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Investigation on the Relationship Between Additional Methylene Groups of Dicarboxylic Acid Derivatives of Triphenyltin(IV) Complex and the Results of Cytotoxicity Tests on Human Promyelocytic Leukemic Cells

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Abstract: The complexes of triphenyltin(IV) derivatives of malonic acid (MaH), succinic acid (ScH), glutaric acid (GtH) and adipic acid (DpH) were successfully synthesized and obtained in solid form. The free ligands and complexes were characterized quantitatively using C, H and Sn elemental analysis as well as spectroscopic methods such as infrared (FTIR) and nuclear magnetic resonance (^{1}H, ^{13}C & ^{119}Sn NMR). Results of the analysis on the free ligands and the complexes showed that the coordination took place via one of the oxygen atoms from the carboxylate group. This indicated that the malonate (Ma), succinate (Sc), glutarate (Gt) and adipate (Dp) anions acted as monodentate ligands. ^{119}Sn NMR data showed that additional methylene groups across the ligands in the complexes 1 to 4 caused the ^{119}Sn peaks of the complexes to be shifted to upfield region. The cytotoxicity of the complexes was tested against promyelocytic leukemic cells. The cytotoxic dose (CD_{50}) was determined using microtitration 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Our results showed that the four complexes synthesized gave CD_{50} values lower than etoposide. Furthermore, the addition of methylene groups to the dicarboxylic ligands causes the CD_{50} to drop gradually from complexes 1 to 4.

Key words: Bis[triphenyltin(IV)] carboxylate complexes, ^{119}Sn NMR, Anticancer properties

INTRODUCTION

The synthesis and study of organotin(IV) carboxylate complexes have received considerable attention in recent years as these complexes display a large array of applications in industries as well as biocidal properties [1-6]. Organotin(IV) carboxylate complexes are well known as biocides, homogeneous catalysts and as stabilizers in the PVC industry [2].

Based on literature review which are well documented over the past 20 years, there are many different types of tri-and diorganotin(IV) carboxylate compounds that have been tested for their *in-vitro* activities against a large array of tumor cell lines [3, 6, 7]. In general, among tri-, di- and mono-organotin(IV) compounds, triorganotin(IV) compounds are found to display a higher biological activity. Among organotin(IV) compounds, organotin(IV) carboxylate complexes derivatives were used as anticancer and antitumor agents *in vitro* [8]. Moreover, structure-activity relationship studies suggest that triorganotin carboxylates with a tetrahedral tin moiety or a *trans*-R_3SnO_2 geometry show significantly greater activity than compounds with the monomeric *cis*-R_3SnO_2 structural type [9]. The function of the anionic carboxylate ligand is to aid the transport of the active organotin cationic group, R_3Sn^+ to the cell or active site (receptor site) [8, 10]. Although the chemistry and crystal structure of bis[triphenyltin(IV)] succinate have been studied [11], the effect of additional methylene ($-CH_2-$) groups across the ligands in the complexes on the cytotoxic properties and ^{119}Sn NMR resonance of this series have not been reported.

In this study, we are interested in the effect of adding methylene groups to the aliphatic chain between the dicarboxylic acid from MaH to DpH on cytotoxic properties and on ^{119}Sn NMR during the complexation

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with triphenyltin(IV). Four bis[triphenyltin(IV)] carboxylate complexes were obtained by condensation of triphenyltin(IV) hydroxide with the respective dicarboxylic acid. The complexes obtained were characterized quantitatively by microanalysis (C, H & Sn) as well as spectroscopic methods such as infrared (FTIR) and nuclear magnetic resonance (NMR). The cytotoxicity of the complexes obtained was tested against promyelocytic leukemic cells.

EXPERIMENTAL

Materials and Instrumentation: Triphenyltin(IV) hydroxide (Ph_3SnOH), glutaric acid (GtH) and adipic acid (DpH) were purchased from Aldrich Chemical Company. Malonic acid (MaH) and Succinic acid (ScH) were obtained from Sigma and Avocado respectively. Elemental C and H analyses were carried out on a Fison EA 1108 CHNS-O analyzer. Tin was determined gravimetrically by igniting a known quantity of each complex to SnO_2. Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded using a Perkin-Elmer FTIR GX spectrophotometer as KBr discs in the frequency range 4000-400 cm^{-1} while the polyethylene nujol mull technique was used for the range 400-200 cm^{-1}. The spectra for ^{1}H and ^{13}C NMR were recorded on a JEOL ECP 400MHz NMR Spectrometer ^{119}Sn NMR was recorded on a Bruker AC-P 400MHz FTNMR Spectrometer using deuterated chloroform, $CDCl_3$ as the solvent and tetramethylsilane, TMS was used as the internal standard.

