Supplementary Information

SUBSTITUTED NAPHTHALENEDIIMIDE COMPOUNDS BIND SELECTIVELY TO TWO HUMAN QUADRUPLEX STRUCTURES WITH PARALLEL TOPOLOGY

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EXPERIMENTAL

Nucleic acid sequences and compounds

Quadruplex DNA (G4) and duplex DNA sequences were annealed from oligonucleotides purchased from Integrated DNA Technologies (Coralville, IA, USA). Biotin-labeled sequences were also purchased for SPR experiments. Biotin-labeled duplex DNA (AAAGAACTTT) was used as a negative control in SPR studies. For quadruplex annealing, the oligonucleotides were resuspended in 10 mM Tris-HCl pH 7.4, 1 mM EDTA and containing 50 mM KCl to yield a final concentration of 1 mM based on theoretical calculation. The stock solution was then incubated at 90°C for 10 min in the thermos bath, then gradually cooled down to room temperature overnight. The stock concentration was determined spectroscopically at 260 nm using the nearest-neighbor method of extinction coefficient. The control was a TERT hairpin duplex model containing the identical sequences of TERT and including the complementary strand as a hairpin (AGG GGA GGG GCT GGG AGG GC CTCT GC CCT CCC AGC CCC TCC CCT). The mixture then was treated with the same procedure for incubation in the thermos bath, as mentioned for quadruplex sequences. The final concentration of the duplex was determined by UV vis at 260 nm using the nearest neighbor method. Compounds were weighed and dissolved in 100% DMSO to the final concentration of 2 mM.

Intrinsic fluorescence titration

Fluorescence measurements were carried out with a Variant Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Satna Clara, CA). Both excitation and emission slits were set to 20 nm. The sample was measured using a 3-mL, four-sided quartz cuvette with the concentration of CM03, SOP1812, and MM41 starting at 30 nM. The amount of G4 oligonucleotide needed for the titration was calculated and corrected for the dilution factor. The final volume change was kept to less than 10% compared to the initial volume to minimize the effect of dilution on the probes. CM03 was excited at 525 nm, and the measurements were taken at a maximum emission wavelength of 570 nm. SOP1812 was excited at 530 nm and recorded at maximum emission wavelength of 580 nm. Each measurement was done in triplicate and averaged. The experimental buffer contained 10 mM Tris-HCl pH 7.4, 50 mM KCl. The dissociation constant (K_D) of the interaction between

molecule and DNA sequences were obtained by fitting the quenching in the total fluorescence intensity upon complex formation to a 1 to 1 binding model [Eq. (1)]:

$$A_{measure} = A_{initial} - (A_{initial} - A_{final}) \frac{K_D + L + D - \sqrt{(K_D + L + D)^2 - 4LD}}{2L} \quad (1)$$

where $A_{initial}$: intensity measured without G4; A_{final}: intensity measured of CM03 fully bounded to G4; K_D: Dissociation constant of the complex; L: total concentration of fluorophore (CM03); D: total concentration of G4.

Fluorescence anisotropy titration

Fluorescence polarization measurements were obtained using a Variant Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, CA). The manual excitation and emission polarizers were inserted in between light source and detector at 90° in the standard configuration. Measurements then were converted into anisotropy value by using the equation:

$$r = \frac{I_{vv} - GF * I_{vh}}{Ivv + 2GF * I_{vh}} \quad (2)$$

Where:

$$GF = \frac{I_{vv}}{I_{vh}} \quad (3)$$

r is the anisotropy value; I_{vv} is the fluorescence intensity of the compound when excitation and emission polarizer align in vertical position; I_{vh} is the fluorescence intensity of compound when excitation and emission polarizers are aligned in vertical and horizontal positions, respectively; I_{hv} is the fluorescence intensity of the compound when excitation and emission polarizer align in horizontal and vertical positions; I_{hh} is the fluorescence intensity of the compound when excitation and emission polarizers align in horizontal positions; GF stands for the grating factor of the polarizers. Fluorophore compounds, MM41, excited at 630 nm and recorded at the maximum emission wavelength of 670 nm, was added into a 3mL, four-sided polished quartz cuvette at a fixed concentration of 50 nM and then G4 was titrated into the cuvette. The amount of G4-RNA was calculated before each titration experiment, and the concentration of fluorophore was corrected after each experiment so that the change in the end volume of titration less than 10% to the

