



Preliminary *in vitro* Cytotoxic Assay on HepG2 and Antibacterial Screening Activity: Synthesis and Characterization of Organotin(IV) Complexes Derivatives of 2-Methyl-3-nitrobenzoic Acid

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Three organotin(IV) carboxylate complexes derivatives of 2-methyl-3-nitrobenzoic acid (HL) have been successfully synthesized and characterized quantitatively and qualitatively. The complexes obtained are screened for their preliminary *in vitro* cytotoxic assay on human liver hepatocellular carcinoma cells (HepG2) and antibacterial screening activity. Results of the infrared and NMR spectroscopy on the acid and complexes showed that the coordination took place *via* oxygen atoms from the carboxylate anions. With the exceptional case, based on the spectroscopy studies indicated that one methanol molecule also take part in the coordination to tin(IV) atom moiety in complex **3** resulting the tin(IV) atom exhibited five coordination in solid state. From the preliminary *in vitro* cytotoxic assay on HepG2 and antibacterial screening activity, triphenyltin(IV) (complex **3**) showed slightly better activity compared to diorganotin(IV) complexes (**1** and **2**).

Key Words: Organotin(IV) complexes, *In vitro* cytotoxic assay, Antibacterial activity.

INTRODUCTION

In general, the coordination chemistry of organotin(IV) complexes are extensively studied due to its coordination geometries as well as structural diversity (monomer, dimeric, hexameric and oligomeric)¹⁻⁵. It was also well-documented that the participation of coordinating solvent molecules to tin(IV) atoms moieties and its coordination sphere will influence the overall structural of organotin(IV) complexes^{3,4,6}. And up-to-date, numerous studies on organotin(IV) complexes have been carried out in order to study its biological properties against bacterial, fungal and cancer cells line to explore its structural-activity relationship⁷⁻¹⁴.

In this paper, we report on the synthesis and structural characterization of new organotin(IV) carboxylate complexes derived from 2-methyl-3-nitrobenzoic acid (HL). Moreover, the preliminary *in vitro* cytotoxic assay on human liver hepatocellular carcinoma cells (HepG2) and antibacterial screening activity of the complexes obtained were carried out and the results were reported herein.

EXPERIMENTAL

All the reagents, starting materials as well as the solvents were purchased commercially and used without any further

purification. The 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 ¹H and ¹¹⁹Sn NMR were recorded on a Bruker AC-P 400 MHz FT NMR spectrometer and ¹³C NMR was recorded on a Bruker AC-P 300 MHz FT NMR spectrometer using deuterated CDCl₃ and *d*₆-DMSO 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 SnO₂. The melting points were determined in an open capillary and were uncorrected.

Preparation of dimethyltin(IV) oxide, Me₂SnO: Dimethyltin(IV) dichloride was dissolved in distilled water and stirred for 16 h. Colourless solution was obtained. Ammonia solution (60 %) was added into the colourless solution and finally white precipitate was obtained. The precipitate was placed in an oven (60 °C) for a few days to dry.

Preparation of sodium salt: The sodium salt of the acid was obtained by heating under reflux a 1:1 molar mixture of sodium hydroxide, NaOH and 2-methyl-3-nitrobenzoic acid in ethanol (50 mL) for 2 h. After a few days, white precipitates were obtained as sodium salt of 2-methyl-3-nitrobenzoic acid:

FTIR as KBr disc (cm^{-1}) selected data: $\nu(\text{COO})_{\text{as}}$ 1629, $\nu(\text{COO})_{\text{s}}$ 1358.

