



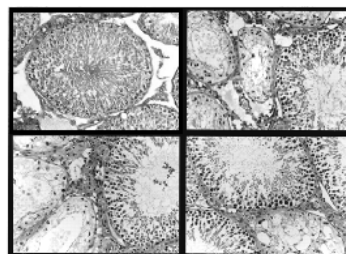
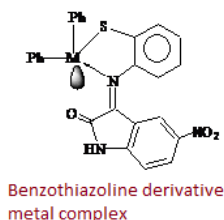
## Synthesis, spectral, antimicrobial and antiandrogenic studies of main group metal complexes with biologically potent benzothiazoline

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### ABSTRACT



New antibacterial and antifungal Schiff's base derived from substituted benzothiazoline ( $N^{\circ}SH$ ), as well as its main group metal complexes incorporating antimony(III), bismuth(III) and arsenic(III) were synthesized with conventional as well as microwave irradiation method, characterized and screened for their *in-vitro* antibacterial as well as antifungal activity against Gram-negative (*Escherichia coli*), Gram-positive (*Staphylococcus aureus*) microbial strains and *Fusarium oxysporum* and *Aspergillus niger* fungal strains. The metal complexes show more antimicrobial potency as compared to the uncomplexed Schiff's base. The ligand and its  $BiCl(N^{\circ}S)_2$  and  $PhAs(N^{\circ}S)_2$  compounds have been screened *in vivo* in male albino rats to test their antifertility property and results indicated that the administration of aforesaid compounds in male rats brought about an interference with spermatogenesis which ultimately caused infertility.

**Keywords:** Benzothiazoline; Main group metal complexes; Antimicrobial activity; Antiandrogenic effects; Microwave assisted synthesis

### INTRODUCTION

Chemical synthesis using reduced amount of organic solvent leads to a clean, efficient and economical technology (green chemistry). Further, safety of handling reaction apparatus is increased and work-up procedure is considerably simplified. In addition, a rapid and homogenous heating using microwave

irradiation in an unconventional method assist in reaction rate acceleration, milder reaction conditions and higher chemical yield.<sup>1</sup>

Organoantimony compounds exhibit significant antimicrobial as well as antitumor activities,<sup>2</sup> by their associated cytostatic activity. In past few years organoantimony complexes have been reported as antispermatogetic agents.<sup>3</sup> Bismuth compounds have been used in medicine for more than two centuries.<sup>4</sup> Their applications have been widespread, due to antiseptic, astringent, protective, antacid, antisecretory, and local gastrointestinal properties of Bismuth.<sup>5</sup> Antimicrobial activities of some arsenic(III) complexes with Schiff bases

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have been evaluated. It has been found that an ideal male contraceptive will effectively and reversibly arrest the production of spermatozoa (or block their fertilizing potential) without affecting hormonal status, libido, or accessory sex organs and their functions.<sup>6</sup> Herein, we reported new range of antimony, arsenic and bismuth Schiff base complexes using conventional and microwave methods, a comparison have also been reported. Further, metal complexes have been biologically evaluated for their antimicrobial and antispermatogenic activities.

## EXPERIMENTAL

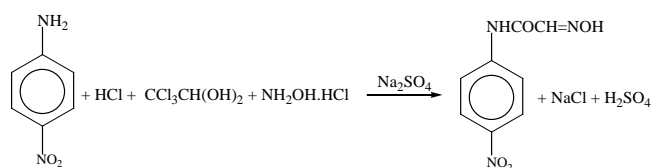
### Reagents and materials

PhSbCl<sub>2</sub>, BiCl<sub>3</sub>, SbCl<sub>3</sub> and Ph<sub>3</sub>As and 2-aminothiophenol were used for the synthesis of the complexes and ligand. PhSbCl<sub>2</sub> was prepared by the reported method.<sup>7</sup> The chlorostibane(III) (SbCl<sub>3</sub>) was purified by distillation before use. All chemicals and solvents used were dried and purified by standard methods.

### Synthesis of the ligand

#### 5-Nitro-1H-indol-2,3-dione

**First Step:** A solution of *p*-nitro aniline (0.01 mol) in HCl (5.5 mL) was added to a solution of chloral hydrate (0.11 mol, 18.12 g) in water (250 mL) followed by addition of a solution of hydroxylamine hydrochloride (0.33 mol, 22.0 g) in water (amount). The reaction mixture was heated to start vigorous boiling within 45 minutes. The boiling was continued for further 10 minutes. The mixture was cooled when coloured needles separated out. These were filtered and recrystallized from ethanol. The synthetic procedure has been shown in Figure 1.

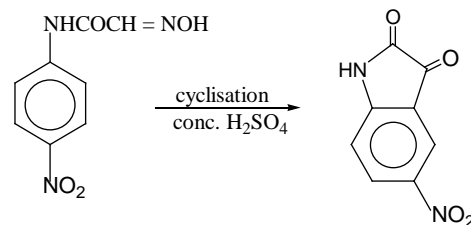


*p*-Nitro aniline

*p*-Nitro-isonitrosoacetanalide

**Figure 1**

**Second Step:** *p*-Nitro-isonitrosoacetanalide (0.05 mol) was added to the concentrated sulphuric acid (50 mL) in about 30 minutes with constant stirring. After the addition was complete, the reaction mixture was heated at 80°C for 10 minutes and poured into ten times of crushed ice. The resultant precipitate was filtered after an hour and dried in air. It was purified by recrystallization from glacial acetic acid. The cyclization reaction has been shown in Figure 2.



*p*-Nitro-isonitrosoacetanalide

5-Nitro-1H-indol-2,3-dione

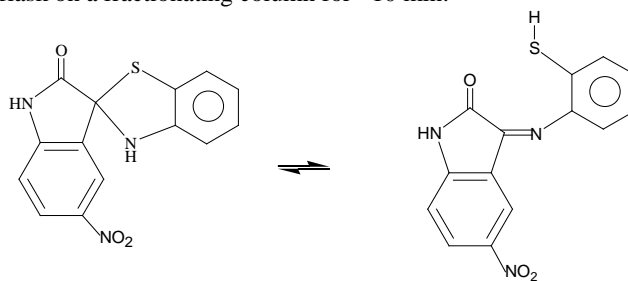
**Figure 2**

### Synthesis of 5-nitro-1H-indol-2,3-dione benzothiazoline (BztH)

Substituted benzothiazoline (BztH) was prepared by the condensation of 5-nitro-1H-indol-2,3-dione with 2-aminothiophenol in 1:1 molar ratio in alcohol. The reaction mixture was stirred magnetically for 3-4 hours, separated out solid was filtered, purified and recrystallized from alcohol and dried *in vacuo* (Figure 2).

#### Preparation of sodium salt of the ligand (BztNa)

Sodium salt of 5-nitro-benzothiazoline (BztNa) was prepared by mixing methanolic solution of BztH (0.81 g, 2.7090 mmol) and sodium metal (0.06g, 2.609 mmol) into 1:1 molar ratio. This mixture was refluxed in into a 100 mL R.B. flask on a fractionating column for ~10 min.



Ring form

Azomethine form

**Figure 3.** Tautomeric forms of the BztH

BztH (C<sub>14</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub>S), Green Solid, 140°C, (%)Yield 85, Found (Calcd.) (%), N 13.91 (14.04), S 10.16 (10.71), Mol. Wt. Found (Calcd.), 286.13 (299.30).

