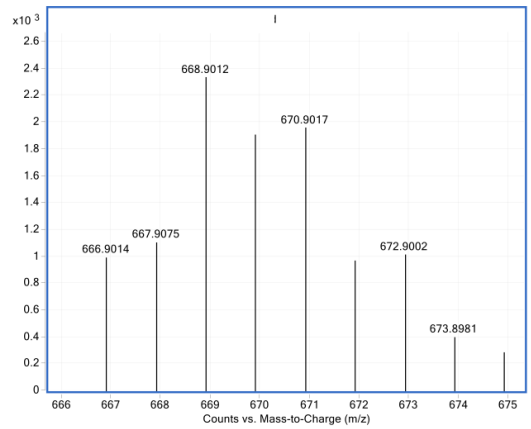
Electronic Supplementary Material (ESI) for Dalton Transactions.

Salphen metal complexes as potential anticancer agents: interaction profile and selectivity studies toward the three G-quadruplex units in the *KIT* promoter

Luisa D'Anna,[a] Simona Rubino,[a] Candida Pipitone,[b] Graziella Serio,[a] Carla Gentile,[a] Antonio Palumbo Piccionello,[a] Francesco Giannici,[b] Giampaolo Barone*[a] and Alessio Terenzi*[a]

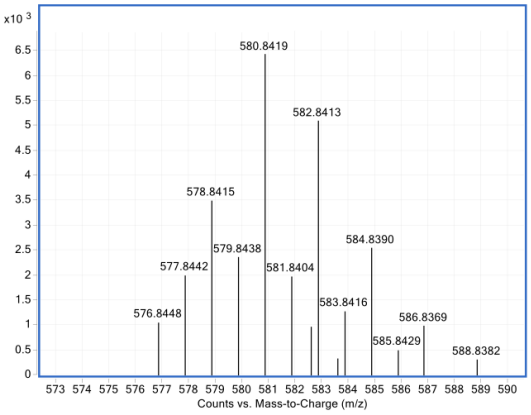
Supplementary Information

Calculated for $C_{20}H_{10}Cl_4N_2O_2Pt + Na^+$



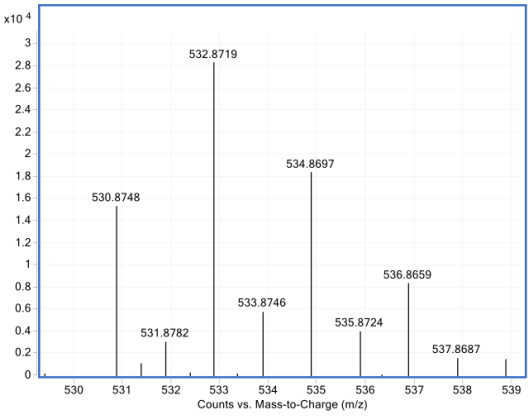
	m/z	Abund (% largest)	Abund (% sum)	Abund (% first)
	662.8988	0.02	0	100
	663.902	0	0	22.55
	664.8998	1.05	0.21	5716.53
	665.903	0.24	0.05	1288.89
	666.9014	44.44	8.91	242850.81
	667.9038	54.24	10.88	296406.25
▸	668.9012	100	20.06	546502.06
	669.9016	77.98	15.64	426169.38
	670.9005	93.59	18.77	511468.84
	671.9001	46.64	9.35	254872.16
	672.8996	46.41	9.31	253623.8
	673.8995	15.33	3.07	83752.4
	674.8986	12.99	2.61	70997.86
	675.8999	3.11	0.62	16968.91
	676.8977	2	0.4	10917.02
	677.9002	0.4	0.08	2166.31
	678.8975	0.15	0.03	797.46
	679.8993	0.03	0.01	144.19
	680.9016	0	0	16.84
*				

Calculated for $C_{20}H_{10}Cl_4N_2O_2Pd + Na^+$



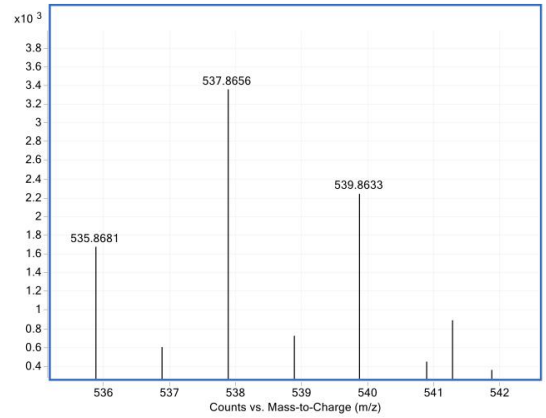
	m/z	Abund (% largest)	Abund (% sum)	Abund (% first)
	574.8445	1.34	0.27	100
	575.8476	0.3	0.06	22.55
	576.8428	16.39	3.27	1222.98
	577.8442	33.03	6.59	2464.66
	578.8421	62.54	12.48	4666.97
	579.842	50.93	10.16	3800.45
▸	580.8409	100	19.95	7462.4
	581.8412	39.5	7.88	2947.95
	582.8398	90.59	18.08	6760.49
	583.8416	23.51	4.69	1754.43
	584.8387	49.23	9.82	3673.38
	585.8413	10.99	2.19	820.32
	586.8372	15.79	3.15	1177.99
	587.84	3.37	0.67	251.77
	588.8357	2.8	0.56	208.88
	589.8382	0.57	0.11	42.89
	590.8348	0.23	0.05	17.28
	591.8366	0.04	0.01	3.19
	592.8389	0.01	0	0.37

Calculated for $C_{20}H_{10}Cl_4N_2O_2Ni + Na^+$



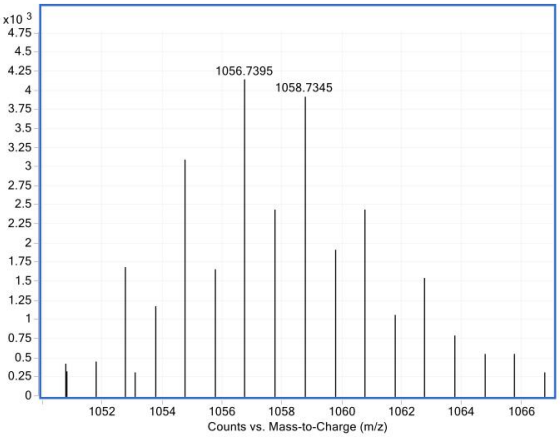
	m/z	Abund (% largest)	Abund (% sum)	Abund (% first)
	530.8742	59.05	17.85	100
	531.8774	13.32	4.02	22.55
▸	532.871	100	30.22	169.34
	533.8739	23.32	7.05	39.48
	534.868	71.56	21.63	121.18
	535.8706	17.01	5.14	28.8
	536.8651	28.8	8.7	48.76
	537.8675	6.81	2.06	11.53
	538.8626	7.47	2.26	12.65
	539.8649	1.7	0.51	2.87
	540.8607	1.37	0.41	2.31
	541.8629	0.29	0.09	0.49
	542.859	0.17	0.05	0.3
	543.8612	0.03	0.01	0.06
	544.8579	0.01	0	0.02

Figure S1. High-Resolution experimental mass spectra of metal complexes **1**, **2**, and **3**. In the tables, the calculated values.



Calculated for $C_{20}H_{10}Cl_4N_2O_2Cu + Na^+$

m/z	Abund (% largest)	Abund (% sum)	Abund (% first)
535.8685	57	18.13	100
536.8716	12.86	4.09	22.55
537.8659	100	31.8	175.43
538.869	22.34	7.1	39.18
539.8635	70.36	22.38	123.43
540.8664	15.49	4.93	27.18
541.8611	25.02	7.96	43.9
542.8638	5.38	1.71	9.44
543.8589	4.6	1.46	8.06
544.8613	0.95	0.3	1.66
545.8576	0.38	0.12	0.67
546.8593	0.07	0.02	0.12
547.8616	0.01	0	0.01



Calculated for $(C_{20}H_{10}Cl_4N_2O_2Zn)_2 + Na^+$

m/z	Abund (% largest)	Abund (% sum)	Abund (% first)
1050.7468	10.5	1.6	100
1051.75	4.74	0.72	45.11
1052.744	40.17	6.13	382.64
1053.7468	19.58	2.99	186.53
1054.7415	78.07	11.91	743.67
1055.744	39.44	6.02	375.72
1056.7392	100	15.25	952.56
1057.7414	50.76	7.74	483.52
1058.737	92.53	14.11	881.43
1059.7389	46.16	7.04	439.7
1060.7348	64.47	9.83	614.08
1061.7365	31.06	4.74	295.82
1062.7327	34.42	5.25	327.83
1063.7342	15.77	2.4	150.19
1064.7307	14.12	2.15	134.48
1065.732	6.07	0.93	57.8
1066.7288	4.42	0.67	42.08
1067.7299	1.76	0.27	16.76
1068.7271	1.04	0.16	9.89
1069.728	0.38	0.06	3.6
1070.7256	0.18	0.03	1.71
1071.7263	0.06	0.01	0.56
1072.7244	0.02	0	0.21
1073.7249	0.01	0	0.06
1074.7239	0	0	0.02

Figure S2. High-Resolution experimental mass spectra of metal complexes **4**, and **5**. In the tables, the calculated values.

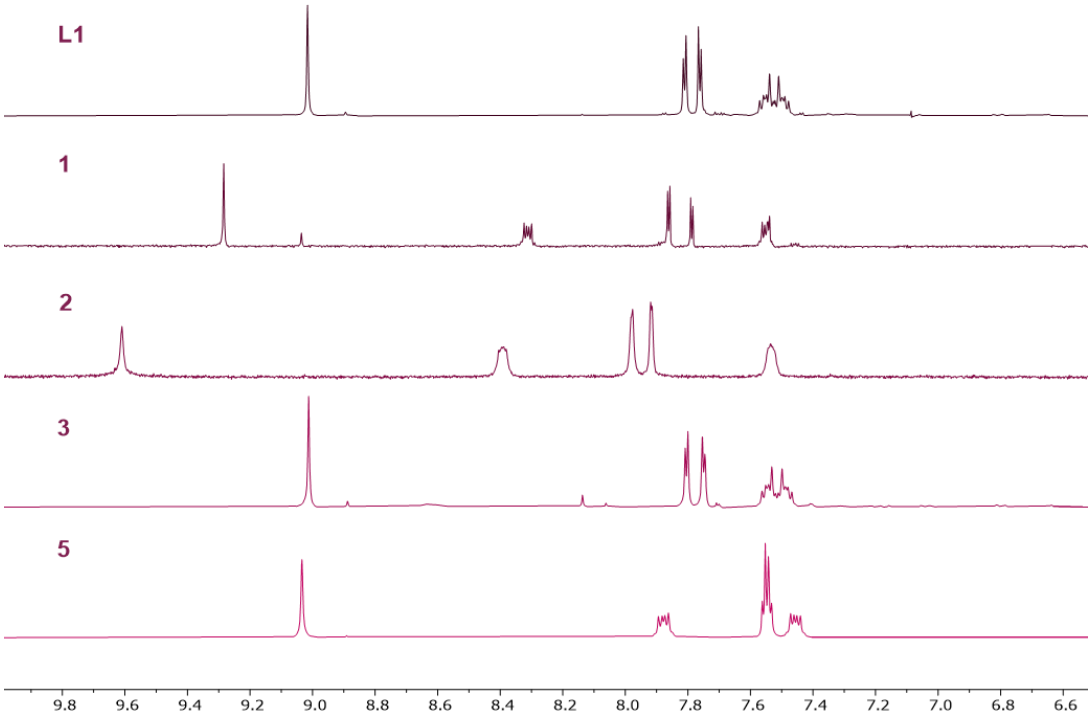


Figure S3. ^{1}H-NMR in DMSO of ligand **L1** and complexes **1**, **2**, **3** and **5**

