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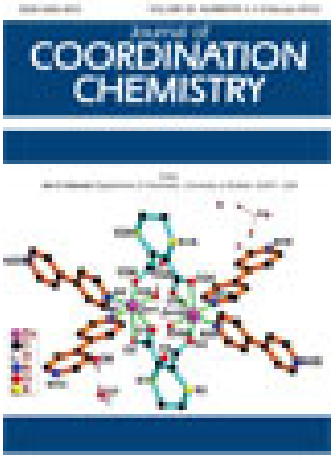

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Synthesis, characterization, semi-empirical study, and biological activities of organotin(IV) complexes with cyclohexylcarbamodithioic acid as biological active ligand

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Synthesis, characterization, semi-empirical study, and biological activities of organotin(IV) complexes with cyclohexylcarbamodithioic acid as biological active ligand

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Cyclohexylcarbamodithioic acid has been synthesized by the reaction of cyclohexylamine with carbon disulfide at room temperature. Its complexes have been synthesized by the reaction of cyclohexylcarbamodithioic acid with organotin(IV) chlorides in 1 : 1/1 : 2 molar ratio. The ligand and complexes have been characterized by elemental analysis, infrared (IR), and multinuclear (^{1}H, ^{13}C, and ^{119}Sn) NMR spectroscopy. Elemental data show good agreement between calculated and found values of carbon, hydrogen, nitrogen, and sulfur. IR data show that the ligand is bidentate and complexes exhibit a five-coordinate geometry in the solid state, which is also confirmed by semi-empirical studies. NMR data show that the complexes exhibit tetrahedral geometry in solution state. The ligand and its complexes were screened for their *in vitro* mutagenic, antimicrobial, MIC, antioxidant activities, and cytotoxicity. Biological screening data demonstrate that complexes show significant activity against various bacterial and fungal strains and are good antioxidants. The cytotoxicity data show positive lethality for complexes as compared to ligand and can play a very significant role in drug development.

Keywords: Organotin(IV) complexes; IR; NMR; Mutagenic; Antimicrobial; MIC; Antioxidant; Cytotoxicity; Semi-empirical study

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1. Introduction

Organotin compounds are among the most widely used organometallic compounds [1]. Industrial use of non-toxic organotin compounds (R_2SnX_2 and $RSnX_3$) account for almost two-thirds of the total world consumption [2], although the other major use of these derivatives is their selectivity for a variety of biological applications including fungicides, anti-fouling agents [3–5], anti-inflammatory agents [6], pesticides [1], and cardiovascular [7]. In addition to extensive biological applications, anti-tumor and anti-cancer activities of such compounds have made them important [6]. The number and diversity of sulfur chelating agents used to prepare new coordination and organometallic compounds have increased rapidly because they provide an interesting series of ligands whose properties can be modified by introducing different organic substituents, thereby causing variation in the donor properties. The interaction of these donors with metal ions gives complexes of different geometries and properties which have potential for biological applications [8]. Metal thiolato complexes have been studied because of their ability to adopt various nuclearities and their relevance in biology [9–12], since they form the inorganic part of the biologically active centers of some metalloproteins and enzymes [13]. Dithiocarbamates are active against fungi [14, 15] and insects [16]. The aim of this work is to prepare new organotin(IV) derivatives with cyclohexylcarbamodithioic acid. All these compounds have been characterized by elemental analyses, infrared (IR), and multinuclear NMR (^{1}H, ^{13}C, and ^{119}Sn) spectroscopy. In addition, these compounds were tested for their *in vitro* mutagenic, antimicrobial, minimum inhibitory concentration (MIC), antioxidant, and hemolytic activities.

2. Experimental

2.1. *Chemicals and instrumentation*

Cyclohexylamine, ethanol, carbon disulfide, and organotin chlorides were purchased from Aldrich Chemical Company (USA). Sodium carbonate (Na_2CO_3), Na_2HPO_4, KH_2PO_4, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and methanol were purchased from Merck (UK). Nutrient agar, nutrient broth, and potato dextrose agar (PDA) were purchased from Oxoid Company (UK). All reagents used were of analytical grade.

The melting points were determined in capillary tube on an electrothermal melting point apparatus, model Stuart (SMP3) (UK) and are uncorrected. IR spectra were recorded using a Perkin-Elmer 1000 FT-IR Spectrophotometer as KBr discs/thin films from 4000 to 250 cm^{-1}. NMR spectra were recorded on a Bruker AM-250 MHz FT-NMR spectrometer (Germany) using deuterated $CDCl_3$ as internal reference. The percentage composition of C, H, N, and S were determined by using a CHNS-932 Leco (USA). The antimicrobial activities of the ligand and organotin(IV) complexes have been performed in an incubator (Sanyo, Germany) and sterilized in an autoclave (Omron, Japan). The MIC and antioxidant activities were determined in a Micro Quant apparatus (BioTek, USA). Centrifuge H-200 NR (Kokusan, Japan) was used to centrifuge the sample solutions and Haemacytometer (Fisher Ultra Plane, Japan) used to count the RBC in cyctotoxic assay. Colony counter GW-92CL, Go Won Scientific Technology Co., Seoul, Korea has been used for counting the colonies in mutagenic activity.

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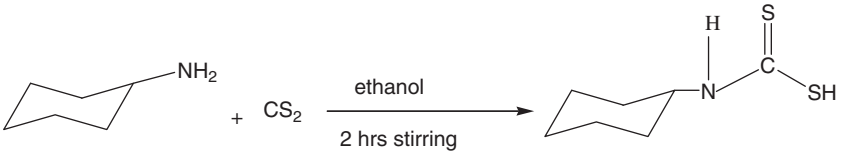
The molecules were modeled by MOPAC2007 [17] program in the gas phase using the PM3 method [18, 19]. Selected parts of the complexes not containing the metal ion were pre-optimized using molecular mechanics methods. Several cycles of energy minimization had to be carried out for each molecule. Geometry was optimized using Eigen Vector. The root-mean-square gradients for molecules were all less than one. Self consistent field was achieved in each case.

