

ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Copper(II) complexes based on tripodal pyridyl amine derivatives as efficient anticancer agents

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1) **Fig. S1.** DNA cleavage by the tested complexes under an inert helium atmosphere. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH₃CN) in water at 37 °C for 1 h under helium atmosphere (solutions were bubbled with helium) in the dark. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means ± SEM of three independent experiments. Electrophoretogram shows a representative result of agarose electrophoresis. * indicates statistical significance as compared to blank (p < 0.05); ** indicates statistical significance as compared to blank (p < 0.01); *** indicates statistical significance as compared to blank (p < 0.001); **** indicates statistical significance as compared to blank (p < 0.0001).

- 2) **Fig. S2.** DNA cleavage by the tested complexes in the presence of hydrogen peroxide and singlet oxygen quencher NaN_3 . Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH_3CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Sodium azide solution was applied together with the tested complexes in equimolar concentration. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means \pm SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis. * indicates statistical significance as compared to blank ($p < 0.05$); **** indicates statistical significance as compared to blank ($p < 0.0001$).
- 3) **Fig. S3.** DNA cleavage by the tested complexes in the presence of hydrogen peroxide, superoxide and hydroxyl scavenger KI. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH_3CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Potassium iodide solution was applied together with the tested complexes in equimolar concentration. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means \pm SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis. * indicates statistical significance as compared to blank ($p < 0.05$); ** indicates statistical significance as compared to blank ($p < 0.01$); **** indicates statistical significance as compared to blank ($p < 0.0001$).
- 4) **Fig. S4.** DNA cleavage by the tested complexes in the presence of hydrogen peroxide and hydroxyl radical scavenger DMSO. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH_3CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Dimethyl sulfoxide was applied together with the tested complexes in equimolar concentration. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means \pm SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis.
- 5) **Fig. S5.** DNA cleavage effect of tested complexes in the presence of hydrogen peroxide and hydroxyl scavenger DMSO applied at the different concentration levels. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at 10 μM concentration or pure solvent (blank, 10% CH_3CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Dimethyl sulfoxide was applied together with the tested complexes in concentrations of 10 μM , 1 mM, and 100 mM. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means \pm SEM of three independent experiments, electrophoretograms show representative results of agarose electrophoresis. **** indicates statistical significance as compared to blank ($p < 0.0001$).
- 6) **Fig. S6.** The changes in the DNA cleavage by the tested complexes in the presence of hydrogen peroxide with the addition of equimolar concentration of EDTA. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH_3CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means \pm SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis. **** indicates statistical significance as compared to blank ($p < 0.0001$).

