Stabilization of Human Telomeric G-Quadruplex and Inhibition of Telomerase Activity by Propeller-Shaped Trinuclear PtII Complexes


Abstract: Two novel propeller-shaped, trigeminal-ligand-containing, flexible trinuclear PtII complexes, [Pt(dien)]_3(1) and [Pt(dpa)]_3(ptp)(NO_3)_6 (2), have been designed and synthesized, and their interactions with G-quadruplex (G4) sequences are characterized. A combination of biophysical and biochemical assays reveals that both PtII complexes exhibit higher affinity for human telomeric (hTel) and c-myc promoter G4 sequences than duplex DNA. Complex 1 binds and stabilizes hTel G4 sequence more effectively than complex 2. Both complexes are found to induce and stabilize either antiparallel or parallel conformation of G4 structures. Molecular docking studies indicate that complex 1 binds into the large groove of the antiparallel hTel G4 structure (PDB ID: 143D) and complex 2 stacks onto the exposed G-quartet of the parallel hTel G4 structure (PDB ID: 1KF1). Telomeric repeat amplification protocol assays demonstrate that both complexes are good telomerase inhibitors, with IC_{50} values of (16.0 ± 0.4) μM and (4.20 ± 0.25) μM for 1 and 2, respectively. Collectively, the results suggest that these propeller-shaped flexible trinuclear PtII complexes are effective and selective G4 binders and good telomerase inhibitors. This work provides valuable information for the interaction between multinuclear metal complexes with G4 DNA.

Keywords: G-quadruplexes · inhibitors · platinum(II) · stabilization

Introduction

Guanine-rich oligonucleotides adopt planar G-quartet configurations stabilized by Hoogsteen hydrogen bonds, and stacking of such G-quartets result in a DNA secondary structure designated as the G-quadruplex (G4).[1] G4 stacking of such G-quartets result in a DNA secondary configurations stabilized by Hoogsteen hydrogen bonds, and Guanine-rich oligonucleotides adopt planar G-quartet conformation, transcription, DNA repair, and recombination.[3] There is great interest in targeting G4 structures in cancer therapy,[4] and a number of small molecules that selectively bind and effectively stabilize G4s have been reported as potential anticancer drugs.[5]

Reported small molecules capable of interacting with G4s include organic molecules and metal complexes, most of which are believed to derive their anticancer activities from stabilizing G4 structures. As G4 binders, metal complexes display a number of superior properties compared with organic molecules, such as versatile structural features (e.g., the possibility of modification for specific geometry), favorable electron-withdrawing properties, the unique photophysical properties of metal centers, and enhanced water solubility. PtII complexes that act as G4 binders have been reported during the past decades. A series of mononuclear PtII complexes, such as Pt-dppz (dppz: dipyrido[3,2-a:2′,3′-c]phenazine), Pt-terpyridine, and Pt-phenanthroline have been shown to preferentially interact with G4 structures. Dinuclear[9] and tetrancular[10,11] PtII complexes have also been demonstrated as effective G4 binders and telomerase inhibitors. However, to the best of our knowledge, studies on flexible trinuclear PtII complexes that target G4 DNA are currently inadequate.

We have previously reported a series of PtII complexes with planar aromatic ligands that act as G4 binders and ex-
Results and Discussion

Selective Binding with G4s by Trinuclear PtII Complexes

SPR experiments were carried out to investigate binding affinity between the trinuclear PtII complexes and different DNA samples.[12] We employed two known G4 sequences, the hTel G4 sequence and a c-myc promoter G4 sequence. In addition, a duplex DNA sequence was also studied as a control.[12b,13] Table 1 presents the kinetic parameters determined using SPR. The results showed that both complexes can selectively and effectively stabilize hTel G4 DNA and show excellent inhibition of telomerase activity.

