Supporting Information

A novel fluorene based "Turn On" fluorescent sensor for the determination of Zinc and Cadmium: Experimental and Theoretical Studies along with live Cell imaging.

Sohini Basu Roy[a], Jesmin Mondal[b], Anisur Rahman Khuda-Bukhsh[b] Kajal Krishna Rajak[a]*

[a]*Inorganic Chemistry Section, Department of Chemistry, Jadavpur University, Kolkata, 700 032, India. E-mail: kajalrajak@hotmail.com, kkrajak@chemistry.jdvu.ac.in*
[b]*Cytogenetics and Molecular Biology Laboratory, Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India*

CONTENTS

11) ***Determination of the fluoresence quantum yield (Table-S1).***

12) ***UV and Fluoresence spectra of the HAFPA ligand with the gradual addition of*** Cu^{2+} ***ion (S19).***

13) a)*Behaviour of the ligand in different Solvent, b)pH analysis of the ligand, Zn complex, Cd complex (S20).*

14) Absorption spectra of the free ligand, ligand complex and ligand complex with anion (S21).

15) Association Constant of the Zn receptor and Cd receptor in presence of EDTA and Sulphide ion (S22).

16) Detection Limit of the Zn receptor and Cd receptor in presence of EDTA and Sulphide ion (S23).

17) Competetion of the Zn receptor and Cd receptor with different anion (S24).

18) Determination of the first order rate constant (S25).

19) Analysis of the logic gate in Bar Diagram (S26).

18) Computational Study (Table-S2) and (Table-S3).

19) Theoretical Spectrum (S27) and (S28).

20) NMR Study over a longer period of time.

^{1}H NMR spectrum of Compound Hydroxy Fluorene:

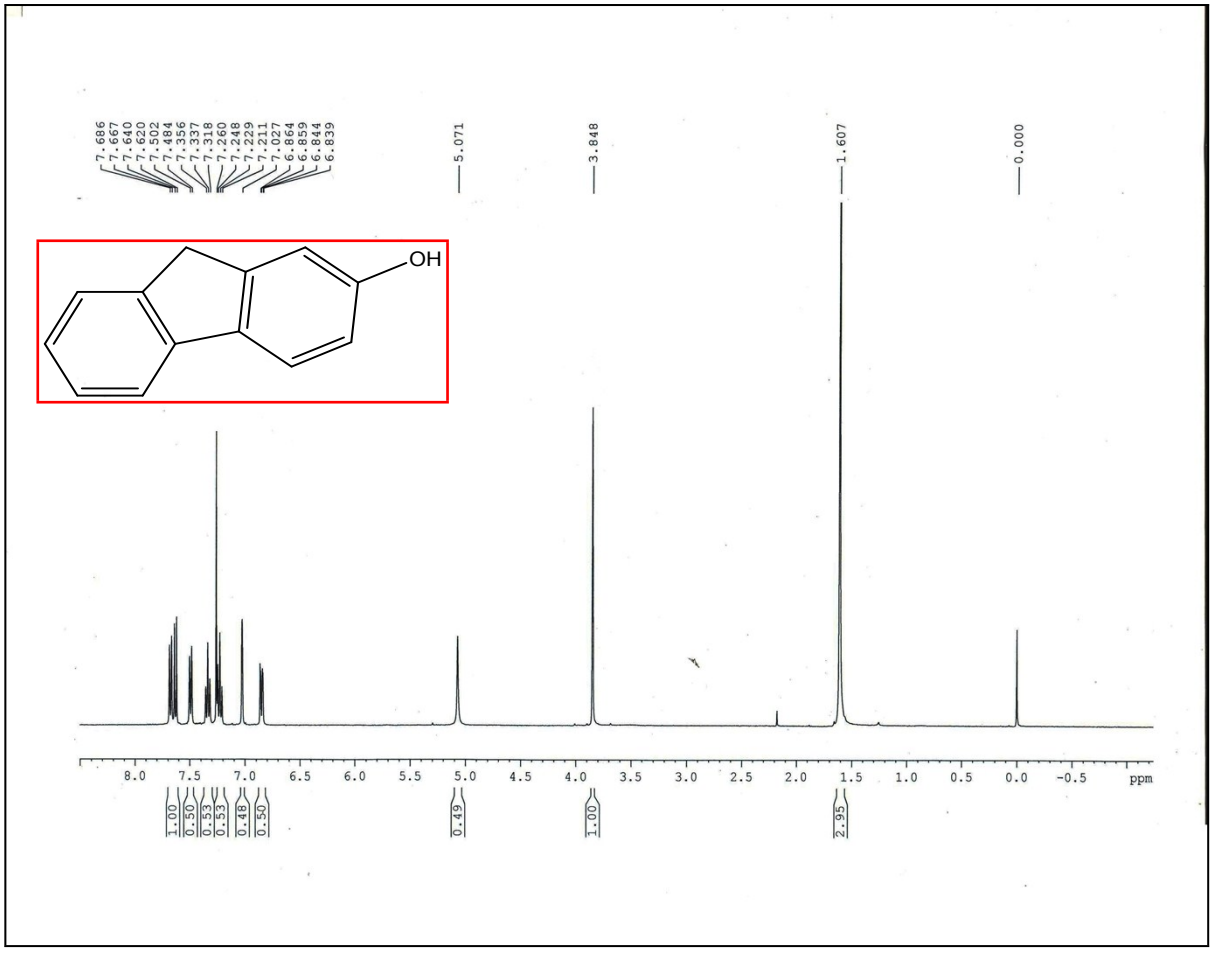


Fig S1. ^{1}H NMR spectrum of Hydroxy Fluorene.

Mass spectrum of compound Hydroxy Fluorene:

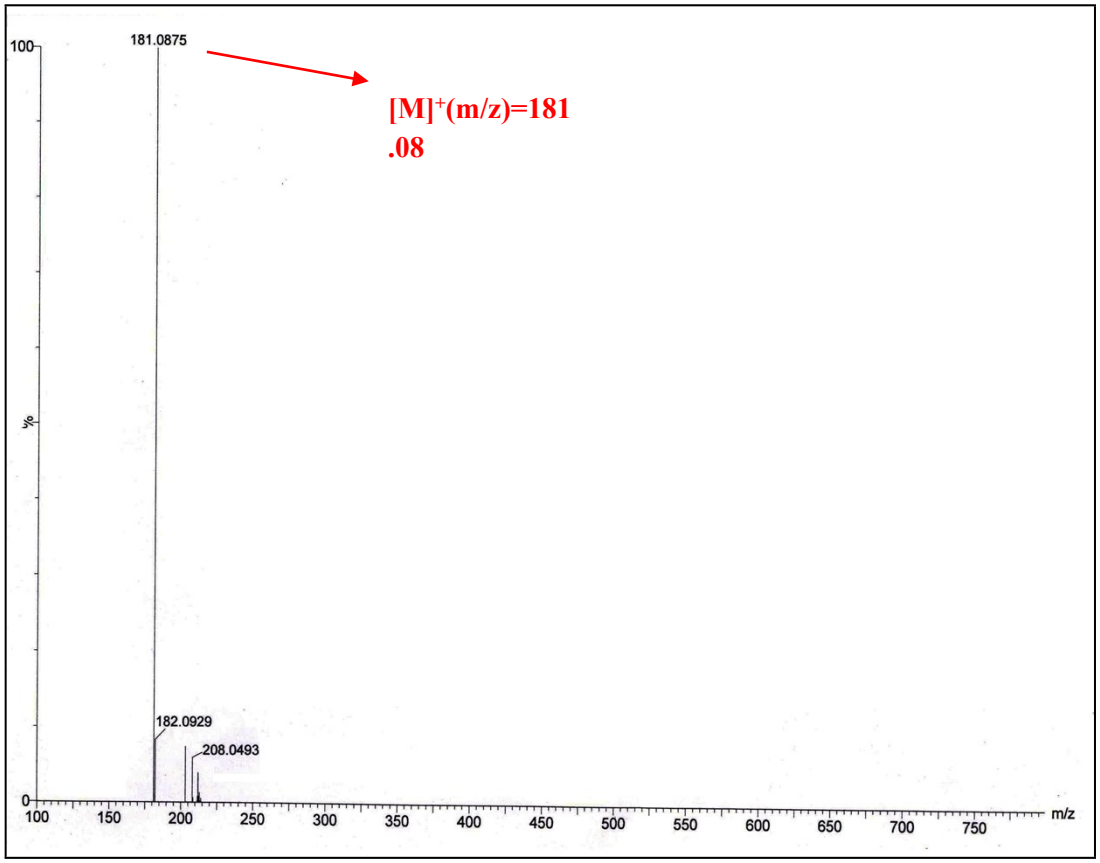
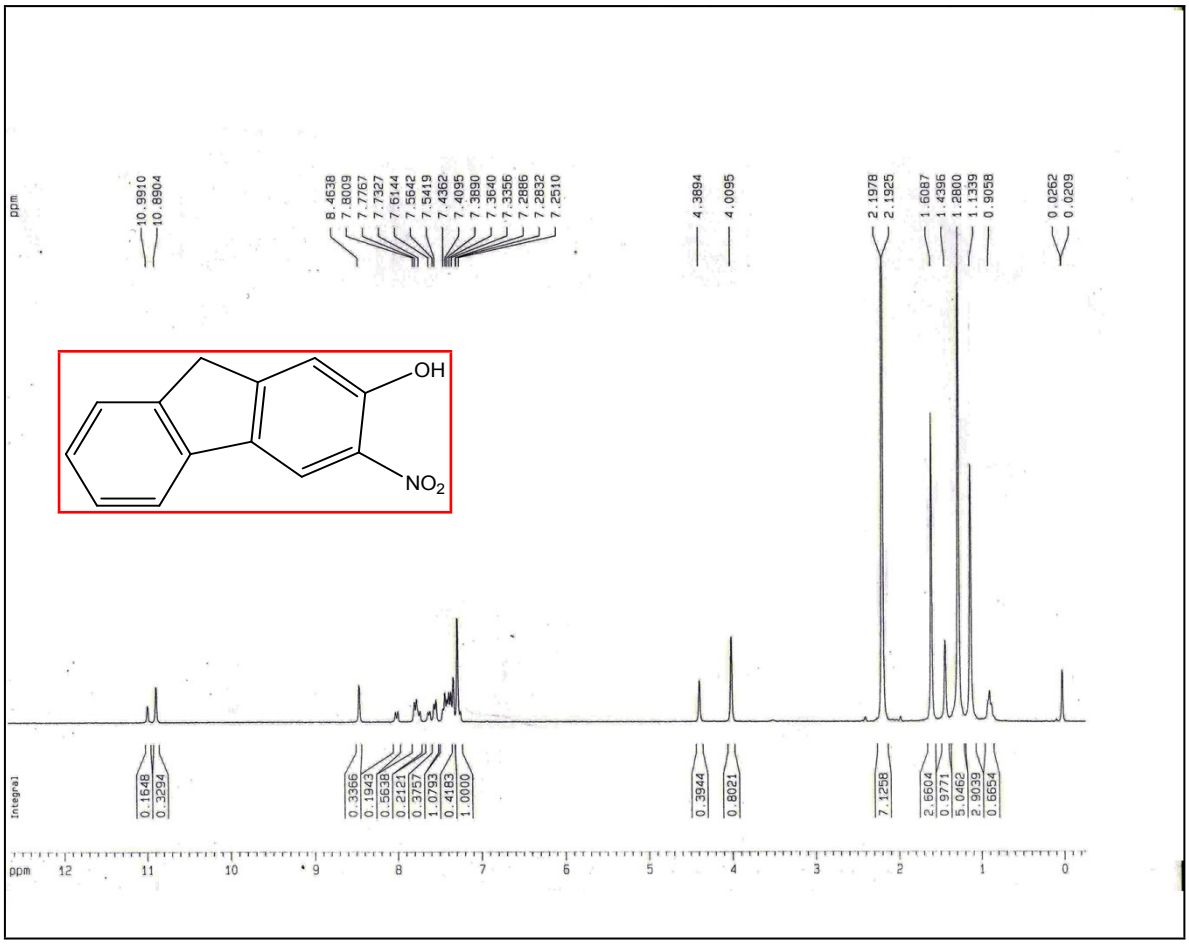


