Supplementary Material (ESI) for Chemical Communications

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

Julie E. Reed, Stephen Neidle* and Ramon Vilar*

Electronic Supplementary Information

Experimental details

Materials, methods and instrumentation. Reactions were carried out using standard Schlenk techniques, under an atmosphere of dry nitrogen or argon. Glassware was dried in an oven at 150^{o}C prior to use. All solvents were distilled over standard drying agents and degassed with nitrogen or argon prior to use, or dried using a solvent purification system (SPS, Innovative-Technology) and degassed with nitrogen or argon prior to use. Infrared spectra were recorded on a Perkin Elmer FTIR 1720 spectrometer as KBr discs between 4000 and 400 cm^{-1}. ^{1}H and ^{13}C spectra were recorded on either a JEOL GS 270 MHz spectrometer, AM-400, Bruker Avance 400, AM-500 or Bruker Avance 500 spectrometer at room temperature and referenced to the residual ^{1}H and ^{13}C signals of the solvents (269.7 MHz and 67.64 MHz for ^{1}H and ^{13}C, respectively). 1,10-phenanthroline-2-carbonyl chloride was prepared according to previously reported experimental procedures.[1,2]

Synthesis of 4-hydroxy-phenyl-1,10-phenanthroline-2-carboxamide (L^1). 1,10-phenanthroline-2-carbonyl chloride (1g, 4.4 mmol) was suspended in dry and degassed CH_2Cl_2 (40 mL) and freshly distilled triethylamine (1.78 mL, 13.5 mmol) was added to this solution. Trimethylsilyl-protected 4-aminophenol (1.6 g, 8.9 mmol) was dissolved in dry and degassed CH_2Cl_2 (10 mL) and added dropwise to the phenanthroline solution under nitrogen. This solution was heated to reflux for 12 hours to give a brown solution with some off-white precipitate. The reaction mixture

was filtered and the resulting dark brown solution was concentrated under reduce pressure. The product was purified by column chromatography using silica and 1% ethanol in 99% ethyl acetate to give **L^1** as yellow solid (0.98 g, 70 %). ^{1}H NMR (DMSO-d$_6$): δ 10.24 (s, 1H), 9.37 (s, 1H) 9.21 (dd, $^3J_{HH}$ = 4.4 Hz, $^4J_{HH}$ = 1.6 Hz, 1H), 8.76 (d, $^3J_{HH}$ = 8.4 Hz, 1H), 8.60 (dd, $^3J_{HH}$ = 8.0, Hz, $^4J_{HH}$ = 1.6 Hz, 1H), 8.48 (d, $^3J_{HH}$ = 8.4 Hz, 1H), 8.13 (m, 2H), 7.89 (dd, $^3J_{HH}$ = 4.4, $^3J_{HH}$ = 8.0 Hz, 1H), 7.73 (d, $^3J_{HH}$ = 8.8 Hz, 2H), 6.83 (d, $^3J_{HH}$ = 8.8 Hz, 2H). ^{13}C NMR (DMSO-d$_6$): δ 162.3, 154.4, 150.4, 149.3, 138.9, 137.8, 131.4, 130.8, 130.4, 129.5, 128.7, 127.2, 124.7, 122.3, 121.7, 115.7. IR (KBr): 3425, 3232, 1648 (C=O), 1595, 1536, 1514, 1439, 1265, 1226, 1134, 1083, 1037, 865, 825, 717. ES(+) MS (*m/z*): 316, [**L^1** + H]$^+$. Anal. Calcd for $C_{19}H_{13}N_3O_2 \cdot C_2H_5O$: C, 69.79; H, 5.30; N, 11.63. Found: C, 69.85, H, 4.99, N, 11.74.

Synthesis of 1,10-phenanthroline-2-carboxamide-phenyl-4-ethoxy-piperidine (L^2). Compound **L^1** (150mg, 0.47 mmol) was suspended in dry and degassed DMF (20 mL). NaH (60% dispersion in mineral oil) (85 mg, 2.3 mmol) was added to this suspension and heated to 110°C. Chloroethylpiperidine (175 mg, 0.95 mmol) was dissolved in DMF (40 mL) and the resulting solution added dropwise to the reaction mixture over a period of 2 hours during which time the reaction mixture turned from deep red to transparent yellow-orange. After the addition was complete the reaction mixture was allowed to cool and left to stir at room temperature for 3 days. The reaction mixture was then filtered to give a yellow solution that was concentrated under reduced pressure yielding a yellow solid. This was solid dissolved in methanol and HCl(aq) and NEt_3 were added until the solution reached pH 7. The resulting solution was concentrated under vacuum and columned on silica using a gradient of

