

Metal-Based Anticancer Agents: *In vitro* DNA Binding, Cleavage and Cytotoxicity

Mala Nath, Nagamani Kompelli, Partha Roy, Snehasish Das

Abstract—Two new metal-based anticancer chemotherapeutic agents, $[(\text{Ph}_2\text{Sn})_2(\text{HG}\text{uO})_2(\text{phen})\text{Cl}_2]$ **1** and $[(\text{Ph}_3\text{Sn})(\text{HG}\text{uO})(\text{phen})]\text{Cl}\cdot\text{CH}_3\text{OH}\cdot\text{H}_2\text{O}$ **2**, were designed, prepared and characterized by analytical and spectral (IR, ESI-Mass, ^1H , ^{13}C and ^{119}Sn NMR) techniques. The proposed geometry of Sn(IV) in **1** and **2** is distorted octahedral and distorted trigonal-bipyramidal, respectively. Both **1** and **2** exhibit potential cytotoxicity *in vitro* against MCF-7, HepG-2 and DU-145 cell lines. The intrinsic binding constant (K_b) values of **1** ($2.33 \times 10^5 \text{ M}^{-1}$) and **2** ($2.46 \times 10^5 \text{ M}^{-1}$) evaluated from UV-Visible absorption studies suggest non-classical electrostatic mode of interaction via phosphate backbone of DNA double helix. The Stern-Volmer quenching constant (K_{sv}) of **1** ($9.74 \times 10^5 \text{ M}^{-1}$) and **2** ($2.9 \times 10^6 \text{ M}^{-1}$) determined by fluorescence studies suggests the groove binding and intercalation mode for **1** and **2**, respectively. Effective cleavage of pBR322 DNA is induced by **1**. Their interaction with DNA of cancer cells may account for potency.

Keywords—Anticancer agents, DNA binding studies, NMR spectroscopy, organotin.

I. INTRODUCTION

SEVERAL research groups [1]–[13] worldwide are working in the field of oncology in search of metal-containing compounds exhibiting potential anticancer activity after the serendipitous discovery of *cis*-platin, a platinum(II) antitumor drug. A variety of second and third generation platinum drugs, *viz.* carboplatin (trade name paraplalin), iproplatin, oxaliplatin, picoplatin, nedaplatin [14]–[16] have been developed, tested, and are currently recommended for clinical use, and the next generation platinum-based antineoplastic agents are now under phase I and II trials. Owing to the limitations such as stringent side effects, high cost, intrinsic and extrinsic resistance of Pt-based drugs, scientists are in quest for a better compound with lesser side effects, higher selectivity and efficacy. Recently, a number of metal-based compounds with significant antitumour activity have been screened and reviewed [17]–[23] and among these, gold and organotins attained particular interest amongst non-platinum chemotherapeutics because of their common activity on mitochondria and strong affinity to thiol groups of proteins and enzymes [24]–[26]. Encouraging cytotoxicity of certain

organotin(IV) complexes *in vitro* [27]–[29] and their debatable mode of action, have led us to focus mainly in this area.

Organotin(IV) compounds have diversified applications in industries, agriculture and pharmaceuticals [27]–[35]. Several organotin(IV) derivatives are known to exhibit good anti-inflammatory [36]–[45] and anti-proliferative [46] activity. Various theories have proposed that the anti-proliferative effects displayed by organotins may be due to their binding with thiol groups of proteins [46] or with sugar-phosphate backbone of DNA [47]–[49]. The binding disposition of organotins with DNA is subjected to the coordination number/stereochemistry, and the nature of groups directly attached to the central tin atom. 1,10-Phenanthroline derivatives are known to have promising activity against cancer, and viral, bacterial, and fungal infections [50]. Early reports for the syntheses of organotins and inorganic tins with 1,10-phenanthroline [51] are available in the literature. Despite an increase in the order of magnitude of cytotoxicity and exact mechanism of action is still a question to be answered. In order to obtain better insight, we aim to develop and study new organotin(IV) complexes of biological interest, with the hope to understand their DNA-interaction.

II. EXPERIMENTAL SECTION

A. Materials and Methods

Triphenyltin(IV) chloride, guanosine (Sigma Aldrich), 1,10-phenanthroline (Qualigens fine chemicals) and diphenyltin(IV) dichloride (Sigma Aldrich) were used as received. All the syntheses were carried out under anhydrous nitrogen atmosphere and precautions were taken to avoid the presence of oxygen at every stage. Tris(hydroxymethyl)aminomethane (Genei), agarose (Hi-media) and pBR322 supercoiled plasmid DNA (Genei), Calf thymus DNA (CT DNA) sodium salt (extrapure) (Sisco Research Laboratories (SRL)) were used as received. All the other chemicals were of reagent grade and used without further purification. CT DNA stock solution prepared in Tris-HCl/NaCl buffer (0.01 M, pH 7.2, 5:50 mM) gave UV absorbance at 260 and 280 nm of 1.9:1 indicating that DNA was free from proteins. The DNA concentration per nucleotide was determined by the UV absorbance at 260 nm [52] using extinction coefficients $6600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$.

