Supplementary Materials

Stabilization of c-KIT G-Quadruplex DNA Structures by the RNA polymerase I inhibitors BMH-21 and BA-41

Stefania Mazzini*, Raimundo Gargallo, Loana Musso, Francesca De Santis, Anna Aviñó, Leonardo Scaglioni, Ramon Eritja, Massimo Di Nicola, Franco Zunino, Annabella Amatulli, Sabrina Dallavalle

Table S1. Binding constants (Kb) and stoichiometries calculated from the fluorimetric titration curves in Figures S3 and S4

Ligand	Overall ligand:DNA stoichiometry	Kb (M^{-1})	
BA-41 (**2**)	3:1	$10^{6.4\pm0.4}$ (1 binding site)	$10^{6.2\pm0.4}$ (2 binding sites)
BMH-21 (**1**)	2:1	$10^{6.4\pm0.44}$ (1 binding site)	$10^{5.9\pm0.24}$ (1 binding site)

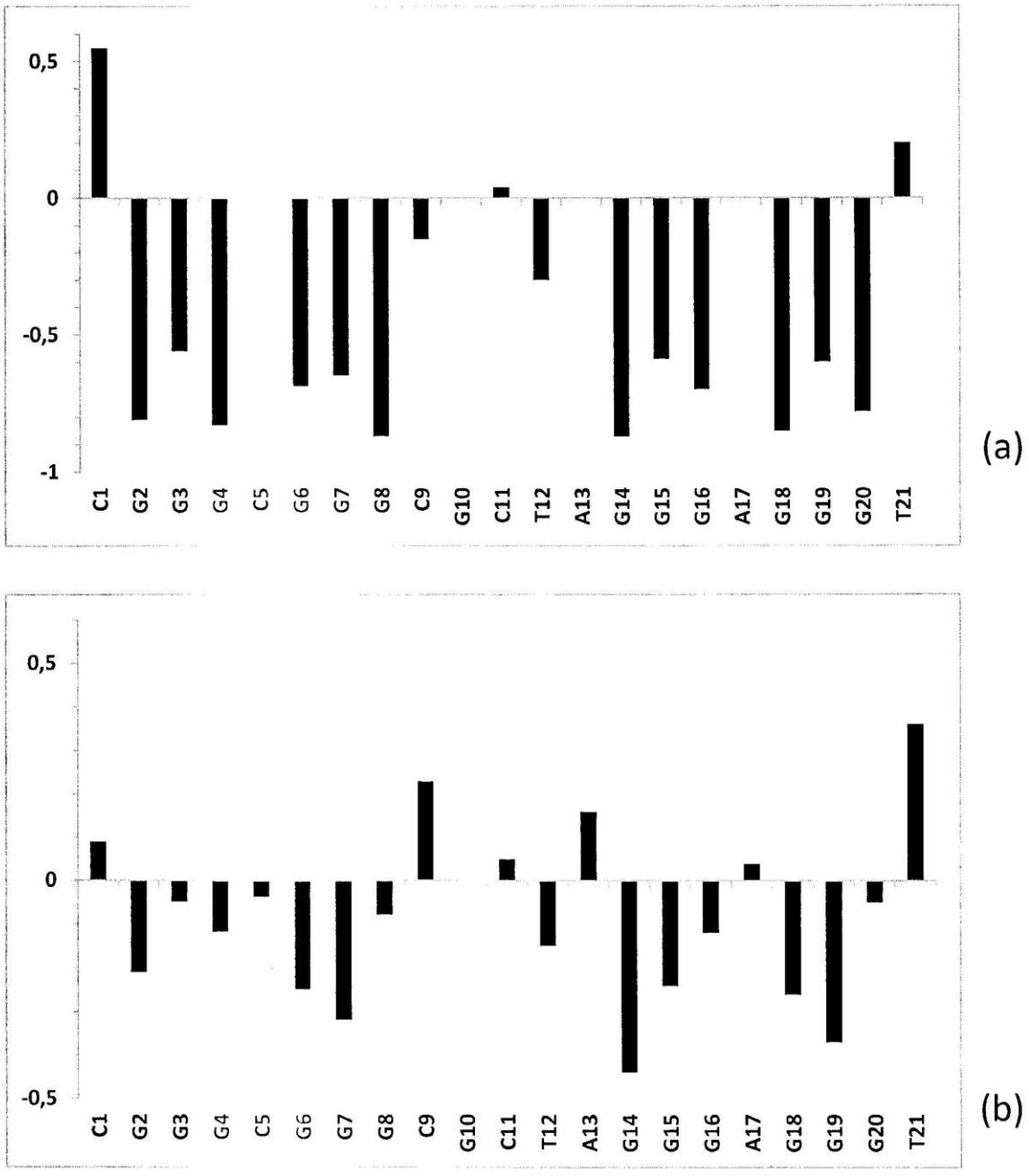
Table S2. Inter-residue NOE interactions of c-kit21T12T21 in the complex with BA-41 (**2**) and BMH-21 (**1**).

