

## Supporting Information

### **Assessment of DNA topoisomerase I unwinding activity, radical scavenging capacity and inhibition of breast cancer cell viability of *N*-alkyl-acridones and *N,N'*-dialkyl-9,9'-biacridylidenes**

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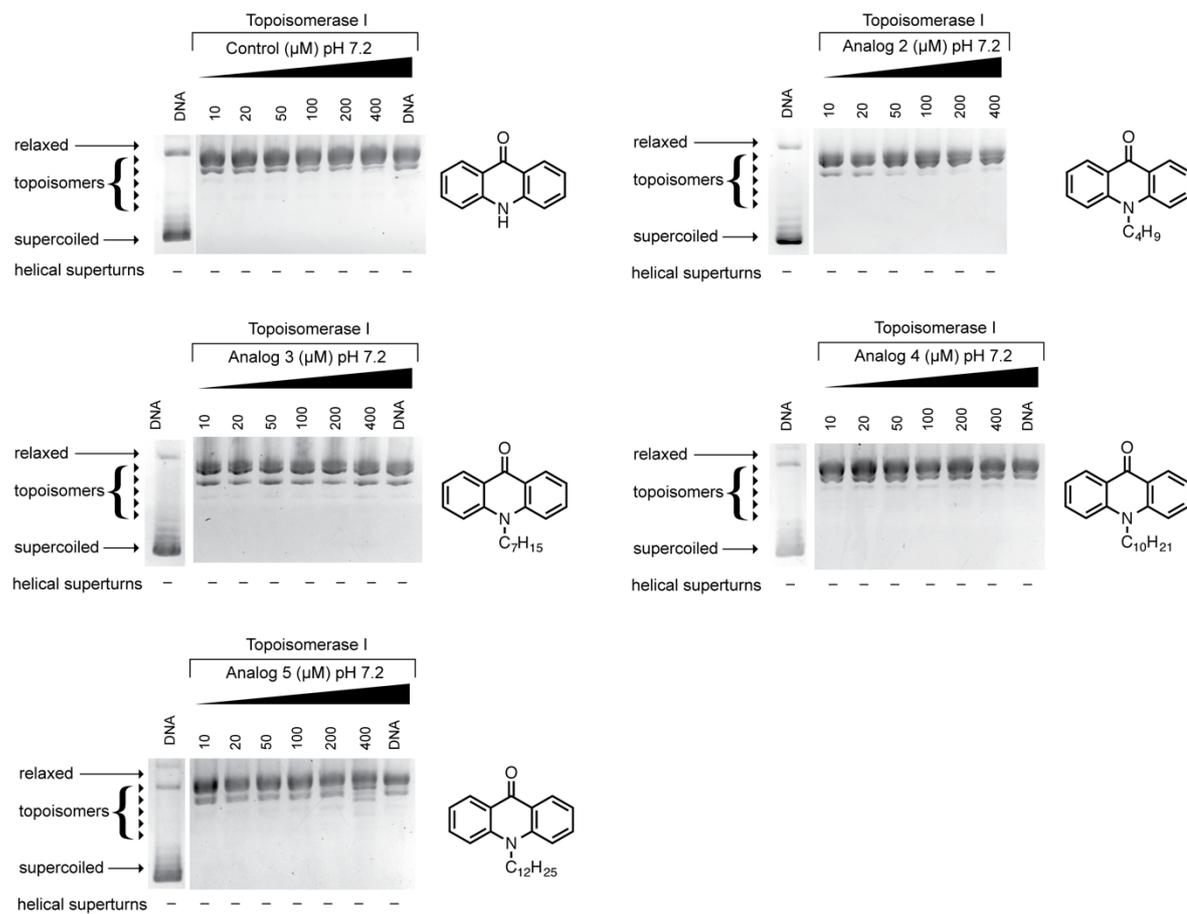
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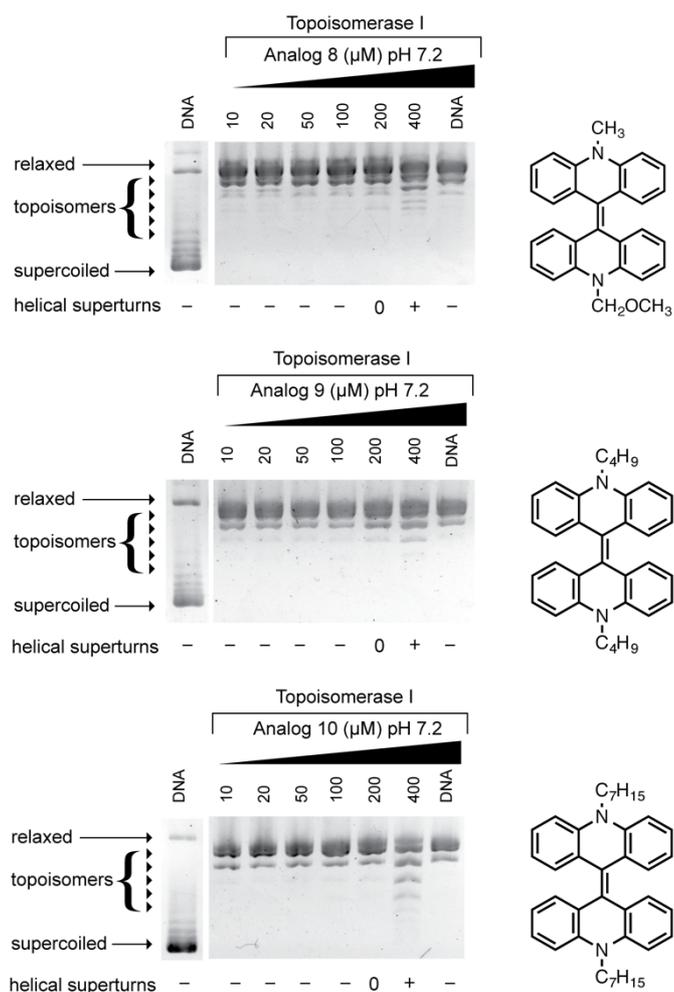
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## 1. Topoisomerase-I mediated DNA relaxation assay



