Supporting Information

Visualization of Parallel G-quadruplexes in Cells with a Series of New Developed Bis(4-aminobenzylidene)acetone Derivatives

Chenlin Yang,[†,∥] Rui Hu,*[,†] Qian Li,[§] Shuang Li,[†,∥] Junfeng Xiang,[§] Xudong Guo,[†] Shuangqing Wang,[†] Yi Zeng,*[,‡,∥] Yi Li,[‡,∥] and Guoqiang Yang*[,†,∥]

[†]Key Laboratory of Photochemistry, Institute of Chemistry, [‡]Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, and [§]State Key Laboratory for Structural Chemistry of Unstable and Stable Species, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

[∥]University of Chinese Academy of Sciences, Beijing 100049, China

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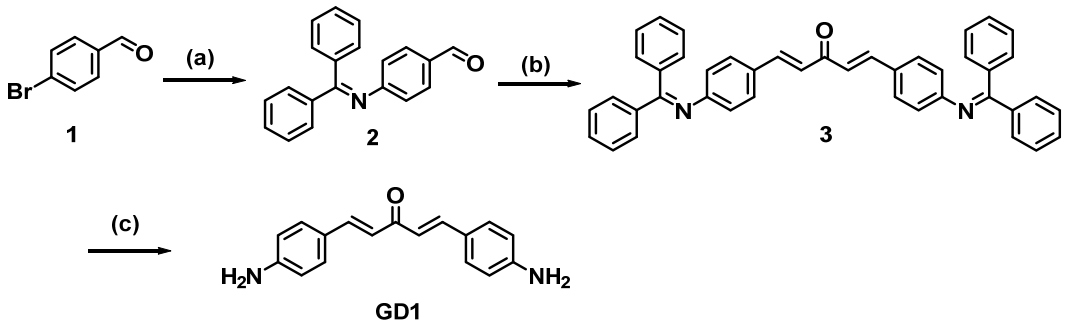
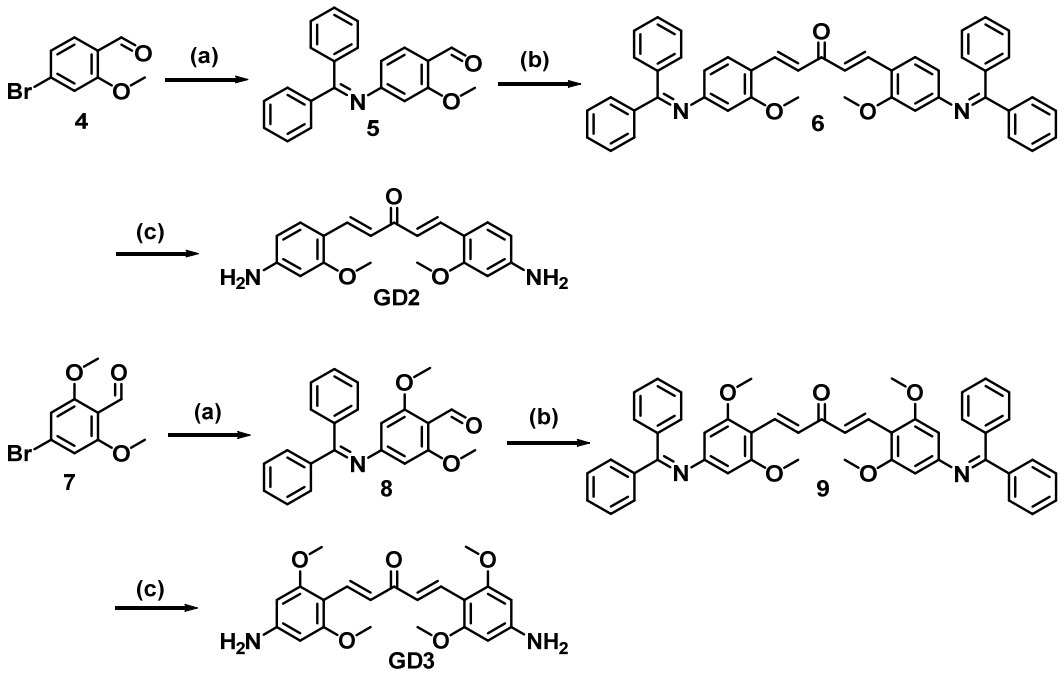
1. Synthesis and Characterization





Scheme S1. The synthesis processes of **GD1** - **GD3**. Reagent and conditions: (a) 2% $Pa(OAc)_2$, 3% BINAP, 1.4eq Cs_2CO_3, 1.2eq benzophenone imine, toluene, 100 °C, 12h. (b) 0.5eq acetone, 1eq aqueous NaOH, ethanol, rt, 1h. (C) aqueous HCl (2.0 M), THF, rt.

Synthesis of compound 2, 5 and 8.

