SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF ORGANOTIN(IV) COMPLEXES OF THIOSEMICARBAZONE LIGAND DERIVED FROM PYRUVIC ACID: X-RAY CRYSTAL STRUCTURE OF [Me_2Sn(PAT)]

(Kajian Sintesis, Pencirian dan Biologi Kompleks Organostannum(IV) bagi Ligan Tiosemikarbazon yang Diterbitkan Daripada Asid Piruvik: Struktur Sinar-X Hablur [Me_2Sn(PAT)])

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Abstract

Six organotin(IV) complexes of pyruvic acid thiosemicarbazone ligand [H_2PAT, (**1**)] with general formula [$RSnCl_{n-1}$ PAT)] [R = Me_2, n = 1 (**2**); R = Bu_2, n = 1 (**3**); R = Ph_2, n = 1 (**4**); R = Me, n = 2 (**5**); R = Bu, n = 2 (**6**); R = Ph, n = 2 (**7**)] were synthesized by direct reaction of thiosemicarbazone ligand (**1**), base and organotin(IV) chloride(s) in absolute methanol under N_2 atmosphere. These organotin(IV) complexes were characterized by elemental analyses, molar conductivity, UV-visible, FTIR, ^{1}H and ^{13}C NMR spectral studies. Among them, dimethyltin(IV) complex (**2**) was also characterized by X-ray crystallography diffraction analyses. The cytotoxicity of the ligand (**1**) as well as its organotin(IV) complexes (**2-7**) were determined by *Artemia salina*, shrimp test lethality bioassay.

Keywords: Thiosemicarbazone, Organotin(IV) complexes, Cytotoxicity

Abstrak

Enam kompleks organostanum(IV) bagi ligan piruvik asid tiosemikarbazon [H_2PAT, (**1**)] dengan formula am [$RSnCl_{n-1}$ PAT)] [R = Me_2, n = 1 (**2**); R = Bu_2, n = 1 (**3**); R = Ph_2, n = 1 (**4**); R = Me, n = 2 (**5**); R = Bu, n = 2 (**6**); R = Ph, n = 2 (**7**)] telah disintesiskan dengan tindak balas terus antara ligan (**1**), bes dan organostannum(IV) klorida dalam methanol kering di bawah persekitaran N_2 tulen. Kompleks-kompleks organostannum(IV) tersebut telah dicirikan dengan analisis keunsuran, kekonduksian molar, ultraungu cahaya-nampak, inframerah, kajian spektrum Resonan Magnetik Nuklear ^{1}H dan ^{13}C. Di antaranya, kompleks dimetilstannum(IV) (**2**) juga telah dicirikan dengan analisis pembelauan kristalografi sinar-X. Ketoksian ligan (**1**) dan kompleks-kompleks organostannum(IV)nya (**2-7**) telah dikaji dengan *Artemia salina*, ujian bioesei kematian anak udang.

Kata kunci: Tiosemikarbazon, kompleks organostannum(IV), ketoksian

Introduction

Thiosemicarbazones have been extensively investigated. This is largely owing to their structural features and potent biological activity [1]. The biological activity of thiosemicarbazones could be enhanced by the functional groups of the parent aldehyde or ketone [2]. Thiosemicarbazones have been evaluated for their potential antitumour [3], antimalarial, antiviral, radioprotector, anticonvulsant properties, as well as their potential as a trypanocidal agent, ulcer inhibitor and anticancer agent [4].

The metal complexes of thiosemicarbazones, especially thiophene-2-carboxaldehyde thiosemicarbazone with transition metals, such as Pd(II) [5-6], Ru(II) [4], Fe(II) [1], Fe(III) [1], Cu(II) [7-8], Zn(II) and Co(II) [9] has been investigated.

However, less studies reported on thiosemicarbazone complexes with main group elements [10-11]. In view of this, our group decided to embark on a study of the thiosemicarbazone complexes of organotin(IV).

Experimental Procedure

General

Thiosemicarbazide, pyruvic acid and organotin(IV) chloride were obtained from Fluka, Merck and Aldrich, respectively. All solvents were reagent grade and purified by a standard method [12]. Elemental analyses were performed with FlashEA® 1112. The melting point was collected using open capillary in Stuart MP3. Molar conductivity was recorded using the molar conductivity meter model Jenway 4510. UV-visible spectroscopy studies were carried out in solution using Perkin Elmer Lambda 25 ranged from 240 to 450 nm in DMF (10^{-4} M). The FT-IR spectra were obtained with a Perkin Elmer Spectrum GX in the range of 4000-370 cm^{-1} using KBr disks. The ^1H and ^{13}C NMR spectra were recorded in acetone-d_6 and DMSO-d_6 on a Joel 500 MHz spectrometer.

Pyruvic acid thiosemicarbazone (H_2PAT) (1)

Pyruvic acid (0.09 g, 1 mmol) was added dropwise to the 20 mL methanolic solution of thiosemicarbazide (0.09 g, 1 mmol), and was heated under reflux for 4 hours, and allowed to cool to ambient temperature. A light brown crystalline solid was formed, filtered, washed and recrystallised with methanol, then dried in vacuo over silica gel. Yield: 0.16g, 89%; mp: 185-186 °C; UV-visible (DMSO) λ_{max}: 306; FT-IR (KBr disc) ν_{max}: 3430 (m, OH), 3407 (s, NH_2), 1702 (m, C=O), 1625 (s, C=N), 1269 (m, C=S), 952 (m, N-N); ^1H NMR (Acetone-d_6) δ: 9.76 (*s*, 1H, COOH), 8.48 (*s*, 1H, HN-C=S), 7.96 (*s*, 2H, NH_2), 2.23 (*s*, 3H, N=C-CH_3); ^{13}C NMR (Acetone-d_6) δ: 181.73 (HN-*C*=S), 165.12 (*C*OOH), 139.72 (*C*=N), 11.60 (*C*H_3). Anal. Calc. for $C_4H_7N_3O_2S$: C, 29.80; H, 4.38; N, 26.08%. Found: C, 29.77; H, 4.37; N, 26.12%.

