Isaindigotone Derivatives: A New Class of Highly Selective Ligands for Telomeric G-quadruplex DNA

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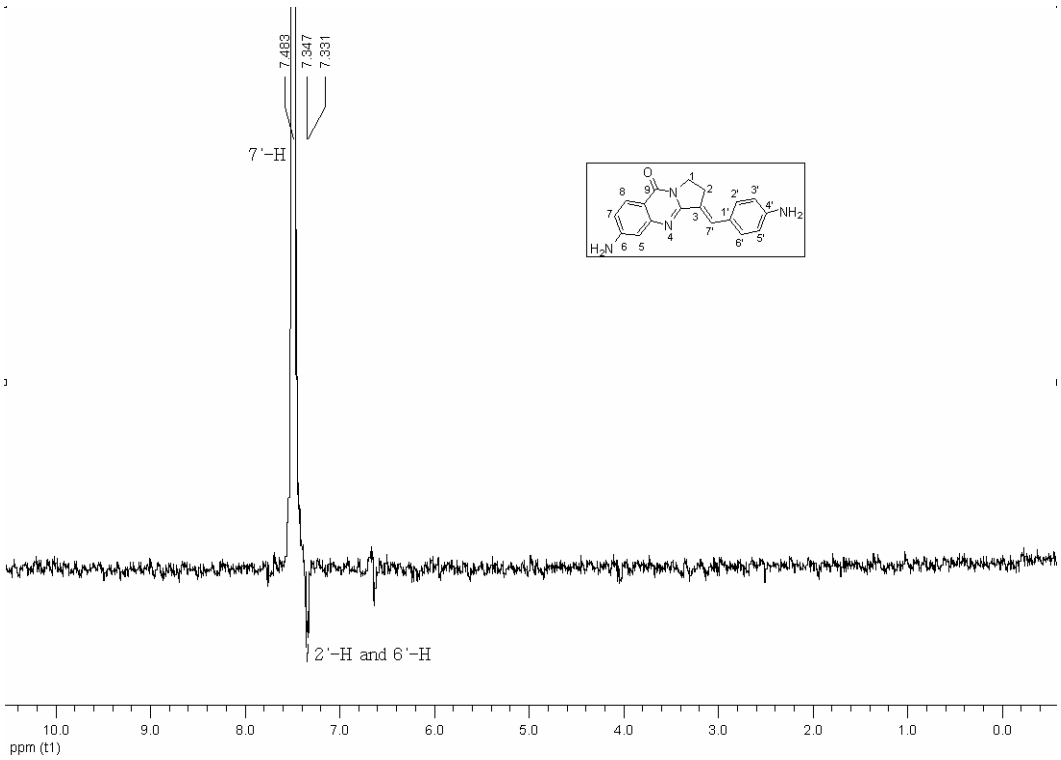
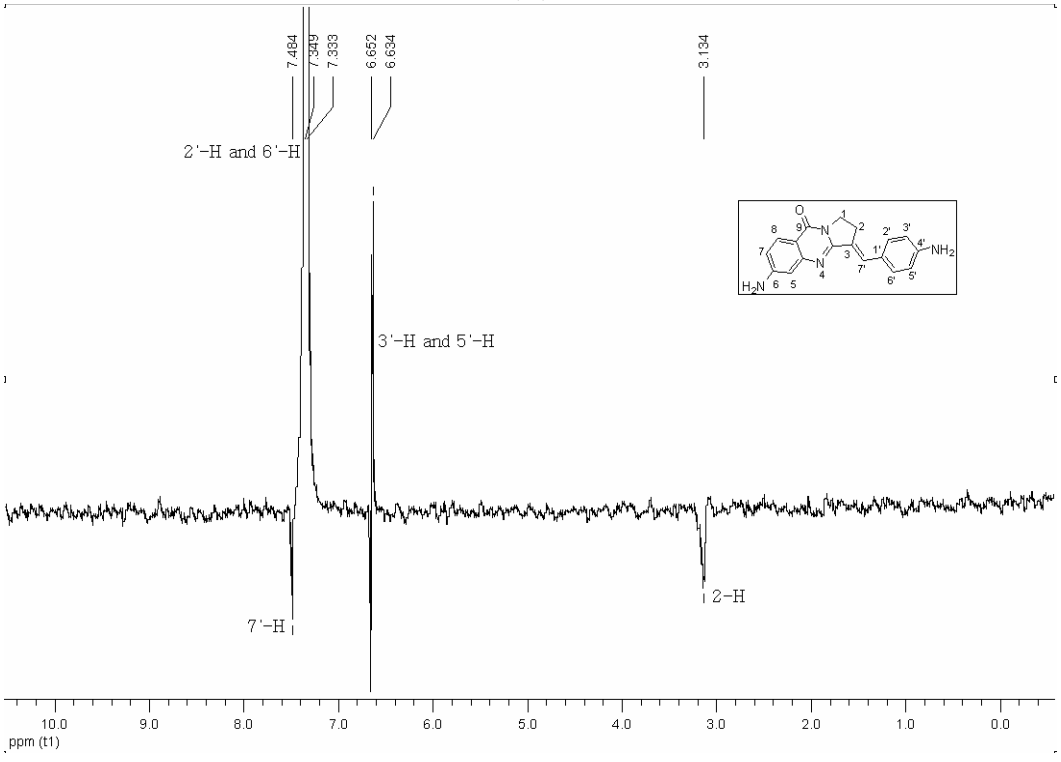
1. NOE Analysis of 3a and 3b

