

Supporting Information

Cascade reaction based rapid and ratiometric detection of H₂S/S²⁻ over bio-thiols with live cell-imaging: demasking of ESIPT approach

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1. Calculation of the detection limit:

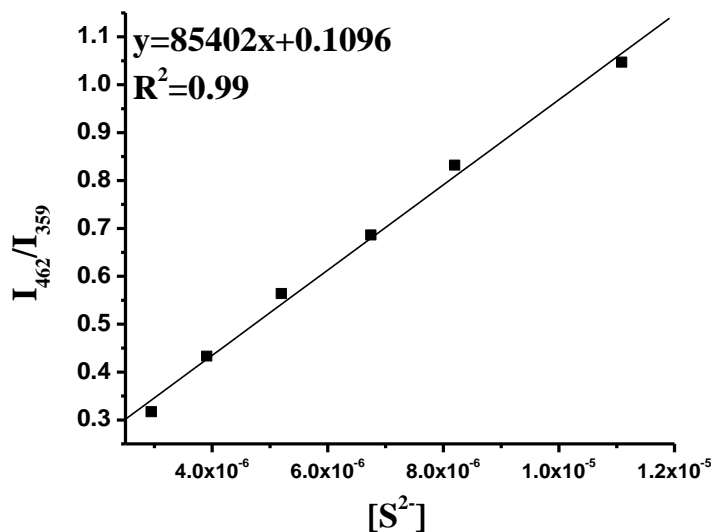


Figure S1: Fl. Intensity ratio ($I_{462 \text{ nm}}/ I_{359 \text{ nm}}$) Vs. Conc. of S^{2-} plot.

The detection limit DL of **FBBP** for S^{2-} was determined from the following equation. [S1]

$$DL = K * Sb1/S$$

Where $K = 2$ or 3 (we take 2 in this case);

$Sb1$ is the standard deviation and S is the slope of the calibration curve.

From the graph we get slope = 85402 , and $Sb1$ value is 0.021958 .

Thus using the formula we get the Detection Limit = $0.51 \mu\text{M}$ i.e. **FBBP** can detect S^{2-} in this minimum level.

2. Calculation of rate constant:

From Fl. Intensity ratio (I_{462}/I_{359}) vs. time (sec.) plot using first order rate equation (Figure S2), we get rate constant $K = \text{slope} \times 2.303 = 0.0086 \times 2.303 = 1.9 \times 10^{-2} \text{ sec}^{-1}$

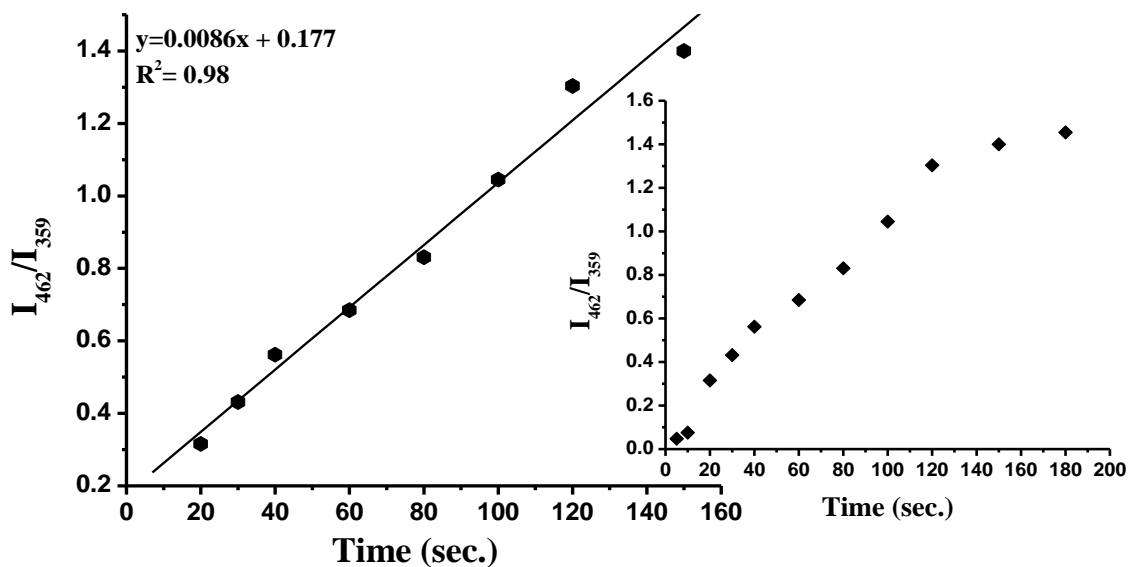


Figure S2: The time vs. Fl. Intensity ratio ($I_{462 \text{ nm}}/I_{359 \text{ nm}}$) plot between 20 to 160 second and the full plot is in inset.

