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Phosphorus, Sulfur, and Silicon and the Related Elements

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In Vitro Antibacterial and Antifungal Activities of Some Sulfur-Nitrogen-Oxygen and Oxygen-Nitrogen-Oxygen Donor Bifunctional Tridentate Schiff Bases and Their Boron(III) Complexes

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IN VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF SOME SULFUR-NITROGEN-OXYGEN AND OXYGEN-NITROGEN-OXYGEN DONOR BIFUNCTIONAL TRIDENTATE SCHIFF BASES AND THEIR BORON(III) COMPLEXES

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Antimicrobial properties of sulfur, nitrogen, and oxygen bonded organoboron (III) complexes with biologically potent ligands viz., 2-hydroxy-N-phenyl benzamide hydrazine carboxamide($HO^{\circ}N^{\circ}OH$), 2-hydroxy-N-phenyl benzamide hydrazine carbothioamide ($HO^{\circ}N^{\circ}SH$), and 2-hydroxy-N-phenyl benzamide hydrazine carbodithioic acid ($HO^{\circ}N^{\circ}SH$), have been studied. The unimolar and bimolar reactions of triisopropoxy borane with dibasic tridentate ligands resulted in the formation of colored solids, which have been characterized by elemental analysis, molecular weight determinations, and conductance measurements. The UV, IR, and NMR (^{1}H , ^{13}C , and ^{11}B) spectral studies indicate a tetra-coordinated geometry for the resulting complexes. The ligands and their complexes have been screened for their fungicidal and bactericidal activities, and the results indicate that they exhibit significant antimicrobial properties.

Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements to view the free supplemental file.

Keywords Bactericidal activities; fungicidal activities; 2-hydroxy-N-phenyl benzamide hydrazine carbodithioic acid; 2-hydroxy-N-phenyl benzamide hydrazine carbothioamide; 2-hydroxy-N-phenyl benzamide hydrazine carboxamide

INTRODUCTION

Interest in metal complexes of sulfur–nitrogen chelating agents, especially those formed from thiosemicarbazide, 4N-substituted thiosemicarbazides,^{1–5} and S-alkyl esters of dithiocarbazic acid,^{6–10} has been stimulated by their interesting physicochemical properties and potentially useful pharmacological properties.^{1,5,7,11} A variety of 5-nitrofuryl semicarbazone derivatives have been developed for the therapy of Chagas disease.¹² Some derivatives of thiosemicarbazides are useful intermediates for drugs and agrochemicals.¹³ The coordinating properties of some semicarbazone metallic complexes have also been studied.¹⁴ Semicarbazones and thiosemicarbazones present a wide range of bioactivities, and their chemistry and pharmacological applications have been extensively investigated. Thiosemicarbazide derivatives have antiviral and antitumor activities.¹⁵ The biological

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	Color		Analys	Mol		
Compound		$Mp \ (^{\circ}C)$	N	S	В	Wt Found/(Calcd.)
L^1H_2	Off white	110-112	20.76 (20.84)	_	_	251.11(270.29)
L^2H_2	Light pink	122-124	19.51 (19.57)	11.13 (11.20)	_	267.46 (286.35)
$L^{3}H_{2}$	Gray	132-133	10.56 (10.68)	16.34 (16.30)	_	407.70 (394.52)
$(OPr^i)B(L^1)$	Light yellow	260-262	16.12 (16.57)	_	3.08 (3.20)	353.30 (338.19)
$(L^1 H)B(L^1)$	Light yellow	280-281	20.23 (20.44)	_	1.12 (1.97)	562.41 (548.39)
$(OPr^i)B(L^2)$	Cream	112-114	15.88 (15.82)	8.89 (9.05)	2.89 (3.05)	338.13 (354.25)
$(L^2 H)B(L^2)$	Pink	134-136	19.01 (19.30)	10.81 (11.05)	1.51 (1.86)	564.27 (580.51)
$(OPr^i)B(L^3)$	Light brown	104-106	8.91 (9.11)	13.51 (13.90)	2.04 (2.34)	474.55 (461.43)
$(L^3 H)B(L^3)$	Brown	120-122	10.15 (10.57)	16.01 (16.14)	1.12 (1.36)	808.11 (794.87)

