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SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF NEW PHENYLTIN(IV) DITHIOCARBAMATE COMPOUNDS

(Sintesis dan Kajian Biologi Sebatian Fenilstanum(IV) ditiokarbamat)

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Abstract

Nine chlorophenyltin(IV) dithiocarbamate compounds of general formula $PhSnCl[S_2CNR'R'']_2$ (R' = CH_3, C_2H_5, C_7H_7 and R" = C_2H_5, C_6H_{11}, iC_3H_7, C_7H_7) were prepared in one pot reaction of various secondary amine, carbon disulphide and phenyltin(1V) trichloride with the ratio of 2:2:1. These compounds have been characterized by elemental analysis, infrared spectroscopy, ultraviolet spectroscopy, ^1H, ^{13}C NMR spectroscopy as well as single crystal X-ray crystallography. Data from X-ray crystallography showed that $(C_6H_5)SnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$ is six coordinated bonded with two chelating dithiocarbamate ligands in bidentate fashion, methyl-C and chloride atom thus form a distorted octahedral geometry. Five selected compounds, $(C_6H_5)SnCl[S_2CN(CH_3)(C_2H_5)]_2$, $(C_6H_5)SnCl[S_2CN(CH_3)(C_6H_{11})]_2$, $(C_6H_5)SnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$, $(C_6H_5)SnCl[S_2CN(C_7H_7)(iC_3H_7)]_2$ and $(C_6H_5)SnCl[S_2CN(C_7H_7)_2]_2$ were screened for anticancer activity against Chang liver cells. These compounds were demonstrated no cytotoxic effect for all concentrations under the condition of the study with no IC_{50} value. The minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC) determinations for these five compounds used *Escherichia coli* and *Pseudomonas aeruginosa* as Gram negative bacteria together with *Staphylococcus aureus* and *Staphylococcus epidermis* as Gram positive bacteria. Compound $(C_6H_5)SnCl[S_2CN(CH_3)(C_2H_5)]_2$ was the most active compared to the other compounds by having the lowest MIC values of 1.25 mg/mL and MBC value of 5.0 mg/mL against *E. Coli* and *S. Aureus* bacteria. Compounds $(C_6H_5)SnCl[S_2CN(C_7H_7)(iC_3H_7)]_2$ and $(C_6H_5)SnCl[S_2CN(C_7H_7)_2]_2$ that having a benzyl group showed their MIC and MBC values slightly higher compared to the other compounds againts certain bacteria indicating their low antibacterial activities.

Keywords: chlorophenyltin(IV), dithiocarbamate, crystal structure, biological studies

Abstrak

Sembilan sebatian fenilklorostanum(IV) ditiokarbamat dengan formula sebatian $PhSnCl[S_2CNR'R'']_2$ (R' = CH_3, C_2H_5, C_7H_7 and R" = C_2H_5, C_6H_{11}, iC_3H_7, C_7H_7) telah disediakan dengan sintesis secara satu pot di antara pelbagai amina sekunder, karbon disulfida dan fenilstanum(IV) triklorida dengan nisbah 2:2:1. Kesemua sebatian ini telah dicirikan dengan kaedah analisis unsur, spektroskopi inframerah, spektroskopi ultralembayung, spektroskopi NMR ^1H, ^{13}C dan kristalografisinar-X. Data daripada kristalografi sinar-X menunjukkan struktur sebatian $(C_6H_5)SnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$ adalah enam koordinatan dan terikat dengan dua ligan ditiokarbamat yang terkelat secara bidentat, metil-C dan atom klorida untuk membentuk geometri oktahedron terherot. Lima sebatian pilihan iaitu $(C_6H_5)SnCl[S_2CN(CH_3)(C_2H_5)]_2$, $(C_6H_5)SnCl[S_2CN(CH_3)(C_6H_{11})]_2$, $(C_6H_5)SnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$, $(C_6H_5)SnCl[S_2CN(C_7H_7)(iC_3H_7)]_2$ dan $(C_6H_5)SnCl[S_2CN(C_7H_7)_2]_2$ telah disaring untuk aktiviti antikanser terhadap sel hati Chang. Sebatian ini tidak menunjukkan sebarang kesan sitotoksik pada setiap kepekatan dan tidak mempunyai nilai IC_{50}. Ujian penentuan nilai kepekatan perencatan minimum (MIC) dan nilai kepekatan minimum bakterisidal (MBC) untuk lima sebatian ini telah menggunakan *Escherichia coli* dan *Pseudomonas aeruginosa* sebagai bakteria Gram negatif serta *Staphylococcus aureus* dan *Staphylococcus epidermis* sebagai bakteria Gram positif. Sebatian

