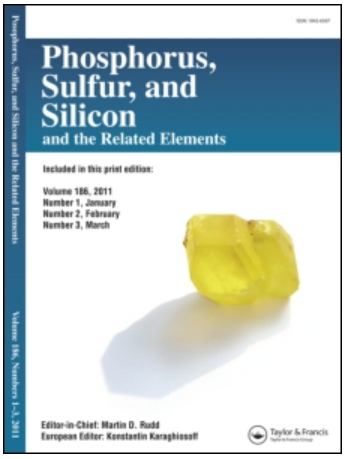

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BIOCHEMICAL BEHAVIOR OF THIO-AZOMETHINE DIFLUORO-BORON COMPOUNDS

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BIOCHEMICAL BEHAVIOR OF THIO-AZOMETHINE DIFLUORO-BORON COMPOUNDS

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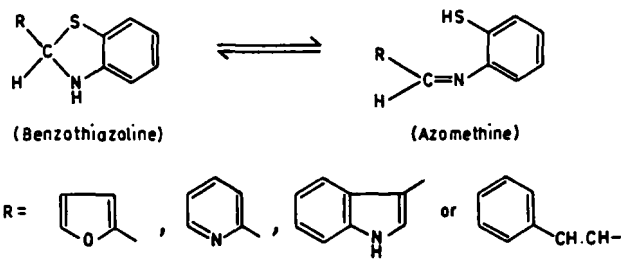
Fluoroboron(III) complexes of the azomethines derived from heterocyclic aldehydes and 2-mercaptoaniline have been characterized on the basis of I.R., ^{1}H, ^{13}C, ^{11}B and ^{19}F N.M.R. spectral studies along with elemental analyses, conductivity measurements and molecular weight determinations. The equimolar reactions between borontrifluoride diacetic acid and these thio-azomethines have afforded biologically active (azomethine) BF_2 compounds in which the central atom appears to be in a tetracoordinated state. These thio-azomethines along with their complexes have been screened in vitro against a number of pathogenic fungi and bacteria to assess their growth inhibiting potential.

Key words: Thio-azomethines; fluoroboron(III) complexes; fungicidal property; bactericidal activity; spectral data.

INTRODUCTION

The study of boron halide-adducts was initiated when Gay-Lussac and Thenard first isolated the ammonia-borontrifluoride complex ($H_3N \cdot BF_3$).[1] The use of borontrifluoride in a variety of chemical reactions has greatly encouraged explorations with these compounds. One use of such adducts has been concerned with the synthesis of heterocyclic boron-oxygen[2,3] compounds and more recently those containing boron-nitrogen[4] bonds. However, fluoroboron heterocycles containing sulfur and nitrogen in the ring are rather scarce.[5] Benzothiazolines, which are biochemically active constitute an important class of nitrogen and sulfur donor ligands.[6,7] Boron-azomethine complexes of these ligands have been found to exhibit conspicuous biocidal activity.[8]

The growing interest in the biochemical applications and greater demand for still better fungicides and bactericides have prompted us to screen all the newly synthesized fluoroboron derivatives with biologically active nitrogen and sulfur donor benzothiazolines for their antifungal and antibacterial activities. In this paper we report such studies of fluoroboron(III) complexes with the following benzothiazolines having NSH donor system:



†Author to whom all correspondence should be directed.

RESULTS AND DISCUSSION

The reactions of borontrifluoride diacetic acid with monobasic bidentate benzothiazolines were carried out in 1:1 molar ratio in dry acetic anhydride. These reactions proceed with the liberation of HF and AcOH.

$$BF_3 \cdot 2AcOH + NSH \xrightarrow{1:1} BF_2(NS) + HF + 2AcOH$$

(where NS represents the donor set of benzothiazoline)

These reactions are quite facile and can be completed in 7–10 hrs of refluxing. The resulting difluoro-boron compounds are slightly soluble in methanol and chloroform and soluble in DMSO. The compounds are monomeric and covalent in nature.

SPECTROSCOPIC STUDIES

The spectroscopic information of the benzothiazolines as well as their corresponding (thio-azomethine)BF_2 compounds is consistent with the formation of proposed structures and some important features of which may be summarized as follows.