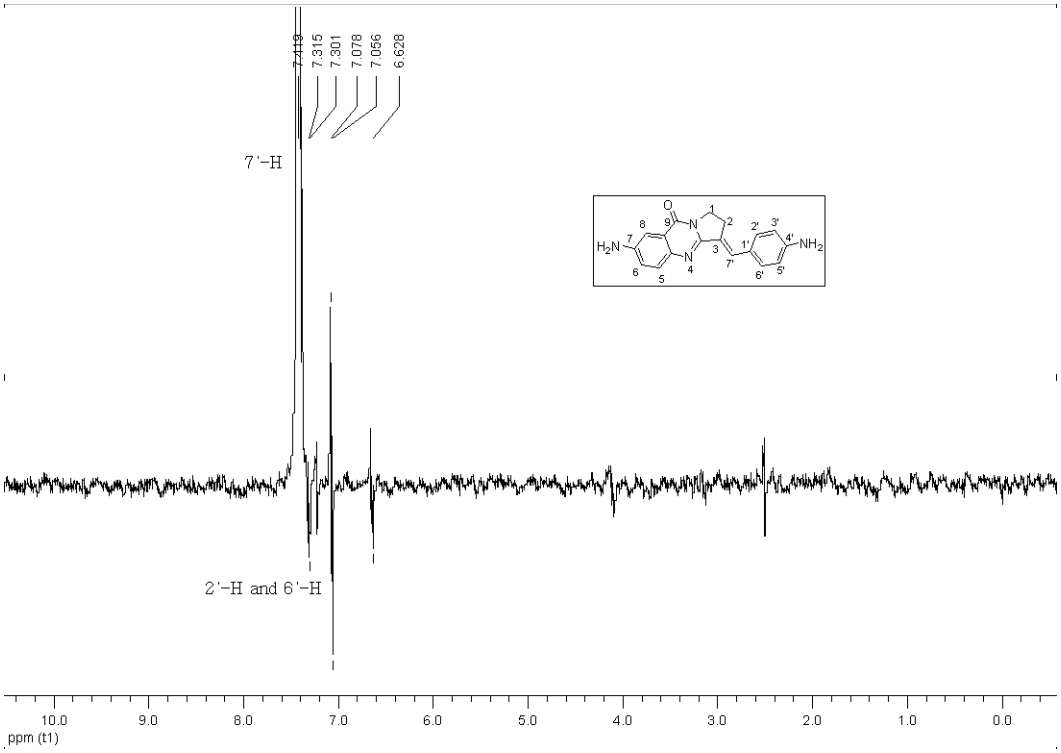


(A)

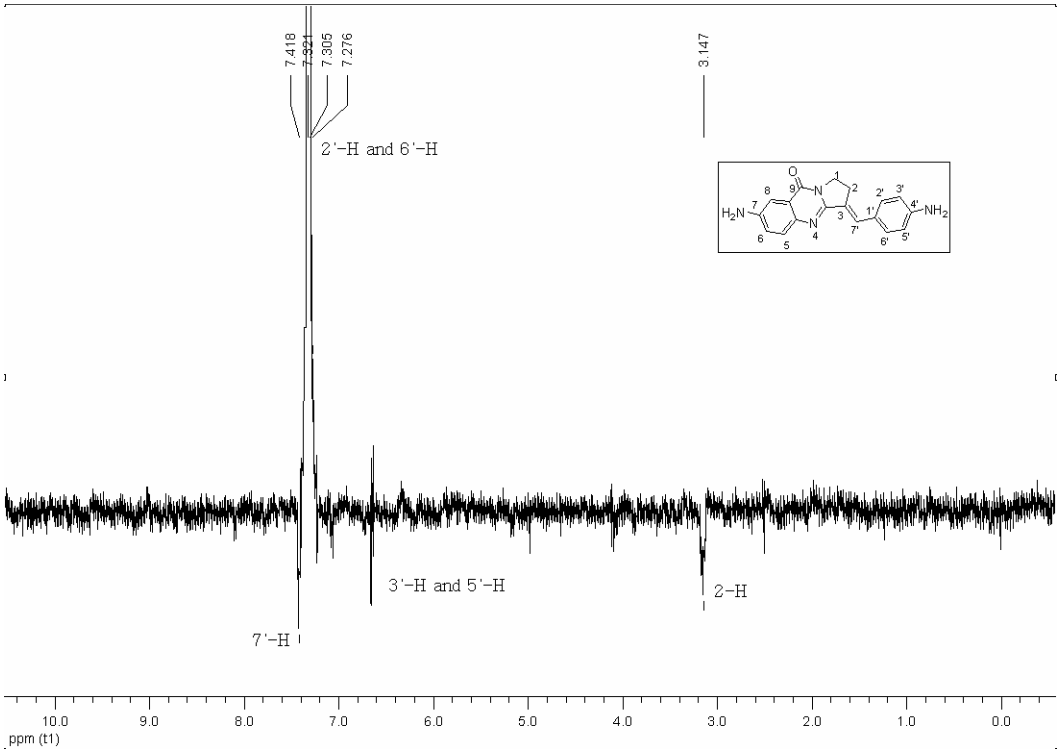


(B)

Figure S1. NOE analysis of **3a**. (A) Upon irradiation of the proton 7'-H, only enhancement of the equivalent protons 2'-H and 6'-H was observed. (B) Upon irradiation of 2'-H and 6'-H, signals of protons 7'-H and 2-H were simultaneously enhanced.



(A)



(B)

Figure S2. NOE analysis of **3b**. (A) Upon irradiation of the proton 7'-H, only enhancement of the equivalent protons 2'-H and 6'-H was observed. The signals of 7'-H and 5-H mostly overlapped, hence H peaks close to 5-H were simultaneously enhanced. (B) Upon irradiation of 2'-H and 6'-H, signals of protons 7'-H and 2-H were simultaneously enhanced.

2. Elemental Analysis Results

Table S1. Elemental analysis results (C, H, N)

Compound	Formula	Calcd	Found
5a (Trihydrochloride salt)	$C_{32}H_{38}N_6O_3 \cdot 3HCl \cdot 3.5H_2O$	C, 52.86; H, 6.65; N, 11.56.	C, 52.83; H, 6.59; N, 11.60.
6a (Trihydrochloride salt)	$C_{34}H_{42}N_6O_3 \cdot 3HCl \cdot 5H_2O$	C, 52.21; H, 7.09; N, 10.74.	C, 52.14; H, 6.95; N, 10.72.
5b (Trihydrochloride salt)	$C_{32}H_{38}N_6O_3 \cdot 3HCl \cdot 1.5H_2O$	C, 55.61; H, 6.42; N, 12.16.	C, 55.85; H, 6.69; N, 11.96.
6b (Trihydrochloride salt)	$C_{34}H_{42}N_6O_3 \cdot 3HCl \cdot 4H_2O$	C, 53.44; H, 6.99; N, 11.00.	C, 53.69; H, 7.13; N, 11.03.

