Supplementary Information

Ruthenium(II)-mercapto Complexes with Anticancer Activity Interact with Topoisomerase IB

Monize M. da Silva,*[,a] Mariana S. de Camargo,[a] Silvia Castelli,[b] Rone A. de Grandis,[c] Eduardo E. Castellano,[d] Victor M. Deflon,[e] Marcia R. Cominetti,[f] Alessandro Desideri[b] and Alzir A. Batista *[,a]

[a]*Departamento de Química, Universidade Federal de São Carlos, CP 676, 13565-905 São Carlos-SP, Brazil*

[b]*Dipartimento di Biologia, Università Tor Vergata di Roma, 00133 Rome, Italy*

[c]*Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista (Unesp), 14801-902 Araraquara-SP, Brazil*

[d]*Instituto de Física de São Carlos, Universidade de São Paulo, CP 369, 13560-970 São Carlos-SP, Brazil*

[e]*Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, 13560-970 São Carlos-SP, Brazil*

[f]*Departamento de Gerontologia, Universidade Federal de São Carlos, CP 676, 13565-905 São Carlos-SP, Brazil*

*e-mail: monize_martins@yahoo.com.br; daab@ufscar.br

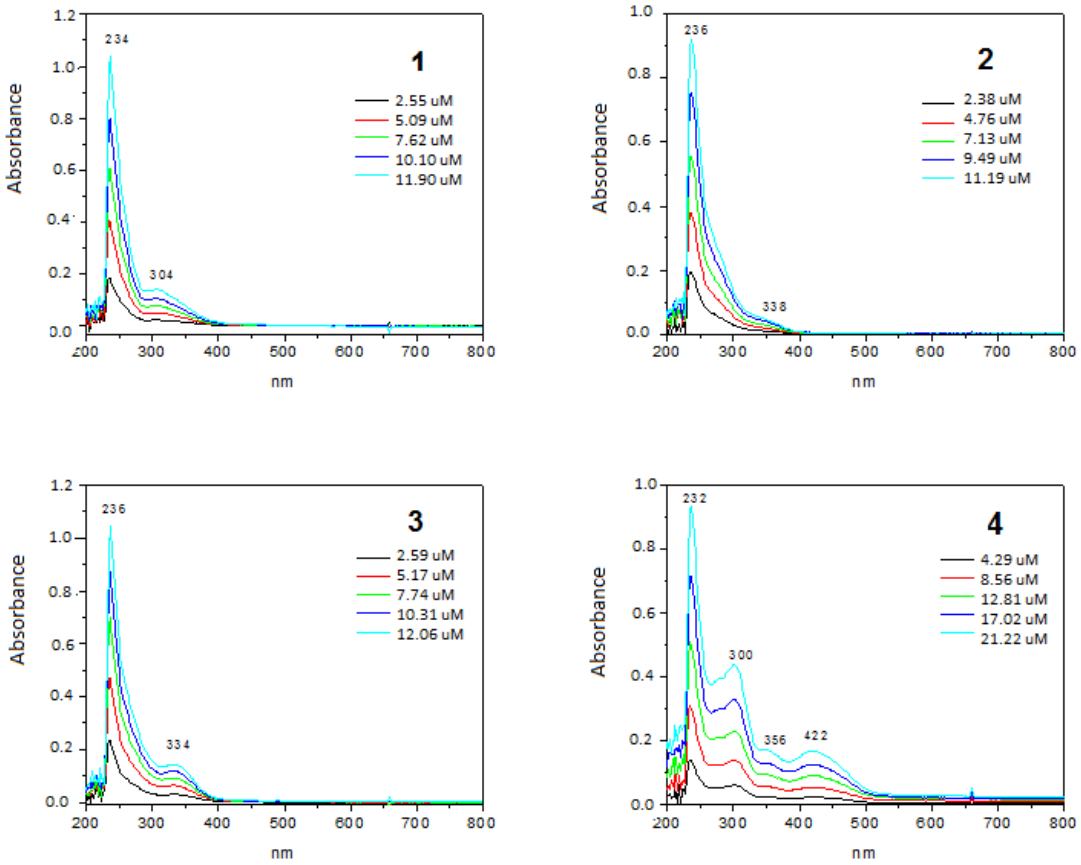


Figure S1. Absorption spectra of the complexes **1-4** in the UV-Vis region for the complexes, in CH_2Cl_2.

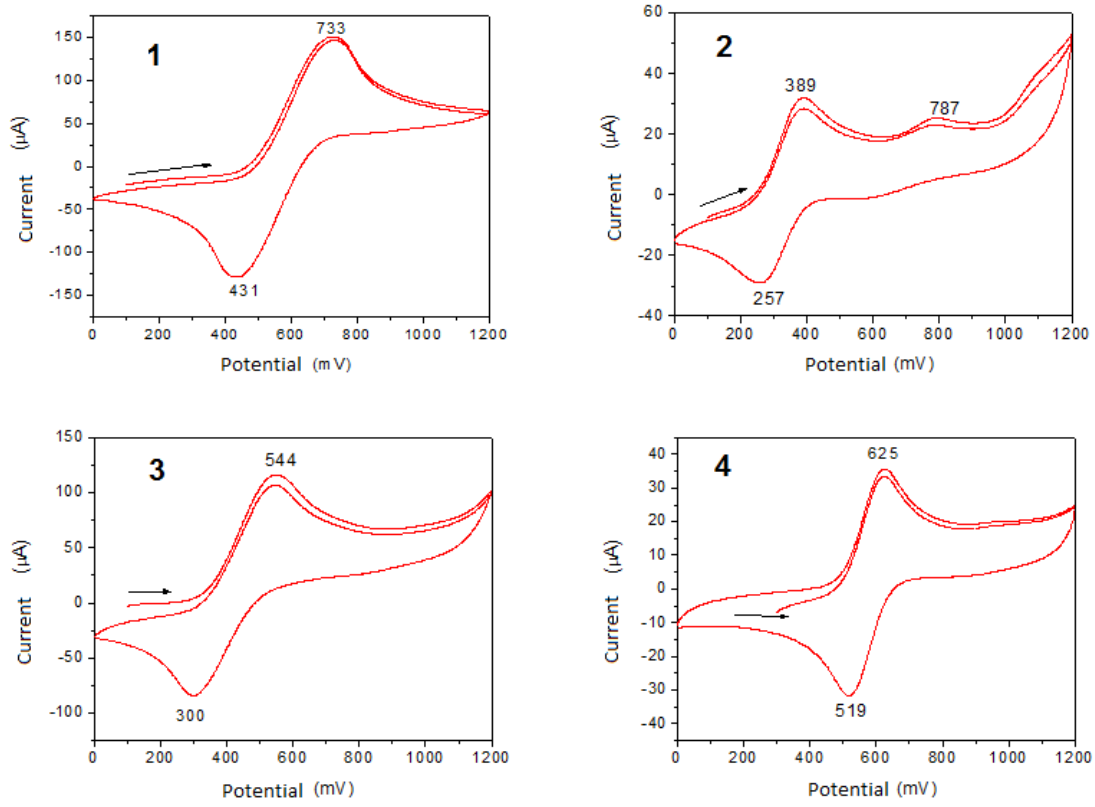


Figure S2. Cyclic voltammograms of the complexes **1**-**4**, in CH_2Cl_2 (0.1 M PTBA, 100 mV s^{-1}, Ag/AgCl), platinum working electrode. Complex **1**: [Ru(mtz)$_2$(dppb)]; complex **2**: [Ru(mmi)$_2$(dppb)]; complex **3**: [Ru(dmp)$_2$(dppb)]; complex **4**: [Ru(mpca)$_2$(dppb)], mtz = 2-mercaptothiazoline; mmi = 2-mercapto-1-methyl-imidazole; dmp = 4,6-diamino-2-mercaptopyrimidine; mpca = 6-mercaptopyridine-3-carboxylic acid; dppb = 1,4-bis(diphenylphosphino)butane.