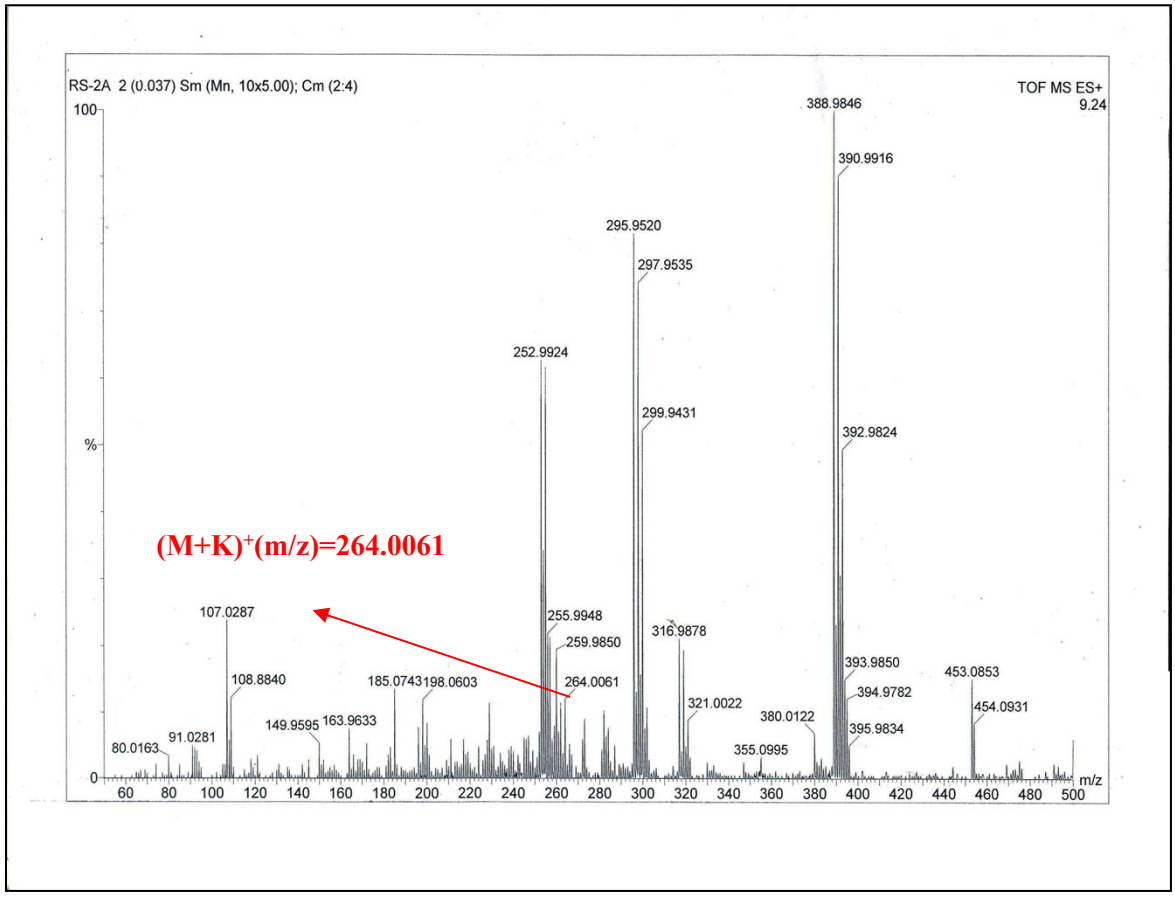
Fig S2. Mass spectrum of Hydroxy Fluorene.

[1]H NMR spectrum of Compound 3 nitro-2Hydroxy Fluorene



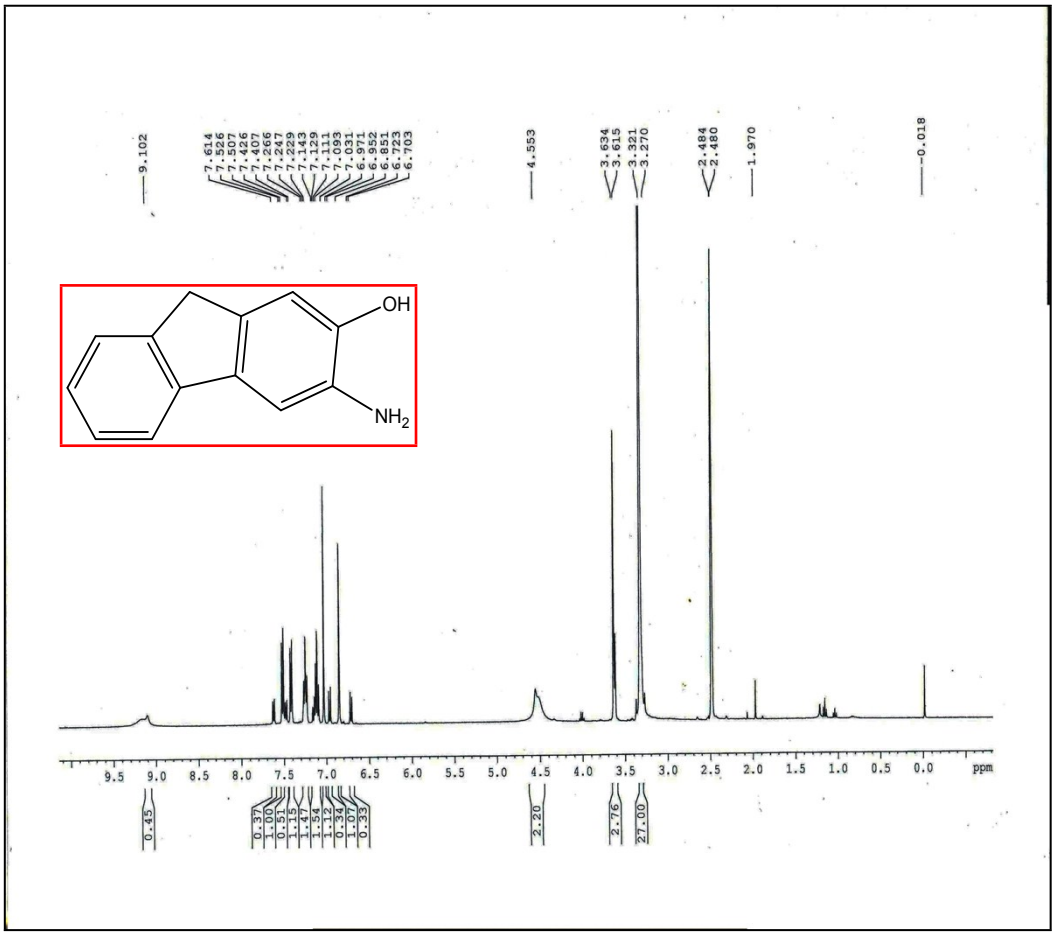
FigS3. [1] H NMR spectrum of 3-nitro 2-Hydroxy Fluorene.

Mass spectrum of compound 3 nitro-2Hydroxy Fluorene



FigS4. Mass spectrum of 3-nitro 2-Hydroxy Fluorene.

^{1}H NMR spectrum of Compound 3 amino-2Hydroxy Fluorene



FigS5. ^{1}H NMR spectrum of 3-amino 2-Hydroxy Fluorene.

Mass spectrum of compound 3 amino-2Hydroxy Fluorene

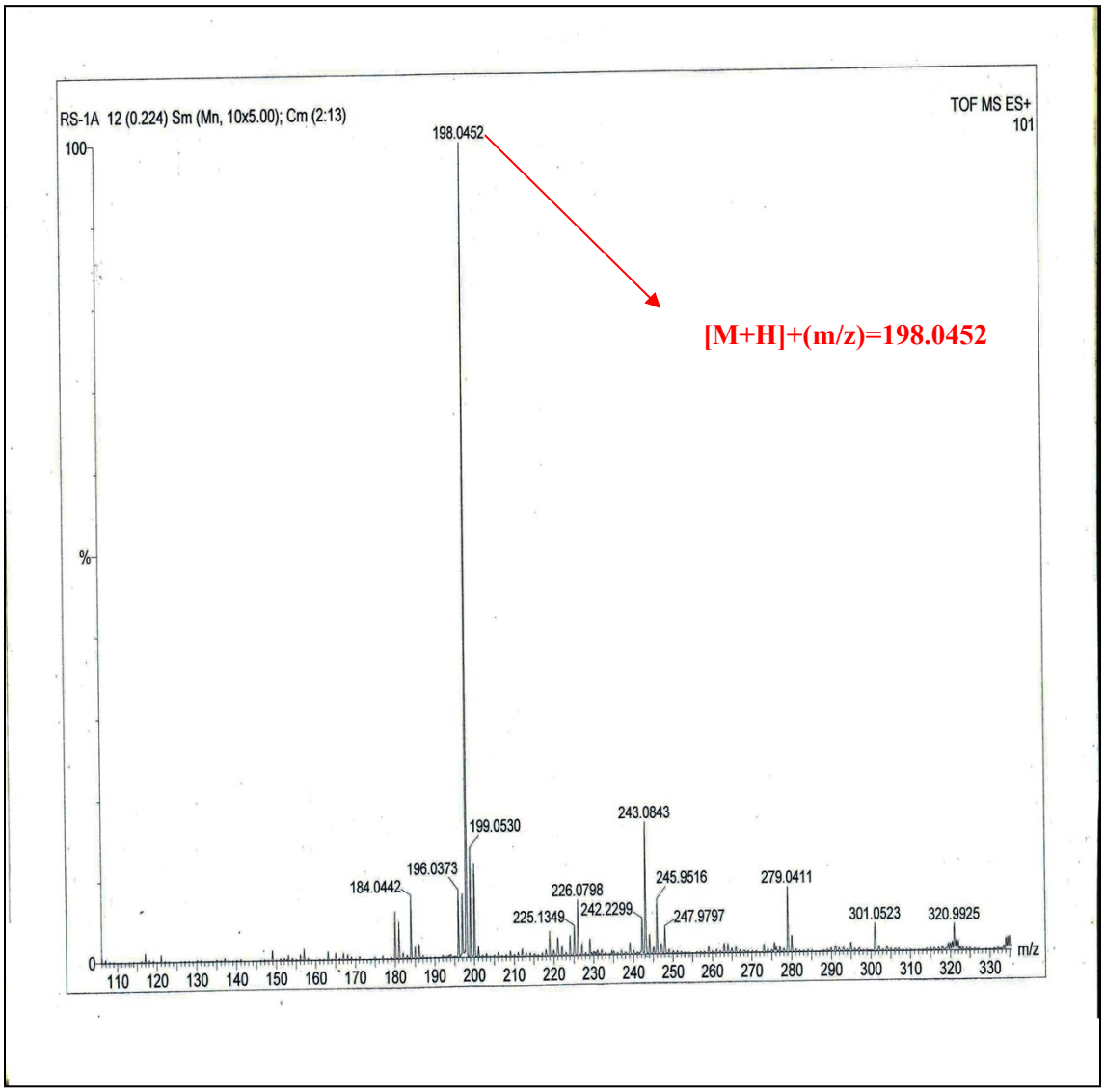
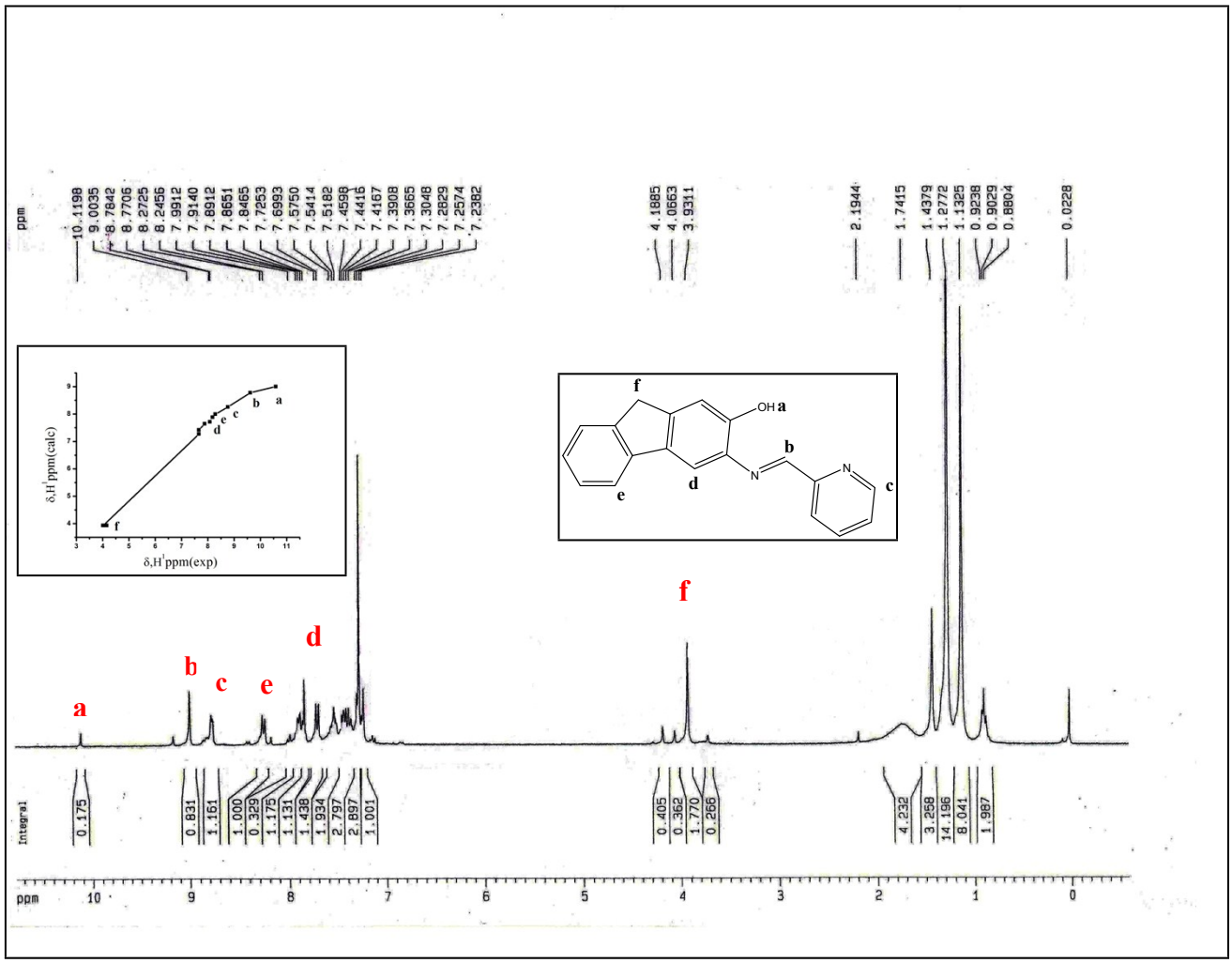


