

Supporting Information

[Ru(η^6 -*p*-cymene)(N[^]O 8-hydroxy quinoline)(pta)] complex as a rising star in medicinal chemistry: synthesis, properties, biomolecular interaction, *in vitro* anti-tumor activity in human brain carcinomas and *in vivo* biodistribution and toxicity in zebrafish model †

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Table S1 Photophysical characterization, solubility, lipophilicity and conductivity study of RAPTA complexes

Complexes	λ_{max} (nm) ^a	λ_{f} (nm) ^b	Stoke's shift	O.D ^c	ϵ (M ⁻¹ cm ⁻¹) ^d	(ϕ_{f}) ^e	Solubility (M) ^f	log P ^g	Λ_{M} ^h (Scm ² mol ⁻¹)	
									DMSO	10% DMSO
[RuL1P TA]	265, 300, 400	375	110, 75	0.03	1000	0.18	0.041	0.45±0.04	120	124
[RuL2P TA]	270, 325, 425	370	100, 45	0.03	1000	0.08	0.044	0.69±0.05	127	128
[RuL3P TA]	280, 350, 425	380	100, 30	0.025	833	0.45	0.046	0.87±0.03	131	134
[RuL4P TA]	275, 350	375	100, 25	0.01	333	0.27	0.045	1.1±0.01	130	132
[RuL5P TA]	285, 340, 425	380	95, 40	0.02	666	0.48	0.052	0.94±0.07	136	138
Cisplatin	-	-	-	-	-	-	0.019	-	36	207
Quinine Sulphate	352	450	98	0.09	3000	0.546	-	-	-	-

^aabsorption maxima, ^bmaximum emission wavelength (λ_{exc} 325 nm), ^coptical density, ^dextinction coefficient, ^equantum yield, ^fDMSO-10% DMEM medium (1:99 v/v, comparable to cell media), ^g Partition Coefficients in n-Octanol/Water, ^hconductance in DMSO and 10% DMSO (3×10^{-5} M)

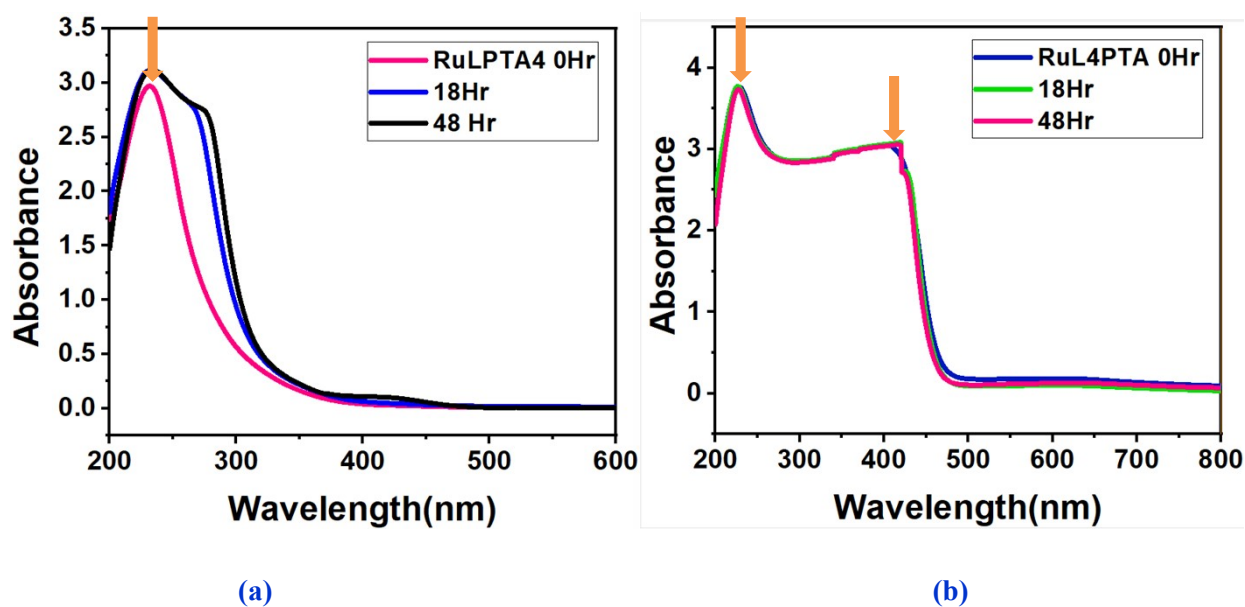


Fig. S1 Stability study of selected complex [RuL4PTA] in (a) GSH (1 mM) (b) MTT Media

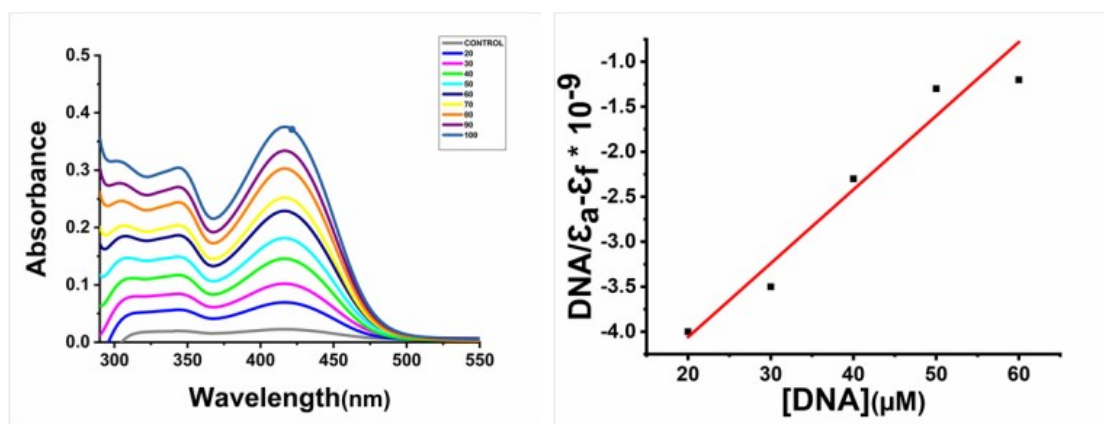


Fig. S2 UV-Visible Spectral pattern of [RuL3PTA] in Tris-HCl-NaCl solution (5 mM) of pH=7.2 with increased concentration of Ct-DNA

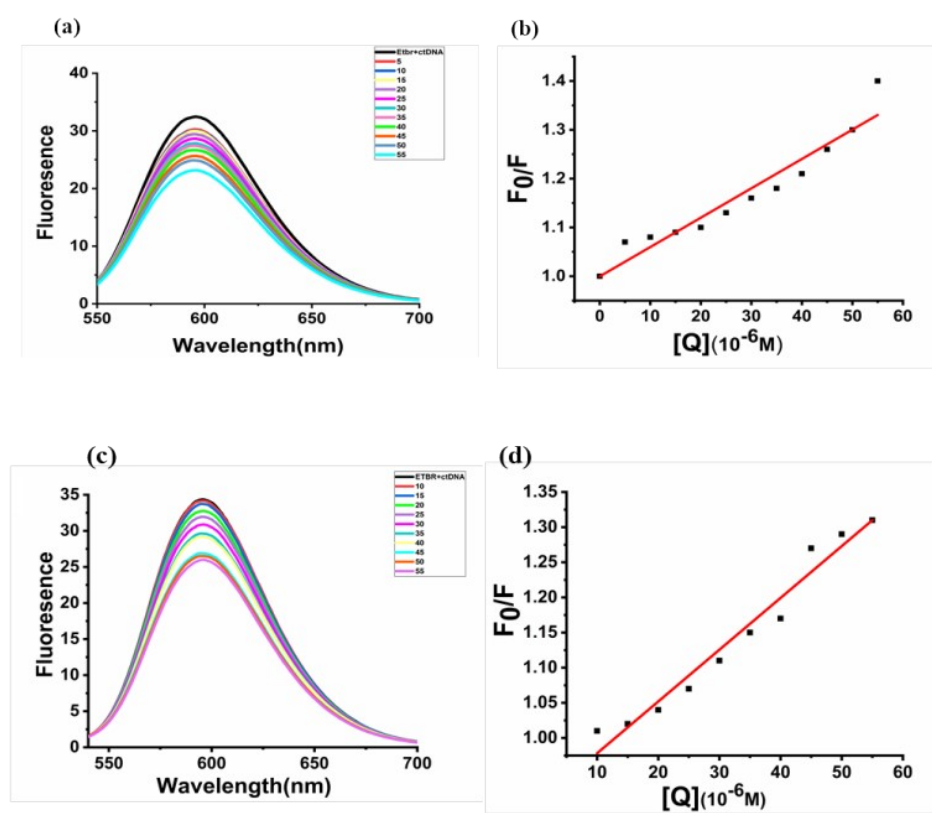


Fig. S3 Fluorescence spectral responses of EtBr bound DNA in the Presence of complexes [RuL3PTA] and [RuL4PTA] in 5 mM Tris-HCl /NaCl buffer of pH 7.2

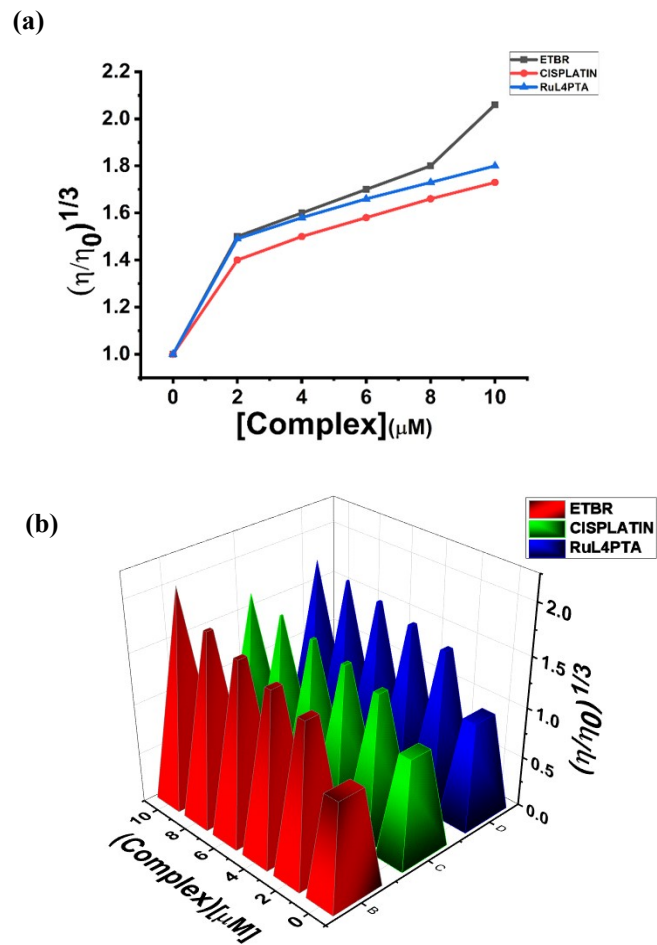


Fig. S4 Effect of increasing amounts of complex, EtBr, and cisplatin on the Viscosity of Ct-DNA at 298 K ($[\text{EtBr}] = 1 \times 10^{-6} \text{ mol/L}$; $[\text{DNA}] = 1 \times 10^{-6} \text{ mol/L}$; $[\text{ligand}] = 1 \times 10^{-3} \text{ mol/L}$)

