

ELECTRONIC SUPPORTING INFORMATION (ESI)

Rhodamine - naphthalene conjugate as a FRET based sensor for Cr³⁺ and Fe³⁺ with cell staining application

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Materials and methods

Rhodamine B, 2-hydroxy naphthaldehyde and diethylenetriamine were purchased from Sigma Aldrich (USA) and used as received. Cr(NO₃)₃·9H₂O and Fe(NO₃)₃·9H₂O were purchased from Merck (India). Spectroscopic grade solvents were used. Milli-Q Millipore 18.2 MΩ cm⁻¹ water was used in all the experiments. All other chemicals used were of analytical grade. A JASCO (model V-570) UV–Vis spectrophotometer was used for recording UV-Vis spectra. FTIR spectra were recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20). Mass spectra were performed on a QTOF Micro YA 263 mass spectrometer in ES positive mode.

¹H NMR spectra were recorded using Bruker Advance 600 (600MHz) in DMSO-d₆. The steady-state fluorescence emission and excitation spectra were recorded with a Hitachi F-4500 spectrofluorimeter. Time-resolved fluorescence life time measurements were performed using a picosecond pulsed diode laser-based time-correlated single photon counting (TCSPC) spectrometer from IBH (UK) at λ_{ex}, 330 nm and MCP-PMT as a detector. The emission from the sample was collected at a right angle to the direction of the excitation beam maintaining

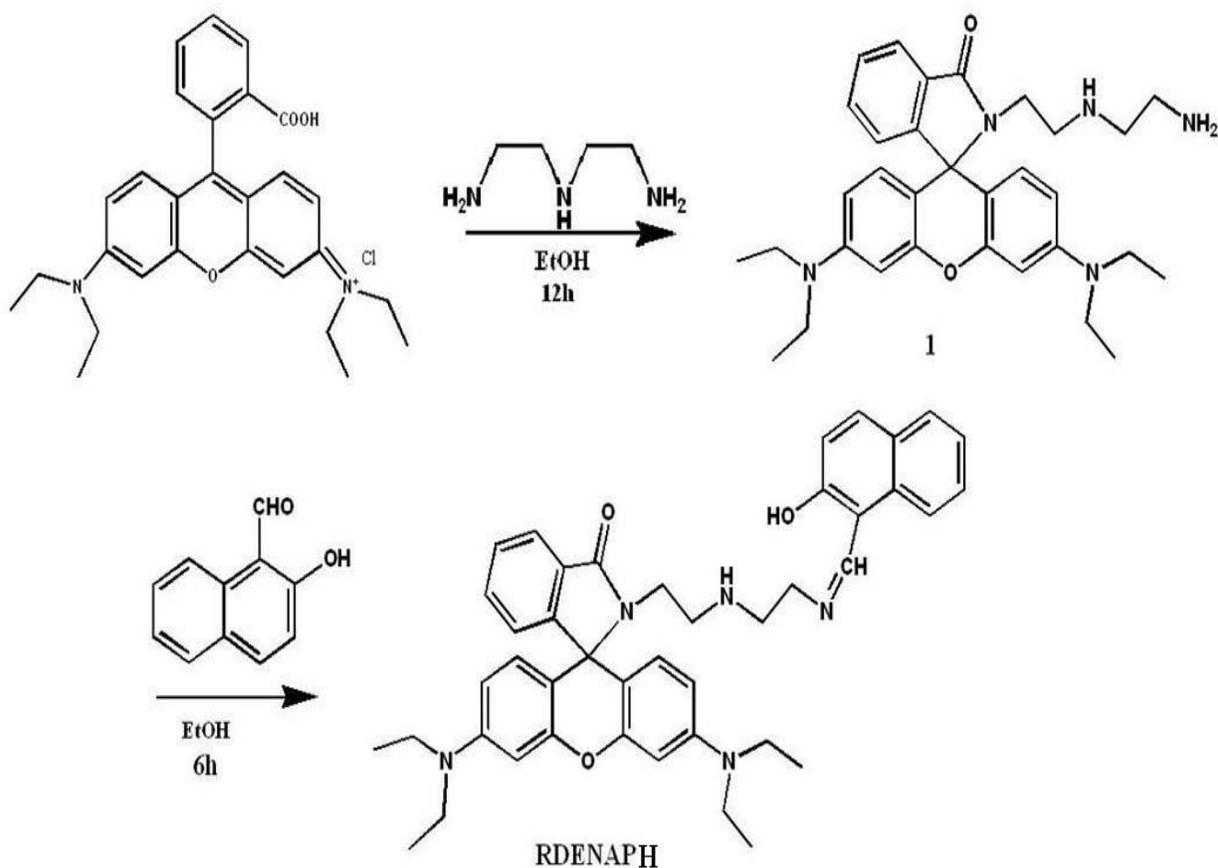
magic angle polarization(54.71).The full width at half maximum (FWHM) of the instrument response function was 250 ps and the resolution was 28 ps per channel. The data were fitted to multi-exponential functions after deconvolution of the instrument response function by an iterative reconvolution technique using IBH DAS 6.2 data analysis software in which reduced χ^2 and weighted residuals serve as parameters for goodness of fit.

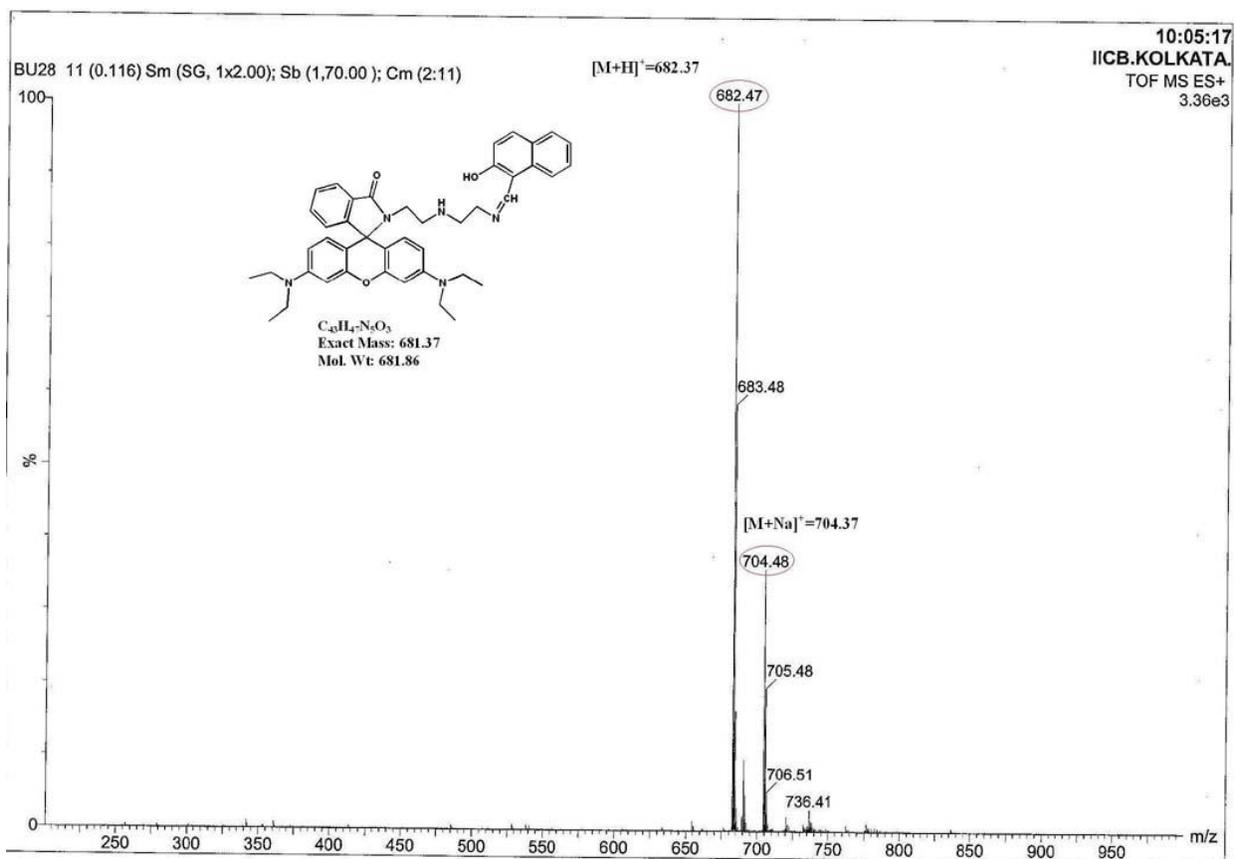
Fluorescence microscopic images were collected from an inverted fluorescence microscope (Leica DM 1000 LED) attached with a digital compact camera (Leica DFC 420C) and an image processor (Leica Application Suite v3.3.0). The microscope was equipped with a mercury 50W lamp.

