

Research Article

Synthesis, Characterization, and Antimicrobial and Antispermato-genic Activity of Bismuth(III) and Arsenic(III) Derivatives of Biologically Potent Nitrogen and Sulfur Donor Ligands

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A series of Bi(III) and As(III) complexes with two N^oS donor ligands, 1-(4-chloro-2-oxo-2H-chromen-3-yl)-methylene-thiosemicarbazide (L¹H) and N'-[1-(2-oxo-2H-chrome-3yl-ethylidene)]-hydrazinecarbodithionic acid benzyl ester (L²H) have been synthesized by the reaction of BiCl₃ and Ph₃As with ligands 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, ¹³C NMR spectroscopic techniques, and X-ray diffraction for structure elucidation. In order to evaluate the effect of metal ions upon chelation, both the ligands and their complexes have been screened for their antimicrobial activity against the various pathogenic bacterial and fungal strains. The metal complexes have shown to be more antimicrobial against the microbial species as compared to free ligands. Both the ligands and their corresponding metal complexes have been tested for their antifertility activity in male albino rats. The marked reduction in sperm motility and density resulted in infertility. Significant alterations were found in biochemical parameters of reproductive organs in treated animals as compared to control group. It is concluded that all these effects may finally impair the fertility of male rats.

1. Introduction

Interest in coordination chemistry is increasing continuously with the preparation of organic ligands containing a variety of donor groups, and it is multiplied manifold when the ligands have biological importance [1]. The number and diversity of nitrogen and sulfur chelating agents used to prepare new coordination and organometallic compounds have increased rapidly during the past few years [2–4]. Sulfur compounds and their metal complexes have antimicrobial activity and showed a high dependence on their substituents [5, 6]. Organic compounds containing –C₆H₄S moiety are well known for their significant biological activities. The activity may be due to the presence of multi-coordination centers having the ability to form stable chelates with the essential metal ions which the organisms need in their

metabolism. Interest in metal complexes of sulfur-nitrogen chelating agents, especially those formed from thiosemicarbazide [7] and S-alkyl/benzyl esters of dithiocarbamic acid has been stimulated by their interesting physicochemical properties and potentially useful pharmacological properties [8].

In the present paper, we describe the synthesis of metal complexes with Schiff bases using microwave-assisted technique. Bismuth compounds have been used in medicine for more than two centuries [9]. Applications have been widespread, due to bismuth's antiseptic, astringent, protective, antacid, antisecretory, and local gastrointestinal properties. Organoarsenicals were the first compounds applied for the successful treatment of syphilis (they have since been replaced by penicillin) and have been used as feed additives in livestock to prevent bacterial and parasitic infections. Organoarsenic(III) compounds are known to be biologically

active and find applications in various fields. Antimicrobial activities of some arsenic(III) complexes with Schiff bases have been evaluated [10].

The focus of our present communication is on the exploration of the studies on the synthetic, structural, and biological aspects of Bi(III) and As(III) complexes of some stereochemical as well as biological interest with monobasic bidentate thiosemicarbazone and hydrazinecarbodithioic acid ligands.

2. Experimental

2.1. Materials and Methods. BiCl₃ and Ph₃As as well as 3-acetylcoumarin and 4-hydroxycoumarin were purchased from Alfa Aesar and used as such. All the chemicals and solvents were dried and purified by standard methods. The reactions were carried out under strictly anhydrous conditions. Molecular weights were determined by the Rast camphor method. Chlorine was estimated by Volhard's method. Bismuth was estimated complexometrically. Arsenic was estimated iodometrically. Nitrogen was estimated by the Kjeldahl method, and sulfur was estimated by the Messenger method [11]. Carbon and hydrogen analyses were performed at the CDRI, Lucknow, India. The electronic spectra were recorded on a Varian-Cary/2390 spectrophotometer at RSIC, IIT, Chennai, India. Infrared spectra of the ligands and their complexes were recorded on a Nicolet Magna FTIR-550 spectrophotometer using KBr pellets. ¹H and ¹³C NMR spectra were recorded on a JEOL-AL-300 FT NMR spectrometer in DMSO-d₆. X-ray powder diffractogram of a representative compound was obtained on a Philip Model PW1840 automatic diffractogram using Cu(K α) target with Mg filter. The wavelength used was 1.540598 Å.

2.2. Preparation of the Ligands. Two different routes, microwave-assisted synthesis and conventional method, were employed for the synthesis of the ligands (see Scheme 1).

3-Formyl-4-chlorocoumarin. Phosphorus oxychloride (10 mL) was added dropwise to a solution of dimethylformamide (DMF) (20 mL) keeping the temperature below 5°C. Solution of 4-hydroxycoumarin (4.0 g) in DMF (10 mL) was then gradually added to the mixture with constant stirring and maintaining the temperature of the reaction mixture below 5°C. The reaction mixture was then allowed to stand at room temperature for 2 h and then heated on a steam bath for 1 h. After cooling, the reaction mixture was poured onto crushed ice and neutralized with sodium carbonate. A solid product was immediately formed which was crystallized from ethanol to give a yellow solid (80%), MP 115°C [12].