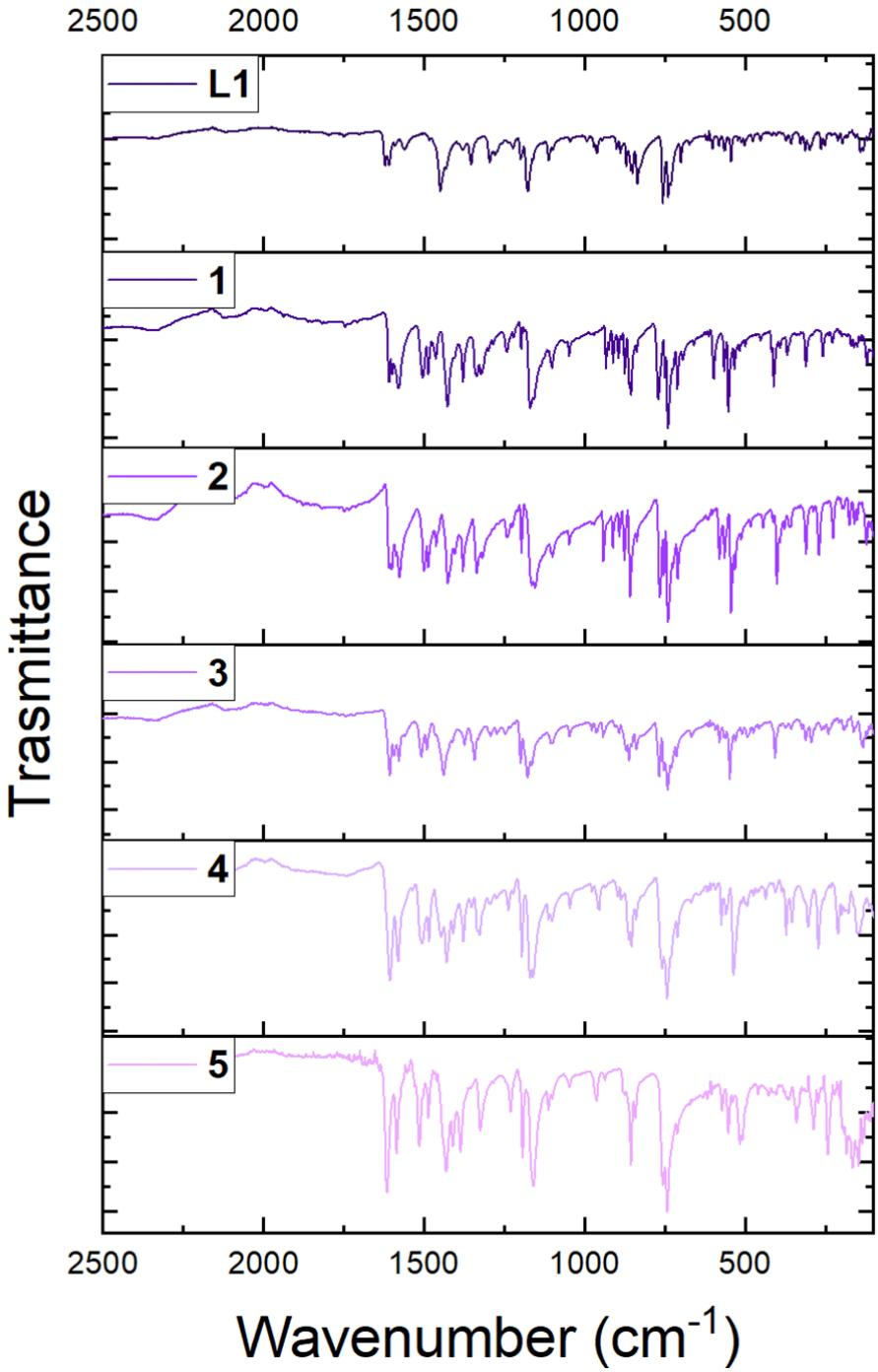


Figure S4. IR spectra of ligand **L1** and complexes **1**-**5**

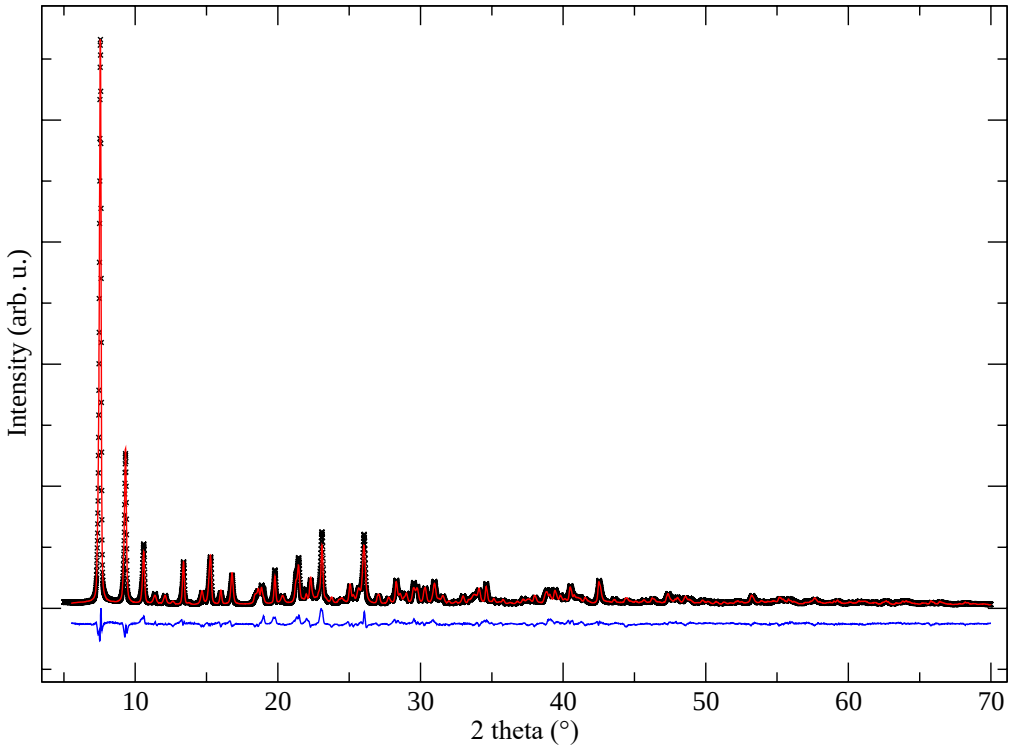


Figure S5. Rietveld refinement of compound **1**.

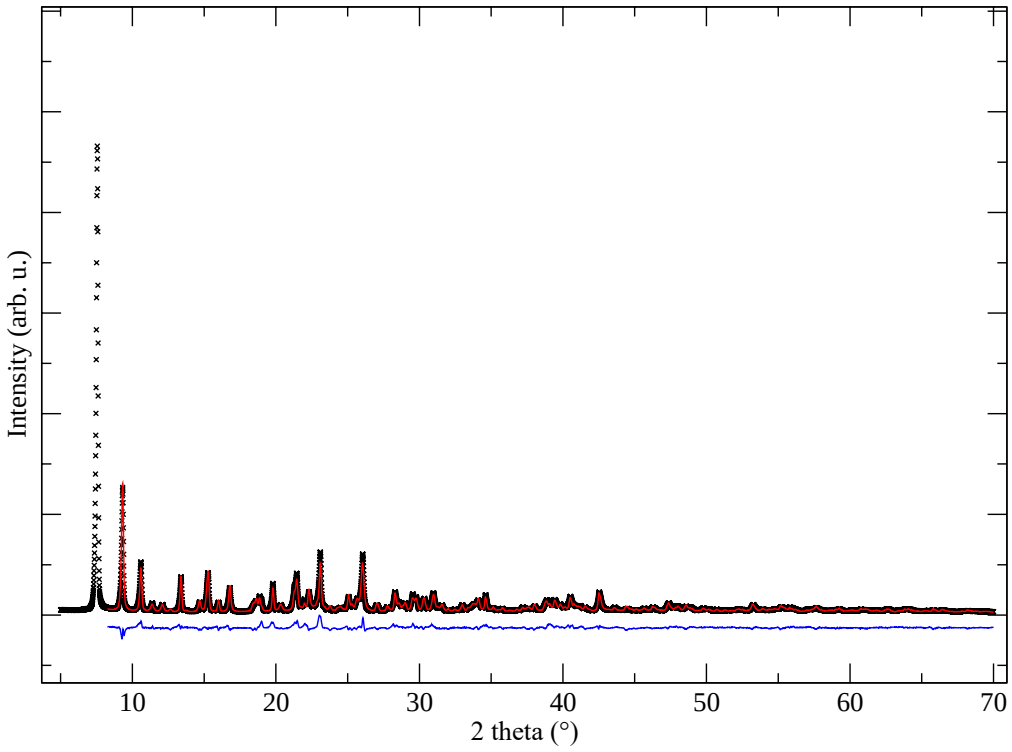


Figure S6. Rietveld refinement of compound **2**.

Structure resolution and refinement was performed with TOPAS Academic on XRPD data collected at room temperature with step size 0.02°. Singular value decomposition was first performed, leading to a monoclinic unit cell in the *P 21/c* (14) space group. The background was modeled by employing Chebychev polynomials of 11th order. The crystal packing in the unit cell was determined with simulated annealing in real space by defining rigid bodies for the ligand-metal complex using the z-matrix notation, translated and rotated four times (from volumetric considerations) in the unit cell, applying reasonable constraints for the bond lengths. This procedure was carried out several times varying the starting position of the rigid body, and the simulated annealing always resulted in the same atomic positions. For the final Rietveld refinement, the peak profiles accounting for size broadening (Gaussian and Lorenzian), axial divergence and Lorenzian anisotropic broadening (2^{nd} order spherical harmonics) parameters were fitted simultaneously, constraining the bond lengths and angles to average values from experimental published data on similar compounds, and allowing the fitting of the C-Cl distance and torsion angles. A common isotropic thermal displacement parameter was used for all atoms in the ligand, and one for the metal atom. For the refinement of compound **2**, the low-angle (100) peak was eventually excluded from refinement, as its intensity was found to be affected by instrumental aberrations and prevented thermal displacement parameters to refine to physically sensible values: the refinement quality improved significantly as a result, while the other structural parameters (lattice size and atomic positions) were not affected.

