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Original article

Synthesis and cytotoxic activity of organotin(IV) diallyldithiocarbamate compounds as anticancer agent towards colon adenocarcinoma cells (HT-29)

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ABSTRACT

Context: Diphenyltin(IV) diallyldithiocarbamate compound (Compound **1**) and triphenyltin(IV) diallyldithiocarbamate compound (Compound **2**) are two newly synthesised compounds of organotin(IV) with diallyldithiocarbamate ligands.
Objective: To assess the cytotoxic effects of two synthesised compounds against HT-29 human colon adenocarcinoma cells and human CCD-18Co normal colon cells.
Materials and methods: Two successfully synthesised compounds were characterised using elemental (carbon, hydrogen, nitrogen, and sulphur) analysis, Fourier-Transform Infrared (FTIR), and ^{1}H, ^{13}C ^{119}Sn Nucleus Magnetic Resonance (NMR) spectroscopies. The single-crystal structure of both compounds was determined by X-ray single-crystal analysis. The cytotoxicity of the compounds was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazholium bromide (MTT) assay upon 24 h of treatment. While the mode of cell death was determined based on the externalisation of phosphatidylserine using a flow cytometer.
Results: The elemental analysis data of the two compounds showed an agreement with the suggested formula of $(C_6H_5)_2Sn[S_2CN(C_3H_5)_2]_2$ for Compound **1** and $(C_6H_5)_3Sn[S_2CN(C_3H_5)_2]$ for Compound **2**. The two major peaks of infrared absorbance, i.e., ν(C = N) and ν(C = S) were detected at the range of 1475–1479 cm^{-1} and 972–977 cm^{-1}, respectively. The chemical shift of carbon in NCS_2 group for Compound **1** and **2** were found at 200.82 and 197.79 ppm. The crystal structure of Compound **1** showed that it is six coordinated and crystallised in monoclinic, $P2_1/c$ space group. While the crystal structure of Compound **2** is five coordinated and crystallised in monoclinic, $P2_1/c$ space group. The cytotoxicity (IC_{50}) of the two compounds against HT-29 cell were 2.36 μM and 0.39 μM. Meanwhile, the percentage of cell death modes between 60% and 75% for compound **1** and compound **2** were mainly due to apoptosis, suggesting that both compounds induced growth arrest.
Conclusion: Our study concluded that the synthesised compounds showed potent cytotoxicity towards HT-29 cell, with the triphenyltin(IV) compound showing the highest effect compared to diphenyltin(IV).

1. Introduction

Currently, colon adenocarcinoma is one of the leading causes of morbidity and death, with nearly 1.4 million cases a year worldwide (Hammond et al., 2016; Zhang et al., 2017). It is also the third most frequently diagnosed cancer among all other types of cancer (Awang et al., 2014). According to the Summary of Malaysian National Cancer Registry Report (2007–2011), colon cancer is the most common type of cancer in Malaysia, with a prevalence of 13.2%. In addition, it is the most common cancer in men and the second most common in women. According to a statement by the World Health Organisation (WHO) in 2018, colon cancer is

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the second cancer that causes many deaths, with a total of 862,000 cases.

Current treatments for colon cancer are surgery, chemotherapy, and radiation therapy (Hagan et al., 2013; Baskar et al., 2012; Phi et al., 2018). Among the three treatments, the most powerful approach to kill the cancer cells that have spread is the use of synthetic drugs through chemotherapy. However, this specific treatment can also lead to treatment failure and the development of cancer (Kamaludin et al., 2017; Adeyemi et al., 2018; Ellahioui et al., 2017). Chemotherapy resistance is a major problem in the treatment of cancer patients, as cancer cells may become resistant to the chemicals used, and the efficacy of chemotherapeutic agents in subsequent treatment may be disrupted (Adeyemi et al., 2018).

Several types of cancer cells have exhibited resistance to current chemotherapeutic drugs such as cisplatin, where new anticancer drugs are needed to fight cancer (Alama et al., 2009). The first inorganic chemotherapeutic agent was cisplatin, which is still used as a primary treatment for testicular, ovarian, and other cancers (Imran et al., 2018). However, the toxic side effects and resistance of cancer cells have minimised the effectiveness of cisplatin treatment and related medications (Imran et al., 2020). To overcome this, cisplatin can be combined with other chemotherapeutic agents such as 5-fluorouracil (5-FU; Ellahioui et al., 2017). Chemotherapy with 5-FU has become the main treatment option, not only for colorectal cancer but for various other cancers such as breast, oesophagus, and stoma. However, many patients develop recurrent cancer after the initial treatment (Zhang et al., 2017). Toxicity associated with 5-FU is a serious issue and is often experienced by many cancer patients, with myelosuppression and gastrointestinal poisoning being the most commonly reported side effects (Tong et al., 2011).

Due to the above problems, the search for new synthetic compounds to be used in cancer chemotherapy is necessary to achieve better targets and reduce side effects on patients. In vitro studies using new synthetic compounds are still the best way to identify new drugs that have the potential to be developed as anticancer agents (Amir et al., 2014). Dithiocarbamate with organotin(IV) is one of the dithiocarbamate metals that has been extensively studied. Studies have shown that some diorganotin(IV) dithiocarbamate compounds that have been characterised and expressed structurally are the most attractive in terms of their structural diversity (Khan et al., 2015). Apart from having the potential to cause cytotoxic effects on various types of cancer cells, it also has various other biological applications such as antimicrobial, larvicidal, anti-malaria, and others (Mohamad et al., 2016).

Organotin(IV) compounds in the bicarbonate have the general formula of $R_nSn(S_2CNR'R'')$ 4-n (n = 1, 2, or 3) and R, R′ and R″ represent alkyl or aryl groups (Awang et al., 2011). The organotin(IV) dithiocarbamate compound has regained attention as a new chemotherapy agent due to its ability to stabilise certain stereochemistry and its good anti-proliferative activity as observed in in vitro studies. These compounds are considered essential due to their diverse applications such as in agriculture, biology, catalysis, and as a single source pioneer for tin sulphide nanoparticles (Kamaludin et al., 2017; Adeyemi et al., 2018).

