Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Screening of DNA G-quadruplex stabilizing ligands by nano differential scanning fluorimetry

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Materials

Phosphoramidites and controlled pore glass supports for DNA synthesis were purchased from Glen Research (Sterling, USA). BRACO-19, pyridostatin, resveratrol, and acridine-9-carboxylic acid (9-Acr-COOH) were purchased from Sigma Aldrich (Merck group), while RHPS4 was obtained from CliniSciences (Italy). All common chemicals, reagents and solvents were purchased from Sigma Aldrich (Merck group) unless otherwise stated.

Methods

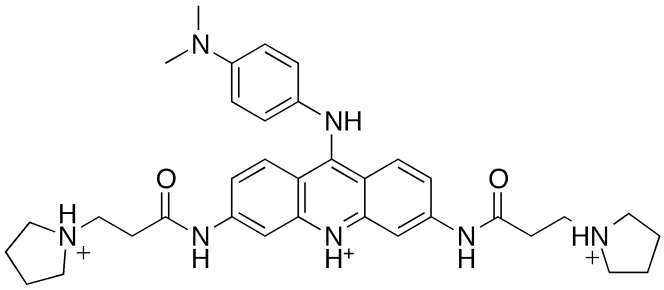
Oligonucleotide Synthesis and Sample Preparation. The oligonucleotide sequences d[TTApGGGT] and d[TTAGGGT] were synthesized on an ABI 394 DNA/RNA synthesizer (Applied Biosystem) using standard β-cyanoethyl phosphoramidite solid phase chemistry at 5 μmol scale. 2-Aminopurine phosphoramidite was efficiently introduced in the d[TTApGGGT] DNA sequence without any change of the standard method. Oligonucleotides detachment from support and deprotection were performed by treatment at 55 °C for 12 h with concentrated ammonia aqueous solution. DNAs purification was performed by high-performance liquid chromatography (HPLC) on a Nucleogel SAX column (Macherey-Nagel, 1000-8/46), using a linear gradient from 100% A to 100% B in 30 min and a flow rate of 1 mL/min (buffer A: 20 mM KH_2PO_4/K_2HPO_4 aqueous solution (pH 7.0), containing 20% (v/v) CH_3CN; buffer B: 1 M KCl, 20 mM KH_2PO_4/K_2HPO_4 aqueous solution (pH 7.0), containing 20% (v/v) CH_3CN). The fractions of purified oligomers were collected and successively desalted by Sep-pak cartridges (C-18), and the isolated oligonucleotides were proved to be >98% pure by NMR. Oligonucleotides concentration was determined by UV adsorption measurements at 90 °C using appropriate molar extinction coefficient values ε (λ = 260 nm) calculated by the nearest-neighbour model. G-quadruplex samples were prepared using a 10 mM KH_2PO_4 buffer containing 35 mM KCl at pH 7.4. All samples were heated at 90 °C for 5 min, then gradually cooled to room temperature overnight, and incubated for 24 h at 4 °C before data acquisition. For each measurement, 80 μM of single stranded DNAs (d[TTApGGGT] or d[TTAGGGT]) was used, which correspond to 20 μM of each $(d[TTApGGGT])_4$ (Tel7Ap) or $(d[TTAGGGT])_4$ (Tel7) G-quadruplex.

Circular dichroism (CD) experiments. CD experiments were recorded on a Jasco J-815 spectropolarimeter equipped with a Jasco PTC-423S temperature controller. CD spectra were recorded in a quartz cuvette with 1 mm path length, in the wavelength range of 220–360 nm, and averaged over three scans. The following parameters were set: 100 nm/min scan rate, 1 s response time and 1 nm bandwidth. Buffer baseline was subtracted from each spectrum.

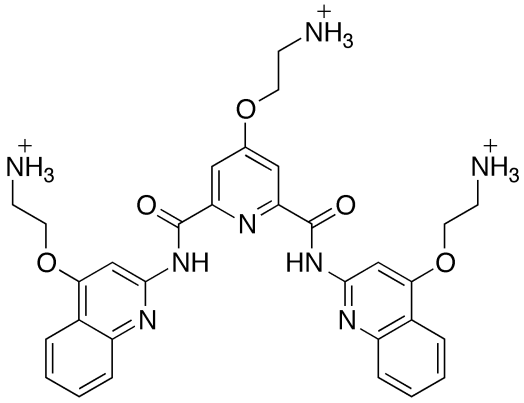
CD melting experiments were acquired in the 20-95 °C or 20-100 °C temperature range, using a heating gradient of 1 °C/min. CD melting curves were obtained by following changes of CD signal at the wavelength of the maximum CD intensity, i.e. 260 nm for both Tel7Ap and Tel7 G-quadruplexes. Apparent melting temperatures ($T_{1/2}$) were determined from curve fit using Origin 7.0 software. $\Delta T_{1/2}$ values were calculated as the difference in the melting temperature of DNA structures with and without ligands. CD melting experiments were performed in triplicate and the values reported are average of three measurements.

