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Supplementary Material

Identification of Effective Anticancer G-Quadruplex-Targeting Chemotypes through the Exploration of a High Diversity Library of Natural Compounds

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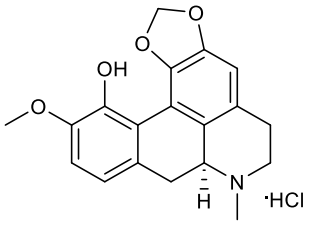
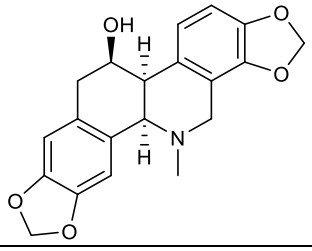
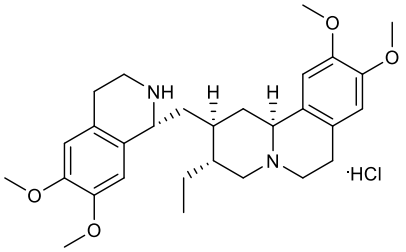
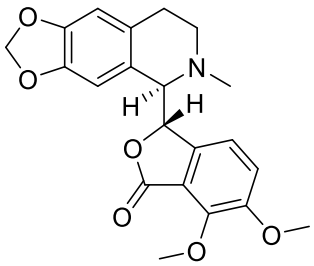
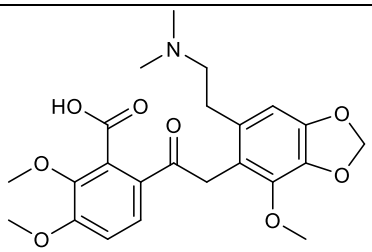
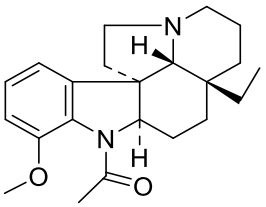
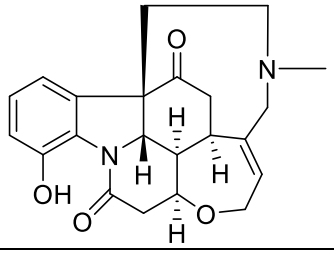
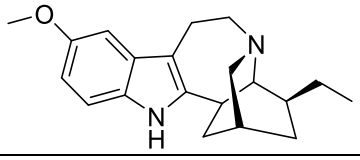
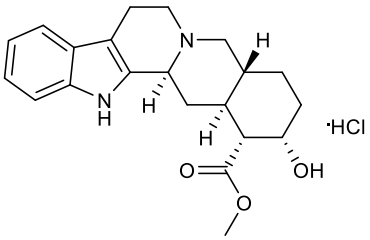
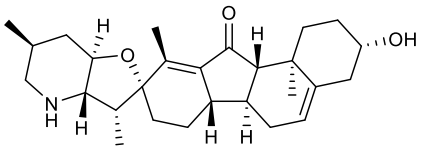
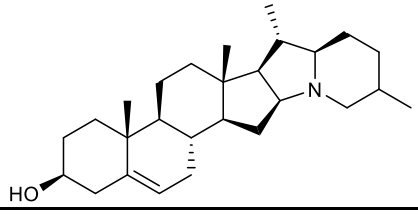
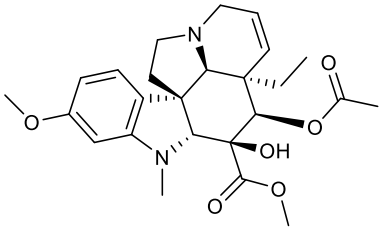
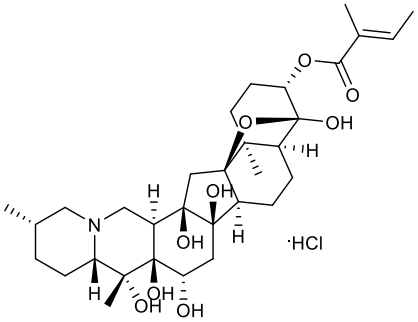
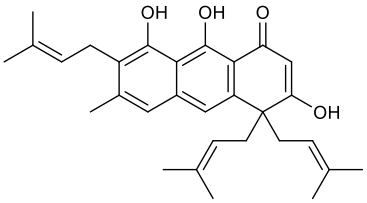
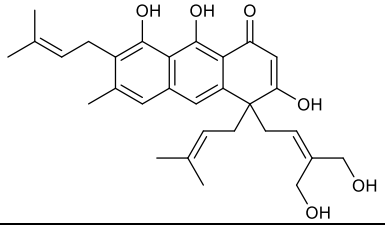
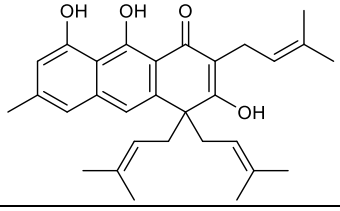
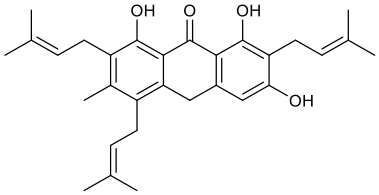
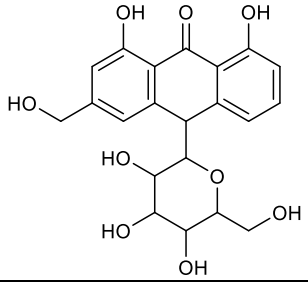
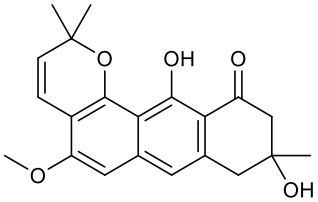
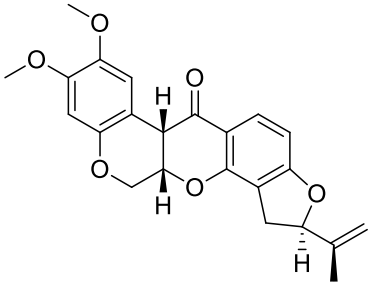
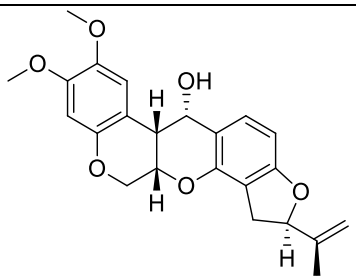
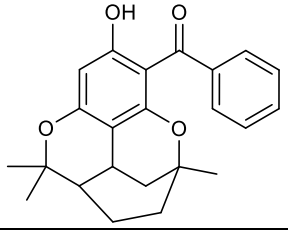
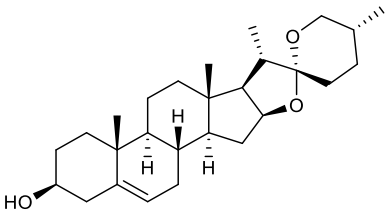
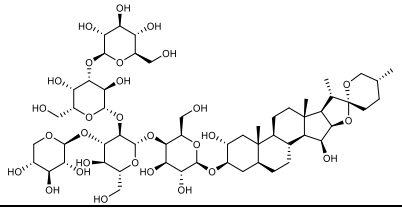
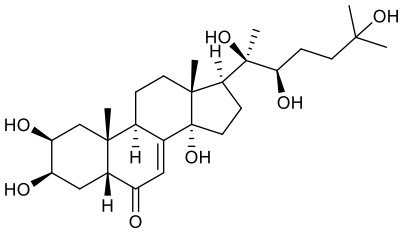
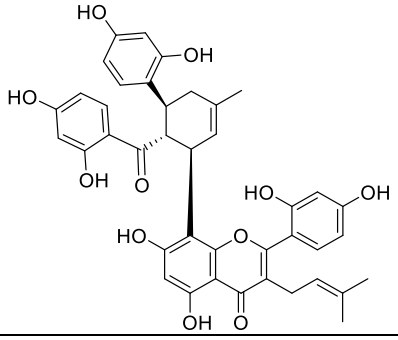
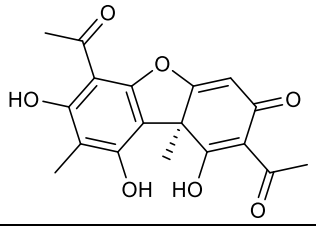
Table S1. Chemical structures, features and natural sources of the 28 natural compounds here investigated.

