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# Synthesis and Structural Characterization of Organotin (IV) Complexes Derived of 4-(Diethylamino)benzoic Acid: Cytotoxic Assay on Human Liver Carcinoma Cells (HepG2)

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**Abstract:** Organotin(IV) carboxylate complexes derived from 4-(diethylamino)benzoic acid, 4-$[N(C_2H_5)_2]C_6H_4COOH$ have been successfully synthesized. Two types of diorganotin(IV) complexes $\{4\text{-}[N(C_2H_5)_2]C_6H_4COO\}_2(R)_2Sn$ (R= methyl **1**, butyl **2**), $[\{4\text{-}[N(C_2H_5)_2]C_6H_4COO(C_4H_9)_2Sn\}_2O]_2$ dimer **3** and $4\text{-}[N(C_2H_5)_2]C_6H_4COO(C_6H_5)_3Sn$ **4** were successfully synthesized and obtained in solid state. The acid and complexes **1**-**4** obtained were characterized quantitatively using C, H, N and Sn elemental analysis as well as spectroscopic methods such as infrared (FTIR) and nuclear magnetic resonance ($^1H$, $^{13}C$ & $^{119}Sn$ NMR). Results of the infrared and NMR spectroscopy on the acid and complexes showed that the coordination took place via oxygen atoms from the carboxylate group. As a result, the tin atom of complex **2** is six-coordinated, complex **4** is four-coordinated and tin atoms of complex **3** are five- and six-coordinated. With the exceptional case the tin atom of complex **1** exhibited five and six coordination in solution state which may be attributed from the dynamic stage or partial disassociation bonding of carboxylate anion. From the cytotoxic assay study, complex **4** revealed a significant result compared to complexes **1**-**3**.

**Key words:** Synthesis, Characterization, Cytotoxic assay

## INTRODUCTION

Although the first organotin(IV) compound was successfully isolated in 1850s, it did not gain any commercial significance in industrial application until almost a hundred years later (Blunden *et al*., 1985). From 1950s onwards, organotin(IV) carboxylate complexes became commercially relevant when the polyvinyl chloride (PVC) industry began to expand tremendously (Blunden *et al*., 1985; Evans and Karpel, 1985). Since then, organotin(IV) carboxylate compounds received tremendous attention due to the large array of applications in industries as well as their biocidal properties (Molloy *et al*., 1984; Poller, 1970; Gielen *et al*., 2000; Tamai *et al*., 2001; Ronconi *et al*., 2002; Han and Yang, 2002; Arkiş and Balköse, 2005). In fact, organotin(IV) complexes are extensively studied due to its coordination geometries as well as structural diversity (monomer, dimeric, hexameric and oligomeric) (Zhang *et al*., 2005; Win *et al*., 2007; Win *et al*., 2008; Amini *et al*., 2009).

As part of our interest and research on organotin(IV) work, we have synthesized and characterized organotin(IV) complexes derived of 4-(diethylamino)benzoic acid. In addition, the cytotoxic assay of the complexes obtained was screened against human liver carcinoma cells, HepG2 and the results are reported herein.

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## MATERIALS AND METHODS

***General and Instrumental*:**

Infrared spectra were recorded using a Perkin-Elmer System 2000 FTIR Spectrophotometer as a KBr disc in the frequency range of 4000-400 $cm^{-1}$. The spectra for $^{1}H$ and $^{119}Sn$ NMR were recorded on a Bruker AC-P 400 MHz FTNMR Spectrometer and $^{13}C$ NMR was recorded on a Bruker AC-P 300MHz FTNMR Spectrometer using deuterated $CDCl_3$ as the solvent and tetramethylsilane, TMS as the internal standard. Elemental C, H and N analyses were carried out on a Perkin-Elmer 2400 CHN Elemental Analyzer. Tin was determined gravimetrically by igniting a known quantity of each complex to $SnO_2$. The melting points were determined in an open capillary and were uncorrected. Triphenyltin(IV) hydroxide, $Ph_3SnOH$ and 4-(diethylamino)benzoic acid, 4-$[N(C_2H_5)_2]C_6H_4COOH$ were purchased from Aldrich Chemical and Merck Schuchardt respectively. Dibutyltin(IV) oxide, $Bu_2SnO$ and dimethyltin(IV) dichloride, $Me_2SnCl_2$ were obtained from Fluka Chemika. All reagents and solvents were purchased commercially and used without any further purification.

