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A comparative DNA binding study for heteroleptic platinum (II) complexes of pip and hpip

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In this study, G-quadruplex DNA (Q-DNA) binding abilities of two platinum complexes ([Pt(bpy)(pip)]$(NO3)_2$ (1) and [Pt(bpy)(hpip)]$(NO3)_2$ (2) (bpy is 2,2′-bipyridine; pip is 2-phenylimidazo[4,5-*f*][1,10]phenanthroline; hpip is 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline) those previously reported were compared with double stranded DNA (ds-DNA) binding abilities. The interactions of both derivatives with human telomere Q-DNA (both the antiparallel basket and the mixed-hybrid G-quadruplex) and ds-DNA have been comparatively studied by UV-visible (UV-Vis), fluorescent intercalator displacement (FID) assays and thermal melting methods. The results show that both derivatives can stabilize Q- DNA and they show different binding affinities for different Q- DNA and ds-DNA. All spectroscopic studies have shown that the derivatives have a modest selectivity for Q-DNA vs ds-DNA. Increase in melting temperature was detected for both DNA forms but increase in Q- DNA melting temperature was significantly higher.

Keywords: Pt(II); G-Quadruplex DNA; ds- DNA; DNA binding

INTRODUCTION

Guanine-rich regions of genomic DNA are suggested to fold into non-canonical secondary structures known as G-quadruplexes (GQ). GQs are formed by π-π stacking of G-quartets, which are composed of four planar guanines held together by Hoogsteen hydrogen-bonds. Since the demonstration of G-quadruplexes and G-tetraplexes telomeric DNA [1-3], the conformations and functions of G-quadruplexes have gained considerable research interest [4]. During the replication in normal cell proliferation, telomeres erode by about 100 bp with each cell division which finally triggering cellular senescence [5, 6]. However, in most cancer cells, telomere cannot be shortened because of high telomerase enzyme activity. The formation of stable G-quadruplex from G-rich strands of telomere inhibits catalytic functions of the telomerase enzyme. Therefore, molecules those can stabilize G-rich strands to form G-quadruplexes have gained considerable research interest because quadruplex stabilizers could serve as antitumor agents [7-9].

As common structural features, G-quadruplex binders generally share a large, flat, aromatic surface, and the presence of protontable side chains. Most known G-quadruplex binders such as BRACO-19, RHSP4, and telomestatin[10-12] are polycyclic planar aromatic compounds with at least one substituent terminating in a cationic group. Some metal complexes also have been used to target G-quadruplexes due to their planar aromatic ligands and cationic metal center [13-15].

Several metal complexes and cationic form of pip and hpip ligands have been synthesized and their ds-DNA binding properties have extensively been studied [16-22]. In this study, double stranded and G-quadruplex DNA (Q-DNA) binding abilities of two platinum complexes (fig.1) those previously synthesized [18] has been discussed.

MATERIALS AND METHODS

All reagents and solvents were of commercial origin and used without further purification unless otherwise noted. Solutions of calf thymus DNA (CT-DNA; purchased from Sigma) in 100 mM KCl, 10 mM Tris (pH 7.0) had a UV–Vis absorbance ratio of 1.8–1.9: 1 at 260 and 280 nm (A_{260}/A_{280} = 1.9), indicating that the DNA was sufficiently free of protein [23]. The concentration of ds-DNA was determined spectrophotometrically using a molar absorptivity of 6600 M^{-1} cm^{-1} (260 nm).

HTG21 (5'-GGGTTAGGGTTAGGGTTAGGG-3') Q-DNA oligomer (purchased from thermo) extinction coefficients were calculated from mononucleotide data using a nearest-neighbour approximation [24]. Double-distilled water was used to prepare buffers. Stock solutions of ds-DNA and Q-DNA were stored at 4 °C and used within 4 days. The formation of intramolecular Q-DNA was

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carried out as follows: the oligonucleotide samples, dissolved in different buffers, were heated to 90 °C for 5 min, gently cooled to room temperature, and then incubated at 4 °C overnight. Solutions of the compounds were prepared by dissolving a weighed amount in 0.5 mL DMSO for solubility reasons and were then diluted (up to 150 times without precipitation) with 100 mM KCl, 20 mM Tris (pH 7.5) to the required concentration.