Table 1. Kinetic parameters determined using SPR.[a,b,c,d]

<table>
<thead>
<tr>
<th></th>
<th>hTel</th>
<th>c-myc</th>
<th>duplex</th>
<th>hTel</th>
<th>c-myc</th>
<th>duplex</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_a ) ([\text{s}^{-1}])</td>
<td>1.92 ( \times 10^3 )</td>
<td>2.18 ( \times 10^3 )</td>
<td>4.66 ( \times 10^3 )</td>
<td>5.80 ( \times 10^3 )</td>
<td>1.95 ( \times 10^3 )</td>
<td>9.29 ( \times 10^3 )</td>
</tr>
<tr>
<td>( k_d ) ([\text{s}^{-1}])</td>
<td>1.06 ( \times 10^{-1} )</td>
<td>1.02 ( \times 10^{-1} )</td>
<td>1.40</td>
<td>1.16 ( \times 10^{-3} )</td>
<td>5.47 ( \times 10^{-2} )</td>
<td>7.33 ( \times 10^{-2} )</td>
</tr>
<tr>
<td>( K_A ) ([\text{M}^{-1}])</td>
<td>5.52 ( \times 10^{-2} )</td>
<td>4.67 ( \times 10^{-2} )</td>
<td>3.00 ( \times 10^{-6} )</td>
<td>3.06 ( \times 10^{-7} )</td>
<td>2.81 ( \times 10^{-7} )</td>
<td>7.88 ( \times 10^{-7} )</td>
</tr>
<tr>
<td>( K_D ) ([\text{M}^{-1}])</td>
<td>1.81 ( \times 10^{-5} )</td>
<td>2.14 ( \times 10^{-5} )</td>
<td>3.33 ( \times 10^{-5} )</td>
<td>3.27 ( \times 10^{-5} )</td>
<td>3.56 ( \times 10^{-5} )</td>
<td>1.27 ( \times 10^{-5} )</td>
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</table>

[a] \( k_a \) is the association rate constant; \( k_d \) is the dissociation rate constant.[b] \( K_A \) was calculated through global fitting of the kinetic data obtained for various concentrations of the complexes by using a two-state binding model; \( K_A \) is given by \( k_a/k_d \). [c] \( K_D \) was determined from \( k_a/k_d \). [c] The \( \chi^2 \) value is a statistical measure of the closeness of the fit. Of all the samples, the hTel DNA was the one that showed the best fit.

Abstract in Chinese:

本文设计和合成两个新的由二元配体联成的螺旋桨型柔丝三核铂(II)配合物[Pt(dien)][(ptp)(NO3)](1)和[Pt(dpa)(ptp)(NO3)](2)，并且开展了它们与G4-DNA的相互作用研究。一系列的生物物理和生物化学实验表明两个铂(II)配合物能够诱导形成并稳定反平行/正平行构型的G4结构，并表现出对人体端粒hTel和启动子c-myc G4序列的亲和力均优于双链DNA。配合物1比配合物2更好地结合并稳定hTel G4。分子对接实验表明配合物1是插入到反平行hTel G4 (PDB ID: 143D)的大沟起作用，配合物2是n-x-n堆积到正平行hTel G4 (PDB ID: 1KFl)裸露的G-四分子体上。端粒重复扩增实验表明二者均有效的端粒酶抑制剂，它们的IC50值分别为16.0 \( \pm \) 0.4 \( \mu \text{M} \)和4.20 \( \pm \) 0.25 \( \mu \text{M} \)。总之，上述实验结果说明这类螺旋桨型柔丝三核铂(II)配合物选择性地结合和稳定G4-DNA是有效的端粒酶抑制剂。本文工作为多核-金属配合物与G4 DNA相互作用的研究提供了有价值的信息。

