Supplementary Information for

New 13-pyridinealkyl berberine analogs intercalate to DNA and induce apoptosis in HepG2 and MCF-7 cells through ROS mediated P53 dependent pathway: biophysical, biochemical and molecular modeling studies

Sabyasachi Chatterjee [a], Sumana Mallick [b], Franco Buzzetti [cc], Gaetano Fiorillo [c], Tanjia Monir Syeda [c], Paolo Lombardi [c], Krishna Das Saha [b] and Gopinatha Suresh Kumar [a*]

[a]Biophysical Chemistry Laboratory, Organic and Medicinal Chemistry Division, CSIR-Indian institute of Chemical Biology, Kolkata 700 032, India

[b]Cancer Biology and Inflammatory Disorder Division, CSIR-Indian Institute of Chemical Biology, Kolkata 700 032, India

[c]Naxospharma srl, Via G. Di Vittorio, 70, 20026 Novate Milanese (MI), Italy

Address for Correspondence

Dr. G. Suresh Kumar, Ph.D

Senior Principal Scientist, Biophysical Chemistry Laboratory

CSIR-Indian Institute of Chemical Biology

4, Raja S.C. Mullick Road, Kolkata 700032, India

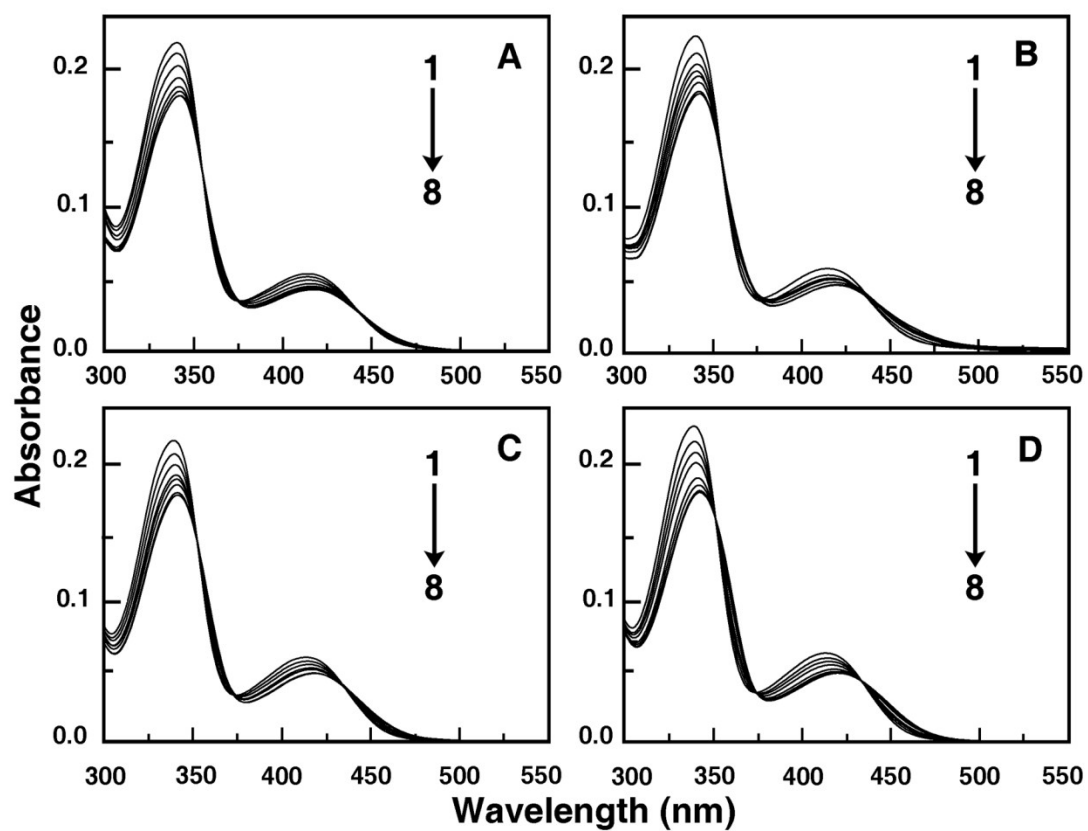Phone: +91 33 2472 4049, Fax : +91 33 2472 3967

Email: gskumar@iicb.res.in

Fig. S1. Absorbance spectra of BER1-4 (11 μM, curve 1 in A-D) with increasing concentration of DNA upto saturation (curves 2-8).

