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Synthesis, Structural Characterization and Cytotoxic Assay on Human Liver Carcinoma Cells (Hepg2) of Organotin(IV) Complexes Derived of 2-amino-5-nitrobenzoic Acid

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Abstract: Organotin(IV) carboxylate complexes derived from 2-amino-5-nitrobenzoic acid, 2-NH_2-5-NO_2-C_6H_3COOH have been successfully synthesized. Two types of diorganotin(IV) complexes $R_2Sn(2\text{-}NH_2\text{-}5\text{-}NO_2\text{-}C_6H_3COO)_2$ (R= methyl **1**, butyl **2**), $\{[(Bu_2Sn(2\text{-}NH_2\text{-}5\text{-}NO_2\text{-}C_6H_3COO)]_2O\}_2$ dimer **3** and $Ph_3Sn(2\text{-}NH_2\text{-}5\text{-}NO_2\text{-}C_6H_3COO)$ **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 spectroscopy on the acid and complexes showed that the coordination took place via oxygen atoms from the carboxylate group. This indicated that the carboxylate anion acted as mono and bidentate ligand. In ^{119}Sn NMR solution study, the tin atom of both complexes **1** and **2** exhibit six-coordination respectively and complex **3** exhibits five- and six-coordination whereas the tin atom of complex **4** exhibits five-coordination. From the cytotoxic assay study, complex **4** revealed a significant result compared to complexes **1**-**3**.

Key words: Organotin(IV) carboxylate, Synthesis, Characterization, Cytotoxic assay

INTRODUCTION

Organotin(IV) complexes are extensively studied due to the applications in industrial as well as biocidal properties (Molloy *et al*., 1984; Willem *et al*., 1997; Gielen *et al*., 2000). Numerous studies on organotin(IV) complexes have been carried out in order to study its biological properties against bacterial, fungus and cancer cells line (Teoh *et al*., 1997; Novelli *et al*., 1999; Gielen *et al*., 2000; Crouse *et al*., 2004). Moreover, the biological activity of organotin(IV) carboxylate complexes are greatly influenced by the structure of the molecule as well as the coordination number of the tin moiety (Parulekar *et al*., 1990). The search for organometallic compounds as a new alternative drug in combating human cancers has been initiated due to certain side-effects of *cis*-platin and carboplatin as antitumour drugs (Khan *et al*., 2000). Hence, organotin(IV) compounds with general formula $R_2SnX_2.L_n$ or R_2SnL_2 (R= alkyl, aryl or phenyl, X= halogen, L= coordinated ligands and n= 1 or 2) belong to the largest group including organotin(IV) carboxylate complexes selected for the anti cancer screening (Gielen *et al*., 2000; Ronconi *et al*., 2002; Pruchnik *et al*., 2003). In general, among tri- di- and mono-organotin(IV) compounds, triorganotin(IV) compounds are found to display higher biological activities due to the ability to bind protein molecules (Baul *et al*., 2001). In addition, structure-activity relationship studies indicated that monomeric triorganotin(IV) carboxylate complexes with *trans*-R_3SnO_2 or tetrahedral geometry show a better activity compared to *cis*-R_3SnO_2 geometry (Baul *et al*., 2002).

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Commonly, diorganotin(IV) carboxylates can be obtained in monomeric or distannoxane-dimeric structure when the reaction of diorganotin(IV) with carboxylic acid is carried out in 1:2 or 1:1 ratio respectively (Yin *et al*., 2005; Win *et al*., 2006; Win *et al*., 2008). Meanwhile triphenyltin(IV) carboxylate complexes exists as monomeric structures with four-coordinate distorted tetrahedral or five-coordinate trigonal bipyramid geometries (Baul *et al*., 2001; Yeap and Teoh, 2003; Win *et al*., 2007).

