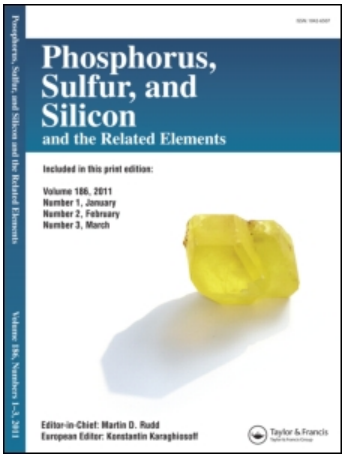

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Structural Studies with Antimicrobial and Antifertility Activity of a Monofunctional Bidentate Ligand with its Boron(III), Palladium(II), and Platinum(II) Complexes

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Structural Studies with Antimicrobial and Antifertility Activity of a Monofunctional Bidentate Ligand with its Boron(III), Palladium(II), and Platinum(II) Complexes

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The reaction of the sulfur donor Schiff base ligand, 7-nitro-3-(indolin-2-one) hydrazinecarbo-thioamide, with phenyldihydroxyboron in benzene, palladium(II)chloride, and platinum(II) chloride, in ethanol, gave the mononuclear tetracoordinated and hexacoordinated complexes. The Schiff base ligand coordinated to the boron atom in 1:1 and 1:2 molar ratios and to the palladium and platinum metals in only 1:2 molar ratios in the presence of an acidic and basic medium. Tentative structural conclusions are drawn for reaction products based upon elemental analysis, electrical conductance, and spectral (electronic, infrared, ^{1}H NMR, ^{13}C NMR, and ^{11}B NMR) data. The antifertility activity of the ligand and its nonmetal / metal complexes are discussed with a comparative study in an effective manner.

INTRODUCTION

Thiosemicarbazones constitute one of the most important class of biologically active ligands providing potential binding sites through nitrogen and sulphur donor atoms. The preparation of a variety of metal complexes from these ligands speak for their spectacular progress in coordination and bioinorganic chemistry. Metal complexes, especially of Pd(II) and Pt(II), with various such heterocyclic compounds have been extensively studied by several researchers due to their antitumour, antiviral, antibacterial, and antifungal activity.[1–4]

Platinum complexes with 2-acetyl pyridine thiosemicarbazone were found to have a completely lethal effect[5] on Gram(+) bacteria, while the same complexes showed no bactericidal effect on Gram(−) bacteria.

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Similarly, boron and its compounds occupy a unique position in the development of inorganic chemistry because of the complexity of the structure and a subtle type of bonding, as boron compounds exhibit structures ranging from a trigonal planar to a tetrahedral to open or close polyhedra. Many boron complexes having B—N bonds have been synthesized due to their use in the industry and agriculture.[6] On the other hand, multifarious roles of transition metals in biochemistry[7] suggest that considerable potential exists for the development of new chemistry with these metals in a ligand system specifically designed to serve these roles. Keeping all these aspects into consideration, we herein reported the synthesis, characterization, and biological activity of a sulfur donor ligand, and its boron, palladium, and platinum complexes.

RESULTS AND DISCUSSION

The elemental analysis and spectral data are consistent with formulations of compounds as $[PhB(OH)(L)]$, $[PhB(L)_2]$, $[M(LH)_2]Cl_2$, and $[M(L)_2]$. Reactions of phenyldihydroxyboron with the ligand (LH) have been carried out in 1:1 and 1:2 molar ratios in dry benzene, and the reactions of palladium chloride and platinum chloride with the ligand (LH) have been carried out in 1:2 molar ratios in ethanol.

The addition products, $[M(LH)_2]Cl_2$, were synthesized in the presence of a few drops of concentrated HCl. However, the substituted products, $[M(L)_2]$, were obtained when the reaction was carried out in the presence of aqueous amonium hydroxide.

$$[PhB(OH)_2] + LH \xrightarrow[\text{Dry Benzene}]{1:1} [PhB(OH)(L)] + H_2O$$

$$[PhB(OH)_2] + 2LH \xrightarrow[\text{Dry Benzene}]{1:2} [PhB(L)_2] + 2H_2O$$

$$[MCl_2] + 2LH \xrightarrow[\text{Ethanol}]{1:2} [M(L)_2]Cl_2$$

$$[MCl_2] + 2LH + 2NH_4OH \xrightarrow[\text{Ethanol}]{1:2} [M(L)_2] + 2NH_4Cl + 2H_2O$$

where M = Pd(II) and Pt(II) and LH = Ligand molecule.

