Electronic Supporting Information

Tunable DNA cleavage activity promoted by copper(II) ternary complexes with N-donor heterocyclic ligands

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Experimental

Materials. All chemicals were reagent grade and used without further purification. All aqueous solutions were prepared using Milli-Q water and sterilized by autoclavation to avoid the contamination by nucleases. Organic solvents such acetontrile and dimethyl sulfoxide (DMSO) or other heat sensitive reagents were sterilized by 0.22 μm pore membrane filtration. The plasmid pBSK II (2961 bp) used for DNA cleavage assays was purchased from Stratagene, transformed into DH5α *Escherichia coli* competent cells and amplified as previously described[1]. The plasmid DNA was extracted from *E. coli* and purified using Qiagen Plasmid Maxi Kit protocol™. For DNA-binding studies, calf thymus DNA (CT-DNA, type XV Sigma) purchased from Sigma (MO, USA) was used. The concentration of DNA in base pairs was estimated by spectrophotometry (ε = 6600 M^{-1} cm^{-1} at 260 nm). The DNA stock solutions presented a ratio of absorbance at 260 and 280 nm of ca. 1.8–1.9:1, indicating that the DNA was sufficiently free of protein[2].

Complexes synthesis. Complexes were obtained as previously published by our group[3].

Circular dichroism. CD studies were carried out on an Jasco J-815 spectropolarimeter to determine the changes on secondary structure of CT-DNA in the presence of **1-4**, as previously described[4] with few alterations. A CT-DNA sample (100 μM) in Tris-HCl buffer (5 mM, pH 7.4) containing 25% CH_3CN were titrated with the complexes to give different ratios of [DNA]/[complex] (r = 0.0-3.0). After each addition of complex, the CT-DNA CD spectra were recorded in a range of 200 to 700 nm and the reagents concentrations were corrected for dilution. The spectrum of the corresponding buffer and of the complexes alone were collected and subtracted from that of the reaction mixture.

DNA cleavage. In general, 330 ng of pBSK II DNA (~25 μM bp) in 10 mM Tris-HCl (pH 7.4) buffer were treated with certain concentrations of complex **1-4** (as described within each figure legend) for up to 4 hours at 37 ºC without any exposure of ambient light. In kinetic assays, different times of reaction were necessary for each complex as showed as data points in figure SX. All assays were conducted including a reaction control (without complex) to serve as a reference of spontaneous plasmid DNA

fragmentation. Thereafter, each reaction was quenched by adding 5 µL of a loading buffer solution (50 mM Tris-HCl pH 7.5, 0.01% bromophenol blue, 50% glycerol and 250 mM EDTA) and then subjected to electrophoresis on a 0.8% agarose gel containing 0.3 µg mL^{-1} of ethidium bromide in 0.5 x TBE buffer (44.5 mM Tris pH 8.0, 44.5 mM boric acid, and 1 mM EDTA) at 90 V for 1.5 h. The resulting gels were visualized and digitized using a DigiDoc-It gel documentation system (UVP, USA). The proportion of plasmid DNA in each band was quantified using KODAK Molecular Imaging Software 5.0 (Carestream Health, USA). The quantification of supercoiled DNA (F I) was corrected by a factor of 1.47, since the ability of ethidium bromide to intercalate into this DNA topoisomeric form is decreased relative to open circular and linear DNA[5].

DNA photocleavage. Essentially, the photocleavage conditions were the same as described to DNA cleavage assays, with minor modifications: the reaction samples were exposed to a UV-light (12 W) lamp for up to 5 min. In kinetic assays, different times of reaction were necessary for each complex as showed as data points in figures S11 and S12. All the subsequent steps of sample preparation to electrophoresis followed the proceedings above described.

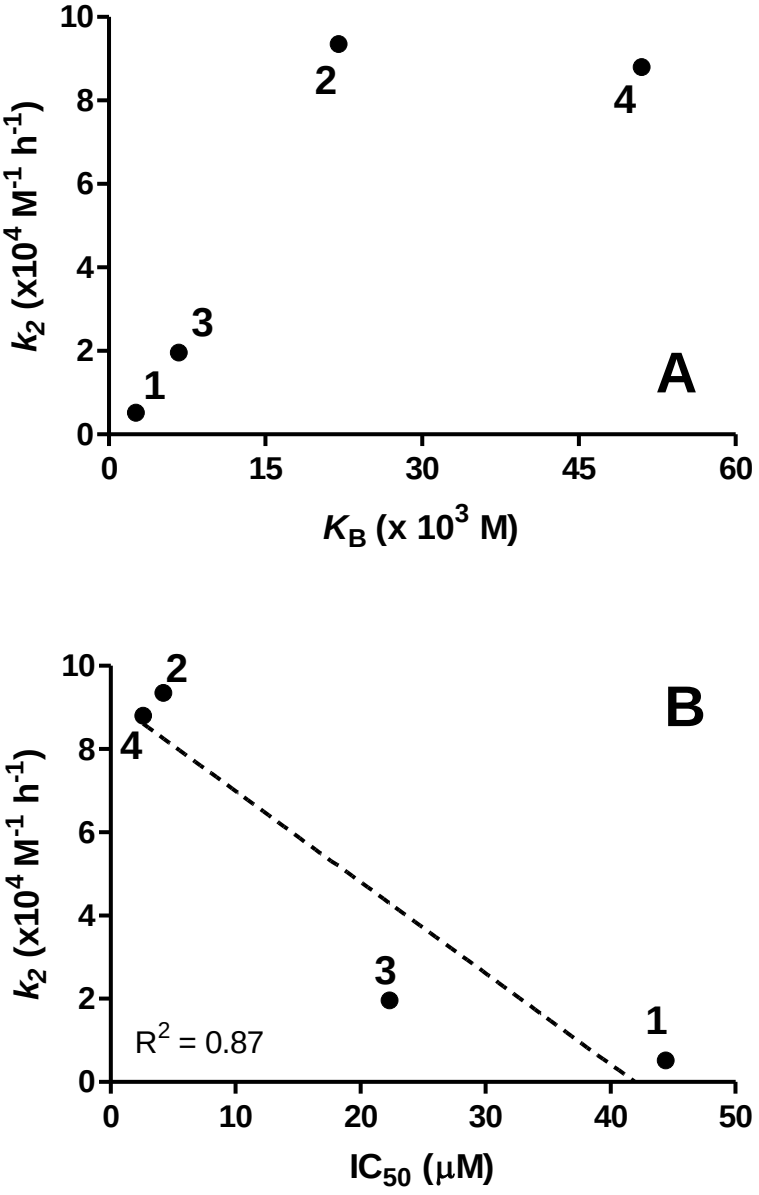

Figure S1. Comparison of different **1-4** parameters: apparent second-order constant of DNA cleavage (as k_2, M^{-1} h^{-1}) *vs.* DNA binding affinity (A) and then cleavage kinetic constants *vs.* antitumor properties (B). Values of K_B and IC_{50} extracted from Silva and co-workers (2014).

