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Supporting Information

A novel square-planar Pt(II) complex as monomeric and dimeric G-quadruplex DNA binder

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Experimental

Generals

^{1}H NMR spectrum was recorded on Bruker Avance 400 MHz Ultrashield NMR spectrometer. ESI-MS and HR-MS were performed on Waters UPLC/Quattro premier XE and Agilent 6460 Triple Quadruple, respectively. IR spectrum was recorded on a Brucker Tensor IR instrument using KBr discs in the range of 400-4000 cm^{-1}. Element analysis for C, H and N were performed on an EA1110 CHNS elemental analyzer. CD spectra and CD-melting experiments were performed on a Chirascan circular dichroism spectrophotomer (Applied Photophysics, UK). UV spectra were performed on a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescent spectra were measured on a Shimadzu RF-5301PC spectrofluorimeter. Polyacrylamide gel electrophoresis (GE) was carried out on a DYY-8C electrophoresis apparatus and DYCZ-24EN electrophoresis capillary (Beijing Liuyi Instrument Factory, Beijing, China). Native GE was analyzed with an Alpha Hp 3400 fluorescent and visible light digitized image analyzer.

The oligonucleotides used in this study were purchased from Shanghai Sangon

Biological Engineering Technology & Services (Shanghai, China). The oligonucleotides G1, G2T1 and Ap-labeled G-quadruplexes (see Table S1), were dissolved in 10 mM Tris-HCl and 100 mM KCl or NaCl (pH 7.0) buffer. Then these oligonucleotides were annealed by heating to 95 °C for 10 min and then cooled to room temperature overnight. Complex **1** was dissolved in DMSO and then further diluted with suitable buffer to the appropriate concentration.

Table S1 DNA sequences used in this study.

DNA	Sequence (from 5' to 3')	Structure
ds 26	CAATCGGATCGAATTCGATCCGATTG + GTTAGCCTAGCTTAAGCTAGGCTAAC	Double stranded
G1	AGGG(TTAGGG)$_3$	G4 (monomeric)
G2T1	AGGG(TTAGGG)$_7$	G4 (dimeric)
A7	AGGGTT*Ap*[a] GGG (TTAGGG)$_2$	G4 (monomeric)
A13	AGGGTTAGGGTT*Ap*GGGTTAGGG	G4 (monomeric)
A19	AGGG(TTAGGG)$_2$TT*Ap*GGG	G4 (monomeric)
Ap7	AGGGTT*Ap*GGG (TTAGGG)$_6$	G4 (dimeric)
Ap13	AGGGTTAGGGTT*Ap*GGG(TTAGGG)$_5$	G4 (dimeric)
Ap19	AGGG(TTAGGG)$_2$TT*Ap*GGG(TTAGGG)$_4$	G4 (dimeric)
Ap31	AGGG(TTAGGG)$_4$TT*Ap*GGG(TTAGGG)$_2$	G4 (dimeric)
Ap37	AGGG(TTAGGG)$_5$TT*Ap*GGGTTAGGG	G4 (dimeric)
Ap43	AGGG(TTAGGG)$_6$TT*Ap*GGG	G4 (dimeric)

[a]*Ap*= 2-aminopurine.

Synthesis of complex 1

Phenanthroimidazole with coumarin derivative **L** (43.6 mg, 0.1 mmol) was mixed with K_2PtCl_4 (50.5 mg, 0.12 mmol) in DMSO aqueous solution (V_{DMSO} = 13 mL and V_{water} = 2 mL), and the resulting mixture was heated at 140 °C for 2 h. The mixture was filtered to obtain a red solid which was washed by DMSO and water. The red solid was mixed with ethylenediamine (EDA, 1 mL) in ethanol (20 mL) and refluxed at 80 °C for 12 h. The

