Supporting Information

FRET based '*red-switch*' for Al^{3+} over ESIPT based '*green-switch*' for Zn^{2+}: Dual channel detection with live-cell imaging on a dyed platform

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CONTENTS

1. General:

The chemicals and solvents were purchased from Sigma-Aldrich Chemicals Private Limited and were used without further purification. NMR spectra were recorded on 400MHz and 600MHz instruments. For NMR spectra, d_6-DMSO was used as solvent with TMS as an internal standard. Chemical shifts are expressed in δ □units and $^1H–^1H$ coupling constants in Hz. Fluorescence experiment was done using PTI fluorescence spectrophotometer with a fluorescence cell of 10 mm path. UV-vis titration experiments were performed on a JASCO UV-V530 spectrophotometer. Crystallographic data were collected at 100K with an Oxford Cyrosystem Cobra low temperature attachment. The live-cell imaging was carried out using Zeiss Axio Observer Fluorescence Microscope equipped with an Apotome apparatus.

2. General method of fluorescence titrations:

For fluorescence titrations, stock solution of the sensor was prepared (c = 2 x 10^{-5} ML^{-1}) in CH_3CN: H_2O (1:1, v/v) at pH 7.5 by using 20 mM HEPES buffer. The solution of the guest anion was prepared (2 x 10^{-4} ML^{-1}) in CH_3CN: H_2O (1:1, v/v). The original volume of the receptor solution is 2 ml. Solutions of the sensor of various concentrations and increasing concentrations of cations were prepared separately. The spectra of these solutions were recorded by means of fluorescence methods.

3. Job plot by fluorescence method:

Stock solutions of same concentration of receptor, zinc chloride and aluminium nitrate were prepared in the order of ≈ 2.0 x 10^{-5} mL^{-1} in CH_3CN : H_2O (1:1, v/v) at pH 7.5 by using 20 mM HEPES buffer. The fluorescence in each case with different *host–guest* ratio but equal in volume was recorded. Job plots are drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of of the intensity spectrum during titration and X_{host} is the mole fraction of the host in each case, respectively).

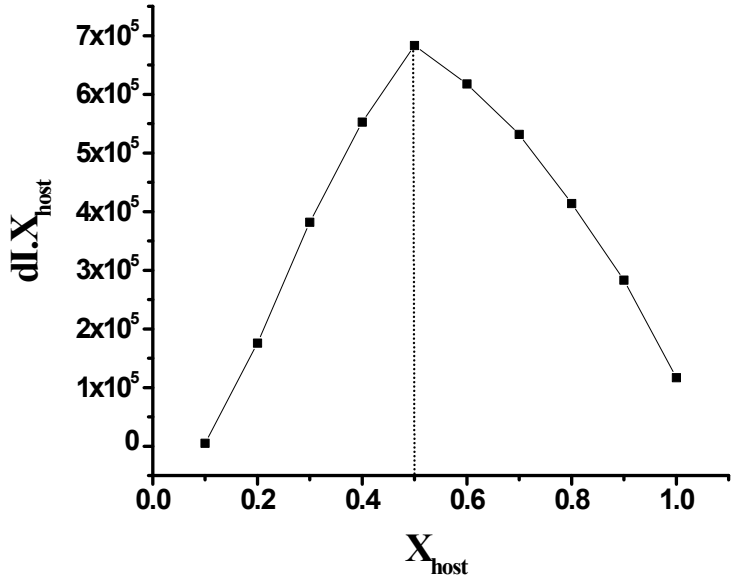


Figure S1: Job plot diagram of RHD with Zn^{2+} (where X_h is the mole fraction of the host and ΔI indicates the change of fluorescence intensity).

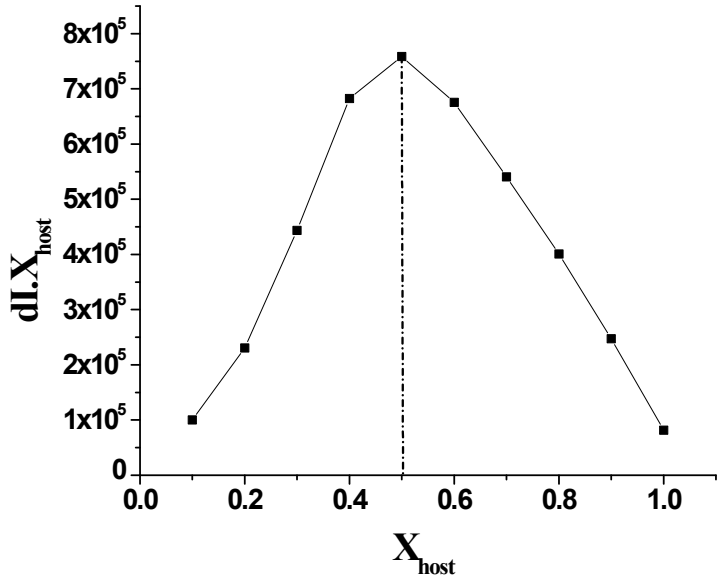
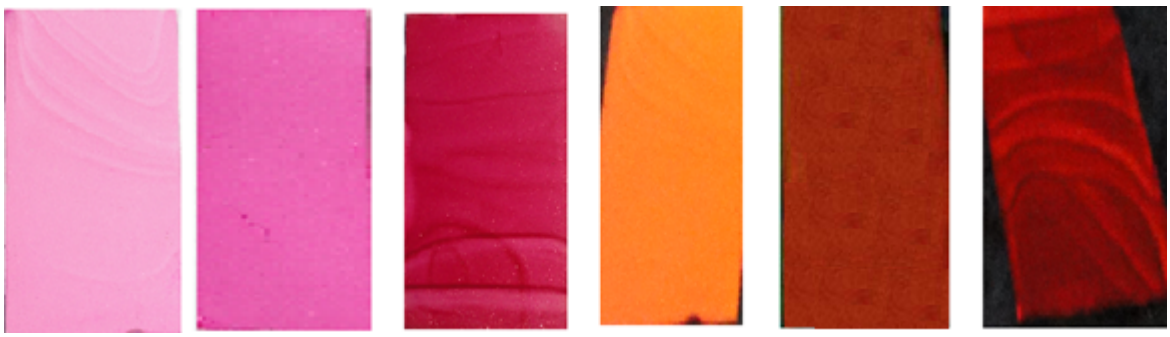


Figure S2: Job plot diagram of RHD with Al^{3+} (where X_h is the mole fraction of the host and ΔI indicates the change of fluorescence intensity).

4. Method for the preparation and application of TLC plate sticks:

It was easily prepared by immersing a TLC plate into the solution of RHD (2 X 10^{-3} M) in CH_3CN and then exposing it to air to evaporate the solvent. The detection of Al^{3+} was carried

out by inserting the TLC plate in the solution of various concentrations of $Al(NO_3)_3.9H_2O$ (from 2 X 10^{-4} M to 2 X 10^{-3}) in aqueous acetonitrile solution and evaporating solvent to dryness. Finally, under sun light and UV light the 'naked-eye' color and fluorescence was checked respectively.



(a) (b) (c) (d) (e) (f)

Figure S3: Picture of TLC plate coated with RHD under sun light (a) and under UV-lamp (d). Effect of Al^{3+} solution is observed using 2 X 10^{-4} (M) Al^{3+} solution under sun light (b) and under uv-lamp (e). Effect of Al^{3+} solution is observed using 2 X 10^{-3} (M) Al^{3+} solution under sun light (c) and under UV-lamp (f).

5. Methods for the preparation of the receptor:

Synthesis of 3-(benzo[d]thiazol-2-yl)-2-hydroxybenzaldehyde: 2-(2-hydroxyphenyl)benzo thiazole (HBT) (550mg, 2.42mmol) was dissolved in toluene (15ml) and acetic acid (25ml). Hexamethylenetriamine (2.80gm, 20 mmol) was added in one portion and the solution was vigorously refluxed for 8 hours. Then the mixture was cooled to room temperature and poured into 6M HCl (40ml) and extracted with ethyl acetate. The combined organic extracts were washed with saturated brine. Next purification was done by column chromatography to get the pure product (50mg) (we used 100-200 mesh silica gel and 0-10% ethyl acetate in petroleum ether as a solvent).

^{1}H NMR (d_6-DMSO, 600 MHz): δ (ppm): 12.97 (s, 1H), 9.98 (s, 1H), 8.40 (d, 1H, J = 9.6 Hz), 8.22 (d, 1H, J = 10.8 Hz), 8.16 (t, 1H, J = 9.6 Hz), 8.12 (t, 1H, J = 7.2 Hz), 7.61 (d, 1H, J = 8.4 Hz), 7.53 (t, 1H, J = 8.4 Hz), 7.47 (d, 1H, J = 7.2 Hz).

Elemental Analysis: C - 65.91%, H – 3.45%, N – 5.47% (Calculated **C - 65.87%, H – 3.55%, N – 5.49%)**

MS (ESI MS): (m/z, %): 256.03 [(2+H^+), 100 %].

Synthesis of Rhodamine-HBT-dyed i.e. RHD: Rhodamine-hydrazide (1gm, 2.19mmol) is mixed with one equivalent 3-(benzo[d]thiazol-2-yl)-2-hydroxybenzaldehyde (561.6mg, 2.2mmol) in methanol at reflux condition for 6hr. to give a red colored ppt. After removing the solvent, the product was directly purified through column chromatography to get the pure product (750mg, yield: 49.4%) (using 100-200 mesh silica gel and DCM as a solvent).

The single crystal of the product is grown in mixed solvent system (DCM: MeOH= 1:1).

^{1}H NMR (d_6-DMSO, 400 MHz): δ (ppm): 12.61 (s, 1H), 9.06 (s, 1H), 8.23 (d, 1H, *J* = 10.4 Hz), 8.14 (d, 1H, *J* = 10 Hz), 8.07 (d, 1H, *J* = 10.8 Hz), 7.97 (d, 1H, *J* = 9.6 Hz), 7.65 (t, 1H, *J* = 11.4 Hz), 7.57 (t, 1H, *J* = 7.4 Hz), 7.49 (t, 1H, *J* = 9.6 Hz), 7.17 (d, 1H, *J* = 10 Hz), 7.07 (m, 1H), 6.50 (d, 1H, *J* = 2.4 Hz), 6.48 (s, 1H), 6.45 (S, 2H), 6.43 (s, 1H), 6.39 (d, 1H, *J* = 3.2 Hz), 6.37 (s, 1H), 2.21 (q, 8H, *J* = 8.4 Hz), 1.08 (t, 12H, *J* = 9 Hz).

Elemental Analysis: C – 72.95%, H – 5.62%, N – 10.04% (Calculated **C - 72.70%, H – 5.67%, N – 10.09%).**

MS (ESI MS): (m/z, %): 694.28 [(RHD+H^+), 100 %]

Synthesis of hydrazone of 3-(benzo[d]thiazol-2-yl)-2-hydroxybenzaldehyde (i.e. "donor" moiety):

Hydrazine (exess) is mixed with 3-(benzo[d]thiazol-2-yl)-2-hydroxybenzaldehyde (255.2mg, 1mmol) in methanol at reflux condition for 4hr. to give a reddish yellow colored ppt. After removing the solvent, the product was directly purified through column chromatography to get the pure product (Using 100-200 mesh silica gel and DCM: MeOH = 4:1 as a solvent).

6. Determination of fluorescence quantum yield:

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

$\varphi_x = \varphi_s (F_x / F_s)(A_s / A_x)(n_x^2/ n_s^2)$

Where,

X & S indicate the unknown and standard solution respectively, φ = quantum yield,

F = area under the emission curve, A = absorbance at the excitation wave length,

n = index of refraction of the solvent. Here φ measurements were performed using anthracene in ethanol as standard [φ = 0.27] (error ~ 10%).

The quantum yield of **RHD** itself is 0.051 which changes into 0.753 in presence of Al^{3+} solution and it becomes 0.654 in presence of Zn^{2+} solution.

7. Calculation of the detection limit:

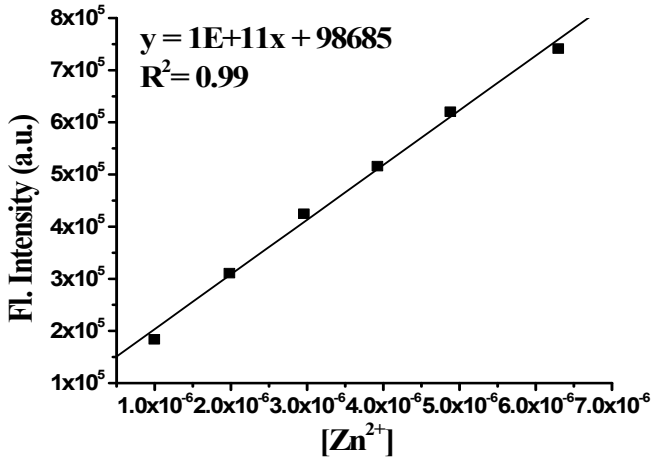


Figure S4: Fl. Intensity Vs. Conc. of Zn^{2+}at 509nm (from 1µM to 7µM).

