† Electronic Supplementary Information (ESI)

Colorimetric and Fluorescent detection of G-quadruplex Nucleic Acids with a Coumarin-benzothiazole Probe

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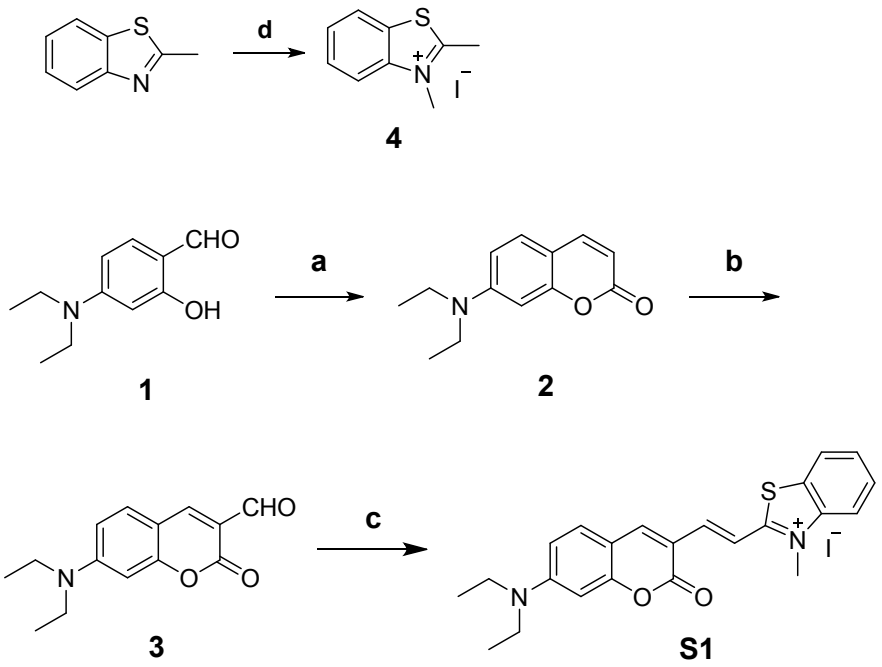
Table of Contents:

1. Experimental Section

1.1 Synthesis and Characterization

Scheme S1. Synthesis of **S1**[a]



[a] Reagents and conditions: (**a)** (i) $CH_2(COOC_2H_5)_2$, piperidine, reflux; (ii) HCl, AcOH, reflux; (**b)** DMF, $POCl_3$, 55 °C; (**c)** compound **4**, AcOH, AcONa, reflux; (**d)** CH_3I, ethanol, 80 °C.

^1H and ^{13}C NMR spectra were recorded using TMS as the internal standard in DMSO-d_6 or $CDCl_3$ with a Bruker BioSpin GmbH spectrometer at 400 MHz and 100 MHz, respectively. Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A instrument with an ESI or ACPI mass selective detector and high resolution mass spectra (HRMS) on Shimadzu LCMSIT-TOF. Melting points (mp) were determined using a SRS OptiMelt automated melting point instrument without correction. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purity of synthesized compound was confirmed to be higher than 95% by using analytical HPLC performed with a dual pump Shimadzu LC-20 AB system equipped with a Ultimate XB-C18 column (4.6 × 250 mm, 5 μm) and eluted with methanol-water (60 : 40) containing 0.1% TFA at a flow rate of 1mL/min. All chemicals were purchased from commercial sources unless otherwise specified. All the solvents were of analytical reagent grade and were used without further purification.

Synthesis of 2, 3-Dimethylbenzothiazolium iodide (4) A solution of 2-methylbenzothiazole (8.95 g, 60.0 mmol) in anhydrous ethanol (20 mL) was treated with iodomethane (20.52 g, 140 mmol, 2.3 equiv). The reaction mixture was heated at 80 °C for 8 h. After cooling to room temperature, the mixture was filtered, washed with ethanol (3 × 5 mL) and dried under vacuum to afford the product as a white solid (**4**, 11.4 g, 65%): ^1H NMR (400 MHz, DMSO) δ 8.44 (d, J = 8.1 Hz, 1H), 8.29 (d, J = 8.4 Hz, 1H), 7.90 (t, J = 7.8 Hz, 1H), 7.81

(t, J = 7.7 Hz, 1H), 4.20 (s, 3H), 3.17 (s, 3H).

Synthesis of 7-(diethylamino)-2H-chromen-2-one (2) A mixture of 4-diethylaminosalicylaldehyde (2.01 g, 10 mmol), piperidine (1 mL) and diethylmalonate (3.20 g, 20 mmol) in an absolute ethanol (30 mL) was stirred for 6 h at 80 °C. After removing ethanol, concentrated HCl (20 mL) and glacial acetic acid (20 mL) were added to hydrolyze the reaction. Then the solution was stirred for another 6 h. After cooling to room temperature, the solution was poured into ice water and then NaOH solution was added to adjust pH of the solution to ~5. The mixture was filtered, washed with water, dried, then was purified on silica gel chromatography (EtOAc : PE=1 : 10) to give desired compound (***2,*** 0.81 g, yield 37.3%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 9.3 Hz, 1H), 7.24 (d, *J* = 8.8 Hz, 1H), 6.56 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.49 (d, *J* = 1.6 Hz, 1H), 6.03 (d, *J* = 9.3 Hz, 1H), 3.41 (q, *J* = 7.1 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H). ESI-MS m/z: 218.1 $[M+H]^+$.

