Electronic Supplementary Material (ESI) for Dalton Transactions.

Copper(I) complexes with phosphine derived from sparfloxacin.

Part II: a first insight into the cytotoxic action mode.

U. K. Komarnicka,[a] R. Starosta,[a] M. Płotek,[b] R. F.M. de Almeida,[c] M. Jeżowska-Bojczuk[a] and A. Kyzioł*[b]*

[a]Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław,

[b]Faculty of Chemistry, Jagiellonian University, R. Ingardena 3, 30-060 Kraków, Poland

[c]Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal

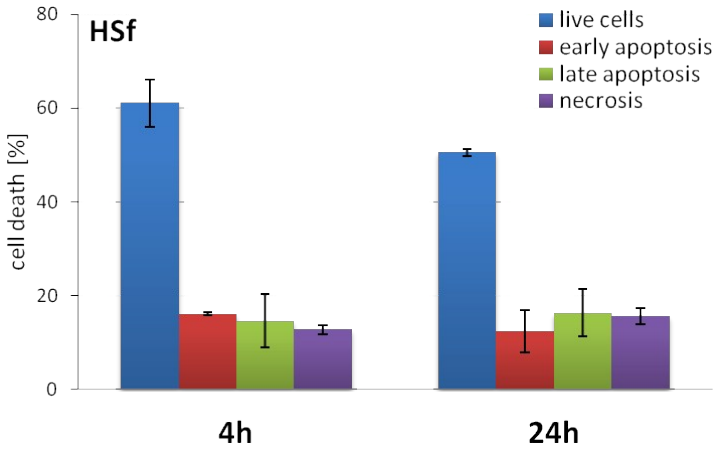


Fig. S1 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line CT26 for **HSf**.

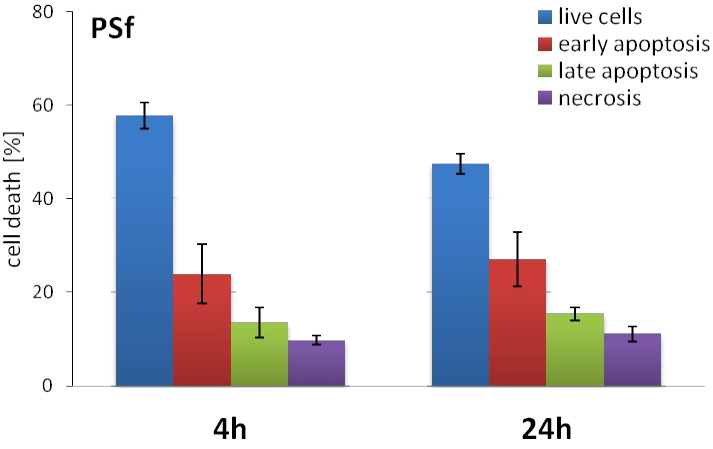


Fig. S2 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line CT26 for **PSf**.

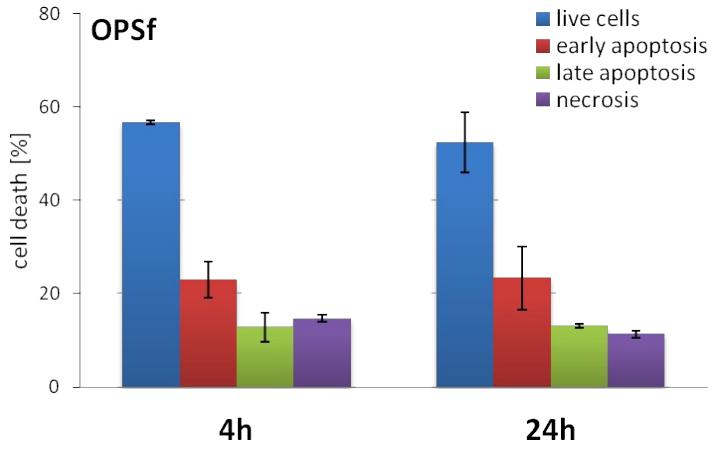


Fig. S3 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line CT26 for **OPSf**.

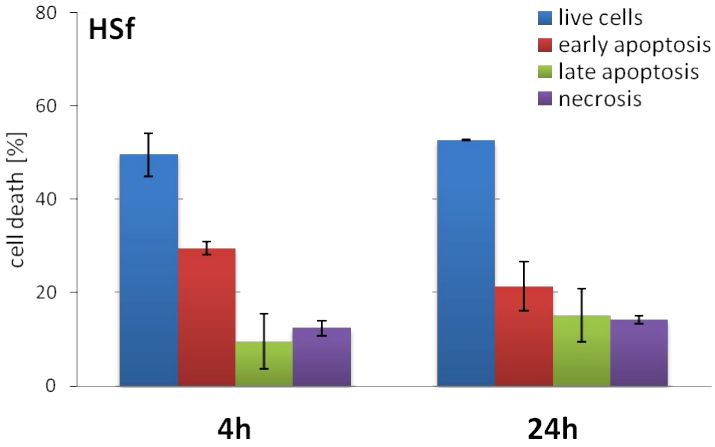


Fig. S4 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line A549 for **HSf**.

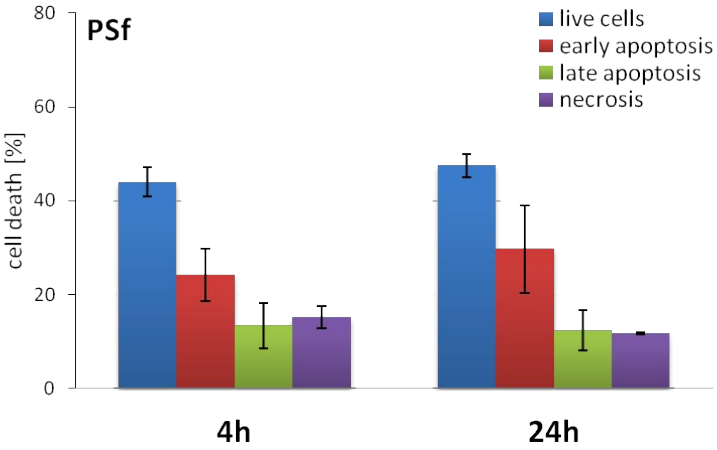


Fig. S5 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line A549 for **PSf**.

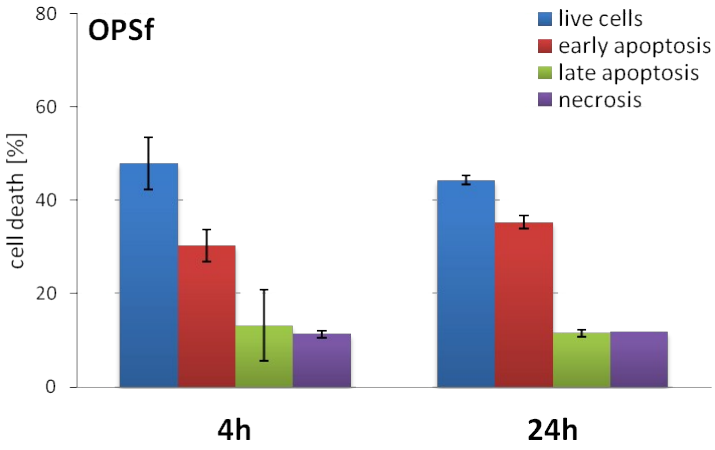


Fig. S6 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line A549 for **OPSf**.

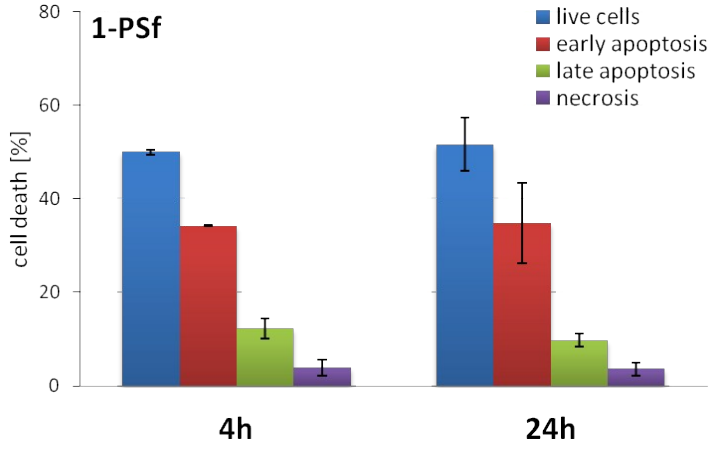


Fig. S7 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line A549 for **1-PSf**.

