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A Pt(II)–Dip complex stabilizes parallel c-myc G-quadruplex†

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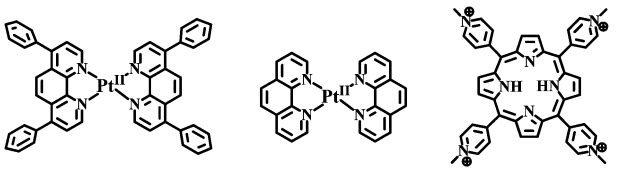
A new G-quadruplex (GQ) stabilizer, $[Pt(Dip)_2](PF_6)_2$ (Dip: 4,7-diphenyl-1,10-phenanthroline), is prepared by the microwave irradiation method. The complex can highly stabilize G-quadruplex, but has negligible interactions with duplex DNA. Aromatic anchors on the polypyridyl ligands bestow the stabilizer with a high binding preference towards parallel GQ.

G-quadruplex (GQ) is a family of four-stranded guanine-rich motifs containing a core stack of planar guanine quartets wrapped by loops with various lengths and 3D orientations.[1] Bioinformatics shows the existence of potential GQ folding sequences (PGS) in many biological significant genomic regions.[2] Human telomeres comprise large numbers of such sequences at the chromosome ends, which protect the telomere from degradation and genomic instability.[3] Besides, G-quadruplexes are found in the promoters of a wide range of genes that are important in cellular signalling pathways and are representatives of all six hallmarks of cancer (for example c-myc, c-kit, and bcl-2).[4] The biological and therapeutic significance of G-quadruplexes is well appreciated and the use of small molecules that promote the formation of and/or stabilize G-quadruplex structures has become an attractive approach towards anticancer drug discovery.[5]

A good G-quadruplex stabilizer should show excellent selectivity over double-stranded DNA, since majority of genomic DNA exists in B-form double helical structures. Furthermore, G-quadruplexes are rich in sequence-dependent structural polymorphism.[6] It is often observed that the same sequence can form multiple topological structures under different buffer conditions. Hence good selectivity among GQ topological structures is necessary for stabilizer molecules to achieve disease specificity on therapeutic effects and to be utilized as personal drugs. To date, several hundreds of small molecules that stabilize G-quadruplexes have been described in the literature and their interactions with G-quadruplexes have been extensively explored.[7] Metal complexes, especially platinum complexes, have been reported as G-quadruplex stabilizers.[8] Pt(II) complexes can achieve a relatively high binding affinity to G-quadruplexes. Nevertheless majority of the complexes show poor selectivity over duplex DNA. The square shape di-ligand coordination to the Pt(II) cation provides the necessary planar plane for good π–π stacking with G-quartets, but at the same time cannot circumvent the intercalation to the base-pair stacking or minor groove binding to duplex DNA. Furthermore, distinction among GQ topology remains a major challenge for both inorganic complexes and organic stabilizers.

A porphyrin GQ binder, TMPyP4 (Fig. 1), which extends the aromatic plane with four cationic pyridine anchors, achieves excellent parallel GQ targeting and also down-regulates c-myc and inhibits the proliferation of tumour cells.[9] Crystal and solution structures show that the specific interactions between the side chains and GQ grooves make a pivotal contribution to the stabilization of parallel c-myc quadruplex.[10] Herein in order to improve the selectivity of the Pt(II) stabilizer not only to global G-quadruplexes, but among GQ polymorphism, we designed and prepared a Pt(II) complex (**1**) (Fig. 1) with two Dip (4,7-diphenyl-1,10-phenanthroline) ligands. Firstly, complex **1** contains the $[Pt(phen)_2]^{2+}$ coordination core to provide aromatic electron conjugation with suitable size for good π–π stacking on G-quartets and hence to achieve good stabilization to the G-quartet core, which constantly exists in all G-quadruplex structures. Secondly, four phenyl groups might endow complex **1** with selectivity to topological GQ structures by interacting with the unique groove and loop arrangements in certain GQ topology, but at the same time, prevent the intercalation/groove binding to duplex DNA. The neutral anchors in $[Pt(Dip)_2]^{2+}$ can avoid the electrostatic interactions with the phosphate backbone in duplex DNA, which is presumably the reason for the nonspecific binding affinity of TMPyP4 to duplex



$[Pt(Dip)_2](PF_6)_2$ (**1**) $[Pt(phen)_2](PF_6)_2$ (**2**) TMPyP4

Fig. 1 Structures of Pt(II) complexes and TMPyP4 as GQ stabilizers.