Synthesis of RDENAPH

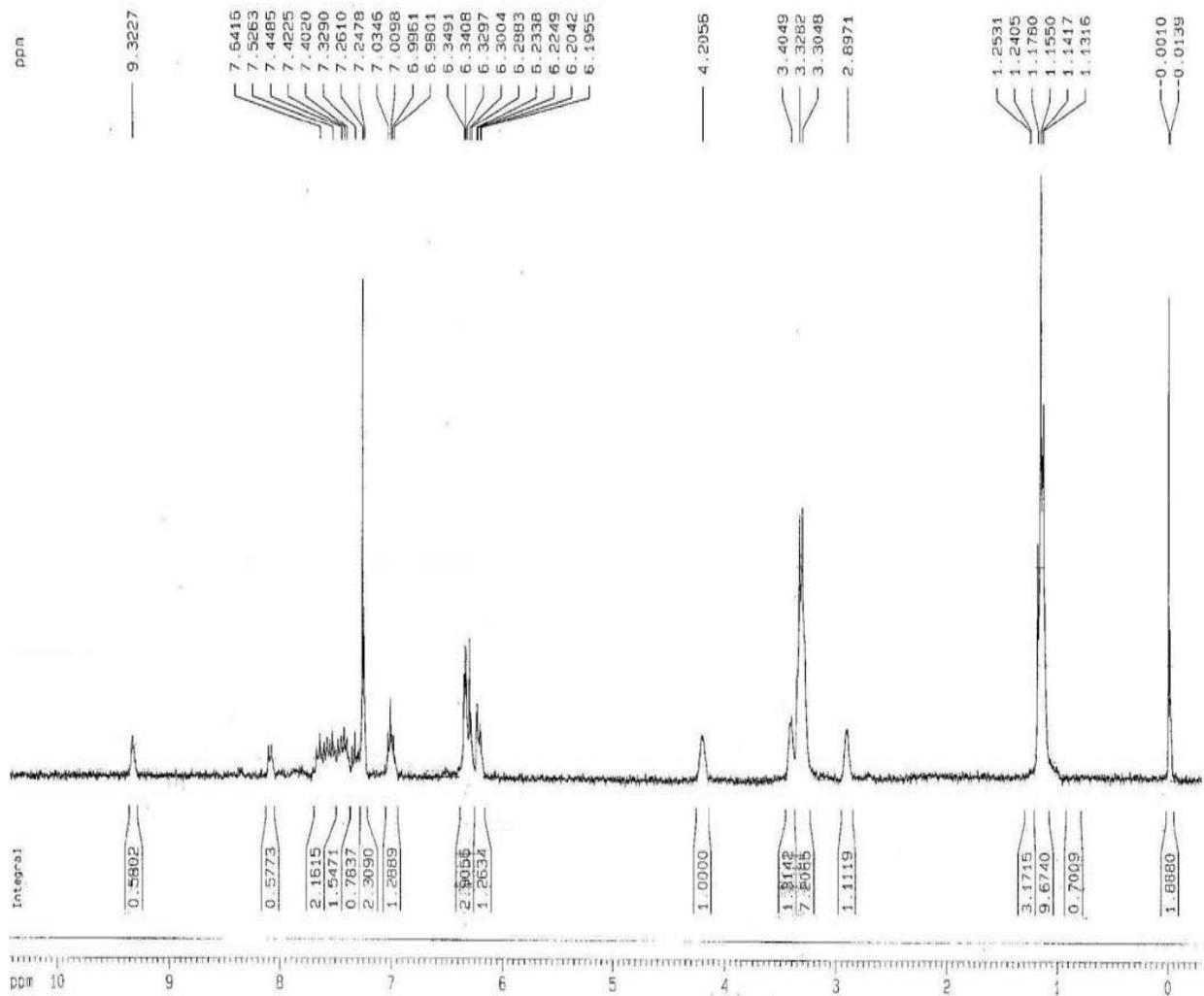
Compound **1** has been prepared by slight modification of the literature procedure.^{S1} Rhodamine B (1916 mg, 4 mmol) was dissolved in 40 mL warm ethanol. Ethanol solution (10 mL) of diethylenetriamine (1.34 mL, 20 mmol) was added drop-wise to Rhodamine B solution. The reaction mixture was refluxed for 8 h when the fluorescence of the solution had disappeared. The reaction mixture was allowed to cool to room temperature and the solvent was removed using rotary evaporator. CH_2Cl_2 (100mL) and water (200 mL) were added to the crude compound and the organic layer was extracted, washed twice with water and dried over anhydrous sodium sulfate. After filtration of sodium sulfate, the solvent was removed under reduced pressure. The resulting pink solid was purified by column chromatography (CH_2Cl_2 : CH_3OH = 5: 1, v/v) to give **1** (pink solid). Then **1** (150mg, 0.284 mmol) was dissolved in 30 mL ethanol followed by addition of 2-hydroxy naphthaldehyde (49mg, 0.248 mmol). The mixture was refluxed for 6 h. The solvent was removed using rotary evaporator. The residue was dissolved in small amount of ethyl acetate to get a clear solution. Addition of small amount of hexane have resulted a light

yellow color product which was filtered and dried in *vacuo*. Yield, 80%. QTOF-MS ES⁺ :
[M+H]⁺ = 682.37, [M + Na]⁺ = 704.37, FTIR (cm⁻¹): $\nu(\text{NH})$ 3223.84, $\nu(\text{C}=\text{N})$ 1614.81, $\nu(\text{OH})$
3406.62; ¹H NMR (300 MHz, DMSO-d₆): δ (ppm): 9.3 (1H, s, J), 8.1 (1H, d, J = 8.2 Hz), 7.6-7.2
(6H, m, J = 9), 7.2 (1H, s), 7 (2H, m, J = 7.4 Hz), 6.3 (5H, m, J = 2.5), 6.2 (2H, m, J = 2.6 Hz),
4.2 (2H, m), 3.4 (2H, m), 3.4 (10H, m, J = 7.2), 2.8 (2H, m), 1.1 (12H, m, J = 3).

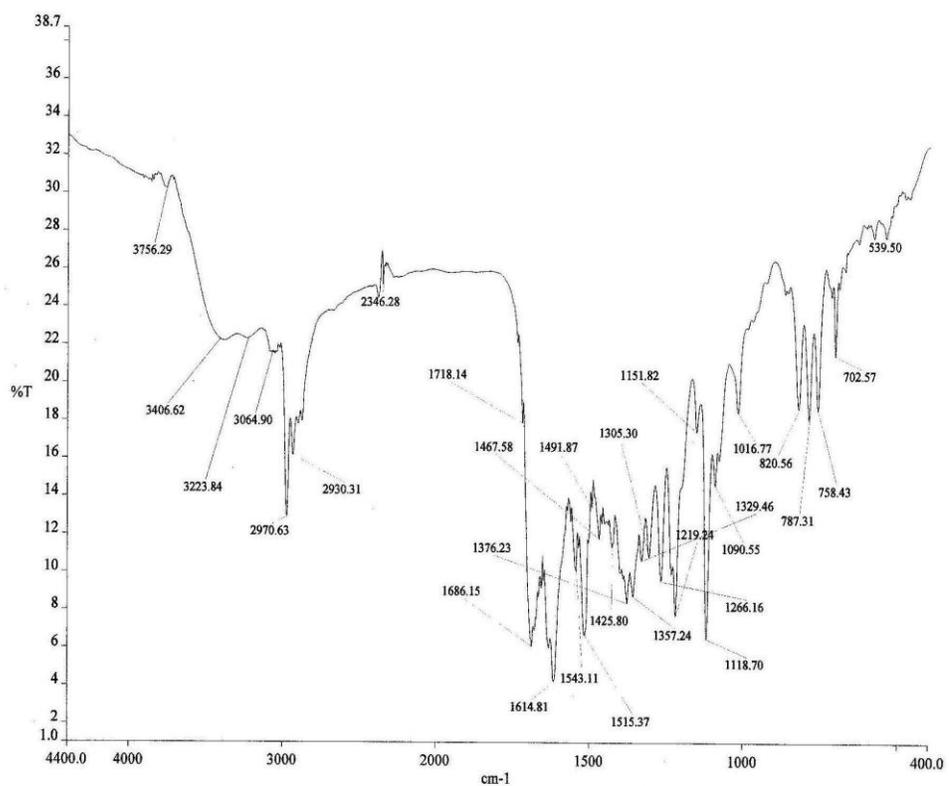




QTOF-MS spectrum of **RDNAPH**



^1H NMR of **RDENAPH** in DMSO-d_6



FTIR spectra of **RDENAPH**

Calculation of Quantum Yield

Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves using the equation,

$$\phi_{\text{sample}} = \frac{\text{OD}_{\text{standard}} \times A_{\text{sample}}}{\text{OD}_{\text{sample}} \times A_{\text{standard}}} \times \phi_{\text{standard}}$$

