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Microwave-assisted synthesis, characterization, biotoxicity and antispermatogenic activity of some antimony(III) complexes with N∩O and N∩S donor ligands

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New series of phenylantimony chloride and antimony chloride with Schiff base ligands, L^1H and L^2H having N∩S and N∩O donor systems were synthesized under microwave irradiation using a domestic microwave oven. The reaction time was brought down from hours to a few seconds with improved yield as compared with conventional heating. All the ligands and their antimony(III) derivatives were characterized on the basis of microanalysis, elemental analyses, molecular weight determinations and spectral studies including IR, NMR (^1H and ^{13}C) and electronic spectral studies. A distorted trigonal bipyramidal and pseudo-octahedral geometry around the antimony atom was tentatively proposed for these derivatives. The compounds were screened *in vitro* against bacteria and fungi to test their antimicrobial property and *in vivo* in male albino rats to test their antifertility properties. The treatment with the ligands and their phenylantimony derivatives at dose levels of 20 mg per rat per day did not cause any significant change in body weight, but a significant reduction in the weights of reproductive organs was observed. Arrest of spermatogenesis was noted at various stages with production of primary spermatocytes (preleptotene and pachytene), secondary spermatocytes and step-19 spermatids found to be decreased. Biochemical parameters of tissues, i.e. protein, sialic acid, cholesterol content of testes and seminal vesicular fructose also showed significant reduction. Further, the serum testosterone concentrations were also decreased after treatment with ligands and their antimony(III) derivatives. **Copyright © 2008 John Wiley & Sons, Ltd.**

Keywords: phenylantimony(III) derivatives; N∩O and N∩S donor ligands; microwave-assisted technique; antispermatogenic activity

Introduction

Microwave dielectric effects are used increasingly in organometallic synthesis.[1] In inorganic chemistry, microwave technology has been used since the late 1970s, while it has only been implemented in organic chemistry since the mid 1980s. Heating a chemical reactor by microwave radiation (just like in a microwave oven) has the advantage that it is rapid and the entire volume of the reactor is heated simultaneously, which can lead to less by-product and/or decomposition products. In this system, it is possible to rapidly increase the temperature to above the conventional boiling point of the solvent used.[2] When we compare this unconventional energy source technology with classical heating we find that the conventional heating process usually starts at the wall of the reactor and, because of this the core takes much longer to achieve the target temperature. Rapid and homogenous heating using an unconventional method (microwave) has many benefits such as: reaction rate acceleration,[3] milder reaction conditions and higher chemical yield;[4] in addition, it is cost effective and less equipment is required. The shorter reaction time and expanded reaction range offered by microwave-assisted synthesis are suited to the increased demand in industry.

The Schiff base complexes of main group elements containing ligands such as semicarbazones and thiosemicarbazones have remained a topic of significant current research interest.[5] This is mainly because of biological applications not only of ligands but also of compounds derived from them. Semi- and thiosemicarbazones can act as neutral or charged ligands. They can show tautomerism and can exist in keto/thione or enol/thiol form. Usual coordination through oxygen or sulfur and azomethine nitrogen is observed[6] in the formation of a five-membered chelate ring.

Semicarbazones and thiosemicarbazones have also attracted attention due to their biological activities. These compounds present a wide variety of biological activities such as antitumoral,[5–8] fungicidal,[9,10] bactericidal[11] and antiviral.[7] It is known that some drugs have increased activity when administered in the form of the metal complexes[12] and a number of metal chelates inhibit tumor growth.[13] In the treatment of cancer, the active species is not the semicarbazones or thiosemicarbazones but their metal chelates.[13,14]

Antimony has quite widespread use in pharmacology for the treatment of syphilis, fever, melancholy, pneumonia, epilepsy

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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] During the last one and half decades, the chemistry of organoantimony(III) derivatives has been extensively pursued in view of the potential fungicidal,[24] bactericidal[25] as well as antitumor activities.[26] These complexes are very interesting due to their fascinating structure,[27] bonding[28] and stereochemical aspects.[29] In respect of the reproductive and developmental toxicity, antimony compounds have also been studied in experimental models.[30] Some compounds of benzothiazolines with antimony (III) were tested for antifertility effects in male rats and were found to show significant activity.[31] A survey of the literature revealed that so far no attention has been paid to comparing the effects of semicarbazone and thiosemicarbazone ligands with antimony (III) metal derivatives on the reproductive system of male rats. In view of this, in the present publication, a comparative study among the effects on the reproductive systems of male albino rats of the corresponding ligands and their antimony(III) derivatives vs control animals are presented and also comparison of the effects of these compounds is made pertaining to their antiandrogenic/antispermatogenic activities.

Experimental

The $PhSbCl_2$ and Ph_2SbCl were prepared according to the literature method.[32] *m*-Nitroaniline was purchased from Lobachemie and used as such. 6-Nitroisatin was prepared in the laboratory. All the chemicals were dried and purified before use and the purity was checked by thin-layer chromatography (TLC).

