Electronic Supporting Information

DNA binding, antitubercular, antibacterial and anticancer studies of newly designed piano-stool ruthenium(II) complexes

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Experimental Section

1. General procedure

1.1. Synthesis of L-1

To the synthesis of L-1, 2 mmol (370 mg) 2-(1-phenylhydrazineyl)pyridine and 2 mmol (412 mg) anthracene-9-carbaldehyde were mixed in a ratio of 1:1 in 10 mL of methanol. The above solution was heated for 1 hour at 50 °C, then cooled to room temperature and stirred. In the solution, a red crystalline precipitate was produced and collected using the filtration process and thoroughly washed with methanol. UV-Vis, FT-IR, and $^{1}H/^{13}C$ NMR spectroscopy techniques were used to characterize the ligand (L-1). Yield: 270 mg, 72.0 %), Selected FT-IR frequency: (potassium bromide, ν/cm^{-1}): 1579 (υ_{imine}), UV-Vis: [DCM (λ_{max}/nm (ε/M^{-1} cm^{-1})]: 8315(405), 6876(309), 44562(260). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 – 8.38 (m, 3H), 8.32 (s, 1H), 8.19 (d, J = 4.2 Hz, 1H), 8.00 (d, J = 7.7 Hz, 2H), 7.78 – 7.70 (m, 3H), 7.65 – 7.61 (m, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.48 (dd, J = 15.6, 7.1 Hz, 6H), 6.85 – 6.80 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.63, 137.77, 136.29, 131.50, 130.79, 130.09, 128.89, 128.24, 128.24, 127.45, 126.31, 125.20, 125.13, 116.07, 109.59. ESI-MS = $[M+H]^+$, m/z = 374.1603. Anal. Calcd. for $C_{26}H_{19}N_3$ (MW=373): C, 83.62; H, 5.13; N, 11.25. Found: C, 83.69; H, 4.99; N, 11.43.

1.2. Synthesis of L-2

To the synthesis of L-2, 2 mmol (370 mg) 2-(1-phenylhydrazineyl)pyridine and 2 mmol (312 mg) 2-naphthaldehyde were mixed in the ratio of 1:1. Then the aforementioned mixture was stirred at room temperature. A pale-white coloured precipitate was appeared in the reaction mixture and isolated using the filtration procedure and thoroughly washed with methanol. Then, L-2 has been characterized by UV-Vis, FT-IR, and $^{1}H/^{13}C$ NMR spectroscopic techniques. Yield: 286 mg, 88.5 %), Selected FT-IR frequency: (potassium bromide, ν/cm^{-1}): 1591 (υ_{imine}), UV-Vis: [DCM, (λ_{max}/ nm (ε/M^{-1} cm^{-1})]: 37050(342), 15069(291), 45207(235). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.19 (d, J = 4.7 Hz, 1H), 8.12 (d, J = 8.6 Hz, 1H), 7.87 (t, J = 8.6 Hz, 3H), 7.81 (dd, J = 11.2, 5.4 Hz, 1H), 7.77 (s, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.67 (t, J = 7.6 Hz, 2H), 7.55 (t, J = 7.5 Hz, 1H), 7.49 (dd, J = 6.0, 3.1 Hz, 2H), 7.44 (s, 1H), 7.34 (d, J = 7.7 Hz, 2H), 6.90 – 6.81 (m, 1H). ^{13}C-NMR(126 MHz, $CDCl_3$) δ 158.44, 147.59, 138.81, 137.73, 137.63, 133.66, 130.51, 129.99, 129.33, 128.41, 128.07, 127.86, 127.58, 126.41, 123.00, 120.11, 116.03, 112.81, 109.87. ESI-MS = $[M+H]^+$, m/z = 324.1478. Anal. Calcd. for $C_{22}H_{17}N_3$ (MW=323): C, 81.71; H, 5.30; N, 12.99. Found: C, 81.93; H, 5.41; N, 12.87.

1.3. Synthesis of complex Ru-1

For the synthesis of **Ru-1**, 0.072 mmol (26.11 mg) of ligand L-1 was added to the solvents dicholoromethane and ethanol in the ratio of 1:1, followed by the addition of 0.036 mmol (22.03 mg) ruthenium precursor, *i.e.*, $[Ru(p\text{-cymene})Cl_2]_2$, The aforementioned solution was stirred at room temperature (RT) up to 2 hours. Further in the same mixture, 0.04 mmol of NH_4PF_6 was added and

agitated for 30-40 minutes. A yellow-coloured precipitate was acquired by a filtration method. Spectroscopic techniques such as UV-Vis, FT-IR, and single-crystal X-ray crystallography were used to validate the molecular structure of Ru-1. Yield: 21.56 mg, 75.90 %), Selected IR frequency: (potassium bromide, ν/cm^{-1}): 1598 (υ_{imine}), UV-Vis: [DCM, (λ_{max}/nm (ε/M^{-1} cm^{-1})]: 7692(395), 7692(374), 875(323), 76346(257). ^{1}H NMR (500 MHz, CD_3CN) δ 8.90 (s, 1H), 8.79 (s, 1H), 8.75 (d, J = 5.8 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.19 (dd, J = 28.8, 8.6 Hz, 2H), 7.95 – 7.85 (m, 3H), 7.83 – 7.65 (m, 6H), 7.62 – 7.56 (m, 1H), 7.12 (t, J = 6.6 Hz, 1H), 6.33 (d, J = 8.7 Hz, 1H), 2.19 (s, 6H), 2.15 (dq, 1H), 1.01 (s, 3H). ^{13}C NMR (126 MHz, CD_3CN) δ 156.35, 153.28, 141.62, 136.23, 133.70, 133.14, 132.14, 131.28, 130.87, 130.48, 129.49, 129.02, 128.42, 127.64, 127.42, 125.11, 119.49, 111.23, 31.32, 21.55, 18.28. ESI-MS = $[M\text{-}H\text{-}PF_6]^+$, m/z = 643.1371. Anal. Calcd. for $C_{36}H_{33}ClF_6N_3PRu$ (MW=789): C, 54.79; H, 4.22; N, 5.32. Found: C, 54.94; H, 4.37; N, 5.17.

1.4. Synthesis of complex Ru-2

For the synthesis of **Ru-2**, 0.082 mmol (25.84 mg) of L-2 was taken in solvents system dichloromethane and ethanol in the ratio of 1:1, followed by the addition of 0.041 mmol (25.10 mg) of ruthenium precursor, *i.e*., $[Ru(p\text{-cymene})Cl_2]_2$, then the aforementioned solution was stirred for 2 hours at RT (25 °C). Again, 0.04 mmol of NH_4PF_6 was added to the same mixture and stirred for 30-40 minutes; a yellow-coloured precipitate was collected by filtration. Spectroscopic techniques such as UV-Vis, FT-IR, ^{1}H/^{13}C NMR, and single crystal X-ray crystallography were used to characterize the molecular structure of **Ru-2**. Yield: 24.26 mg, 80.00 %), Selected FT-IR frequency: (potassium bromide, ν/cm^{-1}): 1604 (υ_{imine}), UV-Vis: [DCM, (λ_{max}/nm (ε/M^{-1} cm^{-1})]: 14553(352), 22832(276), 28758(230). ^{1}H NMR(500 MHz, $CDCl_3$) δ 8.72 (d, J = 10.0 Hz, 2H), 8.21 (s, 1H), 8.18 (d, J = 8.5 Hz, 1H), 8.03 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 7.2 Hz, 2H), 7.68 (t, J = 7.5 Hz, 1H), 7.65 – 7.58 (m, 3H), 7.43 (s, 2H), 7.12 (t, J = 6.5 Hz, 1H), 6.15 (d, J = 8.6 Hz, 1H), 5.49 (d, J = 6.1 Hz, 1H), 5.35 (d, J = 5.8 Hz, 1H), 5.14 (d, J = 6.0 Hz, 1H), 4.53 (d, J = 5.8 Hz, 1H), 2.75 (dq, J = 13.6, 6.8 Hz, 1H), 2.16 (s, 3H), 1.20 (d, J = 6.8 Hz, 3H), 1.06 (d, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.94, 155.06, 153.71, 141.38, 135.45, 134.21, 132.95, 132.47, 131.54, 130.79, 129.28, 128.98, 128.58, 127.95, 126.99, 119.16, 110.05, 88.44, 86.06, 85.87, 31.04, 22.45, 22.05, 18.89. ESI-MS = $[M\text{-}PF_6]^+$, m/z = 594.1207,. Anal. Calcd. for $C_{32}H_{31}ClF_6N_3PRu$ (MW=739): C, 52.00; H, 4.23; N, 5.69. Found: C, 52.41; H, 4.09; N, 5.79.