Synthesis of organotin(IV) complexes

Preparation of (2-CH₃-3-NO₂-C₆H₃COO)₂(CH₃)₂Sn (1): Complex **1** was obtained by heating under reflux a 1:2 molar mixture of dimethyltin(IV) oxide (0.33 g, 2 mmol) and 2-methyl-3-nitrobenzoic acid (0.73 g, 4 mmol) in acetone (50 mL) for 3 h. A clear colourless transparent solution was separated by filtration and kept in a bottle. After few days, yellow solids (0.67 g, 65.2 % yield) were collected. m.p. 223.4–224.1 °C. Analysis for C₁₈H₁₈N₂O₈Sn: C, 42.62; H, 3.10; N, 5.45; Sn, 22.66 %. Calcd. for C₁₈H₁₈N₂O₈Sn: C, 42.47; H, 3.56; N, 5.50; Sn, 23.33 %. FTIR as KBr disc (cm^{-1}): $\nu(\text{C-H})$ aromatic 3089, $\nu(\text{C-H})$ saturated 2993, 2948, 2864; $\nu(\text{COO})_{\text{as}}$ 1618; $\nu(\text{COO})_{\text{s}}$ 1348; $\nu(\text{NO}_2)$ 1526, $\nu(\text{O-Sn-O})$ 613, $\nu(\text{Sn-C})$ 584, $\nu(\text{Sn-O})$ 489. ¹H NMR (ppm) (*d*₆-DMSO): δ : benzene protons 7.46 (t, 7.9 Hz, 2H); 7.86 (d, 3.7 Hz, 2H); 7.89 (s, 2H); CH₃ 2.49 (s, 6H); methyl, CH₃ 0.95 (s, 6H), ²*J*(¹¹⁹Sn-1H) = 102.17 Hz. ¹³C NMR (ppm) (*d*₆-DMSO): δ : benzene carbons 125.97, 127.73, 130.83, 133.66, 138.34, 152.14; CH₃ 16.43; methyl 12.24, ¹*J*(¹¹⁹Sn-¹³C) = 908.6 Hz; COO 172.16. ¹¹⁹Sn-NMR (ppm) (*d*₆-DMSO): δ : -261.95.

Preparation of [{2-CH₃-3-NO₂-C₆H₃COO(C₆H₅)₂Sn]₂O] (2): Complex **2** was obtained by heating under reflux a 1:2 molar mixture of dibutyltin(IV) oxide (0.75 g, 3 mmol) and 2-methyl-3-nitrobenzoic acid (1.10 g, 6 mmol). The reaction was carried out in a mixture of ethanol/hexane (3:2, 50 mL) for 3 h. A clear yellow transparent solution was isolated by filtration and kept in a bottle. After few days, yellow crystals (0.91 g, 72.0 % yield) were collected. m.p. 138.1–138.7 °C. Analysis for C₆₄H₉₆N₄O₁₈Sn₄: C, 45.73; H, 5.63; N, 3.32; Sn, 16.76 %. Calcd. for C₆₄H₉₆N₄O₁₈Sn₄: C, 45.64; H, 5.75; N, 3.33; Sn, 17.09 %. FTIR as KBr disc (cm^{-1}): $\nu(\text{C-H})$ aromatic 3079, $\nu(\text{C-H})$ saturated 2955, 2929, 2869; $\nu(\text{COO})_{\text{as}}$ 1629, 1531; $\nu(\text{COO})_{\text{s}}$ 1341, 1405; $\nu(\text{NO}_2)$ 1561, $\nu(\text{Sn-O-Sn})$ 625, $\nu(\text{Sn-C})$ 577, $\nu(\text{Sn-O})$ 482. ¹H NMR (ppm) (CDCl₃): δ : benzene protons 7.40 (t, 7.8 Hz, 4H); 7.85 (d, 7.9 Hz, 4H); 8.17 (s, 4H); methyl, CH₃ 2.71 (s, 12H); butyl, CH₃ 0.83–0.96 (m, 24H), 1.27–1.49 (m, 16H); CH₂ 1.52–1.65 (m, 16H); CH₂ 1.72–1.87 (m, 16H). ¹³C NMR (ppm) (CDCl₃): δ : benzene carbons 126.25, 127.22, 131.82, 133.68, 134.98, 152.46; CH₃ 16.68; butyl 13.95, 26.07, 26.32, 27.82, 28.03, 28.93, 30.30; COO 176.26, 173.79. ¹¹⁹Sn-NMR (ppm) (CDCl₃): δ : -143.15, -207.94.