2.2.1. Microwave-Assisted Synthesis. The ligands were prepared by the condensation of 3-formyl-4-chlorocoumarin with thiosemicarbazide and 3-acetyl coumarin with S-benzylthiocarbamate in the presence of sodium acetate. The reaction mixture was irradiated in the microwave oven by taking 2-3 mL solvent. The reactions were completed in a short period (5-7 min). The resulting precipitate was

then recrystallized with alcohol and dried under vacuum. These were characterized and analysed before use. Elemental analyses (N and S) were conducted using the methods mentioned above, and their results were found to be in good agreement with the calculated values. The structures of the ligands have been shown in Figure 1 (see Scheme 2).

The above ligands were also synthesized by a thermal method, where instead of few drops of alcohol the starting materials of the ligands were dissolved in ~100 mL of alcohol and the contents were refluxed for nearly 4 to 5 h. The solution was then concentrated under reduced pressure, which on cooling gave crystalline precipitates. These were recrystallized twice in alcohol.

2.3. Preparation of the Metal Complexes

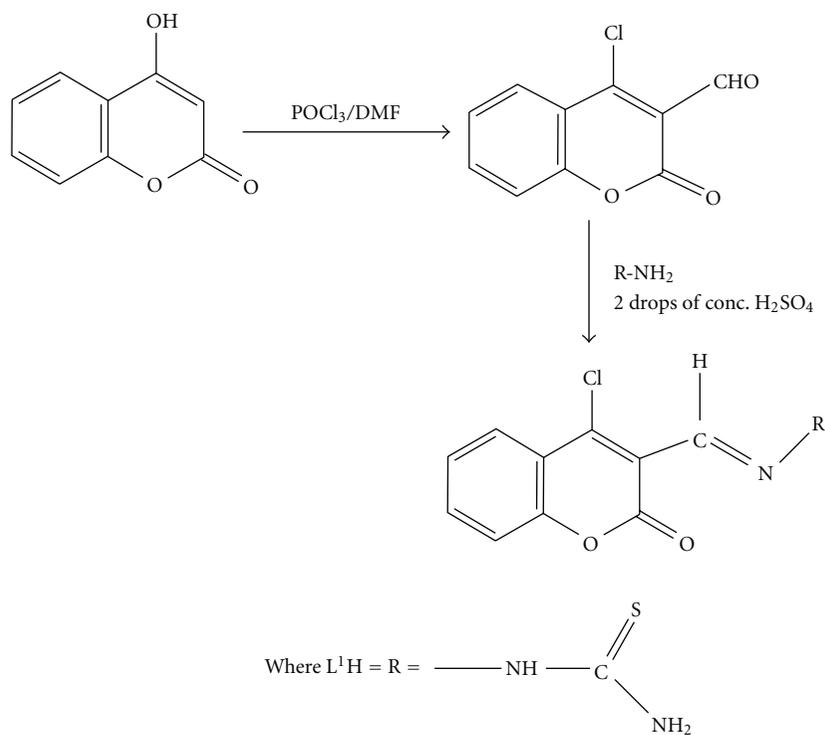
2.3.1. Synthesis of the Bi(III) Complexes. For the synthesis of the complexes, BiCl₃ and sodium salt of the ligands (prepared by adding the corresponding weight of sodium metal to the ligand) in 5 mL of dry methanol in 1 : 1 and 1 : 2 molar ratios were irradiated inside a microwave oven for about 5-8 min. The products were recovered from the microwave oven and dissolved in few mL of dry methanol. The white precipitate of sodium chloride formed during the course of the reaction was removed by filtration, and the filtrate was dried under reduced pressure. The resulting product was repeatedly washed with petroleum ether and then finally dried at 40-60°C/0.5 mmHg for 3-4 h. The purity was further checked by thin layer chromatography using silica gel-G.

Chlorobismuth(III) complexes were also synthesized by the thermal method. The reaction mixtures were heated under reflux for 13-17 h and filtered to remove NaCl, and the solvent was removed by the same procedure mentioned above, which was adopted to get the complexes.

2.3.2. Synthesis of Arsenic(III) Complexes. These complexes were prepared by the reaction of triphenylarsine in dry benzene with equimolar and bimolar amounts of the ligand which was previously dissolved in few mL of benzene. The reaction mixture was heated under reflux for 10-15 h. The complexes were repeatedly washed with dry n-hexane and dry pet-ether and dried at 40-60°C/0.5 mm of Hg pressure for 3-4 h. The purity was further checked by TLC using silica gel-G. The physico-chemical properties and analytical data of these complexes are listed below.

1-(4-Chloro-2-oxo-2H-chromen-3-yl)-methylene)-thiosemicarbazide (L¹H). (C₁₁H₈ClN₃O₂S) colour, brown, mol. wt., 281.35 (281.71), M. P., 128°C, C, 45.23 (46.90), H, 2.09 (3.52), N, 14.33 (14.92), S, 11.13 (11.80), yield 82%T (88% MW).

