Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Material

Naturally occurring quaternary benzo[c]phenanthridine alkaloids selectively stabilize G-quadruplexes

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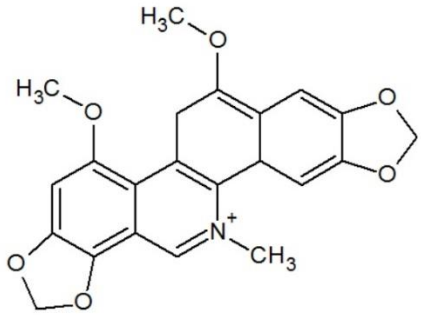
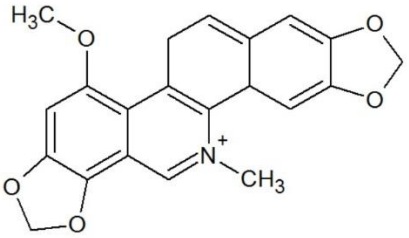
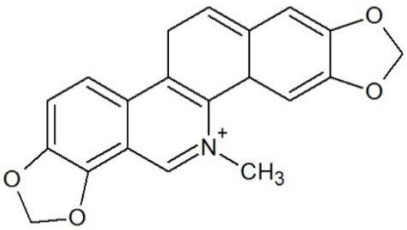
Figure S1. Chemical structures of the alkaloids studied in this work.



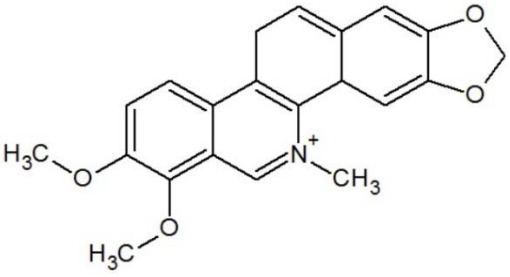
Macarpine (MA)



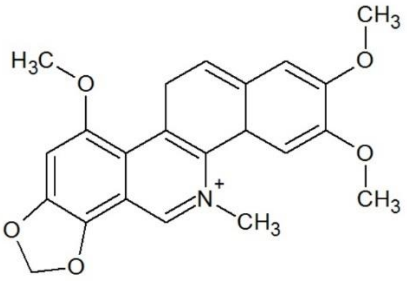
Chelirubine (CHR)



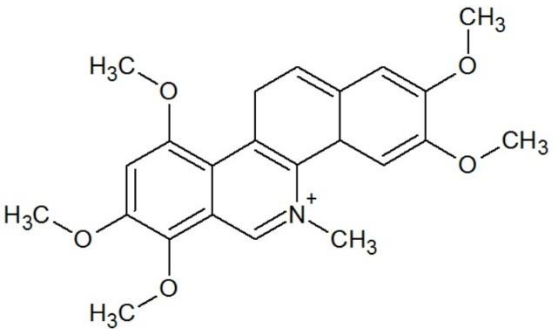
Sanguinarine (SG)



Chelerythrine (CHE)



Sanguirubine (SR)



Sanguilutine (SL)

Figure S2. CD spectra of HT22 and ckit21T21 with ligands.

C_{DNA} = 2 μM, C_{QBA} = 4 μM, 10 mM phosphate buffer, 5 mM KCl. Spectra measured at 20°C. All other experimental conditions as detailed in the main text.