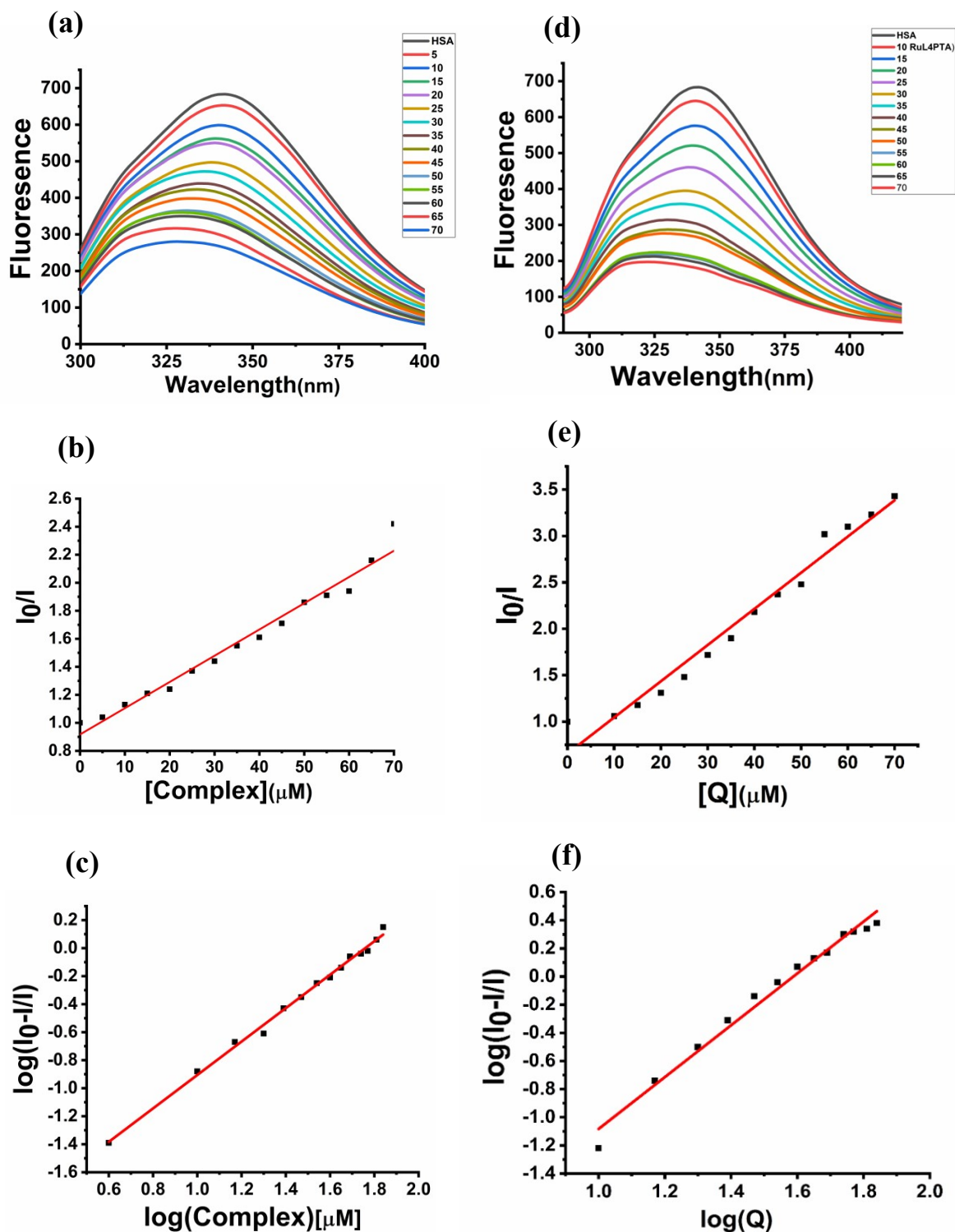


Fig. S5 Fluorescence quenching of HSA on addition of complex (a) [RuL3PTA] (d) [RuL4PTA] in 5 mM TrisHCl/NaCl buffer at pH 7.2 at 298 K ($\lambda_{ex} = 295$; $\lambda_{em} = 350$ nm). Plot of F_0/F vs. concentrations of complex (b) [RuL3PTA] and (e) [RuL4PTA]. Scatchard plot of $\log([F_0-F]/F)$ vs. (c) \log [RuL3PTA] and (f) \log [RuL4PTA]

Table S2 Molecular docking estimated free energy of binding (kcal/mol) and the inhibition constant (K_i) of the RAPTA complexes with the BSA and DNA.

Free Energy of Binding (kcal/mole)					
	[RuL1PTA]	[RuL2PTA]	[RuL3PTA]	[RuL4PTA]	[RuL5PTA]
BSA	-7.35	-7.6	-7.27	-7.49	-7.47
DNA	-9.39	-9.52	-9.19	-9.25	-9.4

Inhibition Constant (K _i)					
	[RuL1PTA]	[RuL2PTA]	[RuL3PTA]	[RuL4PTA]	[RuL5PTA]
BSA	4.10 μM	2.70 μM	4.69 μM	3.23 μM	3.34 μM
DNA	130.66 nM	104.58 nM	182.21 nM	164.73 nM	129.31 nM

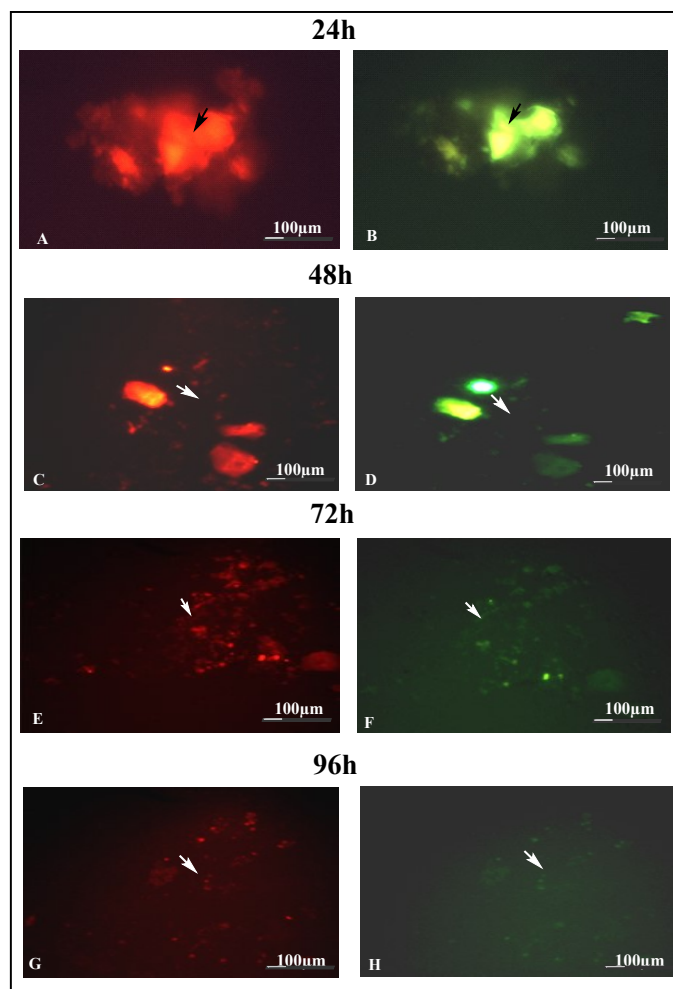
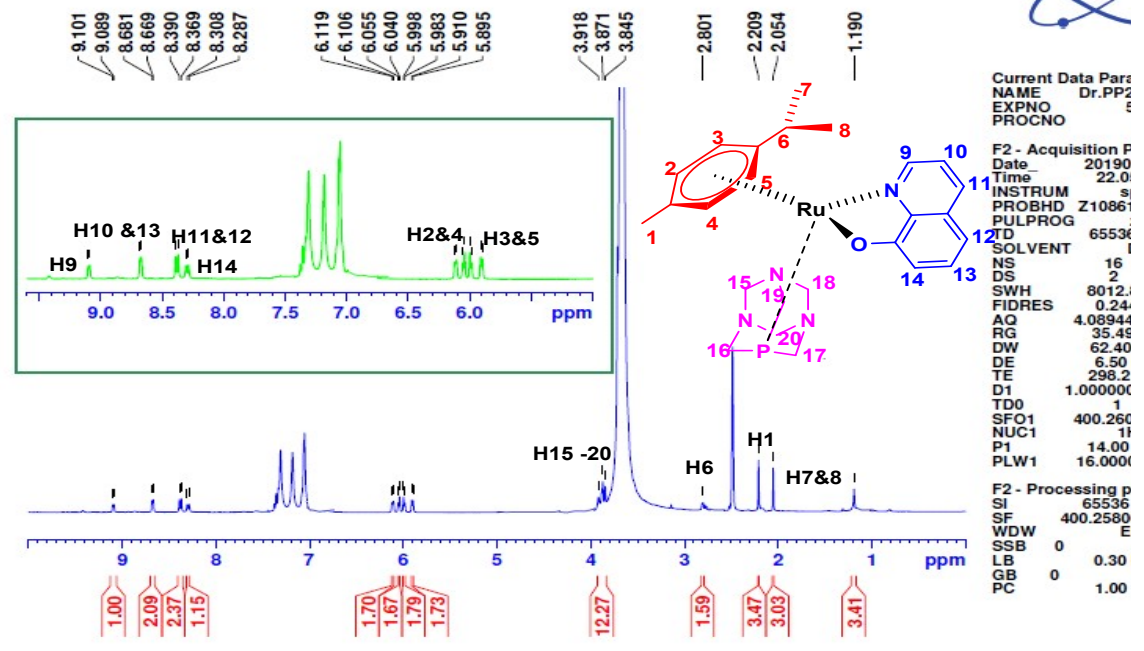


