Supplementary Material (ESI) for Chemical Communications

Supporting Information

Bis(benzimidazole)pyridine derivative as a new class of G-quadruplex inducing and stabilizing ligand

Guorui Li, Jing Huang, Ming Zhang, Yangyang Zhou, Dan Zhang, Zhiguo Wu, Shaoru Wang, Xiaocheng Weng, Xiang Zhou,* and Guangfu Yang

Materials, methods and instrumentation. Anhydrous solvents (DMF, pyridine, $CHCl_3$) were prepared by standard methods. NMR spectra were recorded on a Varian Mercury-VX300 spectrometer at 300 MHz. MS were recorded on a Brucker Daltonics APE XII 47e and VG-707VHF mass spectrometer. CD spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD). Exonuclease I, biotin and TAMRA-labeled oligomers, were purchased from TaKaRa Biotech (Dalian, China). And other oligomers used in this research were purchased from Invitrogen (China). Taq DNA polymerase was purchased from TOYOBO (Japan).

Synthesis of compound 1 and 2.

Synthesis of compound 3a (2,6-Bis(2-benzimidazol-2-yl)pyridine):[1]

Under N_2, 3.35 g (20 mmol) of pyridine-2,6-dicarboxylic acid, 4.7 g (44 mmol) of 1,2-phenylene diamine were added to 160 g of polyphosphoric acid. The mixture was heated at 210 ℃ for 6 h with stirring. The mixture was cooled to 150 ℃ and then poured into 1 L of water. The mixture was then neutralized to pH 8 with ammonium hydroxide. The solid was collected by filtration and repeatedly washed with plenty of water. The solid was dissolved in hot methanol and treated with active charcoal to give compound **3a** (4.9 g, 79%) as a pure white crystal. ^{1}H NMR (300 MHz, [D_6]DMSO) δ(ppm) 13.00 (br, 2 H), 8.36 (d, J = 7.2 Hz， 2 H), 8.19 (t, J = 8.1 Hz, 1 H), 7.78 (m, 4H), 7.34 (m, 4 H).

Synthesis of compound 3b (1,2-Bis(2-benzimidazol-2-yl)benzene):[2]

Compound **3b** was prepared from phthalic acid and 1,2-phenylene diamine as described for **3a**. White crystal, yield 52%. ^{1}H NMR (300 MHz, [D_6]DMSO) δ(ppm) 13.49 (br, 2 H), 8.10 (m, 2 H), 7.67 (m, 2 H), 7.58 (m, 4H), 7.17 (m, 4 H).

Synthesis of compound 4a (2,6-Bis(5-nitro-1H-benzimidazol-2-yl)pyridine):

This compound was obtained by nitration of **3a**. Thus, 2.4 g (7.7 mmol) of **3a** was dissolved into concentrated H_2SO_4 (6 mL), and the solution was cooled in an ice–water bath. A mixture of fuming HNO_3 (1 mL) and concentrated H_2SO_4 (1 mL) was added dropwise. Then the solution was stirred for 4 h and poured into an ice–water mixture. The raw product was filtered, washed with water, neutralized with

sodium carbonate until pH 9, and washed with water to give **4a** (3 g, 97%) as a light yellow solid. ^{1}H NMR (300 MHz, [D_6]DMSO) δ(ppm) 13.69 (br, 2 H), 8.57 (s, 2 H), 8.37 (d, *J* = 7.5 Hz, 2 H), 8.21 (t, *J* = 7.5 Hz, 1 H), 8.09 (m, 2H), 7.89 (m, 2 H).

Synthesis of compound 4b (1,2-Bis(5-nitro-1H-benzimidazol-2-yl)benzene):[3]

This compound was obtained from **3b** using the same method for **4a**. Yellow solid, yield 96%. ^{1}H NMR (300 MHz, [D_6]DMSO) δ(ppm) 13.69 (br, 2 H), 8.50 (s, 2 H), 8.14 (m, 2 H), 7.87 (m, 2 H), 7.76 (d, *J* = 9.6 Hz, 2 H).

