Electronic Supplementary Information

Dual Recognition of Human Telomeric G-quadruplex by Neomycin-anthraquinone Conjugate.

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Materials and Methods.

Chemicals.

All chemicals used were purchased from commercial suppliers (Fisher scientific, Acros Organics, Sigma Aldrich). Neomycin was purchased from MP Biomedicals (solon, OH, USA). Synthesis of neomycin-intercalator conjugates (**1-4**) has been described previously.[1]

Nucleic acids.

Human telomeric DNA oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG) was purchased from MWG Operon (Huntsville, AL) and used without further purification. The DNA was dissolved in buffer 10 mM sodium cacodylate, 0.5 mM EDTA and 100 mM NaCl at pH 7.0 or 10 mM HEPES and 100 mM KCl at pH 7.0. After heating the oligonucleotide at 90 °C for 30 mins, it was allowed to cool back slowly to room temperature. The oligonucleotide was allowed to incubate for at least a week at 4 °C and the G-quadruplex formation was checked by circular dichroism. The stock solution (typically 1-2 mM/strand) was then diluted as desired.

Fluorescence Intercalator Displacement (FID) titrations.

FID experiments were conducted on a Photon Technology International (Lawrenceville, NJ) fluorimeter. The FID experiments were performed at 20 °C. The total volume used for each

experiment was 1.8 mL. A nucleic acid concentration of (0.25 μM/strand) and thiazole orange (TO) (0.5 μM) were added to nucleic acid buffer; 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM NaCl at pH 7.0 and/or 10 mM HEPES and 100 mM KCl at pH 7.0. The mixture was allowed to equilibrate for 20 minutes prior to performing the experiment and five minutes in the instrument to allow for temperature equilibration. The ligands were then serially added until ligand:quadruplex ratio of 2.5:1. Before recording the spectrum, the ligand was allowed to equilibrate for three minutes after each addition. Fluorescence emission spectra were recorded between from 515-600 nm with TO excitation set at 504 nm. Fluorescence readings at the maximum wavelengths (535 nm for buffer containing Na^+ and 539 nm for buffer containing K^+) for each individual titration were used to determine DC_{50} values, molar ratios, and Scatchard analysis. The ligand concentration required to displace 50% of the bound fluorescent probe was determined from a dose response curve using the Origin 5.0 software (MicroCal, Inc.; Northampton, MA) and is expressed as its DC_{50}.

Scatchard Binding Analysis

The binding constant was determined using the following equations where ΔF_x is the change in fluorescence, ΔF_{sat} is the change in fluorescence at saturation, $[DNA_T]$ is the total DNA concentration, [free agent] is the concentration of free agent, and χ is the molar ratio of agent to DNA.[2]

$$\left(\frac{\Delta \mathbf{F_x}}{\Delta \mathbf{F_{sat}}}\right)\frac{\mathbf{1}}{\boldsymbol{x}} = \mathbf{fraction\ of\ DNA - agent\ complex} \quad (\mathbf{1})$$

$$\left[\mathbf{1} - \left(\frac{\Delta \mathbf{F_x}}{\Delta \mathbf{F_{sat}}}\right)\frac{\mathbf{1}}{\boldsymbol{x}}\right] = \mathbf{fraction\ of\ free\ agent} \quad (\mathbf{2})$$

$$[\mathbf{DNA}]_T \left[\chi - \frac{\Delta \mathbf{F_x}}{\Delta \mathbf{F_{sat}}}\right] = [\mathbf{free\ agent}] \quad (\mathbf{3})$$

UV spectroscopy.

All UV spectra were recorded on a Cary 100 Bio UV/Vis spectrophotometer equipped with a thermoelectrically controlled 12-cell holder. Quartz cells with a 1 cm path length were used for all the absorbance studies. Spectrophotometer stability and λ alignment were checked prior to initiation of each melting point experiment. 1 to1 ratios of ligand (2μM) to oligonucleotide 2μM/strand] were heated to 100 °C at a rate of at 0.2 °C/min. Samples were brought back to 20°C after run. All UV melting experiments were monitored at 260 and 295 nm. For the T_m determination, baseline method was used as described by Mergny.[3] Data were recorded every 1.0 °C. The UV melting experiments were performed in buffer 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM NaCl at pH 7.0.

Circular dichroism (CD) spectroscopy.