Fig. S6 Post treatment analysis of neutrosphere *via* fluorescence imaging

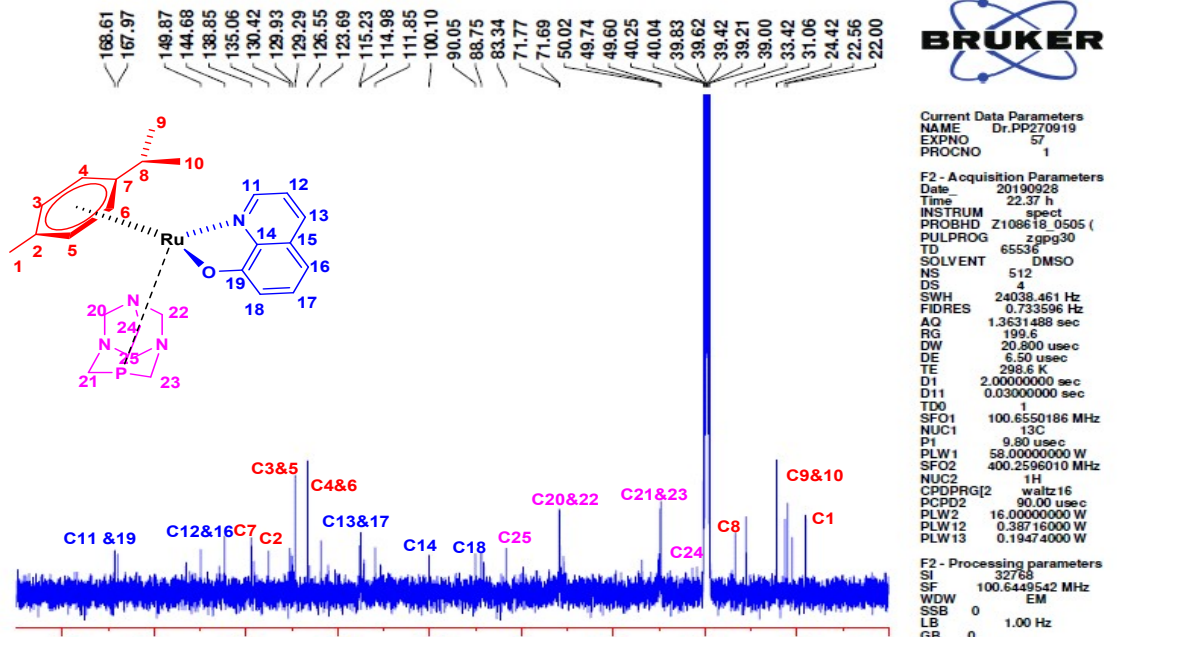
¹H NMR of RuL1PTA

Signature SIF VIT VELLORE
6A



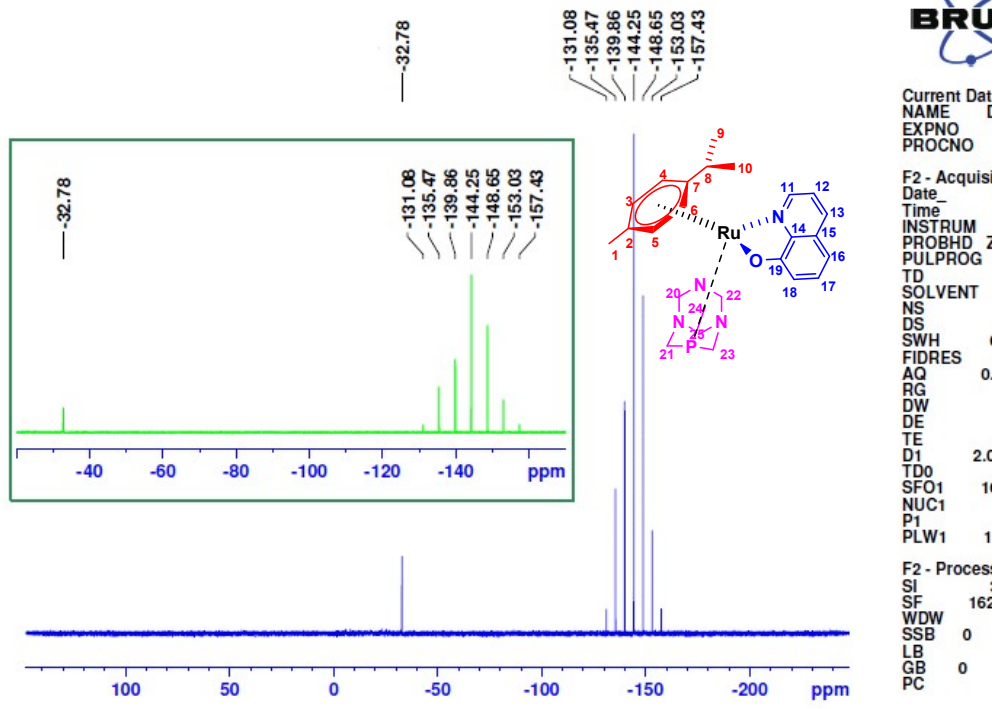
¹³C NMR of RuL1PTA

Signature SIF VIT VELLORE
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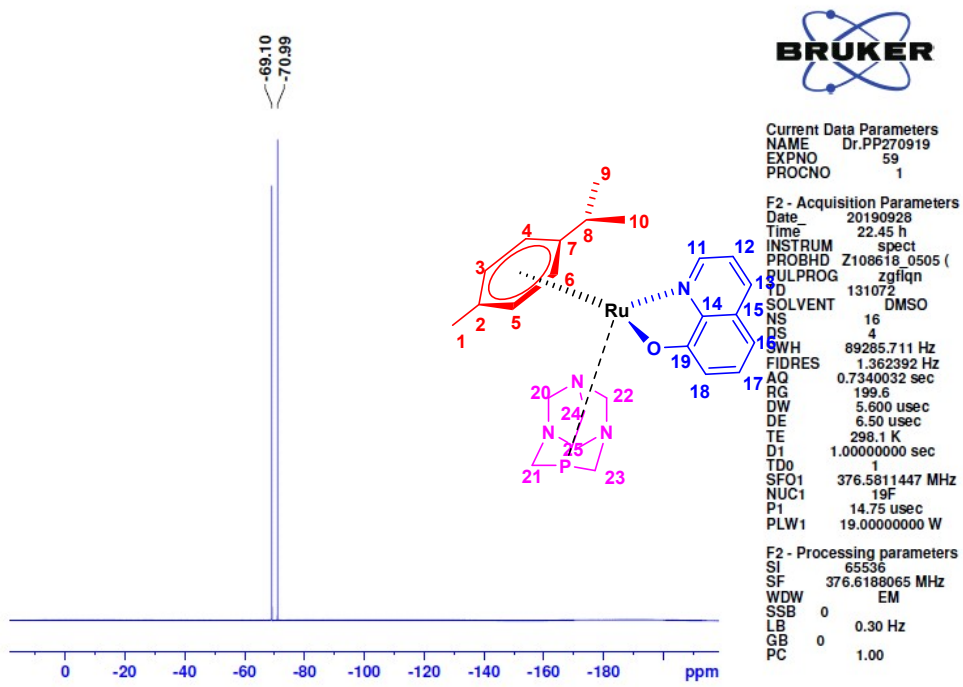
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Signature SIF VIT VELLORE
6A



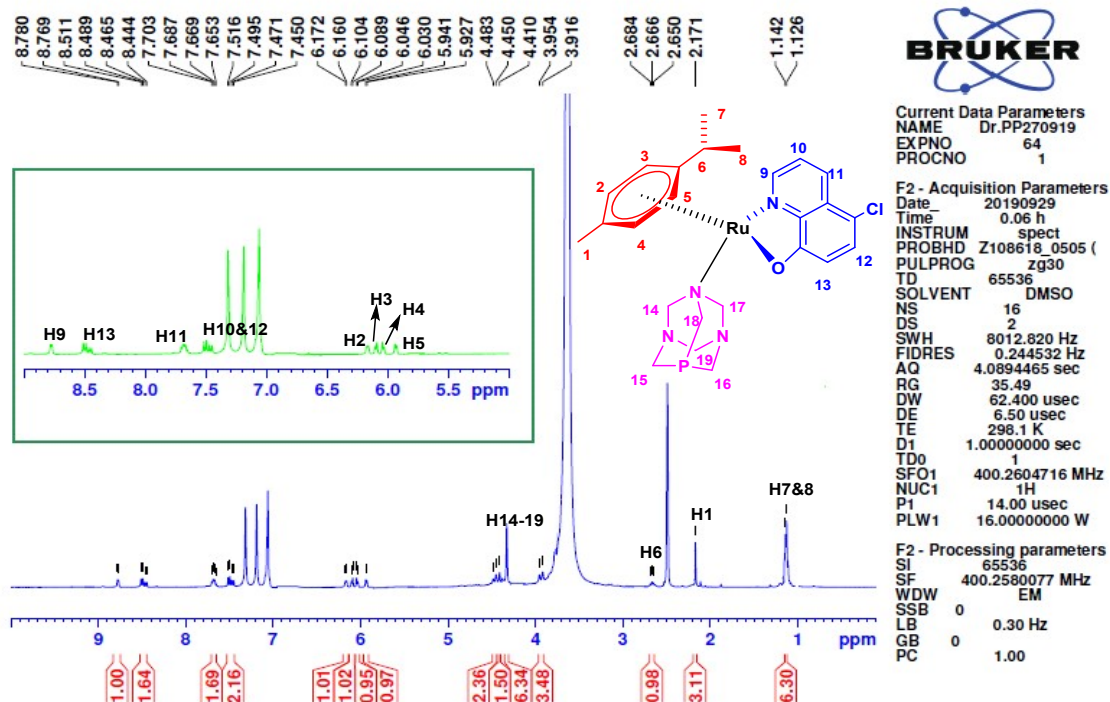
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Signature SIF VIT VELLORE
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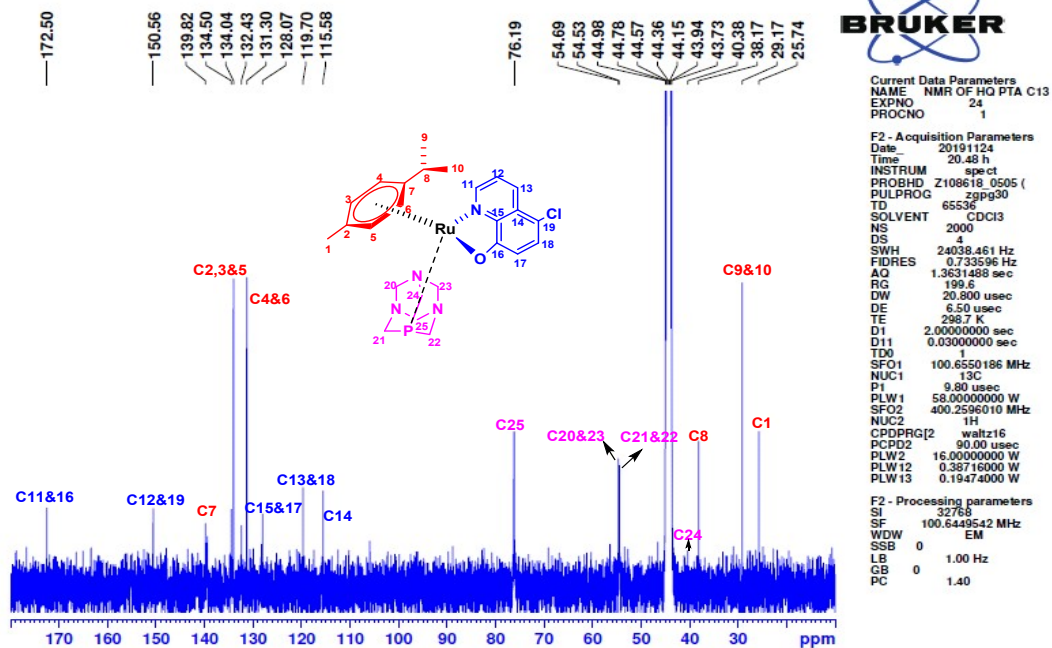
¹H NMR of RuL2PTA

Signature SIF VIT VELLORE
6B



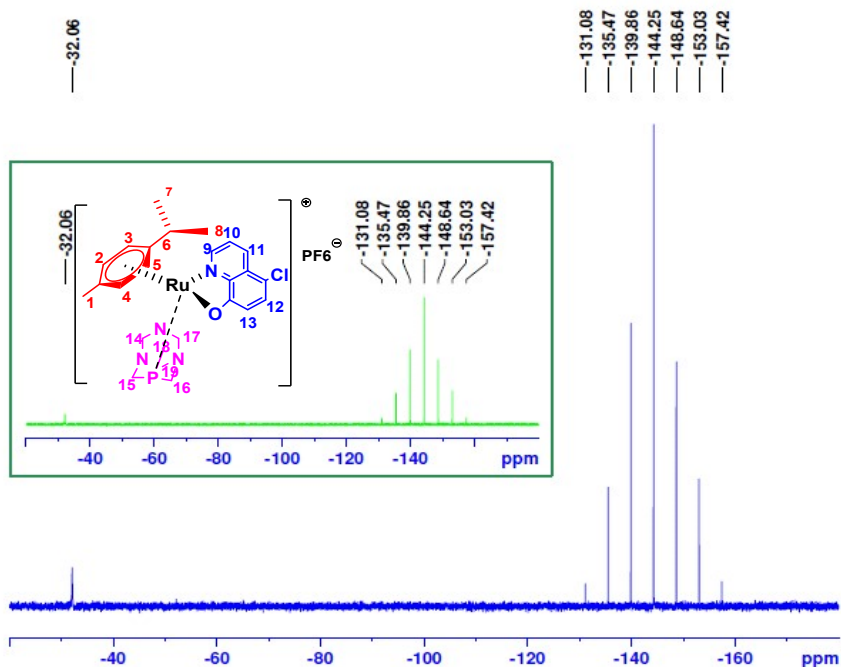
¹³C NMR of RuL2PTA

Signature SIF VIT VELLORE
6B



¹⁹F NMR of RuL2PTA

Signature SIF VIT VELLORE
6B



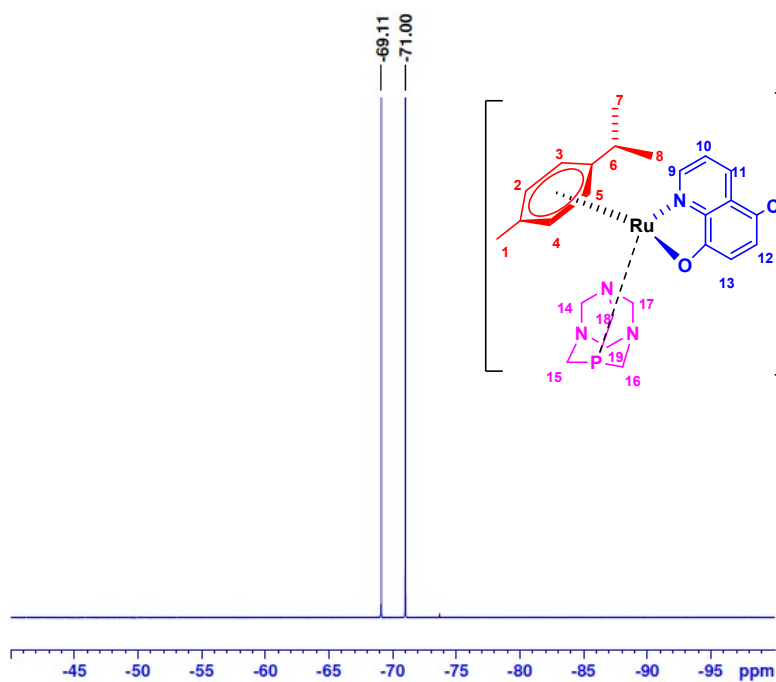
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³¹P NMR of RuL2PTA

Signature SIF VIT VELLORE
6B



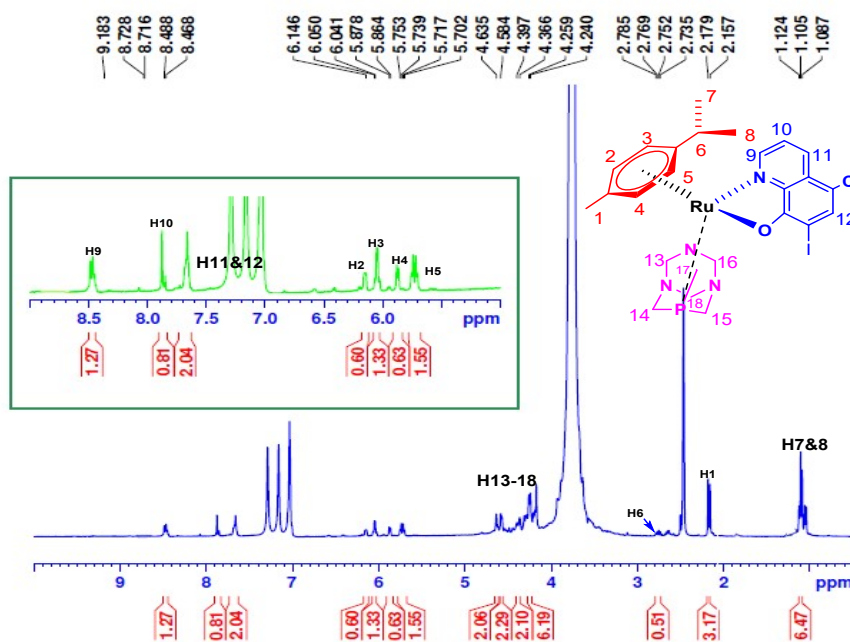
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¹H NMR of RuL3PTA