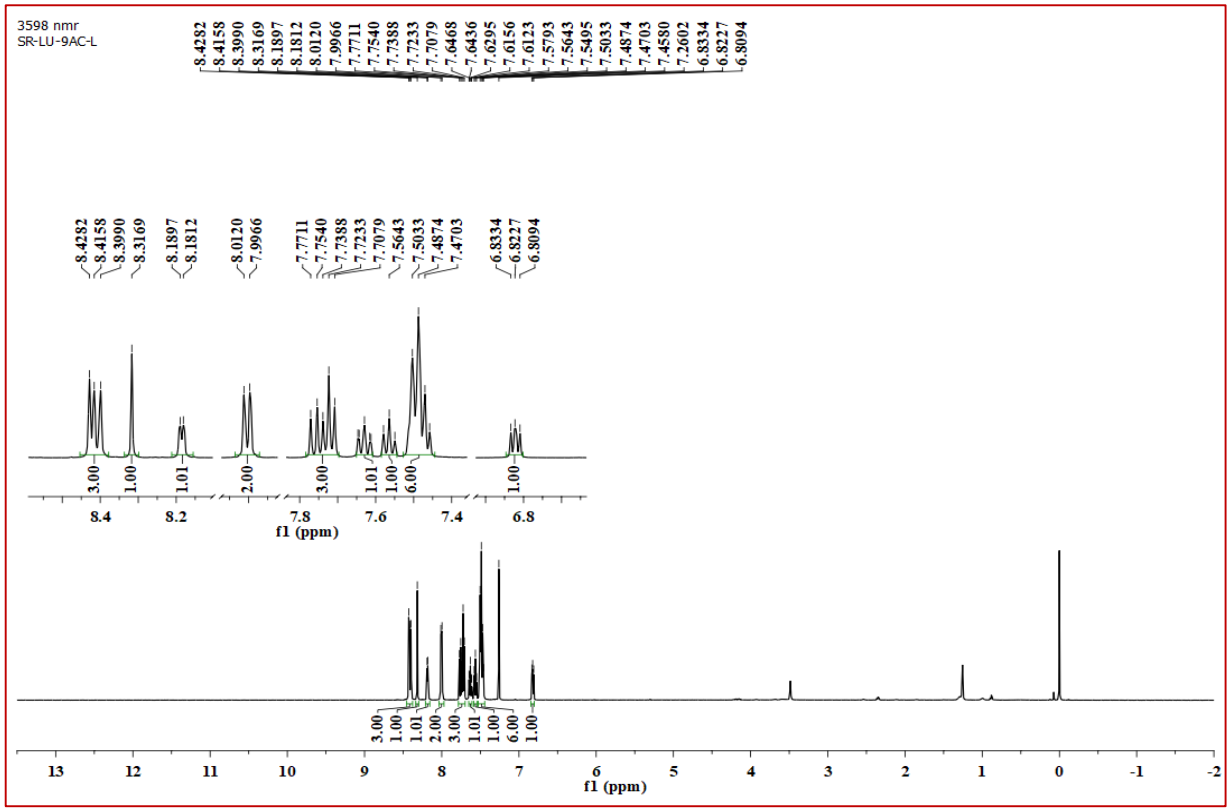

Figure S1. ^{1}H NMR spectrum of L-1 taking $CDCl_3$ as solvent.

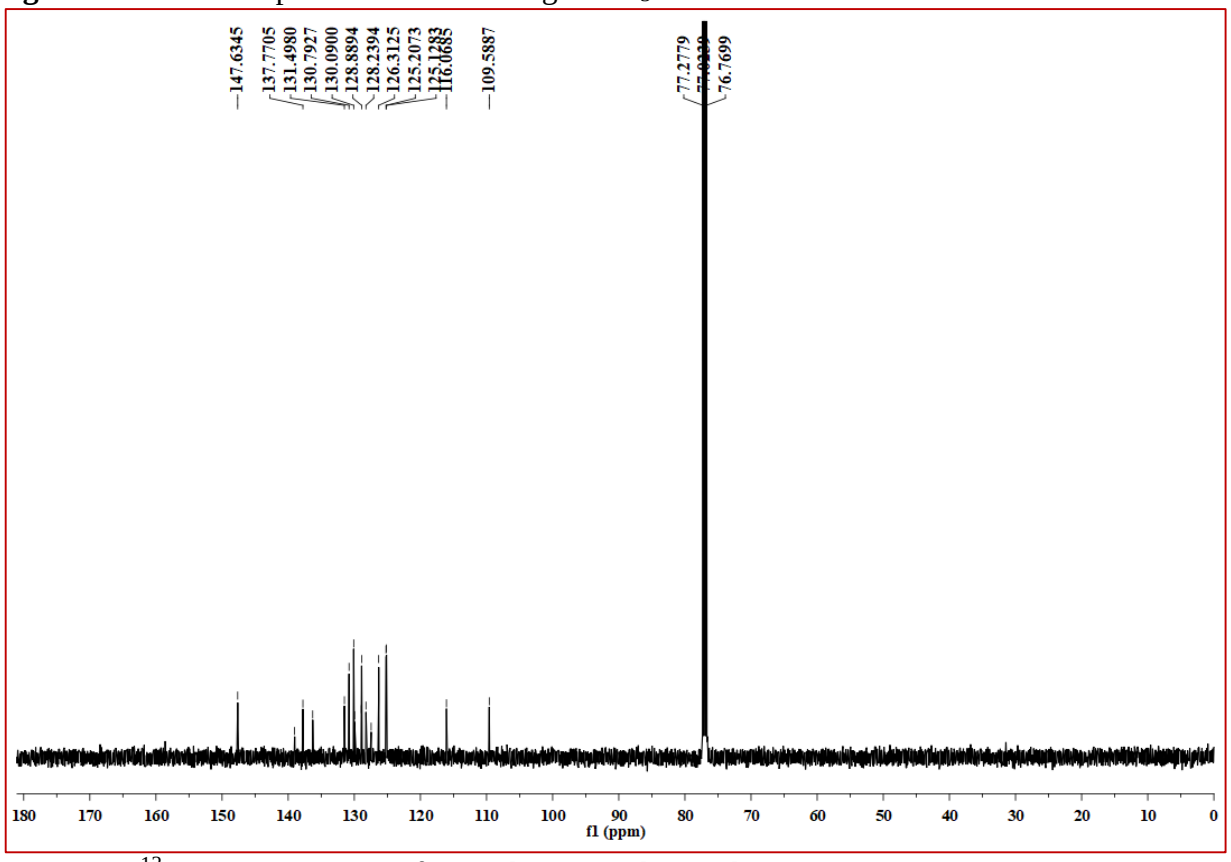

Figure S2. ^{13}C NMR spectrum of L-1 taking $CDCl_3$ as solvent.

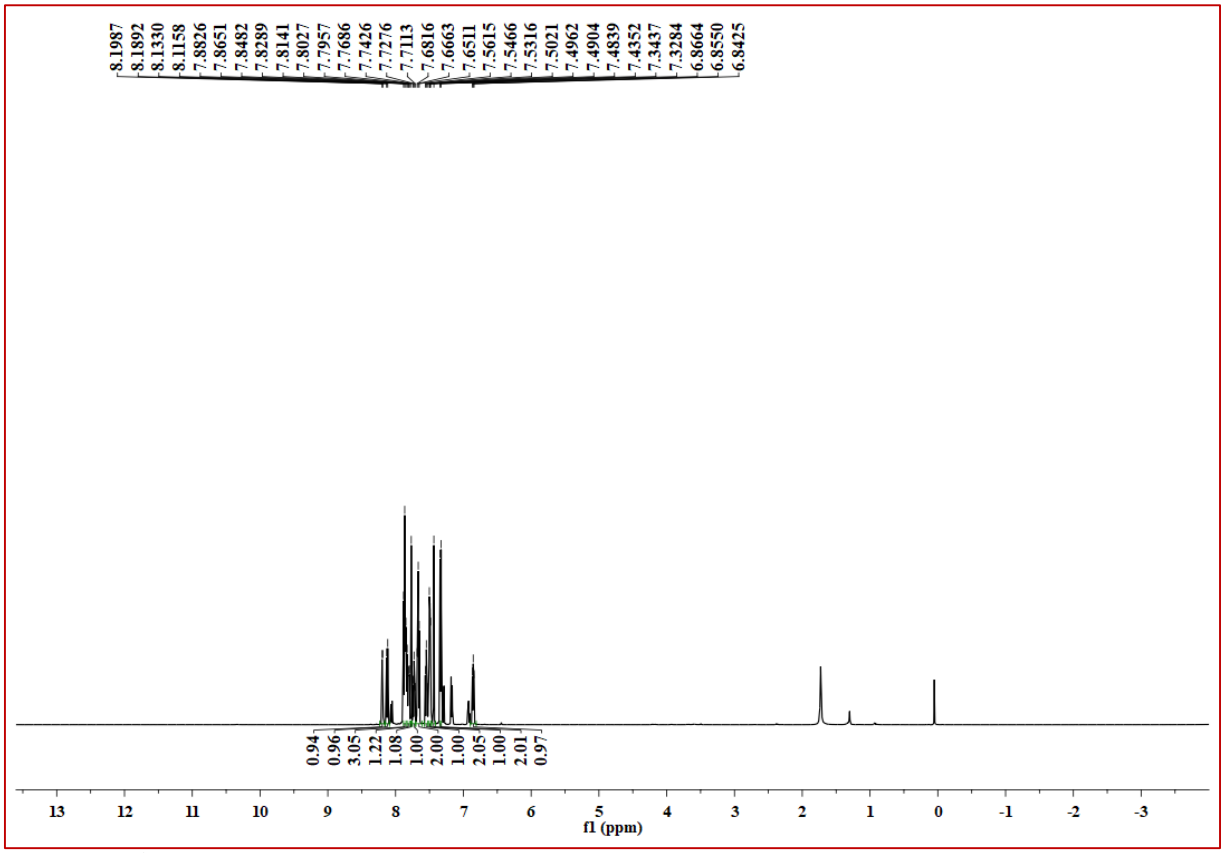

Figure S3. ^{1}H NMR spectrum of L-2 taking $CDCl_3$ as solvent.

