Synthesis, Characterization and Biological Activities of Organotin(IV) Complexes with 2-thioazoline-2-thiol

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Summary: Six complexes of 2-thiazoline-2-thiol (HL) with triorganotin(IV) and chlorodiorganotin(IV), [R_3SnL {R = CH_3 (1), *n*-C_4H_9 (2) C_6H_5 (3)}; R_2SnClL {R = CH_3 (4), *n*-C_4H_9 (5) C_6H_5 (6)}], have been synthesized and characterized by elemental analysis, FT-IR and multinuclear NMR (^1H and ^{13}C) spectroscopy and mass spectrometry. Spectroscopic studies revealed that ligand act as monodentate. NMR data suggested that tin atom is tetra-coordinated in triorganotin(IV) complexes (1-3). The biological screening results show that complexes exhibited strong antibacterial and antifungal activity. Biofilm inhibition activity of these complexes was also found satisfactory. The *in vitro* hemolytic activity data showed that these complexes are least cytotoxic.

Keywords: 2-Thiazoline-2-thiol; Organotin(IV) complexes; IR; NMR; Mass; Biological Activities.

Introduction

Considerable attention has been paid to organotin complexes in recent years, owing to their wide applications in various fields like catalysis, biotechnology, analytical and medicinal chemistry [1-5]. These are also used as agricultural biocides owing to their antifungal properties [6,7].

Recently organotin compounds have been used as reagents in reduction, transmetallation and coupling reactions or as extremely versatile catalysts in organic reactions [8]; the generally high selectivity of organotin reagents allows one to save time and to avoid product losses of protection–deprotection cycles. In industry, organotin compounds are utilized for esterification and trans-esterification reactions, for silicone curing, for the preparation of polyurethanes [9], but the stabilization of poly(vinyl chloride) (PVC) is their largest application so far [10].

A large number of organotin(IV) derivatives have been used to study their antitumor activity and their uses as potential chemotherapy agents [11-14]. These studies seem to demonstrate that not only the organotin moiety is crucial for cytotoxicity but the ligand also plays a key role in this aspect [15]. Moreover, in general, the biological activity of organotin compounds appears to be greatly influenced by the structure of the molecule and likely by the nuclearity of the complexes [16, 17].

The heterocyclic thiones play an important role as ligands in metal coordination chemistry, because of their relevance to biological systems and the versatility in their coordination modes which give rise to complexes with variable nuclearity and wide-ranging structural geometries [18-20]. Interaction of heterocyclic thioamides with metals has been reported [21-23]. These thio ligands also have biochemical significance and have been used as medicines [23,24]. The formation of organotin complexes of this type of ligands may enhance biological activity. More recently, the interaction of the neutral and anionic forms of 2-thiazoline-2-thione with organotin(IV) halides have been investigated by Tarassoli [25].

In continuation with our previous studies on organotin(IV) complexes derived from sulfur donor ligands [26-29], and triggered by the need for potent antimicrobial agents to mitigate or eliminate the infections, we aimed herein to synthesize new complexes of tributyltin(IV), trimethyltin(IV), triphenyltin(IV), chlorodibutyltin(IV), chlorodimethyltin(IV) and chlorodiphenyltin(IV) with 2-thiazoline-2-thiol. To understand the nature of bonding, the synthesized compounds are characterized by EIMS-mass spectrometry, FT-IR and multinuclear NMR (^1H and ^{13}C). Antibacterial, antifungal, hemolytic and biofilm inhibition studies of the complexes and the free ligand were also

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performed in order to evaluate their possible use as potent antibiotic species.

Experimental

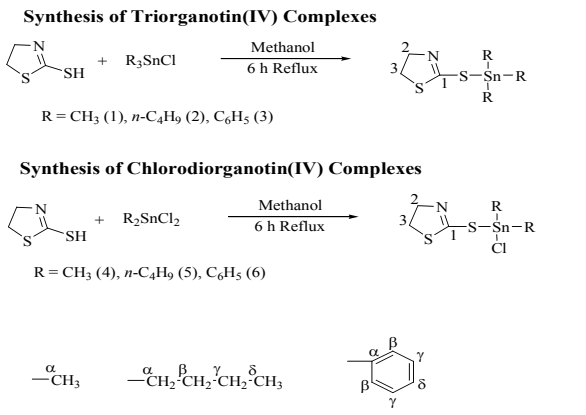
Materials and methods

Organotin(IV) dichlorides and organotin(IV) chlorides were purchased from Sigma-Aldrich (USA) and no further purification was done. The solvents used were obtained from Merck (Germany), Lab-scan and Riedal-de Haen and were dried before use according to literature procedures [30]. The melting points were recorded with melting point apparatus, MP-D Mitamura Rieken Kogyo (Japan). IR spectra were recorded in the range of 4000–250 cm^{-1} as KBr/CsBr pellets on a Perkin-Elmer-100 FTIR spectrophotometer. Elemental analysis was done using a Perkin Elmer 2400 Series II. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARC 300 MHz-FTNMR. Chemical shifts are given in ppm and coupling constants (J) values are given in Hz. The multiplicity of signals in ^{1}H NMR are given with chemical shifts; (s = singlet, d = doublet, t = triplet, m = multiplet). MAT-311A Finnigan (Germany) was used to record electron impact mass spectra (EIMS). The m/z values were investigated assuming that H = 1, C = 12, N = 14, O = 16, Cl = 35 and Sn = 120. Antimicrobial activities were studied in incubator (Sanyo, Germany) and sterilized in autoclave apparatus (Omron, Japan). Micro Quant apparatus (BioTek, USA) was used to find out the minimum inhibitory concentration (MIC). The sample solution was centrifuged in H-200 NR (Kokusan, Japan). In cytotoxicity assay, RBCs were counted by Hemocytometer (Fisher Ultra Plane, Japan).

Synthesis

General Procedure for synthesis of organotin(IV) complexes with 2-thioazoline-2-thiol

Stoichiometric amount of 2-thioazoline-2-thiol was dissolved in dry methanol and then calculated amount of triorganotin(IV) chloride (R_3SnCl:HL = 1:1 molar ratio for complexes, 1-3) and diorganotin dichloride (R_2SnCl_2:HL = 1:1 molar ratio for complexes, 4-6) was added with constant stirring. The resulting mixture was refluxed for 6 h and the solvent was evaporated with rotary evaporator under reduce pressure. The solid product obtained was recrystallized in methanol:petroleum ether (1:2).



Scheme 1: Synthesis of organotin(IV) complexes.

Biological Studies

Antibacterial activity

In vitro antibacterial activity of the ligand and its tri- and chlorodiorganotin(IV) complexes were investigated against four bacterial strains; *B. subtilis, S. aureus, E. coli* and *P. multocida*. Disc diffusion method was adopted for this study [31]. Pure cultures were prepared on nutrient agar medium in the slants and petri plates. Nutrient broth (13 g/L) was added in distilled water, mixed thoroughly and then autoclaved. This autoclaved culture (10 µL) of a bacterial strain was mixed well in the medium and shaked in a shaker at 37 ^{o}C for 24 h. The inocula were placed at 4 ^{o}C. The inocula with 1×10^{8} spores/mL were used for further investigation. Nutrient agar (28 g/L) was mixed well in distilled water and distributed it homogenously. The medium was autoclaved at 121 ^{o}C for 15 minutes for sterilization. Inoculums (100 µL/100 mL) were added to the medium and this medium was poured in sterilized petri plates. After this, small filter paper discs were laid flat on growth medium containing 100 µL of sample (concentration = 10 mg/mL in DMSO). The petri plates were then incubated at 37 ^{o}C for 24 h, for the growth of bacteria. Clear zone obtained due to inhibition of bacterial growth by the ligand and their complexes. The zones of inhibition were measured in millimeters using zone reader [32].