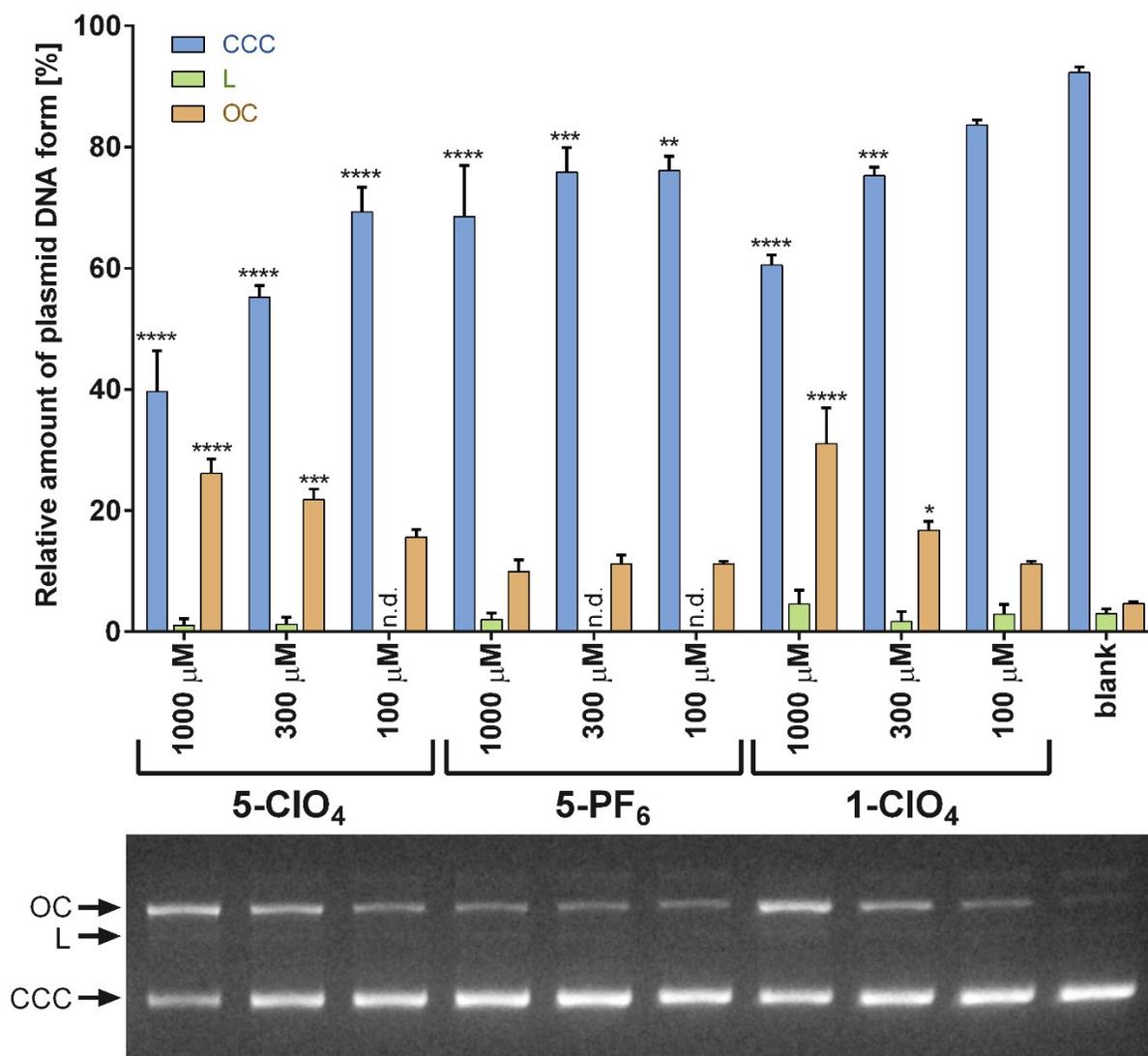


Fig. S1 DNA cleavage by the tested complexes in an inert helium atmosphere. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH₃CN) in water at 37 °C for 1 h under helium atmosphere (all solutions were bubbled with helium) in the dark. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means ± SEM of three independent experiments. Electrophoretogram shows a representative result of agarose electrophoresis. * indicates statistical significance as compared to blank ($p < 0.05$); ** indicates statistical significance as compared to blank ($p < 0.01$); *** indicates statistical significance as compared to blank ($p < 0.001$); **** indicates statistical significance as compared to blank ($p < 0.0001$).

