Electronic Supplementary Information (ESI)

Ni(II)/Cu(II)/Zn(II) 5,5-diethylbarbiturate complexes with 1,10-phenantroline and 2,2'-dipyridylamine: Synthesis, structures, DNA/BSA binding, nuclease activity, molecular docking, cellular uptake, cytotoxicity and the mode of cell death

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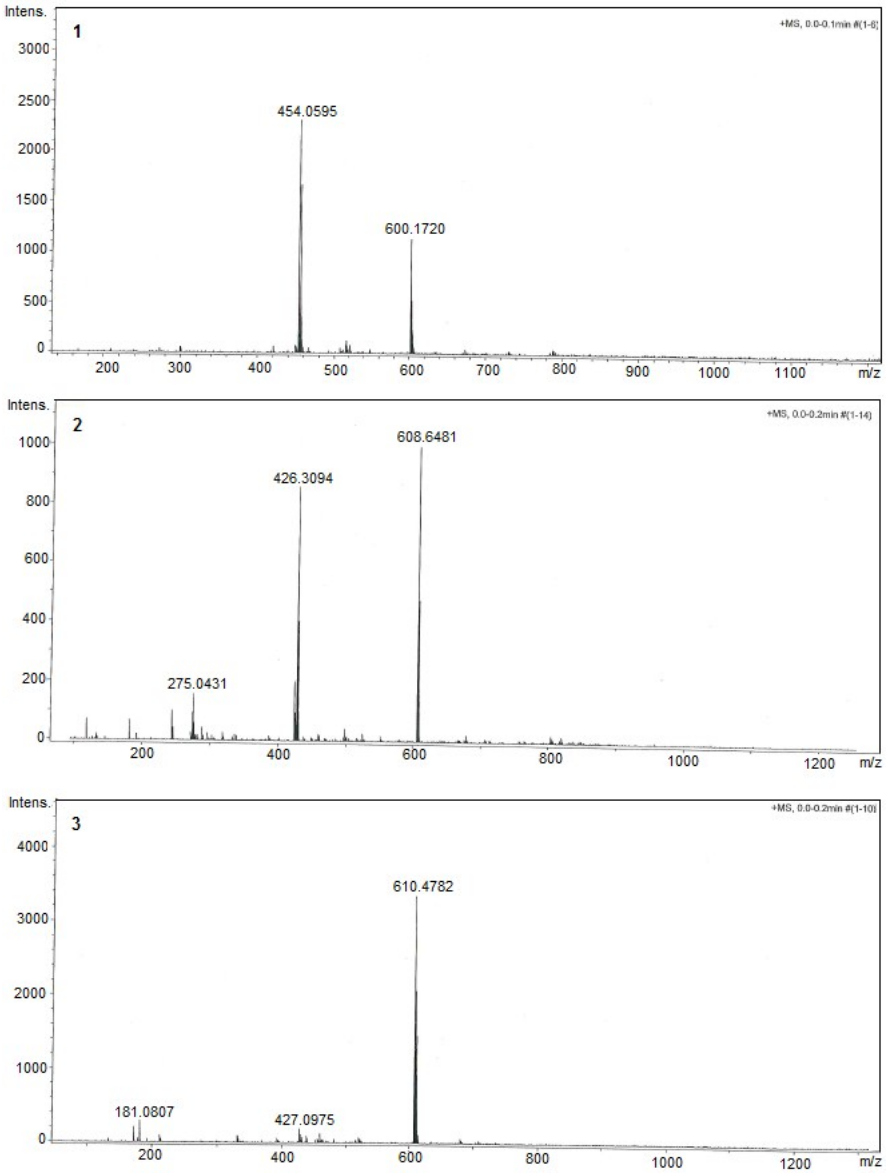


Fig. S1 (Continued)

