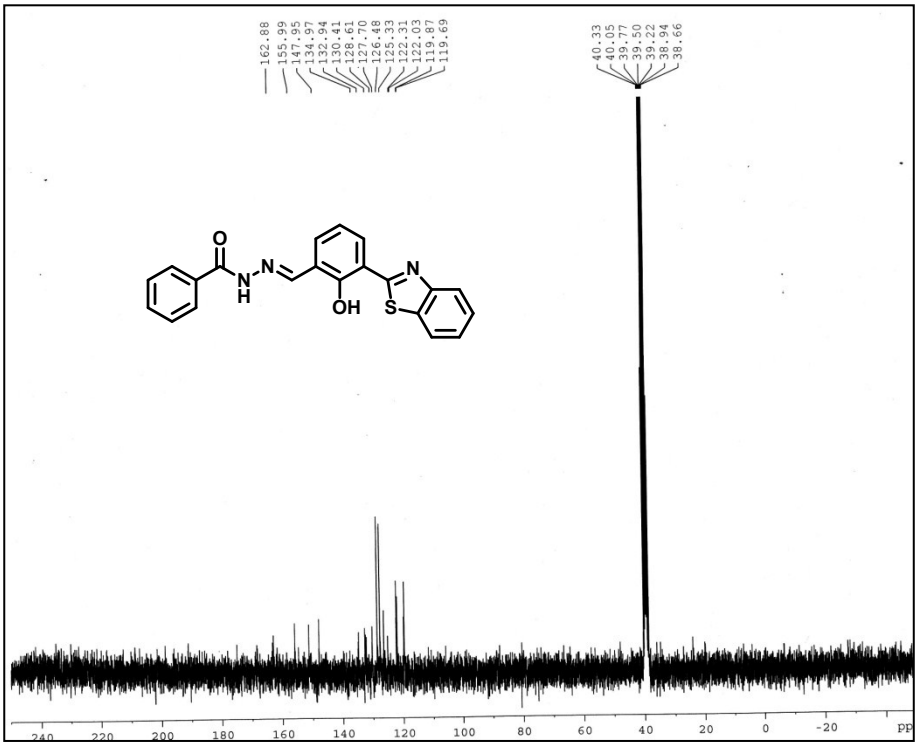


Fig. S14: ^{13}C NMR (100 MHz) spectra of HBB in DMSO-d_6

15. Mass spectrum (HRMS) of HBB:

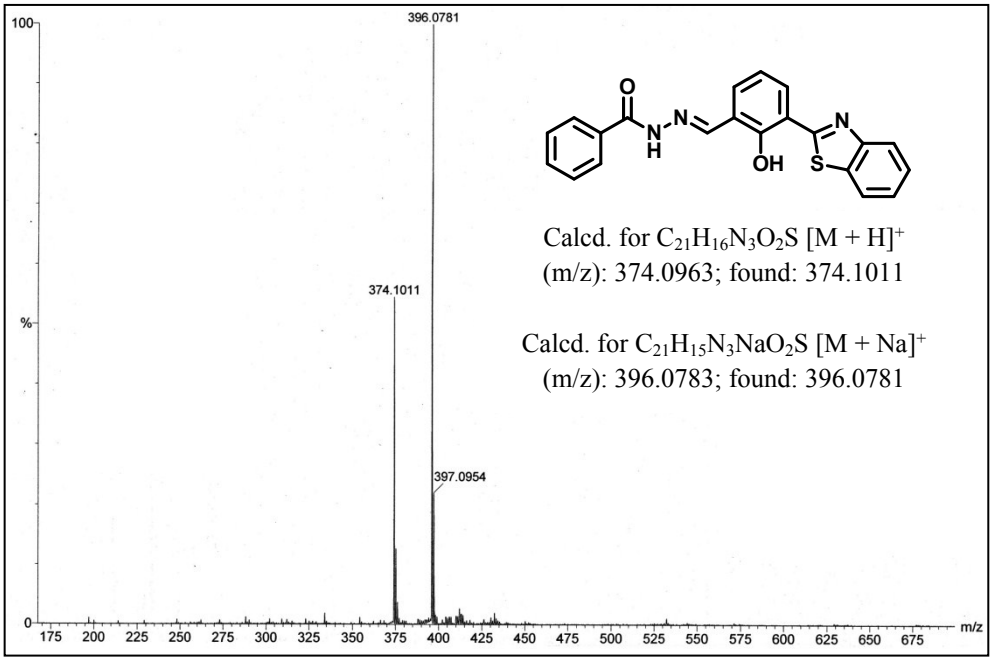


Fig. S15: HRMS of the analogous compound (HBB)

16. ^{1}H NMR titration of HBS with Zn^{2+}:

