Electronic Supplementary Material (ESI) for RSC Advances.

A real time colorimetric 'two in one' kit for tracking ppb level of uric acid and Hg^{2+} in live HeLa S3 cell and Hg^{2+} induced 'keto-enol' tautomerism

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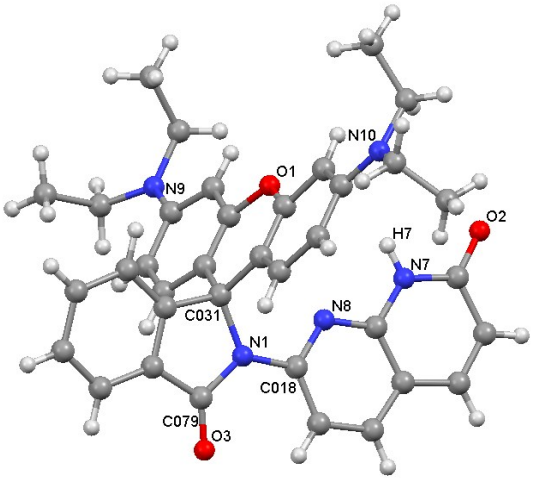
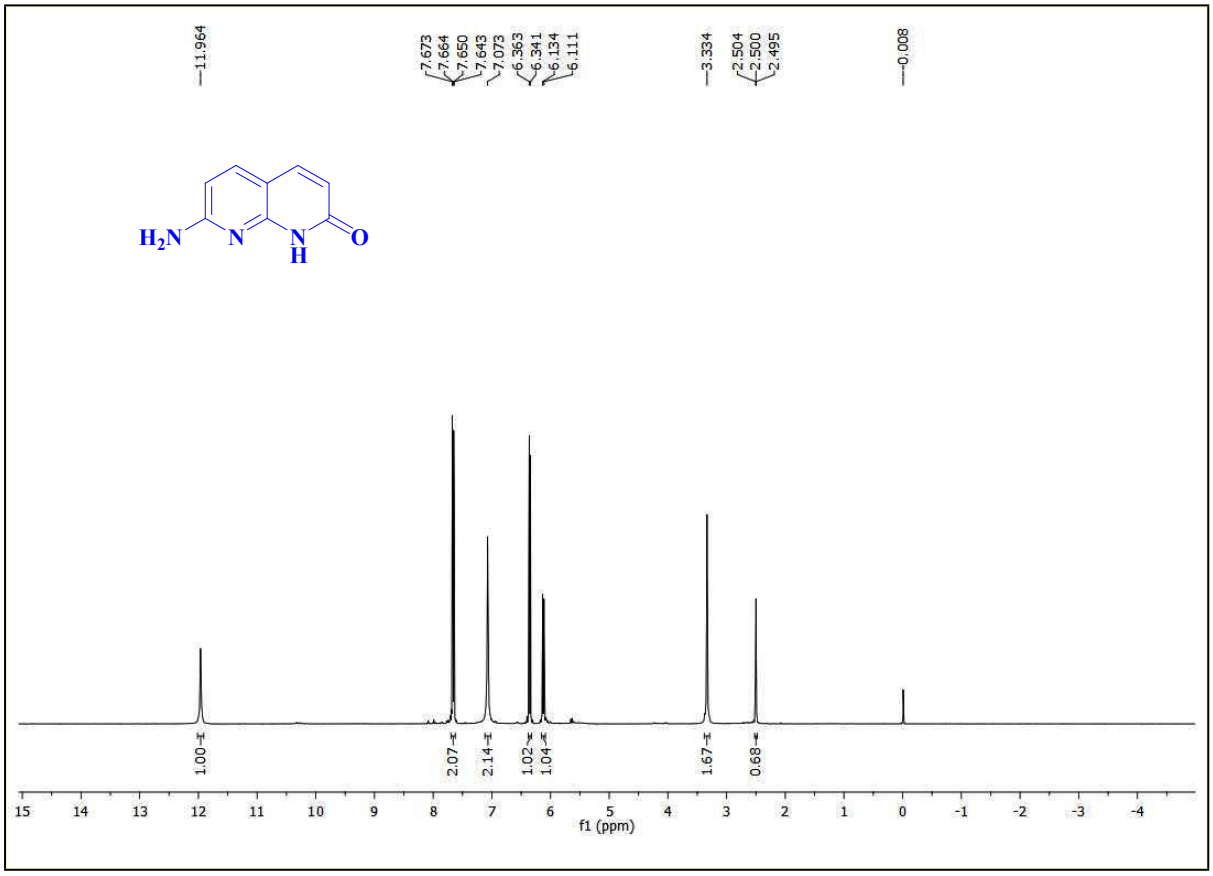


Fig. S1 Crystal structure of **RNpd1**

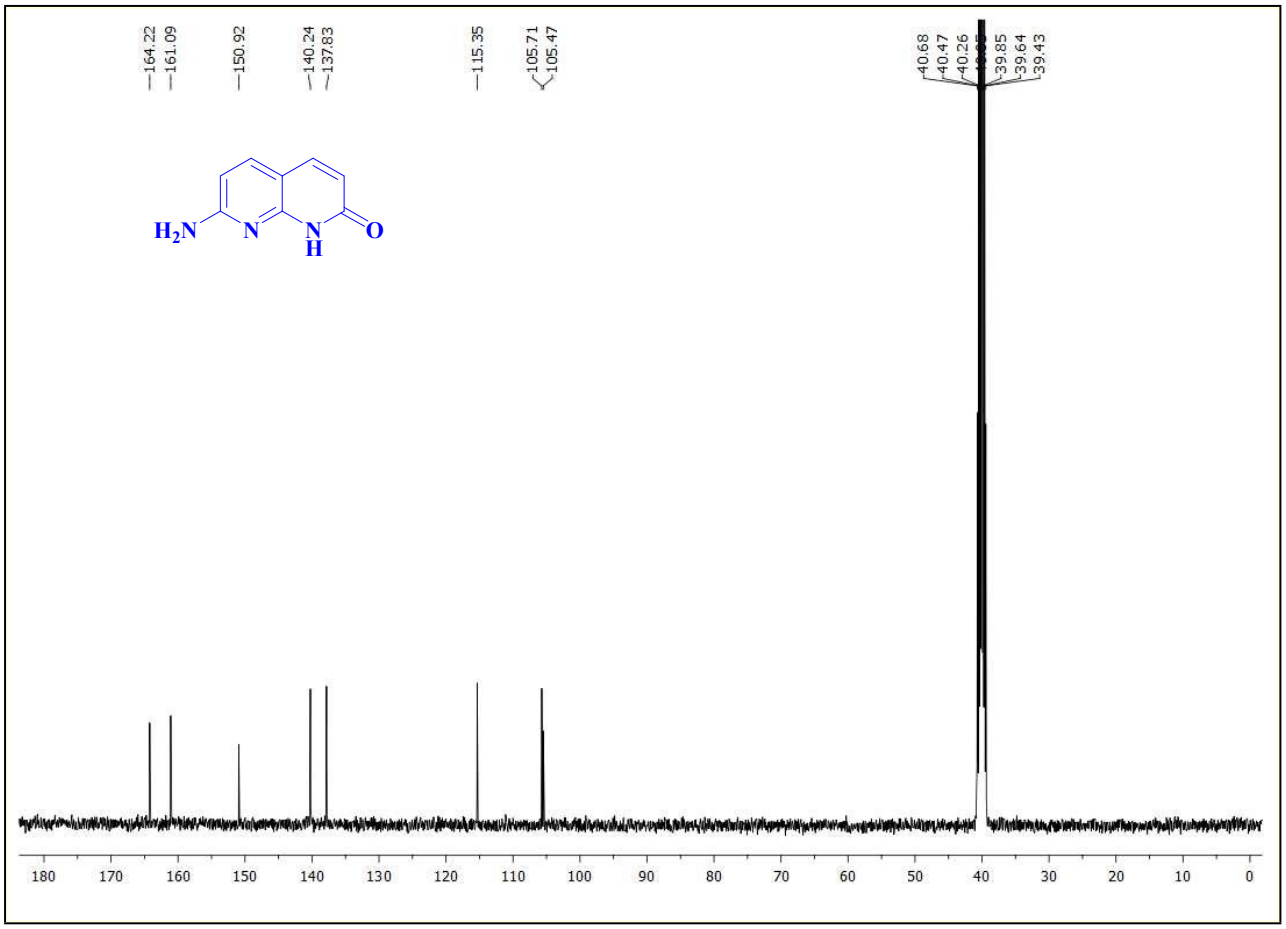
Table S1: ***Crystallographic data for*** **RNpd1**

	RNpd1
CCDC No.	**1420224**
Formula:	$C_{36}H_{35}N_5O_3$
Formula Weight:	585.6948
Crystal System:	Triclinic
Space group:	P -1 (2)
a, b, c [Å]:	16.9751(5), 20.3272(6), 120.5345(6)
α, β, γ (°):	α = 90.308°(2), β = 105.442 °(3), γ = 107.538 °(3)
V [Å]^3:	6484.0(4)
Z:	8
D(calc) [g/cm^3]:	1.001
μ(Mo-Kα) [mm^{-1}]:	0.078
F(000):	2464
Crystal Size [mm]:	0.16 x 0.14 x 0.10
Temperature (K):	293(2)
Radiation [Å]: Mo-Kα	0.71073
$\theta_{Min-Max}$ [°]:	2.712, 27.000
Dataset:	-21≤ h ≤ 21
	-17 ≤ k ≤ 25
	-25 ≤ l ≤ 26
Tot., Uniq. Data, R(int):	52977, 18598, 0.1358
Observed data [I > 2.0 σ(I)]	27747
Refinement: N_{ref}, N_{par}:	27747, 1585
R, wR2, S:	0.1032, 0.2939, 1.035
Max. & Av. Shift/Error:	0.014, 0.001
Min. & Max. Resd. Dens. [e/ Å^3]:	-0.355, 1.001.

