Electronic Supplementary Material (ESI) for RSC Advances.

Ruthenium carboranyl complexes with 2,2'-bipyridine derivatives for potential bimodal therapy application

Ricardo G. Teixeira[a], Fernanda Marques[b], M. Paula Robalo[c,d], Xavier Fontrodona[e], M. Helena Garcia[a], Simonetta Geninatti Crich[f], Clara Viñas[f,*], Andreia Valente[a,*]

[a]Centro de Química Estrutural and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

[b]Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, 2695-006 Bobadela LRS, Portugal.

[c]Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro, 1, 1959-007 Lisboa, Portugal.

[d]Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.

[e]Departament de Química and Serveis Tècnics de Recerca, Universitat de Girona, Campus de Montilivi, 17071 Girona, Spain

[f]Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università di Torino, via Nizza 52, 10126 Torino, Italy.

[g]Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus U.A.B., 08193 Bellaterra, Spain.

INDEX

Page

1. Characterization of RuCB1

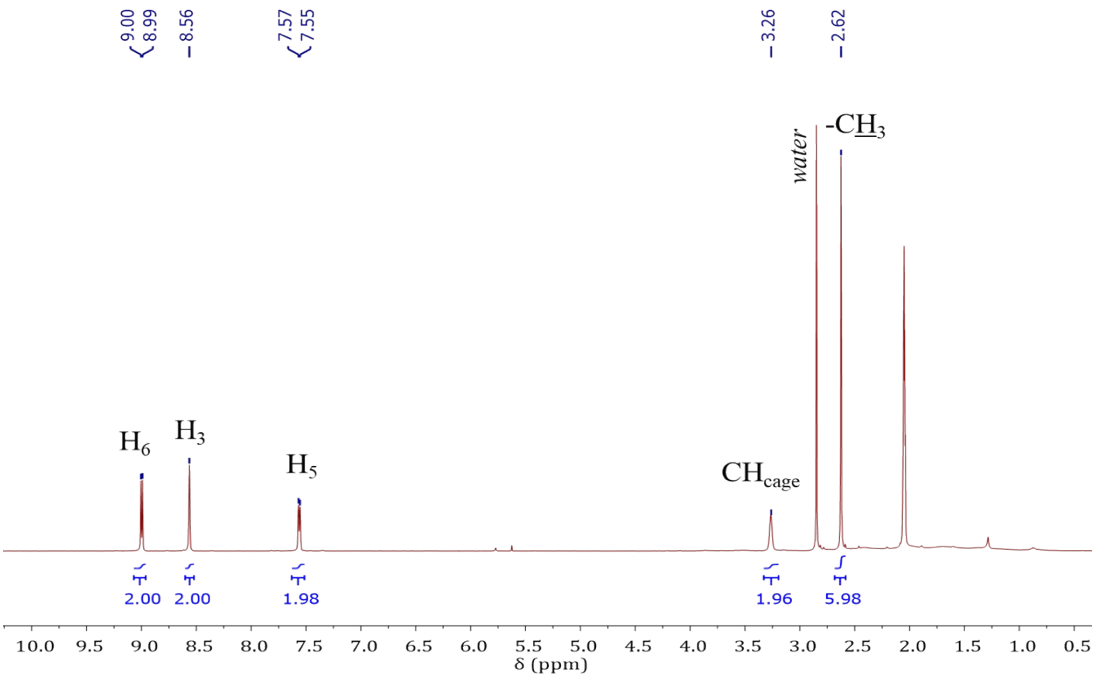

Figure S1. ^{1}H NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-*closo*-3,1,2-$RuC_2B_9H_{11}$] (**RuCB1**) in acetone-d_6 at 298 K (400 MHz) using TMS as reference.