initial volume. The measures were taken at the maximum emission wavelength of MM41 (λ_{em} = 630 nm coordinated to emission wavelength λ_{em} = 670 nm). Both excitation and emission slits were open at 20 nm. Each set of measurement values were taken from a 50-second average reading measurement, PMT = 630 V. Experimental buffer condition was 50 mM KCl, 10 mM Tris-HCl pH 7.3 and 1 mM EDTA. The dissociation constant (K_D) of the molecule to quadruplexes was obtained by fitting the changes in anisotropy values of the compound during the titration, by using the following equation for a 1 to 1 binding reaction, assuming there is no non-specific binding occurring between the molecule and the G4:

$$r_{observed} = r_{\min} + (r_{\max} - r_{\min}) \frac{K_D + L + D - \sqrt{(K_D + L + D)^2 - 4LD}}{2L} \quad (4)$$

Where r_{min} is the anisotropy value of unbound molecule; r_{max} is the anisotropy value of fully bound molecule; L: total concentration of fluorophore (CM03); D: total concentration of G4.

UV Thermal Melting

Measurements were carried out using a Cary UV-Vis 300 spectrophotometer (Agilent Technologies, Satna Clara, CA). For G4 DNAs, the sample contains 5 μM in the experimental buffer, and different concentrations of compounds in compound:DNA ratios of 0:1, 1:1, 2:1 and 4:1. Each sample was mixed and prepared in a 1 mL cuvette. Before the experiment, samples were heated to 95°C for 1 min, then retained to 90°C for 3 min and finally cooled down to 25°C and kept for 15 mins. The change in absorbance at wavelength 295 nm, a signature footprint for a G4, was recorded upon increasing temperature at the rate of 0.5°C/min from 25°C to 95°C. Experiments for the duplex sequence used the same protocol, except for the change in absorbance, which was recorded at 260 nm. The difference in T_m (ΔT_m) was calculated by subtracting the T_m of a sample containing only G4 from a sample containing a mixture of compounds and G4.

Biosensor surface plasmon resonance (SPR)

SPR measurements were carried out with a 4-channel Biacore T200 biosensor instrument (GE Healthcare, Pittsburgh, PA). Biotinylated sequences of interest were immobilized on flow cells 2-4 at low density (~ 250 RU). Flow cell 1 was used as a reference cell. The experimental buffer was 10 mM Tris – HCl pH 7.4, 50 mM KCl, 1

mM EDTA, and 0.05% P20 surfactant. The flow rate was maintained at 100 uM/min to minimize the mass transport effect between the bulk sample and the local environment on the sensor chip. The response in the reference cell was subtracted from the cell containing G4 to give the net signal determining the binding of compounds. The predicted maximum response can be calculated from the known immobilized DNA on the sensor chip, the molecular weights of the G4 and the compound. Equilibrium constants were obtained from fitting the data at steady-state by a single site (K2 = 0) or a two-site model [Eq (5)]:

$$r = \frac{K_1 C_{free} + 2K_1 K_2 C_{free}^2}{1 + K_1 C_{free} + K_1 K_2 C_{free}^2} \quad (5)$$

Where:

$$r = \frac{RU_{obs}}{RU_{\max}} \quad (6)$$

K_1 and K_2 are the equilibrium binding constants and C_{free} is the concentration of the free compound. Data were plotted and fitted using Kaleidagraph 4.1 software.

The concentration of the oligonucleotides can be quantified on the sensor chip, given the known immobilized RU level. We were able to separate the free and bound ligand concentration in the SPR experiment because of the inclusion of a reference flow cell. However, since the fluorescence titration experiment was set up in a free solution environment, therefore the equation needs to be adjusted to account for the difference in the free and bound ligand concentrations.

The nonspecific binding mode of compound SOP1812 is not significant at the higher concentrations used (40-100 nM) since the incentive increment of RUobs is minimal comparing to that at lower compound concentrations (1- 40 nM). Therefore, in this case the predicted value for the RUmax of specific binding between compound and G4-hTERT is statistically reliable. On the other hand, a two-state binding analysis of SOP1812 with G4-hTERT did not improve the outcome, where the fitting results in two similar Kd values and good agreement with Kd value obtained from a single-state binding analysis. CD results also suggested that there are no additional binding interactions rather than the terminal stacking of compound to G-quadruplexes at μM concentrations.

Circular Dichroism (CD) titration – CD studies were performed on the Jasco J-1500 spectrophotometer (Easton, MD) with a 1 cm path-length 2-side clear cuvette at room temperature. The concentration of G4s was kept constant at 5 μM while increasing titration concentration of individual compounds from 0:1 up to a 4:1 ratio of compound to G4. Each final data set was an average of triplicate measurement and subtracted from the blank reference. The scan speed was set to 200 nm/min. Each scan covered a range of wavelengths from 650 to 220 nm.