where A was the area under the fluorescence spectral curve and OD was optical density of the compound at the excitation wavelength. Anthracene was used as quantum yield standard (quantum yield is 0.27 in ethanol) for measuring the quantum yields of ligand and its Cr^{3+} and Fe^{3+} complexes.

S1. Wu, J.-S.; Hwang, I.-C.; Kim, K. S.; Kim, J. S. *Org. Lett.* 2007, **9**, 907.

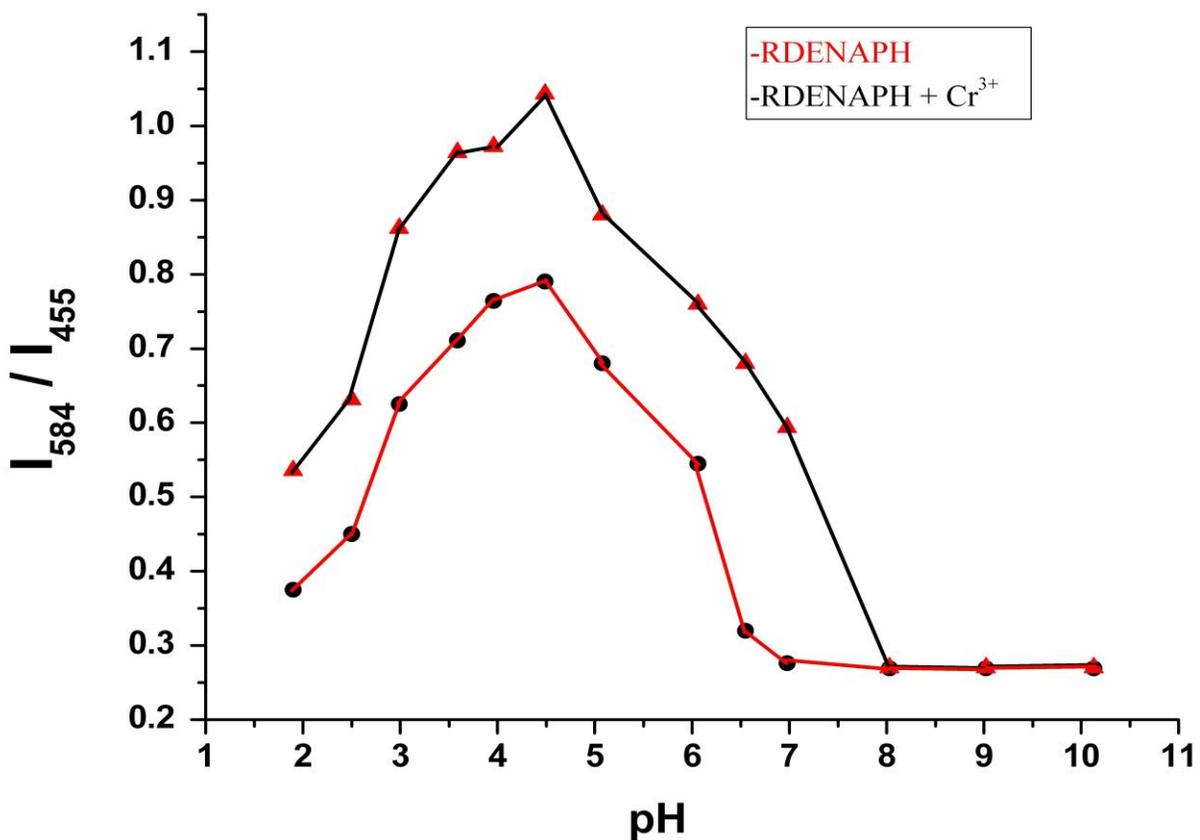


Fig. S1. Effect of pH on the binding efficiency of **RDENAPH** (20 μ M) towards Cr^{3+} (100 μ M) in HEPES buffered (0.1 M) ethanol-water (2:1, v/v, pH-7.0) (ex: 330nm).

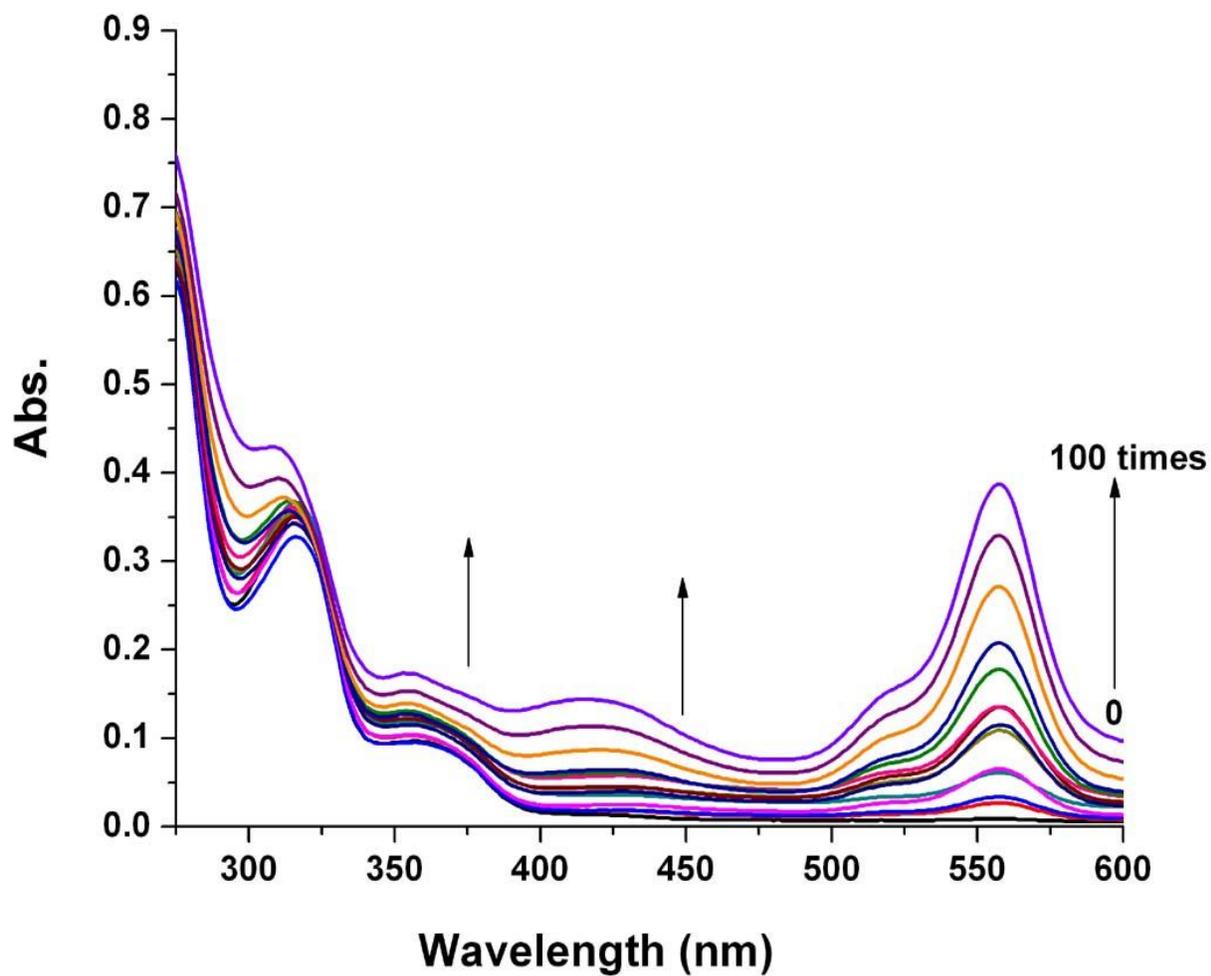


Fig. S2. Changes in the absorption spectra of the **RDENAPH**(20 μM) in HEPES buffered (0.1 M) ethanol-water (2:1, v/v, pH-7.0) with increasing [Cr³⁺] (from 0 to 2000μM). Cell path length, 1 cm.