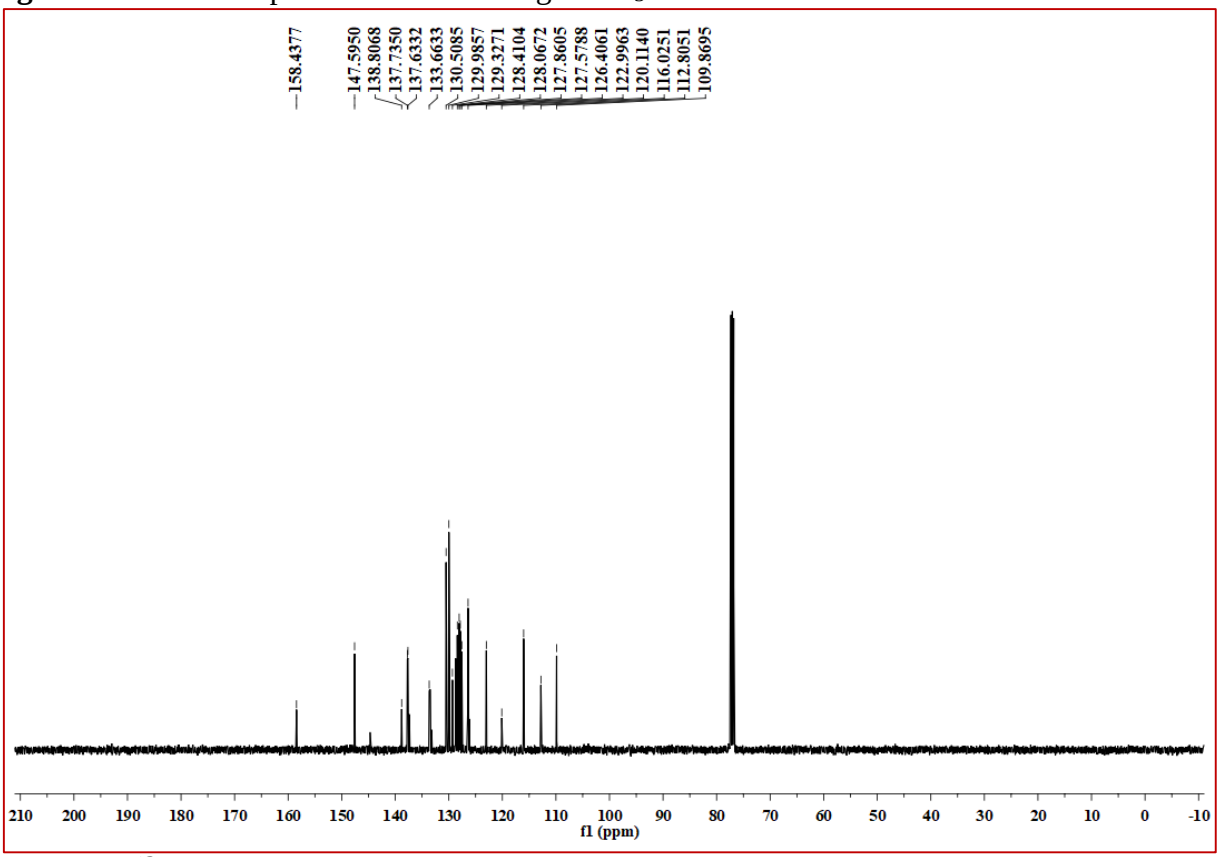

Figure S4. ^{13}C NMR spectrum of L-2 taking $CDCl_3$ as solvent.

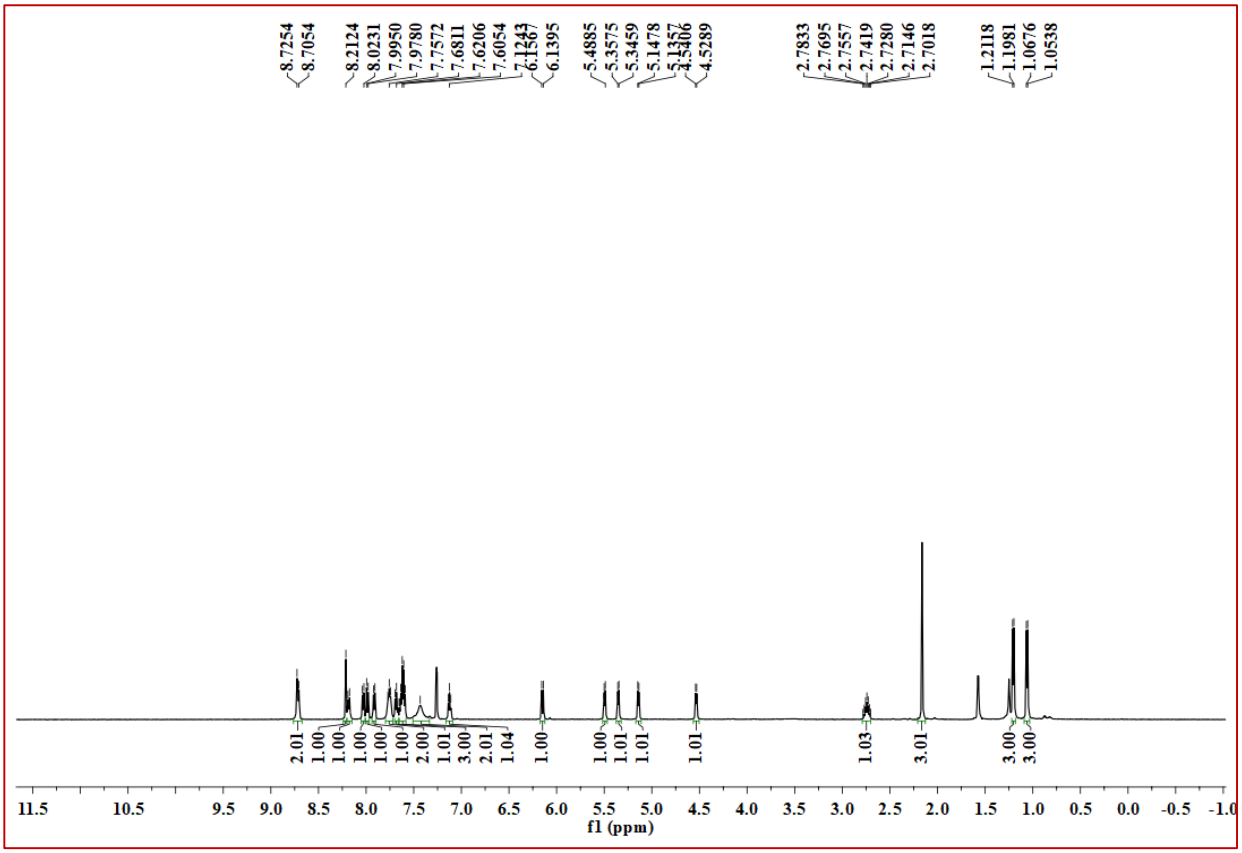

Figure S5. ^{1}H NMR spectrum of Ru-2 taking $CDCl_3$ as solvent.

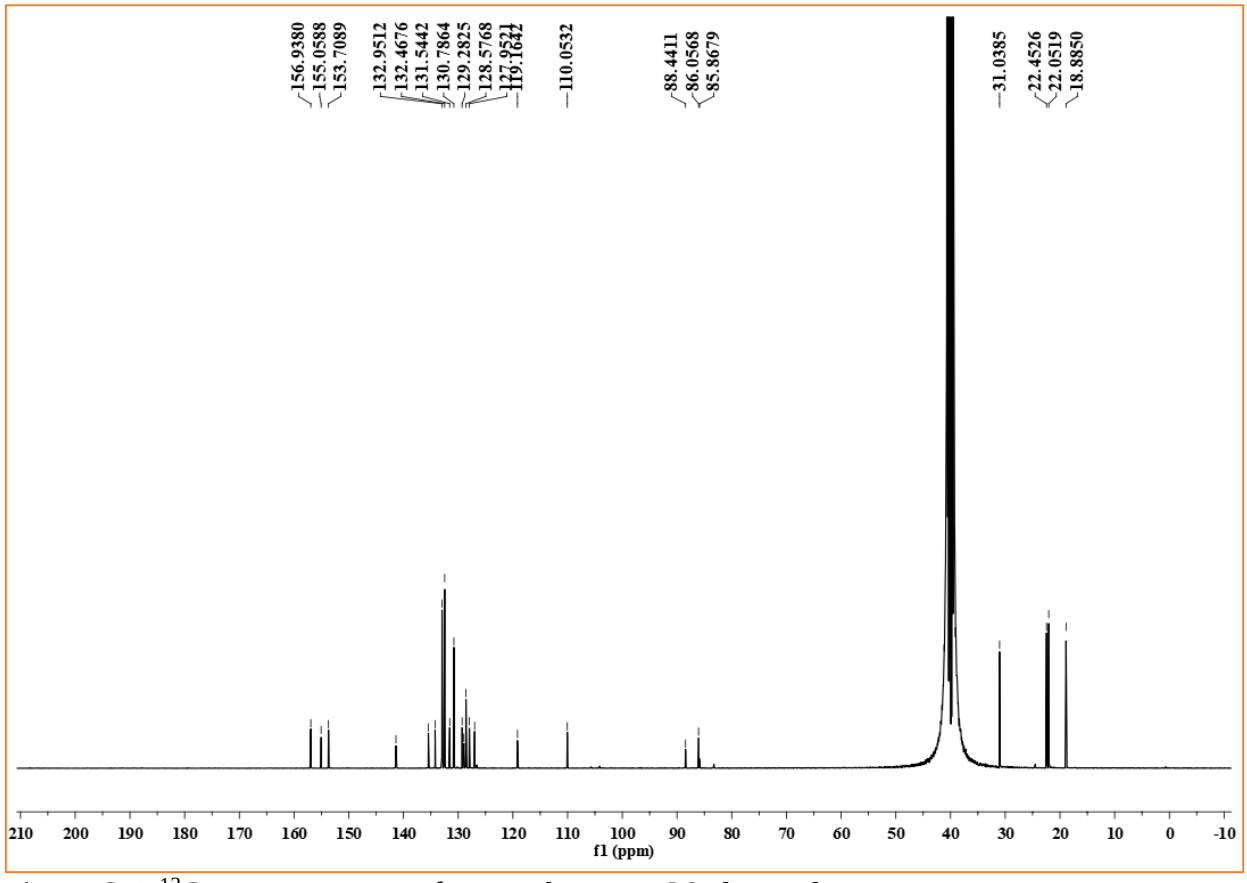

Figure S5[a]. ^{13}C NMR spectrum of Ru-2 taking DMSO-d_6 as solvent.