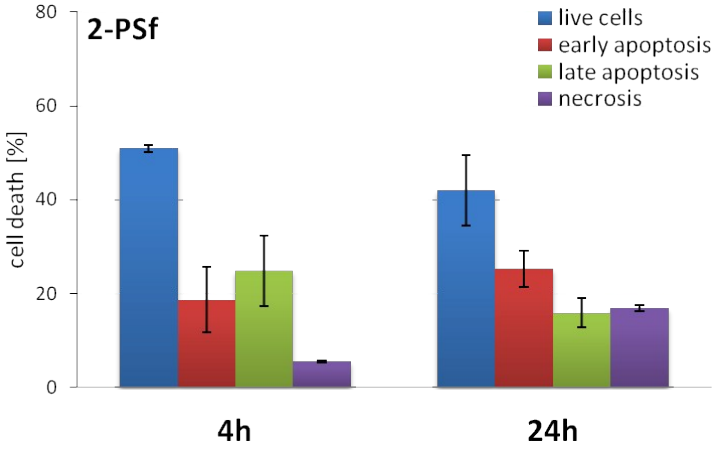


Fig. S8 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line A549 for **2-PSf**.

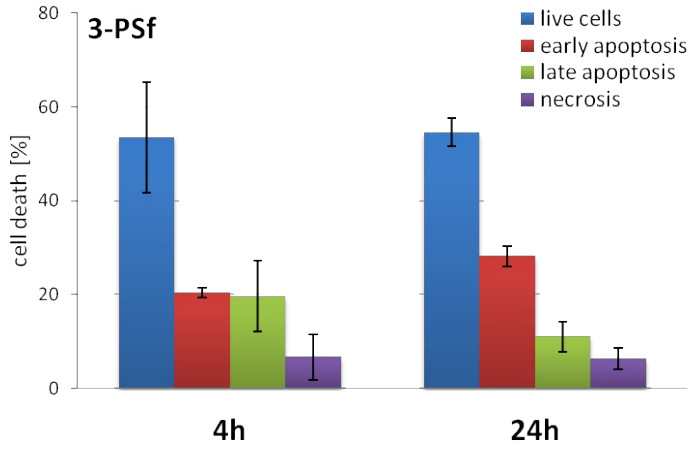


Fig. S9 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line A549 for **3-PSf**.

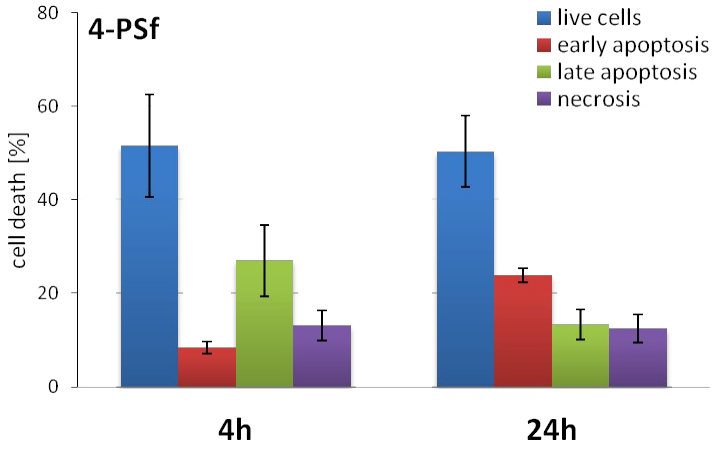


Fig. S10 Graph showing percentage shares of the cell death induced after 4 h and 24 h of incubation with cell line A549 for **4-PSf**.

Table S1 Percentage [%] of normal, apoptotic and necrotic cells after 4 and 24 h of incubation of CT26 cell line with complexes: a) 1-PSf b) 2-PSf, c) 3-PSf and d) 4-PSf in IC50.

	live cells [%]	early apoptosis [%]	late apoptosis [%]	necrosis [%]
	CT26, 4h			
HSf	61.08±5.07	14.13±0.29	13.04±5.68	11.75±0.97
PSf	55.85±2.78	22.91±6.33	12.52±3.15	8.72±0.43
OPSf	55.71±0.42	21.98±3.93	11.78±3.07	10.53±0.70
1-PSf	56.84±7.36	32.32±3.76	9.19±3.56	1.55±0.16
2-PSf	51.70±5.92	19.33±6.94	13.95±0.81	15.02±0.21
3-PSf	50.22±3.38	23.05±1.02	25.35±4.95	1.38±0.56
4-PSf	47.20±6.33	20.29±2.68	20.34±8.25	12.17±4.88
	CT26, 24h			
HSf	54.60±0.76	12.91±4.94	16.85±5.59	15.65±1.70
PSf	47.07±2.18	27.06±5.26	15.01±1.36	10.86±0.28
OPSf	52.31±6.41	23.30±6.75	13.11±0.51	11.28±0.86
1-PSf	53.93±2.19	19.22±8.88	22.17±9.89	4.68±1.25
2-PSf	47.22±3.92	17.44±8.17	25.18±5.82	10.16±4.83
3-PSf	50.28±3.32	24.17±1.64	18.02±5.05	7.53±1.32
4-PSf	50.86±1.69	24.94±0.83	15.78±0.37	8.42±2.29
	A549, 4h			
HSf	49.53±4.63	28.50±1.36	9.55±5.82	12.42±1.60
PSf	44.35±3.04	24.18±5.63	13.36±4.88	15.11±2.37
OPSf	46.93±5.56	30.25±3.42	12.16±7.30	11.33±0.42
1-PSf	49.93±0.52	34.20±0.16	12.30±2.12	3.57±1.76
2-PSf	50.97±0.76	18.70±7.05	24.80±7.56	5.53±0.25
3-PSf	51.51±10.97	8.37±1.24	26.98±10.31	13.14±1.91
4-PSf	53.36±11.79	20.36±1.06	19.63±7.56	6.65±3.17
	A549, 24h			
HSf	51.40±0.14	21.31±5.28	14.13±5.76	13.16±0.84
PSf	47.53±2.50	29.60±9.32	12.42±4.31	10.45±0.19
OPSf	44.26±0.95	34.05±1.36	11.29±0.78	10.40±0.04
1-PSf	51.57±5.66	34.82±8.53	9.75±1.44	3.86±1.42
2-PSf	41.92±7.51	25.26±3.79	15.88±3.08	16.94±0.63
3-PSf	50.33±7.58	23.77±1.53	13.39±8.25	12.51±2.41
4-PSf	54.56±3.00	28.18±2.19	10.97±3.23	6.29±2.94

Table S2. Final intracellular copper concentration expressed by ng Cu/mg protein after 4 and 24 h of incubation with cancer line CT26 for complexes **1-PSf**, **2-PSf**, **3-PSf** and **4-PSf**; in IC_{50}.

	ng Cu/mg protein±SD	
	4h	24h
control	61.67± 9.01	85.83±12.13
1-PSf	411.66±12.97	853.18±33.11
2-PSf	287.92±18.93	906.12±25.01
3-PSf	328.42±23.19	373.01±31.04
4-PSf	419.49±38.24	711.10±47.43

