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Mass spectroscopic and biological activity investigations of bis(triorganotin(IV)) carboxylates with acetylene dicarboxylic acid

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Three bis(trioganotin(IV)) dicarboxylates of the general formulae $R_3SnOOCLCOOSnR_3$ (Where L = C≡C; R = Me (**1**), Bu (**2**), Ph (**3**)) were subjected to mass spectroscopic and biological activity studies. The investigated complexes demonstrated the common mass fragmentation modes due to their structural similarities. Each product was primarily degraded into a trialkyl/triaryltin(IV) cation. The free ligand precursor was biologically inactive against all the tested microbes while the product complexes **1**-**3** were proved to be potent inhibitors of bacteria and fungi. The complexes were also screened for their minimum inhibitory concentration (MIC) evaluation. *In vitro* hemolytic activity studies were also performed on human red blood cells. The organotin(IV) complexes displayed lower cytotoxic effects (12.98-18.85%) as compared to the free ligand (19.68%). The triphenyltin(IV) product **3** showed interaction with salmon sperm DNA and was found to be the most active antimicrobial agent having the lowest hemolytic effects. Its antibacterial/antifungal potential was even higher than that of the standard antimicrobial drugs streptomycin and fluconazole in some cases.

Key words: EIMS, salmon sperm, antibacterial, antifungal, hemolytic

INTRODUCTION

Infectious diseases are the major causes of disability, morbidity and mortality in the world. The death rate of humans due to the infectious diseases is in millions especially in developing countries [1]. The control of bacterial infections has been remarkably effective since the discovery of antimicrobial drugs. However, some of the pathogens are rapidly becoming resistant to many of the first discovered and available drugs. The increasing prevalence of multidrug resistance in pathogenic microorganisms, as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs [2]. In recent years there are numerous studies on organotin(IV) complexes to evaluate their biological potential against fungal and bacterial strains [3]. They are toxic against a variety of microorganisms and are used as active components in various biocidal formulations, finding applications in such diverse areas as fungicides, miticides, mollucides, marine antifouling paints, surface disinfectants, and wood preservatives [4,5]. A large number of organotin compounds are used as pharmaceuticals and pesticides, the activities mainly depends upon the number and nature of organic groups [6,7] as well as the structure of the molecule and coordination number of tin moiety [8,9]. Furthermore the activity will decreases from tetra alkyl substituted tin(IV) compounds to mono alkyl substituted tin(IV) compounds [10]. The biological activity is usually associated with the nature of organic ligand, since the organic ligand assists in the transportation of the complexes across the cell membrane [11]. Several organotin compounds exhibit promising *in vitro* antitumor activities against human tumor cell lines [12]. Currently attempts have been made to search for organometallic compounds as a new alternative drug in combating human cancers. Some organotin derivatives are reported to be even more active than the standard anticancer drug *cis*-platin [13]. It has well been established that organotin(IV) compounds are very important in cancer chemotherapy because of their apoptosis inducing character and received considerable attention as antiproliferative and anticancer drugs [11]. There are also reports on the use of organotin carboxylates as potential antineoplastic and antituberculosis agents [14].

The current study focuses on the mass spectroscopic investigations of bis(trioganotin(IV)) complexes with acetylene dicarboxylic acid. The products were also tested for their DNA binding, antibacterial and antifungal activities. These were also investigated to find their toxic hemolytic effects.

