1,10-Phenanthroline platinum(II) complex: a simple molecule for efficient G-quadruplex stabilization†

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Two simple complexes $[Pt(phen)_2](PF_6)_2$ and $[Pt(bpy)_2](PF_6)_2$ exhibit high stabilization potential for G-quadruplex DNA with $[Pt(phen)_2](PF_6)_2$ possessing higher capability due to its greater planarity.

Biologically significant four-stranded nucleic acid forms G-quadruplex configurations, containing stacks of G-tetrads; these nucleic acids form a planar structure with four guanines in a circle using Hoogsteen hydrogen bonds.[1] Human telomeric DNA includes tandem repeats of the sequence 5′-d(TTAGGG)-3′ that are short and rich in guanine residues.[2] In the presence of metal ions such as K^+ or Na^+, telomeric DNA can form G-quadruplex structures.[3]

Telomerase is active in about 85% of tumors that lead to cancer in an infinite lifetime.[4] Because telomerase needs a single-stranded telomeric DNA primer,[5] the formation of G-quadruplex complexes by telomeric DNA can inhibit the telomerase activity.[6] Thus, the use of small molecules to target and stabilize G-quadruplexes is emerging as a promising way to interfere with telomerase activity in tumor cells and to act as potential anticancer agents.[7]

An effective G-quadruplex stabilizing molecule should possess several attributes: an appropriately large electron deficient π-aromatic surface that can stack on the face of quadruplex; a positively charged area which can reside close to the center of the guanine quartet; positively charged substituents that can interact with both the grooves and loops of the quadruplex and the negatively charged phosphates backbone.[8] So far, only a few Pt(II) complexes have been applied as G-quadruplex stabilizers.[9] Sleiman and co-workers reported that Pt(II) complexes with extended aromatic ligands could provide π-surfaces that were more compatible with G-quartet motif.[9c] Another significant complex, [Pt(dppz-COOH)(N-C)]CF_3SO_3 (dppz-COOH = 11-carboxydipyrido [3,2-*a*:2′,3′-*c*]phenazine, N-CH = 2-phenylpyridine), was demonstrated as an efficient human helomerase inhibitor and luminescent probe for G-quadruplex DNA, as reported by Che and colleagues.[9e] Herein, we use two simple platinum(II) complexes, [Pt(1,10-phenanthroline)]$(PF_6)_2$ (**1**) and [Pt(2,2′-bipyridine]$(PF_6)_2$ (**2**) (see Fig. 1) to investigate their abilities to stabilize G-quadruplexes.

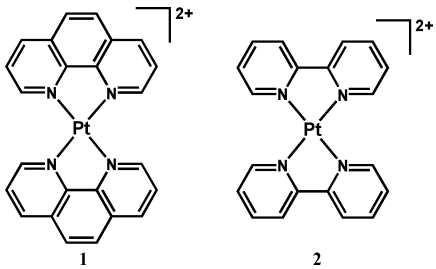

Fig. 1 Schematic drawings of complexes **1** and **2**.

The two complexes are prepared according to previous reports,[10] both containing square π-aromatic surfaces around platinum(II) ions in the center of the complexes.

CD assays have been used to investigate structural conversion between various kinds of human telomeric quadruplexes, including intra- and intermolecularly in parallel, and mixed arrangements, depending on the strand orientation.[11,12] In our studies, a human telomeric DNA (22AG (5′-$AG_3(T_2AG_3)_3$-3′) was titrated with both **1** and **2** and monitored by CD. Fig. 2(a) and 2(b) show that in the presence of potassium cations, the quadruplex molecules exist as a mixture of parallel and antiparallel G-quadruplex conformations.[13] When complexes **1** and **2** were added from 0–4.8 μM and 0–24 μM, respectively, the positive band at about 290 nm and the negative band at about 260 nm significantly increased and reached saturation. These data clearly suggest that both complexes, especially **1**, strongly stabilize antiparallel G-quadruplex conformation.[14] Meanwhile, the peak near 234 nm (Fig. 2(a)), which is typical of a parallel G-quadruplex structure,[13a,15] became ambiguous with increased addition of complex **1**. This interesting trait suggested the conversion of G-quadruplexes from parallel to antiparallel in the presence of complex **1**. As shown in Fig. 2(b), such conversion didn't take place in the presence of complex **2**. On the other hand, in the absence of any salt, the CD spectra indicate the coexistence of single strand, parallel and antiparallel G-quadruplexes;[13] larger changes in the CD spectrum were observed with addition of **1** (0–3.0 μM) and **2** (0–6.0 μM) to 22AG (Fig. 2(c) and Fig. 2(d)). It can be seen that the maximum at 254 nm was gradually suppressed and shifted to 245 nm, while the bands centered at about 293 nm and 263 nm increased sharply along with increases of complexes **1** and **2**. Finally, the induced CD spectra by **1** and **2** virtually resemble that of antiparallel G-quadruplexes. The addition of only 1,10-phenanthroline or 2,2′-bipyridine doesn't induce obvious changes in CD spectra (Fig. S2, ESI†) with or without any salt.

