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Triphenyltin(IV) benzoates with diazenyl/imino scaffold exhibiting remarkable apoptosis mediated by reactive oxygen species

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ABSTRACT

The cytotoxic potency of a series of triphenyltin(IV) compounds of general composition [$Ph_3Sn(L^n)$] (**1–6**) has been probed *in vitro* employing MDA-MB-231 (human breast cancer) and HeLa (human cervical cancer) cell lines, where $L^n = L^{1–3}$; isomeric 2/3/4-{(*E*)-2-[4-(dimethylamino)phenyl]diazenyl}benzoates and $L^{4–6}$ are their corresponding isoelectronic imino analogues 2/3/4-[(*E*)-{[4-(dimethylamino)phenyl]methylidene}amino] benzoates. Compounds **1–6** have been characterized by elemental analysis and their spectroscopic properties were studied using IR and NMR (^1H, ^{13}C, ^{119}Sn) techniques. The molecular structures of a pro-ligand 2-[(*E*)-{[4-(dimethylamino)phenyl]methylidene}amino]benzoic acid (HL^4) and two representative molecules, $Ph_3Sn(L^2)$ **2** and $Ph_3Sn(L^5)$ **5**, have been determined by X-ray crystallography. Structural analyses of **2** and **5** revealed distorted tetrahedral geometries within C_3O donor sets owing to monodentate modes of coordination of the respective carboxylate ligands, close intramolecular Sn…O(carbonyl) interactions notwithstanding. Cytotoxic studies in vitro in MDA-MB-231 and HeLa cell lines revealed high activity, in sub-micromolar range, for all investigated compounds. Among these, **1** and **3** exhibited potent cytotoxicity most effectively towards MDA-MB-231 cells with a IC_{50} value of 1.19 and 1.44 μM, respectively, whereas **5** showed remarkable activity towards HeLa cells with a IC_{50} value of 0.88 μM, yet the series of compounds had minimal cytotoxic effect on normal HEK 293 (human embryonic kidney) cell line. The underlying investigation suggested that the compounds exert potent antitumor effect by elevating intracellular reactive oxygen species generation and cause delay in cell cycle by inhibiting cells at G_2/M phase. The results presented herein suggest further development of this class of triphenyltin(IV) compounds-based drugs as potential anti-cancer therapies should be pursued.

1. Introduction

Cisplatin, *cis*-diammine-dichloroplatinum(II) (CDDP), a square planar Pt(II) complex remains in the frontline of inorganic chemotherapeutic agents for the treatment for cancer [1–4]. The clinical effectiveness of CDDP is limited by considerable side effects and the emergence of drug resistance. Consequently, a number of second- and third-generation CDDP analogues including carboplatin, oxaliplatin, nedaplatin, heptaplatin, lobaplatin and satraplatin were developed for use in the treatment of various cancers. These advances have spurred a surge of investigations to identify new inorganic agents (non-platinum metals) for use in chemotherapy with improved specificity and decreased toxic side effects. Accordingly, non-platinum metallodrugs such as Ru, Cu, Au, Pd, Fe, Co, Ti, Ga, Ni, Rh, Ir, Sn, Os, Zn, V, Ag, Re, Mo and lanthanide complexes have emerged and been shown to exhibit comparable or better cytotoxic properties accompanied by different specificities towards cancer cells, and by a more favourable pharmacological and toxicological profiles [5,6]. Review articles describing the application of transition metal complexes as anti-cancer agents are available in the literatures [7–23].