These reactions proceeded easily; reactions of boron could be completed within 12–24 h of refluxing in benzene, and the reactions of palladium and platinum could be completed within 3–6 h in an ethanol medium. All complexes are soluble in DMF, DMSO, and chloroform. The complexes are monomers as revealed by their molecular weight determinations.

Low molar conductance values (8–12 ohm^{-1} cm^2 mol^{-1}) of these boron derivatives in DMF at 10^{-3} M concentrations show them to be

TABLE I Physical Properties and Analytical Data of LH and Its Complexes

Compound	Color, State	M.p., (°C)	Elemental analysis (%) found (calcd.)					Mol. wt. found (calcd.)
			M	C	H	N	S	
LH	Dim yellow, solid	236	—	40.34 (40.75)	2.72 (2.65)	26.48 (26.40)	12.22 (12.08)	278.36 (265.24)
[PhB(OH)(L)]	Brown, solid	252	2.84 (2.92)	48.22 (48.80)	3.17 (3.27)	18.39 (18.97)	8.39 (8.68)	382.75 (369.15)
$[PhB(L)_2]$	Cock, solid	257	1.34 (1.75)	47.04 (46.76)	2.84 (2.77)	23.14 (22.72)	10.24 (10.40)	628.78 (616.38)
$[Pd(LH)_2]Cl_2$	Brown, solid	267	15.34 (15.03)	30.65 (30.54)	2.17 (1.99)	19.56 (19.78)	9.14 (9.05)	719.68 (707.81)
$[Pd(L)_2]$	Grey, solid	264	16.56 (16.76)	34.18 (34.05)	1.93 (1.90)	21.84 (22.06)	10.03 (10.09)	641.55 (634.89)
$[Pt(LH)_2]Cl_2$	Grey, solid	252	24.65 (24.49)	27.34 (27.14)	1.96 (1.77)	17.36 (17.58)	8.13 (8.05)	782.98 (796.47)
$[Pt(L)_2]$	Dark brown, solid	248	26.82 (26.96)	29.96 (29.58)	1.78 (1.67)	19.43 (19.35)	8.96 (8.86)	738.68 (723.55)

nonelectrolytes, and molar conductance values of 10^{-3} M solutions of $[M(L)_2]$ types of complexes lie in the range of 10–15 ohm^{-1} cm^2 mol^{-1} in dry DMF, indicating their nonelectrolytic behavior. However, the $[M(LH)_2]Cl_2$ complexes are 1:2 electrolytes with conductance values of 200–220 ohm^{-1} cm^2 mol^{-1}. Analytical data of the ligand and its complexes are given in Table I.

Infrared spectra (4000–200 cm^{-1}) were recorded with the help of a Nicolet Magena FT-IR 550 spectrophotometer in KBr pellets. NMR (^1H, ^{13}C, and ^{11}B) spectra were recorded in $CDCl_3$ (^1H) or DMSO-d_6 (^{13}C, ^{11}B) solutions on a JEOL-FX-90Q spectrometer. Molecular weights were determined by the Rast camphor method. Nitrogen was determined by the Kjeldal's method, and sulfur was estimated by the Messanger's method. Boron was determined by the methyl borate procedure,[8] and palladium and platinum were estimated gravimetrically.

Infrared Spectra

IR spectra of the free ligand reveals a band at *ca* 3280 cm^{-1} assigned to NH vibrations of the functional group, which disappears in the corresponding $[M(L)_2]$ type of complexes, indicating possible deprotonation of the functional group upon complexation. The band at 1615 cm^{-1} in LH, due to ν(C=N), is shifted to a higher wave number (ν10–15 cm^{-1}) in the [PhB(OH)L], $[M(LH)_2]Cl_2$, and $[M(L)_2]$ complexes, suggesting

