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Synthesis, Characterization and Biological Activities of Homotrimetallic Complexes contining Sn(IV) with various Oxygen and Sulphur Donor Ligands

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ABSTRACT

Homotrimetallic organotin(IV) dicarboxylates dithiocarbamates, $Me_3SnSSCL^1(SnMe_3)_2$ (1), $Bu_3SnSSCL^1(SnBu_3)_2$ (2) and $Ph_2(Cl)SnSSCL^2[Sn(Cl)Ph_2]_2$ (3) were synthesized by stirring/refluxing 5-amionoisophthalic acid (HL^1K_2) or iminodiacetic acid disodium salt (HL^2Na_2) hydrate with CS_2 and $Me_3SnCl/Bu_3SnCl/Ph_2SnCl_2$ in a 1:1:3 molar ratios in methanol. The products were analyzed by microanalysis (C, H, N, S), FTIR, NMR ($^1H\&^{13}C$) and mass spectrometry. The C, H, N & S analysis verified the molecular composition of the products. FT-IR spectroscopy demonstrated a chelating binding mode of the dithiocarbamate moiety and a bridging coordination behavior of the carboxylate groups. The synthesized complexes change theirsolid state trigonal bipyramdial geometry to a tetrahedral environment around Sn(IV) in solution state. ^1H NMR spectroscopy displayed the excepted signals of organotin(IV) moieties as well as the ligand skeletons. The methylene protons were de-shielded in complex 3 compared to its ligand precursor. ^{13}C NMR spectroscopy verified the ligand-metal coordination. Electron ionization mass spectrum was well agreed with the structure of the product 1. The trinuclear complexes exhibited significant antimicrobial potential as evaluated by disc diffusion method and minimal inhibitory concentration (MIC) measurements. The tributyltin(IV) derivative 2 was found to be a most potent inhibitor against all the fungal strains except *Trichoderma harzianum* while complex 1 was best active against all the tested bacterial strains except *Pasturella multocida*. Complex 3 exhibited an intermediate type of inhibition in most cases. The highest hemolytic activity was displayed by complex 1 (33.06%) while the complex 3 exhibited the lowest hemolytic effects (6.92%) demonstrating its possible safe use in medicine.

Keywords: Homotrimetallic; Organotin(IV); Spectroscopy; FT-IR; NMR; antimicrobial; Hemolytic

1. INTRODUCTION

The coordination of Sn(IV) with oxygen and sulphur donor ligands has been studied extensively from past few decades [1-10]. Organotin(IV) carboxylates and dithiocarboxylates form an important class of organometallic compounds. The structural diversity of these compounds emanates from several features including flexibility in coordination geometries, coordination numbers, and versatility of the ligands to engage in different modes of chelation from monodentate to bidentate [1]. Major structural issues in organotin(IV) chemistry are induced by the high coordination ability of tin [2]; this is because of the availability of empty 5d-orbitals ofsuitable energy in tetravalent tin.It is not uncommon that coordination characteristics of tin and hence the structural features of an organotin compound differ dramatically insolution and solid states [3]. Organotin(IV) carboxylates are of special interest regarding a variety of methods available for their syntheses, spectroscopic/non-spectroscopic characterization and biological activities [4-5]. In recent years organotin(IV) carboxylates have attracted much attention owing to their potential biocidal activity and cytotoxicity [6]. Many organotin carboxylates have been found to be active against various types of cancer cells and display interesting antitumor activities [7]. The chemistry of organotin(IV) derivatives with sulfur ligands has grown with prolific speed on account of multifaceted reasons. Dithiocarbamate anions are highly effective ligands owing to the stability of their complexes with metals [8]. Complexing agents with a dithio functional group have been widely used in industry as rodent repellents, vulcanization additives in rubber manufacturing, additives in lubricants, and in agriculture as fungicides on almond trees, stone fruits, and vegetables [9]. In addition to biological activities, such complexes have also been subjected to thermal and CVD studies [10].

The purpose of this study is to synthesize the homotrimetallic (Sn) complexes from the ligands (HLH_2 and $HLNa_2$) having O and S donor sites. The products were characterized by microanalysis (C, H, N, S), FT-IR, NMR ($H^1\&^{13}C$) and mass spectroscopy (EIMS). The complexes were tested for antibacterial and antifungal activities and their minimum inhibitory concentrations were evaluated. The products were also subjected to hemolytic activities to find their possible safe/unsafe medicinal use. The structure activity relationships were explored.

A tremendous work on simple organotin(IV) and some on their homobimetallic (Sn) derivatives has already been reported or in progress due to their profound applications in biological as well as in non-biological fields. However, the present work will shift the studies from homobimetallic (Sn) to homotrimetallic (Sn) complexation and it may be a good addition to the organobimetallic chemistry as it can help in the development of new bio-active drugs. An attention has been focused in this work on the proper selection of ligands (containing two oxygen and one sulphur donor site). Both the ligand precursors (HLH_2 and $HLNa_2$) have many common features between them: firstly, each ligand is trifunctional and has simultaneously availability of amino and two carboxyl groups; secondly, the two carboxy groups of each ligand have relatively a long distance so there is only a little spatial hindrance for the formation of bis(organotin(IV)) dicarboxylates; thirdly the amino group may also participate in reaction with CS_2 converting the –NH moiety into –NCSSH group resulting in the availability of third donor site (S) for further trimetallic coordination.