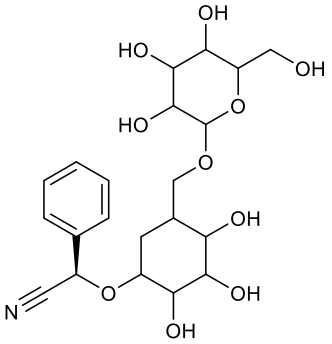
Cpd	Common Name (Library Code)	Chemical Structure	M. W.	Molecular Formula	Source	Reference
		Alkaloids				
1	**Bulbocapnine·HCl (BBN196)**		325.36 361.82 (+HCl)	$C_{19}H_{19}NO_4$·HCl	Species: *Corydalis cava* (Papaveraceae family)	[1]
2	**Chelidonine (BBN192)**		353.37	$C_{20}H_{19}NO_5$	Species: *Chelidonium majus L.* (Papaveraceae family)	[2]
3	**Emetine·HCl (BBN39)**		480.65 517.11 (+HCl)	$C_{29}H_{40}N_2O_4$·HCl	Species: *Psychotria ipecacuanha Stokes* (Rubiaceae family)	[3]
4	**Hydrastine (BBN40)**		383.40	$C_{21}H_{21}NO_6$	Species: *Hydrastis canadensis L.* (Ranunculaceae family)	[4]
5	**Narceine (BBN254)**		445.46	$C_{23}H_{27}NO_8$	Species: *P. somniferum L (Papaveraceae family).*	[5]
6	**Aspidospermine (BBN44)**		354.49	$C_{22}H_{30}N_2O_2$	Aspidosperma species: *Aspidosperma album, Aspidosperma australe, Aspidosperma exalatum, Aspidosperma peroba, Aspidosperma polyneuron,*	[6]

					Aspidosperma pyricollum, *Aspidosperma pyrifolium*, *Aspidosperma quebracho-blanco*, *Aspidosperma quirandy*, *Aspidosperma sessiflorum*, *Aspidosperma rhombeosignatum* (Apocynaceae family)	
7	**Vomicine (BBN186)**		380.44	$C_{22}H_{24}N_2O_4$	Species: *Strychnos icaja* (Loganiaceae family)	[7, 8]
8	**Ibogaine (BBN236)**		310.43	$C_{20}H_{26}N_2O$	Species: *Tabernanthe iboga* (Apocynaceae family)	[9]
9	**Yohimbine· HCl (BBN174)**		354.44 390.90 (+HCl)	$C_{21}H_{26}N_2O_3$· HCl	Species: *Aspidosperma discolor A. DC.*, *Aspidosperma excelsum Benth*, *Aspidosperma eburneum F. Allem*, *Aspidosperma marcgravianum-Woodson*, *Aspidosperma oblongum A. DC* (Apocynaceae family)	[10]
10	**Jervine (BBN47)**		425.60	$C_{27}H_{39}NO_3$	Veratrum species: *V. album; V. dahuricum; V. lobelianum; V. nigrum; V. nigrum var. ussuriense; V. patulum; V. stenophyllum; V. taliense* (Buxaceae family)	[11]
11	**Solanidine (BBN209)**		397.65	$C_{27}H_{43}NO$	Veratrum species: *V. mentzeanum; V. taliense* (Buxaceae family) Fritillaria species: *F.*	[11]

					camtschatcensis; F. tortifolia; F. ussuriensis (Liliaceae family)	
12	**Vindoline (BBN218)**		456.53	$C_{25}H_{32}N_2O_6$	Species: *Catharanthus roseus* (Apocynaceae family)	[12]
13	**Veratrine.HCl (BBN173)**		591.73 628.19 (+HCl)	$C_{32}H_{49}NO_9 \cdot HCl$	Species: *Veratrum lobelianum* (Melanthiaceae family)	[11]
		Phenolic Compounds				
		Anthranoids				
14	**Ferruginin A (BBN240)**		460.60	$C_{30}H_{36}O_4$	Species: *Vismia baccifera var. ferruginea* and *Vismia decipiens* (Hypericaceae family)	[13, 14]
15	**γ, γ'-OH-Ferruginin A (BBN35)**		492.61	$C_{30}H_{36}O_6$	Species: *Vismia guaranirangae* (Hypericaceae family)	[13, 15]
16	**Ferruginin B (BBN161)**		460.61	$C_{30}H_{36}O_4$	Species: *Vismia baccifera var. ferruginea* and *Vismia decipiens* (Hypericaceae family)	[13, 14]
17	**Ferruanthrone (BBN257)**		460.61	$C_{30}H_{36}O_4$	Species: *Vismia baccifera var. ferruginea* and *Vismia decipiens* (Hypericaceae family)	[13, 14]

18	**Aloin (BBN36)**		418.40	$C_{21}H_{22}O_9$	Species: *Aloe vera* (Asphodelaceae family)	[16]
19	**Vismione B (BBN239)**		354.40	$C_{21}H_{22}O_5$	Species: *Vismia baccifera* var *dealdata* (Hypericaceae family)	[17]
		Flavonoids				
		Rotenoids				
20	**Rotenone (BBN255)**		394.42	$C_{23}H_{22}O_6$	Tephrosia species: *T. cathartica Urb.* *T. sp.* *T. grandiflora Pers.* *T. maxima* *T. candida* DC. *T. subtriflora* *T. villosa Pers.* *T. wallichii Graham* *T. kolstii* *T. brachydon* *T. toxicar á* (Sw.) *Pers.* *T. cin ́ea Pers.* *T. purpurea Pers.* *T. spaerospora* *T. noct flora Boj.* *T. vogelii Hook f.* (Fabaceae family)	[18, 19]
21	**Rotenolone (BBN75)**		396.44	$C_{23}H_{24}O_6$	Tephrosia species: *Tephrosia vogelii* (Fabaceae family)	[19]
		Benzophenone				
22	**Clusiacitran B (BBN38)**		364.44	$C_{23}H_{24}O_4$	Species: *Clusia multiflora* (Clusiaceae family)	[20]
		Steroids				

23	**Diosgenin (BBN256)**		414.63	$C_{27}H_{42}O_3$	Species:*Dracaena cambodiana* and *Chlorophytum laxum* (Asparagaceae family) Species:*Balanites aegyptiaca* (Zygophyllaceae or Balanitaceae family)	[21]
24	**Digitonin (BBN253)**		1229.32	$C_{56}H_{92}O_{29}$	Species: *Digitalis lanata* and *Digitalis purpurea* (Scrophulariaceae family)	[22]
25	**20-OH-Ecdysone (BBN258)**		480.64	$C_{27}H_{44}O_7$	Species: *Chenopodium quinoa* Willd. (Chenopodiaceae family)	[23]
		Diels Alder type adduct				
26	**Kuwanon G (BBN99)**		692.72	$C_{40}H_{36}O_{11}$	Species: *Morus nigra* (Moraceae family)	[24]
		Dibenzofuran				
27	**Usnic acid (BBN66)**		344.32	$C_{18}H_{16}O_7$	Species: *Ramalina hierensis* (Ramalinaceae family)	[25]
		Cyanogenic glycoside				

28	**Amygdaline (BBN96)**		455.46	$C_{21}H_{29}NO_{10}$	Seeds of numerous members of Rosaceae family	[26]