Trinuclear PtII Complexes Selectively Target hTel G4 DNA Structure

To investigate the effects of PtII complexes on the stabilization of G4 structures, we used a previously reported FRET assay[14] to monitor thermal denaturation (i.e., melting) of various DNA strands in the presence of K+ ions. Studies were carried out on hTel, three distinct G4-forming promoter sequences (c-myc, bcl2 and c-kit), and a duplex DNA. FRET melting curves (Figure 1) and stabilization temperature \((\Delta T_m)\) values (Table 2) were measured in the presence of PtII complexes 0.5 \( \mu \text{M} \). The results showed that complex 1 had little effect on the duplex DNA (\( \Delta T_m = 0.4^\circ\text{C} \)), but induced an appreciable increase in thermal stability (i.e., positive \( \Delta T_m \)) in G4-forming sequences (Table 2). Similar behavior was observed for 2. Furthermore, the stabilization effect of both PtII complexes for G4 sequences were approximately tenfold higher than those measured for the corresponding duplex DNA sequence (Table 1). This indicates that both PtII complexes bind selectively to G4 structures rather than to a duplex DNA.
is based on the PtII complex rather than the ligand itself because control studies showed that the ptp ligand did not stabilize DNA samples studied herein (Figure S7 in the Supporting Information). More remarkably, FRET competition assays showed that 1 and 2 were able to retain 56 and 48%, respectively, of the G4 stabilizing ability in hTel even in the presence of 100 molar equivalents of the duplex DNA competitor (Figure S8 and Table S1 in the Supporting Information). The FRET melting data indicate that both PtII complexes preferably stabilize G4 structures as compared with duplex DNA. This is consistent with conclusions drawn from the SPR studies (Table 1). It is mentioned that the stabilization temperature ($\Delta T_m$) from FRET and the binding constant ($K_D$) from SPR do not have a straightforward relationship because their physical meanings are different but still relevant.[15]

In comparison with the data from SPR in context, FRET provides additional information that the PtII complexes can not only select G4 structure in preference to duplex DNA, but also distinguish hTel from three other G4-forming sequences derived from promoters. Specifically, complex 1 induced a pronounced increase in the $\Delta T_m$ value of 30.2°C for hTel G4, whereas those for c-myc ($\Delta T_m$ 4.9°C), bcl2 ($\Delta T_m$ 4.2°C), and c-kit ($\Delta T_m$ 18.2°C) were relatively small. Complex 2 showed similar behavior, although its $\Delta T_m$ value with respect to hTel (15.4°C) was lower than that of 1 (30.2°C), and the $\Delta T_m$ differences between hTel and other G4-forming sequences were smaller. As compared with previous reports, 1 displayed a stabilization ability toward hTel that is similar to [[Pt-(dpa)3(dptmp)](PF6)5 (30.5°C at 0.5 μM, FRET; dptmp: 4-[4,6-di(4-pyridyl)-1,3,5-(2-triazinyl)]-1-methylpyridine-1-ium hexafluorophosphate)][9a] and tetranuclear platinum(II) complexes (29.0 and 27.4°C at 0.6 μM, FRET).[10b] Together, these data indicate that the two trinuclear PtII complexes selectively target hTel, with complex 1 showing higher selectivity than 2. Note that the platinum(II) side chain of 1 probably interacts more effectively through either electrostatic interaction or hydrogen bonding with the phosphate backbone of DNA (Scheme 1). To some degree, this may account for the observed difference in G4 structure selectivity between the two complexes.

To further confirm G4 stabilization by the two trinuclear PtII complexes, we carried out a PCR-stop assay[16] on the hTel G4 sequence. In these assays, the formation of a G4 structure within the hTel DNA, which can be stabilized by increasing concentrations of PtII complexes, causes a pause in DNA synthesis and results in a reduction in PCR products, which is detected as the lack of a double-stranded DNA (dsDNA) band. As shown in Figure 2, increasing concentrations of 1 and 2 induce a pronounced decrease in the $\Delta T_m$ value of 30.2°C for hTel G4, whereas those for c-myc (4.9°C), bcl2 (4.2°C), and c-kit (18.2°C) were relatively small. Complex 2 showed similar behavior, although its $\Delta T_m$ value with respect to hTel (15.4°C) was lower than that of 1 (30.2°C), and the $\Delta T_m$ differences between hTel and other G4-forming sequences were smaller. As compared with previous reports, 1 displayed a stabilization ability toward hTel that is similar to [[Pt-(dpa)3(dptmp)](PF6)5 (30.5°C at 0.5 μM, FRET; dptmp: 4-[4,6-di(4-pyridyl)-1,3,5-(2-triazinyl)]-1-methylpyridine-1-ium hexafluorophosphate)][9a] and tetranuclear platinum(II) complexes (29.0 and 27.4°C at 0.6 μM, FRET).[10b] Together, these data indicate that the two trinuclear PtII complexes selectively target hTel, with complex 1 showing higher selectivity than 2. Note that the platinum(II) side chain of 1 probably interacts more effectively through either electrostatic interaction or hydrogen bonding with the phosphate backbone of DNA (Scheme 1). To some degree, this may account for the observed difference in G4 structure selectivity between the two complexes.