3. UV Analysis of 5a and 5b in Solution

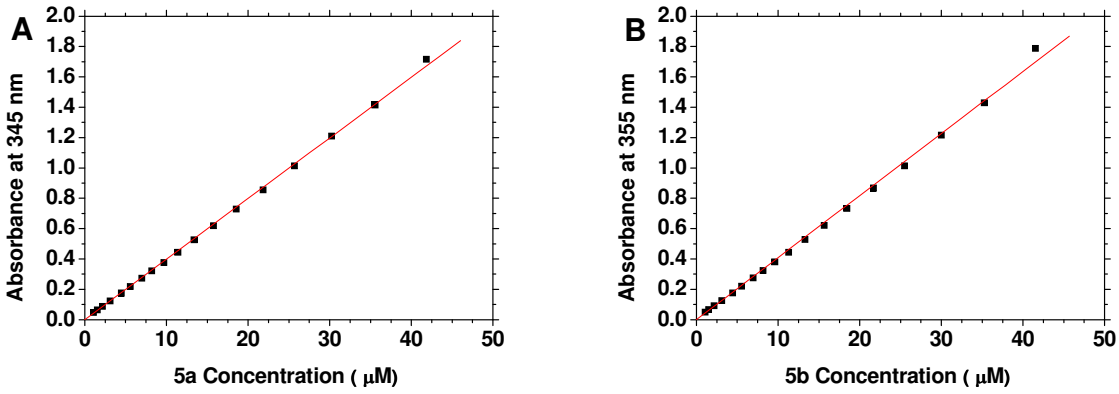


Figure S3. Concentration dependant UV-vis absorbance of **5a** (A) and **5b** (B). All of the spectra were collected in 10 mM Tris-HCl buffer, pH 7.2 using 1 cm path length quartz cuvette on a Shimadzu UV-2450 spectrophotometer. Concentration dependant UV-vis absorbance spectra of **5a** and **5b** showed that the variations follow the Beer-Lambert law. It was thus unlikely that the ligands do dimer or self associate in absence of quadruplex.

4. FRET-melting Results

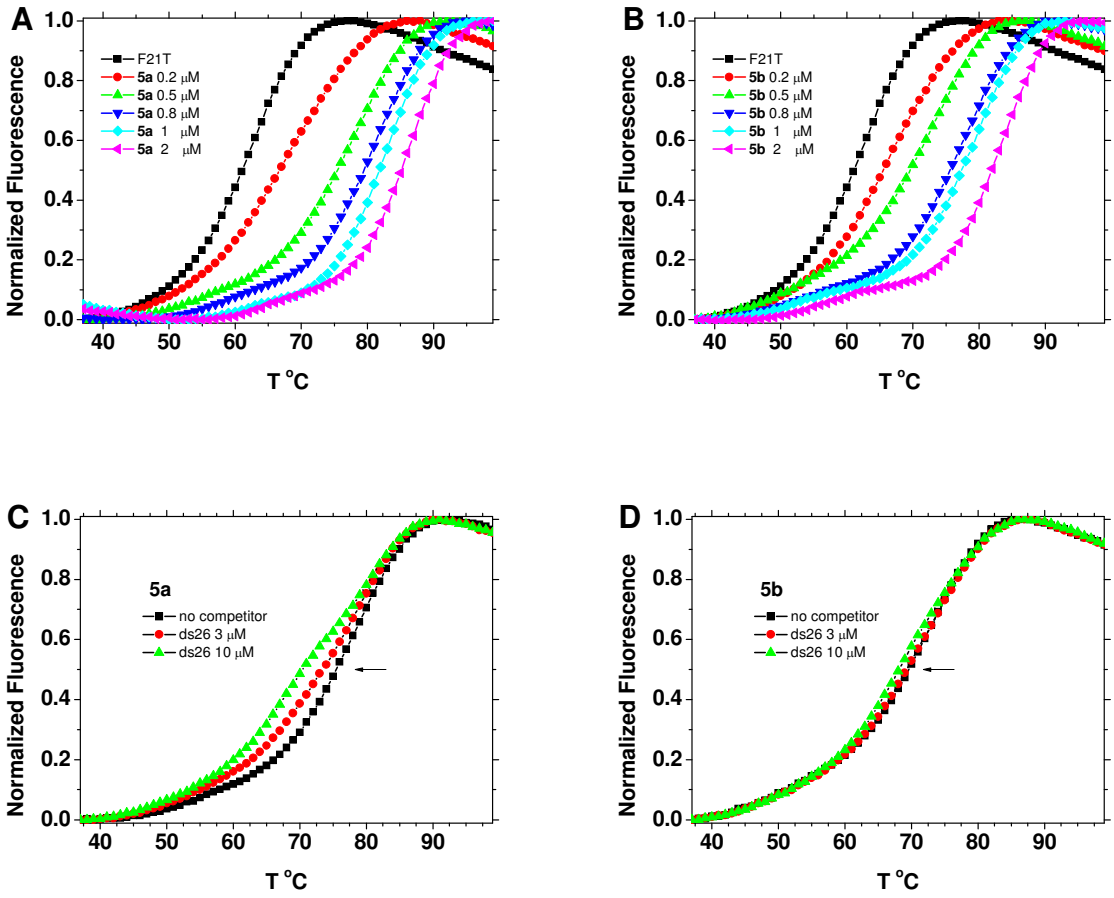


Figure S4. (A) and (B) Representative FRET melting profiles of F21T (0.2 μM) with increasing concentration of **5a** or **5b**. (C) and (D) Representative competitive FRET melting profiles of F21T (0.2 μM) in the presence of **5a** or **5b** (0.5 μM) without (black) and with 15-fold (3 μM, red) or 50-fold (10 μM, green) excess of duplex DNA competitor (ds26).

5. Fluorescence Titration Results

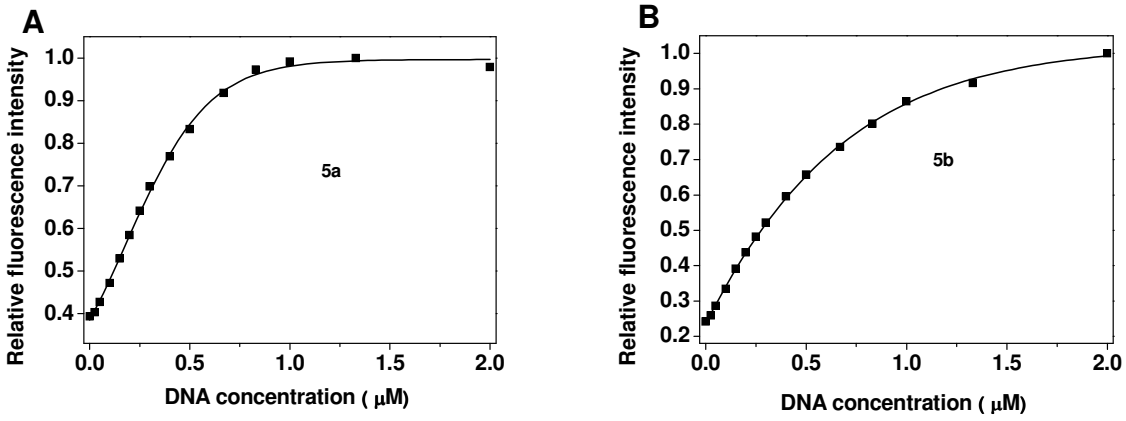


Figure S5. Fluorescence properties of **5a** (A) and **5b** (B) in the presence of quadruplex-forming oligonucleotide HTG21. Excitation was set at 343 nm for **5a** and 353 nm for **5b**. The changes in fluorescence intensity at 448 nm for **5a** and 450 nm for **5b** as a function of DNA concentration were shown.

