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ORIGINAL ARTICLE





Organotin complexes with Schiff's base ligands: insights into their cytotoxic effects on lung cancer cells

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Abstract

Organotin(IV) complexes can be used in chemotherapy due to its lipophilicity which can be affected by the availability of Sn coordination bond and bond stabilization between ligand and Sn(IV). In this study, three types of tri-organotin(IV) complexes which are, Ph_3SnL , Me_3SnL , and Bu_3SnL derived from Schiff base ligand were synthesized by the reaction of methyl dopa with p-dimethyaminobenzaldehyde. All prepared complexes were charechterised using nuclear magnetic resonance (¹H NMR, ¹³C NMR, and ¹¹⁹Sn NMR. The ¹H NMR). The results confirm the coordination of the organotin(IV) moieties to the ligand. The cytotoxicity of tri-organotin(IV) complexes was evaluated against the A549 human lung cancer cell using MTT assay. Ph_3SnL demonstrated a relatively lower effects. These findings highlight the potential of the tri-organotin(IV) complexes, particularly Ph_3SnL and Bu_3SnL , as promising candidates for further modification as anticancer agents. The results obtained from this study can be used to understand the structure–activity of organotin(IV) complexes and their applications as anti-cancer activity.

Keywords Tri-organotin(IV) complexes \cdot Anti-drugs cancer \cdot MTT assay \cdot A549 human cell \cdot Half-maximal inhibitory concentration (IC50)

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1 Introduction

Lung cancer is one of the most lethal malignancies, and it remain a significant health challenge in the world. Therfore the demand to continuous improve the therapeutic agents aganst cancers still in needed [1, 2]. The organotin(IV) complexes have attracted great attention due to its chemical structures and biological activities [3, 4], and it shows a potential cytotoxic agents against a numerous of cancer cell lines study [5]. In addition, Schiff base ligands have demonstrated a significant ability in the synthesis of different metal complexes [6–8]. These ligands possess versatile coordination abilities and have been utilised in the preparation of organotin(IV) complexes [9]. Many studies have been reported the synthesis and characterization of organotin complexes using Schiff base ligands derived from different aldehydes and amines, and evaluated its activity as anticancer drugs [10]. The biological activity affected by organic groups and the coordination geometry around tin atom [11, 12]. The functional groups of selected ligand can modulate the physicochemical properties of the prepared complexes,

which enhance the anti-cancer activity [13]. Therefore, the synthesized organotin(IV) complexes of new Schiff base ligands considered a good candidate for developing of novel anticancer agents. In terms of cytotoxicity evaluation, the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay is a widely used to assess cell viability and proliferation [14, 15]. This assay depends on the reduction of MTT by mitochondrial enzymes to formazan crystals, which can be measured by spectrophotometer. By measuring the reduction in cell viability caused by the complexes, the cytotoxic effects and its efficacy against cancer cells can be determined. Several studies have been done to study the cytotoxic properties of organotin(IV) complexes against various cancer cell lines [16, 17]. For example, a research done by Abbas et al. (2022) reported the synthesis of novel organotin(IV) complexes with Schiff base ligands and evaluated their cytotoxicity in vitro in silico bioactivities [18]. The results demonstrated a cytotoxic effects and emphasized the potential of these complexes as anti-cancer agents. Moreovere, Wang et al. (2022) prepared a different of organotin(IV) complexes with Schiff base ligands and tested their cytotoxic activities against human lung cancer cells [19]. The complexes exhibited a significant cytotoxicity, demonstrating their potential as anti-lung cancer. Based on the studies done before, organotin(IV) complexes derived from Schiff base ligands considered one of the most important drug to be used as anticancer agent [20, 21]. However, further study needed to explain the structure-activity and optimize the design of these complexes to enhance its efficacy in this field. In this study, we synthesized and characterized different organotin(IV) complexes structures derived from Schiff base compounds to assessed its activity against lung carcinoma epithelial cells (A549 cell line).

2 Experimantal part

2.1 Chemicals

Methyl dopa was purchased from Scharlau Company, while ethanol, methanol, glacial acetic acid, organotin compounds and para-dimethyaminobenzaldehyde were obtained from Fluka.

2.2 Charechterization

2.2.1 Fourier transform infrared spectroscopy (FTIR)

FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory (ALPHA II Compact FTIR Spectrometer Bruker) was ustelised in the rang ($4000-400 \text{ cm}^{-1}$) to provid information about functional groups and molecular



Scheme 1 Synthesis of Schiff base ligand (L)

structure by analyze the absorption bands and transmission of infrared light.

2.2.2 Nuclear magnetic resonance (NMR)

NMR spectroscopy (Bruker) was used to provides information about the chemical structure and connectivity of atoms.

2.2.3 Scanning electron microscopy (SEM)

SEM quanta 450 (FEI,USA) was utilised to get image of the surface morphology and microstructure of samples.

2.2.4 Energy-dispersive X-ray spectroscopy (EDX)

EDS is an elemental analysis to identify the compositions of different elements in a specific sample that combines SEM with X-ray spectroscopy.

2.3 Synthesis of Schiff base ligand (L) [22]

A Schiff base was prepared by dissolving 0.25 g (0.1 mol) of methyl dopa and 0.164 g (0.1 mol) of para-dimethylaminobenzaldehyde in 15 mL of ethanol. The mixture was stirred until full dissolve. To catalyzed the reaction, 3 drops of glacial acetic acid were added, and the mixture was refluxed for 3 h as shown in Scheme 1. During this period, a yellowwhite precipitate of Schiff base ligand (L) was formed. Then the reaction mixture was cool down to room temperature and fillterate to get the crude product as yellow-white power. The crude product was recrystallized twice from ethanol to produce pure target product which was used for further characterization and applications.

