Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Bisbenzimidazole to benzobisimidazole: from binding B-form duplex DNA to recognizing different modes of telomere G-quadruplex

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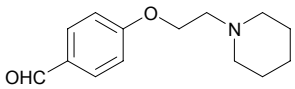
Materials, methods and instrumentation. Anhydrous acetone was prepared by standard methods. NMR spectra were recorded on a Varian Mercury-VX300 spectrometer at 300 MHz. MS were recorded on a Brucker Daltonics APE XII 47e and VG-707VHF mass spectrometer. CD spectra were recorded on a Jasco-810 spectropolarimeter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

General procedure for the synthesis of compounds 5-7.

Under N_2, to a solution of the appropriate hydroxybenzaldehyde (1 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (1.5 mmol for **5**, **6** and 2.15 mmol for **7**) in dry acetone was added K_2CO_3 (2 mmol for **5**, **6** and 4 mmol for **7**) and NaI (0.25 mmol for **5**, **6** and 0.5 mmol for **7**). The reaction mixture was refluxed for 18 h. The mixture was cooled to room temperature and filtered. After evaporation of the solvent, the residue was dissolved in ethyl acetate, washed with saturated K_2CO_3 solution and water, dried (Na_2SO_4), filtered, and evaporated to dryness.

Compound 5 (4-(2-(piperidin-1-yl)ethoxy)benzaldehyde):



Yellow syrup, yield: 81%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 9.88 (s, 1H, CHO), 7.83 (d, 2 H, *J* = 8.4 Hz, arom H), 7.01 (d, 2 H, *J* = 8.4 Hz, arom H), 4.19 (t, 2 H, *J* = 6.0 Hz, CH_2CH_2), 2.80 (t, 2 H, *J* = 6.0 Hz, CH_2CH_2), 2.51 (m, 4 H, CH_2NCH_2), 1.61 (m, 4 H, $CH_2CH_2CH_2$), 1.47 (m, 2 H, $CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) s: 190.9, 164.0, 132.1, 130.0, 115.0, 66.4, 57.8, 55.2, 26.0, 24.2; ESI MS for $C_{14}H_{20}NO_2$ $[M+H]^+$: found 234, calcd 234.

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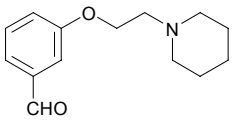
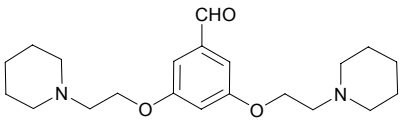
Compound 6 (3-(2-(piperidin-1-yl)ethoxy)benzaldehyde):



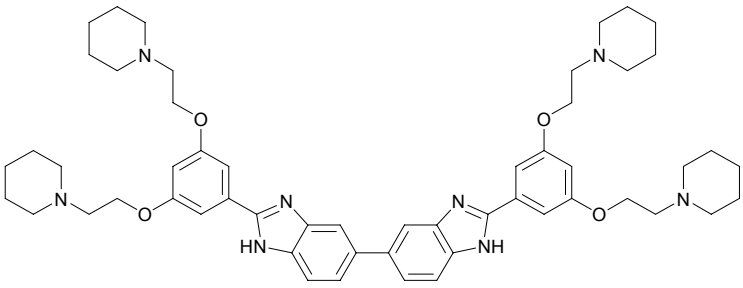
Yellow syrup, yield: 84%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 9.97 (s, 1 H, CHO), 7.46 (m, 2 H, arom H), 7.41 (s, 1 H, arom H), 7.18 (m, 1 H, arom H), 4.18 (t, 2 H, J = 6.0 Hz, CH_2CH_2), 2.82 (t, 2 H, J = 6.0 Hz, CH_2CH_2), 2.54 (m, 4 H, CH_2NCH_2), 1.62 (m, 4 H, $CH_2CH_2CH_2$), 1.46 (m, 2 H, $CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) s: 192.3, 160.0, 137.9, 130.2, 123.6, 122.1, 113.2, 66.3, 57.8, 55.2, 26.1, 24.3; ESI MS for $C_{14}H_{20}NO_2$ $[M+H]^+$: found 234, calcd 234. 7.17 (m, 4 H).

Compound 7 (3,5-bis(2-(piperidin-1-yl)ethoxy)benzaldehyde):



Yellow syrup, yield: 81%.^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 9.76 (s, 1H, CHO), 6.89 (s, 2 H, arom H), 6.63 (s, 1 H, arom H), 4.01 (t, 4 H, J = 6.3 Hz, CH_2CH_2), 2.66 (t, 4 H, J = 5.9 Hz, CH_2CH_2), 2.39 (m, 8 H, CH_2NCH_2), 1.49 (m, 8 H, $CH_2CH_2CH_2$), 1.34 (m, 4 H, $CH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) s: 191.8, 160.6, 138.4, 108.3, 108.0, 66.5, 57.9, 55.2, 26.1, 24.3; ESI MS for $C_{21}H_{33}N_2O_3$ $[M+H]^+$: found 361, calcd 361.

Synthesis of compound 1 (2,2-bis[3',5'-bis(2''-piperidinylethoxy)phenyl]-5,5-bi-1H -benzimidazole):[1]



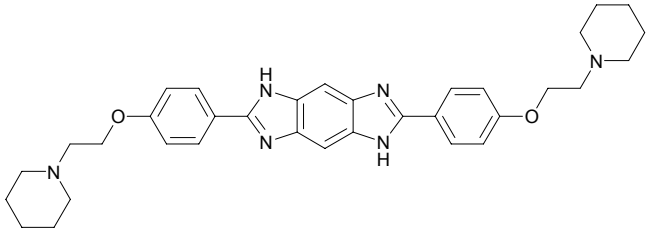
Under N_2, a solution of compound **7** (400 mg, 1.11 mmol), 3, 3'-diaminobenzidine (119 mg, 0.56 mmol), and 1, 4-benzoquinone (120 mg, 1.11 mmol) in ethanol (150 mL) was heated at reflux for 24 h. The reaction mixture was cooled down to room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography by using a mixture of $CH_3OH/CHC1_3$(NH_3 saturated) = 30: 1 as the eluant to afford the compound **1** (105 mg, 21%) as a yellow powder, mp: 187-188 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 7.80-7.54 (m, 6 H, arom H), 7.37 (s, 4 H, arom H), 6.61 (s, 2 H, arom H), 4.12 (m, 8 H, CH_2CH_2), 2.66 (m, 8 H, CH_2CH_2), 2.43 (m, 16 H, CH_2NCH_2), 1.48 (m, 16 H, $CH_2CH_2CH_2$), 1.36 (m, 8 H, $CH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz) 160.7, 160.5, 152.3, 138.9,

136.5, 132.5, 108.3, 107.4, 106.0, 105.6, 103.6, 66.6, 58.0, 55.1, 26.3, 24.6; HRMS (ESI): 895.5591 for $[M+H]^+$ (calcd: 895.5593 for $C_{54}H_{71}N_8O_4$).