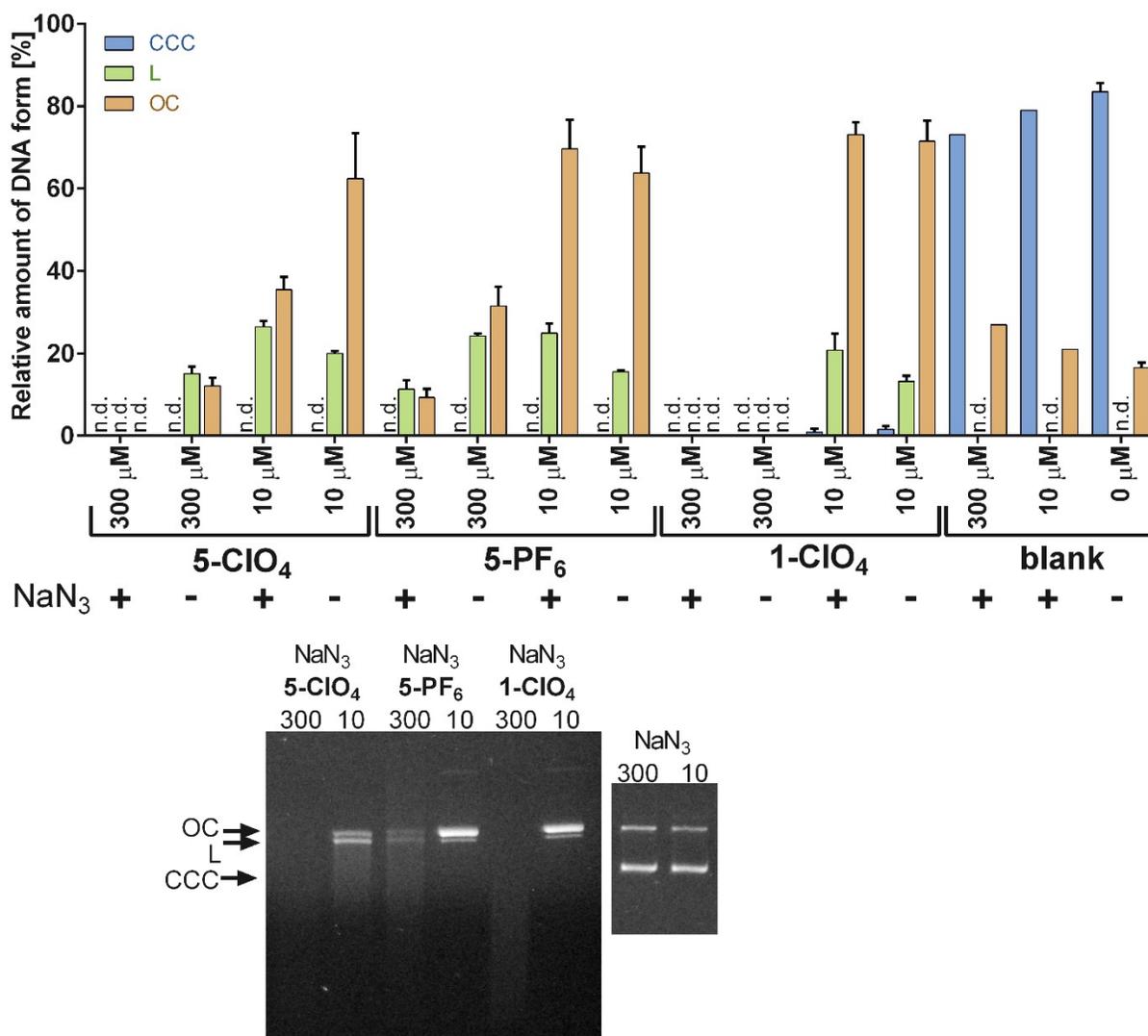


Fig. S2 DNA cleavage by the tested complexes in the presence of hydrogen peroxide and singlet oxygen quencher NaN₃. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH₃CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Sodium azide solution was applied together with the tested complexes in equimolar concentration. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means ± SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis. * indicates statistical significance as compared to blank (p < 0.05); **** indicates statistical significance as compared to blank (p < 0.0001).