A three-neck round flask was charged with $Pa(OAc)_2$ (0.2mmol), BINAP (0.3mmol), Cs_2CO_3 (24mmol) and 4-bromobenzaldehyde (1.85 g, 10mmol) and purged with argon three times, next added with toluene (20mL) containing benzophenone imine (22mmol). The mixture was stirred and heated to 100 °C for 12 hours. After cooled down to room temperature, the solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using hexane-ethyl acetate (10: 1, v/v) as an eluent to give a light yellow solid compound **2** (1.8g, 63%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.68 (m, 2H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.33 – 7.04 (m, 8H), 6.75 (d, *J* = 8.3 Hz, 2H). HRMS(MALDI): Calcd for $[M+H]^+$, 286.12264, Found, 286.12269

Similar procedures were performed for compound 5 (68% yield) and compound 8 (70% yield). For compound 5, ^{1}H NMR (300 MHz, CDCl3) δ 10.19 (s, 1H), 7.71 (m, 2H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.46 – 7.07 (m, 8H), 6.26 (m, 2H), 3.66 (s, 3H). HRMS(MALDI): Calcd for $[M+H]^+$, 316.13321, Found, 316.13315; For compound 8, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.32 (s, 1H), 7.98 (d, *J* = 16.2 Hz, 2H), 7.56 – 7.15 (m, 8H), 5.89 (s, 4H), 5.87 (d, *J* = 16.2 Hz, 2H), 3.68 (s, 6H). HRMS(MALDI): Calcd for $[M+H]^+$, 346.14377, Found, 346.14392.

Synthesis of compound 3, 6 and 9.

Into a round-bottom flask charged with compound 2 (0.855g, 3mmol), was injected ethanol (20mL) and acetone (100μL, 1.5mmol). The reaction was stirred at room temperature for 1 hour, following ultrapure water (100mL) was added. Then the mixture was extracted with dichloromethane (50mL) three times and the organic layer was combined and dried. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using hexane-ethyl acetate (8:1, v/v) as an eluent to give a dark yellow solid compound 3 (0.7g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.2 Hz, 4H), 7.63 (d, *J* = 15.9 Hz, 2H), 7.56 – 7.38 (m, 11H), 7.56 – 7.38 (m, 5H), 7.17 (m, 4H), 6.93 (d, *J* = 15.9 Hz, 2H), 6.78 (d, *J* = 8.2 Hz, 4H). HRMS(MALDI): Calcd for $[M+H]^+$, 593.25874, Found, 593.25899.

Similar procedures were performed for compound 6 (82% yield) and compound 9 (79% yield).For compound 6, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 16.1 Hz, 2H), 7.68 (d, *J* = 7.3 Hz, 4H), 7.44 – 7.29 (m, 9H), 7.22(m, 5H), 7.08 (m, 4H), , 6.95 (d, *J* = 16.1 Hz, 2H), 6.24 (m, 4H), 3.64(s, 6H). HRMS(MALDI): Calcd for $[M+H]^+$, 653.27987, Found, 653.27999; For compound 9, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 16.2 Hz, 2H), 7.76 (d, *J* = 7.3 Hz, 4H), 7.55 – 7.36 (m, 8H), 7.30 (m, 6H), 7.16 (d, *J* = 6.2 Hz, 4H), 5.96 (s, 4H), 3.68 (s, 12H).. HRMS(MALDI): Calcd for $[M+H]^+$, 713.30100, Found, 713.30112. HRMS(MALDI): Calcd for $[M+H]^+$, 346.14377, Found, 346.14392.

Synthesis of compound GD1, GD2 and GD3.

Into a round-bottom flask with compound 3 (0.592g, 1mmol) was injected THF (10 mL) and aqueous HCl (2.0 M, 1 mL). Then the mixture was partitioned between 0.5 M HCl and ethyl acetate. The aqueous layer was separated and made alkaline by adding saturated aqueous $NaHCO_3$. The product was extracted with 50mL dichloromethane three times. The solvent was evaporated to yield orange solid **GD1** (0.26g, 99%). ^{1}H NMR (300 MHz, DMSO) δ 7.54 (d, *J* = 15.8 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 4H), 6.92 (d, *J* = 15.8 Hz, 2H), 6.59 (d, *J* = 8.4 Hz, 4H), 5.79 (s, 4H). HRMS (ESI+): Calcd for $[M+H]^+$, 265.13354, Found, 265.13346.

Similar procedures were performed for **GD2** (99% yield) and **GD3** (99% yield). For compound **GD2**, ^{1}H NMR (400 MHz, DMSO) δ 7.76 (d, *J* = 15.8 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 2H), 6.86 (d, *J* = 15.8 Hz, 2H), 6.23 (s, 4H), 5.87 (s, 4H), 3.80 (s, 6H). HRMS (ESI+): Calcd for $[M+H]^+$, 325.15467, Found, 325.15460. For compound

GD3, ^{1}H NMR (400 MHz, DMSO) δ 7.88 (d, *J* = 15.9 Hz, 2H), 7.06 (d, *J* = 15.9 Hz, 2H), 5.91 (s, 4H), 5.79 (s, 4H), 3.80 (s, 12H). HRMS (ESI+): Calcd for $[M+H]^{+}$, 358.17580, Found, 358.17568.