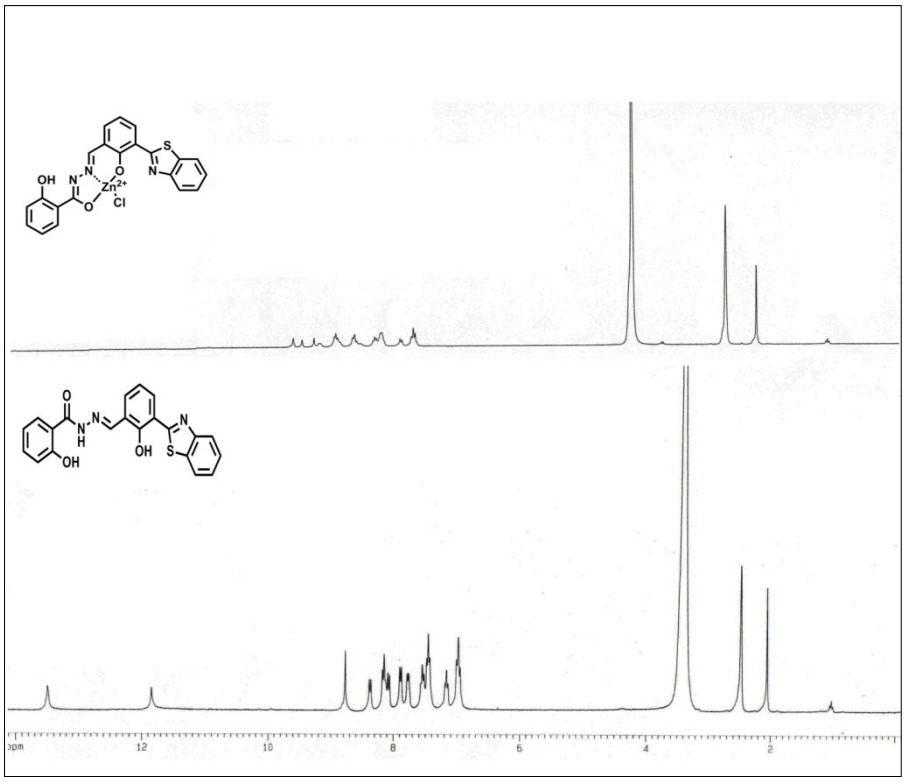


Fig. S16: NMR titration spectra of HBS in presence of Zn^{2+} in DMSO-d_6

17. Mass spectrum (HRMS) of HBS-Zn^{2+}

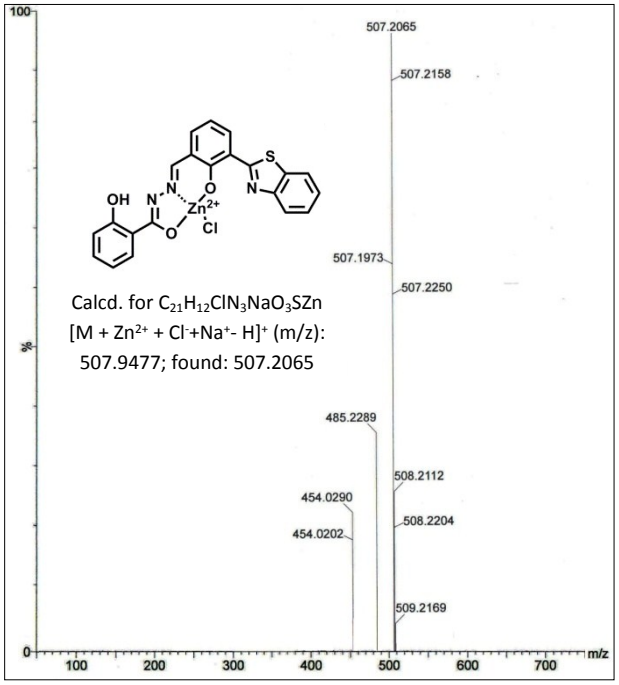


Fig. S17: HRMS of the HBS-Zn^{2+}

18. Computational Study