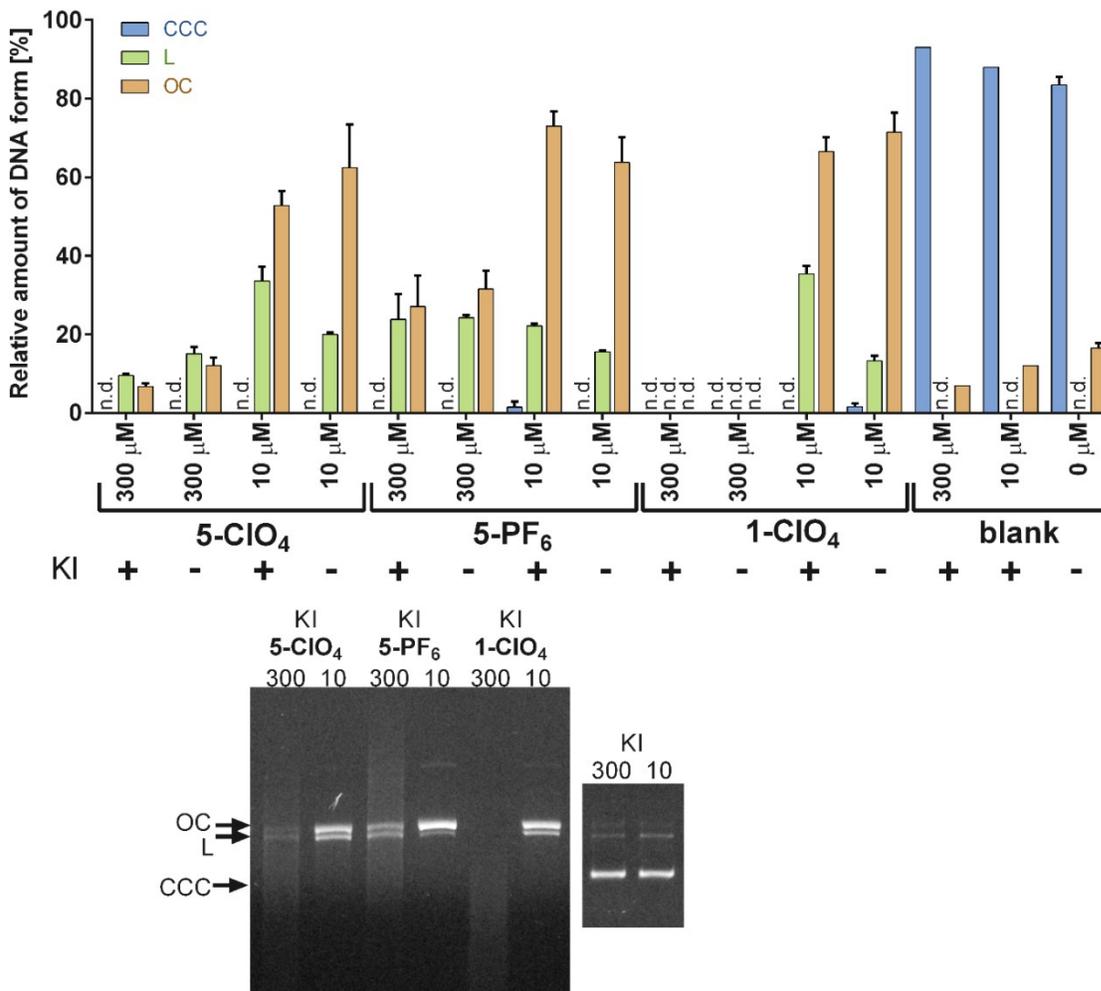


Fig. S3 DNA cleavage by the tested complexes in the presence of hydrogen peroxide, superoxide and hydroxyl scavenger KI. Supercoiled plasmid DNA (CCC) was incubated with the complexes (5-ClO₄, 5-PF₆, 1-ClO₄) applied at different concentrations or pure solvent (blank, 10% CH₃CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Potassium iodide solution was applied together with the tested complexes in equimolar concentration. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means ± SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis. * indicates statistical significance as compared to blank (p < 0.05); ** indicates statistical significance as compared to blank (p < 0.01); **** indicates statistical significance as compared to blank (p < 0.0001).