2. Supplementary Tables and figures

Table S1. Oligonucleotides used in this study purchased from Invitrogen.

NO.	Name	Sequence (5' to 3')	Topology
1	c-myc	TGAGGGTGGGTAGGGTGGGTAA	parallel
2	c-kit2	GGGCGGGCGCGAGGGAGGGG	parallel
3	VEGF	GGGAGGGTTGGGGTGGG	parallel
4	ADAM10	GGGGGACGGGTAGGGGCGGGAGGTAGGGG	Parallel (RNA)
5	22AG	AGGGTTAGGGTTAGGGTTAGGG	hybrid
6	21CTA	GGGCTAGGGCTAGGGCTAGGG	anti-parallel
7	TBA	GGTTGGTGTGGTTGG	anti-parallel
8	dx12	(a) GCGTGAGTTCGG (b) CCGAACTCACGC	duplex
9	Triplex	(a) TTTTTTTTTTTTTTTTTTT (b) AAAAAAAAAAAAAAAAAAA (c) TTTTTTTTTTTTTTTTTTT	triplex
10	ssAf17	CTGAGTTGTATATATTCG	single strand

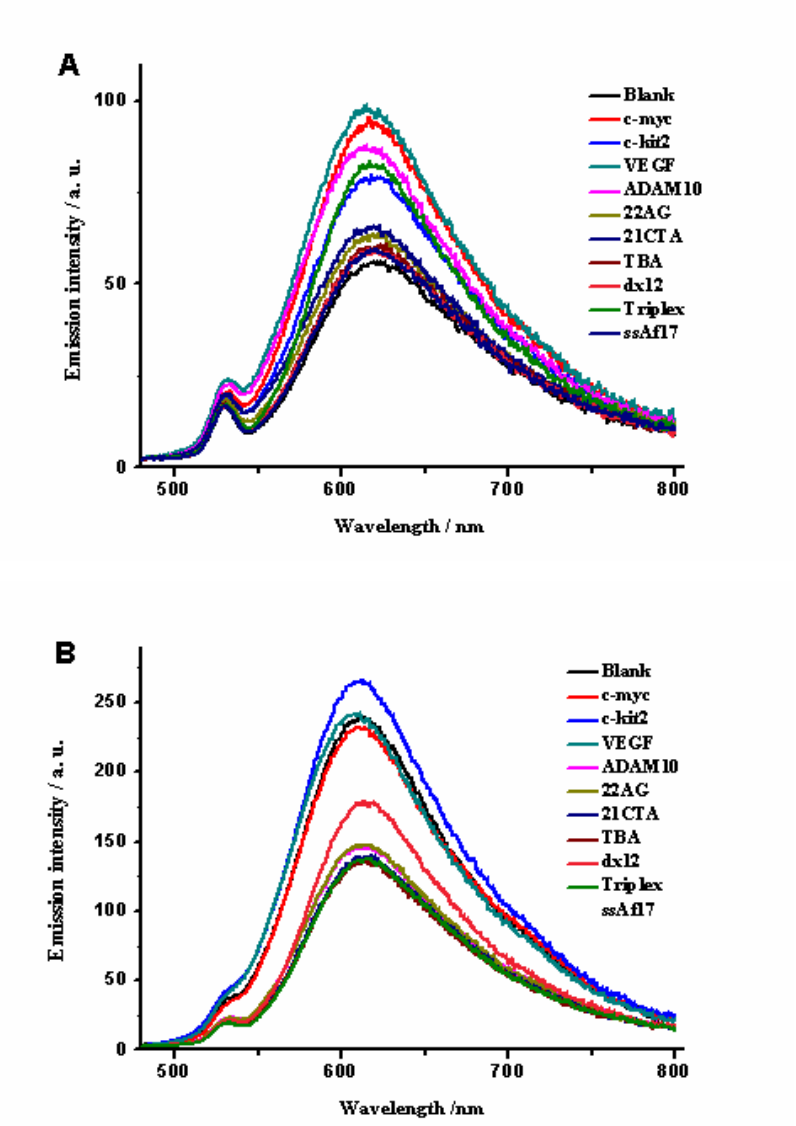


Figure S1. Fluorescence spectra of 2 μM **GD1** (A) and **GD2** (B) with 10 nucleic acid oligomers in Tris-HCl buffer (10 mM, pH = 7.4) containing 50 mM KCl. The excitation was at 450 nm.