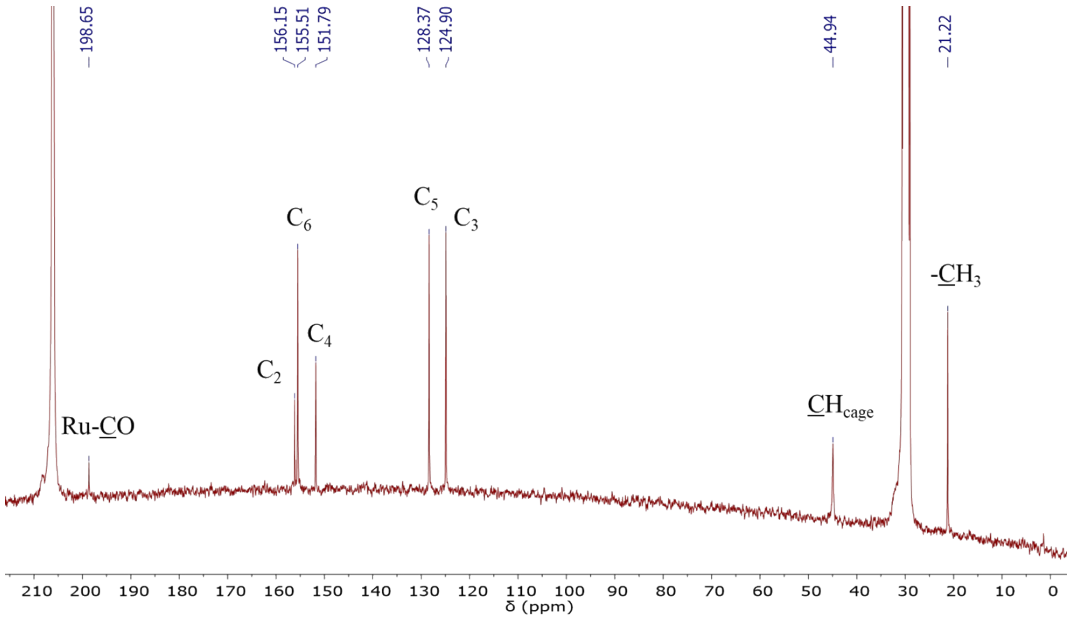

Figure S2. ^{13}C NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-*closo*-3,1,2-$RuC_2B_9H_{11}$] (**RuCB1**) in acetone-d_6 at 298 K (400 MHz) using TMS as reference.

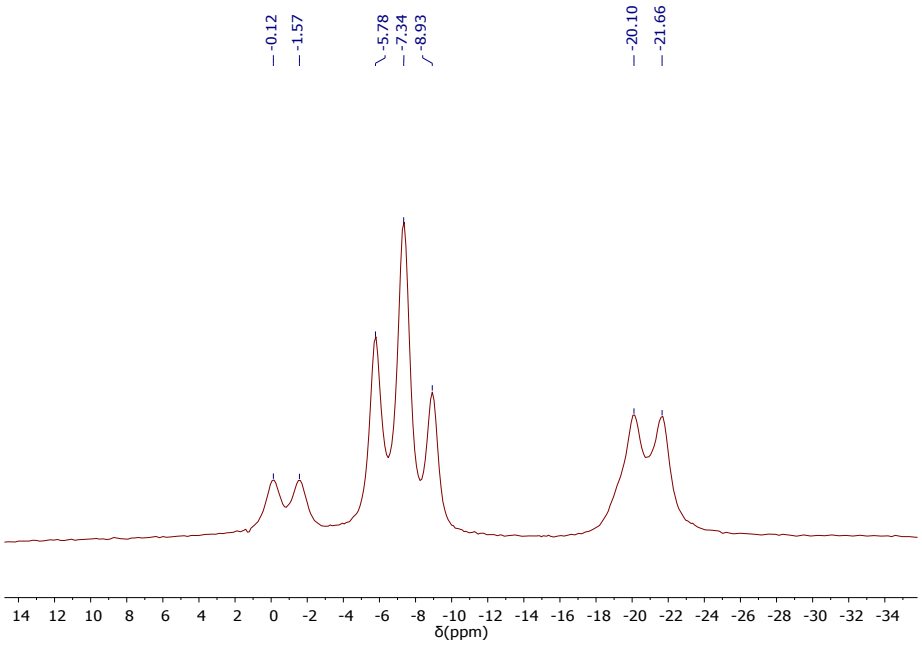

Figure S3. ^{11}B NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-*closo*-3,1,2-$RuC_2B_9H_{11}$] (**RuCB1**) in acetone-d_6 at 298 K (300 MHz) using $BF_3.OEt_2$ as reference.

