Supplementary Information

Discovery of new G-quadruplex binding chemotypes

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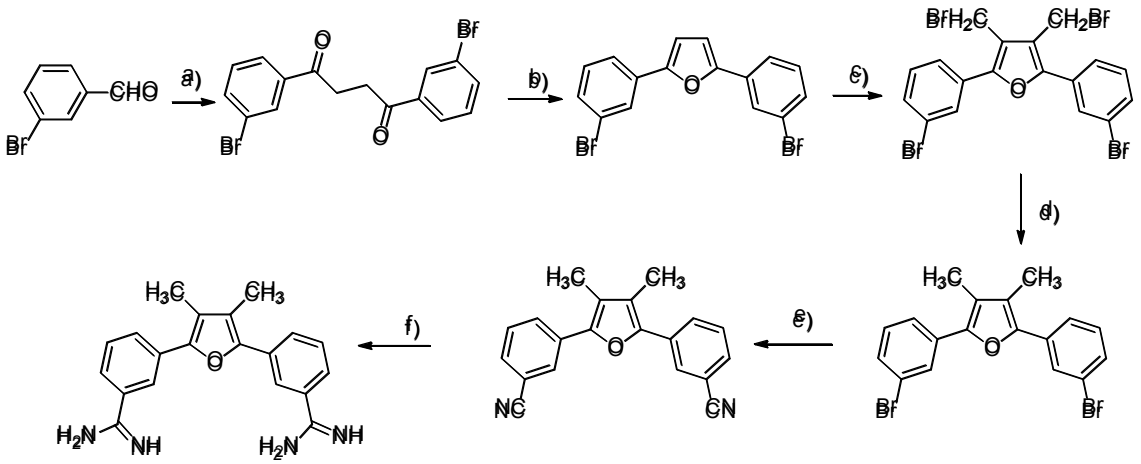
General Methods

Reagents and chemicals were purchased from Sigma-Aldrich and VWR, and were used as supplied without further purification. HPLC analyses were performed using a Gilson apparatus combining a 322 pump and an Agilent 1100 series detector. LCMS analyses were performed using a Waters system combining a 2695 separation module, a Micromass ZQ spectrometer and a 2996 photodiode array detector.

I: Chemistry: synthesis of compound 3

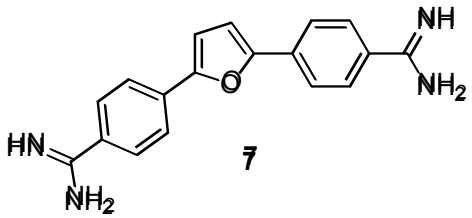


Reagents and conditions: a) divinyl sulfone,NaOAc, 3-benzyl-5-(2-hydroxyethyl)thiazolium chloride, EtOH, reflux b) Ac_2O, H_2SO_4, reflux c) paraformaldehyde, HBr/HOAc, rt d) $LiAlH_4$, ether/THF, rt e) CuCN, NMP, reflux f) i. HCl, EtOH, rt ii. NH_3, EtOH, rt.

2, 5- Bis (3-amidinophenyl)-3, 4-dimethyl-furan dihydrochloride (**3**)

