Article

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Synthesis, Spectroscopy, Semi-empirical and Biological Activities of Organotin(IV) Complexes with *o*-Isopropyl Carbonodithioic Acid

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New organotin(IV) complexes have been synthesized by treating potassium *o*-isopropyl carbonodithioate with R_2SnCl_2/R_3SnCl in 1:2/1:1 M/L ratio. All complexes have been characterized by IR and NMR (^1H, ^{13}C) spectroscopy. IR results shows that ligand acts as bidentate which is also confirmed by semi-empirical study. NMR data reveals four coordinated geometry in solution. Computed positive heat of formation shows that complex **5** is thermodynamically unstable. UV/visible spectroscopy was used to assess the mode of interaction and binding of the complexes with DNA which shows that complex **5** exhibits higher binding constant as compared to complex **3**. In protein kinase inhibition assay, compound **3** was found most active, while other biological activities shows that triorganotin(IV) complexes are biologically more active as compared to diorganotin(IV) complexes.

Keywords: *o*-Isopropyl carbonodithioic acid; Organotin(IV); IR; NMR; Semi-empirical; Enzyme inhibition study; DNA interaction; Antifungal; Antibacterial; Antileishmanial; Cytotoxicity.

INTRODUCTION

The coordination chemistry of sulfur-donor ligands is an area of interest due to their close similarity with biomolecules like amino acids (e.g., methionine, cysteine), peptides such as glutathione, proteins, enzymes and vitamins.[1] The use of sulfur donor ligands, such as dithiophosphates, dithioxanthates, or dithiocarboxylates in triorganotin and diorganotin compounds can be useful to mimic the active sites of some metallic enzymes. This method is sometimes simpler than actually using biological ligands such as peptides or amino acids.[2–4]

Extensive structural analyses were performed by Tiekink and Haiduc[5] which showed that these ligands can coordinate to metal atoms in a monodentate, isobidentate, or anisobidentate fashion. Metal xanthates are extensively used as pesticides[6], corrosion inhibitors,[7] agricultural reagents[8] and quite recently in therapy for HIV infections.[9] Organotin compounds as well as dithiocarbamates ligands[10] are known for their antifungal,[11] antibacterial,[12] and antitumor[13–16] activities. Protein kinases are responsible for the phosphorylation of various proteins, act as regulatory agents in several metabolic pathways.[17]

In continuation of our previous work,[18-21] we have synthesized organotin(IV) complexes with *o*-isopropyl carbonodithioic acid. These complexes were characterized by different spectral techniques and semi-empirical study was also done. The complexes were also screened to check various biological activities.

RESULTS AND DISCUSSION

The synthesized organotin(IV) complexes are solid having sharp melting points and are soluble in common organic solvents. The physical data is given in Table 1.

Infrared spectroscopy

The IR spectrum of the free ligand was compared with the spectra of tri- and diorganotin(IV) complexes to understand the binding mode of the dithiocarbonate ligand. Absence of SH band in the spectra of the complexes in the region 2710 cm^{-1} indicate the deprotonation of carbonodithioic acid group. The IR data of ligand reveals that $(\upsilon C{=}S)_{asym}$ appeared at 1370 cm^{-1} and $(\upsilon C{=}S)_{sym}$ appeared at 1049 cm^{-1} where as in case of complexes **1-3** and **5**, these bands appeared in the range of 1218-1251 and 1070-1085 cm^{-1}, respectively. However for the complex **4**, the corre-

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Table 1. Physical data of organotin(IV) complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	Molecular formula	M.W.	% Yield	Melting point (°C)	Elemental analysis % calculated (found)		
					C	H	S
HL	$C_4H_8OS_2$	136	75	190-193	27.56 (27.50)	4.05 (4.01)	36.79 (36.71)
1	$C_{10}H_{20}O_2S_4Sn$	419	62	170-173	28.65 (28.59)	4.81 (4.75)	30.59 (30.55)
2	$C_{16}H_{32}O_2S_4Sn$	503	58	179-181	38.18 (38.15)	6.41 (6.38)	25.48 (25.45)
3	$C_{20}H_{24}O_2S_4Sn$	543	60	142-146	44.21 (44.16)	4.45 (4.42)	23.60 (23.52)
4	$C_7H_{16}OS_2Sn$	299	55	170-172	45.19 (45.15)	8.06 (8.01)	15.08 (15.02)
5	$C_{22}H_{22}OS_2Sn$	485	52	150-153	54.45 (54.41)	4.57 (4.54)	13.22 (13.19)

sponding bands appeared at 1371 and 1085 cm^{-1}, respectively. The Δν difference between $\nu_{asym}(CSS)^-$ and $\nu_{sym}(CSS)^-$ is important to find out the binding mode of CSS^- moiety with Sn atom. According to literature,[21] Δν greater than 250 cm^{-1} shows a monodentate while a value less than 250 cm^{-1} shows bidentate binding mode of CSS^- moiety with Sn atom. Moreover a Δν value between 150–250 cm^{-1} indicates a bridging behaviour while a value less than 150 cm^{-1} exhibits a chelate structure. The difference Δ υ (CS_2) of υ $(CS_2)_{as}$ and υ $(CS_2)_s$ are 134-286 cm^{-1}, that is an indication of bidentate binding of the ligand to the central tin atom in complex **1-3** and **5** and mondentae mode of ligand is observed in complex **4** probably be due to steric effect of *n*-butyl groups.[48]

The appearance of a new band in the region 367-321 cm^{-1} indicates the formation of Sn-S bond in complexes.[23] The Sn-C band was observed in the range of 579-550 cm^{-1} in complexes **1**, **2** and **4** but at 279 and 260 cm^{-1} for complexes **3** and **5**. The lower frequency of the complexes **3** and **5** could probably be due to the planner structure and conjugate nature of phenyl ring. These values are in close agreement with that observed for a number of similar organotin(IV)-sulfur donor ligands.[48] Based on the infrared spectra analyses of ligands and their organotin(IV) complexes, it is suggested that ligand coordinated to the tin(IV) moiety through the thiolato sulfur atoms. IR data is given in Table 2.