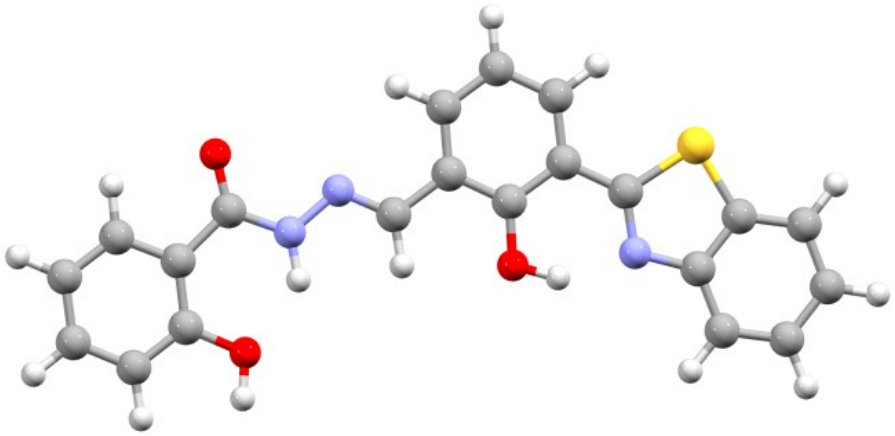


Fig. S18. Optimized structure of HBS by DFT/B3LYP/6-31+G (d)

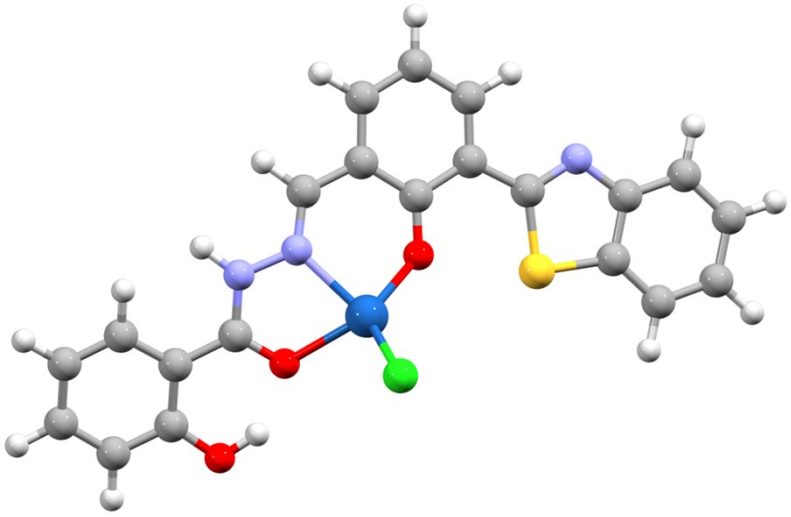


Fig. S19. Optimized structure of HBS-Zn^{2+} complex by DFT/B3LYP/6-31+G (d)/LanL2DZ method

