**Electronic Supplementary Information (ESI)**

# Synthesis, structures, DNA/protein binding, molecular docking, anticancer activity and ROS generation of Ni(II), Cu(II) and Zn(II) 5,5-diethylbarbiturate complexes with bis(2-pyridylmethyl)amine and terpyridine

Veysel T. Yilmaz,*[a] Ceyda Icsel,[a] Feruza Suyunova,[a] Muhittin Aygun,[b] Buse Cevatemre[c] and Engin Ulukaya[d]

[a]Department of Chemistry, Faculty of Arts and Sciences, Uludag University, 16059 Bursa, Turkey.
[b]Department of Physics, Faculty of Sciences, Dokuz Eylul University, 35210 Izmir, Turkey
[c]Department of Biology, Faculty of Arts and Sciences, Uludag University, 16059 Bursa, Turkey.
[d]Department of Medical Biochemistry, Medical School, Uludag University, 16059 Bursa, Turkey

Corresponding Author:

Prof. Dr. Veysel T. Yilmaz
Department of Chemistry
Faculty of Arts and Sciences
Uludag University
16059 Bursa, Turkey

E-mail: vtyilmaz@uludag.edu.tr

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

| EB exchange | | | | | |
|---|---|---|---|---|---|
| Complex | T(K) | $K_{SV}$ ($M^{-1}$) x $10^{-4}$ | ΔG° (kJ $mol^{-1}$) | ΔH° (kJ $mol^{-1}$) | ΔS° ($JK^{-1}$ $mol^{-1}$) |
| **1** | 293 | 0.38 | –28.8 | –14.4 | +49.3 |
| | 297 | 0.33 | –29.0 | | |
| | 300 | 0.29 | –29.2 | | |
| **2** | 293 | 2.46 | –30.5 | –6.5 | +81.9 |
| | 297 | 2.39 | –30.8 | | |
| | 300 | 2.31 | –31.1 | | |
| **3** | 293 | 0.47 | –29.3 | –19.5 | +33.6 |
| | 297 | 0.42 | –29.5 | | |
| | 300 | 0.39 | –29.6 | | |
| **4** | 293 | 1.95 | –29.9 | –6.0 | +81.7 |
| | 297 | 1.90 | –30.3 | | |
| | 300 | 1.84 | –30.5 | | |
| **5** | 293 | 3.69 | –30.6 | –4.9 | +87.7 |
| | 297 | 3.60 | –30.9 | | |
| | 300 | 3.52 | –31.2 | | |
| **6** | 293 | 1.68 | –29.6 | –7.7 | +74.7 |
| | 297 | 1.62 | –29.9 | | |
| | 300 | 1.56 | –30.1 | | |

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

| Complex | T(K) | $K_{SV}$ ($M^{-1}$) x $10^{-4}$ | $K_F$ ($M^{-1}$) x $10^{-5}$ | *n* | ΔG° (kJ $mol^{-1}$) | ΔH° (kJ $mol^{-1}$) | ΔS° ($JK^{-1}mol^{-1}$) |
|---|---|---|---|---|---|---|---|
| **1** | 293 | 1.36 | 3.15 | 1.14 | –33.8 | –40.4 | –22.5 |
| | 297 | 1.39 | 3.27 | 1.15 | –33.7 | | |
| | 300 | 1.44 | 3.48 | 1.17 | –33.6 | | |
| **2** | 293 | 2.41 | 5.09 | 1.17 | –34.1 | –41.3 | –24.6 |
| | 297 | 2.48 | 5.27 | 1.19 | –34.0 | | |
| | 300 | 2.53 | 5.44 | 1.22 | –33.9 | | |
| **3** | 293 | 1.52 | 3.27 | 1.11 | –35.7 | –50.8 | –51.5 |
| | 297 | 1.55 | 3.38 | 1.13 | –35.5 | | |
| | 300 | 1.61 | 3.69 | 1.16 | –35.4 | | |
| **4** | 293 | 1.96 | 3.66 | 1.12 | –34.3 | –29.6 | +16.2 |
| | 297 | 1.84 | 3.51 | 1.10 | –34.4 | | |
| | 300 | 1.76 | 3.49 | 1.08 | –34.5 | | |
| **5** | 293 | 3.03 | 7.24 | 1.18 | –36.2 | –34.9 | +1.5 |
| | 297 | 2.92 | 7.08 | 1.15 | –36.3 | | |
| | 300 | 2.84 | 6.94 | 1.13 | –36.3 | | |
| **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 (D–H) (H····A) | Distance (Å) (H····A, Å) | Binding free energy (kJ $mol^{-1}$) |
|---|---|---|---|
| **1** | N9–H9 (barb)···O6 (DG2) | 2.08 | −28.87 |
| | N9–H9 (barb)···O6 (DG22) | 2.54 | |
| | N1–H1 (bpma)···N7 (DG22) | 2.84 | |
| | N4 (DC3)···O4 (barb) | 2.98 | |
| **2** | N5–H5 (barb)···O3' (DG4) | 2.35 | −30.54 |
| **3** | N5–H5 (barb)···O4 (DT7) | 2.15 | −30.54 |
| | N7–H7 (barb)···O4 (DT19) | 2.32 | |
| | N6 (DA18)··· O5 (barb) | 2.83 | |
| **4** | N1–H1 (barb)···O6 (DG2) | 2.23 | −30.12 |
| | N1-H1(barb)··· O6 (DG22) | 2.87 | |
| **5** | N5–H5 (barb)···O4' (DG4) | 2.40 | −30.12 |
| | N5–H5 (barb)···O2 (DC3) | 2.72 | |
| **6** | N5–H5(barb)···O3' (DA5) | 2.83 | −29.71 |
| | N7–H7(barb)···O4' (DC23) | 2.86 | |
| | N7–H7(barb)···N3 (DG22) | 2.92 | |
| | N5–H5(barb)···OP1 (DA6) | 2.94 | |

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

| Complex | Hydrogen bonding | Distance (Å) | Hydrophobic interaction | Distance (Å) | Binding free energy (kJ $mol^{-1}$) |
|---|---|---|---|---|---|
| **1** | $H_2O$-H2B··· O:ALA291 | 2.40 | LYS199-alkyl···alkyl | 4.04 | −34.31 |
| | ARG257-NH1··· O4-barb | 2.86 | | | |
| **2** | N5-H5 (barb)··· OG:SER192 | 2.53 | ALA215- alkyl ··· alkyl | 3.91 | −34.73 |
| **3** | - | - | Alkyl ···ARG218- alkyl | 3.83 | −35.98 |
| | | | Alkyl ···LEU219- alkyl | 3.95 | |
| | | | ALA215- alkyl ··· alkyl | 3.99 | |
| **4** | - | - | ALA291- alkyl ··· alkyl | 4.08 | −33.47 |
| | | | π ···LYS199- alkyl | 4.08 | |
| | | | Alkyl ···LEU238- alkyl | 4.16 | |
| | | | HIS242-π ···alkyl | 4.63 | |
| | | | HIS288-π ···alkyl | 4.73 | |
| | | | π ···LYS195- alkyl | 4.75 | |
| | | | ARG222:NH2 ··· π (electrost.) | 4.96 | |
| **5** | TYR161:OH··· O1-$H_2O$ | 2.75 | ILE142:CH ··· π | 3.75 | −36.40 |
| | TYR161:OH··· O4-barb | 2.89 | TYR161- π ··· π | 3.80 | |
| | | | Alkyl ···ARG145- alkyl | 3.94 | |
| | | | Alkyl··· ILE142- alkyl | 4.26 | |
| | | | HIS146-π ···alkyl | 4.53 | |
| | | | TYR138-π ··· π | 4.74 | |
| | | | π ···ARG117- alkyl | 4.85 | |
| | | | ARG117:$NH_2$··· π (electrost) | 4.37 | |
| **6** | - | - | ALA215- alkyl ··· alkyl | 4.04 | −31.79 |
| | | | Alkyl ···ARG218- alkyl | 4.16 | |
| | | | π ···LYS195- alkyl | 4.24 | |
| | | | Alkyl ···LEU238- alkyl | 4.43 | |
| | | | HIS288-π ···alkyl | 4.56 | |
| | | | π ···LYS199- alkyl | 4.49 | |
| | | | LYS195:NZ ··· π (electrost.) | 4.74 | |

