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Synthesis, Characterziation, Semi-Empirical Quantum-Mechanical Study and Biological Activity of Organotin(IV) Complexes with 2-Ethylanilinocarbonylpropenoic Acid[1]

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Abstract—Seven different organotin(IV) complexes have been synthesized by reacting 2-ethylanilinocarbonyl-propenoic acid with R_2SnCl_2/R_3SnCl under reflux conditions. The organotin(IV) complexes along with ligand have been characterized by different techniques including elemental analysis, FT-IR and multinuclear NMR (^1H and ^{13}C). IR data show that complexation occurs through –COO site and the ligand is bidentate which is also confirmed by the semi-empirical quantum-mechanical study. ^1H and ^{13}C NMR data confirm the tetrahedral geometry of complexes in solution. The complexes as well as the ligand were also checked for various

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INTRODUCTION

Organotin(IV) form an important series of compounds that get increasing attention in recent years not only because of their intrinsic properties, but also owing to their importance as tin-based anti-tumor drugs; some specimen find wide application as catalysts, stabilizers, and certain derivatives are used as biocides and as antifouling agents [1–4].

The biochemical activity of organotin compounds is also influenced greatly by the structure of the molecule and the coordination number of the tin atom. Therefore, synthesis of new organotin carboxylates with different structural features is beneficial in the development of pharmaceutical organotins and other applications. The synthesis of organotin complexes in research area are of increased interest for inorganic, pharmaceutical, and medicinal chemistry as an approach to the development of new drugs [5].

Research on the structure of organotin(IV) carboxylates continues and, at the same time, some new applications of high importance are discovered which are relevant to ecological medicinal applications. The increasing interest in the chemistry of organotin(IV) compounds has led to the extended studies on their reactions with different biomolecules [6].

On the other hand, organotin(IV) compounds have been tested for their *in vitro* activity against a large variety of tumour cell lines and have been found to be as effective as or better than traditional heavy metal anticancer drugs such as cisplatin.

Organotin(IV) complexes are put to use in various fields and exhibit potential biological applications such as insecticidal, fungicidal, and antitumor activities [5]. Organotin compounds are now the active components in a number of biocidal formulations [7–10] finding applications in such diverse areas as fungicides, miticides, molluscicides, marine antifouling paints, surface disinfectants and wood preservatives [11]. In recent years, investigations have been carried out to

[1] The text was submitted by the authors in English.

test their antitumor activity and it has been observed that several diorganotin and triorganotin species show indeed a potential as antineoplastic agents [12–14].

Having these applications in view, and accounting for our interest in the synthesis, characterization and biological studies of organotin(IV) carboxylates, we synthesized organotin(IV) complexes of 2-ethylanilinocarbonylpropenoic acid and characterized them by elemental analysis, FTIR, multinuclear NMR (^{1}H and ^{13}C) and semi-empirical quantum-mechanical study. These compounds were tested for their *in vitro* antifungal and antibacterial activity. Complexes were screened to check their antioxidant and cytotoxic activities. Antimutagenic activity of selected complexes was checked by Ames test.

EXPERIMENTAL

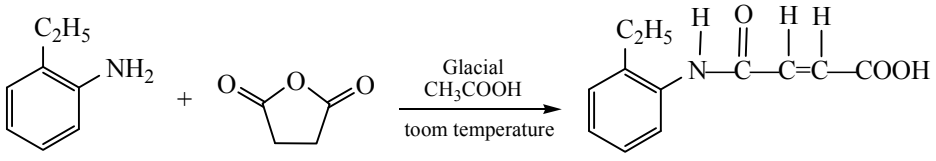
Chemicals and Instrumentation

2-Ethylaniline, dimethyltin dichloride, dibutyltin dichloride, trimethyltin chloride, tributyltin chloride, triphenyltin chloride, and maleic anhydride were purchased from Sigma-Aldrich (UK). DMSO, acetone, acetic acid, *n*-hexane, chloroform, petroleum ether, acetone, and ethanol were of Merck origin. Phosphate buffer saline, Triton-X100, 2,2-diphenyl-1-picryl-1-hydrazyl (DPPH) reagent were purchased from Sigma-Aldrich (UK) and nutrient agar, nutrient broth, potato dextrose agar were of Oxide (UK) origin.

Melting points were determined in a capillary tube using electrothermal melting point apparatus, model stuart (SMP3 of USA). The infrared spectra were recorded as KBr pellets by using a Perkin Elmer 1000 spectrophotometer in the range 4000–250 cm^{-1}. Multinuclear NMR (^{1}H and ^{13}C) spectra were recorded on a Bruker AM 300 MHz, FT-NMR spectrometer using $CDCl_3$ as an internal reference [δ_H ($CDCl_3$) = 7.25 and δ_C ($CDCl_3$) = 77.0]. The percentage composition of C, H, and N was determined by using CHNS-932 Leco (USA). The antimicrobial activities of the ligand and organotin(IV) complexes have been performed in Incubator (Sanyo, Germany) and sterilized in the Autoclave apparatus (Omron, Japan). The minimum inhibitory concentration and antioxidant activity were determined in a Micro Quant apparatus (BioTek, USA). Centrifuge H-200 NR (Kokusan, Japan) was used to centrifuge the sample solutions and Hemacytometer (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.

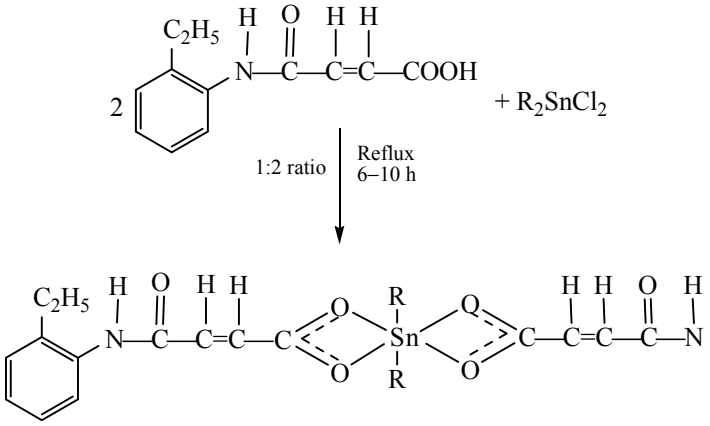
The molecules were modeled by MOPAC 2007 program [15] in gas phase using PM3 method [16,17] which is based on the assumption that atoms prefer an arrangement that has the minimum energy and is hence the most stable. This geometry optimized structures have been found to be in most cases very close to structures found by X-ray diffraction analysis. As the computed molecular model are in three dimensions, with each atoms having distinct *x,y*, and *z* coordinates, it is possible to measure the bond angle, bond lengths, dihedral angles and atomic planes. Selected parts of the complexes not containing the metal ion were pre-optimised using molecular mechanics methods. Several cycles of energy minimization had to be carried for each of the molecules. The structure was optimized by Eigen Vector. The root mean square gradient for molecules was all less than one. Self consistent field was attained in each case [18, 19].

Procedure for the synthesis of ligand. 2-Ethylaniline (1 mmol) was dissolved in glacial acetic acid in a beaker (250 mL) with continuous stirring for 15 min. Solution of maleic anhydride (1 mmol) in glacial acetic acid (15 mL) was added dropwise to the above solution, and the reaction mixture was stirred continuously for 6–8 h. The precipitate formed was filtered off and washed with cold distilled water.