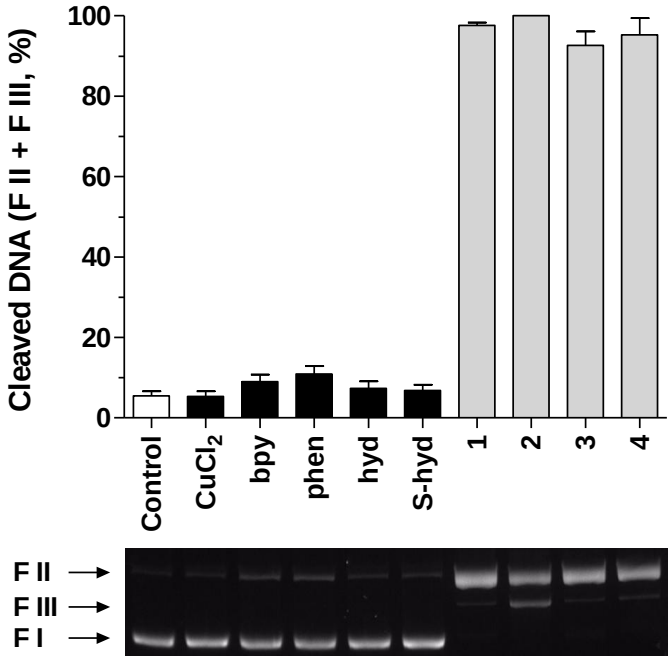

Figure S2. Cleavage of supercoiled DNA by **1**-**4** compared to copper(II) chloride and each complex ligand alone. Reaction conditions: [DNA] = 330 ng, ~ 25 µM; [Buffer] = 10 mM Tris-HCl (pH 7.4); [complex, $CuCl_2$ or ligands] = 20 µM; Time = 5 minutes under UV-light. Representative data from three independent experiments expressed as mean ± standard deviation.

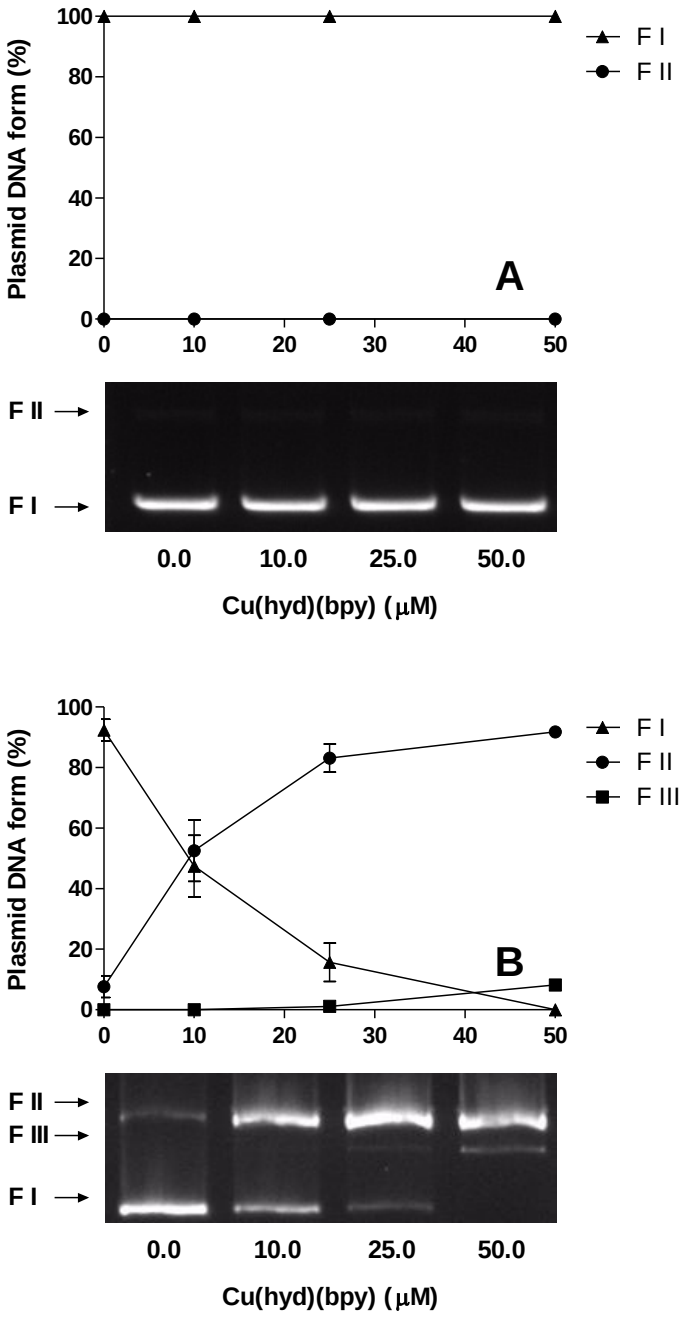

Figure S3. Comparison of DNA cleavage (A) *vs.* photocleavage (B) by **1** for 5 minutes. Reaction conditions: [DNA] = 330 ng, ~ 25 μM; [Buffer] = 10 mM Tris-HCl (pH 7;4); [complex] = 10 to 50 μM. Representative data from three independent experiments expressed as mean ± standard deviation.

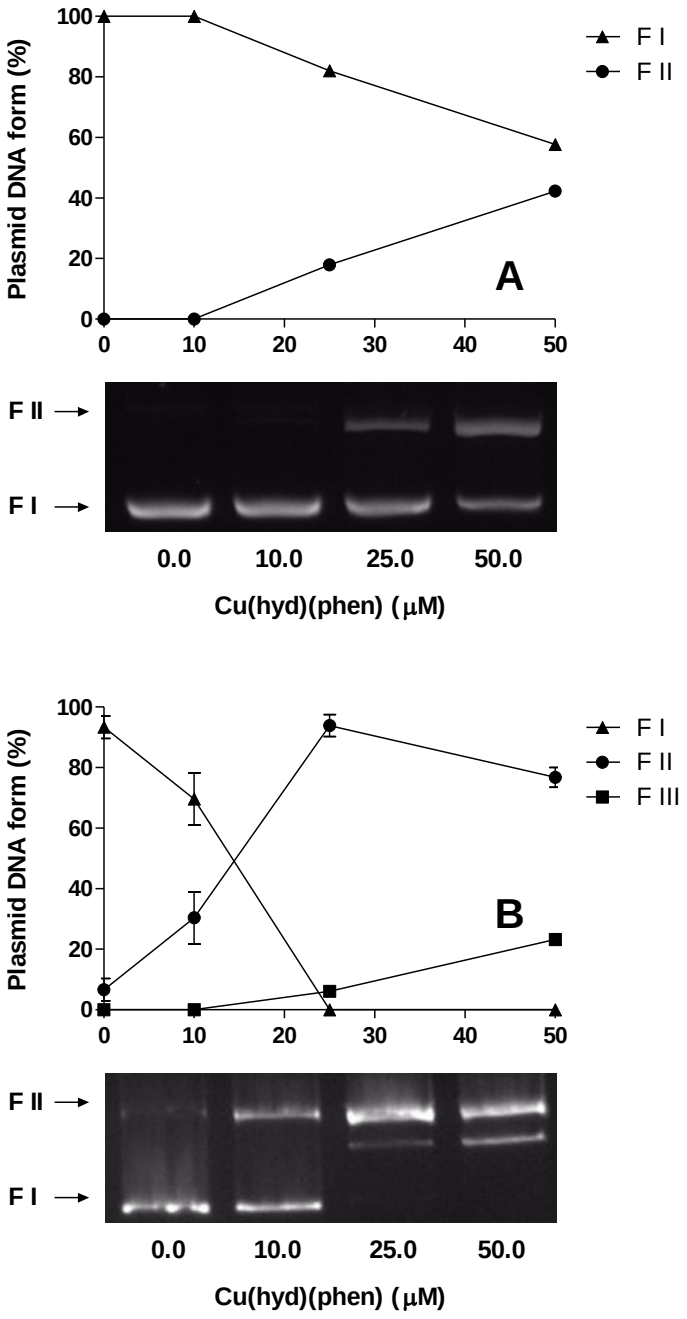

Figure S4. Comparison of DNA cleavage (A) *vs.* photocleavage (B) by **2** for 5 minutes. Reaction conditions: [DNA] = 330 ng, ~ 25 μM; [Buffer] = 10 mM Tris-HCl (pH 7;4); [complex] = 10 to 50 μM. Representative data from three independent experiments expressed as mean ± standard deviation.