Preparation of 2-CH₃-3-NO₂-C₆H₃COO(C₆H₅)₃Sn·CH₃OH (3): The title complex was obtained by heating under reflux a 1:1 molar mixture of triphenyltin(IV) hydroxide (0.73 g, 2 mmol) and 2-methyl-3-nitrobenzoic acid (0.36 g, 2 mmol) in methanol (50 mL) for 1 h. A clear yellow transparent solution was isolated by filtration and kept in a bottle. After few days, yellow crystals (0.87 g, 77.7 % yield) were collected. m.p. 121.3–121.9 °C. Analysis for C₂₇H₂₅N₁O₅Sn: C, 57.53; H, 4.04; N, 2.45; Sn, 21.02 %. Calcd. for C₂₇H₂₅N₁O₅Sn: C, 57.68; H, 4.48; N, 2.49; Sn, 21.11 %. FTIR as KBr disc (cm^{-1}): $\nu(\text{C-H})$ aromatic 3065, 3022; $\nu(\text{C-H})$ saturated 2922, $\nu(\text{COO})_{\text{as}}$ 1608, $\nu(\text{COO})_{\text{s}}$ 1345, $\nu(\text{NO}_2)$ 1520, $\nu(\text{Sn-O})$ 450. ¹H NMR (ppm) (CDCl₃): δ : phenyl protons 7.43–7.50 (m, 9H); 7.78–7.82 (m, 6H); benzene 7.27 (t, 7.9 Hz,

1H); 7.72 (dd, 1.4 Hz, 8.1 Hz, 1H); 8.23 (dd, 1.3 Hz, 7.8 Hz, 1H); methyl, CH₃ 2.61 (s, 3H); CH₃OH 3.35 (s, 3H). ¹³C NMR (ppm) (CDCl₃): δ : phenyl carbons C_{ipso} 138.52 (591.1 Hz), C_{ortho} 137.44 (48.4 Hz), C_{meta} 129.92 (64.5 Hz), C_{para} 130.99 (13.2 Hz); benzene 126.74, 126.91, 130.70, 133.63, 135.08, 151.58; CH₃ 17.09; CH₃OH 51.20; COO 173.19. ¹¹⁹Sn NMR (ppm) (CDCl₃): δ : -106.02.

2-Methyl-3-nitrobenzoic acid (HL): The parent acid, 2-methyl-3-nitrobenzoic acid (HL) was purchased from acros organics and used without any further purification. FTIR as KBr disc (cm^{-1}): Selected data: $\nu(\text{OH})$ 2887–2552, $\nu(\text{COO})_{\text{as}}$ 1699, $\nu(\text{COO})_{\text{s}}$ 1368. ¹H NMR (ppm) (CDCl₃): δ : benzene protons 7.47 (t, 8.0 Hz, 1H); 7.93 (dd, 1.2 Hz, 8.1 Hz, 1H); 8.23 (dd, 1.2 Hz, 7.9 Hz, 1H); methyl 2.74 (s, 3H). ¹³C NMR (ppm) (CDCl₃): δ : benzene carbons 126.25, 127.14, 131.06, 133.29, 134.52, 151.58; methyl 15.61; COO 167.83.

Preliminary *in vitro* cytotoxic assay: The *in vitro* cytotoxic assay was carried out against human liver hepatocellular carcinoma cells line, HepG2. The cells were maintained in Eagle's minimum essential medium supplemented with 2 mM of L-glutamine, 1 mM of sodium pyruvate, 0.1 mM of non-essential amino acid, 1.5 $\mu\text{g}/\text{mL}$ sodium bicarbonate, 100 IU/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin. The cytotoxicity assay was determined using the microtitration 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay^{15,16}. 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)¹⁵. Cytotoxicity was expressed as fifty percent cytotoxic dose (IC₅₀), *i.e.* the concentration causing 50 % inhibition of cell growth with reference to the control (untreated cells). The IC₅₀ and the S.E.M. (standard error of the mean) was determined using Probit Analysis (SPSS, version 12.0.1).