Antifungal activity

The *in vitro* antifungal activity of the ligand and its tri- and chlorodiorganotin(IV) complexes were also tested against four bacterial strains; *A. niger, A. flavus, A. alternata* and *R. solani*. Disc diffusion method was used [32]. Pure cultures were prepared on nutrient agar medium in the slants and

petri plates. Petri plates were presterilized at 180 ^{o}C for 3 h in hot air oven. Potato dextrose agar was used to maintain cultures of the fungi. The multiplication of fungi was done by incubating culture slants at 28 ^{o}C for 3–4 days for the multiplication of fungal strains. This growth medium then placed on the sterilized petri plates. These petri plates were then further incubated at 28 ^{o}C for 48 h. Small filter paper discs were laid flat on growth medium having fungal growth and then 100 µL each sample (10 mg/mL in DMSO) was applied on each disc. The petri plates were again incubated. Active samples gave inhibition zone around the disc. The zones of inhibition were measured in millimeters using zone reader [33].

MIC

Plates were prepared under aseptic condition. A sterile 96 well plate was labeled. A volume of 100 µL of test material in DMSO (10 mg/ml) was pipetted out into the first row of the plate. To all other wells 50 µL of nutrient broth or normal saline was added. Serial dilutions (two fold) were performed. Tips were discarded after use such that each well had 50 µL of the test material in serially descending concentration. Twelve dilutions (two fold) were made with concentration range from 5, 2.5, 1.25, 6.25× 10^{-1}, 3.12 × 10^{-1}, 1.56 × 10^{-1}, 7.81 × 10^{-2}, 3.90 × 10^{-2}, 1.95 × 10^{-2}, 9.76 × 10^{-3}, 4.88 × 10^{-3} and 2.4 × 10^{-3} mg/ml. To each well 10 µL of resazurin indicator solution was added. Using a pipette 30 µL of 3 × strength broth was added to each well to ensure that final volume was single strength of nutrient broth. Finally, 10 µL of bacterial suspension (5 x10^{6} CFUmL^{-1}) was added to achieve a concentration of 5 x 10^{5} CFUmL^{-1}.

Each plate had a set of controls: (i) A column with a broad spectrum antibiotic as positive control; (ii) A column with all solutions except test compound; and (iii) a column with all solutions with the exception of the bacterial solution adding 10 µL of broth instead. Each plate was wrapped to ensure that bacteria did not dehydrate. The plates were then incubated at 28 ^{o}C for 48 h for fungus and at 37 ^{o}C for 24 h for bacteria. The absorbance was recorded at 620 nm by micro quant for fungus and at 500 nm for bacteria. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which the color change occurred was taken as MIC value [33].

Hemolytic activity

Powell's method was used to examine the hemolytic activity of the ligand and synthesized compounds [34]. Freshly obtained heparinized human blood (3 mL) was gently mixed with 2–3 drops of heparin, poured into a sterile 15 mL falcon tube and centrifuged for 5 minutes at 3000 rpm. The supernatant was poured off and viscous pellet was washed three additional times with sterile isotonic phosphate-buffered saline (PBS) solution, and adjusted to pH~ 7.4. To stabilize the pH, the sample was mixed for half an hour at room temperature (25–30 ^{o}C). The washed cells were suspended in the 20 mL chilled, saline PBS buffer. Erythrocytes were counted on a hemacytometer.

The blood cell suspension was maintained on wet ice and diluted with sterile PBS. The cell count should be 7.068 X 10^{8} cells per mL for each assay. Sample (20 µL) in five different solvents was taken in 2 mL Eppendorf tubes. For each assay, 0.1 % Triton X-100 was taken as a positive control, i.e., 100% of blood lysis and PBS was taken for each assay as a negative control, i.e., background (0% lysis). In each 2 mL Eppendorf already containing 20 µL sample, 180 µL diluted blood cell suspension was added and mixed with the help of the pipette tip. Tubes were incubated for 35 minutes at 37 ^{o}C and agitated after 10 minutes. Immediately after incubation; the Eppendorf tubes were placed on ice for 5 minutes and then centrifuged for 5 minutes at 4200 rpm. After centrifugation, 100 µL supernatant was taken from the tubes and diluted with 900 µL chilled PBS. All tubes were maintained on wet ice after dilution. Then 200 µL (10 mg/g) was taken into 96 well plates and three replicates were taken in well plates which contain one positive control (100% of blood lysis) and another negative control (0% of blood lysis). After this, absorbance at 576 nm recorded on micro Quant. Triton X-100 (0.1%) were used as positive control for 100% lysis and PBS buffer as negative control 0% lysis. The experiment was done in triplicate and mean ± SD was calculated by using the following % hemolysis formula:

$$\% \text{ Hemolysis} = \frac{Hb_{ABS}}{Hb_{100\%\ ABS}}$$

Data observed were expressed in % of cell lysis by comparing with 100% hemolysis of the same number of cells using 0.1% Triton X-100 as positive standard.

Antibiofilm activity

Those bacteria that possess good adhesion property to the surface can produce a biofilm. Briefly, a bacterial culture were grown overnight in

the broth media. Then, the cultures were diluted upto 1:100 into fresh medium for biofilm inhibition assay. 200 µL of diluted solution was added to 96-well plate. After the growth phase, the medium was removed carefully with pipette, the wells present in microtitre plates were rinsed three times with 200 µL of sterile PBS. After washing, wells were filled with 96 % ethanol for 15 minutes. The plates were air dried at 37 ^{o}C and to each well, except in the case of positive (growth) controls, 100 µL broth Sabouraud were added, supplemented with concentration 10 mg/ml, the amount of compound used on planktonic form of the same strain. The plates were incubated at 37 ^{o}C for 24 h; after this incubation time the medium was removed. The microtitre plates were dried in air and then, 200 µL of 1 % crystal violet were added for 5 minutes. Then, the microtitre plates were washed with distilled water, and then dried. Finally, 200 µL of 33 % glacial acetic acid were added to the wells and absorbance was measured at 540 nm with an ELISA reader. Data for biofilm formation of all strains were compared with the data obtained for the negative control and positive control. Rifampicine was used as positive control and microbial medium without microorganisms was used as the negative control.

Results and Discussion

Six organotin complexes were prepared, in good yield, by direct reaction between the appropriate triorganotin(IV) chloride/diorganotin(IV) dichloride and 2-thiazoline-2-thiol (HL) in dry methanol. These compounds have sharp melting points. The complexes are air-stable and soluble in common organic solvents. The elemental analysis data agreed very well with the proposed formulas of the complexes. The physical data of the synthesized compounds is given in Table 1.

FT-IR spectroscopy

FT-IR data of HL and its organotin(IV) derivatives is given in Table 2. Valuable structural information could be obtained from the IR spectra of compounds. It has been reported [25] that 2-thiazoline-2-thiol exhibits thione-thiol tautomerism (Figure 1) and both forms coexist in the solid state but thione form is the dominant one.

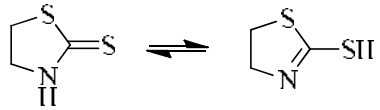


Fig. 1: Tautomeric forms of thione and thiol.