ethanol (0-10%) in ethyl acetate with 0.5% NEt_3. On reduction the combined fractions gave $\mathbf{L^2}$ as a yellow solid (yield = 175 mg, 85%). ^{1}H NMR (DMSO-d_6): δ 10.8 (s, 1H), 9.23 (dd, $^3J_{HH}$ = 4.8 Hz, $^4J_{HH}$ = 1.2 Hz, 1H), 8.77 (d, $^3J_{HH}$ = 6.8 Hz, 1H), 8.60 (dd, $^3J_{HH}$ = 6.8, Hz, $^4J_{HH}$ = 1.2 Hz, 1H), 8.48 (d, $^3J_{HH}$ = 6.8 Hz, 1H), 8.14 (m, 2H), 7.84 (dd, $^3J_{HH}$ = 4.4, $^3J_{HH}$ = 8.0 Hz, 1H), 7.84 (d, $^3J_{HH}$ = 7.2 Hz, 2H), 7.03 (d, $^3J_{HH}$ = 7.2 Hz, 2H), 4.10 (t, $^3J_{HH}$ = 4.8 Hz, 2H), 2.68 (t, $^3J_{HH}$ = 4.8 Hz, 2H), 2.45 (m, 4H), 1.50 (m, 4H), 1.39 (m, 2H) ppm. ^{13}C NMR (DMSO-d_6): δ 162.7, 155.7, 150.8, 145.6, 144.3, 139.0, 137.3, 132.0, 130.5, 129.7, 129.0, 126.9, 124.4, 122.1, 121.6, 115.3, 66.4, 58.3, 54.9, 26.4, 24.6. IR (KBr): 3356 (NH), 2923, 1664 (C=O), 1540, 1509, 1491, 1265, 1242, 1229, 1137, 1043, 1034, 950, 853, 833, 821, 719. ES(+) MS (*m/z*): 427, $[\mathbf{L^2} + H]^+$. Anal. Calcd for $C_{26}H_{26}N_4O_2 \cdot H_2O$: C, 70.25; H, 6.35; N, 12.60. Found: C, 70.07, H, 5.99, N, 12.45.

Synthesis of [Pt($\mathbf{L^1}$)Cl] (1). Compound $\mathbf{L^1}$ (40 mg, 0.12 mmol) was dissolved in ethanol to give a yellow solution. To this an aqueous solution of $K_2[PtCl_4]$ (41 mg, 1.27 mmol) was added and heated to reflux for 12 hours. The resulting brown mixture was filtered to give a brown solid. This was redissolved in DMF and filtered to give a dark red solution which was taken to dryness under reduced pressure at 35°C. The resulting crude solid was washed with water, ethanol and ether to give [Pt($\mathbf{L^1}$)Cl] as a dark red solid (22 mg, 30%). ^{1}H NMR (DMSO-d_6): δ 9.19 (dd, $^3J_{HH}$ = 6 Hz, 1H), 9.14 (s, 1H), 9.00 (m, 2H), 8.31 (m, 2H), 8.18- (dd, $^3J_{HH}$ = 6 Hz, $^3J_{HH}$ = 8.4 Hz 1H), 8.08 (d, 1H), 7.10 (d, $^3J_{HH}$ = 9.2 Hz, 2H), 6.67 (d, $^3J_{HH}$ = 9.2 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 139.4, 129.7, 127.8, 127.5, 127.4, 123.7, 114.4. IR (KBr): 3424, 1626 (C=O), 1603, 1504, 1235. ES(+) MS (*m/z*): 567, $[\mathbf{1} + Na]^+$. Anal. Calcd for $C_{19}H_{12}N_3O_2PtCl$: C, 41.88; H, 2.22; N, 7.71. Found: C, 41.76, H, 1.85, N, 7.36.