Anhydrous ammonia gas was bubbled into a cold suspension of 0.463 g (0.001mol) bis-imidate ester dihydrochloride [prepared as described in Chaires *et al. J. Med. Chem*. 47, 5729 -5742 (**2004**)] in 35 ml anhydrous ethanol. The reaction mixture was stirred at rt for 12 h, solvent removed under reduced pressure, ice water was added (30 ml), filtered, basified with 2N NaOH to pH 10, the precipitated pale yellow solid was filtered, washed with water and dried under reduced pressure. The solid was suspended in 15 ml absolute ethanol, 3 ml of saturated ethanolic HCl was added, the mixture was stirred for 5 h, solvent reduced to ca. 3 ml under reduced pressure, 30 ml of anhydrous ether was added, filtered, washed with ether, dried under reduced pressure (50 °C, 12 h) to yield a pale yellow solid 0.37 g (89%), mp 285-287°C.; ^{1}H NMR (DMSO-d_6): 9.5 (bs, 4H), 9.13 (bs, 4H), 8.13 (s, 2H), 8.06 (d, 2H, J = 10 Hz), 7.73-7.62 (m, 4H), 2.27 (s, 6H); ^{13}C NMR (DMSO-d_6): 165.6, 146.0, 131.6, 130.2, 129.8, 128.7, 126.6, 124.5, 121.4, 9.5; FAB MS: 333 (M^++1); Analysis calc. for $C_{20}H_{22}N_4O.2HCl.1.25\ H_2O$: C, 55.88; H, 6.21; N, 13.03; Found: C, 56.12; H, 5.99; N, 12.91.

Compounds **1**, **2**, **4-6** and **7** (DB75) have previously been reported by the Boykin group.



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II: Fluorescence Resonance Energy Transfer (FRET) Data

Fluorescence Resonance Energy Transfer experiments. The ability of compounds **1-7** to stabilize G-quadruplex DNA sequences was investigated using a fluorescence resonance energy transfer (FRET) assay modified to be used as a high-throughput screen in a 96-well format. The labelled oligonucleotides had attached the donor fluorophore FAM: 6-carboxyfluorescein and the acceptor fluorophore TAMRA: 6-carboxytetramethylrhodamine. The FRET probe sequences were diluted from stock to the correct concentration (400 nM) in a 60 mM potassium cacodylate buffer (pH 7.4) and then annealed by heating to 95 °C for 10 min, followed by cooling to room temperature in the heating block (3 - 3.5 hrs). The compounds were stored as a 1 mM stock solution in 10% DMSO / 90% distilled water; final solutions (at 2× concentration) were prepared using 60 mM potassium cacodylate buffer (pH 7.4). Relevant controls using BRACO-19 (in addition to blank runs) were also performed to check for quality of DNA samples. 96-Well plates (MJ Research, Waltham, MA) were prepared by aliquoting 50 µl of the annealed DNA into each well, followed by 50 µl of the compound solutions. Measurements were made on a DNA Engine Opticon (MJ Research) with excitation at 450–495 nm and detection at 515–545 nm. Fluorescence readings were taken at intervals of 0.5 °C in the range 30–100 °C, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value.

Final analysis of the data was carried out using a script written in the program Origin 7.0 (OriginLab Corp., Northampton, MA). The advanced curve-fitting function in Origin 7.0 was used for calculation of ΔT_m values. Esds in ΔT_m are ± 0.3° C.

III: *In Vitro* Cell Assay Data

Cell culturing. The cell lines A549, ALT, 786-O, Mia-PaCa2, Panc-1, RCC4 (European Collection of Cell Cultures) and WI38 were maintained in monolayer culture in 75 cm^2 flasks (TPP, Switzerland) under a humidified 5 % CO_2 atmosphere at 37 °C. Incubations were also done under these conditions, unless specified otherwise. For the cell lines MCF7 and A549, the medium Dulbecco's MEM (GIBCO 21969, Invitrogen, UK) supplemented with L-glutamine (2 mM, GIBCO 25030, Invitrogen, UK), essential amino acids (1 %, GIBCO 11140, Invitrogen, UK), foetal calf serum (10 %, S1810, Biosera, UK) was used. For MIA-Pa-Ca-2 Dulbecco's MEM, supplemented with L-glutamine (2 mM) and foetal calf serum (10 %) was used. The medium MEM (M2279, Sigma, UK) with added L-glutamine (2 mM), essential amino acids (1 %) and foetal calf serum (10 %) was used for the cell line WI38. Cells were counted using a Neubauer haemocytometer (Assistant, Germany) by microscopy on a suspension of cells obtained by washing with PBS, trypsinisation, centrifugation at 8000 rpm for 3 minutes, and re-suspension in fresh medium.

