Stabilisation of human telomeric quadruplex DNA and inhibition of telomerase by a platinum–phenanthroline complex†

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Two new mono-substituted phenanthroline ligands and their platinum(II) square planar complexes have been prepared; one of the complexes has been shown to induce a high degree of quadruplex DNA stabilisation and to inhibit telomerase.

The telomerase complex enzyme is responsible for the maintenance of the integrity of the ends of chromosomes, preventing critical telomere shortening by catalysing the synthesis of telomeric DNA so that cells cannot reach crisis points of senescence and apoptosis. Telomerase is up-regulated in 80–85% of tumour cells and is a major factor in cancer cell immortalisation.[1] Therefore, there is considerable current interest in finding molecules that can inhibit telomerase and potentially act as anticancer drugs.[2,3] Human telomeric DNA consists of the tandem repeat sequence TTAGGG (having a length of 3–6 KB in cancer cells) with the 3′ terminal 100–200 bases being single-stranded. Crystallographic[4] and NMR[5–7] studies have shown that repeats of this sequence can fold into higher-order guanine-quadruplex DNA structures.[8–10] Since the substrate of telomerase is the 3′-single-stranded overhang of telomeric DNA, the stabilisation of quadruplex DNA structures by small molecules can inhibit telomerase[11] interfering with telomere maintenance in tumour cells.[12]

Over the past few years a wide range of planar heteroaromatic molecules have been investigated as quadruplex DNA stabilisers and evaluated as telomerase inhibitors.[13–19] A few have also been evaluated for anticancer activity.[20,21] From crystallographic and NMR spectroscopic studies, together with computer modelling, it has been possible to develop a rational approach to designing molecules with optimal properties for quadruplex DNA stabilisation. Some of the desired features these molecules should have are: a planar π-delocalised system able to stack on the face of the guanine quartet; a partial positive charge on the molecule positioned at the centre of the quartet; substituents bearing a positive charge to interact with the grooves and loops of DNA and with the negatively charged backbone phosphates.

Most of the molecules reported to date as quadruplex DNA stabilisers and telomerase inhibitors are based on organic heteroaromatic systems. In contrast, very few metal complexes have been studied in this context[17,22–26] in spite of the versatile structural and functional properties that metals can confer on a molecule. We hypothesised that a metal coordinated to heteroaromatic multidentate ligands with a square planar geometry could have important advantages over more "classical" quadruplex DNA binders. The metal can play a major structural role in organising the ligand(s) into an optimal structure for quadruplex DNA interaction. Also, the electropositive metal can in principle be positioned at the centre of the guanine quartet, increasing electrostatic stabilisation by substituting the cationic charge of the potassium or sodium that would normally occupy this site. In addition, metal complexes tend to have interesting optical and magnetic properties which could in turn be employed to probe the interaction between small molecules and quadruplex DNA. As we have recently shown,[24] compounds based on planar nickel(II)–salphen complexes are indeed excellent quadruplex DNA stabilisers and telomerase inhibitors. Herein we present a new quadruplex DNA stabiliser and telomerase inhibitor based on a phenanthroline ligand and its square planar platinum(II) complex (see Scheme 1).

Qualitative computer modelling was initially employed to design small molecules with optimal binding properties to stabilise quadruplex DNA (see Fig. 1). From these studies, it was found that mono-substituted phenanthrolines and their corresponding square planar metal complexes have the appropriate structural features for a good overlap with guanine quartets.

The modelling also suggested that the platinum(II) ion present in the proposed metal complexes would be positioned at the centre of the guanine quartet. Based on this design, the syntheses of $\mathbf{L^1}$ and $\mathbf{L^2}$ and the corresponding platinum complexes **1** and **2** were carried out as outlined in Scheme 1. All the compounds were fully characterised by NMR and IR spectroscopies, mass spectrometry and elemental analysis (see the ESI for full experimental details).

The synthesis of both ligands was based on a previously reported methodology for the formation of mono-substituted phenanthroline amides.[27,28] Complexes **1** and **2** were prepared by reacting the deprotonated version of the corresponding phenanthroline mono-amide with K_2PtCl_4. The products were isolated and purified to yield [Pt($\mathbf{L^1}$)Cl] (**1**) and [Pt($\mathbf{L^2}$)Cl] (**2**) as orange/red solids. The IR spectra of both platinum(II) complexes showed a shift of the CO stretching frequency due to coordination (from 1648 to 1626 cm^{-1} in **1** and from 1664 to 1626 cm^{-1} in **2**) consistent with *N*-coordination of amidato groups. The ^{1}H NMR spectra of **1** and **2** show the expected peaks for $\mathbf{L^1}$ and $\mathbf{L^2}$ respectively, some of the resonances being shifted in comparison to the free ligands. The formulations of both **1** and **2** were confirmed by elemental analyses and ESI(+) mass spectrometry

