SYNTHESIS, CHARACTERIZATION, *IN VITRO* AND *IN VIVO* SCREENING OF UNSYMMETRICAL BOROLE COMPLEXES OF 2-HYDROXY-N-PHENYLBENZAMIDE AND ITS DERIVATIVES

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ABSTRACT

Biochemical aspects, synthesis and characterization of some boron complexes of 2-hydroxy-N-phenylbenzamide (HOPhBenz) and its semicarbazone (HOPhBenz.SCZH) and thiosemicarbazone (HOPhBenz.TSCZH) are described. These derivatives were prepared by the reactions of 2-isopropoxy-4-methyl-1,3,2-dioxaborolane, and 2-isopropoxy-4-methyl-1,3,2-dioxaborinane with 2-hydroxy-N-phenylbenzamide, 1-[(2-hydroxyphenyl)-1-N-phenylamino]hydrazinecarboxamide (HOPhBenz.SCZH), and 1-[(2-hydroxyphenyl)-1-N-phenylamino]hydrazinecarbothioamide (HOPhBenz.TSCZH) in a 1:1 molar ratio. In order to assess the increase of the inhibitor potency, (HOPhBenz), (HOPhBenz.SCZH), (HOPhBenz.TSCZH) and their boron complexes have been tested *in vitro* against a number of pathogenic fungi and bacteria at different concentrations and were found to possess remarkable fungicidal and bactericidal properties. The testicular sperm density, testicular sperm morphology, sperm motility, density of cauda epididymal spermatozoa and fertility in mating trials and biochemical parameters of reproductive organs are discussed.

INTRODUCTION

Boron chemistry has continued to provide the most novel developments in the field of coordination chemistry. Inorganic chemistry of boron is much more diverse and complex than that of any other element in the Periodic Table and this is due to the elucidation of tremendous range of bonding interactions. Several organoboranes find promising applications in the synthesis of insect pheromones¹. Boron azomethine complexes of various ligands have been found to possess conspicuous biocidal activity². Boron-nitrogen compounds act as antivirals, fungicides, pesticides and even traces in medicine³. Bicyclic boron compounds have cytotoxic activity⁴⁻⁶ and are used for the treatment of certain brain tumors⁷. The borohydride complexes of benzothiazepinole derivatives were found to inhibit P₃₈₈ Leukemia cell in mice⁸. There is still a sufficient scope for studies on N and S/O donor systems regarding their biochemical significance. Recently, a variety of azomethine complexes have been synthesized⁹⁻¹². Thiosemicarbazones^{13,14} and semicarbazones^{15,16} are biologically important nitrogen and sulfur/oxygen donor ligands. Keeping all these uses in mind we have synthesized, characterized and screened some unsymmetrical borole complexes, the results of which are discussed in the present paper.

RESULTS AND DISCUSSION

Reactions of unsymmetrical boroles with HOPhBenz, HOPhBenz.SCZH and HOPhBenz.TSCZH have been carried out in a 1:1 molar ratio in dry benzene. These reactions proceed with the liberation of the isopropanol/benzene azeotrope as :

These reactions are quite facile and could be completed in 10-12h of refluxing. The resulting complexes are soluble in methanol, chloroform and DMSO. These are monomeric and non-electrolytes. Spectral studies comprising UV, IR and NMR (¹H, ¹¹B and ¹³C) support the proposed structures of the compounds. The electronic spectra of HOPhBenz, HOPhBenz.SCZH, HOPhBenz.TSCZH and borole complexes were recorded in methanol. The electronic spectra of conjugated bases exhibit three bands at *ca* 230, 270 and 320 nm. The bands at *ca* 230 and 270 nm are possibly due to π - π^* transition within the benzene ring and around 320 nm may be due to n - π^* transitions of the azomethine group. In the borole

complexes these bands remain unaltered. The nitrogen atom of the $>C=N$ group does not take part in the coordination as evidenced by the appearance of this band in the same position.

IR Spectra

On the basis of IR spectral studies an intramolecular hydrogen bonded structure for HOPhBenz has been established. In the case of HOPhBenz.SCZH the coordination can take place either through the -NH phenyl group or through oxygen of the $>C=O$ group. The carbonyl stretching frequency (1685 cm^{-1}) shifts towards the lower frequency side, indicating that coordination has taken place through the carbonyl oxygen. The presence of a benzene ring at the nitrogen atom makes it less available for the coordination to the boron ion. HOPhBenz behaves purely as a bidentate chelating agent. In the IR spectra of conjugated bases broad bands at $3400\text{--}3200\text{ cm}^{-1}$ are assigned to ν_{OH} due to the phenolic OH. This band disappears in the case of complexes indicating the possible loss of proton on complexation and subsequent formation of B-O bond due to the presence of new band in the region of $1345\text{--}1360\text{ cm}^{-1}$ frequencies, respectively.

