

Electronic Supplementary Information

Triazolopyridopyrimidines: an emerging family of effective DNA photocleavers. DNA binding. Antileishmanial activity

Rosa Adam,^a Pablo Bilbao-Ramos,^b Belén Abarca,^{*a} Rafael Ballesteros,^{*a} M. Eugenia González-Rosende,^c M. Auxiliadora Dea-Ayuela,^{b,c} Francisco Estevan,^d Gloria Alzuet-Piña^{*c}

^a*Departament de Química Orgànica, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot (Valencia), Spain*

E-mail: Rafael.Ballesteros@uv.es, Belén.Abarca@uv.es

^b*Departamento de Parasitología, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain*

^c*Departamento de Farmacia, Universidad CEU Cardenal Herrera, Avda. Seminario s/n, 46113 Moncada (Valencia), Spain*

^d*Departament de Química Inorgànica, Facultat de Química, Universitat de València, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain*

^e*Departament de Química Inorgànica, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot (Valencia), Spain*

E-mail: Gloria.Alzuet@uv.es

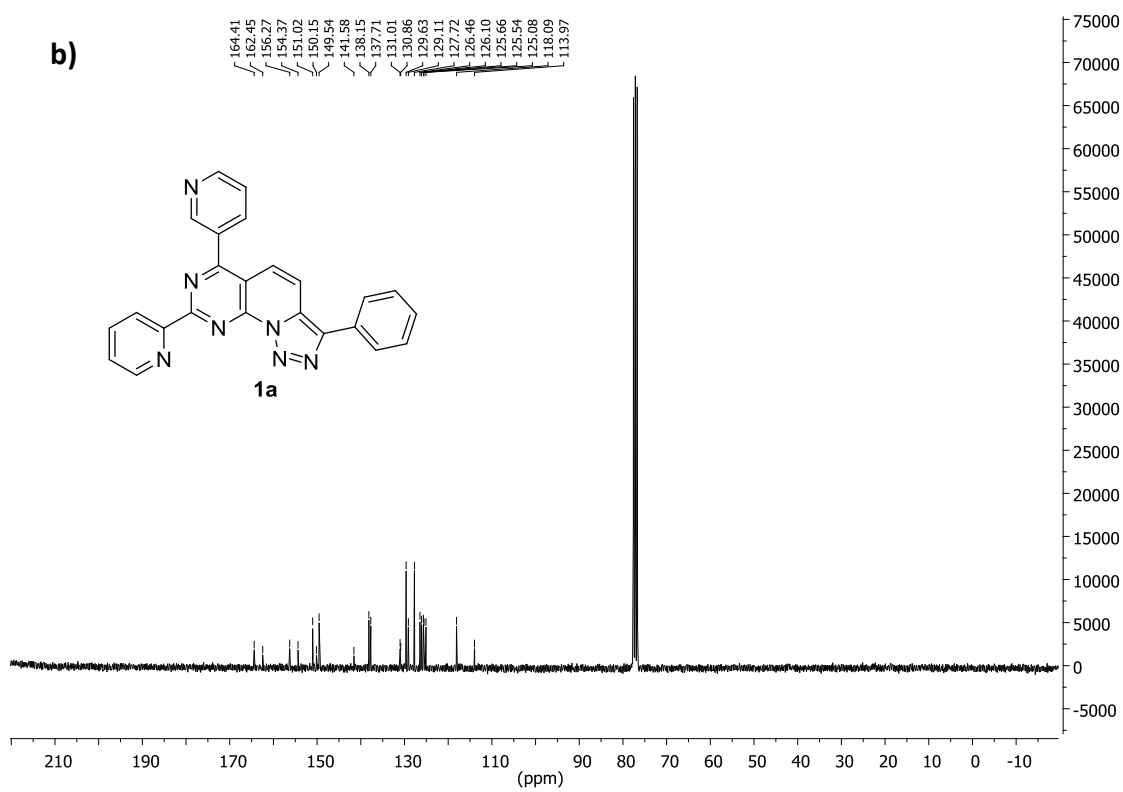
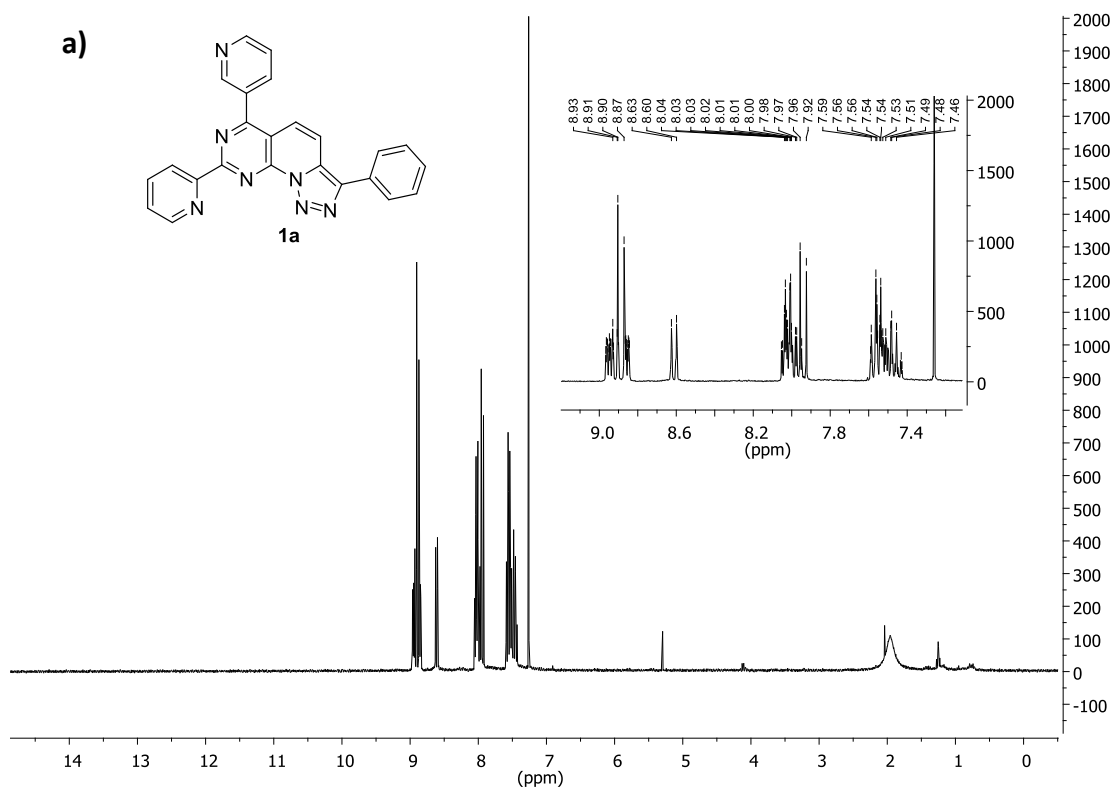