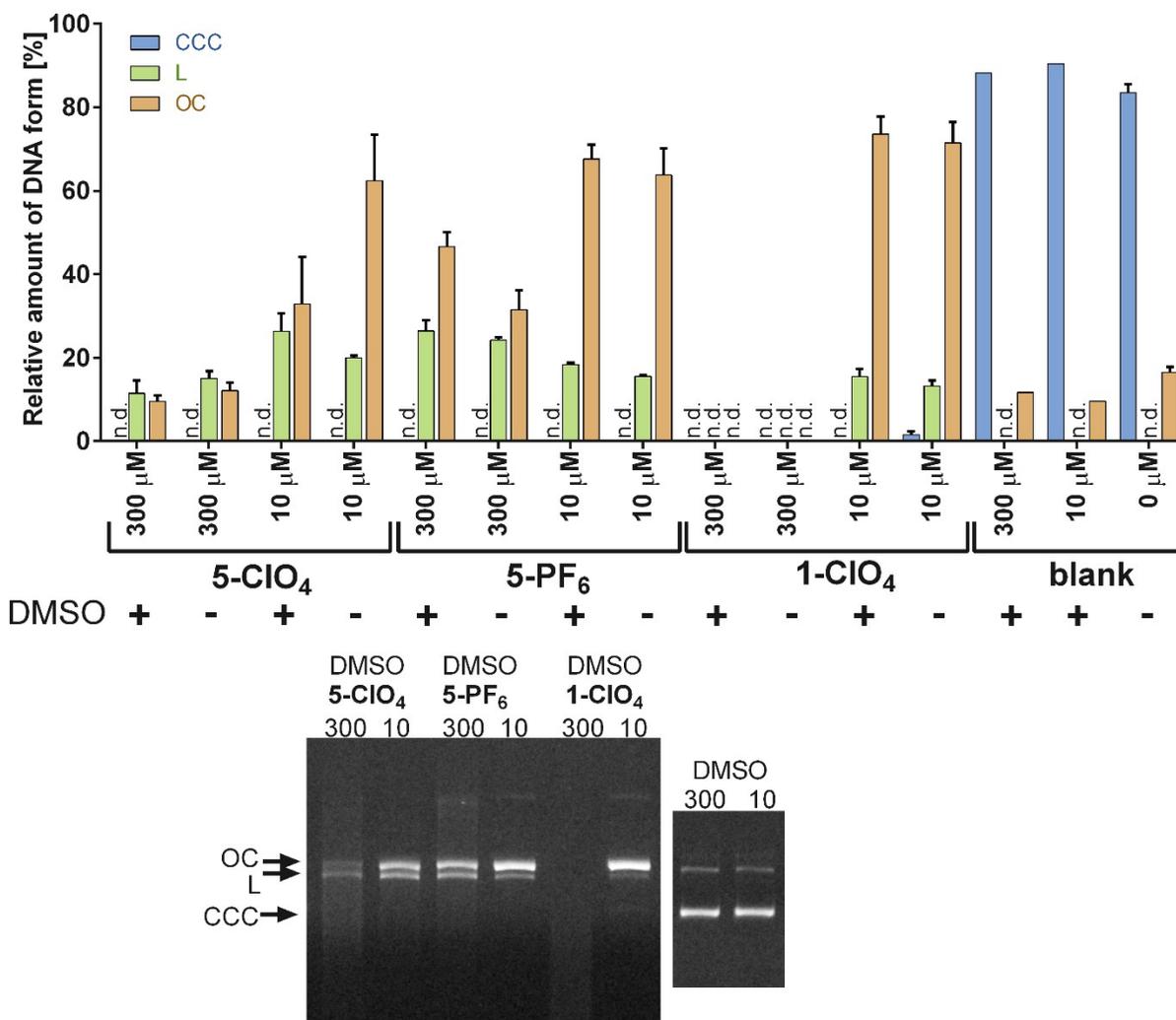


Fig. S4 DNA cleavage by the tested complexes in the presence of hydrogen peroxide and hydroxyl radical scavenger DMSO. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH₃CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Dimethyl sulfoxide was applied together with the tested complexes in equimolar concentration. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means ± SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis.

