Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary material

Piano-stool ruthenium(II) complexes with maleimide and phosphine or phosphite ligands: Synthesis and activity against normal and cancer cells

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Supplementary Materials

Fig. S29. The effect of ruthenium complexes **1**, **2a**, and **3a** on the apoptosis of HL-60 cells. Representative flow cytometric dot plots showing the percentage of cells in viable (Q3), early apoptotic (Q4), late apoptotic (Q2), and necrotic (Q1) stages. The positive control were cells incubated with 20 µM camptothecin (CAM) for 24 h at 37 °C. Data represent means ± SD of 3 experiments.

Fig. S30. The effect of ruthenium complexes **1**, **2a** and **3a** on the apoptosis of HL-60/DR cells. Data represent means ± SD of three experiments. The CAM sample were cells incubated with 20 µM camptothecin for 24 h at 37 °C.

1.5. Docking studies

Fig. S31. Ruthenium(II) complexes **1** (a, b) and **2b** (c, d) with fully complemented DNA (a, c) and with mismatched DNA (b, d).

1.1. X-ray structure determination

Hirshfeld surface analysis

Hirshfeld surfaces were generated with the use of *CrystalExplorer3.0* program [1] using the automatic procedures. They have been mapped with d_{norm}, a parameter which reflects intermolecular distances. Negative values of d_{norm}, indicating contacts shorter than the sum of van der Waals radii are visualized in red, while positive values of contacts longer than the sum of van der Waals radii, are colored in blue. The white color denotes intermolecular distances close to van der Waals contacts. The Hirshfeld surface fingerprint plots were generated using d_i (distance from the surface to the nearest atom in the molecule itself) and d_e (distance from the surface to the nearest atom in another molecule) as a pair of coordinates, at intervals of 0.01 Å, in two-dimensional histograms. Under special investigations were contacts of the type H…O.

Table S1. Selected bond lengths [Å] and angles [°]

Ru1-N1	2.096(1)	2.104(1)
Ru1-P1	2.310(1)	2.285(2)
Ru1-C20	1.869(3)	1.865(2)
Ru1-Cg1	1.882(3)	1.875(2)
Ru1-C11	2.215(2)	2.213(1)
Ru1-C12	2.208(2)	2.251(2)
Ru1-C13	2.243(2)	2.258(2)
Ru1-C14	2.260(2)	2.239(2)
Ru1-C15	2.260(2)	2.201(2)
P1-C21	1.833(2)	1.808(2)
P1-C31	1.830(2)	1.801(1)
P1-C41	1.835(2)	1.808(1)
O20-C20	1.152(3)	1.151(2)
O2-C2	1.216(3)	1.216(2)
O5-C5	1.224(3)	1.222(2)
P1-Ru1-N1	91.4(1)	90.1(1)
P1-Ru1-C20	91.6(2)	87.9(2)
N1-Ru1-C20	93.3(1)	93.6(1)
P1-Ru1-Cg1	123.0(2)	126.7(2)
N1-Ru1-Cg1	122.2(2)	123.1(2)
O2-Ru1-Cg1	126.0(2)	124.9(2)

Ru1-P1-C21	116.9(1)	115.0(1)
Ru1-P1-C31	117.4(1)	113.3(1)
Ru1-P1-C41	112.8(2)	119.5(2)
C21-P1-C31	102.1(1)	103.4(1)
C31-P1-C41	101.1(1)	102.2(1)
C21-P1-C41	104.5(1)	101.2(1)
C5-N1-Ru1	124.2(2)	124.1(2)
C2-N1-Ru1	127.8(1)	127.8(1)
O2-C20-Ru1	172.4(2)	172.4(2)
C5-N1-C20	107.9(1)	107.9(1)
N1-Ru1-P1-C21	11.0(2)	30.4(2)
N1-Ru1-P1-C31	132.9(2)	149.1(2)
N1-Ru1-P1-C41	-110.3(2)	-90.3(2)
C2-N1-Ru1-C20	-9.2(2)	-12.9(2)
C5-N1-Ru1-C20	167.5(2)	161.3(2)

Table S2. Geometric parameters of selected hydrogen bonds–distances [Å] and angles [°]

hydrogen bond	D-H	H…A	D…A	< D-H…A	symmetry
2a					
C26-H26…O2	0.95	2.51	3.199(3)	129	1+x,y,z
C35-H35…O5	0.95	2.46	3.314(3)	149	3/2-x,1-y,-1/2+z
C33-H33…O20	0.95	2.54	3.344(3)	143	1/2-x,1-y,-1/2+z
2b					
C11-H11…O5	0.95	2.43	3.334(2)	159	-1+x,y,z
C12-H12…O2	0.95	2.59	3.469(2)	153	x,-1+y,z
C43-H43…O20	0.95	2.58	3.158(2)	120	1-x, 1-y, 1-z
C44-H44…O2	0.95	2.37	3.311(2)	168	1-x, 1-y, 1-z

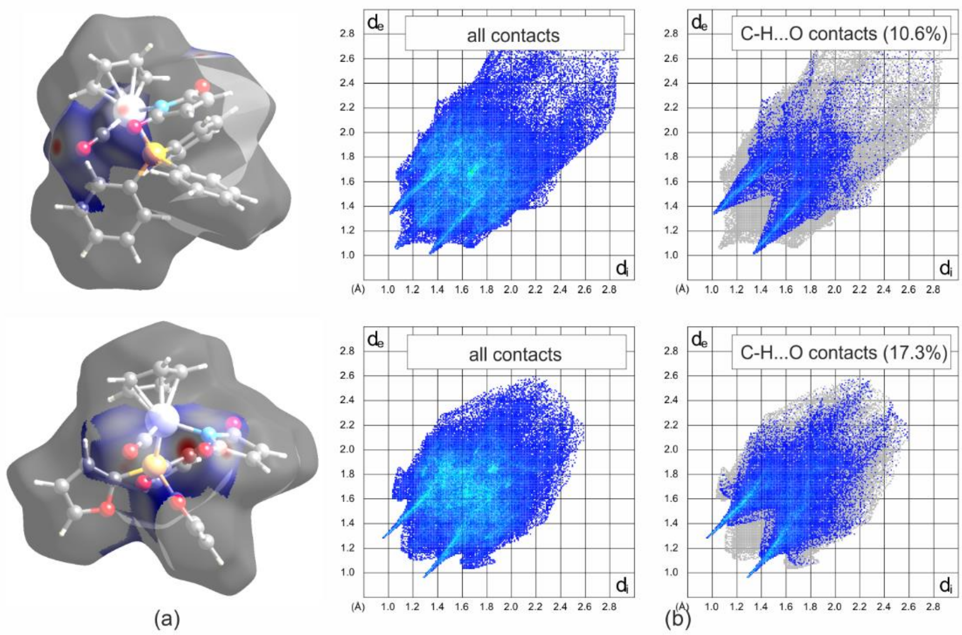


Figure S1. Molecular Hirshfeld surface analysis **2a** (at the top) and **2b** (at the bottom): surfaces mapped with color scale of d_{norm} parameter for C-H…O contacts (a) and corresponding fingerprint plots (b).