2.4 Reaction of Schiff base ligand (L) and organotin(IV) complexes

A 0.20 g of synthesized Schiff base ligand was reacted with Ph₃SnCl (0.22 g), Bu₃SnCl (0.20) g), and Me₃SnCl (0.11g) using 1:1 metal/ligand. The mixtures were refluxed for 6 h at 65 °C in 15 ml methanol as shown in Fig. 2 [23]. The final solutions were filtered, then washing, drying, and recrystallization, to produce Ph₃SnL, Bu₃SnL, and Me₃SnL complexes as off-white powders (Scheme 2). The physical properties of ligand and synthesized organotin complexes are listed in Table 1.

2.5 Cell cultures study

Lung Carcinoma Cells (A549 cell line) was cultured in RPMI-1640 medium with HEPES supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotic and incubated at 37° C in CO₂ (5%) incubator [24]. When cells reached 80-90% confluency, cells were subcultured into new cell culture dishes using the new RPMI-1640 medium (every 2-3 days). The cell line was provided by the Biotechnology Research Center/Al-Nahrain University.

2.6 In vitro cytotoxicity

Scheme 2 Synthesis of the

proposed mechanisum

The cytotoxic potentials of synthesized tri-organotin (IV) complexes were assessed using MTT assay against A549 cells. An aliquot of 200 µL of A549 cells was maintained in 96-well bottom-flat culture plate at a density of 1×10^5

Table 1 The synthesized organotin complexes' physical characteristics

Compounds	Color	Melting point °C	Yield %
L	White	266–268	81
Ph ₃ SnL	Off white	290–292	84
Me ₃ SnL	Off white	294–296	82
Bu ₃ SnL	Off white	297–299	80

cells/mL. Cells were incubated for 24 h to allow the cells to attach and reach the log phase at the time of tri-organotin (IV) complexes exposure. After incubation, cells were treated with a concentrations of (8, 16, 32, 64, 125, 250, 500 and 1000 μ g/mL) of the synthesized complexes for 24 h. After incubation, the medium was removed, and cells were labeled with 50 µL of MTT solution (5 mg/mL in phosphate buffer saline) for 4 h at 37°C. The resulting formazan was solubilized with 100 µL dimethyl sulfoxide. Incubation was continued for further 10 min and absorbance was measured at 570 nm using a microtiter plate reader (Bio-Rad, USA). The viability of A549 cells was expressed as a percentage of the value for control value according to the below equation and IC₅₀ was calculated for each complex depending on viability data [25].

Cell Viability (%) = Sample A570/Control $A570 \times 100\%$

2.7 Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.2 (GraphPad Software Inc., La Jolla, CA) to estimate the effect of variable factors on the main study [26]. One-way ANOVA (Tukey Test) was used to determine whether group variance was significant or not. Data were



expressed as mean \pm standard deviation and statistical difference was considered at the level of p < 0.05.

2.8 Characterization of synthesized organotin(IV) complexes

2.8.1 Scanning electron microscopy (SEM)

SEM was utilized to provides a valuable information about the surface morphology and surface structures of synhesised complexes [27]. Sample prepared by mounted a small amount of ligand and complexes onto a sample holder and sputter-coated with a thin layer of conductive material, such as gold, to improve the conductivity and minimize charging effects during imaging. The SEM images of the ligand was uniform and well-defined particles with surface features, indicating its crystalline nature and regular crystal growth as shown in Fig. 1. The morphology of the ligand appeared to be in the form of amorphous and microcrystalline structures, which showed a characteristic shape and size distribution.

While the SEM image of organotin(IV) complexes showed different particle size and surface texture, indicating the effect of the coordination of Shiff base ligand with organotin(IV) moieties as shown in Fig. 1. These changes in surface morphology may suggest the formation of larger aggregates or the presence of new surface features resulting from the coordinated bonds. In general, SEM analysis of the ligand and its complexes provid a valuable evidence about the surface characteristics and morphological features. This characterization technique added other analytical methods and contributed to a comprehensive understanding of the complexation of ligand and organotin(IV) complexes.



Fig. 1 SEM images of a Ligand (L), b Ph₃SnL complex, c Bu₃SnL complex, and d Me₃SnL complex

2.8.2 Energy dispersive X-ray (EDX)

EDX was used to provide the elemental composition of the prepared complexes. EDX analysis allows the identification and quantification of elements exist in the ligand and synthesised complexes based on their characteristic X-ray emission spectra. A small amount of the samples were mounted on a sample holder, and the EDX detector was positioned to capture the X-ray emissions generated by the interaction of the sample with an electron beam. The detector measures the energy and intensity of the X-rays emitted by the elements present in the prepared samples [28].

EDX analysis of the ligand provided an information about the elements constituting the ligand molecule. The spectrum obtained revealed the presence of carbon, hydrogen, nitrogen, and oxygen, which are typically found in synthesized organic compound (L) as shown in Fig. 2. The relative intensities of the peaks in the spectrum provide insights into the stoichiometry of the synthesized ligand. In the case of the organotin(IV) complexes, EDX analysis confirm the presence of tin (Sn) along with the elements identified in the ligand spectrum as shown in Fig. 2. The relative intensities of the tin peaks compared to the ligand peaks indicated the successful coordination of the ligand with the tin atom in the complexes. The existance of additional elements in the EDX spectrum could provide information about other possible impurities or elements obtained during the synthesis.

