Electronic Supporting Information Materials

Synthesis, characterization and biological evaluation of six highly cytotoxic ruthenium(II) complexes with 4′-substituted-2,2′:6′,2″-terpyridine

Qi-Pin Qin,[†ac] Ting Meng,[†ac] Ming-Xiong Tan,[*ac] Yan-Cheng Liu,[c] Shu-Long Wang,[*ac] Bi-Qun Zou[*b] and Hong Liang [c]

Table S1. Crystal data and structure refinement details for **Ru5**.

Empirical formula	$C_{58.5}H_{60}Cl_4N_6O_{5.5}Ru_2S_4$
Formula weight	1407.31
Temperature/K	293(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	11.5669(4)
b/Å	22.0229(6)
c/Å	24.9620(7)
α/°	90.00
β/°	98.749(3)
γ/°	90.00
Volume/Å^3	6284.7(3)
Z	4
ρ_{calc}mg/mm^3	1.487
m/mm^{-1}	0.835
F(000)	2868.0
Crystal size/mm^3	? × ? × ?
2Θ range for data collection	6.58 to 52.68°
Index ranges	$-14 \le h \le 13$, $-27 \le k \le 16$, $-20 \le l \le 31$
Reflections collected	27591
Independent reflections	12829[R(int) = 0.0325]
Data/restraints/parameters	12829/60/738
Goodness-of-fit on F^2	1.033
Final R indexes [I>=2σ (I)]	$R_1 = 0.0583$, $wR_2 = 0.1559$
Final R indexes [all data]	$R_1 = 0.0897$, $wR_2 = 0.1760$
Largest diff. peak/hole / e Å^{-3}	2.03/-0.77

[a] $R_1 = \Sigma\ ||F_o| - |F_c||/\Sigma|F_o|$; [b] $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

Table S2. Selected bond lengths (Å) for **Ru5**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Ru1	Cl1	2.4440(14)	C9	C18	1.403(8)
Ru1	Cl2	2.4206(15)	C10	C11	1.420(9)
Ru1	S1	2.2190(16)	C11	C12	1.340(12)
Ru1	N1	2.081(5)	C12	C13	1.383(12)
Ru1	N2	1.954(4)	C13	C14	1.412(13)
Ru1	N3	2.079(4)	C13	C18	1.440(8)
Ru2	Cl3	2.4175(14)	C14	C15	1.383(15)
Ru2	Cl4	2.4296(14)	C15	C16	1.387(14)
Ru2	S2	2.2184(15)	C16	C17	1.366(10)
Ru2	N4	2.081(4)	C17	C18	1.413(9)
Ru2	N5	1.949(4)	C19	C20	1.378(7)
Ru2	N6	2.070(4)	C20	C21	1.481(7)
S1	O1	1.461(5)	C21	C22	1.373(8)
S1	C51	1.769(8)	C22	C23	1.370(8)
S1	C52	1.785(8)	C23	C24	1.355(9)
S2	O2	1.467(4)	C24	C25	1.371(9)
S2	C53	1.768(8)	C26	C27	1.375(8)
S2	C54	1.768(8)	C27	C28	1.371(9)
S3	O5	1.466(17)	C28	C29	1.376(8)
S3	C57	1.819(15)	C29	C30	1.376(7)
S3	C58	1.731(14)	C30	C31	1.482(7)
S4	O4	1.435(16)	C31	C32	1.385(7)
S4	C55	1.785(15)	C32	C33	1.391(7)
S4	C56	1.631(14)	C33	C34	1.396(7)
N1	C21	1.344(6)	C33	C41	1.489(7)
N1	C25	1.354(7)	C34	C35	1.382(7)
N2	C6	1.335(6)	C35	C36	1.475(7)
N2	C20	1.343(6)	C36	C37	1.383(7)
N3	C1	1.348(7)	C37	C38	1.371(8)
N3	C5	1.349(6)	C38	C39	1.397(8)
N4	C36	1.359(6)	C39	C40	1.358(8)
N4	C40	1.334(6)	C41	C42	1.372(7)
N5	C31	1.349(6)	C41	C50	1.426(7)
N5	C35	1.338(6)	C42	C43	1.398(8)
N6	C26	1.339(7)	C43	C44	1.328(9)
N6	C30	1.363(6)	C44	C45	1.416(9)
C1	C2	1.382(9)	C45	C46	1.396(10)
C2	C3	1.370(8)	C45	C50	1.421(8)
C3	C4	1.363(8)	C46	C47	1.345(11)
C4	C5	1.393(7)	C47	C48	1.385(11)
C5	C6	1.477(7)	C48	C49	1.364(9)

C6	C7	1.389(7)	C49	C50	1.402(8)
C7	C8	1.387(7)	O6	C60[1]	1.33(4)
C8	C9	1.498(7)	O6	C60	1.47(3)
C8	C19	1.395(7)	C60	O6[1]	1.33(4)
C9	C10	1.368(8)	C60	C60[1]	1.47(7)

Table S3. Selected bond angles (°) for **Ru5**.

Atom	**Atom**	**Atom**	**Angle/°**	**Atom**	**Atom**	**Atom**	**Angle/°**
Cl2	Ru1	Cl1	91.14(6)	C8	C7	C6	119.2(5)
S1	Ru1	Cl1	88.88(6)	C7	C8	C9	119.1(5)
S1	Ru1	Cl2	177.92(6)	C7	C8	C19	119.2(4)
N1	Ru1	Cl1	102.66(12)	C19	C8	C9	121.6(5)
N1	Ru1	Cl2	87.03(13)	C10	C9	C8	118.1(5)
N1	Ru1	S1	90.94(13)	C10	C9	C18	120.9(5)
N2	Ru1	Cl1	177.32(13)	C18	C9	C8	121.1(5)
N2	Ru1	Cl2	87.40(12)	C9	C10	C11	119.9(7)
N2	Ru1	S1	92.67(13)	C12	C11	C10	120.5(8)
N2	Ru1	N1	79.51(17)	C11	C12	C13	121.1(7)
N2	Ru1	N3	79.49(17)	C12	C13	C14	121.3(8)
N3	Ru1	Cl1	98.21(12)	C12	C13	C18	120.0(7)
N3	Ru1	Cl2	87.00(14)	C14	C13	C18	118.7(9)
N3	Ru1	S1	95.06(14)	C15	C14	C13	121.1(9)
N3	Ru1	N1	158.39(17)	C14	C15	C16	120.1(10)
Cl3	Ru2	Cl4	91.51(5)	C17	C16	C15	120.4(10)
S2	Ru2	Cl3	178.37(5)	C16	C17	C18	122.1(8)
S2	Ru2	Cl4	89.37(6)	C9	C18	C13	117.6(6)
N4	Ru2	Cl3	86.91(13)	C9	C18	C17	124.8(5)
N4	Ru2	Cl4	98.37(12)	C17	C18	C13	117.6(6)
N4	Ru2	S2	94.33(13)	C20	C19	C8	119.2(5)
N5	Ru2	Cl3	86.95(12)	N2	C20	C19	120.1(5)
N5	Ru2	Cl4	177.57(13)	N2	C20	C21	113.2(4)
N5	Ru2	S2	92.22(12)	C19	C20	C21	126.6(5)
N5	Ru2	N4	79.68(17)	N1	C21	C20	115.2(5)
N5	Ru2	N6	80.12(16)	N1	C21	C22	121.0(5)
N6	Ru2	Cl3	87.72(12)	C22	C21	C20	123.7(5)
N6	Ru2	Cl4	101.71(12)	C23	C22	C21	119.1(6)
N6	Ru2	S2	90.75(12)	C24	C23	C22	120.6(6)
N6	Ru2	N4	159.34(16)	C23	C24	C25	118.3(6)
O1	S1	Ru1	117.2(2)	N1	C25	C24	122.0(5)
O1	S1	C51	108.6(4)	N6	C26	C27	122.3(5)
O1	S1	C52	105.8(4)	C28	C27	C26	118.9(6)
C51	S1	Ru1	112.0(3)	C27	C28	C29	119.7(6)
C51	S1	C52	99.5(4)	C28	C29	C30	119.3(5)
C52	S1	Ru1	112.1(3)	N6	C30	C29	121.1(5)

O2	S2	Ru2	117.16(19)	N6	C30	C31	114.6(4)
O2	S2	C53	104.9(4)	C29	C30	C31	124.2(5)
O2	S2	C54	107.4(4)	N5	C31	C30	113.6(4)
C53	S2	Ru2	113.5(3)	N5	C31	C32	119.6(5)
C54	S2	Ru2	112.8(3)	C32	C31	C30	126.7(5)
C54	S2	C53	99.3(5)	C31	C32	C33	119.4(5)
O5	S3	C57	112.2(14)	C32	C33	C34	119.1(5)
O5	S3	C58	107.3(14)	C32	C33	C41	121.9(5)
C58	S3	C57	96.3(10)	C34	C33	C41	119.0(5)
O4	S4	C55	107.3(13)	C35	C34	C33	119.6(5)
O4	S4	C56	115.1(14)	N5	C35	C34	119.8(5)
C56	S4	C55	98.2(10)	N5	C35	C36	113.6(4)
C21	N1	Ru1	113.3(3)	C34	C35	C36	126.5(5)
C21	N1	C25	118.8(5)	N4	C36	C35	115.0(5)
C25	N1	Ru1	127.7(4)	N4	C36	C37	121.3(5)
C6	N2	Ru1	118.7(3)	C37	C36	C35	123.7(5)
C6	N2	C20	122.2(4)	C38	C37	C36	119.2(5)
C20	N2	Ru1	118.8(3)	C37	C38	C39	119.2(6)
C1	N3	Ru1	127.7(4)	C40	C39	C38	118.7(5)
C1	N3	C5	118.5(5)	N4	C40	C39	123.0(5)
C5	N3	Ru1	113.2(3)	C42	C41	C33	118.7(5)
C36	N4	Ru2	112.8(3)	C42	C41	C50	119.9(5)
C40	N4	Ru2	128.2(4)	C50	C41	C33	121.4(5)
C40	N4	C36	118.7(5)	C41	C42	C43	120.8(6)
C31	N5	Ru2	118.5(3)	C44	C43	C42	121.0(6)
C35	N5	Ru2	118.9(3)	C43	C44	C45	120.9(6)
C35	N5	C31	122.5(4)	C44	C45	C50	119.5(6)
C26	N6	Ru2	128.0(4)	C46	C45	C44	121.9(7)
C26	N6	C30	118.6(5)	C46	C45	C50	118.5(7)
C30	N6	Ru2	113.3(3)	C47	C46	C45	121.4(7)
N3	C1	C2	121.6(5)	C46	C47	C48	120.5(7)
C3	C2	C1	119.7(6)	C49	C48	C47	120.3(7)
C4	C3	C2	119.1(6)	C48	C49	C50	120.7(6)
C3	C4	C5	119.5(5)	C45	C50	C41	117.8(5)
N3	C5	C4	121.5(5)	C49	C50	C41	123.7(5)
N3	C5	C6	114.9(5)	C49	C50	C45	118.4(5)
C4	C5	C6	123.6(5)	C60[1]	O6	C60	63(3)
N2	C6	C5	113.7(4)	O6[1]	C60	O6	117(3)
N2	C6	C7	119.9(5)	O6[1]	C60	C60[1]	63(3)
C7	C6	C5	126.2(5)	C60[1]	C60	O6	53.7(18)

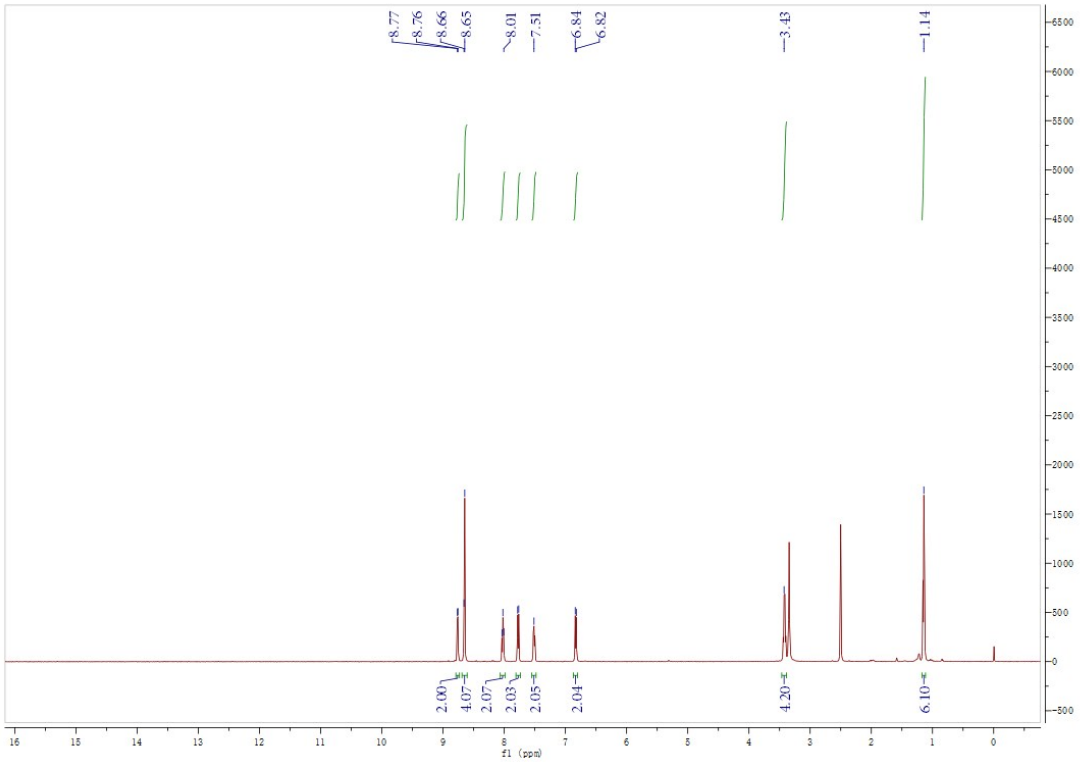


Figure S1. ^{1}H NMR spectrum (500MHz, DMSO-d_6) of 4-EtN-Phtpy.

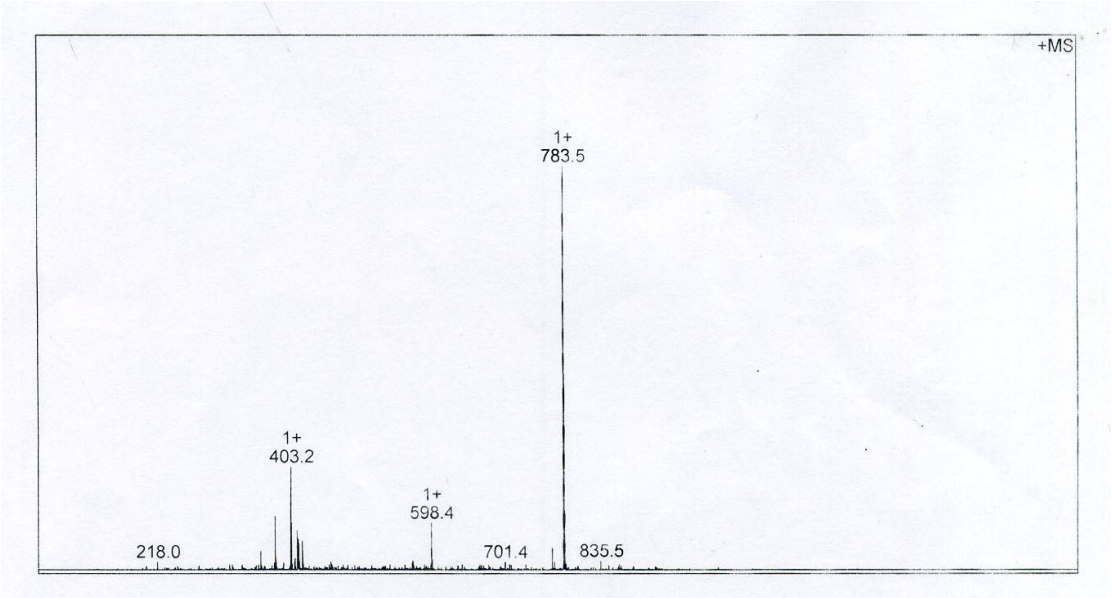


Figure S2. ESI-MS spectrum of 4-EtN-Phtpy.

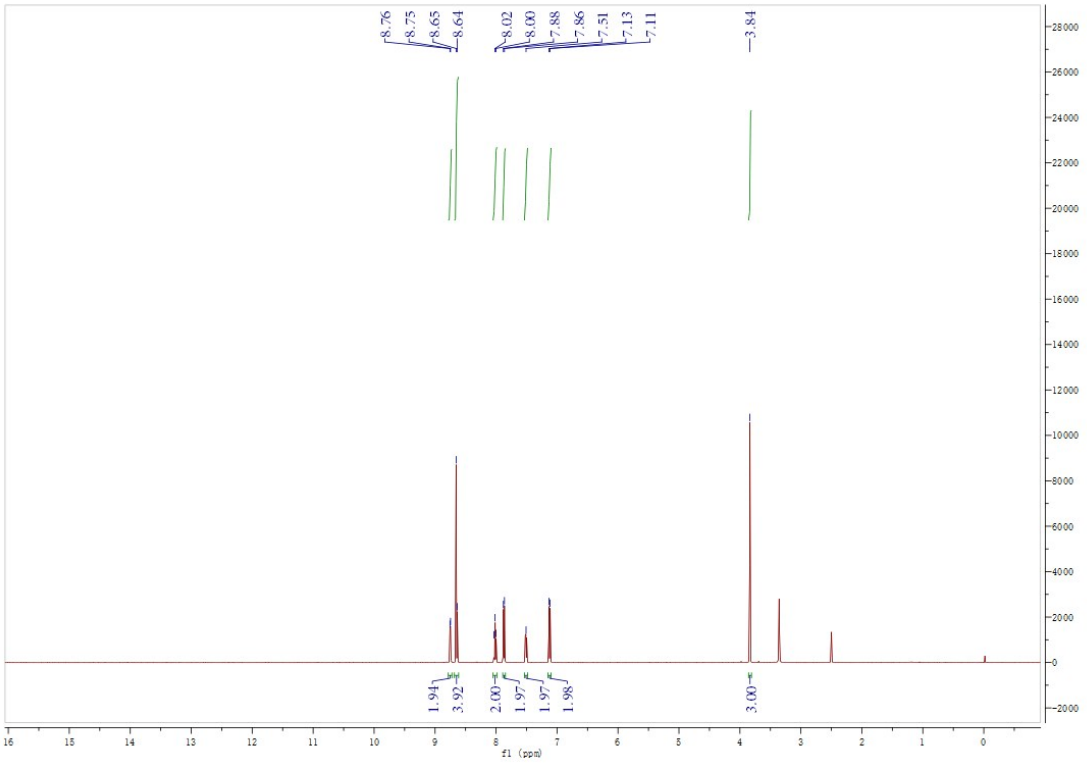


Figure S3. ^{1}H NMR spectrum (500MHz, DMSO-d_6) of 4-MeO-Phtpy.