***In vitro Cytotoxic Assay:***

The *in vitro* cytotoxic assay was carried out on human liver carcinoma cells line, HepG2. The cells were maintained in Eagle's minimum essential medium (MEM) supplemented with 2 mM of L-glutamine, 1 mM of sodium pyruvate, 0.1 mM of non-essential amino acid, 1.5 µg/mL sodium bicarbonate, 100 IU/mL penicillin and 100 µg/mL streptomycin. The cytotoxicity assay was determined using the microtitration 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983; Ali *et al*., 2000). The assay of each concentration for each compound was performed in triplicate. The fraction of surviving cells was measured relative to the untreated cell population by measuring the absorbance values at 570 nm with reference at 630 nm using an ELISA microplate reader (Bio Tek EL 340, USA) (Ali *et al*., 2000). Cytotoxicity was expressed as fifty percent cytotoxic dose ($IC_{50}$), i.e. the concentration causing 50 % inhibition of cell growth with reference to the control (untreated cells). The $IC_{50}$ and the S.E.M. (standard error of the mean) were determined using Probit Analysis (SPSS, version 12.0.1).

***Preparation of Sodium Salt and Dimethyltin(IV) Oxide, $Me_2SnO$*:**

The sodium salt of the acid was obtained by heating under reflux a 1:1 molar mixture of sodium hydroxide, NaOH (0.12 g, 3 mmole) and 4-(diethylamino)benzoic acid, 4-$[N(C_2H_5)_2]C_6H_4COOH$ (0.58 g, 3 mmole) in ethanol (50 mL) for two hours. After a few days, white precipitates were obtained. Dimethyltin(IV) dichloride, $Me_2SnCl_2$ was dissolve in distilled water and stirred overnight which later gave colourless solution. Ammonia solution (60%) was added into the colourless solution and finally fine white precipitates were obtained, filtered and dried in oven (60 °C) for a day.

***Preparation of Complexes:***

***Bis{4-(diethylamino)benzoato}dimethyltin(IV), {4-$[N(C_2H_5)_2]C_6H_4COO\}_2(CH_3)_2Sn$ (1):***

Bis{4-(diethylamino)benzoato}dimethyltin(IV), **1** was obtained by heating under reflux a 1:2 molar mixture of dimethyltin(IV) oxide (0.49 g, 3 mmole) and 4-(diethylamino)benzoic acid (1.16 g, 6 mmole) in ethanol (50 mL) for two hours. A clear colourless transparent solution was isolated by filtration and kept in a bottle. After four days, fine colourless crystals (1.07 g, 67.0 % yield) were collected. Melting point: 185.3-186.1 °C. FTIR as KBr disc ($cm^{-1}$): n(C-H) aromatic 3079, n(C-H) saturated 2970, 2925, 2910, 2871; $\nu(COO)_{as}$ 1605, $\nu(COO)_s$ 1353, ν(C-N) 1272, ν(O-Sn-O) 612, ν(Sn-C) 553, ν(Sn-O) 428. $^{1}H$-NMR (ppm) ($CDCl_3$): d: benzene protons 6.66 (d, 9.0 Hz, 4H); 7.99 (d, 6.6 Hz, 4H); N-$(CH_2CH_3)_2$ 1.22 (t, 7.0 Hz, 12H); 3.45 (q, 7.1 Hz, 8H); methyl, $CH_3$ 1.11 (s, 6H). $^{13}C$-NMR (ppm) ($CDCl_3$): d: benzene carbons 110.54, 115.90, 132.92, 151.62; methyl 5.16, $^{1}J(^{119}Sn - ^{13}C)$= 670.3 Hz; N-$(CH_2CH_3)_2$ 12.90, 44.90; COO 176.76. $^{119}Sn$-NMR (ppm) ($CDCl_3$): δ: -137.25. Analysis for $C_{24}H_{34}N_2O_4Sn$: C, 54.05; H, 6.38; N, 5.15; Sn, 22.06 %. Calculated for $C_{24}H_{34}N_2O_4Sn$: C, 54.05; H, 6.43; N, 5.25; Sn, 22.26 %.

***Bis{4-(diethylamino)benzoato}dibutyltin(IV), {4-$[N(C_2H_5)_2]C_6H_4COO\}_2(C_4H_9)_2Sn$ (2):***

Complex **2** was prepared by a similar method to those described for complex **1**, except substituting dimethyltin(IV) oxide with dibutyltin(IV) oxide. Acetonitrile (50 mL) was used as solvent and the mixture was heated under reflux for three hours. A clear transparent solution was isolated by filtration and kept in a bottle. After twelve days, colourless crystals (1.52 g, 82.0 % yield) were collected. Melting point: 136.9-137.7 °C. FTIR as KBr disc ($cm^{-1}$): ν(C-H) aromatic 3080, ν(C-H) saturated 2969, 2929, 2871, 2856; $\nu(COO)_{as}$ 1603,