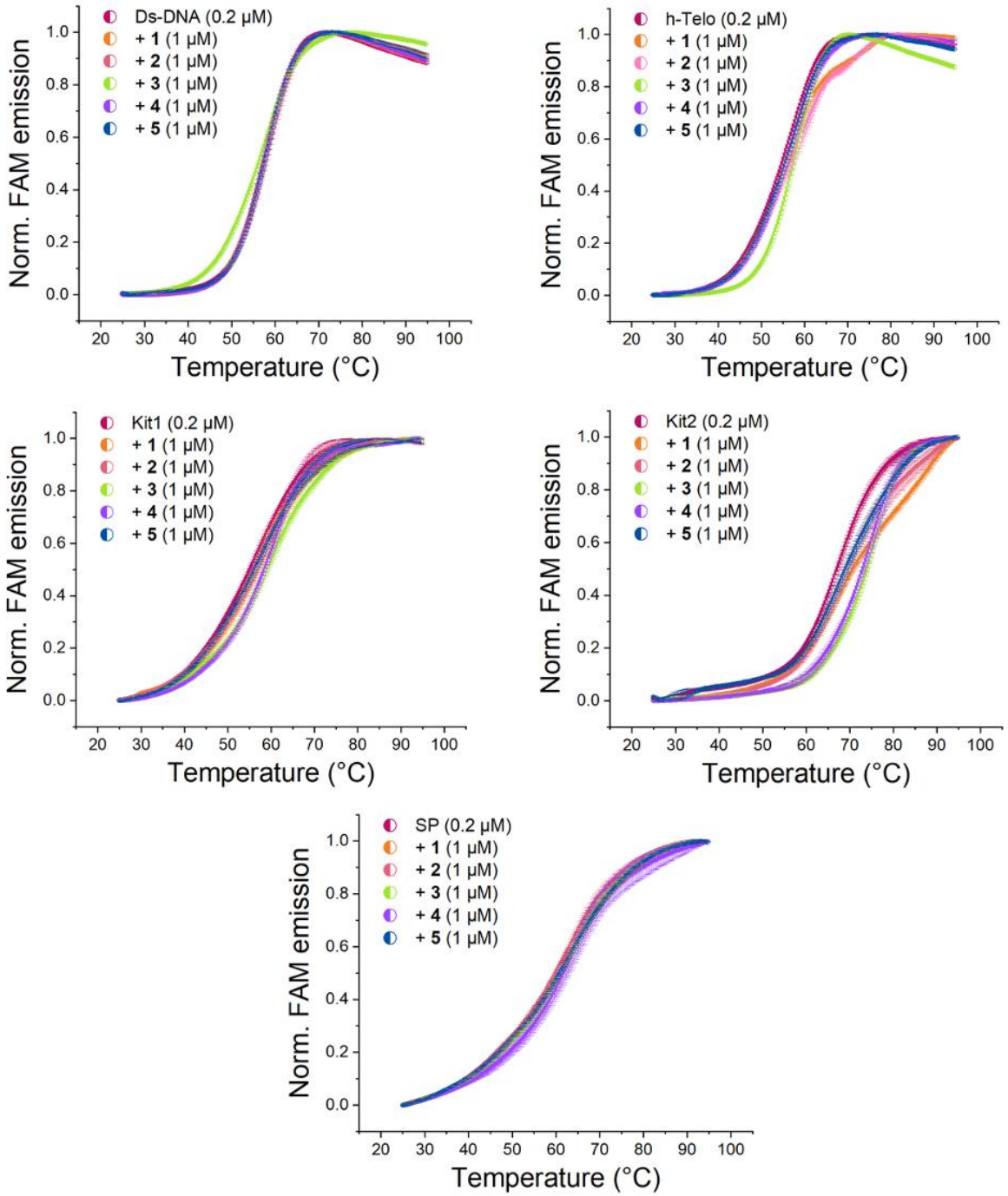


Figure S7. FRET melting profiles of different DNA motif upon interaction with compounds **1**-**5** at the indicated concentrations. Buffer: 60 mM potassium cacodylate, pH 7.4. DNA concentration is indicated per strand.

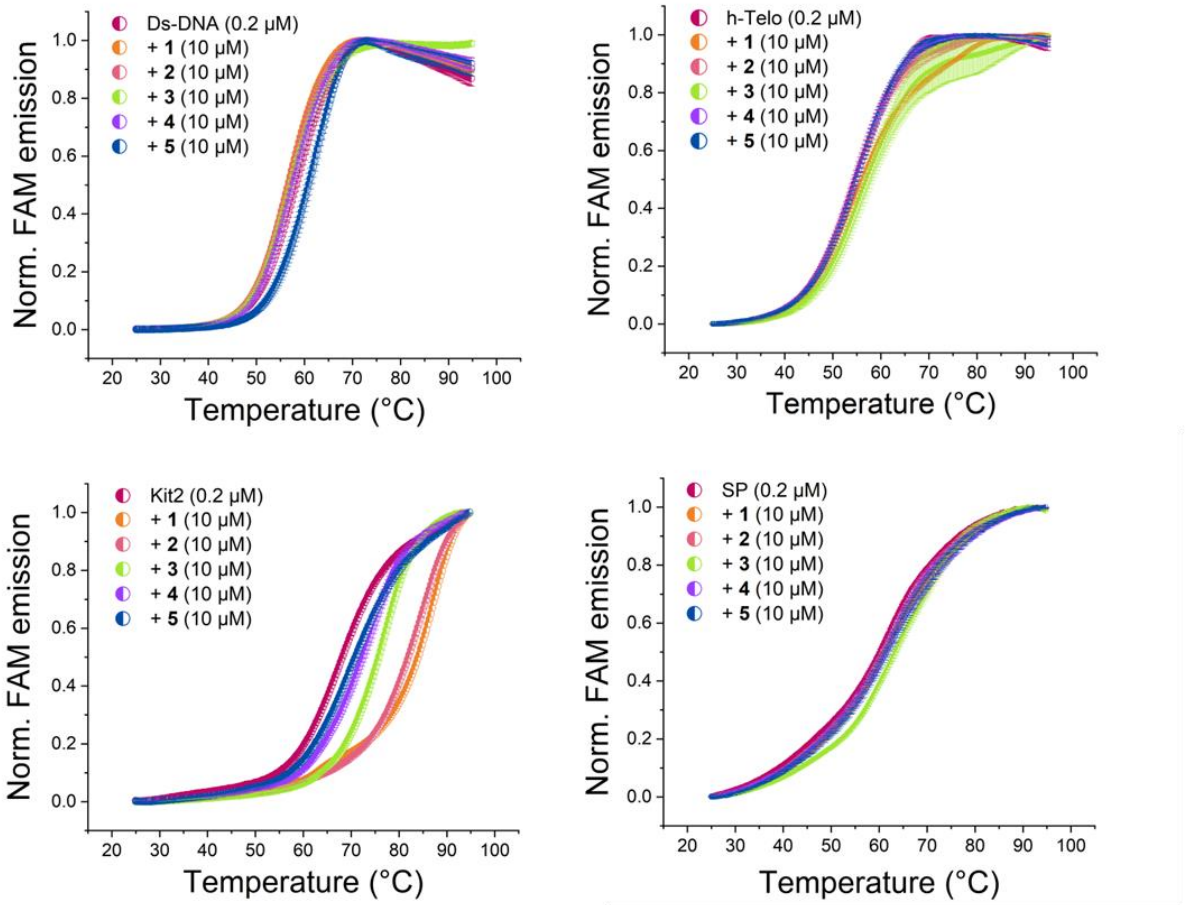


Figure S8. FRET melting profiles of different DNA motif upon interaction with compounds **1**-**5** at the indicated concentrations. Buffer: 60 mM potassium cacodylate, pH 7.4. DNA concentration is indicated per strand.

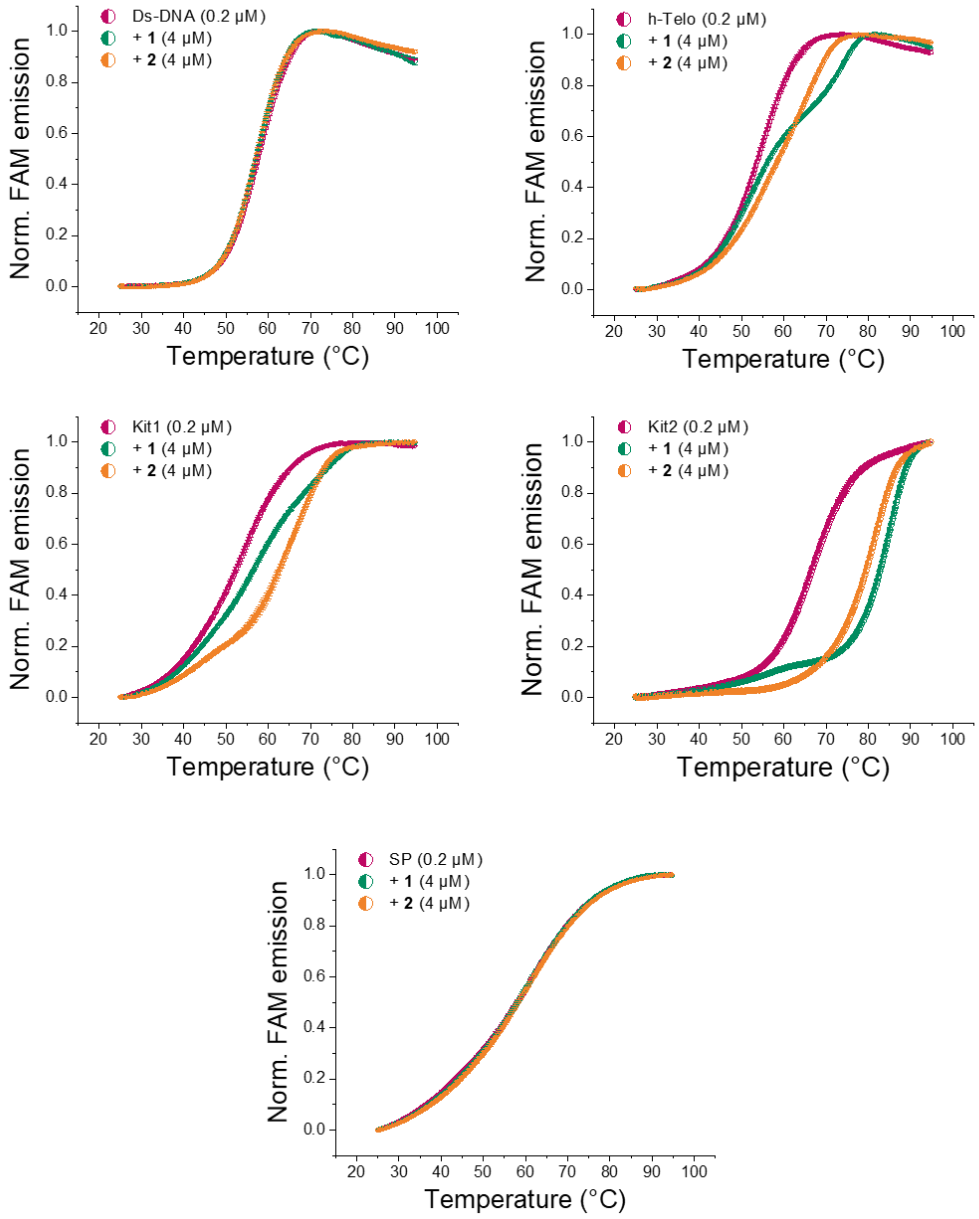


Figure S9. FRET melting profiles of different DNA motif upon interaction with compounds **1** and **2** at the indicated concentrations. Buffer: 60 mM potassium cacodylate, pH 7.4. DNA concentration is indicated per strand.