1H NMR Spectra

The proton magnetic resonance spectral data of HOPhBenz, HOPhBenz.SCZH, HOPhBenz.TSCZH and their boron complexes have been recorded in $DMSO-d_6$. The following structural inferences have been drawn by comparing the spectra of conjugated bases with those of corresponding boron complexes (Table I).

Table I : 1H and ^{11}B NMR Data (δ , ppm/JHz) of Conjugated Bases and Boron Complexes

Compound	-NH- Ph	-OH	-NH	NH_2	Aromatic Protons*				^{11}B
					1	2	3	4	
[HOPhBenz]	10.56s	12.08 bs	-	-	7.36(d) (7.3Hz)	8.08(dd) (7.3Hz) (7.5Hz)	6.80(dd) (7.5Hz) (7.2Hz)	8.40(d) (7.2Hz)	-
[$B(C_3H_6O_2)$ (OPhBenz)]	10.62s	-	-	-	6.96(d) (7.2Hz)	7.92(dd) (7.2Hz) (7.3Hz)	6.86(dd) (7.3Hz) (7.4Hz)	8.57(d) (7.4Hz)	21.53
[$B(C_4H_8O_2)$ (OPhBenz)]	10.66s	-	-	-	7.20(d) (7.3Hz)	7.44(dd) (7.3Hz) (7.7Hz)	6.97(dd) (7.7Hz) (7.5Hz)	8.63(d) (7.5Hz)	26.82
[HOPhBenz. SCZH]	10.42s	12.16 bs	10.00s	2.70bs	7.04(d) (7.1Hz)	6.96(dd) (7.1Hz) (7.4Hz)	7.20(dd) (7.4Hz) (7.2Hz)	8.24(d) (7.2Hz)	-
[$B(C_3H_6O_2)$ (OPh Benz.SCZH)]	10.84s	-	10.34s	2.76bs	7.12(d) (7.5Hz)	6.81(dd) (7.5Hz) (7.2Hz)	6.88(dd) (7.2Hz) (7.3Hz)	8.42(d) (7.3Hz)	19.68
[$B(C_4H_8O_2)$ (OPh Benz.SCZH)]	10.93s	-	10.43s	2.82bs	7.20(d) (7.1Hz)	6.93(dd) (7.1Hz) (7.9Hz)	8.16(dd) (7.9Hz) (7.5Hz)	8.58(d) (7.5Hz)	17.24
[HOPhBenz. TSCZH]	10.60s	12.24 bs	10.32s	3.20 bs	6.88(d) (7.4Hz)	6.80(dd) (7.4Hz) (7.3Hz)	8.24(dd) (7.3Hz) (7.5Hz)	8.32(d) (7.5Hz)	-
[$B(C_3H_6O_2)$ (OPh Benz.TSCZH)]	10.79s	-	10.63s	3.24bs	7.36(d) (7.2Hz)	6.97(dd) (7.2Hz) (7.3Hz)	8.40(dd) (7.3Hz) (7.4Hz)	8.54(d) (7.4Hz)	31.15
[$B(C_4H_8O_2)$ (OPh Benz.TSCZH)]	10.86s	-	10.75s	3.29bs	6.96(d) (7.5Hz)	6.74(dd) (7.5Hz) (7.8Hz)	8.16(dd) (7.8Hz) (7.3Hz)	8.64(d) (7.3Hz)	35.23

The disappearance of the -OH proton signal in case of complexes indicates the deprotonation and complexation through the functional group. The signal due to the NH protons attached to the phenyl ring remain unchanged in the complexes. The disappearance of the NH resonance signals of HOPhBenz.SCZH and HOPhBenz.TSCZH in the case of the complexes provides evidence for the complexation through the functional group. The $-NH_2$ protons remain almost unchanged.

¹³C NMR Spectra

The ¹³C NMR spectral data have been recorded for HOPhBenz, HOPhBenz.SCZH, HOPhBenz.TSCZH and their boron complexes. The shifts of the carbon attached to O and S indicates the involvement of these atoms in coordination (Table II).

Table II : ¹³C NMR Spectral Data (δ, ppm) of Conjugated Bases and Their Boron Complexes