[$Me_2Sn(PAT)$] (2)

The Pyruvic acid thiosemicarbazone (**1**) (0.16 g, 1 mmol) was dissolved in 20 mL of absolute methanol in a Schlenk round bottom flask. Then, 10 mL of methanolic solution of potassium hydroxide (0.11g, 2 mmol) was added in dropwise and the colour of the solution changed to light yellow. The resulting solution was refluxed under nitrogen atmosphere for one hour. Subsequently, 10 mL of methanolic solution of dimethyltin(IV) dichloride (0.44g, 2 mmol) was added in slowly and resulted a colourless solution. The solution was refluxed further for three hours under a nitrogen atmosphere. The potassium chloride was removed by filtration. Colourless single crystals were obtained by recrystallisation from dichloromethane:methanol (1:1 v/v). Yield: 0.22g, 58%; mp: 248-249 °C; Molar conductance (DMF) Ω^{-1} cm^2 mol^{-1}: 2.45; UV-visible (DMSO) λ_{max}: 309, 389; FT-IR (KBr disc) ν_{max}: 3390 (m, NH_2), 1614 [m, $\nu_{as}(COO^-)$], 1590 (s, C=N), 1379 [s, $\nu_{sy}(COO^-)$], 766 (m, C-S), 1008 (w, N-N), 610 (w, Sn-C), 569 (w, Sn-O), 483 (w, Sn-N); ^1H NMR (Acetone-d_6) δ: 7.32 (*s*, 2H, NH_2), 2.23 (*s*, 3H, N=C-CH_3), 0.86 (*s*, 6H, Sn-CH_3); ^{13}C NMR (DMSO-d_6) δ: 175.28 (N=*C*-S^-), 163.80 (*C*OO^-), 150.46 (*C*=N), 14.78 (*C*H_3), 8.72 (Sn-*C*H_3). Anal. Calc. for $C_6H_{11}N_3O_2SSn$: C, 23.40; H, 3.60; N, 13.65%. Found: C, 23.39; H, 3.51; N, 13.50%.

The other complexes (**3-7**) were synthesized by repeating the same procedure as [$Me_2Sn(PAT)$] (**2**) with appropriate organotin(IV) chloride salts.

[$Bu_2Sn(PAT)$] (3)

Yield: 0.33g, 72%; mp: 176-177 °C; Molar conductance (DMF) Ω^{-1} cm^2 mol^{-1}: 5.29; UV-visible (DMSO) λ_{max}: 311, 389; FT-IR (KBr disc) ν_{max}: 3333 (s, NH_2), 1621 [m, $\nu_{as}(COO^-)$], 1592 (s, C=N), 1370 [m, $\nu_{sy}(COO^-)$], 765 (m, C-S), 1006 (w, N-N), 641 (w, Sn-C), 538 (w, Sn-O), 482 (m, Sn-N); ^1H NMR (Acetone-d_6) δ: 7.34 (*s*, 2H, NH_2), 2.33 (*s*, 3H, N=C-CH_3), 2.10-2.07 (*t*, 2H, Sn-C*H*$_2$$CH_2CH_2CH_3$), 1.61-1.55 (*m*, 2H, Sn-CH_2C*H*$_2$$CH_2CH_3$), 1.33-1.27 (*m*, 2H, Sn-CH_2CH_2C*H*$_2$$CH_3$), 0.88-0.85 (*t*, 3H, Sn-$CH_2CH_2CH_2$C*H*$_3$); ^{13}C NMR (DMSO-d_6) δ: 175.79 (N=*C*-S^-), 164.37 (*C*OO^-), 150.61 (*C*=N), 14.85 (*C*H_3), 27.32 (Sn-*C*$H_2CH_2CH_2CH_3$), 26.39 (Sn-CH_2*C*$H_2CH_2CH_3$), 25.77 (Sn-CH_2CH_2*C*H_2CH_3), 13.63 (Sn-$CH_2CH_2CH_2$*C*H_3). Anal. Calc. for $C_{12}H_{23}N_3O_2SSn$: C, 42.88; H, 5.91; N, 10.72%. Found: C, 42.88; H, 5.72; N, 10.58%.

[$Ph_2Sn(PAT)$] (4)

Yield: 0.42g, 84%; mp: 113-114 °C; Molar conductance (DMF) Ω^{-1} cm^2 mol^{-1}: 4.77; UV-visible (DMSO) λ_{max}: 310, 389; FT-IR (KBr disc) ν_{max}: 3295 (m, NH_2), 1663 [m, $\nu_{as}(COO^-)$], 1610 (s, C=N), 1360 [s, $\nu_{sy}(COO^-)$], 764 (m, C-S), 996 (m, N-N), 616 (w, Sn-C), 533 (w, Sn-O), 484 (w, Sn-N); ^1H NMR (Acetone-d_6) δ: 7.92-7.82 (*d*, 2H, *ortho*-H), 7.77-7.70 (*t*, 1H, *para*-H), 7.48-7.43 (*t*, 1H, *meta*-H), 7.32 (*s*, 2H, NH_2), 2.38 (*s*, 3H, N=C-CH_3); ^{13}C NMR (Acetone-d_6) δ: 175.71 (N=*C*-S^-), 163.85 (*C*OO^-), 152.62 (*C*=N), 14.78 (*C*H_3), 141.37, 137.01, 136.75,

136.53, 136.30, 137.07, 131.28, 130.43, 130.56, 129.74, 129.44, 128.73 (Sn-(C_6H_5)$_2$). Anal. Calc. for $C_{16}H_{15}N_3O_2SSn$: C, 44.47; H, 3.50; N, 9.72%. Found: C, 44.35; H, 3.44; N, 9.63%.