Synthesis of 7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (3) DMF (1 mL) was added dropwise to $POCl_3$ (1.5 mL) under nitrogen and stirred at room temperature for 20 minutes. Then ***2*** (0.77 g, 3.5 mmol), dissolved in 4 mL DMF was added dropwise to the above solution. After stirring at 60 ºC for 10 h, the mixture was poured into ice water and then NaOH solution was added to modulate the pH of the mixture to ~7. The precipitate was filtered, washed with water and ethanol, dried under vacuum to afford an orange-yellow solid (**3,** 0.50 g, yield 58.3%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.13 (s, 1H), 8.26 (s, 1H), 7.41 (d, *J* = 9.0 Hz, 1H), 6.64 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 3.48 (q, *J* = 7.1 Hz, 4H), 1.26 (t, *J* = 7.1 Hz, 6H). ESI-MS m/z: 246.1 $[M+H]^+$.

(E)-2-(2-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)vinyl)-3-methylbenzo[d]thiazol-3-ium iodide (S1) A mixture of **4** (0.19 g, 0.65 mmol), **3**(0.12 g, 0.49 mmol) and AcOH (4 mL), sodium acetate (0.02 g) was stirred at reflux temperature for 3 h. After cooling to room temperature, the precipitate was filtered. The crude product was purified by flash column chromatography with CH_2OH/CH_2Cl_2(1 : 50) elution to afford a desired compound (**S1**, 0.19 g, yield 74.9%): ^{1}H NMR (400 MHz, DMSO) δ 8.60 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 8.5 Hz, 1H), 8.09-7.97 (m, 2H), 7.84 (t, *J* = 7.8 Hz, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.58 (d, *J* = 9.0 Hz, 1H), 6.88 (d, *J* = 9.5 Hz, 1H), 6.69 (s, 1H), 4.23 (s, 3H), 3.55 (dd, *J* = 12.9, 5.9 Hz, 4H), 1.18 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 171.48, 159.45, 157.10, 153.23, 148.17, 144.19, 141.88, 131.69, 129.21, 127.96, 127.37, 124.06, 116.41, 111.90, 111.16, 110.84, 108.86, 96.39, 44.64, 35.94, 12.41. Purity: 99.3% by HPLC. HRMS (ESI): calcd for $(M-I)^+$ ($C_{23}H_{23}N_2O_2S^+$) 391.1475, found 391.1459.

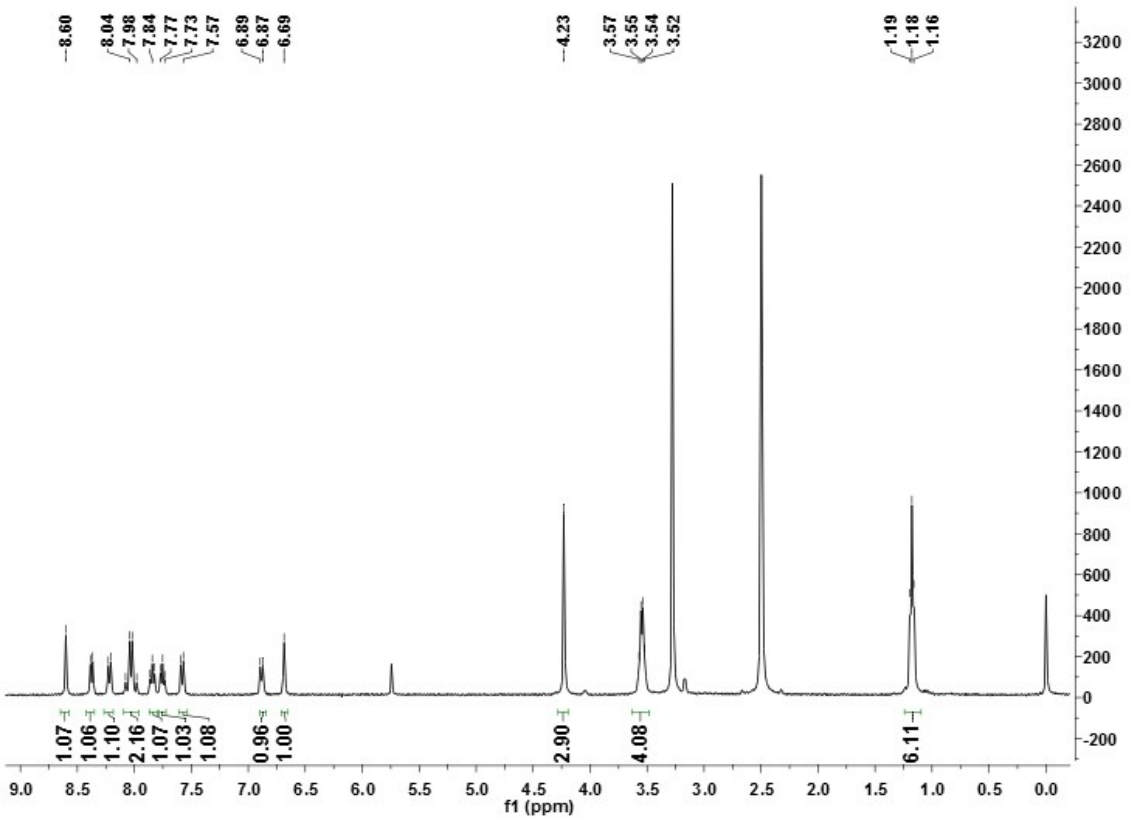


Figure S1. ^{1}H NMR spectrum of **S1**.