The melting points of the synthesized complexes were determined on a Toshniwal capillary melting point apparatus and were uncorrected. Molar conductance was measured at room temperature on a Eutech con 510 electronic conductivity bridge. Elemental analyses of the complexes were obtained on

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
a Vario EL III CHNOS Elemental Analyzer. Thermo Scientific Nicolet 6700 FT-IR Spectrometer was used for recording IR spectra (KBr pellets) in the range of 400–4000 cm^{-1} and also for recording Far-IR spectra (CsI pellets) in the range of 600–190 cm^{-1} . ESI-Mass spectra were recorded on a micrOTOF-Q II 10328 mass spectrometer in acetonitrile. ^1H , ^{13}C and ^{119}Sn NMR spectra were recorded on a Bruker Avance-500 (500 MHz) NMR spectrometer in DMSO-*d*₆ at 25°C. Electronic spectra were recorded on a Shimadzu UV-1800 UV-VIS spectrophotometer in a cell of 1 cm path length. Emission spectra were determined with a Shimadzu RF5301 (2006) fluorescence spectrophotometer. DNA cleavage experiments were performed with the help of Genei submarine electrophoresis supported by Genei power supply with a potential range of 50–250 V, visualized and photographed by Gel documentation system (Bio Rad, USA).

B. Synthesis of $[(\text{Ph}_2\text{Sn})_2(\text{HGuO})_2(\text{phen})\text{Cl}_2]$ (1)

Guanosine (H_2GuO) (0.566 g, 2.0 mmol) was dissolved in the minimum amount (20 mL) of aqueous methanol (1:1 or 50%) under nitrogen. The pH of the solution was maintained at 2–3 by the addition of few drops of conc. HCl. To this was added an aqueous methanol (20 mL, 1:1) solution of diphenyltin(IV) dichloride (0.688 g, 2.0 mmol). The resulting solution was refluxed with constant stirring for ~20 h under nitrogen atmosphere. An aqueous methanolic (20 mL, 1:1) solution of 1,10-phenanthroline (0.18 g, 1.0 mmol) was added to the resulting solution and was refluxed for another ~3 h with constant stirring. After chilling, a pale red solid product thus obtained was washed with water and methanol-hexane or methanol-petroleum ether (b.p. 40–60°C) mixture (1:3 v/v), and dried under vacuum. m.p. 208–210°C (dec.); M (DMSO) 11.0 $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ (non-electrolyte); Anal. Calc. for $[\text{C}_{56}\text{H}_{52}\text{Cl}_2\text{N}_{12}\text{O}_{10}\text{Sn}_2]$ (%): C 49.41, H 3.85, N 12.35. Found: C 49.69, H 4.25, N 11.67; ESI-MS (m/z) 1361.39. IR data (KBr, ν/cm^{-1}): 3564 sbr, 3466 sbr, 3328 sbr, 3213 sbr, $\nu(\text{OH})/\nu(\text{NH}_2)$; 1731 s, 1694 s, 1624 s, $\nu(\text{C}=\text{O})/\nu(\text{C}=\text{N})$; 1578 sh, 1537 s, 1484 s, 1427 s, 1393 m, 1344 m, $\nu(\text{C}=\text{C})/\nu(\text{ring})$; 1250 sh, $\nu(\text{C}-\text{H})$ in plane deformation; 1223 s, 1178 s, 1130 s, 1084 s, 1047 s, $\nu(\text{C}-\text{O})/\nu(\text{C}-\text{N})$ of ribose/1,10-phenanthroline ring (C–H) vibration; 850 s, $\nu(\text{ring})$ out of plane on heterocyclic rings/Ribose pucker; 289 s, 210 m, $\nu_{\text{as}}(\text{Sn}-\text{C})/\nu_{\text{s}}(\text{Sn}-\text{C})$; 460 s, $\nu(\text{Sn}-\text{O})$; 370 w, $\nu(\text{Sn}-\text{N})/\nu(\text{Sn}-\text{N})$; 425 w, 262 s, 1,10-phenanthroline ring deformation. ^1H NMR (DMSO-*d*₆, δ ppm): guanosine protons: 10.64 s, 2H (N1-H); 7.94 s, 2H (H-8); 6.40 s, 4H (NH₂); 5.69 d, 2H, $J = 5.95$ Hz (H-1'); 5.40 sbr, 2H (OH-3'); 5.09 sbr, 2H (OH-5'); 4.39 t, 2H, $J = 5$ Hz (H-2'); 4.08 t, 2H, $J = 3.9$ Hz (H-3'); 3.86 q, 2H, $J = 3.7$ Hz (H-4'); 3.62–3.59 dd, 2H, $J = 3.9$ Hz and 3.53–3.50 dd, 2H, $J = 3.8$ Hz (H-5'); 1,10-phenanthroline protons: 9.32 d, 2H, $J = 1.5$ Hz (H-2, H-9); 8.70 s, 2H (H-4, H-7); 7.98 m, 2H (H-5, H-6); 7.84 s, 2H (H-3, H-8); Sn-phenyl: 8.15–8.58 m, 8H, (H- β); 7.27–7.50 m, 12H (H- γ , H- δ). ^{13}C NMR (DMSO-*d*₆, δ ppm): guanosine carbons: 156.70 (C-6); 153.65 (C-2); 151.26 (C-4); 135.58 (C-8); 116.53 (C-5); 86.36 (C-1'); 85.17 (C-4'); 73.67 (C-2'); 70.32 (C-3'); 61.35 (C-5'); 1,10-phenanthroline carbons: 148.75 (C-9, C-2); 134.13, 133.91 (C-4, C-

