Electronic Supplementary Information

Photobiological properties of 3-psoralenacetic acids

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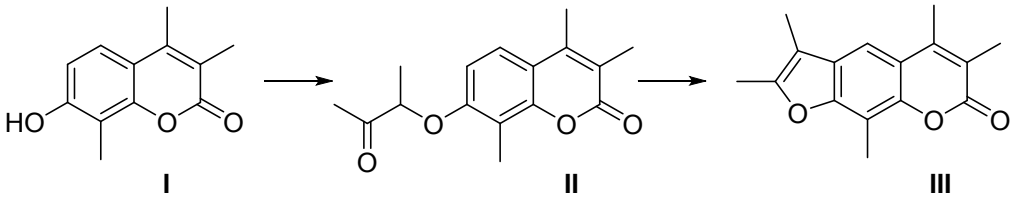
Contents **pag**

Synthesis of compound 7

All commercial chemicals and solvents used were analytical grade and were used without further purification. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates (Merck 60-F-254, 0.25 mm). Melting points were determined on a Gallenkamp MFB-595-010M melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a Bruker 300-AMX spectrometer with TMS as an internal standard. Elemental analysis were performed on a Perkin-Elmer 2400 analyzer. Microwave assisted reactions were performed on a CEM Discover® monomode reactor with the temperature monitored by a built-in infrared sensor and the automatic control of the power; all the reactions were performed in closed devices with pressure control.

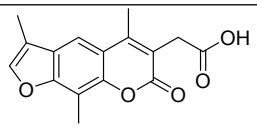
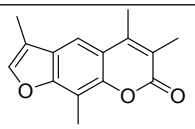
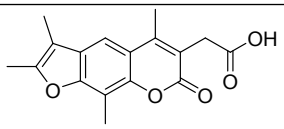
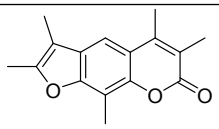
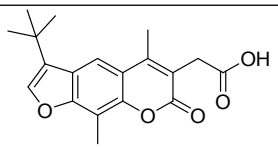
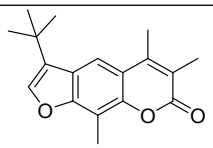
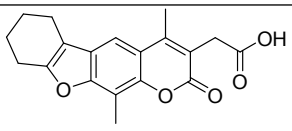
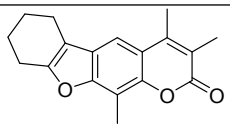


Scheme

3,4,8-Trimethyl-7-[2'-(3'-oxo)butyloxy]coumarin (II). A mixture of 3,4,8-trimethyl-7-hydroxycoumarin (**I**) (1.0 g, 4.9 mmol), 3-chloro-2-butanone (1.1 g, 1.0 mL, 10.0 mmol), TEA (1 mL) and H_2O (1 mL) was microwave irradiated at 130 °C (power set point 200 W; ramp time 2 min; hold time 20 min). After cooling the reaction mixture was poured into 1M HCl (20 mL) and the resulting precipitate was filtered off to give 3,4,8-trimethyl-7-[2'-(3'-oxo)butyloxy]coumarin (1.1 g, 80%) as white solid; mp 105 °C; ^{1}H-NMR ($CDCl_3$): δ 7.35 (d, J = 8.8 Hz, 1H, 5-H), 6.61 (d, J = 8.8 Hz, 1H, 6-H), 4.70 (q, J = 6.8 Hz, 1H, 2'-H), 2.37 (s, 3H, 4-Me), 2.34 (s, 3H, 8-Me), 2.18 (s, 6H, 4'-H and 3-Me), 1.54 (d, J = 6.8 Hz, 3H, 1'-H); Anal. Calcd. for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61. Found: C, 70.04; H, 6.65.