$\nu(COO)_s$ 1352, $\nu$(C-N) 1273, $\nu$(O-Sn-O) 610, $\nu$(Sn-C) 559, $\nu$(Sn-O) 442. $^1$H-NMR (ppm) ($CDCl_3$): $\delta$: benzene protons 6.66 (d, 9.1 Hz, 4H); 8.02 (d, 9.0 Hz, 4H); N-$(CH_2CH_3)_2$ 1.22 (t, 7.0 Hz, 12H); 3.44 (q, 7.0 Hz, 8H); butyl, $CH_3$ 0.91 (t, 7.4 Hz, 6H); $CH_2$ 1.36-1.46 (m, 4H); $CH_2$ 1.68-1.87 (m, 8H). $^{13}$C-NMR (ppm) ($CDCl_3$): $\delta$: benzene carbons 110.43, 119.98, 132.37, 150.74; butyl 14.12, 27.29, 27.92, 28.46; N-$(CH_2CH_3)_2$ 12.95, 44.89; COO 173.44. $^{119}$Sn-NMR (ppm) ($CDCl_3$): $\delta$: -214.50. Analysis for $C_{30}H_{46}N_2O_4Sn$: C, 58.37; H, 7.28; N, 4.61; Sn, 19.05 %. Calculated for $C_{30}H_{46}N_2O_4Sn$: C, 58.36; H, 7.51; N, 4.54; Sn, 19.22 %.

***Bis[4-(diethylamino)benzoato]tetrabutyldistannoxane(IV) dimer, $[\{4\text{-}[N(C_2H_5)_2]C_6H_4COO(C_4H_9)_2Sn\}_2O]_2$ (3):***

Complex **3** was obtained by heating under reflux a 1:1 molar mixture of dibutyltin(IV) oxide (0.49 g, 2 mmole) and 4-(diethylamino)benzoic acid (0.39 g, 2 mmole) in ethanol (50 mL) for two hours. A clear transparent solution was isolated by filtration and kept in a bottle. After five days, colourless crystals (2.96 g, 85.3 % yield) were collected. Melting point: 208.3-209.9 °C. FTIR as KBr disc ($cm^{-1}$): $\nu$(C-H) aromatic 3082, 3052; $\nu$(C-H) saturated 2957, 2925, 2869; $\nu(COO)_{as}$ 1604, 1576; $\nu(COO)_s$ 1350, 1394; $\nu$(C-N) 1269, $\nu$(Sn-O-Sn) 634, $\nu$(Sn-C) 548, $\nu$(Sn-O) 468. $^1$H-NMR (ppm) ($CDCl_3$): $\delta$: benzene protons 6.66 (d, 6.9 Hz, 8H); 7.91 (d, 7.3 Hz, 8H); butyl, $CH_3$ 0.88 (t, 7.3 Hz, 24H); $CH_2$ 1.37-1.41 (m, 16H); $CH_2$ 1.57-1.74 (m, 32H); N-$(CH_2CH_3)_2$ 1.23 (t, 6.5 Hz, 24H); 3.44 (q, 6.8 Hz, 16H). $^{13}$C-NMR (ppm) ($CDCl_3$): $\delta$: benzene carbons 110.43, 120.03, 132.36, 150.78; butyl 14.09, 26.83, 27.13, 27.27, 27.42, 27.90, 28.45; N-$(CH_2CH_3)_2$ 12.95, 44.87; COO 173.44. $^{119}$Sn-NMR (ppm) ($CDCl_3$): $\delta$: -171.65, -221.43. Analysis for $C_{76}H_{128}N_4O_{10}Sn_4$: C, 52.78; H, 7.07; N, 3.19; Sn, 27.37 %. Calculated for $C_{76}H_{128}N_4O_{10}Sn_4$: C, 52.68; H, 7.45; N, 3.23; Sn, 27.41 %.