$(C_6H_5)SnCl[S_2CN(CH_3)(C_2H_5)]_2$ adalah paling aktif berbanding sebatian lain dengan mempunyai nilai MIC yang terendah iaitu 1.25 mg/mL dan nilai MBC 5.0 mg/mL terhadap bakteria *Escherichia coli* dan *Staphylococcus aureus*. Sebatian $(C_6H_5)SnCl[S_2CN(C_7H_7)(iC_3H_7)]_2$ dan $(C_6H_5)SnCl[S_2CN(C_7H_7)_2]_2$ yang mengandungi kumpulan benzil menunjukkan nilai MIC dan MBC yang lebih tinggi berbanding sebatian lain terhadap beberapa jenis bakteria dan menunjukkan aktiviti antibakteria yang lebih rendah.

Kata kunci: fenilklorostanum(IV), ditiokarbamat, struktur hablur, kajian biologi

Introduction

Dithiocarbamates ($R_2NCS_2^-$), sulphur analogs of carbamates ($R_2NCO_2^-$) are dithiocarbonic acid's half amides which through their chemical and biological properties have gained much interest in few industries especially in agriculture, pharmaceutical, medicine and rubber [1,2]. Dithio ligands affects significantly on biological systems by acting as inhibitors of enzymes due to its metal binding [3-5]. One example is diethyldithiocarbamate anion, (Et_2CNS_2) with its extensive usage as copper poisoning antidote in Wilson's disease and nephrotoxicity when platinum-based chemotherapy is used [6]. Due to the stability of heat up to 200 ^oC, mono and dialkyltin were used as heat and light stabilizer additives in polyvinyl chloride (PVC) processing, silicone elastomers and industrial catalysts for polyurethane [7]. Glass manufacturing also used monoorganotins to prevent microfissures and to prevent heat loss through metallic oxide coating [8]. Meanwhile, organotin compounds also show antifungal, antibacterial and anticancer properties [9,10]. Organotin(IV) compounds have strong biological activity and very toxic even in low concentration. Organic groups bounded to central Sn atom is essential as the number and nature of these groups will determines their biological activities [11]. Furthermore, mono organotin compounds have shown the lowest toxic activity compared to the other R_nSnX_{4-n} series as the activity will decreases from tetra alkyl substitute tin(IV) compounds to mono alkyl substituted tin(IV) compound [12]. Due to its stability and possession of an intriguing electrochemical and optical properties, derivatives of dithiocarbamates from secondary amines were most been studied [13].

Dithiocarbamates chemistry grew prolifically especially due to their preferential stabilization of metal compounds with specific stereochemistry owing to dithio ligands can act as a soft donor and have excellent coordination ability [3-5]. Previous study reported two crystal structures of monoorganotin compounds with general formula $PhSn(S_2CNR'_2)_3$ with distorted pentagonal bipyramid geometry and three crystal structures with general formula $PhSn(S_2CNR'_2)_2Cl$ in distorted octahedron geometry were found [14]. Instability has been an issue of corresponding phenyl derivatives of dithiocarbamate compounds leading to few reports for this series. Hence, this paper reports on the synthesis and characterization of nine phenyltin(IV) dithiocarbamate compounds including X-ray structure of $PhSn[S_2CN(Et)(i\text{-}Pr)]_2Cl$ in order to investigate the structural diversity of mono-tin dithiocarbamates compounds [15].

Materials and Methods

Materials

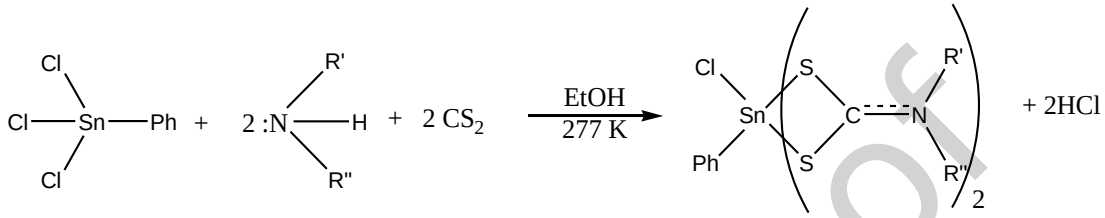
All chemicals and solvents that were used in this experiment were purchased from suppliers without purification and used as received as follows: N-methylethylamine, N-methylcyclohexylamine, N-ethylcyclohexylamine, N-methylisopropylamine, N-ethylisopropylamine, N-methylbenzylamine, N-ethylbenzylamine, N-isopropylbenzylamine, N-dibenzylamine and phenyltin(IV) trichloride together with the carbon disulphide, chloroform, dichloromethane, methanol and ethanol (Merck).

