

Two mixed valence diruthenium (II,III) isomeric complexes show different anticancer properties

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Table S1. Boltzmann populations for the most representative conformations of $[\text{Ru}_2(\text{EB106})_4\text{Cl}]$ and $[\text{Ru}_2(\text{EB776})_4\text{Cl}]$. Distances from closest points on hydrophilic surface to carboxylate oxygens.

Complexes	Conformers	Population, %	Distances from closest points of hydrophilic regions to carboxylate oxygens	
			Within 2.5 Å	Within 3.0 Å
$[\text{Ru}_2(\text{EB106})_4\text{Cl}]$	1	86	-	2.73
	2	6	-	2.75
	3	6	-	2.82, 2.99
$[\text{Ru}_2(\text{EB776})_4\text{Cl}]$	1	24	2.03, 2.14, 2.41	2.58
	2	16	2.19	2.68, 2.76
	3	16	2.48	2.53
	4	11	2.47	2.69
	5	9	2.44	2.61, 2.83
	6	7	2.27	2.66, 2.80
	7	5	-	2.63, 2.91
	8	5	2.39	2.8
	9	3	2.14, 2.35	2.52, 2.96

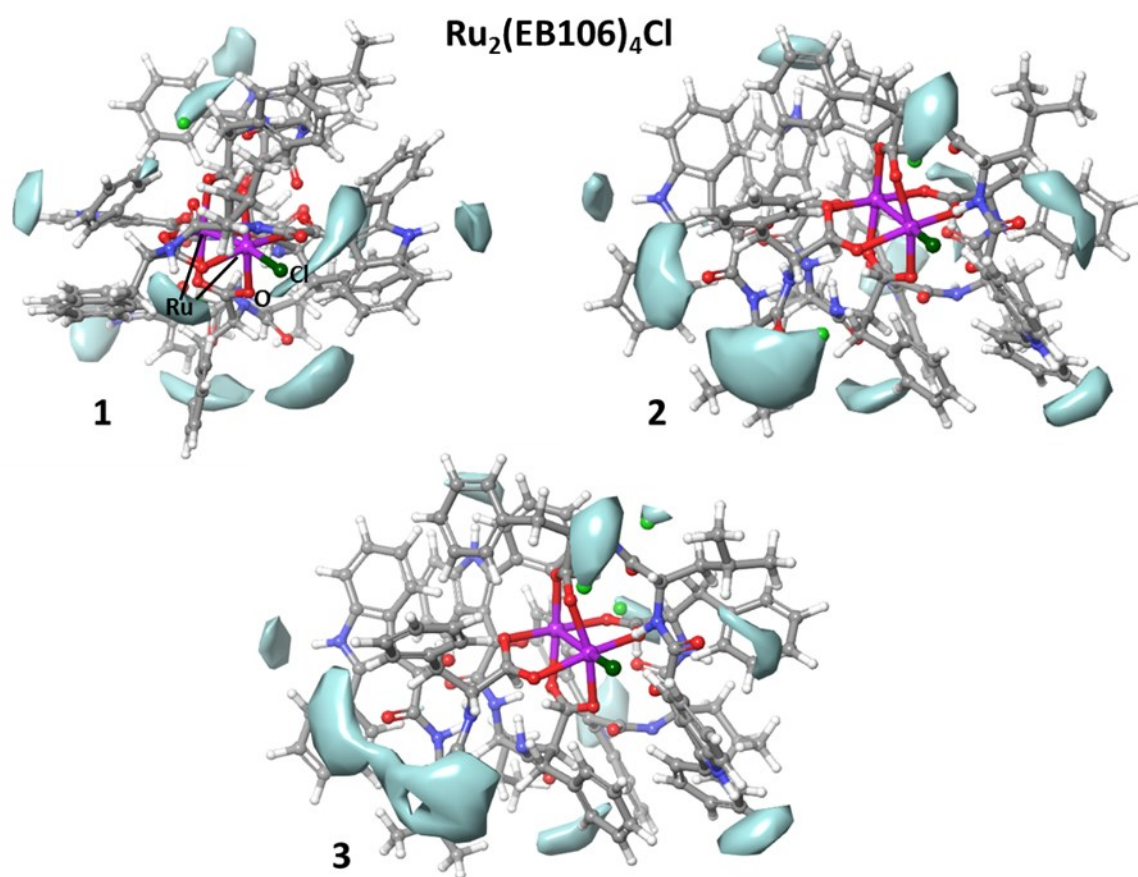


Fig. S1. Analyzed conformers of complex $[\text{Ru}_2(\text{EB106})_4\text{Cl}]$ and their hydrophilic fragments.