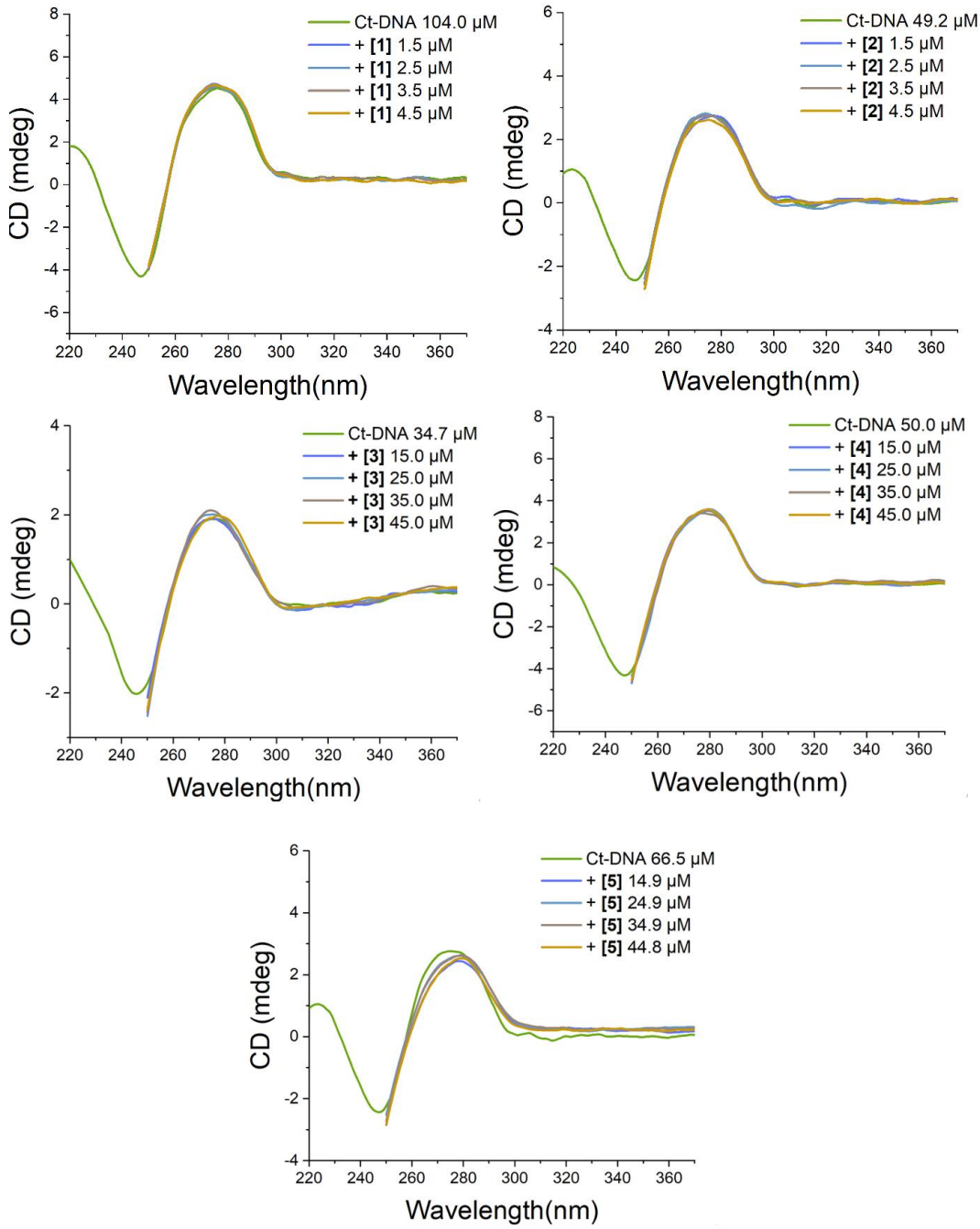


Figure S10. CD spectra of Ct-DNA in the presence of increasing aliquots of compounds **1**-**5** at the indicated concentrations. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Concentration of DNA is reported in bases. When compounds are in solution, the cut-off of the spectra was set at 250 nm due to the presence of DMSO as cosolvent (% < 5%).

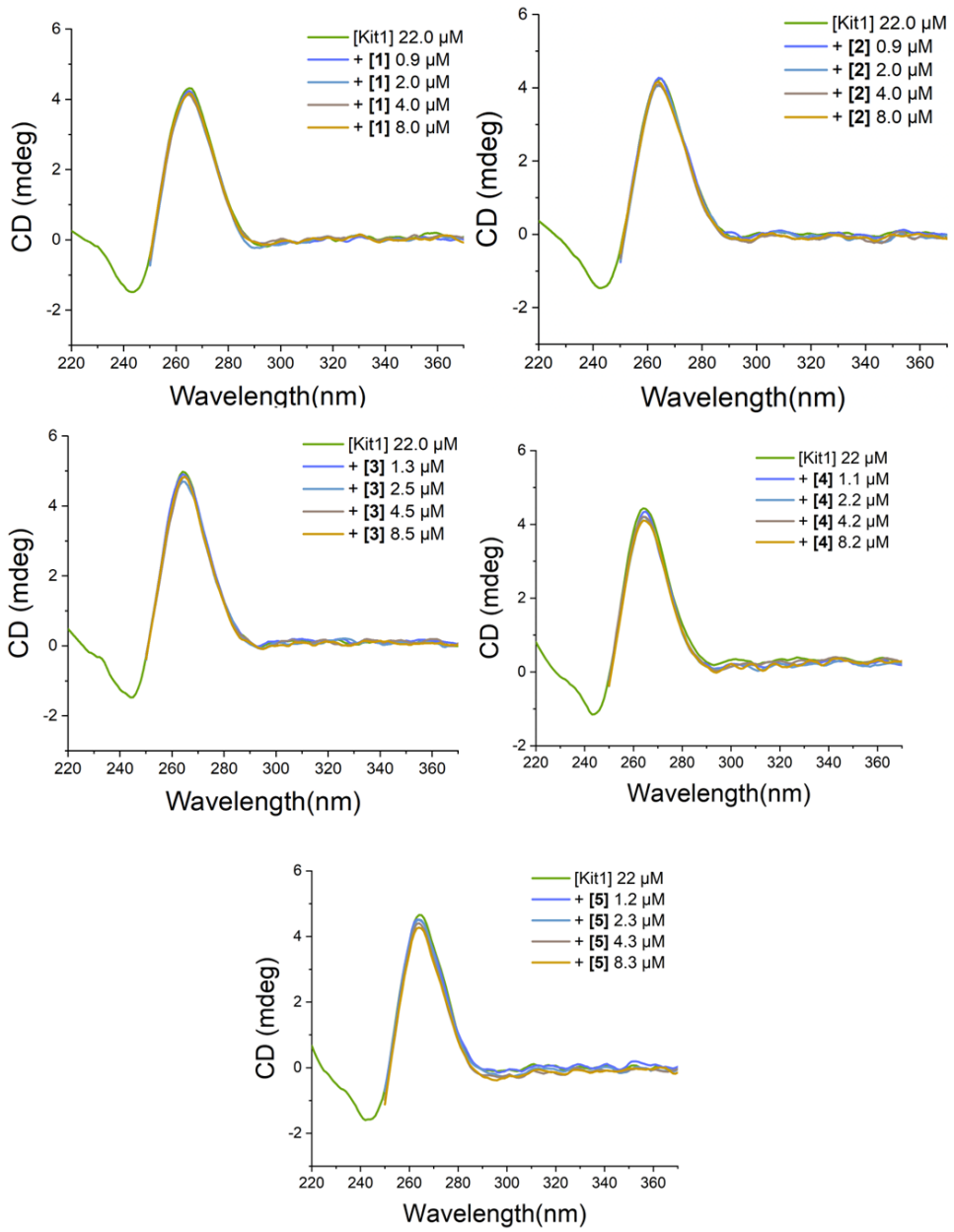


Figure S11. CD spectra of Kit1 G4 in the presence of increasing aliquots of compounds **1**-**5** at the indicated concentrations. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Concentration of DNA is reported in bases. When compounds are in solution, the cut-off of the spectra was set at 250 nm due to the presence of DMSO as cosolvent (% < 5%).

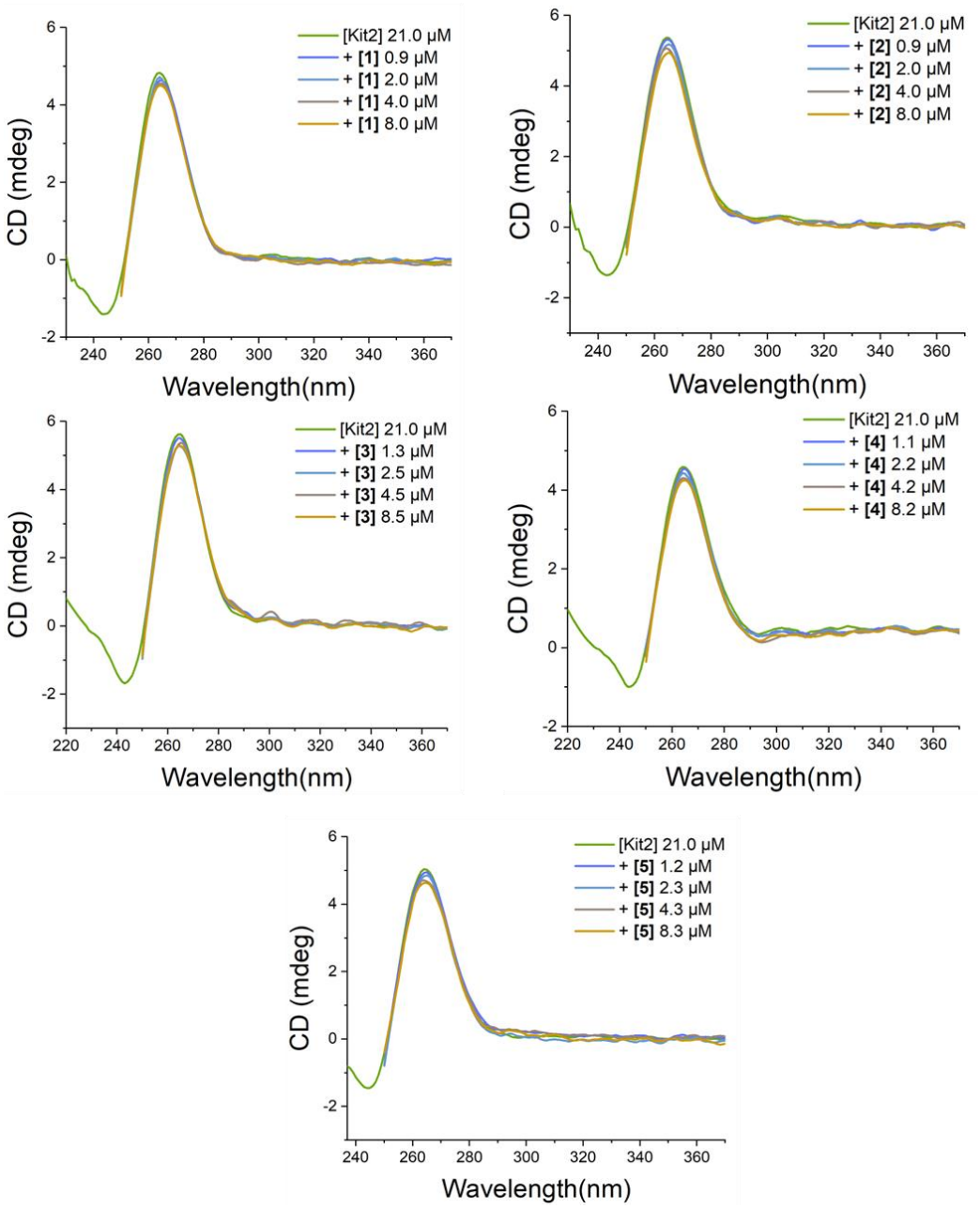


Figure S12. CD spectra of Kit2 G4 in the presence of increasing aliquots of compounds **1**-**5** at the indicated concentrations. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Concentration of DNA is reported in bases. When compounds are in solution, the cut-off of the spectra was set at 250 nm due to the presence of DMSO as cosolvent (% < 5%).