Figure S1. a) **1a** ^1H NMR (CDCl₃). b) **1a** ^{13}C NMR (CDCl₃).

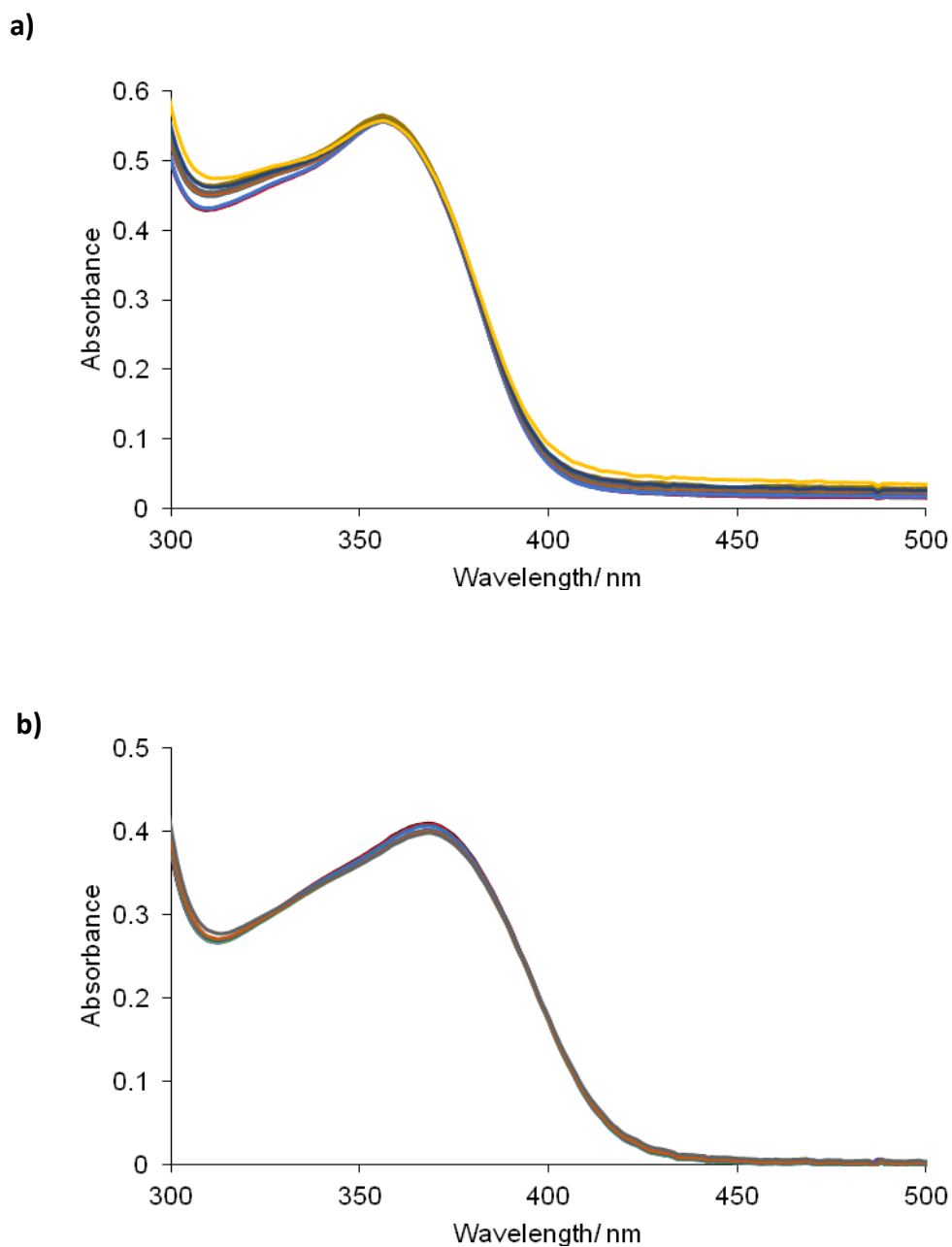


Figure S2. a) **1b** (40 μM) UV spectra in cacodylate buffer 0.1 M, pH= 6.0, 5% DMF in the absence and in the presence of increasing concentrations of CT-DNA (20-160 μM). b) **1c** (40 μM) UV spectra in cacodylate buffer 0.1 M, pH= 6.0, 5% DMF in the absence and in the presence of increasing concentrations of CT-DNA (20-160 μM).