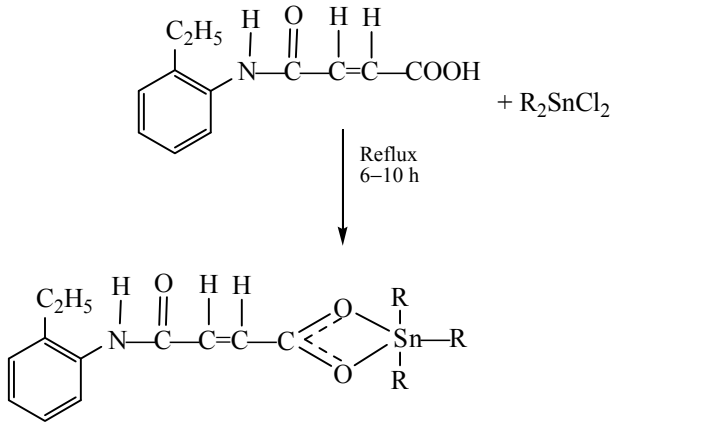
(1)

General procedure for the synthesis of di- and chlorodiorganotin(IV) complexes. 2-Ethylanilinocarbonylpropenoic acid (1 mmol) was dissolved in ethanol (20 ml) under stirring at room temperature. To the above solution, R^2SnCl_2 (1 mmol/2 mmol) was added in portions. Then the reaction mixture was refluxed for 6–10 h. Solvent was evaporated by using rotary evaporator under a reduced pressure. Solid



(2)

R = Me (**I**), *n*-Bu (**III**)

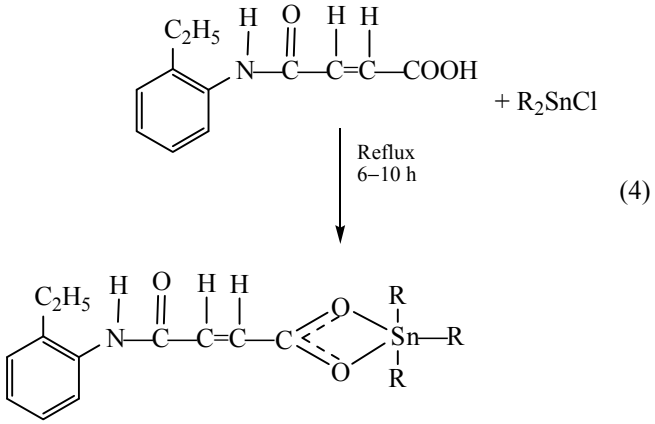


(3)

R = Me (**II**), *n*-Bu (**IV**)

product obtained was dried in air and recrystallized from acetone and petroleum ether (1 : 1).

General procedure for the synthesis of triorganotin(IV) complexes. 2-Ethylanilinocarbonylpropenoic acid (1 mmol) was dissolved in ethanol (20 mL) under continuous stirring at room temperature. To the above solution, R^3SnCl (1 mmol) was added in portions, and the reaction mixture was refluxed for 6–10 h. Solvent was evaporated by using rotary evaporator under a reduced pressure. Solid product obtained was dried in air and recrystallized from acetone and petroleum ether (1 : 1).



(4)

R = Me (**V**), *n*-Bu (**VI**), Ph (**VII**).

Antibacterial activity. The synthesized complexes and parent acid were screened for their *in vitro* antibacterial activity against four bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli,* and *Pasturella multocida* by measuring inhibition zones using disc diffusion method [20].

Pure cultures were maintained on nutrient agar medium in the slants and Petri dishes. For the inocula preparation 13 g/L of nutrient broth was suspended in distilled water, mixed well and autoclaved. 10 μL of pure culture of a bacterial strain was mixed in the medium and placed in shaker for 24 h at 37°C. The inocula were stored at 4°C in refrigerator. The inocula with 1×10^8 spores/mL were used for further analysis.

Nutrient agar 28 g/L was suspended in distilled water, mixed well and distributed homogenously. The medium was sterilized by autoclaving at 121°C for 15 min. Before the medium was transferred to Petri dishes inocula (100 μL/100 mL) were added to the medium and poured in a sterilized Petri dish. After this, small filter paper discs were laid flat on the growth medium containing 100 μL of sample. The Petri dishes were then incubated at 37°C for 24 h for the growth of bacteria. The complexes having antibacterial activity inhibited the bacterial growth and clear zones were formed. The zones of inhibition were measured in millimeters by using zone reader [21].

Antifungal activity. The synthesized complexes and parent acid were screened for their *in vitro* antifungal activity against four fungal strains like *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Rhizopus solani* by using disc diffusion method [20].

Pure cultures of the fungi were maintained on sabouraud dextrose agar (SDA) medium in slant and Petri dishes that were presterilized in a 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. The prepared sterilized growth medium was transferred to the sterilized Petri dishes. The Petri dishes 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 sample was applied on each disc. The Petri plates were again incubated. The sample having antifungal activity exhibited clear zones around the discs. The zone of inhibition was measured in millimeters using zone reader [21].

Minimum inhibitory concentrations (MIC) of synthesized complexes. MIC values were determined against fungal (*Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata*, *Rhizopus solani*) and bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pasturella multocida*) strains [22].

Plates were prepared under aseptic condition and a sterilized 96 well plate was carefully labelled. A volume of 100 μL of test material was pipetted into the first row of the plate. About 50 μL of nutrient broth was poured to all other wells. Dilution was carried out in serially descending concentrations so that each well contained 50 μL of the test material. Used tips were discarded. About 10 μL solution of resazurin indicator was poured to each well. Finally, about 10 μL suspension of bacteria (5×10^6 cfu/mL) was added to obtain a concentration of 5×10^5 cfu/mL. Each plate having an antibiotic (broad spectrum) was used as positive control. Plates were wrapped to avoid the possible dehydration of bacteria. The plates were then incubated at 37°C for bacteria for 24 h and 28°C for fungi for 48 h. Absorbance was measured at 500 nm for bacteria and at 620 nm for fungi by micro Quant Spectrophotometer.

Any color change from purple to pink or colourless was recorded as positive. The lowest concentration at which color changes occur was taken as MIC value [23].

Antioxidant/DPPH radical scavenging ability. The DPPH assay was carried out as described by Bozin's method [24]. The antioxidant activity of synthesized complexes was assessed by measuring their scavenging abilities to 2,2-diphenyl-1-1-picryl-hydrazyl stable radical. 50 μL aliquot of various concentrations of the samples was added to 5 mL of a 0.004 % methanol solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was checked against a blank at 517 nm. The assay was carried out in triplicate. Percent inhibition was calculated by using the following formula:

$$I = A_{\text{blank}} - A_{\text{sample}}A_{\text{blank}}\times100\%, \quad (5)$$

where, A_{blank} is absorbance of 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 of plotted inhibition percentage against sample concentration. The assay was carried out in triplicate.

Hemolytic activity. Hemolytic activity was checked by using the standard Powell's method [25]. About 3 mL of freshly obtained heparinized human blood was gently mixed, poured into a sterile 15 mL falcon tube and centrifuged for 5 min at 4200 rpm. The supernatant was poured off and viscous pellet washed three additional times with 5 mL of chilled (4°C) sterile isotonic phosphate-buffered saline (PBS) solution, adjusted to pH~7.4, to stabilize the pH. The solution 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.