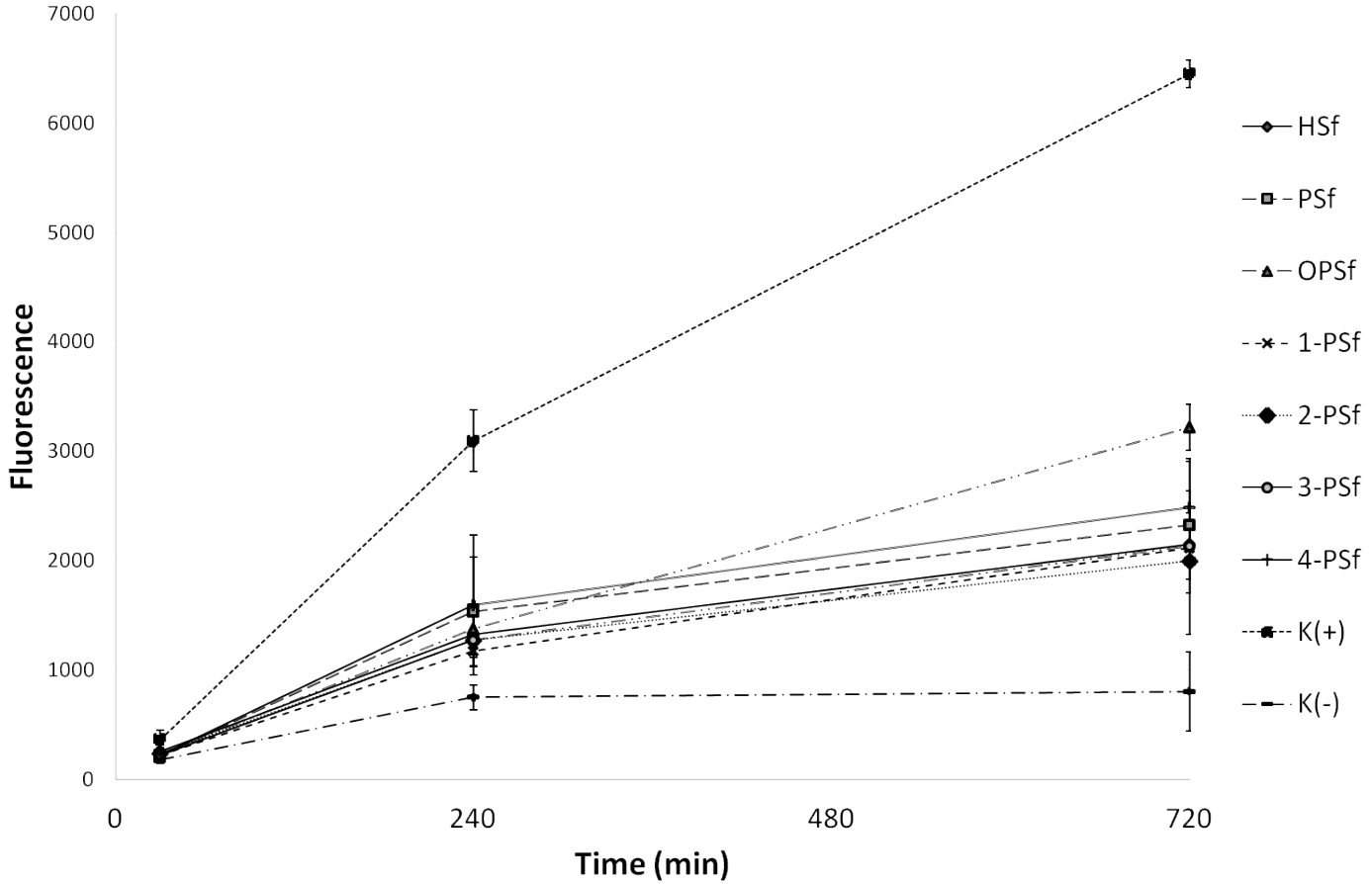


Fig. 11 Oxidative stress induced by the total ROS production in A549 cells after 30 minutes, 4 and 12 h detected by CYTO-ID Hypoxia/Oxidative Stress test for **HSf**, **PSf**, **OPSf**, **1-PSf**, **2-PSf**, **3-PSf** and **4-PSf** K(+) – positive control pyocyanin and K(-) – negative control A549 cells without compounds.

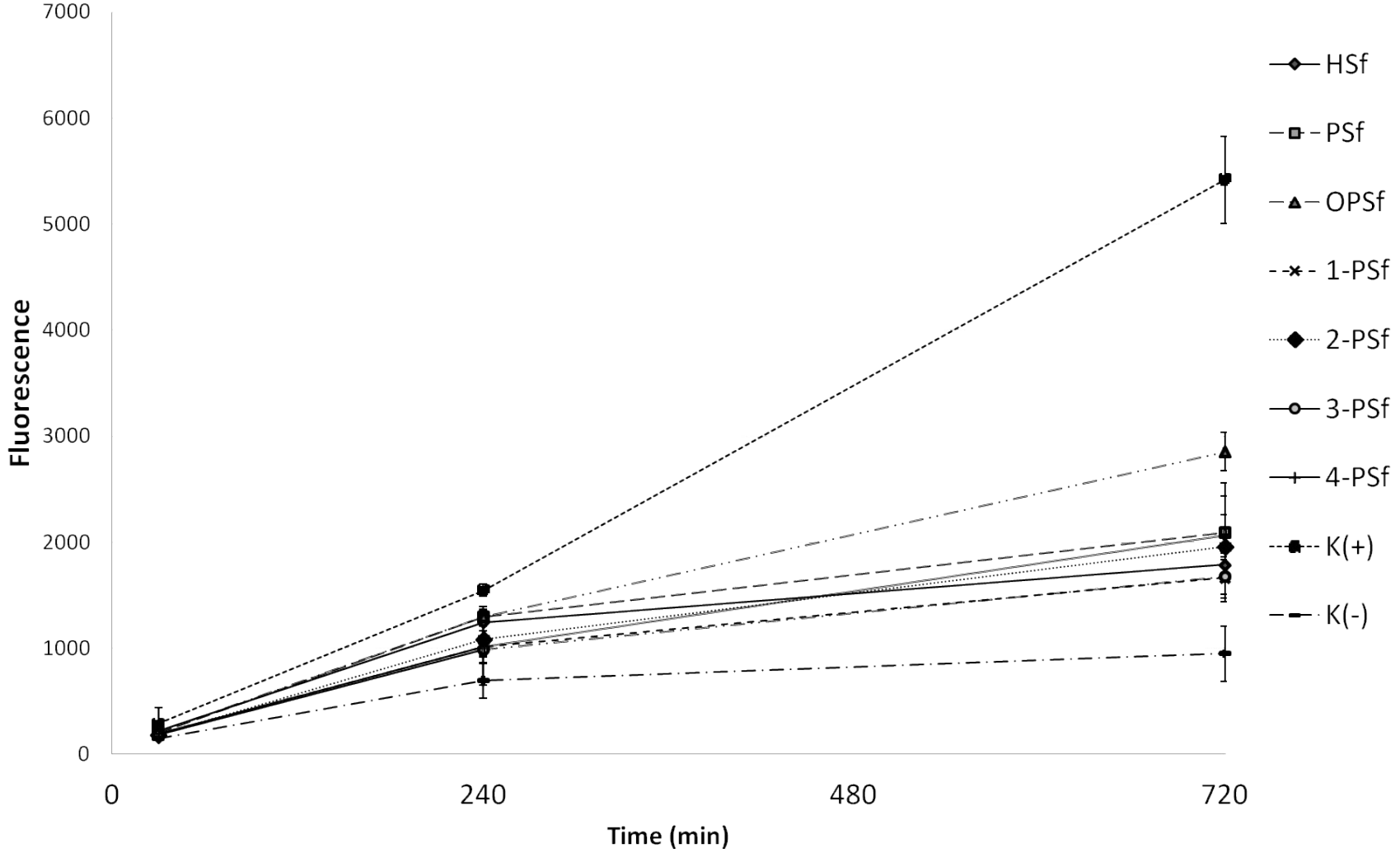


Fig. S12 Oxidative stress induced by the total ROS production in CT26 cells after 30 minutes, 4 and 12 h detected by CYTO-ID Hypoxia/Oxidative Stress test for **HSf**, **PSf**, **OPSf**, **1-PSf**, **2-PSf**, **3-PSf** and **4-PSf** K(+) – positive control pyocyanin and K(-) – negative control CT26 cells without compounds.