2,3,5,6,9-Pentamethyl-7*H*-furo[3,2-*g*][1]benzopyran-7-one (3,4,8,4',5'-pentamethylpsoralen) (III). A solution of 3,4,8-trimethyl-7-[2'-(3'-oxo)butyloxy]coumarin (**II**) (1.0 g, 3.6 mmol) in conc. H_2SO_4 (20 mL) was stirred at room temperature until starting product disappeared (2 h). The solution was diluted with ice and water (200 mL) and the obtained precipitate was filtered, washed with water and dried. The solid was crystallized from MeOH to give 2,3,5,6,9-pentamethyl-7*H*-furo[3,2-*g*][1]benzopyran-7-one (**III**) (0.56 g, 60%) as pale yellow solid: mp 210 °C; ^{1}H-NMR ($CDCl_3$): δ 7.32 (s, 1H, 5-H), 2.55 (s, 3 H, 9-Me), 2.46 (s, 3 H, 5-Me), 2.40 (s, 3 H, 2-Me), 2.23 (s, 3 H, 6-Me), 2.18 (s, 3 H, 3-Me). Anal. Calcd. for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 75.01; H, 6.27.

Table S1. LogD values of psoralen acetic acids **1-4** compared with parent psoralens.

Psoralenacetic acids	logD		Parent psoralens	logD*
	at pH=7.2	at pH=7.4		
	0.10	-0.06		3.66
	0.25	0.09		3.86
	0.69	0.53		4.37
	0.95	0.80		4.70

* For non-carboxylated derivatives, logD is independent from pH (in this case logD=logP)

Mass spectra of photoadducts

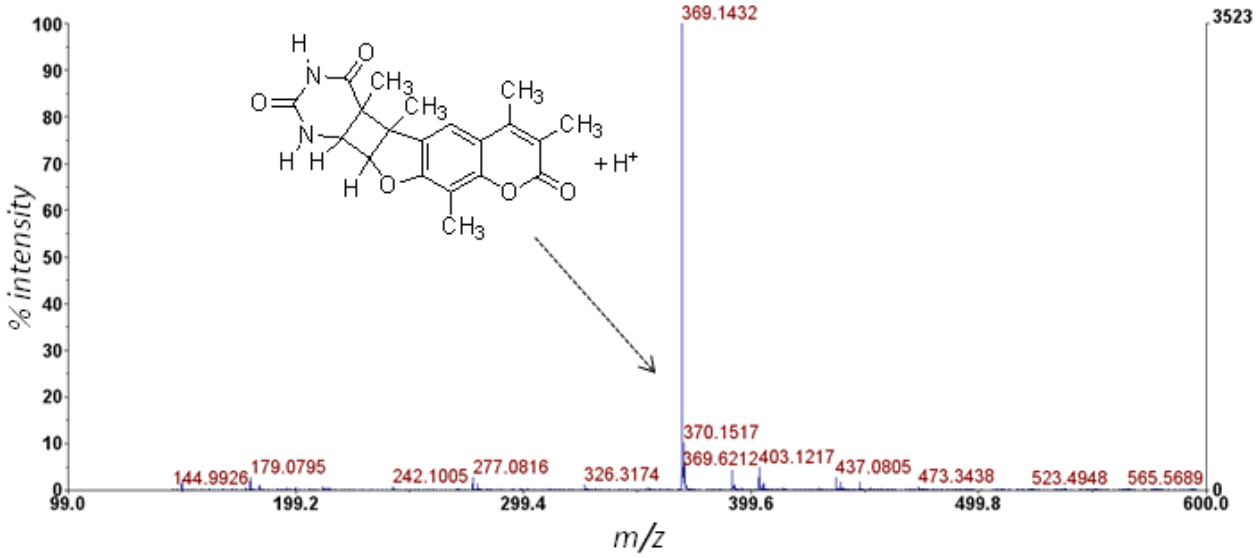


Figure S1. Mass spectrum of compound **5** (photoadduct between compound **1** and thymine).