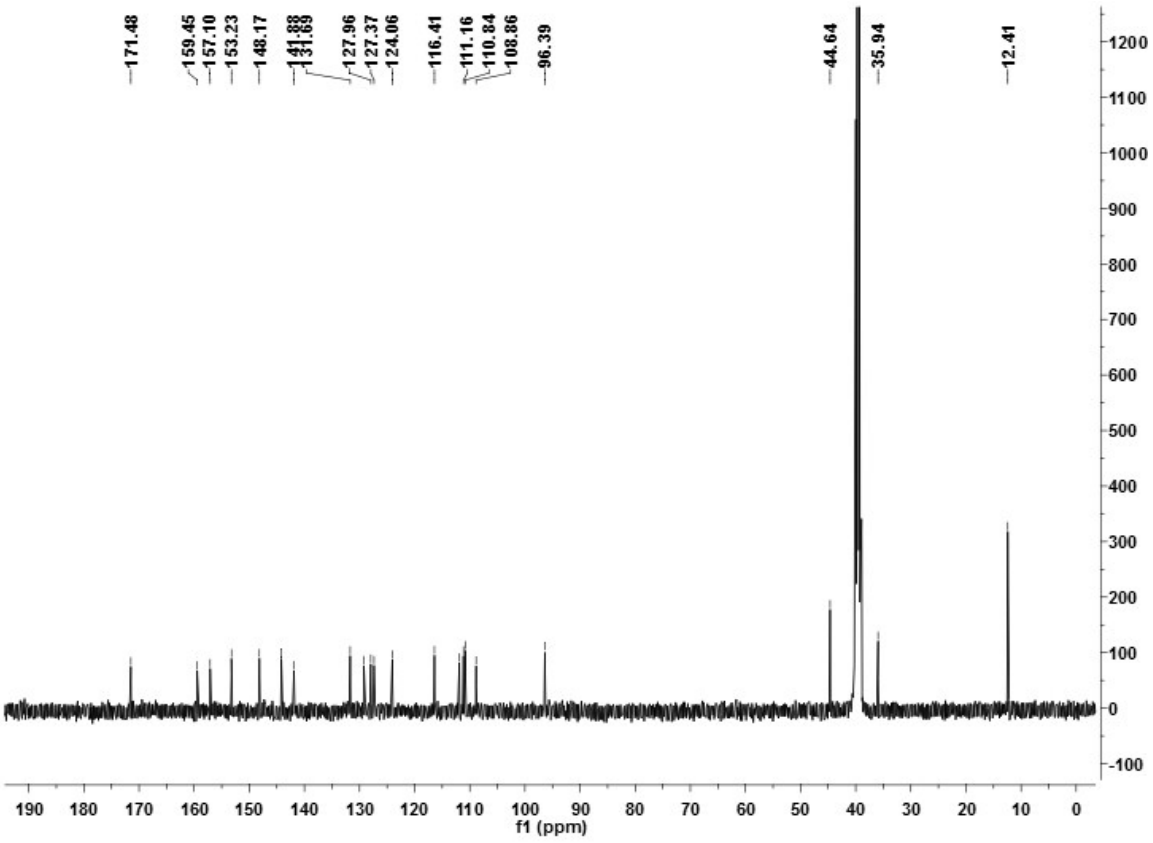


Figure S2. ^{13}C NMR spectrum of **S1**.

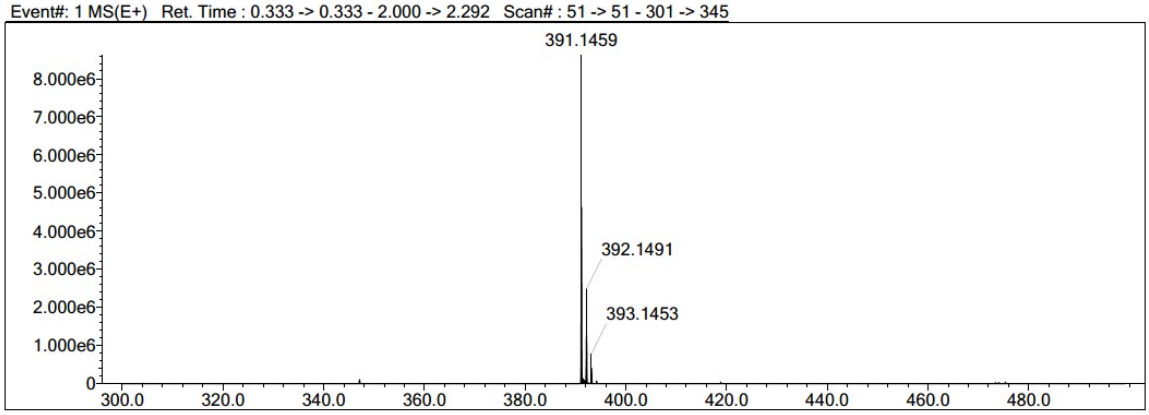
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	30	O	2	0	2	S	2	0	1	Pt	2	0	0	H
B	3	0	0	18O	2	0	0	Cl	1	0	0					Na
C	4	0	25	F	1	0	0	Br	1	0	0					
N	3	0	2	P	3	0	0	I	3	0	0					