CD experiments were performed at 20 °C using a Jasco J-810 spectropolarimeter with a thermo-electrically controlled cell holder. The CD spectra were recorded as an average of three scans. For CD titrations, a concentrated ligand solution was serially added to the preformed quadruplex of human telomeric DNA (10μM/strand) and allowed to equilibrate for five minutes before a scan was taken. The buffer used in the experiments was sodium 10 mM sodium cacodylate, 0.5 mM EDTA and 100 mM NaCl at pH 7.0. Data processing was done using Kaleidagraph 3.5 software.

Molecular surface area calculations.

The surface area of the molecules was calculated using ChemAxon Marvinview, Version 5.11.4 (http://www.chemaxon.com/products/marvin/). The comparison of the surface area (Van der

Waals and polar) was done by keeping only the aromatic moiety of the ligands (**1-4**) capable of making stacking interactions. The chemical structures of the ligand (shown in Table S1) were drawn as .mol files and then imported into MarvinView. The surface areas of the molecules (Van der Waals and polar) were obtained using the van der Waals and polar surface area calculation tools provided in the software.

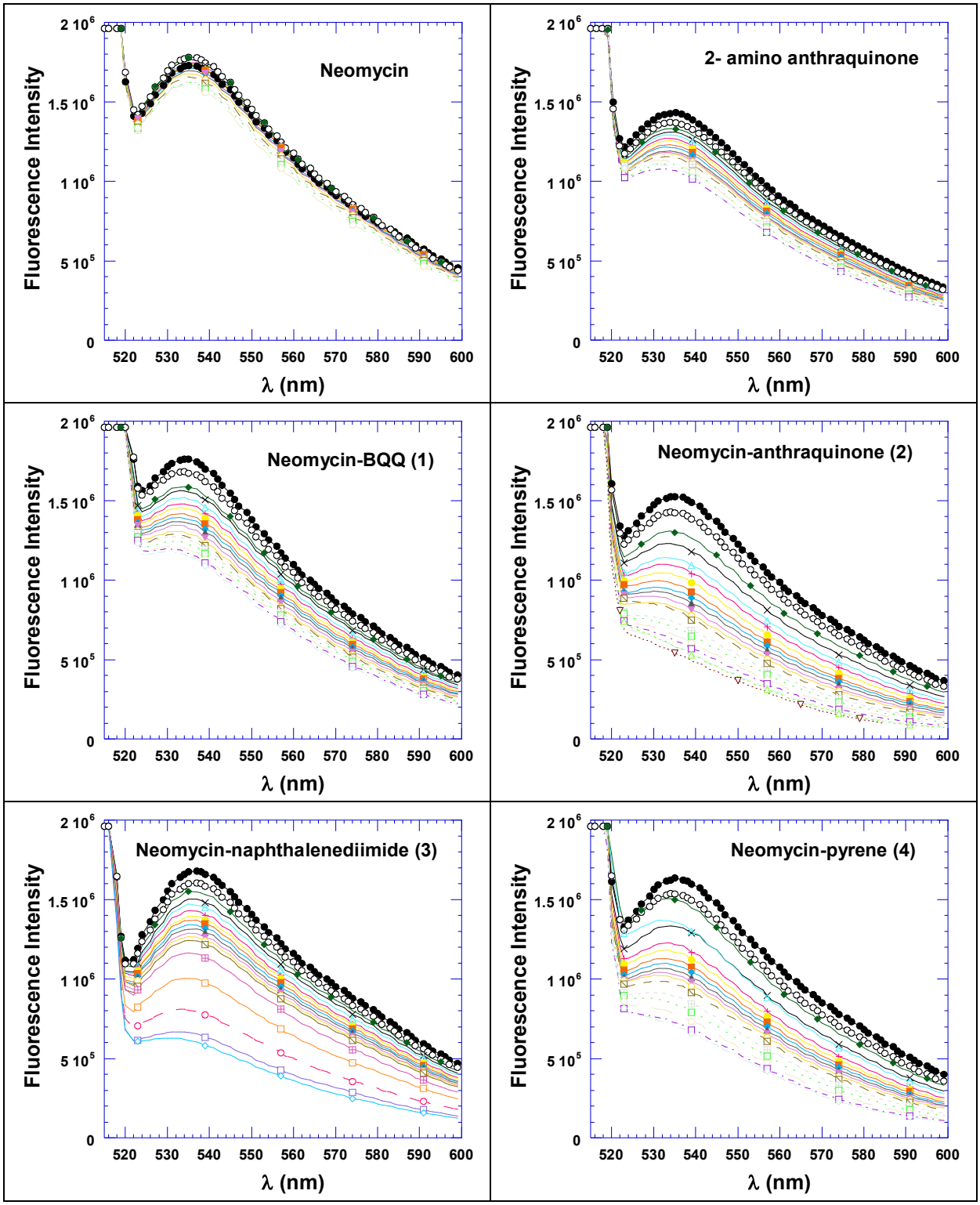
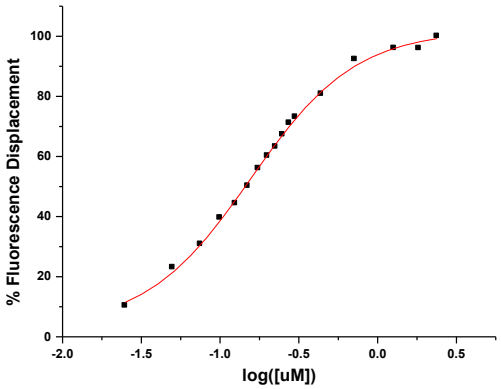


