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Synthesis characterization and biological study of diorganotin(IV) complexes of monomethyl phthalate

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Abstract

The synthesis and characterization of new coordination compounds of some organotin(IV) chlorides with monomethyl phthalate is reported; the ligand molecules appear to be bound to the tin atom through carbonyl oxygen atoms. Their structures have been characterized by elemental analyses, molar conductance, and bonding in these complexes is discussed in terms of their IR, ^{1}H, ^{13}C, ^{119}Sn NMR and ^{119m}Sn Mössbauer spectral studies. The spectroscopic results obtained are in full agreement with the proposed 2:1 stoichiometry. The complexes soluble in DMSO have been screened against a wide spectrum of bacteria and the results obtained are quite promising. The LD_{50} values have also been determined in the albino rats. Some of the complexes also exhibit high anti-inflammatory activity.

Keywords: Organotin(IV); Monomethyl phthalate; Anti-inflammatory; Acute toxic; Cytostatic

1. Introduction

Organotin compounds are of interest in view of their considerable structural diversity. Among the compounds, the most ubiquitous are the carboxylates [1]. The reactions of precursors with carboxylic acids have been studied in considerable detail. Depending on the carboxylic acid used and the stoichiometry of the reactants, several products such as monomers, dimers, tetramers, oligomeric ladders, and hexameric drums have been isolated [1]. The increasing interest in organotin(IV) carboxylates that has arisen in the last few decades is attributed to their significantly important biological properties. Several di- and triorganotin(IV) species have shown potential as antineoplastic and antituberculosis agents [2].

In order to widen the scope of investigations on the coordination behavior of various donor ligands towards organotins, we carried out the investigations on organotin(IV) compounds containing carboxylate ligands and established their bioactivities [3–7]. In view of this we have now synthesized, structurally characterized and determined biological activities of a series of diorganotin(IV) ($R_2Sn(IV)$, R = methyl, ethyl, butyl, phenyl and benzyl) derivatives of monomethyl phthalate, and the results of this study are reported herein.

2. Experimental

All the diorganotin(IV) compounds except dibenzyltin dichloride were purchased from Fluka and were used as such. Dibenzyltin dichloride was synthesized from the literature method [8]. All the reactions were carried out under anhydrous and oxygen-free nitrogen atmosphere. The solvents used were dried before use according to the literature method [9].

The melting points were measured on a Reichert thermometer of F.G. Bode Co., Austria. IR spectra were obtained in KBr using a Perkin Elmer FT IR-1605 spectrophotometer. Elemental analyses were carried out on a Yanaco MT-3 high-speed CHN analyzer with an antipyrene as a reference compound. The amount of tin was determined using an inductively coupled plasma atomic emission spectrometry (ICP-AES) method on ARL 3410. The ^{1}H, ^{13}C and ^{119}Sn NMR spectra were recorded on a multinuclear FT NMR 200 MHz of JEOL using TMS as an internal standard. Some of the ^{13}C spectra were measured on a Bruker AM 270 instruments at 50 MHz with ^{13}C probe. The Mössbauer spectra were recorded at 80 K on a Cryophysics instrument equipped with a 15 mCi Ca $^{119}SnO_3$ source. The

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conductance of the complexes was taken on an Inolab sensor model tetracon 325 WTW conductometer.

2.1. Syntheses

2.1.1. Synthesis of ligand acid (monomethyl phthalate)

The phthalic anhydride 50 mmol (9 g) was refluxed in excess of dry methanol for 8 h under anhydrous conditions; excess of solvent was removed under reduced pressure. The solid product thus obtained was then recrystallized from chloroform.

2.1.2. Synthesis of complexes

To a hot methanolic solution of 10 mmol (1.8 g) of monomethyl phthalate (HL) was added 10 mmol of triethylamine (1.4 g) and this mixture was refluxed for half an hour. To this, 5 mmol of dimethyl (1.1 g), diethyl (1.5 g), dibutyl (1.2 g), diphenyl (1.7 g) or dibenzyltin dichloride (1.8 g) in methanol was added dropwise with constant stirring. The reaction was then refluxed for 8–10 h under nitrogen. The solid mass formed during the reaction as triethylamine chloride was centrifuged. Excess of solvent was evaporated under vacuum, solid product obtained was then recrystallized form chloroform and petroleum ether (40–60 °C). The complexes were placed under nitrogen for further study.