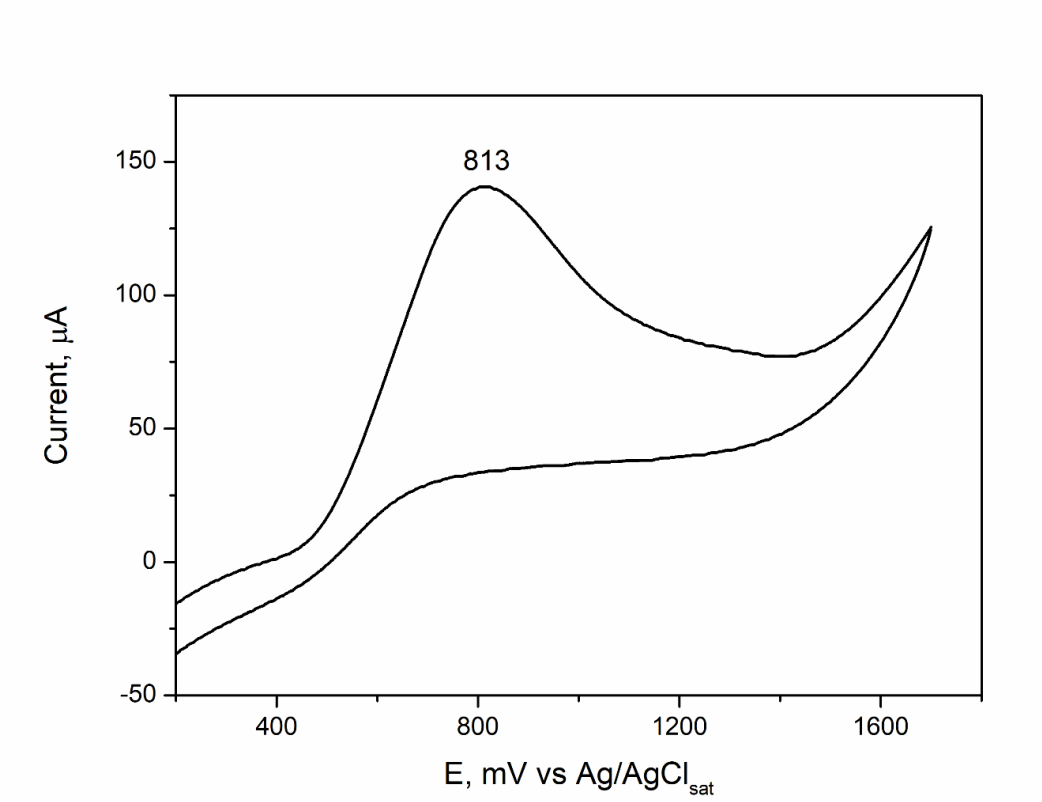
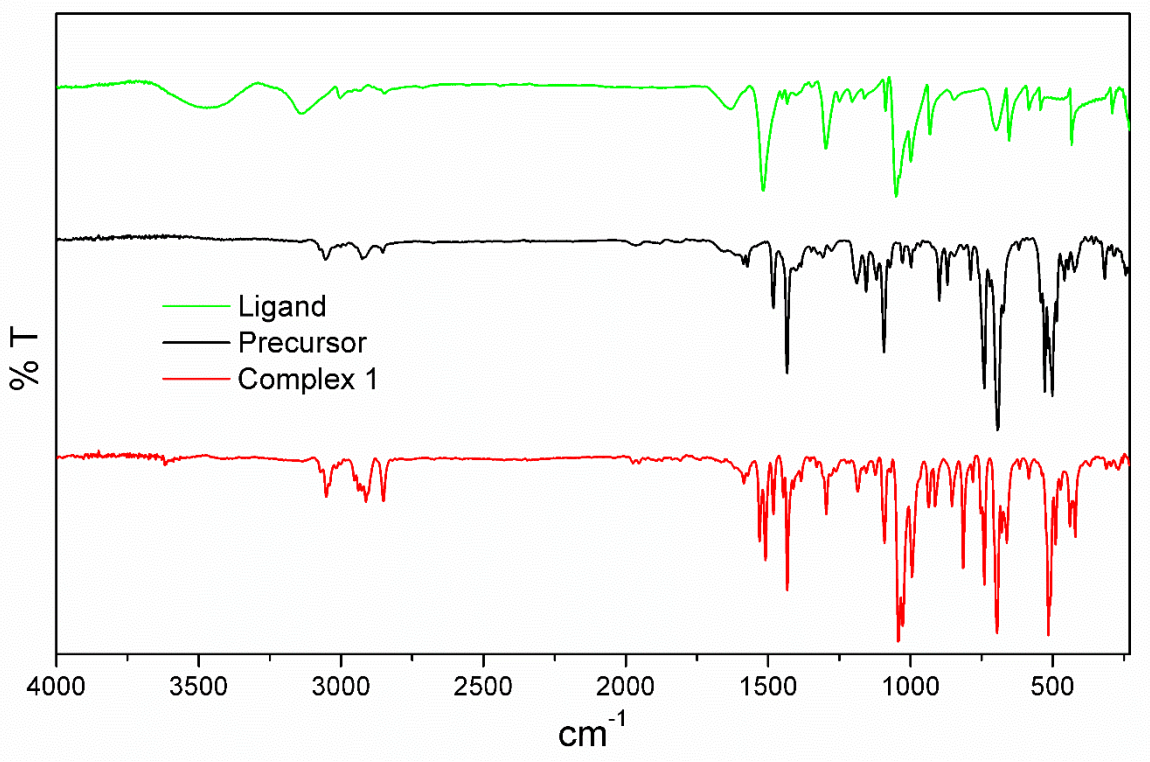
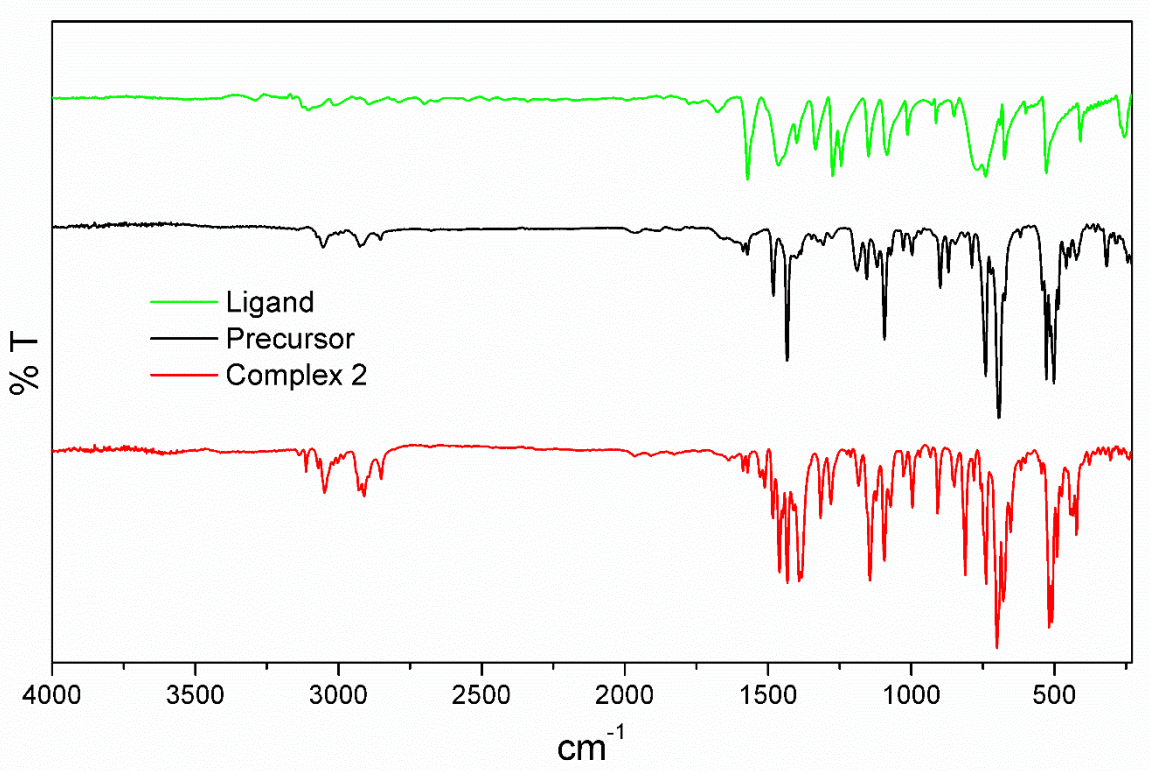


Figure S3. Cyclic voltammogram of the free 2-mercapto-1-methyl-imidazole**,** in CH_2Cl_2 (0.1 M perchlorate tetrabuthyl ammonium (PTBA), 100 mV s^{-1}, Ag/AgCl), platinum working electrode.


