Supplementary Information

Induction of transferrin aggregation by indazolium [tetrachlorobis(1H-indazole)$_2$ruthenate(III)] (KP1019) and its biological implication

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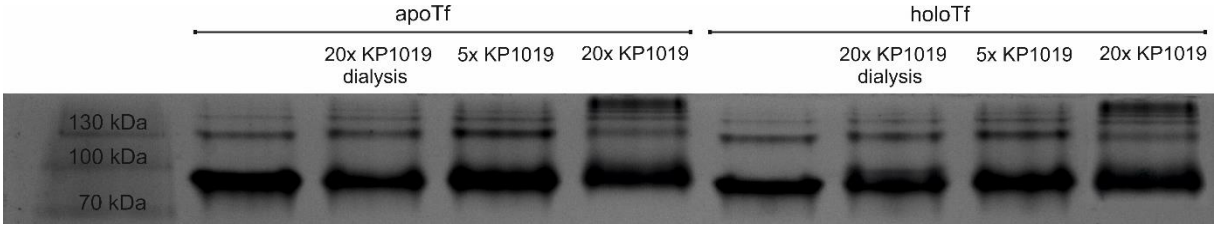

Fig. S1. Native electrophoresis PAGE gel analysis of homogeneity of the reaction mixtures obtained upon incubation of apoTf or holoTf with KP1019 in the following way: dialysis of Tfs in the presence of 20-fold excess of KP1019 (*20×KP1019 dialysis*) or direct reaction between reagents with 5- or 20-fold excess of KP1019 kept over Tf (*5×KP1019* and *20×KP1019 dialysis*, respectively). Incubation conditions: 50 mM HEPES pH 7.4, 100 mM NaCl, 25 mM $NaHCO_3$; 37°C; 24 h. [apoTf] = [holoTf] = 1 mg/ml

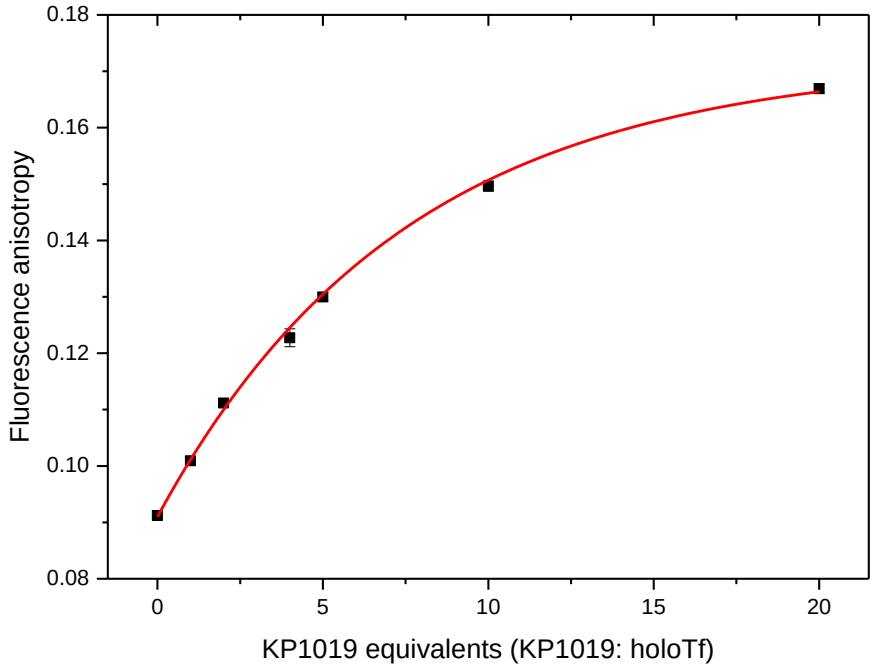

Fig. S2. Fluorescence anisotropy of the holoTfAlexaFluor488 in the presence of increasing concertation of KP1019. HoloTf was incubated for 1 h at 37 °C with KP1019 before measurements. Experimental conditions: [holo-Tf] = 12.6 µM. [KP1019] = 0-252 µM; HEPES pH 7.4; [NaCl] = 0.1 M; [$NaHCO_3$] = 25 mM; T = 25 °C (during the anisotropy measurement).

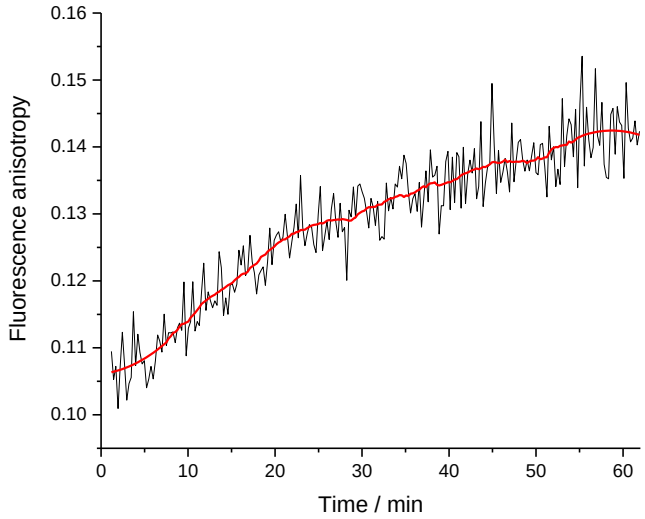

Fig S3. Kinetics of the reaction between holoTf-AlexaFluor488 and 20-fold excess if KP1019 followed by fluorescence anisotropy measurements. Experimental conditions: pH 7.4 (50 mM HEPES, 100 mM NaCl, 25 mM $NaHCO_3$), 37°C. [Tf] = 12.6 µM.