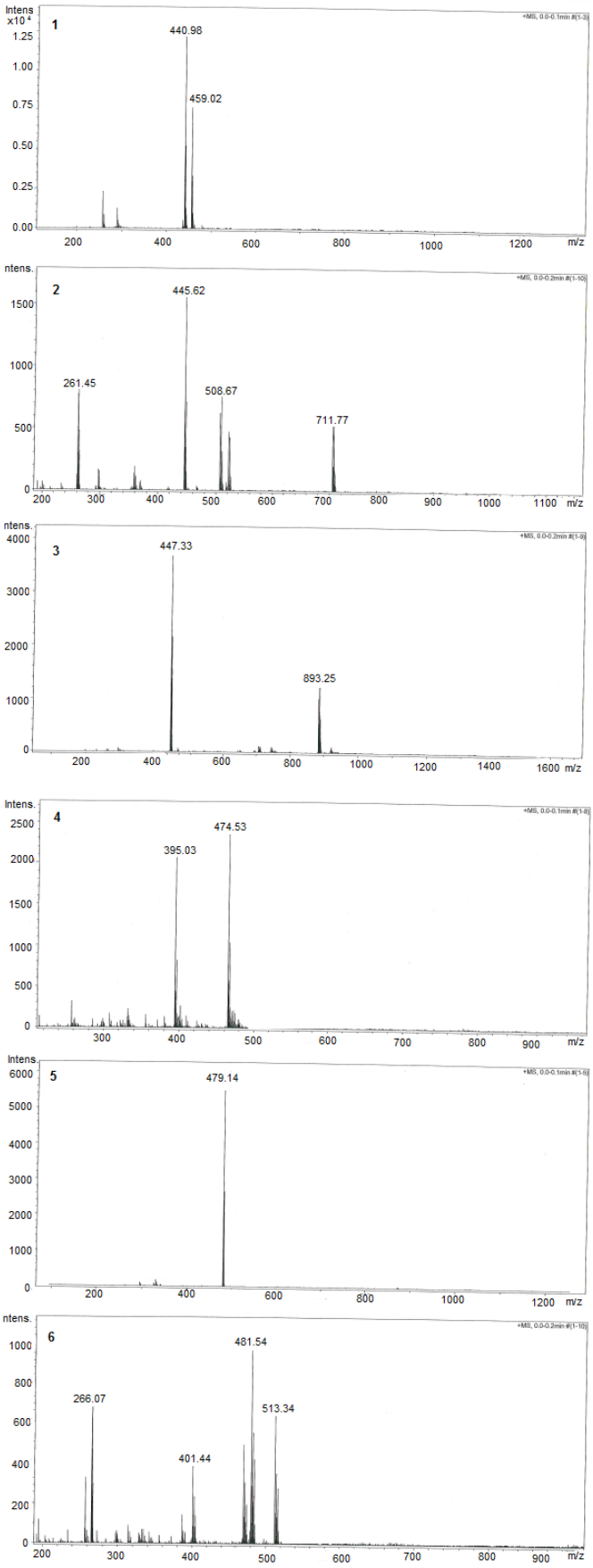



**Fig. S1** ESI-MS spectra of **1–6**.

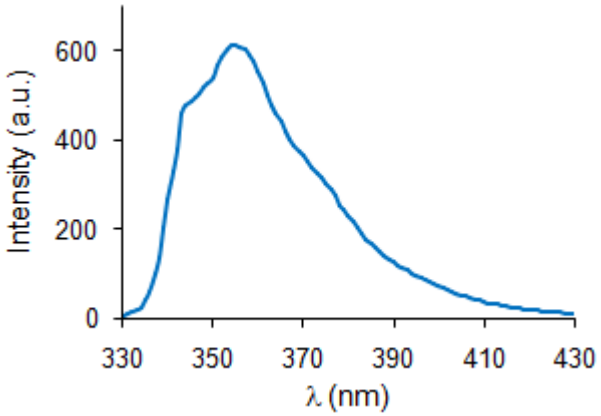



**Fig. S2** The emission spectrum of **6** in MeOH ($1 \times 10^{-4}$ M) at r.t. ($\lambda_{ex}$ = 340 nm). The complex exhibits strong fluorescence and the slits was kept at 2.5.

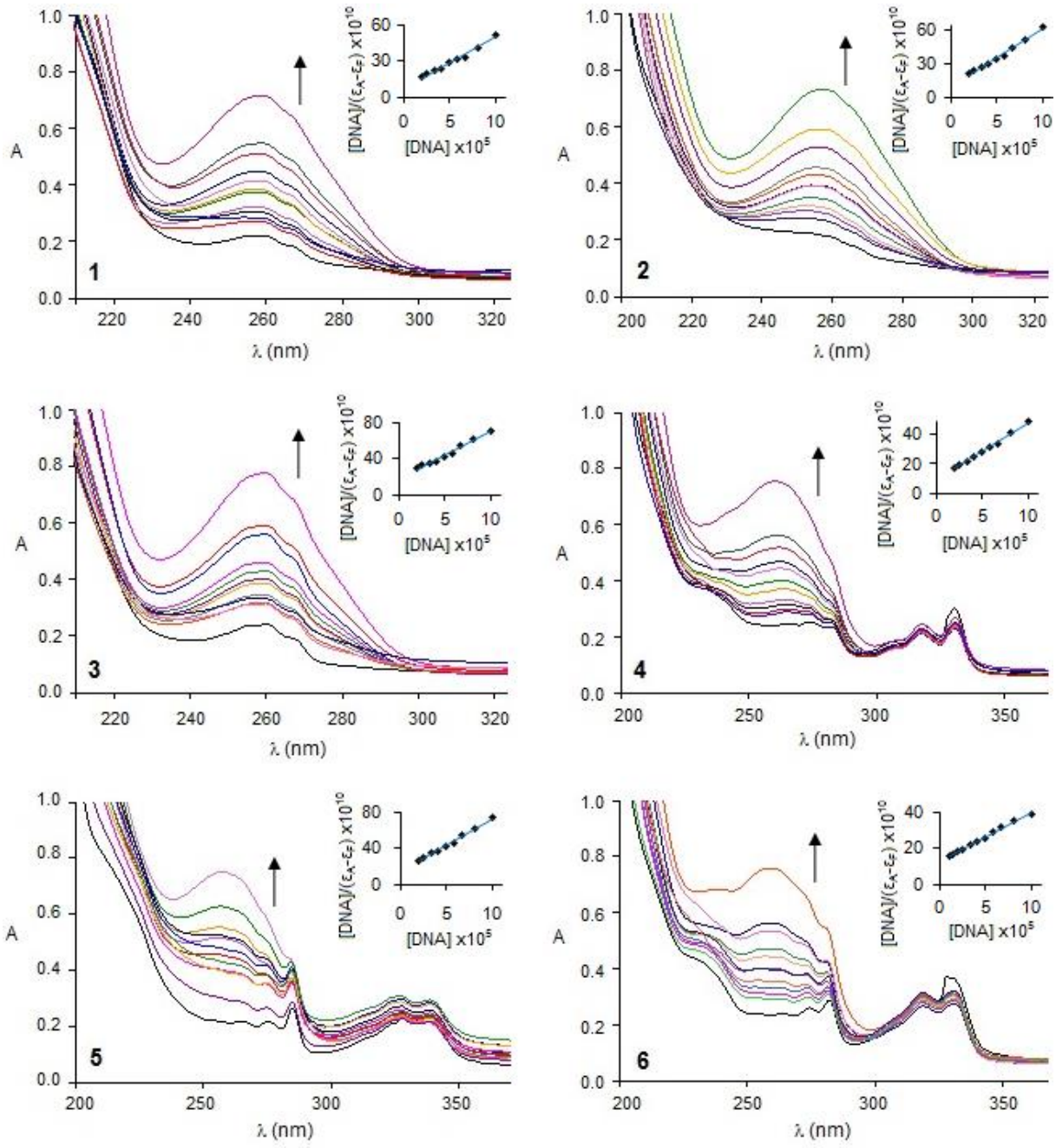



**Fig. S3** UV spectra of solutions containing **1**–**6** (10 μM) upon addition of FS-DNA (0–10 μM) in Tris-HCl. The arrows show the absorbance changes upon increasing FS-DNA concentration. The inset shows the linear fit of [DNA]/($\varepsilon_a - \varepsilon_f$) vs. [DNA].

