Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Benzothiazole-substituted benzofuroquinolinium dye: a selective switch-on fluorescent probe for G-quadruplex

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1. Materials

Calf thymus DNA (Ct-DNA) and Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich (Singapore). The oligonucleotides used in the fluorescence titration (listed in Table S1) were purchased from Sigma-Aldrich or Sangon (China), which had been purified by PAGE (purity 95%). The oligonucleotide Pu24I used in the NMR experiments (listed in Table S1) were purchased from Sigma-Aldrich, purified by HPLC and used without further purification.

Table S1 Sequences of oligonucleotides used in the present study

Name	sequence	description
Telo21	5'-GGG[TTAGGG]$_3$-3'	sequence from telomere DNA
c-myc	5'-TGAG$_3$TG$_3$TAG$_3$TG$_3$TA$_2$-3'	sequence from the promoter of oncogene c-myc
NT6960	5'-G$_3$TG$_2$CG$_7$AGAG$_6$-3	sequence from the nontemplate strand of human ribosomal DNA
ds26	5'-CA$_2$TCG$_2$ATCGA$_2$T$_2$CGATC$_2$GAT$_2$G-3'	self-hybridization duplex DNA

Pu24I	5'-$TGAG_3TG_2IGAG_3TG_4A_2G_2$-3'	sequence derived from the promoter of oncogene *c-myc*
G-quadruplex RNA	5′-$G_3AG_4CG_3UCUG_3$-3′	sequence from the 5′ untranslated regions (UTRs) of mRNAs
dsRNA	5'-$CA_2UCG_2AUCGA_2U_2CGAUC_2GAU_2G$-3'	self-hybridization duplex RNA
ssRNA	5'-$AUACGAUGCU_3ACGGUGCUAU_4G$-3'	single-stranded RNA identified as the JMJD6 substrate

Table S2 Sequences of labeled oligonucleotides used in the FRET-melting assay

Name	sequence	description
Telomere	5'-*FAM*-d(GGG[TTAGGG]$_3$)-*TAMRA*-3'	FAM: 6-carboxyfluorescein TAMRA: 6-carboxy-tetramethylrhodamine
c-myc	5'-*FAM*-d($TGAG_3TG_3TAG_3TG_3TA_2$)-*TAMRA*-3'	
Duplex	5'-*FAM*-dTATAGCTATA-HEG-TATAGCTATA-*TAMRA*-3'	self-hybridization duplex DNA, HEG:hexa(ethylene glycol) linker

2. Preparation of (Z)-5-methyl-11-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)benzofuro[3,2-b]quinolin-5-ium iodide (**1)**

General: ^{1}H NMR and NOESY experiments were performed on a Bruker AvanceIII 600 MHz NMR spectrometer. ^{13}C NMR spectra were recorded on a Bruker DPX-400 MHz NMR spectrometer. Mass spectra were recorded on a VG ZAB-HS (fast atom bombardment) mass spectrometer. High-resolution mass spectra were obtained with a MAT95XP (Thermo) mass spectrometer. Melting point was determined on a Boetius PHMK 05 melting point apparatus. The purity of product was determined by HPLC. The purity was determined on a Shimadzu LC-20AT HPLC system using a ZORBAX SB-C18 analytical column (150 mm × 4.6 mm, 5 μm) in linear gradient solvent mode. The solvent system was acetonitrile/methanol 10: 90 to 40:60 over 30 min at a flow rate of 1 mL/min. Peaks were detected by UV absorption using a diode array detector. All chemicals were purchased from Aldrich or Acros Organic. All the solvents were of analytical reagent grade and were used without further purification.

Synthesis: **1** was synthesized by literature reported procedures for similar compounds[1]. 11-Iodo-5-*N*-methyl-benzofuro[3,2-*b*]quinolinium iodide[2] (0.487 g, 0.001 mol) and *N*-methyl-2-methylbenzothiazolium iodide (0.291 g, 0.001 mol) were dissolved in 80 ml methanol. Sodium bicarbonate (0.18 g, 0.002 mol) dissolved in 5 ml water, was added and stirred at room temperature for 30 min. The reaction mixture was then refluxed for 30 min. After cooling, the content in the flask was poured into 250 mL water with stirring. The precipitate was filtered, washed first with ethanol then with water and air-dried. The resulting precipitate was purified by column chromatography with

dichloromethane/methanol (95: 5) elution to afford 0.454 g of **1** (yield 84%, purity >95%): mp 292°C; ^{1}H NMR (DMSO-d_6, 600 Hz): δ 8.812 (d, J = 7.8 Hz, 1H) 8.650 (d, J = 7.8 Hz, 1H), 8.382 (d, J = 8.4 Hz, 1H), 8.052 (br, 1H), 8.030 (d, J = 8.4 Hz, 1H), 7.936 (m, 2H), 7.774 (br, 1H), 7.732 (d, J = 8.4 Hz, 1H), 7.676 (t, J = 8.0 Hz, 1H), 7.548 (t, J = 7.2 Hz, 1H), 7.390 (t, J = 7.2 Hz, 1H), 6.857 (s, 1H), 4.618 (s, 3H), 4.017 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 Hz): δ 161.9, 156.7, 141.6, 139.5, 138.0, 136.5, 134.6, 133.6, 133.2, 128.4, 127.1, 126.5, 125.9, 125.5, 125.4, 124.9, 123.3, 123.1, 118.4, 118.1, 113.8, 113.4, 85.4, 38.7, 34.6. NOESY experiments showed a correlation between the alkene proton at 6.857 ppm and the *N*-methyl protons at 4.017 ppm of thiazole, thus giving the *E* configuration. FAB-MS *m/z*: 395 [M - I]$^{+}$. FAB-HRMS *m/z*: calcd for $C_{25}H_{19}N_2OS$ [M - I]$^{+}$ 395.1213, found 395.1206.