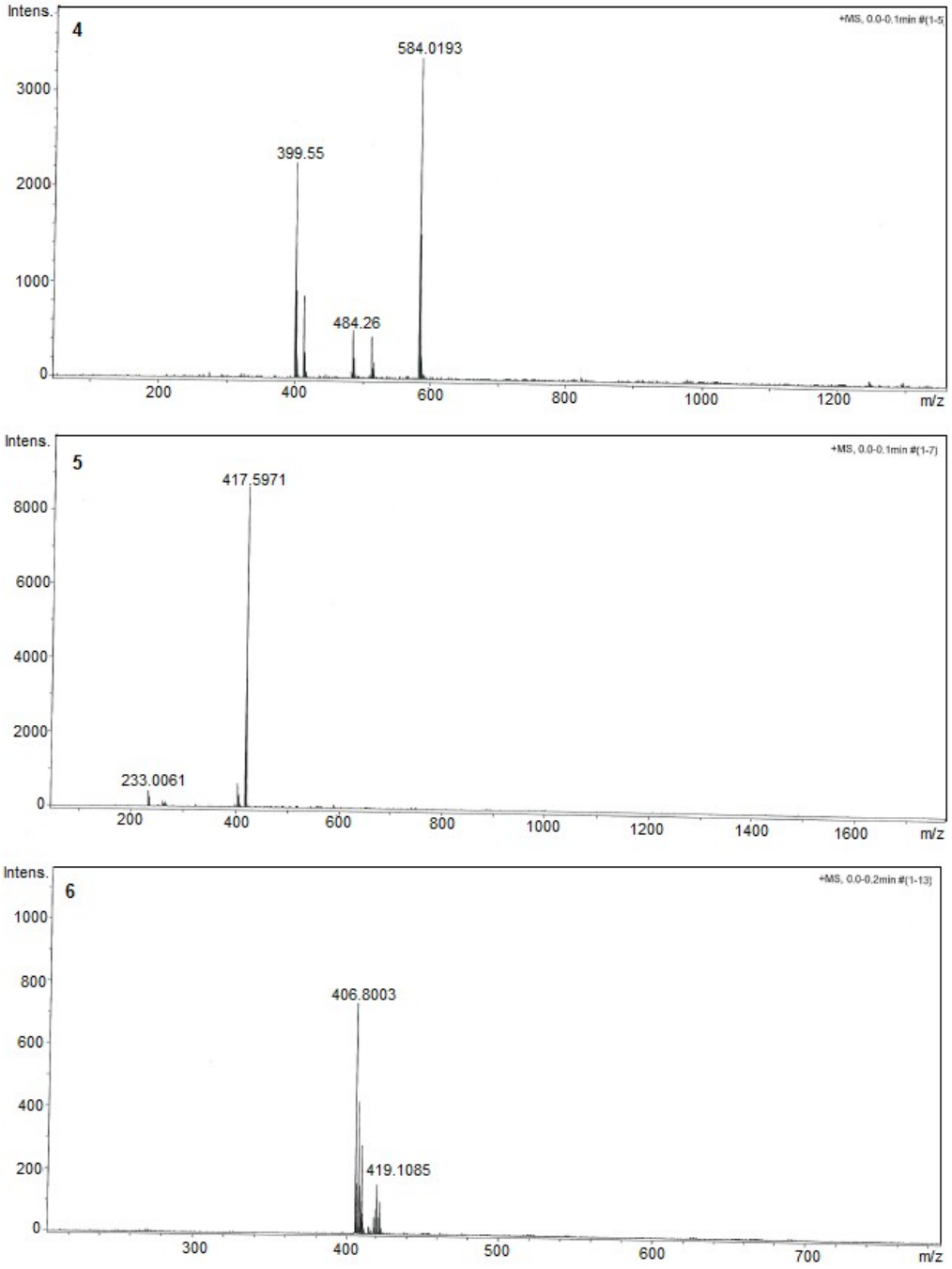


Fig. S1 ESI-MS spectra of **1–6**

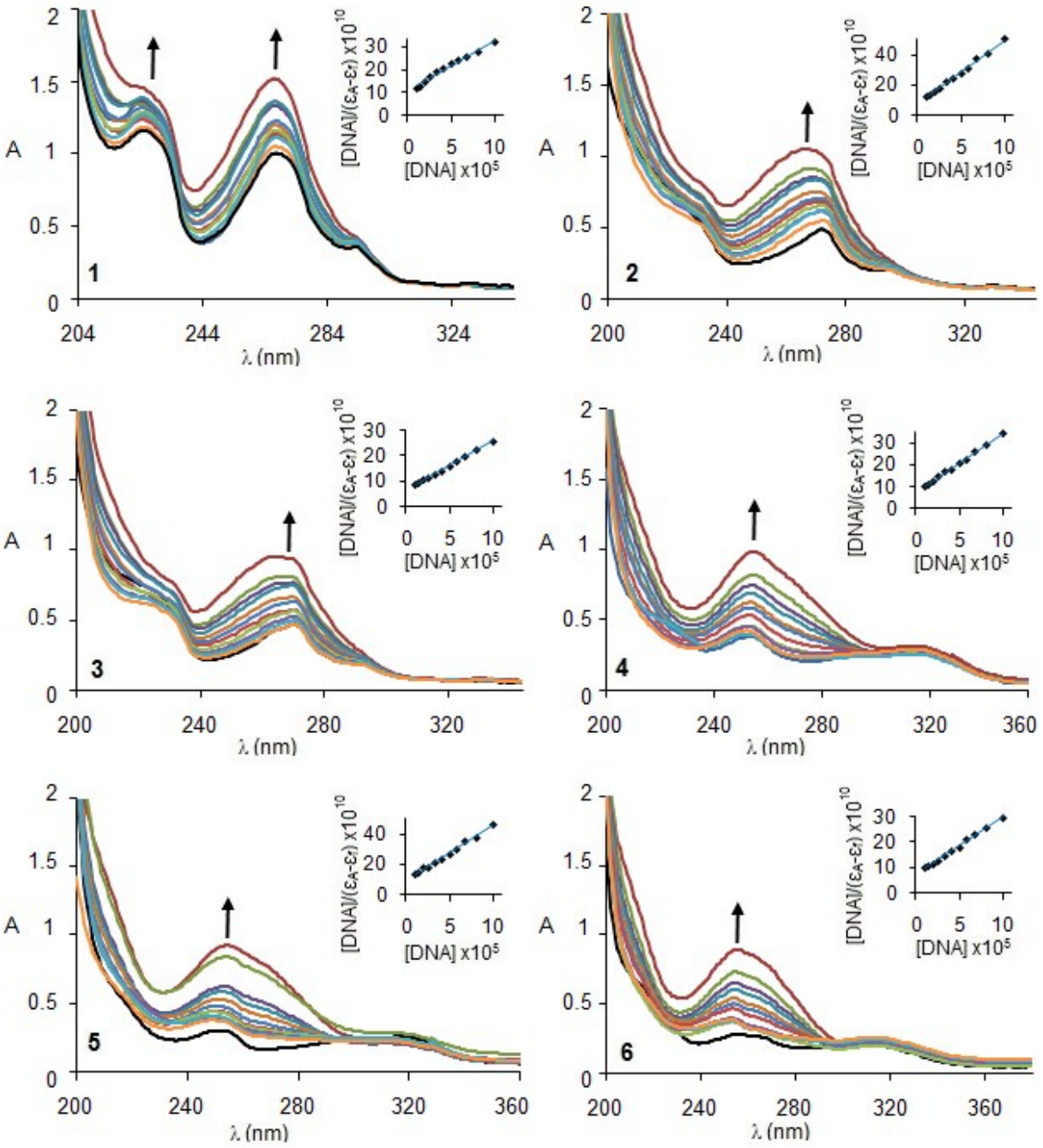


Fig. S2 Absorption spectra of **1**–**6** (10 μM) upon the titration of FS-DNA (0–10 μM) in Tris-HCl buffer. The arrow shows the increases in absorbance with respect to an increase in the FS-DNA concentration. Inset: Plot of [DNA]/(εa − εf) vs. [DNA].

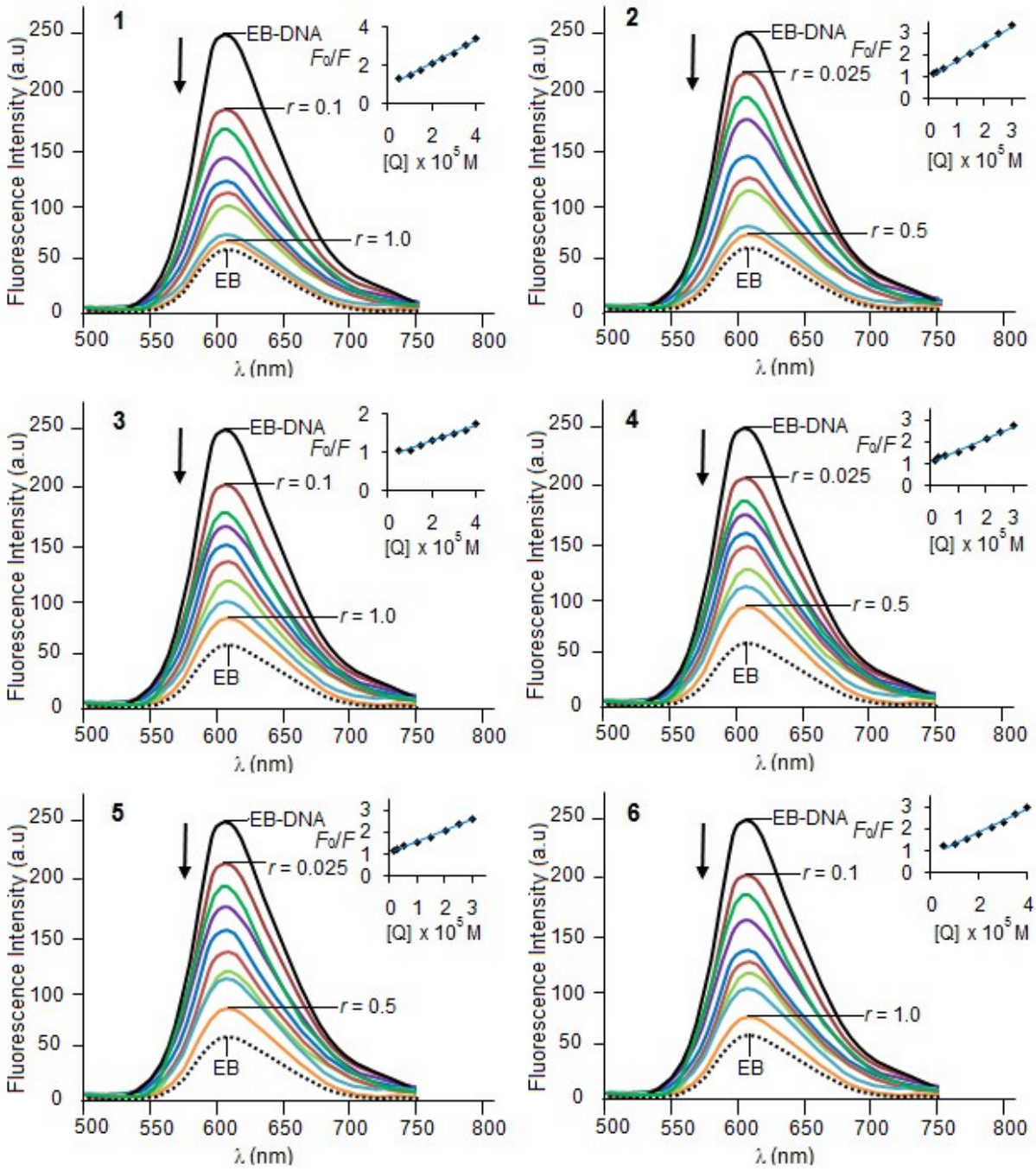


Fig. S3 Emission spectra of EB-bound DNA solutions upon the titration of **1**–**6** (1.25–50 μM) in Tris-HCl buffer. [EB] = 5.0 μM, [DNA] = 50.0 μM. *r* = [complex]/[DNA]. The arrow shows the decreases in emission with increasing the concentration of the complexes. Insets: Stern-Volmer plot of the fluorescence data.

