Supporting Information for

Pt(II)-Dip Complex Stabilizes Parallel c-myc G-Quadruplex

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Contents

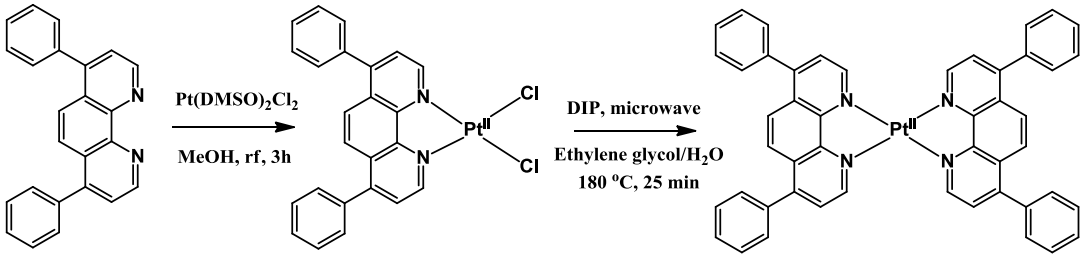
S1. Materials

1,10-phenanthroline (Phen), 4,7-Diphenyl-1,10-phenanthroline (Dip) and K_2PtCl_4 were obtained from Alfa Aesar. Thiazole orange and calf thymus DNA (CT DNA) was purchased from Sigma-Aldrich. TMPyP4 was obtained from TCI. All DNA oligomers (HPLC purified) were purchased from Sangon (Shanghai, China). *Taq* polymerase was obtained from Fermantas and iScript cDNA synthesis kit was purchased from Bio-Rad. Milli-Q water was used in all physical measurement experiments.

Table S1. Sequences of oligomers (primers) used in the manuscript

Oligomer	Sequence
c-myc	5'- TGGGGAGGGTGGGGAGGGTGGGGAAGG -3'
bcl-2	5'-GGGCGCGGGAGGAATTGGGCGGG-3'
HT	5'-GGGTTAGGGTTAGGGTTAGGG-3'
c-myc-rev	5'-ATCGATCGCTTCTCGTCCTTCCCCA-3'
c-myc-mu	5'-TGGTGAGTGTGGTGAGTGTGGTGAAGG -3'
c-myc-mu-rev	5'-ATCGATCGCTTCTCGTCCTTCACCA -3'
ds 26	5'-CAATCGGATCGAATTCGATCCGATTG-3'
ss DNA	5'-TGGTGAGTGTGGTGAGTGTGGTGAAGG -3'
c-myc Forward	5'-GTGGCACCTCTTGAGGACCT-3'
c-myc Reverse	5'-TGGTGCTCCATGAGGAGACA-3'
β-actin Forward	5'-GCATCCTGTCGGCAATGC-3'
β-actin Reverse	5'-GTTGCTATCCAGGCTGTGC-3'

S2. Preparation of complex 1



Pt(Dip)Cl_2

$Pt(DMSO)_2Cl_2$[1] (0.211 g, 0.5 mmol), Dip (0.166 g, 0.5 mmol) was brought to reflux in methanol (25 mL). After 3 hours, the mixture was cool to room temperature and yields yellow precipitates. The crudes were collected by filtration and were washed with methanol . Yield: 0.2838 g, 95%. ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.652 (d, J = 5.7 Hz, 2H, H2/H9), δ 8.131 (d, J = 5.7 Hz, 2H, H3/H8), δ 8.119 (s, 2H, H5/H6), δ 7.666-7.763 (m, 10H, 2 × C_6H_5) ppm.

[Pt(Dip)$_2$](PF_6)$_2$ (1)

$Pt(DIP)Cl_2$ (12 mg, 0.02 mmol), Dip (6.6 mg, 0.02 mmol) and a magnetic stir bar was placed into a microwave vessel. Ethylene glycol (1 mL) and ultrapure water (2 mL) was added. The mixture was heated at 180 °C for 25 minutes under microwave irradiation. Subsequently, the reaction mixture was diluted by 8 mL water and was washed by DCM (3 × 10 mL). A black brown solid was precipitated from the aqueous layer by adding excess amount of NH_4PF_6 and was washed with methanol (2 mL). Yield: 8.4 mg, 37 %. ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.815 (d, J = 6.0 Hz, 2H, H2/H9), δ 8.392 (d, J = 6.4 Hz, 2H, H3/H8), δ 8.324 (s, 2H, H5/H6), δ 7.768-7.845 (m, 10H, 2 × C_6H_5) ppm; HR-MS (Acetone) *m/z* (%): calcd 1004.1917 for $C_{48}H_{32}F_6N_4PPt$, found 1004.1922 for [**1**-PF_6]$^+$; elemental analysis calcd (%) for $C_{48}H_{32}F_{12}N_4P_2Pt$: C 50.14, H 2.81, N 4.87; found: C 50.05, H 2.94, N 4.76.