In the uncoordinated ligand three bands have been observed at 3002, 1600 and 1214 cm^{-1} which can be assigned to the ν(N-H), ν(C=N) and ν(C=S) vibration modes, respectively, indicating the coexistence of both thione and thiol forms in the solid state. The band attributed to the N–H stretching of thione tautomer appeard at low frequency due to the intramolecular hydrogen bond N–H--S, but it is difficult to differentiate between the N–H and C–H stretching vibrations with certainty [11, 35]. Metal-thiolate compounds exhibit two thioamide bands appears in the range 1505-1530 cm^{-1} due to (C=N) stretching vibrations and 1181-1198 cm^{-1} for (C=S). This set of bands is unique since it gave information about the coordination mode of the complexes. These thioamide bands shift to lower wave-numbers (by 16-22 cm^{-1}), which may be due to the evolution of partial double bond character in the thioamide group (–N=C=S–) after its deprotonation and subsequent coordination to tin *via* S and N. The coordination of the metal ion to the thiolate ligand is also confirmed by this shift of thioamide peak which occurred due to increased electron delocalization towards the metal ion upon complexation. In Scheme 1, structural representation of HL and complexes 1–6, the N-C-S bond indicates an increase in the carbon-nitrogen double bond character. Coordination of ligand to organotin species is also confirmed by appearance of new bands in the range of 312-330 cm^{-1} in the far-IR spectra of the newly synthesized the complexes [1]. Further, most of the ring bands shift observed in the case of these complexes can be attributed to the structural changes observed after the coordination of ring nitrogen to tin [35]. On the other hand, in triorganotin(IV) complexes, ligand showed monodentate mode of binding, probably due to steric effect of three organic moieties attached to tin center [36, 37].

The Sn-C band usually occurs in the range of 516-541 cm^{-1} for alkyltin and 241-279 cm^{-1} for aryltin compounds, suggest a non-planar arrangement of C–Sn–C moiety and did not show significant change upon complexation as compared to other compounds reported in the literature [35,38]. The Sn–Cl stretching frequency in complexes (4-6) was observed in the expected region of 352-359 cm^{-1} [39].

Table-1: Physical data of organotin(IV) complexes with 2-thiazoline-2-thiol.

IUPAC Name and Compound No.	Molecular Formula	Molecular Weight	M.P (°C)	Elemental Analysis % Calculated (Found)			
				C	H	N	S
2-Thiazolin-2-thiol (HL)	$C_3H_5NS_2$	119.2	100-1	30.23 (30.26)	4.23 (4.27)	11.75 (11.71)	53.80 (53.84)
2-{(trimethylstannyl)thio}-4,5-dihydrothiazole (1)	$C_6H_{13}NS_2Sn$	282.0	124-5	25.55 (25.60)	4.65 (4.69)	4.97 (5.01)	22.74 (22.78)
2-{(tributylstannyl)thio}-4,5-dihydrothiazole (2)	$C_{15}H_{31}NS_2Sn$	408.2	165-6	44.13 (44.17)	7.65 (7.69)	3.43 (3.47)	15.71 (15.76)
2-{(triphenylstannyl)thio}-4,5-dihydrothiazole (3)	$C_{21}H_{19}NS_2Sn$	468.2	136-7	53.87 (53.83)	4.09 (4.13)	2.99 (2.95)	13.70 (13.74)
2-{(chlorodimethylstannyl)thio}-4,5-dihydrothiazole (4)	$C_5H_{10}ClNS_2Sn$	302.4	141-2	19.86 (19.90)	3.33 (3.37)	4.63 (4.67)	21.24 (21.20)
2-{(dibutylchlorostannyl)thio}-4,5-dihydrothiazole (5)	$C_{11}H_{22}ClNS_2Sn$	386.6	176-7	34.17 (34.21)	5.74 (5.78)	3.62 3.66	16.59 (16.62)
2-{(chlorodiphenylstannyl)thio}-4,5-dihydrothiazole (6)	$C_{15}H_{14}ClNS_2Sn$	426.6	210-1	42.23 (42.27)	3.31 (3.35)	3.28 (3.32)	15.03 (15.07)

Table-2: Characteristic IR (cm^{-1}) data of organotin(IV) complexes with 2-thiazoline-2-thiol.

Comp. No.	υ(N–H)	υ(C=N)	υ(C=S)	υ(Sn-S)	υ(Sn–C)	υ(Sn–Cl)
HL	3002	1600	1214	-	-	-
1	-	1505	1198	326	533	-
2	-	1513	1185	312	541	-
3	-	1530	1181	330	279	-
4	-	1513	1192	323	516	359
5	-	1513	1186	314	530	352
6	-	1519	1194	324	241	358

NMR Spectroscopy

Nuclear magnetic resonance (NMR) is a versatile technique employed to identify the molecular structure. The ^{1}H and ^{13}C NMR spectra of the compounds were recorded using DMSO-d_6 as solvent and TMS as internal standard reference.

^{1}H NMR

The ^{1}H NMR data for the new organotin(IV) complexes is given in Table 3. In the ligand HL, SH gave a singlet at 12.25 ppm. The protons at position 2 (H2) and 3 (H3) appeared as triplet at 3.89 ppm and 3.54 ppm, respectively with $^3J(^1H–^1H)$ coupling of 7.1 Hz. After reaction with diorganotin(IV) dichlorides/triorganotin(IV) chlorides, disappearance of SH peak confirmed the complexation.

According to literature, coordination pattern of tin(IV) in di- and trimethyltin(IV) derivatives with the $^2J[^{119}Sn–^1H]$ coupling constant values are as: in tetracoordinated tin compounds, ^2J values are predicted to be smaller than 59 Hz and for hexacoordinated tin, ^2J values are generally larger than 83 Hz, respectively [40]. Another important parameter that decides the geometry around the tin atom is θ(C-Sn-C angle). Its values are as: for tetracoordinated tin compounds, $\theta \leq 112^o$ and for hexacoordinated tin, $\theta = 129–176^o$ [41].

The methyl protons in trimethyltin(IV) complex (1) gave a characteristic signal at 0.92 ppm with $^2J[^{119/117}Sn–^1H]$ = 56 Hz which indicated 4-coordinated geometry around the tin atom in solution. The butyl groups of *n*-tributyltin(IV) derivative (2) gave multiplet for Hα, Hβ and Hγ protons due to their complex nature, while the protons of methyl carbon atom (Hδ) appear as clear triplets at 0.83 ppm with $^3J(^1H–^1H)$ coupling of 7.6 Hz. The protons of triphenyltin(IV) complex (3) gave two mutiplets for Hβ at 7.73-7.97 ppm, while Hγ and Hδ at 7.37-7.53 ppm. In chloro-*n*-dibutyltin(IV) complex (5) Hα, Hβ and Hγ appeared as multiplet at 1.59-1.70 ppm, 1.50-1.57 ppm and 1.24-1.34 ppm, respectively, while methyl protons of the butyl group gave a triplet at 0.87 ppm. In case of complex (6), Hβ proton gave multiplet at 7.71-8.12 ppm while the Hγ and Hδ protons gave multiplet at 7.24-7.41 ppm. For all complexes (1-6), there is a little downfield shift in the protons at position 2 (H2) and 3 (H3) as shown in Table 3.