Table S2: Vertical electronic excitations of HBS calculated by TDDFT/CPCM method

Energy (eV)	Wave-length (nm)	Osc. strength (f)	Transition	Character
3.3026	375.42	0.6090	(94%) HOMO→LUMO	Lig(π)→Lig(π^*)
3.8026	326.05	0.3175	(88%)HOMO-1→LUMO	Lig(π)→Lig(π^*)
3.9789	311.60	0.6037	(77%) HOMO→LUMO+1	Lig(π)→Lig(π^*)
4.1309	300.14	0.0314	(60%) HOMO-2→LUMO	Lig(π)→Lig(π^*)

Table S3: Vertical electronic excitations of Zn^{2+} complex of HBS calculated by TDDFT/CPCM method

Energy (eV)	Wave-length (nm)	Osc. strength (f)	Transition	Character
3.0519	406.25	0.3637	(97%) HOMO→LUMO	Lig(π)→Lig(π^*)
3.6455	340.10	0.1018	(96%) HOMO→LUMO+1	Lig(π)→Lig(π^*)
3.8225	324.35	0.2874	(47%) HOMO-1→LUMO	Lig(π)→Lig(π^*)
3.8324	323.52	0.0608	(47%) HOMO-2→LUMO	Lig(π)→Lig(π^*)
4.3684	283.82	0.1617	(45%) HOMO-1→LUMO+1	Lig(π)→Lig(π^*)

Table S4. Energy and compositions of some selected molecular orbitals of HBS-Zn^{2+} complex

MO	Energy	% of composition		
		HBS	Cl	Zn
LUMO+5	0.12	98	0	2
LUMO+4	-0	21	3	76
LUMO+3	-0.6	100	0	0
LUMO+2	-1.09	100	0	0
LUMO+1	-1.91	99	0	1
LUMO	-2.42	99	0	1
HOMO	-5.67	99	1	1
HOMO-1	-6.1	100	0	0
HOMO-2	-6.35	98	1	0
HOMO-3	-6.81	92	8	1
HOMO-4	-6.97	62	36	1
HOMO-5	-7.01	45	53	2

HOMO-6	-7.06	6	90	3
HOMO-7	-7.33	97	3	0
HOMO-8	-7.58	97	1	2
HOMO-9	-7.78	92	7	1
HOMO-10	-7.94	100	0	0

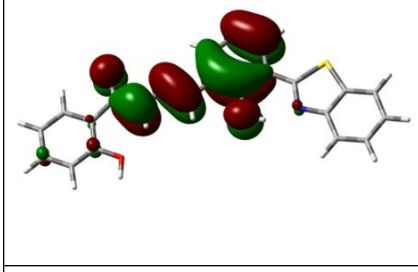
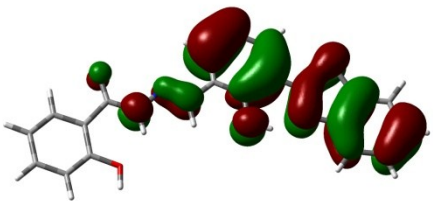
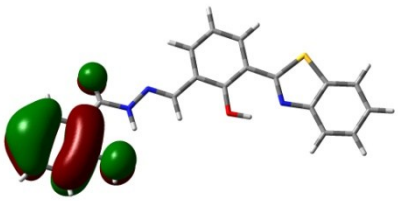
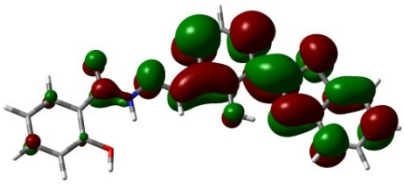
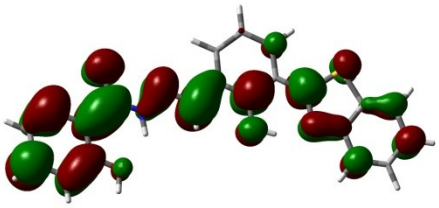
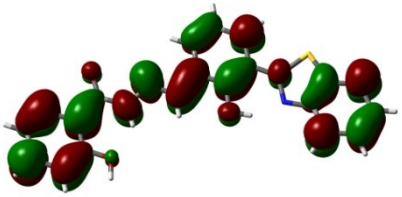
		
