Supplementary Material (ESI) for Chemical Communications

Tetramethylpyridiniumporphyrazines- a new class of G-Quadruplex Inducing and Stabilising Ligands

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Electronic supplementary information

In our studies we used the 5'-GGATTGGGATTGGGATTGGGATTGGG-3' sequence (*Htelo*). The *Htelo* was annealed in a 50 mM Tris (pH 7.4), 150 mM of KCl buffer by heating to 95ºC for five minutes and then slowly cooled to room temperature. The presence of the G-quadruplex was then confirmed by CD spectroscopy.

Circular dichroism experiments, including the DNA melting, were performed on a JASCO model J-810 circular dichroism spectrapolarimeter equipped with a Peltier temperature controller.

UV-Vis spectra were measured using a Carry 400 spectrophotometer. UV-vis spectra were recorded using a 1 cm path-length quartz cell.

Surface plasmon resonance was performed on a BIAcore 3000 instrument, using degassed and filtered Tris.HCl running buffer (50 mM Tris.HCl, 100 mM KCl, pH 7.4). Sensor chips (Type SA, Biacore) were loaded with approximately 600 RU of *Htelo* and duplex DNA. Six serial dilutions of compound were injected at a flow rate of 20 µL min^{-1} and the equilibrium response determined relative to the base line (blank flow cell). The maximum concentration for both porphyrazines was 1.25 µM. Between injections the sensor surface was refreshed with injections of 1 M KCl and buffer. All experiments were carried out in duplicate.

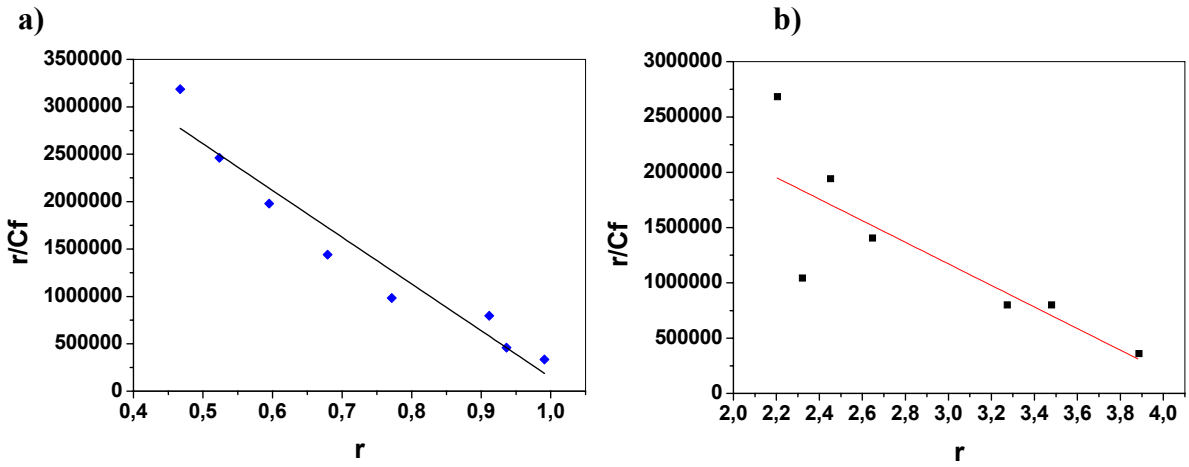

Figure 1. Scatchard binding plots (r/Cf vs r) used to determine the affinity constants for: a) 3,4-TMPyPz; and b) 3,4-TMPyPz Zinc (II), with the *Htelo* G-quadruplex.