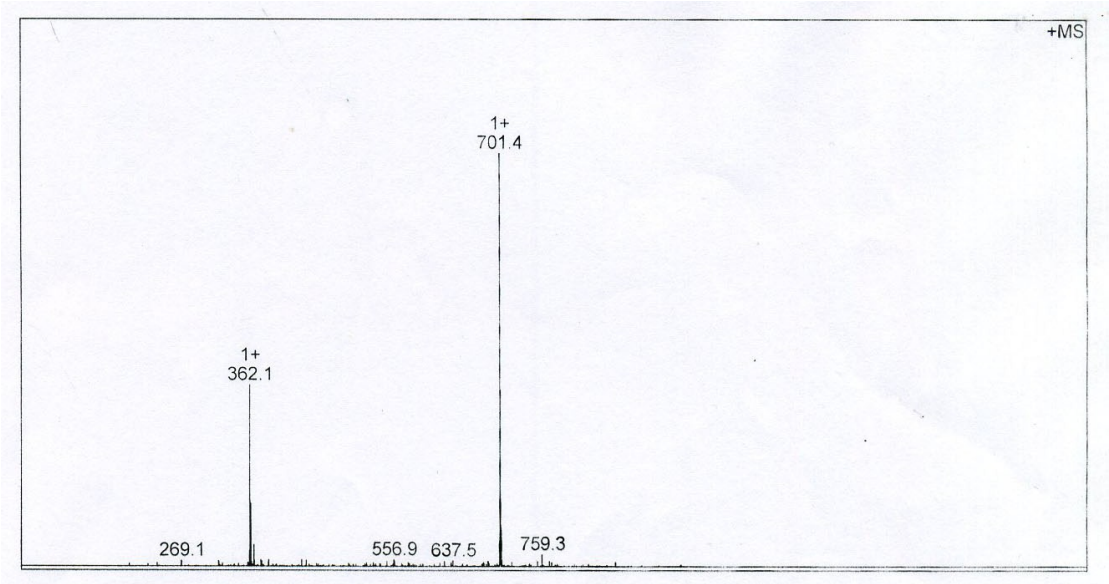


Figure S4. ESI-MS spectrum of 4-MeO-Phtpy.

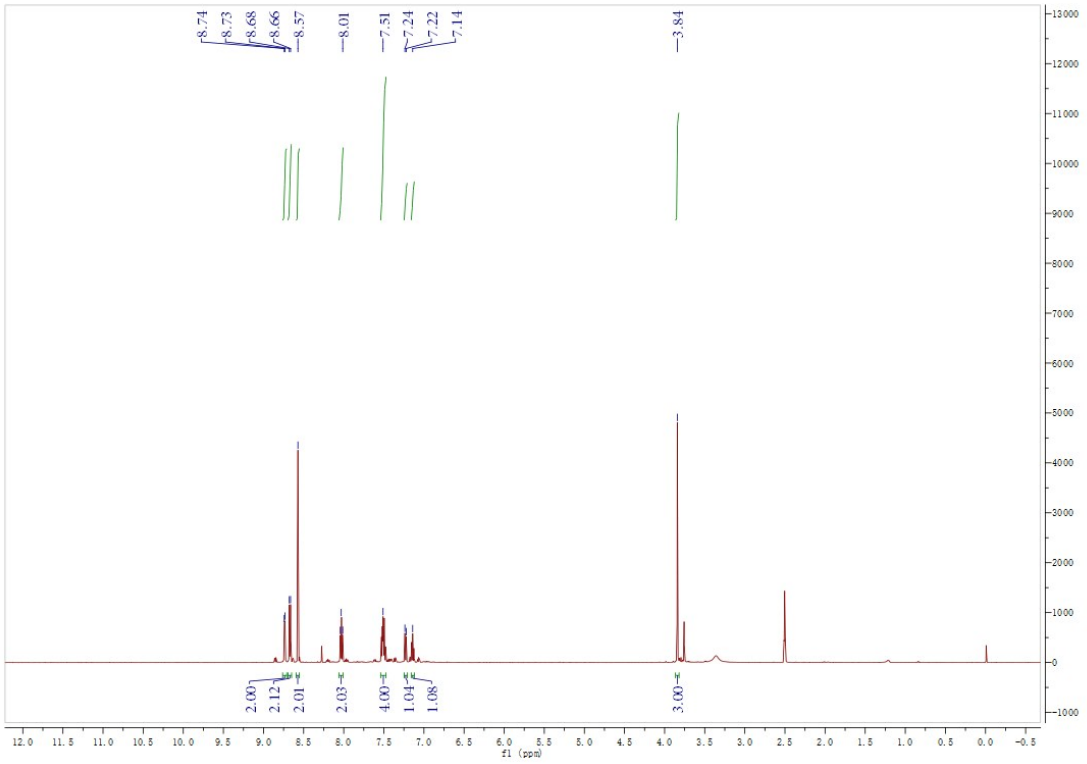


Figure S5. ^{1}H NMR spectrum (500MHz, DMSO-d_6) of 2-MeO-Phtpy.

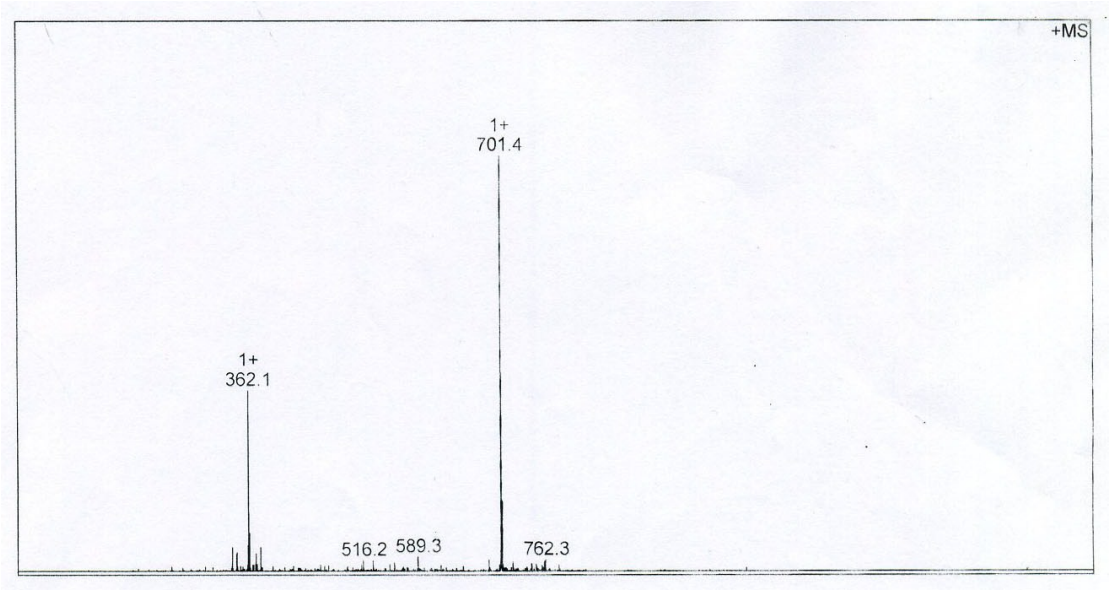


Figure S6. ESI-MS spectrum of 2-MeO-Phtpy.

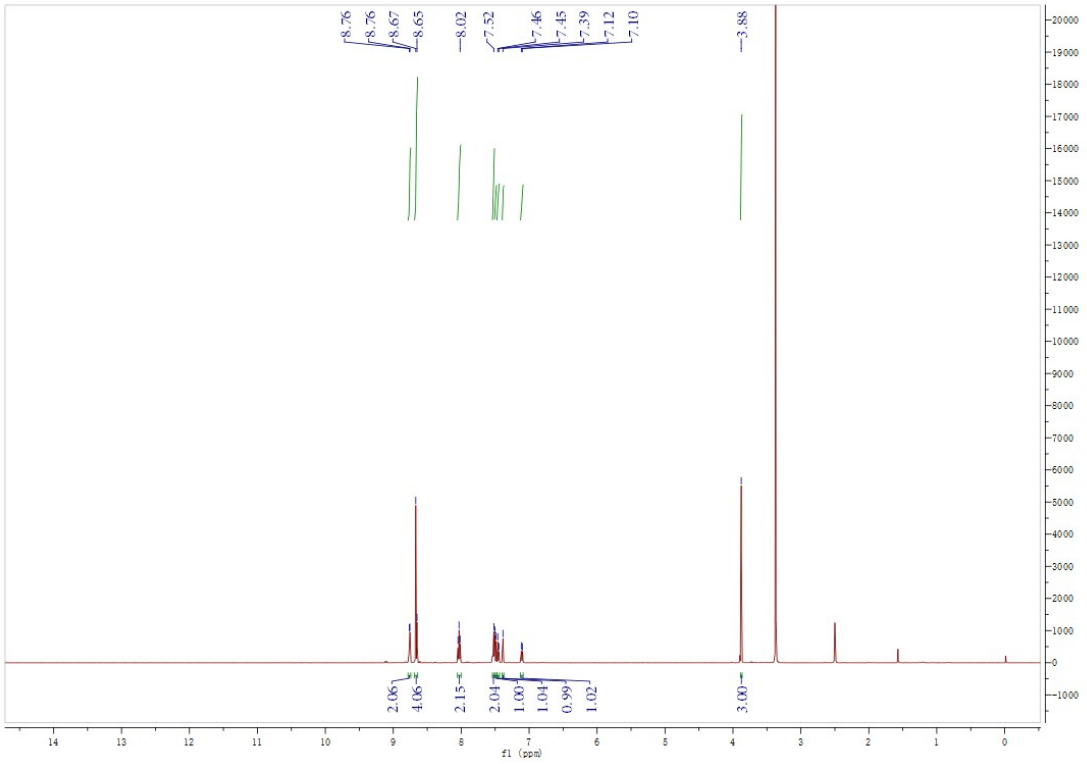


Figure S7. ^{1}H NMR spectrum (500MHz, DMSO-d_6) of 3-MeO-Phtpy.

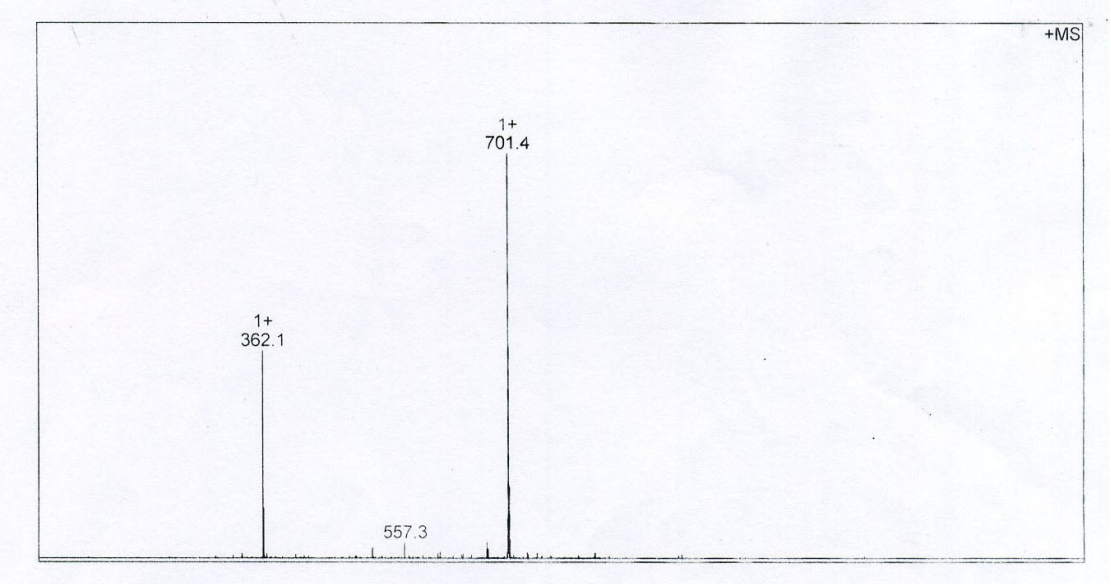


Figure S8. ESI-MS spectrum of 3-MeO-Phtpy.

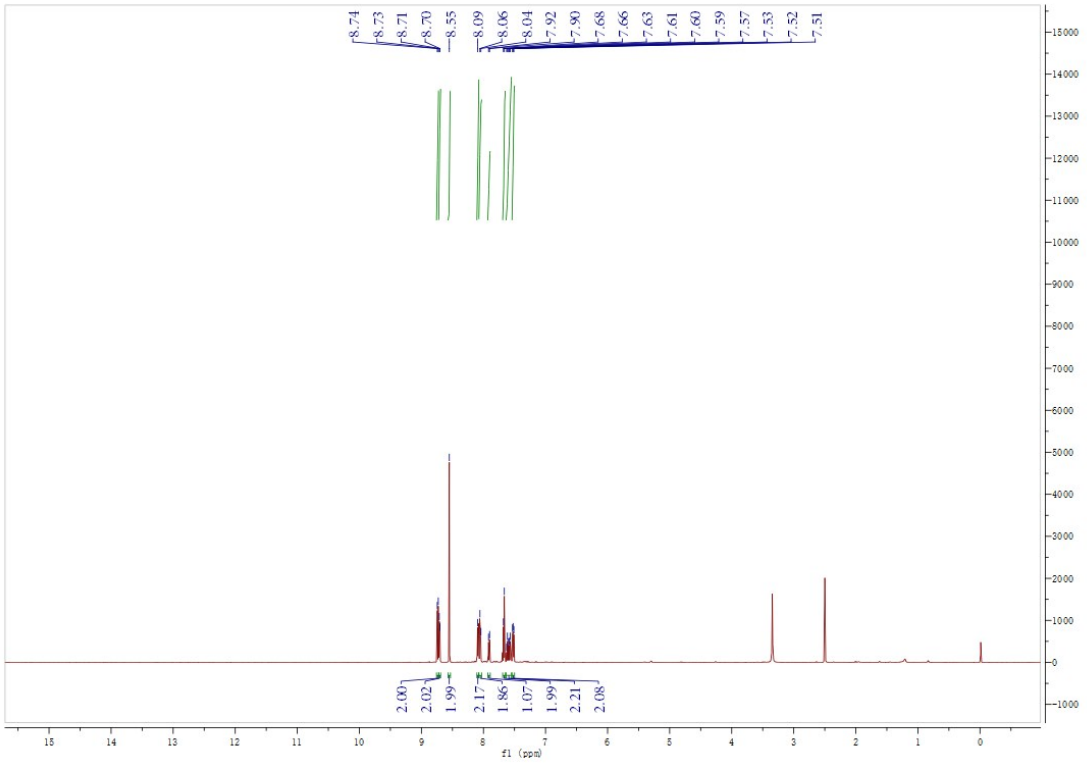


Figure S9. ^{1}H NMR spectrum (500MHz, DMSO-d_6) of 1-Bip-Phtpy.

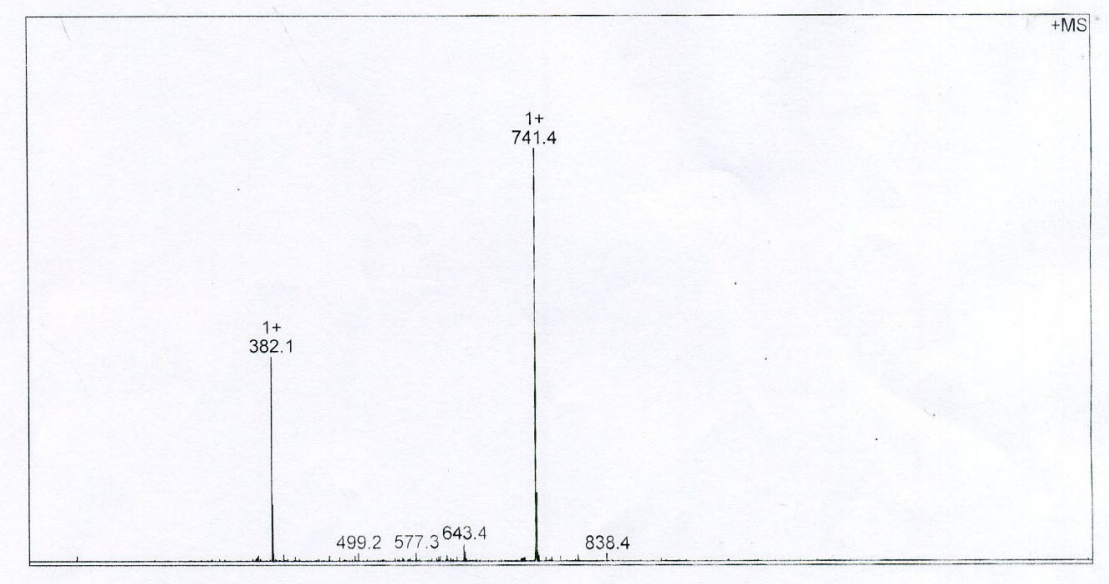


Figure S10. ESI-MS spectrum of 1-Bip-Phtpy.