Sulforhodamine B assay (SRB). The cells were counted and diluted to the required concentration in 20 mL medium. For the cell lines A549, Panc-1, Mia-PaCa2, ALT and RCC4, 1000 - 4000 cells with 160 µL media (WI38: 6000/well) were seeded into each well of a 96 well plate (Nunc, Denmark). After incubation for 24 hours, the compound to be tested, dissolved in 40 µL of medium was added in a range of concentrations, and the cells incubated for 96 hours. The medium was then removed and the cells fixed by incubation with TCA (10 %, Sigma-Aldrich, UK) for 30 min at 4 °C. After removal of the TCA, the cells were washed with deionised water 5 times and dried at 60 °C for 1 h. The cells were then incubated with sulforhodamine B (80 µL, 0.4% in 1 % acetic acid, Acros Organics, UK) for 15 min at RT. The SRB was removed, the wells washed with 1 % acetic acid (200 µL), and dried at 60°C for 1 h. Tris-base (100 µL, 10 mM, Acros Organics, UK) solution was added to each well, and the plates were gently shaken for 5 min. The absorbance at 540 nm was measured with a plate reader (Spectrostar Omega, BMG Labtech, Germany). The data were normalized to the value of 100 for the control experiment (untreated cells), and the IC_{50} values were obtained by interpolation from a plot with Origin (Version 7.0, OriginLab Corp.), as the concentration leading to an absorbance intensity of 50%.

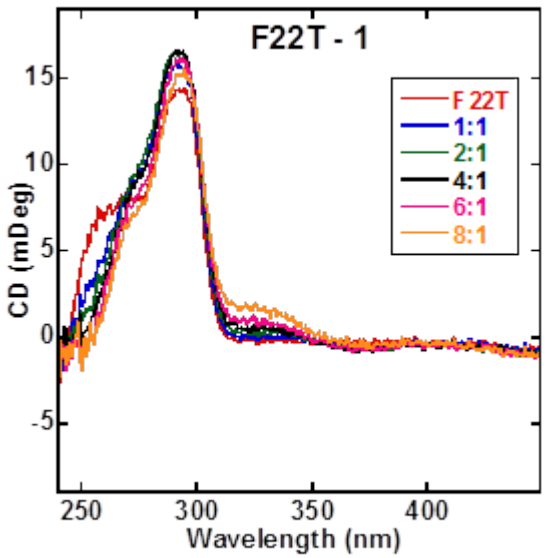
IV: Circular Dichroism (Experimental conditions)

Buffer: 10 mM K_2HPO_4, 100 mM KCl, 1 mM EDTA, pH 7.2

Tel22: 5'-AGGGTTAGGGTTAGGGTTAGGG-3'

AATT: 5'-GCGAATTCGCTCTCGAATTCGC-3' (loop bases are underlined)