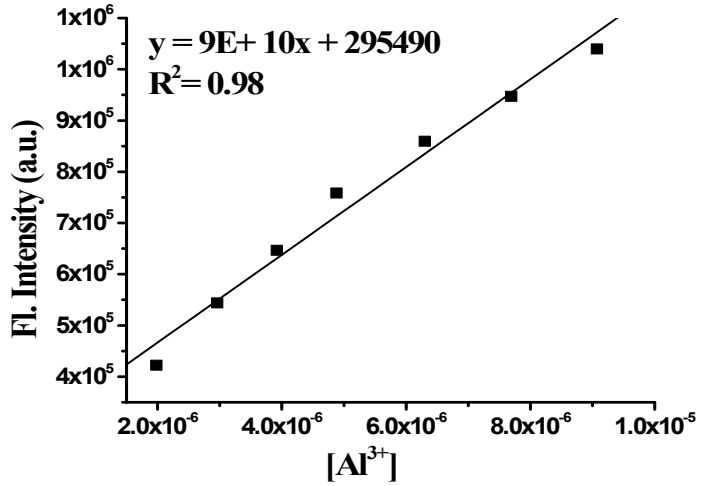


Figure S5: Fl. Intensity Vs. Conc. of Al^{3+} at 581nm (from 2µM to 10µM).

The detection limit DL of **RHD** for Zn^{2+}/Al^{3+} 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 of the blank solution; S is the slope of the calibration curve.

From the graph (figure S1), we get slope = 1E + 11 and Sb1 value is 16486.21097 for Zn^{2+}. Thus using the formula we get the Detection Limit = 0.32 μM i.e. RHD can detect Zn^{2+} in this ppm level. From the graph (figure S2), we get slope = 9E + 10 and Sb1 value is 34257.0383 for Al^{3+}. Thus using the formula we get the Detection Limit = 0.76 μM i.e. RHD can detect Al^{3+} in this ppm level.

8. Calculation of association constant using Emission Titration Data:

From the fluorescence titration data the association constant (K_a) for the formation of respective complex RHD-Al^{3+} and RHD-Zn^{2+} was calculated by nonlinear curve fitting procedure. The non linear curve fitting was done using the following equation (1). [S2]

$$I = I_0 + \frac{I_{lim} - I_0}{2C_H}\left\{C_H + C_G + \frac{1}{K_a} - \left[\left(C_H + C_G + \frac{1}{K_a}\right)^2 - 4C_H C_G\right]^{1/2}\right\} \quad (1)$$

where I0, I, and Ilim are the respective emission intensity of free RHD, RHD present in the form of [RHD-Al^{3+}]/ [RHD-Zn^{2+}] in the complex, and RHD in presence of excess amounts of Al^{3+}/Zn^{2+} ions where the emission intensity reaches a limiting value. C_H and C_G are corresponding concentrations of host and cationic guest; Ka is the binding constant. The binding constant (Ka) and correlation coefficients (R) were obtained from a non-linear least-square analysis of I vs. C_H and C_G.

The association constant (K_a) as determined by fluorescence titration method for **RHD** with Al^{3+} and Zn^{2+} is found to be 5.22 x 10^4 M^{-1} and 4.05 x 10^4 M^{-1} respectively.

9. Fluorescence responses of RHD towards Al^{3+} in presence of various metal ions at 581nm:

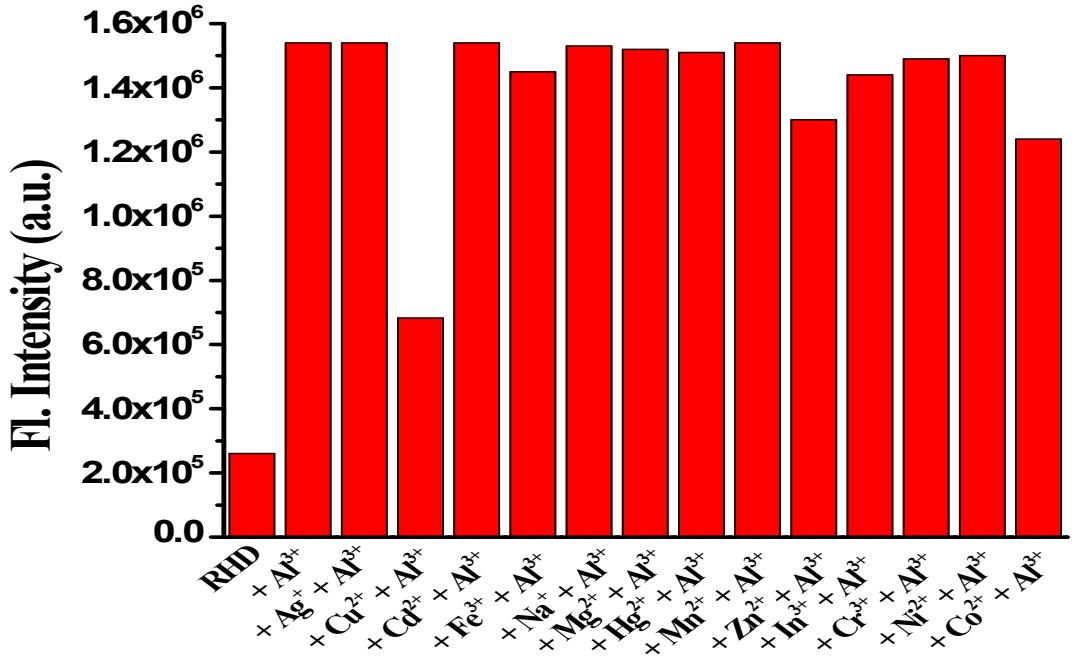
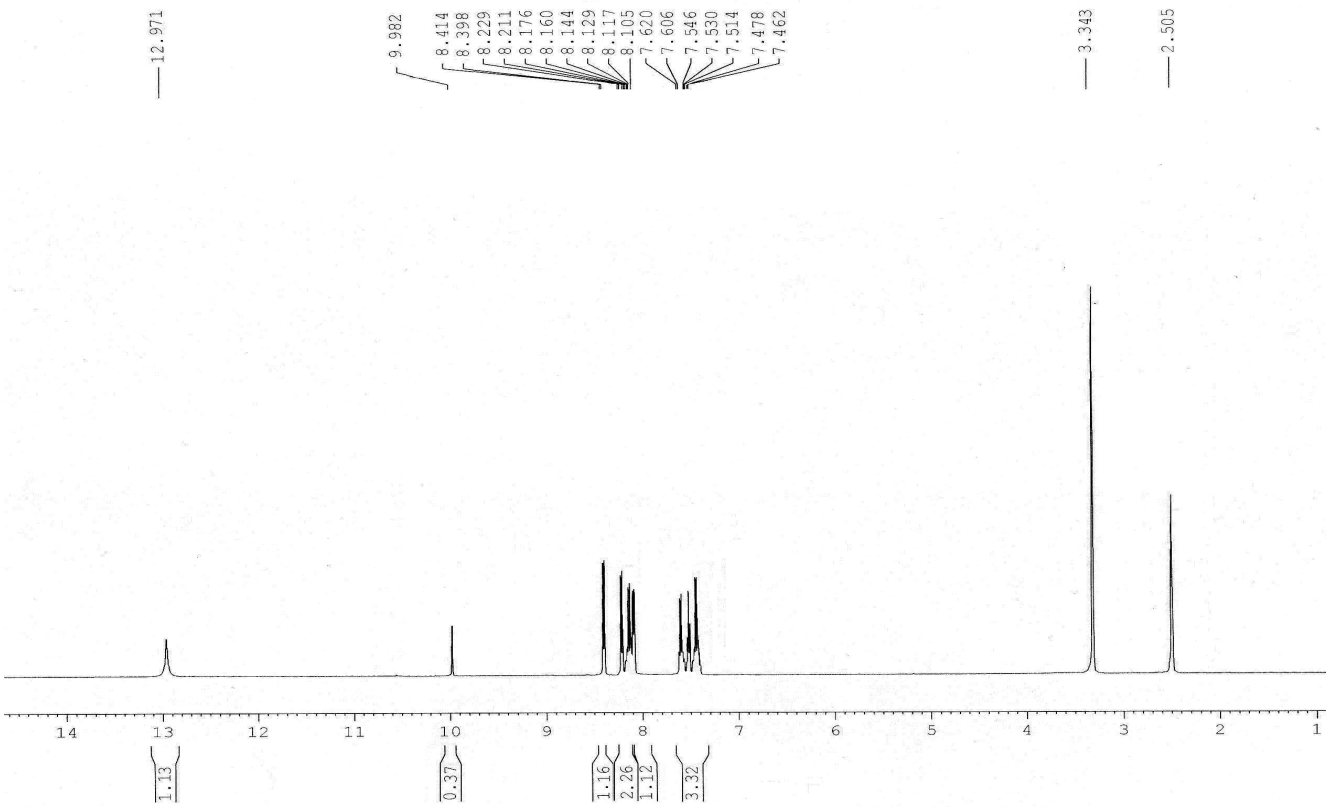


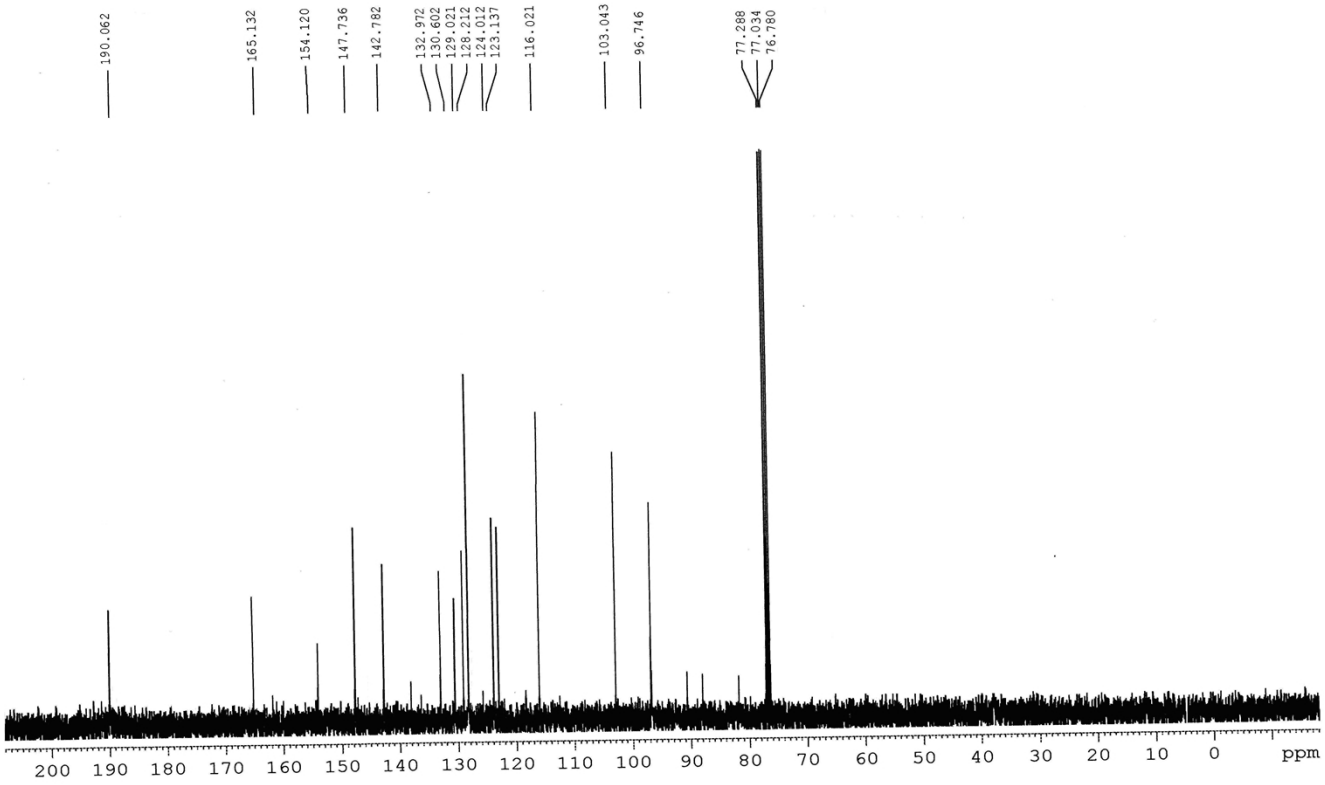
Figure S7: Fluorescence responses of RHD (c = 2.0 x 10^{-5} M) to Al^{3+} (4 equiv.) containing 10 equiv of various metal ions.