General procedure for the synthesis of compounds 2-4.[1]

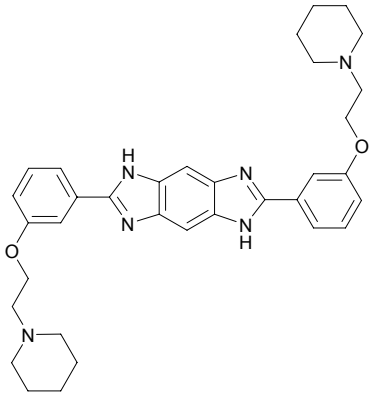
Under N_2, to a solution of NaOH (4 mmol) in ethanol was added 1, 2, 4, 5-benzenetetraamine tetrahydrochloride (1 mmol) and the solution was stirred at room temperature for 10 min. Then the appropriate aldehyde **5-7** (2 mmol), and 1,4-benzoquinone (2 mmol) was added and the mixture was heated at reflux for 18 h. The reaction mixture was cooled to room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography using CH_3OH as the eluant to afford the compound **2-4**.

Compound 2 (2,6-bis[(4'-2''-piperidinylethoxy)phenyl]-benzo[1,2-*d*:4,5-*d'*]bisimidazole):



Brown powder, yield: 41%, mp: >300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.08 (d, 4 H, J = 6.9 Hz, arom H), 7.58 (s, 2 H, arom H), 7.08 (d, 4 H, J = 7.8 Hz, arom H), 4.13 (m, 4 H, CH_2CH_2), 2.66 (m, 4 H, CH_2CH_2), 2.43 (m, 8H, CH_2NCH_2), 1.49 (m, 8 H, $CH_2CH_2CH_2$), 1.37 (m, 4 H, $CH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz) 160.3, 152.2, 128.5, 123.7, 115.6, 107.8, 66.4, 57.9, 55.0, 26.1, 24.5; HRMS (ESI): 565.3290 for $[M+H]^+$ (calcd: 565.3286 for $C_{34}H_{41}N_6O_2$).

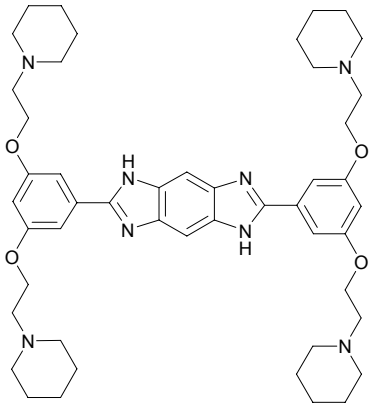
Compound 3 (2,6-bis[(3'-2''-piperidinylethoxy)phenyl]-benzo[1,2-*d*:4,5-*d'*]bisimidazole):



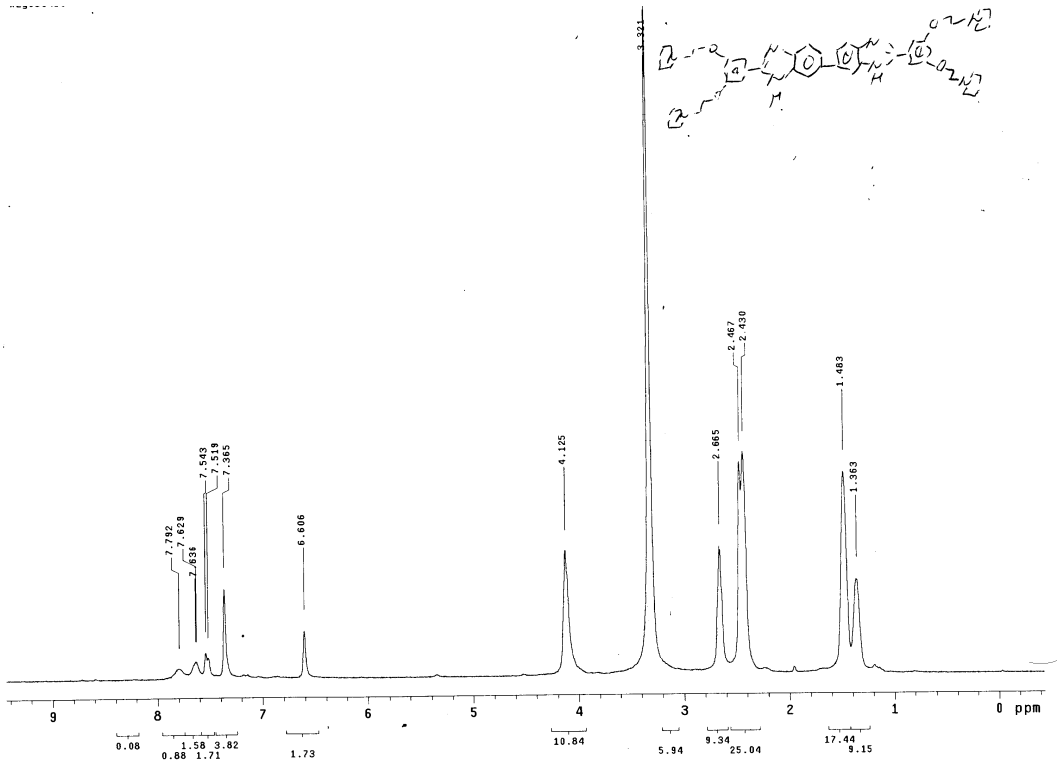
Brown powder, yield: 34%, mp: 189-191°C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 12.73 (s, 2 H, NH), 7.77 (m, 6 H, arom H), 7.42-7.47 (m, 2 H, arom H), 7.04-7.06 (m, 2 H, arom H), 4.18 (m, 4 H, CH_2CH_2), 2.72 (m, 4 H, CH_2CH_2), 2.49 (m, 8H, CH_2NCH_2), 1.52 (m, 8 H, $CH_2CH_2CH_2$), 1.40 (m, 4 H,

