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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (1): 12-21, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.12.21

Synthesis, Characterization and Crystal Structure of Organotin(IV) *N*-Butyl-*N*-Phenyldithiocarbamate Compounds and their Cytotoxicity in Human Leukemia Cell Lines

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Abstract: Organotin complexes are recognized as the biologically active compounds in inducing cancerous cells death at very low doses. To date, organotin compounds currently appear among the most potent candidates in research related to the new anticancer drugs. In this study, new organotin(IV) *N*-butyl-*N*-phenyldithiocarbamate compounds have been successfully synthesized between the reaction of *N*-butylaniline amine with organotin(IV) chloride in 1:2/1:1 molar ratio. All compounds were characterized using the elemental analysis, FT-IR and NMR spectroscopy. The single crystal structure was determined by X-ray single crystal analysis. The elemental analysis showed good agreement with the suggested formula $(C_4H_9)_2Sn[S_2CN(C_4H_9)(C_6H_5)]_2$ (Compound 1 and 2), $(C_6H_5)_2Sn[S_2CN(C_4H_9)(C_6H_5)]_2$ (Compound 3) and $(C_6H_5)_3Sn[S_2CN(C_4H_9)(C_6H_5)]$ (Compound 4). The important infrared absorbance peaks, ν (C = N) and ν(C = S) were detected in range between 1457-1489 cm^{-1} and 951-996 cm^{-1}, respectively. The chemical shift of carbon in NCS_2 group obtained from ^{13}C NMR was found in range 198.86-203.53 ppm. The crystal structure of compound 4 showed that the dithiocarbamate ligand coordinates in a monodentate fashion. It crystallized in monoclinic $P2_1/n$ space group with the crystal cell parameter: a = 10.0488(1)Å, b = 18.0008(2)Å, c = 15.2054(2)Å, β = 102.442(1)° and R = 0.044. The cytotoxicity (IC_{50}) of these compounds against Jurkat E6.1 and K-562 leukemia cells were in the range between 0.4-0.8 and 1.8-5.3 μM, respectively as assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazholium bromide (MTT) assay. In conclusion, our study demonstrate that all compounds showed potent cytotoxicity towards both cell lines tested with the triphenyltin(IV) compound displayed the greatest effect.

Key words: Organotin(IV), dithiocarbamates, butylphenyldithiocarbamate, cytotoxicity, anticancer, leukemia

INTRODUCTION

The introduction of cisplatin as the first inorganic compound approved for clinical use in 1978 obviously gives a great impact in the treatment of cancer and pioneering the use of metal compounds in the cancer treatment (Cepeda *et al*., 2007; Galluzzi *et al*., 2011). Nowadays, cisplatin has been widely employed due to its significant clinical activity in various kinds of solid neoplasms including bladder, testicular, ovarian, head and neck, esophageal, stomach, breast and cervical cancers (Florea and Busselberg, 2011). Unfortunately, the use of cisplatin was also identified to be associated with several disadvantages such as the emergence of cisplatin resistance (Basu Baul *et al*., 2010; Galluzzi *et al*., 2011) and certain side effects primarily nephrotoxicity, neurotoxicity and ototoxicity in treated patients. Hence, carboplatin and oxalipatin, which are also platinum derivative drugs consequently being introduced in 1989 and 2002 (Galluzzi *et al*., 2011). To date, cisplatin and its analogue,

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carboplatin are amongst the most commonly used antitumor drugs today (Cepeda *et al*., 2007).

Besides Pt complexes, other organometallic compounds derived from Sn, Au, Ti, Cu, Pd and Ru have emerged and most of these compounds were found to exhibit potent and comparable cytotoxic properties in different trend of antitumor specificities (Alama *et al*., 2009; Kopf-Maier, 1994). Among the metal complexes with antitumor activity, tin (Sn) derivatives were identified to appear very promising as anticancer drugs (Alama *et al*., 2009). Many studies currently show that the organotin compounds induce cell death at very low doses and have better or similar potential than the drugs that have been approved for clinical use (Yamaguchi *et al*., 2007; Alama *et al*., 2009; Du *et al*., 2011; Ruan *et al*., 2011). Generally, organotin compounds are coordinated to the ligand through atoms or groups that bound to the central metallic atom (Gold *et al*., 1987). This has produced numerous di- and triorganotin(IV) derivatives complexes with strong anti-proliferative effects against various types of cancer cell lines (Gielen and Tiekink, 2005). Interesting findings from previous research have boost up our curiosity to study the potential of organotin(IV) coordinated to dithiocarbamate ligand since dithiocarbamate applications in various fields including chemistry and toxicology have been renowned (Nieuwenhuizen *et al*., 1997; Kaludjerovic *et al*., 2002; Heard, 2005; Robertson and Orrenius, 2000; Li *et al*., 2007).

In inorganic chemistry, dithiocarbamates ($R_2NCS_2^-$) are known as versatile ligands with capability to chelate with various types of metals. Its chelating properties to the metals are greatly influenced by the possession of two donor sulphur atoms present in the ligand (Awang *et al*., 2011a). The formation of bonds linking to the corresponding metal atom allows dithiocarbamates to act as monodentate, bidentate chelating or bidentate bridging ligands (Onwudiwe and Ajibade, 2010), in which these binding properties will determine the structural organization of the resulting metal complexes (Xu *et al*. 2001). In terms of solubility, metal dithiocarbamate complexes are difficult to dissolve in water but can dissolve very well in organic solvents. This property contributes to the lipophilic characteristic which will facilitate the complexes across the cell membrane and leading to the intracellular interaction (Cvek and Dvorak, 2007). This has been proven by previous research which reveal the potential of organometallic dithiocarbamate in inducing cytotoxicity (Nobel *et al*. 1995; Chen *et al*. 2000; Morais *et al*., 2006; Cvek and Dvorak, 2007; Milacic *et al*. 2008; Cao *et al*., 2010).

