Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary data

Interaction and Photo–induced Cleavage Studies of Copper based Chemotherapeutic Drug with Human Serum Albumin: Spectroscopic and Molecular Docking Study

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Experimental Section.

HSA photocleavage experiments.

The photoinduced protein cleavage studies were done using freshly prepared solution of HSA in 50 mM Tris–HCl buffer (pH 7.2). The protein solutions (15 μM) containing complexes **1** with concentrations ranging from 50 to 300 μM were photoirradiated at 365 nm for 20 min and eppendorf vials were used for the UV–A1 light-induced protein cleavage studies. The sample solutions were incubated at 37 °C for 45 min prior to the photoexposure. After exposure, the samples were evaporated to dryness using EYELA centrifugal vaporizer, followed by addition of 5X loading buffer containing SDS (10% w/v), glycerol (4% w/v), Tris–HCl buffer (50 mM, pH 6.8), mercaptoethanol (2% v/v) and bromophenol blue (0.01% w/v). The samples were then boiled for 3 min to denature the protein completely. The solutions were finally loaded into the wells of 10%/3% (separating/stacking) polyacrylamide gel. The gel electrophoresis was done by applying 50 V until the dye passed into the separating gel from the stacking (3%) gel and the voltage was increased to 100 V. Staining was done with Coomassie Brilliant Blue R-250 solution (acetic acid–methanol–water = 1: 2: 7 v/v) and destaining was done with water–methanol–acetic acid mixture (5: 4: 1 v/v) for 4 h. The gels, after destaining, were

visualized and photographed by Vilber–INFINITY gel documentation system. The presence of reactive oxygen species was investigated by carrying out the photo-induced protein cleavage experiments in the presence of various singlet oxygen quenchers like NaN_3 (3 mM), TEMP (3 mM) and hydroxyl radical scavengers like DMSO (20 µL) and KI (3 mM).

Antitumor activity assays.

The following cell lines were used for in vitro antitumor screening; 786–O, A498 (kidney), Zr–75–1 (Breast), SiHa (Cervix), A549, Hop–62 (Lung), SW620, HCT15 (Colon), MIAPACA2 (Pancreatic). Human malignant cell lines were procured and grown in RPMI–1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics to study growth pattern of these cells. The proliferation of the cells upon treatment with chemotherapy was determined by means of the Sulphorhodamine–B (SR–B) semi–automated assay. All cell lines were seeded into 96 well plates and cells were counted and cell count was adjusted according to the titration readings so as to give optical density in the linear range (from 0.5 to 1.8) and were incubated at 37 ºC in CO_2 incubator for 24 h. The stock solution of the complexes were prepared as 100 mg/mL in DMSO and four dilutions i.e. 10 µL, 20 µL, 40 µL, 80 µL, in triplicates were tested, each well receiving 90µL of cell suspension. The plates were labeled properly and were incubated for 48 h. Termination of experiment was done by gently layering the cells with 50 µL of chilled 30% TCA (in case of adherent cells) and 50 % TCA (in case of suspension cell lines) for cell fixation and kept at 4 ºC for 1h. Plates stained with 50 µL of 0.4% SRB for 20 min. All experiments were made in triplicate.