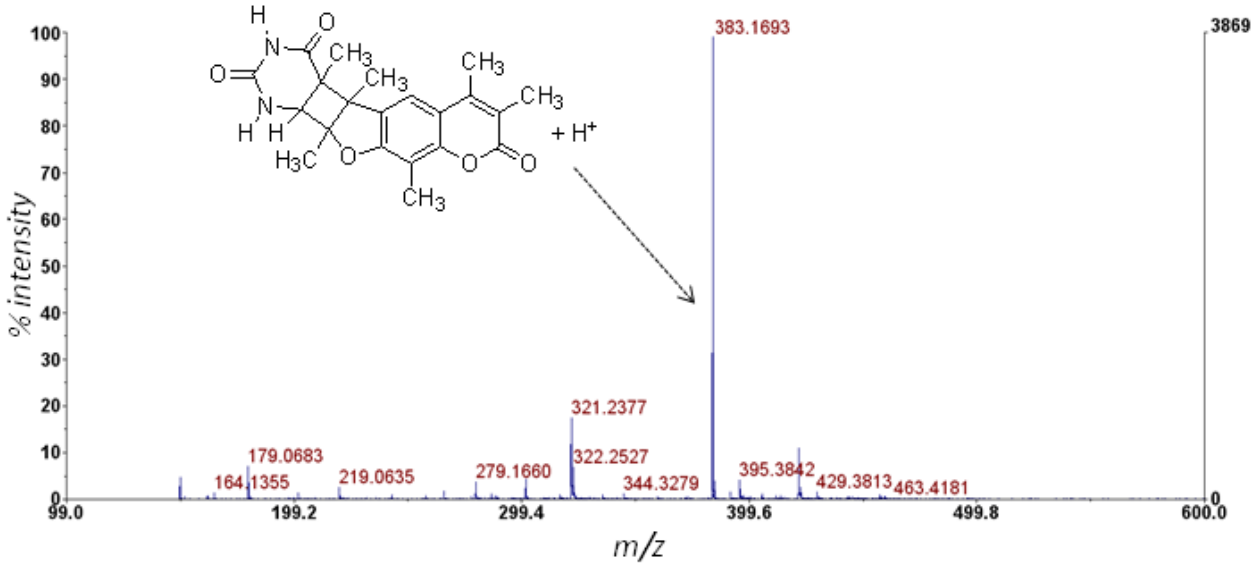


Figure S2. Mass spectrum of compound **6** (photoadduct between compound **2** and thymine).

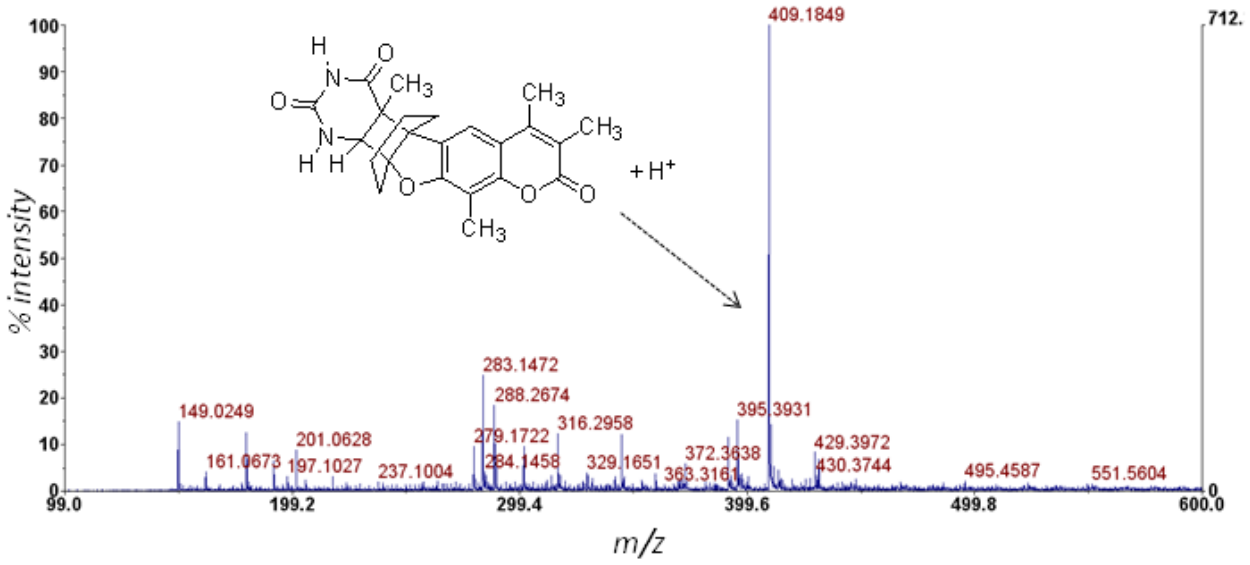


Figure S3. Mass spectrum of photoadduct between compound **4** and thymine.