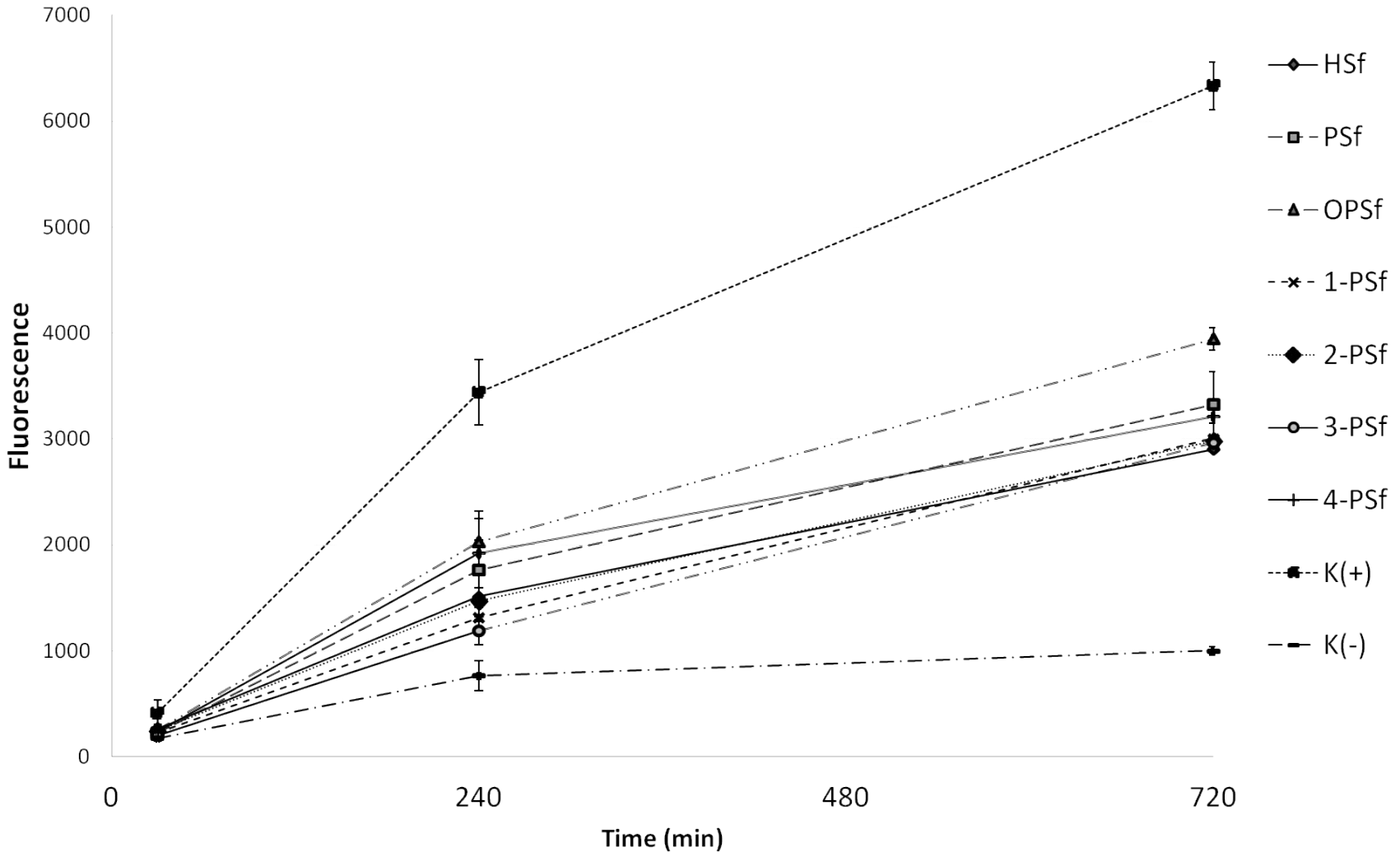


Fig. S13 The increase of ROS production in A549 cells after 30 minutes, 4 and 12 h using H2DCF-DA for **HSf**, **PSf**, **OPSf**, **1-PSf**, **2-PSf**, **3-PSf** and **4-PSf**, K(+) – positive control H_2O_2 and K(-) – negative control A549 cells without compounds.

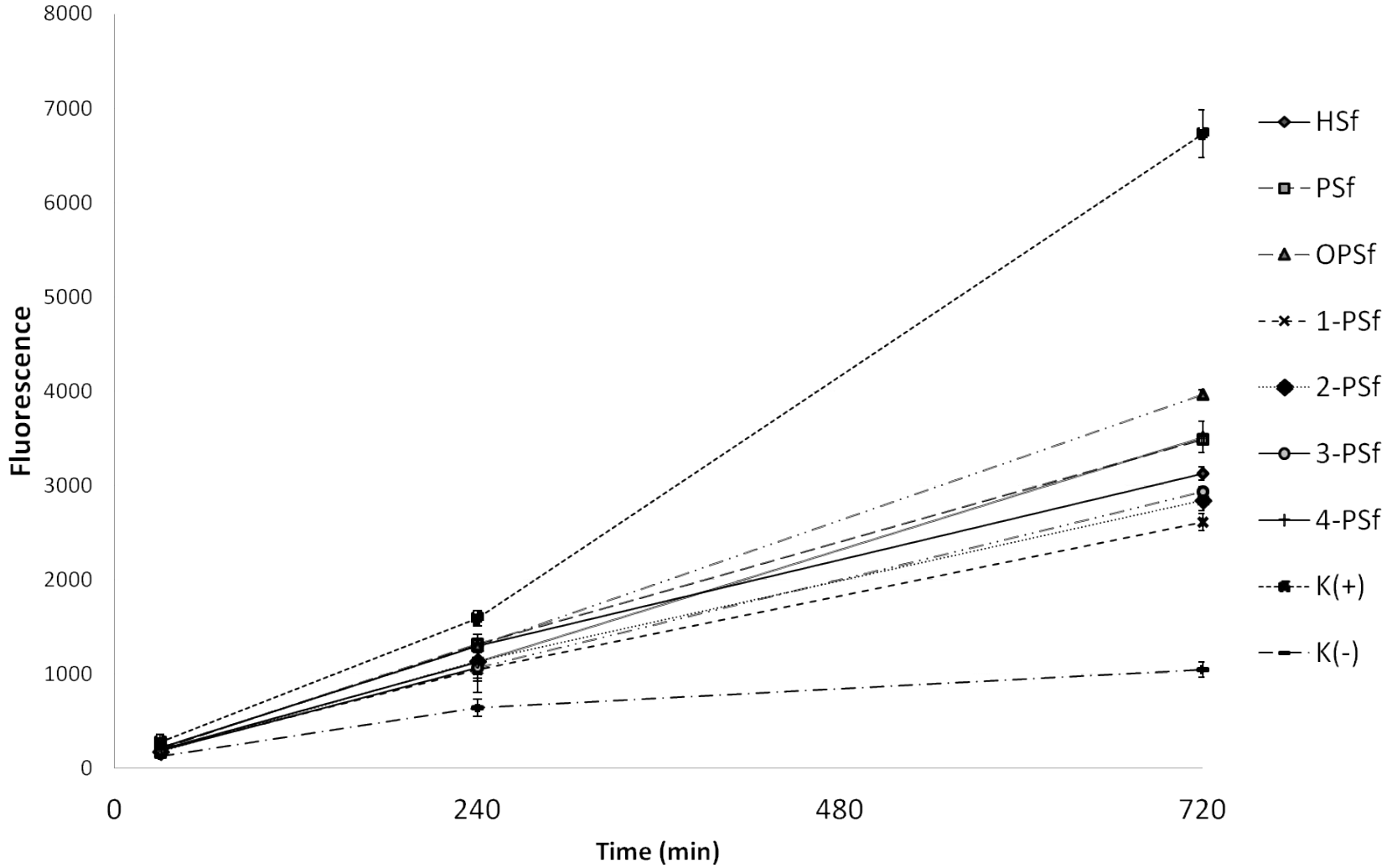


Fig. S14 The increase of ROS production in CT26 cells after 30 minutes, 4 and 12 h using H2DCF-DA for **HSf**, **PSf**, **OPSf**, **1-PSf**, **2-PSf**, **3-PSf** and **4-PSf**, K(+) – positive control H_2O_2 and K(-) – negative control CT26 cells without compounds.