6. EMSA and Thermodynamic Stability Experiments in the Absence of Added Salt

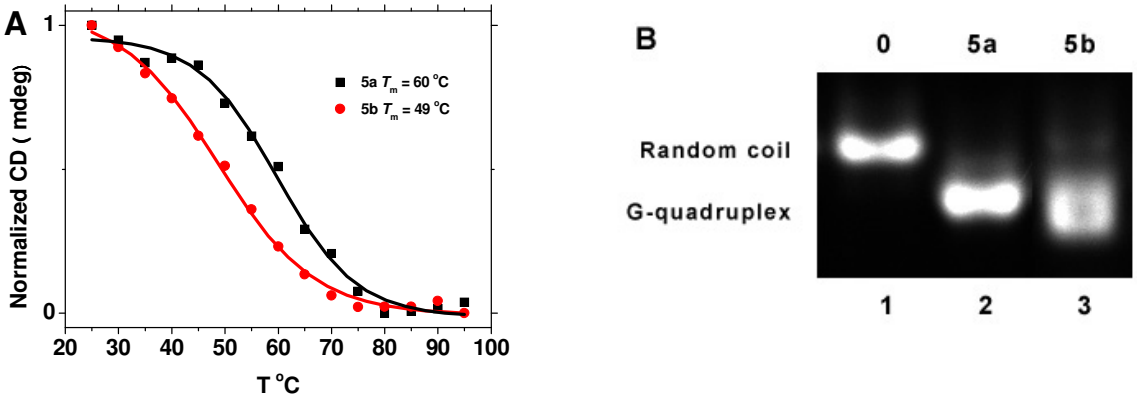


Figure S6. (A) CD melting profiles of HTG21 (12.5 μM) with 50 μM **5a** (black) or **5b** (red) in 10 mM Tris-HCl buffer, pH 7.2, using 1 mm path length quartz cuvette. The quadruplex structures showed thermal stability with T_m = 60 °C and 49 °C in the presence of **5a** and **5b**, respectively. Without ligand or salt, HTG21 oligonucleotide was random coil. (B) Effects of **5a** and **5b** on the formation of G-quadruplex from HTG21 oligonucleotide. End *FAM* (6-carboxyfluorescein) labeled oligonucleotides (5 μM) were incubated with 20 μM **5a** or **5b** in 10 mM Tris-HCl buffer, pH 7.2 for 48 h. Free oligonucleotide and its mixture with ligand were run on a native polyacrylamide gel with 1×TAE buffer without the addition of salt. The new high-mobility bands appeared in lane 2 and lane 3 confirmed the effects of **5a** and **5b** on formation of more compact intramolecular G-quadruplex from randomized HTG21 oligonucleotide.