Table 1. Synthetic and analytical data of antimony (III) compounds of hydrazinecarbothioamide and hydrazinecarboxamide

Sample no.	Reactants in grams: Starting material (a)	Ligand (b)	Sodium (c)	Molar ratio a : b : c	Product and colour	m.p. (°C)	Elemental analysis (%)[a]: C	H	N	S	Cl	Sb	Molecular weight[a]
1	Ph_2SbCl	L^1H	0.06	1 : 1 : 1	($C_{21}H_{16}O_3N_5SSb$)	211	46.11	2.12	11.23	5.11	–	22.65	540.91
	(0.82)	(0.70)			Dark brown		(46.69)	(2.99)	(12.96)	(5.93)		(22.54)	(540.22)
2	$PhSbCl_2$	L^1H	0.06	1 : 1 : 1	($C_{15}H_{11}O_3N_5SClSb$)	198	36.82	2.56	14.91	6.77	8.45	25.01	498.42
	(0.73)	(0.72)			Light brown		(36.14)	(2.22)	(14.05)	(6.45)	(7.11)	(24.42)	(498.56)
3	$PhSbCl_2$	L^1H	0.12	1 : 2 : 2	($C_{24}H_{17}O_6N_{10}S_2Sb$)	242	40.04	2.48	19.97	9.11	–	17.52	728.39
	(0.70)	(1.38)			Dark brown		(39.63)	(2.36)	(19.26)	(8.82)		(16.74)	(727.35)
4	$SbCl_3$	L^1H	–	1 : 1	($C_9H_7O_3N_5SCl_3Sb$)	140	22.78	1.41	14.86	7.10	21.91	24.86	494.00
	(0.98)	(1.14)			Light yellow		(21.91)	(1.43)	(14.20)	(7.92)	(21.56)	(24.68)	(493.37)
5	Ph_2SbCl	L^2H	0.06	1 : 1 : 1	($C_{21}H_{16}O_4N_5Sb$)	130	48.53	30.71	13.12	–	–	23.12	524.82
	(0.82)	(0.65)			Light brown		(48.12)	(30.74)	(13.36)			(23.23)	(524.15)
6	$PhSbCl_2$	L^2H	0.06	1 : 1 : 1	($C_{15}H_{11}O_4N_5ClSb$)	165	37.18	2.49	14.86	–	7.66	26.53	429.98
	(0.72)	(0.66)			Brown		(37.34)	(2.46)	(14.52)		(7.35)	(25.24)	(428.49)
7	$PhSbCl_2$	L^2H	0.14	1 : 2 : 2	($C_{24}H_{17}O_7N_{10}Sb$)	238	42.17	2.22	20.17	–	–	18.54	696.96
	(0.81)	(1.50)			Grey		(41.46)	(2.46)	(20.15)			(17.51)	(695.22)
8	$SbCl_3$	L^2H	–	1 : 1	($C_9H_7O_4N_5Cl_3Sb$)	178	22.58	1.41	14.61	–	23.96	26.42	478.12
	(0.91)	(0.99)			Dark yellow		(22.65)	(1.48)	(14.67)		(22.28)	(25.51)	(477.28)

[a] Calculated values are given in parentheses.

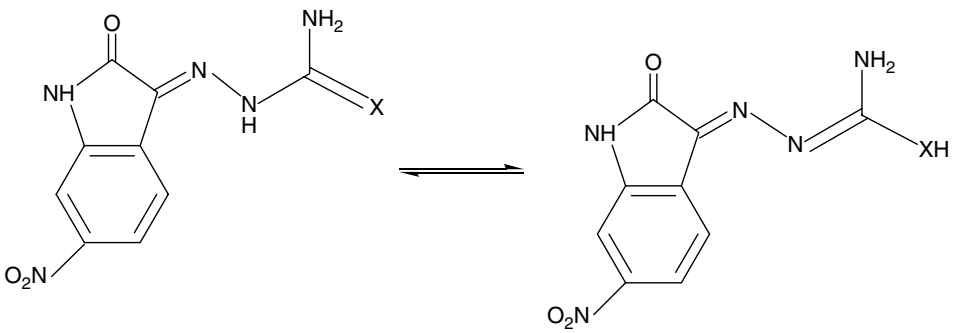

Figure 1. Tautomeric forms of the ligands; X = S (L^1H) and O (L^2H). L^1H, $C_9H_7N_5O_3S$, grey solid, m.p. 221 °C. Analyses (%): found (calcd) N 26.16 (26.40), S 12.24 (12.08); molecular weight: found (calcd.) 263.05 (265.24). L^2H $C_9H_7N_5O_4$, brown solid, m.p. 232 °C. Analyses (%): found (calcd) N 27.86 (28.10); molecular weight: found (calcd) 250.11 (249.18).

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Preparation of the ligands

6-Nitroisatin was synthesized by Sandmeyer isonitrosoacetanilide synthetic method.[33] The corresponding ligands were prepared by the condensation of 6-nitroisatin with thiosemicarbazide and semicarbazide hydrochloride in the presence of sodium acetate in absolute alcohol. The reaction mixture was refluxed over a water bath for 3–4 h and allowed to stand over night. The products were recrystallized from the ethanol and dried *in vacuo*. Their physicochemical properties and analytical data are given in Table 1. The parent ligands exist in the tautomeric forms depicted in Fig. 1.

Preparation of the substitution products [1 and 2(a–c)]

To a weighted amount of Ph_2SbCl and $PhSbCl_2$, the sodium salt of the ligands L^1H and L^2H in 1 : 1 or 1 : 2 molar ratios were added in dry methanol.

