SUPPLEMENTARY INFORMATION FOR

Investigations on Antiproliferative Activity and Apoptosis Mechanism of New Arene Ru(II) Carbazole based Hydrazone Complexes

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CONTENTS

Materials and Methods

Best commercial grade reactants and solvents were used for all the reactions. $RuCl_3.3H_2O$, Benzohydrazide, 4-bromobenzohydrazide and 4-methoxybenzohydrazide were purchased from sigma Aldrich. 2,3,4,9-tetrahydro-1H-carbazol-1-one[1]and dimer ruthenium precursor $[(\eta^6\text{-benzene})RuCl_2]_2$ were prepared[2] from the procedures as specified in the literature. The CHNS elementarVario EL III analyser was used to carry out the elemental analysis of Carbon, Hydrogen, Nitrogen at the Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science and Technology, Cochin. FT-IR spectra were recorded in KBR pellets in the range 4000–400 cm^{-1} with Perkin-Elmer 597 spectrophotometer. Electronic spectra were recorded in chloroform solution using CARY 300 Bio UV-visible Varian spectrometer. The NMR spectral studies were carried out on a Bruker 400MHz spectrometer in presence of $CDCl_3$/[d6]-DMSO solvent using tetramethylsilane (TMS) as internal reference. Gemini A Ultra four-circle diffractometer with monochromated Mo-Kα radiation (λ = 0.71073 Å) was used to collect the X-ray intensity data at room temperature. Lorentz, polarization and empirical absorption correction using spherical harmonics implemented in SCALE3 ABSPACK scaling algorithm[3] were applied. The structure was solved by the Patterson method and subsequently completed by the difference Fourier recycling. All the non-hydrogen atoms were refined anisotropically using the full-matrix, least-squares technique. The Olex2[4] and SHELXS, SHELXL[5] programs were used for all the calculations. Atomic scattering factors were incorporated in the computer programs.

Table S1. Crystal data and structure refinement for the ligands and complexes

Identification code	**HL2**	**HL3**	**Complex 1**	**Complex 2**	**Complex 3**
CCDC number	1872212	1872214	1916478	1872211	1916479
Empirical formula	$C_{19}H_{16}BrN_3O$	$C_{20}H_{21}N_3O_3$	$C_{25}H_{22}ClN_3ORu$	$C_{25}H_{21}BrClN_3ORu$	$C_{26}H_{24}ClN_3O_2Ru$
Formula weight	382.26	351.40	516.97	595.88	547.00
Temperature/K	295(2)	295(2)	295(2)	295(2)	295(2)
Crystal system	orthorhombic	monoclinic	orthorhombic	Monoclinic	monoclinic
Space group	$Pna2_1$	$P2_1/c$	$P2_12_12_1$	$P2_1/n$	$P2_1/n$
a/Å	23.856(4)	10.8358(6)	9.5226(3)	11.6369(7)	11.4635(3)
b/Å	8.3192(18)	7.4023(4)	12.4404(5)	12.9702(7)	12.8709(4)
c/Å	17.006(3)	23.0943(14)	18.1571(7)	15.9325(8)	16.2088(4)
α/°	90	90	90	90	90
β/°	90	96.837(6)	90	109.950(6)	108.314(3)
γ/°	90	90	90	90	90
Volume/Å^3	3375.1(11)	1839.21(18)	2150.96(14)	2260.4(2)	2270.40(12)
Z	8	4	4	4	4
ρ_{calc}g/cm^3	1.505	1.269	1.596	1.751	1.600
μ/mm^{-1}	2.446	0.087	0.876	2.603	0.838
F(000)	1552.0	744.0	1048.0	1184.0	1112.0
Crystal size/mm^3	0.42 × 0.16 × 0.13	0.28 × 0.21 × 0.11	0.24 × 0.17 × 0.11	0.37 × 0.11 × 0.06	0.31 × 0.11 × 0.07
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.832 to 58.928	6.796 to 58.986	6.924 to 58.862	6.846 to 58.866	6.862 to 58.958
Index ranges	-32 ≤ h ≤ 30, -7 ≤ k ≤ 11, -16 ≤ l ≤ 23	-10 ≤ h ≤ 14, -7 ≤ k ≤ 10, -31 ≤ l ≤ 31	-11 ≤ h ≤ 12, -16 ≤ k ≤ 14, -24 ≤ l ≤ 18	-14 ≤ h ≤ 15, -17 ≤ k ≤ 16, -19 ≤ l ≤ 21	-14 ≤ h ≤ 14, -13 ≤ k ≤ 16, -21 ≤ l ≤ 21
Reflections collected	12148	9359	12111	15286	21470
Independent reflections	6459 [R_{int} = 0.0418, R_{sigma} = 0.0618]	4349 [R_{int} = 0.0251, R_{sigma} = 0.0352]	5159 [R_{int} = 0.0280, R_{sigma} = 0.0412]	5510 [R_{int} = 0.0293, R_{sigma} = 0.0370]	5480 [R_{int} = 0.0310, R_{sigma} = 0.0300]
Data/restraints/parameters	6459/1/447	4349/0/252	5159/0/290	5510/0/293	5480/0/299
Goodness-of-fit on F^2	1.029	1.020	1.048	1.049	1.043
Final R indexes [I>=2σ (I)]	R_1 = 0.0564, wR_2 = 0.1281	R_1 = 0.0530, wR_2 = 0.1227	R_1 = 0.0314, wR_2 = 0.0596	R_1 = 0.0326, wR_2 = 0.0670	R_1 = 0.0317, wR_2 = 0.0728
Final R indexes [all data]	R_1 = 0.1077, wR_2 = 0.1554	R_1 = 0.0880, wR_2 = 0.1452	R_1 = 0.0406, wR_2 = 0.0639	R_1 = 0.0495, wR_2 = 0.0741	R_1 = 0.0448, wR_2 = 0.0810
Largest diff. peak/hole / e Å^{-3}	0.57/-0.58	0.32/-0.25	0.66/-0.31	0.42/-0.68	0.63/-0.71

Table S2. Selected bond lengths and bond angles for the complexes **1-3**

Bond length Å / Bond angles °	**Complex 1**	**Complex 2**	**Complex 3**
Ru(1)-O(1)	2.063(3)	2.0632(17)	2.0586(17)
Ru(1)-N(1)	2.112(3)	2.084(2)	2.0928(19)
Ru(1)-Cl(1)	2.414(11)	2.4151(7)	2.4187(7)
Ru-arene centroid	1.669	1.666	1.667
N(1)-N(2)	1.415(4)	1.402(3)	1.402(3)
N(1)-C(8)/C(9)	1.285(5)	1.306(3)	1.303(3)
O(1)-C(1)	1.304(4)	1.296(3)	1.296(3)
O(1)-Ru(1)-centroid$_{benzene}$	127.23	128.58	128.85
O(1)-Ru(1)-N(1)	76.55(11)	76.29(7)	76.28(7)
O(1)-Ru(1)-Cl(1)	86.81(8)	86.57(6)	86.65(5)
O(1)-C(1)-C(2)	117.3(3)	117.3(2)	117.6(2)
O(1)-C(1)-N(2)	125.6(3)	125.4(2)	125.1(2)
N(1)-Ru(1)-centroid$_{benzene}$	130.79	128.16	127.71
N(1)-Ru(1)-Cl(1)	87.76(9)	89.19(6)	89.86(6)
N(1)-N(2)-C(1)	111.5(3)	110.6(2)	110.96(18)
N(1)-C(8)/C(9)-C(9)/ C(10)	122.3(4)	122.0(2)	121.8(2)
N(1)-C(8)/C(9)-C(19)/ C(20)	123.2(3)	123.1(2)	123.1(2)
Cl(1)-Ru(1)-centroid$_{benzene}$	130.04	130.55	130.24
Cl(1)-Ru(1)-centroid$_{metallacycle}$	90.67	91.39	92.03
Centroid$_{benzene}$-Ru(1)-centroid$_{metallacycle}$	139.18	138.04	137.72
Ru(1)-N(1)-N(2)	111.8(2)	113.33(15)	112.65(14)
Ru(1)-N(1)-C(8)/C(9)	132.9(3)	130.12(17)	131.32(15)
Ru(1)-O(1)-C(1)	111.3(2)	111.22(15)	111.49(15)

Table S3. Selected bond lengths and bond angles for the ligands, **HL2** & **HL3**

Bond length Å / Bond angles °	**HL2**	**HL3**
N(1)-N(2)	1.383(9)	1.384(2)
N(2)-C(8)/ N(1)-C(8)	1.30(1)	1.287(2)
O(1)-C(1)	1.22(1)	1.231(2)
C(1)-N(2)/C(1)-N(2)	1.37(1)	1.349(2)
O(2)-C(20)	-	1.399(3)
C(5)-Br(1)	1.913(8)	-
∠N-N-C-Otorsion	-2.57	0.90

Table S4 Hydrogen Bonds for **HL2**

D	**H**	**A**	**d(D-H)/Å**	**d(H-A)/Å**	**d(D-A)/Å**	**D-H-A/°**
N3	H3	O2[1]	0.86	2.04	2.805(8)	147.3
C3	H3A	O2[2]	0.93	2.66	3.285(10)	125.3
C17	H17	O2[1]	0.93	2.72	3.348(11)	125.2
N6	H6A	O1[3]	0.86	2.10	2.866(8)	148.8

[1]-1/2+X,3/2-Y,+Z; [2]-1/2+X,1/2-Y,+Z; [3]1/2+X,1/2-Y,+Z

Table S5 Hydrogen Bonds for **HL3**

D	**H**	**A**	**d(D-H)/Å**	**d(H-A)/Å**	**d(D-A)/Å**	**D-H-A/°**
N3	H3	O3	0.87(2)	2.07(2)	2.924(2)	165.1(18)
O3	H3B	N1	0.81(2)	2.31(3)	2.9467(19)	135(2)
O3	H3C	O1[1]	0.89(2)	1.87(2)	2.7578(17)	175(2)

[1]1-X,1-Y,-Z

Table S6 Hydrogen Bonds for **complexes 1-3**

	D	**H**	**A**	**d(D-H)/Å**	**d(H-A)/Å**	**d(D-A)/Å**	**D-H-A/°**
1	N3	H3	Cl1	0.860	2.440	3.223(4)	151.7
2	N3	H3	Cl1	0.72(3)	2.52(3)	3.213(3)	163.41
3	N3	H3	Cl1	0.860	2.4223	3.203(2)	151.2