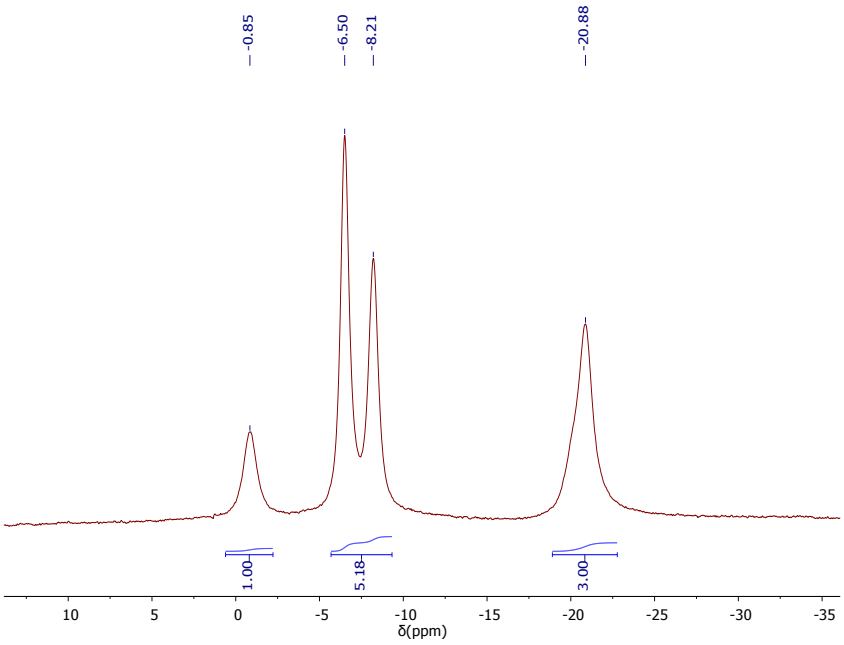

Figure S4. $^{11}B\{^1H\}$ NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-closo-3,1,2-$RuC_2B_9H_{11}$] (**RuCB1**) in acetone-d_6 at 298 K (300 MHz) using $BF_3.OEt_2$ as reference.

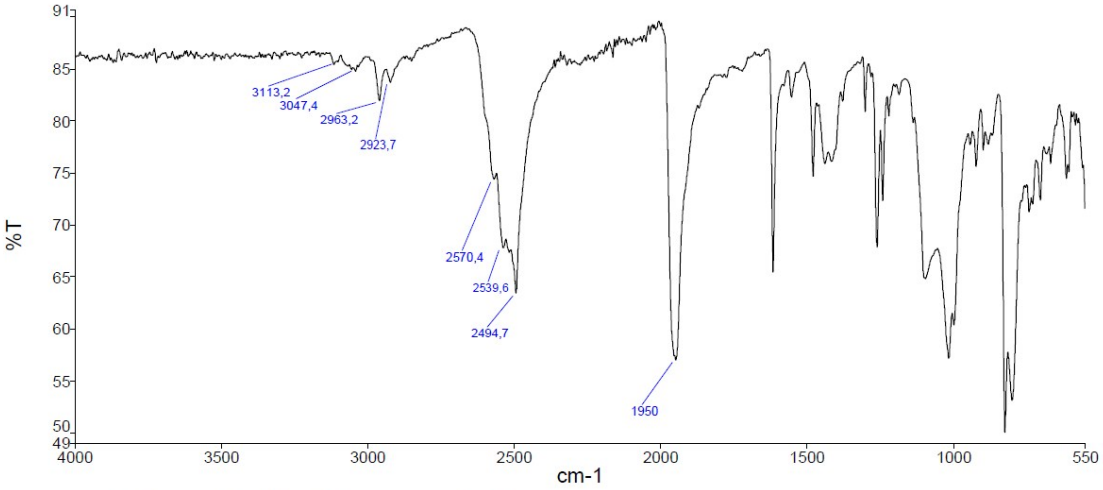

Figure S5. FTIR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-closo-3,1,2-$RuC_2B_9H_{11}$] (**RuCB1**); KBr pellets.