Synthesis of compound 5a (2,6-Bis(5-amino-1H-benzimidazol-2-yl)pyridine):

Compound **4a** (1 g, 2.5 mmol) was dissolved into glacial acetic acid (80 mL). Tin chloride (8.4 g, 37 mmol) was dissolved into concentrated hydrochloric acid (8 mL). Both solutions were heated to boiling, then the acetic acid solution was added dropwise to the tin chloride solution in ca. 30 min. The mixture was refluxed for 6 h and cooled to room temperature. The precipitate was filtered, and neutralized with sodium carbonate until pH 8. The solid was washed with water and dried under vacuum to give **5a** (440 mg, 52%) as a pale yellow solid. ^{1}H NMR (300 MHz, [D_6]DMSO) δ(ppm) 8.11 (d, *J* = 7.2 Hz, 1 H), 8.02 (t, *J* = 7.2 Hz, 2 H), 7.41 (d, *J* = 8.7 Hz, 2 H), 6.85 (s, 2 H), 6.66 (d, *J* = 8.7 Hz, 2H); HRMS(ESI), *m/z*: calculated for: $[M+H]^+$= 342.1462; found:342.1459.

Synthesis of compound 5b (1,2-Bis(5-amino-1H-benzimidazol-2-yl)benzene):[3]

Compound **5b** was prepared from **4b** following the same procedure for **5a**. Yellow solid, yield 48%. ^{1}H NMR (300 MHz, [D_6]DMSO) δ(ppm) 13.20 (br, 2 H), 8.08 (m, 2 H), 7.59 (m, 2 H), 7.33 (m, 2 H), 6.67 (s, 2 H), 6.56 (m, 2 H), 5.00 (br, 4 H); MS(ESI), *m/z*: calculated for: $[M+H]^+$= 341; found:341.

Synthesis of compound 6a (2,6-Bis(5-piperidineacetamide-1H-benzimidazol-2-yl)pyridine):

A solution of 2-chloroacetyl chloride (0.5 mL) in dry DMF (3 mL) at -10℃. was treated dropwise with a solution of **5a** (100 mg, 0.29 mmol) in dry pyridine (2 mL). The reaction mixture was left stirring for 30 min at -10℃. A solution of potassium carbonate (1 M, 5 mL) was then added. A precipitate formed and was filtered off under reduced pressure. Then the yellow powder was dissolved in methanol (150 mL) and treated with excess piperidine. The reaction mixture was left stirring overnight at 30℃. Methanol was removed under vacuum, then a solution of potassium carbonate (1 M, 10 mL) was added, followed by water (60 mL). The precipitate that formed was filtered off and dried under vacuum. The desired compound **6a** was obtained as a yellow powder (57 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 9.41 (s, 2 H), 8.28 (d, *J* = 8.1 Hz, 2 H), 8.09 (s, 2 H), 7.81(t, *J* = 8.1 Hz, 1 H), 7.56 (m, 2 H), 7.01 (m, 2 H),

3.11 (s, 4 H), 2.53 (m, 8 H), 1.62 (m, 8 H), 1.48 (m, 4 H); HRMS(ESI), *m/z*: calculated for: $[M+H]^+$= 592.3143; found:592.3136.

Synthesis of compound 6b (1,2-Bis(5-piperidineacetamide-1H-benzimidazol-2-yl)benzene):

Compound **6b** was prepared from **5b** following the same procedure for **6a**. Yellow solid, yield 14%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 9.44 (s, 2 H), 8.22 (s, 2 H), 7.80 (m, 2 H), 7.67(m, 2 H), 7.21 (m, 2 H), 6.97 (m, 2 H), 3.12 (s, 4 H), 2.59 (m, 8 H), 1.69 (m, 8 H), 1.52 (m, 4 H); HRMS(ESI), *m/z*: calculated for: $[M+H]^+$ = 591.3190; found:591.3187.