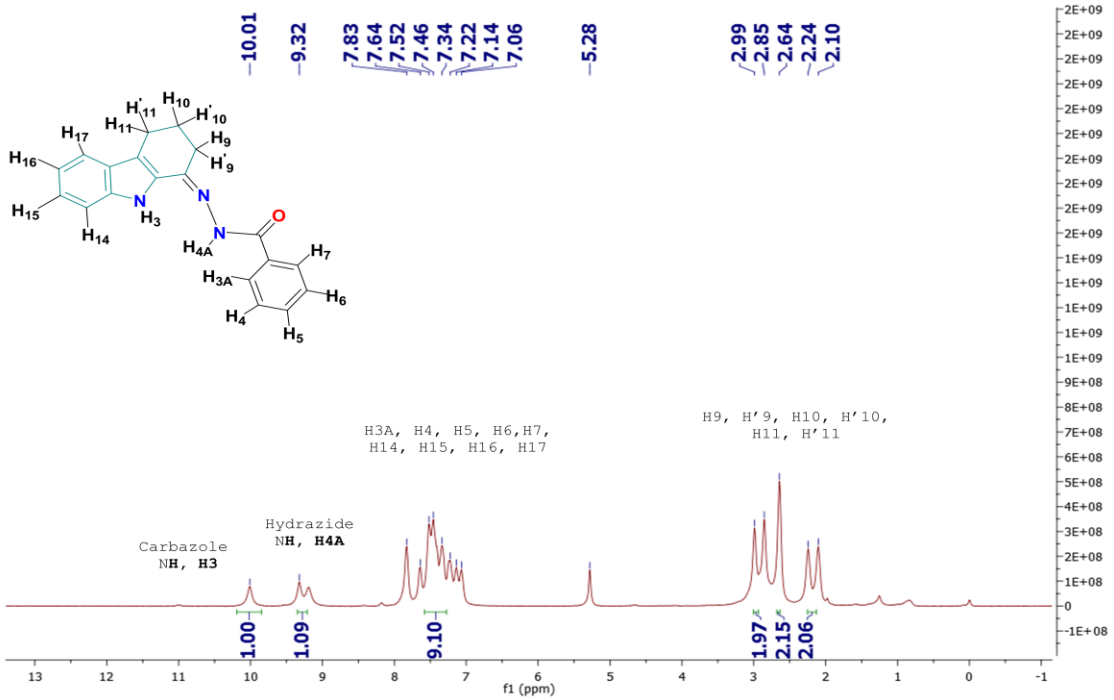

Figure S1.:^{1}H NMR spectrum of **HL1** in$CDCl_3$ (400 MHz, 293 K).

10.01 (1H, s, N-H3$_{carbazole}$), 9.32 (1H, s, N-H4A$_{hydrazide}$), 7.83-7.06 (9H, m, H3A, H4, H5, H6, H7, H14, H15, H16, H17, CH$_{carbazole+hydrazide,}$), 2.99 (2H, s, H9, H'9, CH$_{cyclohexane}$), 2.64 (2H, s, H10, H'10, CH$_{cyclohexane}$), 2.10 (2H, s, H11, H'11, CH$_{cyclohexane}$).

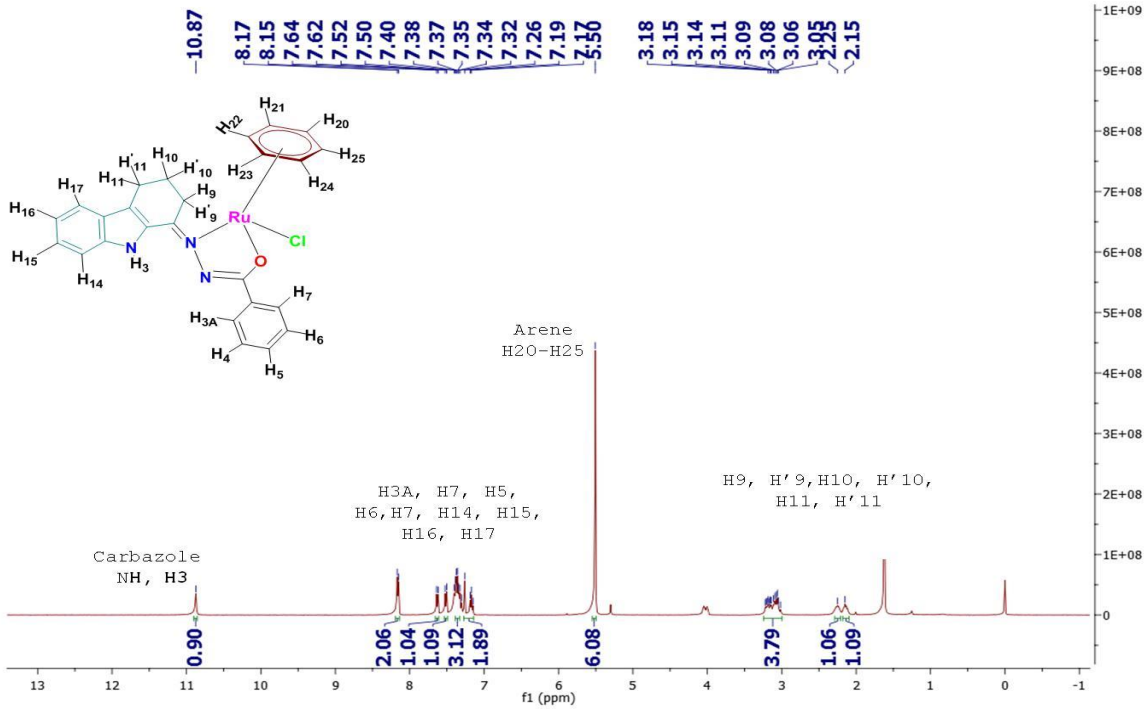

Figure S2. ^{1}H NMR spectrum of [Ru(L1)Cl(η^6-benzene)], **complex 1** in$CDCl_3$ (400 MHz, 293 K).

10.87 (1H, s,N-H3$_{carbazole}$), 8.16 (2H, d,^{3}J = 8.0 Hz, H3A, H7, CH$_{hydrazide}$), 7.63 (H, d, ^{3}J = 8.0 Hz,H17, CH$_{carbazole}$), 7.51 (H, d, ^{3}J = 8.0 Hz,H14, CH$_{carbazole}$), 7.35 (3H, m, H4, H5, H6,CH$_{hydrazide}$), 7.19 (2H, dd, ^{3}J = 8.0 Hz, H15, H16, CH$_{carbazole}$),5.50 (6H, s, H20, H21, H22, H23, H24, H25, CH$_{arene}$), 3.18-3.05 (4H, m, H9, H'9, H10, H'10, CH$_{cyclohexane}$), 2.25 (H, s, H'11, CH$_{cyclohexane}$), 2.15 (H, s, H11, CH$_{cyclohexane}$).

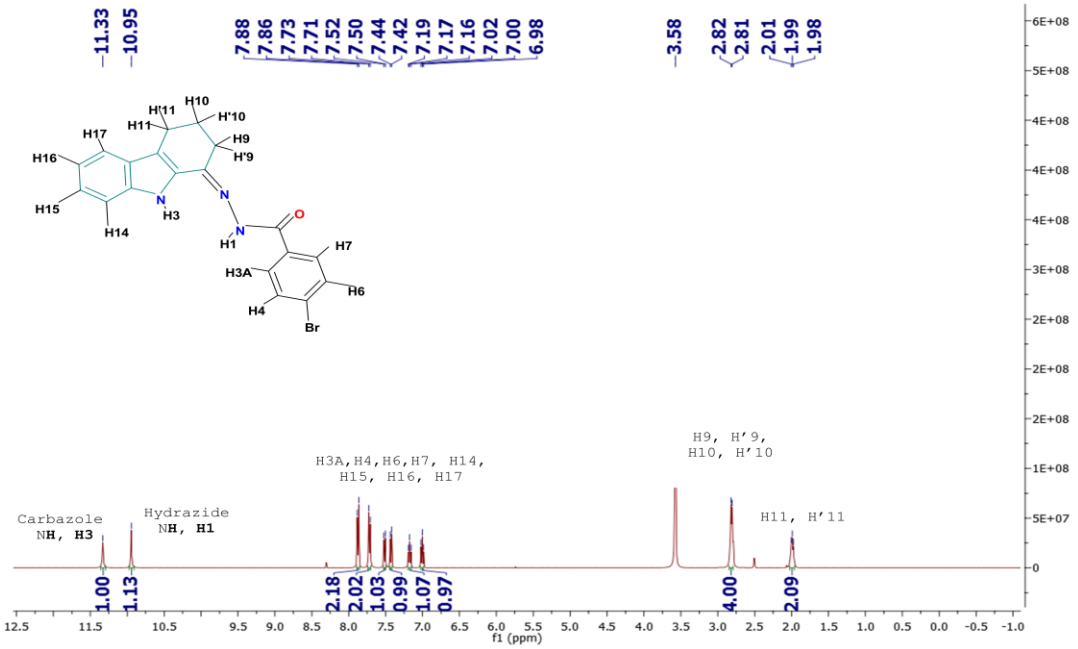

Figure S3. ^{1}H NMR spectrum of **HL2** in DMSO-d_6 (400 MHz, 293 K).

11.33 (1H, s, N-H3$_{carbazole}$), 10.95 (1H, s, N-H1$_{hydrazide}$), 7.87 (2H, d, ^{3}J = 8.0 Hz, H7, H3A, CH$_{hydrazide}$), 7.72 (2H, d, ^{3}J = 8.0 Hz, H4, H6, CH$_{hydrazide}$), 7.51(1H, d, ^{3}J = 8.0 Hz, H17, CH$_{carbazole}$), 7.43 (1H, d, ^{3}J = 8.0 Hz, H14, CH$_{carbazole}$), 7.17 (1H, t, ^{3}J = 8.0, 4.0 Hz, H16, CH$_{carbazole}$), 7.00 (1H, t, ^{3}J = 8.0 Hz, H15, CH$_{carbazole}$), 2.81 (4H, d, ^{3}J = 4 Hz, d, H9, H'9, H10, H'10, CH$_{cyclohexane}$), 1.99 (2H, t, ^{3}J = 8.0, 4.0 Hz, H11, H'11, CH$_{cyclohexane}$).