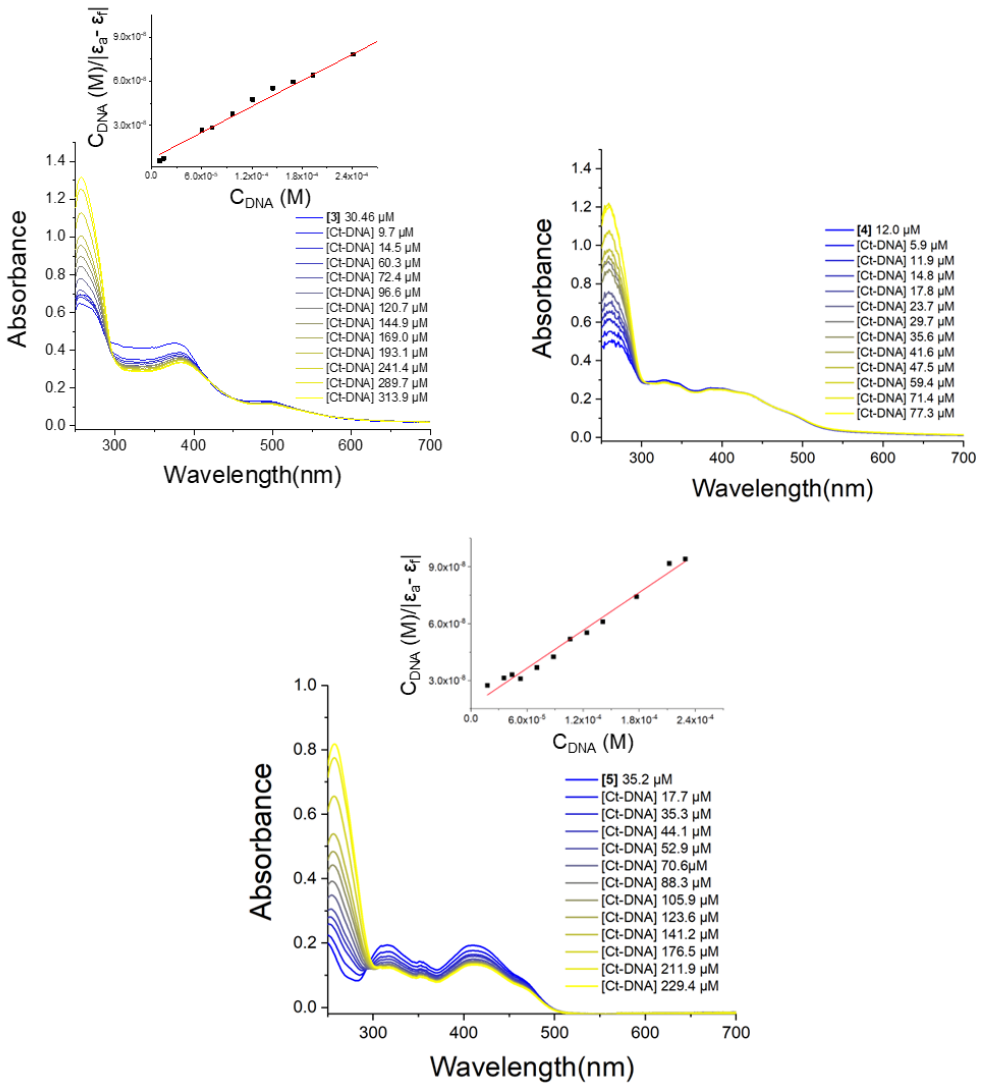


Figure S13. UV-Vis absorption spectrum of compounds **3**, **4** and **5** in combination with increasing amount of Ct-DNA. In the insets the plot and related fit for the calculation of the binding constants (K_b) according to the equation reported in the "Experimental" section. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Concentration of DNA is reported in bases. Cut-off of the spectra was set at 250 nm due to the presence of DMSO as cosolvent (% < 5%).

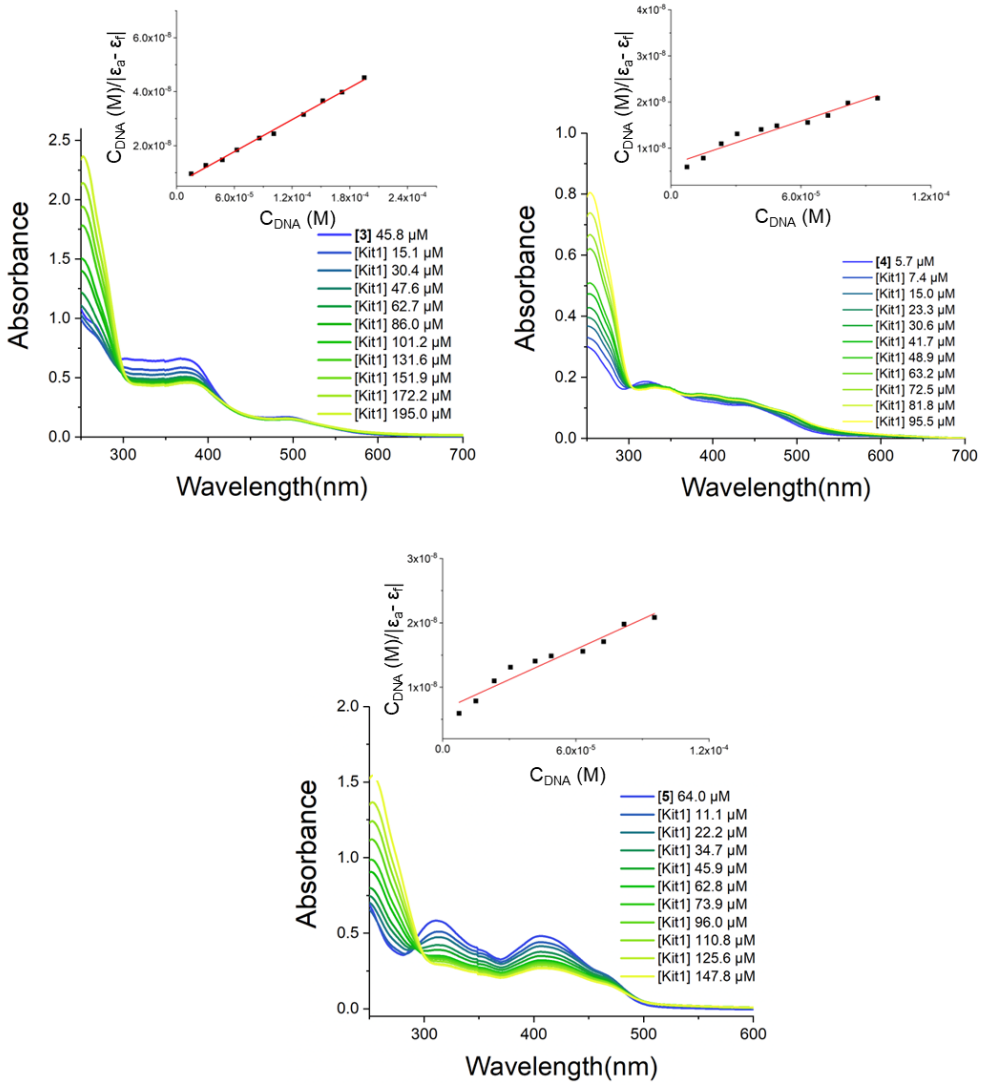


Figure S14. UV-Vis absorption spectrum of compounds **3**, **4** and **5** in combination with increasing amount of Kit1 G4. In the insets the plot and related fit for the calculation of the binding constants (K_b) according to the equation reported in the "Experimental" section. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Concentration of DNA is reported in bases. Cut-off of the spectra was set at 250 due to the presence of DMSO as cosolvent (% < 5%).

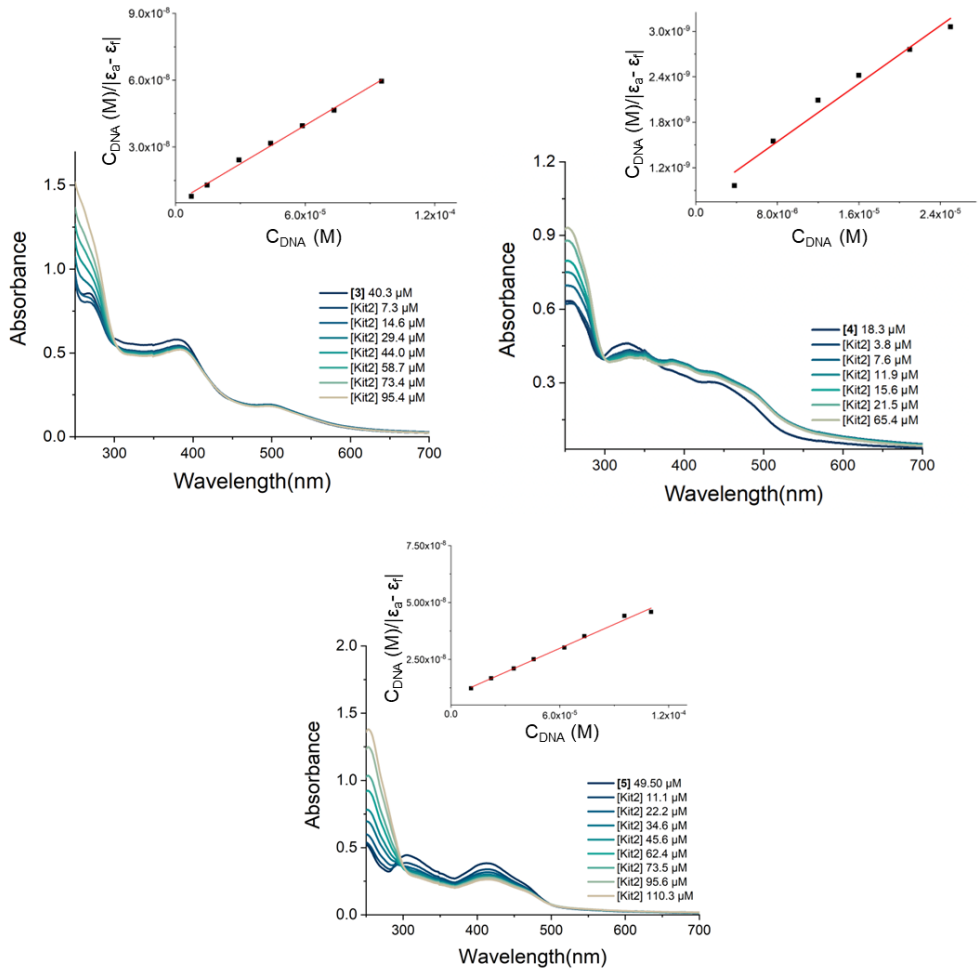


Figure S15. UV-Vis absorption spectrum of compounds **3**, **4** and **5** in combination with increasing amount of Kit2 G4. In the insets the plot and related fit for the calculation of the binding constants (K_b) according to the equation reported in the "Experimental" section. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Concentration of DNA is reported in bases. Cut-off of the spectra was set at 250 nm due to the presence of DMSO as cosolvent (% < 5%).