Preliminary *in vitro* antibacterial screening activity: The synthesized organotin(IV) complexes and acid (HL) were screened for their *in vitro* antibacterial activity against three gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and two gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains, by inhibition zone method using agar well diffusion method. The seeded agar (nutrient agar medium) was prepared by cooling the molten agar to 40 °C and then adding bacterial inoculums containing approximately 10⁴–10⁶ colony forming units (CFU)/mL. The bacterial inoculums were spread on the plate containing agar medium and even coverage was ensured before the agar solidified. The complexes were dissolved in DMSO to prepare 1.0 mg/mL concentration. By using a sterile metallic borer, the wells (6 mm in diameter) were dug and the standard drugs and complexes were introduced into the respective wells. The plates were incubated immediately at 37 °C for 20–24 h. The activity was determined by measuring the diameter of the inhibition zone (in mm).

RESULTS AND DISCUSSION

In this study, complexes **1-3** have been obtained in solid state. The micro-elemental analysis for C, H, N and Sn data

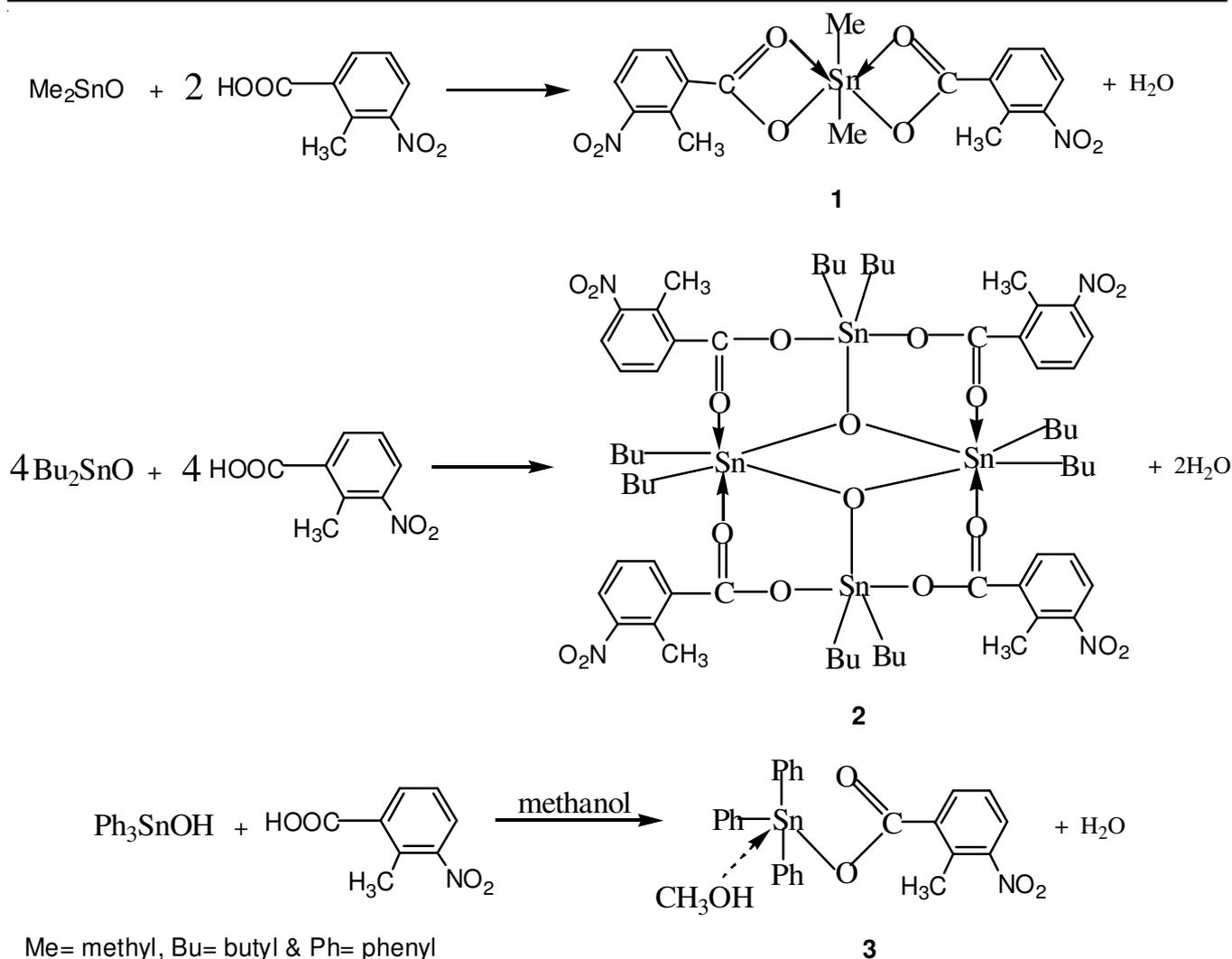


Fig. 1. Proposed structure for complexes 1-3

obtained were in agreement with the predicted formula for complexes 1-3. Based on the elemental analysis, it is believed that a methanol molecule was present in complex 3 which might have been trapped or acted as a solvate in those reported similar complexes^{3,4,6}. This phenomenon has been clarified and the X-ray crystal structure of complex 3 has been reported⁶. Complexes 1-3 gave a sharp melting point indicated the isolation of fairly pure complexes. An outline of the proposed structure for complexes 1-3 are depicted in Fig. 1.

The $\nu(\text{O-H})$ bands for the acid, HL was absent in the infrared spectra of salt and complexes 1-3 showed the deprotonation and coordination of the carboxylate anion. In addition, complexes 1-3 revealed that the $\nu(\text{COO})_{\text{as}}$ was shifted to a lower wavelength number compared to the acid (HL) which signified that the coordination took place *via* the oxygen atoms of the carboxylate anion. Complex 1 was isolated as a monomeric type and its $\Delta\nu$ was 270 cm^{-1} , which was comparable to the sodium salt ($\Delta\nu = 271\text{ cm}^{-1}$) of 2-methyl-3-nitrobenzoic acid, indicating that the carboxylate anions were coordinated to the tin(IV) atom moiety in a bidentate manner¹⁷. From the infrared spectra of complex 2, two $\Delta\nu$ values (288 and 126 cm^{-1}) were observed and both values were either comparable or lower than the $\Delta\nu$ of the sodium salt indicating

that all the carboxylate anions were bonded to the tin(IV) atoms in a bidentate mode¹⁷. As a result, two tin(IV) atoms exhibited a distorted trigonal bipyramidal geometry and while another two tin(IV) atoms exhibited a distorted octahedral geometry in complex 2. For complexes derived from triphenyltin(IV) carboxylate, $\Delta\nu$ greater than 200 cm^{-1} would be expected for the monodentate bonding carboxylate anions¹⁸. Hence, the carboxylate anion in complex 3 would be expected to bond to the tin(IV) atom in monodentate manner since the $\Delta\nu$ above 250 cm^{-1} . Based on the elemental analysis, a methanol molecule was present in complex 3. As a result the absorption bands of the aliphatic and aromatic functional groups centered around 3000 cm^{-1} appeared as though they were sitting on a small hump together with the $\nu(\text{OH})$ band in the spectra of complexes 3. Hence, the tin(IV) atom of complex 3 was five-coordinated and exhibited a trigonal bipyramidal geometry. For further information and structural clarity, the structure of complex 3 has been reported by our research group⁶. The infrared spectrum of complex 3 is depicted in Fig. 2 as a representative.

The $^1\text{H NMR}$ spectra of complexes 1-3 exhibited similarities to their acid, HL. In the upfield regions of the $^1\text{H NMR}$ spectra of the complexes 1 and 2 showed the signal of the

methyl and butyl protons of the organotin(IV) at 0.95 ppm and in the range of 0.83-1.87 ppm respectively. For complex **3**, the resonances appeared as two well separated sets of multiplets in the regions centering around $\delta \approx 7.48$ and 7.80 ppm (downfield) with integration values of 9:6 respectively, ascribed to the aromatic protons of the phenyl group¹⁹. Based on the ¹H NMR spectral studies of complex **3**, the proton resonances originating from the methanol molecule occurred at $\delta = 3.35$ ppm; based on the integration, only one methanol molecule was present in complex **3**.