### Synthesis of the metal complexes

#### Synthesis of the antimony(III) and bismuth(III) substitution complexes

##### Conventional method

PhSbCl<sub>2</sub> (0.72g, 0.61g), SbCl<sub>3</sub> (0.92g, 0.82g) and BiCl<sub>3</sub> (1.27g, 1.31g) metal starting materials were mixed with sodium salt of BztH in 1:1 and 1:2 molar ratios taken in ~15 mL of methanol into a 100 mL round bottom flask. The reaction mixture was refluxed for 10-12 hours and then cooled at the room temperature. After the completion of the reaction the excess of the solvent was distilled off and white precipitate of

Table 1. Synthetic and analytical data of antimony(III), bismuth(III) and arsenic(III) complexes of 5-nitro-benzothiazoline (BztH)

S.No.	Reactants in g			Molar ratio	Compounds / Empirical formula	Product and Color	M.P (°C)	Elemental Analysis (%) <sup>a</sup>				Mol. Wt <sup>a</sup>
	Starting Material I	Ligand C <sub>14</sub> H <sub>9</sub> O <sub>3</sub> N <sub>3</sub> S	Sodium					N	S	Cl	M	
C-1	PhSbCl <sub>2</sub> (0.72)	(0.81)	(0.06)	1:1	[PhSbCl(N <sup>∘</sup> S)] (C <sub>20</sub> H <sub>13</sub> O <sub>3</sub> N <sub>3</sub> SCl)Sb	Light green, Solid	170	7.14 (7.93)	5.78 (6.02)	6.13 (6.66)	22.43 (22.86)	527.24 (532.59)
C-2	PhSbCl <sub>2</sub> (0.61)	(0.68)	(0.10)	1:2	[PhSb(N <sup>∘</sup> S) <sub>2</sub> ] (C <sub>34</sub> H <sub>21</sub> O <sub>6</sub> N <sub>6</sub> S <sub>2</sub> )Sb	Dark green, Solid	120 <sup>b</sup>	10.13 (10.56)	7.46 (8.06)	-	14.97 (15.31)	789.37 (795.43)
C-3	SbCl <sub>3</sub> (0.92)	(1.21)	(0.09)	1:1	[SbCl <sub>2</sub> (N <sup>∘</sup> S)] (C <sub>14</sub> H <sub>8</sub> O <sub>3</sub> N <sub>3</sub> SCl <sub>2</sub> )Sb	Brown, Solid	210	8.04 (8.56)	6.31 (6.53)	14.44 (14.44)	24.16 (24.80)	485.38 (490.95)
C-4	SbCl <sub>3</sub> (0.82)	(1.07)	(0.16)	1:2	[SbCl(N <sup>∘</sup> S) <sub>2</sub> ] (C <sub>28</sub> H <sub>16</sub> O <sub>6</sub> N <sub>6</sub> S <sub>2</sub> Cl)Sb	Grey, Solid	205	10.45 (11.15)	8.16 (8.51)	4.36 (4.65)	15.84 (16.16)	760.25 (753.39)
C-5	SbCl <sub>3</sub> (1.21)	(1.59)	-	1:1	[SbCl <sub>3</sub> (N <sup>∘</sup> SH)] (C <sub>14</sub> H <sub>9</sub> O <sub>3</sub> N <sub>3</sub> SCl <sub>3</sub> )Sb	Light brown, Solid	199	7.19 (7.97)	5.46 (6.08)	19.43 (20.17)	22.85 (23.09)	533.74 (527.41)
C-6	BiCl <sub>3</sub> (1.27)	(1.20)	(0.09)	1:1	[BiCl <sub>2</sub> (N <sup>∘</sup> S)] (C <sub>14</sub> H <sub>8</sub> O <sub>3</sub> N <sub>3</sub> SCl <sub>2</sub> )Bi	Parrot green, Solid	182	7.06 (7.27)	5.07 (5.55)	11.83 (12.26)	35.07 (36.15)	583.13 (578.17)
C-7	BiCl <sub>3</sub> (1.31)	(1.24)	(2.62)	1:2	[BiCl(N <sup>∘</sup> S) <sub>2</sub> ] (C <sub>28</sub> H <sub>16</sub> O <sub>6</sub> N <sub>6</sub> S <sub>2</sub> Cl)Bi	Light brown, Solid	179	9.16 (9.99)	7.16 (7.63)	3.98 (4.22)	24.09 (24.85)	846.43 (841.00)
C-8	BiCl <sub>3</sub> (1.12)	(1.06)	-	1:1	[BiCl <sub>3</sub> (N <sup>∘</sup> SH)] (C <sub>14</sub> H <sub>9</sub> O <sub>3</sub> N <sub>3</sub> SCl <sub>3</sub> )Bi	Reddish brown, Solid	186	6.00 (6.84)	5.13 (5.22)	16.76 (17.30)	33.76 (34.00)	619.39 (614.63)
C-9	Ph <sub>3</sub> As (0.87)	(0.85)	-	1:1	[Ph <sub>2</sub> As(N <sup>∘</sup> S)] (C <sub>26</sub> H <sub>18</sub> O <sub>3</sub> N <sub>3</sub> S)As	Grey, Solid	178 <sup>b</sup>	7.03 (7.99)	5.46 (6.10)	-	13.76 (13.88)	530.73 (525.42)
C-10	Ph <sub>3</sub> As (0.91)	(1.78)	-	1:2	[PhAs(N <sup>∘</sup> S) <sub>2</sub> ] (C <sub>34</sub> H <sub>21</sub> O <sub>6</sub> N <sub>6</sub> S <sub>2</sub> )As	Yellowish green, solid	195	10.46 (11.23)	7.06 (8.56)	-	9.72 (10.00)	753.39 (748.59)

<sup>a</sup>Calculated values are given in parentheses. <sup>b</sup>Decomposition temperature of the compounds.

sodium chloride was removed by filtering it through an alkoxy funnel. Compound was repeatedly washed with n-hexane followed by drying in vacuum for 2-3 hours to get the final purified product. The purity was further checked by TLC using silica gel-G. The physical properties and analytical data are enlisted in Table 1.

#### Microwave method<sup>8</sup>

In microwave-assisted synthesis the reaction mixtures (aforesaid) were taken in 50 mL conical flask, covered with glass wool and then irradiated for 4-7 minutes inside the microwave oven. Anhydrous conditions were attained by using a beaker with silica gel or anhydrous CaCl<sub>2</sub> (the said beaker filled with moisture absorbing substance is known as “Dummy”) which was placed near the reaction vessel during the synthesis. The products were recovered and dissolved in a

1.0 mL dry methanol, where the white precipitate of sodium chloride, formed during the course of the reaction, which further removed by filtration. The products were dried under reduced pressure after repeatedly washing (with n-hexane), dried at 40-60°/ 0.5 mm of Hg pressure for 3-4 hour. The purity was further checked by TLC using silica gel-G. A comparison between two synthetic methods have been reported in Table 2.

#### Synthesis of antimony(III) and bismuth(III) addition products (C-5 and C-8)

##### Conventional method

Antimony(III)chloride (1.21 g) and Bismuth(III)chloride (1.12 g) were mixed with BztH in 1:1 molar ratios. The reaction mixture dissolved in dry CCl<sub>4</sub> (~6 mL) was taken in 100 mL round bottom flask fitted with silica gel guard tube for anhydrous condition and then stirred for ~12 h at room

Table 2. Comparison between conventional and microwave method of synthesis and IR Spectral data (cm<sup>-1</sup>) of (BztH) and its corresponding compounds

Compound	Yield %		Time		ν(>C=N)	νNH	ν(M-N)	ν(M-S)
	Thermal	Microwave	Thermal (h)	Microwave (min)				
BztH	-	-	-	-	-	3315	-	-
[PhSbCl(N <sup>∘</sup> S)]	45	69	12	7	1610	-	410	384
[PhSb(N <sup>∘</sup> S) <sub>2</sub> ]	38	62	11	5	1620	-	417	387
[SbCl(N <sup>∘</sup> S) <sub>2</sub> ]	67	78	14	8	1605	-	426	375
[SbCl <sub>3</sub> (N <sup>∘</sup> SH)]	69	72	8	6	-	3296	450	-
[BiCl <sub>2</sub> (N <sup>∘</sup> S)]	57	85	14	10	1617	-	320	235
[BiCl(N <sup>∘</sup> S) <sub>2</sub> ]	37	65	13	15	1545	-	328	256
[BiCl <sub>3</sub> (N <sup>∘</sup> SH)]	60	85	9	6	-	3307	332	-
[Ph <sub>2</sub> As(N <sup>∘</sup> S)]	-	-	-	-	1569	-	439	422
[PhAs(N <sup>∘</sup> S) <sub>2</sub> ]	-	-	-	-	1570	-	440	435

Table 3. <sup>1</sup>H NMR spectral data (δ, ppm) of BztH and its corresponding complexes

