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Towards the understanding of the biological activity of naphthylisoquinoline alkaloids dioncophylline A, B and C: DNA-binding properties

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1. Photometric and fluorimetric DNA titrations

Preparation of buffer solutions

For the preparation of buffer solutions E-Pure® water (18 MΩ cm) and biochemistry-grade chemicals were used. The buffer solution was filtered through a membrane filter (pore size 0.45 µM; Carl Roth GmbH, Karlsruhe) before use and kept under exclusion of light at 4 °C for a maximum of four weeks. The following concentrations were used:

BPE (biphosphate EDTA) buffer: 6.0 mM Na_2HPO_4, 2.0 mM NaH_2PO_4, 1.0 mM Na_2EDTA; pH 7.0.

ODN buffer: 6.0 mM Na_2HPO_4, 2.0 mM NaH_2PO_4, 1.0 mM Na_2EDTA, 20 mM NaCl; pH 7.0.

Preparation of DNA solutions

The DNA (approximately 1–2 mg/mL) was dissolved in BPE buffer and kept at 4 °C for 24 h under exclusion of light. In the case of ct DNA, the solution was filtered through a membrane filter before use (pore size 0.45 µM; Carl Roth GmbH, Karlsruhe). After dilution with BPE buffer (1:20), the concentration was determined photometrically [ct DNA: ε_{264} = 12824 cm^{-1} M^{-1}, λ = 260 nm; poly(dA-dT)-poly(dA-dT): ε_{264} = 13200 cm^{-1} M^{-1}, λ = 262 nm; poly(dG-dC)-poly(dG-dC) ε_{264} = 16800 cm^{-1} M^{-1}, λ = 262 nm; *c* in base pairs].[8]

Preparation of the oligonucleotide solutions

The oligonucleotides were treated with ODN buffer, heated for 5 min at 90 °C in a water bath and stored at –25 °C; *c* = 500 µM.

Preparation of ligand solutions

For spectroscopic measurements, stock solutions of the ligands **1**, **2** and **3** (*c* = 1.00 mM) were prepared in MeOH and kept under exclusion of light at 4 °C.

Sample preparation, measurement, and measurement parameters

Starting from the stock solution of the derivates **1**, **2** and **3** (in MeOH, c = 1.00 mM) a solution in the corresponding phosphate buffer solution with the concentration of c = 25 µM, c = 30 µM or c = 40 µM was prepared. The experiments were performed with a sample volume of V = 700 µL. To prepare the **TA**, **TX** and **CX** DNA solutions, equal volumes of the two complementary oligonucleotide strands were added to ODN buffer (c = 500 µM) to obtain a final concentration of 200 µM. The solution was heated for 5 min at 90 °C in a water bath, slowly cooled down to room temperature and subsequently used for the titration. Absorbance and fluorescence measurements were performed at the same concentration and initial volume, in order to correct the fluorescence spectra for the change in absorption at the corresponding excitation wavelength. First, an absorption or emission spectrum of the ligand solution was recorded. After addition of the titrant solution and an equilibration time of 1.5 min the solution was analysed with photometric or fluorimetric measurements. After each titration step, an absorption or fluorescence spectrum was recorded, and the concentration of DNA was increased until no more changes in the spectra could be observed. Photometric titrations were measured from 240 nm to 500 nm at a rate of 120 nm min^{-1} at 20 °C. The wavelength range of the fluorimetric titration was selected according to the emission bands of the ligands. Excitation wavelengths were selected based on the photometric titration. Analogous to photometric titration, the emission spectra were recorded at 20 °C with 120 nm min^{-1}. The slit widths for emission and excitation wavelength were 5 nm in all measurements. The detector voltage was between 500 V and 550 V depending on the intensity of the fluorescence. The spectra were modified with the smoothing function "moving average" with a factor of 5 with the Origin software.[9]

Determination of the binding constant K_b

The binding constants, K_b, were determined from binding isotherms extracted from the photometric titration spectra (Fig.S1) and fitting of the experimental data to the theoretical model according to equation 1.[10]

$$\frac{I}{I_0} = 1 + \frac{Q-1}{2}\left(A + xn + 1 - \sqrt{(Q + xn + 1)^2 - 4xn}\right) \qquad \text{(eq. S1)}$$

