Syntheses, Characterization, and Antimicrobial Screening of N-(benzothiazol-2-yl)benzenesulphonamide and its Cu(I), Ni(II), Mn(II), Co(II), and Zn(II) Complexes

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Abstract: N-(benzothiazol-2-yl)benzenesulphonamide (BS2ABT) was synthesized by the condensation (by refluxing) of 2-aminobenzothiazole and benzenesulphonylchloride in acetone at 140°C. The resulting crude precipitates were recrystallized from dimethylformamide (DMF). Five metal complexes of copper(I), nickel(II), manganese(II), cobalt(II) and zinc(II) of the ligands were synthesized. The compounds were characterized using magnetic susceptibility measurements, UV/VIS spectrophotometry, infra red, proton and ¹³C nmr spectroscopies. The antimicrobial tests of the ligands and its metal complexes were carried out on both multi-resistant bacterial strains isolated under clinical conditions and cultured species using agar-well diffusion method. The multi-resistant bacterial strains used were *Escherichia coli*, *Proteus* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus* which were isolated from dogs. The culture species were *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia Coli* (ATCC 25922) *Staphylococcus aureus* (ATCC 25923), and the fungi, *Candida krusei* (ATCC 6258) and *Candida albicans* (ATCC 90028). The tests were both *in vitro* and *in vivo*. Thus the Inhibition Zone Diameter (IZD), the Minimum Inhibitory Concentration (MIC), and the Lethal and Effective Concentrations (LC₅₀ and EC₅₀) were determined. The antimicrobial activities of the compounds were compared with those of Ciprofloxacin and trimethoprim-sulphamethoxazole as antibacterial agents and Fluconazole as an antifungal drug. All the compounds showed varying activities against the cultured typed bacteria and fungi used. However, they were less active than the standard drugs used except Fluconazole which did not show any activity against *Candida krusei* (ATCC 6258) but most of the compounds synthesized were very active against it. The Lethal Concentration (LC₅₀) ranged from 26.25±4.9-1833.88±186.92 ppm. These are within the permissible concentrations.

Key words: N-(benzothiazol-2-yl)benzenesulphonamide, Metal complexes, Antimicrobial.

Introduction

Interest in the coordination chemistry of thiazole and its derivatives with metal ions has risen due to the important role they play in biological systems¹. It is known that metal chelates of ligands with sulphur or nitrogen donor atoms have interesting physicochemical properties as well as physiological activities^{2,3}. Mixed ligand complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) derived from 1,10-phenanthroline and *o*-vanillidene-2-aminobenzothiazole, and Schiff base metal complexes of *o*-vanillidene-2-amino-N-(2-pyridyl)-benzene sulfonamide have been studied. The metal complexes show more potent activities compared with Schiff base ligands⁴. Studies have shown that the metal complexes of sulfa drugs promote rapid healing of skin disorder, for instance, silver(I)sulfadiazine complex is used for human burnt treatment and zinc(II)sulfadiazine in preventing bacterial infections in burnt animals⁵. Mercury(II), and copper(II) complexes of 6-methyl-2-aminobenzothiazole have equally been discovered to show high activity against *Aspergillus niger*, *Alternaria alternate*, *Curvularia plunata*, and *Penicillium fumculorus*⁶. Furthermore, mercury(II) complex of sulfathiazole has been discovered to show high antibacterial activity against *Escherichia Coli*⁷ which causes sepsis and also diarrhea in humans. Obasi *et al* worked on some sulfonyl derivatives of 2-aminothiazole, and the results obtained showed that the compounds were significantly active against *Staphylococcus aureaus* and *Escherichia coli*⁸. Some novel N-(Benzothiazol-2-yl)ethanamides were also synthesized and characterized by Obasi *et al.*, and were screened *in vitro* and *in vivo* for antibacterial activity. The compounds were very stable and showed high antibacterial activities against both gram-positive and gram-negative bacteria tested⁹. The present work is aimed at synthesizing new derivative of 2-aminobenzothiazole and its metal(II) complexes, characterizing them, and investigating how their structural differences affects antimicrobial activities when compared with conventional sulfonamides.