^{13}C NMR

The ^{13}C NMR data of the ligand and complexes were in agreement with the ^{1}H NMR and FT-IR data for the formation of complexes. The small shift in the position of C(1) confirmed the coordination of ligand with Sn *via* sulfur moiety. The positions of C1, C2 and C3 of ligand undergo upfield shift in the complexes as the ligand chelate tin in its anionic form, thiolate. The magnitude for $^nJ[^{119}Sn, ^{13}C]$ coupling were also observed and are given in Table 4. The coupling constant, $^nJ[^{119}Sn, ^{13}C]$ is an important parameter for structural characterization of organotin(IV) compounds. In trimethyltin complex (1), the coupling constant $^1J[^{119}Sn–^{13}C]$ was observed at 390 Hz that falls in the range of 4-coordinated tetrahedral geometry [40]. This is further supported by the C–Sn–-C bond angle (Table 5) calculated from $^2J[^{119}Sn–^1H]$ value using Lockhart equation [41] which is 110° for complex 1 that falls in range of 4-coordinated geometry around the tin. The C–Sn–C angle calculated from $^1J[^{119}Sn–^{13}C]$ is 111° (complex 2) which confirm the 4-coordinated geometry in solution.

Table-3: ^{1}H-NMR data[a-c] of organotin(IV) derivatives of 2-thiazoline-2-thiol.

Proton	HL	1	2	3	4	5	6
—SH	12.25 s	-	-	-	-	-	-
2	3.89 t (7.1)	3.85 t (7.1)	3.85 t (7.1)	3.87 t (7.1)	3.84 t (7.1)	3.85 t (7.1)	3.84 t (7.1)
3	3.54 t (7.1)	3.52 t (7.1)	3.51 t (7.1)	3.51 t (7.1)	3.52 t (7.1)	3.51 t (7.1)	3.52 t (7.1)
α	-	0.92 s [56]	1.46-1.69 m	-	1.38 s	1.59-1.70 m	-
β	-	-	1.46-1.69 m	7.73-7.97 m	-	1.50-1.57 m	7.71-8.12 m
γ	-	-	1.31-1.39 m	7.37-7.53 m	-	1.24-1.34 m	7.24-7.41 m
δ	-	-	0.83 t (7.6)	7.37-7.53 m	-	0.87 t (7.5)	7.24-7.41 m

[a]Chemical shifts (δ) in ppm. [b]Coupling Constants, ^{2}J[^{119}Sn, ^{1}H] and ^{3}J(^{1}H, ^{1}H) in Hz are listed in square brackets and parenthesis, respectively.
[c]Numbering is in accordance with scheme 1.

Table-4: ^{13}C NMR data[a,b] of organotin(IV) derivatives of 2-thiazoline-2-thiol.

Carbon No.	HL	1	2	3	4	5	6
1	199.4	193.5	193.4	193.1	193.2	199.4	199.4
2	33.9	32.8	32.6	32.2	32.2	33.4	33.4
3	51.9	49.8	49.3	49.8	49.4	51.9	49.8
α	-	-2.6 [390]	18.4 [372]	139.2	4.96 [485]	26.0	135.2 [68]
β	-	-	27.1 [28]	137.5 [48]	-	28.1[44]	128.1 [19]
γ	-	-	26.8 [67]	129.6 [64]	-	26.03	127.7
δ	-	-	14.5	130.6 [11]	-	14.2	126.9

[a]Chemical shifts (δ) in ppm. Coupling Constants, ^{n}J[^{119}Sn, ^{13}C] in Hz are listed in square brackets. [b]Numbering is in accordance with scheme 1.

Table-5: (C–Sn–C) angles (°) based on NMR parameters of selected organotin(IV) derivatives.

Comp. No.	^{1}J[$^{119/117}$Sn,^{13}C] (Hz)	^{2}J[$^{119/117}$Sn,^{1}H] (Hz)	Angle (°)	
			^{1}J	^{2}J
1	390	56	111	110
2	372	-	111	-

Mass spectrometry

The conventional EI mass spectral data at 70 eV for the triorganotin(IV) complexes were recorded along with *m/z* and intensity, which are listed in Table 6. In mass spectral data of the compounds, each fragment ion occurred in a group of peaks as a result of tin isotopes. For simplicity, the mass spectral fragmentation data reported here are related to the principal isotope ^{120}Sn peak in each species, and must be regarded as approximate [29]. The molecular ion peaks, M^{+}, are either not observed or if observed have very low intensities in synthesized organotin complexes. Mass spectral data for the complexes (1-3) are consistent with the structures proposed on the basis of other spectroscopic data and consistent with the literature [42]. For the triorganotin compounds, the primary fragmentation from the molecular ion appeared in two ways. The first one was initiated with the loss of R (R = Bu or Ph), whereas the secondary fragmentation was due to the loss of ligand (L). For complexes 1-3, the former pattern predominated and gave $[(C_3H_4NS_2)Sn]^{+}$ species. The $[R_2Sn]^{+}$ formed by the loss of the ligand part, forms a $[Sn]^{+}$ fragment by the successive elimination of the R radical. In addition, the following ions were also observed with reasonable intensities in the mass spectra of all organotin(IV) derivatives $[C_6H_5]^{+}$, $[C_4H_9]^{+}$ and $[CH_3]^{+}$.

Mass spectrum of 1 shows expected peaks at m/z 283 (M^{+}), 238, 152, 150, 135, 120, 118, 76. The peak at 76 was related to the $[C_2H_6NS]^{+}$ group resulting from the decomposition of the ligand. The peak at 15 was due to the methyl group. The peaks at 152, 150, and 135 were attributable to the Sn-containing fragments ^{120}Sn-S, $^{120}Sn(CH_3)_2$, and $^{120}Sn(CH_3)$, respectively. Mass spectrum of 2 showed peaks at m/z 409 (M^{+}), 238, 234, 208, 120, 118, 57. The peak at 118 was attributable to the 2-thiazoline-2-thione. The peak at 57 was due to the butyl group. The peaks at 238 and 208 were related to the Sn-containing ions ^{120}Sn-($C_3H_4NS_2$) and Bu-^{120}Sn-S, respectively. Mass spectrum of 3 exhibited expected ions peaks at m/z: 393, 238, 273, 197, 120, 118 and 77. The peak at 238 is related to the skeleton resulting from $[(C_3H_5NS)Sn]^{+}$. The peak at 77 indicates the presence of the phenyl group, along with a series of less intense peak at m/z 197 was attributable to the Sn-containing ions, ^{120}Sn–Ph moiety.

Table-6: Mass spectral data of organotin(IV) derivatives.