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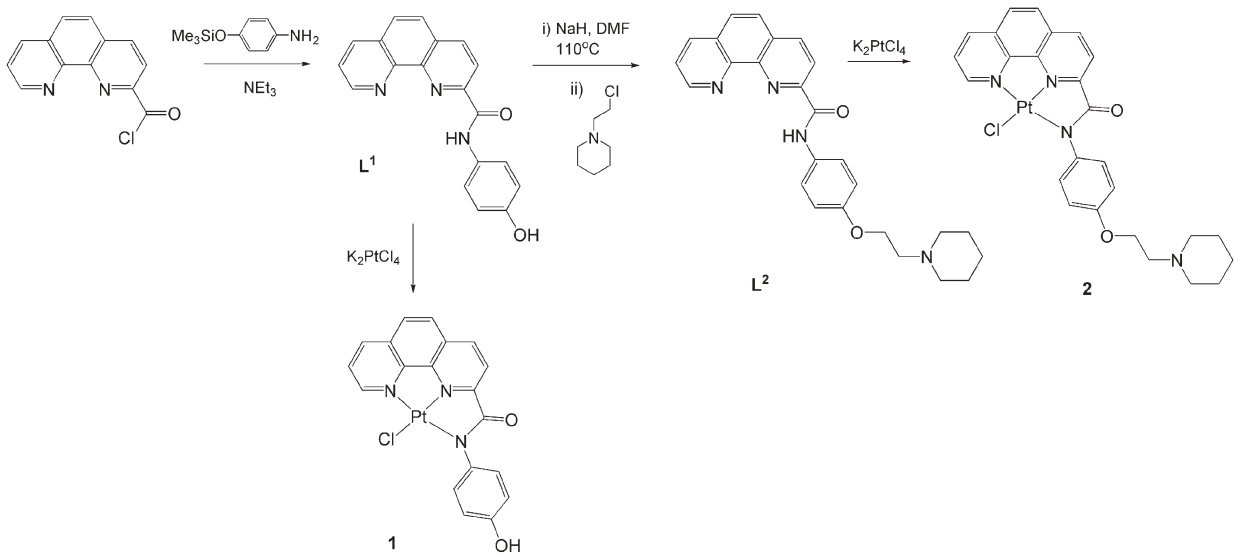

Scheme 1 Synthetic route for the preparation of phenanthroline ligands ($\mathbf{L^1}$ and $\mathbf{L^2}$) and the corresponding square planar platinum complexes (**1** and **2**).

(*m*/*z* = 567 a.m.u. corresponding to $[Pt(\mathbf{L^1})Cl + Na]^+$ and 657 a.m.u. corresponding to $[Pt(\mathbf{L^2})Cl + H]^+$).

The ability of $\mathbf{L^1}$, $\mathbf{L^2}$ and complex **2** to stabilise G-quadruplex DNA (sequence: 5′-*FAM*-d(GGG[TTAGGG]3)-*TAMRA*-3′) was then investigated by a FRET (Fluorescence Resonance Energy Transfer) melting assay.[29] The insolubility of complex **1** in water and water–DMSO mixtures prevented us from evaluating its binding properties towards DNA and therefore no data for this complex are provided.

Complex **2** (see Table 1) induces a high degree of stabilisation for quadruplex DNA with an increase in melting temperature (ΔT_m) at 1 μM of 20 °C. In contrast, at the same concentration of metal complex, the ΔT_m for a duplex DNA sequence (5′-*FAM*-dTATAGCTATA-**HEG**-TATAGCTATA-*TAMRA*-3′; T_m = 60 °C in absence of complex) is negligible, suggesting a ≫ 40-fold selectivity for quadruplex *vs.* duplex DNA. Although the stabilisation of quadruplex DNA by this compound is not as high as that shown by the lead compound BRACO-19 (with a ΔT_m = 27.5 at the same concentration), it is worth pointing out that **2** is more selective for quadruplex DNA *vs.* duplex DNA than BRACO-19.