1.2. Spectra and spectral data

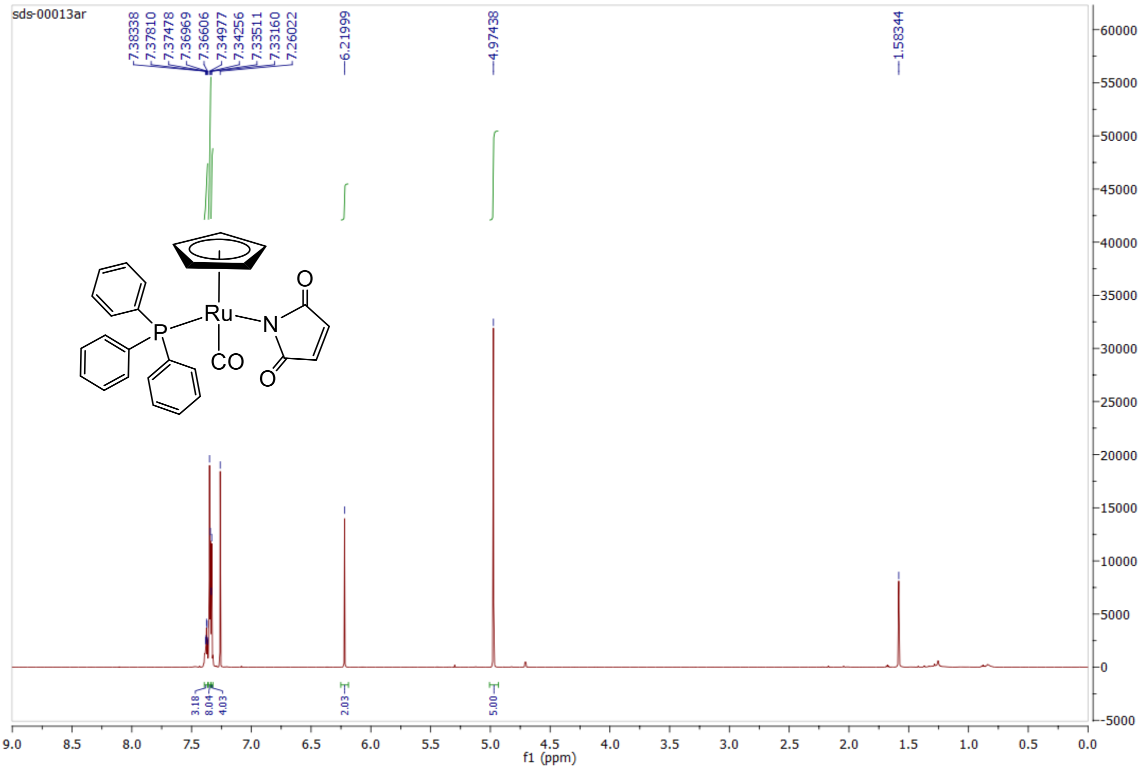


Figure S2. ^{1}H NMR spectra of **2a** in $CDCl_3$

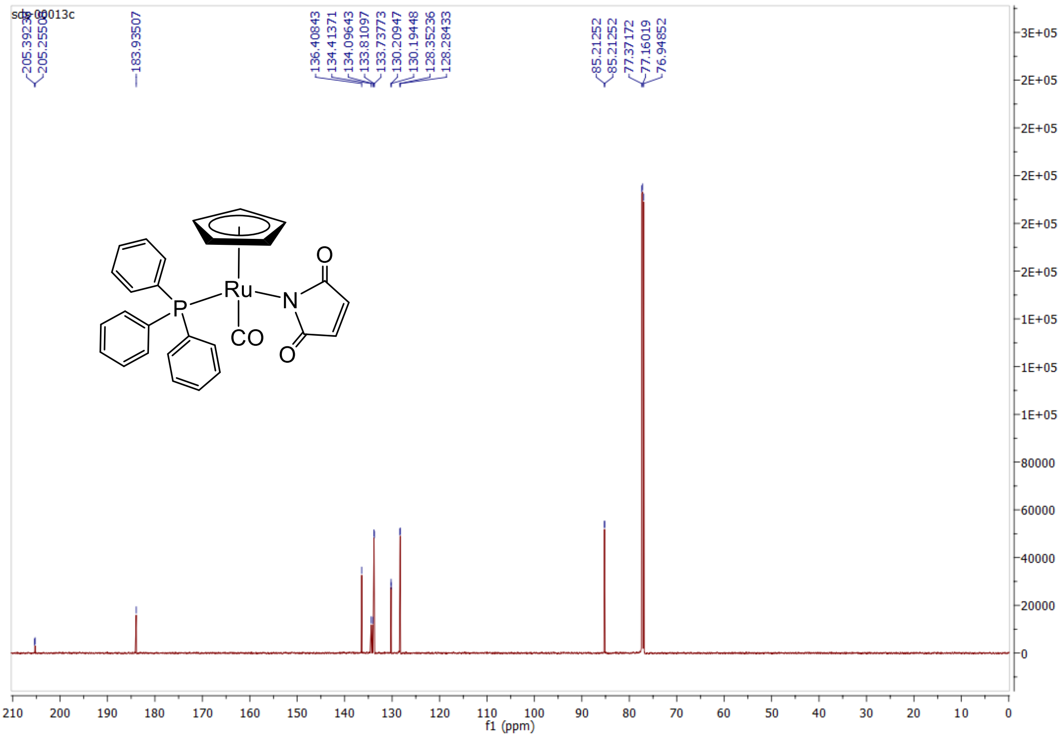


Figure S3. ^{13}C NMR spectra of **2a** in $CDCl_3$

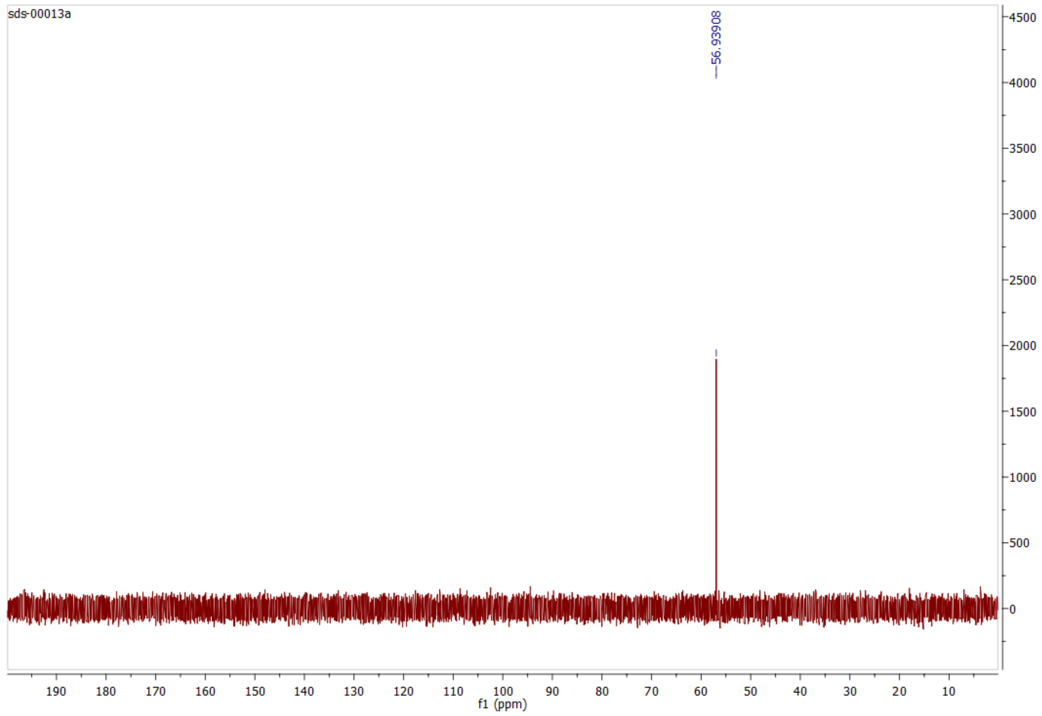


Figure S4. ^{31}P NMR spectra of **2a** in $CDCl_3$

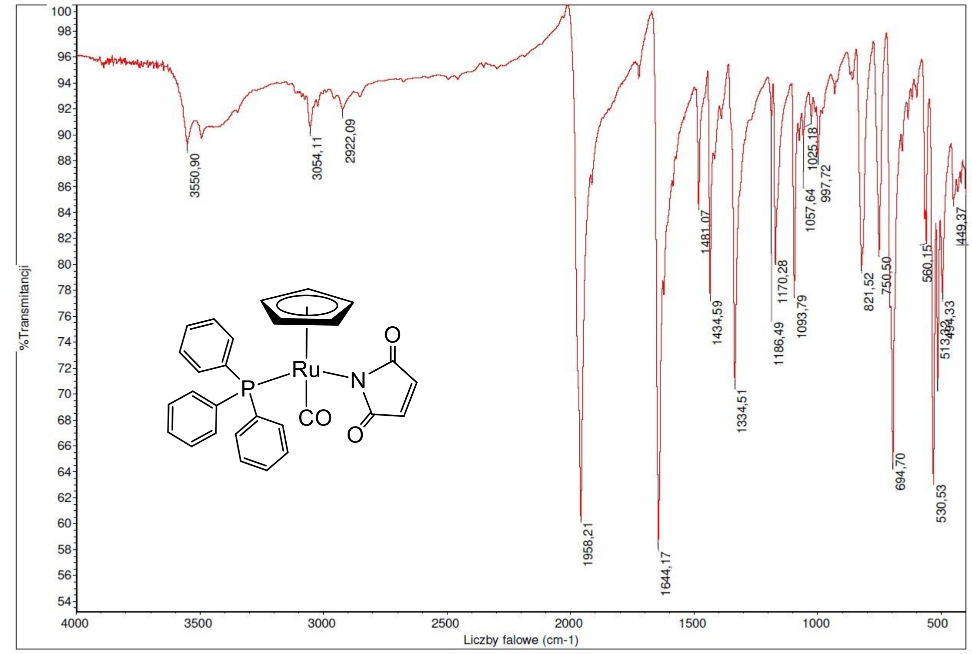


Figure S5. FTIR spectra of **2a**.

