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Fluoro-Boron Complexes as Biocides: Synthetic, Structural and Biological Aspects

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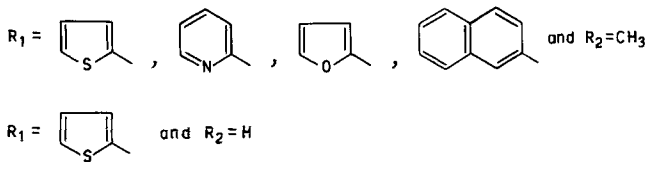
Abstract. The present account deals with the synthesis, stereochemistry and biological properties of coordinatively saturated difluoroboron(III) compounds. The ligands used during the present investigations were prepared by the condensation of [1-(thien-2-yl)-ethanone], [1-(pyridin-2-yl)ethanone], [1-(furan-2-yl)ethanone], [1-(naphthen-2-yl)ethanone] and [(thien-2-yl)methanal] with 2-mercapto-aniline. The unimolar reactions between borontrifluoride-acetic acid and these ligands have produced $BF_2(NS)$ type of biologically active complexes. The quantitative and spectral analyses comprising u.v., i.r. and n.m.r. (^1H, ^{11}B, ^{13}C and ^{19}F) helped in establishing the structures of the resulting complexes. In the quest for better fungicides and bactericides, studies were conducted to assess the growth inhibiting potential of the synthesized complexes against various fungal and bacterial strains. The studies demonstrate that the concentration reached levels which are sufficient to inhibit and kill the pathogens.

The importance of borontrifluoride as industrial chemical stems almost exclusively from its ability to catalyse a wide variety of organic reactions [1,2]. Five membered monocyclic BF_2 chelate rings are generally stable when stabilizing mesomerism in the chelate ring is possible [3]. In view of the interest involved in the stereochemistry and their potential applications, the present situation prompted us to prepare similar five membered BF_2 chelate rings which resulted from the reactions of borontrifluoride-acetic acid and benzothiazolines. Benzothiazolines, which are biologically active, constitute an important set of NSH donor systems [4, 5]. Borontrioazomethine complexes of these ligands have shown prominent biocidal activity [6]. Fungicidal and bactericidal activities of the newly synthesized boron complexes have also been carried out to study the role of coordinated boron atom. the inhibition of fungi and bacteria show greater efficacy for the complexes than the benzothiazolines alone which shows that the bioactivity enhances on undergo complexation with the metal ions [7, 8]. The results of the biological activities have been compared with the conventional fungicide, Bavistin and conventional bactericide, Streptomycin taken as standards for antifungal and antibacterial activities.

The focus of our communication is the synthetic, structural and biological aspects of difluoroboron(III) compounds. The benzothiazolines used can be structurally depicted as follows:

(Benzothiazoline) (Thio-azomethine)

where,



Experimental

Chemicals and solvents used were dried and purified by standard methods and moisture was excluded from glass apparatus using fused $CaCl_2$ drying tubes.

Preparation of Benzothiazolines

Benzothiazolines of [1-(Thien-2-yl)ethanone], [1-(Pyridin-2-yl)-ethanone], [1-(Furan-2-yl)ethanone] and [1-(Naphthen-2-yl)ethanone] were prepared by condensing the unimolar ratio of heterocyclic ketones and 2-mercaptoaniline in dry ethanol on a magnetic stirrer for 3–4 hours. The crystalline product so obtained was filtered, washed with ethanol and dried in vacuo.

Benzothiazoline of [(Thien-2-yl)methanal] was prepared by the condensation of the appropriate aldehyde with 2-mercaptoaniline in a 1:1 molar ratio in benzene. The solution was heated under reflux for 2–3 hours after which the solvent was removed in vacuo. The crude yellow solid obtained was recrystallized from benzene.

Analyses of these ligands for nitrogen and sulphur agreed with the theoretical values within the limits of experimental errors and the physical properties are given in Table 1.