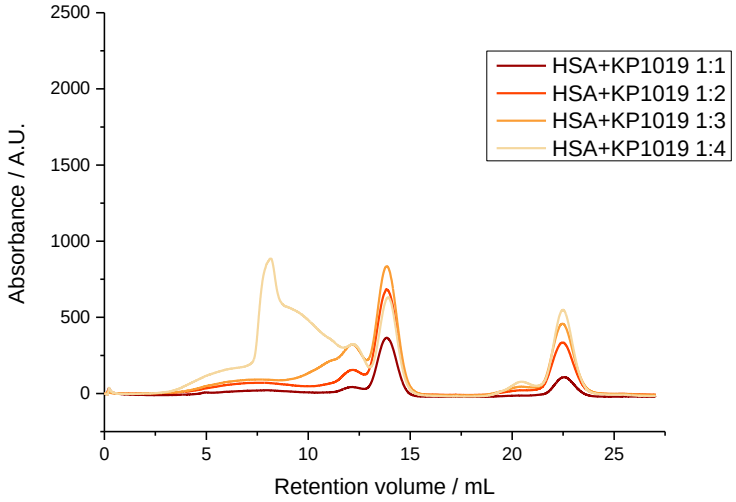

Fig. S4 Chromatograms presenting Size Exclusion Chromatography separation of a reaction mixture resulting from the incubation of HSA with the increasing concentration of KP1019 during 24 h in buffer at pH 7.4 (50 mM HEPES, 100 mM NaCl, 25 mM $NaHCO_3$) at 37°C. [HSA] = 10 mg/ml.

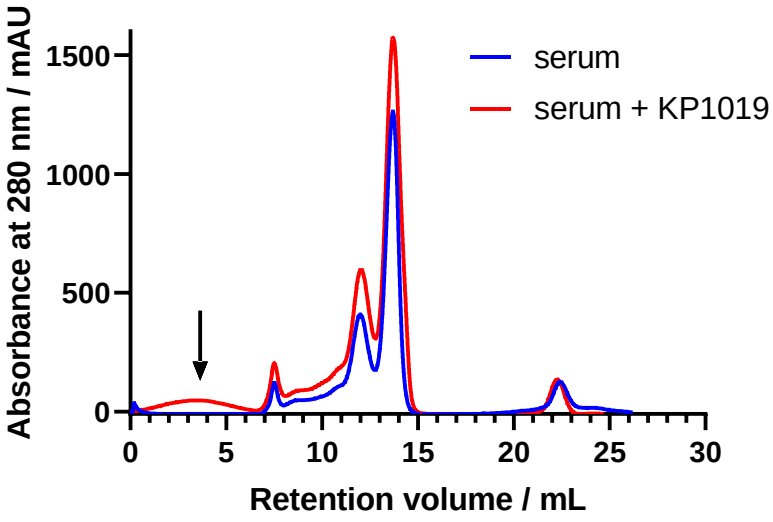

Fig. S5 Chromatograms presenting Size Exclusion Chromatography separation of a reaction mixture resulting from the incubation of control human serum with the KP1019 during 24 h in buffer at pH 7.4 (50 mM HEPES, 100 mM NaCl, 25 mM $NaHCO_3$) at 37°C. Ru concentration equivalent to the Ru concentration after venous application of KP1019 complex (20 mg/L of blood, taken from clinical studies: F. Lentz; Drescher, A.; Lindauer, A.; Henke, M.; Hilger, R. A.; Hartinger, C. G.; Scheulen, M. E.; Dittrich, C.; Keppler, B. K.; Jaehde, U. *Anticancer Drugs.* 2009, 97.)