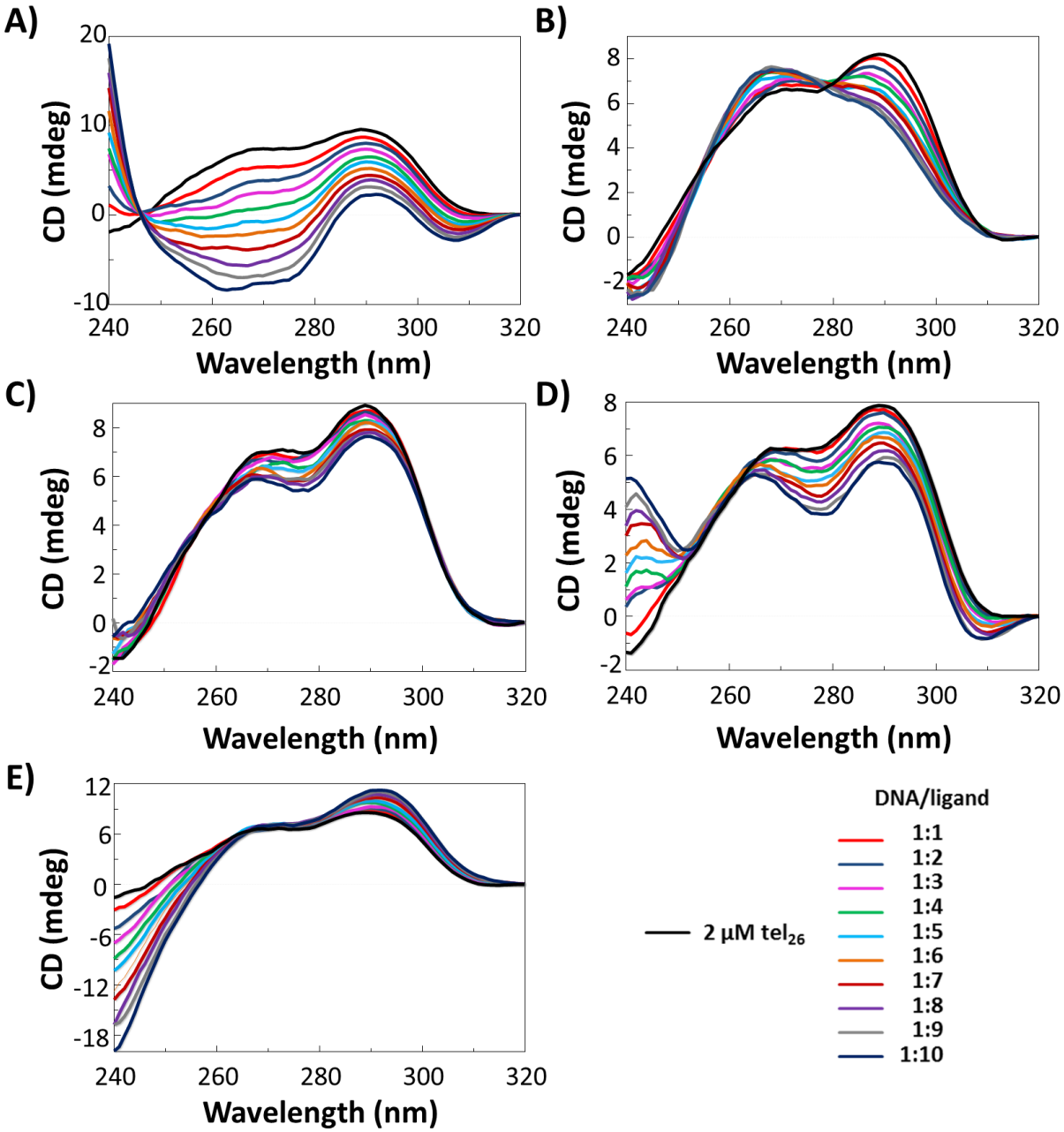


Figure S1. CD spectra of 2 μM solutions of tel_{26} G4 in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7) in the presence of increasing amounts (up to 10 equivalents) of (**A**) Bulbocapnine, (**B**) Chelidonine, (**C**) Ibogaine, (**D**) Rotenone and (**E**) Vomicine.

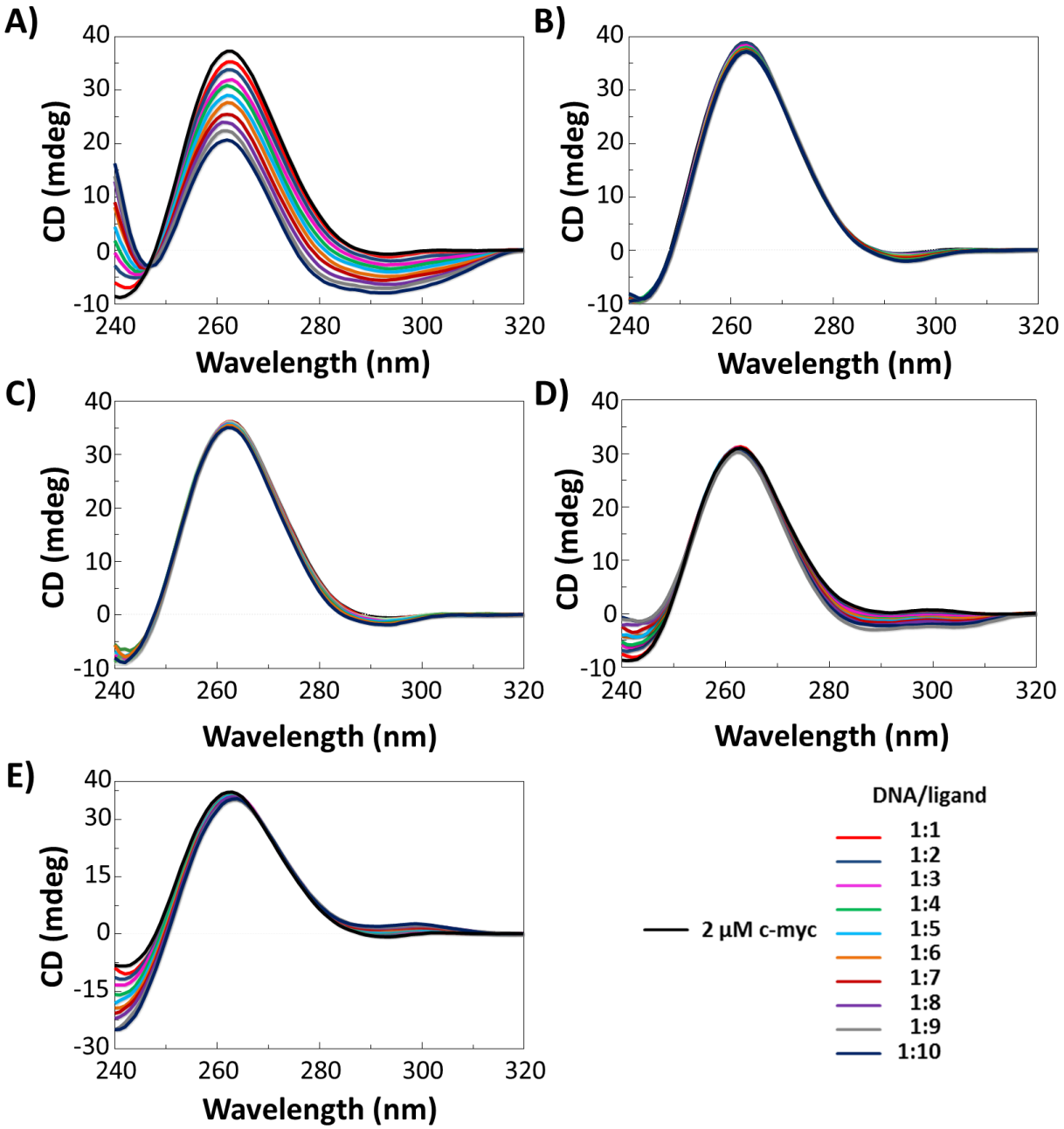


Figure S2. CD spectra of 2 µM solutions of c-myc G4 in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7) in the presence of increasing amounts (up to 10 equivalents) of (**A**) Bulbocapnine, (**B**) Chelidonine, (**C**) Ibogaine, (**D**) Rotenone and (**E**) Vomicine.