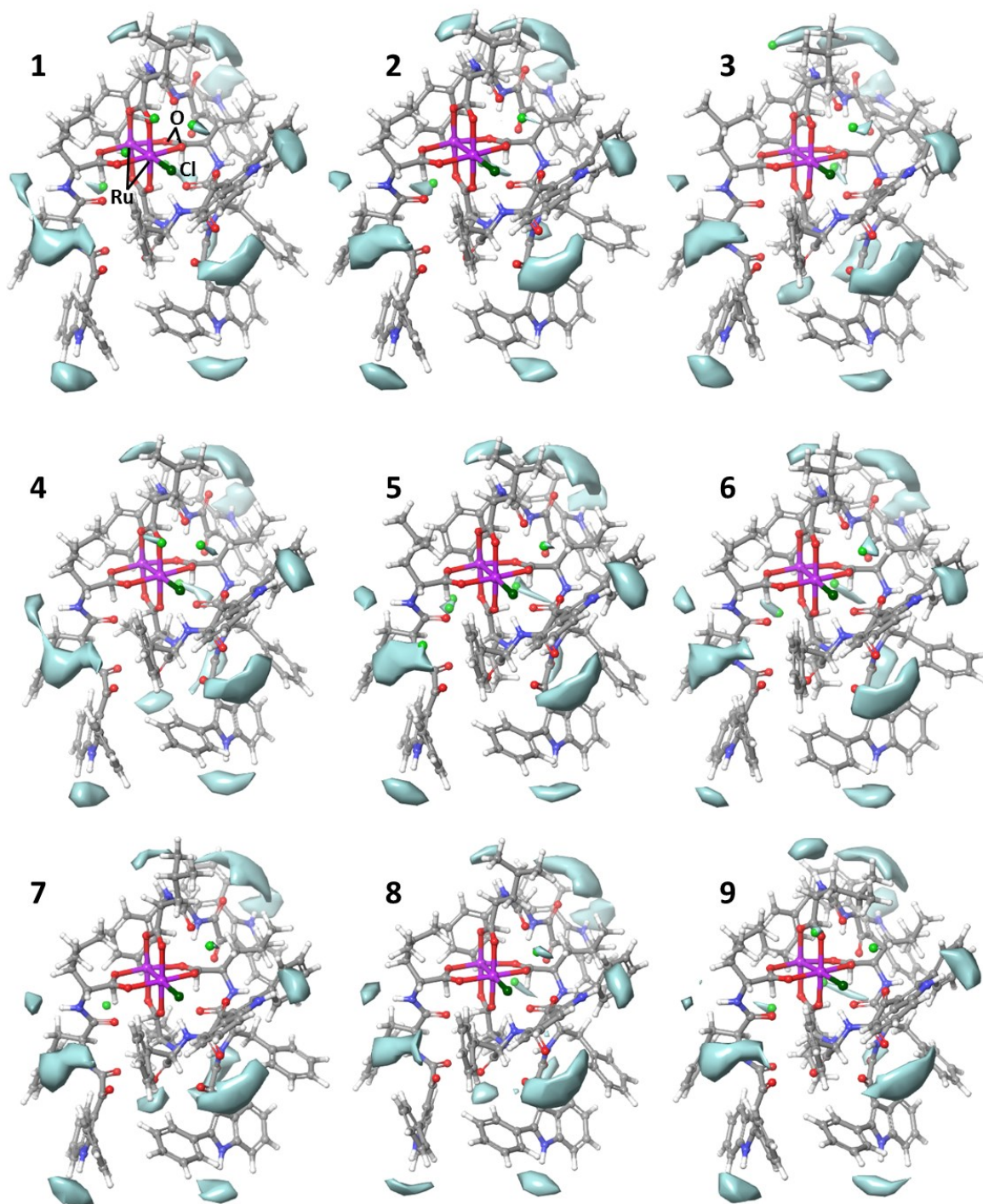


Fig. S2. Analyzed conformers of complex $[\text{Ru}_2(\text{EB776})_4\text{Cl}]$ and their hydrophilic fragments.