Cytotoxic Assay: Cytotoxic assay was carried out against human promyelocetic leukemic cells, HL-60 which was obtained from Institut Pertanian Bogor, Indonesia. The cells were maintained in RMPI-1640 medium supplemented with 10% fetal calf serum and 100 IU/ml penicillin and 100 ig/ml streptomycin. Cytotoxicity was determined using the microtitration 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay [12]. The assay for each concentration of compound was performed in triplicate. Absorbance values at 550 nm was measured with a microplate reader (Bio Tek EL 340, USA) and cytotoxicity was expressed as fifty percent cytotoxic dose (CD_{50}), i.e. the concentration to reduce the absorbance of treated cells by 50% with reference to the control (untreated cells).

Preparation of bis[triphenyltin(IV)] Malonate, $(Ph_3Sn)_2Ma$, (1): The complex bis[triphenyltin(IV)] malonate, $(Ph_3Sn)_2Ma$, 1 was obtained by heating under reflux a 2:1 molar mixture of triphenyltin(IV) hydroxide, Ph_3SnOH (4 mmole, 1.47 g) and malonic acid, MaH (2 mmole, 0.21 g) in acetone (60 mL) for 2 hours. The Dean and Stark apparatus was used to remove the water formed during the reaction. A clear transparent solution was isolated by filtration and kept in a bottle. After 2 weeks a white precipitate (1.04 g, 65% yield) was obtained. M.p.: 146.5-147.7 °C. Anal. Found for $C_{39}H_{32}O_4Sn_2$: C, 57.96; H, 4.35; Sn, 28.73%. Calc. for $C_{39}H_{32}O_4Sn_2$: C, 58.40; H, 4.02; Sn, 29.60%. FTIR as KBr disc (cm^{-1}): $\nu(COO)_{as}$ 1656, $\nu(COO)_s$ 1335, ν(Sn-O) 633, $\nu(Sn\text{-}Ph)_{as}$ 271, $\nu(Sn\text{-}Ph)_s$ 228. ^{1}H-NMR: δ: phenyl protons 7.41-7.48 (18H, m, $H_{meta+para}$); 7.65-7.78 (12H, m, H_{ortho}); CH_2 3.59 (2H, s) ppm. ^{13}C-NMR: δ: phenyl carbons C_{ipso} 137.77, C_{ortho} 136.78, C_{meta} 128.88, C_{para} 130.14, CH_2 41.65, COO 173.34 ppm. ^{119}Sn-NMR: δ:-100.43 ppm

Preparation of bis[triphenyltin(IV)] Succinate, $(Ph_3Sn)_2Sc$, (2): The similar method as in the synthesis of 1 was utilized. Ph_3SnOH (6 mmole, 2.20 g) and substituting succinic acid, ScH (3 mmole, 0.35 g) for malonic acid was applied. After 2 weeks, white crystals (1.18 g, 48% yield) was obtained. M.p.: 154.6-155.2 °C. Anal. Found for $C_{40}H_{34}O_4Sn_2$: C, 58.58; H, 4.17; Sn, 29.75%. Calc. for $C_{40}H_{34}O_4Sn_2$: C, 58.87; H, 4.20; Sn, 29.09%. FTIR as KBr disc (cm^{-1}): $\nu(COO)_{as}$ 1645, $\nu(COO)_s$ 1344, ν(Sn-O) 582, $\nu(Sn\text{-}Ph)_{as}$ 269, $\nu(Sn\text{-}Ph)_s$ 228. ^{1}H-NMR: δ: phenyl protons 7.40-7.42 (18H, m, $H_{meta+para}$); 7.67-7.69 (12H, m, H_{ortho}); CH_2 2.79 (4H, t) ppm. ^{13}C-NMR: δ: phenyl carbons C_{ipso} 138.24, C_{ortho} 136.89, C_{meta} 128.92, C_{para} 130.13, CH_2 30.23, COO 178.98 ppm. ^{119}Sn-NMR: δ:-108.52 ppm