2.8.3 Fourier transform infrared spectroscopy (FTIR)

FTIR was utilized to provide information about the functional groups exist in the prepared complexes by measuring the absorption of infrared by the molecules [29]. The specific band at 3469 cm⁻¹, indicates the presence of O–H stretching, and the bandss at 3097 cm⁻¹ and 2926 cm⁻¹ were assigned to aromatic C–H stretching, while the band at 1636 cm⁻¹ indicated the presence of C=O group. In addition, a band at 1592 cm⁻¹ assigned to the presence of C=N of Schiff bases.

For the Ph₃SnL complex, the FTIR showed similar bands as the ligand, with a small shifts. The O–H stretching bands recognised at 3469 cm⁻¹, while the C–H stretching aromatic was at 3098 cm⁻¹. The C-H stretching aliphatic were observed at 2922 cm⁻¹. The C=O band was at 1640 cm⁻¹, and the C=N observed at 1589 cm⁻¹. A new bands were appeared at 527 cm⁻¹ and 453 cm⁻¹, confirming the presence of Sn–C and Sn–O bonds in the complex. The FTIR of Bu₃SnL complex showed bands attributed to the ligand's functional groups [30]. The O–H stretching band observed at 3472 cm⁻¹, while the C-H stretching aromatic observed at 3097 cm⁻¹. The aliphatic C–H stretching were appered as a very sharp and strong bandss at 2956 cm⁻¹ and 2921 cm⁻¹. The C=O and C=N bands gave two bands at 1637 cm⁻¹ and 1598 cm⁻¹ respectively. The presence of Sn–C and Sn–O bonds were confirmed by specific bands at 525 cm⁻¹ and 451 cm⁻¹ respectively. The FTIR of Me₃SnL complex showed O–H stretching band at 3380 cm⁻¹, while the aromatic C-H stretching gave band at 3095 cm⁻¹. The aliphatic C-H stretching were appeared at 2940 cm⁻¹. The C=O and C=N bands appeared at 1638 cm⁻¹ and 1590 cm⁻¹ respectively. While, bands at 527 cm⁻¹ and 452 cm⁻¹ confirmed the presence of Sn–C and Sn–O bonds in the formation of the complexes [31]. All FTIR data for synthesized ligand and corresponding complexes were summarized in Table 2.

2.8.4 Nuclear magnetic resonance (¹H-NMR)

The ¹H NMR spectrum of the synthesized Schiff base ligand (L) provides detailed information about its structural features and chemical environments as shown in Fig. 3 [32]. 8.43 (s, 1H, N=CH), this peak corresponds to the proton of the imine group (N=CH), indicating the formation of the Schiff base. The singlet (s) nature of the peak suggests that this proton is not coupled to any nearby protons. 8.02 (s, 1H, OH) and 7.82 (s, 1H, OH), these peaks correspond to the hydroxyl (OH) protons, indicating the presence of two hydroxyl groups in the Schiff base. The singlet nature of these peaks suggests that these protons are not coupled to any nearby protons. 7.33 (d, J = 8.5 Hz, 2H, Ar) and 6.63 (d, J = 8.5 Hz, 2H, Ar), these peaks represent the aromatic (Ar) protons. The presence of a doublet (d) indicates that each of these aromatic protons is coupled to two neighboring protons, resulting in a splitting pattern with a coupling constant of 8.5 Hz. Thus 6.53 (m, 2H, Ar) peak corresponds to two aromatic protons that show a multiplet (m) pattern. The multiplet suggests the presence of different neighboring protons, resulting in multiple splitting patterns. While 6.46 (m, 1H, Ar) peak represents a single aromatic proton that exhibits a multiplet pattern, indicating the presence of various neighboring protons. The influence of aromatic substitution on the chemical shift in proton NMR can be explained by the concept of electronic effects. Electron-donating groups, such as hydroxy or amine groups, can donate electron density to the aromatic ring, which leads to shielding of nearby protons. As a result, these protons experience a lower effective magnetic field and appear at lower chemical shift values as shown in Fig. 3.

The peaks at 3.13 (d, J = 12.4 Hz, 1H, CH) and 2.95 (d, J = 12.4 Hz, 1H, CH) correspond to the methylene (CH₂) protons adjacent to a chiral carbon atom. The presence of two doublet peaks arises due to diastereotopicity. In a chiral molecule, the two protons in the CH₂ group experience different chemical environments, leading to non-equivalent coupling patterns. As a result, they give rise to two separate doublet peaks with a coupling constant of 12.4 Hz. 2.90 (s, 6H, 2CH₃), this peak represents the methyl (CH₃) groups,



Fig. 2 EDX patterns of a Ligand (L), b Ph₃SnL complex, c Bu₃SnL complex and d Me₃SnL complex

Table 2FTIR of ligand andcomplexes

Compounds	Wave number cm ⁻¹						
	–OH	C–H Ar	C–H Alph	C=0	C=N	Sn–C	Sn–O
L	3469	3097	2926	1636	1592	_	_
Ph ₃ SnL	3469-3202	3098	2922	1640	1589	540	453
Bu ₃ SnL	3472-3199	3097	2956-2921	1637	1598	525	451
Me ₃ SnL	3380	3095	2940	1638	1590	527	452



Fig. 3 ¹H-NMR spectrum of ligand

indicating the presence of two methyl groups in the Schiff base. The singlet nature of this peak suggests that these protons are not coupled to any neighboring protons. Finally the peak at 1.38 (s, 3H, CH_3) chemical shift corresponds to the methyl (CH_3) group, indicating the presence of a single methyl group. Similar to the previous case, the singlet nature of this peak suggests that these protons are not coupled to any neighboring protons.