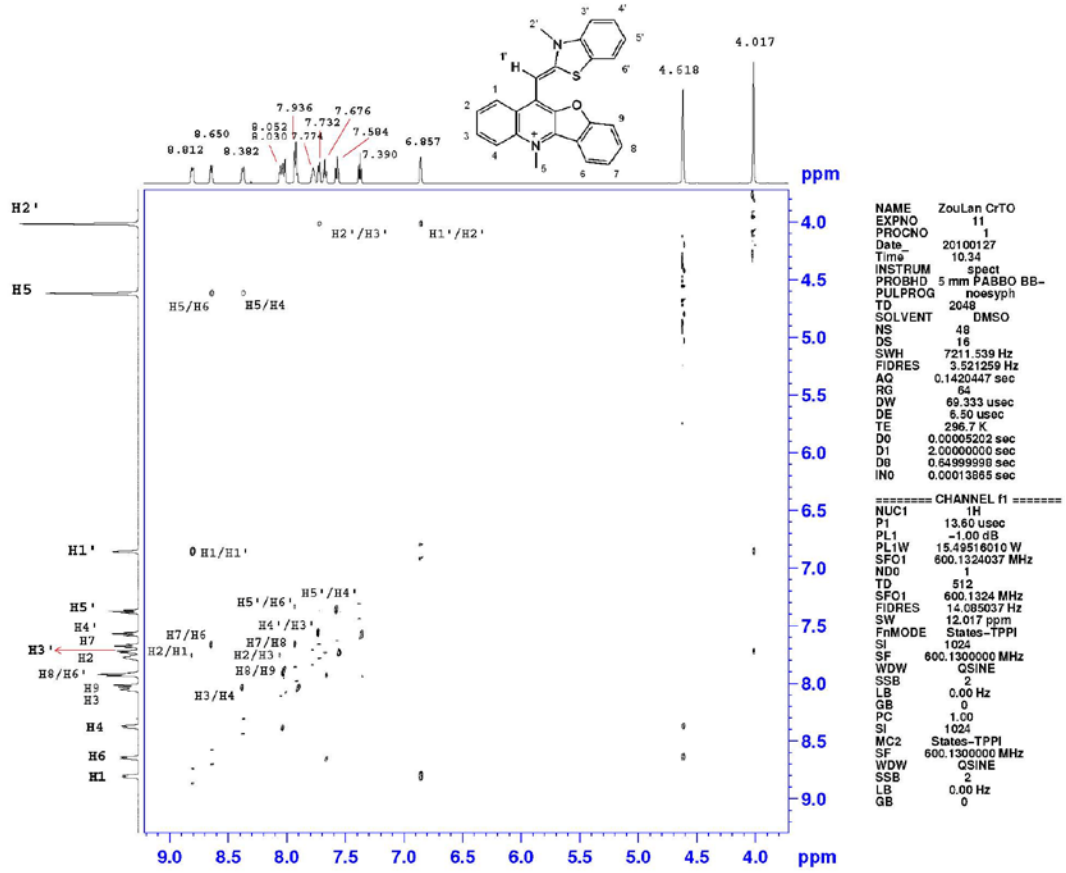


Fig. S1 NOESY spectrum of **1**

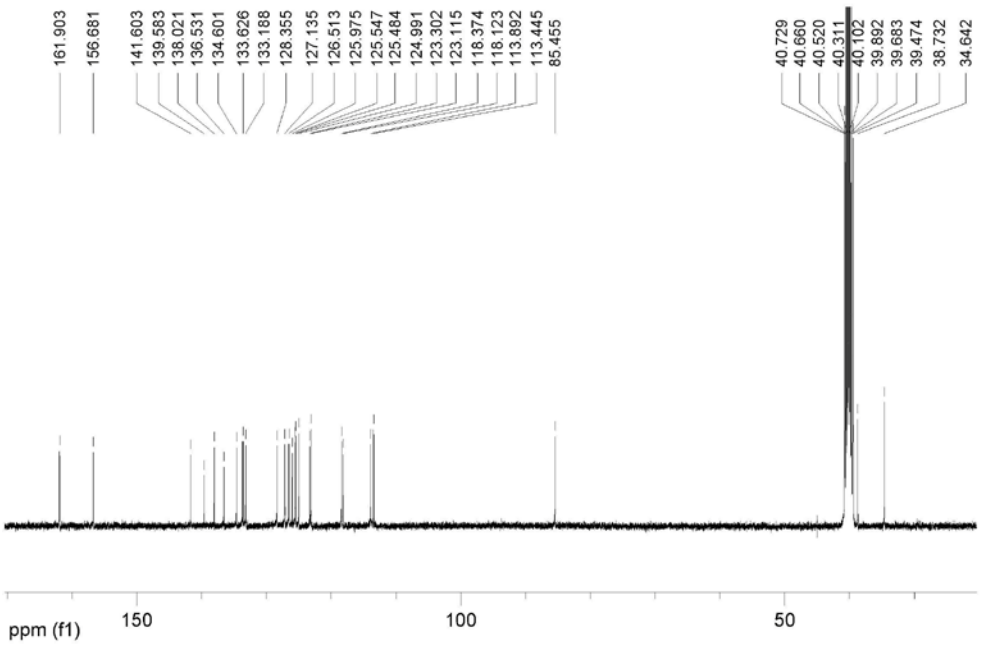


Fig. S2 ^{13}C NMR spectrum of **1**

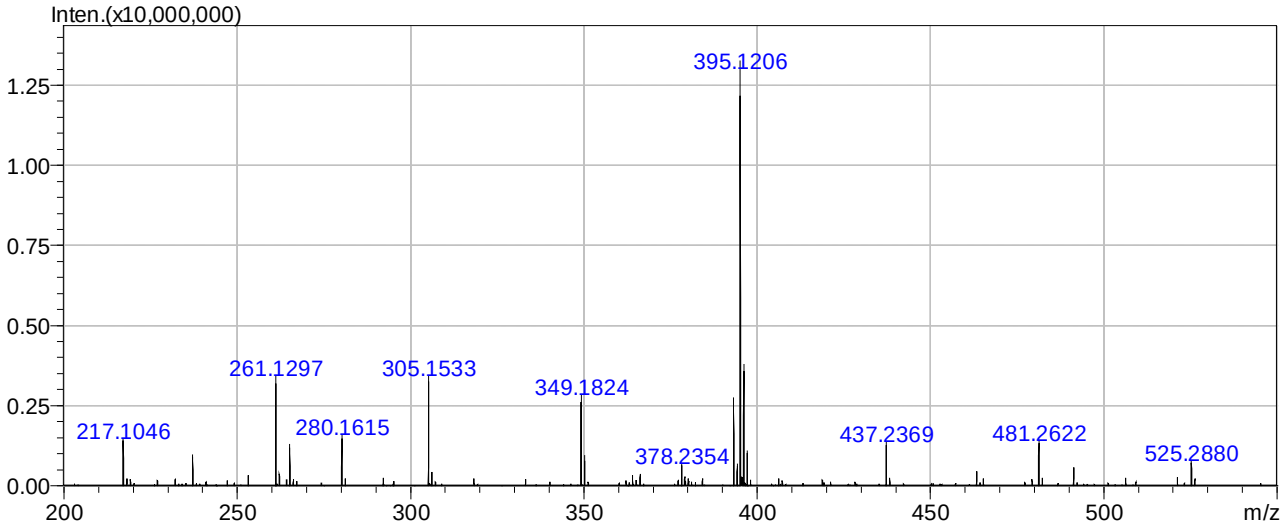
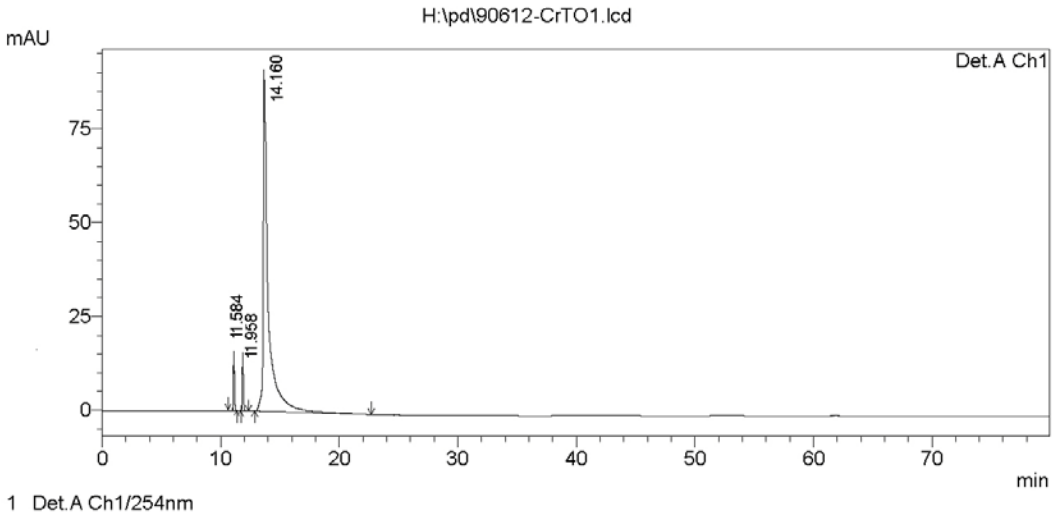


Fig. S3 HRMS spectrum of **1**

<Chromatogram>



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %
1	11.584	62149	17456	2.221
2	11.958	175282	85675	2.424
3	14.160	2868265	91027	95.355
Total		3105696	194518	100.000

Fig. S4 HPLC analysis of **1**

3. Photophysical properties of **1**

All UV-visible spectra were obtained with a LAMBDA 35 UV/vis spectrometer (Perkin Elmer) or a Cary 4000 UV/vis spectrophotometer. Fluorescence emission spectra were obtained with a FluoroLog-3 spectrofluorometer (HORIBA Jobin Yvon), and all samples were excited at the isosbestic point between the corresponding absorbance spectra. The fluorescence quantum yields (Φ_f) of the DNA-bound dye were determined relative to rhodamine 101 in methanol (Φ_f = 0.99) as a reference.