Figure S1. . FID titration plots of human telomeric G-quadruplex interaction with various ligands. The DNA concentration was 0.25 μM/strand and the thiazole orange concentration was

0.5 µM. The experiments were performed in buffer 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM NaCl, at pH 7.0.

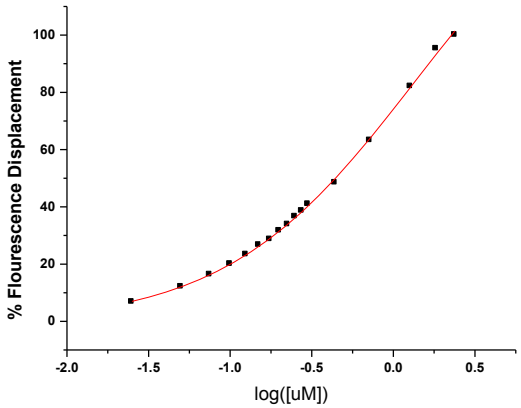


Model: Boltzman

Parameter	Value	Error
Chi^2	1.62614	
Initial(A1)	3.26357	2.47439
Final (A2)	101.94624	1.22598
EC50 (x0)	-0.80542	0.01767
Width (dx)	0.33206	0.01859

Neomycin-anthraquinone (2)

DC_{50} = 0.1565 µM

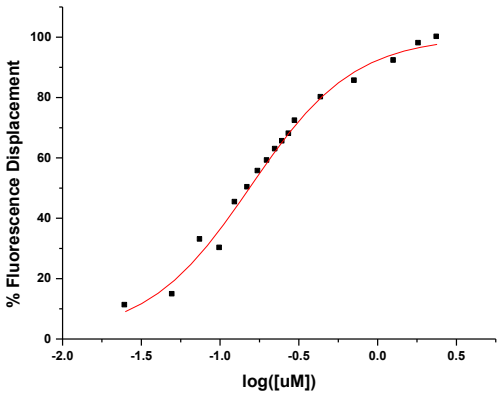


Model: Boltzman

Parameter	Value	Error
Chi^2	0.74596	
Initial(A1)	-1.03903	1.96458
Final (A2)	168.26628	14.86711
EC50 (x0)	0.13033	0.0946
Width (dx)	0.57723	0.04489

Neomycin-naphthalenediimide (3)

DC_{50} = 1.349 µM



Model: Boltzman

Parameter	Value	Error
Chi^2	8.78291	
Initial(A1)	1.03488	5.62422
Final (A2)	100.21891	2.78657
EC50 (x0)	-0.80552	0.03985
Width (dx)	0.32765	0.04167

Neomycin-pyrene (4)

DC_{50} = 0.1634 µM

Figure S2. Sigmoidal fits for determination of $DC_{50\ for}$ human telomeric DNA G-quadruplex-ligand interaction. Buffer conditions: 10 mM sodium cacodylate, 0.5 mM EDTA and 100 mM NaCl at pH 7.0.