Table I Analytical data and physical properties of the ligands and their organoboron (III) complexes

properties of semicarbazones and thiosemicarbazones are often related to metal ion coordination. First, lipophilicity, which controls the rate of entry into the cell, is modified by coordination.¹⁶ Also, the metal complexes can be more active than the free ligands, and some side effects may decrease upon complexation. In addition, the complexes can exhibit bioactivities that are not shown by the free ligands. The mechanism of action can involve binding to a metal in vivo, or the metal complex may be a vehicle for activation of the ligand as the cytotoxic agent. Moreover, coordination may lead to significant reduction of drug resistance.¹⁷ Boron is essential for healthy plants. The biochemical role of boron is not fully understood even 60years after its recognition as an essential element, although it is known to be involved in nucleic acid synthesis, possibly linked to adequate provision of pyrimidine nucleotides. Boron also plays parts in carbohydrate metabolism, hormone action, and membrane formation.¹⁸ Organoboron compounds are used as a source of radicals.¹⁹ In view of these findings, we have synthesized and characterized some new Schiff base complexes of boron with 2-hydroxy-N-phenyl benzamide hydrazine carboxamide, 2-hydroxy-N-phenyl benzamide hydrazine carbothioamide, and 2-hydroxy-N-phenyl benzamide hydrazine carbodithioic acid.

The physicochemical studies of these compounds suggest the tridentate nature of the Schiff base ligands. Spectral analysis and molecular weight measurements have been employed to throw light on the structure of these complexes (Tables I and II).

Compound v(-	-OH)	ν (C=N)	ν (-NH ₂)	v(C=O)	v(C=S)	v(B-N)	$\nu(B=O)$	v(B-S)
	,		(2)	. ,	. ,	· /	. ,	. ,
L ¹ H ₂ 314	40–3256	1612	3478-3367	1685	—	—	—	—
$L^{2}H_{2}$ 31:	50-3250	1620	3480-3372	—	1030	—	—	—
L ³ H ₂ 312	28–3245	1608	3470-3362	—	1040	_		—
$(OPr^i)B(L^1)$	_	1608	3478-3367	1680	_	1550	1340	860
$(L^1 H)B(L^1)$	—	1610–1625	3478-3367	1683	—	1548	1334	854
$(OPr^i)B(L^2)$	_	1616	3480-3372	_	1020	1520	1325	830
$(L^2 H)B(L^2)$	_	1612-1628	3480-3372	_	1025	1514	1321	824
$(OPr^i)B(L^3)$	_	1606	3470-3362	_	1032	1532	1330	840
$(L^3 H)B(L^3)$		1604–1616	3470-3362	—	1036	1530	1328	832

Table II IR spectral data (cm^{-1}) of the ligands and their organoboron (III) complexes

RESULTS AND DISCUSSION

The reactions of triisopropoxy borane with dibasic tridentate 2-hydroxy-N-phenyl benzamide hydrazine carboxamide, 2-hydroxy-N-phenyl benzamide hydrazine carbothioamide, and 2-hydroxy-N-phenyl benzamide hydrazine carbodithioic acid in different molar ratios yielded products according to the general Equations (1) and (2)

$$[B(OPr^{i})_{3}] + HO^{\cap}N^{\cap}XH \rightarrow [(OPr^{i})B(O^{\cap}N^{\cap}X)] + 2Pr^{i}OH$$
(1)

$$[B(OPr^{i})_{3}] + 2HO^{\cap}N^{\cap}XH \rightarrow [(O^{\cap}N^{\cap}XH)B(O^{\cap}N^{\cap}X)] + 3Pr^{i}OH$$
(2)

where, HO^{\cap}N^{\cap}XH represents the donor set of the ligand molecules and X = O or S.