[MeSnCl(PAT)] (5)
Yield: 0.30g, 75%; mp: decomposed > 250 °C; Molar conductance (DMF) Ω^{-1} cm^2 mol^{-1}: 6.51; UV-visible (DMSO) λ_{max}: 310, 384; FT-IR (KBr disc) ν_{max}: 3296 (m, NH_2), 1682 [s, $\nu_{as}(COO^-)$], 1604 (m, C=N), 1381 [m, $\nu_{sy}(COO^-)$], 753 (w, C-S), 1005 (w, N-N), 644 (w, Sn-C), 532 (w, Sn-O), 491 (w, Sn-N); ^{1}H NMR (Acetone-d_6) δ: 7.49 (*s*, 2H, NH_2), 2.27 (*s*, 3H, N=C-CH_3), 1.07 (*s*, 6H, Sn-CH_3); ^{13}C NMR (Actone-d_6) δ: 175.28 (N=*C*-S$^-$), 163.96 (*C*OO$^-$), 146.32 (*C*=N), 13.47 (*C*H_3), 16.17 (Sn-*C*H_3). Anal. Calc. for $C_5H_8N_3O_2SSnCl$: C, 18.29; H, 2.46; N, 12.80%. Found: C, 18.11; H, 2.53; N, 12.70%.

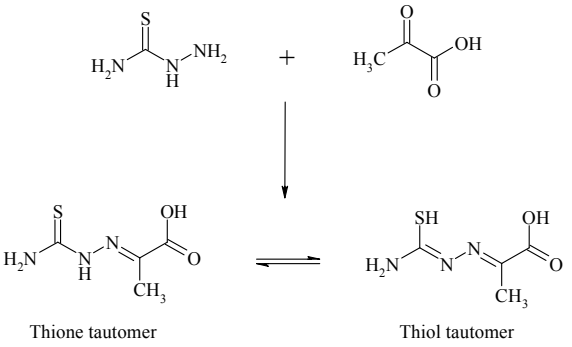
[BuSnCl(PAT)] (6)
Yield: 0.33g, 75%; mp: 183-185 °C; Molar conductance (DMF) Ω^{-1} cm^2 mol^{-1}: 10.72; UV-visible (DMSO) λ_{max}: 332, 384; FT-IR (KBr disc) ν_{max}: 3303 (s, NH_2), 1622 [s, $\nu_{as}(COO^-)$], 1366 (s, C=N), 1594 [s, $\nu_{sy}(COO^-)$], 773 (m, C-S), 1011 (w, N-N), 613 (w, Sn-C), 573 (w, Sn-O), 496 (w, Sn-N); ^{1}H NMR (Acetone-d_6) δ: 7.48 (*s*, 2H, NH_2), 2.26 (*s*, 3H, N=C-CH_3), 2.17-2.13 (*t*, 2H, Sn-C$H_2$$CH_2CH_2CH_3$), 1.80-1.75 (*m*, 2H, Sn-CH_2C$H_2$$CH_2CH_3$), 1.47-1.40 (*m*, 2H, Sn-CH_2CH_2C$H_2$$CH_3$), 0.94-0.91 (*t*, 3H, Sn-$CH_2CH_2CH_2$CH_3); ^{13}C NMR (Acetone-d_6) δ: 175.45 (N=*C*-S$^-$), 164.33 (*C*OO$^-$), 146.35 (*C*=N), 13.92 (*C*H_3), 32.88 (Sn-*C*$H_2CH_2CH_2CH_3$), 28.05 (Sn-CH_2*C*$H_2CH_2CH_3$), 26.30 (Sn-CH_2CH_2*C*H_2CH_3), 13.55 (Sn-$CH_2CH_2CH_2$*C*H_3). Anal. Calc. for $C_8H_{14}N_3O_2SSnCl$: C, 25.94; H, 3.81; N, 9.73%. Found: C, 25.88; H, 3.77; N, 9.58%.

[PhSnCl(PAT)] (7)
Yield: 0.37g, 80%; mp: decomposed > 252 °C; Molar conductance (DMF) Ω^{-1} cm^2 mol^{-1}: 9.37; UV-visible (DMSO) λ_{max}: 332, 384; FT-IR (KBr disc) ν_{max}: 3301 (m, NH_2), 1628 [s, $\nu_{as}(COO^-)$], 1607 (s, C=N), 1371 [m, $\nu_{sy}(COO^-)$], 773 (m, C-S), 1011 (w, N-N), 615 (w, Sn-C), 554 (w, Sn-O), 496 (w, Sn-N); ^{1}H NMR (Acetone-d_6) δ: 7.95-7.83 (*d*, 2H, *ortho*-H), 7.71-7.51 (*t*, 1H, *para*-H), 7.48-7.39 (*t*, 1H, *meta*-H), 7.34 (*s*, 2H, NH_2), 2.32 (*s*, 3H, N=C-CH_3); ^{13}C NMR (Acetone-d_6) δ: 173.15 (N=*C*-S$^-$), 162.65 (*C*OO$^-$), 145.99 (*C*=N), 13.28 (*C*H_3), 133.92, 133.78, 130.23, 129.74, 129.06, 128.62 (Sn-C_6H_5). Anal. Calc. for $C_{10}H_{10}N_3O_2SSnCl$: C, 30.76; H, 2.58; N, 10.77%. Found: C, 30.72; H, 2.50; N, 10.58%.