The diversity of biological activities of organostanum (IV) dithiocarbamate compounds is strongly influenced by the diversity of organotin and the dithiocarbamate molecules found in these compounds (Adeyemi et al., 2018). For example, the presence of two sulphur atoms in a dithiocarbamate ligand that has a strong metal binding capacity has increased the potential of these sulphur donor ligands. Therefore, it can prevent enzymes that eventually affect the biological environment. This has also led to a growing interest in the use of organotin(IV) dithiocarbamate compounds as antibacterial, antifungal, and in some industrial applications such as rubber vulcanisation. In addition, these compounds also show high potential toxic activities against various types of cancer cells, such as lungs, ovaries, melanoma, colon, kidneys, prostate, and breast (Khan et al., 2015; Adeyemi et al., 2018). However, data concerning in vitro testing of normal cells through organotin(IV) cytotoxicity studies that have been performed on cancer cells are scarce (Banti et al., 2019).

Therefore, we have synthesised diphenyltin(IV) diallyldithiocarbamate compound (Compound **1**) (Adeyemi et al., 2019) and triphenyltin(IV) diallyldithiocarbamate compound (Compound **2**) to begin the structural chemistry of organotin(IV) dithiocarbamate compounds. Herein, we report the synthesis and spectral characterisation of the compounds. Fig. 1 and Fig. 2 show the chemical structure of the synthesised compounds. The X-ray crystallographic study of compound **1** and compound **2** was also obtained. After the two compounds were successfully synthesised and characterised, we proceeded with the cytotoxic tests to determine whether these two compounds could potentially exert cytotoxic effects towards cancer cells.

2. Materials and methods

2.1. Materials

Diallylamine, triphenyltin(IV) chloride, diphenyltin(IV) dichloride, MTT salt, and ammonia solution (25%) were purchased from Sigma USA. Whereas, ethyl alcohol, chloroform, and carbon disulphide were supplied by Merck. DMSO was purchased from Fisher Scientific, UK. All reagents and chemicals were used as provided, with no further purification.

2.2. Physical measurements

An OptiMelt MPA100 was used to measure the melting point of each compound. The elemental analysis of carbon, hydrogen, nitrogen, and sulphur was performed using Perkin Elmer 2400, conducted at the Faculty of Health Sciences, Universiti Teknologi Mara. The Thermo Nicolet 6700 spectrophotometer was used to record the infrared spectra using the KBr disk in the spectral range of 400–4000 cm^{-1}. The analysis was performed at the Faculty of Sciences, Universiti Putra Malaysia. Using tetramethylsilane as internal standard, the ^{1}H, ^{13}C, and ^{119}Sn spectra were obtained using Bruker Ascend NMR 400 MHz spectrophotometer in $CdCl_3$. Chemical shift values are given in parts per million (ppm). The measurements were carried out at the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The X-ray single structure determination was reported using the Rigaku XtaLAB Synergy single-crystal X-Ray diffractometer at the Sunway University School of Science and Technology.

2.3. Synthesis of compound 1 and compound 2

The two compounds were prepared using a direct reaction of 30 mmol of carbon disulphide to 30 mmol of diallylamine ethanolic solution. The reaction mixture was stirred at 4 °C for 2 h. An appropriate volume of chloride salt (triphenyltin(IV) chloride and diphenyltin(IV) dichloride) was then applied dropwise in 60 mL of ethanol. The mixture was then stirred for another 2–3 h. The precipitate formed after 3 h was filtered and washed with cold ethanol, and then dried in a desiccator (8) (Adeyemi et al., 2019).

2.4. Recrystallisation and crystallographic study

Crystallisation of the compound synthesized was performed by dissolving the chloroform and ethanol with the compound in a ratio of 1:1 v/v at room temperature. The mixture was allowed

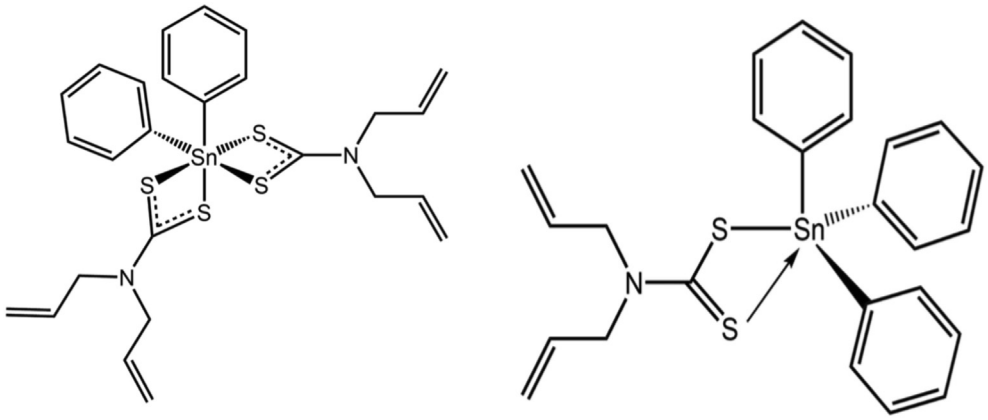
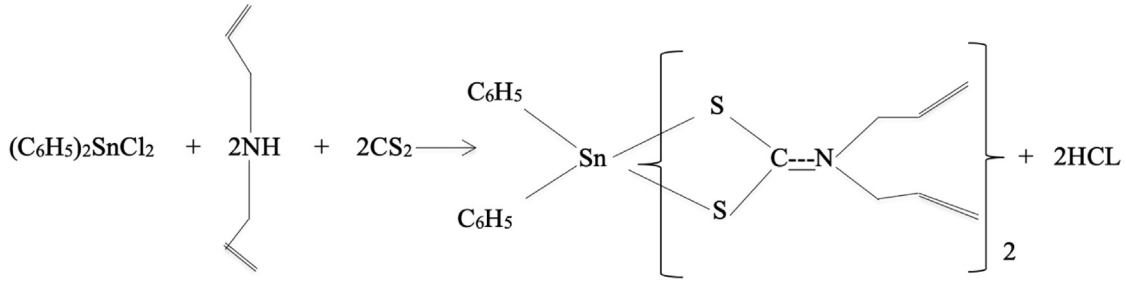


Fig. 1. The chemical structure of Compound **1** and Compound **2** (Haezam et al., 2020).