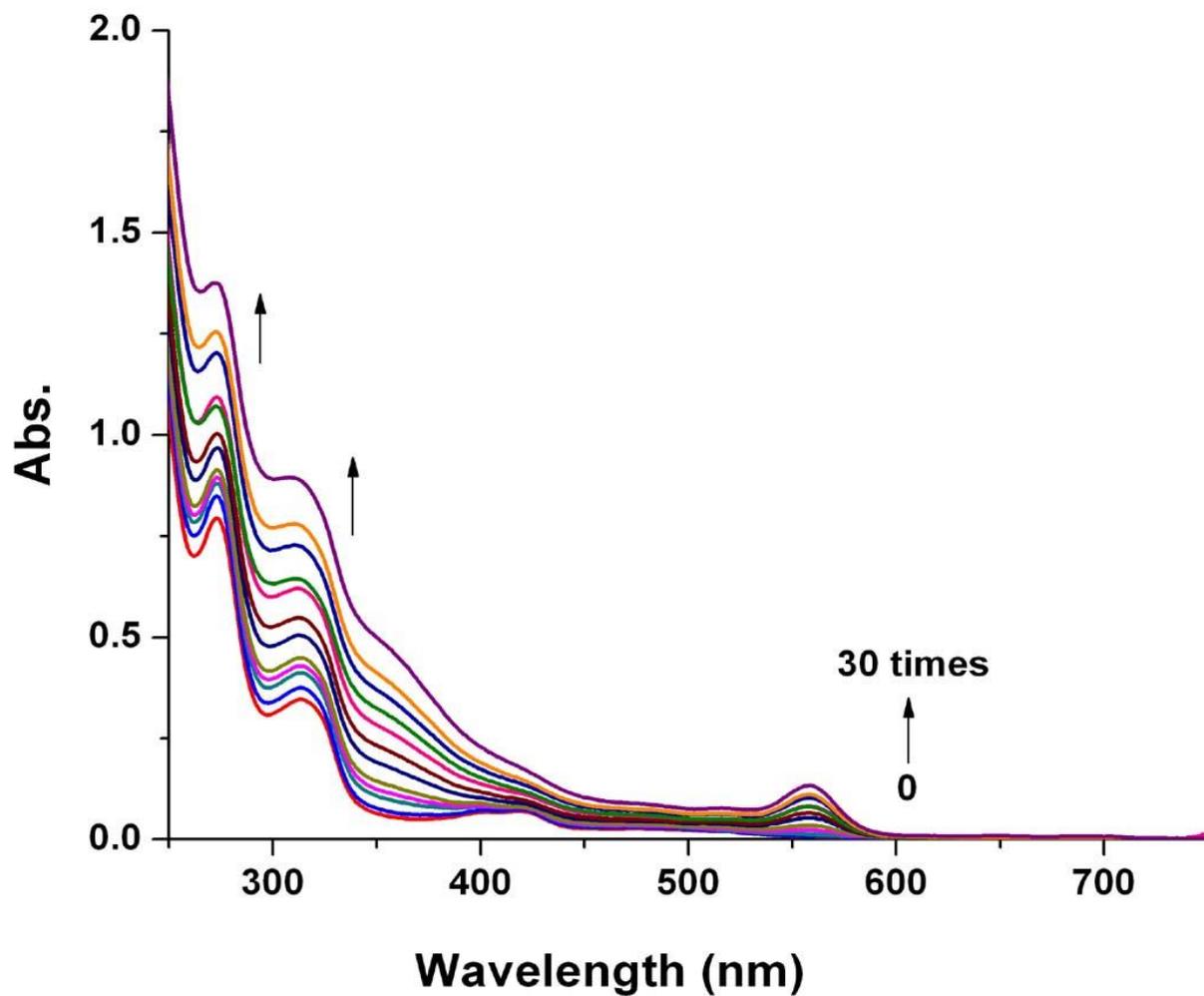


Fig. S3 Changes in the absorption spectrum of the **RDENAPH** (20 μM) in HEPES buffered (0.1 M) ethanol-water (2:1, v/v, pH-7.0) with increasing [Fe³⁺] (from 0 to 600 μM). Cell path length, 1 cm.

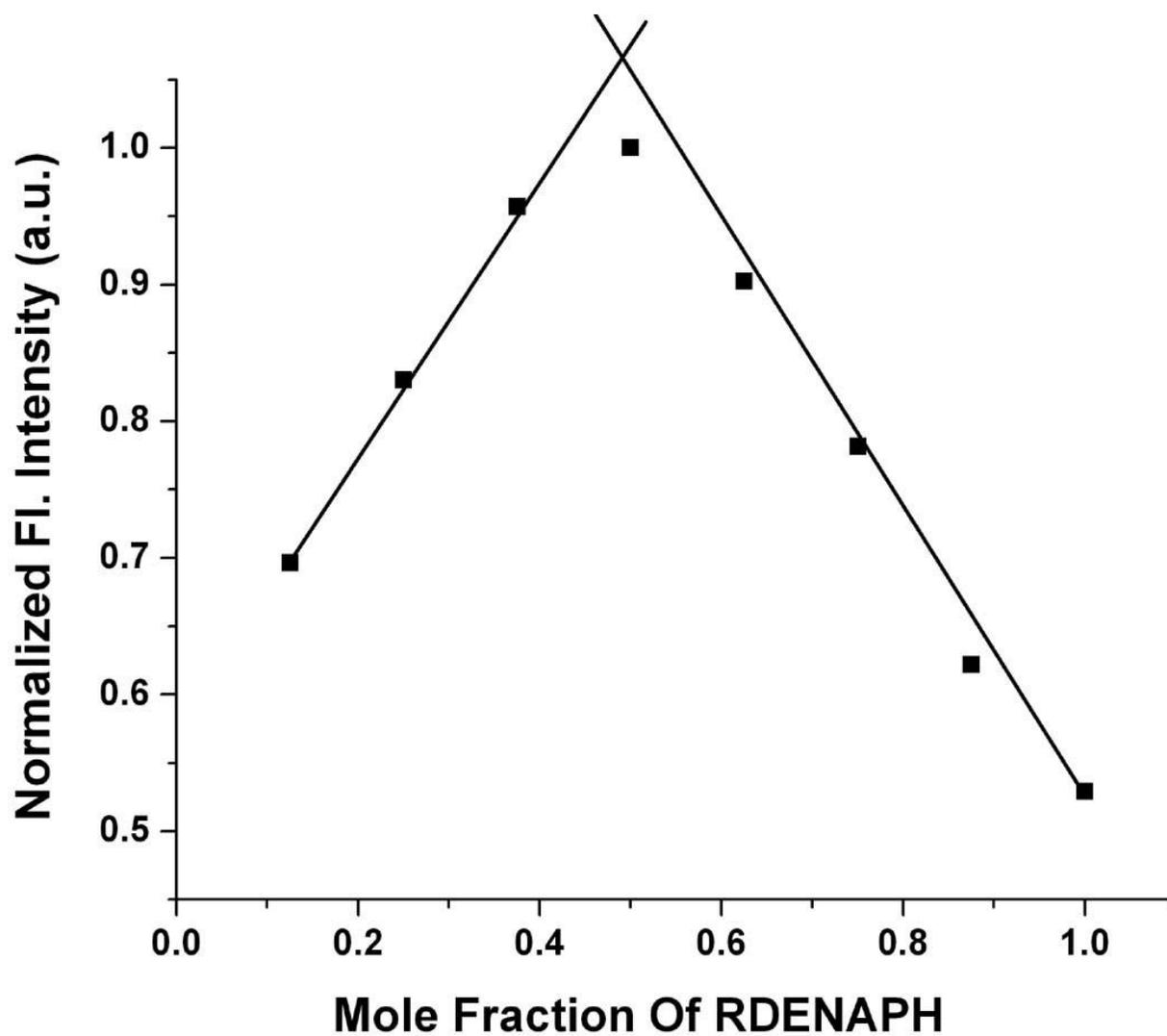


Fig.S4. Job's plot for determination of stoichiometry of [RDENAPH - Cr³⁺] complex.