Compound	-NH (Ring) (bs)	-NH (Free) (bs)	Aromatic proton (m) / *Ph-M
BztH	12.02	4.25	6.65 – 7.20
[PhSbCl(N <sup>∘</sup> S)]	11.98	-	6.74 – 8.02
[PhSb(N <sup>∘</sup> S) <sub>2</sub> ]	11.61	-	6.72 – 8.00
[SbCl <sub>2</sub> (N <sup>∘</sup> S)]	11.23	-	6.78 – 8.09
[SbCl(N <sup>∘</sup> S) <sub>2</sub> ]	11.02	-	6.50 – 8.00
[SbCl <sub>3</sub> (N <sup>∘</sup> SH)]	11.52	5.12	6.70-7.24
[BiCl <sub>2</sub> (N <sup>∘</sup> S)]	12.11	-	6.78-8.35
[BiCl(N <sup>∘</sup> S) <sub>2</sub> ]	12.01	-	6.64-8.24
[BiCl <sub>3</sub> (N <sup>∘</sup> SH)]	12.02	5.02	6.78-8.67
[Ph <sub>2</sub> As(N <sup>∘</sup> S)]	12.09	-	6.35-8.23
[PhAs(N <sup>∘</sup> S) <sub>2</sub> ]	12.08	-	6.89-8.36

Where, bs= broad signal

m= complex patten

\* = merged with aromatic protons,

\*Ph-M = Sb / As

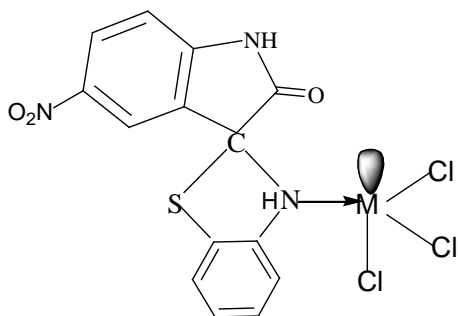
temperature. Light brown colored solid adducts were obtained which further purified and dried using previous method.

#### Microwave method

Antimony and Bismuth adducts have also been synthesized using microwave irradiations. Anhydrous conditions were accompanied by applying the same procedure mentioned in previous section. The completion of the reaction was examined by TLC using silica gel-G. The resulting light brown colored solid adducts were washed with dry n- hexane and dried *in vacuo* for 3-4 h.

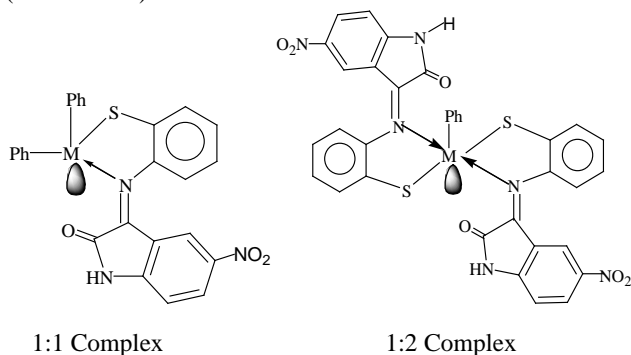
#### Synthesis of arsenic(III) complexes (C-9 and C-10)

These complexes prepared using triphenylarsine (0.87 g and 0.91 g) in dry benzene with equimolar and bimolar amounts of the BztH (0.85 g and 1.78 g) dissolved in benzene. The reaction mixture was heated under reflux for 10-15 hours. The complexes were repeatedly washed with dry n-hexane and dry pet-ether. They have been dried at 40-60°/ 0.5 mm of Hg pressure for 3-4 hour. The purity was further checked by TLC using silica gel-G. The analytical data and physical properties were recorded in Table 1.



Where, M = Sb / Bi, BztH behaving as monodentate donor site

**Figure 4** Proposed structure of the antimony/bismuth adduct (C-5 and C-8)



1:1 Complex

1:2 Complex

**Figure 5-6** Proposed structures of the complexes (C-1 to C-4, C-6 and C-7, C-9 and C-10) Where, M = Sb / Bi / As and S = Sulphur, N = Nitrogen

#### Analytical and physical measurements

The molecular weights were determined by the Rast Camphor method.<sup>9-11</sup> Sulphur and nitrogen were estimated gravimetrically (Messenger's method) as BaSO<sub>4</sub> and by Kjeldahl's method, respectively.<sup>12</sup> Chlorine was determined by Volhard's method. Bismuth was estimated complexometrically. Antimony and arsenic were estimated iodimetrically.<sup>13</sup> Electronic spectra of the complexes were recorded in methanol on a UV-160A, Shimadzu spectrophotometer in the range 200-600 nm. Infrared spectra of the BztH and its complexes were scanned in the range 4000 – 200 cm<sup>-1</sup> with the help of a model Nicolet Magna FTIR-550 spectrophotometer and a model FTIR-8400 S spectrophotometer on KBr/CsCl pellets. NMR spectra were recorded using a JEOL-AL-300 FT NMR spectrometer in DMSO-d<sub>6</sub> using TMS as the internal standard.

#### Pharmacology

Fungicidal and bactericidal activities of the BztH and its corresponding antimony, bismuth and arsenic complexes against different fungi and bacteria have been carried out by the methods reported earlier. The antifungal activity was tested against *Fusarium oxysporum* and *Aspergillus niger* while

antibacterial activity was checked against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacterial strains. Proper temperature (25-30°C), necessary nutrients and growth media free from other micro organisms were employed for the preparation of culture media of fungi and bacteria using aseptic techniques.

#### In Vitro Study

##### Antibacterial activity

(Inhibition Zone Technique)<sup>14</sup>

For the evaluation of degree of inhibitory effects on the growth of a wide spectrum of microorganisms, antibacterial activity was performed. The compounds were dissolved in methanol at 500 and 1000 ppm concentrations. Whatman No. 1 paper with a diameter 5 mm were soaked in these solutions. These discs were placed on the appropriate nutrient medium (0.5% peptone, 0.15% yeast, 0.15% beef extract, 0.35% sodium chloride and 0.13% KH<sub>2</sub>PO<sub>4</sub> in 1000 cm<sup>3</sup> distilled water) was autoclaved for 20 min at 15 psi before inoculation previously seeded organisms in Petri dishes and stored in an incubator at 25 °C. The inhibition zone thus formed around each disc was measured (in mm) after 24 h.

##### Antifungal activity

(Poisoned Food Technique)<sup>15</sup>

Potato dextrose agar medium was prepared in a flask and autoclaved for 20 min at 15 psi before inoculation. The compounds were directly mixed with the medium in 50,100 and 200 ppm (in methanol) concentrations. Aliquots of 15 mL medium were poured in sterilized Petri plates. A culture of test fungus was grown on PDA in 24 h at the 25 ± 2°C temperature for growth. A small disc of the fungus culture was cut with a sterile cork borer and transferred aseptically in the centre of a petri dish containing the medium and incubated for 4 days at 25°C. The colony diameter was measured after the incubation period of growth. The percentage inhibition of growth was calculated by,

$$(C - T) C^{-1} \times 100$$

Where, C = growth in control, T = growth in treatment.

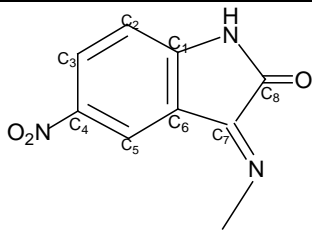
##### Antispermatic activity

The Sprague Dawley albino rats (*Rattus Norvegicus*) obtained from Hamdard University, New Delhi were housed in plastic cages at room temperature (22-25°C) and uniform light (14:10:L:D). They were fed on standard laboratory chow (Aashirwad Food Industries Ltd, Chandigarh, India) and fresh water *ad libitum* access to certified rodent chow and water for the duration of the study. All the procedures involving animals were performed according to the guidelines of the Indian National Science Academy (2000) and the study was approved by the Institutional Animal Ethical Committee of University of Rajasthan, Jaipur, India. Proven fertile healthy male rats (weighing 170-200 g) were divided into four groups of six animals each. In group-I Control animals received vehicle (0.5 mL olive oil per day) only; whereas the animals of groups-II

were administrated orally with BztH (25 mg/Kg b. Wt dissolved in 0.5 mL olive oil) in group III, rats were given

gavage, employing the 25 mg per kg body weight per day dose level for similar durations.