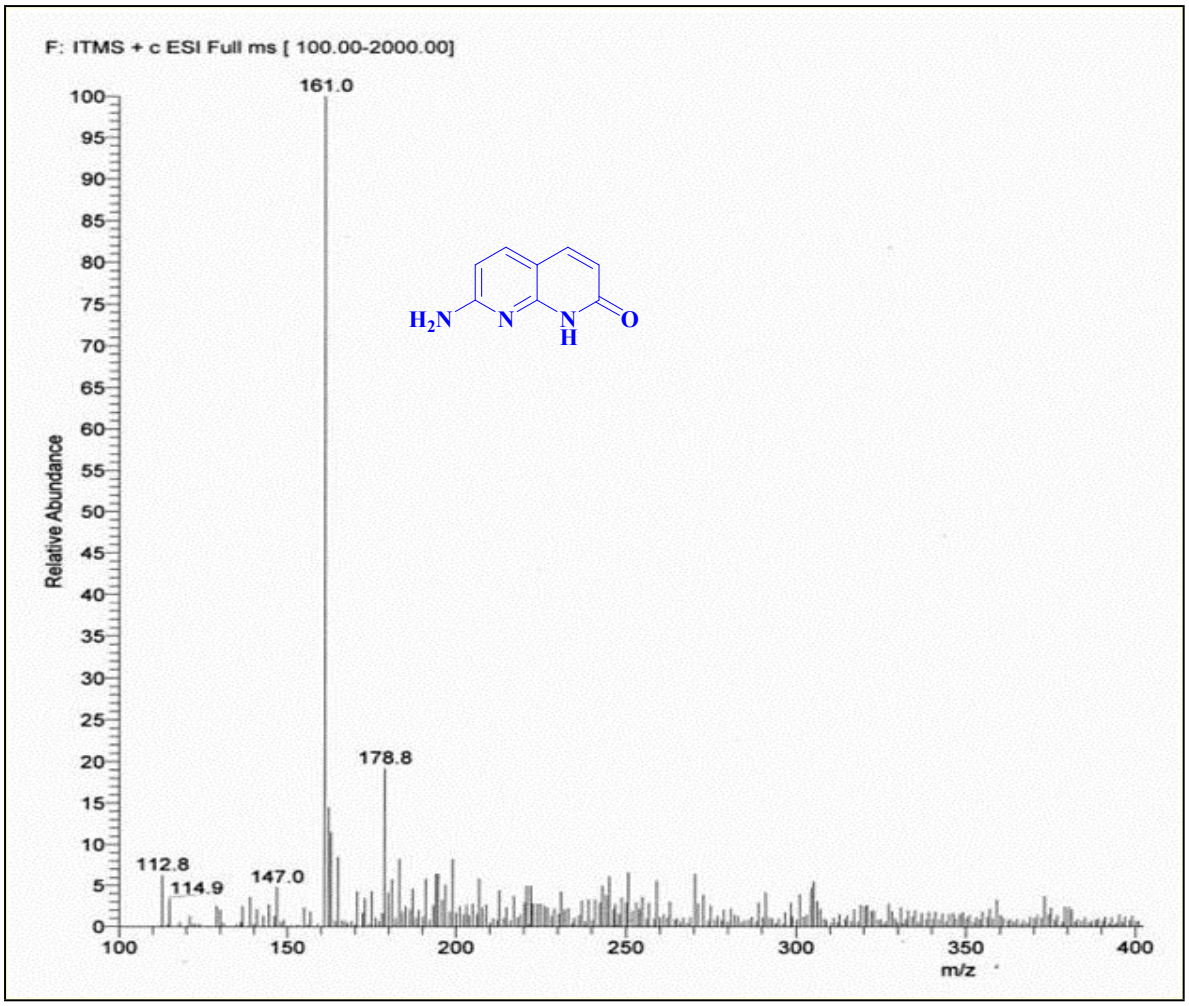
^{1}H-NMR spectrum of Comp A in d_6-DMSO



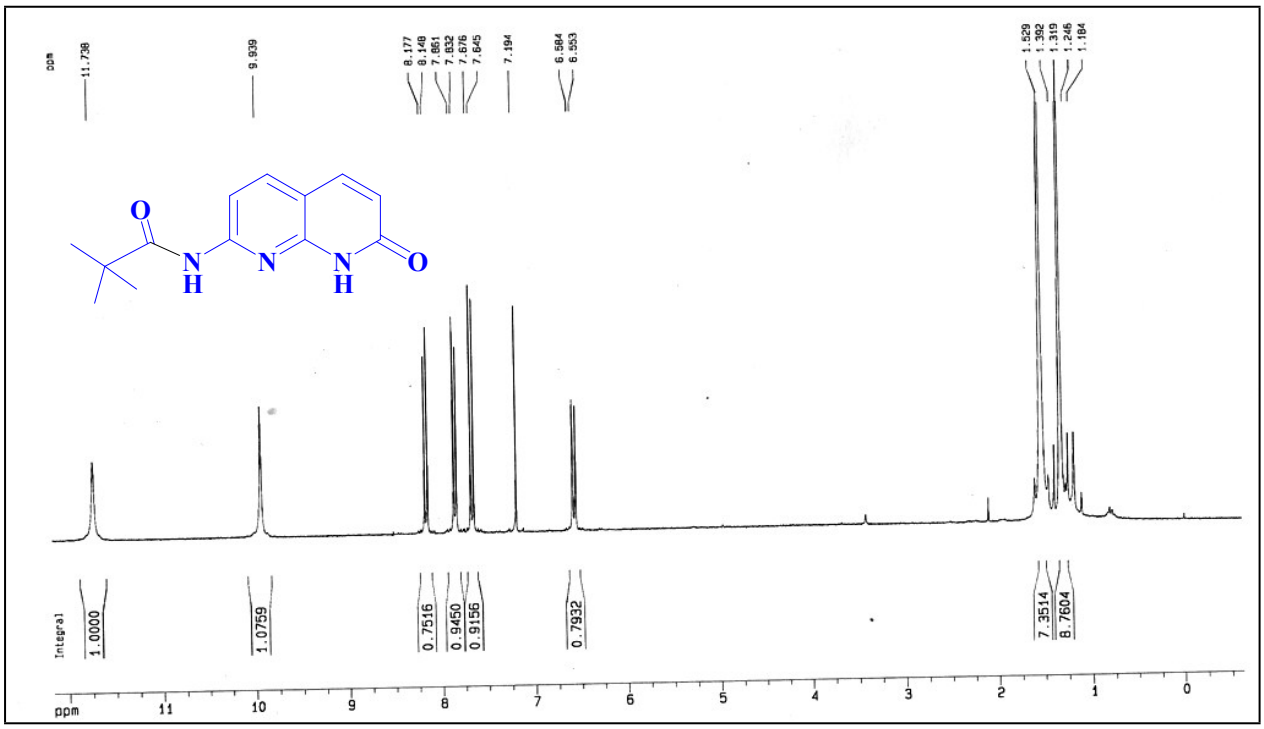
^{13}C-NMR spectrum of RNpd1 in $CDCl_3$