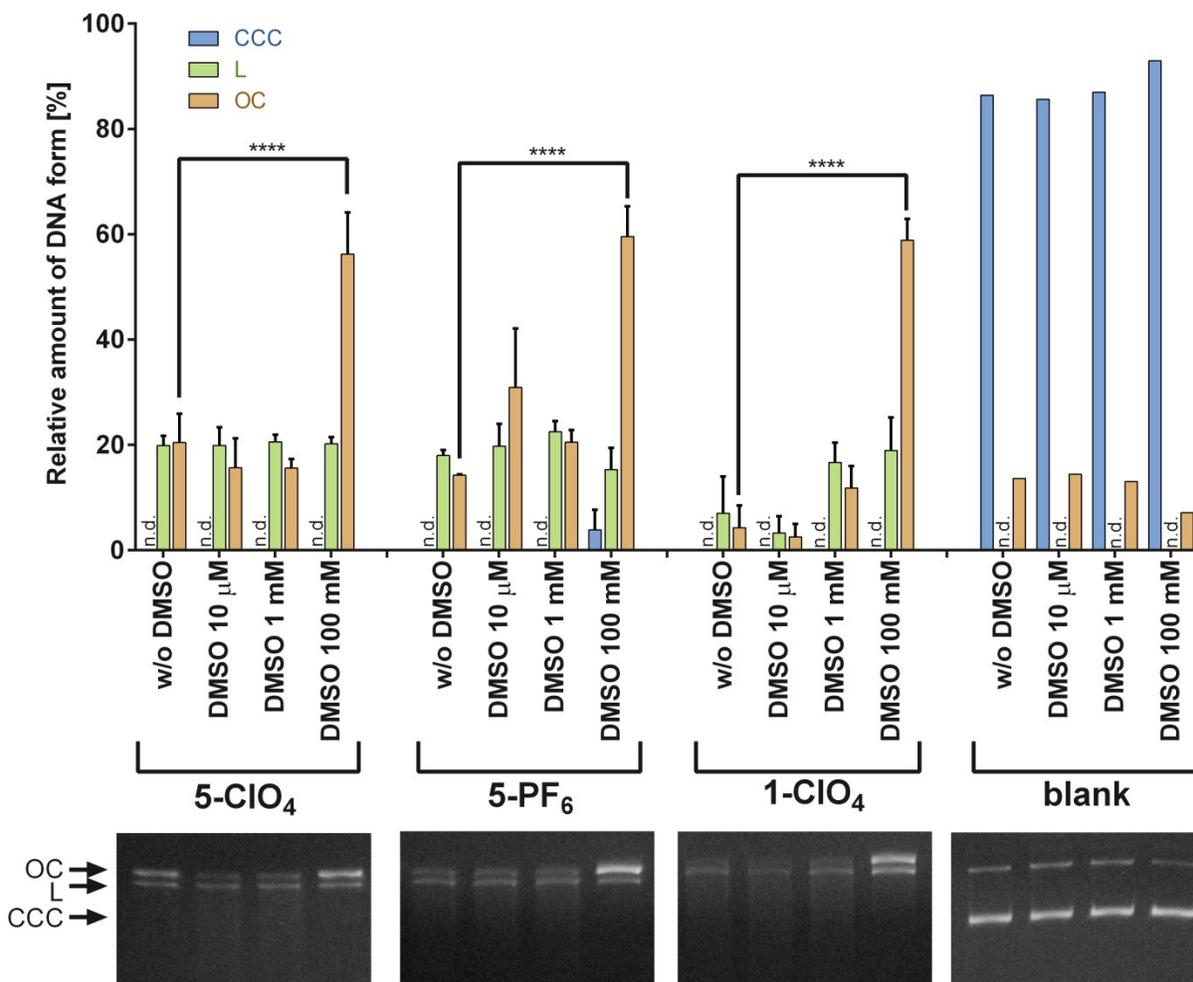


Fig. S5 DNA cleavage effect of tested complexes in the presence of hydrogen peroxide and hydroxyl scavenger DMSO applied at the different concentration levels. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at 10 μM concentration or pure solvent (blank, 10% CH₃CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. Dimethyl sulfoxide was applied together with the tested complexes in concentrations of 10 μM, 1 mM, and 100 mM. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means ± SEM of three independent experiments, electrophoretograms show representative results of agarose electrophoresis. **** indicates statistical significance as compared to blank ($p < 0.0001$).