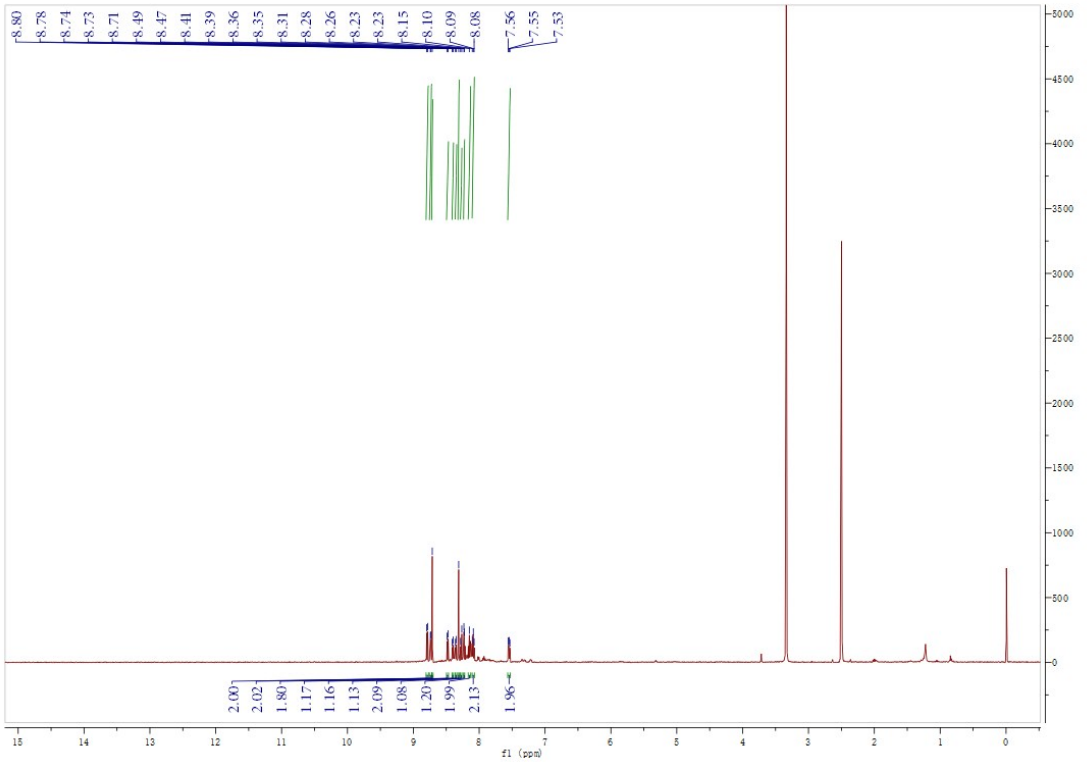


Figure S11. ^{1}H NMR spectrum (500MHz, DMSO-d_6) of 1-Pyr-Phtpy.

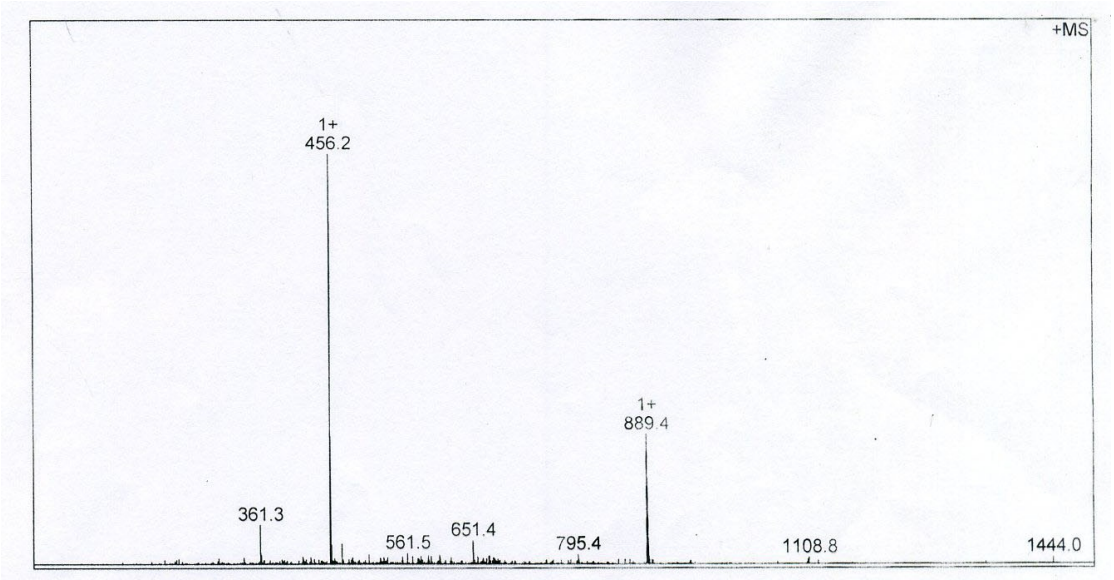


Figure S12. ESI-MS spectrum of 1-Pyr-Phtpy.

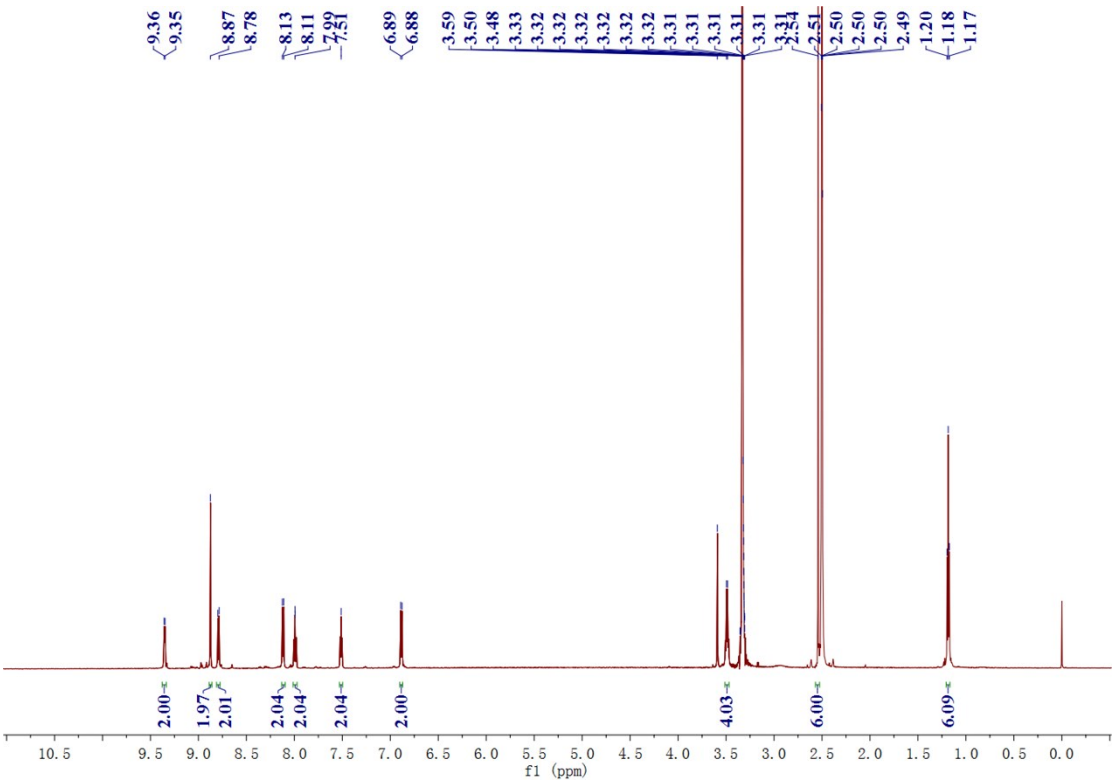


Figure S13. ^{1}H NMR spectrum (600MHz, DMSO-d_6) of **Ru1**.

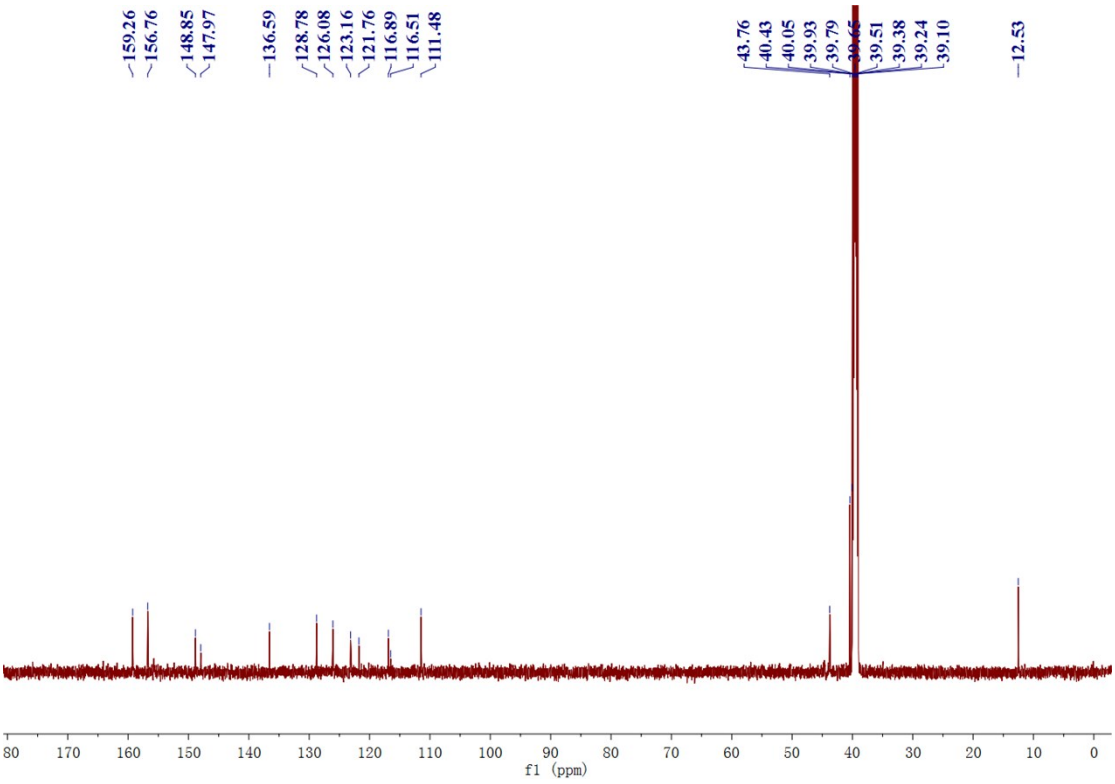


Figure S14. ^{13}C NMR spectrum (151MHz, DMSO-d_6) of **Ru1**.

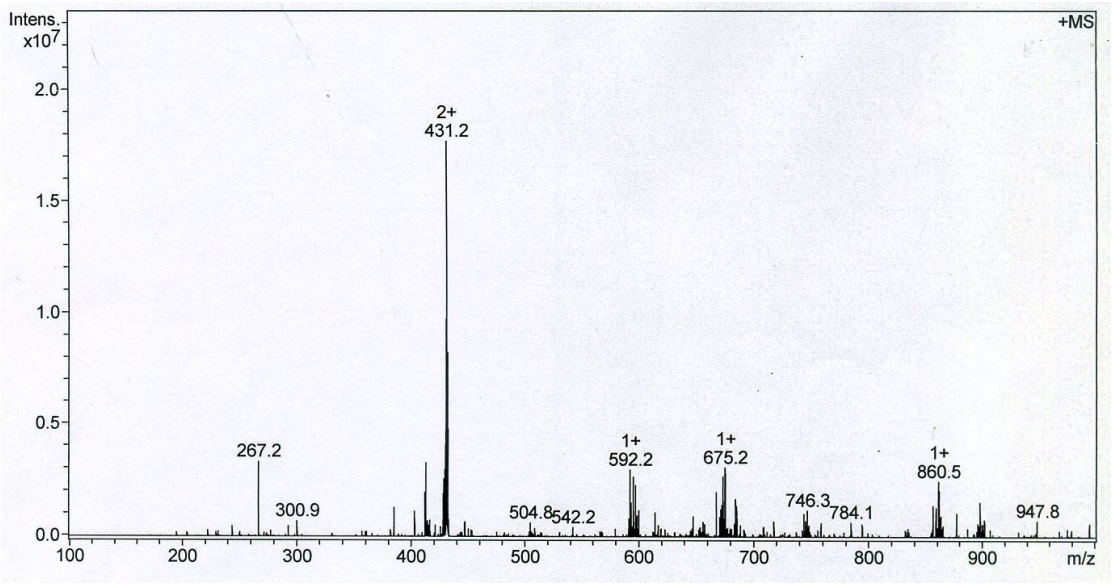


Figure S15. ESI-MS spectrum of **Ru1**.

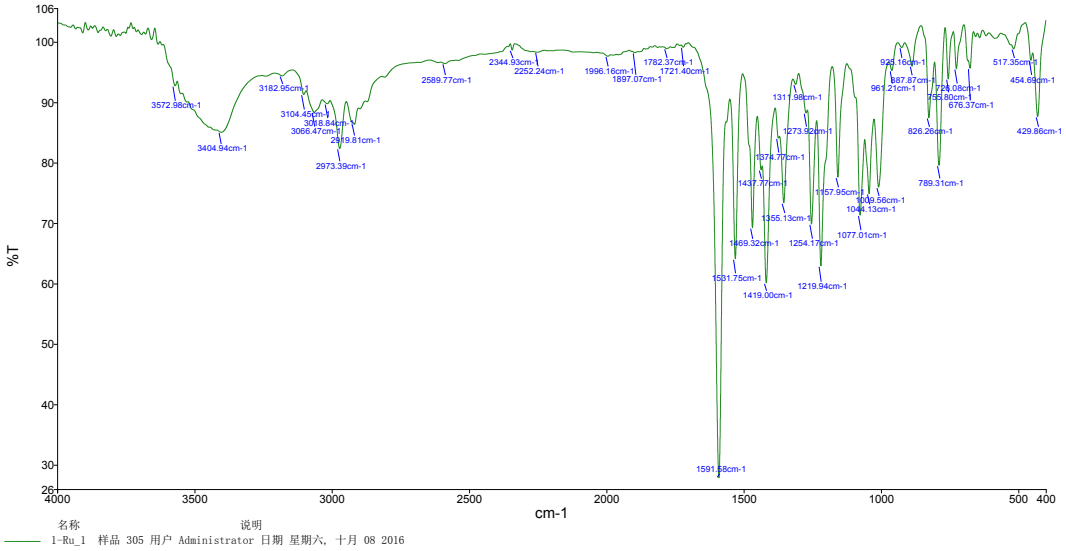


Figure S16. IR (KBr) spectrum of **Ru1**.

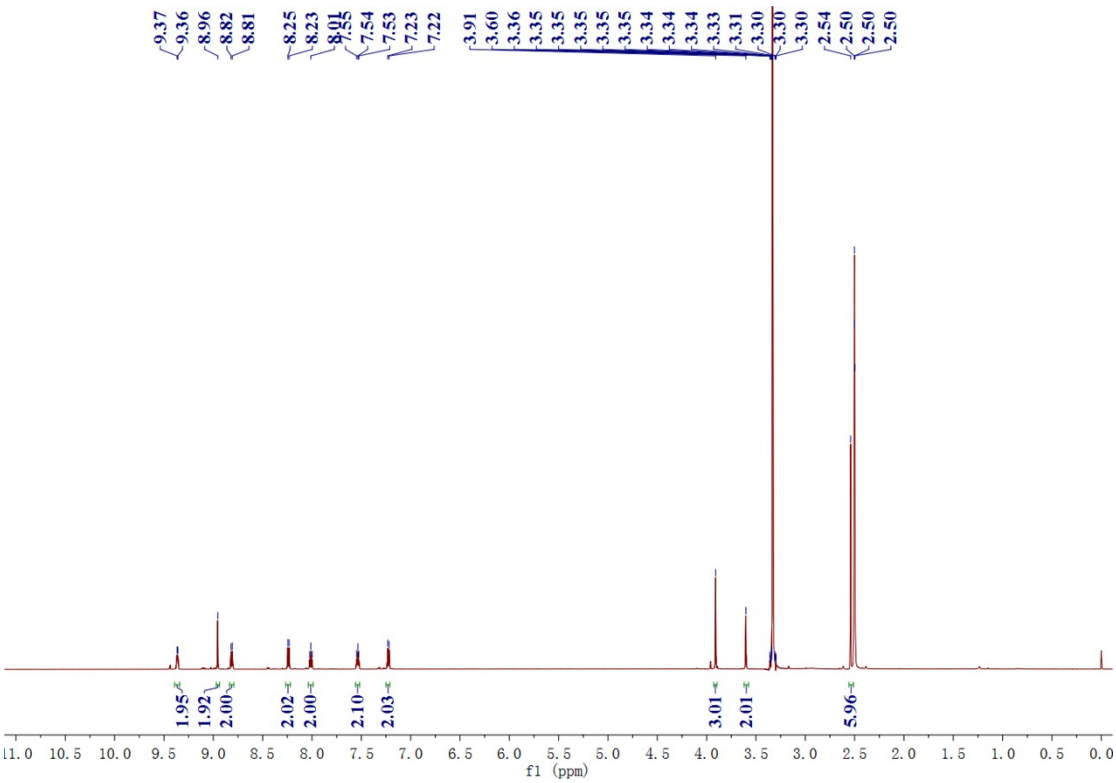


Figure S17. ^{1}H NMR spectrum (600MHz, DMSO-d_6) of **Ru2**.