3. Spectroscopic data

Details are given below for each compound, using the following conventions. *Abbreviations*: s, singlet; t, triplet; m, complex pattern; θ = C–Sn–C angle; n.o., not observed; sr, strong, med, medium; w, weak; br, broad, sh, shoulder; Δ = quadrupole splitting; δ = isomer shift.

3.1. Ligand acid (HL)

Yield: 88%. Physical state: solid; mp = 77–78 °C; Mol. formula: $C_9H_8O_4$; Mol. wt.: 180. CHN analysis: the calculated values are given in parentheses. C: 59.97 (60.00); H: 4.42 (4.44). IR (KBr cm^{-1}): 3027w (νCH); 1715sr (νC=O); 3050br (νOH). ^{1}H NMR ($CDCl_3$)—H-1: 3.80s [3H, OMe]; H-4: 7.75–7.80m [1H, phenyl proton]; H-5: 7.38–7.40m [1H, phenyl proton]; H-6: 7.41–7.43 [1H, phenyl proton]; H-7: 7.55–7.58m [1H, phenyl proton]; H-11: 12.5s [1H, OH]. ^{13}C NMR ($CDCl_3$)—C-1: 50.80; C-2: 165.7; C-3: 130.5; C-4: 126.9; C-5: 130.3; C-6: 128.5; C-7: 128.1; C-8: 129.5; C-9: 168.7. Molar conductance (methanol, 10^{-3} M): 2.4 Ω^{-1} cm^2 mol^{-1}.

3.2. Bis(monomethyl phthalate)dimethylin (*1*)

Yield: 84%. Physical state: solid; mp = 90–91 °C; color: white; Mol. formula: $C_{20}H_{20}O_8Sn$; Mol. wt.: 508. CHN analysis—Anal. Calcd. for $C_{20}H_{20}O_8Sn$ (%): C, 47.20; H, 3.89; Sn, 23.58; C, 47.24; H, 3.93; Sn, 23.62. IR (KBr cm^{-1}): 3038w (νCH aromatic); 1620sr $(\nu OCO)_{asym}$; 1375sr $(\nu OCO)_{sym}$; $\Delta\nu$ = 245; 1722sr (νC=O); 530sh (νSn–C): 470sr (νSn–O). ^{1}H NMR ($CDCl_3$)—H-l: 3.81s [6H, OMe]; H-4: 7.77–7.78m [2H, phenyl protons]; H-5: 7.38–7.40m [2H, phenyl protons]; H-6: 7.42–7.44 [2H, phenyl protons]; H-7: 7.57–7.59m [2H, phenyl protons]; H-α: 2.0s [6H, 2CH_3], $^2J(^{119}Sn–^1H)$ = 95 Hz, θ = 152.4°. ^{13}C NMR ($CDCl_3$)—C-1: 50.81; C-2: 165.9; C-3: 130.6; C-4: 127.0; C-5: 130.4; C-6: 128.7; C-7: 128.3; C-8: 129.8; C-9: 170.9; C-α: 10.2 $^1J(^{119}Sn–^{13}C)$ = 755 Hz. ^{119}Sn NMR ($CDCl_3$): −310.5 ppm. ^{119}Sn Mössbauer ($CDCl_3$, mm s^{-1})—Δ: 3.52 ± 0.05; δ: 1.26 ± 0.01; Γ_1: 0.84; Γ_2: 0.87. Molar conductance (methanol, 10^{-3} M): 3.1 Ω^{-1} cm^2 mol^{-1}.