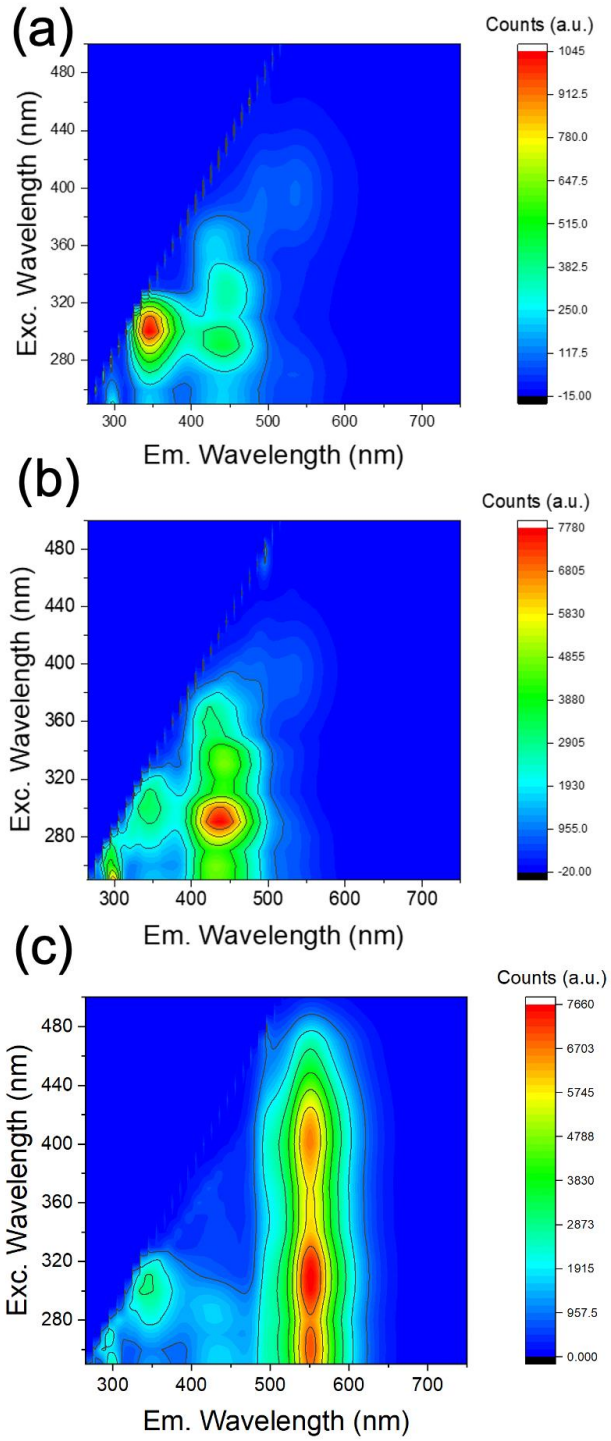


Figure S16. 2D excitation-emission contour map of compound (a) **3**, (b) **4** and (c) **5** obtained by fluorescence spectroscopy. 1st order Rayleigh scattering can be seen as diagonal ridge. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4.

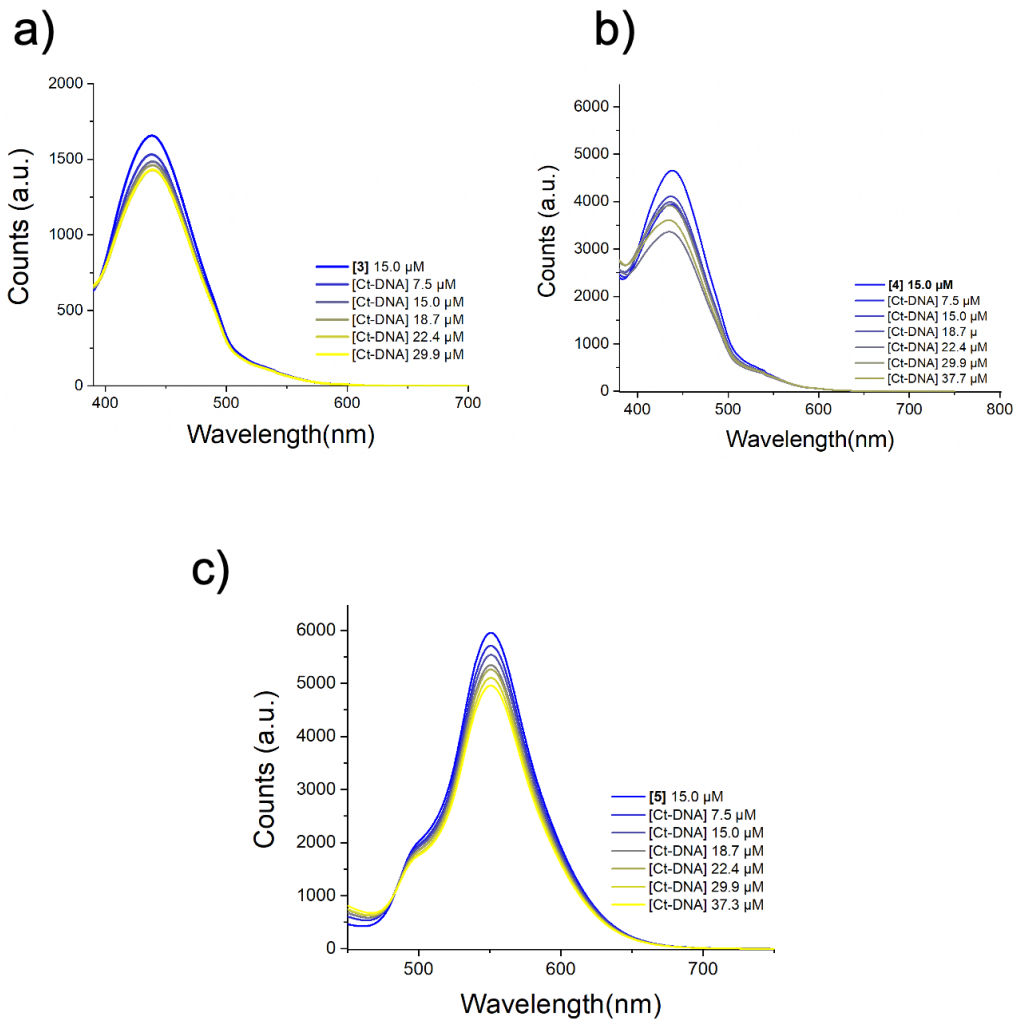


Figure S17. Fluorescence titration of compound **3**, **4** and **5** at the indicated concentrations with increasing amounts of Ct-DNA. (a) λ_{exc} = 300 nm, Slits width = 5.0/5.0 nm; (b) λ_{exc} = 300 nm, Slits width = 10.0/10.0 nm; (c) λ_{exc} = 310 nm, Slits width = 10.0/10.0 nm. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Fluorescence titrations with B-DNA. Concentration of DNA is reported in bases.

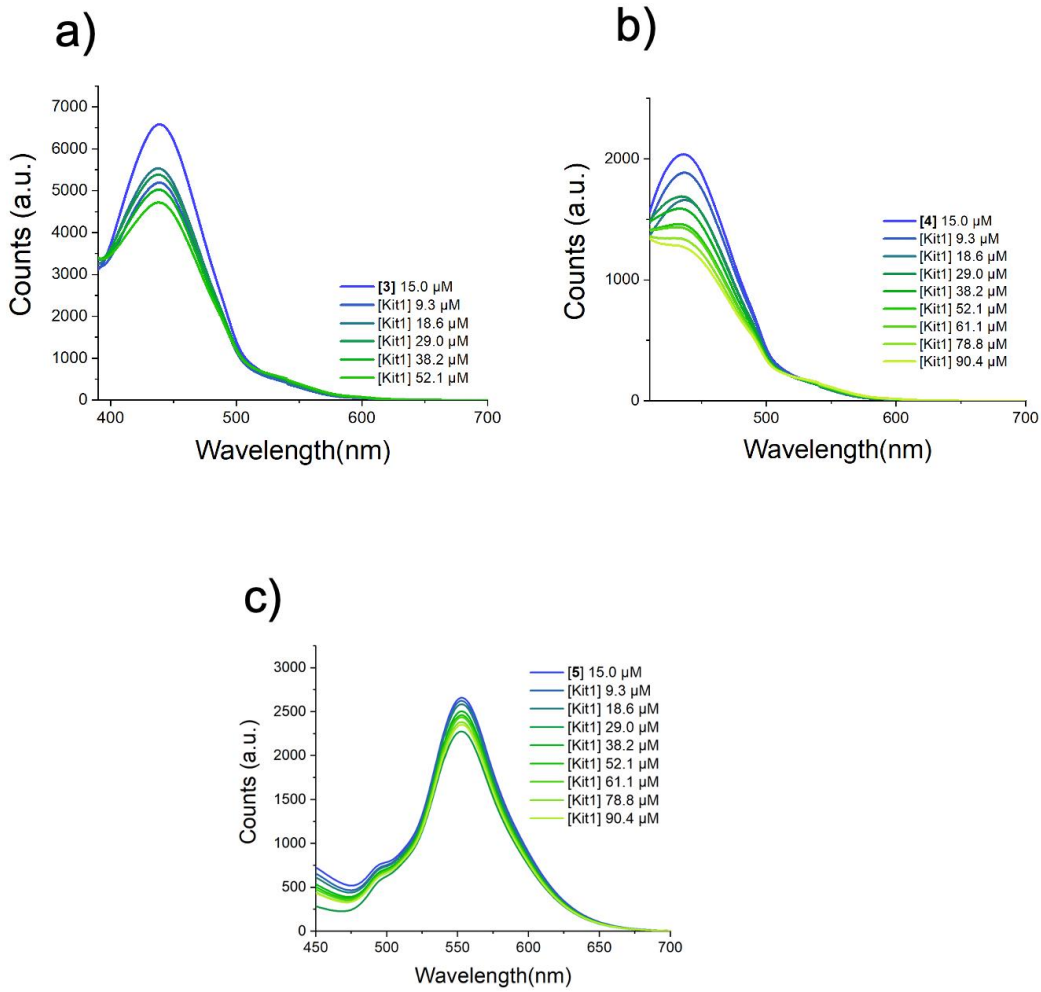


Figure S18. Fluorescence titration of compound **3**, **4** and **5** at the indicated concentrations with increasing amounts of Kit1 G4. (a) λ_{exc} = 300 nm, Slits width = 10.0/10.0 nm; (b) λ_{exc} = 300 nm, Slits width = 10.0/5.0 nm; (c) λ_{exc} = 310 nm, Slits width = 10.0/5.0 nm. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Fluorescence titrations with B-DNA. Concentration of DNA is reported in bases.