Experimental

Reagents and Apparatus

The ligand, N-(benzothiazol-2-yl)benzenesulphonamide (BS2ABT) was prepared based on our modified method from that by Sprague *et al*¹⁰. All reagents were of analytical grade and were used as supplied except otherwise stated. UV-Visible spectra of the ligand and complexes were obtained on UV-2550 UV-VIS Spectrophotometer, (SHIMADZU). FTIR spectra of the compounds were run as Nujol mulls on FTIR-84005 FTIR Spectrophotometer, (SHIMADZU). ¹³C and ¹H NMR spectra were recorded on Bruker-BioSpin 500 MHz NMR Spectrometer (UK) using DMSO and CDCl₃ as solvents respectively. The proton NMR peaks were observed at 500 MHz whereas the carbon-13 spectra were observed at 125 MHz. The magnetic susceptibility of the complexes were determined using Sherwood Scientific Magnetic Susceptibility Balance, Mk1 Model (Cambridge, UK) at Department of Pure and Applied Chemistry, University of Strathclyde, Scotland, UK.

Synthesis of N-(benzothiazol-2-yl)benzenesulphonamide (BS2ABT)

To a solution of 2-aminobenzothiazole (6.0 g; 40 mmole) in acetone (25ml) was added benzenesulphonylchloride (40ml; 40 mmole) with stirring. The mixture was refluxed for 1hr at 140°C. During the refluxing a pinkish white precipitate was formed. The precipitate was recrystallised in DMF.

Synthesis of Bis[N-(benzothiazol-2-yl)benzenesulphonamide]nickel(II) ion (Ni(II)BS2ABT)

To a solution of N-(benzothiazol-2-yl)benzenesulphonamide (0.73 g; 3.00 mmole) in DMF (10ml) was added aqueous solution of nickel(II) chloride hexahydrate (0.28 g; 1.00 mmole). This was refluxed for 30mins at 120°C during which a white crystalline solid was formed. This was filtered and dried in a stream of air and stored in desiccator.

Scheme 2. Synthesis of Ni(II)BS2ABT.

Synthesis of Bis[N-(benzothiazol-2-yl)benzenesulphonamide]dichloro manganese(II) (Mn(II)BS2ABT)

To a solution of N-(benzothiazol-2-yl)benzenesulphonamide (0.73 g; 3.00 mmole) in DMF (10ml) was added aqueous solution of manganese(II) chloride tetrahydrate (0.20g; 1.00 mmole). This was refluxed for 30mins at 120°C during which a white shiny crystalline solid was formed. This was filtered and dried in a stream of air and stored in desiccator.

Synthesis of Bis[N-(benzothiazol-2-yl)benzenesulphonamide]dichloro cobalt(II) (Co(II)BS2ABT)

To a solution of N-(benzothiazol-2-yl) benzenesulphonamide (0.73 g; 3.00 mmole) in DMF (10ml) was added aqueous solution of cobalt(II) chloride hexahydrate (0.30 g; 1.00 mmole). This was refluxed for 30mins at 120°C during which a shiny blue precipitate was formed. This was filtered and dried in a stream of air and stored in desicator.

Synthesis of Bis[N-(benzothiazol-2-yl)benzenesulphonamide] zinc(II)chloride (Zn(II)BS2ABT)

To a solution of N-(benzothiazol-2-yl)benzenesulphonamide (0.73 g; 3.00 mmole) in DMF (10ml) was added aqueous solution of zinc(II) acetate dihydrate (0.29 g; 1.00 mmole). This was refluxed for 30mins at 120°C during which a white precipitate was formed. This was filtered and dried in a stream of air and stored in desicator.

Scheme 5. Synthesis of Zn(II)BS2ABT.

Synthesis of Bis [N-(benzothiazol-2-yl)benzenesulphonamide]copper(I) chloride (Cu(I)BS2ABT)

To a solution of N-(benzothiazol-2-yl)benzenesulphonamide (0.73 g; 3.00 mmole) in DMF (10ml) was added aqueous solution of copper(II) chloride dihydrate (0.25 g; 1.00 mmole). This was refluxed for 30mins at 120°C during which a dirty white precipitate was formed. This was filtered and dried in a stream of air and stored in desicator.

Scheme 6. Synthesis of Cu(II)BS2ABT.

Antimicrobial properties

In vitro Tests

Multi-resistant bacterial strains isolated under clinical conditions and Typed strains (ATCC Cultures) were used in the study. The bacterial strains used were *Escherichia coli* strains (*E. Coli* Strain 1 and *E. Coli* Strain 15), *Proteus* species strains (*Proteus* spp strains 25, *Proteus* spp strains 26), *Pseudomonas aeruginosa* strains 34 and multi-resistant *Staphylococcus aureus* (SR) strain. The bacteria Typed strains (ATCC Cultures) used were *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia Coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923). Fungi Typed strains (ATCC Cultures) used were *Candida Krusei* (ATCC 6258) and *Candida albicans* (ATCC 90028). The Typed strains were obtained from Bioresources Development and Conservation Program (BDGP), International Centre for Ethnomedicine and Drug Development (IntaceEED), Nsukka, Nigeria.

