Supplementary Material

Mechanisms underlying reductant-induced ROS formation by anticancer copper(II) compounds

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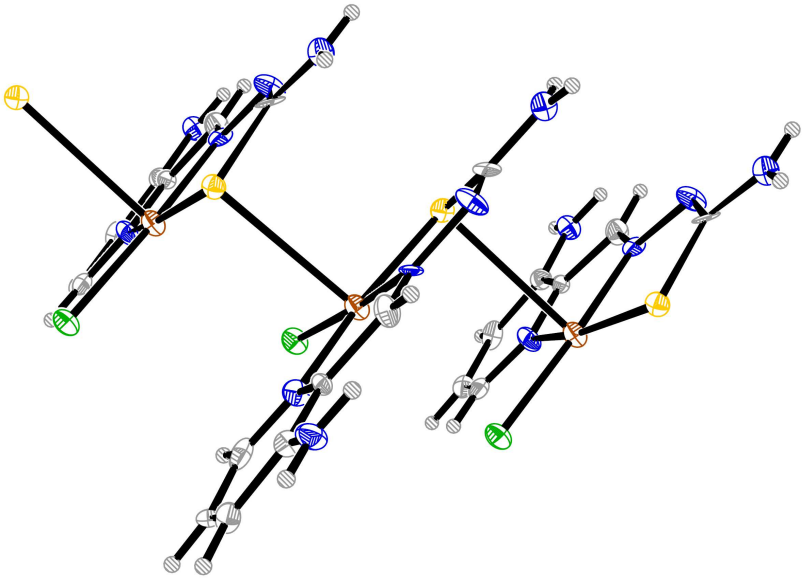

Figure S1. Chain structure of **Cu-Triapine** molecules with thermal ellipsoids depicted at 50% probability level (the $H_3O^+Cl^-$ fragments are omitted). (Cu = brown, Cl = green, S = yellow, N = blue, C and H = grey)

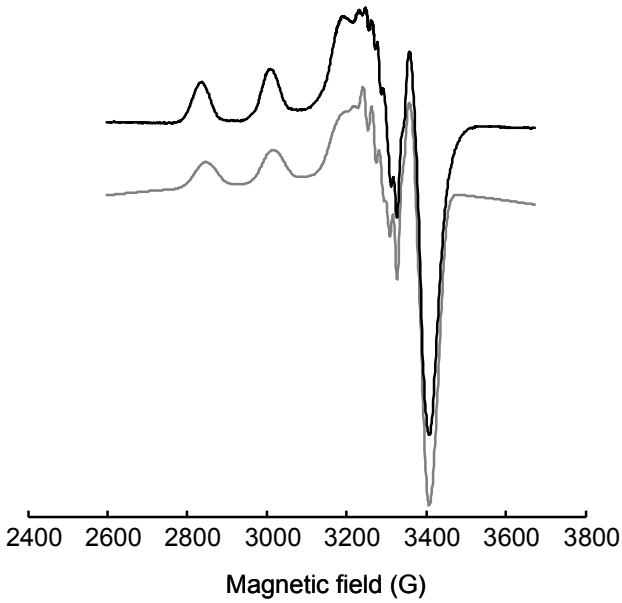

Figure S2. EPR spectrum of 1 mM **Cu-FTSC** (black) in DMSO/DMF (1:3 v/v) and the simulated EPR spectrum (grey). Experimental conditions: $\nu = 9.4$ GHz, $T = 77$ K.