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**Table 2. Stabilization temperatures $\Delta T_m$ of hTel, c-myc, bcl2, c-kit, and duplex DNA stabilized by both complexes from FRET-based thermal melting.[6]**

<table>
<thead>
<tr>
<th></th>
<th>hTel</th>
<th>c-myc</th>
<th>bcl2</th>
<th>c-kit</th>
<th>duplex</th>
</tr>
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<tbody>
<tr>
<td>$\Delta T_m$ [°C]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30.2 ± 1.2</td>
<td>4.9 ± 0.2</td>
<td>18.2 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>1.25</td>
</tr>
<tr>
<td>2</td>
<td>15.4 ± 1.1</td>
<td>2.0 ± 0.1</td>
<td>10.3 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>1.25</td>
</tr>
</tbody>
</table>

[a] Measurements were carried out with 400 nM DNA and 0.5 μM PtII complexes in 60 mM potassium cacodylate buffer (pH 7.4). Fluorescence melting curves were measured by using a Roche LightCycler II real-time PCR machine, and data analysis was carried out by using Origin 8.0 (OriginLab Corp.).
centrations of complex 1 or 2 did decrease the amount of PCR products, with a complete stop observed in the presence of 3.0 mM of complex 1 or 5.0 mM of complex 2. As a control, the ptp ligand itself showed no effect on PCR at concentrations up to 180 mM (Figure S9 in the Supporting Information). In addition, parallel PCR experiments with a DNA with mutated hTel G4 sequence (see the Experimental Section) showed no dependence on the presence of Pt II complexes, which indicated that both Pt II complexes had no effect on the activity of Taq polymerase (Figure S10 in the Supporting Information). Overall, consistent with the FRET assays, the PCR-stop experiments support the conclusion that both Pt II complexes can stabilize the hTel G4 structure, with complex 1 being more effective than 2.

Conformational Transition of G4 Induced by the PtII Complexes

We carried out CD spectroscopy to qualitatively characterize the structures of hTel in the presence of trinuclear PtII complexes. It is known that the hTel G-rich sequence exists in solution as a mixture of various topologies (single strand, parallel, and antiparallel G4s) depending on the presence or absence of Na+ or K+. Addition of PtII complexes can induce transitions between the different conformations. In the absence of metal ions (Figure 3a, left), titration of complex 1 against hTel induced substantial changes in the CD spectra even at a low complex/DNA ratio \( r = 0.2-1.5 \). An increased amount of complex 1 induced a more pronounced \( \lambda = 295 \) nm positive band and \( \lambda = 265 \) nm negative band. The characteristic spectrum of the antiparallel G4 structure has been reported to show a positive band at \( \lambda = 295 \) nm and a negative band at \( \lambda = 265 \) nm. Thus, these data indicate that, in the absence of metal ions, complex 1 induced and stabilized the antiparallel G4 structure. Furthermore, in Na+-containing solution, hTel pre-exists in the antiparallel conformation and titration of 1 did not alter the characteristic antiparallel G4 CD signature (Figure 3b, left). In K+-containing solution, titration of 1 resulted in gradual appearance of the negative \( \lambda = 260 \) nm signal in the CD spectra (Figure 3c, left). Our results fit well with the previously reported relationship between CD spectral changes and G4 conformational transitions in hTel. Moreover, the ptp ligand did not induce any CD spectral change (Figure S11 in the Supporting Information). Overall, we conclude that complex 1 probably induces and stabilizes the antiparallel-stranded topology of the hTel \( d\text{[AG}_{3}	ext{T}_{2}	ext{AG}_{3}] \) sequence.

