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Intramolecular phenylborane complexes with monobasic bidentate Schiff bases

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A series of intramolecular complexes with Schiff base ligands having N∩S and N∩O donor systems were synthesized in an open vessel under microwave irradiation (MWI) using a domestic microwave oven. The reaction time has been brought down from hours to seconds with improved yield as compared with the conventional heating. The complexes have been characterized on the basis of elemental analysis, conductance measurements and spectroscopic analysis. Based on the IR, ^{1}H NMR, ^{11}B NMR and ^{13}C NMR spectroscopic studies, a tetrahedral geometry has been proposed for the resulting complexes. The compounds have been screened *in vitro* against bacteria and fungi to test their antimicrobial property and *in vivo* in male albino rats to test their antifertility property. The testicular sperm density, motility and density of cauda epididymal spermatozoa along with biochemical parameters of reproductive organs have been examined and discussed. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: phenylborane complexes; Schiff base; spectroscopic techniques; antimicrobial and antifertility test

INTRODUCTION

Conventional synthesis suffers from many disadvantages and puts at risk both human health and environment. Green chemistry as applied to chemical processes is environmentally benign (in terms of energy use, auxiliaries, waste, etc.) and always leads to simplification of processes in terms of chemicals used and steps involved. Recently, the acceleration of a wide range of chemical reactions using microwave dielectric heating has been reported.[1] This *in situ* mode of energy conversion has many attractions to the chemist, because its efficiency depends on the properties of the molecules.[2] Studies of new types of chemotherapeutically important Schiff bases and metal coordinated drugs are now attracting much attention.[3] Schiff base metal chelates are important in biological processes, pre-concentration of metal ions and catalysis.[4] After suitable structural modifications, the derivatives of Schiff bases and metal coordinated drugs may be used as bioactive materials for medicines as well as in industry.[5,6] The reason for application of fluoro-organometallic compounds in the pharmaceutical field is their microbial activity.[7] Thiosemicarbazones[8] are biologically active pharmacophores, besides having good complexing ability, and their activity enhances on complexation with metal ions.[9,10] The biological properties of semicarbazones and thiosemicarbazones are often related to the metal ion coordination. Borane complexes of Schiff base ligands are known to function as antimicrobial and antifertility agents.[11] There is an immense scope for undertaking systematic studies including the biochemical applications[12] of the borane complexes with a variety of azomethines. Organoboron compounds are attracting attention currently because of their importance as synthetic intermediates.[13,14] Organoboron compounds are used as a source of radicals.[15] Boron may play an active role in human brain function[16] there is additional evidence that boron is an essential nutrient for humans. The present situation prompted us to prepare complexes of monobasic bidentate Schiff base ligands. Antifungal and antibacterial activities of these boron complexes have been carried out to study the role of boron in tetrahedral surroundings. The focus of our present communication is the

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exploration of the studies on synthetic, structural and biological aspects of boron complexes.

EXPERIMENTAL

All the chemicals were dried and purified before use and the purity was checked by thin layer chromatography (TLC).

Preparation of the ligands

Ligands 1,3-dihydro-3-[2-(4-fluorophenyl)-2-oxo-ethylidene]-2H-indol-2-one hydrazine carbothioamide and 1,3-dihydro-3-[2-(4-fluoro-3-methyl-phenyl)-2-oxo-ethylidene]-2H-indol-2-one hydrazine carbothioamide as well as 1,3-dihydro-3-[2-(4-fluorophenyl)-2-oxo-ethylidene]-2H-indol-2-one hydrazine carboxamide and 1,3-dihydro-3-[2-(4-fluoro-3-methyl-phenyl)-2-oxo-ethylidene]-2H-indol-2-one hydrazine carboxamide were prepared by reaction of hydrazine carbothioamide and hydrazine carboxamide with the respective ketones in ethanolic medium. The reaction mixture was refluxed for 1 h. On cooling, the products were recrystallized from the ethanol and dried *in vacuo*. Their physical properties and analytical data are given in Table 1. The parent ligands exist in tautomeric forms.

Preparation of the complexes

Traditional method

A calculated amount of the ligand dissolved in dry toluene was added to the dihydroxyphenylborane in unimolar and bimolar ratios. The reaction mixture was refluxed for 10–12 h on a fractionating column and the progress of the reaction was monitored by the liberation of azeotrope water–toluene. After the completion of the reaction, excess solvent was distilled off and products were dried. The resulting products were washed with dry cyclohexane and then finally dried *in vacuo* for 3–4 h.