**Figure S1:** Topoisomerase-I mediated DNA relaxation assay in the presence of increasing concentrations (10-400  $\mu\text{M}$ ) of control acridone and *N*-alkylacridones **2-5** at pH 7.2.



**Figure S2:** Topoisomerase-I mediated DNA relaxation assay in the presence of increasing concentrations (10-400  $\mu\text{M}$ ) of *N,N'*-dialkyl-9,9'-biacridylidenes **8**, **9** and **10** at pH 7.2.

## 2. Regression equations parameters of radical scavenging activity of DPPH and APTS assays

**Table S1:** Free radical-scavenging activity of *N,N'*-dialkyl-9,9'-biacridylidenes derivatives along with their corresponding regression equations parameters calculated by the DPPH-assay. Results were expressed as IC<sub>50</sub>± standard deviation or TEAC values.

<b>Analog</b>	<b>Lineal range (µM)</b>	<b>Slope</b>	<b>correlation coefficient (r<sup>2</sup>)</b>	<b>Radical scavenging activity</b> IC <sub>50</sub> ± sd (TEAC)
<b>7</b>	5.0-25	2.7975	0.9943	16.98±0.88 (1.03)
<b>8</b>	10-50	2.6325	0.9824	33.7±2.1 (2.00)
<b>9</b>	10-50	2.346	0.9711	28.350 ± 3.1(1.69)
<b>10</b>	10-50	1.587	0.9739	28.350 ± 3.1 (1.69)
<b>11</b>	5.0-20	2.7361	0.9643	17.73±0.53 (1.05)
<b>12</b>	5.0-20	3.0161	0.956	16.02±0.56 (0.96)
Caffeic acid	1.0-10.0	6.7494	0.9835	6.95±0.46 (0.42)
Trolox	2.5-50.0	2.8673	0.9781	16.76±0.54 (1.0)

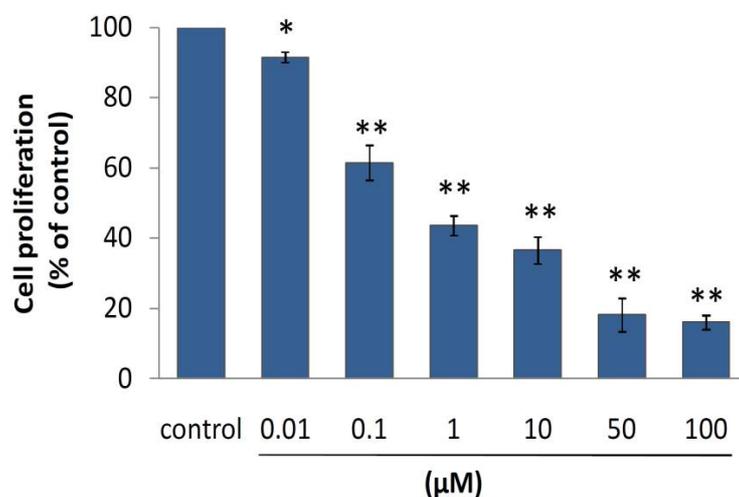
Equations were calculated using five different concentrations assayed in triplicate. All equations followed a linear regression model. TEAC-values were calculated by dividing the IC<sub>50</sub> value of each analog through the IC<sub>50</sub> value of trolox.