2. EXPERIMENTAL

2.1. Materials and Methods

Trimethyltin chloride, Tributyltin chloride, diphenyltin dichloride and carbon disulfide were procured from Sigma-Aldrich (USA) and used without any further purification. 5-amionoisophthalic acid (HLH_2) and iminodiacetic acid disodium salt ($HLNa_2$) hydrate were purchased from Merck (Germany). AR grade solvents of Merck (methanol), Lab-scan (DMSO) and Riedel-de Haen (petroleum ether) origin were used. The solvents were dried before use by standard procedures [11].

The melting points of samples were measured in capillary tubes by an electrochemical melting point apparatus Stuart SMP3 and are uncorrected. Infrared spectral measurements were performed using theKBr discs in a range of 4000-250 cm^{-1} by the Thermo Nicolet-6700 FT-IR Spectrophotometer. The ^{1}H &^{13}C NMR spectra were recorded in DMSO-d_6 by a Bruker ARC FT-NMR spectrometer operating at 300 MHz and 75 MHz, respectively. The mass spectrometry (EIMS) was performed using a Thermo Fisher Exactive Orbitrap instrument.

The ligand and the synthesized complexes were tested for their antimicrobial activities against various strains of bacteria (*Escherichia coli, Bacillus subtilis, Staphlococcus aureus* and *Pasturella multocida*) and fungi (*Alternaria alternata, Ganoderma lucidum, Penicillium notatum, Trichoderma harzianum* and *Aspergillus niger*) by disc diffusion method [12] and minimum inhibitory concentration (MIC) [13] evaluations. Streptomycin and fluconazole were used as standard drugs for antibacterial and antifungal screening tests, respectively. The *in vitro* hemolytic bioassays [14] of the compounds were reported with respect to the triton X-100 as a positive control and PBS as a negative control.

2.2. Syntheses

2.2.1. Procedure for the Syntheses of Complexes 1 & 2

A mixture of KOH (2 mmol), 5-amionoisophthalic acid (**HL^1H_2**) (1 mmol) and CS_2 (1 mmol) was vigorously stirred for 1 h in a methanol (50 ml) at room temperature in a round bottom flask (250 ml). The reaction mixture was added slowly to the solution of Me_3SnCl/Bu_3SnCl (3 mmol) in methanol (30 ml) in a round bottom two necked (250 ml) flask. After 5 h stirring, the precipitated KCl formed was filtered off. The filtrate was rotary evaporated and the product was dried and recrystallized from methanol after adding few drops of petroleum ether (Scheme 1).

HL^1H_2. M.p. >300 °C. IR (cm^{-1}): 3266w υ(NH), 3671b υ(OH), 1718s υ(OCO)asym, 1445m υ(OCO)sym, (Δυ = 273 cm^{-1}).

Complex 1. Yield: 83%. M.p. 145-147 °C. Anal. Calc. for $C_{18}H_{31}NO_4S_2Sn_3$: C, 28.99; H, 4.19; N, 1.88; S, 8.60. Found: C, 28.96; H, 4.15; N, 1.92; S, 8.65%. IR (cm^{-1}): 1681m υ(COO)asym, 1458 υ(COO)sym, (Δυ = 223 cm^{-1}), 1560m υ(C=N), 1007s υ(C-S), 591s, 555m υ(Sn-C), 441m υ(Sn-O), 361m υ(Sn-S).). ^{1}H NMR (DMSO-d_6, ppm):6.94 (s, $H_{3,3'}$, 2H), 7.32 (s, H_5, 1H), 3.18 (s, H_α, 18H), 0.19 (s, $H_{\alpha'}$, 9H); $^{2}J(^{119/117}Sn, ^{1}H)$ = 52 Hz.

Complex 2. Yield: 87%. M.p. 279-282 °C. Anal. Calc. for $C_{45}H_{85}NO_4S_2Sn_3$: C, 48.07; H, 7.62; N, 1.25; S, 5.70. Found: C, 48.11; H, 7.66; N, 1.29; S, 5.73%. IR (cm^{-1}): 1694s υ(OCO)asym, 1455m υ(OCO)sym, (Δυ = 239 cm^{-1}), 1538sυ(C=N), 1019s υ(C-S), 589s, 552s υ(Sn-C), 438m υ(Sn-O), 366s υ(Sn-S). ^{1}H NMR (DMSO-d_6, ppm):7.38 (s, $H_{3,3'}$, 2H), 7.68 (s, H_5, 1H), 1.07-1.12 (m, $H_{\alpha,\alpha'}$, 18H), 1.53-1.61 (m, $H_{\beta,\beta'}$, 18H), 1.22-1.32 (m, $H_{\gamma,\gamma'}$, 18H), 0.85 (t, $H_{\delta,\delta'}$ 27H). ^{13}C NMR (DMSO-d_6, ppm):149.6 (C-2), 118.6 (C-3,3′), 132.1 (C-4,4′), 117.9 (C-5), 167.7 (C-6,6′), 26.7 (C-α), 28.2 (C-β), 27.2 (C-γ), 14.1 (C-δ).

