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Synthesis, characterization, antimicrobial and DNA cleavage study of organoantimony(III) and organoarsenic(III) complexes with monofunctional bidentate Schiff base

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A series of mononuclear organoantimony(III) and organoarsenic(III) complexes of bidentate Schiff base [2-(2-fluorophenyl)methylene]hydrazinecarbothioamide (LH) have been synthesized by the reaction of Ph_3Sb and Ph_3As with ligand in 1:1 and 1:2 molar ratios. All the synthesized compounds were characterized by elemental analyses, melting point determinations and a combination of electronic, IR, ^1H NMR, ^{13}C NMR and X-ray diffraction studies. These studies have shown that the ligand coordinated to the antimony and arsenic in a monobasic bidentate manner through sulfur and nitrogen donor system. Thus, a tetra-coordinated and a penta-coordinated environment around the antimony and arsenic atom have been proposed for 1:1 and 1:2 complexes, respectively. All the complexes and parent ligand have been screened for their antimicrobial activity on several pathogenic fungi and bacteria and are found to possess appreciable fungicidal and bactericidal properties. Further, the ligand and its corresponding metal complexes have been tested for their DNA cleavage activity by the gel electrophoresis and the results revealed that the complexes are better cleaving agents than ligand.

Keywords: Antimicrobial activity, DNA cleavage activity, Organoantimony(III) and Organoarsenic(III) complexes, Sulfur and nitrogen donor system

In recent years, the coordination behaviour of organometallic compounds of Schiff bases with oxygen, sulfur and nitrogen containing ligands have received great attention as a consequence of their biological property[1]. The toxicity as well as therapeutic value of organometallics is well known. The use of organometallic medicinal is widespread. Schiff-base complexes of main group elements containing thiosemicarbazones have remained a topic of research interest,[2] this is mainly due to the biological applications of ligands and compounds derived from them. The metal complexes of fluoroimines are biologically active and used in pharmaceutical field[3,4]. The compounds of antimony and arsenic present a wide variety of biological activities such as antitumoral[5–7], fungicidal[8,9], bactericidal[10] and antiviral. It is known that some drugs have increased activity when administered in the form of the metal complexes[11] and a number of metal chelates inhibit tumor growth[12].

In the treatment of cancer, the active species is not the thiosemicarbazones but their metal chelates[13]. Arsenic is a metal, known to the ancients with toxic as well as medicinal properties. Recently interest has also been renewed in low temperature ionic liquid technology using organoarsenic derivatives for electrodeposition of actinides of relevance to nuclear fuel processing[14]. Antimony has use in pharmacology for the treatment of syphilis, fever, melancholy, pneumonia, epilepsy and inflammatory conditions[15]. Antimony potassium tartrate and stibophen are known to be antiprotozoal and anthelmintic agents[16]. Organic antimony salts are used medically to treat some tropical diseases[17], especially in the treatment of all types of leishmenasis[18]. The pharmacological activity of antimony compounds has been developed ever since the advent of rational chemotherapy[19,20]. Early studies sought to develop this element as an anticancer compound with the current reports of the *in vitro* cancer properties of diphenylantimony compounds[21,22]. Their anticarcinogenic properties are reported to be much lower than those of platinum and palladium complexes[23]. In respect of the reproductive and developmental toxicity, antimony compounds have also been studied in experimental models[24].

In view of this, the present article, is on the exploration of the studies on the synthesis, structural and biological aspects of Sb(III) and As(III) complexes of stereochemical as well as biological interest with Schiff base ligand. The results were indeed positive.

Materials and Methods

Materials

Moisture was carefully excluded throughout the experimental manipulations. All the chemicals used were of reagent grade. Solvents were carefully dried by standard methods before use.

Preparation of ligand

The ligand was prepared by the condensation of o-flourobenzaldehyde (4 g, 0.03 mol) with thiosemicarbazide (2.7 g, 0.03 mol) in absolute alcohol. The reaction mixture was refluxed over a water bath for 3–4 h and allowed to stand overnight. The products were recrystallized from the ethanol and dried *in vacuo*. Their physicochemical properties and analytical data are given in Table 1. The parent ligand exists in the tautomeric forms depicted in Scheme 1.