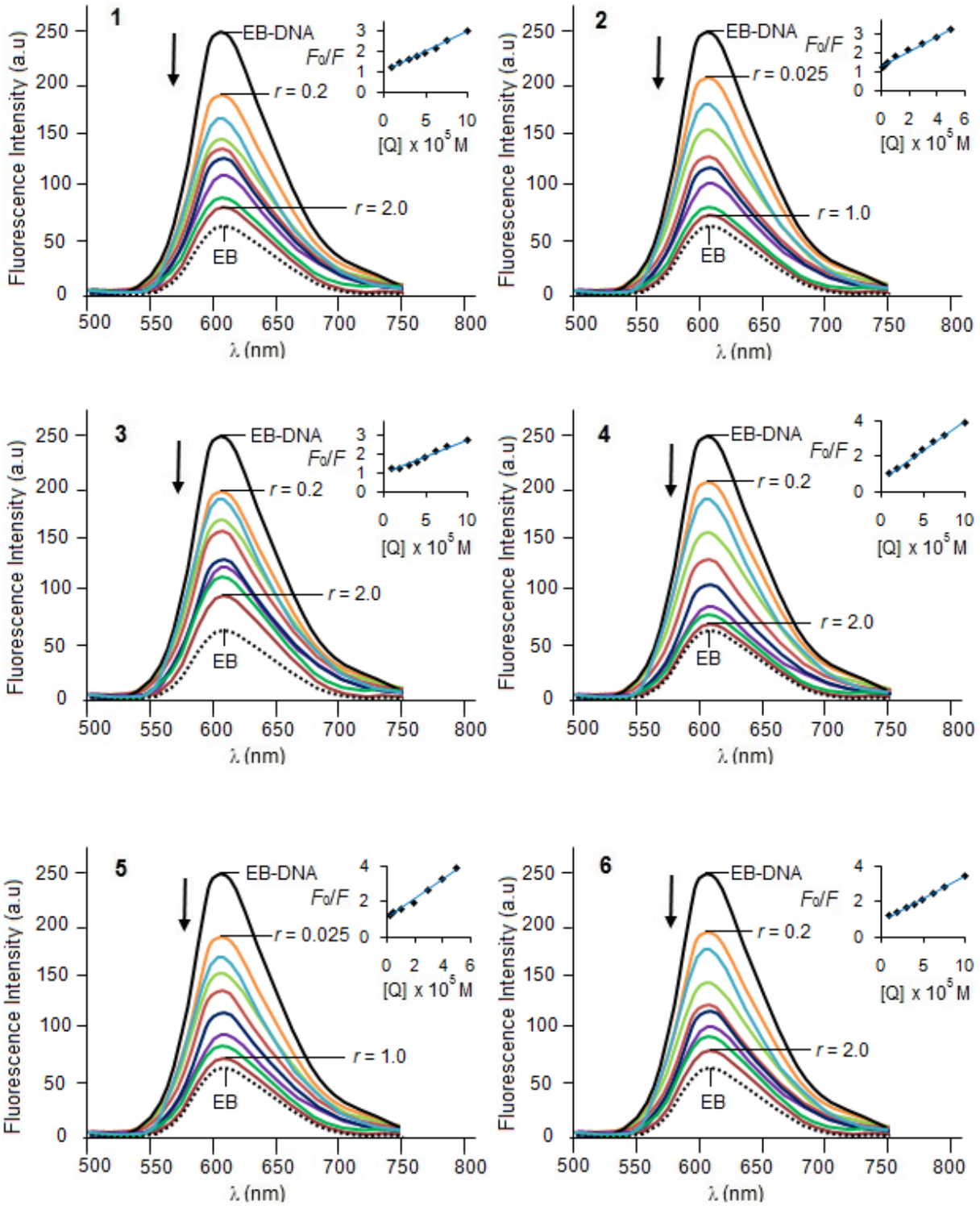



**Fig. S4** Emission spectra of EB-bound and Hoechst 33258-bound DNA solutions in the absence and presence of increasing concentrations of **1**–**6** in Tris-HCl. [EB] = 5.0 μM, [DNA] 50.0 μM. *r* = [complex]/[DNA]. The arrows show the changes in intensity upon increasing amounts of the complexes. Insets: Stern-Volmer plot of the fluorescence data.

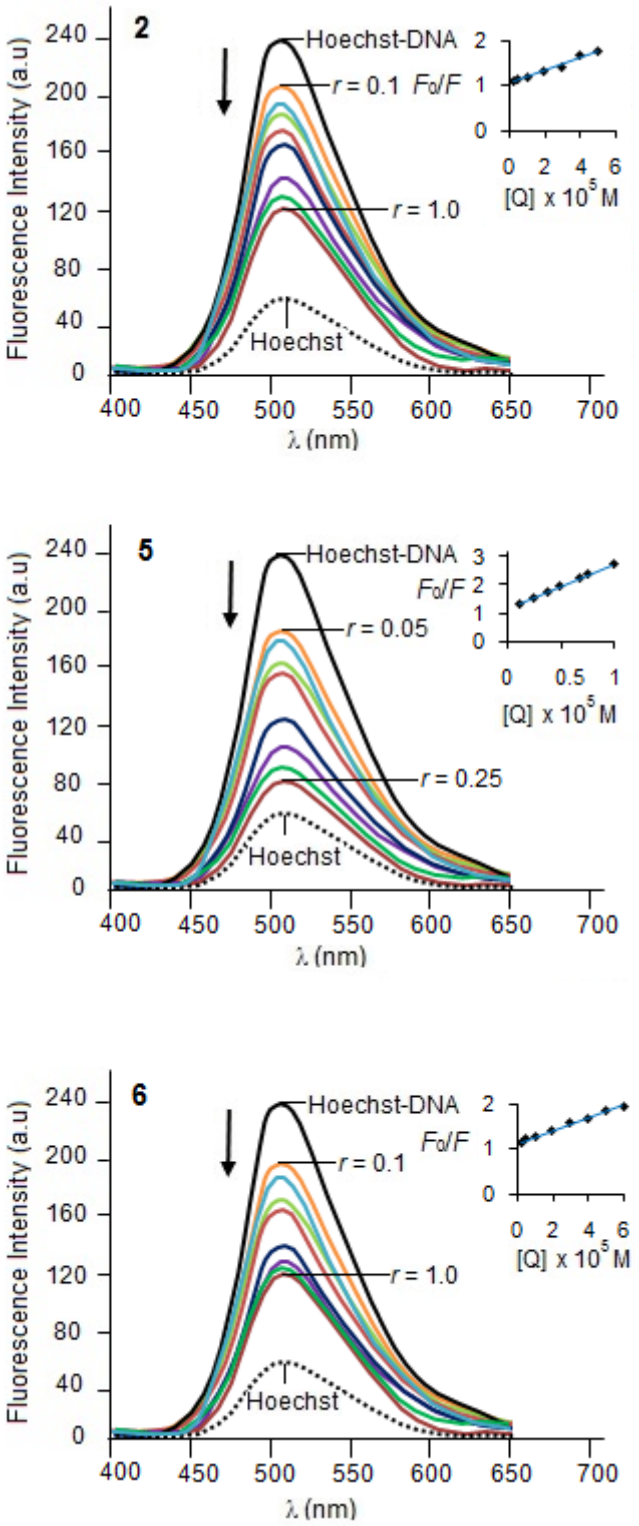



**Fig. S5** Emission spectra of Hoechst 33258-bound DNA solutions in the absence and presence of increasing concentrations of **2**, **5**, **6** in Tris-HCl buffer. [Hoechst 33258] = 5.0 µM, [DNA] 50.0 µM. The arrows show the changes in intensity upon increasing amounts of the complexes. Insets: Stern-Volmer plot of the fluorescence data.

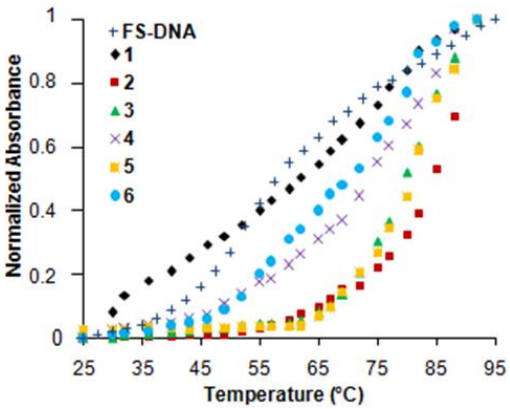



**Figure S6** Thermal denaturation profiles of FS-DNA (100 μM) in the absence and in the presence of **1**–**6** (50 μM) in a Tris-HCl.