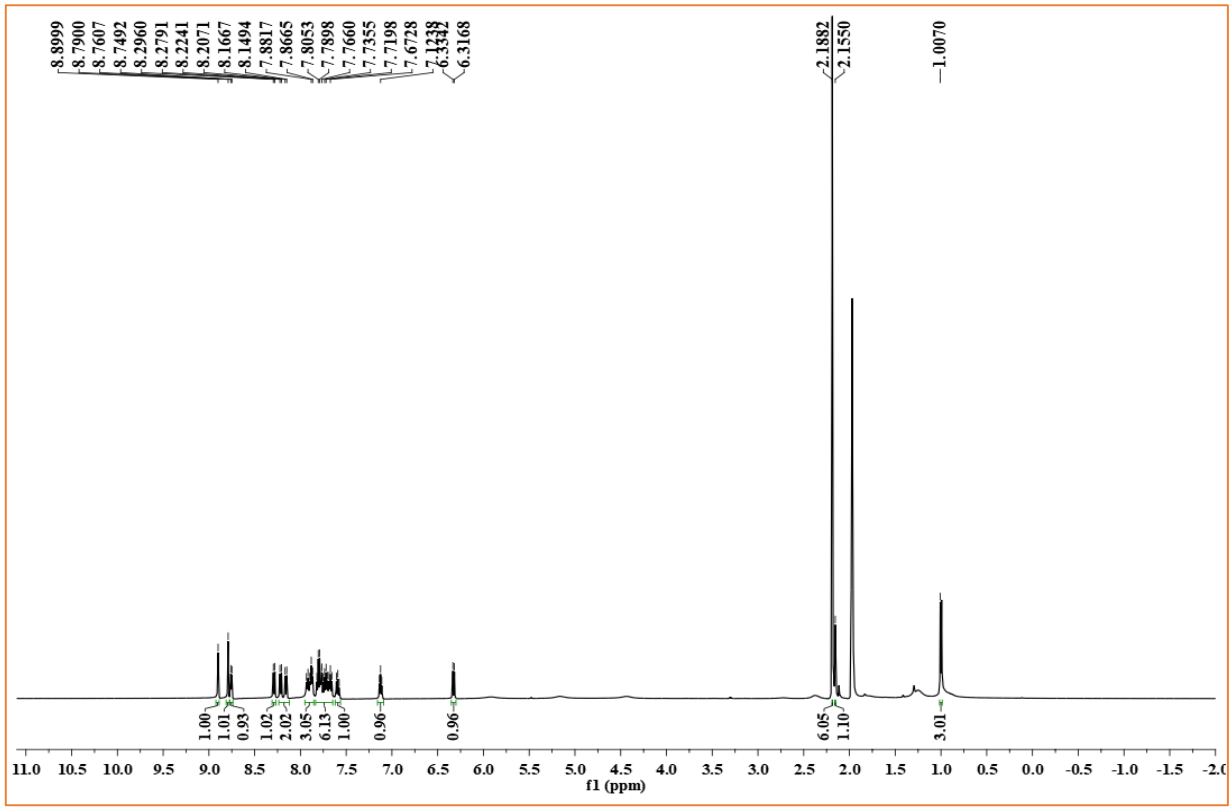

Figure S5[b]. ^{1}H NMR spectrum of Ru-1 taking CD_3CN as solvent.

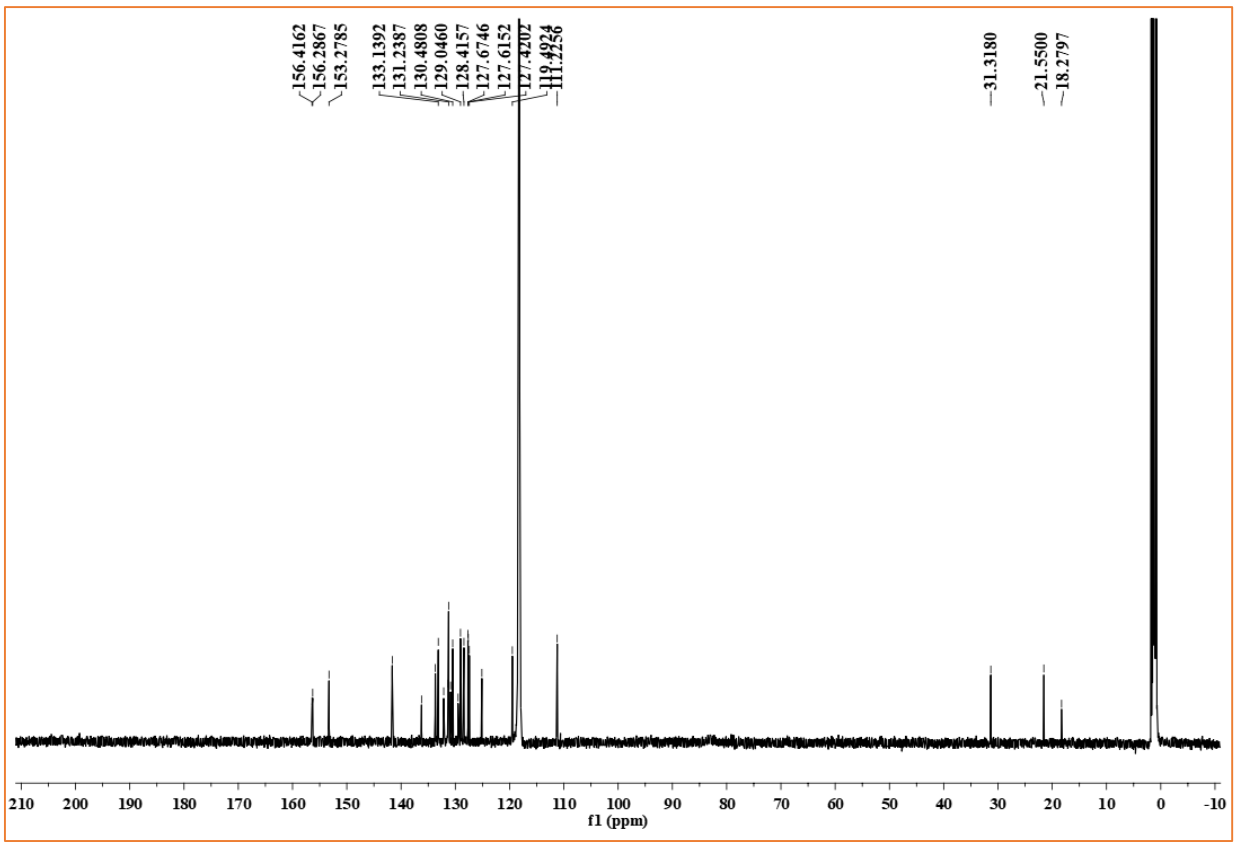

Figure S5[c]. ^{13}C NMR spectrum of Ru-1 taking CD_3CN as solvent.

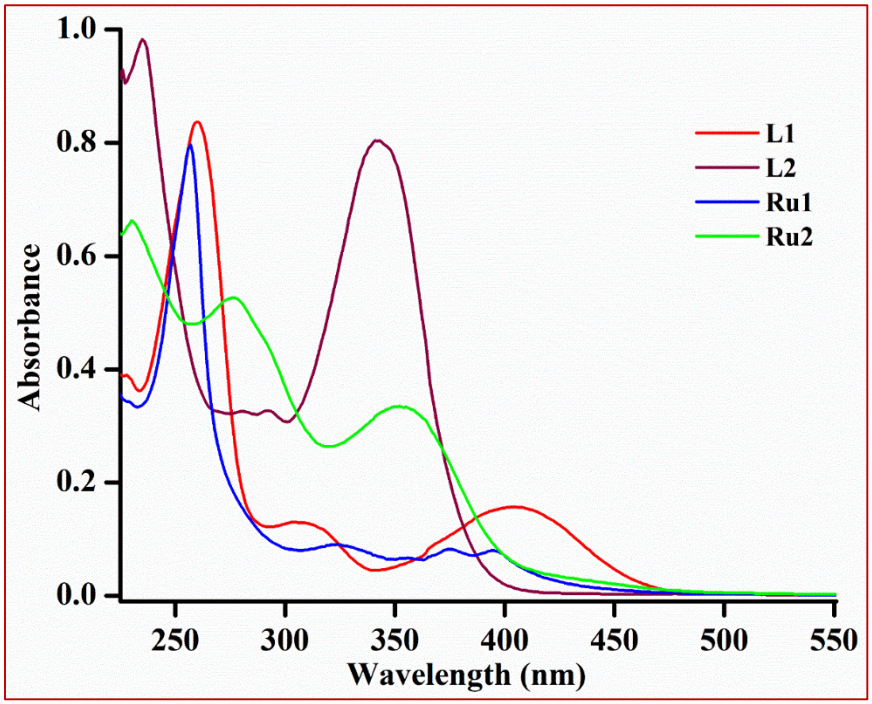

Figure S6. Electronic absorption spectra of ligands (L-1 and L-2) and complexes (Ru-1 and Ru-2) in CH_2Cl_2 solution