In this paper, we are focus on synthesis and structural characterization of new organotin(IV) carboxylate complexes derived from 2-amino-5-nitrobenzoic acid. In addition, the cytotoxic assay of the complexes obtained was screened against human liver carcinoma cells, HepG2.

MATERIALS AND METHODS

***General and Instrumental*:**

Triphenyltin(IV) hydroxide, Ph_3SnOH and 2-amino-5-nitrobenzoic acid, 2-NH_2-5-NO_2-C_6H_3COOH were purchased from Aldrich Chemical and Acros Organics 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. The melting points were determined in an open capillary and are uncorrected. 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. Infrared spectra were recorded using a Perkin-Elmer System 2000 FTIR Spectrophotometer as a KBr disc in the frequency range 4000-400 cm^{-1}. The spectra for ^1H 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 d_6-DMSO as the solvent and tetramethylsilane, TMS as the internal standard.

***Preparation of dimethyltin(IV) oxide, Me_2SnO and salt*:**

Dimethyltin(IV) dichloride, Me_2SnCl_2 was dissolve in distilled water and stirred for overnight. Colourless solution was obtained. Ammonia solution (60%) was added into the colourless solution and finally fine white precipitate was obtained and filtered. The precipitate was dried in oven (60 °C) for a day until dry white precipitate was obtained. 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 2-amino-5-nitrobenzoic acid, NH_2-5-NO_2-C_6H_3COOH (0.55 g, 3 mmole) in ethanol (50 mL) for two hours. After a few days, yellowish precipitate was obtained.

Preparation of Organotin(IV) Complexes:

Bis(2-amino-5-nitrobenzoato) dimethy ltin(IV), $Me_2Sn(2\text{-}NH_2\text{-}5\text{-}NO_2\text{-}C_6H_3COO)_2$ (**1**)

Complex **1** was obtained by heating under reflux a 1:2 molar mixture of dimethy ltin(IV) oxide (0.17 g, 1 mmole) and acid (0.363 g, 2 mmole) in ethanol (50 mL) for an hour. A clear yellow transparent solution was separated by filtration and kept in a bottle. After four days, fine yellow solids (0.45 g, 88.0% yield) were collected. Melting point: >300 °C (decomposed). Analysis for $C_{16}H_{16}N_4O_8Sn$: C, 37.66; H, 3.01; N, 11.04; Sn, 23.26%. Calculated for $C_{16}H_{16}N_4O_8Sn$: C, 37.61; H, 3.16; N, 10.96; Sn, 23.23 %. FTIR as KBr disc (cm^{-1}): ν(NH_2) 3468, 3358; ν(C-H) aromatic 3091, ν(C-H) saturated 2955, ν$(COO)_{as}$ 1626, ν$(COO)_s$ 1315, ν(NO_2) 1552, n(O-Sn-O) 644, ν(Sn-C) 506, ν(Sn-O) 404. ^1H-NMR (ppm) (d_6-DMSO): δ: benzene protons 6.82 (d, 9.3 Hz, 2H); 8.03 (dd, 2.8 Hz, 9.2 Hz, 2H); 8.63 (d, 2.8 Hz, 2H); methyl, CH_3 0.93 (s, 6H), $^2J(^{119}Sn - ^1H)$= 95.2 Hz. ^{13}C-NMR (ppm) (d_6-DMSO): δ: benzene carbons 112.43, 116.91, 128.94, 129.99, 135.98, 156.82; methyl 12.25; COO 172.24. ^{119}Sn-NMR (ppm) (d_6-DMSO): δ: -281.39.