Solution behavior of $[\text{Ru}_2(\text{EB776})_4\text{Cl}]$

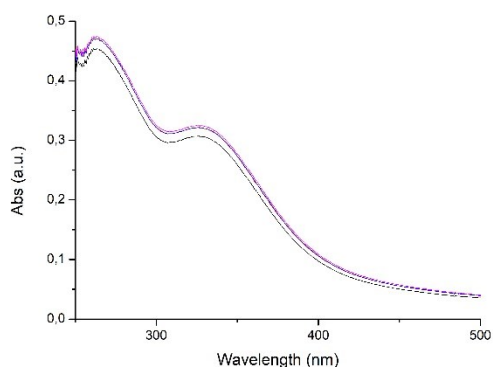


Fig. S3. Time dependent UV-Vis spectral profiles of $[\text{Ru}_2(\text{EB776})_4\text{Cl}]$, 10^{-5} M in 10 mM phosphate buffer in presence of 40 % DMSO (pH = 7.4) followed for 72 h.

ESI-MS experiments on $[\text{Ru}_2(\text{EB106})_4\text{Cl}]$ and $[\text{Ru}_2(\text{EB776})_4\text{Cl}]$ in presence of lysozyme and HSA

ESI-MS interactions experiments were carried out at different protein to complex ratio (10:1 and 5:1) after 72 h incubation. Figures S4-S7 show the incubation with Human Serum Albumin (HSA) while figures S8-S11 incubation with lysozyme. 66 KDa=HSA; 14.3 KDa Lysozyme. No adducts formation was detected. Experiments were carried out following the protocol reported in ref. 20 of main manuscript (*Barresi et al., Dalton Trans., 49, 2020, 14520-14527*), i.e. using a TripleTOF[®] 5600+ mass spectrometer (Sciex, Framingham, MA, U.S.A.), equipped with a DuoSpray[®] interface operating with an ESI probe in positive polarity.