McGhee−von Hippel equation:

Analysis of the Binding Data. Scatchard plots (r/Cf versus r) were constructed from the binding data obtained from spectrophotometric titrations. The Scatchard isotherms with positive slope at low r values were analyzed using the following McGhee−von Hippel equation for cooperative binding.

$$\frac{r}{C_f} = K(1-nr) \times \left(\frac{(2\omega+1)(1-nr)+(r-R)}{2(\omega-1)(1-nr)} \right)^{(n-1)} \left(\frac{1-(n+1)r+R}{2(1-nr)} \right)^2 \qquad \text{(Eqn. S1)}$$

where, $R = \{[1-(n+1)r]^2 + 4\omega r(1-nr)\}^{\frac{1}{2}}$,

Scatchard plots with negative slopes at low r values were analyzed by the non-cooperative binding model of McGhee and von Hippel as per the following equation

$$r/C_f = K (1-nr)[(1-nr)/\{1-(n-1)r\}]^{(n-1)} \qquad \text{(Eqn. S2)}$$

Here, K is the intrinsic binding constant to an isolated binding site, n is the number of base pairs excluded by the binding of a single alkaloid molecule and ω is the cooperativity factor. All the binding data were analyzed using the Origin 7.0 software (Origin Labs, Northampton, MA, USA) that determines the best-fit parameters to K_i, n and ω to equation (Eqn. S1) and K and n to equation (Eqn. S2).
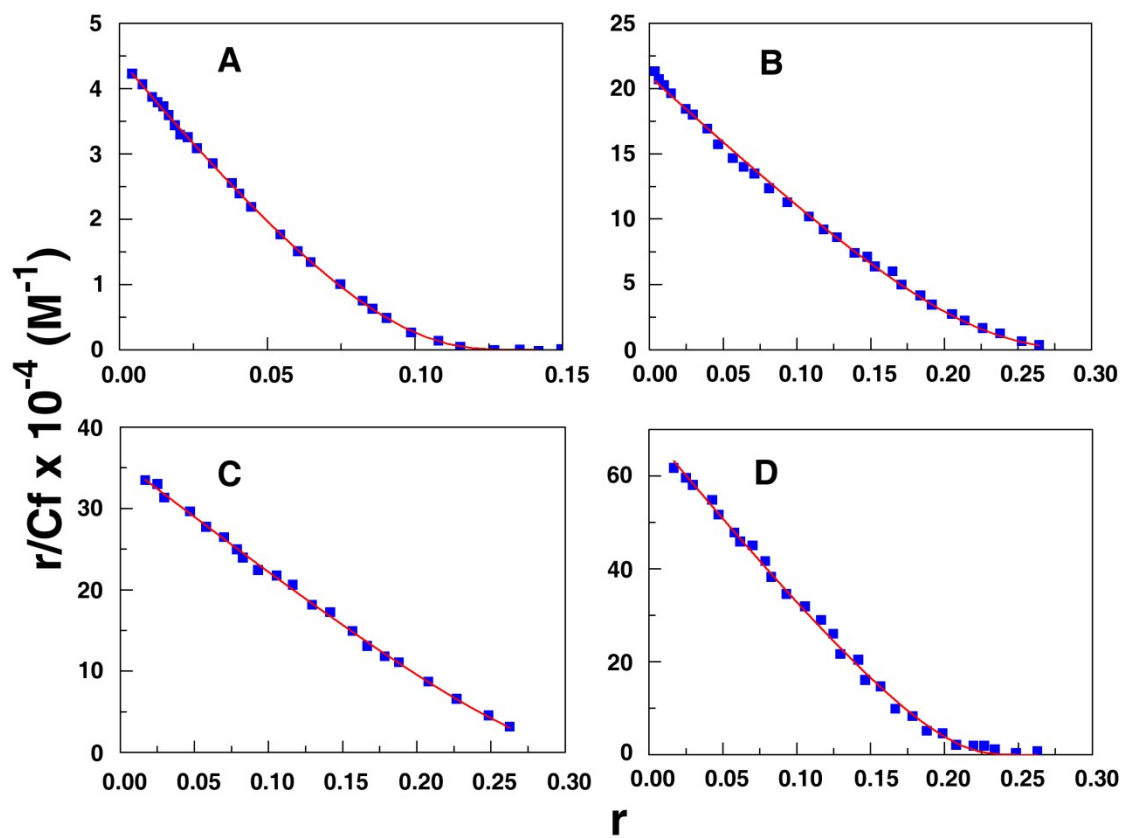