Our recent study mainly focusing on the assessment of organotin (IV) dithiocarbamate compounds with the intention to be developed as new anticancer drugs. In this paper, we present the synthesis of a new series of compounds where the *N*-butyl-*N*-phenyldithiocarbamate ligands were substituted with dibutyltin(IV), di-tert-butyltin(IV), diphenyltin(IV) and triphenyltin(IV). The general formula for these compounds is $R_nSn[S_2CN(C_4H_9)(C_6H_5)]_{4-n}$ (where R = Bu and Ph for n = 2; R = Ph for n = 3). All compounds were characterized using the elemental analysis and spectroscopic techniques. Single crystal structure obtained from compound 4 was determined by X-ray single crystal analysis. The preliminary in vitro cytotoxicity test was conducted against two types of leukemia cell lines, Jurkat E6.1 and K-562.

MATERIALS AND METHODS

Materials: *N*-butylaniline, organotin(IV) chloride, MTT salt and doxorubicin hydrochloride were purchased from Sigma, USA, whereas ethyl alcohol and chloroform were supplied by Merck. DMSO was obtained from Fisher Scientific, UK. Ammonia solution (25%) was purchased from BDH Chemicals, whereas carbon disulphide was provided by Panreac (Spain). All reagents and chemicals were used as received without further purification.

Physical measurements: The melting points of each compound were determined using Electrothermal IA 9100. Carbon, hydrogen, nitrogen and sulphur elemental analysis were performed with Fison EA 1108. FT-IR spectrophotometer with samples investigated as KBr discs. IR spectra in range 4000-400 cm^{-1} were recorded using a Perkin Elmer GX spectrophotometer. The ^1H and ^{13}C NMR spectra were obtained on Joel JNM-LA 400 in $CdCl_3$ using TMS as internal standard. Chemical shifts are given in ppm (δ scale) relative to tetramethylsilane. All measurements were conducted at School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia. The X-ray single structure determination was recorded using Bruker SMART CCD. This analysis was done at Universiti Putra Malaysia.

Synthesis of compound 1-4: All compounds were prepared using direct reaction method. To a mixture of ethanolic solution consisting 30 mM of *N*-butylaniline and a little amount of ammonia solution (12 mL) at temperature below 4°C, ice cold of carbon disulphide (30 mM) was added. The yellowish mixture was stirred for 6-8 h. After 6-8 h of stirring, the organotin(IV) chloride solution was added to the mixture and stirred for another 3 h. The white precipitate formed was filtered and rinsed several times

with cold ethanol. Then, the precipitate was dried in vacuo over silica gel. The yield and melting points of each synthesized compounds were recorded.

Recrystallization and crystallography study: Recrystallization process was carried out by dissolving the compound with chloroform and ethanol in 1:1 v/v ratio. This mixture was allowed to slowly evaporate at room temperature for 3-4 days. The crystals formed were carefully collected and the structural determination was done using X-ray analysis. Data collection: CrysAlis PRO (Oxford Diffraction, 2010). Cell refinement: CrysAlis PRO. Data reduction: CrysAlis PRO. The program used to solve the structure: SHELXS97 (Sheldrick, 2008). The program used to refine the structure: SHELXL97 (Sheldrick, 2008). Molecular graphics: ORTEP-3 (Farrugia, 1997) and DIAMOND (Brandenburg, 2006).

Cells and cell culture: Acute T cell leukemia (Jurkat E6.1) and chronic myelogenus leukemia (K-562) cell lines of human origins were purchased from American Type Culture Collection (ATCC). Briefly, Jurkat E6.1 and K-562 cell lines were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) (Gibco, USA) and Iscove's Modified Dulbecco's Medium (IMDM) (Sigma-Aldrich, USA), respectively. Both media were supplemented with 1 % penicillin/streptomycin (PAA Laboratories, GmbH) and 10% fetal bovine serum (JR Scientific, USA). Cells were maintained at 37°C with humidified atmosphere containing 5% CO_2. Subculturing of cells was done according to protocol provided by ATCC.

Cytotoxicity screening: The synthesized compounds were screened against both cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazholium bromide (MTT) assay as described by Mosmann (1983). The serial dilution method was performed in each compound up to seven concentrations using 10 μM as the highest concentration. Doxorubicin hydrochloride was used as positive control whereas non-treated cells represented as negative control. Cells Jurkat E6.1 (1×10^6 cells mL^{-1}) and K-562 (5×10^5 cells mL^{-1}) were seeded in 96-well plate. Each treatment was established as triplicate. Following 24 h of incubation (37°C in 5% CO_2), 20 μL of MTT (5 mg mL^{-1} in PBS solution) were added into each well in dark condition. The cells were then further incubated for 4 h. After 4 h, 180 μL of supernatant in each well was removed and 180 μL DMSO was added to dissolve formazan crystals formed. After 15 to 20 min of incubation, the cytotoxicity of compounds were detected using ELISA microplate reader (iMark) at 570 nm. The mean absorbance of each compound concentrations and untreated cells were expressed as percentage. The graph were plotted as percentage of viable cells versus compound concentrations. The IC_{50} values were determined based on the plotted graphs where the IC_{50} values represent the reduction of 50% cells population in treated cells comparing to untreated cells.

RESULTS

Synthesis of compound 1-4: All compounds were successfully synthesized using *in situ* method. The general reaction scheme of diorganotin (IV) butylphenyldithiocarbamate preparation was given in Fig. 1. The physical and analytical data for compound 1-4 were given in Table 1.