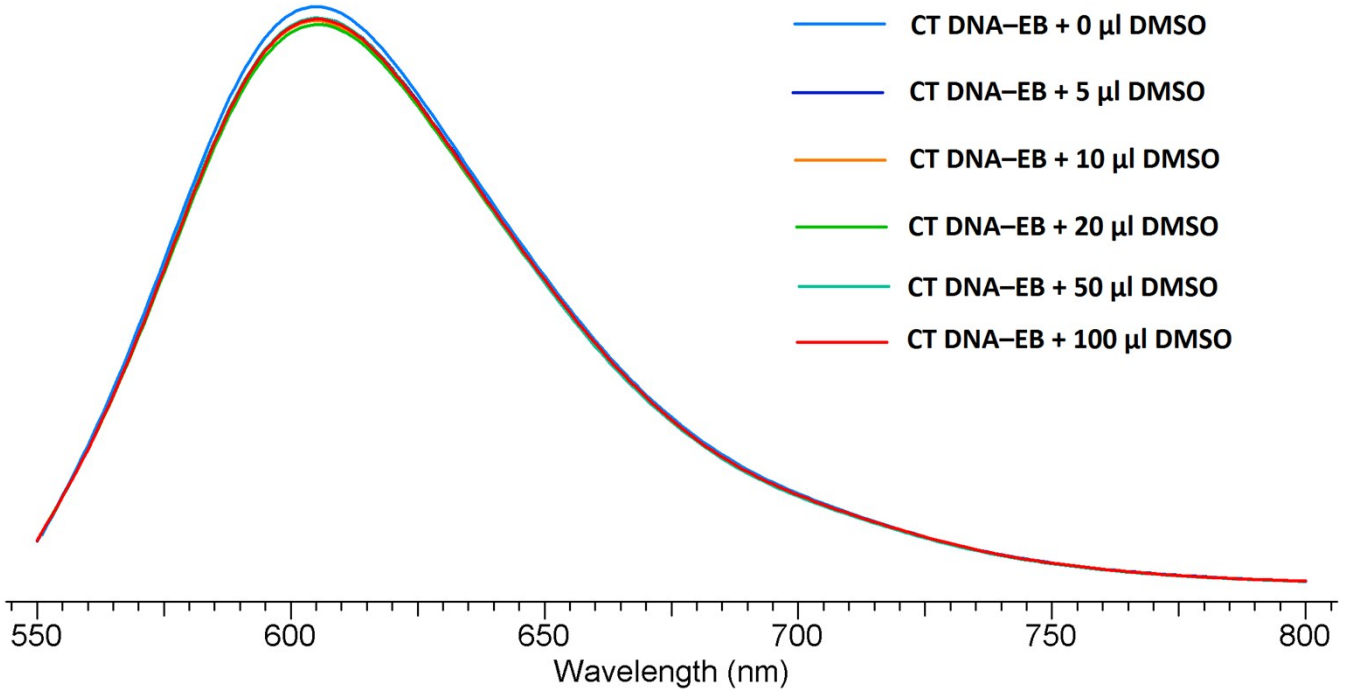
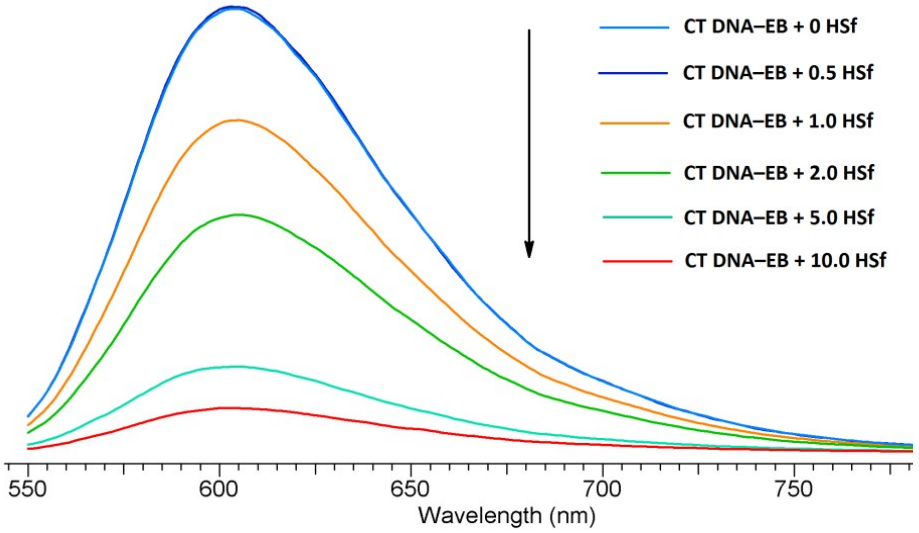
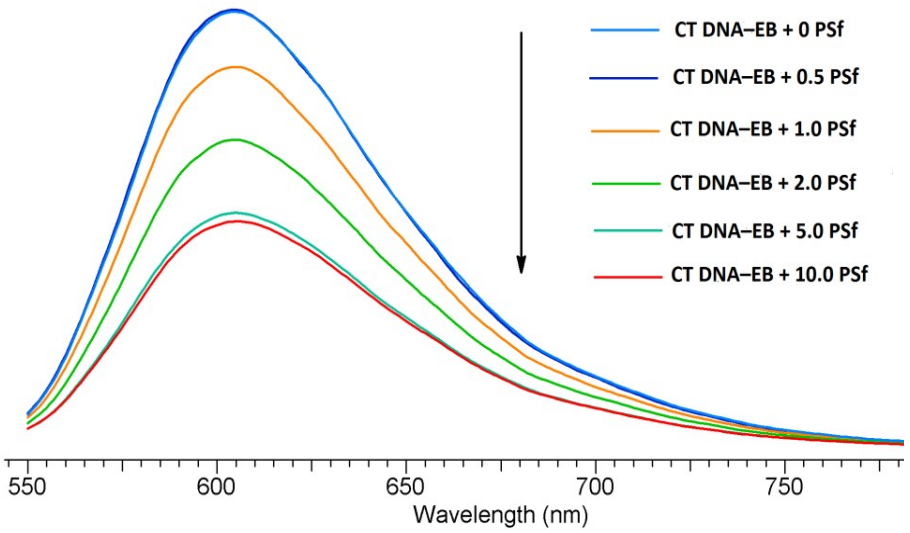


Fig. S15 Fluorescence quenching of EB–CT DNA by the increasing volumes of DMSO (3ml of EB–CT DNA solution in buffered (pH = 7.4) water solution).





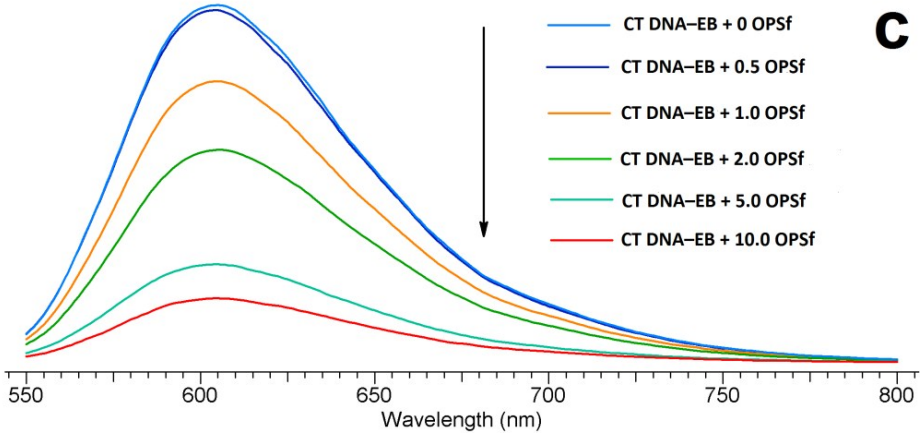


Fig. S16 Fluorescence quenching of EB–CT DNA (c= $5 \cdot 10^{-5}$M) by a) **HSf**, b) **PSf**, c) **OPSf** (molar ratios 0.5; 1.0; 2.0; 5.0 and 10.0) in 50 mM pH 7.4 buffer.

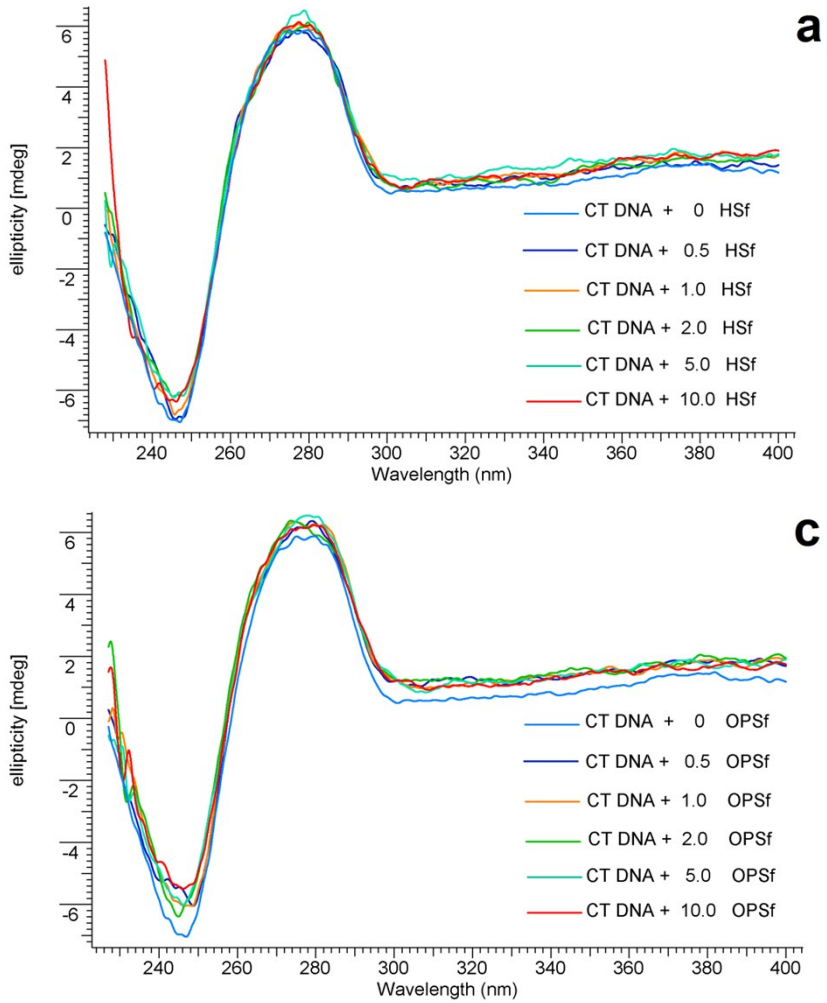


Fig. S17 Circular dichroism spectra of CT DNA (c= $5 \cdot 10^{-5}$M) with a) **HSf**, b) **PSf**, c) **OPSf** (molar ratios 0; 0.5; 1.0; 2.0; 5.0 and 10.0) in 50 mM pH 7.4 buffer.