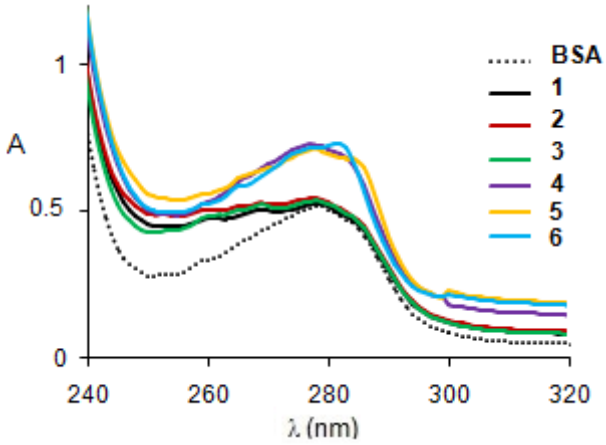



**Fig. S7** UV-vis absorption spectra of BSA (10 μM) in Tris-HCl in the presence of **1**–**6** (5 μM).

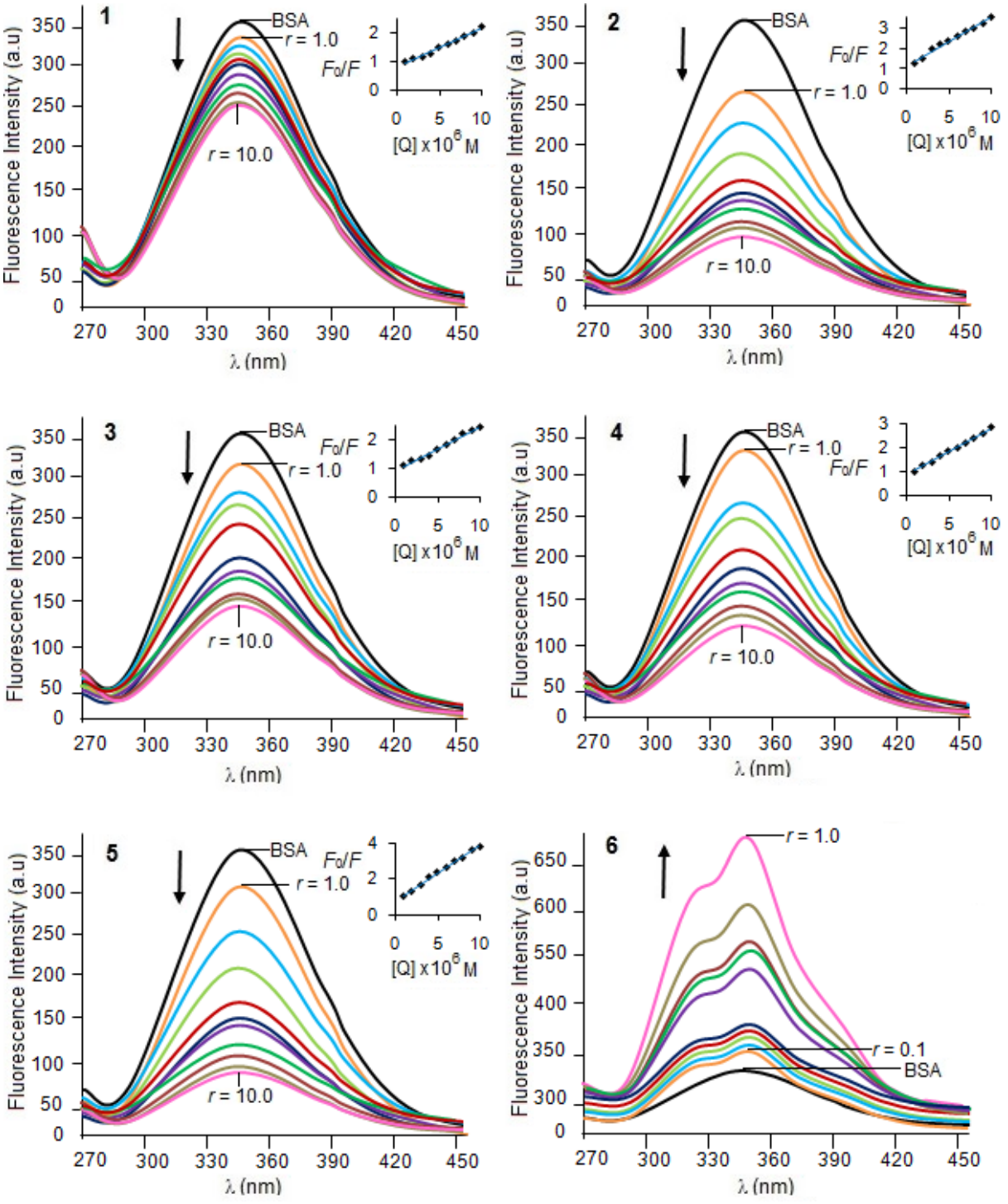



**Fig. S8** Emission spectra of BSA (1.0 µM; λex = 280 nm) in presence of **1–5** (0-10.0 µM) and **6** (0-1.0 µM). The arrow shows the emission intensity changes upon increasing complex concentration. Insets: Stern-Volmer plot of the fluorescence data.