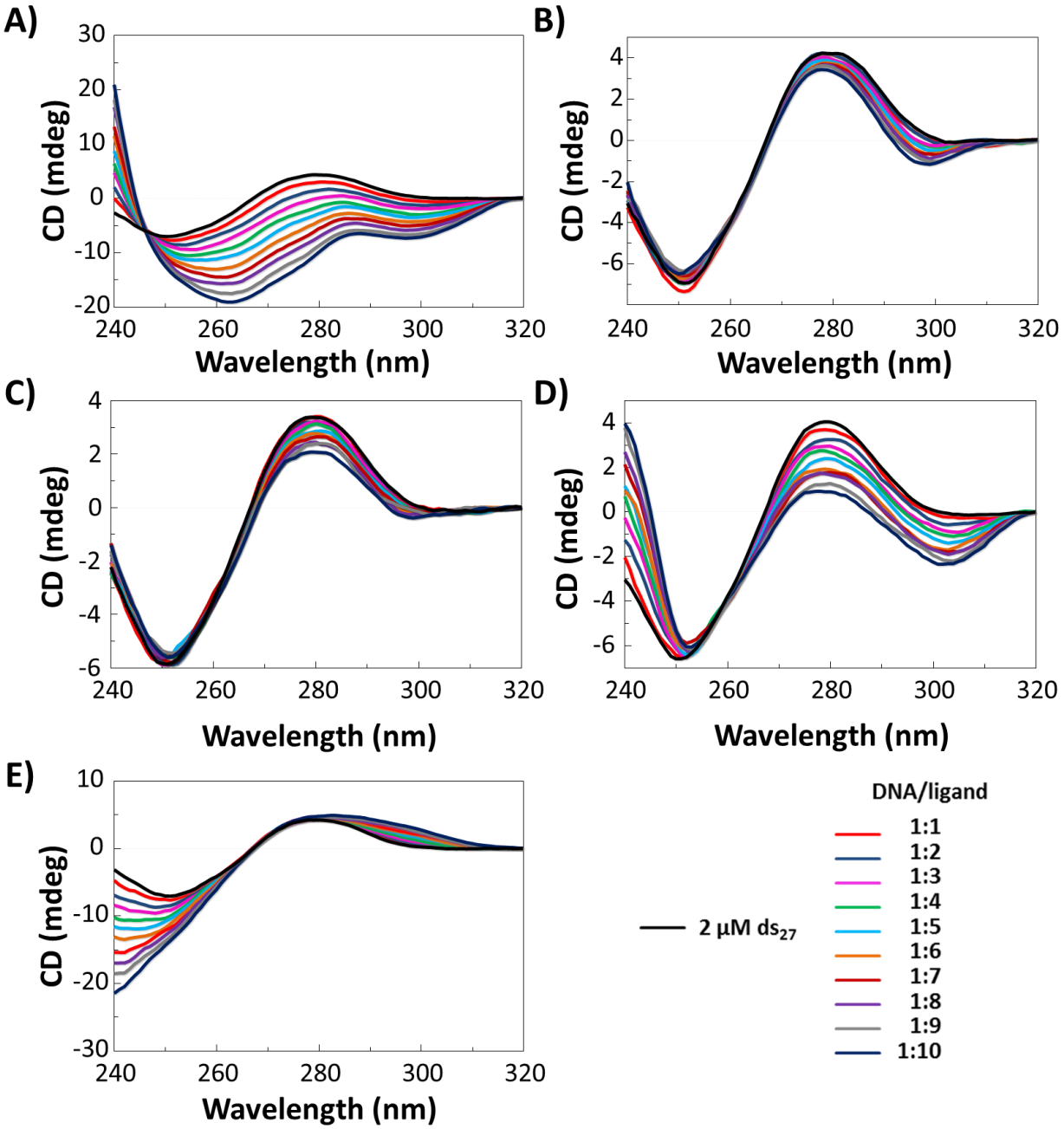


Figure S3. CD spectra of 2 μM solutions of ds_{27} duplex in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7) in the presence of increasing amounts (up to 10 equivalents) of (**A**) Bulbocapnine, (**B**) Chelidonine, (**C**) Ibogaine, (**D**) Rotenone and (**E**) Vomicine.

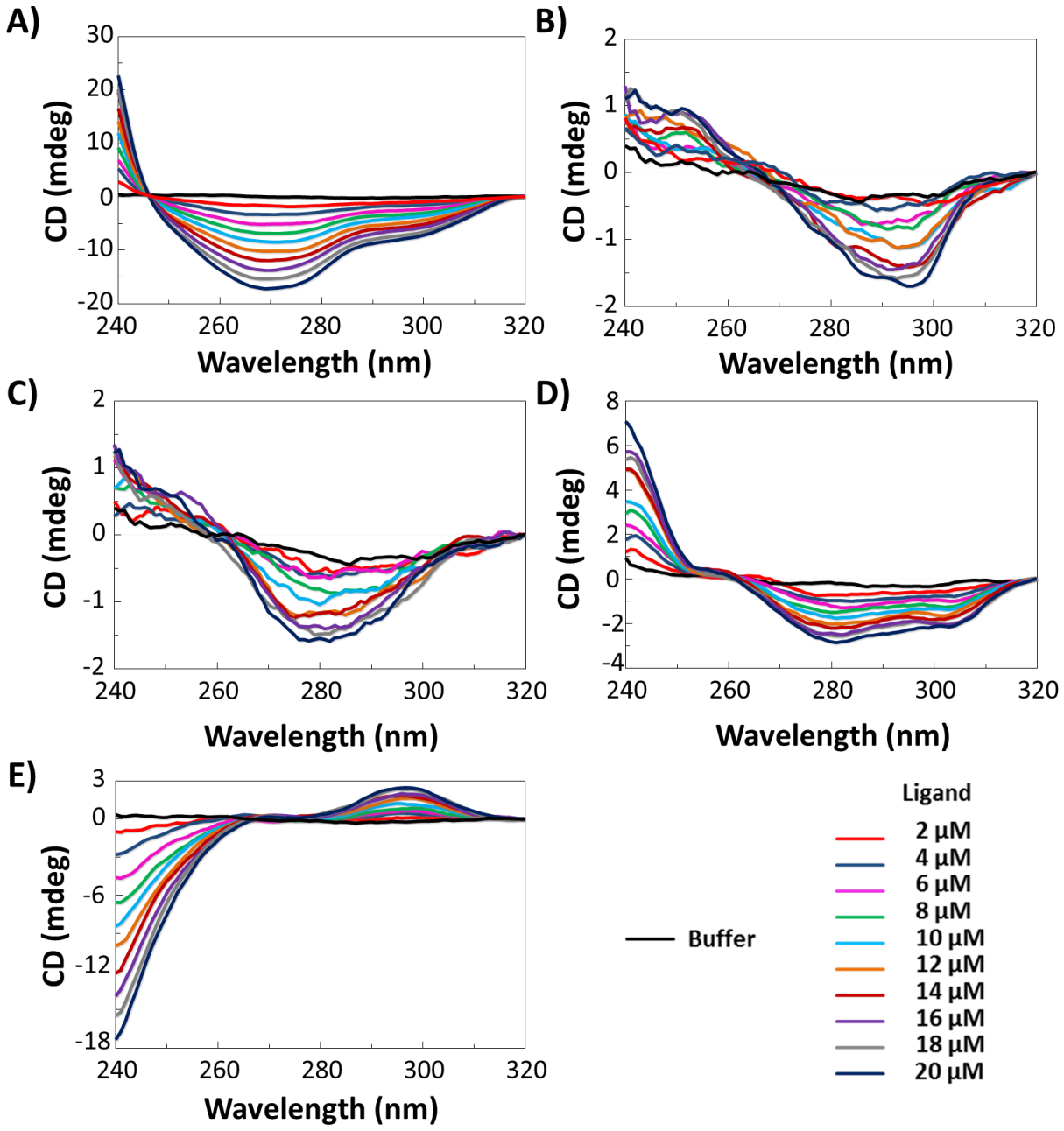


Figure S4. CD spectra of solutions (from 2 to 20 µM) of (**A**) Bulbocapnine, (**B**) Chelidonine, (**C**) Ibogaine, (**D**) Rotenone and (**E**) Vomicine in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7).