Preparation of bis[triphenyltin(IV)] Glutarate, $(Ph_3Sn)_2Gt$, (3): The similar method as in the synthesis of 1 was utilized, substituting glutaric acid (2 mmole, 0.26 g) for malonic acid. After a month, a white precipitate (0.78 g, 47% yield) was obtained. M.p.: 115.5-116.8°C. Anal. Found for $C_{41}H_{36}O_4Sn_2$: C, 59.54; H, 4.76; Sn, 28.12%. Calc. for $C_{41}H_{36}O_4Sn_2$: C, 59.32; H, 4.37; Sn, 28.60%. FTIR as KBr disc (cm^{-1}): $\nu(COO)_{as}$ 1531, $\nu(COO)_s$ 1332, ν(Sn-O) 534, $\nu(Sn\text{-}Ph)_{as}$ 272, $\nu(Sn\text{-}Ph)_s$ 234. ^{1}H-NMR: δ: phenyl protons 7.42-7.45 (18H, m, $H_{meta+para}$); 7.71-7.73 (12H, m); CH_2 1.98-2.02 (2H, m, H_{ortho}), 2.46 (4H, t) ppm. ^{13}C-NMR: δ: phenyl carbons C_{ipso} 138.24, C_{ortho} 136.84, C_{meta} 128.86, C_{para} 130.08, CH_2 21.56, 33.28; COO 179.75 ppm. ^{119}Sn-NMR: δ:-110.78 ppm.

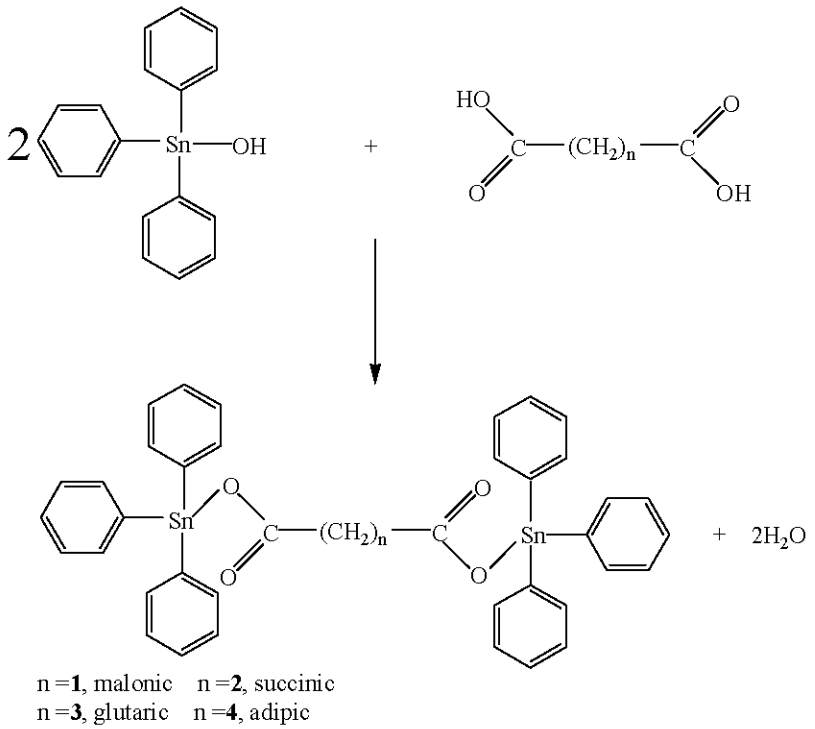


Fig. 1: Reaction scheme for the syntheses of 1-4.

Preparation of bis[triphenyltin(IV)] adipate, $(Ph_3Sn)_2Dp$, (4): The similar method as in the synthesis of 1 was utilized, substituting adipic acid (2 mmole, 0.29 g) for malonic acid. After heating under reflux, a white precipitate (0.81 g, 48% yield) was obtained and washed with acetone (20 mL) and dried in a desicator. M.p.: 146.7-147.3 °C. Anal. Found for $C_{42}H_{38}O_4Sn_2$: C, 59.76; H, 5.27; Sn, 29.33%. Calc. for $C_{42}H_{38}O_4Sn_2$: C, 59.76; H, 4.54; Sn, 28.12%. FTIR as KBr disc (cm^{-1}): $\nu(COO)_{as}$ 1633, $\nu(COO)_s$ 1334, ν(Sn-O) 557, $\nu(Sn\text{-}Ph)_{as}$ 269, $\nu(Sn\text{-}Ph)_s$ 224. ^{1}H-NMR: δ: phenyl protons 7.42-7.44 (18H, m, $H_{meta+para}$); 7.71-7.74 (12H, m, H_{ortho}); CH_2 1.66-1.68 (4H, m), 2.41 (4H, t) ppm. ^{13}C-NMR: δ: phenyl carbons C_{ipso} 138.52, C_{ortho} 137.06, C_{meta} 129.07, C_{para} 130.29, CH_2 25.37, 33.92; COO 180.42 ppm. ^{119}Sn-NMR: δ:-112.19 ppm An outline of the reaction scheme for complexes 1-4 is given in Figure 1.