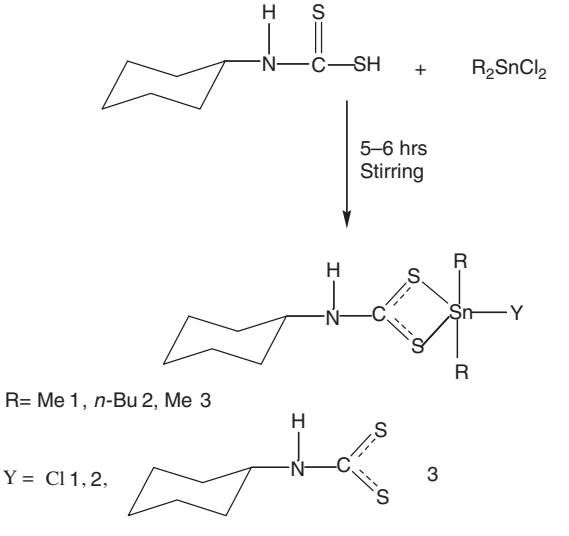
2.2. *Synthesis of cyclohexylcarbamodithioic acid*

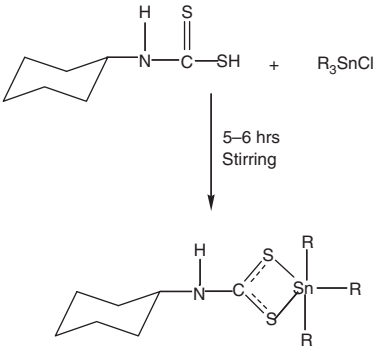
Cyclohexylamine (0.09 mL, 1 mmol) was dissolved in ethanol (25 mL) in a round bottom flask and stirred for 15 min at room temperature. Then carbon disulfide (0.06 mL, 1 mmol) was added dropwise to the above solution with constant stirring. The reaction mixture was continuously stirred for 2 h at room temperature. The off-white precipitates formed were filtered, washed with ethanol, and air dried. Purity was checked by TLC.



2.3. *General procedure for synthesis of organotin(IV) complexes*

Cyclohexylcarbamodithioic acid (1 mmol) was dissolved in chloroform (90 mL) in a round bottom flask under continuous stirring for 15 min at room temperature. Then R_2SnCl_2 (1 : 1/1 : 2)/R_3SnCl and L (1 : 1) ratio was added in portions to the reaction flask and the reaction mixture was stirred for 5–6 h. The light yellow precipitates formed were filtered off and dried in open air. Purity was checked by TLC.





R= Me **4**, n-Bu **5**, Ph **6**

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2.4. *Antibacterial assay*

2.4.1. Bacterial growth medium, cultures, and inoculums preparation. Pure cultures were maintained on nutrient agar medium in petri plates. For the inoculums preparation, 13 g L^{-1} of nutrient broth was suspended in distilled water, mixed well, and distributed homogeneously. Then autoclaved 10 μL of pure culture of a bacterial strain was mixed in the medium and placed in a shaker for 24 h at 37°C. The inocula were stored at 4°C. The inocula with 1×10^8 spores mL^{-1} were used for further analysis.

2.4.2. Antibacterial assay by the disc diffusion method. Antibacterial activity of samples was determined by using the disc diffusion method [20, 21]. Nutrient agar 28 g L^{-1} was suspended in distilled water, mixed well, and distributed homogeneously. The medium was sterilized by autoclaving at 121°C for 15 min. Before the medium was transferred to petri plates, inoculums (100 μL/100 mL) were added to the medium and poured in sterilized petri plates.

After this, small filter paper discs were laid flat on the growth medium containing 100 μL of sample solution. The petri plates were then incubated at 37°C for 24 h, for the growth of bacteria. The sample having antibacterial activity inhibited the bacterial growth and clear zones were formed. The zones of inhibition were measured in millimeters using a zone reader [22].

2.5. *Antifungal assay*

2.5.1. Growth medium, cultures, and inoculum preparation. Pure cultures of the fungi were maintained on PDA medium in slant or petri plates that were pre-sterilized in hot air oven at 180°C for 3 h. These culture slants were incubated at 28°C for 3–4 days for the multiplication of fungal strains.

2.5.2. Antifungal assay by the disc diffusion method. The prepared sterilized growth medium was transferred to the sterilized petri plates. The petri plates were then

incubated at 28°C for 48 h, for the growth of fungus. Small filter paper discs were laid flat on the growth medium having fungal growth and 100 µL of each sample was applied on each disc. The petri plates were again incubated. The samples having antifungal activity exhibit clear zones around the discs. The zones of inhibition were measured in millimeters using a zone reader [22].

2.6. *Minimum inhibitory concentration*

The MIC was determined by the modified resazurin assay utilizing microtiter-plate [23].

2.6.1. Preparation of bacterial and fungal culture. Using aseptic technique a single colony was transferred to 100 mL bottle of nutrient broth, capped and placed in an incubator overnight at 37°C. After 12–18 h of incubation using aseptic preparation and the aid of a centrifuge, a clean sample of bacteria was prepared. The broth was spun down using the centrifuge at 4000 rpm for 5 min. The supernatant was discarded into an appropriately labeled and contaminated waste beaker.

The pellet was re-suspended using 20 mL of sterile normal saline and centrifuged again at 4000 rpm for 5 min. This step was repeated until the supernatant was clear. The pellet was suspended in 20 mL normal saline and was labeled. The optical density of bacterial culture was recorded at 500 nm and serial dilutions were carried out with appropriate aseptic technique until the optical density was in the range 0.5–1.0. The actual number of colony forming units was calculated from the viability graph. The dilution factor needed was calculated and the dilution was carried out to obtain a concentration of 5×10^6 CFU mL^{-1}. The fungal culture was prepared by the same procedure as described in antifungal activity.

2.6.2. Procedure for MIC. Plates were prepared under aseptic condition. A sterile 96 well plate was labeled. A volume of 100 µL of test material was pipetted out into the first row of the plate. To all other wells 50 µL of nutrient broth was added. Serial dilutions were performed. Tips were discarded after use such that each well had 50 µL of the test material in serially descending concentration. To each well 10 µL of resazurin indicator solution was added. Finally, 10 µL of bacterial suspension (5×10^6 CFU mL^{-1}) was added to achieve a concentration of 5×10^5 CFU mL^{-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°C for 48 h for fungus and at 37°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 [23].

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2.7. *DPPH radical scavenging assay*

The DPPH assay was carried out as described by Bozin [24]. The antioxidant activity of samples was assessed by measuring their scavenging abilities of the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Aliquot (50 μL) of various concentrations of the samples was added to 5 mL of a 0.004% methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The assay was carried out in triplicate.

$$I(\%) = (A_{\text{blank}} - A_{\text{sample}}A_{\text{blank}}) \times 100$$

A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph which was obtained by plotting inhibition percentage against sample concentration.

2.8. *Hemolytic activity*

Hemolytic activity was checked by using Powell's method [25]. 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 min 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°C). The washed cells were suspended in the 20 mL chilled, saline PBS buffer. Erythrocytes were counted on a haemacytometer.

The blood cell suspension was maintained on wet ice and diluted with sterile PBS. The cell count should be 7.068×10^8 cells per mL for each assay. 20 μL of sample solution (10%) was taken in 2 mL Apendoff 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 Apendoff 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 min at 37°C and agitated after 10 min. Immediately after incubation, the Apendoff tubes were placed on ice for 5 min and then centrifuged for 5 min 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 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:

$$\%\ \text{hemolysis} = \frac{\text{Hb}_{\text{ABS}}}{\text{Hb}_{100\ \%\text{ABS}}} \times 100.$$

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 [25].

2.9. *Statistical analysis*

The values obtained are given as means ± SD of each measurement. Where appropriate, the data were tested by one-way ANOVA using Minitab 13. Pearson correlation coefficients and *p*-values were used to show correlations and their significance. Differences of $p < 0.05$ were considered significant [26].