Instrumentations

Melting point was measured using Electrothermal IA 9100. Elemental analysis was carried out and recorded by Fison EA 1108. Perkin Elmer Model GX was used to record the infrared spectra by using KBr disc for the range 400-4000 cm^{-1} and nujol with polyethylene tablets for the range 400-250 cm^{-1}. Ultraviolet spectra were recorded on Cary 100 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Joel JNM-LA 400 by using TMS as internal standard. Bruker SMART APEX2 was used to record X-ray structure determination and had been carried out at Department of Chemistry, University of Malaya.

General Synthesis

All compounds from phenyltin(IV) dithiocarbamate were prepared using direct reaction by mixing of metal and ligand with 1:2 molar ratio. The ligand was prepared by the addition of carbon disulphide, CS_2 to an ethanolic solution of respective amine in the same molar ratio. The mixture was stirred for one hour at 277 K. After one hour of stirring, the solution was added dropwise to a solution of phenyltin(IV) trichloride in 20 mL of ethanol. The mixture was stirred again for one hour at same temperature and the white precipitate was filtered and washed with cold ethanol and dried in desiccator. Recrystallization was carried out by using mixture of methanol:dichloromethane at ratio 1:2. The general reaction between secondary amine, carbon disulphide and phenyltin(IV) trichloride was illustrated in Figure 1.



(R' = CH_3, C_2H_5, iC_3H_7, C_7H_7 and R" = C_2H_5, C_6H_{11}, iC_3H_7, C_7H_7,)

Figure 1. General reaction between secondary amine, carbon disulphide and phenyltin(IV) trichloride.

Synthesis of $PhSnCl[S_2CN(CH_3)(C_2H_5)]_2$ (1)

An ethanolic solution of N-ethylmethylamine (1.3 mL, 15 mmol) was stirred for an hour together with CS_2 (0.91 mL, 15 mmol). This ligand was treated with phenyltin(IV) trichloride (0.82 g, 5 mmol) in 20 mL of ethanol. The solution was stirred at temperature below 277 K for another 1 hour to obtain a white precipitate. The product was vacuum filtered, washed with cold ethanol and dried in desiccator. The compound $PhSnCl[S_2CN(CH_3)(C_2H_5)]_2$ was white in colour. Yield: 89%, m.p. 86-88 ˚C. Elemental Analysis %: Anal.(calculated): C, 33.54 (3.51); H, 3.64 (4.62); N, 5.27 (5.58); S, 23.03 (25.56); Sn, 21.56 (23.66). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.8288-7.2500 m (Sn–C_6H_5); 3.3037 s (N–CH_3); 3.7717 q (Et–CH_2); 1.2412 t (Et–CH_3).^{13}C NMR ($CDCl_3$, ppm): δ_C 157.2694, 130.7985, 128.6500, 128.2605 (Sn–C_6H_5); 42.7535 (N–CH_3); 11.8327 (Et–CH_3); 53.5311 (Et–CH_2); 201.6076 (NCS_2). IR (KBr, cm^{-1}): 1511(C–N), 997, 957(C-S), 318(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max} : 254 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(CH_3)(C_6H_{11})]_2$ (2)

The synthesis of compound **2** was prepared by using the same procedure as the preparation of compound **1** with N-methylcyclohexylamine (1.98 mL, 15 mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(CH_3)(C_6H_{11})]_2$ was white in colour. Yield: 80%, m.p. 94-96 ˚C. Elemental Analysis %: Anal.(calculated): C, 43.40 (43.32); H, 5.63 (5.78); N, 5.38 (5.59); S, 20.24 (21.03); Sn, 18.42 (19.46). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.4437-7.2505 m (Sn–C_6H_5); 4.3847 m (Cy–CH), 3.2123 s (N–CH_3); 1.8843-1.3531 m (Cy–CH_2). ^{13}C NMR ($CDCl_3$, ppm): δ_C 156.2448, 135.7838, 131.6549, 128.3136 (Sn–C_6H_5); 36.7666 (N–CH_3); 66.2883 (Cy–CH); 30.0601, 25.3663, 24.1751 (Cy–CH_2); 197.1821 (NCS_2). IR (KBr, cm^{-1}): 1481(C–N), 970(C-S), 320(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max}: 253 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(C_2H_5)(C_6H_{11})]_2$ (3)