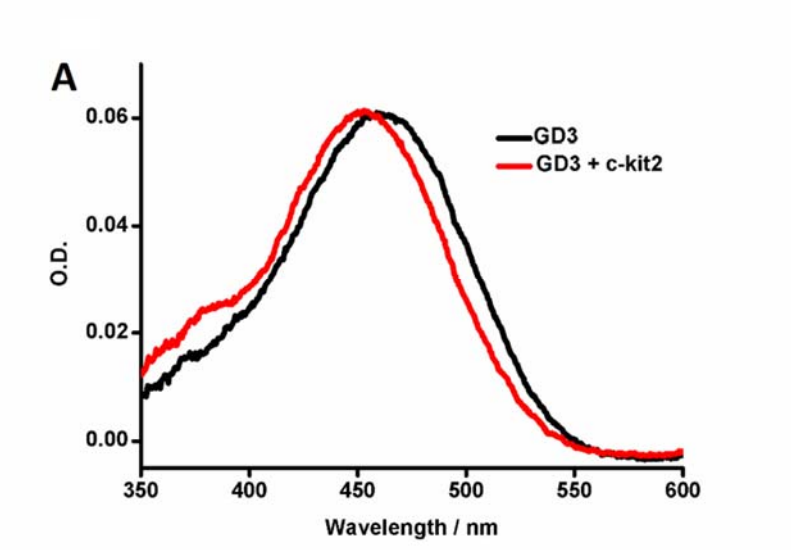


Figure S2. Absorption spectra of **GD3 (**2 μM) with and without 1.0 eq of c-kit2

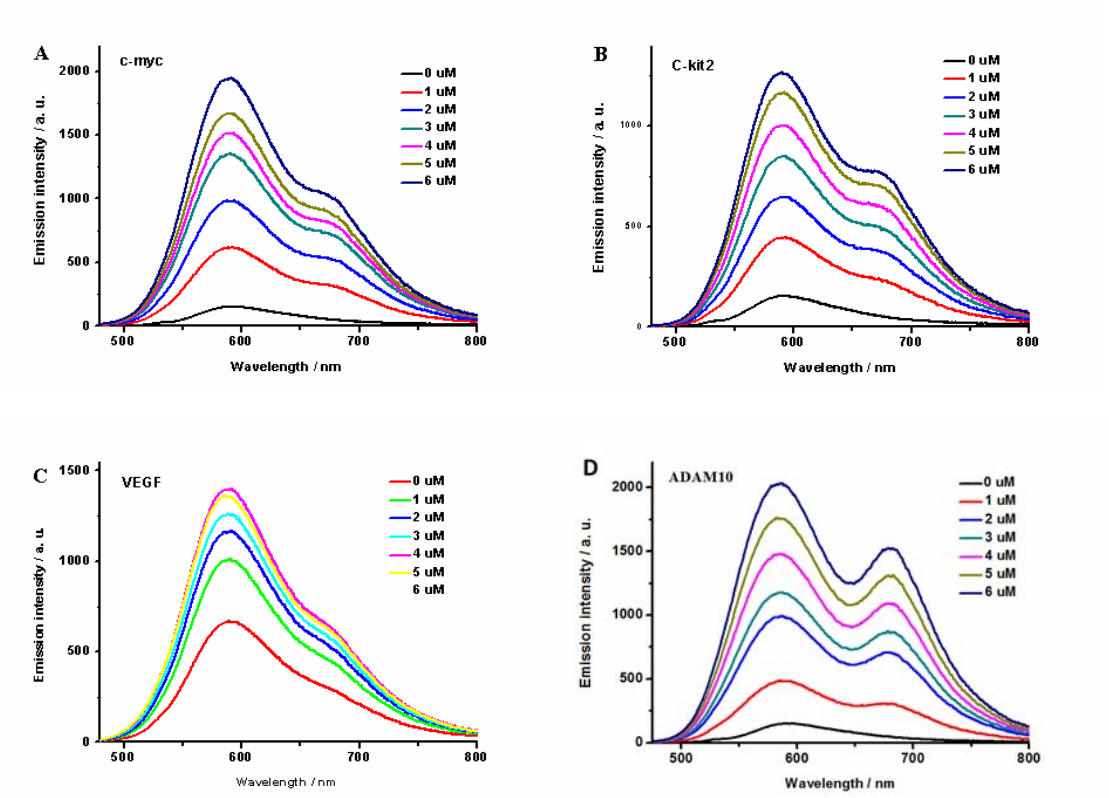


Figure S3. Fluorimetric titration of **GD3 (**2 μM) with different amounts of c-myc(A), c-kit2(B), VEGF(C) and ADAM10 (D) in Tris-HCl buffer (10 mM, pH = 7.4) containing 50 mM KCl. The excitation wavelength was 450 nm.