Compound	>C=S	>C=O/ >C=N	Ar-OH/ Ar-O	Ph-NH	Aromatic Carbons
[HOPhBenz]	-	166.40	160.10	138.68	C ₁ , 134.89; C ₂ , 129.97; C ₃ , 129.35; C ₄ , 125.66; C ₅ , 122.58; C ₆ , 120.74; C ₇ , 118.89; C ₈ , 118.96
[B(C ₃ H ₆ O ₂)(OPhBenz)]	-	165.85	156.89	139.12	C ₁ , 135.05; C ₂ , 131.25; C ₃ , 129.87; C ₄ , 124.76; C ₅ , 124.17; C ₆ , 120.98; C ₇ , 119.62; C ₈ , 118.68
[B(C ₄ H ₈ O ₂)(OPhBenz)]	-	165.39	155.93	139.28	C ₁ , 134.90; C ₂ , 130.45; C ₃ , 130.23; C ₄ , 126.05; C ₅ , 124.53; C ₆ , 121.15; C ₇ , 118.75; C ₈ , 119.16
[HOPhBenz.SCZH]	-	165.78	157.70	137.23	C ₁ , 133.22; C ₂ , 129.15; C ₃ , 128.18; C ₄ , 125.41; C ₅ , 121.22; C ₆ , 120.21; C ₇ , 116.53; C ₈ , 116.42
[B(C ₃ H ₆ O ₂)(OPhBenz.SCZH)]	-	162.82	153.25	135.64	C ₁ , 133.87; C ₂ , 131.05; C ₃ , 127.43; C ₄ , 125.79; C ₅ , 122.64; C ₆ , 122.85; C ₇ , 117.09; C ₈ , 115.57
[B(C ₄ H ₈ O ₂)(OPhBenz.SCZH)]	-	160.98	151.35	134.49	C ₁ , 134.62; C ₂ , 131.64; C ₃ , 128.98; C ₄ , 126.38; C ₅ , 121.88; C ₆ , 121.56 C ₇ , 116.05; C ₈ , 115.42
[HOPhBenz.TSCZH]	179.52	168.34	160.86	138.99	C ₁ , 130.18; C ₂ , 129.79; C ₃ , 129.75; C ₄ , 125.58; C ₅ , 122.64; C ₆ , 120.68 C ₇ , 119.99; C ₈ , 118.94
[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	167.47	166.45	153.72	137.76	C ₁ , 130.89; C ₂ , 130.12; C ₃ , 129.51; C ₄ , 126.03; C ₅ , 123.15; C ₆ , 121.82 C ₇ , 118.73; C ₈ , 118.65
[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	166.55	162.67	151.56	137.46	C ₁ , 131.18; C ₂ , 129.88; C ₃ , 130.23; C ₄ , 125.84; C ₅ , 124.33; C ₆ , 120.85 C ₇ , 119.32; C ₈ , 119.74

X-Ray Powder Diffraction Spectra

The X-ray powder diffraction studies of two representative complexes [B(C₄H₈O₂)(OPhBenz.)] and [B(C₃H₆O₂)(OPhBenz.TSCZH)] have also been carried out. The results, show that the compounds belong to the orthorhombic crystal systems. The interplaner spacing values (dA°) and the miller indices h,k,l and a, b, c are listed in Tables III and IV.

¹¹B NMR Spectra

The ¹¹B Nuclear magnetic resonance were observed between δ17.24 and 35.23 ppm (Table I) and which clearly supports a tricoordinated environment around the boron atom.

On the basis of the spectral evidences, the boron derivatives have been assigned the following structure with tricoordinated boron atom.

Where, R =

PhNHCO,
 PhNHC:NN:C(OH)NH₂ and PhNHC:NN:C(SH)NH₂
 n = 1 or 2

Table III : X-Ray Powder Diffraction Data of [B(C₄H₈O₂)(OPhBenz.)]

Peak No.	2θ(deg.)(obs.)	d-spacing (obs.)(Å ^o)	h	k	l
1	7.8	14.2420	0	0	2
2	8.64	12.860	1	1	1
3	16.3	6.8330	0	2	3
4	17.75	6.2963	0	3	3
5	19.4	5.7492	0	0	5
6	21.2	5.2659	2	1	5
7	23.15	4.8297	0	0	6
8	23.45	4.7688	1	6	5
9	24.6	4.5471	3	0	5
10	25.4	4.4062	4	1	0
11	26.0	4.3062	2	7	4
12	27.15	4.1285	1	8	4
13	29.0	3.8688	0	6	8
14	32.4	3.4721	3	5	7
15	36.7	3.0769	1	8	7
16	37.3	3.0276	2	9	5
17	38.5	2.9381	3	7	6
18	43.5	2.6141	2	4	11

Refined value = a = 10.8256, b = 23.7350, c = 29.1565 (Orthorhombic system)

Table IV : X-Ray Powder Diffraction Data of (B(C₃H₆O₂)(OPhBenz.TSCZH)]

Peak No.	2θ(deg.)(obs.)	d-spacing (obs.)(Å ^o)	h	k	l
1	8.54	13.0101	1	0	0
2	12.4	8.9693	1	3	1
3	18.6	5.9942	0	3	2
4	21.0	5.3155	0	5	5
5	22.0	5.0767	2	1	3
6	23.2	4.8174	1	1	4
7	24.7	4.5290	2	1	4
8	25.4	4.4062	1	5	3
9	26.2	4.2739	0	2	5
10	27.2	4.1195	0	5	4
11	28.0	4.0041	2	1	5
12	29.2	3.8429	4	4	1
13	31.6	3.5577	0	6	4
14	32.8	3.4309	1	5	5
15	33.0	3.4107	5	3	0
16	33.74	3.3380	3	8	0
17	34.8	3.2393	1	2	7
18	36.86	3.0688	5	5	2
19	37.4	3.0213	3	9	1
20	38.6	2.9308	0	9	4