Ecofriendly method

A mixture of dihydroxyphenylborane (11.91–17.11 g) and the ligand (6.88–9.13 g) dissolved in dry toluene was taken in an open borosil beaker and irradiated inside a microwave oven for 4–8 min. A drastic reduction in reaction time was thus observed due to the rapid heating capability of microwaves. The completion of the reaction was examined by TLC (each after 1 min). Finally the product was worked up as described in method A and found to be pure by TLC.

Analytical methods and physical measurements

Nitrogen and sulfur[17] were estimated by Kjeldahl's and Messenger's methods, respectively. Boron was estimated volumetrically as boric acid. The UV spectra were recorded on an Hitachi-U-2000 spectrophotometer. The IR spectra with KBr optics were obtained using the Perkin–Elmer 577 grating spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 using TMS as the standard on a Jeol AL 300 FT NMR. ^{11}B NMR spectra were scanned using $BF_3^{\bullet}Et_2O$ as an external standard. The molecular weights of the compounds were determined ebullioscopically.

RESULTS AND DISCUSSION

Reactions of phenylboronic acid with monobasic bidentate ligands were carried out in dry toluene and under microwave irradiation. These reactions may be represented as follows:

$$PhB(OH)_2 + N^{\cap}XH \longrightarrow PhB(OH)(N^{\cap}X) + H_2O$$

$$PhB(OH)_2 + 2N^{\cap}XH \longrightarrow PhB(N^{\cap}X)_2 + 2\,H_2O$$

where $N^{\cap}X$ is the donor system of the reacting ligand moiety and X = S or O.

The resulting colored solids are soluble in DMF and DMSO. The UV, IR and NMR spectra support the proposed structures. Their low molar conductivity values (10–15 $ohm^{-1}\ cm^2\ mol^{-1}$) show that they are non-electrolytes in nature.

SPECTROSCOPIC STUDIES

UV spectra

The UV spectra of the ligands and their complexes show bands at ca.275 and 300 nm assignable to $\pi-\pi^*$ transitions. These remain almost unchanged in the complexes. Another band due to >C═N is observed at 370 nm in the spectra of the ligands, which shifts to the higher wavelength (380–385 nm) for organoboron derivatives due to donation of the lone pair of electrons by the nitrogen of the ligand to central boron atom, indicating the delocalization of the electronic charge within the chelate ring and thus the stabilizing of the resulting complexes.

IR spectra

The band due to ν(C═N)[18] at 1590–1620 cm^{-1} registers a substantial change (20–30 cm^{-1}) in the boron complexes as a result of increase in bond order showing coordination of the azomethine nitrogen to the boron atom. In 1 : 2 complexes a band due to uncoordinated (C═N) appears at ~1595 cm^{-1}. The bands due to ν(C═O) and ν(C═S) groups in the spectra of the ligands were observed at 1680–1690 and 1020–1050 cm^{-1}, respectively. These bands disappeared in the spectra of the complexes, suggesting thereby enolization of the ligands and their chelation through amido oxygen and thiolic sulfur.[19] In the solid-state IR spectra of the ligands, bands observed in the region 3250–3100 cm^{-1} are due to ν NH,[19] which do not appear in the spectra of the complexes showing the deprotonation of this group. However, in the solution spectra of the ligands an additional band due to ν (SH)/(OH) also appears and thus shows tautomerization (Scheme 1). The ν (OH) band in the case of 1 : 1 boron complexes appears at ca. 3450 cm^{-1}. Two sharp bands around 3360 and 3420 cm^{-1}