The blood cell suspension was maintained on wet ice and diluted with sterile PBS, the cell count should be 7.068×10^8 cell per mL for each test. 20 μL of

sample in five different solvents was taken in 2 mL Apendoff tubes. For each assay, 0.1% Triton X-100 was taken as a positive control, 100% of blood lysis and phosphate buffer saline (PBS) was taken for each assay as a negative control, background (0% lysis). In each 2 mL Apendoff tube already containing 20 μL sample was added 180 μL of diluted blood cell suspension and mixed it with the help of the pipette tip. Tubes were incubated for 35 min at 37°C and agitated immediately for 10 min after incubation. The tubes were placed on ice for 5 min then centrifuged for 5 min at 4200 rpm. After centrifugation, 100 μL supernatant was taken from the tubes and diluted with 900 μL of chilled PBS. All tubes were maintained on wet ice after dilution. Then 200 μL was poured into 96 well plates and three replicates were taken in well plate which contain one positive control and other negative control. After this, absorbance at 576 nm was taken at μ Quant Spectrophotometer. Triton-X 100 (0.1 %) was used as positive control (100% of blood lysis) and PBS buffer as negative control (0% of blood lysis). The experiment was done in triplicate. The % hemolysis values were obtained by using the following formula:

$$\% \text{ hemolysis} = \frac{\text{Hb}_{\text{ABS}}}{\text{Hb}_{100\%\ \text{ABS}}} \times 100. \quad (6)$$

Mutagenicity testing. Mutagenicity of synthesized complexes was determined by using Ames test through plate incorporation method [26]. In these experiments, mutagenicity of selected complexes was checked using two strains of the bacterium *Salmonella typhimurium* TA-98 and TA-100. Negative and positive controls were also used for each test and each experiment was done in triplicate.

Top agar tubes containing 2 mL top agar were prepared and autoclaved. Tubes were heated to melt the top agar at 45°C. The 0.5 mM histidine/biotin (his/bio) solution was prepared and filter sterilized. Then 200 μL of this solution was added to the top agar tubes. 100 μl of the test sample was added to these test tubes. Then 100 μL of overnight grown culture of test strain containing approximately 3×10^7 cells/mL was added. The tube is vortexed and then its content is immediately poured onto the minimal medium (V.B) plates. The plates are quickly tilted and rotated for even spreading of top agar mixture over the whole surface of the plate. Plates were then placed on a level surface to harden.

The small amount of histidine in the growth medium allows the bacterial strains to grow for an initial time and have the opportunity to mutate. When the histidine is depleted in the medium, only bacteria that have mutated to gain the ability to produce its own histidine will survive. The plate is incubated for 48 h. Mutagenicity index (M.I) was calculated by the following formula:

$$\text{M.I} = \text{no.of revertant colonies in test plate} \times \text{no.of revertant colonies in negative control plate.} \quad (7)$$

RESULTS AND DISCUSSION

2-Ethylanilinocarbonylpropenoic acid HL was prepared by stirring 2-ethylaniline with maleic anhydride in glacial acetic acid. The complexes are soluble in organic solvents and have sharp melting points. Elemental analyses (C, H, and N) show good agreement between the calculated and found values. Physical data are given in Table 1.

Table 1. Physical data of organotin(IV) complexes

Comp. no.	Molecular formula	Molecular weight	Melting point, °C	C, % calculated (found)	H, % calculated (found)	N, % calculated (found)
HL	$C_{12}H_{13}O_3N$	219.0	103–105	65.75 (65.71)	5.90 (5.94)	6.30 (6.34)
I	$C_{26}H_{30}O_6N_2Sn$	584.0	92–93	53.42 (65.46)	5.13 (5.17)	4.80 (6.84)
II	$C_{32}H_{44}O_6N_2Sn$	644.0	89–91	60.00 (60.04)	6.80 (6.84)	4.30 (4.34)
III	$C_{14}H_{18}O_3NSnCl$	401.5	79–80	42.00 (42.04)	4.50 (4.46)	3.50 (3.46)
IV	$C_{20}H_{22}O_3NSnCl$	461.5	95–96	52.00 (51.98)	4.80 (4.84)	3.00 (3.04)
V	$C_{30}H_{27}O_3NSn$	567.0	70–71	47.24 (47.20)	5.50 (5.54)	3.70 (3.66)
VI	$C_{24}H_{40}O_3NSn$	471.0	86–88	61.14 (61.18)	8.50 (8.46)	2.97 (2.93)
VII	$C_{15}H_{21}O_3NSn$	381.0	72–73	42.24 (42.20)	5.50 (5.54)	3.70 (3.74)

Table 2. Assignment of characteristics of FT-IR vibrations (cm^{-1}) of organotin(IV) complexes

Comp. no.	$\nu(COO)_{asym}$	$\nu(COO)_{sym}$	$\Delta\nu$	ν(C=O)	ν(N–H)	ν(Sn–C)	ν(Sn–O)	ν(Sn–Cl)
HL	1560	1320	240	1730	3290	–	–	–
I	1540	1350	190	1700	3291	570	480	–
II	1575	1380	195	1700	3290	545	440	312
III	1540	1370	170	1700	3290	570	470	–
IV	1590	1485	105	1700	3290	590	460	309
V	1570	1380	190	1720	3291	580	490	–
VI	1580	1460	120	1700	3290	585	430	–
VII	1530	1390	140	1720	3291	–	420	–

Table 3. ^{1}H NMR spectral data[a] (ppm) of 2-ethylanilinocarbonylpropenoic acid and organotin(IV) compounds

Proton	Chemical shift, ppm							
	HL	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
CH_2CH_3	1.22 t (7.2) 2.67 q (9.3) 7.23–7.44 m	1.15 t (7.3) 2.49 q (9.3) 7.09–7.43 m	1.14 t (7.2) 2.48 q (9.3) 7.08–7.39 m	1.15 t (7.3) 2.49 q (9.3) 7.09–7.45 m	1.17 t (7.2) 2.49 q (9.3) 7.09–7.43 m	1.20 t (7.2) 2.65 q (9.2) 7.08–7.43 m	1.24 t (7.3) 2.66 q (9.3) 7.22–7.45 m	1.24 t (7.3) 2.66 q (9.3) 7.28–7.46 m
NH	9.86 s	9.38 s	9.78 s	9.28 s	9.98 s	9.84 s	9.70 s	9.61 s
CCHH	6.41d (7.6) 6.91 d (7.6)	6.41 d (7.8) 6.91 d (7.8)	6.30 d (7.2) 6.80 d (7.2)	6.49 d (7.8) 6.88 d (7.8)	6.41 d (7.6) 6.89 d (7.6)	6.38 d (7.3) 6.88 d (7.3)	6.41 d (7.6) 6.897 d (7.6)	6.41 d (7.6) 6.87 d (7.6)
R	–	0.28 t (80)	1.18 s	0.97 t (7.9) 1.22–1.80 m	0.95 t (7.7) 1.38–1.82 m	1.21 s	0.98 t (7.7) 1.38–1.7 8m	7.53–7.86 m

[a] Chemical shifts (δ) in ppm. $^{2}J[^{117/119}Sn, ^{1}H]$; $^{2}J[^{119}Sn, ^{1}H]$, and $^{3}J(^{1}H, ^{1}H)$ in Hz are listed in parenthesis.

Infrared spectroscopy. The infrared spectra for newly synthesized ligand acid and compounds **I–VII** were recorded in the range 4000–250 cm^{-1} using KBr/CsBr pellets. The assignments were made on the basis of earlier work and important data are listed in Table 2.

The complexation of tin with the ligand is through –COO as confirmed by the absence of a broad –OH band in the region of 2650 cm^{-1}. The –NH group does not participate in the interactions via intra- or intermolecular modes as confirmed by the presence of characteristic strong band at 3290–3291 cm^{-1} which does not show any shift after complexation [27].

Infrared COO stretching frequencies have been used to identify the monodentate or bridging nature of bonding of the carboxy group. The COO-groups in organotin(IV) derivatives generally adopt a bridged structure in the solid state unless the organic substituents at tin are bulky or the carboxylate group is branched at the α-carbon. The assignment of the coordination of the complexes was based on difference between (COO)sym and (COO)asym and the corresponding band position. Complexes are divided into three groups on the basis of Δν [28]:

(a) In compounds where $\Delta\nu(COO) > 350$ the compounds contain, with high probability, the monodentate carboxylate group.