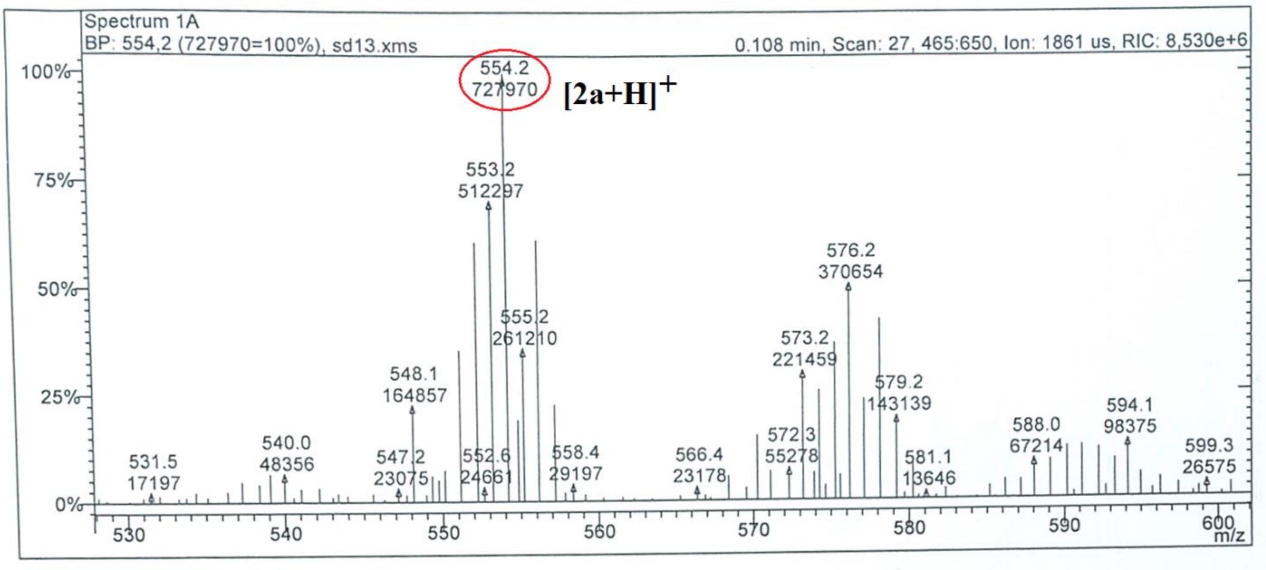


Figure S6. ESI-MS spectra of **2a**

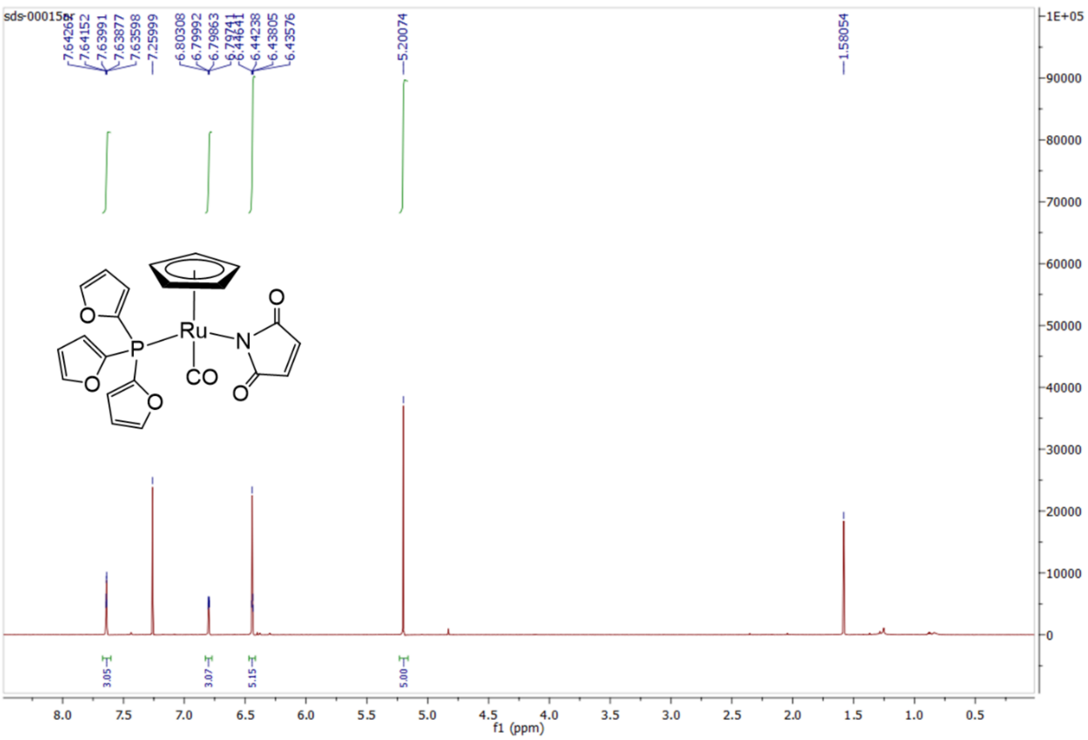


Figure S7. ^{1}H NMR spectra of **2b** in $CDCl_3$

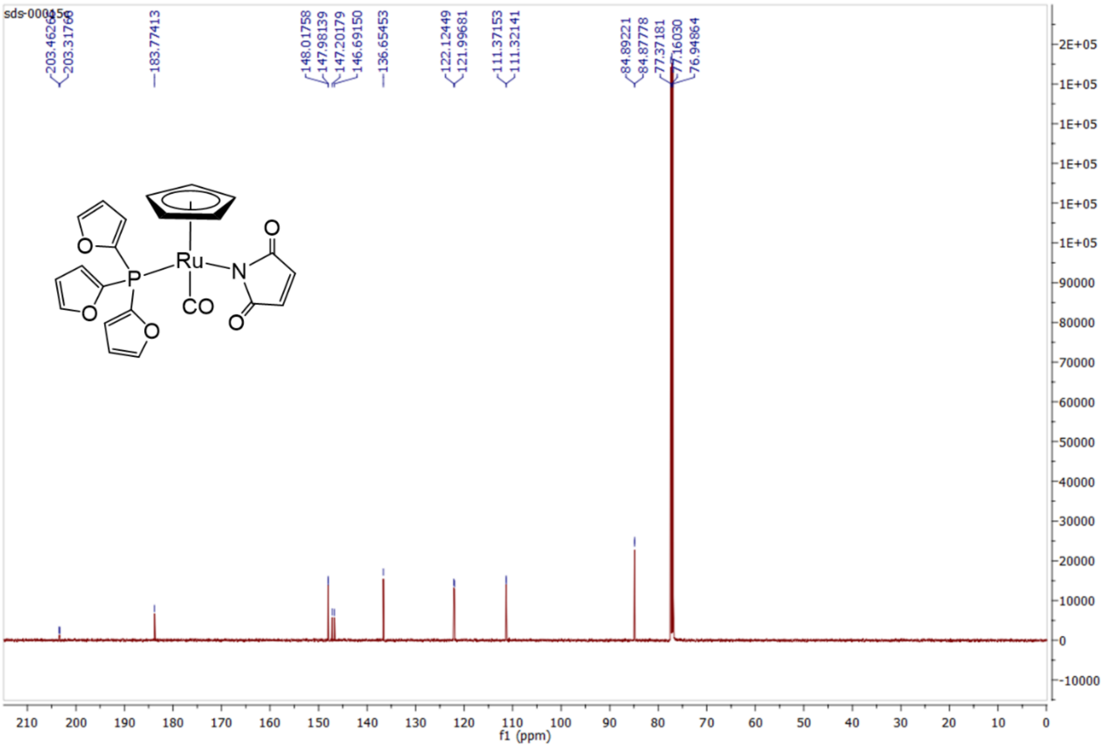


Figure S8. ^{13}C NMR spectra of **2b** in $CDCl_3$

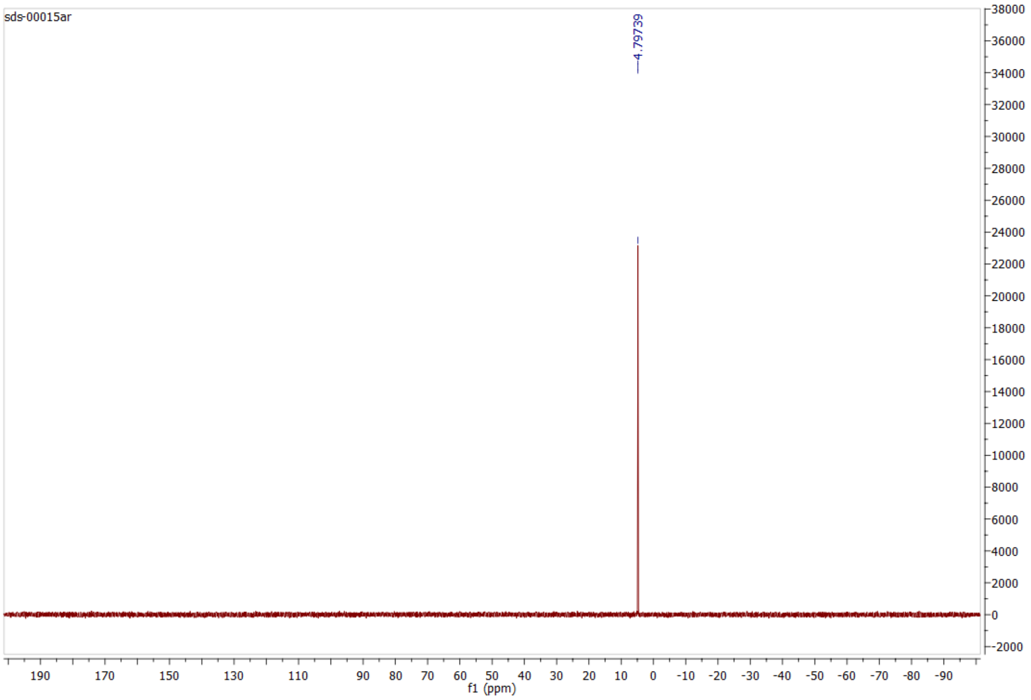


Figure S9. ^{31}P NMR spectra of **2b** in $CDCl_3$

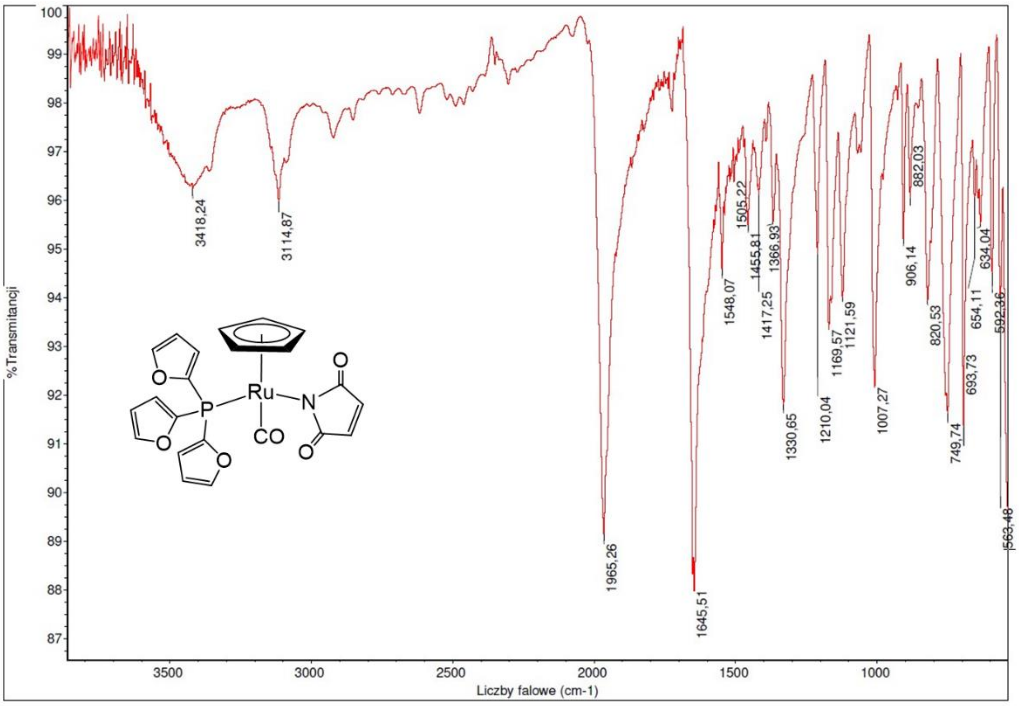


Figure S10. FTIR spectra of **2b**.