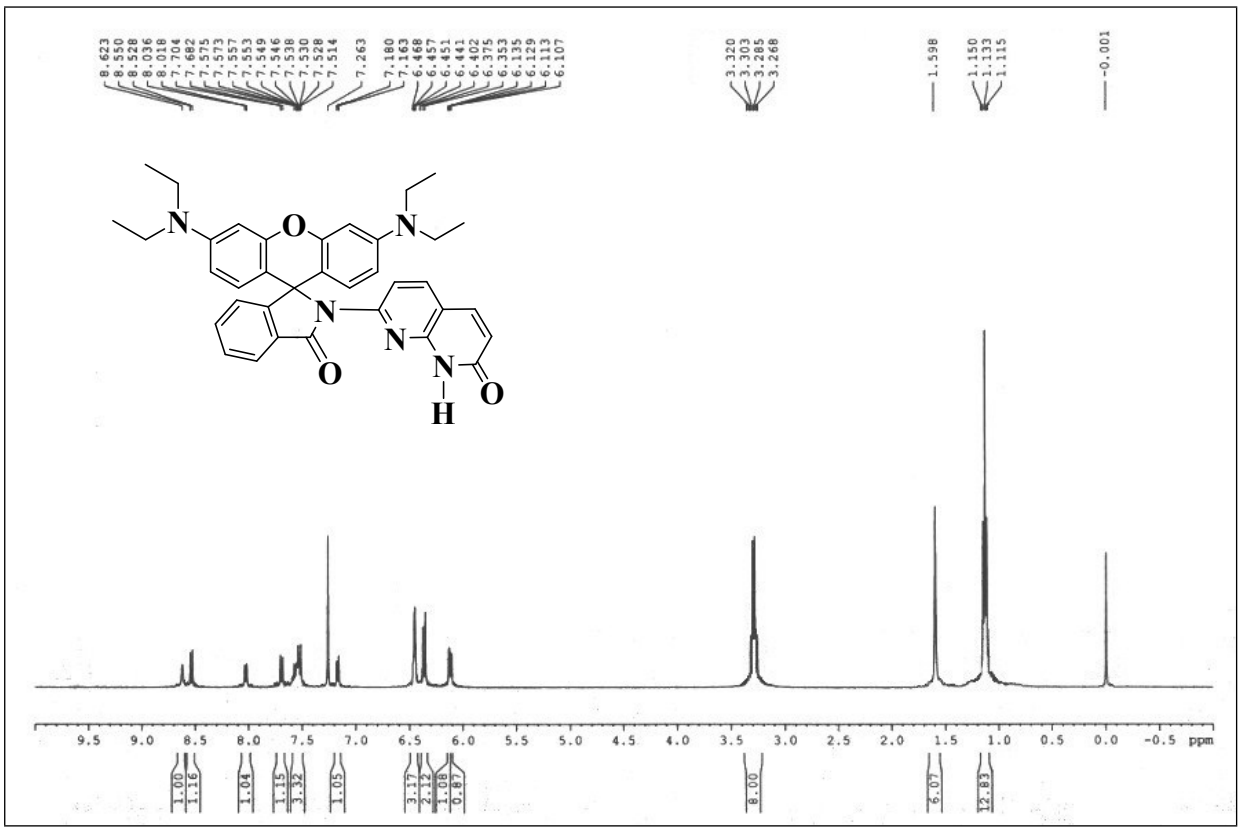
Mass Spectra of comp A



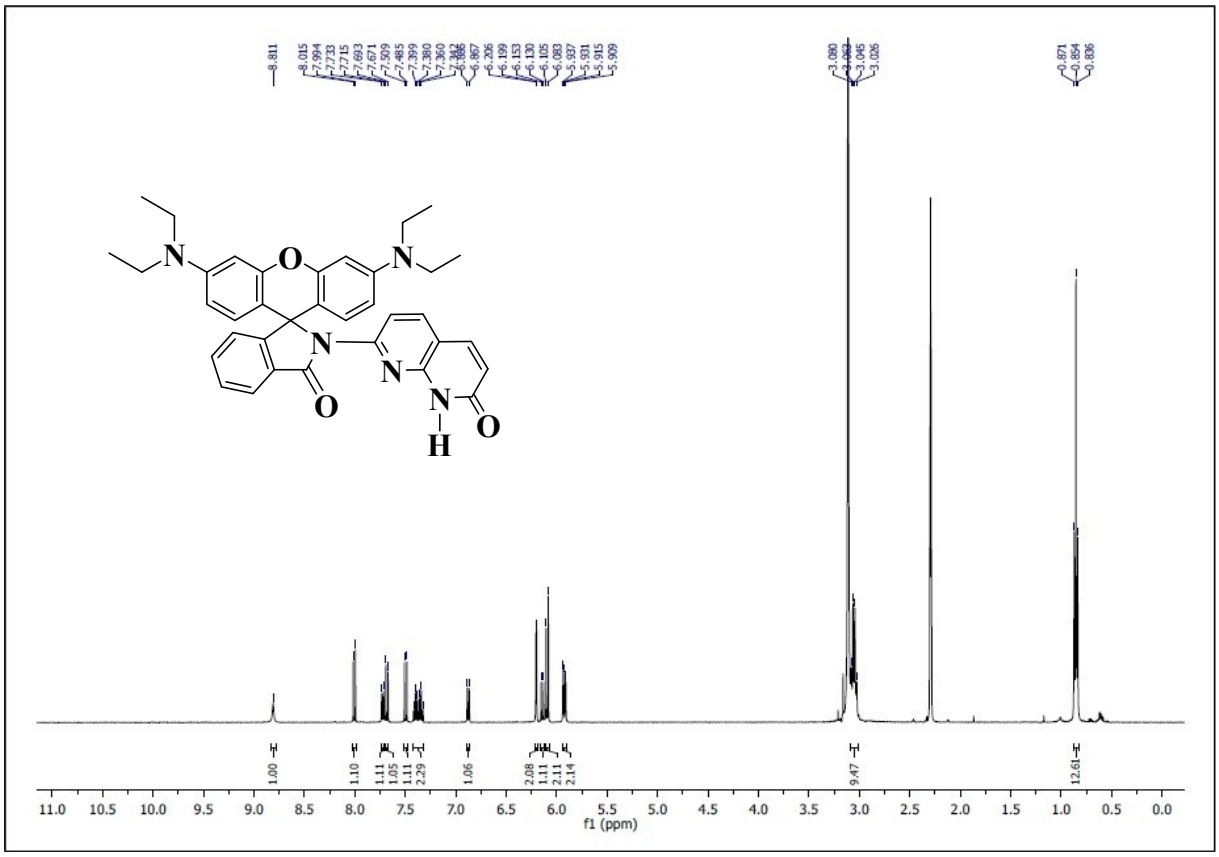
^{1}H-NMR spectrum of Comp B in $CDCl_3$:



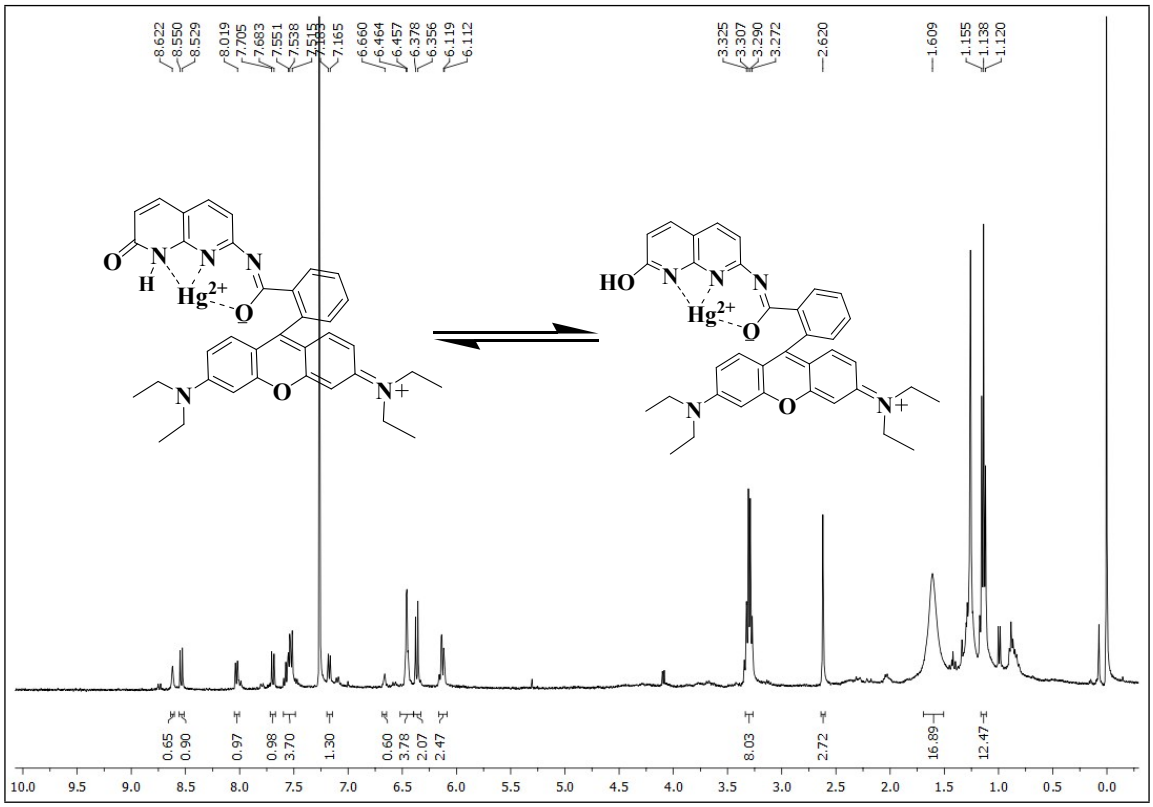
^{1}H-NMR spectrum of RNpd1 in $CDCl_3$



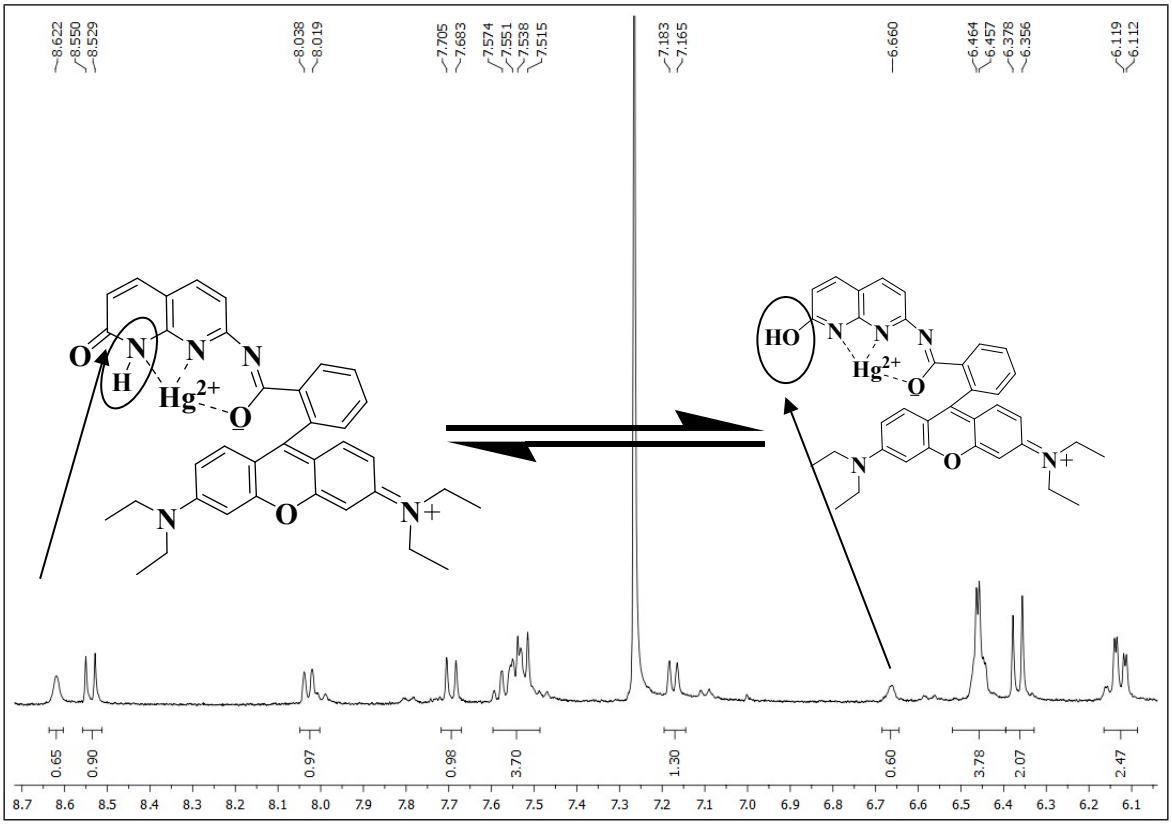
^{1}H-NMR spectrum of RNpd1 in d_6-DMSO



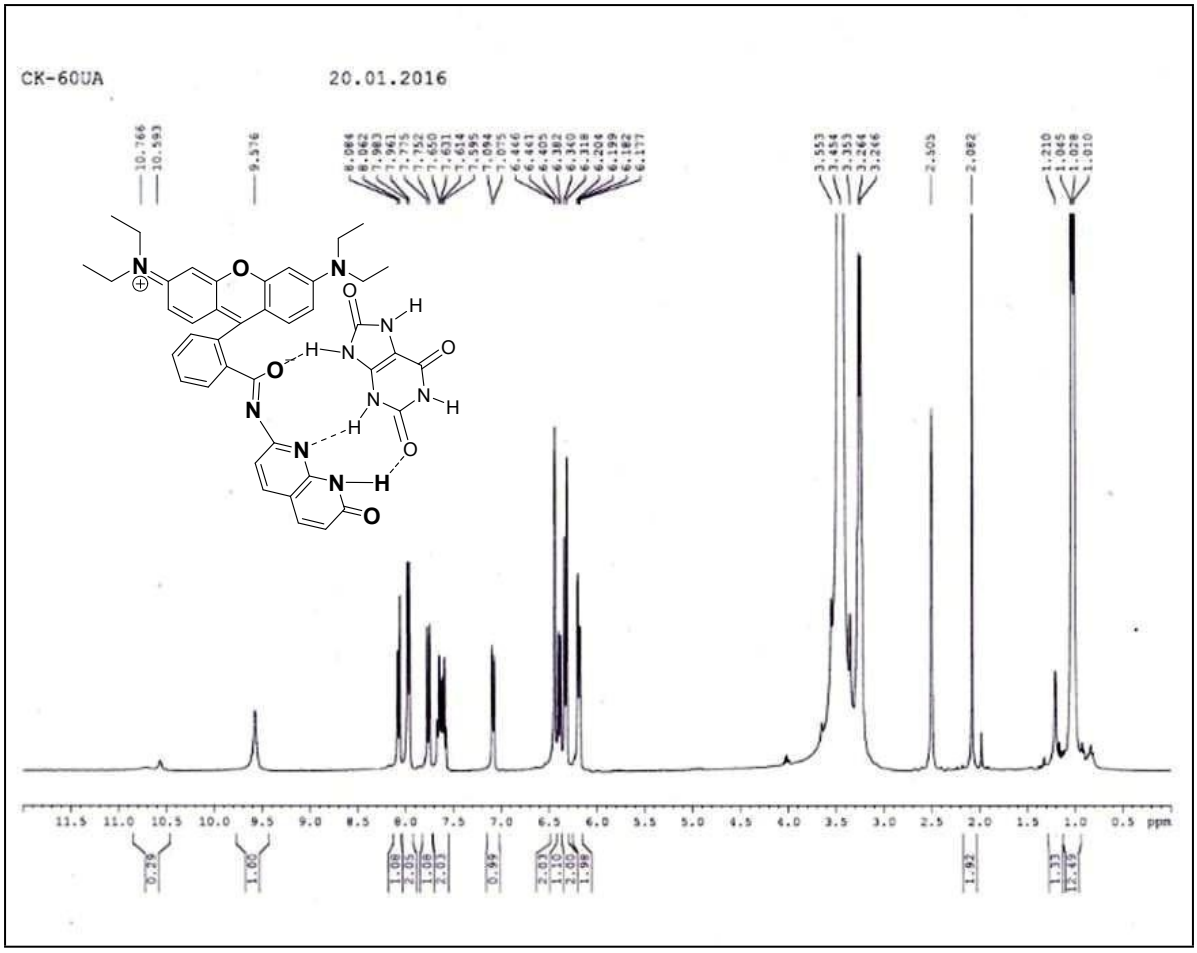
^{1}H-NMR spectrum of RNpd1:Hg^{2+} in $CDCl_3$



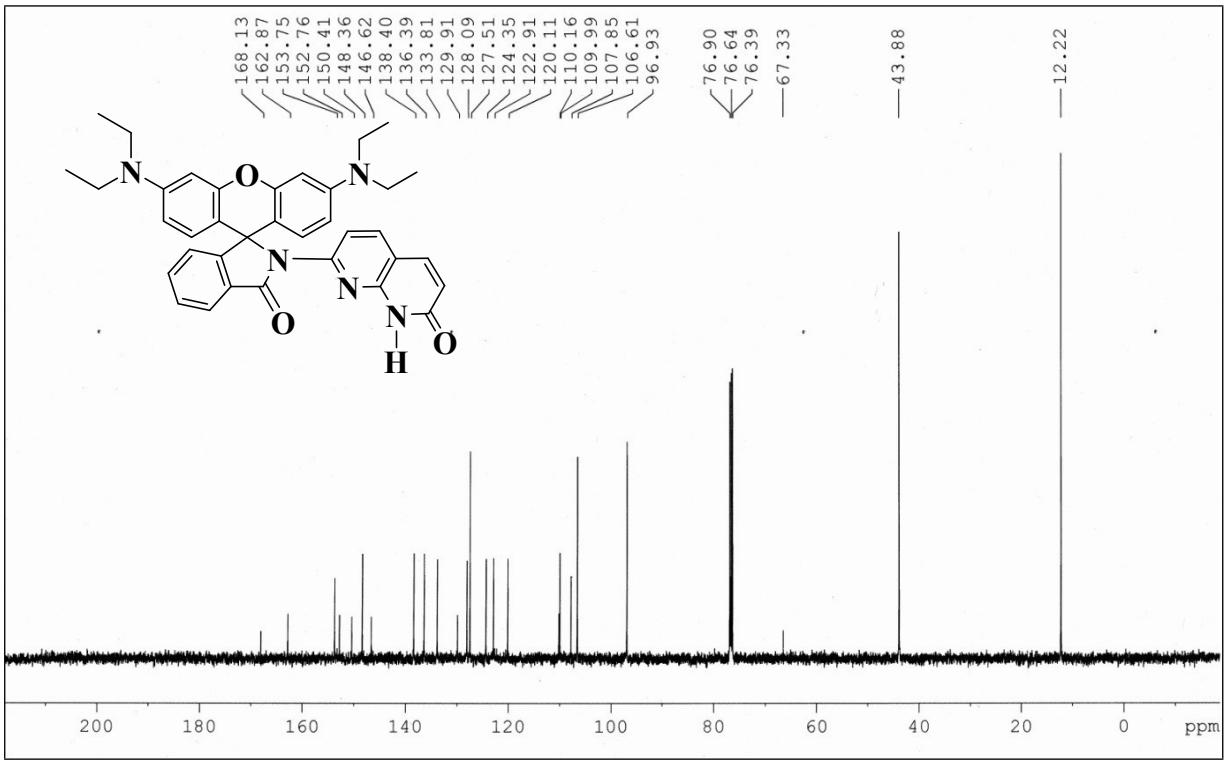
^{1}H-NMR spectrum of RNpd1:Hg^{2+} in $CDCl_3$ (Expansion) (Keto-enol tautomerism)



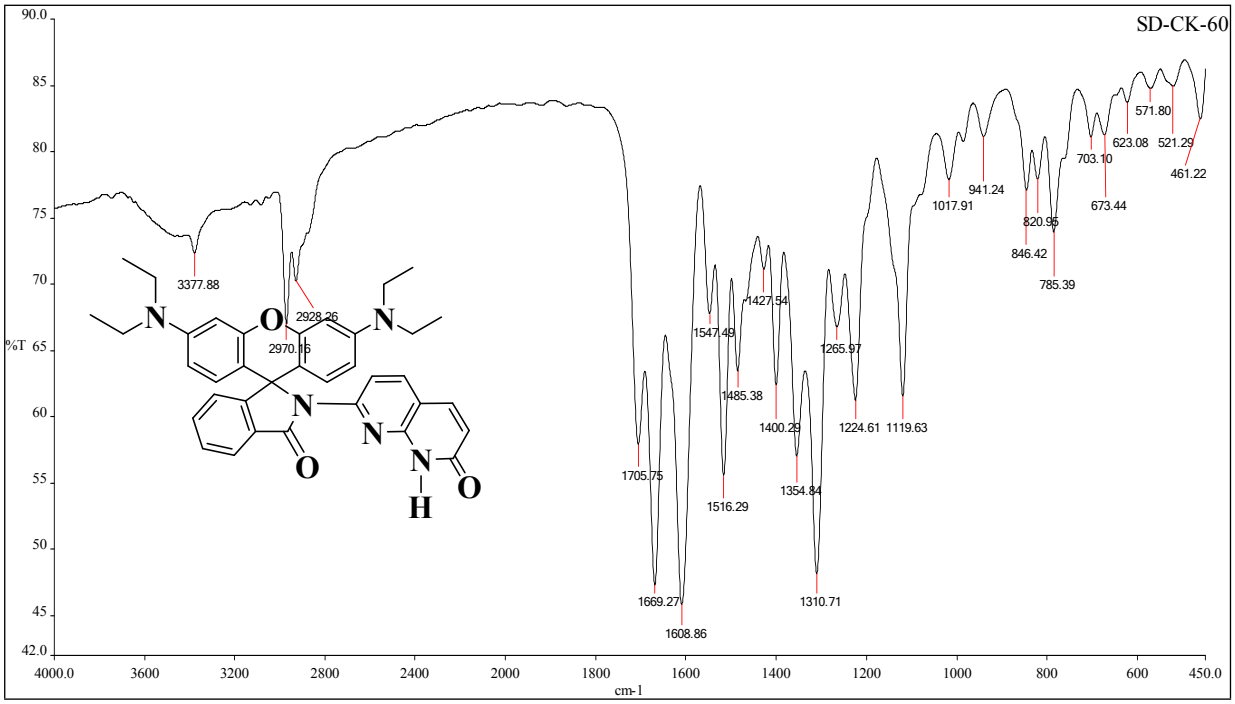
^{1}H-NMR spectrum of RNpd1:UA in d_6-DMSO