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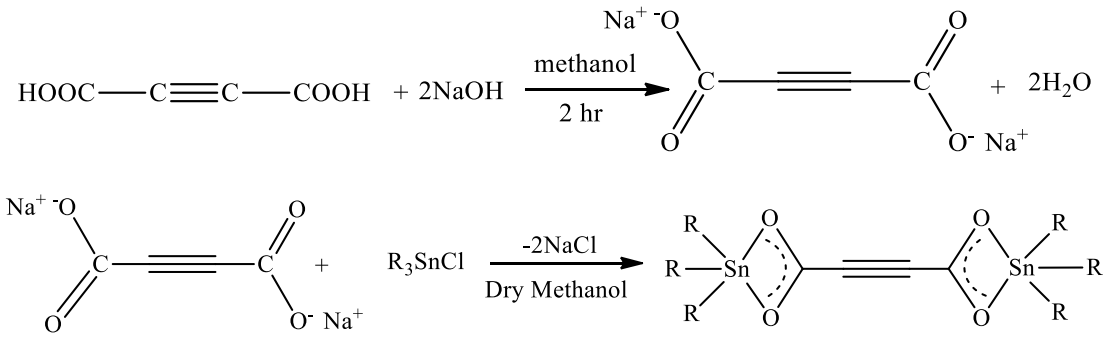
EXPERIMENTAL

Materials and Methods

Triorganotin chloride precursors (Me_3SnCl, Bu_3SnCl & Ph_3SnCl) and sodium hydroxide (NaOH) were purchased from Aldrich (Germany). Acetylene dicarboxylic acid (HLH) and solvents (methanol, petroleum ether & DMSO) were of Merck (Germany) origin. The methanol was dried before use by a standard procedure [15]. Melting points were noted by taking the compound in a capillary tube and using the electrochemical melting point apparatus model MP-D Mitamura Rikero Kogyo (Japan) and are uncorrected. Elemental analyses were performed on the CHN-932 elemental analyzer Leco Corporation USA. Infrared spectra were recorded in the range of 4000-400 cm^{-1} on a Perkin-Elmer-1000 FTIR spectrophotometer. The ^1H and ^{13}C NMR spectral measurements were performed on a Bruker ARC 300 MHz-FT-NMR spectrometer. The electron ionization mass spectra (EIMS) were recorded using a Thermo Fisher Exactive Orbitrap instrument.

The complexes were investigated for their interaction with salmon sperm DNA [16,17]. The pure pathogenic (bacterial and fungal) cultures were obtained from the Biochemistry Department of the University of Agriculture, Faisalabad. The origin and identification of these microorganisms is given below: *E. coli* (ATCC 25922), *B. subtilis* (JS-2004); *S. aureus* (API Staph TAC-6736152); *P. multocida* (Local isolate); *A. alternate* (ATCC-19575); *G. lucidum* (Local isolate); *P. notatum* (FCBP-PTF0111); *T. harzianum* (E-58).

The ligand and the products were compared for their antimicrobial activities against various bacterial (*Escherichia coli, Bacillus subtilis, Staphilocuccus aureus* and *Pasturella multocida*) and fungal (*Alternaria alternata, Ganoderma lucidum, Penicillium notatum, Trichoderma harzianum* and *Aspergillus niger*) strains by the disc diffusion method [18]. The complexes were also evaluated for their minimum inhibitory concentrations (MIC) [19]. Fluconazole and streptomycin were used as standard drugs for antibacterial and antifungal screening tests, respectively. The *in vitro* hemolytic bioassays [20] of the products were investigated with respect to Triton X-100 as a positive control and PBS as a negative control. The complexes **1**-**3** were synthesized by using a two-step reported procedure (Scheme 1) [21].



Scheme 1. Synthesis of complexes **1**-**3** where R = Me (**1**), *n*-Bu (**2**), Ph (**3**)

RESULTS AND DISCUSSION

The products have shown sharp melting points and are soluble in common organic solvents, i.e., DMSO, MeOH and $CHCl_3$. The synthesized products were characterized by microanalysis, IR and NMR (^1H &^{13}C) and EIMS.

IR and NMR Spectroscopy

The data obtained by IR, ^1H NMR and ^{13}C NMR spectroscopic analyses were found identical to those reported elsewhere [21] for the same complexes.

Mass Spectrometry

The electron ionization mass spectra (EI-MS) were recorded for the investigated complexes. The fragment ions are shown in Table 1. The degradation pattern of the products is shown in Scheme 2. The mass spectroscopic patterns verified the structural similarities between the three products **1**-**3**. The EI-mass spectrum of a representative complex **3** is shown in Figure 1.

