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An *in vitro* comparative assessment with a series of new triphenyltin(IV) 2-/4-[(*E*)-2-(aryl)-1-diazenyl]benzoates endowed with anticancer activities: Structural modifications, analysis of efficacy and cytotoxicity involving human tumor cell lines

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ABSTRACT

Four new triphenyltin(IV) complexes of composition Ph_3SnLH (where LH = 2-/4-[(*E*)-2-(aryl)-1-diazenyl] benzoate) (1–4) were synthesized and characterized by spectroscopic (^1H, ^{13}C and ^{119}Sn NMR, IR, ^{119}Sn Mössbauer) techniques in combination with elemental analysis. The ^{119}Sn NMR spectroscopic data indicate a tetrahedral coordination geometry in non-coordinating solvents. The crystal structures of three complexes, Ph_3SnL^1H (1), Ph_3SnL^3H (3), Ph_3SnL^4H (4), were determined. All display an essentially tetrahedral geometry with angles ranging from 93.50(8) to 124.5(2)°; ^{119}Sn Mössbauer spectral data support this assignment. The cytotoxicity studies were performed with complexes 1–4, along with a previously reported complex (5) *in vitro* across a panel of human tumor cell lines *viz*., A498, EVSA-T, H226, IGROV, M19 MEL, MCF-7 and WIDR. The screening results were compared with the results from other related triphenyltin(IV) complexes (6–7) and tributyltin(IV) complexes (8–11) having 2-/4-[(*E*)-2-(aryl)-1-diazenyl]benzoates framework. In general, the complexes exhibit stronger cytotoxic activity. The results obtained for 1–3 are also comparable to those of its *o*-analogs i.e. 4–7, except 5, but the advantage is the former set of complexes demonstrated two folds more cytotoxic activity for the cell line MCF-7 with ID_{50} values in the range 41–53 ng/ml. Undoubtedly, the cytotoxic results of complexes 1–3 are far superior to CDDP, 5-FU and ETO, and related tributyltin(IV) complexes 8–11. The quantitative structure-activity relationship (QSAR) studies for the cytotoxicity of triphenyltin (IV) complexes 1–7 and tributyltin(IV) complexes 8–11 is also discussed against a panel of human tumor cell lines.

1. Introduction

Organotin(IV) compounds are a widely studied class of metal-based anti-tumor drugs and their intensive investigation has led to the discovery of compounds with excellent *in vitro* anti-tumor activity, but in many cases disappointingly low *in vivo* potency or high *in vivo* toxicity [1–3]. It is well established that organotin(IV) compounds are very important in cancer chemotherapy because of their apoptosis-inducing character [4,5]. The design of improved organotin(IV) anti-tumor agents occupies a significant place in cancer chemotherapy, as revealed in their remarkable therapeutic potential reflected in recent research reports [6–17]. Consequently, a large number of organotin(IV) carboxylates have been investigated for their anti-tumor potential. Among organotin(IV) carboxylates, triorganotin(IV) carboxylates are quite well known for exceptionally high *in vitro* anti-tumor activities, *e.g.*, triphenyltin(IV) -benzoates, -salicylates [18], -3,6-dioxaheptanoate, -3,6,9-trioxadecanoate [19], -4-carboxybenzo-15-crown-5, -4-carboxybenzo-18-crown-6 [19,20], -steroidcarboxylate [21], -terebate [22,23,24] and -aminoacetates (Schiff bases) [25,26]. From these examples, it is clear that the compounds can be developed with high *in vitro* antitumor activity and sufficient water solubility. The most important point remains the activity. The organotin(IV) compounds containing the diazenyl group show not only high *in vitro* antitumor activity, but also displayed interesting interactions with various enzymes (see below). In the present study, attempts have been made to improve the water solubility by the systematic study of various

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structural changes. In general, organotin(IV) compounds are dissolved in DMSO and diluted with test medium prior to the in vitro testing. The limited solubility needs further improvement in a way comparable to cisplatin which shows limited water solubility too.