(b) When $\Delta\nu(COO) < 200$ the carboxylate groups of these compounds can be regarded as practically bidentate.

(c) Compounds where $\Delta\nu(COO) < 350$ and > 200 are considered as intermediate between monodentate and bidentate, which is called anisobidentate state [29].

In all synthesized complexes Δν is less then 200 cm^{-1} which confirms the bidentate nature of ligand. In the spectra of the compounds, new bands that appeared in the region 490–358 cm^{-1}, 590–540 cm^{-1}, and 309–312 cm^{-1} were assigned to Sn–O, Sn–C, and Sn–Cl

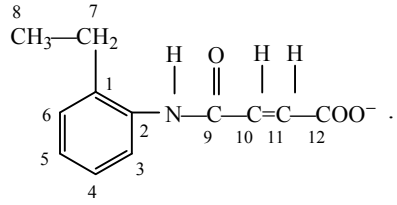
Table 4. ^{13}C NMR data[a–c] of 2-ethylanilinocarbonylpropenoic acid and organotin(IV) complexes

Carbon no.	HL	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
1	136.01	136.21	135.89	135.98	135.01	135.93	136.16	136.55
2	133.08	132.71	134.48	134.45	134.44	133.05	134.42	133.32
3	129.94	129.40	129.92	129.88	129.88	129.93	129.86	129.94
4	127.81	127.13	127.20	127.97	127.13	127.40	127.54	127.03
5	132.67	132.25	132.70	132.73	132.77	132.69	132.86	132.75
6	136.58	134.44	135.31	135.89	137.69	137.37	137.92	137.41
7	24.18	24.13	24.16	24.16	24.19	24.16	24.18	24.24
8	14.22	14.28	14.16	14.16	14.28	14.21	14.21	14.26
9	165.78	165.92	165.67	165.67	165.75	165.76	165.68	165.68
10	138.85	138.24	138.73	138.03	137.69	138.84	138.69	138.68
11	129.13	129.02	129.42	129.47	129.39	129.42	129.12	129.28
12	166.71	170.15	170.22	170.16	170.13	170.82	170.53	171.20

[a] **I**: Sn–CH_3, (C^{α}) 13.01. **II**: Sn–CH_3, (C^{α}) 8.77. **III**: Sn–$CH_2CH_2CH_2CH_3$, (C^{α}) 23.85, (C^{β}) 27.18, (C^{γ}) 26.88, (C^{δ}) 13.59. **IV**: Sn–$CH_2CH_2CH_2CH_3$, (C^{α}) 23.85, (C^{β}) 29.80, (C^{γ}) 27.09, (C^{δ}) 13.39. **V**: Sn–CH_3, (C^{α}) 13.85. **VI**: Sn–$CH_2CH_2CH_2CH_3$, (C^{α}) 24.47, (C^{β}) 27.87, (C^{γ}) 26.90, (C^{δ}) 13.68. **VII**: Sn–C_6H_5, (C^{α}) 136.22, (C^{β}) 129.12, (C^{γ}) 128.28, (C^{δ}) 130.62.

[b] Chemical shifts (δ) in ppm: $^{n}J[^{119}Sn, ^{13}C]$ in Hz is listed in parenthesis.

[c]



bonds, respectively, which also confirmed the complexation [30].

^{1}H NMR spectroscopy. The ^{1}H NMR spectral data of reported complexes recorded in $CDCl_3$, are presented in Table 3. The signals are assigned by the peak multiplicity, integration and intensity pattern.

The –COOH resonance signal of the ligand is absent in ^{1}H NMR spectra of all the complexes which indicates the replacement of carboxylic proton by the tin(IV) moiety. Insignificant shift of the –NH signal suggests that this group is not involved in bonding to organotin moiety or in the inter/intramolecular hydrogen bonding. According to the earlier reported work [28], magnetically nonequivalent alkyl protons of the ligands undergo the diamagnetic shielding upon complexation.

In complex **I** methyl protons appeared as singlet at δ 0.28 ppm while that of complex **II** and **V** appeared as sharp singlet at 0.28 ppm and 1.21 ppm, respectively. Compounds **III**, **IV**, and **VI** gave a complex pattern which is in accordance with the earlier reports [30], while the protons of α-carbon show a triplet at 0.95–0.98 ppm with $^{n}J(^{1}H–^{1}H)$ of 7.7–7.9 Hz. Triphenyltin(IV) complex gave rise to a multiplet in the aromatic region of 7.53–7.86 ppm. The position and number of all protons present in the synthesized compounds **I–VII** have been identified by incremental method [31].

^{13}C NMR spectroscopy. ^{13}C NMR spectra were recorded in $CDCl_3$ and the data are given in Table 4. The geometry of diorganotin dicarboxylates **I** and **III** could not be determined clearly due to the fluxional behavior of the carboxylate oxygen. In organotin(IV) compounds the resonance signal of carboxylic carbon (C^{12}) shows a shift (171.20–170.13 ppm) as compared to the ligand (166.71 ppm) confirming the coordination of the ligand through the carboxylic oxygen to the organotin(IV) moiety [30].

Carbons of alkyl group (CH_3 and *n*-Bu) attached to the tin appear in the range 8.77–13.85 ppm and 29.80–13.39 ppm, respectively as reported earlier [32]. The resonance signals of aromatic carbons were assigned

Table 5. Selected bond lengths (Å) and bond angles (deg) for organotin(IV) complexes

I	II	III	IV	V	VI	VII
Selected bond lengths, Å						
Sn^1–O^2 2.71 Sn^1–O^{29} 2.02	Sn^1–O^2 2.68 Sn^1–O^{29} 2.01	Sn^1–O^2 2.71 Sn^1–O^{29} 2.02	Sn^1–O^2 2.68 Sn^1–O^{29} 2.02	Sn^1–O^2 2.73 Sn^1–O^{29} 2.02	Sn^1–O^2 2.76 Sn^1–O^{29} 2.04	Sn^1–O^2 2.02 Sn^1–O^{29} 2.73
C^{30}–Sn^1 2.08 C^{34}–Sn^1 2.08	C^{31}–Sn^1 2.09 Cl^{30}–Sn^1 2.36	C^{30}–Sn^1 2.11 C^{43}–Sn^1 2.11	C^{30}–Sn^1 2.12 Cl^{56}–Sn^1 2.37	C^{30}–Sn^1 2.10 C^{34}–Sn^1 2.11	C^{30}–Sn 2.14 C^{43}–Sn^1 2.15	C^{30}–Sn^1 2.06 C^{41}–Sn^1 2.07
C^3–O^{29} 1.32 C^3–O^2 1.24	C^3–O^{29} 1.32 C^3–O^2 1.23	C^3–O^{29} 1.32 C^3–O^2 1.24	C^3–O^{29} 1.32 C^3–O^2 1.24	C^3–O^{29} 1.32 C^3–O^2 1.24	C^3–O^{29} 1.32 C^3–O^2 1.23	C^3–O^{29} 1.34 C^3–O^2 1.24
Selected bond angles, deg						
O^2–Sn^1–O^{29} 50.1	O^2–Sn^1–O^{29} 50.6	O^2–Sn^1–O^{29} 50.3	O^2–Sn^1–O^{29} 50.6	O^2–Sn^1–O^{29} 49.9	O^2–Sn^1–O^{29} 49.5	O^2–Sn^1–O^{29} 50.0
O^{29}–C^3–O^2 110.7	O^{29}–C^3–O^2 110.0	O^{29}–C^3–O^2 110.8	O^{29}–C^3–O^2 110.1	O^{29}–C^3–O^2 112.2	O^{29}–C^3–O^2 111.5	O^{29}–C^3–O^2 111.0
C^{30}–Sn^1–C^{34} 115.8	C^{31}–Sn^1–C^{35} 114.8	C^{30}–Sn^1–C^{34} 115.8	C^{30}–Sn^1–C^{34} 113.7	C^{30}–Sn^1–C^{34} 110.5	C^{30}–Sn^1–C^{34} 107.7	C^{30}–Sn^1–C^{341} 17.2
–	Cl^{30}–Sn^1–C^{31} 105.7	–	Cl^{56}–Sn^1–C^{30} 106.5	–	–	–

by the assessment of experimental chemical shift with those calculated by the incremental method [31] and by comparison with the published values [33].