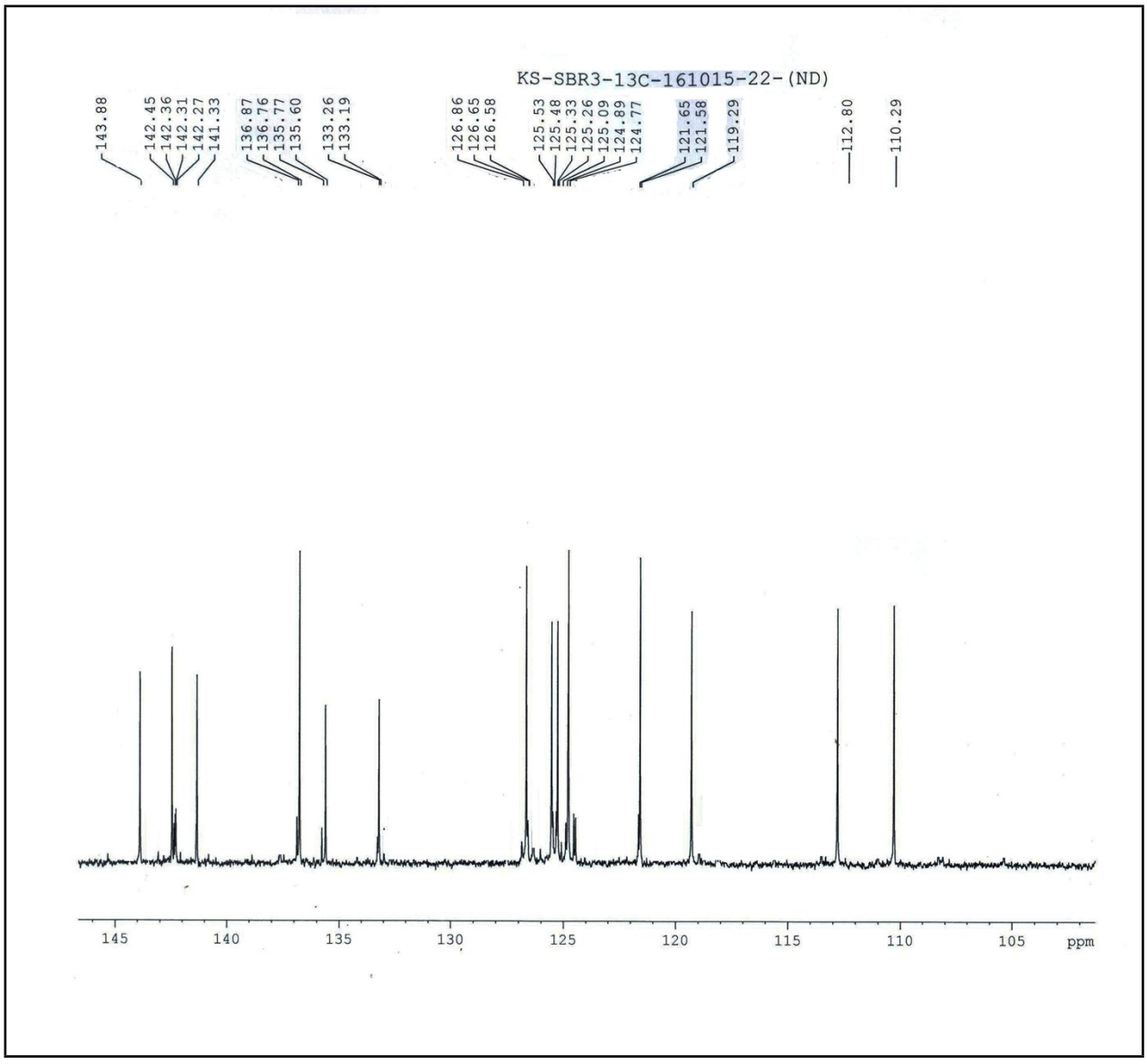
Fig S6. Mass spectrum of 3-amino 2-Hydroxy Fluorene.

^{1}H NMR spectrum of Compound HAFPA:



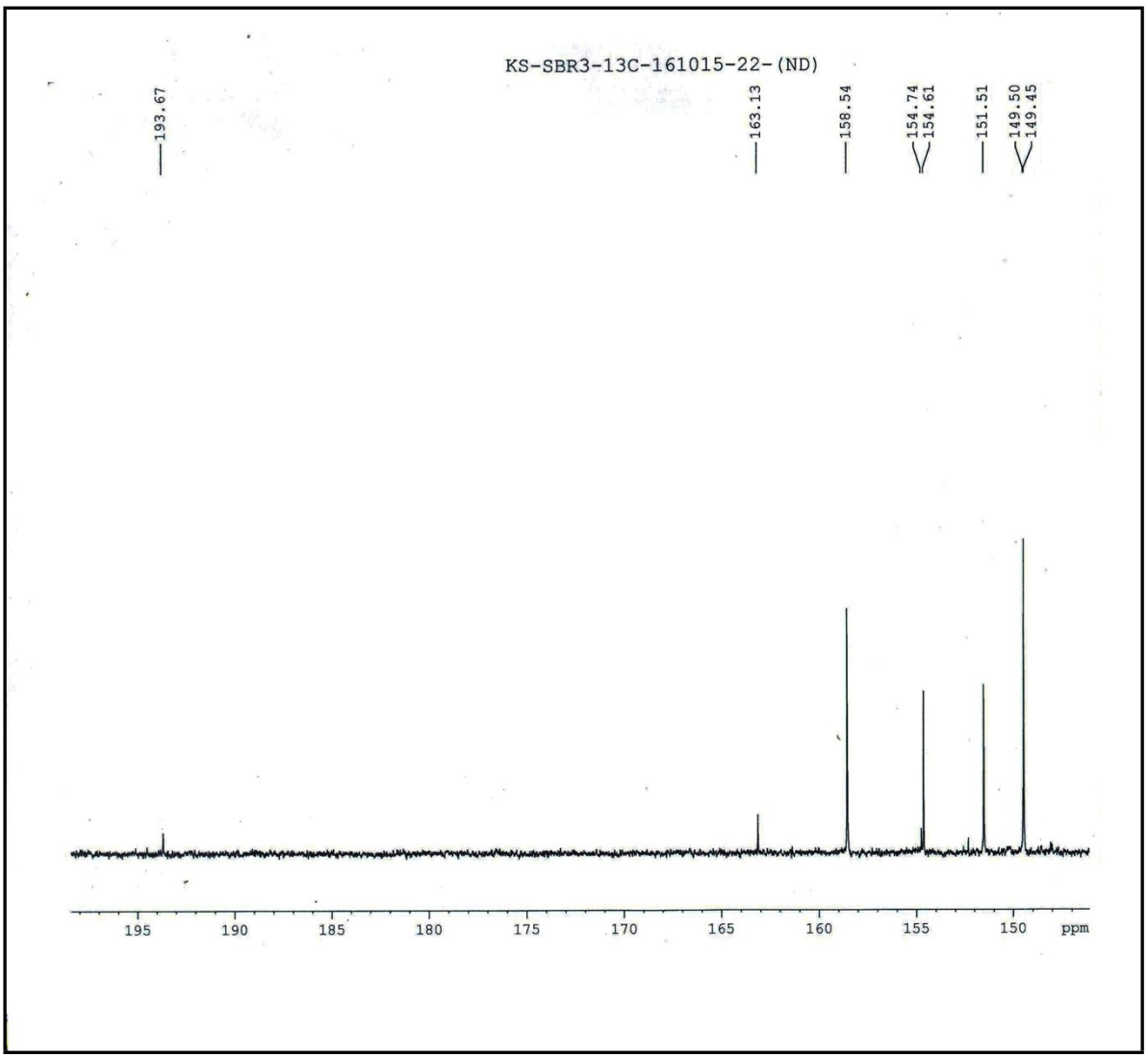
FigS7. ^{1}H NMR spectrum of HAFPA. The Linear correlation between the experimental and Calculated 1H NMR chemical shifts of HAFPA (inset).

^{13}C NMR spectrum of Compound HAFPA



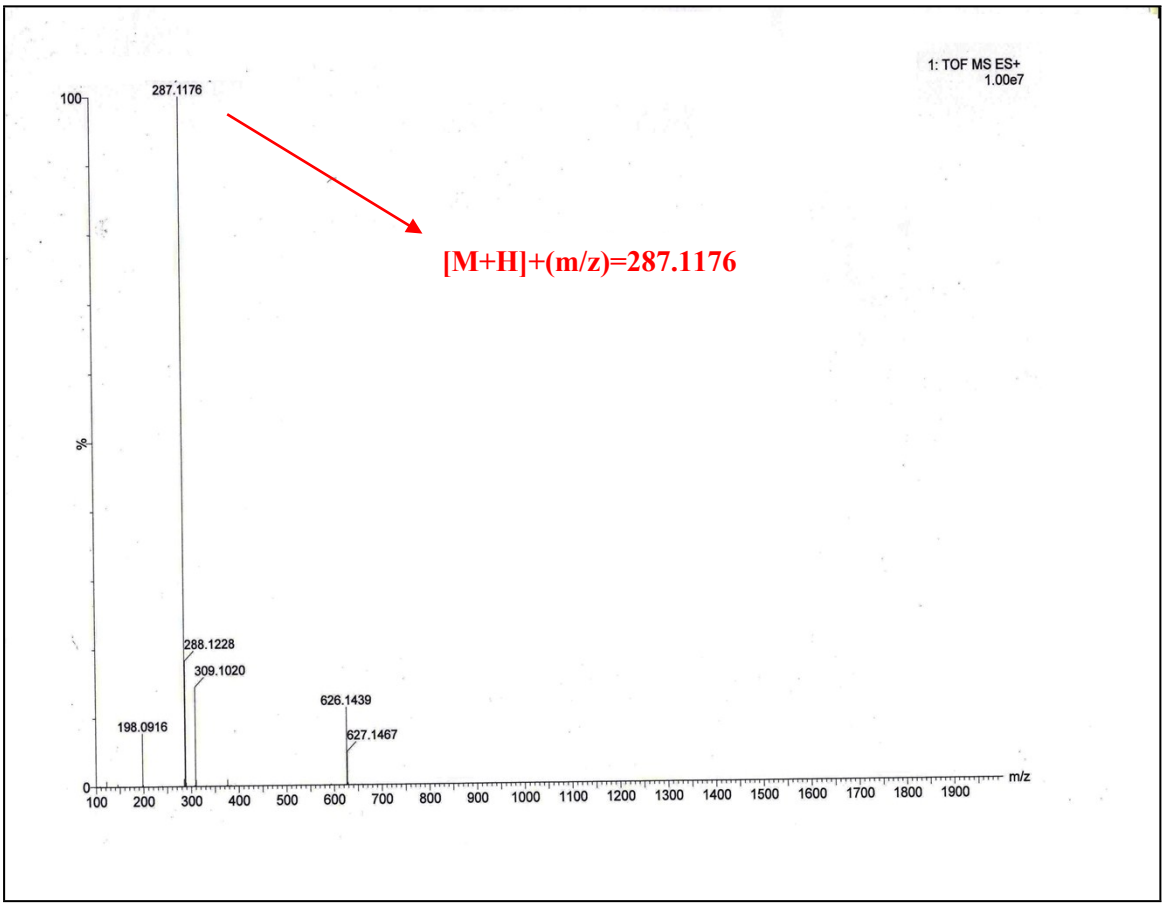
FigS8. ^{13}C NMR spectrum of Compound HAFPA.

