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Investigation of diorganotin(IV) complexes: Synthesis, characterization, *in vitro* DNA binding studies and cytotoxicity assessment of di-*n*-butyltin(IV) complex

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ABSTRACT

Diorganotin(IV) complexes of general formula R_2SnL (R = Me, **1**; Bu, **2**; Ph, **3**) with tridentate ONO donor Schiff base ligand were synthesized and structurally characterized by adopting various spectroscopic (IR, ^1H ^{13}C and ^{119}Sn NMR, UV–Vis, ESI MS, XRD) and analytical techniques. *In vitro* DNA binding profile of **1**–**3** were carried out by various biophysical methods *viz*., UV–Vis titrations, fluorescence, circular dichroic and viscosity measurements which revealed the electrostatic mode of interaction *via* phosphodiester backbone of DNA duplex. The intrinsic binding constant K_b values of **L** and **1**–**3** were found to be 7.53×10^3, 2.98×10^4, 5.74×10^4 and $3.64 \times 10^4\ M^{-1}$, respectively suggesting the higher binding propensity of **2**, di-*n*-butyltin(IV) complex as compared to **1** and **3**. Further, the computer-aided molecular docking technique was carried out to validate and rationalize the observed binding affinities towards the molecular target DNA. The resulting binding energies of docked complexes were found to be −212.2, −317.8 and −286.0 KJ mol^{-1}, respectively. In addition, complex **2** was found remarkably effective against U373MG (CNS), PC3 (prostrate), Hop62 (lung), HL60 (leukemia), HCT15 (colon), SK-OV-3 (ovarian), HeLa (cervix) and MCF7 (breast) cancer cell lines with GI_{50} values <10 μg/ml.

1. Introduction

Cancer as a leading cause of death worldwide, accounting for 7.6 million deaths in 2008 and anticipates ~13 million more deaths by 2030 [1]. Cancer chemotherapy is the primary treatment modality against cancer, which was initially fueled by the serendipitous discovery of antitumor drug- cisplatin, *cis*-diamminedichloroplatinum(II) in 1969 [2]. Potentially, cisplatin and its analogues exhibit a broad antineoplastic spectrum and in combination with other chemotherapeutic agents, can be highly effective in the treatment of many cancers [3,4]. Platinum drugs are believed to induce cytotoxicity by cross-linking DNA, causing changes to the DNA structure that inhibit replication and protein synthesis which would ultimately lead to induction of apoptosis [5]. However, serious adverse effects, such as dose limiting, nephrotoxicity, peripheral neuropathy and hearing loss, are well known [6]. These limitations have stimulated an extensive search for unconventional chemotherapeutic strategies. In this context, organometallic compounds are the promising chemotherapeutics candidates in cancer therapy, which offer ample opportunities in the design of novel classes of medicinal compounds, potentially with new metal-specific modes of action [7–10]. Interestingly, among the organometallics, organotin(IV) compounds are recognized as an effective alternative to platinum drugs, affording different mechanisms of action than those of cisplatin and its analogues, thus leading to alternative therapeutic protocols showing advantages both in terms of lower toxicity and platinum induced resistance [11–13]. In particular, a large number of organotin derivatives have been prepared and were tested *in vitro* and *in vivo* against different panels of human cell lines, which exhibited remarkable antiproliferative properties [14]. In general, organotin(IV) moieties bind to glycoproteins or to cellular proteins, and directly interact with DNA, causing cell death by apoptotic mechanisms [15,16].

Ligands can significantly alter the biological properties by modifying reactivity or substitution inertness. The role of tailored ligand framework introduced into the metal-based medicinal agents is of considerable importance in tuning the cytotoxic characteristics of the complex as it not only mutes the potential toxicity of metallodrugs but also significantly alters the reactivity of the metal ion [17,18]. In recent years, polydentate proligand 2-amino-2-methyl-1,3-propanediol ($ampdH_2$) have been a center of attention and attraction for chemists and biologists because of

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their wide structural and pharmacological relevance [19–21]. The ligand with mixed N and O donor atoms like ampdH_2 was selected because its Schiff base derivatives are versatile ligands in terms of donor properties and so are able to form higher nuclearity compounds (Scheme 1).

DNA is the main intracellular target for many anticancer drugs [22] and metal complexes which can bind with specificity to DNA are of importance in the development of new anticancer agents. DNA binding is a major criterion for the designing of novel anticancer agents as many molecules exert their anticancer activities by binding with DNA, causing alteration in DNA replication and thereby inhibiting the tumor cells growth [23,24]. In fact, the cytotoxic activity of metallodrugs has often been correlated to their DNA-binding properties [25]. The phosphate group of the DNA sugar backbone acts as an anchoring site and the binding of the nitrogen of the DNA base are extremely effective; this often results in the stabilization of the tin center as an octahedral species [12].