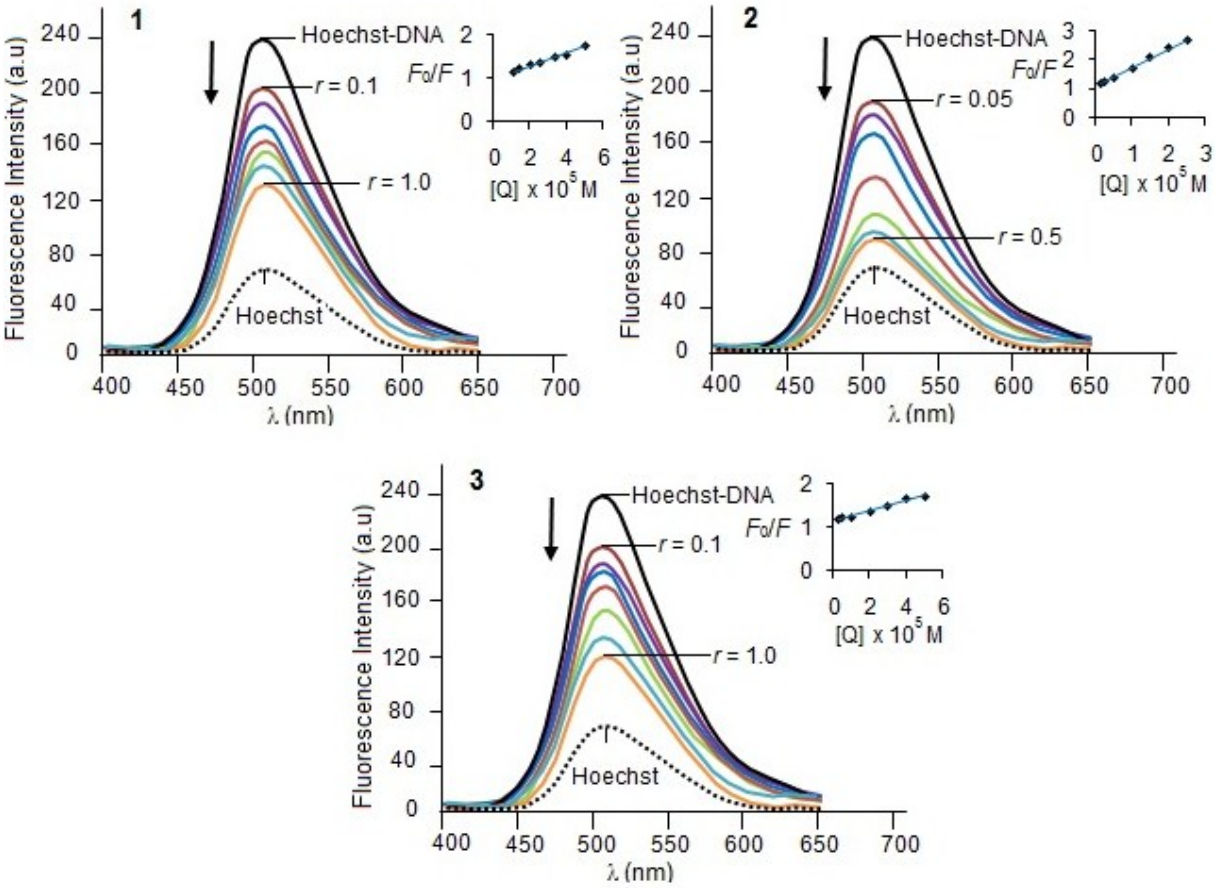


Fig. S4 Emission spectra of Hoechst 33258-bound DNA solutions upon the titration of **1**–**3** (2.5–50 µM) in Tris-HCl buffer. [[Hoechst 33258] = 5.0 µM, [DNA] = 50.0 µM. *r* = [complex]/[DNA]. The arrow shows the decreases in emission with increasing the concentration of the complexes. Insets: Stern-Volmer plot of the fluorescence data.

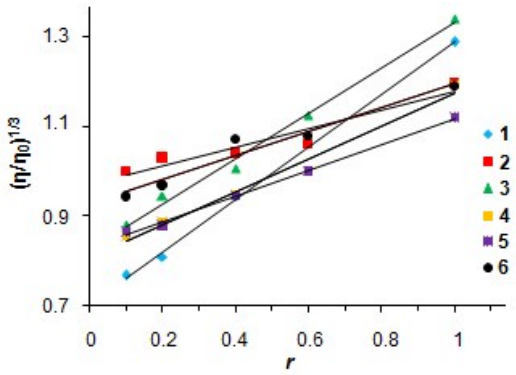


Fig. S5 The relative viscosity of FS-DNA upon addition of increasing amounts of complexes 1−6 in Tris-HCl buffer.

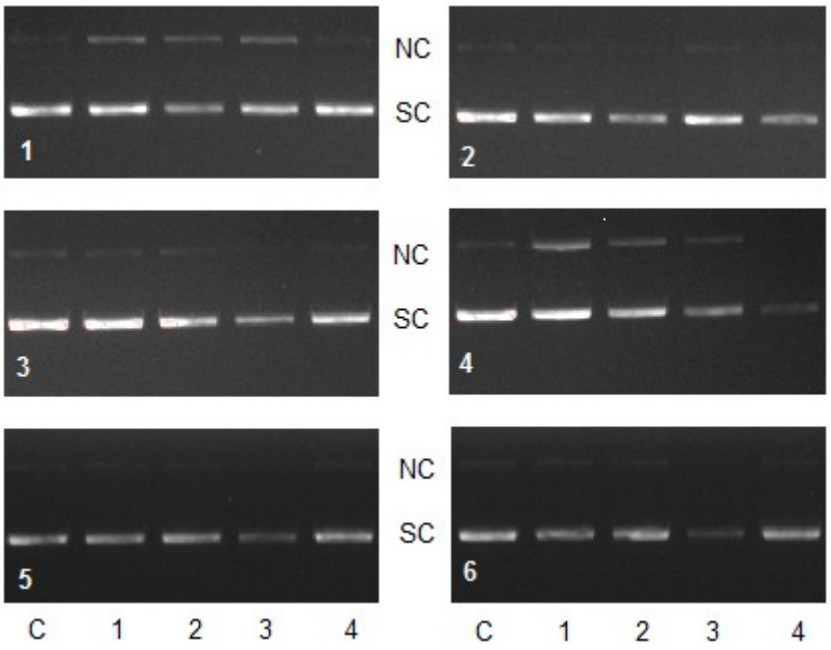


Fig. S6 Gel electrophoresis images of supercoiled 10 μM pUC19 and **1**–**6** (25-200 μM) in 5 mM Tris-HCl/50 mM NaCl buffer (pH 7.2) at 37 °C for 1 h with increasing concentrations of **1**–**6**. Lane C: pure plasmid DNA; lanes 1-4: DNA + complexes at 25, 50, 100 and 200 μM.