mixture was then poured into water (20 mL) and filtered to remove insoluable substances. The obtained filtrate was added into $CHCl_3$ and appeared the flocculent precipitation which filtered to obtain complex **1** as a red solid (37 mg, 39%). ^{1}H NMR(CD_3OD, 400 MHz): δ 8.94 (s, 2H), 8.68 (s, 2H), 8.51 (s, 1H), 8.32 (s, 2H), 8.21 (s, 1H), 7.70 (s, 2H), 7.61 (s, 2H), 6.85 (d, J = 8.0Hz, 1H), 6.60 (s, 1H), 5.36 (s, 1H), 3.58 (m, 4H), 2.97 (s, 4H), 1.30 (t, J = 6.0Hz, 6H). Main IR (KBr disc, cm^{-1}): v 3435 (m, N-H), 3152, 2323, 1633 (s, C=O), 1400 (s, C=N), 683, 654. ESI-MS: *m/z* 689.2 ([**1**-2Cl-H]$^+$) and HR-MS for $C_{28}H_{29}Cl_2N_7O_2Pt$ ([**1**-2Cl]$^{2+}$) calcd: 345.1010, found: 345.1006. Elemental analysis calcd (%) for $C_{28}H_{29}Cl_2N_7O_2Pt\cdot 0.5EDA\cdot 0.5CHCl_3\cdot 6H_2O$: C 36.97, H 4.68, N 11.69; found: C 36.95, H 4.53, N 11.91. Ligand **L** was prepared according to reported protocols.[1, 2]

CD procedures

CD spectra were measured in a strain-free 10 mm×2 mm rectangular cell path length cuvette. The CD spectra were measured in the spectral range of 500~200 nm. The following CD spectra were recorded: (1) CD spectra of annealed G2T1 (3.0 μM) in 10 mM Tris-HCl and 100 mM NaCl (pH 7.0) with complex **1** (0, 6.0, 12 and 24 μM, respectively); (2) CD spectra of annealed G2T1 (3.0 μM) in 10 mM Tris-HCl and 100 mM KCl (pH 7.0) with complex **1** (0, 6.0, 12 and 24 μM, respectively).

CD melting assays were performed at fixed concentration of G2T1 (3.0 μM) with complex **1** (0, 12 and 24 μM, respectively) in10 mM Tris-HCl and 100 mM KCl (pH 7.0). CD melting assays were performed at fixed concentration of G1 (6.0 μM) and ds 26 (3.0 μM) with complex **1** (0 and 12.0 μM, respectively) in 10 mM Tris-HCl and 100 mM KCl (pH 7.0). CD-melting was recorded at 290 nm for mixed-type G-quadruplexes and at 275 nm for ds 26 at intervals of 5 °C from 25 °C to 95 °C with a heating rate of 1 °C/min. The experiments were repeated three times, and the normalized CD values of the average ± SD were calculated. The melting temperature (T_m) was determined from the melting profiles with the software origin 8.0.

Gel electrophoresis

In order to prepare the final loading sample, complex **1** (100 μM) was mixed with annealed G2T1 and G1 (20 μM) in Tris-HCl buffer (10 mM, 100 mM KCl and pH 7.0) and incubated at 4 °C for 3 h. Native gel electrophoresis was carried out on acrylamide gel (15%), run at 0 °C in 1×TBE buffer (pH 8.3) and was stained by ethidium bromide. DNA binding selectivity was analyzed with Alpha Hp 3400 fluorescent and visible light digitized image analyzer.

UV-Vis titration

Complex **1** (20 μM) was titrated with concentrated solutions of annealed G2T1, G1 and CT DNA (500 μM) in 100 mM KCl buffer. UV-Vis spectra were recorded at room temperature. The data from spectrophotometric titrations were analyzed to give the binding constants (*K*'s) of complex **1** towards mixed-type G1 and G2T1 by linear fitting to Equation (1a).[3]

$$r/C_f = nk - rK \quad (1a)$$

$$r = C_b / C_{DNA} \quad (1b)$$

$$C_b = C_t\,(A\text{-}A_0)/(A_{max} - A_0) \quad (1c)$$

Here, C_t is the total complex concentration, C_b is bound complex concentration, C_f is free complex concentration, A and A_{max} are the observed and maximum absorption values of complex **1** at *ca.* 481 nm with addition of DNA, and A_0 is the observed absorption values of complex **1** at *ca.* 481 nm without addition of DNA. In Eq. 1a, r represents the number of moles of bound complex per mole of DNA, D_f represents the concentration of unbound complex, K is the binding constant, and n is the number of binding sites. The data from spectrophotometric titrations were analyzed to give the binding constant (*K*'s) of complex **1** towards CT DNA by linear fitting to Equation (2).[4]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/[K(\varepsilon_b - \varepsilon_f)] \quad (2)$$

Here, the concentration of DNA is determined by measuring the absorption at 260 nm, the apparent molar extinction coefficient $\varepsilon_a = A_{observed}/[compound]$, ε_f and ε_b correspond to the extinction coefficient of free and DNA-bound compound, respectively. The experiments were

repeated three times, and the *K* values of the average ± SD were calculated.