2.10. *Mutagenic activity*

The mutagenic potential was evaluated by the Ames test [27] through the plate incorporation method. The plate incorporation test consists of combining the test compound and bacterial tester strain in soft top agar which is poured onto the minimal agar plates; positive and negative controls are also included in each assay. After incubation at 37°C for 48 h revertant colonies are counted. The same method was followed by just increasing the time of incubation because the size of revertant colonies after 48 h remained too small to be counted easily. So, the time of incubation was increased to 60 h. Top agar tubes containing 2 mL top agar were prepared and autoclaved. Tubes were heated to melt the top agar and were placed in a heating block at 45°C. Histidine/biotin (his/bio) 0.5 mmol L^{-1} solution was prepared and filter sterilized by using autoclaved filter assemblies with 0.22 μmol L^{-1} filter paper discs. Filtered samples were used for testing their mutagenicity. 100 μL of test sample was added to these tubes. Then 100 μL of overnight grown culture of test strain containing approximately 3×10^7 cell mL^{-1} and OD 0.7–0.8 at 590 nm was added. The tube was vortexed and then immediately poured onto the medium plates. The plates were tilted and rotated quickly for even spreading of top agar mixture over the whole surface of the plates, which were then placed on a level surface to harden. For each experiment plates were used in triplicate. All samples were checked with TA98 and TA100. Positive and negative controls were also used for each test. As a negative control 100 μL autoclaved DMSO was used. As a positive control, potassium dichromate (10 μg/plate) was used for TA98 and the same amount of sodium azide was used for TA100. This process was carried out with great care under aseptic conditions by using laminar air flow and gas burner. Plates were incubated at 37°C for 60 h and the revertant colonies on test and control plates were counted on the colony counter. The presence of background lawn on the negative control plate is evidence of bacterial toxicity. In the negative control, spontaneously revertant colonies were also found for both strains. Single-lines were streaked on nutrient agar plates by sterilized wire loop from all test samples and solutions for sterility testing and from bacterial cultures for their viability. Mutagenicity index (MI) was calculated by the following formula:

$$\text{Mutagenicity index (MI)} = \frac{\text{No. of revertant colonies in test plate}}{\text{No. of revertant colonies in negative control plate}}$$

3. Results and discussion

The synthesized cyclohexylcarbamodithioic acid and organotin(IV) complexes are solids and stable in air. They have sharp melting points and are soluble in common

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Table 1. Physical data of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.

Compound number	Molecular formula	m.w.	m.p. (°C)	Yield (%)	Elemental analysis % C Calcd (found)		% H Calcd (found)		% N Calcd (found)		% S Calcd (found)	
HL	$C_7H_{13}NS_2$	175.32	181	64	47.96	(47.92)	7.47	(7.51)	7.99	(7.95)	36.58	(36.62)
1	$C_9H_{18}NS_2SnCl$	358.54	155	96	30.15	(30.11)	5.16	(5.02)	3.91	(3.95)	17.89	(17.93)
2	$C_{15}H_{30}NS_2SnCl$	442.2	201	77	40.77	(41.74)	6.83	(6.87)	3.16	(3.12)	14.49	(14.53)
3	$C_{16}H_{30}N_2S_4Sn$	497.4	130	54	38.64	(38.68)	6.08	(6.94)	5.63	(5.59)	25.79	(25.74)
4	$C_{10}H_{21}NS_2Sn$	338.19	195	55	35.52	(35.56)	6.26	(6.22)	4.14	(4.18)	18.97	(18.93)
5	$C_{19}H_{39}NS_2Sn$	464.36	209	64	49.14	(49.10)	8.47	(8.51)	3.02	(3.06)	13.81	(13.77)
6	$C_{25}H_{27}NS_2Sn$	524.33	111	93	57.27	(57.31)	5.19	(5.15)	2.67	(2.63)	12.23	(12.27)

Table 2. Characteristic IR bands (cm^{-1}) of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.

Compound number	ν(N–H)	ν(S–H)	ν(C–N)	ν(C=S)	ν(C–S)	ν(Sn–C)	ν(Sn–S)	ν(Sn–Cl)
HL	3463	2749	1446	1087	943	–	–	–
1	3467	–	1447	1082	982	558	453	368
2	3368	–	1444	1078	960	551	449	358
3	3467	–	1447	1079	982	550	450	–
4	3367	–	1446	1073	954	551	440	–
5	3468	–	1444	1085	920	555	456	–
6	3466	–	1449	1072	920	342	440	–

organic solvents. Elemental analysis was done to compare the observed values of percentage of carbon, hydrogen, nitrogen, and sulfur in the cyclohexylcarbamodithioic acid and its complexes with those of predicted values. The observed values are in good agreement with the calculated values. The physical data of cyclohexylcarbamodithioic acid and organotin(IV) complexes are summarized in table 1.

3.1. *IR spectroscopy*

IR spectra of HL and complexes were recorded as KBr discs/thin films from 4000 to 250 cm^{-1}. The values of different vibrational frequencies were assigned to different bonds by comparing spectra of ligand and complexes. Complexation through sulfur was confirmed by disappearance of ν_{S-H} in spectra of the complexes [28]. This band appears at 2749 cm^{-1} in spectra of the ligand. The appearance of medium intensity bands due to ν_{Sn-S} and ν_{Sn-Cl} in the complexes at 456–440 cm^{-1} and 368–358 cm^{-1}, respectively, further confirm complexation. The presence of a single band at 1072–1085 cm^{-1} is assigned to ν_{C-S} and its relatively high value suggests that dithiocarbamate is linked to Sn bidentate. The complexes do not show any significant shift in ν_{N-H}, confirming that complexation does not take place through nitrogen.

The medium to sharp intensity band that appears at 558–342 cm^{-1} was assigned to ν_{Sn-C} (table 2). The band at 982–920 cm^{-1} in spectra of complexes was assigned to ν_{C-S} and was important to confirm the bidentate mode of the ligand [29].

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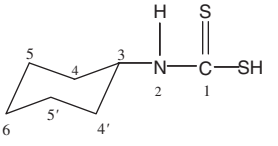
Table 3. ^{1}H NMR data[a–c] of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.