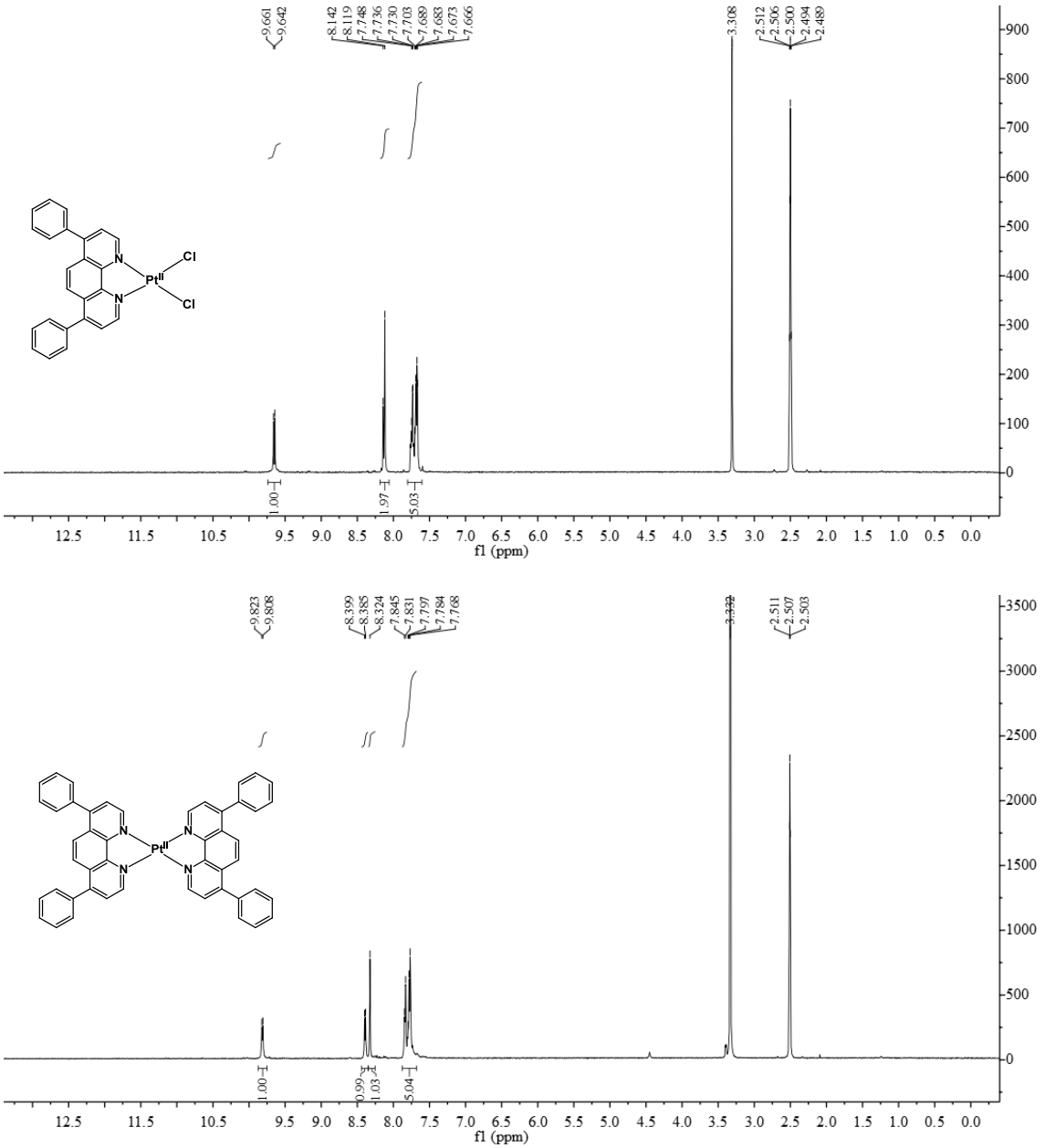


Figure. S1 ^{1}H-NMR spectra of Pt(Dip)Cl_2 and complex **1**.