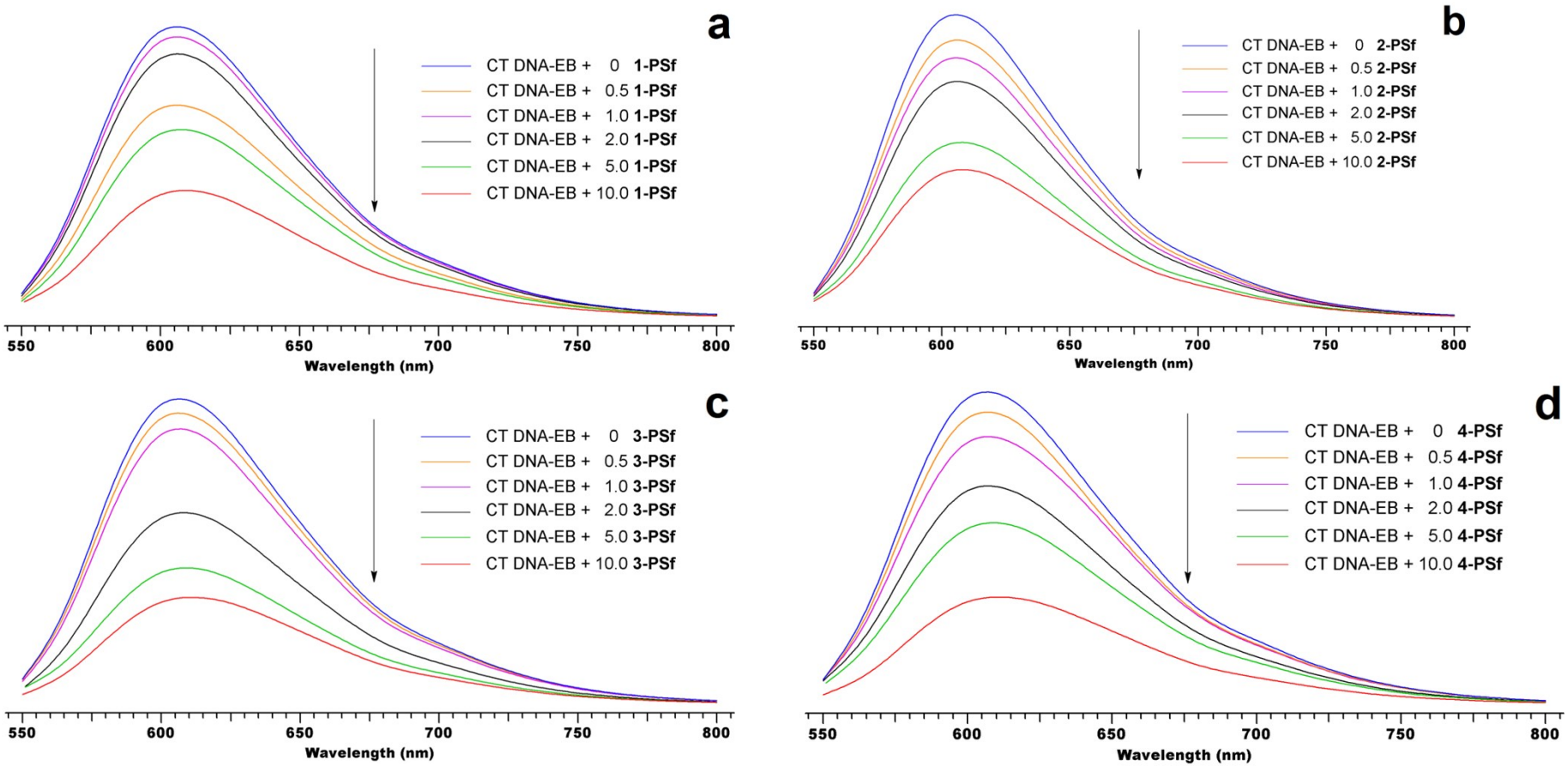


Fig. S18 Fluorescence quenching of EB–CT DNA (c= $5 \cdot 10^{-5}$M) by a) **1-PSf**, b) 2-**PSf**, c) **3-PSf**, d) **4-PSf** (molar ratios 0.5; 1.0; 2.0; 5.0 and 10.0) in 50 mM pH 7.4 buffer.

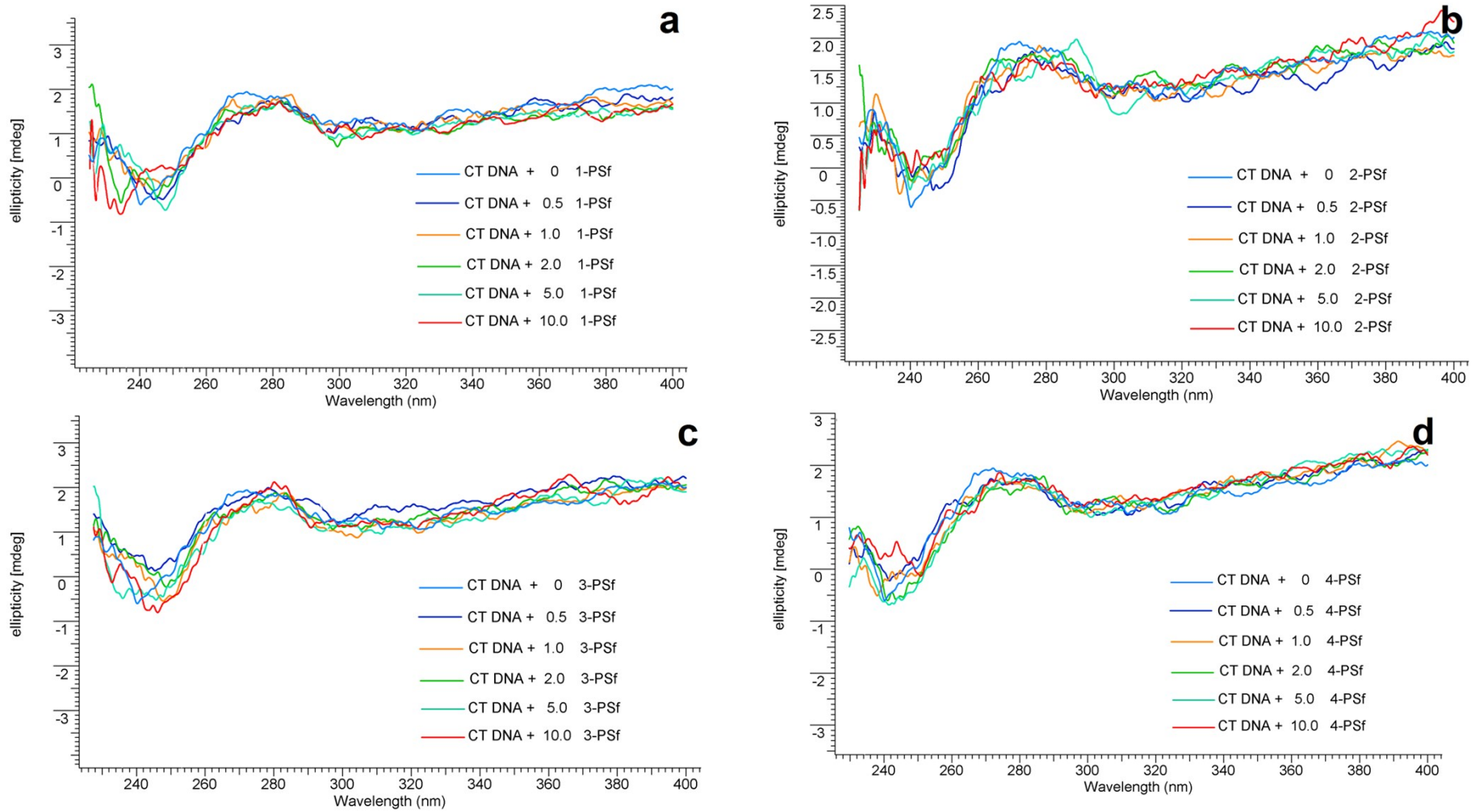
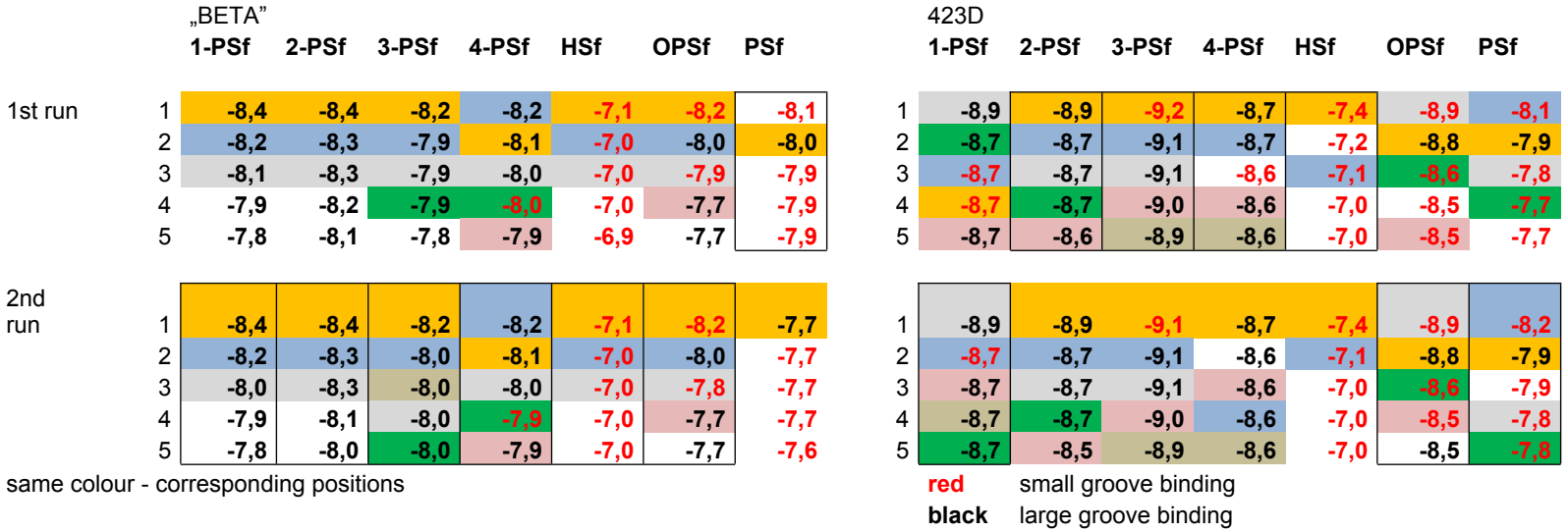