All measurements were recorded both in the absence and presence of ligands (BRACO-19, pyridostatin and RHPS4) added to the folded DNA structures. G-quadruplex/ligand mixtures were obtained by adding 1 and 5 mol equiv. of each compound to the G-quadruplex solution (20 μM). Experiments with negative controls (resveratrol and 9-Acr-COOH) were performed by adding 5 molar equivalents of each compound. Stock solutions of all ligands were 10 mM in DMSO.

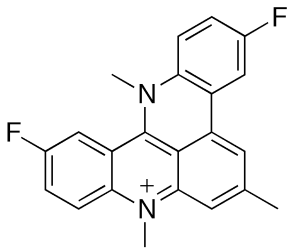
Nano-DSF experiments. The Prometheus NT.48 instrument (NanoTemper Technologies) was used to determine melting temperature ($T_{1/2}$) values of Tel7Ap G-quadruplex both in the absence and presence of ligands. Samples (10 µL per sample) were manually loaded into nanoDSF High Sensitivity Capillaries (NanoTemper Technologies). Thermal unfolding was detected by using a linear heating ramp of 1 °C/min from 20 to 95 °C with an excitation power of 98%. Unfolding transition points were determined from changes in the emission wavelengths of the 2-aminopurine fluorescence signals at 330 nm, 350 nm and their ratios. Data were analysed with Prometheus Stability Analysis software (NanoTemper Technologies) where the transition midpoints were determined automatically from the second derivative of the averaged unfolding profiles. Each measurement was performed at least in triplicate, and the standard deviation between repeats was calculated.



BRACO-19



Pyridostatin



RHPS4

Fig. S1 Chemical structures of the G-quadruplex ligands.

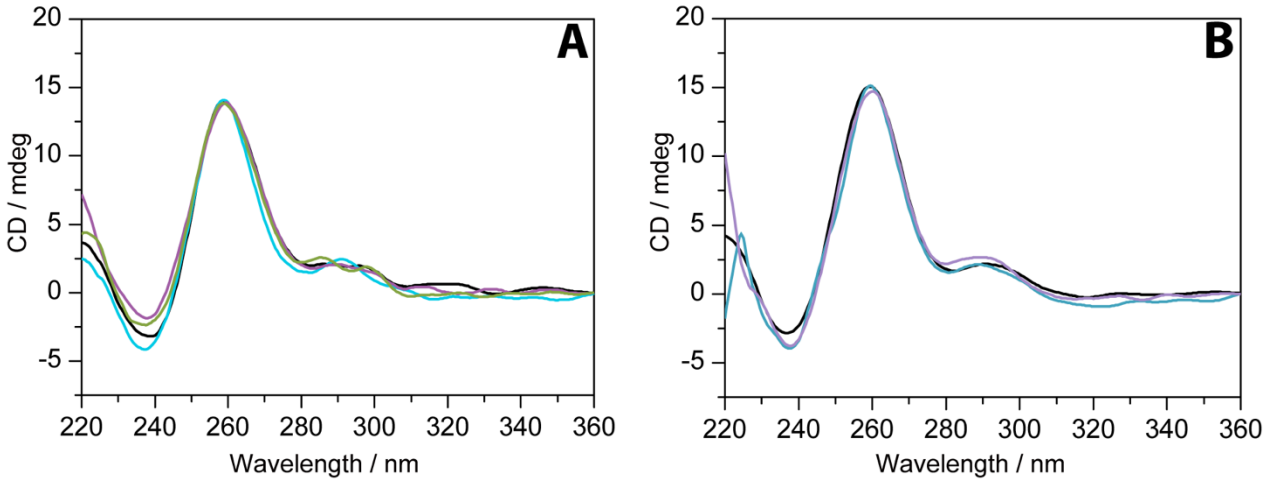
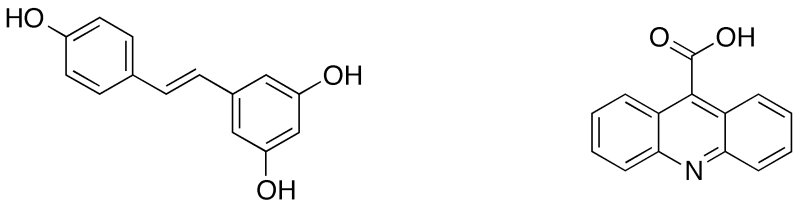


Fig. S2 CD spectra of Tel7Ap G-quadruplex recorded at 20 °C in the absence (black lines) and presence of (**A**) BRACO-19 (cyan line), pyridostatin (purple line), RHPS4 (olive line); and (**B**) resveratrol (violet line), and 9-Acr-COOH (light blue line).



Resveratrol **9-Acr-COOH**

Fig. S3 Chemical structures of resveratrol and acridine-9-carboxylic acid (9-Acr-COOH).