RESULTS AND DISCUSSION

The complexes 1, 3 and 4 were obtained as white precipitate while 2 was isolated as single crystals. Molecular sieves were added during the heating under reflux to remove water formed by product [11]. Besides, the water liberated in the reaction was removed by azeotropic dehydration using the Dean and Stark apparatus. Elemental analysis C, H and Sn data obtained were in agreement with the predicted formula. Complexes 1-4 gave sharp melting points indicating the isolation of fairly pure complexes.

The infrared spectra of the complexes 1-4 show distinct differences from the free ligands. The ν(O-H) bands which appeared in the range 2924-2954 cm^{-1} for the free ligands, are absent in the infrared spectra of complexes 1-4. The $\nu(COO)_{as}$ band of the complexes are shifted to lower wavenumber compared to the free ligands, an observation also reported by others [13-15]. Complexes 1-4 show $\nu(COO)_{as}$ and $\nu(COO)_s$ in the range of 1656-1531 and 1332-1344 cm^{-1} respectively. For triorganotin(IV) carboxylate complexes, the values of $\Delta\nu = [\nu(COO)_{as}\text{-}\nu(COO)_s]$ which are in the range greater than 200 cm^{-1} indicating a monodentate bonding mode for the carboxylate moiety [9, 16-18]. For bridging and chelating behaviour, the magnitude of $\Delta\nu$ values would be expected to be smaller or equal to 150 cm^{-1} [9, 16-20]. For triphenyltin(IV) carboxylate complexes, a value greater than 200 cm^{-1} for $\Delta\nu$, has been reported for monodentically coordinated carboxylate group wirh respect to a single tin atom [17, 21-23]. This phenomenon has been attributed the steric effect by the triphenyltin(IV) moiety [24]. Hence, the value for $\Delta\nu$ found for 1, 2, 3 and

Table 1: Important infrared data for the free ligands and complexes 1-4 (cm^{-1})

Complexes	ν(OH)	$\nu(COO)_{as}$	$\nu(COO)_s$	Δν	ν(Sn-O)	$\nu(Sn\text{-}Ph)_{as}$	$\nu(Sn\text{-}Ph)_s$
MaH	2947	1728	1315	413	-	-	-
ScH	2933	1693	1311	382	-	-	-
GtH	2954	1708	1305	403	-	-	-
DpH	2924	1703	1355	348	-	-	-
1	-	1656	1335	321	633	271	228
2	-	1645	1344	301	582	269	228
3	-	1531	1332	199	534	272	234
4	-	1633	1334	299	557	269	224

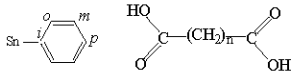
$\Delta\nu = [\nu(COO)_{as} - \nu(COO)_s]$

Table 2: ^{1}H, ^{13}C and ^{119}Sn NMR data for complexes 1-4 (ppm)