HSA_ $[\text{Ru}_2(\text{EB106})_4\text{Cl}]$ _10:1_5x10⁻⁷M_AmAc2mM_72h_

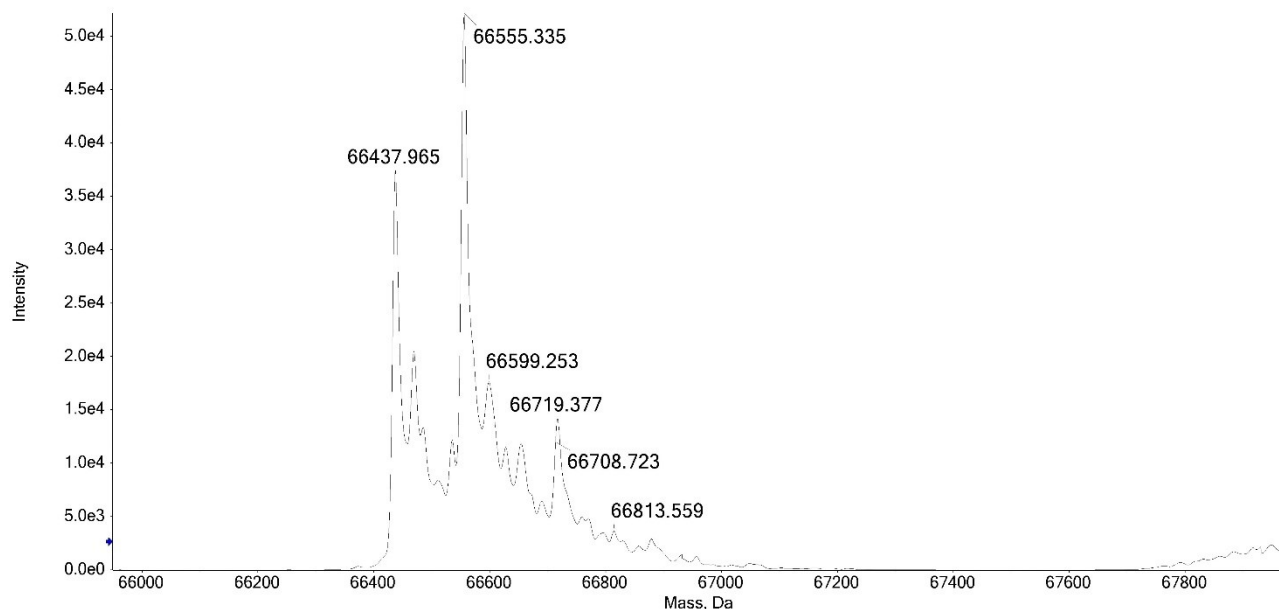


Fig. S4.

HSA_[Ru₂(EB106)₄Cl]_5:1_5x10⁻⁷M_AmAc2mM_72h_

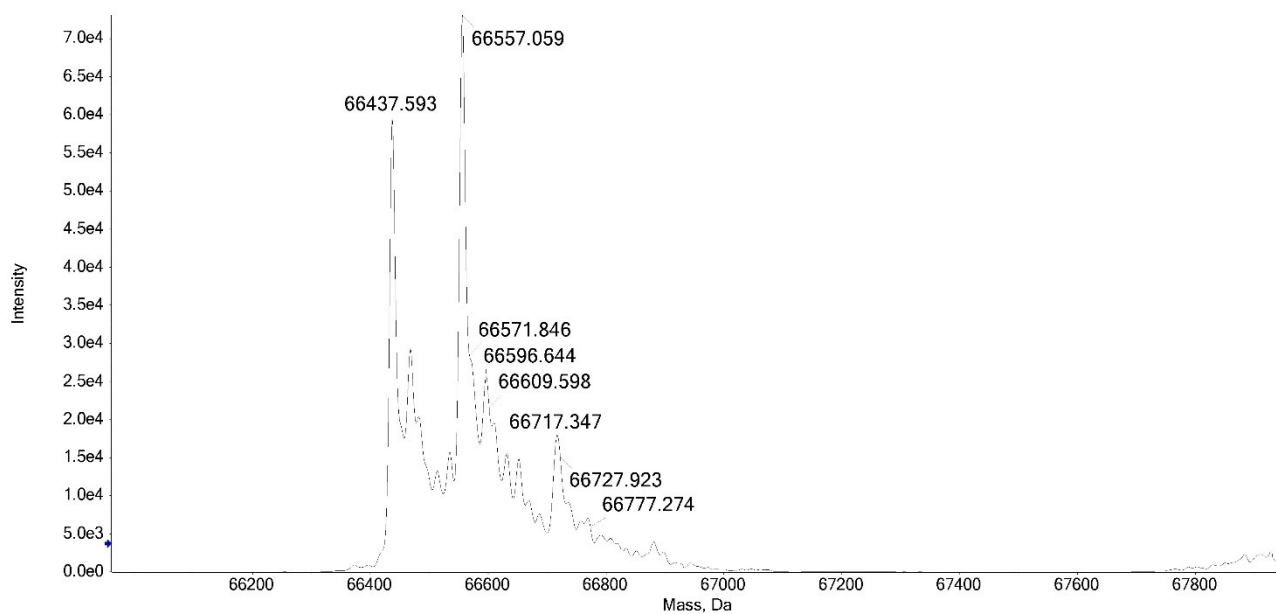


Fig. S5.