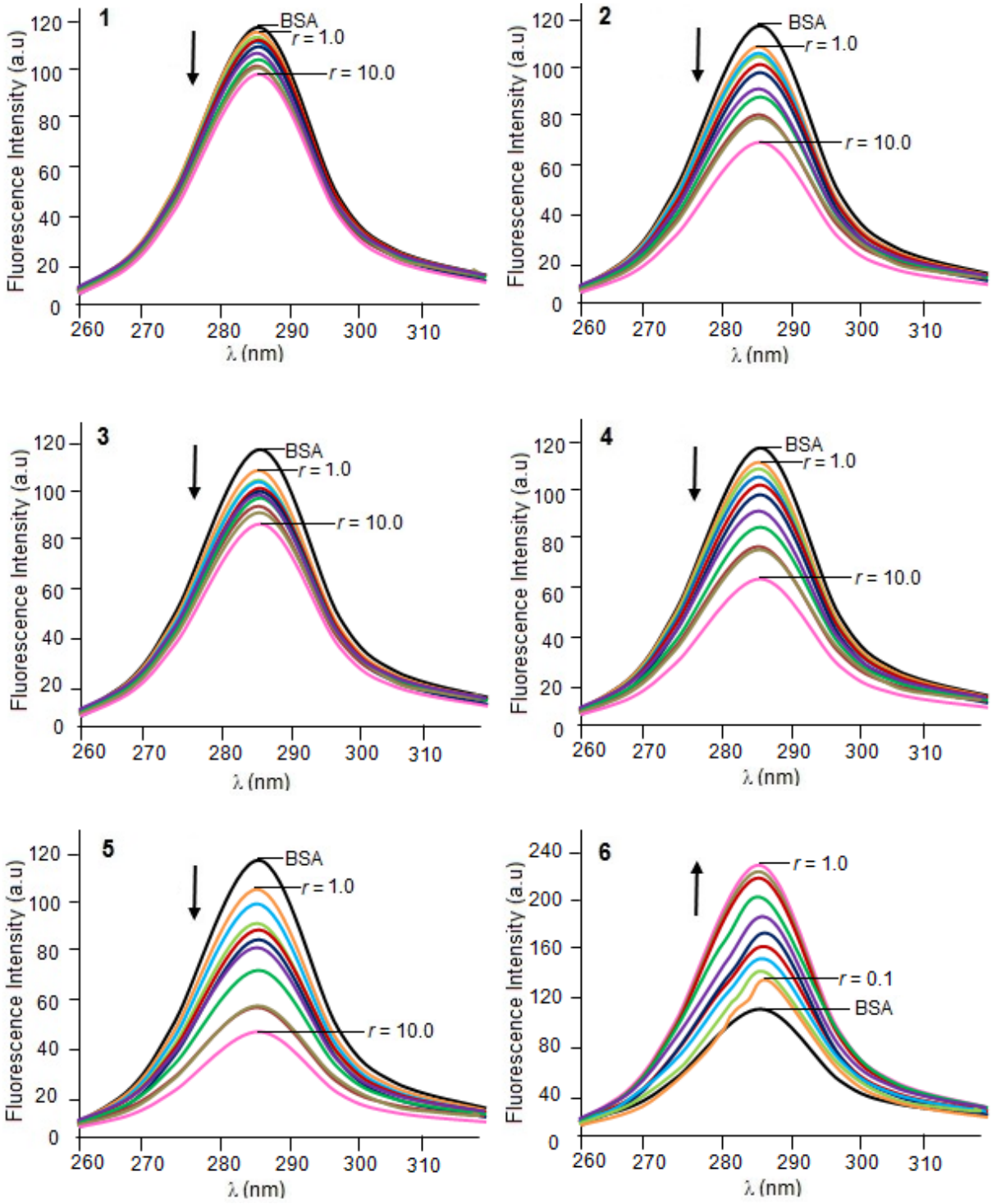
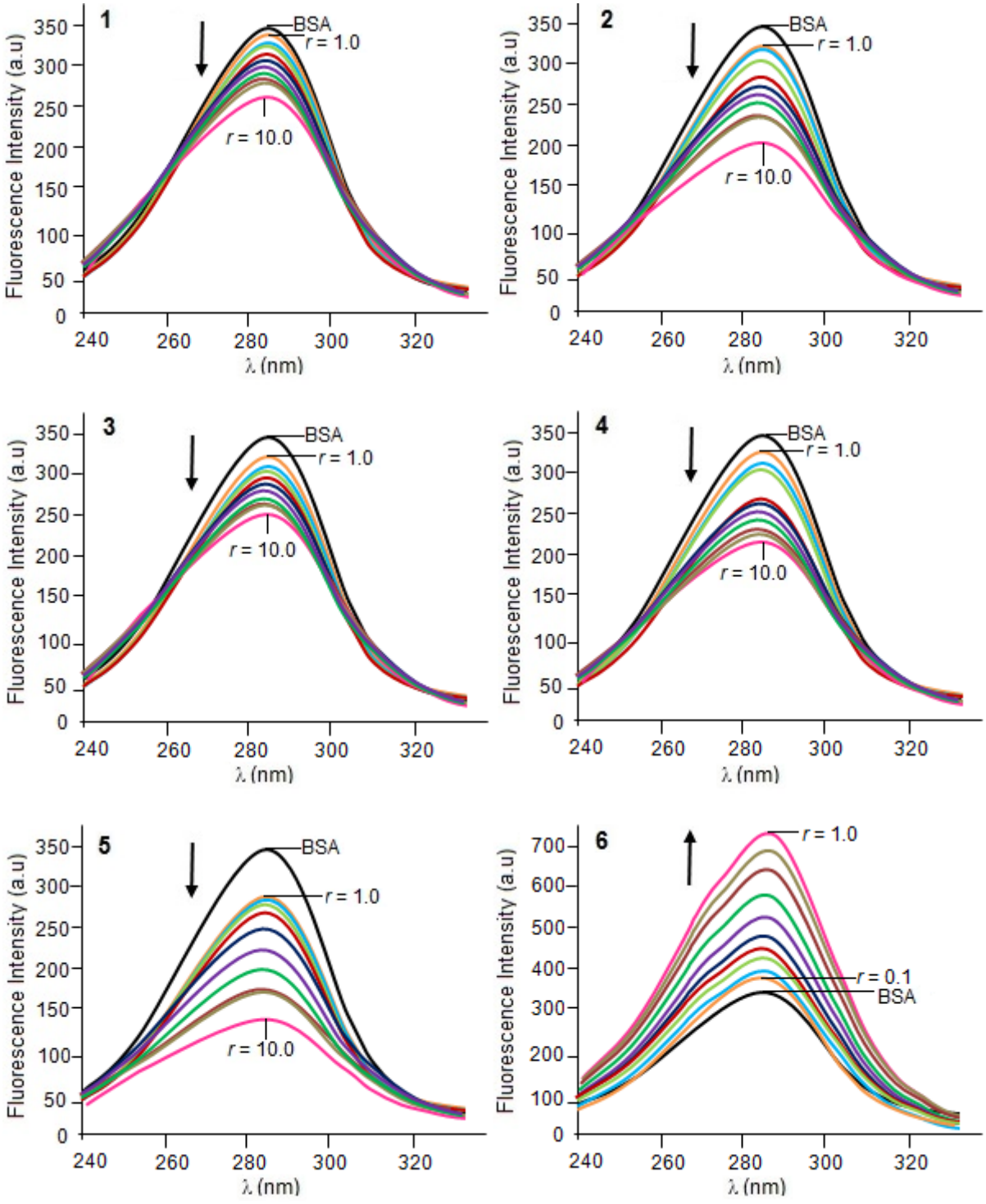



**Fig. S9** (**a**) Continued




(**b**)

**Fig. S9** Synchronous spectra of BSA (1.0 μM) in presence of **1–5** (0-10.0 μM) and **6** (0-1.0 μM). at Δλ = 15 nm (**a**) and Δλ = 60 nm (**b**). Arrows show the emission intensity changes upon increasing concentration of **1**–**6**.