The antibacterial and antifungal activities of the ligand, BS2ABT, and its complexes, Ni(II)BS2ABT, Mn(II)BS2ABT, Co(II)BS2ABT, Zn(II)BS2ABT, Cu(I)BS2ABT against these multi-resistant bacteria were determined using the agar well diffusion method as described by Chah *et al.*¹¹. Mueller-Hinton agar plates were inoculated with 0.1 ml of 3 hr broth culture of the test bacteria. Using a cork borer, wells (7 mm in diameter and 2.5 mm deep) were bored into the inoculated agar. The test compounds were solubilised in 20 % v/v dimethyl sulfoxide (DMSO) and 0.05 ml of each compound at a concentration of 20 mg/ml were delivered into the wells. One of the wells contained 20% v/v DMSO and served as control. The plates for antibacterial screening were incubated at 37°C for 24 hours while the fungi were incubated at 30°C for 48 hrs and assessment of activity was based on the measurement of the diameter of inhibition zone (IZD) around the wells. The test was performed in triplicates, mean IZD was recorded to the nearest whole millimetre.

The minimum inhibitory concentrations (MICs) of the test compounds were determined using the agar dilution method as described by Ojo *et al.*¹². Two-fold serial dilutions of test compounds were made in 20% v/v DMSO. One millilitre of each serial dilution was added to 19 ml of sterile Mueller-Hinton agar maintained at 45°C, thoroughly mixed and poured into a sterile plate and the medium allowed to solidify. The final concentrations of the compounds ranged from 20 mg/ml to 1.25 mg/ml. Amended media were incubated overnight at 37°C to check for sterility. Overnight nutrient broth cultures of the test bacteria were adjusted to contain approximately 10⁸cfu/ml and 0.025 ml of each of the test organisms was spot-inoculated on the amended culture media. Inoculated plates were incubated at 37°C for 24 hours and observed for presence of visible growth. The minimum inhibition concentration was determined as the value of the lowest concentration that completely suppressed growth of the organisms.

In vivo Tests [Brine Shrimps Lethality Test (BSLT)]

The method of McLaughlin and coworkers was used to study the bioactivity of the synthesized compounds¹³. *Artemia salina* eggs obtained from a pet shop in Davis California was incubated in natural sea water (from Bar Beach, Lagos, Nigeria) in a dam-well under room condition. About ten (10) 48h- shrimp nauplii in 1mL of autoclaved sea water were put into Bijou bottles using a Pasteur pipette under a stereo-microscope with a light source. They were separated into 7 groups in triplicate. Increasing concentrations (10, 100, 1000 ppm) of the synthesized compounds were added into each of the triplicate and distilled water was added into the control group. The nauplii were incubated at room temperature (37°C) for 24h after which the survivors in each well were counted. The results were analysed using Finney Probit Analysis (MS-DOS-Computer-Program) to determine the LC₅₀ at 95% confidence interval. Weak nauplii were noted as an indication of central nervous system depression.

Results and Discussion

The equations of reactions for the syntheses of the ligand, BS2ABT and its metal complexes are represented in Schemes 1-6.

Physical Properties of the Compounds

Table 1 shows some physical properties of both ligand and its complexes. The melting point of the ligand, BS2ABT is 130-132°C while those of the complexes range from 181-271 °C. The ligand and its copper complex are dirty white, the nickel and zinc complexes are white, while the manganese and cobalt complexes are shiny white and shiny blue respectively. The ligand, BS2ABT is amorphous; its nickel, manganese and cobalt complexes are crystalline while the copper complex is powdery.

Electronic Spectra

The electronic transition result of the compounds synthesized are recorded in Table 2. Two bands were observed for the ligand at 201 nm and 264 nm. They are due to $\pi \rightarrow \pi^*$ transitions.