7); 127.70, 127.43 (C-6, C-5); 125.01, 124.90 (C-3, C-8); 129.00 (C-4a, C-6a/C-4b, C-6b); Sn-phenyl: 129 (C- α), 137.5 (C- β), 128.8 (C- γ , C- δ). ^{119}Sn NMR (DMSO-*d*₆, δ ppm): –362.28.

C. Synthesis of $[\text{Ph}_3\text{Sn}(\text{HGuO})(\text{Phen})]\text{Cl} \cdot \text{CH}_3\text{OH} \cdot \text{H}_2\text{O}$ (2)

The procedure was same as described in section B, except triphenyltin(IV) chloride (0.385 g, 1.0 mmol), guanosine (H_2GuO) (0.283 g, 1.0 mmol) and 1,10-phenanthroline (0.18 g, 1.0 mmol) were added in the ratio of 1:1:1. m.p. 150–152°C (dec.); Anal. Calc. for $[\text{C}_{41}\text{H}_{40}\text{ClN}_7\text{O}_7\text{Sn}]$ (%): C 54.90, H 4.49, N 10.93. Found: C 53.39, H 3.99, N 9.69; ESI-MS (m/z) 846.90. IR data (KBr, ν/cm^{-1}): 3571 s, 3469 sbr, 3323 sbr, 3211 sbr, $\nu(\text{OH})/\nu(\text{NH}_2)$; 1733 s, 1694 s, 1627 m, $\nu(\text{C}=\text{O})/\nu(\text{C}=\text{N})$; 1571 s, 1537 s, 1486 s, 1425 s, 1396 s, 1338 sh, $\nu(\text{C}=\text{C})/\nu(\text{ring})$; 1250 s, $\nu(\text{C}-\text{H})$ in plane deformation; 1180 s, 1130 s, 1084 s, 1046 sh, $\nu(\text{C}-\text{O})/\nu(\text{C}-\text{N})$ of ribose/1,10-phenanthroline ring (C–H) vibration; 883 s, $\nu(\text{ring})$ out of plane on heterocyclic rings/Ribose pucker; 273 s, 211 s, $\nu_{\text{as}}(\text{Sn}-\text{C})/\nu_{\text{s}}(\text{Sn}-\text{C})$; 446 s, $\nu(\text{Sn}-\text{O})$; 335 s, $\nu(\text{Sn}-\text{N})/\nu(\text{Sn}-\text{N})$; 427 m, 260 sh, 243 s, 1,10-phenanthroline ring deformation. ^1H NMR (DMSO-*d*₆, δ ppm): guanosine protons: 10.69 s, 1H (N1-H); 7.98 s, 1H (H-8); 6.50 sbr, 2H (NH₂); 5.70 d, 1H, $J = 5.95$ Hz (H-1'); 5.60–4.90 br, 2H (OH-5' and OH-3'); 4.39 t, 1H, $J = 5.4$ Hz (H-2'); 4.08 t, 1H, $J = 4.2$ Hz (H-3'); 3.88–3.85 q, 1H, $J = 3.75$ Hz (H-4'); 3.62–3.59 dd, 1H, $J = 4.0$ Hz and 3.53–3.50 dd, 1H, $J = 4.0$ Hz (H-5'); 1,10-phenanthroline protons: 9.21 d, 2H, $J = 2.95$ Hz (H-2, H-9); 8.8 d, 2H, $J = 8.0$ Hz (H-4, H-7); 8.22–8.13 m, 2H (H-5, H-6); 8.02–7.99 m, 2H (H-3, H-8); Sn-phenyl: 7.87–7.79 m, 6H (H- β); 7.44–7.38 m, 9H (H- γ , H- δ). ^{13}C NMR (DMSO-*d*₆, δ ppm): guanosine carbons: 156.59 (C-6); 153.68 (C-2); 151.20 (C-4); 135.54 (C-8); 116.32 (C-5); 86.38 (C-1'); 85.16 (C-4'); 73.67 (C-2'); 70.28 (C-3'); 61.30 (C-5'); 1,10-phenanthroline carbons: 148.59 (C-9, C-2); 136.05 (C-4, C-7); 127.13 (C-6, C-5); 124.56 (C-3, C-8); Sn-phenyl: 129.04 (C- α), 139.13 (C- β), 128.19 (C- γ), 128.81 (C- δ). ^{119}Sn NMR (DMSO-*d*₆, δ ppm): –253.11. 