7. Molecular Modeling Studies of Interactions between Ligand and G-quadruplex

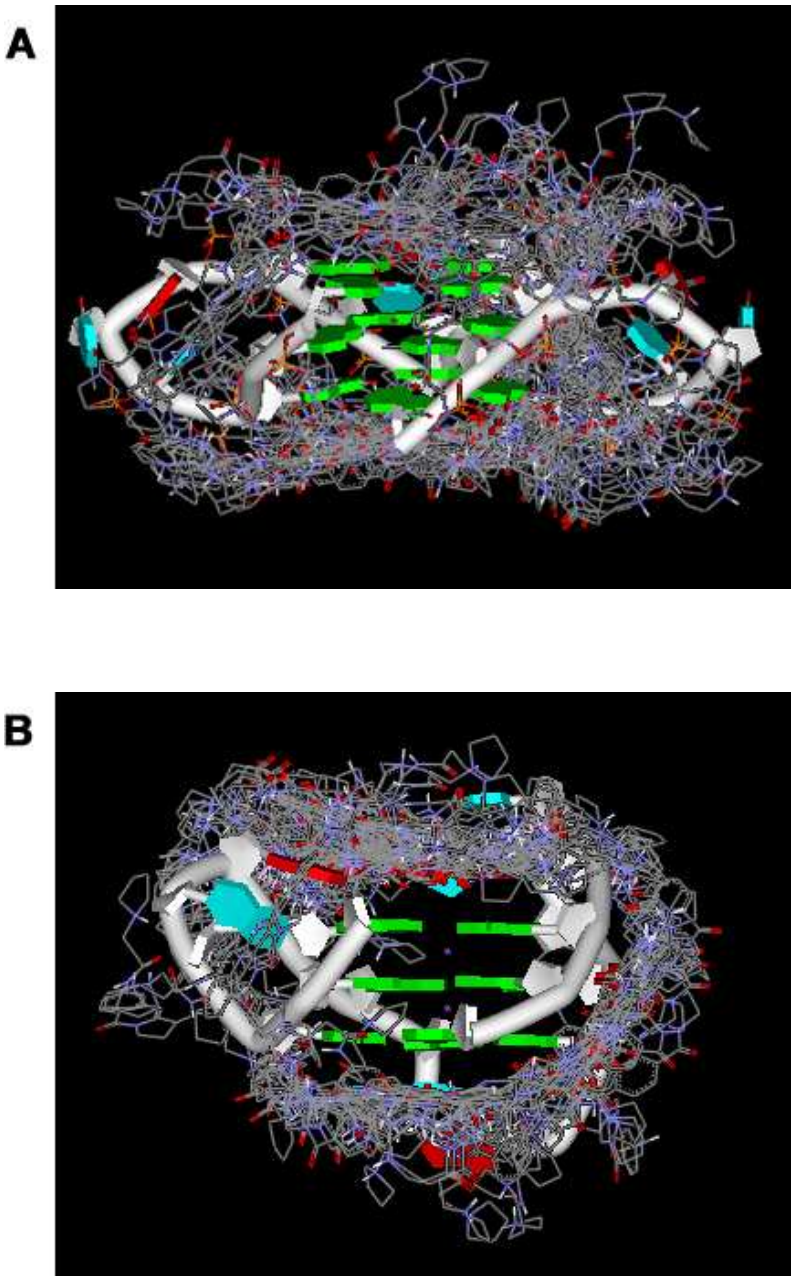
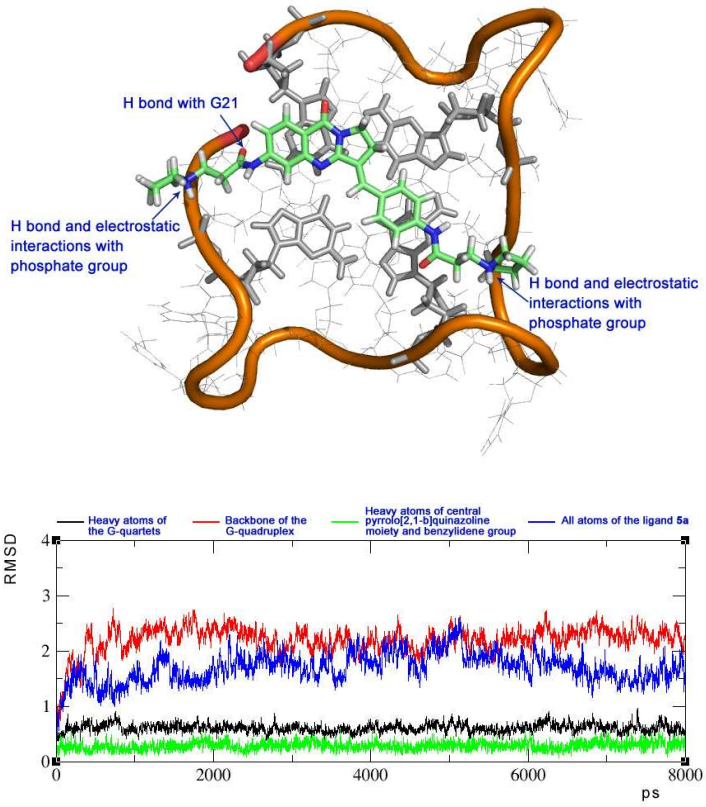
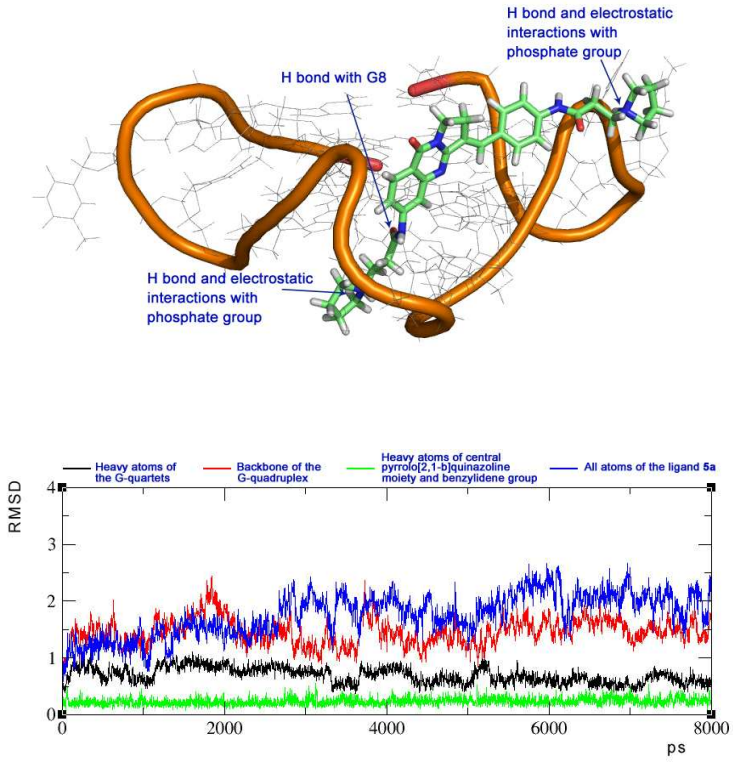
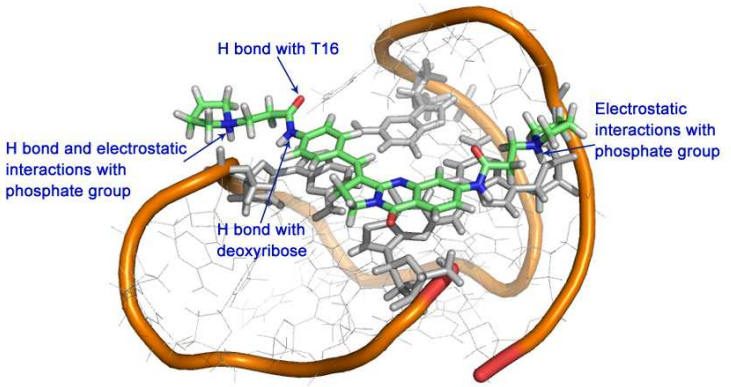
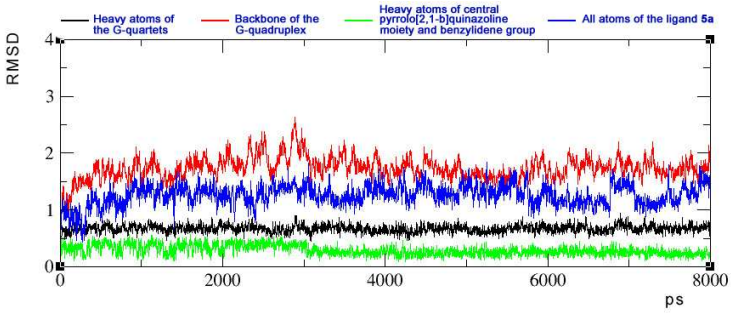
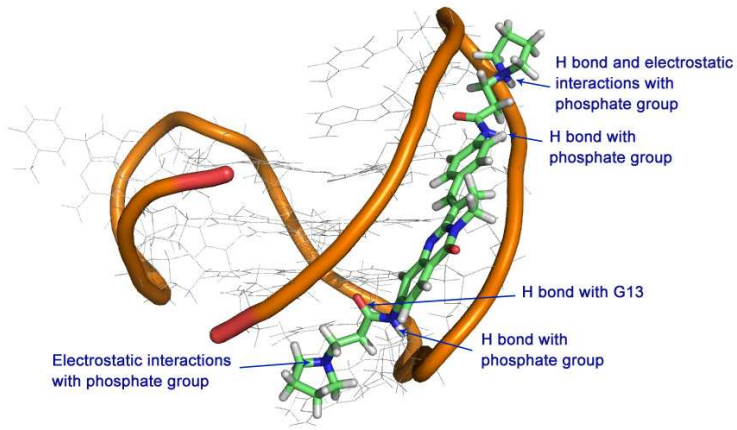
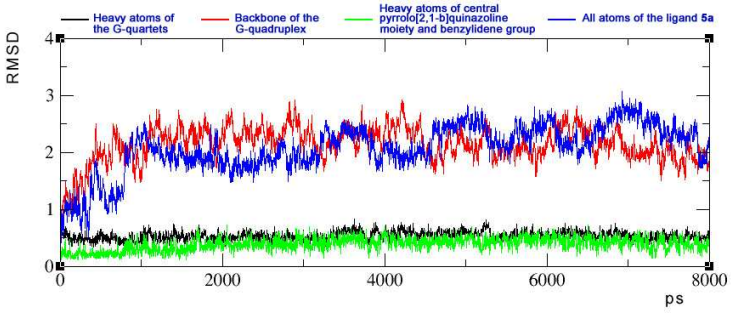
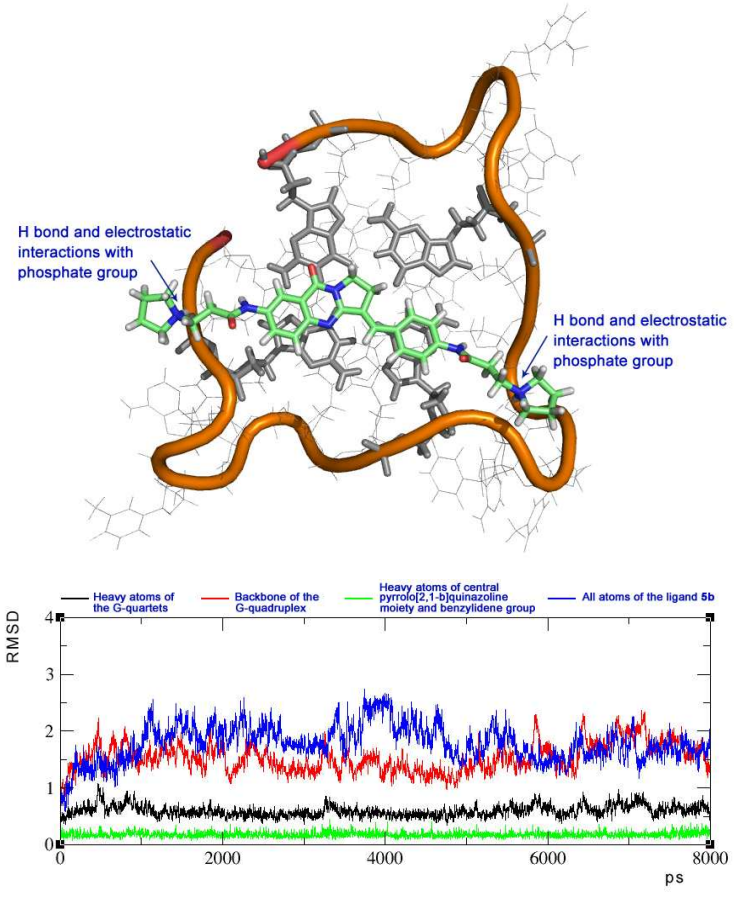
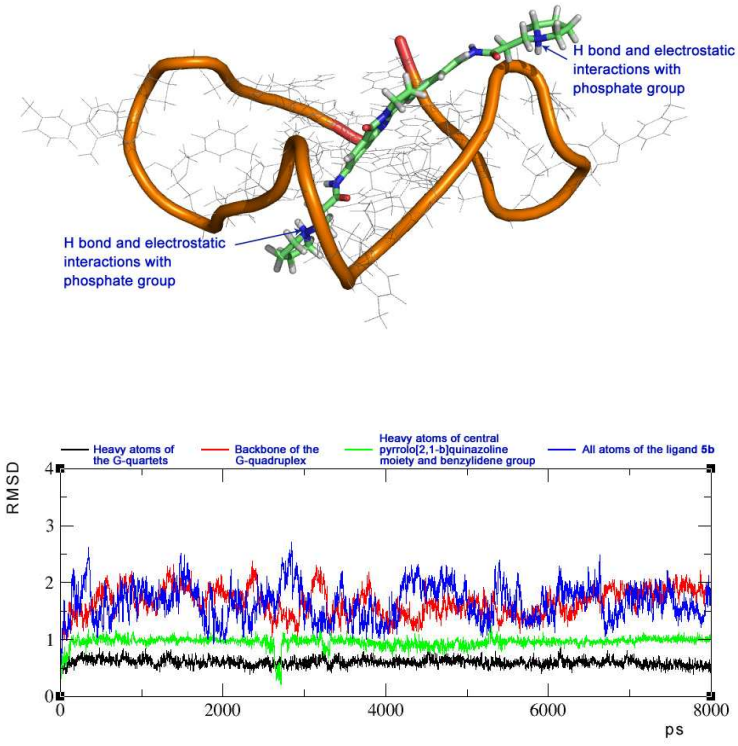