Signature SIF VIT VELLORE
6C



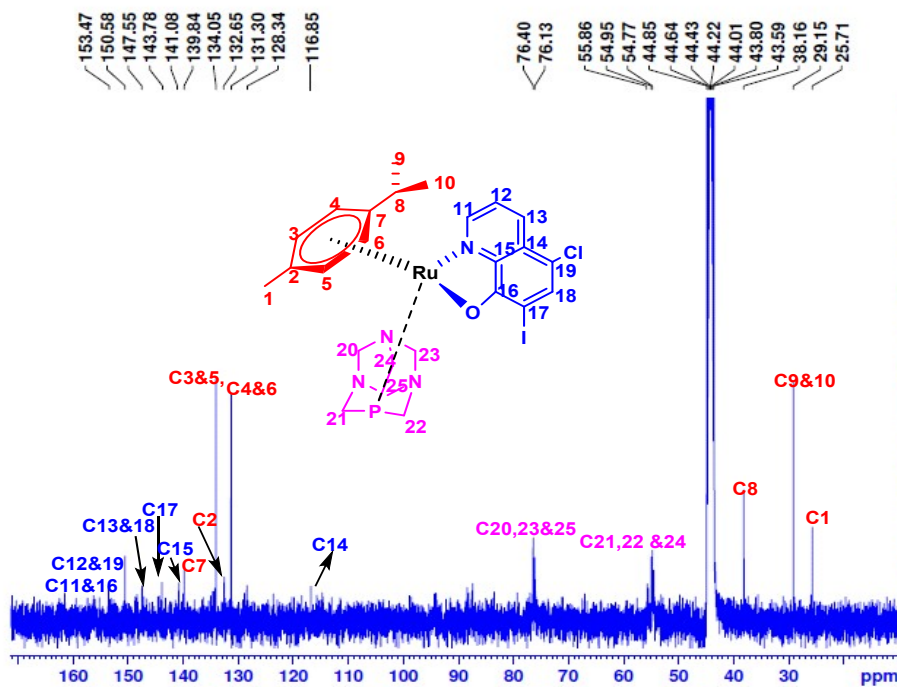
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¹³C NMR of RuL3PTA

Signature SIF VIT VELLORE
6C



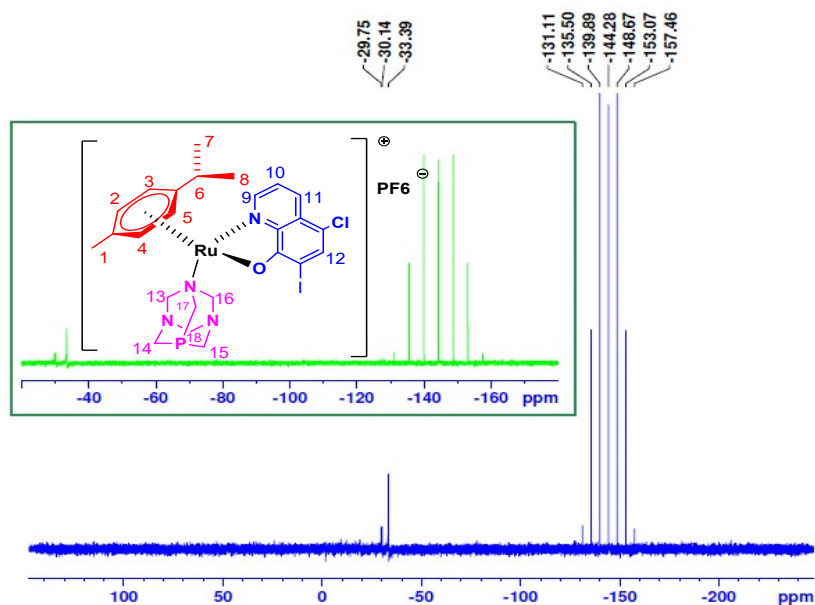
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¹⁹F NMR of RuL3PTA

Signature SIF VIT VELLORE
6C



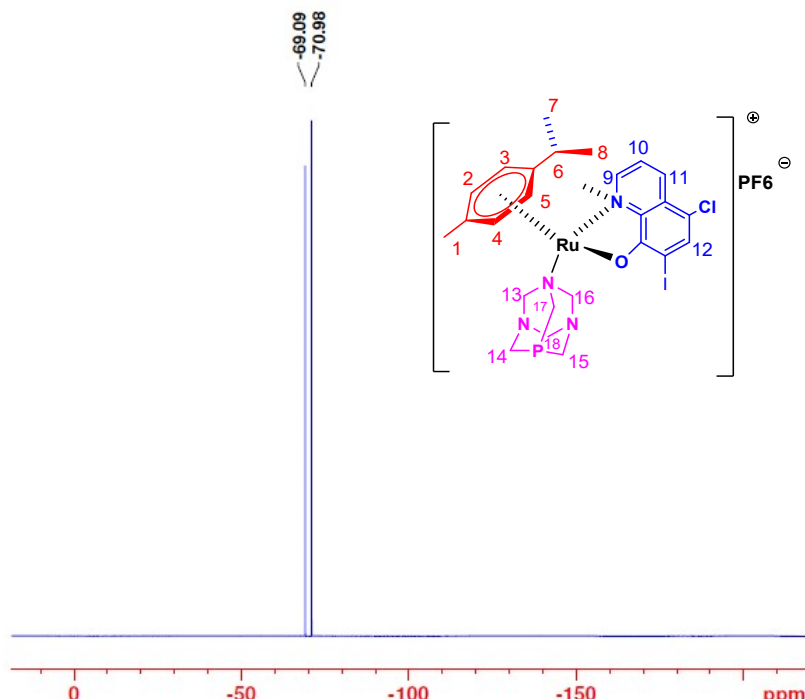
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³¹P NMR of RuL3PTA

Signature SIF VIT VELLORE
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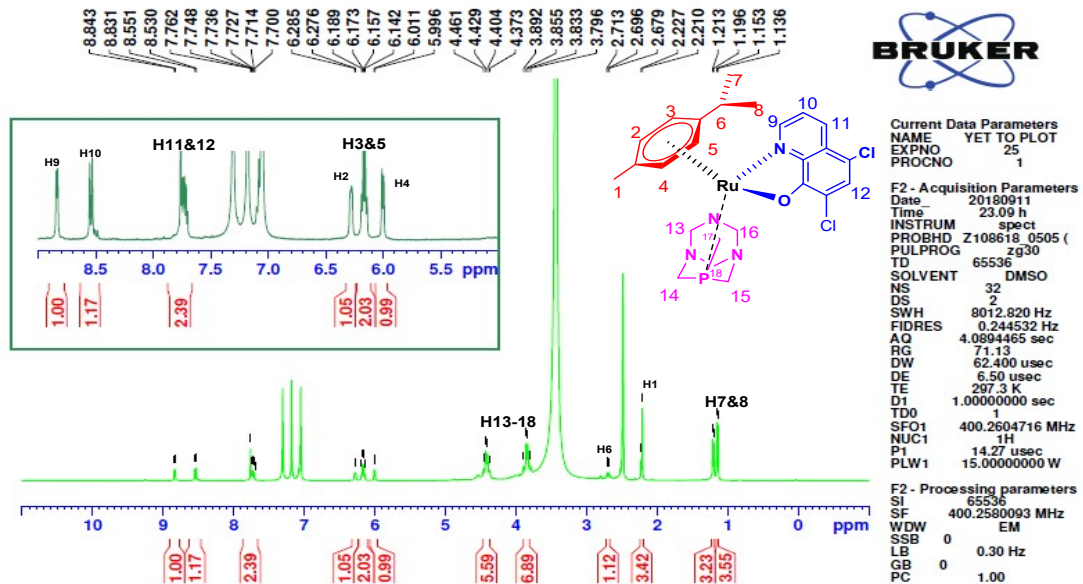
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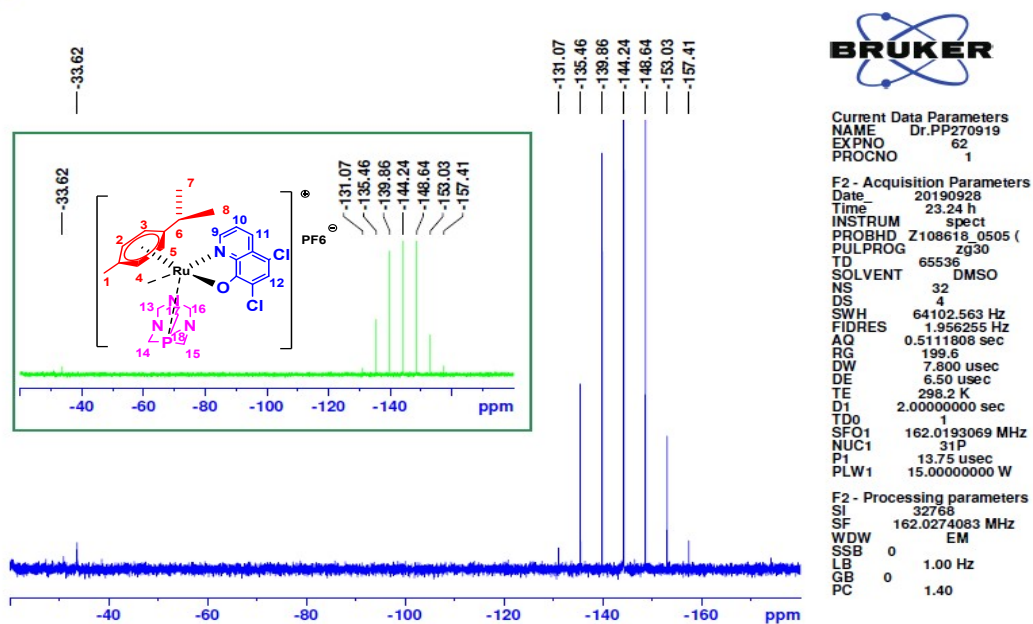
¹H NMR of RuL4PTA

Signature SIF VIT VELLORE
6D



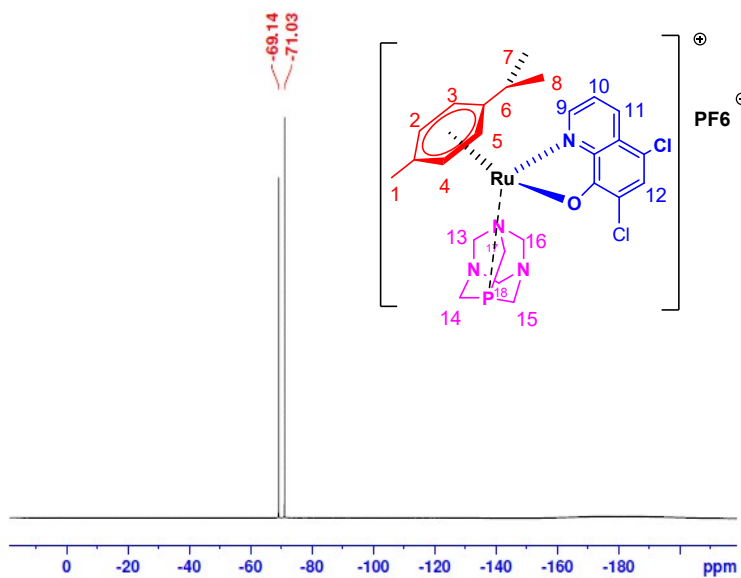
¹⁹F NMR of RuL4PTA

Signature SIF VIT VELLORE
6d



³¹P NMR of RuL4PTA

Signature SIF VIT VELLORE
6D



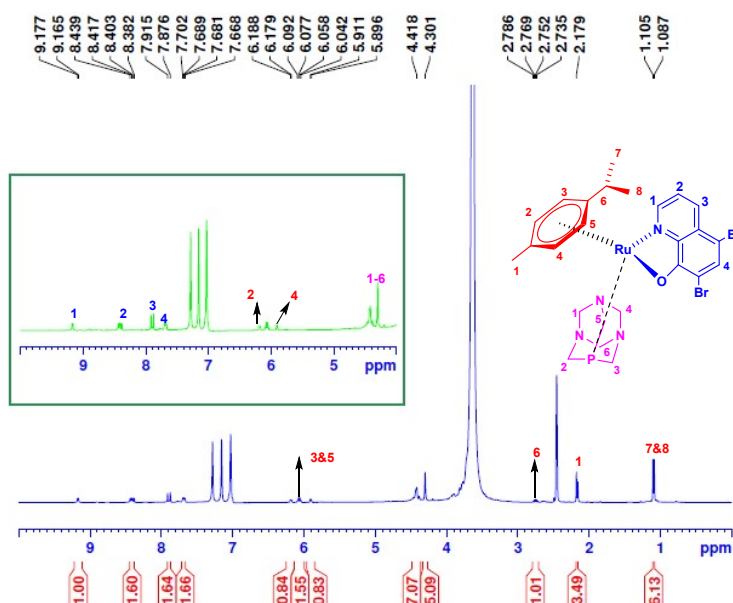
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¹H NMR of RuL5PTA