Synthesis of Difluoroboron(III) Complexes

To a weighed amount of borontrifluoride-acetic acid ($BF_3 \cdot CH_3COOH$) in dry acetic anhydride (~ 40 ml) was added the requisite amount of ligand. The contents were refluxed over the fractionating column for six to nine hours and the reaction proceeded smoothly with the elimination of HF and AcOH. The excess of the solvent was removed under reduced pressure and the complexes were dried for 3–4 hours after repeated washings with dry ether in vacuo. Their analyses and physical properties are given in Table 2.

Analytical Methods and Physical Measurements

Carbon and hydrogen analyses were performed at the micro-analytical laboratory of the department. Nitrogen and sulphur were estimated by the Kjeldahl's and Messenger's methods respectively. Boron was estimated as boric acid in presence of mannitol using phenolphthalein as an indicator. The conductance was measured at 24±1 °C using Systronics Conductivity Bridge (Model 305). The molecular weights were determined by the Rast-Camphor method. The electronic spectra were recorded on a Pye-Unicam SP-8-100 ultraviolet Spectrophotometer in the range, 200–500 nm. The i.r. spectra were recorded on a Perkin-Elmer 577 Grating Spectrophotometer using KBR pellets. The ^1H, ^{11}B, ^{13}C and ^{19}F n.m.r. spectra were recorded on a Jeol FX 90Q Spectrometer in DMSO-d_6 and dry DMSO for ^{13}C n.m.r. spectra. TMS was used as an internal reference for ^1H and ^{13}C n.m.r. spectra and $BF_3 \cdot Et_2O$ and C_6F_6 as the external reference for ^{11}B and ^{19}F n.m.r. spectra, respectively.

Biological Aspects

The synthesized ligands and their difluoroboron (III) compounds were tested for the in vitro growth inhibitory activity against pathogenic fungi, viz., *Macrophomina phaseolina*, *Fusarium oxysporum* and *Aspergillus niger* and bacteria, viz., *Staphylococcus aureus*, *Klebsiella aerogenous*, *Escherichia coli* and *Pseudomonas cepacicola*. Proper temperature, necessary nutrients and growth media free from other microorganisms were employed for the preparation of cultures of fungi and bacteria using aseptic techniques [9]. The Radial Growth Method and Paper-Disc plate method were employed to evaluate the antifungal and antibacterial activities, respectively [10].

Antifungal Activity

A culture of test fungus was grown on PDA medium (glucose, starch, agar-agar and 1000 ml of H_2O) at 25±2 °C and the compounds after being dissolved in 50, 100 and 200 ppm concentrations in methanol were mixed in the medium. The linear growth of the fungus was obtained by measuring the diameter of the colony in petriplates after four days, and the percentage inhibition was calculated by the following relationship : % inhibition = $(C-T)\times C^{-1}$, where C and T are the diameters of the fungus colony in check and test plate, respectively.

Antibacterial Activity

The nutrient ager medium (peptone, beef extract, agar-agar and NaCl) and 5 mm diameter paper discs of Whatman No.

Table 1 Physical properties of the ligands

Ligand		Colour	m.p. (°C)
2-[{1-(Thien-2-yl)ethylidene}amino]benzenethiol	L_1H	Yellow	85
2-[{1-(Pyridin-2-yl)ethylidene}amino]benzenethiol	L_2H	Yellow	87
2-[{1-(Furan-2-yl)ethylidene}amino]benzenethiol	L_3H	Brown	84
2-[{1-(Naphthen-2-yl)ethylidene}amino]benzenethiol	L_4H	Yellow	88
2-[(Thien-2-ylmethylene)amino]benzenethiol	L_5H	Dark brown	91

Table 2 Syntheses, analyses and physical properties of difluoroboron(III) compounds