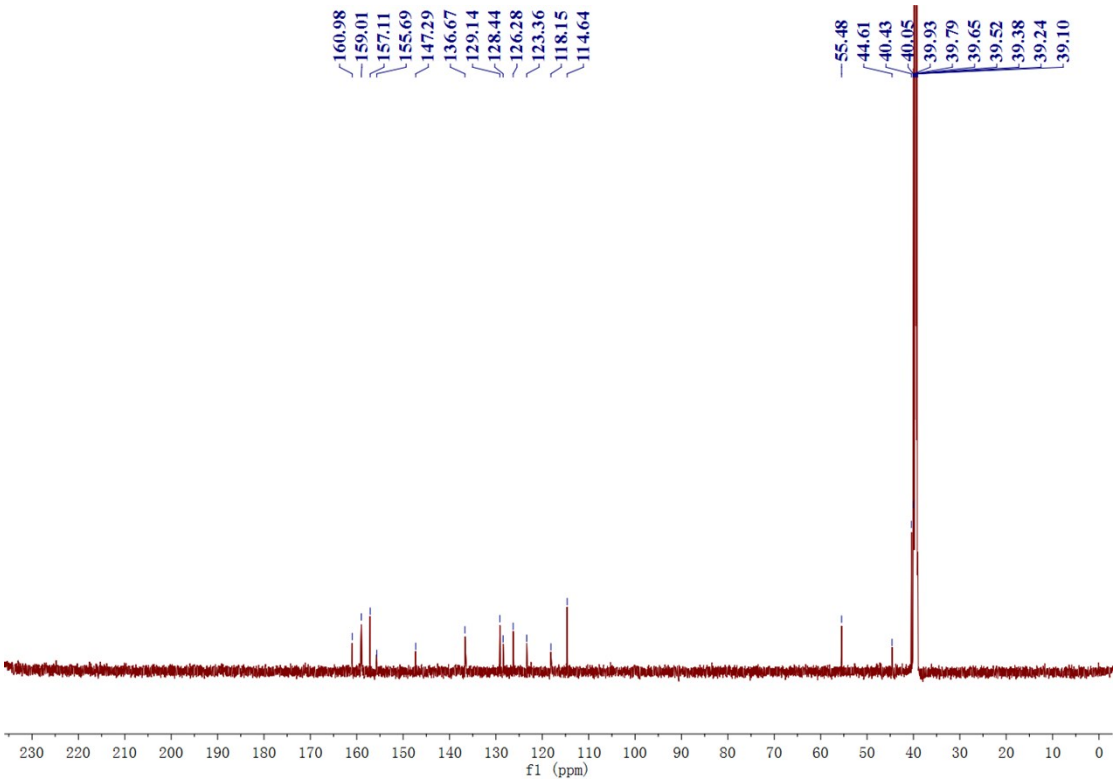


Figure S18. ^{13}C NMR spectrum (151MHz, DMSO-d_6) of **Ru2**.

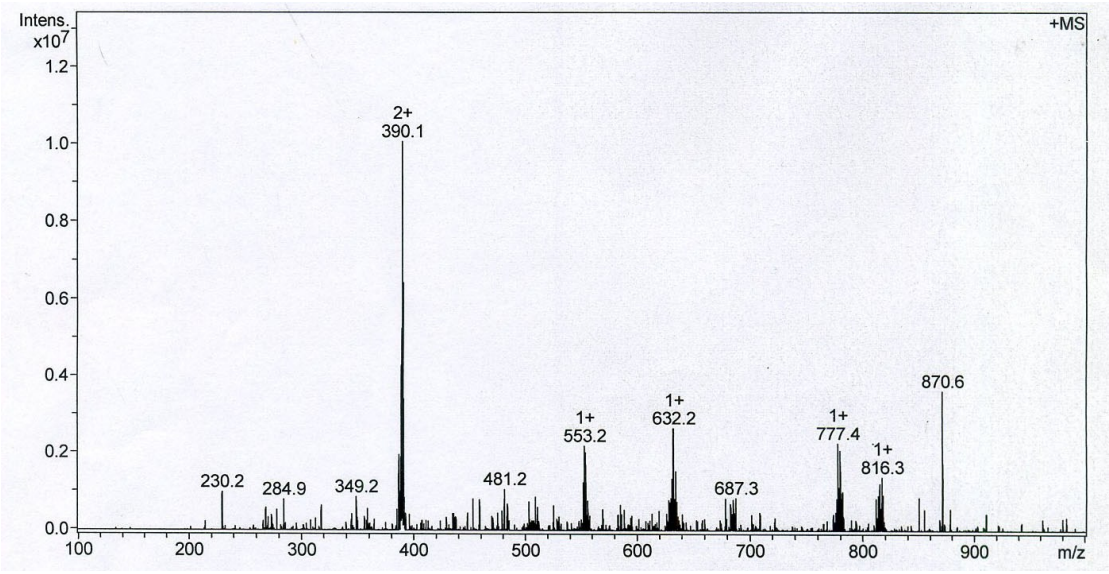


Figure S19. ESI-MS spectrum of **Ru2**.

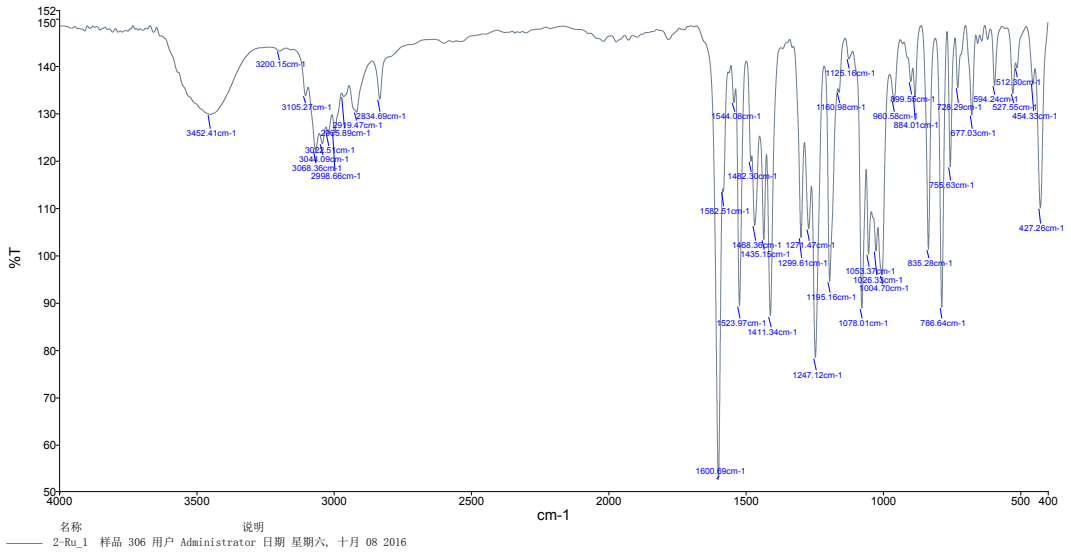


Figure S20. IR (KBr) spectrum of **Ru2**.

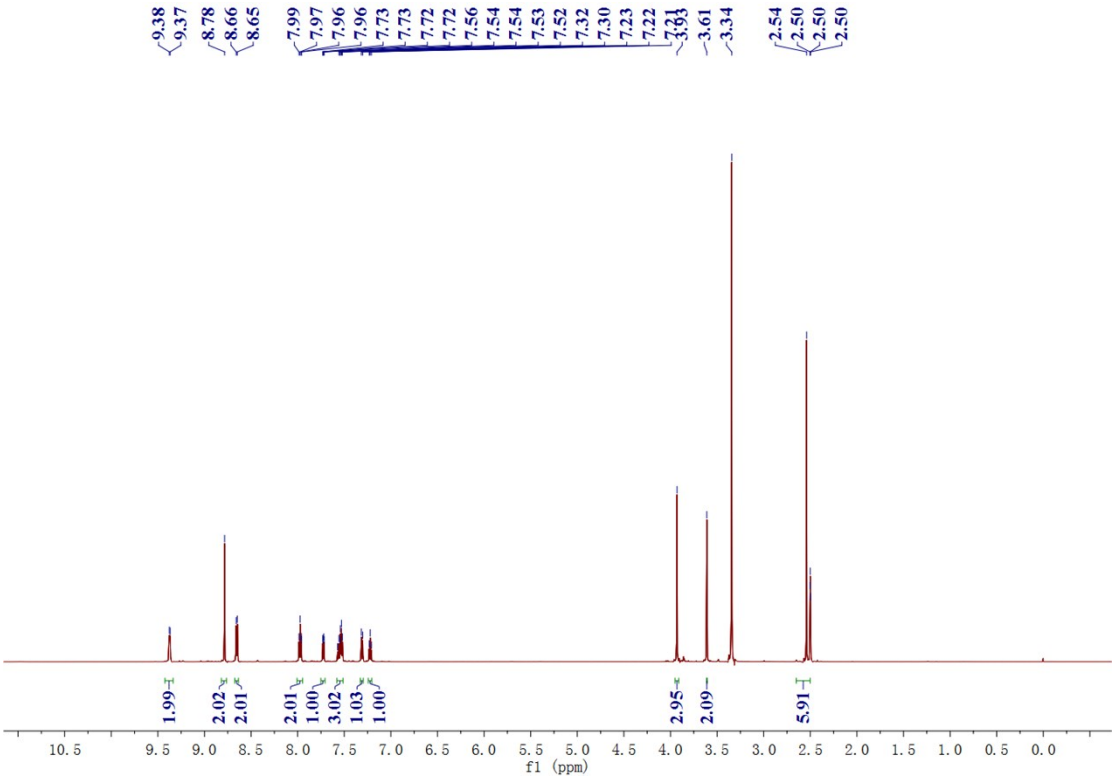


Figure S21. ^{1}H NMR spectrum (600MHz, DMSO-d_6) of **Ru3**.

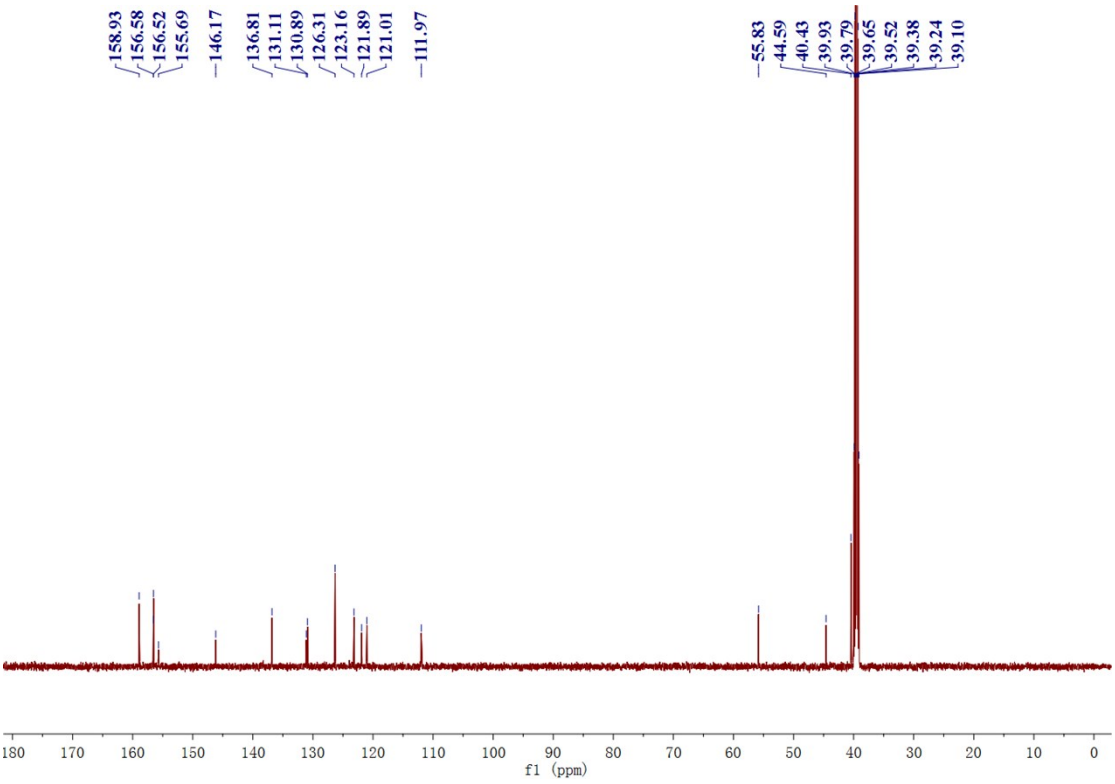


Figure S22. ^{13}C NMR spectrum (151MHz, DMSO-d_6) of **Ru3**.

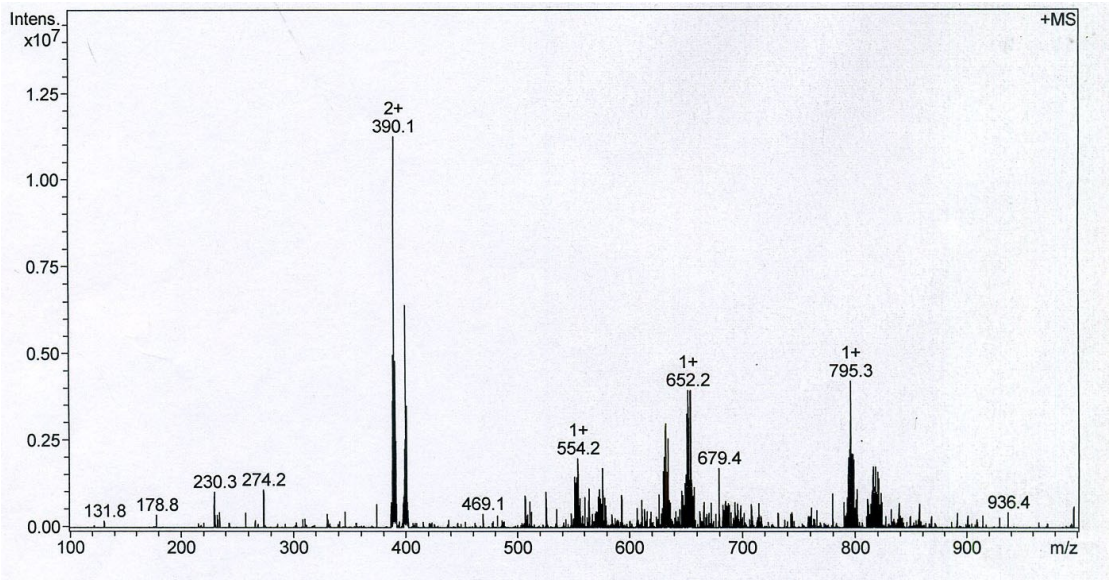


Figure S23. ESI-MS spectrum of **Ru3**.

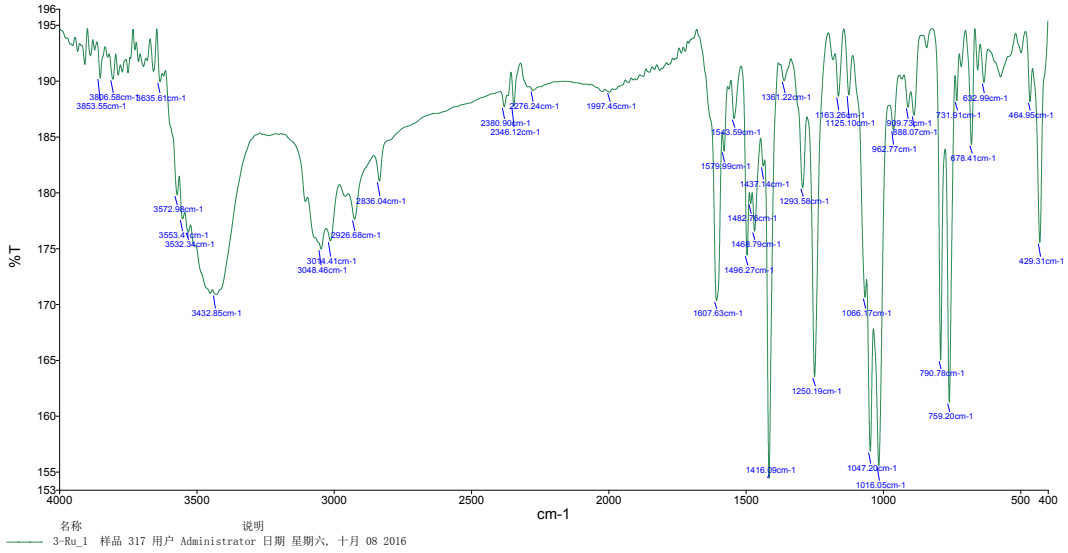


Figure S24. IR (KBr) spectrum of **Ru3**.

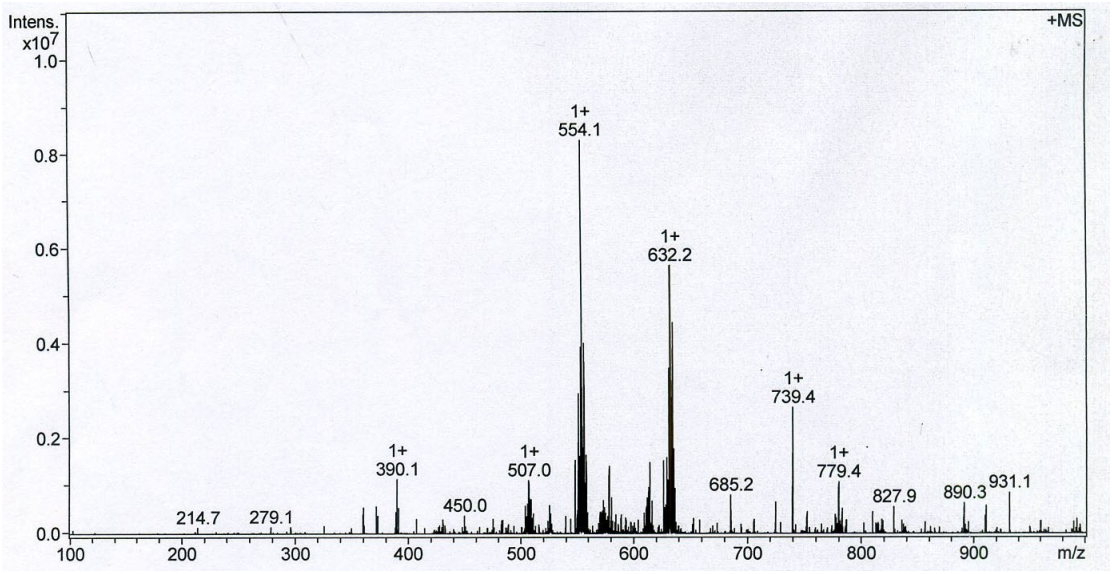


Figure S25. ESI-MS spectrum of **Ru4**.