Physical measurements

UV–Vis spectra were recorded with a Varian Cary 100 spectrophotometer and emission spectra were recorded with a PerkinElmer LS 55 spectrofluorophotometer at room temperature.

Absorption titrations

For the absorption and emission titrations, compounds were dissolved in a minimum amount of DMSO, and were then diluted in buffer (100 mM KCl, 20 mM Tris (pH 7.5)) to a final concentration of 20 μM. Titrations were performed in a 10-mm stoppered quartz cell by using a fixed concentration of the complexes (20 μM), to which the CT-DNA stock solution was added in increments of 1 μL to a DNA-to-compound concentration ratio of 2.4:1 for ds-DNA and 0.003:1 for Q-DNA. Complex–DNA solutions were incubated for 10 min each time before the spectra were recorded. A control solution of 20 μM compound in the same buffer was also treated in the same manner. Cell compartments were thermostated at 25 ± 0.1 °C.

UV melting studies

For UV thermal melting studies, solutions of the Q-DNA and ds-DNA in the absence and presence of the complexes [DNA/complex = 1/1] were prepared in a buffer solution (20 mM Tris-HCl, pH 7.5, 100 mM KCl). The temperature of the solution was increased at a 1 °C min^{-1} interval, and the absorbance at 295 nm was continuously monitored. The T_m values were determined graphically from the plots of absorbance vs temperature.

FID studies

The competitive behavior of each compound with thiazole orange (TO) was investigated by fluorescence spectroscopy in order to examine whether the compound is able to displace TO from the Q-DNA-TO complex.

DNA was pretreated with TO at a TO to DNA concentration ratio of 2:1 for 30 min at 27 °C to prepare the initial complex. The intercalating effect of the complexes with the Q-DNA-TO complex was studied by adding a certain amount of a solution of the compound in increments to the solution of the Q-DNA-TO complex. The influence of each addition of complex to the solution of the Q-DNA-TO complex was obtained by recording the change in the fluorescence spectrum. To study the competitive binding of the compound with TO, TO was excited at 504 nm.

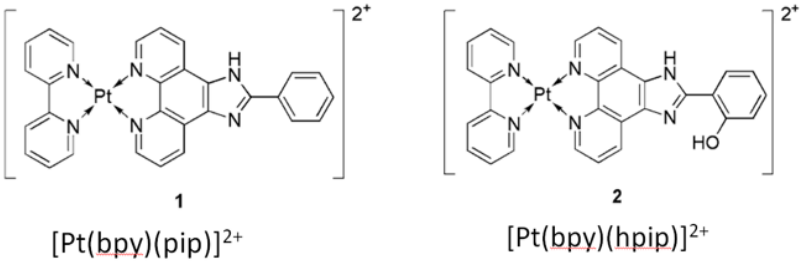

Fig. 1. Platinum complexes used in this study (([Pt(bpy)(pip)]$(NO_3)_2$**(1)** and [Pt(bpy)(hpip)]$(NO_3)_2$**(2)**).

