

Supporting information for

Design, synthesis, and evaluation of 2,9-bis[(substituted-aminomethyl)phenyl]-1,10-phenanthroline derivatives as G-quadruplex ligands

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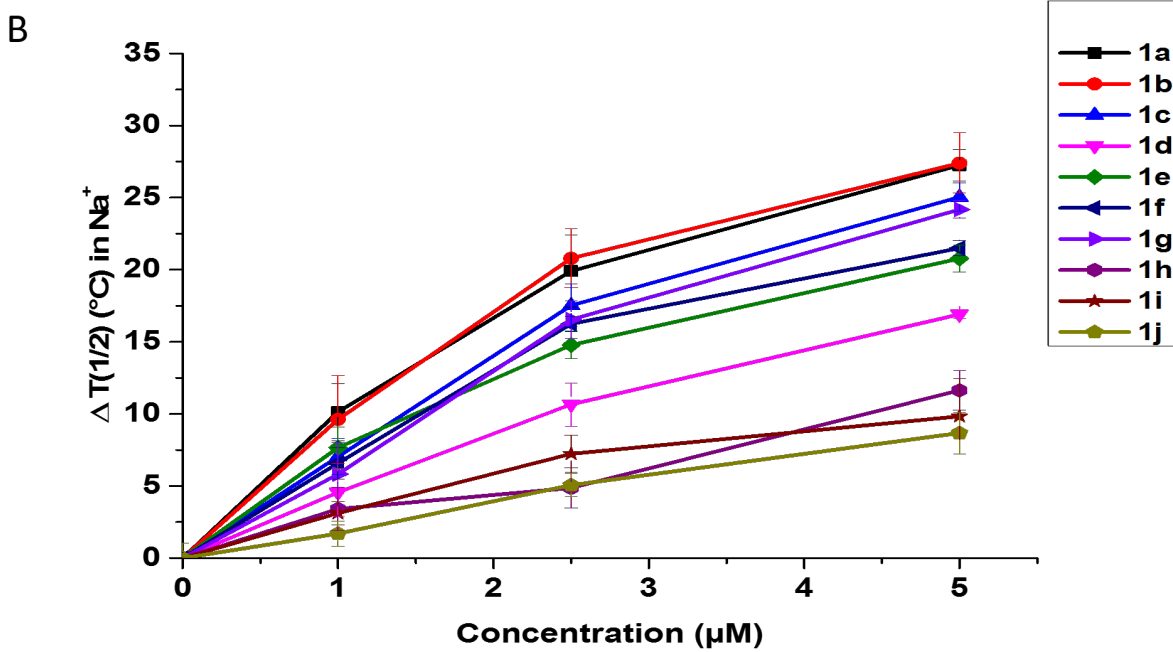
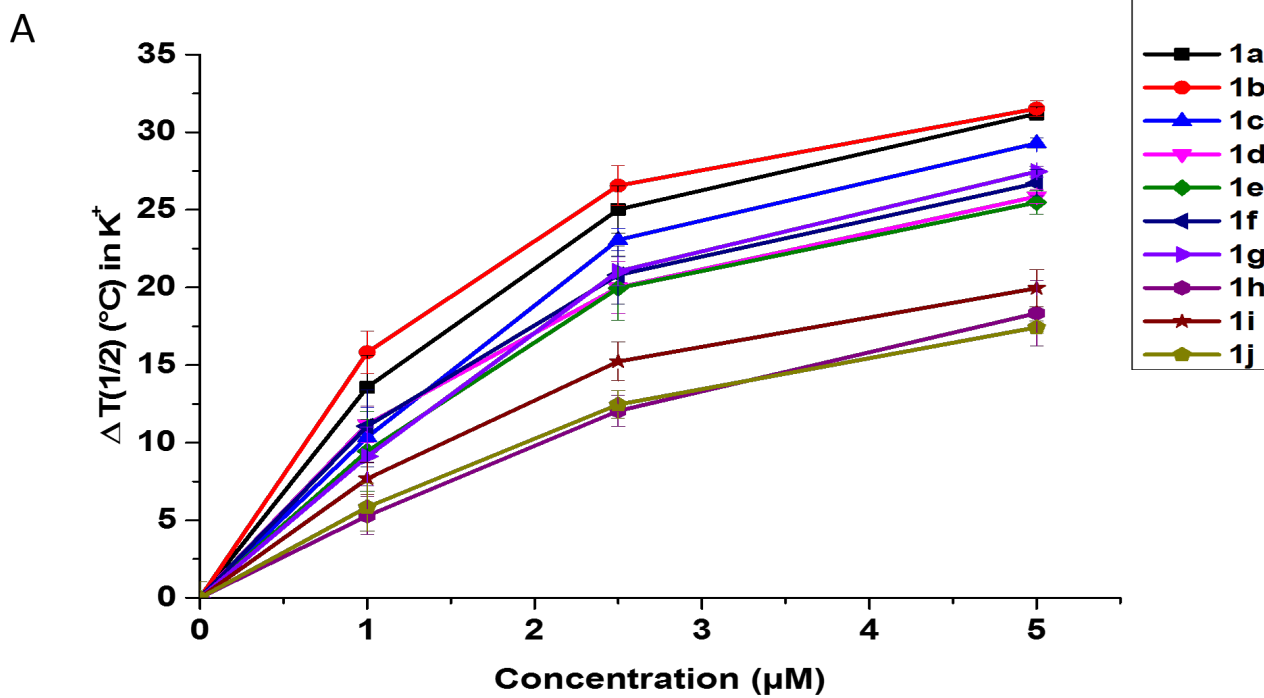
Supplementary Table 1. List of DNA sequences