Boron compound (g)	Ligand (g)	Complex and colour	Yield (%)	m.p. (°C)	Analyses (%) C Found (Calcd.)	H Found (Calcd.)	N Found (Calcd.)	S Found (Calcd.)	B Found (Calcd.)	Mol. wt. Found (Calcd.)
$BF_3 \cdot 2AcOH$	L_1H	$BF_2(L_1)$	72	112	51.18	3.45	4.85	22.68	3.64	253
0.86	1.07	Brown			(51.26)	(3.58)	(4.98)	(22.81)	(3.85)	(281)
$BF_3 \cdot 2AcOH$	L_2H	$BF_2(L_2)$	66	129	56.71	4.11	10.21	11.72	3.98	294
1.12	1.36	Brown			(56.55)	(4.01)	(10.15)	(11.61)	(3.92)	(276)
$BF_3 \cdot 2AcOH$	L_3H	$BF_2(L_3)$	70	98d	54.42	3.92	5.36	12.15	4.12	291
0.71	0.82	Yellow brown			(54.37)	(3.80)	(5.28)	(12.10)	(4.08)	(265)
$BF_3 \cdot 2AcOH$	L_4H	$BF_2(L_4)$	64	131	66.54	4.38	4.25	9.79	3.29	340
0.69	1.02	Brown			(66.48)	(4.34)	(4.31)	(9.86)	(3.32)	(325)
$BF_3 \cdot 2AcOH$	L_5H	$BF_2(L_5)$	76	105	49.58	3.11	5.18	24.12	4.11	294
0.88	1.03	Brown			(49.46)	(3.02)	(5.24)	(24.00)	(4.05)	(267)

1 were used to evaluate bactericidal activity. The compounds were dissolved in dry methanol in 500 and 1000 ppm concentrations. The filter paper discs were soaked in different solutions of the compounds, dried and then placed in the petriplates previously seeded with the test organism. The plates were incubated for 24–30 hours at 30±1 °C and the inhibition around each disc was measured.

Results and Discussion

The 1:1 complexes of the thio-azomethines have been obtained as dark coloured solids soluble in DMSO and slightly soluble in methanol and chloroform. The complexes are monomeric in nature as indicated by the molecular weight determinations. The low molar conductance values (11–14 ohm^{-1} cm^2 mol^{-1}) reveal the non-electrolytic nature of the synthesized complexes.

U.v. spectra

The electronic spectra of benzothiazolines consist of two broad and strong bands around 270 and 310 nm, characteristic of the cyclic from of the ligands and these may be attributed to the ϕ–ϕ^* and π–π^* (benzenoid) transitions, respectively [11, 12]. These bands remain unaltered in the difluoroboron(III) complexes. An additional band in the complexes is also observed around 400 nm due to n–π^* electronic transitions of the azomethine group. This suggests the formation of azomethine grouping on complexation and subsequent isomerisation of the ligands into the azomethine form[13].

I.r. spectra

The free ligands show an NH stretching band at 3350–3150 cm^{-1}, but no bands of ν (SH) at 2600–2500 cm^{-1} and ν (C=N) at 1630–1600 cm^{-1}. This is indicative of the benzothiazoline rather than the Schiff base structure [14]. In the spectra of complexes, bands due to ν N-H vibrations disappear indicating the chelation of nitrogen with the boron atom, and a new band at $\sim$ 1600 cm^{-1} is observed, which may be assigned to >C=N vibrations. The appearance of this band suggests that the complexes are boron-Schiff base derivatives, as the benzothiazoline ring opens to give the Schiff base structure in presence of boron atom. Several new bands in the complexes in the region 1560–1535 cm^{-1}, 785–760 cm^{-1} and 1240–1210 cm^{-1} may be assigned to ν (B←N), ν (B–S) and ν (B–F) and thus lending support to the proposed coordination in the complexes [15].

^1H n.m.r. spectra

The proton magnetic resonance spectra of all the benzothiazolines and their difluoroboron(III) complexes have been recorded in Table 3. The broad signals due to NH protons in the ligands disappear in the case of complexes and thus substantiating the coordination of boron with nitrogen and sulphur. The -CH_3 protons and -CH- proton signals in the ligands undergo deshielding and appear at a downfield shifted position in the spectra of complexes, indicating the coordination of nitrogen to the boron atom. The complex multiplet for the aromatic protons also show a slight downfield shift in the spectra of difluoroboron(III) complexes.