Nickel Complexes of all the Ligand

For most octahedral and tetrahedral Ni(II) complexes, three bands are expected, however for some tetrahedral Ni(II) complexes like $[\text{NiL}_4]^{2-}$ two broad bands may be found^{14, 15}. Thus three bands were observed for Ni(II) complex of BS2ABT (20,700 cm^{-1} , 31,750 cm^{-1} , and 33,160 cm^{-1}), and the transitions are assigned as follows;

$$\begin{aligned} \nu_1 &= {}^3\text{T}_2(\text{F}) \leftarrow {}^3\text{A}_2(\text{F}) \\ \nu_2 &= {}^3\text{T}_1(\text{F}) \leftarrow {}^3\text{A}_2(\text{F}) \\ \nu_3 &= {}^3\text{T}_1(\text{P}) \leftarrow {}^3\text{A}_2(\text{F}) \text{ transitions,} \end{aligned}$$

Manganese Complexes of all the Ligand

Three bands were observed for the Mn(II) complex synthesized (13,620 cm^{-1} , 20,560 cm^{-1} , and 37,740 cm^{-1}), the transitions are assigned:

$$\begin{aligned} \nu_1 &= {}^2\text{T}_{1g}(\text{H}) \leftarrow {}^2\text{A}_{1g} \\ \nu_2 &= {}^2\text{E}_g(\text{H}) \leftarrow {}^2\text{A}_{1g} \text{ transitions of octahedral geometry.} \end{aligned}$$

Cobalt Complexes of the Ligand

In a cubic field, three spin-allowed transitions are anticipated because of the splitting of the free-ion ground ${}^4\text{F}$ term and the accompanying ${}^4\text{P}$ term. Of course it is essentially a 2-electron transition from $t_{2g}^5 e_g^2$ to $t_{2g}^3 e_g^4$. Two bands were observed for the Cobalt complex (11,460 cm^{-1} and 13,610 cm^{-1}), it is assigned:

$$\begin{aligned} \nu_1 &= {}^2\text{T}_{2g}(\text{H}) \leftarrow {}^2\text{T}_{1g}(\text{H}) \\ \nu_2 &= {}^2\text{A}_{2g}(\text{H}) \leftarrow {}^2\text{T}_{1g}(\text{H}) \text{ transitions of octahedral geometry.} \end{aligned}$$

Zn(II) Complexes of the Ligand

Three bands were observed for the Zn(II) complex synthesized, they are probably due to Metal-Ligand Charge Transfer (MLCT) transition. We deduced a tetrahedral geometry for the Zn(II) complex.

Table 1. Physical properties of the ligand, BS2ABT and of its metal Complexes, and the Magnetic Properties of the Complexes.

S/n	SAMPLES	Melting Point (°C)	Colour	Texture	Mw	μ_{eff} (BM)	No. of Electrons	Properties
1	BS2ABT	130-132	Dirty white	Amorphous	290.00	-	-	-
2	Ni(II)BS2ABT	256-258	White	Crystalline	709.70	2.88	2	Paramagnetic
3	Mn(II)BS2ABT	243-245	Shiny white	Crystalline	706.00	1.79	1	Paramagnetic
4	Co(II)BS2ABT	269-271	Shiny blue	Crystalline	710.00	2.28	1	Paramagnetic
5	Zn(II)BS2ABT	181-183	White	Crystalline	645.40	0.67	0	Diamagnetic
6	Cu(I)BS2ABT	184-186	Dirty white	Powdery	679.00	1.13	0	Diamagnetic

Table 2. UV/Visible spectral Result of BS2ABT and its complexes.

Samples	λ_{max} (nm)	ν_1 (cm^{-1}) I_1	ν_2 (cm^{-1})	ν_3 (cm^{-1})	$10^{-3}\epsilon_1$	$10^{-4}\epsilon_2$	$10^{-2}\epsilon_3$	Assignment
BS2ABT	201 264				1.82	1.99		$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$
Ni(II)BS2ABT	483.0 315.0 301.5	20 700	31 750	33 160	36.9	83.5	8.81	$\nu_1 = {}^3T_2(F) \leftarrow {}^3A_2(F)$ $\nu_2 = {}^3T_1(F) \leftarrow {}^3A_2(F)$ $\nu_3 = {}^3T_1(P) \leftarrow {}^3A_2(F)$
Mn(II)BS2ABT	734.0 486.5 265.0	13 620	20 560	37 740	20.7	25.0	17.9	$\nu_1 = {}^2T_{1g}(H) \leftarrow {}^2A_{1g}$ $\nu_2 = {}^2E_g(H) \leftarrow {}^2A_{1g}$
Co(II)BS2ABT	872.5 735.0	11 460	13 610		19.6	1.85		$\nu_1 = {}^2T_{2g}(H) \leftarrow {}^2T_{1g}(H)$ $\nu_2 = {}^2A_{2g}(H) \leftarrow {}^2T_{1g}(H)$
Zn(II)BS2ABT	302.0 297.5 291.0	33 110	33 610	34 360	1487	151	15.4	MLCT
Cu(I)BS2ABT	896.5 734.5	11 150		13 620	25.7		0.13	MLCT

Table 4. ¹H NMR Spectra of the BS2ABT and of its complexes in ppm.