Results from these CD experiments suggest that **1** and **2** can convert the preformed hybrid-type G-quadruplexes structure into antiparallel G-quadruplexes; that is to say, they interact

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† Electronic supplementary information (ESI) available: Syntheses of complexes **1** and **2**. Experimental procedures for CD spectra, FRET and PCR stop assay. See DOI: 10.1039/c0dt00211a

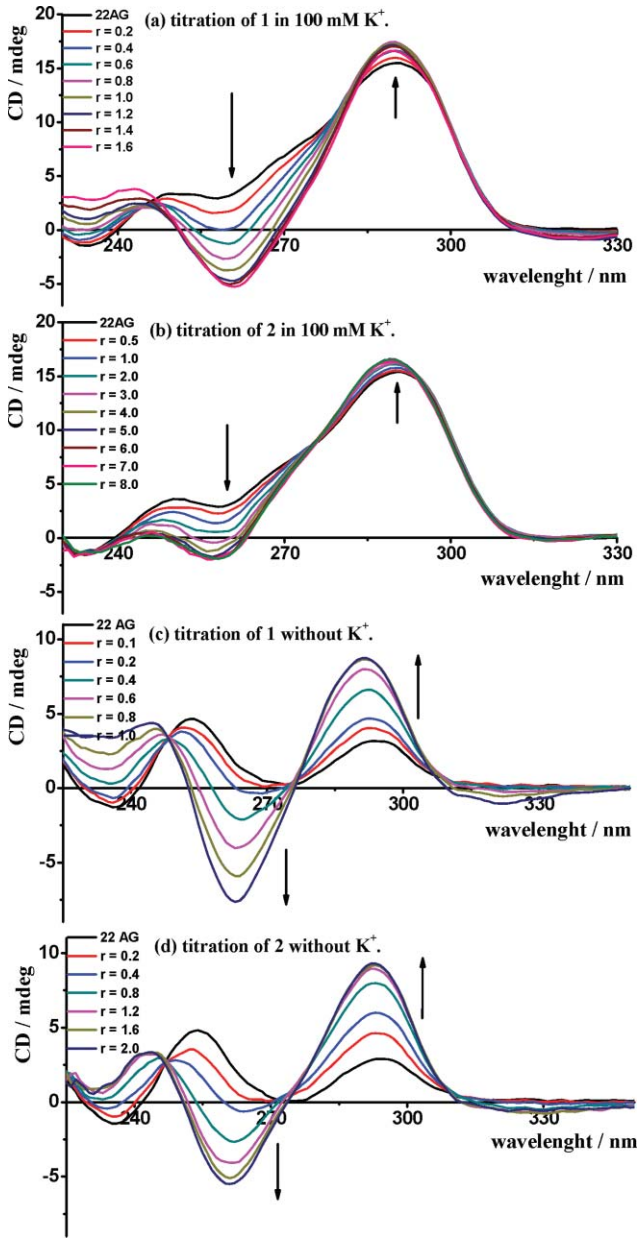
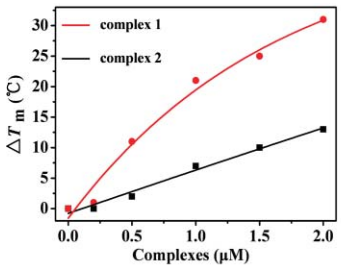


Fig. 2 CD titration spectra of 22AG quadruplex (3 μM) at increasing concentrations of **1** and **2** in 10 mM Tris-HCl buffer, pH 7.4, 100 mM KCl and no metal cations, rt. The arrows indicate the increasing amounts of complexes (r = compound/DNA strand concentration). (a) Complex **1**: r = 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 in 100 mM KCl; (b) complex **2**: r = 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 in 100 mM KCl; (c) complex **1**: r = 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 in the absence of salt; (d) complex **2**: r = 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 in the absence of salt.

preferentially with the antiparallel quadruplexes. Compound **1** is especially more efficient at inducing the formation of antiparallel G-quadruplexes at very low concentration (< 4.0 μM), and even leads to the conversion of parallel G-quadruplexes to antiparallel G-quadruplexes. To the best of our knowledge, this is a very high level CD signal conversion and is rare among the molecules that stabilize G-quadruplexes.[14]