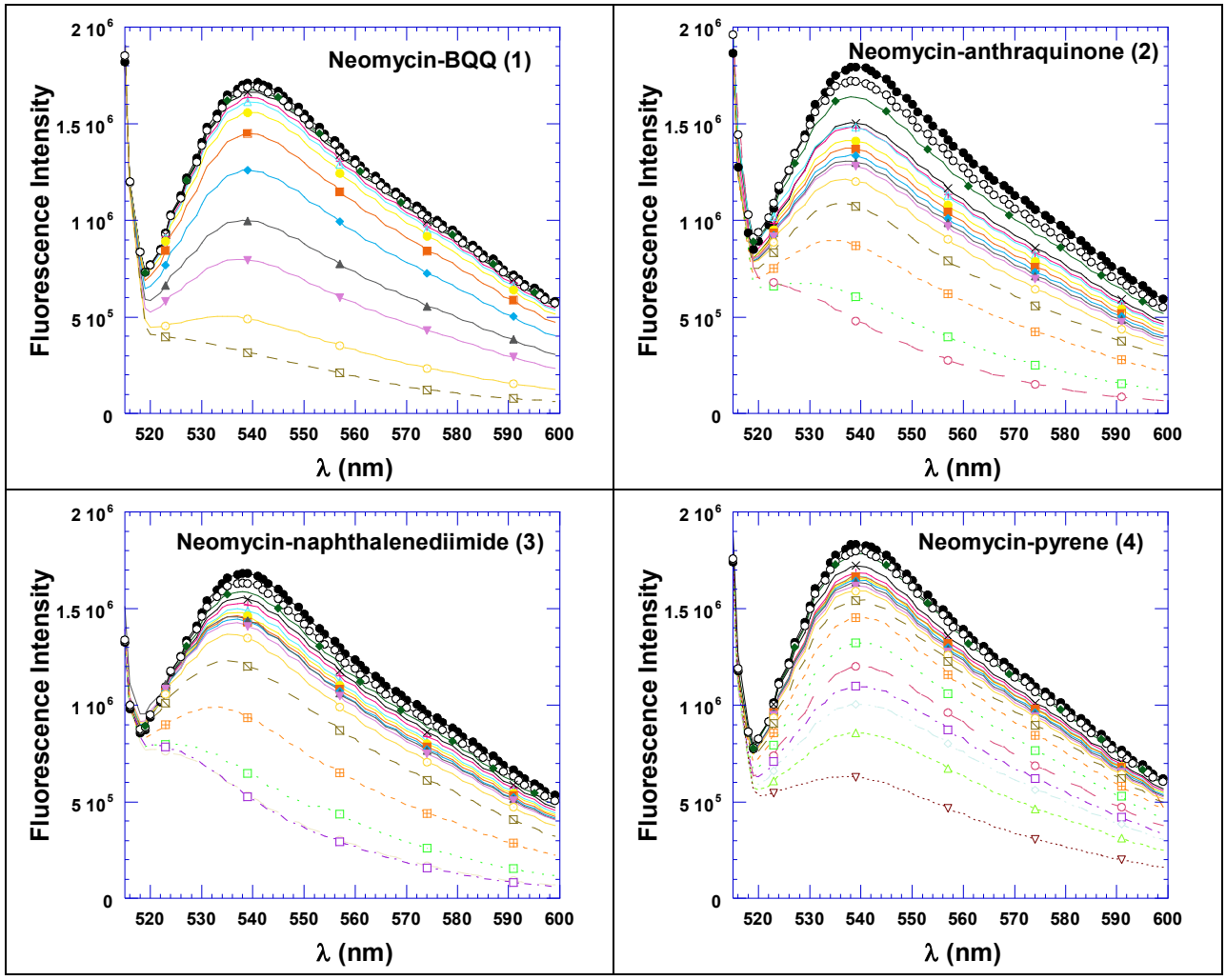
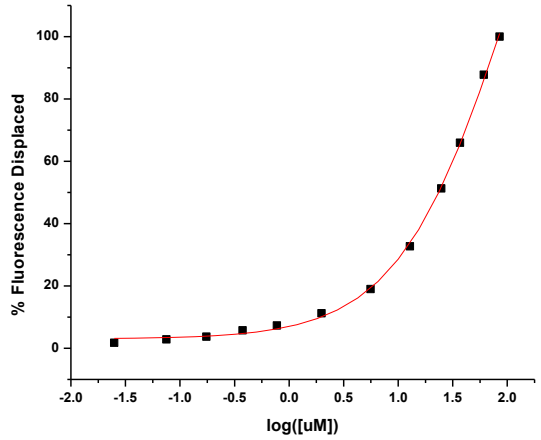
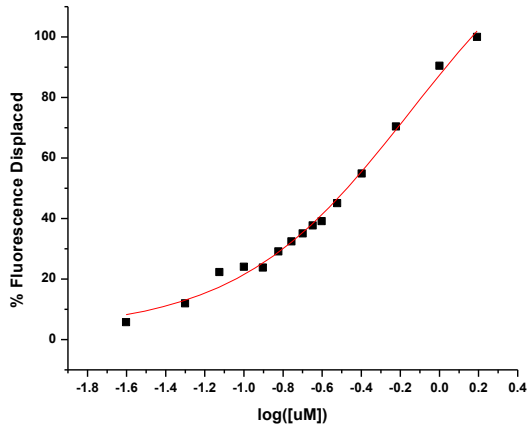
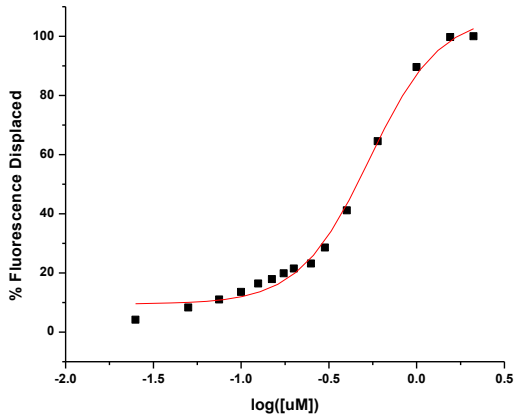
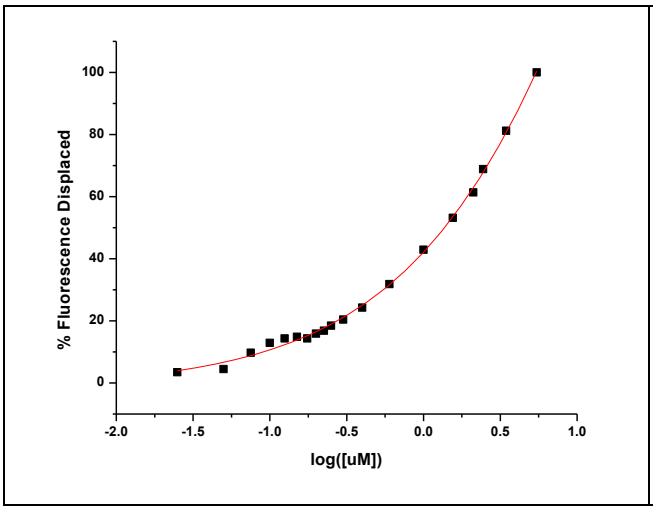