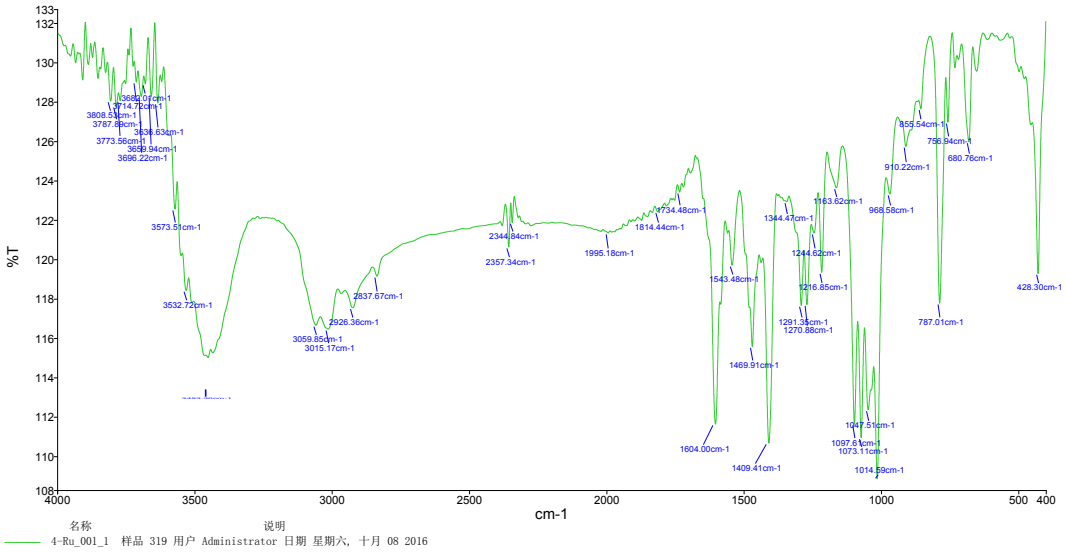


Figure S26. IR (KBr) spectrum of **Ru4**.

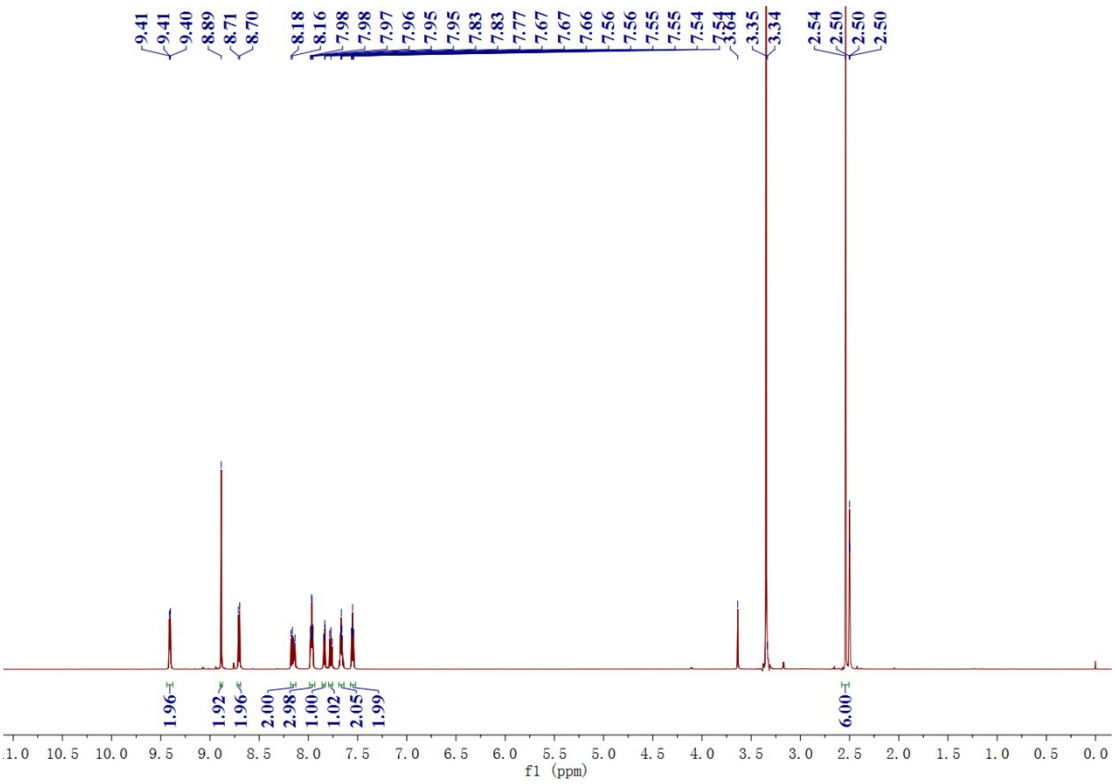


Figure S27. ^{1}H NMR spectrum (600MHz, DMSO-d_6) of **Ru5**.

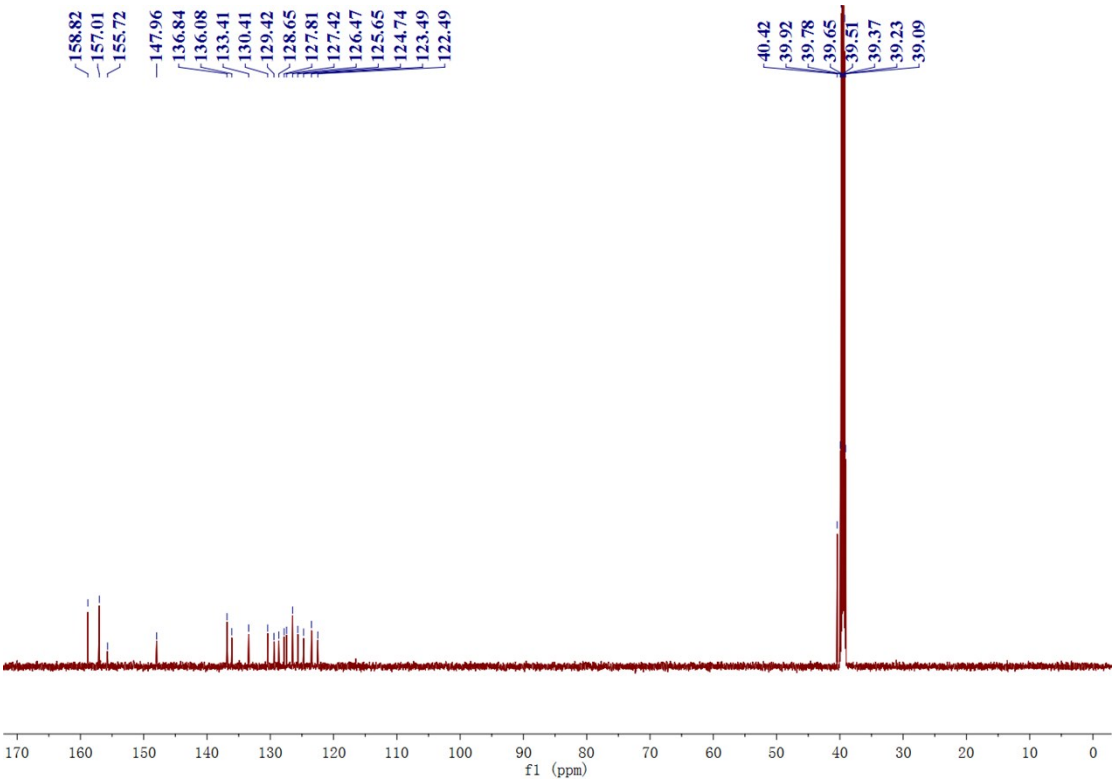


Figure S28. ^{13}C NMR spectrum (151MHz, DMSO-d_6) of **Ru5**.

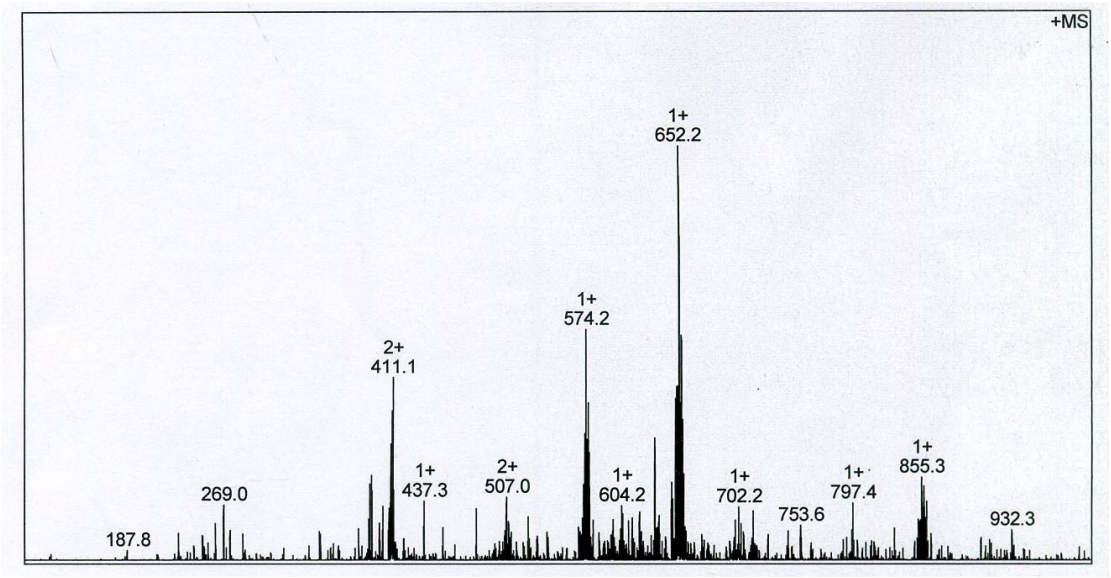


Figure S29. ESI-MS spectrum of **Ru5**.

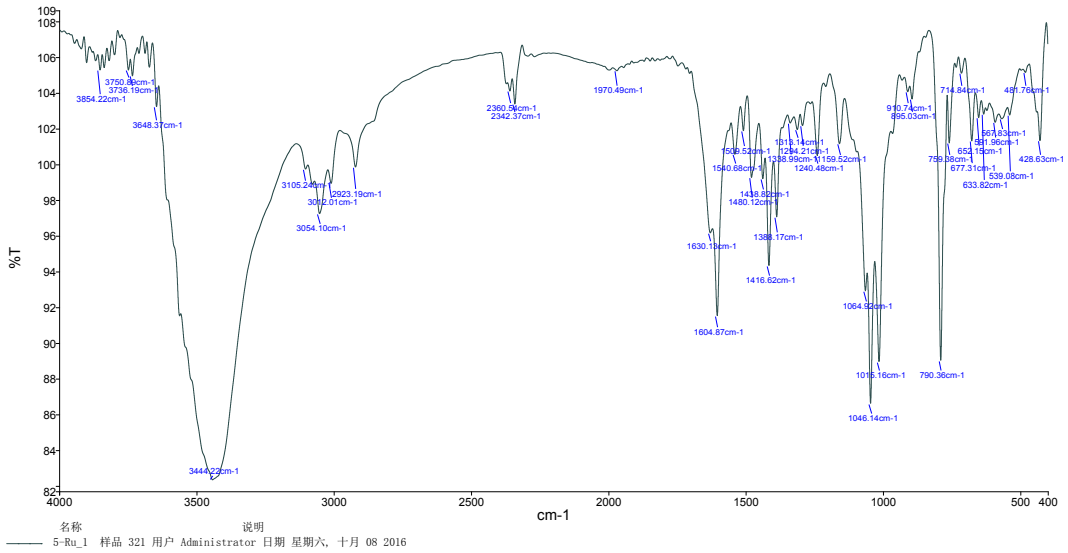


Figure S30. IR (KBr) spectrum of **Ru5**.

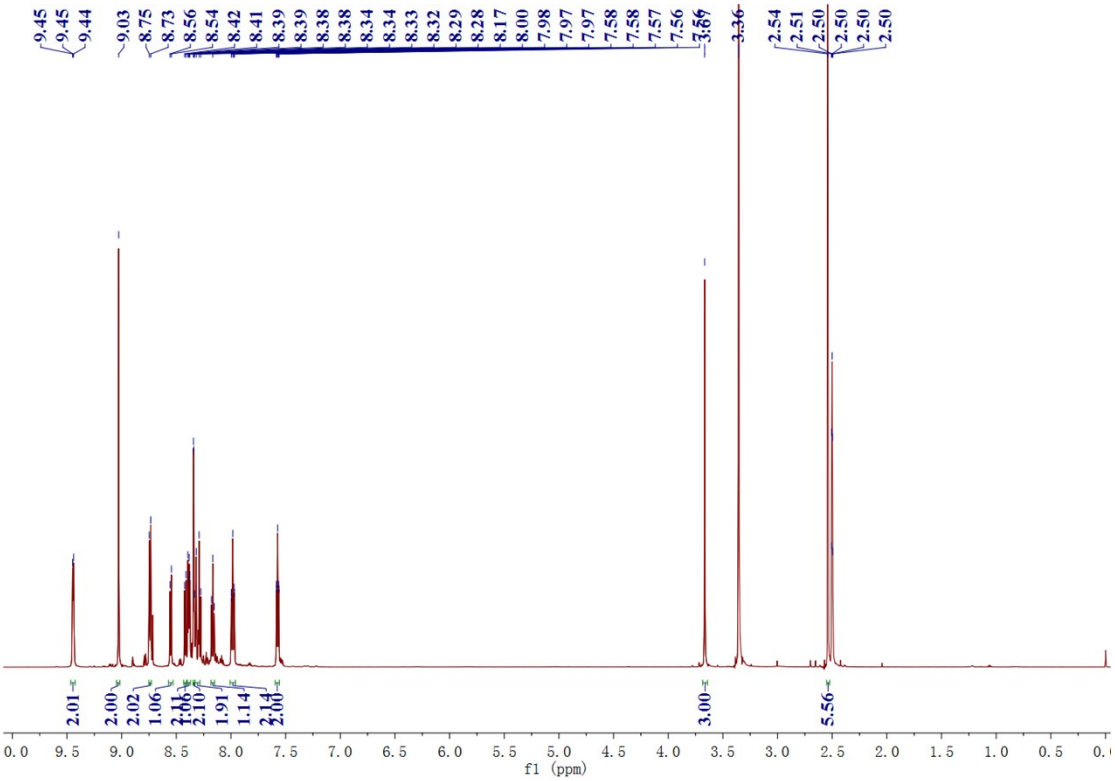


Figure S31. ^{1}H NMR spectrum (600MHz, DMSO-d_6) of **Ru6**.

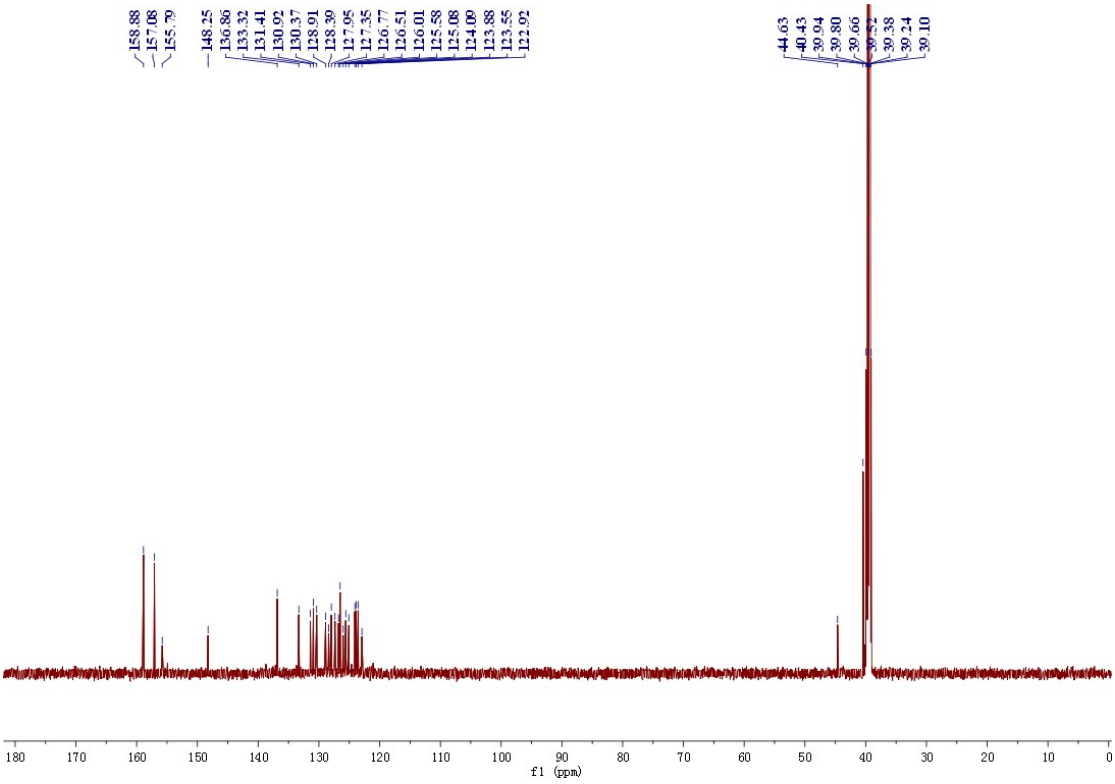


Figure S32. ^{13}C NMR spectrum (151MHz, DMSO-d_6) of **Ru6**.