In view of the remarkable activity of the triphenyltin(IV) carboxylates, triphenyltin(IV) carboxylates containing the 2-[(*E*)-2-(3-formyl-4-hydroxyphenyl)-1-diazenyl]benzoate and 2-[(*E*)-2-(4-hydroxy-5-methylphenyl)-1-diazenyl]benzoate skeletons have recently been investigated, showing encouraging cytotoxic activity across a panel of cell lines [27]. As a result of these promising cytotoxic activities, the mechanistic role of the compounds was investigated to determine the influence of the azo group nitrogen. Docking studies were performed with some of the key enzymes, such as ribonucleotide reductase, thymidylate synthase, thymidylate phosphorylase and topoisomerase II, which take part in the synthesis of raw materials for DNA and its replication [28]. The docking studies indicated that the azo group nitrogen atoms and formyl, carbonyl and ester oxygen atoms in the ligand moiety play an important role. They exhibit hydrogen bonding interactions with the active site of amino acids of the aforementioned enzymes. The higher activity was attributed to the presence of the azo group nitrogen atoms in the molecules of triphenyltin (IV) complexes [27]. As a continuation of our previous work in this area, we report some new triphenyltin(IV) complexes, $Ph_3SnL^{1-4}H$ (**1**–**4**), of related systems where the ligand skeletal framework has been modified (Scheme 1) in an attempt to improve the dissolution properties and thereby influence cytotoxicity. The carboxylate ligands selected herein have variations in the position of the carboxylate functionality in the diazo part and also have variations of the nuclear substituents in the coupling moieties of the molecule. The newly synthesized complexes (**1**–**4**) were characterized by spectroscopic (^{1}H, ^{13}C and ^{119}Sn NMR, IR, ^{119}Sn Mössbauer) techniques. Complete characterization was accomplished from the crystal structure determination of some representative complexes Ph_3SnL^1H (**1**), Ph_3SnL^3H (**3**) and Ph_3SnL^4H (**4**). The newly synthesized triphenyltin(IV) complexes (**1**–**4**) and one previously reported triphenyltin(IV) complexes Ph_3SnL^5H (**5**) [29] were tested across a panel of human tumor cell lines consisting of A498 (renal cancer), EVSA-T (mammary cancer), H226 (non-small-cell lung cancer), IGROV (ovarian cancer), M19 MEL (melanoma), MCF-7 (mammary cancer) and WIDR (colon cancer) and the results were compared with analogous Ph_3SnL^6H (**6**), Ph_3SnL^7H (**7**) [27] and related tributyltin(IV) complexes (**8**–**11**) (see Scheme 1 for complex description).

2. Experimental

2.1. Materials

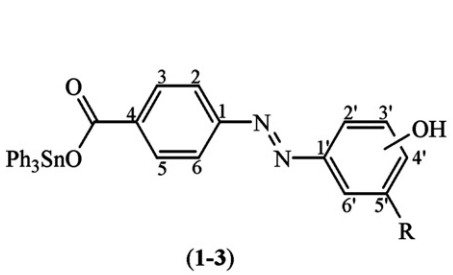
Ph_3SnOH was prepared from Ph_3SnCl (Fluka) by following the literature method [30]. $(Ph_3Sn)_2O$ (Fluka), 2-hydroxybenzaldehyde (Sisco), 2-methylphenol, 4-methylphenol, 4-*tert*-butylphenol (Merck), anthranilic acid (Spectrochem), and 4-aminobenzoic acid (Hi Media) were used without further purification. The solvents used in the reactions were of AR grade and were dried using standard procedures. Toluene was distilled from sodium benzophenone ketyl.

