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Cooperative 2:1 binding of a Bisphenothiazine to duplex DNA

Electronic Supplementary Information

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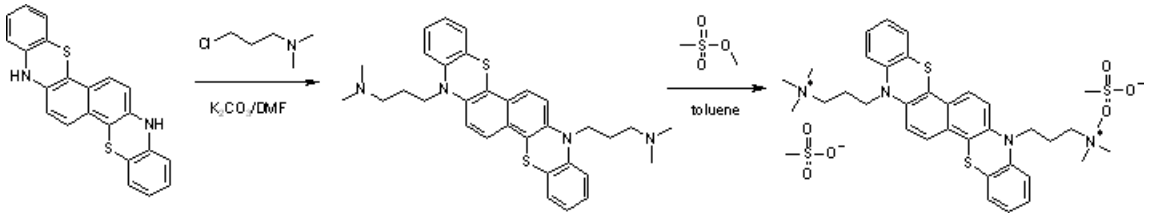
Contents:

Materials and Methods

Compound RP12274

The synthesis of RP12274 has been previously described. It was prepared in two steps from 8,16-dihydro-phenothiazino[4,3-c]phenothiazine [1], according to scheme 1.



Scheme 1.

DNA oligonucleotides

All DNA sequences were purchased from Eurogentec (Seraing, Belgium). The oligonucleotide self-complementary dodecamer duplexes used in electrospray mass spectrometry binding experiments and circular dichroism are named as follows: DK33 = $(CGTAAATTTACG)_2$, DK66 = $(CGCGAATTCGCG)_2$, DK100 = $(CGCGGGCCCGCG)_2$ and $(CGCGATATCGCG)_2$. Duplexes are formed by diluting the corresponding single strands to 100 µM in 100 mM NH_4OAc, annealing the solution, which results in 50 µM stock duplex solutions.

The oligonucleotide hairpins used for surface plasmon resonance experiments are named as follows: [AATT] = d<u>CGAATTCG</u>TCTC<u>CGAATTCG</u>, predicted melting temperature using DINAMelt [2] software was T_m = 66.1 °C in 150 mM NaCl, $[CG]_4$ = d<u>CGCGCGCG</u>TTTT<u>CGCGCGCG</u> predicted T_m = 91.9 °C in 150 mM NaCl.[2] The underlined sequences form double helical stem of the hairpin. The biotin is attached to the 5' end.

CD Spectroscopy

CD spectra were obtained using a JASCO J810 spectropolarimeter. A quartz cell (Hellma Inc.) of 10 mm path cell was used. 0.5 mm intervals from 220 to 500 nm were used. Spectra result from the averaging of four scans, followed by subtraction of the baseline using a solution of 0.1 M aqueous NH4OAc. The temperature was set to 20°C.

References:

1. Zander M. et al, *Chem. Berichte*, 1969, 102(8), 2728-2738.

2. Markham, N. R. et al., Nucleic Acids Res., 2005, 33, W577-W581.

Supplementary Results

Electrospray mass spectrometry.