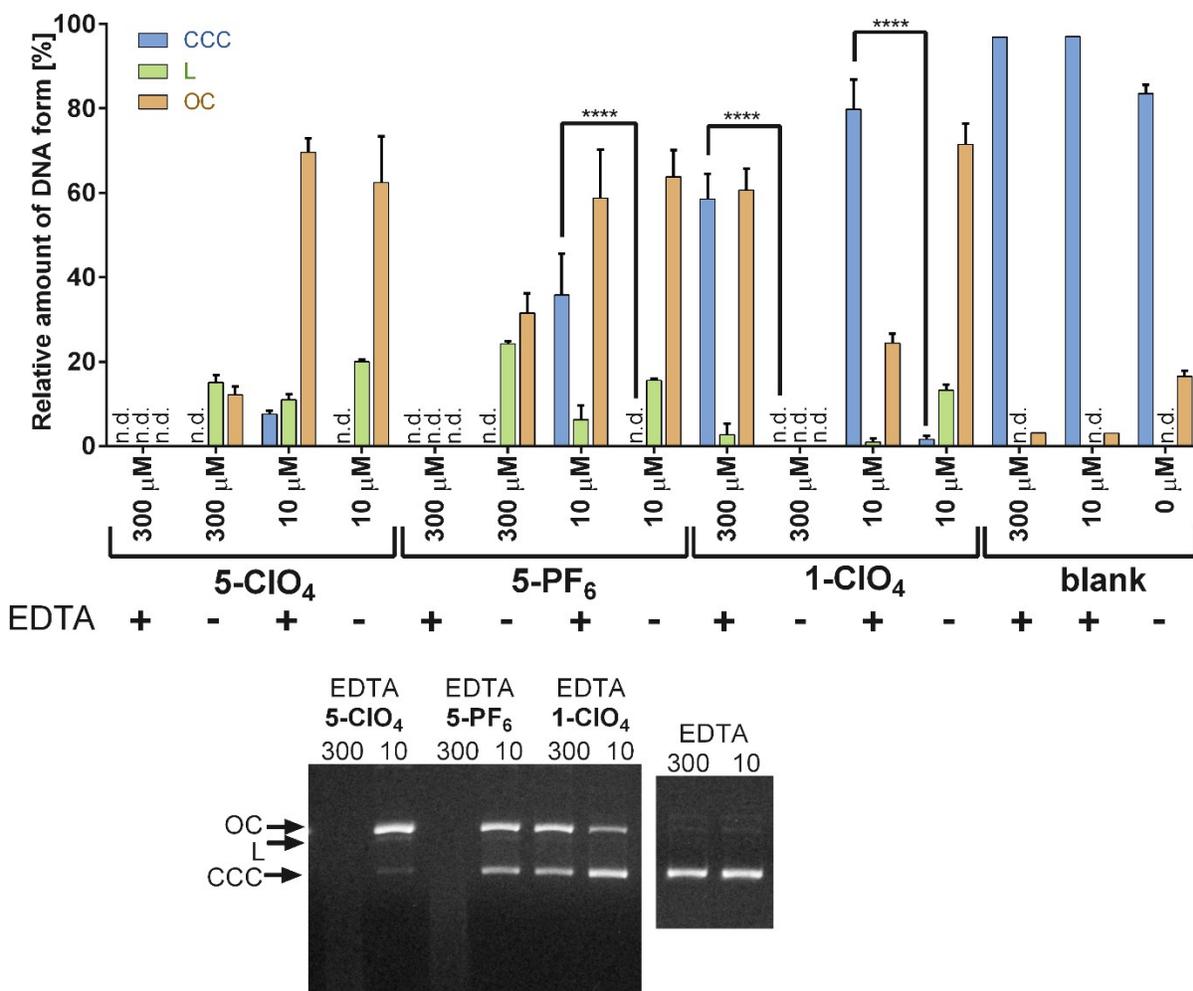


Fig. S6 The changes in the DNA cleavage by the tested complexes in the presence of hydrogen peroxide with the addition of equimolar concentration of EDTA. Supercoiled plasmid DNA (CCC) was incubated with the complexes (**5-ClO₄**, **5-PF₆**, **1-ClO₄**) applied at different concentrations or pure solvent (blank, 10% CH₃CN) at 37 °C for 1 h with the addition of 0.66 mM hydrogen peroxide. After the incubation, the amount of generated open circle (OC) and linear (L) form of plasmid was evaluated by densitometric analysis of agarose gel electrophoretograms. Graphs indicate means ± SEM of three independent experiments. Electrophoretograms show representative results of agarose electrophoresis. **** indicates statistical significance as compared to blank (p < 0.0001).