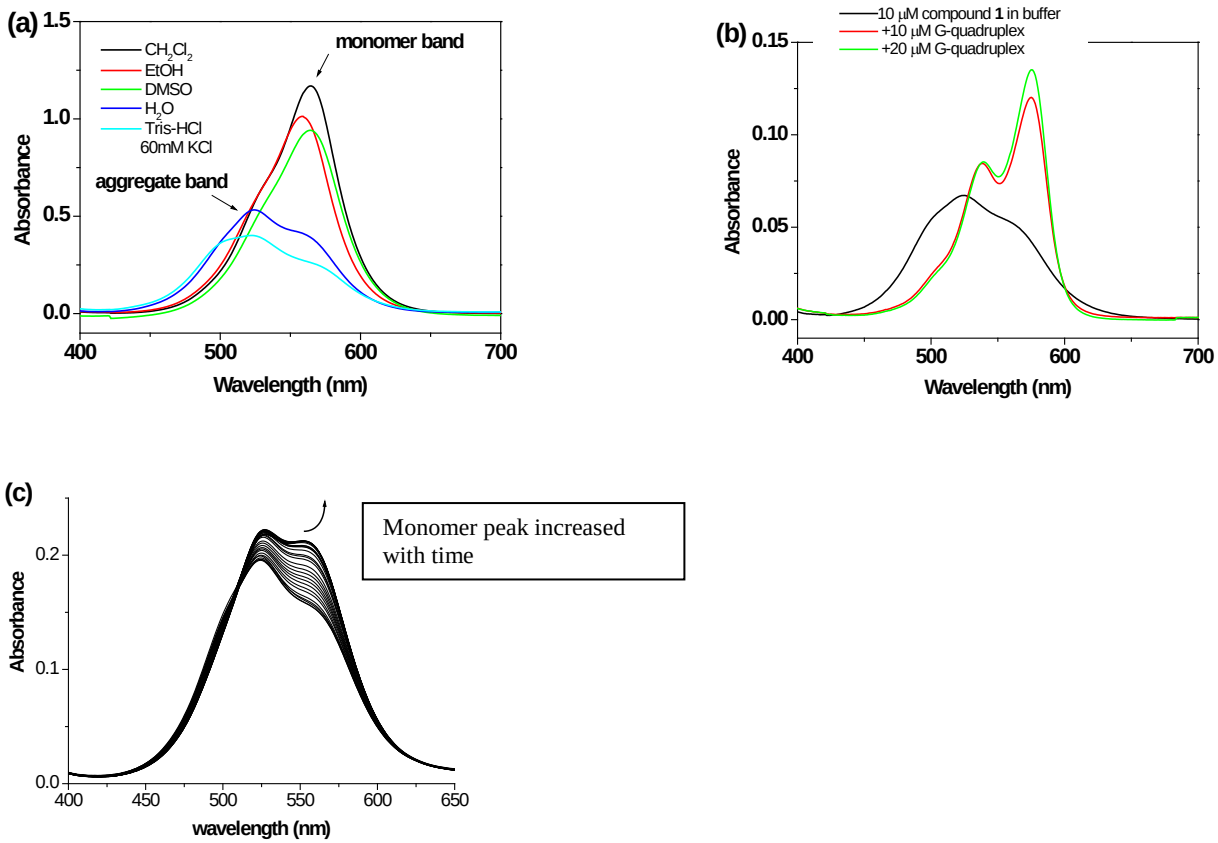


Fig. S5 (a) UV-vis spectra of 25 μM compound **1** in CH_2Cl_2 (black curve), EtOH (red curve), DMSO (green curve), water (blue curve), and 10 mM Tris-HCl buffer containing 60 mM KCl (cyan curve); (b) UV-vis spectra of 10 μM compound **1** in the presence of G-quadruplex DNA in 10 mM Tris- HCl buffer containing 60 mM KCl; (c) UV-vis spectra of 15 μM compound **1** in Tris-HCl buffer following 20-fold dilution with buffer showing its disaggregation as a function of time over 5 hours; the spectra were recorded at 15 min intervals.[3]

Table S3 Photophysical properties of **1**

	λ_{max} (nm)		Φ_f
	absorption	emission [a]	
H_2O	530	570	0.0012
ethanol	560	N. D.[b]	N. D.[b]
85% glycerol	563	580	0.0173
telo21	576	589	0.2466
c-myc	576	587	0.1839

[a] Emission determined by excitation at 527 nm. [b] Value not determinable.

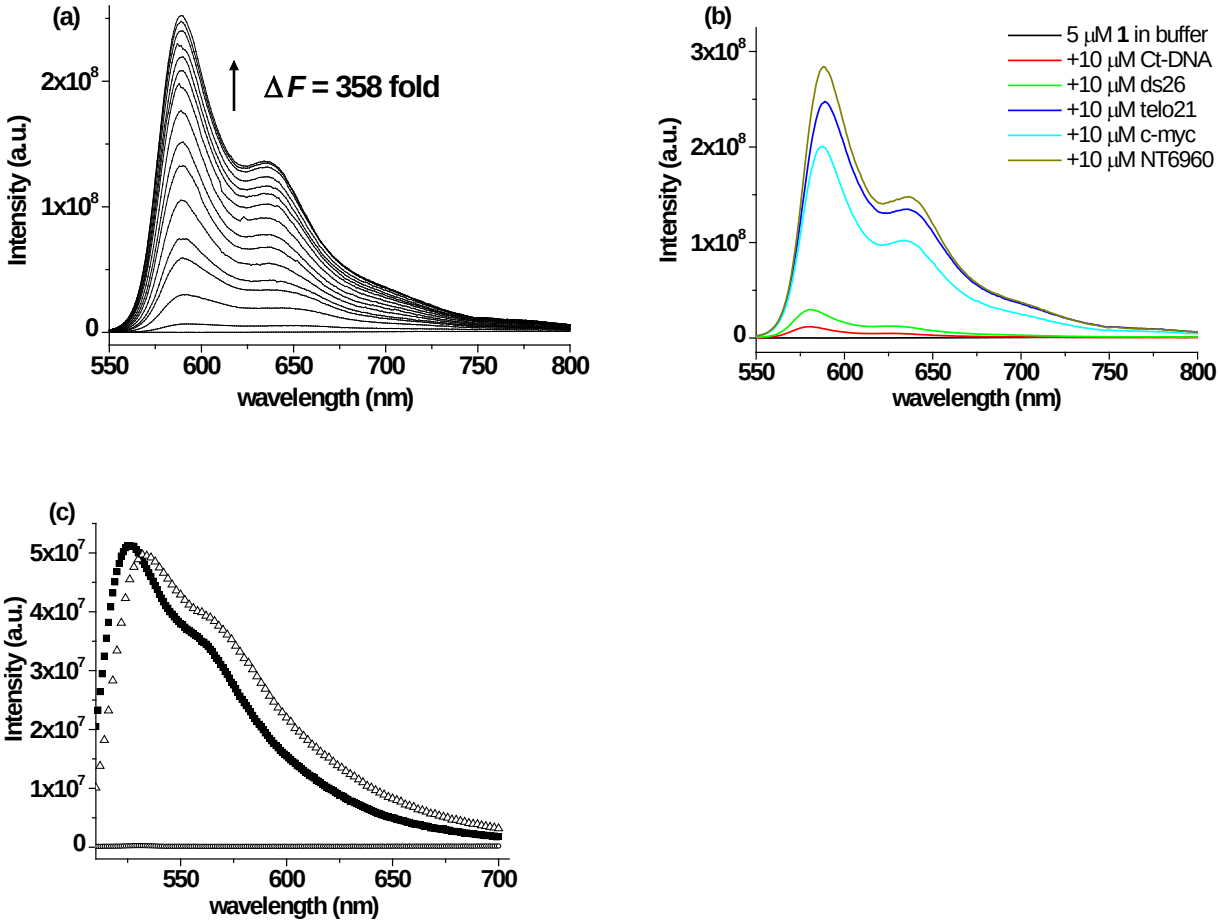
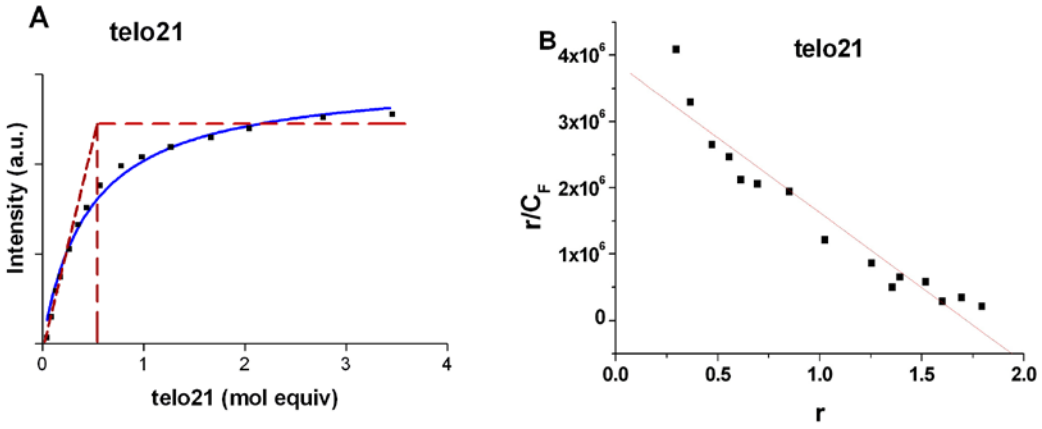