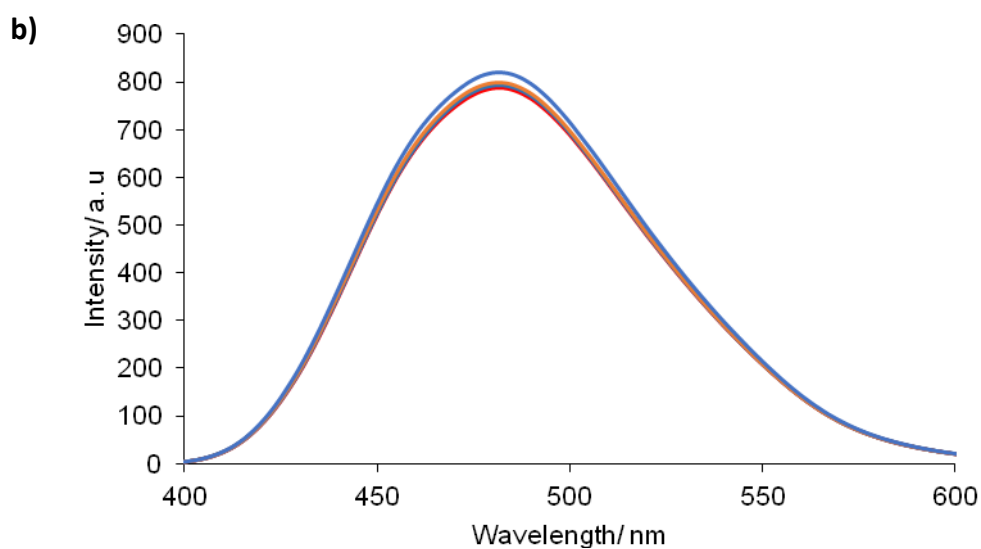
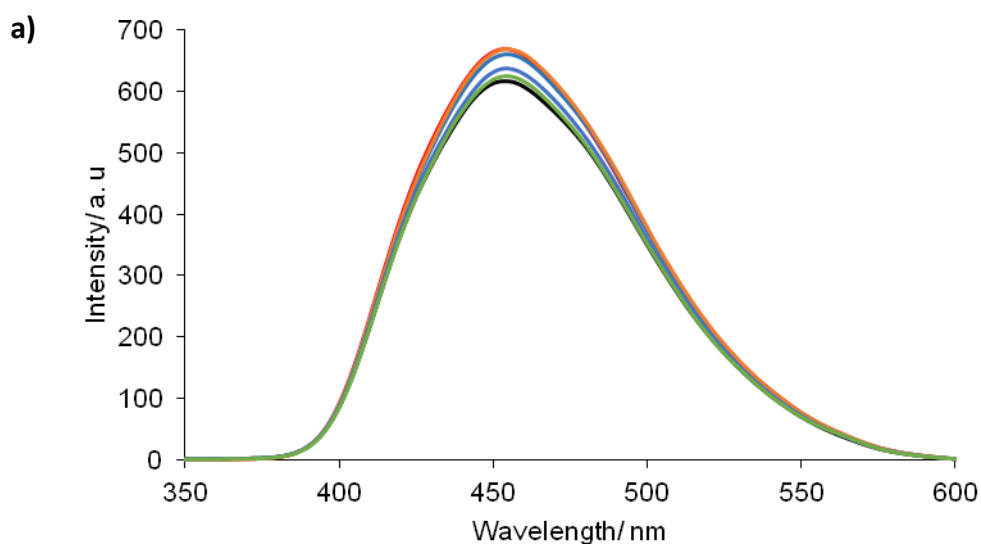


Figure S3. a) **1b** (5 μM) fluorescence emission spectra in cacodylate buffer 0.1 M, pH= 6.0, 5% DMF in the absence and in the presence of increasing concentrations of CT-DNA (2.5-30 μM). b) **1c** (10 μM) fluorescence emission spectra in cacodylate buffer 0.1 M, pH= 6.0, 5% DMF in the absence and in the presence of increasing concentrations of CT-DNA (10-60 μM).