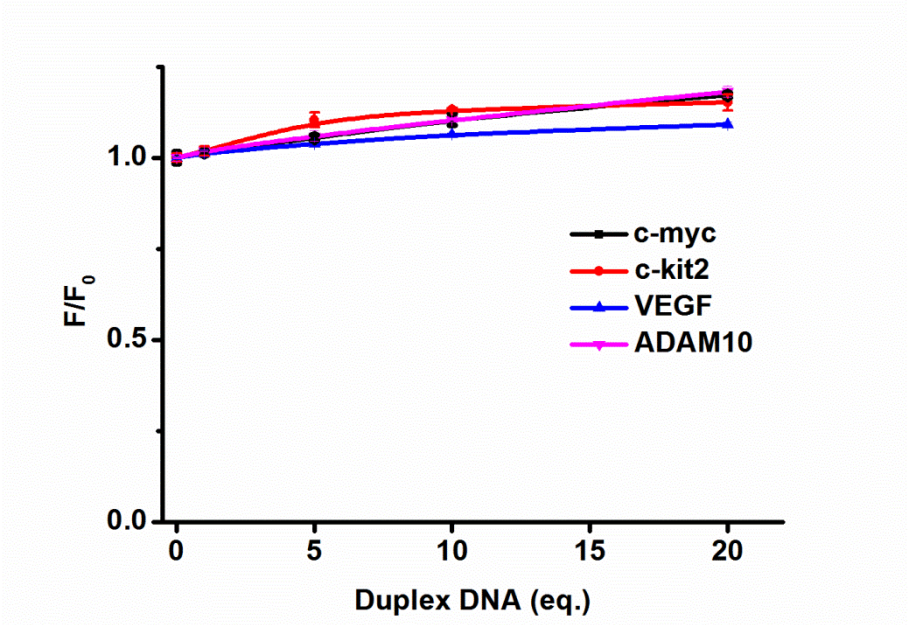


Figure S4. The selectivity of GD3 to G4s against the increasing concentration of duplex DNA in Tris-HCl buffer (10 mM, pH = 7.4) containing 50 mM KCl at room temperature. The concentration of duplex DNA was increased from 0 to 20 mol. equiv of GD3/G4 complex (1:1).

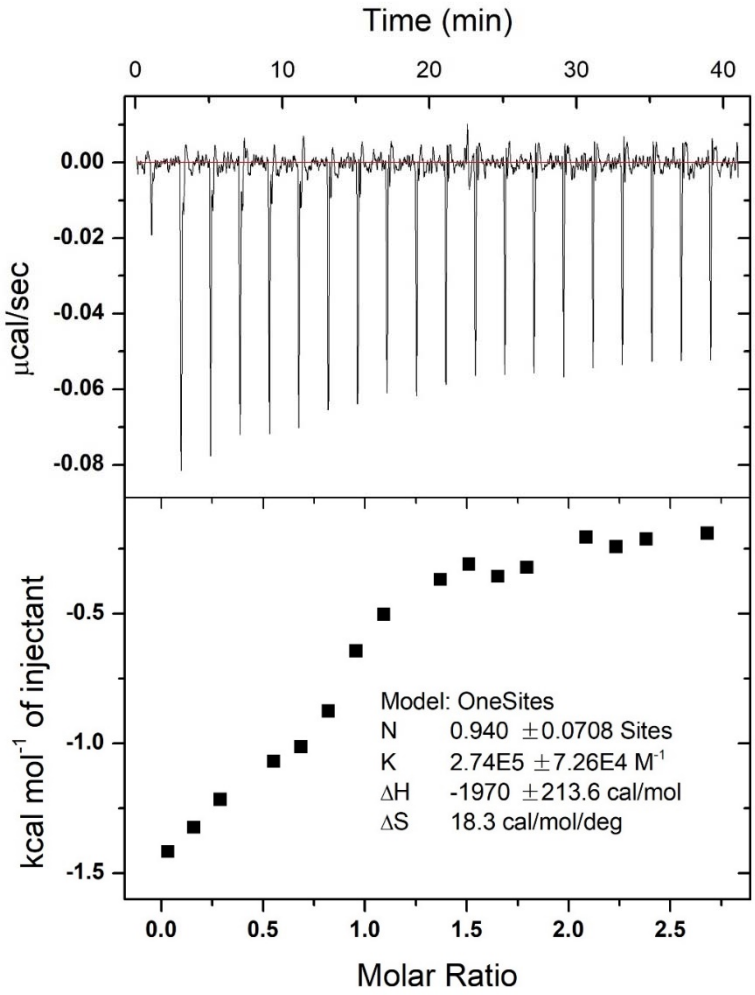


Figure S5. ITC analysis of GD3 binding affinity to G4-structure VEGF in Tris-HCl buffer (10 mM, pH = 7.4) containing 50 mM KCl at 25 °C. (TOP) the ITC raw data of titration of VEGF (100 μM) into GD3 (10 μM); (Bottom) Enthalpogram retrieved from A, corrected for the heat of dilution; the line represents the least-squares fit to the single-site binding model. The binding constant (K_b) was calculated as $2.74\times10^5 \pm 7.26\times10^4$ M^{-1}