N'-[1-(2-oxo-2H-chrome-3-yl-ethylidene)]hydrazine-carbthionic acid benzyl ester (L²H). (C₁₉H₁₆N₂O₂S₂) colour, red, mol. wt., 366.85 (368.47), MP, 155°C, C, 60.91 (61.93), H, 4.19 (4.38), N, 6.56 (7.60), S, 16.54 (17.40), yield 77%T (86% MW).



SCHEME 1

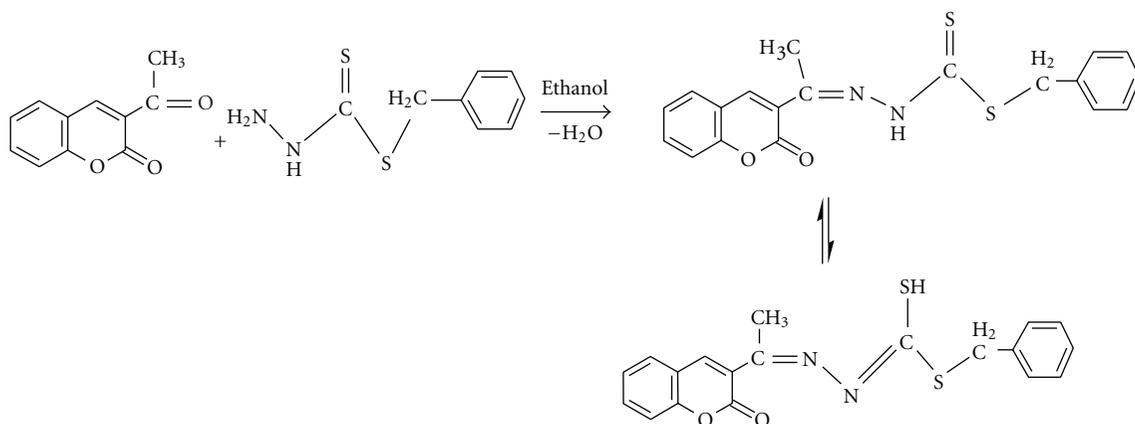
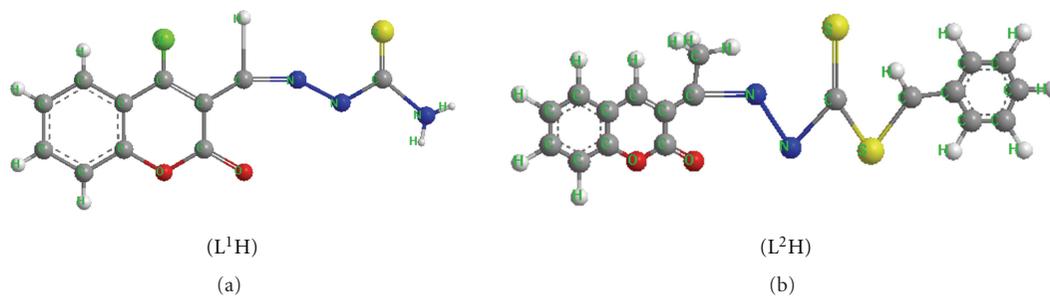
SCHEME 2: Synthesis of N' -[1-(2-oxo-2H-, chrome-3yl-ethylidene)]-hydrazinecarbodithionic acid benzyl ester (L^2H).

FIGURE 1: 3D molecular structures of the ligands.

Bismuth(III) Complexes with L¹H and L²H (Monofunctional Bidentate). [Cl₂Bi(L¹)], colour, dark brown, mol. wt., 558.14 (560.6), MP, 150°C, C, 22.56 (23.57), H, 1.18 (1.26), N, 6.56 (7.50), Cl, 17.25 (18.97), Bi, 36.99 (37.28), yield 75%T (89% MW).

[ClBi(L¹)₂], colour, brown, mol.wt. 782.56 (785.44), MP, 183°C, C, 34.25 (35.17), H, 2.00 (2.18), N, 9.12 (10.70), Cl, 8.23 (9.03), Bi, 25.63 (26.61), yield 73%T (90% MW).

[Cl₂Bi(L²)], colour, light green, mol. wt., 645.32 (647.35), MP, 105°C, C, 33.59 (35.25), H, 1.99 (2.34), N, 3.56 (4.33), S, 8.65 (9.91) Bi, 31.99 (32.28), yield 78%T (91% MW).

[ClBi(L²)₂], colour, light yellow, mol.wt. 978.25 (979.36), MP, 210°C, C, 45.25 (46.40), H, 2.85 (3.09), N, 4.82 (5.72), S, 12.36 (13.10) Bi, 20.63 (21.34), yield 77%T (90% MW).

Arsenic(III) Complexes with L¹H and L²H (Monofunctional Bidentate). Ph₂As(L¹) colour, yellow, mol. wt. 508.45 (509.84), MP, 96°C, C, 53.78 (54.18), H, 3.25 (3.36), N, 7.95 (8.24), S, 6.00.26 (6.29) As, 13.99 (14.70), yield 67%.

PhAs(L¹)₂, colour, orange, mol.wt. 612.65 (613.45), MP, 116°C, C, 46.78 (47.14), H, 2.25 (2.68), N, 9.12 (11.78), S, 7.56 (8.99) As, 9.97 (10.50), yield 68%.