3. Fluorescence responses of FBBP + various species + S^{2-} :

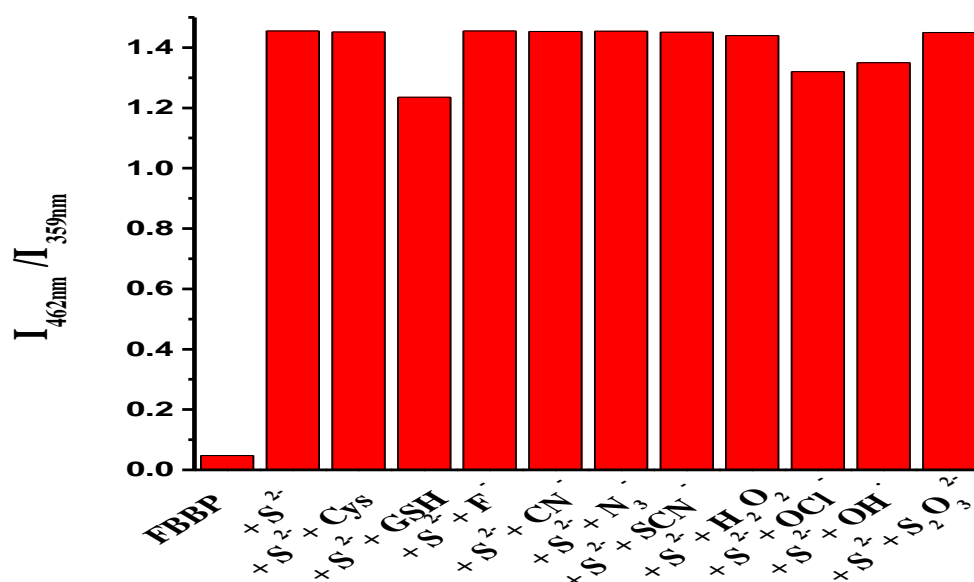
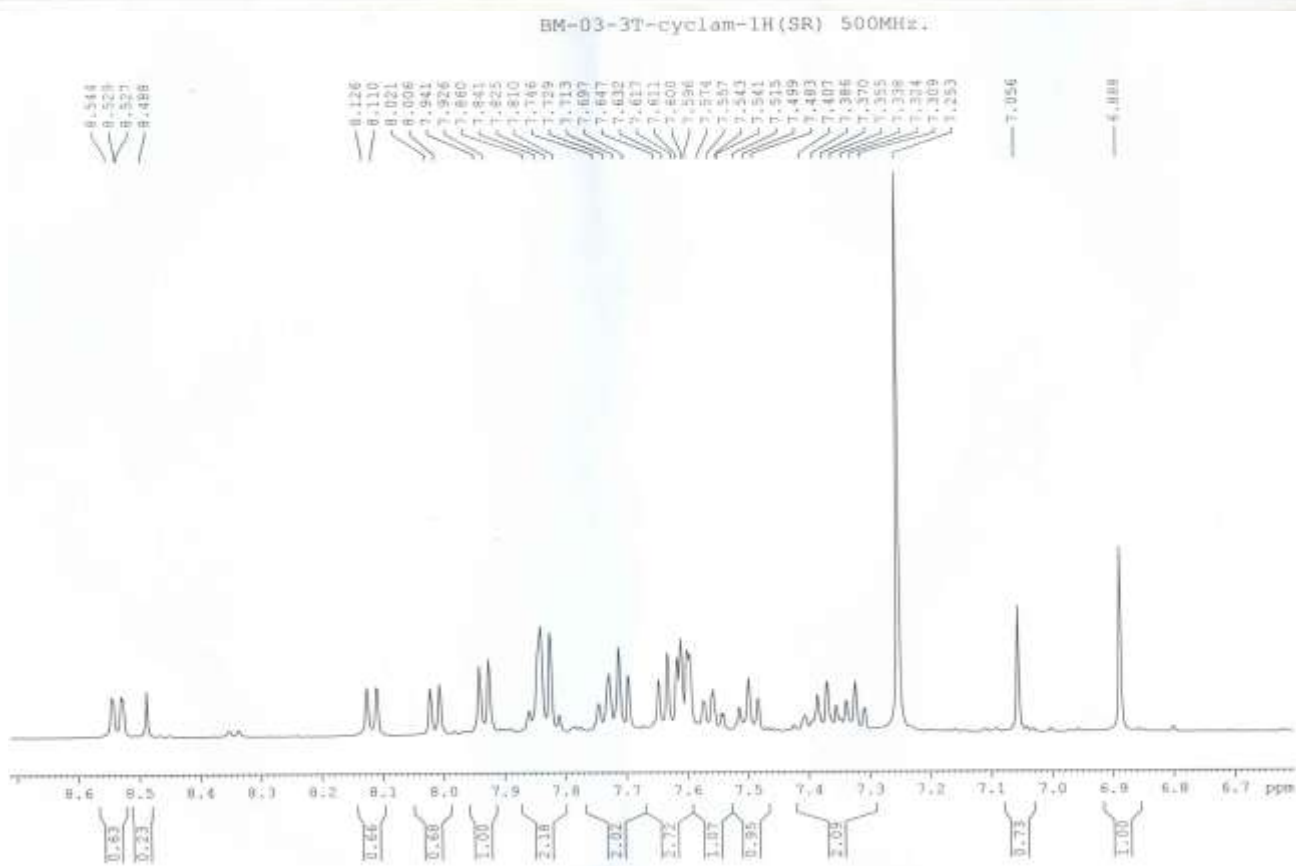
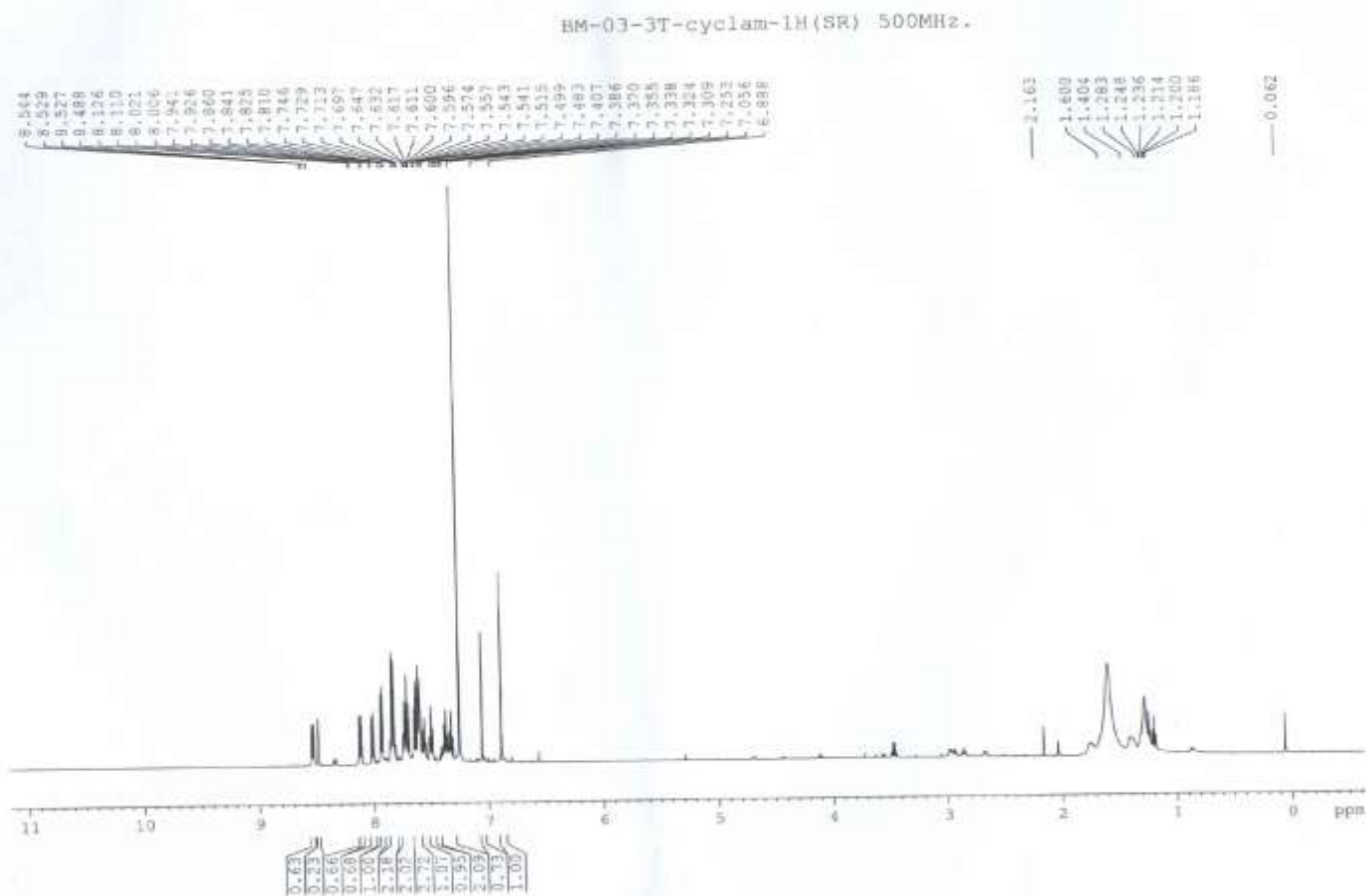
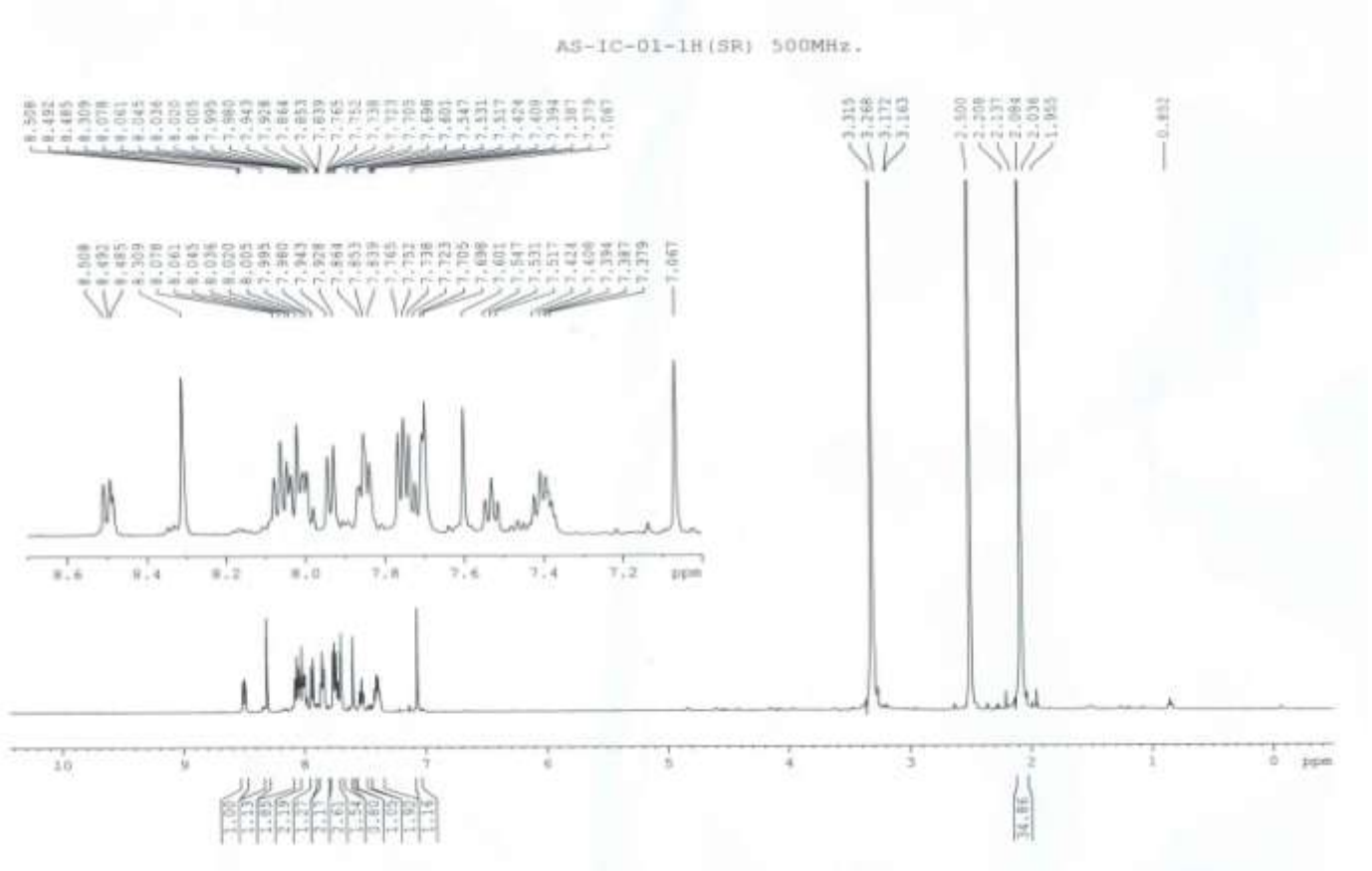


