Supporting Information for

Platinum(II)-Triarylpyridines Complexes with Electropositive Pendants as Efficient G-Quadruplex Binders

Jin-Tao Wang, Yi Li, Jia-Heng Tan, Liang-Nian Ji and, Zong-Wan Mao*

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S1. Synthesis of L^1 - L^3 and 1-3.

Preparation of ligands

4'-(4-(trimethylamino)methylphenyl)-2,2':6',2''-terpyridine bromide (L¹Br · H₂O). 4'-(4-Bromomethylphenyl)-2,2':6',2"-terpyridine¹ (0.402 g, 1.0 mmol) and 33% aqueous dimethylamine (0.537 g, 3.0 mmol) were mixed in 10.0 mL ethanol at room temperature for 4 h. The solvent was evaporated and 20.0 ml diethyl ether was added. Then the mixture was stirred overnight and precipitate was formed finally. The residue was filtered and the resulting white powder was washed with diethyl ether and dried in vacuum (0.428 g, 89.4%). ¹H NMR (300 MHz, [*D*₆]DMSO, 25 °C): δ = 8.72 (d, 2 H, *J* = 4.2), 8.70 (s, 2 H), 8.64 (d, 2 H, *J* = 7.8), 8.05-7.80 (m, 4 H), 7.40 (d, 2 H, *J* = 8.4), 7.53-7.49 (m, 2 H), 4.64 (s, 2 H), 3.09 (s, 9 H); ¹³C NMR (75 MHz, [*D*₆]DMSO, 25 °C): δ = 156.36, 155.32, 149.94, 149.30, 139.93, 138.27, 134.44, 130.02, 128.14, 125.35, 121.71, 118.86, 68.15, 52.72; ESI-MS: *m/z* (%): 381.2 (100) [L¹]⁺, 322.4 (23) [L¹-N(CH₃)₃]⁺; elemental analysis calcd (%) for C₂₅H₂₇BrN₄O (479.41): C 62.63, H 5.68, N 11.69; found: C 62.80, H 5.68, N 11.53.

4'-(4-(triethylammonio)methylphenyl)-2,2':6',2''-terpyridine bromide (L^2Br **2H₂O).** 4'-(4-Bromomethylphenyl)-2,2':6',2''-terpyridine (0.402 g, 1.0 mmol) and triethylamine (0.152 g, 1.5 mmol) were mixed in 10.0 mL ethanol at 60 °C for 6 h. The solvent was evaporated and 20.0 ml diethyl ether was added. Then the mixture was stirred overnight and precipitate was formed finally. The residue was filtered and the resulting white powder was washed with diethyl ether and dried in vacuum (0.443 g, 82.1%). ¹H NMR (300 MHz, [D₆]DMSO, 25 °C): $\delta = 8.72$ (d, 2 H, J = 3.9), 8.69 (s, 2 H), 8.63 (d, 2 H, J = 8.1), 8.04-7.99 (m, 4 H), 7.10 (d, 2 H, J = 8.1), 7.53-7.49 (m, 2 H), 4.57 (s, 2 H), 3.21 (m, 6 H), 1.34 (t, 9 H, J = 7.2); ¹³C NMR (100 MHz, [D₆]DMSO, 25 °C): $\delta = 156.41$, 155.36, 149.98, 149.22, 139.86, 138.21, 134.19, 129.57, 128.19, 125.33, 121.68, 118.81, 59.94, 53.02, 8.45 ppm; ESI-MS: m/z (%): 423.2 (100) [L^2]⁺, 322.4 (23) [L^2 -N(CH₂CH₃)₃]⁺; elemental analysis calcd (%) for C₂₈H₃₅BrN₄O₂ (539.51): C 62.33, H 6.54, N 10.38; found: C 62.50, H 6.38, N 10.36.

