Supplementary Material

Constrained bisantrrene derivatives as G-quadruplex binders

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COMPOUNDS CHARACTERIZATION

NMR Spectra

Ant1,5-Ri

Figure S1. 1H-NMR (DMSO)

Figure S2. 13C-NMR (DMSO)
Ant1,5-Ro

Figure S3. 1H-NMR (CDCl₃)

Figure S4. 13C-NMR (DMSO)
S3

Detailed assignation of $^1$H NMR signals

![Diagram of Ant1,5-Ri and Ant1,5-Ro with NMR assignments]

**Figure S5.** NMR assignation
HRMS
Experiments were carried out on a Mariner ESI-TOF (Perceptive Biosystems Inc.) in positive ionization mode.

\textit{Ant1,5-Ri} \ [(M+2H^+)/2]^+ 

\textbf{Figure S6. HRMS spectra}
S5

**Tandem Mass Spectrometry**
Experiments were carried out on a Thermo OrbiTrap LTQ Velos in positive ionization mode. HCD fragmentation experiments (13-16 eV) were performed using a 10 µM solution of the compound of interest in 150 mM ammonium acetate.

*Figure S7. HRMS fragmentation spectra*

Parent peak is marked with a star and the structures of possible fragments are reported next to their relative peaks.
Preparation of DNA samples for Mass Spectrometry (MS) analysis
Oligonucleotides were purchased from IDT, USA. The telomeric sequence “GQ” (5’ - AGG GTT AGG GTT AGG GTT AGG GT - 3’, average mass 7270.774) was dissolved in water and allowed to stand in an ice bath for 2 hours. The obtained solutions were poured into two 3000 Da cut-off centrifuge tubes and 4 mL of 150 mM ammonium acetate were added. The tubes were centrifuged for 1 hour at 4°C and the cleaning procedure was repeated another time with fresh ammonium acetate solution and for the third time with water. The final concentration of the cleaned solution obtained was measured with NanoDrop 2000c UV-Vis spectrophotometer (Thermo). The samples were then diluted to 100 µM with ammonium acetate (150 mM) and stored in a 4°C fridge for three weeks. Samples for the experiments were prepared just before the analysis from opportune dilutions of stock solutions. The presence of the G-quadruplex structure in solution without any ligand was verified through direct analysis (negative ionization mode) of these samples after a dilution to 5 µM in 150 mM ammonium acetate.
Figure S8. In the spectrum above naked oligonucleotide (1453 and 1816, charge state -5 and -4 respectively), mono (1456 and 1820, charge state -5 and -4 respectively) and di
ammonium (1459 and 1825, charge state -5 and -4 respectively) adducts are clearly detectable. Considering the -4 charge state the (GQ +2NH4⁰)⁻ peak (1825), that can be assumed as the one representative for the quadruplex structure, is the most intense of the group.
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Mass Spectrometry (MS) binding studies
Experiments were performed on a Thermo OrbiTrap LTQ Velos. Samples were prepared just before the analysis from opportune dilutions of stock solutions. The final concentration of the oligonucleotide was 3 µM in 150 mM ammonium acetate, with a ratio compound/oligo of 5:1 if a binding study was being performed. Samples were prepared just before the analysis and experiments were recorded in negative ionization mode.

Compounds were tested singularly towards GQ DNA (spectrum b reported below, example for Ant1,5) and then a more comprehensive experiment was designed in order to evaluate at the same time the competitive binding capability of the three molecules; the three molecules were mixed together with the GQ DNA sequence before the analysis and the resulting mixture was then analyzed (spectrum a).

Figure S9. Comparison of binding experiments
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IN SILICO CONFORMATIONAL SEARCH

An *in silico* conformational search was carried out on the three compounds Ant1,5, Ant1,5-Ri and Ant1,5-Ro using Avogadro$^{1,2}$. The most stable conformations detected are reported in the picture below.

![Conformational search](image)

*Figure S10. Conformational search*
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