Ph₂As(L²) colour, orange, mol. wt. 595.46 (596.59), MP, 166°C, C, 61.71 (62.41), H, 3.96 (4.22), N, 4.12 (4.70), S, 9.26 (10.75) As, 11.99 (12.56), yield 69%.

PhAs(L²)₂, colour, red, mol.wt. 885.25 (886.95), MP, 174°C, C, 58.32 (59.58), H, 2.89 (3.98), N, 5.62 (6.32), S, 13.21 (14.46) As, 7.97 (8.45), yield 70%.

3. Biological Assay

All the compounds were evaluated for their antimicrobial properties. MIC was recorded as minimum concentration which inhibits the growth of microorganism. The results obtained were compared with those of the standard drug streptomycin for bacteria and bavistin for fungi. The microorganisms used were *Escherichia coli* (ATCC25922), *Bacillus subtilis* (ATCC6633), *Fusarium oxysporum* (ATCC7808) and *Rhizopus nigricans* (ATCC6227b). Both ligands and their corresponding metal complexes have been tested in vivo for their antifertility activity in male albino rats.

3.1. In Vitro Study

3.1.1. Antibacterial Screening. The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC25922) and *Bacillus subtilis* (ATCC6633) by paper disc plate method [13]. Each compound was dissolved in DMSO, and solutions of the concentrations (500 and 1000 ppm) were prepared separately. Paper discs of Whatman filter paper (no. 42) of uniform diameter (5 mm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solutions were placed aseptically in the petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with *Escherichia coli* (ATCC25922) and *Bacillus subtilis* (ATCC6633) separately. The petri dishes were

incubated at 37°C, and the inhibition zones were recorded after 24 h of incubation. The antibacterial activity of standard antibiotic streptomycin was also recorded using the same procedure. The medium with DMSO as a solvent was used as a negative control. The experiments were performed in triplicates. The % activity index for the complex was calculated by the formula as follows:

% Activity Index

$$= \frac{\text{zone of inhibition by test compound (diameter)} \times 100}{\text{zone of inhibition by standard (diameter)}} \quad (1)$$

3.1.2. Antifungal Screening. The antifungal activities of the standard fungicide (Bavistin), ligands, and complexes were tested for their effect on the growth of microbial cultures and studied for their interaction with *Fusarium oxysporum* (ATCC7808) and *Rhizopus nigricans* (ATCC6227b) using Czapek's agar medium [14] having the composition, glucose 20 g, starch 20 g, agar-agar 20 g, and distilled water 1000 mL. To this medium was added requisite amount of the compounds after being dissolved in methanol so as to get the certain concentrations (100 and 200 ppm). The medium then was poured into Petri Plates, and the spores of fungi were placed on the medium with the help of inoculum's needle. These Petri Plates were wrapped in polythene bags containing a few drops of alcohol and were placed in an incubator at ± 30°C. The controls were also run, and three replicates were used in each case. The linear growth of the fungus was recorded by measuring the diameter of the fungal colony after 96 h and the percentage inhibition was calculated by the equation

$$\% \text{ of inhibition} = \frac{C - T}{C} \times 100, \quad (2)$$

where *C* and *T* are the diameters of the fungal colony in the control and the test plates, respectively.

3.1.3. Determination of Minimum Inhibitory Concentration (MIC). The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation [15]. The determination of the MIC involves a semiquantitative test procedure, which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. The minimum inhibitory concentration was determined by microbroth dilution method [16]. Inoculum of the overnight culture was prepared. In a series of tubes, 1 mL each of complex solution with different concentrations was taken and 0.4 mL of the inoculum was added to each tube. Further 3.5 mL of the sterile water was added to each of the test tubes. These test tubes were incubated for 18 h and observed for the presence of turbidity. The absorbance of the suspension of the inoculum was observed with spectrophotometer at 555 nm. The end result of the test was the minimum concentration of the antimicrobial agent (test materials) which gave a clear solution, that is, no visual growth [17, 18].

3.2. In Vivo Study

3.2.1. Antifertility Activity. The activity of the synthetic products towards the biological systems is an important feature of the current research, and the Schiff base metal complexes play a significant role in this direction. In view of such potential interest in these biologically active compounds, the antifertility activity of some selected compounds has been studied on male albino rats. Proven-fertile male albino rats of the Wistar strain, weighing 190–200 g (90–100 days old), were used. They were housed in clean, polypropylene cages and maintained under standard conditions (12 h light/12 h dark; $20 \pm 5^\circ\text{C}$; 35–60% relative humidity). Rat feed (Ashirwad Industries Ltd., Chandigarh, India) and water were provided *ad libitum*. Animals were divided into seven groups of six animals each. Group A served as a vehicle (olive oil) treated control. In the groups Band C, the ligands L^1H and L^2H , and in animals of groups D, E, F, and G the compounds $[\text{Ph}_2\text{As}(L^1)]$, $[\text{PhAs}(L^1)_2]$, $[\text{Cl}_2\text{Bi}(L^2)]$ and $[\text{ClBi}(L^2)_2]$ (30 mg/kg b.wt) suspended in olive oil were given orally for a period of 60 days. At the end of experiment, all the animals were screened for fertility test and autopsied. The reproductive organs were removed, washed with distilled water, dried weight, and processed for biochemical examination. Sperm mobility in cauda epididymis and sperm density in testes and cauda epididymis were assessed. The protein, sialic acid, glycogen, acid and alkaline phosphatase of testes, and serum testosterone were determined by standard laboratory techniques. Results were analysed statistically using student's *t*-test.