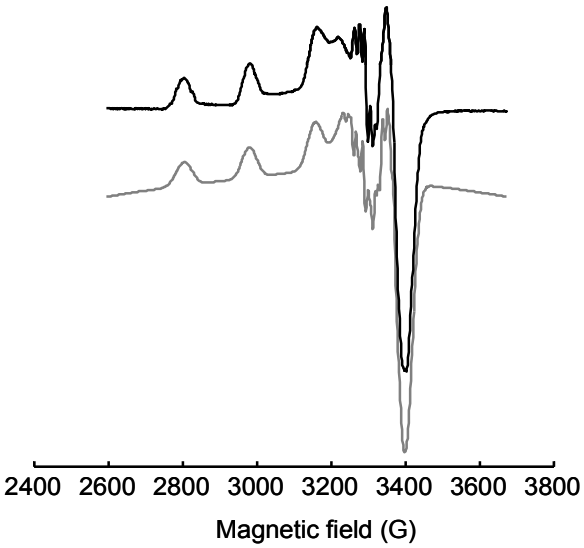

Figure S3. EPR spectrum of 1 mM **Cu(OAc)-Triapine** (black) in DMSO/DMF (1:3 v/v) and the simulated EPR spectrum (grey). Experimental conditions: ν = 9.4 GHz, T = 77 K.

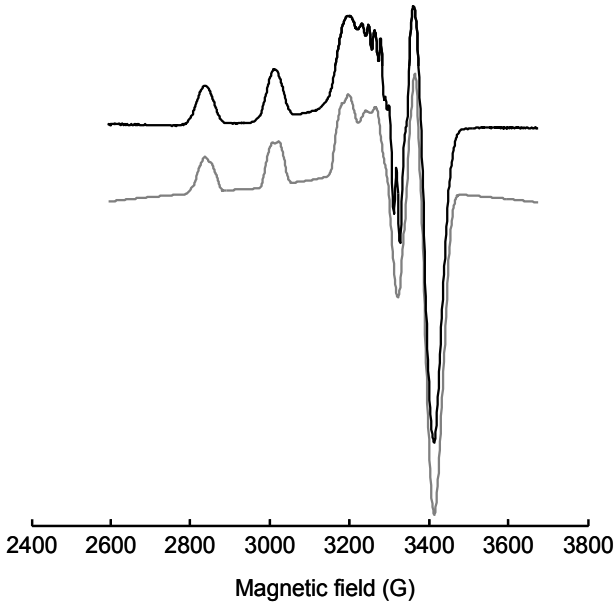

Figure S4. EPR spectrum of 1 mM **Cu-APTSC** (black) in DMSO/DMF (1:3 v/v) and the simulated EPR spectrum (grey). Experimental conditions: ν = 9.4 GHz, T = 77 K.

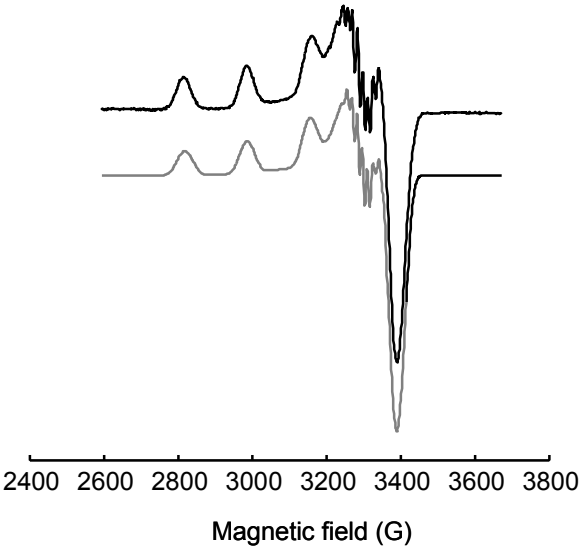

Figure S5. EPR spectrum of 1 mM **Cu-BPYTA** (black) in DMSO/DMF (1:3 v/v) and the simulated EPR spectrum (grey). Experimental conditions: ν = 9.4 GHz, T = 77 K.