Fluorescent spectroscopy

Annealed Ap-labeled oligonucleotide (2 μM) was titrated with a concentrated solution of complex **1** (1 mM) in Tris-HCl buffer (10 mM, 100 mM KCl and pH 7.0). Their fluorescence spectra were measured at $\lambda_{ex}/\lambda_{em}$ = 305/370 nm. The experiments were repeated three times, and F/F_0 values of the average ± SD were calculated. Here, F is the fluorescence intensity of Ap-labeled oligonucleotide in the presence of complex **1**, and F_0 is the fluorescence intensity Ap-labeled oligonucleotide without complex **1**.

Molecular modeling studies

Molecular docking studies were carried out using Schrodinger software package (Schrodinger, LLC, New York, NY, USA). The coordinates of telomeric G-quadruplex were generated on based of the NMR structure (PDB ID: 2MB3) and subjected to preparation with the protein preparation wizard. The docking compound was drawn in Maestro and prepared with LigPrep. The docking simulations were performed using Glidewiththe active site generated throughout the G-quadruplex.

References

1. X. Yi, J. Zhao, W. Wu, D. Huang, S. Ji and J. Sun, *Dalton Trans.,* 2012, **41**, 8931.
2. J. Sun, W. Wu, H. Guo and J. Zhao, *Eur. J. Inorg. Chem.,* 2011, **21**, 3165.
3. L. -N. Zhu, B. Wu and D. -M. Kong, *Nucleic Acids Res.,* 2013, **41**, 4324.
4. Z. -Q. Li, T. -C. Liao, C. Dong, J. -W. Yang, X. -J. Chen, L. Liu, Y. -Y. Liang, W. -H. Chen and C. -Q. Zhou, *Org. Biomol. Chem.,* 2017, **15**, 10221.