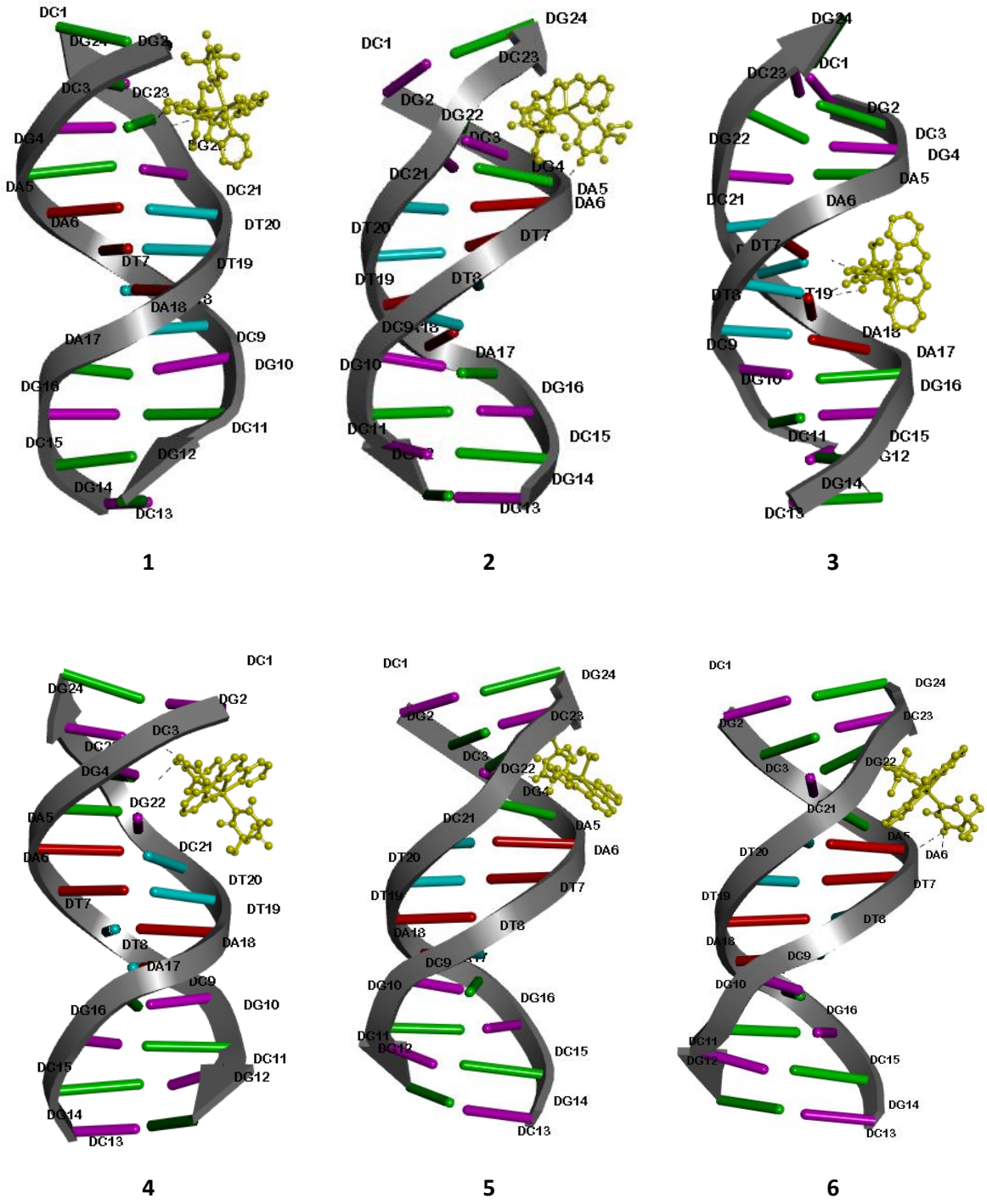



**Fig. S10** Molecular docking of **1**–**4** with DNA.

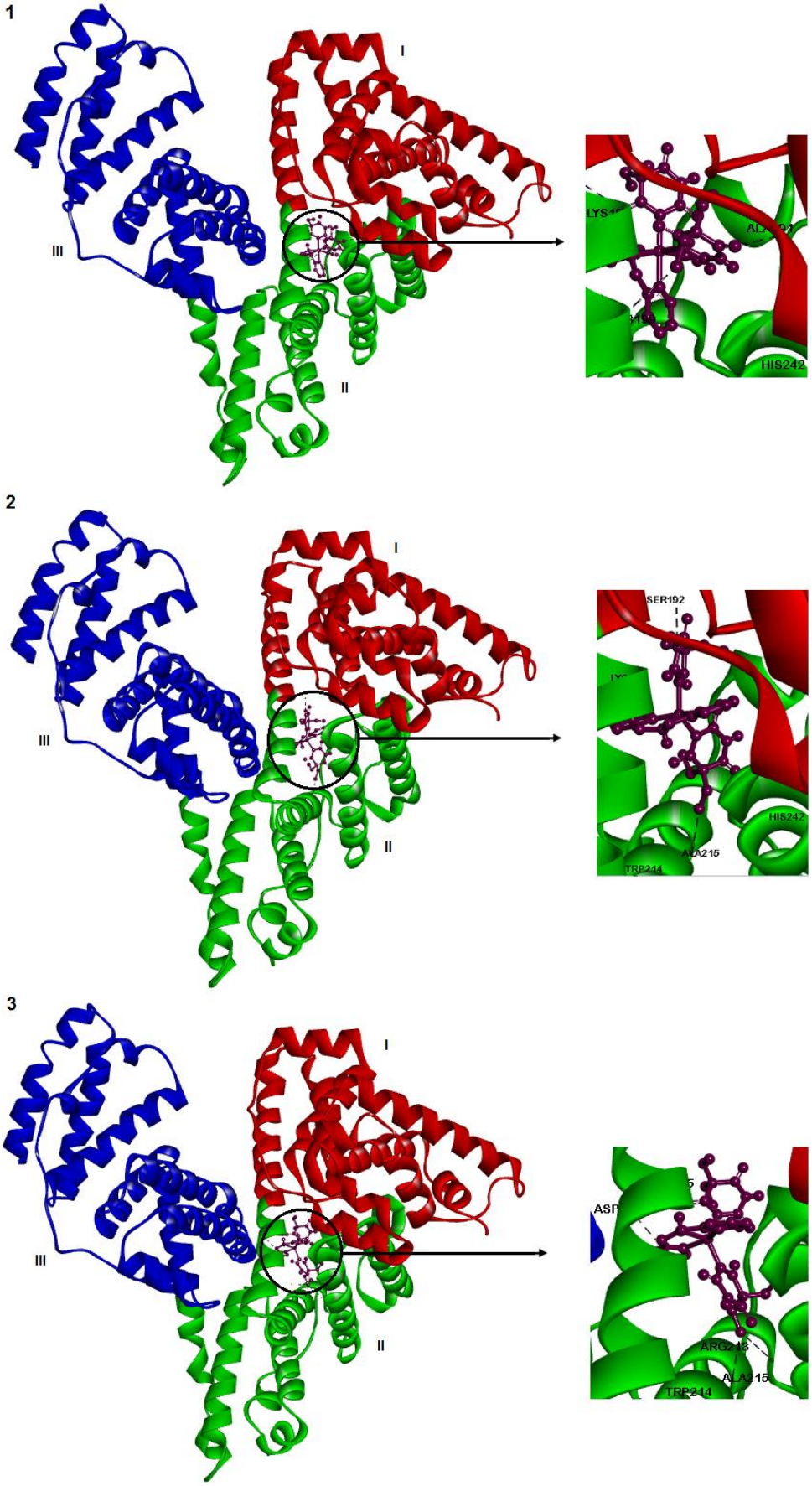



**Fig. S11** Continued

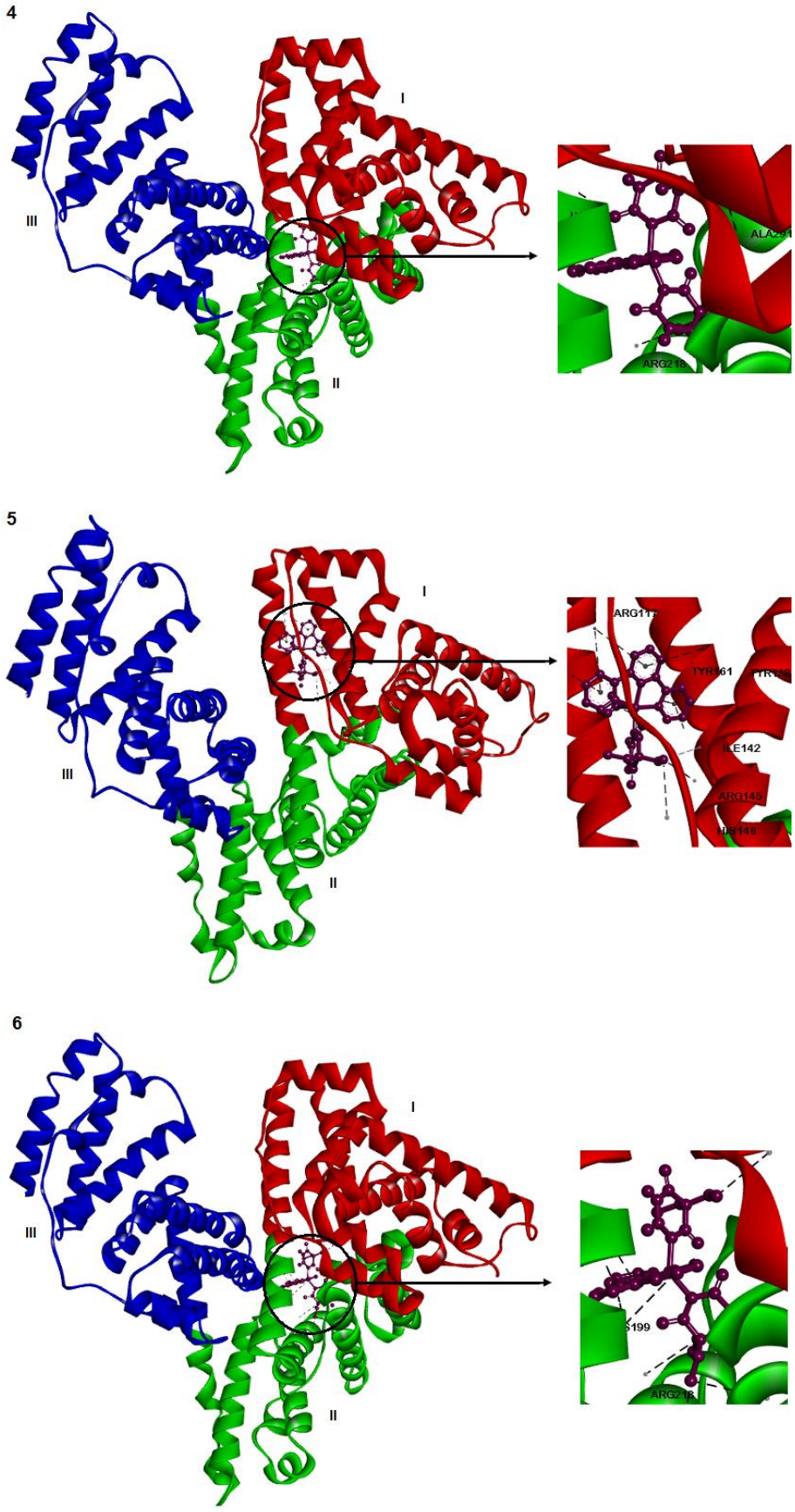