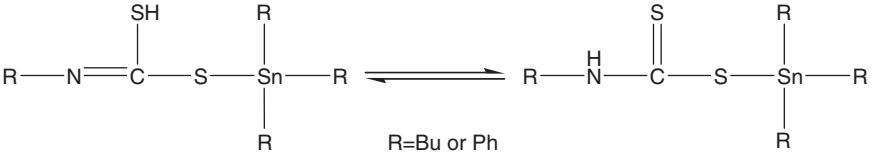
	Chemical shift (ppm)						
Proton	HL	**1**	**2**	**3**	**4**	**5**	**6**
2	3.14 s	3.14 s	3.13 s	3.14 s	3.13 s	3.14bs	3.14bs
3	3.61–3.65 m	3.60–3.66 m	3.61–3.65 m	3.61–3.66 m	3.60–3.66 m	3.60–3.66 m	3.61–3.65 m
4,4′	1.74–1.79 m	1.74–1.79 m	1.74–1.79 m	1.74–1.78 m	1.73–1.78 m	1.74–1.79 m	1.74–1.78 m
5,5′	1.58–1.65 m	1.58–1.66 m	1.58–1.65 m	1.57–1.66 m	1.58–1.65 m	1.58–1.65 m	1.57–1.65 m
6	1.39–1.47 m	1.39–1.47 m	1.39–1.47 m	1.39–1.46 m	1.39–1.49 m	1.39–1.47 m	1.39–1.47 m

[a](**1**) Sn–CH_3Cl, 1.55 s ^{2}J[96]; (**2**) Sn–$CH_2CH_2CH_2CH_3Cl$, 0.88–0.93 m, 0.23 t (7.2); (**3**) Sn–CH_3, 1.35 s ^{2}J[90]; (**4**) Sn–CH_3, 0.51 s ^{2}J[82]; (**5**) Sn–$CH_2CH_2CH_2CH_3$, 0.64–1.1 m, 0.23 t (7.2); (**6**) Sn–C_6H_5, 7.91 d ^{2}J[57.2], 7.50–7.53 m, 7.42–7.50 m.
[b]Chemical shifts (δ) in ppm. $^{2}J[^{119}Sn,^{1}H]$ and $^{3}J(^{1}H,^{1}H)$ in Hz are listed in square brackets and parenthesis, respectively. Multiplicity is given as: s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet.



3.2. *NMR spectroscopy*

3.2.1. ^{1}H NMR. The ^{1}H NMR spectral data of the ligand and complexes are given in table 3 with their coupling constants. All protons present in the complexes have been identified at position and number with protons calculated from the incremental method [30]. The SH peak at 1.58 ppm in the ligand is absent in all the complexes, showing deprotonation of the ligand during complexation. As reported earlier, when bulky groups like *n*-C_4H_9 or C_6H_5 are attached in $R_3SnOOCR'$, the carboxylate is monodentate [31–33]. Probably there is a fast tautomerism in the ligand as shown below, giving a broad signal at 3.14 ppm.



Protons of the dithiolate give a multiplet at 1.39–3.65 ppm which does not show significant shift upon complexation. In **2** protons of the butyl group appear as multiplet at 0.88–0.93 ppm. The terminal CH_3 group of butyl is a triplet at 0.23 ppm with $^{3}J[^{1}H–^{1}H]$ coupling of 7.2 Hz. The proton chemical shift of the methyl in **4** attached to the Sn gives a singlet at 0.51 ppm. The $^{2}J[^{119}Sn–^{1}H]$ coupling constant value for this complex is 82 Hz, in the range expected for the five-coordinate tin atom and consistent with the C–Sn–C angle of 126.5°.

3.2.2. ^{13}C NMR. ^{13}C and ^{119}Sn NMR spectral data of the complexes are listed in table 4. The assignment of ^{13}C signals are made by comparison with related compounds from the incremental method [30]. The ^{13}C NMR chemical shifts due to phenyl attached to Sn were observed at positions comparable to related compounds [34].

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Table 4. ^{13}C and ^{119}Sn NMR data[a–c] of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.

	Chemical shift (ppm)						
Carbon	HL	**1**	**2**	**3**	**4**	**5**	**6**
1	211.7	197.6	195.8	195.7	195.5	198.9	195.4
3	51.4	51.2	51.5	51.6	51.2	51.1	51.9
4,4′	59.7	59.7	59.8	59.6	59.7	59.6	59.5
5,5′	24.7	24.6	24.2	24.8	24.3	24.9	24.6
6	32.0	32.2	32.1	32.7	32.5	32.8	32.3

[a](**1**) Sn–CH_3Cl, 10.5; δ $^{119}Sn = -336.2$; (**2**) Sn–$CH_2CH_2CH_2CH_3Cl$, (C–α) 22.78 ^1J[341.0], (C–β) 28.69, (C–γ) 27.08, (C–δ) 13.70; δ $^{119}Sn = -129.4$; (**3**) Sn–CH_3, 2.2 ^1J[395.6]; δ $^{119}Sn = -201.2$; (**4**) Sn–CH_3, (C–α) –2.0 ^1J[571]; (**5**) Sn–$CH_2CH_2CH_2CH_3$, (C–α) 22.6 ^1J[578.6], (C–β) 28.7 ^2J[34.29], (C–γ) 27.6 ^3J[87.2], (C–δ) 14.2; (**6**) Sn–C_6H_5, (C–α) 136.75, (C–β) 129.11, (C–γ) 128.52, (C–δ) 128.11; δ $^{119}Sn = -80.0$.
[b]Chemical shifts (δ) in ppm. $^nJ[^{119}Sn,^{13}C]$ in Hz is listed in parenthesis.

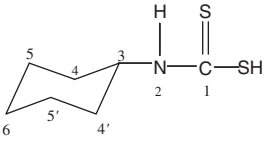

Table 5. C–Sn–C angles (°) calculated from NMR.

Compound number	$^1J[^{119}Sn–^{13}C]$ (Hz)	$^2J[^{119}Sn–^1H]$ (Hz)	C–Sn–C angles (°)	
			^1J	^2J
2	341.0	–		106.6
4	571.0	82	126.8	126.5
5	578.0	–	127.5	

The carbon position of C=S resonance shifted upfield upon complexation, attributed to a lowering of C=S bond order upon coordination and a shift of N→C electron density producing a partial double bond character in the C–N bond.

To gain further information regarding possible coordination geometries in solution, $^1J[^{119}Sn–^{13}C]$ and $^2J[^{119}Sn–^1H]$ coupling constants examined as C–Sn–C bond angles can be determined [35, 36]. The C–Sn–C angles (°) based on NMR parameters for the complexes are given in table 5. The geometric data calculated (table 5) are consistent with five- and six-coordinate geometry [37]. In triorganotin(IV) complexes, ^{119}Sn chemical shifts lie in the tetrahedral environment whereas the diorganotin(IV) compounds show higher coordination, probably five or six.

3.3. *Antibacterial activity*

Antibacterial activities of ligand and complexes were determined using $1\,mg\,mL^{-1}$ DMSO solutions by the disc diffusion method [20, 21]. The inhibition zone (IZ) diameters were measured in mm.

HL and organotin complexes were tested for *in vitro* antibacterial activity using two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pasteurella multocida*). The results were

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Table 6. Antibacterial activities[a–d] of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.