Bis(2-amino-5-nitrobenzoato)dibutyltin(IV), $Bu_2Sn(2\text{-}NH_2\text{-}5\text{-}NO_2\text{-}C_6H_3COO)_2$ (**2**)

Complex **2** was obtained by heating under reflux a 1:2 molar mixture of dibutyltin(IV) oxide (0.75 g, 3 mmole) and acid (1.09 g, 6 mmole) in methanol (50 mL) for four hours. After two weeks, yellow crystals (1.60 g, 90.0% yield) were collected. Melting point: 209.3-209.7 °C. Analysis for $C_{22}H_{28}N_4O_8Sn$: C, 44.42; H, 4.19; N, 9.38; Sn, 19.91%. Calculated for $C_{22}H_{28}N_4O_8Sn$: C, 44.39; H, 4.74; N, 9.41; Sn, 19.94%. FTIR as KBr disc (cm^{-1}): ν(NH_2) 3484, 3313, 3334; ν(C-H) aromatic 3059, ν(C-H) saturated 2951, 2922, 2854; ν$(COO)_{as}$ 1619, ν$(COO)_s$ 1314, ν(NO_2) 1541, ν(O-Sn-O) 642, ν(Sn-C) 515, ν(Sn-O) 420. ^1H-NMR (ppm) (d_6-DMSO): δ: benzene protons 6.94 (d, 9.3 Hz, 2H); 8.15 (dd, 2.8 Hz, 9.3 Hz, 2H); 8.73 (d, 2.8 Hz, 2H); butyl, CH_3 0.91 (t, 7.3 Hz, 6H); CH_2 1.40 (sx, 7.1 Hz, 4H); CH_2 1.64-1.80 (m, 8H). ^{13}C-NMR (ppm) (d_6-DMSO): δ: benzene carbons 111.84, 116.98, 129.15, 130.04, 135.93, 156.81; butyl 14.42, 26.47, 27.72, 31.02; COO 173.05. ^{119}Sn-NMR (ppm) (d_6-DMSO): δ: -310.76.

Bis(2-amino-5-nitrobenzoato)tetrabutyldistannoxane(IV) dimer, $\{[(Bu_2Sn(2\text{-}NH_2\text{-}5\text{-}NO_2\text{-}C_6H_3COO)]_2O\}_2$ (**3**)

Complex **3** was prepared from a 1:1 molar mixture of dibutyltin(IV) oxide (0.50 g, 2 mmole) and 2-amino-5-nitrobenzoic acid (0.36 g, 2 mmole) in ethanol (50 mL). Dibutyltin(IV) oxide was first dissolved in ethanol (20 mL) and heated for an hour until clear solution was obtained. Then, 2-amino-5-nitrobenzoic acid dissolved in ethanol (30 mL) added to the dibutyltin(IV) oxide solution. The resulting mixture was heated under reflux for two hours. A clear yellow transparent solution was isolated by filtration and kept in a bottle. After four days, yellow solids (0.61 g, 73.0% yield) were collected. Melting point: 240.7-241.5 °C. Analysis for $C_{60}H_{92}N_8O_{18}Sn_4$: C, 42.74; H, 5.79; N, 6.57; Sn, 27.98%. Calculated for $C_{60}H_{92}N_8O_{18}Sn_4$: C, 42.66; H, 5.49; N, 6.64; Sn, 28.12%. FTIR as KBr disc (cm^{-1}): n(NH_2) 3457, 3344, 3314; n(C-H) aromatic 3059, n(C-H) saturated 2956, 2926, 2870; ν$(COO)_{as}$ 1622, ν$(COO)_s$ 1310, ν(NO_2) 1537, ν(Sn-O-Sn) 630, ν(Sn-C) 531, ν(Sn-O) 391. ^{1}H-NMR (ppm) (d_6-DMSO): δ: benzene protons 6.92 (d, 9.3 Hz, 4H); 8.12 (dd, 2.4 Hz, 9.2 Hz, 4H); 8.72 (s, 4H); butyl, CH_3 0.84 (t, 7.3 Hz, 12H); 0.90 (t, 7.3 Hz, 12H); CH_2 1.28-1.43 (m, 32H); CH_2 1.64-1.80 (m, 16H). ^{13}C-NMR (ppm) (d_6-DMSO): δ: benzene carbons 112.27, 116.50, 128.49, 129.47, 135.55, 156.49; butyl 13.71, 13.91, 26.09, 26.77, 27.02, 27.29, 29.90; COO 172.11. ^{119}Sn-NMR (ppm) (d_6-DMSO): δ: -173.87, -213.71.