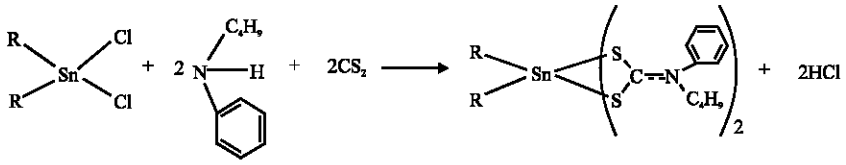

Fig. 1: The general reaction between N-butylaniline amine, carbon disulfide and diorganotin (IV) dichloride

Table 1: Physical and elemental analysis data of organotin (IV) butylphenyldithiocarbamate compounds

				Found (calculated) (%)			
Molecular formula	Colour	Yield (%)	Melting point (°C)	C	H	N	S
$(C_4H_9)_2Sn[S_2CN(C_4H_9)(C_6H_5)]_2$	White	71.0	72.1-73.8	53.47	7.67	4.00	16.52
(Compound 1)				52.86	6.80	4.11	18.82
$(C_4H_9)_2Sn[S_2CN(C_4H_9)(C_6H_5)]_2$	White	47.3	114.6-115.3	49.03	7.02	3.94	17.62
(Compound 2)				52.86	6.80	4.11	18.82
$(C_6H_5)_2Sn[S_2CN(C_4H_9)(C_6H_5)]_2$	White	82.1	102.1-104.0	56.15	5.89	3.77	16.81
(Compound 3)				56.59	5.31	3.88	17.77
$(C_6H_5)_3Sn[S_2CN(C_4H_9)(C_6H_5)]$	White	34.0	101.0-102.0	60.54	5.08	2.34	11.31
(Compound 4)				60.64	5.09	2.44	11.17

Spectroscopic analysis: The important absorbance peaks obtained from FT-IR spectrophotometer for compound 1-4 were showed in Table 2. Dithiocarbamate compounds can be identified via the presence of certain absorbance peaks primarily v(C- - N) and v(C- - S). The vibration of v(C- - N), also known as "thioureida band" and v(C- - S) of all compounds were observed in range between 1457-1489 cm^{-1} and 951 – 996 cm^{-1}, respectively. Other than the stretching between C with N and S elements, another important peak observed specifically in organotin (IV) compounds is v(Sn-C). The absorbance peaks within the range of 567-597 and 258-272 cm^{-1} indicate the stretching of Sn-C elements for compounds with alkyl and aryl groups, respectively. Based on the IR spectra recorded from the FT-IR analysis, all synthesized compounds showed the presence of those significant absorbance peaks, thus confirmed the formation of suggested compounds.

Table 2: Important infrared absorption bands of compound 1-4

Compounds	v(C-H)	v(C- - N)	v(N-C)	v(C- - S)	v(Sn-C)
1	2927.84	1487.42	1136.37	951.14	567.97
2	2955.77	1489.74	1138.85	959.81	597.45
3	2955.95	1457.81	1137.32	996.86	258.69
4	2960.37	1478.81	1138.65	996.96	272.92

Table 3: The ^{13}C NMR chemical shifts (δ, ppm) of compound 1-4

Compounds	CS_2	N-C_4H_9	N-C_6H_5
1	203.53	58.31	145.40
2	202.83	58.58	145.78
3	202.03	59.53	150.32
4	198.86	59.77	145.69

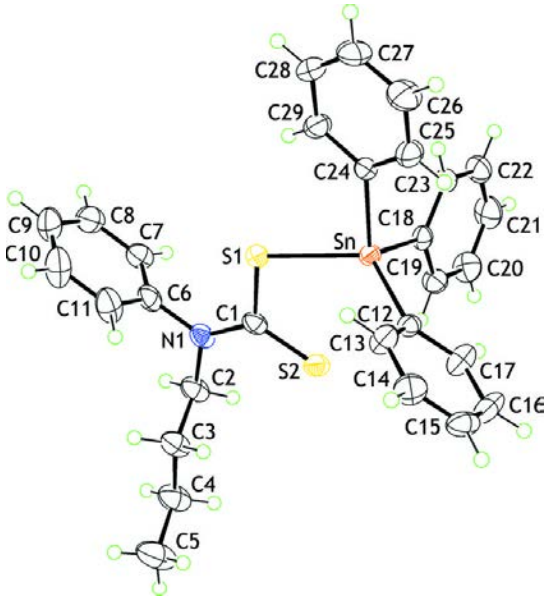

Fig. 2: ORTEP plot of compound 4 at the 50% probability level

In $CdCl_3$ solution, the ^{13}C NMR spectra exhibited the expected signals indicate the presence of significant bonds between the elements in the compounds as well as the CS_2 peak that characterize the existence of dithiocarbamate (-NCS_2) group. The important peaks observed in ^{13}C NMR spectra have been shown in Table 3.

Recrystallization and crystallography study: Single crystal of compound 4 obtained by slow evaporation of ethanol and chloroform mixture in room temperature was analyzed using X-ray analysis. The final R ($I>2/\sigma(I)$) was 0.044. The details of the crystal data including the refinement and selected geometric parameters are summarized in Table 4 and 5, respectively. Figure 2 shows the molecular structure of compound 4 showing the displacement ellipsoids at the 50% probability level, whereas Fig. 3 displays the crystal packing of compound 4 which shows the helical supramolecular chains mediated by C-H.....S interactions.

Table 4: Crystallographic data and refinement parameters of compound 4

Parameters	Compound 4
Empirical Formula	$C_{29}H_{29}NS_2Sn$
Formula weight	574.34
Crystal system	Monoclinic
Space group	$P2_1/n$
Crystal size (mm)	0.24 × 0.22 × 0.10
a (Å)	10.0488 (1)
b (Å)	18.0008 (2)
c (Å)	15.2054 (2)
β (°)	102.442 (1)
V (Å^3)	2685.85 (5)
Z	4
D (Mg m^{-3})	1.420
μ (mm^{-1})	1.12
F (000)	1168
Colour	Colourless
Temperature (K)	150
θ range (°)	2.2 - 27.5
Limiting indices (±h, ±k,±,l)	-13/13, -23/23, -19/19
Reflections collected/unique	34109/6099 [R_{int} = 0.044]
Refinement method	Full-matrix least-squares on F^2
Data/ restraints/ parameters	6099/0/299
Goodness-of-fit on F^2	1.02
Final R indices I > 2σ(I)	R_1 = 0.024
	wR_2 = 0.060
Largest diff. peak and hole (e Å^{-3})	0.49 and -0.27