Ecofriendly method

In microwave-assisted synthesis the reaction mixtures were taken into 50 ml conical flasks, covered with glass wool and then irradiated for 4–7 min inside the microwave oven. Anhydrous conditions were attained using a beaker with silica gel or anhydrous $CaCl_2$ (the said beaker filled with a moisture-absorbing substance is known as a 'dummy'), which was placed near the reaction vessel during the compound formation inside the microwave oven. The products were recovered from the microwave oven and dissolved in a few millilitres of dry methanol, where the white precipitate of sodium chloride, formed during the course of the reaction, was removed by filtration and the products were dried under reduced pressure. The resulting products were repeatedly washed with *n*-hexane 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 (each after 1 min).

Conventional method

These organoantimony(III) complexes were also synthesized by the thermal method. The contents were boiled under reflux for 14–16 h on the fractionating column. In the thermal method, instead of 4–7 min, reactions were completed in 14–16 h, and the yield of the products was also less than obtained in the microwave-assisted synthesis. A comparative study was also done between microwave technique and the conventional method, and the results are summarized in Table 2.

Preparation of the addition products

A calculated amount of the ligands (L^1H and L^2H) dissolved in dry CCl_4 was added to the antimony trichloride in unimolar ratio.

Ecofriendly method

In microwave-assisted synthesis the reaction mixture was irradiated inside the microwave oven for 2–3 min, and so a drastic reduction in reaction time was observed due to the rapid heating capability of microwaves. Anhydrous conditions were accompanied by applying the same procedure as in the previous section. The completion of the reaction was examined by TLC using silica gel-G (each after 1 min). The resulting yellow-creamish colour adducts were washed with dry *n*-hexane and then finally dried *in vacuo* for 3–4 h.

Conventional method

A calculated amount of the ligands (L^1H and L^2H) dissolved in dry CCl_4 (~6 ml) was added to the antimony(III) trichloride. The reaction mixture was taken into a 100 ml round-bottom flask fitted with a silica gel guard tube for anhydrous conditions and then stirred for ~12 h at room temperature. After completion of the reaction, a yellow-creamish colour adduct was obtained, which was washed with *n*-hexane and then finally dried *in vacuo* for 3–4 h.

Physical measurements and analytical methods

The molecular weights were determined by the Rast camphor method as well as ebulliometerically. Sulfur and nitrogen were estimated gravimetrically (Messenger's method) as $BaSO_4$ and by Kjeldahl's method. Chlorine was determined by Volhard's method.[34] Antimony was estimated by oxidation of Sb(III) to Sb(V) on heating with $KMnO_4$, the excess of which was decolourized

Table 2. Comparison between conventional and microwave method of synthesis and ^1H NMR Spectral data (δ, ppm) of the ligands and their corresponding antimony(III) derivatives

	Yield %		Solvent (ml)		Time					
Compound	Thermal	Microwave	Thermal	Microwave	Thermal (h)	Microwave (min)	–NH (ring) (bs)	–NH (free) (bs)	$-NH_2$ (bs)	Aromatic proton (m)/Ph-Sb[a]
L^1H	–	–	–	–	–	–	11.92	11.24	3.50	6.12–7.29
1a	71	89	30	2	12	8	11.96	–	3.52	6.13–7.33
1b	69	87	30	3	14	9	11.98	–	3.51	6.45–7.82
1c	65	82	30	3	13	8	12.00	–	3.52	6.50–8.21
1d	74	92	20	2	12	5	11.99	11.28	3.51	6.88–7.32
L^2H	–	–	–	–	–	–	11.96	11.26	3.41	6.59–7.84
2a	86	83	30	3	14	8	11.97	–	3.42	6.72–7.92
2b	89	79	30	3	15	8	11.98	–	3.43	6.81–8.23
2c	90	88	30	2	13	9	12.02	–	3.42	6.83–8.10
2d	76	93	20	2	12	6	11.97	11.27	3.43	6.91–7.93

[a] Merged with aromatic protons. bs = Broad singlet, m = complex pattern.

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Table 3. Antifungal and antibacterial screening data of the ligands and their antimony(III) derivatives

	Alternaria alternata (concentration in ppm)			*Fusarium oxysporum* (concentration in ppm)			*Rhizoctonia bataticola* (concentration in ppm)			*Escherichia coli* (concentration in ppm)		*Pseudomonas cepacicola* (−) (concentration in ppm)	
Compound	50	100	200	50	100	200	50	100	200	500	1000	500	1000
L^1H	43	51	58	48	56	62	42	48	56	5	8	3	4
1a	69	71	74	68	70	72	–	–	61	6	9	4	9
1b	72	73	79	71	73	75	72	73	77	7	10	6	11
1c	74	76	82	75	77	79	76	78	83	8	11	8	15
1d	73	75	81	72	74	77	74	76	81	7	12	7	13
L^2H	37	45	52	42	52	57	42	52	57	6	8	5	6
2a	59	59	61	57	61	63	69	70	69	7	9	6	7
2b	68	72	78	72	76	77	–	75	79	10	12	9	10
2c	69	73	80	76	79	80	74	78	82	11	14	8	8
2d	70	77	80	72	77	79	69	76	81	12	15	9	12

with H_2O_2. The remaining H_2O_2 was decomposed and Sb(V) then determined iodimetrically.[35] Carbon and hydrogen analyses were performed at the Central Drug Research Institute (CDRI), Lucknow. 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 ligands and their complexes were scanned in the range 4000–200 cm^{-1} with the help of a model Nicolet Megna FTIR-550 spectrophotometer and a model FT IR-8400 S spectrophotometer on 'KBr optics as well as Nujol mulls'. NMR spectra were recorded using a Jeol-AL-300 FT NMR spectrometer in DMSO-d_6 using Tetra Methyl Silane as the internal standard. The conductivity of the resulting derivatives was determined at the room temperature in dry DMF by the Systronics conductivity bridge (model 305) using a cell having a cell constant of 0.5 cm^{-1}.