2-Amino-5-nitrobenzoatotriphenyltin(IV), $Ph_3Sn(2\text{-}NH_2\text{-}5\text{-}NO_2\text{-}C_6H_3COO)$ (**4**)

Complex **4** was obtained by heating under reflux a 1:1 molar mixture of triphenyltin(IV) hydroxide (0.73 g, 2 mmole) and 2-amino-5-nitrobenzoic acid (0.36 g, 2 mmole) in methanol (60 mL) for an hour. A clear yellow transparent solution was separated by filtration and kept in a bottle. After six days, yellow crystals (0.51 g, 96.0% yield) were collected. Melting point: 208.5-208.9 °C. Analysis for $C_{25}H_{20}N_2O_4Sn$: C, 56.41; H, 3.48; N, 5.23; Sn, 22.03%. Calculated for $C_{25}H_{20}N_2O_4Sn$: C, 56.53; H, 3.80; N, 5.27; Sn, 22.35%. FTIR as KBr disc (cm^{-1}): ν(NH_2) 3442, 3328; ν(C-H) aromatic 3058, ν$(COO)_{as}$ 1618, ν$(COO)_s$ 1310, ν(NO_2) 1556, ν(Sn-O) 443. ^{1}H-NMR (ppm) (d_6-DMSO): δ: phenyl protons 7.53-7.59 (m, 9H); 7.95-7.97 *(m, 6H); benzene 6.82 (d, 9.2 Hz, 1H); 8.07 (dd, 2.8 Hz, 9.2 Hz, 1H); 8.73 (d, 2.9 Hz, 1H). ^{13}C-NMR (ppm) (d_6-DMSO): δ: phenyl carbons C_{ipso} 142.87 (839.3 Hz), C_{ortho} 136.11 (45.6 Hz), C_{meta} 128.43, C_{para} 129.03 (18.4 Hz); benzene 112.77, 115.75, 127.72, 127.97, 134.89, 155.92; COO 169.52. ^{119}Sn-NMR (ppm) (d_6-DMSO): d: -265.89.

In Vitro Cytotoxic Assay:

The *in vitro* cytotoxic assay was carried out against 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; Crouse *et al*., 2004). 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 a 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) was determined using Probit Analysis (SPSS, version 12.0.1).

RESULT AND DISCUSSION

Physical And Elemental Analysis:

Complexes **2** and **4** were obtained as single yellow crystals and X-ray structure of both complexes have been reported (Win *et al*., 2006; Win *et al*., 2007). Complexes **1** and **3** were obtained as fine yellowish powder and precipitate respectively. Molecular sieves were added during the heating under reflux for complexes **2** and **4** to remove water formed during the reaction. Besides, the water liberated in the reaction for complexes **1-4** was removed by azeotropic dehydration using the Dean-Stark apparatus. Elemental analysis C, H, N and Sn data obtained were in agreement with the predicted formula. Complex **1** decomposed when the temperature raise up to 300 °C. Moreover, the complexes **2-4** gave sharp melting points indicating the isolation of fairly pure complexes. An outline of the reaction scheme and proposed structure for complexes **1-4** are depicted in Figure 1.