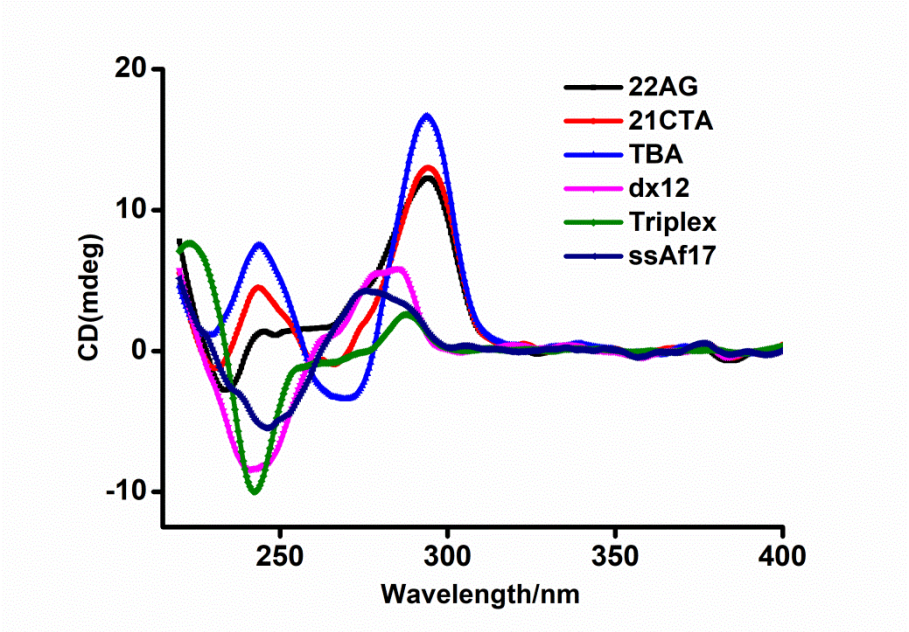


Figure S6. The CD spectra of oligonucleotides 22AG, 21CTA, TBA, dx12, Triplex and ssAf17 (5 μM), in Tris-HCl buffer with 50 mM KCl (10 mM, pH = 7.4).

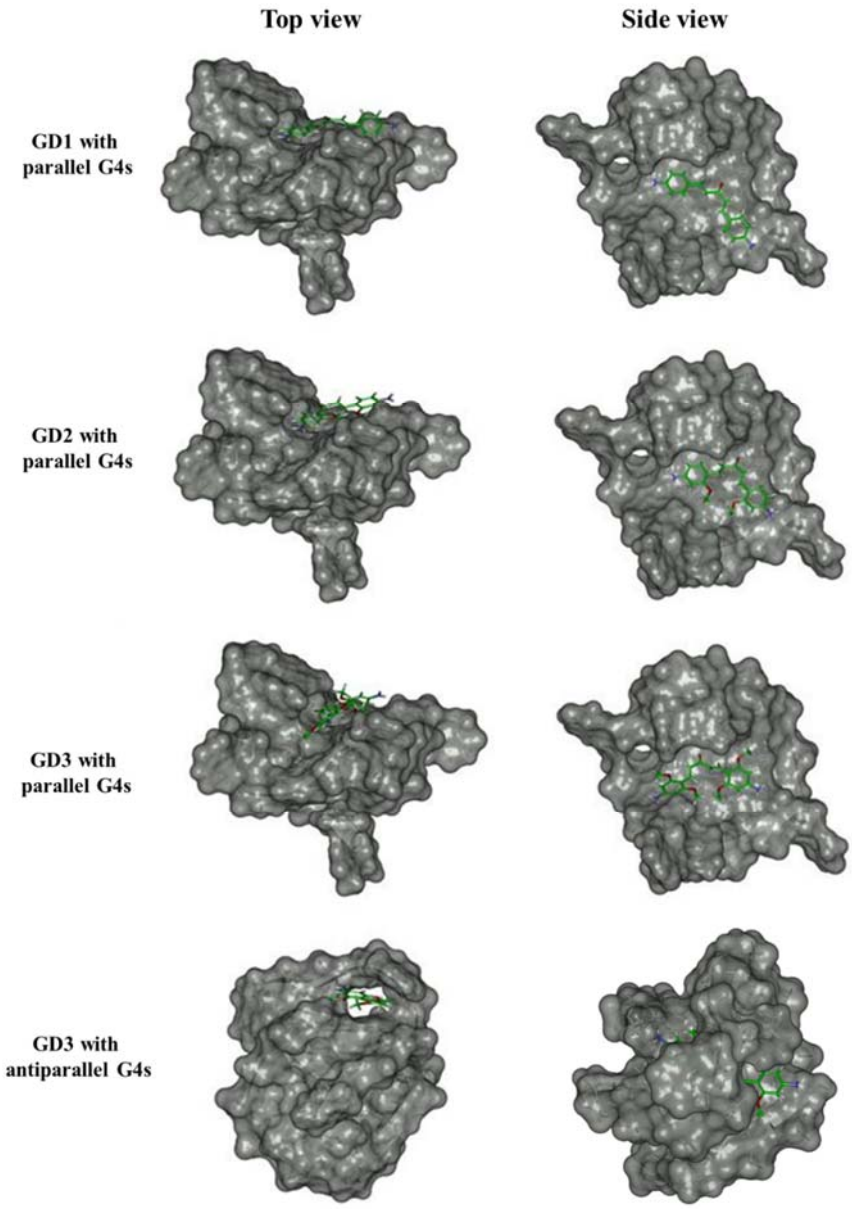


Figure S7. Molecular docking results of GD1, GD2, and GD3 with parallel G4 structure as well as GD3 with antiparallel G4 structure from top view and side view.