Refined value = a = 13.0202, b = 22.4347, c = 17.0650 (Orthorhombic system)

BIOCHEMICAL ASPECTS

All the conjugated bases and their corresponding boron complexes were tested for the *in vitro* growth inhibitory activity against pathogenic fungi viz. *Fusarium oxysporum*, *Alternaria alternata*, *Rhizoctonia bataticola* and bacteria viz. *Staphylococcus aureus* and *Xanthomonas compestris*. Proper temperature, necessary nutrient and growth media free from other microorganism were employed for the preparation of cultures of fungi and bacteria using aseptic techniques. The antifungal activity of the complexes was evaluated by the Agar Plate Technique and antibacterial activity was evaluated by Paper Disc Plate Method¹⁷. The data of fungicidal and bactericidal activities of conjugated bases and their respective boron complexes against pathogenic fungi and bacteria were recorded in Tables V and VI.

Table V : Fungicidal Screening Data of Conjugated Bases and Their Boron Complexes

Treatment (Compound)	Average Percentage Inhibition after 4 Days.											
	<i>Alternaria alternata</i>				<i>Rhizoctoniabaticola</i>				<i>Fusarium oxysporum</i>			
	(Conc. in ppm)				(Conc. in ppm)				(Conc. in ppm)			
	25	50	100	200	25	50	100	200	25	50	100	200
[HOPhBenz]	57	62	68	72	61	66	70	78	61	66	70	78
[B(C ₃ H ₆ O ₂)(OPhBenz)]	63	65	72	76	73	78	81	85	67	71	77	82
[B(C ₄ H ₈ O ₂)(OPhBenz)]	65	67	74	80	75	82	87	88	72	78	85	86
[HOPhBenz.SCZH]	62	64	74	80	62	68	72	80	62	68	72	80
[B(C ₃ H ₆ O ₂)(OPhBenz.SCZH)]	69	72	2	84	74	75	79	84	66	74	75	85
[B(C ₄ H ₈ O ₂)(OPhBenz.SCZH)]	72	78	85	86	77	79	82	86	69	77	80	88
[HOPhBenz.TSCZH]	67	72	81	85	65	70	76	80	65	70	76	80
[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	73	78	84	87	72	78	83	86	72	77	79	84
[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	77	82	86	88	75	82	87	88	76	80	82	86

Table VI : Bactericidal Screening Data of Conjugated Bases and Their Boron Complexes

Treatment (Compound)	<i>Staphylococcus aureus</i>		<i>Xanthomonas compestris</i>	
	500 ppm	1000 ppm	500 ppm	1000 ppm
[HOPhBenz]	6.0	7.0	3.0	4.0
[B(C ₃ H ₆ O ₂)(OPhBenz)]	8.0	9.5	4.0	6.0
[B(C ₄ H ₈ O ₂)(OPhBenz)]	8.5	10.0	5.5	7.5
[HOPhBenz.SCZH]	7.0	8.5	4.0	5.0
[B(C ₃ H ₆ O ₂)(OPhBenz.SCZH)]	10.0	9.0	6.5	7.5
[B(C ₄ H ₈ O ₂)(OPhBenz.SCZH)]	10.5	10.5	7.0	9.0
[HOPhBenz.TSCZH]	8.0	9.0	4.5	6.0
[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	9.5	11.0	6.0	7.5
[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	10.0	11.5	7.0	9.0

Percent Disease Incidence Technique

In this method compounds were tested in the field for controlling the disease caused by the casual organism. Two concentrations i.e. 100 and 200 ppm were used in different plates and observations were recorded.

$$\text{Percent Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total no. of Plants observed}} \times 100$$

The fungus *Alternaria alternata* in brinjal plants was used for these studies (Tables VIIa, VIIb, VIIc).

Hanging Drop Method

In this technique total number of spores, germinated spores, and number of ungerminated spores were counted. LD₅₀ values have been calculated to compare HOPhBenz, HOPhBenz.TSCZH and their boron complexes by plotting graphs between concentration of conjugated bases or their complexes with the number of germinated spores.

Antifertility Activity

Fourty two male albino rats were used in present study. Rats having weight between 175 to 200 g were divided into seven groups of six animals each. Animals were housed in polypropylene cages measuring 12"x10"x8" under controlled environmental conditions and fed with rat feed pellets and free access to water.

Table VII (a) : Efficacy of HOPhBenz, HOPhBenzTSCZH and B(C₄H₈O₂)(OPhBenzTSCZH) Against Leaf Spot of Brinjal Plant caused by *Alternaria alternata* by Percent Disease Incidence Method.