Mass spectra of photolysis products

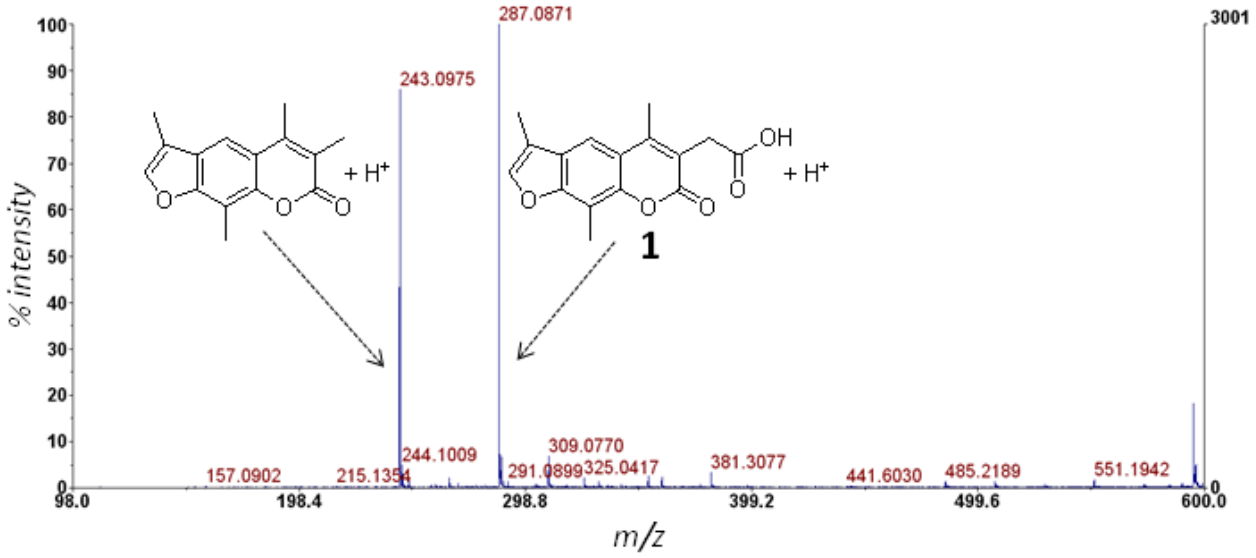


Figure S4. Mass spectrum of photolysis products from compound **1**.

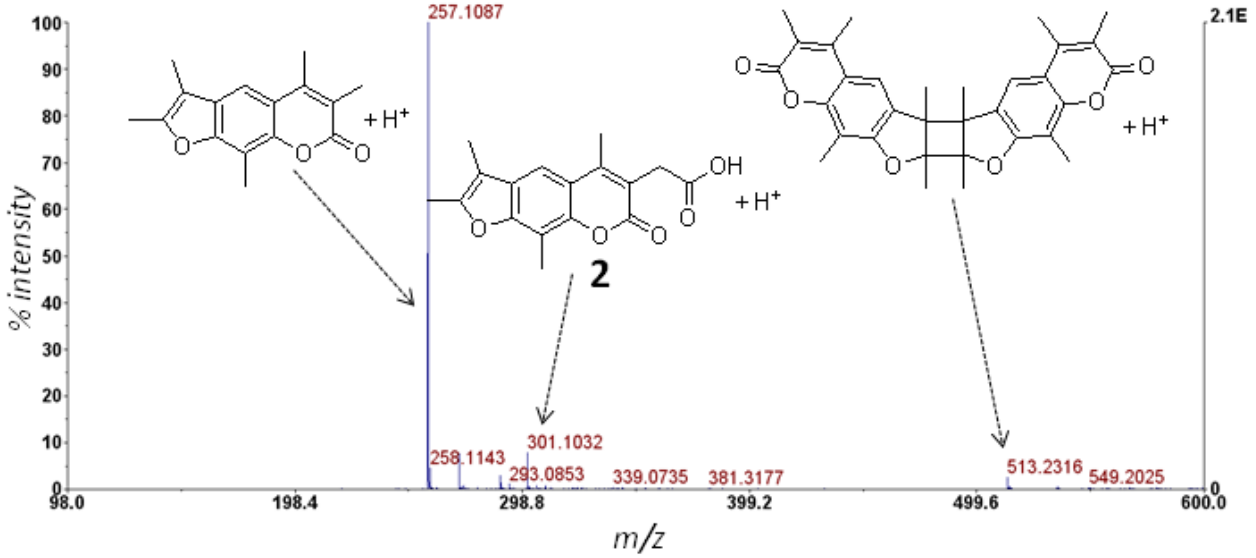


Figure S5. Mass spectrum of photolysis products from compound **2**.