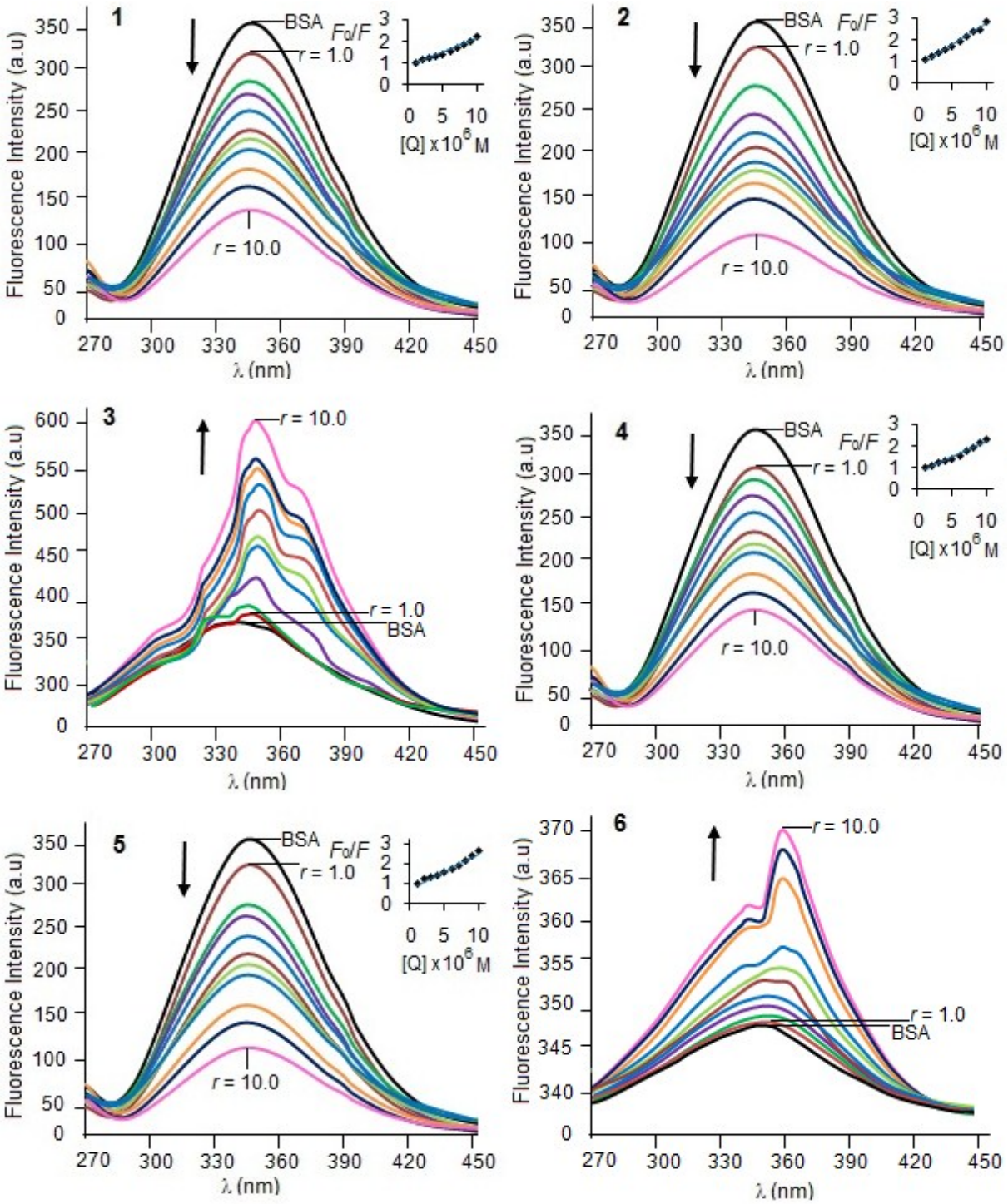
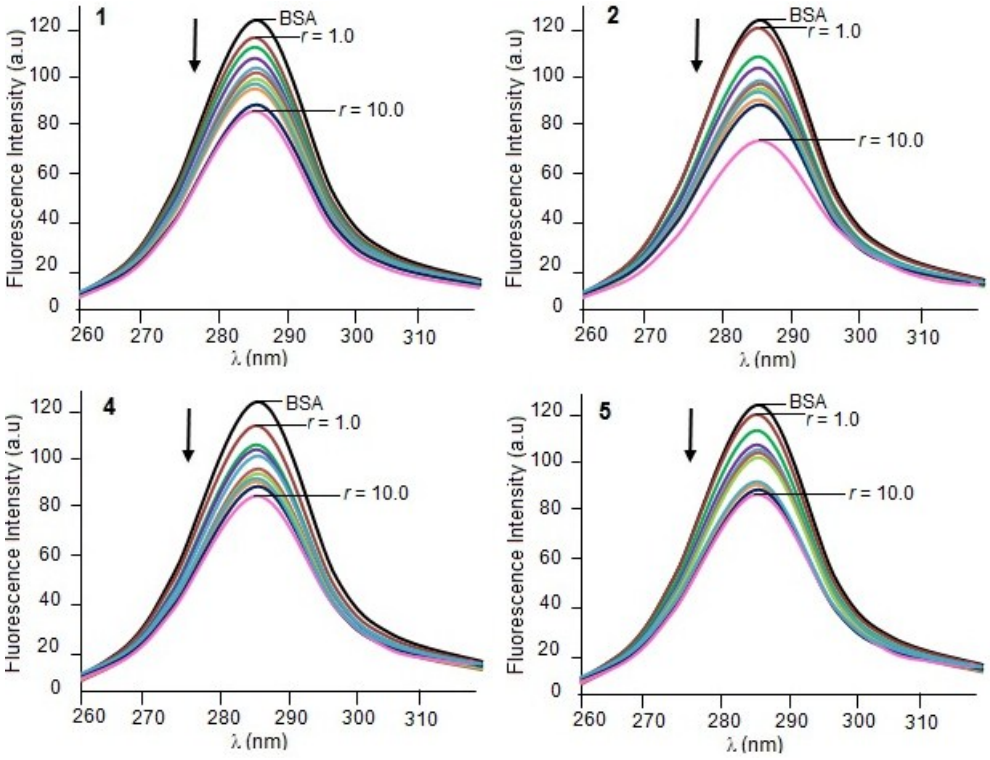
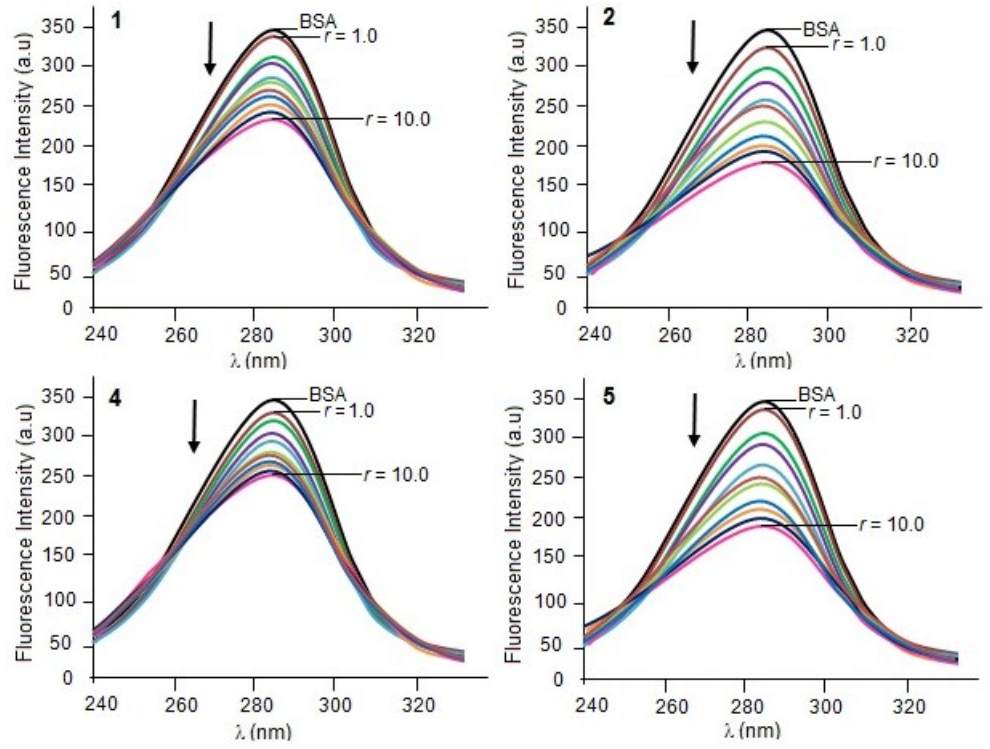