Semi-empirical quantum-mechanical study. Among organotin(IV) complexes, different types of geometries are observed depending on coordination number of Sn. Complexes **I** and **III** are hexacoordinated, they exhibit highly distorted octahedral geometry and can be best described as having a skew-trapezoidal planar geometry [34]. In compound **I** and **III**, two carboxylate ligands are attached to the Sn atom, with Sn–O bond distances 2.02, 2.71 Å and 2.02, 2.69 Å, respectively. The C–O bonds are also unequal being 1.24, 1.32 Å for one carboxylate and 1.25, 1.31 Å for the second carboxylate ligand. In complex **I**, two methyl groups complete the coordination sphere around Sn being equidistant (2.08 Å) and the CH_3–Sn–CH_3 angle is 115.8°. The O–C–O and O–Sn–O angles are 110.7° and 50.5°, respectively, for both the carboxylate ligands.

Compounds **II** and **IV** are pentacoordinated with distorted trigonal bipyramidal geometry, in which Sn atom is attached to O of the carboxylic group with distances of 2.01 and 2.68 Å, respectively. The two methyl groups and one chloride complete the coordination sphere around Sn in complex **III**. The Sn–C bond length is 2.09 Å and Sn–Cl bond length is

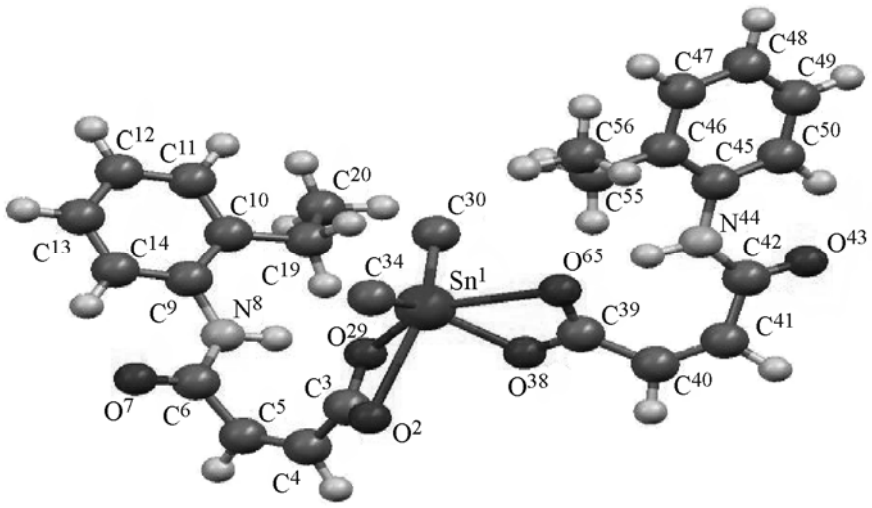


Fig. 1. Geometry optimized structure of complex **I**.

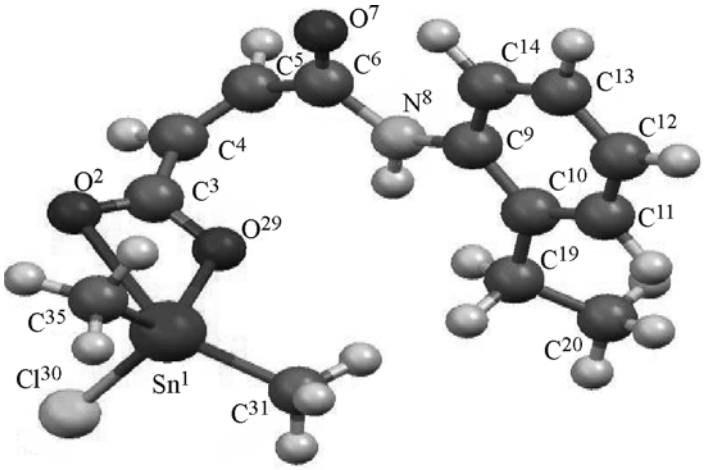


Fig. 2. Geometry optimized structure of complex **II.**

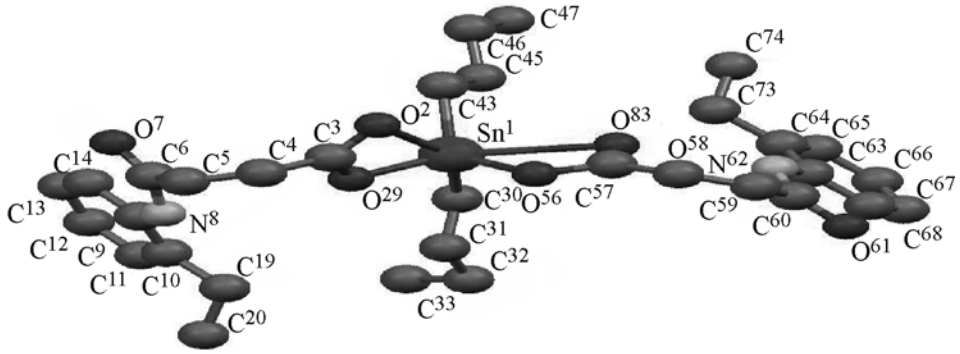


Fig. 3. Geometry optimized structure of complex **III.**

2.36 Å. The Me–Sn–Me angle is 114.8° and Me–Sn–Cl, 105.7°.The O–Sn–O and O–C–O angles are 50.6° and 110.0°, respectively. But in complex **IV**, Sn–C bond lengths are equal (2.12 Å) and Sn–Cl bond length is 2.37 Å. The Bu–Sn–Bu angle is 113.0°, while Bu–Sn–Cl is 106.5°. The O–Sn–O and O–C–O angles are 50.6° and 110.1°, respectively.

Complexes **V–VII** are also pentacoordinated with distorted trigonal bipyramidal geometry and the Sn-O bond distances of (2.02–2.04 Å) and (2.73–2.76 Å). Three R (CH_3, *n*-Bu, Ph) groups are bonded to the Sn atom with essentially identical bond distances (Sn–CH_3 = 2.10, Sn–Bu = 2.14, Sn–Ph = 2.07 Å). Geometry optimized structures of complexes **I–VII** are presented in Figs. 1–7.