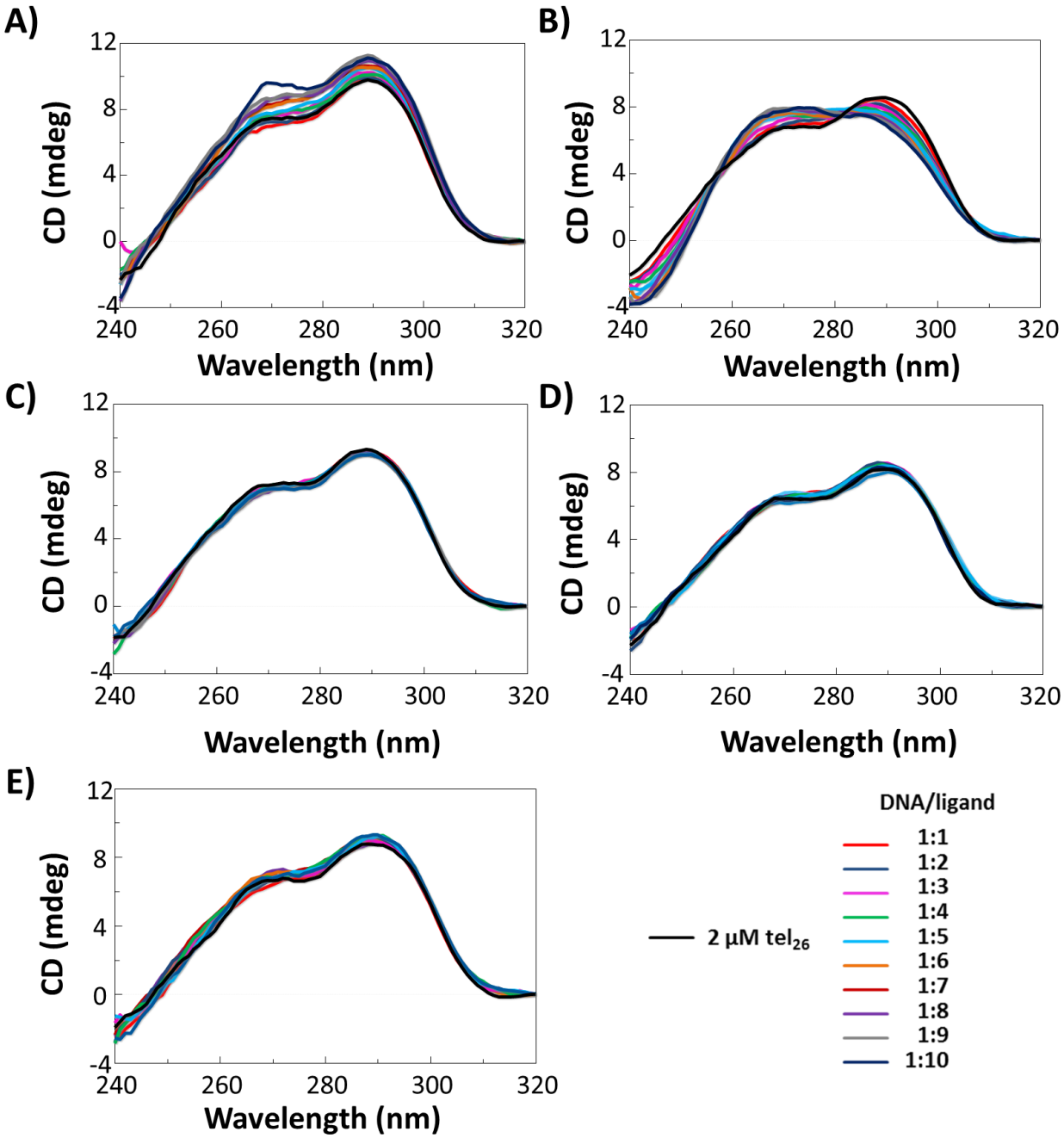


Figure S5. CD spectra (after ligand contribution subtraction) of 2 μM solutions of tel_{26} G4 in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7) with increasing amounts (up to 10 molar equivalents) of (**A**) Bulbocapnine, (**B**) Chelidonine, (**C**) Ibogaine, (**D**) Rotenone and (**E**) Vomicine.

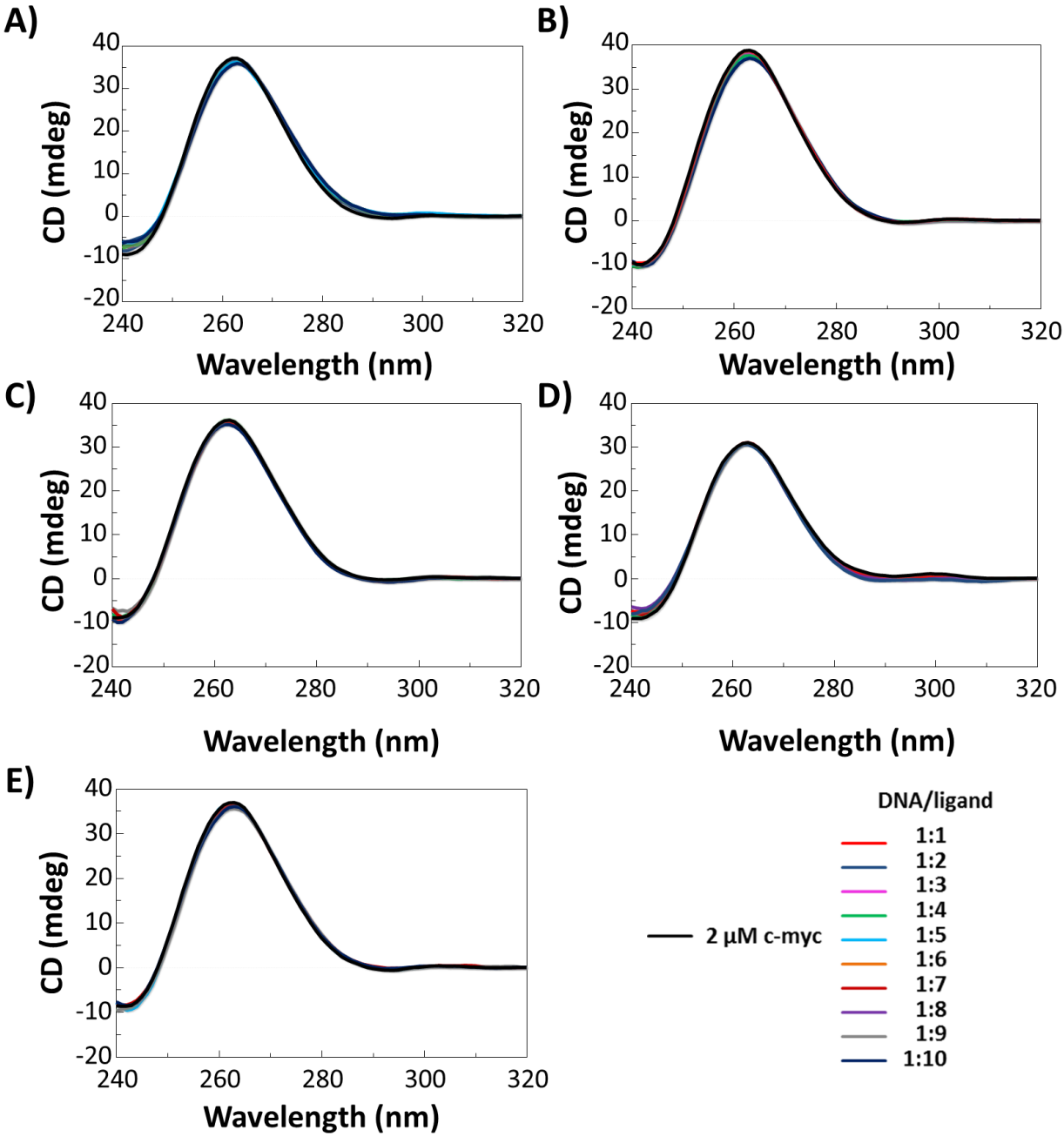


Figure S6. CD spectra (after ligand contribution subtraction) of 2 µM solutions of c-myc G4 in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7) with increasing amounts (up to 10 molar equivalents) of (**A**) Bulbocapnine, (**B**) Chelidonine, (**C**) Ibogaine, (**D**) Rotenone and (**E**) Vomicine.