$Q = I_\infty / I_0$ = Minimal absorbance in the presence of excess ligand

n = Number of independent binding sites per DNA

$A = 1 / (K_b \times c_{Ligand})$

$x = c_{DNA} / c_{Ligand}$ = Titration variable

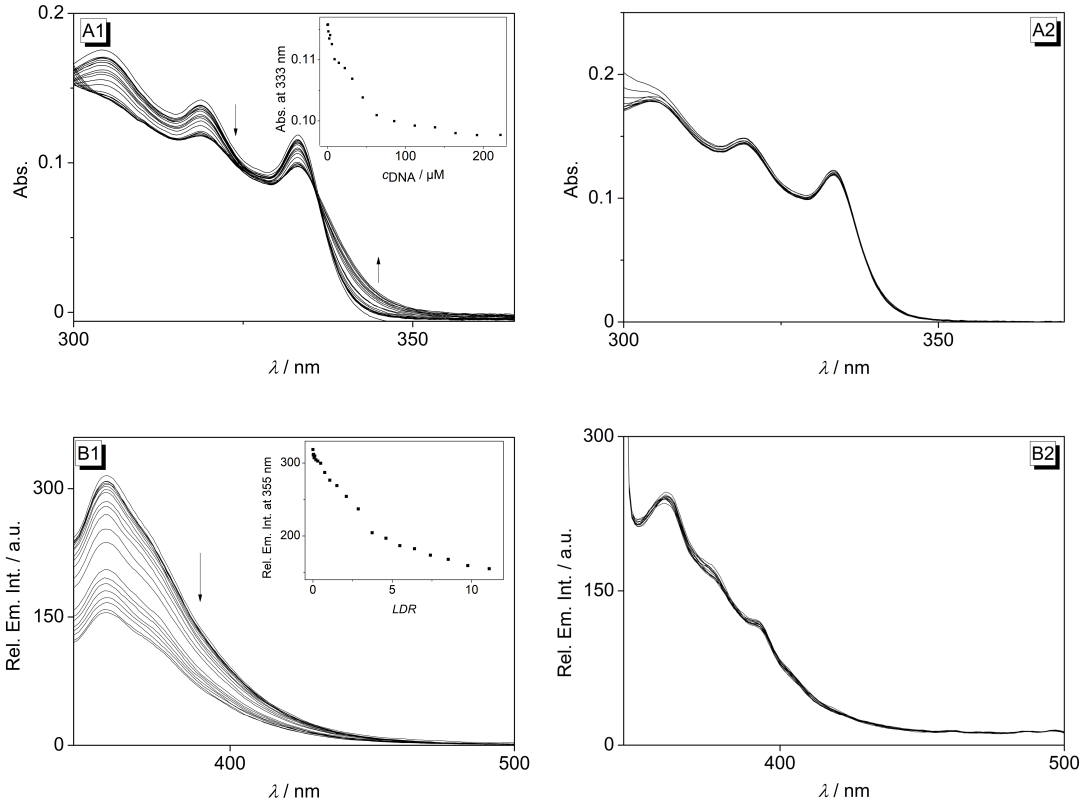

Fig. S1. Photometric (A) and fluorimetric (B) titration of **1** (c = 30 µM) with poly(dA-dT)-poly(dA-dT) (1) and poly(dG-dC)-poly(dG-dC) (2) in BPE buffer (c_{Na+} = 16 mM, pH = 7.0). The arrows indicate the development of the absorption and emission bands during the titration. Inset (A): Plot of the absorption at λ = 333 nm or λ = 337 nm versus c_{DNA}. Inset (B): Plot of the emission at λ = 355 nm versus *LDR*.