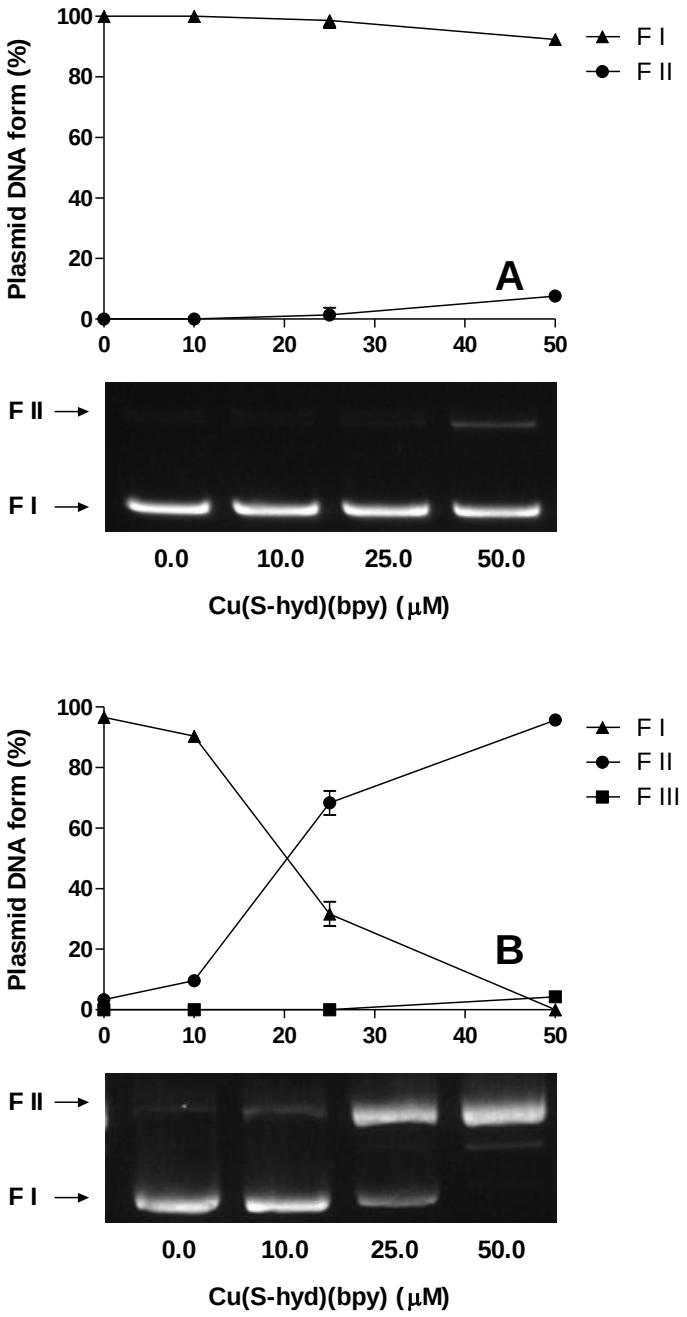

Figure S5. Comparison of DNA cleavage (A) *vs.* photocleavage (B) by **3** for 5 minutes. Reaction conditions: [DNA] = 330 ng, ~ 25 μM; [Buffer] = 10 mM Tris-HCl (pH 7;4); [complex] = 10 to 50 μM. Representative data from three independent experiments expressed as mean ± standard deviation.

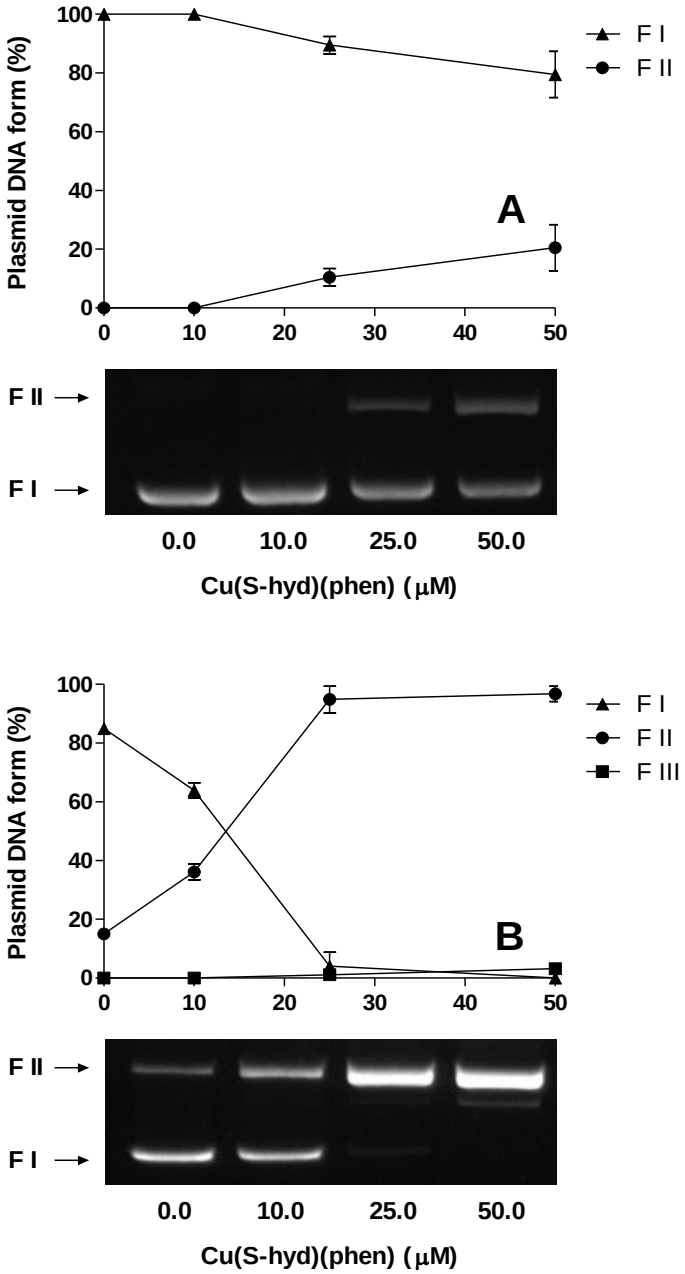

Figure S6. Comparison of DNA cleavage (A) *vs.* photocleavage (B) by **4** for 5 minutes. Reaction conditions: [DNA] = 330 ng, ~ 25 μM; [Buffer] = 10 mM Tris-HCl (pH 7;4); [complex] = 10 to 50 μM. Representative data from three independent experiments expressed as mean ± standard deviation.