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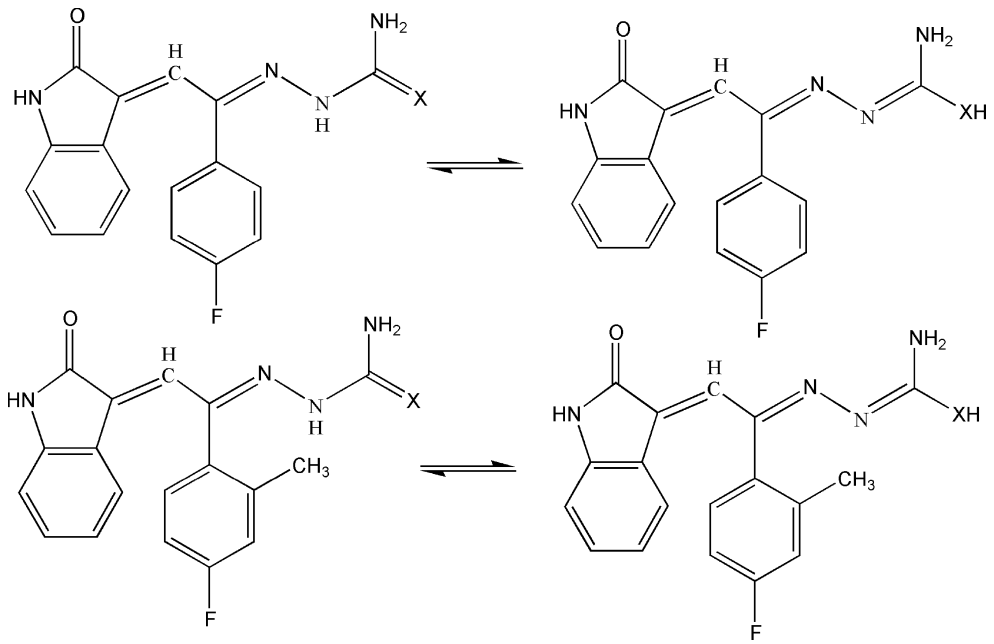
Table 1. Synthetic and analytical data of the ligands and their boron complexes

Reactants, g (mmol)		Molar ratio	Compound	Colour	M.P. (°C)	Time/yield (h)/(%) Traditional method	Time/yield (min)/(%) Ecofriendly method	Elemental analysis (%)[a]			Mol. wt[a]
SM	Ligand							N	S	B	
—	—	—	L^1H ($C_{17}H_{13}N_4OSF$)	Orange	185	—	—	16.15 (16.48)	9.13 (9.43)	—	335 (340)
PhB $(OH)_2$ 0.24 (1.97)	L^1H 0.64 (1.97)	1 : 1	$C_{23}H_{18}N_4O_2SFB$	Orange red	203	8/56	4/83	12.59 (12.62)	7.15 (7.22)	2.38 (2.43)	440 (443)
PhB $(OH)_2$ 0.24 (1.97)	L^1H 1.28 (3.95)	1 : 2	$C_{40}H_{29}N_8O_2S_2F_2B$	Brown	295	11/54	6/89	14.52 (14.61)	8.24 (8.36)	1.39 (1.41)	762 (766)
—	—	—	L^2H ($C_{18}H_{15}N_4OSF$)	Orange	165	—	—	15.72 (15.83)	9.01 (9.06)	—	350 (354)
PhB $(OH)_2$ 0.38 (3.12)	L^2H 1.05 (3.11)	1 : 1	$C_{24}H_{20}N_4O_2SFB$	Red brown	205	12/62	5/78	11.91 (12.23)	6.88 (7.00)	2.29 (2.36)	456 (457)
PhB $(OH)_2$ 0.38 (3.12)	L^2H 2.10 (6.21)	1 : 2	$C_{42}H_{33}N_8O_2S_2F_2B$	Orange	225	10/64	8/75	13. 95 (14.10)	7.85 (8.07)	1.32 (1.36)	789 (794)
—	—	—	L^3H ($C_{17}H_{13}N_4O_2F$)	Orange	153	—	—	17.11 (17.29)	—	—	322 (324)
PhB $(OH)_2$ 0.26 (2.13)	L^3H 0.72 (2.12)	1 : 1	$C_{23}H_{18}N_4O_3FB$	Orange	195	10/65	7/76	12.98 (13.09)	—	2.49 (2.52)	425 (427)
PhB $(OH)_2$ 0.26 (2.13)	L^3H 1.44 (4.23)	1 : 2	$C_{40}H_{29}N_8O_4F_2B$	Red	222	12/52	8/68	14.42 (14.83)	—	1.43 (1.47)	730 (734)
—	—	—	L^4H ($C_{18}H_{15}N_4O_2F$)	Orange	160	—	—	16.48 (16.58)	—	—	336 (338)
PhB $(OH)_2$ 0.21 (1.72)	L^4H 0.61 (1.72)	1 : 1	$C_{24}H_{20}N_4O_3FB$	Orange red	198	10/55	6/75	12.42 (12.67)	—	2.39 (2.44)	438 (441)
PhB $(OH)_2$ 0.21 (1.72)	L^4H 1.22 (3.45)	1 : 2	$C_{42}H_{33}N_8O_4F_2B$	Brick red	210	10/58	8/75	14.53 (14.69)	—	1.39 (1.41)	760 (762)

[a] Calculated values are given in parentheses.