Sequence name	Sequence(5'-3')	Technic	Function
F21T	Fam- G ₃ TTAG ₃ TTAG ₃ TTAG ₃ -Tamra	FRET melting	Labelled G4 (DNA)
ds26	CAATCGGATCGAATTCGATCCGATTG	FRET and CD melting	Competitor (DNA) Hairpin, duplex
Fbom17T	Fam- G ₂ TTAG ₂ TTAG ₂ TTG ₂ -Tamra	FRET melting	Labelled G4 (DNA)
FdxT	Fam- TATAGCTAT-hexaethyleneglycol-TATAGCTATA -Tamra	FRET melting	Labelled G4 (DNA)
FcmycT	Fam- TTGAG ₃ TG ₃ TAG ₃ TG ₃ TAA -Tamra	FRET melting	Labelled G4 (DNA)
F21ctaT	Fam- G ₃ CTAG ₃ CTAG ₃ CTAG ₃ -Tamra	FRET melting	Labelled G4 (DNA)
F25cebT	Fam- AG ₃ TG ₃ TGTAAGTGTG ₃ TG ₃ T -Tamra	FRET melting	Labelled G4 (DNA)
FtbaT	Fam- G ₂ TTG ₂ TGT G ₂ TTG-Tamra	FRET melting	Labelled G4 (DNA)
F21RT	Fam-G ₃ UUAG ₃ UUAG ₃ UUAG ₃ -Tamra	FRET melting	Labelled G4 (RNA)
c-myc	TTGAG ₃ TG ₃ TAG ₃ TG ₃ TAA	CD	G4 (DNA)
22AG	AG ₃ TTAG ₃ TTAG ₃ TTAG ₃	CD	G4 (DNA)
24TTG	TTGGGTTAGGGTTAGGGTTAGGGA	ESI-MS and CD	G4 (DNA)
22CTA	AGGGCTAGGGCTAGGGCTAGGG	CD	G4 (DNA)
Ss-Fam	CTCTGCTCAACGGAA-FAM	Helicase assay	Fluorescent strand
S-mut	A ₁₁ TGGTGTGTAGTGTGGTTTATTCGGTTGAGCAGAG-Dabeyl	Helicase assay	Control mutated
Ss-dx	AACCACACTACACACC	Helicase assay	Control duplex
Compl-mut	CTCTGCTCAACGGAAATAAACACACTACACACCAT ₁₁	Helicase assay	Complementary strand to mut and dx
S-Cmyc	A ₁₁ -G ₃ TG ₃ TAG ₃ TG ₃ -TATTCCGTTGAGCAGAG-Dabeyl	Helicase assay	G4 forming strand
Compl-myc	CTCTGCTCAACGGAAATACCCACCTACCCACCC-T ₁₁	Helicase assay	Complementary to cmyc strand
S-25ceb	A ₁₁ . AG ₃ TG ₃ TGTAAGTGTG ₃ TG ₃ T TATTCCGTTGAGCAGAG-Dabeyl	Helicase assay	G4 forming strand
Compl-25ceb	CTCTGCTCAACGGAAATA ACCCACCCACACTTACACCCACCC-T ₁₁	Helicase assay	Complementary strand
S-tba	A ₁₁ -G ₂ TTG ₂ TGT G ₂ TTG TATTCCGTTGAGCAGAG-Dabeyl	Helicase assay	G4 forming strand
Compl-tba	CTCTGCTCAACGGAAATA CAACCACACCAACC- T ₁₁	Helicase assay	Complementary strand
Compl-Ss-Fam	TTCCGTTGAGCAGAG	Helicase assay	Trap the Ss-FAM