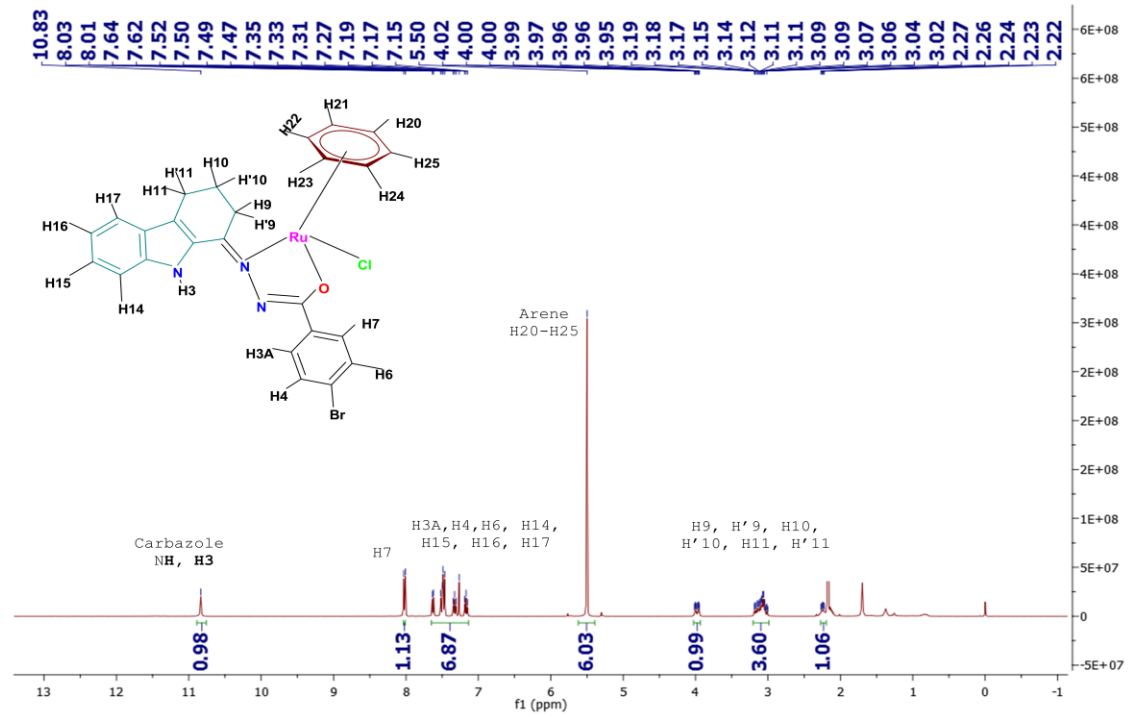

Figure S4. ^{1}H NMR spectrum of [Ru(L2)Cl(η^6-benzene)], **complex 2** in$CDCl_3$ (400 MHz, 293 K).

10.83 (1H, s, N-H3$_{carbazole}$), 8.02 (H, d, ^{3}J = 8.0 Hz, H7, CH$_{hydrazide}$), 8.03-7.15 (7H, m, H3A, H4, H6, H14, H15, H16, H17,CH$_{carbazole+hydrazide}$), 5.50 (6H, s, H20, H21, H22, H23,H24, H25, CH$_{arene}$), 4.02-2.22 (6H, m, H9, H'9, H10, H'10, H11, H'11, CH$_{cyclohexane}$).

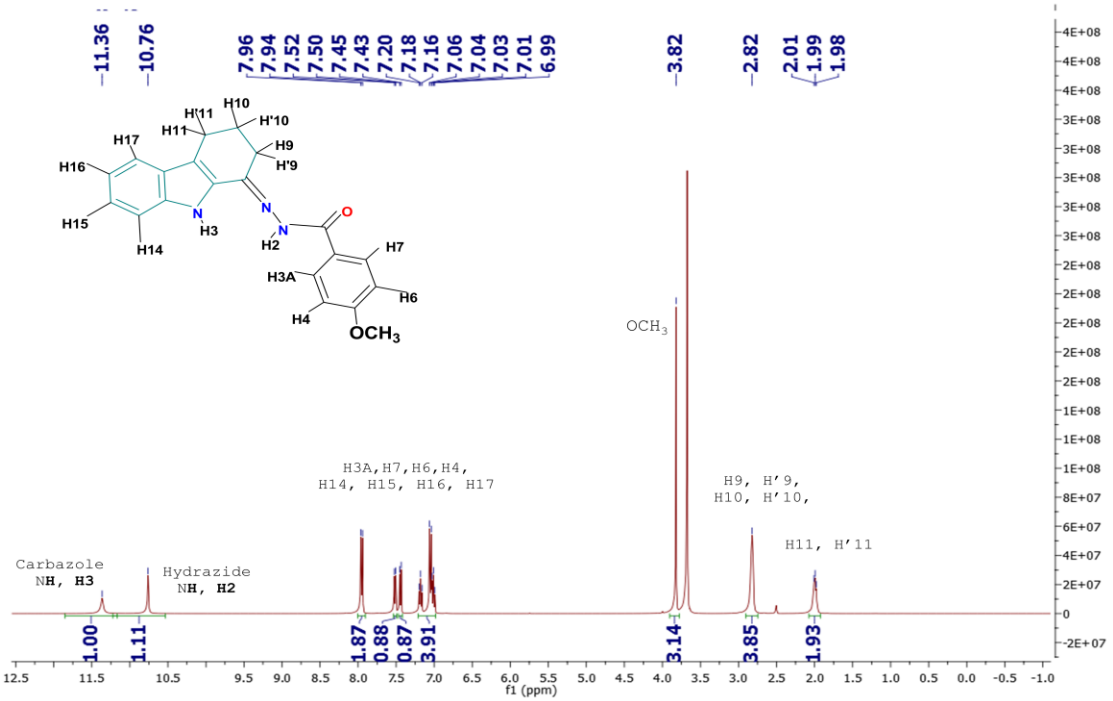

Figure S5. ^{1}H NMR spectrum of **HL3** in DMSO-d_6(400 MHz, 293 K).

11.36 (1H, s,N-H3$_{carbazole}$), 10.76 (1H, s, N-H2$_{hydrazide}$), 7.95 (2H, d, ^{3}J = 8.0 Hz, H7, H3A, CH$_{hydrazide}$), 7.51 (H, d, ^{3}J = 8.0 Hz, H6, CH$_{hydrazide}$), 7.44 (H, d, ^{3}J = 8.0 Hz, H4, CH$_{hydrazide}$), 7.20-6.99 (4H, m, H14, H15, H16, H17, CH$_{carbazole}$), 3.82 (3H, s, OCH$_3$), 2.82 (4H, s, H9, H'9, H10, H'10, CH$_{cyclohexane}$), 1.99 (2H, t, ^{3}J = 8.0, 4.0 Hz, H11, H'11, CH$_{cyclohexane}$).

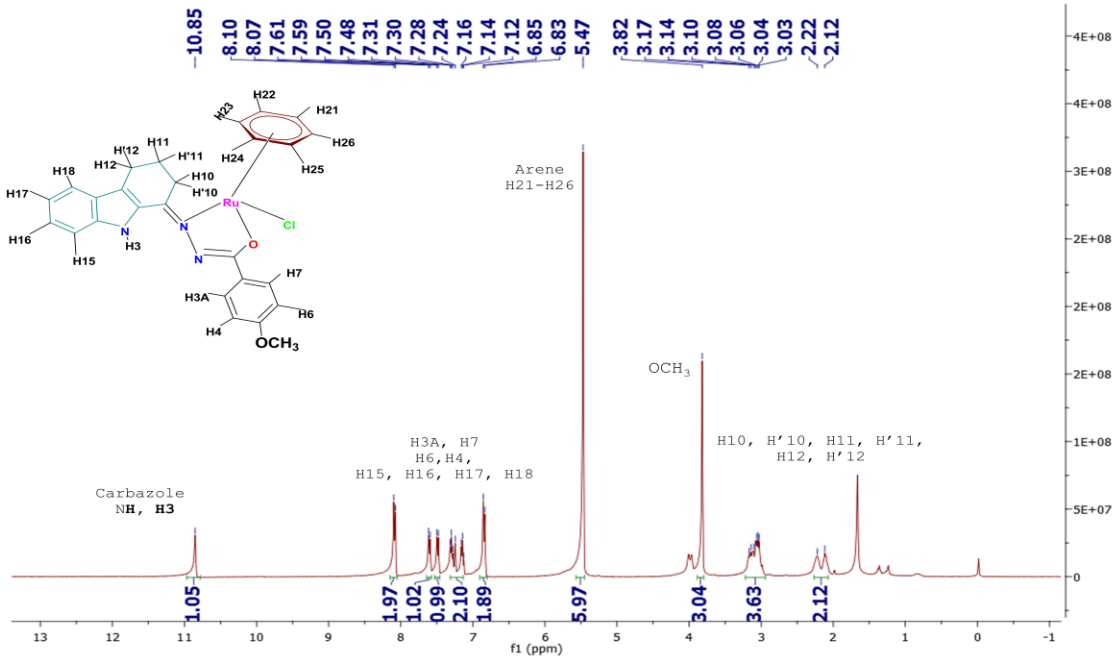

Figure S6. ^{1}H NMR spectrum of [Ru(L3)Cl(η^6-benzene)], **complex3** in$CDCl_3$ (400 MHz, 293 K).10.85 (1H, s, N-H3$_{carbazole}$), 8.09 (2H, d, ^{3}J = 12.0 Hz, H7, H3A, CH$_{hydrazide}$), 7.60 (H, d, ^{3}J = 8.0 Hz, H6, CH$_{hydrazide}$), 7.49 (H, d, ^{3}J = 8.0 Hz, H4, CH$_{hydrazide}$), 7.31-7.12 (2H, m, H16, H17, CH$_{carbazole}$), 6.84 (2H, d, ^{3}J = 8.0 Hz, H15, H18, CH$_{carbazole}$), 5.47 (6H, s, H21, H22, H23,H24, H25, H26, CH$_{arene}$), 3.82 (3H, s, OCH$_3$), 3.03-3.17 (4H, m, H10, H'10, H11, H'11, CH$_{cyclohexane}$), 2.22 (H, s, H'12, CH$_{cyclohexane}$), 2.12 (H, s, H12, CH$_{cyclohexane}$).