3.3. Bis(monomethyl phthalate)diethyltin (*2*)

Yield: 77%. Physical state: solid; mp = 126–128 °C; color: white; Mol. formula: $C_{22}H_{24}O_8Sn$; Mol. wt.: 508. CHN analysis—Anal. Calcd. for $C_{22}H_{24}O_8Sn$ (%): C, 49.21; H, 4.43; Sn, 22.34; C, 49.25; H, 4.47; Sn, 22.38. IR (KBr cm^{-1}): 3042w (νCH aromatic); 1635sr $(\nu OCO)_{asym}$; 1385sr $(\nu OCO)_{sym}$; $\Delta\nu$ = 250; 1720sr (νC=O); 530sh (νSn–C): 470sr (νSn–O). ^{1}H NMR ($CDCl_3$)—H-l: 3.81s [6H, OMe]; H-4: 7.77–7.78m [2H, phenyl protons]; H-5: 7.38–7.40m [2H, phenyl protons]; H-6: 7.42–7.44 [2H, phenyl protons]; H-7: 7.57–7.59m [2H, phenyl protons]; H-α: 1.20q [6H, 2 OMe], $^2J(^{119}Sn–^1H)$ = 98 Hz, θ = 157.7°; H-β: 1.32t [9H, 3CH_3]. ^{13}C NMR ($CDCl_3$)—C-1: 50.83; C-2: 166.0; C-3: 130.70; C-4: 127.6; C-5: 130.8; C-6: 129.1; C-7: 128.7; C-8: 130.1; C-9: 171.2; C-α: 7.5 $^1J(^{119}Sn–^{13}C)$ = 770 Hz; C-β: 9.7 $^2J(^{119}Sn–^{13}C)$ = 25 Hz. ^{119}Sn NMR ($CDCl_3$): −305.8 ppm. ^{119}Sn Mössbauer ($CDCl_3$, mm s^{-1})—Δ: 3.54 ± 0.05; δ: 1.28 ± 0.01; Γ_1: 0.92; Γ_2: 0.98. Molar conductance (methanol, 10^{-3} M): 3.5 Ω^{-1} cm^2 mol^{-1}.

3.4. Bis(monomethyl phthalate)dibutyltin (*3*)

Yield: 67%. Physical state: solid; mp = 139–141 °C; color: white; Mol. formula: $C_{26}H_{32}O_8Sn$; Mol. wt.: 592. CHN analysis—Anal. Calcd. for $C_{26}H_{32}O_8Sn$ (%): C, 52.66; H, 5.35; Sn, 20.23; C, 52.70; H, 5.40; Sn, 20.27. IR (KBr cm^{-1}): 3040w (νCH aromatic); 1640sr $(\nu OCO)_{asym}$; 1393 sr $(\nu OCO)_{sym}$; $\Delta\nu$ = 247; 1718sr (νC=O); 535sh (νSn–C): 488sr (νSn–O). ^{1}H NMR ($CDCl_3$)—H-l: 3.83s [6H, OMe]; H-4: 7.77–7.79m [2H, phenyl protons]; H-5: 7.38–7.40m [2H, phenyl protons]; H-6: 7.42–7.44 [2H, phenyl protons]; H-7: 7.58–7.60m [2H, phenyl protons]; H-α: 1.72–1.74m [4H, 2 CH_2]; H-β: 1.66–1.68m [4H, 2CH_3]; H-γ: 1.58–1.60m; H-δ: 0.90t [6H, 2CH_3]. ^{13}C NMR ($CDCl_3$)—C-1: 50.95; C-2: 166.7; C-3: 131.5; C-4: 128.1; C-5: 131.3; C-6: 129.4; C-7: 128.7; C-8: 130.3; C-9: 171.4; C-α: 24.2 $^1J(^{119}Sn–^{13}C)$ = 795 Hz; C-β: 22.9 $^2J(^{119}Sn–^{13}C)$ = 29 Hz; C-γ: 22.7 $^3J(^{119}Sn–^{13}C)$ = 90 Hz; C-δ: 13.6 $^4J(^{119}Sn–^{13}C)$ = n.o. ^{119}Sn NMR ($CDCl_3$): −295.5 ppm. ^{119}Sn Mössbauer ($CDCl_3$, mm s^{-1})—Δ: 3.58 ± 0.05; δ: 1.34 ± 0.01; Γ_1: 0.93; Γ_2: 0.99. Molar conductance (methanol, 10^{-3} M): 3.3 Ω^{-1} cm^2 mol^{-1}.