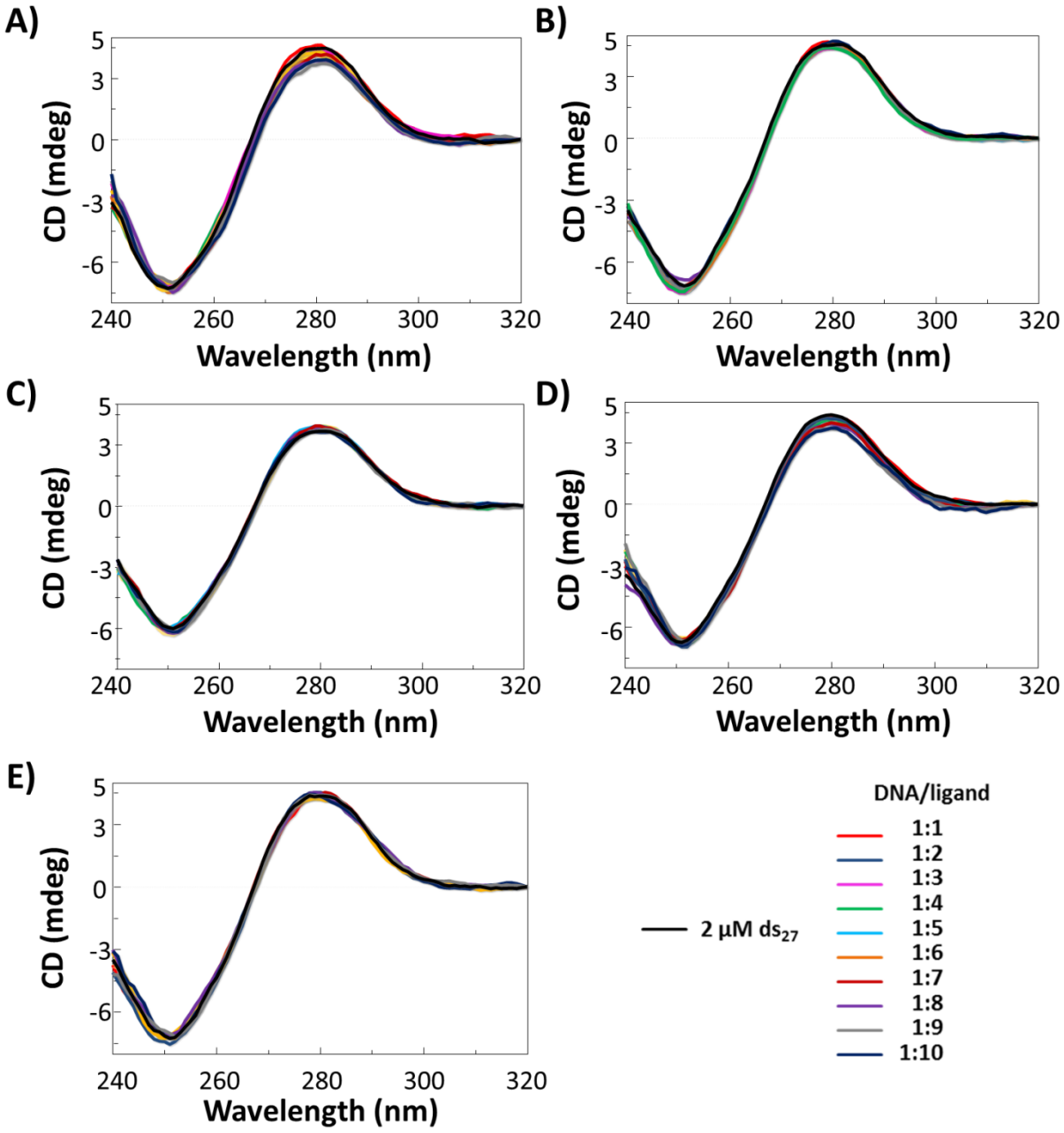


Figure S7. CD spectra (after ligand contribution subtraction) of 2 μM solutions of ds_{27} duplex in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7) with increasing amounts (up to 10 molar equivalents) of (**A**) Bulbocapnine, (**B**) Chelidonine, (**C**) Ibogaine, (**D**) Rotenone and (**E**) Vomicine.

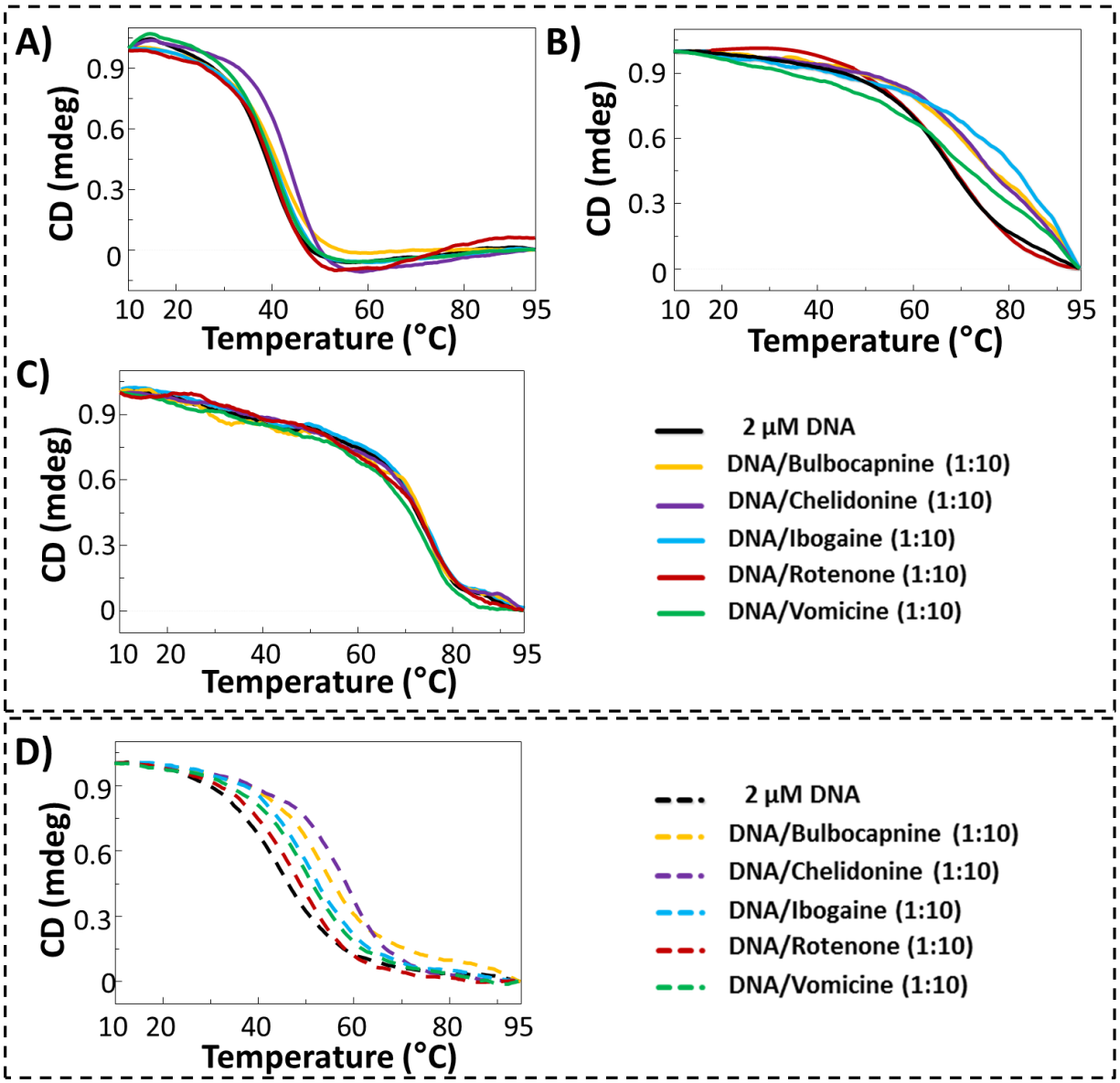


Figure S8. CD-melting curves (solid lines) for (**A**) tel$_{26}$/ligand, (**B**) c-myc/ligand and (**C**) ds$_{27}$/ligand mixtures (1:10) in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7), recorded at 290, 262 and 251 nm, respectively, and CD-melting curves (dashed lines) for (**D**) c-myc/ligand mixtures (1:10) in 0.5 mM KCl, 0.5 mM phosphate buffer, 5% DMSO (pH 7), recorded at 262 nm.