***4-(Diethylamino)benzoatotriphenyltin(IV), 4-$[N(C_2H_5)_2]C_6H_4COO(C_6H_5)_3Sn$ (4):***

Complex **4** was prepared by heating under reflux a 1:1 molar mixture of triphenyltin(IV) hydroxide (0.73 g, 2 mmole) and 4-(diethylamino)benzoic acid (0.39 g, 2 mmole) in acetonitrile (50 mL) for two hours. A clear transparent solution was isolated by filtration and kept in a bottle. After four days, colourless crystals (1.30 g, 79.9 % yield) were obtained. Melting point: 113.5-113.8 °C. FTIR as KBr disc ($cm^{-1}$): $\nu$(C-H) aromatic 3067, 3053; $\nu$(C-H) saturated 2967, 2927, 2903, 2869; $\nu(COO)_{as}$ 1603, $\nu(COO)_s$ 1347, $\nu$(C-N) 1270, $\nu$(Sn-O) 448. $^1$H-NMR (ppm) ($CDCl_3$): $\delta$: phenyl protons 7.48-7.53 (m, 9H); 7.86-7.88 (m, 6H); benzene 6.66 (d, 8.9 Hz, 2H); 8.06 (d, 9.1 Hz, 2H); N-$(CH_2CH_3)_2$ 1.22 (t, 7.2 Hz, 6H); 3.43 (q, 7.1 Hz, 4H). $^{13}$C-NMR (ppm) ($CDCl_3$): $\delta$: phenyl carbons $C_{ipso}$ 139.75, $C_{ortho}$ 137.39 (48.1 Hz), $C_{meta}$ 129.20 (63.2 Hz), $C_{para}$ 130.28; benzene 110.53, 116.29, 133.25, 151.41; N-$(CH_2CH_3)_2$ 12.92, 44.92; COO 173.95. $^{119}$Sn-NMR (ppm) ($CDCl_3$): $\delta$: -127.33. Analysis for $C_{29}H_{29}N_1O_2Sn$: C, 64.28; H, 5.00; N, 2.63; Sn, 21.31 %. Calculated for $C_{29}H_{29}N_1O_2Sn$: C, 64.24; H, 5.39; N, 2.58; Sn, 21.89 %.

## RESULT AND DISCUSSION

***Physical and Elemental Analysis:***

During the heating under reflux of complexes **1-4**, molecular sieves and Dean-Stark apparatus were applied to remove the water liberated from the reaction. All the complexes obtained as colourless crystals and only crystals from complex **4** were suitable for the single crystal X-ray structure determination. The crystal structure of complex **4** has been reported earlier (Win *et al*., 2007). Elemental analysis C, H, N and Sn data obtained were in agreement with the predicted formula and complexes **1-4** gave sharp melting points which indicate the isolation of fairly pure complexes. An outline of the reaction scheme and proposed structure for complexes **1-4** are depicted in Figure 1.

***Infrared Spectroscopy:***

The $\nu$(O-H) bands which appeared in the range 2899-2545 $cm^{-1}$ for the acid, were absent in the infrared spectra of salt and complexes **1-4** showed the deprotonation and coordination of the carboxylate anion. The infrared spectra of complexes **1-4** revealed that the $\nu(COO)_{as}$ was shifted to a lower wave length number compared to the parent acid which signify that the coordination took place via the oxygen atoms of the carboxylate anion. The assignment of important infrared data for acid, sodium salt and complexes **1-4** are listed in Table 1. Complexes **1-4** showed the $\nu(COO)_{as}$ and $\nu(COO)_s$ are in the range of 1605-1579 and 1394-1347 $cm^{-1}$ respectively.

The magnitude of $\Delta\nu = [\nu(COO)_{as} - \nu(COO)_s]$ is a useful indicator in the correlation of the coordination modes of the carboxylate anion to the tin atoms. Sandhu and Verma have shown that the $\Delta\nu$ value of complexes greater by 65-90 $cm^{-1}$ than in their sodium salts indicates either asymmetric or monodentate bonding