D. DNA Binding Studies

Absorption spectral titration experiments were performed in 10% DMSO/Tris–HCl buffer by diluting an appropriate amount of metal complex solution and DNA stock solutions while maintaining the total volume constant. The concentration of **1** and **2** were maintained constant (8.24×10^{-6} M and 3.14×10^{-5} M, respectively) and the DNA concentration (2.46 – 19.4×10^{-6} M) is varied, resulting in a series of solutions. Absorbance (A) was recorded with successive addition of CT DNA to both the compound solution and the reference solution to eliminate the absorbance of the DNA itself. The intrinsic binding constant K_b of the complex to CT DNA was determined from (2) through a plot of $[\text{DNA}]/(e_a - e_f)$ vs $[\text{DNA}]$, where $[\text{DNA}]$ is the concentration of the DNA in base pairs; e_a , e_f and e_b , the apparent extinction coefficient (A_{obs}/M), the extinction coefficient for the free metal (M) and the extinction coefficient for the metal complex in the fully

bound form, respectively. In plots of $[DNA]/(e_a - e_f)$ vs $[DNA]$, K_b is given by the ratio of the slope to the intercept.

Fluorescence spectral measurements were carried out using Shimadzu RF5301 (2006) fluorescence spectrophotometer. Tris-HCl/NaCl buffer was used as a blank to make preliminary adjustments. The excitation wavelength was fixed and the emission range was adjusted before measurements. The fluorescence quenching constants ' K_{sv} ' were obtained by titrating the fixed amount of ethidium bromide (EB)-bound CT DNA [0.125 mM for **1**; 0.006 mM for **2**] with increasing amount of metal complexes $[0-50 \times 10^{-6} \text{ M}]$. An excitation wavelength of 263 nm was used and the total fluorescence emission was monitored at 603 nm. The measured fluorescence was normalized to 100% relative fluorescence.

E. Gel Electrophoresis

DNA cleavage experiments were executed using pBR322 plasmid DNA. The supercoiled DNA in buffer solution (5:50 mM Tris-HCl/NaCl, pH 7.2) was treated with different concentrations of **1** and **2** (5–50 μM , $r = 0.1-1.0$, where $r = [\text{complex}]/[\text{DNA}]$) and diluted with the buffer to a total volume of 10 μL . The samples were incubated at 37°C for 2.5 h. The loading buffer (30 mM EDTA, 0.05% (w/v) glycerol, 36% (v/v) bromophenol blue) (3 μL) was added to the samples after incubation and were loaded onto agarose gel (0.8%) containing EB (0.5 $\mu\text{g}/\text{mL}$). The gel was run in 0.5X TBE buffer at 50 V/cm for 2 h. Bands were visualized and photographed by Gel documentation system (Bio Rad, USA).

F. Cytotoxicity Assay (MTT Assay)

1 and **2** were evaluated *in vitro* against three cancer cell lines (purchased from National Center for Cell Science (NCCS) Pune, India) of human origin, viz. MCF-7 (mammary cancer), HepG-2 (liver cancer), DU-145 (prostate cancer). Cell viability was determined through a colorimetric method based on the tetrazolium salt MTT (Mosmann, 1983), which yielded purple formazan crystals on reduction in the cells. Stock solutions were prepared by dissolving the complexes in DMSO (HPLC Grade). On first day of experiment, approximately 5×10^3 cells in 200 μL of media were seeded in 96-well flat bottom microtitre plates (Corning, New York, USA) and were preincubated at 37°C for 66 h, in 5% CO_2 , for the cells to adhere. On the second day, serial dilutions of the stock ranging from 0–200 mM in DMSO were prepared. On day 3, vehicle control (or negative control) (with same concentration of DMSO as used for preparing dilutions) and 50 μL of each dilution (test and reference) were added in triplicate to three consecutive wells. The cell cultures were then incubated at 37°C for 24 h. On day 4, cultures were assayed by adding 10 μL of 5 mg/mL MTT and again incubating the plates for 4 h at 37°C. Later, the MTT containing media was aspirated and 200 μL DMSO and 25 μL sorenson glycine buffer (0.1 M of glycine and 0.1 M of NaCl, at pH 10.5) were added to lyse the cells and solubilise the water insoluble purple formazan. The absorbance values of the cell lysates were measured on a Dynatech MR 5000

microplate reader at 584 nm. The % inhibition was calculated according to (1):

$$\% \text{ Inhibition} = \frac{\text{Mean OD of negative control} - \text{Mean OD of treated cells}}{\text{Mean OD of negative control}} \times 100 \quad (1)$$