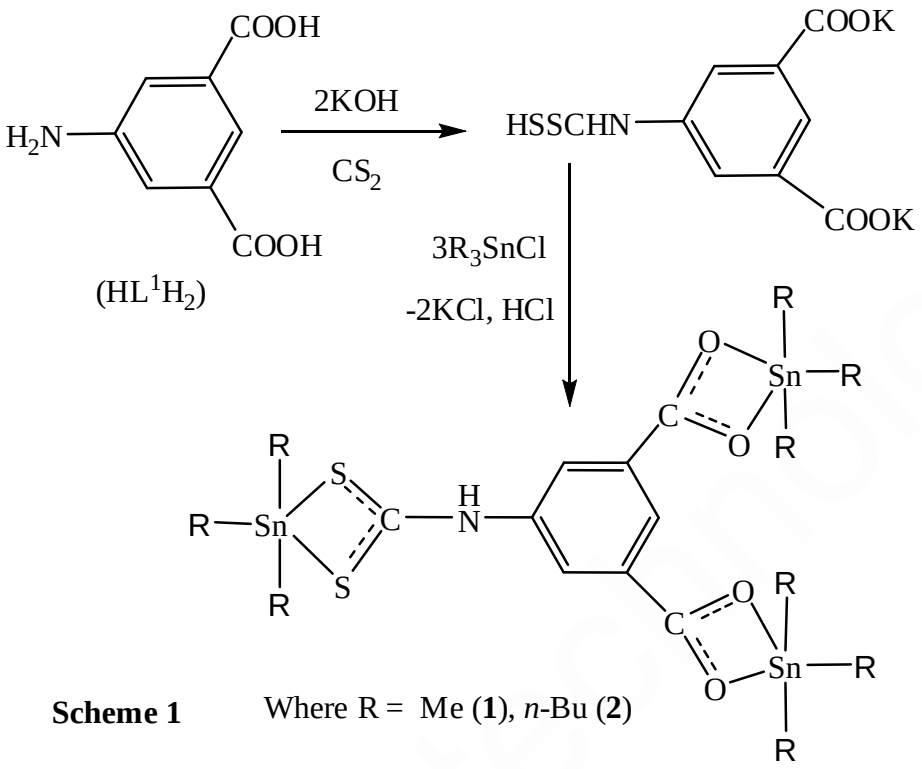
2.2.2. Procedure for the Synthesis of Complex 3

1 mmol of iminodiacetic acid disodium salt (HL^2Na_2) hydrate was stirred with 1 mmol of CS_2 for 1 h in a methanol (50 ml) at room temperature in a round bottom flask (250 ml). Then Ph_2SnCl_2 (3 mmol) was added and the reaction mixture was refluxed for 5 h. The precipitated NaCl formed was filtered off. The filtrate was rotary evaporated and the solid product was recrystallized from methanol after adding few drops of petroleum ether (Scheme 2).

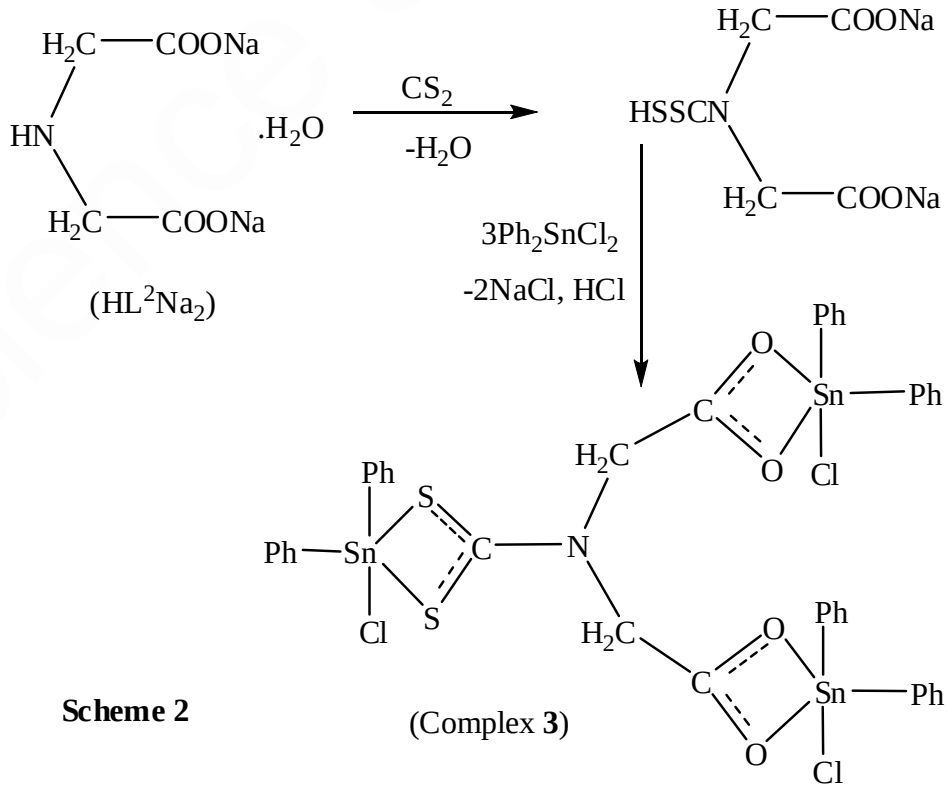
HL^2Na_2. M.p. 180 °C. IR (cm^{-1}): 3285b υ(N-H), 1659s υ(OCO)asym, 1412s υ(OCO)sym, (Δυ = 247 cm^{-1}), 3439b υ(O-H), 897m $\upsilon\rho_r(H_2O)$, 691m υ $\rho_w(H_2O)$. ^{1}H NMR (D_2O, ppm): 3.03 (S, $H_{2,2'}$, 4H), 4.70 (S, H_4, 2H). ^{13}C NMR (D_2O, ppm): 179.3 (C-1,1′), 51.9 (C-2,2′).