Compound number	Bacterial IZ (mm)			
	B. subtilis Mean ± SD	*P. multocida* Mean ± SD	*S. aureus* Mean ± SD	*E. coli* Mean ± SD
HL	$6.0^{c} \pm 0.5$	$5.0^{c} \pm 0.4$	$7.0^{c} \pm 0.6$	$4.0^{c} \pm 0.8$
1	$13^{b} \pm 1$	$8.5^{b} \pm 0.9$	$9^{b} \pm 1$	$12^{b} \pm 1$
2	$13^{b} \pm 1$	$9.5^{b} \pm 0.6$	$14.5^{b} \pm 0.7$	$12.0^{b} \pm 0.5$
3	$24^{a,b} \pm 1$	$7.0^{b} \pm 0.5$	$14.5^{b} \pm 0.9$	$12.5^{b} \pm 0.8$
4	$11^{b} \pm 1$	$12^{b} \pm 1$	$14^{b} \pm 1$	$6^{b} \pm 1$
5	$14.5^{b} \pm 0.5$	$7.5^{b} \pm 0.4$	$13.0^{b} \pm 0.6$	$10.0^{b} \pm 0.8$
6	$10^{b,c} \pm 1$	$12^{a} \pm 1$	$11^{a,b} \pm 1$	$9.5^{c} \pm 0.7$
Standard	$26^{a} \pm 1$	$19^{a} \pm 1$	$26^{a} \pm 2$	$17^{a} \pm 2$

[a]Concentration = 1 mg mL^{-1} in DMSO.
[b]Standard = Rifampicin.
[c]0 = No activity, 5–10 = [a]ctivity present, 10–20 = moderate activity, 20–30 = strong activity.
[d]Antibacterial values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.

obtained as IZ diameters and compared against the results of rifampicin (positive control).

The activities of the complexes were less than the standard drug, but higher than the ligand [26]. Complexes **1**–**5** show moderate activity against all bacterial strains but relatively high against Gram-positive bacteria, *B. subtilis*. This can be attributed to the difference in the structure of the cell walls. The walls of Gram (−) cells are more complex than those of Gram (+) cells. The lipopolysaccharide forms an outer-lipid membrane and contributes to antigenic specificity for the Gram (−) cells. The results obtained (IZ) are summarized in table 6.

3.4. *Antifungal activity*

Many organotin compounds are antifungal agents. So HL and compounds were also tested for antifungal activities against *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata*, and *Rhizopus solani*. Fluconazole (positive control) was used as standard drug to calculate the growth IZs of ligand and complexes [38]. The result shows that coordination of tin causes higher activity of complexes than ligand.

The complexes were more potent inhibitors of fungal growth as compared to bacterial culture. This may be due to the antifungal nature of sulfur present in the complexes [39]. The antifungal activity of the complexes against *A. niger* was comparable to that of the standard drug. Thus, further studies of these complexes as fungicides could lead to more interesting results. The IZ diameters of antifungal activity are given in table 7.

3.5. *Minimum inhibitory concentration*

The MICs of ligand and complexes were determined by the modified resazurin assay utilizing microtiter-plates [24]. Resazurin is an oxidation–reduction indicator used for evaluation of cell growth, particularly in various cytotoxicity assays. It is a blue

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Table 7. Antifungal activities[a–d] of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.

Compound number	Fungal IZ (mm)			
	A. alternate Mean ± SD	*A. niger* Mean ± SD	*R. solani* Mean ± SD	*A. flavus* Mean ± SD
HL	$4.0^{c} \pm 0.3$	$7.0^{c} \pm 0.8$	$8.0^{c} \pm 0.9$	$5.0^{c} \pm 0.4$
1	$13^{b} \pm 1$	$13^{a,b} \pm 1$	$12^{b} \pm 1$	$9^{b} \pm 0.8$
2	$16^{a,b} \pm 1$	$10^{a,b} \pm 1$	$12^{b} \pm 1$	$9^{b} \pm 1$
3	$8.5^{b} \pm 0.5$	$11.5^{a,b} \pm 0.4$	$22^{a} \pm 1$	$11^{a,b} \pm 0.5$
4	$14^{b} \pm 1$	$12^{a,b} \pm 1$	$10^{b} \pm 1$	$11^{a,b} \pm 1$
5	$11^{b} \pm 1$	$19^{a} \pm 1$	$21^{b} \pm 2$	$12^{a,b} \pm 1$
6	$13^{a,b} \pm 1$	$12^{a,b} \pm 1$	$15^{a,b} \pm 1$	$9.5^{b} \pm 0.7$
Standard	$18^{a} \pm 2$	$14^{a} \pm 1$	$24^{a} \pm 2$	$13^{a} \pm 1$

[a]Concentration = 1 mg mL^{-1} in DMSO; [b]Standard = Fluconazole; [c]0 = no activity, 5–10 = [a]ctivity present, 10–20 = moderate activity, 20–30 = strong activity; [d]Antifungal values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.

Table 8. MICs[a–c] of cyclohexylcarbamodithioic acid and its organotin(IV) complexes against selected fungal strains.

Compound number	*A. alternate* Mean ± SD (μg mL^{-1})	*A. niger* Mean ± SD (μg mL^{-1})	*R. solani* Mean ± SD (μg mL^{-1})	*A. flavus* Mean ± SD (μg mL^{-1})
HL	$3000^{c} \pm 1$	$750^{c} \pm 1$	$1500^{c} \pm 1$	$3000^{c} \pm 1$
1	$375^{b} \pm 1$	$187^{b} \pm 1$	$750^{b} \pm 1$	$750^{b} \pm 1$
2	$187^{a,b} \pm 1$	$375^{b} \pm 1$	$750^{b} \pm 1$	$1500^{b} \pm 1$
3	$750^{b} \pm 1$	$375^{b} \pm 1$	$187^{b} \pm 1$	$750^{b} \pm 1$
4	$375^{b} \pm 1$	$750^{b} \pm 1$	$187^{a} \pm 1$	$750^{b} \pm 1$
5	$750^{b} \pm 1$	$187^{b} \pm 1$	$375^{a} \pm 1$	$750^{b} \pm 1$
6	$750^{b} \pm 1$	$375^{b} \pm 1$	$187^{a} \pm 1$	$750^{b} \pm 1$
Standard	$187^{a} \pm 1$	$93^{a} \pm 1$	$375^{b} \pm 1$	$46^{a} \pm 1$

[a]Concentration = 6 mg mL^{-1} in DMSO; [b]Standard = Fluconazole; [c]MIC values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.

non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductase within viable cells. The effectiveness of this modified resazurin assay has been demonstrated with ligand and complexes.

Rifampicin was used as a standard drug for bacterial strains and fluconazole was used as antifungal standard drug. The complexes show higher MIC values than standards but lower than ligand. For antifungal activity the minimum value of MIC of **5** was against *A. niger* (187 μg mL^{-1}) and maximum was against *A. flavus* and *R. solani* (750 μg mL^{-1}) as shown in table 8. Similarly maximum MIC of **5** was against *S. aureus* (1250 μg mL^{-1}) and minimum against *B. subtilis* (156 μg mL^{-1}) for bacterial strains which show higher activity against *B. subtilis* (table 9). The MIC results agree with antibacterial and antifungal activity results.

3.6. *Antioxidant activity*

3.6.1. DPPH radical scavenging assay. Antioxidants are substances or nutrients which can prevent or slow oxidative damage to our body. Health problems such as

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Table 9. MICs[a–c] of cyclohexylcarbamodithioic acid and its organotin(IV) complexes against selected bacterial strains.