L^nH = L^1H, L^2H, L^3H and L^4H
where n = 1, 2, 3, 4
X = S, L^1H, L^2H
X = O, L^3H, L^4H

Scheme 1.

due to the NH_2 group remain unaltered in the spectra of the complexes. A strong band observed in the spectra of the boron complexes in the region 1280–1245 cm^{-1} can reasonably be assigned to Ph-B grouping. The appearance of ν (B ← N), ν (B–S) and ν (B–O) bands at 1535, 870 and 1024 cm^{-1}, respectively, indicate that the azomethine nitrogen, thiolo sulfur and enol oxygen are in coordinative interaction at the boron centre.[20]

^{1}H NMR spectra

The ^{1}H NMR spectra of the free ligands[19] and the complexes were recorded in DMSO-d_6. The spectra of the ligands exhibit signals due to the –NH of the isatin ring and the –NH of the thiosemicarbazone and semicarbazone. The disappearance of the –NH signal of the thiosemicarbazone and semicarbazone in the complexes indicates coordination of the azomethine nitrogen as well as covalent bond formation between boron and sulfur/oxygen. Further, in the spectra of the complexes, a slight downfield shift in the position of aromatic protons also indicates the coordination of the azomethine nitrogen to the boron atom. The appearance of a signal due to NH_2 group at about same position in the ligand and the boron complexes shows the non-involvement of this group in coordination. Data are given in Table 2.

Table 2. ^{1}H NMR spectroscopic data (δ, ppm) of the ligands and their boron complexes

Compound	–NH (ring)	–NH (free)	NH_2	>C=N	Aromatic/ Ph–B[a]
L^1H ($C_{17}H_{13}N_4OSF$)	11.08	10.04	3.16	8.08	7.94–7.54
$C_{23}H_{18}N_4O_2SFB$	11.22	—	3.56	8.22	8.32–7.96
$C_{40}H_{29}N_8O_2S_2F_2B$	11.36	—	3.66	8.34	8.28–7.89
L_2H ($C_{18}H_{15}N_4OSF$)	11.12	10.24	2.64	8.12	7.72–6.34
$C_{24}H_{20}N_4O_2SFB$	11.32	—	2.72	8.26	7.84–6.46
$C_{42}H_{33}N_8O_2S_2F_2B$	11.38	—	2.76	8.42	8.04–6.89
L_3H ($C_{17}H_{13}N_4O_2F$)	10.04	9.72	3.04	7.16	6.95–6.16
$C_{23}H_{18}N_4O_3FB$	10.36	—	3.26	7.54	7.14–6.64
$C_{40}H_{29}N_8O_4F_2B$	10.28	—	3.34	7.82	7.24–6.78
L_4H ($C_{18}H_{15}N_4O_2F$)	10.12	10.08	2.98	8.08	7.68–6.65
$C_{24}H_{20}N4O_3FB$	10.64	—	3.08	8.26	7.50–6.16
$C_{42}H_{33}N_8O_4F_2B$	10.69	—	3.04	8.34	7.20–6.36

[a] Merged with aromatic protons.

^{13}C NMR spectra

The chemical shift values of the carbon atom attached to the azomethine nitrogen, thiolic sulfur or amido oxygen lends further support to the proposed coordination in these complexes. The heterocylic moiety carbon signals, especially those of the carbon atoms directly bonded to the heteroatom, undergo slight upfield shifts relative to the other carbon atoms which remain almost undisturbed. The upfield shift of the thiolo carbon and azomethine carbon in the complexes indicate participation of these groups in coordination to the

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Table 3. ^{13}C NMR spectroscopic data (δ, ppm) of the ligands and their boron complexes

