Supplementary Material

Interaction with Blood Proteins of a Ruthenium(II) Nitrofuryl Semicarbazone Complex: Effect on the Antitumoral Activity

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Figure S1. UV-Vis spectroscopy for the system RuNTF—HSA over time: isotropic absorption spectra of solutions of RuNTF complex in the absence (green, red) and in the presence (blue, orange) of HSA after (a) 16h and (b) 96 h of incubation at 37°C [in 2%DMSO/PBS buffer, spectra recorded at room temperature (20.0 \pm 0.5)°C, C_{RuNTF} = 60 µM)].



Figure S2. UV-Vis spectroscopy for the system L—HSA over time: (a) CD spectra of solutions of the 5nitro-2-furaldehyde semicarbazone ligand L and HSA; and (b) their corresponding isotropic absorption spectra; the incubation time at 37°C is indicated. [Conditions: 1%DMSO/PBS buffer, spectra recorded at room temperature (20.0 ± 0.5)°C, C_{HSA} = C_{RuNTF} = 200μ M)].



Figure S3. Far-UV CD spectrum for the system RuNTF—**HSA over time: (a)** Far-UV CD spectrum of solutions containing HSA (red, 5 μ M) and HSA-RuNTF at a 1:4 molar ratio (blue) with increasing incubation time; **(b)** CD signal intensity normalized at $\lambda^{(\cdot)}_{max}$ = 208 nm for HSA-RuNTF 1:4 molar ratio recorded at increasing incubation times up to 68h [C_{HSA}= 100 μ M = constant; C_{RUNTF}: 20 μ M; samples in PBS pH7.4 incubated at 37°C; spectra recorded at room temperature (20.0 ± 0.5)°C].



Figure S4. Effect of increasing concentrations of RuNTF on the secondary structure of HSA: (a) Far-UV spectra of HSA in the presence (colored lines) and absence (red line, open circles) of increasing concentrations of RuNTF complex following a 68h incubation period (b) CD spectra with $\Delta \epsilon$ values

normalized at $\lambda^{(\cdot)}_{max}$ =208 nm [ChSA= 5 μ M, kept constant; Crunte: 0-50 μ M; samples in PBS pH7.4 incubation at 37°C; spectra recorded at room temperature (20.0 \pm 0.5)°C.].



Figure S5. Cytotoxicity of RuNTF complex against non-cancer cell lines: dose-response curve obtained for the determination of the IC50 value in the V79 lung fibroblasts upon 72h incubation with RuNTF and cisplatin (CisPt). (IC₅₀ > 200 μ M; results are average values from 2 independent experiments with a minimum of 6 replicates.)