III. RESULTS AND DISCUSSION

Reaction(s) of Ph_2SnCl_2 and Ph_3SnCl with guanosine (H_2GuO) and 1,10-phenanthroline (phen) in aqueous methanol (50%) at 2:2:1/1:1:1, respectively, led to the formation of new mixed-ligand complexes of general formulae, $[(\text{R}_2\text{Sn})_2(\text{HGGuO})_2(\text{phen})\text{Cl}_2]$ (**1**) and $[(\text{R}_3\text{Sn})(\text{HGGuO})(\text{phen})]\text{Cl} \cdot \text{CH}_3\text{OH} \cdot \text{H}_2\text{O}$ (**2**). Both the complexes obtained are pale red solid and are stable towards air and moisture. In common organic solvents, they are insoluble but readily soluble in DMSO.

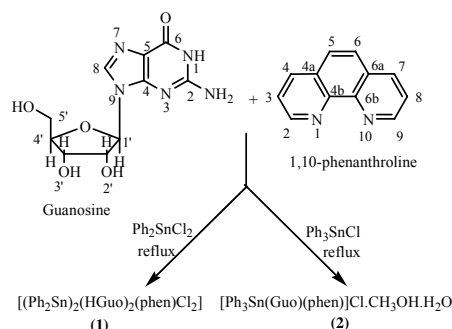


Fig. 1 Labelling and schematic representation of formation of complex **1** and **2**

A. IR and Far IR Spectroscopy

The characteristic IR and Far IR frequencies (cm^{-1}) and their assignments of **1** and **2** are presented in Experimental section. A minor shift of OH/NH_2 ($3466/3469 \text{ cm}^{-1}$) and $\nu(\text{C}=\text{O})$ ($1731, 1694/1733, 1694 \text{ cm}^{-1}$) of the nitrogen base unit for **1** and **2** is observed indicating that base ring of guanosine is not involved in complexation. For complex **1**, $\nu(\text{C}-\text{O})$ (1223 cm^{-1}) of ribose $-\text{OH}$ shifted a little towards lower frequency indicating its interaction with Sn. The $\nu(\text{ring})$ characteristic of the ribose pucker shows a considerable shift towards lower wave number for **1** (850 cm^{-1}) and higher frequency for **2** (883 cm^{-1}), which suggests that furanose $-\text{OH}$ of guanosine may be involved in bonding with tin. The appearance of new bands in Far IR region (in cm^{-1}) for **1** and **2** at 289 ($\nu_{\text{as}}(\text{Sn}-\text{C})$), 273 ($\nu_{\text{s}}(\text{Sn}-\text{C})$), 460 ($\text{Sn}-\text{O}$), 370 ($\text{Sn}-\text{N}/\text{Sn}-\text{N}$) and 210 ($\nu_{\text{as}}(\text{Sn}-\text{C})$), 211 ($\nu_{\text{s}}(\text{Sn}-\text{C})$), 446 ($\text{Sn}-\text{O}$), 335 ($\text{Sn}-\text{N}/\text{Sn}-\text{N}$), respectively, indicate that the new $\text{Sn}-\text{X}$ ($\text{X} = \text{O}/\text{N}$) bonds were formed with oxygen of guanosine and nitrogen of 1,10-phenanthroline. A significant shift towards higher wave number for $\nu(\text{C}=\text{N})$ at $1624/1627 \text{ cm}^{-1}$ and appearance of ring deformation peaks ($425, 262/427, 260, 243 \text{ cm}^{-1}$) of 1,10-phenanthroline for **1** and **2**, respectively, clearly show that a change in 1,10-phenanthroline-ring might have occurred due to bond formation.

B. NMR Spectroscopy

The ^1H , ^{13}C and ^{119}Sn NMR spectra of **1** and **2** were recorded in $\text{DMSO}-d_6$. The $\text{N}(1)\text{H}$ chemical shift of guanosine

base moiety drifted downfield but the H-8 and NH₂ showed negligible upfield shift. All the protons and hydroxyl protons (–OH) of furanose ring shifted upfield except for H-5' protons, clearly indicate that coordination to tin occurs via ribose sugar. A downfield shift of all protons is observed for 1,10-phenanthroline moiety, show that electron fluctuation might have occurred due to reorganisation for new Sn-N (phen) bond. The phenyl-Sn resonances in aromatic region (δ 7.79–8.58 (*o*) and δ 7.27–7.50 (*m*, *p*) ppm) appeared in multiplet pattern, hence, $^2J [^{119}\text{Sn}-^1\text{H}]$ could not be resolved. Similarly, in ^{13}C NMR, a negligible downfield shift is observed for all carbon atoms, and $^1J [^{119}\text{Sn}-^{13}\text{C}]$ value could not be resolved. Therefore, ^{119}Sn NMR spectrum is incumbent for proposing the geometry. The diphenyltin complex (**1**) showed a ^{119}Sn chemical shift at δ –362.28 ppm, which may suggest octahedral geometry around tin. ^{119}Sn NMR spectrum of triphenyltin complex (**2**) showed a resonance at δ –253.11 ppm indicating the presence of distorted trigonal-bipyramidal geometry around tin.