Figure S3: Ratiometric fluorescence responses ($I_{462\text{nm}}/I_{359\text{nm}}$) of FBBP ($c = 1.0 \times 10^{-5} \text{ M}$) to S^{2-} (1 equiv) containing 10 equiv of various metal species.

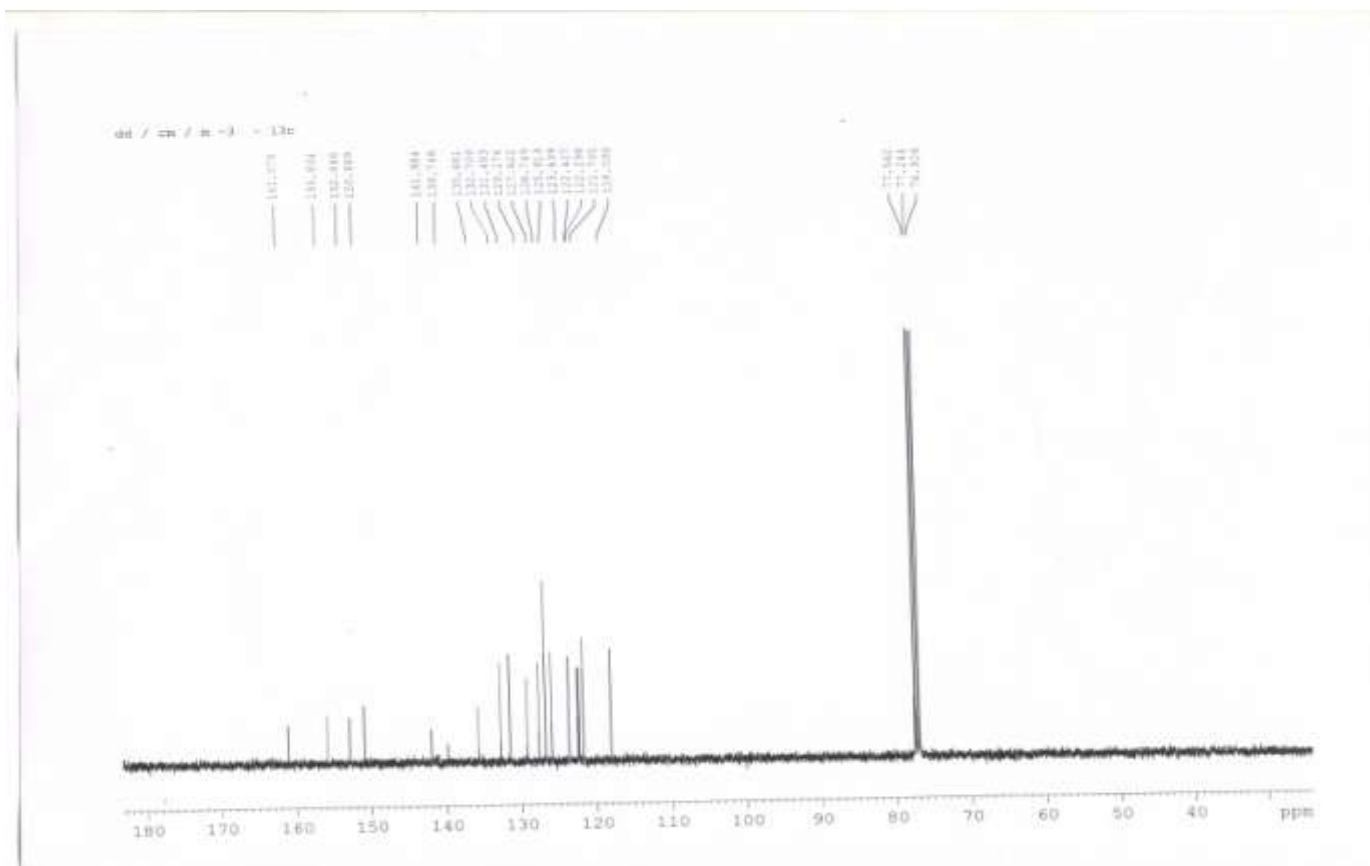
4. ^1H NMR, ^{13}C NMR and ESI MS spectra:
 ^1H NMR spectrum and its expansion of Receptor i.e. FBBP in CDCl_3 :