Table 1. Mass spectral data[#] of the complexes

Compound No.	MS, m/z (%)
1	$[C_{10}H_{18}O_4Sn_2]^+$ 442 (n.o.)[#], $[C_{10}H_{18}O_3Sn_2]^+$ 423 (5), $[C_9H_{15}OSn_2]^+$ 379 (25), $[C_6H_9OSn_2]^+$ 337 (100), $[C_5H_5Sn_2]^+$ 305 (16), $[C_7H_9O_4Sn]^+$ 277 (10), $[C_6H_6O_4Sn]^+$ 262 (1), $[C_4O_4Sn]^+$ 232 (2), $[C_7H_9OSn]^+$ 229 (4), $[C_5H_6O_2Sn]^+$ 218 (12), $[C_3H_9Sn]^+$ 165 (68), $[C_2H_6Sn]^+$ 150 (21), $[CH_3Sn]^+$ 135 (61), $[Sn]^+$ 120 (35), $[C_4H_2O_2]^+$ 82 (10).
2	$[C_{40}H_{30}O_4Sn_2]^+$ 814 (n.o.)[#], $[C_{22}H_{15}O_4Sn]^+$ 463 (1), $[C_{16}H_{10}O_4Sn]^+$ 386 (3), $[C_{18}H_{15}Sn]^+$ 351 (4), $[C_{10}H_5O_4Sn]^+$ 309 (100), $[C_{12}H_{10}Sn]^+$ 273 (2), $[C_4O_4Sn]^+$ 231 (7), $[C_6H_5Sn]^+$ 197 (16), $[C_{12}H_{10}]^+$ 154 (86), $[Sn]^+$ 120 (5), $[C_6H_5]^+$ 77 (12), $[C_4H_3]^+$ 51(7).
3	$[C_{28}H_{54}O_4Sn_2]^+$ 694 (n.o.)[#], $[C_{16}H_{27}O_4Sn]^+$ 403 (2), $[C_{12}H_{18}O_4Sn]^+$ 346 (18), $[C_{12}H_{27}Sn]^+$ 291 (100), $[C_8H_{18}Sn]^+$ 234 (44), $[C_4O_4Sn]^+$ 232 (43), $[C_4H_9Sn]^+$ 177 (19), $[Sn]^+$ 120 (7), $[C_4H_9]^+$ 57 (81)

[#] Molecular ion peak (M^+); n.o. = not observed.