Synthesis of compound 1 (2,6-Bis(N-methyl-5-piperidineacetamide-1H-benzimidazol-2-yl)pyridine):

Compound **6a** (20 mg, 0.034 mmol) was dissolved in $CHCl_3$ (50 ml), and excess CH_3I (1 ml) was added into the solution and the reaction mixture was stirred at room temperature in dark for a whole night. The precipitate that formed was filtered off and washed with dry Et_2O to give compound **1** (25 mg, 85%) as a yellow solid. ^{1}H NMR (300 MHz, $[D_6]$DMSO) δ (ppm) 10.69 (s, 2 H), 8.34 (m, 2 H), 8.24 (m, 3 H), 7.78 (m, 2 H), 7.43 (m, 2 H), 4.39 (s, 4 H), 3.66 (s, 6 H), 3.60 (m, 8 H) 1.90 (m, 8 H), 1.61 (m, 4 H); HRMS(ESI), *m/z*: calculated for: [M-2I]/2 = 310.6764, [M-I] = 748.2579; found:310.6771, 748.2575.

Synthesis of compound 2 (1,2-Bis(N-methyl-5-piperidineacetamide-1H-benzimidazol-2-yl)benzene):

Compound **2** was prepared from **6b** following the same procedure for **1**. Yellow solid, yield 90%. ^{1}H NMR (300 MHz, $[D_6]$DMSO) δ (ppm) 10.61 (s, 2 H), 8.01 (m, 4 H), 7.78 (m, 2 H), 7.56 (m, 2 H), 7.30 (m, 2 H), 4.33 (s, 4 H), 3.61 (s, 6 H), 3.55 (m, 8 H) 1.86 (m, 8 H), 1.58 (m, 4 H); HRMS(ESI), *m/z*: calculated for: [M-I] = 747.2626; found:747.2622.

References:

1. A. W. Addison and P. J. Burke, *J. Heterocyclic. Chem.*, 1981, **18**, 803.
2. H. Vogel and C. S. Marvel, *J. Polym. Sci.*, 1961, **50**, 511.
3. M. Berrada, F. Carriere, Y. Abboud, A. Abourriche, A. Benamara, N. Lajrhed, M. Kabbajc and M. Berrada, *J. Mater. Chem.*, 2002, **12**, 3551.

Molecular Modelling.

The models of these two compounds were built manually and optimized by Gaussian03[1] using DFT method at B3LYP/6-31G** level. Figure S1 shows the optimized conformations.