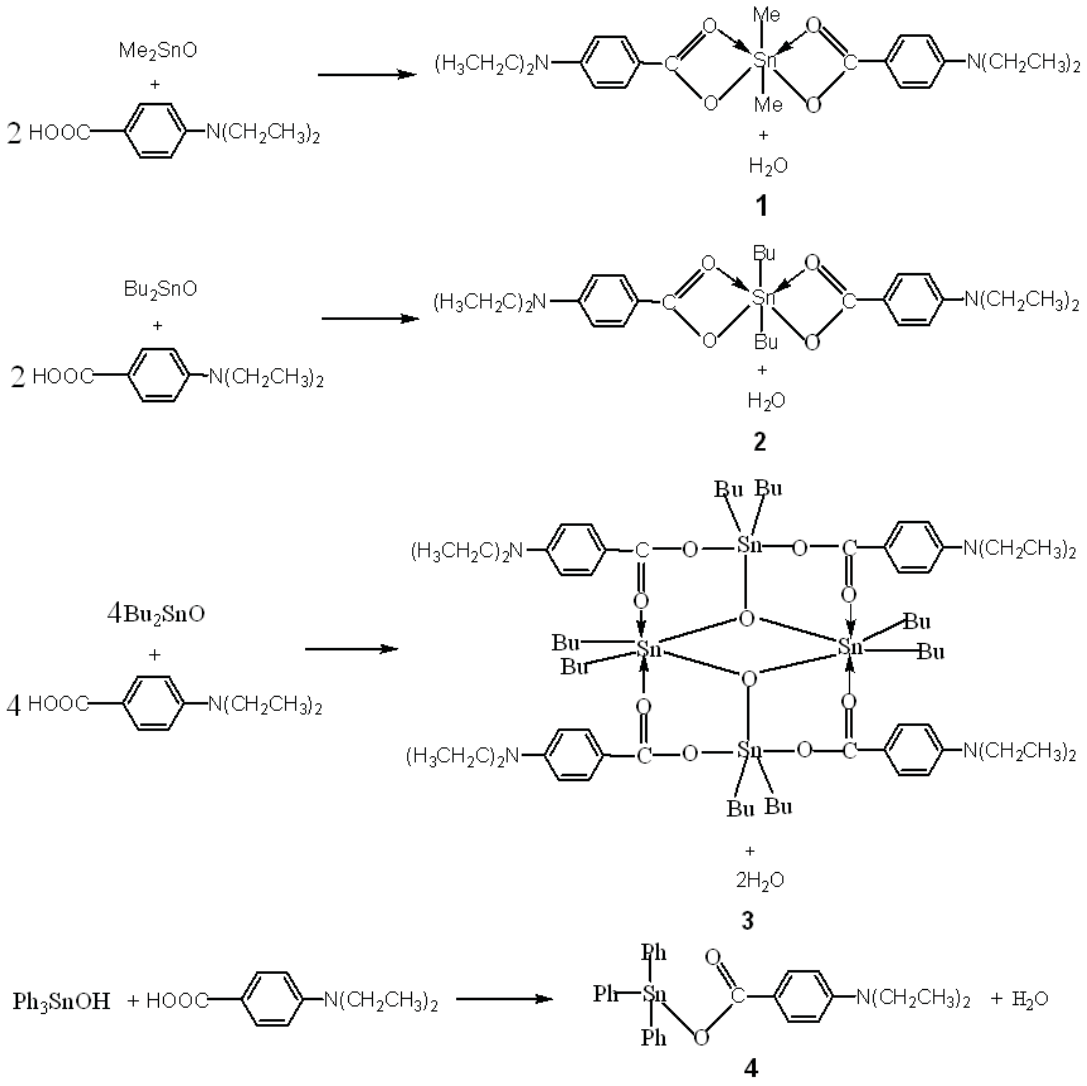



**Fig. 1:** An outline of proposed structure for complexes **1-4** and their reaction scheme.

of the carboxylate group to tin(IV) atom (Sandhu and Verma, 1987). Complexes **1-3** showed that the Dν is comparable to the sodium salt of the acid indicating bidentate bonding of the carboxylate group to tin(IV) atom. Moreover, for complexes derived from triphenyltin(IV) carboxylate, Δν below 200 $cm^{-1}$ would be expected for bridging or chelating carboxylates, but greater than 200 $cm^{-1}$ for the monodentate bonding carboxylate anions (Yeap and Teoh, 2003). Hence, carboxylate anion in complex **4** would be expected to bond to the tin atom in monodentate manner since the Δν above 200 $cm^{-1}$. Based on the infrared spectroscopy study, complex **4** exhibited four-coordinated tin atom; complex **3** exhibited five- and six-coordinated tin atoms whereas complexes **1** and **2** exhibited six-coordinated tin atom.

Further evidence for the coordination to Sn via O atoms was revealed by the presence of the Sn-O stretching bands in the spectra of complexes **1-3** in the region of 468-428 $cm^{-1}$. The carboxylate anion was bonded to the tin cation via *pπ-pπ* and *pπ-dπ* during complexation (Poller, 1970). Bonding via *pπ-dπ* occurred due to the transfer of a pair of electrons from the carbonyl group to the vacant 5*d* orbitals of the tin atom (Poller, 1970). The presence of a medium absorption band at 634-610 $cm^{-1}$ in the infrared spectra of complex **1-3** was ascribed to ν(Sn-O-Sn)/ν(O-Sn-O) which further supported the coordination of the oxygen atoms to

the tin atom moiety (Gielen *et al*., 1989; Win *et al*., 2008). Generally, ν(Sn-O-Sn) band was fit in the assignment of additional Sn-O bonding in organodistannoxane dimer type since the centrosymmetry of the complexs were occupied by $Sn_2O_2$ (complex **3**). Complex **4** [triphenyltin(IV)] revealed the presence of the ν(Sn-O) band at 448 $cm^{-1}$, hence *pπ-pπ* was involved in the complexation of complex **4** (Harrison, 1989).