**Table 4.**  $^{13}\text{C}$ NMR spectral data ( $\delta$ , ppm) of the BztH and its antimony(III), bismuth(III) and arsenic(III) complexes

Compound	Azomet hine Carbon C <sub>7</sub>	Thiolo Carbon	Aromatic Carbon											
													*Ph-Sb / *Ph-As	
			C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C(i)	C(o)	C(m)	C(p)		
BztH	169.82	160.23	142.23	129.23	132.17	135.15	132.74	131.23	-	-	-	-		
[PhSbCl(N $\text{S}$ )]	176.67	167.67	142.58	129.94	132.98	136.78	132.89	131.45	140.06	134.26	132.39	131.50		
[PhSb(N $\text{S}$ ) <sub>2</sub> ]	171.34	170.45	142.92	129.38	133.76	136.97	133.79	132.41	142.28	133.62	131.10	130.72		
[BiCl <sub>2</sub> (N $\text{S}$ )]	172.98	165.82	143.45	129.99	132.68	136.79	132.93	131.74	-	-	-	-		
[BiCl(N $\text{S}$ ) <sub>2</sub> ]	169.92	163.26	143.78	130.11	132.69	136.69	132.89	131.79	-	-	-	-		
[Ph <sub>2</sub> As(N $\text{S}$ )]	182.79	171.37	142.84	130.23	133.07	136.56	132.92	131.50	141.30	134.20	131.49	130.59		
[PhAs(N $\text{S}$ ) <sub>2</sub> ]	183.77	172.56	142.90	130.09	132.28	136.43	133.06	132.40	140.40	132.50	130.39	131.61		

\*Antimony / arsenic phenyl carbon values are given in order C(i), C(o), C(m) and C(p), respectively.

**Table 5.** Antifungal and Antibacterial screening data of BztH and its corresponding antimony(III), bismuth(III) and arsenic(III) complexes.

Compound	Antifungal Screening						Antibacterial Screening			
	Average inhibition (%) after 96 h (conc. in ppm.)						Diameter of inhibition zone (mm) after 24 h (conc. in ppm)			
	<i>Fusariumoxysporum</i>			<i>Aspergillusniger</i>			<i>Staphylococcus aureus</i> (+)		<i>Escherichia coli</i> (-)	
	50	100	200	50	100	200	500	1000	500	1000
BztH	34	38	47	23	35	53	5	7	3	6
C-1	62	70	82	63	70	74	6	7	5	7
C-2	65	74	86	65	73	79	8	8	7	9
C-3	42	49	52	34	42	56	7	9	6	8
C-4	44	50	55	39	45	59	9	11	7	6
C-6	67	75	86	65	76	82	10	12	6	7
C-7	70	76	88	68	78	84	11	14	7	8
C-9	69	78	89	67	79	92	10	15	9	10
C-10	72	80	92	70	82	94	12	17	10	13

compound C-7 (25 mg per kg body weight per day) and in group IV, rats were given compound C-10 (25 mg per kg body weight per day) for the period of 60 days. Each test compounds were dissolved in olive oil (vehicle) and was given by oral

The fertility test of each experimental animal was done before and on the 55<sup>th</sup> day of treatment, by a natural mating exposure test. The male rats were cohabitated with proestrous females in 1: 4 ratios. Vaginal smears were checked for

**Table 6.** LD<sub>50</sub> for the compounds

*Sample No.	Dose (mg / day / b.wt)	No. of animals	Death
1	100	10	10
2	90	10	10
3	80	10	10
4	70	10	5

\*Sample No. showing the dose level of BztH and its complexes (C-7 and C-10) given to different sets of animal group to check their mortality rate (LD<sub>50</sub>).

**Table 7.** Alternation in body weight of reproductive organs after treatment with BztH and its C-7 and C-10 complexes.

Group/treatment	Body weight (g)		Reproductive organs weight (mg/100 g b.wt)			
	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
Group-I / Control (Vehicle treated)	175±10	185±13	1220±70	490 ± 28	375 ± 25	260 ± 14
Group-II / BztH 25 mg in 0.5 mL Olive oil/kg.b.wt./ day for 60 days	182 ± 9	205±10	1025±45*	395 ± 40*	300 ± 18*	210 ± 9*
Group-III / C-7 25 mg in 0.5 mL Olive oil/kg.b.wt./ day for 60 days	190±12	210 ± 9	815±60*	300 ± 30*	230 ± 15*	170±10*
Group-IV / C-10 25 mg in 0.5 mL Olive oil/kg.b.wt./ day for 60 days	185±10	198 ± 7	720±62**	240 ± 28**	160 ± 10**	140±5**

(Mean ± SEM of 6 animals) \* = P ≤ 0.05 = Significant  
 \*\* = P ≤ 0.001 = highly significant ns = Non-significant  
 All groups compared with control

positive mating. The inseminated females were separated and the number of litters delivered recorded. Fertility was calculated in control as well as in treated groups. Individual body weight data were recorded on the first day of the treatment, weekly thereafter and on the day of autopsy. Experimental male rats were autopsied on day 61 using light ether anesthesia. Blood was collected from cardiac puncture; serum was separated from blood by centrifugation at 3000 rpm

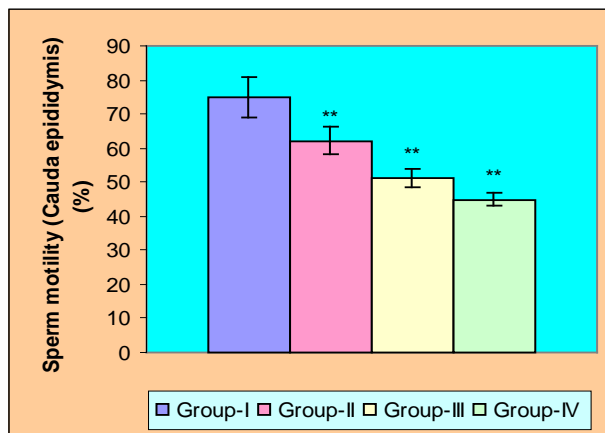


Figure 7

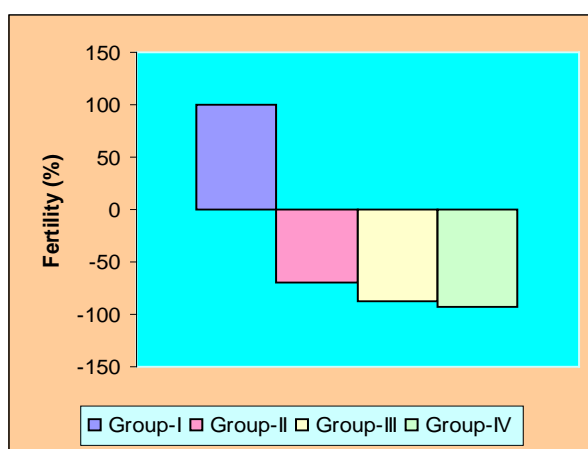


Figure 8

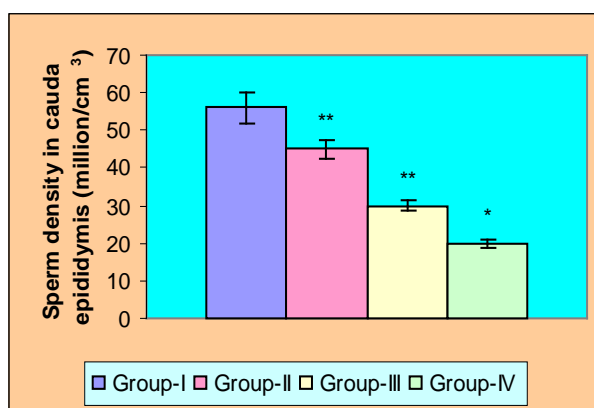


Figure 9

and stored at -20°C for the serum hormonal assay. Sperm motility in caudaepididymides and sperm density in testes and caudaepididymides were observed by the method of Prasad *et al.*<sup>16</sup> The weights of the testes and other sex organs were recorded after removing the adherent tissue and frozen for