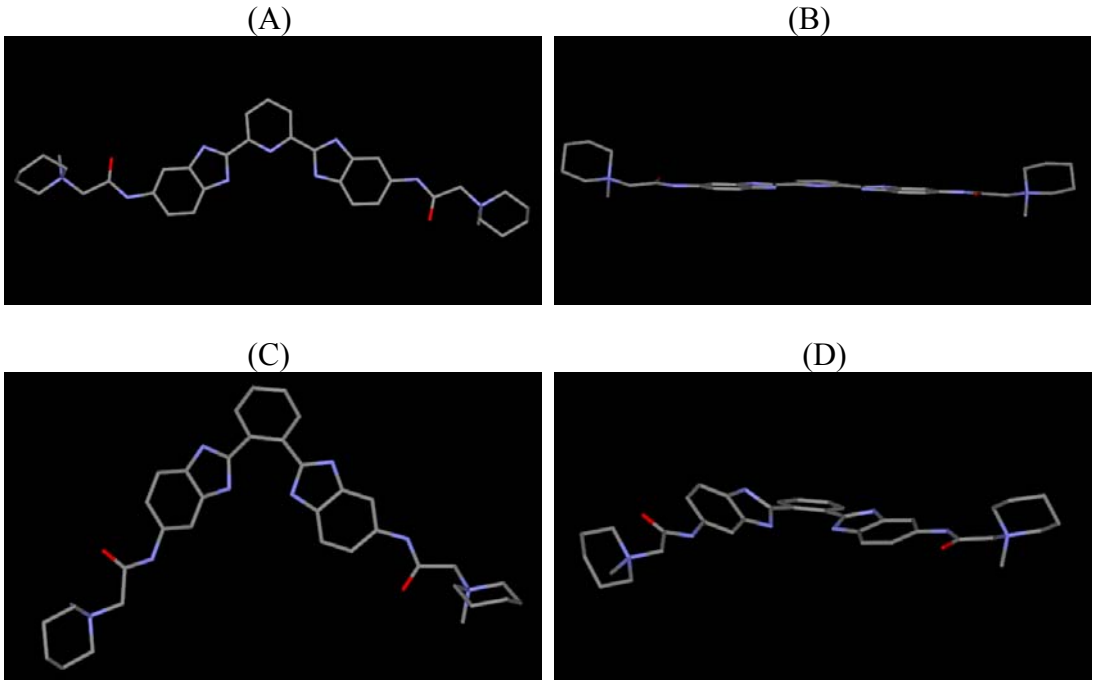


Figure S1 (A) Top view of compound **1**; (B) Side view of compound **1**; (C) Top view of compound **2**; (D) Side view of compound **2**. Molecules rendered as stick style and carbon atoms are colored in gray, nitrogens are in blue, oxygens are in red. Hydrogens are omitted for clarity.

Reference:

1. Gaussian 03, Revision C.02, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, Jr., T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K. G.; Voth, A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A. Gaussian, Inc., Wallingford CT, 2004.

Exonuclease I hydrolysis assay. Two TAMRA-5'-end-labeled oligonucleotides, $T_{24}(G_3T_2A)_3G_3$ and T_{24}GTGTGAGTGGAGGTGTGAGGT denoted as T24G21 and T24RG21, respectively, were used as substrates. Exonuclease I hydrolysis experiment was carried out in 10 μl reaction mixture containing 67mM Tris–HCl, pH 7.4, 6.7mM $MgCl_2$, 10mM β-mercaptoethanol, 0.1 mg/ml BSA and 0.1μM oligonucleotides. Before the addition of exonuclease I, samples were heated at 95℃ for 5 min, slowly cooled down to room temperature, then the compounds were added and incubate for 30 min at 37□. The reaction was initiated by addition of 0.1U Exonuclease I at 37℃. 30min later, the reactions were stopped by adding 10μl stop solution (10mM EDTA, 10mM NaOH and 0.1% bromphenol blue in formamide solution). Samples were electrophoresed on 19% denaturing polyacrylamide gel containing 7M urea for 30 min.

Circular dichroism (CD) spectroscopy and CD-melting assay.

CD spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD) using a quartz cell of 1 mm optical path length and an instrument scanning speed of 100 nm/min with a response time of 1 s, and over a wavelength range of 220-350 nm. All CD spectra were baseline-corrected for signal contributions due to the buffer.

CD melting curves were obtained on a Jasco-810 spectropolarimeter (Jasco, Easton, MD) using a quartz cell of 1 mm optical path length with a digital circulating water bath, while the temperature was ramped from 25 to 95°C at about 1.5°C/min.

Ds DNA: ds 5'-GCATTGGTAACTGTCAGACC-3'

3'-CGTAACCATTGACAGTCTGG-5'

Table S1 G-quadruplexes and duplex DNA stabilization by **1** and **2** determined by CD melting experiments (compound/DNA strand concentration=5)

	ΔTm (°C)		
	G4(Na^+)[a]	G4(K^+)[b]	dup[c]
Compound **1**	13.1	9.0	3.0
Compound **2**	10.3	5.6	0.3

[a] CD Tm of 10 μM G4 in 10 mM Tris-HCl at pH 7.4, 100 mM NaCl, 1 mM EDTA buffer. [b] CD Tm of 10 μM G4 in 10 mM Tris-HCl at pH 7.4, 100 mM KCl, 1 mM EDTA buffer. [c] CD Tm of 5 μM ds DNA in 10 mM Tris-HCl at pH 7.4, 100 mM KCl, 1 mM EDTA buffer.