The synthesis of compound **3** was also prepared from the same procedure as the preparation of compound **1** with N-ethylcyclohexylamine (2.24 mL, 15 mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(C_2H_5)(C_6H_{11})]_2$ was white in colour. Yield: 83%, m.p. 98-100˚C. Elemental Analysis %: Anal.(calculated): C, 45.28 (45.18); H, 6.03 (6.16); N, 5.06 (4.39); S, 18.28 (20.10); Sn, 17.42 (18.61). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.4740-7.2844 m (Sn–C_6H_5); 1.2853 t (Et–CH_3); 3.6941 q (Et–CH_2); 4.3847 m (Cy–CH), 1.9685-1.3210 m (Cy–CH_2). ^{13}C NMR ($CDCl_3$, ppm): δ_C 156.3519, 135.8144, 131.6778, 128.2983 (Sn–C_6H_5); 13.7824 (Et–CH_3); 45.3762 (Et–CH_2); 30.7033, 25.2363, 25.6874 (Cy–CH_2); 66.8847 (Cy–CH); 196.9680 (NCS_2). IR (KBr, cm^{-1}): 1477(C–N), 995(C-S) 318(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max}: 256 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(CH_3)(iC_3H_7)]_2$ (4)

The synthesis of compound **4** was prepared from the same procedure as the preparation of compound **1** with N-methylisopropylamine (1.56 mL, 15 mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(CH_3)(iC_3H_7)]_2$ was white in colour. Yield: 79%, m.p. 104-106˚C. Elemental Analysis %: Anal.(calculated): C, 35.48 (36.27); H, 4.51 (5.14); N, 6.34 (5.29); S, 22.64 (24.21); Sn, 21.37 (22.41). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.9085-7.2528 m (Sn–C_6H_5); 3.1571 s (N–CH_3); 1.2284 d (*i*-Pr–CH_3); 4.8478 m (*i*-Pr–CH). ^{13}C NMR ($CDCl_3$, ppm): δ_C 156.1989, 135.8067, 131.6855, 128.3411 (Sn–C_6H_5); 35.5356 (N–CH_3); 19.6546 (*i*-Pr–CH_3); 58.0993 (*i*-Pr–CH); 196.9680 (NCS_2). IR (KBr, cm^{-1}): 1484(C–N), 967(C-S), 320(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max} : 247 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$(5)

The synthesis of compound **5** was prepared from the same procedure as the preparation of compound **1** with N-ethylisopropylamine (1.82 mL, 15 mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$ was white in colour. Yield: 81%, m.p. 130-132 ˚C. Elemental Analysis %: Anal.(calculated): C, 37.79 (38.75); H, 5.76 (5.60); N, 5.32 (5.02); S, 20.32 (22.99); Sn, 20.56 (21.28). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.8810-7.2931 m (Sn–C_6H_5); 1.3081 t (Et–CH_3); 3.6279 qt (Et–CH_2); 4.7956 m (*i*-Pr–CH); 1.2467 d (*i*-Pr–CH_3).^{13}C NMR ($CDCl_3$, ppm): δ_C 156.2830, 135.7991, 131.6626, 128.3289 (Sn–C_6H_5); 13.8130 (Et–CH_3); 44.4510 (Et–CH_2); 20.3504 (*i*-Pr–CH_3); 58.6804 (*i*-Pr–CH); 197.2356 (NCS_2). IR (KBr, cm^{-1}): 1480(C–N), 991(C-S), 328(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max} : 253 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(CH_3)(C_7H_7)]_2$ (6)

The synthesis of compound **6** was prepared using the same procedure as the preparation of compound **1** with N-methylbenzylamine (1.93 mL, 15 mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(CH_3)(C_7H_7)]_2$ was white in colour. Yield: 72%, m.p. 156-158 ˚C. Elemental Analysis %: Anal.(calculated): C, 45.17 (46.05); H, 4.58 (4.35); N, 3.93 (4.48); S, 18.24 (20.49); Sn, 16.46 (18.97). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.3901-7.2537 m (Sn–C_6H_5); 3.2744 s (N–CH_3); 4.9733 s (Ben–CH_2); 7.3901-7.2537 m (Ben-C_6H_5). ^{13}C NMR ($CDCl_3$, ppm): δ_C 155.8319, 133.9182, 131.6396, 127.9711 (Sn–C_6H_5); 43.1511 (N–CH_3); 62.4805 (Ben–CH_2); 133.9182, 131.6396, 129.2082, 127.9711 (Ben–C_6H_5); 196.9347 (NCS_2). IR (KBr, cm^{-1}): 1511(C–N), 964(C-S), 320(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max}: 255 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(C_2H_5)(C_7H_7)]_2$ (7)