$CH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz) 159.6, 152.2, 132 .3, 130.7, 119.3, 116.8, 112.4, 66.4, 58.0, 55.1, 26.3, 24.6; HRMS (ESI): 565.3285 for $[M+H]^+$ (calcd: 565.3286 for $C_{34}H_{41}N_6O_2$).

Compound 4 (2,6-bis[3',5'-bis(2''-piperidinylethoxy)phenyl]-benzo[1,2-*d*:4,5-*d'*]bisimidazole):



Yellow powder, yield: 28%, mp: 221-222 °C. ^1H NMR (DMSO-d_6, 300 MHz) δ(ppm) 12.69-12.71 (m, 2H, NH), 7.67 (s, 2 H, arom H), 7.36 (s, 4 H, arom H), 6.60 (s, 2 H, arom H), 4.14 (m, 8 H, CH_2CH_2), 2.68 (m, 8 H, CH_2CH_2), 2.48 (m, 16 H, CH_2NCH_2), 1.50 (m, 16 H, $CH_2CH_2CH_2$), 1.38 (m, 8 H, $CH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 125 MHz) 160.7, 132.8, 105.8, 105.4, 103.5, 79.2, 79.4, 66.7, 58.1, 55.1, 26.3, 24.6; HRMS (ESI): 819.5287 for $[M+H]^+$ (calcd: 819.5280 for $C_{48}H_{67}N_8O_4$).



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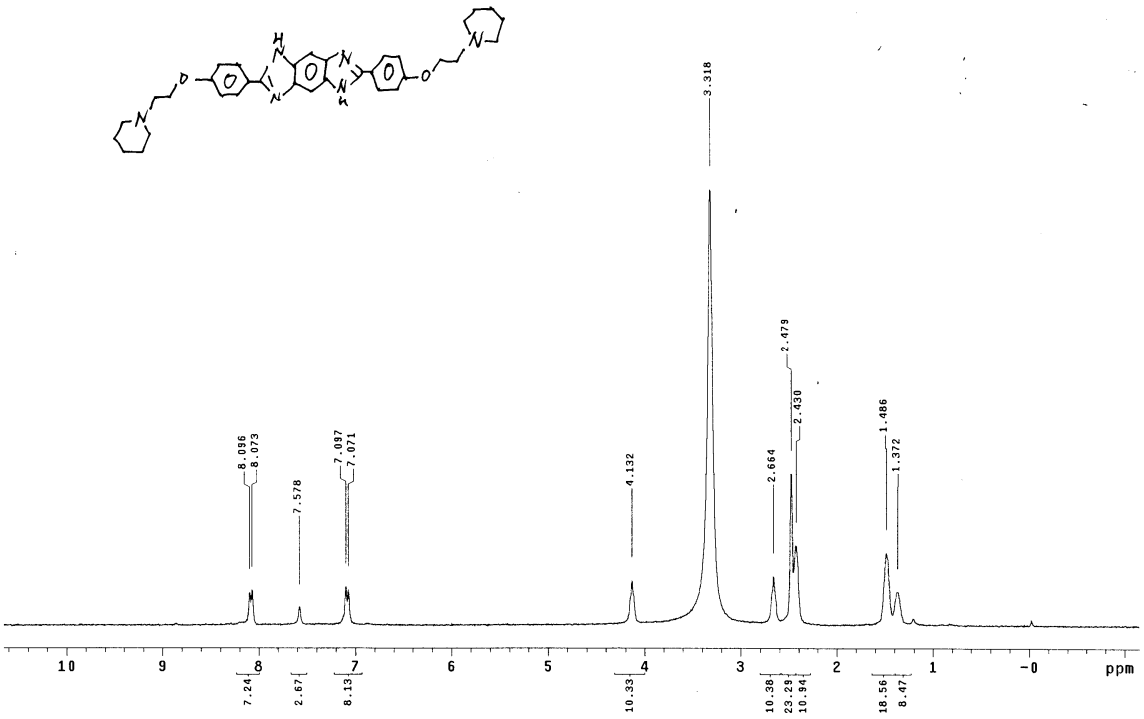