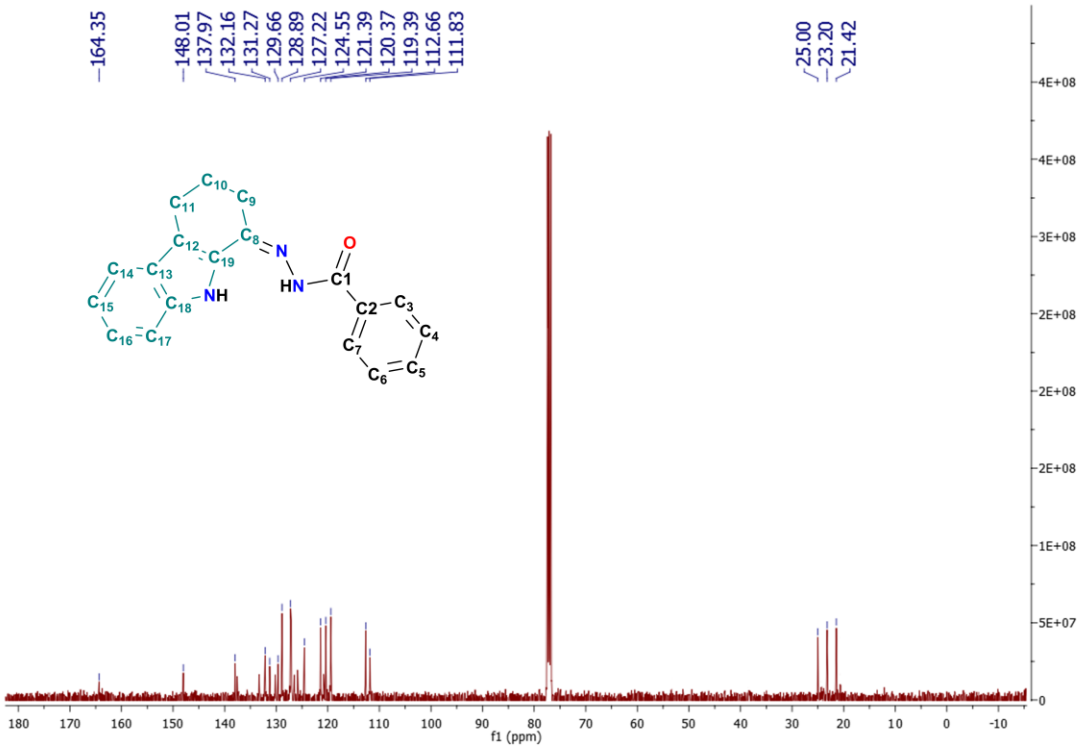

Figure S7. ^{13}C NMR spectrum of **HL1** in $CDCl_3$ (100 MHz, 293 K).

164.3(C1), 148.0(C8), 137.9(C2), 132.2(C19), 131.3(C18), 129.7(C3,C7), 128.9(C4,C6), 127.2(C5), 124.5(C13), 121.4(C16), 120.4(C15), 119.4(C14), 112.7(C17), 111.8(C12), 25.0(C9), 23.2(C10), 21.4(C11)

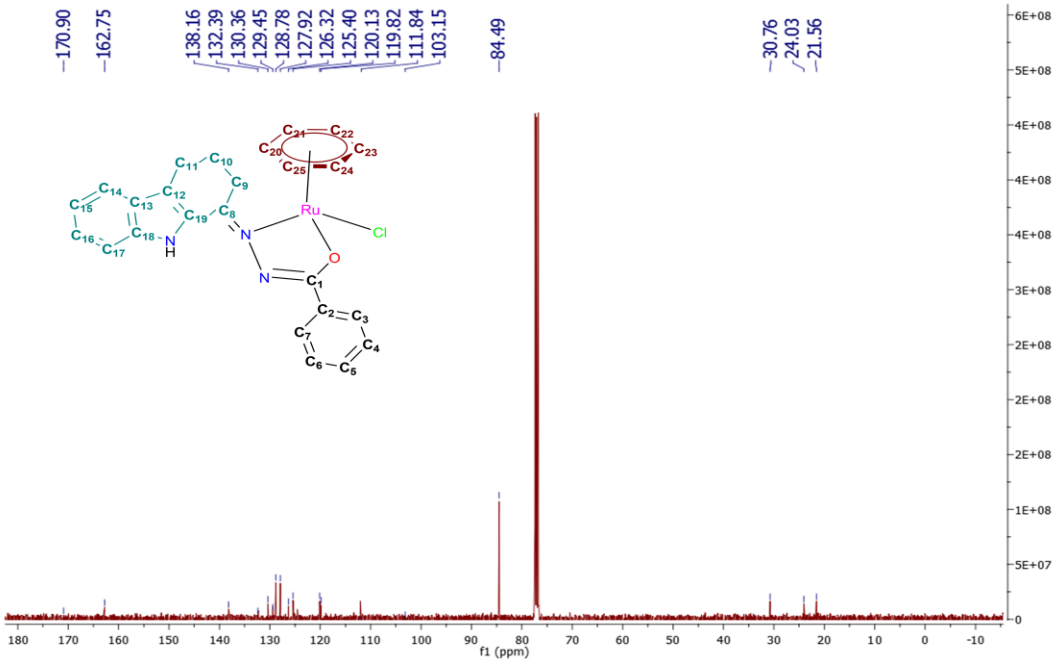

Figure S8. ^{13}C NMR spectrum of **complex1** in $CDCl_3$ (100 MHz, 293 K).

170.9(C1), 162.7(C8), 138.2(C2), 132.4(C19), 130.4(C18), 129.4(C3,C7), 128.8(C4,C6), 127.9(C5), 126.3(C13), 125.4(C15, C16), 120.1(C17), 119.8(C14), 111.8(C12), 84.5(C20,C21,C22,C23,C24,C25), 30.8(C9), 24.0(C10), 21.6(C11)

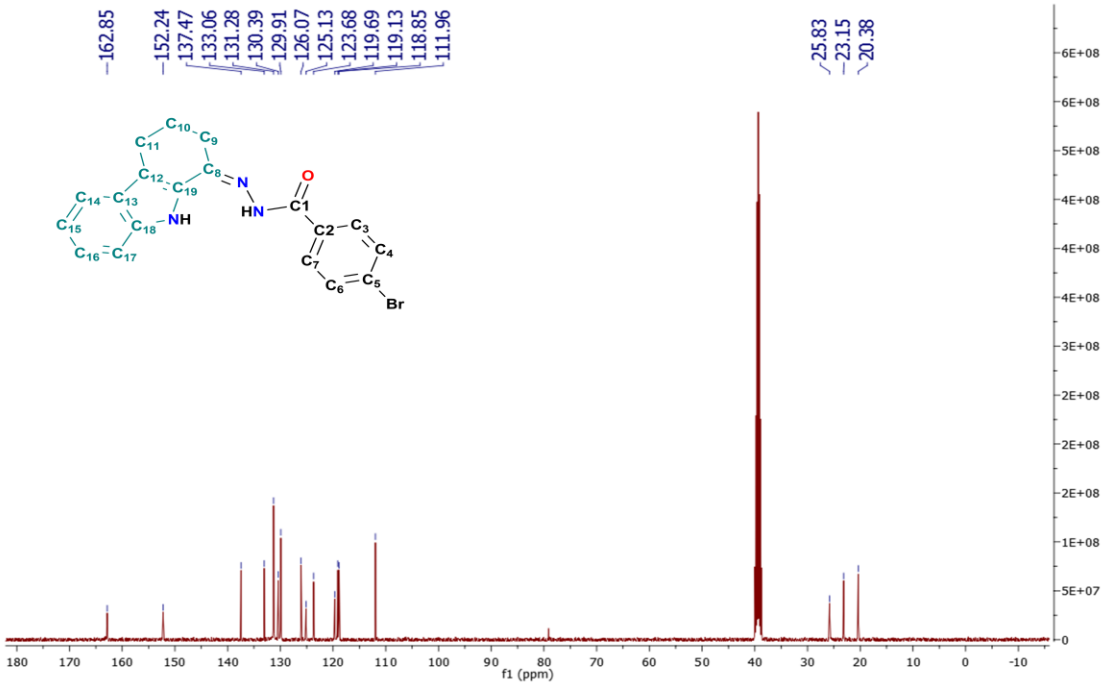

Figure S9. ^{13}C NMR spectrum of **HL2** in DMSO-d_6 (100 MHz, 293 K).

162.8(C1), 152.2(C8), 137.5(C2), 133.1(C5), 131.3(C19), 130.4(C18), 129.9(C3,C7), 126.1(C6,C4), 125.1(C13), 123.7(C16), 119.7(C15), 119.1(C14), 118.8(C17), 111.9(C12), 25.8(C9), 23.1(C10), 20.4 (C11)

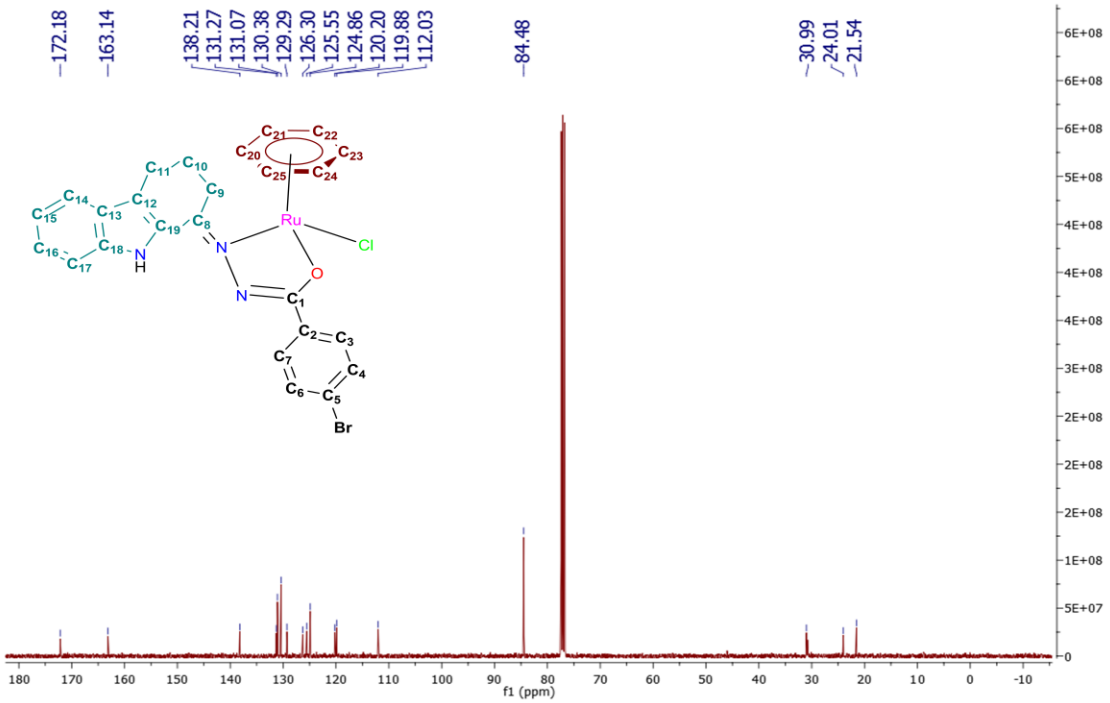

Figure S10. ^{13}C NMR spectrum of **complex 2** in $CDCl_3$(100 MHz, 293 K).