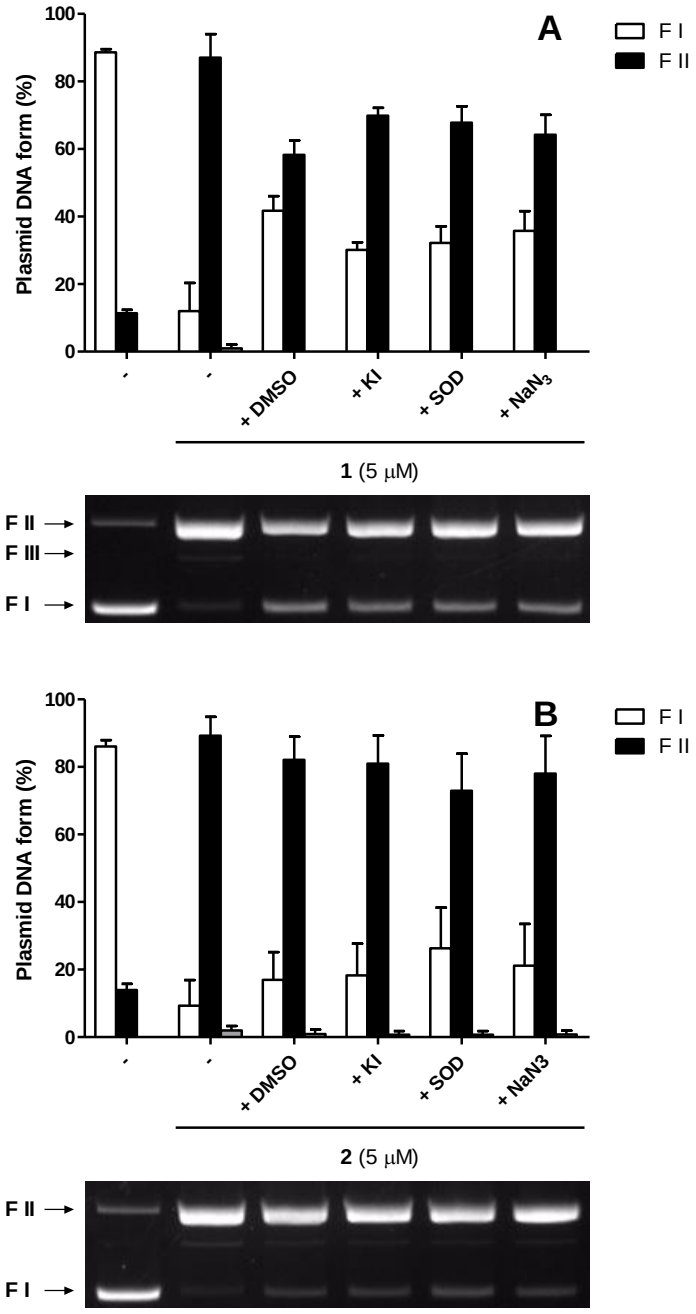

Figure S7. Effect of ROS scavengers on the cleavage of supercoiled DNA by **1** (A) and **2** (B). Reaction conditions: [DNA] = 330 ng, ~ 25 μM; [Buffer] = 10 mM Tris-HCl (pH 7;4); [complex] = 5.0 μM; [DMSO] = 2 M; [SOD] = 20 Units; [KI] = 0.5 mM; [NaN_3] = 0.5 mM; Time = 5 min under UV-light. Representative data from three independent experiments expressed as mean ± standard deviation.

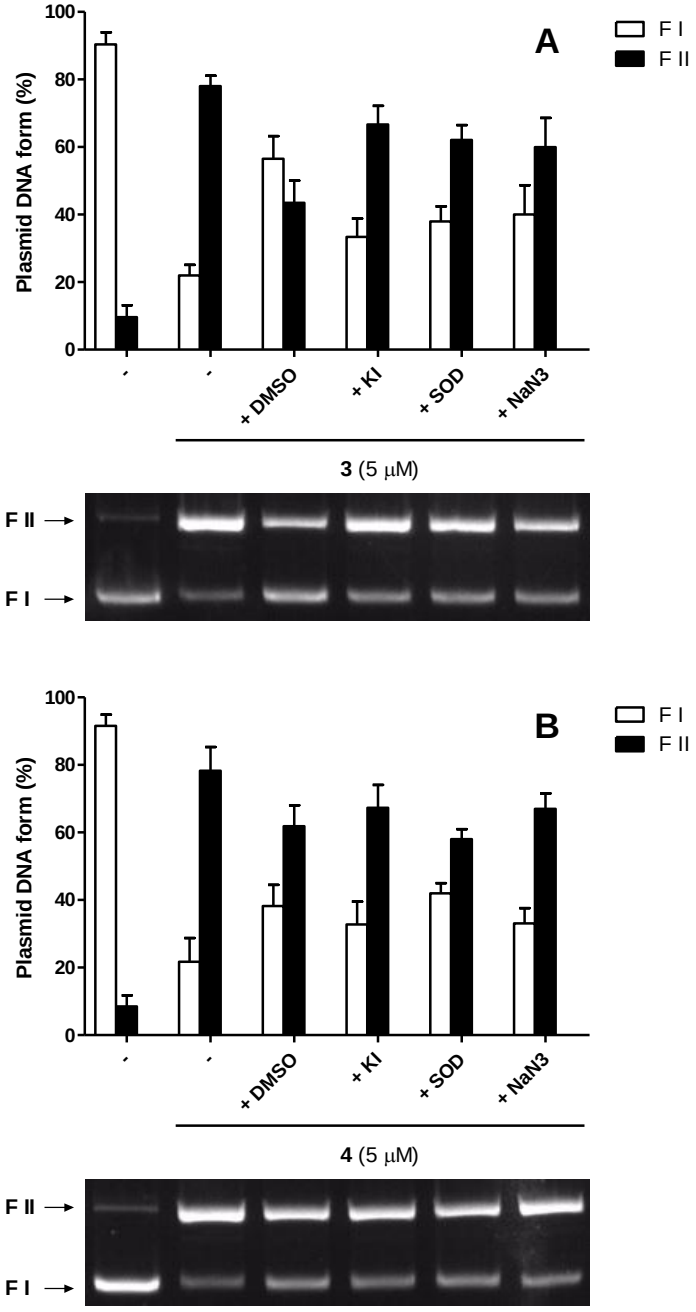

Figure S8. Effect of ROS scavengers on the cleavage of supercoiled DNA by **3** (A) and **4** (B). Reaction conditions: [DNA] = 330 ng, ~ 25 μM; [Buffer] = 10 mM Tris-HCl (pH 7;4); [complex] = 5.0 μM; [DMSO] = 2 M; [SOD] = 20 Units; [KI] = 0.5 mM; [NaN_3] = 0.5 mM; Time = 5 min under UV-light. Representative data from three independent experiments expressed as mean ± standard deviation.