Fig. S2. Scatchard plots fitted to the McGhee-von Hippel analysis for the binding of BER1 (A), BER2 (B), BER3 (C) and BER4 (D) to CT DNA obtained from spectrophotometric titration data. The symbols represent the actual data points and the continuous line is the best fit to the data.
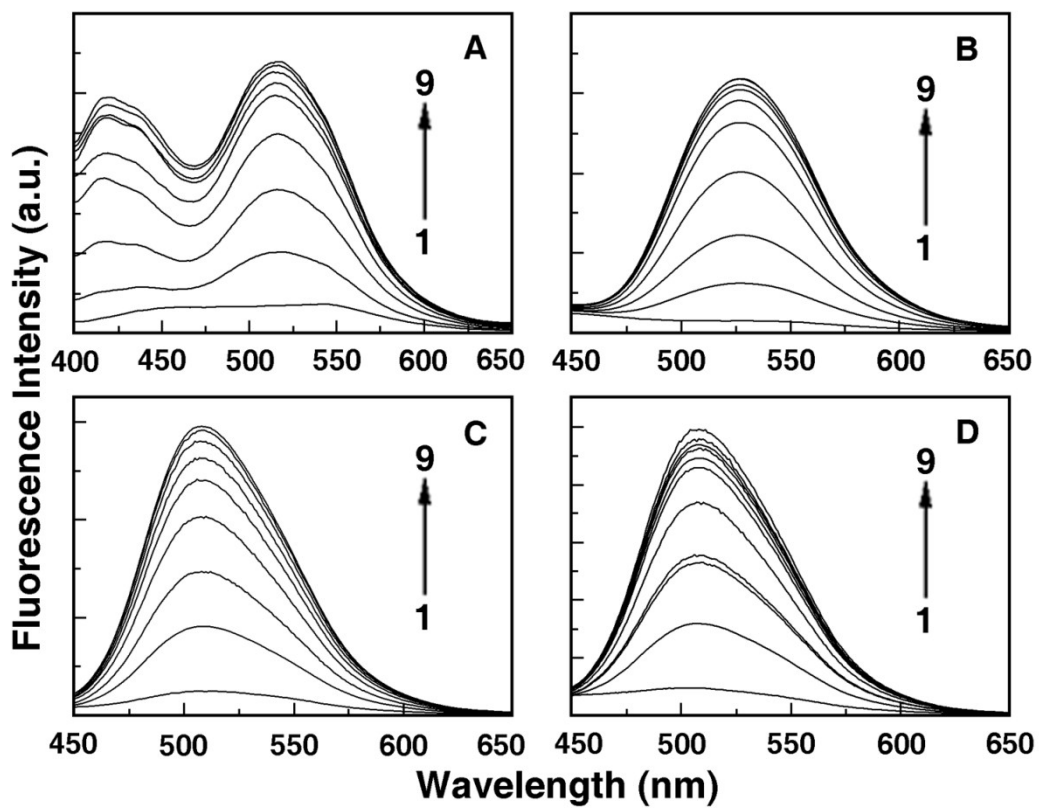
Fig. S3. Fluorescence spectral titration of BER1-4 (6 μM, curve 1 in A-D) with increasing concentration of DNA upto saturation (curves 2-9).
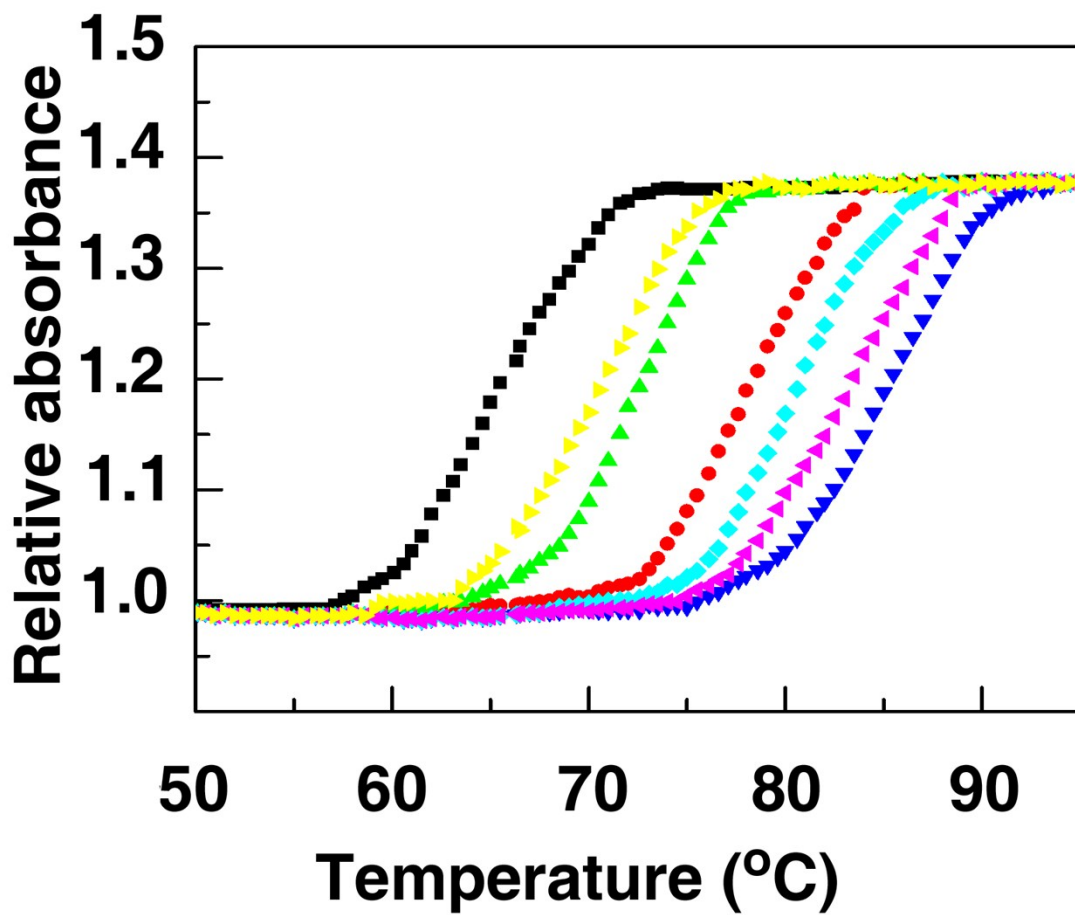
Fig. S4. Thermal melting profiles (relative absorbance change at 260 nm versus temperature) of CT DNA (◯) and complex with BER (▲), BER1 (▲), BER2 (ဢ), BER3 (◯), BER4 (▲) and BER5 (▲).