Microbial assay

Antifungal activity

The antifungal activity was evaluated against *Alternaria alternata, Fusarium oxysporum* and *Rhizoctonia bataticola* 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[36] (Table 3).

Antibacterial activity

Antibacterial activity was evaluated against *Escherichia coli* and *Pseudomonas cepacicola* (−) 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 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[37] (Table 3).

Antifertility activity

The Sprague–Dawley albino rats (*Rattus norvegicus*) obtained from Hamdard University, New Delhi were housed in plastic cages at room temperature (20±5 °C) and uniform light (14 : 10 light : dark). They were fed on standard laboratory chow (Aashirwad Food Industries Ltd, Chandigrah, India) and fresh water *ad libitum*. Proven fertile healthy male rats (weighing 170–200 g) were divided into seven groups of six animals each. The control group A received vehicle (olive oil) whereas the animals of groups B and C were administrated orally with the ligands L^1H and L^2H (20 mg kg^{-1} body weight dissolved in 0.5 ml olive oil) respectively for the period of 60 days. The animals of groups D, E, F and G received the same doses of compounds **1b, c** and **2b, c**, respectively, for the same period.

The rats were cohabited with normal adult proestrous females in the ratio of 1 : 4 and successful mating was confirmed by the presence of sperms in the vaginal smears. Females were separated and resultant pregnancies were noted when dams gave birth. The number and weights of litters were recorded. Fertility was calculated in control as well as in treated groups. The animals were weighed and autopsied under light ether anesthesia. Sperm motility in the cauda epididymis and density of testicular and epididymis suspended sperms were calculated.[38] The weights of the testes and other sex organs were recorded after removing the adherent tissue and frozen for cholesterol, protein, sialic acid and fructose. The right testis of each animal was fixed in Bouin's fluid for histopathological studies. The evaluation of cell population dynamics was based on the count of each cell type per cross tubular section. Various cell components were quantitatively analysed using spherically appearing sections. Abercrombie's correcting factor was introduced.[39] Serum was separated from blood by centrifugation at 3000 rpm and stored at −20 °C. Testosterone concentration was measured by radioimmunoassay.[40] The data were analysed statistically using 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.*[38] using a White Blood Corpuscles (WBC) counting Neubauer chamber of a haemocytometer, and were expressed as million spermatozoa per milliliter suspension.

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Table 4. LD_{50} for the compounds

Sample no.	Dose (mg kg^{-1} body weight per day)	No. of animals	Death
1	100	20	20
2	90	20	20
3	80	20	20
4	70	20	20
5	60	20	10

Biochemical studies

Protein was estimated by the reported procedure.[41] Sialic acid was estimated by the procedure given by Warren.[42] Cholesterol was determined as per the method of Zlatkis *et al*.[43] Fructose was determined by the method of Foreman *et al*.[44] 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 a hypodermic syringe with a pearl point needle, for 60 days and withdrawal (recovery) for 30 days.

The LD_{50} (Table 4) is statistically derived single dose of a substance that can be expected to cause death in 50% of the animals.[45] In a prohibited analysis method of LD_{50}, the selected dose levels should bracket the expected LD_{50} value with at least one dose level higher than the expected LD_{50} but not causing 100% mortality and one dose level below the expected LD_{50} but not causing 0% mortality. Toxicity of the complexes was determined by calculating the LD_{50} values. Symptoms of poisoning and mortality were observed and results of toxicity were analysed for determination of LD_{50} values of the complexes.

Results and Discussion

The reactions of Ph_2SbCl and $PhSbCl_2$ with the ligands (L^1H and L^2H) were carried out in unimolar and bimolar ratios in dry methanol. The successive replacement of chloride resulted in the formation of $Ph_2Sb(N^{\cap}X)$, $PhSbCl(N^{\cap}X)$ and $PhSb(N^{\cap}X)_2$ products. Similarly the reactions of $SbCl_3$ with the ligands were carried out in 1 : 1 molar ratio, giving addition products in dry carbon tetrachloride. The resulting addition products are creamish-yellow solids and non-electrolytic in nature. The molar conductances of 10^{-3} Molar solutions of the complexes in dry DMF lie in the 7–12 ohm^{-1} cm^2 mol^{-1} range, indicating that they are non-electrolytes. The complexes isolated are presented in Table 1 together with their analytical data.