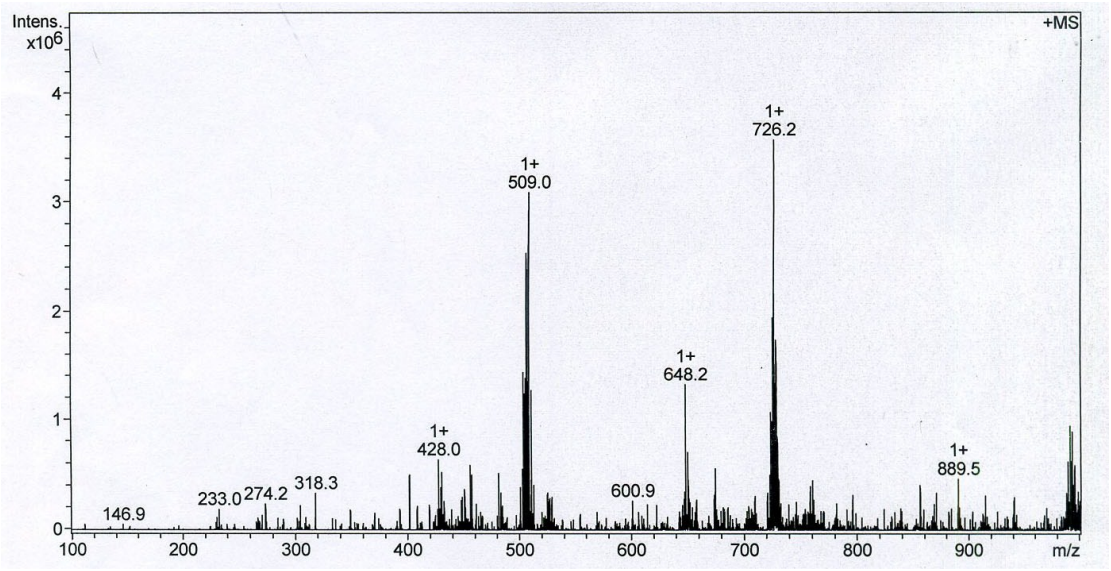


Figure S33. ESI-MS spectrum of **Ru6**.

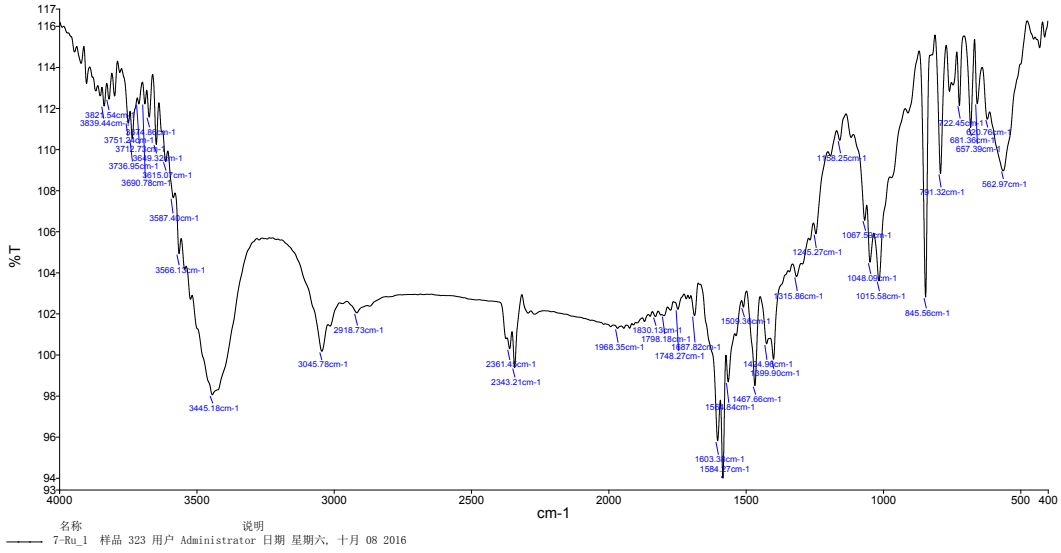


Figure S34. IR (KBr) spectrum of **Ru6**.

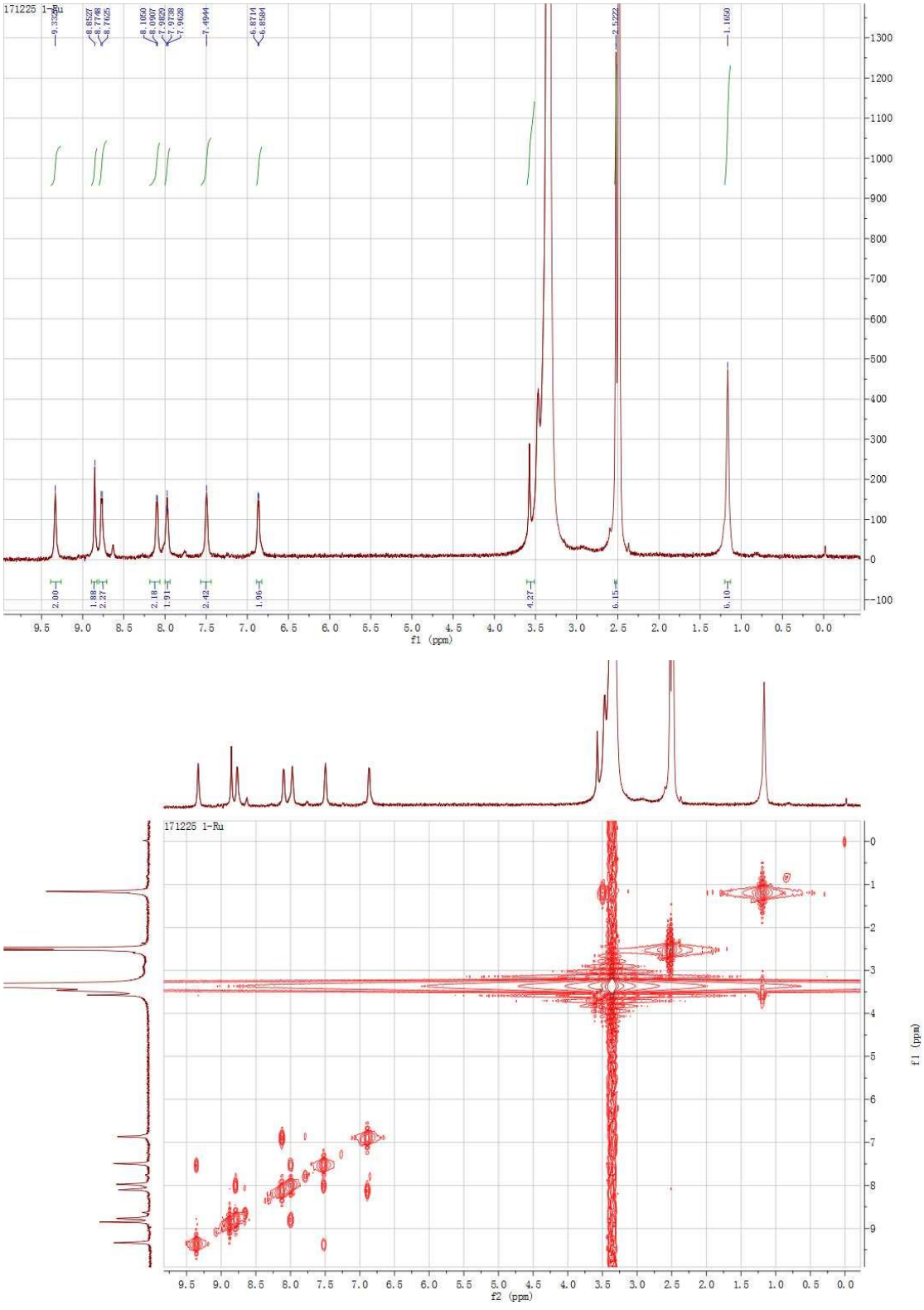


Figure S35. The experiment of ^{1}H NMR spectra and ^{1}H-^{1}H COSY NMR spectra for **Ru1**.

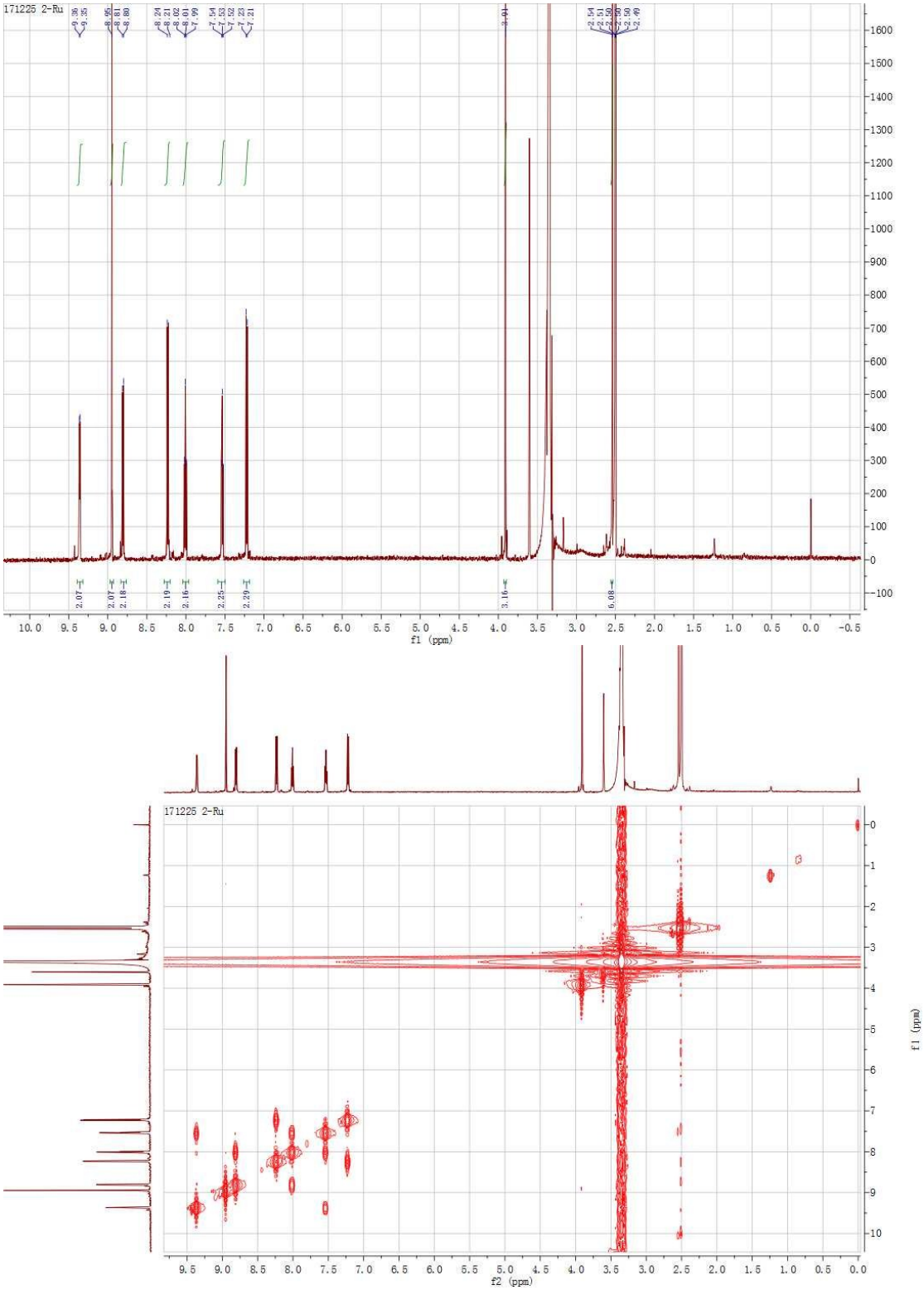


Figure S36. The experiment of ^{1}H NMR spectra and ^{1}H-^{1}H COSY NMR spectra for **Ru2**.

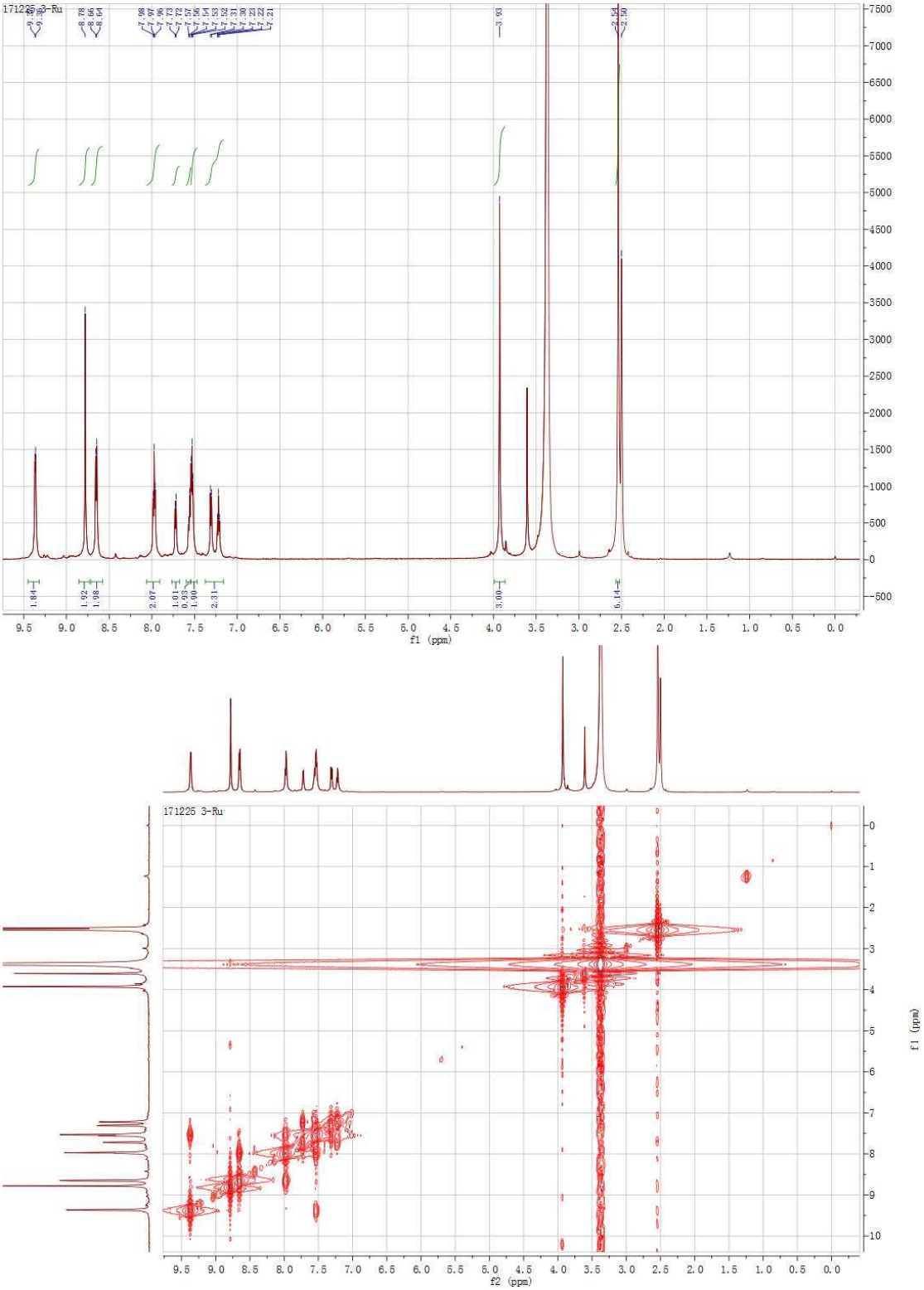


Figure S37. The experiment of ^{1}H NMR spectra and ^{1}H-^{1}H COSY NMR spectra for **Ru3**.

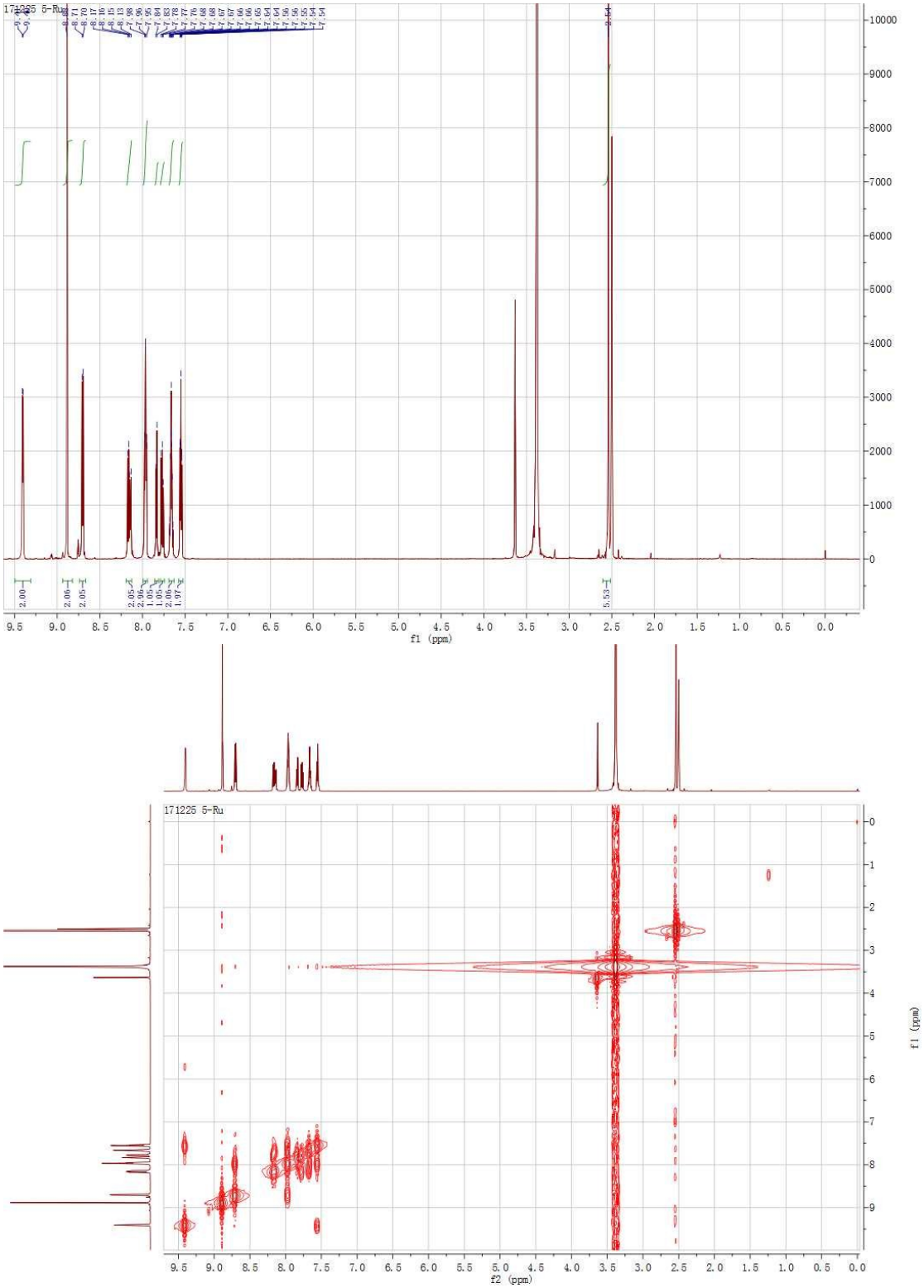


Figure S38. The experiment of ^{1}H NMR spectra and ^{1}H-^{1}H COSY NMR spectra for **Ru5**.