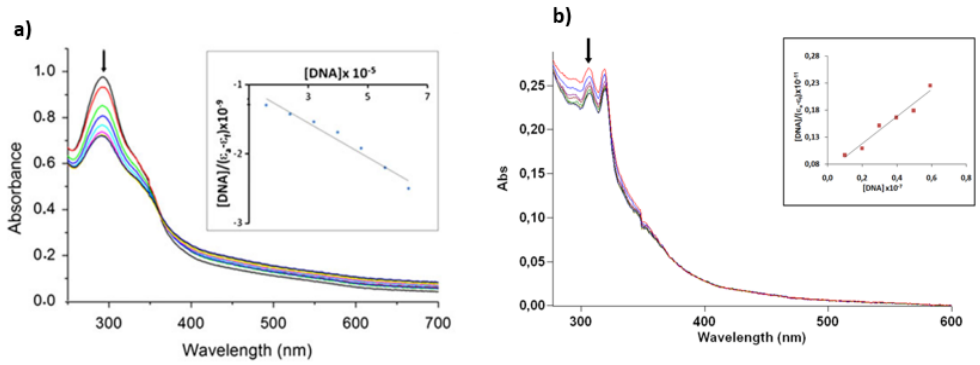

Fig. 2. UV-vis spectra of **1** (20 μM) in Tris/KCl buffer (100 mM KCl, 20 mM Tris-HCl, pH 7.5) with increasing amounts of ds (a) Q-DNA (b) ds-DNA.

RESULTS AND DISCUSSION

Synthesis of ligands and complexes

Synthesis and characterization of ligands and complexes was discussed in detail in our previous study [18].

Electronic absorption titration

The application of electronic absorption spectroscopy in DNA binding studies is one of the most useful techniques. Electronic spectra are a useful way to investigate the interactions of complexes with DNA. A complex bound to DNA through intercalation usually results in hypochromism and red shift (bathochromism) owing to the intercalation mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The extent of the hypochromism in the visible metal-to-ligand charge transfer band is commonly consistent with the strength of the intercalative interaction [25].

The high energy band around 292 nm is attributed to the π→π* transitions corresponding to the phenanthroline moiety of the ligands. Absorption spectra titrations were performed to determine the binding affinity of complex to Q-DNA. DNA sample was added in aliquots sequentially to complex solutions, with absorbance spectra recorded after each addition. The changes in the spectral profiles during titration were shown in Fig. 2. When both Q-DNA and ds-DNA is added into complex **1** solutions, significant hypochromism is observed in absorption spectra. Similar hypochromism is observed for complex **1** upon addition of the lower rate of Q-DNA than ds-DNA under the same conditions. R_{max} values can be seen in table 1. When the amount of DNA was increased, the decreases in the intensities of the π–π* transitions of complex **1** were 27.9 % for ds-DNA at a DNA-to-Pt concentration ratio of 4.8 and 12.2 % for Q-DNA at a DNA-to-Pt concentration ratio of 0.03. The similar case could be seen for complex **2**. Hypochromism at low R values for Q-DNA indicated strong interactions between the G-quadruplex form of DNA and the complexes. In order to compare quantitatively the binding strength of complexes to Q- DNA and ds-DNA, the intrinsic binding constants K_b with each DNA at 25 °C were obtained using the following equation;

$$[DNA]/(\varepsilon_A - \varepsilon_f) = [DNA]/(\varepsilon_B - \varepsilon_f) + 1/K_b\,(\varepsilon_B - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of the nucleic acid in base pairs, ε_a is the apparent absorption coefficient obtained by calculating A_{obs}/[Pt], and ε_f and ε_B are the absorption coefficients of the free and the fully bound platinum complex, respectively. In the [DNA]/(ε_A - ε_f) versus [DNA] plot, K_b is given by the ratio of the slope to the intercept. The values of the intrinsic binding constants K_b of **1** were derived to be 2.88 (± 0.4) x 10^4 and 3.58 (± 0.3) x 10^7 for ds-DNA and Q-DNA respectively. As seen in results, K_b for Q-DNA is bigger than ds-DNA. This indicates that complex **1** is more selective for G-quadruplex form of DNA. K_b of **2** were derived to be 5.38 (± 0.4) x 10^4 and 3.83 (± 0.5) x 10^7 for ds-DNA and Q-DNA respectively.

Similar binding trend to complex **1** has been observed for complex **2** with a greater binding constant value for Q-DNA (Fig. 3). Both complexes can bind to G-quadruplex form more selective. The difference between K_b values of complexes for different DNA forms was approximately 10^3 results from complexes can interact with Q-DNA at much lower R values than ds-DNA.