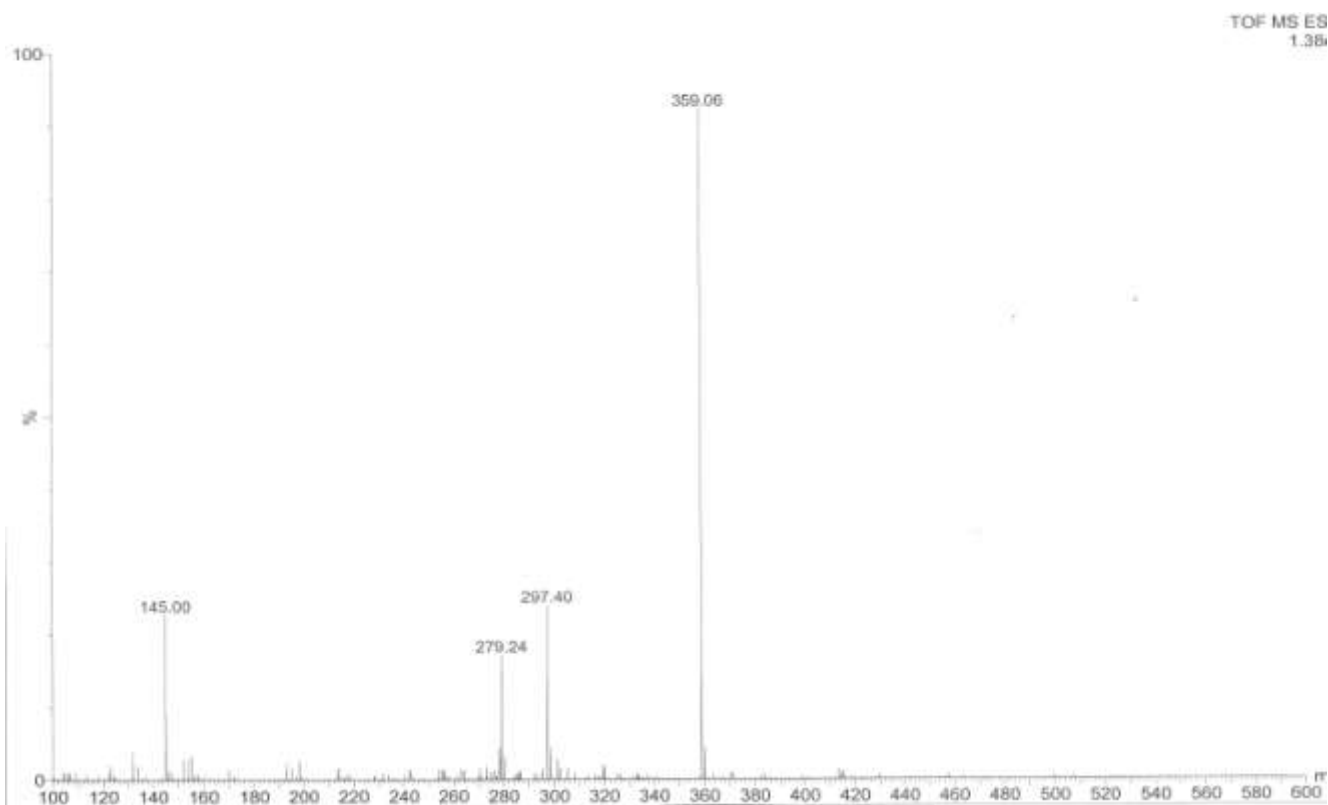
¹H NMR spectrum and its expansion of Receptor i.e. FBBP in *d*₆-DMSO:



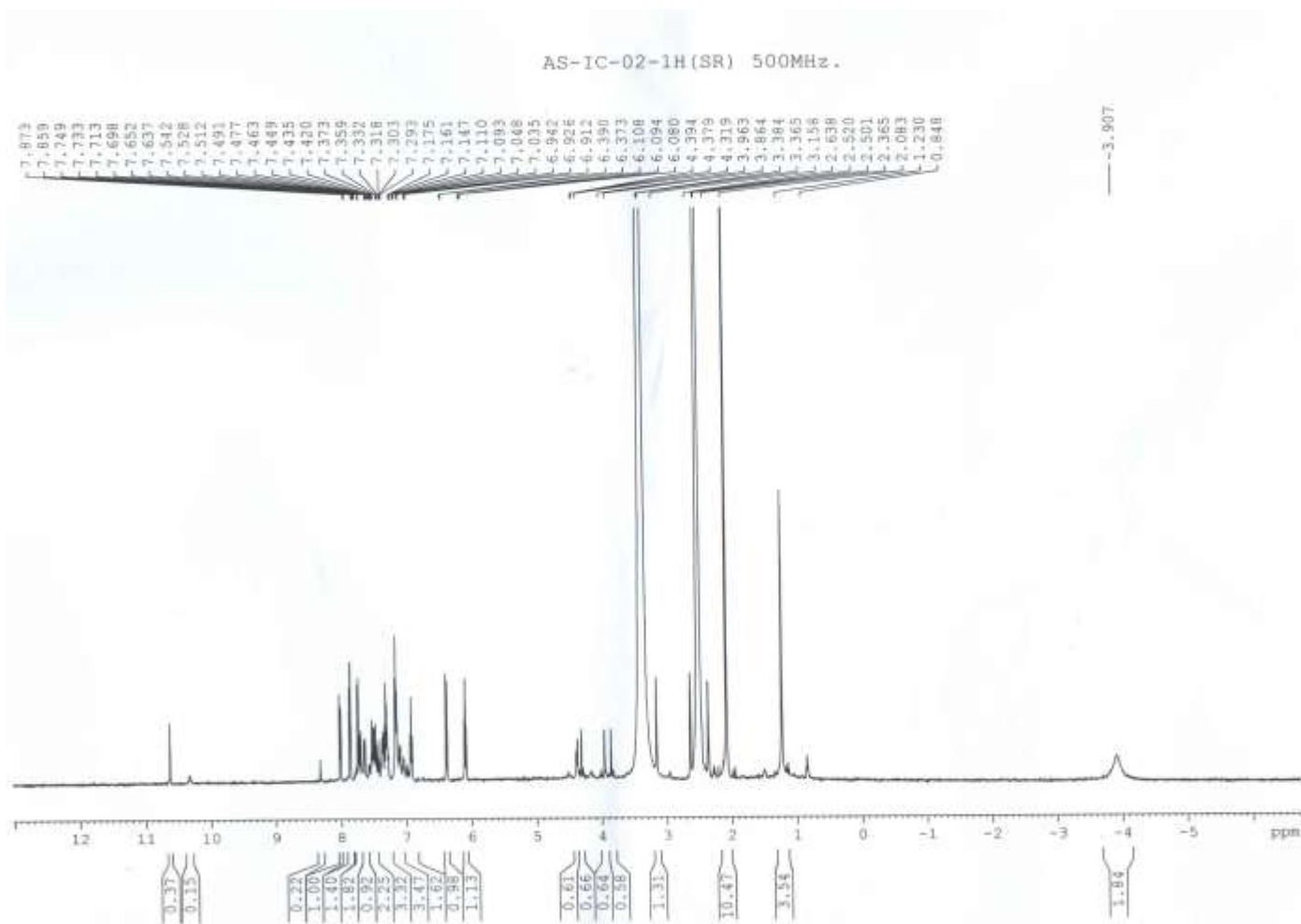
¹³C NMR spectrum of Receptor i.e. FBBP:

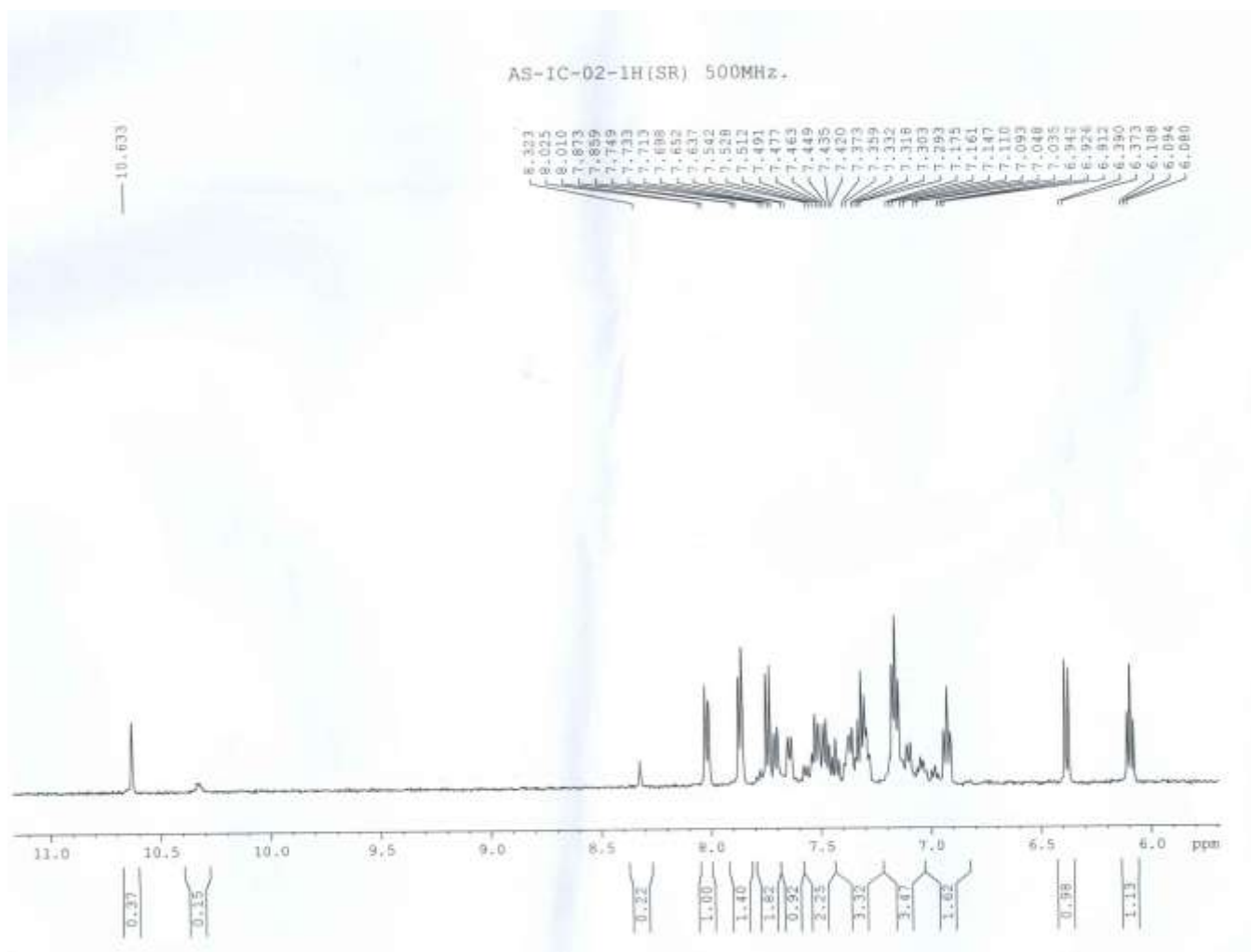


ESI MS Mass Spectrum of FBBP:

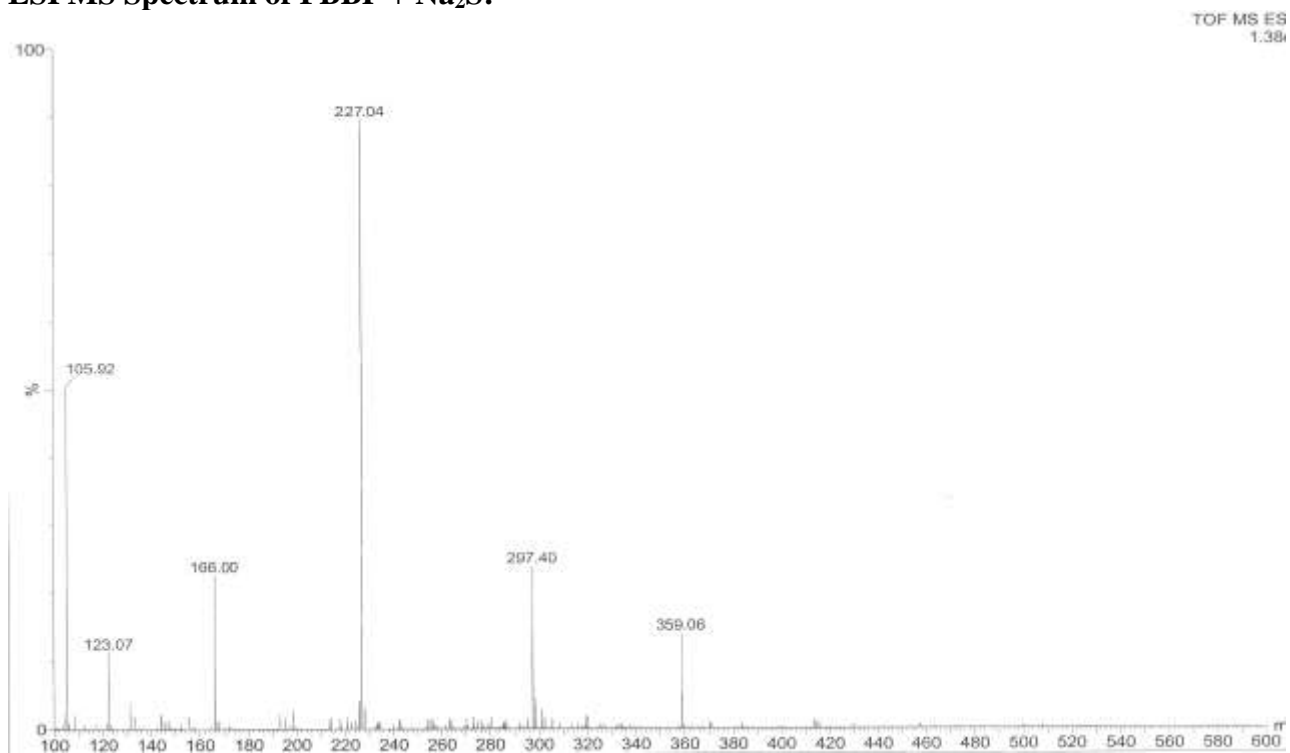


¹H NMR spectrum and its expansion of FBBP + Na₂S in d₆-DMSO:

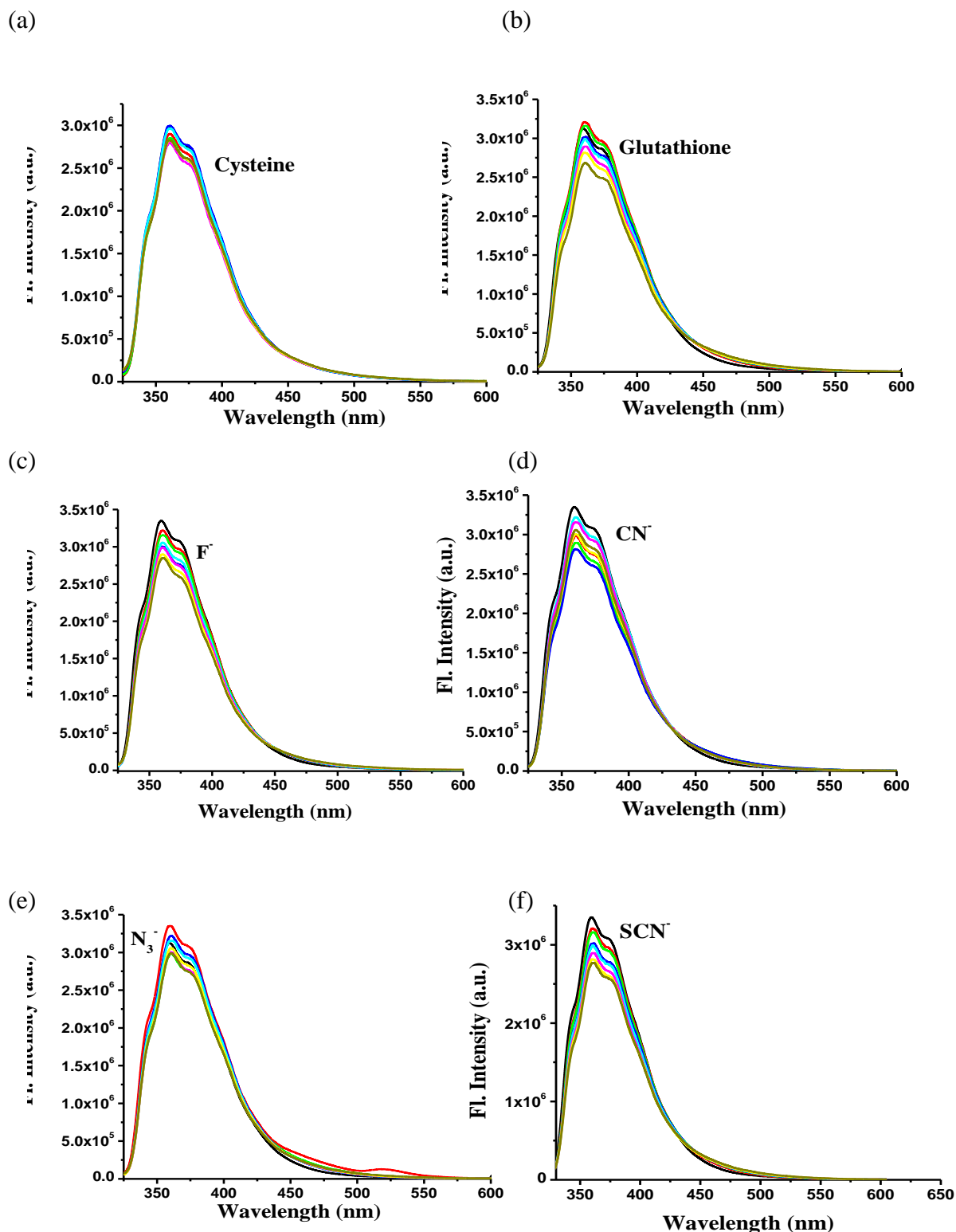




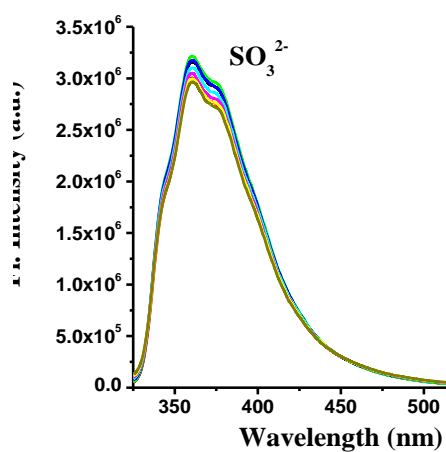
ESI MS Spectrum of FBBP + Na₂S:



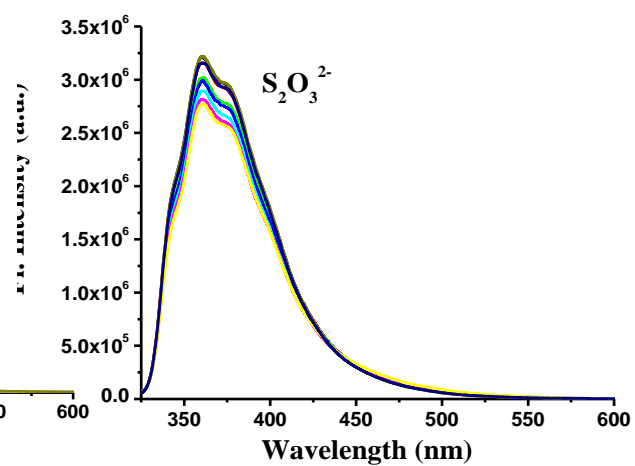
5. Fluorescence emission spectra of FBBP with different species such as Cysteine, Glutathione, F^- , CN^- , N_3^- , SCN^- , SO_3^{2-} , $S_2O_3^{2-}$, H_2O_2 , HSO_3^- , Hydroxyl radical, Hypochlorite, Ascorbic Acid, NH_4^+ , Co^{2+} , Al^{3+} , Hg^{2+} and Cu^{2+} in $CH_3CN : H_2O$ (2:8, v/v) (The solutions of anions and oxidants were prepared from Cysteine, Glutathione, TBAF, KCN, NaN_3 , KSCN, KSO_3 , $K_2S_2O_3$, $NaHSO_3$, H_2O_2 , Femton's reagent, $NaOCl$, Ascorbic Acid, NH_4Cl , $CoCl_2$, $Al(NO_3)_3 \cdot 9H_2O$, $HgCl_2$ and $CuCl_2$ respectively in H_2O).