Ligand
Precursor
Complex 1
% T
4000
3500
3000
2500
2000
1500
1000
500
cm-1


Ligand
Precursor
Complex 2
% T
4000
3500
3000
2500
2000
1500
1000
500
cm-1

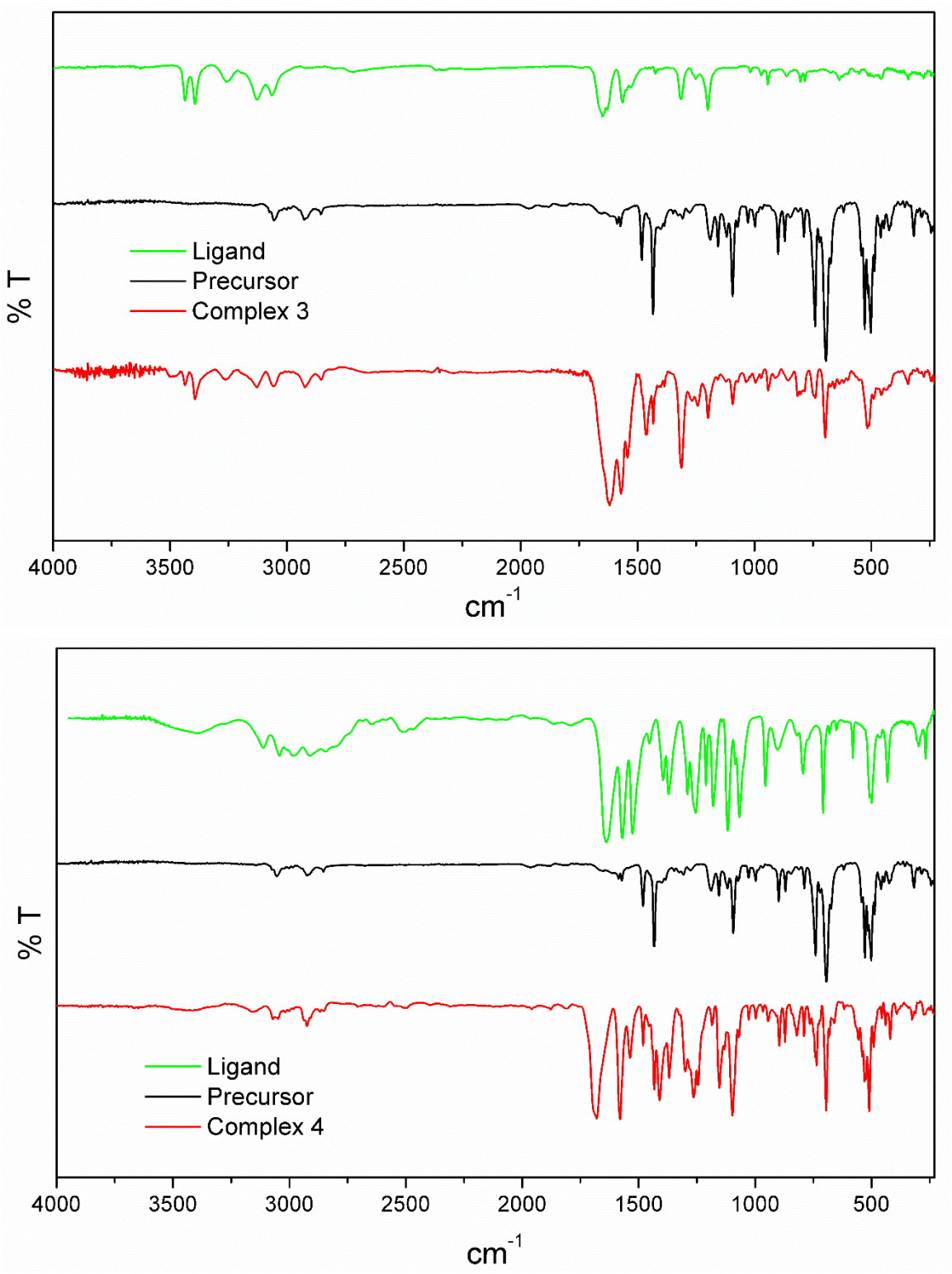


Figure S4. IR spectra of the **1-4** complexes, in CsI pellets. Complex **1**: $[Ru(mtz)_2(dppb)]$; complex **2**: $[Ru(mmi)_2(dppb)]$; complex **3**: $[Ru(dmp)_2(dppb)]$; complex **4**: $[Ru(mpca)_2(dppb)]$.

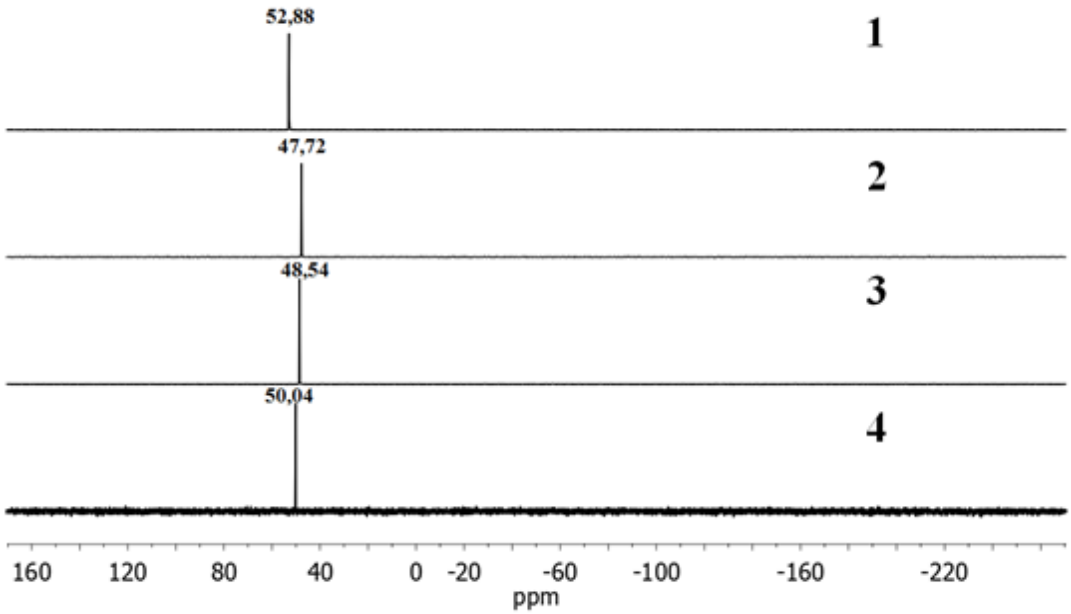


Figure S5. $^{31}P\{^{1}H\}$ (162 MHz, CH_2Cl_2/D_2O) NMR spectra of the complexes **1**-**4**.