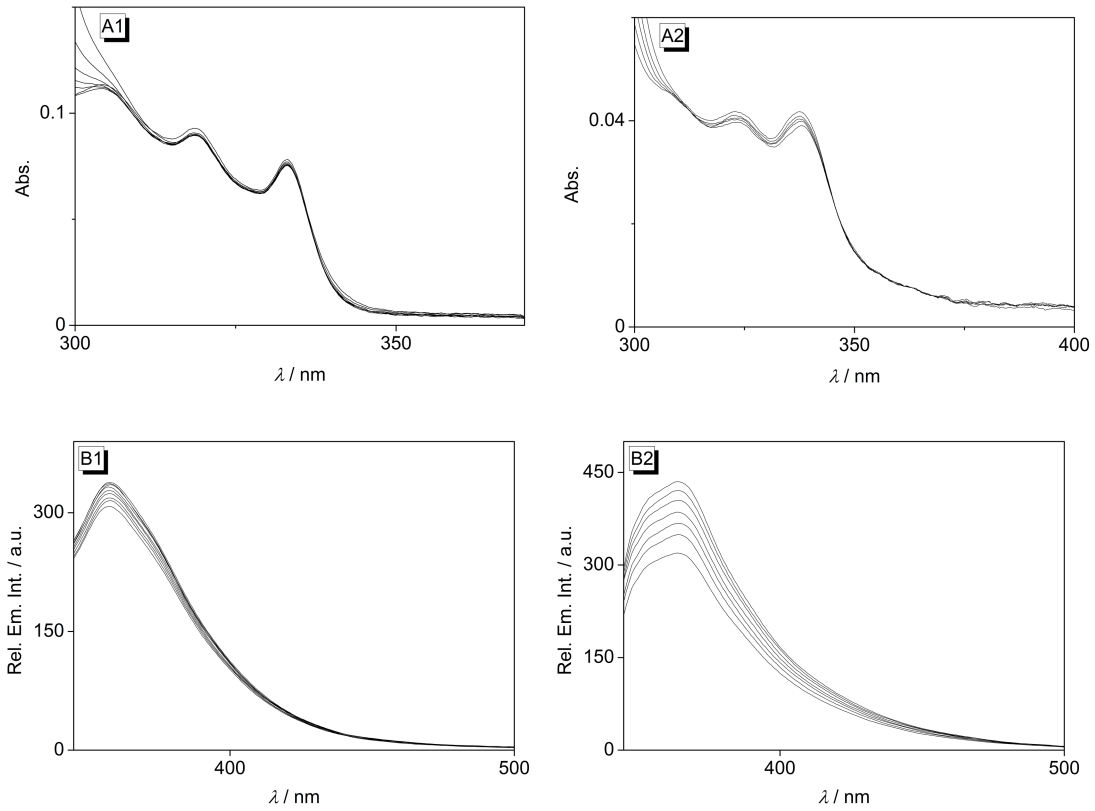

Fig. S2. Photometric (A) and fluorimetric (B) titration of **1** (1) (c = 30 μM) and **2** (2) (c = 25 μM) with **TA** in ODN buffer (c_{Na+} = 38.1 mM, pH = 7.0). The arrows indicate the development of the absorption and emission bands during the titration.