2. Characterization of RuCB2

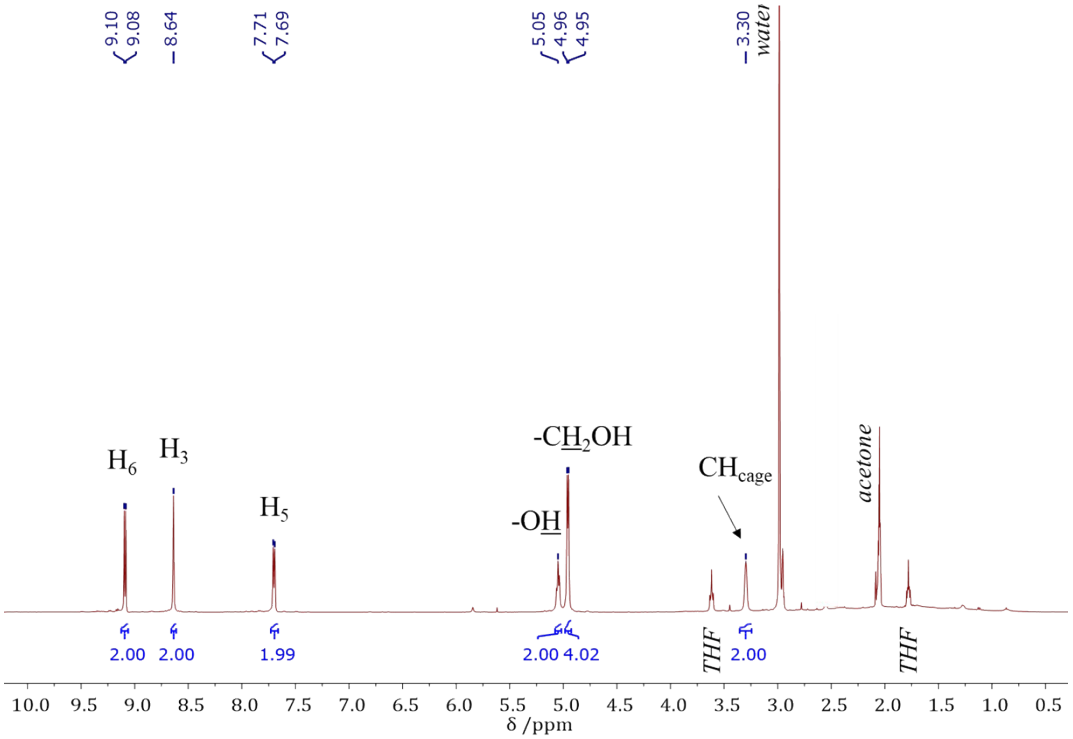

Figure S6. ^{1}H NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-closo-3,1,2-$RuC_2B_9H_{11}$] (**RuCB2**) in acetone-d_6 at 298 K (400 MHz) using TMS as reference.

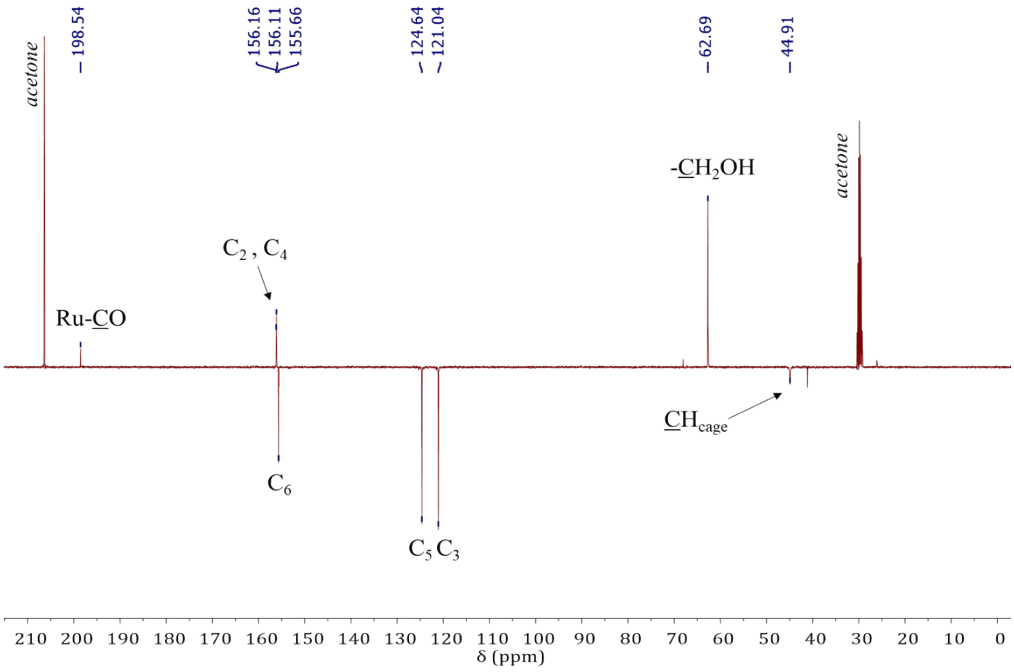

Figure S7. ^{13}C NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-closo-3,1,2-$RuC_2B_9H_{11}$] (**RuCB2**) in acetone-d_6 at 298 K (400 MHz) using TMS as reference.