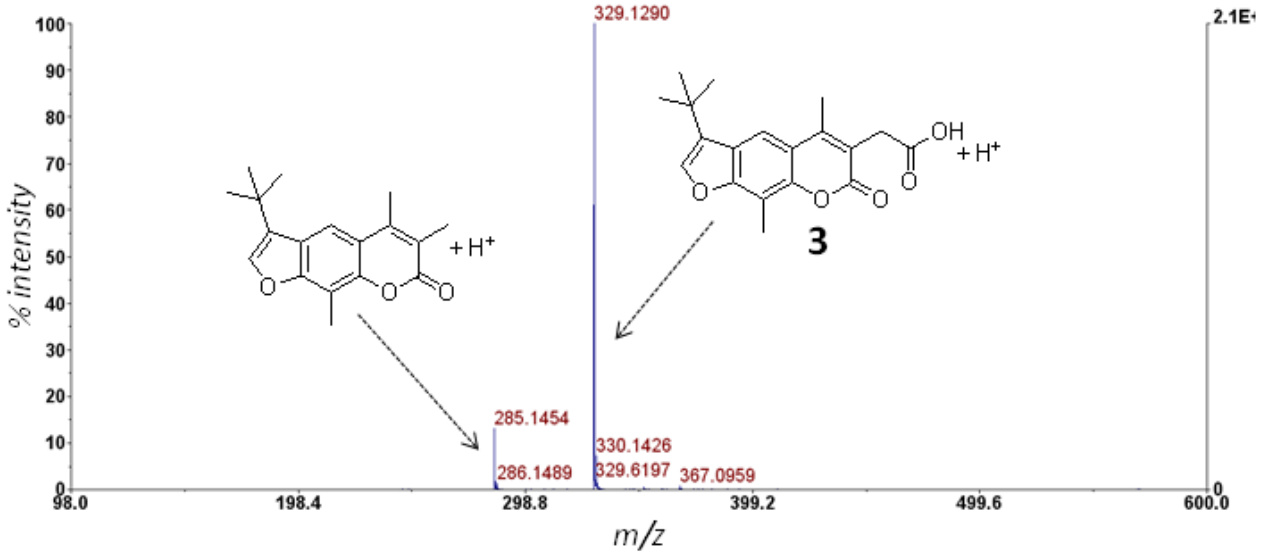


Figure S6. Mass spectrum of photolysis products from compound **3**.