Fluorescence quenching studies.

Quenching studies were carried out with the anionic quencher $[Fe(CN)_6]^{4-}$. Quenching experiments were performed by mixing, in different ratios, two solutions, one containing KCl and the other containing $K_4[Fe(CN)_6]$, in addition to the normal buffer components, at a fixed total ionic strength. Experiments were performed at a constant P/D (DNA base pair/alkaloid molar ratio) monitoring fluorescence intensity as a function of increasing the concentration of the ferrocyanide ions as described in details previously. The data were plotted as Stern-Volmer plots of relative fluorescence intensity (F_o/F) versus $[Fe(CN)_6]^{4-}$.

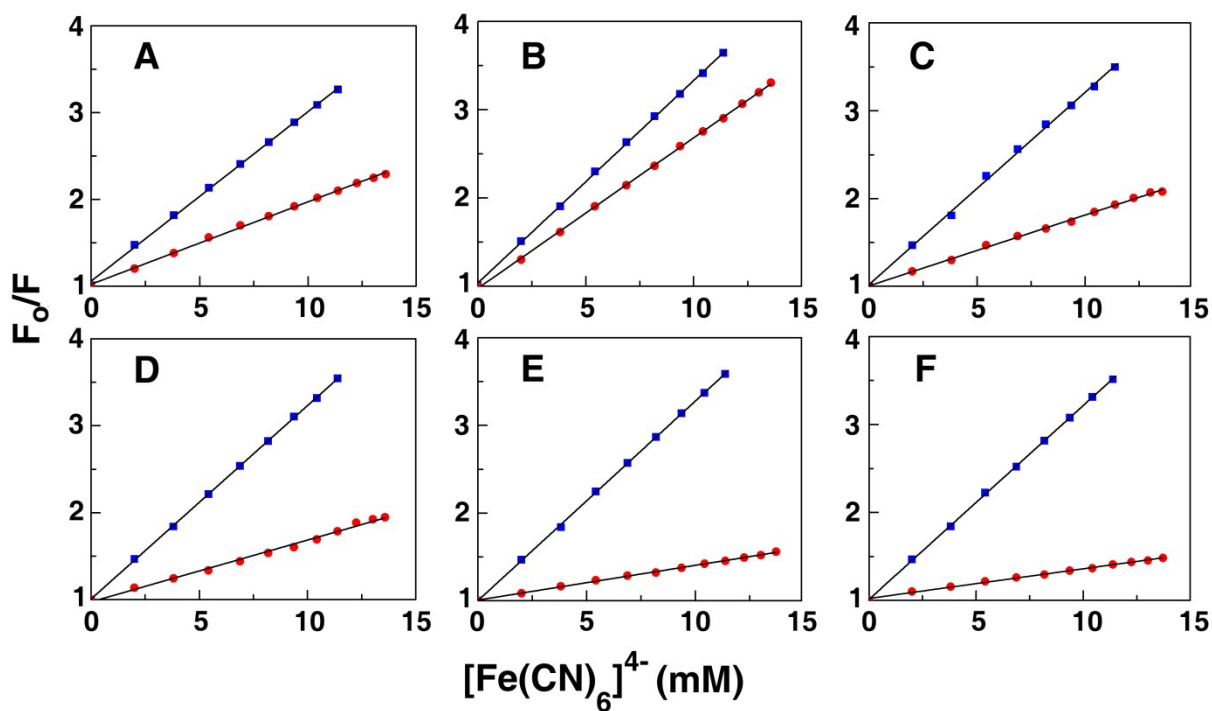**Fig. S5.** Stern-Volmer plots for quenching of the fluorescence intensity versus concentration of $[Fe(CN)_6]^{-4}$ of BER (A), BER1 (B), BER2 (C), BER3 (D), BER4 (E), and BER5 (F) in absence (O) and presence (∞) of CT DNA.

Hydrodynamic studies.