The synthesis of compound **7** was also prepared from the same procedure as the preparation of compound **1** with N-ethylbenzylamine (2.20 mL, 15 mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(C_2H_5)(C_7H_7)]_2$ was white in colour. Yield: 76 %, m.p. 161-163˚C. Elemental Analysis %: Anal.(calculated): C, 48.31 (47.75); H, 4.20 (4.78); N, 4.81 (4.28); S, 17.61 (19.61); Sn, 17.54 (18.15). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.9680-7.2802 m (Sn–C_6H_5); 1.2485 t (Et–CH_3); 3.7131 qt (Et–CH_2); 4.9678 s (Ben–CH_2); 7.9680-7.2802 m (Ben-C_6H_5). ^{13}C NMR ($CDCl_3$, ppm): δ_C 11.8097 (Et-CH_2); 50.6979 (Et–CH_3); 155.9722, 135.3012, 129.1317, 127.9160 (Sn–C_6H_5); 59.3762 (Ben–CH_2); 134.1399, 131.6549, 129.1317, 127.9160 (Ben–C_6H_5); 199.2466 (NCS_2). IR (KBr, cm^{-1}): 1491(C–N), 974(C-S),322(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max}: 256 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(C_7H_7)(iC_3H_7)]_2$ (8)

The synthesis of compound **8** was prepared from the same procedure as the preparation of compound **1** with N-benzylisopropylamine (2.51 mL, 15mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(C_7H_7)(iC_3H_7)]_2$ was white in colour. Yield: 73%, m.p. 183-185˚C. Elemental Analysis %: Anal.(calculated): C, 49.19 (49.31); H, 5.79 (5.17); N, 4.52 (4.11); S, 16.62 (18.81); Sn, 16.42 (17.41). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.660-7.199 m (Sn–C_6H_5); 5.010 s (Ben–CH_2); 7.660-7.199 m (Ben-C_6H_5); 1.208 d (*i*-Pr–CH_3); 4.987 m (*i*-Pr–CH). ^{13}C NMR ($CDCl_3$, ppm): δ_C 155.7172, 130.4239, 128.8106, 126.6161 (Sn–C_6H_5); 20.6176 (*i*-Pr–CH_3); 53.1599 (*i*-Pr–CH); 58.9327 (Ben–CH_2); 135.7142, 130.4239, 128.8106, 121.6161 (Ben–C_6H_5); 200.2405 (NCS_2). IR (KBr, cm^{-1}): 1458(C–N), 995, 964(C-S), 324(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max} : 256 (π-π* transition for NCS_2).

Synthesis of $PhSnCl[S_2CN(C_7H_7)_2]_2$ (9)

The synthesis of compound **9** was prepared from the same procedure as the preparation of compound **1** with N-dibenzylamine (2.88 mL, 15 mmol) was used instead of N-ethylmethylamine. The compound $PhSnCl[S_2CN(C_7H_7)_2]_2$ was white in colour. Yield: 71%, m.p. 202-204˚C. Elemental Analysis %: Anal.(calculated): C, 56.65 (55.57); H, 6.51 (4.53); N, 3.59 (3.60); S, 14.34 (16.48); Sn, 14.37 (15.26). ^{1}H NMR ($CDCl_3$, ppm): δ_H 7.4928-7.3123 m (Sn–C_6H_5); 4.9275 s (Ben–CH_2); 7.4928-7.3123 m (Ben-C_6H_5).^{13}C NMR ($CDCl_3$, ppm): δ_C 155.6943, 133.8304, 129.2464, 128.1377 (Sn–C_6H_5); 57.8393 (Ben–CH_2); 133.8304, 131.6931, 129.2464, 128.1377 (Ben–C_6H_5); 201.4028 (NCS_2).IR (KBr, cm^{-1}): 1486(C–N), 994(C-S), 326(Sn–S). UV–Vis ($CHCl_3$, nm) λ_{max} : 257 (π-π* transition for NCS_2).