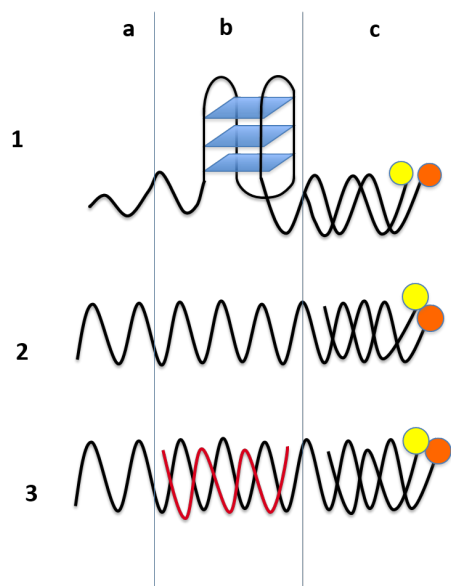
Supplementary Table 2. $T_{1/2}$ values obtained by CD melting experiments.

	$T_{1/2}$ without ligand	$T_{1/2}$ with 3 eq of 1a
ds26	77 ± 1 °C	78 ± 1 °C
c-myc	73.5 ± 2 °C	> 86 °C
22AG in Na⁺ condition	56.2 ± 1 °C	68.4 ± 2 °C

$T_{1/2}$ values are reported at 3 μ M unlabelled DNA concentration and 9 μ M of **1a** in 10 mM lithium cacodylate buffer (pH 7.2), 90 mM LiCl, and 10 mM KCl or for the 22AG sequence in Na⁺ condition in 10 mM lithium cacodylate buffer (pH 7.2), 100 mM NaCl.