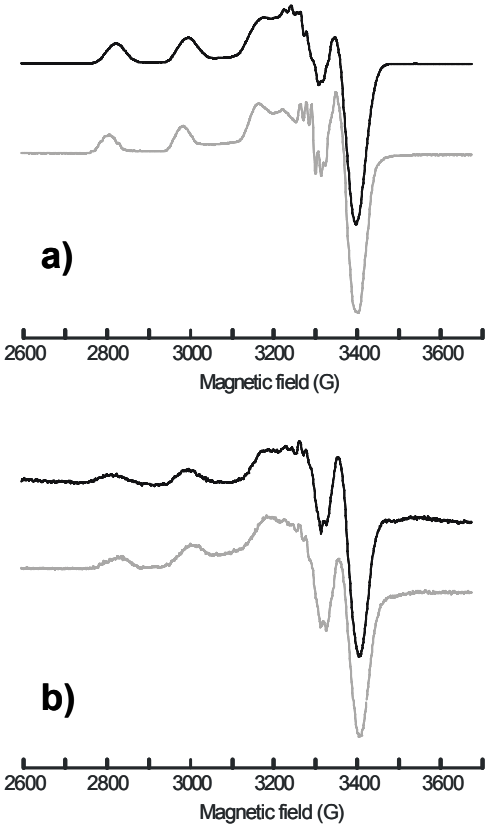

Figure S6. a) EPR spectra of 1mM **Cu-Triapine** (black) and **Cu(OAc)-Triapine** (grey) in DMSO/DMF (1:3 v/v) and **b)** 1mM **Cu-Triapine** (black) and **Cu(OAc)-Triapine** (grey) in DMSO/PBS pH 7.4 (1:1 v/v). Experimental conditions: ν = 9.4 GHz, T = 77 K.