Fig. S6 (a) Fluorescence titration of **1** (5 μM in Tris-HCl buffer, 60 mM KCl, λ_{ex} = 527 nm) upon addition of increasing amounts (0-12 μM) of telomere G-quadruplex (telo21). (b) Fluorescence spectra (λ_{ex} = 527nm) of **1** (5 μM) in the absence (black curve) or presence of 2 equivalents of Ct-DNA (red curve), ds26 (green curve), telo21 (blue curve), c-myc (cyan curve), or NT6960 (brown curve). (c) Fluorescence spectra of thiazole orange (5 μM in Tris-HCl buffer, 60 mM KCl, λ_{ex} = 490 nm) in the absence (○) or presence of 2 equivalents of ds26 (■) or telo21 (Δ).



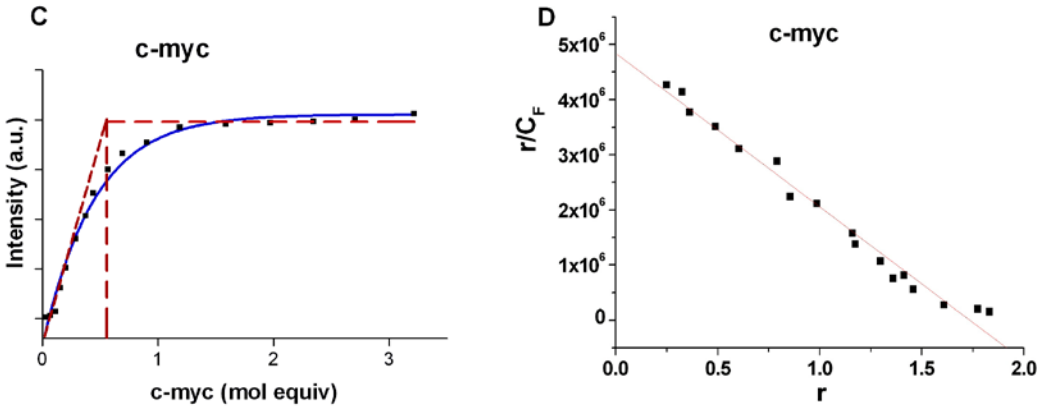
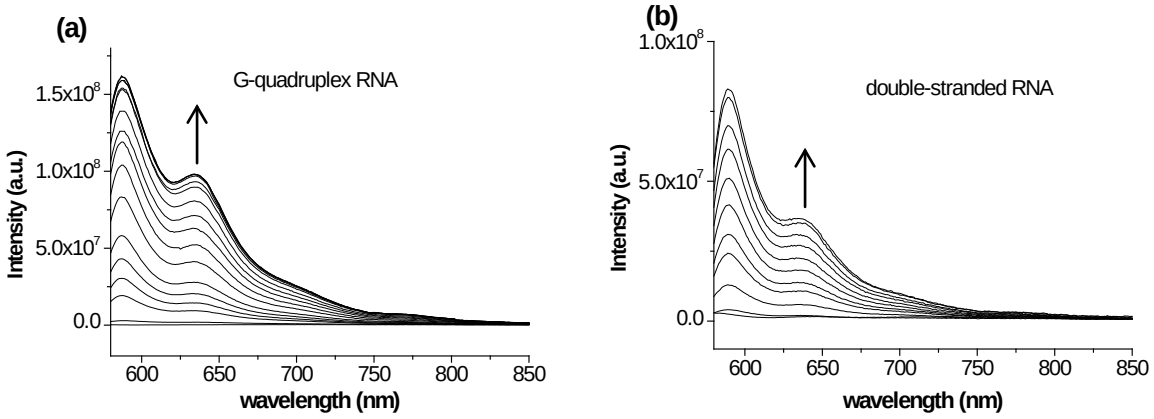


Fig. S7 Enhancement of the fluorescence intensity (λ_{ex} = 527nm) of **1** (5 μM) upon addition of increasing amounts of telomere G-quadruplex telo21 (A) and oncogene promoter G-quadruplex DNA c-myc (C). Scatchard analysis of the fluorescence titration data of telo21 (B) and c-myc (D). The binding stoichiometry has been determined to be 2:1 (ligand: DNA).