coordination through the azomethine nitrogen atom to the central atom, but in the case of the [PhB(L)$_2$] type of complex, it shifted to a lower wave number at 1605 cm^{-1} and a higher wave number at 1630 cm^{-1}, suggesting the uncoordinated and coordinated (C=N) groups, respectively, to the boron atom. The band at 1020 cm^{-1}, due to ν(C=S), is shifted toward lower frequencies in complexes, indicating the coordination of sulfur to the central atom and the formation of B/Pd/Pt–S bonds. In addition, new weak to strong intensity bands are observed in the far IR spectra of the complexes. Bands in the regions of 830–836 cm^{-1}, 1520–1524 cm^{-1}, and 1230–1238 cm^{-1} in boron complexes are due to ν(B – S), ν(B $\leftarrow$ N), and ν(Ph–B) frequency,[8] respectively, and 364–369, 440–445, and 308–314 cm^{-1} can be assigned to ν(M-S), ν(M $\leftarrow$ N), and ν(M-Cl) binds[9] in the spectra of the [M(LH)$_2$]Cl$_2$ complexes.

The IR spectra of free ligands display two sharp bands at *ca* 3446 and 3325 cm^{-1} assignable to asymmetric and symmetric NH_2 group modes, respectively, which remain at almost the same positions in the complexes, suggesting that the amino group is not involved in chelation.

^{1}H NMR Spectra

^{1}H NMR spectra of the free ligand and its compelxes were recorded in DMSO-d$_6$. The free ligand shows a sharp singlet for the –NH proton at δ11.24 ppm, which disappears from the spectra of [PhB(OH)(L)], [PhB(L)$_2$], and [M(L)$_2$], suggesting that this proton has been lost via the thioenolization of the (>C=S) group and the coordination of the sulfur atom to the central atom. This signal however, suffers a downfield shift in the complexes [M(LH)$_2$]Cl$_2$ (δ11.52–11.58 ppm) as a consequence of their deshielding due to the coordination of the ligand to the metal atom. The ligand shows multiplets in the region of δ6.70–8.30 ppm attributable to aromatic protons, which appear almost in the same position as in the respective complexes. The $-NH_2$ group gives a singlet of δ3.36–3.44 ppm in the free ligand as well as in the complexes. It shows that the $-NH_2$ group is not taking part in the complexes (Table II).

^{13}C NMR Spectra

^{13}C NMR spectral data also support the authenticity of the proposed structures. Considerable shifts in positions of carbon atoms adjacent to the azomethine nitrogen (δ157.28–163.76 ppm) and thiolic sulfur (δ169.42–171.85 ppm) support the proposed coordination in the complexes.

TABLE II ^{1}H NMR Spectral Data of the Ligand and Its Complexes

	^{1}H NMR (δ, ppm)				
Compound	—NH_2	—NH(1)	—NH(2)	—OH	Aromatic protons
LH	3.36	11.24	11.92	4.20	6.70–8.26
[PhB(OH)(L)]	3.42	—	11.94	4.52	6.76–8.30
[$PhB(L)_2$]	3.44	—	11.95	—	6.75–8.28
[$Pd(LH)_2$]Cl_2	3.38	11.52	11.93	—	6.72–8.20
[$Pd(L)_2$]	3.40	—	11.95	—	6.75–8.28
[$Pt(LH)_2$]Cl_2	3.39	11.58	11.94	—	6.73–8.24
[$Pt(L)_2$]	3.42	—	11.96	—	6.76–8.28

^{11}B NMR Spectra

^{11}B NMR spectra of boron complexes have signals in DMSO-d_6 in the region of δ2.5–5.8 ppm, which unequivocally suggests a tetracoordinated environment around the boron atom and the presence of a B ← N coordinate bond. The driving force for the formation of this coordinate bond is the ability of a trivalent $PhB(OH)_2$ to accept a pair of electrons from a suitable donor atom.

X-Ray Spectra

The X-ray diffraction spectrum of one powdered sample has been recorded in order to further substantiate the lattice of the complexes. The observed interplanar spacing values d(Å) have been measured from the differactogram of the complex PhB(L)OH, and the Miller Indices h, k, and l have been assigned to each "d" value. The data suggest an "orthorhombic" lattice to this derivative having unit cell dimensions; a = 9.9777Å, b = 6.7131Å, c = 7.4079Å; the Miller Indices h, k, and l are recorded in Table III and $\alpha = \beta = \gamma = 90^\circ$ (Table III).