**Table S2:** Free radical-scavenging activity of *N,N'*-dialkyl-9,9'-biacridylidenes derivatives along with their corresponding regression equations parameters calculated by the APTS-assay. Results were expressed as IC<sub>50</sub>± standard deviation or TEAC values.

<b>Antioxidant</b>	<b>Linear range (μM)</b>	<b>Slope</b>	<b>Correlation coefficients (r<sup>2</sup>)</b>	<b>Radical scavenging activity</b> IC <sub>50</sub> ± sd (TEAC)
<b>7</b>	5.0-50	1.3787	0.9766	32.32±1.90 (3.68)
<b>8</b>	10-50	2.2452	0.9824	9.33±0.42 (1.07)
<b>9</b>	10-50	2.845	0.9722	21.45±1.15 (1.14)
<b>10</b>	10-50	2.7576	0.9725	16.88±1.25 (1.92)
<b>11</b>	5.0-25	3.5243	0.9788	12.43±0.86 (1.42)
<b>12</b>	5.0-50	1.7882	0.9778	24.1±1.11 (1.52)
Caffeic acid	2.5-15	5.5325	0.9923	8.67±0.36 (0.99)
Trolox	2.5-12.5	5.8934	0.9961	8.78±0.29 (1.0)

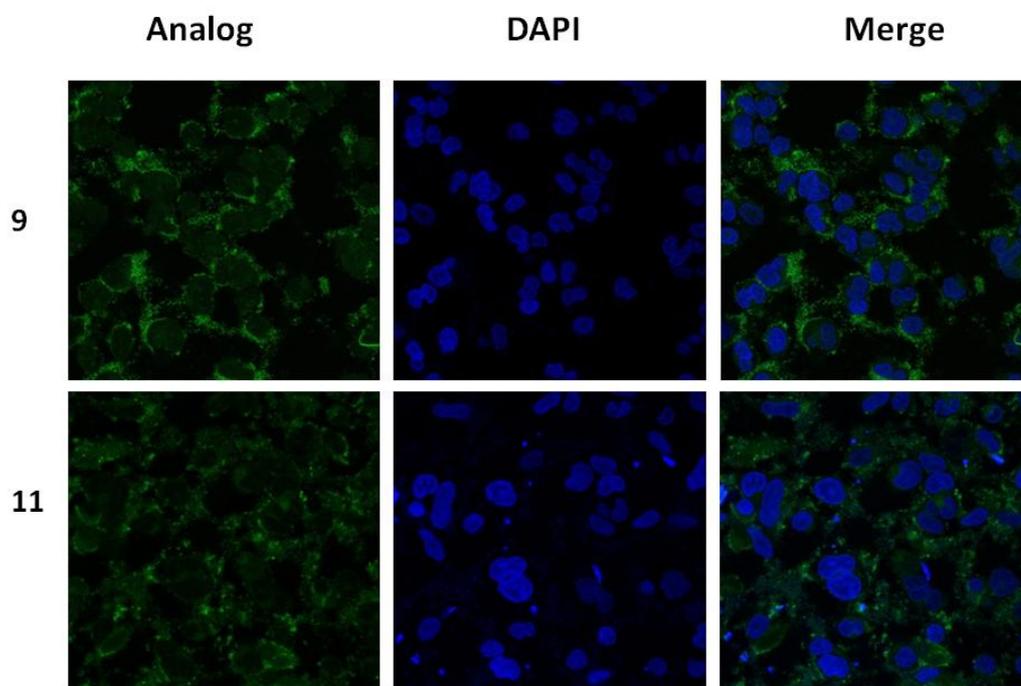
Equations were calculated using five different concentrations assayed in triplicate. All equations followed a linear regression model. TEAC-values were calculated by dividing the IC<sub>50</sub> value of each analog through the IC<sub>50</sub> value of trolox.

### 3. MTT assay of control acridone



**Figure S3:** Dose-dependent response of MCF-7 epithelial breast cancer cells to acridone (0.01 to 100  $\mu\text{M}$ ) for 24 h in serum containing medium. The results are presented as percentage of growth in respect to control cells. Each point represents the mean  $\pm$  standard deviation from experiments in triplicate. Asterisks mark the statistically significant levels using the Student t-test: \* $p < 0.05$ , \*\* $p < 0.01$ , respectively, as compared to control.

#### 4. Intracellular distribution of 9 and 11 after 24 h



**Figure S4:** Determination of intracellular distribution of analogs **9** and **11** in MCF-7 breast cancer cells. Cells were treated with 1  $\mu$ M of each derivative and after 24 h incubation cells were imaged by confocal microscopy. Blue is DAPI (4',6-diamidino-2-phenylindole) nuclear stain.