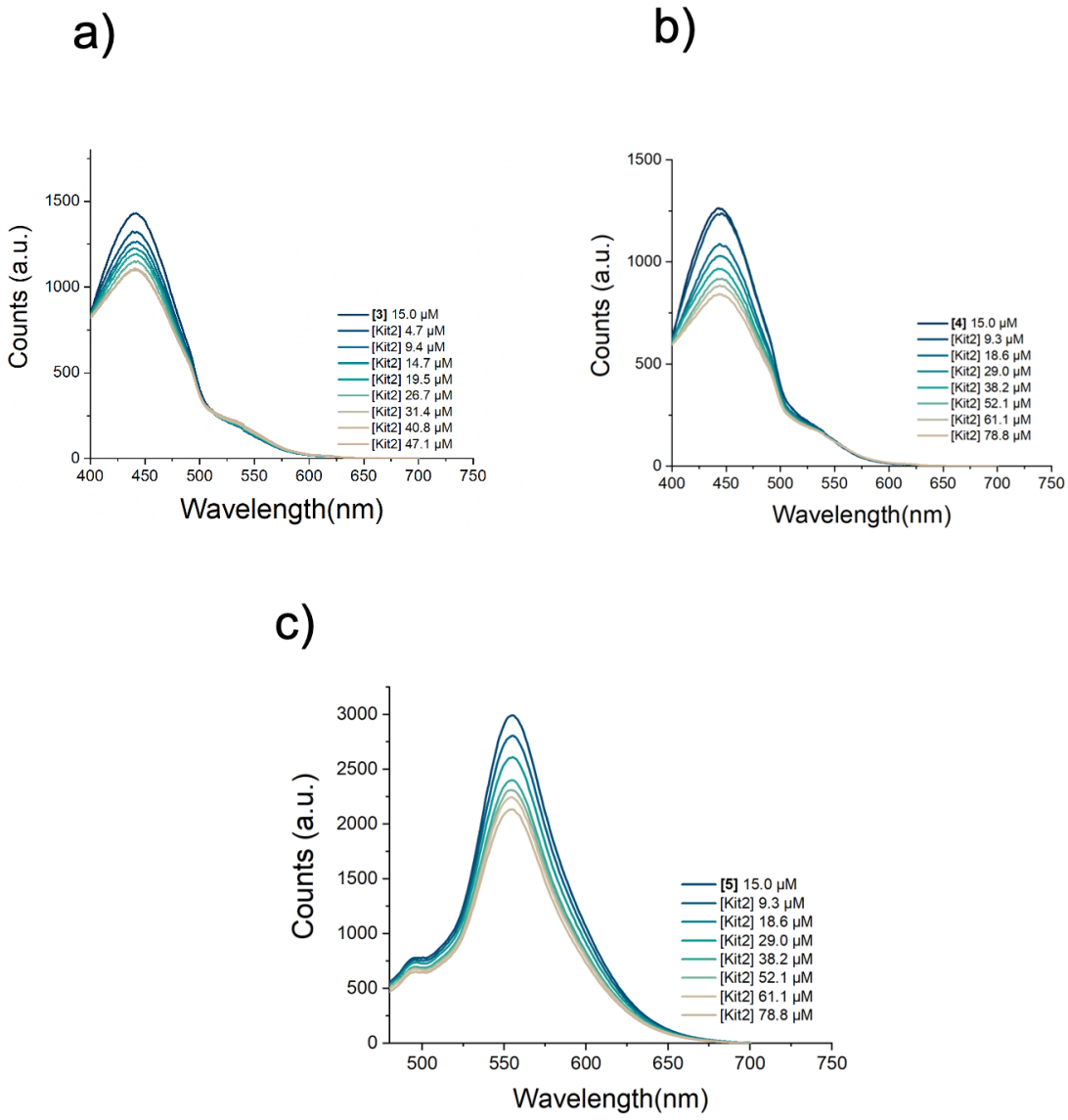


Figure S19. Fluorescence titration of compound **3**, **4** and **5** at the indicated concentrations with increasing amounts of Kit2 G4. (a) λ_{exc} = 300 nm, Slits width = 10.0/5.0 nm; (b) λ_{exc} = 300 nm, Slits width = 10.0/5.0 nm; (c) λ_{exc} = 310 nm, Slits width = 10.0/5.0 nm. Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Fluorescence titrations with B-DNA. Concentration of DNA is reported in bases.

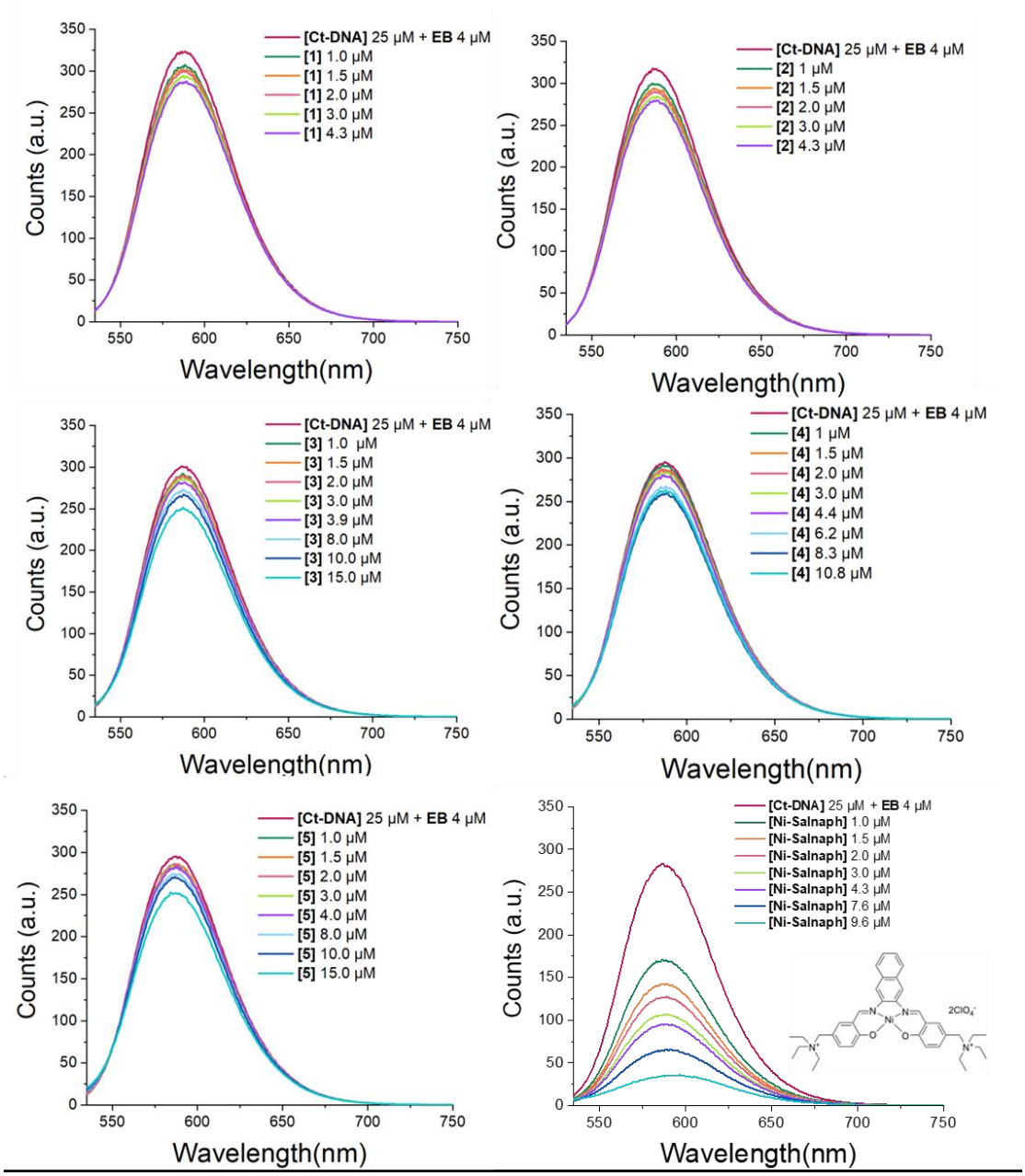


Figure S20. Fluorescence titration of Ct-DNA in complex with EB with increasing amounts of metal complexes **1**-**5** and Ni-Salnaph as control. λ_{exc} = 510 nm, Slits width = 5.0/5.0 nm; Buffer: Tris-HCl 50 mM, KCl 100 mM, pH=7.4. Concentration of DNA is reported in bases

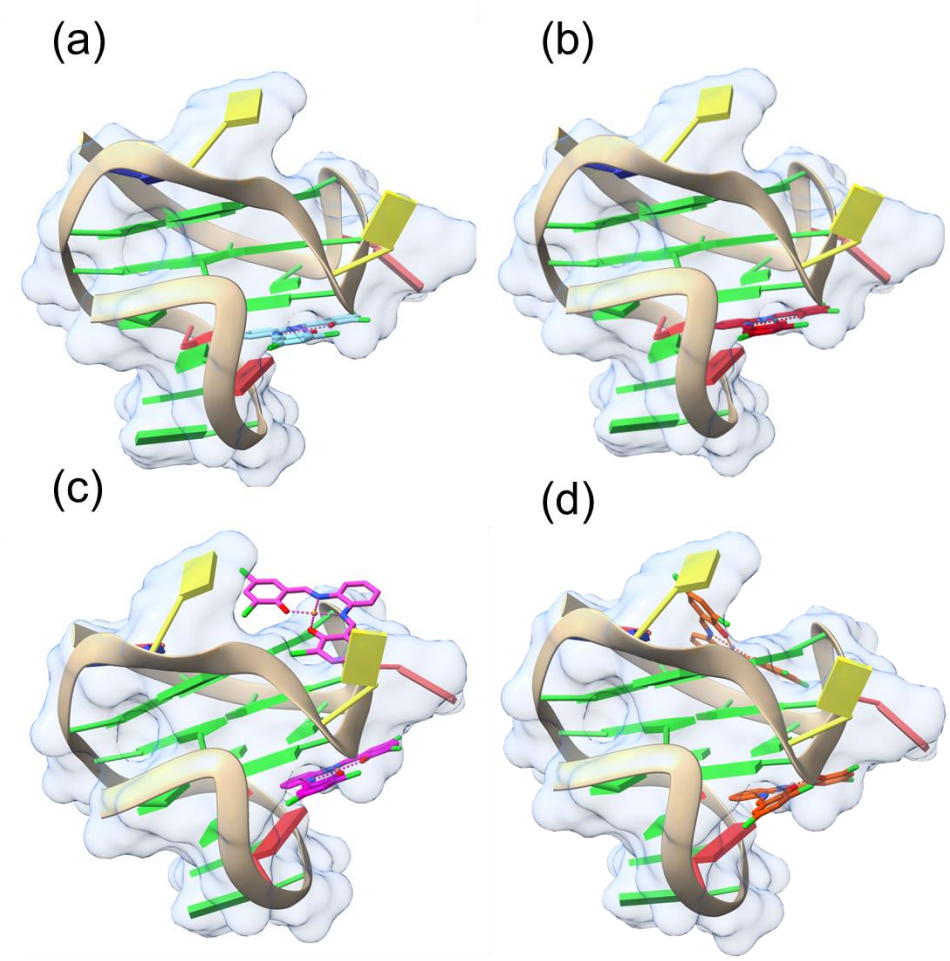


Figure S21. Cartoons showing possible binding sites of (a) **1**, (b) **2**, (c) **4** and (d) **5** with Kit1 G4 motif (PDB id: 2O3M). (Base colours: G = green, C = yellow, T = blue, A = red).