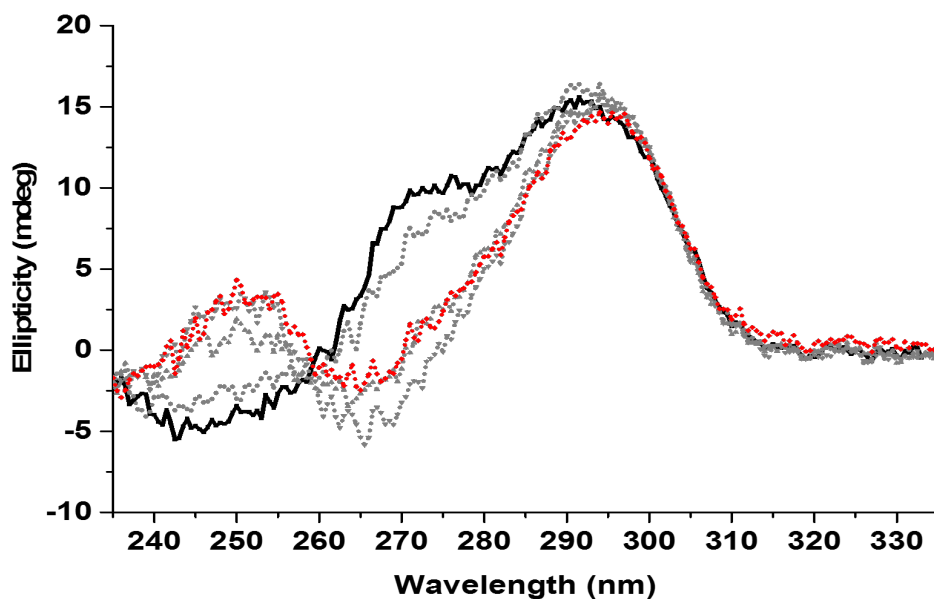


Supplementary Figure S1: FRET-melting curves obtained for ligands 1a-j with F21T (0.2 μM) in (a) K⁺ conditions and (b) Na⁺ conditions.

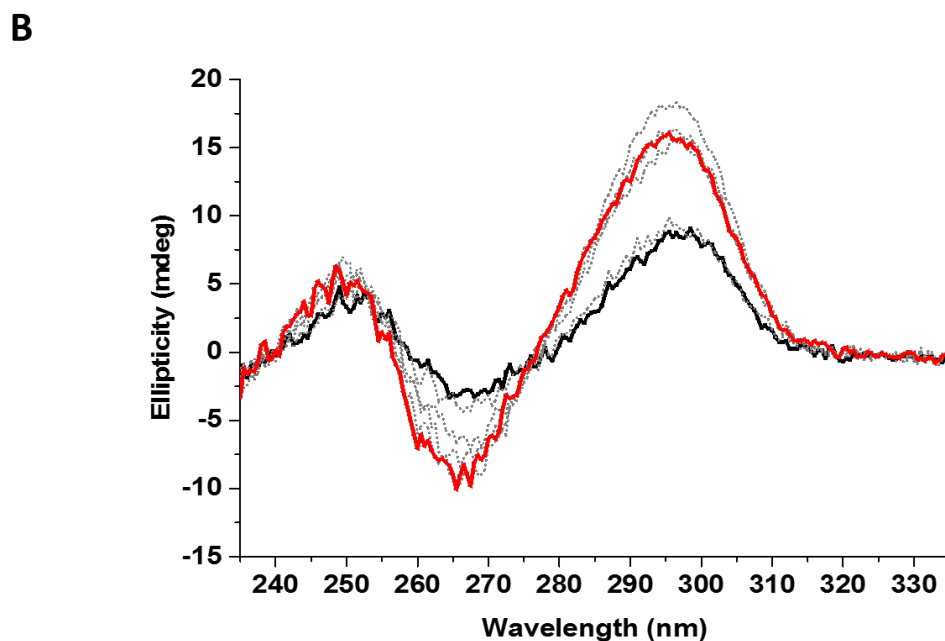
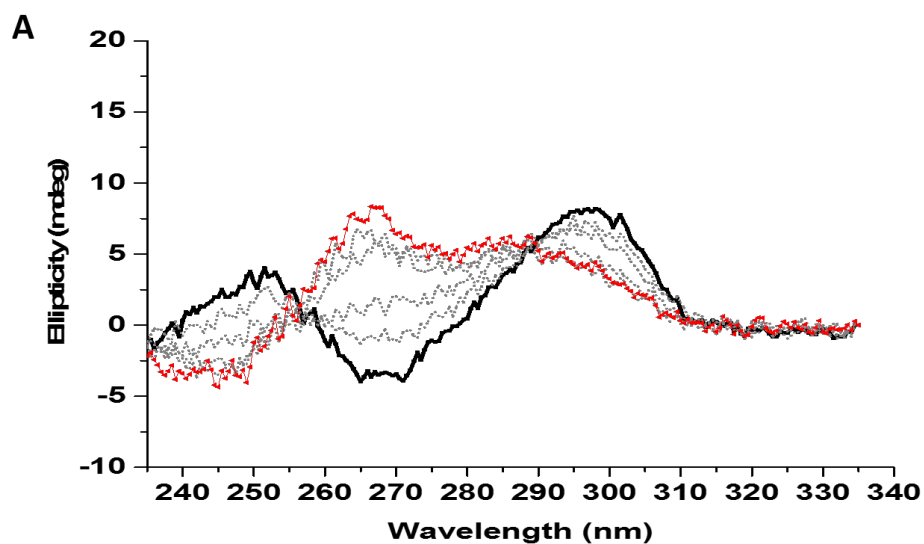


Supplementary Scheme 1. Representations of the DNA systems used in the helicase assay.