Compound	m/z (%)
1	$[(C_3H_4NS_2)Sn(CH_3)_3]^{+}$ 283 (11.2), $[(C_3H_4NS_2)Sn]^{+}$ 238 (44.6), $[Sn(CH_3)_2]^{+}$ 150 (13.6), $[SnCH_3]^{+}$ 135 (9.7), $[SSn]^{+}$ 152 (10.9), $[C_3H_4NS_2]^{+}$ 118 (61.5), $[C_2H_6NS]^{+}$ 76 (4.8), $[Sn]^{+}$ 120.0 (100.0)
2	$[(C_3H_4NS_2)Sn(C_4H_9)_3]^{+}$ 409 (6.7), $[(C_3H_4NS_2)Sn]^{+}$ 238 (14.7), $[Sn(C_4H_9)_2]^{+}$ 234 (12.0), $[SSnC_4H_9]^{+}$ 208 (3.4), $[Sn]^{+}$ 120 (5.9), $[C_3H_4NS_2]^{+}$ 118 (35.7), $[C_4H_9]^{+}$ 57 (100.0)
3	$[(C_3H_4NS_2)Sn(C_6H_5)_2]^{+}$ 393 (2.6), $[(C_3H_5NS)Sn]^{+}$ 238 (8.1), $Sn(C_6H_5)_2]^{+}$ 273 (5.6), $[SnC_6H_5]^{+}$ 197 (39.4), $[Sn]^{+}$ 120 (6.9), $[C_3H_4NS_2]^{+}$ 118 (100.0), $[C_6H_5]^{+}$ 77 (35.2)

Biological activities

Antibacterial activity

The need for more potent and specific antimicrobial agents continues to grow, since the pathogenic microbes develop resistance against such compounds due to gene mutation. Thus, the ligand

and synthesized organotin(IV) complexes (1-6) were screened for their antibacterial activity against two gram positive (*B. subtilis, S. aureus*) and two gram negative bacteria (*E. coli*, *P. multocida*). The results of present investigation are presented in Table 7. These complexes (1-6) exhibited elevated inhibitory activity against gram positive bacteria as their polyglycane outer layer is loosely packed to facilitate deep penetration of the complex inside the cell to interact with the cytoplasmic membrane. On the other hand, a gram-negative bacterial cell with a bilayer phospholipid structure protects the inner cytoplasmic membrane to a greater degree against the inhibitory action of the organotin(IV) complex. Tributyltin(IV) complex (2) was found to be the most potent bactericide and showed the highest antibacterial activity against *B. subtilis* and *S. aureus*. This complex was even found more active than the reference drug. Other triorganotin(IV) complexes (1 and 3) showed significant antibacterial activity against all tested bacteria. Chlorodiphenyltin(IV) complex (6) exhibited mild toxicity to all bacterial strains. However, details of bacterial resistance mechanisms are still poorly understood [43]. The bacterial growth inhibition may be due to bactericide effects or bacteriostatic effects of compounds. The inhibitory action of organotin(IV) derivatives can be understood by considering chelation theory and is believed to be due to their ability to inhibit cellular respiration and ATP synthesis. The parent ligand on chelation reduces the polarity of the central tin atom primarily because of the partial sharing of its positive charge with the donor groups and possible p-electron delocalization within the whole chelate ring. The lipophilic nature of the central Sn atom increases, which favors the permeation of the complexes through the lipid layer of the cell membrane. The alkyl groups bonded to the tin atom also play a significant role in the diffusion of metal complex through the bacterial cell wall [44].

Table-7: Antibacterial activity data[a] of organotin(IV) complexes.

Compound No.	Bacterial inhibition zone (mm)			
	Escherichia Coli	*Pasteurella Multocida*	*Bacillus Subtilis*	*Staphylococcus aureus*
HL	-	-	-	-
1	19 ± 0.6	-	24 ± 0.5	22 ± 0.7
2	19 ± 0.4	18 ± 0.7	26 ± 0.7	25 ± 0.5
3	15 ± 0.6	17 ± 0.7	-	20 ± 0.6
4	14 ± 0.7	16 ± 0.4	17 ± 0.6	20 ± 0.7
5	10 ± 0.6	9 ± 0.6	8 ± 0.7	8 ± 0.4
6	-	9 ± 0.5	6 ± 0.4	7 ± 0.6
Rifampicine	25 ± 0.7	27 ± 0.6	25 ± 0.5	24 ± 0.5

[a]0 = No activity, 5-10 = Activity present, 11-25 = Moderate activity, 26-40 = Strong activity.

Antifungal activity

The *in vitro* antifungal activity of ligand and its organotin(IV) complexes (1-6) were evaluated against four fungal strains including *A. niger, A. flavus, R. solani* and *A. alternate*, using the agar tube dilution test. Fluconazole was taken as a reference drug. The results are given in Table 8. The ligand exhibited insignificant activity against all fungal strains. All the complexes were active against *A. alternate* and the highest antifungal activity was shown by tributyltin(IV) complex (2). However, all the compounds have lower activity than the standard drug. The increase in antifungal activity of metal ions in complexes is due to their chelation with ligand. A direct relation between the activity and the coordination environment of the metal has also been observed. All complexes which generate tetrahedral geometry in solution are more active [45,46]. The activity of compounds is also affected by the nature of substituent attached with tin(IV) which decides lipophilicity of a complex. The mechanism of antifungal activity of organotin(IV) complexes is not fully understood. However, antifungal activity of these complexes may be due to their ability to form secondary intermolecular interactions with the cell constituents of the microorganisms, which in turn block the synthesis of protein by inhibiting the movement of ribosome along with RNA. This would inhibit synthesis of DNA in the cell nucleus [47]. Ligand also plays an important role in determining the degree of activity of organotin compounds [48].

Table-8: Antifungal activity data[a] of organotin(IV) complexes.

Compound No.	Fungal inhibition zone (mm)			
	Aspergillus Niger	*Aspergillus flavus*	*Rhizoctonia solani*	*Alternaria alternate*
HL	3 ± 0.6	4 ± 0.5	-	3 ± 0.4
1	-	21 ± 0.6	16 ± 0.6	19 ± 0.6
2	21 ± 0.8	20 ± 0.7	22 ± 0.5	24 ± 0.6
3	17 ± 0.5	-	18 ± 0.5	19 ± 0.5
4	12 ± 0.6	11 ± 0.7	10 ± 0.5	13 ± 0.6
5	-	9 ± 0.4	12 ± 0.7	11 ± 0.7
6	11 ± 0.7	12 ± 0.5	10 ± 0.6	10 ± 0.6
Fluconazole	23 ± 0.6	23 ± 0.5	24 ± 0.4	25 ± 0.7

[a]0 = No activity, 5-10 = Activity present, 11-25 = Moderate activity, 26-40 = Strong activity.

Minimum inhibitory concentration

The MICs of ligand and complexes were determined by the modified resazurin assay utilizing microtiter-plates [26]. Resazurin is an oxidation-reduction indicator used for evaluation of cell growth, particularly in various cytotoxicity assays. The minimum inhibitory concentrations of parent acid and synthesized complexes were evaluated against representative bacterial and fungal strains. Results are given in Tables 9 and 10 for bacterial and fungal strains, respectively. Rifapmicin and fluconazole were used as standard drugs for bacteria and fungi, respectively. The complexes showed higher MIC values than standards but lower than

ligand. In particular, complexes (2 and 3) were found to possess least MIC values against *P. multocida* at concentrations ranging from 104-119 µg/mL. Strong antibacterial properties were shown by complex (2) with MIC = 126 µg/mL and 130 µg/mL against *B. subtilis* and *S. aureus*, respectively. Among all the reported complexes, the tri-*n*-butyltin derivatives possess the most significant activity, being particularly effective against both Gram positive and negative bacteria.

Complex (2) displayed better minimum inhibitory concentrations (134 µg/mL to 172 µg/mL) against all fungal strains. Trimethyltin(IV) derivative (1) was also found to be effective against *A. flavus* (MIC 167 µg/mL). Among all the complexes, the tri-organotin derivatives possessed the most significant activity than chlorodiorganotin compounds and are most effective against fungal strains as reported earlier [49]. The MIC results agreed with antibacterial and antifungal activities results.

Table-9: MIC (bacterial) data of organotin(IV) complexes.