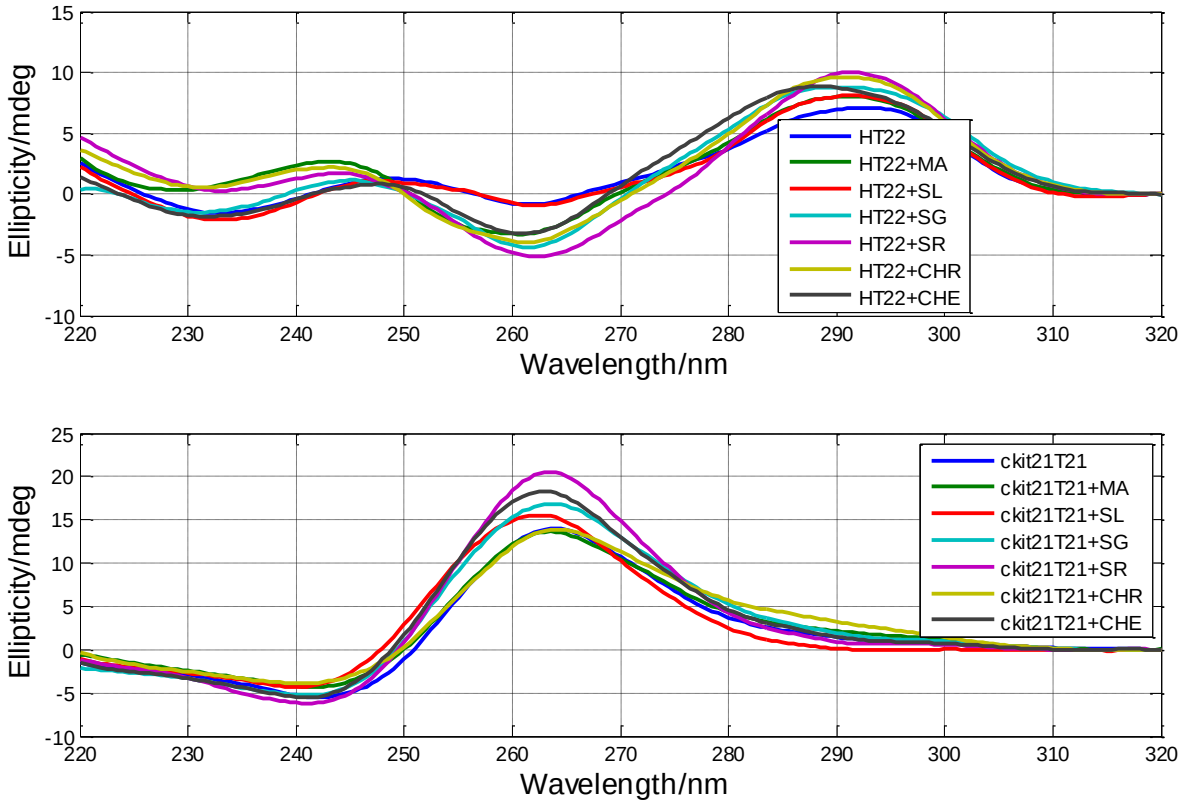


Figure S3. Examples of CD spectra recorded in the wavelength range from 220 to 600 nm.

C_{DNA} = 2 μM, C_{QBA} = 4 μM, 10 mM phosphate buffer, 5 mM KCl. Spectra measured at 20°C. All other experimental conditions as detailed in the main text. In all cases, there is not an evidence of any induced CD signal in the visible region due to the interaction of the ligand with either HT22 or ckit21T21 GQ structures.