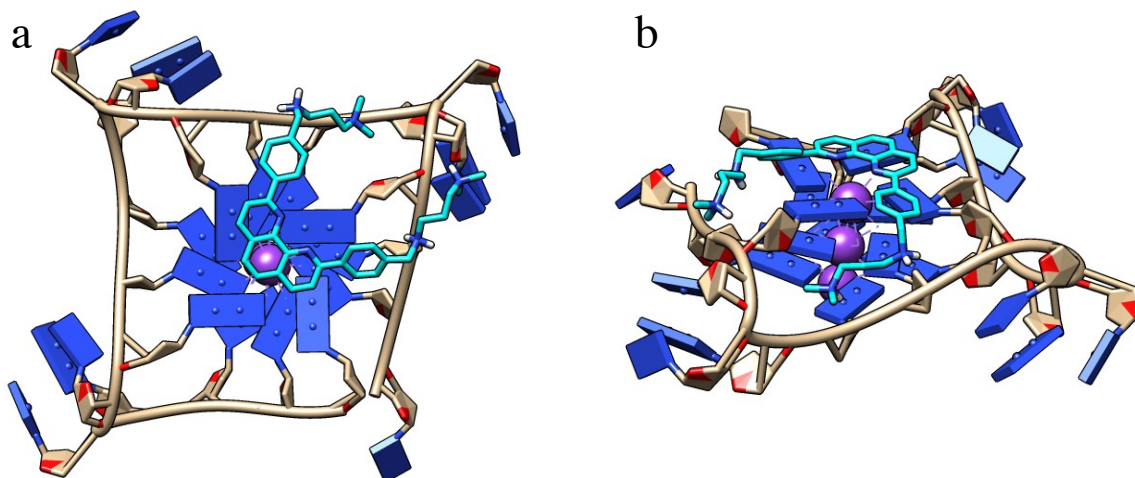
Each system contains three regions: “a” and “c” represent fixed regions where “a” is a 5’ overhang needed for Pif1 activity and “c” is a duplex labeled by FAM and Dabcyl enabling monitoring by FRET. The region “b” is variable: in “1” it corresponds to an intramolecular G-quadruplex structure, in “2” it corresponds to a single strand, and in “3” it corresponds to a duplex conformation. When the system is hybridized the FAM fluorescence emission is quenched by the Dabcyl proximity. When the system is unfolded, FAM fluorescence emission is enhanced.