The viscosity of the DNA-alkaloid complexes was determined by measuring the time needed to flow through a Cannon-Manning semi micro size 75 capillary viscometer (Cannon Instruments Company, State College, PA, USA) that was submerged in a thermostated bath (20 ± 1 $^{\circ}$C) as reported previously. Flow times were measured in triplicate to an accuracy of $\pm$ 0.01 seconds with an electronic stopwatch Casio Model HS-30W (Casio Computer Co. Ltd, Tokyo, Japan). Relative viscosities for DNA either in the presence or absence of the alkaloids were calculated from the relation.

$$\eta'_{sp} / \eta_{sp} = \{(t_{complex} - t_o) / t_o\} / \{(t_{control} - t_o) / t_o\} \qquad \text{(Eqn. S3)}$$

where, η'_{sp} and η_{sp} are specific viscosities of the alkaloid-DNA complex and the DNA respectively; $t_{complex}$, $t_{control}$, and t_o are the average flow times for the DNA-alkaloid complex, free DNA and buffer, respectively. The relative increase in length of DNA, L/L_o, is obtained from a corresponding increase in relative viscosity using the following equation.

$$L/L_o = (\eta/ \eta_o)^{1/3} = 1 + \beta r \qquad \text{(Eqn. S4)}$$

where L and L_o are the contour lengths of DNA in presence and absence of the alkaloid and η and η_o are the corresponding values of intrinsic viscosity (approximated by the reduced viscosity $\eta = \eta_{sp}/C$ where C is the DNA concentration) and β is the slope of the plot of L/L_o versus r.
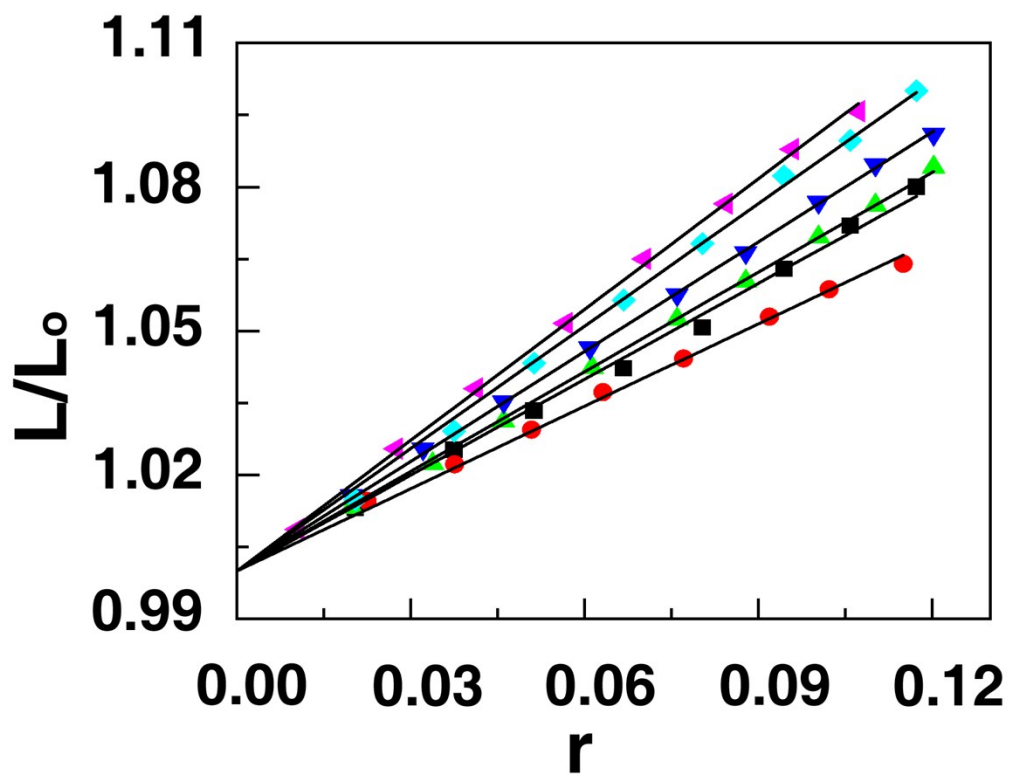
Fig. S6. Plot of increase in helix contour length (L/Lo) versus 'r' for the complexation of BER (O), BER1 (∞), BER2 (▲), BER3 (▲), BER4 (O), BER5 (▲) with DNA.
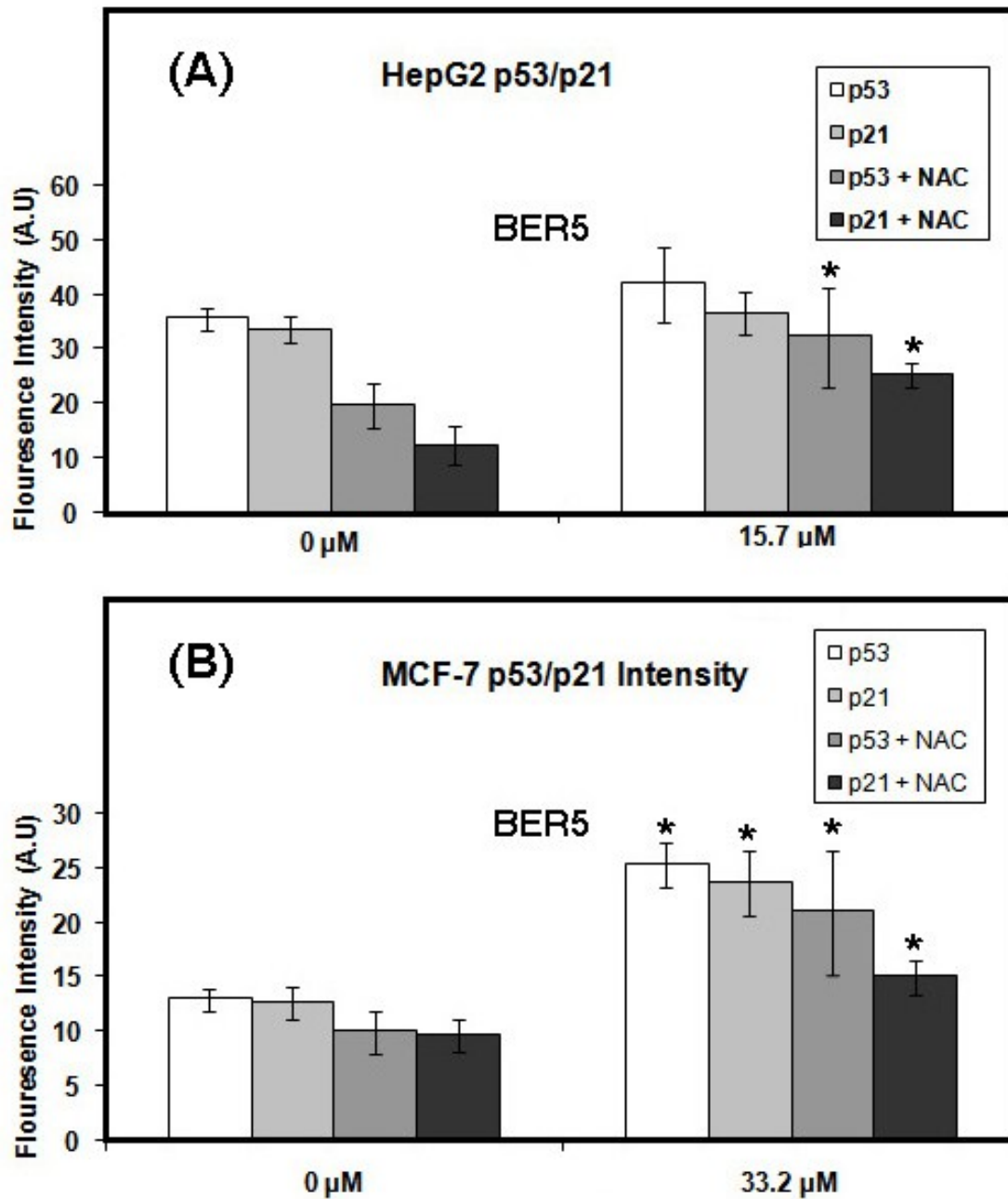
Fig. S7. (A) Fluorescence intensity graph for the expression of p53 and p21 in HepG2 cells following treatment with BER5 after 24 h. (B) Fluorescence intensity graph for the expression of p53 and p21 in MCF-7 cells following treatment with BER5 after 24 h. Values are mean ± S.D and represent one of the three representative experiments. *P<0.05.