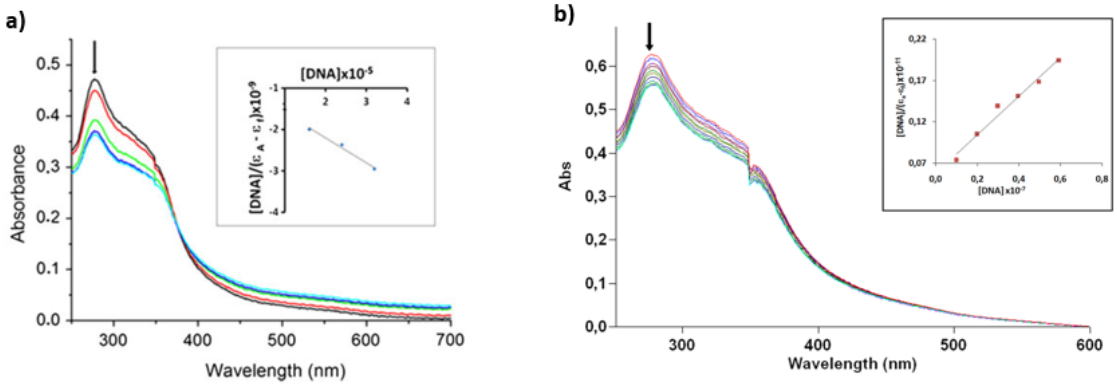

Fig. 3. UV-vis spectra of 2 (20 μM) in Tris/KCl buffer (100 mM KCl, 20 mM Tris-HCl, pH 7.5) with increasing amounts of ds (a) Q-DNA (b) ds-DNA

Table 1. UV titration results

	Titration results for ds-DNA			*Titration results for Q-DNA*		
Complex	ΔA (%)	K_b (M^{-1})	R	ΔA (%)	K_b (M^{-1})	R
1	27.9	2.88 (±0.4) x 10^4	4.8	12.2	3.58 (± 0.3) x 10^7	0.03
2	23.4	5.38 (±0.4) x 10^4	2.4	11.5	3.83 (± 0.5) x 10^7	0.03

UV melting studies

The DNA thermal melting is a measure of the stability of the DNA double helix with temperature an increase in the thermal melting temperature (T_m) indicates an interaction between ds-DNA and the metal complex [26]. Like ds-DNA, an increase in T_m of Q- DNA can be observed as a result of interaction between Q- DNA and metal complexes [27]. In the present case, thermal melting studies were carried out and T_m values were determined by monitoring the absorbance of ds-DNA at 260 nm and Q-DNA at 295 nm as a function of temperature. Usually, classical intercalation gives rise to higher ΔT_m values than either groove binding or outside stacking [28]. The Tm of ds-DNA in the absence of any added complex was found to be 78 °C ± 0.20, under our experimental conditions. Under the same set of conditions, ds-DNA structure degraded at 82 °C ± 0.32 and 83 °C ± 0.24 in the presence of complexes 1 and 2, respectively. The observed Δ*T*m value of 2 °C and 3 °C for **1** and **2** respectively indicates that complex **2** binds to ds-DNA stronger than complex **1**. These are consistent with the determined K_b values, further stressing that intercalation took place (Fig. 4).

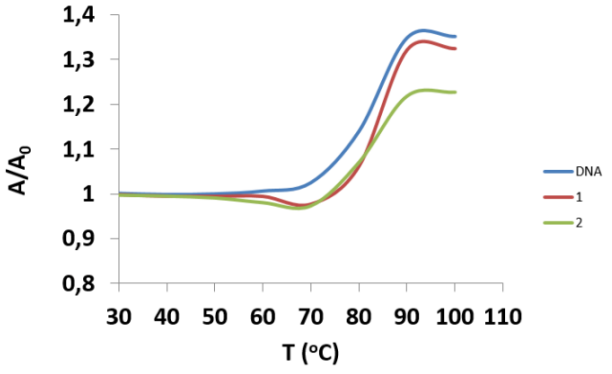

Fig. 4. UV melting curves for (—) ct-DNA, (—) ct-DNA + **1** and (—) ct-DNA + **2**.