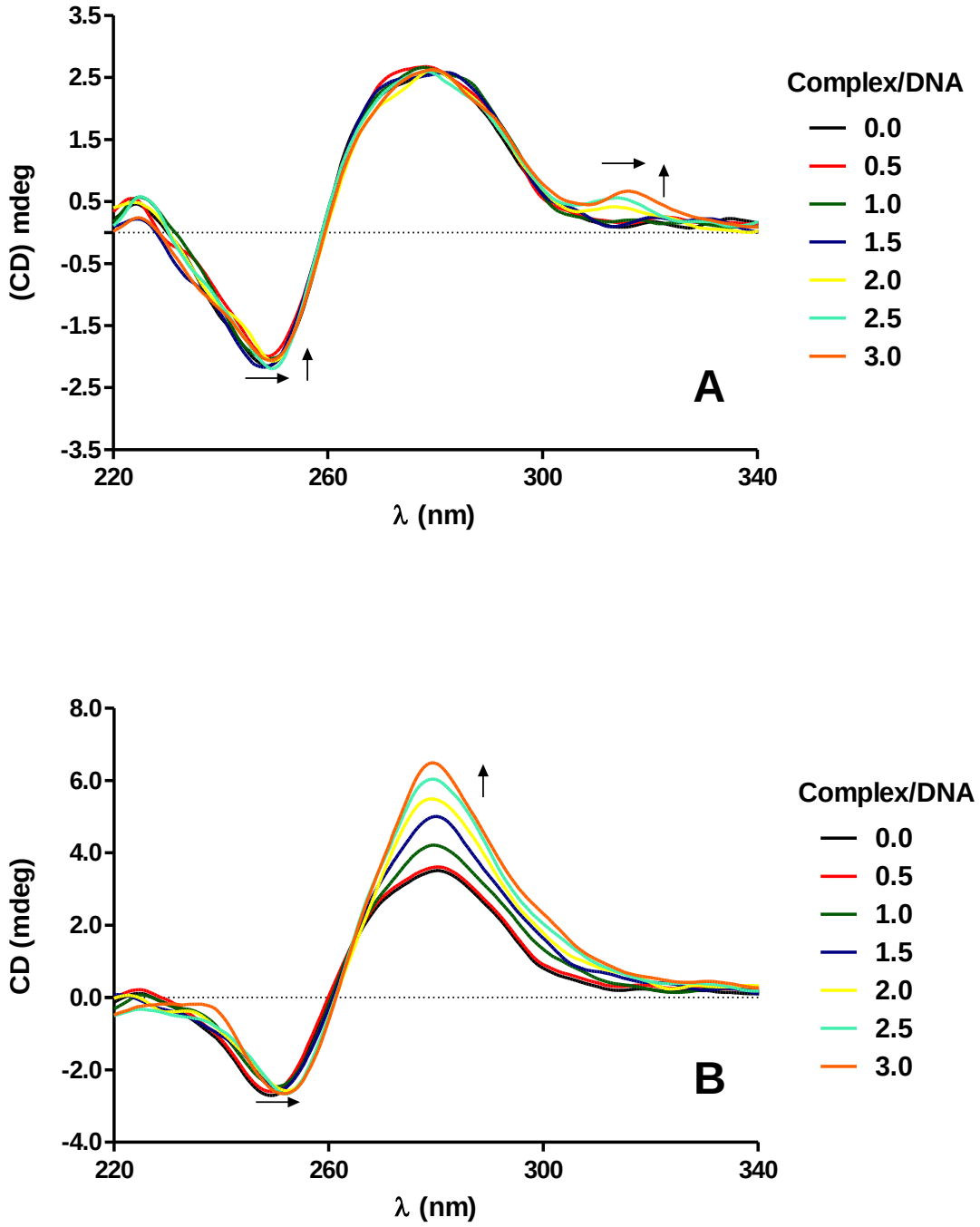

Figure S9. CD spectra of CT-DNA in absence and presence of increasing amounts of **1** (A) and **2** (B). Experimental conditions: [CT-DNA] = 100 μM in base pairs, [buffer] = 10 mM Tris-HCl (pH 7.4); [complex] = 0 to 300 μM, temperature = 37° C

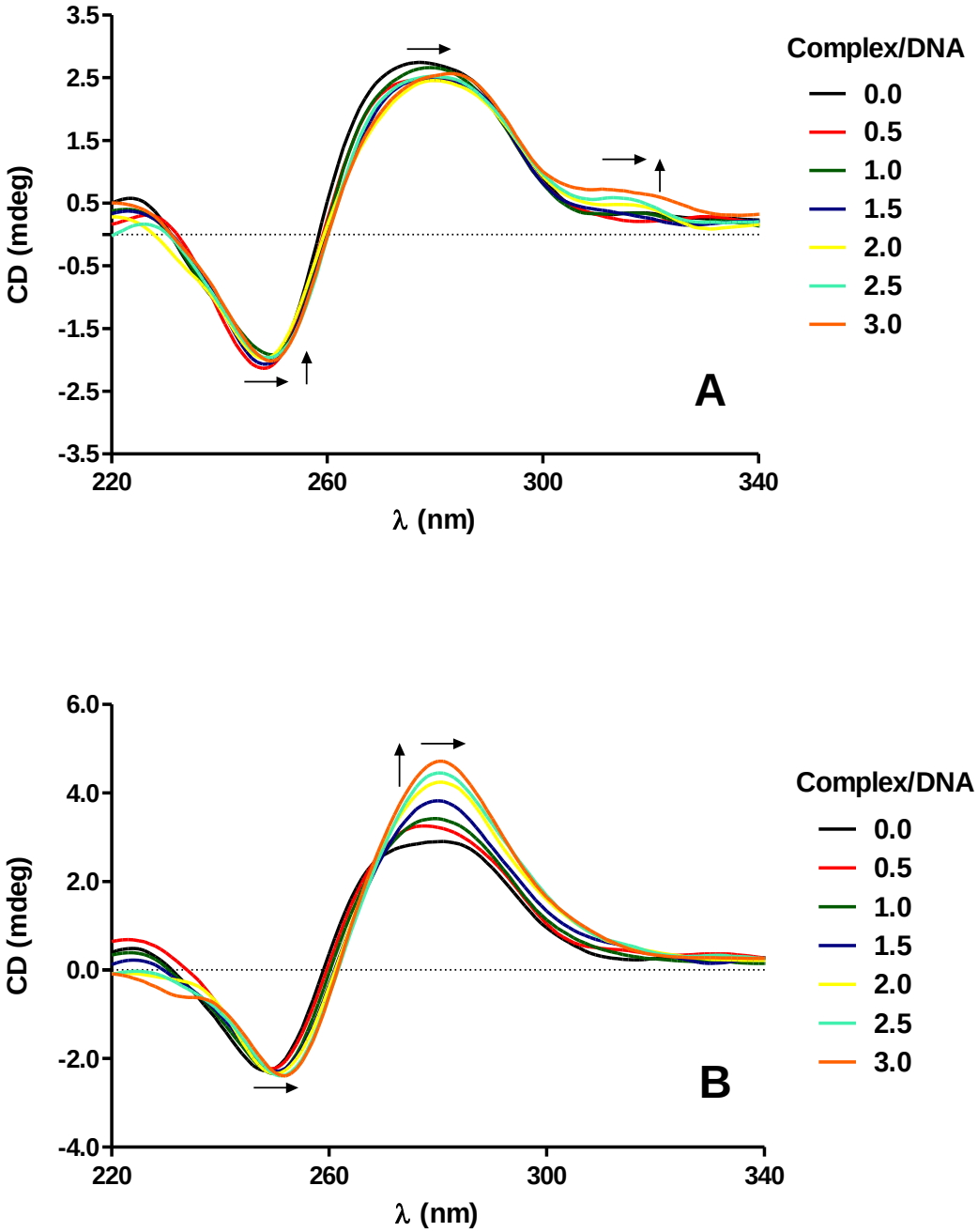

Figure S10. CD spectra of CT-DNA in absence and presence of increasing amounts of **3** (A) and **4** (B). Experimental conditions: [CT-DNA] = 100 μM in base pairs, [buffer] = 10 mM Tris-HCl (pH 7.4); [complex] = 0 to 300 μM, temperature = 37° C.