	^{1}H NMR		^{13}C NMR			
Complexes	δH(phenyl)	$\delta H(CH_2)_n$	δC(phenyl), $^{n}J(^{119}Sn\text{-}^{13}C)$	$\delta C(CH_2)_n$	δC(COO)	^{119}Sn NMR
1	7.41-7.48 (18H, m) 7.65-7.78 (12H, m)	n= 1; 3.59 (2H, s)	C_i; 137.77 (^{1}J= a) C_m; 128.88 (^{2}J= 49.20 Hz) C_o;136.78 (^{3}J= 63.80 Hz) C_p;130.14 (^{4}J= a)	n= 1; 41.65	173.34	-100.43
2	7.40-7.42 (18H, m) 7.67-7.69 (12H, m)	n= 2; 2.79 (4H, s)	C_i;138.24 (^{1}J= 647.27 Hz) C_m;128.92 (^{2}J= 48.43 Hz) C_o;136.89 (^{3}J= 63.04 Hz) C_p;130.13 (^{4}J= 13.07 Hz)	n= 2; 30.23	178.98	-108.52
3	7.42-7.45 (18H, m) 7.71-7.73 (12H, m)	n= 3; 1.98-2.01 (2H, m) 2.46 (4H, t)	C_i;138.24 (^{1}J= a) C_m;128.86 (^{2}J= 47.78 Hz) C_o;136.84 (^{3}J= 64.74 Hz) C_p;130.08 (^{4}J= 12.33 Hz)	n= 3; 21.56 33.28	179.75	-110.78
4	7.42-7.44 (18H, m) 7.71-7.74 (12H, m)	n= 4; 1.66-1.68 (4H, s) 2.41 (4H, s)	C_i;138.52 (^{1}J= a) C_m;129.07 (^{2}J= 47.66 Hz) C_o;137.06 (^{3}J= 63.04 Hz) C_p;130.29 (^{4}J= 13.07 Hz)	n= 4; 25.37 33.92	180.42	-112.19
GtH_2	-	n= 3; 2.03-2.07 (2H, m) 2.47 (4H, t)	-	n= 3; 19.89 33.11	178.19	-

s= singlet, t= triplet, m= multiplet, a= not observed

i= ipso, *o*= ortho, *m*=meta, *p*=para



4 which were 321, 301, 199 and 299 cm^{-1} respectively, greater or equal to 200 cm^{-1}, could signify the presence of monodentically chelated carboxylates. The assignment of important infrared bands for the free ligands and complexes are presented in Table 1.

The relevant data obtained from the ^{1}H and ^{13}C NMR spectra for the complexes 1-4 are presented in Table 2. Chemical shift values are relative to an internal standard, tetramethylsilane (TMS). The ^{1}H NMR spectra for the complexes 1-4 and the integration of peaks concurred with the number of protons postulated from the structures proposed for the complexes. The ^{13}C NMR spectra of complexes 1-4 show that the chemical shifts of the $\delta(^{13}C)_{ipso}$ lie in the range 137.77-138.52 ppm indicative of a tetrahedrally coordinated Sn atom [24]. The chemical shifts $\delta(^{119}Sn)$ for triphenyltin(IV) carboxylate lie in a broad range from-40 to-260 ppm [25], but four-coordinated triphenyltin(IV) carboxylate complexes display chemical shifts $\delta(^{119}Sn)$ in the range-40 to-120 ppm [23-24] as it is well known that the $\delta(^{119}Sn)$ values in the ^{119}Sn NMR are markedly dependent on the coordination properties around the tin atom in the triphenyltin(IV) carboxylate complexes. For complexes 1 and 2, sharp peaks were observed at-100.43 and-108.52 ppm, respectively.

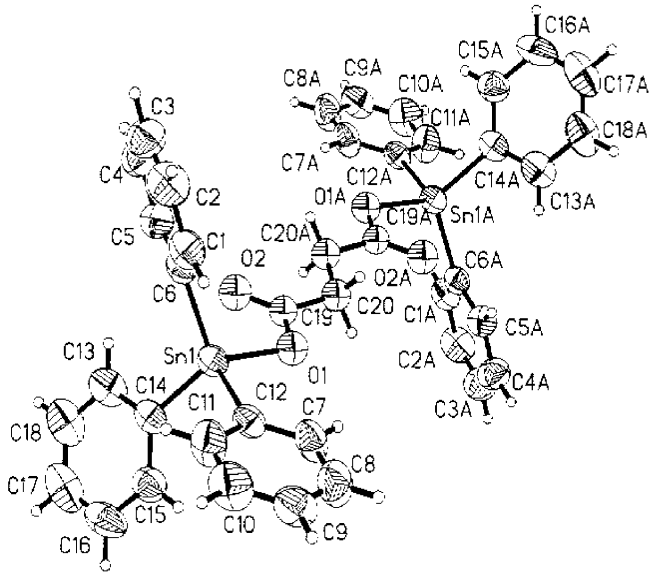


Fig. 2: *ORTEP* plot for complex 2 at 50% probability level with atom numbering scheme

Complexes 3 and 4 showed signals at-110.78 and-112.19 ppm, respectively. All the ^{119}Sn signals for the complexes 1-4 indicate that the tin atom is four-coordinated. The $^{n}J(^{119}Sn-^{13}C)$ data for complexes 1-4 in $CDCl_3$ solution are also in accord with triphenyltin compounds with four-coordinated tin [23-24]. The ^{119}Sn NMR data for complexes 1-4 in $CDCl_3$ solution are listed in Table 2.