Table S1. Binding data of berberine analogues derived from McGhee-von Hippel analysis of the Scatchard plots from spectrophotometric titration data.

Alkaloid analogue	K (M^{-1})	n
BER1	4.45×10^4	7.01
BER2	2.08×10^5	3.30
BER3	3.58×10^5	3.20
BER4	6.90×10^5	3.20
BER5	1.02×10^6	3.21

Table S2. The melting temperature of DNA-alkaloid analogue complexes derived from UV-thermal melting and DSC studies.

System	Melting temperature (°C) (Optical Melting)	Melting temperature (°C) (DSC)
DNA	65.01	65.30
BER	73.03	72.34
BER1	69.20	70.24
BER2	78.34	78.00
BER3	81.76	80.40
BER4	82.95	83.02
BER5	84.58	85.02

Table S3. ITC derived thermodynamic parameters for the binding of berberine analogue BER5 with DNA at different salt conditions.[a]

[Na+]	$K \times 10^5$	$(\Delta_r H^o)$	$(T\Delta_r S^o)$	$(\Delta_r G^o)$	$(\Delta_r G^o{}_{pe})$	$(\Delta_r G^o{}_t)$
mM	(M^{-1})	(kcal/mol)	(kcal/mol)	(kcal/mol)	(kcal/mol)	(kcal/mol)
10	9.96±0.34	-1.04±0.5	7.00	-8.00	-1.84	-6.16
20	6.31±0.42	-0.98±0.7	6.79	-7.72	-1.57	-6.15
30	4.61±0.11	-0.86±0.1	6.73	-7.53	-1.40	-6.13

[a]The ITC experiments were conducted at a pressure of 101.10 kPa at 20 °C. K, the binding constant, N, the binding stoichiometry and $\Delta_r H^o$, the standard molar enthalpy change were evaluated from ITC profiles fitting to Origin 7.0 software using the 'one set of binding sites' model. The values of $\Delta_r G^o$, standard molar Gibbs energy change and $T\Delta_r S^o$, the standard molar entropy contribution were determined using the equations $\Delta_r G^o = \Delta_r H^o - T\Delta_r S^o = -RT\ln K$. R is the universal gas constant (1.98722 cal K^{-1} · mol^{-1}.

Table S4. ITC derived thermodynamic parameters for the binding of berberine analogue BER5 with DNA at three temperatures.[a]

Temperature (°C)	K x10^5 (M^{-1})	$\Delta_\mathrm{r}H^o$ (kcal/mol)	$T\Delta_\mathrm{r}S^o$ (kcal/mol)	$\Delta_\mathrm{r}G^o$ (kcal/mol)
10	14.80±0.85	-0.75±0.02	7.27	-7.97±1.02
20	9.96±0.77	-1.04±0.02	7.00	-8.04±0.55
30	4.91±0.22	-1.27±0.01	6.57	-7.88±0.65

constant, N, the binding stoichiometry and $\Delta_\mathrm{r}H^o$, the standard molar enthalpy change were evaluated from ITC profiles fitting to Origin 7.0 software using the 'one set of binding sites' model. The values of $\Delta_\mathrm{r}G^o$, standard molar Gibbs energy change and $T\Delta_\mathrm{r}S^o$, the standard molar entropy contribution were determined using the equations $\Delta_\mathrm{r}G^o = \Delta_\mathrm{r}H^o - T\Delta_\mathrm{r}S^o = -RT\ln K$. R is the universal gas constant (1.98722 cal K^{-1} · mol^{-1}.