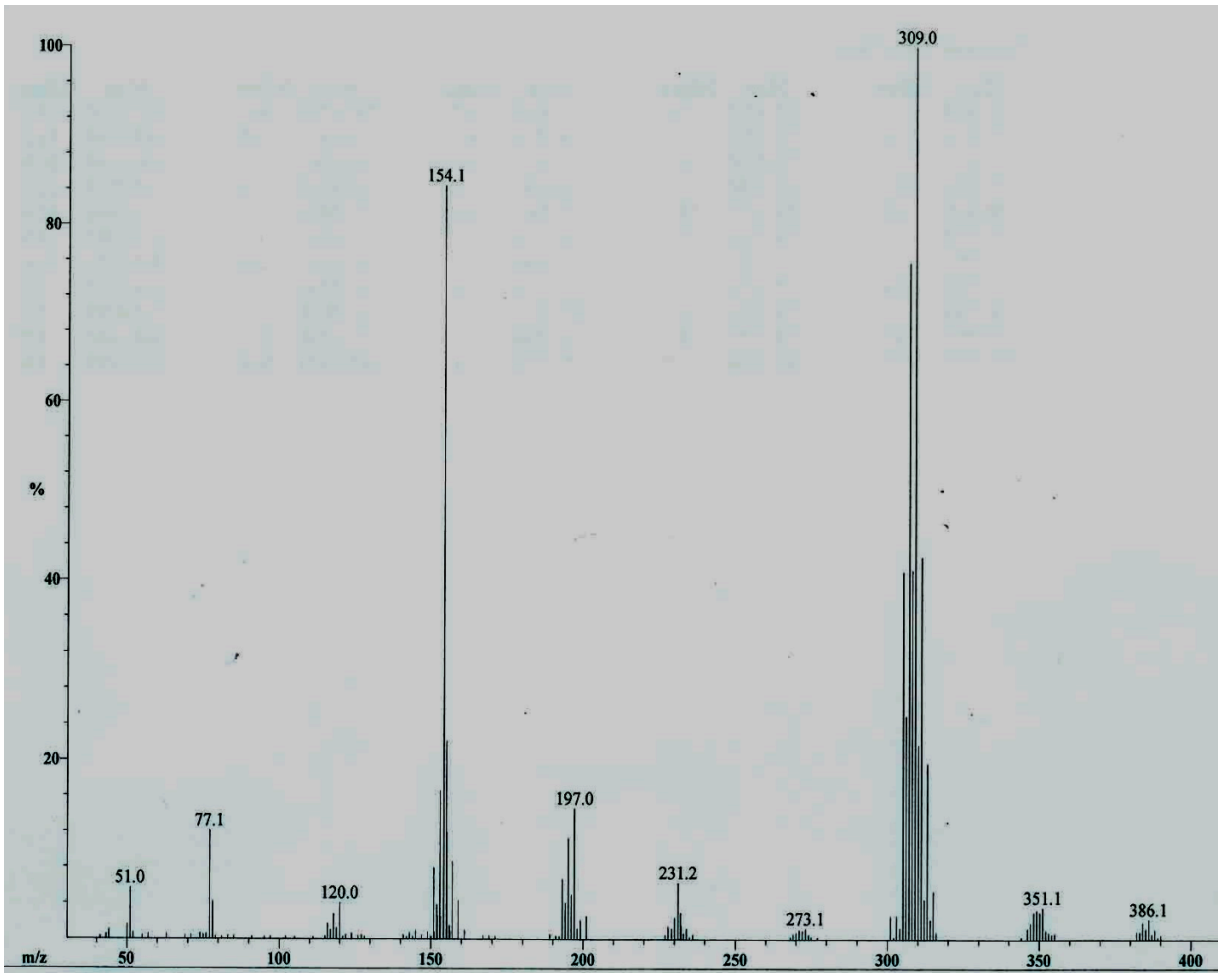


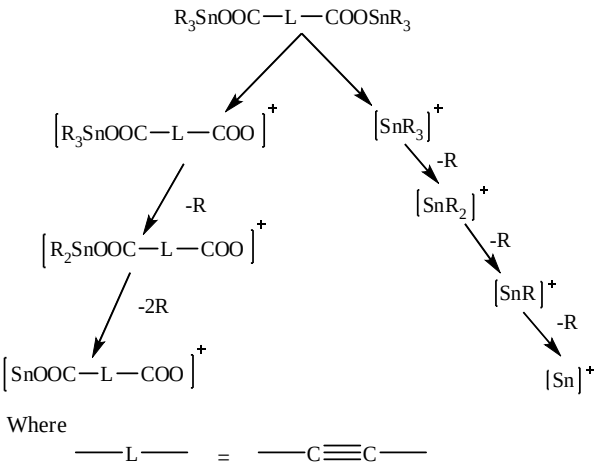
Figure 1. EI-mass spectrum of complex **3**

Mass-spectral data showed the rich distribution of ions but the fragments containing the tin are particularly important in structural characterization of organotin(IV) complexes. Such fragments are easily identified from the characteristic isotopic peak patterns for tin. The mass spectra displayed a series of peaks very close to each other due to isotopic effects of Sn, i.e., tin has 10 naturally occurring isotopes. The most intense peak in each group of ions corresponds to the most abundant isotope of tin (Sn-120). The spectra showed no molecular ion ($M^{+\bullet}$) peak; the absence of molecular ion peak in the mass spectra is a common observation in most organometallic complexes

