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COMMUNICATION

Platinum(II)-triarylpyridines complexes with electropositive pendants as efficient G-quadruplex binders†

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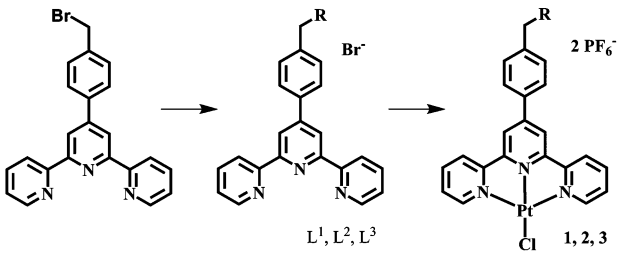
Herein we reported three new platinum(II)-triarylpyridines complexes with peralkylated ammonium pendants that strongly stabilize G-quadruplex DNA.

G-quadruplex DNA sequences, formed by biologically significant four-stranded nucleic acid, have received considerable attention in several research fields including chemistry, biology, and pharmacology in the last decade.[1] Such DNA sequences have an unusual planar structure with four guanines in a circle through Hoogsteen hydrogen bonds.[2] In human telomeric DNA, the G-quadruplex DNA has just a short sequence 5′-d(TTAGGG)-3 and is tandemly repeated in rich guanine residues domains. The formation of G-quadruplex complexes can prevent telomerase elongation of telomeres by interrupting the interaction between the enzyme and unfolded guanine-rich single strand.[3] Therefore, the use of small molecules that promote the formation and/or stabilize G-quadruplex structures has become attractive for anticancer drug design.[4]

Many reports have shown that an effective G-quadruplex-stabilizing molecule exhibits unique characteristics, including a large electron-deficient π-aromatic surface that can stack on the quadruplex, a positively charged area that can reside closely to the center of the guanine quartet, or positively charged substituents that can interact with both the grooves and loops of the quadruplex and negatively charged phosphates backbone.[5] In order to optimize the above-mentioned features, metal complexes are usually employed. For example, a platinum(II) ion has positive divalent charges and usually forms a planar square complex with an appropriate ligand. These features make them desirable for G-Quadruplex stabilization. However, only a few Pt(II) complexes have been applied in this field.[6] Sleiman and co-workers found Pt(II) complexes with extended aromatic ligands to provide π-surfaces that were more compatible with G-quartet motif.[6d] The same case also took place in the Pt(II) complexes reported by us.[6i]

Recently, a triarylpyridine ligand family has been found to have a general capacity to interact with G-quadruplex DNA sequences.[7] Their elementary unit is composed of four aromatic rings, which form a large planar π-conjugated surface. To become more electrophilic and extend planarity to the ligands, herein, we add different peralkylated ammonium pendants to the triarylpyridine ligand and synthesize their platinum(II) complexes, $[Pt(L^1)Cl](PF_6)_2 \cdot H_2O$ (**1**), $[Pt(L^2)Cl](PF_6)_2 \cdot H_2O$ (**2**), and $[Pt(L^3)Cl](PF_6)_2 \cdot 2H_2O$ (**3**), where L^1 = 4′-(4-(trimethylamino)methylphenyl)-2,2′:6′,2″-terpyridine, L^2 = 4′-(4-(triethylammonio)methylphenyl)-2,2′:6′,2″-terpyridine, and L^3 = 4′-(4-(tributylammonio)methylphenyl)-2,2′:6′,2″-terpyridine (see Scheme 1).



L^1 and **1** : R=$N(CH_3)_3$; L^2 and **2** : R=$N(CH_2CH_3)_3$; L^3 and **3** R=$N(CH_2CH_2CH_2CH_3)_3$

Scheme 1 Synthesis of the ligands L^1–L^3 and Pt(II) complexes **1**–**3**.

These complexes were obtained through a synthetic route summarized in Scheme 1. 4′-(4-Bromomethylphenyl)-2,2′:6′,2″-terpyridine, as a reactant,[8] directly reacts with trimethylamine, triethylamine, and tributylamine to give L^1, L^2, and L^3, respectively. The reaction of the ligands with $Pt(DMSO)_2Cl_2$ gave the final products **1**, **2** and **3** with suitable yields. Detailed experimental procedures for the preparation of ligands and complexes can be found in the ESI.†

During the investigation of stabilizing G-quadruplexes, structural conversion between various kinds of the human telomeric quadruplexes, including intra- and intermolecularly in parallel, and mixed arrangements, depending on the strand orientation were initially observed.[9] In our current studies, a human telomeric DNA sequence (22AG, 5′-$AG_3(T_2AG_3)_3$-3′) is applied to study the stabilization of G-quadruplex by CD titration with ligands and their Pt(II) complexes that are monitored by CD assays. Obtained results are compared in Fig. 1 and S1.† Evidenced by