^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded in DMSO-d_6 to find the behavior of magnetically nonequivalent protons. The data is given in Table 3. The numbers of protons calculated by integration of peaks are in excellent agreement to those theoretically calculated by incremental method.[24] The absence of SH signal in the complexes suggested the deprotonation of carbonodithioic acid group for S→M coordination through CSS^- anions. A doublet appeared at 1.16 ppm for methyl protons and heptet at 5.45 ppm for methylene proton in the free ligand **HL** exhibited downfield shift in the range 1.17-5.85 ppm in the complexes **1**-**5**. The downfield shift is due to dieshielding of these protons upon coordination of both sulphur atoms with the metal.[48] These observation are in agreement with the reported metal dithiocarboxylates.[49] In complex **1**, characteristic signal of methyl protons which are directly coupled with tin metal gives singlet at 1.42 ppm with coupling ^2J ($^{119/117}$Sn-^{1}H = 79, 76 Hz). In compound **2**, the protons of butyl group appeared as multiplet in the region 1.45 (4H, m), 1.89 (4H, m), 2.04 (4H, m), while in compound **4**, the proton of butyl group appeared as multiplet in the region 1.30 (6H, m), 1.36 (6H, m), 1.60 (6H, m). The terminal CH_3 group of butyl gives triplet at 0.96 and 0.90 ppm with $^3J[^1H–^1H]$ value of 7.3 and 7.2 Hz in complexes **2** and **4**, respectively.

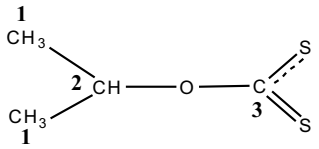
Table 2. IR spectral data (cm^{-1}) of organotin complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	υC=S	Δν	υC-S	υSn-S	υSn-C
HL	1370, 1049	321	1014	-	-
1	1219, 1085	134	981	319	546
2	1251, 1070	181	959	361	507
3	1218, 1082	136	960	321	279
4	1371, 1085	286	961	367	550
5	1219, 1081	138	995	329	260

Table 3. ^{1}H NMR data (ppm)[a-c] of organotin complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	H-1	H-2	R
HL	1.16 (3H, d, ^{3}J = 6.3 Hz)	5.45 (1H, heptet, ^{2}J = 6.3 Hz)	-
1	1.43 (12H, d, ^{3}J = 6.3 Hz)	5.44 (2H, heptet, ^{3}J = 6.3 Hz)	1.42 (6H, s), ^{2}J ($^{119/117}$Sn-^{1}H = 79,76 Hz)
2	1.41 (12H, d, ^{3}J = 6.3 Hz)	5.44 (2H, heptet, ^{3}J = 6.3 Hz)	0.96 (6H, t, ^{3}J = 7.3 Hz), 1.45 (4H, m), 1.89 (4H, m), 2.04 (4H, m)
3	1.15 (12H, d, ^{3}J = 6.3 Hz)	5.29 (2H, heptet, ^{3}J = 6.3 Hz)	7.51 (2H, m), 7.54 (4H, m), 8.05 (4H, m)
4	1.38 (6H, m)	5.58 (1H, heptet, ^{3}J = 6.3 Hz)	0.90 (9H, t, ^{3}J = 7.2 Hz), 1.30 (6H, m), 1.36 (6H, m), 1.60 (6H, m)
5	1.98 (6H, d, ^{3}J = 6.3 Hz)	5.41 (1H, heptet, ^{3}J = 6.3 Hz)	7.42 (3H, m), 7.45 (6H, m), 7.70 (6H, m), 7.51 (2H, m)

[a]



[b] Chemical Shifts (δ) in ppm.
[c] Multiplicity is given as, s = singlet, t = triplet, m = multiplet.

The signals for the protons of the phenyl groups attached to the Sn were distinguishable as two sets in complexes **3** and **5**. The ortho protons were observed upfield at 7.42 and 7.51 ppm, the meta and para protons downfield (7.45-8.05 ppm) and were assigned according to literature.[25]

^{13}C NMR spectroscopy

^{13}C NMR data is given in Table 4. The ^{13}C NMR chemical shifts due to the phenyl groups are observed at positions comparable to other similar compounds.[26] In compound **3**, the meta carbon was observed at 135.62 ppm with ^{3}J(^{119}Sn-^{13}C 56.65 Hz) and para carbon was observed at 130.29 ppm with ^{4}J(^{119}Sn-^{13}C 17.96 Hz). The ortho carbon was observed at 129.20 ppm with ^{2}J($^{119/117}$Sn-^{13}C 85.90, 82.95 Hz) and ipso carbon C-5 at 141.63 with ^{1}J($^{119/117}$Sn-^{13}C 832, 795.58 Hz), while in compound **5**, the meta carbon was observed at 136.57 ppm with ^{3}J(^{119}Sn-^{13}C 44.36 Hz) and para carbon at 129.94 ppm with ^{4}J(^{119}Sn-^{13}C 12.55 Hz). The ortho carbon was observed at 128.9 ppm with ^{2}J($^{119/117}$Sn-^{13}C 61.40, 59.02 Hz) and ipso carbon at ^{1}J($^{119/117}$Sn-^{13}C 583.15, 557.33 Hz).