1.2

These reactions are quite facile and could be completed within 12–24 h of refluxing in benzene. The resulting products are colored solids and insoluble in common organic solvents but soluble in DMSO, THF, and DMF. The complexes are quite stable. The molecular weight determinations indicate their monomeric nature. The low molar conductance values $(8-12 \ \Omega^{-1} \ cm^2 \ mol^{-1})$ of these derivatives in DMF at 10^{-3} M concentrations show them to be nonelectrolytes.

Electronic Spectra

The electronic spectra of the ligands $L^{1}H_{2}$, $L^{2}H_{2}$, and $L^{3}H_{2}$ exhibit three bands at 237, 272, and 332 nm. The bands in the regions 235 nm and 275 nm are assignable to $\pi - \pi^{*}$ transitions of the azomethine group. The considerable hypsochromic shifting of the third band in the spectra of boron complexes may be attributed to the coordination of the azomethine nitrogen to the boron atom.

Infrared Spectra

The IR spectra of the free ligands and their complexes were scanned in the form of KBr pellets. IR spectra of the free ligands show a medium intensity band at 3260–3128 cm^{-1} due to v NH/v OH vibrations, which is absent in the spectra of the complexes. The bands due to ν (C=O) and ν (C=S) modes in the spectra of the ligands are observed at 1705 cm⁻¹ and 1035 cm⁻¹, respectively.²⁰ In the ligand $L^{3}H_{2}$, a doublet at 2900 cm⁻¹ and 2950 cm⁻¹ is assigned to symmetric and asymmetric vibrations of S-CH₂-C₆H₅ grouping and is reduced to a weak doublet in the spectra of the complexes. These bands disappeared in the spectra of the boron compounds, suggesting the enolization and thioenolization of the ligands and their chelation through the amido oxygen and thiolic sulfur, respectively. This fact is supported by the observation of the bands due to ν (C=O) and ν (C=S) modes at lower frequencies in the spectra of the boron compounds. In the ligands $L^{1}H_{2}$, $L^{2}H_{2}$, and $L^{3}H_{2}$, the most significant band in the region, 1620 cm⁻¹ assignable to the ν (C=N)^{21,22} group, shifts to the lower frequency in the complexes, suggesting the coordination^{23,24} of the azomethine nitrogen to the boron atom. The absorption at ca. 1612 cm⁻¹, characteristic of the azomethine $(>C=N)^{25}$ group in the spectra of the ligands, gets split into two sharp bands at $ca 1612 \text{ cm}^{-1}$ and 1625 cm^{-1} in 1:2 complexes. This splitting of the bands suggests that the azomethine group is in different chemical environments. The shifting of the bands at ca. 1625cm⁻¹ (higher wavenumber side) suggest the coordination of the azomethine nitrogen to the boron atom, whereas the band at ca. 1612 cm^{-1} is assigned to the uncoordinated azomethine group. There are no changes in the ν sym and ν asym modes of the NH₂ group²⁶ appearing at ca 3360 cm⁻¹ and 3480 cm⁻¹, respectively, indicating the noninvolvement of this amino group in chelation. The coordination through the azomethine nitrogen atom and the enolic oxygen/thiolo sulfur is further substantiated by the appearance of new bands in the regions 1320–1340 cm⁻¹, 830–860 cm⁻¹, and 1510–1550 cm⁻¹ in the boron complexes due to ν (B-O), ν (B-S),²⁷ and ν (B \leftarrow N)²⁸ frequencies, respectively.