S3. CD Melting and ICD spectra

CD measurements were recorded on a Jasco J-810 CD spectropolarimeter using a 1 cm path length cuvette. For CD melting, 400 μL of c-myc (10 μM in 10 mM lithium cacodylate buffer, pH 7.4, without KCl), HT (10 μM in 10 mM lithium cacodylate buffer, pH 7.4, 5 mM KCl), bcl-2(10 μM in 10 mM lithium cacodylate buffer, pH 7.4, 5 mM KCl) and ds26 (10 μM in 10 mM lithium cacodylate buffer, pH 7.4, 5 mM KCl) were annealed by heating at 92 °C for 5 min followed by gradual cooling to room temperature, respectively. After that, 400 μL of 10 μM complexes **1** and **2** prepared in corresponding buffer according to different DNA were mixed with the annealed DNA. The final concentrations of DNA and complexes were both 5 μM. Thermal melting was monitored at 261, 290, 261 and 282 nm for c-myc, HT, bcl-2 and ds26 respectively, at the heating rate of 1 °C/min. Final analysis of the data was carried out using Origin 8.0 (OriginLab Corp.). The melting results are the average of two replicates.

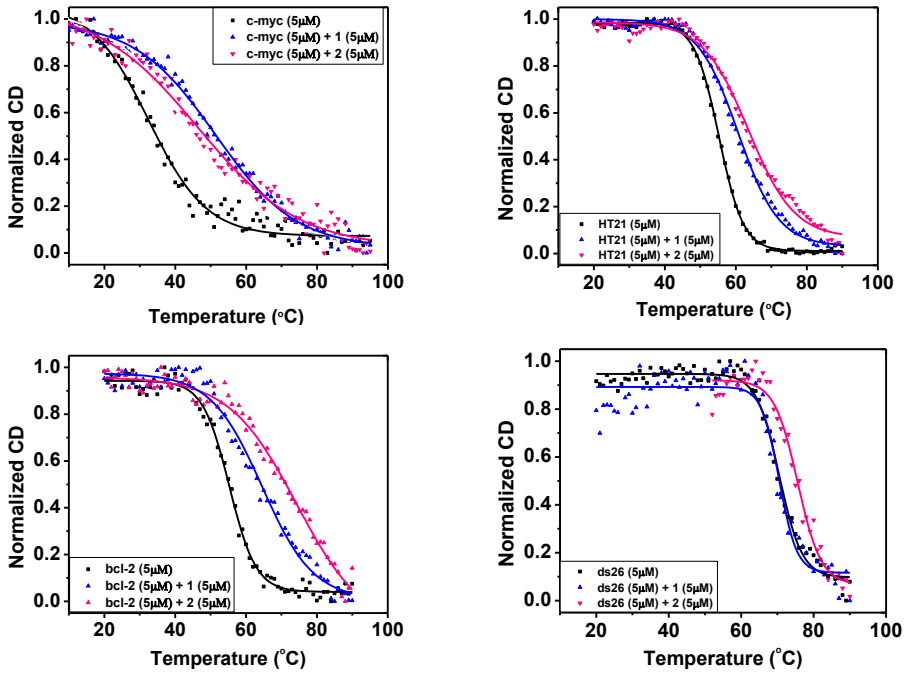
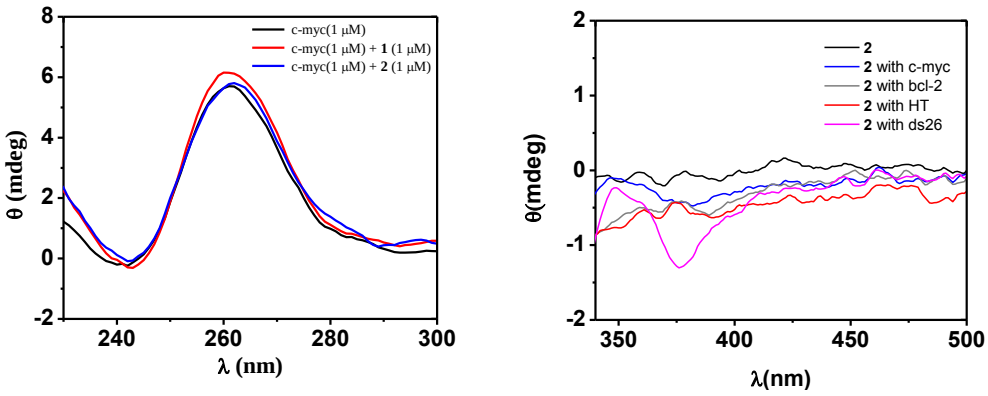