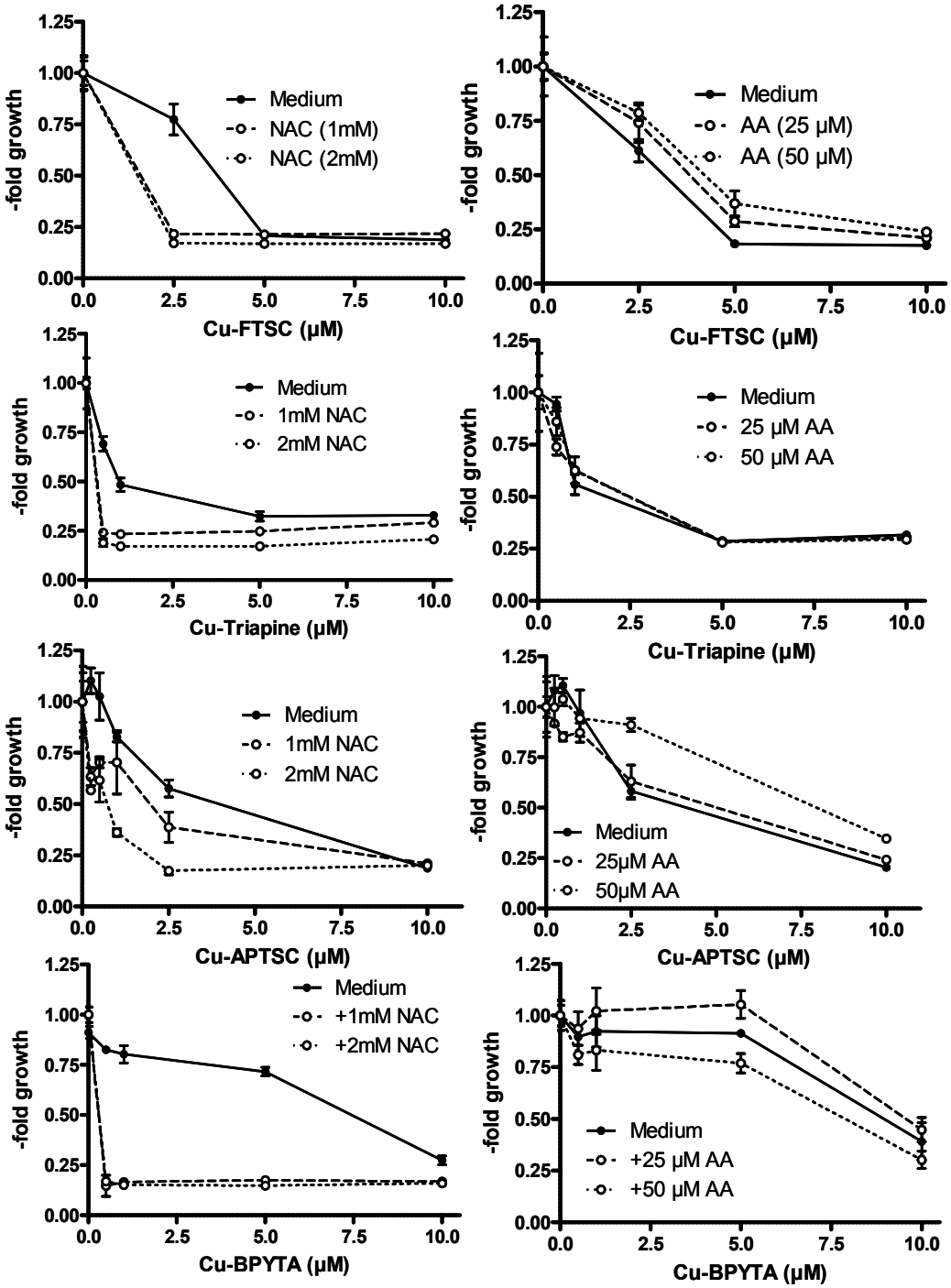

Figure S7. Impact of reductants on the anticancer activity of the copper complexes in HL60 cells. To evaluate the effects of reductants, the GSH precursor NAC as well as the anti-oxidant ascorbic acid were used. Briefly, after 30 min preincubation with NAC (1 and 2 mM) or AA (25 and 50 μM), HL60 cells were treated for 72 h with the indicated concentrations of the Cu complexes. Vitality was determined using MTT assay. Values given are means ± standard deviation of three determinations out of three experiments.

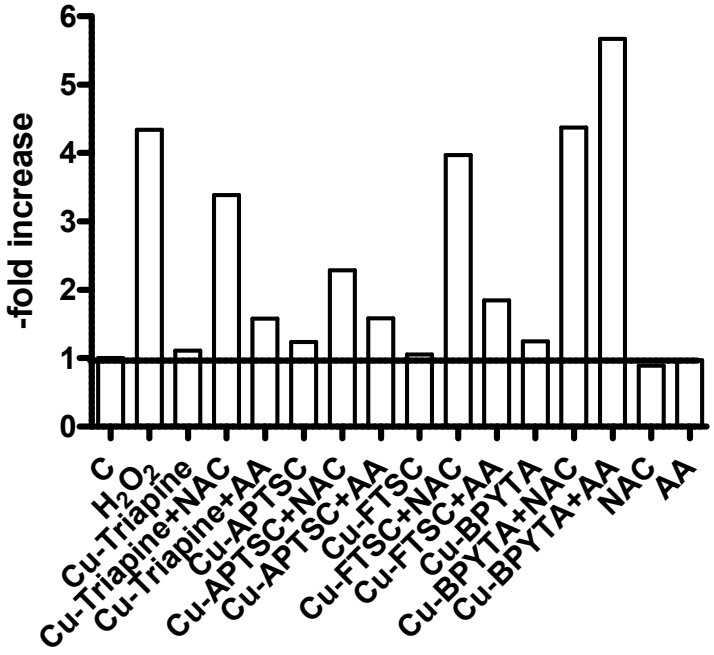

Figure S8. Reductant-induced ROS generation by the Cu complexes. Influence of pretreatment with 2 mM NAC or 50 μM AA on the intracellular ROS levels in HL60 cells after incubation with the Cu complexes (5 μM) was determined using the ROS indicator DCF-DA. Fluorescence was measured by flow cytometry. One representative experiment out of two delivering comparable results is shown.