Fig. S19 Circular dichroism spectra of CT DNA (c= 5•10-5M) with a) **1-PSf**, b) **2-PSf**, c) **3-PSf**, d) **4-PSf** (molar ratios 0; 0.5; 1.0; 2.0; 5.0 and 10.0) in 50 mM pH 7.4 buffer.



		„BETA" 1-PSf	2-PSf	3-PSf	4-PSf	HSf	OPSf	PSf	423D 1-PSf	2-PSf	3-PSf	4-PSf	HSf	OPSf	PSf
1st run	1	-8,4	-8,4	-8,2	-8,2	-7,1	-8,2	-8,1	-8,9	-8,9	-9,2	-8,7	-7,4	-8,9	-8,1
	2	-8,2	-8,3	-7,9	-8,1	-7,0	-8,0	-8,0	-8,7	-8,7	-9,1	-8,7	-7,2	-8,8	-7,9
	3	-8,1	-8,3	-7,9	-8,0	-7,0	-7,9	-7,9	-8,7	-8,7	-9,1	-8,6	-7,1	-8,6	-7,8
	4	-7,9	-8,2	-7,9	-8,0	-7,0	-7,7	-7,9	-8,7	-8,7	-9,0	-8,6	-7,0	-8,5	-7,7
	5	-7,8	-8,1	-7,8	-7,9	-6,9	-7,7	-7,9	-8,7	-8,6	-8,9	-8,6	-7,0	-8,5	-7,7
2nd run	1	-8,4	-8,4	-8,2	-8,2	-7,1	-8,2	-7,7	-8,9	-8,9	-9,1	-8,7	-7,4	-8,9	-8,2
	2	-8,2	-8,3	-8,0	-8,1	-7,0	-8,0	-7,7	-8,7	-8,7	-9,1	-8,6	-7,1	-8,8	-7,9
	3	-8,0	-8,3	-8,0	-8,0	-7,0	-7,8	-7,7	-8,7	-8,7	-9,1	-8,6	-7,0	-8,6	-7,9
	4	-7,9	-8,1	-8,0	-7,9	-7,0	-7,7	-7,7	-8,7	-8,7	-9,0	-8,6	-7,0	-8,5	-7,8
	5	-7,8	-8,0	-8,0	-7,9	-7,0	-7,7	-7,6	-8,7	-8,5	-8,9	-8,6	-7,0	-8,5	-7,8

same colour - corresponding positions

red small groove binding

black large groove binding

Table S3. The binding free energies [kcal/mol] of the lowest energy conformers with the comments for all the tested compounds interacting with the double-stranded helical DNA (an optimized synthetic canonical B form of the DNA ds hexadecanucleotide ("BETA", ATATCGCGATATCGCG) and DNA ds dodecanucleotide (PDB ID 423D, ACCGACGTCGGT)[44]). The calculations were performed twice (1st run and 2nd run), but only one (marked by a black frame) was taken into the considerations.

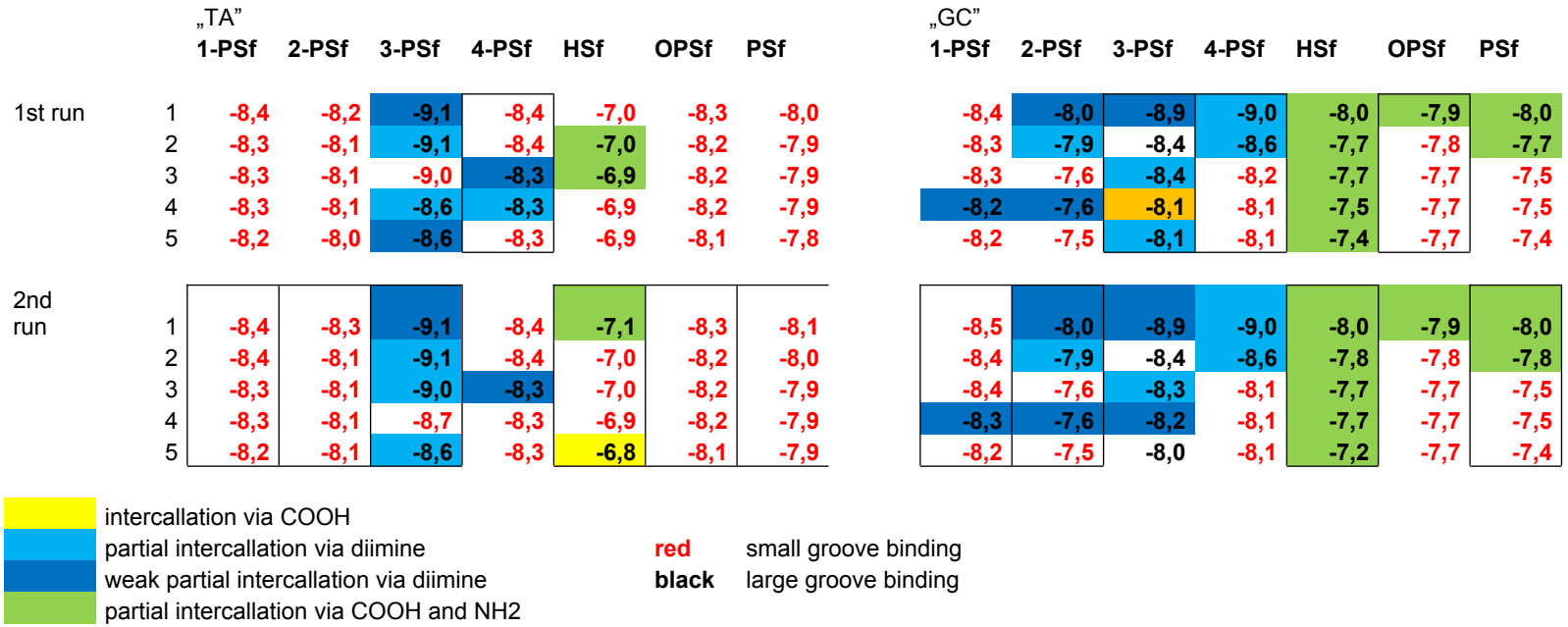


Table S4. The binding free energies [kcal/mol] of the lowest energy conformers with the comments for all the tested compounds interacting with the double-stranded helical DNA with two different optimized structures of the ATATCGCGATATCGCG ds hexadecanucleotide with pre-formed intercalating gaps between the 6th and 7th base pairs ("GC" gap) or between the 10th and 11th base pairs ("TA" gap)[45]. The calculations were performed twice (1st run and 2nd run), but only one (marked by a black frame) was taken into the considerations.