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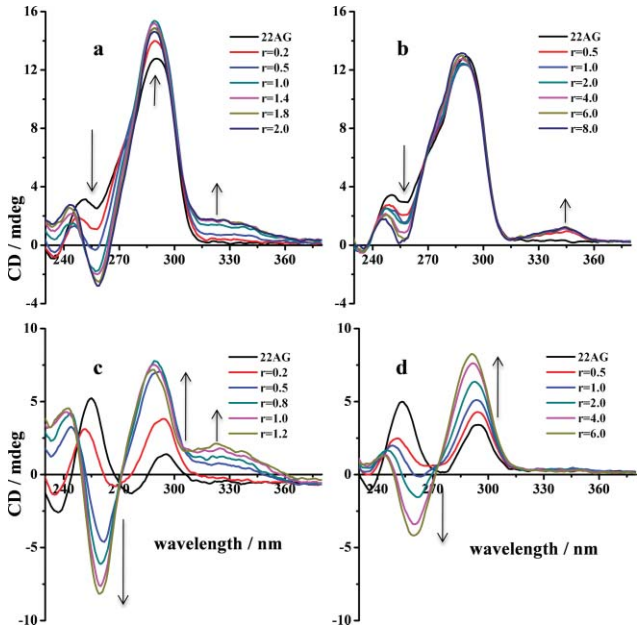

Fig. 1 CD titration spectra of 22AG sequence (3 μM) at increasing concentrations of **2** and L^2 in 10 mM Tris-HCl buffer, pH 7.4, 100 mM KCl and no metal cations, rt. Arrows indicate the increasing amounts of complexes (r = compound/DNA strand concentration). (a) **2**: r of 0.2, 0.5, 1.0, 1.2, 1.4, 1.8 and 2.0 in 100 mM KCl; (b) L^2: r of 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 in the absence of KCl; (c) **2**: L^2: r of 0.2, 0.5, 0.8, 1.0 and 1.2 in 100 mM KCl; (d) L^2: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl.

the CD spectra in Fig. 1a, in the presence of potassium cations, the quadruplex molecules exist as a mixture of parallel and antiparallel G-quadruplex conformations at the very beginning. When **2** is added at 0–6.0 μM, a positive band at about 290 nm and a negative band at about 260 nm are observed and reach saturation. These changes suggest that **2** strongly stabilizes antiparallel G-quadruplex conformation.[10] In the absence of any salt, the quadruplex molecules not only exist as a mixture of parallel and antiparallel G-quadruplex conformations, but also are dissociated partially to single-stranded molecules.[9] Therefore, larger band changes are observed in the CD spectrum with the addition of **2** (0–3.6 μM) to 22AG sequence. As illustrated in Fig. 1c, the maximum at 254 nm is gradually suppressed and shifted to 245 nm, while the bands centred at about 293 nm and 263 nm increasing sharply along with the addictions of **2**. Finally, the induced CD spectra of **2** virtually resemble those of antiparallel G-quadruplexes. Relative to **2**, smaller band changes induced by L^2 and require higher compound concentrations, 24.0 and 18.0 μM, to reach saturation, are observed in both the presence and absence of K^+ ions. The results indicate that the complex **2** is more efficient at inducing the formation of antiparallel G-quadruplexes than its ligand L^2. The same results are also obtained during the investigation on **1** and **3** and their ligands (Fig. S1). As expected, complexes containing larger square π-aromatic surfaces with Pt(II) present more effective interaction with G-quadruplex DNA sequences. In principle, the positively charged substituents provide potential interactions with the negatively charged sugar–phosphate backbone, the grooves and loops of the quadruplex.[5,7a,10c] Two hints concerning such interactions also can be found from the CD spectra. Raised induced circular dichroism

Table 1 Stabilization temperatures (ΔT_m) determined by FRET experiment

	ΔT_m/°C at 1 μM compound concentration[a]	
Compounds	F21T	F10T
L^1	21.0	2.0
L^2	23.2	2.0
L^3	20.0	1.9
1	26.2	5.6
2	30.5	6.2
3	25.3	3.8

[a] $\Delta T_m = T_m$ (DNA + compound) – T_m (DNA). The concentrations of F21T and F10T were both 0.2 μM.

(ICD) signals in the region of 310–360 nm (Fig. 1a and 1c) and a minor positive peak at near 345 nm (Fig. 1b) were observed. These ICD signals indicated that besides the end-stacking mode, other binding modes (groove binding, loop binding, or phosphates backbone binding) are also possible. Considering the multiple binding modes, the sizes of the peralkylated ammonium groups may affect the binding abilities of these compounds. So the potential reason that the complexes **1** and **2** show stronger capability than **3** is that steric hindrance from the largest group tributylamine inhibits the interaction between **3** and G-quadruplexes.