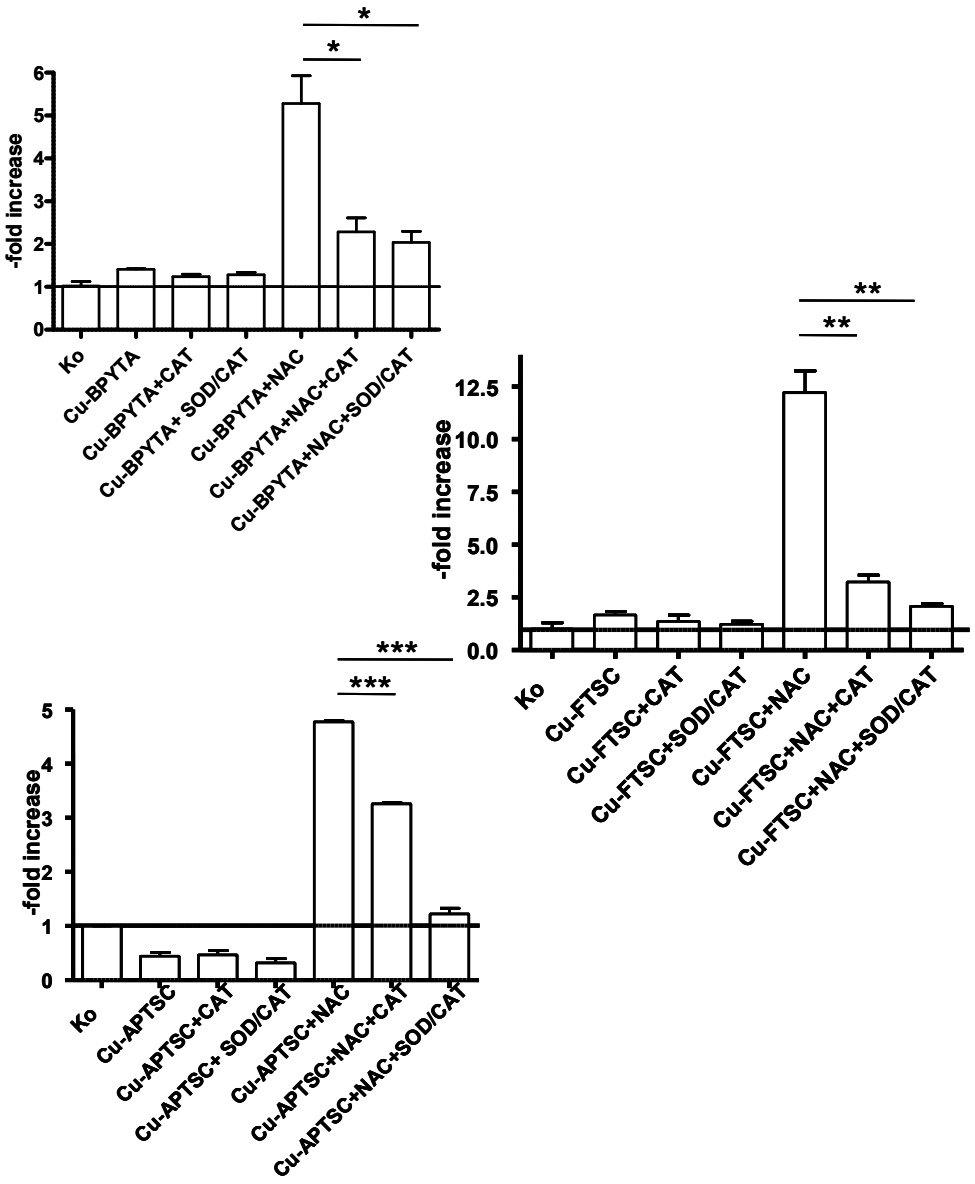

Figure S9. Effect of extracellular SOD and CAT on thiol-induced ROS generation. Influence of SOD and CAT cotreatment (100 units/ml) on the NAC-induced (2 mM) ROS-formation of A) Cu-BPYTA, B) Cu-FTSC, C) Cu-APTSC (25 µM) in HL60 cells was determined using the H_2O_2-detecting dye DCF-DA. Fluorescence was measured by flow cytometry. One representative experiment out of three delivering comparable results is shown.