Compound	Concentration in ppm	% Inhibition (Replication)			Average Statistical Values
HOPhBenz	100	25	17	15	(24.57)
	200	15	11	14	(21.38)
HOPhBenzTSCZH	100	17	19	12	(23.45)
	200	13	12	10	(19.94)
[B(C ₄ H ₈ O ₂)(OPhBenzTSCZH)]	100	12	13	11	(17.50)
	200	9	7	8	(16.55)
Bavistin (0.2%)	-	8	11	9	(17.75)
Control (water spray)	-	34	39	42	(38.24)

S.Em = 0.94

CD at 5%= 2.68

Table VII (b) : Antifungal Screening Data of Conjugated Bases and Their Complexes by Spore Germination Method

Compound	Conc. in (ppm)	<i>Alternaria alternata</i>			<i>Helminthosporium gramineum</i>		
		Total No. of Spores	No. of Germinated Spores	No. of Ungerminated Spores	Total No. of Spores	No. of Germinated Spores	No. of Ungerminated Spores
[HOPhBenz]	500	10	1	9	12	2	10
	250	10	3	7	12	4	8
	125	10	6	4	12	5	7
	62.5	10	7	3	12	7	5
[HOPhBenz.TSCZH]	500	8	2	6	10	1	9
	250	8	3	5	10	3	7
	125	8	5	3	10	4	6
	62.5	8	7	1	10	6	4
[B(C ₃ H ₆ O ₂)(OPhBenz)]	500	12	1	11	10	1	9
	250	12	3	9	10	2	8
	125	12	7	5	10	3	7
	62.5	12	9	3	10	6	4
[B(C ₄ H ₈ O ₂)(OPhBenz)]	500	8	1	7	12	2	10
	250	8	2	6	12	4	8
	125	8	3	5	12	6	6
	62.5	8	5	3	12	8	4
[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	500	8	2	6	10	2	8
	250	8	4	4	10	5	5
	125	8	5	3	10	6	4
	62.5	8	6	2	10	7	3
[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	500	8	1	7	12	2	10
	250	8	2	6	12	3	9
	125	8	5	3	12	5	7
	62.5	8	7	1	12	7	5

Table VII (c) : Comparison of LD₅₀ Value

Compound	LD ₅₀ Value in <i>Alternaria alternata</i>	LD ₅₀ value in <i>Helminthosporium gramineum</i>
(HOPhBenz)	160 ppm	89 ppm
(HOPhBenz.TSCZH)	172 ppm	86 ppm
[B(C ₃ H ₆ O ₂)(OPhBenz)]	152 ppm	80 ppm
[B(C ₄ H ₈ O ₂)(OPhBenz)]	145 ppm	72 ppm
[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	162 ppm	83 ppm
[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	156 ppm	75 ppm

The group A served as vehicle (olive oil) treated control. In the group B, starting material, i.e., HOPhBenz. 50 mg/kg b.wt. suspended in olive oil was given orally for a period of 60 days. The animals of groups C,D,E,F and G received the same amount of HOPhBenz.TSCZH, [B(C₃H₆O₂)(OPhBenz.)], [B(C₃H₆O₂)

(OPhBenz.TSCZH)], [B(C₄H₈O₂)(OPhBenz)] and [B(C₄H₈O₂)(OPhBenz.TSCZH)] compounds, respectively for the same period. These animals were screened for fertility test and autopsied for detail biochemical changes. Reproductive organs were excised, blotted free of blood, weighed and were frozen for biochemical estimations. The sperm motility and density of cauda epididymal spermatozoa, total cholesterol, total protein, sialic acid, fructose and acid phosphatase were determined by Standard Laboratory Techniques.

Table VIII : Effects of HOPhBenz, HOPhBenz.TSCZH and Their Boron Complexes on Body Weight of Organ Weights of Male Rats

Group	Treatment	Body Weight (g)		Testes	Epididymis	Seminal vesicle	Ventral prostate
		Initial	Final				
A	Control	180.0 ± 20.0	210.0 ± 15.0	120.0 ± 30.5	415.0 ± 25.0	330.0 ± 20.70	250.0 ± 32.0
B	[HOPhBenz]	185.0 ± 20.0	220.0 ± 15.0 ^c	850.0 ± 30.0 ^b	310.0 ± 20.0 ^b	250.0 ± 10.5 ^b	240.0 ± 30.70 ^c
C	[HOPhBenz.TSCZH]	175.0 ± 13.0	190.0 ± 10.0 ^c	810.0 ± 15.0 ^a	290.0 ± 5.0 ^b	210.0 ± 7.5 ^a	170.0 ± 7.8 ^c
D	[B(C ₃ H ₆ O ₂)(OPhBenz)]	190.0 ± 15.0	225.0 ± 17.0 ^c	750.0 ± 15.0 ^a	270.0 ± 12.0 ^a	215.0 ± 18.0 ^a	180.0 ± 5.9 ^a
E	[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	186.0 ± 12.0	218.0 ± 15.0 ^c	650.0 ± 18.0 ^b	220.0 ± 10.0 ^b	190.0 ± 8.0 ^b	150.0 ± 6.8 ^b
F	[B(C ₄ H ₈ O ₂)(OPhBenz)]	195.0 ± 20.0	230.0 ± 20.0 ^c	730.0 ± 18.0 ^a	250.0 ± 15.0 ^a	210.0 ± 9.0 ^a	168.0 ± 9.8 ^a
G	[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	188.0 ± 15.0	222.0 ± 18.0 ^c	630.0 ± 20.0 ^b	210.0 ± 12.0	285.0 ± 10.0 ^b	140.0 ± 9.6 ^b