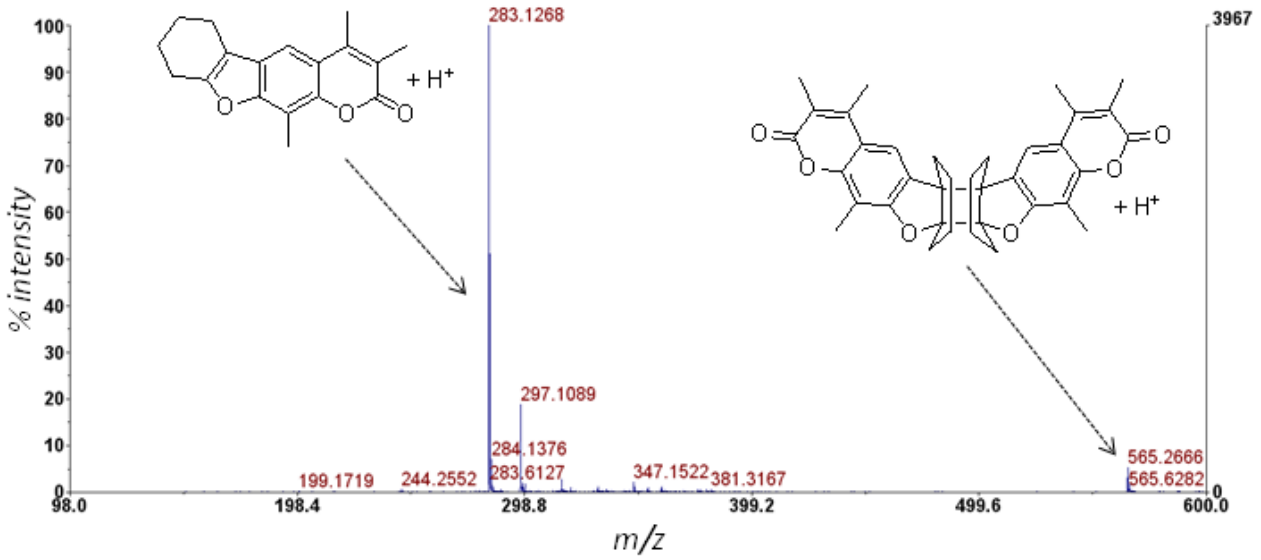


Figure S7. Mass spectrum of photolysis products from compound **4**.

Effect of compounds 2 and 7 on Topoisomerase II relaxation activity

The effect on the relaxation of supercoiled plasmid pBR322 DNA mediated by topoisomerase II is reported in Figure S8. The enzyme converted plasmid DNA (DNA) to a series of relaxed forms (Topo II) and this activity appeared unaffected by UVA irradiation (Topo II + 7.5 J/cm^2). The incubation with 100 μM compound **2** did not affect the catalytic activity in the dark. Conversely, upon UVA irradiation, the psoralen derivative exerted a notable inhibitory effect on topoisomerase II relaxation, as demonstrated by the disappearance of the majority of topoisomers and the concurrent appearance of the band corresponding to supercoiled DNA (compound **2** + UVA). A very similar behaviour was observed for the corresponding decarboxylated derivative **7.**

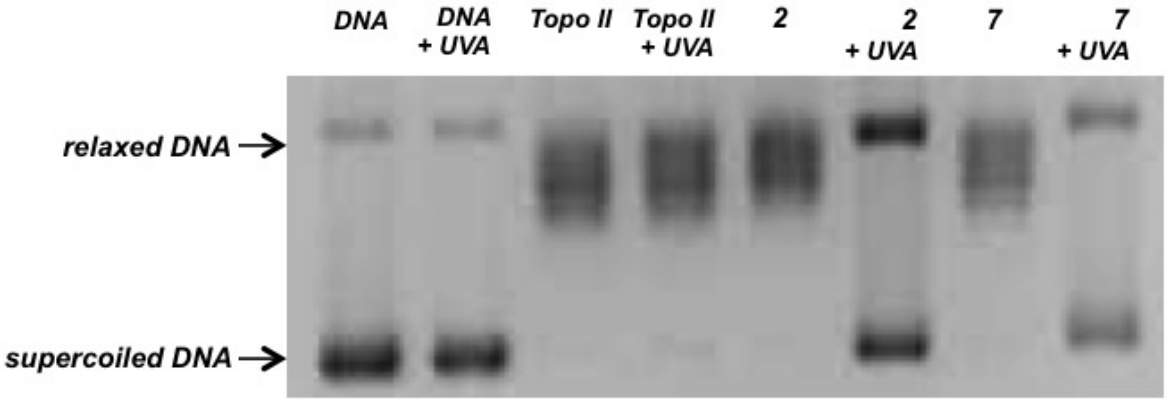


Fig. S8. Effect of compounds **2** and **7** on relaxation of supercoiled pBR322 DNA mediated by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated with topoisomerase II in the absence (Topo II) and presence of **2** and **7** at 100μM concentration. Where indicated, UVA irradiation (7.5 J/cm^2) was performed.

Moreover, the inhibition exerted by both compounds **2** and **7** on DNA relaxation mediated by the enzyme was strongly dependent on the dose of UVA irradiation (Fig. S9). Indeed, by increasing the UVA radiant energy from 0 to 7.5 J/cm^2, a progressive disappearance of relaxed DNA can be observed, paralleled by the increase of the supercoiled form.

Under these conditions a difference could be seen between the two derivatives: at the lower UVA doses (3 and 5 J/cm^2) compound **2** seems more effective in damaging plasmid DNA and consequently is able to inhibit more efficiently the enzyme activity than **7** (see the presence of more topoisomers in the samples with **7** at 3 and 5 J/cm^2 in Fig. S9).