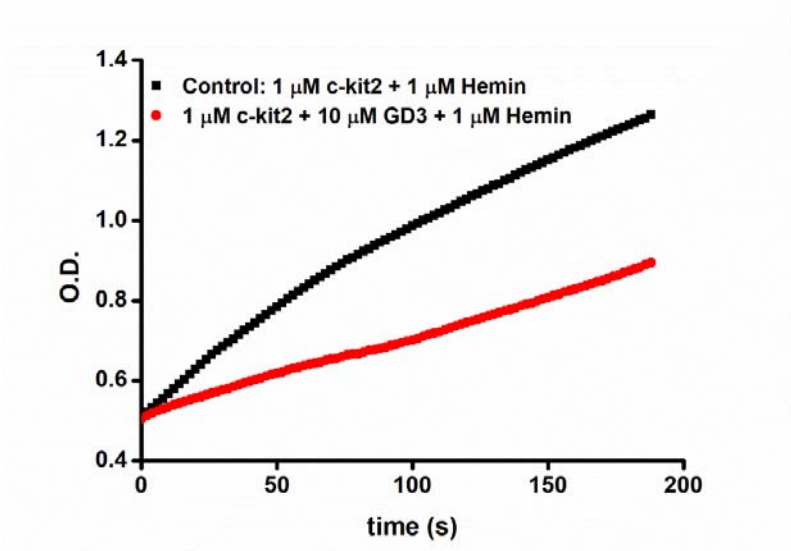
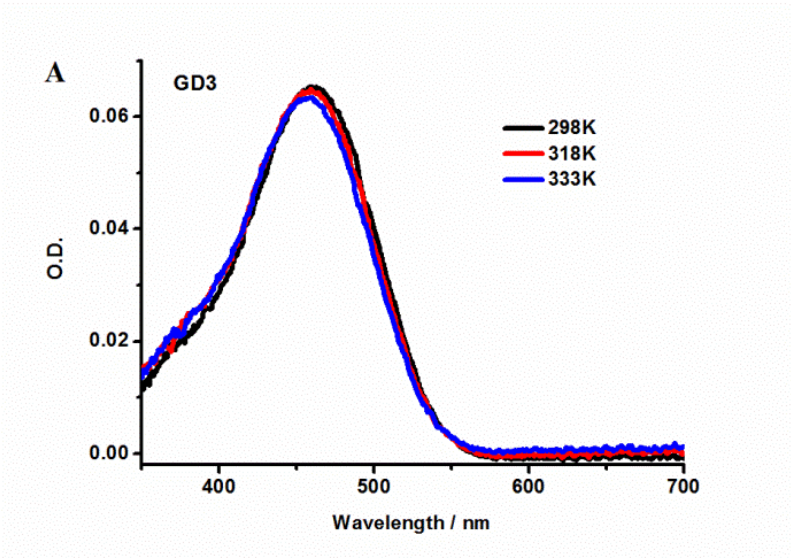


Figure S8. Inhibition activity of GD3 on the peroxidase activity of c-kit2 (G4)/hemin complexes. The curves represent the change of the product concentration (absorbance at 415 nm) of c-kit2/hemin peroxidase within 200 s at room temperature. The c-kit2/hemin peroxidase reaction in the absence of GD3 was used as control. The final concentrations were as follows: c-kit2 (1 μM), GD3 (10 μM), hemin (1 μM), ABTS (2 mM), and H_2O_2 (2 mM).



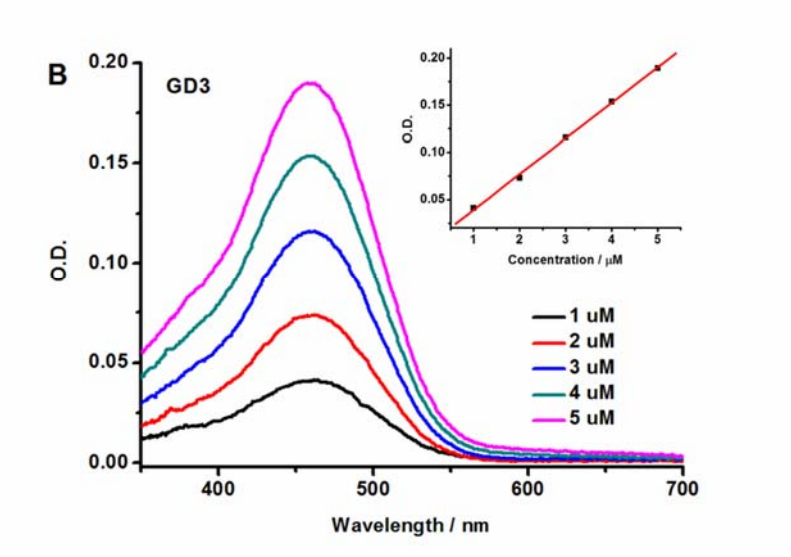


Figure S9. (A) Absorption spectra of GD3 (2 μM) in experimental buffer at various temperatures; (B) Absorption spectra of GD3 in experimental buffer with different concentrations (T = 273 K); Inset: The linear fitting of the absorption maximum *versus* GD3 concentrations (R^2 = 0.9998).