In compounds **2** and **4**, the methyl carbon at sigma position was observed in the upfield region at 13.74 and 13.67 ppm, respectively, while methylene carbon at gamma position was observed at 26.39 ppm with ^{3}J($^{119/117}$Sn-^{13}C 117.33, 112.61 Hz) and 27.06 ppm with ^{3}J($^{119/117}$Sn-^{13}C 62.71, 64.94 Hz). Methylene carbon at beta position was observed at 28.80 ppm with ^{2}J(^{119}Sn-^{13}C = 39.06 Hz) and 28.67 with ^{2}J(^{119}Sn-^{13}C = 21.90 Hz) and methylene carbon at alpha position was observed at 30.48 ppm with ^{1}J($^{119/117}$Sn-^{13}C 541.11, 516.34 Hz) and 15.61, ^{1}J($^{119/117}$Sn-^{13}C 334.95, 320.09 Hz). The difference in coupling constant in **2** and **4** attributes to four and five coordination geometry, respectively.[50] The C-3 was observed in the range 213.51-222.06 ppm in compounds **1**-**5**.

Protein kinase inhibition assay

Kinase phosphorylation activity is usually associated

Table 4. ^{13}C NMR data (ppm) of organotin complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	C-1	C-2	C-3	R
HL	22.37	72.53	229.76	-
1	21.33	80.63	220.76	10.43, ^{1}J($^{119/117}$Sn-^{13}C = 593.46, 567.70 Hz)
2	21.34	80.52	222.06	13.74, 26.39, ^{3}J($^{119/117}$Sn-^{13}C117.33, 112.61 Hz), 28.80, ^{2}J (^{119}Sn-^{13}C = 39.06 Hz), 30.48, ^{1}J($^{119/117}$Sn-^{13}C 541.11, 516.34 Hz)
3	20.99	81.41	217.43	141.63, ^{1}J($^{119/117}$Sn-^{13}C 832, 795.58 Hz), 129.20, ^{2}J($^{119/117}$Sn-^{13}C 85.90, 82.95 Hz), 135.62, ^{3}J(^{119}Sn-^{13}C 56.65 Hz), 130.29, ^{4}J(^{119}Sn-^{13}C 17.96 Hz)
4	21.31	78.69	216.62	13.67, 27.06, ^{3}J($^{119/117}$Sn-^{13}C 62.71, 64.94 Hz), 28.67, ^{2}J (^{119}Sn-^{13}C = 21.90 Hz), 15.61, ^{1}J($^{119/117}$Sn-^{13}C 334.95, 320.09 Hz)
5	20.68	80.03	213.51	138.06, ^{1}J($^{119/117}$Sn-^{13}C 583.15, 557.33 Hz), 128.99, ^{2}J($^{119/117}$Sn-^{13}C 61.40, 59.02 Hz), 136.57, ^{3}J(^{119}Sn-^{13}C 44.36 Hz), 129.94, ^{4}J(^{119}Sn-^{13}C 12.55 Hz)

with a number of disorders e.g. cancer, cardiovascular disorders, diabetes, infectious diseases, inflammatory disorders, cell survival and growth. Protein kinases are most important and functionally diverse enzymes. These are key regulators of different cellular and extracellular processes. In our study, *Streptomyces* 85E., by using Serine-threonine specific protein kinases (STPKs) strain, are considered to have prominent role in cell survival, division and cellular apoptosis. This filamentous *Sreptomyces* utilizes ser/ther protein kinases for their hyphae formation, branching and polar growth. This strain is internationally recommended to be used in kinase inhibition assay and is widely used to identify large number of eukaryotic kinase modulators as their enzymes are potent to the highly specific eukaryotic counter-parts. It can ascertain the cytotoxic potential of the synthetic compounds.[51] In protein kinase inhibition assay, compounds **1-5** shows a varying degree of inhibition and produced the zones of inhibition ranging from 5.0 ± 0.075-17.0 ± 1.25 mm. Compound **3** was found to be most effective as it produced the maximum zone of inhibition on the culture plates. The exact mechanism is still not known but it is proposed that it blocks the aerial hyphae formation of *Streptomyces species* thus may be hypothesized to inhibit the cancer cell proliferation. The data showed that compound **3** may be considered as an effective candidate to inhibit the tumor initiation. The data is given in Table 5.

Antifungal activity

The synthesized compounds **1-5** were tested for antifungal activity against five different strains including *A. flavis, A. niger, F. solani, A. fumigates* and *Mucor specie* by disc diffusion method.[27] The results are summarized in Table 6. It was evident from data that the synthesized complexes exhibited variety of fungicidal activity as compared to ligand **HL**. The complex **4** exhibited maximum activity (30.0 mm) against *A. fumigates.* Complexes **2** and **3** showed lowest zone (3.0 ± 0.001) and (4.0 ± 0.075) against *A. niger* and *A. fumigates*, respectively. From the data it is evident that triorganotin(IV) complexes exhibits significant fungicidal activity as compared to diorganotin complexes.