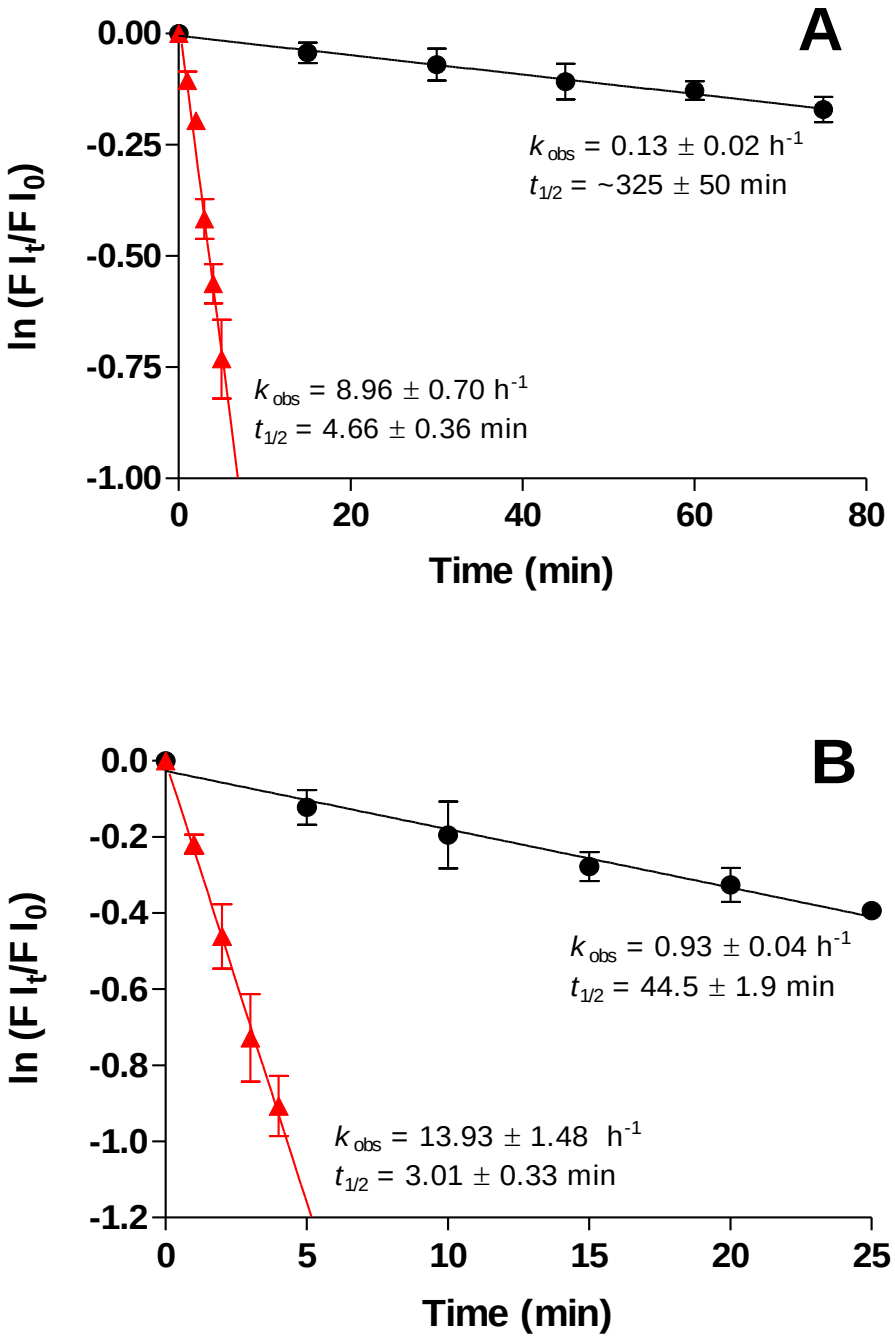

Figure S11. Pseudo-first-order kinetics of DNA cleavage (dark line) and photocleavage (red line) by **1** (A) and **2** (B). Reaction conditions (A): [complex] = 25 µM (cleavage conditions) and 10 µM (photocleavage conditions); Time = 0 to 75 minutes without light and 0 to 4 minutes under UV-light (A); Reaction conditions (B): [complex] = 10 µM (cleavage and photocleavage conditions); Time = 0 to 25 minutes without light and 0 to 4 minutes under UV-light.