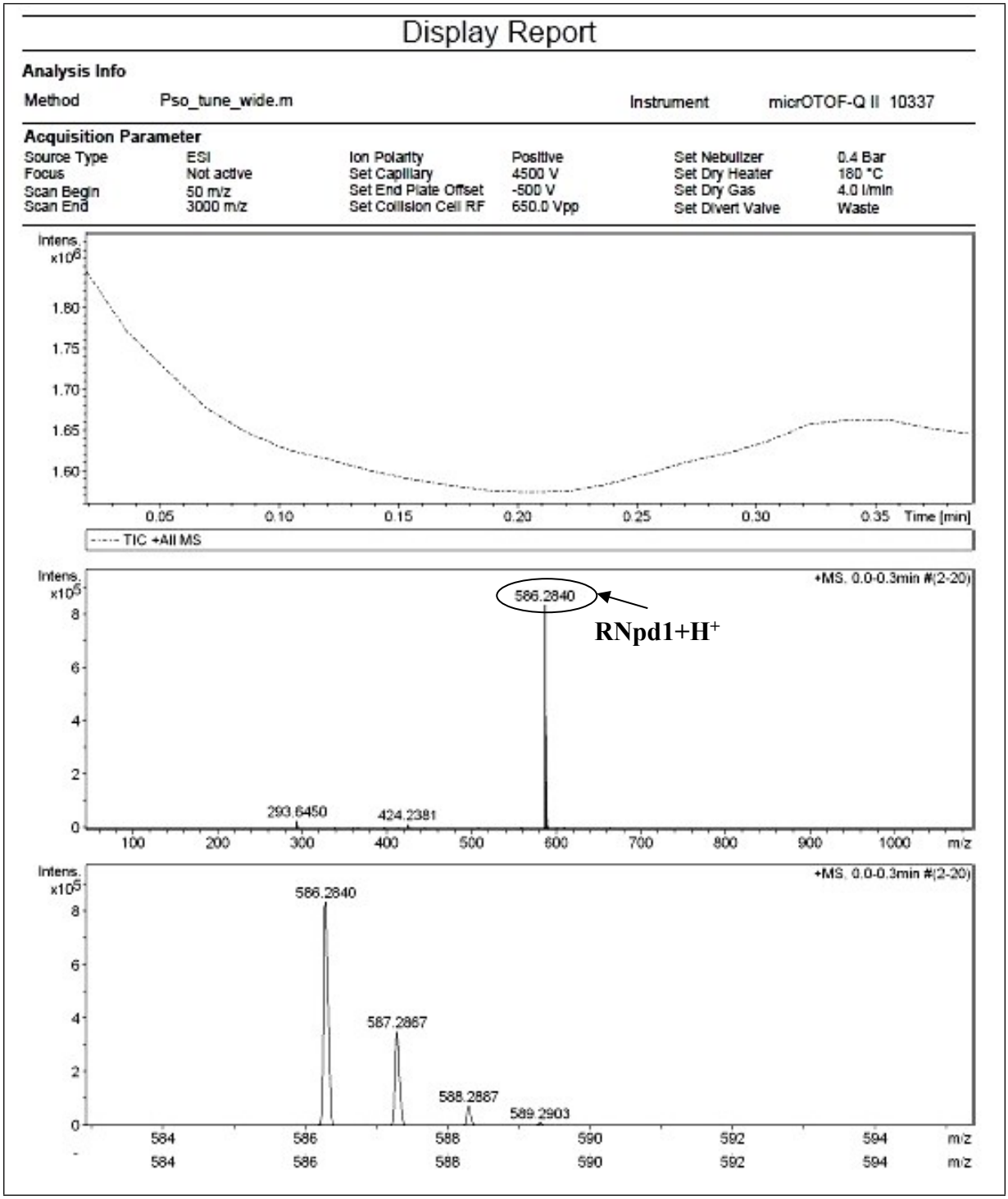
^{13}C-NMR spectrum of RNpd1 in $CDCl_3$



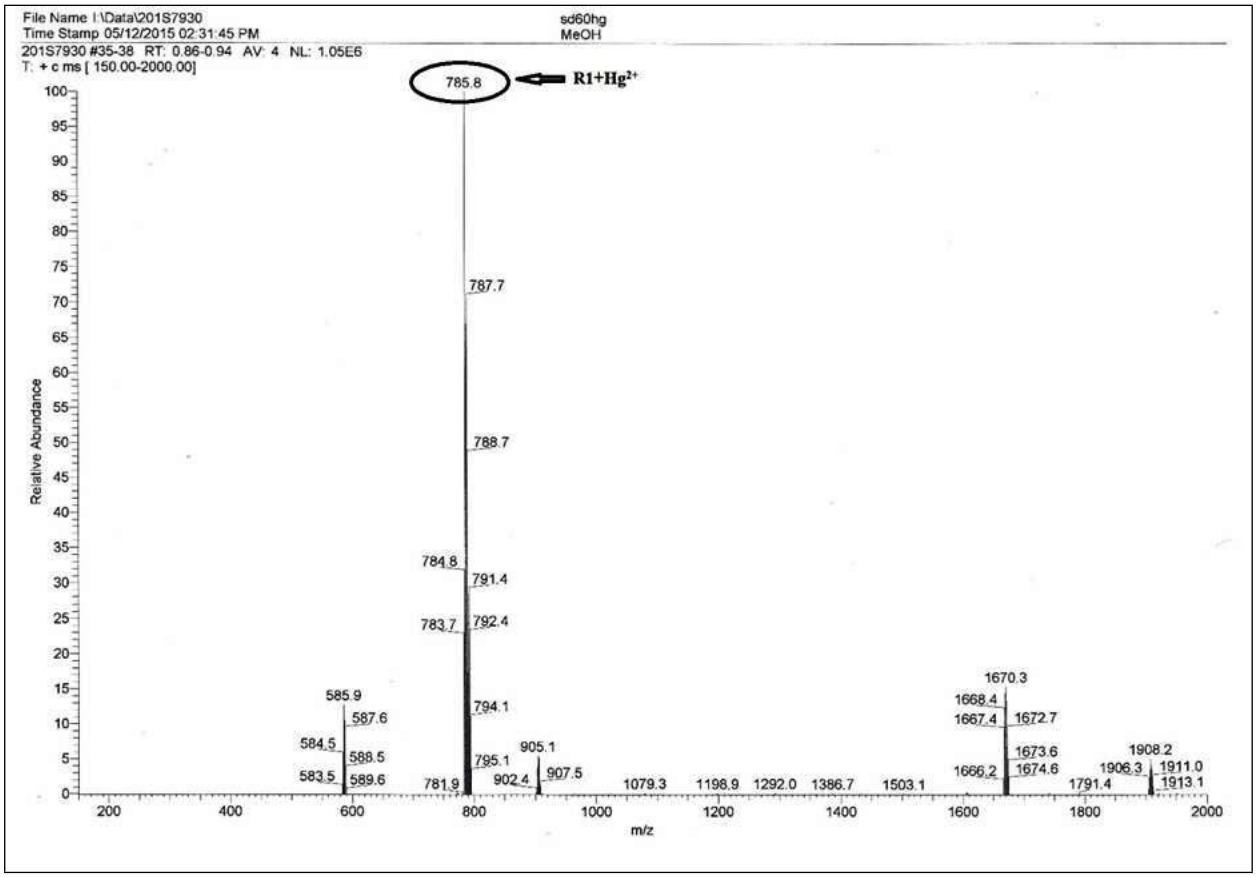
IR spectrum of RNpd1



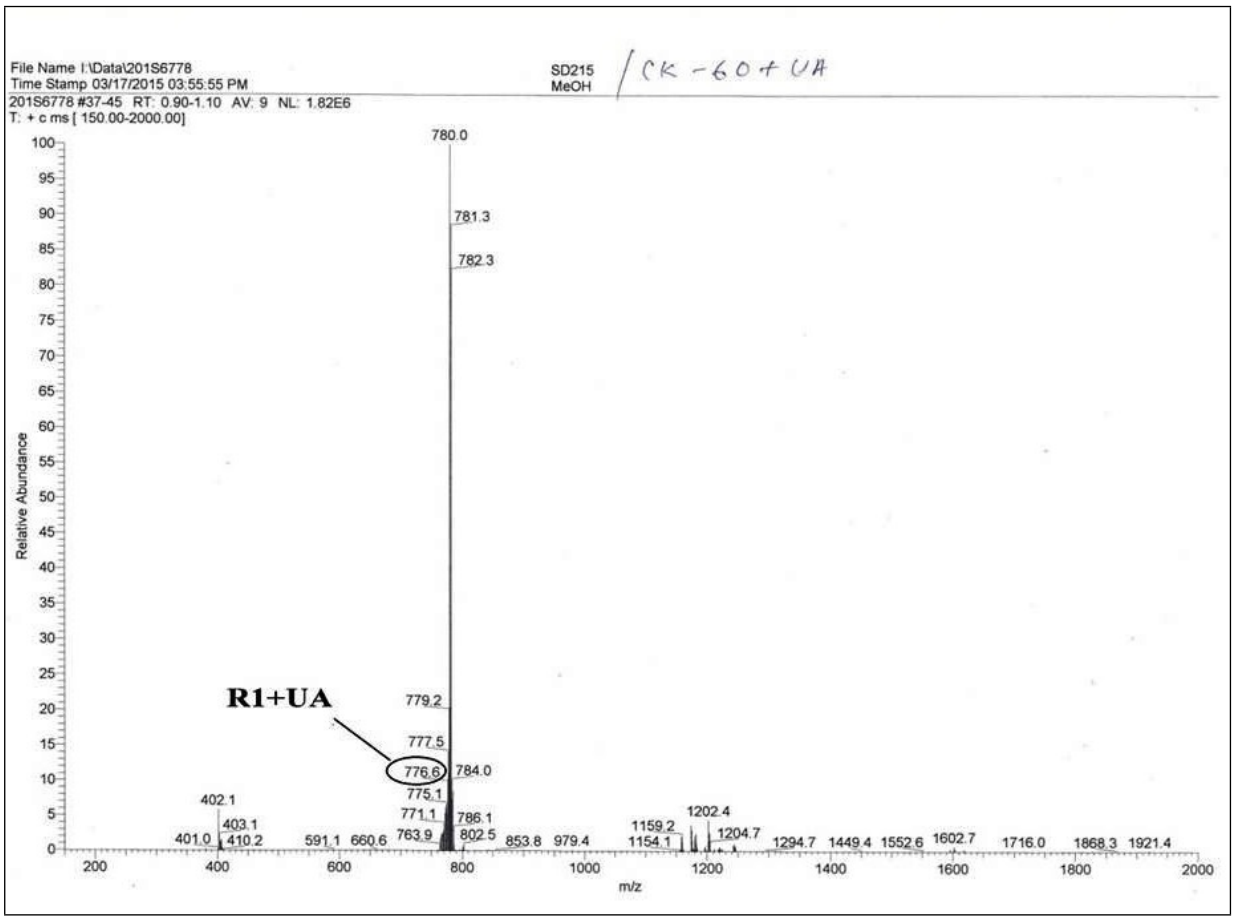
Mass spectrum of RNpd1



Mass spectrum of RNpd1:Hg^{2+} complex



Mass spectrum of RNpd1:UA complex



Visual change of RNpd1with different metal ions

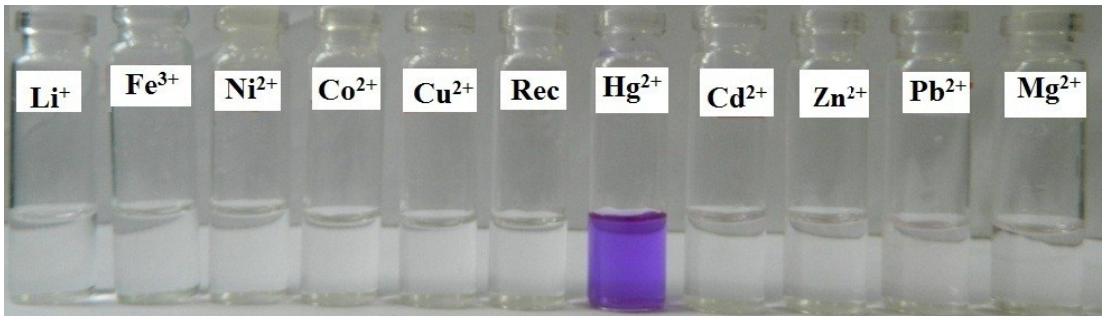


Fig. S2 Visual colour change of **RNpd1** (10 μM) with different metal ions (Li^{+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Mg^{2+} in pH 7.0 phosphate buffer/CH_3OH (1:2, v/v).