Crystallographic studies

Suitable crystal for X-Ray analysis of compound **4** was obtained by slow evaporation of the mixture of ethanol and chloroform solution with ratio 1:2. Data collection: APEX2; cell refinement: SAINT; data reduction: SAINT; programmes used to solve structure: SHELXS97; programs used to refine structure: SHELXL97; molecular graphics: XSEED; software used to prepare material for publication.

Cytotoxicity Test (MTT assay)

A dose response cytotoxicity of compounds **1**, **2**, **5**, **8** and **9** were evaluated in Chang liver cells. The cells were treated with the concentration of 6.25, 12.5, 25, 50, 100 and 200 µg/mL of the test substance or compounds for 24 hours. The cyctotoxicity was determined by assessing the cell viability through the reduction of tetrazolium salts (MTT). Cell viability was obtained by dividing the mean optical density (OD) values of the test substances with the mean OD of negative control and multiplied by 100.

Minimum inhibitory concentration (MIC) and minimum bacteria concentrations (MBC) of the compounds

Compounds **1**, **2**, **5**, **8** and **9** were tested for antibacterial activities by the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) methods. Microorganism were tested for their ability to produce visible growth on microplate wells of broth and dilutions of the compounds from the concentration of 20.00, 10.00, 5.00, 2.50, 1.25, 0.63, 0.31 0.16 and 0.08 mg/mL. MIC was determined by the lowest concentration of selected compounds (antimicrobial agent) that will inhibit the visible growth of microorganism. MBC was carried out by using the sample with no visible growth from the same plates used for MIC test. The sample were subcultured on nutrient agar medium and incubated at 37^{o}C for 24 hours. MBC was taken as the lowest concentration of compounds at which all bacteria are killed. The test microorganism chosen for the antibacterial activities each two from gram positive and gram negative were *S.epidermis, S aureus, E.coli and P.aeruginosa*.

Results and Discussion

Nine new series of phenyl dithiocarbamate compounds with the general formula $PhSnCl[S_2CNR'R'']_2$ (R' = CH_3, C_2H_5, C_7H_7 and R" = C_2H_5, C_6H_{11}, iC_3H_7, C_7H_7) had been successfully prepared by using *in-situ* method between various amine with CS_2 in alkaline solution. All compounds were stable with the melting point ranging from 86 to 202 ˚C and yield was above 70%. These compounds were easily soluble in the mixture of dichloromethane and methanol with ratio 2:1. A strong to moderate signal of infrared spectra for dithiocarbamate compounds focused in three main regions to identify structural differences in each compounds which are ν(C$\equiv$S), ν(C$\equiv$N) and Sn-S bond absorption [16]. The band for ν(C$\equiv$S) or also known as thiocarbonyl group were detected in the range of 957-1098 cm^{-1}. Vibrations in these ranges have been used effectively to differentiate between monodentate and bidentate dithiocarbamate ligands [17]. A single peak in this range showed that the ligand coordinated to metal atom through sulfur donor in symmetrical bidentate fashion. Meanwhile, having a splitted peak about 20 cm^{-1} was because due to the asymmetric bidentate fashion of S-C-S fragment [18]. Only single peak was detected for all compounds except **1**, **3** and **8** in the range of 967-994 cm^{-1} to assume a completely symmetrically bonding of the dithiocarbamate ligand. Acting in a bidentate fashion followed by compounds **1**, **3** and **8** that having doublet peak which implying the unsymmetrical bound bidentate ligand. Coordination via the C-S group through complexation is proven by the shifting of this band to the higher frequency [19]. A thioureide band or ν(C$\equiv$N) band can be seen in the region 1480-1511 cm^{-1} which suggested a considerable double bond character in the C-N bond vibration of the S_2C–NR_2 group and due to the presence of electron rich sulfur atoms that delocalization of electrons occurs over the

entire NCS_2 region [20]. The Sn-S band were detected at lower frequencies in far infrared region (318-328 cm^{-1}) to indicate the existence of metal-ligand bond [21].

^1H NMR showed an expected pattern for all of the compounds confirming the formation of dithiocarbamates compounds especially for the resonance frequency. A chemical shift range from δ_H 7.9689-7.1990 ppm was attributed for phenyl group at the metal centre region. The deshielding of proton from CH-N group was due to the releasing of electron from nitrogen atom to the sulfur atom via thioureide to form compoundation [22]. The data of ^{13}C chemical shifts of NCS_2 moiety appeared between δ_C 196.9347 and 201.6076 ppm. A signal in this region was observed due to presence of NCS_2 carbon atoms of dithiocarbamate moieties [23]. The increase of double bond character in NCS_2 moiety and the promotion of unshared electron pair in nitrogen atom of dithiocarbamate to the metal atom caused the high value of NCS_2 chemical shifts [24].