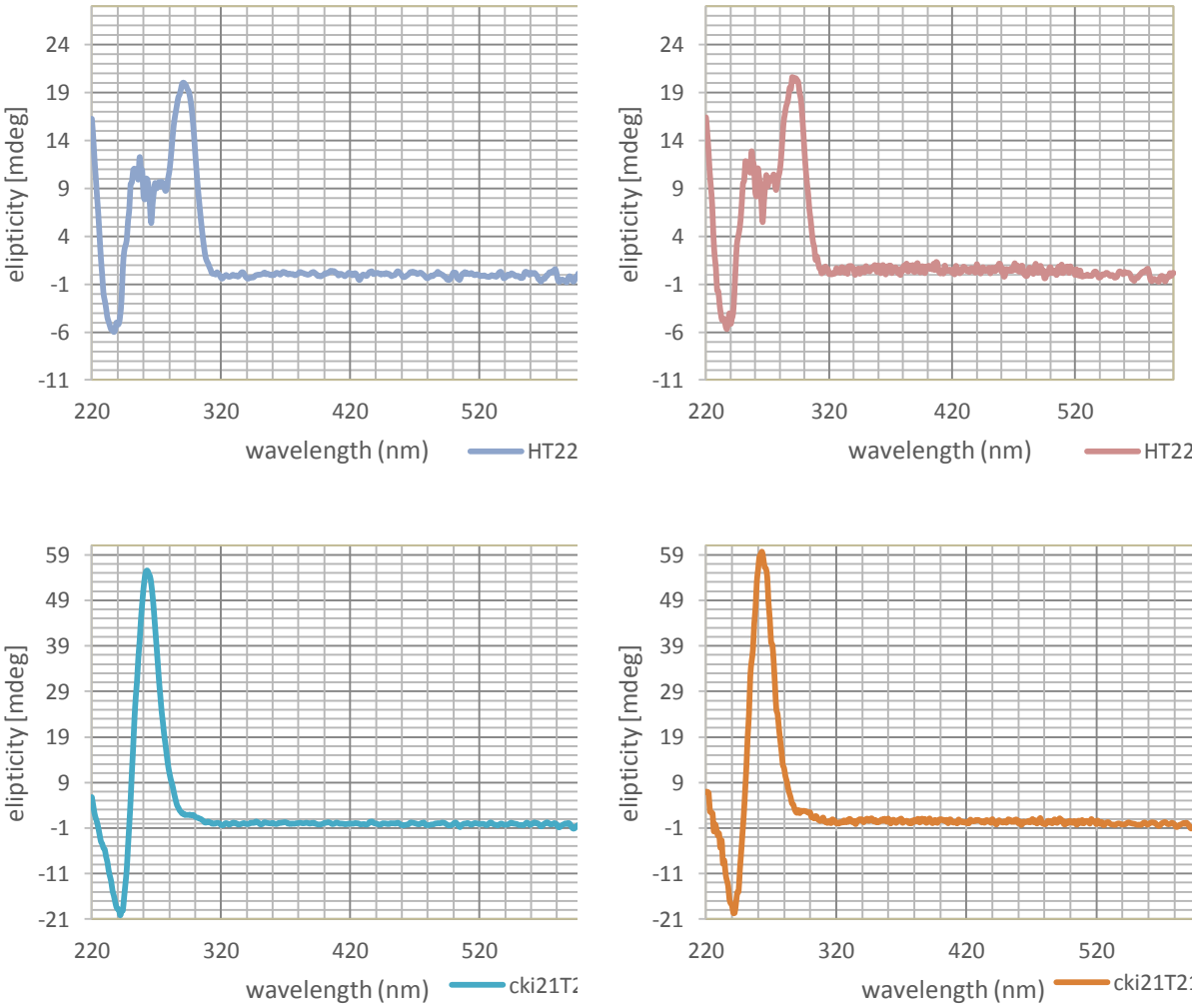
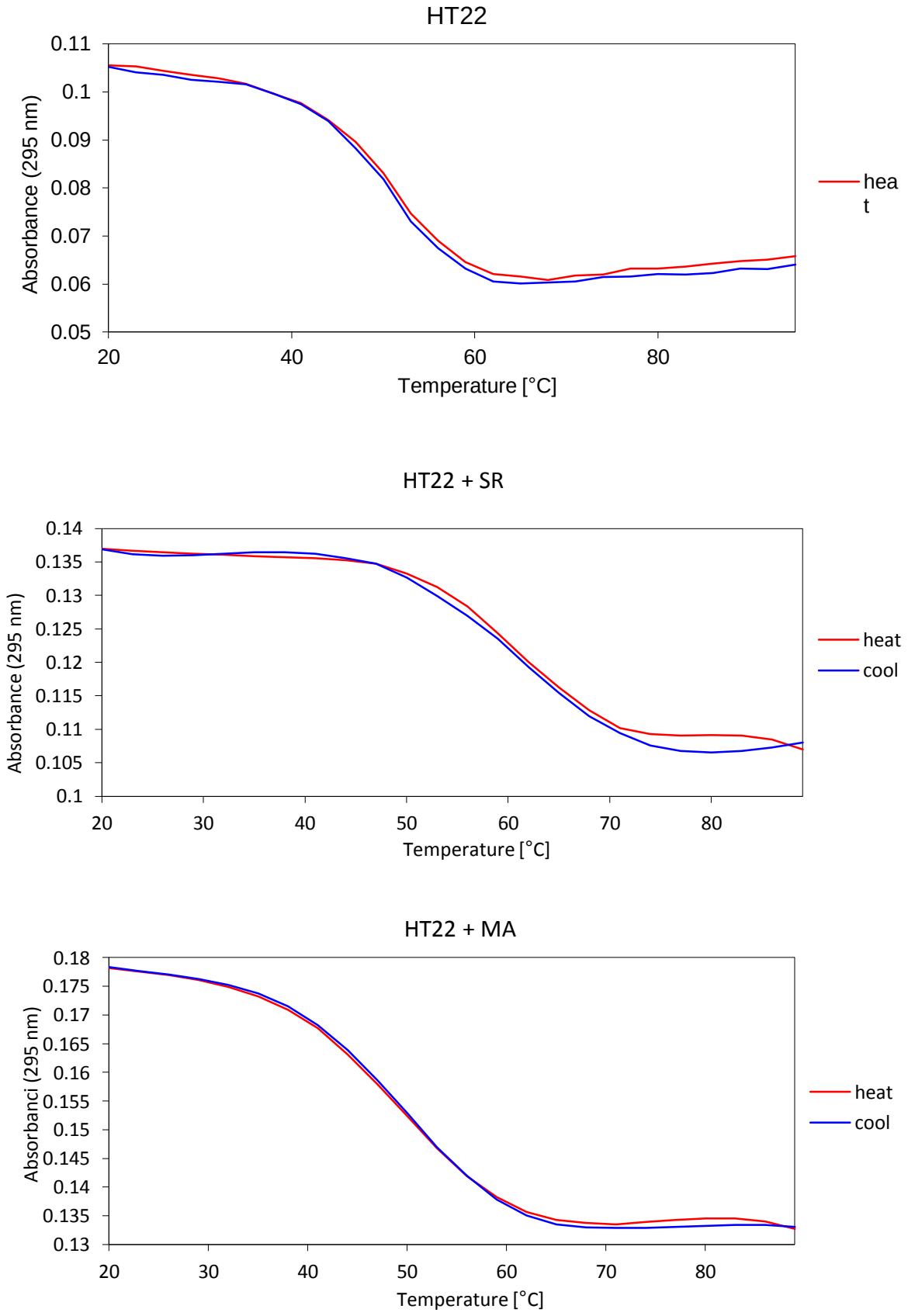