Figure S3. FID titration plots of human telomeric G-quadruplex interaction with various ligands. The DNA concentration was 0.25 μM/strand and the thiazole orange concentration was 0.5 μM. The experiments were performed in buffer 10 mM HEPES and 100 mM KCl at pH 7.0 (T = 20 °C).

	Chi^2/DoF 1.36409 Lower 2.995660.68199 Upper 217.87166 35.95591 LogEC50 2.019630.15582 Slope 0.852970.06453 Neomycin-BQQ (1)
	Chi^2/DoF 5.63432 Lower 2.979634.06515 Upper 147.97808 23.7573 LogEC50 -0.14734 0.14029 Slope 0.980160.17122 Neomycin-anthraquinone (2)
	Chi^2/DoF 8.06187 Lower 9.493171.56705 Upper 107.33335 3.76592 LogEC50 -0.26931 0.02555 Slope 2.176220.22126 Neomycin-naphthalenediimide (3)



Chi^2/DoF	1.63401	
Lower	-1.53672	2.34607
Upper	469.09292	291.11185
LogEC50	1.69091	0.68423
Slope	0.58567	0.07542

Neomycin-pyrene (4)

Figure S4. Sigmoidal fits for determination of $DC_{50\ for}$ human telomeric DNA G-quadruplex-ligand interaction. Buffer conditions: 10 mM HEPES, 100 mM KCl, at pH 7.0.