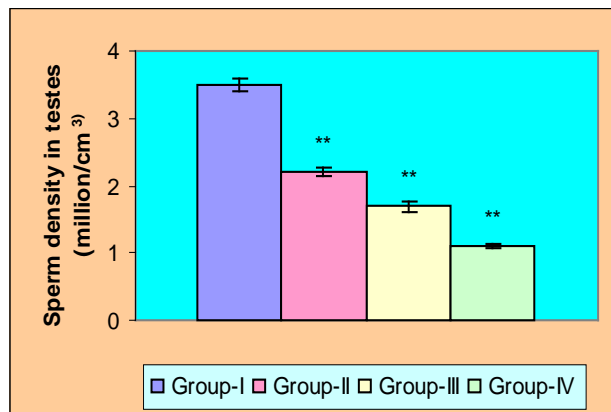


Figure 10

**Figure 7-10.** Altered sperm dynamics and fertility test after treatment with BztH and its C-7 and C-10 complexes

and stored at  $-20^{\circ}\text{C}$  for the serum hormonal assay. Sperm motility in caudaepididymides and sperm density in testes and caudaepididymides were observed by the method of Prasad *et al.*<sup>16</sup> The weights of the testes and other sex organs were recorded after removing the adherent tissue and frozen for cholesterol, protein, sialic acid and glycogen. The right testis of each animal was placed into Bouin's fixative for 48 h for histopathological studies. After immersion in the fixative, slabs, perpendicular to the longest axis of the organ, were then cut and dehydrated in graded series of alcohol and embedded in paraffin and bee's wax; 5 m-thick sections were cut and stained with hematoxylin and eosin. Histological changes were studied at microscopic level and were supplemented by histometric study. The relative number of each variety of germ cells of the seminiferous epithelium cycle was ascertained for the histomorphological changes, according to the standard method.<sup>17</sup> The evaluation of cell population dynamics was based on the count of each cell type per cross tubular section. Various cell components were quantitatively analyzed using spherically appearing sections. Testosterone concentration was measured by radio immuno assay.<sup>18</sup> The group count of all the germ types was designated by Abercrombie.<sup>19</sup> Results are expressed as arithmetic means with their standard error ( $\pm\text{SEM}$ ). The limit of significance set at  $p < 0.01$  was followed by unpaired Student's *t*-test.

#### Body and organ weights

The body weights of each animal were measured both before and after the treatment.

#### Spermatozoa motility and count

The spermatozoa motility was determined according to the method of Prasad *et al* using a WBC counting Neubauer chamber of a haemocytometer and was expressed as million spermatozoa / mL suspension.

#### Bio-chemical studies

Protein was estimated by the reported procedure.<sup>20</sup> Sialic acid was estimated by the procedure given by Warren.<sup>21</sup> Cholesterol analysis was done as per the method of Zlatkis *et al.*<sup>22</sup> Glycogen was estimated by the method of Montgomery.<sup>23</sup> The values for the body weight, organ weight, sperm dynamics and biochemical estimations were averaged, standard error of the mean values were calculated and student's 't' test was applied for the standard comparisons. In these investigations doses of the compounds mixed in vehicle (olive oil) were given orally with the help of hypodermic syringe having pearl point needle, for 60 days and withdrawal (recovery) for 30 days. The  $\text{LD}_{50}$  is statistically derived single dose of a substance that can be expected to cause death in 50% of the animals. In a prohibited analysis method of  $\text{LD}_{50}$ , the selected dose levels should bracket the expected  $\text{LD}_{50}$  value with at least one dose level higher than the expected  $\text{LD}_{50}$  but not causing 100% mortality and one dose level below the expected  $\text{LD}_{50}$  but not causing 0% mortality. Toxicity of the complexes was determined by calculating the  $\text{LD}_{50}$  values. Symptoms of poisoning and mortality were observed and results of toxicity were analyzed for determination of  $\text{LD}_{50}$  values of the complexes.

## RESULTS AND DISCUSSION

Complexes were colored, hygroscopic solids, stable. All the derivatives were found to be soluble in common organic solvents, moisture sensitive, and some of them found decomposed on heating. Elemental analysis and spectral data confirms their synthesis and proposed structures.

#### Uv-vis. spectra

The electronic spectra of BztH consists of two bands around 250-270nm and 300-325 nm attributable to  $\phi\text{-}\phi^*$  and  $\pi\text{-}\pi^*$  transitions which remain unchanged in the complexes. An additional band is also observed around 370-395 nm due to  $n\text{-}\pi^*$  electronic transitions of the azomethine, which indicates isomerization of the ligands on complexation. All the complexes show absorption maxima at 545 nm in the visible region, which probably arises from ligand to metal charge transfer.

#### I.R. spectra

In IR spectra of the free BztH, the -NH stretching band appear at  $3315\text{ cm}^{-1}$ . The absence of  $\nu(\text{SH})$  band<sup>24</sup> in the range of  $2600\text{-}2500\text{ cm}^{-1}$  and  $\nu(\text{C}=\text{N})$  band at  $1545\text{-}1625\text{ cm}^{-1}$  range, is a strong evidence for the existence of benzothiazoline structure rather than the Schiff base structure. In the spectra of metal complexes  $\nu(\text{NH})$  bands disappear suggesting the deprotonation of the BztH on chelation. A strong and sharp band in the region  $1545\text{-}1625\text{ cm}^{-1}$  ascribed to  $\nu(\text{C}=\text{N})$ , is observed in the metal complexes confirming that the ligand adopts the Schiff base form in complexes.

In case of the adducts  $\text{SbCl}_3\text{.}(\text{N}^{\ominus}\text{SH})$ , and  $\text{BiCl}_3\text{.}(\text{N}^{\ominus}\text{SH})$ , the presence of  $\nu(\text{NH})$  mode of vibration indicates that benzothiazoline ring remains intact<sup>25</sup> in the said adducts. This band due to  $\nu(\text{NH})$  mode appears with a lowering of  $\sim 20\text{-}25$



**Table 8.** Biochemical changes in the reproductive organs after oral administration of BztH and its C-7 and C-10 complexes.

Group / treatment	Protein (mg / g)				Sialic acid (mg / g)				Testicular Cholesterol (mg / g)	Testicular Glycogen (mg / g)
	Testes	Epididymis	Ventral Prostate	Seminal Vesicle	Testes	Epididymides	Seminal Vesicle	Ventral Prostate		
Group-I / Control	195 ±10	260 ±10	245 ±9	235 ±6	8.4 ±0.8	7.4 ±0.7	7.7 ±0.6	8.2 ±0.9	8.5 ±0.7	2.50 ±0.6
Group-II / Ligand (N <sup>o</sup> SH)	145 ±12*	205 ±8*	185 ±7*	175 ±8**	7.3 ±0.5*	6.1 ±0.5*	6.3 ±0.4**	6.9 ±0.8*	10.3 ±0.6*	1.9 ±0.4**
Group-III / [BiCl(N <sup>o</sup> S) <sub>2</sub> ]	100 ±7	145 ±7**	130 ±6**	130 ±5**	5.6 ±0.4**	4.5 ±0.4**	5.1 ±0.3**	5.3 ±0.4**	12.8 ±0.5**	1.3 ±0.3*
Group-IV [PhAs(N <sup>o</sup> S) <sub>2</sub> ]	102 ±6	120 ±6**	105 ±3**	108 ±4**	4.1 ±0.3**	3.8 ±0.3**	3.7 ±0.5**	3.2 ±0.3**	12.9 ±0.1**	1.2 ±0.2**

(Mean ± SEM of 6 animals) \* = P ≤ 0.05 = Significant \*\* = P ≤ 0.001 = Highly significant ns = Non-significant All groups compared with control.

cm<sup>-1</sup> in its position in the synthesized adducts. This indicates the participation of the -NH group in bonding. This is further supported by the appearance of a new M←N band. This confirms the coordination in these adducts, taking place through nitrogen only and also showing the monodentate behavior of the BztH.

Some new bands observed in the regions 410–450, 375–387, 320–332, 235–256, and 439–440 and 422–435 cm<sup>-1</sup> for ν(Sb←N), ν(Sb–S), ν(Bi←N), ν(Bi–S) and ν(As←N), ν(As–S),<sup>25</sup> respectively. The band in 450–470 cm<sup>-1</sup> range may be assigned to ν(Sb–Ph)<sup>26</sup> and ν(As–Ph) vibrations in the respective complexes. This may be interpreted in terms of the benzothiazoline ring opening and the formation of Schiff base complexes by the rearrangement of the ring.