Reaction scheme of Compound 1 (Diphenyltin(IV) diallyldithiocarbamate compound)



Reaction scheme of Compound 2 (Triphenyltin(IV) diallyldithiocarbamate compound)

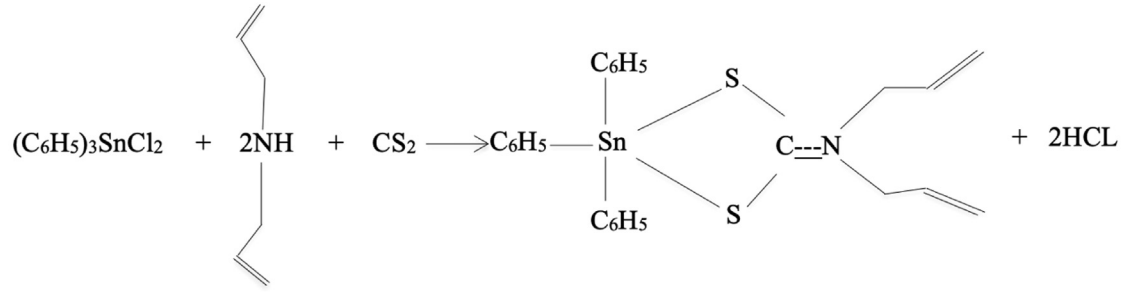


Fig. 2. The general reaction between chloride salt, diallylamine, and carbon disulphide.

to slowly evaporate for three to four days. The crystals formed were then carefully collected for structure determination using X-ray analysis.

2.5. Cell culture and reagents

Adenocarcinoma colon cell (HT-29) and normal colon cell (CCD-18Co) were purchased from American Type Culture Collection (ATCC). HT-29 cells were cultured in McCoy's 5a Medium Modified (Sigma-Aldrich, USA), while CCD-18Co cells were cultured in Eagle's Minimum Essential Medium (Sigma-Aldrich, USA). Both media were supplemented with 1% penicillin/streptomycin and 10% foetal bovine serum.

2.6. MTT cytotoxicity assay

Both compounds that have been synthesised were screened on both HT-29 cell and CCD-18Co cell using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazholium bromide (MTT) assay (Mosmann, 1983). Through this MTT assay, a serial dilution of both compounds (Compound 1 and Compound 2) was made up to seven concentrations which are 0.16 μM, 0.32 μM, 0.63 μM, 1.25 μM, 2.5 μM, 5 μM with 10 μM as the highest concentration which are. The positive control used in this assay was menadione, while the normal colon cell (CCD-18Co cell) was used as a negative control. Both HT-29 cells and CCD-18Co cells were seeded in 96-well microplate at a density of 5×10^4 mL^{-1} and incubated at 37 °C in 5% CO_2. After 24 h of incubation, the medium in each well was removed and replaced with synthesised compounds. Following 24 h of incubation for cell treatment (37 °C in 5% CO_2), 20 μL of MTT (5 mg mL^{-1} in PBS solution) was added into each well in dark condition. Then, the cells were incubated for 4 h. At the end of the incubation period, 180 μL DMSO was added to dissolve the formazan crystals formed. Then, the 96-well plate was incubated again for 15 min. After that, the cytotoxicity of the compounds was detected using the BIO-RAD microplate reader (iMark) at 570 nm. The graph was plotted as the percentage of viable cells against compound concentrations. Based on the plotted graphs, the IC_{50} values were

determined, where the IC_{50} value represents the reduction of 50% cells population in treated cells compared to untreated cells.

2.7. Modes of cell death

The modes of cell death either through apoptosis or necrosis, were distinguished through Annexin V-FITC/PI test according to the method described by Chan et al., (2012), with slight modifications. HT-29 cells must be seeded first into a sterile 6-well plate at a density 5×10^4 cells mL^{-1}. After 24 h of incubation, the medium in each well was removed and replaced with synthesised compounds at IC_{50} concentrations. Then, the 6-well plate was incubated for another 24 h. After 24 h, the media in each well were collected into different centrifuge tubes. The attached cells were washed using cold PBS and collected into a centrifuge tube. The remaining attached cells were then trypsinised for 1 min to collect the cells suspension into the same tube. After all the cells have been collected, it was centrifuged at 1500 rpm for 3 min. Then, the cell pellet was washed twice using cold PBS after the supernatant was removed. After all the supernatant was removed, the cells were resuspended in 100 μL of Annexin V binding buffer. Then, the cells were stained with 2.5 μL of Annexin V-FITC (BD Pharmingen) for 15 min at room temperature and 5 μL of propidium iodide (BD Pharmingen) for 5 min. Both staining processes were done in a dark condition. Before all the samples were transferred into a Falcon tube, the cells were resuspended in another 400 μL Annexin V binding buffer. Lastly, the cells were analysed using a BDFACSCanto II flow cytometer.