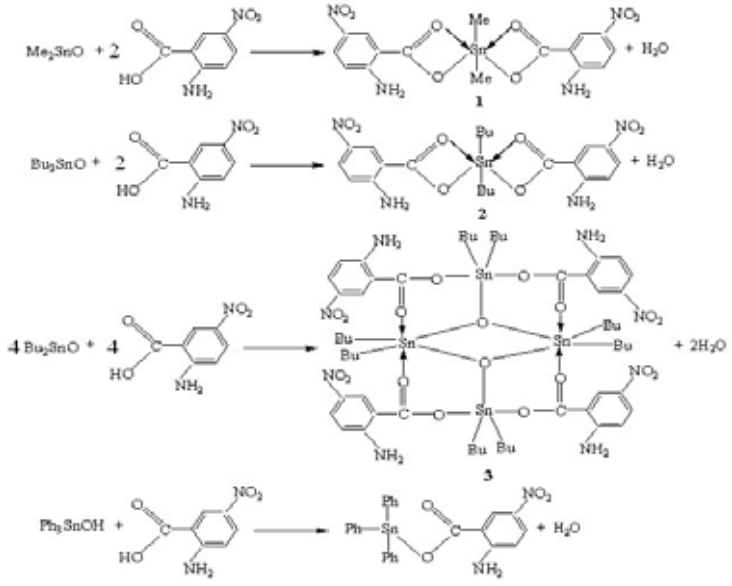


Fig. 1: An outline of the reaction scheme and proposed structure for complexes 1-4.

Infrared Spectroscopy:

The assignment of important infrared data for acid, sodium salt and complexes **1-4** are listed in Table 1. The ν(O-H) bands which appeared in the range 2892-2616 cm^{-1} for the acid, were absent in the infrared spectra of salt and complexes **1-4** showing the deprotonation and coordination of the carboxylate anion. The $\nu(COO)_{as}$ band of the complexes are shifted to lower wave number compared to the acid. Complexes **1-4** showed the $\nu(COO)_{as}$ and $\nu(COO)_{s}$ in the range of 1618-1626 and 1310-1315 cm^{-1} respectively. Generally, the $D\Delta\nu = [\nu(COO)_{as} - \nu(COO)_{s}]$ value is used to determine the bonding properties of carboxylate anion to tin atom in organotin(IV) carboxylate complexes. Sandhu and Verma in their studies and reports have shown that the DΔν value of complexes greater by 65-90 cm^{-1} than in their sodium salts indicates either asymmetric or monodentate bonding of the carboxylate group to tin(IV) atom (Sandhu and Verma, 1987). Complexes **1**-**3** showed that the Dn 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, Dn 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 DΔν above 200 cm^{-1}. Based on the infrared spectroscopy study, both complexes **1** and **2** exhibit six-coordinated tin atom; complex **3** exhibits five- and six-coordinated whereas complex **4** exhibits four-coordinated tin atom.

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

Compounds	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	2892-2616	1685	1330	355	-	-	-
salt	-	1637	1334	303	-	-	-
1	-	1626	1315	311	404	644	506
2	-	1619	1314	305	420	642	515
3	-	1622	1310	312	391	630	531
4	-	1618	1310	308	443	-	-

Overall, a band was observed in the region of 391-443 cm^{-1} in complexes **1-4** was assigned to the Sn-O stretching (Sandhu and Verma, 1987). The presence of the band indicates that the Sn atom is bonded to the oxygen atom from the carboxylate group of the acid. Disappearance of the ν(O-H), shifting of ν(COO) and occurrence of ν(Sn-O) bands in complexes **1-4** indicates that the carboxylate group is coordinated to the Sn atom.

^{1}H, ^{13}C and ^{119}Sn spectroscopy:

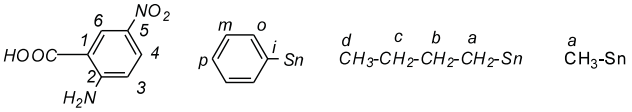
The relevant data obtained from the ^{1}H NMR spectra of complexes **1-4** was presented in Table 2, ^{13}C and ^{119}Sn NMR data are presented in Table 3. The ^{1}H NMR spectra of complexes **1-4** only showed two sets of peaks. The benzene protons appear in the downfield region in the range of 6.82-8.73 ppm in the spectra of complexes and acid. The resonances appearing as two well separated sets of multiplets in the regions centering around d≈ 7.55 ppm and 7.96 ppm ascribed to phenyl protons for complex **4**. At the low field arising from *ortho* and at higher field arising from *meta* and *para* phenyl protons respectively (Sau and Holmes, 1981).