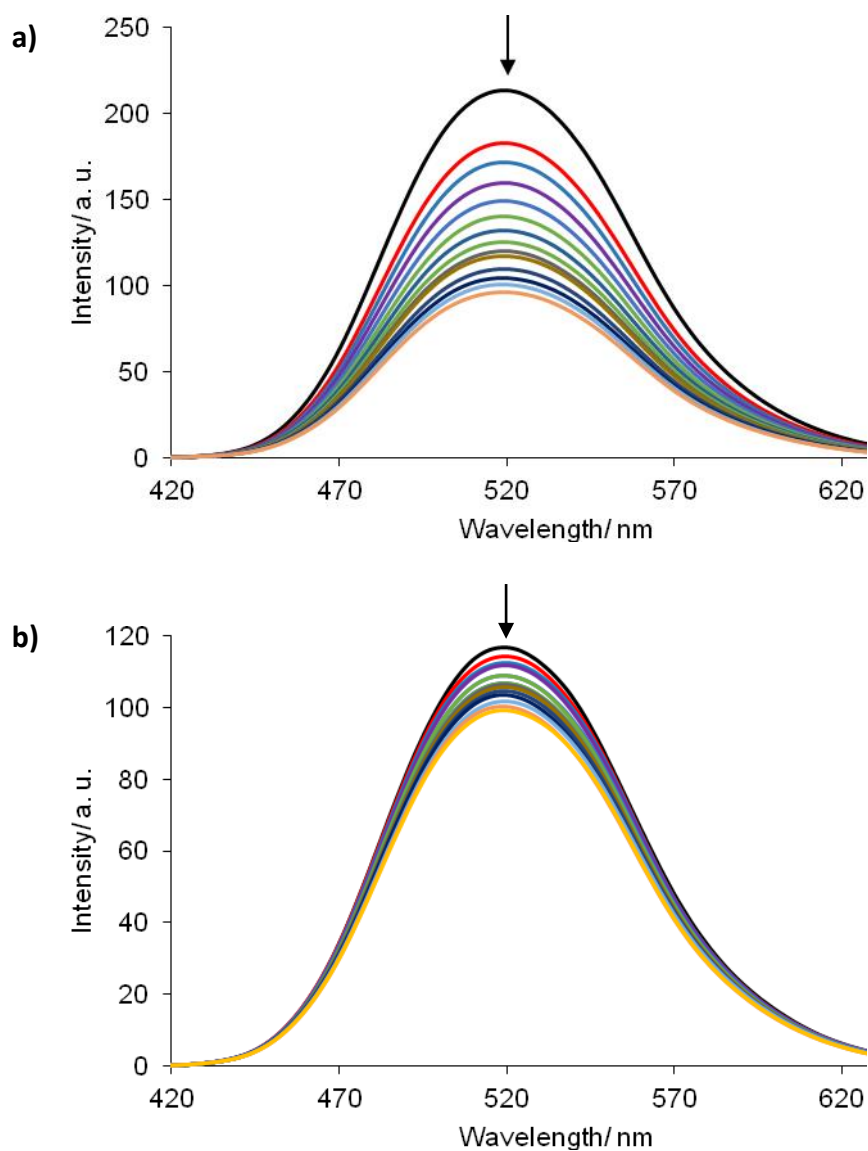


Figure S4. a) Fluorescence emission spectra of **1a** (15 μM) in cacodylate buffer 0.1 M, pH= 6.0, 5% DMF in the absence and the presence of increasing amounts of $[\text{K}_4\text{Fe}(\text{CN})_6]$ from 0.15 μM to 3 μM . b) Fluorescence emission spectra of **1a** (15 μM) + CT-DNA (90 μM) in cacodylate buffer 0.1 M, pH= 6.0, 5% DMF in the absence and in the presence of increasing amounts of $[\text{K}_4\text{Fe}(\text{CN})_6]$ from 0.15 μM to 3 μM . Arrows indicate the effect of $[\text{K}_4\text{Fe}(\text{CN})_6]$ in fluorescence emission.