Titration of complex 2 against hTel showed different CD spectral changes compared with those observed for complex 1. In the absence of metal ions, increasing concentrations of 2 resulted in a more and more pronounced \( \lambda = 240 \) nm negative band and \( \lambda = 275 \) nm positive band (Figure 3a, right), both are signatures of a parallel G4 structure. In the presence of Na+ solution (Figure 3b, right), hTel pre-existed in

![Figure 3. CD spectra of 1 (left) and 2 (right) with a 3.0 µM solution of G-quadruplex in the presence and absence of metal cations (10 mM TrisHCl, pH 7.4, RT; \( r = C_{\text{Pt}}/C_{\text{DNA}} \). a) In the absence of metal ions; b) in 100 mM NaCl; c) in 100 mM KCl.](https://www.chemasianj.org)
the antiparallel conformation in the absence of PtII complexes, and titration of 2 weakened the antiparallel signatures (i.e., negative $\lambda = 265$ nm band and positive $\lambda = 295$ nm band), with a concomitant rise in the parallel signatures (i.e., negative $\lambda = 240$ nm band and positive $\lambda = 275$ nm band). Finally, in the presence of the K$^+$ solution (Figure 3c, right), a parallel G4 signature again appeared as complex 2 was increased in concentration. Overall, CD data suggest that 2 might induce the antiparallel-to-parallel transition for G4 structure and stabilize parallel G4 conformation, which has also been observed for other PtII complexes.$^{[10]}$

Last but not least, the stoichiometries of the PtII complexes binding to the hTel G4 structure were obtained from the CD titration plotting curves, that is, the changes in the molar ellipticity values of the characteristic CD band as a function of titrated PtII complexes.$^{[12b,20]}$ Analysis showed that 1 has a complex/G4 stoichiometric ratio of 1.5, whereas that of 2 is 2.5 (Figure S12 in the Supporting Information). These values fall within the range previously reported for other PtII complexes.$^{[20]}$

### Molecular Docking Analysis

Molecular docking$^{[11]}$ studies were carried out to study the binding modes between the PtII complexes and hTel G4 structures. We focused on the interaction between the two PtII complexes and hTel because hTel is one of the most important targets in developing anticancer drugs, and both complexes showed a strong selectivity in targeting hTel (Figures 1 and 2, Table 2). The antiparallel basket-type NMR spectroscopy structure (Protein Data Bank (PDB) ID: 143D$^{[1a]}$ and the parallel crystal structure (PDB ID: 1KF1$^{[1b]}$) of hTel DNA sequencing d(AG$_3$[T$_2$AG$_3$]$_3$) were employed as the receptors in our modeling. The simulations showed that complex 1 preferentially binds into the large groove of the antiparallel G4 structure, with the positively charged PtII side chains interacting with the negatively charged sugar phosphate backbone (Figure 4a). On the other hand, complex 2 preferably stacks onto the exposed G-quartet of the parallel G4 structure, with the charged PtII side chains penetrating the G-quadruplex grooves to establish electrostatic and hydrogen-bonding interactions with the DNA (Figure 4d). Furthermore, the simulations show that with the antiparallel G4, the total score of 1 is higher than that of 2 (11.15 and 8.35 for 1 and 2, respectively), whereas for the parallel G4, 2 has a higher score than 1 (9.96 and 12.46 for 1 and 2, respectively). This supports the notion that 1 has higher binding affinity for antiparallel hTel G4 and 2 preferentially binds to parallel hTel G4. These data are consistent with the results from the CD studies.

### Inhibition of Telomerase Activity

TRAP assays have been widely used to evaluate the inhibition of telomerase.$^{[21,22]}$ As previously reported, the scientific procedure includes three steps; an initial primer elongation by telomerase in the absence or presence of small molecules, removal of the small molecules, and PCR amplification of the telomeric products.$^{[22]}$ Here we carried out modified three-step TRAP-LIG$^{[22]}$ assays to assess the ability of these two trinuclear PtII complexes to target telomerase activity and inhibit telomere elongation. The results clearly showed that both PtII complexes exhibit effective inhibition towards telomerase activity in a concentration-dependent manner (Figure 5). The $IC_{50}$ values were determined to be (16.0±0.4) $\mu$m and (4.20±0.25) $\mu$m for 1 and 2, respectively. Both PtII complexes displayed inhibitory activity towards telomerase comparable to nickel(II) and copper(II) salphen complexes.$^{[22d]}$