Compound	Amido/thiolo	Azomethine carbon	Aromatic carbons
L^1H ($C_{17}H_{13}N_4OSF$)	178.62	157.66	148.08, 144.05, 136.25, 137.71, 133.22, 132.13
$C_{23}H_{18}N_4O_2SFB$	165.48	154.63	148.40, 144.51, 136.76, 137.22, 133.05, 132.30.
$C_{40}H_{29}N_8O_2S_2F_2B$	166.49	156.72	147.20, 144.10, 136.90, 137.52, 132.64, 132.99
L_2H ($C_{18}H_{15}N_4OSF$)	172.52	155.12	147.24, 144.28, 136.92, 135.72,130.22, 129.71
$C_{24}H_{20}N_4O_2SFB$	165.47	148.35	146.58, 144.12, 136.52, 135.40, 130.05, 128.62
$C_{42}H_{33}N_8O_2S_2F_2B$	165.86	149.72	146.34, 143.35, 136.09, 135.18, 129.62, 128.62
L_3H ($C_{17}H_{13}N_4O_2F$)	167.65	158.25	148.80, 146.51, 132.40, 130.10, 129.70, 121.80
$C_{23}H_{18}N_4O_3FB$	161.58	151.38	148.45, 144.87, 132.14, 129.89, 129.56, 121.45
$C_{40}H_{29}N_8O_4F_2B$	162.05	153.10	148.67, 144.62, 132.33, 130.05, 129.47, 121.28
$C_{24}H_{20}N4O_3FB$	160.45	155.38	143.49, 127.56, 126.08, 123.14, 121.34, 120.07
L_4H ($C_{18}H_{15}N_4O_2F$)	170.20	160.24	143.66, 127.85, 126.54, 123.32, 122.36, 120.66
$C_{42}H_{33}N_8O_4F_2B$	162.32	154.12	143.35, 127.38, 125.67, 123.04, 121.23, 120.05

boron atom (Table 3).

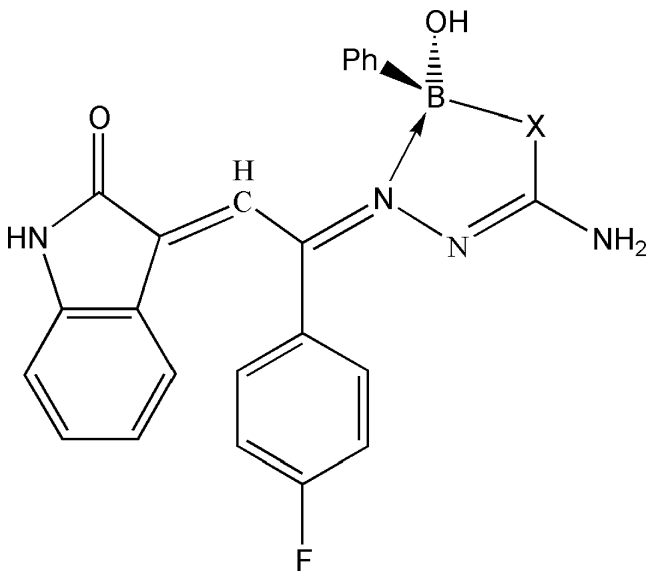
^{11}B NMR spectra[21]

The signals in the ^{11}B NMR spectra of the complexes were observed in the range δ2.1–6.4 ppm, which suggest a tetracoordinated environment around the boron atom and formation of a coordinate bond.

On the basis of the results discussed so far, including analytical and spectroscopic data, a tetracoordinated geometry is suggested for the boron complexes (Scheme 2).

MICROBIAL ASSAY

Fungicidal and bactericidal activities of the ligands and their corresponding boron complexes against different fungi and bacteria have been recorded in Tables 4 and 5 by the methods reported earlier.[22] On the basis of these studies, it may be concluded that fungitoxicity and bacteriostatic properties of a compound may be slightly enhanced on chelation with the boron ion.



Scheme 2.

The free ligands (L^1H, L^2H, L^3H and L^4H) and their complexes were tested against fungi and bacteria to see their growth inhibitory potential towards the test organisms. The antifungal activity was tested against *Fusarium oxysporum* and *Aspergillus niger*, and the bacteria used were *Escherichia coli* and *Staphylococcus aureus*. 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: fungicidal screening (poisoned food technique)

Potato dextrose agar medium was prepared in flask and sterilized. Accurate amounts of all the compounds were added after being dissolved in methanol so as to obtain certain final concentrations of 50,100 and 200 ppm. Aliquots of 15 ml medium were poured in sterilized petriplates. A culture of test fungus was grown on PDA for certain days at the optimum temperature for growth. A small disc of the fungus culture was cut with a sterile cork borer and transferred aseptically into the centre of petridisc containing the medium with a certain amount of fungi and incubated for 4 days at 25 ± 2 °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.