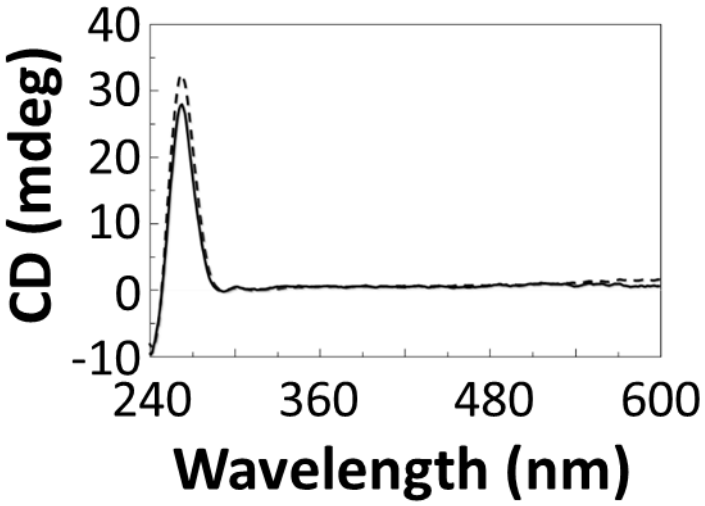


Figure S9. CD spectra for 2 μM solutions of c-myc G4 in 5 mM KCl, 5 mM phosphate buffer, 5% DMSO (pH 7) (dashed line) or in 0.5 mM KCl, 0.5 mM phosphate buffer, 5% DMSO (pH 7) (solid line).

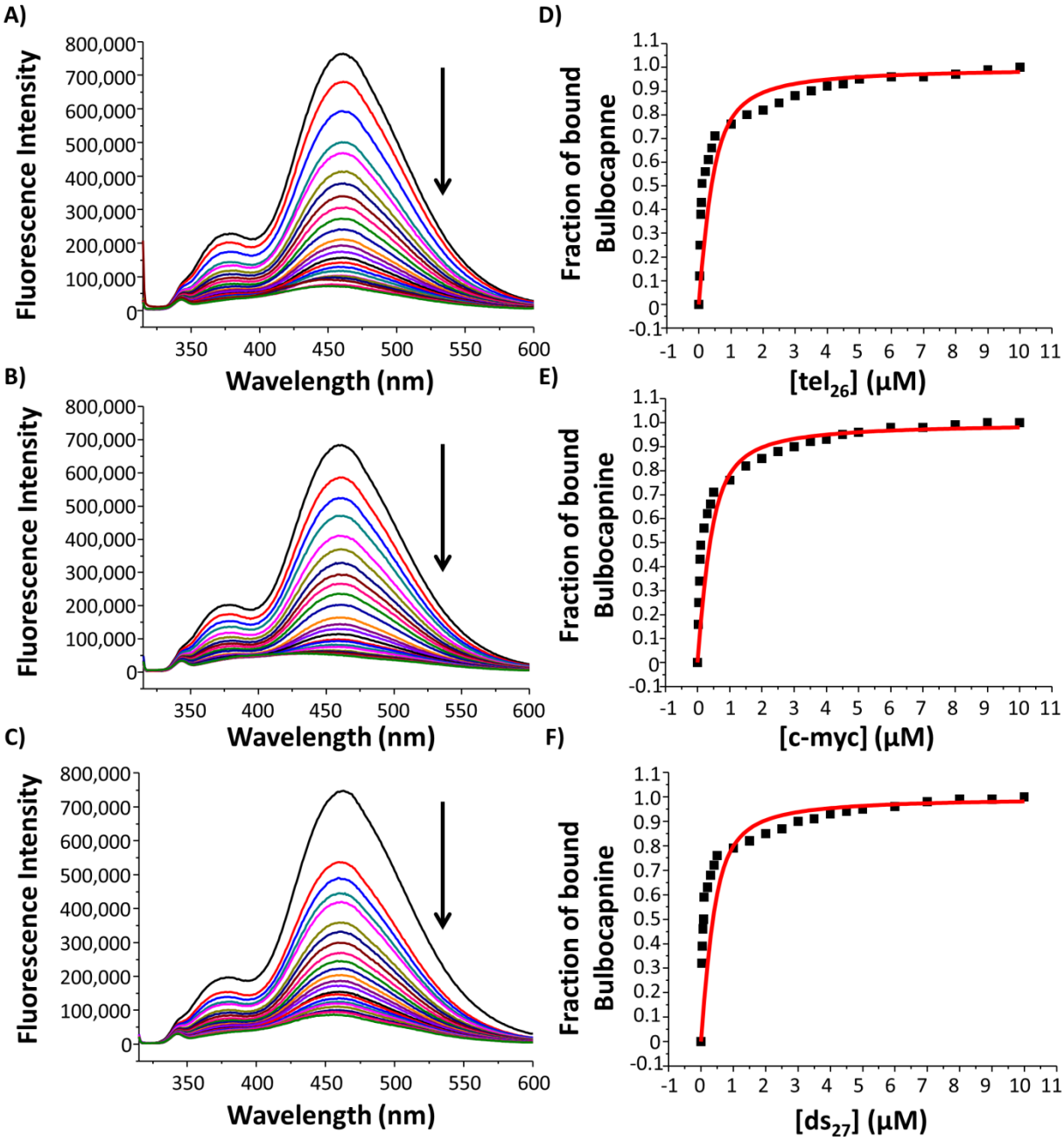


Figure S10. Left: Fluorescence emission spectra obtained by adding increasing amounts of (**A**) tel$_{26}$ G4, (**B**) c-myc G4 and (**C**) ds$_{27}$ duplex to 2 μM solutions of Bulbocapnine. Arrows indicate the variation of fluorescence intensity on increasing DNA concentration. Right: Representative binding curves obtained by plotting the fraction of bound Bulbocapnine to (**D**) tel$_{26}$ G4, (**E**) c-myc G4 and (**F**) ds$_{27}$ duplex as a function of the DNA concentration. The black squares represent the experimental data; the red lines represent the best fit obtained using an independent and equivalent-sites model.