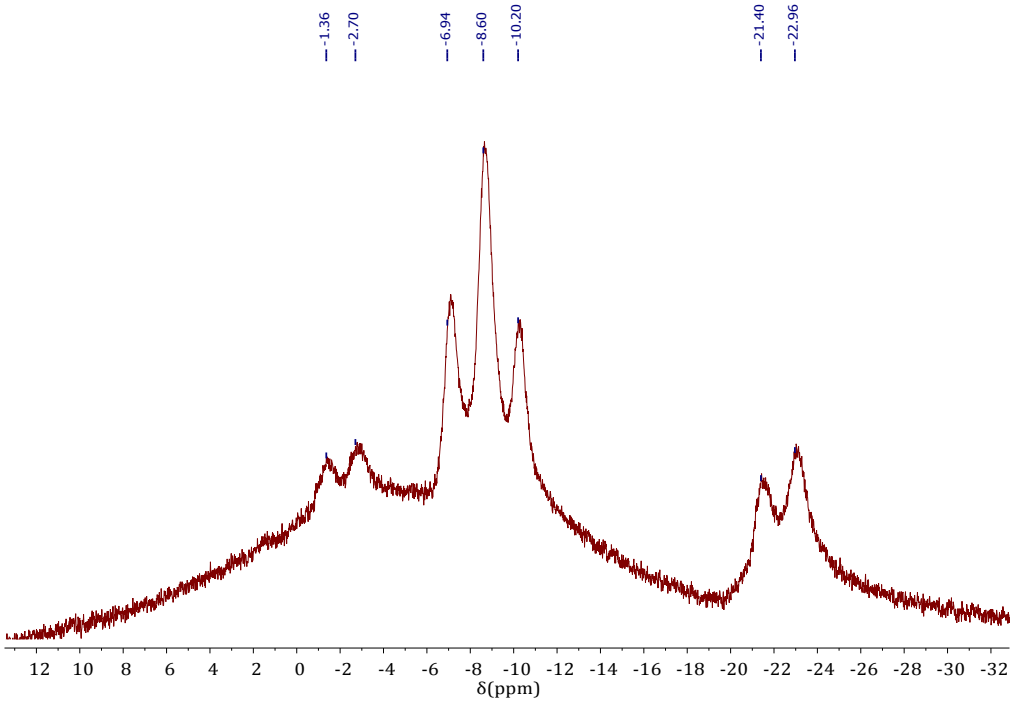

Figure S8. ^{11}B NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-closo-3,1,2-$RuC_2B_9H_{11}$] (**RuCB2**) in acetone-d_6 at 298 K (300 MHz) using $BF_3.OEt_2$ as reference.