Bactericidal screening (inhibition zone technique)

Flat-bottomed petridisc were used and nearly 15 ml of the beef extract medium (peptone 5 g, beef extract 5 g, NaCl 5 g, agar–agar 20 g and distilled water 1000ml) were pipetted out into the petridisc. Then bacterial suspension was added and after some time bacterial growth was seen in the medium. The

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Table 4. Antifungal screening data of the ligands and their corresponding complexes. Average inhibition (%) after 96 h (concentration 25, 50 and 100 ppm)

	Fusarium oxysporum			*Aspergillus niger*		
Compound	50 ppm	100 ppm	200 ppm	50 ppm	100 ppm	200 ppm
L^1H ($C_{17}H_{13}N_4OSF$)	34	46	53	54	62	68
$C_{23}H_{18}N_4O_2SFB$	38	52	60	58	64	69
$C_{40}H_{29}N_8O_2S_2F_2B$	39	54	62	58	66	72
L_2H ($C_{18}H_{15}N_4OSF$)	48	57	60	55	66	70
$C_{24}H_{20}N_4O_2SFB$	50	58	67	56	61	68
$C_{42}H_{33}N_8O_2S_2F_2B$	56	64	70	59	66	69
L_3H ($C_{17}H_{13}N_4O_2F$)	23	34	45	54	60	65
$C_{23}H_{18}N_4O_3FB$	34	39	50	56	63	67
$C_{40}H_{29}N_8O_4F_2B$	40	48	54	62	68	74
L_4H ($C_{18}H_{15}N_4O_2F$)	28	38	55	53	62	66
$C_{24}H_{20}N4O_3FB$	38	44	57	54	67	71
$C_{42}H_{33}N_8O_4F_2B$	48	64	70	62	68	72
Standard (Bavistin)	91	100	100	86	98	100

test compounds were dissolved in methanol to give 500 and 1000 ppm final concentrations. Paper discs of Whatman no. 1 filter paper of 5 mm diameter were soaked in these solutions of varied concentrations. The discs were dried and placed on the medium with organism in Petri disc at suitable distances. These Petri discs were incubated for 24 h at $25 \pm 2\,^{\circ}C$ and zone of inhibition was measured in mm.

Results of antimicrobial studies

Fungicidal and bactericidal screening data show that under identical experimental conditions the compounds possess antimicrobial properties. However, a few compounds possess good activity against microorganisms. It is also noteworthy that the complexes are more active than their parent ligands against the same microorganism. This increase in biocidal activity of the complexes can be explained by the chelation theory.[23] The chelation reduces the polarity of the central ion because of partial sharing of its positive charge with the donor groups and possible π electron delocalization over the whole chelate ring. Such chelation increases the lipophilic character of the central atom in complex. The increase in the lipophilic nature results in an increase in permeability of the complex through the lipid layer of the cell membrane of microbes. It is seen that concentration plays an important role in inhibiting the growth of microorganisms. At lower concentration, inhibition is less severe. Owing to this fact the activities of the organisms will be slowed down, while at higher concentrations, more enzymes will become inhibited, leading to a quicker death of the organisms.

Biological aspects

Experimental design

The healthy male albino rats were used for the present study. The animals were given commercial pelleted feed and tap water *ad libitum*. The temperature of their houses was kept at $24 \pm 3\,^{\circ}C$. No rat mortality occurred during the study period. The rats were weighed weekly and changes recorded. The mating exposure test was done on day 55th of the experiment. The rats were sacrificed 24 h after the last dose (61st day) to perform various tests. The weighed rats were divided into three groups and each group comprised six rats.

Group I was selected as control and treated with olive oil, 0.5 ml/day, which was the vehicle chosen to administer the synthesized compounds. Group II was treated with L_1H 5 mg in 0.5 ml/day olive oil and Group III was treated with $C_{23}H_{18}N_4O_2SFB$ 5 mg in 0.5 ml/day olive oil. All the compounds were given regularly for 60 days.

Table 5. Antibacterial screening data of the ligands and their corresponding complexes. Diameter of Inhibition zone (mm) after 24 h (concentration 500 and 1000 ppm)

	Staphylococcus aureus		*Escherichia coli*	
Compound	500	1000	500	1000
L^1H ($C_{17}H_{13}N_4OSF$)	7	9	6	7
$C_{23}H_{18}N_4O_2SFB$	8	11	7	10
$C_{40}H_{29}N_8O_2S_2F_2B$	10	14	9	11
L_2H ($C_{18}H_{15}N_4OSF$)	8	10	5	6
$C_{24}H_{20}N_4O_2SFB$	10	14	9	13
$C_{42}H_{33}N_8O_2S_2F_2B$	11	13	9	14
L_3H ($C_{17}H_{13}N_4O_2F$)	3	5	5	6
$C_{23}H_{18}N_4O_3FB$	7	9	7	9
$C_{40}H_{29}N_8O_4F_2B$	10	12	10	14
L_4H ($C_{18}H_{15}N_4O_2F$)	4	7	4	6
$C_{24}H_{20}N_4O_3FB$	7	10	6	13
$C_{42}H_{33}N_8O_4F_2B$	9	14	7	14
Standard (Streptomycin)	15	17	17	18