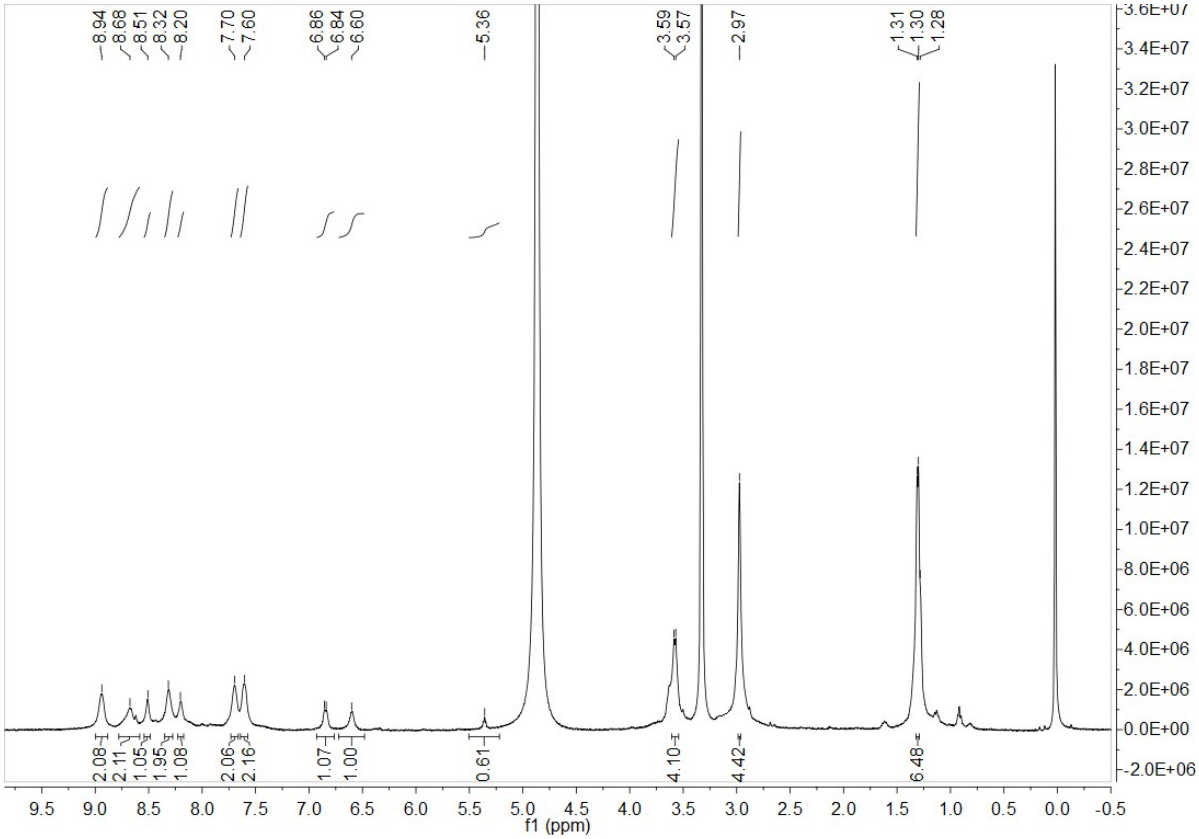

Fig. S1 ^{1}H NMR (400 MHz) of complex **1** in CD_3OD.

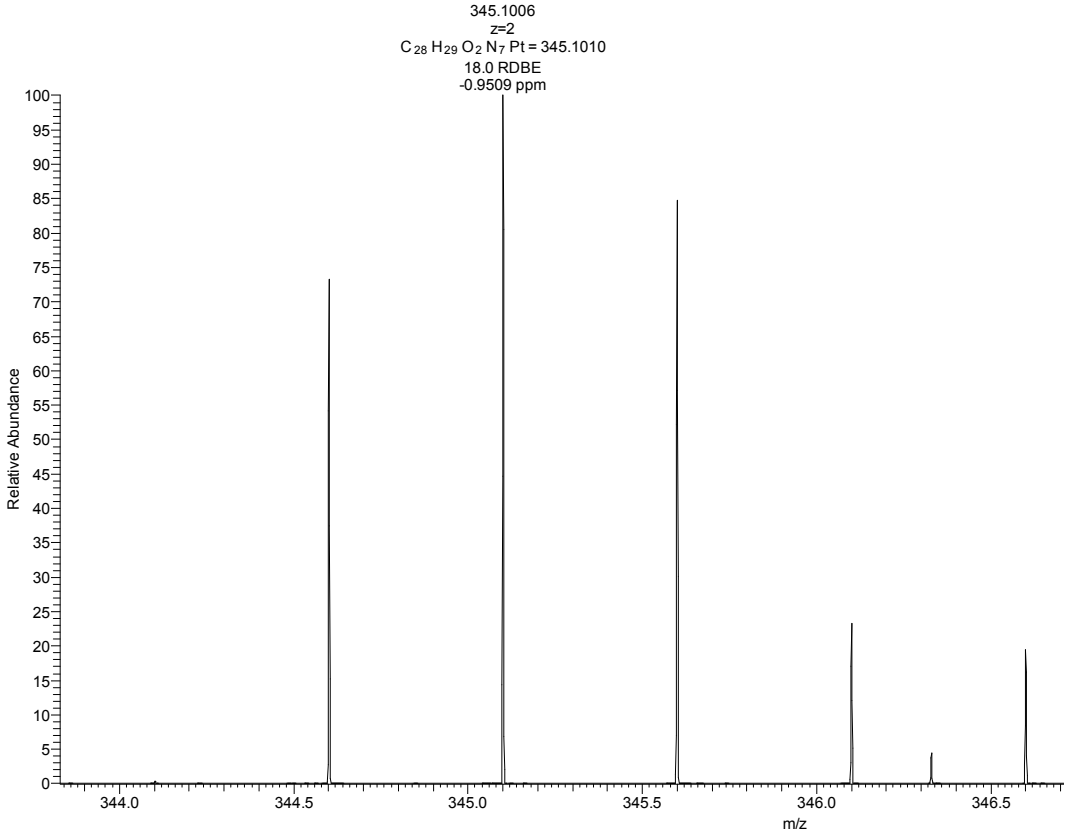

Fig. S2 HR-ESI-MS of complex **1**.