### Conclusion

We have synthesized and characterized two novel propeller-shaped flexible trinuclear PtII complexes. Biophysical and biochemical analysis confirmed that both PtII complexes preferred binding to G4 sequences and were capable of inducing and stabilizing particular conformations of G4 structures. Interestingly, complex 1 exhibited a high selectivity in targeting the hTel G4 sequence, which might arise from its ability to bind into the large groove of the G4 structure as revealed by molecular docking. Furthermore, both PtII complexes displayed strong inhibition towards telomerase activity. These data established the flexible trinuclear PtII complex as a new class of complexes in targeting G4 sequences, particularly those residing at the telomere. This study lays a strong foundation for further optimization and structure–function relationship investigation in the development of more efficient anticancer drugs.
Figure 5. Inhibition of telomerase activity by 1 and 2 assessed in vitro by using a three-step TRAP-LIG. Activity was measured by the telomeric ladders produced by PCR amplification of oligonucleotides generated by the action of telomerase on a TS primer, with a longer ladder indicating a higher telomerase activity. The lower band marked as ITAS is an internal control primer. Each single assay was carried out using 1.0 mL of telomerase extract (1000 cells), prepared from HeLa cells with NP-40 lysis buffer. Negative controls 1 and 2, which accompanied every assay, were obtained by using either heat-inculated cell lysate or NP-40 lysis buffer only, respectively (see the Experimental Section). The control assay was performed without adding any complex.

Experimental Section

Materials

All chemicals and solvents were commercially available and used without further purification.

Synthesis of ptp

The ptp ligand was synthesized according to literature procedures (yield: 50%). The obtained product was confirmed by 1H NMR spectroscopy, ESI-MS, and elemental analysis. 1H NMR (300 MHz, CDCl 3, 25°C, TMS): δ = 8.32 (d, J = 9.0 Hz, 1H, H 3), 9.00 (s, 1H, H 5), 5.80 (d, J = 9.0 Hz, 2H), 8.04 (d, J = 9.0 Hz, 1H), 7.93 (s, 2H), 7.51–7.44 ppm (m, 3H); ESI-MS (CHCl 3): m/z calecd for C 20H 14N 4: 311.35 ppm, with K 2PtCl 4 as the internal ref-

Synthesis of Complex 1

[Pt(dien)Cl]Cl 2 (97.7 mg, 0.2646 mmol) and AgNO 3 (89.0 mg, 0.53 mmol) in water (10 mL) were stirred in a stoppered flask in darkness for 48 h at 45°C and then filtered to remove AgCl. The clear filtrate was subsequently transferred into another stoppered flask, in which ptp (28.63 mg, 0.09 mmol) was added, followed by heating at 80°C for 48 h under N 2. The whole experiment was conducted in the dark. The reaction solution was collected by filtration and then concentrated to about 0.5 mL, after which excess ethyl alcohol (about 80 mL) was added. The resulting mixture was concentrated under vacuum until the pale yellow product precipitated. Finally, the resulting slurry was filtered and the product was dried under vacuum (yield: 85%, 60%). 1H NMR (400 MHz, D 2O, 25°C, TMS): δ = 9.42 (s, 2H), 9.22 (s, 1H), 8.76 (d, J = 8.0 Hz, 3H), 8.67 (d, J = 8.0 Hz, 2H), 8.42 (d, J = 8.0 Hz, 1H), 8.24 (s, 2H), 7.65 (t, J = 8.0 Hz, 3H), 3.25–2.78 ppm (m, 24H); 13C NMR (500 MHz, D 2O, 25°C, TMS): δ = 135.9, 152.8, 152.7, 152.1, 151.0, 146.2, 138.7, 138.6, 137.3, 136.4, 127.3, 127.1, 120.5, 53.9, 50.3 ppm; 195Pt NMR (1H, 400 MHz, D 2O, 25°C): δ = −121.87 ppm, with K 2PtCl 4 as the internal ref-