Figure. S2 Melting curves of c-myc GQ, HT GQ, bcl-2 GQ and ds26 in the presence of complexes **1** and **2**.

For ICD spectra, 20 μM complexes and 40 μM annealed DNA were mixed in a 10 mM lithium cacodylate buffer, 100 mM KCl, pH 7.4 buffer. Spectra were recorded from 500–320 nm at a scan rate of 200 nm/min and a response time of 2.0 s with an average of six scans. Final analysis of the data was carried out using Origin 8.0 (OriginLab Corp.).



(a) (b)

Figure. S3 (a) CD spectra of c-myc, c-myc+**1** and c-myc+**2.** [c-myc GQ] = 1 μM; [complex] = 1 μM if added. (b) Induced CD spectra of **2** in the presence of c-myc, HT, bcl-2 and ds26. [**2**] = 20 μM and [DNA] = 40 μM.

S4. UV/Vis Absorption Titration

Absorption spectra were recorded on a Cary 100 UV/Vis spectrophotometer. A Tris/KCl buffer (100 mM KCl, 10 mM Tris HCl, pH 7.4) was used and UV/Vis spectra were recorded after each addition of concentrated DNA stock to 20 μm Pt(II) complex solutions in a quartz cuvette (path length = 1 cm) at 25 °C. Binding data was determined with Equation (1)[2]

$$D/\Delta\varepsilon_{ap} = D/\Delta\varepsilon + 1/[(\Delta\varepsilon)\times K] \qquad (1)$$

where D is the concentration of DNA, $\Delta\varepsilon_{ap} = |\varepsilon_A - \varepsilon_F|$, $\varepsilon_A = A_{obs}/[complex]$, $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$, and ε_B and ε_F correspond to the extinction coefficients of the DNA-complex adduct and free complex that is in solution, respectively.

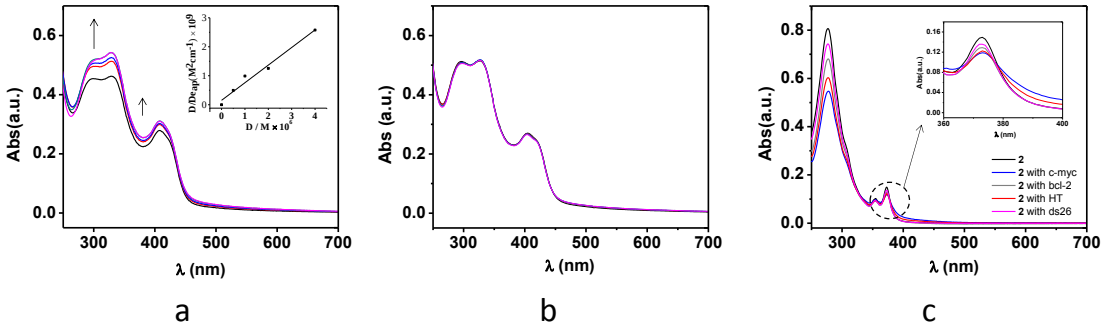
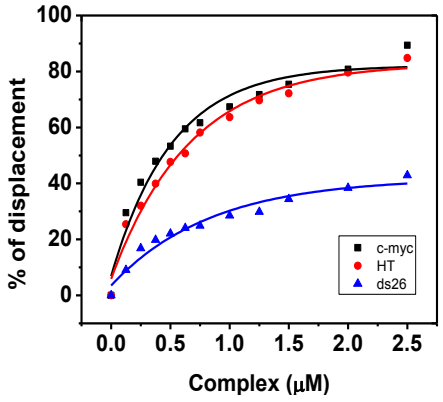


Figure. S4 (a,b) UV-vis spectra of **1** (20 μM) with increasing amounts of c-myc (a, 0-4 μM) and ds26 (b, 0-100 μM). Inset: $D/\Delta\varepsilon_{ap}$ vs D. (c) Absorption spectra of **2** (20 μM) in the presence of c-myc GQ, bcl-2 GQ, HT GQ and ds26. DNA concentrations are 40 μM. Insert: Spectra zoom in at 360-400 nm to highlight the MLCT hypochromicity of complex **2** upon interaction with various DNA structures.