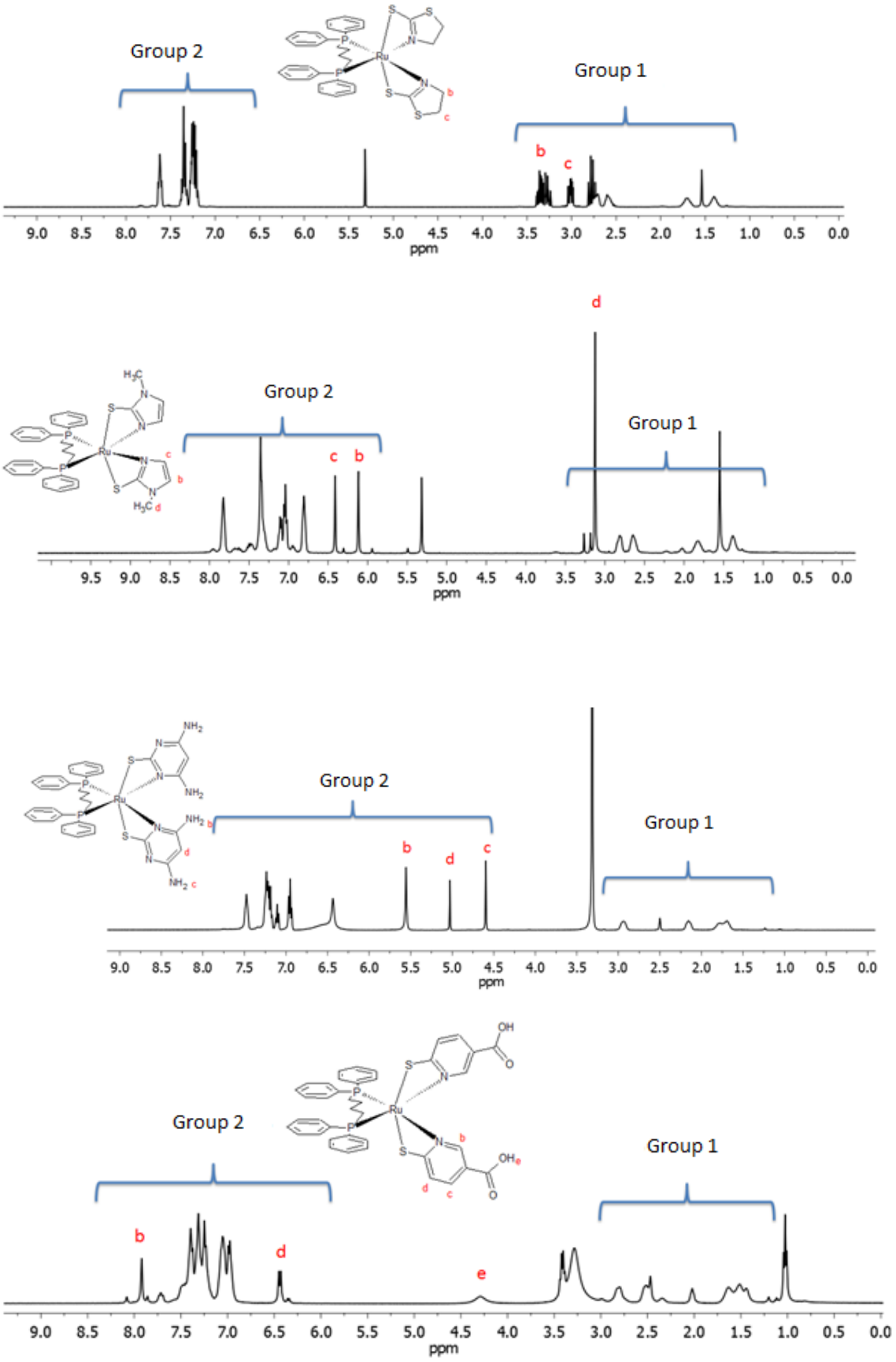
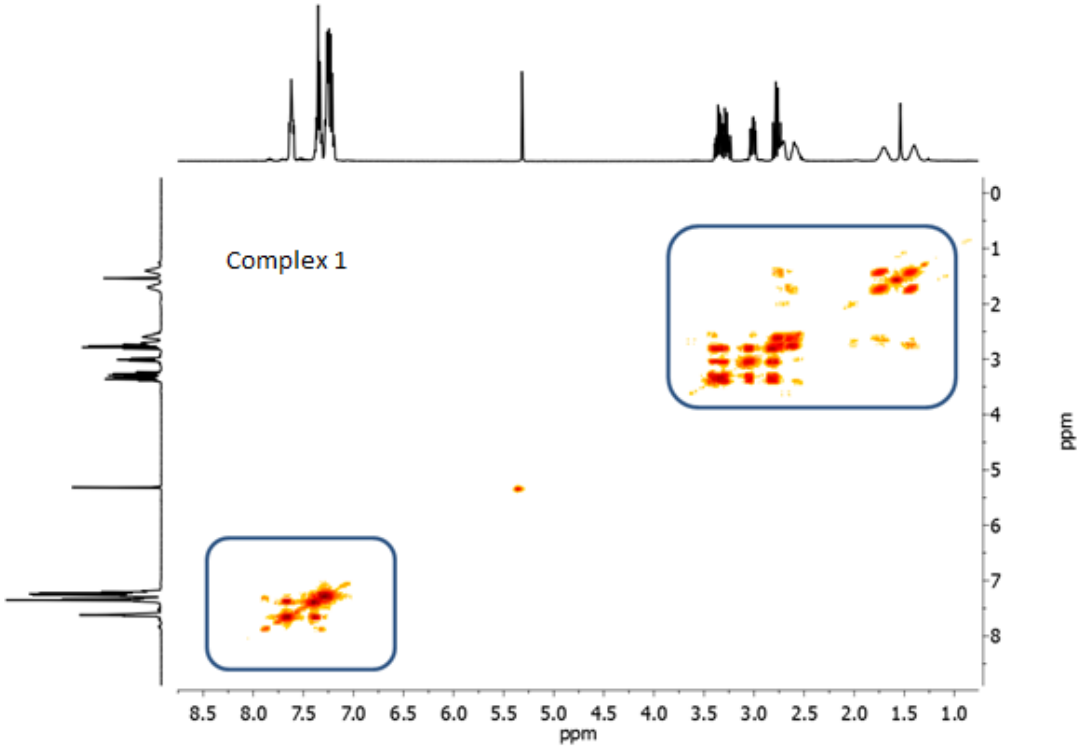
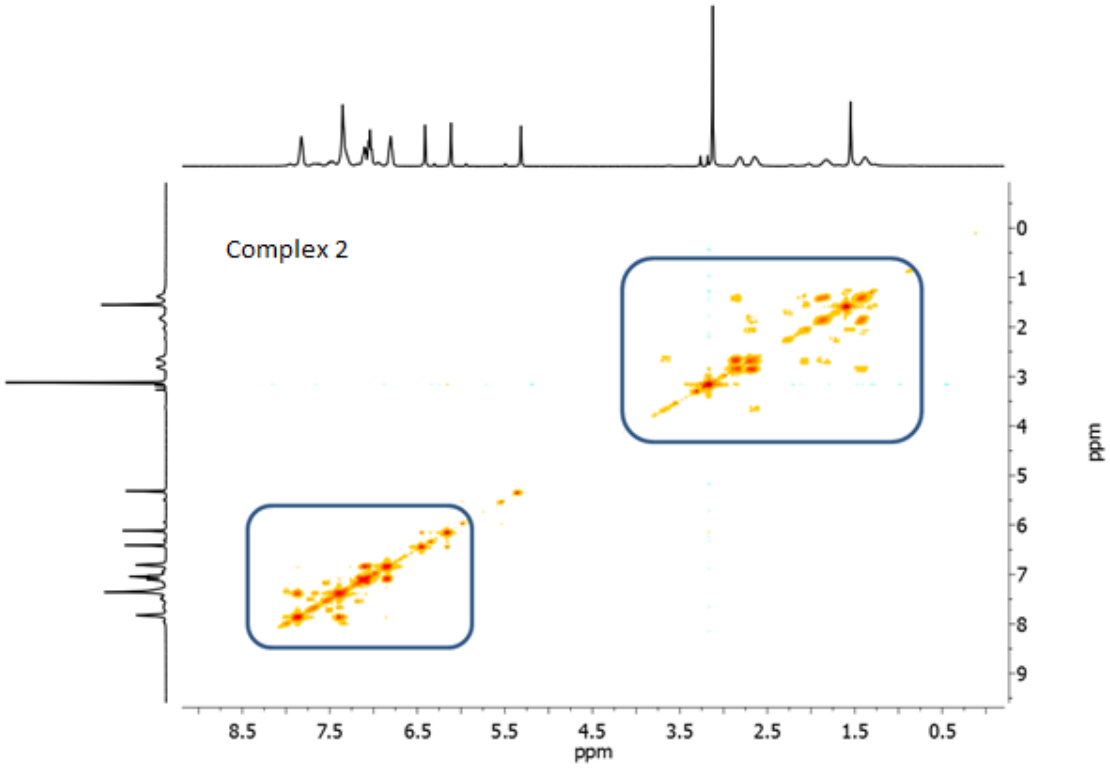


Figure S6. ^{1}H NMR (400 MHz) spectra of the complexes in CD_2Cl_2 (complexes **1** and **2**) and in DMSO-d_6 (complexes **3** and **4**).


Complex 1
0
1
2
3
4
5
6
7
8
ppm
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm


Complex 2
0
1
2
3
4
5
6
7
8
9
ppm
8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
ppm

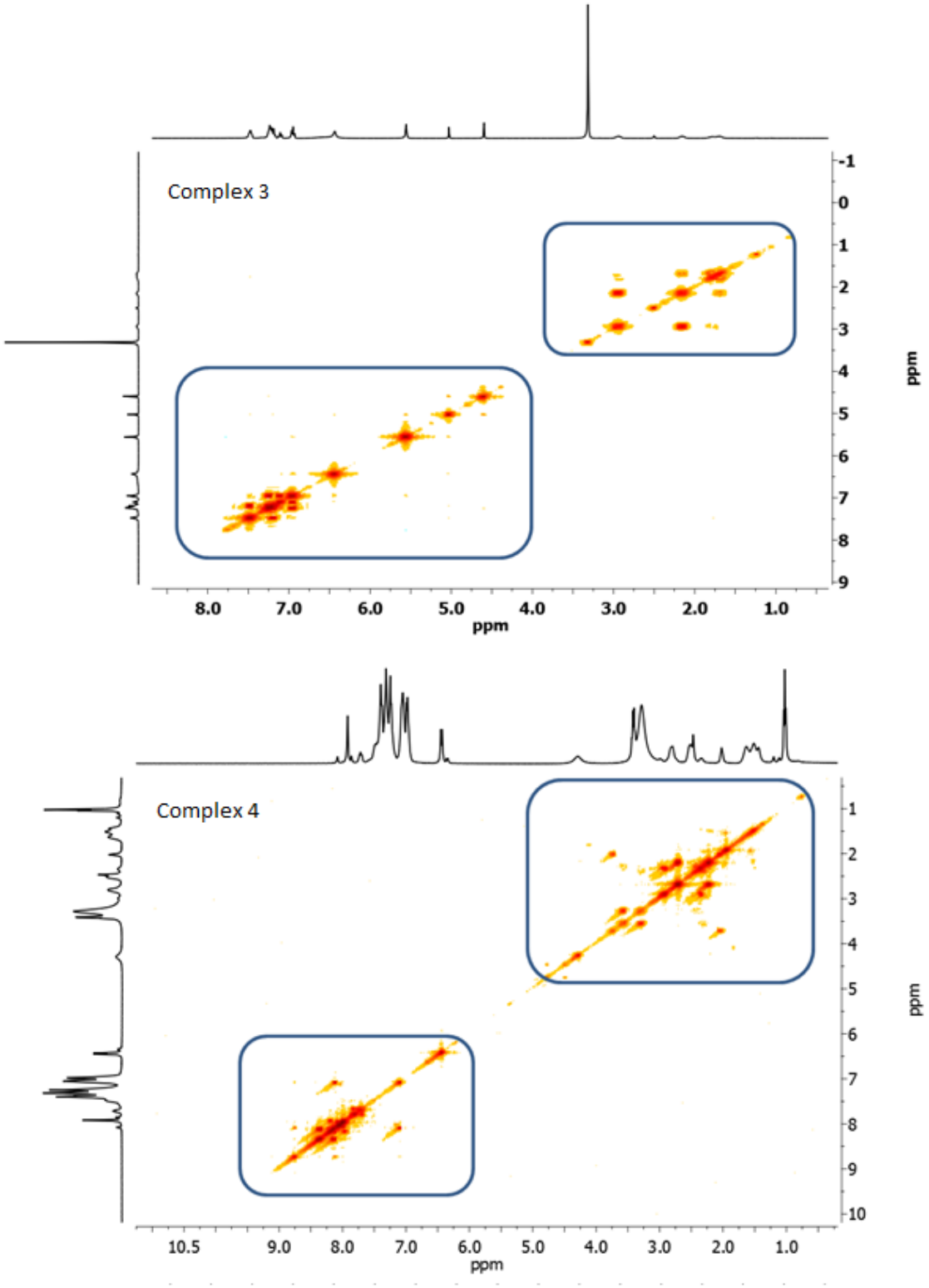


Figure S7. Contour map of correlation spectroscopy (COSY) (^{1}H-^{1}H) for complexes in CD_2Cl_2 (complexes **1** and **2**) and DMSO-d_6 (complexes **3** and **4**).