UV spectra

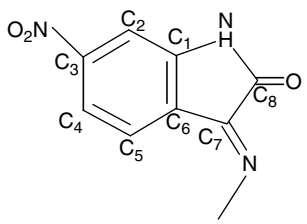
The electronic spectra of the ligands display two maxima at ~270 and ~320 nm which are due to $\pi-\pi^*$ electronic transitions and remain almost unchanged in the spectra of the metal complexes. The band around 370 nm is due to the $n-\pi^*$ transitions of the → C═N chromophore and shows a bathochromic shift of 20–30 nm after the coordination of the azomethine nitrogen to the metal atom, indicating the delocalization of the electronic charge within the chelate ring and thereby stabilizing the resulting complexes. These imine complexes were synthesized by reacting the ligands (L^1H and L^2H) having $N^{\cap}XH$ donor sites with Ph_2SbCl, $PhSbCl_2$ and $SbCl_3$ in unimolar and bimolar ratios in methanol (or in CCl_4 in case of adduct formation) to form products (or adduct) of the type $[Ph_2Sb(N^{\cap}X)]$, $[PhSbCl(N^{\cap}X)]$, $[PhSb(N^{\cap}X)_2]$ and $[Cl_3Sb.(N^{\cap}XH)]$.

IR spectra

The tentative assignments of the important characteristic bands in the IR spectra of these new antimony(III) derivatives were made by comparing them with the IR spectral data of the corresponding

Table 5. ^{13}CNMR spectral data (δ, ppm) of the ligands and their antimony(III) derivatives

Aromatic carbon



*Ph–sb

Compound	Amido/thiolo	Azomethine carbon, C_7	C_1	C_2	C_3	C_4	C_5	C_6	C(i)	C(o)	C(m)	C(p)
L^1H	165.85	156.24	142.12	124.19	128.93	127.16	127.91	123.03	–	–	–	–
1a	168.72	161.12	142.36	124.72	128.98	127.34	127.96	123.26	141.15	135.38	132.34	130.67
1b	170.83	161.86	142.38	124.63	128.93	127.36	127.82	123.36	141.37	134.77	131.89	130.88
1c	172.23	161.24	143.29	124.77	129.92	128.38	128.88	124.42	142.27	134.12	131.16	130.16
1d	171.13	161.33	142.32	124.76	129.31	126.12	126.21	122.18	–	–	–	–
L^2H	179.35	159.16	141.65	123.24	126.18	125.39	125.15	124.76	–	–	–	–
2a	168.80	163.11	140.71	124.13	127.18	125.12	126.39	124.72	142.17	135.26	132.18	130.03
2b	167.22	163.25	142.88	124.34	127.91	126.17	125.13	124.77	141.78	133.26	131.28	130.12
2c	167.45	164.12	141.79	125.33	127.09	125.89	126.38	124.79	142.16	133.27	132.68	130.74
2d	166.52	164.37	142.84	127.45	136.16	130.52	135.71	128.22	–	–	–	–

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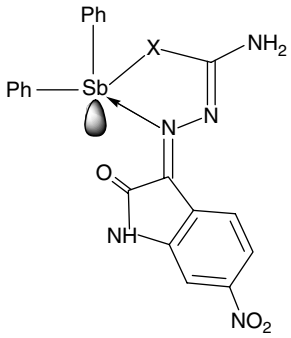
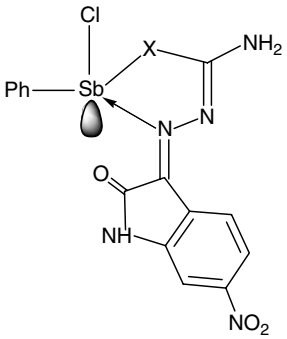


1:1 Complex



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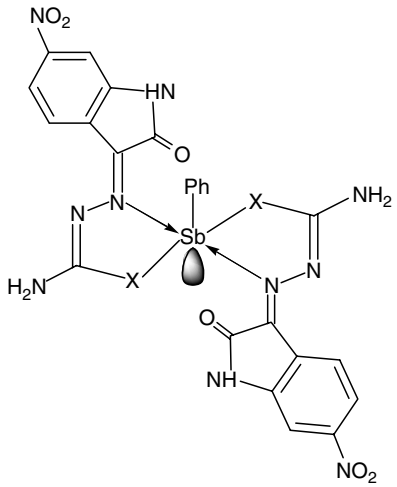

1:2 Complex

Figure 2. Proposed structures of the complexes (**1** and **2a–c**).

ligands. The presence of NH vibrations at *ca* 3150 and 3180 cm^{-1} in ligands and the disappearance (**1–2a–c**) or shifting (**1–2d**) show the participation of this group in complex formation. The shifting of → C═N[46] (10–15 cm^{-1}) and → C═O/→ C═S group bands further supports the participation of these groups in

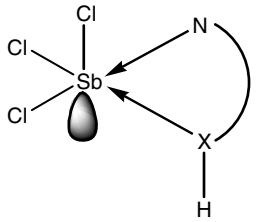

Figure 3. Proposed structure of the antimony adducts (**1–2d**); N∩XH = L^1H and L^2H, X = O/S.

complexation. The appearance of some new bands at *ca* 410–435, 510–520, 360–395, 450–440 and 250–350 cm^{-1} can be assigned to ν(Sb ← N),[47] ν(Sb–O),[48] ν(Sb–S),[49] deformation (Ph–Sb)[50] and Sb–Cl, respectively. The lack of a significant shift in the position of ring C═O (*ca* 1670 cm^{-1}) and NH_2 group (*ca* 3430–3350 cm^{-1}) suggests the non-involvement of these groups in bonding.