The ¹H NMR analysis of the synthesized Schiff base ligand (L) confirm the presence of different functional groups. The observations of singlets, doublets, multiplets, and diastereotopic splitting patterns support in the identification of specific proton and the characterization of the ligand's structural features. Table 3 summarized the ¹H NMR spectrums data for the synthesized ligand and its complexes.

The ¹H NMR spectrum of the synthesized Ph₃SnL complex provides insights into the changes in chemical shifts compared to the ligand (L) alone. Let's discuss the spectrum in more detail and compare it to the ligand data see Table 3 and Fig. 4. The peak at 8.76 (s, 1H, N=CH) corresponds to the proton of the imine group (N=CH) in the complex. The chemical shift is slightly upfield compared to the ligand, indicating a change in the electronic environment due to coordination with the tin atom. Furthermore, the peak at 8.58 (s, 1H, OH) which assigned to the hydroxyl (OH) proton in the synthesized complex. Similarly, the imine group, the chemical shift is slightly upfield compared to the ligand, proposing the effect of the Sn coordination on the electronic field of the OH group. While, the peaks at 7.52-7.30 (m, 1H, OH; 2H, Ar; 15H, 3Ph groups), were assigned to the aromatic protons in the prepared complex. The multiplet (m) pattern indicates the existance of different neighboring protons. The chemical shifts of the aromatic protons show slight variations compared to the ligand, which demonstrate





an impact of Sn coordination on their electronic surroundings. The integration of this peak shows 18 hydrogen atoms 15 of them belongs to the three phenyl groups linked to the tin atom. 6.75-6.58 (m, 5H, Ar, from L part) peaks represent the aromatic protons originating from the ligand portion. The multiplet pattern predict the existence of different adjusing protons. Similarly, the aromatic protons in the prepared complex, the chemical shifts of these protons show slight differences compared to the ligand, which predict a limited impact of Sn coordination. Peaks at 3.28 (d, J=12.4 Hz, 1H, CH) and 3.01 (d, J = 12.4 Hz, 1H, CH): were attributed to the methylene (CH₂) protons next to to the chiral carbon atom in the complex. The existence of doublets predict the coupling with one adjacent proton, and the chemical shifts are similar to those in the ligand, proposing that the Sn coordination has a minimal effect on these protons. The peak at 2.90 (s, 6H, 2CH₃), attributed to the methyl (CH₃) groups in the prepared complex. The chemical shift was similar compared to the ligand, demonstrating that the Sn coordination does not affect the electronic surroundings of these groups. The peak at 1.53 (s, 3H, CH_3), attributed to the methyl (CH_3) group, and the chemical shift as same as the ligand, demonstrating that the Sn coordination has minimal impact on the electronic field of this group.

Generally, the ¹H NMR of the synthesized Ph_3SnL complex shows slight changes in chemical shifts compared to the ligand, which predicting some electronic changes accourding to the coordination with the Sn atom. On the other hand, the overall structural features and chemical structure of the ligand are conserved in the complex. The aromatic protons and the methylene protons adjacent to the chiral carbon show slight differences in their chemical shifts, which predict an impact of Sn coordination on their electronic surroundings. The -CH₃ groups show no changes, predicting that they are no influnced by Sn atom.

The ¹H NMR analysis of the Bu_3SnL complex gave information about the changes in chemical shifts related to the ligand (L) (Fig. 5). The peak at 8.56 (s, 1H, N=CH attributed



Fig. 4 ¹H-NMR spectrum of Ph₃SnL complex

to the proton of the imine group (N=CH) in the prepared complex. The chemical shift is similar to the ligand, proposing that the electronic fields of this group is slightly affected by the coordination with the butyltin group. While the peak at 8.17 (s, 1H, OH) and 7.86 (s, 1H, OH assigned to the hydroxyl (–OH) protons in the synthesized complex. The chemical shifts are quite similar to those in the ligand, predicting that the coordination with the butyltin group has negligible effect on the electronic fields of the -OH groups. The peak at 7.33 (d, J=8.5 Hz, 2H, Ar) attributed to the aromatic protons in the prepared complex.

The existance of a doublet pattern propose coupling with two adjusting protons, and the chemical shift is similar to the ligand, predicting that the Sn coordination has a negligible impact on the electronic field of protons. The peak at 6.68-6.52 (m, 5H, Ar) attributed to the aromatic protons in the complex. The multiplet pattern proposed the presence of different neighboring protons. The chemical shifts are similar to those in the ligand, indicating that the tin coordination does not significantly affect the electronic environment of these protons. 3.42 (d, J = 12.4 Hz, 1H, CH): This peak corresponds to the methylene (CH₂) proton adjacent to the chiral carbon atom in the complex. The presence of a doublet indicates coupling with one neighboring proton. The chemical shift is slightly upfield compared to the ligand, indicating some electronic modifications upon coordination with the butyltin group. 2.99 (d, J = 12.4 Hz, 1H, CH): This peak represents another methylene (CH₂) proton adjacent to the chiral carbon atom in the complex. The presence of a doublet indicates coupling with one neighboring proton. The chemical shift is comparable to the ligand, suggesting that the tin coordination has minimal influence on the electronic environment of this proton. 2.88 (s, 6H, 2CH₃): This peak represents the methyl (CH₃) groups in the complex. The chemical shift remains unchanged compared to the ligand, indicating that the tin coordination does not significantly affect the electronic environment of these groups. 1.59 (s, 3H, CH_3): This peak corresponds to the methyl (CH_3) group. The chemical shift remains the same as in the ligand, indicating that the tin coordination has minimal influence on the electronic environment of this group. ¹H NMR also showed 1.45-1.25 (m, 12H, 6CH₂), 1.12-0.96 (m, 9H, 3CH₂, 1CH₃), and 0.66 (m, 6H, 2CH₃) peaks represent the CH₂ and CH₃ groups of the three butyl groups linked to tin atom with integration all togather 27 hydrogen atom which is exactly represents the butyl groups.