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 0.93 and 0.84-1.80 ppm respectively. In addition, complex **1** showed $^{2}J(^{119}\text{Sn-}^{1}\text{H})$ at 95.2 Hz and based on the application of the Lockhart-Manders equation, the C-Sn-C angle is 153.61° (Lockhart and Manders, 1986). Based on the $^{2}J(^{119}\text{Sn-}^{1}\text{H})$ and C-Sn-C angle, the tin atom of complex **1** is believed to exist in distorted octahedral geometry and six-coordinated. In general, complex **3** is one of the distannoxane dimer types and should exhibited 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). However, complex **3** only showed two unresolved sets of CH_3 signal at 0.84 and 0.90 ppm respectively and two set of methylene signal of butyl group in the range of 1.28-1.43 and 1.64-1.80 ppm in the spectra. This may due to a very similar environment or overlapping of methylene signals multiplicity in the NMR spectra. In general, the complexes **1-4** obtained were found to exhibit no additional resonances and thus reflect the purity of the complexes. The integration of peaks concurs with the number of protons postulated from the structures proposed for the complexes.

Evidence of the formation of the complexes is clearly displayed in the ^{13}C NMR spectra. The ^{13}C NMR spectra of complexes **1-4** showed the δ(COO) signal shifted to the downfield region which is lower compared to that of the acid (168.95 ppm) indicating the carboxylate anion is bonded to tin atom upon complexation. The occurrence of six resonances in the range of 109.31-156.99 ppm in the ^{13}C NMR spectra of the complexes and acid are due to the presence of benzene carbons.

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

Compounds	Chemical Shift, δ (ppm)	
	Benzene	Sn-R (R= Me, Bu & Ph)
Acid	6.96 (d, 9.3 Hz, 1H) H3 8.17 (dd, 2.8 Hz, 9.3 Hz, 1H) H4 8.68 (d, 2.8 Hz, 1H) H6	-
1	6.82 (d, 9.3 Hz, 2H) H3 8.03 (dd, 2.8 Hz, 9.2 Hz, 2H) H4 8.63 (d, 2.8 Hz, 2H) H6	0.93 (s, 6H) H*a* $^{2}J(^{119}\text{Sn-}^{1}\text{H})$= 95.2 Hz
2	6.94 (d, 9.3 Hz, 2H) H3 8.15 (dd, 2.8 Hz, 9.3 Hz, 2H) H4 8.73 (d, 2.8 Hz, 2H) H6	0.91 (t, 7.3 Hz, 6H) H*d* 1.40 (sx, 7.1 Hz, 4H) H*c* 1.64-1.80 *(m, 8H) H*a* & H*b*
3	6.92 (d, 9.3 Hz, 4H) H3 8.12 (dd, 2.4 Hz, 9.2 Hz, 4H) H4 8.72 (s, 4H) H6	0.84 (t, 7.3 Hz, 12H) H*d* 0.90 (t, 7.3 Hz, 12H) H*d* 1.28-1.43 *(m, 32H) H*b* & H*c* 1.64-1.80 *(m, 16H) H*a*
4	6.82 (d, 9.2 Hz, 1H) H3 8.07 (dd, 2.8 Hz, 9.2 Hz, 1H) H4 8.73 (d, 2.9 Hz, 1H) H6	7.53-7.59 *(m, 9H) H*m* & H*p* 7.95-7.97 *(m, 6H) H*o*



s= singlet, d= doublet, t= triplet, sx= sextet, dd= doublet of doublet,
m= multiplet; *o*= ortho, *m*= meta, *p*= para; Coupling constant= Hz, *= overlap