10. NMR and MS spectra:

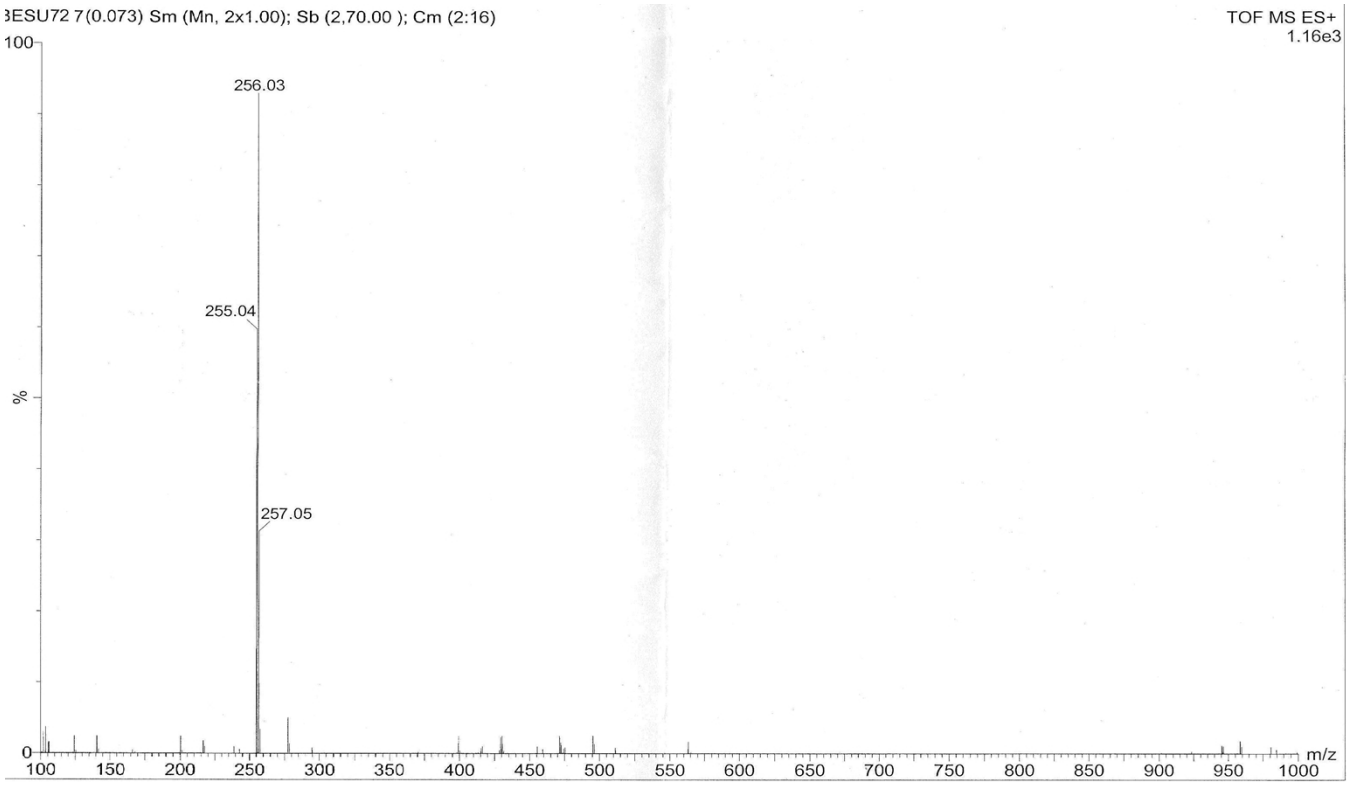
^{1}H NMR spectrum of 3-(benzo[d]thiazol-2-yl)-2-hydroxybenzaldehyde:



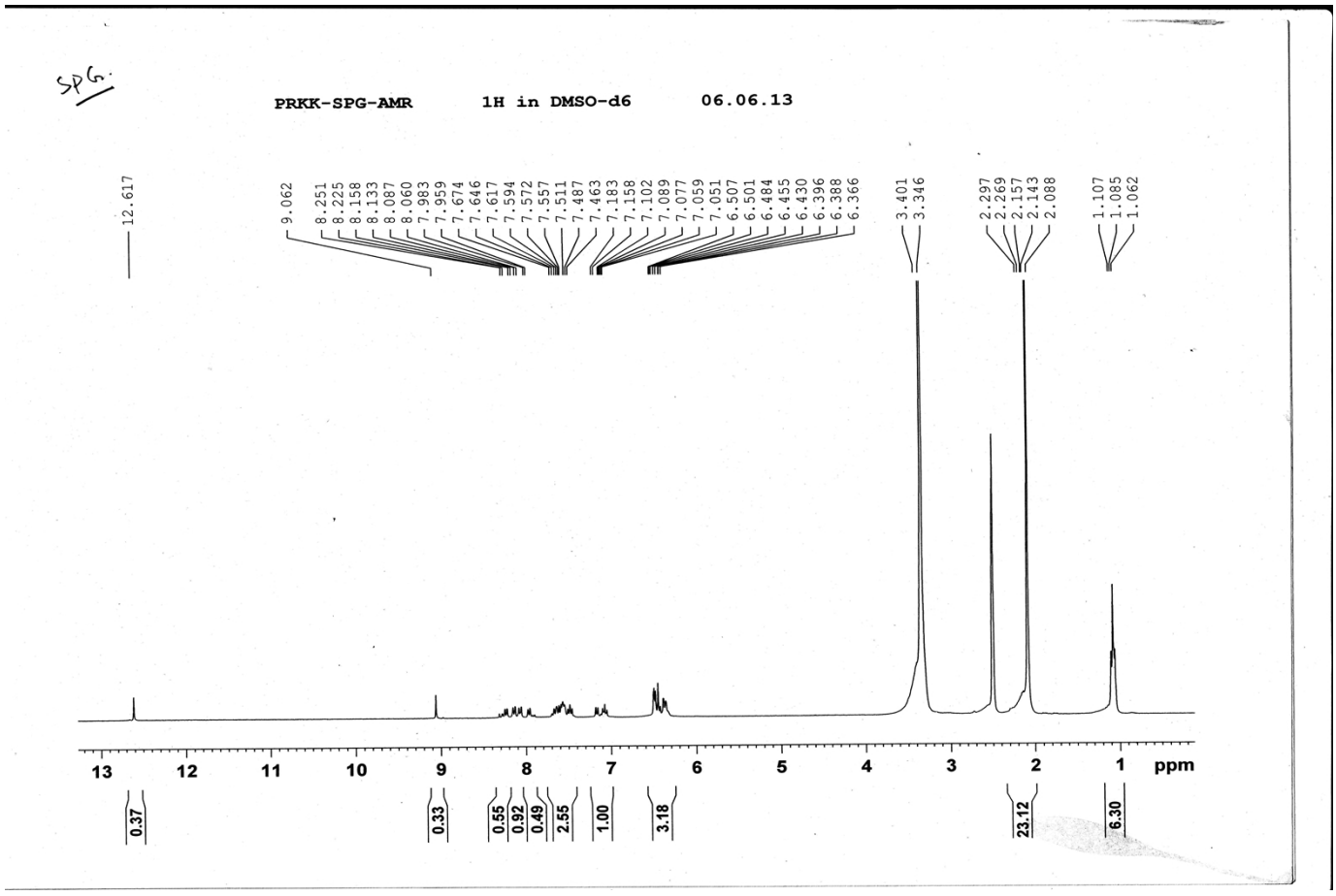
^{13}C NMR spectrum of 3-(benzo[d]thiazol-2-yl)-2-hydroxybenzaldehyde:



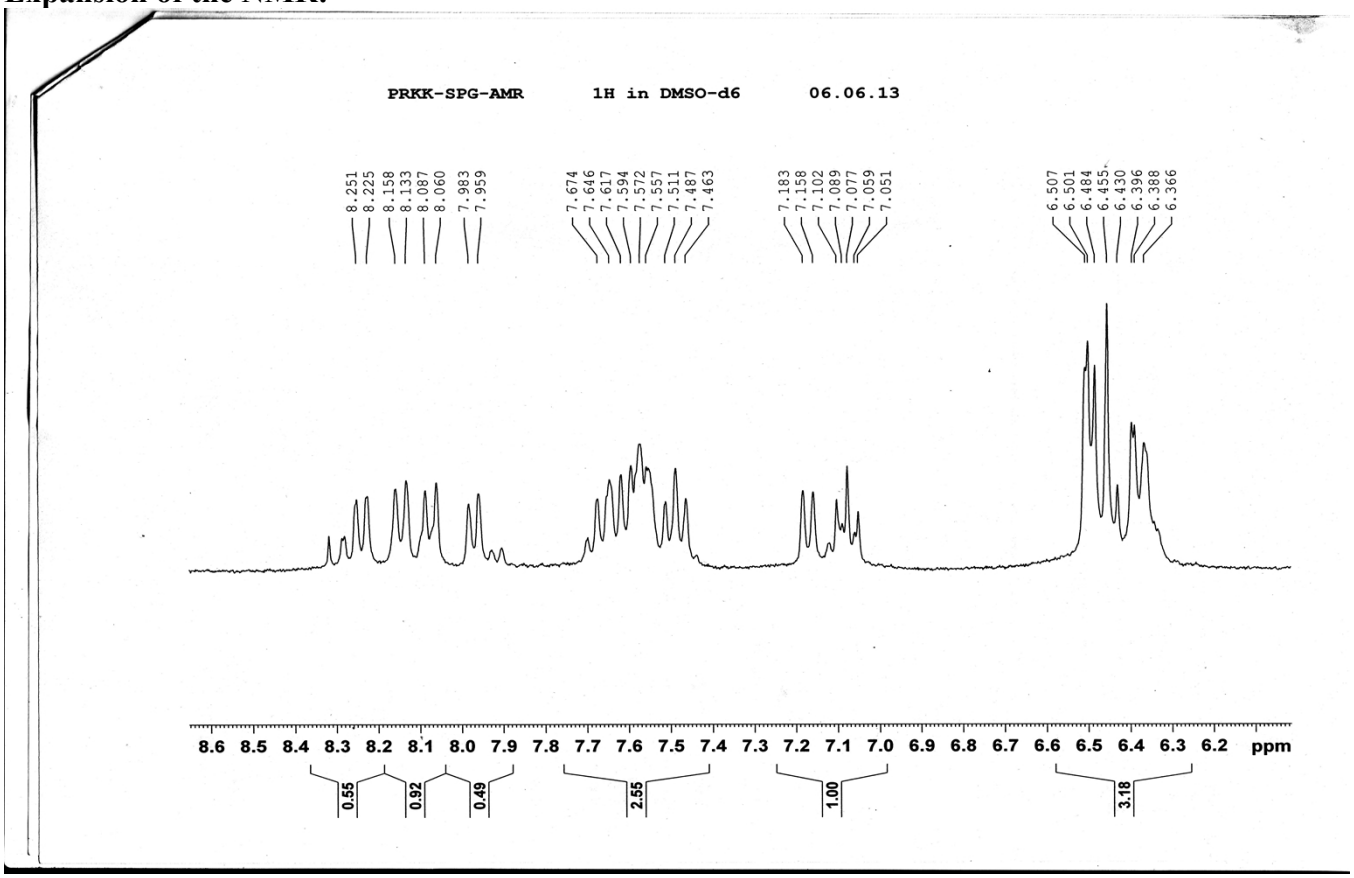
ESI MS Mass Spectra of 3-(benzo[d]thiazol-2-yl)-2-hydroxybenzaldehyde:



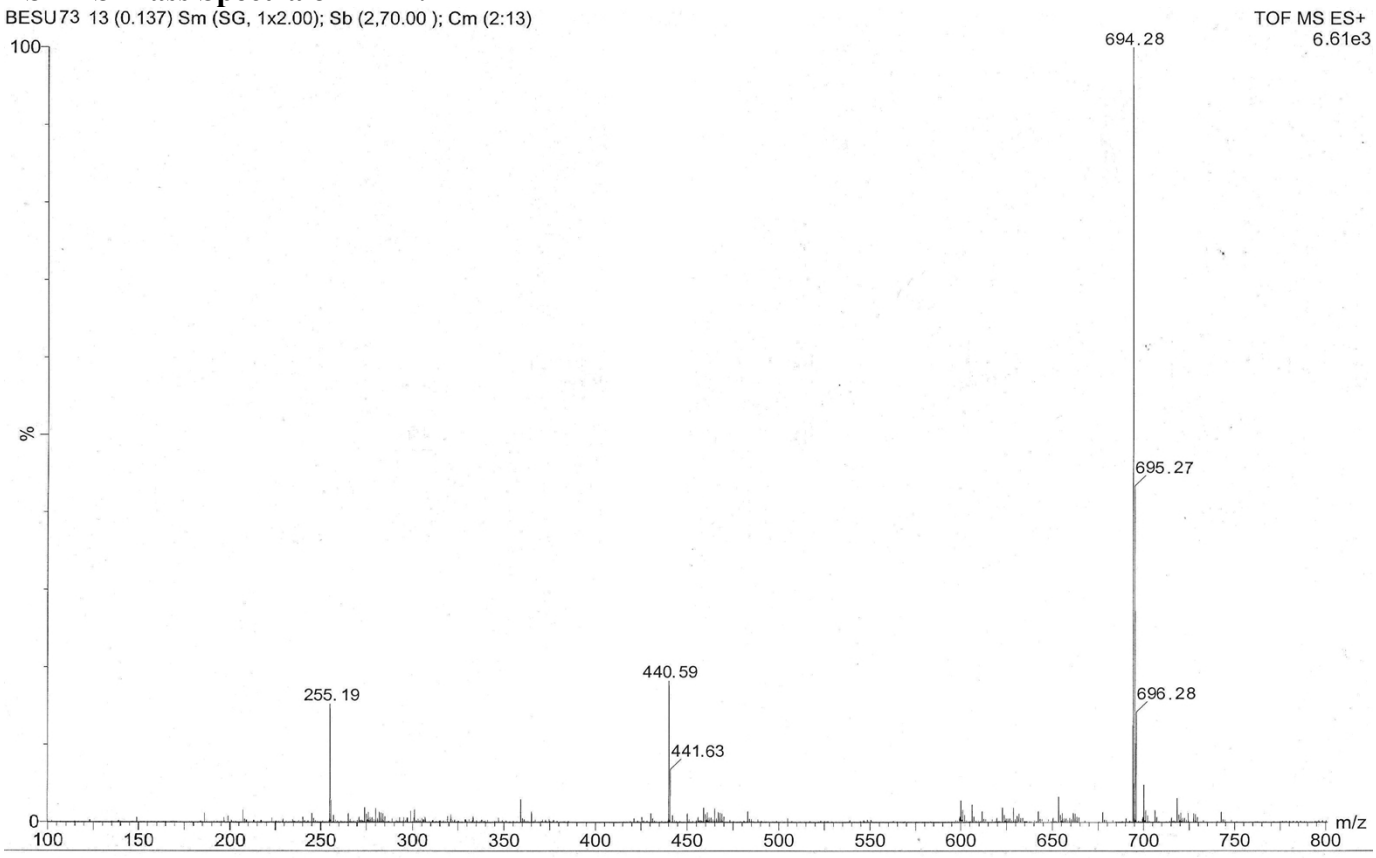
^{1}H NMR spectrum of Receptor i.e. RHD:



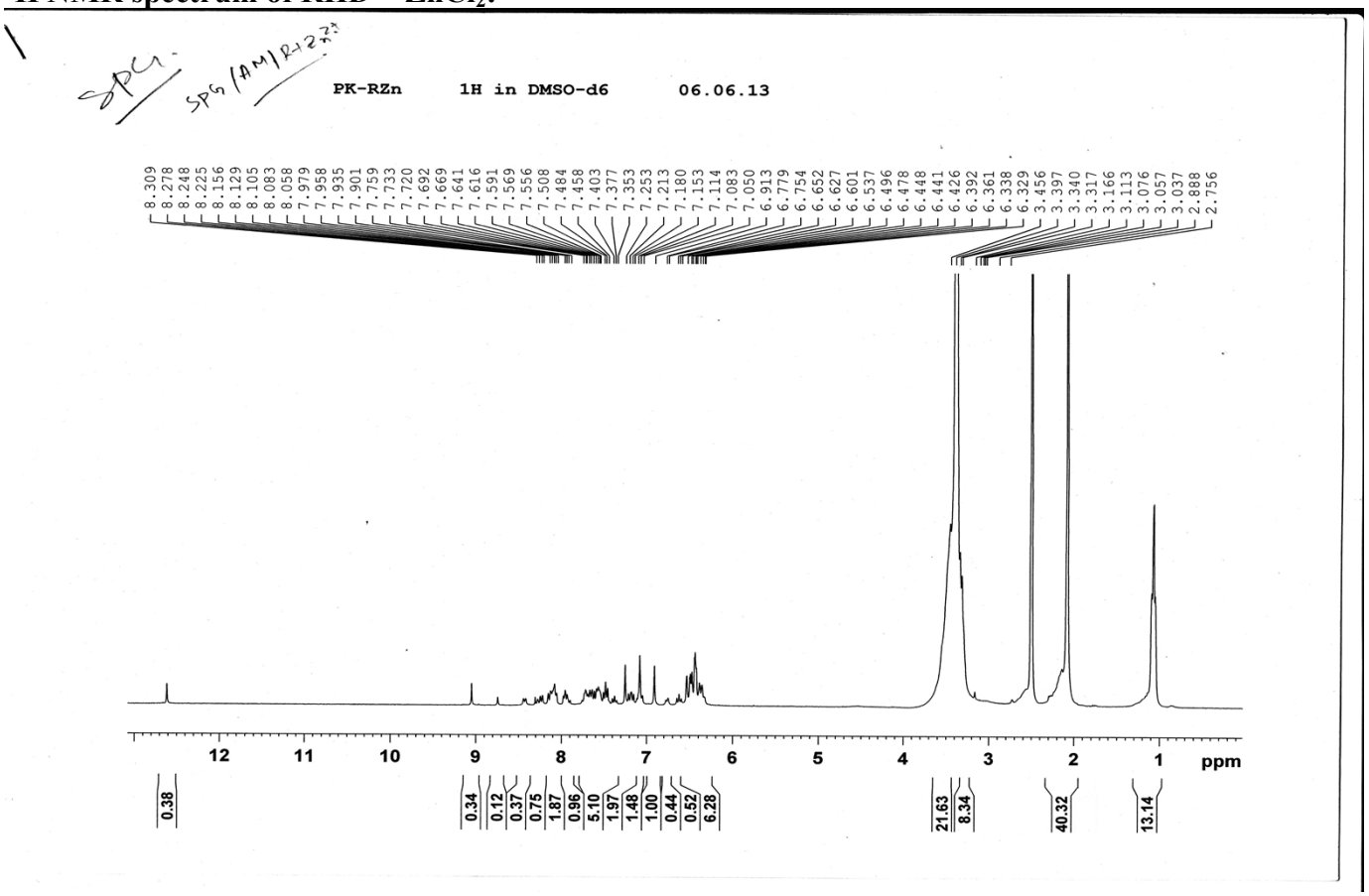
Expansion of the NMR:



ESI MS Mass Spectra of RHD:



^{1}H NMR spectrum of RHD + $ZnCl_2$:



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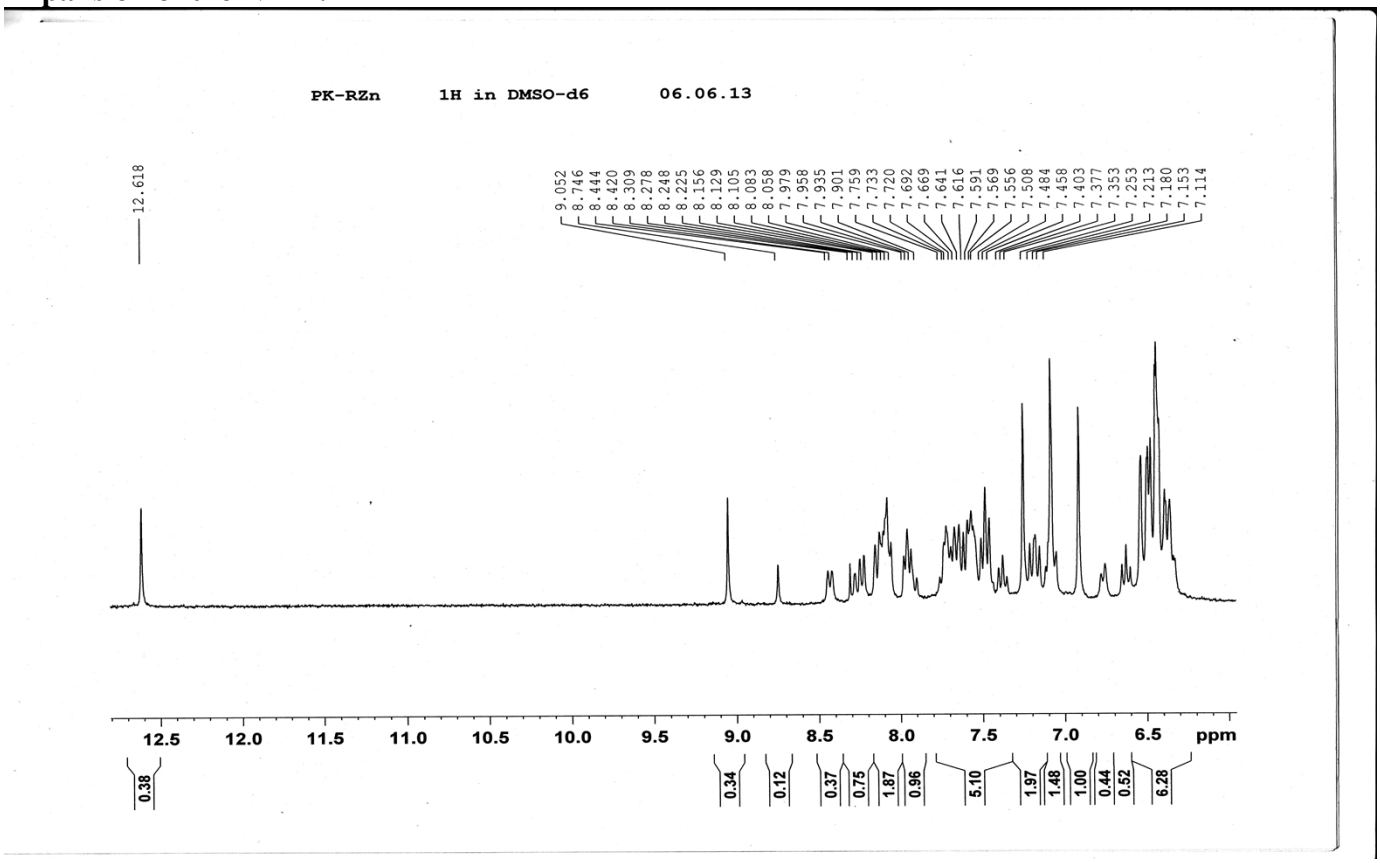