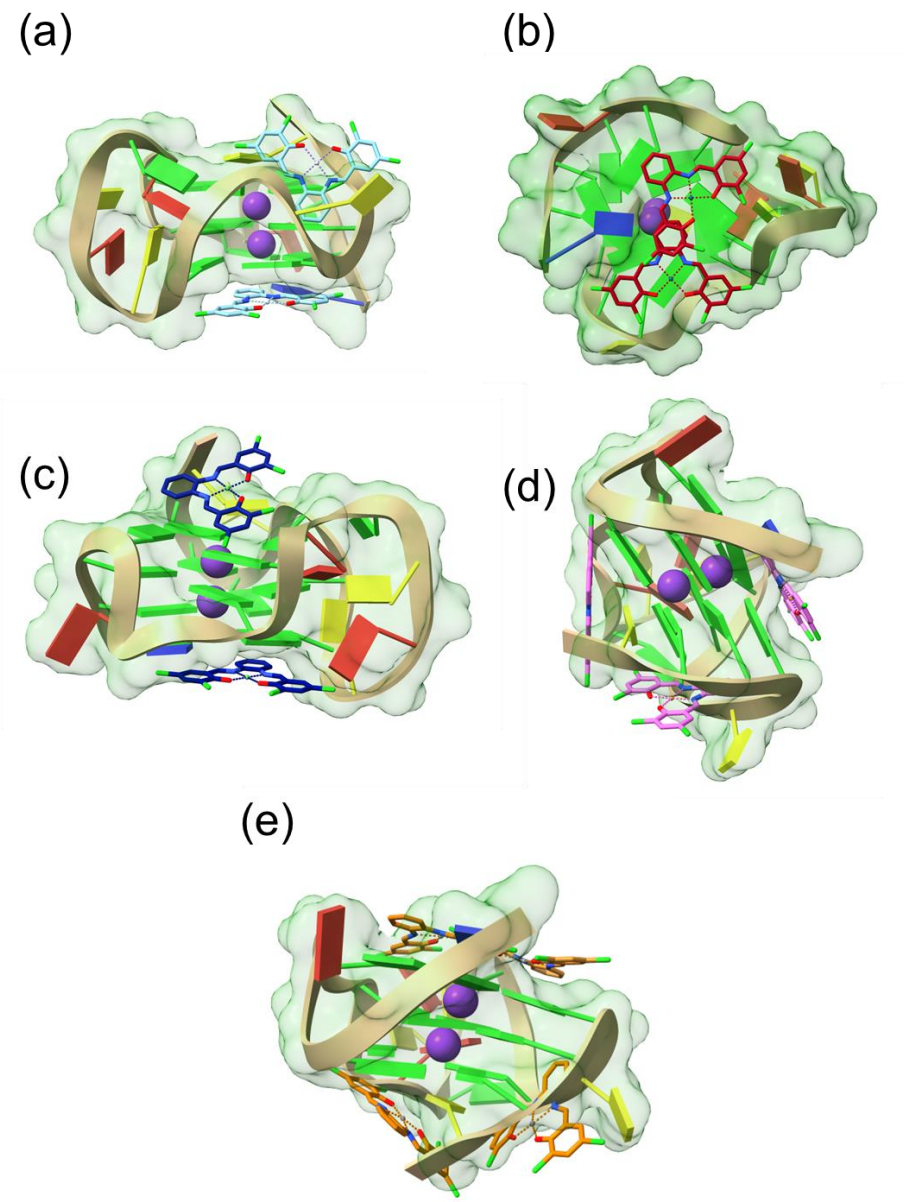


Figure S22. Cartoons showing possible binding sites of (a) **1**, (b) **2**, (c) **3**, (d) **4** and (e) **5** with Kit2 G4 motif (PDB id: 2KQG). (Base colours: G = green, C = yellow, T = blue, A = red).

Table S1. Results of Rietveld refinement of compounds **1** and **2**. Uncertainty in parentheses.

	1	**2**
Formula	$C_{20}H_{10}N_2O_2Cl_2Pt$	$C_{20}H_{10}N_2O_2Cl_2Pd$
System	monoclinic	monoclinic
M (g/mol)	2488.665	2134.033
Space group	*P* $2_1/c$ (14)	*P* $2_1/c$ (14)
a (Å)	13.3874(5)	13.3341(6)
b (Å)	8.6772(5)	8.6691(5)
c (Å)	19.2131(8)	19.183(1)
β (°)	120.931(3)	120.719(3)
Volume (Å^3)	1914.5(2)	1906.3(2)
Z	4	4
U_{iso} Pt/Pd (Å^2)	0.021(1)	0.021(1)
U_{iso} ligand (Å^2)	0.020(2)	0.0311(2)
R_p, R_{wp}	8.6, 11.3	9.9, 12.6
R_{Bragg}	6.4	5.8
2θ range (°)	5-70	8-70
Torsion angle C7-N1-C1-C3 (°)	-5.2(5)	-0.6(5)
C-Cl (Å)	1.716(6)	1.701(5)

Table S2. Fractional atomic coordinates of compound **1**. Uncertainty is on the last digit.

	x	y	z
Pt	0.015	0.685	0.041
N1	0.175	0.625	0.077
N2	0.030	0.802	0.960
C1	0.218	0.691	0.030
C2	0.140	0.786	0.967
C3	0.329	0.667	0.043
C4	0.174	0.857	0.917
C5	0.362	0.738	0.993
C6	0.284	0.833	0.930
H3	0.383	0.602	0.086
H4	0.120	0.922	0.874
H5	0.438	0.721	0.002
H6	0.307	0.881	0.896
C7	0.234	0.524	0.135
C8	0.193	0.452	0.180

C9	0.272	0.349	0.238
C10	0.242	0.269	0.288
C11	0.134	0.292	0.280
C12	0.056	0.395	0.222
C13	0.085	0.475	0.173
O1	0.011	0.571	0.119
Cl1	0.340	0.142	0.359
Cl2	0.922	0.424	0.213
H7	0.311	0.498	0.150
H9	0.346	0.333	0.243
H11	0.114	0.238	0.314
C14	0.949	0.896	0.908
C15	0.841	0.920	0.900
C16	0.771	0.025	0.841
C17	0.661	0.060	0.827
C18	0.621	0.989	0.873
C19	0.691	0.883	0.932
C20	0.801	0.849	0.946
O12	0.867	0.750	0.002
Cl3	0.575	0.190	0.753
Cl4	0.642	0.795	0.989
H14	0.961	0.950	0.870
H16	0.799	0.074	0.809
H18	0.546	0.012	0.863

Table S3. Fractional atomic coordinates of compound **2**. Uncertainty is on the last digit.

	x	y	z
Pd	0.015	0.687	0.041
N1	0.181	0.623	0.078
N2	0.030	0.809	0.957
C1	0.224	0.689	0.031
C2	0.141	0.792	0.964
C3	0.335	0.665	0.043
C4	0.174	0.863	0.914
C5	0.368	0.736	0.993
C6	0.285	0.838	0.926
H3	0.388	0.599	0.086
H4	0.121	0.928	0.871

H5	0.444	0.719	0.002
H6	0.308	0.886	0.892
C7	0.239	0.523	0.136
C8	0.199	0.451	0.181
C9	0.278	0.349	0.239
C10	0.249	0.270	0.289
C11	0.140	0.293	0.282
C12	0.062	0.396	0.224
C13	0.091	0.475	0.174
O1	0.017	0.571	0.120
Cl1	0.348	0.140	0.361
Cl2	0.925	0.425	0.215
H7	0.317	0.497	0.150
H9	0.352	0.333	0.244
H11	0.121	0.239	0.316
C14	0.949	0.902	0.905
C15	0.841	0.926	0.898
C16	0.771	0.032	0.838
C17	0.661	0.066	0.824
C18	0.621	0.996	0.871
C19	0.691	0.890	0.930
C20	0.801	0.856	0.944
O12	0.867	0.757	1.000
Cl3	0.572	0.199	0.749
Cl4	0.641	0.801	0.989
H14	0.961	0.957	0.867
H16	0.799	0.080	0.807
H18	0.545	0.019	0.861

Table S4. 5’-3’ DNA sequences used in this study. In Ds-DNA, Heg linker is $[(-CH_2-CH_2-O-)_6$

Name	Sequence
Ds-DNA (FRET)	*FAM*-TAT AGC TA-Heg-TATA GCT ATA-*TAMRA*
h-Telo (FRET)	*FAM*-AGG GTT AGG GTT AGG GTT AGG G-*TAMRA*
Kit1 (FRET)	*FAM*-AGG GTT AGG GTT AGG GTT AGG G-*TAMRA*
SP (FRET)	*FAM*-GGC GAG GAG GGG CGT GGC CGG C-*TAMRA*
Kit2 (FRET)	*FAM*-CGG GCG GGC GCG AGG GAG GGG-*TAMRA*
h-Telo	AGG GTT AGG GTT AGG GTT AGG G
Kit1	AGG GAG GGC GCT GGG AGG AGG G
Kit2	CGG GCG GGC GCG AGG GAG GGG

Table S5. $\Delta T_{1/2}$ values of 0.2 μM ds-DNA and G4s upon interaction with metal complexes at 1.0 μM concentration. Uncertainty is ≤ 0.5 for the $\Delta T_{1/2}$ reported. Concentration of DNA is reported in strand.

	ds-DNA	h-Telo	Kit1	SP	Kit2
1	0	1.9	1.5	0.0	3.7
2	0.1	1.8	1.3	-0.0	2.3
3	0.8	2.6	4.0	1.1	6.9
4	-0.1	0.9	2.6	2	5.9
5	0.0	0.7	1.1	1.0	2

Table S6. K_b values calculated by UV-Vis titrations.

	Ct-DNA	Kit1	Kit2
1	na	na	na
2	na	na	na
3	$(4.0 \pm 0.9) * 10^4$	$(3.3 \pm 3.4) * 10^3$	$(1.1 \pm 0.3) * 10^5$
4	nd	$(2.4 \pm 0.5) * 10^4$	$(1.2 \pm 0.2) * 10^5$
5	$(2.0 \pm 0.4) * 10^4$	$(1.3 \pm 0.2) * 10^4$	$(4.0 \pm 0.5) * 10^4$

na = not available due to solubility issues. nd = not determined due to the absence of hypochromic effects.

Table S7. Docking free energies of binding with Kit1 G4.

	kcal/mol (1st pose)	kcal/mol (2nd pose)
1	-7.51	
2	-6.85	
3	-6.9	-6.71
4	-6.89	-6.75
5	-8.09	-8.07

Table S8. Docking free energies of binding with Kit2 G4.

	kcal/mol (1st pose)	kcal/mol (2nd pose)	kcal/mol (3rd pose)	kcal/mol (4th pose)
1	-6.91	-6.82		
2	-6.69	-6.66		
3	-6.77	-6.13		
4	-6.88	-6.73	-6.09	
5	-7.71	-7.68	-7.36	-6.53