4'-(4-(tributylammonio)methylphenyl)-2,2':6',2''-terpyridine bromide (L³Br · **H**₂**O)** 4'-(4-Bromomethylphenyl)-2,2':6',2''-terpyridine (0.402 g, 1.0 mmol) and tributylamine (0.280 g, 1.5 mmol) were mixed in 10.0 mL ethanol and heated at reflux for 16 h. The solvent was evaporated and 20.0 ml diethyl ether was added. Then the mixture was stirred overnight and precipitate was formed finally. The residue was filtered and the resulting white powder was washed with diethyl ether and dried in vacuum (0.460 g, 75.9%). ¹H NMR (300 MHz, CDCl₃, 25 °C): $\delta = 8.62$ (d, 2 H, J = 4.8), 8.57 (s, 2 H), 8.52 (d, 2 H, J = 7.8), 7.87-7.76 (m, 4 H), 7.67 (d, 2 H, J = 8.1), 7.29-7.27 (m, 2 H), 5.05 (s, 2 H), 3.33 (m, 6 H, J = 7.2), 1.84 (m, 6 H, J = 7.2), 1.43 (m, 6 H), 1.01 (t, 9 H, J = 7.5); ¹³C NMR (100 MHz, CDCl₃, 25 °C): $\delta = 156.12$, 155.87, 149.19, 148.51, 140.89, 136.95, 133.47, 128.26, 128.12, 124.04, 121.42, 118.78, 63.12, 59.20, 25.02, 20.22, 14.09 ppm; ESI-MS: m/z (%): 507.3 (100) [L³]⁺, 322.4 (19) [L³-N(CH₂CH₂CH₂CH₃)₃]⁺; elemental analysis calcd (%) for C₃₄H₄₅BrN₄O (605.65): C 67.43, H 7.49, N 9.25; found: C 67.36, H 7.53, N 9.15.

Preparation of Pt(II) complexes

General Procedure for the Synthesis of complexes 1-3

A total of 1 mmol of the ligands and 1 mmol of $Pt(DMSO)_2Cl_2^2$ was suspended in 40 mL of chloroform, and the mixture was refluxed for 24 h. After that, the reaction mixture was concentrated and cooled down to room temperature. The solid was then filtered out and dissolved in a small amount of DMF. Saturated NaPF₆ aqueous solution was added, and the mixture was stirred at room temperature for another 3 h. The resultant solid was filtered out; washed with chloroform and ether; and dried.

[Pt(L¹)Cl](PF₆)₂·H₂O (1) Yield as light yellow powder (83.6%). ¹H NMR (300 MHz, $[D_6]$ DMSO, 25 °C): $\delta = 8.92$ (s, 2 H), 8.75-8.69 (m, 4 H), 8.45 (d, 2 H, J = 7.8), 8.27 (d, 2 H, J = 7.8), 7.87-7.81 (m, 4 H), 4.63 (s, 2 H), 3.10 (s, 9 H); elemental analysis calcd (%) for C₂₅H₂₇ClF₁₂N₄OP₂Pt (919.97): C 32.64, H 2.96, N 6.09; found: C 32.46, H 2.95, N 6.06.

[Pt(L²)Cl](PF₆)₂·H₂O (2) Yield as yellow powder (80.8%). ¹H NMR (300 MHz, [D₆]DMSO, 25 °C): δ = 8.92 (s, 2 H), 8.74-8.68 (m, 4 H), 8.45 (d, 2 H, *J* = 7.8), 8.25 (d, 2 H, *J* = 7.8), 7.86-7.77 (m, 4 H), 4.59 (s, 2 H), 3.24 (m, 6 H), 1.37 (s, 9 H, *J* = 7.2); elemental analysis calcd (%) for C₂₈H₃₃ClF₁₂N₄OP₂Pt (962.05): C 34.96, H 3.46, N 5.82; found: C 35.17, H 3.63, N 6.07.

[Pt(L³)Cl](PF₆)₂·2H₂O (3) Yield as red powder (77.7%). ¹H NMR (300 MHz, [D₆]DMSO, 25 °C): $\delta = 8.95$ (s, 2 H), 8.79-8.71 (m, 4 H), 8.46 (d, 2 H, J = 7.8), 8.32 (d, 2 H, J = 7.8), 7.87-7.74 (m, 4 H), 4.64 (s, 2 H), 3.17 (m, 6 H, J = 7.2), 1.88 (m, 6 H, J = 7.2), 1.35 (m, 6 H), 0.99 (t, 9 H, J = 7.5); elemental analysis calcd (%) for C₃₄H₄₇ClF₁₂N₄O₂P₂Pt (1064.22): C 38.37, H 4.45, N 5.26; found: C 38.46, H 4.23, N 5.33.

S2. CD spectroscopy.

CD measurements were recorded on a Jasco J-810 CD spectropolarimeter at room temperature using a cell length of 1 cm, and over a wavelength range of 200-400 nm. The oligomer 22AG (5'-AG₃(T₂AG₃)₃-3') at a final concentration of 3 μ M was resuspended in Tris-HCl buffer (10 mM, pH 7.4) containing 100 mM K⁺ or not and the complexes to be tested. The samples were heated to 95 °C for 5 min, then gradually cooled to room temperature and incubated at 4 °C overnight. CD spectra were baseline-corrected for signal contributions due to the buffer. CD titration was performed at a fixed 22AG concentration (3 μ M) with various concentrations of the complexes (3 mM in DMSO). After each addition of complex, the reaction was stirred and allowed to equilibrate for at least 10 min (until no elliptic changes were observed) and a CD spectrum was collected at least five scans. Final analysis of the data was carried out using Origin 7.5 (OriginLab Corp.).