On the basis of the previously discussed spectral studies, suitable structures have been suggested for new complexes (Figure 1).

Biocidal Screening

Antifungal and antibacterial activities of the ligand and its complexes have been evaluated. Results reveal that the activity increases on complexation. Newly synthesized complexes have indeed been found to be more active in inhibiting the growth of fungi and bacteria than the ligand itself. The greater toxicity of metal/non-metal complexes than the ligand can be explained on the basis of the chelation theory. Chelation reduces the polarity of the metal ion mainly because of the partial

TABLE III X-Ray Powder Diffraction Data of [PhB(OH)(L)]

Peak	2θ (deg.)	d-spacing (Å)	h	k	l
1	14.6022	7.4564	0	0	1
2	21.8468	5.0379	0	1	1
3	30.3523	3.6619	0	0	2
4	33.0726	3.3483	0	2	0
5	34.5589	3.2317	0	1	2
6	36.3355	3.0801	1	1	2
7	40.2568	2.7933	2	2	0
8	52.3622	2.1829	1	3	0
9	58.1818	1.9228	3	0	3
10	72.98264	1.62264	3	0	4
11	86.67176	1.40729	5	2	3

sharing of its positive charge with the donor groups and possible π-electron-delocalization over the whole chelate ring. This increases the lipophilic character of the metal complexes, which subsequently favors its permeation through the lipid layers of the organism cell membrane and normal cell process being impaired. The results are discussed and reported in Tables IV and V.

Antifertility Activity

Weight Response

There were no significant differences in body weights. However, the weights of testes, the epididymis, the ventral prostate, and the seminal vesicle were decreased significantly ($P \leq 0.01$ to $P \leq 0.001$) after the treatment with the ligand and its boron, palladium, and platinum complexes (Table VI).

TABLE IV Fungicidal Screening Data of the Ligand and Its Metal Complexes

	Inhibition after 96 h (%) (Conc. in ppm)					
	Macrophomina phaseolina			*Fusarium oxysporum*		
Compound	50	100	200	50	100	200
LH	30	38	46	29	38	45
[PhB(OH)(L)]	40	50	59	41	51	60
[PhB(L)2]	42	54	62	42	55	62
[Pd(LH)2]Cl2	49	54	62	48	53	62
[Pd(L)2]	47	52	61	46	54	63
[Pt(LH)2]Cl2	52	58	65	52	58	64
[Pt(L)2]	56	60	68	55	60	68