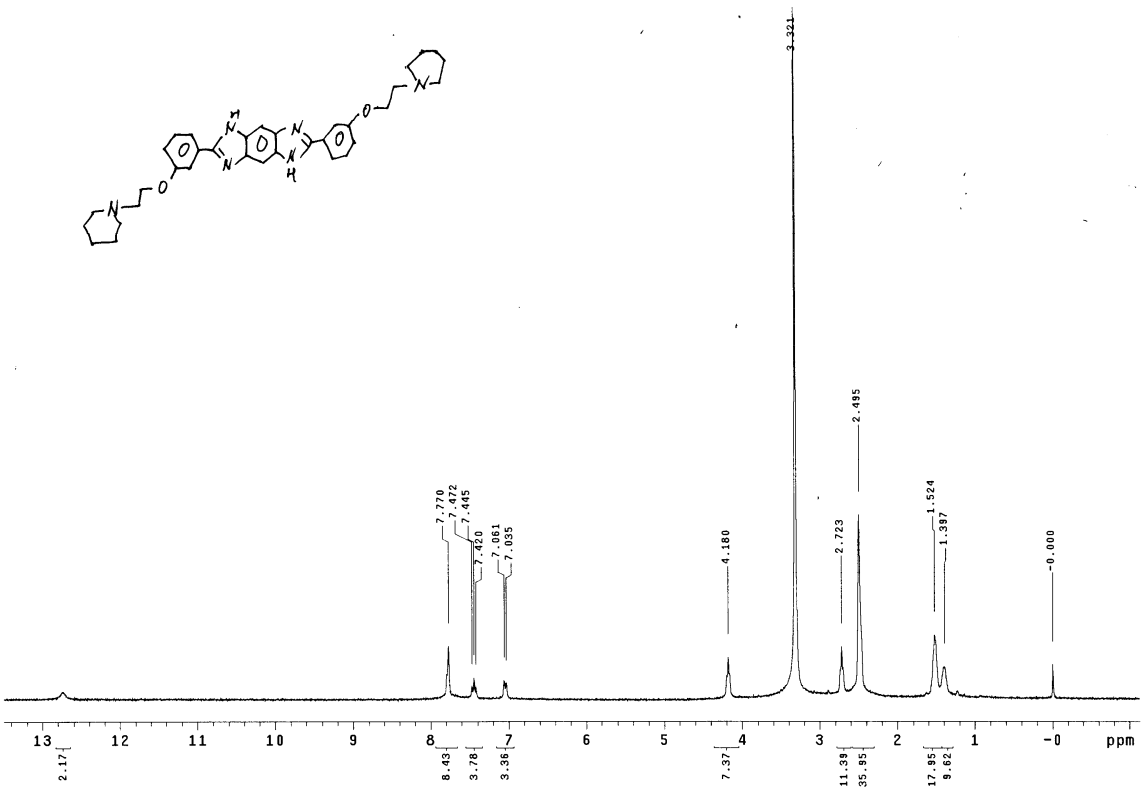

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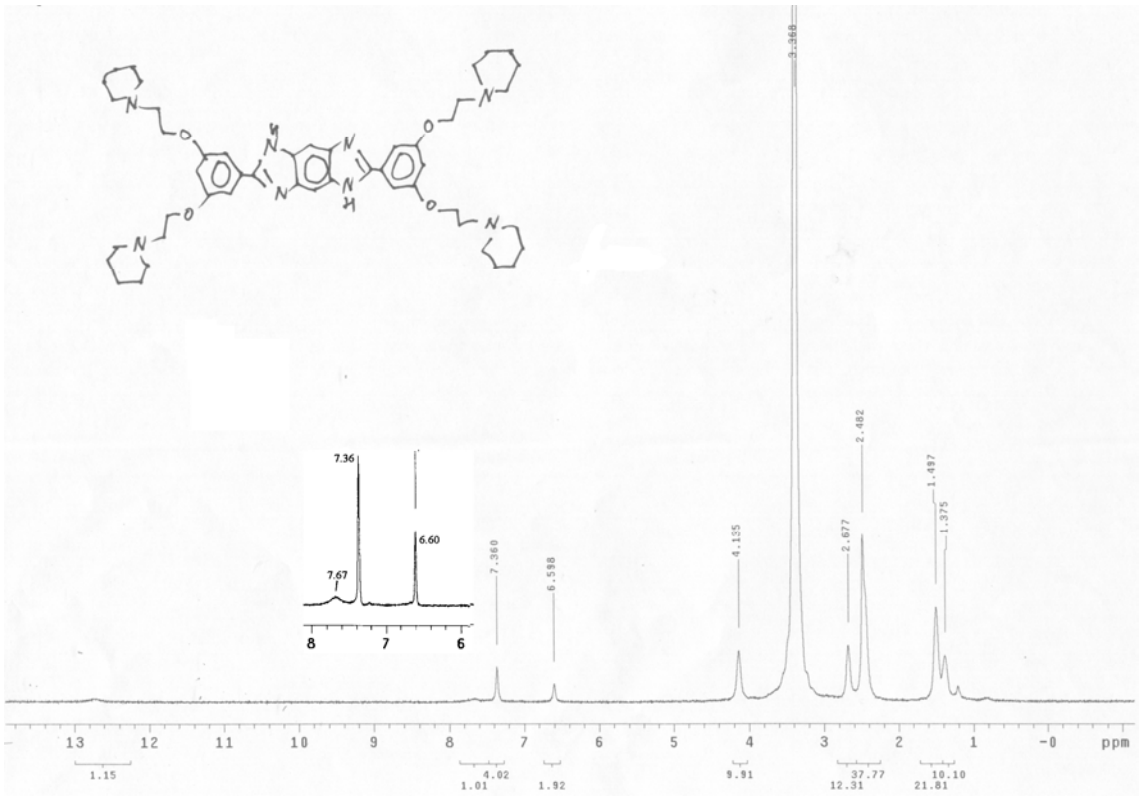
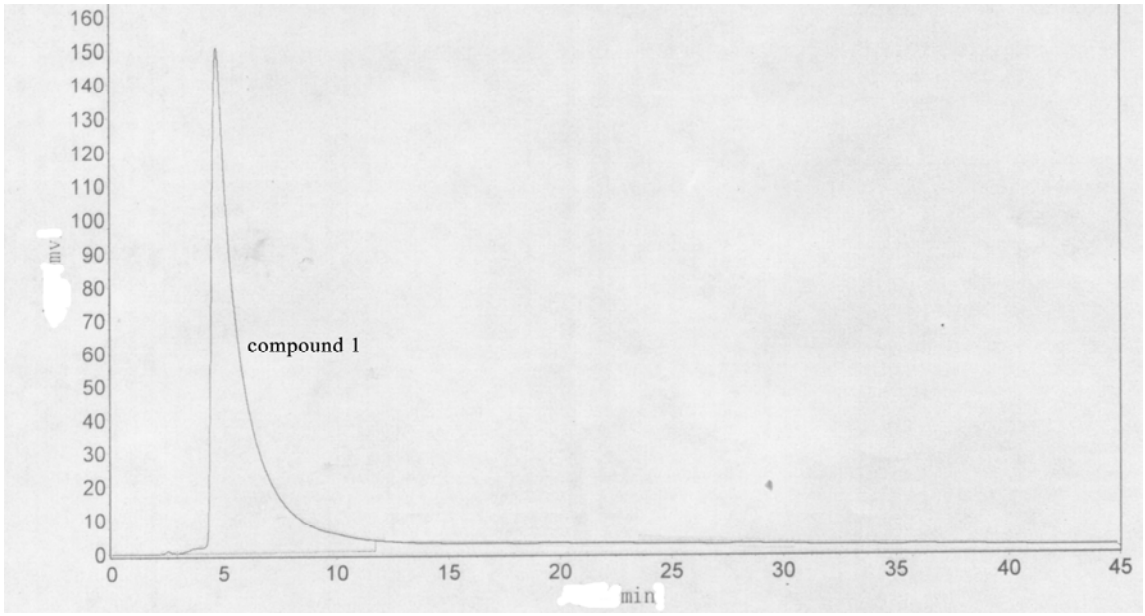


Fig. S1. The ^{1}H NMR spectra of compound **1**, **2**, **3**, and **4**.