Complex 3. Yield: 86%. M.p. 255°C. Anal. Calc. for $C_{41}H_{34}Cl_3NO_4S_2Sn_3$: C, 43.53; H, 3.03; N, 1.24; S, 5.67. Found: C, 44.49; H, 3.07; N, 1.27; S, 5.63%. IR (cm^{-1}): 1646m υ(OCO)asym, 1431m υ(OCO)sym, (Δυ = 215 cm^{-1}), 1587m υ(C=N), 1022s υ(C-S), 253s, 236s υ(Sn-C), 444m υ(Sn-O), 396w υ(Sn-S), 307m υ(Sn-Cl). ^{1}H NMR (DMSO-d_6, ppm): 3.59-3.72 (m, H_2, 2H), 3.24-3.43 (m, $H_{2'}$, 2H), 7.96 (d, H_β, 8H),

7.88 (d, $H_{\beta'}$, 4H), 7.73-7.77 (m, $H_{\gamma,\delta}$, 12H), 7.33-7.43 (m, $H_{\gamma',\delta'}$, 6H). ^{13}C NMR (DMSO-d_6, ppm): 170.2 (C-1), 169.1 (C-1′), 53.3 (C-2), 52.6 (C-2′), 136.8 (C-α), 135.2 (C-β), 134.5 (C-γ), 133.7 (C-δ), 129.4 (C-α′), 128.8 (C-β′), 128.4 (C-γ′), 127.5 (C-δ′).



Scheme 1 Where R = Me (**1**), *n*-Bu (**2**)



Scheme 2 (Complex **3**)

2.3. Antimicrobial activities

2.3.1. Growth medium, culture and inoculum preparation

Pure strains of bacteria were cultured overnight at 37 °C in nutrient agar medium. Then 10 µL bacterial culture was added to a 100 mL of sterilized nutrient broth medium prepared in distilled water (concentration = 13g/1L) and the mixture was incubated in a shaker (140 rpm) at 37 °C for 24 hours. The prepared inocula were stored in a refrigerator at 4 °C. The inoculum with 1×10^8 spores/mL was used for further analysis [15]. The fungal strains were cultured in potato dextrose agar medium (concentration = 39g/L in distilled water) overnight at 28 °C. These cultures were incubated at 28 °C for 3-4 days for their further multiplication [15].

2.3.2. Antimicrobial assay by disc diffusion method

100 mL aqueous suspension of 2.8 g of nutrient agar (for antibacterial activities) or 3.9 g of potato dextrose agar (for antifungal activities) was autoclaved (for sterilization) at 121 °C for 15 minutes. It was then mixed well with 100 µL inoculum of a microbial strain at room temperature and transferred into sterilized petri plates (diameter = 10''). Small filter paper discs (size, 9 mm) each soaked with 100 µL solution of a standard drug/test sample, were placed flat on the growth medium. The plates were finally wrapped in Al foil and then placed in an incubator for 24 hours at 37 °C (for bacterial growth) or 28°C (for fungal growth). Zones of inhibition were formed by biologically active samples; these zones were measured by a zone reader [12,15].

2.3.3. Antimicrobial assay by minimum inhibitory concentration (MIC)

Sterilized 96 well plates were taken. Then 100 µL solution of a test sample was added to a separate well of the first row of a sterilized 96 well plate while 50 µL of nutrient broth medium was added to the wells of remaining rows. Serial dilutions were done by a multichannel pipette starting from the first row so that each well had a 50 µL test material. Then 20 µL resazurin solution in distilled water (270mg/40 mL) was added to each well. Finally, 10 µL bacterial suspension was added to each well. Then the plate was loosely wrapped with Al foil to prevent dehydration of microbes. Each plate contained a set of controls: a column having all the solutions except the bacteria because 10 µL of nutrient broth was added instead, a column with all the solutions except the test compound and a column with the standard drug. The plates were prepared in triplicate and placed in an incubator at 37 °C for 18–24 hours. The color changes from purple to colorless or pink were taken as the bacterial growth. The lowest concentration of a compound, at which the color was changed, was considered as MIC value [13,15].

MIC measurements with the fungi were performed by the same methodology. However, the sabouraud dextrose agar (SDA) was used as a medium in this procedure. Moreover, resazurin indicator was not involved. The lowest active concentration of each test sample was assessed visually and was taken as MIC value [13,15].