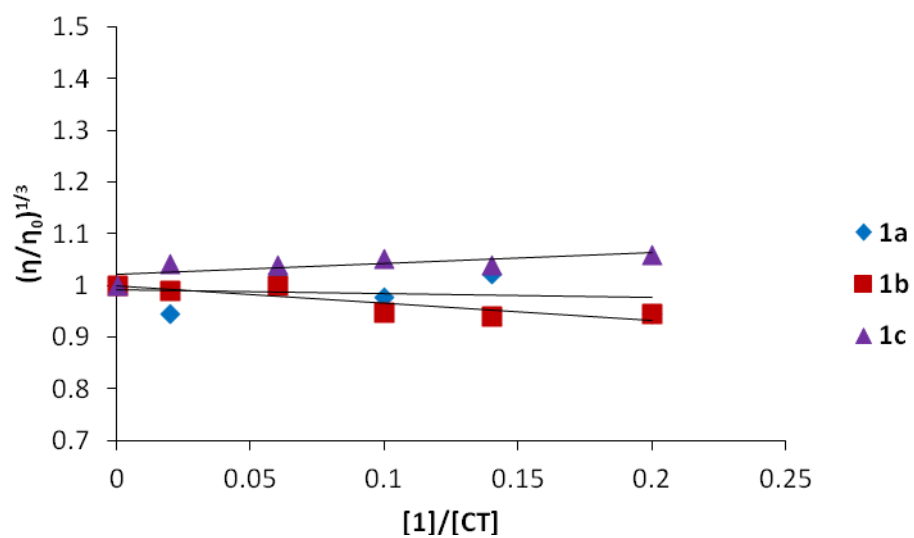


Figure S5. Effect of increasing amounts of **1a** (blue diamond); **1b** (red square) and **1c** (violet triangle) on the relative viscosity of CT-DNA.

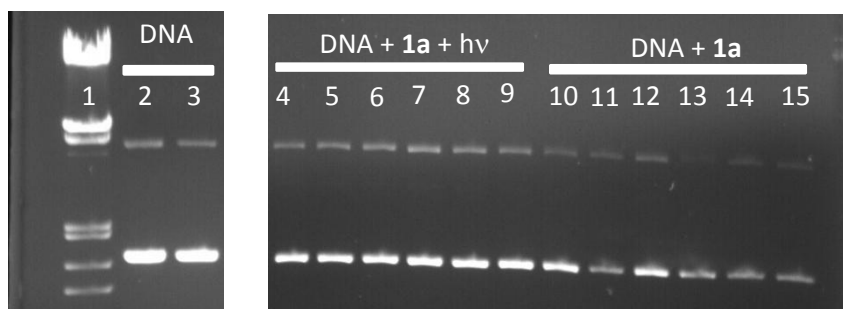


Figure S6. Agarose gel electrophoresis of pUC18 of 80 μ M compound **1a** upon photo-irradiation at 365 nm for 2 h. 1: λ DNA/EcoR1+HindIII Marker; 2: No irradiated DNA control; 3: Irradiated DNA control; 4: **1a** 5 μ M; 5: **1a** 7.5 μ M; 6: **1a** 10 μ M ; 7: **1a** 12.5 μ M; 8: **1a** 15 μ M; 9: **1a** 18 μ M; 10: **1a** 5 μ M no irradiated; 11: **1a** 7.5 μ M no irradiated; 12: **1a** 10 μ M no irradiated; 13: **1a** 12.5 μ M no irradiated; 14: **1a** 15 μ M no irradiated; 15: **1a** 18 μ M no irradiated.

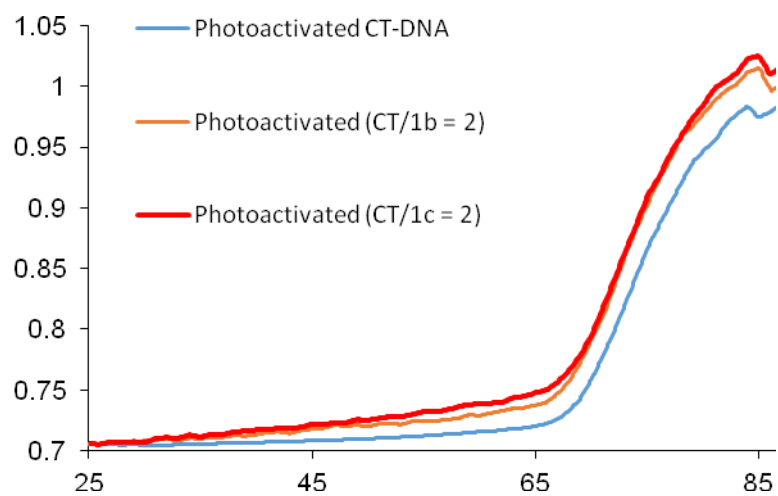


Figure S7. Thermal denaturation curves of CT-DNA 100 μ M (blue) in cacodylate buffer 0.1 M, pH= 6.0, 5% DMF, with 50 μ M **1b** (orange) or 50 μ M **1c** (red) upon photoirradiation.