Preparation of the complexes

The organoantimony(III) and organoarsenic(III) complexes were synthesized by the unimolar and bimolar reactions of triphenylantimony and triphenylarsine with the monobasic bidentate ligand in benzene. The contents were boiled under reflux for 14–16 h on the fractionating column. The completion of the reaction was examined by TLC using silica gel-G. The resulting coloured complexes were washed with dry *n*-hexane and then finally dried *in vacuo* for 3–4 h. Their physicochemical properties and analytical data are given in Table 1.

Microbial assay

Antifungal activity

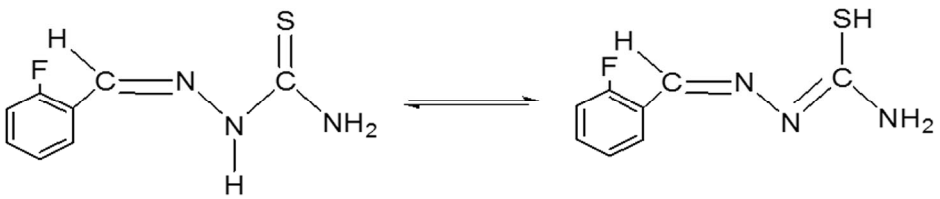
The antifungal activity was evaluated against *Aspergillus niger and Fusarium oxysporum* using agar plate technique. The linear growth of the fungus was recorded by measuring the diameter of the fungus colony after 96 h and the percentage inhibition was calculated as 100 $(C - T)/C$, where C and T are the diameters of the fungus colony in the control and the test plates, respectively[25]. The activity of compound was compared with standard fungicide (Flucanazole).

Antibacterial activity

Antibacterial activity was evaluated against *Gram-positive (Pseudomonas aeruginosa) and Gram-negative (Escherichia coli)* by the paper disc plate method. The nutrient agar medium (peptone, beef extract, NaCl and agar–agar) and 5 mm diameter paper disc of Whatman no. 1 were used. The

Table 1 — Analytical data and physical properties of the ligand and its complexes

Ligand/ Complex	Complex formula	Colour	Melting Point (ºC)	Elemental analysis Found (Calculated) (%)					Molar mass Found (Calc.)	Yield (%)
				C	H	N	S	M		
LH		White	190	48.60 (48.71)	4.00 (4.08)	21.09 (21.30)	16.20 (16.25)	-	197.18 (197.22)	86
1	$[Ph_2Sb(L)];C_{20}H_{17}N_3SFSb$	Yellow	177	50.32 (50.88)	3.59 (3.62)	8.86 (8.90)	6.68 (6.79)	25.69 (25.78)	472.02 (472.08)	75
2	$[PhSb(L)_2];C_{22}H_{19}N_6S_2F_2Sb$	White	146	44.63 (44.70)	3.19 (3.23)	14.13 (14.21)	10.64 (10.84)	20.47 (20.59)	591.04 (591.09)	73
3	$[Cl_2Sb(L)];C_8H_7N_3Cl_2SFSb$	Light yellow	180	24.45 (24.71)	1.16 (1.81)	10.56 (10.80)	8.06 (8.24)	31.15 (31.31)	388.43 (388.77)	82
4	$[ClSb(L)_2];C_{16}H_{14}N_6S_2F_2ClSb$	White	150	34.50 (34.97)	2.19 (2.56)	15.19 (15.29)	11.36 (11.67)	22.05 (22.15)	549.34 (549.44)	79
5	$[Ph_2As(L)];C_{20}H_{17}N_3SFAs$	Light yellow	162	56.09 (56.48)	3.97 (4.02)	9.76 (9.88)	7.36 (7.54)	17.45 (17.61)	425.10 (425.25)	70
6	$[PhAs(L)_2];C_{22}H_{19}N_6S_2F_2As$	Light yellow	160	48.29 (48.54)	3.18 (3.51)	15.24 (15.44)	11.56 (11.78)	13.65 (13.76)	544.18 (544.27)	78



Scheme 1 — Schematic representation for the tautomeric forms of the ligand

compounds were dissolved in methanol in 500 and 1000 ppm concentrations. The filter paper discs were soaked in different solutions of the compounds, dried and then placed in Petri plates previously seeded with the test organisms. The plates were incubated for 20–30 h at 28 ± 2 °C and the inhibition zone around each disc was measured[26]. The antibacterial activity of Streptomycin was also recorded using the same procedure, concentrations and solvent.