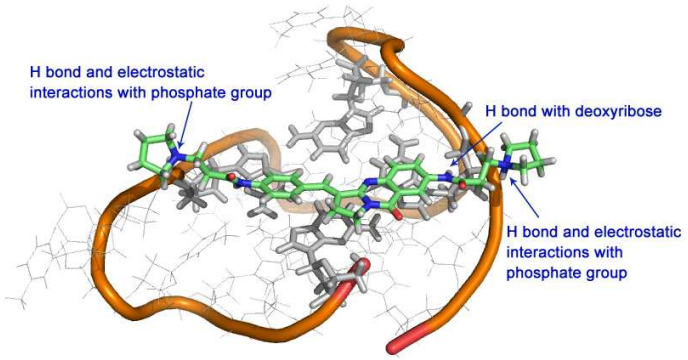
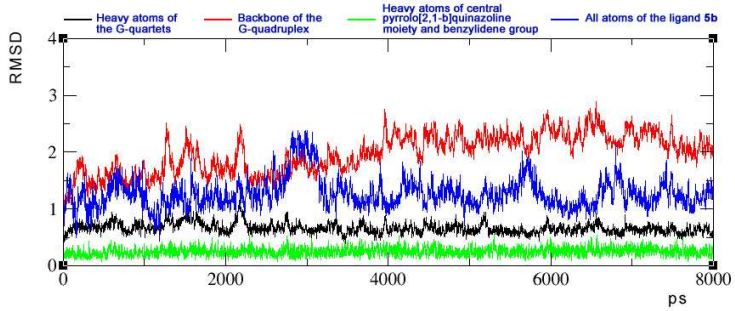
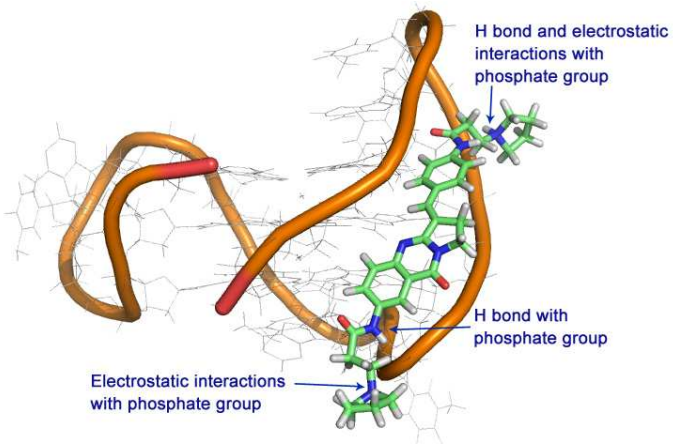
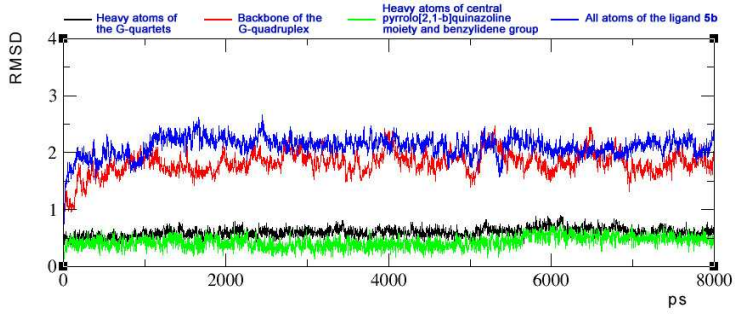
Figure S7. One hundred docking conformers of **5a** and G-quadruplex complexes. (A) Propeller-type G-quadruplex. (B) Hybrid-type G-quadruplex.