Surface plasmon resonance (SPR) analysis.

Two 5'-biotin-labeled oligomers were used in SPR studies:

[G4]: 5'biotin-$AG_3(TTAG_3)_3$-3';

Duplex DNA: 5'-biotin-GGGCATAGTGCGTGGGCGTTAGC-3';

Its complementary strand: 5'-TAACGCCCACGCACTATGCC-3'.

Immobilization of DNA and surface plasmon resonance binding: Biotinylated DNA was immobilized on a CM5 sensor chip (BIAcore, Switzerland) with streptavidin by using a BIAcore 3000 optical biosensor.

Streptavidin was coupled to the carboxymethylated dextran matrix covering the surface of the sensor chip by using an amine coupling kit (BIAcore, Switzerland) according to the manufacturer's instruction. Biotinylated oligonucleotide (0.5 μM) in coupling buffer (10 mM Tris, pH 7.4, 1 mM EDTA, and 150 mM LiCl) was heated at 95°C for 5 min and cooled slowly to room temperature. Then biotin–DNA (30 μL) was injected at a flow rate of 5 μL/min. One of the flow cells was used to immobilize the DNA and another served as a blank reference.

All the samples were dissolved in DMSO (10 mM) and prepared in freshly filtered and degassed running buffer (HBS-EP/KCl buffer-pH 7.4, 0.01M HEPES, 0.2M KCl, 3 mM EDTA, 0.005% (v/v) surfactant P20) by serial dilutions from the stock solutions. This DNA folded in running buffer with K^+ (200 mM) and formed a quadruplex during extended flow in the SPR experiments.

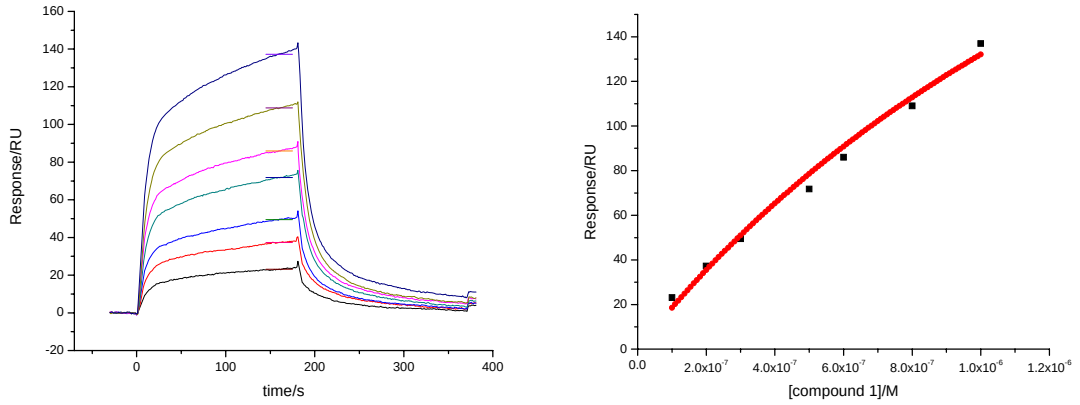
The double-stranded DNA (dsDNA) consisted of biotin–oligoDNA, hybridized with its complementary sequence. The sensor chip was regenerated by injection of NaOH/NaCl (20 mM/1M, 5 μL). All procedures used repetitive cycles of the same injection and regeneration.

DNA-binding experiments were carried out in the running buffer (pH 7.4, 0.01M HEPES, 0.2M KCl, 3 mM EDTA, 0.005% (v/v) surfactant P20) at a flow rate of 10 μL/min.