Compound No.	Minimum Inhibitory Concentration (µg/ml)			
	Escherichia Coli	*Pasteurella Multocida*	*Bacillus Subtilis*	*Staphylococcus aureus*
HL	-	-	-	-
1	147 ± 0.8	-	133 ± 0.8	148 ± 0.6
2	129 ± 0.6	104 ± 0.7	126 ± 0.7	130 ± 0.6
3	138 ± 0.7	129 ± 0.6	-	135 ± 0.7
4	134 ± 0.8	119 ± 0.7	120 ± 0.6	126 ± 0.8
5	141 ± 0.8	136 ± 0.8	126 ± 0.6	132 ± 0.7
6	-	148 ± 0.7	135 ± 0.8	134 ± 0.6
Rifampicine	125 ± 0.7	87 ± 0.6	112 ± 0.8	124 ± 0.7

Table-10: MIC (antifungal) data of organotin(IV) complexes.

Compound No.	Minimum Inhibitory Concentration (µg/ml)			
	Aspergillus niger	*Aspergillus flavus*	*Rhizoctonia solani*	*Alternaria alternate*
HL	326 ± 0.4	414 ± 0.6	-	404 ± 0.5
1	-	167 ± 0.5	190 ± 0.4	194 ± 0.5
2	156 ± 0.4	134 ± 0.5	169 ± 0.4	172 ± 0.4
3	208 ± 0.6	-	151 ± 0.5	183 ± 0.4
4	178 ± 0.5	181 ± 0.4	165 ± 0.5	159 ± 0.6
5	-	240 ± 0.4	235 ± 0.6	242 ± 0.4
6	251 ± 0.6	267 ± 0.4	286 ± 0.5	260 ± 0.4
Fluconazole	134 ± 0.6	132 ± 0.5	122 ± 0.5	114 ± 0.6

Biofilm inhibition activity

Antibiofilm properties of the ligand and its complexes have been tested against a preformed biofilm of two bacterial strains *B. subtilis* and *E. coli*. Results are shown in the Figure 2. The obtained results showed that the tested compounds inhibited the ability of bacterial cells to colonize the inert substratum represented by the plastic wells, as compared with the reference drug (Rifampicine) and antibiofilm activity is reported as percentage inhibition.

Almost all the synthesized complexes showed good antibacterial activity values against the planktonic form of bacteria, but a weak activity has been observed against biofilm of the same strains. However, the ligand showed maximum biofilm inhibition activity (73 % for *B. subtilis* and 69 % for *E. coli*). Triorganotin(IV) complexes which exhibited potent antibacterial activity have also been proved active against bacterial biofilm development. These compounds can found their potential use as antibiofilm agents.

The ability of metal complexes to inhibit the formation of biofilms on inert substrata can be justified that the tested compounds interfere with the synthesis of glycocalyx, the polysaccharidic substance which can form an organized structure called capsule, or an amorphous layer called slime, implicated in the formation of biofilms on inert surfaces. Tweedy's chelation theory [50] offers an explanation for the increased antimicrobial activity of the metal complexes. In the chelated complex, the positive charge of the metal ion is partially shared with the donor atoms of the ligand and - electron delocalization occurs over the whole chelate ring. In this way, the lipophilic character of the metal chelate is increasing and favoring its permeation through the lipid layers of the bacterial membranes. The antibiofilm activity of the tested substances could be explained by the inhibition of bacterial metabolic pathways, implicated in the synthesis of different microbial components, including those implicated in different phases of the adherence and biofilm development processes [51].

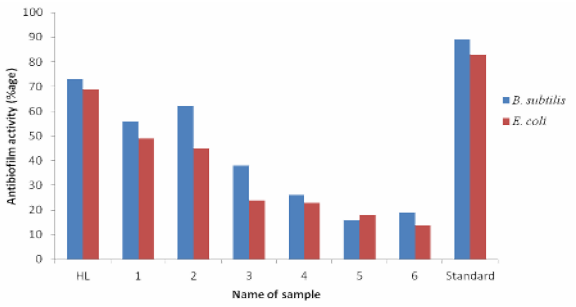


Fig. 2: Antibiofilm activity of organotin(IV) complexes. Concentration: 10 mg/mL in DMSO. *Standard: Rifampicine*

Hemolytic activity

The *in vitro* hemolytic activity (or cytotoxicity assay) of HL and the synthesized compounds were performed to check their effect on human blood by the hemolytic method [52]. The results were obtained by comparison with a positive control (Triton X-100) and a negative control (Phosphate Buffered Saline, PBS). The results are shown in Figure 3. The hemolytic activity exhibited by ligand, negative control and positive control are 4.21 %, 1.5 % and 97.0 %. The hemolytic activity of the complexes was found higher than that of the ligand and close to PBS, but not to Triton X-100. The lowest hemolytic activity was found for the complex 1 (9.45 %) and the highest for complex 4 (33.73 %). Hence the toxicity of these complexes may be very low to human blood. By comparing these results with antimicrobial activities can be concluded that these complexes showed poor hemolytic activity, but good antimicrobial activities, and could be further investigated as medicinal agents against different microorganisms with minor side effect.

Hemolytic event defined operationally by release of hemoglobin in the supernant of red blood cell suspension, is a complex phenomenon dictated by their convergence of more than one type of biochemical damage. Hemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer [52]. Membrane destruction occurs by formation of pores and lesions in it. Results reported support the concept that tributyltin(IV) induced hemolysis *via* direct membrane disruption [53]. Red blood cells have more complex nature as compared to phospholipid vesicles. The destruction properties of organotin(IV) are due to in part to their effect on membrane hydration. The effect of triphenyltin(IV) was also similar to tributyltin(IV) but less pronounced. This hemolysis relates to potency and chemical composition of the complex [54].

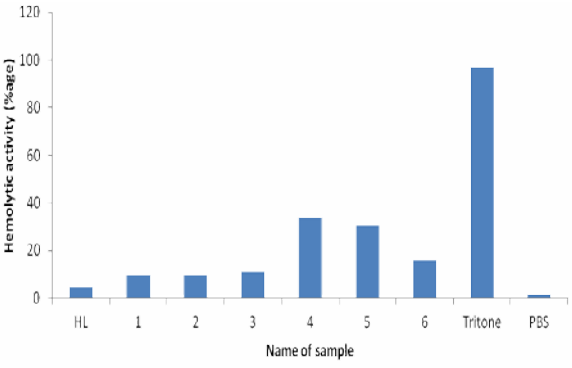


Fig. 3: Hemolytic activity of organotin(IV) complexes. Concentration: 10 mg/mL in DMSO.

Triton X-100 taken as positive control (97 % lysis) and PBS as negative control (1.5 % lysis).

Conclusion

Chlorodiorganotin(IV) and trioganotin(IV) derivatives of 2-tiazoline-2-thiol are prepared and characterized by FT-IR, NMR, mass spectroscopy and elemental analysis. Ligand coordinates to tin atom *via* sulphur atom of the thiazol group in solution. The complexes exhibit 4-coordinated geometry in solid and solution state. These compounds are found active against studied bacteria, and fungi. These are also found good inhibitor of biofilms of bacterial pathogens. The hemolytic activity of the synthesized compounds proved that these compounds can be used as drugs in safe way after further research in future.