[22].Owing to their large sizes, the coordination products underwent an extensive fragmentation so a complex mass spectral pattern was observed. The metal complexes have shown many common features (Scheme 2) in their fragmentation patterns, due to the close structural similarities between the products. Each of these bimetallic complexes was split into a triorganotin cation (R_3Sn^+) and $[R_3SnOOCLCOO]^+$ (where R = Me, Bu, Ph; L = CC); these fragment ions lost the methyl, butyl or phenyl radicals and degraded into $[Sn]^+$ and $[SnOOCLCOO^+]$ ions in the former and the latter case, respectively.

DNA Interaction Studies

DNA is generally considered as the target agent for most of the anticancer compounds. So the investigated complexes **1**-**3** were also studied for their binding with DNA using absorption spectroscopy in the wavelength range of 250-400 cm^{-1}.



Scheme 2. Common EIMS pattern of **1** (R = Me), **2** (R = Ph) and **3** (R = Bu)

The absorbance and shifts were noted in the presence and absence of SS-DNA. Each measurement was performed at the same concentration of the complex (2 mM) while varying the concentration of DNA (Figure 2) [14].

It was found that the alkyltin(IV) derivatives **1** and **2** did not show any affinity for DNA; only the triphenyltin(IV) derivative **3** expressed its binding with DNA. These results are in complete agreement with the earlier findings [23, 24] that only the phenyltin(IV) complexes exhibit interaction with DNA. Actually, the phenyl group in an orgnotin(IV) species facilitates its interaction with the double stranded DNA [14].

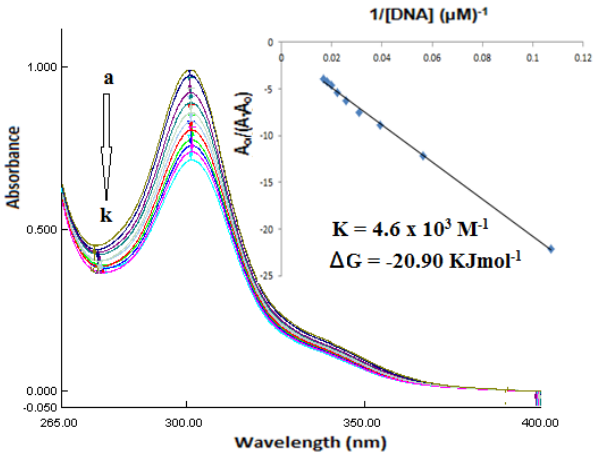


Figure 2. Absorption spectra of the complex **3** (2 mM) in the absence (a) and presence of 10 μM (b), 19 μM (c), 27 μM (d), 35 μM (e), 42 μM (f), 48 μM (g), 54 μM (h), 59 μM (i), 64 μM (j) and 69 μM (k) DNA. The direction of the arrow demonstrates increasing concentrations of DNA. Inset graph is the plot of $A_o/(A-A_o)$ *vs.* 1/[DNA] to find the Gibbs free energy and the binding constant of the DNA-complex adduct.

The pure complex (2 mM) displayed very strong absorption at 302 nm which may be attributed to the π-π* transitions of the phenyl ring. After the addition of DNA and then increasing its concentration during each successive measurement, the absorption was lowered (hypsochromism) with a minor red shift (Fig. 1). The extent of hypsochromism demonstrates the strength of intercalative binding between complex and DNA [14, 23]. After intercalation, the π* orbital of the intercalated ligand could couple with the π orbital of the base pairs of DNA, which results in a decrease of the π-π* transition energy. The bathochromism (red shift) may be observed when the DNA duplex is stabilized [25].

After 24 h, the experiment was repeated to produce again the visible spectra which displayed the identical results thus verifying the stability of the drug-DNA adduct.