^{13}C n.m.r. spectra

The ^{13}C n.m.r. spectra of benzothiazolines and their corresponding complexes, recorded in dry DMSO are given in Table 4. A considerable change in the chemical shift of carbons attached to nitrogen and sulphur is indicative of the role of these elements in coordination.

Table 3 ^1H n.m.r. spectral data (δ ppm) of benzothiazolines and their corresponding difluoroboron(III) complexes

Compound	-NH(s)	H–C–N/H_3C–C–N (s) or or H–C=N/H_3C–C=N	Aromatic (m)
L_1H	4.32	*	7.36–6.44
$BF_2(L_1)$	–	3.26	7.48–6.52
L_2H	5.40	3.36	7.28–6.36
$BF_2(L_2)$	–	3.42	7.40–6.40
L_3H	5.44	3.40	7.24–6.32
$BF_2(L_3)$	–	3.52	7.36–6.48
L_4H	5.52	3.42	7.32–6.40
$BF_2(L_4)$	–	3.58	7.52–6.46
L_5H	4.52	7.90	7.26–6.98
$BF_2(L_5)$	–	8.12	7.32–6.98

s = singlet, m = multiplet, * = merged with -NH proton.

Table 4 ^{13}C n.m.r. spectral data (δ ppm) of ligands and their corresponding difluoroboron(III) complexes

Compound	C-N/C=N	C-S	-CH_3	Aromatic
L_2H	151.24	139.68	13.15	123.19, 121.75, 120.40, 121.80, 125.81, 126.51, 125.73, 125.63, 125.70, 126.90
$BF_2(L_2)$	170.22	154.13	14.41	124.26, 121.54, 121.13, 121.98, 126.61, 126.64, 126.24, 125.97, 125.63, 126.65
L_3H	148.71	134.46	11.26	123.44, 120.84, 115.56, 115.18, 113.88, 124.54, 126.38, 128.82, 130.18
$BF_2(L_3)$	166.70	152.28	18.64	124.01, 120.70, 121.08, 116.31, 115.86, 122.24, 123.67, 124.00, 125.25

^{11}B n.m.r. spectra

The ^{11}B nuclear resonance is observed in the region δ 0.70–2.37 ppm (Table 5). This speaks in favour of a tetracoordinated environment [16] around the boron atom and the presence of a (B←N) dative bond. The driving force for the formation of this coordinate bond is the ability of the boron to accept a share of electrons from a suitable donor atom (nitrogen in the present case) in order to complete its outer shell of electrons and thus achieve a stable higher coordination state. This type of bonding confirms the conclusion drawn earlier on the basis of u.v., i.r., ^{1}H and ^{13}C n.m.r. spectra, regarding the coordination of azomethine nitrogen to the boron atom.

^{19}F n.m.r. spectra

The ^{19}F shifts of the BF_2 entity are found in the range between δ −142.47 to −147.16 ppm. The ^{19}F[JHz] coupling constant data have also been calculated (Table 5).

On the basis of the spectra, it can be inferred that the imines derived from heterocyclic ketones/aldehyde and 2-mercaptoaniline behave as monobasic bidentate ligands and form complexes of the following structure.

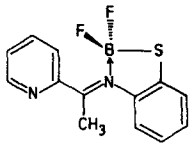


Table 5 ^{11}B and ^{19}F n.m.r. spectral data in (δ ppm) and Hz of BF_3·2AcOH and its compounds

Compound	^{11}B	^{19}F [JHz]
BF_3·2AcOH	1.19	141.45
$BF_2(L_1)$	0.70	170.77
$BF_2(L_2)$	2.04	112.22
$BF_2(L_3)$	2.37	198.99
$BF_2(L_4)$	2.28	56.86
$BF_2(L_5)$	2.37	165.89

Biological Aspects

Antifungal and Antibacterial activities of heterocyclic benzothiazolines and their corresponding difluoroboron(III) complexes against different fungi and bacteria and recorded in Tables 6 and 7.