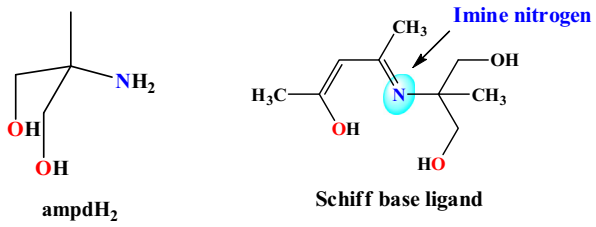
In lieu of above and in continuation of our previous work [26], herein, we report the synthesis and structural characterization of new diorganotin(IV) complexes by using an amino polyalcohol as a ligand backbone. Furthermore, the interaction studies of the complexes **1**–**3** with CT DNA were explored by employing UV absorption, emission and circular dichoric spectroscopic techniques. Specifically, the cytotoxicity of di-*n*-butyltin(IV) complex, **2** were preliminarily investigated by SRB assay against the panel of human tumor cells, demonstrating that the complex could be a potent therapeutic agent for the treatment of cancer.

2. Experimental

2.1. Materials and instrumentation

Reagent grade chemicals were used without further purification for all syntheses and experiments. Acetylacetone (acac) (Merck), 2-amino-2-methylpropane-1,3-diol (ampdH_2), dimethyltin(IV) dichloride, di-*n*-butyltin(IV) dichloride, diphenyltin(IV) dichloride, triethylamine (Et_3N), tris (hydroxymethyl)aminomethane or Tris (Sigma–Aldrich) and supercoiled plasmid pBR322 DNA (Genei) were utilized. Disodium salt of calf thymus DNA (CT DNA) purchased from Sigma Chem. Co. and was stored at 4 °C.

The ^{1}H, ^{13}C and ^{119}Sn NMR spectra were obtained on a Bruker DRX-400 spectrometer operating at 400, 100 and 150 MHz, respectively. Molar conductance was measured at room temperature on Eutech con 510 electronic conductivity bridge. Infrared spectra were recorded on Interspec 2020 FTIR spectrometer in KBr pellets from 400–4000 cm^{-1}. Electrospray mass spectra were recorded on Micromass Quattro II triple quadrupol mass spectrometer. Microanalyses (C, H and N) were performed on a Elementar Vario EL III. XRD were recorded on Rikagu mini Flex II instrument. UV–Vis spectra were recorded at room temperature on a Perkin–Elmer Lambda 25 spectrometer. Fluorescence measurements were made on Shimadzu RF–5301PC Spectrofluorophotometer. The SEM images were recorded with JEOL JSM-6510LV Scanning Electron Microscope. CD spectra were measured by a Jasco-J-815 spectrometer using a quartz cell (0.1 cm) at 0.1 nm intervals, adjusting the band width to 1.0 nm and the scan speed to 20 nm min^{-1}. Viscosity measurements were carried out from observed flow time of CT DNA containing solution (t > 100 s) corrected for the flow time of buffer alone (t_0), using Ostwald's viscometer at 25 ± 0.01 °C. Flow time was measured with a digital stopwatch.



Scheme 1. Ligand used in the synthesis of diorganotins.

2.2. Synthesis

2.2.1. Synthesis of ligand (*L*)

To a methanolic solution of ampdH_2 (1.051 g, 10 mmol) the stoichiometric amounts of acetylacetone (1.03 ml, 10 mmol) was added drop wise. The reaction mixture was stirred and refluxed for about 6–7 h during which the color of the solution turned to pale-yellow, the reaction was monitored by TLC till its completion. The solvent was removed by using a rotavapor which afforded the product in the form of an oily yellow viscous liquid followed by repeated washings with petroleum ether.

2.2.2. Synthesis of organotin(IV) complexes R_2SnL with R = Me (*1*), n-Bu (*2*), Ph (*3*)

Diorganotin(IV) complexes were synthesized by refluxing equivalent molar ratio of R_2SnCl_2 (2 mmol; R = Me, 0.438 g for **1**; R = *n*-Bu, 0.606 g for **2**; R = Ph, 0.686 g for **3**) and ligand L (0.374 g, 2 mmol) in the presence of Et_3N (0.276 ml, 2 mmol) in dry methanol. The resulting solution was refluxed for 4–5 h and subsequently concentrated to dryness under reduced pressure to get the final product. The product obtained was washed with hexane in order to remove impurities and dried in *vacuo*.