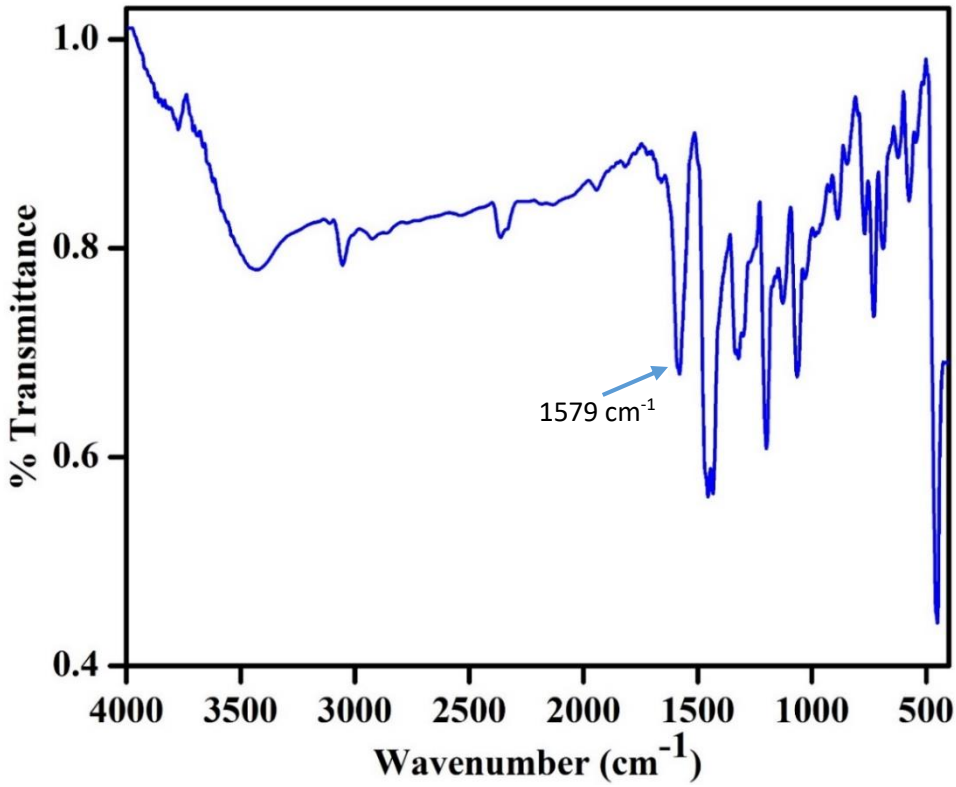

Figure S7. FT-IR spectrum of free ligand L-1

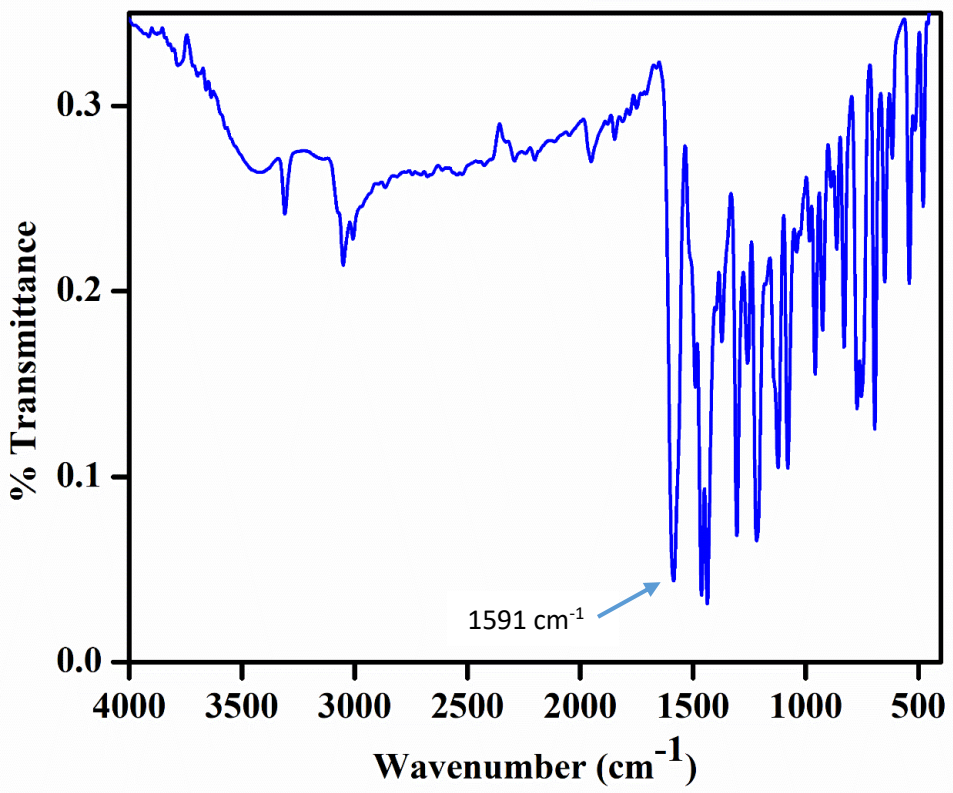

Figure S7[a]. FT-IR spectrum of free ligand L-2

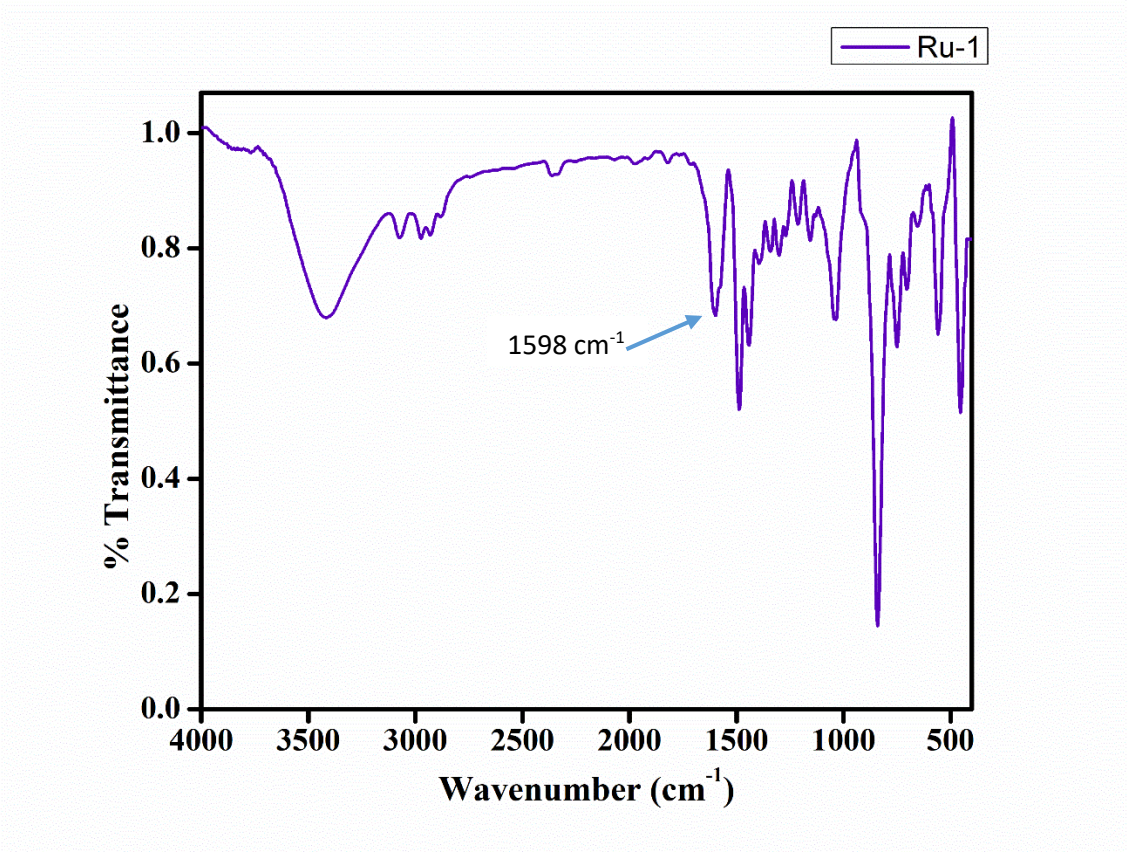

Figure S8. FT-IR spectrum of free ligand Ru-1.