2.8. Selectivity index (SI)

The selectivity index (SI) was determined to assess the ability of two synthesised compounds either to kill selectively between normal cells and cancer cells or to indicate the general cytotoxicity of the compound. Thus, the value of the SI was calculated as suggested by Badisa et al. (2009) using the equation below:

$$\text{Selectivity index (SI)} = \frac{\text{IC50 value of synthesised compound against cancer cells}}{\text{IC50 value of synthesised compound against normal cell}}$$

2.9. Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). The data were analysed using one-way ANOVA using Statistical Package for Social Sciences (SPSS) and considered statistically significant when $p < 0.05$.

3. Results

3.1. Synthesis of compound 1 and compound 2

Adeyemi and friends (2019) have successfully synthesized Compound **2**. However, the two compounds were successfully synthesised using in situ method. Fig. 2 below shows the general reaction schemes for the preparation of two successfully synthesised compounds (diphenyltin(IV) diallyldithiocarbamate and triphenyltin(IV) diallyldithiocarbamate). Meanwhile, the physical and analytical data for compound **1** and compound **2** are presented in Tables 1 and 2.

3.2. Spectroscopic analysis

Table 2 below shows the important vibrational bands obtained from FT-IR spectroscopy for compound **1** and compound **2**. Dithiocarbamate compounds can be identified via the presence of certain important vibrational bands, i.e., ν(C-N) and ν(C = S). The vibrational bands ν(C-N), which is also known as thiourea band and ν (C = S) of two synthesised compounds are observed in the range between 1475 and 1479 cm^{-1} and 972–977 cm^{-1}. Meanwhile, organotin(IV) compounds can be identified via the presence of vibrational bands, ν(Sn-C). The vibrational bands with range 545–546 cm^{-1} indicates the stretching of Sn-C elements for compounds with aryl groups. Based on the IR spectra recorded from the FT-IR analysis, all synthesised compounds showed the presence of the significant vibrational bands; thus, confirming the formation of suggested compounds.

The ^1H and ^{13}C NMR data displayed the signals as expected. Tables 3 and 4 show the important chemical shifts of ^1H and ^{13}C NMR, respectively. Table 3 presents the resonance signals observed in the ^1H NMR spectra for both compounds. Meanwhile, Table 4 shows the resonance signals observed in the ^{13}C NMR spectra. This spectrum exhibited the expected signals, which indicated the presence of significant bonds between the elements in the compounds and CS_2 resonance signals that characterise the existence of dithiocarbamate (-NCS_2) group. The data for single-crystal analysis obtained for compound **1** are shown in Tables 5 and 6, whereas Tables 7 and 8 present the data for compound **2**. The ORTEP plot of compound **1** and compound **2** is given in Figs. 3 and 4, respectively.

3.3. Cytotoxicity of Organotin(IV) diallyldithiocarbamate compounds in HT-29 and CCD-18Co cells

Table 9 shows the IC_{50} values for compounds **1** (diphenyltin(V) diallyldithiocarbamate) and 2 (triphenyltin(IV) diallyldithiocarbamate). Both compounds showed potent cytotoxicity towards HT-29 cells. Based on the graph in Fig. 5, the IC_{50} value for compound **1** is 2.36 μM, while the IC_{50} for compound **2** is lower than compound **1**, i.e., 0.39 μM. Based on the IC_{50} value, compound 2 has higher toxicity compared to compound **1**. This showed that the HT-29 cells had higher sensitivity towards compound **2** compared to compound **1**. The statistical analysis for bot compounds showed significant differences between the percentage of viability of treated and untreated cells ($p < 0.05$) at all concentrations. Table 10 shows the IC_{50} values of CCD-18Co cell for compound **2** and its SI index. Based on the table, compound 2 exhibits high cytotoxicity towards CCD-18Co cells, with an IC_{50} value of 3.52 μM upon 24 h of treatment.

3.4. Selectivity index (SI)

Compound **2** shows good selectivity against HT-29 and CCD-18Co cell lines, with an SI value of more than 2 (Table 10). The high SI value (greater than2) of a compound indicates selectivity towards cancer cells. Meanwhile, a compound with SI value < 2 is considered generally toxic to normal cells (Badisa et al., 2009).

3.5. Mode of cell death on HT-29 cells upon treated with Organotin(IV) diallyldithiocarbamate compounds

Fig. 6 shows the percentage of externalisation of phosphatidylserine in HT-29 cells treated with IC_{50} concentration of both compounds. Based on the graph, both compound **1** and compound **2** showed the percentage of apoptotic cell death as 64.2% and 73.87%, respectively. Whereby the total cell death is 3.9% for compound **1** and 4.57% for compound **2**. The remaining percentage is comprised of viable cells, i.e., 31.87% and 21.6% for both compound **1** and compound **2**, respectively. No significant difference is observed among the percentage of live cells in treated and untreated cells.

Table 1
Analysis data of physical and elemental for Compound **1** and **2.**

Molecular formula	Colour	Yield percentage (%)	Melting point (°C)	Found (calculated) (%)			
				C	H	N	S
C1 $(C_6H_5)_2Sn[S_2CN(C_3H_5)_2]_2$	White	51.84	59.3–61.2	50.20 (50.59)	4.80 (4.86)	4.20 (4.54)	16.85 (20.78)
C2 $(C_6H_5)_3Sn[S_2CN(C_3H_5)_2]$	White	44.52	181.6–183.0	55.48 (57.51)	4.93 (4.79)	3.40 (2.68)	11.12 (12.28)

Table 2
Important infrared vibrational bands of compound **1** and compound **2.**

Compounds	ν(C-H)	ν(C=N)	ν(N-C)	ν(C—S)	ν(Sn-C)	ν(Sn-S)
1	3012.81	1475.54	1230.58	977.91	545.85	441.70
2	3043.67	1479.40	1236.37	972.12	555.50	445.56

Table 3
^{1}H NMR spectra data of compound 1 and compound 2 (δ, ppm).