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Table 1 Pt(II) stabilizers increase melting temperatures (T_m) of DNA structures

	ΔT_m[a] (°C)			
Complexes	c-myc	bcl-2	HT	ds26
1	18.9(±0.7)	9.5(±0.3)	6.3(±0.4)	−0.1(±0.1)
2	14.5(±0.6)	16.0(±0.2)	8.0(±0.2)	4.8(±0.2)

[a] $\Delta T_m = T_m$ (5 μM DNA + 5 μM complex) − T_m (5 μM DNA).

DNA *via* the cationic pyridine rings.[11] Complex **2** with the same aromatic planar core as **1**, but no anchors, is also prepared as a reference molecule.[12]

Complex **1** was synthesized by the microwave method under high temperature and pressure, since the conventional synthetic method, which was used to prepare complex **2** by simply incubating Pt(Phen)Cl_2 and Phen in boiling water, cannot work for the synthesis of **1**, due to the low water solubility of Pt(Dip)Cl_2 and the Dip ligand. More remarkably, the reaction time was shortened from overnight to less than 30 minutes and this is the first example of using microwave irradiation to assist the synthesis of the Pt(II)–polypyridinyl complex.

Thermal stabilization of G-quadruplexes in the presence of complexes **1** and **2** were studied by performing CD melting experiments. As shown in Table 1, complex **1** shows stabilizing effects only to GQ structures, with the highest T_m elevation, 18.9 °C, obtained in the case of GQ c-myc. No T_m enhancement of duplex DNA by complex **1** is observed. Whereas, comparable T_m elevations in telomeric GQ (HT) and duplex DNA (ds26) are observed in the case of complex **2**. This selectivity of complex **1** is further confirmed by G-quadruplex fluorescent intercalator displacement assay (G4-FID) and competitive dialysis.[13,14] $^{G4}DC_{50}$ of **1** from G4-FID (this concentration of **1** is needed to displace 50% thiazole orange (TO) from GQ c-myc) is 0.44 μM, while $^{ds}DC_{50}$ cannot be achieved even with the addition of 10 fold excess of the complex (Fig. S5, ESI†). The efficient TO-displacement implies a stable π–π stacking between G-quartets and **1**, but not between duplex DNA and **1**, since majority of TO molecules interact with GQ and duplex DNA *via* π–π stacking. In the competitive dialysis (Fig. S6, ESI†), 6.1- and 29.0-folds higher in bound concentration of complex **1** is observed on GQ c-myc than on duplex and single-stranded DNA, respectively. Hence, complex **1** shows excellent stabilizing selectivity to GQ structures over duplex DNA, while the reference complex **2** without the phenyl anchors can achieve only moderate selectivity between GQ and duplex (Fig. S2 and S6, ESI†).

More remarkably, **1** exhibits enhanced topological selectivity to GQ c-myc over HT and bcl-2 G-quadruplexes, compared to the reference molecule **2**. In the presence of **1**, the T_m of GQ c-myc is 9.4 °C and 12.6 °C higher than those of GQ bcl-2 and HT, which indicates the strong preference of **1** to stabilize GQ c-myc over the other two GQ topology. A similar trend is absent in the case of **2**, which induces comparable enhancements in the T_m of GQ c-myc and bcl-2, though a moderate selectivity over HT is observed for **2**. In the DNA titration experiments[15] (Fig. S4a, ESI†), an apparent hyperchromism of the MCLT band of complex **1** is observed upon the addition of GQ c-myc and yields a K_B as high as 4.01(±0.6) × 10^6 M^{-1}. Whereas, the titration of HT, bcl-2 and duplex DNA to the solution of **1** induces little alterations in the MLCT band (Fig. 2a), suggesting that **1** is unlikely to form strong π–π stacking with GQ HT and bcl-2, which would result in a similar hypochroism on MCLT band to those observed in the case of complex **2** with duplex and GQ