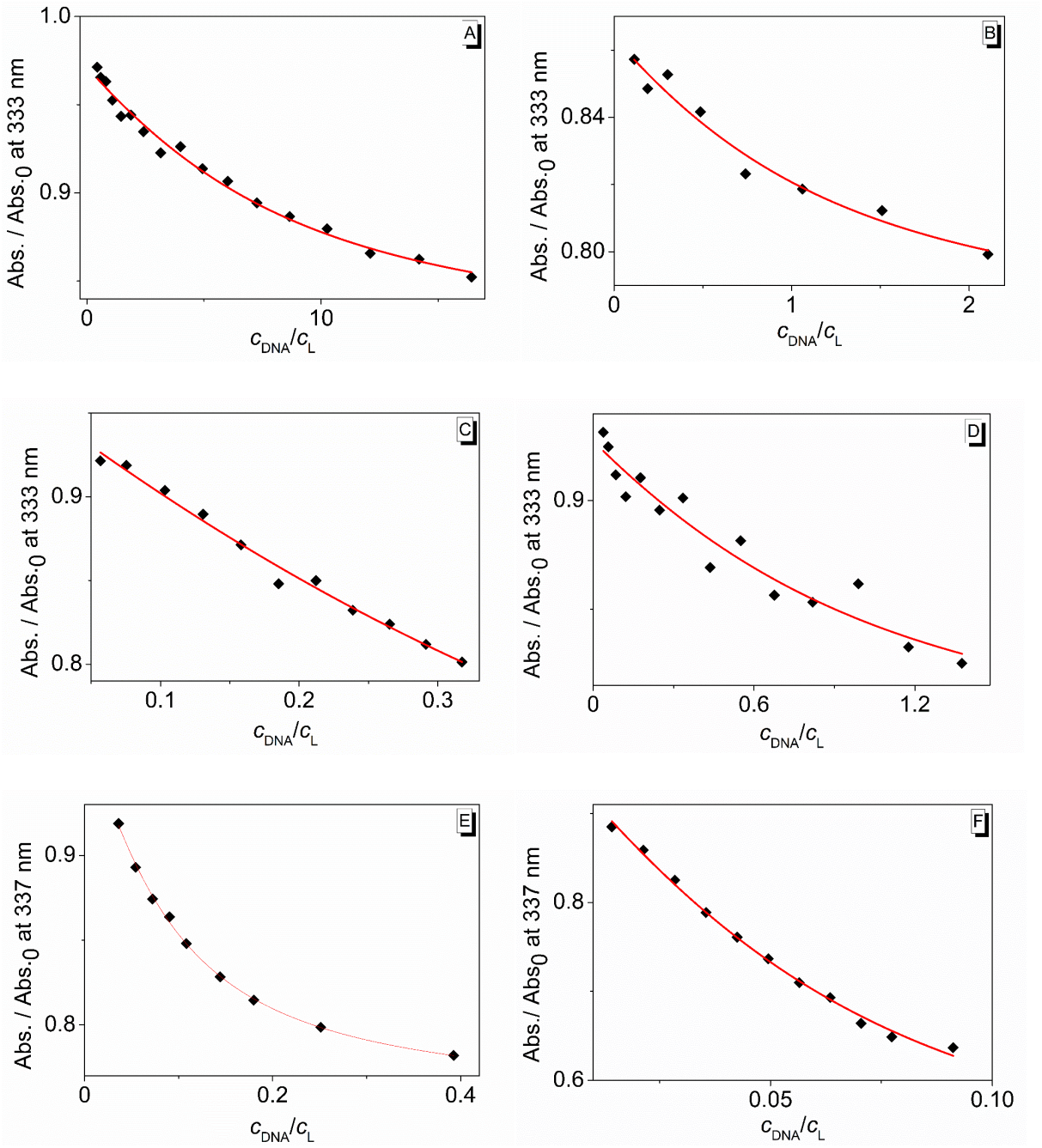

Fig. S3. Fitting curves of binding isotherms resulting from photometric titrations of **1** with ct DNA (A), poly(dA-dT)-poly(dA-dT) (B), **TX** (C), and **CX** (D), and from titrations of **2** with ct DNA (E) and with **TX** (F). Red lines represent the best fits of the experimental data to the theoretical model (eq. S1).

2. CD- and LD-spectroscopic analysis

For CD- and LD-spectroscopic analysis, a stock solution of DNA in BPE buffer with a concentration of 200 µM was prepared. The ligand solution was taken directly from the stock solution (c = 1.00 mM) in MeOH and the solvent was removed with a vigorous N_2 gas stream. For the CD measurement seven samples with constant DNA concentration (20 µM) and increasing ligand concentrations (*LDV*: 0.20, 0.40, 0.60, 0.80, 1.00, 1.60, 2.00) were prepared in BPE buffer (Table S1 and S2). A spectrum of the pure ligand in BPE buffer was also recorded. For the LD measurement seven samples with constant DNA concentration (20 µM) and increasing ligand concentrations (*LDV*: 0.20, 0.40, 0.60, 0.80, 1.00, 1.60, 2.00) were prepared in BPE buffer (Table S3).

Table S1. Composition of the solutions for CD-spectroscopic investigations of ligand-DNA interactions with dioncophylline A (**1**).

Sample[a]	c_{Ligand} /µM	V_{Ligand} / µL[b]	*LDR*[c]
1	0	0	0
2	10	20	0.20
3	20	40	0.40
4	30	60	0.60
5	40	80	0.80
6	50	100	1.00
7	80	160	1.60

[a]c_{DNA} = 20 µM, V_{DNA} = 200 µL, V_{buffer} = 1.800 mL; [b]c_0 (ligand) = 1.00 mM in BPE buffer; [c]LDR = ligand DNA ratio.

Table S2. Composition of the solutions for CD-spectroscopic investigations of ligand-DNA interactions with dioncophylline B (**2**).

Sample[a]	c_{Ligand} /µM	V_{Ligand} / µL[b]	LDR[c]
1	0	0	0
2	1	2	0.05
3	4	8	0.20
4	10	20	0.50
5	20	40	1.00
6	30	60	1.50
7	40	80	2.00

[a]c_{DNA} = 20 µM, V_{DNA} = 200 µL, V_{buffer} = 1.800 mL; [b]c_0 (ligand) = 1.00 mM in BPE buffer; [c]LDR = ligand DNA ratio.