The ¹H NMR spectrum of the synthesized Bu_3SnL complex shows minor changes in chemical shifts compared to the ligand, indicating some electronic modifications upon coordination with the butyltin group. However, the overall



Fig. 5 ¹H-NMR spectrum of Bu₃SnL complex

structural features and chemical environments of the ligand are preserved in the complex. The imine group, hydroxyl groups, aromatic protons, and the methylene groups exhibit chemical shifts similar to those in the ligand, suggesting a limited influence of tin coordination on their electronic environments. But, the methylene proton adjacent to the chiral carbon atom shows a slightly upfield shift, indicating some electronic modifications due to the coordination with the butyltin group.

The ¹H NMR analysis Me₃SnL complex gave changes in chemical shifts related to the ligand (L). The peak at 8.28 (s, 1H, N=CH corresponds to the imine group (N=CH) proton in the complex. The chemical shift is somewhat downfield compared to the ligand, proposing an electronic change due to coordination with the methyltin group. While the peak at 8.03 (s, 1H, OH) and 7.87 (s, 1H, OH): attributed to the hydroxyl (OH) protons in the complex. The chemical shifts are similar to the ligand, predicting that the coordination with the methyltin group has negligible impact on the electronic field of the -OH groups. The peak at 7.28 (d, J = 8 Hz, 2H, Ar) corresponds to the aromatic protons in the prepared complex. The existance of a doublet indicates coupling with two adjusing protons, and the chemical shift is similar to the ligand, predicting that the Sn coordination does not effect on the electronic field of these protons. Moreovere, the peak at 6.72-6.51 (m, 5H, Ar) represent the aromatic protons in the complex. The multiplet pattern suggests the existence of different adjusing protons. The chemical shifts are similar to those in the ligand, indicating that the Sn coordination has slight effect on the electronic field of protons. Peaks at 3.35 (d, J = 16 Hz, 1H, CH) and 3.02 (d, J = 16 Hz, 1H, CH) assigned to the methylene $(-CH_2)$ protons in the complex. The presence of doublets confirms coupling with one adjusing proton, and the chemical shifts are equal to the ligand, proposing that the Sn coordination has a minimal effect on the electronic surroundings of these protons. Furthermore, the peaks at 2.89 (s, 6H, 2CH₃) represents the methyl ($-CH_3$) groups next to N atom in the complex. The chemical shift remains the same as in the ligand, which indicate the Sn coordination does not influnce the electronic environment of these groups. The peak at 1.46 (s, 3H, CH₃) attributed to the methyl $(-CH_3)$ group. The chemical shift is similar to the ligand, which predicthat the Sn coordination has a negligible impact on the electronic field of this group. Finally, the peak at 1.36 (s, 9H, 3CH₃): This peak represents the three methyl (CH₃) groups in the complex connected to the Sn atom. In general, the ¹H NMR analysis of the synthesized Me₃SnL complex demonstrate a slight changes in chemical shifts related to the ligand, indicating some electronic changs upon coordination with the methyltin group (Fig. 6).



Fig. 6 ¹H-NMR spectrum of Me₃SnL complex

2.8.5 Nuclear magnetic resonance (¹³C-NMR)

The synthesized ligand (L) and its tin complexes (Ph₃SnL, Bu₃SnL, and Me₃SnL) were characterized using ¹³C NMR spectroscopy to gain insight into their structural features and confirm the formation of the desired compounds. The ¹³C NMR spectra provide information about the carbon atoms present in the compounds and their chemical environments. Here is a brief discussion of the results [33, 34].

The ¹³C NMR spectrum of the ligand (L) would show signals corresponding to the carbon atoms in the methyl dopa and p-dimethylaminobenzaldehyde moieties. The chemical shifts in the ligand spectrum influenced by the substituents and structural arrangement (Table 4). The ¹³C NMR analysis of the Schiff base gave different peaks, demonstrating the presence of different types of carbon atoms within the ligand structure. The ligand gave peaks at 177.15 and 173.33 ppm, which can be assigned to carbon atoms in carbonyl (-C=O) and azo-mthine groups (-C=N). The chemical shifts predict the presence of electron-withdrawing groups or strong deshielding effects on these carbon atoms. A number of peaks in the range of (155.08 – 131.26) ppm indicate the presence of carbon atoms within the aromatic rings. The chemical shifts can vary depending on the substitution and adjusting functional groups. These carbon atoms in the aromatic rings contribute to the overall aromaticity of the ligand and play a vital role in its chemical featurs. The chemical shifts at 59.27, 45.39, 41.90, and 21.24 ppm correspond to carbon atoms in aliphatic area, and these carbon atoms are a part of the ligand's backbone or side chains. The differences in the chemical shifts reveal to the differences in the local electronic fields and potential adjusting groups attached to these aliphatic carbon atoms. The detected chemical shifts provide understandings into the ligand's structure and chemical environment. The existance of carbonyl groups proposes the participation of the ligand in coordination chemistry. The aromatic rings is importantin the ligand's stability and π -conjugation effects. On the other hand, the aliphatic areas provide information about the contribution to the ligand's solubility and reactivity. Through theses results, the we can confirm the formation of the compound and gain valuable information about its structural features. These understandings are vital for further studies and the succeeding formation of Sn complexes.