**Table 1:** Important infrared data of acid, salt and complexes 1-4

| Complexes | Wavelength ($cm^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | ν(OH) | $\nu(COO)_{as}$ | $\nu(COO)_{s}$ | Δν | ν(Sn-O) | ν(O-Sn-O)/ ν(Sn-O-Sn) | ν(Sn-C) |
| Acid | 2899 - 2545 | 1663 | 1357 | 306 | - | - | - |
| salt | - | 1604 | 1356 | 248 | - | - | - |
| 1 | - | 1605 | 1353 | 253 | 428 | 612 | 553 |
| 2 | - | 1603 | 1352 | 251 | 442 | 610 | 559 |
| 3 | - | 1604 | 1350 | 254 | 468 | 634 | 548 |
| | | 1576 | 1394 | 182 | | | |
| 4 | - | 1603 | 1347 | 256 | 448 | - | - |

### *$^{1}H$, $^{13}C$ and $^{119}Sn$ Spectroscopy:*

The $^{1}H$ NMR spectral data of complexes **1-4** are summarized in Table 2, $^{13}C$ and $^{119}Sn$ NMR data are listed in Table 3. The $^{1}H$ NMR spectra of complexes **1-4** revealed similarities to their parent acid, 4-(diethylamino)benzoic acid. The $^{1}H$ NMR spectrum of 4-(diethylamino)benzoic acid exhibited two sets of signals [6.66 and 7.97 ppm] with integration values of 2:2 and another two set of signals [1.22 and 3.44 ppm] with integration values of 6:4. Both signals were also observed in the $^{1}H$ NMR spectra of complexes **1-4** arising from the aromatic protons of the benzene ring and the -ethylamino protons attached at the *para* position at the benzene ring.

The upfield regions of the $^{1}H$ NMR spectra of the complexes **1-3** showed the signal of the methyl and butyl protons in the range of 1.11 and 0.88-1.87 ppm respectively. In general, complex **3** (organodistannoxane dimer) should exhibit two unresolved sets of butyl signals, one of the butyl groups linked to the endo-cyclic tin atom and the other one linked to the exo-cyclic tin atom respectively (Danish *et al*., 1995). Due to a very similar environment or overlapping of methylene signals multiplicity of butyl group in the NMR spectra, complex **3** only showed one unresolved set of $CH_3$ signal at 0.88 ppm and two sets of methylene signals in the range of 1.37-1.41 and 1.57-1.74 ppm. For complex **4**, the resonances appeared as two well separated sets of multiplets in the regions centering around δ≈ 7.50 and 7.87 ppm ascribed to phenyl protons. At the low field arising from *ortho* and at higher field arising from *meta* and *para* phenyl protons respectively (Sau and Holmes, 1981). In general, the complexes **1-4** obtained exhibited no additional resonances and thus reflect the purity of the complexes.

The formation of the complexes were evident from the δ(COO) value in the $^{13}C$ NMR spectra. All the complexes exhibited a δ(COO) signal in the range of 173.44-176.76 ppm. The $^{13}C$ NMR spectra of complexes **1-4** showed that the chemical shift of the δ(COO) signal in each complex was shifted downfield compared to that of their parent acids (173.02 ppm), indicating the participation of the carboxylate anions in the coordination to the tin(IV) atom (Win *et al*., 2007; Win *et al*., 2008). This phenomenon resulted from the decrease of the electron density in the carboxylate anions upon coordinated to the tin atom moiety during complexation. The occurrence of four resonances in the range of 110.43-151.62 ppm and two resonances in the range of 12.88-44.93 ppm in the $^{13}C$ NMR spectra of the complexes and acid defined as benzene and -ethylamino carbons signals respectively.

Complex **1** exhibited a sharp signal at 5.16 ppm with the $^{1}J(^{119}Sn\text{-}^{13}C)$ value of 670.3 Hz and the C-Sn-C angle was 135.6°. Hence, the tin atom in complex **1** was six-coordinated and exhibited a distorted octahedral geometry (Lockhart and Manders, 1986). Complexes **2** and **3** showed the occurrence of $CH_3$ and $CH_2$ in the range of 14.09-14.12 and 26.83-28.46 ppm respectively (Danish *et al*., 1995; Win *et al*., 2008). In addition, complex **3** exhibited two sets of butyl signals in $^{13}C$ NMR spectra which attributed to the butyl groups linked to the exo- and endo-cyclic tin atom respectively. Complex **4** revealed the chemical shifts of the $\delta(^{13}C)_{ipso}$ at 139.75 ppm indicative of a four-coordinated Sn atom (Holeček *et al*., 1983a, Holeček *et al*., 1983b, Baul *et al*., 2001). Five-coordinated triphenyltin(IV) carboxylate complexes the $\delta(^{13}C)_{ipso}$ will be observed at approximately 4 ppm higher (Baul *et al*., 2001). Generally, the $^{13}C$ NMR spectra of the complexes obtain were found to exhibit no additional resonance and thus reflects the purity of the complexes.