HSA_[Ru₂(EB776)₄Cl]_10:1_5x10⁻⁷M_AmAc2mM_72h_

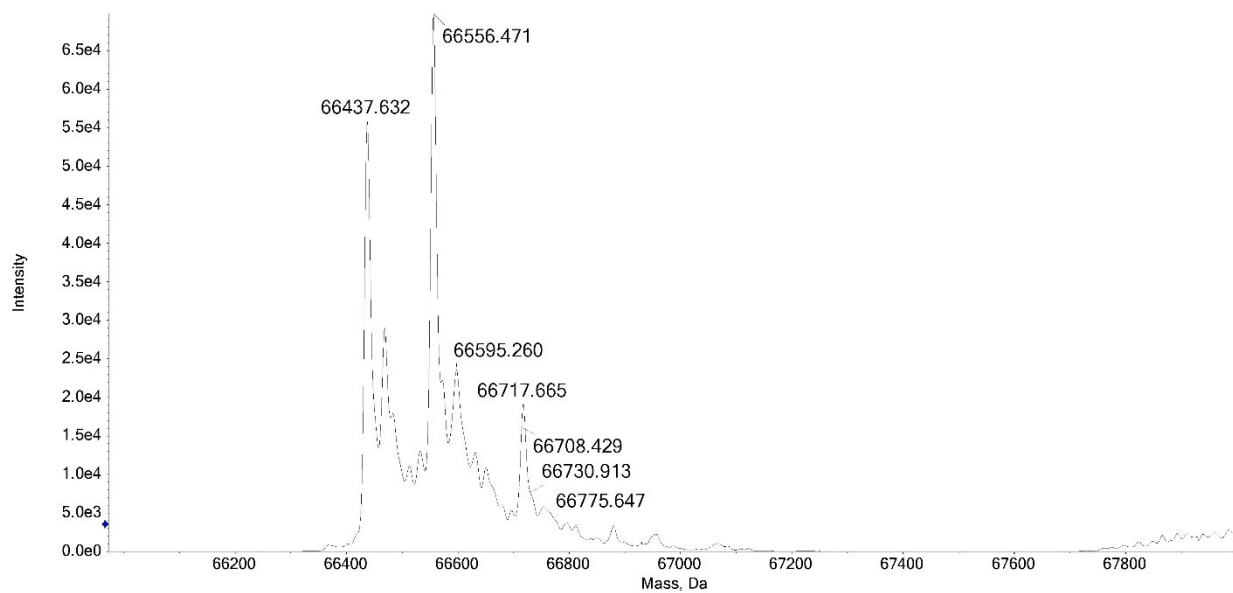


Fig. S6.