¹H NMR Spectra

The ¹H NMR spectra of the ligands and their complexes have been recorded in DMSO–d₆- using TMS as an internal standard. In the ligands, the signal in the region δ 12.10–12.14 ppm, due to –OH, disappears in the complexes, and this confirms the deprotonation and complexation. The signal due to the –NH proton attached to the phenyl ring remains unaltered in the complexes. The NH₂ group gives singlet at δ 2.80–2.95 ppm in the ligands (L¹H₂ and L²H₂) and their complexes. This shows that the NH₂ group is not taking part in the complexation. The signal of the –NH proton in the ligands in the range δ 10.72–10.86 ppm that disappears²⁹ in the spectra of the corresponding (1:1) complexes and appears in 1:2 complexes is assigned to the uncoordinated –NH group. The free ligands show multiplets in the region δ 6.67–8.36 ppm attributable to aromatic protons.³⁰ The resonance due to the SCH₂ and aromatic protons in the complexes appears in almost the same positions as in the respective free ligands. Chemical shift values of all the complexes are listed in Table III.

¹³C NMR Spectra

The ¹³C NMR spectral data also support the authenticity of the proposed structures. The considerable shifts in the positions of carbon atoms adjacent to the azomethine nitrogen (δ 167.15–174.36 ppm) and thiolic sulfur/enolic oxygen (δ 176.24–184.20 ppm) support the proposed coordination in the complexes. Thus the shifts in the position of carbon atoms adjacent to the coordinating atoms clearly suggest the bonding of the azomethine nitrogen and amido oxygen to the boron atom (Table IV).

Compound	—ОН (s)	—NH (bs)	-NH ₂ (s)	-S-CH ₂ (s)	φ-NH (s)	Aromatic protons (m)
L ¹ H ₂	12.14	10.86	2.92		10.60	6.68-8.36
L^2H_2	12.12	10.84	2.80	_	10.64	6.75-8.32
$L^{3}H_{2}$	12.10	10.72	_	1.82	10.75	6.70-8.19
OPr ⁱ)B(L ¹)	_	_	2.94	_	10.61	6.67-8.31
$(L^1 H)B(L^1)$		10.89	2.95	_	10.62	6.68-8.33
OPr ⁱ)B(L ²)	_	_	2.82	_	10.65	6.74-8.30
$(L^2 H)B(L^2)$		10.87	2.83	_	10.66	6.75-8.31
OPr ⁱ)B(L ³)	_	_	_	1.90	10.73	6.69-8.12
$(L^3 H)B(L^3)$	—	10.76	_	1.92	10.74	6.70-8.14

Table III ¹H NMR spectral data (δ , ppm) of the ligands and their organoboron (III) complexes

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Compound	>C=O/ >C=S	>C=N/ C-N	Aromatic carbons	¹¹ B chemical shift
L^1H_2	180.03	167.15	160.72, 138.92, 130.15, 129.83 129.88 125.42, 122.57, 120.51, 118.77, 119.79	
L^2H_2	179.52	168.01	160.86, 138.99, 130.18, 129.79, 129.95, 125.58, 122.64, 120.68, 118.94, 119.99	—
$L^{3}H_{2}$	176.24	168.28	160.72, 138.84, 132.81, 129.84, 129.92, 125.46, 122.66, 120.72, 118.92, 118.98	—
$(OPr^i)B((L^1)$	182.28	168.53	158.43, 137.56, 129.26, 129.31, 129.63, 125.37, 121.79, 120.19, 118.08, 118.27	3.98
$(L^1 H)B(L^1)$	184.20	169.98	159.32, 137.03, 130.25, 130.46, 129.62, 128.56, 121.31, 120.86, 118.83, 118.74	4.84
$(OPr^i)B(L^2)$	181.42	170.28	158.01, 137.12, 129.46, 129.12, 129.11, 124.02, 121.01, 120.11, 117.91, 118.02	3.54
$(L^2 H)B(L^2)$	183.35	173.12	158.28, 137.67, 129.97, 129.86, 129.42, 125.32, 121.49, 120.22, 118.01, 118.22	4.42
(OPr ⁱ)B(L ³)	178.24	171.11	158.44, 137.33, 131.65, 128.99, 128.98, 125.36, 121.34, 120.43, 118.89, 118.93	4.25
$(L^3 H)B(L^3)$	179.35	174.36	159.33, 137.54, 130.23, 129.72, 128.96, 125.64, 121.52, 120.61, 118.93, 118.89	4.29