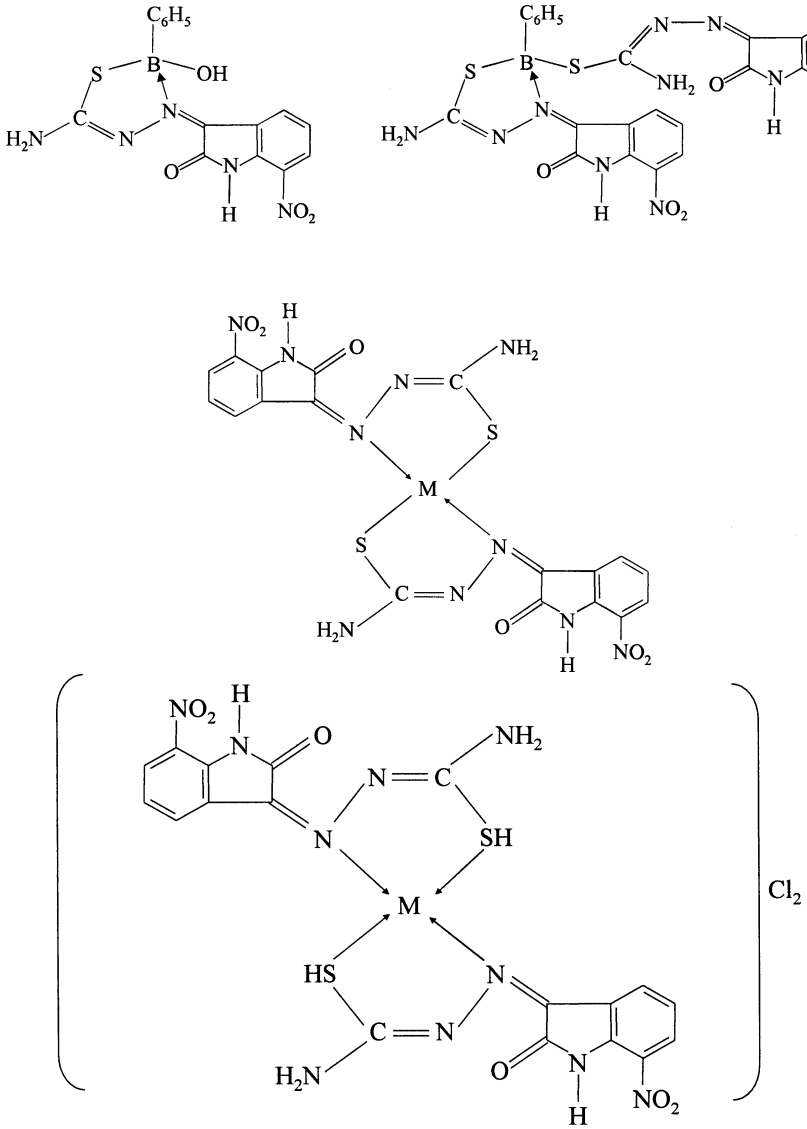


FIGURE 1 Structures of complexes where M = Pd (II) and Pt (II).

Sperm Dynamics and Fertility

The sperm motility in the cauda epididymis and sperm density in testes and the cauda epididymis were decreased significantly after treatment with the ligand and its various complexes (Table VII).

TABLE V Antibacterial Screening Data of the Ligand and Its Complexes

	Diameter of Inhibition zone (mm) (Conc. in ppm)			
	Bacillus subtilis		*Salmonella species*	
Compound	500	1000	500	1000
LH	7	10	6	9
[PhB(OH)(L)]	9	11	9	11
$[PhB(L)_2]$	10	12	10	13
$[Pd(LH)_2]Cl_2$	10	12	9	14
$[Pd(L)_2]$	9	11	9	13
$[Pt(LH)_2]Cl_2$	11	14	11	13
$[Pt(L)_2]$	13	16	12	15

Biochemical Findings

The ligand and its boron, palladium, and platinum complexes bring about a significant decrease in the testicular, total protein, and total cholesterol contents. A significant increase was observed in testicular glycogen, cholesterol, acid, and alkaline phosphatase activity after the treatment with the various compounds (Table VIII).

The administration of the ligand (LH) and its boron, palladium, and platinum complexes bring about a reduction in the weights of testes and sex accessories. The weight of testes are largely dependent on the mass of differentiated spermatogenic cells, and the reduction in weight may be due to the decreased number of germ cells and elongated spermatids.[10] The observed reduction in the weight of accessory sex organs may be due to reduced bioavailability and the estrogenic and/or antiandrogenic activities of the compounds.[11] Low causal epididymal sperm density may be due to the alteration in the androgen metabolism. The physiological and biochemical integrity of the epididymis is dependent on androgens.[12] The 50–95% negative fertility test may be attributed to the lack of forward progression and reduction in the density of spermatozoa and altered biochemical milieu of the cauda épididymis.[13] The treatment with the ligand (LH) and its boron, palladium, and platinum complexes also changes the testicular biochemical parameters. The reduction in testicular sialic acid contents may be due to the absence of spermatozoa or reduced androgen production.[14,15] The increased level of testicular cholesterol contents is attributed to the decreased androgen concentration, which resulted in impaired spermatogenesis.[16] An increase in the testicular glycogen of the ligand and its complexes treated animals might be associated

TABLE VI Effects of the Ligand (LH) and Its Boron, Palladium, and Platinum Complexes on Body and Reproductive Organ Weights of Male Rats