Error Margin (ppm): 500
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 90.00

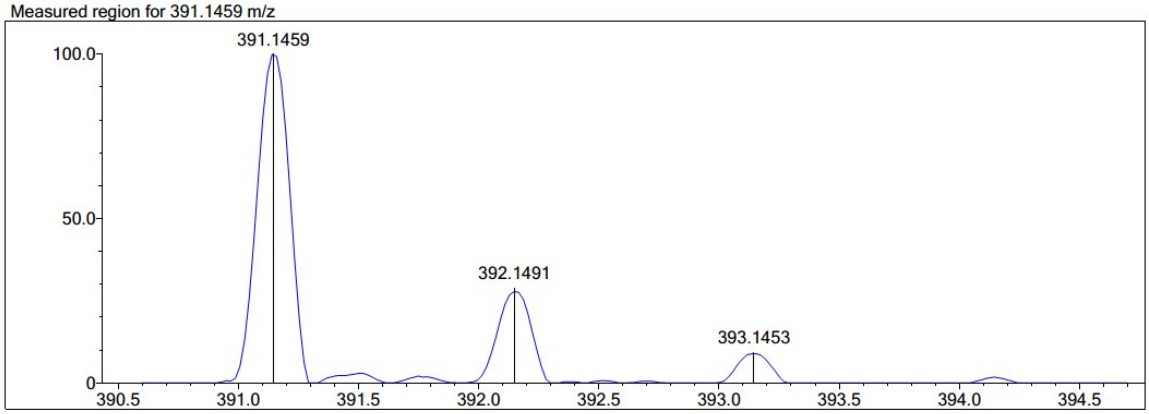
DBE Range: 0.0 - 3000.0
Apply N Rule: yes
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: yes
Isotope Res: 10000
Max Results: 500

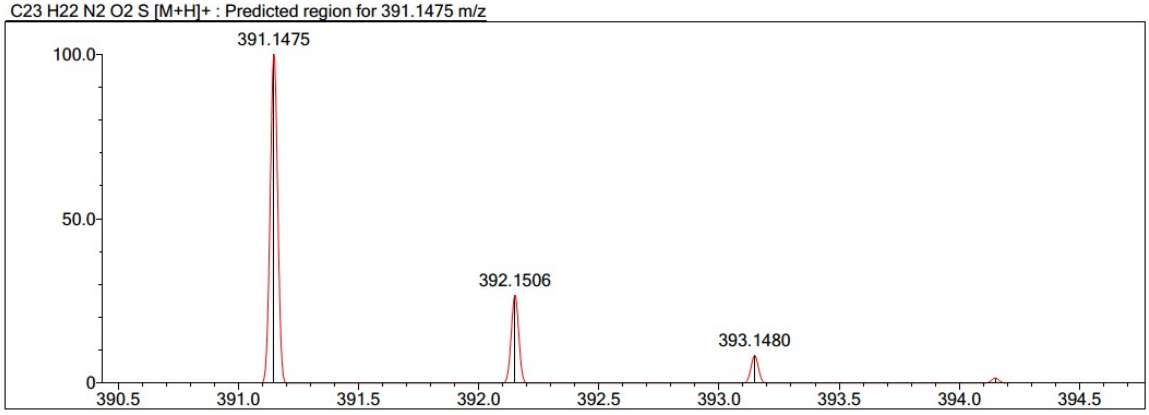
Event#: 1 MS(E+) Ret. Time : 0.333 -> 0.333 - 2.000 -> 2.292 Scan# : 51 -> 51 - 301 -> 345



Measured region for 391.1459 m/z



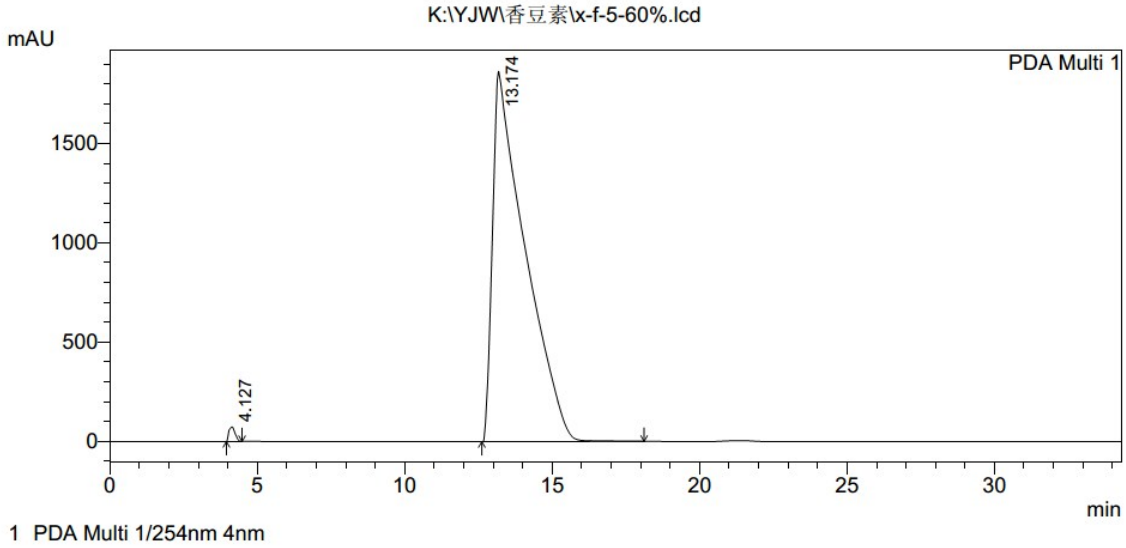
C23 H22 N2 O2 S [M+H]+ : Predicted region for 391.1475 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	67.73	C23 H22 N2 O2 S	[M+H]+	391.1459	391.1475	-1.6	-4.09	73.40	14.0