BS2ABT	Ni(II)BS2ABT	Mn(II)BS2ABT	Co(II)BS2ABT	Zn(II)BS2ABT	Cu(II)BS2ABT	Assignments
9.73(1H, s)	-	-	-	8.32(1H, s)	8.32(1H, s)	N-H protons
7.26 (5H, m)	-	-	-	7.31(5H, m)	7.31(5H, m)	Phenyl protons
7.88 (4H, m)	-	-	-	7.80(4H, m)	7.80(4H, m)	Benzothiazole protons

Table 5. ¹³C NMR Spectra of the BS2ABT and of its complexes in ppm.

BS2ABT	Ni(II)BS2ABT	Mn(II)BS2ABT	Co(II)BS2ABT	Zn(II)BS2ABT	Cu(II)BS2ABT	Assignments
136.9	167.6	167.6	167.6	167.6	167.6	Benzothiazole carbon (C1)
129.8	136.8	136.7	132.9	132.8	136.7	Benzothiazole carbon (C2)
133.1	142.6	142.5	142.6	142.7	142.5	Benzothiazole carbon (C3)
128.4	129.7	133.0	129.7	132.2	129.7	Benzothiazole carbon (C4)
126.0	125.3	127.8	125.4	127.6	126.1	Benzothiazole carbon (C5)
125.0	124.2	126.4	124.2	126.3	125.3	Benzothiazole carbon (C6)

Table 6a. Antimicrobial activity of the ligand and of their metal complexes against multi-resistant bacterial strains isolated under clinical conditions.

Multi-resistant bacterial strains isolated from clinical conditions															
S/n	Samples	strains				Escherichia coli		Proteus species strains				Pseudomonas aeruginosa strains 34		multi-resistant Staphylococcus aureus (SR) strain	
		E. Coli Strain 1		E. Coli Strain 15		Proteus spp strains 25		Proteus spp Strains 26							
		IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)
1	BS2ABT	00	00	10	10	00	00	00	00	00	00	10	10	00	00
2	Ni(II)BS2ABT	00	00	00	00	00	00	00	00	00	00	00	00	00	00
3	Mn(II)BS2ABT	00	00	00	00	00	00	00	00	00	00	00	00	00	00
4	Co(II)BS2ABT	00	00	00	00	00	00	00	00	00	00	00	00	00	00
5	Zn(II)BS2ABT	00	00	00	00	00	00	00	00	00	00	00	00	00	00
6	Cu(I)BS2ABT	00	00	00	00	00	00	00	00	00	00	00	00	00	00
7	Ciprofloxacin	00	0.05	00	0.05	00	0.05	25	0.05	27	0.05	00	0.05	00	0.05
8	Trimethoprim-sulphamethoxazole		0.025		0.025		0.025		0.025		0.025		0.025		0.025

Table 6b. Antimicrobial activity of the compounds against Typed Strains (ATCC Cultures) microorganisms.
Typed strains (ATCC Cultures)

S/n	Samples	<i>Pseudomonas aeruginosa</i> (ATCC 27853)		<i>Escherichia coli</i> (ATCC 25922)		<i>Staphylococcus aureus</i> (ATCC 25923)		<i>Candida krusei</i> (ATCC 6258)		<i>Candida albicans</i> (ATCC 90028)	
		IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)
1	BS2ABT	11	10	00	00	10	2.5	13	20	11	10
2	Ni(II)BS2ABT	00	00	00	00	10	5	00	00	00	00
3	Mn(II)BS2ABT	00	00	00	00	13	10	00	00	00	00
4	Co(II)BS2ABT	00	00	00	00	13	10	00	00	00	00
5	Zn(II)BS2ABT	00	00	00	00	12	10	00	00	00	00
6	Cu(I)BS2ABT	00	00	00	00	15	10	00	00	00	00
7	Ciprofloxacin	25	0.005	18	0.005	17	0.005	-	-	-	-
8	Trimethoprim-sulphamethoxazole		0.025		0.025		0.025	-	-	-	-
9	Fluconazole disk	-	-	-	-	-	-	00	00	20	10

Table 7. Lethal Concentration (LC_{50}) and Effective Concentration (EC_{50}) Results in ppm (Cytotoxic test).