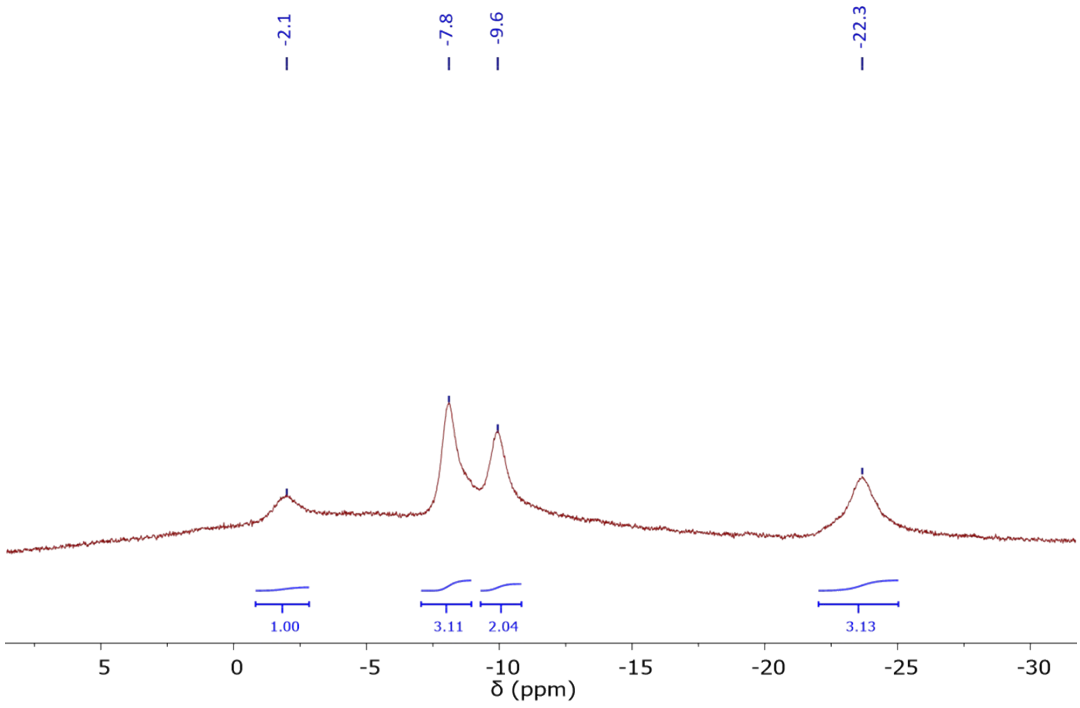

Figure S9. $^{11}B\{^1H\}$ NMR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_3)_2$-2,2'-bipy}-closo-3,1,2-$RuC_2B_9H_{11}$] (**RuCB2**) in acetone-d_6 at 298 K (300 MHz) using $BF_3.OEt_2$ as reference.

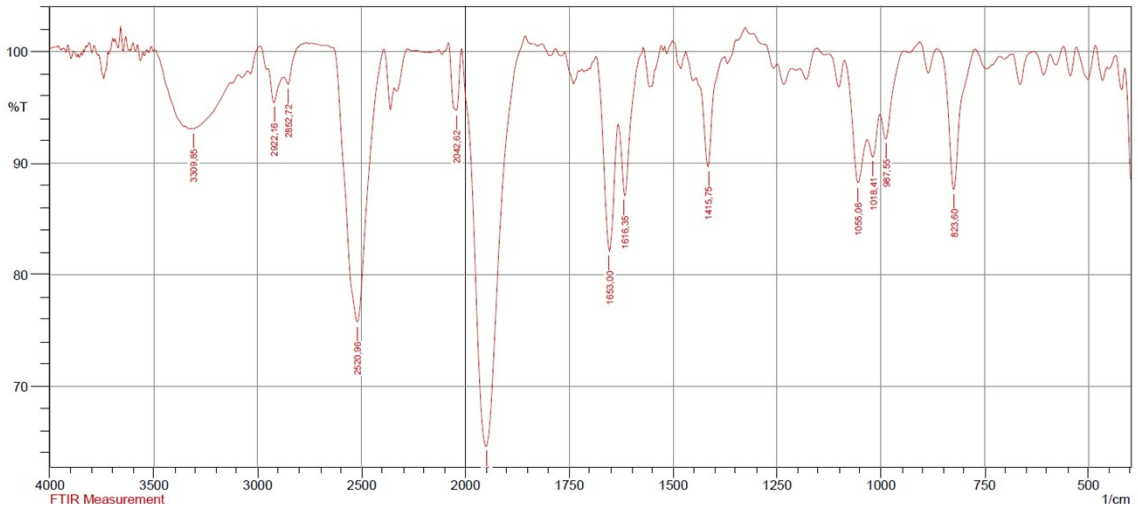

Figure S10. FTIR spectrum of [3-CO-3,3-{k^2-4,4'-$(CH_2OH)_2$-2,2'-bipy}-closo-3,1,2-$RuC_2B_9H_{11}$] (**RuCB2**); KBr pellets.

Table S1. ^{1}H NMR data (ppm) in acetone-d_6 for compounds **RuCB1** and **RuCB2**, respective bipyridyl ligands and ruthenacarborane precursor.

Compound	Carboranyl	Bipyridine			
	cage	H_3	H_5	H_6	R
4,4'-dimethyl-2,2'-bipyridyl	-	8.30	7.22	8.51	2.43
4,4'-dihydroxymethyl-2,2'-bipyridyl	-	8.50	7.39	8.60	4.79
[3,3,3-(CO)$_3$-*closo*-3,1,2-RuC$_2$B$_9$H$_{11}$]	4.15	-	-	-	-
RuCB1	3.26	8.56	7.56	9.00	2.62
RuCB2	3.30	8.64	7.70	9.09	4.96

Table S2. $^{11}B\{^{1}H\}$ NMR data in acetone-d$_6$ for compounds **RuCB1** and **RuCB2**. In parenthesis, the correspondent integral area of each $^{11}B\{^{1}H\}$ NMR peak.

Compound	$^{11}B\{^{1}H\}$ NMR	<δ>
[3,3,3-(CO)$_3$-*closo*-3,1,2-RuC$_2$B$_9$H$_{11}$]	8.6 (1B), -4.2 (3B), -7.6 (2B), -16.7 (3B)	-7.7
RuCB1	-0.9 (1B), -6.5 (3B), -8.21 (2B), -20.9 (3B)	-11.1
RuCB2	-2.1 (1B), -7.8 (3B), -9.6 (2B), -22.3 (3B)	-12.4

Table S3. Optical spectral data for complexes **RuCB1** and **RuCB2** in different solvents. Measurements were performed at room temperature using 10^{-4}-10^{-5} M solutions. (Sh = Shoulder).