S5. FID assay[3]

FID assay was performed on Varian Cary Eclipse fluorescence spectrophotometer in a 100 mM KCl, 10 mM lithium cacodylate, pH 7.4 buffer. The concentration of all DNA used was 0.25 μM. Quadruplex DNA were added with 2 molar equivalents of thiazole orange (TO, 0.5 μM) and ds26 were added with 3 molar equivalents of TO (0.75 μM) according to the binding stoichiometry. Metal complexes were titrated to displace TO from DNA by following a 11-steps gradual addition (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0 and 10.0 molar equivalents of DNA). Fluorescence spectra are recorded after each addition and the TO displacement is evaluated by measuring the fluorescence area (FA, from 510 nm to 750 nm, excitation wavelength is 501 nm). The percentage of TO displacement (^{TO}D) is determined using the following equation: $^{TO}D_x = 100 - ((FA_i/FA_1) \times 100)$, with $1< i <12$, where FA_1 is the fluorescence area of thiazole orange upon binding to DNA and FA_i are the fluorescence area of TO under various ratios of [Pt]/[TO-DNA]. The percentage of TO displacement is then plotted as a function of the concentration of **1**.



DNA	DC_{50} (μM)
c-myc	0.44
HT	0.56
ds26	>2.5

Figure. S5 Left: Plot of TO displacement *vs*. concentrations of **1** with different DNA. Right: DC_{50} (the concentration of **1** needed to displace 50% of thiazole orange (TO) values for different DNA.

S6. Competition Dialysis Assay[4]

75 μM of different DNA structures, including random coil, duplex and G-quadruplexes (i.e. ssDNA, CT-DNA, ds26, HT, c-myc, c-kit1 and bcl-2) in buffer (10 mM sodium cacodylate with 190 mM NaCl, pH 7.4) were annealed by heating at 95 °C for 5 min and slowly cooling down to room temperature before use. 100 μL of each oligonucleotide sample was incubated within Slide-a-Lyzer MINI dialysis unit which contains semipermeable membrane, held by floatation device, in contact with 1 μM of complexes in a beaker. After incubation for 12 hours, 90 μL of each oligonucleotide solution was taken out, and 10 μL of 10 % sodium dodecyl sulphate (SDS) was added into each solution to dissociate the complexes from the oligonucleotides. The SDS treated solutions and the free complexes in the dialysis buffer were then analysed by UV/Vis spectroscopy to determine the amount of complexes present inside and outside of the dialysis membrane.

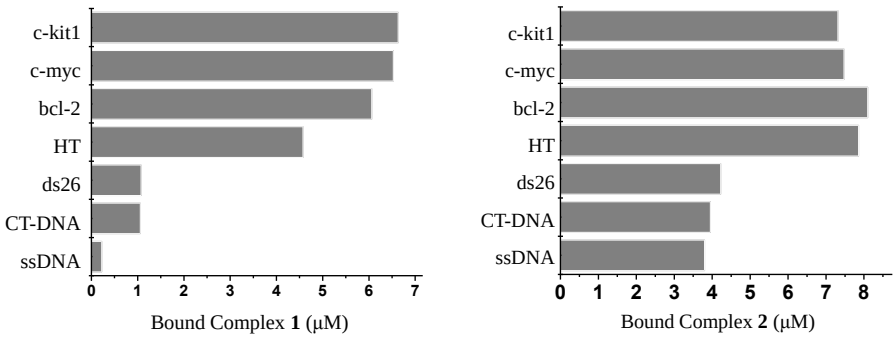


Figure. S6 Competition dialysis of complexes **1** and **2** on various DNA secondary structures.

S7. PCR stop assay[5]

The oligonucleotide c-myc (c-myc-rev) and c-myc-mu (c-myc-mu-rev) were used here. The reactions were performed in 1× PCR buffer, containing 8 pmol of each oligonucleotide, 0.2 mM dNTPs, 2.5 U *Taq* polymerase, and different concentrations of complexes. Reaction mixtures were incubated in a Takara TP600 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. PCR products were then analysed on 15% native polyacrylamide gels in 1× TBE and EB stained, then Typhoo scanner (Trio variable mode imager V5.0) was used for imaging.