Figure S3. HRMS spectrum of **S1**.

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.127	1071515	73645	0.746	3.800
2	13.174	142575333	1864241	99.254	96.200
Total		143646848	1937886	100.000	100.000

Figure S4. HPLC analysis of **S1**.

1.2 Materials

All oligonucleotides used in this study were purchased from Invitrogen (Shanghai, China) and Sangon (Shanghai, China). Calf thymus DNA (CT-DNA), bovine serum albumin (BSA) and DAPI were purchased from Sigma-Aldrich (Singapore). Antibody against nucleolin was purchased from Abcam (USA). Normal rabbit IgG was purchased from Santa Cruz Biotechnology (USA). Tumor cell lines were obtained from the American Type Culture Collection. All the oligonucleotides, CT-DNA and BSA were dissolved in relevant buffer. Their concentrations were determined from the absorbance at 260 nm and 279 nm for DNA and BSA, respectively on the basis of respective molar extinction coefficients using NanoDrop 1000 Spectrophotometer (Thermo Scientific). Stock solutions of **S1** (10 mM) were dissolved in DMSO and stored at -80 °C. Further dilutions of samples and **S1** to working concentrations were made with relevant buffer immediately prior to use.

Table S1. Sequences of oligonucleotides used in the present study

Name	Oligonucleotide Sequence
dA21	5′-d[AAAAAAAAAAAAAAAAAAAA]-3′
dT21	5′-d[TTTTTTTTTTTTTTTTTTTT]-3′
htg21	5′-d[GGGTTAGGGTTAGGGTTAGGG]-3′
pu18	5′-d[AGGGTGGGGAGGGTGGGG]-3′
dimer	5′-d[GGGGTTTTGGGG]-3′
tetramer	5′-d[TGGGGT]-3′

rDNA-2957	5′-d[GGGTCGGGGGGTGGGGCCCGGGCCGGGG]-3′
rDNA-5701	5′-d[AGGGAGGGAGACGGGGGGG]-3′
rDNA-13079	5′-d[GGGGTGGGGGGGAGGG]-3′
SPR- HTG	5′-biotin-d[GTTAGGGTTAGGGTTAGGGTTAGGGTTAGG]-3′
SPR-c-MYC	5′-biotin-d[ACGTACGTGGGGAGGGTGGGGAGGGTGGGGAAGGTGGGG]-3′
SPR-c-KIT	5′-biotin-d[AGGGAGGGCGCTGGGAGGAGGG]-3′
SPR-Single	5′-biotin-d[AGTTAGAGTTAGAGTTAGAGTTAGAGTTAG]-3′
SPR-Duplex	5′-biotin-d[TTCGCGCGCGTTTTCGCGCGCG]-3′

1.3 UV-Vis Spectroscopic Studies

UV-Vis spectroscopic studies were performed on a UV-2450 spectrophotometer (Shimadzu, Japan) using 1 cm path length quartz cuvette. For titration experiment, all oligonucleotides were firstly prepared through heating at 95 °C for 10 min followed with slow cooling to room temperature. Small aliquots of a stock solution of sample (oligonucleotides, CT-DNA and BSA) were added into the solution containing **S1** at fixed concentration (5 μM) in Tris-HCl buffer (10 mM, pH 7.4) with 60 mM KCl. The final concentration of sample was varied from 0 to 15 μM. After each addition of sample, the reaction was stirred and allowed to equilibrate for at least 2 min and absorbance measurement was taken.