[Me_2SnL], **1**: Yield: 68%. M.p. = 220 °C. % *Anal.* Calc. for $C_{11}H_{21}NO_3Sn$ (334.0): C, 39.56; H, 6.34; N, 4.19. Found: C, 39.53; H, 6.26; N, 4.11%. Selected IR data (cm^{-1}/ν): 3617 ν(O–H), 1640 ν(C=N), 687 ν(Sn–C); 532 ν(Sn–O); 442 ν(Sn–N). Molar Conductance, Λ_M (10^{-3} M, DMSO): 21 Ω^{-1} cm^2 mol^{-1} (non electrolyte). UV–Vis (DMSO, λ_{max}, nm): 284. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm); 5.08 (C–C*H*=C, s, 1H), 3.20 and 3.13 (–CH_2, s, 4H), 2.9 (CH_2–O*H*, s, 1H), 2.25 (O–C–CH_3, s, 3H), 1.82 (N=C–CH_3, s, 3H), 0.84 (Sn–CH_3, s, 6H, ^2J[^{119}Sn–^{1}H] = 76 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 190.2 (C=N), 170.0 (O–C=), 99.8 (=CH–), 63.8 (CH_2–OH), 58.4 (CH_2–O), 49.4 (C$_{ter}$), 27.8 (CH_3–C=N), 18.5 (Sn–CH_3, ^1J[^{119}Sn–^{13}C] = 652 Hz). ^{119}Sn NMR (DMSO-d_6, 146 MHz) δ (ppm): –162.2. ESI–MS (m/z$^+$): [$C_{11}H_{21}NO_3Sn$]$^+$ 334.0.

[*n*-Bu_2SnL], **2**: Yield: 70%. M.p. = 191 °C. % *Anal.* Calc. for $C_{17}H_{33}NO_3Sn$ (418.16): C, 48.83; H, 7.95; N, 3.35. Found: C, 48.78; H, 7.87; N, 3.29%. Selected IR data (cm^{-1}/ν): 3640 ν(O–H), 1647 ν(C=N), 679 ν(Sn–C); 548 ν(Sn–O); 439 ν(Sn–N). Molar Conductance, Λ_M (10^{-3} M, DMSO): 30 Ω^{-1} cm^2 mol^{-1} (non electrolyte). UV–Vis (DMSO, λ_{max}, nm): 283. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm); 5.31 (C–C*H*=C, s, 1H), 3.43 and 3.34 (–CH_2, s, 4H), 3.09 (CH_2–O*H*, s, 1H), 2.33 (O–C–CH_3, s, 3H), 2.11 (N=C–CH_3, s, 3H), 2.06 (αCH_2, t, 4H, ^2J[^{119}Sn–^{1}H] = 75 Hz), 1.99–1.77 (βCH_2, m, 4H), 1.14 (γCH_2, m, 4H), 1.06 (δCH_3, t, ^5J[^{119}Sn–^{1}H] = 12 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 192.3 (C=N), 171.2 (O–C=), 97.4 (=CH–), 64.5 (CH_2–OH), 58.2 (CH_2–O), 48.5 (C$_{ter}$), 31.4–24.5 (CH_3–C=N + CH_3–C=CH + αCH_2), 23.3 (βCH_2), 20.5 (γCH_2), 17.4 (δCH_3). ^{119}Sn NMR (DMSO-d_6, 146 MHz) δ (ppm): –171.9. ESI–MS (m/z^+): [$C_{17}H_{33}NO_3Sn$+H]$^+$ 419.1, [$C_{17}H_{33}NO_3Sn$–C_4H_9]$^+$ 361.4, [$C_{17}H_{33}NO_3Sn$–2C_4H_9]$^+$ 303.2.

[Ph_2SnL], **3**: Yield: 76%. M.p. = 263 °C. % *Anal.* Calc. for $C_{21}H_{25}NO_3Sn$ (458.14): C, 55.05; H, 5.50; N, 3.06. Found: C, 55.00; H, 5.43; N, 3.01%. Selected IR data (cm^{-1}/ν): 3595 ν(O–H), 1643 ν(C=N), 681 ν(Sn–C); 550 ν(Sn–O); 447 ν(Sn–N). Molar Conductance, Λ_M (10^{-3} M, DMSO): 28 Ω^{-1}cm^2 mol^{-1} (non electrolyte). UV–Vis (DMSO, λ_{max}, nm): 284. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm); 7.77–7.01 (m, C_6H_5, 10H), 5.23 (C–C*H*=C, s, 1H), 3.24–3.01 (–CH_2, s, 4H), 2.32 (CH_2–O*H*, s, 1H), 2.19 (O–C–CH_3, s, 3H),