The UV-visible absorption spectra of all compounds were recorded in chlorofom, 1 cm^3 cuvette with concentration $1x10^{-5}$ M to 1×10^{-3} M. In general, all compounds exhibited strong absorptions at λ_{max} 247-257 nm due to intramolecular π-π* transition of N ═══ **C** ═══ S group [25]. This absorption showed the existence of NCS_2 group in each compounds [26]. Absorption for NCS_2 group that involve in complexation were shifted to a shorter wavelength [27].

Suitable single crystal of compound **5** was obtained by slow evaporation process of dichloromethane: methanol 2:1 mixture and it took approximately about five days at room temperature to produce good crystal for X-ray diffraction analysis. The ORTEP plot of $PhSnCl[S_2CN(Et)(i\text{-}Pr)]_2$**(5)** at the 50% probability level is shown in Figure 2. The crystallographic data and refinement detail for compound **5** are given in the Table 1 and selected geometric parameter (Å, °) is listed in Table 2.

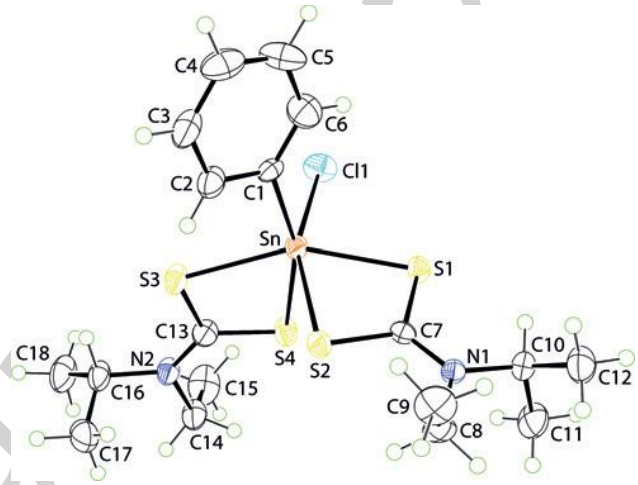


Figure 2. ORTEP plot of $PhSn[S_2CN(Et)(i\text{-}Pr)]_2Cl$ at the 50% probability level.

By looking at the ORTEP plot, S1 is *cis* to S4 and S3 is *cis* to the *ipso*-C atom and this compound are six coordinated bonded with two chelating dithiocarbamate ligands, methyl-C and chloride atom thus form a distorted octahedral geometry. Dithiocarbamate ligands are coordinated to central tin atom in bidentate fashion proven by the almost similar Sn-S bond length which Sn1-S1 = 2.5191(11) Å and Sn1-S2 = 2.6012(12)Å. The differences in Sn-S bond length, Δ(Sn–S) = Sn–S(long) – Sn–S(short) for both S1 and S3 dithiocarbamate ligand are 0.082 Å and 0.004 Å respectively. The decreased of Sn-S bond length may caused by the presence of more electronegative phenyl substituent. The Sn-S stronger bonds formed in this compound due to the enhances of Lewis acidity in tin atom caused by the reduction of electron density. This compound matches with the prototype structure of $PhSn(S_2Cpyrrole)_2Cl$ that had been reported before, whereby one dithiocarbamate ligand is opposite to the organic substituent and the second one is opposite to the chloride atom [28]. The bond length in this compound almost

similar to the previous reported compounds such as $PhSn[S_2CN(R)_2]_2Cl$ (R = Et, *n*-Bu, *i*-Bu) in which Sn-S bond length are quite symmetric and fall in the range of 2.5-2.6 Å and 2.43-2.48 Å for Sn-Cl bond [29-31].