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Fertility test

The experimental rats were cohabited with normal adult proestrous females in the ratio of 1 : 4. Successful mating was confirmed by the presence of sperm in vaginal smears. Females were separated and resultant pregnancies were noted, when dams gave birth. The number and size of litters delivered were recorded. Fertility was calculated in control as well as in treated animals.

Body and organ weight measurements

The animals were sacrificed under light ether anaesthesia. The reproductive and accessory sex organs (testes, epididymis, seminal vesicle, ventral prostate and vas deferens) were carefully dissected out and freed from adherent tissues. The liver was also dissected and separated. The weight of each organ was measured with an electronic weighing machine, with a sensitivity of 0.01 g.

Sperm motility and sperm density

The epididymis exposed by a scrotal incision. A cut was made at the distal end of the cauda epididymis and spermatozoa were expressed by gentle pressure into a fixed amount of physiological saline to make a sperm suspension. The sperm suspension was then placed on a glass slide and observed for forward motility. At least 100 spermatozoa per rat were observed under a microscope using a pre-calibrated micrometer.

The sperm suspension was placed on a Neubauers chamber of haemocytometer and allowed to settle for 1 h. The numbers of spermatozoa in appropriate squares were counted under light microscope at 100× magnification. Then with the help of standard formulae, counts per millilitre were calculated.

Haematology

Blood collected in preheparinized tubes by cardiac puncture. Total red blood corpuscles, haematocrit, percentage haemoglobin, MCV, MCH and MCHC were calculated using the standard methods.

RESULTS

Body and organ weight

No significant change in the body weight was observed throughout the experimental duration in all these groups. On the contrary weight of testes, epididymis, seminal vesicle, ventral prostate and vas deferens was significantly ($p < 0.001$) reduced in groups II and III compared with the control group (Table 6).

Sperm motility and sperm density[24]

There was 43.15 and 41.73% reduction in the sperm motility of the groups II and III, respectively, as compared with the control group. Sperm density in cauda epididymis was found to be only 8.99 and 6.11% of that in control groups II and III,

Table 6. Effect of various compounds on body and organ weights of male rats

Treatment	Body weight (g)			Tissue weight (mg/100 g body weight)			
	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate	Vas deferens
Control (vehicle treated) 0.5 ml olive oil/kg body wt/day for 60 days	189.00 ± 07.24	193.00 ± 05.24	1275.00 ± 39.28	406 ± 07.80	563.75 ± 08.63	263.20 ± 26.62	157.66 ± 12.63
$C_{17}H_{13}N_4OSF$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	185.00^{ns} ± 06.65	192.00^{ns} ± 05.64	795.63^{ns} ± 41.65	387.45^{ns} ± 06.19	528.10^{ns} ± 09.69	159.62^{ns} ± 17.65	121.01^{ns} ± 12.02
$C_{23}H_{18}N_4O_2SFB$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	186.00^{ns} ± 07.01	191.00^{ns} ± 05.04	729.71^{ns} ± 47.79	336.33^{ns} ± 06.99	519.04^{ns} ± 08.32	132.23^{ns} ± 21.63	118.24^{ns} ± 11.65

Mean ± SEM of six animals.
^{ns} = Non-significant.
* $p \leq 0.01$ = significant.
** $p \leq 0.001$ = highly significant.
All groups compared with control.

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Table 7. Effects of various compounds on sperm dynamics and fertility of male rats

Compound	Sperm motility (*Cauda epididymis*) (%)	Sperm density (*Cauda epididymis*) (million/cm^3)	Fertility test (%)
Control (vehicle treated) 0.5 ml olive oil/kg body wt/day for 60 days	68.65 ± 02.20	35.70 ± 02.90	98 (+ve)
$C_{17}H_{13}N_4OSF$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	29.62** ± 03.05	03.21** ± 00.06	43 (−)
$C_{23}H_{18}N_4O_2SFB$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	28.65** ± 05.20	2.18** ± 00.30	68 (−)

Mean ± SEM of six animals.
ns = Non-significant.
* $p \leq 0.01$ = significant.
** $p \leq 0.001$ = highly significant.
All groups compared with control.