Oligonucleotides

The employed FRET probes included a fluorescently labeled hTel oligonucleotide that mimics the human telomeric repeat (5′-FAM-[GGGTAGGTAGGTAGGTAGG]-TAMRA-3′ (FAM: 6-carboxy-fluorescein, TAMRA: 6-carboxytetramethylrhodamine), HPLC purified, Sangon, Shanghai, China), three promoter sequences (c-myc: 5′-FAM-[TGGGGAGGGTGAGGGTGGGG]-TAMRA-3′; bcl2: 5′-FAM-[AGGGGCGGGCGCGGGAGGGGAGGGGAGGGG]-GCTG-TAMRA-3′; c-kit: 5′-FAM-[AGGGGCGGGCGCTGGGGAGGGA]-GCTG-TAMRA-3′, HPLC purified, Sangon, Shanghai, China), and a duplex DNA sequence (5′-FAM-TATAGCTATA-HEG-TATAGCCTA-

Surface Plasmon Resonance Studies

SPL measurements were conducted by using a ProteOn XPR36 protein interaction array system (Bio-Rad Laboratories, Hercules, CA, USA) with a Neutravidin-coated GLM sensor chip. Biosensor experiments were conducted in filtered and degassed running buffer (50 m TrisHCl, 0.005% Tween-20, pH 7.4) at 25°C. DNA samples involved in these assays were captured (approximately 1200 RU) in flow cells 1, 2, and dptmp, leaving the fourth flow cell as a blank. Ligand solutions (in these assays were captured (approximately 1200 RU) in flow cells 1, 2,

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FLUORESCENCE RESONANCE ENERGY TRANSFER STUDIES

The FRET probes were diluted in Tris-HCl buffer (10 mM, pH 7.4) that contained potassium cacodylate (60 mM) and annealed by heating to 95°C for 5 min, then gradually cooled to RT overnight. Fluorescence melting curves were measured by using a Roche LightCycler 2.0 real-time PCR machine in a total reaction volume of 20 μL, which contained the complexes (0.5 μM) and labeled oligonucleotides (0.4 μM) in buffer solution. Measurements were conducted with excitation at λ = 470 nm and detection at λ = 530 nm. Fluorescence data were recorded at intervals of 1°C over a temperature range of 37–99°C, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable signal. Fluorescence melting curves were determined by using a Roche LightCycler II real-time PCR machine, and final analysis of the data was carried out by using Origin 8.0 (OriginLab Corp.).

Polymerase Chain Reaction Stop Assays

The reactions were performed in 1 × PCR buffer, which contained 10 pmol of each pair of oligonucleotides, 0.2 mM dNTPs (TaKaRa), 2.5 U of Taq polymerase (TaKaRa), and different concentrations of the complexes. The reaction mixtures were incubated in a Bio-Rad S1000 (Bio-Rad Inc., USA) thermal cycler under 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. The amplified PCR products were then analyzed on a 1% agarose gel in 1 × TBE (Tris-Borate/EDTA) buffer, following by silver staining. A parallel experiment was performed by using a mutated oligomer, hTel-mu, with its corresponding complementary sequence hTel-mu-rev instead of hTel and hTel-rev under the same conditions.

CIRCULAR DICHROISM MEASUREMENTS

CD spectra were measured by using a J-810 spectropolarimeter (JASCO, Japan) with a 1 cm long quartz cell, over a wavelength range of 190–360 nm. CD spectra were measured by using a mutated oligomer, hTel-mu, with its corresponding complementary sequence hTel-mu-rev instead of hTel and hTel-rev under the same conditions.

MOLECULAR MODELING METHODS

Molecular docking procedures were performed by using the Surflex-Dock suite (SFXC) module in SYBYL-X 1.0 (Tripos, St. Louis, MO, USA). Molecular models of both PtII complexes were built by geometry optimizations by using the Gaussian 09 program, in which the B3LYP density functional method with the basis set LanL2DZ and the relativistic pseudopotential LanL2DZ for the PtII atoms, and 6-31G* for the other atoms, were utilized. Their electrostatic potential fitting charges were also optimized at the same level. Receptors G4 DNA were extracted from the NMR structure of antiparallel G4 and the crystal structure of parallel G4 from human telomeric DNA sequencing dA(dT)22. The Surflex-Dock total score and patented search engine (Surflex-Dock) were used during the docking procedure. The images in the manuscript were created from SYBYL with the intercalation into the large groove of the G4 structure.