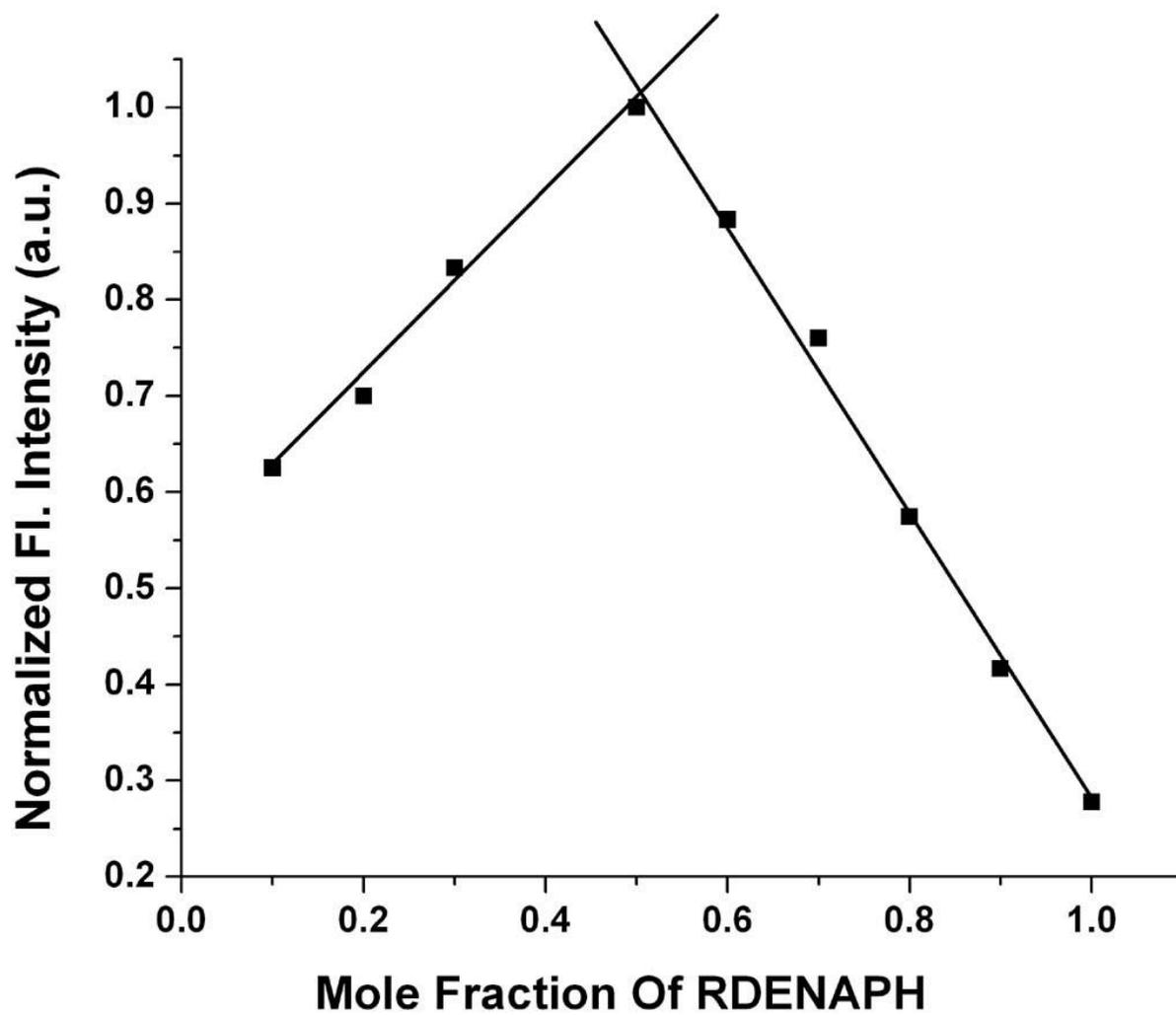


Fig.S5. Job's plot for determination of stoichiometry of [RDENAPH - Fe³⁺] complex.

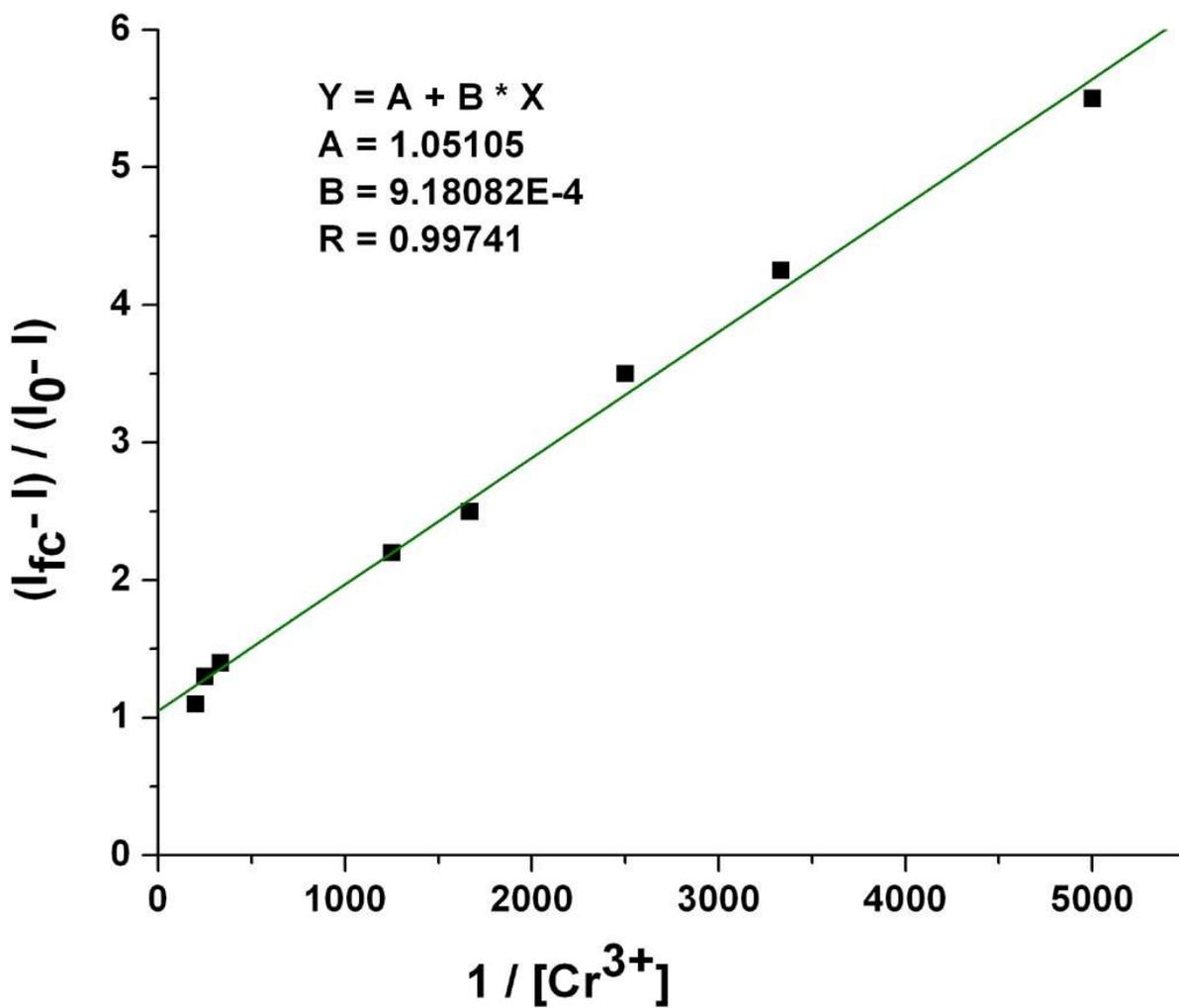


Fig.S6. Determination of binding constant of **RDENAPH** with Cr^{3+} using Benesi-Hildebrand method (fluorescence).