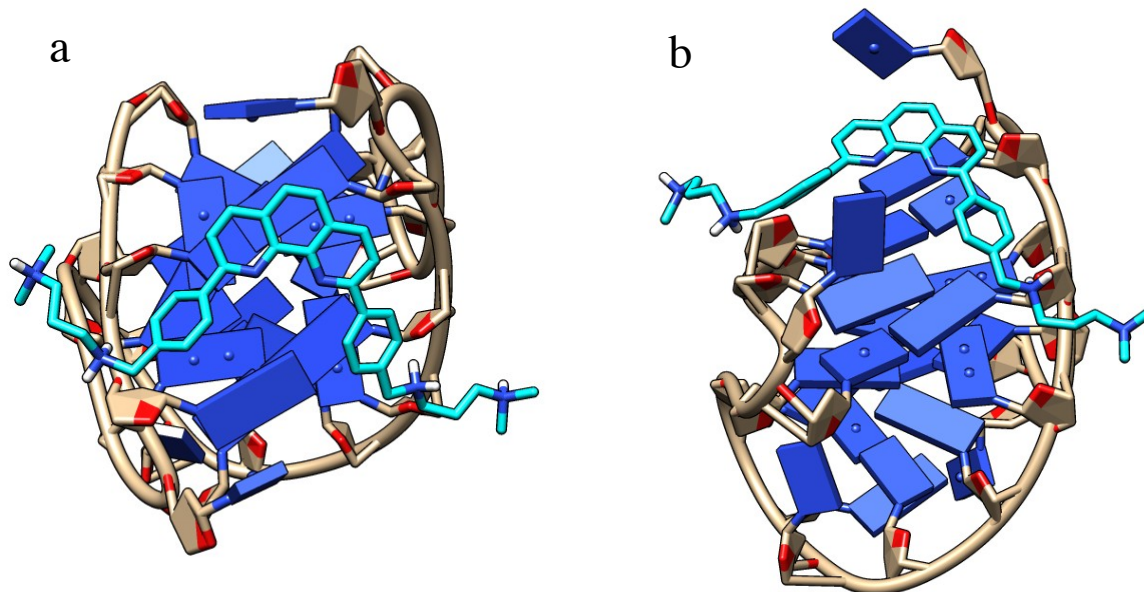
Supplementary Figure S2: CD titration of 24TTG (3 μ M) with the ligand **PhenDC3**. CD spectra were recorded in 10 mM lithium cacodylate (pH 7.3), 90 mM LiCl, and 10mM KCl. Black line corresponds to the CD profile of the sequence in absence of **PhenDC3**, dotted grey lines correspond to the spectra after additions of 1, 2 or 3 eq of **PhenDC3**, and red line corresponds to the final CD profile after addition of the fourth equivalent of **PhenDC3**.



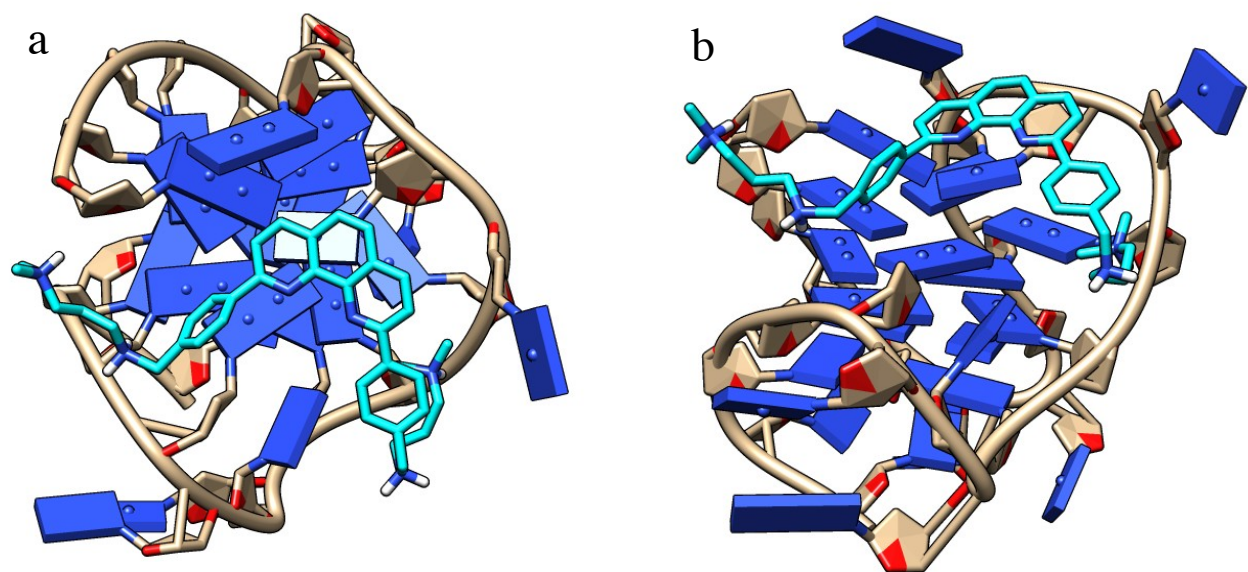
Supplementary Figure S3: CD titration of 22CTA (3 μ M) by (a) ligand **1a** and (b) **PhenDC3**. CD spectra were recorded in 10 mM lithium cacodylate (pH 7,3), 90 mM LiCl, and 10mM KCl. Black lines correspond to the CD profile of the sequence in absence of ligand, dotted grey lines correspond to the addition of 4 equivalents of ligand, 1 eq each time, red lines correspond to the CD profile after the addition of the fifth equivalent of ligand.