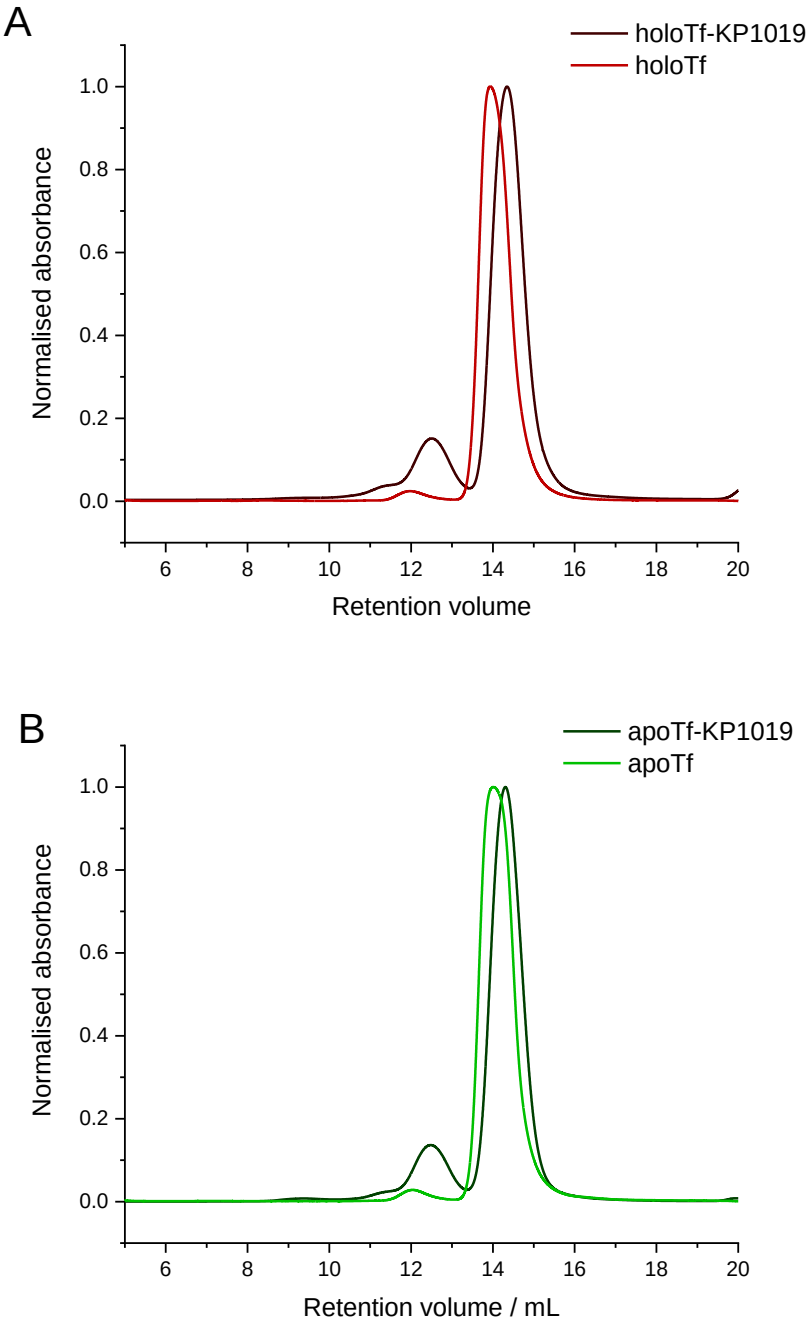

Fig. S6. Chromatograms presenting Size Exclusion Chromatography separation of adducts obtained by dialysis of apoTf A).and holoTf B) in the presence of 20-fold excess of KP1019 dissolved in buffer (PBS, pH 7.4) followed by dialysis to PBS. For adduct formation [apoTf] = [holoTf] = 10 mg/ml.

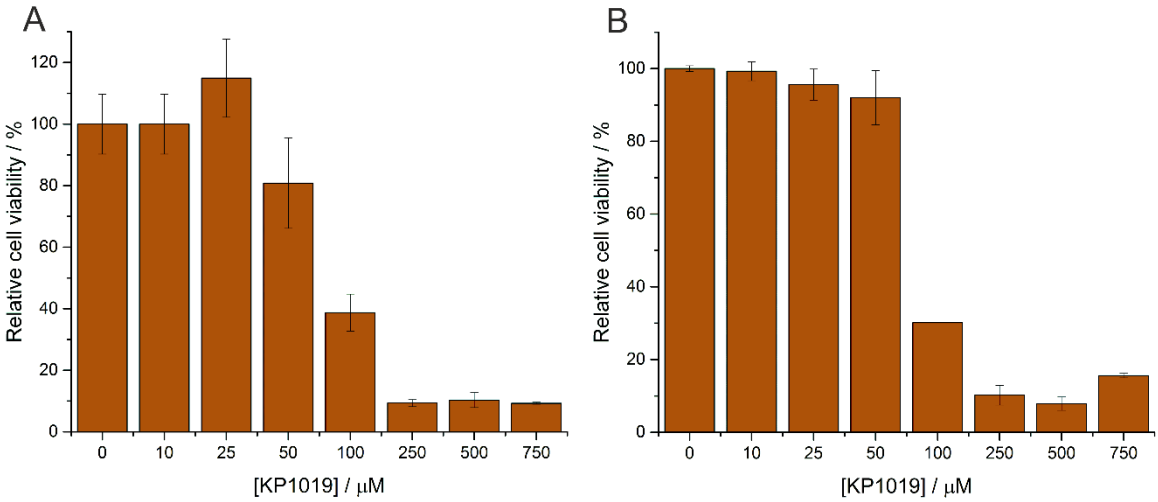

Fig. S7. The cytotoxic effect of KP1019 on human keratinocyte cells after 48 h incubation evaluated by A) MTT and B) Alamar Blue test.