4. Results and Discussion

The elemental analysis and spectral data suggested the formation of the ligands (L^1H and L^2H) and their complexes of the type $[\text{Cl}_2\text{Bi}(L^1)]$, $[\text{ClBi}(L^1)_2]$, $[\text{Cl}_2\text{Bi}(L^2)]$, $[\text{ClBi}(L^2)_2]$, $[\text{Ph}_2\text{As}(L^1)]$, $[\text{PhAs}(L^1)_2]$, $[\text{Ph}_2\text{As}(L^2)]$, and $[\text{PhAs}(L^2)_2]$. The resulting complexes are colored solid, which were found to be soluble in DMF and DMSO. Molecular weight determinations showed that they are monomeric in nature. The molar conductances of 10^{-3} M solutions of the complexes in dry DMF lie in the $12\text{--}15\text{ ohm}^{-1}\text{ cm}^2\text{ mol}^{-1}$ range, indicating that they are nonelectrolytes.

4.1. UV Spectra. The electronic spectra of the ligands show a band due to the $>\text{C}=\text{N}$ chromophore observed at 365 nm that shifts to the lower wavelength region in the spectra of the complexes. Such a shift in the $n\text{-}\pi^*$ band is probably due to the donation of the lone pair of electrons by the nitrogen of the ligand to the central metal atom. Two bands at 319–320 and 298–300 nm assigned to $\pi\text{-}\pi^*$ transitions, within the benzene and coumarin rings and azomethine linkage also show some shifting in their position due to bond formation.

4.2. IR Spectra. The IR spectra of the free ligands L^1H and L^2H display absorption bands at 3150–3250, 1630–1635, and 1050–1080 cm^{-1} assigned to ($-\text{NH}$), ($>\text{C}=\text{N}$), and ($>\text{C}=\text{S}$), respectively. The bands at ca. 1735 cm^{-1} due to ($>\text{C}=\text{O}$) of

lactone moiety of the ligands remain almost unchanged in the complexes indicating their non-involvement in complexation. The broad band, due to ($-\text{NH}$) vibrations, disappears in the spectra of the complexes, indicating the deprotonation of this group on coordination with the metal atom. The marked shift ($10\text{--}20\text{ cm}^{-1}$) of ($>\text{C}=\text{N}$) band observed in all the complexes indicates the involvement of azomethine nitrogen upon complexation. The band due to ($>\text{C}=\text{S}$) is shifted towards lower frequencies in the complexes indicating coordination of sulfur to the central metal atom. The spectra of the free ligands display two sharp bands at 3340–3500 and 3350–3490 cm^{-1} due to asym. and sym. vibrations of NH_2 group, respectively, which remain at almost the same positions in the spectra of the complexes, suggesting that the NH_2 group is not involved in chelation. Some new bands observed in the regions 439–442 and 422–425 cm^{-1} for $\nu(\text{As}-\text{N})$, $\nu(\text{As}-\text{S})$, respectively. The band in 450–470 cm^{-1} range may be assigned to $\nu(\text{As}-\text{Ph})$ vibrations in the respective complexes.

4.3. ^1H NMR and ^{13}C NMR Spectra. Further evidence for the coordinating mode of the ligands was obtained from ^1H NMR spectra. The ^1H NMR spectra of the ligands recorded in DMSO-d_6 exhibit a broad peak at δ 8.59–8.30 ppm due to $-\text{NH}$ proton. The $-\text{NH}$ proton signal of the ligands disappears in the complexes. The absence of this signal in these complexes suggests that this proton has been lost via thioenolization of $>\text{C}=\text{S}$ group and coordination of sulfur to the metal atoms, respectively, has taken place. The $>\text{CH}=\text{N}$ proton moves downfield in the complexes in comparison with its original positions in the ligands due to coordination of $>\text{C}=\text{N}$ to the metal atom. The ^1H NMR spectrum of the ligand (L^2H) exhibits $-\text{CH}_2$ proton signals at δ 4.15–4.16 ppm, and aromatic proton signals at δ 6.38–7.50 ppm and these remain at the same position in the spectra of the metal complexes. In the case of ^{13}C NMR spectra, the considerable shifts in the position of the carbon atoms attached to the different participating groups clearly indicate the bonding of azomethine nitrogen to the metal atom. The signal due to lactone $>\text{C}=\text{O}$ carbon atoms remains almost same position in the ^{13}C NMR spectra of the metal complexes while mark shifts in the position of the carbon atoms attached to the sulfur atom shows that proton is lost via thioenolization. Spectral data of the ligands and their metal complexes are given below.