Mode of Action

The toxicity of difluoroboron(III) complexes can well be understood by considering the chelation theory. The chelation reduces the polarity of the central ion mainly because of the partial sharing of its positive charge with the donor groups and possible π-electron delocalisation over the whole chelate ring. Such chelation increases the lipophilic character of the central atom, which subsequently favours its permeation through the lipid layer of the membrane [17].

Though, these compounds are stable in open atmosphere and sparingly soluble in H_2O but on prolonged keeping in H_2O they occupy $[BF_2{\cdot}L(OH]^-$ (LH = ligand molecule) type of structure and which is later on accumulated by the fungal cells [18]. These ions are denaturing the proteins. Enzymes are proteins and it is expected that nonmetal inactive these catalysts. However, not all enzymes are equally inactivated by low concentrations of these non-metallic complexes, therefore, low concentration seems to be less effective against growth.

In bactericidal activity, it is observed that the complexes were more toxic towards Gram (+) stain as compared to Gram (−) stain. The reason is the difference in the structures of the cell walls. The walls of Gram (−) cells are more complex than those of Gram (+) cells. The lipopolysaccharide forms an outer lipid membrane and contributes to the complex antigenic specificity of the Gram (−) cells. On comparison with the conventional bactericide, Streptomycin, all of the ligands and their complexes were found to be more toxic for the

Table 6 Fungicidal screening data of benzothiazolines and their respective difluoroboron(III) complexes. Average percentage inhibition after 96 hours

Compound	*Macrophomina phaseolina*			*Fusarium oxysporum*			*Aspergillus niger*		
	conc. in ppm.								
	50	100	200	50	100	200	50	100	200
L_1H	23	31	52	31	35	41	38	46	58
$BF_2(L_1)$	32	56	68	41	60	74	47	62	78
L_2H	28	37	52	24	35	58	30	41	54
$BF_2(L_2)$	41	52	73	40	64	76	46	60	80
L_3H	15	24	40	17	27	52	23	31	50
$BF_2(L_3)$	23	40	58	29	37	69	34	47	70
L_4H	28	43	56	40	52	64	42	58	69
$BF_2(L_4)$	46	62	75	64	74	82	66	79	86
L_5H	28	52	67	29	41	64	30	54	68
$BF_2(L_5)$	32	65	75	35	62	82	42	69	84
Bavistin	82	100	100	86	100	100	91	100	100

Table 7 Bactericidal screening data of benzothiazolines and their respective difluoroboron(III) complexes. Inhibition after 24 hours

Compound	Diameter of inhibition zone (mm)							
	Staphylococcus aureus (+)		*Klebsiella aerogenous (–)*		*Escherichia coli (–)*		*Pseudomonas cepacicola ( )*	
	500	1000	500	1000	500	1000	500	1000
L_1H	6	9	4	5	4	8	5	8
$BF_2(L_1)$	10	13	6	8	5	12	7	10
L_2H	6	8	3	5	5	7	4	6
$BF_2(L_2)$	7	10	5	6	8	10	6	9
L_3H	4	6	2	3	3	4	2	3
$BF_2(L_3)$	5	8	3	4	4	6	3	5
L_4H	7	9	4	7	5	8	4	7
$BF_2(L_4)$	9	14	8	11	6	12	8	11
L_5H	6	9	6	8	4	8	5	9
$BF_2(L_5)$	10	13	10	12	9	13	9	13
Streptomycin	15	17	3	5	1	2	2	3

Gram (–) stain. On the contrary, for Gram (+) stain none of the ligands nor their complexes reached the toxicity level of the standard. However, it can be inferred that the complexes were more toxic than the ligands and their toxicity for Gram (–) stain is remarkable.

In fungicidal activity, it is observed that through the bioactivity increased on undergoing complexation, but it could not reach the efficacy/effectiveness of the conventional fungicide, Bavistin, at lower concentration. However, at higher ppm concentration, the results achieved were satisfactory

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