The T_m of Q-DNA in the absence of any added complex was found to be 45 °C ± 0.40, under our experimental conditions. It is uprised to 62 °C ± 0.34 and 67 °C ± 0.28 by addition of complex **1** and **2**, respectively (fig.5). The great increase in T_m values indicates that both complexes bind to Q-DNA stronger than ds-DNA. Also ΔT_m value of 22 °C for complex **2** shows that it can stabilize the Q-DNA better than complex **1** with a ΔT_m value of 17 °C.

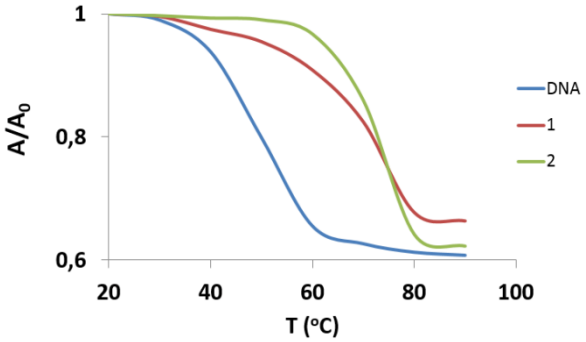

Fig. 5. UV melting curves for (—) HTG21, (—) HTG21 + **1** and (—) HTG21 + **2**.

FID studies

To further clarify the nature of the interaction between the complex and Q-DNA and ds-DNA FID was carried out. FID is a simple and fast method to evaluate the affinity of a compound for Q-DNA [29, 30]. This assay is based on the loss of fluorescence of thiazole orange (TO) upon competitive displacement from DNA by a putative ligand. Upon interaction with Q-DNA, TO exhibits high affinity (K = 3.0 × 10^6 M^{-1}) [31] and displays a significant increase in its fluorescence, whereas when free in solution, the fluorescence is quenched. Therefore, displacement of TO by another molecule provides an approximate measure of the affinity of the given compound for duplex and quadruplex DNA by evaluating the DC_{50} which corresponds to the required concentration of complex to induce a 50% fluorescence decrease.

We were first interested in comparing the Q-DNA binding abilities of the complex **1**. The emission spectra of TO bound to ds-DNA and Q-DNA in the absence and the presence of complex **1** are shown in Fig. 6.

DC_{50} value of complex **1** for Q-DNA that can be seen in table 2 was lower than ds-DNA. This indicates that **1** can kick out TO molecules from Q-DNA easier due to stronger binding. The emission spectra of TO bound to ds-DNA and Q-DNA in the absence and the presence of complex **2** are shown in Fig. 7. DC_{50} of complex **2** for Q-DNA is lower than DC_{50} for ds-DNA. To discuss the G-

quadruplex selectivity of complexes, DC_{50} values can be compared.

The selectivity of both complexes for Q-DNA versus ds-DNA was calculated and both complexes showed prominent G-quadruplex binding affinity, a modest selectivity for G-quadruplex over double stranded was observed. However, calculated selectivity of complex **2** is nearly 2 times better than complex **1**.

Table 2. DC_{50} values

Complex	DC_{50} (ds-DNA (μM))	DC_{50} (Q-DNA (μM))	Selectivity (DC_{50}-ds/ DC_{50}-Q)
1	11.70	7.87	1.49
2	11.23	4.53	2.65

CONCLUSION

Q-DNA selectivities of two previously synthesized platinum complexes have been compared with different spectroscopic methods. All methods are consistent with each other and demonstrate that complex **2** is a more selective complex for Q-DNA as supposed. Such a difference in the DNA-binding affinities between **1** and **2** can be reasonably explained by the intramolecular hydrogen bond between the ortho phenolic group and the nitrogen atom of the imidazole ring. The extended co-planarity of the hpip ligand leads to enhanced DNA-binding affinity of the hpip complex. Q-DNA binding affinity was more significantly enhanced because Q-DNA has a larger surface area due to four guanine bases.