Table 8. Effect of various compounds on biochemical parameters of male rats

Treatment	Cholesterol (mg/g)		Glycogen (mg/g)	
	Testes	Liver	Testes	Liver
Control (vehicle treated) 0.5 ml olive oil/kg body wt/day for 60 days	8.05 ± 00.06	14.05 ± 00.18	03.84 ± 00.10	06.85 ± 00.39
$C_{17}H_{13}N_4OSF$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	09.36** ± 00.02	14.62ns ± 00.15	01.18** ± 00.12	05.92ns ± 01.30
$C_{23}H_{18}N_4O_2SFB$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	09.85** ± 00.02	14.91** ± 00.44	01.11** ± 00.08	05.10ns ± 02.20

Mean ± SEM of six animals.
ns = non-significant.
* $p \leq 0.01$ = Significant
** $p \leq 0.001$ = Highly significant
All groups compared with control

respectively. The motility of sperm in groups II and III was found to be sluggish without forward progression compared with the controls. The results are summarized in Table 7.

Biochemical parameters[25]

Highly significant ($p < 0.001$) increase in the testicular cholesterol levels was observed in groups II and III with respect to the control. On the other hand, the level of cholesterol in hepatic tissue did not show significant change. A highly significant decrease ($p < 0.001$) was observed in the glycogen level of the testes in groups II and III with respect to the control. A non-significant change in the liver glycogen was observed in all groups (Table 8).

Haematology

The change in haematological parameters[26] was found to be insignificant in all the groups (Table 9).

DISCUSSION

The weight of testes largely depends on the mass of spermatogenic cells. The reduction in weight may be the result of the decreased number of germ cells and elongated spermatids[27] caused by the ligand and its boron complex. The decrease in weight of accessory sex organs may be due to reduced oestrogenic and/or antiandrogenic[28] activities of the compounds. The negative fertility test may be attributed to the lack of forward progression and the reduction in the density of spermatozoa.[29] Changes in testicular biochemical parameters occurred on treatment with the ligand and its complex. Cholesterol is the precursor for the synthesis of testosterone hormones. Any inhibition of androgen synthesis[30] will lead to an increase in the cholesterol due to its less local utilization. This is reflected in our study as groups II and III had significant increases in testicular cholesterol. An increase in testicular glycogen of treated animals may be due to poor utilization of glycogen for energy due to decrease in phosphorylase activity. These findings match the fact that total weight is decreased due to the loss of active cells in testes. No changes in hepatic cholesterol and hepatic glycogen indicate that these compounds are not hepatotoxic in the given dosages. It may be of value that these are also not showing haematological toxicity.

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Table 9. Effect of various compounds on haematology of male rats

Compound	RBC counts (million/mm^3)	WBC counts (million/mm^3)	Haematocrit (%)	Hemoglobin (gm %)	MCV (%)	MCH (%)	MCHC (%)
Control (vehicle treated) 0.5 ml olive oil/kg.body wt/day for 60 days	05.06 ± 00.39	8817.77 ± 189.53	41.00 ± 00.65	13.96 ± 00.62	81.02 ± 00.62	27.58 ± 00.68	34.04 ± 00.95
$C_{17}H_{13}N_4OSF$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	04.65^{ns} ± 00.27	8802.65^{ns} ± 190.00	39.48^{ns} ± 00.98	14.50^{ns} ± 01.28	84.90^{ns} ± 00.51	31.18^{ns} ± 00.76	36.72^{ns} ± 00.91
$C_{23}H_{18}N_4O_2SFB$ 5 mg in 0.5 ml olive oil/kg body wt/day for 60 days	04.89^{ns} ± 00.28	8813.01^{ns} ± 201.13	41.38^{ns} ± 00.38	14.63^{ns} ± 02.00	84.62^{ns} ± 00.61	29.91^{ns} ± 00.73	35.35^{ns} ± 00.52

Mean ± SEM of six animals.
^{ns} = non-significant.
* $p \leq 0.01$ = significant
** $p \leq 0.001$ = highly significant.
All groups compared with control.

All the above results indicate the effects of organoboron (III) complexes on the male reproductive system of rats and other physiological parameters. Furthermore, the current study strongly demonstrates that the boron complex is a more effective antimicrobial and antifertility agent than the parent ligand.

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