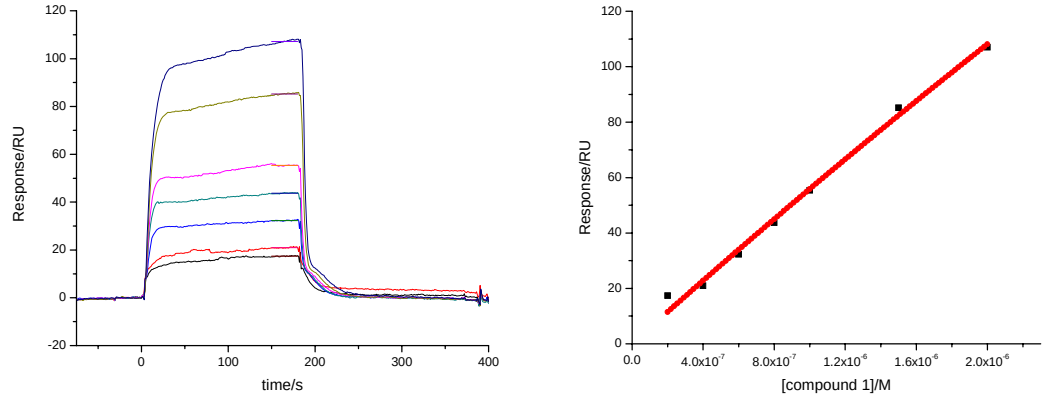
Table S2 Equilibrium Binding constants determined by SPR using general fit and the kinetic constants determined from BIAcore analysis of 1:1 Langmuir global fitting with mass transfer

	DNA	KA(1/M)[a]	ka(1/Ms)[b]	kd(1/S)[b]	KA(1/M)[c]
Compound **1**	[G4]	4.68×10^{5}	1.46×10^{4}	1.57×10^{-2}	9.28×10^{5}
	dsDNA	3.41×10^{4}	4.99×10^{3}	9.58×10^{-2}	5.21×10^{4}
Compound **2**	[G4]	3.05×10^{4}	9.04×10^{2}	5.63×10^{-3}	1.61×10^{5}
	dsDNA	5.02×10^{3}	6.80×10^{2}	1.44×10^{-2}	4.71×10^{4}

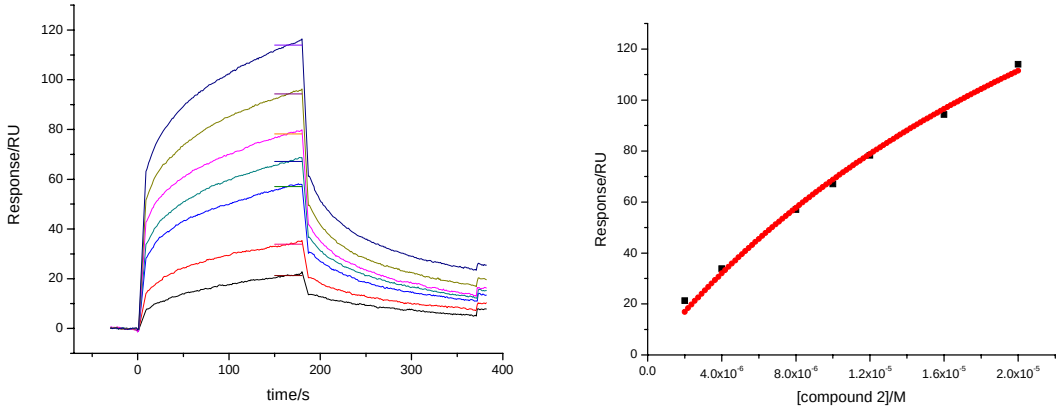
[a] Equilibrium Binding constants determined by SPR using general fit of 180s association in 30s steady-state region. [b] Kinetic constants determined from BIAcore analysis of 1: 1 Langmuir global fitting with mass transfer. [c] Determined by ka/kd.