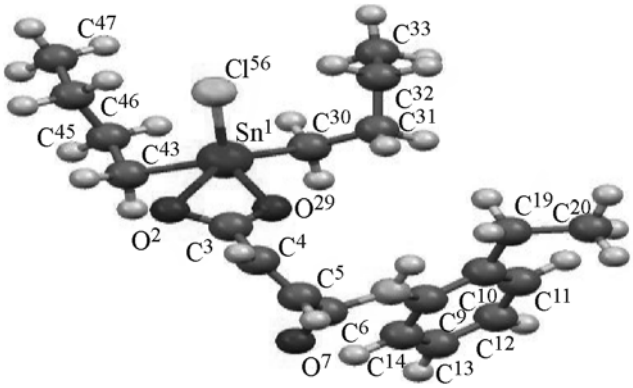


Fig. 4. Geometry optimized structure of complex **IV.**

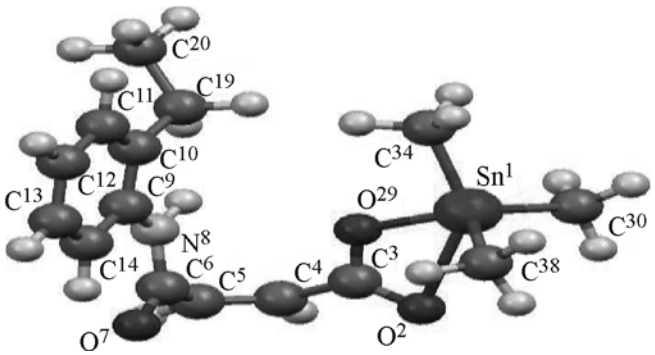


Fig. 5. Geometry optimized structure of complex **V.**

Table 6. Antibacterial activitya (mm) data of complexes against different strains of bacteria[a]

Complex no.	*Bacillus subtilis*	*Styphylococcus aureus*	*Escherichia coli*	*Pasturella multocida*
HL	6.0+	7.0++	5.0	4.0+
I	20.0++	14.5++	12.5++	5.5+
II	16.0++	10.0+	11.0+	12.5++
III	20.5+++	19.0++	9.0+	6.0+
IV	23.5+++	15.5++	7.0+	9.0+
V	5.0+	7.0+	8.5+	10.5+
VI	14.0++	14.0++	8.0+	13.0++
VII	18.5++	18.5++	17.0++	9.5+
Rifapmicin	24.0+++	23.0+++	26.0+++	28.0+++

[a] (+): low activity, (++): moderate activity, (+++): strong activity.

In vitro antibacterial activity. The newly synthesized ligand and complexes **I–VII** were screened for their *in vitro* antibacterial activity against four bacterial strains. Two of which are Gram prositive (*B. subtilis, S. aureus*) while the other two are Gram negative (*E. coli, P. multocida*). Results are summarized in Table 6. Rifampicin was used as a standard drug.

The synthesized complexes showed higher activity than ligand but lower activity than the standard drug. The studies on structure/activity correlation of organotin(IV) compounds reveal that the following structural features characterize the active compounds [27].

(1) The accessibility of coordination site of Sn;

(2) The occurrence of comparatively stable ligand–tin bond via Sn-O interaction .

Complexes **I** and **IV** show low activity against *P. multocida* and *E. coli* while complex **V** expressed very low activity against *B. subtilis* and *S. aureus*. Among the synthesized complexes, the dimethyltin and dibutyltin **I** and **III** were found to be significantly active. The enhanced activity of organotin(IV) complexes may be due the increased lipophilic nature of the complexes arising due to the chelation [7].

It is observed that all reported complexes show significant antibacterial activity against Gram (+) bacteria *(Basillus subtilis*) and slightly lower with other bacterial strains. The significant results may be due to the simple cell wall composition as compared to the Gram (–) cells in which cell wall complexity contributes to the complex antigenic specificity to Gram (–) cells [27].

In vitro **antifungal activity.** Preliminary in vitro tests for antifungal screening activity of ligand and complexes have been carried out by disc diffusion method [20]. Fluconazole is used as a standard drug. The results (Table 7) show that all complexes exhibit the significant antifungal activity as compared to the ligand. The increased activity of organotin(IV)

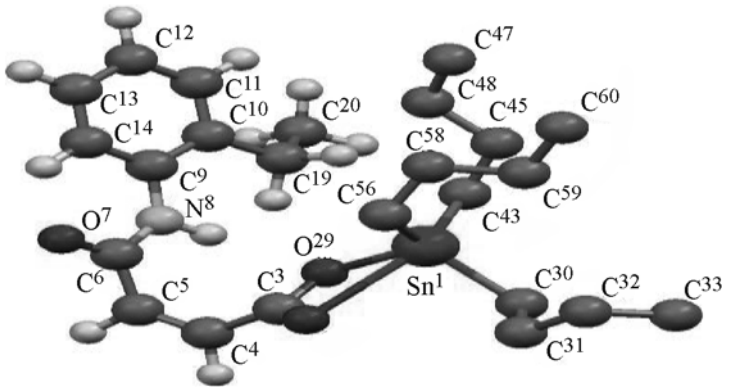


Fig. 6. Geometry optimized structure of complex **VI.**

Table 7. Antifungal activitya (mm) data of complexes against different strains of fungi[a]

Complex no.	*Alternaria alternata*	*Aspergillus niger*	*Rhizopus solani*	*Aspergillus flavus*
HL	10.4+	10.0+	11.5++	10.0+
I	15.0++	14.5++	12.0++	10.5+
II	18.5++	15.0++	12.5++	14.5++
III	12.5++	14.0++	9.5+	11.0+
IV	11.5++	11.0+	13.0++	15.0++
V	15.5++	12.5++	15.0++	12.5++
VI	14.5++	11.5++	9.5+	13.0++
VII	18.0++	15.5++	15.5++	14.0++
Fluconazole	26.0+++	25.0+++	23.0+++	20.0++

[a] (+): low activity, (++): moderate activity, (+++): strong activity.

Table 8. Minimum inhibitory concentration (MIC) data of synthesized organotin(IV) complexes against bacterial strains

Complex no.	*Bacillus subtilis*	*Styphylococcus aureus*	*Escherichia coli*	*Pasturella multocida*
HL	1500	1500	750	750
I	93	187	187	750
II	187	375	187	46
III	93	93	375	375
IV	46	375	750	187
V	750	750	375	93
VI	375	187	375	46
VII	187	187	46	93
Rifapmicin	23	46	23	23

complexes may be due to the coordination and polarity of tin(IV) atom with oxygen of the ligand [35].

Complexes **II** and **IV** showed significant fungicidal character against *Alternaria alternata* and *Aspergillus flavus*, respectively. Complex **VII** also shows remarkable activity against *Aspergillus nigar* and *Rhizopus solani*, while complexes **III** and **IV** were found to be less effective against *Aspergillus flavus* and *Aspergillus nigar*, respectively.

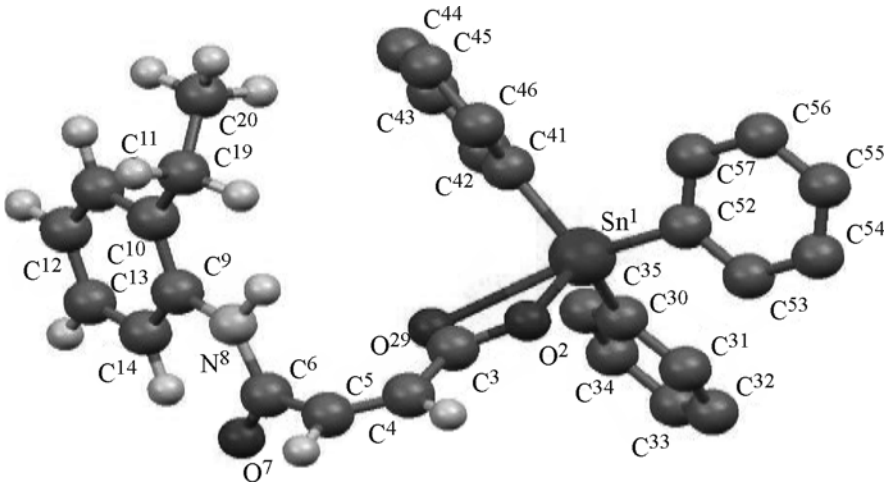


Fig. 7. Geometry optimized structure of complex **VII.**

Table 9. Minimum inhibitory concentration (MIC) data of synthesized organotin(IV) complexes against fungal strains

Complex no.	*Alternaria alternata*	*Aspergillus niger*	*Rhizopus solani*	*Aspergillus flavus*
HL	1250	2500	1250	1250
I	312	156	312	625
II	78	156	312	156
III	625	312	652	625
IV	625	625	312	456
V	312	312	156	312
VI	312	312	625	312
VII	156	78	156	312
Fluconazole	39	39	78	78

Among the triorganotin(IV) complexes the antifungal activity increases in the order $CH_3 < C_4H_9 < C_6H_5$ with the few exception, suggesting the more bulky/longer the R (R = CH_3, C_4H_9 and C_6H_5) group, the higher is the biological activity [36]. In diorganotin(IV) series, complex **II**, which is chlorodimethyltin(IV) derivative, was more effective against *Alternaria alternata*.