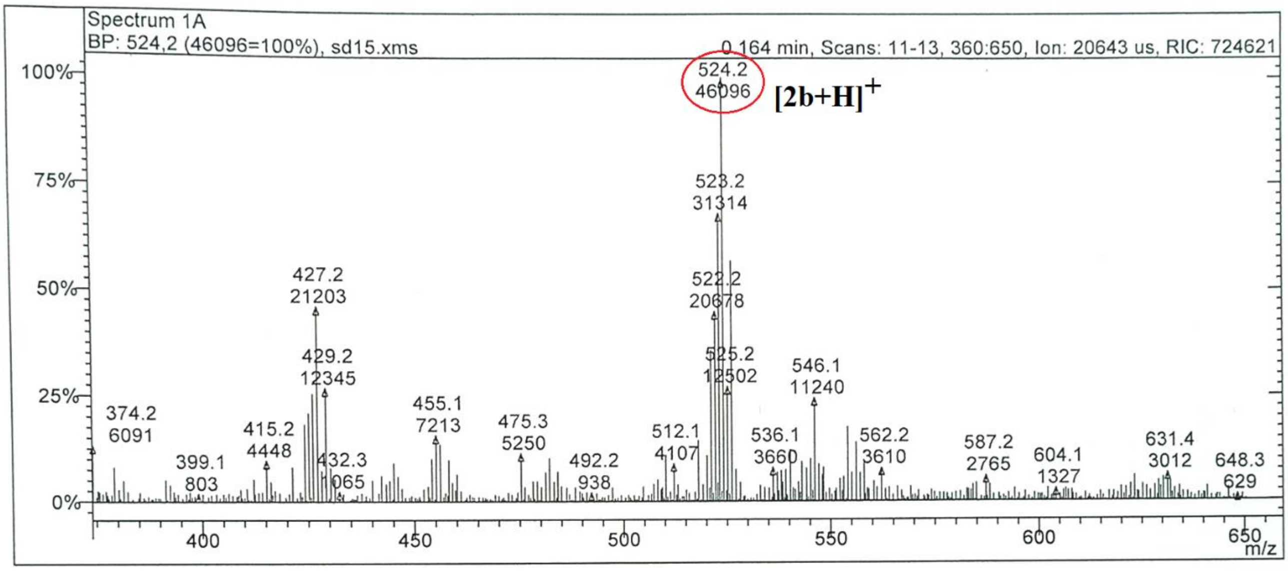


Figure S11. ESI-MS spectra of **2b**

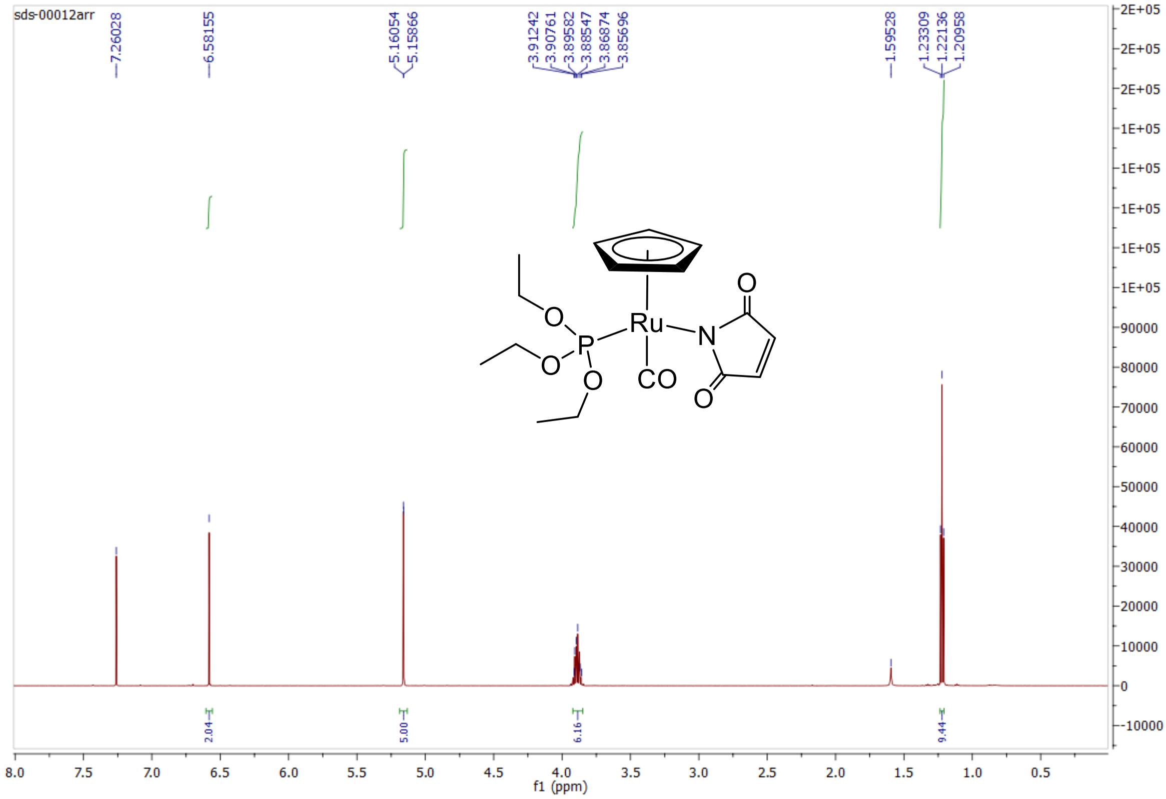


Figure S12. ^{1}H NMR spectra of **3a** in $CDCl_3$

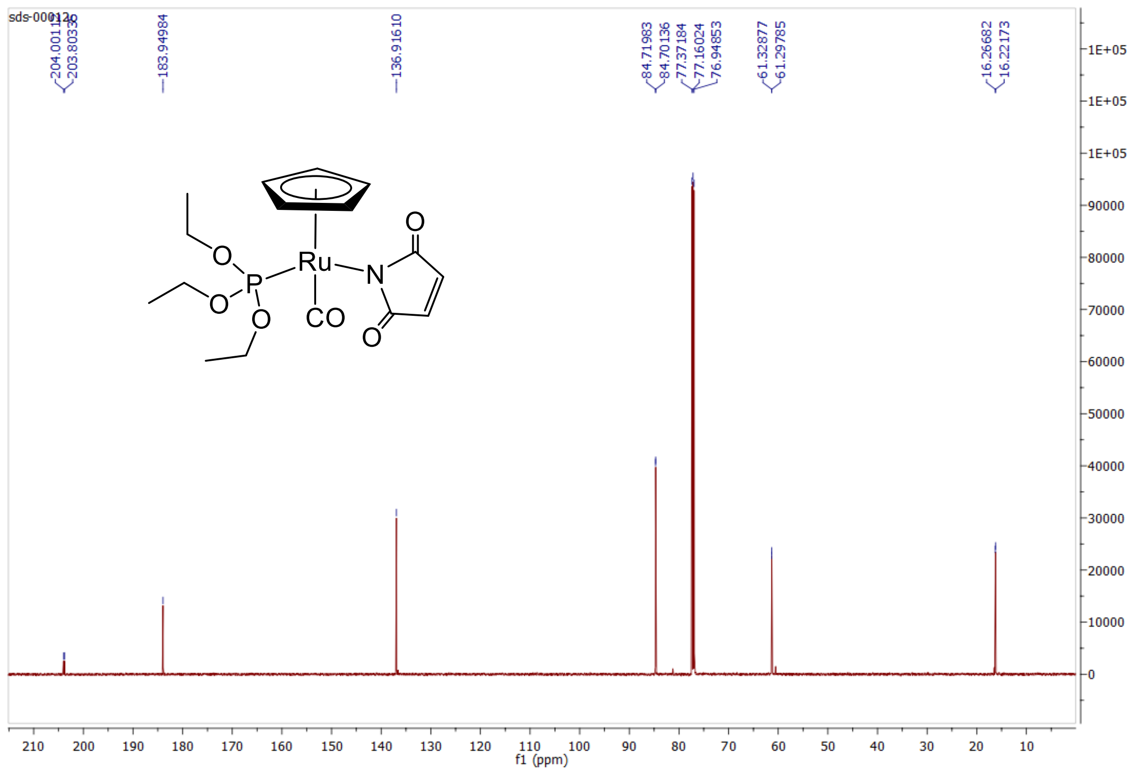


Figure S13. ^{13}C NMR spectra of **3a** in $CDCl_3$

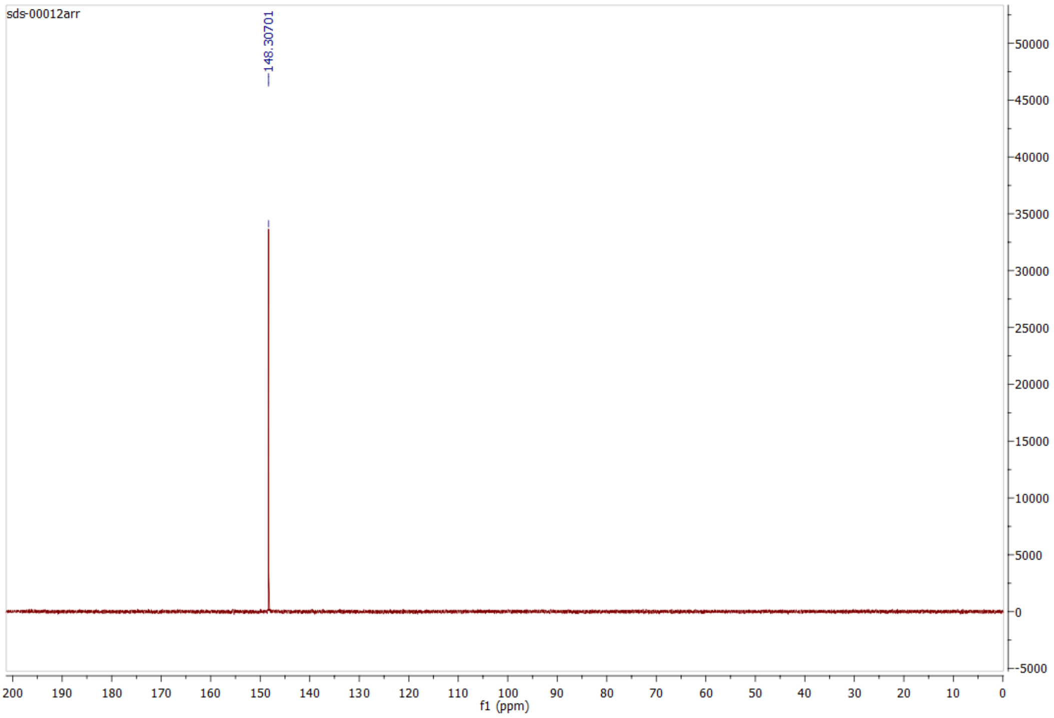


Figure S14. ^{31}P NMR spectra of **3a** in $CDCl_3$

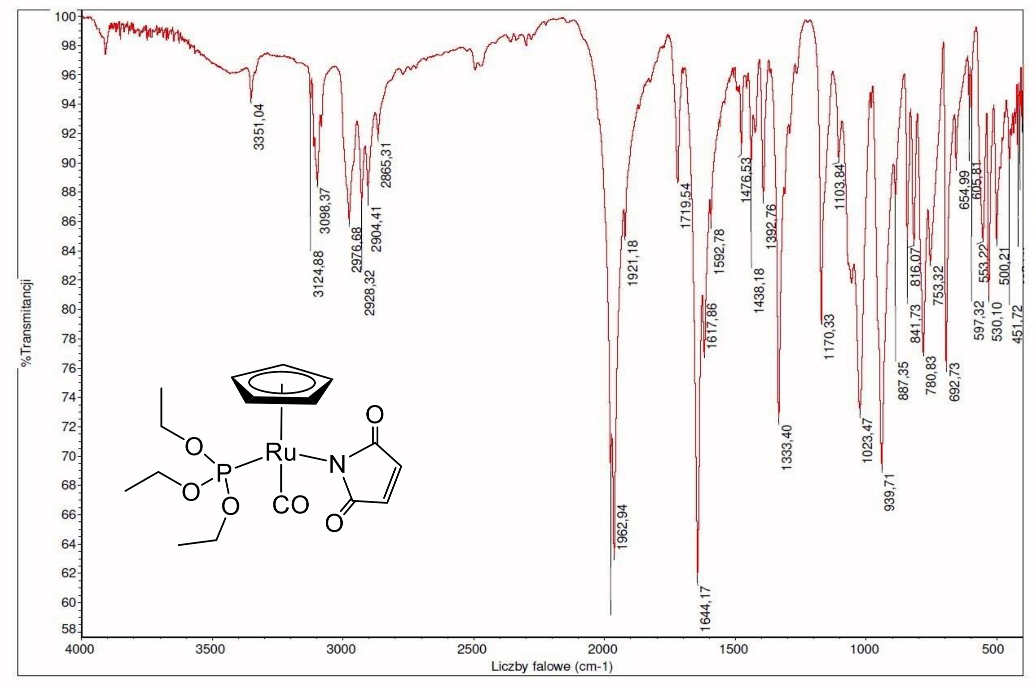


Figure S15. FTIR spectra of **3a**.

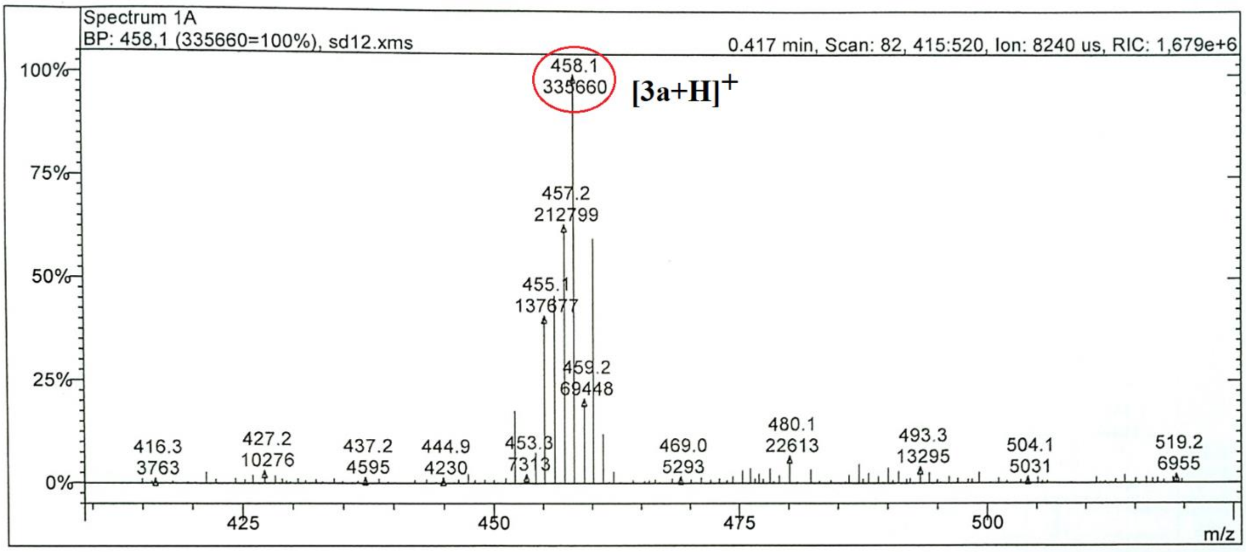


Figure S16. ESI-MS spectra of **3a**.