HSA_[Ru₂(EB776)₄Cl]₅:1_5x10⁻⁷M_AmAc2mM_72h_

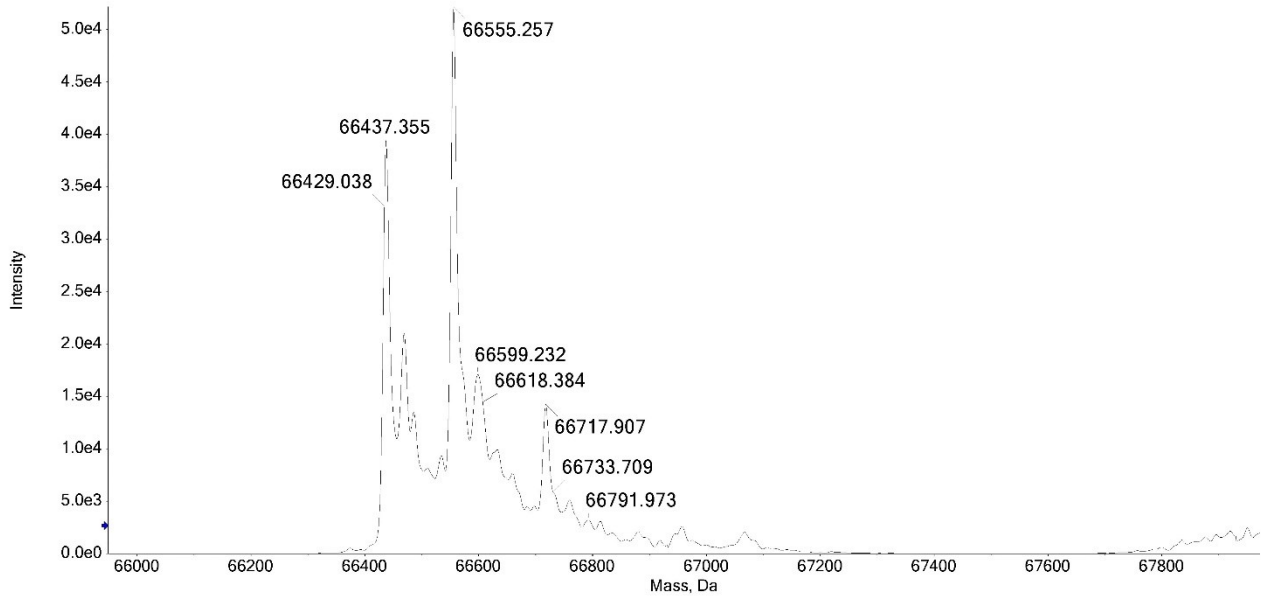


Fig. S7.

Lys_[Ru₂(EB106)₄Cl]₁₀:1_10⁻⁷M_AmAc2mM_72h_

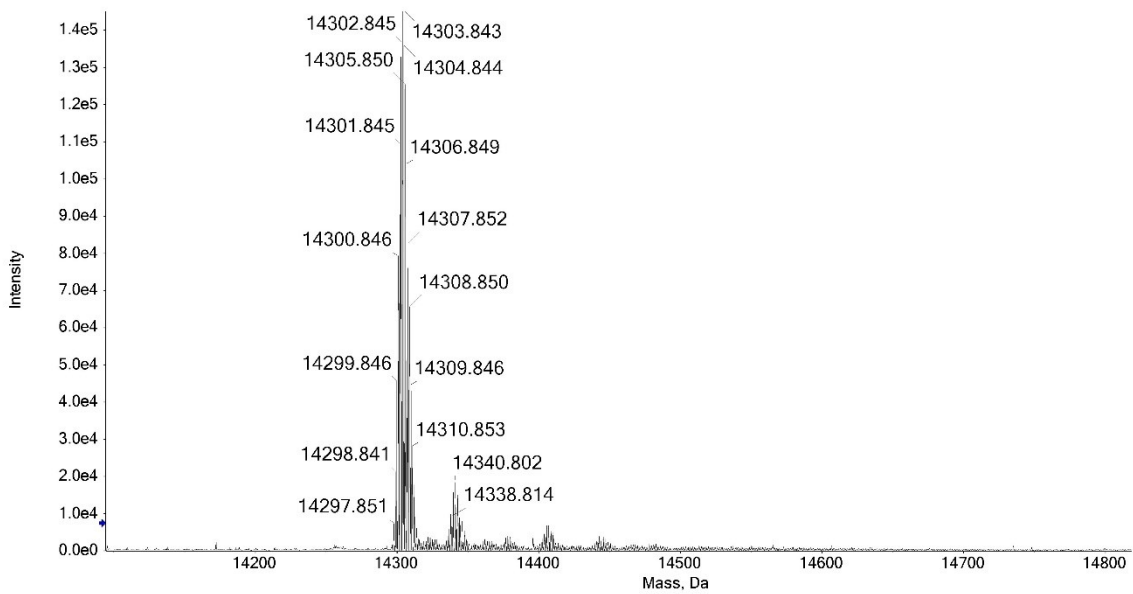


Fig. S8.