C. ESI-Mass Spectroscopy

The complex **1** and **2** have been unambiguously characterized through mass spectral analysis. The ESI-Mass spectrum of complex **1** and **2** showed the molecular ion peak *m/z* at 1361.39 and 846.9, which was assigned to $[\text{C}_{28}\text{H}_{26}\text{ClN}_6\text{O}_5\text{Sn}]_2$ and $[\text{C}_{41}\text{H}_{40}\text{ClN}_7\text{O}_7\text{Sn}]$, respectively.

D. DNA Binding Studies by UV-Visible Absorption Spectrometry

The absorption spectra of the complexes show intense absorption bands arising from the intraligand transitions located at 263 nm and 330 nm. Upon increasing the concentration of DNA, a considerable increase in the absorptivity with apparent red shift of 2 nm for complex **1** and blue shift of 0.5 nm upto 9.8×10^{-6} M concentration and later red shift of 0.4 nm for complex **2** was observed as shown in Figs. 2 (a) and (c), respectively. The hyperchromism in the spectra of the complex **1** was found to be correlated with the non-classical electrostatic binding mode with DNA and may arise due to the preferential binding of the Sn(IV) to dinegative phosphate group of DNA backbone because of its hard Lewis acidic property. The complex does not exhibit any pronounced sequence or base preference while interacting with DNA which elicits electrostatic binding of the complex. For complex **2**, a decrease in the intensity suggests that the complex can unwind DNA helix and leads to the loss of helicity, perturbing the secondary structure of DNA. As complexes **1** and **2** are non-planar, the presence of two/three phenyl groups induces steric effects which also promote partial intercalative binding of complexes to DNA. The intrinsic binding constant determined according to (2) for the complex **1** and **2** was found to be $2.33 \times 10^5 \text{ M}^{-1}$ and $2.46 \times 10^5 \text{ M}^{-1}$, respectively, indicating pronounced binding with DNA.

$$\frac{[\text{DNA}]}{[e_a - e_f]} = \frac{[\text{DNA}]}{[e_b - e_f]} + \frac{1}{[e_b - e_f]K_b} \quad (2)$$

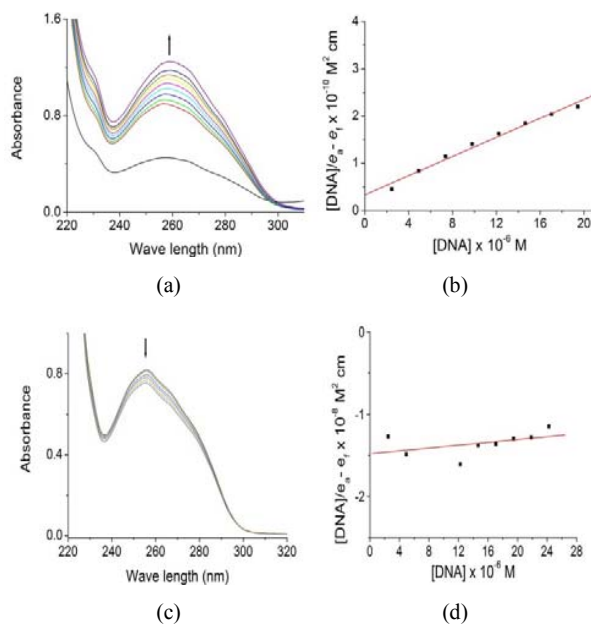


Fig. 2 Variation of UV-visible absorption for complex **1**: (a) (8.24×10^{-6} M); **2**: (c) (3.14×10^{-5} M) with increase in the concentration of CT DNA (2.46 – 19.4×10^{-6} M) in buffer (5 mM Tris–HCl/50 mM NaCl, pH = 7.2) at room temperature. Arrows indicate the change in absorbance upon increasing the [DNA]. (b) and (d) are plots of $[\text{DNA}]/(e_a - e_f)$ vs $[\text{DNA}]$ for the titration of CT DNA with complex

E. Fluorescence Studies

The binding of the complexes to CT DNA was studied by evaluating the fluorescence emission intensity of the ethidium bromide (EB)–DNA system upon the addition of series of concentrations of both the complexes. The emission spectra of **1** and **2** in the absence of DNA and presence of DNA are presented in Figs. 3 (a) and (b), respectively, when excited at 263 nm and the emission wave length is 603 nm. As evident from the spectra, the intensity of the emission ($\lambda = 603$ nm) decreases for **1** because it replaces the EB. Emission intensity increases in case of **2** appreciably in presence of DNA indicative of the binding of the complex **2** to the hydrophobic pockets of DNA. This is due to the fact that the metal complex is bound in a relatively non-polar environment compared to water [53]. Although the emission enhancement could not be regarded as criterion for binding mode, they are related to the extent to which the complex gets into the hydrophobic environment inside the DNA and avoid or reduce the accessibility of solvent molecules to the complex.