Table S3. Composition of the solutions for LD-spectroscopic investigations of ligand-DNA interactions with ligand **1** and **2**.

Sample[a]	c_{Ligand} /µM	V_{Ligand} / µL[b]	LDR[c]
1	0	0	0
2	1	2	0.05
3	4	8	0.20
4	10	20	0.50
5	20	40	1.00
6	30	60	1.50
7	40	80	2.00
8	50	100	2.50
9	60	120	3.00
10	70	140	3.50

[a]c_{DNA} = 20 µM, V_{DNA} = 200 µL, V_{buffer} = 1.800 mL; [b]c_0 (ligand) = 1.00 mM in BPE buffer; [c]LDR = ligand DNA ratio.

The CD and LD spectra were recorded from 200 to 500 nm with a measuring speed of 1.0 nm/s at 20 °C in a cuvette with 10 mm (CD) path length, or a rotating cuvette (LD) with 1 mm path length. In LD experiments, the shear gradient was 1200 s^{-1}. The spectra were processed with the software Origin with the implemented smoothing function “moving average” with a factor of 5.[9]

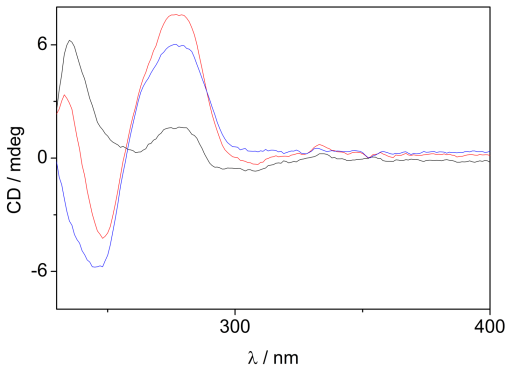

Fig. S4 Comparison of CD spectra of the free and ct DNA-bound ligand **1** (*c* = 20 µM) in a difference spectrum in BPE buffer. (cNa+ = 16 mM, pH = 7.0). Black line: Pure ligand 1; red line: LDV 1.00; blue line: difference spectrum.

3. Fluorescent indicator displacement (FID) analysis

To prepare the solutions of hybridized oligonucleotides **TX**, equal volumes of the two complementary ODN strands were added to ODN buffer (c = 500 µM) with a final concentration of 10 µM. The solution was heated for 5 min at 90 °C in a water bath, slowly cooled to room temperature and subsequently used for the titration. The solution contained berberine in the same concentration as the DNA strand (c = 10 µM) and after an equilibrium time of 30 min, a spectrum was recorded. Compound **1** (50 µM in ODN buffer or BPE buffer) was added with an equilibration time of 3 min, and after each addition a fluorescence spectrum was recorded with an excitation wavelength of λ_{ex} = 366 nm. The fluorescence spectra were recorded at a slit width of 5 nm, each for the excitation and the emission in a range from 342 nm to 600 nm. The measuring speed was 120 nm min^{-1} and the detector voltage was 800 V. The spectra were processed with the smoothing function “moving average” with the factor 10 or 5.[9]

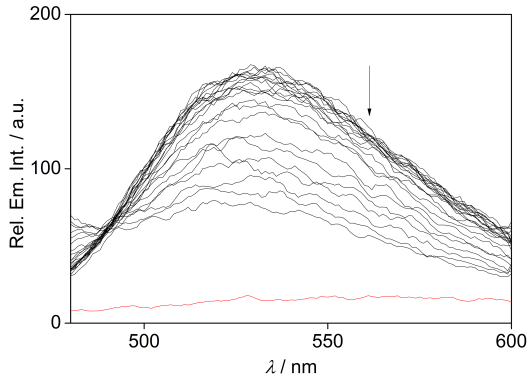

Figure S5. Fluorimetric monitoring of the displacement of berberine (c = 10 µM) from **TX** (c = 10 µM) in ODN buffer (c_{Na+} = 38.1 mM, pH = 7.0) by ligand **1** (c = 50 µM). The arrows indicate the change of the emission during the titration. Red line: Pure ligand solution of berberine (10 µM).

4. References

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