HOMO (E= -5.57 eV)	HOMO-1 (E= -6.03 eV)	HOMO-2 (E= -6.48 eV)
		
LUMO (E= -1.85 eV)	LUMO+1 (E= -1.23 eV)	LUMO+2 (E= -0.5 eV)

Fig. S20: Contour plot of some selected molecular orbitals of HBS

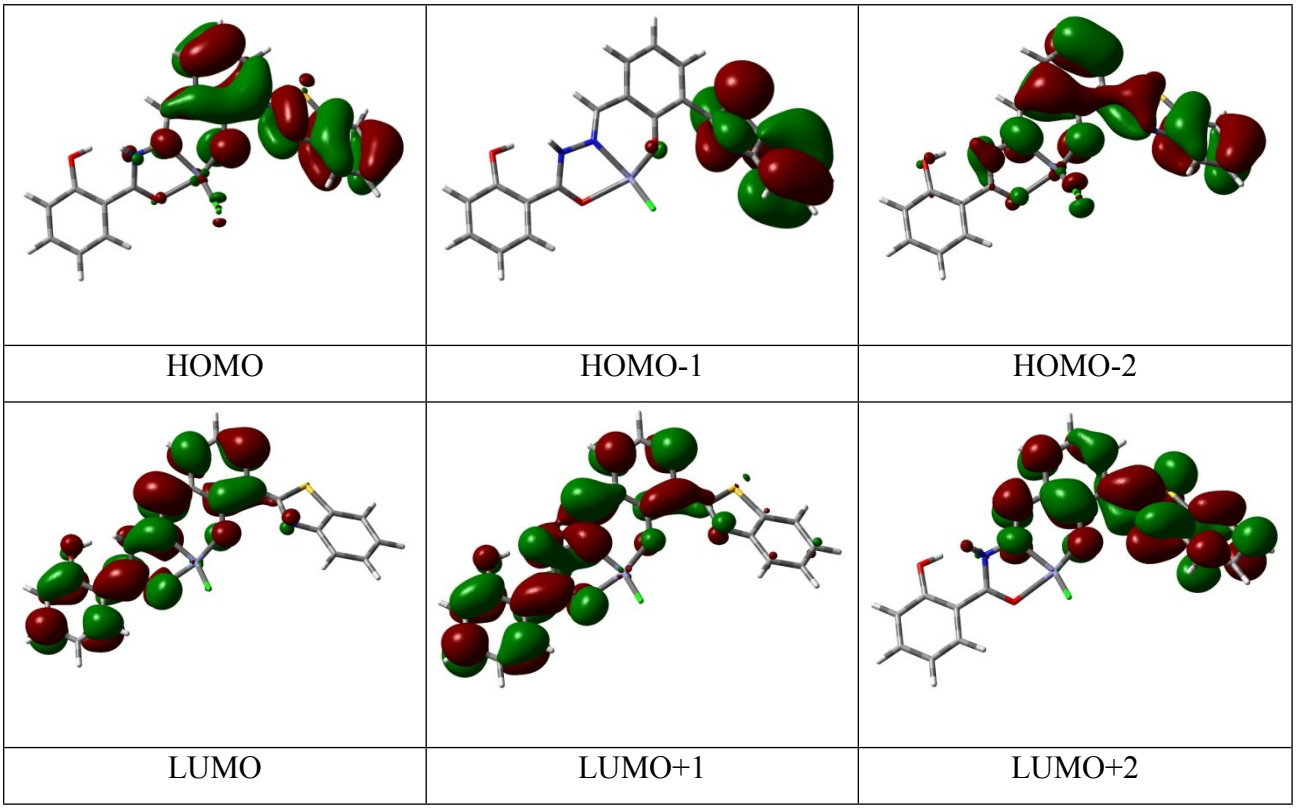


Fig. S21: Contour plot of some selected molecular orbitals of HBS-Zn^{2+}

19. Cell-bio imaging

MTT assay

Human breast cancer cell lines MCF-7 were evaluated for cytotoxicity with HBS and HBS-Zn^{2+} complex by the following protocol as described by Ray et.al.[13] MCF-7 cell lines were obtained from National centre for cell science, Pune, India and maintained in Minimum Essential Media Earle's (MEM) (Gibco, life technologies) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, life technologies, USA) and stored at 37°C in a humidified incubator under 5% CO_2 atmosphere. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well and cultured in CO_2 incubator for 24 h. The cells were separately treated with increasing doses of HBS and HBS-Zn^{2+} complex concentrations (5, 10, 20, 25, 30, 50, 75, 100, 125) μM, along with control. Zn^{2+} was treated in aqueous medium while the receptor HBS was dissolved in DMSO but final concentration of DMSO was maintained below 1%. After 24 h, methyl tetrazolium dye (MTT) (5 mg/ml) solution was added to each well (10 μl/well). The plates were incubated in the dark at 37°C for 2 h. 100 μL of DMSO was added to each well and allowed to stand for 1 h in vortex shaker. Cell viability determination was