X-Ray crystal structure determinations
A transparent single-crystal of complex (**2**) (0.48 x 0.29 x 0.24 mm) was grown from dichloromethane-methanol (1:1 v/v) mixture at room temperature. The measurements were performed at 298(2) K on a Siemens SMART CCD diffractometer at Universiti Kebangsaan Malaysia using a graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). Orientation matrix and unit cell parameters were obtained from the setting angles of 25-centered reflection. The crystals are monoclinic, space group P2(1)/c with a = 6.7332(11), *b* = 9.3504(16), *c* = 9.5267(16), α = 67.264(2), β = 70.731(2), γ = 86.450(2), V = 520.70(15) Å^3, Z = 2, D_{calc} = 1.964 Mgm^{-3}, μ = 1.279 mm^{-1}. The diffraction intensities were collected by ω scans (2.37 - 25.50°). A total of 1938 reflections were collected ($-8 \le h \le 8$, $-11 \le k \le 11$, $-11 \le l \le 11$). The structure was solved using direct methods and refined using the full-matrix least-square method on F^2_{obs} using the SHELXTF [13] software package. All non-hydrogen atoms were refined with anisotropic thermal parameters. All H atoms were placed in calculated positions and treated as riding atoms on the parent C atoms, with C-H distances = 0.97 Å.

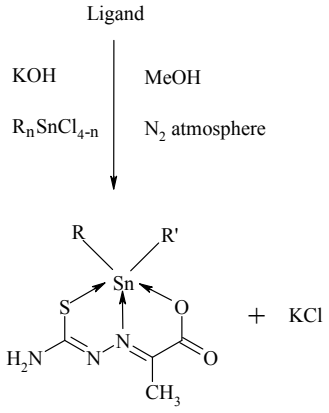
Results and discussion

Synthesis
The pyruvic acid thiosemicarbazone ligand (**1**) contains SNO donor atoms; it can be synthesized by the condensation reaction of equimolar of thiosemicarbazide and pyruvic acid in absolute methanol. Based on the outcome of the characterizations, the ligand exists in the thione tautomer when it is in solid form; and in the thiol tautomer when it is in solution.



Scheme 1: Tautomerization of the thiosemicarbazone ligand.

Potassium hydroxide was added during the complexation process to deprotonate the protons at the thiol (SH) and the carboxylic (COOH) groups. This caused the ligand to coordinate to the organotin(IV) moiety in its deprotonated thiol form. All complexes (**2-7**) were synthesized by direct reaction of ligand (**1**) with appropriate organotin(IV) salts under N_2 atmosphere. The coordination mode of the ligand (**1**) in all the complexes (**2-7**) is the same as the dinegative tridentate fashion.



R= Me	R'= Me	n=2	(**2**)
R= Bu	R'= Bu	n=2	(**3**)
R= Ph	R'= Ph	n=2	(**4**)
R= Me	R'= Cl	n=1	(**5**)
R= Bu	R'= Cl	n=1	(**6**)
R= Ph	R'= Cl	n=1	(**7**)

Scheme 2. Organotin(IV) complexes (**2-7**) of ligand (**1**).

Molar conductivity

The molar conductivity of the complexes (**2-7**) was determined in DMF (10^{-4} M) at room temperature. The molar conductance values of all the complexes (**2-7**) lie in the range of 2.45-10.72 Ω^{-1} cm^2 mol^{-1}, thus indicating that all of them are non-electrolytes [14].

UV-visible spectra

There is only one band lying around 298 nm in the ligand (**1**) spectrum and this peak corresponds to the NH-C=S group in the ligand (**1**). After complexation, two bands were observed around 272-285 and 324-331 nm and these are assigned to n-π^* of carbonyl (from pyruvic acid) and the ligand metal charge transfer (LMCT) band. The band which is attributed to the carbonyl n-π^* of ligand at 272-285 nm is not observed in the ligand spectrum as it might be covered by the NH-C=S band which has a stronger absorption around 298 nm. Upon complexation, tautomerization took place and all the thione is transformed to the thiol form and coordinated to the organotin(IV) moiety in its deprotonated thiolato form. The conversion to the thiolato tautomer uncovered the carbonyl band around 272-285 nm, so the carbonyl band only appears in the complexes (**2-7**) spectra.

Infrared spectroscopy

Several characteristic bands are available in the spectrum of ligand (**1**) at 3430, 3407, 1702, 1625, 1269 and 952 cm^{-1}; they are attributed to ν(OH), ν(NH_2), ν(C=O), ν(C=N), ν(C=S) and ν(N-N), respectively.
The band situated at 3407 cm^{-1} in the spectrum of ligand (**1**) is assigned to ν(NH_2), thus proving that the amine (H_2N-C=S) group was not involved in the condensation reaction during the ligand synthesis. The presence of the carboxylic group (–COOH) in the ligand (**1**) can be traced via the appearance of the ν(OH) (3430 cm^{-1}) and ν(C=O) (1702 cm^{-1}) bands.

Based on the structure of the ligand (**1**), there is a proton adjacent to the thione group (C=S), so tautomerization might take place and the ligand (**1**) may be present in the thione or thiol tautomer. However, the absence of S-H stretching vibration band (3100-3162 cm^{-1}) in the spectrum has confirmed that the ligand (**1**) exists in thione form when it is in the solid form. A medium band is observed around 1625 cm^{-1} and this is attributed to the azomethine in the ligand (**1**), while the hydrazinic band appeared at 952 cm^{-1}. The characteristic bands observed are in accordance with those reported [15].