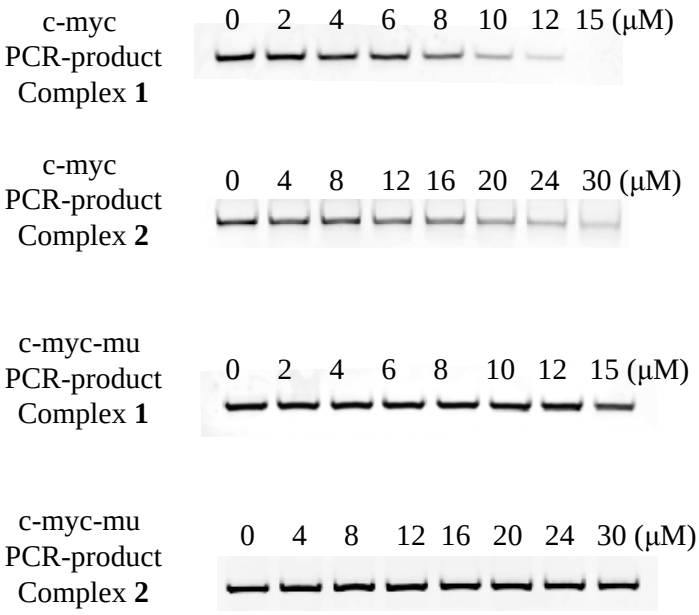


Figure. S7 Inhibitory effects of complex **1** and **2** on c-myc and c-myc-mu templates extension in PCR-stop assay.

S8. Molecular docking of Pt complexes to GQ topological structures

Molecular modeling is carried out to dock complex **1** to parallel (PDB:1XAV), antiparallel (PDB:143D) and mixparallel (PDB:2F8U) G-quadruplexes, respectively, using Glide[6a,b] of the Maestro suite in extra-precision (XP) mode. During flexible docking, T1, G2, and A3 at the 5' end in parallel DNA, and G5 and C6 at the 5' end in mixparallel DNA are allowed to move freely. The relaxation of all-atom docking structures obtained is then implemented under MacroModel[6c] from Schrödinger using Steepest Descent followed by Truncated Newton Conjugate Gradient until the root mean square (RMS) of the energy gradient reaches a value of 0.01 kcal/mol/Å in an OPLS 2005 force field and a distance-dependent dielectric. The calculated core structure of complex **1** (Figure S8c) is close to the reported crystal structure of complex **2**.[7]

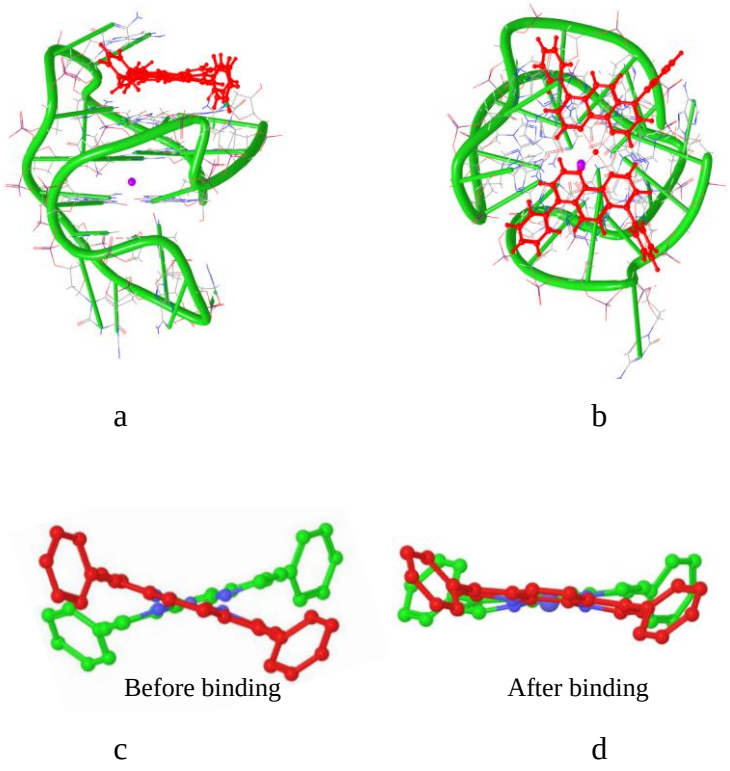


Figure S8. Molecular docking of **1** (red) on mixparallel GQ (backbone: green; base: dim stick), bcl-2 (PDB: 2F8U). (a). side view; (b). top view. (c) and (d) are the structures of complex **1** before and after binding to c-myc GQ.