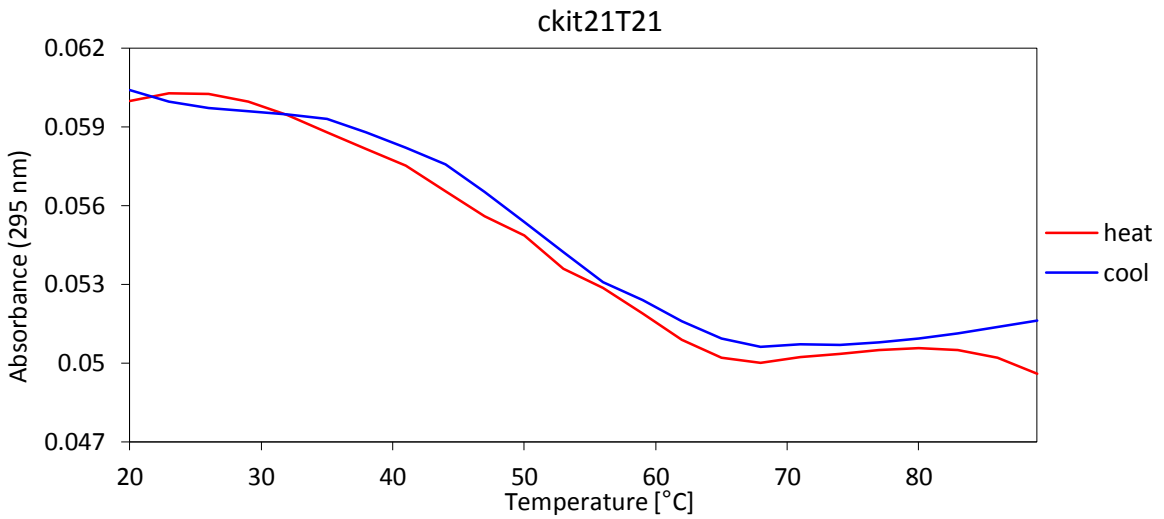
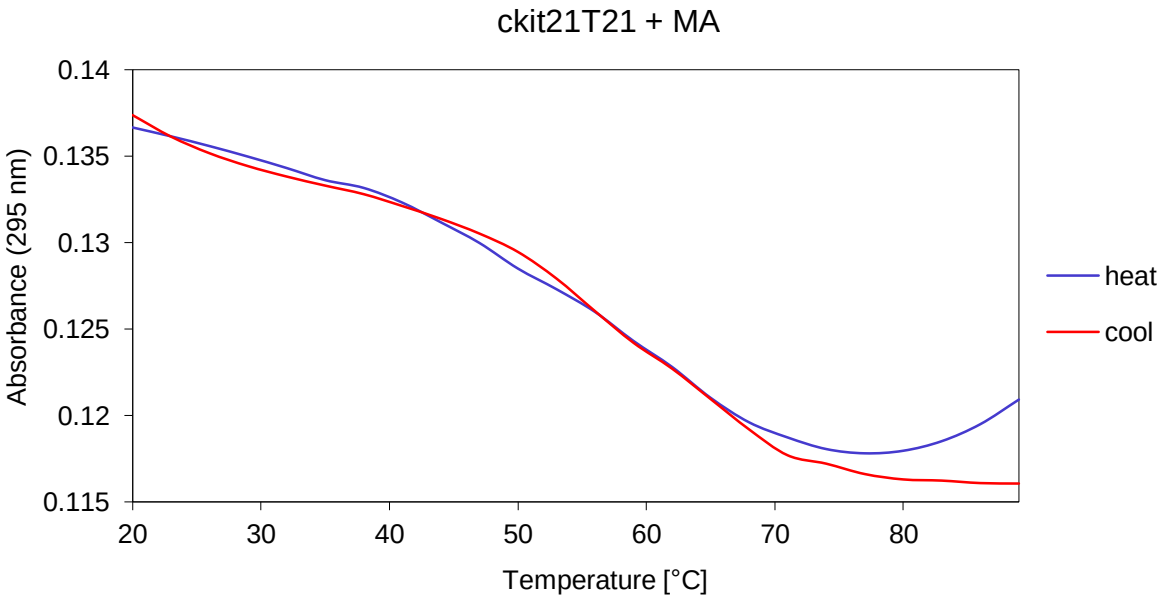