2.4. Hemolytic activities

Fresh heparinized human blood (3 ml) was obtained from a volunteer after consent. Then it was mixed gently, transferred into a sterile polystyrene screw-cap tube and centrifuged for 5 minutes at 850 g. The resultant supernatant mass was poured off and the viscous pellet was washed 3 times with 5 mL of sterile chilled (4 °C) isotonic phosphate-buffered saline (PBS) of pH 7.4. The washed cells were suspended in 20 mL sterile chilled PBS and counted on a hemacytometer. The blood cell suspension retained on wet ice, was diluted for each assay with sterile PBS to 7.068 × 108 cells mL^{-1}. Aliquots of 20 µL of each sample solutions were placed aseptically into 2.0 mL microfuge tubes. For each assay, 0.1% Triton-X 100 was used as a positive control (100% lysis) and PBS as a negative control (0% lysis). Aliquot of 180 µL diluted blood cell suspension was aseptically placed into each 2 mL tube and mixed gently with a wide mouth pipette tip three times. The tubes were incubated (80 revolutions per minute) at 37 °C for 35 minutes and then placed for 5 minutes on ice followed by centrifugation at 1310 g for 5 minutes. Aliquots of 100 µL of supernatant were collected, placed into a sterile 1.5 mL microfuge tube and then diluted with 900 µL chilled and sterile PBS. All the tubes were maintained on wet ice after dilution. Absorbance was then noted on a microquant at 576 nm. The experiment was performed in triplicate. Percent hemolysis was calculated by following formula [14,15]:

% hemolysis = Abs (sample absorbance)/Abs (control absorbance) × 100

3. RESULT AND DISCUSSION

The room temperature reaction between 5-amionoisophthalic acid (HL^1H_2), KOH, CS_2 and Me_3SnCl/Bu_3SnCl in a 1:2:1:3 molar ratios in methanol formed the homotrimetallic products 1 and 2. The homotrinuclear organotin(IV) product 3 was produced by stirring the iminodiacetic acid disodium salt (HL^2Na_2) hydrate with CS_2 followed by refluxing with Ph_2SnCl_2 in a 1:1:3 molar ratios in methanol. The synthesized compounds are stable in air and have sharp melting points. These have white solid crystalline form and have good

solubility in common organic solvents. The elemental analysis data (CHNS) agrees well with the proposed composition of the products; the calculated and experimental values have been reported in Experimental Section 2.2.

3.1. IR Spectroscopy

Infrared spectra were recorded in a range of 4000-250 cm^{-1} and the most important bands have been summarized in Experimental Section 2.2.

A broad band at 3671 cm^{-1} in the free amino acid precursor (HL^1H_2) was assigned to υOH stretching vibrations, along with the stronger hydrogen bonding [15]. This band was disappeared in the coordination products (1-2) due to deprotonation of the carboxylic acid groups for their coordination with the metal. The carbonyl stretching frequencies, $\upsilon COO_{(asym)}$ of the free ligands HL^1H_2 (1718 cm^{-1}) and HL^2Na_2 (1659 cm^{-1}) were shifted to a lower wave number in the complexes 1-2 (1582-1600 cm^{-1}) and product 3 (1646 cm^{-1}) which is ascribed to COO-Sn interaction according to literature [16]. The value of $\Delta\upsilon = \upsilon COO_{(asym)}-\upsilon COO_{(sym)}$ is varied depending upon the coordination mode of carboxylate group; the Δυ value of 215-239 cm^{-1} in products 1-3 evidently supports a bridging coordination mode of the COO^- group [17]. Δυ value in the complex 3 (215 cm^{-1}) is relatively small compared to the products 1-2 (223-239 cm^{-1}) demonstrating the role of ligand and coordinated organotin(IV) moieties in elucidating the coordination modes of the carboxylate group.υNH bands occur at 3266 cm^{-1} and 3285 cm^{-1} in the free ligands HL^1H_2 and HL^2Na_2, respectively; both these bands disappeared in the products 1-3 due to insertion of CS_2 into amino moieties.

The observed υ(C-N) vibrations (1538-1587 cm^{-1}) in products 1-3 are almost intermediate between the ranges of 1250–1360 cm^{-1} (for C-N single bond) and 1640–1690 cm^{-1} (for C=N double bond). It suggests that the C-N bond in the complexes has some partial double-bond character which would result in some partial double bond character in the C-S bonds and in a bidentate linkage of the dithiocarboxylate group to the metal [18]. The bidentate coordination behavior is also verified by the existence of medium strength solitary C–S vibrations at 1007-1022 cm^{-1} [19]. So a penta-coordinated environment around each of the oxygen and sulfur bonded tin in the solid state was suggested. Two medium to sharp intensity Sn-C bands were observed at 589-591 cm^{-1} & 552-555 cm^{-1} (products 1-2) and 253 & 236 cm^{-1} (product 3) due to two kinds of organotin(IV) moieties (oxygen and sulfur bond) in each complex. The Sn-O bond absorbed at 438-444 cm^{-1} and Sn-S at 361-396 cm^{-1}. Chlorodiphenyltin(IV) derivative 3 produced a medium intensity band at 307 cm^{-1} representing Sn-Cl bond.

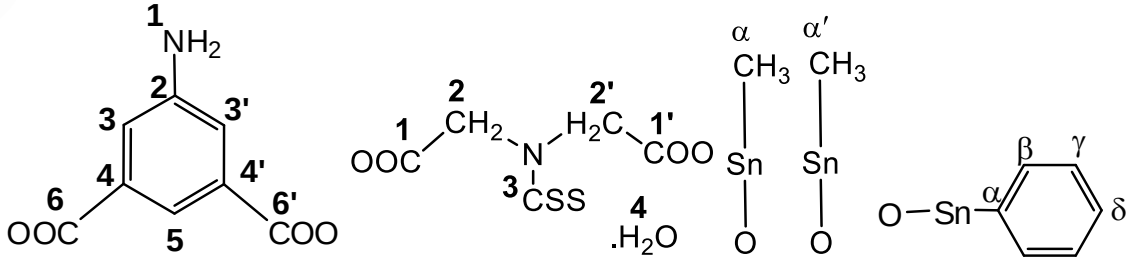
The free ligand HL^2Na_2 exhibited a broad band at 3439 cm^{-1} for υ_a(OH) and υ_s(OH) vibrations while bands at 897 cm^{-1} and 691 cm^{-1} were assigned to rocking and wagging modes of incorporated water.