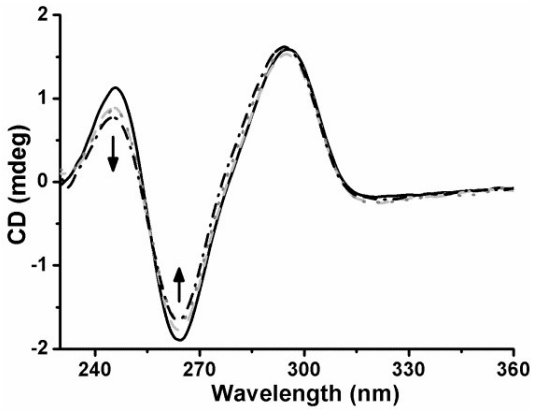

Fig. S3 CD spectra of G2T1 (3.0 μM) in 10 mM Tris-HCl and 100 mM NaCl (pH 7.0) with complex **1** (0~20 μM).

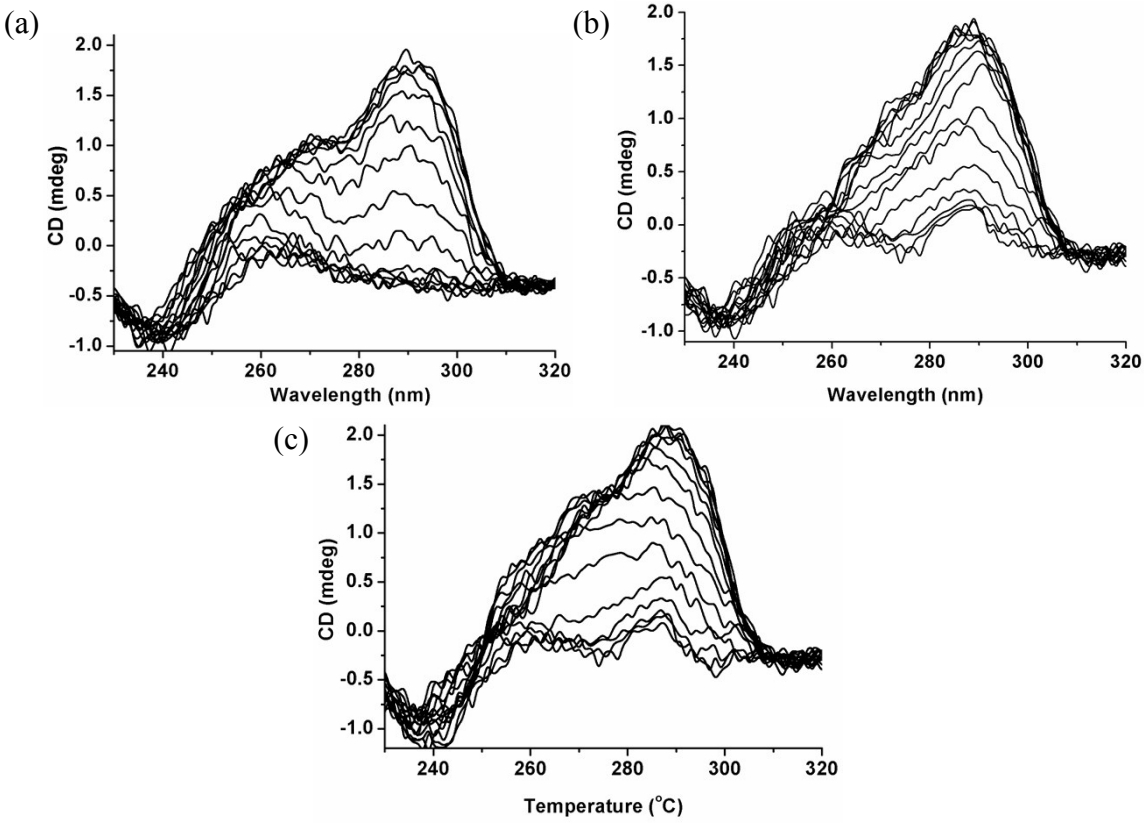

Fig. S4 CD spectra of G2T1 (3.0 μM) from 20 °C to 95 °C without complex **1** (a), with 12.0 μM complex **1** (b) and with 24 μM complex **1** (c) in the buffer of 10 mM Tris-HCl and 100 mM KCl (pH 7.0).