Figure S4. Examples of heating and cooling traces for HT22, ckitG1T21 and several QBA:GQ mixtures.




ckit21T21
0.062
0.059
0.056
0.053
0.05
0.047
Absorbance (295 nm)
20
30
40
50
60
70
80
Temperature [°C]
heat
cool


ckit21T21 + MA
0.14
0.135
0.13
0.125
0.12
0.115
Absorbance (295 nm)
20
30
40
50
60
70
80
Temperature [°C]
heat
cool

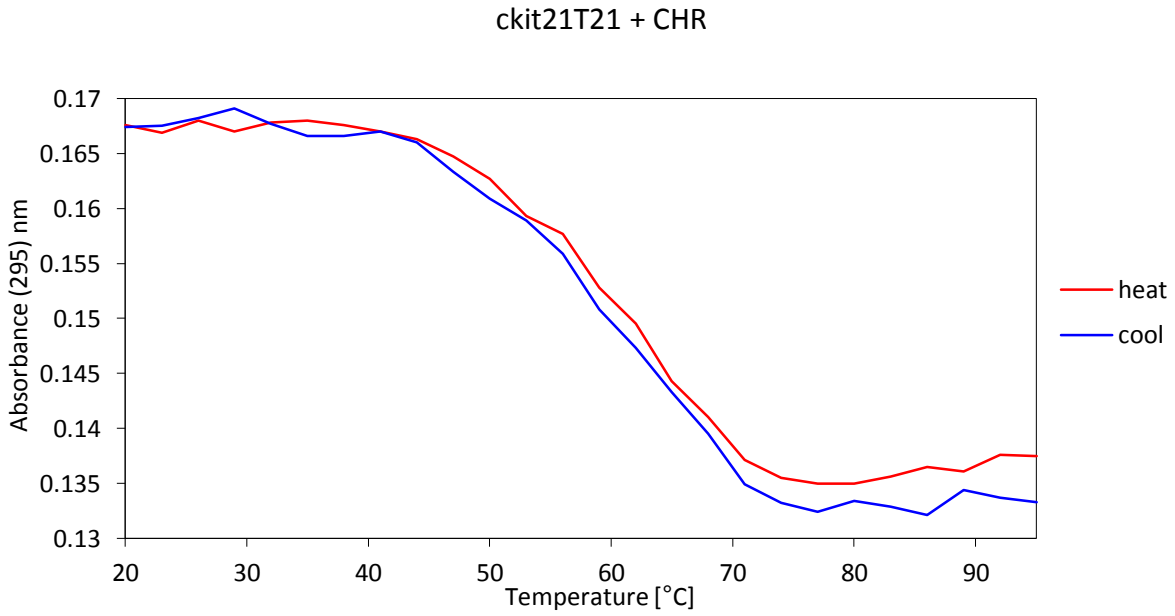

ckit21T21 + CHR
Absorbance (295) nm
0.17
0.165
0.16
0.155
0.15
0.145
0.14
0.135
0.13
20
30
40
50
60
70
80
90
Temperature [°C]
heat
cool

Figure S5. CD-monitored unfolding experiment of ckit21T21.