Fig. S7 Emission spectra of BSA (1.0 μM; λex = 280 nm) in presence of **1**–**6** (0-10.0 μM). The arrow shows the emission intensity changes upon increasing complex concentration. Insets: Stern-Volmer plot of the fluorescence data.



(a)



(b)

Fig. S8 Synchronous spectra of BSA (1.0 μM) in presence of the complexes (0-10.0 μM) at Δλ = 15 nm (**a**) and Δλ = 60 nm (**b**). The arrow shows the emission intensity changes upon increasing complex concentration.

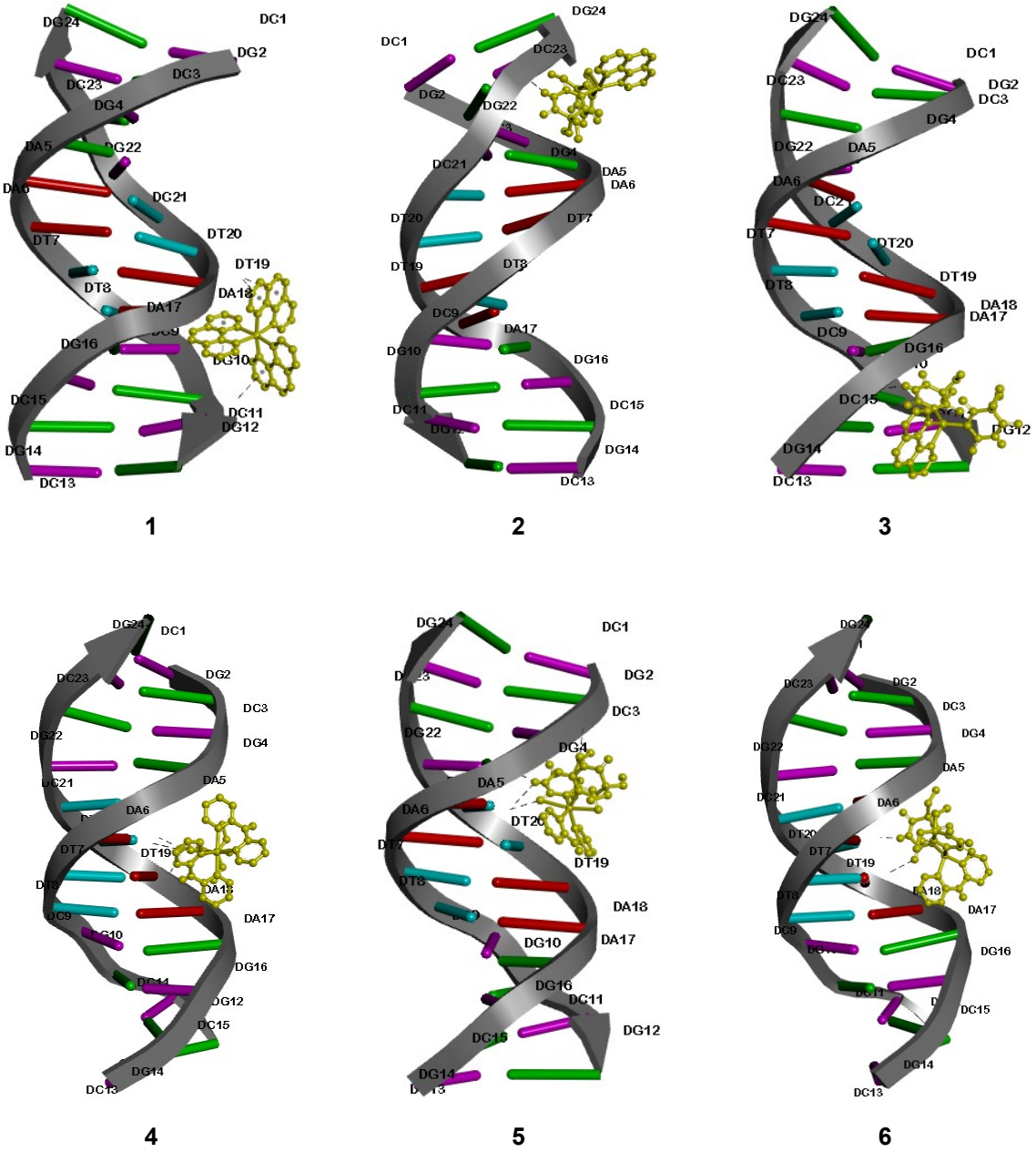


Fig. S9 Molecular docking of **1**–**6** with DNA.