Reversed-phase HPLC analysis

The samples were analyzed on an AT.ChromC18 column, SHIMADZU LC-10AT*VP*.



Detection at 338 nm, CH_3OH: H_2O 40: 60, 1 mL/min. retention time: 4.67 min; purity: 99.4%.

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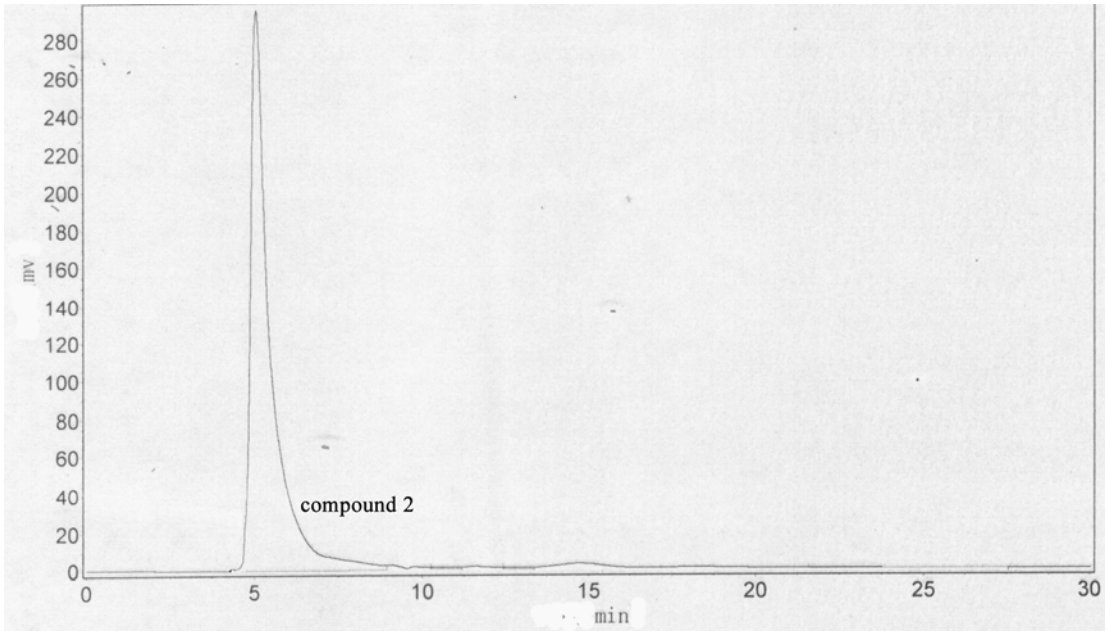
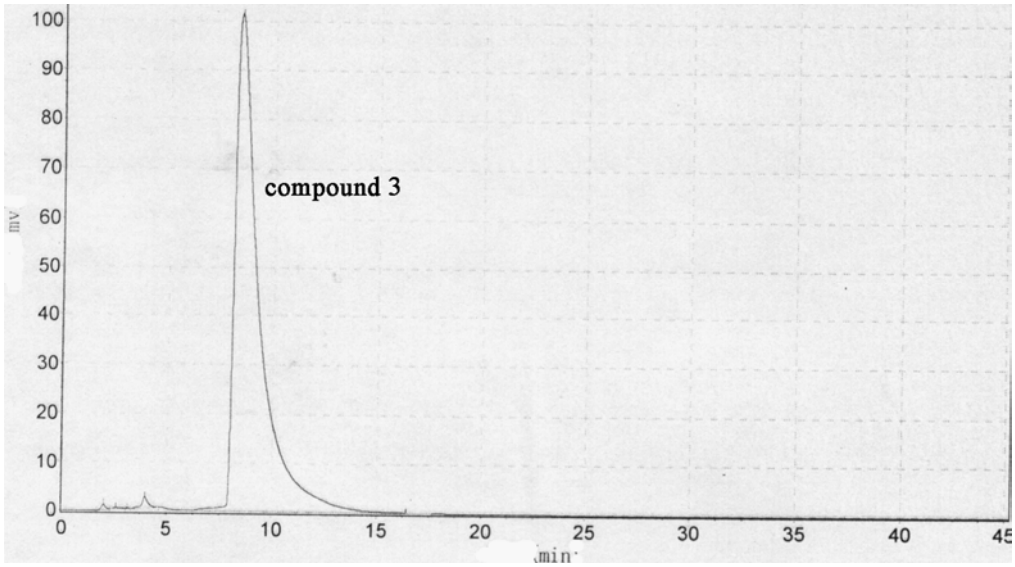
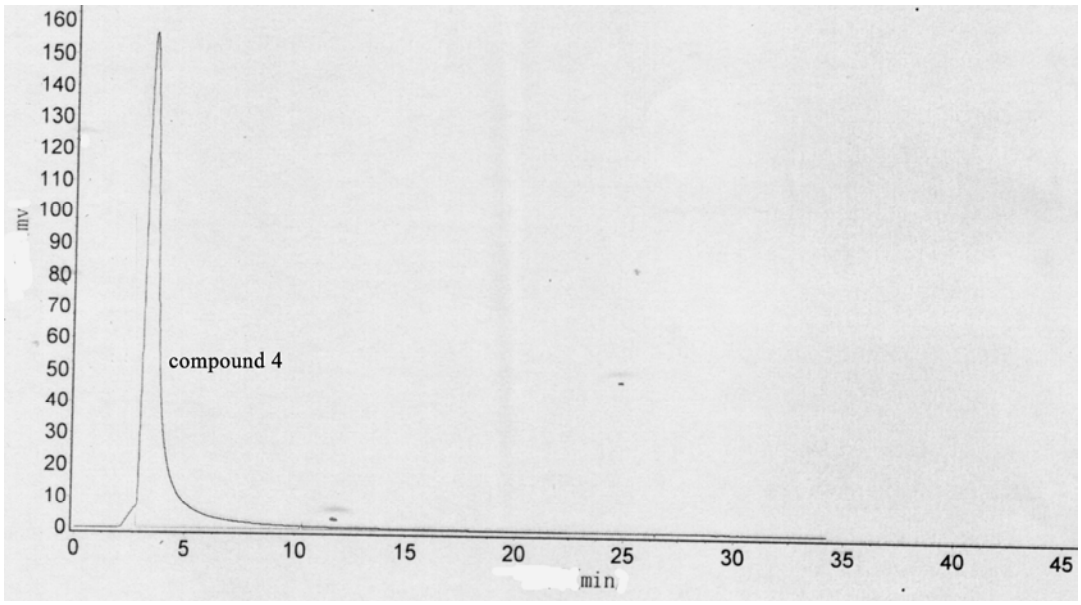