G-tetrad I	*G-tetrad II*	*Tetrad III*
G4H1…..G8H8	G3H1….G7H8	G18H1….G2H8
G8H1…G16H8	G7H1...G15H8	G14H1….G18H8
G16H1…G20H8	G15H1…G19H8	G6H1….G14H8
G20H1…G4H8	G19H1…G3H8	G2H1…..G6H8

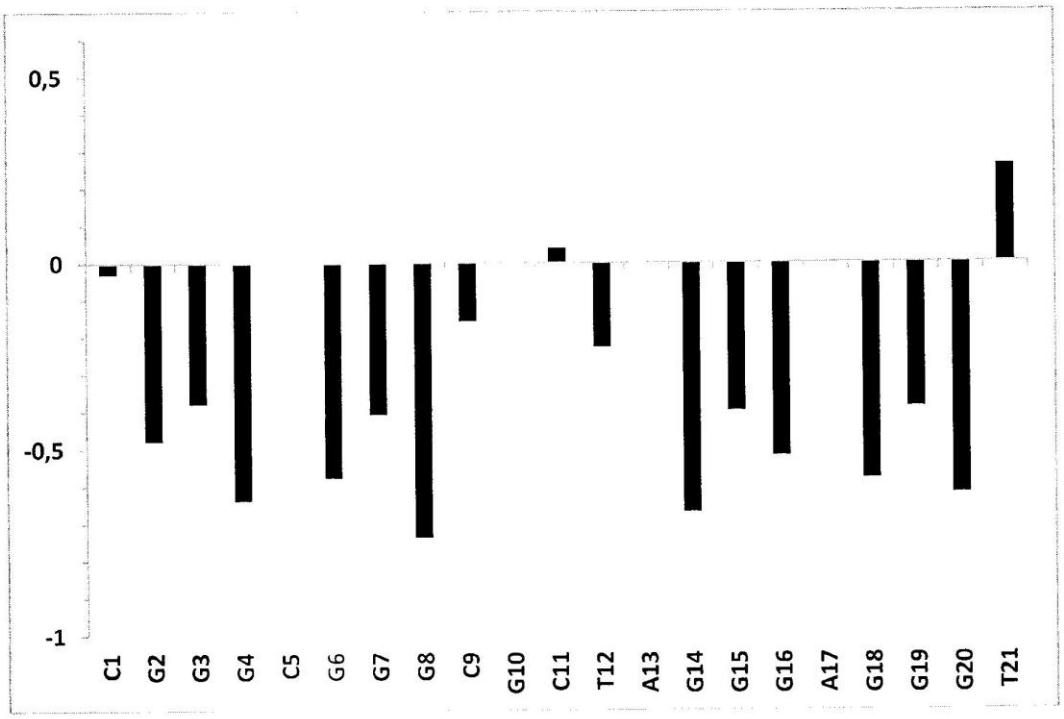
Table S3. ^{1}H chemical shift values for the complex of BA-41 (**2**) with c-kit21T12T21.[a]