CD studies were conducted using a Jasco J-810 spectrophotometer with a 1 cm path length quartz cuvette at 25 °C. DNA solutions (5 μM) were annealed in experimental buffer overnight prior to the collection of spectra. Appropriate amount of the ligands were sequentially titrated into the DNA solution and the spectra were collected with a scanning speed of 50 nm/min and a response time of 1 second over the wavelength range of 230 nm to 550 nm. The spectra were collected until no further change in the induced CD signal was observed upon further ligand titration. The spectra were averaged over four scans, and a buffer scan collected in the same cuvette was subtracted from the average scan of each ratio. Data were processed and plotted using Kaleidagraph 4.0 software.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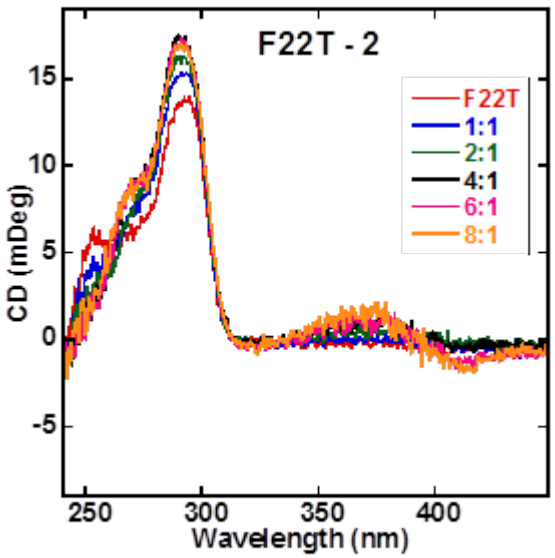
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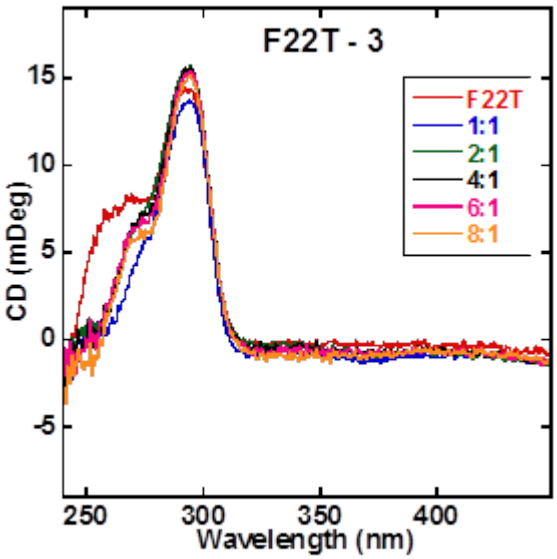
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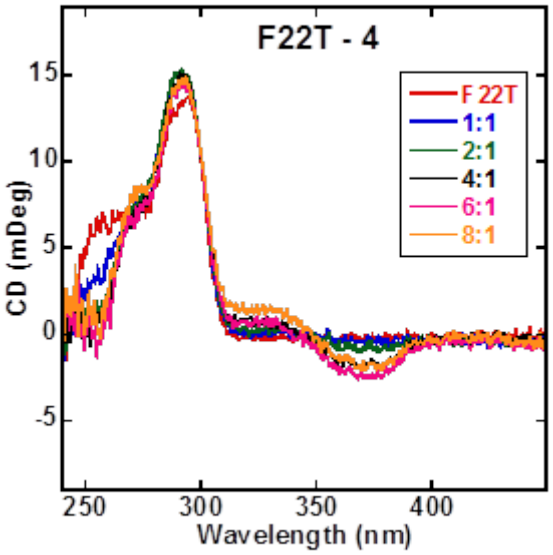
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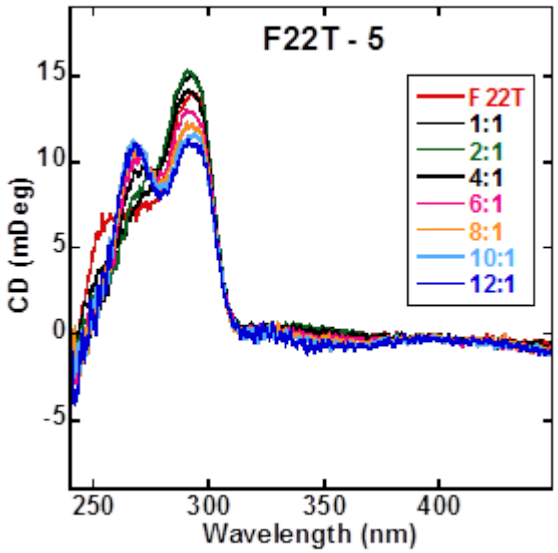
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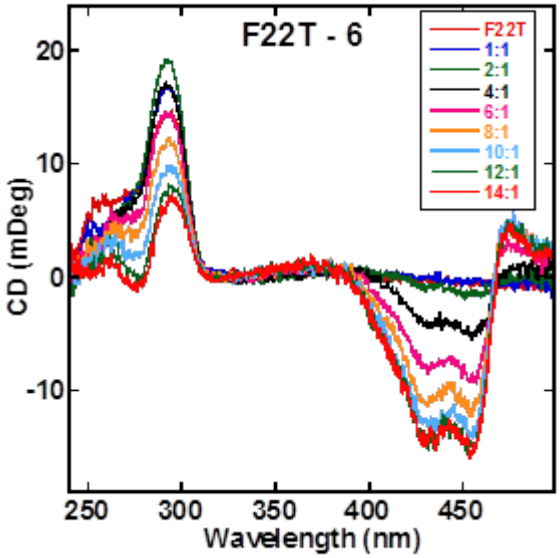