Antibacterial activity

In vitro antibacterial activity of ligand **HL** and organotin(IV) complexes was carried out by disc diffusion method[27] using 30 μg/disc. The results are shown in Table 7. Criteria for activity is based on zone of inhibition (mm); inhibition zone more than 20 mm shows significant activity, for 18-20 mm inhibition activity is good, 15-17 mm is low, and below 11-14 mm is non-significant.[26]

Complex **1** showed good activity against *S. aureus* and *M. luteus*. The microorganism against which compounds **2** and **3** presented highest activity was *S. typhimurium*, followed by *B.bronchiseptica* and *E. erogens*. Complexes **4** and **5** have comparable activity to reference drug against *Micrococcus luteus, S. aureus and S. typhimurium.* The triorganotin(IV) compounds showed greater antibacterial activity as compared to diorganotin complexes which may be due to greater lipophilicity and permeability through the cell membrane which is consistent with literature.[28,29]

Table 5. Protein kinase inhibition assay of complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	Zone of inhibition (mm)
HL	-
1	10.0 ± 0.97
2	15.0 ± 1.17
3	17.0 ± 1.25
4	5.0 ± 0.075
5	13.0 ± 1.00
Standard drug; Tyrab	18.0

Table 6. Antifungal activity data of organotin complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	Zone of inhibition (mm)				
	A. flavus	*A. niger*	*F. solani*	*A. fumigates*	*M. specie*
HL	-	-	-	-	-
1	-	-	-	-	-
2	-	3.0 ± 0.001	-	-	-
3	3.0 ± 0.001	-	-	4.0 ± 0.075	-
4	26.0	22.0	18.0	30.0	26.0
5	12.0 ± 1.00	12.0 ± 1.001	16.0 ± 1.755	14.0 ± 1.01	14.0 ± 0.75
Standard drug Terbinafine	16.0	16.0	16.0	16.0	16.0

Table 7. Antibacterial activity data of organotin complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	Zone of Inhibition (mm)				
	M. luteus	*B. bronchiseptica*	*S. aureus*	*S. typhimurium*	*E. erogens*
HL	16.0 ± 0.707	25.0 ± 2.121	0	20.0 ± 2.12	10.0 ± 1.0
1	19.0 ± 7.53	14.0 ± 1.414	21.0 ± 6.65	11.0 ± 0.707	10.0 ± 0.707
2	6.0 ± 0.707	20.0 ± 2.121	0	21.0 ± 0.70	20.0 ± 2.12
3	17.0 ± 0.707	15.0 ± 0.707	0	18.0 ± 2.12	15.0 ± 0.707
4	20.0 ± 0.707	16.0 ± 1.41	11.0 ± 3.21	17.0 ± 0.707	16.0 ± 1.41
5	25.0 ± 0.707	15.0 ± 0.707	12.0 ± 1.10	19.0 ± 1.41	15.0 ± 0.707
Standard drug Roxithromycin and Cefixime	20.0	20.0	20.0	20.0	20.0

Antileishmanial assay

In vitro antileishmanial assay of the compounds **1-5** was performed and the results are summarized in Table 8. All the compounds significantly inhibit the parasite. The LC_{50} data showed that compound **4** causes inhibition at very reduce concentration (0.00001 μg/ml) as compared to standard drug, Amphotericin. B (0.01 μg/ml) followed by compound **2** (0.0013 μg/ml), compound **3** (0.024 μg/ml), compound **1** (0.029 μg/ml) and compound **5** (0.27 μg/ml).

Cytotoxicity assay

The cytotoxicity data of ligand **HL** and complexes **1-5** was studied *in vitro* against the brine-shrimps and the results are summarized in Table 9. The data is based on mean value of two replicates each of 16.12, 31.25, 62.5, 125, 250, 500 and 1000 μgml^{-1}. The LD_{50} data showed that ligand **HL** and organotin(IV) complexes are toxic having values in the range 35-50 μgml^{-1}.

Table 8. Antileishmanial activity data of organotin complexes with *o*-isopropyl carbonodithioic acid

Antileishmanial activity (% mortality)							
Concentration used (μg/mL)	HL	1	2	3	4	5	Standard drug Amp. B
0.00002	0	0	0	0	88	0	0
0.0002	0	0	0	0	96	0	0
0.002	0	0	61	0	100	0	20
0.02	0	36	90	47	100	0	64
0.2	0	100	100	84	100	40	100
2	40	100	100	100	100	100	100
20	78	100	100	100	100	100	100
200	97	100	100	100	100	100	100
LC_{50} (μg/ml)	3.10	0.029	0.0013	0.024	0.0001	0.27	0.010

Table 9. Cytotoxicity data of organotin complexes with *o*-isopropyl carbonodithioic acid

Comp. No.	Lethality %							LD_{50} (μg/ml)
	Concentration (ppm)							
	1000	500	250	125	62.5	31.25	16.12	
HL	100	100	95	55	50	40	35	38.8
1	100	100	100	45	40	35	30	50
2	100	100	95	60	55	45	40	49
3	100	100	100	55	45	35	35	50
4	100	100	100	55	45	45	45	35
5	100	100	100	50	45	40	40	41
Vehicle control	0	0	0	0	0	0	0	0

DNA interaction study

The mode of interaction of organotin(IV) complexes with DNA was determined by UV/visible spectroscopy which is one of the useful techniques for this purpose.[30] The comparison of absorbance and shift in the wavelength of ligand and complexes with and without SS-DNA give the idea about the interaction of ligand or metal complexes with DNA.[31] The absorption spectra of ligand **HL** and organotin(IV) complexes **3** and **5** was taken as a representative one and spectra have been recorded at different concentration of DNA by keeping concentration of ligand **HL** and complex constant.