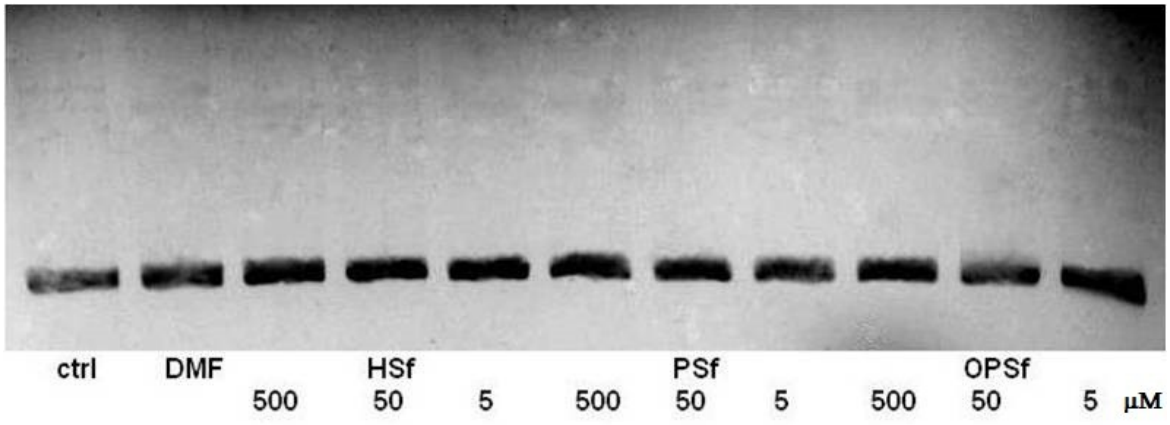


Fig. S20 Agarose gel electrophoresis of pBluescsriptSK+ plasmid DNA cleavage by **HSf**, **PSf** and **OPSf** (each in the 10% DMF). Lanes from left: 1, control plasmid (in the buffer solution); 2, plasmid in the 10% DMF/buffer solution; 3, plasmid+ 500 μM **HSf**; 4, plasmid+ 50 μM **HSf**; 5, plasmid+ 5 μM **HSf**; 6, plasmid+ 500 μM **PSf**; 7, plasmid+ 50 μM **PSf**; 8, plasmid+ 5 μM **PSf**; 9, plasmid+ 500 μM **OPSf**; 10, plasmid+ 50 μM **OPSf**; 11, plasmid+ 5 μM **OPSf**.

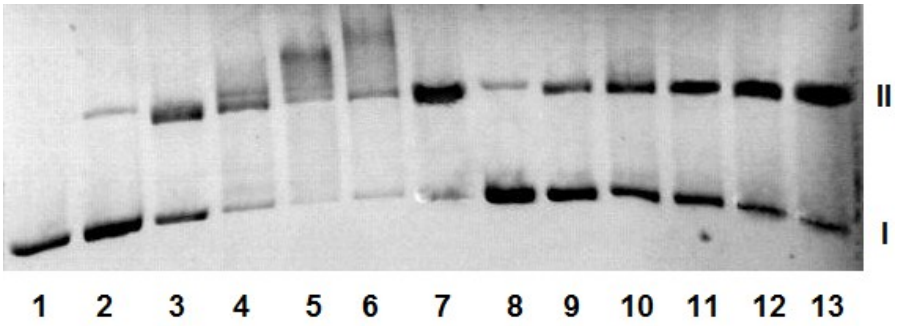


Fig. S21 Agarose gel electrophoresis of pBR322 plasmid cleavage by copper(I) complexes in a DMF (each in the 10% DMF) solution. Lanes: 1, plasmid + DMF; 2, plasmid + 10 μM **1-PSf**; 3, plasmid + 20 μM **1-PSf**; 4, plasmid + 50 μM **1-PSf**; 5, plasmid + 100 μM **1-PSf**; 6, plasmid + 200 μM **1-PSf**; 7, plasmid + 500 μM **1-PSf**; 8, plasmid + 10 μM **2-PSf**; 9, plasmid + 20 μM **2-PSf**; 10, plasmid + 50 μM **2-PSf**; 11, plasmid + 100 μM **2-PSf**; 12, plasmid + 200 μM **2-PSf**; 13, plasmid + 500 μM **2-PSf.**

Table S5. Densitometric analysis of plasmid cleavage by **1-PSf** and **2-PSf**, % plasmid forms.

Lines	Concentrations [μM]	Form I [%]	Form II [%]
1-PSf			
2	10	78.269	21.731
3	20	47.085	52.915
4	50	26.509	73.491
5	100	19.351	80.649
6	200	14.547	85.453
7	500	12.945	87.055
2-PSf			
8	10	79.898	20.102
9	20	64.074	35.926
10	50	49.149	50.851
11	100	42.994	57.006
12	200	32.557	67.443
13	500	30.059	69.941

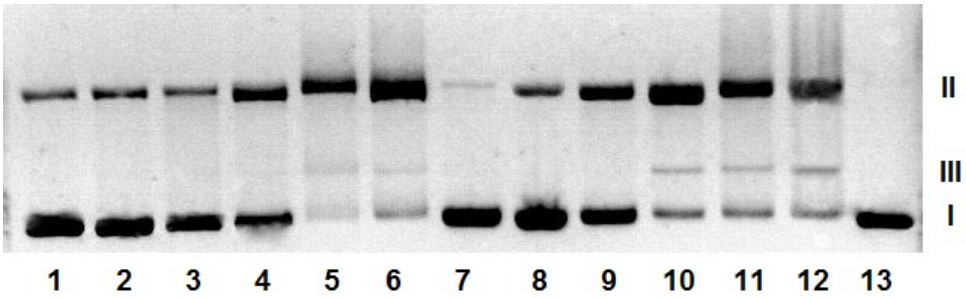


Fig. S22 Agarose gel electrophoresis of pBR322 plasmid cleavage by copper(I) complexes in a DMF (each in the 10% DMF) solution. Lanes: 1, plasmid + 10 μM **3-PSf**; 2, plasmid + 20 μM **3-PSf**; 3, plasmid + 50 μM **3-PSf**; 4, plasmid + 100 μM **3-PSf**; 5, plasmid + 200 μM **3-PSf**; 6, plasmid + 500 μM **3-PSf**; 7, plasmid + 10 μM **4-PSf**; 8, plasmid + 20 μM **4-PSf**; 9, plasmid + 50 μM **4-PSf**; 10, plasmid + 100 μM **4-PSf**; 11, plasmid + 200 μM **4-PSf**; 12, plasmid + 500 μM **4-PSf.** 13, plasmid + control.

Table S6. Densitometric analysis of plasmid cleavage by **3-PSf** and **4-PSf** , % plasmid forms

Lines	Concentrations [μM]	Form I [%]	Form II [%]	Form III [%]
3-PSf				
1	10	79.285	20.715	0
2	20	55.689	44.311	0
3	50	41.193	58.807	0
4	100	30.972	63.028	6.000
5	200	12.490	65.747	21.763
6	500	4.488	68.898	26.614
4-PSf				
7	10	61.355	38.645	0
8	20	59.796	40.204	0
9	50	44.449	55.551	0
10	100	24.610	57.579	17.811
11	200	20.257	59.309	20.434
12	500	16.065	61.795	22.139