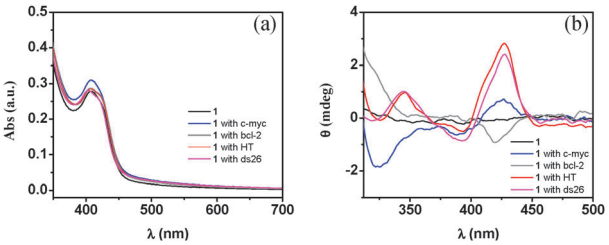

Fig. 2 Absorption and induced CD spectra of **1** in the presence of c-myc, bcl-2, HT and ds26. [**1**] = 20 μM and [DNA] = 40 μM.

DNA (Fig. S4c, ESI†). The unique enhancement of MCLT absorption suggests that, besides the usual π–π stacking mode acquired by the planar Pt(II)–polypyridyl complexes, Pt(Dip)$_2$ may also harness alternative interactions with GQ c-myc, presumably from the contributions from the interactions between the four phenyl anchors and GQ groove/loops. Furthermore, in a polymerase chain reaction stop assay (PCR-stop),[16] which demonstrates the inhibitory ability of GQ stabilizers on the replication of a template containing a G-rich c-myc sequence, complex **1** exhibits a complete inhibition of c-myc template extension at 15 μM, while no apparent polymerization arrest is observed with 30 μM of complex **2** (Fig. S7, ESI†).

To better understand the origin of the topological selectivity of complex **1**, CD spectra were used to observe the structural alternation of both DNA and the complex upon interaction with each other. The characteristic CD peak of GQ c-myc at 260 nm is enhanced upon the binding of complex **1**, but not in the case of binding of **2** (Fig. S3a, ESI†). Fig. 2b shows the induced CD spectra (ICD) in the MLCT region (310–500 nm) of complex **1**. Since absorption of DNA in this region can be neglected, ICD spectra represent only the conformational changes of the complexes upon binding to various DNA structures. In the presence of GQ c-myc, **1** exhibits a strong negative peak at 325 nm and a moderate positive peak at 425 nm, whereas both HT21 and ds26 induce a strong and a weak positive peak at 425 nm and 345 nm, respectively. Upon addition of GQ bcl-2, **1** generates a weak negative peak around 420 nm. The ICD spectra suggest that the interaction modes between **1** and GQ c-myc are distinct to those between **1** and GQ HT, bcl-2 or duplex DNA. The strong negative ICD in the Soret region indicates that π–π end-stacking and/or intercalating interactions could be the dominant interaction mode(s) and the reason for high affinity/selectivity between **1** and GQ c-myc.[17] In contrast, GQ HT and duplex DNA can only induce positive ICD, indicating no stable stacking/intercalating modes, but the external binding between the two structures and **1**.[18] Since intercalation to duplex DNA is achieved by **2** inducing negative ICD spectra (Fig. S3b, ESI†), the four phenyl anchors on **1** should be the cause for preventing **1** from stabilizing GQ HT and duplex DNA *via* strong stacking/intercalating modes.

Molecular docking of complex **1** onto several GQ topological structures, c-myc (PDB code: 1XAV), HT (PDB code: 143D) and bcl-2 (PDB code: 2F8U), provides further explanation for the topological selectivity of complex **1**. GQ c-myc folds into parallel GQ topology, which contains only side loops and four grooves.[19a] Docking **1** on the parallel GQ results in favourable stacking of the aromatic coordination core on the G-quartet, while the four phenyl groups are well accommodated in the four side grooves to lock the stabilizer into GQ c-myc (Fig. 3A). The limited rotation of phenyl groups in GQ grooves

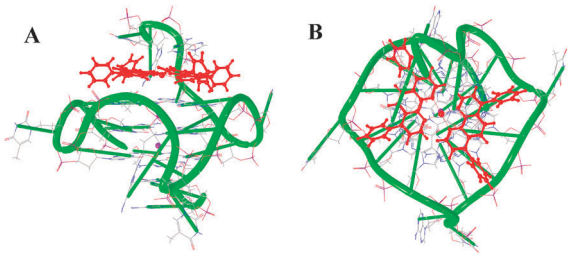

Fig. 3 Molecular docking of **1** (red) on parallel GQ (backbone and base orientation: green), c-myc (PDB: 1XAV). (A) Side view; (B) top view.