The UV/vis study of ligand **HL** and compounds **3** and **5** show maximum absorption at 309, 310 and 310 nm which is mainly due to the n-π* transition of the C=S bond. Upon the addition of various concentration of DNA this peak results in hypochromism (decrease in absorbance or molar absorptivity) as well as hypsochromic shift along with minor blue shift upto 1 nm (Figs. 1-3). Hypochromism indicates the intercalative mode involving a stacking interaction between an aromatic chromophore and the base pair of DNA. In intercalation, after binding to DNA, the p orbital of the binding ligand could couple with the p orbital of base pairs in the DNA. The coupling p orbital is partially filled by electrons, thus decreasing the transition probabilities, and hence resulting in the hypochromicity.[32] The intrinsic binding constant K of the ligand **HL** and complexes **3** and **5** were calculated in order to compare binding strengths of ligand-DNA and complex-DNA by using Benesi-Hildebrand equation.[33]

$$\frac{A_0}{A-A_0}=\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}+\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K[DNA]}$$

where K is the association constant, A_0 and A are absorbance of the drug and its complex with DNA, respectively, and ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of the drug and drug-DNA complex, respectively. In order to compare quantitatively, the binding strength of the ligand **HL** and its complexes with DNA, the intrinsic binding constants (K or Kb) were obtained by examining the changes in the absorbance for the complexes with increasing concentration of DNA. Kb was obtained from the ratio of slope to the intercept from the plot of 1/[DNA] versus Ao/A–Ao. The intrinsic binding constant values (Kb) for the **HL**, complexes **3** and **5** were found to be 9.0×10^3, 3.6×10^5 and 3.8×10^5 M^{-1}, respectively. The interaction with DNA is a spontaneous process as indicated by their negative values of ΔG (-16.92, -20.20, -20.66 kJmol^{-1} for **HL**, compounds

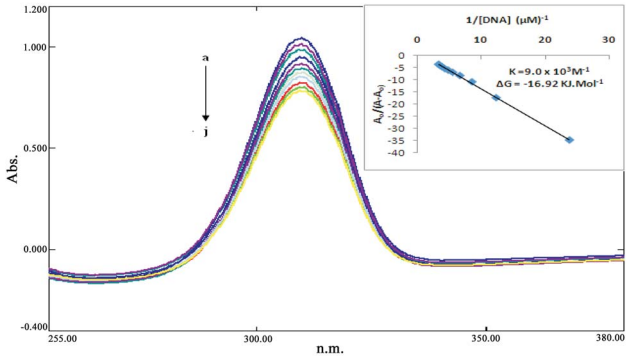


Fig. 1. DNA binding study of **HL** by UV/visible spectroscopy (a) without DNA (b) 10 μM (c) 15 μM (d) 20 μM (e) 25 μM (f) 30 μM (g) 40 μM (h) 45 μM (i) 50 μM (λ_{max} at 309 nm, 1 nm blue shift).

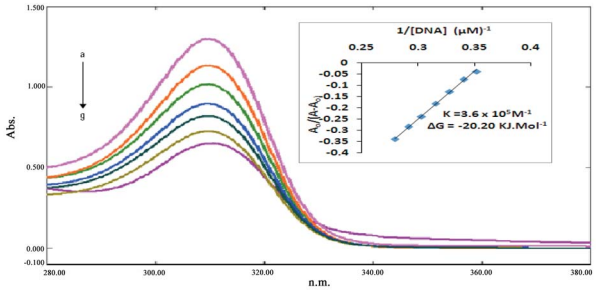


Fig. 2. DNA binding study of complex **3** by UV/visible spectroscopy (a) without DNA (b) 10 μM (c) 15 μM (d) 20 μM (e) 25 μM (f) 30 μM (g) 40 μM (h) 45 μM (i) 50 μM (λ_{max} at 310 nm, 1 nm blue shift).

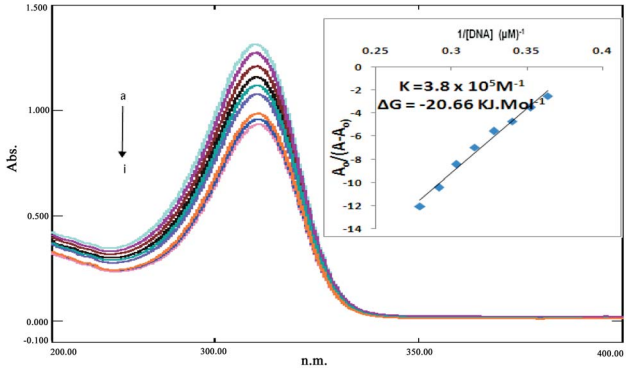


Fig. 3. DNA binding study of complex **5** by UV/visible spectroscopy (a) without DNA (b) 10 μM (c) 15 μM (d) 20 μM (e) 25 μM (f) 30 μM (g) 40 μM (h) 45 μM (i) 50 μM (λ_{max} at 310 nm, 1 nm blue shift).