studied by recording absorbance at 570 nm for each well using a microplate reader (Tecan, infinite M200). Untreated cells were served as 100% viable.

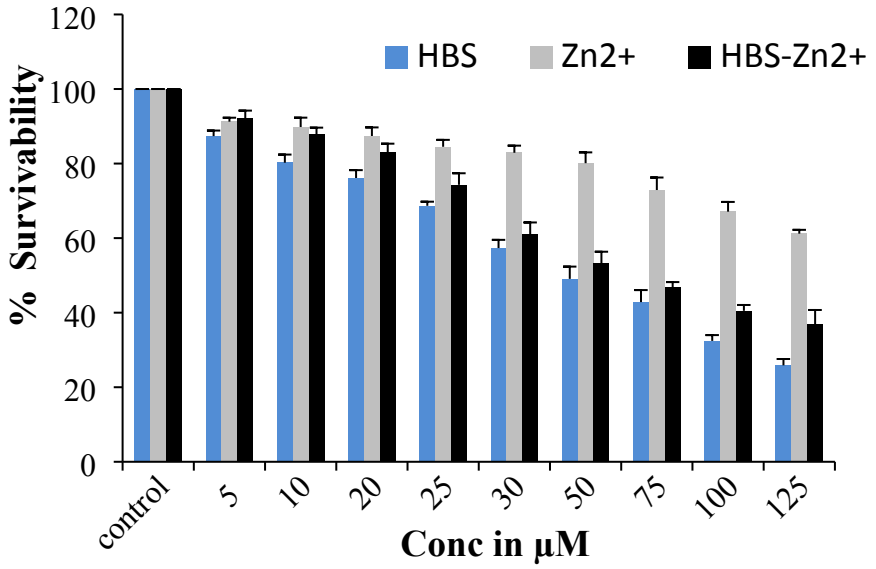


Fig. S22. MTT assay of HBS and HBS-Zn^{2+} complex on MCF-7 cell lines

20. Determination of fluorescence quantum yield

The luminescence quantum yield was determined using coumarin 153 as reference dye. The compounds and the reference dye were excited at the same wavelength, maintaining nearly equal absorbance (~0.1), and the emission spectra were recorded. The area of the emission spectrum was integrated using the software available in the instrument and the quantum yield is calculated according to the following equation:

$$\phi_S/\phi_R = [A_S / A_R] \times [(Abs)_R /(Abs)_S] \times [n_S^2/n_R^2]$$

Here, ϕ_S and ϕ_R are the luminescence quantum yields of the sample and reference, respectively. A_S and A_R are the area under the emission spectra of the sample and the reference respectively, $(Abs)_S$ and $(Abs)_R$ are the respective optical densities of the sample and the reference solution at the wavelength of excitation, and n_S and n_R are the values of refractive index for the respective solvent used for the sample and reference.

We calculated the quantum yields of HBS and HBS-Zn^{2+} using the above mentioned equation; the values are 0.155 and 0.167 respectively.