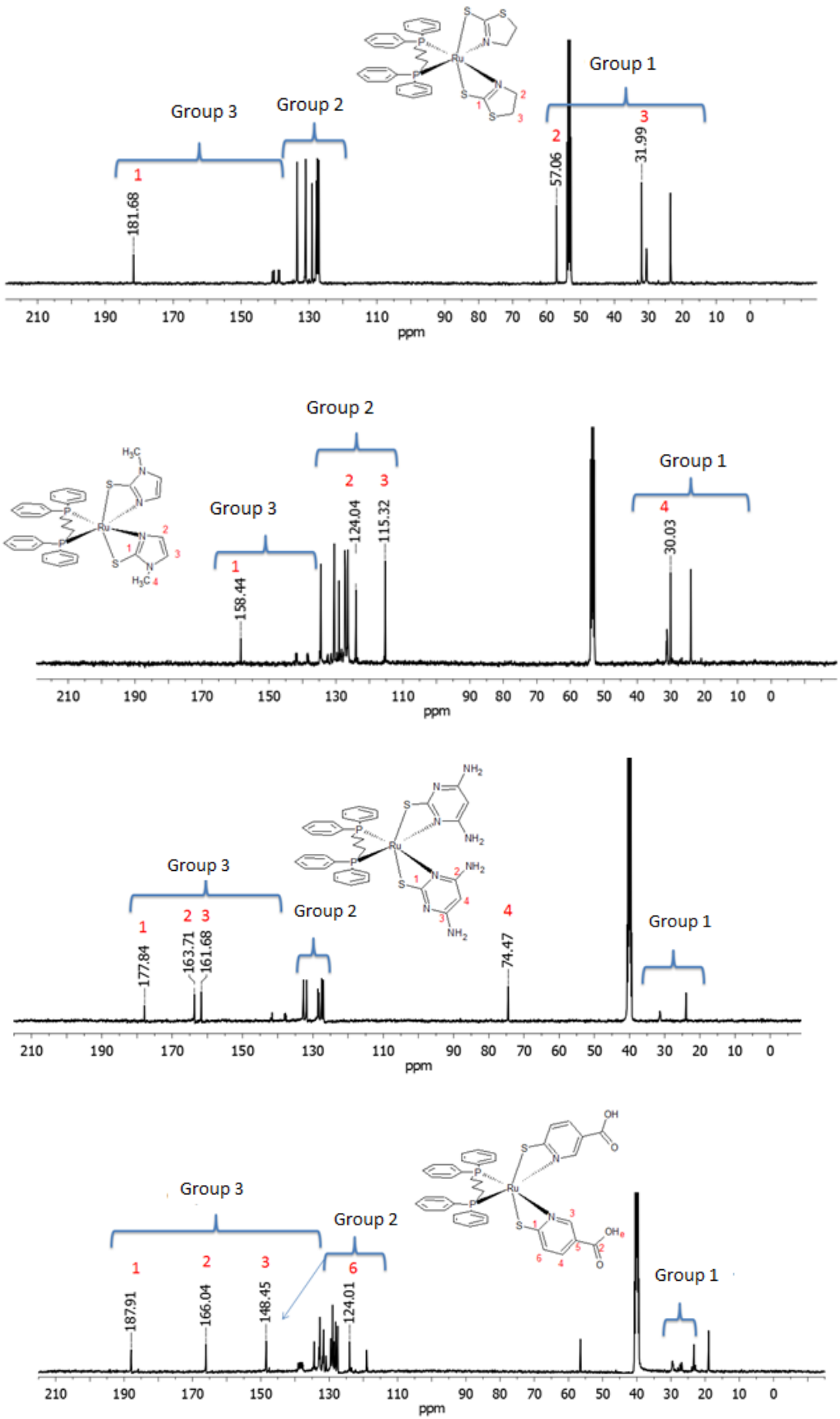
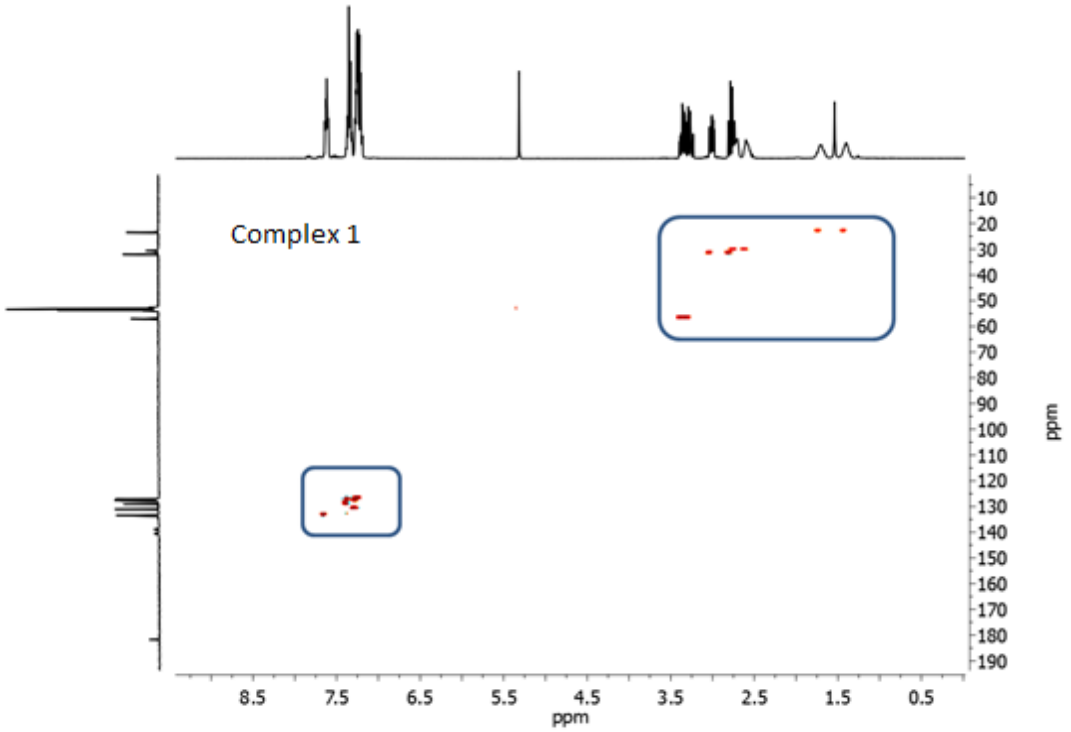
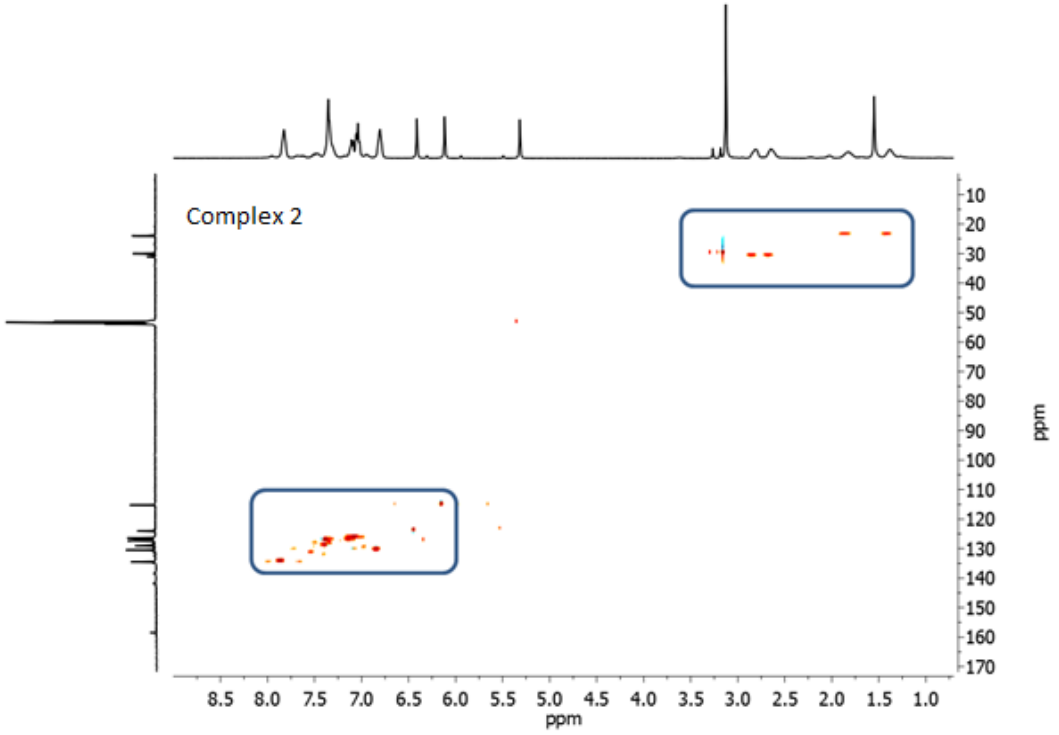


Figure S8. ^{13}C NMR (100 MHz) spectra for complexes in CD_2Cl_2 (complexes **1** and **2**) and DMSO-d_6 (complexes **3** and **4**).