Compound	λ_{max}/nm (ε x 10^3 / $M^{-1}cm^{-1}$)	
	Dichloromethane	**Dimethylsulfoxide**
RuCB1	246 (11.28), 287 (10.25), 311 (Sh), 362 (2.74), 451 (0.60)	283 (18.71), 311 (13.01), 350 (5.80), 438 (1.35)
RuCB2	245 (18.92), 289 (16.84), 314 (Sh), 368 (4.33), 453 (1.07)	284 (16.06), 313 (10.45), 347 (4.32), 434 (1.08)

Table S4. Electrochemical data for complexes **[3,3,3-(CO)$_3$-closo-3,1,2-RuC$_2$B$_9$H$_{11}$], RuCB1** and **RuCB2** in acetonitrile and dichloromethane (all values *vs.* SCE, v = 100 mVs^{-1}).

Compound	E_{pa} (V)	E_{pc} (V)	$E_{1/2}$ (V)	$E_{pa} - E_{pc}$ (mV)	I_c/I_a
Acetonitrile					
[3,3,3-(CO)$_3$-closo-3,1,2-RuC$_2$B$_9$H$_{11}$]	--	-1.36	--	--	--
RuCB1	1.12	--	--	--	--
	--	-1.45	--	--	--
	-1.50	-1.58	-1.54	80	0.9[a]
RuCB2	1.16	--	--	--	--
	--	-1.50	--	--	--
	--	-1.59	--	--	--
Dichloromethane					
RuCB1	1.12	1.00	1.06	120	0.5
	--	-1.60	--	--	--
	--	-1.69	--	--	--
RuCB2	1.17	--	--	--	--
	--	-1.60	--	--	--
	--	--	--	--	--

[a] I_a/I_c

Table S5. Crystallographic Data and Structural Refinement Details for **RuCB1** and **RuCB2**.

	RuCB1	**RuCB2**
Empirical formula	C15 H23 B9 N2 O Ru	C18 H26 B9 N2 O3 Ru
Formula weight	445.71	516.77
Temperature (K)	193(2)	193(2)
Crystal system	Monoclinic	Monoclinic
space group	P 2_1/n	P 2_1/c
a (Å)	12.589(2)	6.8160(14)
b (Å)	11.463(2)	25.702(5)
c (Å)	14.575(2)	12.914(3)
β (deg)	111.301(2)	92.384(4)
Volume (Å^3)	1959.7(6)	2260.4(8)
Z	4	4
Calculated density (g cm^{-3})	1.511	1.518
Absorption coeficient (mm^{-1})	0.808	0.718
Goodness-of-fit	1.031	1.064
R_1 [I>2σ(I)]	0.0308	0.0391
wR_2 [I>2σ(I)]	0.0745	0.1149