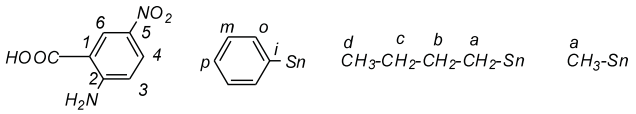
It is known that the chemical shifts $\delta(^{13}\text{C})_{ipso}$ of the phenyl groups in the $SnPh_3$ moiety lie in the range of 135.95-138.70 ppm, corresponding to the value shown by tetrahedrally coordinated Sn atom (Holeček *et al*., 1983a, Holeček *et al*., 1983b, Baul *et al*., 2001). For five-coordinated triphenyltin(IV) carboxylate complexes the $\delta(^{13}\text{C})_{ipso}$ will be observed at approximately 4 ppm higher (Baul *et al*., 2001). The ^{13}C NMR spectra of complexes **4** showed that the chemical shifts of the $\delta(^{13}\text{C})_{ipso}$ at 142.87 ppm indicative of a five-coordinated Sn atom. Complex **1** showed a sharp signal at 12.25 ppm indicated the present of methyl groups in the $SnMe_2$ moiety. In the upfield region of ^{13}C NMR spectra, complexes **2** and **3** showed the occurrence of CH_3 and CH_2

in the range of 13.71-14.42 and 26.09-29.90 ppm respectively (Danish *et al*., 1995, Holeček *et al*., 1986). In addition, complex **3** exhibited two sets of butyl signals in ^{13}C NMR spectra. This attributed to the butyl groups linked to the exo- and endo-cyclic tin atom respectively. Generally, the ^{13}C NMR spectra of the complexes obtain were found to exhibit no additional resonances and thus reflects the purity of the complexes.

Holeček and his coworkers in their studies and reports have shown that the coordination number of tin in triphenyltin(IV) carboxylate could be determined by the studies of $^{1}J(^{119}Sn\text{-}^{13}C)$ coupling constant (Holeček *et al*., 1983a, Holeček *et al*., 1983b, Baul *et al*., 2001). Normally, triphenyltin compounds with five-coordinated tin atom will show the coupling in the range of 600-850 Hz and the chemical shifts $\delta(^{119}Sn)$ lie in the range of -180 to -260 ppm (Holeček *et al*., 1983b). Basically, the tin atom of triphenyltin(IV) compounds with higher $\delta(^{119}Sn)$ and $^{1}J(^{119}Sn\text{-}^{13}C)$ value lie in the range of -200 to -260 ppm and 750-850 Hz respectively are believed to exhibit five-coordinated and in trigonal bipyramid geometry of the substituents and ligand, $Ph_3SnX{\bullet}L$ and L is a monodentate ligand (Holeček *et al*., 1983b). Holeček and his coworkers proposed that the substituent, X and the ligand, L lie in the axial positions and the three phenyl groups lie in the equatorial positions to form *trans*-trigonal bipyramid geometry (Holeček *et al*., 1983b). Complex **4** showed that the $\delta(^{119}Sn)$ and $^{1}J(^{119}Sn\text{-}^{13}C)$ are -265.89 ppm and 839.3 Hz respectively lie in the range of -200 to -260 ppm and 750-850 Hz, hence, this indicated that the tin atom in complex **4** is five-coordinated and having *trans*-trigonal bipyramid geometry. This due to one d_6-DMSO molecule coordinated to the tin atom in complex **4** resulting the complex exhibited five-coordinated tin atom. Complexes **1** and **2** showed that the $\delta(^{119}Sn)$ are -281.39 and -310.76 ppm respectively, hence indicated that the tin atom in complexes **1** and **2** are six-coordinated. Moreover, complex **3** showed two well separated resonances of $\delta(^{119}Sn)$ at -173.87 and -213.71 ppm respectively. These two low- and high-field resonances respectively are attributed to the exo- and endo-cyclic tin atoms in complex **3** as observed in distannoxane dimer (Danish *et al*., 1995). Hence, complex **3** showed that the exo- and endo-cyclic tin atoms are five- and six-coordinated respectively (Danish *et al*., 1995, Holeček *et al*., 1986).