Compound number	*B. subtilis* Mean ± SD ($\mu g\,mL^{-1}$)	*P. multocida* Mean ± SD ($\mu g\,mL^{-1}$)	*S. aureus* Mean ± SD ($\mu g\,mL^{-1}$)	*E. coli* Mean ± SD ($\mu g\,mL^{-1}$)
HL	$625^{b} \pm 1$	$1250^{c} \pm 1$	$625^{c} \pm 1$	$2500^{b} \pm 1$
1	$156^{b} \pm 1$	$312^{b} \pm 1$	$312^{b} \pm 1$	$625^{b} \pm 1$
2	$312^{b} \pm 1$	$625^{b} \pm 1$	$156^{b} \pm 1$	$625^{b} \pm 1$
3	$78^{b} \pm 1$	$1250^{b} \pm 1$	$156^{b} \pm 1$	$312^{b} \pm 1$
4	$625^{b} \pm 1$	$312^{b} \pm 1$	$156^{b} \pm 1$	$1250^{b} \pm 1$
5	$312^{b} \pm 1$	$156^{a} \pm 1$	$1250^{c} \pm 1$	$312^{b} \pm 1$
6	$1250^{c} \pm 1$	$156^{a,b} \pm 1$	$312^{b} \pm 1$	$312^{b,c} \pm 1$
Standard	$39^{a} \pm 1$	$156^{a} \pm 1$	$39^{a} \pm 1$	$78^{a} \pm 1$

[a]Concentration = 5 mg mL^{-1} in DMSO; [b]Standard = Rifampicin; [c]MIC values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.

Table 10. IC_{50} of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.[a,b]

Compound number	IC_{50} Mean ± SD ($\mu g\,mL^{-1}$)
HL	76 ± 2
1	$48^{b} \pm 2$
2	$48^{b} \pm 2$
3	$44^{a} \pm 2$
4	$49^{b} \pm 2$
5	$75^{b} \pm 2$
6	$49^{b} \pm 2$
Standard	$44^{a} \pm 2$

[a]Standard = butylated hydroxytoluene.
[b]IC_{50} values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.

heart disease, diabetes, cancer, are all contributed by oxidative damage. Antioxidant activities or free radical scavenging activities of complexes and ligand were determined using the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH·). In its radical form DPPH· has an absorption at 517 nm with deep violet color which changes to pale yellow upon reduction by an antiradical compound. The free radical scavenging capacity increased with increasing concentration.

The % inhibition values and hence IC_{50} values of complexes and ligand were calculated and results were compared with the antioxidant butylated hydroxytoluene (BHT) ($44 \pm 2\,\mu g\,mL^{-1}$). The IC_{50} (the sample concentration providing 50% inhibition) values were used as end point to investigate the antioxidant activity, i.e., the greater the IC_{50} value, the lower will be the antioxidant activity [40]. The results obtained (table 10) were significant with $R^2 = 0.9099$ and $p < 0.05$.

The results show that the ligand itself acts as an antioxidant agent (with IC_{50} value of $76 \pm 2\,\mu g\,mL^{-1}$) but to lower extent than BHT (with IC_{50} value of $44 \pm 2\,\mu g\,mL^{-1}$). The activity of the ligand was increased on coordination with tin due to the presence of the proton donor capacity of the ligand [41].

3.6.2. Hemolytic activity. The *in vitro* hemolytic activities (or cytotoxicity assay) of HL and compounds were performed to check their effect on human blood by the

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Table 11. Cytotoxicities of cyclohexylcarbamodithioic acid and its organotin(IV) complexes.[a–c]

Compound No.	% Hemolysis Mean ± SD (%)
HL	$6.8^{b,c} \pm 0.1$
1	$7.1^{b} \pm 0.1$
2	$7.3^{b} \pm 0.1$
3	$8.7^{b} \pm 0.5$
4	$8.3^{b} \pm 0.1$
5	$7^{b,c} \pm 1$
6	$8.7^{b} \pm 0.1$
PBS	$0.01^{c} \pm 0.0$
Triton X-100	$99^{a} \pm 1$

[a]PBS = Negative control standard drug.
[b]Triton X-100 = positive control standard drug.
[c]Cytotoxicity activities are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.

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hemolytic method [25]. The results were obtained by comparison with a positive control (Triton X-100) and a negative control (PBS). The results are given in table 11. The mean values obtained are 7.1 ± 0.1% (**1**), 6.8 ± 0.1% (ligand), 0.01 ± 0.0% (negative control), and 99 ± 1% (positive control).

The hemolytic activities of the complexes were higher than that of the ligand (6.8 ± 0.1%) and close to PBS (negative control), but not to Triton X-100 (positive control). Hence the toxicity of the complexes was very low to human blood. By comparing these results with antimicrobial and antioxidant activities, it can be concluded that the reported complexes (1 mg mL^{-1}) show poor hemolytic activity, but good antimicrobial and antioxidant activities, and could be further investigated as medicinal agents against different microorganisms with minor side effect.

3.6.3. Mutagenic activity. In the Ames test [27], mutagenic potential of **6** was evaluated by using *Salmonella typhimurium*, TA 98 and TA 100. Experiments were performed in triplicate for both complexes. Negative and positive controls were also carried out simultaneously. For TA-98, $K_2Cr_2O_7$, and TA-100, sodium azide (NaN_3) (10–35 μg/plate) were used as positive controls in experiments along with known mutagens like MNNG (12 μg/plate) and benzo (a) pyrene (35 μg/plate). Distilled water served as negative control and DMSO as vehicle control. In the Ames test, with various concentrations of synthesized compounds (10,000, 1000, and 500 μg/plate), the mutation frequencies did not change significantly when compared to the positive control.

Only a few revertant colonies were observed for the complex (table 12), probably due to the toxic or inhibitory effect of the complex which may inhibit the growth of strains TA-100 and TA-98. It may also be due to the antibacterial property of the reported complex. This result is consistent with an earlier report [42] that complexation with organotin compounds led to complexes devoid of mutagenicity. This behavior suggests that structural factors imposed by the organotin moieties may be responsible for the absence of activity.

Table 12. Mutagenic activity of **6** by plate incorporation assay.

		His^+ revertants/plate (Number of colonies)							
		TA98				TA100			
Compound number	Amount tested (μL/plate)	1	2	Mean	M.I	1	2	Mean	MI
6	100	0	22	11	0.21	12	6	15	0.27
Negative control									
				Mean				Mean	
Distilled water	100	34	68	50	51	55	35	71	54
DMSO	100	0	13	32	21	5	12	14	10
Positive control									
NaN_3	10	0	0	0		53	66	75	
$K_2Cr_2O_7$	10	38	66	66		0	0	0	
MNNG	3	35	42	81		58	58	115	
B(a)P	10	84	95	110		0	0	0	
B(a)P	5	0	0	0		84	65	87	

$NaN_3 = 35\,\mu g$/plate; $K_2Cr_2O_7 = 35\,\mu g$/plate; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) = 12 μg/plate; Benzo[a] pyrene[B(a)P] = 35 μg/plate; Test compound = 0.01 gmm$L^{-1}$of DMSO (500 and 1000 μg/plate).