complex of **BA-41**/c-kit21T12T21[b]						
c-kit	H1/H2/H5/CH_3	$\Delta\delta^c$	H6/H8	$\Delta\delta^c$	H1'	H2'-H2''
C1	6.10	+0.55	7.59	+0.09		
G2	11.15	-0.81	7.99	-0.21	6.01	
G3	10.74	-0.56	7.63	-0.05	6.03	
G4	10.32	-0.83	7.63	-0.12		
C5	6.20	0.00	8.00	-0.04	6.48	2.40, 2.70
G6	10.83	-0.69	7.77	-0.25	5.94	
G7	10.93	-0.65	7.70	-0.32	5.93	
G8	10.44	-0.87	7.71	-0.08		
C9	5.95	-0.15	7.85	+0.23	6.00	
G10	n.d.	-	n.d.			
C11	6.10	+0.04	7.95	+0.05	6.32	
T12	1.60	-0.30	7.45	-0.15	5.99	1.90, 2.13
A13	n.d.	-	8.26	+0.16	6.24	2.70,2.90
G14	11.07	-0.87	7.82	-0.44	6.01	
G15	10.79	-0.59	7.63	-0.24		
G16	10.34	-0.70	7.68	-0.12	6.33	
A17	n.d.	-	8.55	+0.04	6.62	2.92, 2.92
G18	10.97	-0.85	7.83	-0.26		
G19	10.96	-0.60	7.72	-0.37		
G20	10.47	-0.78	7.65	-0.05	6.24	
T21	1.70	+0.20	7.51	+0.36	6.33	2.30, 2.30

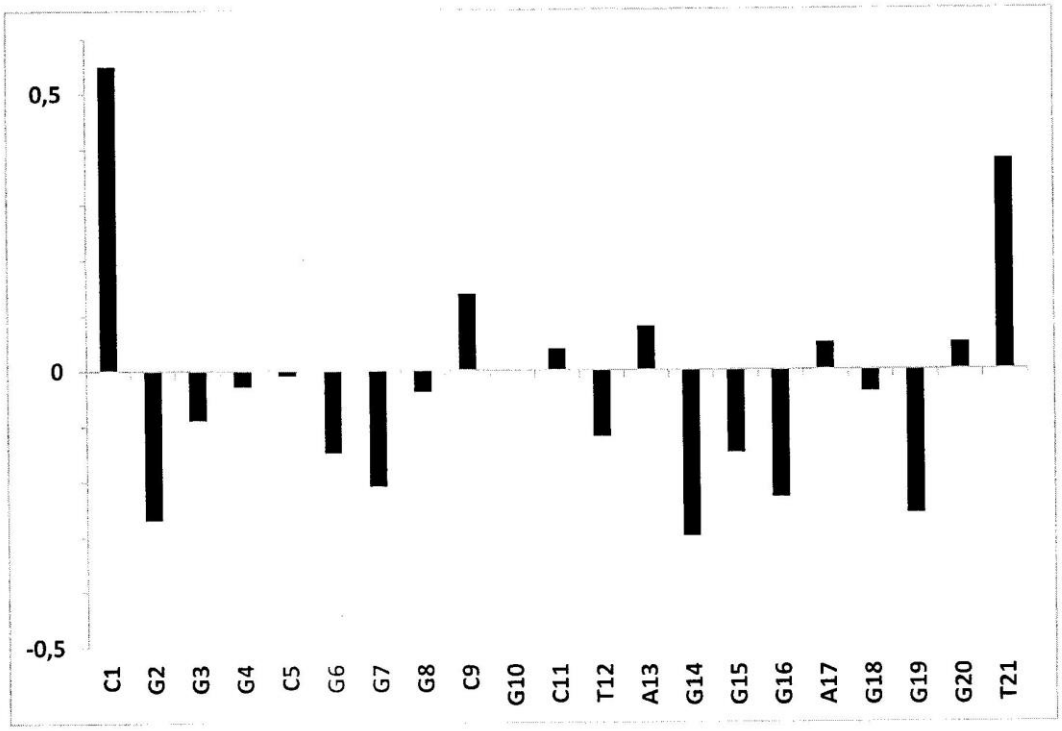
[a] Measured at 25°C in ppm (δ) from external DSS. Solvent H_2O-D_2O (90:10 v/v), 5 mM phosphate buffer, 20 mM KCl, pH 6.9. [b] R = 3.0. [c] $\Delta\delta = \delta_{bound} - \delta_{free}$