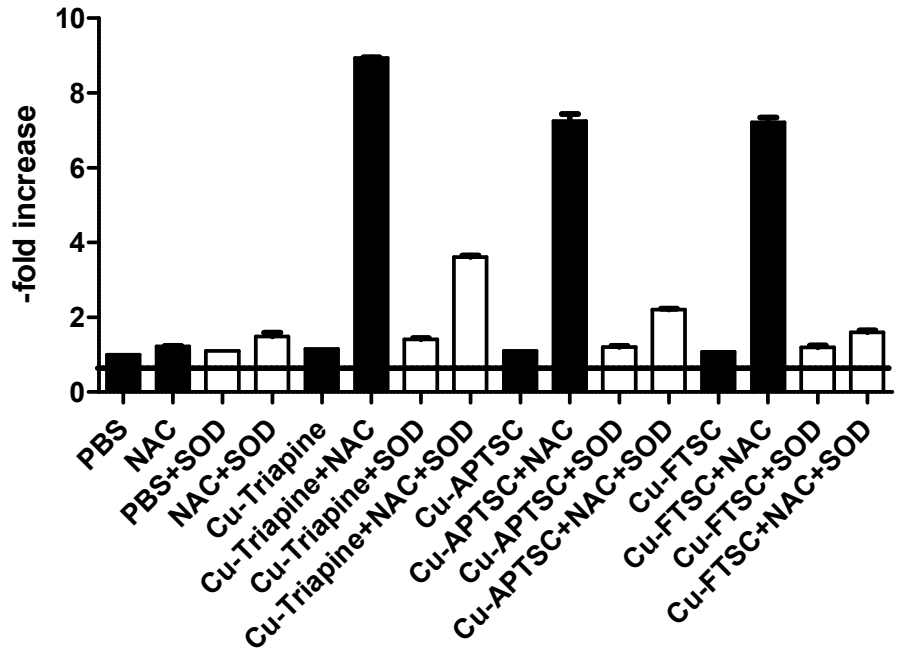

Figure S10. Impact of SOD cotreatment in the $O_2^{\bullet-}$ generation ability of the Cu complexes in the presence of NAC and AA. Levels of Cu complex-generated $O_2^{\bullet-}$ in dependence of NAC (2 mM) and AA (50 μM) were determined measuring the reduction of NBT spectrophotometrically in presence or absence of SOD (5 units/mL; Superoxide Dismutase from bovine erythrocytes, Sigma-Aldrich). The copper complexes were used at concentrations of 25 μM. Values given are means ± standard deviation of three determinations.

X-Ray crystallographic data

Table S1. Crystal data, data collection parameters and structure refinement details for Cu-Triapine.

	Cu-Triapine
Empirical	$C_7H_{11}Cl_2CuN_5OS$
Formula weight	347.71
Space group	$P2_1/c$
a [Å]	17.798(18)
b [Å]	9.686(9)
c [Å]	7.109(7)
α [deg]	
β [deg]	97.59(3)
γ [deg]	
V [Å^3]	1215(2)
Z	4
λ [Å]	0.71073
ρ_{calcd} [g cm^{-3}]	1.901
Crystal size [mm]	0.30 × 0.13 × 0.03
T [K]	100
μ [mm^{-1}]	2.399
R_1[a]	0.0680
wR_2[b]	0.2632
GOF[c]	1.002

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. [c] GOF = $\{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.