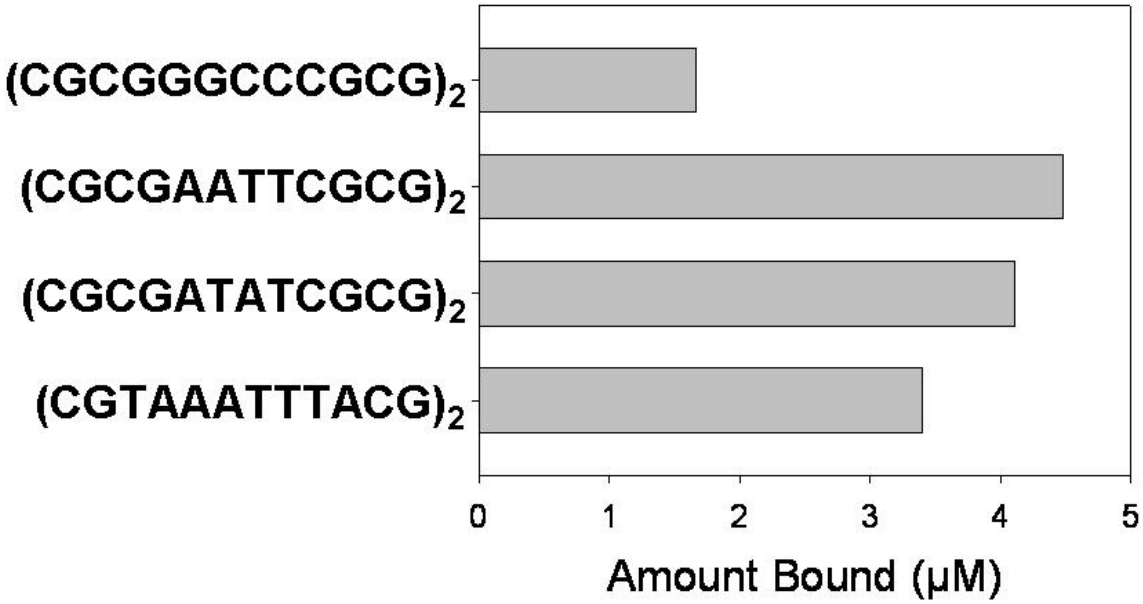


Figure S1: ESI-MS screening of ligand-duplex binding. Relative ligand affinities for the different DNA duplex: Concentration of ligand bound (in µM bound out of 8 µM total ligand added to 5 µM DNA) to duplexes. A larger proportion of RPR12274 is bound to the AT-rich duplexes.

Surface plasmon resonance (SPR) experiments in HBS buffer

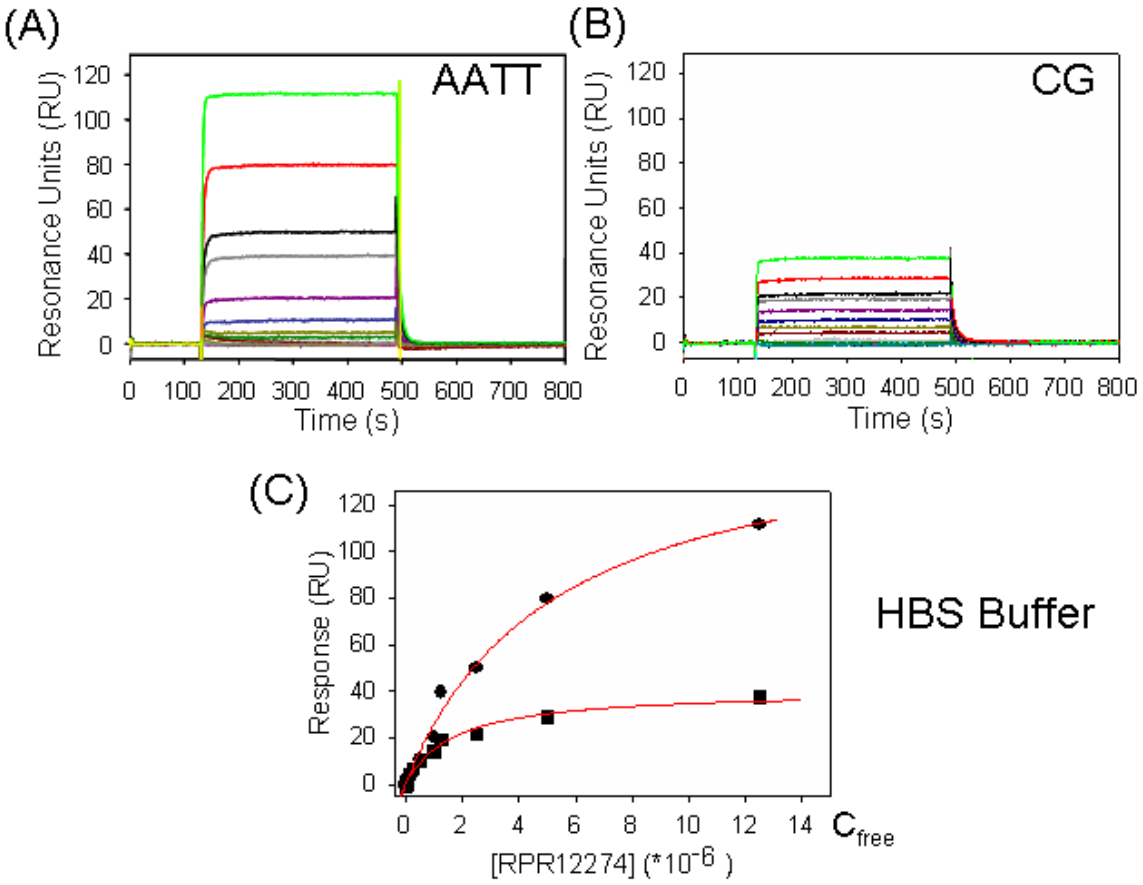


Figure S2: SPR sensograms for binding of RPR12274 to (a) AATT, (b) [CG]4 in **HBS-EP** buffer at 20 °C. The concentration of unbound ligand in the flow solution varies from 7.5 nM for the lowest curve to 12.5 µM for the top curve. (c) Binding curves used to determine the equilibrium binding constants for RPR12274 interacting with (●) AATT duplex and (■) CG duplex. HBS-EP buffer is composed of 0.01M HEPES, 0.15M NaCl, 3mM EDTA, a,d 0.005% polysorbate 20 (v/v) (pH=7.4). The values of the binding constants were for [ATT] oligonucleotide: $K_{1[AATT]}$ = 2.1 10^5 M^{-1} $K_{2[AATT]}$ = 1.2 10^6 M^{-1}. For $[CG]_4$ oligonucleotide: K_1 = 5.9 10^5 M^{-1}. K_2 was equal to 0.