172.2(C1), 163.1(C8), 138.2(C2), 131.3(C5), 131.1(C19), 130.4(C18), 129.3(C6,C4), 126.3(C3,C7), 125.5(C13), 124.9(C15,C16) 120.2(C14), 119.9(C17), 112.0(C12), 84.5(C20,C21,C22,C23,C24,C25), 30.9(C9), 24.0(C10), 21.5(C11)

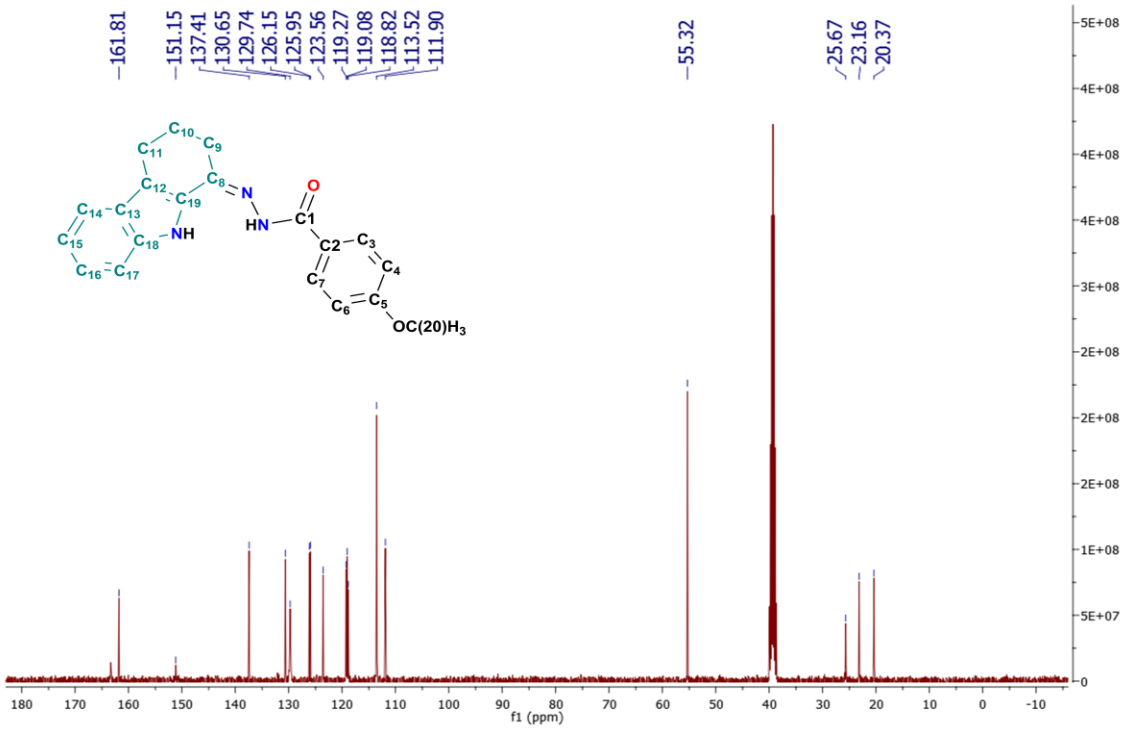

Figure S11. ^{13}C NMR spectrum of **HL3** in DMSO-d_6 (100 MHz, 293 K).

161.8(C1), 151.1(C8), 137.4(C2), 130.6(C5), 129.7(C19), 126.1(C18), 125.9(C3,C7), 123.6(C6,C4), 119.3(C13), 119.1(C15, C16), 118.8(C14), 113.5(C17), 111.9(C12), 55.3(C20), 25.7(C9), 23.2(C10),20.4(C11)

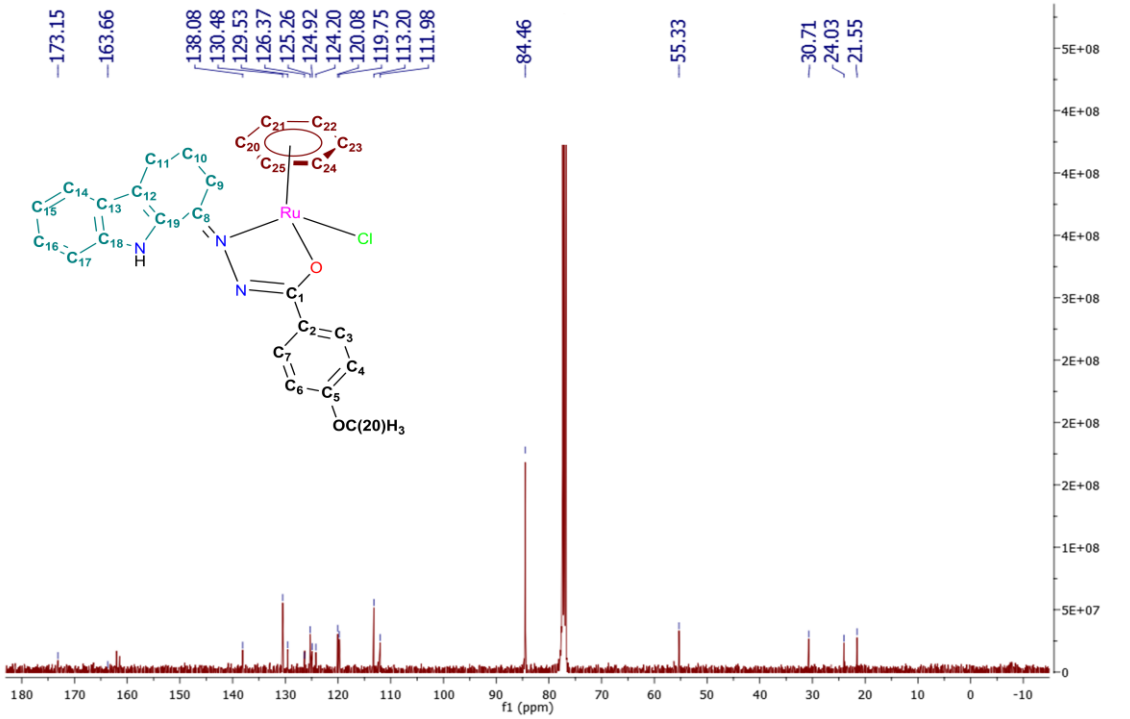

Figure S12. ^{13}C NMR spectrum of **complex 3** in $CDCl_3$(100 MHz, 293 K).

173.1(C1), 163.7(C8), 138.1(C2), 130.5(C5), 129.5(C19), 126.4(C18), 125.3(C3,C7), 124.9 (C6,C4), 124.2(C13), 120.1(C15, C16),119.7(C14), 113.2(C17), 111.9(C12), 84.5(C20,C21,C22,C23,C24,C25), 55.3(C20), 30.7(C9), 24.0(C10), 21.5(C11)

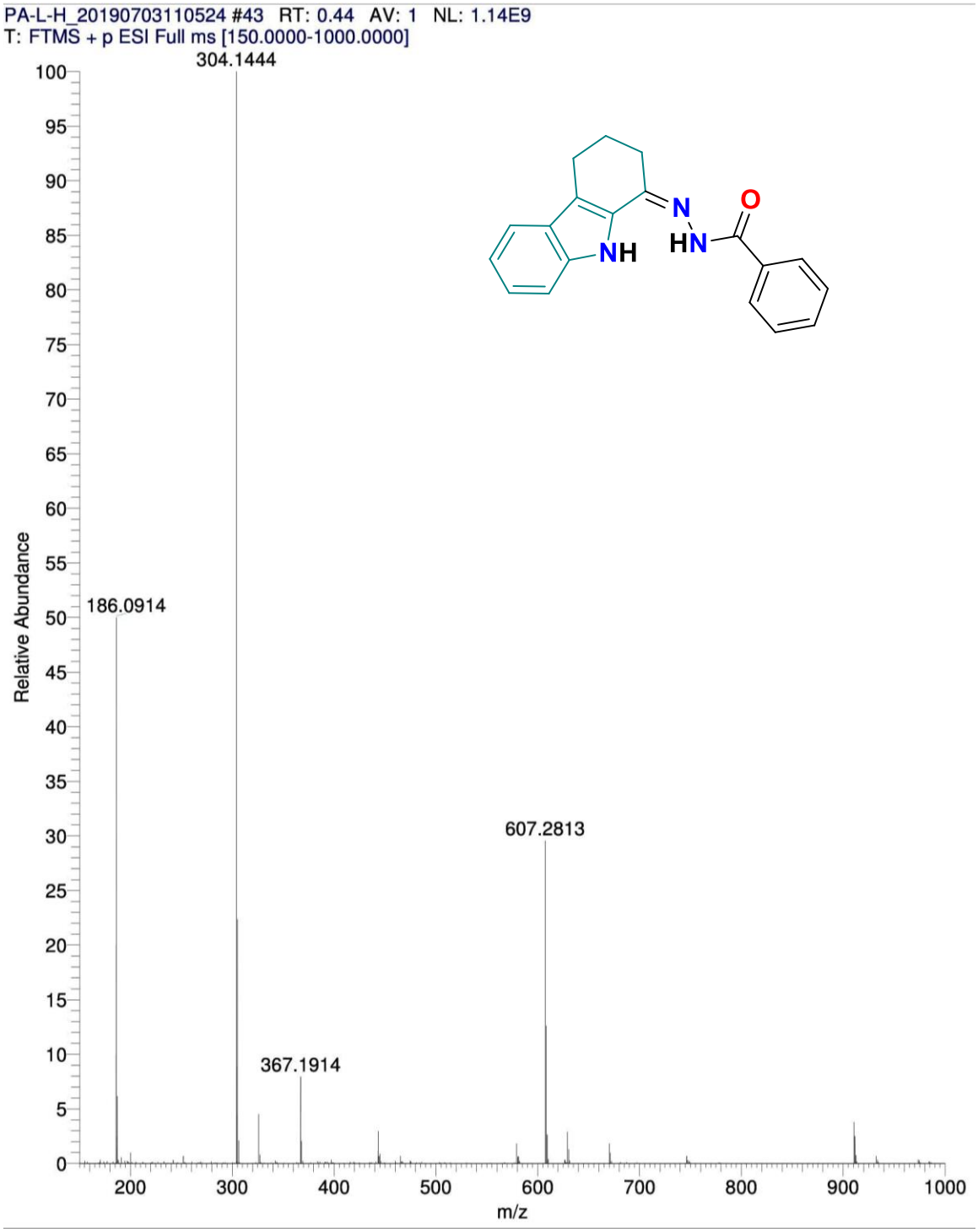

Figure S13. Mass spectrum of **HL1**in (CH_3OH + CH_3CN)
m/z: Calculated for $C_{19}H_{17}N_3O$: 303.14 $[M]^+$; Observed: 304.1444 $[M+H]^+$; 367.1914$[M+Na+CH_3CN]^+$; 607.2813 $[2M+H]^+$