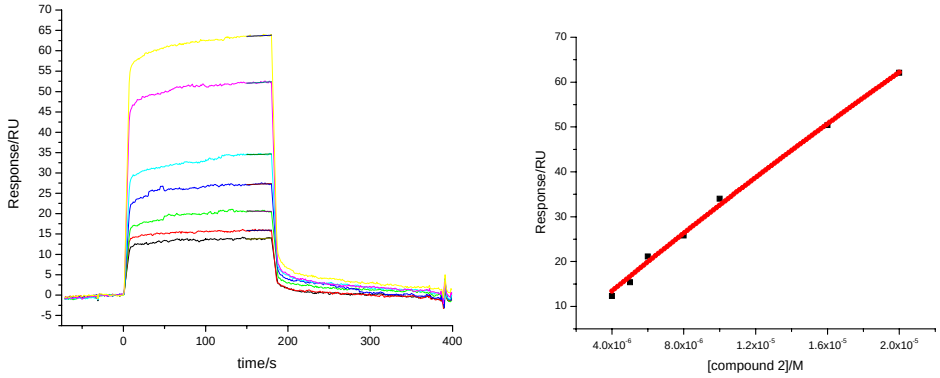
A



B



C



D

Figure S2. SPR sensorgram overlay for binding of ligands to different sequences DNA at 25°C used for general fit of 30s steay-state region and the corresponding fitting curves.
(A) [G4] sequence. The unbound compound **1** concentrations in the flow solution were 100, 200, 300, 500, 600, 800, 1000 nM from the lowest curve to the top curve.
(B) DNA duplex sequence. The unbound compound **1** concentrations in the flow solution were 200, 400, 600, 800, 1000, 1500, 2000nM from the lowest curve to the top curve.
(C) [G4] sequence. The unbound compound **2** concentrations in the flow solution were 2, 4, 8, 10, 12, 16, 20μM from the lowest curve to the top curve.
(D) DNA duplex sequence. The unbound compound **2** concentrations in the flow solution were 4, 5, 6, 8, 10, 16, 20μM from the lowest curve to the top curve.

PCR Stop Assay

In this assay, the tested oligomer 21G (5'-GGGTTAGGGTTAGGGTTAGGG-3') and the corresponding complementary sequence Rev21G (5'-TCTCGTCTTCCCTAA-3') was used. The assay reaction was performed in a final volume of 12.5 mL, in a 10 mM Tris, pH 8.3, buffer with 50 mM KCl, 1.5 mM $MgCl_2$, 400 nM of each pair of oligomers, 200 nM dNTP, 2.5 U Taq polymerase, and corresponding amount of the compounds. Reaction mixtures were incubated in a thermocycler, with the following cycling conditions: 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. Amplified products were resolved on 16% nondenaturing polyacrylamide gels in 1×TBE and EB stained. The parallel experiment was performed using a mutated oligomer 21Gmu (5'-GGGTTAGAATTAGGGTTAGGG-3') instead of 21G in identical conditions.