Signature SIF VIT VELLORI
6E



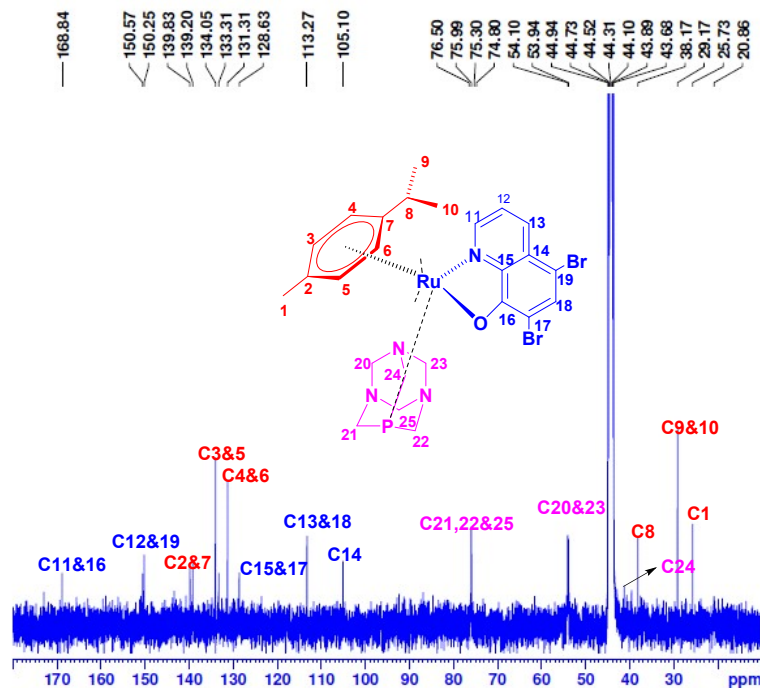
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¹³C NMR of RuL5PTA

Signature SIF VIT VELLORI
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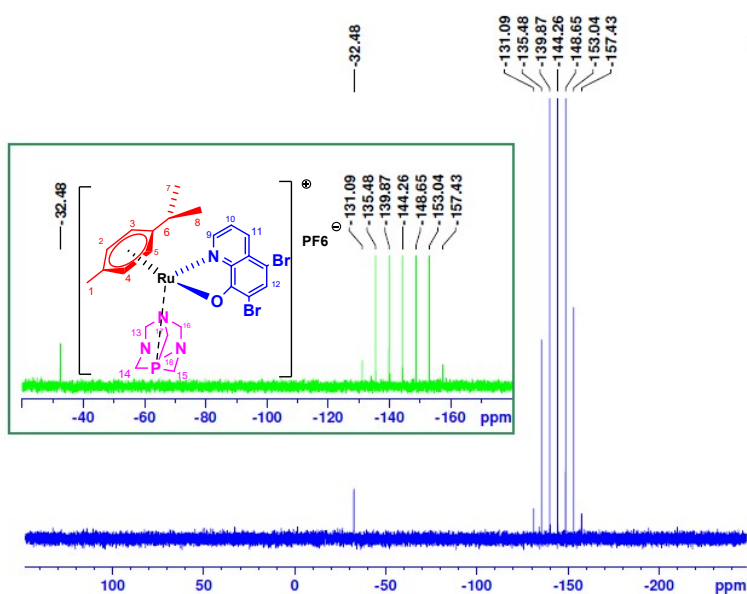
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¹⁹F NMR of RuL5PTA

Signature SIF VIT VELLORE
6E



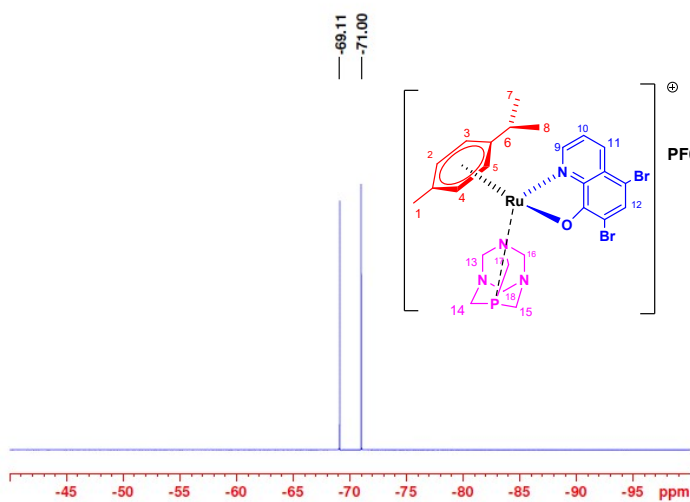
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³¹P NMR of RuL5PTA

Signature SIF VIT VELLORE
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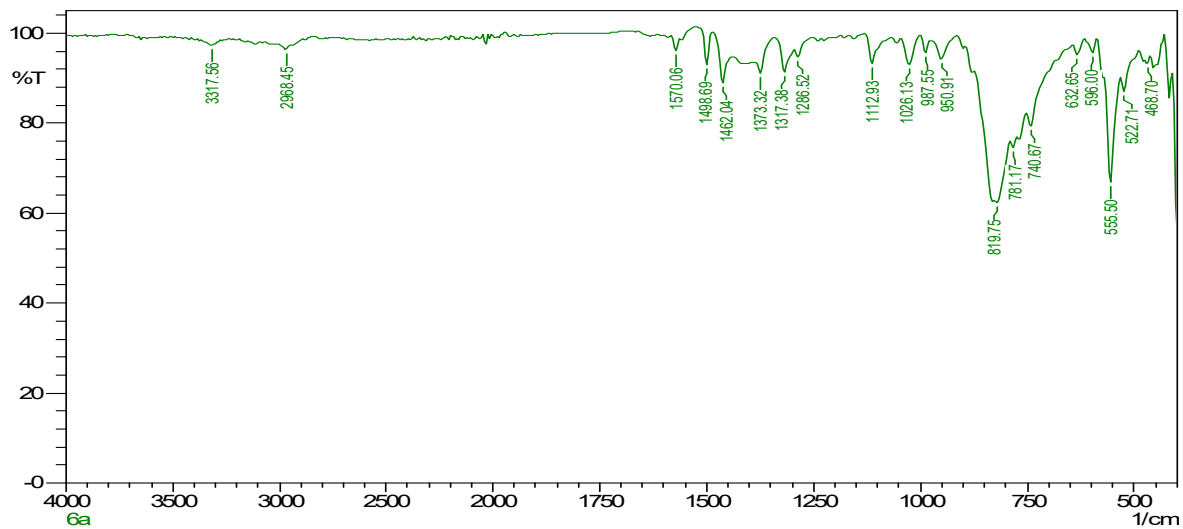
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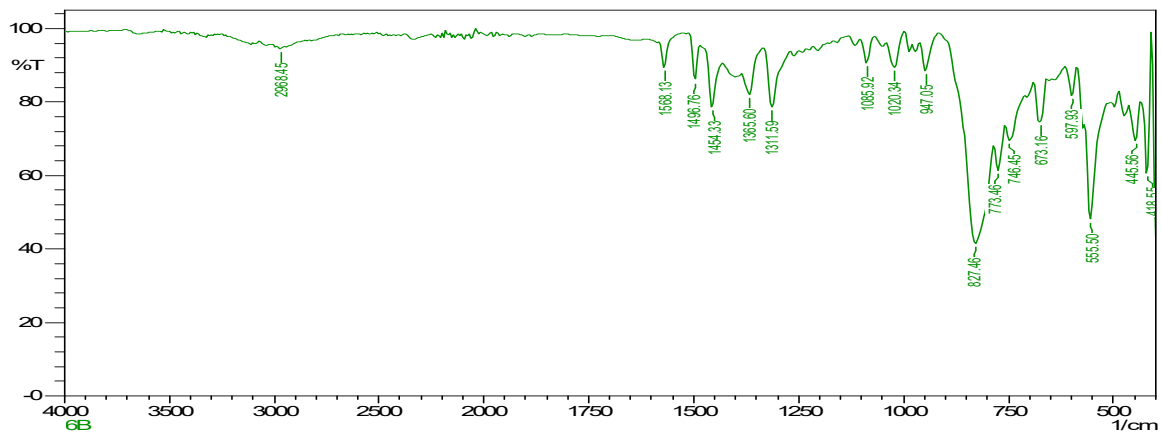
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FT-IR Spectra