Lys_ [Ru₂(EB106)₄Cl]_5:1_10-7M_AmAc2mM_72h_

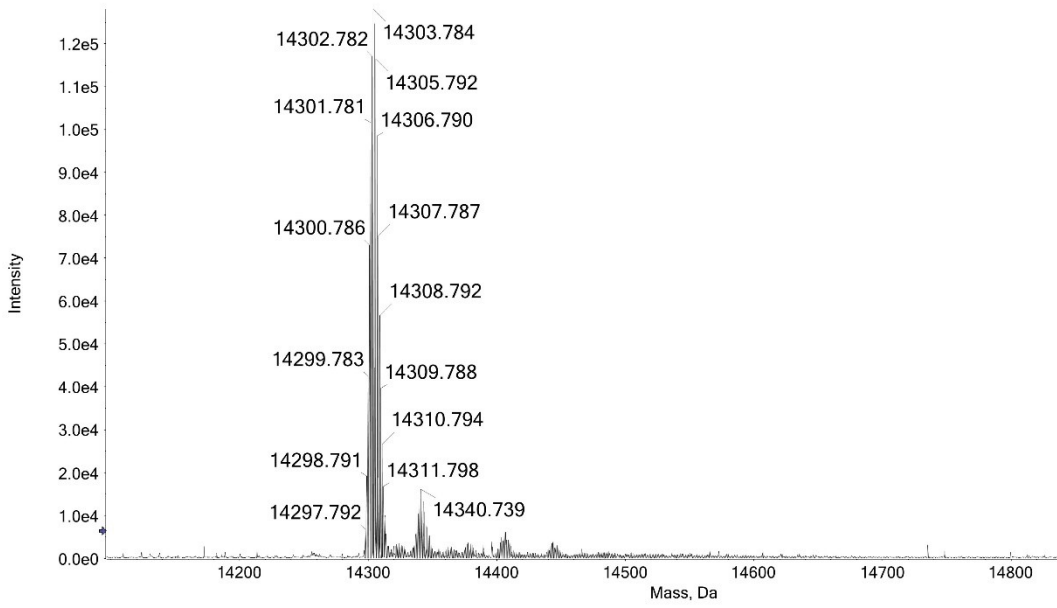


Fig. S9.

Lys_ [Ru₂(EB776)₄Cl]_10:1_10-7M_AmAc2mM_72h_

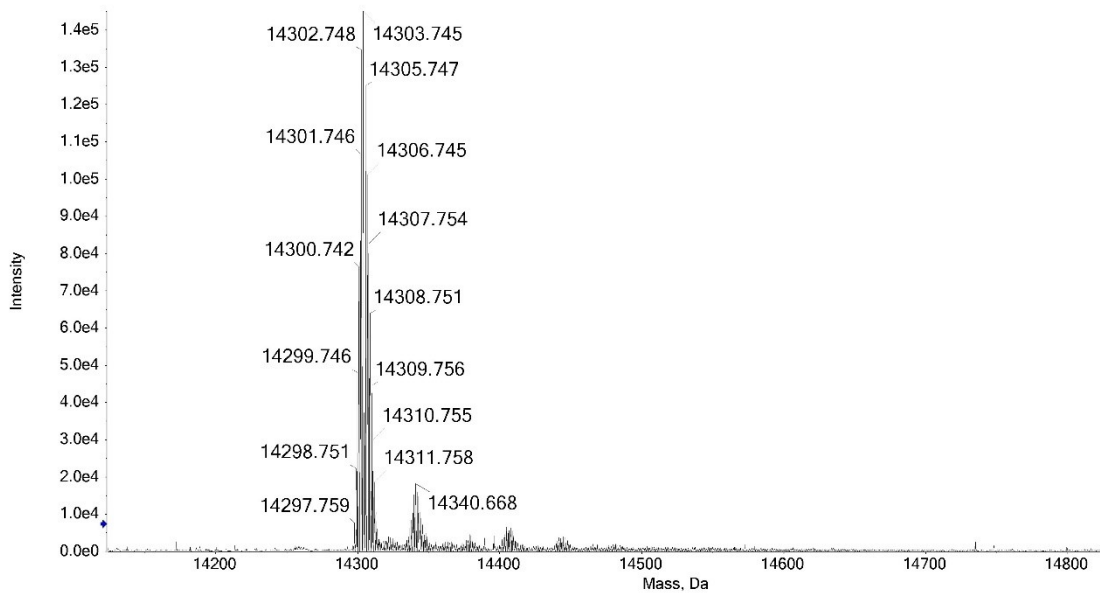


Fig. S10.