4. Fluorescence titration with RNA

The change in fluorescence intensity of **1** with increasing amount of G-quadruplex RNA, double-stranded RNA, single-stranded RNA (see Table S1), and total RNA from cell lysate were recorded.



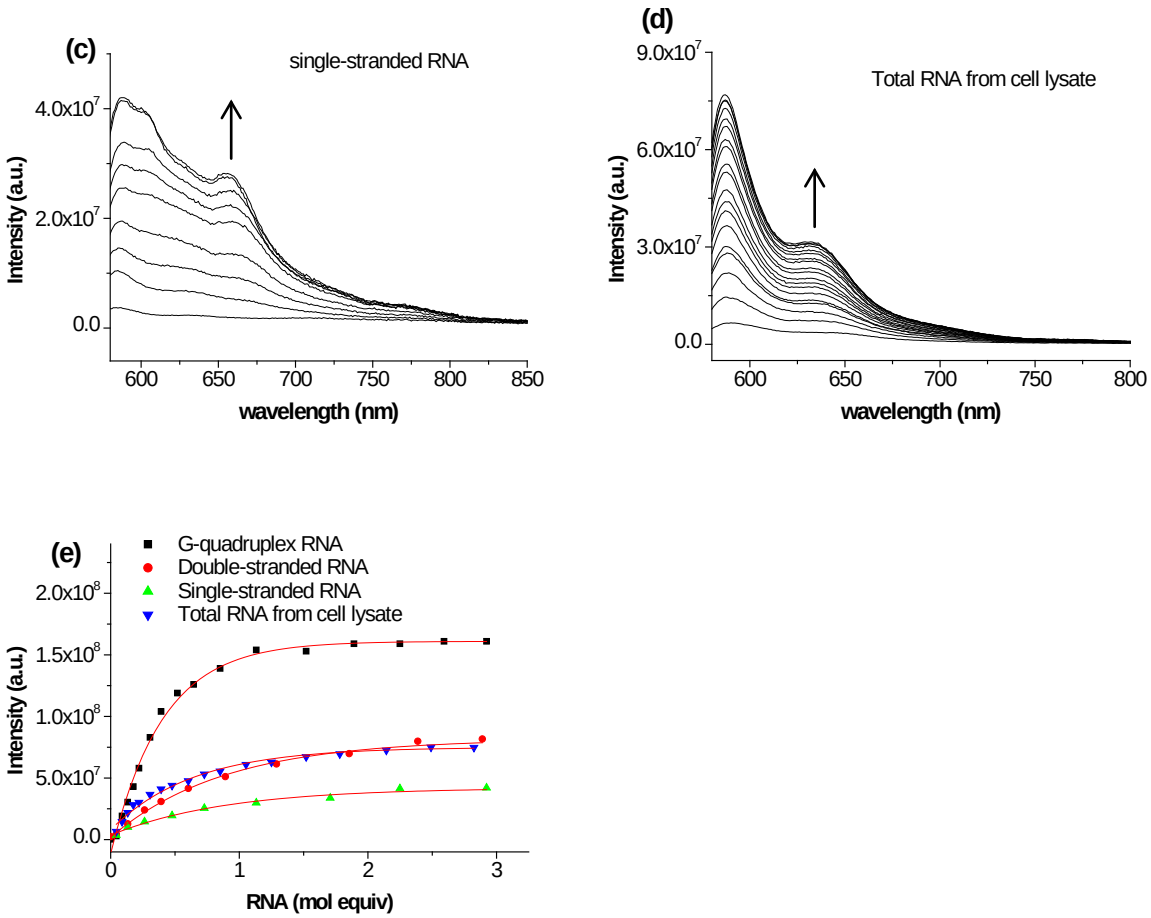


Fig. S8 Fluorescence titration of **1** (5 μM in Tris-HCl buffer, 60 mM KCl, λ_{ex} = 527 nm) upon addition of increasing amount of G-quadruplex RNA (a), double-stranded RNA (b), single-stranded RNA (c), and total RNA from cell lysate (d); (e) shows the plots of fluorescence intensity at 588nm versus the amount of RNA added.

5. FRET-melting assays[4]

FRET measurements were performed using a BIORAD iQ™ 5 multicolor real-time PCR detection system (Bio-Rad laboratories, Hercules, CA) with excitation at 475–495 nm and emission at 515–545 nm. Fluorescence readings were taken at 1 ºC intervals over the temperature range 20–95ºC, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value.

DNA was prepared as a 20 μM stock solution. All dilutions were carried out with 10 mM sodium cacodylate buffer (pH 7.2) containing 0.1 M LiCl. The ability of compound **1** to stabilize G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) assay modified to be used in a 96-well format. The labeled oligonucleotide telomere, c-myc and duplex (Table S2) were diluted from the stock solution to 400 nM with sodium cacodylate buffer and then annealed by heating to 92ºC for 5 min, followed by cooling to room temperature. The PCR

tube strips (BIO-RAD) were prepared by aliquoting 10 μL of the annealed DNA into each well, followed by 10 μL of a solution of compound **1**. Final analysis of the data was carried out using Microcal Origin 6.0. Emission of *FAM* was normalized between 0 and 1, and $T_{1/2}$ was defined as the temperature for which the normalized emission is 0.5. $\Delta T_{1/2}$ values are the mean of three experiments.