I.R. Spectra

In the I.R. spectra of benzothiazolines, the absence of ν(SH) at 2600–2500 cm^{-1} and ν(C=N) at 1630–1600 cm^{-1} is strong evidence for a ring structure.[9] After substitution reaction, the bands at 3250–3100 cm^{-1} due to NH[10] stretching vibrations of the ligands disappear due to chelation of nitrogen to boron and a new band at ~1600 cm^{-1} is observed due to ν(C=N) vibrations. The chelation of ligands through nitrogen and sulfur are supported by the appearance of new bands at 1565–1535 cm^{-1} and at 780–760 cm^{-1} in the spectra of compounds due to ν(B←N)[11] and ν(B—S)[12] vibrations. Apart from this, the band at 1240–1210 cm^{-1} may be assigned to ν(B—F)[13] vibrations.

^{1}H N.M.R. Spectra

The ^{1}H N.M.R. spectra of benzothiazolines and their (thio-azomethine)BF_2 compounds have been recorded in DMSO-d_6 (Table I) using TMS as an internal standards.

The disappearance of —NH proton signals from the ^{1}H N.M.R. spectra of fluoroboron compounds clearly indicates the deprotonation of the NH group after substitution reaction. Further, the complex multiplet for the aromatic protons shows a slight downfield shifting in the spectra of fluoroboron compounds. This shift supports the coordination of the nitrogen of the azomethine group to the boron atom. The change in the positions of the H—C=N protons of the benzothiazolines

in the spectra of compounds is a strong evidence of the formation of a coordinate linkage between nitrogen and boron.

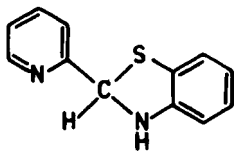
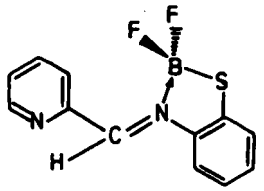
TABLE I

^{1}H N.M.R. spectral data (δ, ppm) of ligands and their (thio-azomethine)BF_2 compounds

Compound	-NH(S)	H-C-N/H-C=N(S)	Aromatic (m)
Furf-2-ald.BztH	4.34	7.92	7.20-6.82
(Furf-2-ald.Bzt)BF_2	-	8.06	7.34-6.88
Pyd-2-ald.BztH	4.72	7.84	7.10-6.74
(Pyd-2-ald.Bzt)BF_2	-	7.96	7.24-6.88
Ind-3-ald.BztH	4.41	7.83	7.40-7.08
(Ind-3-ald.Bzt)BF_2	-	7.98	7.52-7.26
Cin ald.BztH	4.68	7.88	7.32-6.96
(Cin ald.Bzt)BF_2	-	8.02	7.44-7.10

TABLE II

^{13}C N.M.R. spectral data (δ, ppm) of 2-[(2-Pyridinylmethylene)-amino] benzenethiol and its (thio-azomethine)BF_2 compound

Compound	C-N/C=N	C-S	Aromatic Carbons
	149.57	136.92	126.77 125.42 125.61 125.95 126.17 125.52 121.19 120.64 121.89 123.46
	168.37	148.87	126.84 125.51 125.73 125.98 126.74 125.68 121.23 120.75 121.91 123.54

^{13}C N.M.R. Spectra

The ^{13}C N.M.R. spectra of one benzothiazoline and its corresponding (thio-azomethine)BF_2 compound were recorded in dry DMSO (Table II). The shift in the position of the carbons attached to S and N indicate their coordination with the boron atom.

TABLE III

^{11}B and ^{19}F N.M.R. spectral data (δ, ppm) of $BF_3 \cdot 2AcOH$ and its (thio-azomethine)BF_2 compounds

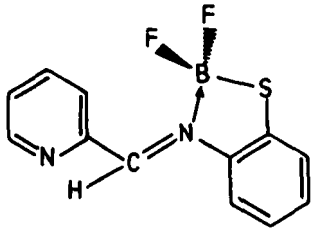
Compound	^{11}B	^{19}F
$BF_3.2AcOH$	1.19	-144.21
(Furf-2-ald.Bzt)BF_2	2.04	-159.15
(Pyd-2-ald.Bzt)BF_2	0.58	-146.76
(Ind-3-ald.Bzt)BF_2	0.86	-144.67
(Cin ald.Bzt)BF_2	2.24	-147.39

^{11}B and ^{19}F N.M.R. Spectra

The ^{11}B and ^{19}F N.M.R. spectra of $BF_3 \cdot 2AcOH$ and its (thio-azomethine)BF_2 compounds, recorded in DMSO-d_6 using $BF_3 \cdot Et_2O$ and C_6F_6 as external standards for boron and fluorine, respectively, are listed in Table III.