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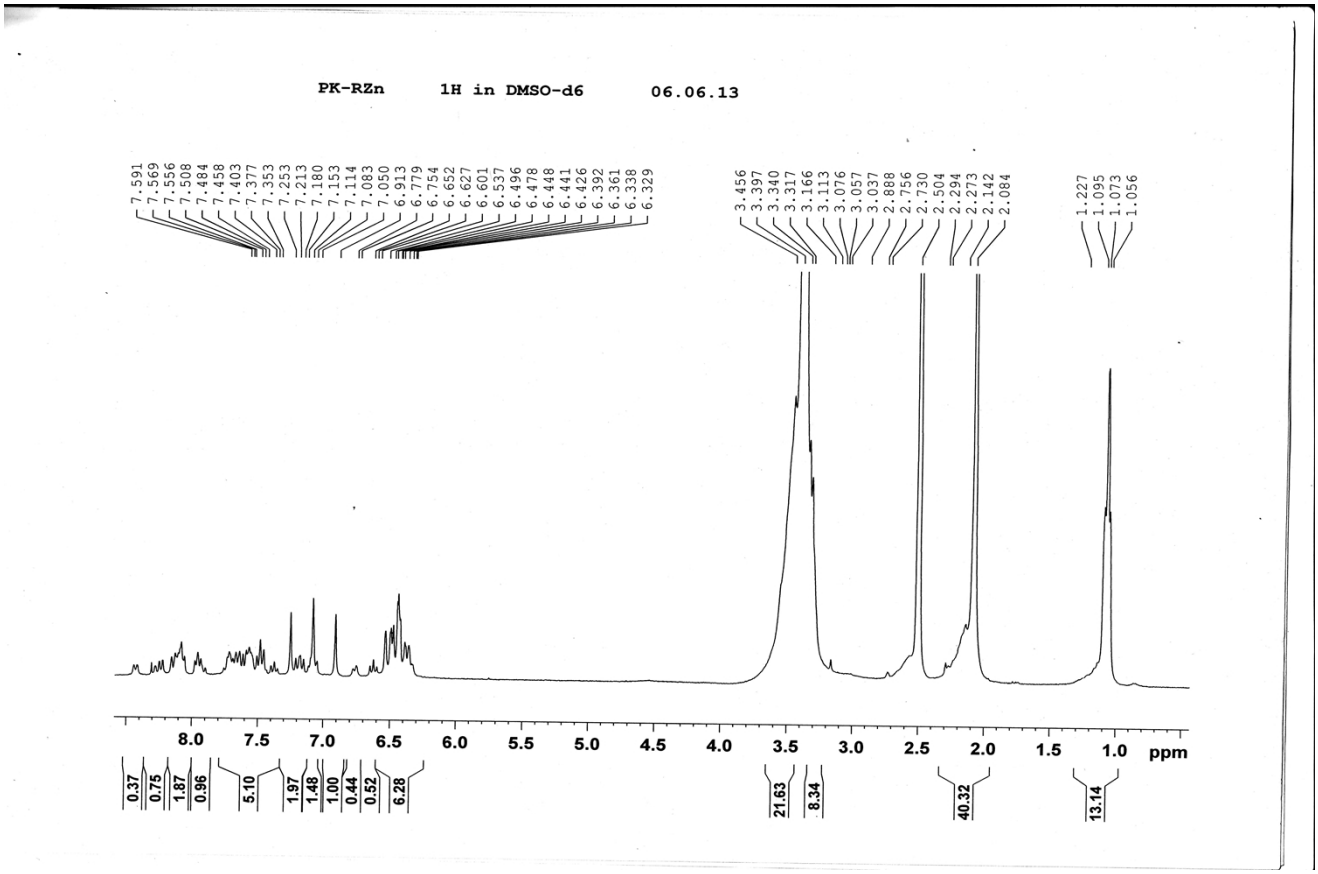
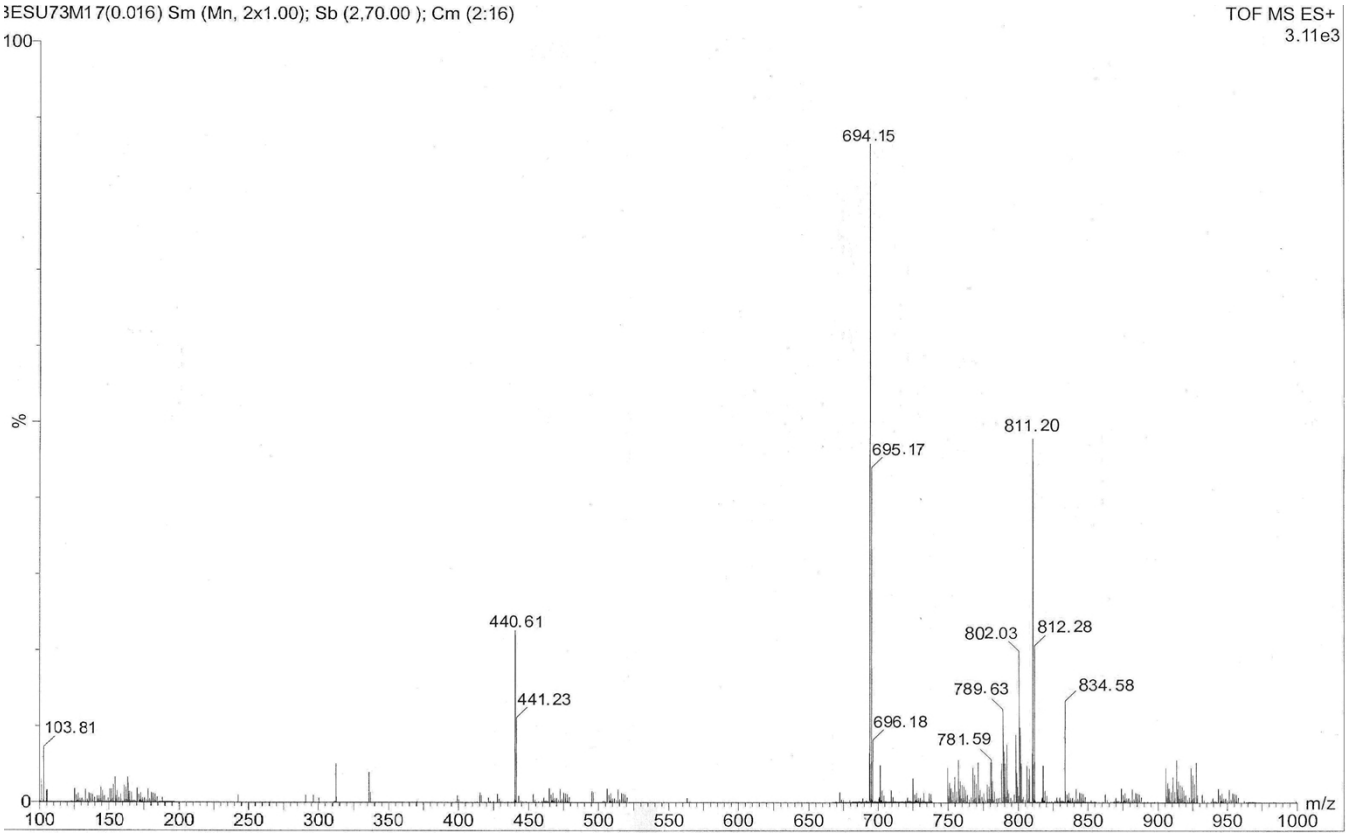
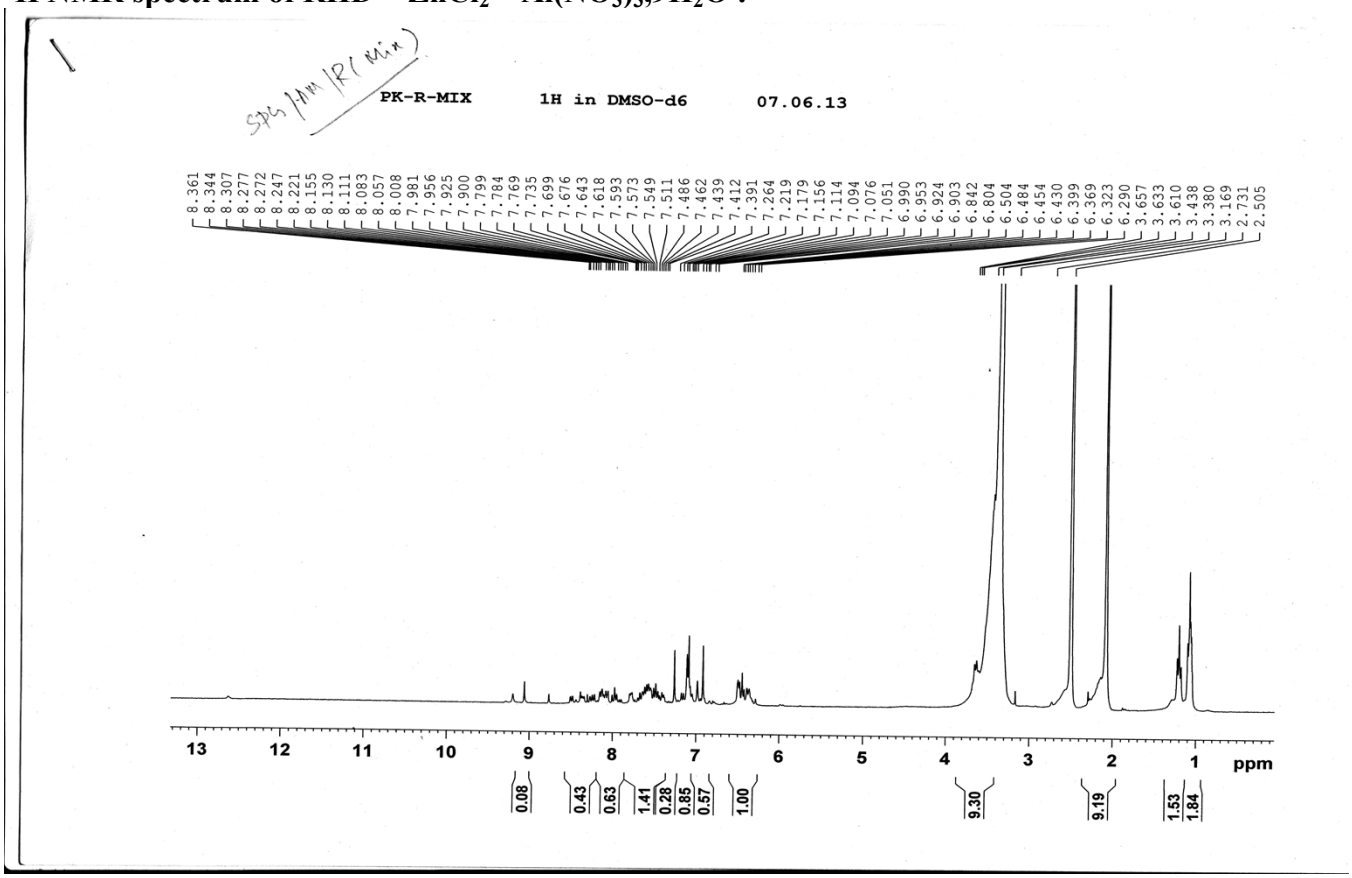


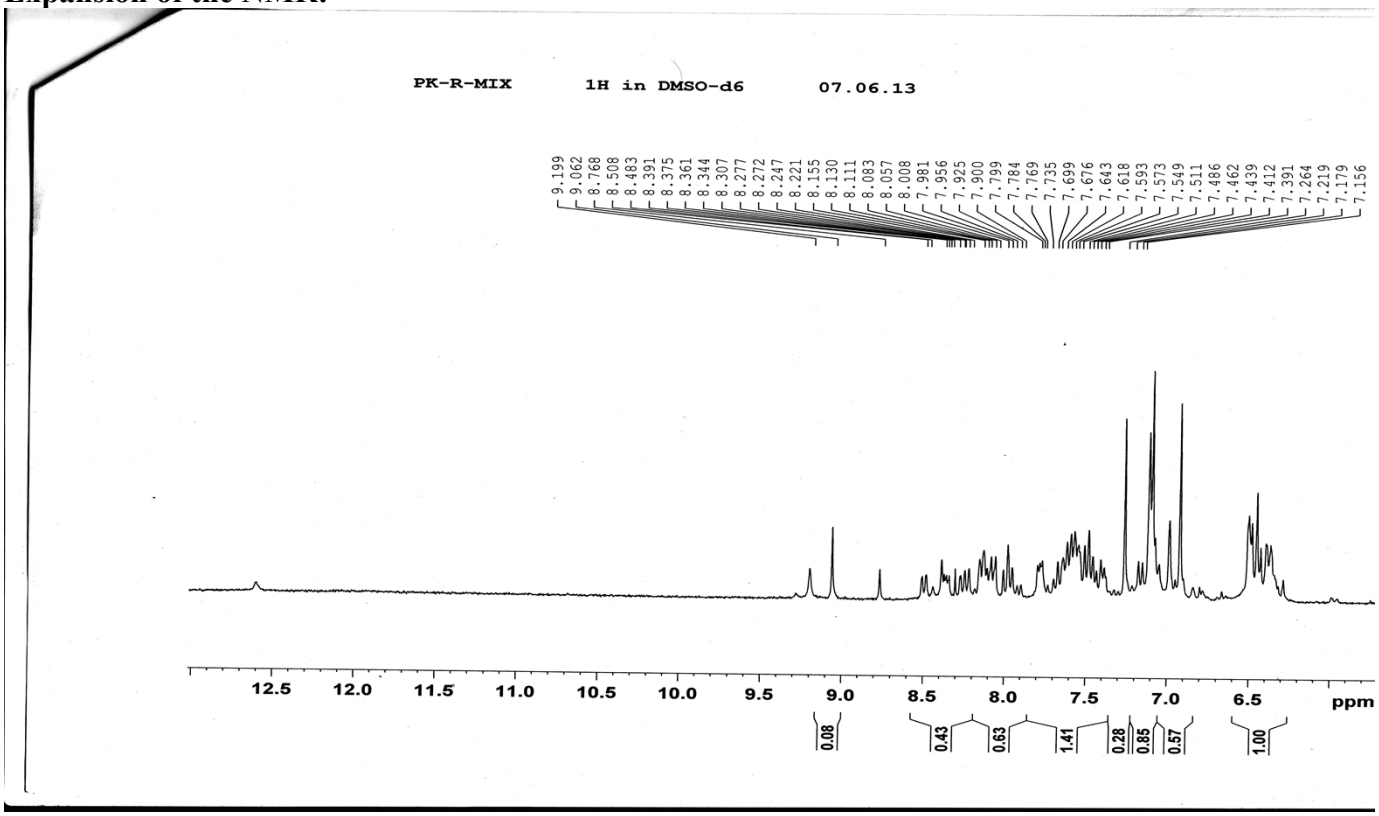
ESI MS Spectra of RHD + $ZnCl_2$:



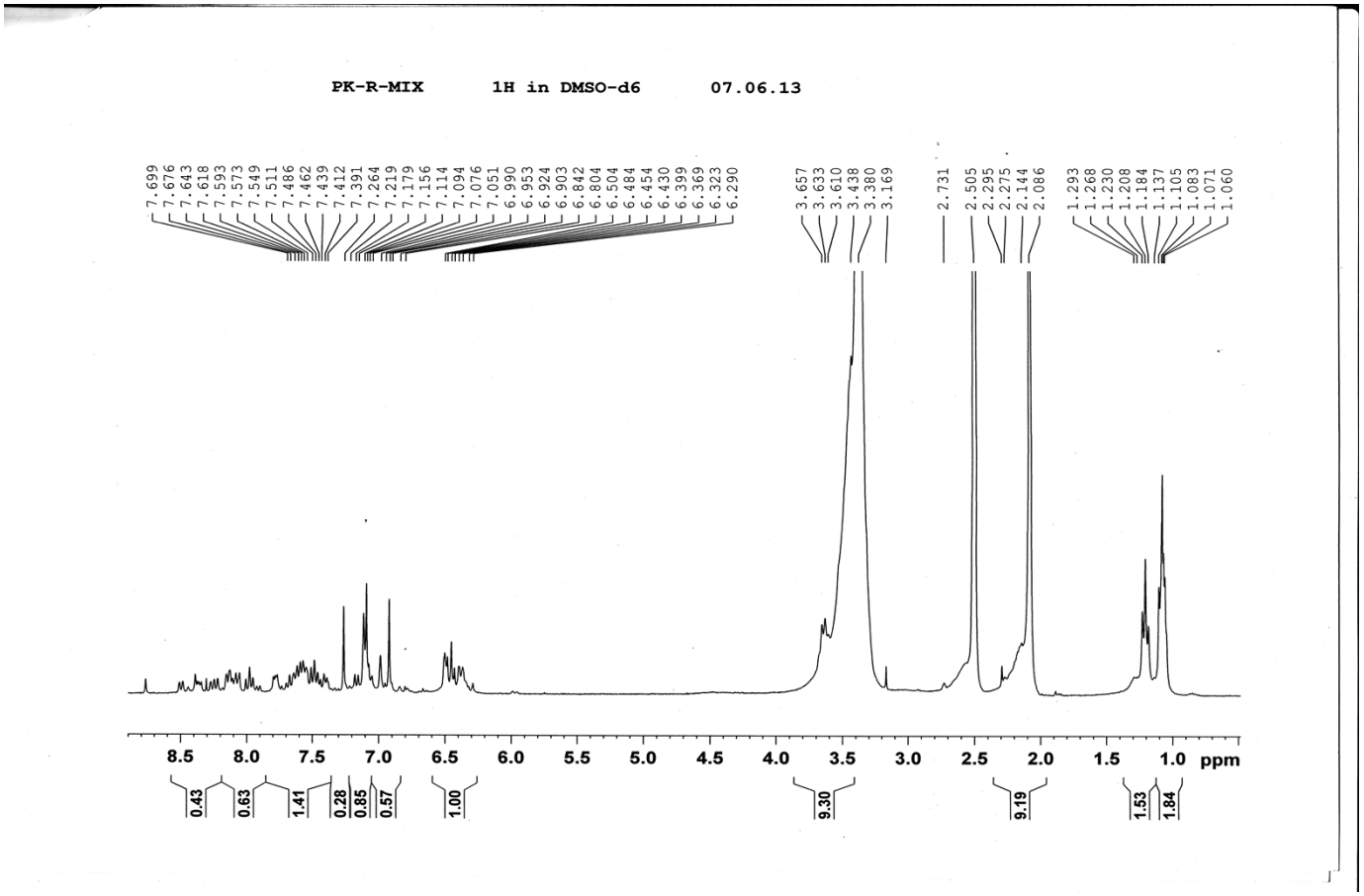
^{1}H NMR spectrum of RHD + $ZnCl_2$ + $Al(NO_3)_3$,$9H_2O$:



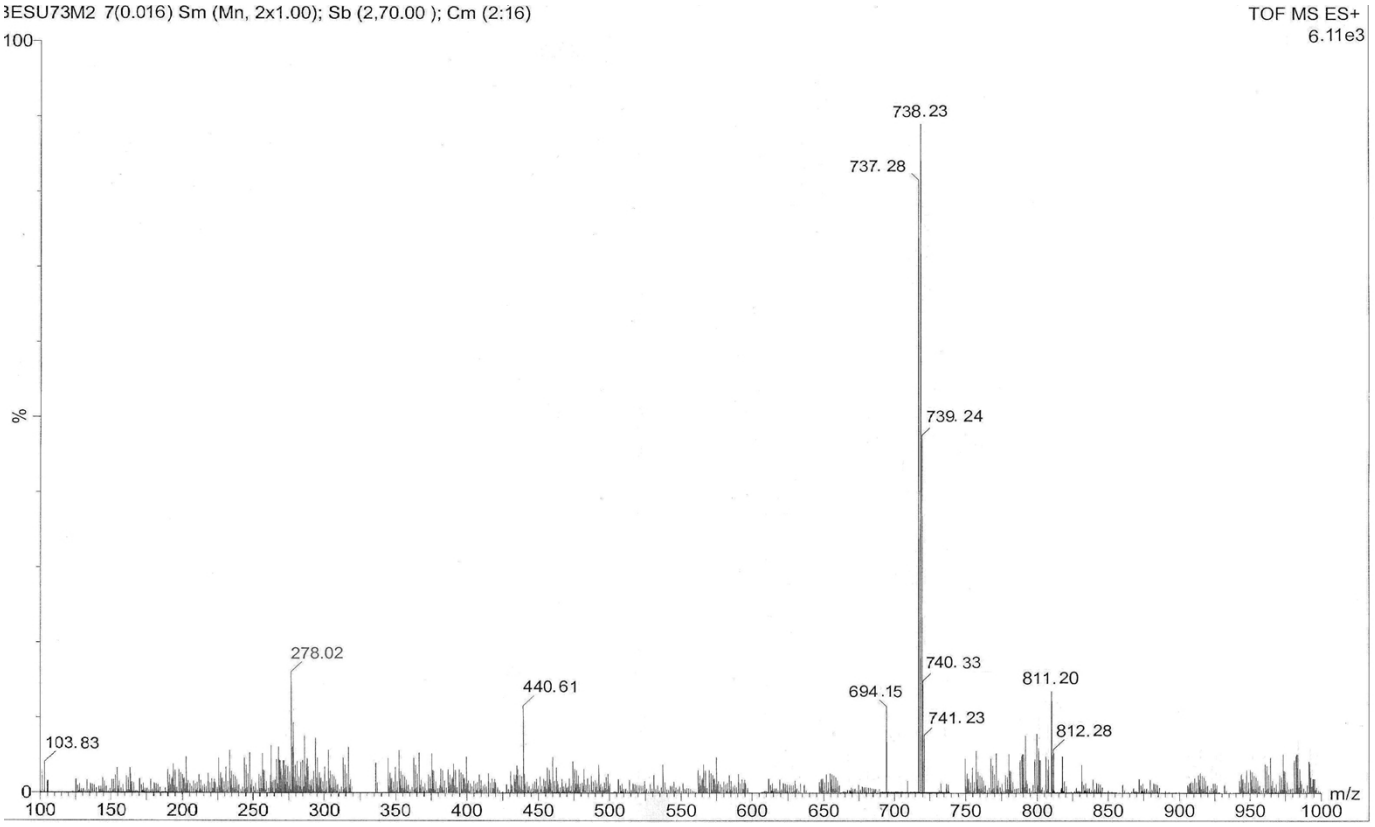
Expansion of the NMR:



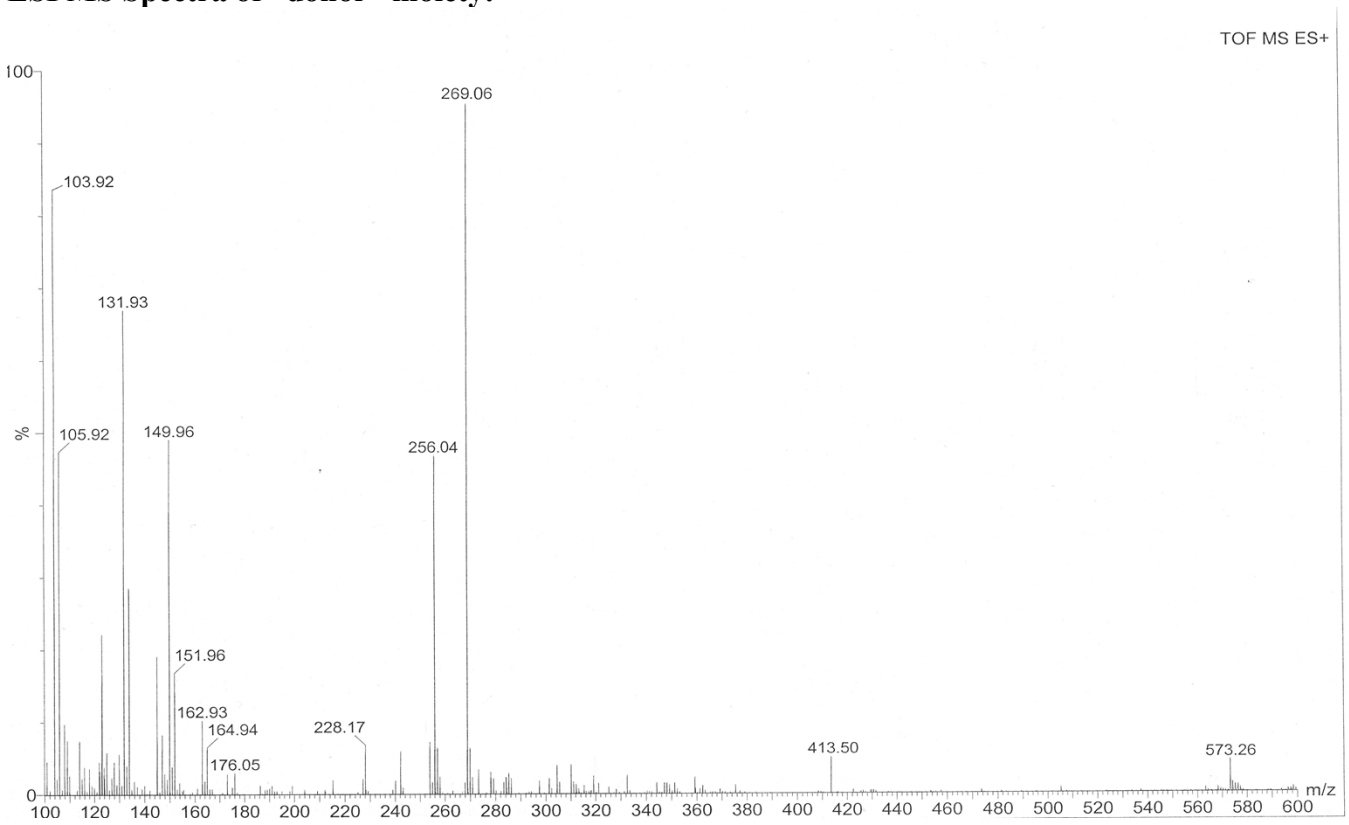
Expansion of the NMR:



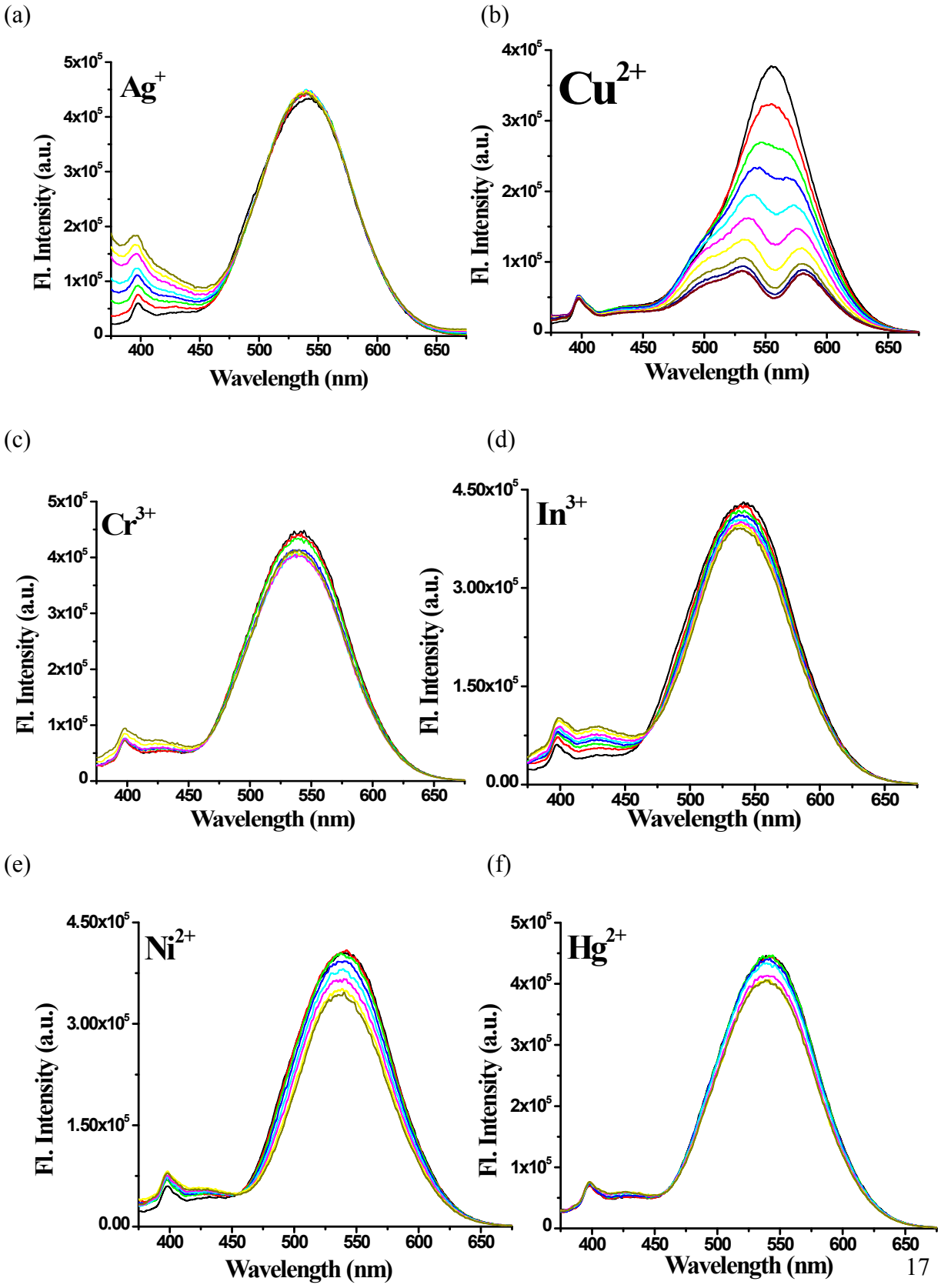
ESI MS Spectra of RHD + $ZnCl_2$+ $Al(NO_3)_3.9H_2O$:



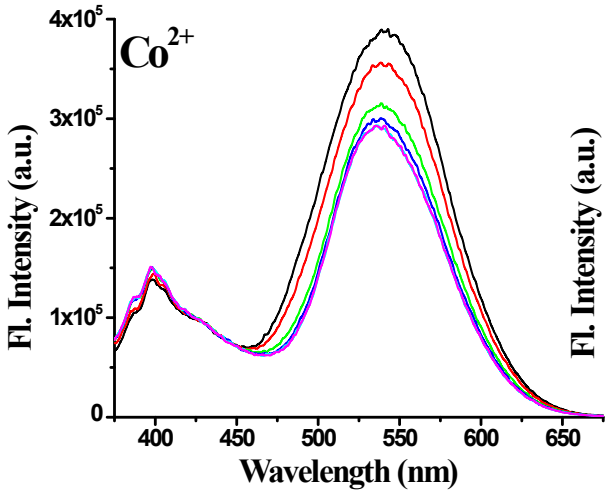
ESI MS Spectra of "donor" moiety:



11. Fluorescence emission spectra of RHD with different cations as Ag^{+}, Cu^{2+} , Cr^{3+}, In^{3+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Cd^{2+}, Fe^{3+}, Na^{+}, Pb^{2+}, Mn^{2+}, Ga^{3+}, Mg^{2+}, in CH_3CN : H_2O (1:1, v/v) at pH 7.5 by using 20 mM HEPES buffer (The solutions of cations were prepared from AgNO3, $Cu(ClO_4)_2 \cdot 6H_2O$, $CrCl_3$, $InCl_3$, $Ni(ClO_4)_2 \cdot 6H_2O$, $HgCl_2$, $Co(ClO_4)_2 \cdot 6H_2O$, $CdCl_2$, $FeCl_3$, NaCl, $Pb(ClO_4)_2.3H_2O$, $Mn(ClO_4)_2.6H_2O$, $GaCl_3$, $Mg(ClO_4)_2.6H_2O$, respectively in CH_3CN-H_2O).



(g)


Co2+
Fl. Intensity (a.u.)
Wavelength (nm)
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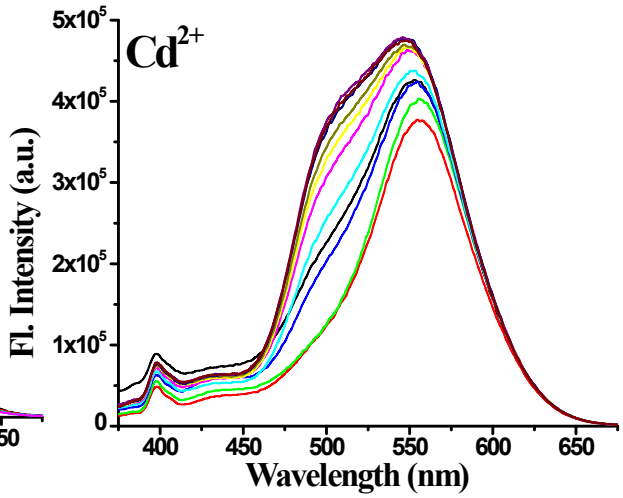
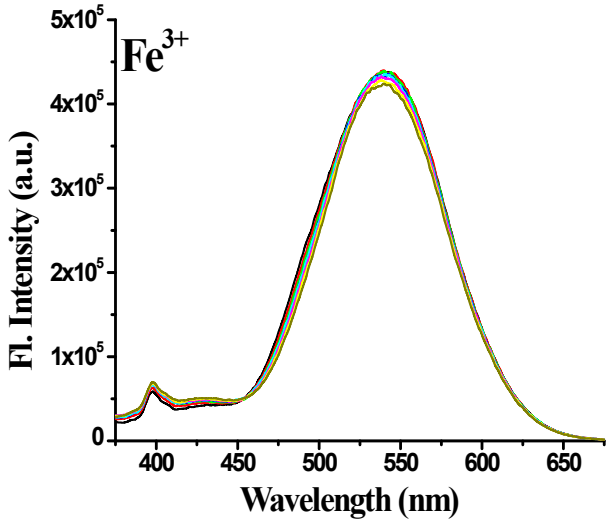
3x10^5
2x10^5
1x10^5
0
400
450
500
550
600
650

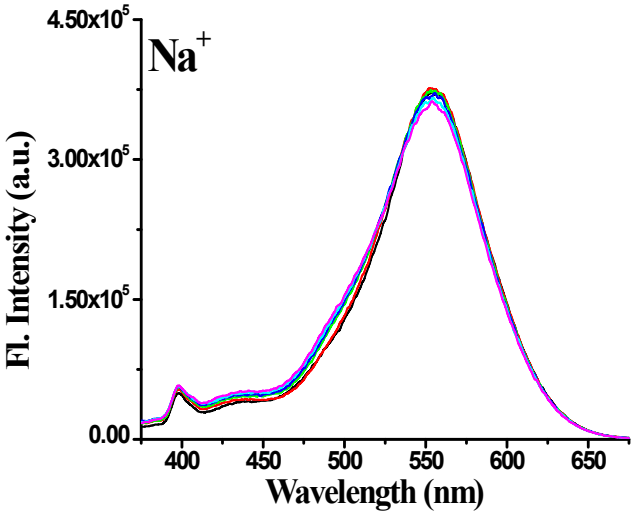
(h)


Cd2+
Fl. Intensity (a.u.)
Wavelength (nm)
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
400
450
500
550
600
650

(i)


Fe3+
Fl. Intensity (a.u.)
Wavelength (nm)
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
400
450
500
550
600
650

(j)


Na+
Fl. Intensity (a.u.)
Wavelength (nm)
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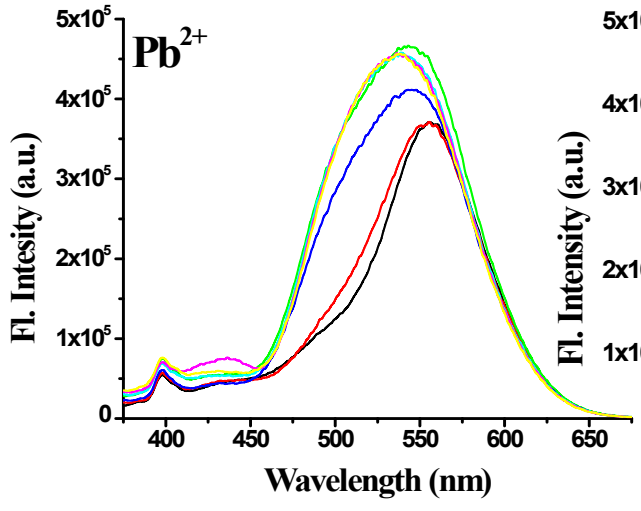
3.00x10^5
1.50x10^5
0.00
400
450
500
550
600
650

(k)


Pb2+
Fl. Intesity (a.u.)
Wavelength (nm)
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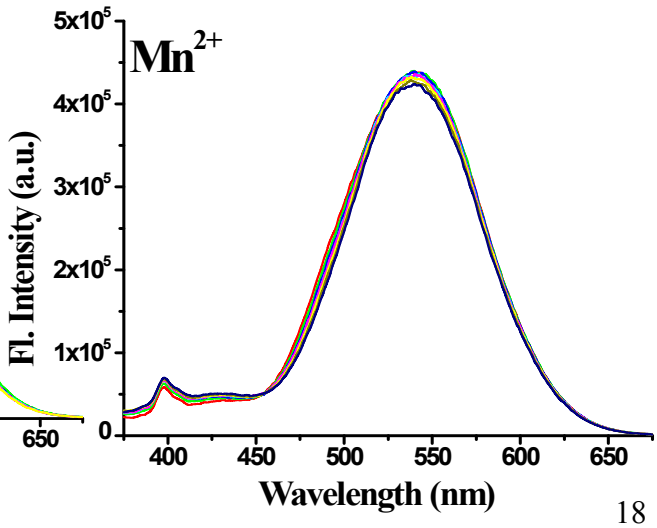
3x10^5
2x10^5
1x10^5
0
400
450
500
550
600
650

(l)


Mn2+
Fl. Intensity (a.u.)
Wavelength (nm)
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2x10^5
1x10^5
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400
450
500
550
600
650

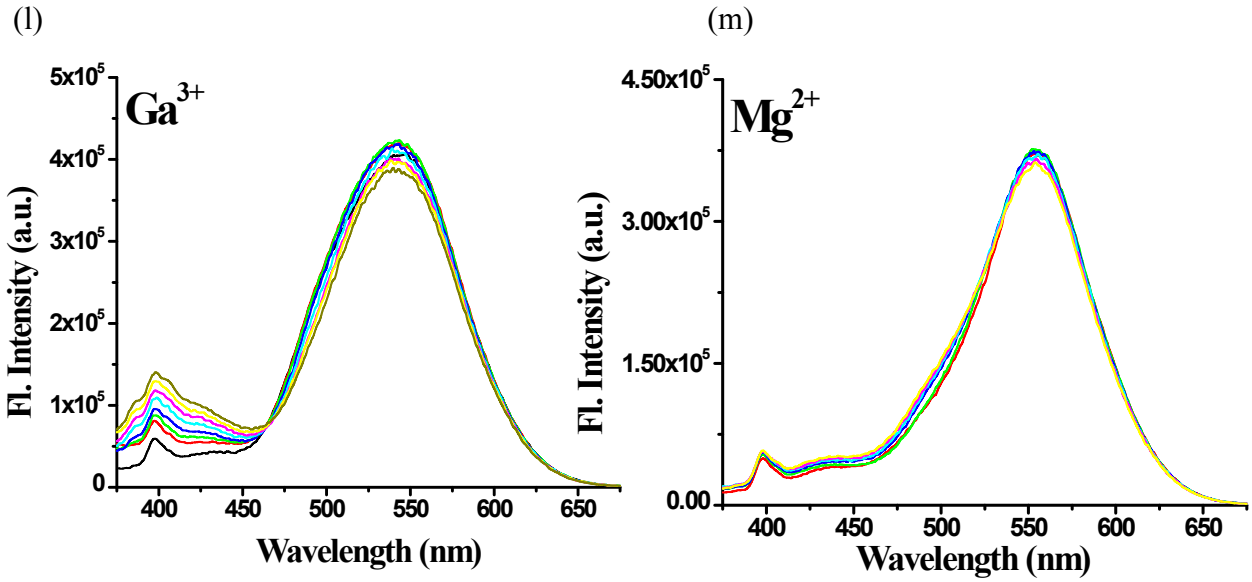

Figure S8-S21: Fluorescence emission spectra of RHD (c = 2.0 x 10^{-5} M) in presence of different cations Ag^{+}, Cu^{2+}, Cr^{3+}, In^{3+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Cd^{2+}, Fe^{3+}, Na^{+}, Pb^{2+}, Mn^{2+}, Ga^{3+}, Mg^{2+} (c = 2.0 x 10^{-4} M) respectively at pH 7.5 in CH_3CN : H_2O (1:1, v/v).