Upon complexation, the structure of the ligand (**1**) is altered; all alterations can be observed through the shift of the characteristic bands. The most remarkable change is the disappearance of ν(OH) (3430 cm^{-1}). This is attributable to $\nu_{as}(COO^-)$ and $\nu_{sy}(COO^-)$ existing in the spectra of all the complexes (**2-7**), thus supporting the deprotonation of carboxylic group and coordination of its carboxylic oxygen to the tin(IV) ion. The magnitude of Δν [$\nu_{as}(COO^-)$ – $\nu_{sy}(COO^-)$] for the complexes (**2-7**) falls in the range of 303-235 cm^{-1}, indicating the carboxyl group in all the complexes (**2-7**) is bound in monodentate form [15].

The band which represents ν(C=S) is out of sight in all the complexes' spectra (**2-7**), whilst, a new band which is attributed to ν(C-S) appears around 773-753 cm^{-1}. This evidence concurs with the occurrence of the thiol tautomer bound in its deprotonated form to the tin(IV) ion. The absorption frequency of all characteristic bands of ligand (**1**) decreases upon complexation except the hydrazinic ν(N-N) band; compared to the free ligand (**1**), the absorption frequency of this band has increased to 1011-996 cm^{-1}. The principle behind this phenomenon is due to the donation of the unpaired electrons from one of the nitrogen ones to the tin(IV) ion, incidentally deflating the repulsion force between the two adjacent nitrogen electrons. This decreases the distance between the two nitrogen ones, subsequently, shifting the absorption frequency to a higher value.
In all the complexes' spectra (**2-7**), there are some new bands which can be traced in the finger print area. There are three new bands around 644-610, 573-532 and 496-482 cm^{-1}, assigned to the ν(Sn-C), ν(Sn-O) and ν(Sn-N), respectively. The appearance of these new bands indicates the coordination of ligand (**1**) to the center tin (IV) ion via its carboxylic-O, azomethine-N and thiolato-S.

^{1}H NMR spectra

Four proton signals are available in the free ligand (**1**) spectrum; they are around 9.76, 8.48, 7.96 and 2.23 ppm. These signals are assigned to COO*H*, CNS*H*, NH_2 and N=C-CH_3, respectively. Upon complication, the band at 9.76 and 8.48 ppm which indicate the protons of the COO*H* and S=C-N*H* (throne tautomer), respectively disappeared, implying that the tautomerization has occurred. At the same time, the azomethine in all complexes (**2-7**) is shifted 0.15-0.03 ppm downfield compared to the free ligand (**1**). These pieces of evidence reveal that the organotin(IV) adducts are formed via carboxylic-O, thiol-S and azomethine-N.

The NH_2 in the ligand (**1**) is not involved in coordination as the band attributed to NH_2 appeared in all the complexes (**2-7**) spectra. Nevertheless, the NH_2 band of all complexes (**2-7**) is 0.64-0.47 ppm more upfield compared to the free ligand (**1**). This might due to the steric hindrance from the bulky groups attached to the organotin(IV) core.

The methyl group(s) attached to the organotin(IV) moiety in mono/dimethyltin(IV) complexes gives a singlet band at a very upfield region. For complex (**2**), the two methyl groups that are attached to the tin(IV) core appear at 0.86 ppm with $^2J(^{119}Sn\text{-}^1H)$ satellite equal to 68 Hz, while for complex (**5**), the Sn-CH_3 appears around 1.07 ppm.
The organo groups that are attached to the organo tin(IV) moiety of the complex (**3**) are two butyl groups, whereas only one butyl group is attached at the tin(IV) core in complex (**6**). In complex (**3**), the Sn-$CH_2CH_2CH_2CH_3$ gave four signals namely, 2.10-2.07 ppm (triplet, Sn-C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.61-1.55 ppm (multiplet, Sn-CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.33-1.27 ppm (multiplet, Sn-CH$_2$CH$_2$C*H*$_2$CH$_3$) and 0.88-0.85 ppm (triplet, Sn-CH$_2$CH$_2$CH$_2$C*H*$_3$). While for complex (**6**), there are four sets of data as well, which are around 2.17-2.13 ppm (triplet, Sn-C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.80-1.75 ppm (multiplet, Sn-CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.47-1.40 ppm (multiplet, Sn-CH$_2$CH$_2$C*H*$_2$CH$_3$) and 0.94-0.91 ppm (triplet, Sn-CH$_2$CH$_2$CH$_2$C*H*$_3$).

The organotin(IV) moieties of complex (**4**) and (**7**) are attached with diphenyltin(IV) and monophenyltin(IV), respectively. Three sets of signals which are attributed to the proton in the benzene ring can be seen in the spectrum of complex (**4**); 7.92-7.82 ppm (duplet, 2H, *Ortho*-H), 7.70-7.77 (triplet, 1H, *Para*-H) and 7.48-7.43 (triplet, 2H, *Meta*-H). On the other hand, the complex (**7**) also has three set of signals lying around 7.95-7.83 ppm (duplet, 2H, *Ortho*-H), 7.71-7.51 (triplet, 1H, *Para*-H) and 7.48-7.39 (triplet, 2H, *Meta*-H).

^{13}C NMR spectra
Due to the solubility limitation, two types of deuterated solvents were used to obtain the ^{13}C NMR results of ligand (**1**) and its complexes. The spectra of ligand (**1**) as well as its complexes (**4-6**) were recorded with acetone-d_6, while the complexes (**2**), (**3**) and (**7**) were recorded with DMSO-d_6. From the spectra of the ligand (**1**), four set of signals were observed around 181.73, 165.12, 139.72 and 11.60 ppm. These signals are attributed to the carbon of HN-*C*=S, *C*OOH, *C*=N and *C*H$_3$, respectively.