Compound	C = CH_2	N-CH_2	Sn-R (R = C_6H_5)
1	5.32	4.37	7.38, 7.40, 7.49, 7.51, 7.89
2	5.29	4.46	7.42, 7.43, 7.48, 7.49, 7.50

4. Discussions

4.1. Synthesis of compound 1 and compound 2

Two organotin(IV) diallyldithiocarbamate compounds have been successfully synthesised with a relatively high percentage of yield. This in situ method is performed by the direct and stoichiometric reaction between solutions of metal saline compounds and secondary amines corresponding to the presence of carbon disulfide in 99.8% ethanol solvent. In addition, the reaction between the secondary amine ligands with the aliphatic group R should be performed in cold conditions (-4 °C). This is due to the exothermic reaction (heat release) that occurs due to the production of dithiocarbamic acid, as a result of the reaction between carbon disulphide and secondary amine solution (Baba and Raya 2010).

The results of the elemental analysis performed of the successfully synthesised organotin(IV) compounds confirmed the structural formula of the proposed compound. The structural formula of these compounds is $R_mSn\,[S_2CNR'R'']_{4-m}$ where R′ = C_6H_5 (diphenyl), C_6H_5 (triphenyl), R″ = C_3H_5.

4.2. Spectroscopic analysis

Infrared spectroscopy (FTIR) analysis was performed to determine the coordination mode and bond properties inherent in the synthesised compound. Infrared spectroscopy is a powerful analysis to detect changes caused by ligands in biomolecules because they have different signals and provide different levels of structural features (Kumar, 2010). The dithiocarbamate compounds can be identified through the presence of specific absorption peaks, especially ν(C---N) and ν(C---S) (Kartina et al., 2019). The absorption peaks given at the vibrations ν(C---N) or named as thioreide bands on organotin(IV) diallyldithiocarbamate compounds in this study are between 1475 and 1479 cm^{-1}, where the thioreide absorption band is at the highest wavenumber. The results of this study are in line with the study by Adeyemi and colleagues (2020) who stated that the compounds performed by infrared spectroscopy analysis were also at 1503–1478 cm^{-1} due to the removal of electrons to the tin centre of the compound. The absorption peaks will move to a higher frequency due to its sensitivity to the replacement of tin atoms, with the introduction of more electronegative substituents (Muthalib et al., 2011).

Another important absorption peak in dithiocarbamate compounds is ν(C---S). A single band at the absorption peak of 1008–996 cm^{-1} is associated with the ν(C---S) absorption band, which indicates the bidentate coordination mode (Adeyemi et al., 2020). The presence of ν(C---S) absorption bands located at 960–1005 cm^{-1} also concludes that the ligand is attached as bidentate (Muthalib et al., 2011). In this study, the ν(C---S) absorption band of the compounds is at a strongly absorbed intensity between 972 and 978 cm^{-1} as a result of the carbon–sulphur bond stretching, with the wavenumber range showing that the bond has a double feature, which is C --- S. Thus, the results of the study obtained from this study met the criteria proposed in the previous study.

The ^{13}C NMR signalling for the CS_2 group is simple and is given in the range of δ 197–200 ppm, indicating that sulphur is coordinated with the Sn atom organotin(IV) diallyldithiocarbamate compounds. The results of this study are congruent to a previous study, where the ^{13}C spectrum showed a weak signal of thioreide carbon (–NCS_2) for all diorganotinIV) benzyldithiocarbamate compounds at δ 202–200 ppm (Adeyemi et al., 2020). Therefore, the results obtained in this study have been further strengthened with the results of the study.

The study of the organotin (IV) diallyldithiocarbamate by Adeyemi and colleagues (2019) also presented the determination of the crystal structure. Using different organotin(IV) series in this study, compound **1** (diphenyltin[IV] diallyldithiocarbamate) and **2** (triphenyltin[IV] diallyldithiocarbamate) also produced single colourless crystals. The crystal size is 0.19 × 0.14 × 0.07 mm and 0.13 × 0.10 × 0.04 mm with the mass being 617.45 g and 522.27 g, respectively. Both compounds have a monoclinic crystal system with a $P2_{1/c}$ space. The parameters of compound crystal units 4 are a = 9.6160 (1) Å, b = 30.4216 (2) Å, c = 19.1928 (1) Å,

Table 4
^{13}C NMR spectra data of compound 1 and compound 2 (δ, ppm).

Compound	CH = CH_2	N-CH_2	N-CS2	Sn-R (R = C_6H_5)
1	141.92	57.00	200.82	119.00, 128.25, 129.12, 130.51, 134.29, 135.75
2	142.24	57.28	197.79	119.02, 128.54, 129.18, 130.51, 136.16, 136.75

Table 5
Crystallographic data and refinement parameters for compound **1**.

Compound **1**	
Empirical formula	$Sn(C_6H_5)_2(C_7H_{10}NS_2)_2$
Formula weight	617.45
Crystal system	Monoclinic
Space group	$P2_1/c$
Crystal size (mm)	0.19 × 0.14 × 0.07
a (Å)	9.6160 (1)
b (Å)	30.4216 (2)
c (Å)	19.1928 (1)
β (°)	100.019 (1)
V (Å)	5528.93 (8)
Z	8
D/Mgm^{-3}	1.484
μ (mm^{-1})	10.30
F (000)	1080
Colour	Colourless
Temperature (K)	100
θ range (°)	2.9–76.3
Index ranges (±h, ±k, ±1)	$-11\leq h\leq 11$, $-36\leq k\leq 25$, $-22\leq l\leq 22$
Reflection collected	67,735
Independent reflections	9876 [R(int) = 0.037]
Reflections with $I > 2\sigma(I)$	9370
Refined parameters	595
Largest diff. peak and hole (e Å^{-3})	1.23 dan −0.73