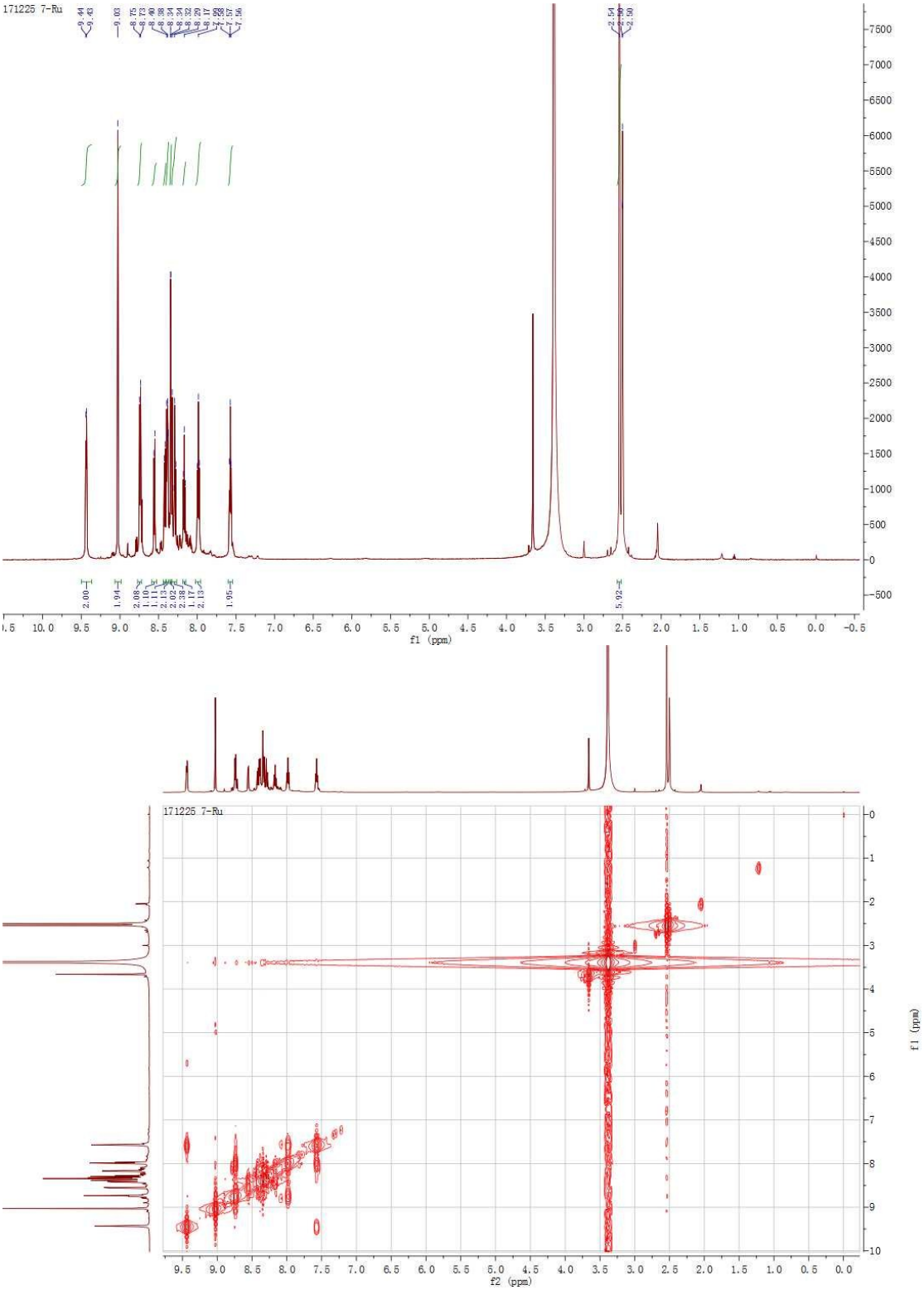
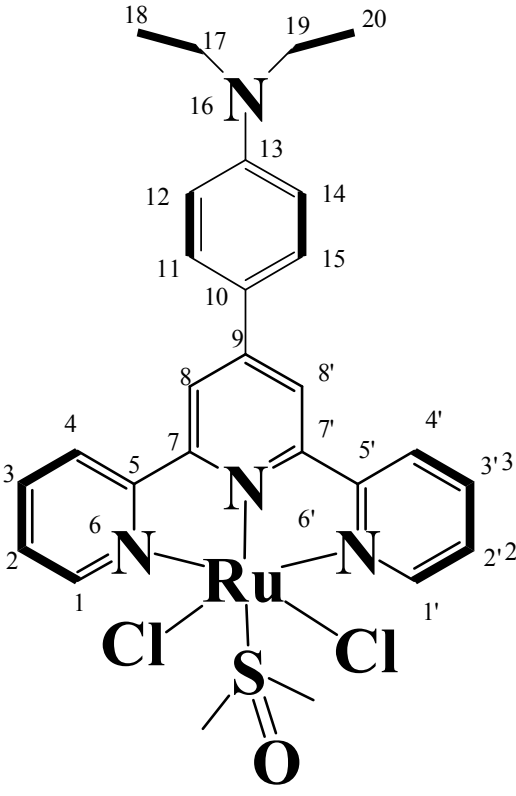
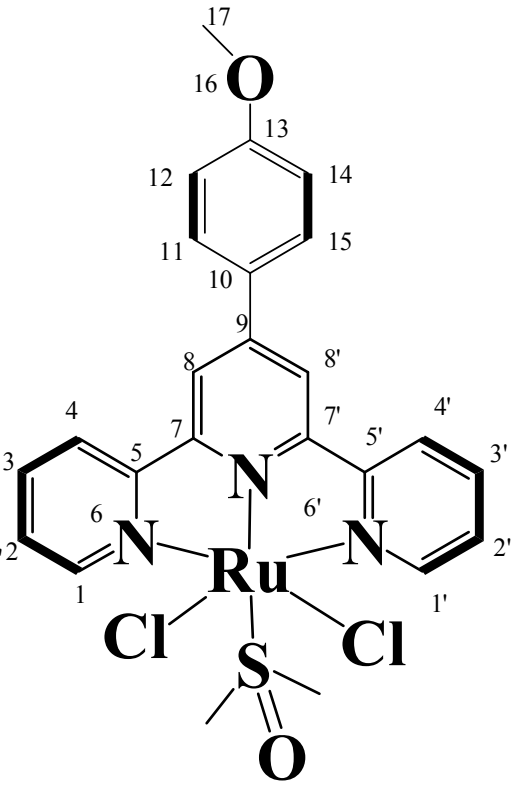


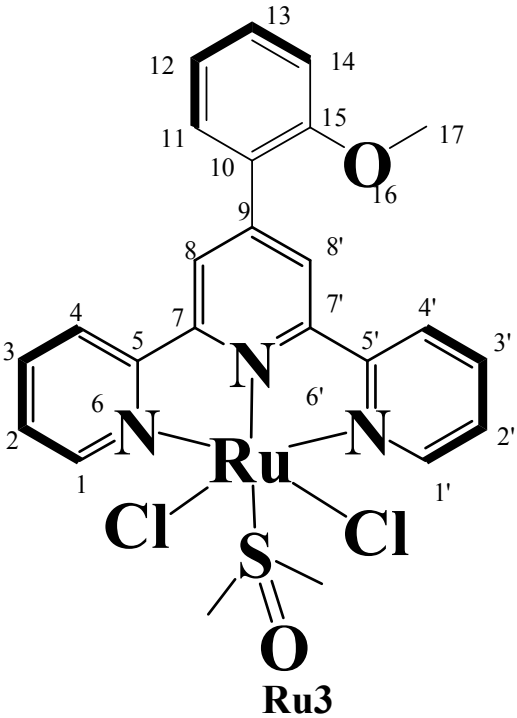
Figure S39. The experiment of ^{1}H NMR spectra and ^{1}H-^{1}H COSY NMR spectra for **Ru6**.