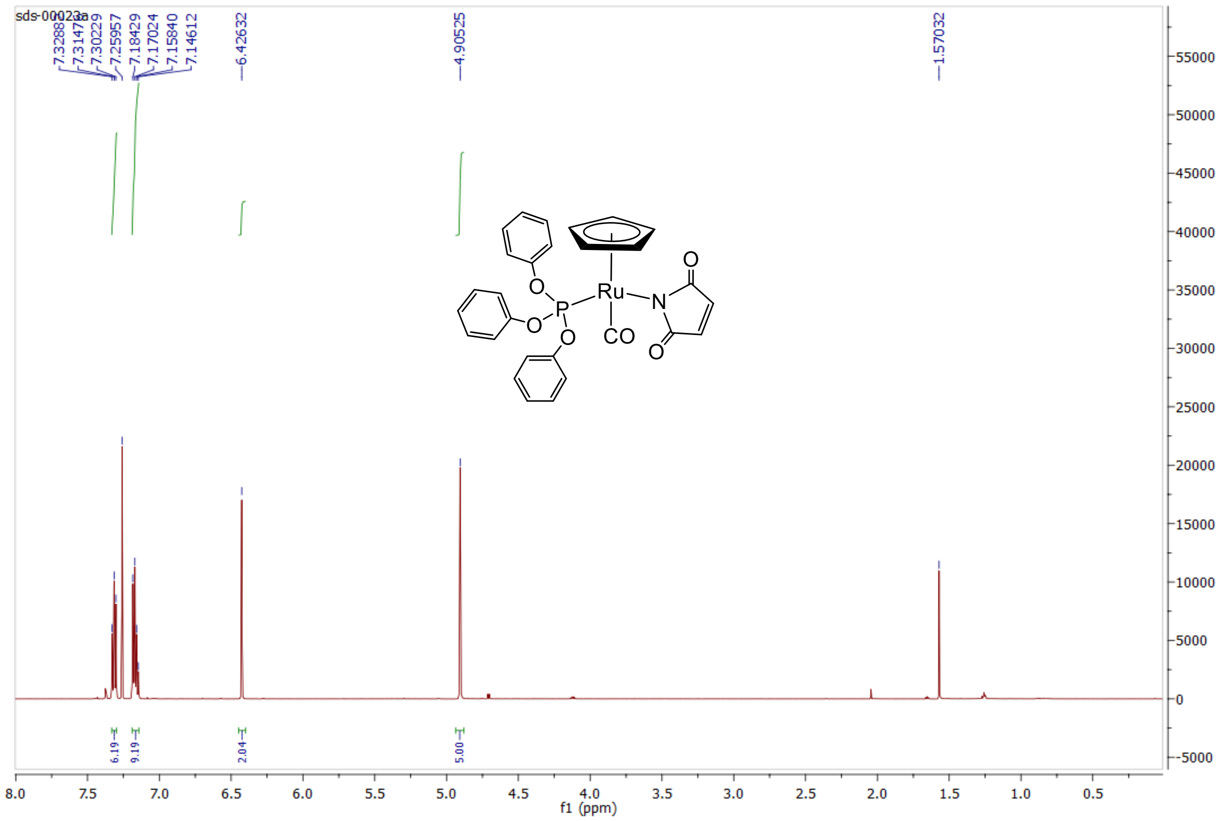


Figure S17. ^{1}H NMR spectra of **3b** in $CDCl_3$

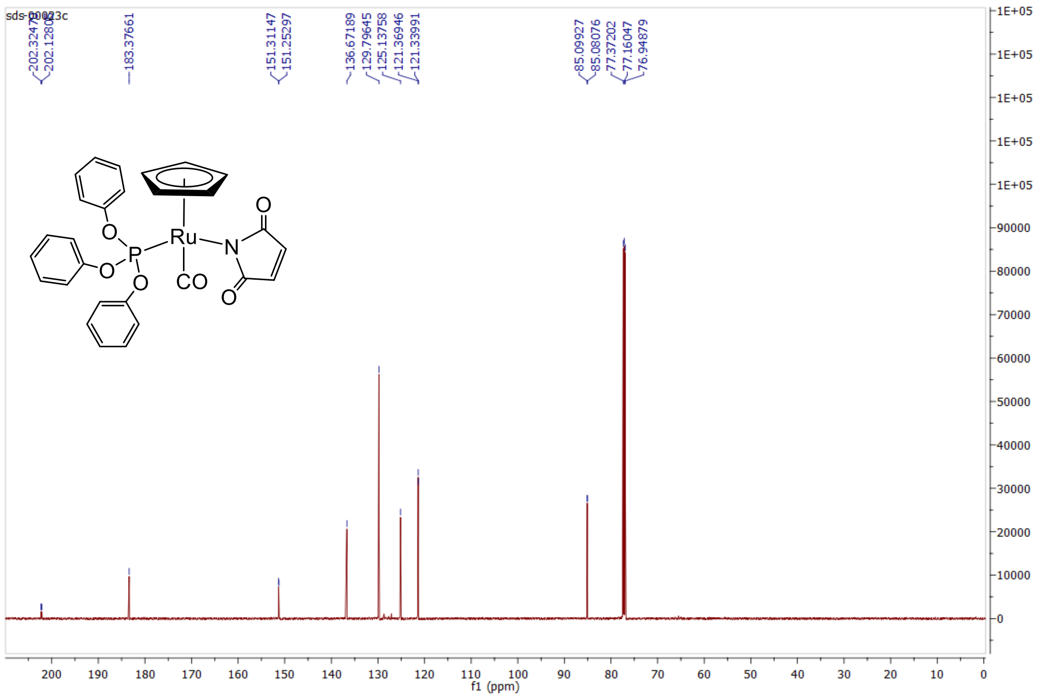


Figure S18. ^{13}C NMR spectra of **3b** in $CDCl_3$

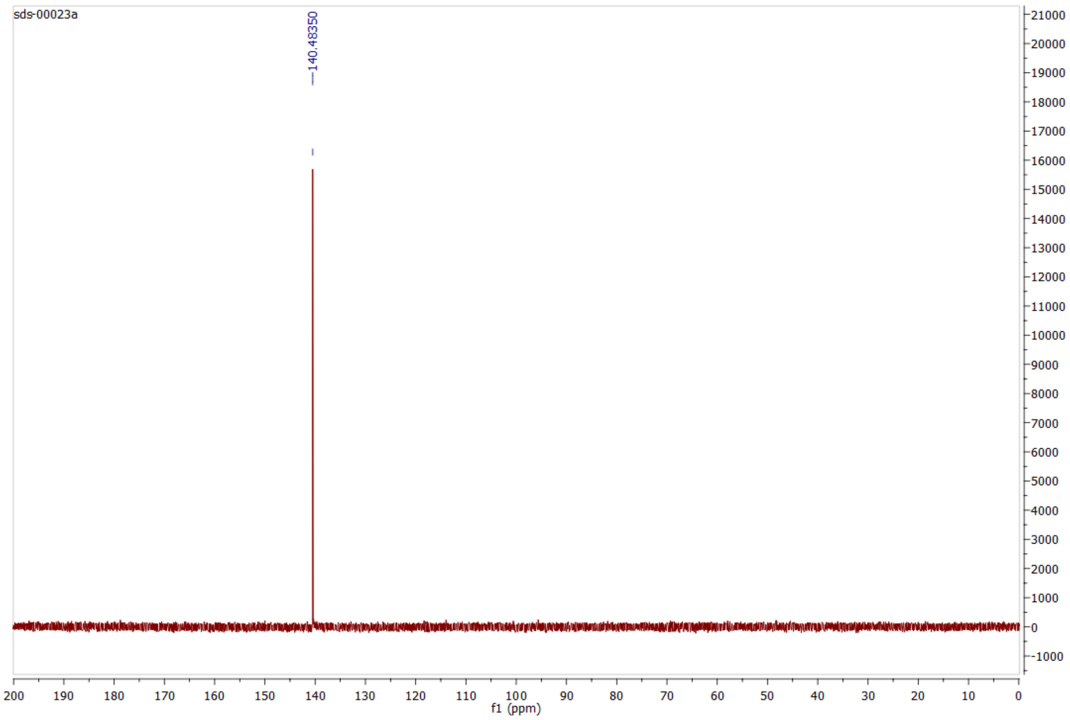


Figure S19. ^{31}P NMR spectra of **3b** in $CDCl_3$

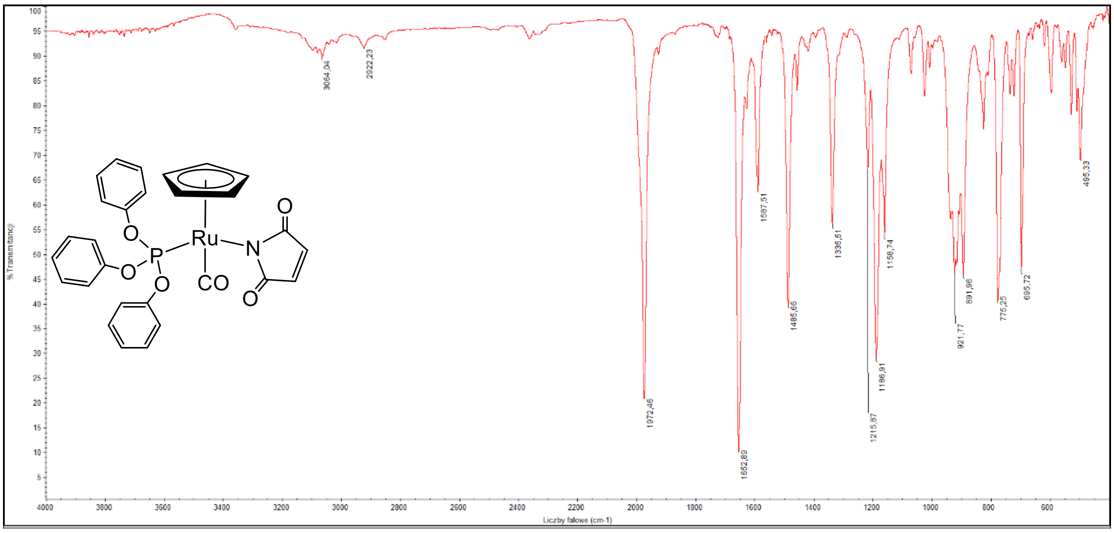


Figure S20. FTIR spectra of **3b**.

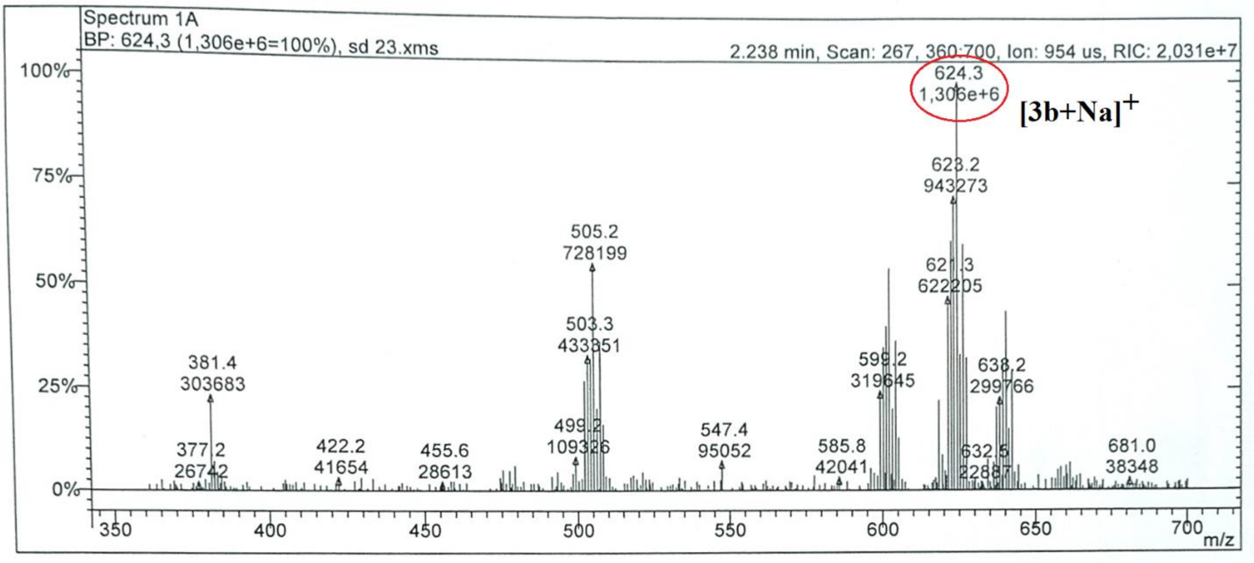


Figure S21. ESI-MS spectra of **3b**.