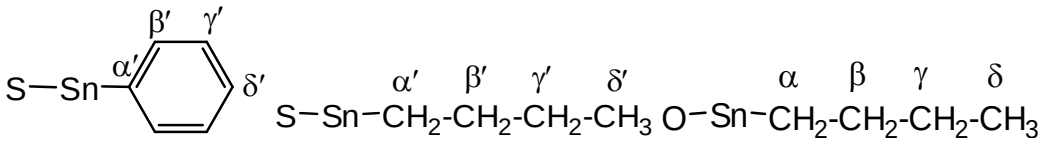
3.2 ^{1}H NMR Spectroscopy

The ^{1}H NMR spectra of the free ligand HL^2Na_2 and the complexes 1-3 were recorded in deuterated water and DMSO, respectively at room temperature. The number of protons found by integration of peaks in the spectra agreed very well with those calculated from the expected composition. The spectroscopic data are reported in Experimental Section 2.2.

The ^{1}H NMR spectra of the products 1-3 displayed no –SH signal indicating coordinated dithiocarbamate moieties. The methylene protons of the ligand precursor HL^2Na_2 exhibited a downfield shift from 3.03 ppm (singlet) to 3.24-3.72 ppm (multiplet) in the consequent complex 3. It means that the $–CH_2-$ protons were deshielded due to flow of electronic charge density from carboxylate ion towards the metal [20].The oxygen and sulfur bound trimethyltin(IV) moieties appeared as singlets at 3.18 ppm and 0.19 ppm, respectively. The C-Sn-C bond angle (109.2^0) calculated [21] from $^2J(^{119/117}Sn, ^1H)$ value of 52 Hz clearly demonstrates a tetrahedral geometry of Sn(IV) ion in solution state of compound 1.

Despite the complex pattern of tri-*n*-butyl fragments in complex 2, a clear triplet due to terminal methyl group was appeared at 0.85 ppm with $^3J(^1H, ^1H)$ = 7.2 Hz. Ortho protons absorbed downfield as compared to meta and para protons in phenyltin(IV) derivative 3 [22]. A singlet resonance at 4.70 ppm confirmed the presence of water in the free ligand (HL^2Na_2); however, this signal was disappeared in the product 3; these findings thus completely support the evidences concluded from IR spectroscopy.





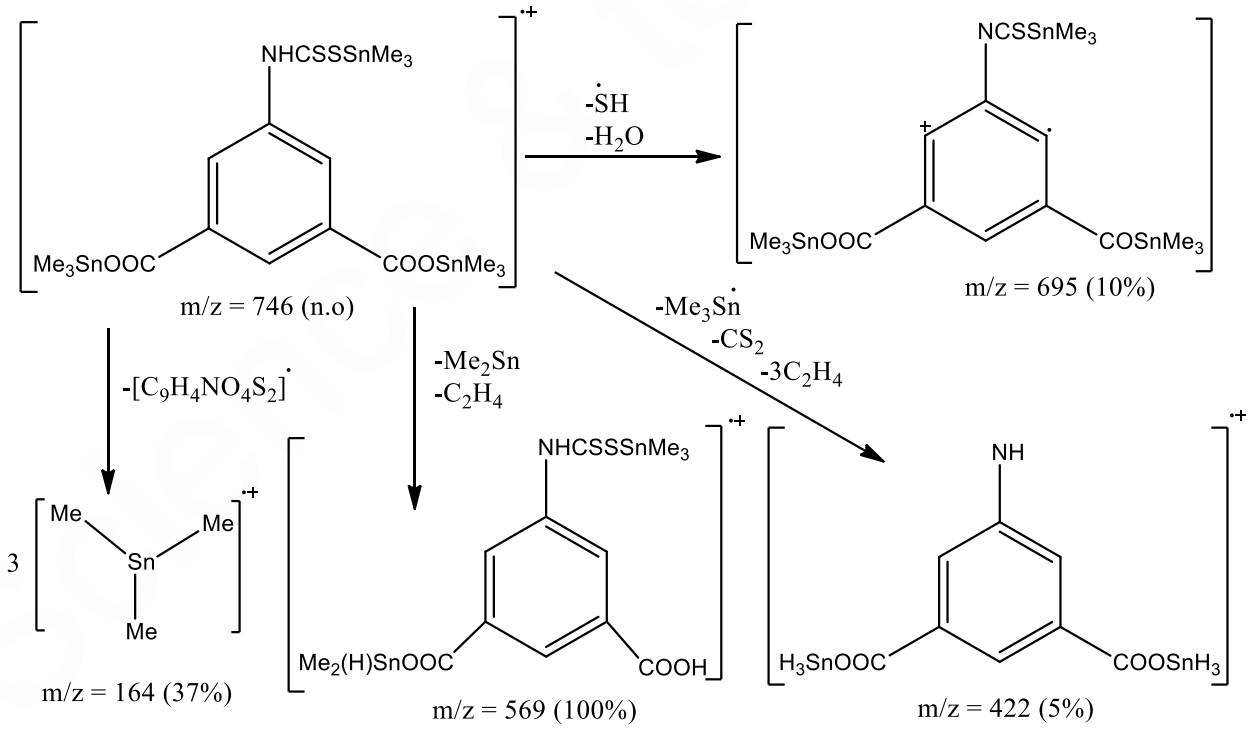
Scheme 3 NMR numbering scheme of the ligand part and organotin(IV) moieties in the complexes