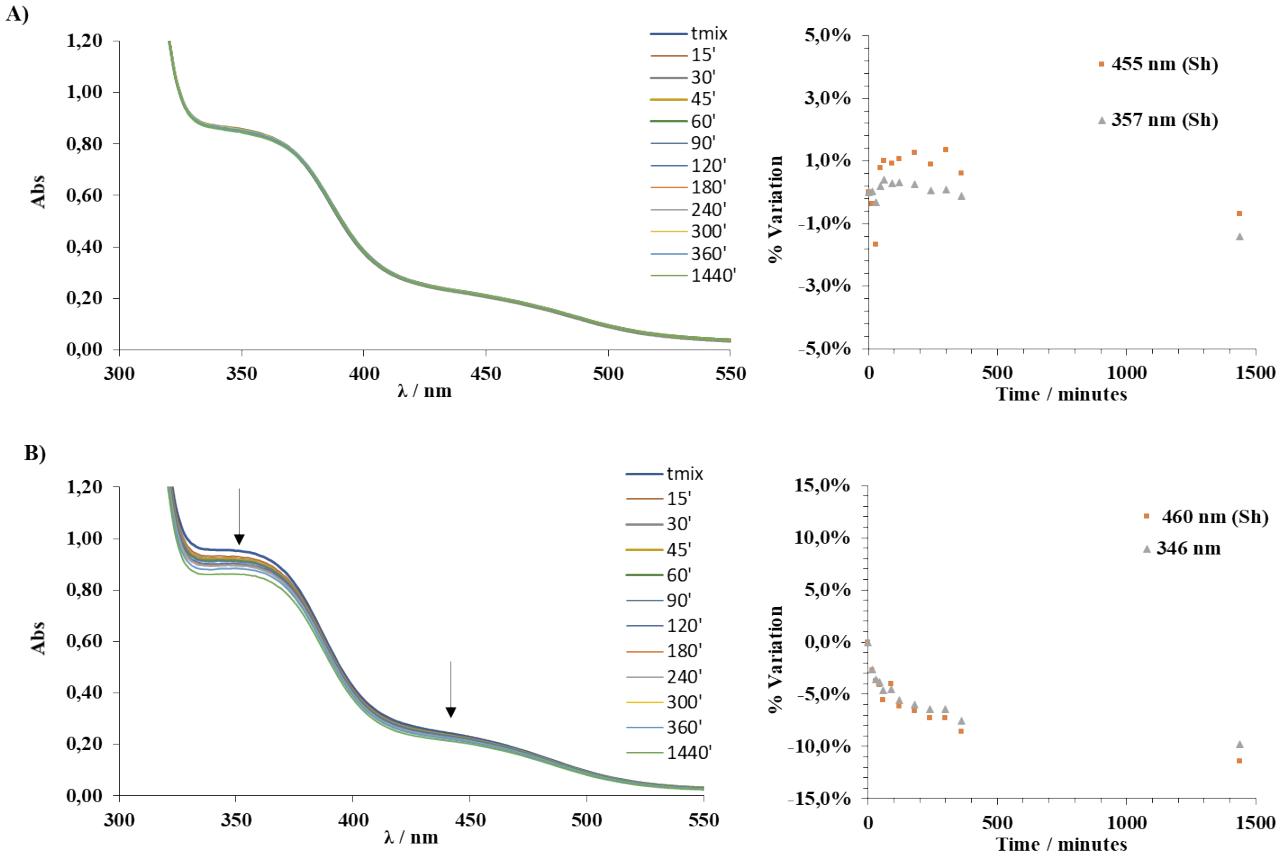

Figure S11. Stability studies in cellular media, 3% DMSO / 97 % DMEM for compounds **RuCB1 (A)** and **RuCB2 (B)**. On the right are represented the UV-Vis spectra along the 24 h of the study and on the left the percentage of variation for fixed wavelengths along time.

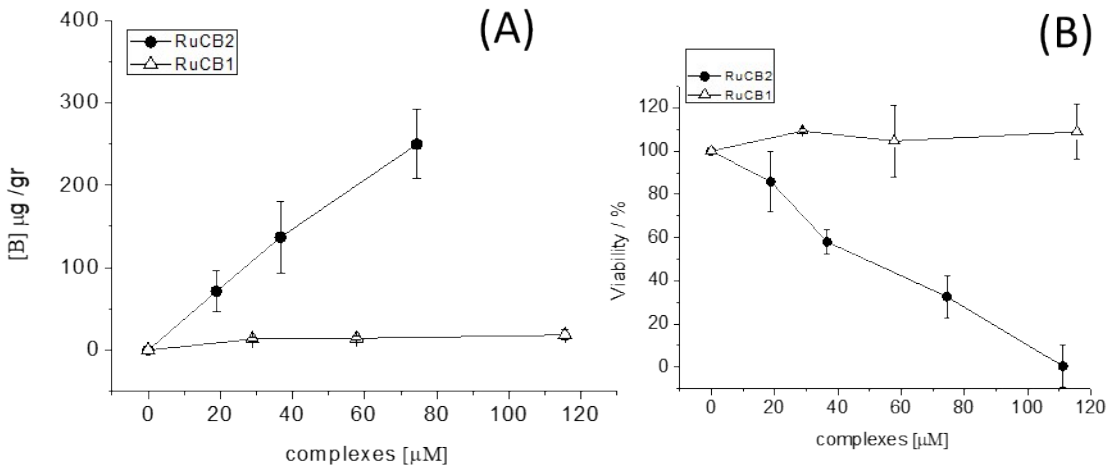

Figure S12. (A) *In vitro* uptake experiments on A375 cells that were incubated for 24 h at 37 °C in the presence of increasing amounts of **RuCB1** and **RuCB2**. B content in the cell samples was determined by ICP-MS, and values were normalized to the protein content of each cell sample. **(B)** Cells % viability evaluated by measuring the protein content for each treated cell samples with respect to a not treated control sample. Errors bars report the standard deviation (SD) of the data.