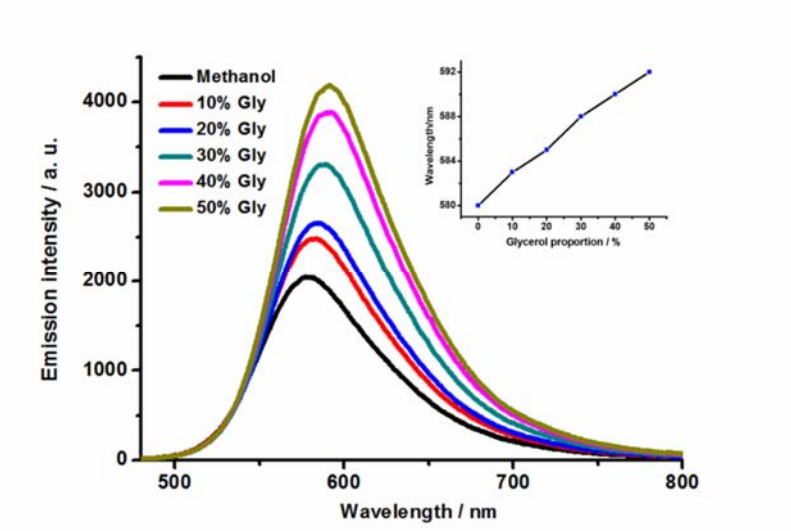


Figure S10. Fluorescence spectra of **GD3** (2 μM) in in glycerol/methanol mixtures with different glycerol proportions; Inset: the wavelength of GD3 emission maximum at different glycerol proportions.

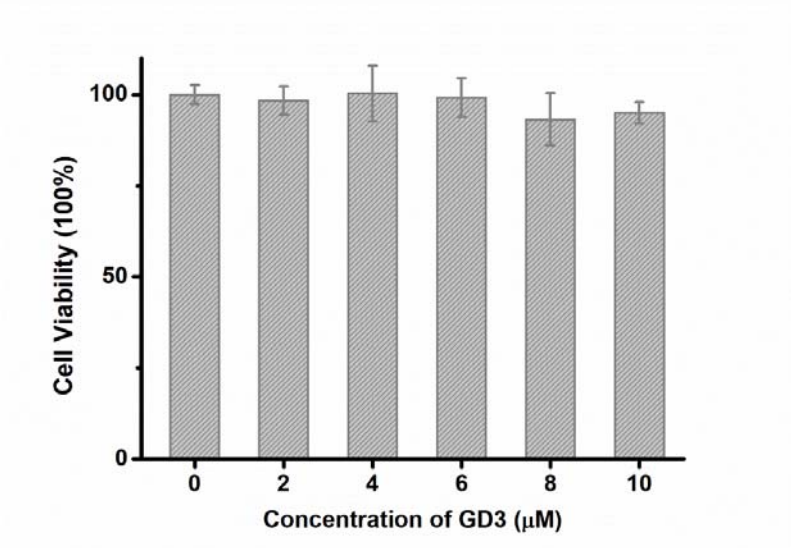


Figure S11. Effect of GD3 at varied concentrations on the viability of ARPE-19 cells. The viability of cells without GD3 is defined as 100%. The results were expressed as the mean ± standard deviation of four separate measurements.

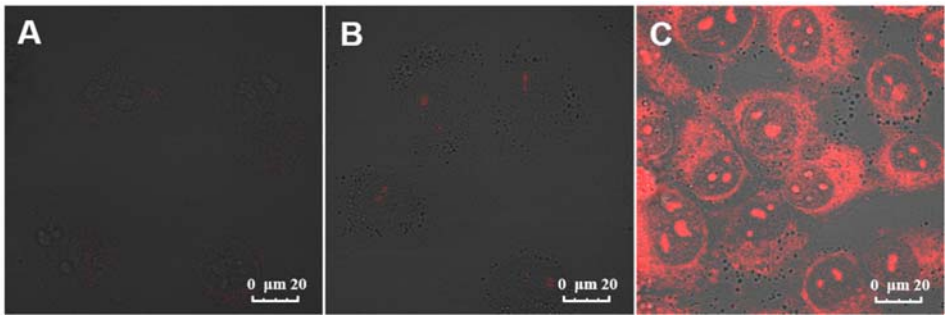


Figure S12. Confocal imaging of ARPE-19 cells (fixed) stained 1 μM **GD1** (a), **GD2** (b), and **GD3** (c), respectively.