A Small Molecule that Disrupts G-Quadruplex DNA Structure and Enhances Gene Expression

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SUPPORTING INFORMATION for an article for the

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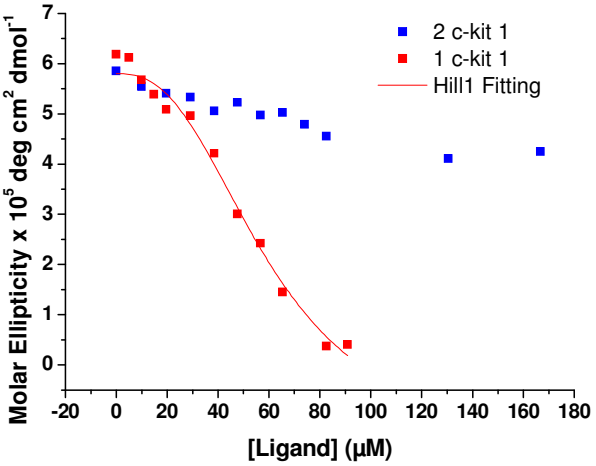
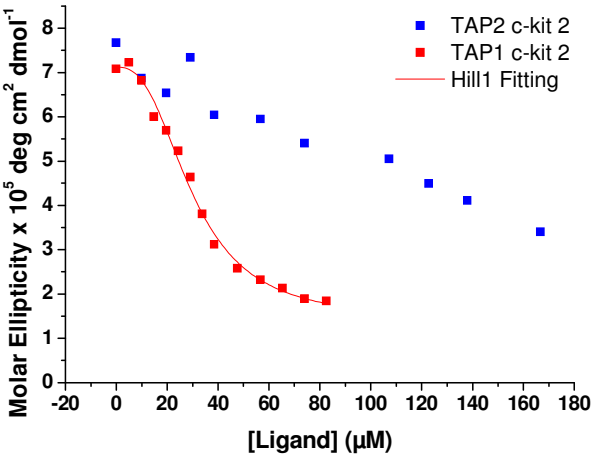
Hill Fitting Parameters for CD Data

Figure 1 shows the plot of molar ellipticity against the concentration of ligands **1** and **2** for c-kit 1 and includes the Hill parameters used to obtain sigmoidal curve fitting. Figure 2 shows the plot of molar ellipticity against the concentration of ligands **1** and **2** for c-kit 2 and includes the Hill parameters used to obtain sigmoidal curve fitting.



Model		Hill1		
Equation		y = START + (END - START) * x^n / (k^n + x^n)		
Reduced Chi-Sqr		0.07877		
Adj. R-Square		0.9832		
			Value	Standard Error
c-kit 1	1	START	5.79838	0.20248
c-kit 1	1	END	-1.76497	1.63964
c-kit 1	1	k	60.2837	11.09822
c-kit 1	1	n	2.57533	0.66305

Figure 1. Plot of molar ellipticity against ligand concentration for c-kit 1 and the associated fitting parameters



Model		Hill1		
Equation		y = START + (END - START) * x^n / (k^n + x^n)		
Reduced Chi-Sqr		0.03466		
Adj. R-Square		0.99157		
			Value	Standard Error
c-kit 2	1	START	7.12047	0.17719
c-kit 2	1	END	1.41058	0.26323
c-kit 2	1	k	29.98969	1.41797
c-kit 2	1	n	2.62198	0.34864

Figure 2. Plot of molar ellipticity against ligand concentration for c-kit 2 and the associated fitting parameters

UV Spectroscopy

UV-Vis absorption was used to investigate the behaviour of a ligand **1** in solution**.** Figure 3 shows the titration of **1** in water. Absorption spectra were recorded on a Cary 400 UV/Vis spectrophotometer in the 200–400 nm range at 20 °C in a 1 cm path length quartz cuvette. Aliquots of a concentrated solution of **1** were added to water and scanned immediately up to a total concentration of 45 μM. Figure 4 shows a plot of absorbance at 285 nm against concentration of **1**. The linear correlation shows that the solution obeys Beer-Lambert law and thus aggregation of ligand does not occur at such concentrations.

Analogous experiments were then performed in the presence of 10 μM of c-kit 2 in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4 to investigate the behaviour of a ligand **1** in the presence of DNA. Figure 5 shows the titration of **1** into a solution of c-kit

2 in buffer. Figure 6 shows a plot of absorbance at 285 nm against concentration of **1**. The linear correlation shows that the solution obeys Beer-Lambert law and thus aggregation of ligand does not occur in the presence of DNA.