Furthermore, the relative binding propensity of the complexes to CT DNA was determined by the classical Stern-Volmer equation according to (3) [54].

$$\frac{I_0}{I} = 1 + K_{sv}r \quad (3)$$

I and I_0 are fluorescence intensities in absence and presence of quencher, respectively; K_{sv} is the linear Stern-Volmer quenching constant, dependent on r_{bE} (the bound concentration of EB to the [DNA]) and r is the ratio of [quencher] to that of [DNA]. The Stern-Volmer quenching

constants can be used to evaluate the nature of DNA-binding modes; a value above 10^6 M^{-1} is an indication of intercalation, while values in the range 10^4 – 10^5 M^{-1} imply the groove binding mode [55]–[59]. The Stern-Volmer quenching constant (K_{sv}) of complex **1** and **2** is 9.74×10^5 and $2.9 \times 10^6 \text{ M}^{-1}$, respectively. Therefore, from K_{sv} values, the DNA-binding mode of **1** might be by the groove binding and that of **2** by intercalation.

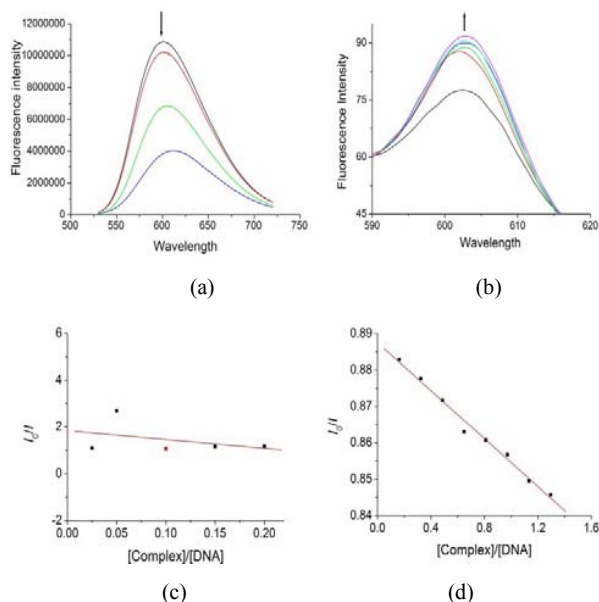


Fig. 3 Emission spectra of complex **1**: (a); **2** (b) in Tris-HCl buffer (pH = 7.2) in the absence and presence of CT DNA. [Complex] = 0 – $50 \times 10^{-6} \text{ M}$, [DNA] = 0.125 mM for **1**; [DNA] = 0.006 mM for **2**. Arrows indicate the change in emission intensity upon increasing the [Complex]; (c) and (d) Stern-Volmer quenching plots of EB bound to DNA by complex **1** and **2**

F. Gel Electrophoresis

To assess the DNA cleavage ability of the **1** and **2**, pBR322 DNA was incubated with different concentrations of the complexes (5 – $50 \mu\text{M}$) in 5 mM Tris-HCl/ 50 mM NaCl buffer at pH 7.2, for 2.5 h. When circular plasmid DNA was subjected to gel electrophoretic mobility assays, relatively fast migration was observed for intact supercoiled Form I. However, if scission of DNA occurs at one strand, the supercoiled DNA converted in to a slower moving open/nicked circular Form II. If both strands are cleaved, a linear Form III was generated [60].

Upon gel electrophoresis, as depicted from Fig. 4 (a), there is an enhancement of the intensity of Form II upon increasing concentration of complex **1** up to $r = 0.5$, followed by a slight decrease in Form II at $r = 1$. This clearly indicates that complex **1** promotes the conversion of DNA from supercoiled Form I to nicked circular form II, which clearly implicates the role of organotin in the process of DNA cleavage. In case of complex **2**, there is a change in the intensities of Form I to Form II as compared to control with increase in the

concentration of complex up to $r = 0.5$ (Fig. 4 (b)), indicating that complex **2** also promotes DNA cleavage to some extent.