Complex 1
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
ppm
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
ppm


Complex 2
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

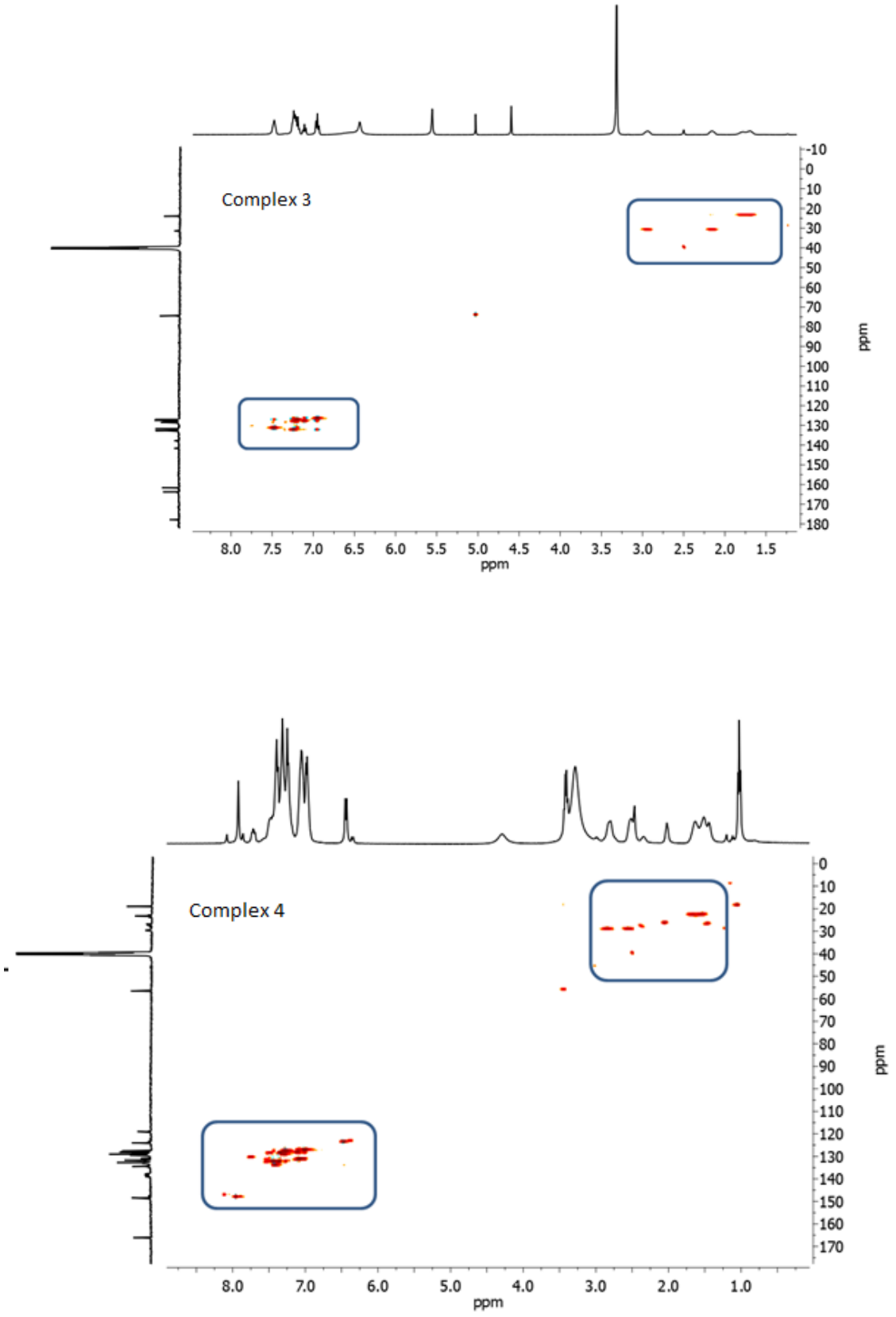
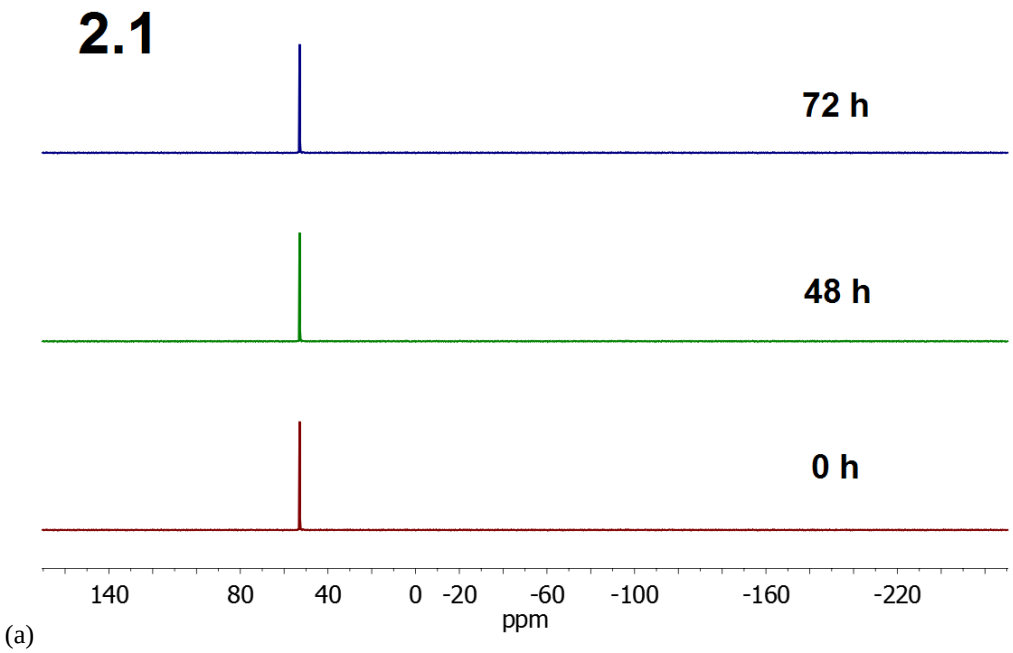
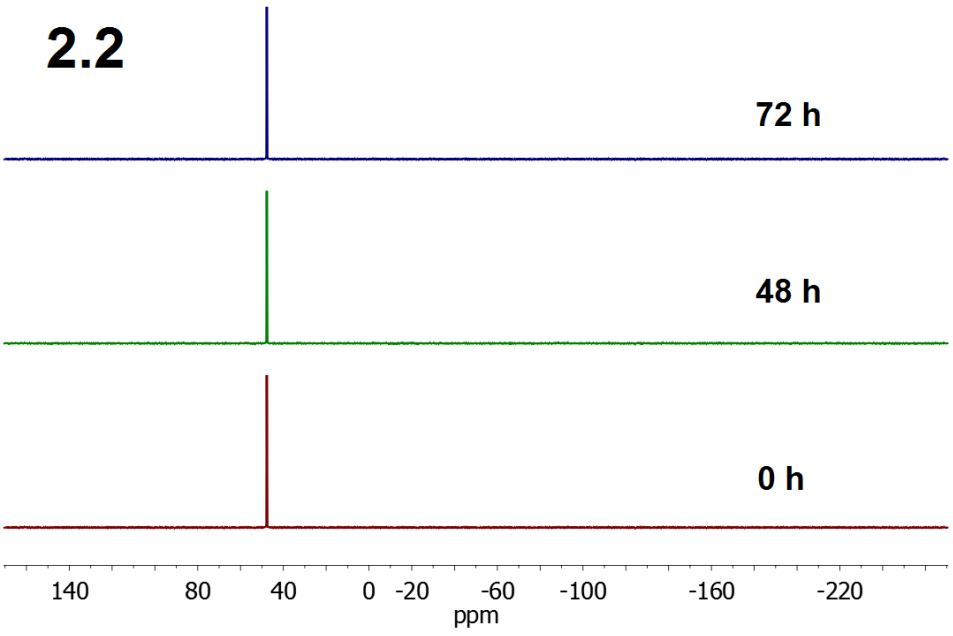