Expanded ^{13}C NMR spectrum of Compound HAFPA



FigS9. Expanded ^{13}C NMR spectrum of HAFPA.

Mass spectrum of compound HAFPA:



FigS10. Mass spectrum of HAFPA.

[1]HNMR spectrum of HAFPA-Zn Complex:

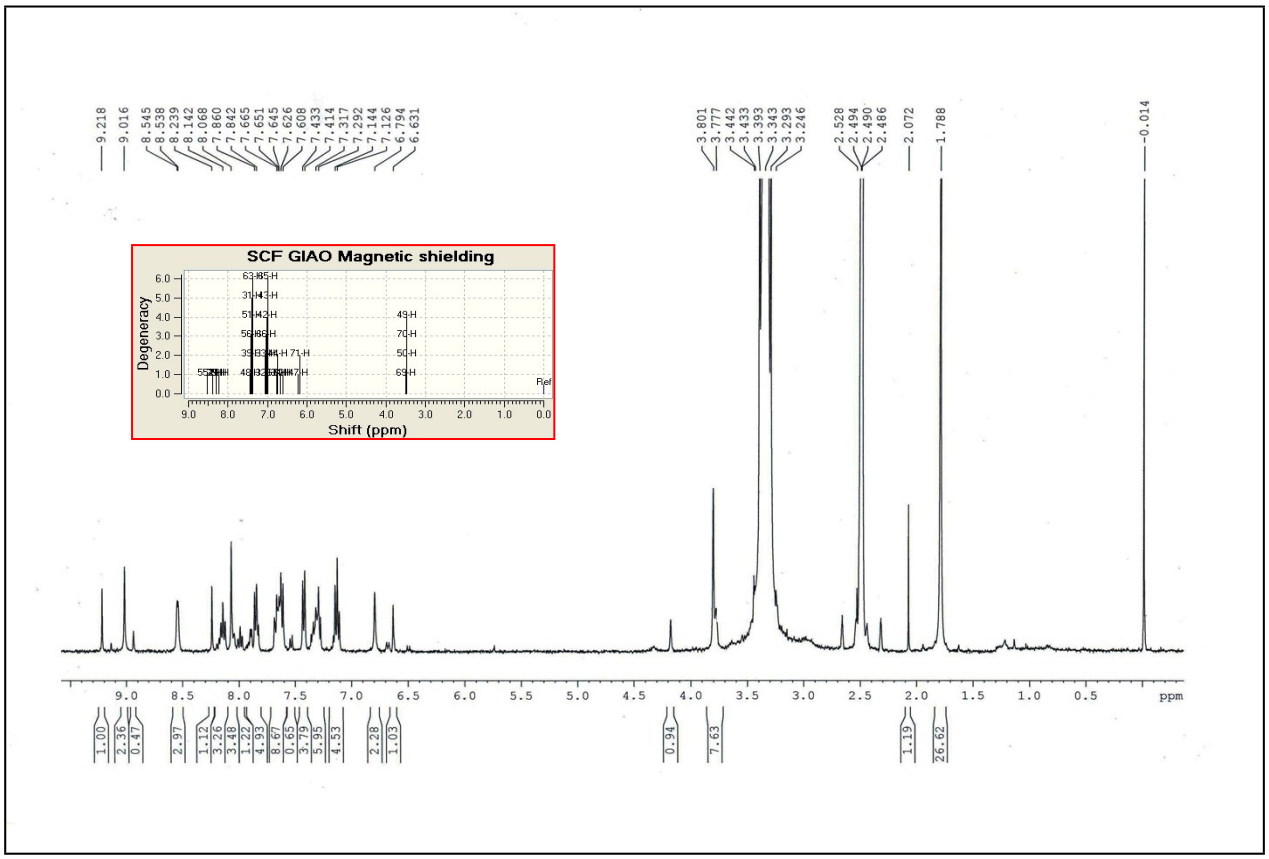
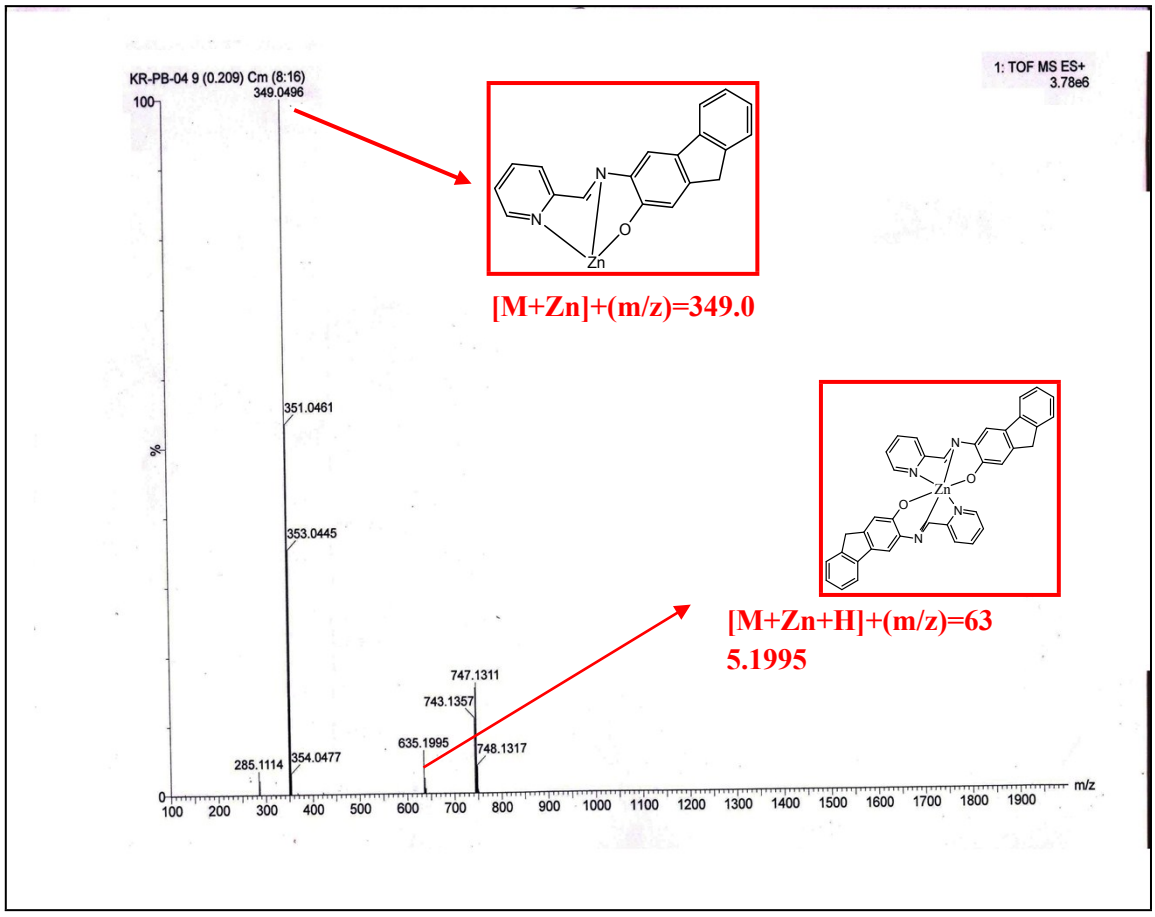


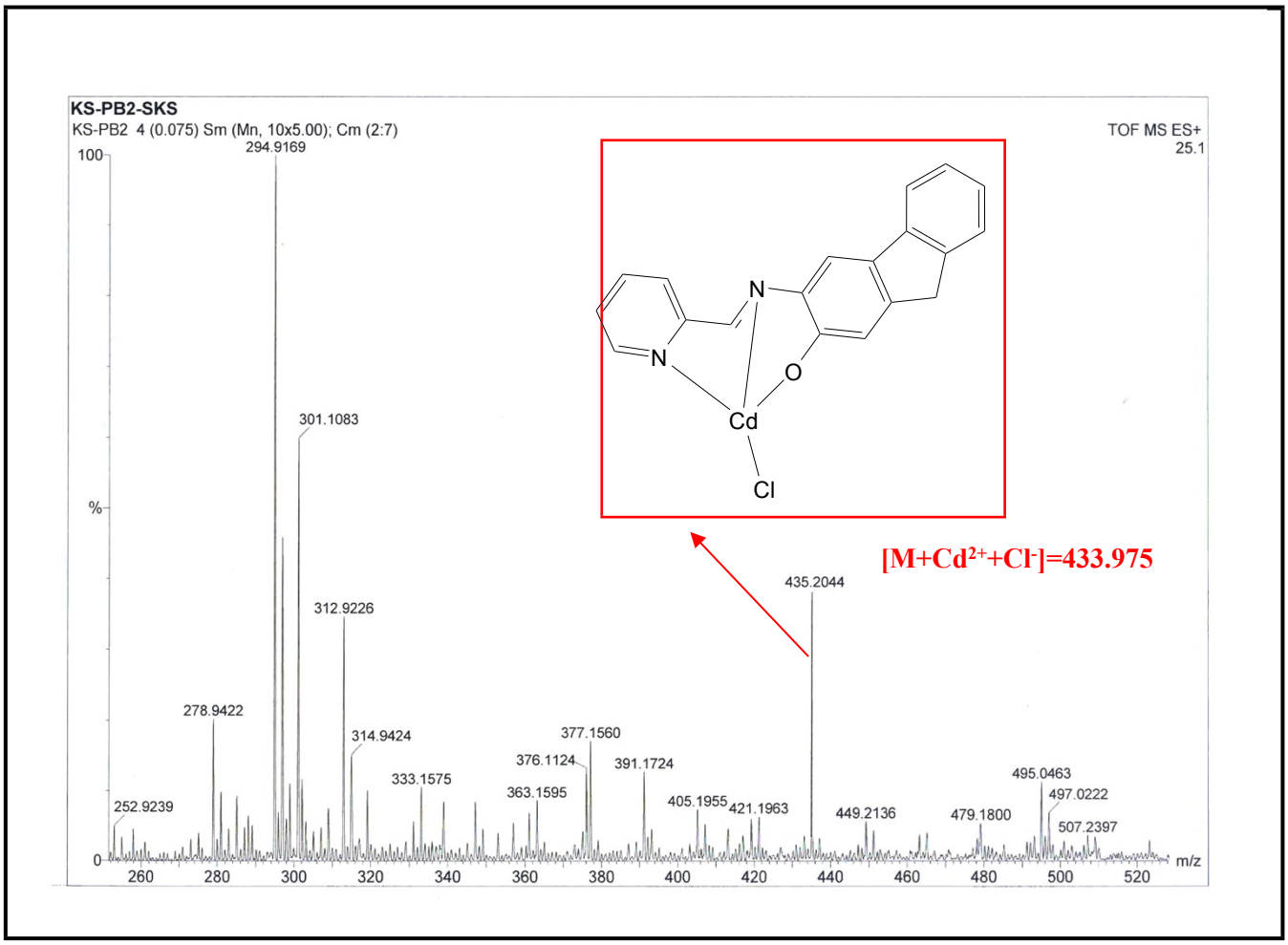
Fig S11. [1]H NMR spectrum of Zinc-HAFPA Complex.

Mass spectrum of HAFPA- Zn complex :



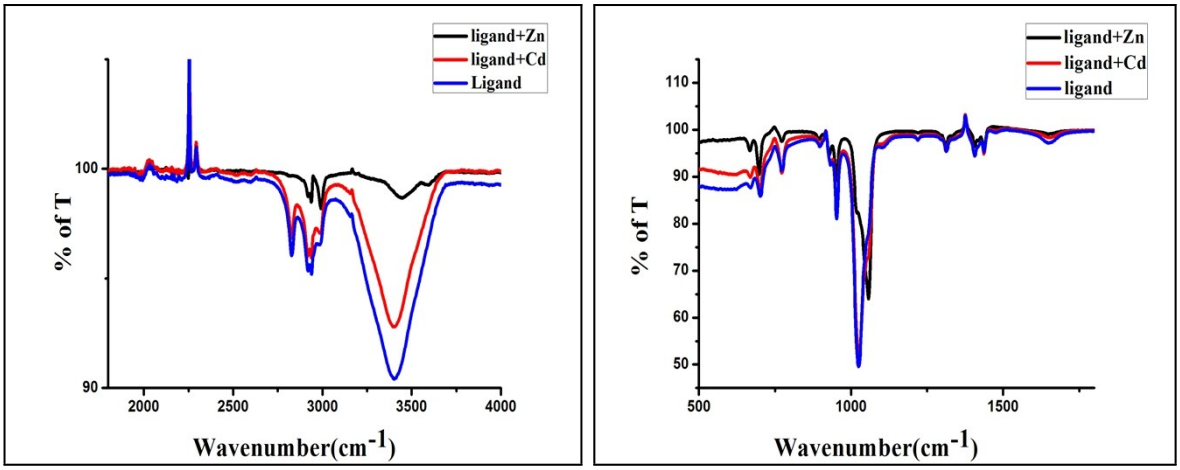
FigS12. Mass spectrum of HAFPA- Zn complex.