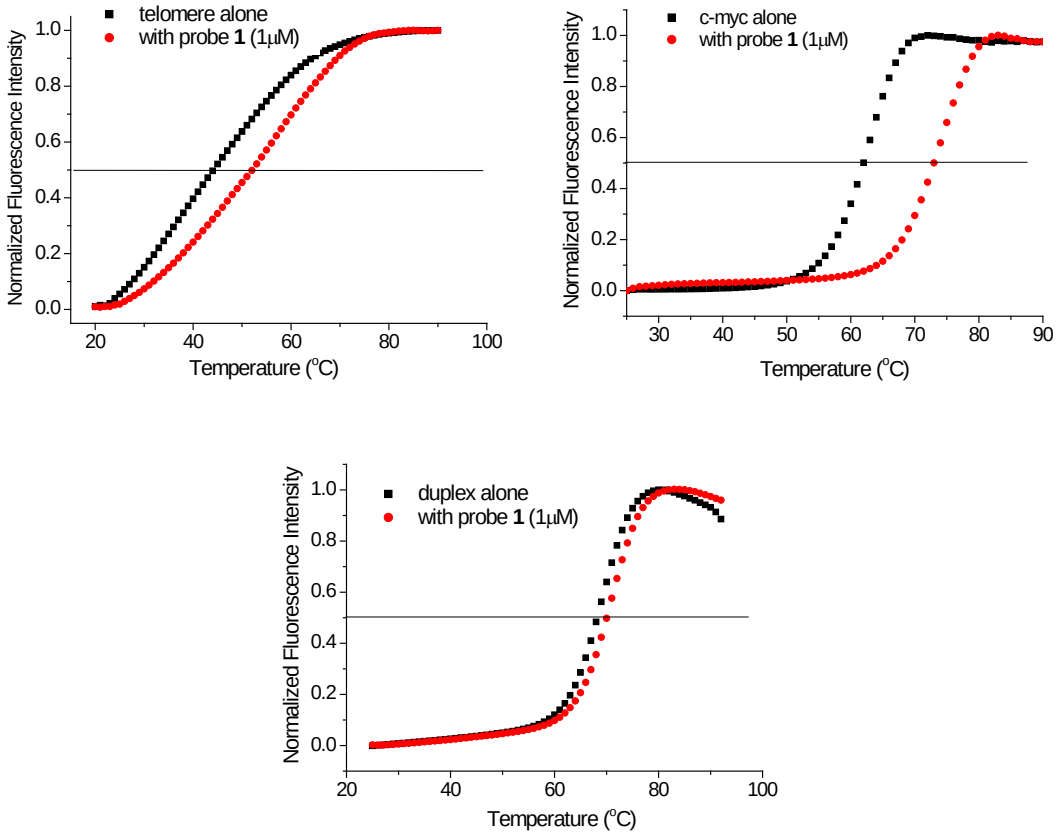


Table S4 Stabilization of DNA G-quadruplexes and duplex by **1** as determined by FRET melting assays[a]

	ΔT_m at 1 μM ligand concentration (°C)		
Ligand	Telomere	c-myc	Duplex
1	7.9	11.5	1.0

[a] Obtained in a 10 mM sodium cacodylate buffer, pH 7.2, containing 0.1 M LiCl.

6. Gel electrophoresis stained by **1**

Different concentrations (0.5, 1.0, 2.0 and 5.0 μM) of DNA samples (both duplex- (ds26) and quadruplex-DNA (c-myc) were loaded onto a 16% bisacrylamide gel in 1X TBE buffer and were electrophoresed at 20°C. Oligonucleotides were stained by **1** (2 μM, 15 mins), and by conventional staining agent GelRed (2 μM, 15 mins). DNA fragments were visualized under UV light and photographed using a ChemiDoc System (Bio-Rad Laboratories).

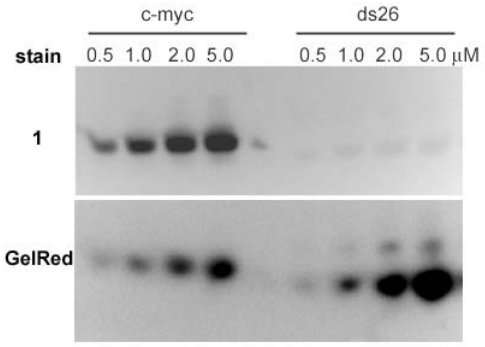


Fig. S9 Gel electrophresis (16% arcylamide in 1×TBE) of c-myc G-quadruplex (left lanes) and duplex DNA ds26 (right lanes) at the concentration from 0.5 to 5.0 μM stained by **1** (top) and GelRed (bottom).

7. Cell culture and microscopy

7.1 Fixed cell staining

The MCF 7 cell line was purchased from ATCC and grown in Dulbecco's modified Eagle's media (DMEM, Gibco) with 10% fetal bovine serum (Gibco), 1% penicillin/streptomycin (Invitrogen) at 37°C with 5% CO_2 atmosphere. Cells were seeded in Lab-Tek chambered coverglass slide (Nunc) and grew overnight. Cells were fixed with 4% paraformaldehyde in PBS, stained with 2 μM of **1** in PBS for 15 minutes at room temperature. The cells were subsequently stained with 2 μM DAPI for 20 minutes at room temperature. The cells stained with **1** and DAPI were imaged with a Leica DMRB fluorescence microscope, equipped with a UV filter for observation of DAPI signal and a green fluorescent protein/fluorescein isothiocyanate filter.

7.2 Living cell staining

The MCF 7 cell line was grown in DMEM media with 10% fetal bovine serum, 1% penicillin/streptomycin at 37°C with 5% CO_2 atmosphere. Cells were seeded in Lab-Tek chambered coverglass slide (Nunc) and grew overnight. Cells were incubated with 2 μM of **1** in media for 15 minutes at room temperature and imaged directly without washing with a Leica DMRB fluorescence microscope, equipped with a UV filter for observation of a green fluorescent protein/fluorescein isothiocyanate filter.