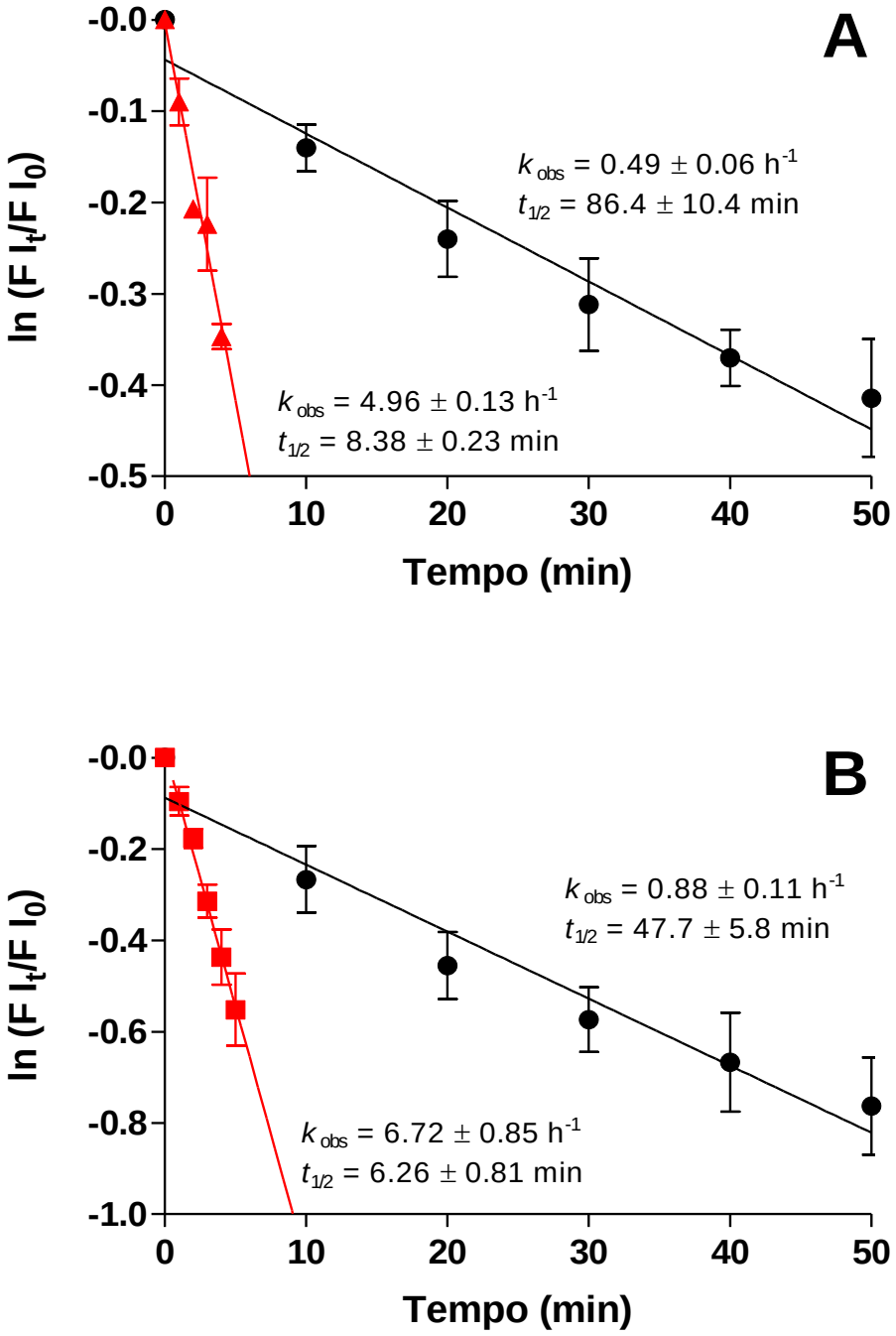

Figure S12. Pseudo-first-order kinetics of DNA cleavage (dark line) and photocleavage (red line) by **3** (A) and **4** (B). Reaction conditions (A): [complex] = 25 µM (cleavage conditions) and 10 µM (photocleavage conditions); Time = 0 to 50 minutes without light and 0 to 4 minutes under UV-light (A); Reaction conditions (B): [complex] = 10 µM (cleavage and photocleavage conditions); Time = 0 to 25 minutes without light and 0 to 5 minutes under UV-light.

Figure S13. Full-length sequence of the 49-mer oligonucleotide used as DNA substrate in denaturing PAGE assays. The hybridized version of this hairpin DNA show a 21-bp region containing two distinctive sequence sites: a AT-rich site (in red) and a GC-rich site (in blue). The TTTT hairpin segment is highlighted in green. The "F" denotes the fluorescein dye at 5'-terminal.

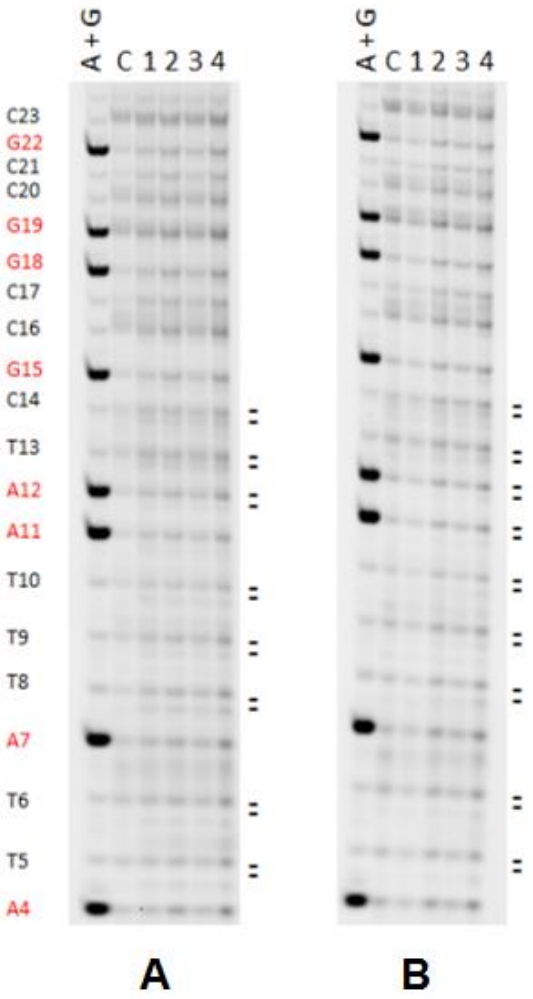

Figure S14. Cleavage (A) and photocleavage (B) of a 5'-FAM labeled 49-mer oligonucleotide by **1-4**. The gel image shows the representative area corresponding to the DNA fragments from C23 to A4 of oligonucleotide sequence. Full-length gel image are showed in SI (Figure S14). The "C" lane of each gel represents the control reaction without complex and the A+G lane stands for the Adenine + Guanine ladder by Maxam-Gilbert reaction. Beside the gels are noted the terminal nucleotide of each fragment from 49-mer to 4-mer long as well in red the A+G ones. The double "-" at certain bands indicate two different 3'-termini: the 3'-phosphate (upper band) and 3'-phosphoglycolate (lower band). Reaction conditions: [DNA] = 50 pmol; [Buffer] = 10 mM Tris-HCl (pH 7.4); [complex] = 50 µM; Incubation = 24 h in the dark or 5 min under UV-light (12 W). Representative gel from two independent assays.

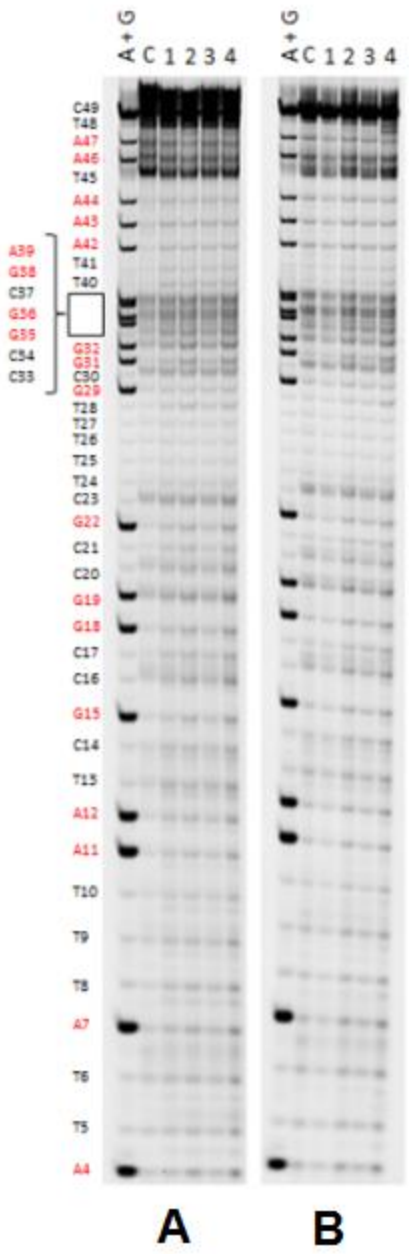

Figure S15. Full-length image of the representative gel shown in Figure S14.