Detection at 348 nm, CH_3OH: H_2O 40: 60, 1 mL/min. retention time: 5.06 min; purity: 98.8%.



Detection at 348 nm, CH_3OH: H_2O 30: 70, 1 mL/min. retention time: 8.40 min, purity: 98.1%.



Detection at 348 nm, CH_3OH: H_2O 40: 60, 1 mL/min. retention time: 3.59 min; purity: 98.7%.

Fig. S2. The HPLC spectra of compound **1**, **2**, **3**, and **4**.

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

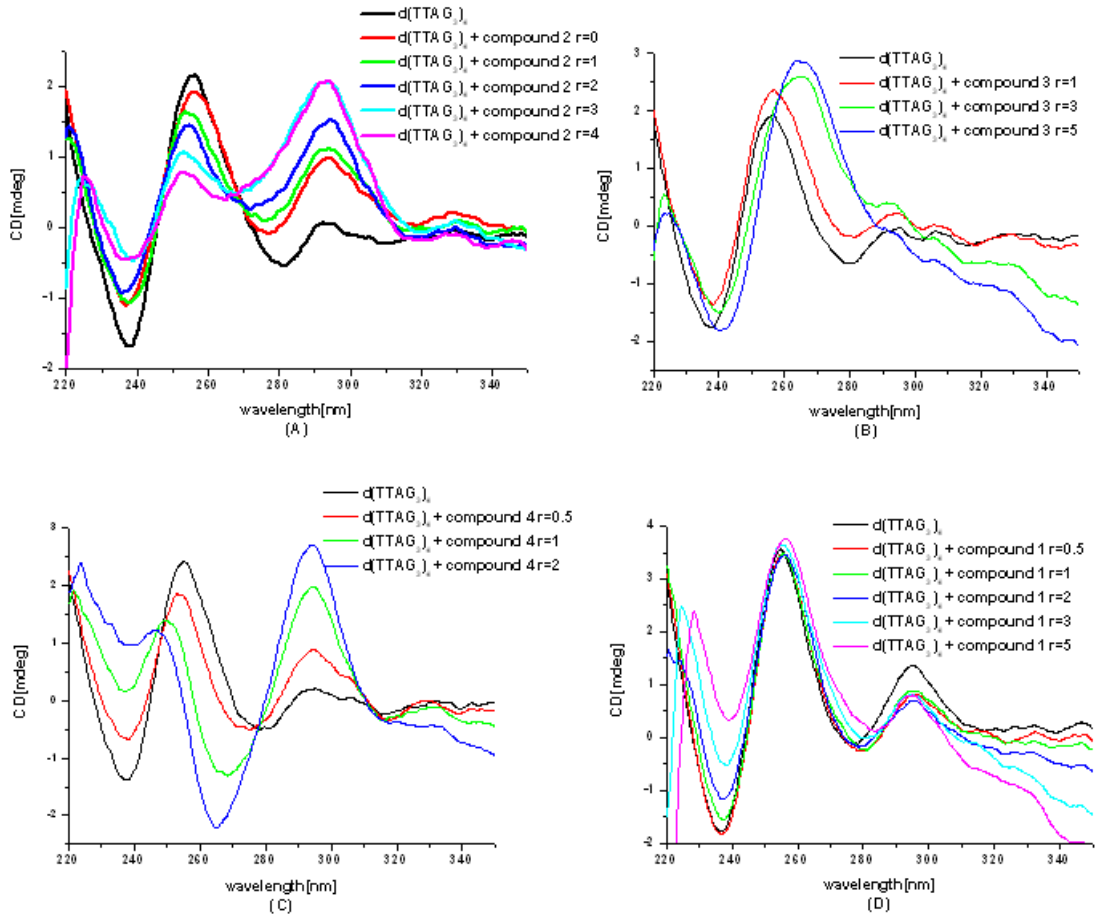


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Fig. S3 CD titration of d[T_2AG_3]$_4$(12.5 μM) with compounds **1**, **2**, **3** and **4** in 10mM Tris-HCl, 1mM EDTA buffer at pH 7.4 (r = compound /DNA strand concentration). CD spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD) at room temperature.