^{1}H NMR spectra

^{1}H NMR spectral data of ligands and their antimony(III) derivatives were recorded in Table 2. The spectra of free ligands exhibit a sharp singlet at δ11.24–11.28 ppm due to an NH proton which disappears (**1–2a–c**) or shifts[51] (**1–2d**) in the corresponding antimony(III) derivatives. No significant changes were observed in NH_2 (δ3.41–3.52 ppm) and ring NH (δ11.92–12.02 ppm) protons, showing their non-involvement in the bonding. The aromatic protons of ligand and phenyl group (attached to Sb) were observed as a complex pattern in the range of δ6.12–8.23 ppm.

^{13}C NMR spectra

A comparison of the ^{13}C NMR spectra (Table 5) of the antimony(III) complexes with those of the ligands provides very useful information about the mode of bonding. Significant shifts[52] (~5–15 ppm) were observed in the position of azomethine (δ156.24 and 159.16 ppm), thiolo (165.85 ppm) and amido (179.35 ppm) group carbon atoms in antimony(III) derivatives, further supporting the involvement of these groups in complexation. A new set of four signals observed in the spectra of the complexes in the range

Table 6. Alteration in the body weight and weights of the reproductive organs after treatment with the ligands and their antimony(III) complexes

		Body weight (g)		Organ weight (mg per 100 g^{-1} body weight)			
Group	Treatment	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
A	Control	185.0 ± 3.90	198.0 ± 4.80[c]	1370.0 ± 20.7	445.0 ± 7.8	485.0 ± 10.7	380.0 ± 9.7
B	L^1H	178.0 ± 4.70	195.0 ± 3.80[c]	1150.0 ± 22.6[b]	390.0 ± 6.7[a]	445.0 ± 12.3[a]	342.0 ± 6.5[a]
C	L^2H	190.0 ± 5.30	205.0 ± 4.70[c]	1070.0 ± 26.5[b]	380.0 ± 9.7[b]	435.0 ± 15.3[a]	315.0 ± 7.3[b]
D	**1b**	195.0 ± 5.90	210.0 ± 5.70[c]	950.0 ± 18.7[b]	265.0 ± 6.9[b]	335.0 ± 10.6[b]	208.0 ± 6.9[b]
E	**1c**	172.0 ± 4.90	193.0 ± 6.40[c]	870.0 ± 21.5[b]	275.0 ± 7.3[b]	327.0 ± 9.7[b]	215.0 ± 7.8[b]
F	**2b**	180.0 ± 5.60	200.0 ± 4.80[c]	860.0 ± 17.5[b]	245.0 ± 9.6[b]	317.0 ± 6.7[b]	205.0 ± 6.3[b]
G	**2c**	175.0 ± 7.80	197.0 ± 6.50[c]	845.0 ± 16.9[b]	255.0 ± 7.8[b]	280.0 ± 6.9[b]	195.0 ± 5.8[b]

Values are means ± SEM of six determinations.
[a] $p \leq 0.01$.
[b] $p \leq 0.001$.
[c] NS, non-significant.
Groups B and C compared with group A.
Groups D and E compared with group B.
Groups F and G compared with group C.

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Table 7. Effects of ligands and their antimony(III) complexes on sperm dynamics of male rats

Group	Treatment	Sperm motility (%) cauda epididymis	Sperm density (million ml^{-1}) Testes	Sperm density (million ml^{-1}) Cauda epididymis	Fertility(%)	Testosterone (mg dl^{-1})
A	Control	75.0 ± 2.7	4.85 ± 0.25	50.30 ± 2.60	100 (+ve)	5.40 ± 0.40
B	L^1H	55.0 ± 2.6[b]	3.10 ± 0.19[b]	25.00 ± 2.70[b]	78 (−ve)	3.10 ± 0.50[b]
C	L^2H	61.0 ± 3.5[b]	3.25 ± 0.13[b]	22.40 ± 2.40[b]	80 (−ve)	2.90 ± 0.30[b]
D	1b	35.7 ± 4.7[b]	2.10 ± 0.14[b]	15.30 ± 2.21[b]	93 (−ve)	1.50 ± 0.10[b]
E	1c	30.5 ± 3.9[b]	1.95 ± 0.13[b]	10.70 ± 1.90[b]	95 (−ve)	1.40 ± 0.15[b]
F	2b	42.0 ± 3.6[b]	1.90 ± 0.17[a]	13.30 ± 1.80[a]	94 (−ve)	1.30 ± 0.18[b]
G	2c	32.5 ± 4.3[b]	1.45 ± 0.19[b]	10.20 ± 1.40[b]	96 (−ve)	1.01 ± 0.15[b]

Values are means ± SEM of six determinations.
[a] $p \leq 0.01$.
[b] $p \leq 0.001$.
[c] NS, non-significant.
Groups B and C compared with group A.
Groups D and E compared with group B.
Groups F and G compared with group C.

δ130.03–142.27 ppm (**1a–c** and **2a–c**) was assigned to the phenyl carbons attached to the antimony atom. Further, only one set of signals for the phenyl carbons of Ph_2Sb group was observed, which indicates that the two phenyl groups are chemically equivalent.[53] The signal for carbonyl carbon (C═O) present in heterocyclic ring appears in the range δ165.26–168.26 ppm.

In view of the bidentate nature of the ligands, 1:1 and 1:2 derivatives of antimony were found to be tetra-[54] and penta-coordinated (Fig. 2), but due to the presence of a stereochemically active[29] lone pair of electrons, these derivatives were assigned to pseudo trigonal bipyramidal and pseudo octahedral geometries, respectively. The tentative structures for the antimony trichloride adducts[55] have also been suggested (Fig. 3).