		Body weight (gm)		Organ weight (mg)			
Groups	Treatment	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
A	Control	208.0 ± 13.2	228.0 ± 11.5^{c}	1350.0 ± 35.9	490.0 ± 18.3	518.0 ± 20.2	495.0 ± 11.8
B	LH	215.0 ± 15.2	235.0 ± 13.3^{c}	1120.0 ± 36.5^{a}	395.0 ± 38.5^{a}	450.0 ± 18.7^{a}	425.0 ± 12.4^{a}
C	[PhB(OH)(L)]	210.0 ± 14.5	227.0 ± 12.3^{c}	980.0 ± 31.9^{a}	320.0 ± 20.6^{a}	390.5 ± 17.4^{a}	370.0 ± 11.1^{a}
D	$[PhB(L)_2]$	220.0 ± 11.8	238.0 ± 10.5^{c}	910.0 ± 15.7^{b}	310.0 ± 18.4^{b}	350.0 ± 11.5^{b}	360.0 ± 17.5^{b}
E	$[Pd(LH)_2]Cl_2$	205.0 ± 10.9	222.0 ± 13.4^{c}	990.0 ± 35.4^{a}	335.0 ± 18.7^{b}	370.5 ± 14.2^{b}	373.0 ± 17.4^{a}
F	$[Pd(L)_2]$	212.0 ± 11.8	234.0 ± 11.9^{c}	890.0 ± 31.3^{b}	305.0 ± 17.4^{b}	340.0 ± 11.8^{b}	305.0 ± 14.3^{b}
G	$[Pt(LH)_2]Cl_2$	218.0 ± 12.3	239.0 ± 11.8^{c}	950.0 ± 29.8^{a}	330.0 ± 14.9^{b}	355.0 ± 17.4^{b}	340.0 ± 11.6^{b}
H	$[Pt(L)_2]$	221.0 ± 17.3	240.0 ± 14.3^{c}	840.0 ± 18.6^{b}	295.0 ± 13.7^{b}	315.0 ± 14.1^{b}	300.0 ± 10.8^{b}

Group B was compared with Group A.
Groups C, D, E, F, G, and H were compared with Group A.
Mean $\pm$ SEM of five animals; $a = P \leq 0.01$; $b = P \leq 0.001$; $c = \leq$ ns.

TABLE VII Effects of the Ligand (LH) and Its Boron, Palladium, and Platinum Complexes on Body and Reproductive Organ Weights on Male Rats

Group	Treatment	Sperm motility Cauda epididymis (%)	Sperm density million/cm^3		Fertility test (%)
			Testes	Cauda epididymis	
A	Control	85.0 ± 4.6	5.1 ± 0.60	57.5 ± 3.6	100% positive
B	LH	65.0 ± 4.5^a	4.1 ± 6.30^a	47.3 ± 1.5^a	50% negative
C	[PhB(OH)(L)]	50.0 ± 3.8^a	3.1 ± 0.20^a	35.8 ± 1.8^a	55% negative
D	$[PhB(L)_2]$	42.0 ± 3.5^b	2.2 ± 0.10^b	30.0 ± 1.6^b	60% negative
E	$[Pd(LH)_2]Cl_2$	41.0 ± 2.9^b	2.9 ± 0.25^a	31.0 ± 1.7^b	65% negative
F	$[Pd(L)_2]$	35.0 ± 3.1^b	1.9 ± 0.10^b	28.0 ± 2.1^b	80% negative
G	$[Pt(LH)_2]Cl_2$	34.1 ± 4.1^b	2.1 ± 0.15^b	25.0 ± 2.5^b	90% negative
H	$[Pt(L)_2]$	27.0 ± 2.1^b	1.4 ± 0.10^b	19.0 ± 1.1^b	95% negative

Group B was compared with Group A.
Groups C, D, E, F, G, and H were compared with Group A.
Mean ± SEM of five animals; $a = P \leq 0.01$; $b = P \leq 0.001$.

with the poor utilization of glycogen due to a decrease in phosphorylase activity.[17] Furthermore, an increase in testicular acid and alkaline phosphatase activities indicate an impairment of the functional integrity of testes.[18] From these results, it can be conducted that the addition of boron, palladium, or platinum moiety to the ligand enhances its activity, and the compounds of platinum are more effective in regulating the fertility in male rats.

EXPERIMENTAL

All syntheses and manipulations were carried out under strict exclusion of moisture using an apparatus made up of standard interchangeable quick-fit joints. The $[PhB(OH)_2]$, $PdCl_2$, $PtCl_2$, and *o*-nitroaniline were purchased from L obachemie and used as such. 7-nitroisatin was prepared in the laboratory. All preparations were carried out under acidic or basic conditions. All solvents were dried and distilled before use.