The ^{11}B N.M.R. spectra give signals in the region δ 0.58 to 2.24 ppm and the ^{19}F shifts of the BF_2 entity are found in the range between δ −144.67 to −159.15 ppm. Both values are in good agreement with previously reported values,[14-16] indicating coordination number four and presence of fluorine atoms, in the difluoroboron compounds around the boron atom. Nöth and coworkers have also reported similar data for a number of boron complexes with N and S donor ligands.[17]

Based on the foregoing studies, it can be deduced that imines derived from heterocyclic aldehydes and 2-mercaptoaniline behave as monobasic bidentate ligands. The tentative structure of the difluoroboron complex with tetracoordinated environment around boron atom and with 2-[(2-Pyridinylmethylene)-amino]benzenethiol as the ligand molecule is shown below:



MICROBIAL ASSAY

Fungicidal and bactericidal activities of heterocyclic benzothiazolines and their corresponding (thio-azomethine)BF_2 compounds against different fungi and bacteria have been recorded in Tables IV and V by methods reported earlier.[11]

TABLE IV

Fungicidal screening data of ligands and their (thio-azomethine)BF_2 compounds

Compound	Average percentage inhibition after 96 hours					
	Macrophomina phaseolina			Fusarium oxysporum		
	Conc. in ppm					
	50	100	200	50	100	200
Furf-2-ald.BztH	23	32	58	29	37	55
(Furf-2-ald.Bzt)BF_2	32	52	65	41	60	69
Pyd-2-ald.BztH	27	42	52	23	35	51
(Pyd-2-ald.Bzt)BF_2	34	57	65	31	47	66
Ind-3-ald.BztH	35	47	56	34	41	60
(Ind-3-ald.Bzt)BF_2	52	68	75	55	64	86
Cin ald.BztH	27	32	48	30	51	60
(Cin ald.Bzt)BF_2	40	52	67	47	64	80

TABLE V

Bactericidal screening data of ligands and their (thio-azomethine)BF_2 compounds

Compound	Diameter of inhibition zone after 24 hours (mm)					
	Pseudomonas cepacicola(-)		Klebsiella aerogenous(-)		Escherichia coli(-)	
	Conc. in ppm					
	500	1000	500	1000	500	1000
Furf-2-ald.BztH	2	4	4	6	5	8
(Furf-2-ald.Bzt)BF_2	4	5	6	7	7	11
Pyd-2-ald.BztH	3	4	5	6	3	7
(Pyd-2-ald.Bzt)BF_2	5	7	7	9	5	9
Ind-3-ald.BztH	7	10	5	8	4	6
(Ind-3-ald.Bzt)BF_2	9	13	7	11	5	9
Cin ald.BztH	5	8	5	7	4	5
(Cin ald.Bzt)BF_2	8	10	7	9	6	8

Mode of Action

It is seen that the compounds are inhibiting the growth of fungi and bacteria to a greater extent as the concentration is increased. Potato dextrose media (PDA) rich

in carbohydrates serves as the major nutrient source and is utilized by the fungus or bacteria with the help of various enzymes (viz. amylase, pectinase, cellulase etc.). These extracellular enzymes[18] secreted by these microorganisms diffuse out from the membrane of fungus or bacteria into the medium and lead to the breakdown of complex polysaccharides into simpler monosaccharides. Further, these enzymes turn complex protein into simpler proteins. These are then utilized by the respective bacteria or fungus.

Since the complexes are inhibiting the growth of microorganisms, it is assumed that the complexes are affecting the production of these enzymes. As a result of it the fungus or bacteria is unable to draw nutrition for itself or the intake of nutrients in simpler, suitable forms is decreased and consequently the growth ceases. At lower concentrations when the enzyme leaches out, the growth of microorganism is arrested. Though the enzyme production is being affected, the little amount produced is sufficient to meet the need of the microorganism to grow. Higher concentration proves toxic or fatal to the microorganisms. The higher concentration destroys the enzyme mechanism by blocking any of the metabolic pathway (viz. lipid, carbohydrate, amino acid etc.) and due to the lack of availability of proper food the organism dies.

The mechanism of toxicity of these complexes to microorganisms may also be due to the inhibition of energy production or ATP production[19]; for instance by inhibition of respiration or by uncoupling of oxidative phosphorylation. The energy producing processes are located partly in the cytoplasm and partly in the mitochondria. Strong inhibition of such processes will eventually have a fungicidal and bactericidal effect.

Fungal and bacterial cells accumulate the water soluble non-metal complex which later on dissociates to give free non-metal or non-metal-complex ion. These non-metal ions are denaturing the proteins. Enzymes are proteins and it is expected that the central atoms deactivate these catalysts. However, not all enzymes are equally deactivated by low concentrations of these complexes, therefore, low concentration seems to be less effective against growth.