L^1H . IR (KBr, $\nu\text{ cm}^{-1}$): 3150 cm^{-1} ($-\text{NH}$ stretching), 1635 cm^{-1} ($>\text{C}=\text{N}$), 1080 cm^{-1} ($>\text{C}=\text{S}$), 1735 cm^{-1} , ($>\text{C}=\text{O}$); ^1H NMR (600 MHz, DMSO-d_6 , δ ppm), δ 8.69 ppm (1H, $-\text{NH}$), δ 6.70 ppm (1H, $\text{HC}=\text{N}$) δ 3.46 ppm ($-\text{NH}_2$), δ 6.72–8.15 ppm (ArH); ^{13}C NMR: 165.65 ($>\text{C}=\text{N}$), 170.86 ($>\text{C}=\text{S}$).

L^2H . IR (KBr, $\nu\text{ cm}^{-1}$): 3250 cm^{-1} ($-\text{NH}$ stretching), 1622 cm^{-1} ($>\text{C}=\text{N}$), 1050 cm^{-1} ($>\text{C}=\text{S}$), 1735 cm^{-1} ($>\text{C}=\text{O}$); ^1H NMR (600 MHz, DMSO-d_6 , δ ppm): δ 8.30 ppm (1H, $-\text{NH}$), δ 4.15 ppm ($-\text{S}-\text{CH}_2$), δ 7.50–6.38 ppm (ArH); ^{13}C NMR: 166.55 ($>\text{C}=\text{N}$), 172.66 ($>\text{C}=\text{S}$).

$[Cl_2Bi(L^1)]$. IR (KBr, ν cm⁻¹): 1645 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 6.80 ppm (1H, HC=N) δ 3.47 ppm (-NH₂), δ 6.73–8.09 ppm (ArH); ¹³C NMR: 169.20 (>C=N), 162.56 (>C=S).

$[ClBi(L^1)_2]$. IR (KBr, ν cm⁻¹): 1643 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 6.90 ppm (1H, HC=N) δ 3.43 ppm (-NH₂), δ 6.70–8.10 ppm (ArH); ¹³C NMR: 170.40 (>C=N), 160.80 (>C=S).

$[Cl_2Bi(L^2)]$: IR (KBr, ν cm⁻¹): 1628 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 3.41 ppm (-NH₂), δ 4.15 ppm (-S-CH₂), δ 6.73–8.09 ppm (ArH); ¹³C NMR: 168.10 (>C=N), 161.66 (>C=S).

$[ClBi(L^2)_2]$. IR (KBr, ν cm⁻¹): 1630 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 3.42 ppm (-NH₂), δ 4.16 ppm (-S-CH₂), δ 6.70–8.00 ppm (ArH); ¹³C NMR: 167.10 (>C=N), 160.86 (>C=S).

$[Ph_2As(L^1)]$. IR (KBr, ν cm⁻¹): 1625 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O), 438 cm⁻¹ (As-S), 423 cm⁻¹ (As ← N); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 6.90 ppm (1H, HC=N) δ 3.45 ppm (-NH₂), δ 6.35–8.23 ppm (ArH); ¹³C NMR: 160.10 (>C=N) 162.50 (>C=S).

$[PhAs(L^1)_2]$. IR (KBr, ν cm⁻¹): 1628 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O), 439 cm⁻¹ (As-S), 422 cm⁻¹ (As ← N); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 6.80 ppm (1H, HC=N) δ 3.43 ppm (-NH₂), δ 6.89–8.36 (ArH); ¹³C NMR: 161.10 (>C=N), 160.00 (>C=S).

$[Ph_2As(L^2)]$. IR (KBr, ν cm⁻¹): 1615 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O), 440 cm⁻¹ (As-S), 422 cm⁻¹ (As ← N); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 3.41 ppm (-NH₂), δ 4.16 ppm (-S-CH₂), δ 6.50–8.00 ppm (ArH); ¹³C NMR: 159.30 (>C=N), 162.86 (>C=S).

$[PhAs(L^2)_2]$. IR (KBr, ν cm⁻¹): 1610 cm⁻¹ (>C=N), 1735 cm⁻¹ (>C=O), 441 cm⁻¹ (As-S), 420 cm⁻¹ (As ← N); ¹H NMR (600 MHz, DMSO-d₆, δ ppm): δ 3.40 ppm (-NH₂), δ 4.15 ppm (-S-CH₂), δ 6.80–8.30 (ArH); ¹³C NMR: 158.70 (>C=N), 160.46 (>C=S).

4.4. X-Ray Structure Determination. The possible lattice dynamics of the finely powdered product $[ClBi(L^1)_2]$ has been deduced on the basis of X-ray powder diffraction studies. The observed interplanar spacing values (“*d*” 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 unit cell parameters as *a* = 12.2578,

b = 18.2154, *c* = 8.359, maximum deviation of 2-theta = 0.028 and alpha = 90, beta = 90, gamma = 90 at the wavelength = 1.540598.

On the basis of the above discussion, the structures shown in Figures 2 and 3 have been proposed for the complexes.

5. Biological Results and Discussion

The results of antimicrobial activity are shown in Figures 3 and 4. Both the ligands and their metal complexes were sensitive against all the fungal and bacterial strains. The antimicrobial screening data indicate that the metal complexes are more potent antimicrobial agents than the free ligands.