Two peaks observed at 177.15 and 173.33 ppm, which are attributed to carbonyl carbon atoms (-C=O) and imine (-C=N) groups of the Schiff base, these peaks undergo a significant downfield shift to 171.41 and 170.95 ppm, respectively in the prepared Ph₃SnL complex. This shift proposes a change in the electronic environment around





the carbonyl groups upon coordination to the Sn atom. The peaks detected in the aromatic region of the spectrum (155.08, 145.47, 144.30, 143.45, 134.37, 131.26, 131.03, 129.40, 127.04, 122.97, 121.71, and 117.97 ppm) assigned to carbon atoms of the 's aromatic rings of ligand. These peaks come in accourdance Ph_3SnL with the ligand's data, indicating that the coordination of the Sn atom does not significantly change the electronic field of the aromatic rings. The presence of the Sn atom in the Ph_3SnL complex is confirmed by the presence of a peak at 58.72 ppm, which attributed to the carbon atom bonded to Sn atom, and this peak does not apeared in the ligand's spectrum, which indicate

the characteristic feature of the Sn complex. While, the aliphatic group of the Ph_3SnL (45.13, 41.90, and 22.65 ppm) shows peaks that come in accourdance with the ligand's data. These peaks represent carbon atoms within aliphatic group of the ligand, prospective part of the ligand's backbone or side chains. Similarly, the chemical shifts suggests that coordination to the Sn atom does not influence the electronic environment of these carbon atoms.

The ¹³C NMR analysis of Bu₃SnLprovides valuable information about the structural features of the synthesized complex. Upon comparesion with the ligand, the peaks in the Bu₃SnL spectrum to the ligand's data, several changes can be observed. These changes provide information of the effects of Sn atom on the ligand's electronic environment. The ligand shows peaks at 177.15 and 173.33 ppm, attributed to the carbonyl carbon atoms (-C=O) and imine (-C=N) groups in the Schiff base ligand. These peaks show downfield shifts to 171.41 and 170.66 ppm, respectively. This shift proposes a change in the electronic environment around the carbonyl groups upon coordination to the Sn atom. The aromatic area of the spectrum (155.08, 145.47, 144.30, 131.26, 127.04, 122.97, 121.71, and 117.97 ppm) shows peaks that come in accourdance with the ligand's data. This indicates that coordination to the Sn atom does not has a significantly effect on the electronic environment of the ligand's aromatic rings. The presence of the Sn atom is confirmed by the existance of peak at 58.72 ppm, which assigned to the carbon atom bonded to the Sn atom. This peak is disappeared in the ligand's spectrum, indicating that it is a characteristic feature of the Sn complex. The aliphatic area of the Bu₃SnL spectrum (45.13, 41.90, 28.55, 24.29, 22.65, 14.01, and 10.74 ppm) also exhibits shifts a chemical shifts compared to the ligand's. These shifts propose changes in the electronic field of the ligand's aliphatic carbon atoms upon coordination with Sn atom. Remarkably, the presence of additional peaks in this region suggests the formation of butyl groups from the tributyltin moiety in the complex.

The ¹³C NMR analysis of Me₃SnL shoes Sn complex formation by Schiff base ligand with trimethyltin chloride (Me₃SnCl), which provides a valuable knowledge of structural changes by Sn coordination. In comparesion with the ligand's spectrum, we can investigate the effects of Sn coordination on the ligand's electronic environment. The ¹³C NMR show signals corresponding to the ligand carbon atoms, the methyl groups, and the Sn core center. The ligand carbon atoms might behas a minor chemical shift changes due to coordination with Sn atom. Upon analysis the peaks in the Me₃SnL spectrum and compared to the ligand's data, we can observe several changes in chemical shifts, predicting the impact of Sn coordination on the ligand's structure. Generally, the ligand's spectrum, the presence of carbonyl carbon atoms (-C=O) and Schiff base (-C=N) groups in the Schiff base are confirmed by peaks at 177.15 and 173.33 ppm. Nevertheless, in the Me₃SnL spectrum, these peaks undergo remarkable higher chemical shifts to 171.82 and 171.41 ppm, respectively.

This suggests that tin coordination induces changes in the electronic environment around the aromatic carbon atoms next to high electronegative atoms such as oxygen and nitrogen. The aromatic region of the spectrum (155.08, 145.47, 144.30, 131.26, 127.04, 122.97, 121.71, and 117.97 ppm) exhibits peaks that align well with the ligand's data, suggesting that the coordination to the Sn atom does not significantly affect the ligand's aromatic rings. The presence of the tin atom in Me₃SnL is confirmed by the appearance of a peak

at 58.72 ppm, corresponding to the chiral carbon atom. In the aliphatic region of the Me₃SnL spectrum (45.13, 41.90, 22.65, and 1.95 ppm), we observe shifts in chemical shifts compared to the ligand's data. These shifts indicate changes in the electronic environment of the ligand's aliphatic carbon atoms due to tin coordination. The appearance of additional peaks in this region suggests the involvement of the methyl groups from the trimethyltin moiety in complex formation. The peak at 1.95 ppm, disappeared in the ligand's spectrum, confirming that it is a characteristic feature of the Sn complex. The ¹³C NMR spectrum of Me₃SnL demonstrates a significant changes in chemical shifts in comparable with the ligand's spectrum, pecially for the carbonyl carbon atoms involved in Sn coordination, and the aliphatic carbon atoms affected by the presence of the methyl groups. On the other hand, the aromatic area shows good agreement between the complex and the ligand, indicating a negligible impact on the electronic environment of the ligand's aromatic rings. These results provide a valuable insights into the structural and electronic changes induced by tin coordination and contribute to the comprehensive characterization of the complex.