Table 5: Selected geometric parameter (Å, °) for compound 4

Compounds	Value	Compounds	Value
Bond length (Å)			
Sn-S1	2.4772 (5)	Sn-C24	2.1521 (18)
Sn-S2	3.1048 (5)	S1-C1	1.758 (2)
Sn-C12	2.1286 (18)	S2-C1	1.675 (2)
Sn-C18	2.1380 (19)		
Bond angle (°)			
C12-Sn-C18	113.76 (7)	C12-Sn-S1	117.26 (5)
C12-Sn-C24	107.51 (7)	C18-Sn-S1	115.54 (5)
C18-Sn-C24	107.45 (7)	C24-Sn-S1	92.18 (5)

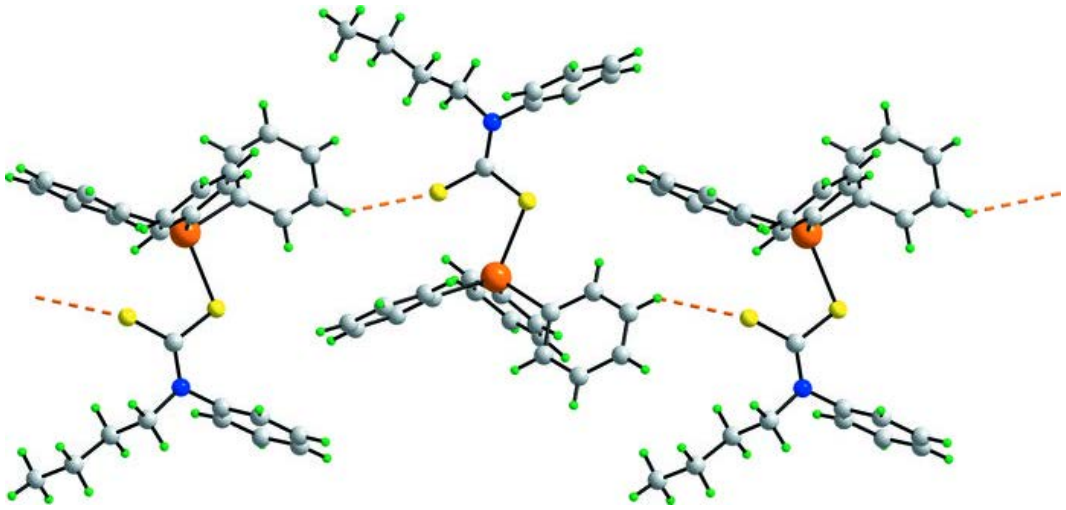

Fig. 3: Helical supramolecular chain of compound 4 mediated by C-H.....S interactions (dashed lines) along the b axis

Table 6: *In vitro* cytotoxicity data of compound 1-4

	IC_{50} values (μM)	
Compounds	Jurkat E6.1	K-562
1	0.8	5.3
2	0.5	3.9
3	1.3	9.2
4	0.4	1.9
DOX	0.1	11.0

DISCUSSION

Synthesis of compound 1-4: Organotin(IV) compounds with dithiocarbamate ligand are extensively being synthesized due to their potential in various fields including chemotherapeutic drugs. Organotin(IV) compounds derived from butylphenyldithiocarbamate series have not yet been synthesized, characterized and currently reported by any researchers. In this study, four types of organotin(IV) butylphenyldithiocarbamate compounds were successfully synthesized using direct method in 1:2 and 1:1 mole ratios for diorganotin (IV) and triorganotin (IV) compounds, respectively. All compounds were in agreement with the suggested molecular formula:

$$R_nSn[S_2CN(C_4H_9)(C_6H_5)]_{4-n}$$

where, R = Bu and Ph for n = 2; R = Ph for n = 3, as shown in Table 1, characterized by elemental analysis. The ligand is unstable at room temperature and the temperature must be kept below 4°C during the whole process to avoid the breakage of bonds between elements. Due to the instability of this ligand, we were unable to synthesize the ligand in solid form, as obtained by Onwudiwe and Ajibade, (2010), thus direct reaction with tin (IV) solution was proposed as the best solution to form the complexes. All synthesized complexes were formed as air stable compounds at room temperature and soluble in organic solvents such as chloroform and ethyl acetate. The purities of compounds were determined based on melting point, elemental analysis and NMR spectroscopy. The ranges of melting point of each compound were around 1-2°C indicated that the compounds are in good purities.

Spectroscopic analysis: The most important band observed in IR spectra, known as thioureide band, ν(C- - N) was detected in range 1457-1489 cm^{-1}. The band appears intermediate within C and N single bond (C-N: 1063-1261 cm^{-1}) and double bond (C=N: 1640-1690 cm^{-1}) wave numbers. This band shows the partial double bond feature that characterized the formation of dithiocarbamate (S_2 C- - NR_2). The stretching vibration from this partial double bond is due to the partial delocalization of electron density within the dithiocarbamate (Nabipour *et al*., 2010). As mentioned earlier, the stretching vibration between C and S elements are another significant band point to the dithiocarbamate characteristic, and the band was detected in the region 950-1050 cm^{-1} attributed to the v(C- - S) band (Johnson *et al*., 1969; Bonati and Ugo, 1967). According to Bonati and Ugo (1967), the chelating modes of the ligand to the central Sn(IV) atom can be determined via IR spectrum, by referring to the number of peaks appear within the v(C- - S) band region. Through this principle, the chelating modes of each compound in this experiment was determined. The IR spectra of three

compounds (C1,C2 and C4) gave two peaks within this region, therefore indicating that the dithiocarbamate chelate to the central tin (IV) atom as monodentate ligands. Furthermore, the chelating mode of C4 has been proven via X-ray single crystal analysis (Fig. 2), thus, confirm the formation of monodentate chelating mode to the central Sn (IV) atom. Meanwhile, compound 3 showed only single peak within the same range which characterize the formation of dithiocarbamate as bidentate chelating ligand. Other than these bands, there is another one important band specially used to characterized the formation of organotin (IV) compounds, that is v(Sn-C). This band, which occurs due to the stretching vibration between Sn (IV) and C atoms prove the presence of bonds between these two elements, which is detected at wave number within the range of 500-600 cm^{-1} (Nath *et al*., 2001) in tin (IV) compounds associated with alkyl groups. All spectra showed the absence of bands around 3422 and 1601 cm^{-1}, where the bands that appear within these regions are attributed to v(N-H) and σ(N-H) in the ligand, thus confirm the displacement of ammonium ion with metal atom (Onwudiwe and Ajibade, 2010).