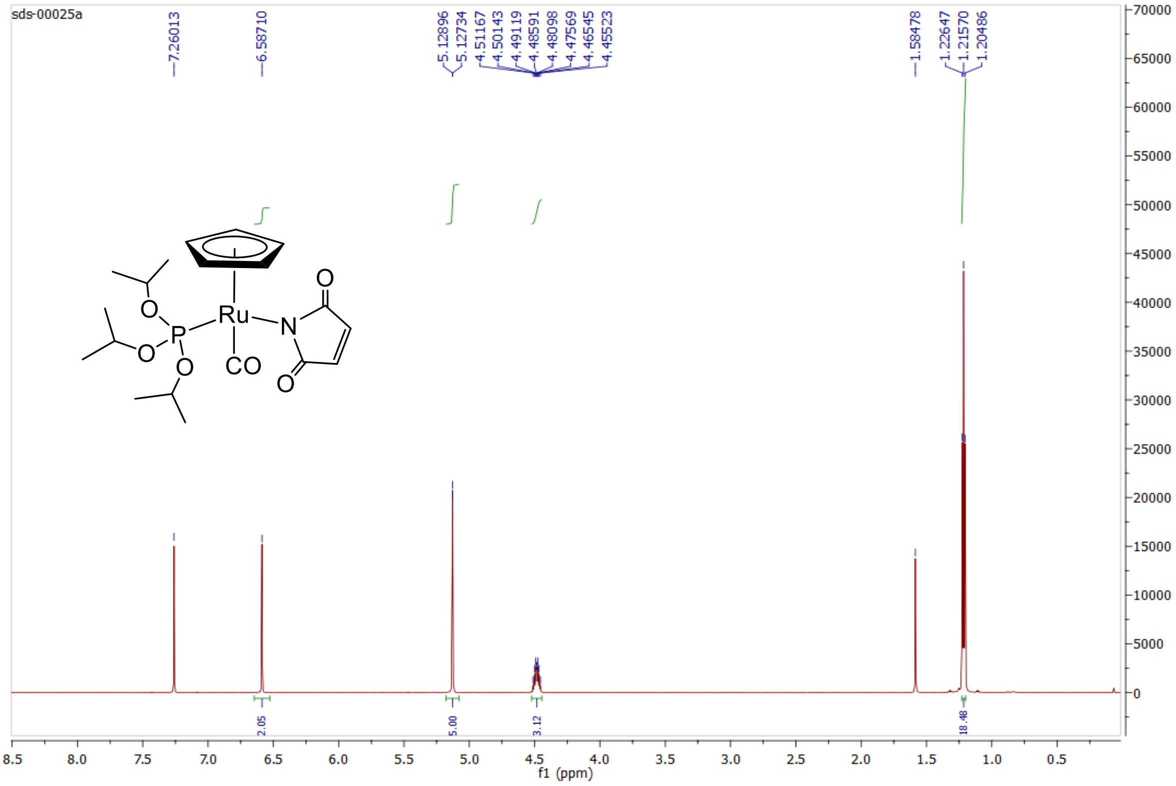


Figure S22. ^{1}H NMR spectra of **3c** in $CDCl_3$

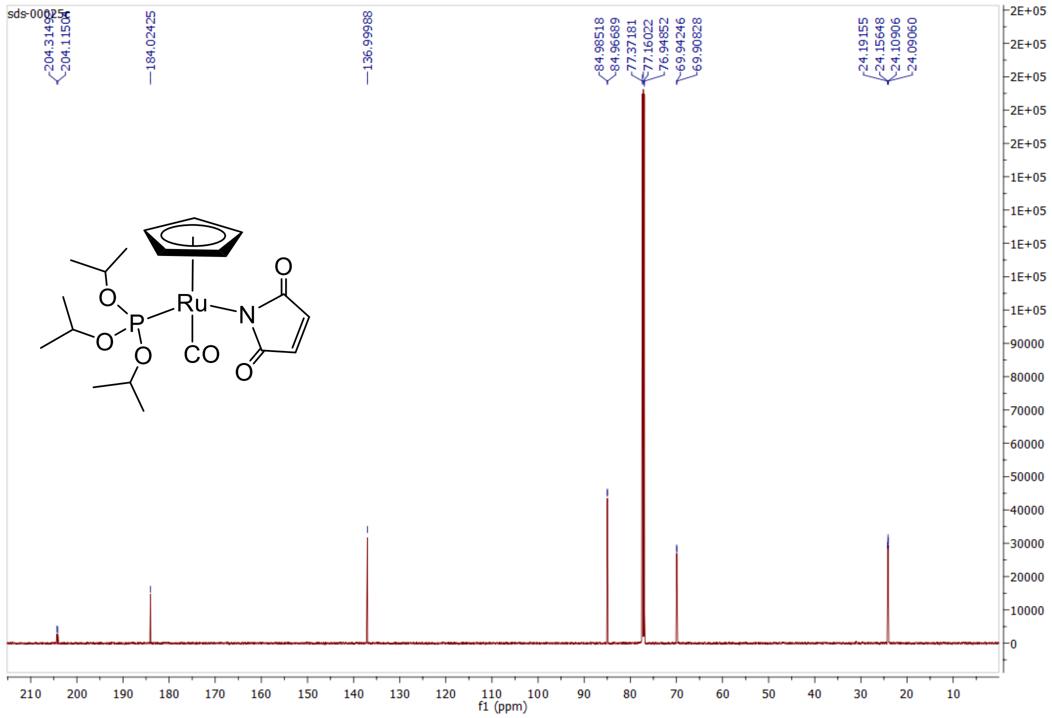


Figure S23. ^{13}C NMR spectra of **3c** in $CDCl_3$

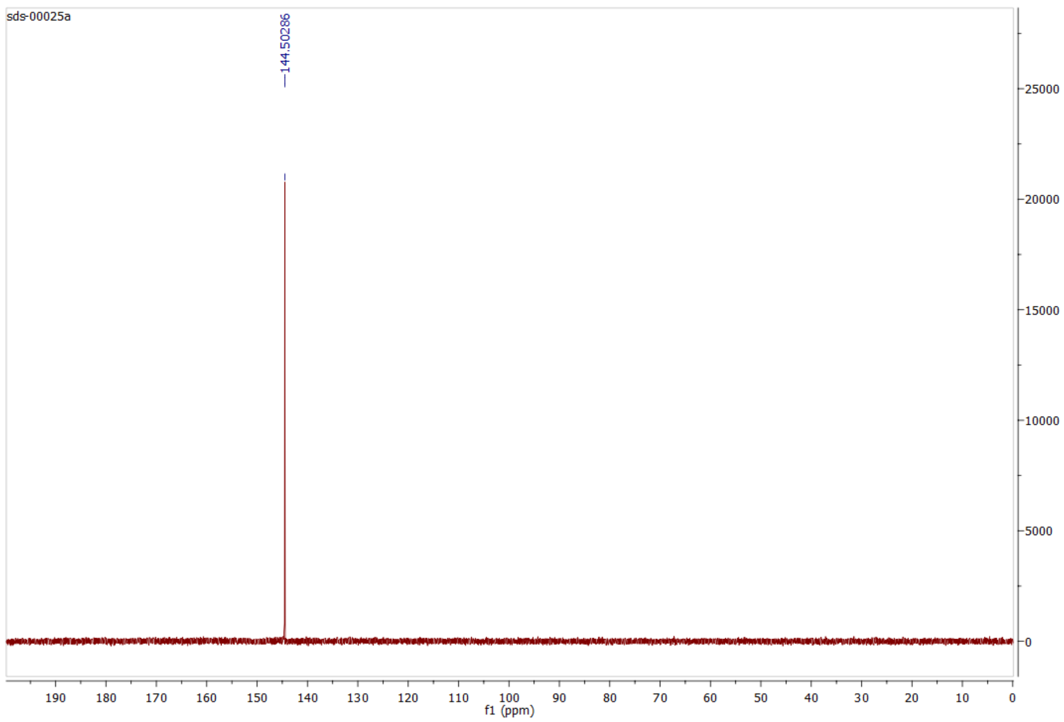


Figure S24. [31]P NMR spectra of **3c** in $CDCl_3$

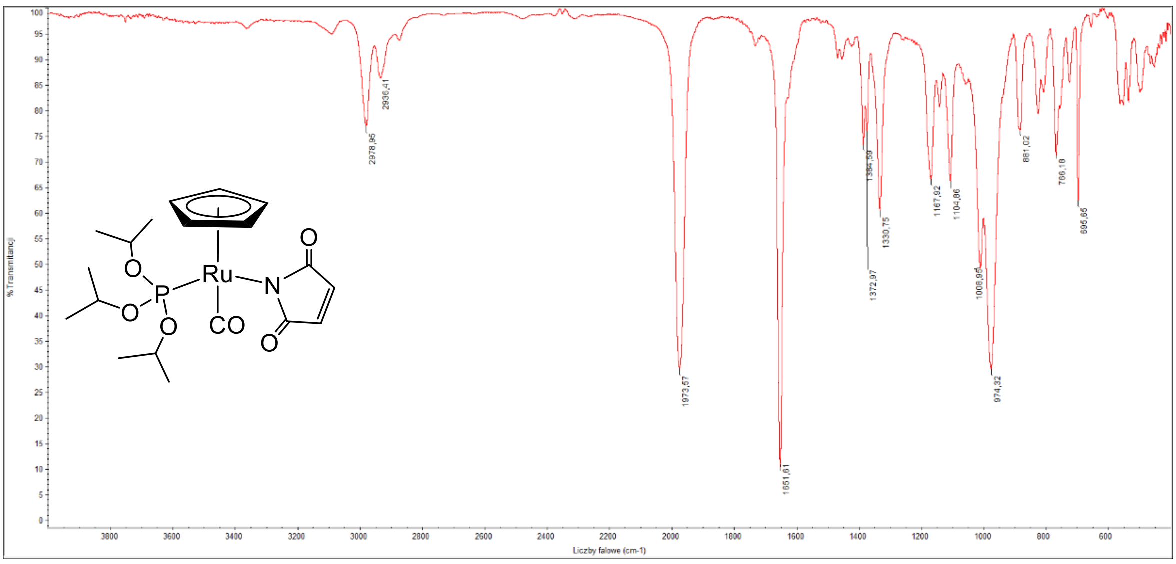


Figure S25. FTIR spectra of **3c**.

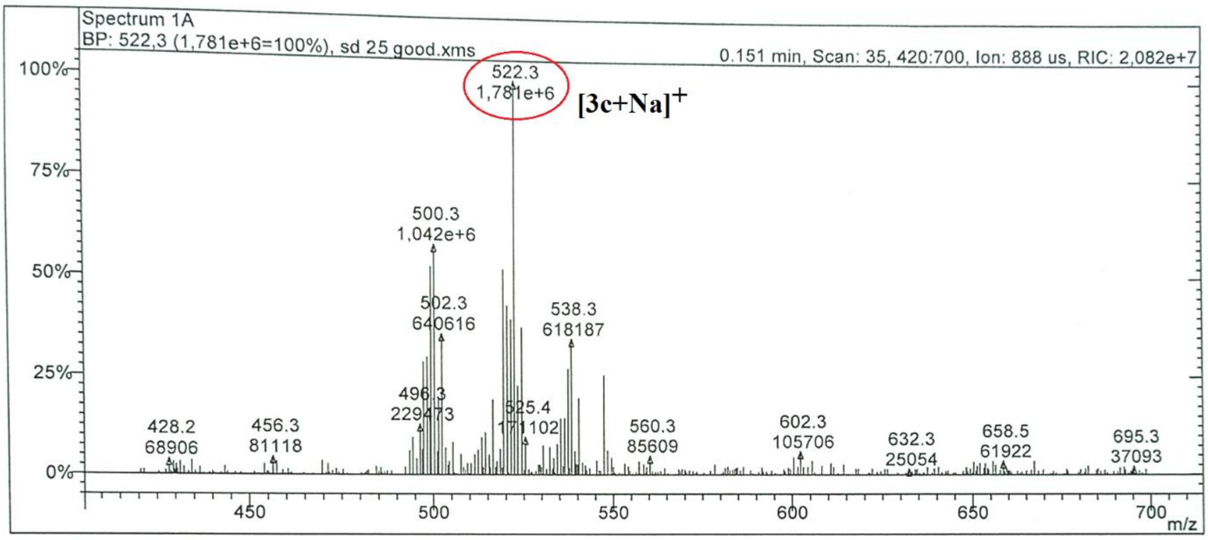


Figure S26. ESI-MS spectra of **3c**.

1.3. Absorption and Emission studies:

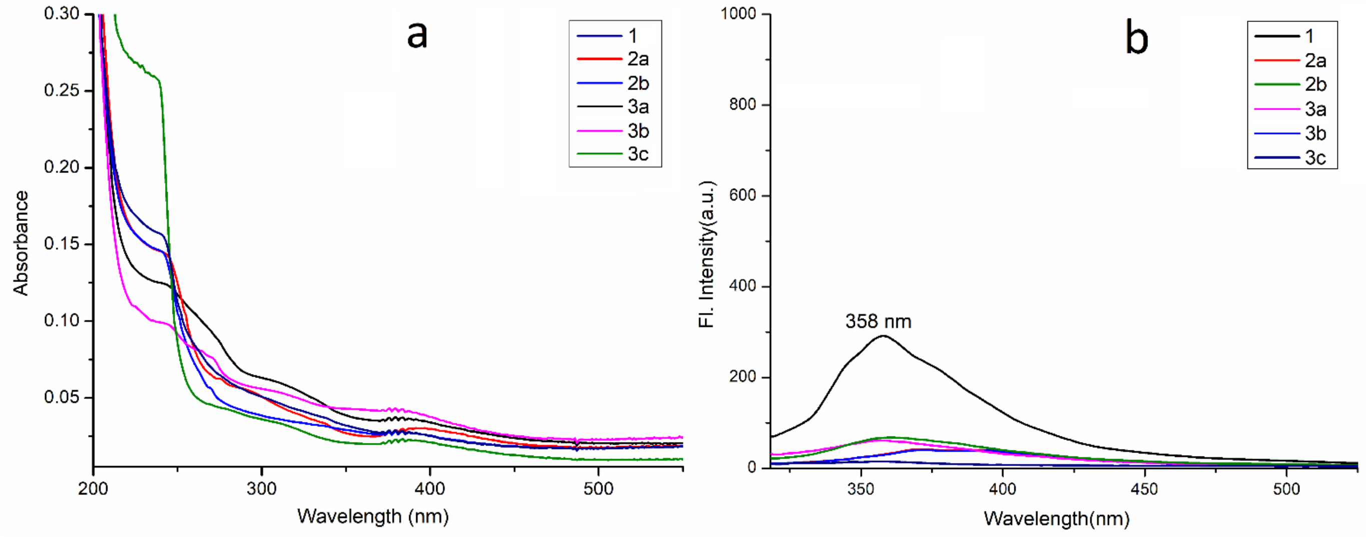


Figure S27. Comparative absorbance (a) and emission (b) spectra of **1**, **2a**, **2b**, **3a**, **3b** and **3c** in Chloroform. Excitations were recorded at 300 nm.