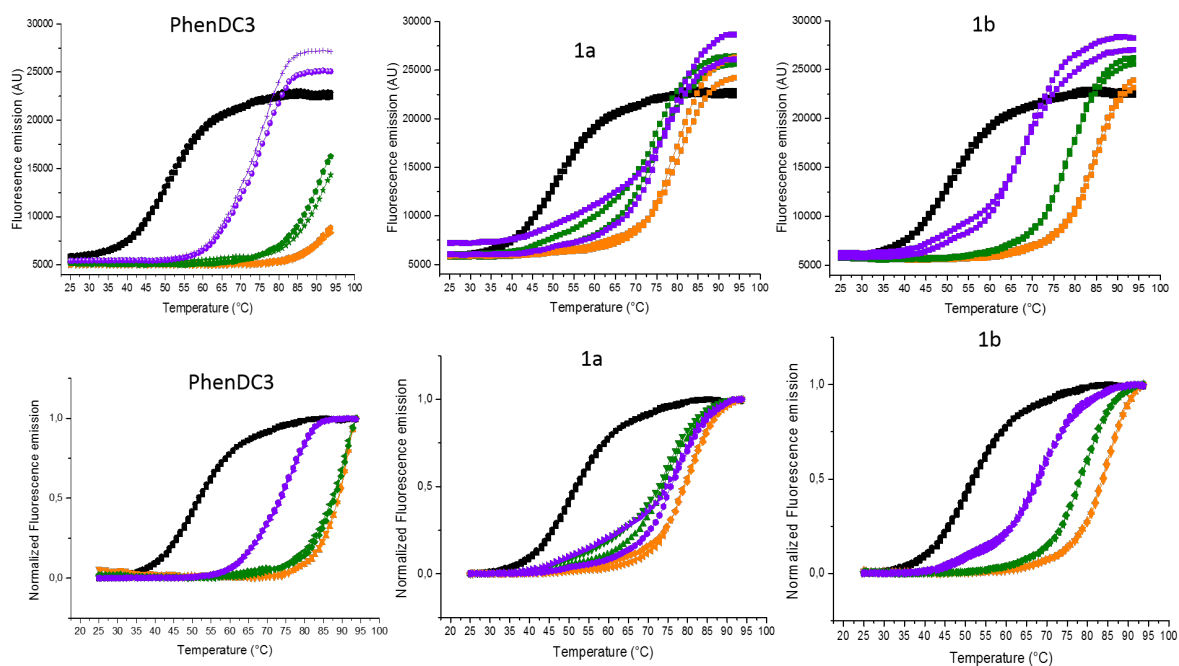
Supplementary Figure S4. Binding of **1a** to a 22-mer human DNA telomeric sequence. (a) Top view and (b) side view of the binding mode of **1a** on the G4 PDB ID1KF1 obtained by docking.



Supplementary Figure S5. Binding of **1a** to an antiparallel DNA telomeric sequence 22CTA (2KM3). (a) Top view and (b) side view of the best binding mode of **1a** on the G4 obtained by docking.



Supplementary Figure S6. Binding of **1a** to a hybrid 24TTG hybrid telomeric (2GKU) sequence. (a) Top view and (b) side view of the best binding mode of **1a** on the G4 obtained by docking.



Supplementary Figure S7. Original melting curves obtained for ligands **PhenDC3**, **1a** and **1b** with F21T (0.2 μM) in K⁺ conditions. On the top are represented the raw data and in the bottom the normalized data. Black curves correspond to F21T melting profile in absence of ligand, purple curves correspond to F21T melting profile in presence of 1 μM ligand, green curves correspond to F21T melting profile in presence of 2.5 μM and orange curves correspond to F21T melting profile in presence of 5 μM ligand.