3 and **5**, respectively.

Semi-empirical study

In geometrically optimized structures of two representative complexes **1** and **5**, both sulphur atoms of ligand **HL** bonds to tin in a bidentate way which is also confirmed by FT-IR data. The other positions are occupied by methyl or phenyl groups. The Sn-C and Sn-S bonds are typical.[34] The bond angles and bond lengths are given in Tables 10 and 11, respectively.

It is well known that a large HOMO-LUMO gap indicates stable molecule with chemical reactivity, while a small E_{HOMO} is associated with unstable molecule with high chemical reactivity. The ability of the molecule to donate electrons, (ionization potential), E_{LUMO} represents (electron affinity), and electrophilicity values ($\omega = \mu^2/2\eta$),[35] chemical potential values $\mu = -(I+A)/2$,[36] global hardness ($\eta = I-A/2$) values[37] and global softness values ($S = 1/2\eta$)[37] have been calculated in each case (Table 12).

Computed positive heats of formation indicate that compound **5** is thermodynamically unstable as compared to compound **1** (Table 10). The calculated HOMO and LUMO orbitals of complexes **1** and **5** are shown in Figs. 4 and 5, respectively. In organotin complexes, the HOMO orbital is primarily located on a sulphur moiety. The calculated HOMO and LUMO energies are given in Table 13.

Potential applications

The investigated compounds have proved to have potential against bacterial, fungal, viral infections, cytotoxic, DNA binding and protein kinase activities.

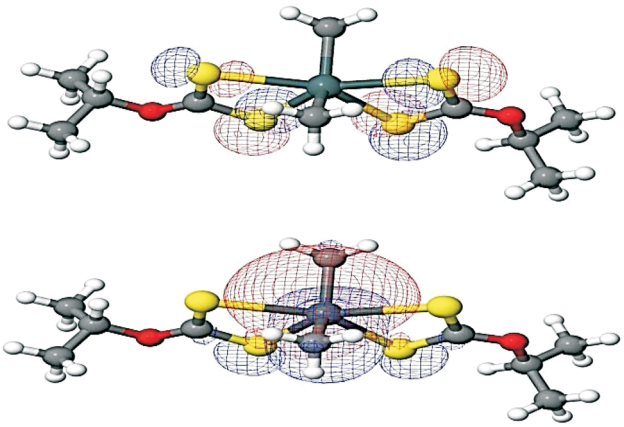


Fig. 4. HOMO-LUMO of complex **1**.

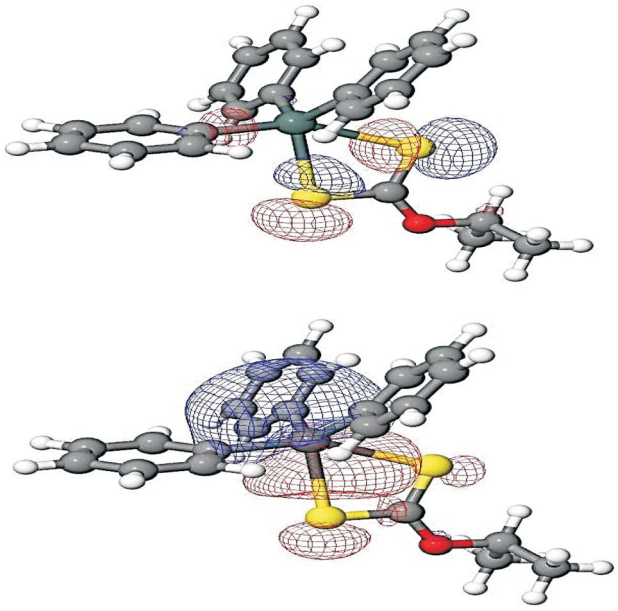


Fig. 5. HOMO-LUMO of complex **5**.

Table 10. Selected Bond angles (°) of complexes **1** and **5**

	1	**5**
S-C-S	116.0, 116.4	115.1
C-Sn-C	132.3	105.7,117.7,106.2

Table 11. Selected Bond Lengths (Å) of complexes **1** and **5**

	1	**5**
Sn-S	2.82, 2.66	2.66
C-O	1.34, 1.46, 1.46, 1.34	1.34, 1.46
C-S	1.74, 1.69, 1.74, 1.69	1.73, 1.68

Table 12. Computed molecular descriptors

Comp. No.	**1**	**5**
HOMO energy (eV)	-9.20307	-9.17988
LUMO energy (eV)	-2.38196	-1.92413
HOMO-LUMO (eV)	-6.82111	-7.25575
Global hardness (η, eV)	3.410555	3.627875
Global softness (S, eV^{-1})	0.146604	0.137822
Chemical potential (μ, eV)	-5.79252	-5.55201
Electrophilicity (ω)	4.919028	4.248322
Dipole moment (debyes)	1.366	1.577