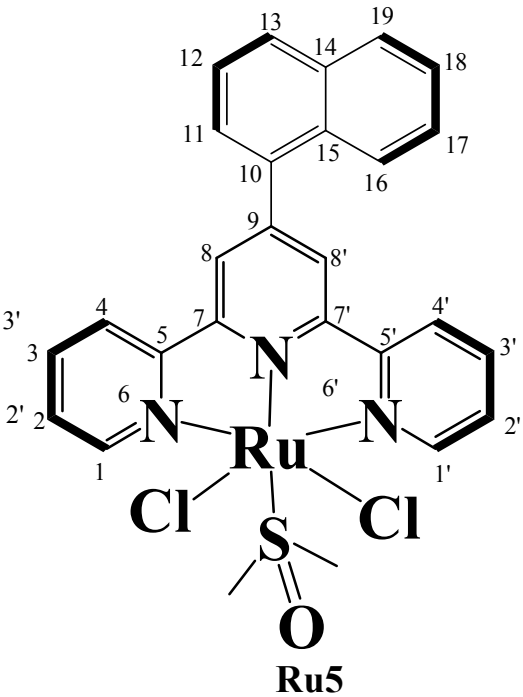
Ru1



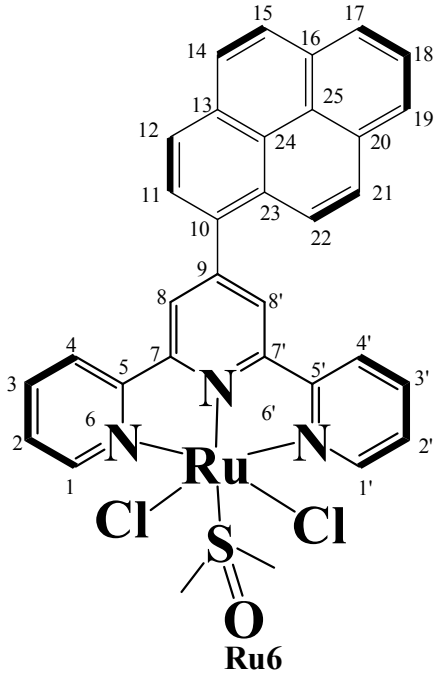
Ru2



Ru3



Ru5

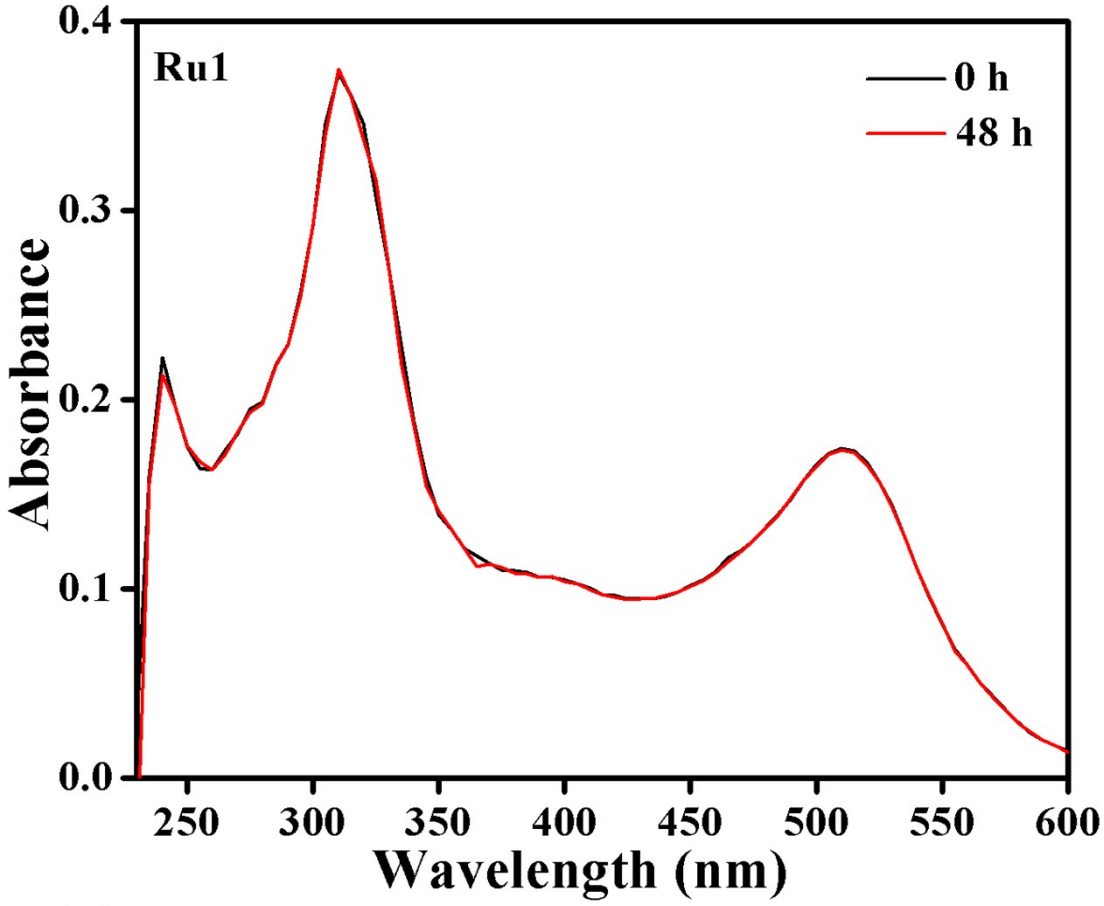


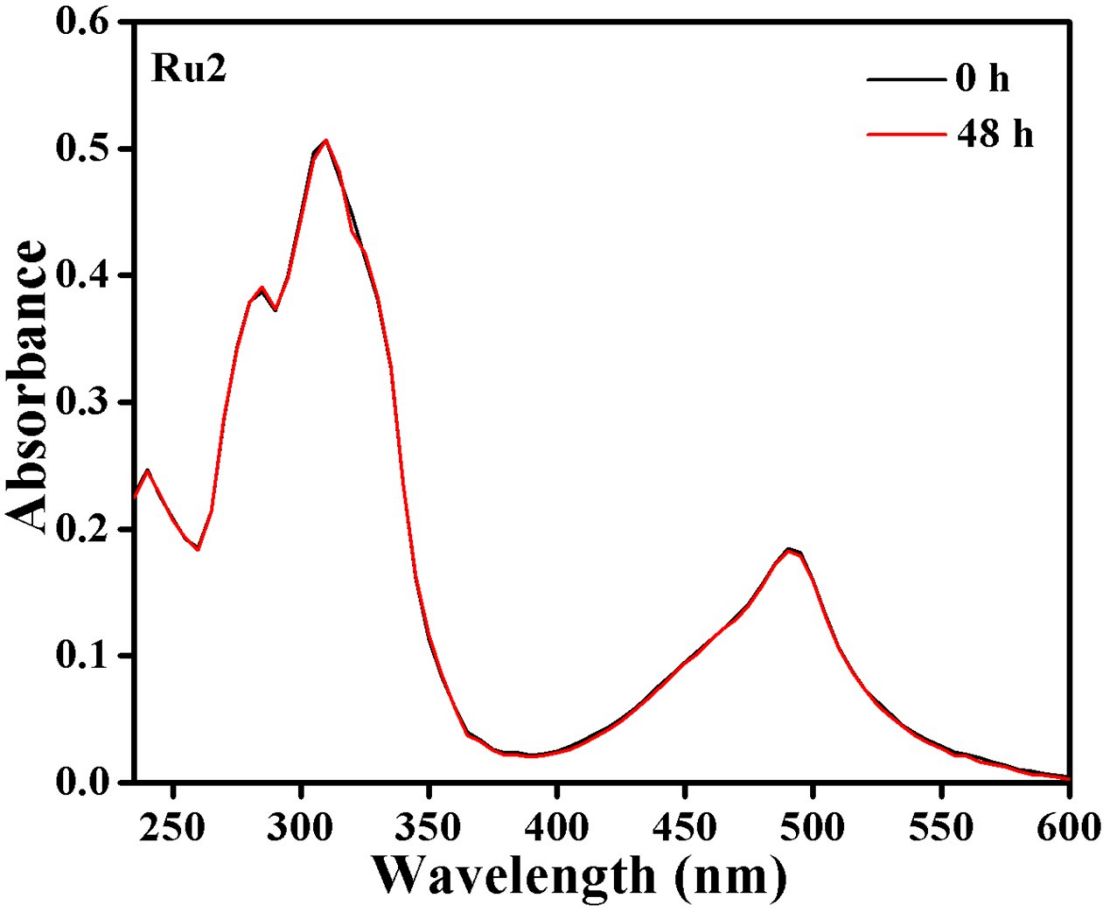
Ru6

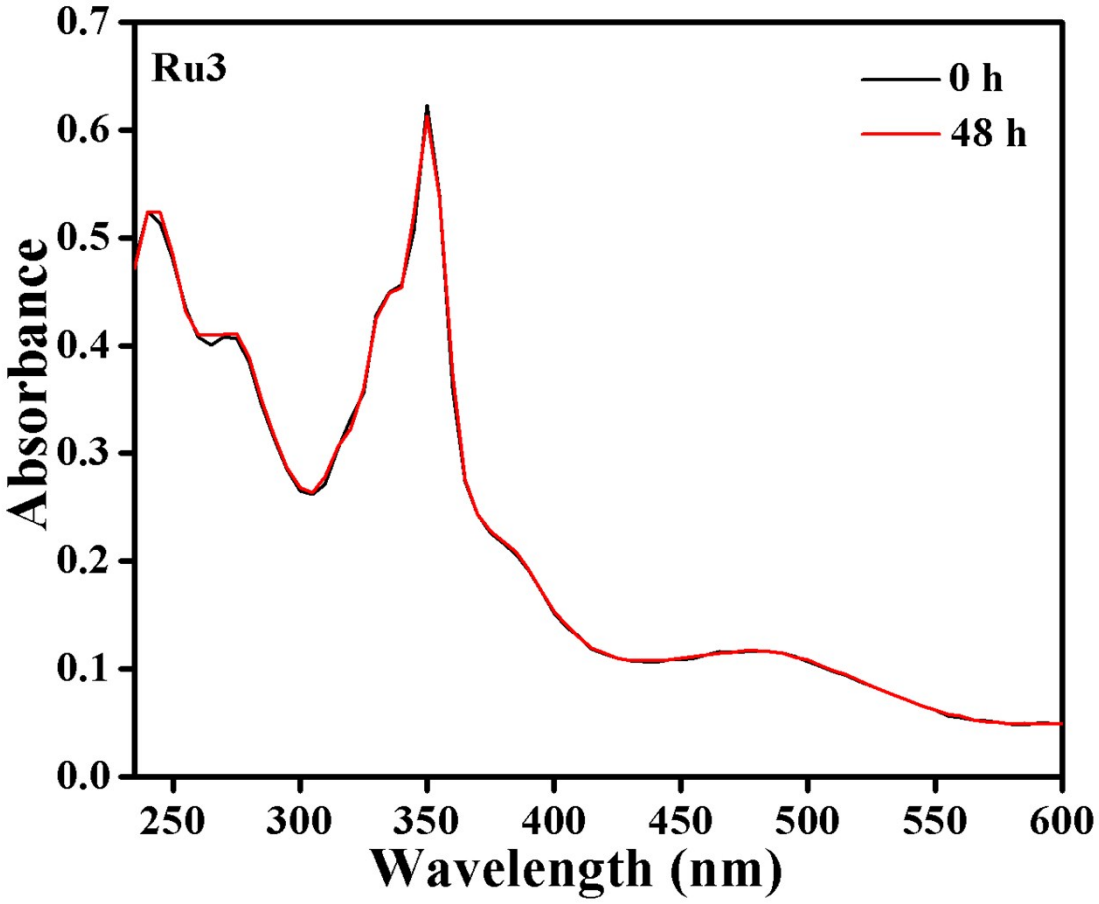
Figure S40. The ^{1}H-^{1}H COSY (bond) correlations of complexes **Ru1**–**Ru6**.

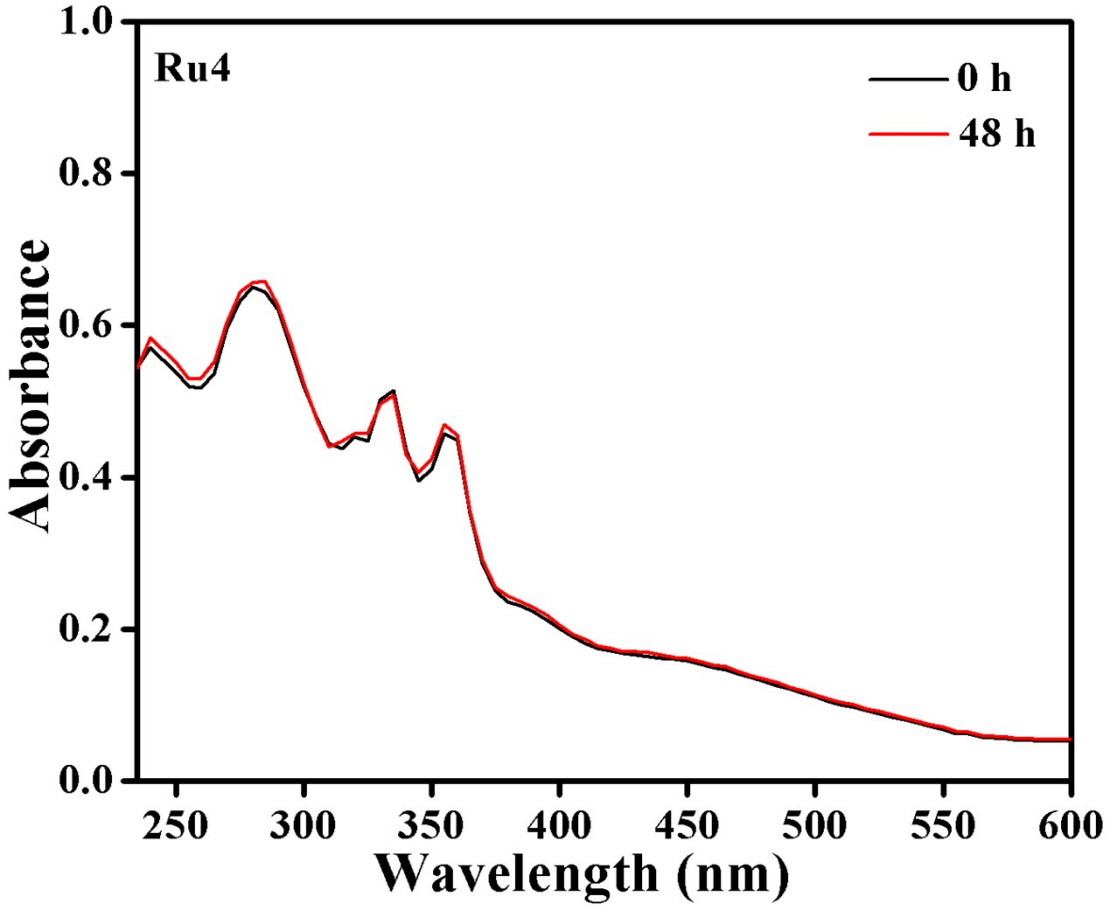
Table S4. ESI-MS spectrum of **Ru1**–**Ru6**.

complexes	m/z						
Ru1	429.6(+2)	430.1(+2)	430.6(+2)	431.2(+2)	431.7(+2)	432.1(+2)	432.6(+2)
Ru2	389.0(+2)	389.6(+2)	390.1(+2)	390.5(+2)	391.1(+2)	391.6(+2)	
Ru3	388.6(+2)	389.0(+2)	389.5(+2)	390.1(+2)	390.6(+2)	391.1(+2)	
Ru4	551.1(+1)	553.1(+1)	554.1(+1)	555.0(+1)	556.1(+1)		
Ru5	649.3(+1)	650.2(+1)	651.2(+1)	652.2(+1)	653.2(+1)	654.2(+1)	
Ru6	725.2(+1)	726.2(+1)	727.2(+1)	728.2(+1)			
	504.0(+1)	506.0(+1)	507.0(+1)	508.0(+1)	509.0(+1)		


Ru1
0 h
48 h
Absorbance
Wavelength (nm)
0.0
0.1
0.2
0.3
0.4
250
300
350
400
450
500
550
600


Ru2
0 h
48 h
Absorbance
Wavelength (nm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
250
300
350
400
450
500
550
600


Ru3
0 h
48 h
Absorbance
Wavelength (nm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
250
300
350
400
450
500
550
600


Ru4
0 h
48 h
Absorbance
Wavelength (nm)
0.0
0.2
0.4
0.6
0.8
1.0
250
300
350
400
450
500
550
600

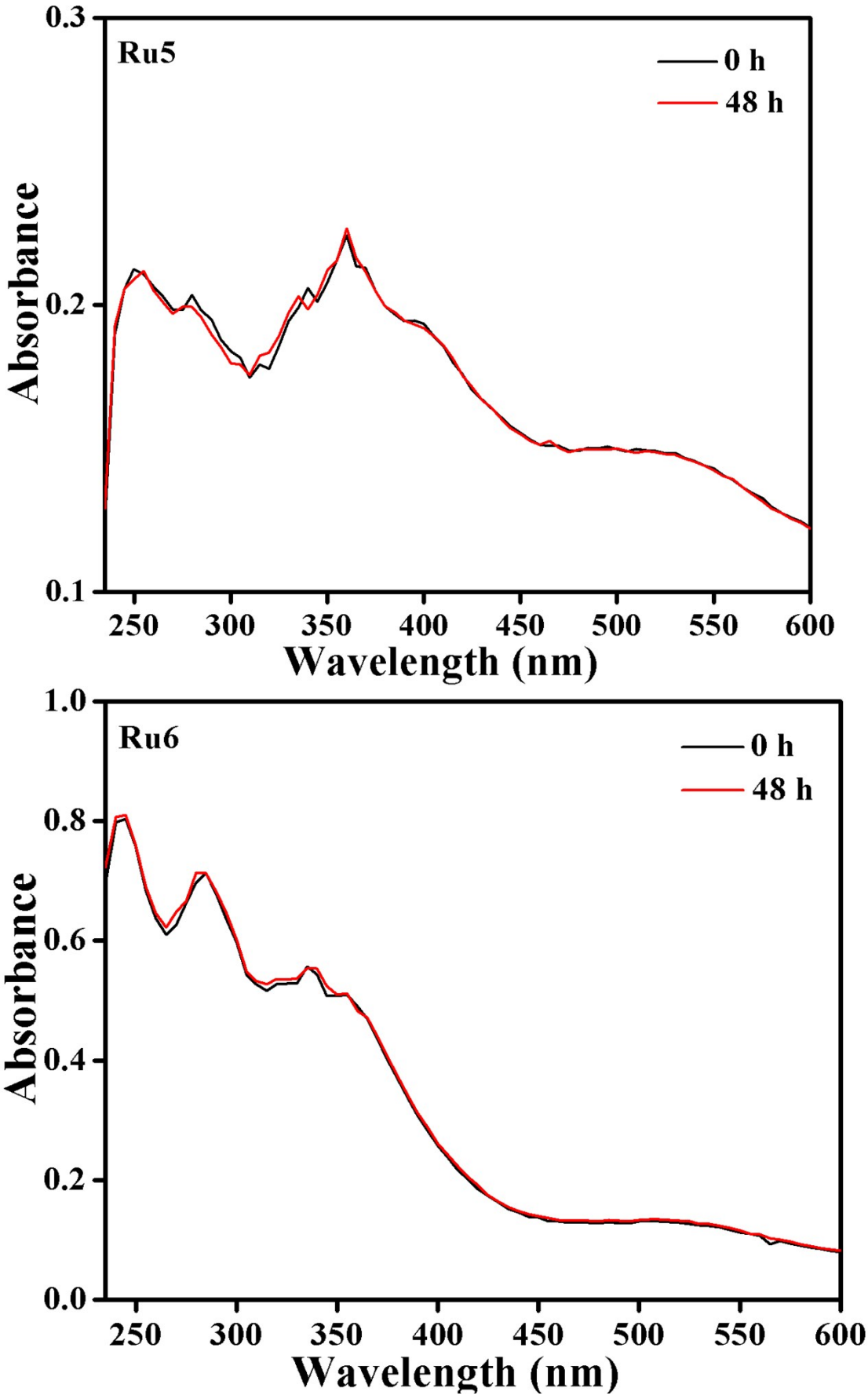


Figure S41. UV-Vis absorption spectra of **Ru1**–**Ru6** (2.0×10^{-5} M) in Tris-KCl solution

in a 48 h time course, respectively.

Table S5. UV-Vis absorption spectra of **Ru1**–**Ru6** (2.0×10^{-5} M) in Tris-KCl solution in a 48 h time course, respectively.

complexes	λ (nm)			
Ru1	511	310	239	
Ru2	490	310	285	240
Ru3	482	350	273	240
Ru4	355	335	282	240
Ru5	527	360	279	254
Ru6	337	283	244	

Table S6. The inhibitory ratios (%) of 4′-substituted-2,2′:6′,2″-terpyridine, **Ru1**–**Ru6**, cis-$Pt(DMSO)_2Cl_2$ and cisplatin towards five tumor cell lines and the normal liver HL-7702 for 48 h.

Compds.	BEL-7404	Hep-G2	NCI-H460	MGC80-3	HL-7702
4-EtN-Phtpy	47.96±0.92	58.07±1.45	60.13±1.58	58.69±0.45	42.78±2.01
Ru1	75.03±0.56	82.01±0.39	75.17±0.62	70.34±1.77	45.04±1.13
4-MeO-Phtpy	26.59±0.61	44.29±0.35	40.33±1.85	39.79±0.89	54.20±0.99
Ru2	52.09±1.16	65.49±1.04	62.07±1.01	55.24±1.59	58.98±1.92
2-MeO-Phtpy	37.11±0.83	47.17±1.35	49.91±1.08	45.11±1.53	55.99±1.09
Ru3	58.96±0.56	70.15±0.53	67.09±1.77	60.23±0.79	56.01±0.64
3-MeO-Phtpy	37.88±1.05	48.85±0.71	52.98±0.29	48.13±0.28	33.69±0.85
Ru4	65.89±1.34	72.18±1.00	68.98±0.79	63.08±1.42	40.16±1.67
1-Bip-Phtpy	40.22±0.61	51.06±1.09	55.23±1.67	50.67±1.39	48.98±1.37
Ru5	69.72±0.89	75.82±1.55	70.16±0.46	65.96±0.51	43.09±1.03
1-Pyr-Phtpy	43.03±1.82	53.78±1.19	59.05±1.08	55.01±0.61	45.95±1.22
Ru6	70.83±0.43	78.90±0.78	71.47±1.64	68.92±1.59	46.05±0.86
cis-$Ru(DMSO)_4Cl_2$ [b]	19.91±1.52	25.93±2.90	15.09±1.08	30.23±1.67	17.49±1.29

Cisplatin [a,c]	62.67±1.06	60.13±0.62	58.56±1.26	58.12±0.65	61.09±2.10

Results represent mean ± SD of at least six independent experiments. SD represents the standard deviation. [a] The concentration is 20 μM. [b] The concentration is 100 μM. [c] Cisplatin was dissolved at a concentration of 1 mM in 0.154 M NaCl.

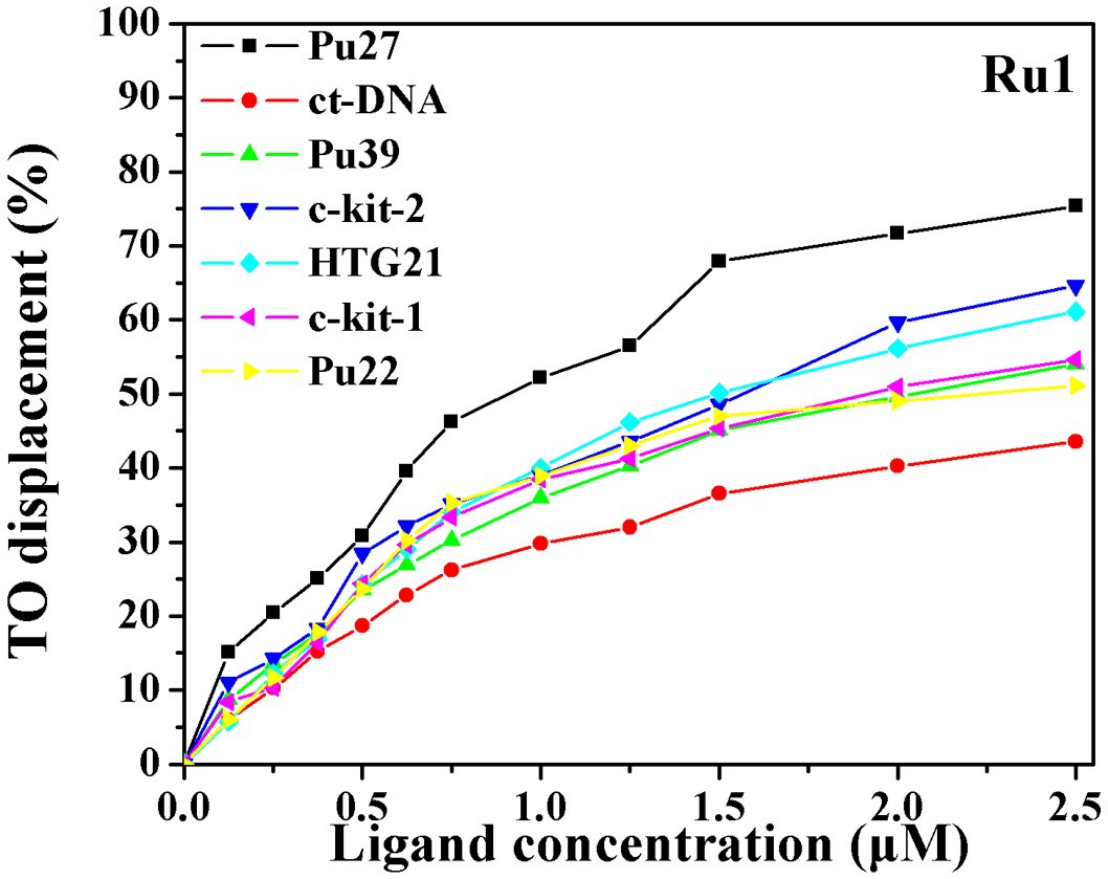


Figure S42. FID Assay for **Ru1** on DNA

Table S7. Sequences of oligomers (primers) used in this work

oligomer	sequence
Pu27	5′-TGGGGAGGGTGGGGAGGGTGGGGAAGG-3′
Pu39	5′-AGGGGCGGGCGCGGGAGGAAGGGGGCGGGAGCGGGGCTG-3′
c-kit-2	5′-CGGGCGGGCGCTAGGGAGGGT-3′
HTG21	5′-GGGTTAGGGTTAGGGTTAGGG-3′
c-kit-1	5′-CGGGCGGGCACGAGGGAGGGT-3′
Pu22	5′-TGAGGGTGGGTAGGGTGGGTAA-3′