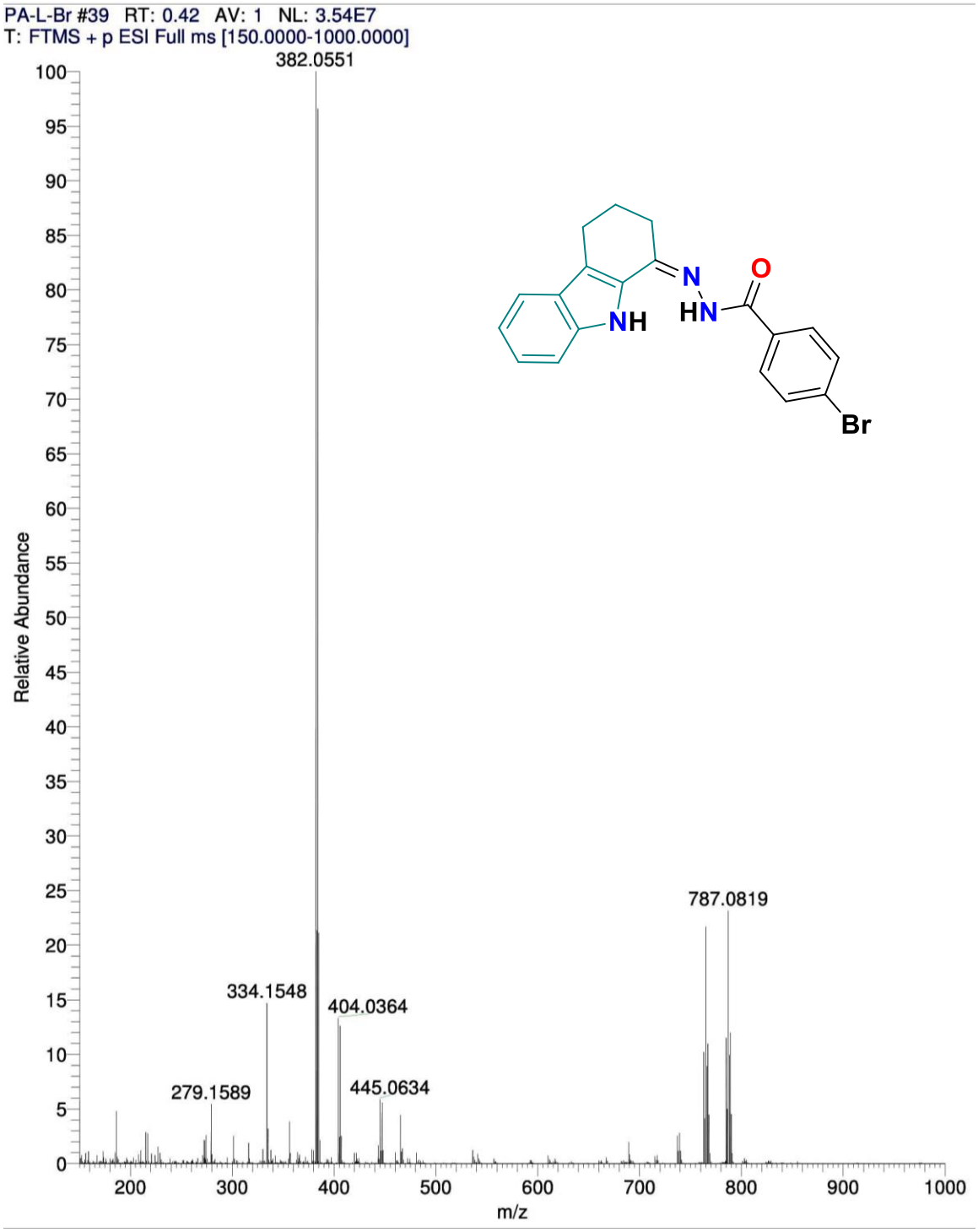

Figure S14. Mass spectrum of **HL2**in (CH_3OH + CH_3CN)
m/z: Calculated for $C_{19}H_{16}BrN_3O$: 381.05 $[M]^+$; Observed: 382.05 $[M+H]^+$.
404.04 $[M+Na]^+$; 445.06 $[M+Na+CH_3CN]^+$; 787.08 $[2M+Na+2H]^+$

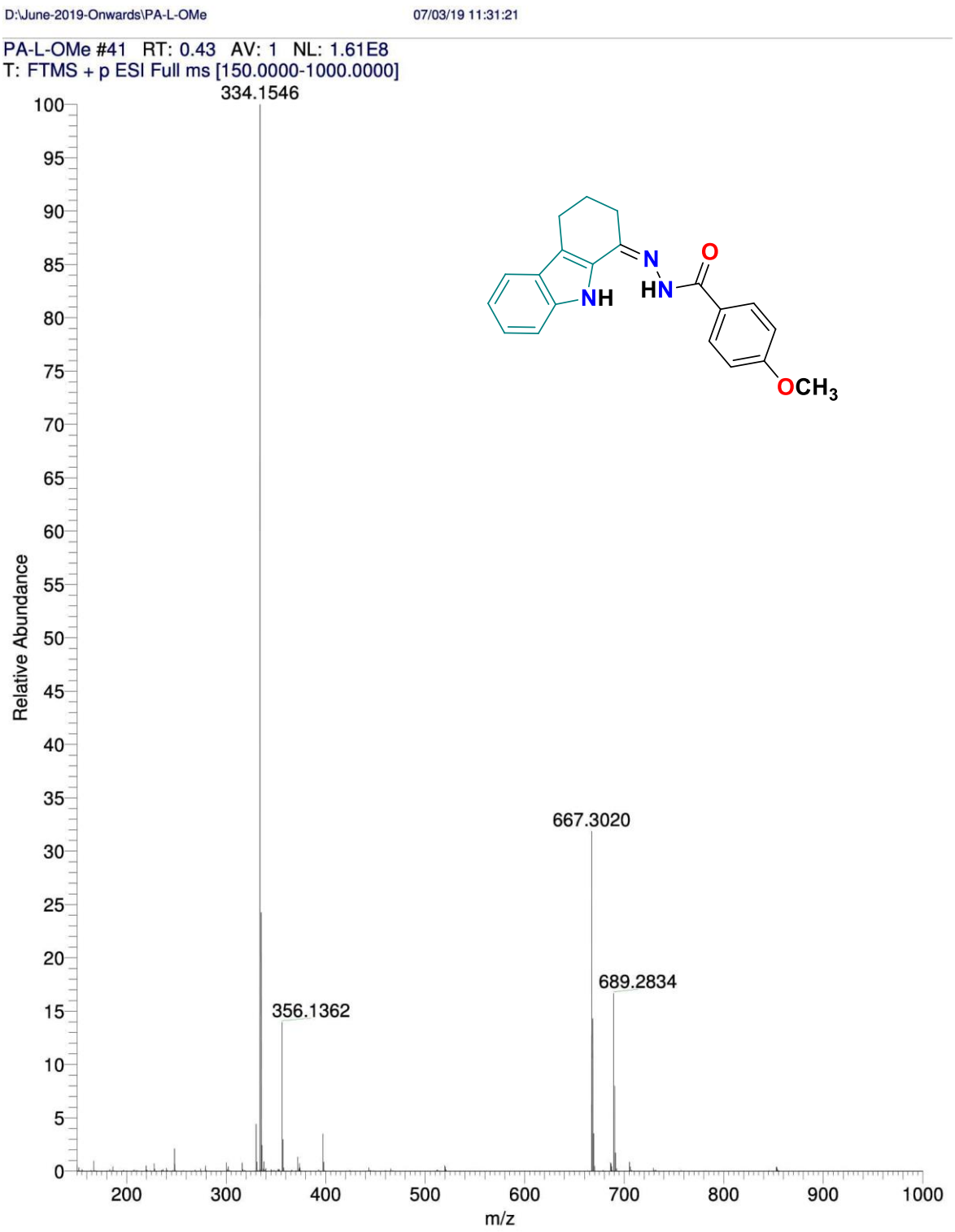

Figure S15. Mass spectrum of **HL3**in (CH_3OH + CH_3CN)
m/z: Calculated for $C_{20}H_{19}N_3O_2$: 333.15 $[M]^+$; Observed: 334.15 $[M+H]^+$; 356.13$[M+Na]^+$; 667.30 $[2M+H]^+$; 689.28 $[2M+Na]^+$.

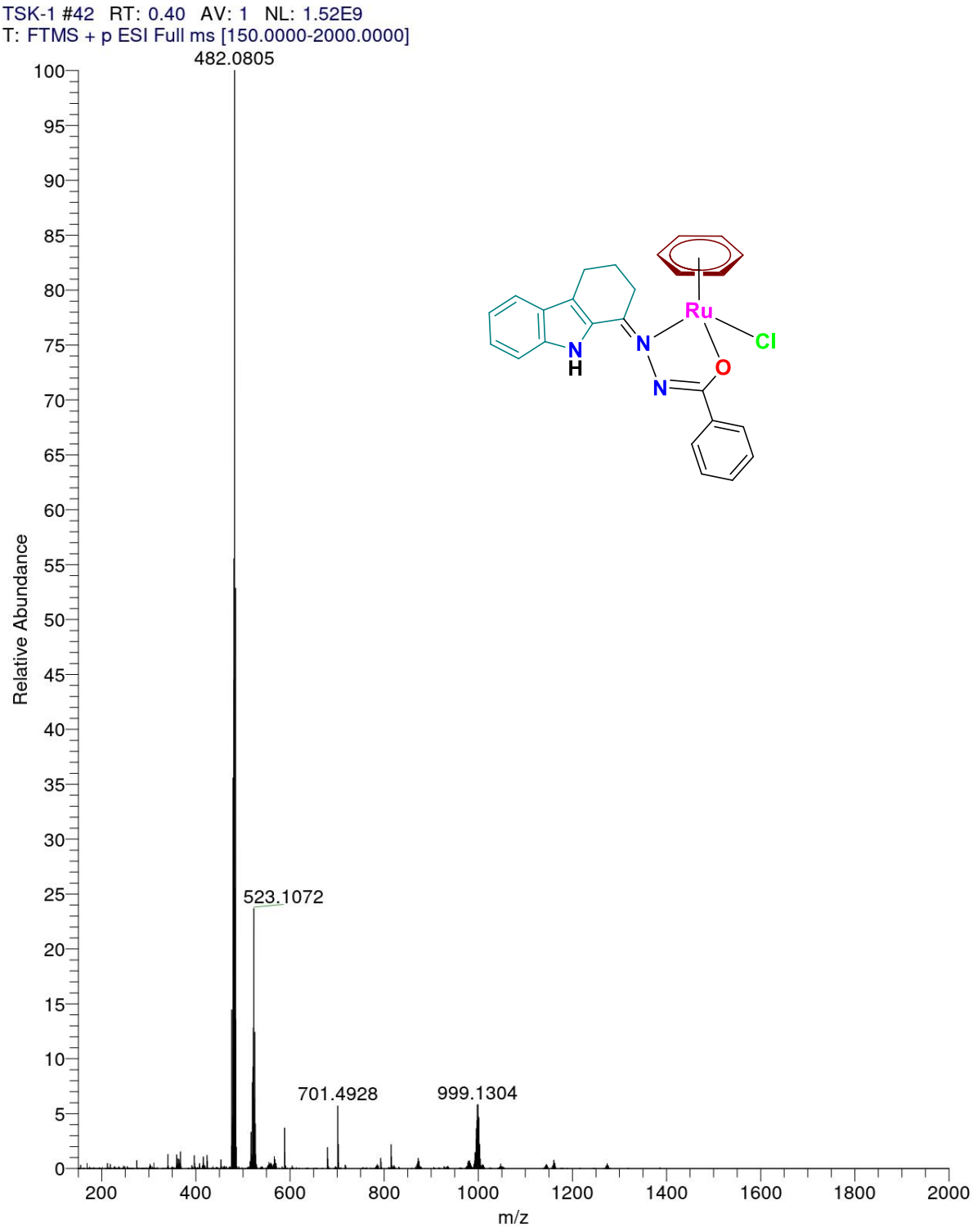

Figure S16. Mass spectrum of **complex 1**in CH_3OH + CH_3CN
m/z: Calculated for $C_{25}H_{22}ClN_3ORu$: 517.05 $[M]^+$; Observed: 482.08 $[M\text{-}Cl]^+$.
523.10 $[M\text{-}Cl+CH_3CN]^+$; 999.13$[2M\text{-}2Cl+CH_3OH+3H]^+$;