Mass spectrum of HAFPA- Cd complex :



FigS13. Mass spectrum of HAFPA- Cd complex.

FT-IR Data:



FigS14 . FT IR spectra of (a) HAFPA and its complex with Zn^{2+} and Cd^{2+} (b) same in expansion mode.

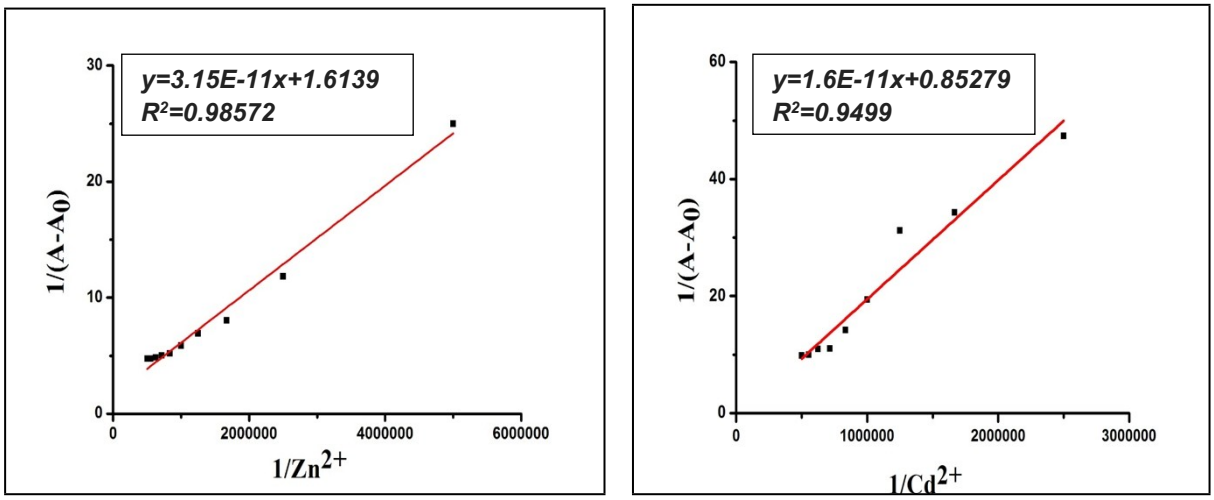
Determination of Association Constant (K_a):

By UV-vis method:

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

$1/(A\text{-}A_o) = 1/\{K(A_{max}–A_o)\ [M^{x+}]^n\} + 1/[A_{max}\text{-}A_o]$

Here A_o is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, A_{max} is absorbance in presence of added $[M^{x+}]_{max}$ and K_a is the association constant, where $[M^{x+}]$ is $[Zn^{2+}]$ and $[Cd^{2+}]$. The association constant (K_a) could be determined from the slope of the straight line of the plot of $1/(A\text{-}A_o)$ against $1/[M^{x+}]$ and is found to be 1.34×10^4 M^{-1} for Zn^{2+} and $6.4X10^4$ M^{-1} for Cd^{2+}.



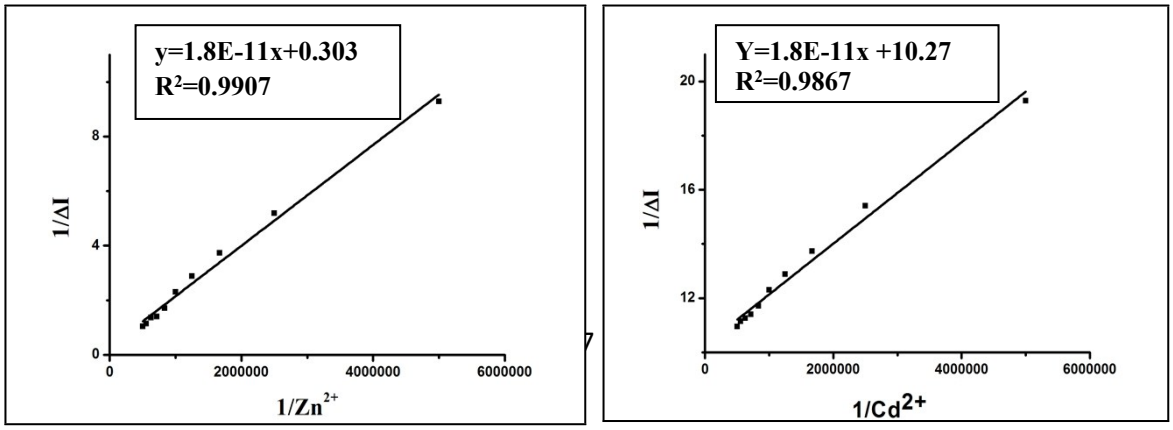
FigS15. Benesi-Hildebrand plot from absorption titration data of receptor (10 μM) with Zn^{2+} and Cd^{2+}.

By fluorescence method:

The binding constant value of Zn^{2+} and Cd^{2+} with receptor has been determined from the emission intensity data following the modified Benesi–Hildebrand equation,

$$1/\Delta I = 1/\Delta I_{max} + (1/K_a[C])(1/\Delta I_{max}).$$

Here $\Delta I = I - I_{min}$ and $\Delta I_{max} = I_{max} - I_{min}$, where Imin, I, and Imax are the emission intensities of receptor considered in the absence of metal, at an intermediate metal concentration, and at a concentration of complete saturation where K is the binding constant and [C] is the M^{x+}concentration respectively. From the plot of $[1 / (I - I_{min})]$ against $1/M^{x+}$ for receptor, the value of K has been determined from the slope. The association constant (K_a) as determined by fluorescence titration method for the receptor with Zn^{2+} is found to be 8.8×10^4 $M^{-1}$and Cd^{2+} is $8.8 \times 10^4 M^{-1}$.



FigS16. Benesi–Hildebrand plot from fluorescence titration data of receptor (10 μM) with Zn^{2+} and Cd^{2+}.

Calculation of the detection limit:

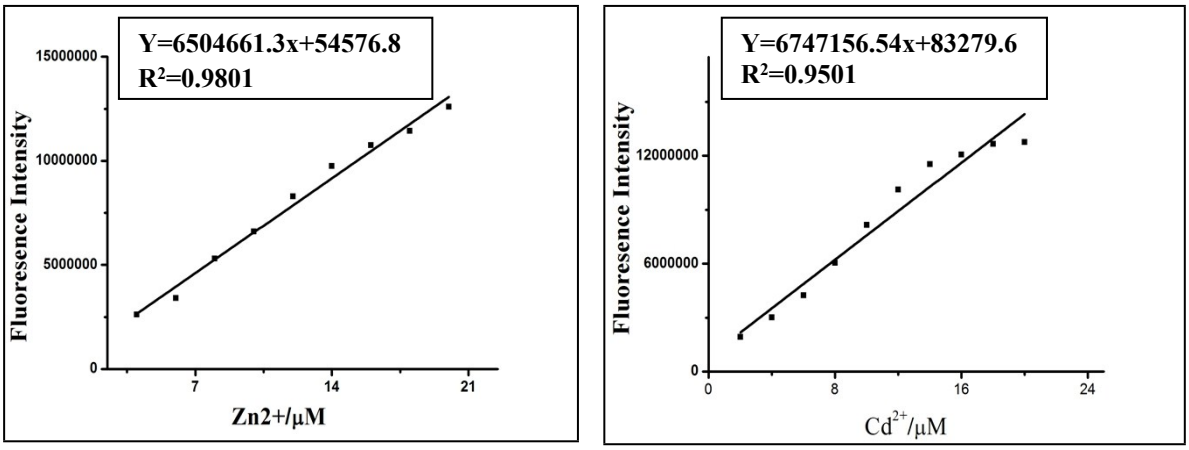
The detection limits DL of HAFPA for Zn^{2+} and Cd^{2+} were determined from the following equation1:

DL = K* Sb1/S

Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

From the graph (a) we get slope = 650461.35, and Sb1 value is 13.442 and from graph (b) we get slope = 674715.54 and Sb1 value is 11.96.

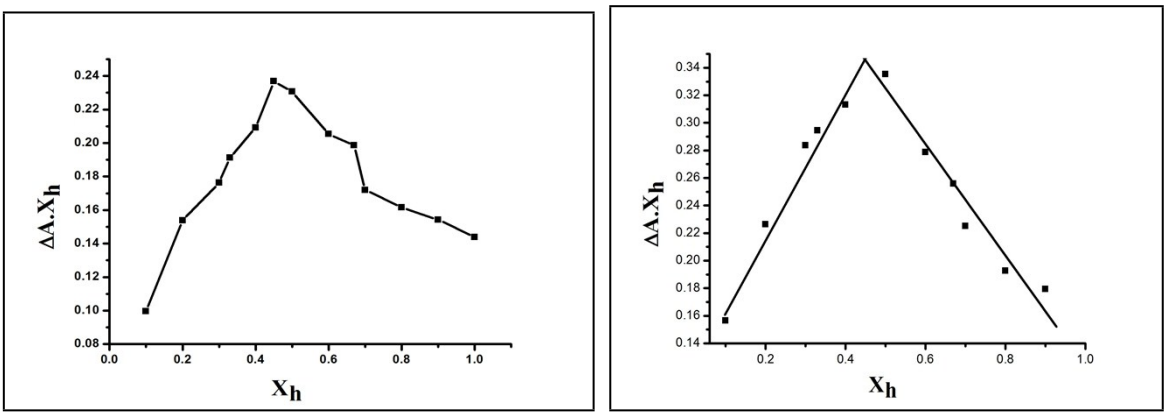
Thus using the formula we get the Detection Limit for Zn^{2+} is 0.61 μM i.e. HAFPA can detect Zn^{2+} ion in this minimum concentration and Detection Limit for Cd^{2+} is 0.53 μM.



FigS17. The linear change of fluorescence intensity as a function of (a)[Zn^{2+}] at 485nm and (b)[Cd^{2+}] at 485 nm.

Job's plot by absorbance method:

Stock solution of same concentration of sensor and Zn^{2+} and Cd^{2+} was prepared in the order of 10 μM in (at 25 °C) at pH 7.4 in HEPES buffer. The absorption spectrum in each case with different host–guest ratio but equal in volume was recorded. Job's plots were drawn by plotting $\Delta I.X_{host}$ vs X_{host} (ΔI = change of intensity of the emission spectrum during titration and X_{host} is the mole fraction of the host in each case, respectively).



FigS18. Jobs plot diagram of HAFPA for a) Zn^{2+} and b) Cd^{2+}(where Xh is the mole fraction of host and ΔA indicates the change of the absorbance).

The highest peak at 0.45 indicates the formation of both 1:1 and 1:2 complexes for Zn^{2+} whereas the peak at 0.5 indicates the formation of only 1:1 complex for Cd^{2+}.

Determination of the fluorescence quantum yield

Here, the quantum yield φ was measured using the following equation:

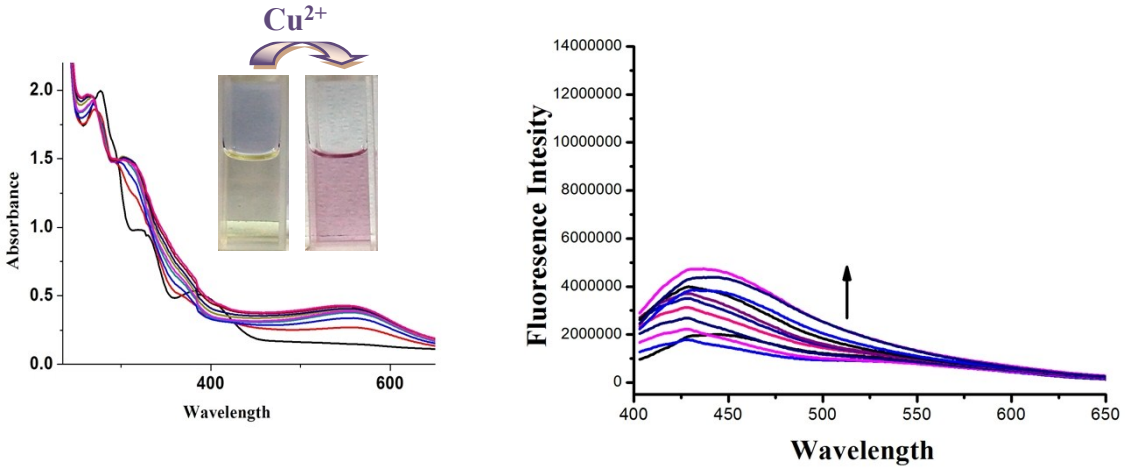
$$\Phi_X = \Phi_S \text{ X } (I_x/I_s) \text{ X } (A_x/A_s) \text{ X } (n_x/n_s)^2$$

where X and S indicate the unknown and standard solution respectively, φ = quantum yield, I = area under the emission curve, A = absorbance at the excitation wavelength, and n =index of refraction of the solvent. Here φ measurements were performed using quinine sulphate in ethanol as a standard [φ =0.54] (error ~ 10%).