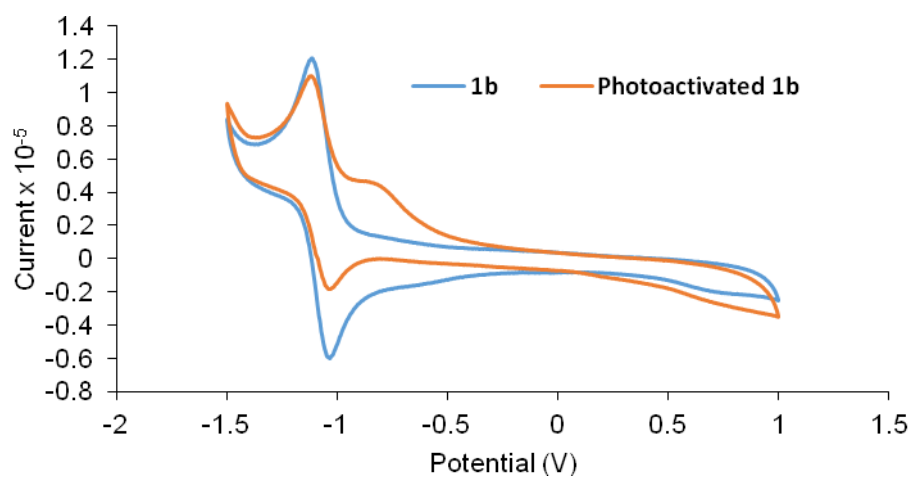


Figure S8. Cyclic voltammogram of compound **1b** (2.5 mM in DMF) (blue line) and compound **1b** (2.5 mM in DMF) after photoirradiation at 365 nm for 2 hours (orange line). Scan rate 50 mV/s.

Table S1. Relative amounts of the three forms of plasmid DNA after irradiation in the presence of **1b**.

	UVA Irradiation	Form I (%)± SD	Form II (%)± SD	Form III (%)	S^a
DNA	✗	94.2 ± 1.2	5.8 ± 1.2	-	
DNA	✓	95.5 ± 0.8	4.5 ± 0.8	-	
1b 25 μM	✓	46.7 ± 0.9	52.8 ± 0.2	traces	0.76
1b 30 μM	✓	39.3 ± 0.4	60.2 ± 0.3	traces	0.93
1b 35 μM	✓	40.3 ± 0.4	59.2 ± 0.3	traces	0.91
1b 40 μM	✓	38.6 ± 0.9	60.8 ± 0.2	traces	0.95
1b 45 μM	✓	36.3 ± 0.5	63.1 ± 0.2	traces	1.01
1b 50 μM	✓	28.0 ± 0.0	71.5 ± 0.7	traces	1.27
1b 60 μM	✓	27.6 ± 0.6	71.9 ± 1.3	traces	1.29
1b 70 μM	✓	8.4 ± 0.6	91.1 ± 0.1	traces	2.48
1b 80 μM	✓	3.5 ± 0.7	96.0 ± 0.1	traces	3.35
1b 90 μM	✓	4.6 ± 0.9	94.9 ± 0.2	traces	3.10
1b 100 μM	✓	3.5 ± 0.8	96.0 ± 0.1	traces	3.35
1b 90 μM	✗	99.5 ± 0.7	0.5 ± 0.7	-	
1b 100 μM	✗	98.8 ± 1.2	1.2 ± 1.2	-	

S= mean number of single-strand scissions per DNA molecule.

Table S2. Relative amounts of the three forms of plasmid DNA after irradiation in the presence of **1c**.