MIC values for the ligands and their complexes are shown in Figure 5. Minimum inhibitory concentration of the ligands and their metal complexes was determined against four tested strains. Compounds $[ClBi(L^2)_2]$ and $[PhAs(L^2)_2]$ show low MIC values at 10 µg/mL for bacterial strain *Bacillus subtilis* and at 16 µg/mL for fungal strain *Fusarium oxysporum*. Metal complexes are more active against fungal strains in comparison to bacterial strains.

The biological activity of the ligands exhibited a marked enhancement on coordination with the metal ions against all the test bacterial/fungal strains which shows that metal chelates are more active than the ligands. This may be explained by Tweedy’s chelation theory [19] according to which chelation reduces the polarity of the central metal atom because of the partial sharing of its positive charge with the ligand [20], which favours permeation of the complexes through the lipid layer of cell membrane [21]. Other factors such as solubility, conductivity, and dipole moment which are affected by the presence of metal ions may also be possible reasons for increasing the biological activity of the metal complexes as compared to the corresponding ligands.

The results of antifertility showed that administration of ligands and its metal complexes did not affect the body weights of treated animals. However, a significant reduction was observed in the weights of testes, epididymis, and accessory sex organs (seminal vesicles and ventral prostate) in rats treated with these compounds than those of the control group.

A significant decline in sperm density in testes and epididymis were noticed in rats treated with ligand and its metal complexes. Sperm motility in cauda epididymis was also decreased significantly in experimental animals. Decrease in sperm motility and density could compromise the fertility.

The study revealed a marked reduction in sialic acid and glycogen content in testes. However, testicular cholesterol and protein as well as acid and alkaline phosphatase activity were increased. The levels of serum testosterone were decreased significantly after ligands (L¹H) and (L²H) and their Bi and As complexes.

Our results showed that the weight of testes and accessory sex organs were significantly reduced in rats treated with ligands (L¹H), (L²H) and their Bi and As complexes treated

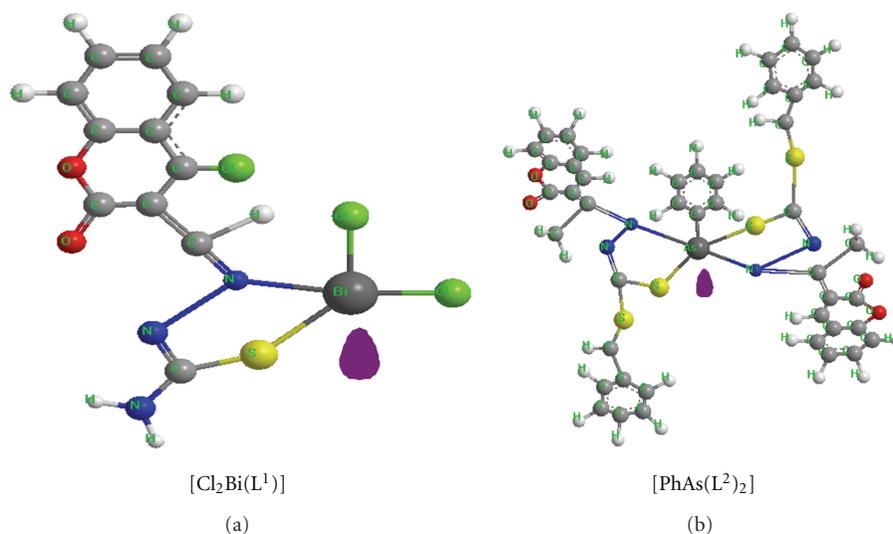


FIGURE 2: 3-D Molecular structures of the metal complexes.

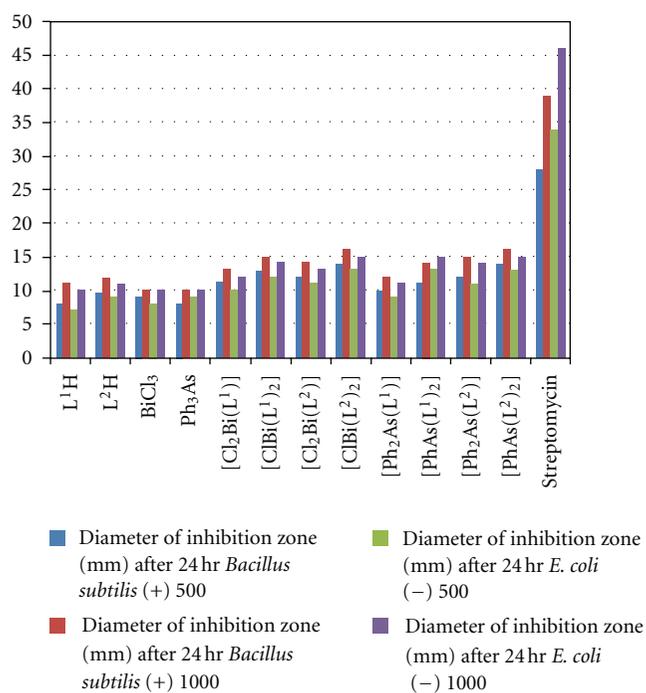


FIGURE 3: Antibacterial screening of the ligands and their complexes.