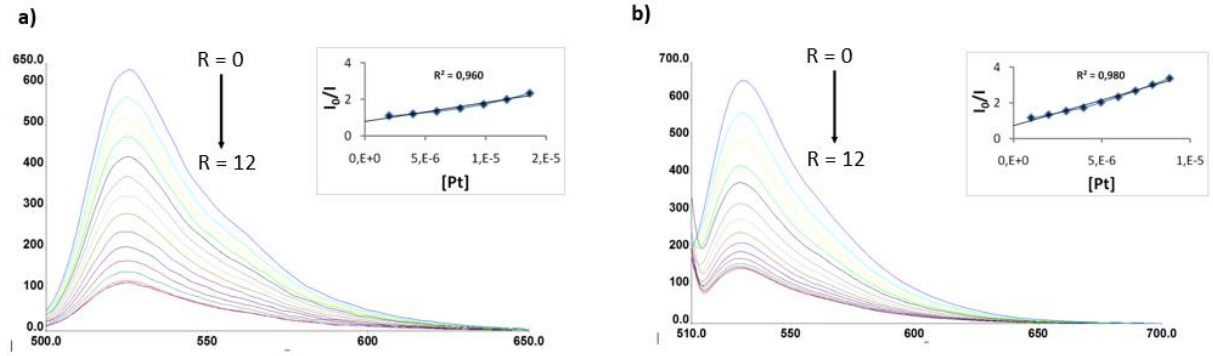

Fig. 6. Flurescence spectra of TO (2 μM) with (a) ds-DNA (30 μM) (b) Q-DNA (1 μM) in Tris/KCl buffer (100 mM KCl, 20 mM Tris-HCl, pH 7.5) with increasing amounts of **1**.

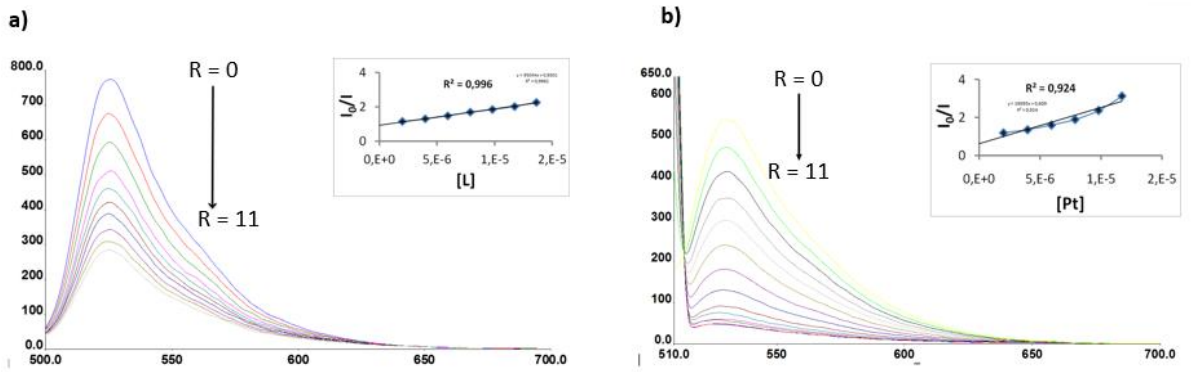

Fig. 7. Flurescence spectra of TO (2 μM) with (a) ds-DNA (30 μM) (b) Q-DNA (1 μM) in Tris/KCl buffer (100 mM KCl, 20 mM Tris-HCl, pH 7.5) with increasing amounts of **2**.

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