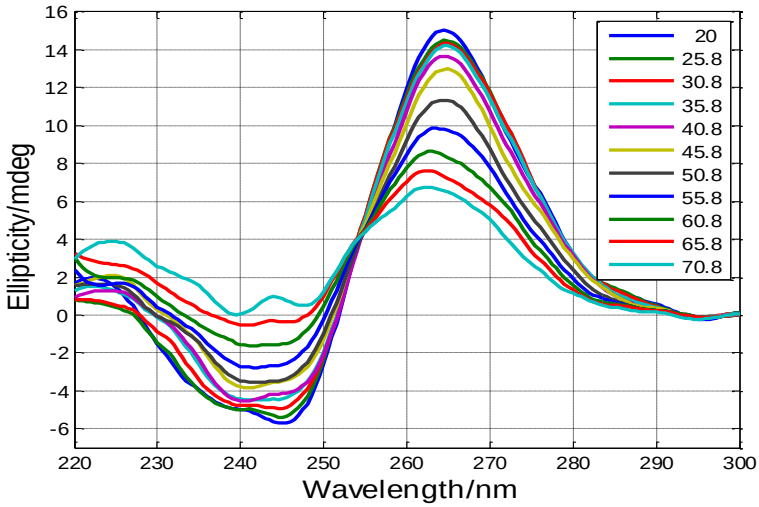
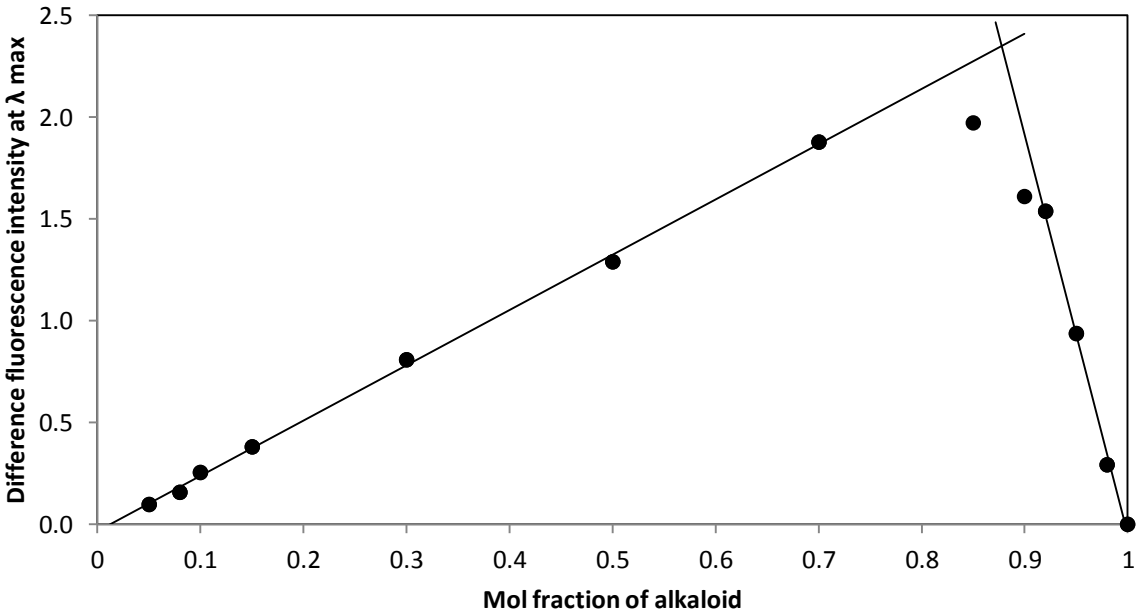


Figure S6. Examples of estimation of binding stoichiometries by means of Job plots. (a) HT22 + chelirubine (b) ckit21T21 + sanguinarine