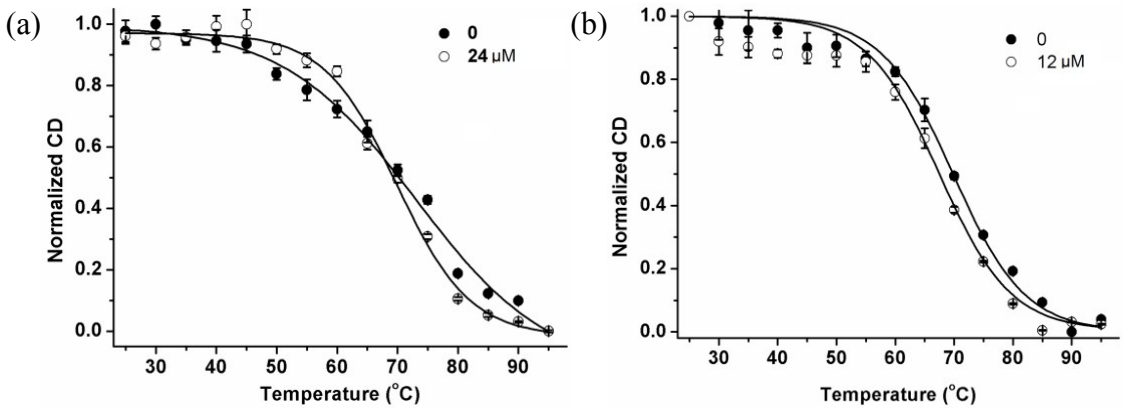

Fig. S5 (a) CD-melting profiles at 290 nm for G1 (6.0 μM) in the absence and presence of complex **1** (24 μM). (b) CD-melting profiles at 275 nm for ds DNA (3.0 μM) in the absence and presence of complex **1** (12 μM). Values are the average ± SD of three independent measurements.

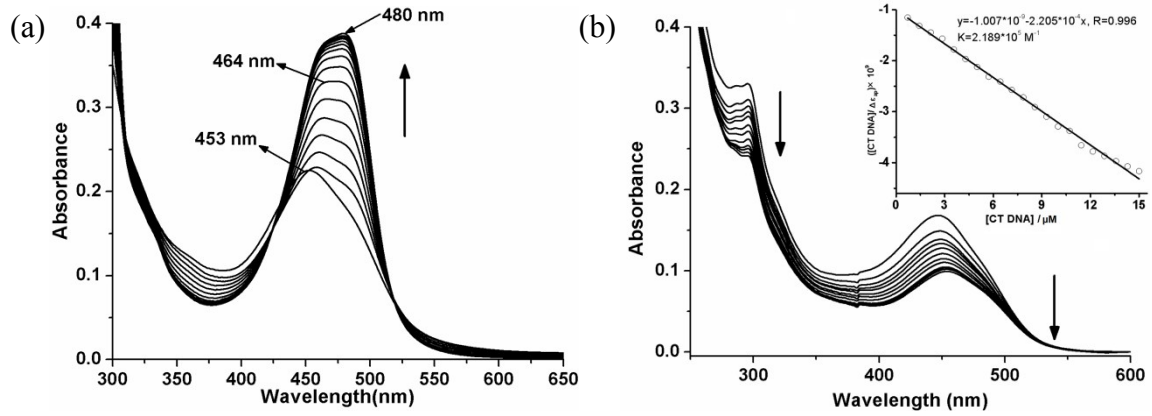

Fig. S6 UV-Vis titration of 20 μM complex **1** with G1 (a) and CT DNA (b) (from 0~25 μM). (b) Inset: a reciprocal plot of ([CT DNA]/$\Delta\varepsilon_{ap}$)×10^9 versus [CT DNA]/μM, $\Delta\varepsilon_{ap}$= ($A_{observed}$−$A_{free\ complex}$) / [complex].