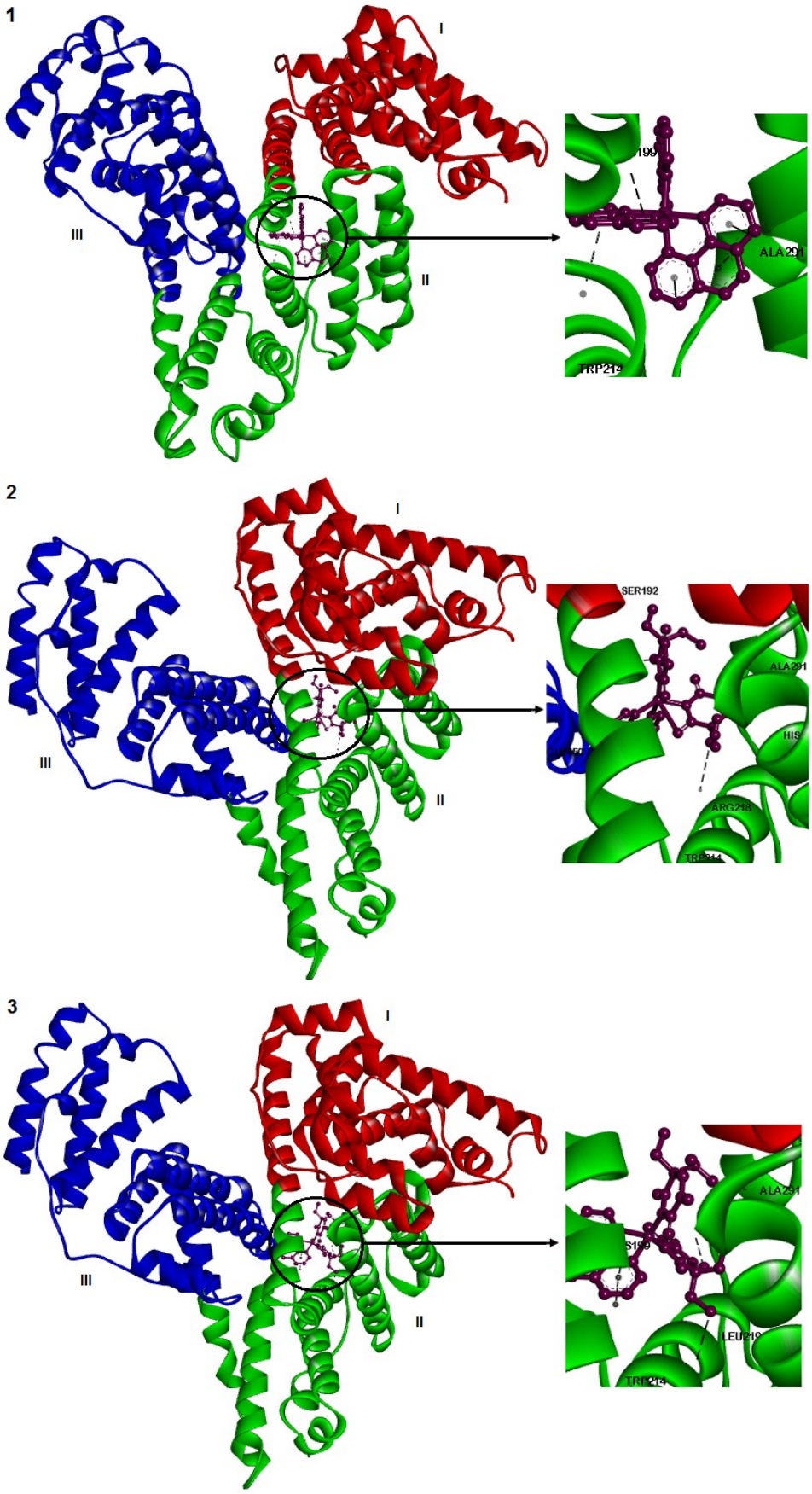


Fig. S10 (continued)

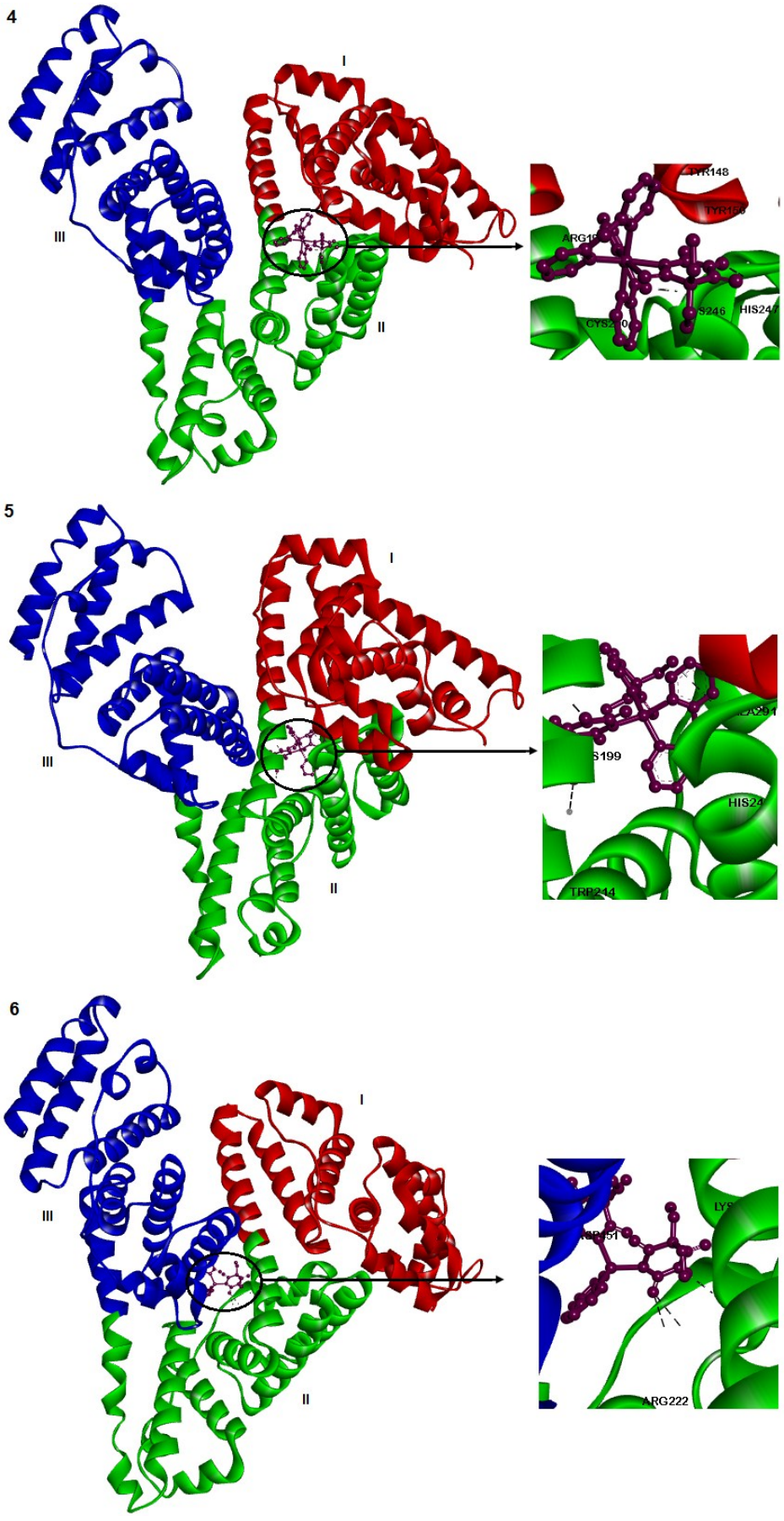


Fig. S10 Molecular docking of **1**–**6** with HSA.