1.4 Fluorescence Studies

Fluorescence studies were performed on a LS-55 luminescence spectrophotometer (Perkin-Elmer, USA). A quartz cuvette with 1 cm × 1 cm path length was used for the spectra recorded at 10 nm excitation and emission slit widths unless otherwise specified. The fluorescence quantum yield (Φ_F) of **S1** was calculated relative to a standard solution of sulforhodamine 101 in ethanol (Φ_F = 0.95) and was determined using the following formula: $\Phi_u = \Phi_s(I_u A_s/I_s A_u)\times(n_u/n_s)^2$, where Φ is the fluorescence quantum yield, I is the measured integrated emission intensity, n is the refraction index of the solvents, and A is the optical density (absorbance). The u refers to the compound (**S1**) of unknown quantum yield, and s refers to the reference compound (sulforhodamine 101) of known quantum yield. In the titration experiment, all oligonucleotides were firstly prepared through heating at 95 °C for 10 min followed with slow cooling to room temperature. Small aliquots of a stock solution of sample (oligonucleotides, CT-DNA and BSA) were added into the solution containing **S1** at fixed concentration (0.2 μM) in Tris-HCl buffer (10 mM, pH 7.4) with 60 mM KCl. The final concentration of sample was varied from 0 to 1 μM. After each addition of sample, the reaction was stirred and allowed to equilibrate for at least 2 min and fluorescence measurement was taken at Ex 550 nm. According to our previous study (*Analyst*, 2015, 140, 4616–4625), the LOD values were calculated on the basis of the equation LOD = $K \times S_b/m$. Fluorescence decays in solution were measured on a Horiba Fluorolog-TCSPC spectrophotometer (excitation: 605 nm; measurement range: 100 ns).

1.5 SPR Studies

SPR measurements were performed on a ProteOn XPR36 Protein Interaction Array system (Bio-Rad Laboratories, Hercules, CA) using a Streptavidin-coated GLH sensor chip. Biotinylated oligonucleotides

(SPR- HTG, SPR-c-MYC, SPR-c-KIT, SPR-Single, SPR-Duplex) were attached to the chip. In a typical experiment, biotinylated DNA was folded in filtered and degassed running buffer (50 mM Tris-HCl, 100 mM KCl, pH 7.4). The DNA samples were then captured (~1000 RU) in five flow cell, leaving one flow cell as a blank. Ligand solutions were prepared with running buffer through serial dilutions of stock solution. Five concentrations were injected simultaneously at a flow rate of 50 μL/min^{-1} for 400 s of association phase, followed with 400 s of dissociation phase at 25 °C. The GLH sensor chip was regenerated with short injection of 50 mM NaOH between consecutive measurements. The final graphs were obtained by subtracting blank sensorgrams from different DNA sensorgrams. Data were analyzed with ProteOn manager software, using the Langmuir model for fitting kinetic data.

1.6 Fixed Cell Staining Experiment

The cells were grown in DMEM media containing 10% fetal bovine serum, 100 U/mL penicillin with 100 μg/mL streptomycin at 37 °C with 5% CO_2 atmosphere. 2.0×10^5 Cells were seeded in 3 cm Petri dishes and grew overnight. Cells were fixed with 4% paraformaldehyde in PBS for 15 min (rinsing with 0.1 M PBS twice), then were subsequently incubated with 5 μM of **S1** in PBS for 30 min at 37 °C (rinsed with 0.1 M PBS twice), and subsequently stained with 14 μM (5 μg/mL) DAPI for 20 min at 37 °C (rinsed with 0.1 M PBS three times). The cells were imaged using a LSM 710 laser scanning confocal microscope (Carl Zeiss, Germany) with a 60× objective lens. The excitation wavelengths were 405 nm for DAPI and 543 nm for **S1**, respectively. Duplicated experiments were performed.

1.7 ChIP Assay

ChIP was performed using the Pierce Agarose ChIP Kit (Thermo) following the protocol described in the kit. Briefly, A549 cells were fixed with 1% formaldehyde for 10 min and lysed. Chromatin was sheared to an average size of 200-500 bp using nuclease (provided by the kit). 10% of lysate was used as input. 3 μg antibody against nucleolin (abcam: ab13541) was used to immunoprecipitate chromatin. Normal rabbit IgG (SantaCruz: sc-3888) was used as negative control. ChIP was performed overnight at 4 °C. Immunoprecipitated DNA samples were amplified by using PCR. The sequences of primers used in the PCR are as follows: -48, 5'-CCCGGGGGAGGTATATCTTT-3' and 5'-CCAACCTCTCC-GACGACA-3'; +2907, 5'-GACGTG-TGGCGTGGGTCGAC-3' and 5'-GACGG-GAGGCAGCGACCGG-3'; +5645, 5'-GTTCGCTCGCTCGTTCGTTC-3' and 5'-CAACGACACGCCCTTCTTTC-3'; and +12855, 5'-ACCTGGCGCTAAACCATTCGT-3' and 5'-GGACAAA- CCCTTGTGTCGAGG-3'. The primers were used to amplify the rDNA promoter region without PQS and three PQS-containing regions (rDNA-2957: 5'-GGGTCGGGGGGTGGGGCCCGGGCCGGGG-3', rDNA-5701: 5'- AGGGAGGGAGACGGGGGGG-3' and rDNA-13079: 5'- GGGGTGGGGGGGAGGG-3') in the nontemplate strand of rDNA. The amplified products were separated on a 1.5% agarose gel, and photos were taken on a Gel Doc 2000 Imager System. Duplicated experiments were carried out.