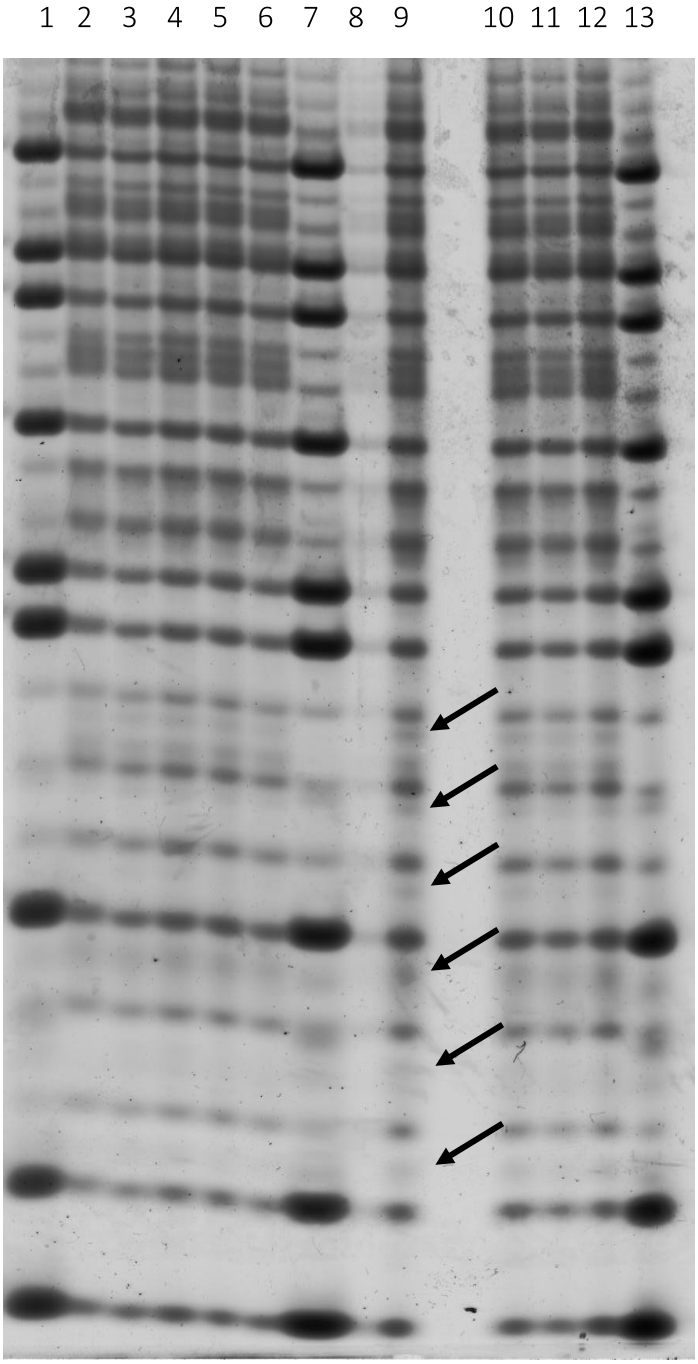

Figure S16. Photocleavage of a 5'-FAM labeled 49-mer oligonucleotide by **1-4**. The gel image shows the representative area corresponding to the DNA fragments from T25 to G3 of the oligonucleotide sequence (see Figure S14 and S15 for reference The lanes of the gel are as follows: 1) Maxam-Gilbert Adenine + Guanine ladder; 2) negative control; 3 to 6) **1** to **4** at 50μM; 7) Maxam-Gilbert Adenine + Guanine ladder; 8) negative control; 9 to12) **1** to **4** at 100μ; 13) Maxam-Gilbert Adenine + Guanine ladder. The double bands indicated by arrows indicate two different 3'-termini: the 3'-phosphate (upper band) and 3'-phosphoglycolate (lower band). Reaction conditions: [DNA] = 50 pmol; [Buffer] = 10 mM Tris-HCl (pH 7.4); [complex] = 50 or 100μM; Incubation 5 min under UV-light (12 W).

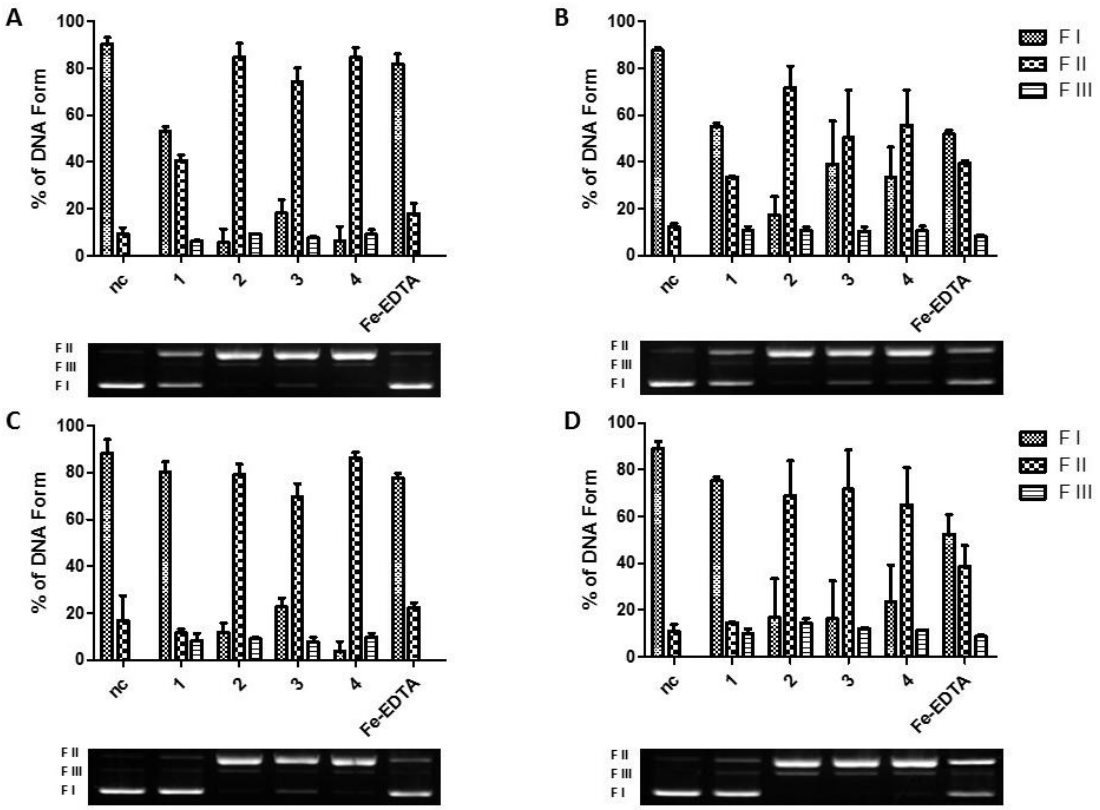

Figure S17: Complexes **1**, **2**, **3** and **4** activity under argon or oxygen atmosphere in the presence of UV light (A and B, respectively, 1 min exposure) or in the absence of light (C and D, respectively, 1 hour exposure). The results show that the mechanism of reaction is oxygen independent in both conditions. Complexes concentration was 100 μM. "Fe-EDTA" – control reaction for the presence of oxygen, and "nc" – negative control.

SI References

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