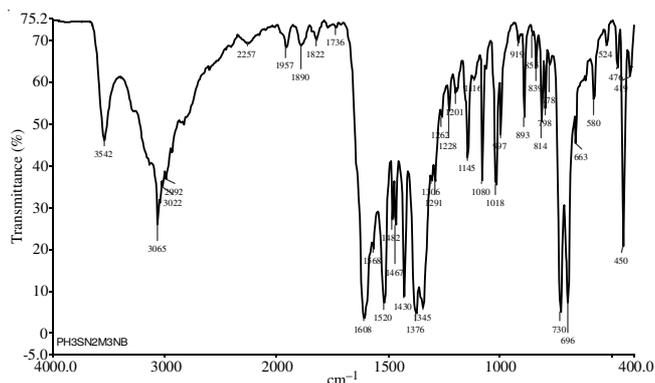


Fig. 2. Infrared spectrum of complex **3**

Evidence of the formation of the complexes is displayed in the ¹³C NMR spectra. The ¹³C NMR spectra of complexes **1-3** showed the $\delta(\text{COO})$ signal shifted to the downfield region which is lower compared to that of the acid, HL indicating the carboxylate anion is bonded to tin(IV) atom. Complex **1** exhibited a sharp signal at 12.24 ppm indicating the presence of the methyl groups in the SnMe₂ moiety whereas complex **2** was derivatives of organodistannoxane dimer types exhibited two sets of signals corresponding to the butyl groups in the ¹³C NMR spectra. These two sets of signals were attributed to the butyl groups linked to the exo- and endo-cyclic tin(IV) atom respectively²⁰. Complex **3** revealed the chemical shifts of the $\delta(^{13}\text{C})_{\text{ipso}}$ at 138.52 ppm respectively indicative of a four-coordinated tin(IV) atom²¹⁻²³. Complex **3** also showed that the ¹J(¹¹⁹Sn-¹³C) value of 591.1 Hz lie in the range of 550-660 Hz, thus indicating that the tin(IV) atom in complex **3** was four-coordinated and has a distorted tetrahedral geometry. In addition, in the ¹³C NMR spectra of complex **3**, the signals due to the methanol molecule was located at the upfield region at $\delta = 51.20$ ppm as observed.

The $\delta(^{119}\text{Sn})$ value of the four-coordinated complexes fall in the range between +200 to -60 ppm; the five-coordinated complexes between -90 to -190 ppm and the six-coordinated complexes between -210 to -400 ppm²⁴. Complexes derivatives of organodistannoxane dimer types usually exhibit two well resolved $\delta(^{119}\text{Sn})$ signals (complex **2** = -143.15, -207.94 ppm). These two low- and high-field resonances were attributed to the exo- and endo-cyclic tin(IV) atoms^{14,20}. Based on the ¹¹⁹Sn NMR spectra, all the tin(IV) atoms in complex **2** were five-coordinated and each exhibited a distorted trigonal bipyramidal geometry. This maybe due to the disassociation of the bidentate bonds upon dilution during the preparation of the NMR sample in solution form. The $\delta(^{119}\text{Sn})$ value of complex **1** was -261.95

ppm indicating that the tin(IV) atom was remained six-coordinated and exhibited a distorted octahedral geometry. Complex **3** showed that the $\delta(^{119}\text{Sn})$ value at -106.02 ppm, which lie in the range of -40 to -120 ppm [for triphenyltin(IV) complexes], hence, indicating that the tin(IV) atom in complex **3** was four-coordinated with a distorted tetrahedral geometry^{21,22}. From the ¹¹⁹Sn NMR spectral studies, it is believed that methanol molecule was disassociated upon dilution during the NMR solution preparation resultant tin(IV) atom in complex **3** became four-coordinated.