In general, the organotin(IV) complexes exhibit remarkable potential in inhibiting the growth of bacteria and fungi. Therefore these results can be used for further intensive studies of these complexes in order to develop antibacterial and antifungal agents for the applications in various fields.

MIC of organotin(IV) complexes against bacteria and fungi. The minimum inhibitory concentration (MIC) of newly synthesized ligand and complexes were evaluated against selected strains of bacteria and fungi. MIC values are summarized in

Tables 8 and 9 for bacteria and fungi, respectively. Rifapmicin and Fluconazole were used as standard drugs for bacteria and fungi, respectively.

Data showed that complexes exhibit more potent growth inhibitory effect than the ligand but lesser than that of standard drug (for both bacterial and fungal strains). Increased activity may be due to metallation in the complexes which is in accordance with earlier reports [36, 37].

Complexes **III** and **IV** showed strong antibacterial activity with lower MIC values (MIC, 93 and 46 μg/mL) against *Styphlococcus aureus* and *Bacillus subtillus*, respectively. Complex **II** and **VII** were found to be more effective against bactaria (*Pasturella multocida*, *Escherichia coli*) with MIC value of 46 μg/mL. From overall results, it was concluded that the diorganotin(IV) derivatives showed the strong activity [7].

Result of MIC for fungal strains showed that complex **II** exhibited strong antifungal activity (MIC 78 and 156 μg/mL) against *Altermaria alternata* and *Aspergillus flavus* and this was due to the nature of chlorodimethyltin(IV) derivatives [13]. Complex **VII** was found to be more effective (MIC 78 and 156 μg/mL) against *Aspergillus niger* and *Rhizopus solani* because it contains triphenyltin moiety that showed strong growth inhibitory effect [38].

Antioxidant/DPPH radical scavenging activity. The DPPH scavenging activity of ligand and complexes was assessed by measuring their scavenging abilities to 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) stable radical. This mechanism involves transfer of hydrogen to DPPH. The ligand and complexes behave as donors of hydrogen that corresponds to the transformation of DPPH to reduced (DPPH–H).

BHT was used as standard drug. Antioxidant activity increases with the increase in the concentration of the complex because as the concentration increases, their IC_{50} index decreases. So, the lower the IC_{50} values, the higher the antioxidant activity [39].

The IC_{50} values for ligand and complexes **I–VII** are listed in Table 10. From data it was found that all complexes show significant antioxidant activity as compared to the ligand which is consistent with the literature [37] suggesting that metal moiety will increase antioxidant activity of ligand. This information confirms that the observed antioxidant activity is increased by the presence of Sn(IV) metal center.

Table 10. IC^{50} data for organotin(IV) complexes with 2-ehyl-anilinocarbonylpropenoic acid

Complex no.	IC_{50} Mean±S.D
HL	290.06±4.17
I	89.72±1.436
II	287.11±8.61
III	72.07±1.35
IV	125.56±2.43
V	152.48±4.57
VI	63.36±1.17
VII	153.83±4.13
BHT (Standard)	44.60±1.00

Table 11. Hemolytic (%) of 2-ehylanilinocarbonylpropenoic acid and organotin(IV) complexes

Complex no.	Hemolysis, %
HL	4.867±0.75
I	14.700±2.3
II	9.133±1.2
III	6.2±1.5
IV	9.900±1.6
V	11.033±2.00
VI	5.800±1.0
VII	9.533±1.5
PBS	3.737±0.73
Tx-100	98.367±2.9

Complex **VI** with IC_{50} value 63.36 μg/mL expressed significant free radical scavenging activity as compared to other complexes. Complex **VI** is more active than diorganotin(IV) derivative [38], while complex **II** showed poor antioxidant activity with higher IC_{50} value 287.11μg/mL, which is slightly lower than the ligand. This may be due to chlorine atom attached to tin moiety.

Hemolytic activity. Complexes **I–VII** were examined to determined cytotoxicity against the human red blood cells (RBC) by hemolytic method. Results are given in Table 11. Triton Tx-100 (positive control) and PBS (negative control) were the standard drugs used for comparison [40]. Results show that the % hemolytic values for all complexes **I–VII** were less than the reference cytotoxic compound Tx-100

Table 12. Mutagenicity testing of selected complexes by Ames test

Comp. no.	Amount tested (μL/plate)	His^+ revertants/plate (no. of colonies)							
		TA-98				TA-100			
		I	**II**	Mean	M.I	**I**	**II**	Mean	M.I
V	100	0	0	–	–	0	0	–	–
VII	100	0	0	–	–	0	0	–	–
Negative control									
				Mean				Mean	
DW	100	68		51	–	55	71	54	–
DMSO	100	13		21	–	5	14	10	–
Positive Control									
				Mean				Mean	
NaN_3	10	xx		xx	–	53	75	64.6	–
$K_2Cr_2O_7$	10	66		56.6	–	–	–	–	–
MNNG	3	42		52.6	–	58	115	77	–
B(a)P	10	95		96.3	–	–	–	–	–
B(a)P	5	–		–	–	84	87	78.6	–

(98.36%) but slightly higher than ligand. These results also support the previously suggested theory that organotins are membrane effectors because of their comparatively high hydrophobic, lipid partitioning properties. The relatively lipophilic compound, triphenyltin chloride, appeared to be anomalous because it did not readily promote hemolysis or induce the formation of intramembranous aggregates in human erythrocytes [40].

Complex **I** exhibits slightly higher cytotoxic activity with % hemolysis value (14.700 ± 2.30) as com-pared to ligand and other complexes, while complex **VI** was found to be least cytotoxic (5.800 ± 1.00).

Mutagenicity testing. In Ames test, mutagenic potential of newly synthesized selected complexes **V** and **VII** were 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) was used as positive control in experiments along with known mutagens like Methylnitronitrosoguanidine (MNNG) 12 μg/plate and Benzo[*a*]pyrene (35 μg/plate). Distilled water served as negative control and DMSO was used as vehicle control. In Ames test it was shown that at various concentrations of synthesized compounds (10 000, 1000, and 500 μg/plate), the mutation frequencies did not change significantly as compared to the positive control.

No revertant colonies were observed for the complexes **V** and **VII** (Table 12). This is probably due to toxic or inhibitory effect of complexes which may inhibit the growth of strains TA-100 and TA-98. It may also be due to antibacterial property of our complexes **V** and **VII**, therefore further work related to antimutagenic potential of these complexes by Ames test was not done. These results revealed that the isolated complexes at concentration (1000–500 μg/plate) were found to be toxic and not suitable for mutagenicity testing by Ames test. These results are consistent with earlier report [41] that structural factors (including steric and electronic constraints imposed by the organometallic moieties) may be responsible for the absence of activity.