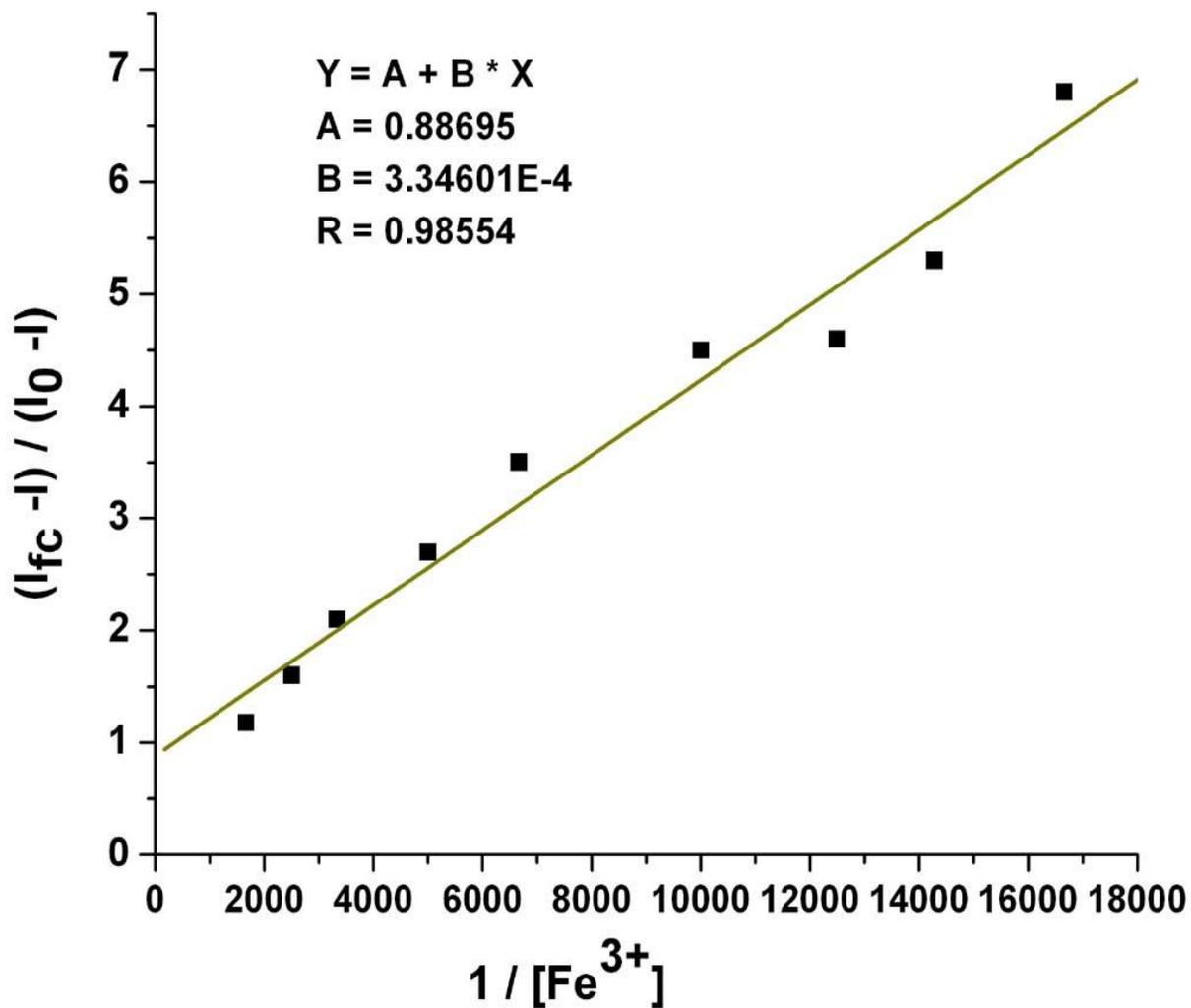


Fig.S7. Determination of binding constant of **RDENAPH** with Fe^{3+} using Benesi-Hildebrand method (fluorescence).

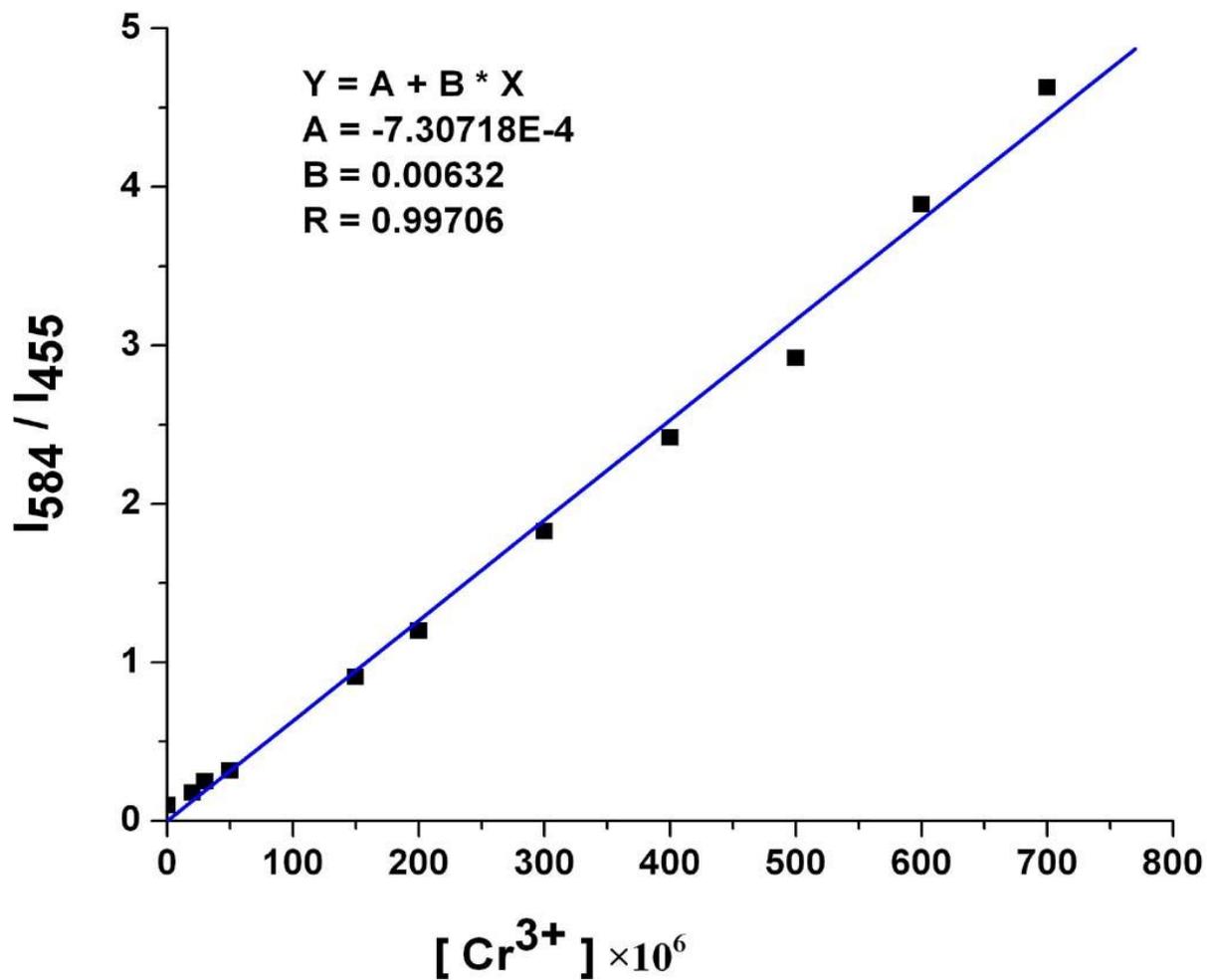


Fig.S8. Plot of emission intensities ratio of **RDENAPH** (20 μ M) as a function of externally added Cr^{3+}