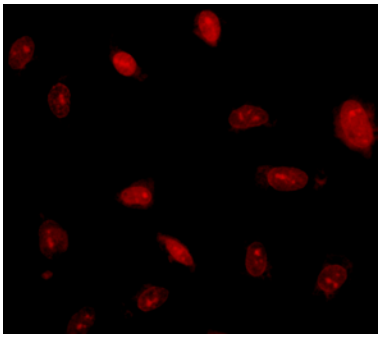


Fig. S10 Fluorescence microscopic images of living MCF7 cells incubated with **1** (2 μM) for 15 mins.

8. NMR experiments

^{1}H NMR experiments were performed with a Bruker AV 600 spectrometer. Acquisition conditions for a ^{1}H NMR spectrum were 45^o pulse length, 2.0 s relaxation delay, 16K data point, 16-32 transients, and PU24I in 90% H_2O/10% D_2O with 150 mM KCl, 25 mM KH_2PO4, 1 mM EDTA (pH 7.0). Aliquots of stock solutions of **1** were titrated directly into the DNA solution inside an NMR tube. Spectra were recorded at 300K utilizing a standard jump-return pulse sequence for water suppression with a relaxation delay of 2 s.

An intramolecular quadruplex Pu24I derived from c-myc was chosen for this study, since it has clearly assigned G-tetrad imino proton signals[5]. Upon addition of **1** to a solution of Pu24I (1: 1 mole ratio), the imino proton resonances of the 5' terminal G-tetrad residues (G4, G8, G13 and G17) shift downfield slightly. These results reveal that **1** is likely to interact with G-quadruplex DNA by the end-stacking mode.

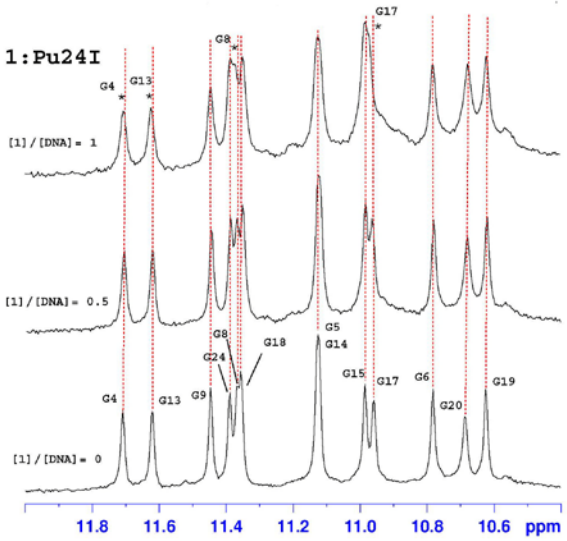


Fig. S11 NMR titration of G-quadruplex Pu24I with **1** at various ratios of [**1**]/[Pu24I]. The imino proton resonances of the residues in the G-tetrad were assigned based on the data from literature.[5]

9. Molecular modeling

The NMR structure of intramolecular quadruplex Pu24I (PDB 2A5R)[5] and the crystal structure of the 6-mer duplex (PDB 3FT6) were used as initial modeling templates to probe the binding modes of **1** with G-quadruplex or duplex DNA. Molecular docking was performed with the ICM-Pro 3.6-1f program (Molsoft)[6]. Before starting a docking stimulation, **1** was assigned ECEPP/3 potential, mmff partial charges and energy-minimized, and the G-quadruplex or duplex structure was converted into an ICM object. All water molecules, metal ions and the original ligand were removed from the PDB file. All atom types, hydrogen and missing heavy atoms were added to the receptor structure. According to the ICM local energy minimization method, the molecular conformation was represented by the internal coordinate variables. Energy calculations were based on a modified version of the ECEPP/3 force field with a distance-dependent dielectric function. Docking was conducted by a biased probability Monte Carlo (BPMC) minimization procedure. During the docking stimulation, grid energy, continuum electrostatic and entropy terms were used to

evaluate the binding energy of **1** with the DNA structure and the internal conformational energy of **1** in order to find the most favorable orientation of **1**. Each independent docking was repeated 3 times. The resulting complexes between **1** and DNA were rapidly refined with both ligand and receptor side chains flexible and the binding energies were computed.

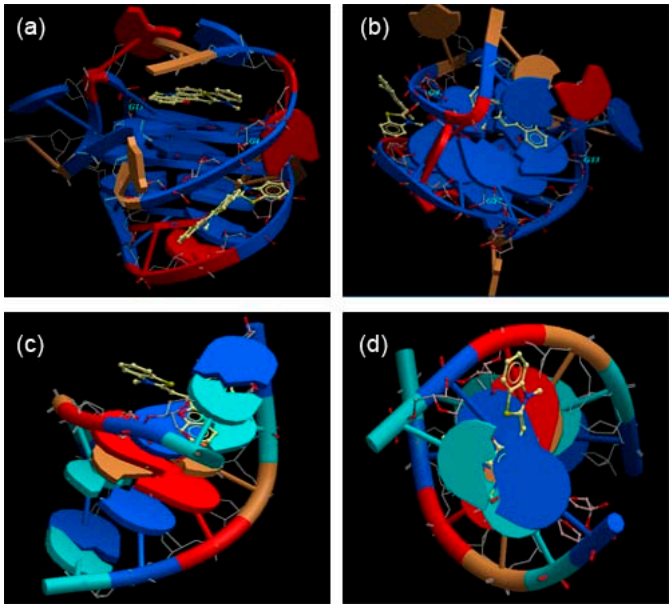


Fig. S12 Molecular models showing the interaction of ligand **1** with G-quadruplex DNA Pu24I and duplex DNA: side view (a) and top view (b) of **1** with Pu24I via multiple binding modes in a 2:1 stoichiometry; side view (c) and top view (d) of **1** with duplex DNA.

Notes and references

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