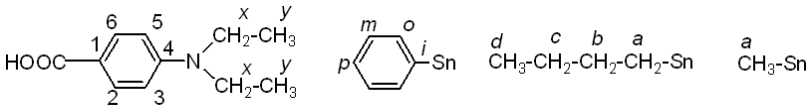
**Table 2:** $^{1}$H NMR data of acid and complexes 1-4.

| Compounds | Chemical Shift, $\delta$ (ppm) | | |
|---|---|---|---|
| | Benzene | Amino $-N(CH_2CH_3)_2$ | Sn-R (R= Me, Bu & Ph) |
| Acid | 6.66 (d, 9.1 Hz, 2H) H3 & H5 | 1.22 (t, 7.1 Hz, 6H) H*y* | - |
| | 7.97 (d, 9.1 Hz, 2H) H2 & H6 | 3.44 (q, 7.1 Hz, 4H) H*x* | |
| 1 | 6.66 (d, 9.0 Hz, 4H) H3 & H5 | 1.22 (t, 7.0 Hz, 12H) H*y* | 1.11 (s, 6H) H*a* |
| | 7.99 (d, 6.6 Hz, 4H) H2 & H6 | 3.45 (q, 7.1 Hz, 8H) H*x* | |
| 2 | 6.66 (d, 9.1 Hz, 4H) H3 & H5 | 1.22 (t, 7.0 Hz, 12H) H*y* | 0.91 (t, 7.4 Hz, 6H) H*d* |
| | 8.02 (d, 9.0 Hz, 4H) H2 & H6 | 3.44 (q, 7.0 Hz, 8H) H*x* | 1.36-1.46 *(m, 4H) H*c* |
| | | | 1.68-1.87 *(m, 8H) H*a* & H*b* |
| 3 | 6.66 (d, 6.9 Hz, 8H) H3 & H5 | 1.23 (t, 6.5 Hz, 24H) H*y* | |
| | 7.91 (d, 7.3 Hz, 8H) H2 & H6 | 3.44 (q, 6.8 Hz, 16H) H*x* | 0.88 (t, 7.3 Hz, 24H) H*d* |
| | | | 1.37-1.41 *(m, 16H) H*c* |
| | | | 1.57-1.74 *(m, 32H) H*a* & H*b* |
| 4 | 6.66 (d, 8.9 Hz, 2H) H3 & H5 | 1.22 (t, 7.2 Hz, 6H) H*y* | 7.48-7.53 *(m, 9H) H*m* & H*p* |
| | 8.06 (d, 9.1 Hz, 2H) H2 & H6 | 3.43 (q, 7.1 Hz, 4H) H*x* | 7.86-7.88 *(m, 6H) H*o* |

s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet; *o*= ortho, *m*= meta, *p*= para; Coupling constant= Hz, *= overlap

**Table 3:** $^{119}$Sn and $^{13}$C NMR data of complexes 1-4.

| Compounds | Chemical Shift (ppm) | | | | |
|---|---|---|---|---|---|
| | $^{119}$Sn | Benzene | Amino $N(CH_2CH_3)_2$ | Sn-R (R= Me, Bu & Ph) $^{n}J(^{119}Sn\text{-}^{13}C)$ (n=1, 2, 3 & 4) | COO |
| Acid | - | 110.54 (C3 & C5), 115.45 (C1), 132.73 (C2 & C6), 151.96 (C4) | 12.88 (C*y*), 44.93 (C*x*) | - | 173.02 |
| 1 | -137.25 | 110.54 (C3 & C5), 115.90 (C1), 132.92 (C2 & C6), 151.62 (C4) | 12.90 (C*y*) 44.90 (C*x*) | 5.16 ($^{1}J$= 670.3 Hz) (C*a*) | 176.76 |
| 2 | -214.50 | 110.43 (C3 & C5), 119.98 (C1), 132.37 (C2 & C6), 150.74 (C4) | 12.97 (C*y*) 44.89 (C*x*) | 14.12 (C*d*), 27.29 (C*b*) 27.92 (C*c*), 28.46 (C*a*) | 173.44 |
| 3 | -171.65 -221.43 | 110.43 (C3 & C5), 120.03 (C1), 132.36 (C2 & C6), 150.78 (C4) | 12.95 (C*y*) 44.87 (C*x*) | 14.09 (C*d*), 26.83 (C*c*), 27.13 (C*c*), 27.27 (C*b*), 27.42 (C*b*), 27.90 (C*a*), 28.45 (C*a*) | 173.44 |
| 4 | -127.33 | 110.53 (C3 & C5), 116.29 (C1), 133.25 (C2 & C6), 151.41 (C4) | 12.92 (C*y*) 44.92 (C*x*) | 139.75 (C*i*), 137.39 ($^{2}J$= 48.1 Hz) (C*o*) 129.20 ($^{3}J$= 63.2 Hz) (C*m*), 130.28 (C*p*) | 173.95 |