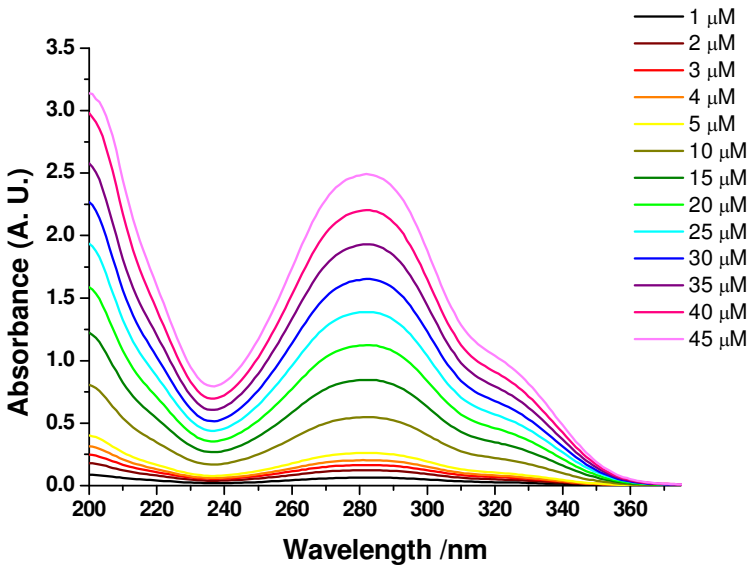


Figure 3. UV titration of **1** in water

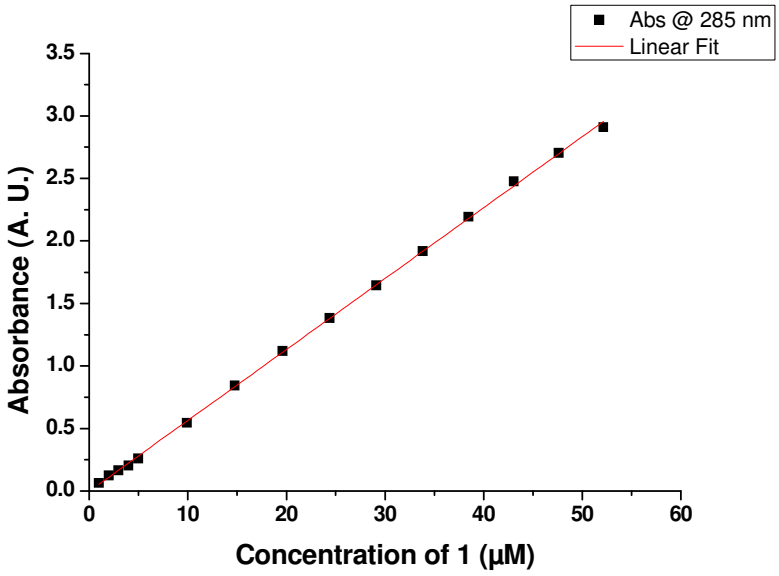


Figure 4. Beer-Lambert plot of the concentration of **1** against absorbance

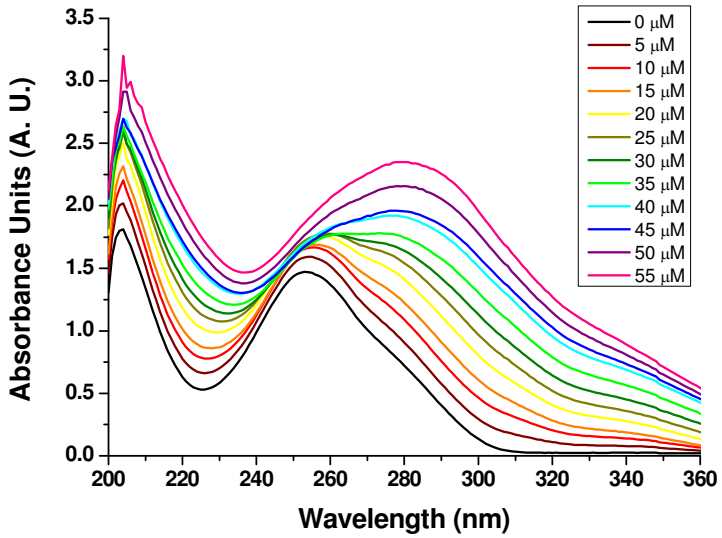


Figure 5. UV titration of **1** into a solution of c-kit 2 (10 μM) in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4