Table 6
Selected bond lengths (Å) and angles (°) for compound **1.**

Bond lengths (Å)		Bond angles (°)	
Sn1-S3	2.5726 (7)	C21-Sn1-C15	99.84 (9)
Sn1-S4	2.6754 (6)	C15-Sn1-S1	104.01 (7)
Sn1-S1	2.5501 (6)	C21-Sn1-S1	92.77 (7)
Sn1-S2	2.7393 (7)	C15-Sn1-S2	91.81 (7)
Sn1-C15	2.159 (3)	C21-Sn1-S3	101.09 (7)
Sn1-C21	2.174 (2)	S1-Sn1-S3	154.38 (2)
S1-C1	1.742 (3)	C15-Sn1-S4	160.59 (7)
S2-C1	1.710 (3)	C21-Sn1-S4	92.52 (7)
S3-C8	1.738 (3)	S1-Sn1-S4	90.18 (2)
S4-C8	1.715 (3)	S4-Sn1-S2	67.824 (19)
N1-C1	1.326 (3)	S3-Sn1-S4	67.97 (2)
N1-C2	1.465 (3)	Sn1-S3-C8	89.22 (9)
N1-C5	1.481 (3)	Sn1-S4-C8	86.37 (9)
N2-C8	1.318 (3)	C8-N2-C12	122.5 (2)
N2-C12	1.477 (3)	C8-N2-C9	122.6 (2)
N2-C9	1.486 (3)	C1-N1-C5	122.6 (2)
C12-C13	1.496 (5)	C1-N1-C2	122.5 (2)
C9-C10	1.496 (4)	N1-C2-C3	112.5 (2)
C5-C6	1.498 (4)	N1-C1-S2	123.62 (19)
C21-C26	1.391 (4)	N1-C1-S1	118.58 (19)
C15-C20	1.392 (4)	S2-C1-S1	117.77 (14)

and β = 100.019 (1)°. Whereas, *a* = 8.0650 (1) Å, *b* = 11.4490 (1) Å, *c* = 25.8875 (2) Å, and β = 98.282 (1)° are the case parameters for compound **2** (Haezam et al., 2020).

The results of the study of crystallography of compound **1**, based on the appropriate length and angle of binding indicated that the four sulphur atoms of the ligand dithiocarbamate coordinate unequally on the tin atoms. Two parts of the ligand dithiocarbamate are bound to the stanum atom in organotin(IV) through two bidentate sulphur atoms of the ligand. Thus, the structure of these compounds produces an octahedral environment around the tin atom (Adeyemi et al., 2018). The structure uniformly has sulphur atoms that form a shorter Sn-S bond to one side of the SnS_4 plane, and this explains the geometry of this structure being skew-bipyramidal. This indicates that the dithiocarbamate ligand is asymmetrically coordinated with the phenyl substituent bound to the stanum atom.

While for compound **2**, it was found that the results for the bond length of Sn-S1 are 2.4749 (4) Å, while the bond length of Sn-S2 is 2.9456 (7)Å (Haezam et al., 2020). The short Sn-S bond distance falls in the range of 2.45–2.48 Å, while the long Sn-S bond distance falls in the range of 2.92–3.24 Å (Tiekink 2008). Thus, there is a large difference in the separation of Sn-S, where (Sn-S) = [(Sn-S long) - (Sn-S short)] = 0.47 Å, which indicates that the interaction of Sn - S2 is weak. On the other hand, if the coordinate geometry is considered tetrahedral C_3S_2, i.e. the weak Sn-S2 bond is ignored then the tetrahedral angle range is 91.01 (5)°, for S1 - Sn - C31, to 128.76 (5)°, for S1-Sn-C11. Sulphur atoms are involved in forming a longer Sn---S bond that occupies one of the axial positions. Thus, the dithiocarbamate ligand for the crystals of this compound is asymmetrically coordinated, where C_3S_2 determines the geometry between the square pyramid and the bipyramidal trigonal.

Table 7
Crystallographic data and refinement parameters for compound **2.**

Compound **2**	
Empirical formula	$Sn(C_6H_5)3(C_7H_{10}NS_2)]$
Formula weight	522.27
Crystal system	Monoclinic
Space group	$P2_1/c$
Crystal size (mm)	0.13 × 0.10 × 0.04
a (Å)	8.0650 (1)
b (Å)	11.4490 (1)
c (Å)	25.8775 (2)
β (°)	98.282 (1)
V (Å)	2364.51 (4)
Z	4
D/Mgm^{-3}	1.467
μ (mm^{-1})	10.32
F (000)	1056
Colour	Colourless
Temperature (K)	100
θ range (°)	3.4–76.3
Index ranges (±h, ±k, ±1)	$-9\leq h\leq 9$, $-13\leq k\leq 13$, $-26\leq l\leq 30$
Reflection collected	55,086
Independent reflections	4226 [R(int) = 0.044]
Reflections with $I > 2\sigma(I)$	4083
Refined parameters	262
Largest diff. peak and hole (e Å^{-3})	0.82 dan −0.50

Table 8
Selected bond lengths (Å) and angles (°) for compound **2**.

Bond lengths (Å)		Bond angles (°)	
Sn-S1	2.4749 (4)	C31-Sn-S1	91.01 (5)
Sn-S2	2.9456 (5)	C11-Sn-S1	128.76 (5)
Sn-C31	2.1673 (19)	C21-Sn-S1	110.28 (5)
Sn-C11	2.1427 (19)	C32-C31-Sn	121.84 (15)
Sn-C21	2.130 (2)	C12-C11-Sn	118.09 (14)
S1-C1	1.7559 (19)	C22-C21-Sn	122.33 (15)
S2-C1	1.6894 (19)	S1-Sn-S2	65.470 (14)
C1-N1	1.330 (3)	S1-C1-S2	118.29 (11)
N1-C2	1.468 (3)	S1-C1-N1	117.96 (14)
N1-C5	1.477 (2)	S2-C1-N1	123.75 (14)
C2-C3	1.502 (3)	C1-N1-C2	123.08 (16)
C5-C6	1.493 (3)	C1-N1-C5	121.51 (16)
C31-C32	1.395 (3)	N1-C2-C3	112.04 (17)
C11-C12	1.393 (3)	N1-C5-C6	110.80 (17)
C21-C22	1.395 (3)	C5-C6-C7	124.0 (2)