DNA cleavage activity

Preparation of culture media

The DNA-binding and cleavage experiments were performed at room temperature**.** Agarose gel electrophoresis was used to study the DNA cleavage activity of complexes **1** [$Ph_2Sb(L)$] and **2** [$PhSb(L)_2$]. *Pseudomonas aeruginosa* plasmid was cultured, isolated and used as DNA for the experiment. Nutrient broth (peptone-5 g; beef extract-3 g; NaCl-5 g; distilled water -1000 mL; pH-7.0 autoclaved at 121 °C and pressure- 15 psi). The media was inoculated with the *Pseudomonas aeruginosa (ATCC 27853)*.

Isolation of DNA

The fresh bacterial culture (1000 µL) was centrifuged (6000 rpm for 10 min) to obtain the pellet, which was then dissolved in 250 µL of cell lysis buffer. To this 250 µL of saturated phenol, chloroform and isoamyl alcohol was added in the ratio 25:24:1 and incubated at -20 °C for 1 h. Then, it was centrifuged at 10,000 rpm for 10 min and the upper aqueous layer was collected. To this supernatant, two volumes of chilled absolute alcohol and 50 µL of sodium acetate was added. The precipitated DNA was separated by centrifugation. The pellet was dried, collected and dissolved in TE buffer (10 mmol L^{-1} Tris pH 8.0, 1 mmol L^{-1} EDTA) and stored in cold conditions.

Agarose gel electrophoresis

The agarose gel electrophoresis method was used for the analysis of cleavage products. Test samples (1 mg/mL) were prepared in DMF. The samples (25 µg) were added to the isolated DNA of *Pseudomonas aeruginosa*. The samples were incubated for 2 h at 37 °C and then 20 µL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 m EDTA/L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. The gel was removed and stained with 10.0 g/mL ethidium bromide for 10-15 min, and the bands were observed under a UV transilluminator and photographed to determine the extent of DNA cleavage, and the results were compared with standard DNA marker.

Characterization

Molecular weights were determined by the Rast camphor method. Chlorine was estimated by Volhard's method. Arsenic and antimony was estimated iodimetrically. Nitrogen was estimated by the Kjeldahl method, and sulfur was estimated by the Messenger method. Carbon and hydrogen analyses were performed at the Saurashtra University, Gujarat, India. The infrared (IR) spectra have been recorded on a Nicolet Megna FTIR- 550 spectrophotometer using KBr pellets. Absorption spectra were recorded on a Bio-Age/752 spectrophotometer in the Department of Chemistry, University of Rajasthan, Jaipur. ^1H and ^{13}C NMR were recorded at room temperature using a JEOL-AL-300 FT NMR spectrometer in DMSO-d6, with TMS as internal standard.

Results and Discussion

Metal ions interact with the monobasic bidentate ligand having $N^{\cap}S$ donor set in 1:1 and 1:2 molar ratios with the formation of $[Ph_2M(N^{\cap}S)]$, $[PhM(N^{\cap}S)_2]$ (where , M=Sb/As) and $[Cl_2Sb(N^{\cap}S)]$, $[ClSb(N^{\cap}S)_2]$ type of complexes. These reactions can be represented by the following general equations:

$$Ph_3M + N^{\cap}SH \xrightarrow[Benzene]{1:1} [Ph_2M(N^{\cap}S)] + C_6H_6 \quad \ldots(1)$$

$$Ph_3M + 2N^{\cap}SH \xrightarrow[Benzene]{1:2} [PhM(N^{\cap}S)2] + 2C_6H_6 \quad \ldots(2)$$

Where, $M = Sb\ \&\ As$.

$$SbCl_3 + N^{\cap}S.Na \xrightarrow[MeOH]{1:1} [Cl_2Sb(N^{\cap}S)] + NaCl \quad \ldots(3)$$

$$SbCl_3 + 2N^{\cap}S.Na \xrightarrow[MeOH]{1:2} [ClSb(N^{\cap}S)_2] + 2NaCl \quad \ldots(4)$$

Where, $N^{\cap}S$ doner system of the ligand.