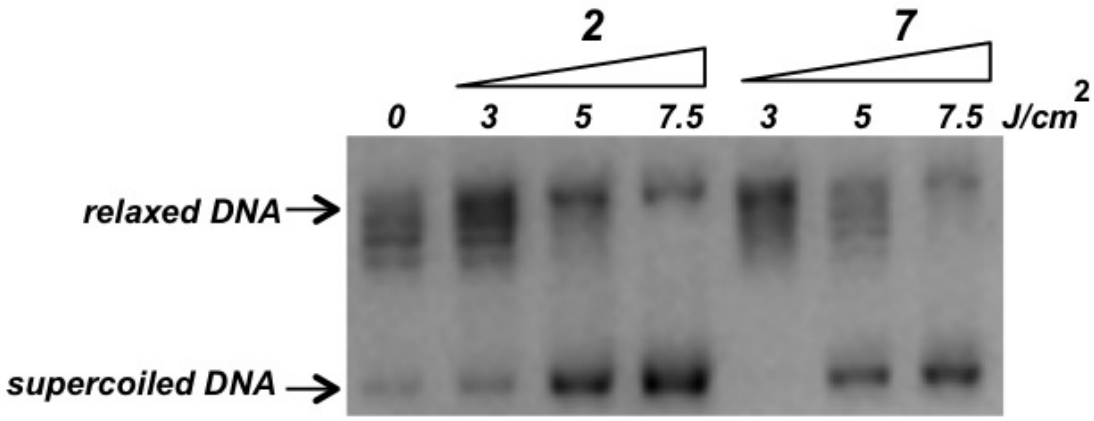


Fig. S9. Effect different doses of UVA light (3.0, 5.0, 7.5 J/cm^2) on relaxation of supercoiled pBR322 DNA mediated by human recombinant topoisomerase II. In each sample supercoiled DNA was incubated with topoisomerase II in the presence of **2** and **7** at 100 μM concentration

Furthermore, the UVA-mediated effect of the same test compound on topoisomerase II catalytic activity was concentration dependent. In fact, the UVA irradiation of a solution containing the enzyme and 25 μM compound **2** and **7** induced only a partial inhibitory effect on DNA relaxation, that became significantly more pronounced at 50 μM concentration (Fig. S10).

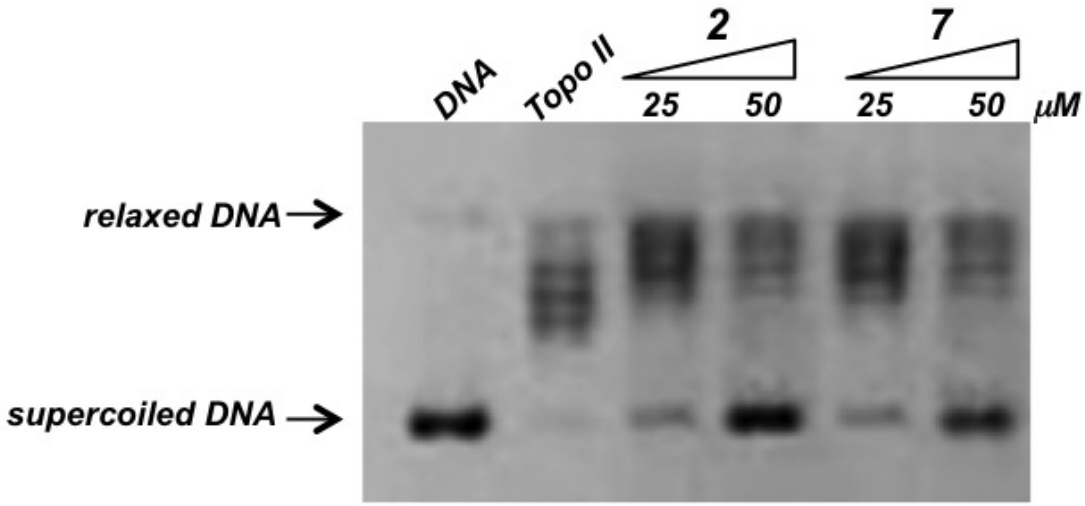


Fig. S10. Concentration-dependent effect of compounds **2** and **7** (0, 25 and 50 μM) on relaxation of supercoiled pBR322 DNA mediated by human recombinant topoisomerase II upon UVA irradiation (7.5 J/cm^2). Supercoiled DNA (DNA) was incubated with topoisomerase II in the absence (Topo II) and in the presence of **2** and **7** at the indicated concentrations.