Figures

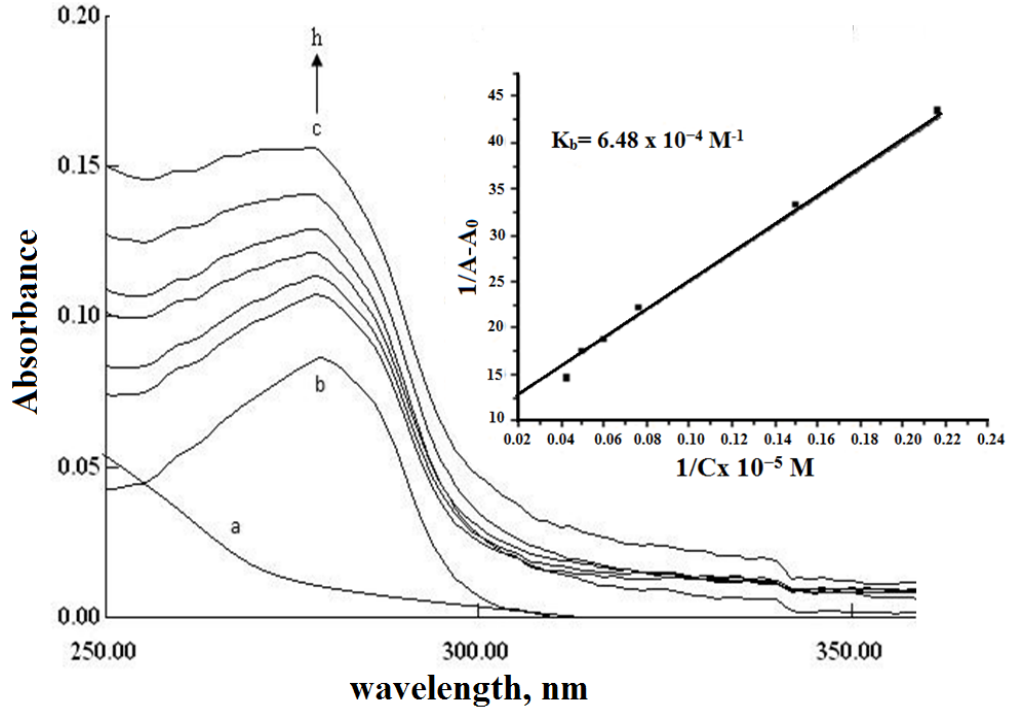

Fig. S1. UV absorption spectra of the HSA–complex **1** conjugate system obtained in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4, at room temperature: (a) complex **1**, 0.60 x 10^{-5} M; (b) HSA, 1.80 x 10^{-6} M; (c–h) complex **1**–HSA, the complex **1** concentrations were 0.67, 1.0, 1.33, 1.67, 2.00, 2.33 x 10^{-5} M, respectively. Arrows show the intensity changes upon increasing concentration of the complex **1**. Inset: Plot of $1/A–A_o$ vs. 1/ [Complex **1**].

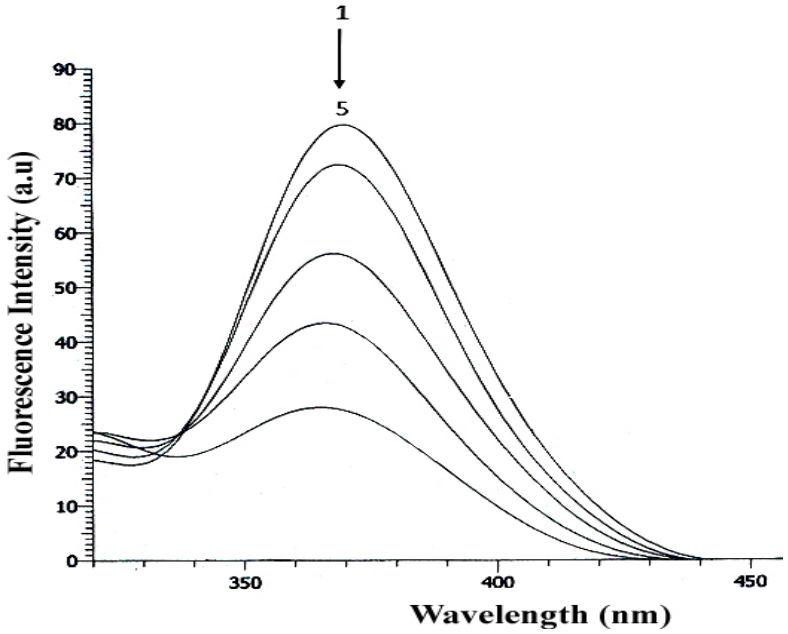

Fig. S2. The fluorescence quenching spectra of HSA by different concentrations of complex **1** with the excitation wavelength at 295 nm in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4, at room temperature: [HSA], 1.0 x10^{-6} M; (1–5) the concentration of complex **1** corresponding to 0, 0.60, 1.00, 2.00, 3.33 x 10^{-5} M, respectively. Arrow shows the intensity changes upon increasing concentration of the quencher.

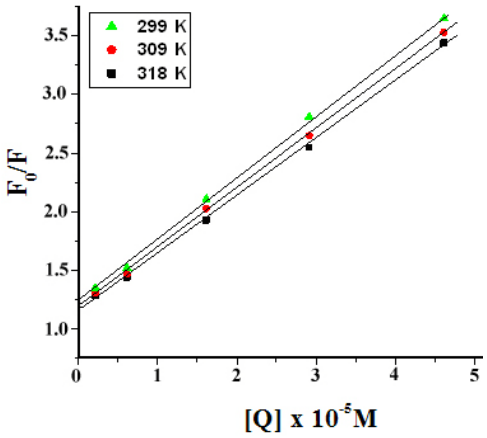

Fig. S3. Stern–Volmer plots showing HSA tryptophan quenching caused by complex **1** at three different temperatures (pH 7.40, λ_{ex} = 295 nm, λ_{em} = 370 nm).