After removing the solvent under reduced pressure, coloured solid compounds were obtained, which were

found to be soluble in benzene, choloroform and DMSO. Molecular weight determinations show them to be monomeric. The molar conductances of 10^{-3} M solutions of the complexes in dry DMF lie in the 12–15 ohm^{-1} cm^2 mol^{-1} range, indicating that they are nonelectrolytes.

Electronic spectra

The electronic spectra of ligand (LH) show bands around at 270 and 300 nm due to π -π* electronic transitions which appear almost in the same region in the spectra of their antimony(III) and arsenic(III) derivatives. However, a band around 365-370 nm due to n-π* electronic transitions within the azomethine group in fluoroimines which undergoes bathochromic shift due to the polarization within the >C=N chromophore caused by the metal-ligand interaction. i.e. due to the donation of the lone pairs of electrons by the nitrogen of the fluoro ligand to the central metal atom.

IR spectra

In the IR spectra of the ligand, absorption bands in the region 3280 cm^{-1} appeared due to ν(NH) vibrations, which disappear in the spectra of antimony(III) and arsenic(III) complexes, indicating a possible deportation of the functional group upon complexation. The non-involvement of the NH_2 group in chelation has been confirmed by the appearance of its bands due to asymmetric and symmetric modes at 3440 and 3320 cm^{-1}, respectively, in the same position as in the spectra of complexes. The band arround 1610 cm^{-1}, characteristic of azomethine (>C=N) group in the spectrum of fluoroimine, is shifted to the lower frequency by 15-20 cm^{-1} in case of antimony and arsenic complexes indicating the coordination of azomethine nitrogen to the central metal atom. The $\nu_{C=S}$ band in LH appear around 820 cm^{-1}, is suffer a negative shift upon chelation. The appearance of a new sharp band in the complexes simultaneously in the region 700–600 cm^{-1} due to ν_{C-S} is evidence of the ligand coordinating via the thioenol structure. The appearance of some new bands arround 410-435, 360-378, 450-440 and 250-350 cm^{-1} can be assigned to ν(Sb←N)[27], ν(Sb-S)[28], deformation (Ph-Sb)[29] and Sb-Cl, respectively. The spectra of complexes exhibit new bands in the range 439–442 cm^{-1} and 365–368 cm^{-1} due to ν(As←N), and ν(As–S)[30] vibrations, respectively. The band in 450–470 cm^{-1} range may be assigned to ν (As–Ph)[31] vibrations in the respective complexes. The IR spectral data of the ligand and its complexes are listed in Table 2. The IR spectra are provided in Supplementary Data.

^{1}H NMR spectra

To confirm the bonding pattern, ^{1}H NMR spectra of fluoro substituted ligand along with its metal complexes have also been recorded in DMSO-d_6 using TMS as internal standard. The ^{1}H NMR spectra of the free ligand exhibit –NH proton signal at δ 11.23 ppm which disappears in the spectra of the complexes indicating the bond formation through thiolic sulfur atom to the metal atom after enolization and deprotonation of the ligand molecule. The proton signals due to the azomethine proton is shifted downfield in the spectra of the metal complexes due to the coordination through the nitrogen atom of the azomethine group. The appearance of a signal due to -NH_2 group (δ 2.16 ppm) at almost the same position in the spectra of ligand and its corresponding antimony(III) and arsenic(III) complexes show its non-involvement in complex formation. A multiplet due to aromatic protons in the spectra of metal complexes resonates nearly at the same position as that of free ligand (Table 3). The ^{1}H NMR spectra are provided in supplementary data.