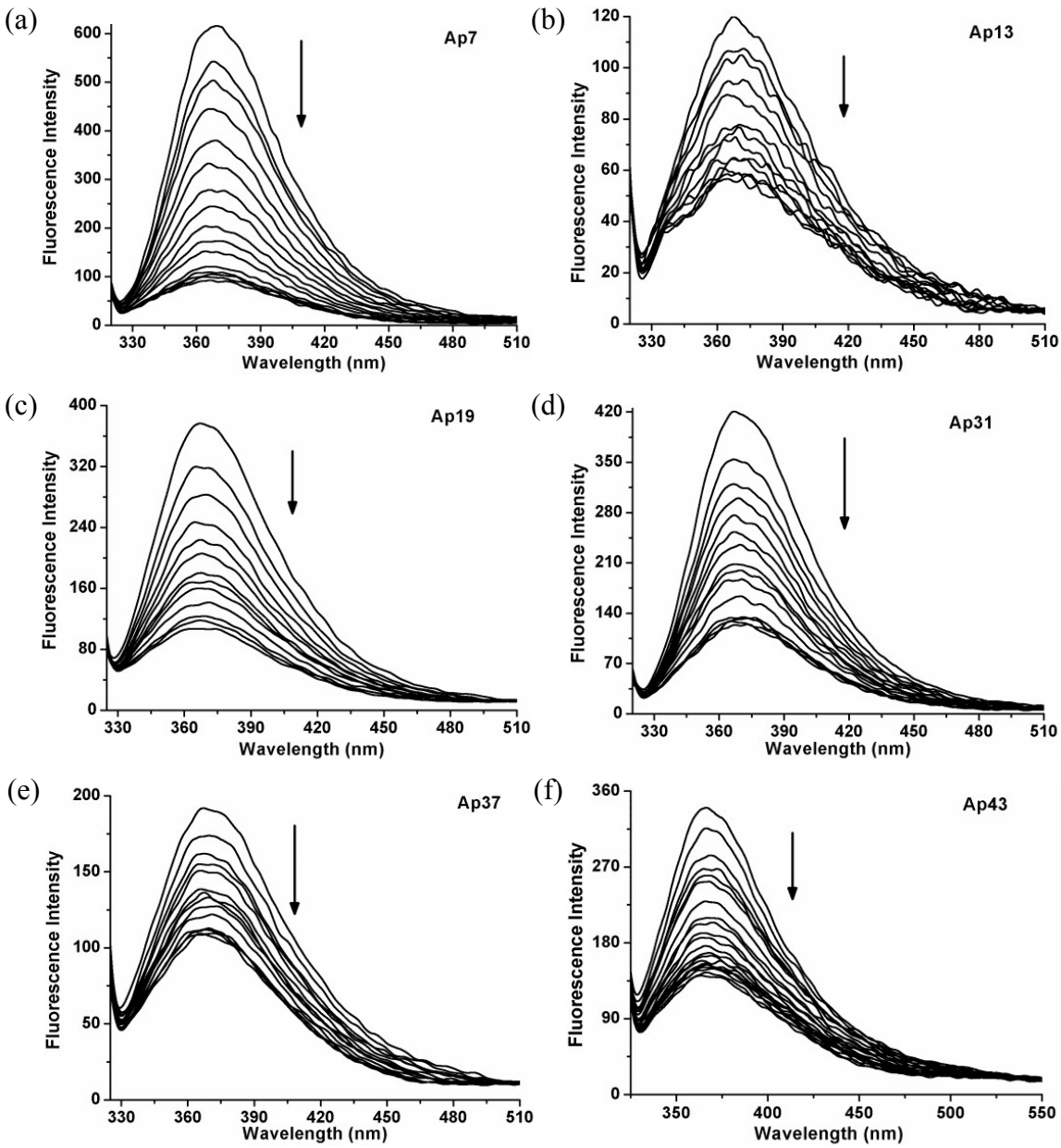

Fig. S7 Fluorescence emission spectra of Ap7 (a), Ap13 (b), Ap19 (c), Ap31 (d), Ap37 (e) and Ap43 (f), respectively, titrated with complex **1**.