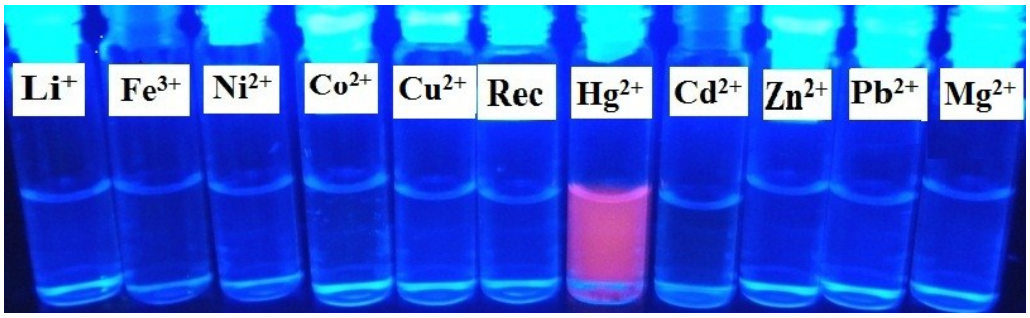


Fig. S3 Visual fluorescence change under long wavelength UV light of **RNpd1** (10μM) with different metal ions (Li^{+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Rec, Hg^{2+},Cd^{2+}, Zn^{2+}, Pb^{2+}, Mg^{2+}) in pH 7.0 phosphate buffer/CH_3OH (1:2, v/v).

Binding constant calculation by UV-Vis and fluorescence titration method using linear regreation analysis

The reciprocal of intensity difference ($1/\Delta I$), where $\Delta I = (I - I_0)$, was plotted against the reciprocal of concentration of guest ($1/[G]$) for the calculation of association constant (K_a) using Benesi-Hildebrand linear regression analysis following equation (i) where association constant K_a = intercept/slope.

$$1/(I - I_0) = 1/(I_\infty - I_0)K_a[G] + 1/(I_\infty - I_0) \quad \text{..... eqn. (i)}$$

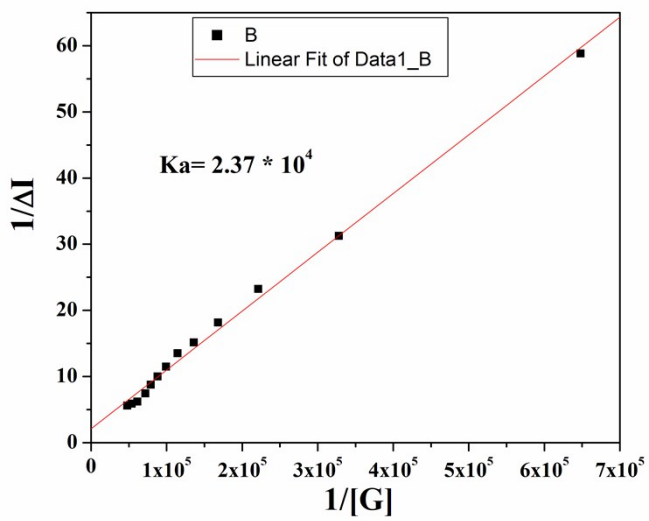


Fig. S4 Binding constant calculation graph of **RNpd1** with Hg^{2+} using UV-vis titration.

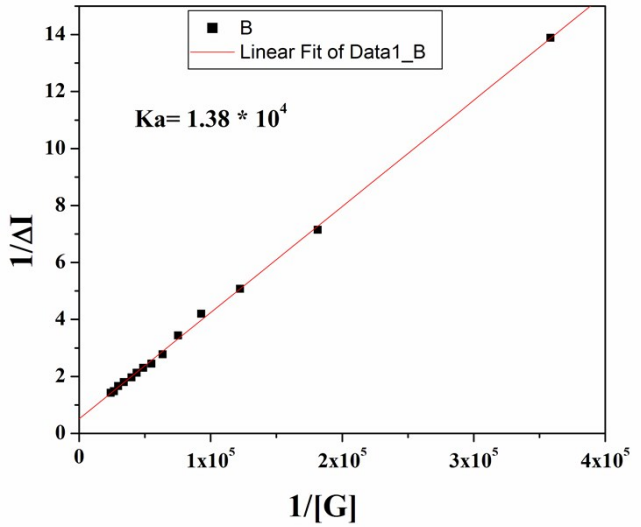


Fig. S5 Binding constant calculation graph of **RNpd1** with **UA** using UV-vis titration.

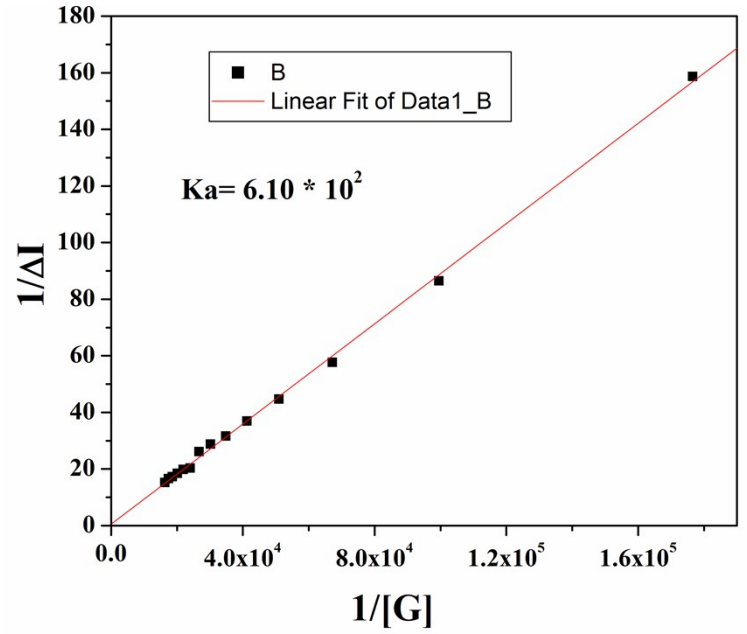


Fig. S6 Binding constant calculation graph of **RNpd1** with **adenine** using UV-vis titration.

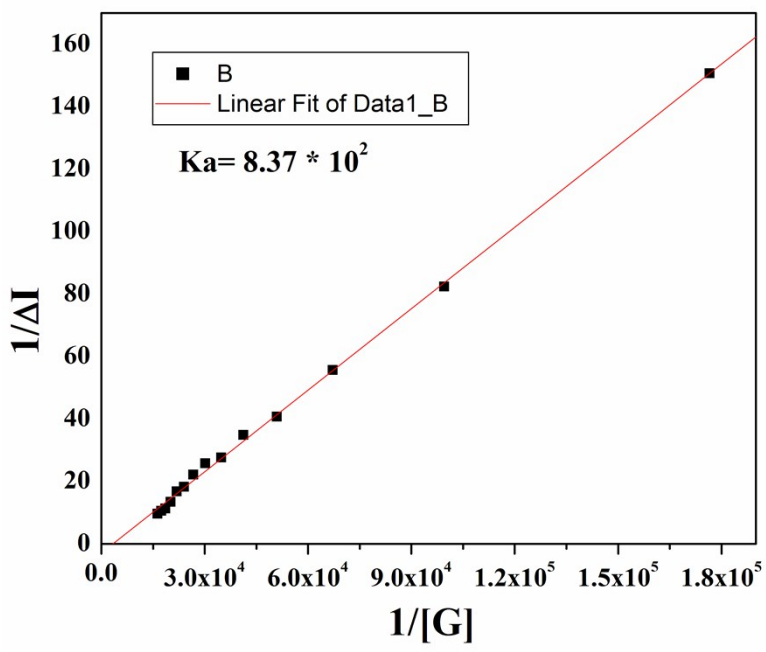


Fig. S7 Binding constant calculation graph of **RNpd1** with **guanine** using UV-vis titration.

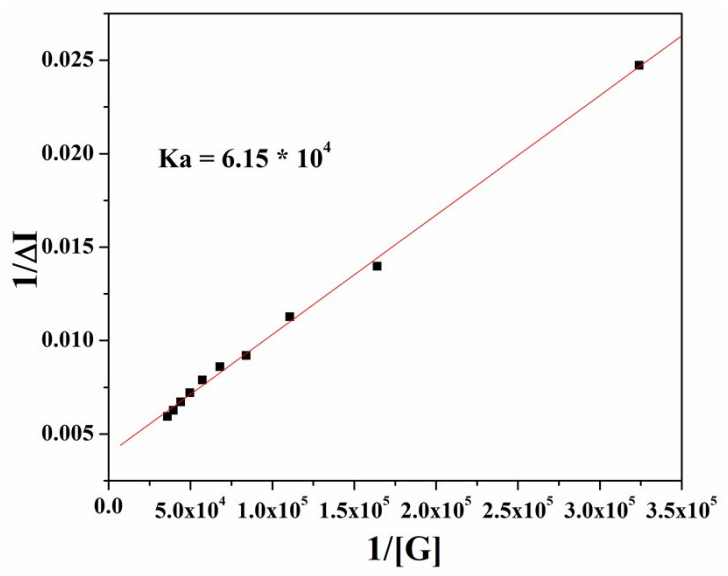


Fig. S8 Binding constant calculation graph of **RNpd1** with **Hg^{2+}** using fluorescence titration.