S9. Reverse transcriptase–polymerase chain reaction (RT-PCR)[8]

Cell Culture: 5×10^5 Hela cells were seeded into each well of the 6 well plate with 1 mL DMEM (10% FBS, 1% L-glutamine and 1% P/S). The cells were cultured at 37 ^oC, 5% CO_2 incubator for 24 hours. After incubation, the treatment groups received 1mL DMEM containing 10 μL complex **1** from different DMSO stock solutions to achieve the indicated concentrations, while the negative control group was treated with 10 μL DMSO, and the positive control group was treated with a final concentration of 100 μM TmPyp4. The cells were incubated in the same condition for another 24 hours. After 24 hours, the cells were harvested for further analysis.

RNA Extraction and RT-PCR: Total RNA was extracted from cells using TRIzol reagent. Reverse-transcription was performed with Iscript cDNA synthesis kit, according to the manufacturer's instructions. PCR reactions were performed using the GoTaq colourless master mix (Promega) and Takara TP600 thermal cycler. The thermal cycle condition was: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 59 °C for 30 s, 72 °C for 30 s, then a terminal extension at 72 °C for 7 min. The PCR products were subjected to electrophoresis on 1.5% agarose gels stained with ethidium bromide. The bands were scanned by a UV scanner (Typhoon) and β-actin was employed as the internal control.

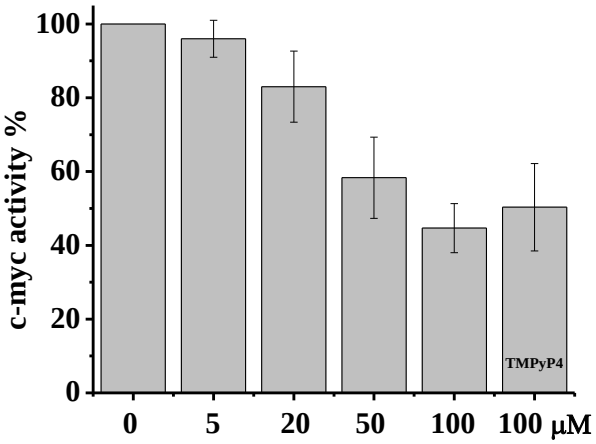


Figure. S9 Percentage of c-myc mRNA level in Hela cells treated with 0-100 μM of complex **1** and 100 μM TMPyP4.

S10. Cytotoxicity test

The cells were seeded in a 96-well flat-bottomed microplate at 5000 cell per well in growth medium solution (100 μL DMEM, 10% FBS, 1% L-glutamine and 1 % P/S). The microplate was incubated at 37 °C with 5% CO_2 in a humidified incubator for 24 hours. The medium was then changed to growth medium with or without complexes with serial concentrations. The microplate was then incubated at 37 °C with 5% CO_2 in a humidified incubator for 48 hours. After 48 hours, MTT (20 μL, 5 mg/mL) was added to each well. The microplate was reincubated at 37 °C in 5% CO_2 for another 4 hours. Then the medium was carried out and 150 μL DMSO was added into each well, the microplates were shaken for 10 mins. The absorbance at the wavelength of 570 nm was measured by a microplate reader (reference wavelength: 630 nm). The IC_{50} value of each complex (the concnetration that is required to reduce the absorbance by 50% relatively to the controls) was analyzed and calculated by SPSS.

Table S2. Cytotoxicities of compounds towards four carcinoma cell lines (MDA-MB-231, HT1080, Hela, HepG2)

Compound	IC_{50}			
	MDA-MB-231	HT1080	Hela	HepG2
1	2.2 (0.4)	2.1 (0.1)	2.9 (0.6)	6.9 (1.3)
2	47.5 (2.8)	11.5 (0.5)	53.0 (2.9)	92.3 (6.5)
TMPyP4	40.9 (5.4)	38.2 (6.3)	38.0 (4.4)	4.9 (0.1)

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