Preliminary *in vitro* cytotoxic assay and antibacterial screening activity: The preliminary *in vitro* cytotoxic assay of acid (HL) and its organotin(IV) complexes **1-3** are given in Table-1. Based on the data given in Table-1, it was found that both the acid and complex **1** were inactive against HepG2 cell lines. In addition, complex **3** (0.143 $\mu\text{g}/\text{mL}$) was more active compared to complex **2** (0.171 $\mu\text{g}/\text{mL}$). This is due to complex **2** were obtained as organodistannoxane dimer type (bulky molecules) hence the aid of ligands on organotin(IV) to the receptor (active) sites of the cells was inhibited. In general, complex **3** was derivatives of triorganotin(IV), which is more active compared to the diorganotin(IV)²⁵⁻²⁷. In addition, in solution form, complex **3** was four-coordinated and exhibited distorted tetrahedral geometry (*sp*³) causing them to be more active²⁰. However, their activities were lower compared to the vincristine sulphate (reference drug).

TABLE-1
PRELIMINARY CYTOTOXIC ASSAYS, IC₅₀ VALUE OF
ACID (HL) AND ITS ORGANOTIN(IV) COMPLEXES 1-3

Complexes	IC ₅₀ ($\mu\text{g}/\text{mL}$)
	Human liver hepatocellular carcinoma cells, HepG2
HL	Inactive (start at 1.0)
1	Inactive (start at 1.0)
2	0.171 \pm 0.011
3	0.143 \pm 0.013
Vincristine sulphate (reference)	0.042 \pm 0.031

IC₅₀ ($\mu\text{g}/\text{mL}$) = concentration that yields 50 % inhibition of the cell compared with untreated control; The cytotoxicity values are expressed as mean \pm S.E.M. from the triplicate

The preliminary *in vitro* antibacterial screening activity of acid (HL) and its organotin(IV) complexes **1-3** are given in Table-2. Inhibition zones with a diameter less than 10 mm are considered as weak; larger than 10 mm but less than 16 mm are considered as moderate and finally larger than 16 mm and above are active^{13,28}. Based on this study, complex **3** which was derivatives of triphenyltin(IV) were found to be more active compared to the diorganotin(IV) complexes (**1** and **2**) and acid (HL) against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* but lower compared to the reference drugs. Based on the structural-activity study, complex **3** was found to be more active due to complex **3** was obtained as a simple monomer, four-coordinated and exhibited tetrahedral (*sp*³) in solution form whereas complexes **1** (distorted octahedral) and **3** (bulky molecule) obtained as a more complicated structure which in turn restrict their mobility to the target cell or active site. Moreover, based on the data in Table-2 showed that complex **3** was completely inactive against *Klebsiella*

TABLE-2
PRELIMINARY *IN VITRO* ANTIBACTERIAL SCREENING ACTIVITY OF ACID (HL) AND ITS ORGANOTIN(IV) COMPLEXES 1-3

Complexes	Inhibition zone (mm)				
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
HL	12	9	13	12	12
1	14	10	11	14	14
2	11	10	11	9	11
3	16	8	-	23	17
Chloramphenicol	29	-	23	34	30
Doxycycline	34	24	21	40	28
Rifampicin	25	24	23	29	37

Agar well diffusion method (*in vitro*) = 1.0 mg/mL; Reference drug = chloramphenicol, doxycycline and rifampicin

pneumoniae bacterial strains in this study indicated that complex **3** was selective against certain bacterial strains.

Conclusion

Complexes **1-3** have been successfully synthesized. The structural as well as the coordination number of tin(IV) atoms moieties of complexes **1-3** have been successfully characterized quantitatively and qualitatively. Based on the preliminary *in vitro* cytotoxic assay on human liver hepatocellular carcinoma cells (HepG2) and antibacterial screening activity, complex **3** [triphenyltin(IV)] showed better activity compared to complexes **1** and **2** [diorganotin(IV)] but lower activity compared to the reference drugs.