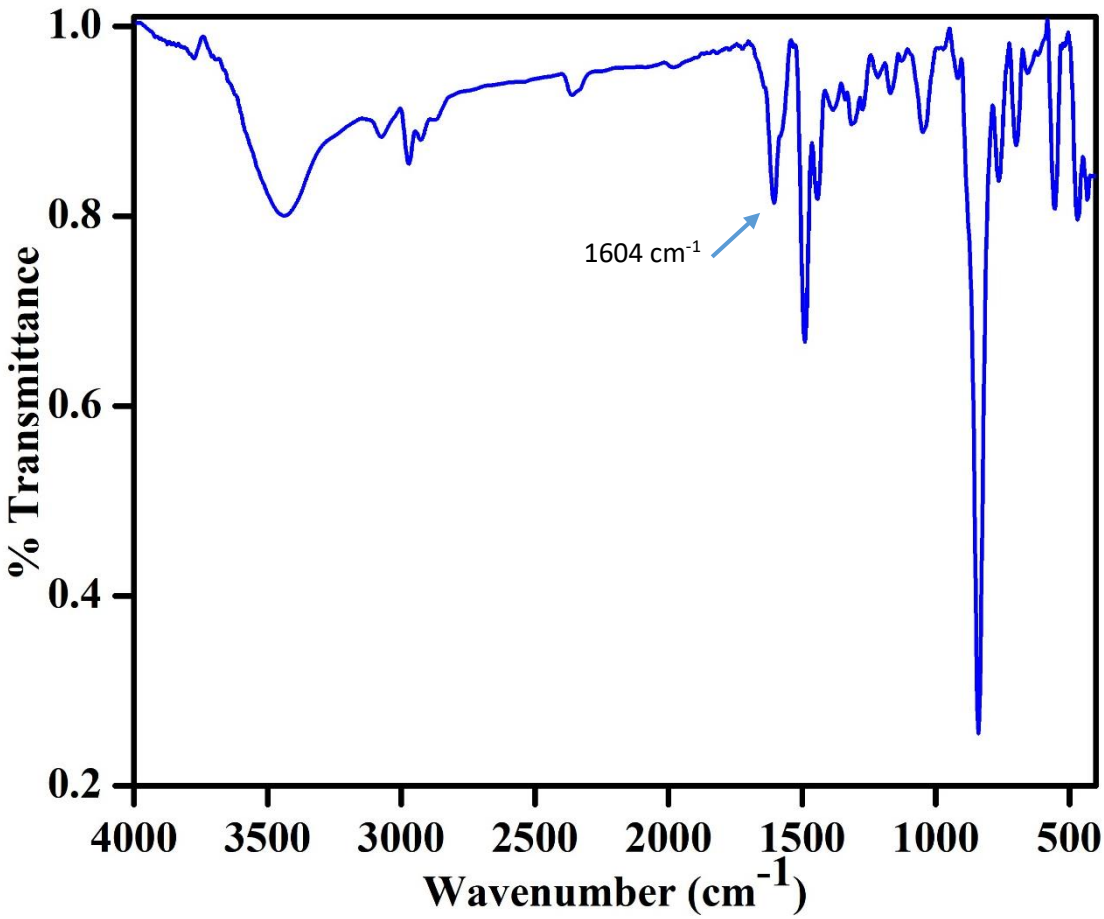

Figure S8[a]. FT-IR spectrum of free ligand Ru-2

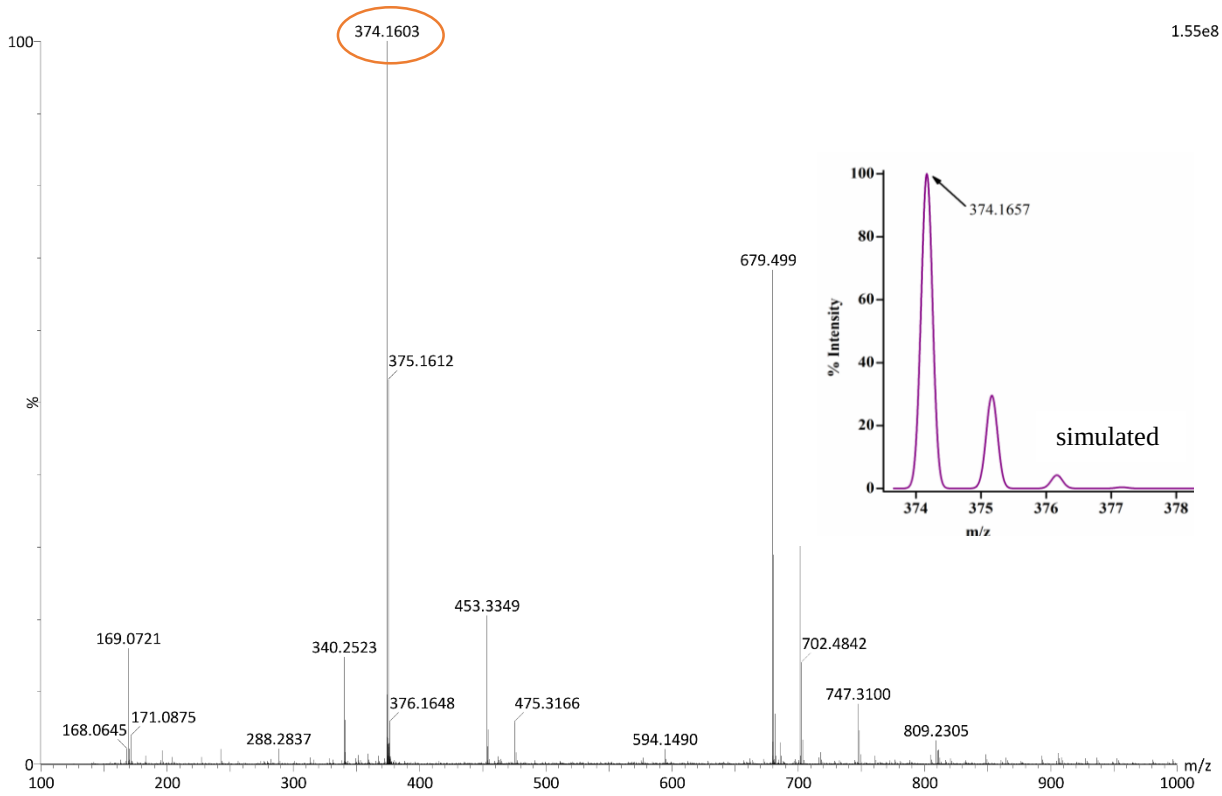

Figure S9. ESI-MS of ligand L-1, $[M+H]^+$, m/z = 374.160, taking CH_3CN as solvent.

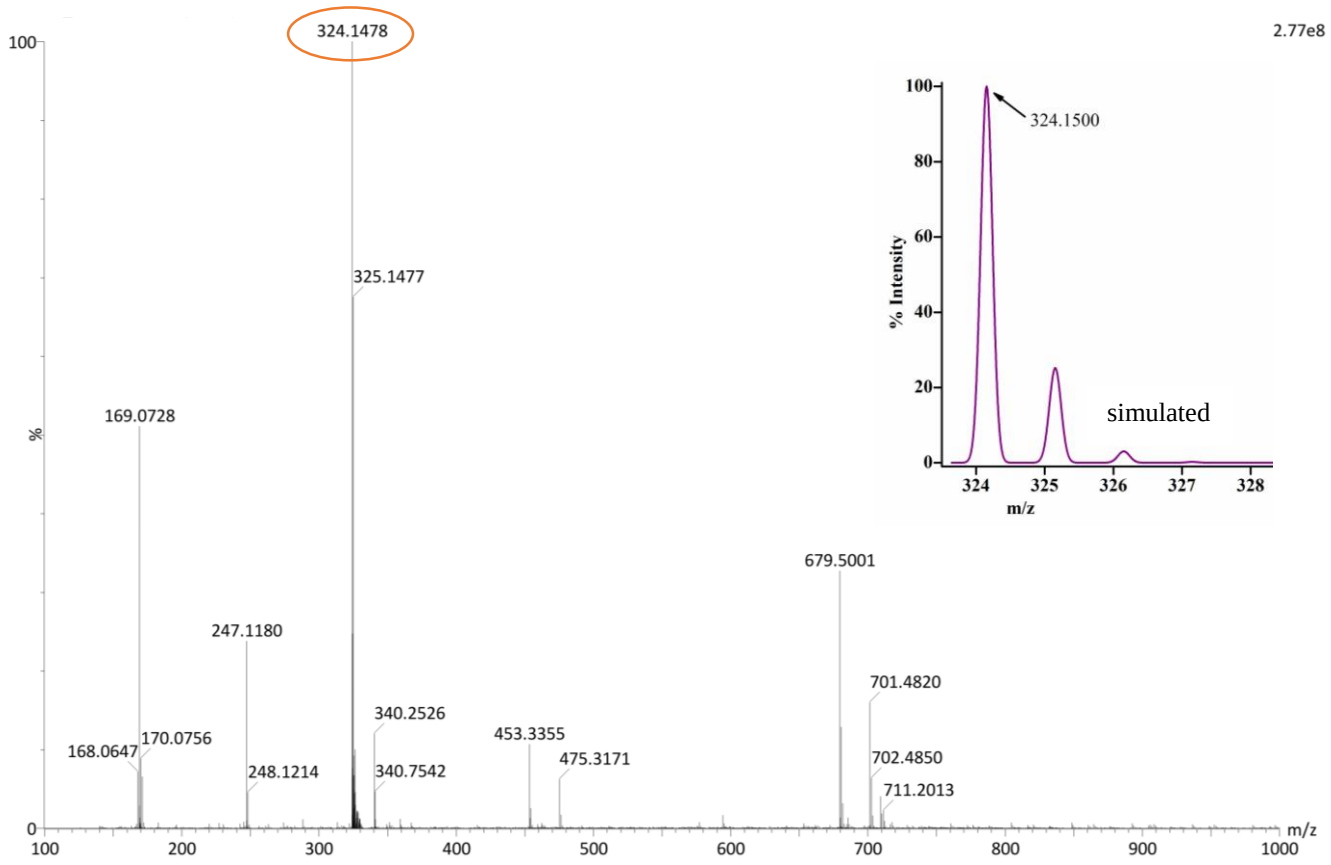

Figure S9[a]. ESI-MS of ligand L-2, $[M+H]^+$, m/z = 324.1478, taking CH_3CN as solvent.

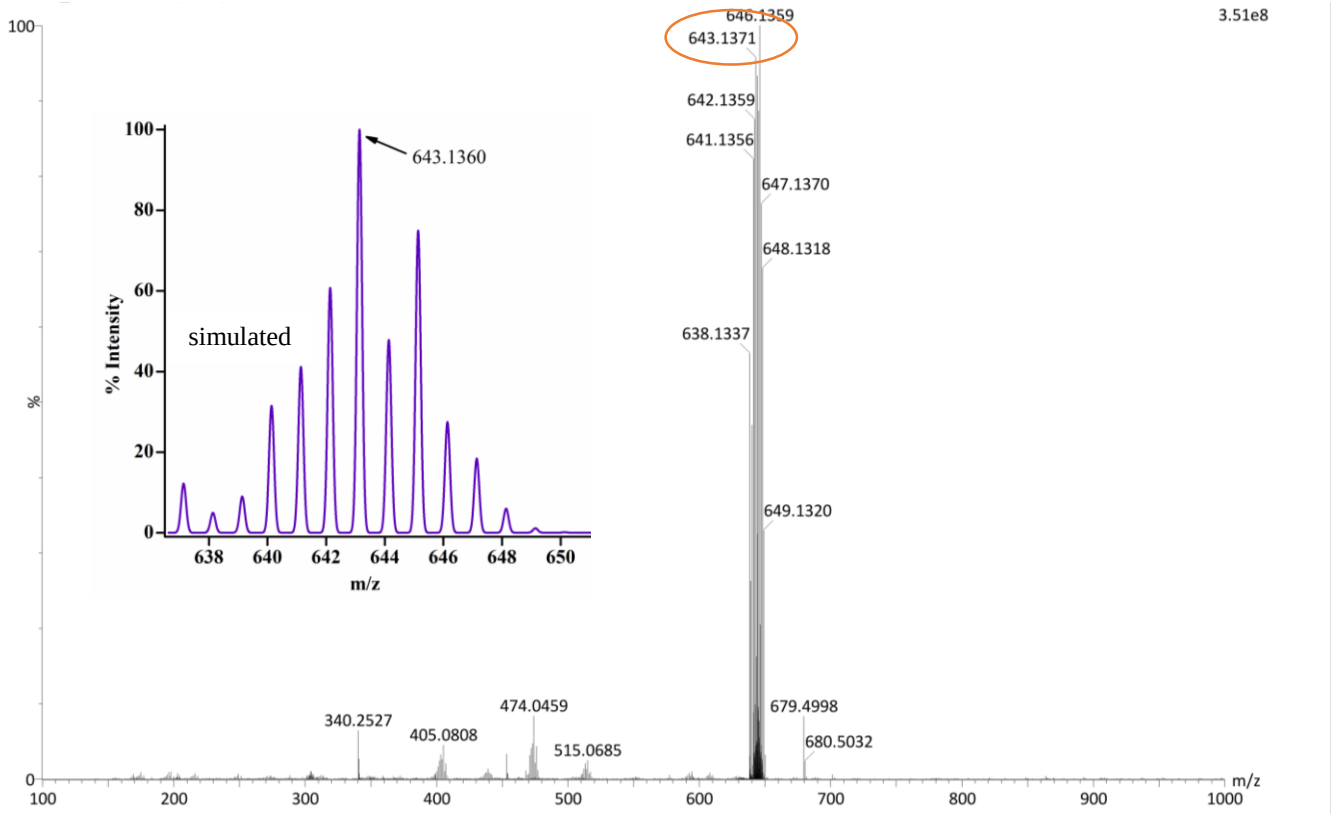

Figure S10. ESI-MS of Ru-1, $[M\text{-}H\text{-}PF_6]^+$, m/z = 643.1371, taking CH_3CN as solvent.