3.3 ^{13}C NMR Spectroscopy

The ^{13}C NMR spectroscopy displayed the expected signals of the ligand constituent and Sn–R skeletons of the complexes. The spectroscopic data have been reported in Experimental Section 2.2.There was a significant upfield shift of the –COO^- signal from δ = 179.3 ppm in the free ligand (HL^2Na_2) to δ = 169.1-170.2 ppm in the complex 3; this shift evidently supports tin-carboxylate coordination. In product 2, the carboxylate signal occurred at still higher field (167.7 ppm) due to more electron withdrawing nature of its ligand skeleton (5-aminoisophthalte ion). The methylenic carbon(51.9) of the free ligand (HL^2Na_2) was appeared downfield in the complex 3 producing two signals at 52.6 and 53.3ppm; this shift may be rendered to the flow of electronic charge from adjacent dithiocarbamate moiety towards tin(IV) ion.

3.4 Mass spectrometry

The electron ionization mass spectrum (EI-MS) (Figure 1) was recorded for the synthesized complex **1**; the most important fragment ions have been shown in Scheme 4. As the tin has ten and palladium has nine naturally occurring isotopes, each metal ion appeared in the mass spectrum as a series of peaks close to each other due to isotopic effects. The fragment ions completely verify the molecular skeleton of the complex. However, the spectrum displayed no molecular ion ($M^{+\bullet}$) peak; the data thus supports the earlier reports [15,23] thatin the mass spectra of the organotin(IV) compounds, the molecular ion peak [M+•] is generally not observed or it is of very low intensity.



Scheme 4 The mass fragmentation pattern of complex 1

3.5. Antimicrobial activities

The free ligands and the synthesized products were screened to see their *in vitro* response against bacterial (*Escherichia coli, Bacillus subtilis, Staphlococcus aureus* and *Pasturella multocida*) and fungal (*Alternaria alternata, Ganoderma lucidum, Penicillium notatum,Trichoderma harzianum* and *Aspergillus niger*) strains. The activities were carried out by disc diffusion method [12] and

minimum inhibitory concentration (MIC) [13] evaluations. Fluconazole and streptomycin were used as the standard drugs for antifungal and antibacterial screening tests respectively. The solutions of the test samples in DMSO and those of reference drugs in water (concentration = 1mg/1ml) were introduced into the small paper discs or wells. The zones of inhibition of discs were measured in millimeters while the wells exhibiting MICs were noted visually while. The data have been summarized in Tables 1-4.

The free ligands (HL^1H_2 &HL^2Na_2) were found biologically inactive against all the tested bacterial as well as fungal strains. However, the trinuclear coordination (Sn-O, Sn-O, Sn-S) of the metal with oxygen and sulfur donor sites of the ligands has induced significant antifungal/antibacterial activities in the consequent complexes. The increased activity in going from the ligands to the complexes 1-3 may be rendered to the coordination and polarity of tin(IV) atom with the donor sites of a ligand [24]. Some products exhibited their antibacterial/antifungal potential very close to the standard drugs (streptomycin and fluconazole); Such a high potential may be attributed to the multinuclear coordination in the complexes. The synthesized complexes show strong differences in their activities and MIC values depending upon the nature of substituent present on the Sn metal and the nature of ligand. Comparison of activities of both the complexes (1&2) with HL^1H_2clarifies that the tributyltin(IV) derivative 2 is a most potent inhibitor against all the fungal strains except *Trichoderma harzianum* while complex 1 is best active against all the tested bacterial strains except *Pasturella multocida*. Complex 3 exhibited an intermediate type of inhibition in most cases. The ligand supports transport of the organotin moiety to the site of action where it is released by hydrolysis [25].

Table 1 Antifungal activity data[a]

Comp. No.	*A. alternata*	*G. lucidum*	*P. notatum*	*T. harzianum*	*A. niger*
HL^1H_2	-	-	-	-	-
HL^2Na_2	-	-	-	-	-
1	$22^{c}\pm0.11$	$32^{bc}\pm0.21$	$20^{c}\pm0.15$	$48^{a}\pm0.36$	$19^{c}\pm0.12$
2	$40^{a}\pm0.29$	$38^{ab}\pm0.25$	$30^{bc}\pm0.22$	$29^{c}\pm0.27$	$28^{b}\pm0.21$
3	$26^{bc}\pm0.17$	$25^{c}\pm0.19$	$29^{bc}\pm0.23$	-	$22^{bc}\pm0.16$
Fluconazole	$38^{ab}\pm0.29$	$41^{a}\pm0.21$	$45^{a}\pm0.31$	-	$37^{a}\pm0.24$

[a]Concentration of complex (in DMSO) or that of a ligand/standard drug (in water) = 1mg/ml in a solvent; Data are expressed as the mean ± standard deviation of samples analyzed individually in triplicate at $p<0.1$; Values having same letters in superscripts do not differ significantly; 0 = No activity, 5-10 = Activity present, 11-25 = Moderate activity, 26-40 = Strong activity