12. Details of X-ray Crystallography:

Crystallographic data were collected at 100K with an Oxford Cyrosystem Cobra low temperature attachment. A single crystal of 0.498 x 0.197 x 0.131 mm^3 in size was mounted on a glass fibre with epoxy cement for X-ray crystallographic study. The data was collected using a Bruker Apex 2 CCD diffractometer with graphite monochromatic MoKα radiation (λ

= 0.71073 Å) at a detector distance of 5 cm and an APEX2 software [S3]. The collected data was reduced using SAINT [S3] program and the empirical absorption corrections were performed using SADABS program [S3]. The structure was solved by using the direct method whereas it was refined using the least-square method from the SHELXTL software package [S4]. Materials for publication were prepared using SHELXTL and PLATON [S4, S5]. All non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined isotropically. The O-bound hydrogen atom was first located in difference Fourier maps, and then fixed in calculated sites, with (H) = 1.5 U_{eq} (O) and d(O—H) = 0.9804 Å. The remaining hydrogen atoms were positioned geometrically with U_{iso} (H) = 1.2 or 1.5 U_{eq} (C). A rotating group model was applied to the methyl groups. One of the ethyl groups (atoms C35 and C36) is disordered over two sites with a refined occupancy ratio of 0.694(13) : 0.306(13). Full crystallographic data, in CIF format, can be obtained from the Cambridge Crystallographic Data Centre (CCDC 947172) *via* http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx.

The crystal data and structure refinement of FS239 is summarized in Table 1 and has been deposited with the Cambridge Crystallographic Data Center No. CCDC 947172. The single-crystal X-ray diffraction analysis reveals that FS239 was crystallized in the orthorhombic space group *P*bca. In the structure of FS239, the xanthene ring system is approximately planar, with a maximum deviation of 0.045 (3) Å, and its mean plane makes dihedral angles of 75.92 (13), 78.15 (9) and 89.45 (9) ° with the benzene ring, benzothiazole and isoindoline ring systems, respectively. The benzene ring forms dihedral angles of 7.30 (13) and 3.68 (14)° with the mean planes of isoindoline (maximum deviation = 0.019 Å) and benzothiazole (maximum deviation = 0.034 Å) ring systems, respectively. The dihedral angle between isoindoline and benzothiazole ring systems is 19.65 (11)°. The molecular structure is stabilized by intramolecular O3—H1*O*3···N5 and C21—H21*A*···O2 hydrogen bonds (Table 2), which generate S(6) ring motifs (Fig. S22). There are no classical intermolecular hydrogen bonds observed in the crystal packing which features short C36A···O3 [2.88 (2) Å] contacts and weak C—H···π interactions (Table 2). A short intermolecular contact between the two benzene rings (C8–C13 & C14–C19), with a centroid–centroid distance of 3.7929(18) Å, indicates a weak aromatic π–π interaction. Molecules are stacked down the *b* axis caused by these interactions (Fig. S23).

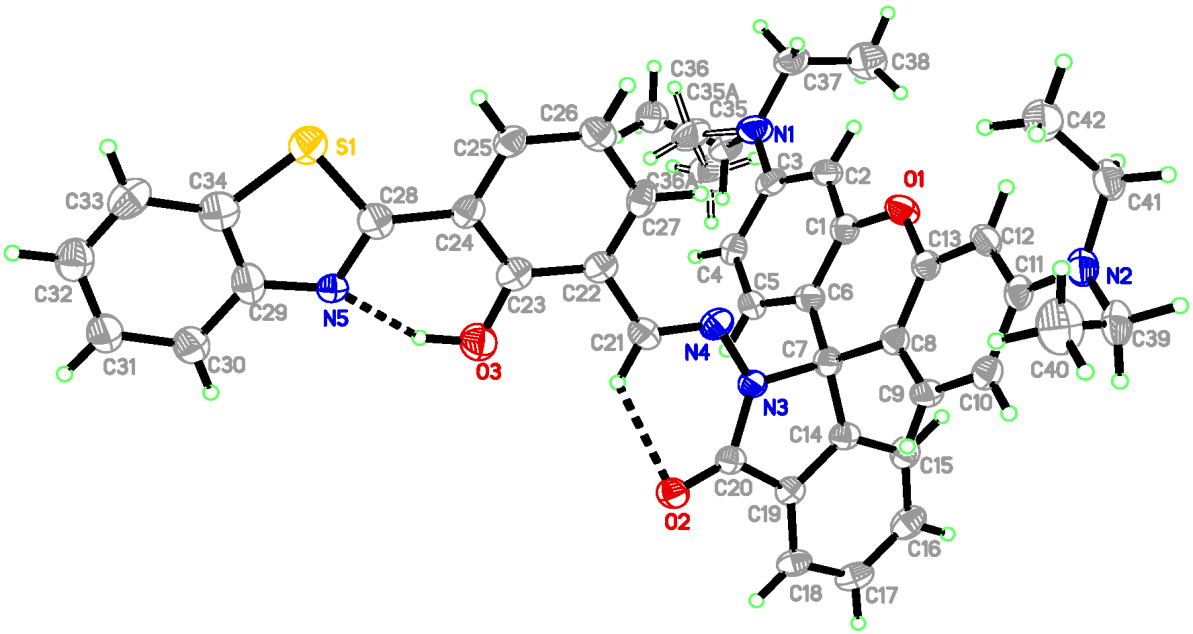

Figure S22: The molecular structure of **RHD**, showing 50% probability displacement ellipsoids for non-H atoms. Intramolecular hydrogen bonds and minor component of disorder are shown as dashed line and open bonds, respectively.

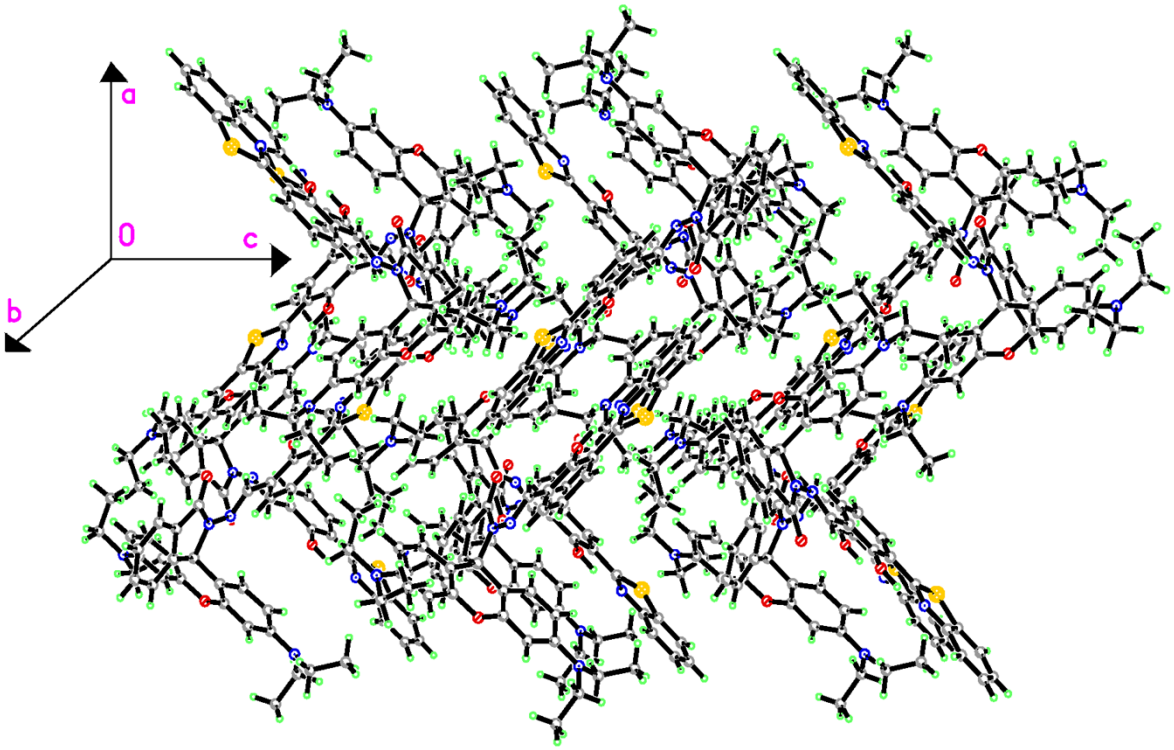

Figure S23: The crystal structure of **RHD**, viewed along [010]. Only the major disorder component is shown.

Table 1: X-ray crystallographic data

Compounds	**RHD** (FS239) (CCDC 947172)
Formula	$C_{42}H39N_5O_3S$
Formula Weight	693.84
Crystal System	Orthorhombic
Space Group	*Pbca*
T, K	100
Z	8
a,Å	20.0319 (9)
b,Å	14.3160 (6)
c,Å	24.1507 (9)
α,deg	90
β,deg	90
γ,deg	90
V, Å^3	6925.9 (5)
d_{calcd}, g/cm^3	1.331
μ, mm^{-1}	0.14
Reflections with $I > 2\sigma(I)$	4442
Independent reflections	6089
θ range, deg	1.9–25.0
hkl range	$h = -23 \rightarrow 23$ $k = -17 \rightarrow 17$ $l = -28 \rightarrow 17$
GOF (F^2)	1.02
R_1 (wR_2), %	0.069, 0.201
Completeness, %	99.8
T_{min}, T_{max}	0.982, 0.932

Table 2 Hydrogen-bond geometry (Å, º)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O3—H1*O*3···N5	0.98	1.70	2.621 (4)	155
C21—H21*A*···O2	0.95	2.19	2.871 (4)	128
C18—H18*A*···*Cg*1^{i}	0.95	2.86	3.676	145
C31—H31*A*···*Cg*2^{ii}	0.95	2.59	3.416	146

Symmetry codes: (i) *x*, −*y*−1/2, *z*−1/2; (ii) −*x*−1, *y*+1/2, −*z*+5/2.

* Cg1 and Cg2 are the centroids of the C8-C13 and C14-C19 rings

13. Details of cell culture and fluorescence microscopy:

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and penicillin-streptomycin (0.5 U/ml of penicillin and 0.5 µg/ml streptomycin) on cover slip in 35 mm dishes at 37^{o}C in an atmosphere of air with 5% CO_2 and constant humidity. The cells were initially incubated with the addition of 50 µM of Zn^{2+} in the growth medium for 30 minutes. After washing three times with phosphate buffered saline (PBS) fresh growth medium containing 50 µM of the receptor (RHD) was added and the cells were further incubated for 30 minutes. Following the incubation one set of the cells was washed three times with PBS and the imaging was carried out using Zeiss AxioObserver Fluorescence Microscope equipped with an Apotome apparatus. And the other set of cells were further washed three times with PBS, fresh growth medium containing 50 µM of Al^{3+} was added incubated for 30 minutes. After washing three times with PBS, the imaging was carried out using Zeiss AxioObserver Fluorescence Microscope equipped with an Apotome apparatus.

14. UV-absorption spectra of RHD with addition of Al^{3+}:

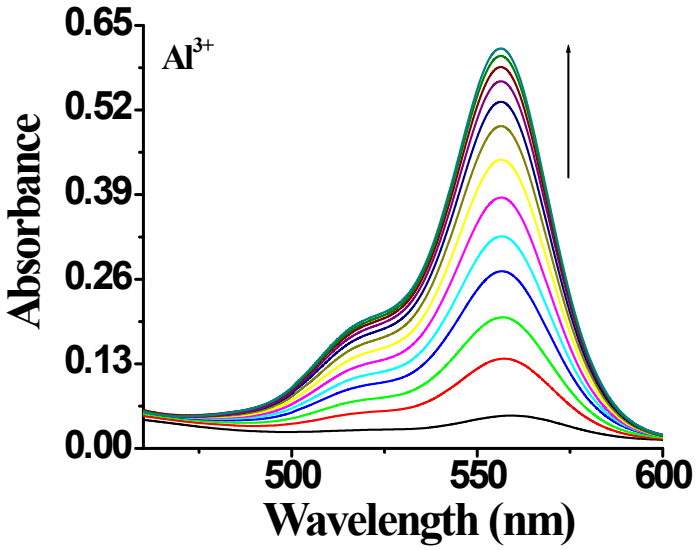

Figure S24: UV-absorption titration spectra of RHD (c = 2.0 x 10^{-5} M) in presence of Al^{3+} (c = 2.0 x 10^{-4} M) at pH 7.5 in $CH_3CN:H_2O$ = 1:1 (v/v).

15. References:

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