After complexation, both of the carbons assigned to S-C=N and COO^- were shifted approximately 6.02-6.45 and 1.27-0.79 ppm upfield, respectively. So, the δ value of carbon in the S-C=N group for the complexes (**4-6**) were located at 175.28-175.71 ppm, while both C=N and CH_3 in complexes (**4-6**) are shifted downfield. Upon complexation, the new C=N value in complexes is 152.62-146.32 ppm. Concurrently, the CH_3 is shifted to 14.78-13.47 ppm. Besides, new bands which are attributed to the carbons in the two phenyl rings in complex (**4**) appear at 141.37-129.44 ppm, while in complex (**6**), the butyl group can be supported via the four bands at δ 32.88 (Sn-*C*H$_2$CH$_2$CH$_2$CH$_3$), 28.05 (Sn-CH$_2$*C*H$_2$CH$_2$CH$_3$), 26.30 (Sn-CH$_2$CH$_2$*C*H$_2$CH$_3$) and 13.55 (Sn-CH$_2$CH$_2$CH$_2$*C*H$_3$). Lastly, the methyl group attached to the tin(IV) core in complex (**5**) appeared at 16.67 ppm.

X-ray crystallography
The molecular structure of dimethyltin(IV) complex (**2**) of ligand (**1**) with relevant atom numbering is given in Figure 1. Also, important information as well as the bond lengths and bond angles of complex (**2**) are depicted in Table 1 and Table 2, respectively. Based on the ORTEP diagram, the ligand (**1**) (Figure 1) is bound with the organotin(IV) core in dinegative tridentate pattern; which involved the thiol sulfur (S1), hydrazinic nitrogen (N3) and carboxyl oxygen (O2). The coordination number of the tin(IV) moiety is five.

According to the value of several essential bond lengths, such as Sn1-O2, Sn1-N3 and Sn1-S1, the ligand (**1**) is bound with the tin(IV) moiety in a moderate-loose mode. The Sn1-S1 of the dimethyltin(IV) complex (**2**) is reported to be 2.56 Å, and this value is 0.14 Å longer than the sum of non-polar covalent radii of tin and sulfur. Besides, it is bound more loosely compared to $SnPh_2Cl$(tctcs) [10] and $[PhSn(Hapt)Cl_2]\cdot C_2H_5OH$ [11]. The sum of non-polar covalent radii of tin and oxygen is 2.10 Å, while the Sn1-O2 reported here is 2.16 Å. This indicates that the tin-oxygen is bound more loosely than the normal circumstance. Besides, the bond length of Sn1-N3 reported here is also longer than the sum of the non-polar covalent radii of tin and nitrogen (2.14 Å). The bond lengths of Sn1-C5 and Sn1-C6 in this work are 2.105 and 2.101 Å, respectively. These values are slightly shorter than the sum

of non-polar covalent radii of tin and carbon (2.17 Å) and are comparable with the published organotin(IV) complexes [16-17].

The sum of the angles of (N3-Sn1-C5), (C5-Sn1-C6) and (C6-Sn1-N3) is 359.91°, which is almost planar; supports that the meridional plane is taken up by the two methyl groups from the tin(IV) core and the hydrazinic nitrogen (N3) from the ligand (**1**). On the other hand, the axial plane is occupied by the thiol sulfur (S1) and the carboxyl oxygen (O2) from the ligand (**1**), nevertheless, they are not bound in 180°, in fact their angle is 147.90°, which is 32.1° deviated from the perfect axial.

A perfect meridional plane of the trigonal bipyramidal should have 120° for each of the atom located at the three edges. However, the dimethyltin(IV) complex (**2**) does not have 120° for its three atoms located at the three edges (C5, C6 and N3), in fact it possesses 112.72° (N3-Sn1-C5), 136.24° (C5-Sn1-C6) and 110.95° (C6-Sn1-N3). The angles of N3-Sn1-C5 and C6-Sn1-N3 are slightly smaller than 120°, while the angle of C5-Sn1-C6 is slightly bigger than the 120°, this phenomenon might be due to the repulsion introduced by the two methyl groups bound to the tin(IV). Furthermore the steric hindrance from the two methyl groups at the tin(IV) core also cause the ligand (**1**) to not be able to bind nearer with the hydrazinic (N3), so this has caused the axial deviation from the linearity. Based on all the values obtained from the X-ray crystallography diffraction analyses, the dimethyltin(IV) complex (**2**) of ligand (**1**) can be identified as a distorted trigonal bipyramidal complex.

The crystal packing pattern of the dimethyltin(IV) complex (**2**) is shown as Figure 2. Some intermolecular hydrogen bonding can be seen from the diagram. This kind of intermolecular interaction that takes place is greatly due to the presence of the free NH_2 group in the complex. Furthermore, the size of dimethyltin(IV) is small and simple; hence there is less chance for two molecules to have great repulsion. Based on the diagram, the hydrogen bonding form at several points in a complex (**2**) molecule; the two hydrogen from the aminic nitrogen (N1) are able to form hydrogen bonding with carboxyl oxygen (O1) ($NH_2\cdots O$) and hydrazinic nitrogen (N2) ($NH_2\cdots N$) from the adjacent molecule.

Table 1. The crystallography data of the complex (**2**).