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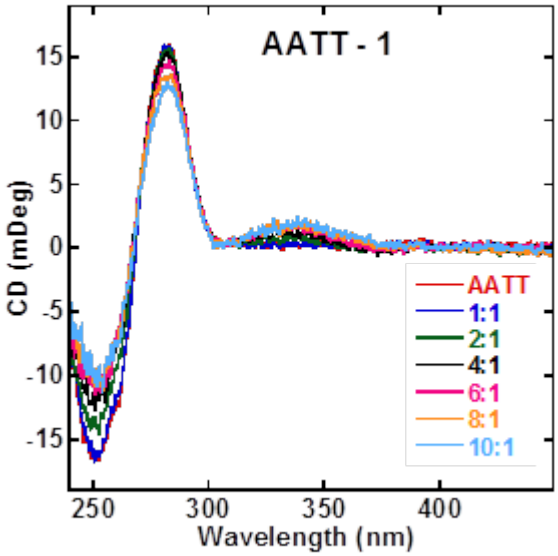
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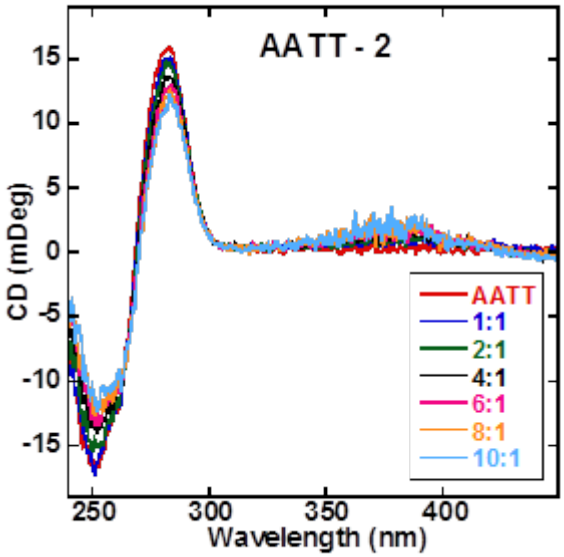
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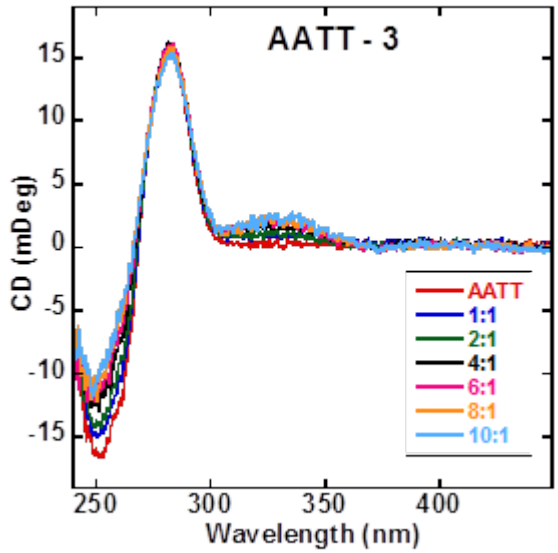
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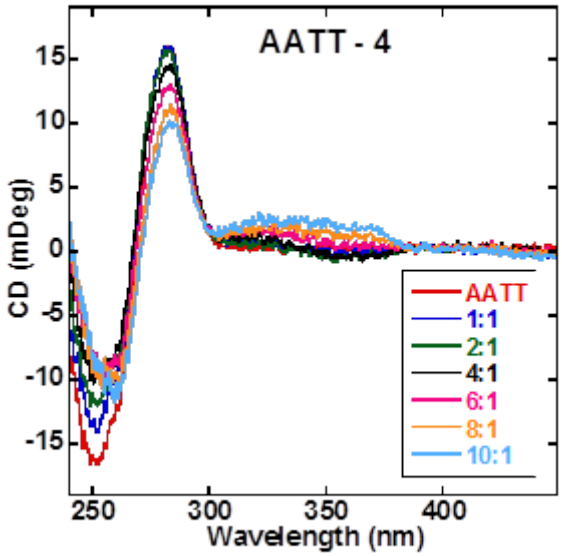
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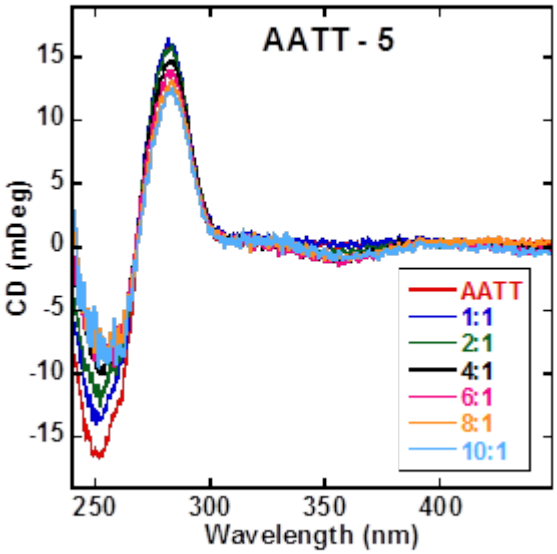
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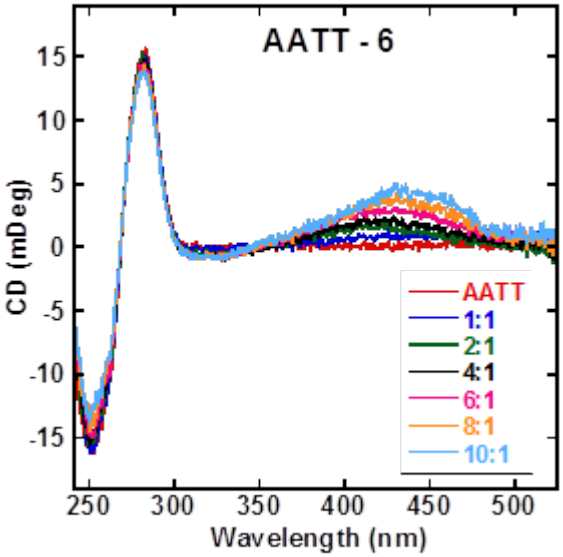
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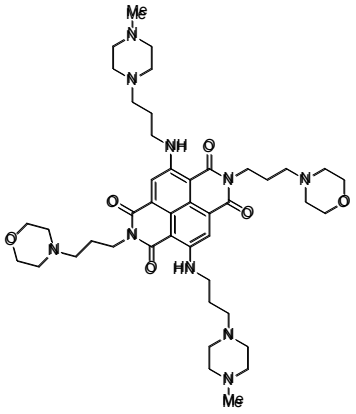

Compound **8**