a



b

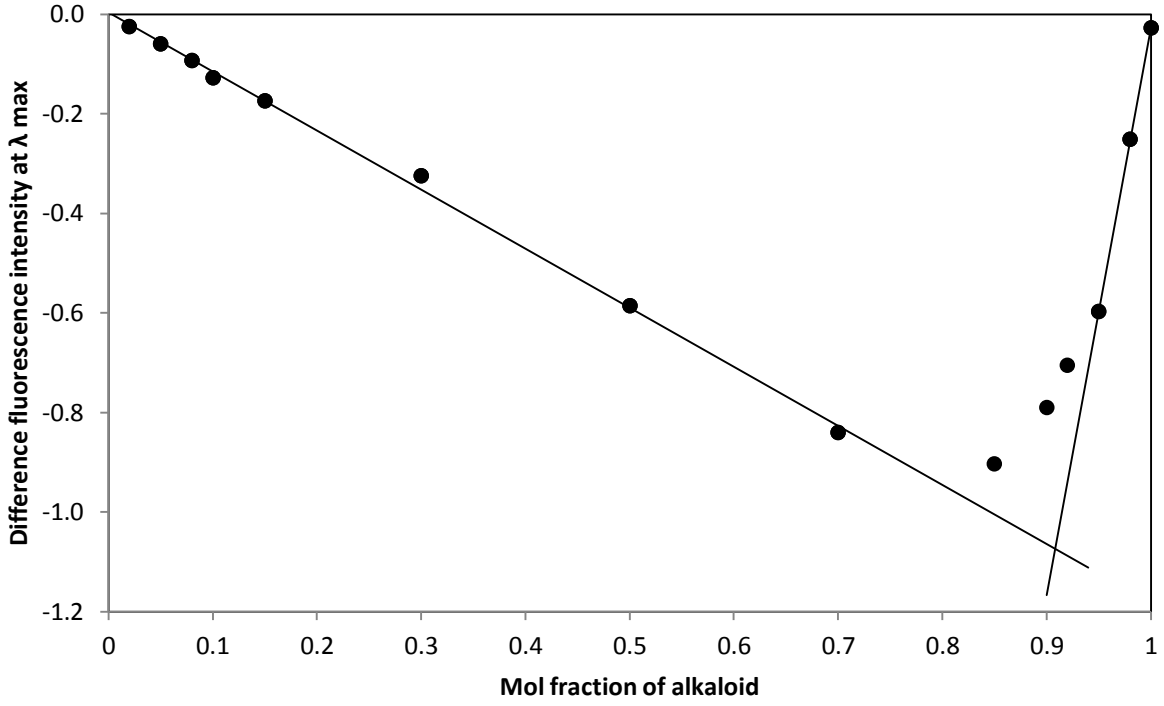

0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
Difference fluorescence intensity at λ max
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Mol fraction of alkaloid

Table S1. ^{1}H chemical shift values for the complex of sanguilutine with Pu22-T14T23.[a]

	H1/H2/Me	Δδ[b]	H6/H8	Δδ[b]
T4	n.d.	-	n.d.	-
G5	n.d.	-	n.d.	-
A6	n.d.	-	7.95	+0.30
G7	11.04	- 0.72	8.08	+0.08
G8	10.76	- 0.48	7.66	- 0.08
G9	10.10	- 0.55	7.67	- 0.07
T10	n.d.	-	n.d.	-
G11	10.95	- 0.76	n.d.	-
G12	10.90	- 0.60	7.66	-0.02
G13	10.36	- 0.69	7.73	- 0.18
T14	1.98	+0.06	7.68	+0.03
A15	8.38	+0.11	8.57	+0.02
G16	n.d.	-	8.19	+0.08
G17	10.80	- 0.45	7.60	- 0.20
G18	10.28	- 0.74	7.73	- 0.06
T19	2.01	+0.02	7.88	-0.02
G20	11.08	- 0.20	7.85	- 0.04
G21	11.08	- 0.29	7.83	- 0.07
G22	10.43	- 0.61	7.71	-0.10
T23	1.35	- 0.13	6.92	- 0.22
A24	n.d.	-	7.92	+0.15
A25	7.49	+0.10	7.37	-0.13

[a] Measured at 25°C in ppm (δ) from external DSS. Solvent H_2O-D_2O (90:10 v/v), 25 mM phosphate buffer, 70 mM KCl, pH 6.9, R = 3. Other ribose protons showing significant shift variations: T23 H-1'= -0.34. [b] $\Delta\delta = \delta_{bound} - \delta_{free}$.