Values ± SE of Six determinations

a = p ≤ 0.05 Group B compared with Group A
 b = p ≤ 0.001 Group C compared with Group B
 c = p = NS Groups D and F compared with Group B
 Groups E and G compared with Group C

Table IX : Sperm Dynamics and Fertility Test after Treatment With HOPhBenz, HOPhBenz.TSCZH and Their Boron Complexes

Group	Treatment	Sperm Motility	Sperm Density (million/ml)		Fertility Test %
		Cauda epididymis	Testes	Epididymis	
A	Control	80.0 ± 5.1	1.95 ± 0.10	55.5 ± 1.75	99% + ve
B	[HOPhBenz]	55.0 ± 3.1 ^b	0.89 ± 0.11 ^b	37.5 ± 0.90 ^b	71% - ve
C	[HOPhBenz.TSCZH]	40.0 ± 4.1 ^b	0.70 ± 0.10 ^b	30.0 ± 0.95 ^b	85% - ve
D	[B(C ₃ H ₆ O ₂)(OPhBenz)]	45.0 ± 2.1 ^a	0.65 ± 0.12 ^b	28.0 ± 0.91 ^b	88% - ve
E	[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	35.0 ± 3.1 ^b	0.55 ± 0.10 ^b	26.0 ± 0.90 ^b	90% - ve
F	[B(C ₄ H ₈ O ₂)(OPhBenz)]	44.0 ± 3.1 ^a	0.68 ± 0.15 ^b	27.0 ± 0.80 ^b	92% - ve
G	[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	29.0 ± 2.1 ^b	0.65 ± 0.12 ^b	25.0 ± 0.91 ^b	93% - ve

Values means ± SE of Six determinations.

a = p ≤ 0.05 Group B compared with Group A
 b = p ≤ 0.001 Group C compared with Group B
 c = p = NS Groups D and F compared with Group B
 Groups E and G compared with Group C

- (i) **Body and Organ Weights** : Body weight of rats were not affected after HOPhBenz, HOPhBenz.TSCZH and their boron complexes administration, However, the weight of testes, epididymis, seminal vesicle and ventral prostate were significantly described (Table VIII).
- (ii) **Fertility Test** : The sluggish motile spermatazoa were unable to fertilize normal cyclic females. The test was 71 to 93% negative in rats treated with these compounds.
- (iii) **Sperm Motility** : The sperm motility declined significantly after treatment with the compounds.

- (iv) **Sperm Density** : The sperm density in testes and cauda epididymis were declined significantly after treatment (Table IX).

EXPERIMENTAL

All the chemicals and solvents were dried and purified by standard methods. The reactions were carried out under anhydrous conditions.

Preparation of Hydrazinecarboxamide and Hydrazinecarbothioamide

Hydrazinecarboxamide and Hydrazinecarbothioamide of 2-hydroxy-N-phenyl-benzamide were prepared by the condensation of (HOPhBenz) with semicarbazide hydrochloride (in presence of sodium acetate) and thiosemicarbazide in a 1:1 molar ratio in alcoholic medium. The mixture was refluxed for 2-3 hours. On cooling, crystals were separated out which were recrystallized in the same solvent and finally dried in vacuum. The physical properties of these moieties are as follows :

Conjugated bases, Colour and M.P. (°C).

2-Hydroxy-N-phenylbenzamide (HOPhBenz), Light brown, 132-134

1-[(2-Hydroxyphenyl)-1-N-phenylamino]hydrazinecarboxamide (HOPhBenz.SCZH), Off white, 109-110

1-[(2-Hydroxyphenyl)-1-N-phenylamino]hydrazinecarbothioamide(HOPhBenz.TSCZH),Light pink,123-125

Preparation of the Complexes

The unimolar reactions of unsymmetrical boroles with 2-hydroxy-N-phenylbenzamide and its derivatives were carried out in dry benzene. The reaction mixture was refluxed for 10-12h on a fractionating column and the progress of the reaction was monitored by the liberation of azeotropic isopropanol/benzene. After the completion of the reaction, the excess of the solvent was distilled off and the product was dried in vacuo. It was repeatedly washed with dry cyclohexane and again dried for 3-4h. These complexes were recrystallized in (1:1) solution of benzene and ether. The important physical properties and analytical data are given in Table X.