Table 13. Computed thermodynamic parameters

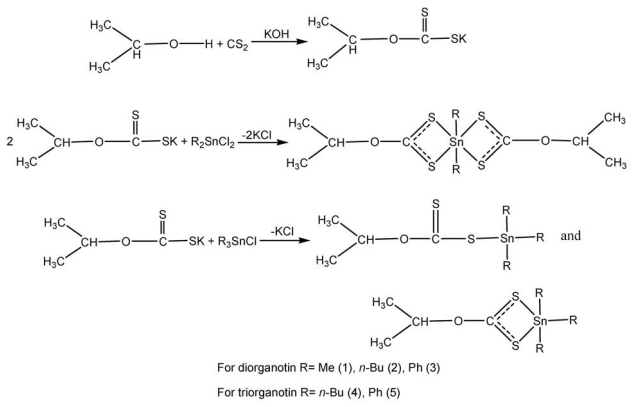
Parameters at 298K	**1**	**5**
Heat of formation (KCal/mole)	-31.835	87.097
Enthalpy (KCal/mole-K)	12217.8013	12549.8310
Entropy (KCal/mole-K)	136.9073	137.5329
Heat capacity (Cp) (Cal/mole-K)	73.4253	83.1424

EXPERIMENTAL

Chemicals and Instrumentation: All organotin(IV) chlorides, isopropanol, potassium hydroxide, carbon disulfide, *p*-nitrophenylphosphate hexahydrate (*p*-NPP), diethanolamine and magnesium chloride were purchased from Sigma Aldrich and used as such. Solvents such as ethanol, chloroform, toluene, diethyl ether, and petroleum ether were purified before use according to reported methods.[38,39] Melting points were recorded with a Bio-Cote Model SMP10 melting point apparatus and are uncorrected. Infrared spectra were recorded in the range 4000-250 cm^{-1} with Thermo Scientific Nicolet-6700 FTIR as KBr/CsBr pellets, whereas NMR spectra were obtained on Bruker Avance 300 MHz NMR spectrometers. Absorption spectra were measured on a UV/visible spectrophotometer, Shimadzu 1800, at 25 ± 1 °C. Sodium salt of DNA (Acros) was used as received. Solutions of DNA in 10 $mmolL^{-1}$ phosphate buffer (pH = 7.0) gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.8–1.9, indicating the purity of DNA. Concentrated stock solution of DNA was prepared in 10 $mmolL^{-1}$ phosphate buffer (pH = 7.0) and the concentration was determined by UV absorbance at 260 nm after 1:100 dilutions. The molar absorption coefficient has been taken as 6600 $mol^{-1}cm^{-1}$. Stock solutions were stored below 4 °C and used within 4 days.

Procedure for the synthesis of Potassium *o*-isopropyl carbonodithioate HL: Isopropanol (1 mmol), potassium hydroxide (1 mmol) and carbon disulfide (1 mmol) were continuously stirred in round bottom flask (250 ml) at room temperature for 4 hr (Scheme 1). Precipitate obtained were filtered off and dried in air.

Scheme 1



General procedure for the synthesis of organotin(IV) complexes with Potassium *o*-isopropyl carbonodithioate 1-5: The potassium *o*-isopropyl carbonodithioate (1 mmol) was dissolved in toluene (100 ml) in a round bottom two necked flask (250 ml) with continuous stirring. Then R_2SnCl_2/R_3SnCl (2 mmol/1 mmol) was added in portions as solid in above solution and reaction mixture was refluxed for 8 hr (Scheme 1). The potassium chloride formed was filtered off and the solvent was evaporated through rotary evaporator under reduced pressure. The solid product obtained was recrystallized from chloroform and *n*-hexane (1:1).

UV-Vis spectroscopy: SS-DNA (salmon sperm-DNA) (0.3 g) was dissolved in double deionized water (100 ml) and kept stirring at 4 °C for 2 days. Doubly distilled water was used to prepare buffer (20 mM phosphate buffer (NaH_2PO_4–Na_2HPO_4), pH = 7.3). A solution of (SS-DNA) in the buffer gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.8, indicating that DNA was sufficiently free of protein.[40] The DNA concentration was determined *via* absorption spectroscopy using the molar absorption coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm,[41] and was found as 1.76×10^{-4} M. From this stock solution, 20, 40, 63, 83, 105, 125 and 170 μM working solutions were prepared by dilution method. The complexes were dissolved in 10% DMSO at a concentration of 3×10^{-5} M. The UV absorption titrations were performed by keeping the complexes concentration fixed while varying the concentration of DNA. Equivalent solutions of DNA were added to the complex and reference solutions to eliminate the absorbance of DNA itself. Compound-DNA solutions were allowed to incubate for 30 minutes at ambient temperature before measurements were made. Absorption spectra were recorded using cuvettes of 1 cm path length. The DNA-binding constants of the compounds were calculated by UV/vis spectroscopy using following equation:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \frac{1}{K[DNA]}$$

Intercept to slope ratio of the plot 1/[DNA] versus $A_o/A–A_o$ gives the value of binding constant K.[42]

Protein kinase inhibition study: Protein kinase inhibition was performed by disc diffusion method.[27] Streptomycin (Actinobacter) culture was used for determination of protein kinase inhibition potential of samples. Tryptic soya broth (Merck) was autoclaved at 121 °C for 15 minutes and the strain was refreshed in the sterilized media for 24 hours at 30 °C. Mineral media are self prepares media with combination of different salts and agar for *Streptomyces growth* and it was autoclaved at the same specifications as that of TSB. Media was poured in pre-sterilized petri plates and allowed to solidify at room temperature. After setting, activated streptomycin strain was swabbed on solidified media

with the help of sterilized cotton plug. Then test samples were prepared in DMSO (20 mM) and the 5 µl of the solution was placed on the sterilized filter paper disc at the final concentration of 100 µM. The discs were placed above media at their respective labeled positions. These plates were incubated at 28 °C for 48-72 hours. After incubation period, zone around each disc with no growth were measured for determination of protein kinase inhibition activity of the samples. Assay was performed in triplicate and the average reading was taken.