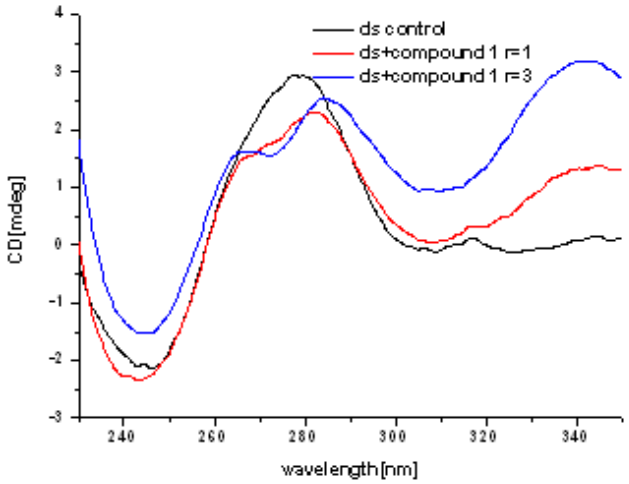

Fig. S4 CD titration of ds DNA(5 μM) with compounds **1** in 10mM Tris-HCl, 1mM EDTA buffer at pH 7.4 (r = compound /DNA strand concentration). CD spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD) at room temperature.

Table S1 G-quadruplexes and duplex DNA stabilization by **1**, **2**, **3** AND **4** determined by CD melting experiments (compound/DNA strand concentration=3)

	ΔTm / °C	
compound	G-quadruplex DNA[a]	duplex DNA[b]
1	4.1	17.1
2	9.2	4.0
3	9.0	3.3
4	13.6	5.3

[a] CD Tm of 10 μM G4 in 10 mM Tris-HCl at pH 7.4, 100 mM KCl, 1 mM EDTA buffer. The G-quadruplex DNA *T*m in the buffer without ligand is 54.4 °C.
[b] CD Tm of 5 μM ds DNA in 10 mM Tris-HCl at pH 7.4, 100 mM KCl, 1 mM EDTA buffer. The duplex DNA *T*m in the buffer without ligand is 68.6 °C.

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Optimized conformations

The models of these two compounds were built manually and optimized by Gaussian03[2] using DFT method at B3LYP/6-31G** level.

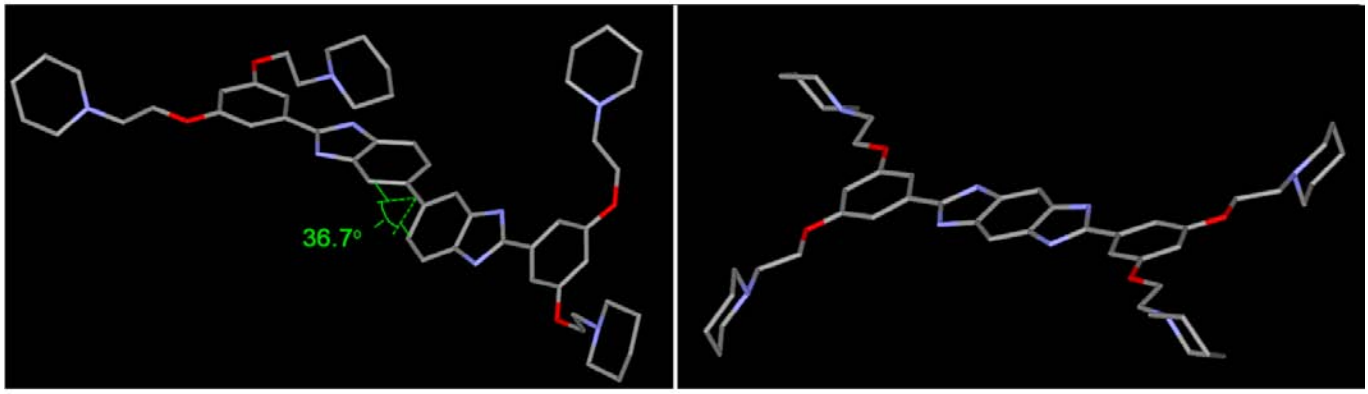
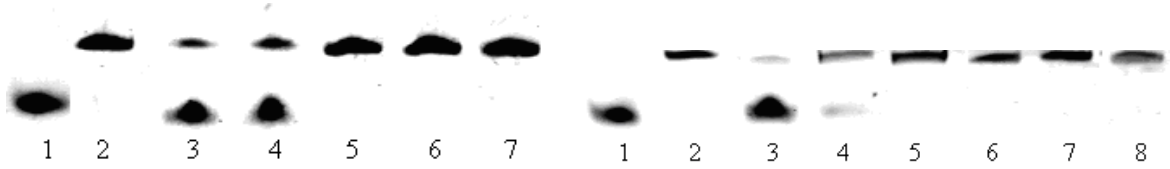


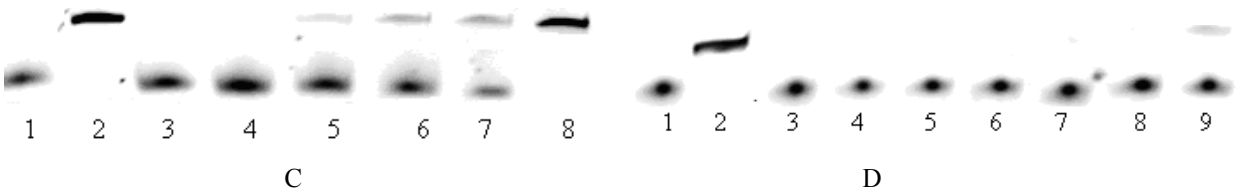
Fig. S5 The DFT geometry optimization of compound **1** and compound **4**. Molecules rendered as stick style and carbon atoms are colored in gray, nitrogens are in blue, oxygens are in red. Hydrogens are omitted for clarity.

Exonuclease I hydrolysis assay.

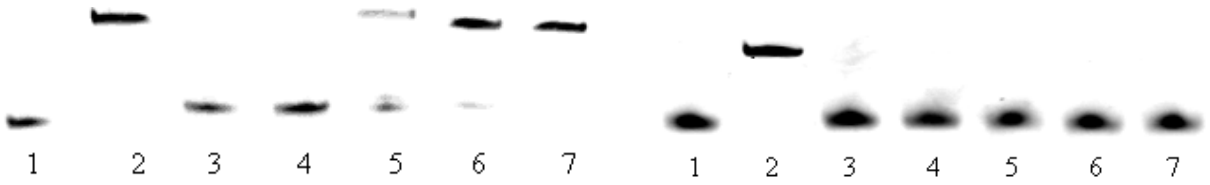
The TAMRA-5'-end-labeled oligonucleotides, T24G21 and T24RG21, were used as substrates. Exonuclease I hydrolysis experiment was carried out as described previously.[8]



(A):Lane 1: T24G21 treated with exonuclease I; Lane 2: T24G21 control; Lanes 3-7: T24G21 treated with 0.1 μM, 1 μM, 10μM, 20μM, 25 μM of compound **1**; (B) Hydrolysis of T24RG21 by exonuclease I of compound **1**. Lane 1: T24RG21 treated with exonuclease I; Lane 2: T24RG21 control; Lanes 3-8: T24RG21 treated with 0.1 μM, 1 μM, 10μM, 20μM, 25 μM, 50 μM of compound **1**.