Scheme S1. Models and RMSD values of **5a**-quadurplx complex and **5b**-quadruplex complex with 1:1 stoichiometry.

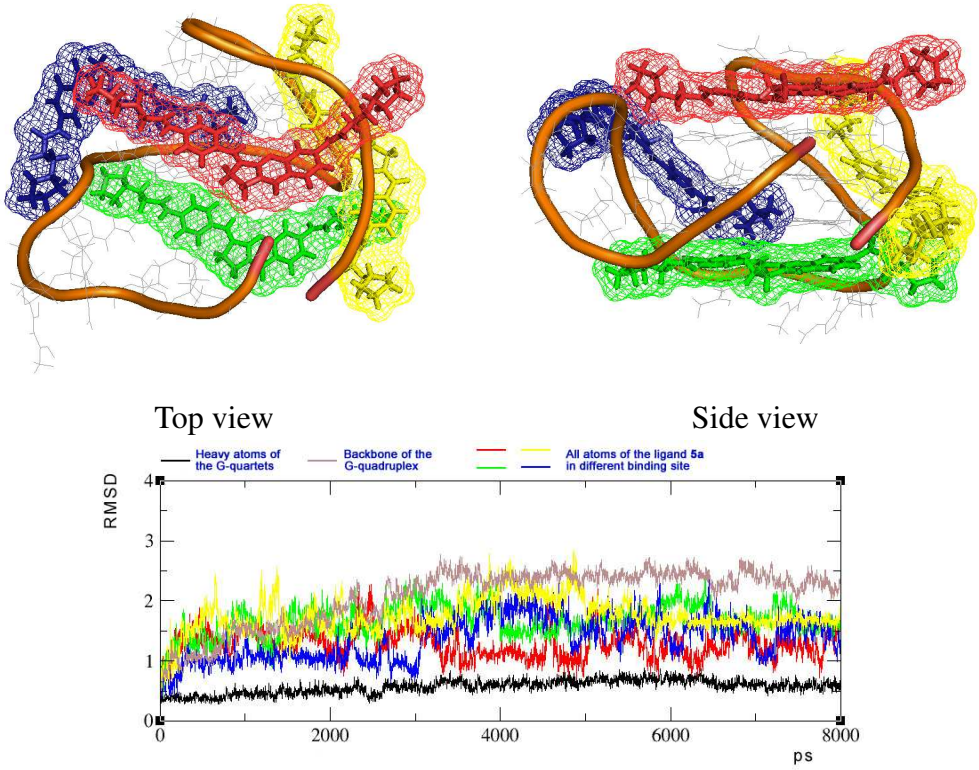
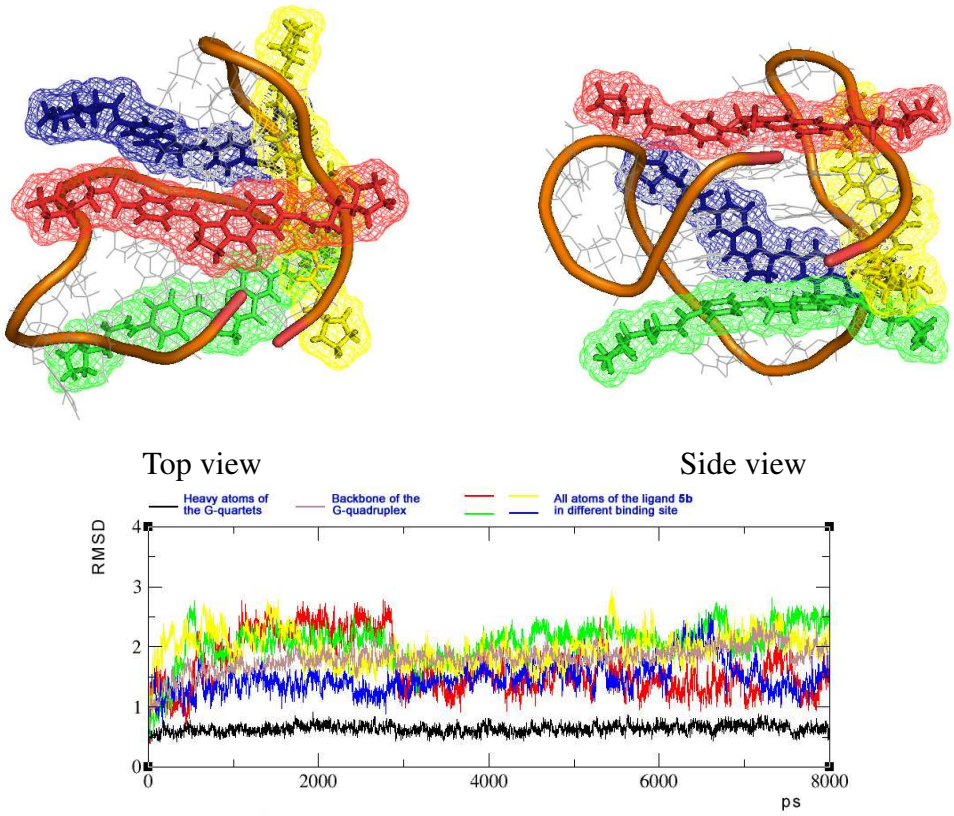
Compd.	G4	Binding Mode	Model and RMSD Value
5a	Propeller-type	End-stacking	
		Groove-binding	

5a	Hybrid-type	End-stacking	 
		Groove-binding	 

5b	Propeller-type	End-stacking	
		Groove-binding	

5b	Hybrid-type	End-stacking	 
		Groove-binding	 

Scheme S2. Models and RMSD values of **5a** and **5b** binding to the hybrid-type G-quadruplex via multiple binding modes with 4:1 stoichiometry.