Graph S1: Chemical shift difference of DNA protons between the complex of c-kit21T12T21 with **(2)** R=3.0 and the free c-kit21T12T21: (a) H1/H2/H5/CH_3, (b) H6/H8



(a)



(b)

Graph S2: Chemical shift difference of DNA protons between the complex of c-kit21T12T21 with **(1)** R=2.0 and the free c-kit21T12T21: (a) H1/H2/H5/CH_3, (b) H6/H8

Table S4. ^{1}H chemical shift values for the complex of BMH-21 (**1**) with c-kit21T12T21.[a]

complex of **BMH21**/c-kit21T12T21[b]				
c-kit	H1/H2/H5/CH_3	$\Delta\delta^c$	H6/H8	$\Delta\delta^c$
C1	6.10	-0.03	7.53	+0.55
G2	11.44	-0.48	7.93	-0.27
G3	10.92	-0.38	7.69	-0.09
G4	10.51	-0.64	7.72	-0.03
C5	6.20	0.00	8.03	-0.01
G6	11.06	-0.58	7.87	-0.15
G7	11.17	-0.41	7.81	-0.21
G8	10.57	-0.74	7.75	-0.04
C9	5.94	-0.16	7.76	+0.14
G10	n.d.	-	n.d.	-
C11	6.10	+0.04	7.94	+0.04
T12	1.67	-0.23	7.48	-0.12
A13	n.d.	-	8.25	+0.08
G14	11.27	-0.67	7.96	-0.30
G15	10.98	-0.40	7.72	-0.15
G16	10.52	-0.52	7.75	-0.23
A17	n.d.	-	8.56	+0.05
G18	11.40	-0.58	8.05	-0.04
G19	11.17	-0.39	7.83	-0.26
G20	10.63	-0.62	7.75	+0.05
T21	1.76	+0.26	7.53	+0.38

[a] Measured at 25°C in ppm (δ) from external DSS. Solvent H_2O-D_2O (90:10 v/v), 5 mM phosphate buffer, 20 mM KCl, pH 6.9. [b] R=2.0. [c] $\Delta\delta = \delta_{bound} - \delta_{free}$

Table S5. Guanine residues involved in the pi-pi interactions with the polycyclic system of compounds BMH-21 (**1)** and BA-41 (**2**)

rings	A	B	C	D
BMH-21 (**1**)	G16	G8, G16	G4, G8	G4
BA-41 (**2**)	G8, G16	G8, G16	G4	G4

Table S6. Chemical shift values of BA-41 in the complex with c-kit21T12T21.[a]

complex of BA-41/c-kit21T12T21	
H1	7.10
H2	6.83
H3	6.94
H4	7.16
H7	7.98
H8	6.70
H9	8.22

[a] Measured at 25°C in ppm (δ) from external DSS. Solvent H_2O-D_2O (90:10 v/v), 5 mM K-phosphate buffer, 20 mM KCl, pH 6.9, R = 3.0.

	MW	log(MW)	tR exp.	tR calc.	Variat. (%)
c-kit21T12T21	6609.3	3.82	10.41	10.92	-4.64%
	6609.3	3.82	11.02	10.92	0.95%
Pu22T14T23	7041.6	3.85	10.76	10.86	-0.92%
	7041.6	3.85	11.61	10.86	6.90%

Retention times and molecular weights of the c-kit21T12T21 and Pu22T14T23 according to the calibration with Tx standards.