Preparation of the Ligands

The Synthesis of 7-nitroisatin

7-nitroisatin can be synthesized conveniently by Sandmeyer isonitroso-acetanilide synthesis. *o*-nitroaniline-HCl, chloralhydrate-Na_2SO_4, and hydroxylamine hydrochloride are dissolved in water, and the solution is heated for a few minutes. The solution is shaken

TABLE VIII Testicular Biochemistry of the Ligand (LH) and Its Boron, Palladium, and Platinum Complexes

Group	Treatment	Glycogen (mg/gm)	Total protein (mg/gm)	Total cholesterol (mg/gm)	Sialic acid (mg/gm)	Phosphatase mg/ip/g/h	
						Acid	Alkaline
A	Control	$3.95 + 0.57$	$220.0 + 15.6$	$5.90 + 0.26$	$5.40 + 0.21$	$3.15 + 0.19$	$10.50 + 0.80$
B	LH	4.91 ± 0.10^a	180.0 ± 13.4^a	6.70 ± 0.12^a	4.50 ± 0.19^a	4.15 ± 0.15^a	13.40 ± 0.70^a
C	[PhB(OH)(L)]	5.60 ± 0.11^a	150.0 ± 12.3^a	7.30 ± 0.10^a	3.9 ± 0.25^a	5.15 ± 0.11^b	16.10 ± 0.50^b
D	$[PhB(L)_2]$	5.70 ± 0.15^a	145.0 ± 13.3^a	7.45 ± 0.15^a	3.20 ± 0.18^b	5.65 ± 0.12^b	17.1 ± 0.56^b
E	$[Pd(LH)_2]Cl_2$	5.95 ± 0.15^b	142.5 ± 10.2^a	8.10 ± 0.17^b	3.85 ± 0.15^a	5.50 ± 0.10^b	15.4 ± 0.10^b
F	$[Pd(L)_2]$	6.25 ± 0.18^b	130.0 ± 11.4^b	8.25 ± 0.19^b	3.25 ± 0.17^b	5.90 ± 0.11^b	17.50 ± 0.13^b
G	$[Pt(LH)_2]Cl_2$	6.15 ± 0.10^b	132.0 ± 10.5^b	8.45 ± 0.20^b	3.00 ± 0.10^b	5.70 ± 0.11^b	16.90 ± 0.14^b
H.	$[Pt(L)_2]$	6.73 ± 0.23^b	110.0 ± 9.5^b	9.40 ± 0.50^b	2.75 ± 0.12^b	6.10 ± 0.15^b	17.89 ± 0.15^b

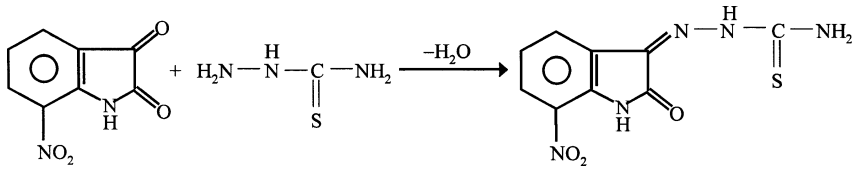
Group B was compared with Group A.
Groups C, D, E, F, G, and H were compared with Group A.
Mean $\pm$ SEM of five animals; $a = P \leq 0.01$; $b = P \leq 0.001$.

vigorously and cooled whereby crystals of isonitrosoacetanilide are obtained. The isonitrosoacetanilide is filtered and dried.

The dried isonitrosoacetanilide is then cyclized in conc. H_2SO_4 at 60–80°C to yield 7-nitroisatin. The crude isatin is then recrystallized from glacial acetic acid or purified by dissolving in aq. NaOH and reprecipitating with HCl.

The Synthesis of LH of 7-nitroisatin

The ligand LH was prepared by the condensation of 7-nitroisatin with hydrazinecarbothioamide in the presence of sodium acetate in a 1:1 molar ratio, in absolute alcohol. The reaction mixture was refluxed over a water bath for 3–4 h and allowed to stand over night (Scheme 1). The product was recrystallized from the ethanol and dried in vacuo. Its physico-chemical properties and analytical data are given in Table I.