Compd.	Model and RMSD Value
5a	
5b	

8. Molecular Modeling Studies of Interactions between Ligand and Duplex DNA

A self-complementary duplex DNA was built in SYBYL using Biopolymer package from the sequence d[$(TA)_2GC(TA)_2$]. The duplex model was stable in 6 ns MD runs (Figure S8A). Major and minor groove binding models were studied using Autodock package and the pseudointercalation models were built at the GC-step.

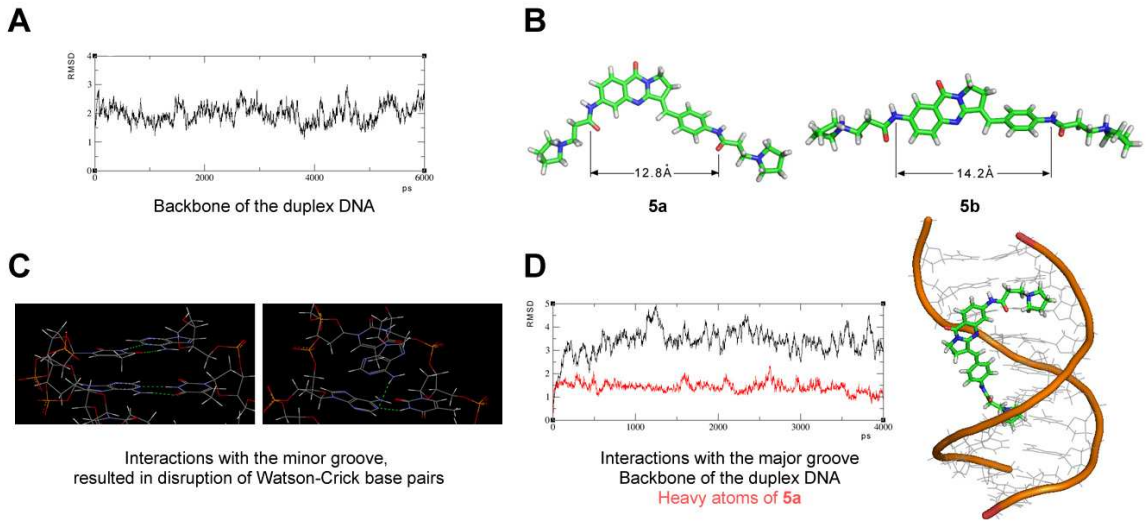


Figure S8. Molecular modeling studies of interactions between ligand and duplex DNA.

Table S2. Estimated Free Energy of Binding (ΔG, in kcal·mol^{-1}) in MM-PBSA Calculations

	ΔG / kcal·mol^{-1} (interactions with duplex DNA)		
Compd	Intercalation	Minor groove	Major groove
5a	nr[a]	nr[b]	-31.81
5b	nr[a]	nr[b]	-30.06

[a] nr: no result due to ligand escaping from its primary pseudointercalation site during dynamic runs. [b] nr: no result due to duplex DNA destabilization.

It was found that the putatively coplanar chromophore of compound **5a** and **5b** was sterically too large (about 12.8-14.2 Å) for the pseudointercalation site in the duplex DNA (about 10.7 Å) to allow significant DNA intercalation (Figure S8B). In further MD runs, ligands were found to escape from their primary pseudointercalation site accompanied by the migration towards groove region. On the other hand, the ligands were well accommodated in

the duplex DNA grooves. However, interactions with the minor groove resulted in disruption of Watson-Crick base pairs, leading to duplex DNA destabilization (Figure S8C). These results were in agreement with previous published studies.[1,2] Hence, interactions with the DNA pseudointercalation site and minor groove were discounted on structural stability grounds.[2] Estimated free energy of binding in MM-PBSA calculations was shown in Table S2.

9. Telomerase Inhibition

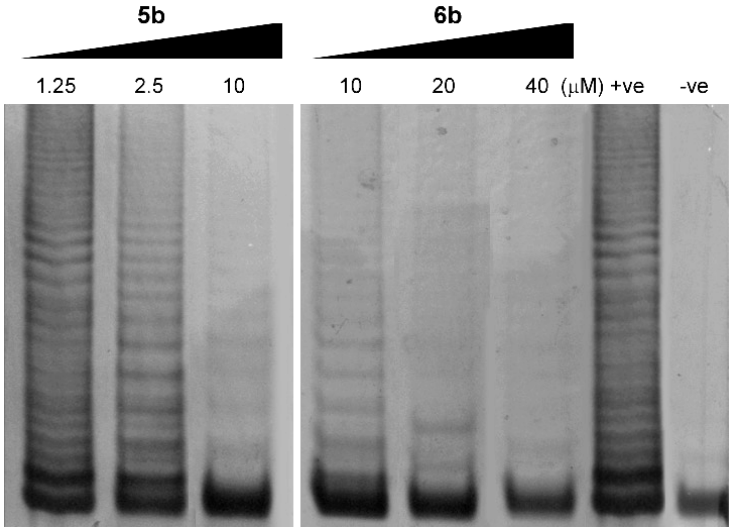


Figure S9. TRAP gel for ligand **5b** and **6b**.

Reference

1. Wheelhouse, R. T.; Jennings, S. A.; Phillips, V. A.; Pletsas, D.; Murphy, P. M.; Garbett, N. C.; Chaires, J. B.; Jenkins, T. C. Design, synthesis, and evaluation of novel biarylpyrimidines: a new class of ligand for unusual nucleic acid structures. *J. Med. Chem.* **2006**, *49*, 5187-5198.

2. Drewe, W. C.; Nanjunda, R.; Gunaratnam, M.; Beltran, M.; Parkinson, G. N.; Reszka, A. P.; Wilson, W. D.; Neidle, S. Rational design of substituted diarylureas: a scaffold for binding to G-quadruplex motifs. *J. Med. Chem.* **2008**, *51*, 7751-7767.