Table 2 Antibacterial activity data[a]

Comp. No.	Bacterial inhibition zone (mm)			
	E. coli	*B. subtilis*	*S. aureus*	*P. multocida*
HL^1H_2	-	-	-	-
HL^2Na_2	-	-	-	-
1	$28^{b}\pm0.23$	$30^{ab}\pm0.25$	$27^{ab}\pm0.19$	$15^{c}\pm0.08$
2	$15^{c}\pm0.11$	$20^{c}\pm0.15$	$26^{b}\pm0.17$	$22^{b}\pm0.14$
3	$40^{a}\pm0.27$	$22^{bc}\pm0.20$	$22^{c}\pm0.19$	$20^{bc}\pm0.16$
Streptomycin	$30^{ab}\pm0.17$	$31^{a}\pm0.28$	$31^{a}\pm0.31$	$29^{a}\pm0.28$

[a]Concentration of complex (in DMSO) or that of a ligand/standard drug (in water) = 1mg/ml in a solvent; Data are expressed as the mean ± standard deviation of samples analyzed individually in triplicate at p<0.1; Values having same letters in superscripts do not differ significantly; 0 = No activity, 5-10 = Activity present, 11-25 = Moderate activity, 26-40 = Strong activity

Table 3 MIC (fungal) data[a] (mg/well)

Comp. No.	*A. alternata*	*G. lucidum*	*P. notatum*	*T. harzianum*	*A. niger*
HL^1H_2	-	-	-	-	-
HL^2Na_2	-	-	-	-	-
1	3.12	1.56	$3.90x10^{-1}$	$>2.44x10^{-2}$	1.56
2	$4.88x10^{-2}$	$1.95x10^{-1}$	$4.88x10^{-2}$	$1.95x10^{-1}$	$1.95x10^{-1}$
3	$1.95x10^{-1}$	3.12	$4.88x10^{-2}$	-	1.56
Fluconazole	$9.76x10^{-2}$	$4.88x10^{-2}$	$>2.44x10^{-2}$	-	$9.76x10^{-2}$

[a]Concentration of a complex = $1mgmL^{-1}$ in DMSO

Table 4 MIC (bacterial) data[a] (mg/well)

Comp. No.	*E. coli*	*B. subtilis*	*S. aureus*	*P. multocida*
HL^1H_2	-	-	-	-
HL^2Na_2	-	-	-	-
1	$9.7x10^{-2}$	$3.9\ x10^{-1}$	$1.95x10^{-1}$	25
2	12.5	12.5	$3.9\ x10^{-1}$	1.56
3	$4.88x10^{-2}$	3.12	1.56	3.12
Streptomycin	$9.7x10^{-2}$	$1.95x10^{-1}$	$1.95x10^{-1}$	$3.9\ x10^{-1}$

[a]Concentration of a complex = $1mgmL^{-1}$ in DMSO

3.6. Hemolytic activity

Hemolytic activity studies were performed to see the possible toxic hemolytic effects of the novel complexes. The literature [26] shows that even if a novel compound possesses strong antimicrobial activities, its use in medicine is prohibited in the presence of hemolytic effects. Thus *in vitro* hemolytic bioassays of the complexes 1-3 and their ligand precursors (HL^1H_2 &HL^2Na_2) were performed with human red blood cells [14,15] and the average lysis was reported with respect to the triton X-100 as a positive control (100% lysis) and PBS as a negative control (0% lysis). The data has been reported in Table 5.

The complexes possessed their hemolytic activities which were sufficiently lower as compared to triton X-100 and higher than PBS. The highest hemolytic activity was displayed by complex 1(33.06%) while the complex 3 exhibited the lowest hemolytic effects (6.92%) demonstrating its possible safe use in medicine. All other compounds presented their hemolytic effects between this range of minimum and maximum values. The activity of complex 3 was found even less compared to its ligand precursor HL^2Na_2. The compound showing the highest activity may be considered for antitumor activity [27].

Table 5 Hemolytic activities of complexes

Comp. No.	HL^1H_2	HL^2Na_2	Triton-X 100	PBS
% of Hemolysis	14.72±0.05	7.36±0.07	99.53 ±0.00	0.00 ±0.00
Comp. No.	**DMSO**	**1**	**2**	**3**
% of Hemolysis	9.71±0.05	33.06±0.04	13.43±0.01	6.92±0.05

4. CONCLUSION

Trinuclear organotin(IV) dicarboxylates dithiocarbamates, can be synthesized by stirring/refluxing the dipotassium/disodium salt of an amino dicarboxylate ligand precursor with CS_2 and $Me_3SnCl/Bu_3SnCl/Ph_2SnCl_2$ in a 1:1:3 molar ratios. There is a chelating mode of the dithiocarbamate moiety and a bridging coordination behavior of the carboxylate groups for binding with tin(IV). The complexes change their solid state trigonal bipyramdial geometry to tetrahedral in solution state. The methylene protons of HL^2Na_2 ion are significantly de-shielded upon NCSS-Sn coordination in complex 3. The synthesized complexes show strong differences in their activities and MIC values depending upon the substituent present on the Sn metal and the nature of ligand. The complex 1 displays the highest hemolytic activity (33.06%), while the complex 3 has the lowest hemolytic effects (6.92%) demonstrating the possible safe use of the later in medicine.

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