3.5. Bis(monomethyl phthalate)diphenyltin (*4*)

Yield: 82%. Physical state: solid; mp = 179–180 °C; color: white; Mol. formula: $C_{28}H_{24}O_8Sn$; Mol. wt.: 608. CHN analysis—Anal. Calcd. for $C_{28}H_{24}O_8Sn$ (%): C,

55.22; H, 3.90; Sn, 19.70; C, 55.26; H, 3.94; Sn, 19.73. IR ($KBr\,cm^{-1}$): 3046w (νCH aromatic); 1658sr ($\nu OCO)_{asym}$; 1405sr ($\nu OCO)_{sym}$; $\Delta\nu = 253$; 1725sr (νC=O); 545sh (νSn–C): 492sr (νSn–O). ^{1}H NMR ($CDCl_3$): H-l: 3.85s [6H, OMe]; H-4: 7.78–7.79m [2H, phenyl protons]; H-5: 7.62–7.64m [2H, phenyl protons]; H-6: 7.45–7.47 [2H, phenyl protons]; H-7: 7.58–7.61m [2H, phenyl protons]; H-α, H-β, H-γ, H-8: 7.65–7.70m [10H, 2 phenyl protons]. ^{13}C NMR ($CDCl_3$)—C-1: 50.99; C-2: 166.8; C-3: 131.8; C-4: 128.3; C-5: 131.6; C-6: 129.6; C-7: 128.9; C-8: 130.6; C-9: 172.4; C-α: 126.2 $^1J(^{119}Sn–^{13}C) = 850$ Hz; C-β: 130.5 $^2J(^{119}Sn–^{13}C) = 20$ Hz; C-γ: 129.1 $^2J(^{119}Sn–^{13}C)$ 54 Hz; C-8: 128.2 $^3J(^{119}Sn–^{13}C)$ = n.o. ^{119}Sn NMR ($CDCl_3$): −222.7 ppm. ^{119}Sn Mössbauer ($CDCl_3$, $mm\,s^{-1}$)—Δ: 3.80 ± 0.05; δ: 1.32 ± 0.01; Γ_1: 0.90; Γ_2: 0.95. Molar conductance (methanol, 10^{-3} M): $4.1\ \Omega^{-1}\,cm^2\,mol^{-1}$.

3.6. Bis(monomethyl phthalate)dibenzyltin (*5*)

Yield: 73%. Physical state: solid; mp = 155–157 °C; color: white; Mol. formula: $C_{32}H_{32}O_8Sn$; Mol. wt.: 660. CHN analysis—Anal. Calcd. for $C_{32}H_{32}O_8Sn$ (%): C, 58.13; H, 4.20; C, 58.18; Sn, 18.14; H, 4.24; Sn, 18.18. IR ($KBr\,cm^{-1}$): 3035w (νCH aromatic); 1646sr ($\nu OCO)_{asym}$; 1398sr ($\nu OCO)_{sym}$; $\Delta\nu = 248$; 1718sr (νC=O); 538sh (νSn–C): 485sr (νSn–O). ^{1}H NMR ($CDCl_3$)—H-l: 3.83s [6H, OMe]; H-4: 7.70–7.71m [2H, phenyl protons]; H-5: 7.48–7.52m [2H, phenyl protons]; H-6: 7.40–7.42 [2H, phenyl protons]; H-7: 7.55–7.58m [2H, phenyl protons]; H-α: 2.58 s [4H, $2CH_2$], $^2J(^{119}Sn–^1H) = 105$ Hz, $\theta = 171.2°$; H-β: 7.53–7.54m [2H, phenyl protons]; H-γ: 7.20–7.22m [2H, phenyl protons]; H-δ: 7.25–7.27 [2H, phenyl protons]; H-ω: 7.30–7.32 [2H, phenyl protons]. ^{13}C NMR ($CDCl_3$)—C-1: 50.85; C-2: 165.8; C-3: 131.3; C-4: 127.8; C-5: 131.1; C-6: 129.1; C-7: 128.4; C-8: 130.2; C-9: 171.6; C-α: 30.3 $^1(^{119}Sn–^{13}C) = 759$ Hz; C-β: 135.6 $^2J(^{119}Sn–^{13}C) = 20$ Hz; C-γ: 129.3 $^3J(^{119}Sn–^{13}C) = 53$ Hz; C-δ: 127.5 $^4J(^{119}Sn–^{13}C) = 12$ Hz; C-ω: 124.2 $^5J(^{119}Sn\text{-}^{13}C)$ = n.o. ^{119}Sn NMR ($CDCl_3$): −290.8 ppm. ^{119}Sn Mössbauer ($CDCl_3$, $mm\,s^{-1}$)—Δ: 3.50 ± 0.05; δ: 1.22 ± 0.01; Γ_1: 0.86; Γ_2: 0.88. Molar conductance (methanol, 10^{-3} M): $3.4\ Q^{-1}\,cm^2\,mol^{-1}$.