On the other hand, organotin(IV) compounds have displayed diverse medicinal applications such as anti-viral, anti-microbial, anti-

Abbreviations: ROS, reactive oxygen species; IC_{50}, compound concentrations that produce 50% of cell growth inhibition; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DCFH-DA, dichlorodihydrofluorescein diacetate; PI, propidium iodide; CDDP, cisplatin, *cis*-diammine-dichloroplatinum(II); FACS, fluorescence-activated cell sorting; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; ORTEP, Oak Ridge thermal ellipsoid plot program (molecular modeling); PBS, phosphate-buffered saline; DCFH, 2,7-dichlorodihydrofluorescein; DCF, 2,7-dichlorofluorescein; FACS, fluorescence-activated cell sorting

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parasitic, anti-hypertensive, anti-hyperbilirubinemia and anti-cancer activities, apart from their standard uses as biocides, catalyzers and stabilizers [24–29]. Compared to standard drugs, the majority of organotin(IV) carboxylates discussed in the literature have shown superior anticancer activity against various cell lines. Additionally, some of them have demonstrated distinct anticancer activity even against CDDP-resistive cancer cells. Organotin(IV) compounds with pronounced medicinal properties also exhibit drawbacks such as reproductive toxicity, neurotoxicity and other toxic effects apart from poor water solubility; however, these can be circumvented by a rational design of the structures of the compounds, but the vital point still remains the activity. In practice, organotin(IV) compounds are dissolved in DMSO and diluted with test medium prior to *in vitro* testing. Nevertheless, the limited solubility needs to be further enhanced in a way comparable to CDDP, which also shows limited water solubility. It is therefore important to find more effective and safer organotin(IV) compounds for therapeutic use by designing and synthesizing new compounds and to find suitable means of their delivery to the biotargets. In this pursuit, recently a novel biocompatible strategy of drug delivery employing nanostructured silica-based material loaded with triphenyltin(IV) compound was used. This resulted in an increase in efficacy of the drug and the pattern of action leads to the non-aggressive suppression of melanoma tumor growth with non-recognizable toxicity towards normal tissue [30]. While the potency of a drug is a very important consideration, drug selectivity towards cancer cells is key to ensuring both safety and effectiveness [31]. Additionally, the mechanism of action of organotin(IV) compounds in achieving cell death remains uncertain, and hence additional work is essential to identify the actual apoptotic or necrotic pathways.

In view of the above, careful functionalization of a series of organotin compounds was undertaken. In this endeavour, six pro-ligands (i) isomeric 2-, 3- and 4-{(*E*)-2-[4-(dimethylamino)phenyl] diazenyl}benzoic acids and their isoelectronic imino counterparts, i.e. (ii) 2-, 3- and 4-[(*E*)-{[4-(dimethylamino)phenyl]methylidene}amino] benzoic acid, have been designed and their triphenyltin(IV) esters prepared (Fig. 1). In the first three pro-ligands ($HL^1 - HL^3$), the aryl moieties are linked by a diazenyl skeleton while in the others (HL^{4-6}) by an imino group, substitution patterns which offer flexibility in the molecules. The presence of the carboxylate group is vital for aqueous solubility and results in increased cellular accumulation [32,33]. The incorporation of the triphenyltin(IV) moiety in the molecule offers much higher activities when compared with titanocene derivatives and CDDP [34–36]. Thus, it is expected that the modification of the carboxylate ligands in the current series by incorporating $SnPh_3$ can optimize the cytotoxicity [37,38] while varying the location of the triphenyltin ester can modulate the activity at a specific target. In this context, cytotoxic properties of a series of $[Ph_3Sn(L^n)]$ complexes [$L^n = L^{1-3}$ (**1–3**) and L^{4-6} (**4–6**)], were determined towards the MDA-MB-231 (human breast cancer) and HeLa (human cervical cancer) cell lines. To realize the mechanistic/mode of cell death objective, an additional series of experiments were conducted: (i) the cytotoxic activity was examined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (ii) the ability of the test compounds to generate ROS (reactive oxygen species) in the cells was examined using DCFH-DA (dichlorodihydro-fluorescein diacetate) dye (iii) apoptosis or mode of cell death was detected by Hoechst 33342/PI (propidium iodide) and annexin V-PI assay and (iv) cell cycle arrest by the compounds were examined by FACS (fluorescence-activated cell sorting) studies. The biological results indicated that the triphenyltin(IV) compounds show potent cytotoxicity which can be modulated either by varying the positions of the triphenyltin(IV) ester across a series or switching from triphenyltin(IV)- diazobenzoato- to -iminobenzoato-ligands. The present studies demonstrated that the triphenyltin(IV) compounds exert their cytotoxic effect by elevating intracellular ROS generation as discussed below.