CONCLUSIONS

The results obtained by IR spectroscopy suggest bidentate nature of the ligand and the complexes exhibits octahedral geometry for diorganotin dicarboxylate and trigonal bipyramidal geometry for chloro-diorganotin and triorganotin complexes which is also confirmed by semi-empirical quantum-mechanical study. ^1H and ^{13}C NMR data show tetracoordinated geometry in solution.

Biological screening of the complexes reveals that the di-organotin(IV) complexes exhibit the significant antibacterial activity while clorodiorganotin and triorganotin show higher antifungal activity as compared to free ligand. All complexes show good antioxidant activity. Hemolytic activity was also studied for complexes and it was found that the activity was very low as compared to standard drug. By comparing the results of antimicrobial and antioxidant activities with hemolytic results, it can be predicted that these complexes can be used as medicinal agent in future against pathogens. Mutagenicity results show the inhibitory effect of complexes which may be due to antibacterial activity of reported complexes.

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REFERENCES

1. Cusack, P.A., *Applied Organometallic Chemistry*; 2nd ed., London: Blackie Academic and Professional, 1998.
2. David, A., Gielen, M., Pannell, K., and Tiekink, E., *Tin Chemistry*; John Wiley, 2008.
3. Molloy, K.C., Percell, T.G., Hahn, R., Schuman, H., and Zand Zuckerman, J.J., *Organometallics,* 1986, vol. 5, p. 85.
4. Smith, P.J., Crow, A.J., and Hill, R., *Laboratory Evaluation of Tributyltin(IV) Compounds as Wood Preservatives; Publication 559*, International Tin Research Institute, London, 1979.
5. Shahzadi, S., Ali, S., and Fettouhi, M., *J. Chem. Cryst.,* 2008, vol. 38, p. 273.
6. Aldridge, W.N. and Zuckerman, J.J., *Organotin Compounds*: *New Chemistry and Applications. Advanced Chemistry;* American Chemical Society, Washington, 1976.
7. Hussain, M., Hanif, M., Ali, S., Shahzadi, S., Ahmad, M.S., Mirza, B., and Evans, H.S., *J. Iran. Chem. Soc.,* 2010, vol. 7, p. 155.
8. Penninks, A.H., Punt, P.M., Bol-Schoenmakers, M., Van Rooijen, H.J.M., and Seinen, W., *Silicon, Germanium, Tin and Lead Comp.,* 1986, vol. 9, p. 367.

9. Blunden, S.J., Cusak, P.A., and Hill, R., *The Industrial Uses of Tin Chemicals*, London: The Royal Society of Chemistry, 1985.
10. Hon, D.N.S. and Shiraishi, N., *Preservation of Wood, Wood and Cellulosic Chemistry*, New York, 2001.
11. Gielen, M., Willem, R., Holecek, J., and Lycka, A., *Main Group Met. Chem*., 1993, vol. 16, p. 29.
12. Pellerito, C., Nagy, L., Pellerito, L., and Szorcsik, A., *J. Organomet. Chem.,* 2006, vol. 691, p. 1733.
13. Valla, V. and Christianopoulou, M.B., *Synth. React. Inorg. Met. Org. Nano-Met. Chem.,* 2006, vol. 36, p. 765.
14. Affan, M.A., Fasihuddin, B.A., Liew, Y.Z., Foo, S.W., and Ismail, J., *J. Sci. Res.,* 2009, vol. 1, p. 306.
15. Stewart, J.J.P., *J. Comput. Chem.*, 1989, vol. 10, p. 209.
16. Stewart, J J.P., *J. Comput. Chem.,* 1991, vol. 12, p. 320.
17. Stewart, J.J.P., *J. Mol. Modeling.,* 2007, vol. 13, p. 1173.
18. *http://openmopac.net/manual/Eigenfollowing.html.*
19. *http://openmopac.net/manual/scf_test.html.*
20. CLSI (The Clinical Laboratory Standards Institute), *J. Clin. Microbio*., 2007, vol. 45, p. 2758.
21. Huang, G., Moore, S., Jiaxin, J., and Dehui, D., *J. Food Sci. Techn.,* 2001, vol. 11(1), p. 25.
22. Huynh, Q.K., Borgmeyer, J.R., Smith, C.E., Bell, L.D., and Shah, D.M., *J. Bio. Chem.,* 2001, vol. 316, p. 723.
23. Sarker, S.D., Nharb, L., and Kumarsamyc, Y., *Methods,* 2007, vol. 42, p. 321.
24. Bozin, B., Mimica-Dukic, N., Simin, N., and Anackov, G., *J. Agri. Food Chem.,* 2006, vol. 54, p. 1822.
25. Powell, W.A., Catranis, C.M., and Maynard, C.A., *Lett. Appl. Microbiol.,* 2001, vol. 31, p. 163.
26. Ames, B.N., Lee, F.D., and Durston, W.E., *Proc. Nat. Acad. Sci.*, USA, 1973, vol. 70, p. 782.
27. Shahzadi, S., Shahid, K., Ali, S., Mazhar, M., and Khan, K.M., *J. Iran. Chem. Soc*., 2005, vol. 2, p. 277.
28. Rehman, W., Baloch, M.K., Badshah, A., and Ali, S., *J. Chin. Chem. Soc.,* 2005, vol. 52, p. 231.
29. Santillan, G.A. and Carrano, C.J., *Inorg. Chem.,* 2008, vol. 47, p. 930.
30. Shahzadi, S., Shahid, K., Ali, S., Yousaf, M., Sharma, S.K., and Qanungo, K., *J. Chin. Chem. Soc.,* 2010, vol. 57, p. 659.
31. Kalinowski, H.O., Berger, S., B and raun, S., *13C-NMR Spektroskopie*, Stuttgart: ThiemeVerlag, 1984.
32. Sadiq-ur-Rehman., Ali, S., and Shahzadi, S., *Hetero-atom Chem.,* 2008, vol. 19, p. 612.
33. Shahzadi, S., Shahid, K., Ali, S., and Bakhtiar, M., *Turk. J. Chem*., 2008, vol. 32, p.333.
34. Shahzadi, S. and Ali, S., *J. Iran. Chem. Soc.,* 2008, vol. 5, p. 16.
35. Singh, N., Gupta, S., and Nath, G., *Cent. Nat. De La Rech. Scien.,* (CAT.INIST), 2000, vol. 14, p. 484.
36. Masood, M.T., Ali, S., Danish, M., and Mazhar, M., *Synth. React. Inorg. Met. Org. Nano-Met. Chem.,* 2002, vol. 32, p. 9.
37. Bonire, J.J., Ayoko, G.A., Olurinola, P.F., Ehinmindu, J.O., Jalil, N.S.N., and Omachi, A.A., *Metal-Based Drugs,* 1998, vol. 5, p. 231.
38. Jamil, K., Bakhtiar, M., Khan, A.R., Rubina, F., Rehana, R., Rehman, W., Qaisar, R.M., Khan, A.F., Khan, A.K., Danish, M., Awais, M., Bhatti, Z.A., Rizwan, M., Naveed, A., Hussani, M., and Pervez, A., *Afri. J. Pure and Appl. Chem.,* 2009, vol. 3, p. 066.
39. Sanshez, M., Hostettmann, K., and Potterat, O., *Helv. Chim. Acta,* 1999, vol. 80, p. 1144.
40. Bukhari, S.B., Memon, S., Mahroof-Tahir, M., and Bhanger, M.I., *Spectrochim. Acta A,* 2009, vol. 71, p. 1901.
41. Gray, B.H., Porvaznik, M., Flemming, C., and Lee, L.H., *Cell Biol. and Toxico.,* 1987, vol. 3, p. 23.