The intrinsic binding constant 'K' was calculated for the investigated DNA active product **3** by using Benesi-Hildebrand equation 1 [26]:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_0} + \frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_G} \times \frac{1}{K[DNA]} \quad \text{(Eqn. 1)}$$

where A = absorbance of the drug and its adduct with DNA; A_0 = absorbance of the drug; $\varepsilon_{H\text{-}G}$ = absorption coefficient of the drug–DNA adduct; ε_G = absorption coefficient of the drug.

The association constant was obtained from the intercept-to-slope ratios of $A_0/(A\text{-}A_0)$ *vs.* 1/[DNA] plot. The binding constant (K) was found to be 4.6 × 10^3 M^{-1}.

The Gibbs free energy (ΔG) was calculated by using equation 2:

$$\Delta G = -RT \ln K \qquad \text{(Eqn. 2)}$$

where T is the temperature (298 K) and *R* is general gas constant (8.314 J $K^{-1}mol^{-1}$). The Gibbs free energy was found to be −20.9 kJ mol^{-1}. The negative value of ΔG suggests that the interaction of the compounds with DNA is a spontaneous process.

Biological Activity Studies

The free ligand and the investigated bis(trioganotin(IV)) dicarboxylates were evaluated for their antimicrobial activities by the disc diffusion method [18]. The samples were tested against various strains of fungi (*Alternaria alternata, Ganoderma lucidum, Penicillium notatum* and *Trichoderma harzianum*) and bacteria (*Escherichia coli, Bacillus subtilis, Staphlocuccus aureu s*and *Pasturella multocida*). Fluconazole and streptomycin were used as standard drugs for antifungal and antibacterial screening tests. One mg of a test sample was dissolved in 1 ml of the solvent (water for the ligand/reference drug and DMSO for the complex) and 100 μL of this solution was applied to soak 9 mm filter paper discs. The zones of inhibition of the discs were measured in mm. The compounds were also tested to find their minimum inhibitory concentration (MIC) using a standard procedure [19]. The wells exhibiting MICs were visually noted. The data are summarized in Tables 2-5.

The organotin(IV) products were found to be biologically active against the tested microbes. The coordination of the ligand with trialkyltin(IV) or triphenyltin(IV) moieties has appreciably induced antimicrobial activities in the product complexes **1-3**. Thus, the homobimetallic complexes of tin displayed considerably higher inhibitory effects as compared to the free ligand HLH. The antifungal/antibacterial activities of some complexes were even higher than those of the standard drugs (Tables 2 and 3). The minimum inhibitory concentration values of the complexes are given in Tables 4 and 5. The biological activities varied according to the nature of the substituent at tin [27]. The inhibitory action of organotin(IV) compounds is mainly due to their ability to interact with DNA and proteins. They can also damage mitochondria, thus causing the death of microorganisms [28].

Hemolytic Activities of Complexes

The disodium salt of the free ligand (**Na_2L**) and the complexes **1-3** were evaluated for their toxic hemolytic effects by a reported procedure [20]. Human red blood cells were obtained from volunteers and the average lysis was reported with respect to Triton X-100 and PBS as a positive (100% lysis) and PBS as negative (0% lysis) controls, respectively. The results obtained are summarized in Table 6.

Table 2. Antibacterial activity data[a] of the ligand (HLH) and the complexes 1-3

Compound No.	Bacterial inhibition zone (mm)			
	E. coli	*B. subtilis*	*S. aureus*	*P. multocida*
HLH	--	--	--	-
1	$30^{a}\pm0.14$	$26^{ab}\pm0.37$	$30^{c}\pm0.28$	$22^{c}\pm0.21$
2	$30^{a}\pm0.19$	$22^{bc}\pm0.32$	$32^{a}\pm0.14$	$30^{ab}\pm0.46$
3	$28^{c}\pm0.28$	$18^{c}\pm0.51$	$31^{b}\pm0.42$	$34^{a}\pm0.38$
Streptomycin	$30^{a}\pm0.17$	$31^{a}\pm0.28$	$31^{b}\pm0.31$	$29^{ab}\pm0.28$