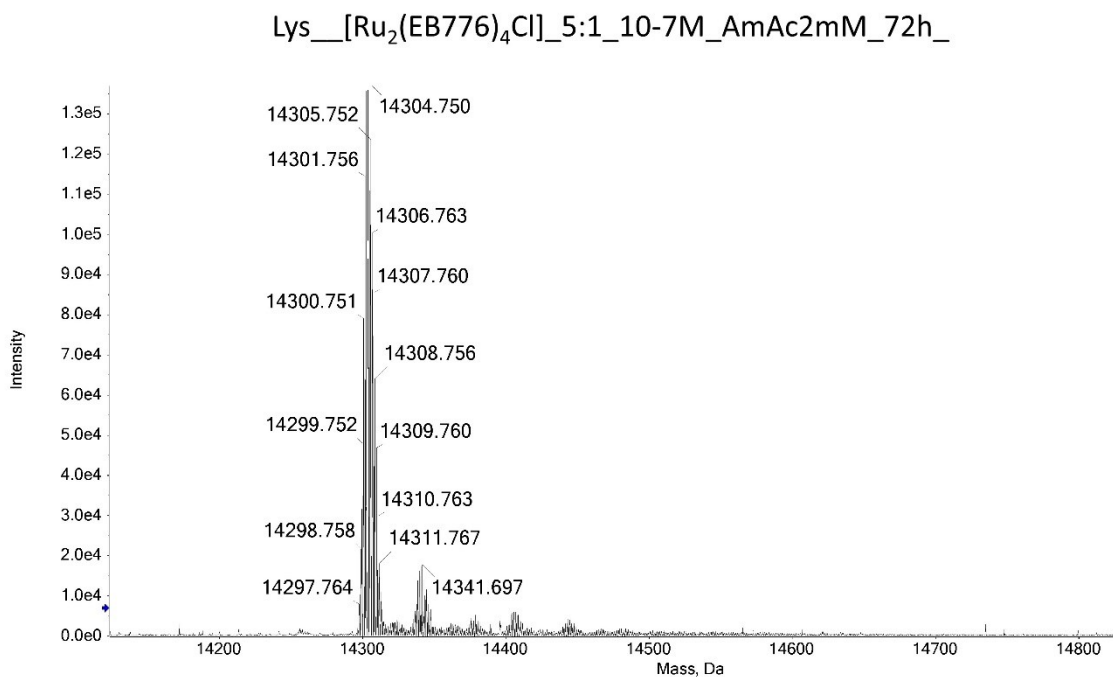


Fig. S11.

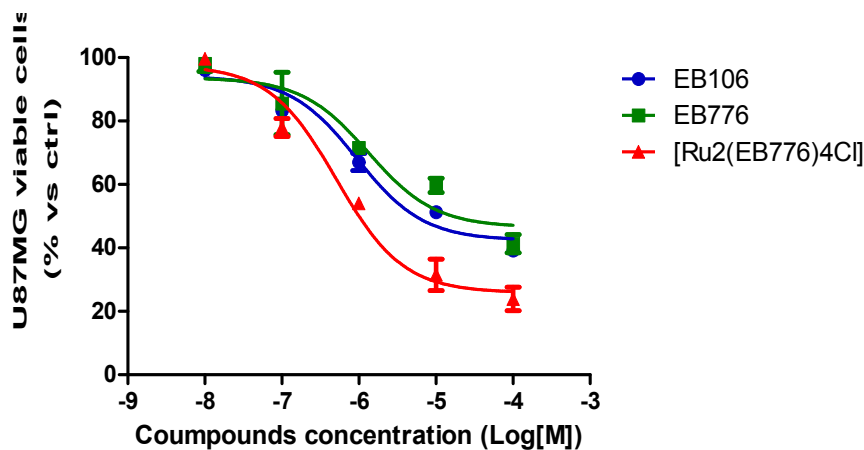


Fig. S12. Evaluation of in vitro anti-proliferative effect: U87MG cells were treated with increasing concentrations of the EB106, EB776 and [Ru₂(EB776)₄Cl] compounds; after 72 h of treatment cellular viability was measured by MTS assay. Data were expressed as percentage of compound-treated viable cells respect to control viable cells. Curves were generated using a sigmoidal dose-response curve model (GraphPad Prism 5 software) from which the IC₅₀ values were derived. Data represent the mean ± SEM of three different experiments. Each experiment was performed in triplicate.