1.4. Biological studies

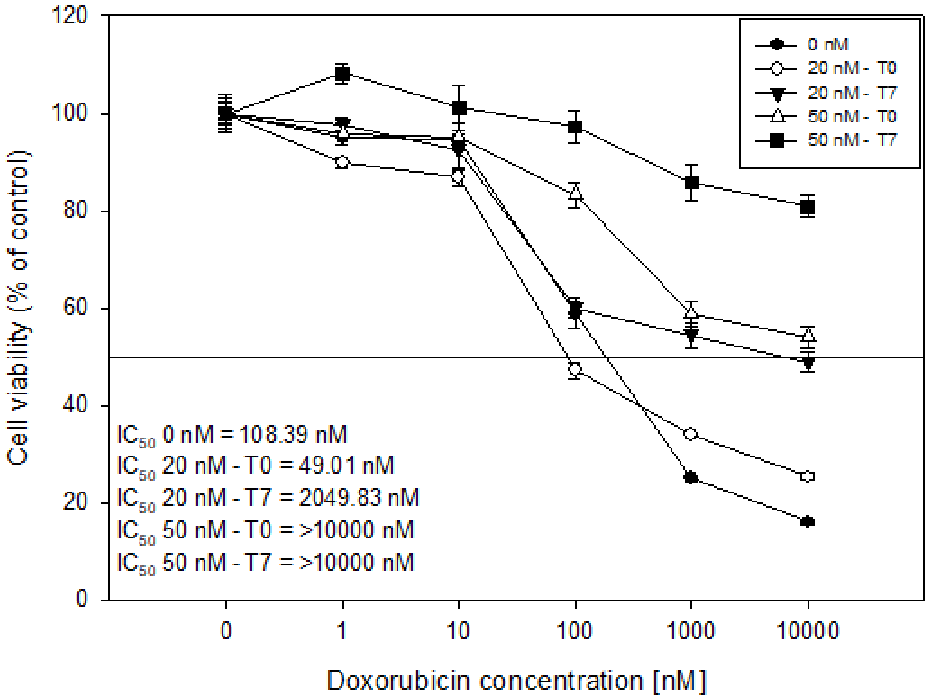


Figure S28. The process of derivation of doxorubicin resistant HL-60 cells line (HL-60/DR). First, HL-60 cells were treated with doxorubicin concentration from 1 to 10000 nM (0 nM). Then cells were treated for 6 days with 20 nM doxorubicin, as was described in the Materials and Methods section (20 nM – T0). Next cells were incubated with pure IMDM medium like HL-60 cells (20 nM – T7). Then cells were incubated for 6 days with 50 nM doxorubicin (50 nM – T0). After this time cells were harvested like normal HL-60 cells for one week (50 nM – T7). Then cells were used for experiments as HL-60/DR. The horizontal line represents viability at a level of 50%.

Table S3. The viability of HL-60 cells, HL-60/DR cells and PBM cells after 2 h incubation with ruthenium complexes. The viability for individual samples were calculated relative to control ± SD

			HL-60 cells			
Concentration (µM)	1	2a	2b	3a	3b	3c
0.5	97.38±1.5 * ↓	101.56±0.29	101.67±2.77	101.8±0.53	99.94±1.63	101.42±1.73
1	96.29±1.35 ** ↓	98.96±2.71	100.93±1.56	99.92±2.88	98.64±2.04	98.55±1.78
2.5	91.46±2.13 *** ↓	99.75±1.02	99.97±2.7	99.05±1.64	99.03±1.38	101.01±0.9
5	91.62±1.52 *** ↓	99.36±2.36	99.89±0.56	101.79±2.37	101.16±1.74	102±1.18
10	87.03±0.28 *** ↓	99.94±2.31	99.59±0.35	101.83±1.95	109.95±0.43 ***↑	101.11±2.28
25	73.25±2.01 *** ↓	99.96±0.67	99.4±3.31	98.62±3.29	123.89±1.87 ***↑	122.72±4.07 ***↑
50	69.16±1.74 *** ↓	98.85±0.48	93.77±1.6 *** ↓	92.26±0.96 *** ↓	106.79±3.9 ***↑	108.46±3.83 ***↑
100	59.99±0.31 *** ↓	91±0.71 *** ↓	84.6±1.89 *** ↓	81.85±2.67 *** ↓	100.13±1.35	106.61±1.6 ***↑
250	16.23±0.45 *** ↓	94.34±0.57 *** ↓	47.82±4.51 *** ↓	87.8±0.25 *** ↓	88.71±0.58 ***↓	102.1±0.99
			HL-60/DR cells			
0.5	96.31±4.88	108.17±1.73 ***↑	105.67±0.44 ***↑	98.29±2.43	96.29±3.52 * ↑	99.43 ± 1.25
1	94.18±1.61 *** ↓	105.04±4	103.85±2.49 * ↑	95.44±2.61	96.15±2.42 * ↑	97.69±1.84
2.5	94.72±2.8 *** ↓	101.96±5.35	103.11±1.1 ** ↑	96.38±2.1	97.52±2.65	97.39±3.57
5	95.14±1.19 *** ↓	104.61±2.75 ***↑	102.92±3.24	93.24±1.96 *** ↓	100.65±3.12	96.52±3.69
10	92.67±0.98 *** ↓	110.07±4.59 ***↑	102.33±2.8	94.8±4.91	103.56±1.6	101.91±2.75
25	92.87±2.68 *** ↓	114.54±3.8 ***↑	101.95±1.71	95.92±5.31	110.73±1.87 ***↑	115.31±3.89 ***↑

50	91.62±1.12 *** ↓	122.85±3.33 ***↑	103.22±0.98 ** ↑	95.57±3.32	117.61±1.1 ***↑	120.96±2.73 ***↑
100	93.61±2.73 *** ↓	123.91±3.86 ***↑	105 56±1.78 ***↑	100.71±2.7	121.13±1.64 ***↑	135.41±2.97 ***↑
250	91.02±2.79 *** ↓	133.47±5.23 ***↑	115.28±2 ***↑	112.35±4 *** ↑	125.55±1.91 ***↑	147.02±3.43 ***↑
PBM cells						
0.5	104.94±0.7 ***↑	99.88±0.88	100.6±0.47	101.18±2.13	101.45±0.56	99.94±1.65
1	108.97±0.32 ***↑	99.15±0.58	99.51±0.71	99.79±0.91	99.72±0.76	99.28±1.24
2.5	112.87±0.31 ***↑	100.97±0.36	99.73±1.01	99.3±0.27	99.22±1.29	99.32±0.97
5	117.79±1.3 ***↑	100.88±0.76	100.76±0.74	101.44±0.74	99.76±1.75	103.9±1.14
10	117.63±1.53 ***↑	106.48±1.01 ***↑	104.49±0.84 ***↑	104.25±1.17 ***↑	103.71±0.58 ***↑	105.04±0.53 ***↑
25	113.84±1.03 ***↑	133.7±0.47 ***↑	106.24±2.59 ***↑	112.33±0.8 ***↑	123.75±2.23 ***↑	111.46±1.72 ***↑
50	110±1.1 ***↑	138.73±0.49 ***↑	121.9±9.7 ***↑	118.28±1.4 ***↑	166.14±0.29 ***↑	117.65±0.21 ***↑
100	107.17±1.13 ***↑	142.29±1.86 ***↑	134.1±4.19 ***↑	117.94±0.55 ***↑	171.97±2.77 ***↑	114.18±0.42 ***↑
250	96.68±0.27 ***↓	116.44±0.4 ***↑	153.43±2.01 ***↑	131.03±0.29 ***↑	159.28±0.79 ***↑	166.21±2.62 ***↑

Cell viability in the control was taken as 100%. * p < 0.05. ** p < 0.01. *** p < 0.001. ↑ – increase. ↓ – decrease

Table S4. The viability of HL-60 cells, HL-60/DR cells and PBM cells after 24 h incubation with ruthenium complexes. The viability for individual samples were calculated relative to control ± SD.

Concentration (μM)	**1**	**2a**	**2b**	**3a**	**3b**	**3c**
HL-60 cells						
0.5	108.6±0.49***↑	100.58±1.25	101.04±0.36	104.06±0.9***↑	99.54±2.15	100.44±1.9
1	109.83±3.3***↑	99.88±2.69	101.74±1.24	105.49±0.8***↑	104.42±2.8**↑	100.96±1.68
2.5	103.77±1.7***↑	98.95±2.14	103.88±2.16**↑	106.57±1.2***↑	102.39±3.48	102.81±1.38
5	56.77±1.77***↓	99.89±1.58	102.86±1	106.32±1.9***↑	102.19±2.39	104.3±1.02***↑
10	38.45±1.38***↓	101.36±1.63	99.74±0.83	72.02±7.4***↓	62.11±1.66***↓	98.55±3.27
25	36.46±0.32***↓	48.93±2.18***↓	55.44±4.36***↓	36.56±1.03***↓	63.73±3.53***↓	42.26±1.07***↓
50	8.14±0.44***↓	39.02±1.65***↓	34.01±0.3*** ↓	37.36±0.58***↓	46.62±1.01***↓	40±0.8***↓
100	4.1±0.28***↓	38.19±1.24***↓	35.27±1.17***↓	33.49±1.38***↓	42.31±1.02***↓	25.5±1.11***↓
250	2.14±0.12***↓	28.23±0.19***↓	4.96±0.17***↓	9.31±0.45***↓	44.05±0.52***↓	15.33±2.71***↓
HL-60/DR cells						
0.5	99.31±4.37	102.34±1.99	98.58±1.55	96.33±4.74	104.4±1.22**↑	97.79±9.71
1	95.75±4.99	96.98±3.8	97.63±2.02	97.36±4.66	101.9±3.23	104.08±1.27**↑
2.5	95.68±7.05	96.52±1.48	97.44±2.3	97.74±1.86	101.29±2.56	102.42±2.55
5	95.4±4.21	91.63±9.53	97.29±2.4	94.5±0.87***↓	102.02±1.75	102.08±1.86
10	92.72±3.09***↓	103.87±3.88	95.18±8.51	93.16±4.89***↓	107.78±6.08	105.42±4.15
25	91.18±2.04***↓	116.68±5.2***↑	101.41±5.79	93.18±3.98***↓	96.98±3.27	118.92±1.8***↑
50	89.09±2.71***↓	112.51±2.6***↑	101.85±4.07	92.17±2.88***↓	58.63±0.7***↓	128.04±1.0***↑
100	78.25±0.84***↓	102.81 ± 3.54	72.21±2.65	91.43±2.0***↓	52.8±1.19***↓	88.87±4.6***↓
250	46.9±1.36***↓	53.6±11.79***↓	26.75±0.68***↓	66.38±2.18***↓	36.42±2.03***↓	5.21±0.1***↓
PBM cells						
0.5	108.4±2.48***↑	100.44±0.73	103.79±2.53*↑	103.32±0.75**↑	99.82±2.86	94.2±4.03***↓
1	119.9±3.65***↑	101.22±3.01	101.37±0.51	103.08±2.06*↑	96.6±0.18**↓	91.26±3.15***↓
2.5	112.9±0.56***↑	101.83±2.7	104.07±1.51**↑	104.83±2.1**↑	97.55±4.03	92.39±3.49***↓
5	103.41±1.68**↑	100.18±1.98	93.14±0.85***↓	108.4±1.81***↑	88.3±2.63***↓	80.62±0.84***↓
10	83.3±0.65***↓	44.63±0.13***↓	47.94±2***↓	106.8±2.71***↑	19.74±1.91***↓	55.19±2.08***↓