Synthesis of [Pt(L^2)Cl] (2). Compound L^2 (40 mg, 0.09 mmol) was dissolved in ethanol (15 mL) to give a pale yellow solution. To this, an aqueous solution of $K_2[PtCl_4]$ (30 mg, 0.09 mmol) was added and the resulting mixture heated to reflux for 6 hours to give a red-brown colored solution. This was left to stir at room temperature overnight. The reaction mixture was filtered to give a red solution which was taken to dryness under reduced pressure to yield a bright red solid. This was washed twice with small quantities of methanol and once with diethyl ether to give [Pt(L^2)Cl] as a bright orange solid (25 mg, 44%). ^{1}H NMR (DMSO-d_6): δ 9.23 (dd, H_a, 1H), 9.01 (m, 2H), 8.29 (m, 2H), 8.13 (dd, H_b, 1H), 8.08 (d, H_f, 2H), 7.22 (d, H_i, 2H), 6.89 (d, H_j, 2H), 4.40 (br m, 2H), 3.51 (br m, 4H), 3.04 (br m, 2H), 1.80 (br m, 4H), 1.41 (br m, 2H). ^{13}C NMR (DMSO-d_6): δ 150.9, 139.8, 130.3, 129.0, 127.7, 127.4, 124.2, 114.0, 65.4, 63.0, 52.8, 22.4. IR (KBr): 3424 (NH), 1626 (C=O), 1603, 1504, 1235. ES(+) MS (*m/z*): 657, [**2** + H]$^+$. Anal. Calcd for $C_{26}H_{25}N_4O_2PtCl{\cdot}3KCl$: C, 35.50; H, 2.86; N, 6.36. Found: C, 35.10, H, 2.94, N, 6.11.

Fluorescence resonance energy transfer (**FRET) studies**. All oligonucleotides and their fluorescent conjugates were purchased from Eurogentec (UK). DNA was dissolved as a stock 20 μM solution. All dilutions were carried out with 50 mM potassium cacodylate buffer (pH 7.4). The ability of the compounds to stabilise G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) assay modified to be used as a high-throughput screen in a 96-well format. The appropriate labelled oligonucleotide (F21T, 5-*FAM*-dGGG(TTAGGG)3-*TAMRA*-3 or DS, 5-FAM-duplex sequence-TAMRA-3; donor fluorophore *FAM*: 6-carboxyfluorescein; acceptor fluorophore *TAMRA*: 6-carboxy-tetramethylrhodamine) used as the FRET probe was diluted from stock to the correct concentration (400 nM)

in a 50 mM potassium cacodylate buffer (pH 7.4) and then annealed by heating to 92ºC for 5 min, followed by cooling to room temperature in the heating block. Compounds were prepared from stock solutions (**L**^{2} and **2**, 10 mM in water (30%) and DMSO (70%); **L**^{1}, 10 mM in DMSO) on the day of use. Final solutions were prepared using DMSO in the initial 1:10 dilution, after which 50 mM potassium cacodylate buffer (pH 7.4) was used in all subsequent steps. The maximum HCl concentration in the reaction volume (at a compound concentration of 20 μM) is thus 200 μM, well within the range of the buffer used. 96-well plates were prepared using a dilution robot resulting in 200 nM DNA concentration in each well and the appropriate compound concentration. Measurements were made on a DNA Engine Opticon (MJ Research) with excitation at 450–495 nm and detection at 515–545 nm. Fluorescence readings were taken at intervals of 0.5°C over the range 30–100°C, with a constant temperature being maintained for 30 seconds prior to each reading to ensure a stable value. Final analysis of the data was carried out using a script written in the program Origin 7.0 (OriginLab Corp., Northampton, MA). The advanced curve-fitting function in Origin7.0 was also used to obtain the relevant curves and values of interest were read directly from this graph.

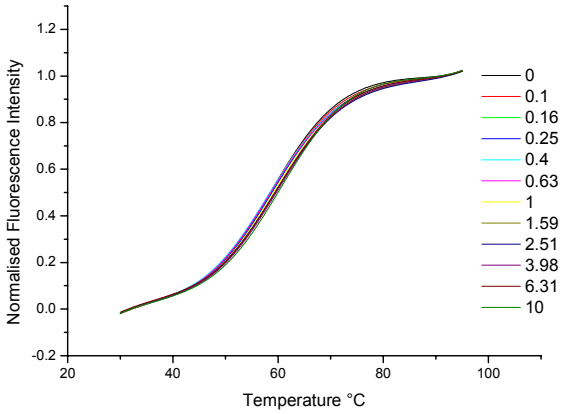

Figure S1 – FRET melting curves for **L**^{1} (concentrations in the inset of the graph are in μM).