	UVA Irradiation	Form I (%) ± SD	Form II (%)± SD	Form III (%)	S^a
DNA	✗	96.9 ± 0.1	3.1 ± 0.1	-	
DNA	✓	96.3 ± 0.9	3.7 ± 0.9	-	
1c 25 μM	✓	73.1 ± 0.2	26.8 ± 0.2	-	0.31
1c 30 μM	✓	59.2 ± 0.4	40.7 ± 0.4	-	0.52
1c 35 μM	✓	65.5 ± 0.7	34.5 ± 0.7	-	0.42
1c 40 μM	✓	66.7 ± 0.3	33.2 ± 0.3	-	0.40
1c 45 μM	✓	59.4 ± 0.7	40.5 ± 0.7	-	0.52
1c 50 μM	✓	56.3 ± 0.5	43.7 ± 0.5	-	0.57
1c 60 μM	✓	49.2 ± 1.6	50.3 ± 0.9	traces	0.71
1c 70 μM	✓	46.7 ± 0.9	52.8 ± 0.3	traces	0.76
1c 80 μM	✓	40.0 ± 1.4	59.5 ± 0.7	traces	0.92
1c 90 μM	✓	38.6 ± 0.9	60.9 ± 0.1	traces	0.95
1c 100 μM	✓	33.4 ± 0.6	66.1 ± 0.1	traces	1.10
1c 90 μM	✗	95.2 ± 0.3	4.8 ± 0.3	-	
1c 100 μM	✗	99.0 ± 0.0	0.97 ± 0.1	-	

S= mean number of single-strand scissions per DNA molecule.

Table S3. Relative amounts of the three forms of plasmid DNA after irradiation in the presence of **1b** and different inhibitors.

	Form I (%)± SD	Form II (%)± SD	Form III (%)
DNA	96.8 ± 2.5	3.2 ± 2.5	-
1b 80 μM	8.8 ± 0.3	90.7 ± 1.0	traces
D₂O^a	-	-	-
Sodium formate	49.4 ± 0.8	50.1 ± 1.5	traces
KI	98.0 ± 0.8	2.0 ± 0.8	-
DMSO	72.5 ± 0.7	25.5 ± 0.7	-
TMP	59.0 ± 0.0	40.5 ± 0.7	traces
DABCO	68.5 ± 0.6	30.9 ± 1.3	traces
NaN₃	59.7 ± 0.4	39.8 ± 1.0	traces
Methyl green 3 μM	9.8 ± 0.7	89.1 ± 1.2	traces
Methyl green 6 μM	10.6 ± 1.3	87.1 ± 0.5	traces
Hoescht 8 μM	10.8 ± 3.3	88.7 ± 2.5	traces
Hoescht 16 μM	12.5 ± 3.5	86.9 ± 2.6	traces
Tiron 10 μM	95.6 ± 3.7	4.3 ± 0.4	-

^aData quantification was not possible due to the presence of smearing.

Table S4. Relative amounts of the three forms of plasmid DNA after irradiation in the presence of **1c** and different inhibitors.

	Form I (%)± SD	Form II (%)± SD	Form III (%)± SD
DNA	96.8 ± 1.3	4.7 ± 1.3	-
1c 90 μM	42.3 ± 0.5	57.8 ± 0.3	traces
D₂O	-	54.7 ± 0.2	45.3 ± 0.2
Sodium formate	5.9 ± 2.8	40.1 ± 2.8	-
KI	89.3 ± 4.7	10.6 ± 0.6	-
DMSO	54.7 ± 0.2	44.8 ± 0.6	traces
TMP	39.1 ± 2.8	60.4 ± 3.1	traces
DABCO	56.6 ± 1.0	42.9 ± 1.4	traces
NaN₃	46.5 ± 0.3	52.7 ± 0.2	traces
Methyl green 6 μM	44.1 ± 0.1	56.4 ± 0.3	traces
Hoescht 16 μM	40.6 ± 1.7	58.2 ± 2.0	traces
Tiron 10 μM	87.6 ± 1.7	12.4 ± 1.7	-