Table 13. Selected bond lengths (Å) and angles (°) for **1**, **3**, and **4**.

Compound **1**			
S2–C3–1.70		C3–S4	1.74
S2–Sn1–2.74		Cl24–Sn1	2.40
C25–Sn1–2.12		C29–Sn1	2.12
S2–Sn1–Cl24	154.9	S2–Sn1–C25	93.4
C3–S2–Sn1	88.1	S2–C3–S4	115.4
S2–C3–N5	125.2	S4–C3–N5	119.4
Compound **3**			
S2–C3–1.74		C3–S4	1.70
S2–Sn1–2.57		C24–Sn1	2.13
C28–Sn1–2.13		Sn1–S4	2.84
S2–Sn1–C24	107.6	S2–Sn1–C28	107.7
S2–Sn1–S4	65.2	S2–Sn1–S32	145.9
S2–Sn1–S34	80.9	C24–Sn1–C28	133.8
Compound **4**			
Sn1–S4–2.76		C3–S4	1.70
S2–Sn1–2.67		C24–Sn1	2.13
C28–Sn1–2.13		C32–Sn1	2.14
S2–Sn1–C24	119.5	S2–Sn1–C28	118.7
S2–Sn1–C32	87.8	S2–Sn1–S4	64.3
C24–Sn1–C28	114.7	C24–Sn1–C32	104.6

3.7. *Structure–activity relationship*

The different methods by which complexes can exert their action as antimicrobial agents can include the effect of resonating structure such as potentially reactive groupings, the introduction of a lipophilic substituent, either aryl or alkyl group, or the hydrolysis of amine or reactive halogens to form microbial inhibitor growth compounds [40].

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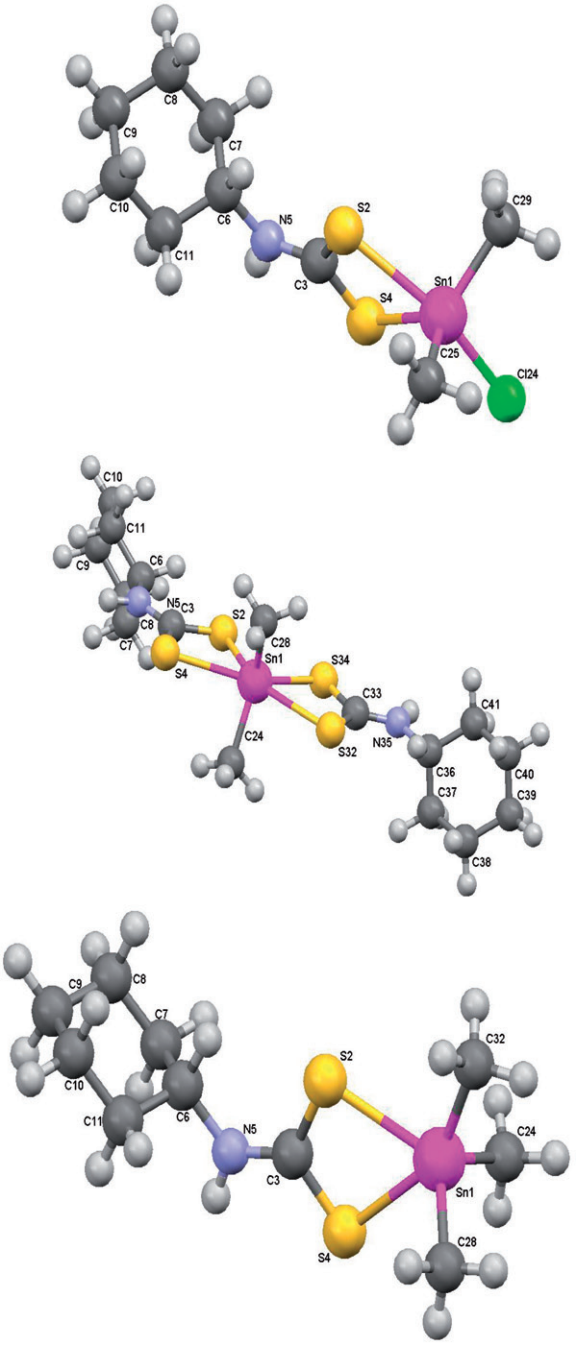

Figure 1. Optimized structures of **1**, **3**, and **4**.

Degradative enzymes produced by the microorganism are important in host infection, food deterioration, and breakdown of organic matter. Since the organotin(IV) complexes inhibit the growth of organisms, it is assumed that the production of the enzymes is being affected; hence the organisms are unable to utilize food and, consequently, growth ceases.

Although, it is difficult to make out an exact structure–activity relationship between the microbial activity and the structure of complexes, chelation as well as addition of a substrate enhances the activities of the complexes. The variation in the toxicity of different antibacterial agents against different organisms depends either on the impermeability of the cell or difference in ribosomes to the antimicrobial agent.

Though the results suggest that the ligand also has some toxicity, the complexes inhibit the growth of organisms to a greater extent. Further, the greater activity of the complexes can also be explained on the basis of their higher solubility [41].

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3.8. *Semi-empirical study*

In **1**, the ligand binds bidentate to two Sn(IV) atoms. The two-methyl groups, Cl and the dithio group are arranged in a distorted trigonal bipyramid. The Sn–S bond distances are 2.63 and 2.74 Å. The Sn–C bond lengths are 2.12, 2.12 Å, Sn–Cl distance is 2.40 Å, and the C–S bond distances are 1.74 and 1.70 Å. The Sn–S–C bond angles are 88.1° and 91.1° and S–C–S bond angle is 115.4°, while the S–Sn–S angle is 65.5°.

Two ligands bind bidentate to Sn(IV) in **3**. The two-methyl groups and the two dithio groups are arranged in a distorted octahedron. The Sn–S bond distances are 2.57, 2.84, 2.57, and 2.84 Å. The Sn–C bond lengths are 2.13 and 2.13 Å and the C–S bond distances are 1.74, 1.70, 1.75, and 1.69 Å. The Sn–S–C bond angles are 85.0°, 93.2°, 85.0°, and 93.3° and S–C–S bond angles are 116.6° and 116.7°, while the S–Sn–S angles are 65.2° and 65.1°.

In **4**, the ligand binds bidentate to Sn(IV). The three-methyl groups and the dithio group form a distorted trigonal bipyramid. The Sn–S bond distances are 2.67 and 2.76 Å. The Sn–C bond lengths are 2.13, 2.13, and 2.14 Å and the C–S bond distances are 1.73 and 1.70. The Sn–S–C bond angles are 89.2° and 91.8° and S–C–S bond angle is 114.7°, while the S–Sn–S angle is 64.3°.

All bond lengths and angles are comparable to literature values [43]. The selected bond lengths and angles of the optimized structures are tabulated in table 13. The optimized structures are given in figure 1.