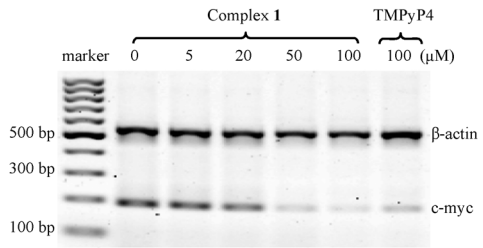

Fig. 4 Down-regulation of c-myc transcription upon the treatment of complex **1** and TMPyP4, as the positive control.

could be correlated to the emerging positive ICD signals. Furthermore, docking results show that **1** may provide extra stability to the parallel GQ structures *via* π stacking between 3′-terminal purines. Upon binding to the parallel GQ, the coordination plane in complex **1** becomes flatter (Fig. S8c and d, ESI†), which may result in the unique hyperchromism of the MLCT band observed in the titration of GQ c-myc to **1**, since disruption of the coordination plane often dampens the MLCT band.[20] Since GQ HT adopts a basket form structure,[19b] the rigid diagonal loop cannot open to yield an entry pathway for the propeller anchors on **1**. No stable docking mode can be achieved. A similar situation occurs in GQ bcl-2,[19c] which folds into a 3 + 1 mix topology with two lateral and one side loops. Similar to the diagonal loop in antiparallel GQ, the lateral loop in GQ bcl-2 repels the phenyl anchor and hence **1** can only partially stack on the terminal G-quartet (Fig. S8, ESI†), which explains the small increase in the T_m of GQ bcl-2 upon binding to **1**.

Reverse-transcription PCR assay (RT-PCR) has shown the potent biological activity of **1** *via* stabilizing GQ in the promoter of c-myc.[21] RT-PCR was performed to determine the impact of complex **1** on the mRNA level of c-myc oncogene in Hela cells. Fig. 4 shows that down regulation of c-myc mRNA is observed with the incubation of 20 μM of complex **1**. Comparable inhibitory efficacy is obtained for both **1** and TMPyP4 at a concentration of 100 μM (Fig. S9, ESI.† The efficiency of TMPyP4 in our study is consistent with previous results in the literature[9a]). The results indicate that **1**, as a stabilizer to parallel GQ structure, can inhibit c-myc expression in cancerous cell lines.

The cytotoxicities of **1** and **2** against Hela (human cervical epithelioid carcinoma), MDA-MB-231 (human breast carcinoma), HT-1080 (human fibrosarcoma) and HepG2 (human hepato-cellular carcinoma) cancer cell lines were determined by MTT assay. IC_{50} of the complexes after incubation for 48 hours are shown in Table S2 (ESI†). **1** exhibits micromolar IC_{50} against all the cell lines (2–7 μM), which can be twenty-fold more potent than **2** and TMPyP4.

In conclusion, we have synthesized $[Pt(Dip)_2](PF_6)_2$ by the novel microwave method with good yield and short reaction time. Both *in vitro* and biological studies indicate that complex **1** with four phenyl anchors shows excellent binding affinity to G-quadruplex and selectivity over duplex DNA. Furthermore, significantly high binding preference to parallel GQ, especially the c-myc promoter, is achieved by **1**, due to the distinct binding modes between **1** and different GQ topologies. **1** exhibits a higher potency to various cancer cell lines than the two reference molecules, **2** and TMPyP4. Hence, our results suggest that appropriately extending aromatic anchors from the aromatic Pt(II) coordination plane could be a promising strategy for developing Pt(II) polypyridyl complexes as stabilizers for specific GQ topology, if not necessary.

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