4.3. Cytotoxicity of Organotin(IV) diallyldithiocarbamate compounds in HT-29 and CCD-18Co cells

Cytotoxic activity shows that the IC_{50} value of compound **2** is lower than compound **1**, which indicates that the cytotoxic effects of compound 2 are higher than compound 1. The results of this study can be proved by previous studies by Pellerito and colleagues (2006), who stated that the cytotoxic effects of organotin(IV) compounds are dependent on their structure. The toxicity

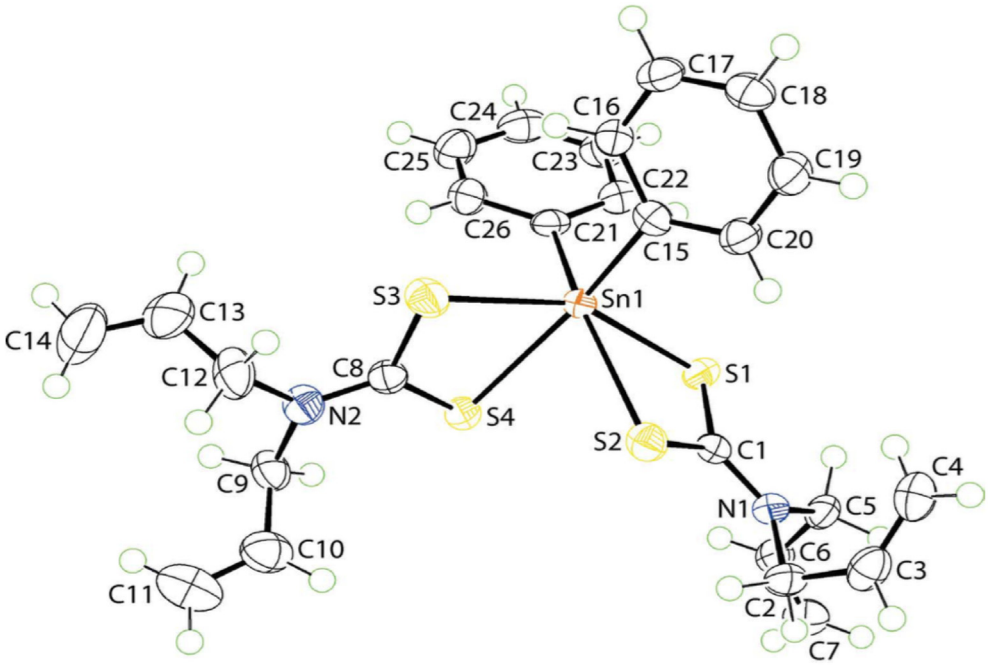


Fig. 3. ORTEP plot of compound **1** at 50% probability level.

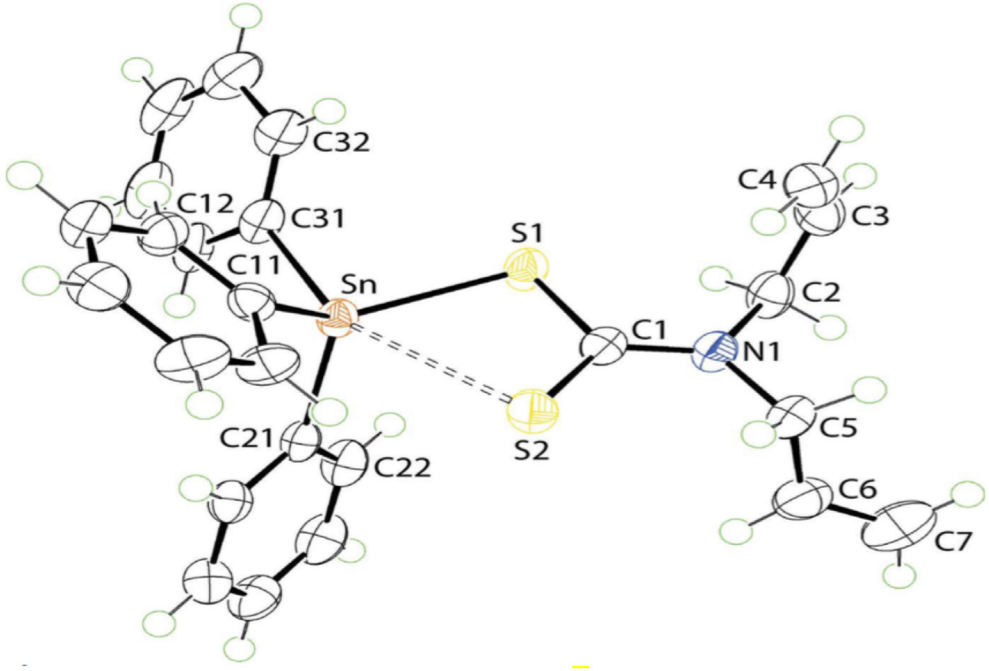


Fig. 4. ORTEP plot of compound **2** at 50% probability level.

Table 9
IC_{50} value of compound **1** and compound **2** against HT-29 cells.

Compound	IC_{50} (μM)
1	2.36
2	0.39

properties of an organotin(IV) compound can be influenced by the properties of the compound and the number of alkyl groups attached to the stanum atom (Hadjikakou and Hadjiliadis, 2009; Awang et al., 2011). The functional group R on the stanum atom is believed to induce and increase in vitro toxicity to the tested cells. For the same ligand, the same structure, but with different organic groups, the difference is very significant in in vitro activity. Generally, organotin sequence activity is a group of RSn_3 + <$R2Sn_2$ + <R_3Sn + (Adeyemi et al., 2019). Alkyl groups bound to the stanum atomic centre play an important role in their toxicity (Hong et al., 2015). Thus, certain functional elements or groups in organotin(IV) compounds in dithiocarbamate provide antiproliferative effects on treated cells (Pellerito et al., 2006).