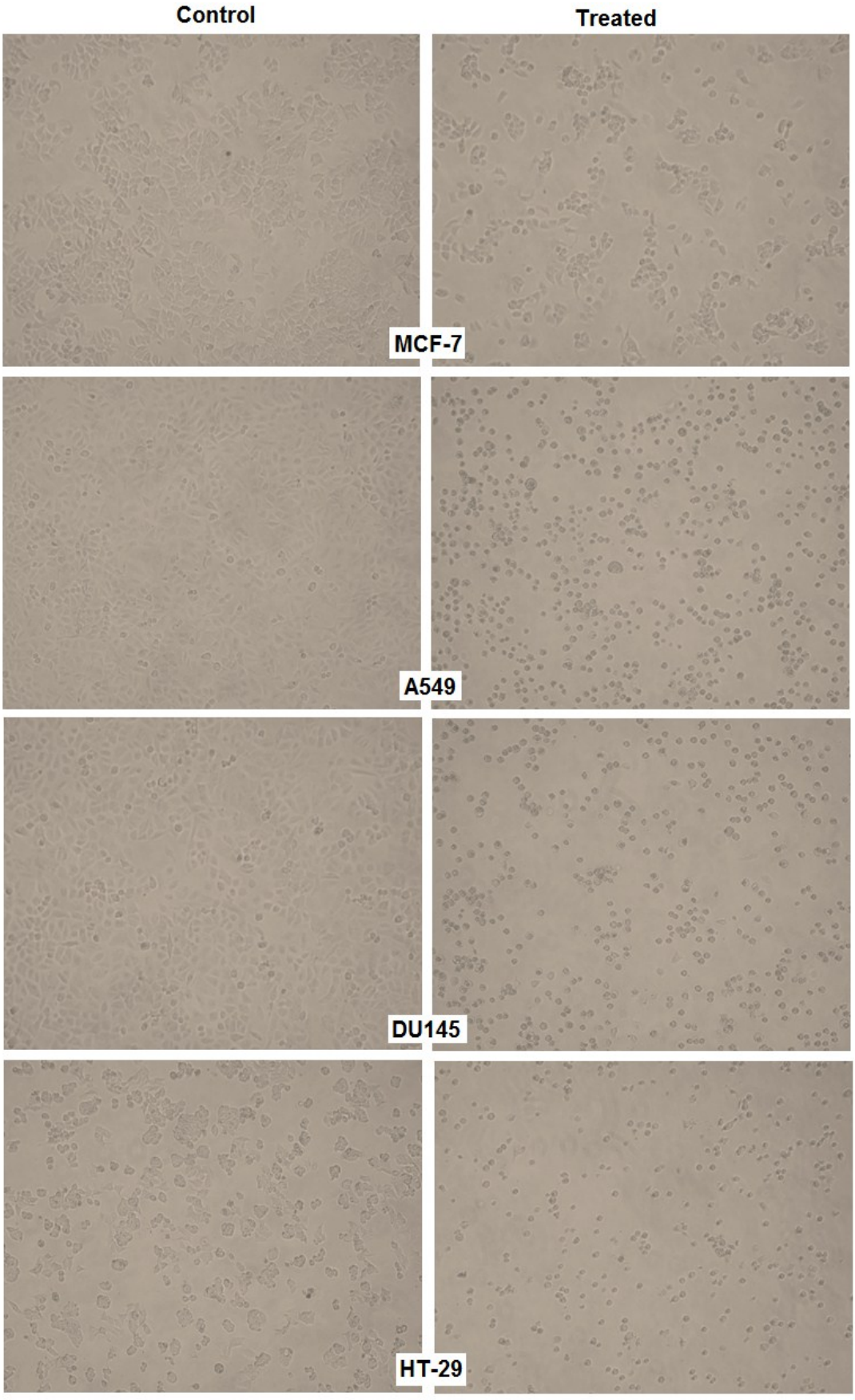


Fig. S11 Phase contrast microscopy images of the cancer cells treated with **2** (10 µM) for 24 h.

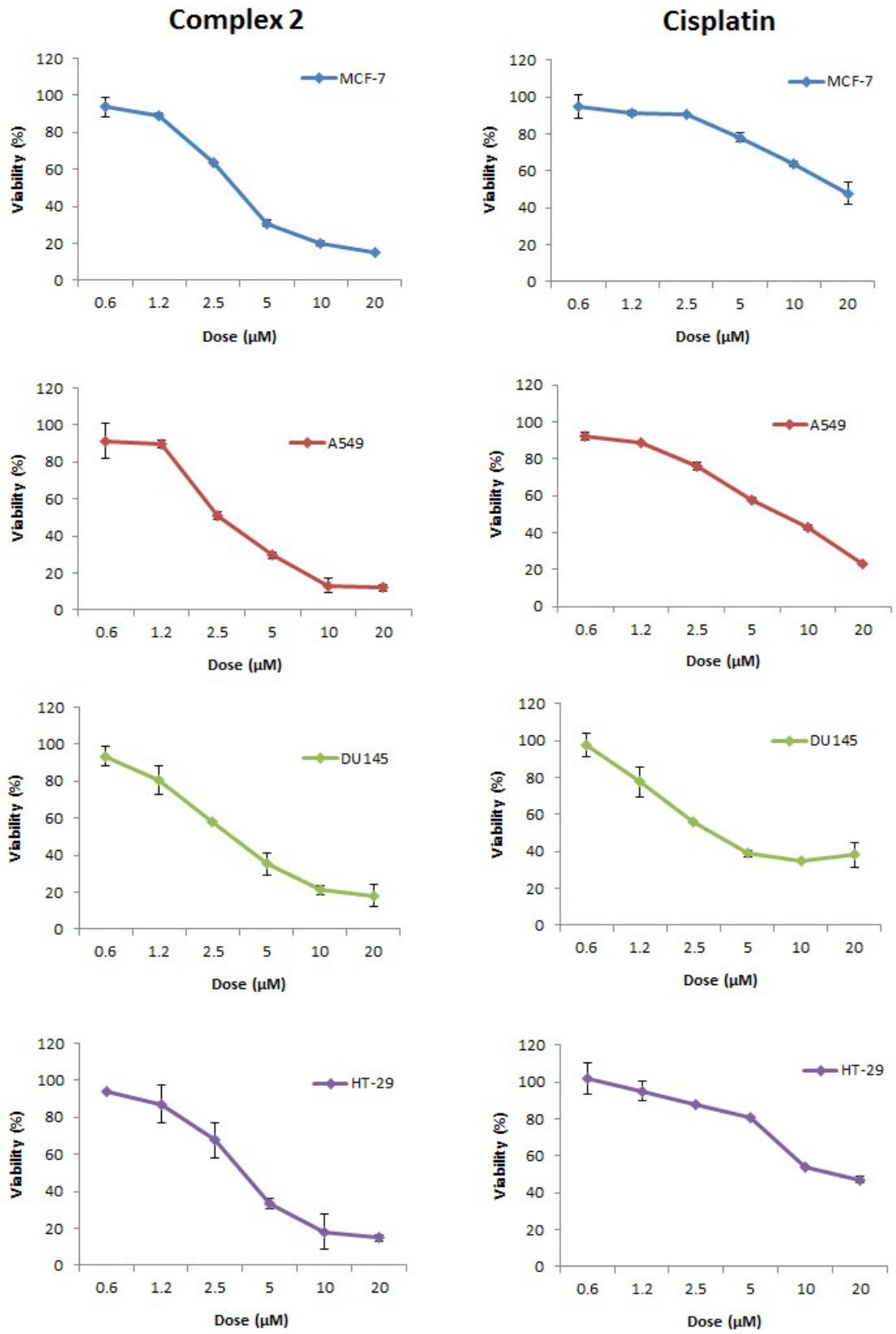


Fig. S12 The dose–response graphics for **2**, showing the effect of the complex on the growth of the cell lines after 48 h of treatment.