Other Supplementary Spectra and Graphs

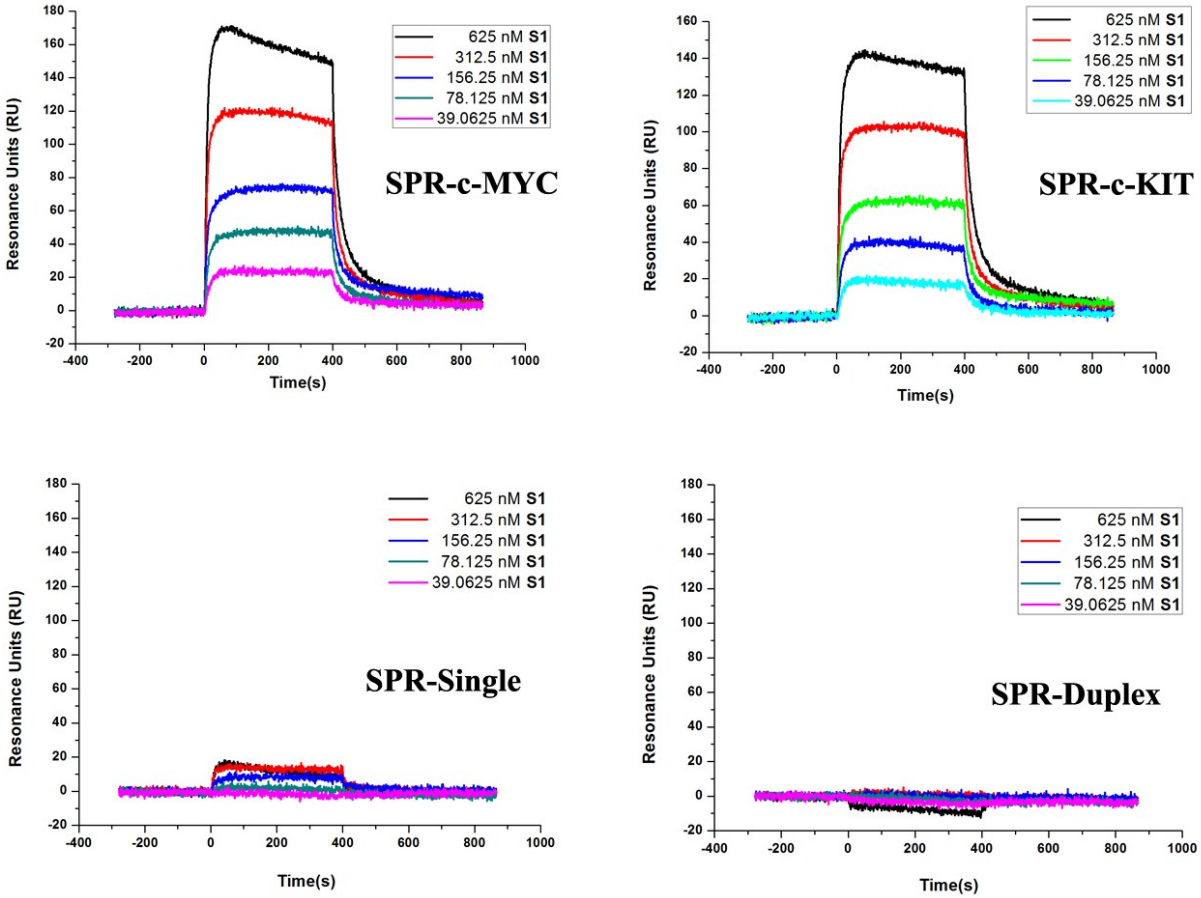


Fig. S5. SPR sensorgrams for binding of **S1** to the immobilized G-quadruplex DNA (SPR-c-MYC, SPR-c-KIT), single-strand DNA(SPR-Single), and double-strand DNA(SPR-Duplex). In the plot, the concentrations of **S1** in the flow solutions were 39.0625, 78.125, 156.25, 312.5, and 625 nM.

Table. S2 Equilibrium binding constants (K_D) of **S1** binding with different DNA measured with SPR. —[a] No significant binding was found for addition of up to 10 μM **S1**, which might indicate no specific interactions between **S1** with the duplex-strand and single-strand DNA.

	K_D/μM				
	c-myc	**c-kit**	**htg**	**duplex**	**Single**
S1	0.397	0.431	0.825	—[a]	—[a]

Table S3. The detection limits and linear ranges of **S1** for different G-quadruplexes

G-quadruplex	LOD (nM)	Linear range (nM)
htg21	11.3	0–120
pu18	16.8	0–200
dimer	13.9	0–180
tetramer	9.8	0–120