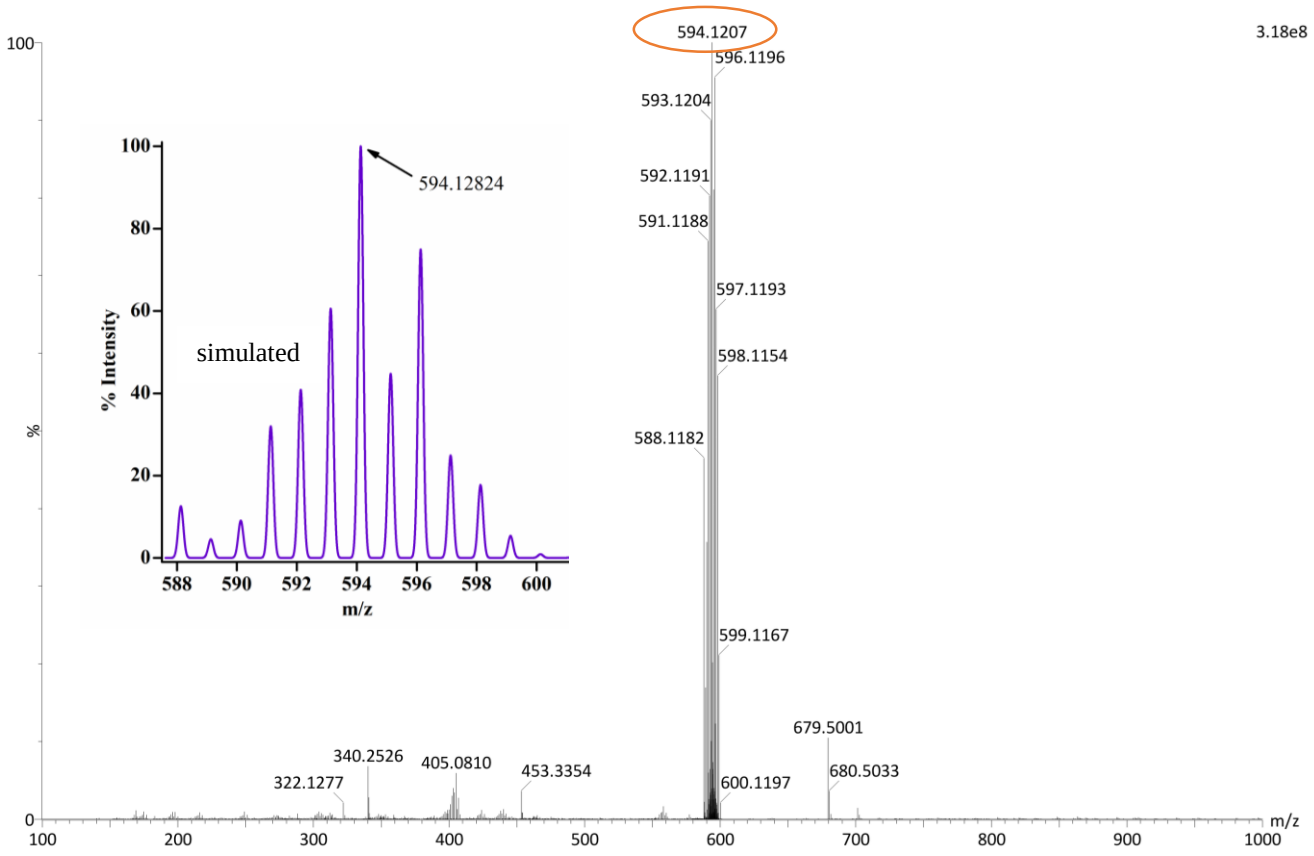

Figure S10[a]. ESI-MS of ligand Ru-2, $[M\text{-}PF_6]^+$, m/z = 594.1207, taking CH_3CN as solvent.

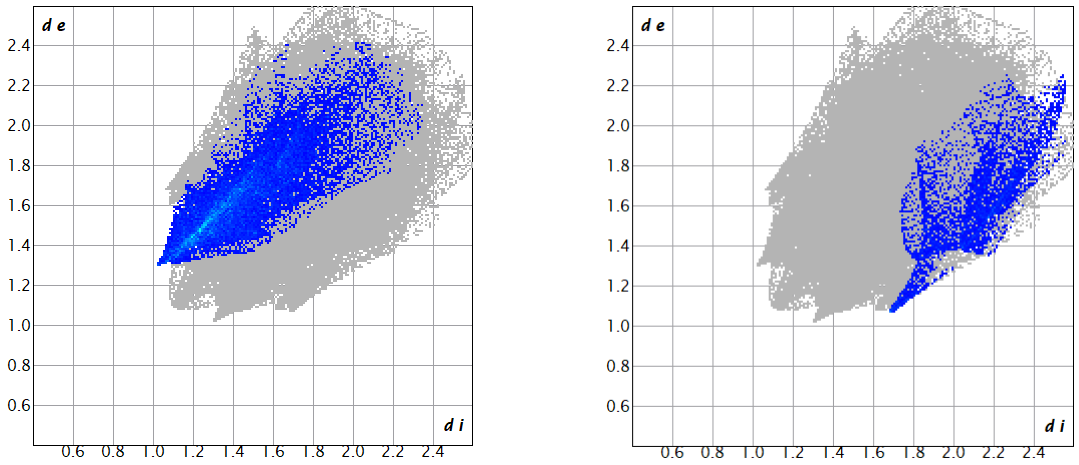

Figure S11. Hirshfeld surface analysis for weak interactions in Ru-2: (H-F type of interaction 12.6 %, left side) and (H-Cl type of interaction 3.6 %, right side)

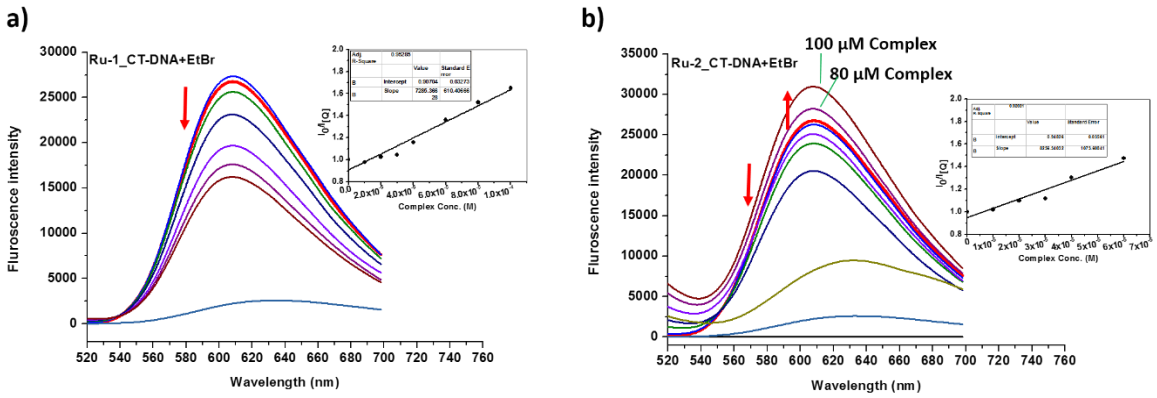

Figure S12. Fluorescence emission spectra (quenching curve) of CT-DNA+EtBr upon addition of Ru-1 (a) and Ru-2 (b) complexes. Inset: Stern-Volmer plots of fluorescence quenching of CT-DNA+EtBr with Ru-complexes. (K_{SV} for Ru-1: 8.03×10^3 M^{-1}; and Ru-2: 8.71×10^3 M^{-1}).

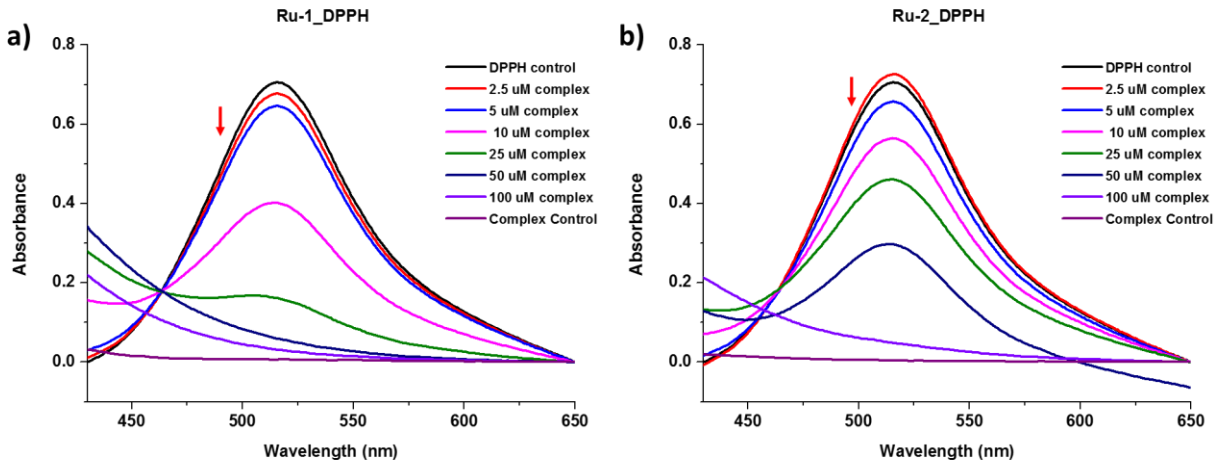

Figure S13. Antioxidant activity: DPPH assay fluorescence scan for Ru-1 (a) and Ru-2 (b).