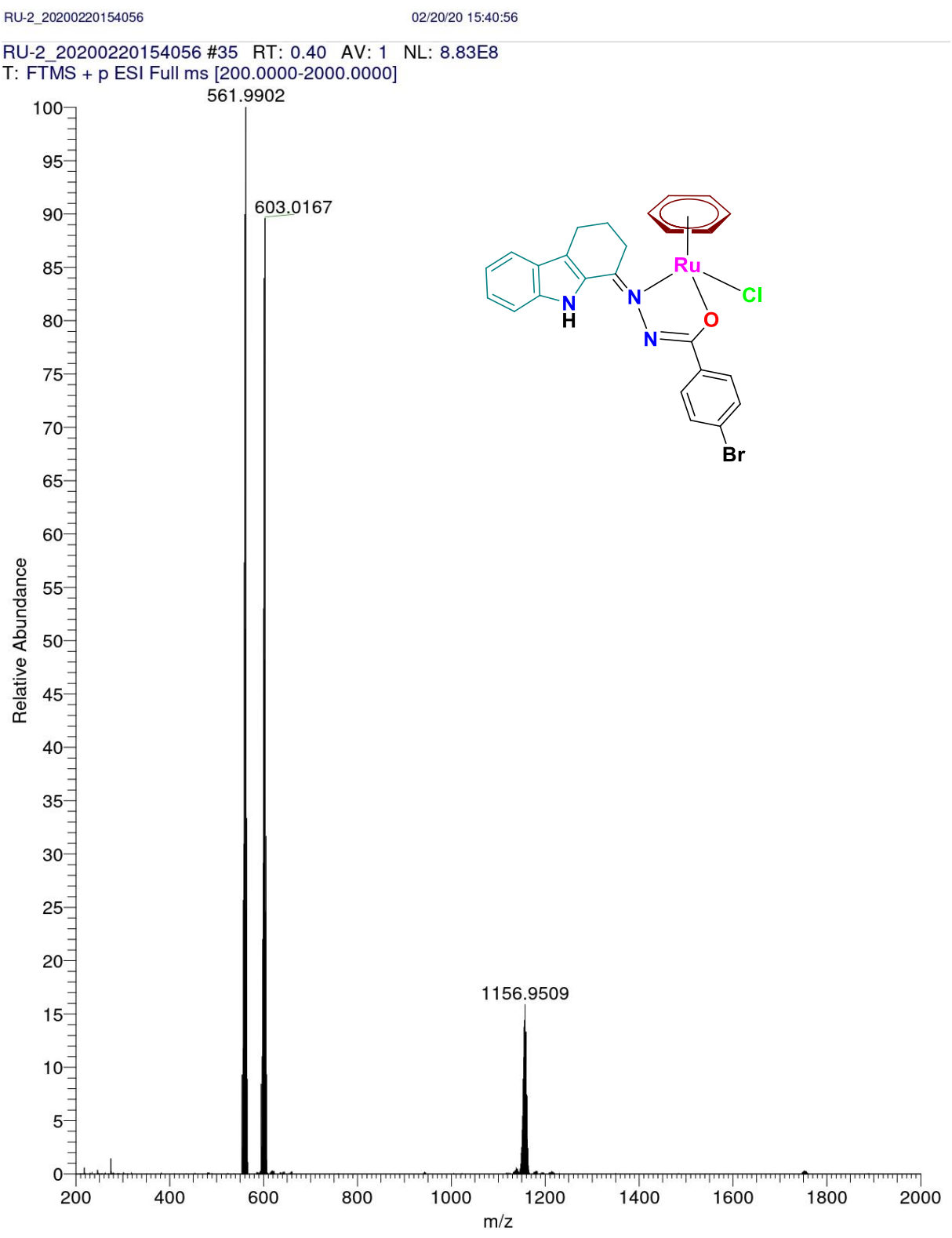

Figure S17. Mass spectrum of **complex 2**inCH_3OH + CH_3CN
m/z: Calculated for $C_{25}H_{21}BrClN_3ORu$: 594.96 $[M]^+$; Observed: 561.99 $[M\text{-}Cl+2H]^+$. 603.02 $[M\text{-}Cl+CH_3CN]^+$; 1156.95 $[2M\text{-}2Cl+CH_3OH+4H]^+$.

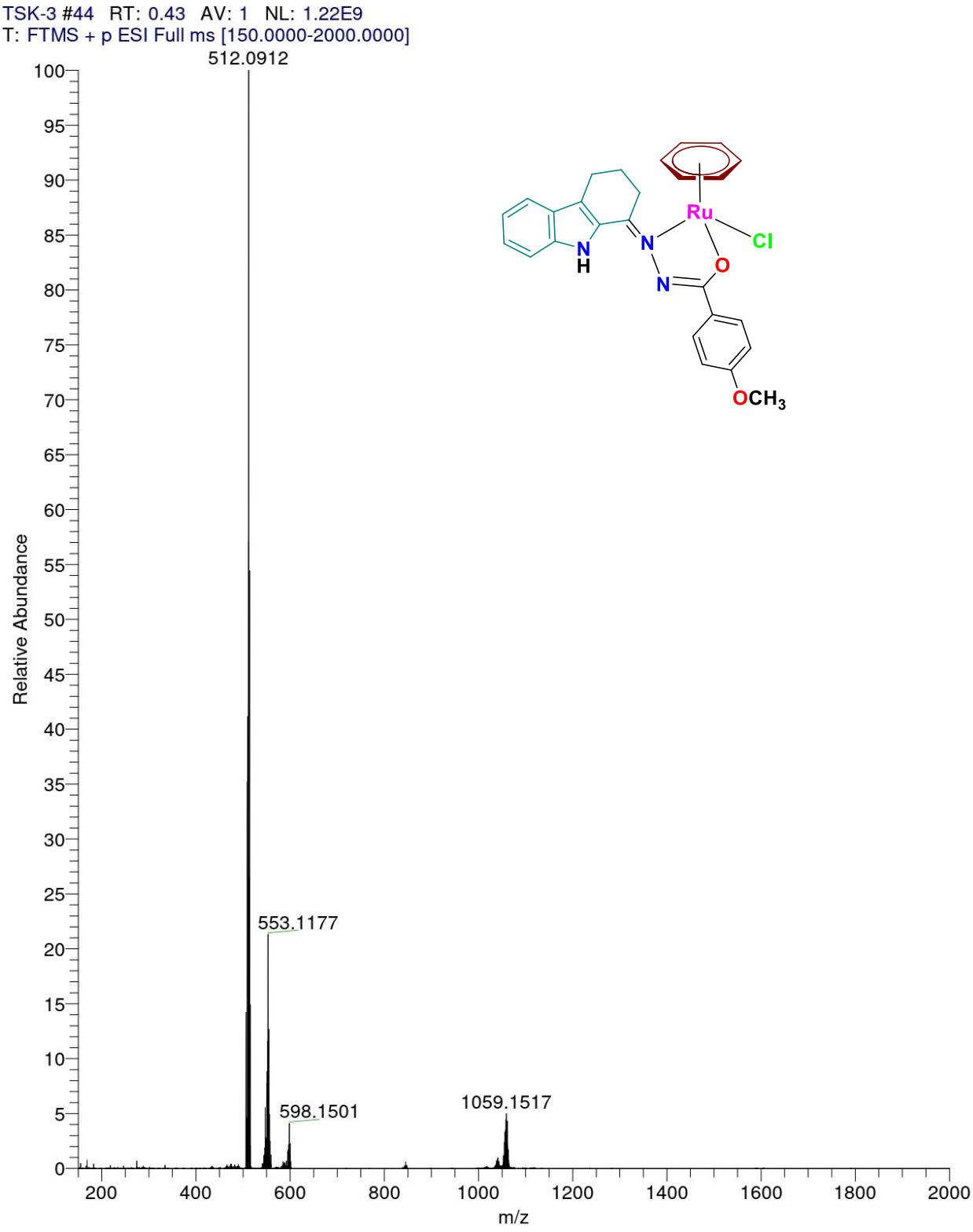

Figure S18. Mass spectrum of **complex 3** in CH_3OH + CH_3CN
m/z: Calculated for $C_{26}H_{24}ClN_3O_2Ru$: 547.06 $[M]^+$; Observed: 512.09 $[M\text{-}Cl]^+$.
553.12 $[M\text{-}Cl+CH_3CN]^+$; 598.15 $[M\text{-}Cl+CH_3CN+4H]^+$; 1059.15 $[2M\text{-}2Cl+CH_3OH+3H]$.