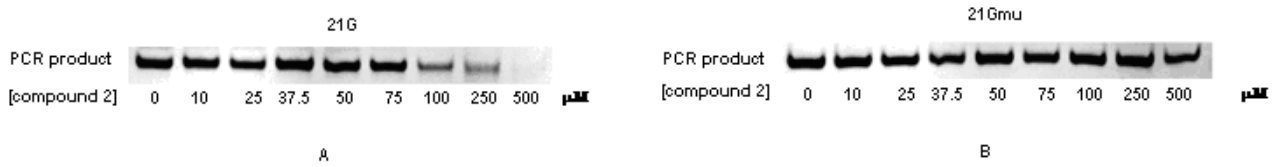
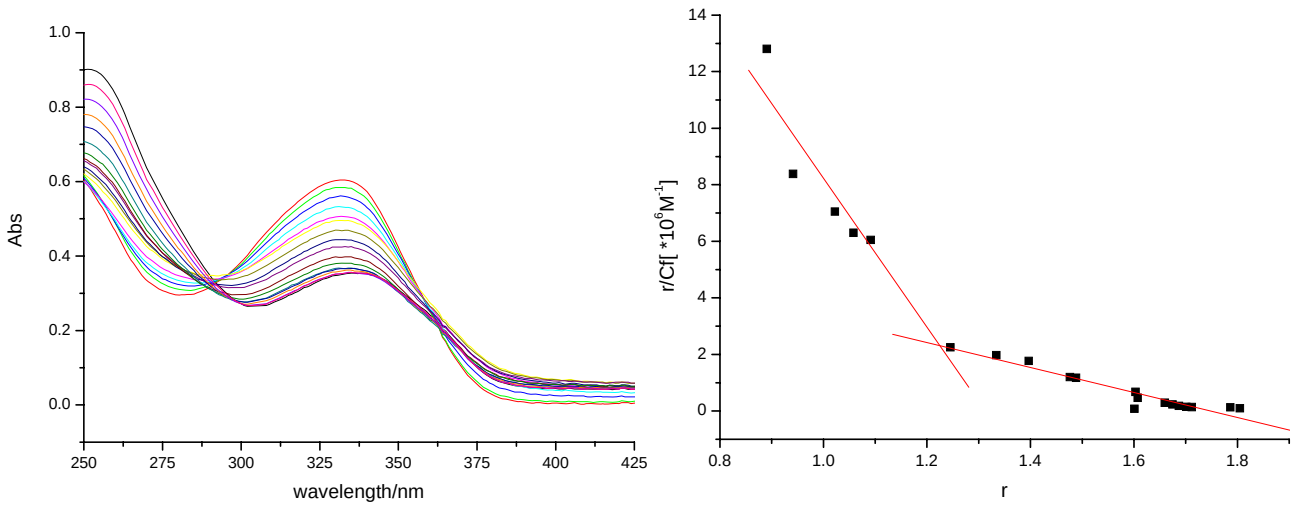


Figure S3 Effect of compound **2** on the formation of the PCR-stop assay with G-quadruplex forming 21G oligomer (A) or with control mutated Rev21G oligomer (B).

UV-vis absorption titration

Absorption spectra were measured on a Shimadzu 1901UV-vis double beam spectrophotometer with a 1 cm path-length quarter cell. The reference solution was 10 mM Tris-HCl, 1 mM Na_2EDTA, and 100 mM KCl at pH 7.4. UV-vis absorption titrations were carried out by the stepwise addition of G-quadruplex ($d[T_2AG_3]_4$) solutions at room temperature. The titration was terminated when the wavelength and intensity of the absorption band for compound **1** did not change any more. The UV-vis absorption titration data were analyzed by Scatchard equation according to previous reports.[1,2] The data were fit via the simple Scatchard model: $r/Cf) K(n - r)$, where K is the equilibrium binding constant and n represents the number of ligands bound per DNA.

The $d[T_2AG_3]_4$ G-quadruplexes produce the red shifts(9nm) and great degrees of hypochromicities(42%) in the band of compound **1** (332 nm). From the Scatchard analysis, an inflection point observed in Scatchard plots is r = 1.2.



(A) (B)

Figure S4 (A)UV-vis absorption titration spectra of 20μM compound **1** with d[T_2AG_3]$_4$ G-quadruplexes in 10 mM Tris-HCl, 1 mM Na_2EDTA, and 100 mM KCl buffer at pH 7.4. (B) Scatchard plots for compound **1** with d[T_2AG_3]$_4$ G-quadruplexes.

References:

1 C.-Y. Wei, G.-Q. Jia, J.-L. Yuan, Z.-C. Feng, and C. Li, *Biochemistry,* 2006, **45**, 6681.

2 L.R. Keating and Veronika A. Szalai, *Biochemistry* 2004**, 43**, 15891.