Circular Dichroism

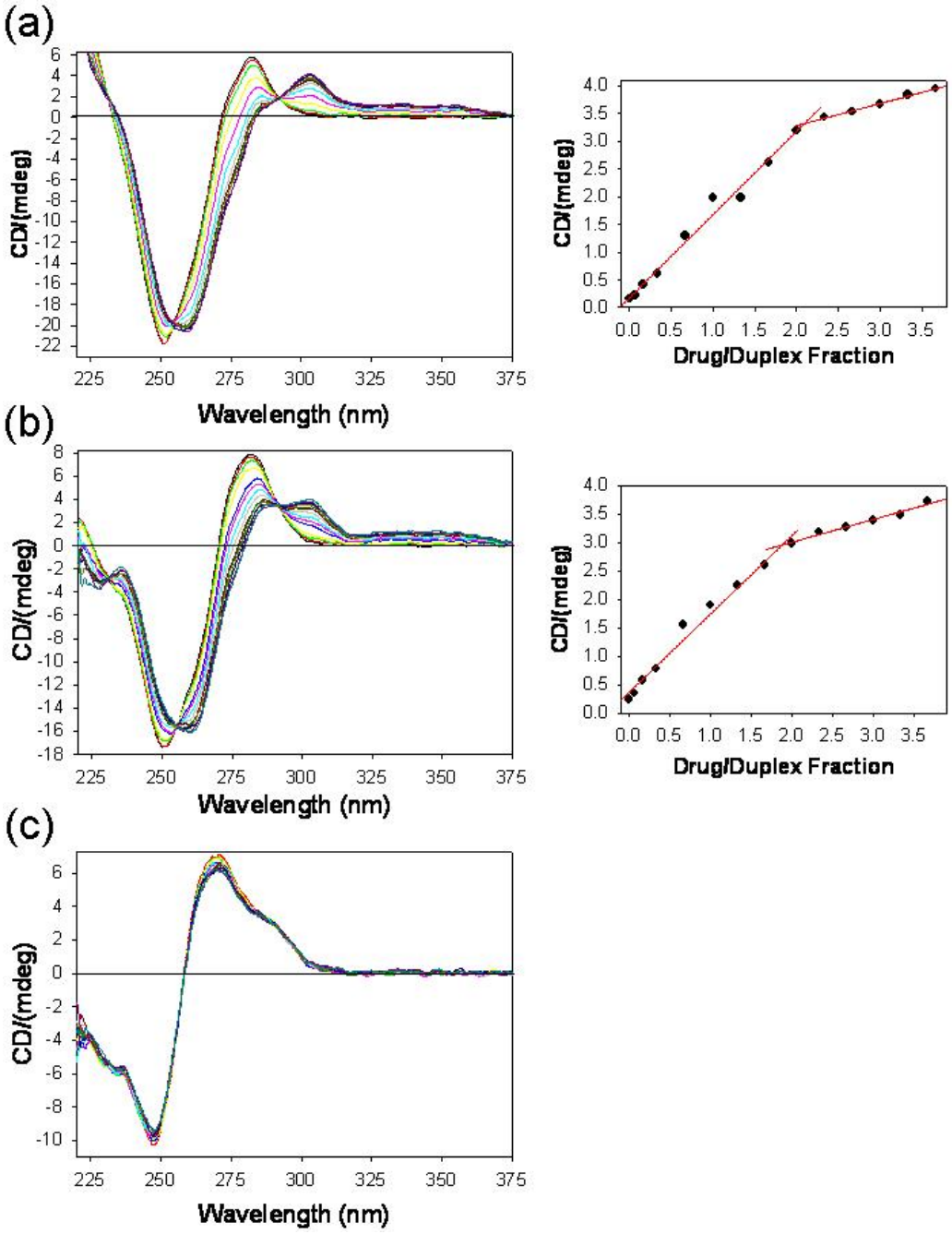


Figure S3: Circular dichroism spectra obtained after titration of RPR12274 with : (a) duplex $(CGTAAATTTAGC)_2$, (b) duplex $(CGCGAATTCGCG)_2$, (c) duplex $(CGCGGGCCCGCG)_2$ in ammonium acetate 100 mM. The starting concentration in duplex was 3 µM. The CD change at 305 nm as a function of the drug/duplex fraction ***r*** is plotted on the right.

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