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

omplexes	Chemical Shift (ppm)			
	Benzene	Aliphatic & phenyl (Sn-R, R= Me, Bu & Ph)	COO	^{119}Sn
Acid	109.31 (C1), 117.34 (C3), 129.54 (C4), 129.58 (C6), 135.87 (C5), 156.99 (C2)	-	168.95	-
1	112.43 (C1), 116.91 (C3), 128.94 (C4), 129.99 (C6), 135.98 (C5), 156.82 (C2)	12.25 (C*a*)	172.24	-281.39
2	111.84 (C1), 116.98 (C3), 129.15 (C4), 130.04 (C6), 135.93 (C5), 156.81 (C2)	14.42 (C*d*), 26.47 (C*c*), 27.72 (C*b*), 31.02 (C*a*)	173.05	-310.76
3	112.27 (C1), 116.50 (C3), 128.49 (C4), 129.47 (C6), 135.55 (C5), 156.49 (C2)	13.71 (C*d*), 13.91 (C*d*), 26.09 (C*c*), 26.77 (C*c*), 27.02 (C*b*), 27.29 (C*b*), 29.90 (C*a*)	172.11	-173.87 -213.71
4	112.77 (C1), 115.75 (C3), 127.72 (C4), 127.97 (C6), 134.89 (C5), 155.92 (C2)	142.87 (^{1}J= 839.3 Hz) (C*i*), 136.11 (^{2}J= 45.6 Hz) (C*o*), 128.43 (C*m*), 129.03 (^{4}J= 18.4 Hz) (C*p*)	169.52	-265.89



In Vitro Cytotoxic Assay:

The cytotoxic activity of acid and complexes **1-4** are given in Table 4. It was found that the parent acid and complex **1** are inactive against HepG2 cells. Complex **4** showed a significant cytotoxic activity with a lower IC_{50} value of 0.121 µg/mL compared to complexes **2** (0.310 µg/mL) and **3** (0.245 µg/mL). This is due to complex **4** is derived of triphenyltin(IV) (triorganotin) which is more active compared to the diorganotin(IV) derivatives (complexes **1-3**). In addition, based on NMR solution studies, complex **4** existed as monomer and the tin moiety was five-coordinated with *trans*-trigonal bipyramid geometry consequently enhanced the cytotoxic activity. However, the cytotoxic activity of complex **4** is lower compared to the reference drug (vincristine sulphate).

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

Complexes	IC_{50} (µg/mL)
	Human liver hepatocellular carcinoma cells, HepG2
Acid	Inactive (start at 1.0)
1	Inactive (start at 1.0)
2	0.310 ± 0.018
3	0.245 ± 0.012
4	0.121 ± 0.011
Vincristine sulphate	0.042 ± 0.013

IC_{50}(mg/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

Conclusion:

Complexes **1**- **4** have been successfully synthesized. Elemental analysis C, H, N and Sn data obtained are in agreement with the predicted formula. The infrared spectra of these complexes show the presence of monodentate and bidentate carboxylate ligand. The ^{1}H NMR spectra showed that the calculated number of protons for each functional group in the complexes is equal to the number predicted from the molecular formula. Moreover, the ^{1}H and ^{13}C NMR spectra of the complexes obtained were found to exhibit no additional resonances, thus reflects the purity of the complexes. In addition, ^{119}Sn NMR spectra of the complexes indicated that the tin atom of complexes **1** and **2** are six-coordinated, complexes **3** are five- and six-coordinated and complex **4** is five-coordinated in the solution state. Based on the cytotoxic activity, complex **4** showed significant activity compared to complexes **1-3** but lower compared to reference drug.

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