Methodology: The H-atom or electron-donating ability of the metal complex was measured from the bleaching of the purple-coloured methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH) as per the previous report.[1] This spectrophotometric assay uses the stable radical DPPH as a reagent. Ru-1 and Ru-2 complexes (5, 10, 25, 50, 100 and 150 µg mL-1) were prepared in methanol solution, 1.6 mL of complex solution was added to 2.4 mL of (0.004 % w/v or 0.1 mM) DPPH solution in methanol. Reactions were mixed well and incubated in the dark for 30 min at RT. The absorbance scan was taken against the blank. The absorbance at 517 nm was monitored.

Ref.

1. M. H. Shaikh, D. D. Subhedar, B. B. Shingate, F. A. Kalam Khan, J. N. Sangshetti, V. M. Khedkar, L. Nawale, D. Sarkar, G. R. Navale and S. S. Shinde, Med. Chem. Res., 2016, 25, 790–804.

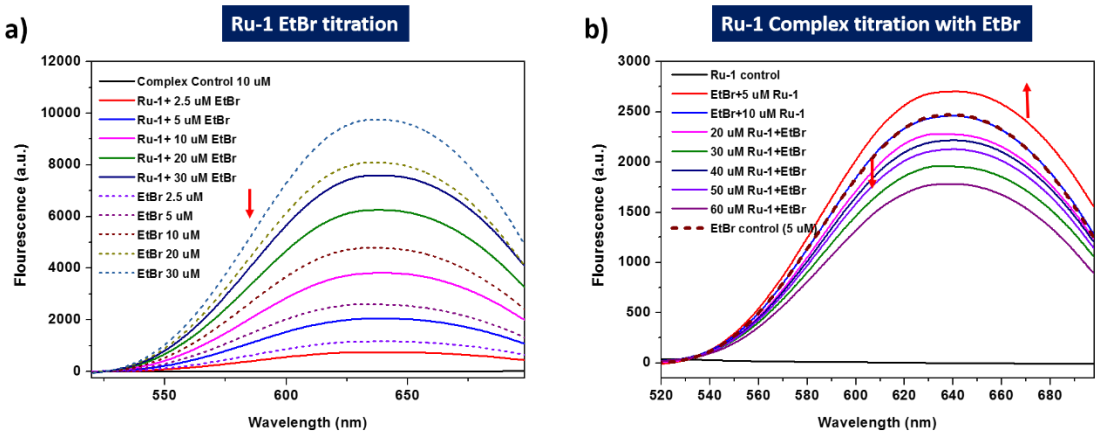

Figure S14. Fluorescence emission spectra of titration of EtBr with Ru-1 complex (**a**) [Ru-1: 10 µM and EtBr (0-30 µM)]; and Ru-1 complex with EtBr(**b**) [EtBr: 5 µM and complex (10-60 µM)].

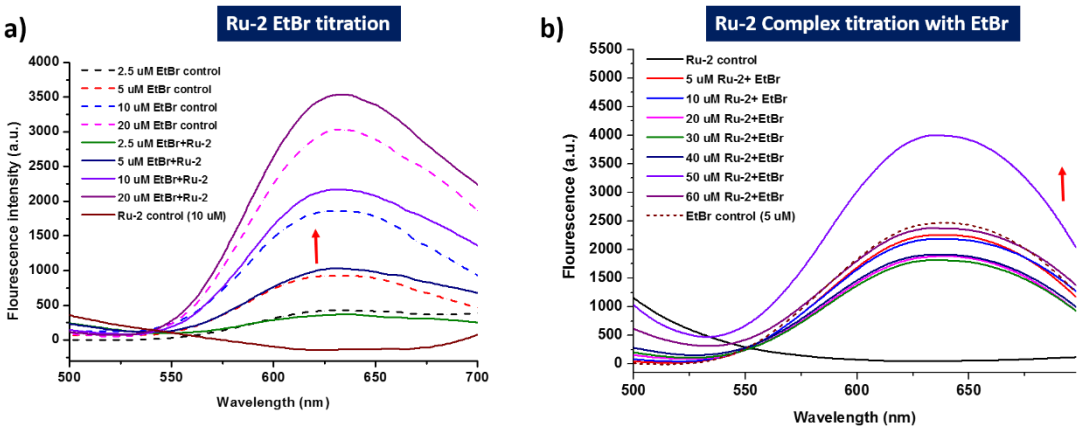

Figure S15. Fluorescence emission spectra of titration of EtBr with Ru-2 complex (**a**) [Ru-2: 10 µM and EtBr (0-30 µM)]; and Ru-2 complex with EtBr (**b**) [EtBr: 5 µM and complex (10-60 µM)].

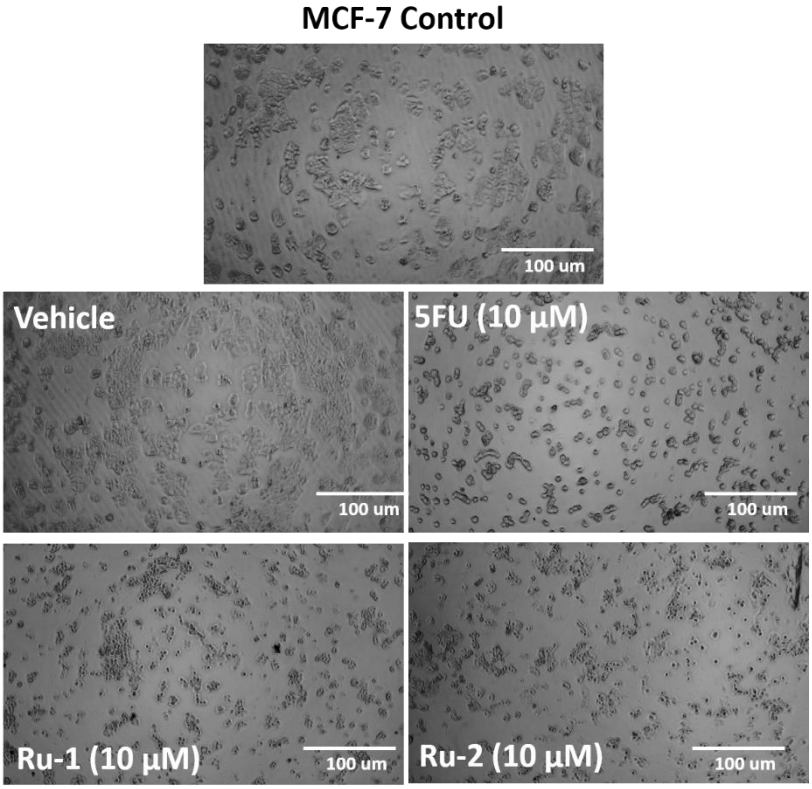

Figure S16. Observation of MCF-7 cells after treatment of 10 µM of Ru-complexes and standard drug 5FU (10 µM).

Table S1. Selected bond lengths (Å) for complex Ru-2		
S. N.	**Atoms**	**Bond Length**
1	**Ru1-Cl1**	2.3960(6)
2	**Ru1-N3(py)**	2.066(2)
3	**Ru1-N1(imine)**	2.122(2)
4	**Ru1-C1**	2.215(2)
5	**Ru1-C2**	2.177(2)
6	**Ru1-C3**	2.187(2)
7	**Ru1-C4**	2.253(2)
8	**Ru1-C5**	2.242(2)
9	**Ru1-C6**	2.177(2)

Table S2. Selected bond angles (º) for complex Ru-2			
Atoms	**Bond Angles**	**Atoms**	**Bond Angles**
Cl1-Ru1-N3	83.73(5)	**N1-Ru1-N3**	76.47(6)
Cl1-Ru1-N1	89.09(5)	**N3-Ru1-C5**	165.36(7)
Cl1-Ru1-C5	110.80(5)	**N3-Ru1-C3**	111.19(7)
Cl1-Ru1-C3	96.62(5)	**N3-Ru1-C2**	90.83(7)
Cl1-Ru1-C2	127.13(6)	**N3-Ru1-C1**	97.78(7)
Cl1-Ru1-C1	164.61(5)	**N3-Ru1-C4**	147.13(7)
Cl1-Ru1-C4	89.89(5)	**N3-Ru1-C6**	128.53(7)
Cl1-Ru1-C6	146.80(6)	**N1-Ru1-C5**	104.63(7)
N1-Ru1-C3	17083(7)	**N1-Ru1-C2**	140.44(7)
N1-Ru1-C1	106.19(7)	**N1-Ru1-C4**	135.81(7)

Table 3. Crystal data and structural refinement parameters for complex Ru-2			
Empirical Formula	$C_{32}H_{31}ClN_3Ru$, F_6P	**Z**	4
Colour	Yellow	**ρcalc(gcm^{-3})**	1.600
Formula Weight	739	**F(000)**	1496.0
Temperature (K)	100 K	**θ range for data collection**	2.590 – 32.742
λ (Å) (Mo-Kα)	0.71073	**Index ranges**	-19<h<19, -21<k<21, -24<l<24
Crystal System	monoclinic	**Refinement method**	Full matrix least squares on F^2
Space Group	P 21/c	**Data/restraint/ parameters**	10758/0/400
a(Å)	12.6841(3)	**GOF[a] on F^2**	1.159
b(Å)	14.4742(3)	**R1[b] [I > 2σ(I)]**	0.0409
c(Å)	16.9097(4)	**R1 (all data)**	0.0497
α(º)	90	**wR2[c] (I > 2σ(I))**	0.0960
β(º)	98.756(2)	**wR_2 (all data)**	0.1041(10758)
γ(º)	90		

[a]GOF = $[\Sigma[w(F_o^2-F_c^2)^2]/M-N)]^{1/2}$ (M = number of reflections, N = number of parameters refined). [b]R1 = $\Sigma \| F_o | - | F_c \| / \Sigma | F_o$, [c]$wR_2 = [\Sigma[w(F_o^2-F_c^2)^2]/\Sigma [w(F_o^2)^2]]^{1/2}$