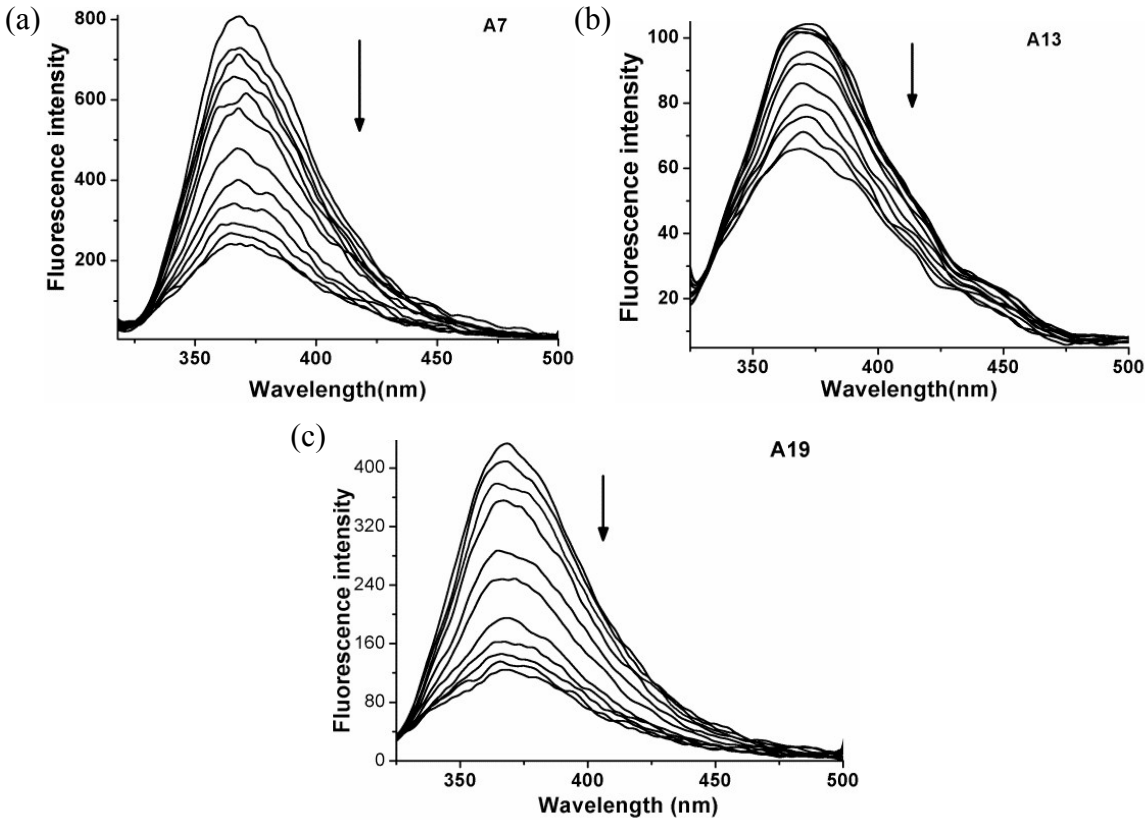

Fig. S8 Fluorescence emission spectra of A7 (a), A13 (b) and A19 (c), respectively, titrated with complex **1**.

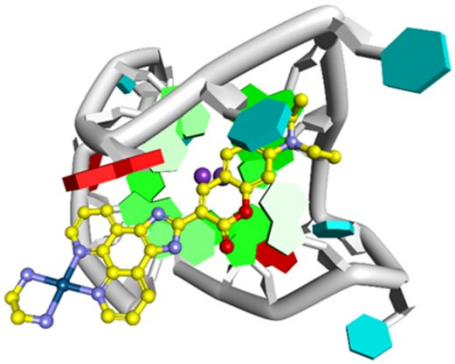

Fig. S9 Docked model of complex **1** with G1 (Hybrid-1) at the 3′-terminal.