#### <sup>1</sup>H NMR spectra

The <sup>1</sup>H NMR spectrum of the free BztH and its metal derivatives were recorded in DMSO-d<sub>6</sub>. The chemical shifts of different proton are given in Table 3. The signal at δ4.25 is assigned to the -NH proton. The signal disappears in the metal complexes, C-1, C-2, C-3, C-4, C-6, C-7, C-9 and C-10 indicating the deprotonation of this functional group on complexation. The free BztH shows a complex multiplet at δ6.65–7.20 ppm for the aromatic protons and it remains more or less at the same position in the spectra of the complexes. The spectra of free ligand display a broad signal at 12.02 ppm due –

NH proton of indole ring which remain in the same position in the complexes showing its non-involvement in the complexation.

In the spectra of addition products C-5 and C-8 the -NH proton in spite of disappearing, gets deshielded and shows a downfield shift. This supports the formation of M←NH bond during adduct formation. On the basis of these spectral studies for the metal complexes, the pseudo-trigonalbipyramidal and pseudo-octahedral geometries respectively have been suggested.

#### <sup>13</sup>C NMR spectra

<sup>13</sup>C NMR spectrum of BztH and its corresponding metal complexes were also recorded in dry DMSO and assigned peak positions are listed in Table 4. The noticeable shift in the position of carbon attached to azomethine nitrogen and thiolic sulphur in the spectra of metal complexes confirms the inferences drawn earlier on the basis of IR and <sup>1</sup>H NMR spectra concerning the participation of nitrogen and sulphur in bonding with metal atom.

#### Antimicrobial activity

The synthesized BztH and its metal complexes have been screened against some pathogenic fungi and bacteria, the results are recorded in Table 5. The results reveal that there is considerable increase in the toxicity of the complexes as compared to the BztH. On giving a closer look at these results a

common feature which appears is that the bioactivity enhances due to the points given below:

1. 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<sup>27</sup>. Due chelation after complex formation, complexes are showing better results than its BztH ligand. Though C-2, C-4, C-7 and C-10 are showing considerable good antimicrobial activity than C-1, C-3, C-6 and C-9 because they have more chelation.
2. It has been found that the gram (+)ve bacteria are more affected than the gram (-)ve bacteria. BztH showed better antibacterial activity with gram positive bacteria and this toxicity is further enhanced in the complexes. Lawrence *et al.*<sup>28</sup> have suggested that the toxicity of antibacterial compounds against different species of bacteria depends either on the difference in ribosome or the impermeability of the cell to the antimicrobial agent. The bonding of the chelated metals to the nitrogen bases of DNA, RNA and the inhibition of DNA synthesis through the blockage of the enzyme ribonucleotide diphosphate reductase (RDR).<sup>29</sup>
3. The results also indicated that complexes of different metals behave differently towards these microorganisms taken for the experiments. Bismuth complexes C-6 and C-7 are showing better results<sup>30</sup> as compared to antimony complexes (C-1 to C-4) while organoarsenic(III) complexes (C-9 and C-10) are best antimicrobial agents among the all reported complexes.

#### **Antispermatic activity**

Male rats exposed to BztH and its bismuth(III) and arsenic(III) complexes C-7 and C-10 (25 mg/ Kg b.Wt / day) respectively for a period of 60 days showed following alternation in reproductive function of male rats.

#### **Effects on animal body weights**

Oral administration of BztH (Group-II) and its C-7 (Group-III) and C-10 (Group-IV) complexes to male rats (25 mg/rat per day) for the period of 60 days did not affect the body weights of male rats during the period of exposure.

#### **Effects on organs weights**

However, the weights of testes, epididymis, seminal vesicle and ventral prostate were reduced significantly. Weights of accessory sex glands were also reduced significantly (Table 7). In the BztH and compounds treated rats (groups II and IV), reduction in the accessory sex glands was more than that of control group and compound treated group. The significant reduction in testes weight after the treatment with C-7 and C-10 can be attributed to decrease in number of spermatogenic elements and spermatogonia that is cell death which leads to regression in these organs.<sup>31</sup>

#### **Antispermatic effects**

A significant decrease in sperm motility in caudaepididymis was noticed after treatment with BztH and its bismuth and

arsenic complexes (C-7 and C-10). Sperm motility was decreased by in groups II, III and IV respectively, in comparison to control animals (Figure 7). Sperm density in testes and epididymides were also reduced after various treatments (Figure 8-9). Sperm density was highly suppressed in the testes and caudaepididymides of all the treated rats when compared with the control group. The number of spermatozoa, in group-IV animals, was lower than that in groups-II and III (Table 9). The marked reduction in sperm count in our experiment also suggests a disturbed testicular and epididymal microenvironment. The treatment with compounds C-7 and C-10 exerted a strong inhibitory effect on epididymides, seminal vesicle and ventral prostate.

#### **Effects on cell population dynamics (Table 9)**

The total number of sertoli cells and seminiferous tubular diameter were reduced after treatment with BztH and its C-7 and C-10 complexes. A significant decrease in spermatogonia, proleptotene spermatocytes, pachytene spermatocytes and secondary spermatocytes were observed after various treatments. These compounds may have direct effect on sertoli cell function which appears to be involved in the control of spermiation and when disturbed caused epithelial disorganization and subsequent tubular atrophy. Reduction in the sertoli cell population and secondary spermatocytes may be due to antiandrogenic nature of the compounds as these stages are completely androgen dependent.<sup>32</sup> Further, it is supported by reduction in the serum testosterone levels which clearly demonstrated the inhibitory effect of BztH and its bismuth and arsenic complexes. Testicular glycogen contents were low. Antifertility effects of complexes seemed to be mediated by disturbances in testicular somatic cells functions (Leydig and Sertoli cells) resulting in the physio-morphological events of spermatogenesis.

#### **Effects on testosterone, FSH and LH concentrations**

Serum Testosterone concentration was significantly reduced after treatment with BztH and its complexes C-7 and C-10 (Figure11). FSH and LH concentrations were also decreased significantly in all the treated groups in comparison with control animals (Figure 12-13). The reduced population of leydig cells directly established that testosterone suppresses the circulating levels of FSH and LH either by inhibiting the synthesis or blocking the release.<sup>33</sup> It is known that differentiation of primordial germ cells into spermatogonia and the consequent appearance of spermatogenic cycles are under the control of gonadotrophins<sup>34</sup> and testosterone. These are mediated possibly by sertoli cells, which regulate cell cycle kinetics and influence both spermatogonia and preleptotene spermatocyte. The quantitative production of sperm generally requires the presence of FSH, LH and testosterone. The regulation of spermatogenesis depends primarily on an interaction between FSH and testosterone. FSH plays a key role in the development of the immature testes by stimulating sertoli cell proliferation and later progression of spermatogenesis.

Testosterone alone can maintain complete spermatogenesis, but the synergistic action of FSH is necessary to normalize quantitative aspects of spermatogenesis. LH stimulates the production of testosterone in leydig cells.

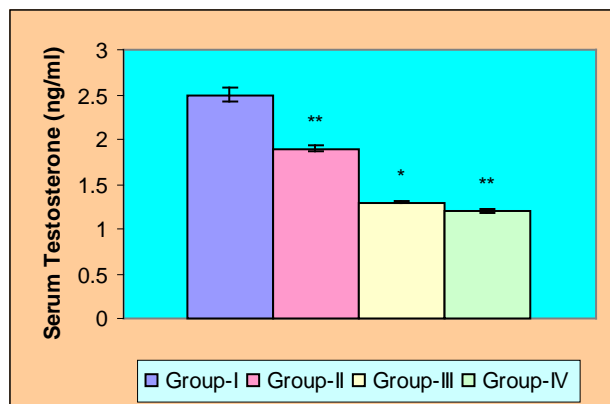


Figure 11

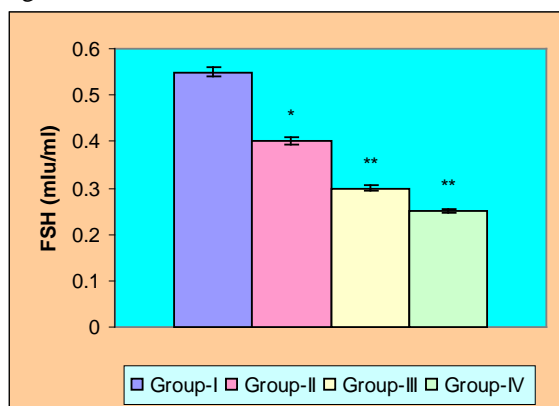


Figure 12

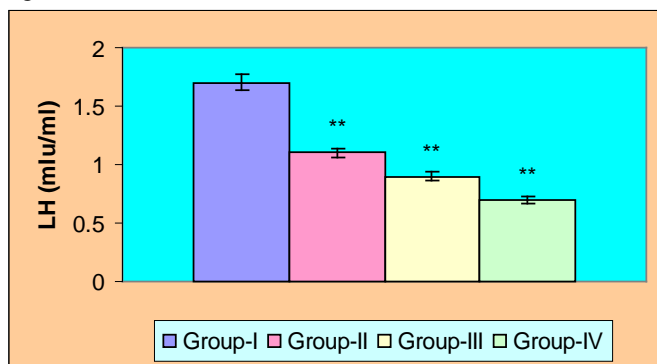


Figure 13

**Figure 11-13.** Testosterone, FSH and LH concentration after oral administration of BztH and its C-7 and C-10 complexes.