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References

1. M. Nath, S. Pokharia and R. Yadav, Organotin(IV) complexes of amino acids and peptides. *Coord. Chem. Rev*; **215**, 99 (2001).
2. L. Pellerito and L. Nagy, Organotin(IV)$^{n+}$ complexes formed with biologically active ligands: equilibrium and structural studies, and some biological aspects, *Coord. Chem. Rev*; **224**, 111 (2002).
3. P. Yang and M. Guo, Interactions of organometallic anticancer agents with nucleotides and DNA, *Coord. Chem. Rev*; **185**, 189 (1999).
4. V. I. Bregadze, S. A. Glazun, P. V. Petrovskii, Z. A. Starikova, V. Y. Rochev, H. Dalil, M. Biesemans, R. Willem, M. Gielen and D. de Vos, Synthesis, characterization, X-ray crystal structure *in vitro* tumor activity of bis(1,2-dicarbocloso-dodecacarborane-carboxylato)di-*n*-butyltin, *Appl. Organomet. Chem*; **17**, 453 (2003).
5. S. Barreiro, M. L. Dur-an-Carril, J. Viqueira, A. Sousa-Pedrares, García-V-azquez and J. A. J. Romero, Structural studies and bioactivity of diorganotin(IV) complexes of pyridin-2-thionato derivatives, *J. Organomet. Chem*; **791**, 155 (2015).
6. M. Dylag, H. Pruchnik, F. Pruchnik, Majkowska-Skrobek and S. Ulaszewsk, Antifungal activity of organotin compounds with functionalized carboxylates evaluated by the microdilution bioassay *in vitro*, *Med. Mycol*; **48**, 373 (2010).

7. M. N. Xanthopoulou, S. K. Hadjikakou, N. Hadjiliadis, E. R. Milaeva, J. A. Gracheva, V. Y. Tyurin, N. Kourkoumelis, K. C. Christoforidis, A. K. Metsios, S. Karkabounas and K. Charalabopoulos, Biological studies of new organotin(IV) complexes of thioamide ligands, *Eur. J. Med. Chem*; **43**, 327 (2008).
8. S. Durand, K. Sakamoto, T. Fukuyama, A. Orita, J. Otera, A. Duthie, D. Dakternieks, M. Schulte and K. Jurkschat, Cationic organotin clusters for highly efficient alcohol acetylation catalysts, *Organometallics*; **19**, 3220 (2000).
9. S. H. L. Thoonen, B. J. Deelman and G. V. Koten, Synthetic aspects of tetraorganotins and organotin(IV) halides, *J. Organomet. Chem*; **689**, 2145 (2004).
10. C. J. Evans, *PVC for carbonated drinks, Tin and its uses*. International tin research and development council. Tin Research Institute, Greenford, England, (1981).
11. M. Nath, Sulaxna, X. Song, G. Eng and A. Kumar, Synthesis and spectral studies of organotin(IV) 4-amino-3-alkyl-1,2,4-triazole-5-thionates: *In vitro* antimicrobial activity, *Spectrochimica Acta Part A*; **70**, 766 (2008).
12. M. N. Xanthopoulou, S. K. Hadjikakou, N. Hadjiliadis, M. Schuermann, K. Jurkschat, A. Michaelides, S. Skoulika, T. Bakas, B. J. J. Binolis, S. Karkabounas and C. Haralampopoulos, Synthesis, structural characterization and *in vitro* cytotoxicity of organotin(IV) derivatives of heterocyclic thioamides, 2-mercaptobenzothiazole, 5-chloro-2-mercaptobenzothiazole, 3-methyl-2-mercaptobenzothiazole and 2-mercaptonicotinic acid, *J. Inorg. Biochem*; **96***,* 425 (2003).
13. Y. Li, Z. Wang, P. Guo, L. Tang, R. Ge, S. Ban, Q. Chai, L. Niu and Q. Li, Diorganotin(IV) derivatives of substituted N-hydroxybenzamides with selective cytotoxicity *in vitro* and potent antitumor activity *in vivo*, *J. Inorg. Biochem*; **133**, 1 (2014).
14. A. Alama, B. Tasso, F. Novelli and F. Sparatore, Organometallic compounds in oncology: implications of organotins as antitumor agents, *Drug Discovery Today*; **14(9-10)**, 500 (2009).
15. X. Song, A. Zapata and G. Eng, Organotins and quantitative-structure activity/property relationships, *J. Organomet. Chem*; **691**, 1756 (2006).
16. T. Baul, C. Masharing, G. Ruisi, R. Jir-asko, M. Holcapek, D. de-Vos, Wolstenholme and A. Linden, Self-assembly of extended Schiff base amino acetate skeletons, 2-{[(2Z)-(3-hydroxy-1-methyl-2-butenylidene)]amino}phenylpropionateand 2-{[(E)-1-(2-hydroxyaryl)-alkylidene]amino}phenylpropionate skeletons incorporating organotin(IV) moieties: Synthesis, spectroscopic characterization, crystal structures, and *in vitro* cytotoxic activity, *J. Organomet. Chem*; **692**, 4849 (2007).
17. E. Katsoulakou, M. Tiliakos, G. Papaefstathiou, A. Terzis, C. Raptopoulou, G. Geromichalos, K. Papazisis, R. Papi, A. Pantazaki, D. Kyriakidis, P. Cordopatis, and E. Zoupa, Diorganotin(IV) complexes of dipeptides containing the α-aminoisobutyryl residue (Aib): Preparation, structural characterization, antibacterial and antiproliferative activities of $[(n\text{-Bu})_2Sn(H^1L)]$ (LH = H-Aib-L-Leu-OH, H-Aib-L-Ala-OH), *J. Inorg. Biochem*; **102**, 1397 (2008).
18. E. S. Raper, Complexes of heterocyclic thionates Part 2: Complexes of bridging ligands, *Coord. Chem. Rev*; **165**, 475 (1997).
19. B. Krebs and G. Henkel, Transition-metal thiolates: from molecular fragments of sulfidic solids to models for active centers in biomolecules, *Angew Chem*; **30**, 769 (1991).
20. J. R. Dilworth and J. Hu, Complexes of sterically hindered thiolate ligands, *Adv. Inorg. Chem*; **40**, 411 (1993).
21. P. D. Akrivos, Recent studies in the coordination chemistry of heterocyclic thiones and thionates, *Coord. Chem. Rev*; **213**, 181 (2001).
22. J. A. García-Vazquez, J. Romero and A. Sousa, Electrochemical synthesis of metallic complexes of bidentate thiolates containing nitrogen as an additional donor atom, *Coord. Chem. Rev*; **193**, 691 (1999).
23. D. Katiyar, V. K. Tiwari, R. P. Tripathi, A. Srivastava, V. Chaturvedi, R. Srivastava and B. S. Srivastava, Synthesis and antimycobacterial activity of 3,5-disubstituted thiadiazine thiones, *Bioorg. Med. Chem*; **11**, 4369 (2003).
24. A. G. Davies, M. Gielen, K. H. Pannell and E. R. T. Tiekink, *Tin Chemistry, Fundamental, Frontiers, and Applications* (Eds.), John Wiley, London (2008).
25. A. Tarassoli and S. Azizi-Talooky, Synthesis and characterization of complexes of organotin(IV) with 2-thiazoline-2-thione, *J. Coord. Chem*; **65**, 3395 (2012).
26. S. Jabbar, I. Shahzadi, R. Rehman, H. Iqbal, Qurat-ul-Ain, A. Jamil, R. Kousar, S. Ali, S. Shahzadi, M. A. Choudhary, M. Shahid, Q. M. Khan, S. K. Sharma and K. Qanungo, Synthesis, characterization, semi-empirical study, and biological activities of organotin(IV) complexes with cyclohexylcarbamodithioic acid as biological active ligand, *J. Coord. Chem*; **65**, 572 (2012).