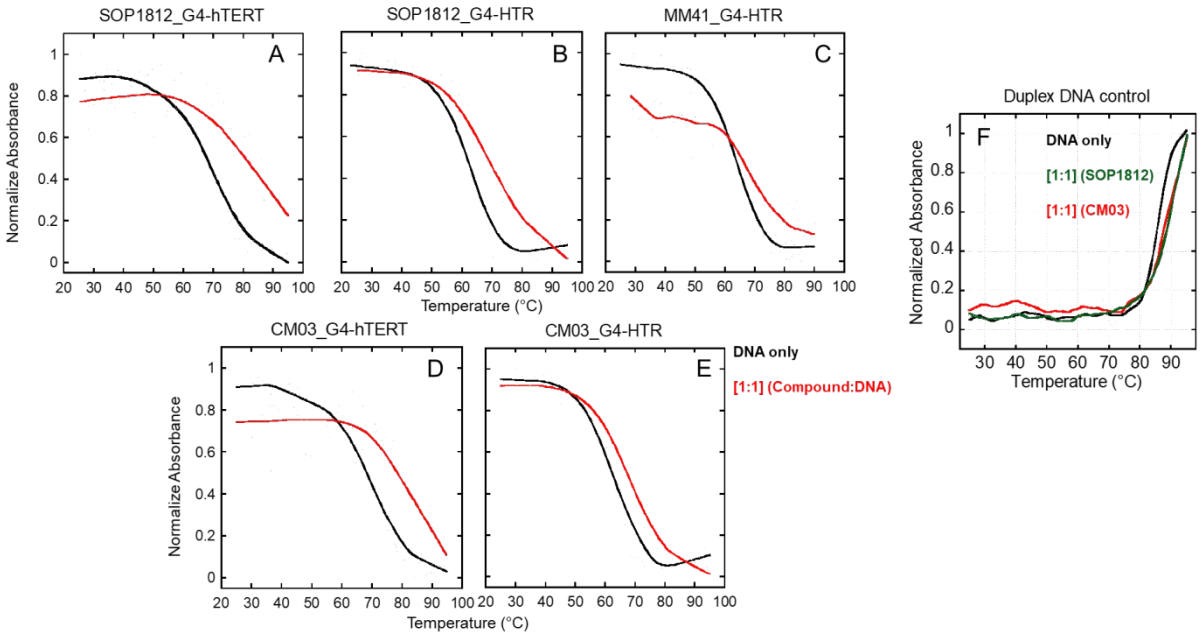

Figure S1. Representative UV thermal melting curves of SOP1812 (A,B) and CM03 (C,D) with different G4-DNA sequences. A, SOP1812 with G4-hTERT, B, SOP1812 with G4-HTR, C, MM41 with G4-HTR, D, CM03 with G4-hTERT and E, CM03 with G4-HTR. F, UV thermal melting of SOP1812 and CM03 with duplex DNA model.