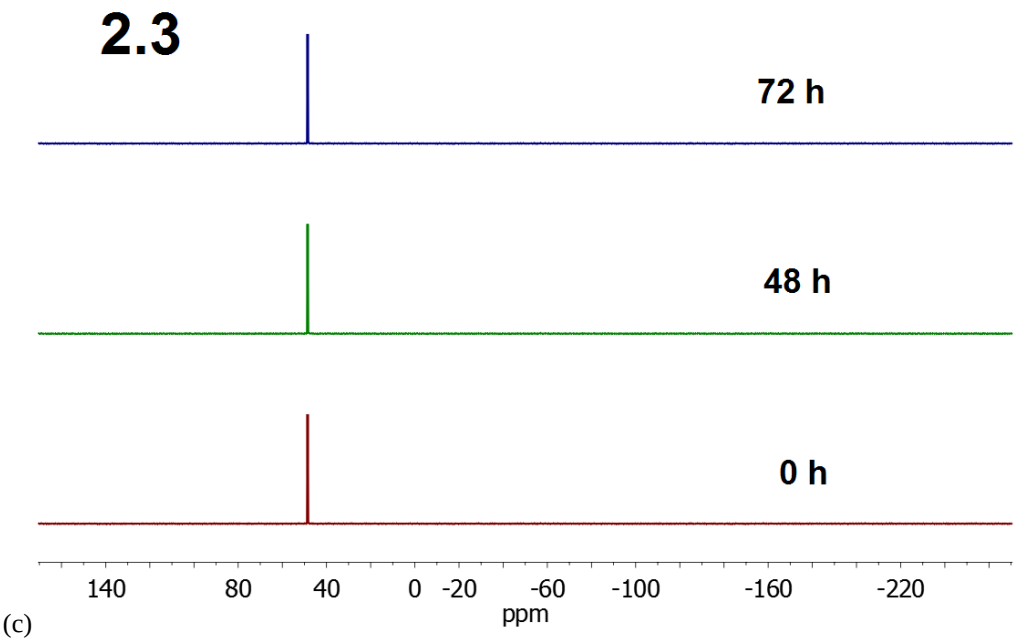
Figure S9. Contour map of heteronuclear single quantum coherence spectroscopy (HSQC) (^{1}H-^{13}C) for complexes in CD_2Cl_2 (complexes **1** and **2**) and DMSO-d_6 (complexes **3** and **4**).


2.1
72 h
48 h
0 h
140
80
40
0
-20
-60
-100
-160
-220
ppm

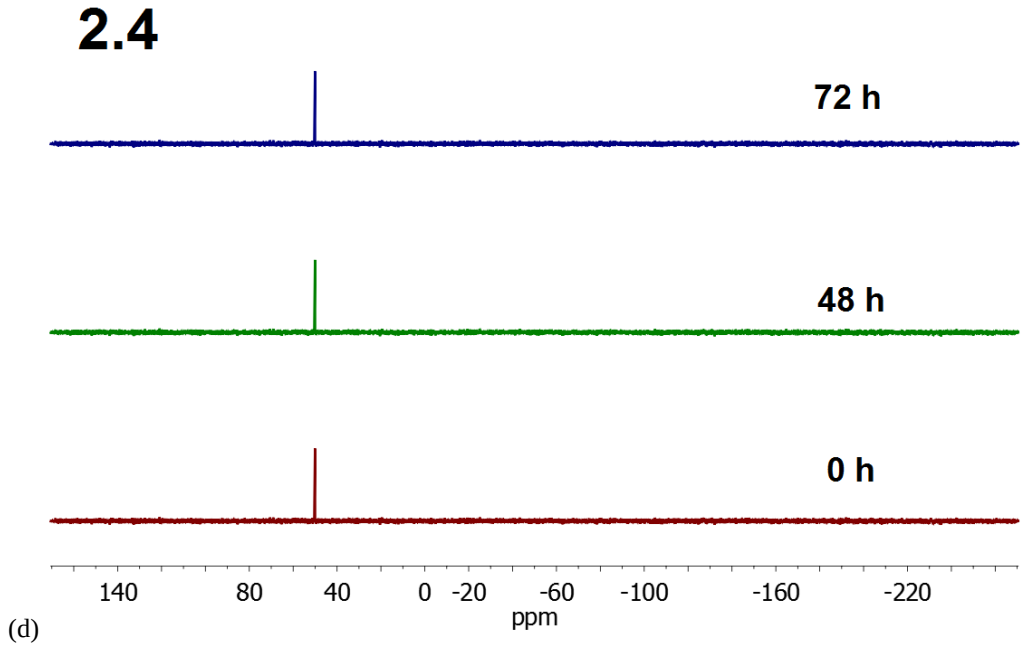
(a)


2.2
72 h
48 h
0 h
140
80
40
0
-20
-60
-100
-160
-220
ppm

(b)

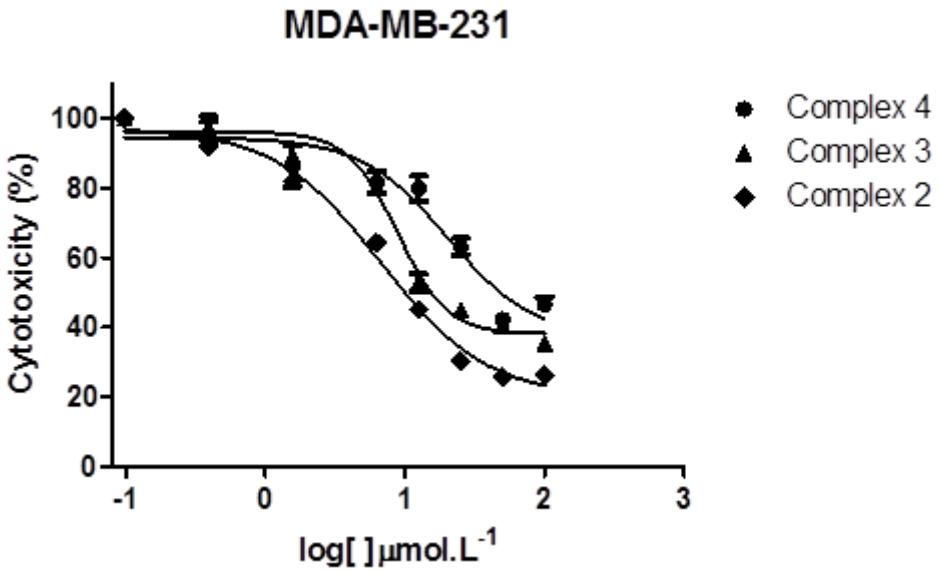


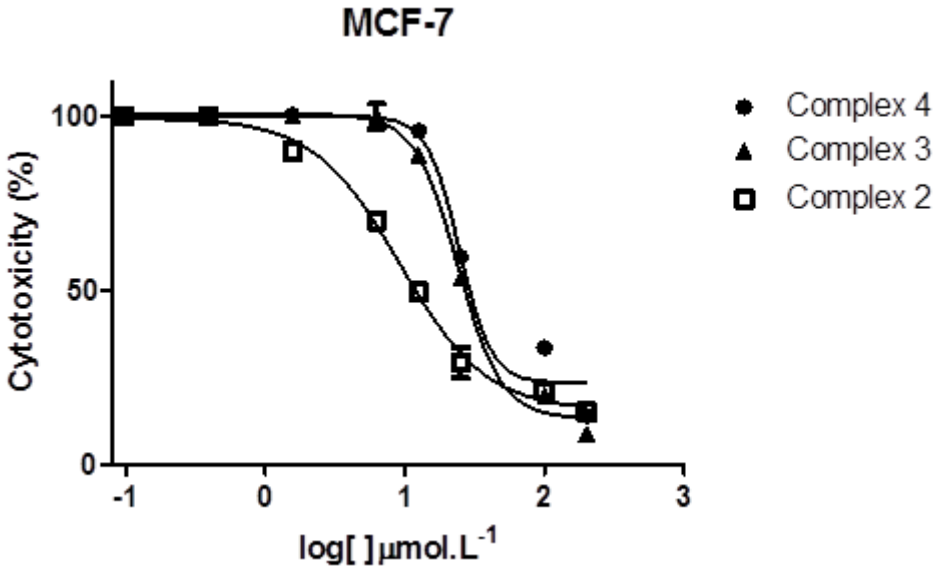
(c)



(d)

Figure S10. Stability of the complexes, for 72 h, in DMSO (0.1%)/DMEM solutions. ^{31}P NMR (162 MHz) (a) complex **1**: [Ru(mtz)$_2$(dppb)]; (b) complex **2**: [Ru(mmi)$_2$(dppb)]; (c) complex **3**: [Ru(dmp)$_2$(dppb)]; (d) complex **4**: [Ru(mpca)$_2$(dppb)].