### Tissue biochemistry (Table 8)

**Protein:** Protein contents of testes, epididymis, seminal vesicle and ventral prostate were reduced significantly in compound treated rats when compared with control. Reduction in the protein contents of testes and other accessory sex organs may be due to the absence of spermatogenic stages<sup>35</sup> in the testes. The accumulation of cholesterol in testes is a direct evidence of antiandrogenic action.<sup>36</sup>

**Sialic acid:** Sialic acid contents of testes, epididymis, seminal vesicle and ventral prostate were decreased after treatment with BztH and its C-7 and C-10 complexes. These declined levels of sialic acid may be correlated with the loss of androgen.<sup>37</sup> Reduced androgen production was reflected in low levels of sialic acid in the testes, epididymides and seminal vesicles.

**Cholesterol:** Testicular cholesterol contents were increased significantly in rats treated with BztH and its C-7 and C-10 complexes.

**Glycogen:** Testicular glycogen level was found to be low.

### Effects on fertility

Sperm motility and density directly correlate with fertility and any change in them may cause infertility. The fertility index was decreased by 70%, 88% and 92% in group II, III and IV-treated rats respectively, in comparison with control animals (Figure 8).

**Table 9.** Testicular cell population dynamics in rats following BztH and its C-7 and C-10 complexes.

Group / treatment	Testicular Cell Counts (number / cross sections)					
	Sertoli Cells	Spermatogonia	Spermatocytes (primary)	Spermatocytes (Secondary)	Spermatides	Spermatozoa
Group-I / Control	6.2 ± 0.62	10.9 ± 2.1	48 ± 5.0	85 ± 9.0	58 ± 5.0	(++++)
Group-II / BztH	5.1 ± 0.5**	7.5 ± 1.2**	10.2 ± 1.2**	19 ± 0.8**	28 ± 3.0**	(+)
Group-III / C-7	4.2 ± 0.3	6.3 ± 1.1**	6.8 ± 1.5**	5.0 ± 0.3**	22 ± 2.0**	(-)
Group-IV / C-10	3.9 ± 0.2	6.1 ± 1.2**	6.4 ± 0.9**	3.0 ± 0.2**	20 ± 3.0**	(-)

(Mean ± SEM of 6 animals) \* = P ≤ 0.05 = Significant

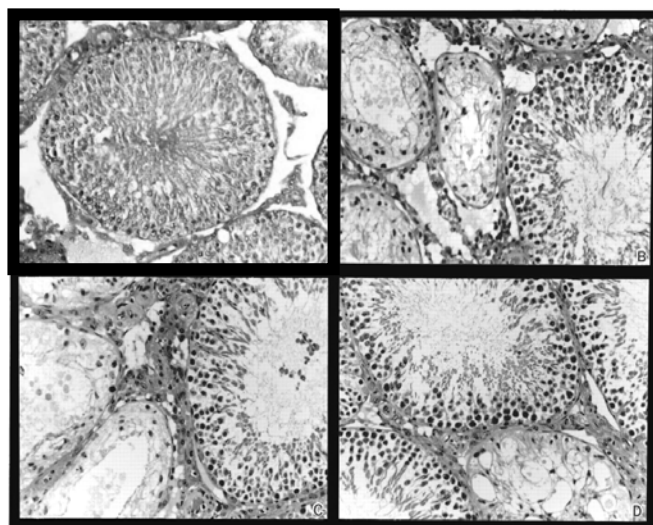
\*\* = P ≤ 0.001 = Highly significant ns = Non-significant

All groups compared with control.

### Histopathology

The testes of rats (treated with BztH, C-7 and C-10 complexes) showed marked histopathological changes and spermatogenesis was disturbed. Testes showed disorganized

seminiferous tubules. The tubular lumen had clear irregular spaces devoid of sperm. The seminiferous tubules became irregular in shape and size, and were reduced in diameter. Decreased seminiferous tubular diameter reflects tubular shrinkage which may occur due to cell death or sloughing of epithelial cells. Treatment of compounds caused degeneration in spermatogenic cells and in Sertoli cells. The count of Sertoli cells decreased in all the treated groups. The number of spermatogonia, primary and secondary spermatocytes and rounded spermatids was also decreased. Following the administration of BztH, C-7 and C-10 complexes in comparison with the control group (Table 9). Diameter of seminiferous tubules reduced when compared with control animals (Figure 11). The quantitative production of sperm generally requires the presence of FSH and testosterone. The regulation of spermatogenesis depends primarily on an interaction between FSH and testosterone. FSH plays a key role in the development of the immature testis by stimulating Sertoli cell proliferation and later progression of spermatogenesis. Testosterone alone can maintain complete spermatogenesis, but the synergistic action of FSH is necessary to normalize quantitative aspects of spermatogenesis. LH stimulates the production of testosterone in Leydig cells. Administration of the test compounds decreased the serum testosterone concentration significantly in male rats, which might be due to the altered hormonal milieu of the testes and simultaneously testosterone inhibition affected the growth, maintenance and development of male reproductive organs. Significant reduction



**Figure 14.** (A) **Control:** Micrograph of control (vehicle treated) rat testis showing histological features, with all the successive stages of spermatogenesis. Lumen filled with spermatozoa. Leydig cells are also present (Final magnification 120X). (B) Histology of rat testes treated with BztH showing degeneration. (C) & (D) Histology of rat testes treated with C-7 and C-10 complexes for 60 days, showing lumen filled with debris.

in the relative weights of the testes indicates low biosynthesis of androgens, which would not be sufficient to maintain the structural and functional integrity of male gonads and accessory organs.

Seminiferous tubular diameter is one of the most important indications of testosterone level. Decrease in diameter of seminiferous tubules reflects tubular shrinkage. Seminiferous tubules contain Sertoli cells and germ cells. Spermatogenesis appears to be particularly dependent on the interaction between germ cells and Sertoli cells. Therefore, reduced number of Sertoli cells could affect the production of sperm. Reduced count of germ cells, i.e. spermatogonia, primary and secondary spermatocytes and spermatids, indicates low concentration of FSH and LH. Decreased sperm production correlates well with decrease in germ cells, testicular weight and disturbed testicular microenvironment. Treatment of adult male rats with the test compounds caused a reduction in the sperm motility in the cauda epididymides. The epididymis contributes to the initiation of sperm motility by providing them with a unique environment along the length, by secreting proteins and ions. Inhibition in sperm motility resulted in abnormal sperm functions, which ultimately gave rise to complete sterility.

## CONCLUSION

Microwave (MW) irradiation is an efficient method to accomplish various inorganic syntheses to afford products in higher yields in shorter reaction periods. All the complexes obtained by 1:1 as well as 1:2 molar reactions were found to be tetra- and penta-coordinated but, due to stereochemically active lone pair of electrons present on the metal atom, pseudo-trigonal bipyramidal and pseudo-octahedral geometries have been tentatively proposed for the said complexes. Antimicrobial activity of the complexes and the ligand showed that the former are more active than the parent ligand. The results of antiandrogenic studies indicated that the test compounds are capable of suppressing the process of spermatogenesis by inhibiting the serum testosterone, FSH and LH levels. The fertility of male animals was suppressed by 70% with BztH treated rats and 88%, 92% with C-7 and C-10 compounds treated rats respectively. In conclusion our study suggested that the addition of the bismuth and arsenic metal moiety to BztH enhances their antiandrogenic efficiency.