Table 2 — IR spectral data (cm^{-1}) of the ligand and its metal complexes

Ligand / Complex	ν(-NH)	ν (>C=N)	ν -NH_2 (Sym & Asym)	ν(Sb←N)	ν(As←N)
LH (N⌒SH)	3280	1610	3320, 3440	-	-
$Ph_2Sb(L)$	-	1590	3321, 3441	412	-
$PhSb(L)_2$	-	1595	3318, 3440	420	-
$Cl_2Sb(L)$	-	1588	3320,3440	428	-
$ClSb(L)_2$	-	1585	3322,3442	435	-
$Ph_2As(L)$	-	1592	3321,3443	-	442
$PhAs(L)_2$	-	1590	3319,3440	-	439

Table 3 — The ^{1}H NMR spectral data (ppm) of the ligand and its complexes

Ligand / Complex	-CH=N	-NH (Free) (bs)	-NH_2 (bs)	Aromatic proton (m)
LH (N⌒SH)	8.35	11.23	2.16	7.68-6.70
$Ph_2Sb(L)$	8.57	-	2.18	8.15-6.37
$PhSb(L)_2$	8.58	-	2.19	8.12-6.36
$Cl_2Sb(L)$	8.59	-	2.17	7.78-6.70
$ClSb(L)_2$	8.57	-	2.16	8.28-6.72
$Ph_2As(L)$	8.40	-	2.15	8.14-6.98
$PhAs(L)_2$	8.46	-	2.17	6.92-8.28

^{13}C NMR spectra

To support the proposed structures, ^{13}C NMR spectra of the ligand and its complexes were recorded in $CDCl_3$ and DMSO-d_6. A comparison of the spectra of the antimony and arsenic derivatives with the ligand (LH) provide very useful information about the mode of bonding. The signal observed for >C=N group carbon at δ 157.38 ppm in the spectrum of free ligand has been observed at δ 150.26–152.75 ppm in the spectra of these derivatives. This downfield shifting in the position of >C=N group signal suggests the participation of this group in bonding by the formation of As←N and Sb←N bonds. A marked shift in the position of the carbon atoms attached to the sulfur atom as compared to the ligand (δ 157.38) shows the participation of thiol group in bonding. A new set of four signals observed in the spectra of the complexes in the range δ 130.33–141.45 ppm was assigned to the phenyl carbons attached to the antimony atom. The phenyl carbons attached to the arsenic atom appear in the region δ 128.32–155.88 ppm. Further, only one set of signals for the phenyl carbons of Ph_2Sb/Ph_2As group was observed, which indicates that the two phenyl groups are chemically equivalent. The ^{13}C NMR spectra are provided in supplementary data.

X-Ray diffraction studies

The possible lattice dynamics of the finely powdered product, [Ph_2Sb (L)] has been deduced on the basis of X-ray powder diffraction studies. The observed inter planer spacing values (d-value in Å) have been measured from the diffractogram of the compound and the Miller indices h, k and l have been assigned to each d-value and 2-theta angles are reported. The results show that the compound belongs to orthorhombic crystal system having cell parameter a= 9.3453 Å, b= 10.2312 Å, c= 25.645 Å and Alpha=90º, Beta=90º, Gama=90º at the wavelength=1.540598 Å (Table 4 and Fig. 1).

On the basis of spectral data, it is suggested that the central metal ion acquire the coordination number four and five and the most plausible geometry of the complexes is distorted trigonal bipyramidal and distorted octahedral, respectively. Suggested structures of antimony complexes are drawn using ChemDraw 3D Ultra as shown in Fig. 2.

Biological evaluation

The synthesized ligand and its metal complexes were screened against some pathogenic fungi and bacteria, and the results are shown in Fig. 3 (a and b) The results revealed that there was a significant increase in the toxicity of the complexes as compared with the ligand because the chelation reduces the polarity and increases the lipophilic nature of the central metal atom, which subsequently favours its permeation through the lipid layer of the cell membrane. This can be well ascribed to Tweedy's chelation theory[32]. It was found that the Gram-positive bacteria are more affected than the Gram-negative bacteria.