Empirical formula	$C_6\ H_{11}\ N_3\ O_2\ S\ Sn$
Formula weight	307.93
Temperature (K)	298(2)
Wavelength (Å)	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)/c
a (Å)	6.7332(11)
b (Å)	9.3504(16)
c (Å)	9.5267(16)
β (°)	70.731(2)
Volume ($Å^3$)	520.70(15)
Z	2
Calculated density (Mgm^{-3})	1.964
F(000)	300
Crystal size (mm)	0.48 x 0.29 x 0.24
Scan range θ (°)	2.37 - 25.50
Total/ Unique/ Rint	5068/ 1938/ 0.0174
Goodness-of-fit on F^2	1.082
Refined parameters	254
Final R indices [I> 2σ(I)]	R_1=0.0167, wR_2 =0.0440
R indices (all data)	R_1=0.0173, wR_2 =0.0443
μ (mm^{-1})	1.279
ρ_{max}/ρ_{min} (eA^{-3})	0.5712/ 0.3652

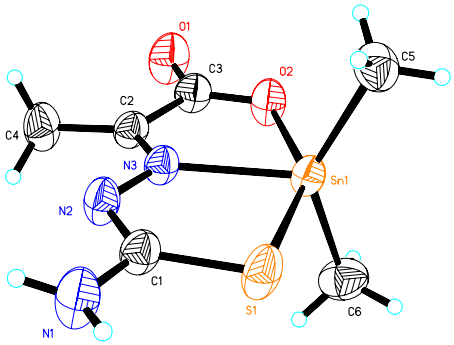


Figure 1. The asymmetric unit of the complex (**2**).

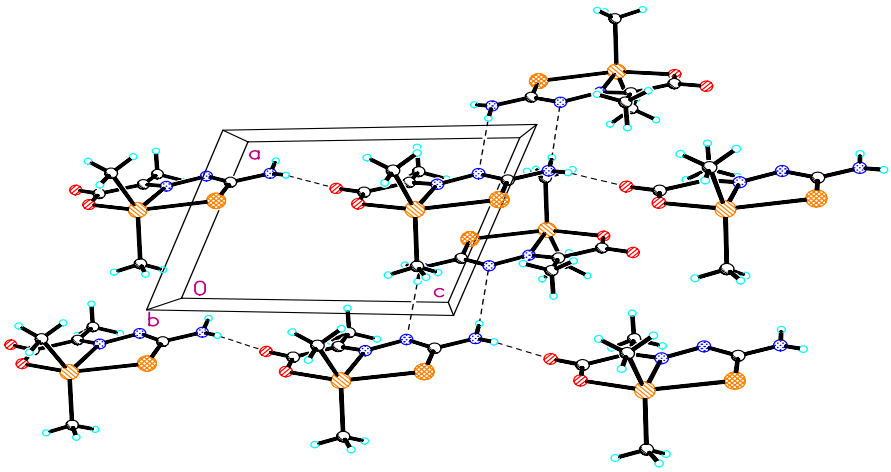


Figure 2. The crystal packing pattern of the complex (**2**).

Table 2. The selected bond lengths and bond angles of the dimethyltin(IV) complex (**2**).

Bond lengths (Å)			
Sn1-O2	2.1625(17)	N2-N3	1.370(3)
Sn1-N3	2.2394(18)	N2-C1	1.318(3)
Sn1-S1	2.5643(8)	N1-C1	1.334(3)
Sn1-C5	2.105(3)	N3-C2	1.288(3)
Sn1-C6	2.101(3)	O1- C3	1.221(3)
S1-C1	1.727(2)	O2- C3	1.278(3)
Bond angles (°)			
O2-Sn1-N3	72.81(6)	S1-Sn1-C5	96.79(8)
O2-Sn1-C5	95.14(10)	S1-Sn1-C6	96.78(8)
O2-Sn1-C6	94.91(10)	N3-Sn1-C5	112.72(9)
O2-Sn1-S1	147.90(5)	C6-Sn1-C5	136.24(12)
S1-Sn1-N3	75.10(5)	C6-Sn1-N3	110.95(10)

Cytotoxicity

Generally, the brine shrimp bioassay of the ligand (**1**) and its complexes (**2-7**) were conducted utilizing the published method [18] with some modifications. Brine shrimp eggs were hatched in treated sea water collected from Pantai Puteri (Kuching, Sarawak). Before the sea water was used, it had to undergo a series of treatment processes; after it was collected from the coastal area, filtered with Whatman filter paper grade 1, autoclaved and the salinity was adjusted to 20 psu.

A stock solution of 5000 ppm of the ligand (**1**) and its organotin(IV) complexes (**2-7**) was prepared in dimethyl sulphoxide. Basically, the stock solutions were further diluted into seven different concentrations, which were 1, 5, 10, 50, 100, 150 and 300 ppm. Under certain circumstances, some of the complexes might be tested up to 1000 ppm in order to obtain an accurate LC_{50} sigmoidal curve. There was one negative control and three replicates per concentration for each of the ligand (**1**) and its organotin(IV) complexes (**2-7**). All solutions were prepared in 5 mL with different levels of dose in small plastic mugs.

The hatching process was carried out for 24 hours, and a pinch of *Artemia salina* cysts were placed in a beaker with 100 mL of treated sea water. Throughout the hatching process, aeration and light was provided at ambient temperature. After 24 hours, the hatched nauplii were attracted by the light source and tend to stay at the surface of the sea water, while the shells and remaining cysts were deposited at the bottom of the sea water. The shrimps were transferred to fresh sea water in a petri dish for ease of calculation.