4. Antibacterial activity

The antibacterial activities were determined by using the agar well diffusion method [10]. The wells were dug in the media with a sterile borer and 8-h-old bacterial inocula containing ca. 10^4 to 10^6 colony-forming units (CFU)/mL were spread on the surface of the nutrient agar using a sterile cotton swab. The recommended concentration of the test sample (2 mg/mL in DMSO) was introduced into the respective well. Other wells containing DMSO and the reference antibacterial drug served as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 20 h. The activity was determined by measuring the diameter of the inhibition zone (in mm) showing complete inhibition. Growth inhibition was calculated with reference to the positive control.

5. Anti-inflammatory activity

A freshly prepared suspension of carrageenin (0.2 mL, 1.0% in 0.9% saline solution) was injected subcutaneously into the plantar aponeurosis of the hind paw of the rats of both sexes (body weight 120/160 g) by the method of Winter et al. [11]. One group of five rats was kept as a control and the animals of the other group of five; each was pretreated with the test drugs given orally 30 min before the carrageenin injection. The paw volume was measured by a water plethysmometer socrel at the time of treatment and then at an interval of 1 h for 4 h. The mean increase of paw volume at each time interval was compared with that of control group (five rats treated with carrageenin, but not treated with test compounds) and percent anti-inflammatory value was calculated as given below:

$$\%\ \text{anti-inflammatory} = (1 - DT/DC) \times 100$$

where DT and DC are the volumes of paw edema in drug treated and control groups, respectively.

6. Acute toxicity study

ALD_{50} (average lethal dose at 50% survival) of the compounds was determined in albino mice. The mice of either sex (body weight 20–25 g) were used. The test compound was injected intraperitoneally at different dose levels in groups of 10 animals and percent mortality in each group was observed after 24 h of drug administration. The ALD_{50} value ($1\ mg\,kg^{-1}$) was calculated from the data obtained by the method of Smith [12].

7. Cytostatic activity

Cytostatic activity was assayed against the established cell line KB, which derives from a human oral epidermoid carcinoma. Stock cultures were grown in 25 cm^3 flasks containing 10 mL of buffered Eagle's minimum essential medium (MEM) supplemented with glutamine, non-essential amino acids (1%) and new born calf serum (10%), according the literature [13]. The cell population doubling time was approximately 24 h. The cells were dissociated with 0.05% trypsin solution, plated at density of 5×10^5 cells per well in 24-well cell culture clusters (Costar) containing 1.0 mL of MEM per well, and preincubated for 24 h to allow adhesion to the substrate. Subsequently, the compounds to be tested were dissolved immediately before use in DMSO and these solutions were diluted with the growth medium to the desired concentrations before addition to the wells. At least five concentrations of each compound were used, with eight cell culture wells for each concentration. Each compound was assayed on at least three separate occasions. Each assay included a blank containing complete medium without cells.

The cells were incubated with the compounds to be tested at 37 °C in an atmosphere that was 5% CO_2 and had a relative humidity of 100%. The incubation time was 72 h, during which period the control cells showed exponential growth.