Table S2. ^{1}H chemical shift values for the complex of chelerythrine with Pu22-T14T23.[a]

	H1/H2/Me	Δδ[b]	H6/H8	Δδ[b]	H1'	Δδ[b]
T4	n.d.	-	n.d.	-	n.d.	-
G5	n.d.	-	n.d.	-	n.d.	-
A6	n.d.	-	n.d.	-	n.d.	-
G7	11.10	- 0.66	8.02	0.00	6.03	-0.03
G8	10.72	- 0.50	7.60	- 0.15	6.03	-0.10
G9	10.09	- 0.56	7.63	- 0.12	n.d.	-
T10	1.95	-0.04	7.68	-0.14	6.28	-0.24
G11	n.d.	-	n.d.	-	n.d.	-
G12	10.84	- 0.66	7.63	- 0.27	n.d.	-
G13	10.52	- 0.53	7.72	- 0.14	n.d.	-
T14	1.89	-0.03	7.58	-0.07	6.25	+0.02
A15	8.35	-0.18	8.55	-0.02	6.64	-0.04
G16	10.63	- 1.36	8.18	+0.07	6.22	+0.05
G17	10.92	- 0.33	7.82	+0.02	n.d.	-
G18	10.52	- 0.50	7.60	- 0.18	6.03	-0.39
T19	2.00	+0.01	7.89	+0.03	6.54	+0.02
G20	10.92	- 0.36	n.d.	-	n.d.	-
G21	10.98	- 0.39	7.91	0.00	5.80	-0.24
G22	10.28	- 0.76	7.79	+0.18	6.00	-0.14
T23	1.45	- 0.03	7.03	- 0.11	5.63	- 0.27
A24	n.d.	-	7.93	+0.16	5.68	- 0.07
A25	n.d.	-	7.33	+0.03	5.40	-0.20

[a,b] See footnotes (a) and (b) of **Table S1**.

Table S3. Chemical shift values of chelerythrine in the complex with Pu22-T14T23 and ckit21T12T21.

Chelerythrine	Pu22-T14T23[a]	Δδ[b]	ckit21T12T21[c]	Δδ[b]
H1	6.54	-0.58	6.54	-0.58
2,3 O-CH_2-O	5.91	-0.31	5.91	-0.31
H4	7.27	-0.53	7.30	-0.50
H6	9.11	-0.50	9.20	-0.41
H9	n.d.	-	n.d.	-
H10	n.d.	-	n.d.	-
H11	7.68	-0.42	7.58	-0.52
H12	7.23	-0.57	7.24	-0.56
NCH_3	4.45	-0.45	4.46	-0.54
7-OCH_3	3.90	-0.10	n.d.	-
8-OCH_3	3.90	-0.30	n.d.	-

[a] Measured at 25°C in ppm (δ) from external DSS. Solvent H_2O-D_2O(90:10 v/v), 25 mM phosphate buffer, 70 mM KCl. For Sanguilutine complex aromatic proton H6 and NCH_3 lie at 9.10 ppm and 4.55 ppm respectively. Other aromatic protons were not assigned and lie around 6.8/7.2 ppm. [b] $\Delta\delta = \delta_{bound} - \delta_{free}$. [c] 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.

Table S4. Fluorescence intensity of each QBA:DNA complex– competitive dialysis.

	SG (559 nm)	CHE (554 nm)	CHR (596 nm)	MA (589 nm)	SL (593 nm)	SR (591 nm)
Blank	0.9	0.6	0.2	0.3	0.3	0.5
HT22	13.5	10.0	3.7	12.6	14.4	5.7
ckit21T21	**18.1**	15.3	4.3	**17.0**	**19.9**	8.9
T20	2.8	3.0	0.5	2.4	1.0	1.4
ds26	7.0	6.8	2.0	8.6	8.5	4.1
Dickerson	8.5	6.6	2.0	9.9	8.3	2.6

Table S5. Inter-residue NOE interactions of ckit21T12T21 in the complex with chelerythrine.

Solvent H_2O-D_2O (90:10 v/v), 5 mM phosphate buffer, 20 mM KCl, pH 6.9, R = 3.

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

Table S6. Inter-residue NOE interactions of Pu22-T14T23 in the complexes with sanguilutine and chelerythrine.[a]

G-tetrad I	*G-tetrad II*	*Tetrad III*
G11H1…G16H8	G8H1….G12H8	G9H1….G13H8
G20H1…G7H8	G12H1...G17H8	G13H1…G18H8
G16H1…G20H8[b]	G17H1…G21H8	G18H1…G22H8
G7H1…G11H8[c]	G21H1…G8H8	G22H1…G9H8

[a] Acquired at 25°C in H_2O-D_2O (90:10 v/v), 25 mM phosphate buffer, 70 mM KCl, pH 6.9.

[b] Not detect in sanguilutine complex.

[c] Not detect in chelerythrine complex.