**Fig. S11** Molecular docking of **1**–**6** with HSA.

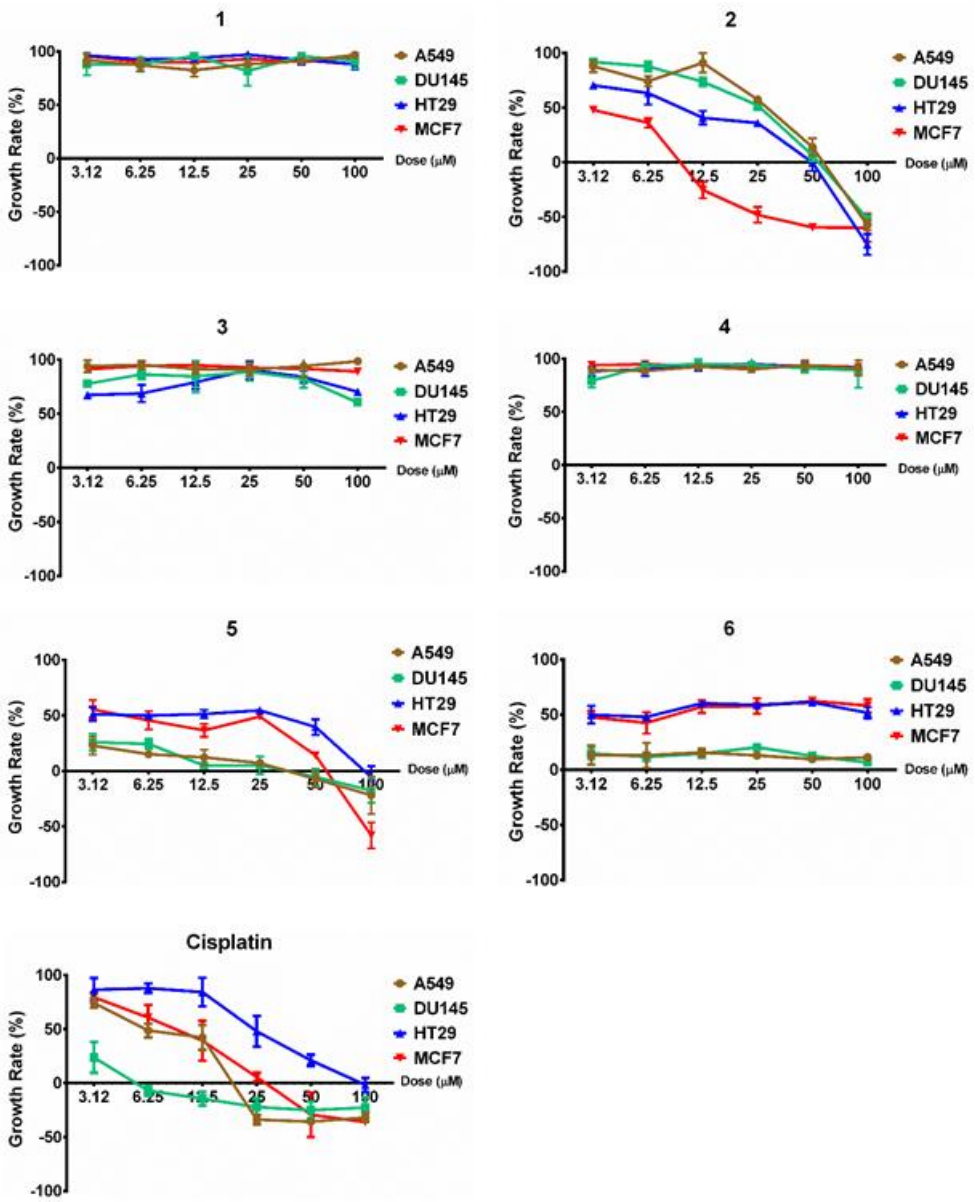



**Fig. S12** The dose-response graphics for **1**–**6** obtained from SRB assay, showing the effect of the complexes on the growth of the cell lines after 48 h of treatment.