Fig. S1 CD titration spectra of 22AG quadruplex (3 μ M) at increasing concentrations of L¹, L³, **1** and **3** in 10 mM Tris-HCl buffer, pH 7.4, 100 mM KCl and no metal cations, rt. The arrows indicate the increasing amounts of complexes (r = compound/DNA strand concentration). (1a) 1: r of 0.2, 0.5, 1.0, 1.2, 1.4, 1.8 and 2.0 in 100 mM KCl; (1b) L¹: r of 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 in the absence of KCl; (1c) 1: L²: r of 0.2, 0.5, 0.8, 1.0 and 1.2 in 100 mM KCl; (1d) L¹: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2a) 3: r of 0.2, 0.5, 1.0, 1.2, 1.4, 1.8 and 2.0 in 100 mM KCl; (2b) L³: r of 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl; (2d) L³: r of 0.5, 1.0, 2.0, 4.0 and 6.0 in the absence of KCl.

S3. Fluorescence resonance energy transfer (FRET) studies.

The fluorescent labeled oligonucleotide F21T (5'-*FAM*-G₃[T₂AG₃]₃-*TAMRA*-3', FAM: 6-carboxyfluorescein, TAMRA: 6-carboxy-tetramethylrhodamine) and F10T (5'-*FAM*-dTATAGCTATA-HEG-TATAGCTATA-*TAMRA*-3', HEG linker is [(-CH2-CH2-O-)₆]) used as the FRET probes were diluted in 60 mM potassium cacodylate buffer (pH 7.4) and then annealed by heating to 92 °C for 5 min, followed by cooling slowly to room temperature overnight. Fluorescence melting curves were determined with a Roche LightCycler 2 real-time PCR machine, using a total reaction volume of 20 μ L, with 0.2 μ M of labeled oligonucleotide and 1 μ M complexes. Measurements were made on a RT-PCR with excitation at 470 nm and detection at 530 nm. Fluorescence readings were taken at intervals of 1 °C over the range 37-99 °C, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value. Final analysis of the data was carried out using Origin 7.5 (OriginLab Corp.).



Fig. S2. FRET-melting curves obtained with F21T (a) and F10T (b) alone (\blacksquare) and with 1µM of L¹(\bullet), L² (\blacktriangle), L³ (\bigtriangledown), 1(\checkmark), 2(\triangleright) and 3().

S4. PCR stop assay.

The oligonucleotide HTG21 (5'-GGGTTAGGGTTAGGGTTAGGG-3') and the corresponding complementary sequence (5'-ATCGCTTCTCGTCCCTAACC-3', HTG21rev) were used here. The reactions were performed in $1 \times PCR$ buffer, containing 10 pmol of each oligonucleotide, 0.2 mM dNTP, 2.5 U Taq polymerase, and different concentrations of complexes. Reaction mixtures were incubated in a thermocycler with the following cycling conditions: 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. PCR products were then analysed on 15% nondenaturing polyacrylamide gels in 1× TBE and silver stained. The parallel experiment was performed using а mutated oligmer HTG21mu (5' -GAGTTAGAGTTAGAGTTAGAG-3') with its corresponding complementary sequence HTG21murev (5'-ATCGCTTCTCGTCTCTAACT-3') instead of HTG21 in identical conditions.



Fig. S3. Effect of L^1 , L^2 , L^3 , (0 - 8.0 μ M) on the hybridization of HTG21 and effect of **1**, **2**, **3**, L^1 , L^2 , L^3 , (8.0 μ M) on the hybridization of HTG21mu in the PCR-stop assay.

S5. Molecular Modeling

An automated docking analysis of complex **2** was performed into the crystallographic structure parallel 22-mer telomeric G-quadruplex (PDB ID 1KF1), using the Surflex-Dock suite³ (SYBYL 7.3.5, Tripos, Inc.). Anion $2(PF_6)^-$ was negligible in complex **2** and water molecules were removed from the PDB file. Herein the Surflex-Dock uses an empirical scoring function and a patented search engine to dock the complexes into telomeric G-quadruplex for finding the most stable and favourable orientation. The images in the manuscript were created with ChemBio3D Ultra 11.0.

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L1 #45-48 RT: 1.40-1.49 AV: 4 SB: 13 0.01-0.39 NL: 5.31E6 T: + c Full ms [50.00-2000.00]















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