Photophysical parameters of the complexes in acetonirile solution at room temperature.Table(S1):

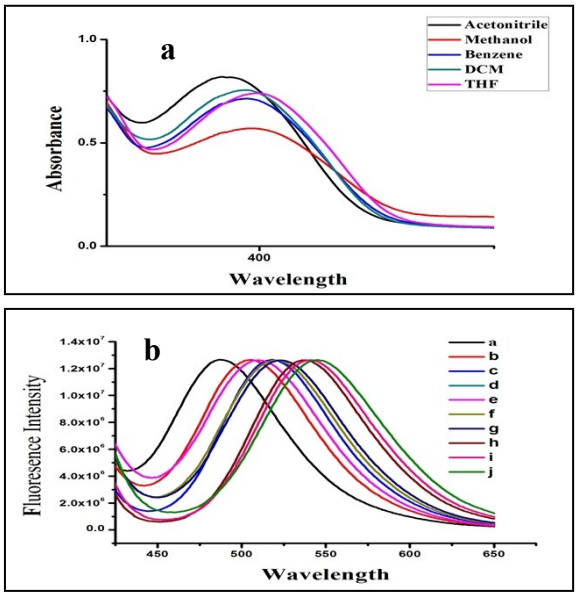
Sample	$\lambda_{max,}$ nm (ε, M^{-1} cm^{-1})	$\lambda_{emi,}$ nm	Φ ($\times 10^{-3}$)	k_r, s^{-1} ($\times 10^6$)	k_{nr}, s^{-1} ($\times 10^8$)	τ_1, ns	τ_2, ns
Ligand	440(2193371), 540(1086772)	380	42	9.110	2.0079	1.16	4.610
Zn Complex	485(12635645)	485	557	103.91	8.26	5.36	1.03
Cd Complex	495(12590869)	495	996	113.2	12.2	9.89	4.87

UV and Fluoresence spectra of the HAFPA ligand with the gradual addition of Cu2+ ion:

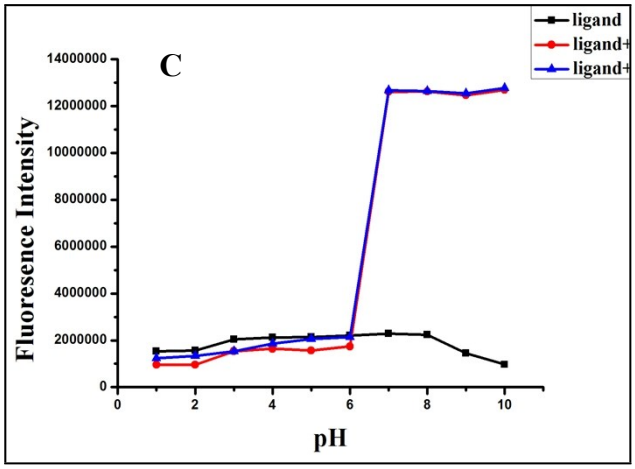


FigS19. Absorption and Fluoresence emission spectra of the ligand($2x10^{-5}$) with subsequent addition of Cu^{2+}($2x10^{-4}$).

Solvent Study:



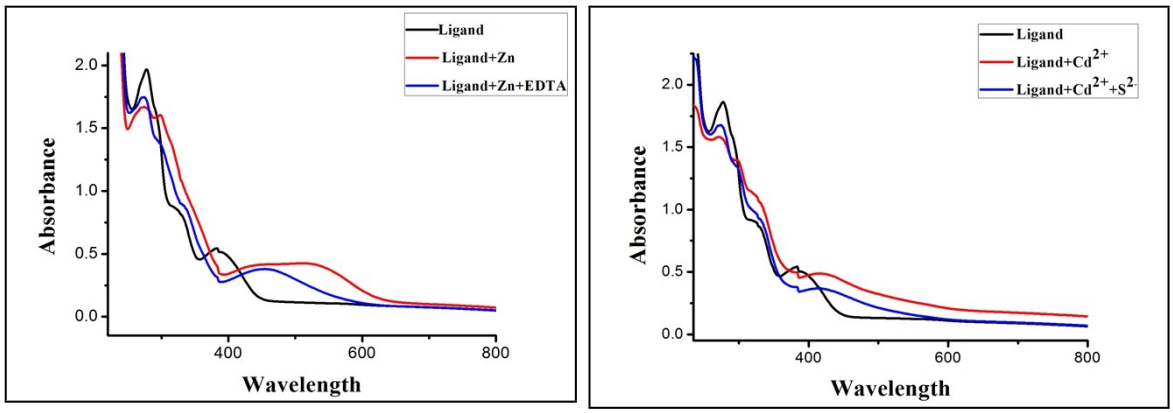
pH Study:



FigS20. a) Absorption spectra of the ligand ($1X10^{-5}$) in different solvent as depicted in the picture. **b)** Fluorescence spectra of the receptor HAFPA (c = $1x10^{-5}$ M) with 1.0 equiv of zinc (c = $2x10^{-4}$ M) in different proportions of water in CH_3CN at pH =7.4: (a)10% H_2O (b) 20% H_2O (c) 30% H_2O (d) 40% H_2O e) 50% H_2O f) 60% H_2O g)70% H_2O h)80% H_2O i)90% H_2O j)99.5% H_2O.

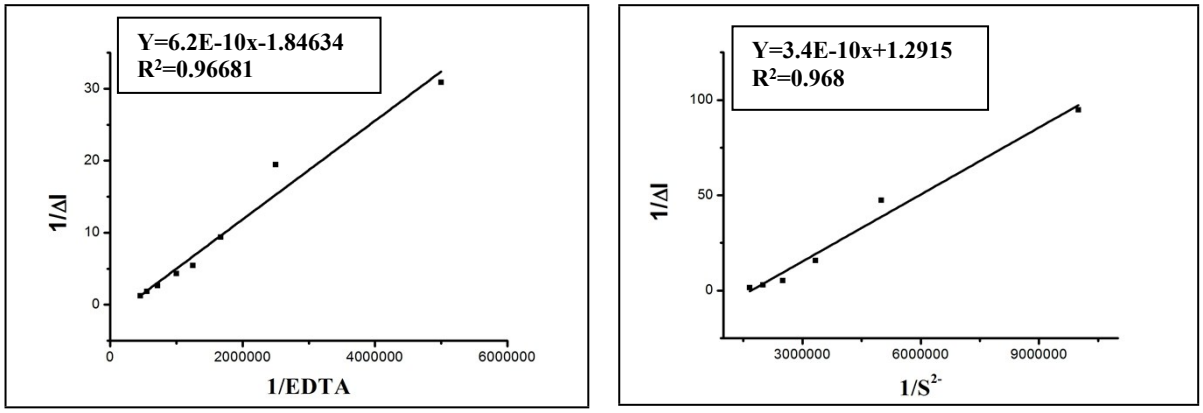
C) Fluorescence intensity of HAFPA (c = 2×10^{-5} M) at various pH values in water medium in the absence and presence of Zn^{2+} (c = 2.0 $x10^{-4}$M) and Cd^{2+} adjusted by using aqueous solutions of 1M HCl and 1M NaOH.

Reversibility Study:



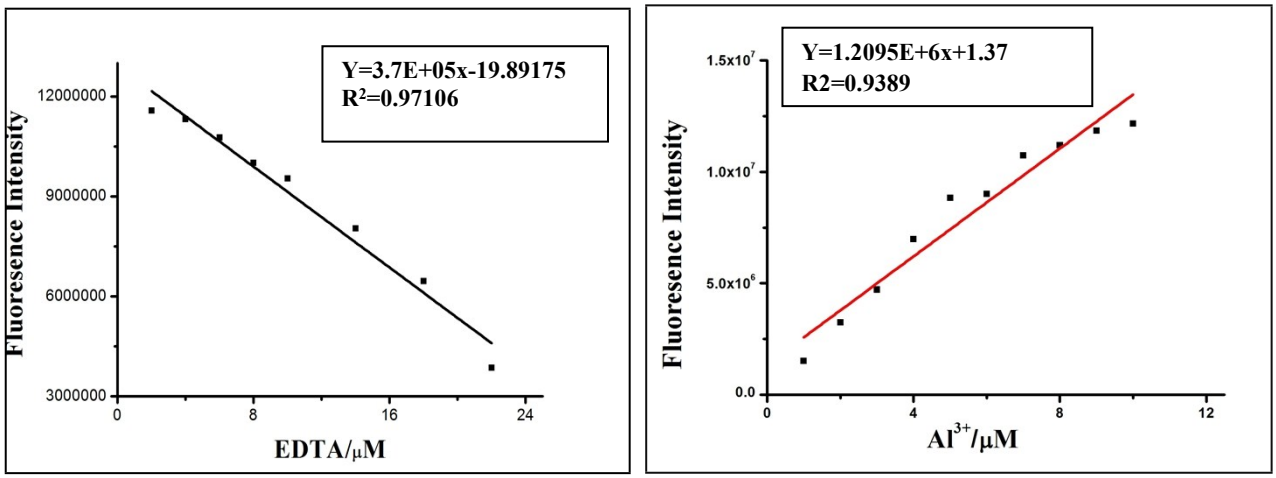
FigS21. UV-vis absorption spectra of HAFPA(c = 1.0×10^{-5}M) in CH_3CN-HEPES buffer (8/2, v/v, 25 º C) by alternative addition of a)Zn^{2+} and EDTA and b)Cd^{2+} and S^{2-}.

Calculation of the Association Constant:



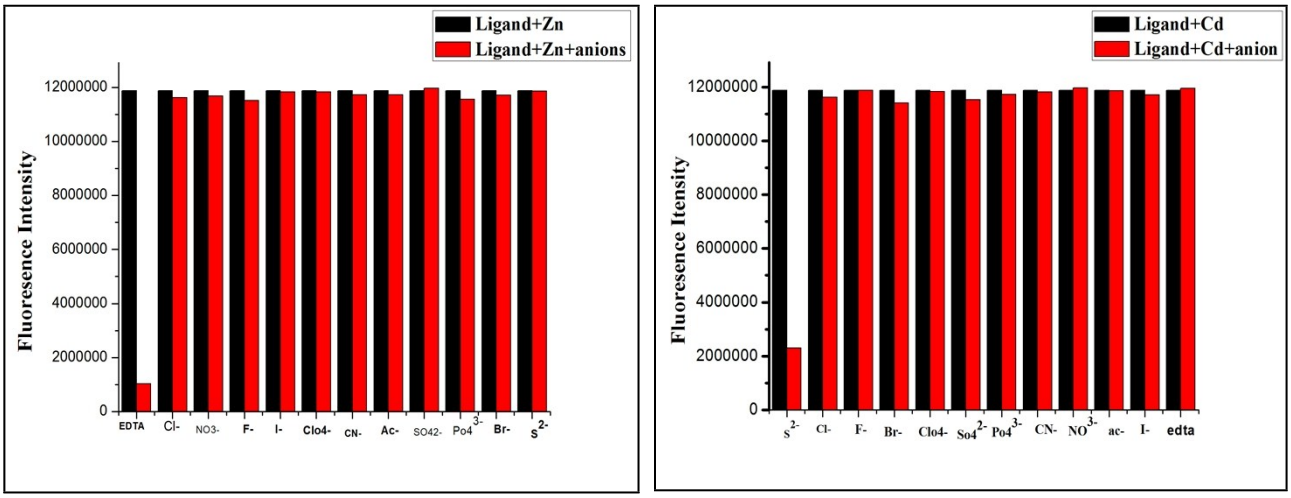
FigS22. Determination of association constant of a)HAFPA-Zn receptor and b)HAFPA-Cd receptor for EDTA and S^{2-} respectively from fluorescent titration data(For determination of association constant of EDTA and S^{2-} with the receptor, K_a is found to be $2.41x10^4$ and $4.41x10^4$respectively.)