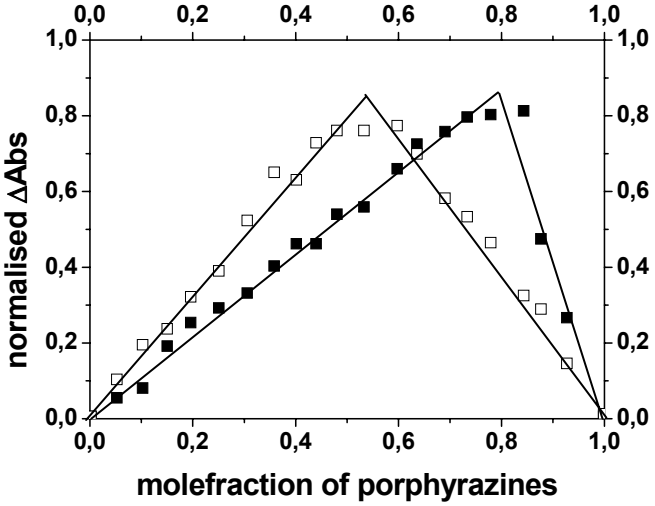

Figure 2. Job plots for the binding of the 3,4-TMPyPz (□) and of the 3,4-TmPyPz Zinc (II) (■)with the *Htelo* G-Quadruplex in a 50 mM Tris (pH 7.4), 150 mM of KCl buffer at 20ºC. The sum of porphyrazines and *Htelo* G-Quadruplex concentrations were fixed at 5 μM. Optical absorbances changes of the bound 3,4-TMPyPz at 388 nm, and at 398 nm for the 3,4-TMPyPz Zinc (II) were normalised to the maximum increase in each case. The Y-axis represents the differences in absorbance for mole fractions of ligand (χL) in G-quadruplex and in buffer alone. Intercept of the mole fraction values were determined from least-squares fits to the linear data points, giving χint values of 0.52 (1.1:1 stoichiometry) for 3,4-TmPyPz and 0.80 (4.2:1 stoichiometry) for the 3,4-TMPyPz Zinc (II), respectively.

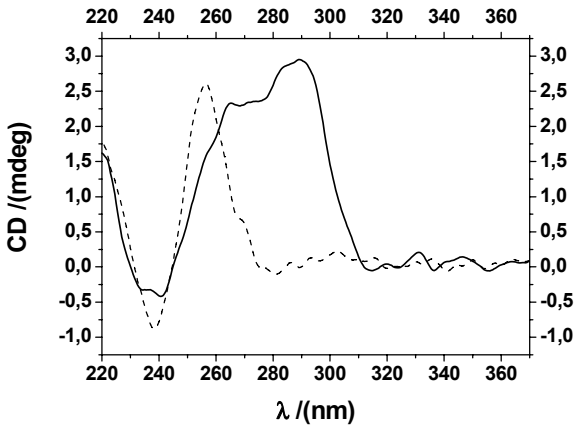

Figure 3. CD spectra of 10 μM of annealed *Htelo* DNA in a 50 mM Tris (pH 7.4), 150 mM of KCl buffer (G-quadruplex) (solid line), showing its two characteristic parallel (at around 265 nm) and anti-parallel (at around 290 nm) conformations. Together with the CD spectra of 10 μM of non-annealed *Htelo* (dashed line) in a 50 mM Tris (pH 7.4) buffer, showing its characteristic positive peak at around 255 nm. All experiments were carried out at 20ºC.

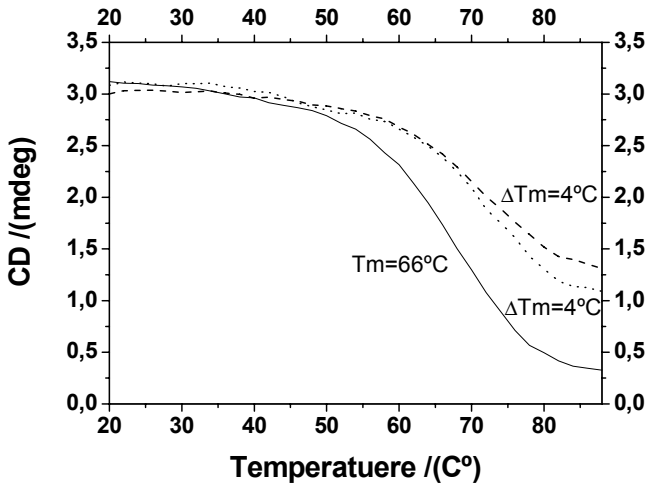

Figure 4. CD melting profiles monitored at 295 nm of 10 μM of Htelo G-quadruplex in a 50 mM Tris (pH 7.4), 150 mM of KCl buffer (solid line) in the presence of 9 equiv. of 3,4-TmPyPz (dotted line) and of 9 equiv. of 3,4-TmPyPz Zinc (II) (dashed line) porphyrazines.