The δ($^{119}$Sn) values define the region with different coordination number of the tin atom moiety. For diorganotin(IV) carboxylate complexes, the δ($^{119}$Sn) value for four-coordinated complexes fall in the range between +200 to -60 ppm; for five-coordinated complexes between -90 to -190 ppm and for six-coordinated complexes between -210 to -400 ppm (Holeček *et al*., 1986). For complex **1**, the δ($^{119}$Sn) value was -137.25 ppm, indicating that the tin atom was five-coordinated and exhibited distorted trigonal bipyramid geometry. Upon dilution, one of the bonds in complex **1** disassociated resulting in the tin atom to exhibit a five-coordinated geometry. Complex **2** exhibited a well resolved resonance of δ($^{119}$Sn) at -214.50 ppm respectively, indicating that the tin atom was six-coordinated and occupied a distorted octahedral geometry. Complex **3** derivatives of the organodistannoxane dimer type exhibited two well resolved δ($^{119}$Sn) signals attributed to the exo- and endo-cyclic tin atoms (Danish *et al*., 1995). Complex **3** exhibited two well resolved signals, one lying in the range of a five-coordinated geometry and the other in the range of a six-coordinated geometry, indicating that the carboxylate anions remained in a bidentate manner in the coordination to the tin atoms in solution state. Complex **4** exhibited the δ($^{119}$Sn) values at -127.33 ppm which lie in the range of -40 to -120 ppm [for triphenyltin(IV) complexes], hence, indicating that the tin atom in complex **4** is four-coordinated with a distorted tetrahedral geometry (Holeček *et al*., 1983a; Holeček *et al*., 1983b). Moreover, the δ($^{119}$Sn) value of complex **4** obtained is slightly in the upfield region of -40 to -120 ppm and did not lie in the range of five-coordinated tin atom [-180 to -260 ppm for triphenyltin(IV) complexes] indicating the tin atom of complex **4** was still four-coordinated with distorted tetrahedral geometry.

***In vitro Cytotoxic Assay:***

The $IC_{50}$ values for the acid and complexes **1-4** are given in Table 4. From the data in Table 4, it was found that 4-(diethylamino)benzoic acid is inactive against HepG2 cells compared to complexes **1-4**. Complex **4** showed a significant cytotoxic activity with a lower $IC_{50}$ value of 0.151 µg/mL compared to complexes **1** (0.747 µg/mL), **2** (0.565 µg/mL) and **3** (0.722 µg/mL). This was due to complex **4** is derived from triphenyltin(IV) (triorganotin) which is more active compared to the diorganotin(IV) derivatives (complexes **1-3**). In addition, complex **4** existed as monomer and the tin moiety was four-coordinated with distorted tetrahedral geometry consequently enhanced the cytotoxic activity (Ashfaq *et al*., 2004).

**Table 4:** Cytotoxicity assays, $IC_{50}$ of acid and complexes 1-4

| Complexes | $IC_{50}$ (µg/mL)<br>Human liver hepatocellular carcinoma cells, HepG2 |
|---|---|
| Acid | Inactive (start at 1.0) |
| 1 | 0.747 ± 0.037 |
| 2 | 0.565 ± 0.031 |
| 3 | 0.722 ± 0.036 |
| 4 | 0.151 ± 0.007 |
| Vincristine sulphate | 0.042 ± 0.013I |

$IC_{50}$(µg/mL)= the concentration that yields 50% inhibition of the cell compared with untreated control.
The cytotoxicity values are expressed as mean ± S.E.M. from the triplicate. Reference drug= Vincristine sulphate

Complex **2** is found to be more active compared to complexes **1** and **3**. This may due to complex **3** (organodistannoxane dimer) existed as a bulky structure which in turn perturb the transportation ability towards the targeted cell; the lipophilicity and membrane activity of complex **2** enhanced by the butyl groups attached to the tin atom moiety compared to complex **1** (methyl) resulted in higher cytotoxic activity in complex **2**. Hence the biological activities of organotin(IV) obtained in this present study could be arranged as: triorganotin(IV) [tetrahedral] > diorganotin(IV) [monomer] > diorganotin(IV) [organodistannoxane dimer].

***Conclusion:***

The title complexes have been successfully synthesized and characterized. Elemental analysis C, H, N and Sn data obtained were in agreement with the predicted formula. Results of the infrared and NMR spectroscopy on the acid and complexes showed that the coordination took place via oxygen atoms from the carboxylate group. As a result, the tin atom of complex **2** is six-coordinated, complex **4** is four-coordinated and tin atoms of complex **3** are five- and six-coordinated. With the exceptional case the tin atom of complex **1** exhibited five and six coordination in solution state with may attributed from the dynamic stage or partial disassociation bonding of carboxylate anion. Based on the cytotoxic activity, complex **4** showed significant cytotoxic activity compared to complexes **1-3** but lower compared to reference drug.

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