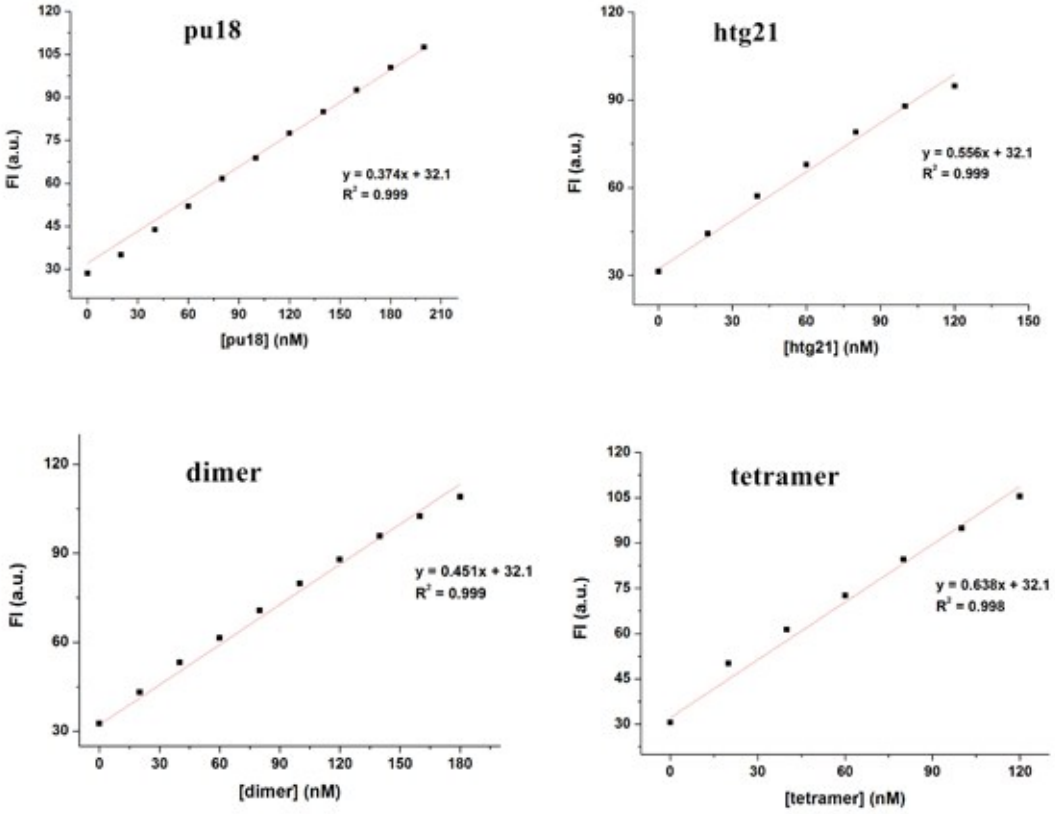


Figure S6. Linear fit equations for calculating LOD values of **S1** for G-quadruplexes. The concentration of **S1** as fixed at 0.2 M in 10 mM Tris-HCl buffer, 60 mM KCl, and pH 7.4. Fluorescence intensities at 625 nm were collected.

Table S4. Fluorescence lifetimes of **S1** alone and in the presence of G-quadruplexes, for excitation at 605 nm.

	τ_f (ns) at 625 nm
S1 alone	0.37
htg21	2.5
Pu8	2.6
tetramer	2.3

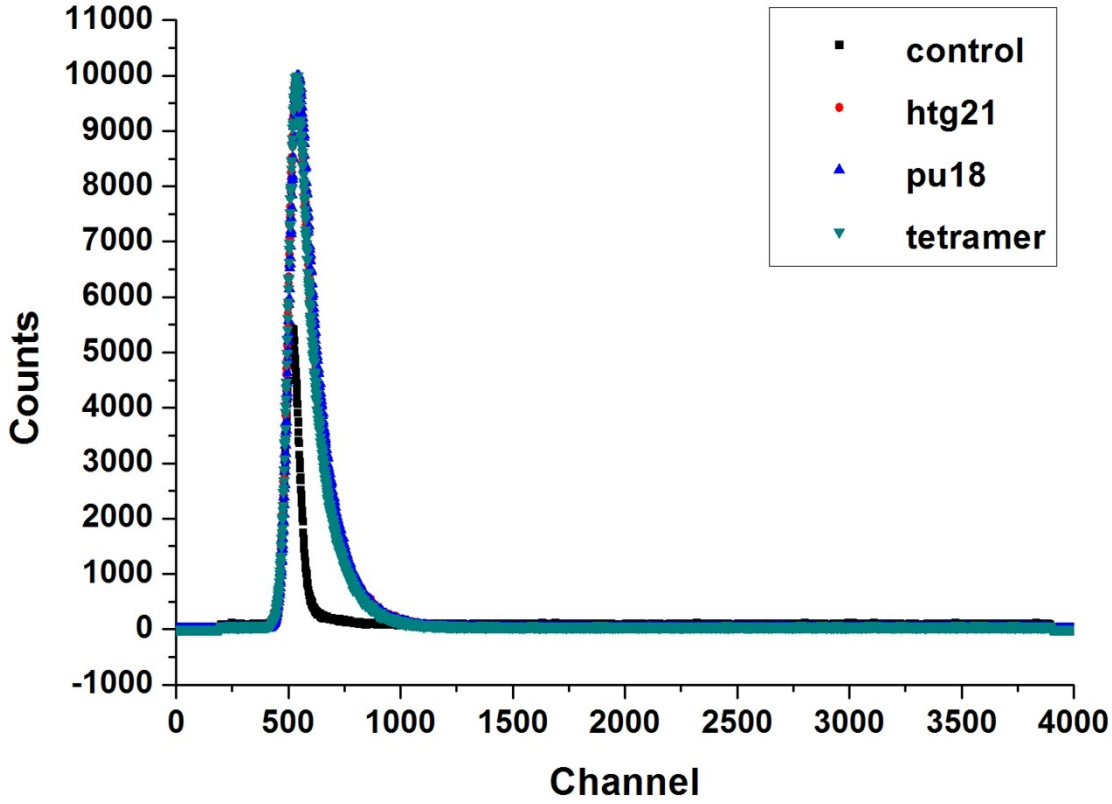


Figure S7. Fluorescence decay of **S1**alone and in the presence of G-quadruplexes (htg21, pu18 and tetramer)