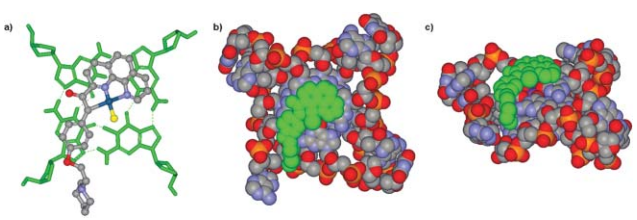

Fig. 1 Qualitative *in silico* docking of complex **2** with a guanine quartet (using DS viewer Pro); a) a stick and ball model showing overlap of the complex with a quartet of guanine nucelosides; b) a CPK model showing a top view of the complex stacked on top of a guanine quadruplex (structural details obtained from X-ray crystal structure of parallel quadruplexes from human telomeric DNA[4]); c) a side view of the quadruplex–complex interaction showing the piperidine side arm of the complex fitting neatly into the pocket created by a TTA loop of the quadruplex.

To evaluate the effect of the metal on quadruplex DNA stabilisation the corresponding free ligand ($\mathbf{L^2}$) was also analysed (Table 1, second entry). In this case the ΔT_m at 1 μM of $\mathbf{L^2}$ is 9 °C, considerably lower than the ΔT_m obtained with complex **2**. This suggests that the metal plays an important role in aiding quadruplex DNA stabilisation. To evaluate the importance of the ethylpiperidine substituent (in $\mathbf{L^2}$), $\mathbf{L^1}$ was also studied (Table 1, first entry). This ligand is a poor quadruplex DNA stabiliser, indicating that the piperidine substituent plays an important role in increasing the strength of the interaction between planar molecules, such as the phenanthrolines under study, and quadruplex DNA.

The encouraging results obtained by FRET for complex **2** prompted us to investigate if this compound would also show telomerase inhibition in the two-step TRAP assay. We have substantially modified the assay to eliminate the possibility of ligand being carried over to the second PCR step in the assay,[30] when it can then itself bind to PCR products and so give false positive values for telomerase inhibitory activity. Complex **2** showed 50% telomerase inhibition at 49.5 μM concentration (see Fig. 2). Although this $^{Tel}EC_{50}$ value is greater than those observed for well-established telomerase inhibitors such as BRACO-19 and tetra-*N*-methylpyridylporphyrin (TMPyP4) (3.1 and 8.9 μM respectively using the same modified TRAP assay) it shows that complex **2** is active as an inhibitor for telomerase.

Table 1 Stabilisation temperatures (determined by FRET) of quadruplex and duplex DNA. The last column shows the concentration of compound required to induce a ΔT_m of 20 °C

	ΔT_m at 1 μM (°C)		Conc. (μM) ΔT_m = 20 °C
Compound	G4 DNA	dsDNA	G4 DNA
$\mathbf{L^1}$	0.5	0	>10.0
$\mathbf{L^2}$	9.0	0	8.0
2	20.0	0.5	1.0
BRACO-19[a]	27.5	14.5	0.7

[a] Data reported in reference 29.

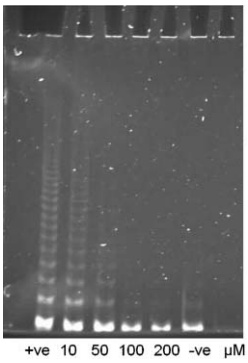

Fig. 2 TRAP gel from compound **2** showing the characteristic ladders by PCR amplification of the oligonucleotides generated by the activity of telomerase on a TS primer. With increasing concentrations of **2** (values in the figure are μM concentrations) a decrease in the intensity of the ladder is observed (*i.e.* increase in telomerase inhibition).

Since the FRET assay showed large differences in quadruplex DNA thermal stabilisation between the metal-free ligands and the metal complex, the TRAP assay was carried out for both ligands (again, complex **1** could not be evaluated due to its low solubility).

Both $\mathbf{L^1}$ and $\mathbf{L^2}$ show significantly less activity than **2**: the piperidine-substituted ligand $\mathbf{L^2}$ shows activity at approximately 200 μM while $\mathbf{L^1}$ shows no activity at the concentrations tested. These results are in agreement with the FRET studies, strongly suggesting a correlation between quadruplex DNA stabilisation and telomerase inhibition.

In summary, two new mono-substituted phenanthrolines have been synthesised and used as tridentate *N*,*N*,*N*-ligands to coordinate to platinum(II). The interactions between these compounds and DNA (both duplex and quadruplex) have been studied by FRET methods. From these studies we have identified a compound that induces high stabilisation of telomeric quadruplex DNA (ΔT_m = 20 °C at 1 μM) and high selectivity for quadruplex *vs.* duplex DNA. In order to examine the ability to bind strongly to telomeric quadruplex DNA, TRAP assays with the two ligands and complex **2** have been carried out. These studies and the FRET results indicate a significant correlation, with the metal complex being a much more potent telomerase inhibitor than the free phenanthroline ligands.

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