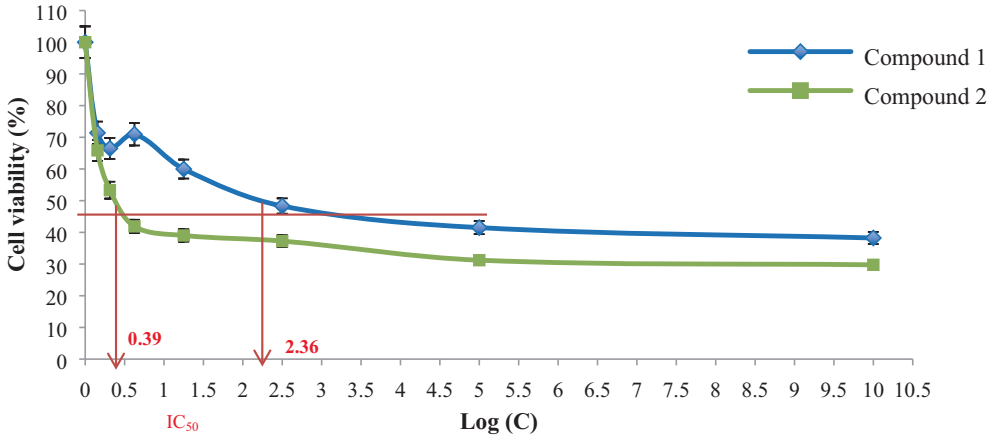


Fig. 5. The cytotoxicity of organotin(IV) diallyldithiocarbamate compounds against HT-29 cells upon 24 h of treatment.

Table 10
IC_{50} value of compound 2 against CCD-18Co cells.

	Compound **2**
IC_{50}, CCD-18Co	3.52
IC_{50}, HT-29	0.39
SI	9.03

Compound 2 was tested on CCD-18Co cells to find the value of the selectivity index (SI). It was found that compound **2** (triphenyltin[IV] diallyldithiocarbamate) have a selectivity index value of more than 2. Thus, compound **2** is categorised as a selective drug because the compounds can induce the response of this drug to select colon cancer cells (HT-29) and not normal cells (CCD-18Co). The SI can indicate the differentiation activity of compounds, where the higher the SI value, the more selective the compound against cancer cells (Badisa et al., 2009). Therefore, this selective cytotoxicity is very important to identify the effectiveness of anticancer drugs (Attanzio et al., 2019).

Based on the results of Annexin V-FITC/PI test, the treatment effect of organotin (IV) dithiocarbamate compounds showed a high apoptotic effect during the 24-hour treatment period, especially for compounds **1** and **2**. Both compounds had high percentage of cell death by apoptosis and low percentage of cell death by necrosis. These two compounds also have good potential when affecting more than 50% HT-29 cell mortality after treatment using IC_{50} concentrations for 24 h.

5. Conclusions

Two organotin(IV) diallyldithiocarbamate compounds have been successfully synthesized using in situ method and were characterised using elemental and spectroscopic analysis. The structure of two compounds (diphenyltin(IV) diallyldithiocarbamate and triphenyltin(IV) diallyldithiocarbamate) were also confirmed through X-ray single crystal crystallography studies. Triphenyltin (IV) diallyldithiocarbamate (Compound **2**) compound showed strong cytotoxicity in HT-29 cells and showed good selectivity between HT-29 cells and CCD-18Co cells. Other than that, upon treatment with IC_{50} concentration of both compounds, its efficacy to kill HT-29 cells were as expected, where 64–74% of cells died through apoptosis, while only 3–5% died through necrosis. Hence, this study suggested that the mode of cell death induced by these synthesised compounds is selective towards cancer cells.

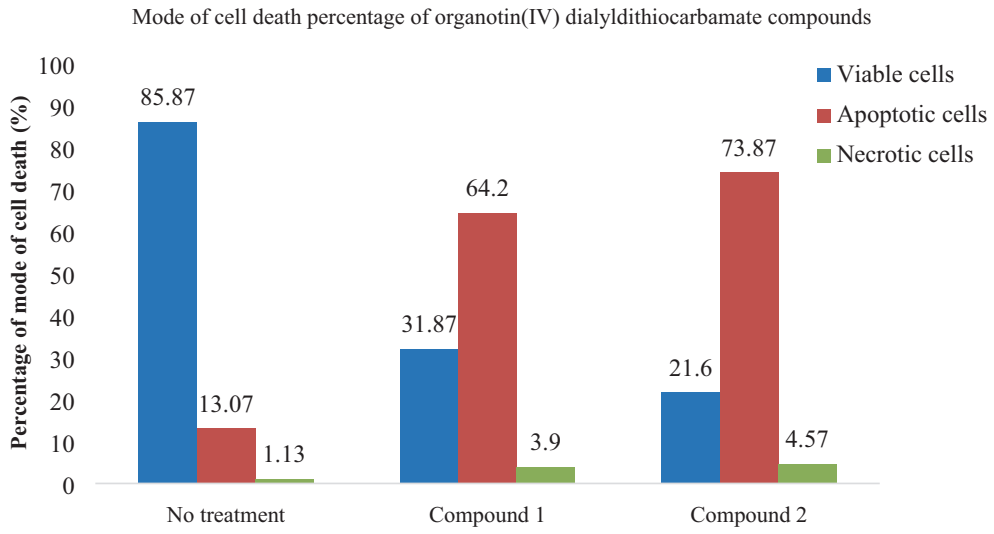


Fig. 6. The percentage of viable, apoptotic and necrotic cells in HT-29 cells upon treated with compound **1** and compound **2** at IC_{50} concentration for 24 h.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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