Antifungal activity: The antifungal activity was determined by using five fungal strains i.e., *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Fusarium solani* and *mucor specie*. The disc diffusion method was employed.[27] Sabouraud dextrose agar (SDA) (Sigma-Aldrich Germany) medium was autoclaved at 121 °C for 15 minutes. The media was allowed to cool at 50 °C and 25 ml quantity of it was poured in pre-sterilized petri plates. Plates were then allowed to solidify in upright position at room temperature. Each fungal culture was streaked on the petri plates. Test samples were prepared in DMSO (20 mM) and 5 µl of the solution was placed on the sterilized filter paper disc at the final concentration of 100 µM. The discs were placed on the media and the plates were incubated at 37 °C for 24 hr. DMSO and clotrimazole (1 $mgml^{-1}$) were used as negative and positive control, respectively. After period of incubation, diameter of the clear zones with no fungal growth around each disc was measured. Triplicate plates were prepared for each fungal strain. Then average of these three plates was taken.

Antibacterial activity: The ligand and organotin(IV) complexes were tested against five bacterial strains; Two gram positive strains (*M. luteus*, *S. aureus*) and three gram negative (*B. bronchiseptica*, *S. typhimurium*, *A. aerogens*). The disc diffusion method was used for the determination of antibacterial activity.[27] Each complex (10 mg) was dissolved in 1 ml of DMSO, for the production of stock solution of each complex. Final concentration of 30 µg/disc of each complex was used in assay. Bacterial inoculum was prepared in nutrient broth (Sigma-Aldrich Germany). It was prepared by dissolving 2 g of nutrient broth in 100 ml of water and pH was maintained to 7. Filter paper discs of 6 mm in size ware prepared from whatman filter paper no.1. Media, filter paper discs along with other apparatus required in this assay were autoclaved for sterilization. After autoclaving, whole experiment was carried out in microbiological safety cabinet. Solidified plates of nutrient agar were labeled and respective bacterial strain was streaked. Then complex solution (10 µl) was absorbed on disc. These discs were placed on respective place in petri plate. These petri plates were incubated for 24 hr at 28 °C. Cefixime-USP and roxithromycin were used as standard drugs. Stock of each standard drug was prepared by dissolving 4 mg/1 ml of DMSO. DMSO was used as negative control. Zones of inhibition were measured after 24 hr.

Antileishmanial assay: Leishamania tropica kwh23 strain was maintained in fresh medium 199 Merck (Merck KGaA, Germany) at 24 °C for 7 days to get log phase of growth. The medium contained Fetal Bovine Serum at concentration of 10%. The experiment was performed as per procedure of Nabi *et al.*, with slight modification.[43] Stock solutions of 10, 000 ppm of the compounds were prepared in DMSO. The assay was carried out at different concentrations (200, 20, 2, 0.2, 0.02, 0.002, 0.0002 and 0.00002 ppm). The medium containing culture were inoculated in 96 well plates and incubated with test samples at 24 °C for 24 hr after serial dilution. Positive (Amphotericin. B) and negative (DMSO) control of the assay were also maintained. The experiment was performed in triplicates. After required time of incubation, the live parasites of leishmania were counted using improved neubauer chamber under compound microscope. The data was statistically analyzed through Graph Pad Prism software 5.0.

Cytotoxicity assay: Cytotoxicity was study by the brine-shrimp lethality assay method.[44,45] Brine-shrimp (*Artemia salina*) eggs were hatched in artificial sea water (3.8 g sea salt/L) at room temperature (22–29 °C). After two days, these shrimps were transferred to vials containing 5 ml of artificial sea water (8 shrimps per vial) with 1000, 500, 250, 125, 31 and 16.5 µg/ml in DMSO. After 24 h number of surviving shrimps was counted. Data was analyzed with a slide write program.

Semi-empirical study: The semi-empirical study was done by MOPAC 2007[46] program in gas phase using PM3 method.[47] Selected parts of the complexes not containing the metal ion were pre-optimized using molecular mechanics methods. Several cycles of energy minimization had to be carried for each of the molecules. Geometry was optimized using Eigen Vector. The Root Mean Square Gradient for molecules was all less than one. Self-Consistent Field was achieved in each case. Absences of imaginary frequencies were checked consistently.

CONCLUSIONS

The ligand was treated with different di- and tri-organotin(IV) chlorides to form the corresponding complexes which coordinates through sulphur. IR data showed the bidentate nature of ligand in complexes **1-3** and **5** and monodentate mode in case of complex **4**, which was also confirmed by NMR and semi-empirical studies. NMR data showed 6-coordinated (compexes **1-3**), 4-coordinated (complex **4**) and **5**-coordinated geometry (complex **5**) in solution. HOMO-LUMO calculations showed that com-

plex **5** is thermodynamically unstable as compared to complex **1**. DNA interaction study showed the hypochromic shift due to intercalative mode involving stacking interaction between an aromatic chromophore and base pair of DNA. The negative values of Gibb's free energy change confirmed the spontaneity of these interactions. Antimicrobial activity data showed that triorganotin complexes exhibited greater antimicrobial activity as compared to diorganotin complexes. LC_{50} data revealed that complexes can cause inhibition at lower concentration as compared to standard drug while LD_{50} data proved that all complexes are toxic.

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