With the intention to further confirm the formation of compounds according to the suggested molecular formula, ^{1}H and ^{13}C NMR analysis were carried out. The ^{1}H NMR chemical shifts of complexes around δ_H 3.98-4.16 ppm, which contain the triplet signals attributed to the protons in methylene carbon linked directly with N atom in dithiocarbamate ligands. There is a downfield shift of about 2 ppm, from the normal chemical shift of methylene protons which usually found at δ_H 1.2-1.4 ppm (Pavia *et al*., 2001) as a consequences to the electronegativity of N atom in the butylphenyldithiocarbamate ligands. The ^{1}H NMR chemical shifts found using the same ligand bonded to the Zn, Cd and Hg by Onwudiwe and Ajibade (2011) were δ_H 3.97-4.21 ppm, also ascribed to the protons in methylene carbon linked directly to the N atom within the ligands. These findings were also supported by Onwudiwe and Ajibade (2010) which found a sharp singlet in the region δ_H 3.82-3.64 ppm for methyl carbon linked directly with N atoms in methylphenyldithiocarbamate complexes. Whilst, the multiplet resonances observed in all spectra within the region of δ_H 7.18-7.73 ppm ascribed to the protons in phenyl rings that attach to the N atoms in butylphenyldithiocarbamate ligands, in agreement with previously reported data (Onwudiwe and Ajibade, 2010). The signals of aromatic protons especially exhibited by C3 and C4 were observed as multiplet resonances due to the overlapping of proton signals in aromatic group, referring to the phenyl ring attached to the N atom in the ligand and also phenyl groups bonded to the central Sn(IV) atom.

In conjunction to ^{1}H NMR data, the ^{13}C NMR analysis of all compounds exhibited all carbon signals as expected. The spectra of C1-C4 showed CS_2 peaks at δ_C 198-203 ppm, which indicate the presence of dithiocarbamate (-NCS_2) group, thus confirm the formation of dithiocarbamate compounds. The chemical shift of CS_2 peak from -NCS_2 group is identified as the most significant shift to characterize dithiocarbamate moieties. This peak normally found in the range of δ_C 185-220 ppm (Van Gaal *et al*., 1979). Furthermore, single peak in C1-C4 was recorded at δ_C 58-59 ppm, signifying the C from butyl group that linked directly to the N atom. In normal condition, this methylene C can be observed within δ 15-55 ppm (Pavia *et al*., 2001), but in this present study, the methylene C signals showed downfield shift as a result of electronegative effect from N atom. The electronegativity affect ^{13}C chemical shifts in almost similar trend as they affect ^{1}H chemical shifts, but the electronegative elements such as N will produces a large downfield shift of about 20 times larger in ^{13}C as compared to ^{1}H chemical shifts (Macomber, 1991). The presence of methylene C that linked directly to the N atom is in agreement with the presence of chemical shift from methylene hydrogens in ^{1}H NMR as discussed above. Other two methylene groups in each compounds were found between δ 19-31 ppm, shifted to the higher field as the distance to the N atom is increasing. Meanwhile, the peaks correspond to the end methyl group were recorded at δ 13.72-13.97 ppm. Other than these two peaks, the chemical shifts obtained in the range of δ 145-150 ppm are also important to characterize the formation of butylphenyldithiocarbamate, where these correspond to C from phenyl ring that attached directly to the N atom. The other C in phenyl ring were found at δ 127-145 ppm. Thus, the presence of all these peaks in ^{13}C NMR spectra had confirmed the formation of organotin (IV) butylphenyldithiocarbamate synthesized.

Recrystallization and crystallography study: The recrystallization process was done to all compounds but out of four compounds, only C4 has yielded the crystal. The molecule crystallized in monoclinic crystal system with space group $P2_1/n$ and unit cell a = 10.0488(1)Å, b = 18.0008(2)Å, c = 15.2054(2)Å, β = 102.442(1)° and R = 0.044 (Table 4). The molecule features a tetrahedrally coordinated Sn atom, in which the dithiocarbamate ligands coordinated in a monodentate fashion, in agreement with the dissimilarity in the C-S bond distances where S1-C1 is 1.758 (2) Å and S2-C1 is 1.675 (2) Å (Table 5). Additionally, there is also a large disparity of

bond lengths between Sn-S1 (2.4772 (5) Å) and Sn-S2 (3.1048 (5) Å), hence revealed the monodentate chelating mode of the ligand to the central Sn atom. The X-ray structure of compound 4 showed that the molecule adopts distorted tetrahedral geometry. The range of the tetrahedral angles is 92.18 (5)° to 117.26 (5)°, where the wider angles are assigned to the influence of the nearest S2 atom. Therefore, the distortion of the Sn atom from ideal tetrahedral geometry (109.5°) is mainly due to the proximity of the non-coordinating thione S atom (Sn-S2 = 3.1048 (5) Å), whose C12-Sn-S1 is 117.26 (5)°. The bond lengths of three phenyl rings attached to Sn(IV) atom are in normal ranges and in agreement with the previous molecules that have been reported (Kamaludin *et al*., 2012a; Du *et al*., 2011; Liu *et al*., 2011; Dokorou *et al*., 2011; Gomez-Ruiz *et al*., 2010). The bond length of C1-N1 (1.337 (2) Å) is shorter compared to the normal C-N bond length (mean value 1.45 Å) (Sieber *et al*., 1974) which characterized the partially double bond feature in most dithiocarbamate complexes (Kamaludin *et al*., 2012a, b; Fuentes-Martinez *et al*., 2009; Baba and Raya, 2010). This is also in agreement with the ν(C- - N) bands as observed in the IR spectra. The crystal packing of this molecule (Fig. 3) display helical supramolecular chains along the *b* axis, which is sustained by C-H····S interactions. It is linked by C28-H28.....S2 bond forming one dimensional network.