The binding of all the ligands and complexes to G-quadruplex DNA F21T (sequence: 5′-*FAM*-$G_3[T_2AG_3]_3$-*TAMRA*-3′, mimicing the human telomeric repeat) and a hairpin duplex DNA F10T (5′-*FAM*-dTATAGCTATA-HEG-TATAGCTATA-*TAMRA*-3′) were also investigated by a FRET (Fluorescence Resonance Energy Transfer) melting assay.[11] Table 1 provides the effect of the ligands and complexes on the enhanced melting temperature (ΔT_m) of two labelled oligonucleotides in K^+ solution.

All of the ligands and complexes have high ΔT_m values. At 1.0 μM of them, the ΔT_m values are in the range of 20–30 °C. On the other hand, none of these compounds are observed to increase the melting temperature of F10T significantly, suggesting their poor binding to the duplex DNA. Likewise, The FRET-melting data demonstrate that the complexes are more capable than the ligands, and all the three complexes are better G-quadruplexes binders than another Pt(II) complex (11.3 °C at 1.0 μM, FRET) coordinated by a similar ligand but lacking of any electropositive pendants, Pt-ttpy (ttpy = *p*-tolyl-terpyridine).[6b] This reveals that the introduced electropositive pendants are beneficial to G-quadruplex stabilization by these Pt(II) complexes. Besides, a consistent result from this experiment is that L^2 and **2** show little higher ΔT_m values than other two ligands and complexes. This may implied that moderate triethylamine group can better favor the binding to G-quadruplex.

In order to further evaluate how well the ligands and the complexes stabilize G-quadruplex DNA, a polymerase chain reaction (PCR)-stop assay was used to ascertain whether these compounds are bound to a test oligomer (5′-$G_3[T_2AG_3]_3$-3′) and therefore stabilize the G-quadruplex structure.[12] Fig. 2 illustrates that in the presence of the complexes, the template sequence forms G-quadruplex structures undetectable to the PCR product. The inhibitory effect of these complexes are enhanced clearly as the concentration increases from 1.0 to 8.0 μM with even no

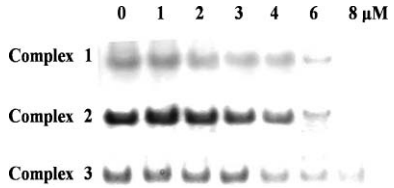

Fig. 2 Effect of complexes **1**, **2** and **3** (0–8.0 μM) on the hybridization of HTG21 in a PCR-stop assay.

PCR product detected at 8.0 μM. But the three ligands hardly inhibit the appearance of the PCR product (Fig. S3†). This data also suggest all the complexes are efficient G-quadruplexes binders. Besides, in order to exclude the possibility of enzyme inhibition by the ligands and the platinum complexes, the parallel experiment was performed using a mutated oligomer (5′-GAG[T_2AGAG]$_3$-3′, HTG21 mu) instead of HTG21 in identical conditions. And the result indicates that these compounds cannot obviously inhibit the *Taq* polymerase at comparable concentration (Fig. S3†).

Finally, Molecular Docking was performed to study the binding interaction of **2** with human telomeric DNA (PDB code 1KF1) to gain more structural insights. The data (Fig. 3) indicate that **2** contains a square π-aromatic surface that is in the center of a terminal G-quartet, with the charged side chains extending into the groove of the quadruplex. This result implies that multiple binding modes co-exist between **2** and the quadruplex. Such results may explain why platinum(II)-triarylpyridines complexes with positively charged tetraalkylammonium groups are efficient G-quadruplex binders.

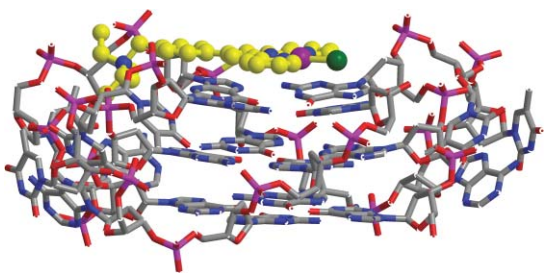

Fig. 3 Predicted interaction between **2** and the intermolecular G-quadruplex. The Pt(II) complex is in yellow (shown as a stick and ball model).

In this work, our purpose was to design and evaluate a series of platinum(II)-triarylpyridines complexes with tetraalkylammonium pendants that would show effective binding to G-quadruplex. All the experimental results clearly show that these ligands and complexes are capable of inducing the stabilization of G-quadruplexes, while the complexes are more efficient than their free ligands. Furthermore, complex **2** exhibits highest stabilization potential for G-quadruplex due to its appropriate triethylamine pendant. Our experiments also suggest that Pt(II) complexes could provide planar π-surfaces that promise them with G-quadruplex binding potentials, therefore the introduction of proper positively charged pendants is a practical approach for G-quadruplex binder design.

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