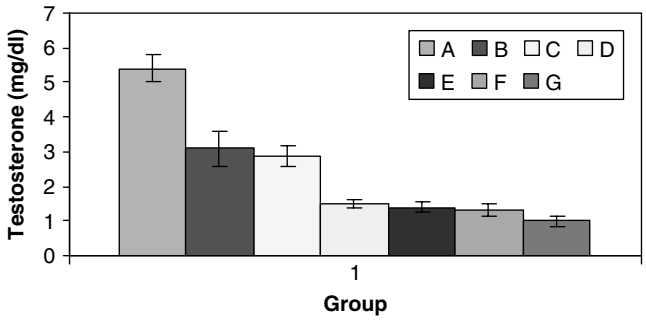

Figure 4. Changes in the testosterone concentrations after treatment with ligands and their antimony(III) derivatives.

Biological evaluation

Antimicrobial assay

The synthesized ligands and their metal complexes were screened against some pathogenic fungi and bacteria, and the results are recorded in Table 3. The results reveal that there was a significant increase in the toxicity of the complexes as compared with the ligands. On taking a closer look at these results, a common feature which appears is that the bioactivity is enhanced because:

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[56] theory.
2. It is also evident that the complexes having sulfur as a donor atom in the ligand system display higher activity than those which do not have.[57]
3. It was found that the Gram-positive bacteria are more affected than the Gram-negative bacteria. The ligands are much more toxic to Gram-positive bacteria and this toxicity is further enhanced in the complexes.[58]
4. Solubility, concentration and fineness of the particle size of the metal ion[59] as well as the presence of the bulkier organic moieties affect the growth of organisms.
5. The effect of resonating rings on toxicity may be appraised by electronic theory (Gilman) and activation theory (Arrhenius).[60]
6. Hydrolysis plays a very vital role in this matter. Fungal and bacterial cells accumulate the water-soluble metal complexes, which later dissociate to give a free central atom or its complex ion. These ions denature the proteins. Enzymes are proteins, and it is expected that the central atoms inactivate these catalysts, as a result of which normal cellular processes are impaired.

Antifertility activity

Male rats exposed to ligands L^1H and L^2H and their antimony complexes **1b–c** and **2b–c** (20 mg kg^{-1} body weight per day) for a period of 60 days showed the following alterations in reproductive function of male rats.

1. *Body and organ weights*: no significant change was observed in the body weights of male rats after treatment with ligands L^1H and L^2H and their complexes (**1b–c** and **2b–c**). However, the weights of testes, epididymis, seminal vesicle and ventral prostate were reduced significantly. Significant decline in the weight of testes may be due to a decrease in number of spermatogenic elements and spermatogonia,[61] that is cell death, which leads to regression in these organs[62,63] (Table 6).
2. *Sperm dynamics and fertility*: a significant decrease in sperm motility in the cauda epididymis was noticed after treatment with ligands L^1H and L^2H and their antimony complexes

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Table 8. Biochemical changes in the reproductive organs after oral administration of the ligands and their antimony(III) complexes

Group	Treatment	Protein (mg g^{-1})				Sialic acid (mg g^{-1})				Testicular cholesterol (mg g^{-1})	Seminal fructose (mg g^{-1})
		Testes	Epididymis	Seminal vesicle	Ventral prostate	Testes	Epididymis	Seminal vesicle	Ventral prostate		
A	Control	245.0 ± 7.5	220.0 ± 3.7	198.0 ± 4.7	188.0 ± 7.5	5.30 ± 0.59	5.9 ± 00.61	5.80 ± 0.81	6.00 ± 0.72	7.80 ± 0.92	470.0 ± 35.0
B	L^1H	190.0 ± 8.6[a]	180.0 ± 3.4[a]	158.0 ± 4.8[a]	147.0 ± 6.3[a]	4.18 ± 0.70[b]	4.49 ± 0.63[b]	4.40 ± 0.32[b]	4.89 ± 0.32[b]	8.40 ± 0.12[a]	385.0 ± 17.0[b]
C	L^2H	185.0 ± 3.9[b]	189.0 ± 3.2[a]	148.0 ± 4.3[b]	151.0 ± 6.4[a]	4.27 ± 0.68[b]	4.41 ± 0.38[b]	4.21 ± 0.84[b]	4.60 ± 0.18[b]	8.80 ± 0.19[a]	375.0 ± 15.8[b]
D	**1b**	140.0 ± 6.5[a]	150.0 ± 3.9[a]	130.0 ± 3.2[a]	123.0 ± 3.4[a]	3.60 ± 0.40[b]	2.70 ± 0.42[b]	3.41 ± 0.14[b]	3.21 ± 0.38[b]	9.19 ± 0.13[a]	290.0 ± 13.7[b]
E	**1c**	148.0 ± 3.2[a]	138.0 ± 2.9[b]	121.0 ± 3.9[b]	108.0 ± 3.7[b]	3.30 ± 0.47[b]	2.55 ± 0.44[b]	2.91 ± 0.53[b]	3.75 ± 0.35[b]	9.94 ± 0.11[a]	275.0 ± 17.4[b]
F	**2b**	129.0 ± 3.0[b]	118.0 ± 3.4[b]	115.0 ± 3.1[a]	127.0 ± 3.9[a]	2.90 ± 0.41[a]	2.15 ± 0.42[b]	2.60 ± 0.39[b]	2.98 ± 0.57[b]	10.32 ± 1.20[b]	260.0 ± 18.3[b]
G	**2c**	138.0 ± 6.5[b]	108.0 ± 4.7[b]	118.0 ± 2.9[a]	112.0 ± 3.3[b]	2.78 ± 0.71[a]	2.75 ± 0.33[b]	2.10 ± 0.19[b]	2.49 ± 0.33[b]	10.88 ± 1.38[b]	251.0 ± 18.8[b]

Values are means ± SEM of six determinations.
[a] $p \leq 0.01$.
[b] $p \leq 0.001$.
[c] NS, non-significant.
Groups B and C compared with group A.
Groups D and E compared with group B.
Groups F and G compared with group C.