Results of electrophoresis analysis

DNA cleavage studies of the synthesized ligand and corresponding antimony metal complexes [$Ph_2Sb(N^{\cap}S)$] and [$PhSb(N^{\cap}S)_2$] have been carried out against *Pseudomonas aeruginosa* (ATCC 27853) by agarose gel electrophoresis. Gel electrophoresis works on the migration of DNA under the influence of electric potential. Gel electrophoresis in the control

Table 4 — XRD data of the complex **1** [Ph_2Sb(L)]

h k l	2θ (º) (Exp.)	2θ (º) (Calc.)	d (Å) (Exp.)	d (Å) (Calc.)	Intensity
0 2 0	24.014	24.039	3.70276	3.69904	57.46
0 3 10	30.883	30.913	2.89305	2.89037	36.99
0 7 7	39.241	39.242	2.29400	2.29395	178.42
0 6 2	36.687	36.689	2.44760	2.44750	30.05
0 7 1	38.428	38.419	2.34065	2.34120	19.79
0 8 7	40.854	40.884	2.20707	2.20552	20.40
1 0 7	42.690	42.697	2.11630	2.11599	17.64
1 1 7	44.244	44.276	2.04551	2.04413	27.33
1 4 6	49.754	49.790	1.83113	1.82987	8.61
1 5 8	51.119	51.110	1.78538	1.78568	13.88
3 5 5	58.049	58.030	1.58764	1.58812	8.26
5 0 7	66.149	66.116	1.41150	1.41213	10.71
5 4 2	68.510	68.501	1.36850	1.36866	3.36

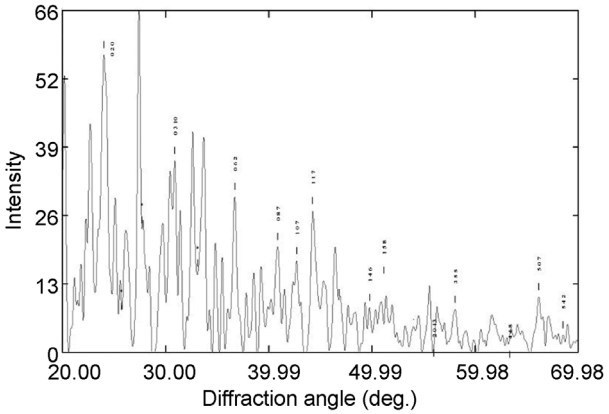


Fig. 1 — X-ray diffractogram of the complex [Ph_2Sb(L)]

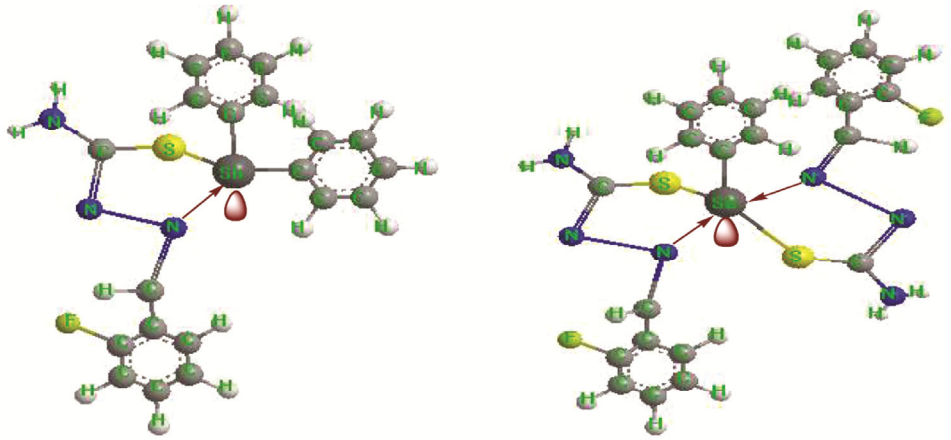


Fig. 2 — Suggested structures of antimony complexes drawn using ChemDraw 3D Ultra