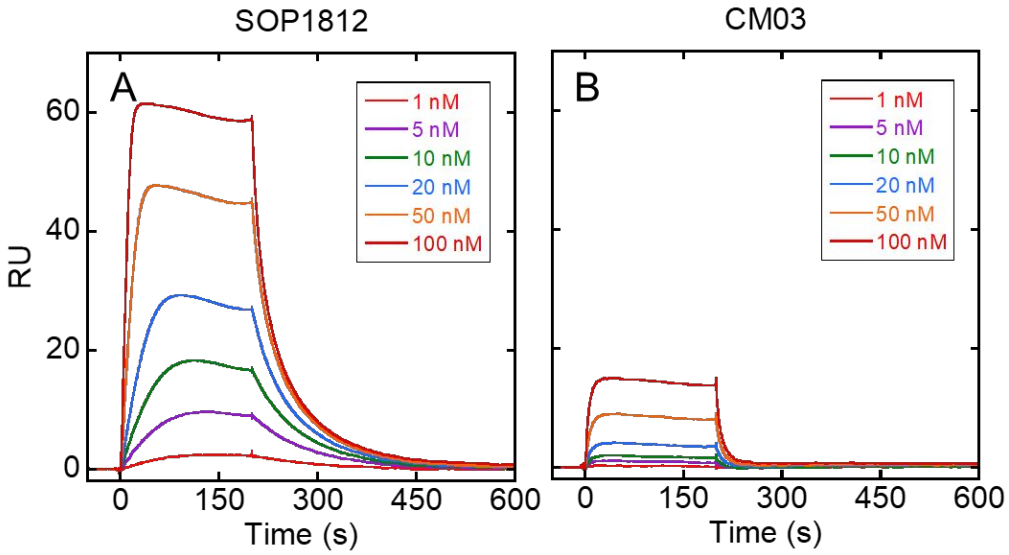

Figure S2. Representative SPR sensorgrams for SOP1812 (A) and CM03 (B) with duplex DNA model (AAAGAACTTT). Both compounds are injected from 1 nM to 100 nM concentrations. Nonspecific binding showed through rapid kinetic (fast-on rate and rapid off-rate), and the steady state did not reach saturation at the theoretical binding for single site. For a specific single binding site, the theoretical saturation RU for a one-to-one complexes is around 20 RU for TRO1812 and 17 RU for CM03. The sensorgrams show a nonspecific binding for both compounds with a guestimate KD in 100 nM range.

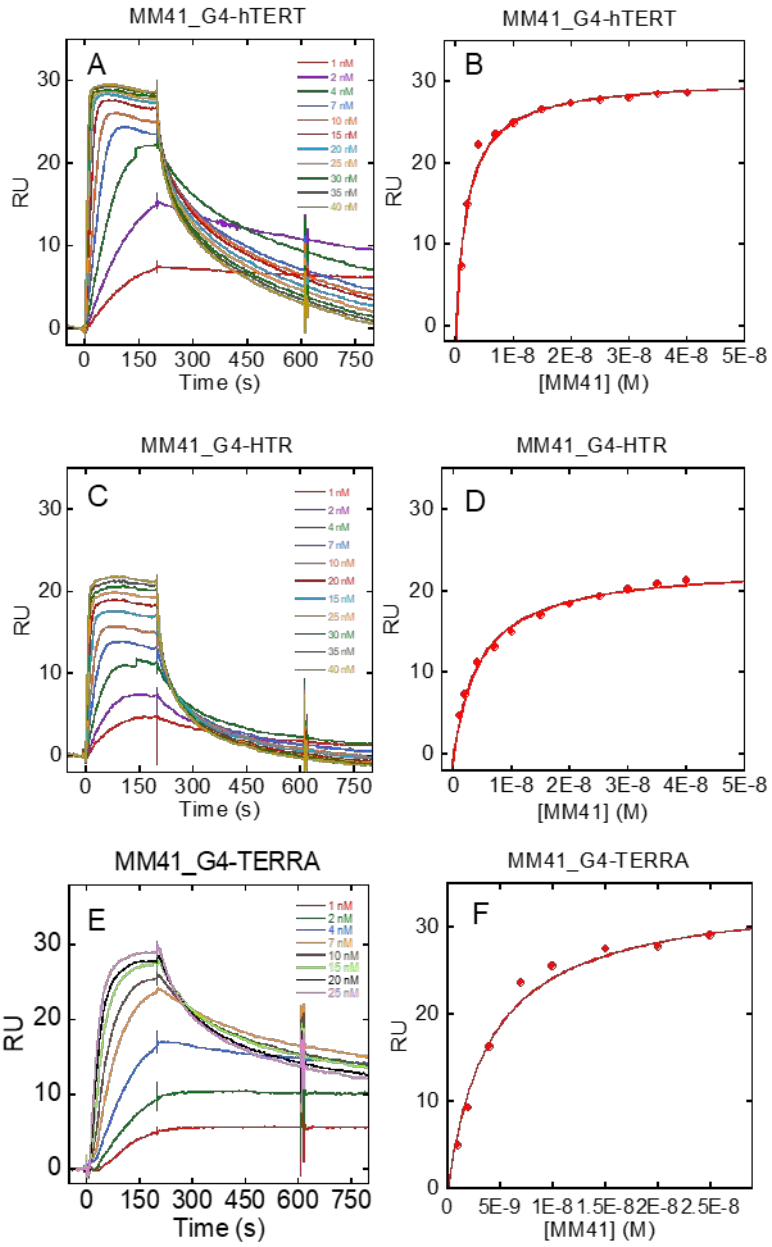

Figure S3. Representative sensorgrams (left column) and steady state analysis (right column) of MM41 with different G4 sequences. A-B, MM41 with G4-hTERT, C-D, MM41 with G4-HTR, E-F, MM41 with G4-TERRA. Steady state analysis was fitted with a 1-to-1 binding ratio and the equilibrium dissociation constant values was reported in Table 2.