Table 1. Crystal data for compound $PhSnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$ **(5)**

Compound	$PhSnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$ **(5)**
Empirical Formula	$C_{18}H_{29}ClN_2S_4Sn$
Formula weight	555.81
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	13.3542(18)
b (Å)	10.0355(14)
c (Å)	18.257(3)
α (˚)	90
β (˚)	92.904(2)
γ (˚)	90
V ($Å^3$)	2443.6(6)
Z	4
D/Mgm^{-3}	1.511
μ (mm^{-1})	1.503
F (000)	1128
Temperature (K)	173
θ range (˚)	2.2 – 26.4
Final R indices [$I > 2\sigma(I)$]	3536

Table 2. Selected geometric parameter (Å, °) for $PhSnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$ **(5)**

Compounds	$PhSnCl[S_2CN(Et)(i\text{-}Pr)]_2$ **(5)**
Sn-S1	2.5191(11)
Sn-S2	2.6012(12)
Sn-S3	2.5682(11)
Sn-S4	2.5724(12)
Sn-Cl	2.4550(12)
C-Sn-Cl	93.18(12)
S1-Sn-S4	90.30(4)
S2-Sn-Cl	158.02(4)
S3-Sn-C	92.04(11)

The basis of choosing selected compounds to be applied in the biological studies was based on the different alkyl or aryl group bonded to the nitrogen group. The different substituent group varies from methyl, ethyl, cyclohexyl, isopropyl and benzyl group. The initial theory may indicate that different substituent group may give a different result in biological studies. Cytoxicity study on selected compounds i.e. **1**, **2**, **5**, **8** and **9** were tested againts Chang liver cells. The cytotoxicity were determined by assessing the cell viability through the reduction of tetrazolium salts (MTT). The study had been carried out and all the selected compounds did not inhibit the viability of Chang liver cells following 24 hours exposure. Thus proved that these compounds did not demonstrate any cytotoxic effect at all concentrations under the condition of the study. *Escherichia coli* and *Pseudomonasaeruginosa* as negative

bacteria together with *Staphylococcus aureus* and *Staphylococcus epidermis* as positive bacteria were used for the minimun inhibition concentration (MIC) and minimum bactericidal concentrations (MBC) test. *Penincillin* and *Kanamycin* were used as commercial antibacterial agent each for gram positive and gram negative bacteria respectively. The result of these two test for compounds **1**, **2**, **5**, **8** and **9** were evaluated and presented in Table 3. It showed that compound **1** was active compared to the other compounds by having the lowest MIC values of 1.25 mg/mL and MBC value of 5.0 mg/mL against *E. Coli* and *S. Aureus* bacteria. Compounds **8** and **9** that having a benzyl group showed their MIC and MBC slightly higher compared to compounds **1**, **2** and **5** againts certain bacteria indicating their low antibacterial activities. The reasons behind a non cytotoxic effect in these compounds may agreed with the previous research reported that triorganotin compounds have the highest toxicity effect followed by diorganotin and while monoorganotin compounds having the lowest effects. The number of organic group attached to the tin atom together with their nature playing an important role to distinguish their cytotoxic effect. Organic ligand having ethyl group is the most toxic followed by methyl group, propyl, phenyl and hexyl group [32]. This explains why compound **1** that has ethyl methyl dithiocarbamate ligand was the most toxic compound for MIC and MBC test.

Table 3. MIC and MBC values (mg/mL) for dithiocarbamate compounds.

No	**Compounds**	***E. coli***		***P. aeruginosa***		***S. epidermidis***		***S. aureus***	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	$(C_6H_5)SnCl[S_2CN(CH_3)(C_2H_5)]_2$	1.25	5.0	2.5	>20.0	5.0	5.0	1.25	5.0
2	$(C_6H_5)SnCl[S_2CN(CH_3)(C_6H_{11})]_2$	5.0	>20.0	10.0	>20.0	10.0	20.0	5.0	10.0
5	$(C_6H_5)SnCl[S_2CN(C_2H_5)(iC_3H_7)]_2$	5.0	>20.0	5.0	>20.0	5.0	20.0	5.0	10.0
8	$(C_6H_5)SnCl[S_2CN(C_7H_7)(iC_3H_7)]_2$	>20.0	ND	20.0	>20.0	>20.0	ND	5.0	>20.0
9	$(C_6H_5)SnCl[S_2CN(C_7H_7)_2]_2$	>20.0	ND	2.5	10.0	20.0	20.0	10.0	10.0

Conclusion

Nine new phenyltin(IV) dithiocarbamate were successfully synthesized and characterized. The crystal structure of $PhSnCl[S_2CN(Et)(iPr)]_2$ (5) showed that this compound was six coordinated bonded with two chelating dithiocarbamate ligands, methyl-C and chloride atom thus form a distorted octahedral geometry. Compounds **1**, **2**, **5**, **8** and **9** did not demonstrate any cytotoxic effect againts Chang liver cell. Whilst compound **1** showed the highest antibacterial activities by having the lowest MIC and MBC values againts *E. Coli* and *S. Aureus* bacteria respectively.

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