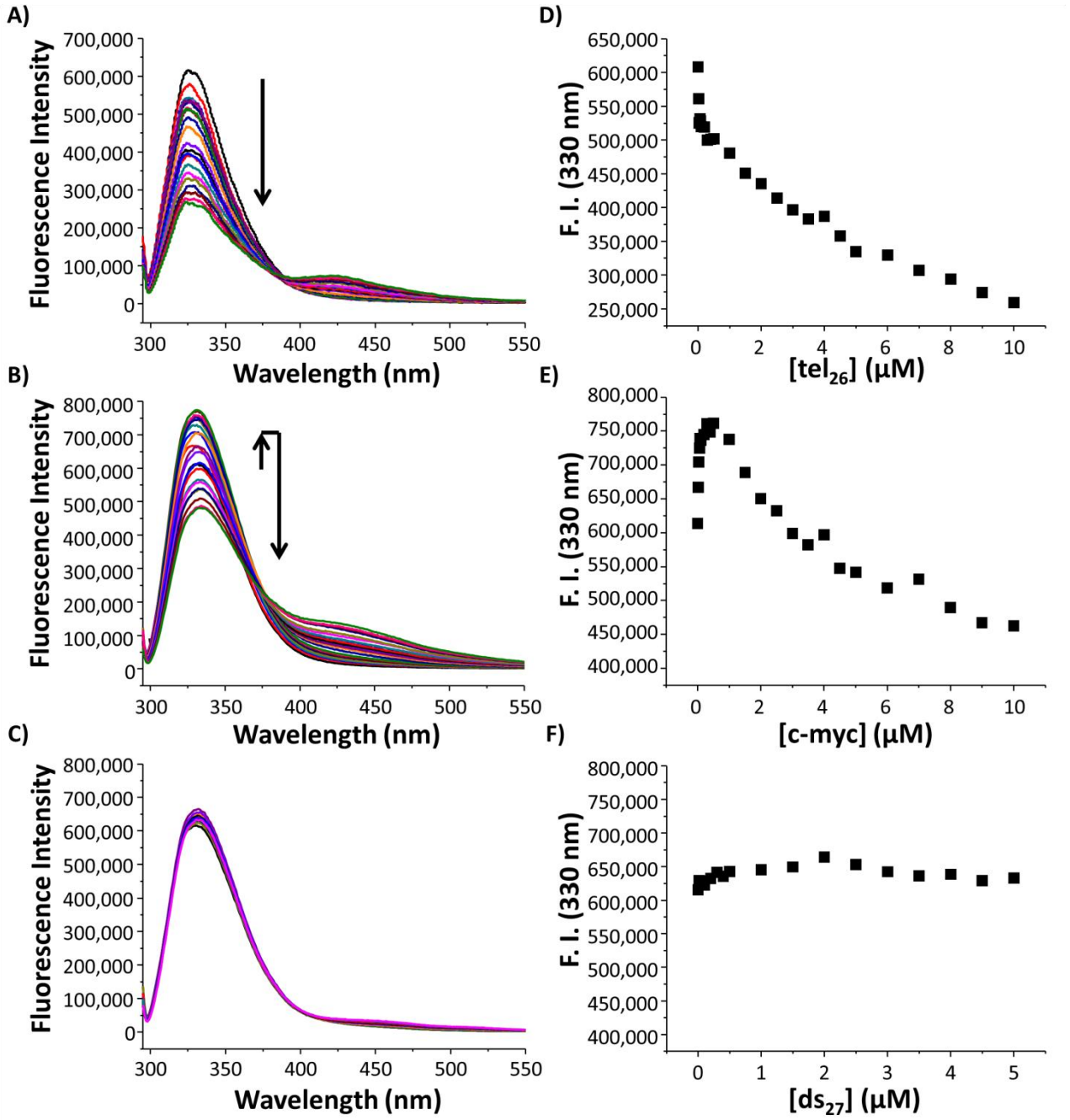


Figure S11. Left: Fluorescence emission spectra obtained by adding increasing amounts of (**A**) tel26 G4, (**B**) c-myc G4 and (**C**) ds27 duplex to 2 μM solutions of Chelidonine. Arrows indicate the variation of fluorescence intensity on increasing DNA concentration. Right: Fluorescence intensity at 330 nm vs. concentration of (**D**) tel26 G4, (**E**) c-myc G4 and (**F**) ds27 duplex.

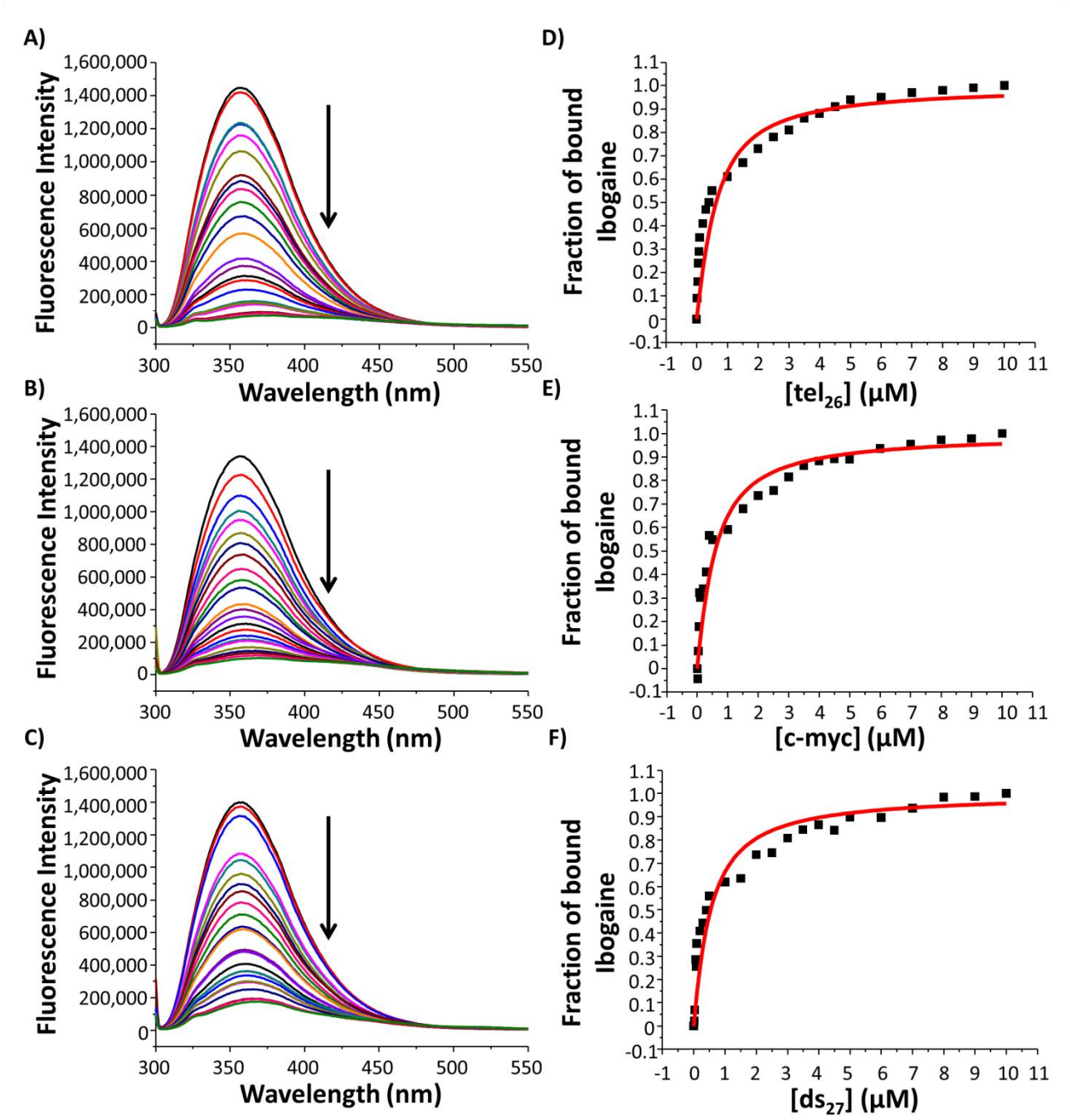


Figure S12. Left: Fluorescence emission spectra obtained by adding increasing amounts of (**A**) tel$_{26}$ G4, (**B**) c-myc G4 and (**C**) ds$_{27}$ duplex to 2 μM solutions of Ibogaine. Arrows indicate the variation of fluorescence intensity on increasing DNA concentration. Right: Representative binding curves obtained by plotting the fraction of bound Ibogaine to (**D**) tel$_{26}$ G4, (**E**) c-myc G4 and (**F**) ds$_{27}$ duplex as a function of the DNA concentration. The black squares represent the experimental data; the red lines represent the best fit obtained using an independent and equivalent-sites model.

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