27. M. Jabeen, S. Ali, S. Shahzadi, M. Shahid, Q. M. Khan, S. K. Sharma and K. Qanungo, Homobimetallic complexes of ligand having O- and S-donor sites with same and different di- and trialkyl/aryltin(IV) moiety: their synthesis, spectral characterization and biological activities, *J. Iran. Chem. Soc*; **9**, 307 (2012).
28. M. Rafiq, S. Ali, S. Shahzadi, M. Shahid, S. K. Sharma, K. Qanungo, Synthesis, characterization, and biological activities of homo- and heterobimetallic complexes of Sn(IV) and Pd(II) with 2-mercapto-5-methyl benzimidazole, *J. Iran. Chem. Soc*; **11**, 169 (2014).
29. B. Parveen, I. H. Bukhari, S. Shahzadi, S. Ali, S. Hussain, K. G. Ali and M. Shahid, Synthesis and spectroscopic characterization of mononuclear/binuclear organotin(IV) complexes with 1*H*-1,2,4-triazole-3-thiol: Comparative studies of their antibacterial/antifungal potencies, *J. Serb. Chem. Soc*; **80**, 755 (2015).
30. W. L. F. Armarego and C. L. L. Chai, *Purification of laboratory chemicals*, 5th Edition, Butterworth Heinemann, London (2003).
31. CLSI (The Clinical Laboratory Standards Institute), *J. Clin. Microbiol*; **45**, 2758 (2007).
32. G. Huang, S. J. Moore, Jiaxin and D. Dehui, Antioxidative and antibacterial activity of the methanol extract of *Artemeisia anomala*, *Food Sci. Technol*; **11**, 25 (2001).
33. S. D. Sarker, L. Nahar and Y. Kumarasamy, Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals, *Methods*; **42**, 321 (2007).
34. W. A. Powell, C. M. Catranis and C. A. Maynard, Design of self processing antimicrobial peptide for plant protection, *Lett. Appl. Microbiol*; **31**, 163 (2000).
35. M. Nath, Sulaxna, X. Song and G. Eng, Synthesis, spectral and thermal studies of some organotin(IV) derivatives of 5-amino-3H-1,3,4-thiadiazole-2-thione, *Spectrochim. Acta Part A*; **64**, 148 (2006).
36. Q. Wang, J. Zhang and J. Sun, A series of new tin compounds derived from 4-mercaptophenylacetic acid: Synthesis and characterization, *J. Organomet. Chem*; **803**, 128 (2016).
37. M. Sirajuddin, S. Ali, F. Shah, M. Ahmad and M. N. Tahir, Potential bioactive Vanillin–schiff base di- and tri-organotin(IV) complexes of 4-((3,5-dimethylphenylimino)methyl)-2-methoxyphenol: synthesis, characterization and biological screenings, *J. Iran. Chem. Soc*; **11**, 297 (2014).
38. M. Sirajuddin and S. Ali, *Synthesis and characterization of potential bioactive organotins*, LAP, Germany (2013).
39. M. Sirajuddin, S. Ali and M. N. Tahir, Pharmacological investigation of mono-, di- and triorganotin(IV) derivatives of carbodithioates: Design, spectroscopic characterization, interaction with SS-DNA and POM analyses, *Inorg. Chim. Acta*; **439**, 145 (2016).
40. J. Holecek, M. Nadvornik, K. Handlir and A. Lycka, ^{13}C and ^{119}Sn NMR spectra of Di-*n*-butyltin(IV) compounds, *J. Organomet. Chem*; **315**, 299 (1986).
41. T. P. Lockhart, W.F. Manders and E.M. Holts, Structure determination by NMR spectroscopy. Dependence of $|^2J(^{119}Sn,^1H)|$ on the Me-Sn-Me angle in methyltin(IV) compounds, *J. Am. Chem. Soc*; **108**, 6611 (1986).
42. A. Tarassoli, T. Sedaghat, B. Neumuller and M. Ghassemzadeh, Synthesis, spectroscopic investigations and crystal structures of organotin(IV) derivatives of 2-amino-1-cyclopentene-1-carbodithioic acid, *Inorg. Chim. Acta*; **318**, 15 (2001).
43. S. Shujah, Z. Rehman, N. Muhammad, A. Shah, S. Ali, A. Meetsma and Z. Hussain, Homobimetallic organotin(IV) complexes with hexadentate Schiff base: Synthesis, crystal structure and antimicrobial studies, *J. Organomet. Chem*; **759**, 19 (2014).
44. N. Muhammad, Zia-ur-Rehaman, S. Shujah, A. Shah, S. Ali, A. Meetsma and Z. Hussain, Syntheses, structural characteristics, and antimicrobial activities of new organotin(IV) 3-(4-bromophenyl)-2-ethylacrylates, *J. Coord. Chem*; **65***,* 3766 (2012).
45. K. Tahira, S. Ali, S. Shahzadi, S. K. Sharma and K. Qanungo, Bimetallic organotin(IV) complexes with ferrocene-based azomethines: synthesis, characterization, semi-empirical study, and antibacterial activity, *J. Coord. Chem*; **64**, 1871 (2011).
46. M. K. Samota and G. Seth, Synthesis, characterization, and antimicrobial activity of palladium(II) and platinum(II) complexes with 2-substituted benzoxazole ligands, *Heteroatom Chem*; **21**, 44 (2010).
47. Zia-ur-Rehman, N. Muhammad, A. Shah, S. Ali and E. Khan, New supramolecular triorganotin(IV) dithiocarboxylates as potential antibacterial agents, *Heteroatom Chem*; **23**, 560 (2012).
48. S. Hussain, S. Ali, S. Shahzadi and M. Shahid, Heterobimetallic complexes containing Sn(IV) and Pd(II) with 4-(2-Hydroxyethyl)piperazine-1-carbodithioic acid: Synthesis, characterization

and biological activities, *Cogent Chem*; **1**, 1029038 (2015).

49. M. Ashfaq, A. Majeed, A. Rauf, A. W. K. Khazanda, W. U. Shah and M. I. Ansari, Organotin esters of *N*-phthaloylamino acids: synthesis, spectroscopic characterization, and biological activities, *Bull. Chem. Soc. Japan*; **72**, 2073 (1999).
50. L. V. Tamayo, A. E. Burgos and P. F. B. Brandão, Synthesis, characterization, and antimicrobial activity of the ligand 3-methylpyrazole-4-carboxaldehyde thiosemicarbazone and its Pd(II) complex, *Phosphorus, Sulfur, and Silicon and Rela. Elem*; **189**, 52 (2014).
51. T. Vladkova, D. Akuzov, A. Kloppel and F. Brummer, Current approaches to reduction of marine biofilm formation, *J. Chem. Metallurgy*; **4**, 345 (2014).
52. M. Zohra and A. Fawzia, Hemolytic activity of different herbal extracts used in Algeria, *Inter. J. Pharma Sci. Res*; **5**, 495 (2014).
53. A. Ortiz, Effect of triorganotin(IV) compounds or membrane permeability, *Biochim. et Phys. Acta*; **1720**, 137 (2005).
54. A. Pagliarani, F. Trombetti and V. Ventrell, *Biochemical and biological effects of organotins(IV)*, Bentham Science Publishers, USA (2012).