25	77±2.17***↓	10.31±1***↓	24.54±0.49***↓	85.14±1.42***↓	10.06±0.34***↓	28.77±2.03***↓
50	61.47±1.24***↓	9.87±0.25***↓	24.12±0.67***↓	83.76±0.55***↓	6.55±0.14***↓	15.11±0.25***↓
100	45.15±1.4***↓	10.97±0.39***↓	21.28±4.36***↓	64.48±0.7***↓	7.22±1.23***↓	13.77±0.2***↓
250	18.46±0.43***↓	9.36±0.72***↓	7.09±0.38***↓	56.73±0.35***↓	6.12±0.9***↓	8.34±0.25***↓

Cell viability in the control was taken as 100%. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. ↑ – increase, ↓ – decrease

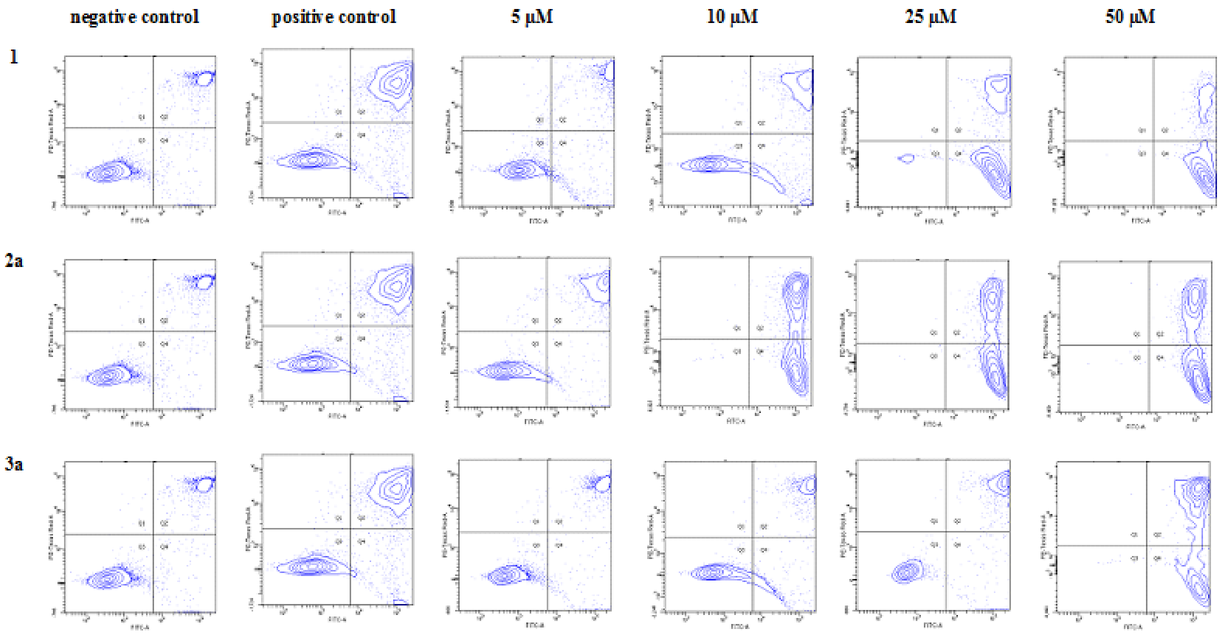


Figure S29. The effect of ruthenium complexes **1**, **2a** and **3a** on the apoptosis of HL-60 cells. Representative flow cytometric dot plots showing the percentage of cells in viable (Q3), early apoptotic (Q4), late apoptotic (Q2) and necrotic (Q1) stages. The positive control were cells incubated with 20 µM camptothecin (CAM) for 24 h at 37 °C. Data represent means ± SD of 3 experiments.

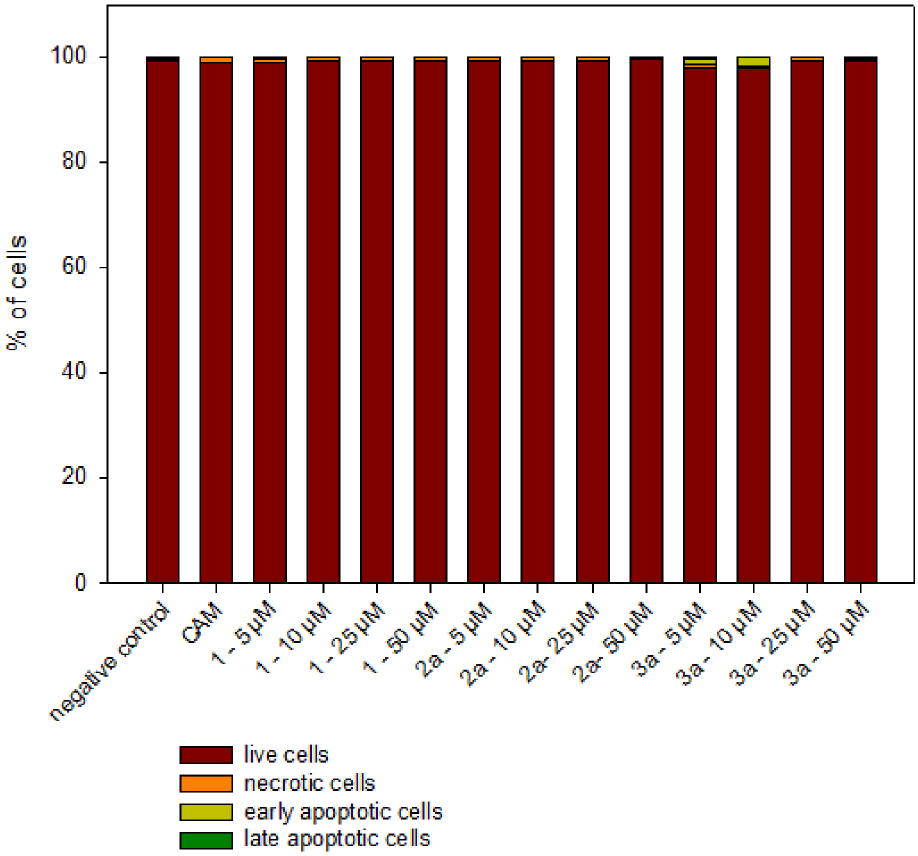
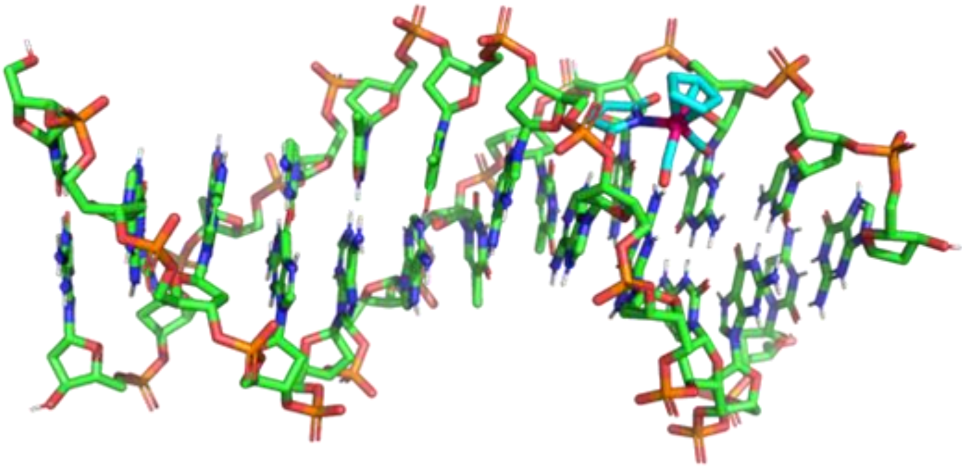


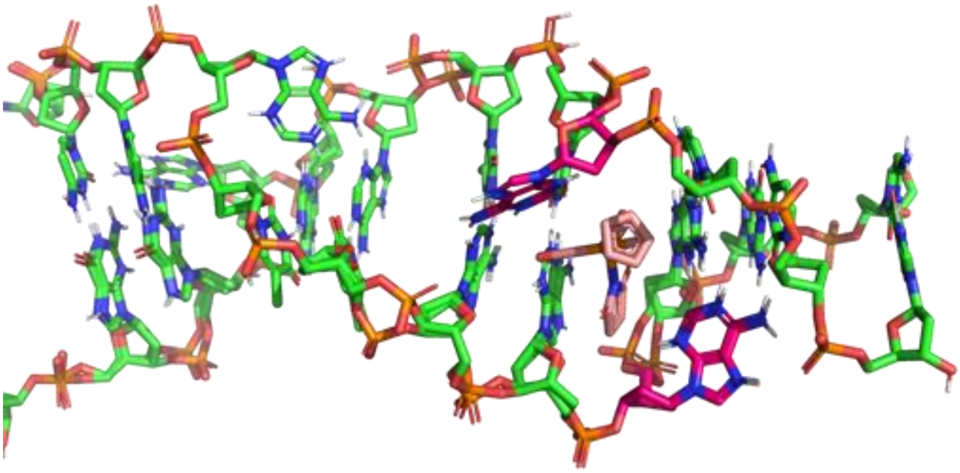
Figure S30. The effect of ruthenium complexes **1**, **2a** and **3a** on the apoptosis of HL-60/DR cells. Data represent means ± SD of three experiments. The CAM sample were cells incubated with 20 µM camptothecin for 24 h at 37 °C.

1.5. Docking studies

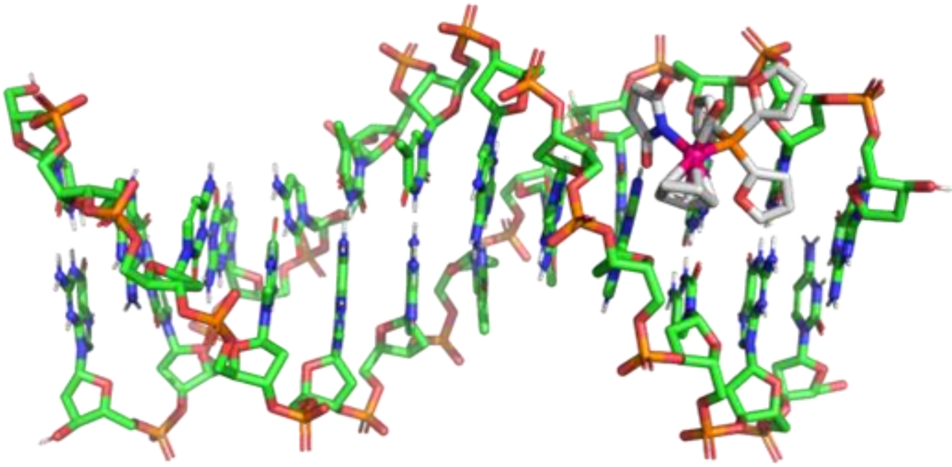
a



b



c



d

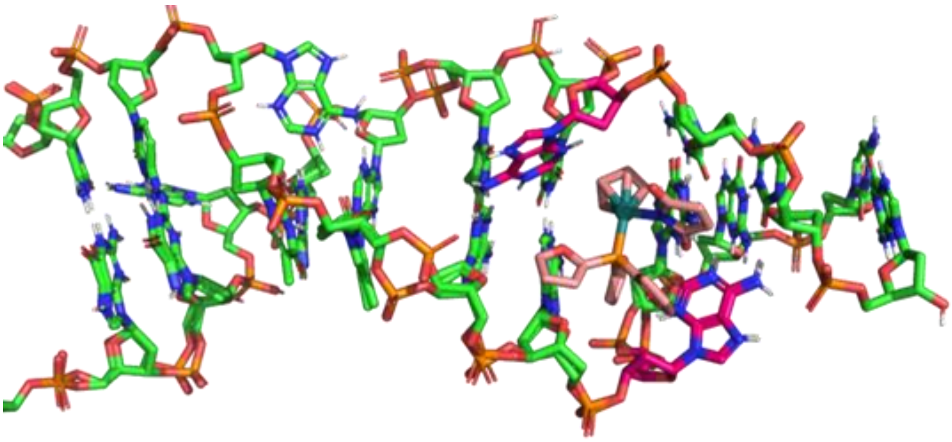


Figure S31. Ruthenium(II) complexes **1** (a, b) and **2b** (c, d) with fully complemented DNA (a, c) and with mismatched DNA (b, d).