2.8.6 Nuclear magnetic resonance (¹¹⁹Sn-NMR)

The ¹¹⁹Sn NMR analysis provides an essential information about the electronic properties and coordination of Sn complexes, these complexes are, Ph₃SnL, Bu₃SnL, and Me₃SnL. In comparesion the chemical shifts of the Sn atom in these three complexes, we can found the behaiviour of the Sn coordenated-ligand and the impact of different organotin groups on the coordination. The ¹¹⁹Sn NMR spectra, showed a chemical shifts of the Sn atom in the complexes at – 217.31 ppm, – 235.57 ppm and – 259.57 ppm for Ph₃SnL, Bu₃SnL and Me₃SnL respectively. These chemical shifts are downfield compared to the reference compound, which indicate the effect of the ligand coordination on the electronic surroundings around the Sn atom [35]. The detected downfield shifts in the ¹¹⁹Sn NMR spectra can be attributed to the electronic effects of the different organotin groups and ligand. The ligand, resulting from the Schiff base formation between methyl dopa and p-dimethylaminobenzaldehyde, which contains different functional groups that can contribute to the coordination chemistry with Sn atom. The electron-donating and electron-withdrawing behaiviour of these groups can influence the electron density around the Sn center, resulting in changes in the ¹¹⁹Sn chemical shifts. By comparing the chemical shifts of the Sn atom in the prepared complexes, we can found the arrangement: Ph₃SnL $(-217.31 \text{ ppm}) > \text{Bu}_3\text{SnL} (-235.57 \text{ ppm}) > \text{Me}_3\text{SnL} ($ - 259.57 ppm). This arrangement proposes that the electronic fields around the Sn atom becomes more shielded as we move from Ph₃SnL to Bu₃SnL and then to Me₃SnL. This can be attributed to the changing in electronic properties and steric effects of the different organotin groups.

2.9 Anticancer drugs

2.9.1 Cytotoxic effect of organotin(IV) complexes on A549 human cell

MTT assay can be utilised to evaluate the cytotoxicity of the synthesized tri-organotin(IV) complexes on human lung cancer cells (A549). This assay is based on the ability of living cells to inhibit a yellow tetrazolium salt (MTT) to a purple formazan creation via the activity of mitochondrial dehydrogenases [15, 36]. The colorimetric change reveal the viability of the cells, with a reduction in color that indicating cell death due to apoptosis. In this study, the tri-organotin(IV) complexes and the standard drug doxorubicin were dissolved in acidic medium of isopropanol solvent and diluted with culture medium. Different concentrations (0, 8, 16, 32, 62.5, 125, 250, 500, and 1000 µg/mL) of the complexes and doxorubicin were added to the A549 cells and incubated for 48 h. Then, MTT solution was added and further incubated for 4 h in the dark. The absorbance of the producing formazan was measured at 570 nm.

The inhibition of the tri-organotin(IV) complexes was measured by determining the half maximal inhibitory concentration (IC₅₀). The IC₅₀ value represents the concentration of the compounds that inhibits 50% of cell proliferation compared to the normal cells [37]. This parameter gave a valuable information about the influence of the complexes as anti-cancer (Tables 5 and 6). The results can be used to assess the cytotoxicity of the tri-organotin(IV) complexes against human A549 cancer cells. A lower IC₅₀ value indicates a higher strength of the complexes to inhibit cell proliferation. The comparison with doxorubicin, as a chemotherapeutic drug, permits for evaluating the relative efficacy of the synthesized complexes in comparison to the standard [38].

The IC₅₀ values obtained for the combination of the synthesized Schiff base ligand with different tri-organotin(IV) complexes which found to be 16.84 μ g/mL for Ph₃SnL, 980.1 µg/mL for Me₃SnL, and 11.63 µg/mL for Bu₃SnL. The I_{C50} value reflects the concentration of the compounds required to inhibit 50% of cell proliferation compared to untreated cells [39]. In this circumstance, a lower I_{C50} value of (16.84 µg/mL) indicates a higher effectiveness and stronger cytotoxic effect of the compounds on the A549 lung cancer cells. The results demonstrate that the combination of the Schiff base ligand with Ph₃SnL showed the most powerful cytotoxic effect. This suggests a synergistic interaction between the ligand and Sn atom, which enhance a cytotoxic against the A549 cancer cells [40]. On the other hand, the Me₃SnL showed a higher I_{C50} value of (980.1 µg/ mL), indicating a less cytotoxic effect associated to the other compounds. This proposes that the communication between ligand and Me₃SnCl may have a weaker synergistic effect on the A549 cells. Similarly, Bu₃SnL exhibited a relatively low I_{C50} value of about (11.63 µg/mL), indicating a significant cytotoxic effect. This indicates that Bu₃SnL possesses a strong cytotoxic properties towards human A549 cancer cells and may give a promise anti-cancer agent. These results gives the ability of tri-organotin(IV) complexes, especially Ph₃SnL and Bu₃SnL, as anti-lung cancer. Further studies are needed to discover their mechanism of action inside the human bodyto evaluate their activity towards cancer cells, and assess their therapeutic clinically. Statistical analysis and IC_{50} values are shown in Table 7 and Fig. 7.