Cells growth was terminated by in situ fixation and followed by staining with the protein-binding dye sulforhodamine

B (SRB) [14]. Specifically, adherent cell cultures were fixed in situ by addition of 250 μL of cold 50% (w/v) trichloroacetic acid (TCA) and were kept for 60 min at 4 °C. The supernatant was then discarded and the plates were washed three times with deionized water and dried. SRB solution (500 μL, 0.4% (w/v) in 1% AcOH) was added to each well, and the cells were allowed to stain for 20–30 min at room temperature. Unbound SRB was removed by washing three times with 1% AcOH, and the plates were then air dried while bound stain was solubilized with unbuffered Tris base [tris(hydoxymethyl)aminomethane]. Optical densities at 565 nm were read on a Perkin-Elmer 550 SE spectrophotometer.

Cytostatic activity was evaluated from the inhibition of cell growth in the treated cultures with respect to the controls. IC_{50}, the concentration of the test compound at which cell proliferation was 50% of that observed in control cultures, was determined by linear regression analysis. The statistical significance of these results was estimated by means of Student's *t*-test ($P < 0.01$).

8. Results and discussion

Reactions of R_2SnCl_2 with monomethyl phthalate and triethylamine in 1:2 molar ratios, respectively, led to the formation of complexes according to Eq. (1).

The above reactions were found to be quite facile and were completed within 8–9 h of refluxing. The resulting complexes were obtained in good yield (70–90%). The resulting complexes were white solids, stable in air and soluble in methanol, chloroform, DMSO and DMF. The analytical data are in good agreement with the proposed stoichiometry of the complexes. The molar conductance values of 10^{-3} M solution of the complexes in methanol are in the range of 2.4–4.4 Ω^{-1} cm^2 mol^{-1}, indicating their non-electrolytic nature [15]. Structural proposals are based on FT-IR, ^{1}H NMR, ^{13}C NMR, ^{119}Sn and ^{119m}Sn Mössbauer studies:

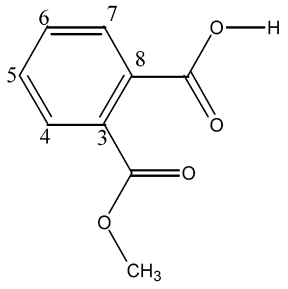
$$R_2SnCl_2 + 2HL + 2Et_3N \rightarrow R_2SnL_2 + 2Et_3N \cdot HCl \qquad (1)$$

where R = methyl, ethyl, butyl, phenyl and benzyl (Scheme 1).

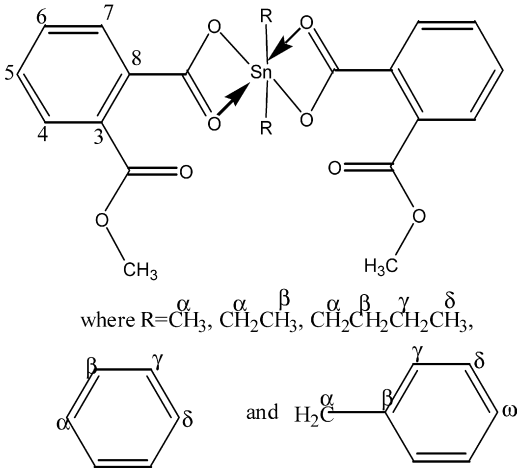
9. Spectroscopy

9.1. Infrared

Infrared O–C=O stretching frequencies were used to distinguish from coordinated from non-coordinated carboxyl groups, and also to identify the nature of bonding of carboxylic group. The carboxylic group in organotin(IV) derivatives generally adopt a bridged structure in the solid state unless the organic substituents at tin are bulky or unless the carboxylate group is branched at the α-carbon [16]. The strong band at 3015 cm^{-1} for ligand is absent for all organotin complexes indicating the deprotonation of carboxylic oxygen of the monomethyl phthalate upon complexation with the tin metal, as expected. Further, the ν_{asym}(OCO) and ν_{sym}(OCO) stretches have been detected and the magnitude of $\Delta\nu$(OCO) is in the range 245–250 cm^{-1}



Hydrogen monomethylphthalate HL



where R=$\overset{\alpha}{C}H_3$, $\overset{\alpha}{C}H_2\overset{\beta}{C}H_3$, $\overset{\alpha}{C}H_2\overset{\beta}{C}H_2\overset{\gamma}{C}H_2\overset{\delta}{C}H_3$,

Scheme 1.

which is characteristic of ester-type carboxylate groups [17]. It has been further confirmed by the presence of bands in the range 528–538 and 466–492 cm^{-1} for Sn–C and Sn–O, respectively [18,19]. Thus the IR data provide reasonable evidence for complexation through carboxylate group.