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Table 9. Testicular cell population dynamics in rats following ligands and their antimony(III) complexes

Group	Treatment	Testicular cell counts (number per 10 cross sections)					Seminiferous tubular diameter (μm)
		Sertoli Cells	Spermatogonia	Proleptotene spermtocytes	Pachytene spermatocytes	Secondary spermatocytes	
A	Control	2.90 ± 0.06	7.37 ± 0.81	24.25 ± 1.51	39.0 ± 2.10	50.30 ± 2.95	265.0 ± 7.50
B	L^1H	2.49 ± 0.09	6.19 ± 0.65[b]	17.20 ± 1.32[b]	25.0 ± 1.70[b]	38.0 ± 2.15[b]	220.0 ± 7.40[a]
C	L^2H	2.36 ± 0.08[b]	6.12 ± 0.15[b]	18.10 ± 1.54[b]	27.0 ± 0.90[b]	35.0 ± 1.97[b]	200.5 ± 6.90[b]
D	**1b**	2.02 ± 0.05[b]	4.85 ± 0.91[b]	12.1 ± 2.30[b]	20.4 ± 6.80[a]	20.5 ± 1.65[b]	160.0 ± 7.40[b]
E	**1c**	2.05 ± 0.04[b]	4.70 ± 0.75[b]	12.8 ± 1.41[b]	18.5 ± 0.50[b]	22.1 ± 1.30[b]	150.0 ± 6.30[b]
F	**2b**	2.09 ± 0.03[b]	4.10 ± 0.35[b]	14.15 ± 1.21[a]	21.5 ± 0.30[b]	21.5 ± 1.70[b]	145.0 ± 3.70[b]
G	**2c**	2.00 ± 0.06[b]	4.30 ± 0.42[b]	12.40 ± 1.25[a]	17.8 ± 0.40[b]	20.3 ± 1.42[b]	125.0 ± 3.80[b]

Values are means ± SEM of six determinations.
[a] $p \leq 0.01$.
[b] $p \leq 0.001$.
[c] NS, non-significant.
Groups B and C compared with group A.
Groups D and E compared with group B.
Groups F and G compared with group C.

(**1b–c** and **2b–c**). Sperm density in testes and epididymis were also reduced after various treatments (Table 7). Low cauda epididymis sperm density may be due to alteration in androgen metabolism.[64,65] Further, low motility and negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milleu of the cauda epididymis.[66]

3. *Testosterone*: serum testosterone concentration was significantly reduced after treatment with ligands L^1H and L^2H and their antimony complexes **1b–c** and **2b–c** (Fig. 4). Reduction in the weight of accessory reproductive organs directly supports the reduced availability of androgens.[67,68] Shrinkage in the seminiferous tubular diameter may be attributed to a decline in the testosterone production.[69]
4. *Biochemical change* (Table 8): (a) *protein* – the protein contents of testes, epididymis, seminal vesicle and ventral prostate were significantly reduced in compound-treated rats when compared with the control. Reduction in the protein contents of testes and other accessory sex organs may be due to the absence of spermatogenic stages[70] in the testes. The accumulation of cholesterol in testes is direct evidence of antiandrogenic action.[68] (b) *Sialic acid* – the sialic acid contents of testes, epididymis, seminal vesicle and ventral prostate were decreased after treatment with ligands L^1H and L^2H and their complexes; these decreased levels of sialic acid may be correlated with the loss of androgen.[71] (c) *Cholesterol* – testicular cholesterol contents were significantly increased in rats treated with ligands L^1H and L^2H and their antimony complexes. (d) *Fructose* – seminal vesicular fructose contents were decreased in all experimental groups when compared with control.
5. *Cell population dynamics* (Table 9) – the total number of Sertoli cells and seminiferous tubular diameter was reduced after treatment with ligands L^1H and L^2H and their antimony complexes. A significant decrease in spermatogonia, proleptotene spermatocytes, pachytene spermatocytes and secondary spermatocytes was 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 the antiandrogenic nature of the compounds as these stages are completely androgen dependent.[72] Further, it is supported by reduction in the serum testosterone levels, which clearly demonstrates the inhibitory effect of ligands L^1H and L^2H and their antimony complexes.

Conclusions

The results of this study indicate that the test compounds are capable of suppressing the process of spermatogenesis by inhibiting the serum testosterone levels. The fertility of male animals was suppressed by 78 and 80% with ligand-treated rats and 93, 95, 94 and 96% with antimony(III) compounds-treated rats. In conclusion, our study suggests that the addition of the antimony(III) moiety to ligands L^1H and L^2H enhances their fertility regulatory efficiency.

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