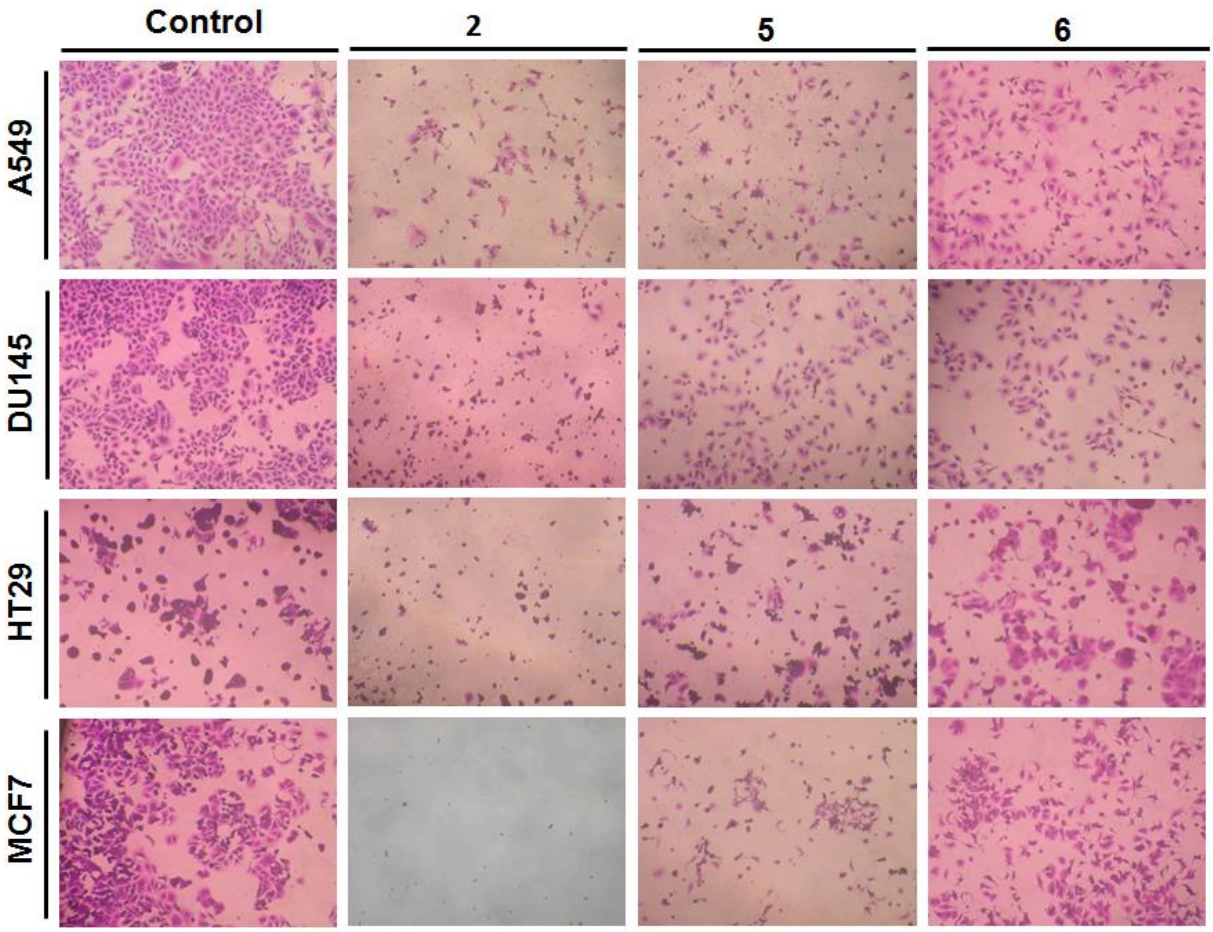



**Fig. S13** Phase contrast microscopy images of the cancer cells treated with **2**, **5** and **6** (50 µM) for 48 h.

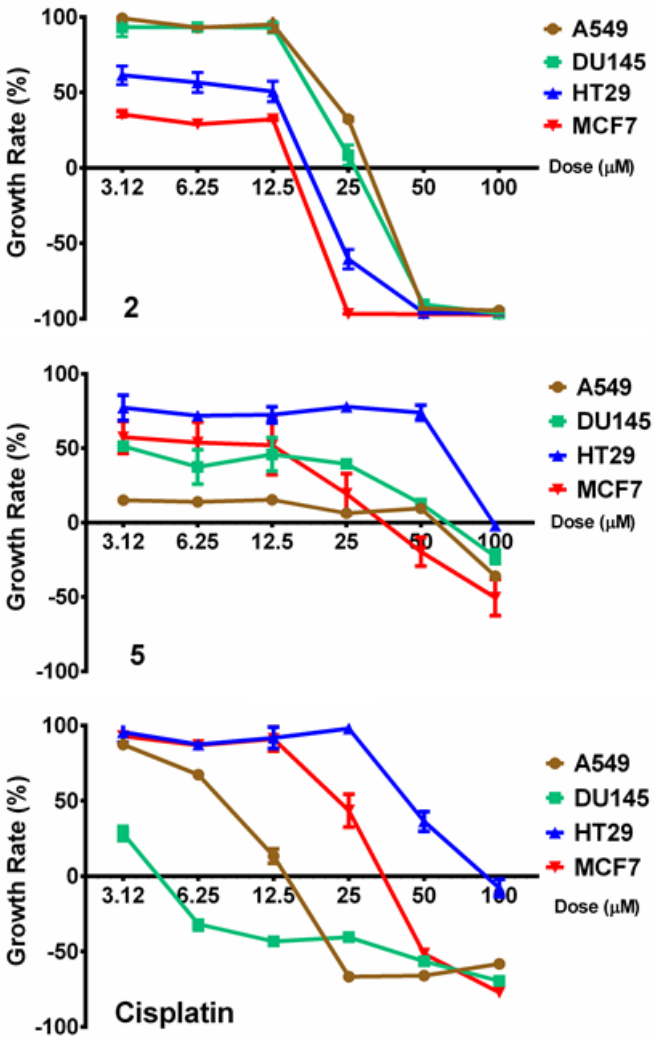
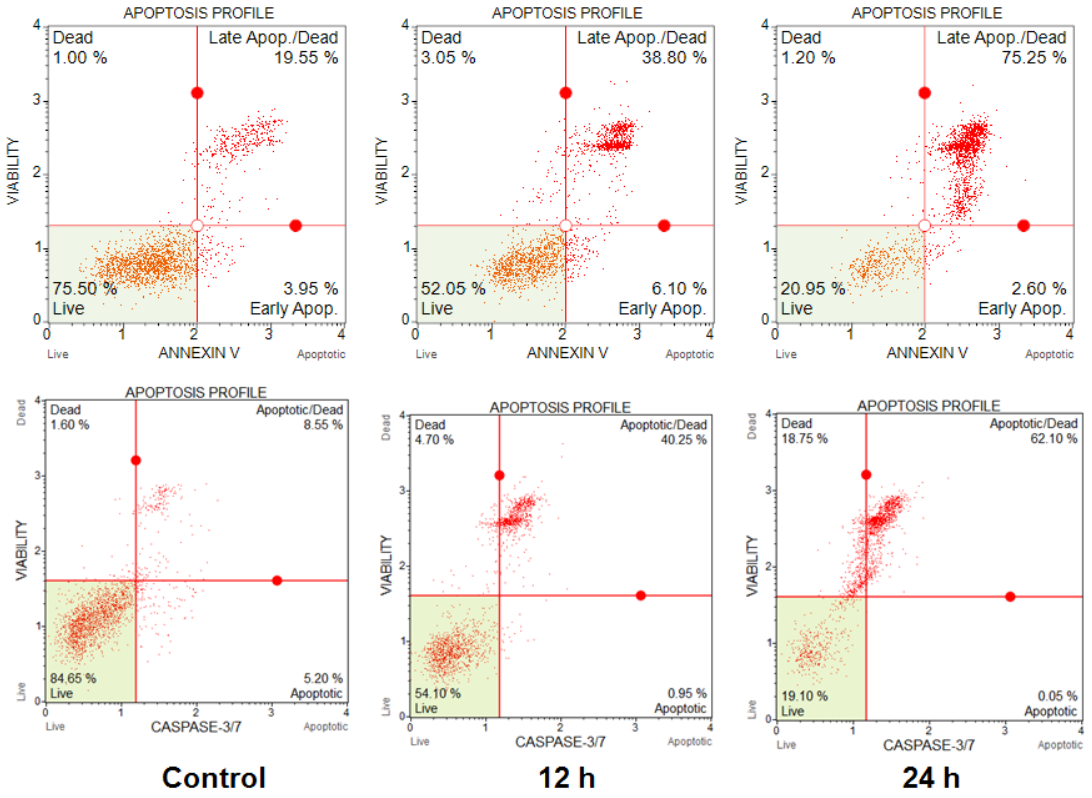
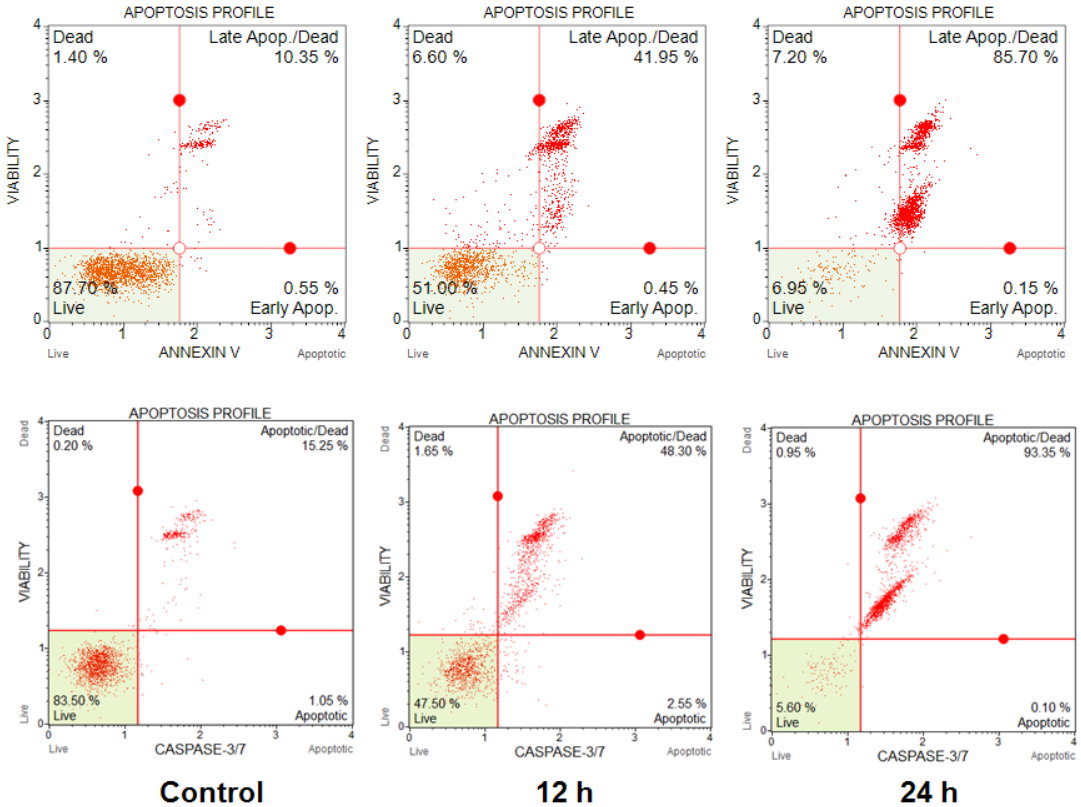



**Fig. S14** The Growth rate of A549, DU145, HT29, MCF-7 cells treated with six different doses (3.12–100 μM) of **2**, **5** and cisplatin for 48 h. Growth rate curves were obtained by the ATP assay.




**(a)**




**(b)**

**Fig. S15** Flow cytometry analysis of apoptosis of MCF-7 cells treated with $LC_{90}$ concentrations of **2** (**a**) and **5** (**b**) using Annexin V and caspase-3/7 assays.