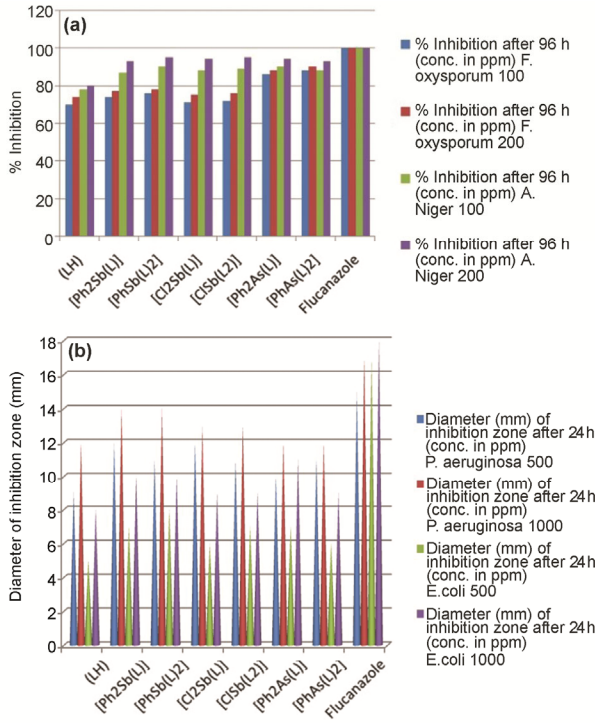


Fig. 3 — (a) Antifungal and (b) antibacterial screening data of fluoroimine and its metal complexes

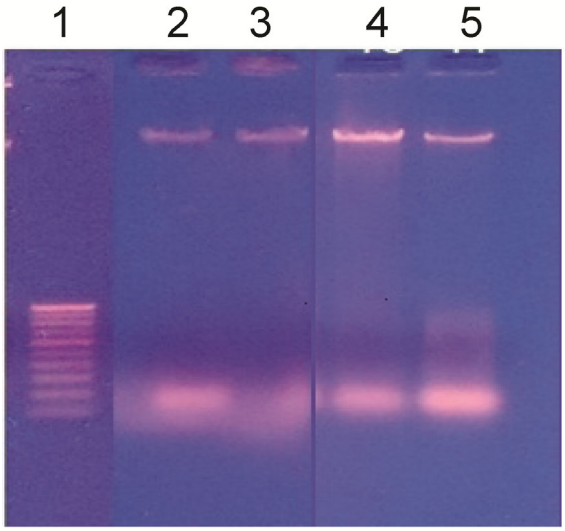


Fig. 4 — DNA cleavage diagram of synthesized compounds: Lane 1, standard molecular weight marker; Lane 2, control DNA of *P. Aeruginosa*; Lanes 3, *P.Aeruginosa* DNA treated with the ligand LH; Lane 4 & 5 *P.Aeruginosa* DNA treated with complexes 1;[$Ph_2Sb(L)$] and 2;[$PhSb(L)_2$], respectively

experiments as shown in Fig. 4, clearly depict that the untreated DNA of standard *Pseudomonas aeruginosa* does not show any cleavage (Lane 2), whereas all the metal complexes along with the ligand exhibit a remarkable cleavage activity on DNA. The difference in migration was observed in the Lanes 3, 4 and 5 of ligand and antimony complexes, respectively, compared to the control DNA of *Pseudomonas aeruginosa* (Lane 2). The cleavage activity was confirmed by the tailing in the DNA bands. The complexes converted the double stranded or super-coiled DNA into single stranded or open circular DNA. In this work, the comparative data of DNA cleavage of ligand and its antimony complexes showed that the metal complexes exhibited better results than the free ligand.

Conclusions

Synthesis, characterization and biological activity of Sb(III) and As(III) complexes have been described. On the basis of spectral data, it is suggested that the

central metal ion acquire the coordination number four and five and the most plausible geometry of the complexes is distorted trigonal bipyramidal and distorted octahedral, respectively. The antimicrobial results indicated that the complexes showed promising antifungal and antibacterial activity. The DNA cleavage results revealed that the complexes were better cleaving agents than ligand.

Supplementary Data

Supplementary Data associated with this article are available in the electronic form at http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A(03)341-347_Suppl Data.pdf.

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