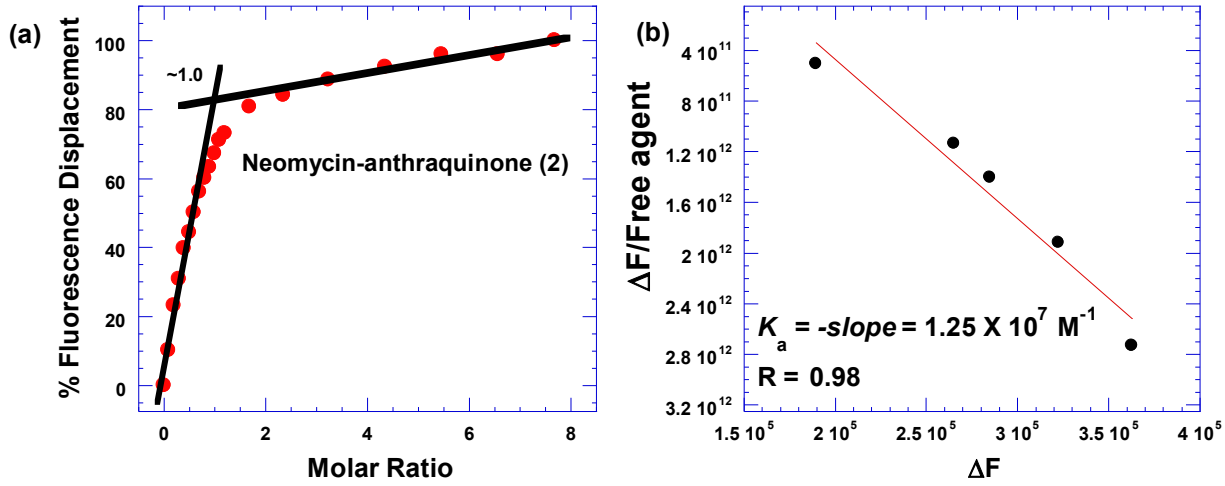
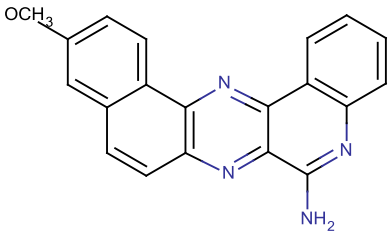
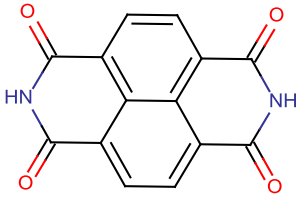
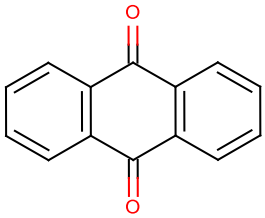
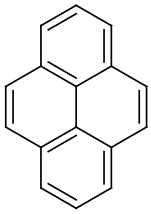


Figure S5. (a) Binding stoichiometry plot for the titration of neomycin-anthraquinone **(2)** into human telomeric quadruplex (0.25 μM) with thiazole orange (0.5 μM). The experiment was performed in buffer 10 mM sodium cacodylate, 0.5 mM EDTA, and 100 mM NaCl at pH 7.0. (b) Determination of association constant of human telomeric quadruplex -neomycin-anthraquinone **(2)** interaction using Scatchard analysis.

Table S1. Molecular surface area calculations.

Molecule	Van der Waals Surface Area ($Å^2$)	Polar Surface Area ($Å^2$)
BQQ 	427.80	73.92
Naphthalenediimide 	290.20	92.34
Anthraquinone 	269.16	34.14
Pyrene 	279.09	0.00

REFERENCES

1 Xue, L.and Xi, H.and Kumar, S.and Gray, D.and Davis, E.and Hamilton, P.and Skriba, M., Arya, D. P., *Biochemistry,* 2010, *49,* pp. 5540-5552.

2 Boger, D. L.and Fink, B. E.and Brunette, S. R.and Tse, W. C., Hendrick, M. P., *J. Am. Chem. Soc.,* 2001, *123,* pp. 5878-5891.

3 Mergny, J. L., Lacroix, L., *Oligonucleotides,* 2003, *13,* pp. 515-537.