MDA-MB-231
Cytotoxicity (%)
100
80
60
40
20
0
-1
0
1
2
3
log[ ]µmol.L-1
Complex 4
Complex 3
Complex 2


MCF-7
Cytotoxicity (%)
100
50
0
-1
0
1
2
3
log[ ]µmol.L-1
Complex 4
Complex 3
Complex 2

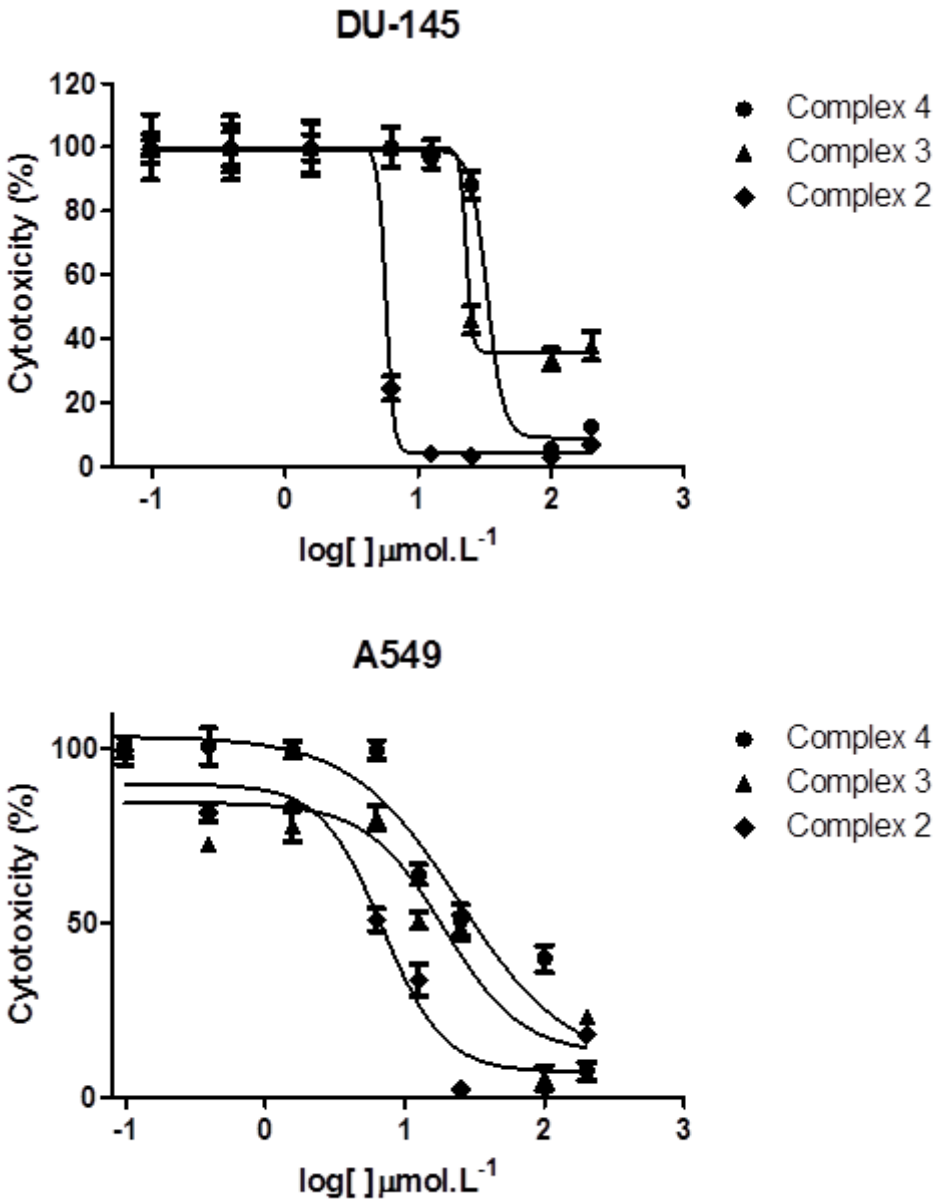


Figure S11. Graphics of half maximal inhibitory concentration (IC_{50}) for the complexes (**2**-**4**) with respect different cell lines. The complex **1** was not cytotoxic.

Table S1. Crystal and refinement data for (**1**), (**2**), (**3**) and (**4**)

Compound	**1**	**2**	**3**	**4**
Empirical formula	$C_{34}H_{36}N_2P_2RuS_4$	$C_{36}H_{38}N_4P_2RuS_2$	$C_{36}H_{38}N_8P_2RuS_2$	$C_{40}H_{36}N_2O_4P_2RuS_2$
Formula weight	763.90	753.83	809.87	835.84
Temperature / K	293(2)	293(2)	296(2)	296(2)
Wavelength / Å	0.71073	0.71073	0.71073	0.71073
Crystal system	monoclinic	triclinic	monoclinic	triclinic
Space group	$P2_1/n$	$P\bar{1}$	C2/c	$P\bar{1}$
Unit cell dimensions / Å	*a* = 15.0555(2)	*a* = 10.1920(7)	*a* = 20.053(2)	*a* = 9.9027(13)
	b = 11.7774(2)	*b* = 11.3782(10)	*b* = 32.567(4)	*b* = 15.142(2)
	c = 20.6408(3)	*c* = 16.6300(13)	*c* = 15.2562(15)	*c* = 15.427(2)
α / degree	90	98.703(4)	90	86.416(3)
β / degree	108.4750(10)	104.127(5)	120.872(6)	78.599(3)
γ / degree	90	103.997(4)	90	75.717(3)
Volume / $Å^3$	3471.29(9)	1768.7(2)	8551.8(18)	2197.1(5)
Z	4	2	8	2
Density (calculated) / (mg m^{-3})	1.462	1.415	1.258	1.263
Absorption coefficient / mm^{-1}	0.811	0.683	0.572	0.562
F(000)	1568	776	3328	856
Crystal size / mm^3	0.322 × 0.222 × 0.164	0.218 × 0.160 × 0.024	0.090 × 0.060 × 0.030	0.350 × 0.110 × 0.030
Theta range for data collection / degree	2.71 to 26.00	2.644 to 25.671	1.251 to 24.997	1.347 to 26.434
Index ranges	$-17 \le h \le 18$	$-11 \le h \le 12$	$-23 \le h \le 23$	$-12 \le h \le 12$
	$-14 \le k \le 14$	$-13 \le k \le 13$	$-38 \le k \le 38$	$-18 \le k \le 18$
	$-25 \le l \le 25$	$-20 \le l \le 19$	$-18 \le l \le 15$	$-19 \le l \le 19$
Reflections collected	47703	14516	51358	47497
Independent reflections	6819 [R(int) = 0.1258]	6198 [R(int) = 0.0449]	7527 [R(int) = 0.1548]	8981 [R(int) = 0.0784]
Completeness to theta = 25.242° / %	99.8	94.3	99.9	100.0
Absorption correction	Gaussian	Gaussian	Multi-scan	Multi-scan
Max. and min. transmission	0.8817 and 0.7836	0.9832 and 0.8919	0.7452 and 0.5266	0.7454 and 0.6556
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2

Table S1. Crystal and refinement data for (**1**), (**2**), (**3**) and (**4**) (cont.)

Compound	**1**	**2**	**3**	**4**
Data / restraints / parameters	6819 / 0 / 388	6198 / 0 / 408	7527 / 0 / 436	8981 / 0 / 462
Goodness-of-fit on F^2	1.032	1.102	0.999	1.038
Final R indices [I > 2sigma(I)]	R1 = 0.0448, wR2 = 0.1153	R1 = 0.0605, wR2 = 0.1458	R1 = 0.0589, wR2 = 0.1403	R1 = 0.0459, wR2 = 0.0935
R indices (all data)	R1 = 0.0586, wR2 = 0.1273	R1 = 0.0824, wR2 = 0.1611	R1 = 0.1155, wR2 = 0.1771	R1 = 0.0874, wR2 = 0.1086
Largest diff. peak and hole / (e $Å^{-3}$)	1.010 and –1.021	0.956 and –0.812	0.895 and –0.803	0.551 and –0.835

F(000): structure factor in the zeroth-order case; R(int): internal R-value; F^2: squared structure factor.

Table S2. Data of the electronic spectra of the complexes (CH_2Cl_2 solutions)

Complex	λ / nm	log ε	Transition
1	234	4.89	IL (π→π*)
	304	4.04	MLCT
2	236	4.88	IL (π→π*)
	276	4.36	IL (π→π*)
	338	3.66	MLCT
3	236	4.91	IL (π→π*)
	266	4.47	IL (π→π*)
	334	4.05	MLCT
4	232	4.64	IL (π→π*)
	298	4.32	IL (π→π*)
	356	3.90	MLCT
	422	3.90	MLCT

IL: intra ligand charge transfer; MLCT: metal ligand charge transfer.

Table S3. Assignments of the vibrational frequencies of the complexes

	Vibrational frequency / cm^{-1}			
	$[Ru(mtz)_2(dppb)]$	$[Ru(mmi)_2(dppb)]$	$[Ru(dmp)_2(dppb)]$	$[Ru(mpca)_2(dppb)]$
νNH_2			3437/3394	
νC–H	3137/3051	3114/3051	3127/3058	3152/3046
νCH_2	2912/2853	2909/2851	2917/2852	2919/2849
ν_{as}COOH				1683
νC=O				
νC=N	1588	1590	1619	1580
νC=C+ C=N	1531	1527	1547	1538
ν_sCOOH				1364
νC–S	1294	1282	1312	1264
νC–S	1183	1143	1159	1153
νP–C_{anel}	1088	1092	1091	1094
νP–C_{alif}	740	741	742	738
νP–C	512	515	516	510
νRu–S	440	438	460	442
νRu–N	423	421	430	420

mtz: 2-mercaptothiazoline; mmi: 2-mercapto-1-methyl-imidazole; dmp: 4,6-diamino-2-mercaptopyrimidine; mpca: 6-mercaptopyridine-3-carboxylic acid; dppb: 1,4-bis(diphenylphosphino)butane.