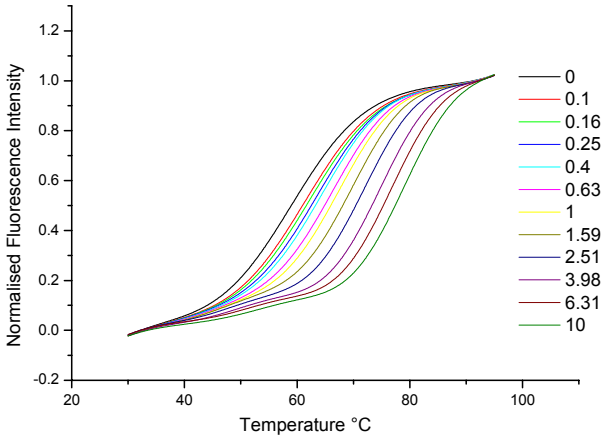

Figure S2 – FRET melting curves for $\mathbf{L^2}$ (concentrations in the inset of the graph are in μM).

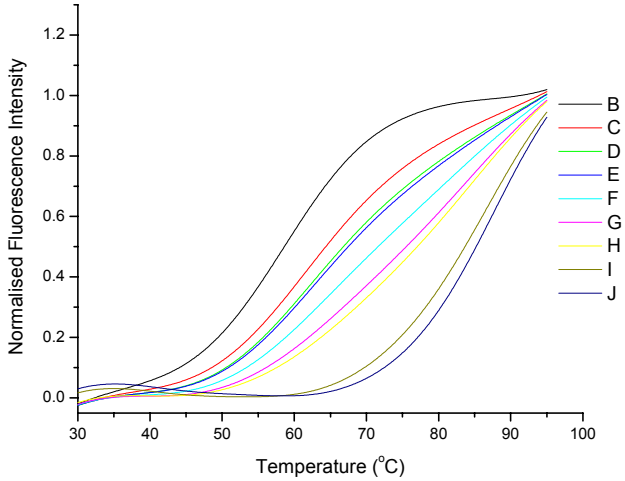

Figure S3 – FRET melting curves for complex **2** (concentrations in the inset of the graph are in μM).

Modified TRAP assay. Telomerase activity in the presence of the compounds was assessed using a modified version (steps 2 and 3, see below) of previously published TRAP protocols, with cell extract from exponentially growing A2780 human ovarian carcinoma cells used as the enzyme source. The TRAP assay was carried out in two main steps with an initial primer-elongation step and subsequent PCR amplification of the telomerase products to enable detection. *Step 1*: a master reaction mixture (40 μl) was prepared containing the TS forward primer (0.1 μg; 5'-AATCCGTCGAGCAGAGTT-3'), TRAP buffer (20 mM Tris-HCl [pH 8.3], 68 mM

KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.05% v/v Tween-20), BSA (0.05 μg), and dNTPs (125 μM each). Protein (1 μg) was then incubated with the reaction mixture with or without the compound to be tested (made up in solution as the HCl salt) for 10 min at 30 ºC. *Step 2*: Following heat inactivation of telomerase at 94 ºC for 4 min and cooling to 20ºC, purification of the telomerase products was performed using QIAquick nucleotide removal spintubes, following the protocol described for their use with the exception that the elution stage was performed with 40 μL PCR-grade water. Samples were then dried using a SpeedVac centrifuge. *Step 3*: A master mix (50μL) of (TS forward primer (0.1 μg; 5'-AATCCGTCGAGCAGAGTT-3'), TRAP buffer (20 mM Tris-HCl [pH 8.3], 68 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.05% v/v Tween-20), BSA (0.05 μg), and dNTPs (125 μM each) ACX primer (0.1 μg; 5'-GTG$[CCCTTA]_3$CCCTAA-3') and 2μM *Taq* polymerase (RedHot, ABgene, Surrey, UK) were added to each tube to start the PCR protocol. Thermal cycling was carried out in three parts following an initial 5 min denaturing period at 94 ºC (30 cycles of 94 ºC for 30 s, 61 ºC for 60 s, 72 ºC for 60 s). PCR-amplified reaction products were then run out on a 10% w/v non-denaturing PAGE gel and visualised by staining with SYBR Green I (Sigma). $^{tel}EC_{50}$ values were calculated by quantitating the TRAP product using a gel scanner and GeneTools software (Syngene, Cambridge, UK), Measurements were made with respect to a negative control run using the equivalent TRAP-PCR conditions but omitting the protein extract, thus ensuring that the ladders observed were not due to artefacts of the PCR reaction.

References:

1. E. J. Corey, A. L. Borror, T. Foglia *J. Org. Chem.* 1965, *30,* 288-290.
2. J. F. J. Engbersen, A. Koudijs, M. H. A. Joosten, H. C. Van der Plas *J. Heterocycl. Chem.* 1986, **23***,* 989-990.