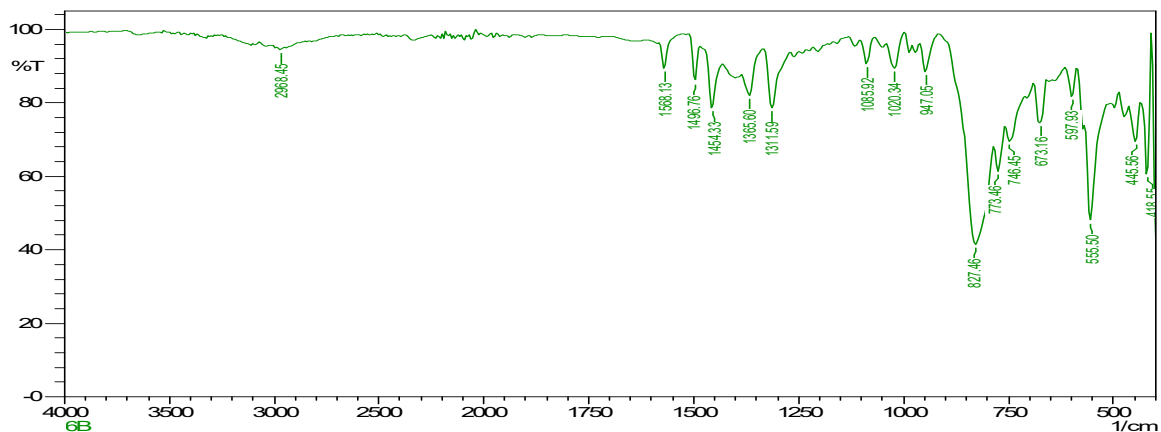
RuL1PTA



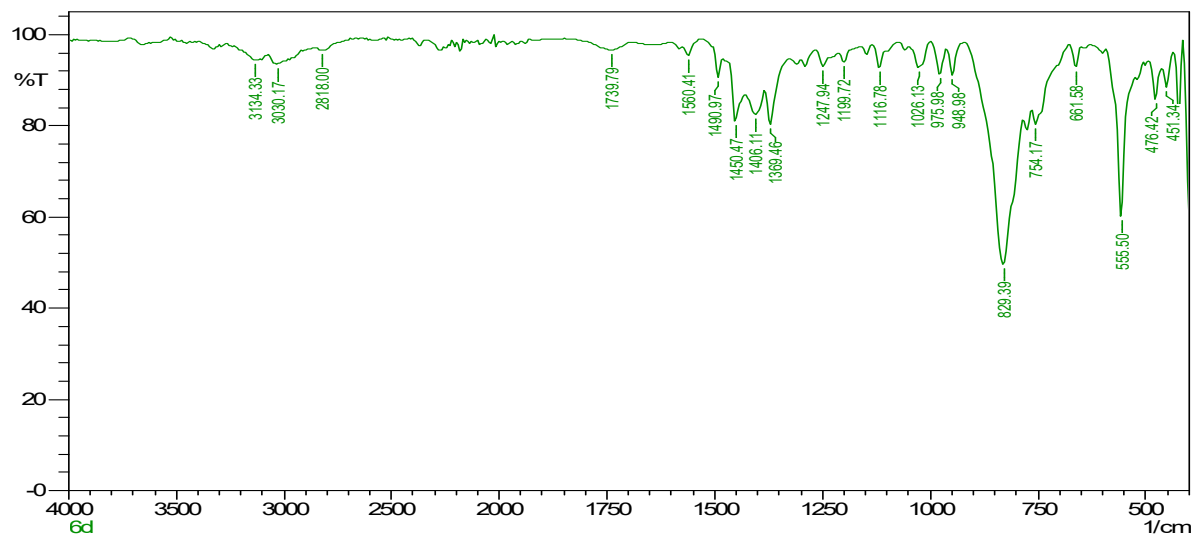
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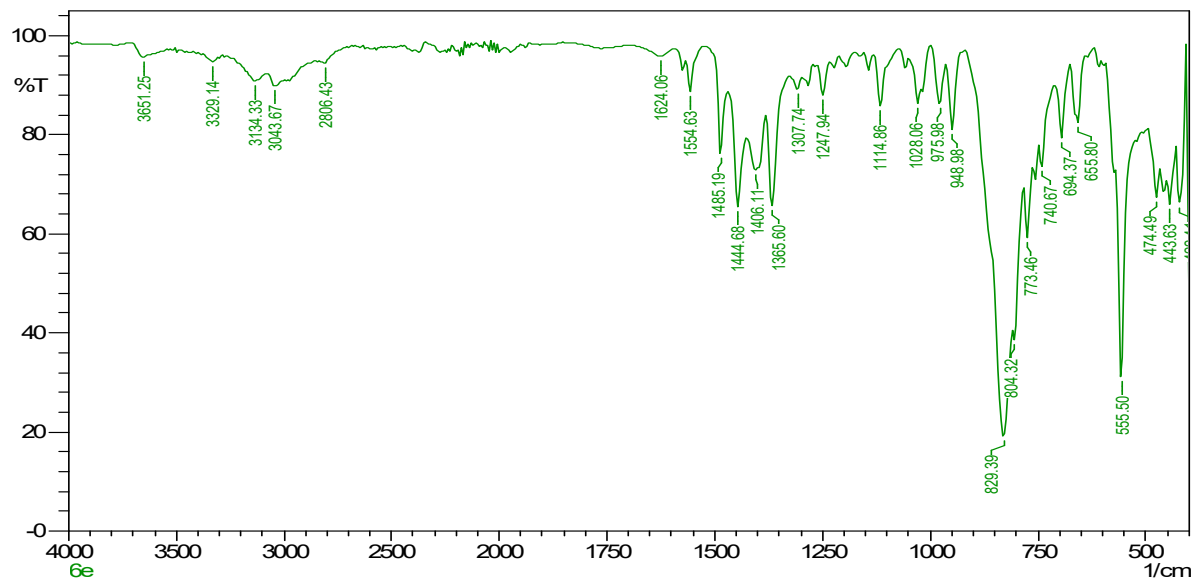
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RuL4PTA

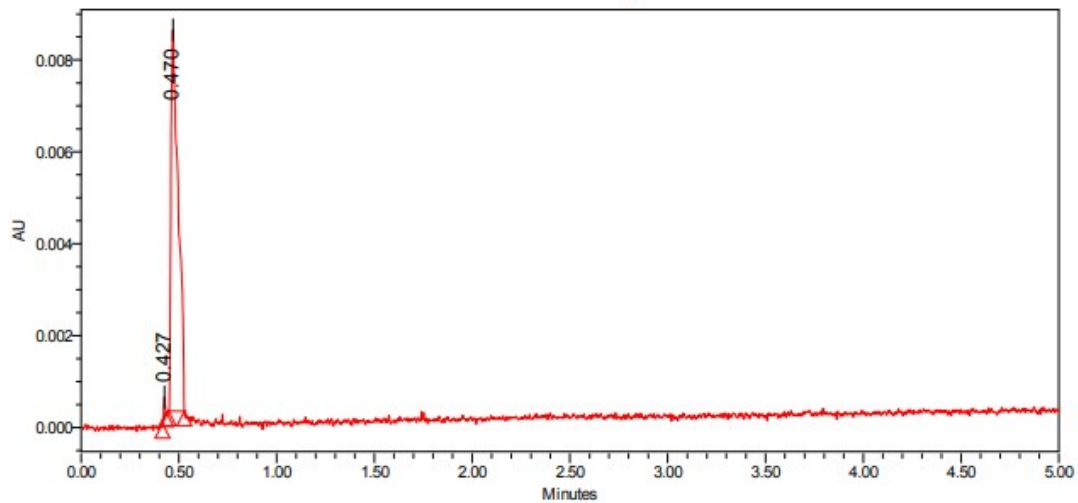


RuL5PTA



Purity (UPLC of RuL4PTA)

Auto-Scaled Chromatogram

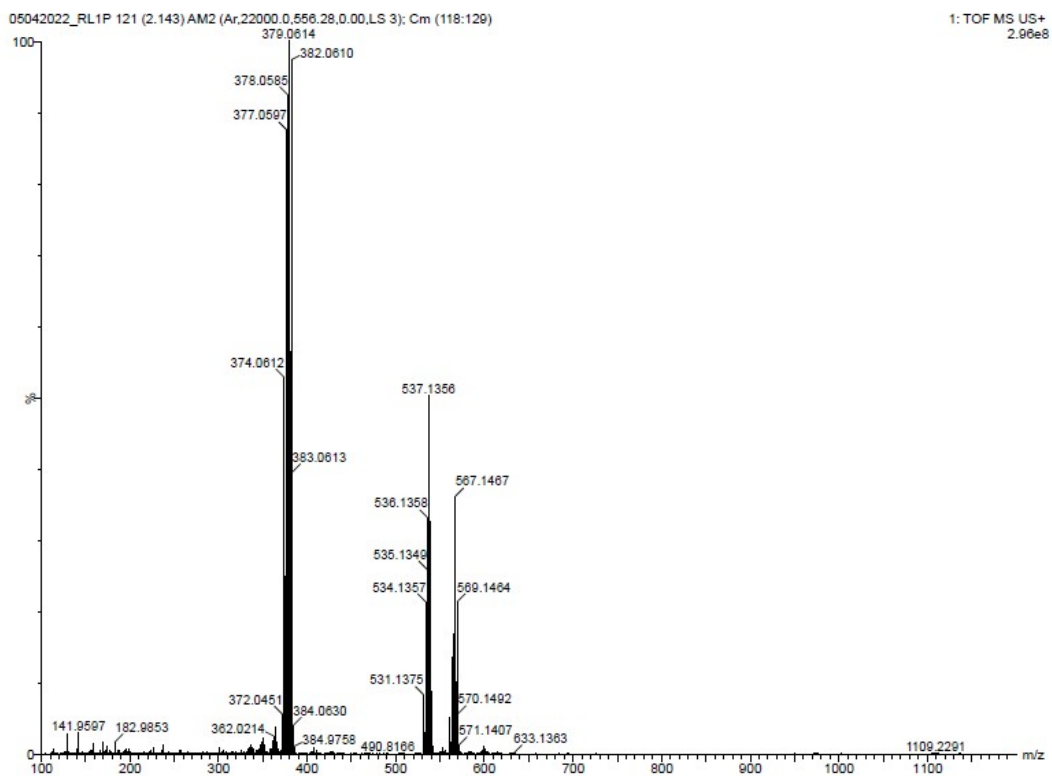


Peak Results

Name	RT	Area	% Area
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ESI-MS (HRMS) :

RuL1PTA

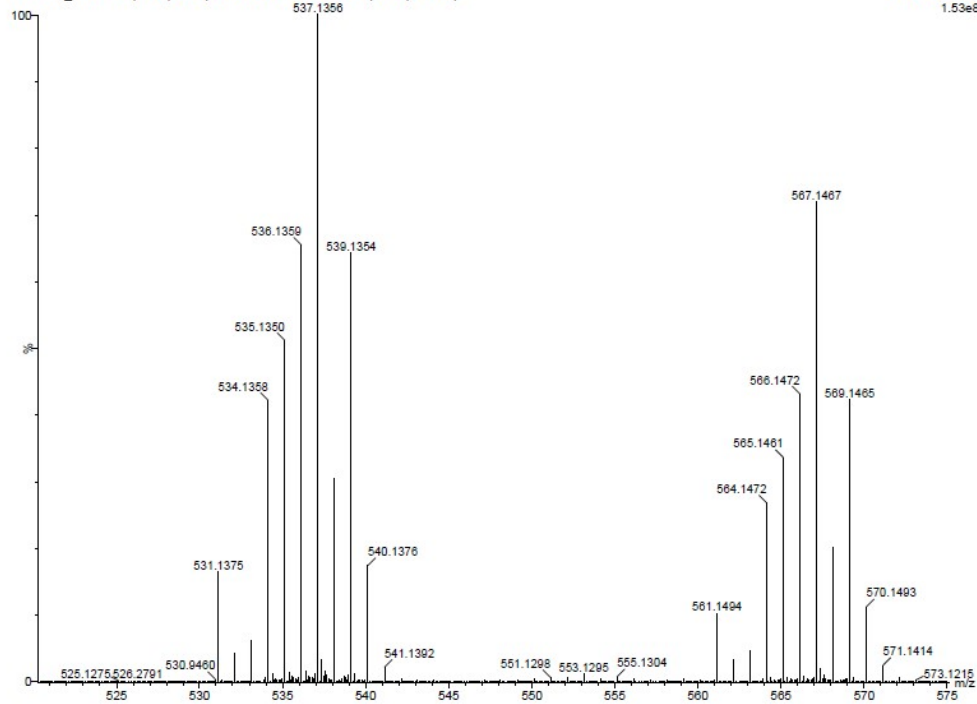


Isotopic distribution

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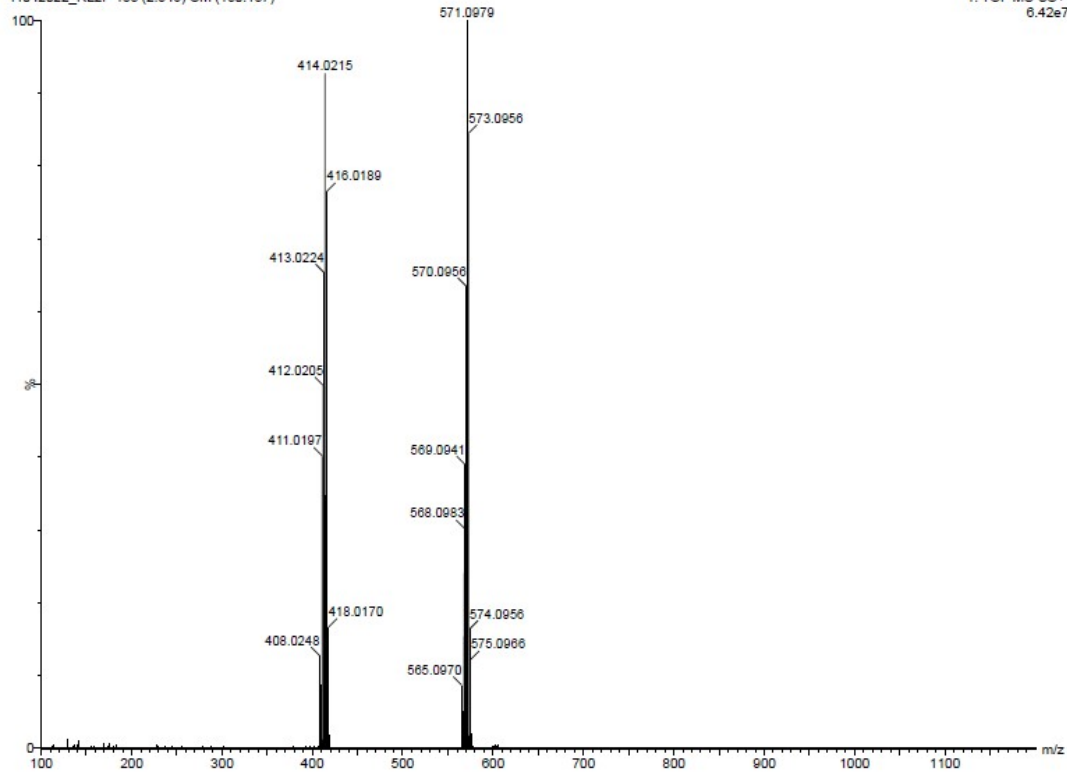
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RuL2PTA