2. Experimental

2.1. Materials and physical measurements

Ph_3SnOH (Aldrich), anthranilic acid (Sigma-Aldrich), 3-aminobenzoic acid, 4-aminobenzoic acid, 4-(dimethylamino)benzaldehyde (SRL) and dimethylaniline (sd fine-chem) were used without further purification. The solvents used in the reactions were of AR grade and were dried using standard procedures. Toluene was distilled from sodium. Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) (Cellclone, Genetix Biotech Asia), penicillin 1000 IU, streptomycin 10 mg mL^{-1}, trypsin, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide dye (MTT) (Himedia), dimethyl sulphoxide (DMSO), RNAse (GeNie, Merck), 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), Hoechst 33342 (Sigma), propidium iodide (PI) (EMD Millipore-Calbiochem), Annexin V Alexa flour 488 conjugate (Life Technologies; Invitrogen Bioservices India Pvt. Ltd.), cytoplatin (Cipla; generic name- cisplatin), Triton X-100 and other analytical grade chemicals (Lobachemie) were used.

Melting points were measured using a Büchi melting point apparatus M-560 and are uncorrected. Carbon, hydrogen and nitrogen analyses were performed with a Perkin-Elmer 2400 series II instrument. IR spectra in the range 4000–400 cm^{-1} were obtained on a Perkin Elmer Spectrum BX series FT-IR spectrophotometer with samples investigated as KBr discs. Absorption measurements of compounds **1–6** were carried out on a Perkin-Elmer Lambda25 spectrophotometer at ambient temperature in benzene and DMSO solutions. ^1H and $^{13}C\{^1H\}$ NMR spectra, measured at 400.13 and 100.62 MHz respectively, were recorded on a Bruker AMX 400 spectrometer. ^{119}Sn NMR spectra were measured on a Jeol GX 270 spectrometer at 100.75 MHz. The ^1H, ^{13}C and ^{119}Sn chemical shifts were referenced to Me_4Si (δ 0.00 ppm), $CDCl_3$ (δ 77.00 ppm), and Me_4Sn (δ 0.00 ppm), respectively. Equipment such as ELISA plate reader, Microscan (MS5608A), inverted fluorescence microscopy (Nikkon E800) and Inverted fluorescent microscope (Evos FL, Life technologies, AMF4300) were used for biological work.

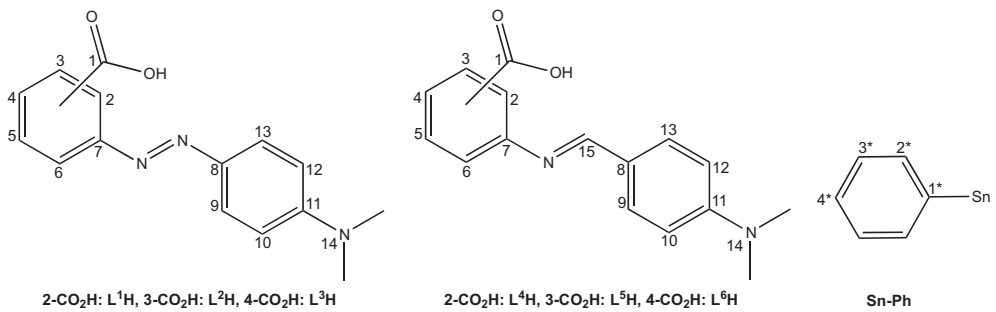

Fig. 1. Generic structures with numbering protocol of pro-ligands HL^n (n = 1–6) and tin phenyl moiety.