30 shrimps were placed in each and every mug, after 24 hours of incubation under direct light at room temperature, the mortality of the shrimps were observed under the binocular microscope. The immobile shrimps in each mug were counted as dead individuals. The mortality was computed and corrected for the natural death observed in the negative control using Abbott's formula [18]:

$$p = \frac{p_i - C}{l - C}$$

Where p_i denotes the observed mortality in the sample solutions; C is the natural mortality observed from the negative control; l is the number of individuals in each replicate. The percentage of mortalities could be calculated by multiplying p by 100 %.

Lastly, all results of the mortality were expressed in percentages and plotted in an allosteric sigmoidal graph with a GraphPad Prism, and the LD_{50} could be obtained from the graph.

Organotin(IV) compounds are ecotoxicants whose action depends on their structure [19]. Obviously, the outcome of the *Artemia* cytotoxicity test (Table 3) reveals that the diorganotin(IV) complexes (**2-4**) are more toxic compared to monoorganotin(IV) complexes (**5-6**). The presence of chloride in complexes (**2-4**) makes it more polar than complexes (**5-7**), thus decreasing the permeability through the cell [10]. On the other hand, the charge of the Sn cation shared the positive charge of the donor atoms during complexation and consequent π-electron delocalization over the entire chelate ring manifold [10]. The delocalization phenomenon works synergistically while the bulky organo group is indeed magnifying the lipophilic character of the diorganotin(IV) complexes and enhances the permeation of the compound through the membrane cell. In the diorganotin(IV) family, the complex (**2**) is much less toxic compared to complex (**3-4**) as the methyl group is a weaker electron donor compared to butyl group in complex (**3**), thus it might not accelerate much the π-electron delocalization and subsequently decreases its cytotoxicity.

Table 3. LC_{50} of the ligand (**1**) and its complexes (**2-7**).

Complexes		LC_{50} (ppm)
H_2PAT	(**1**)	184.60
$[Me_2Sn(PAT)]$	(**2**)	305.50
$[Bu_2Sn(PAT)]$	(**3**)	13.34
$[Ph_2Sn(PAT)]$	(**4**)	10.53
[MeSnCl(PAT)]	(**5**)	352.30
[BuSnCl(PAT)]	(**6**)	212.20
[PhSnCl(PAT)]	(**7**)	121.10

Conclusions

The COOH and N=C-SH in ligand (**1**) were deprotonated during complexation. Subsequently, the ligand (**1**) was coordinated to the organotin(IV) moiety in the dinegative tridentate nature via its thiolato-S, azomethine-N and carboxyl-O. Since the results of characterization are uniform with respect to each other, all the complexes (**2-7**) are concluded to have a similar configuration as trigonal bipyramidal geometry. Among ligand (**1**) and its organotin(IV) complexes, the diphenyltin(IV) complex (**4**) showed highest cytotoxicity; where the LC_{50} value was 10.53 ppm.

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References

1. Hong, W. S., Wu, C. Y., Lee, C. S., Hwang, W. S., Chiang, M. Y. **2004**. *J. Organomet. Chem. 689*, 277-285.
2. Belicchi Ferrari, M., Bisceglie, F., Pelosi, G., Tarasconi, P., Albertini, R., Pinelli, S. *J. Inorg. Biochem.* **2001**, *87*, 137-147.
3. Gielen, M. **2003**. *J. Braz. Chem. Soc. 14*, 870-877.
4. Singh, S., Athar, F., Maurya, M. R., Azam, A. **2006**. *Europ. J. Med. Chem. 41*, 592-598.
5. Bharti, N., Shailendra, Sharma, S., Naqvi, F., Azam, A. **2003**. *Bioorg. Med. Chem. 11*, 2923-2929.
6. Shailendra, Bharti, N., Naqvi, F., Azam, A. **2003**. *Bioorg. Med. Chem. Lett. 13*, 689-692.
7. Mohamed, G. G., Omar, M. M., Hindy, A. M. M. **2005**. *Spec. Act. A. 62*, 1140-1150.
8. Sharma. S., Athar, F., Maurya, M. R., Azam, A. **2005**. *Europ. J. Med. Chem. 40*, 1414-1419.
9. Belicchi Ferrari, M., Gasparri Fava, G., Pelosi, G., Tarasconi, P. **2000**. *Polyhedron*, *19*, 1895-1901.
10. Teoh, S. G., Ang, S. H., Fun, H. K., Ong, C. W. **1999**. *J. Organomet. Chem. 580*, 17-21.
11. Carcelli, M., Fochi, A., Pelagatti, P., Pelizzi, G., Russo, U. **2001**. *J. Organomet. Chem. 626*, 161-167.
12. Armarego, W. L. F., Perrin, D. D. **1996**. *Purification of Laboratory Chemicals*, *4th edition*, Butterworth Heinemann, Oxford, USA.
13. Sheldrick, G. M. **1997**. *SHELXTL V5 I Software Reference Manual Bruker AXS*, Inc. Madison, WI: USA.
14. Sharaby, C. M. **2007**. *Spectrochim. Act. A. 66*, 1271-1278.
15. Yin, H. D., Chen, S. W., Li, L. W., Wang, D. Q. **2007**. *Inorg. Chim. Act. 360*, 2215-2223.
16. Ma, C. L., Tian, G. R., Zhang, R. F. **2007**. *Inorg. Chim. Act. 360*, 1762-1766.
17. Shahzadi, S., Ali, S., Bhatti, M. H., Fettouhi, M., Athar, M. **2006**. *J. Organomet. Chem. 691*, 1797-1802.
18. Martl, M., Humpf. H. U. **2000**. *Food Chem. Toxi. 38*, 1097-1102.
19. Pellerito, C., Nagy, L., Pellerito, L., Szorcsik, A. **2006**. *J. Organomet. Chem.* 691, 1733-1747.