The *ORTEP* plot for complex 2 is shown in Figure 2. Crystallographic studies show that the crystal structure obtained for complex 2 is similar to the crystal structure reported by Ng (1998) [26]. However, in this study the crystal of complex 2 was obtained from acetone instead of ethyl acetate which has been used by Ng (1998) [26]. The crystal structure of complex 2 shows that each carboxylate anion of the succinic acid is bonded to each tin atom in monodentate mode. Hence, the tin atom moiety of complex 2 are four coordinated and exhibits distorted tetrahedral geometry with sp^3 hybrid orbitals [21]. On the basis of crystal structure, infrared and ^{13}C and ^{119}Sn NMR spectroscopic studies we can concluded that the carboxylate anions in complex 2 are monodentically bonded to the tin atom. Due to the similar spectroscopic data (infrared and NMR), we conclude that complexes 1, 3 and 4 have the same structural characteristics as complex 2.

The cytotoxic activity of the complexes 1-4 were evaluated against human promyelocytic leukemic cells, HL-60 which was obtained from Institut Pertanian Bogor, Indonesia using the standard MTT assay. The CD_{50} value for complexes 1-4 are given in Table 3. The CD_{50} value for complexes 1-4 were 0.28, 0.30, 0.32 and 0.37 μg/mL respectively. All the CD_{50} values for complexes 1-4 were less than the reference cytotoxic compound etoposide (0.6 μg/mL). Hence, complexes 1-4 have been demonstrated to possess potent cytotoxic properties against human promyelocytic leukemic cells, HL-60. In this study, we observed that when the ^{119}Sn signals shifted slightly to upfield region across complexes 1 to 4, there were a slightly decreased in cytotoxic activity. From complexes 1 to 4, the ^{119}Sn peak has moved slightly toward upfield in the NMR spectra in the sequence of 2 ppm. This may due to shielding phenomena attributed from the addition of methylene ($-CH_2-$) group across complexes 1 to 4 [10]. This phenomenon is attributed to the electron back-donating of methylene group to tin atom in conjunction with the additional one methylene group from complexes 1 to 4. The upfield shift can be explained in terms of increase in the electron density on the central tin atom and finally the increase of its s-character, [15, 25],

Table 3: CD_{50} value for complexes 1-4

	CD_{50} (μg/mL)	
Complexes	Human promyelocetic leukemic cells, HL-60	δ(^{119}Sn), ppm
1	0.28	-100.43
2	0.30	-108.52
3	0.32	-110.78
4	0.37	-112.19
Etoposide (reference)	0.60	

hence forming relatively more stable Sn-O bonding upon complexation. As a results, our study showed a slightly decreased cytotoxic activity from complexes 1 to 4 may due to strong Sn-O bonding upon complexation leading to the decrease of hydrolytic properties of complexes to form Ph_3Sn^+ cations across complexes 1 to 4 and finally trigger cell apoptosis. Generally, all the four complexes obtained show promising cytotoxic activity compared to the reference drug (etoposide).

CONCLUSION

The complexes 1-4 have been successfully synthesized. Elemental analysis C, H and Sn data obtained were in agreement with the predicted formula. The complexes gave sharp melting point indicating the isolation of fairly pure complexes. The infrared spectra showed that the carbonyl group of the complexes was shifted to lower wave number compared to the free acid. The value $\Delta\nu = [\nu(COO)_{as}\text{-}\nu(COO)_s]$ found for complexes 1-4 are greater than 200 cm^{-1} signifies the presence of monodentate carboxylates. The ^1H NMR spectra showed that the number of protons for each functional group in the complexes calculated from the integration curves were equal to the number of protons predicted from the molecular formula of the complexes. The $\delta(^{13}C)_{ipso}$ and ^{119}Sn NMR spectra of the complexes 1-4 showed that the complexes obtained were tetrahedral in geometry and the tin atom moiety was four-coordinated thus indicating that the free ligands acted in a monodentate manner in the complexes. From the cytotoxicity study, complexes 1-4 showed positive cytotoxic properties against leukemia cells and may have potential role in future anticancer treatment of myeloid leukemia even showed a slightly decreased in cytotoxic activity across complexes 1 to 4.

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