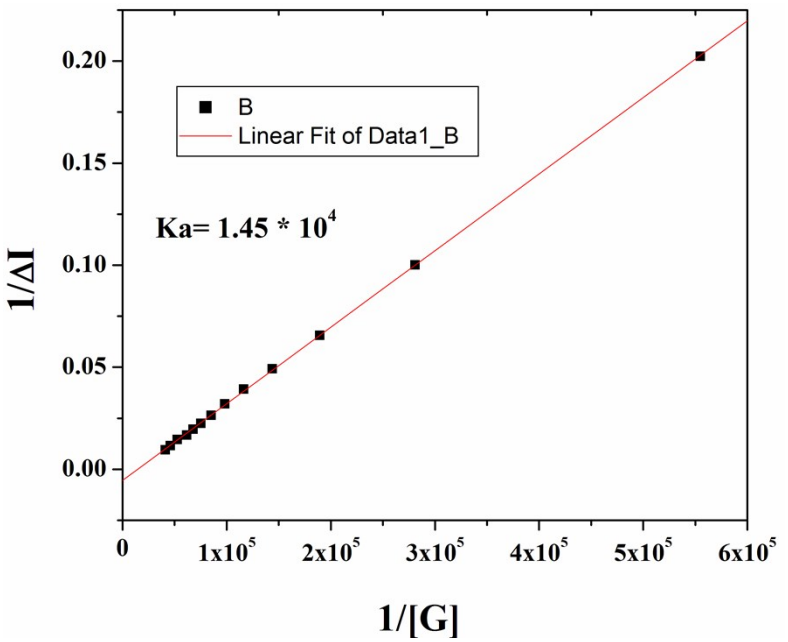


Fig. S9 Binding constant calculation graph of **RNpd1** with **UA** using fluorescence titration.

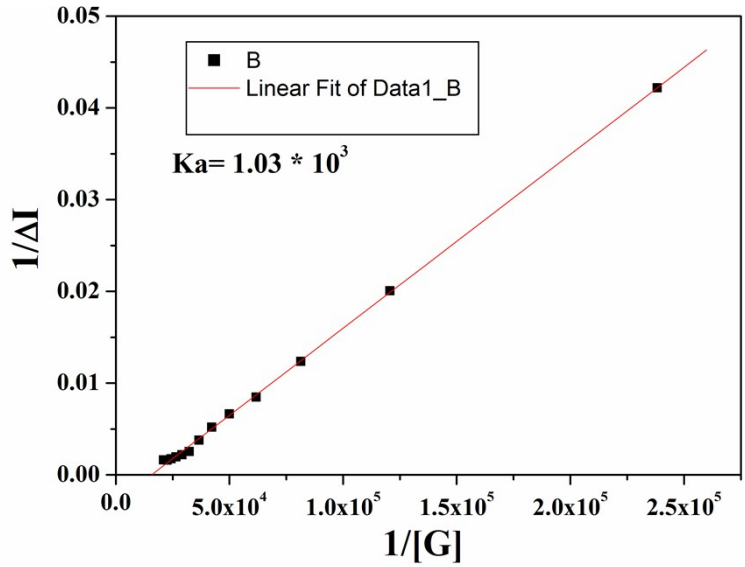


Fig. S10 Binding constant calculation graph of **RNpd1** with adenine using fluorescence titration.

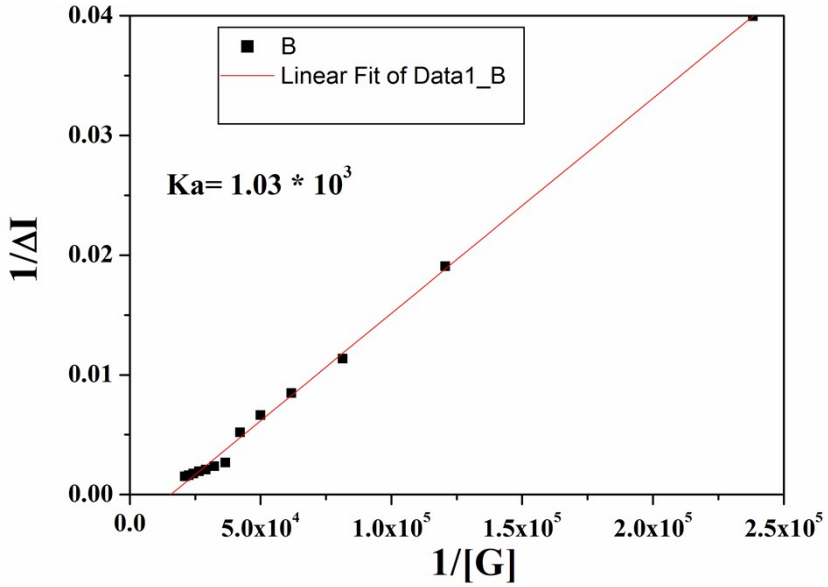


Fig. S11 Binding constant calculation graph of **RNpd1** with guanine using fluorescence titration.

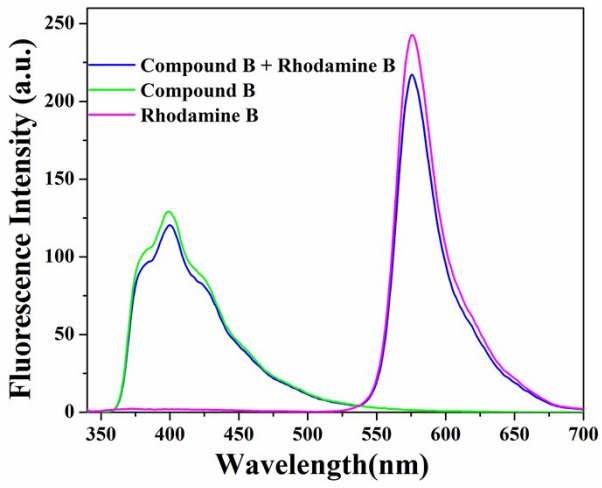


Fig. S12 Comparision of Fluorescence specctra of comp B and Rhodamine B in pH 7.0 phosphate buffer/CH_3OH (1:2, v/v).

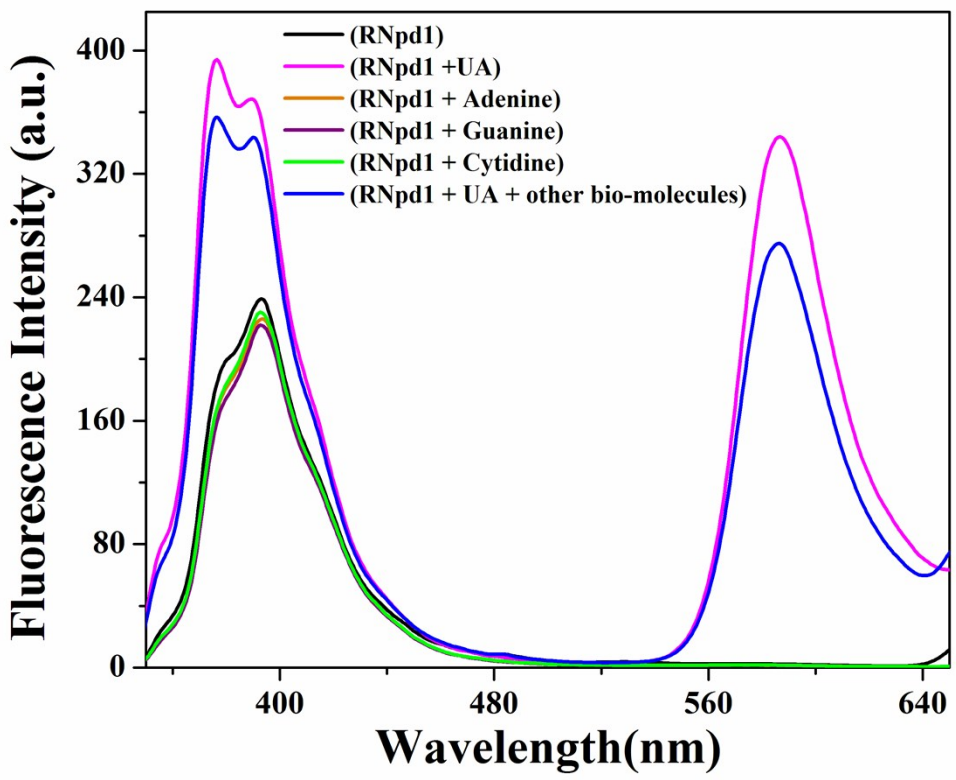


Fig. S13 Competitive Fluorescence specctra of RNpd1 with UA and other biomolecules in pH 7.0 phosphate buffer/CH_3OH (1:2, v/v).

Quantum yield calculation:

Fluorescence quantum yields (Φ) were calculated using Equation given below (1) (Wu, D.; Huang, W.; Duan, C.; Lin, Z.; Meng, Q. *Inorg. Chem.* **2007**, *46*, 1538) using Rhodamine B ($\Phi_f = 0.49$ in ethanol) as standards.

$$\Phi_u = \Phi_s \times \frac{I_u}{I_s} \times \frac{A_s}{A_u} \times \left(\frac{\eta_u}{\eta_s}\right)^2$$

Where Φ_u and Φ_s are the fluorescence quantum yields of the sample and standard, I_u and I_s are the integrated emission intensities of the sample and standard, A_u and A_s are the absorbance of the sample and standard at the excitation wavelength (568 nm), and η_u and η_s are the refractive indices of the sample and standard solutions, respectively.