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

Table 3. Antifungal activity data[a] of the ligand (HLH) and the complexes 1-3

Compound No.	*A. alternata*	*G. lucidum*	*P. notatum*	*T. harzianum*
HLH	-	-	-	$20^{c}\pm0.19$
1	$41^{ab}\pm0.21$	$28^{bc}\pm0.13$	$26^{c}\pm0.11$	$24^{bc}\pm0.14$
2	$30^{c}\pm0.22$	$25^{c}\pm0.12$	$30^{bc}\pm0.23$	$26^{bc}\pm0.19$
3	$42^{a}\pm0.18$	$32^{bc}\pm0.25$	$34^{bc}\pm0.12$	$33^{a}\pm0.21$
Fluconazole	$38^{ab}\pm0.29$	$41^{a}\pm0.21$	$45^{a}\pm0.31$	-

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

Table 4. MIC (bacterial) of the ligand (HLH) and the complexes 1-3 (mg/well)

Compound No.	*E. coli*	*B. subtilis*	*S. aureus*	*P. multocida*
HLH	3.12	25	25	25
1	3.90×10^{-1}	7.81×10^{-1}	3.90×10^{-1}	3.12
2	3.90×10^{-1}	6.25	3.12	6.25
3	7.81×10^{-1}	7.81×10^{-1}	3.12	6.25
Streptomycin	9.7×10^{-2}	1.95×10^{-1}	6.25	3.12

Table 5. MIC (fungal) data of the ligand (HLH) and the complexes 1-3 (mg/well)

Compound No.	*A. alternata*	*G. lucidum*	*P. notatum*	*T. harzianum*
HLH	-	-	-	6.25
1	3.90×10^{-1}	1.95×10^{-1}	1.95×10^{-1}	1.95×10^{-1}
2	3.90×10^{-1}	1.95×10^{-1}	$>2.44\times10^{-2}$	$>2.44\times10^{-2}$
3	1.95×10^{-1}	9.76×10^{-2}	1.95×10^{-1}	$>2.44\times10^{-2}$
Fluconazole	1.56	1.56	$>2.44\times10^{-2}$	25

Table 6. Hemolytic activities of the disodium salt of the ligand (Na_2L) and the complexes 1-3

Compound No.	Na_2L	1	2	3	Triton-X 100	PBS
% of Hemolysis	19.68±0.05	17.31±0.06	18.85±0.03	12.98±0.06	99.53±0.00	0.00±0.00

All the coordinated products possessed lower hemolytic activity as compared to **Na_2L**, thus coordination with the metal ion has reduced the cytotoxic hemolytic effects of the resultant species. The lowest activity (12.98%) was reported for the complex **3** and the highest value (18.85%) was recorded for the complex **2**. All other compounds presented their hemolytic activities within this range of minimum and maximum values. The synthesized complexes possessed hemolytic activities much lower as compared to Triton X-100 and much closer to PBS.

CONCLUSIONS

Bis(trioganotin(IV)) derivatives of acetylene dicarboxylic acid demonstrate the common mass fragmentation mode due to their structural similarities. The free ligand precursor was biologically inactive against all the tested microbes but a proficient antimicrobial potential was developed after its coordination with tin. The complexes were also screened for their minimum inhibitory concentration (MIC) evaluations. The *in vitro* hemolytic activity studies were also performed on human red blood cells. All the tin(IV) coordinated products displayed lower cytotoxic effects (12.98-18.85%) as compared to the free ligand (19.68%). The triphenyltin(IV) derivative **3** was unique in the sense that (i) it was the most active biological agent (ii) it showed interaction with salmon sperm DNA (iii) it possesses less toxic hemolytic effects compared to all three complexes. Its antibacterial/antifungal potential was even higher than those of the standard antimicrobial drugs streptomycin and fluconazole.

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