This indicates its strong potential as an effective anticancer agent. Bu₃SnL also showed significant cytotoxicity with an I_{C50} value of 11.63 µg/mL, suggesting its potential as an alternative candidate for further development. Bu₃SnL also showed significant cytotoxicity with an I_{C50} value of 11.63 µg/mL, suggesting its potential as an alternative candidate for further development.

3 Conclusions

In conclusion, tri-organotin(IV) complexes were synthesised by the reaction with Schiff base ligand which produce by the reaction of methyl dopa with p-dimethyaminobenzaldehyde.

Conc	SB+Ph3SnL		SB+Me3SnL		SB+Bu3SnL	
	Mean	SD	Mean	SD	Mean	SD
1,000	11.79	0.53	49.87	3.50	11.87	0.29
500	13.06	0.59	77.11	3.94	12.55	0.64
250	15.69	2.67	80.59	8.41	14.59	0.29
125	17.73	4.23	90.11	5.07	15.35	1.28
62.5	20.36	5.33	96.51	1.93	17.81	0.44
32	34.27	5.24	96.51	1.93	24.94	5.51
16	59.54	5.95	97.16	1.35	50.98	7.47
8	82.36	11.20	94.29	1.07	86.51	10.11

Table 5 Effect of complexesand doxorubicin on A549 cellsviability

Table 6	Tukey's	multiple	comparisons	test

Tukey's multiple compari- sons test	Below thresh- old	Summary	Adjusted P value
16			
$SB + Ph_3SnL$ vs. $SB + Me_3SnL$	Yes	**	< 0.0001
$SB + Ph_3SnL vs.$ $SB + Bu_3SnL$	Yes	*	0.0327
$SB + Me_3SnL$ vs. $SB + Bu_3SnL$	Yes	**	< 0.0001
32			
$SB + Ph_3SnL$ vs. $SB + Me_3SnL$	Yes	**	< 0.0001
$SB + Ph_3SnL$ vs. $SB + Bu_3SnL$	Yes	*	0.0184
$SB + Me_3SnL$ vs. $SB + Bu_3SnL$	Yes	**	< 0.0001
62.5			
$SB + Ph_3SnL$ vs. $SB + Me_3SnL$	Yes	**	< 0.0001
$SB + Ph_3SnL$ vs. $SB + Bu_3SnL$	No	ns	0.7199
$SB + Me_3SnL$ vs. $SB + Bu_3SnL$	Yes	**	< 0.0001
125			
$SB + Ph_3SnL vs.$ $SB + Me_3SnL$	Yes	**	< 0.0001
$SB + Ph_3SnL$ vs. $SB + Bu_2SnL$	No	ns	0.7508
$SB + Me_3SnL vs.$ $SB + Bu_3SnL$	Yes	**	< 0.0001
250			
$SB + Ph_3SnL$ vs. $SB + Me_3SnL$	Yes	**	< 0.0001
$SB + Ph_3SnL$ vs. $SB + Bu_3SnL$	No	ns	0.9397
$SB + Me_3SnL$ vs. $SB + Bu_3SnL$	Yes	**	< 0.0001
500			
$SB + Ph_3SnL$ vs. $SB + Me_3SnL$	Yes	**	< 0.0001
$SB + Ph_3SnL$ vs. $SB + Bu_3SnL$	No	ns	0.9868
$SB + Me_3SnL$ vs. $SB + Bu_3SnL$	Yes	**	< 0.0001
1000			
$SB + Ph_3SnL$ vs. $SB + Me_3SnL$	Yes	**	< 0.0001
$SB + Ph_3SnL$ vs. $SB + Bu_3SnL$	No	ns	0.9996
$SB + Me_3SnL$ vs. $SB + Bu_3SnL$	Yes	**	< 0.0001

NS non-significant

 $p^* < 0.01$

 $p^* < 0.05$



۵ 2 1 3 Log Concentration in µg mL-1

Fig. 7 Effect of complexes and doxorubicin in mean of A549 cells viability

The characterization of these complexes was acquired by diffenet analysis techniques, such as FTIR, SEM, ¹H NMR, ¹³C NMR, and ¹¹⁹Sn NMR. The resulted spectra gave a valuable information about the structure and coordination of organotin(IV) complexes. The cytotoxicity was evaluated towards A549 human lung cancer cell which demonstrate its activity as anti-cancer agents. The MTT assay shown concentration-dependent growth inhibition of the cancer cells, with Ph₃SnL exhibiting the maximum cytotoxic effect among other synthesized complexes, which confirm by lowest IC₅₀ value of about (16.84 µg/mL). Bu₃SnL shown a lower cytotoxicity with an IC₅₀ value of (11.63 μ g/mL), while Me₃SnL exhibited a relatively lower cytotoxic effect, with an IC₅₀ value of (980.1 μ g/mL). The results highlighted the significance of the ligand structure and the behaiviour of organotin(IV) complexes. The presence of different organotin(IV) groups have an effect towards anti-cancer agents. The observed cytotoxicity may be attributed to the ability of these complexes to impact with a vital cellular processes or to persuade apoptosis of cancer cells. Further investigations are necessary to demonstrate the mechanisms of action of these complexes, and their selectivity towards cancer cells, and their prospective for combination therapy.

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Author contributions F I, A A, A H and A Z A: experimental work, E Y, M B and N H: conceptualization, M A, A H J, A Z A and H H: writing-original draft, F I, H H, A B, A H and EY: review and editing.

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Data availability Data is available upon reasonable request from the corresponding author.

Declarations

Conflict of interest The authors declare no confict of interest.

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