Table S2: Quantum yield calculation and molar extinction coefficient value

	λ_{max} (Absorption)	λ_{max} (Emission)	Quantum Yield	Molar Extinction coefficient
RNpd1	328	380	0.017	3.26
RNpd1:Hg^{2+}	568	617	0.353	5.86
RNpd1:UA	561	582	0.251	4.95

Calculation of limit of detection (LOD):

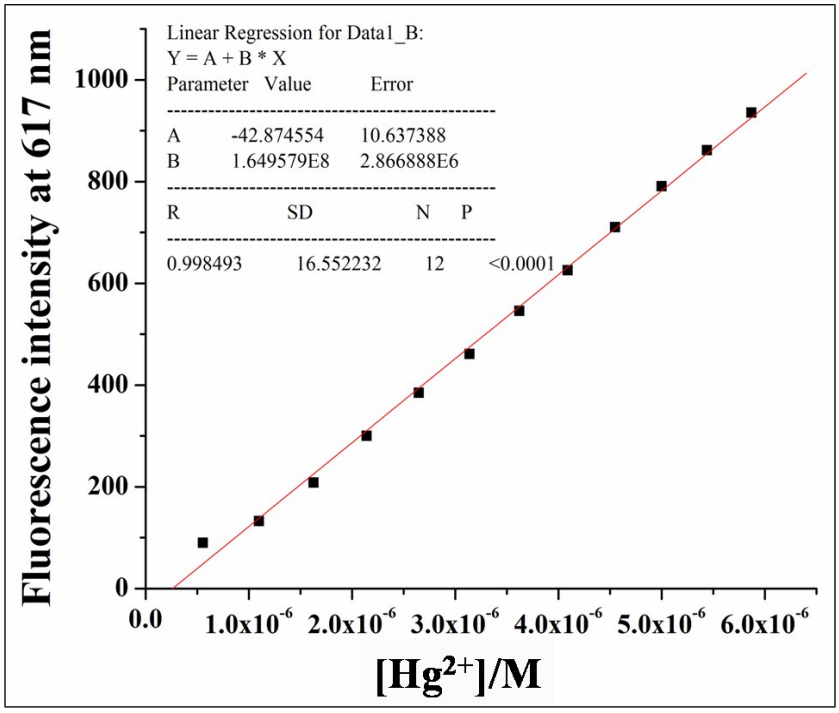
The detection limit of **RNpd1** with **Hg^{2+}** and **UA** were calculated on the basis of fluorescence titration data. To determine the standard deviation for the fluorescence intensity, the emission intensity of the individual receptors without any anion was measured by 10 times and the standard deviation of blank measurements was calculated. The limit of detection (LOD) of the receptor for sensing **Hg^{2+}** and **UA** were determined from the following equation:

LOD = K × SD/S

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor (**RNpd1**) solution; S is the slope of the calibration curve.

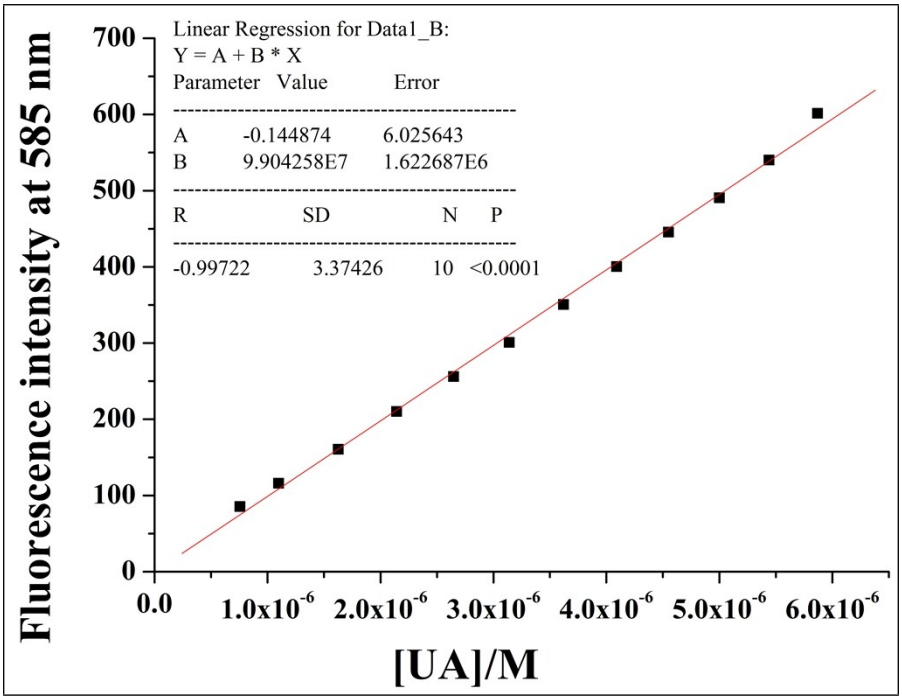
For RNpd1 with Hg^{2+}:

From the linear fit graph we get slope = 1.64×10^{8}, and SD value is 0.51. Thus using the above formula we get the Limit of Detection = 9.32×10^{-9} M i.e. **RNpd1** can detect **Hg^{2+}** up to this very lower concentration by fluorescence techniques.



For RNpd1 with UA:

From the linear fit graph we get slope = 9.90×10^{7}, and SD value is 0.51. Thus using the above formula we get the Limit of Detection = 1.54×10^{-8} M i.e. **RNpd1** can detect **UA** up to this very lower concentration by fluorescence techniques.



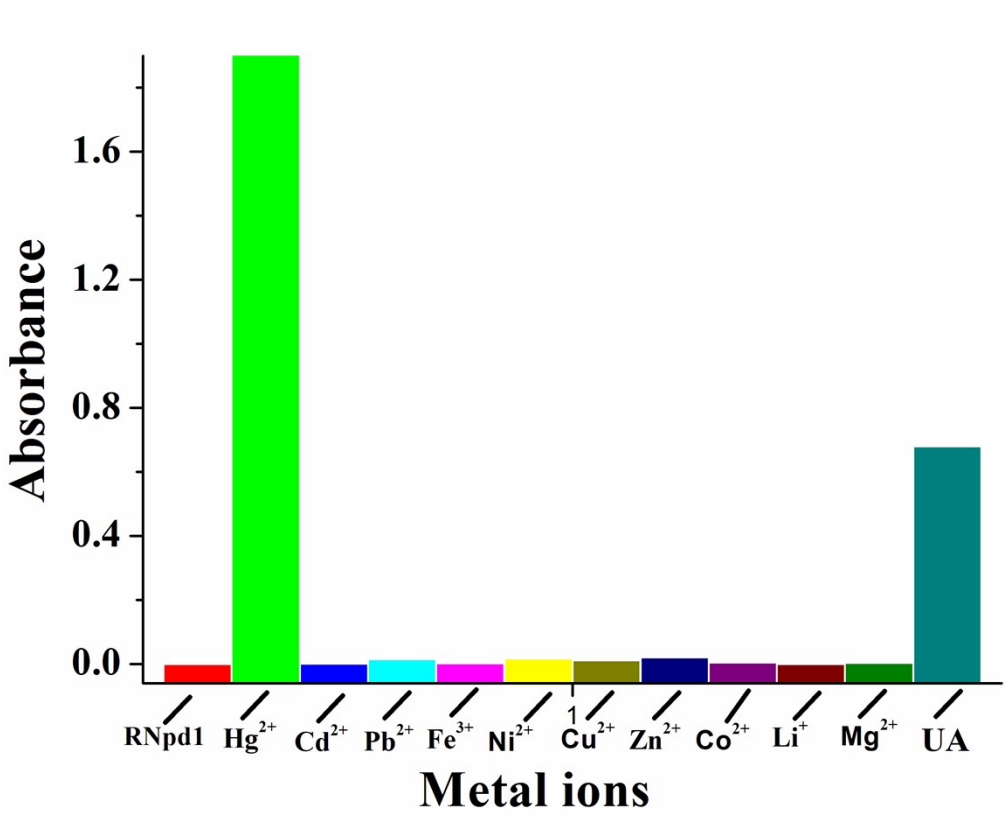


Fig. S14 Bar diagram of RNpd1 with different metal ions and Uric acid in pH 7.0 phosphate buffer/CH_3OH (1:2, v/v).

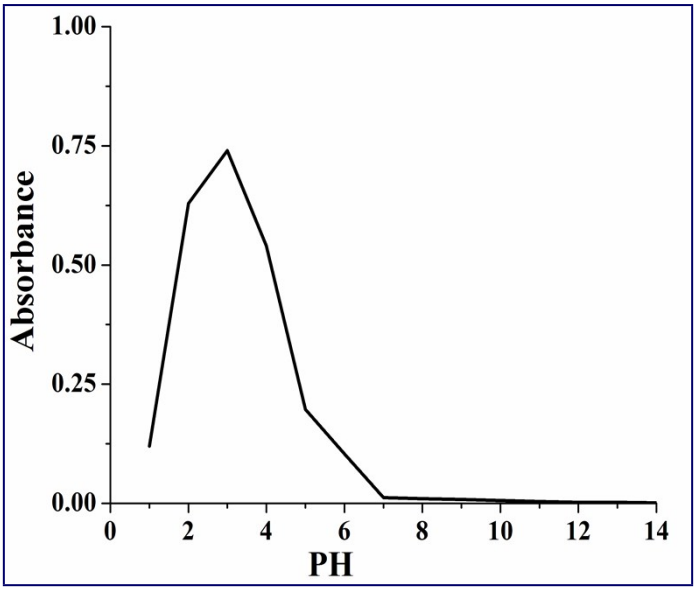


Fig. S15 Influence of pH in absorbance of **RNpd1** (558 nm) in pH 7.0 phosphate buffer/CH_3OH (1:2, v/v).

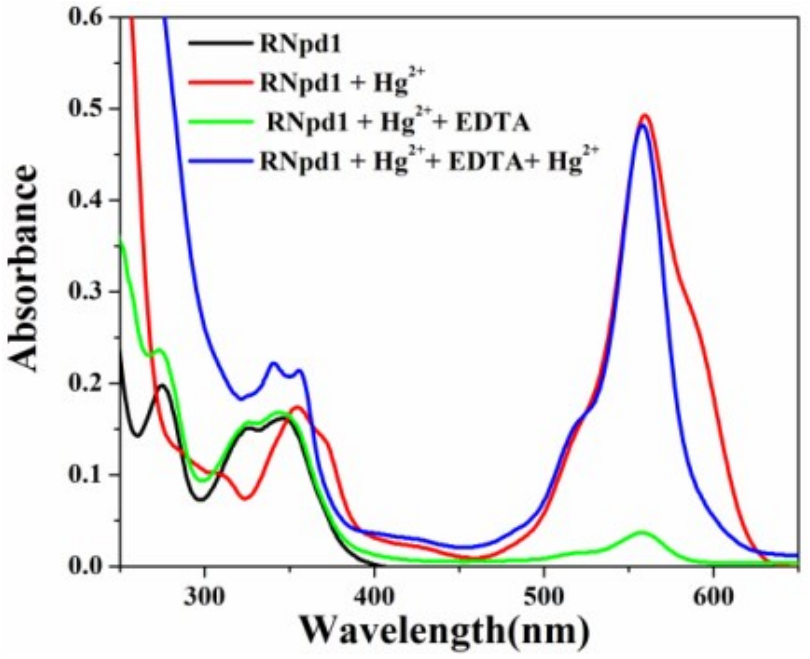


Fig. S16 Reversibility of **RNpd1:Hg^{2+}**complex with EDTA in pH 7.0 phosphate buffer/CH_3OH (1:2, v/v).