***In vitro* cytotoxicity test:** Inorganic compounds consisting of various types of metals have been screened to evaluate their potential to be used as anticancer drugs, and organotin is included to be one of them. The butylphenyldithiocarbamate derivatives that bound to tin(IV) have not yet been studied, thus the potential of the newly synthesized organotin (IV) butylphenyldithiocarbamate compounds as anticancer drugs are still not revealed. Previous studies carried out by our group and others have shown that organotin (IV) compounds were among the most potent candidates to be developed as new anticancer drugs (Awang *et al*., 2010; Alama *et al*., 2009; Gielen and Tiekink, 2005; Hadjikakou and Hadjiliadis, 2007). In order to evaluate the potential of this series of compounds, we performed the preliminary in vitro test using human leukemia cell lines comprise of Jurkat E6.1 and K-562 cells (Table 6). All compounds, with an exception of C3, can be classified as strongly active against both cell lines, as they showed high cytotoxicity with the IC_{50} values of less than 5.0 μg cm^{-3} (< 8.70 μM) (How *et al*., 2008). In contrast to its potential in Jurkat E6.1 cell, C3 gave higher IC_{50} value (9.2 μM) in K-562 cell line, hence can be classified as moderately active (How *et al*., 2008). In Jurkat E6.1 cell, the IC_{50} values of all compounds are comparable with the positive control used, doxorubicin hydrochloride (DOX), meanwhile all compounds of this series exhibited higher cytotoxicity against K-562 cell as compared to DOX.

Dithiocarbamate moieties itself or organotin(IV) dithiocarbamate complexes are believed to be a part of significant factors that lead to the various degrees of cytotoxicity in the cell lines tested. This could be due to the role of ligand which serve as a carrier, as well as its lipophilic properties which facilitate the transportation of metal to the cellular environment and finally inducing cytotoxicity (Milacic *et al*., 2008; Cvek and Dvorak, 2007). Other than that, the diversity of organotin(IV) moieties could also be a relevant factor. It has been suggested that the organotin toxicity are depending on the number and length of the alkyl chain in the substituted group (Ray *et al*., 2000; Pellerito *et al*., 2006). By referring to the results obtained in this study, C4 with three phenyl groups attached to the central Sn(IV) atom gave the highest toxicity in both cell lines as compared to C1-C3, which are diorganotin(IV) derivative compounds. This finding was supported by previous studies which have also shown that triorganotin(IV) displayed higher toxicity in various types of cell lines than diorganotin(IV) compounds, with decreasing toxicity as the length of the alkyl chain is increasing (Ray *et al*., 2000; Casas *et al*., 1999; Awang *et al*., 2011b; Gielen *et al*., 2000). According to Jenkins *et al*. (2004), the organotin(IV) toxicity is directly related to the number and nature of the organic moiety, in which the highly substituted organotin compounds are more toxic where the increasing alkyl chain length will reduce its toxicity. This present finding showed that among diorganotin (IV) compounds (C1-C3), C3 with two phenyl rings (six carbon of each ring) exhibited lowest toxicities in both cell lines as compared to C1 and C2 which have two butyl groups (four carbon of each group), hence in agreement with the factors suggested in influencing the organotin (IV) toxicity.

CONCLUSION

The structures of the novel organotin (IV) butylphenyldithiocarbamate compounds have been proven via elemental analysis, IR and NMR spectroscopy. The X-ray crystal structure of C4 displayed distorted tetrahedral geometry and coordinated in monodentate fashion. The results obtained in this study demonstrate that all compounds showed potent cytotoxicity towards leukemia cell lines tested where the triphenyltin(IV) compound exhibited the most potent effect. The underlying mechanism of actions and the upstream signaling pathways of these compounds are being investigated.

ACKNOWLEDGMENT

We would like to thank the Malaysia Ministry of Higher Education (UKM-NN-FRGS0091-2010) and Universiti Kebangsaan Malaysia for supporting this study. We also like to thank Universiti Putra Malaysia and Universiti Malaya for crystallographic study. Technical support from laboratory assistants of Faculty of Science and Technology and Faculty of Health Sciences, Universiti Kebangsaan Malaysia are gratefully acknowledged.

REFERENCES

Alama, A., B. Tasso, F. Novelli and F. Sparatore, 2009. Organometallic compounds in oncology: Implications of novel organotins as antitumor agents. Drug Discovery Today, 14: 500-508.

Awang, N., I. Baba, N.S.A.M. Yousof and N.F. Kamaludin, 2010. Synthesis and characterization of organotin(IV) N-benzyl-N-isopropyldithiocarbamate compounds: cytotoxic assay on human hepatocarcinoma cells (HepG2) Am. J. Applied Sci., 7: 1047-1052.

Awang, N., I. Baba, B.M. Yamin, M.S. Othman and N.F. Kamaludin, 2011a. Synthesis, characterization and biological activities of organotin (IV) methylcyclohexyldithiocarbamate compounds. Am. J. Applied Sci., 8: 310-317.

Awang, N., N.F. Kamaludin and A.R. Ghazali, 2011b. Cytotoxic effect of organotin(IV) benzylisopropyldithiocarbamate compounds on change liver cell and hepatocarcinoma HepG2 cell. Pak. J. Biol. Sci., 14: 768-774.

Baba, I. and I. Raya, 2010. Praseodymium dithiocarbamate 1,10 phenantroline complexes. Sains Malaysiana, 39: 45-50.

Basu Baul, T.S., A. Paul, L. Pellerito, M. Scopelliti and C. Pellerito *et al*., 2010. Molecular basis of the interaction of novel tributyltin (IV) 2/4-[(E)-2-(aryl)-1-diazenyl] benzoates endowed with an improved cytotoxic profile: Synthesis, structure, biological efficacy and QSAR studies. J. Inorganic Biochem., 104: 950-966.