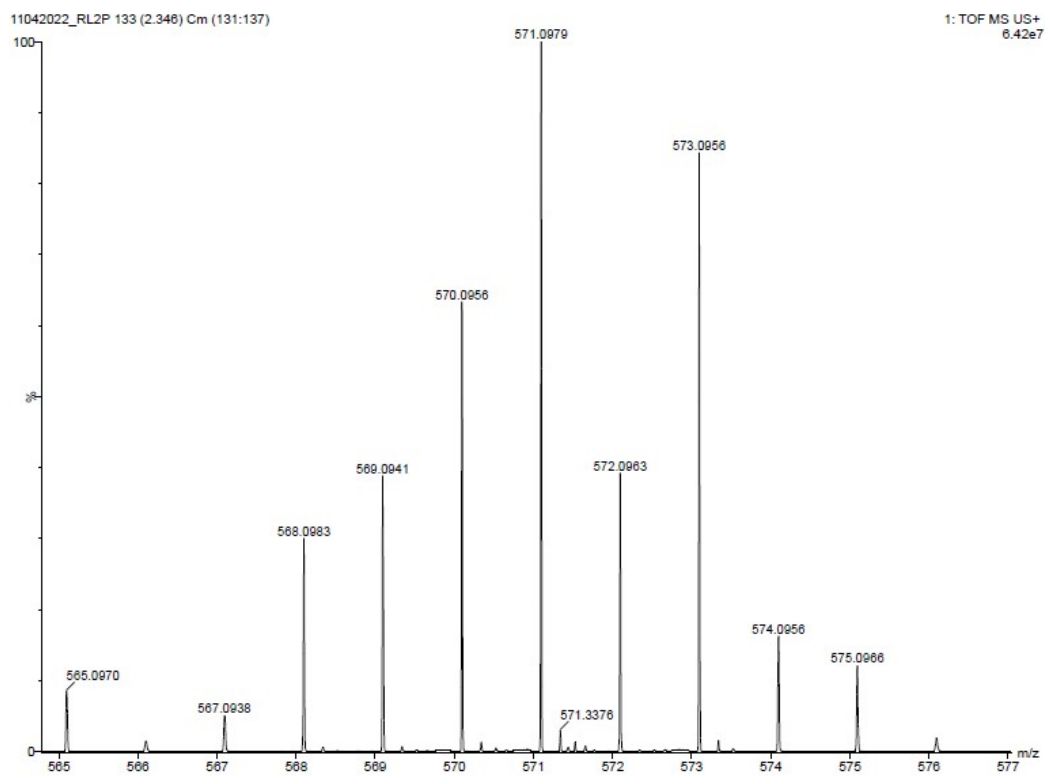
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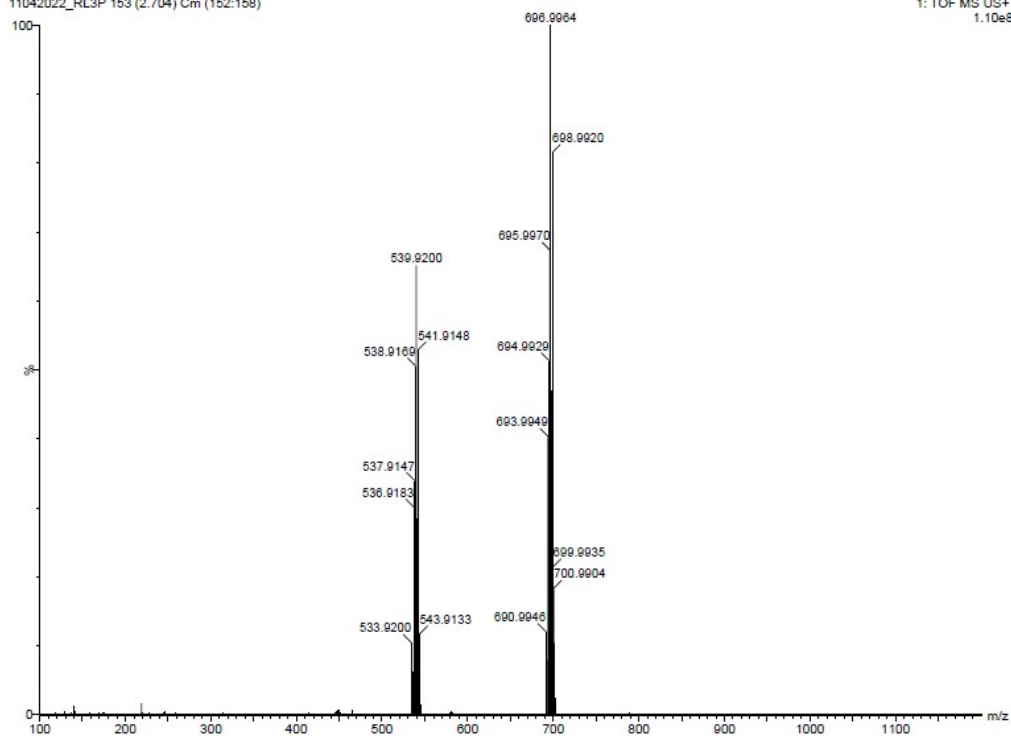


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Error = 0.00019 % (1.926 ppm)

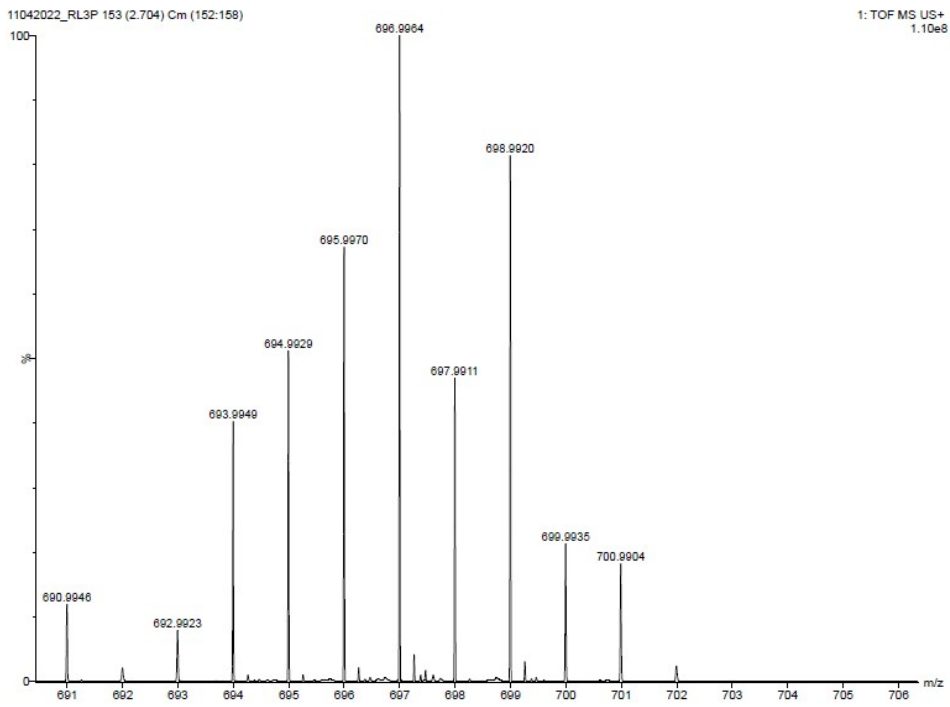


RuL3PTA

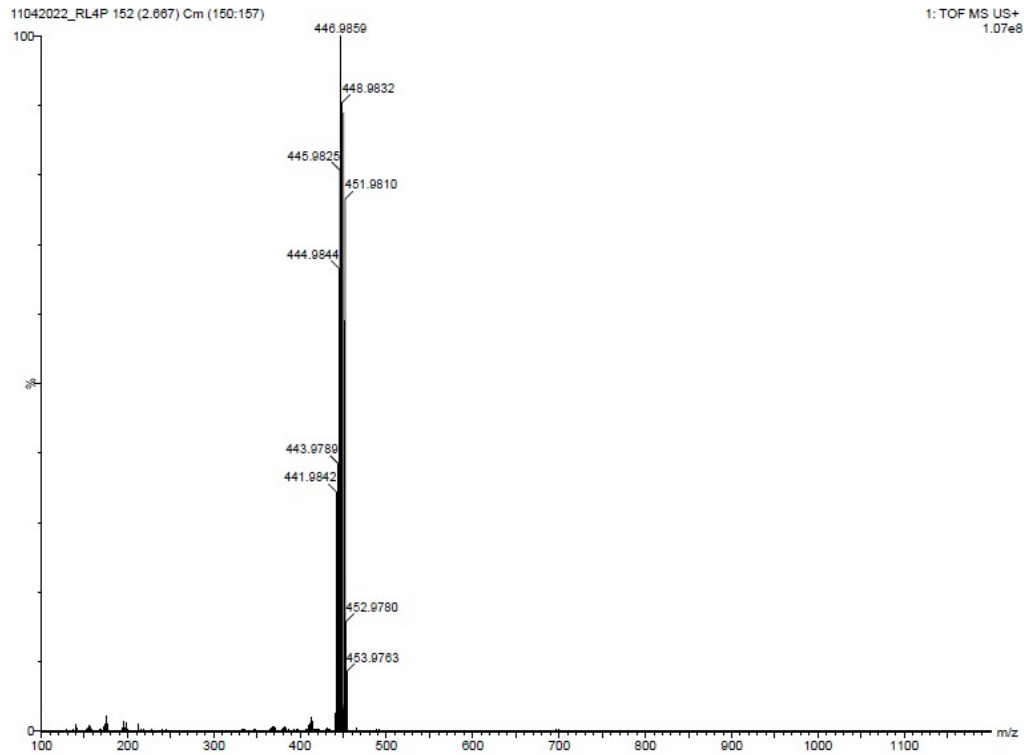


Isotopic distribution

Error = 0.00043 % (4.30 ppm)