(C):Lane 1: T24G21 treated with exonuclease I; Lane 2: T24G21 control; Lanes 3-8: T24G21 treated with 0.1 μM, 1 μM, 10μM, 20μM, 50 μM, 75 μM of compound **2**; (D) Hydrolysis of T24RG21 by exonuclease I of compound **2**. Lane 1: T24RG21 treated with exonuclease I; Lane 2: T24RG21 control; Lanes 3-9: T24RG21 treated with 0.1 μM, 1 μM, 5μM, 10μM, 25 μM, 50 μM, 100 μM of compound **2**.



E F

(E):Lane 1: T24G21 treated with exonuclease I; Lane 2: T24G21 control; Lanes 3-7: T24G21 treated with 0.1 μM, 1 μM, 10μM, 25 μM, 50 μM of compound **3**; (F) Hydrolysis of T24RG21 by exonuclease I of compound **3**. Lane 1: T24RG21 treated with exonuclease I; Lane 2: T24RG21 control; Lanes 3-7: T24RG21 treated with 0.1 μM, 1 μM, 10μM, 25 μM, 50 μM of compound **3**.

G H

(G):Lane 1: T24G21 treated with exonuclease I; Lane 2: T24G21 control; Lanes 3-8: T24G21 treated with 0.1 μM, 1 μM, 10μM, 20μM, 25 μM, 50 μM of compound **4**; (D) Hydrolysis of T24RG21 by exonuclease I of compound **4**. Lane 1: T24RG21 treated with exonuclease I; Lane 2: T24RG21 control; Lanes 3-8: T24RG21 treated with 0.1 μM, 1 μM, 5μM, 10μM, 25 μM, and 50 μM of compound **4**.

Fig. S6. Hydrolysis of T24G21 and T24RG21 by exonuclease I of compounds.

TRAP–LIG assay

The telomerase protein was extracted from exponentially growing gastric carcinoma cell line SGC7901. The cells lysis buffer contains 0.5% Chaps, 10 mM Tris–HCl [pH 7.5], 1 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 5 mM β-mercaptoethanol, 10 U/ml RNasin and 0.1 mM PMSF.

There were three steps in TRAP–LIG procedure according to the report[18]: After elongation of initial primer TS (5'-AAT CCG TCG AGC AGA GTT-3') for 30 min at 30 °C, the telomerase was inactivated at 94 °C for 5 min. 50μL telomerase extension products were purified by QIA quick nucleotide purification kit (Qiagen). The purified elongated products, removal of compounds, was used as template for PCR step, following 35 cycles of 94 °C for 30 s, of 61 °C for 1 min, and of 72 °C for 1 min. The PCR products were analyzed by 12% nondenaturing polyacrylamide gel, stained by EB. The gel was recorded and quantitated on Chemilmager 5500. The quantitation graphs were fitted to dose–response curves using the GraphPad Prism 4 software package.

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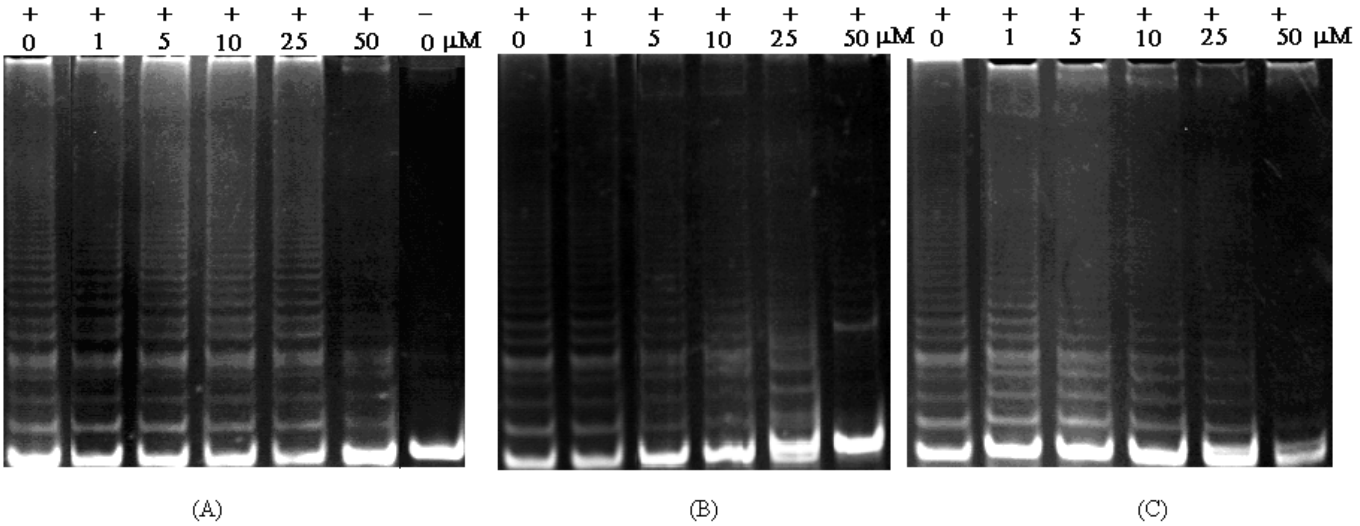

Figure S7. Inhibitory activity of compound **1** (A), compound **2** (B) and compound **3** (C) on telomerase. Increasing concentrations of these compounds (1-25μM) were added in a TRAP-LIG assay. + indicate telomerase extracts were added, - indicate without telomerase extracts.

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