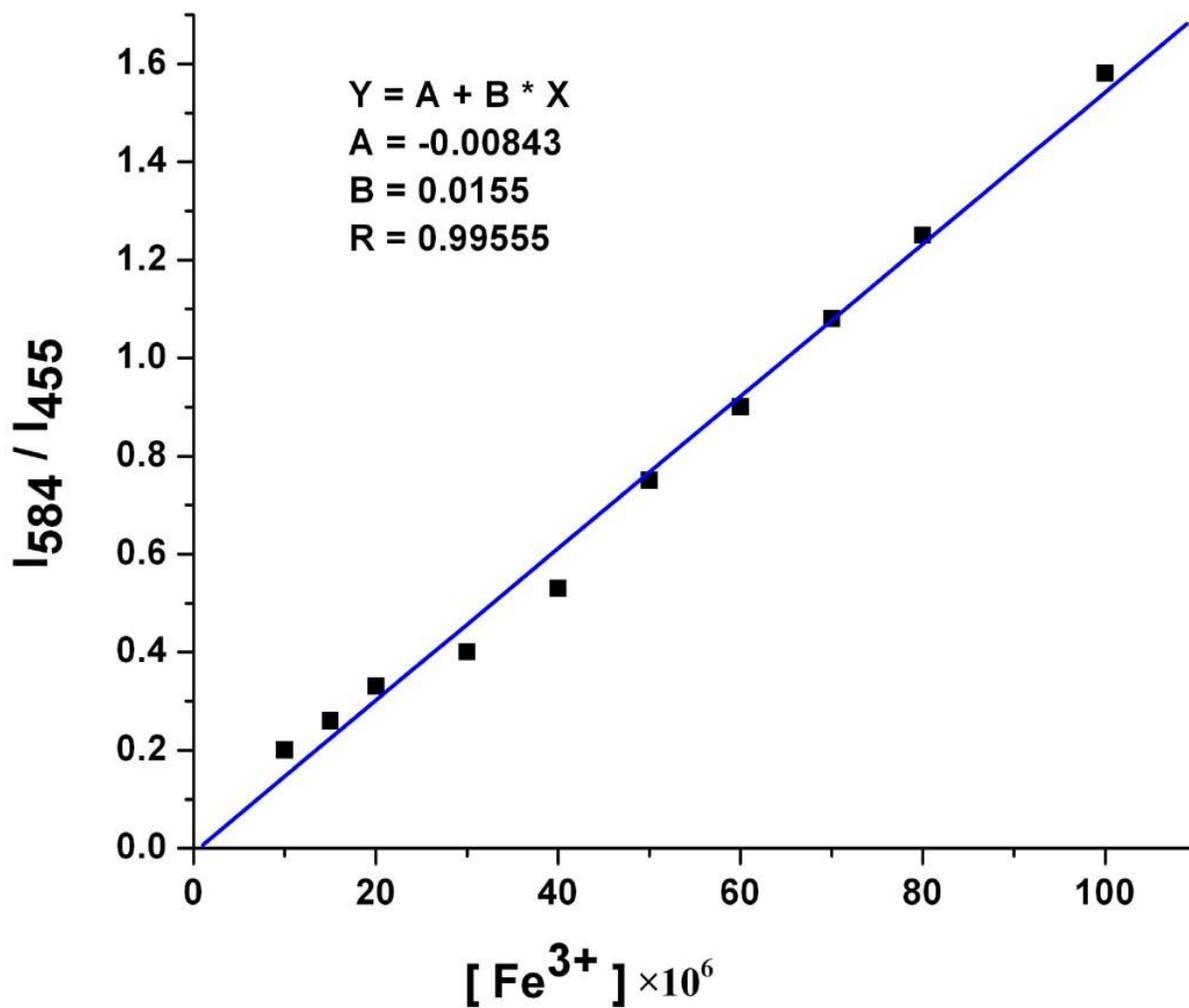


Fig. S9. Plot of emission intensities ratio of **RDENAPH** (20 μ M) as a function of externally added Fe^{3+}

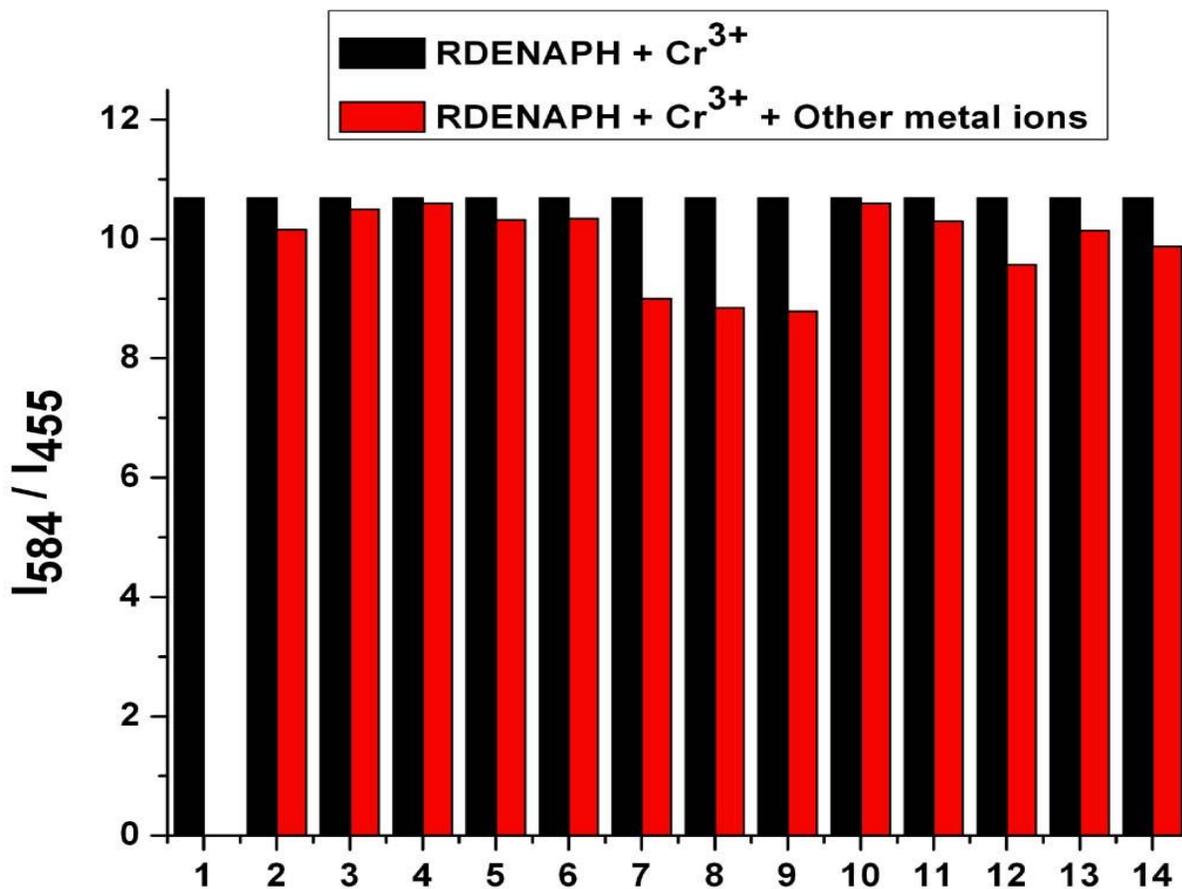


Fig. S10. Variation of I_{584}/I_{455} of **RDNAPH** (20 μM) in presence of other common metal ions in HEPES buffered (0.1 M) ethanol-water (2:1, v/v, pH-7.0). $\lambda_{\text{Ex.}} = 330 \text{ nm}$. $[\text{M}^{n+}] = 100 [\text{Cr}^{3+}]$ and $[\text{Cr}^{3+}] = 8000 \mu\text{M}$; 2 = Mn^{2+} , 3 = Co^{2+} , 4 = Ni^{2+} , 5 = Fe^{3+} , 6 = Cu^{2+} , 7 = Zn^{2+} , 8 = Cd^{2+} , 9 = Hg^{2+} , 10 = Ag^{+} , 11 = Na^{+} , 12 = K^{+} , 13 = Ca^{2+} , 14 = Mg^{2+} .