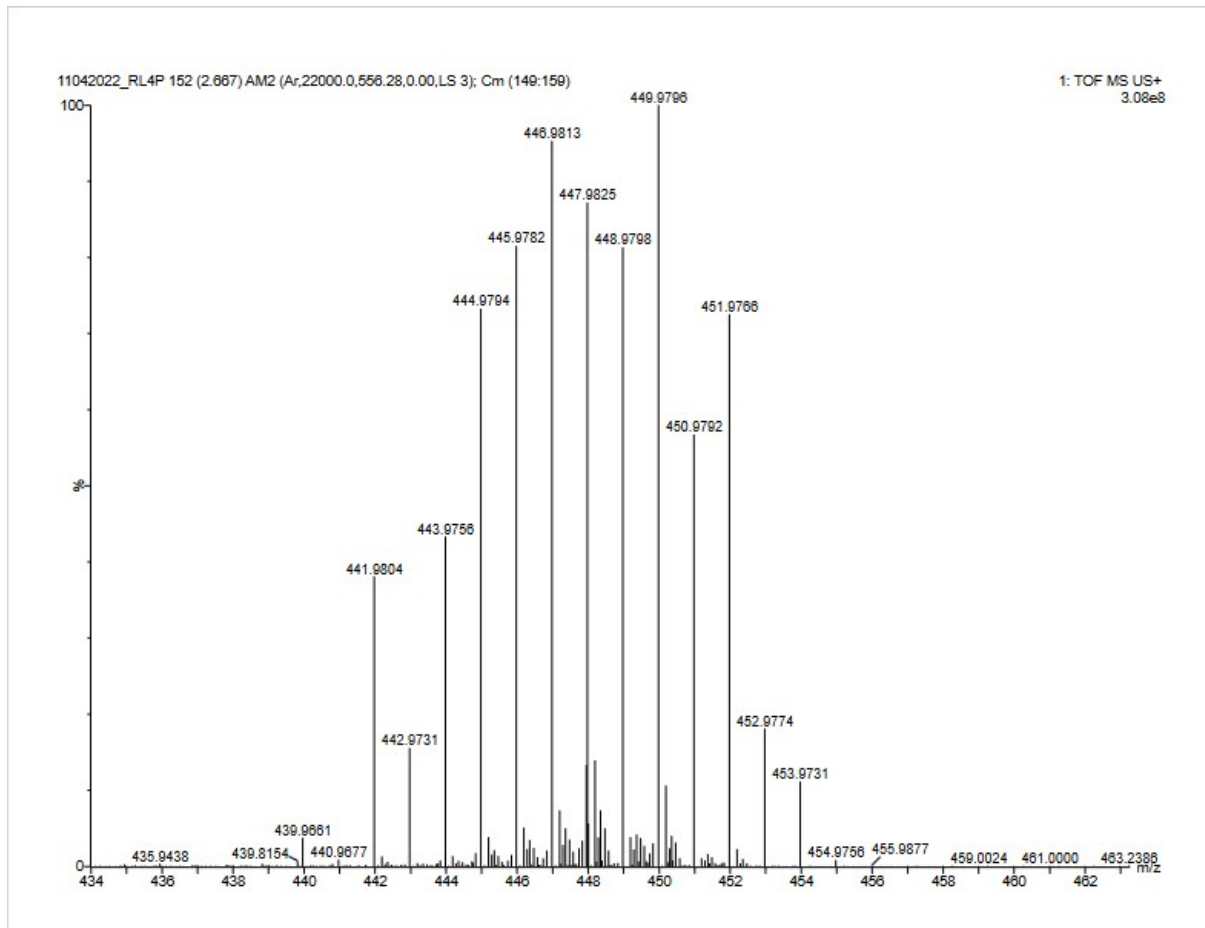


RuL4PTA

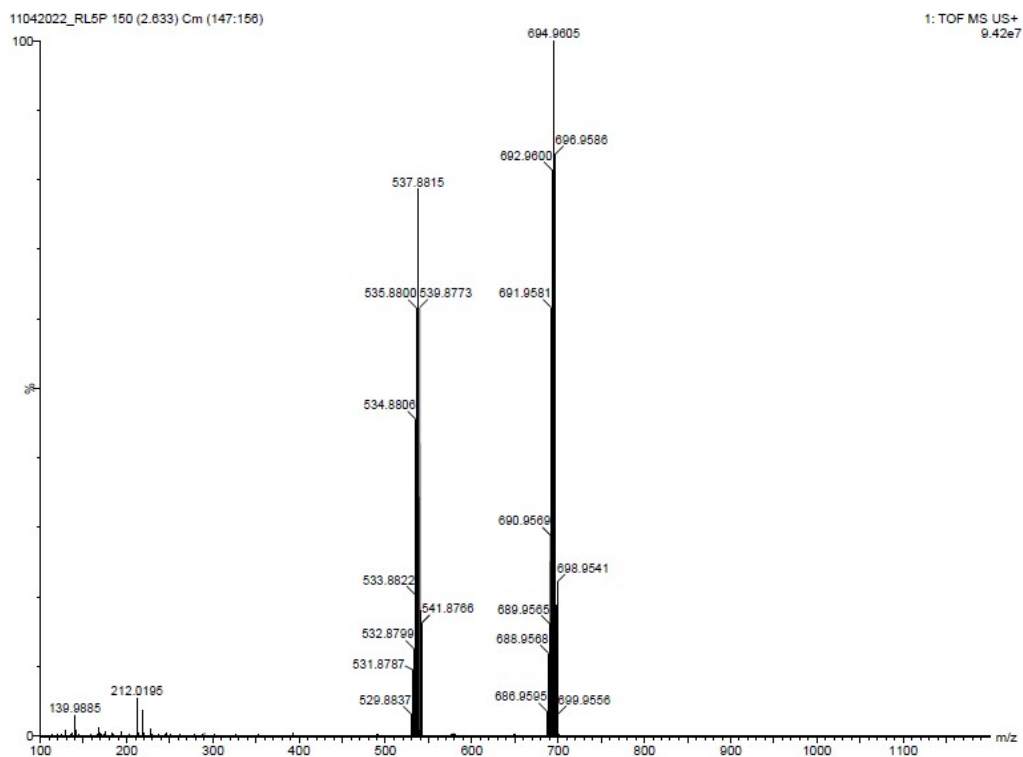


Isotopic distribution

Error = 0.00286 % (28.6 ppm)

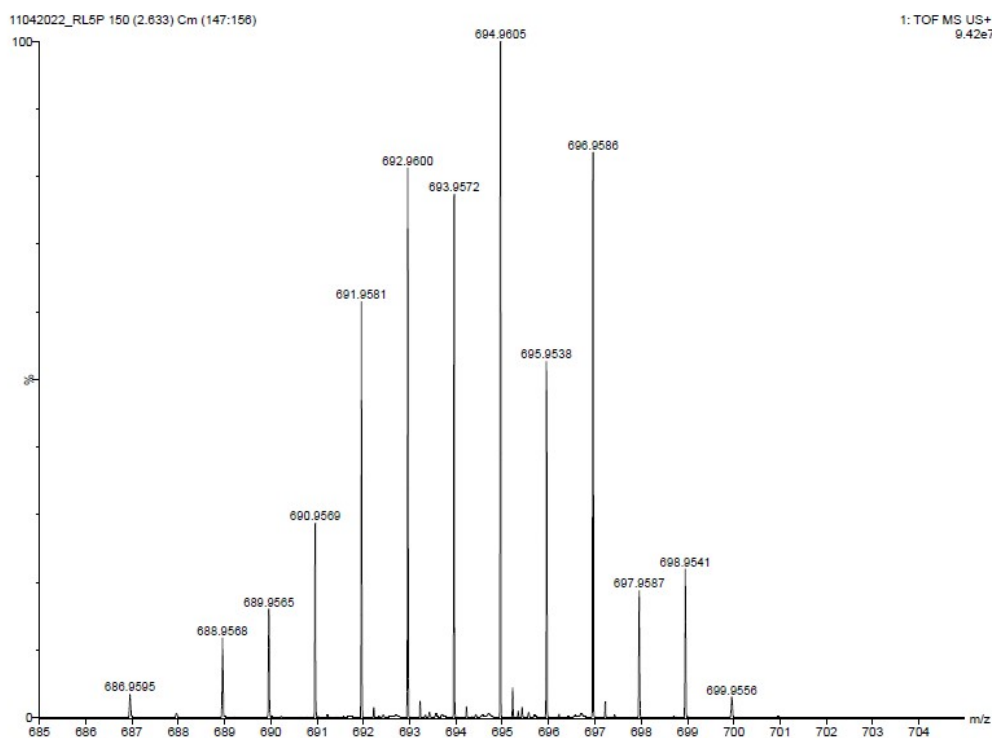


RuL5PTA



Isotopic distribution

Error = 0.000834 % (8.34 ppm)



Experimental Section

Stability study

The stability of the RAPTA complex, **[RuL4PTA]** were performed in aqueous DMSO (H₂O: DMSO = 9:1), GSH (1mM) medium.

DNA binding study

Electronic absorption spectroscopy was employed to study the binding capacity of the complexes with calf-thymus DNA (Ct-DNA) and competitive binding assay as studied using ethidium bromide (EtBr) as quencher by fluorescence spectroscopy.

UV–visible studies¹

DNA binding assay was carried out by using complexes **[RuL3PTA]** and **[RuL4PTA]** in Tris-HCl buffer (5 mM Tris-HCl in water, pH 7.4) in aqueous medium. The concentration of Ct-DNA was calculated from its absorbance intensity at 260 nm and its known molar absorption coefficient value of 6600 M⁻¹ cm⁻¹. Equal amount of DNA was added in both the

sample and reference in cuvettes. Titration was carried out by increasing concentration of CT-DNA. On the eve of each measurement, sample was equilibrated with CT-DNA for about 5 min and then absorbance of the complex was measured. The intrinsic DNA binding constant (K_b) was calculated using the equation (i):

$$\frac{[DNA]}{(\epsilon_a - \epsilon_f)} = \frac{[DNA]}{(\epsilon_b - \epsilon_f)} + \frac{1}{K_b(\epsilon_a - \epsilon_f)}L \quad (i)$$

Where $[DNA]$ is the concentration of DNA in the base pairs, ϵ_a is the apparent extinction coefficient observed for the complex, ϵ_f corresponds to the extinction coefficient of the complex in its free form, and ϵ_b refers to the extinction coefficient of the complex when fully bound to DNA. The resultant data were plotted using Origin Lab, version 8.5 to obtain the $[DNA]/(\epsilon_a - \epsilon_f)$ vs. $[DNA]$ linear plot. The ratio of the slope to intercept from the linear fit gave the values of the intrinsic binding constants (K_b).

UV and Fluorescence study

UV and Fluorescence study of all these RAPTA complexes were executed in 10 % DMSO solution. Then the fluorescence quantum yields (Φ) were calculated by applying the comparative William's method which involves the use of well-characterized standard with known quantum yield value using 10% DMSO solution.² Quinine sulphate was used as a standard. Quantum yield was calculated according to the equation (ii):

$$\phi = \phi_R \times \frac{I_S}{I_R} \times \frac{OD_R}{OD_S} \times \frac{\eta_S}{\eta_R} \dots \dots (ii)$$

Where, ϕ = quantum yield, I = peak area, OD = absorbance at λ_{max} , η = refractive index of solvent (s) and reference (R). Here, we have used quinine sulphate as a standard for calculating the quantum yield.

Ethidium bromide displacement assay

The ethidium bromide (EtBr) displacement assay was conducted to illustrate the mode of binding between the potent compounds with DNA.³ The apparent binding constant (K_{app}) of the complexes **[RuL3PTA]** and **[RuL4PTA]** to Ct-DNA were calculated using ethidium bromide (EtBr) as a spectral probe in 5 mM Tris-HCl buffer (pH 7.4). EtBr do not show any fluorescence in its free state as its fluorescence is quenched by the solvent molecules. Nevertheless, its fluorescence intensity was radially increases with increase the concentration

of Ct-DNA, which suggested the intercalative mode of binding of EtBr with DNA grooves. The fluorescence intensity was found to decrease with further increase in concentration of the complexes. According to the displacement theory, it can be said that the complexes displaced EtBr from CT-DNA grooves and then bound to the DNA base pairs. The values of the apparent binding constant (K_{app}) were obtained by using the equation (iii):

$$K_{app} \times [Complex]_{50} = k_{EtBr} \times [EtBr] \dots \dots \dots (iii)$$

Where K_{EtBr} is the EtBr binding constant ($K_{EtBr} = 1.0 \times 10^7 \text{ M}^{-1}$), and $[EtBr] = 8 \times 10^{-6} \text{ M}$. Stern-Volmer equation was followed for quantitative determination of the Stern-Volmer quenching constant (K_{SV}).⁴ Origin (8.5) software was used to plot the fluorescence data to obtain linear plot of I_0/I vs. [complex]. The value of K_{SV} was calculated from the following equation.

$$I_0/I = 1 + K_{SV} [Q] \dots \dots \dots (iv)$$

Where I_0 = fluorescence intensity in absence of complex and I = fluorescence intensities in presence of complex of concentration [Q].

Protein binding studies

We are acquainted with the fact that serum albumin proteins are the main component. It is well known in blood plasma proteins and plays important roles in drug transport and metabolism, interaction of the drug with human serum albumin (HSA) was studied from tryptophan emission quenching experiment.⁵ Tryptophan emission quenching experiment was performed to detect the interaction of the RAPTA complexes, [RuL3PTA] and [RuL4PTA] with protein HSA. Initially, HSA solution ($2 \times 10^{-6} \text{ M}$) was prepared in Tris-HCl/NaCl buffer. The aqueous solutions of the complexes were subsequently added to HSA solution with gradual increase of their concentrations. After each addition, the solutions were shaken slowly for 5 min before recording the fluorescence at a wavelength of 295 nm ($\lambda_{ex} = 295 \text{ nm}$). A gradual decrease in fluorescence intensity of HSA at $\lambda = 340 \text{ nm}$ was observed upon increasing the concentration of complex, which confirmed that the interaction between the complex and HSA was happened. Stern-Volmer equation was employed to quantitatively determine the quenching constant (K_{HSA}). Origin Lab, version 8.5 was used to plot the emission spectral data to obtain linear plot of I_0/I vs. [complex] using the equation (v) given below:

$$I_0/I = 1 + K_{BSA} [Q] = 1 + k_q \tau_0 [Q] \quad (v)$$

Where I_0 is the fluorescence intensity of HSA in absence of complex and I indicates the fluorescence intensity of HSA in presence of complex of concentration $[Q]$, τ_0 = lifetime of the tryptophan in HSA found as 1×10^{-8} and k_q is the quenching constant. Scatchard equation (vi) gives the binding properties of the complexes.⁶ Where K = binding constant and n = number of binding sites.

$$\log(I_0 - I/I) = \log K + n \log [Q] \quad (vi)$$

Conductivity measurement⁷

For authenticating the interaction of the complexes with DMSO and aqueous DMSO, conductivity of the prepared complexes were performed using conductivity-TDS meter-307 (Systronics, India) and cell constant 1.0 cm^{-1} . Rate of conductivity was also estimated in different pH medium. Time dependent conductivity measurement was also carried out.

n-Octanol–water partition coefficient ($\log P_{o/w}$)⁸

The $\log P_{o/w}$ of the iridium complexes were adhering to shake flask method using the previously published procedure. A known amount of each RAPTA complexes was suspended in water (pre-saturated with n-octanol) and shaken for 48 h on an orbital shaker. To allow the phase separation, the solution was centrifuged for 10 min at 3000 rpm. To obtain the partition coefficient, different ratios (0.5: 1, 1: 1, and 2: 1) of the saturated solutions were shaken with pre-saturated n-octanol for 20 min on an orbital shaker and followed the same procedure. Aliquots of the aqueous and octanol layers were pipetted out separately and the absorbances were measured with UV-Vis spectrophotometer using proper dilution. Each set was performed in triplicate, concentration of the substances in each layer was calculated using the respective molar extinction coefficients and the partition coefficient ($\log P_{o/w}$) values were obtained from the ratio.

Notes and References

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