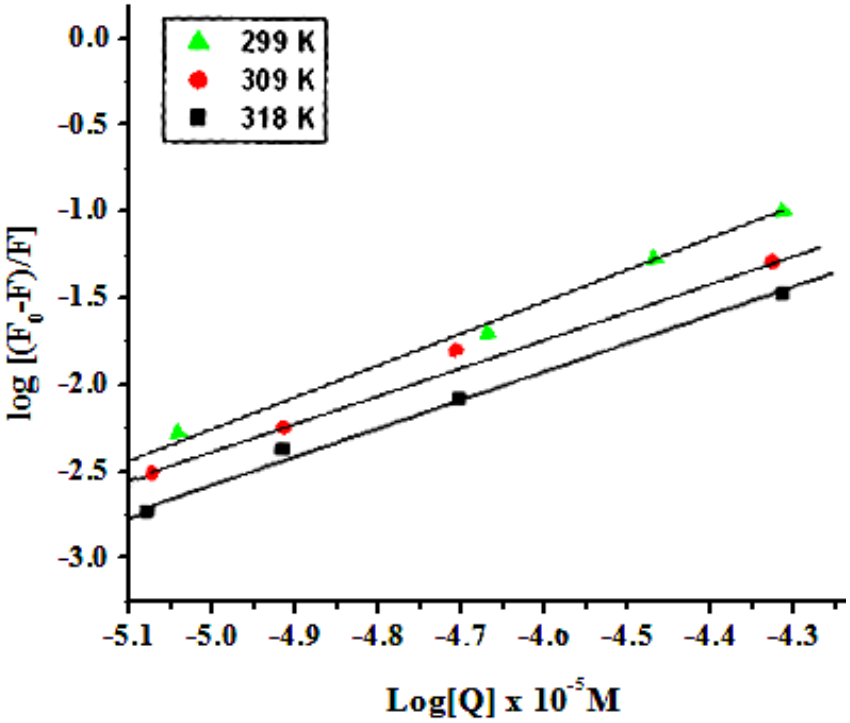

Fig. S4. Logarithmic plot of the fluorescence quenching of HSA at different temperatures.

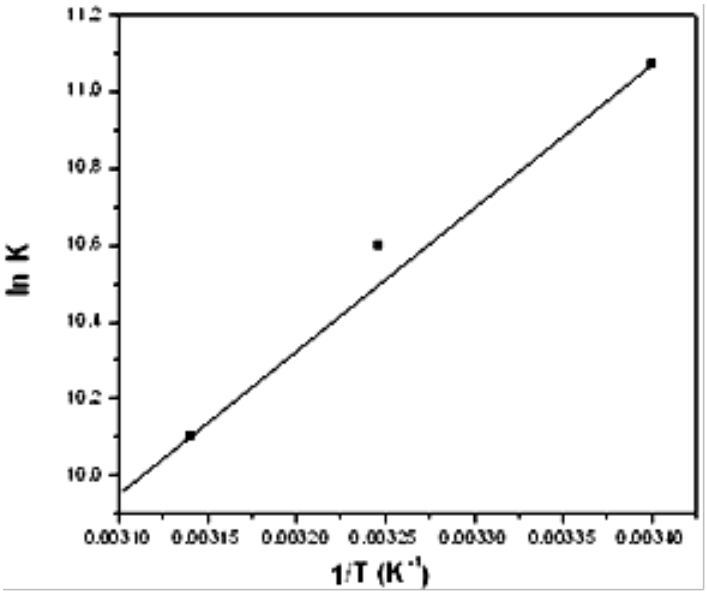

Fig. S5. Van't Hoff plot for the interaction of complex **1** and HSA.

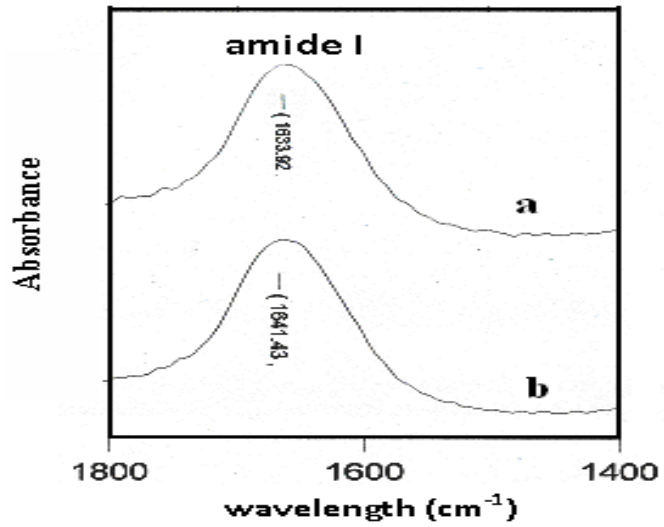

Fig. S6. FT-IR spectra of (a) free HSA; (b) different spectra [(HSA solution + complex **1** solution)–complex **1** solution)] in 5 mM Tris-HCl/50 mM NaCl buffer, pH 7.4, at room temperature in the region of 1750–1400 cm^{-1}, [HSA], 1.5 x 10^{-5} M; [complex **1**], 3.0 x 10^{-5} M.

Tables:

Table S1: Binding constant (K) and the number of binding sites (n) for the complex **1**–HSA system at different temperatures.

pH	T (K)	K($10^4 M^{-1}$)	n	R
	299	6.48	1.68	0.998
7.40	309	5.02	1.71	0.995
	318	3.62	2.10	0.981

Table S2: The binding constant according to Stern–Volmer curves at different NaCl concentrations in Tris–HCl buffer (pH 7.4).

NaCl (mM)	T (K)	K_{sv}(HSA) ($10^4 M^{-1}$)	R
33	299	1.301	0.995
83	309	0.958	0.997
116	318	0.762	0.999