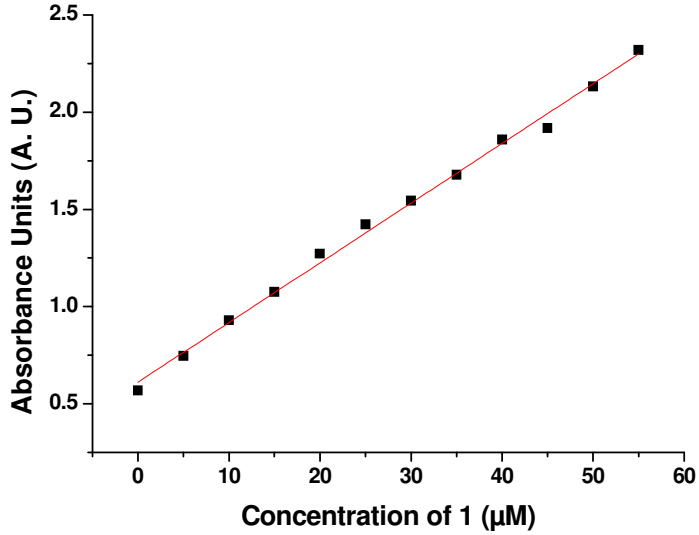


Figure 6. Beer-Lambert plot of the concentration of **1** against absorbance of the ligand-DNA complex

CD Spectroscopy of c-kit 2 21T Mutant

The NMR titration was performed with the 21T mutant c-kit 2 sequence so a control CD titration of ligand **1** with c-kit 2 21T d[CGGGCGGGCGCGAGGGAGGGT] was performed in the same buffer conditions. We titrated the ligand **1** into a pre-annealed solution of c-kit 2 21T in buffer containing 10 mM potassium phosphate at pH 7.0, mixed thoroughly, and acquired a CD spectrum immediately throughout the titration series. A reduction in the signal at 260 nm was observed, consistent with the results with the native (wild-type) sequence.

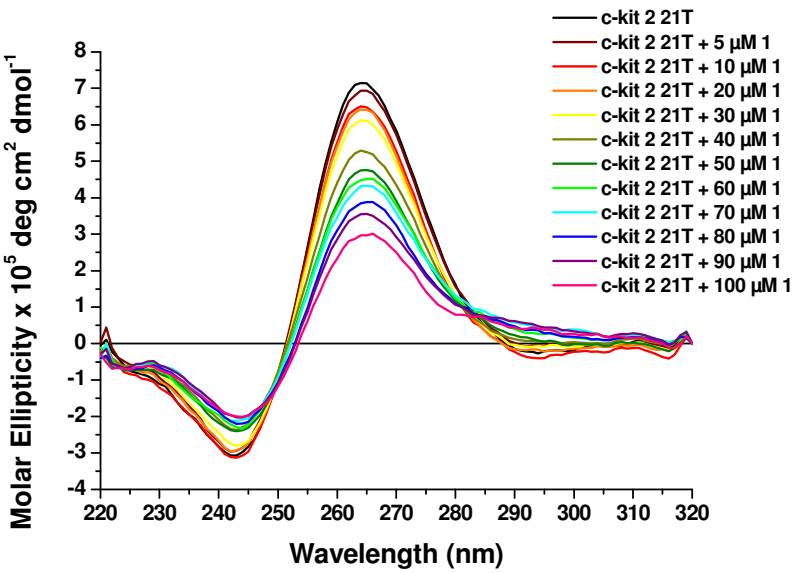


Figure 7. CD spectra of titration experiments of 0 – 100 µM of ligand **1** with 10 µM c-kit 2 21T DNA

NMR Spectroscopy

To investigate the structural heterogeneity of c-kit 2 21T in the absence and presence of ligand **1** we employed 1D jump-return Hahn echo spectroscopy (Figure 8). The spectra

were recorded under identical conditions with echo delays ranging from 1.5 to 40 ms. The loss of observed signals is due to the transverse relaxation processes of individual protons: systems that tumble slower in solution and/or undergoing conformation exchanging process will relax faster, i.e., less residual signals over a given relaxation period, T. Comparison of the two series indicate that c-kit2 21T in the liganded form relaxes faster than the free form, suggesting conformational heterogeneity, since changes in molecular size has been precluded by diffusion measurements (see Figure 9).