10. NMR studies

The ^{1}H NMR chemical shift assignments of the diorganotin moiety are straightforward from the multiplicity pattern and/or resonance intensities; whereas the ligand skeleton was assigned by multiplicity patterns and/or resonance intensities. The absence of a signal due to the hydroxyl proton at 12.5 ppm suggests deprotonation of carboxylic oxygen atom of the ligand upon complexation. For dimethyl, diethyl and dibenzyltin derivatives $^2J(^{119}Sn–^1H) = 95$, 98 and 105 Hz, respectively, which falls in the range of a octahedral environment around the tin atom which is further supported by C–Sn–C angle (θ), which was calculated using Lockhart's equation [20]. In case of *n*-butyl and phenyl derivatives, $^nJ(^{119}Sn–^1H)$ couplings are not visible due to a complex multiplet pattern. The signals for the alkyl protons attached to the tin(IV) atom are obtained as expected [21].

^{1}H NMR measurements for all the complexes were also performed in DMSO solution, showing that no dissociation occurs in this solvent over 48 h, contrary to the findings for analogous compounds [22].

The characteristic resonance peaks in ^{13}C NMR spectra of the complexes were recorded in $CDCl_3$. The conclusions drawn

from the IR and ^{1}H NMR spectra are concurrent with ^{13}C spectral data regarding the authenticity of the proposed structures. Various carbons have been successfully assigned. The spectra of organotin(IV) derivatives are consistent with the following observations:

- The resonance of the carboxylic carbon in organotin(IV) compounds are observed at larger δ (δ 170.9–172.4 ppm) than in the ligand (δ 168.7 ppm), suggesting the coordination of ligand through the carboxylic oxygen, to the organotin(IV) moiety [23].
- The ^{13}C chemical shifts of alkyl groups attached to tin are observed at positions comparable with other, similar compounds [23].
- Coordination of the tin atom in organotins has been related to the ^{1}J(^{119}Sn–^{13}C) coupling constants. The ^{1}J(^{119}Sn–^{13}C) coupling constants for the synthesized compounds ranged from 755 to 850 Hz which is indicative of six-coordinated compounds [24].

In order to obtain further structural evidence, the ^{119}Sn NMR spectra of the representative complexes were recorded. As the electron releasing power of alkyl group bonded to the tin increases, tin atom becomes progressively more shielded and ^{119}Sn chemical shift moves to higher field [25]. According to the literature reported work ^{119}Sn chemical shift is directly linked to the coordination, tin shifts are normally higher with phenyl compared to that of alkyl substituents [26]. The results further confirm the octahedral structure to synthesized diorganotin(IV) complexes.

11. ^{119m}Sn Mössbauer study

The ^{119m}Sn Mössbauer parameters have been utilized as a diagnostic tool for proposing the structure that a particular complex can adopt in the solid state. The spectra of the complexes display a characteristic doublet absorption indicating a single tin site. The R_2Sn derivatives show isomer shift (δ) values typical of quadrivalent organotin derivatives, similar in alkyl$_2$Sn moieties and higher with respect to Ph_2Sn. The quadrupole splitting (Δ) values of complexes are in the range 3.72–3.97 mm s^{-1} in alkyl$_2$Sn and 3.61 mm s^{-1} in Ph_2Sn derivatives, suggesting *trans*-R_2Sn octahedral structure [27].

12. Biological studies

Ligand acid (HL) and its organotin(IV) derivatives exhibit varying degrees of inhibitory effects on the growth of a wide spectrum of microorganisms. All the complexes were very active against all of microorganisms used.