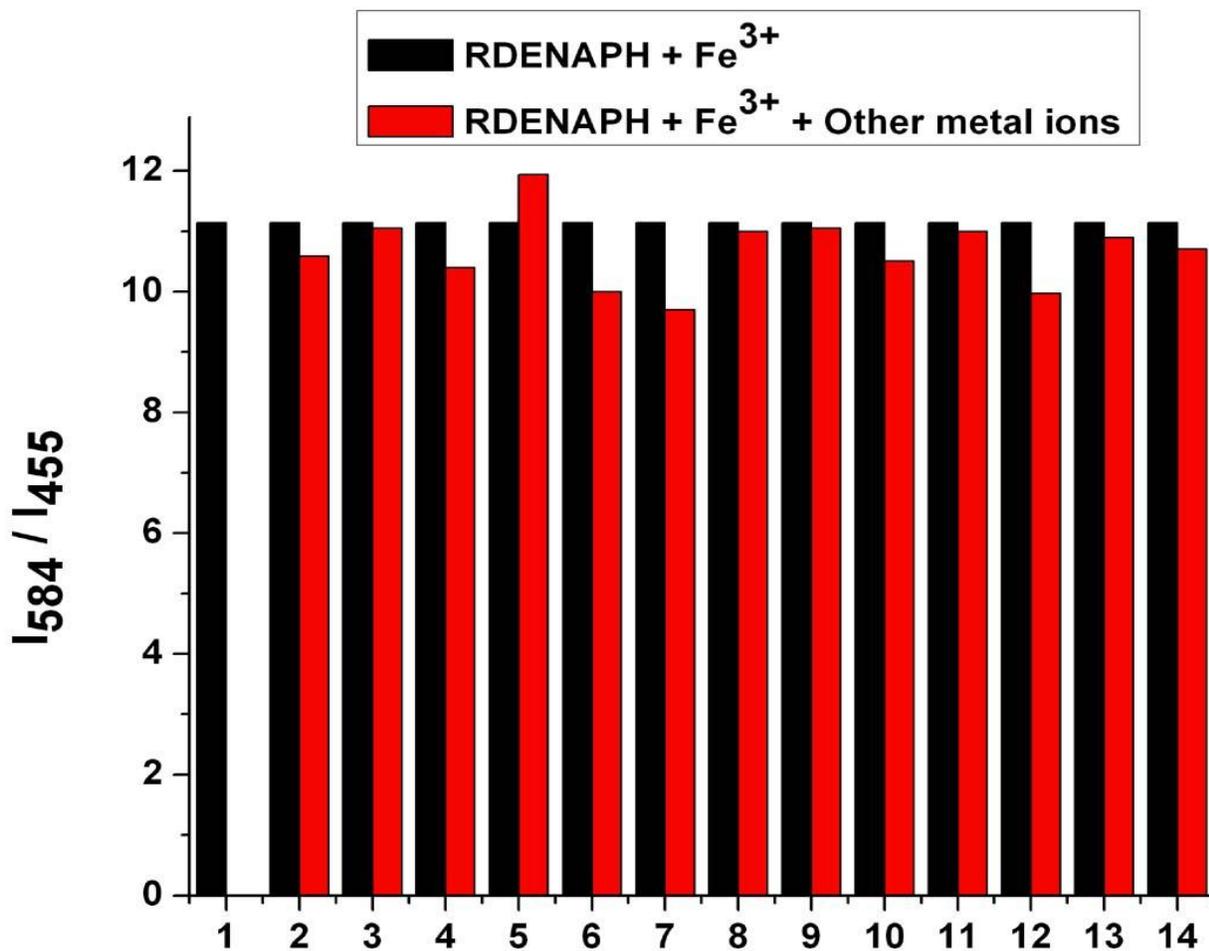


Fig. S11. Variation of I_{584}/I_{455} of **RDENAPH** (20 μM) in presence of other common metal ions in HEPES buffered (0.1 M) ethanol-water (2:1, v/v, pH-7.0). $\lambda_{\text{Ex.}} = 330 \text{ nm}$. $[\text{M}^{\text{n+}}] = 100 [\text{Fe}^{3+}]$ and $[\text{Fe}^{3+}] = 8000 \mu\text{M}$, $[\text{Fe}^{3+}] (600 \mu\text{M})$. 2 = Mn^{2+} , 3 = Co^{2+} , 4 = Ni^{2+} , 5 = Cr^{3+} , 6 = Cu^{2+} , 7 = Zn^{2+} , 8 = Cd^{2+} , 9 = Hg^{2+} , 10 = Ag^+ , 11 = Na^+ , 12 = K^+ , 13 = Ca^{2+} , 14 = Mg^{2+} .

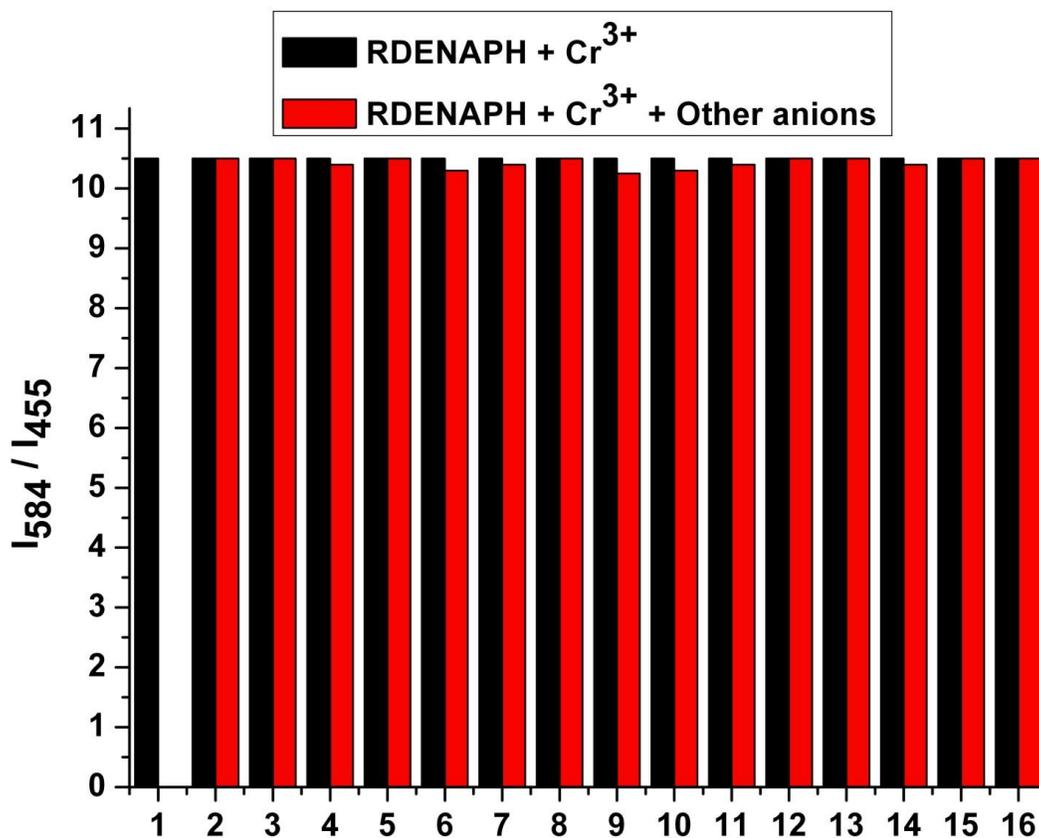


Fig. S12. Effect of different anions on I_{584}/I_{455} of $[\text{RDENAPH}-\text{Cr}^{3+}]$ system in HEPES buffered (0.1 M) ethanol-water (2:1, v/v, pH-7.0). $\lambda_{\text{EX}} = 330 \text{ nm}$. ($[\text{RDENAPH}] = 20 \mu\text{M}$, $[\text{Cr}^{3+}] = 8000 \mu\text{M}$; $[\text{anions}] = 100 [\text{Cr}^{3+}]$; F⁻ (2), Cl⁻ (3), Br⁻ (4), N₃⁻ (5), NCO⁻ (6), NO₂⁻ (7), NO₃⁻ (8), SCN⁻ (9), CN⁻ (10), CH₃COO⁻ (11), SO₄²⁻ (12), ClO₄⁻ (13), H₂PO₄⁻ (14), I⁻ (15), AsO₄³⁻ (16).