Table IV ¹³C NMR and ¹¹B spectral data (δ , ppm) of the ligands and their organoboron (III) complexes

¹¹B NMR Spectra

The ¹¹B NMR spectra of the boron complexes have signals in DMSO-d₆ at δ 3.0–5.0 ppm, which unequivocally suggests a tetracoordinated environment around the boron atom and the presence of a B \leftarrow N coordinate bond.^{31,32}



1:2 Complex

Where X= O,S and R=NH₂,S-CH₂-C₆H₅

Where X= O,S and R=NH₂,S-CH₂-C₆H₅

MICROBIAL ASSAY

Antifungal Screening

The complexes were screened against *Alternaria alternata* and *Fusarium oxysporum* fungi. The antifungal activity of synthesized compounds was evaluated by the radial growth method.³³ (See the Supplemental Materials online, Tables 5S and 6S).

Antibacterial Screening

Various methods are available for the evaluation of the antibacterial activity of different types of compounds. In the present work, activities of synthesized compounds were evaluated by the disc-diffusion method using inhibition zone technique.³⁴ The complexes were screened against *Staphylococcus aureus* and *Xanthomonas compestris* bacteria (see the Supplemental Materials).

EXPERIMENTAL

The boron compound, boric acid, 2-hydroxy-N-phenylbenzamide, hydrazine hydrochloride, and hydrazinecarbothioamides were purchased from Sisco, Lancaster, and Lobachemie and used as such. The triisopropoxy borane was prepared by the reaction of boric acid with isopropanol in benzene medium.³⁶ All the chemicals and solvents were dried and purified by standard methods. The reactions were carried out under strictly anhydrous conditions. Boron was estimated as boric acid in the presence of mannitol using phenolphthalein as an indicator. Conductivity measurements were made with a Systronics model 305 conductivity bridge in dry dimethylformamide. Molecular weights were determined by the Rast Camphor method. Nitrogen was estimated by the Kjeldahl's method, and sulfur was estimated by the Messenger's method. The electronic spectra were recorded on a Varian–Cary/2390 spectrophotometer at RSIC, I.I.T., Chennai. Infrared spectra of the ligands and their complexes were recorded on a Nicolet Megna FTIR-550 spectrophotometer using KBr pellets. ¹H, ¹³C, and ¹¹B NMR spectra were recorded on a JEOL-AL-300 FT NMR spectrometer in DMSO-d₆.

Preparation of the Ligands

The ligands $(L^{1}H_{2})$ and $(L^{2}H_{2})$ were prepared as reported.³⁷ The ligand $(L^{3}H_{2})$ was prepared by the reaction of 2-hydroxy-N-phenylbenzamide with S-benzyldithiocarbazate in a 1:1 molar ratio, in absolute ethanol in a unimolar ratio. This reaction mixture was then refluxed over a water bath for 3–4 h and allowed to stand overnight. The ligands $(L^{1}H_{2}, L^{2}H_{2}, \text{ and } L^{3}H_{2})$ that separated out were purified by recrystallization from the same solvent and analyzed before use. The parent ligands exist in the tautomeric forms (Figure 1).

Preparation of the Complexes

The triisopropoxy borane was dissolved in dry benzene in a 100-mL RB flask, and to this the requisite amounts (1:1 and 1:2 molar ratios) of the ligands were added. The resulting mixture was refluxed for 12–24 h. The progress of the reaction was monitored by the liberation of isopropanol azeotropically. The solid product was dried in vacuo. The physical properties and analytical data of these complexes are listed in Table I. The



 $L^{1}H_{2}$ and $L^{2}H_{2}$ (HO^NXH) X= O, S



$L^{3}H_{2}$ (HO ^{N}SH)

Figure 1 Schiff base ligands.

purity of the compounds was checked by TLC on silica gel-G using anhydrous methanol and tetrahydrofuran (1:1) as a solvent. Each of the compounds moves as a single spot, indicating the presence of only one component, hence validating their purity.

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