Table X : Physical Properties and Analytical Data of the Boron Complexes

Compound	Colour	M.P. (°C)	Analysis % Found (Calcd.)					Mol. Wt. Found (Calcd.)
			C	H	N	S	B	
[B(C ₃ H ₆ O ₂)(OPhBenz)]	Cream	214-	64.33	5.23	4.15	-	3.50	332
		218	(64.62)	(5.42)	(4.71)	-	(3.65)	(297)
[B(C ₄ H ₈ O ₂)(OPhBenz)]	Cream	220-	65.42	5.59	4.13	-	3.35	345
		224	(65.63)	(5.83)	(4.50)	-	(3.47)	(311)
[B(C ₃ H ₆ O ₂)(OPhBenzSCZH)]	Cream	180-	57.47	5.21	15.10	-	3.01	398
		185	(57.64)	(5.40)	(15.81)	-	(3.05)	(354)
[B(C ₄ H ₈ O ₂)(OPhBenzSCZH)]	Cream	202-	58.32	5.25	14.88	-	2.80	405
		205	(58.75)	(5.74)	(15.23)	-	(2.93)	(368)
[B(C ₃ H ₆ O ₂)(OPhBenzTSCZH)]	Light brown	170-	56.03	5.11	14.13	7.99	2.70	425
		174	(56.26)	(5.50)	(14.58)	(8.34)	(2.81)	(384)
[B(C ₄ H ₈ O ₂)(OPhBenzTSCZH)]	Brown	161-	55.01	4.98	14.85	8.23	2.83	417
		164	(55.15)	(5.17)	(15.13)	(8.66)	(2.91)	(370)

Analytical Methods and Physical Measurements

IR spectra were recorded as KBr pellets or Nujol Mulls on a FTIR Spectrophotometer, Model Megna IR 550. ¹H NMR spectra were recorded on Jeol FX 90Q Spectrometer in DMSO-d₆ using TMS as the internal standard at 89.55 MHz. ¹¹B NMR spectra were recorded on the same Spectrometer. The electronic spectra were recorded in methanol on a Pye Unicam SP8-100 Spectrometer. Boron(III) was estimated volumetrically as boric acid¹⁸. Carbon and hydrogen analyses were carried out at the Microanalytical Laboratory of the Department. Nitrogen and Sulfur¹⁹ were determined by Kjeldahl's and Messenger's methods, respectively. Conductivity measurements in dry DMF were made by a Systronics Conductivity Bridge Type 305 and molecular weights were determined by the Rast Camphor method.

The XRD measurements were performed on Phillips X-ray diffractometer (model PW-1840) having Fe-K α target and operated at 30 KV and 40mA. The diffraction data were refined using a least-squares refinement computer programme to obtain structural parameters. The programme essentially uses the least squares method of fitting of d_{hkl} values for the different reflections. The intensity of Fe-K α radiation diffracted from the powder specimen was detected by a solid state detector and recorded as function of 2 θ , where θ is the angle of incidence.

Biochemical Changes

Total protein and sialic acid contents of testes, epididymis, ventral prostate and seminal vesicle were depleted significantly after treatment with starting materials and their boron complexes. The acid phosphatase levels of testes epididymis and ventral prostate were also reduced significantly. A significant decrease in seminal vascular fructose contents was also noticed whereas the testicular cholesterol contents was increased significantly after the treatment with various compounds (Tables XI and XII).

Table XI : Effects of HOPhBenz, HOPhBenzTSCZH and Their Boron Complexes on Biochemical Parameters of Reproductive Organs of Male Rats

Group	Treatment	Total protein (mg/gm)				Sialic acid (mg/gm)			
		Testes	Epididymis	Ventral prostate	Seminal vesicle	Testes	Epididymis	Seminal Vesicle	Ventral prostate
A	Control	225.0 ± 18.0	208.0 ± 20.0	245.0 ± 15.0	260.0 ± 17.0	7.80 ± 0.9	6.90 ± 0.8	6.75 ± 0.9	7.10 ± 0.5
B	[HOPhBenz]	165.0 ± 15.0 ^b	170.0 ± 10.0 ^a	195.0 ± 10.0 ^b	210.0 ± 16.8 ^b	5.1 ± 0.6 ^b	5.0 ± 0.4 ^b	5.6 ± 0.3 ^b	5.3 ± 0.4 ^b
C	[HOPhBenz.TSCZH]	140.0 ± 15.0 ^a	150.0 ± 7.8 ^a	155.0 ± 6.5 ^a	160.0 ± 9.6 ^b	4.1 ± 0.8 ^a	4.0 ± 0.3 ^a	4.8 ± 0.3 ^a	4.6 ± 0.3 ^a
D	[B(C ₃ H ₆ O ₂)(OPhBenz)]	120.5 ± 10.0 ^a	128.0 ± 9.0 ^a	150.0 ± 6.0 ^a	170.0 ± 10.0 ^b	4.0 ± 0.3 ^a	3.9 ± 0.4 ^a	4.5 ± 0.4 ^a	4.1 ± 0.5 ^a
E	[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	100.0 ± 12.0 ^a	110.0 ± 8.0 ^a	120.0 ± 7.5 ^b	102.0 ± 5.0 ^b	3.9 ± 0.2 ^b	2.9 ± 0.5 ^b	3.5 ± 0.4 ^b	3.2 ± 0.2 ^b
F	[B(C ₄ H ₈ O ₂)(OPhBenz)]	125.7 ± 12.0 ^a	130.0 ± 10.0 ^a	145.0 ± 8.0 ^a	165.0 ± 8.0 ^b	4.2 ± 0.6 ^a	3.7 ± 0.6 ^a	4.3 ± 0.5 ^a	4.4 ± 0.2 ^a
G	[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	105.0 ± 10.3 ^a	112.0 ± 9.0 ^a	121.0 ± 9.7 ^b	108.0 ± 9.0 ^b	4.1 ± 0.8 ^a	3.0 ± 0.6 ^a	3.6 ± 0.5 ^b	3.1 ± 0.1 ^b