Calculation of the Detection Limit:



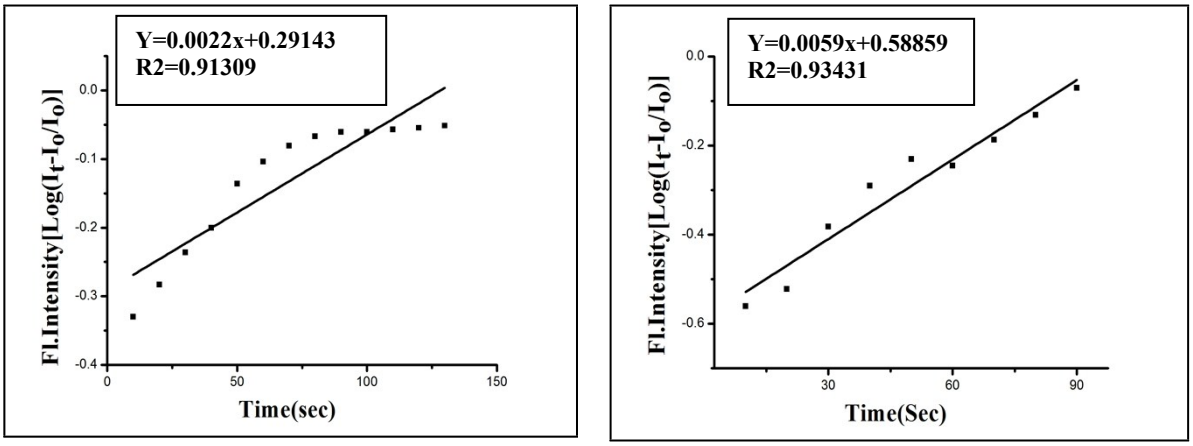
FigS23. From the graph (a) we get slope = 378324, and Sb1 value is 11.32 and from graph (b) we get slope = 1209565 and Sb1 value is 26.32.Thus using the formula we get the Detection Limit for EDTA is $8.97X10^{-5}$ M and Detection Limit for S^{2-} is $6.5X10^{-5}$M.

Competetion Experiment of Different anions with Receptor :



FigS24. Anion selectivity profile of the sensor(a) HAFPA–Zn and (b) HAFPA-Cd (Receptor) : (black bar) change of the emission intensity of the ligand with Zn and Cd(Receptor); (red bar) change of the emission intensity of Receptor + 5.0 equiv. of different anions, at 485 nm.

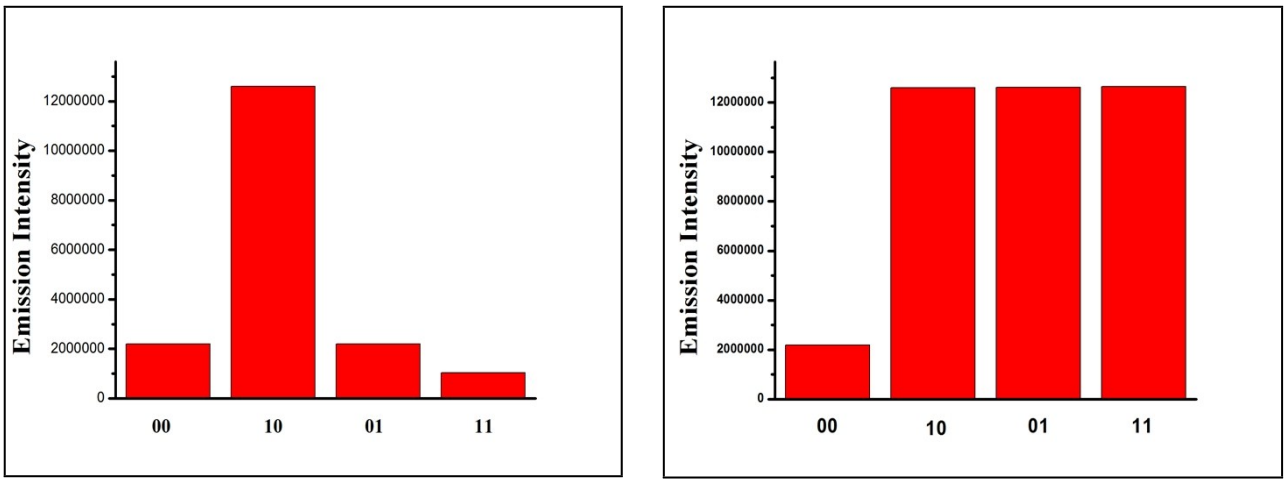
Calculation of the first order rate constant of HAFPA at different time interval by addition of Zn2+ and Cd2+:



FigS25. The first order rate equation by using Time vs. fluorescence plot at 490 nm (I_t =Maximum intensity, I_0 = Initial Intensity) for a) Zn^{2+} and b)Cd^{2+}.

From the time vs. fluorescence plot Fig. (a) and (b) at fixed wavelength at 490 mm by using first order rate equation, we get the rate constant K=slope x 2.303. From the plot (a), Slope=0.0022, K=5.066X10^{-3}. (b), Slope=0.00595, K=1.37X10^{-2}.

Logic Gate:



FigS26. (**a**) Fluorescence output of HAFPA (c = 1 × 10^{-5} M) at 490 nm (λex = 380 nm) in the presence of chemical inputs, Zn^{2+} (c = 2 × 10^{-4} M) and EDTA (c =2 × 10^{-4} M) at pH = 7.4.(**b**) Fluorescence output of HAFPA (c = 1 × 10^{-5} M) at 490 nm (λex = 380 nm) in the presence of chemical inputs, Zn2+ and Cd2+ (c = 2 × 10^{-4} M) and finally both at pH = 7.4.

Similar experiment is carried with Cd^{2+} and S^{2-} for the plot (a), it shows the same result.

Computational Method

Full geometry optimizations were carried out using the density functional theory (DFT) method at the Becke-3-Lee-Yang-Parr(B3LYP) 24 level for the ligand HAFPA and its octahedral Zn^{2+} and square planar Cd^{2+} complexes. The 6-31+G (d,p) basis set was assigned for all the elements. All calculations were performed with Gaussian03program with the aid of the Gauss View visualization program.

TableS2. Vertical electronic excitations of HAFPA, HAFPA-Zn^{2+} and HAFPA- Cd^{2+} calculated by TDDFT/B3LYP/CPCM method.

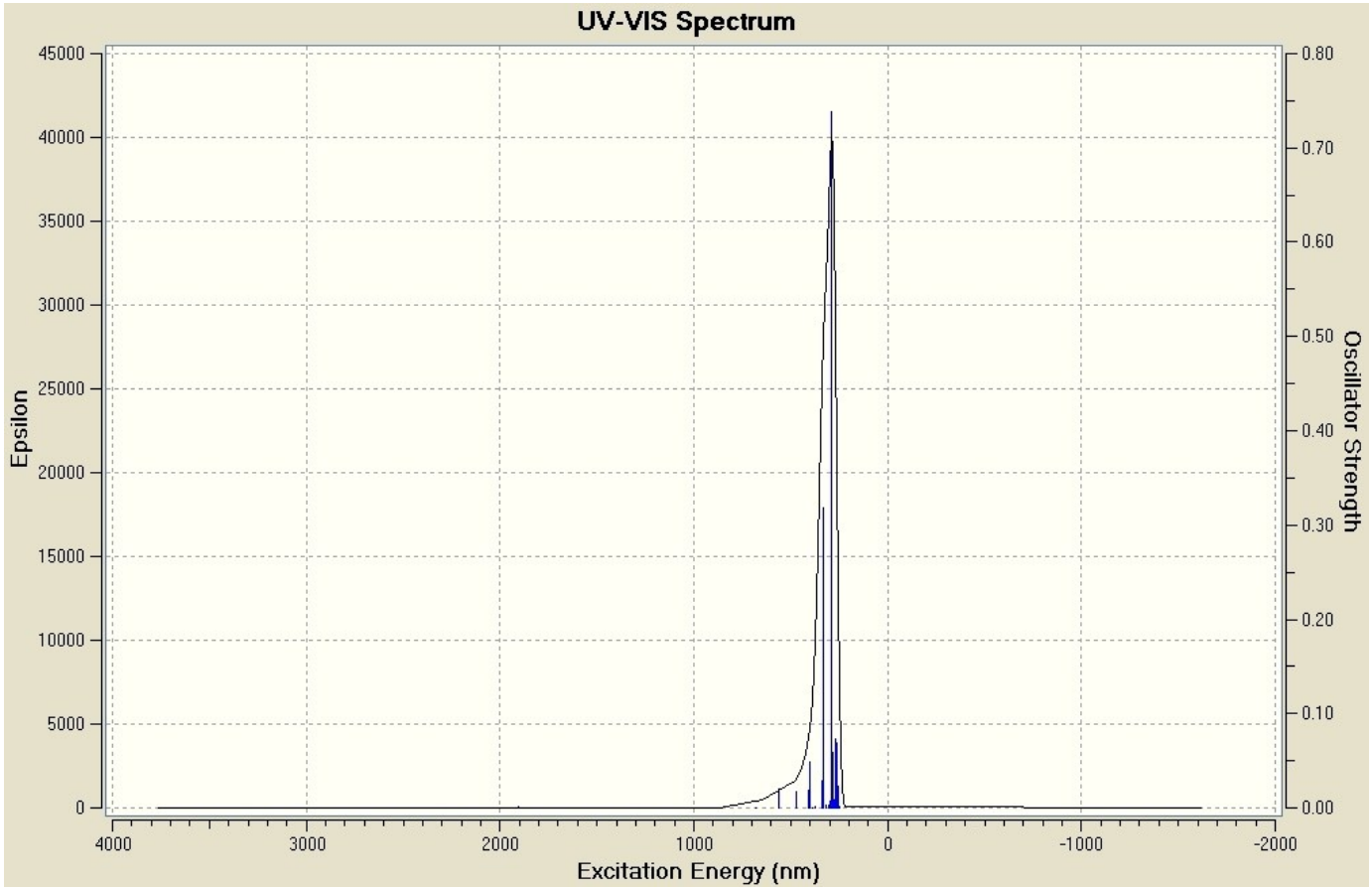
Compoud	Excitation	Theoretical Wavelength(nm)	CI	Λexpt. (nm)
HAFPA	HOMO-1 → LUMO HOMO → LUMO+3 HOMO-3 → LUMO	398 384 376	0.0490 0.0005 0.0014	380
	HOMO-2 → LUMO	330	0.49851	328
HAFPA-Cd2+	HOMO → LUMO	562	0.70461	480
	HOMO → LUMO+1 HOMO-1 → LUMO	363 358	0.0159 0.56239	385
	HOMO → LUMO+2 HOMO-2 → LUMO	320 318	0.46848 0.5304	324
HAFPA-Zn2+(2:1)	HOMO-1 → LUMO+1 HOMO-1 → LUMO	552 553	0.65904 0.64670	500

	HOMO → LUMO+2 HOMO → LUMO+3	371 369	0.69929 0.69304	385
	HOMO-2 → LUMO+2	266	0.12290	275
HAFPA-Zn(1:1)	HOMO ⟶ LUMO	582	0.70367	500
	HOMO-1 → LUMO HOMO → LUMO+2	369 358	0.65227 0.65045	380
	HOMO-3 ⟶ LUMO	331	0.69055	320
	HOMO-2 → LUMO+3	282	0.69033	275

TableS3. Calculated Triplet excited state of Zn and Cd Complex in Acetonitrile based on the lowest lying triplet state geometry.