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## REFERENCES

1. R.S. Varma, V.V. Namboodiri. Solvent-free preparation of ionic liquids using a household microwave oven. *Pure Appl. Chem.*, **2001**, 73, 1309.
2. C. Silvestru, M. Curtui, I. Haiduc, M.J. Begley, D.B. Sowerby. Phenylantimony(III) diorganophosphorodithioates: the crystal structure of diphenylantimony(III) di-isopropylphosphorodithioate,  $\text{Ph}_2\text{SbS}_2\text{P}(\text{O}i\text{Pr})_2$ ; unusual polymerisation through semi bonding. *J. Organomet. Chem.*, **1992**, 426, 49.

3. R. Johar, R. Kumar, A.K. Aggarwal. Tailoring methodologies for the architecture of organometallic frame works of Bi(V) derived from antibiotics: Spectral, MS, XRPD and molecular modeling with antifungal effectiveness. *J. Integr. Sci. Technol.*, **2013**, 1(1), 54-64 (b)
- R. Iffl and, H.G. Seiler, A. Sigel, H. Sigel (ED.), Handbook on metals in clinical and analytical chemistry. Marcel Dekker, Inc., New York, 1994.
4. Slikkerveer. Bismuth biokinetics toxicity and experimental therapy of overdose. Koninklijke Bibliotheek, Hague, Netherlands, 1992.
5. J.S. Thayer. Organometallic compounds and living organisms, Academic Press, New York, 1984.
6. N. Subirán, L. Casis, J. Irazusta. Regulation of male fertility by the opioid system. *Mol Med.* **2011**, 17, 846-853.
7. D.D. Perin, W.L.F. Armarego, D.R. Perrin. Purification of Laboratory Chemical, third ed., Pergamon Press, Oxford, 1986.
8. K. Mahajan, M. Swami, S.C. Joshi, R.V. Singh. Microwave-assisted synthesis, characterization, biotoxicity and antispermatogenic activity of some antimony(III) complexes with N<sup>1</sup>O and N<sup>1</sup>S donor ligands. *Appl. Organomet. Chem.*, **2008**, 22, 359.
9. A.I. Vogel. A Text Book of Quantitative Inorganic Analysis, fifth ed., Longmans, London, 1989.
10. C.L. Wilson, D.W. Wilson, EDS, Comprehensive Analytical Chemistry, Elsevier, New York, 1960,
11. E.J. Cowles, M.T. Pike. Semimicro modification of the Rast method for determining molecular weights. *J. Chem. Educ.* **1963**, 40, 422.
12. R. Grappin, W. Horwitz. Determination of nitrogen content in milk by the Kjeldahl method using copper sulfate: interlaboratory study. *J. Assoc. Off Anal Chem.* **1988**, 71, 893.
13. J. Mendham, R.C. Denney, J.D. Barnes, M.J.K. Thomas, Vogel's Quantitative Chemical Analysis (6th ed.), New York: Prentice Hall, ISBN 0-582-22628-7, 2000.
14. M. Saggi, A. Sanna, F. Cottiglia, L. Chisu, L. Casu, L. Bonsignore, A. De Logu. Antiherpesvirus activity of Artemisia arborescens essential oil and inhibition of lateral diffusion in Vero cells. *Ann Clin. Microbiol. Antimicrob.* **2007**, 6, 10.
15. G.N. Agrios. Control of plant diseases. Plant pathology. 4th edition. California, 1997.
16. M.R.N. Prasad, N.J. Chinoy, K.M. Kadam. Changes in succinate dehydrogenase levels in the rat epididymis under normal and altered physiological conditions. *Fertil. Steril.* **1972**, 23, 186.
17. C.L. Winchester, R.K. Ferrier, Sermoni A, et al. Characterization of the expression of *DMPK* and *SIX5* in the human eye and implications for pathogenesis in myotonic dystrophy. *Hum. Mol. Genet.* **1999**, 8, 481.
18. B.S. Chhikara, S. Kumar, N. Jain, A. Kumar, R. Kumar. Prospectivity of bifunctional chelating agents in chemical, biological and biomedical applications. *Chem. Biol. Lett.* **2014**, 1(2), 77.
19. M. Abercrombie. Estimation of nuclear populations from microtome sections. *Anat. Rec.* **1946**, 94, 238.
20. R. Milo. What is the total number of protein molecules per cell volume? A call to rethink some published values, *Bioessays. Dec.* **2013**, 35, 1050.
21. L. Warren. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.* **1959**, 234, 1971.
22. Zlatkis, B. Zak, J.A. Boyle. A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med.* **1953**, 41, 486.
23. R. Montgomery. Determination of Glycogen Arch. *Biochem. Biophys.* **1957**, 67, 378.
24. J.R. Dilworth, N. Wheatley. The preparation and coordination chemistry of phosphorus-sulfur donor ligands. *Coord. Chem.* **2000**, 199, 89-158.
25. A.D. Adams. Metal-Ligand and Related Vibration; Edward Arnold Ltd.: London, 1967, 238.
26. C.N.R. Rao. Chemical application of infrared spectroscopy, Academic press, New York, 1963.
27. B.G. Tweedy. Possible mechanism for reduction of elemental sulfur by moniliniafructicola. *Phytopathology.* **1964**, 55, 910-4.
28. J.C. Lawrence. The use of iodine as an antiseptic agent. *J. Wound Care.* **1998**, 7, 421.
29. M.K. Pulatova, V.L. Sharygin, I.N. Todorov. The activation of ribonucleotide reductase in animal organs as the cellular response against the treatment with DNA-damaging factors and the influence of radioprotectors on this effect. *Biochimica et Biophysica. Acta. (BBA) - Molecular Basis of Disease.* **1999**, 1453, 321.
30. U. Dittes, E. Vogel, B.K. Keppeler. Overview on bismuth (III) and bismuth (V) complexes with activity against *Helicobacter pylori*. *Coord. Chem. Rev.* **1997**, 163, 345.
31. A.P.S. Hikim, C. Wang, Y.H. Lue, L. Johnson, X.H. Wang, R.S. Swerdloff. Spontaneous germ cell apoptosis in humans: evidence for ethnic differences in the susceptibility of germ cells to programmed cell death. *J. Clin. Endocrinol. Metab.* **1998**, 83, 152.
32. J.B. Kerr, M. Millar, S. Maddocks, R.M. Sharpe. Stage-dependent changes in spermatogenesis and Sertoli cells in relation to the onset of spermatogenic failure following withdrawal of testosterone. *Anat. Rec.* **1993**, 235, 547.
33. C.H. Mortimer, G.M. Besser, A.S. Mc Neilly, W.M.C. Tunbridge, A. Gomez-Pan, R. Hall. Interaction between secretion of the gonadotrophins, prolactin, growth hormone, thyrotrophin and corticosteroids in man: the effects of LH/FSH-RH, TRH and hypoglycaemia alone and in combination. *Clin. Endocrinol.* **1993**, 2, 317.
34. W. Rüdiger, Schulz1, T. Miura. Spermatogenesis and its endocrine regulation. *Fish Physiology and Biochemistry*, **2002**, 26, 43.
35. J.H. van Rooijen, M.P. Ooms, R.F. Weber, A.O. Brinkmann, J.A. Grootegoed, J.T. Vreeburg. Comparison of the response of rat testis and accessory sex organs to treatment with testosterone and the synthetic androgen methyltrienolone (R1881), *J. Androl.* **1997**, 18, 51.
36. M.T. Yakubu, M.A. Akanji, A.T. Oladiji. Evaluation of antiandrogenic potentials of aqueous extract of *Chromolaena odoratum* (L.) K. R. leaves in male rats. *Andrologia.* **2007**, 39, 235.
37. R.S. Gupta, R.K. Yadav, V.P. Dixit, M.P. Dobhal. Antifertility studies of *Colebrookia oppositifolia* leaf extract in male rats with special reference to testicular cell population dynamics. *Fitoterapia.* **2001**, 72, 236.