2.2. Physical measurements

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 disks. The ^{1}H and ^{13}C spectra were recorded on a Bruker AMX 400 spectrometer and measured at 400.13 and 100.62 MHz, respectively, while ^{119}Sn NMR spectra were recorded on a Jeol GX 270 spectrometer and measured at 100.75 MHz. The ^{1}H, ^{13}C and ^{119}Sn chemical shifts were referenced to Me_4Si, $CDCl_3$ and Me_4Sn set at 0.00, 77.0 and 0.00 ppm, respectively. The ^{119}Sn Mössbauer spectra (Table 1) were recorded at liquid nitrogen temperature with a conventional instrument in transmission mode, constituted by a multichannel analyzer (TAKES Mod.269, Ponteranica, Italy) and the following Wissenschaftliche Elektronik system (MWE, München, Germany): MR250 driving unit, FG2 digital function generator and MA250 velocity transducer, moving at linear velocity, constant acceleration, in a triangular waveform. The organotin(IV) samples (**1**–**4**) were maintained at liquid nitrogen temperature in a Cryo NDR-1258-MD liquid nitrogen cryostat (Cryo Industries of America, Inc. Atkinson, NH, USA) with a Cryo sample holder. The 77.3 ± 0.1 K temperature was controlled with an Oxford Instruments ITC 502 temperature controller (Oxford, UK). The multichannel calibration was performed with an enriched iron foil (α^{57}Fe, 4 μm thick, RITVERC GmbH, St. Petersburg, Russia), at room temperature, by using a ^{57}Co/Rh source (10 mCi, RITVERC GmbH, St. Petersburg, Russia), while the zero point of the Doppler velocity scale was determined, at room temperature, through absorption spectra of natural $CaSnO_3$ (^{119}Sn = 0.5 mg cm^{-2}) and a $Ba^{119}SnO_3$ source (10 mCi, RITVERC GmbH). The obtained $5 \cdot 10^5$ count spectra were interpreted by means of non-linear least square analysis as a sum of Lorentzian doublets, to obtain the isomer shift, $\delta \pm 0.03$ mm s^{-1}, the nuclear quadrupole splitting, $|\Delta_{exp}| \pm 0.03$ mm s^{-1} and the average full width at half height, Γ_{av}, ± 0.03 mm s^{-1}.

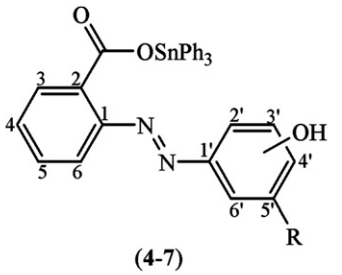
2.3. Synthesis of ligands and complexes

Ligands 4-[(*E*)-2-(4-hydroxy-3-methylphenyl)-1-diazenyl]benzoic acid (L^2HH') [31], 4-[(*E*)-(5-*tert*-butyl-2-hydroxyphenyl)diazenyl]benzoic acid (L^3HH') [31,32], 2-[(*E*)-(5-*tert*-butyl-2-hydroxyphenyl)diazenyl]benzoic acid (L^4HH') [31,33], and complex **5** [29] were prepared by the methods described in our earlier reports and purities were established from melting point, elemental analysis and ^{1}H NMR spectroscopy.



(1-3)

L^1H = 2'-OH, R = Me : Ph_3SnL^1H (**1**)
L^2H = 4'-OH, R = Me : Ph_3SnL^2H (**2**)
L^3H = 2'-OH, R = t-Bu : Ph_3SnL^3H (**3**)



(4-7)

L^4H = 2'-OH, R = t-Bu : Ph_3SnL^4H (**4**)
L^5H = 2'-OH, R = Me : Ph_3SnL^5H (**5**)
L^6H = 4'-OH, R = Me : Ph_3SnL^6H (**6**)
L^7H = 4'-OH, R = CHO : Ph_3SnL^7H (**7**)

Scheme 1. Structures and numbering protocol of triphenyltin(IV) complexes $Ph_3SnL^{1-7}H$ (**1**–**7**).