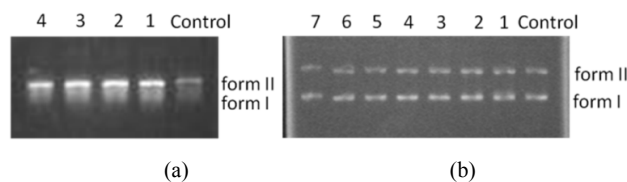


Fig. 4 Agarose gel (0.8%) electrophoretograms of cleavage of pBR322 DNA plasmid with complex **1**: (a); **2**: (b) incubated for 2.5 h at 37°C in $5:50 \text{ mM}$ Tris-HCl/NaCl buffer, pH 7.2. From right to left: (a) DNA control; lane 1, $r = 0.1$; lane 2, $r = 0.2$; lane 3, $r = 0.5$; lane 4, $r = 1$; (b) DNA control; lane 1, $r = 0.1$; lane 2, $r = 0.2$; lane 3, $r = 0.3$; lane 4, $r = 0.5$; lane 5, $r = 0.7$; lane 6, $r = 0.9$; lane 7, $r = 1$, where [complex] = 5 – $50 \mu\text{M}$; $r = [\text{complex}]/[\text{DNA}]$

G. Cytotoxicity Assay (MTT assays)

Both the complexes exhibited *in vitro* potential cytotoxic activity against MCF-7 (mammary cancer), HepG-2 (liver cancer) and DU-145 (prostate cancer) cell lines (Table I). Further, both complexes are more active in comparison to *cis*-platin but less active than 5-fluorouracil against HepG-2 cell line. They exhibit comparable cytotoxicity against DU-145 in comparison to *cis*-platin but their activity is lower than that of 5-fluorouracil. Both complexes are less active than *cis*-platin and much less active than 5-fluorouracil against MCF-7 cell line. Furthermore, complex **1** is more active than **2**.

TABLE I
IC₅₀ (μM) VALUES OF (*IN VITRO* ANTI-PROLIFERATIVE ACTIVITY) OF COMPLEXES

Complex	MCF-7	HepG-2	DU-145
1	38.232 ± 0.031	4.645 ± 0.087	20.726 ± 0.141
2	> 40.0	11.609 ± 0.104	21.267 ± 0.193
<i>Cis</i> -platin	29.79 ± 0.10	19.83 ± 0.10	19.894 ± 0.05
5-Fluorouracil	< 4.97	< 4.97	10.782 ± 0.05

50% growth inhibition as determined by MTT assay (24 h drug exposure); Compounds tested in triplicate; data expressed as Standard error of the mean value (SEM) of three independent experiments.

It has been reported [50] that 1,10-phenanthroline derivatives exhibit promising activity against cancer, and viral, bacterial, and fungal infections. Several R_2SnX_2 -phen (R = Ph, Me, Et, Pr, Bu, Oct; X = F, Cl, I, NCS; phen = 1,10-phenanthroline) adducts were screened for *in vivo* antitumour activity against P388 Lymphocytic Leukaemia in mice; out of which ethyl-, propyl- and butyltin adducts were found to be active whereas methyl-, phenyl- and octyltin adducts were inactive [7]. Further, Bu_2SnAd_2 and Ph_2SnAd_2 (Ad = (adeninato)⁻¹) [61] and diphenyl- and dibutyltin adducts of adenosine monophosphate were also active against p388 lymphocytic leukaemia in mice [7], whereas, diethyl- and dimethyltin adducts of adenosine monophosphate were inactive [7]. Antitumor activities of organotin complexes/adducts of guanine, guanosine and guanosine phosphates have not been reported so far. Encouraged by the serendipitous discovery of *cis*-platin, an anticancer drug, which crosslinks the two strands of double helix of DNA via N7 nitrogen atoms of guanine base and/or the adjacent N7 atoms of guanine of a

single strand, the studies on binding modes of organotin(IV) moiety with guanine/and or guanosine along with 1,10-phenanthroline are indispensable. Organotin complexes of guanine have limited solubility in aqueous medium as well as in various organic solvents [43]. Therefore, it has been planned to study the antitumor activity and to explore the possible ways of the mode of action of organotin(IV)-mixed ligand (guanosine and 1,10-phenanthroline) derivatives. It has been proposed that the anti-proliferative activity displayed by organotin derivatives may be due to their binding with thiol groups of proteins [46] or with sugar-phosphate backbone of DNA [47]–[49]. Since the studied complex **1** and **2** show significant binding with CT DNA, therefore, their potential antitumor activity may be due to their interaction with DNA in cancer cells as one of the reason, although other possibilities such as their interaction with proteins and mitochondria could not be ruled out. Hence, further studies should be addressed to explore the other possible modes of action.

IV. CONCLUSION

Two new organotin-based chemotherapeutic agents have been designed and prepared. Both exhibit potential *in vitro* cytotoxic activity against mammary, liver and prostate cancer cell lines. Both of them are effectively bound with DNA double helix, which might be the reason for their potential anti-cancer activity. Further studies such as enzyme activity, comet assay and DNA fragmentation of the cancer cell lines would be more helpful in understanding their mode of action.

ACKNOWLEDGMENT

Ms. Nagamani Kompelli is thankful to the Ministry of Human Resources development, India, for the award of Junior/Senior Research Fellowship.

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