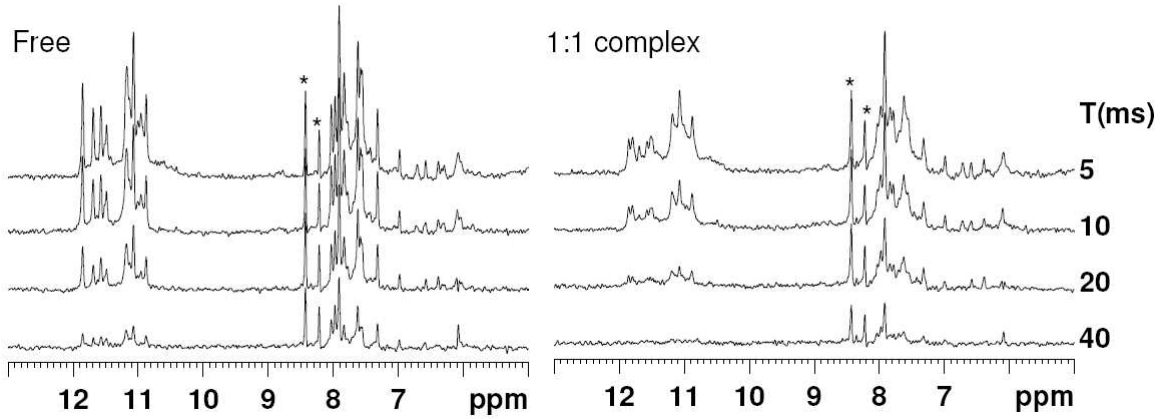


Figure 8. 1D jump-return Hahn echo spectra of c-kit2 21T in the absence (left) and in the presence of one equivalent of **1** (right). The length of the echo delays is given on the right.

To rule out the presence of oligomerization we compared the apparent hydrodynamic radius of c-kit2 21T in the presence and absence of ligand **1** by means of diffusion measurements using Pulse Field Gradient NMR Spectroscopy (Figure 9). There is no indication of oligomerization of c-kit2 21T upon binding to ligand **1**, confirming that the increased R_2 relaxation rate is predominately caused by conformational heterogeneity.

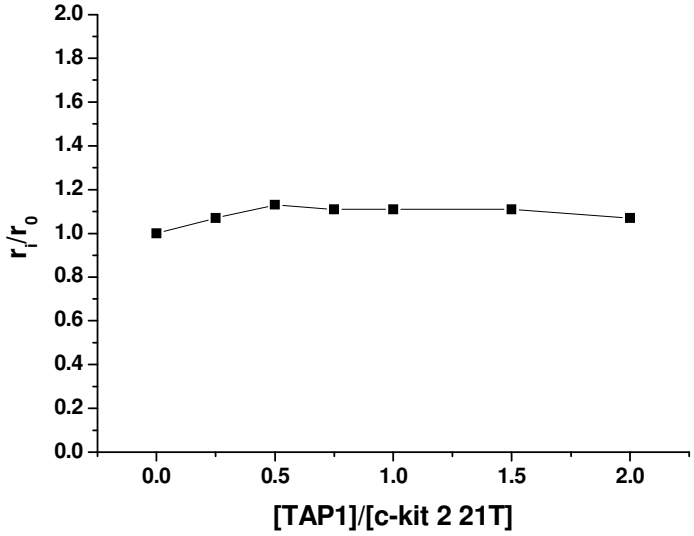


Figure 9. Relative apparent hydrodynamic radii of c-kit2 21T obtained by Pulse Field Gradient NMR Spectroscopy. The molar ratio of ligand **1** with respect to c-kit2 21T is indicated along the horizontal axis.