4. Conclusion

FT-IR data of the complexes clearly demonstrate that the organotin(IV) moiety reacts with [S,S] of the ligand in a bidentate manner. NMR data show tetrahedral geometry in solution. The antimicrobial assay of ligand and complexes against different bacterial and fungal strains show that the complexes are more active than the ligand; mutagenicity results show that the antibacterial property of **6** is coupled with lack of mutagenicity. The complexes also show higher antioxidant activities than the ligand. The hemolytic activities of the complexes and ligand were very low compared to the

free ligand. By comparing the results of mutagenic and hemolytic activities with the results of antimicrobial and antioxidant activities, it can be predicted that the complexes have large inhibitory effect and might indeed be potential antimicrobial agents and be used in biomedical and pharmaceutical fields.

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References

[1] K.E. Apple. *Drug Metabol. Rev.*, **36**, 763 (2004).
[2] M. Jain, S. Maanju, R.V. Singh. *Appl. Organomet. Chem.*, **18**, 471 (2004).
[3] D.D. Nhan, D.T. Loan, I. Tolosa, S.J. De Mora. *Appl. Organomet. Chem.*, **19**, 811 (2005).
[4] A. Chaudhary, M. Agarwal, R.V. Singh. *Appl. Organomet. Chem.*, **20**, 295 (2006).
[5] W. Rehman, M.K. Baloch, B. Muhammad, A. Badshah, K.M. Khan. *Chin. Sci. Bull.*, **49**, 119 (2004).
[6] L. Pellerito, L. Nagy. *Coord. Chem. Rev.*, **224**, 111 (2002).
[7] M. Nath, S. Pokharia, G. Eng, X. Song, A. Kumar. *Eur. J. Med. Chem.*, **40**, 289 (2005).
[8] M.T.H. Tarafder. *Indian J. Chem.*, **28A**, 531 (1989).
[9] D.C. Menezes. *Main Group Met. Chem.*, **31**, 21 (2008).
[10] D.C. Menezes. *Appl. Organomet. Chem.*, **22**, 221 (2008).
[11] J.P. Fuentes-Martinez. *Polyhedron*, **28**, 3953 (2009).
[12] A. Tarassoli. *J. Coord. Chem.*, **56**, 1179 (2003).
[13] E.I. Stiefel, K. Matsumoto. *Transition Metal Sulfur Chemistry*, ACS Symposium Series 653, American Chemical Society, Washington, DC (1995).
[14] B. Kerbs, G. Henkel. *Angew. Chem. Int. Ed. Engl.*, **30**, 769 (1991).
[15] K.S. Siddiqi, R.I. Qureshi, N.H. Khan, S.A.A. Zaidi. *Indian J. Chem. A*, **24A**, 578 (1985).
[16] T.N. Srivastava, V. Kumar, D.P. Srivastava. *Natl Acad. Sci. Lett.*, **1**, 97 (1978).
[17] J.J.P. Stewart. *MOPAC2007 (Version: 7.334W)*, Stewart Computational Chemistry.
[18] H, C, N, O, Cl, S (PM3): J.J.P. Stewart. *J. Comp. Chem.*, **10**, 209 (1989).
[19] Sn: (PM3): J.J.P. Stewart. *J. Comp. Chem.*, **12**, 320 (1991).
[20] T.R. Fritsche, P.F. McDermott, T.R. Shryock, R.D. Walker. *J. Clin. Microbiol.*, **45**, 2758 (2007).
[21] G. Huang, S. Moore, J. Jiaxin, D. Dehui. *Food Sci. Tech.*, **11**, 25 (2001).
[22] Q.K. Huynh, J.R. Borgmeyer, C.E. Smith, L.D. Bell, D.M. Shah. *J. Biol. Chem.*, **316**, 723 (2001).
[23] S.D. Sarker, L. Naharb, Y. Kumarasamy. *Methods*, **42**, 321 (2007).
[24] B. Bozin, N.M. Dukic, I. Samojlik, A. Goran, R. Igic. *Food Chem.*, **111**, 925 (2008).
[25] W.A. Powell, C.M. Catranis, C.A. Maynard. *Lett. Appl. Microbio.*, **31**, 163 (2000).
[26] F.G.D. Steel, J.H. Torrie, D.A. Dikey. *Principle and Procedure of Biomaterials Approach*, 3rd Edn, WC McGraw-Hill, New York, USA (1997).
[27] B.N. Ames, W.E. Dusston, E. Yamasaki, F.D. Lee. *Proc. Natl Acad. Sci. USA*, **70**, 2281 (1973).
[28] Zia-ur-Rehman, S. Shahzadi, S. Ali, G.-X. Jin. *Turk. J. Chem.*, **31**, 435 (2007).
[29] Q. Xie, Z.Q. Yang, Z.X. Zhang, D.K. Zhang. *Appl. Organomet. Chem.*, **6**, 193 (1992).
[30] H.O. Kalinowski, S. Berger, S. Brown. *^{13}C-NMR Spectroskopie*, Vol. 56, p. 133, Thieme, Stuttgart, Germany (1984).
[31] M. Danish, M.N. Tahir, N. Ahmad, S. Ali, A. Badshah. *Acta Cryst.*, **E65**, m1614 (2009).
[32] M.M. Amini, T. Hajiashrafi, A.N. Kharat, S.W. Ng. *Acta Cryst.*, **E64**, m1403 (2008).
[33] T.S. Basu Baul, A. Paul, A. Linden. *J. Organomet. Chem.*, **696**, 4229 (2012).
[34] M. Nadvornik, J. Holecek, A. Lycka. *J. Organomet. Chem.*, **275**, 43 (1984).
[35] T.P. Lockhart, W.F. Manders. *Inorg. Chem.*, **25**, 892 (1986).
[36] J. Holecek, M. Nadvornik, K. Handlir, A. Lycka. *J. Organomet. Chem.*, **315**, 299 (1986).
[37] S. Shahzadi, S. Ali, M.H. Bhatti, M. Fettouhi, M. Athar. *J. Organomet. Chem.*, **691**, 1797 (2006).
[38] L. Dostal, A. Ruzicka, R. Jambor, V. Buchta, P. Kubanova, J. Holecek. *Met.-Based Drugs*, **9**, 91 (2002).

[39] H.L. Singh, A.K. Varshney. *Appl. Organomet. Chem.*, **15**, 762 (2001).
[40] G. Miliauskas, P.R. Venskutonis, T.A.V. Beek. *Food Chem.*, **85**, 231 (2004).
[41] A.C. Bustamante, V.J.M.V. Paredes, A.F. Parra, A.L.P. Campos, F.J.M. Martinez, M.T.S. Martinez, A.R. Organillo. *Molecules*, **15**, 5445 (2010).
[42] A. Bacchi, M. Carcelli, P. Pelagatti, G. Pelizzi, M.C. Rodriguez-Arguelles, D. Rogolino, C. Solinas, F.J. Zani. *J. Inorg. Biochem.*, **99**, 397 (2005).
[43] S. Shahzadi, S. Ali. *J. Iran. Chem. Soc.*, **5**, 16 (2008).

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