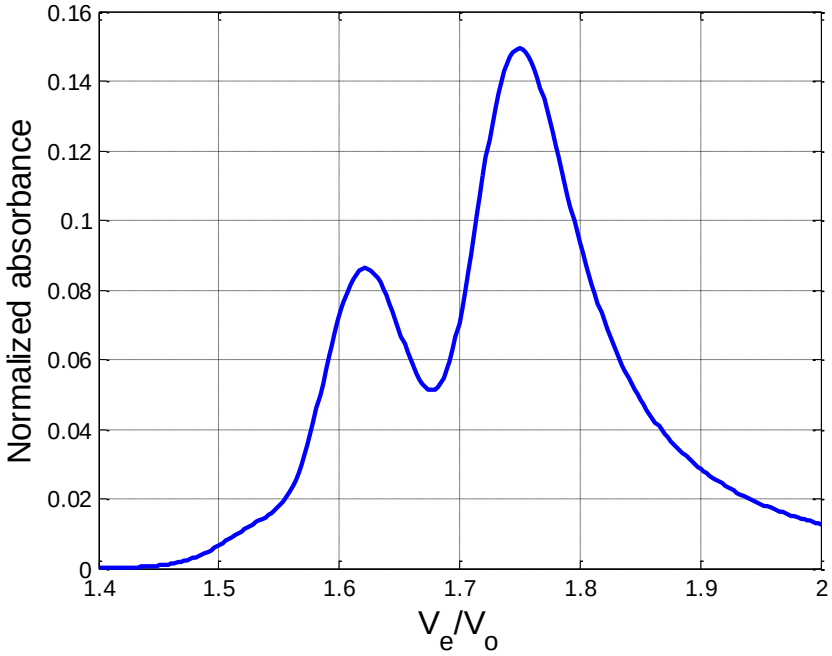


Figure S1. SEC profile recorded for Pu22T14T23. The peaks at V_e/V_0 ratios equal to 1.62 and 1.75 were related to linear and monomeric structure, respectively, according to reference [32]. The void volume was 5.30 mL.

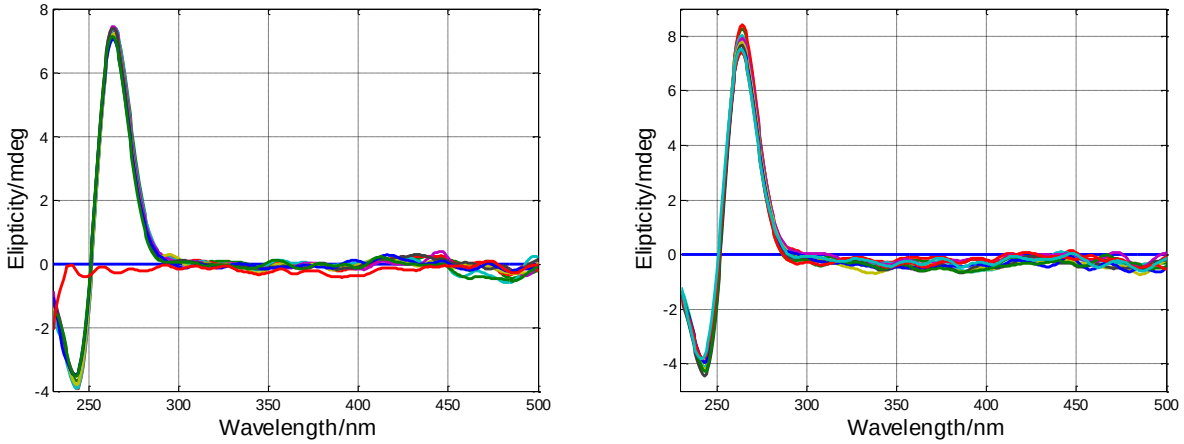


Figure S2. CD spectra recorded along the titration of Pu22T14T23 with (a) BMH-21 (**1**) and (b) BA-41 (**2**). In both cases, the medium consisted of 20 mM phosphate buffer (pH 7.0) and 5 mM KCl. DNA concentration was 1 µM. Ligand concentration ranged from 0 to 3 µM. Experiments were carried out at 25 ^{o}C.