SCHEME 1

The Preparation of [PhB(OH)(L)] and [PhB(L)$_2$] Complexes

Phenylboronic acid was dissolved in dry benzene in a 100-mL RB flask, and to this the requisite amounts (1:1 or 1:2 molar ratios) of the ligand were added. The resulting mixture was refluxed for 12–24 h. The progress of the reaction was monitored azeotropically by the liberation of water. The solid product was dried in vacuo. It was then washed several times with dry cyclohexane and again dried in vacuo for 3–4 h. It is interesting to note that if the same reactions were carried out under microwave irridation, reactions completed within 5 min. This shows that the microwave technology is more useful than the conventional method.

The Preparation of [M(LH)$_2$]Cl$_2$ Complexes

These complexes were prepared by dissolving MCl_2(0.01 mol) in ethanol and then adding an ethanolic solution of a the ligand (0.02 mol) to this solution in 1:2 molar ratios. The reaction mixture was heated under reflux for about 1 h in the presence of a few drops of concentrated HCl.

On cooling, complexes separated out, which were filtered and washed several times with alcohol and dried in vacuo.

The Preparation of $[M(L)_2]$ Complexes

The ethanol solution of MCl_2 (0.01 mol) was mixed with an ethanolic solution of a ligand (0.02 mol) in 1:2 molar ratios. Aqueous NH_4OH was added dropwise to the reaction mixture until it was weakly alkaline (pH *ca*. 8.0). The mixture was then stirred on a magnetic stirrer for 2–3 h, and the resulting product was recovered by filtration, washed with ethanol, and dried in vacuo. Physical properties and analytical data of these complexes are listed in Table I.

BIOCIDAL ACTIVITY

Antimicrobial Activity

The antifungal activity was evaluated against *Macrophomina phaseolina* and *Fusarium oxysporum* by the agar plate technique. Solutions of compounds in different concentrations (50, 100, and 200 ppm) in DMF were used. The linear growth of the fungus was recorded by measuring the diameter of the colony after 96 h, and the percentage inhibition was calculated as 10^2 $(d_c - d_T)/d_c$, where d_c and d_T were the diameters of the fungus colony in the control and test plates, respectively.

The antibacterial activity was screened by the paper-disc plate method. The nutrient agar medium (Peptone, beef extract, NaCl, and agar-agar) and 5-mm diameter paper discs (Whatman No. 1) were used. Compounds were dissolved in DMF in 500 and 1000 ppm concentrations. Filter paper discs were soaked in different solutions of compounds, dried, and then placed in petri dishes previously seeded with the test organism (*Bacillus subtilis* and *salmonella species*). Plates were incubated for 24–30 h at $28 \pm 2^{\circ}C$, and the inhibition zone around each disc was measured.

Antifertility Activity

Male rats obtained from ICMR, New Delhi were used. Animals were housed in steel cages and maintained under standard conditions (14 h light/10 h dark cycle; $25 \pm 3^{\circ}C$, 35–50% humidity); water and food were given ad libitum. Proven fertile male rats were taken and divided into 8 groups of 6 each. Group A served as a vehicle (olive oil) treated control. Groups B, C, D, E, F, G, and H were respective to ligand, boron (1:1 and 1:2), palladium (1:2 add. and 1:2 sub.), and platinum (1:2 add. and

1:2 sub.), respectively, for the same period and dose. On the 61st day, these animals were autopsied, and testes, the epididymis, the seminal, the vesicle, and the ventral prostate were removed; fat and connective tissue was cleared off and kept at -20°C until they were assayed for total protein, sialic acid, cholesterol, frustose, and glycogen, by standard laboratory techniques.

Fertility Test

The mating exposure test of all animals was performed. They were cohabited with progesterone females in the ratio of 1:3. The vaginal plug and presence of sperm in the vaginal smear was checked for positive mating. Females were separated, and resultant pregnancies were noted when dams gave birth. Fertility was calculated in control as well as treated groups. Animals were weighed and autopsized under light ether anesthesia. The sperm motility in cauda epididymis and density of testis and cauda epididymis suspended sperm were calculated.[19] The weight of testes, the epididymis, the ventral prostate, and the seminal vesicle were recorded, testes were frozen for the measurements of glycogen, total protein, total cholesterol, sialic acid, acid, and alkaline phosphatase, by standard laboratory techniques. The difference between control and treated groups were evaluated statistically using a student's "t" test. Data were expressed as mean ± 5 EM. The significance was set at $P \leq 0.01$ and $P \leq 0.001$.

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