Bonati, F. and R. Ugo, 1967. Organotin (IV) N,N-disubstituted dithiocarbamates. J. Organomet. Chem., 10: 257-268.

Brandenburg, K., 2006. Diamond. Crystal Impact GbR, Bonn, Germany.

Cao, S.L., Y. Wang, L. Zhu, J. Liao and Y.W. Guo *et al*., 2010. Synthesis and cytotoxic activity of *N*-((2-methyl-4(3*H*)-quinazolinon-6-yl)methyl)dithiocarbamates. Eur. J. Med. Chem., 45: 3850-3857.

Casas, J.S., A. Castineiras, M.C. Rodriguez-Arguelles, A. Sanchez and J. Sordo *et al*., 1999. Synthesis, structure, spectroscopic properties and biological activity of mixed diorganotin (IV) complexes containing pyridine-2-carbaldehydethiosemicarbazonato and diphenyldithiophosphinato ligands. J. Inorganic Biochem., 76: 277-284.

Cepeda, V., M.A. Fuertes, J. Castilla, C. Alonso, C. Quevedo and J.M. Perez, 2007. Biochemical mechanisms of cisplatin cytotoxicity. Anticancer Agents Med. Chem., 7: 3-18.

Chen, S.H., S.H. Liu, Y.C. Liang, J.K. Lin and S.Y. Lin-Shiau, 2000. Death signaling pathway induced by pyrrolidine dithiocarbamate-Cu^{2+} complex in the cultured rat cortical astrocytes. Glia, 31: 249-261.

Cvek, B. and Z. Dvorak, 2007. Targeting nuclear factor-κB and proteasome by dithiocarbamate complexes with metals. Current Pharm. Design, 13: 1-13.

Dokorou, V., A. Primikiri and D. Kovala-Demertzi, 2011. The triphenyltin(VI) complexes of NSAIDs and derivatives. Synthesis, crystal structure and antiproliferative activity. Potent anticancer agents. J. Inorganic Biochem., 105: 195-201.

Du, D., Z. Jiang, C. Liu, A.M. Sakho, D. Zhu and L. Xu, 2011. Macrocyclic organotin(IV) carboxylates based on benzenedicarboxylic acid derivatives: Syntheses, crystal structures and antitumor activities. J. Organomet. Chem., 696: 2549-2558.

Farrugia, L.J., 1997. *ORTEP*-3 for windows-a version of *ORTEP*-III with a Graphical User Interface (GUI). J. Applied Cryst., 30: 565-565.

Florea, A.M. and D. Busselberg, 2011. Cisplatin as an anti-tumor drug: Cellular mechanism of activity, drug resistance and induced side effects. Cancers, 3: 1351-1371.

Fuentes-Martinez, J.P., I. Toledo-Martinez, P. Roman-Bravo, P.G. Garcia, C. Godoy-Alcantar, M. Lopez-Cardoso and H. Morales-Rojas, 2009. Diorganotin(IV) dithiocarbamate complexes as chromogenic sensors of anion binding. Polyhedron, 28: 3953-3966.

Galluzzi, L., L. Senovilla, I. Vitale, J. Michels and I. Martins *et al*., 2011. Molecular mechanisms of cisplatin resistance. Oncogene, 31: 1869-1883.

Gielen, M., M. Biesemans, D. De Vos and R. Willem, 2000. Synthesis, characterization and *in vitro* antitumor activity of di- and triorganotin derivatives of polyoxa- and biologically relevant carboxylic acids. J. Inorg. Biochem., 79: 139-145.

Gielen, M. and E.R.T. Tiekink, 2005. Tin Compounds and their Therapeutic Potential. In: Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine, Gielen, M. and E.R.T. Tiekink (Eds.). John Wiley and Sons, New York, pp: 421-439.

Gold, V., K.L. Loening, A.D. McNaught and P. Sehmi, 1987. International Union of Pure and Applied Chemistry Compendium of Chemical Terminology. Blackwell Scientific Publications, Oxford, UK.

Gomez-Ruiz, S., S. Prashar, T. Walther, M. Fajardo, R. Paschke, G.N. Kaluderovic and D. Steinborn, 2010. Cyclopentadienyltin(IV) derivatives: Synthesis, characterization and study of their cytotoxic activities. Polyhedron, 29: 16-23.

Hadjikakou, S.K. and N. Hadjiliadis, 2007. Antiproliferative and anti-tumor activity of organotin compounds. Coord. Chem. Rev., 253: 235-249.

Heard, P.J., 2005. Main Group Dithiocarbamate Complexes. In: Progress in Inorganic Chemistry, Karlin, K.D. (Eds.). Vol. 53. John Wiley and Sons Inc., London, UK., ISBN: 9780471463702, pp: 2-68.

How, F.N.F., K.A. Crouse, M.I.M. Tahir, M.T.H. Tarafder and A.R. Cowley, 2008. Synthesis, characterization and biological studies of S-benzyl-&beta-N-(benzoyl) dithiocarbazate and its metal complexes. Polyhedron, 27: 3325-3329.

Jenkins, S.M., K. Ehman and S. Barone, Jr., 2004. Structure-activity comparison of organotin species: Dibutyltin is a developmental neurotoxicant *in vitro* and *in vivo*. Dev. Brain Res., 151: 1-12.

Johnson, B.F.G., K.H. Al-Obalidi and J.A. Mecleverty, 1969. Transition-metal nitrosyl compounds. Part III. (*NN*-dialkyldithiocarbamato)nitrosyl compounds of molybdenum and tungsten. J. Am. Chem. Soc. A, 19: 1668-1670.

Kaludjerovic, G.N., V.M. Djinovic, S.R. Trifunovic, I.M. Hodzic and T.J. Sabo, 2002. Synthesis and characterization of tris[butyl-(1-methyl-3-phenyl-propyl)-dithiocarbamato]-cobalt(III) seskvitoluene. J. Serb. Chem. Soc., 67: 123-126.