The binding abilities of **1** and **2** to G-quadruplex DNA F21T (sequence: *FAM*-G$_3$[T$_2$AG$_3$]$_3$-*TAMRA*, mimicking the human telomeric repeat) were also investigated by FRET (fluorescence resonance energy transfer) melting assay.[16] As shown in Fig. 3, complex **1** induces a high degree of stabilization for quadruplex-DNA, as demonstrated by an increase in melting temperature (ΔT_m) of 21 °C at 1 μM. This value is competitive with another two platinum(II) complexes, such as modified phenanthroline-platinum complex (20 °C at 1 μM, FRET)[9a] and [Pt(dppz-COOH)(N-C)]CF$_3$SO$_3$ (14 °C, UV melting study);[9e] however, this result was inferior to another tetranuclear platinum(II) complex [Pt(en)(4,4′-dipyridyl)]$_4$(NO$_3$)$_8$ (34.5 °C at 0.75 μM, FRET).[9b] When compared to **1**, complex **2** (1 μM, ΔT_m = 7 °C) is not a very effective quadruplex-DNA stabilizer, and this activity difference is consistent with CD studies. Likewise, the ligands themselves cannot increase the melting temperature of F21T (1 °C and 0 °C for 1 μM phen and bpy, respectively (Fig. S3, ESI†)).



Complexes (μM)	ΔTm(℃) 1	2
0.2	1	0
0.5	11	1
▲ 1.0	21	7
1.5	25	10
2.0	31	13

(b)

Fig. 3 (a) Concentration dependent melting curves (ΔT_m *vs.* complex concentration) for **1** and **2** upon binding to DNA F21T. (b) ΔT_m values at various concentrations of **1** and **2**.

In order to further evaluate the ability of complexes **1** and **2** to stabilize G-quadruplex DNA, polymerase chain reaction (PCR) stop assay was used to ascertain whether complexes are bound to the test oligomer (5′-G$_3$(T$_2$AG$_3$)$_3$-3′) and stabilized the G-quadruplex structure.[17] Fig. 4 shows that in the presence of complexes **1** and **2**, the template sequence formed into G-quadruplex structures that the PCR product could not detect. The inhibitory effect of **1** is clearly enhanced as the concentration is increased from 3.0 to 12.0 μM with no PCR product detected at 12.0 μM (Fig. 4, left). Meanwhile, more than 20 μM of **2** could completely inhibit the appearance of PCR product, which also indicates that complex **1** is a better G-quadruplex binder. Furthermore, the ligands 1,10-phenanthroline and 2,2′-bipyridine cannot inhibit the appearance of PCR product at all (Fig. S4, ESI†).

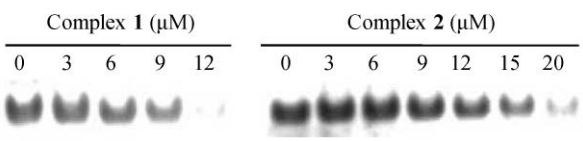


Fig. 4 Effect of complexes **1** (0–12 μM, left) and **2** (0–20 μM, right) on the hybridization of HTG 21 in the PCR-stop assay.

All the above results have shown that complex **1** is a good G-quadruplex inducing and stabilizing molecule, and better than complex **2**. As complex **1** itself is a very simple molecule, structural analysis is helpful to reveal the relations between the complex and G-quadruplex. Fig. 5 shows some useful distances of complex

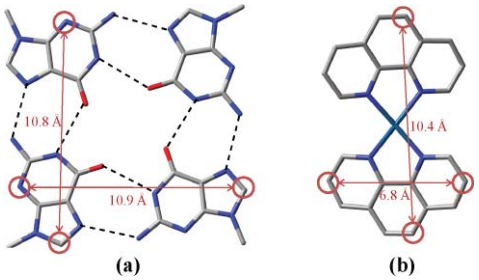


Fig. 5 (a) Structure of stabilized G-quadruplexes. Selected distances of the structure of the parallel 22-mer telomeric G-quadruplex (PDB code 1KF1) are evaluated by GaussView program. (b) Structure of complex **1** and the selected distances are from the reported X-ray crystal structure.

1[18] and telomeric G-quadruplex.[11] Complex **1** contains a square π-aromatic surface with the cation located in the center of the structure. In addition, the maximal length of **1** is 10.4 Å which is very close to the 10.8 Å of the G-quartet. This may explain why complex **1** can effectively induce the formation of G-quadruplex. As the structural rigidity was a key parameter for quadruplex recognition,[19] it is understandable that complex **1** was a better G-quadruplex stabilizer than complex **2**.

In conclusion, we have described a complex that significantly stabilizes G-quadruplex and can even convert parallel G-quadruplex to antiparallel G-quadruplex. The difference in activities between complexes **1** and **2** convinced us that the planarity of the molecules is vital for G-quadruplex recognition in binding. Complex **1** is also easily prepared, which we believe is an important asset for every potential drug. Furthermore, we expect that some modifications of this simple molecule, such as adding protonated substituents around the ligand, would generate a better G-quadruplex stabilizer.

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