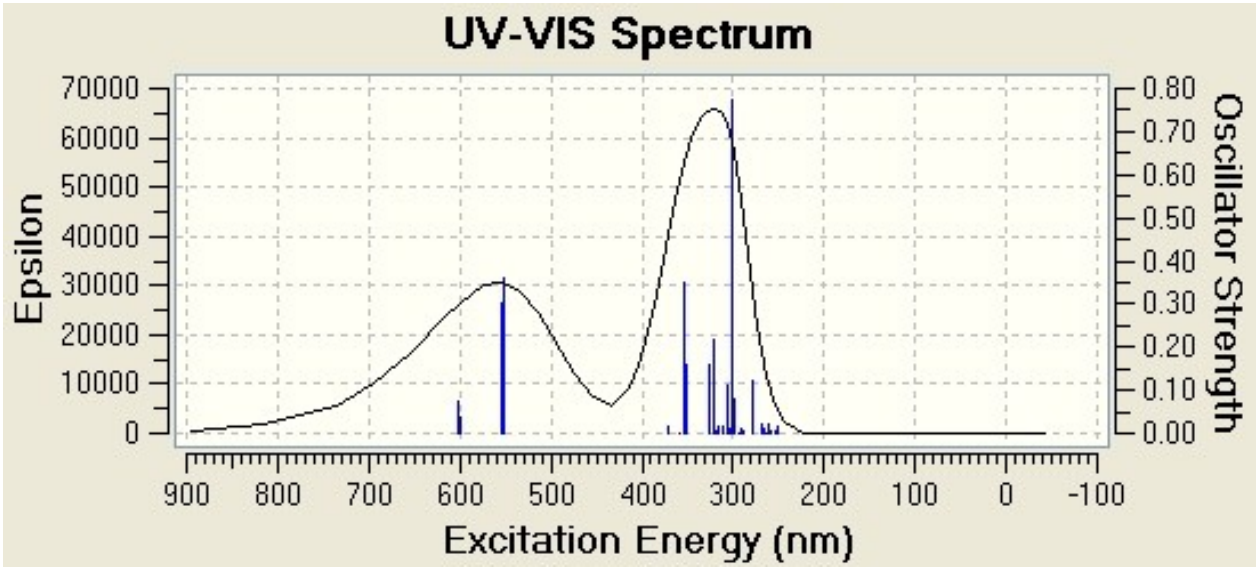
Complex	Excitation	Composition	E(eV) (λ_{theo} nm)	CI	λ_{exp} (nm)
Zn-HAFPA	1(380nm)	HOMO-1 →LUMO+1 HOMO →LUMO+3 HOMO-1 → LUMO HOMO-1→ LUMO+2	514 484 480 442	0.49696 0.83590 0.8190 0.57066	485 440
Cd-HAFPA	1(380nm)	HOMO →LUMO+3 HOMO-3 → LUMO	488 485	0.66255 0.52357	490

Theoretical Spectrum

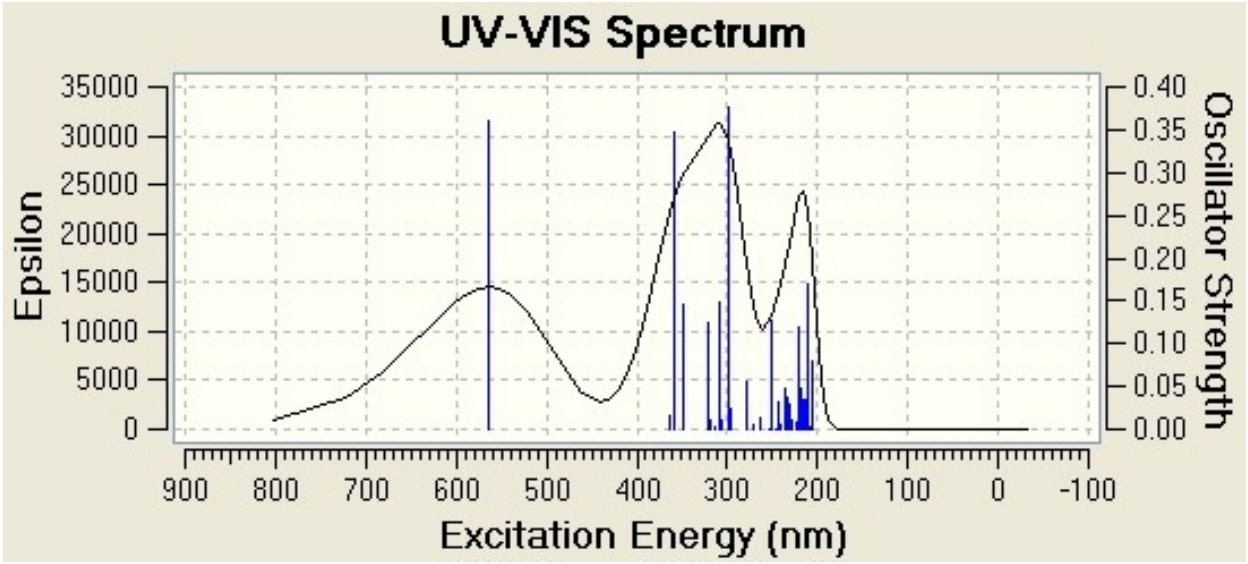


FigS27. The theoretical UV-Vis spectra for HAFPA.

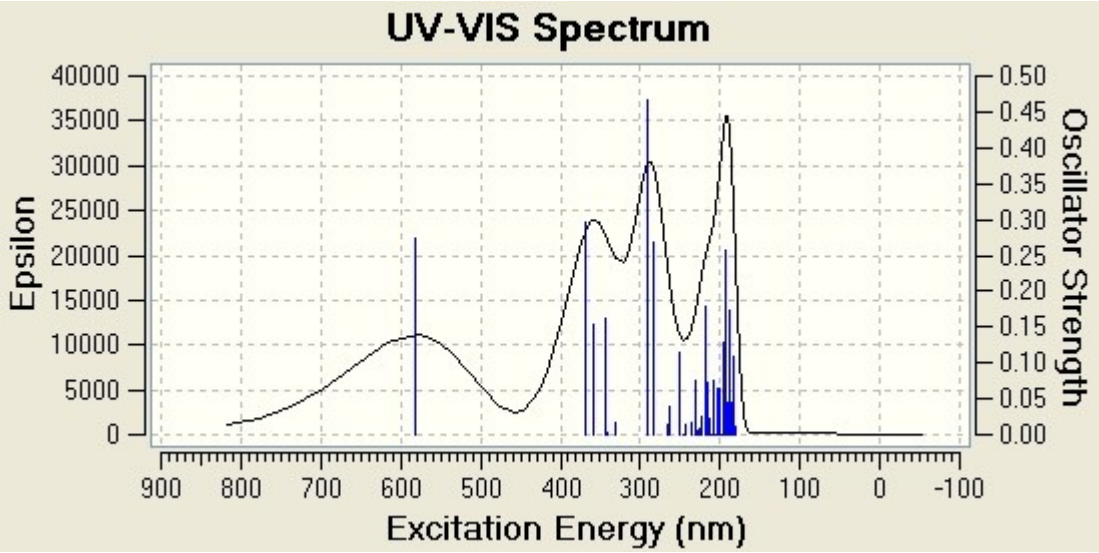
a)


UV-VIS Spectrum
Epsilon
70000
60000
50000
40000
30000
20000
10000
0
Oscillator Strength
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
900 800 700 600 500 400 300 200 100 0 -100
Excitation Energy (nm)

b)

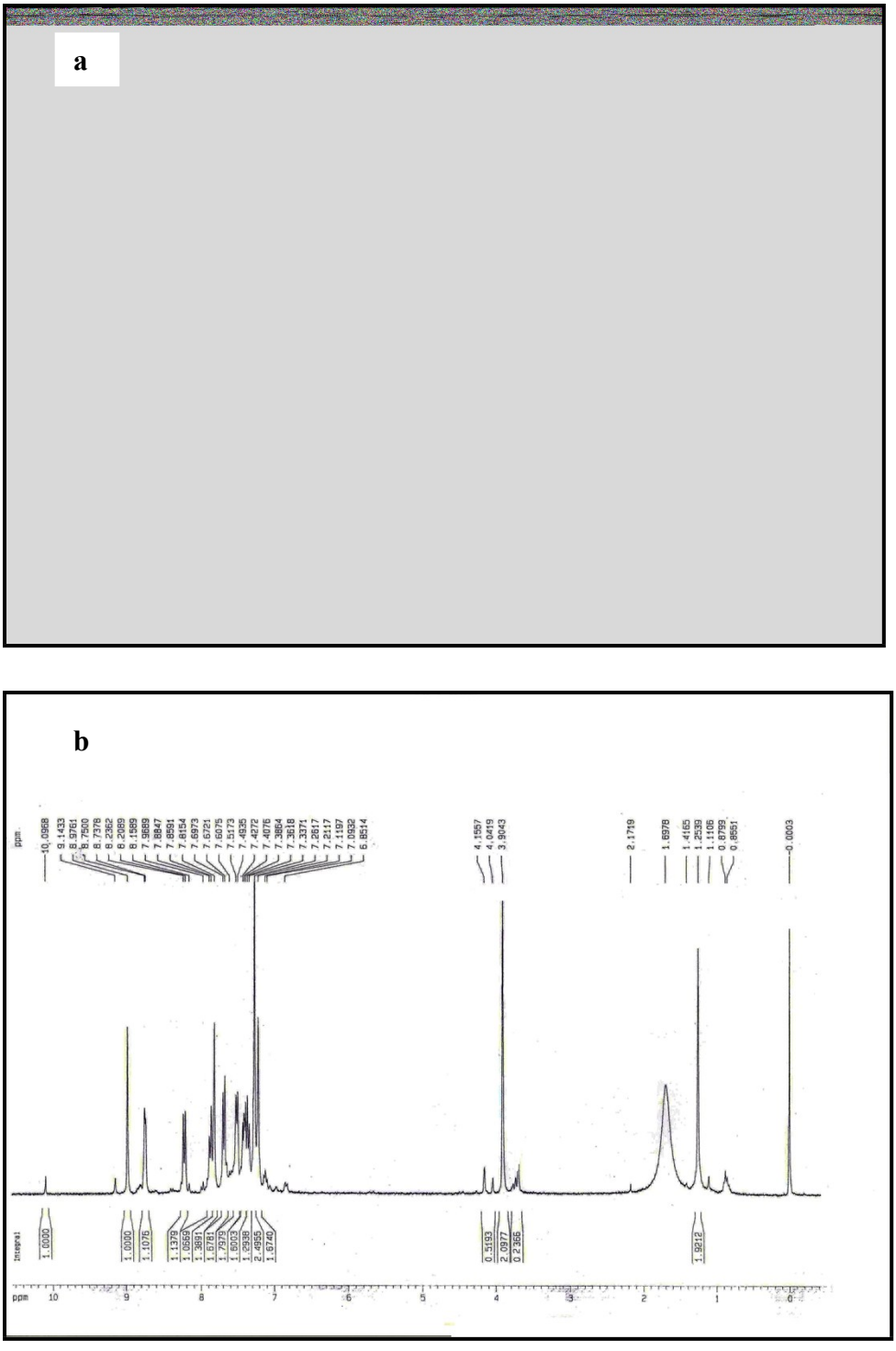

UV-VIS Spectrum
Epsilon
35000
30000
25000
20000
15000
10000
5000
0
Oscillator Strength
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
900 800 700 600 500 400 300 200 100 0 -100
Excitation Energy (nm)

c)



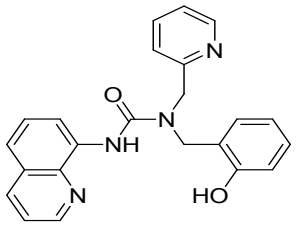
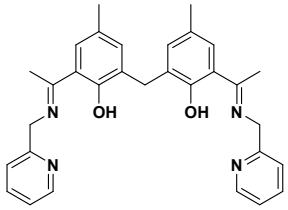
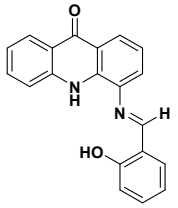
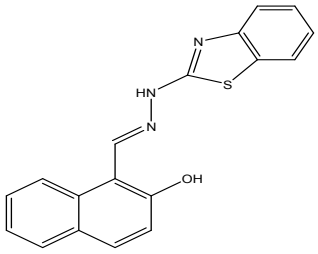
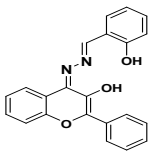
FigS28. The theoretical UV-Vis spectra for a)HAFPA-Zn (2:1)complex, b)HAFPA-Cd complex and c)HAFPA-Zn(1:1).

NMR Study over a longer period of time



FigS29. (a)[1]H NMR taken after 1hr(b) [1]H NMR taken after 24hr.

Table S4. Comparison of Zinc and Cadmium metal sensing aptitude of some reported chemosensor.

References	**Experimental Medium**	**Sensed Metal ion with LOD**	**In vivo application**
 Dalton Trans., 2013, 42, 15514	Acetonitrile	Zn^{2+} sensor with **LOD-** 66nM Cd^{2+} sensor with **LOD-** 120nM	Al^{3+} imaging
 RSC Adv,2015,5,63338-63344	MeOH-H_2O(99:1)	Al^{3+} sensor with **LOD** - 0.7μM	-
 RSC Adv,2015,5,33878	0.1 M water – methanol, 97.5 :2.5 ,v/v, pH=7.4)	Zn^{2+} sensor with **LOD** 0.1nM	Zn^{2+} imaging
 Sensors andActuatorsB202(2014)788–794	MeOH /aqueous HEPES Buffer (5mM, pH7.3; 7:3 v/v).	Zn^{2+} sensor with **LOD-** 6.5×10−7 M Cd^{2+} sensor with **LOD-** 2.1×10−6 M	
 Analytica Chimica Acta, 829(2014), 54-59	Ethanol: H2O= 9:1 in 10mM HEPES buffer at pH=7.0	Al^{3+} sensor with **LOD** 0.29μM	Al^{3+} imaging