Kamaludin, N.F., I. Baba, N. Awang, M.I. Mohamed Tahir and E.R.T. Tiekink, 2012a. (N-Ethyl-N-phenyldithiocarbamato-êS)triphenyltin(IV). Acta Cryst., 68: 62-63.

Kamaludin, N.F., I. Baba, N. Awang, M.I.M. Tahir and E.R.T. Tiekink, 2012b. Di-n-butylbis(N-ethyl-N-phenyldithiocarbamato-κS)tin(IV). Acta Cryst. Sect. E, 68: m79-m80.

Kopf-Maier, P., 1994. Complexes of metals other than platinum as antitumour agents. Eur. J. Clin. Pharmacol., 47: 1-16.

Li, H., C.S. Lai, J. Wu, P.C. Ho, D. de Vos and E.R.T. Tiekink, 2007. Cytotoxicity, Qualitative Structure-Activity Relationship (QSAR) and anti-tumor activity of bismuth dithiocarbamate complexes. J. Inorganic Biochem., 101: 809-816.

Liu, C., S. Liu, D. Du, D. Zhu and L. Xu, 2011. Organotin(IV) carboxylates of (E)-3-(2-nitrophenyl) propenoic acid: Synthesis, spectroscopic characterization, crystal structure and antitumor activity. J. Mol. Struct., 1003: 134-140.

Macomber, R.S., 1991. Proton-carbon chemical shift correlations. J. Chem. Educ., 68: 284-285.

Milacic, V., D. Chen, L. Giovagnini, A. Diez, D. Fregona and Q.P. Dou, 2008. Pyrrolidine dithiocarbamate-zinc(II) and -copper(II) complexes induce apoptosis in tumor cells by inhibiting the proteasomal activity. Toxicol. Applied Pharmacol., 231: 24-33.

Morais, C., B. Pat, G. Gobe, D.W. Johnson and H. Healy, 2006. Pyrrolidine dithiocarbamate exerts anti-proliferative and pro-apoptotic effects in renal cell carcinoma cell lines. Nephrol. Dial Transplant, 21: 3377-3388.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.

Nabipour, H., S. Ghammamy, S. Ashuri and Z.S. Aghbolagh, 2010. Synthesis of a new dithiocarbamate compound and study of its biological properties. Org. Chem. J., 2: 75-80.

Nath, M., S. Pokharia and R. Yadav, 2001. Organotin(IV) complexes of amino acids and peptides. Coord. Chem. Rev., 215: 99-149.

Nieuwenhuizen, P.J., J. Reedijk, M. van Duin and W.J. McGill, 1997. Thiuram-and dithiocarbamate-accelerated sulfur vulcanization from the chemist's perspective: Methods, materials and mechanisms reviewed. Rubber Chem. Technol., 70: 368-429.

Nobel, C.I., M. Kimland, B. Lind, S. Orrenius and A.F. Slater, 1995. Dithiocarbamates induce apoptosis in thymocytes by raising the intracellular level of redox-active copper. J. Biol. Chem., 270: 26202-26208.

Onwudiwe, D.C. and P.A. Ajibade, 2010. Synthesis and characterization of metal complexes of N-alkyl-N-phenyl dithiocarbamates. Polyhedron, 29: 1431-1436.

Onwudiwe, D.C. and P.A. Ajibade, 2011. Synthesis, characterization and thermal studies of Zn(II), Cd(II) and Hg(II) complexes of N-methyl-N-phenyldithiocarbamate: The single crystal structure of $[(C_6H_5)(CH_3)NCS_2]_4Hg_2$. Int. J. Mol. Sci., 12: 1964-1978.

Oxford Diffraction, 2010. CrysAlis PRO. Oxford Diffraction Ltd., Yarnton, England.

Pavia, D.L., G.M. Lampman and S.G. Kriz, 2001. Introduction to Spectrochemistry. 3rd Edn., Thomson Learning, USA., Pages: 30.

Pellerito, C., L. Nagy, L. Pellerito and A. Szorcsik, 2006. Biological activity studies on organotin(IV)$^{n+}$ complexes and parent compounds. J. Organomet. Chem., 691: 1733-1747.

Ray, D., K.D. Sarma and A. Antony, 2000. Differential effects of tri-n-butylstannyl benzoates on induction of apoptosis in K562 and MCF-7 cells. IUBMB Life, 49: 519-525.

Robertson, J.D. and S. Orrenius, 2000. Molecular mechanisms of apoptosis induced by cytotoxic chemicals. Crit. Rev. Toxicol., 30: 609-627.

Ruan, B., Y. Tian, H. Zhou, J. Wu and R. Hu *et al*., 2011. Synthesis, characterization and in vitro antitumor activity of three organotin(IV) complexes with carbazole ligand. Inorg. Chim. Acta, 365: 302-308.

Sheldrick, G.M., 2008. A short history of SHELX. Acta Cryst., 64A: 112-122.

Sieber, K., L. Kutschabsky and S. Kulpe, 1974. The molecular and crystal structure of bis-dimethyl pentamethine cyanine perchlorate, $C_9H_{17}N_2Cl0_4$. Krist. Tech., 9: 1111-1122.

Van Gaal, H.L.M., J.W. Diesveld, F.W. Pijpers and J.G.M. Van der Linden, 1979. Carbon-13 Nmr spectra of dithiocarbamates Chemical shifts, carbon-nitrogen stretching vibration frequencies, and bonding in the NCS_2 fragment. Inorg. Chem., 18: 3251-3260.

Xu, L.Z., P.S. Zhao and S.S. Zhang, 2001. Crystal structure and characterization of Pd(II) bis(diisopropyldithiocarbamate) complex. Chin. J. Chem., 19: 436-440.

Yamaguchi, A., K. Tomiyama, Y. Sayama, T. Kuriyama, H. Nakashima, Y. Matsuda and Y. Arakawa, 2007. Mechanism on the cell death of T-lymphocytes induced by organotin in vitro. Trace Nutr. Res., 24: 90-97.