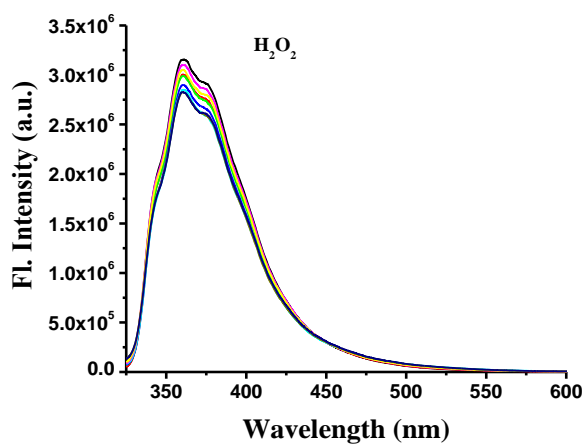
(g)



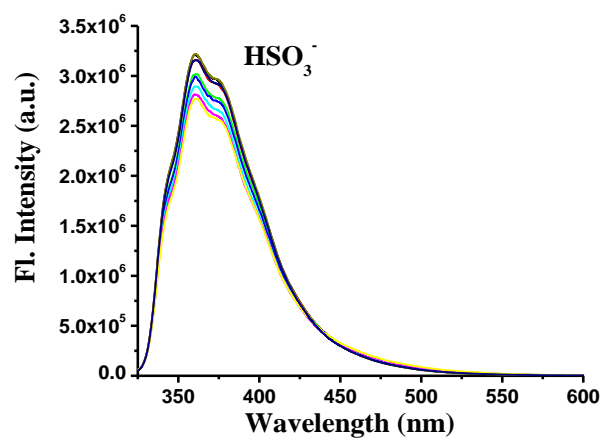
(h)



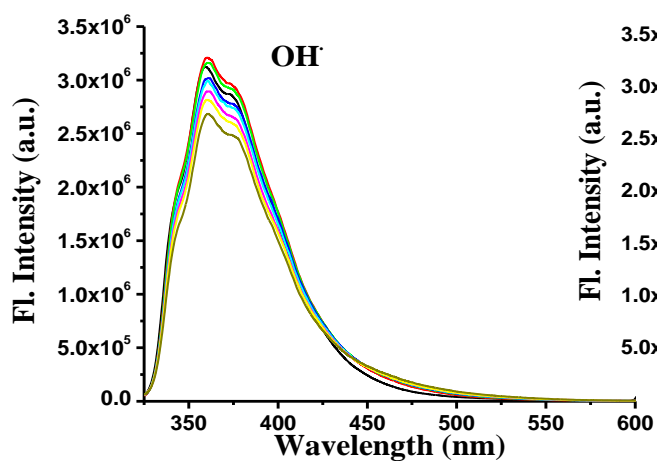
(i)



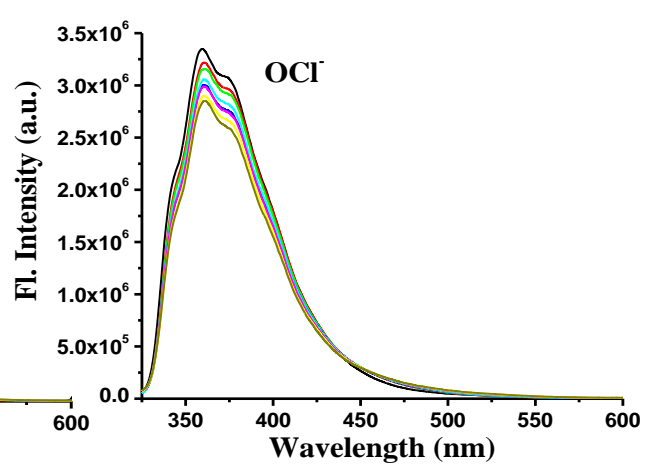
(j)



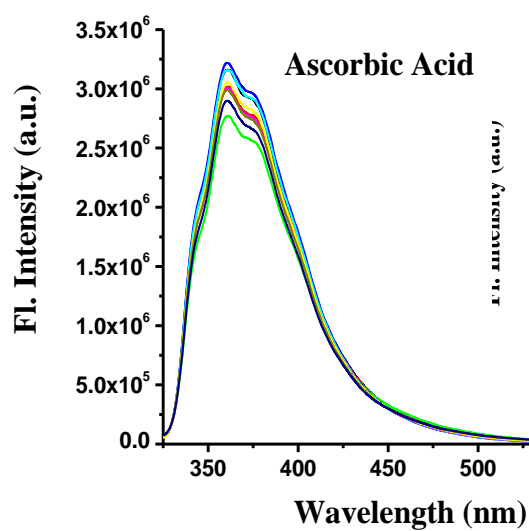
(k)



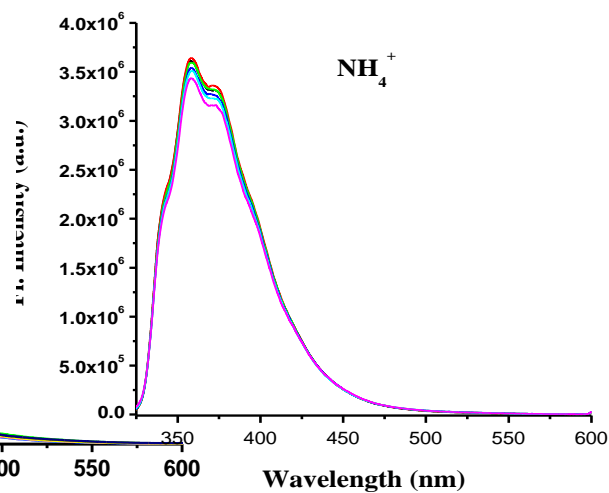
(l)



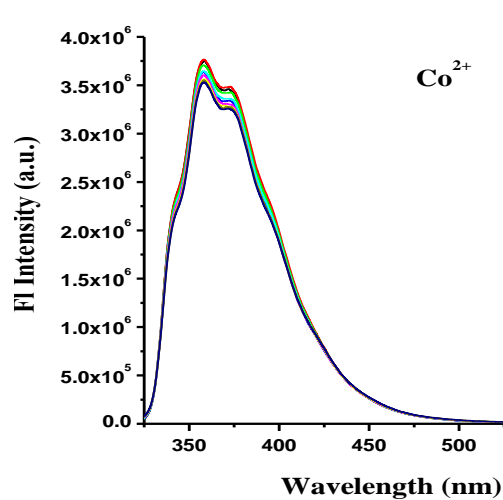
(m)