The enzymes which require free sulfhydryl (—SH) groups for activity appear to be especially susceptible to deactivation by ions of the non-metal. Due to greater lipoid solubility, complexes facilitate their diffusion through the spore membrane to the site of action within spores and ultimately killing them by combining with sulfhydryl (—SH) groups of certain cell enzymes.[20] As the non-metal boron preferentially binds to —SH group, it is logical to assume that the complexes screened are involved in competitive equlibria involving the —SH group of the cell enzyme on one hand and the coordinated ligand on the other. If this were the case, complexes of softer acids which are expected to bind to the —SH group of the cell enzymes more strongly should have lower MIC (minimum inhibitory concentration) values than complexes of relatively harder acids. Boron trifluoride moiety being the harder acid shows leser bioactivity.

In general the greater fungitoxicity of the sulfur containing compounds suggests the better suitability and possibility of organic sulfur compounds as fungicides. The better fungitoxicity than bacteriotoxicity of these compounds may be ascribed to the lipophilic nature.

On the basis of these studies, it may be concluded that fungitoxicity and bac-

teriotoxicity of a compound may be significantly enhanced on chelation with the metal ion (Boron in the present case). The softer the metal ion coordinated, the more effective is the resulting complex as a toxic agent.

EXPERIMENTAL

All the chemicals were dried and purified before use and the reactions were carried out with a ratiohead (distillation assembly), fitted with condenser and protected from moisture using fused $CaCl_2$ drying tubes.

Preparation of ligands. The ligands are prepared by the procedure reported in earlier.[21]

Synthesis of (thio-azomethine)BF_2 compounds. The (thio-azomethine)BF_2 compounds were prepared by reaction of borontrifluoride diacetic acid with the ligands in 1:1 molar ratio in the presence of dry acetic anhydride. The mixture was refluxed for 7–10 hrs and the reaction proceeded smoothly with the elimination of HF and AcOH. The resulting products were recrystallised from methanol-ether (1:1) mixture and finally dried in vacuo. The important physical properties and analytical data of difluoro-boron complexes are given in Table VI.

Analytical methods and physical measurements. Nitrogen and sulfur were estimated by 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 with a conductivity bridge type 304 systronics model and the molecular weights were determined by Rast-Camphor method. Infrared spectra with KBr optics were obtained using Perkin-Elmer 557 grating Spectrophotometer. 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 using TMS as the internal reference for ^1H and ^{13}C N.M.R. and $BF_3 \cdot Et_2O$ and C_6F_6 as the external reference for ^{11}B and ^{19}F N.M.R., respectively.

Biological screening. The synthesized ligands and their (thio-azomethine)BF_2 compounds were tested for the in vitro growth inhibitory activity against pathogenic fungi, viz., Macrophomina phaseolina, Fusarium oxysporum and bacteria, viz., Pseudomonas cepacicola, Klebsiella aerogenous and Escherichia coli. Proper temperature, necessary nutrients and growth media free from other microorganisms were employed for the preparation of cultures of fungi and bacteria using aspetic techniques.[22]

TABLE VI

Analytical data and physical properties of (thio-azomethine)BF_2 compounds

Product formed and colour	M.P. (°C)	Yield (%)	Analyses (%) B Found (Calcd.)	N Found (Calcd.)	S Found (Calcd.)	Mol.wt. Found (Calcd.)
(Furf-2-ald.Bzt)BF_2 Brown	79-81d	67	4.30 (4.31)	5.55 (5.58)	12.84 (12.77)	225 (251)
(Pyd-2-ald.Bzt)BF_2 Dark brown	115-117	72	4.08 (4.12)	10.64 (10.69)	12.19 (12.23)	231 (262)
(Ind-3-ald.Bzt)BF_2 Dark brown	124-125	74	3.58 (3.60)	9.36 (9.33)	10.72 (10.68)	335 (300)
(Cin ald.Bzt)BF_2 Dark brown	86-89d	65	3.75 (3.77)	4.82 (4.88)	11.13 (11.17)	244 (287)

Antifungal activity. The fungi were grown in agar medium (glucose, starch, agar-agar and 1000 ml of water) 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 colony in petriplates after four days and the percentage inhibition was calculated by the relationship (C—T) × 100 × C $^{-1}$. C and T are the diameters of the fungus colony in control and test plate, respectively.

Antibacterial activity. The bactericidal activity was evaluated by the paper-disc plate method. The nutrient agar medium (peptone, Beef extract, NaCl and agar agar) and 5 mm diameter paper discs of Whatman No. 1 were used. 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 hrs at 30 ± 1°C and the inhibition around each disc was measured.

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