Fluorescence Spectroscopy

In our previous study (Waller, Z. A. E.; Shirude, P. S.; Rodriguez, R.; Balasubramanian, S. *Chem. Commun.*, **2008**, 1467) we used FRET-melting experiments to determine the stabilization temperature of triarylpyridine ligands. This technique can be complicated by ligand-fluorophore interaction (De Cian, A.; Guittat, L.; Kaiser, M.; Saccà, B.; Amrane, S.; Bourdoncle, A.; Alberti, P.; Teulade-Fichou, M.-P.; Lacroix, L.; Mergny, J.-L. *Methods*. **2007**, 183-195). Given the apparent unfolding effect indicated by the CD and NMR experiments we considered the possibility that the ΔT_m might be complicated by ligand-fluorophore interactions. To further investigate the interaction of ligand **1** with the dual-labelled FRET oligonucleotide we performed a fluorescence titration. In the folded conformation excitation of FAM results in FRET to the TAMRA fluorophore thus the fluorescence spectrum of dual-labeled c-kit 2 (sequence 5□-FAM-

d($G_3CG_3CGCGAG_3AG_4$)-TAMRA-3□) excited at 483 nm gives rise to an emission spectrum with maxima at 522 nm and 587 nm arising from the fluorophores FAM and TAMRA respectively. We titrated the ligand **1** into a pre-annealed solution of dual-labled c-kit 2 in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4, mixed thoroughly, and acquired a fluorescence spectrum immediately throughout the titration series (Figure 10). Upon titration of up to **1** into a solution of dual-labeled c-kit 2 the maxima at 587 nm was observed to increase without change in the intensity at 522 nm. This is indicative that ligand **1** interacts with the TAMRA fluorophore, causing an increase in fluorescence intensity. Thus the ΔT_m value is likely to be complicated by such an interaction and could be a false positive.

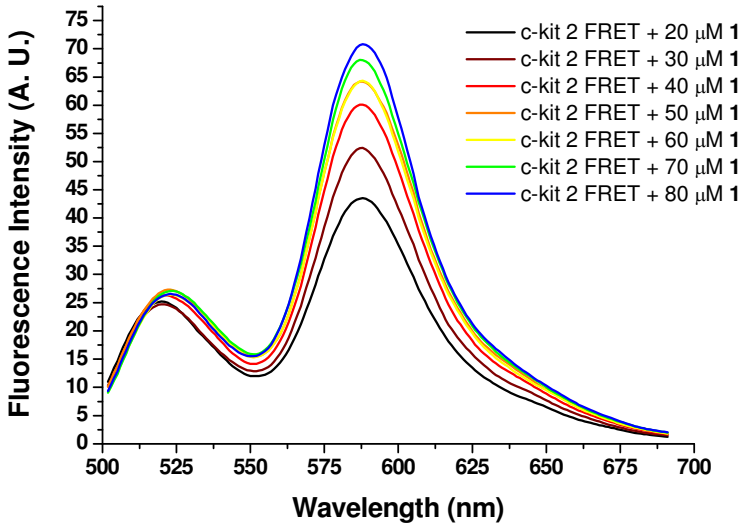


Figure 10. Fluorescence titration of **1** into a solution of dual-labeled c-kit 2 (10 μM) in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4. Excitation wavelength was 483 nm.

A CD titration with the same dual-labeled oligonucleotide in identical buffer conditions showed a dose-dependant decrease in the intensity at 260 nm (Figure 11).

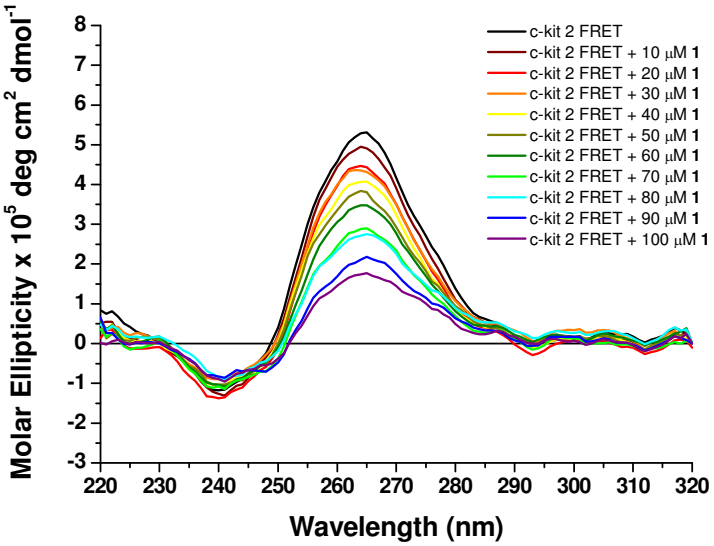


Figure 11. CD spectra of titration experiments of 0 – 100 μM of ligand **1** with 10 μM dual-labeled c-kit 2 21T DNA