Antibacterial activity was performed against two Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and four Gram-negative (*Escherichia coli*, *Schigella flexenari*, *Pseudomonas aeruginosa*, *Salmonella typhi*) bacteria and the results are summarized in Table 1. In order to compare the results obtained the Imipinem is used as standard drug. All the synthesized compounds show higher activity than the ligand but slightly lower than the standard drug.

Table 1
Anti bacterial bioassay results[a,b] for R_2SnL_2 (inhibition zone in mm)

Micro-organism	HL	**1**	**2**	**3**	**4**	**5**	Imipinem[b]
Gram-positive							
Bacillus subtilis	15	18	22	20	25	20	31
Staphlococcus aureus	16	n.a.	20	25	34	19	43
Gram-negative							
Escherichia coli	n.a.	16	21	22	25	18	30
Schigella flexenari	n.a.	n.a.	n.a.	n.a.	22	16	33
Pseudomonas aeruginosa	12	12	12	14	21	18	25
Salmonella typhi	15	18	20	20	35	20	41

[a] Concentration used: 1.00 mg/1.00 mL of DMSO. Size of well: 6 mm (diameter). n.a: no activity.
[b] Standard drug.

The anti-inflammatory activity (% inhibition) of the synthesized complexes was conducted on adult albino rats (body weight 120–160 g) of Froster Charles species against carrageenin-induced edema in the doses of 50 mg kg^{-1} given orally and the acute toxicity (ALD50) was studied on albino mice (body weight 20–25 g) of either sex. The results are presented in Table 2. The activity of the standard drug, phenyl butazone, is used for the comparison.

The studies on the structure–activity correlation of organotin(IV) compounds reveal that the following structural features characterize the active compounds:

(i) the availability of coordination positions at tin;
(ii) the occurrence of stable ligand–Sn bonds viz., Sn–O bond.

Among the synthesized complexes, as revealed from the data presented in Table 2, the diphenyltin(IV) complex is found to be more active than the others. Lower activity of dialkyltin complexes except diphenyltin(IV) complex is probably due to the fact that the former form the most stable bonds upon complexation than the latter ones. The higher activity of diphenyltin(IV) complexes is also probably due to less number of coordination sites/bonds, which facilitate the easier formation of Ph_2Sn^{2+}(IV) moiety as a part of inhibition. It may be due to stronger interactions in dialkyltin(IV) complexes except diphenyl tin(IV) complex in which there are weaker interactions of ligand with tin, thereby, regulating the formation of R_2Sn^{2+} (IV) moiety.

Table 2
ALD_{50} (in mg kg^{-1}), anti-inflammatory activity of diorganotin (IV) complexes

Cpd	ALD_{50} (mg kg^{-1})	Anti-inflammatory activity (% inhibition) 50 mg kg^{-1} po
Phenyl butazone[a]	–	38.4
HL	<400	15.5
1	<500	20.4
2	>500	22.5
3	>500	25.7
4	>500	32.2
5	>500	28.5

[a] Standard drug.

Table 3
Results of in vitro cytostatic assays against cell line KB

Compound	IC_{50} ($\mu g\,mL^{-1}$ medium)	IC_{50} (μM)
HL	0.10	0.18
1	0.21	0.70
2	3.30	6.50
3	0.14	0.34
4	0.36	0.62
5	0.20	0.38
Cis-$[PtCl_2(NH_3)_2]$[a]	0.11	0.37

[a] Standard drug.

ALD_{50} values of the studied diorganotin(IV) derivatives is greater than $500\,mg\,kg^{-1}$ (the maximum dose tested), whereas ALD_{50} value for the ligand is $<400\,mg\,kg^{-1}$ indicating that the bigger bimolecules lower the toxicities but increase the activities of the resulting organotin(IV) complexes.

The results of cytostatic activity are summarized in Table 3. IC_{50} values of the compounds are expressed in μM, together with that of *cis*-$[PtCl_2(NH_3)_2]$ for comparison. All complexes show significant cytostatic activity. In particular, dibutyltin complex is even more active than the *cis*-$[PtCl_2(NH_3)2]$.

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