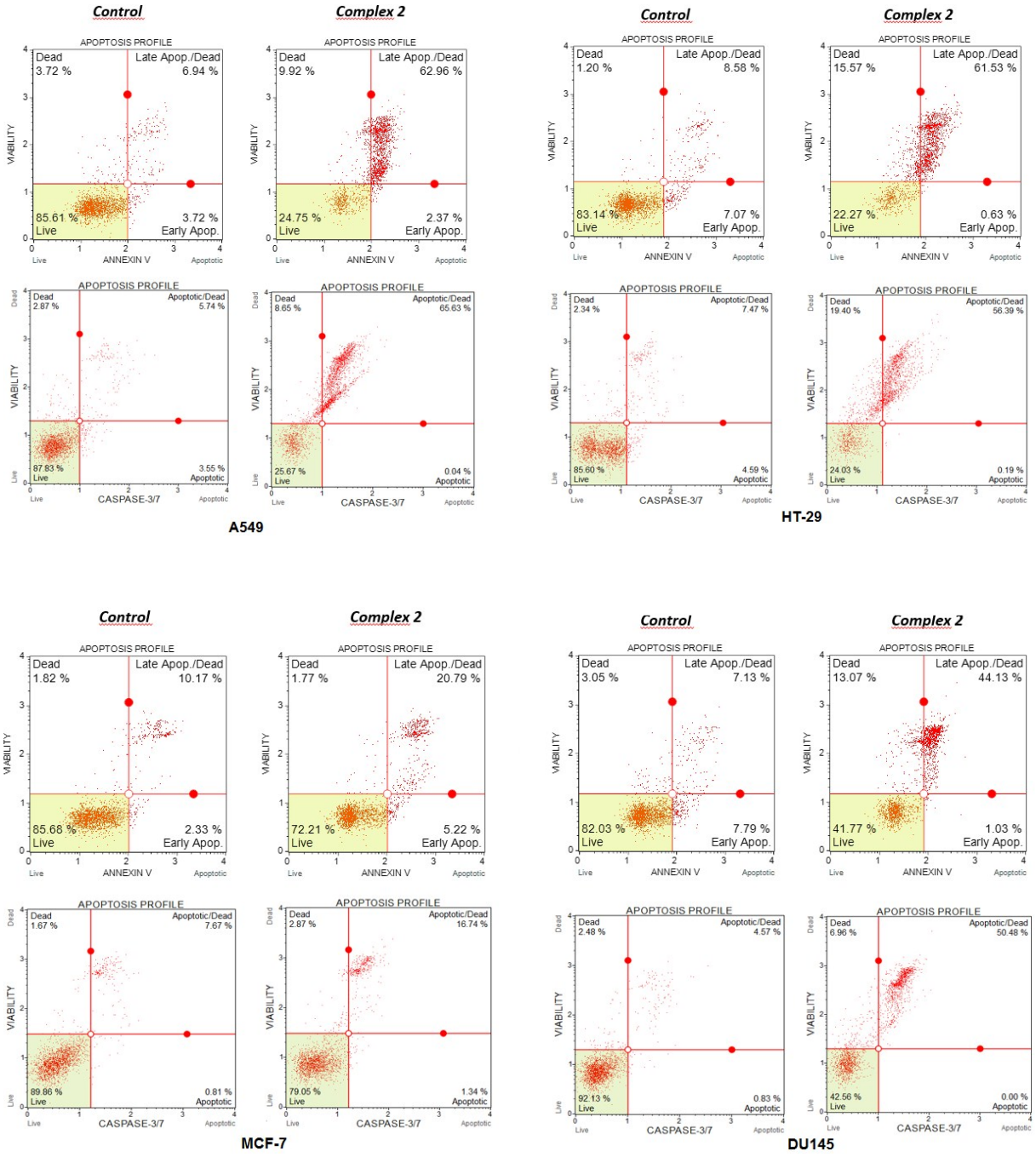


Fig. S13 Flow cytometric analysis of A549, HT-29, MCF-7 and DU145 cells treated with 2 (10 µM) for 24 h, using Annexin V-FITC/PI and caspase 3/7 staining. Percentages of cells in each quadrant are given.

Table S1 Temperature dependent fluorescence emission titration data for the interaction of **1**-**6** with FS-DNA.

Complexes	T (K)	K_{SV} (M^{-1}) x 10^{-4}	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/K x mol)
1	293	6.16	–27.3	+6.8	+116.4
	297	6.01	–27.8		
	300	5.86	–28.1		
2	293	7.55	–28.1	+5.0	+112.8
	297	7.43	–28.5		
	300	7.32	–28.8		
3	293	2.56	–26.4	+8.9	+120.6
	297	2.39	–26.9		
	300	2.18	–27.3		
4	293	5.25	–27.4	+7.1	+117.9
	297	5.13	–27.9		
	300	5.02	–28.3		
5	293	5.96	–27.9	+4.7	+111.1
	297	5.82	–28.3		
	300	5.71	–28.6		
6	293	2.75	–26.1	+6.3	+110.6
	297	2.64	–26.5		
	300	2.50	–26.9		

Table S2 Temperature dependent fluorescence emission titration data for the interaction of **1**-**6** with BSA.

Complexes	T (K)	K_{SV} (M^{-1}) x 10^{-4}	K_F (M^{-1}) x 10^{-5}	*n*	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/K x mol)
1	293	1.44	1.66	1.04	–29.2	+53.3	+281.7
	297	1.37	3.97	1.15	–30.3		
	300	1.22	5.98	1.18	–31.2		
2	293	2.05	7.09	1.13	–32.7	+15.9	+166.1
	297	2.01	8.57	1.15	–33.4		
	300	1.93	10.4	1.22	–33.9		
3	–	–	–	–	–	–	–
4	293	1.49	3.46	1.08	–31.0	+13.5	+151.9
	297	1.34	3.91	1.10	–31.6		
	300	1.16	4.79	1.11	–32.1		
5	293	1.66	4.62	1.12	–31.8	+5.2	+126.3
	297	1.52	5.08	1.13	–32.3		
	300	1.44	5.24	1.15	–32.7		
6	–	–	–	–	–	–	–

Table S3 Hydrogen bonding and van der Waals interactions and the binding free energy of the most stable docking conformations for complexes **1**–**6** docked into DNA.

Complex	Hydrogen bonding	Distance (Å)	Electrostatic interaction	Distance (Å)	ΔG (kJ mol^{-1})
1	-	-	DT19:OP1···π	3.56	−28.96
			DC11:OP1···π	3.73	
			DG10:OP1···π	3.80	
2	N6-H6 (barb)···O2(DC23)	2.82	-	-	−29.71
3	N4–H4 (barb)···O2 (DC15)	2.21	-	-	−27.61
4	N1–H1 (barb)···O4 (DT19)	2.48	-	-	−28.45
	N1-H1(barb)···N7 (DA18)	2.72			
	N6–H62 (DA6)···O2 (barb)	2.81			
5	N1–H1 (barb)···O6 (DG4)	2.31	-	-	−28.87
	N4–H4 (barb)···O5’ (DC3)	2.64			
	N1–H1 (barb)···O4 (DT20)	2.75			
	N6–H61 (DA5)···O1 (barb)	2.82			
6	N5-H5(barb)···O4 (DT19)	2.02	-	-	−27.03
	N6–H61 (DA18)···O5 (barb)	2.98			

Table S4 Hydrogen bonding, binding sites and the binding free energy of the most stable docking conformations for complexes **1**–**6** docked into HAS.

Complex	Hydrogen bonding	Distance (Å)	Hydrophobic interaction	Distance (Å)	Binding free energy (kJmol^{-1})
1	-	-	ALA291-CH···π	3.70	-31.24
			LYS199-CB-CH···π	3.80	
			TRP214-π···π	3.94	
			π···ALA291-alkyl	4.02	
2	N4-H4 (barb)···O:ALA291	2.14	Alkyl···ARG218- alkyl	4.01	-33.89
	LYS195-NZ···O4-barb	2.94			
3	ARG222:NH2···O5-barb	2.87	ALA215- alkyl ··· alkyl	3.74	-32.64
			LYS199-CB-CH···π	3.79	
			TRP214-π···π	3.84, 3.95	
			ALA291- alkyl···alkyl	3.96	
4	N4-H4 (dpya)···O:CYS246	2.04	-	-	-30.96
	N1-H1(barb)···O: HIS247	2.52			
5	N1-H1 (barb)···O: LYS195	1.93	CH···TRP214-π	3.59	-32.21
			ALA291- alkyl···alkyl	4.02	
6	N5-H5(barb)···O:ALA291	2.27	-	-	-31.79
	ARG222:NH2···O5-barb	2.91			
	ARG222:NH1···O5-barb	2.98			