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REFERENCES

- R. Zhang, J. Sun and C. Ma, *J. Organomet. Chem.*, **690**, 4366 (2005).
- Y.F. Win, S.G. Teoh, J.B.-J. Teh, H.K. Fun and L. Zakaria, *Acta Cryst.*, **E63**, m323 (2007).
- Y.-F. Win, S.G. Teoh, M.R. Vikneswaran, J.H. Goh and H.K. Fun, *Acta Cryst.*, **E66**, m695 (2010).
- Y.-F. Win, C.-S. Choong, S.-T. Ha, C.K. Quah and H.K. Fun, *Acta Cryst.*, **E67**, m535 (2011).
- Y.-F. Win, C.-S. Choong, S.-T. Ha, S.-G. Teoh and N.E. Eltayeb, *Asian J. Chem.*, **25**, 972 (2013).
- Y.-F. Win, S.G. Teoh, B.M. Yamin and S.W. Ng, *Acta Cryst.*, **E66**, m1164 (2010).
- K.C. Molloy, T.G. Purcell, K. Quill and I.W. Nowell, *J. Organomet. Chem.*, **267**, 237 (1984).
- R. Willem, A. Bouhdid, B. Mahieu, L. Ghys, M. Biesmans, E.R.T. Tiekink, D. de Vos and M. Gielen, *J. Organomet. Chem.*, **531**, 151 (1997).
- S.G. Teoh, S.H. Ang, S.B. Teo, H.K. Fun, K.L. Khew and C.W. Ong, *J. Chem. Soc., Dalton Trans.*, **4**, 465 (1997).
- F. Novelli, M. Recine, F. Sparatore and C. Juliano, *IL Farmaco*, **54**, 237 (1999).
- M. Gielen, M. Biesemans, D.D. Vos and R. Willem, *J. Inorg. Biochem.*, **79**, 139 (2000).
- K.A. Crouse, K.-B. Chew, M.T.H. Tarafder, A. Kasbollah, A.M. Ali, B.M. Yamin and H.K. Fun, *Polyhedron*, **23**, 161 (2004).
- Y.-F. Win, S.-G. Teoh, M.R. Vikneswaran, S.-T. Ha and I. Pazilah, *Int. J. Phys. Sci.*, **5**, 1263 (2010).
- Y.-F. Win, S.-G. Teoh, S.-T. Ha, L.G.-A. Ong and T.-S. Tengku-Muhammad, *Int. J. Phys. Sci.*, **6**, 1463 (2011).
- T. Mosmann, *J. Immunol. Methods.*, **65**, 55 (1983).
- A.M. Ali, N.H. Ismail, M.M. Mackeen, L.S. Yazan, S.M. Mohamed, A.S.H. Ho and N.H. Lajis, *Pharmaceut. Biol.*, **38**, 298 (2000).
- G.K. Sandhu and S.P. Verma, *Polyhedron*, **6**, 587 (1987).
- L.L. Yeap and S.G. Teoh, *J. Coord. Chem.*, **56**, 701 (2003).
- A. Sau and R.R. Holmes, *J. Organomet. Chem.*, **217**, 157 (1981).
- M. Danish, H.G. Alt, A. Badshah, S. Ali, M. Mazhar and N. Islam, *J. Organomet. Chem.*, **486**, 51 (1995).
- J. Holecek, K. Handlír, M. Nádvorník and A. Lycka, *J. Organomet. Chem.*, **258**, 147 (1983).
- J. Holecek, M. Nádvorník, K. Handlír and A. Lycka, *J. Organomet. Chem.*, **241**, 177 (1983).
- T.S.B. Baul, S. Dhar, S.M. Pyke, E.R.T. Tiekink, E. Rivarola, R. Butcher and F.E. Smith, *J. Organomet. Chem.*, **633**, 7 (2001).
- J. Holecek, M. Nádvorník, K. Handlír and A. Lycka, *J. Organomet. Chem.*, **315**, 299 (1986).
- W. Rehman, M.K. Baloch and A. Badshah, *J. Braz. Chem. Soc.*, **4**, 827 (2005).
- K. Shahid, S. Shahzadi, S. Ali and M. Mazhar, *Bull. Korean Chem. Soc.*, **27**, 44 (2006).
- M.S. Ahmad, M. Hussain, M. Hanif, S. Ali and B. Mirza, *Molecules*, **12**, 2348 (2007).
- Z.H. Chohan, M. Arif, M.M. Akhtar and C.T. Supuran, *Bioinorg. Chem. Appl.*, **2006**, 83131 (2006).