rats in comparison to control rats. The decreased weight of testes may be due to reduced tubule size spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells, a site of steroid biosynthesis. The observed reduction in weight of accessory sex organs may be due to antiandrogenic activity of the compounds [22]. Suppression of gonadotropins might have caused decrease in sperm density in testes [23]. The negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and

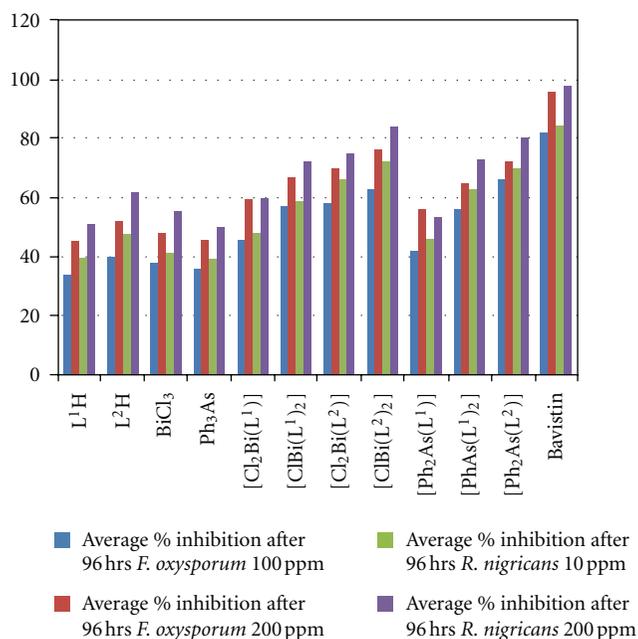


FIGURE 4: Antifungal screening of the ligands and their complexes.

altered biochemical milieu of cauda epididymis. Ligands (L¹H) and (L²H) and their Bi(III) and As(III) complexes induces biochemical changes in testes. A significant decrease in testicular sialic acid content in present study indicated the suppressed activity of androgen and gonadotropin. Low levels of sialic acid concentration in treated rats might be due to loss of viability and fertilizing ability of spermatozoa. The elevation in the testicular protein may be due to hepatic detoxification activities caused by these compounds which results in inhibitory effect on the activities of enzymes involved in the androgen biotransformation. A significant increase in testicular cholesterol after ligands (L¹H) and

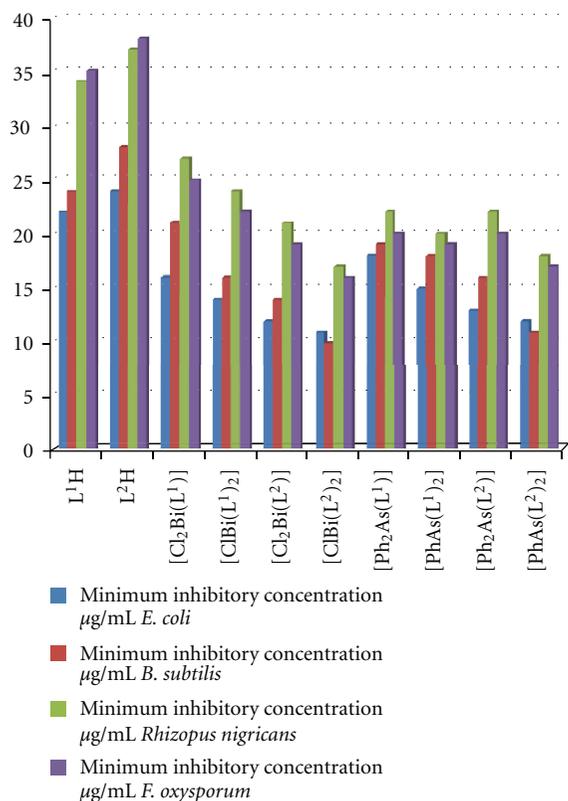


FIGURE 5: Minimum inhibitory concentration of the ligands and their complexes.

(L²H) and their Bi(III) and As(III) complexes treatment suggests suppressed androgen biosynthesis.

A fall in glycogen levels in testes eventually decreases spermatogenic process [24]. Furthermore, increases in the acid and alkaline phosphatase activity in testes indicate an impairment of functional integrity of the testes.

6. Conclusion

We describe the synthesis, characterization, and biological activity of Bi(III) and As(III) complexes. On the basis of analytical and spectral data, tetra- and pentacoordinated geometries have been proposed for the Bi(III) and As(III) complexes. The antimicrobial activity results indicated that the complexes showed promising antibacterial and antifungal activities, but compounds [ClBi(L¹)₂] and [PhAs(L²)₂] showed highest activity against bacterial strain *B. subtilis* ATCC6633 (MIC = 10 µg/mL) and fungal strain *F. oxysporum* ATCC7808 (MIC = 16 µg/mL). The results suggested that the ligand (L²H) is most effective in reducing fertility, and addition of bismuth and arsenic moiety to this ligand enhanced its activity.

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