Values means ± SE of Six determinations.

a = $p \leq 0.05$

b = $p \leq 0.001$

c = $p = \text{NS}$

Group B compared with Group A

Group C compared with Group B

Groups D and F compared with Group B

Groups E and G compared with Group C

Inferences

The present study revealed that administration of the starting materials, HOPhBenz and HOPhBenz.TSCZH and their boron complexes caused a significant reduction in the weights of testes and other sex accessory glands. The development and function of male sex accessory glands the prostate and seminal vesicle are a well documented androgen dependent process.²⁰ The reduction in the weights of these organs could be due to reduced androgen level²¹. Sperm motility is considered as an important parameters in evaluating the fertility potential. HOPhBenz, HOPhBenz.TSCZH and their boron complexes significantly reduced the fertility of male rats. Since a number of androgen sensitive parameters (protein, sialic acid, fructose, acid phosphatase and total cholesterol) in target organs were found to be altered by these compounds, it is expected that the structure and function of epididymis and other sex accessory organs are changed. Most of the activities of HOPhBenz.TSCZH, and its effect on fertility is due to the presence of thio group²². Earlier it has been reported that boron exposure results in infertility, oligospermia and decreased libido in occupationally exposed men and rats. Our finding further suggested that addition of the boron moiety to the starting materials HOPhBenz or HOPhBenz.TSCZH increased their activity, and the boron complexes of HOPhBenz.TSCZH being indeed more effective fertility inhibitors.

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Table XII : Effects of HOPhBenz, HOPhBenzTSCZH and Their Boron Complexes on Biochemical Parameters of Reproductive Organs of Male Rats

Group	Treatment	Acid phosphatase (mg/ip Liberated/hr/mg tissue)			Fructose (mg/gm)	Cholesterol (mg/gm)
		Testes	Epididymis	Ventral prostate	Seminal vesicle	Testes
A	Control	3.9 ± 0.30	5.45 ± 0.06	3.0 ± 0.07	445.0 ± 30.0	7.60 ± 0.50
B	[HOPhBenz]	2.5 ± 0.11 ^b	3.41 ± 0.04 ^b	2.0 ± 0.01 ^a	380.0 ± 15.0 ^a	8.50 ± 0.5 ^a
C	[HOPhBenz.TSCZH]	2.1 ± 0.003 ^a	2.20 ± 0.04 ^a	1.70 ± 0.001 ^a	320.0 ± 17.0 ^b	9.1 ± 0.2 ^a
D	[B(C ₃ H ₆ O ₂)(OPhBenz)]	2.0 ± 0.01 ^a	2.30 ± 0.05 ^a	1.68 ± 0.001 ^a	325.0 ± 15.0 ^b	9.7 ± 0.3 ^a
E	[B(C ₃ H ₆ O ₂)(OPhBenz.TSCZH)]	1.9 ± 0.01 ^a	1.8 ± 0.02 ^a	1.3 ± 0.001 ^a	280.0 ± 10.0 ^b	9.9 ± 0.4 ^a
F	[B(C ₄ H ₈ O ₂)(OPhBenz)]	2.2 ± 0.01 ^a	2.25 ± 0.03 ^a	1.59 ± 0.002 ^a	300.0 ± 18.0 ^b	9.8 ± 0.4 ^a
G	[B(C ₄ H ₈ O ₂)(OPhBenz.TSCZH)]	2.0 ± 0.01 ^a	1.9 ± 0.03 ^a	1.4 ± 0.001 ^a	295.0 ± 15.0 ^b	9.78 ± 0.3 ^a

Values means ± SE of Six determinations.

a = p ≤ 0.05

Group B compared with Group A

b = p ≤ 0.001

Group C compared with Group B

c = p = NS

Groups D and F compared with Group B

Groups E and G compared with Group C

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