Stability studies of the complexes

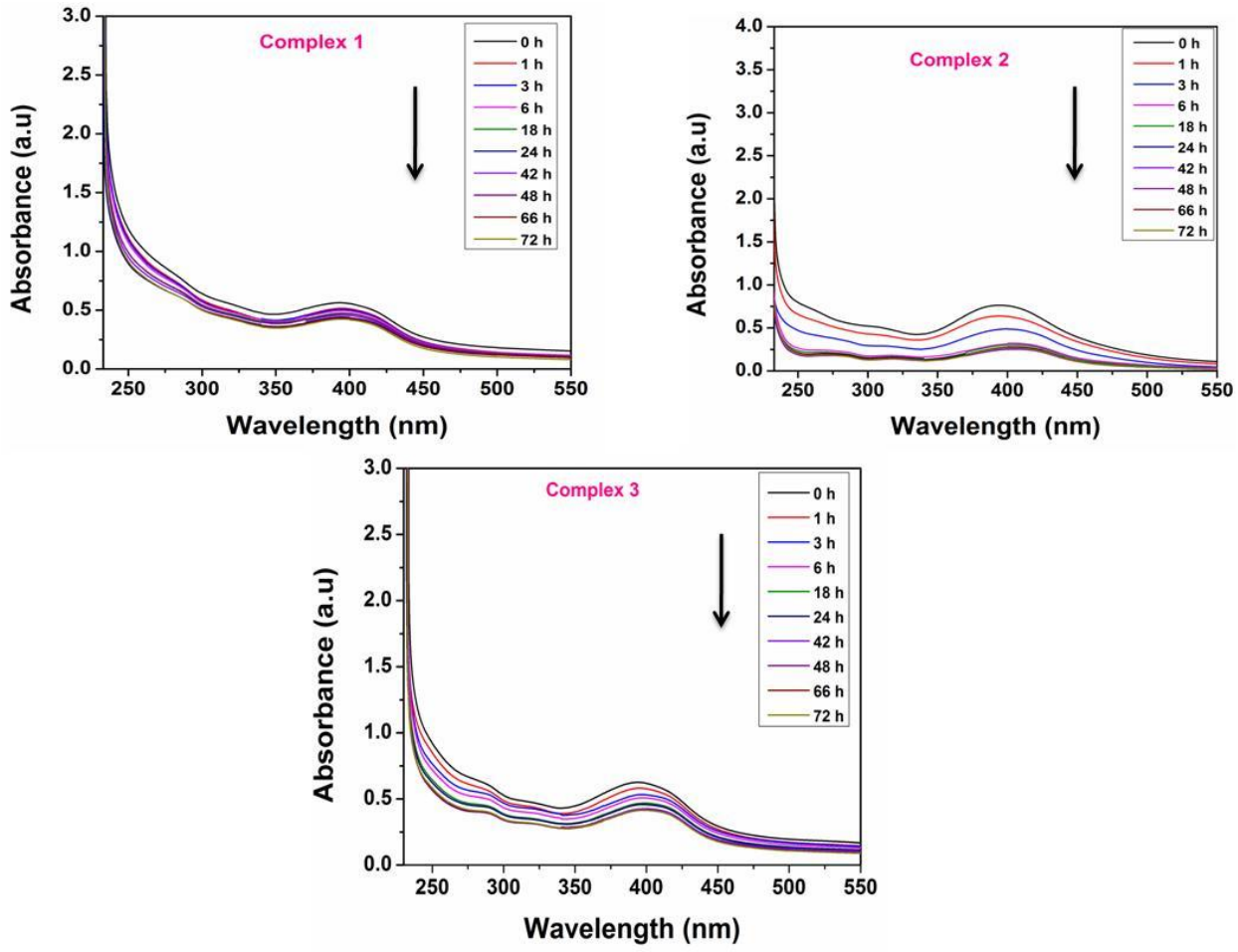

Figure S19. UV-vis spectrum of complexes **1-3** in 1% DMSO in phosphate buffer at 293K over various time intervals. [complex] = 5 μM. Arrows indicate the steady decrease in absorption intensity

In vitro cancer cell growth inhibition by MTT assay

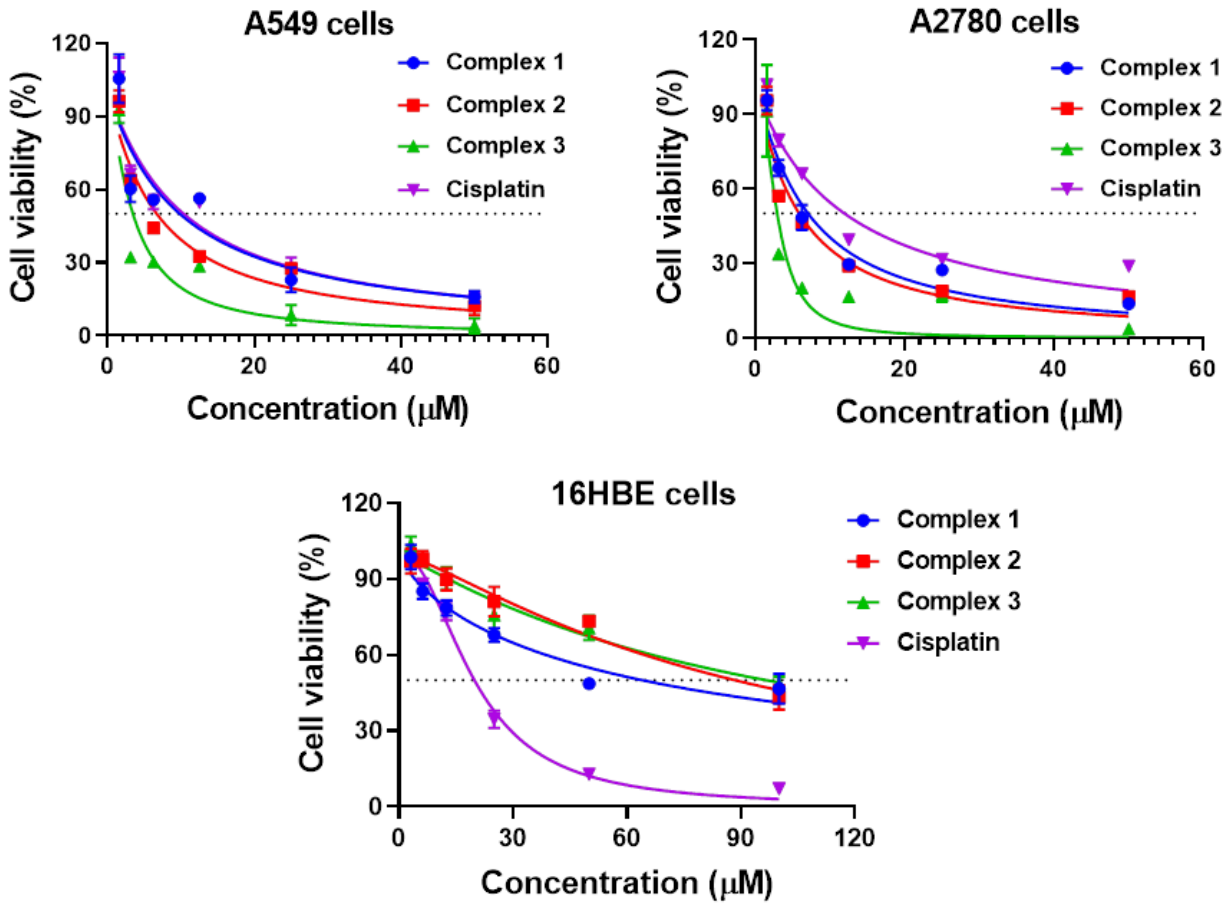

Figure S20. Effect of concentrations of the complexes **1-3** and cisplatin on % cell viability

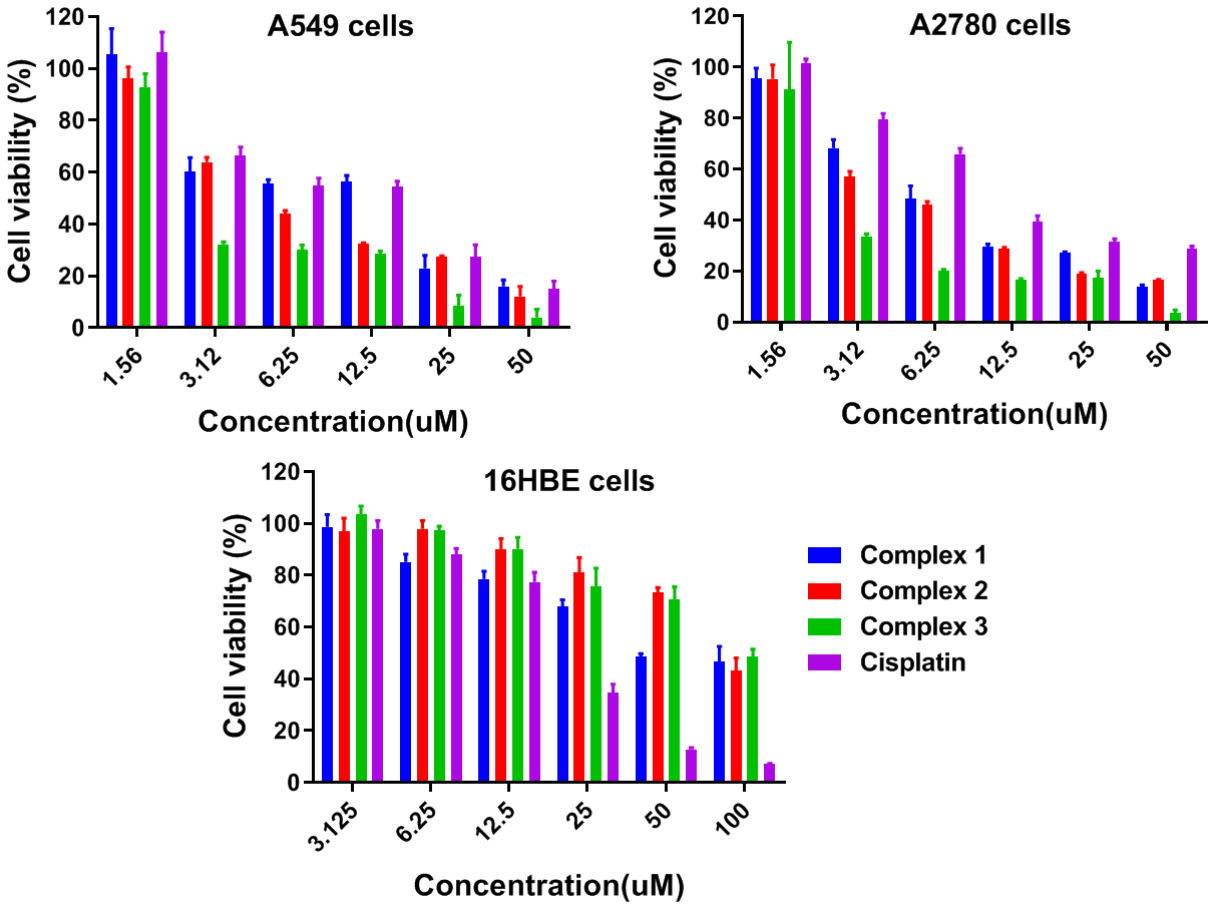

Figure S21. Cytotoxic effects of the complexes **1-3** assessed by MTT assay after 48 h of incubation period

References

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