## **Supplementary Material**

## Heteroleptic mononuclear compounds of ruthenium(II): synthesis, structural analyses, in vitro antitumor activity and in vivo toxicity on zebrafish embryos

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Figure S1 Molecular structure for the cation of complex **4a**. Ellipsoids drawn at the 50%. Hydrogen atoms have been omitted for clarity reasons.



Figure S2. Molecular structure for the cation of complex **5a**. Ellipsoids drawn at the 50%. Hydrogen atoms have been omitted for clarity reasons.



Figure S3. Effect of compounds **1a-5a** exposure on A549, Fibroblasts, HCT116 and K562 cell viability. Cells were exposed to:  $1 - 100 \mu$ M of each compound or DMSO (vehicle control) for 72 h. The results are expressed as mean ± SEM fold-change compared to controls from at least three independent experiments.

Table S1. Relative  $IC_{50}$  (concentration that inhibits the proliferation of 50 % of the cell population) values determined after 72 h of incubation of A529, K562, HCT116 cancer cells and normal primary fibroblasts with compounds 1a-5a. The results showed are expressed as mean ± SEM of three independent assays.

IC <sub>50</sub> (μM)	1a	<b>2</b> a	3a	4a	5a
A529	>100	>100	>100	>100	>100
Fibroblasts	>100	>100	>100	>100	>100
K562	>100	>100	>100	>100	>100
HCT116	>100	67.97 ± 0.23	44.49 ± 0.11	70.12 ± 0.18	76.04 ± 0.25



Figure S4. Effect of increasing concentrations of compound A1) **2a**, B1) **3a**, C1) **4a** and D1) **5a** in the *in vitro* pUC19 DNA cleavage. pDNA hydrolysis with *HindIII* was used as a control for the Linear form. Ctrl – pUC19 DNA control; Agarose gel electrophoresis (0.8 % (w/v)) showing the cleavage of pUC19 DNA (100 ng) incubated with increasing concentrations of compound A2) **2a**, B2) **3a**, C2) **4a** and D2) **5a**. M- DNA Ladder; HindIII – pUC19 hydrolysis with HindIII (linear form).



Figure S5. Survival of the introduced Danio rerio as observed under presence of compound 1a.



Figure S6. Survival of the introduced Danio rerio as observed under presence of compound **3a**.



Figure S7. Survival of the introduced Danio rerio as observed under presence of compound 4a.



Figure S8. Survival of the introduced Danio rerio as observed under presence of compound 5a.



Figure S9. Concentration-effect curve showing the influence of compound **1a** on survival of the introduced zebrafish as observed after 72 h.



Figure S10. Concentration-effect curve showing the influence of compound **3a** on survival of the introduced zebrafish as observed after 72 h.



Figure S11. Concentration-effect curve showing the influence of compound **4a** on survival of the introduced zebrafish as observed after 72 h.



Figure S12. Concentration-effect curve showing the influence of compound **5a** on survival of the introduced zebrafish as observed after 72 h.