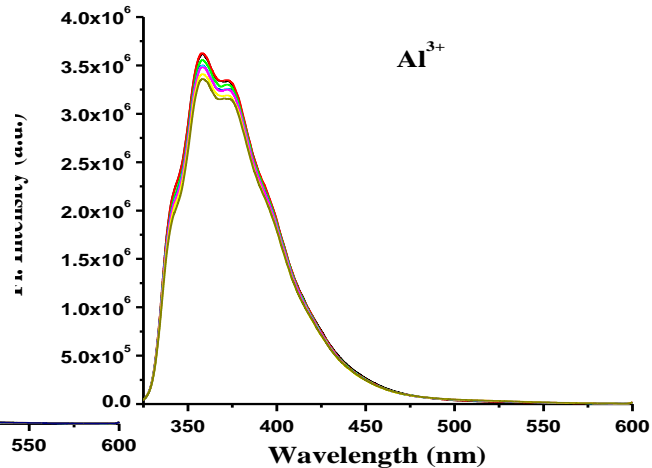
(n)



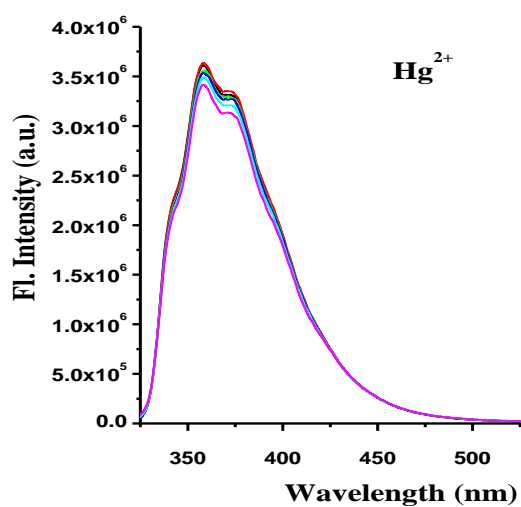
(o)



(p)



(q)



(r)

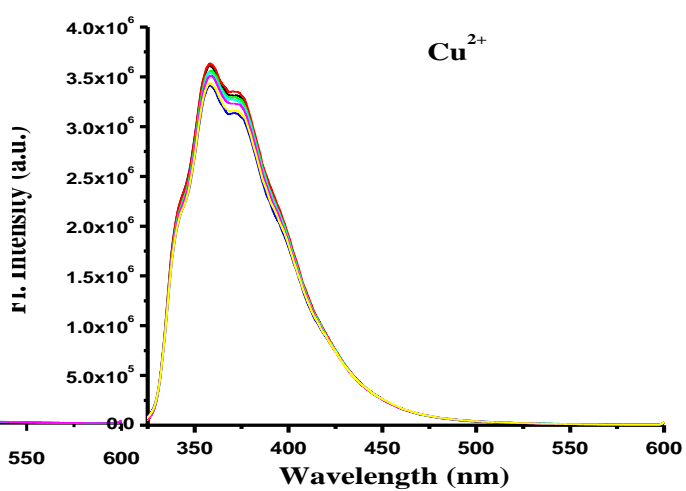


Figure S4-S22: Fluorescence emission spectra of FBBP with different species such as Cysteine, Glutathione, F⁻, CN⁻, N₃⁻, SCN⁻, SO₃²⁻, S₂O₃²⁻, H₂O₂, HSO₃⁻, Hydroxyl radical, Hypochlorite, Ascorbic Acid, NH₄⁺, Co²⁺, Al³⁺, Hg²⁺ and Cu²⁺ in CH₃CN : H₂O (2:8, v/v) respectively.

6. Fluorescence emission spectra of FBBP with Na₂S (excitation at 405 nm):

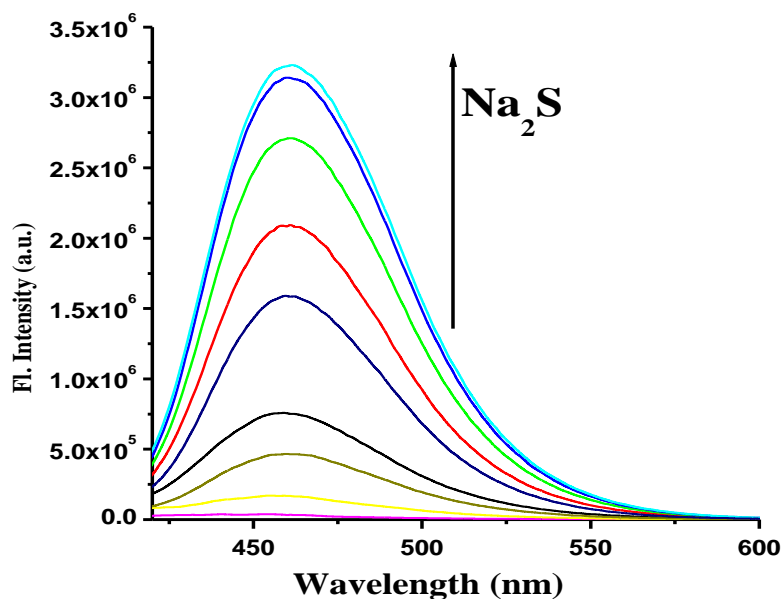


Figure S23: Fluorescence emission spectra of FBBP ($c = 1.0 \times 10^{-5}$ M) with Na₂S ($c = 1.0 \times 10^{-5}$ M) at pH 7.5 in CH₃CN: H₂O (2:8, v/v) (excitation at 405 nm).

7. MTT assay of the probe FBBP:

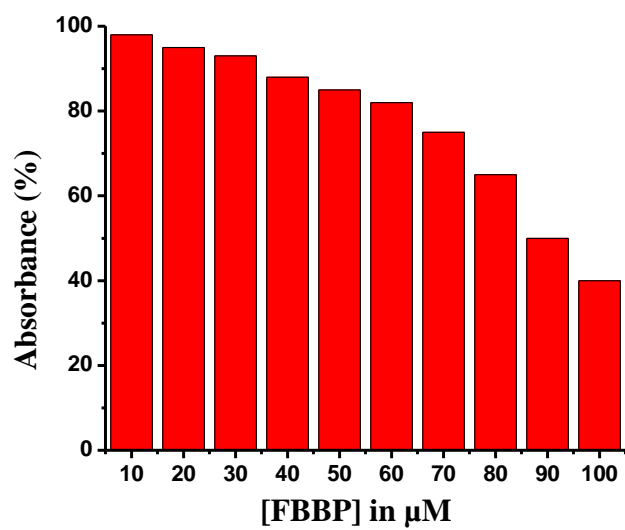


Figure S24: MTT assay with different concentration of probe (FBBP) after 24h.

8. References:

[S1] M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang, D. Zhu, *Org. Lett.* 2008, **10**, 1481-1484.