S/N	SAMPLES	LC_{50} (ppm)	EC_{50} (ppm)
1	BS2ABT	26.25 ± 4.9	2.6
2	Ni(II)BS2ABT	176.07 ± 21.62	17.6
3	Mn(II)BS2ABT	337.56 ± 46.81	33.8
4	Co(II)BS2ABT	1833.88 ± 186.92	183.4
5	Zn(II)BS2ABT	215.00 ± 16.7	21.5
6	Cu(I)BS2ABT	78.90 ± 18.0	7.9

Cu(I) Complexes of the Ligand

Two bands were observed for the Cu(I) complex of the ligand synthesized ($11,150\text{cm}^{-1}$ and $13,620\text{cm}^{-1}$). Based on the fact that the Cu(II) complex was reduced to Cu(I) in this synthesis, there are no $d \leftarrow d$ transitions¹⁶. With this fact, coupled with the colour of the complex, we presumed that the bands observed are as a result of charge transfer transitions. We therefore proposed tetrahedral geometry for the copper complex synthesized. The molar absorptivities of the ligand and its complexes are also shown in Table 2.

IR Spectra of the BS2ABT and of Its Complexes

Table 3 gives the essential peaks of the ligand and complexes and presents a scheme for determining the mode of ligation of the ligand. The broad peaks at 3464cm^{-1} , 3380cm^{-1} , and 3400cm^{-1} were assigned N-H Stretching vibrations of the ligand BS2ABT, and its Co(II), Ni(II), Mn(II), Zn(II) and Cu(I) complexes respectively. It was also observed that there was a reduction in the stretching vibration in (N-H) of the metal complexes compared to the ligand's values. This indicates that coordination may have taken place via the N-H of the ligand. The strong peaks around $2850\text{--}2985\text{cm}^{-1}$ are assigned the C-H stretching vibrations in both the ligand, BS2ABT and its metal complexes. The strong peaks around $1603\text{--}1650\text{cm}^{-1}$ were assigned the C=C stretching vibration of the aromatic ring. The two strong peaks observed in the ligand and metal complexes around $1450\text{--}1560\text{cm}^{-1}$ were assigned C=N stretching vibration of benzothiazole ring. It was also observed that there was a reduction of the frequencies to about 55cm^{-1} in the complexes when compared with the ligand. We therefore state that there was coordination through the C=N of the benzothiazole ring. The strong to medium peaks around $1305\text{--}1385\text{cm}^{-1}$ were assigned to SO_2 stretching vibration. The two medium to weak peaks observed for the ligand and the metal complexes between $818\text{--}961\text{cm}^{-1}$ were assigned C-H to bending vibration of substituted benzene ring. However the peaks around $576\text{--}657\text{cm}^{-1}$ were assigned C-S-C stretching vibration of thiazole ring. The two peaks each observed in the complexes around $325\text{--}360\text{cm}^{-1}$ were assigned the metal-coordinating atom, M-N, and M-Cl stretching vibrations, where N is the nitrogen atom in N-H and thiazole ring.

 ^1H and ^{13}C NMR Spectral Data

^1H and ^{13}C NMR Spectra data of the ligand and its complexes are shown on Tables 4 and 5 respectively. The peaks at 9.73 ppm (1H, s) for BS2ABT, 8.32 ppm (1H, s) for Zn(II)BS2ABT and Cu(I)BS2ABT are assigned to N-H protons. The peaks at 7.26 ppm (5H, m) for BS2ABT, 7.31 ppm (5H, m) for Zn(II)BS2ABT and Cu(I)BS2ABT are assigned to phenyl protons. The peaks at 7.88 ppm (4H, m) for BS2ABT, 7.80 ppm (4H, m) for Zn(II)BS2ABT and Cu(I)BS2ABT are assigned to benzothiazole protons. Proton nmr of the complexes of Mn(II)BS2ABT, Ni(II)BS2ABT, and Co(II)BS2ABT were non-definite and incomprehensible. This is indicative of paramagnetic nature of these complexes.

Zn(II)BS2ABT and Cu(I)BS2ABT showed the same peak patterns, and the peaks at 3.34 ppm (singlet) and 4.13 ppm (triplet) observed in their spectra may be due interference of magnetic field from the metal ions in the complexes.

Peaks at 136.9 ppm in the ligand and 167.6 ppm in the metal complexes are assigned benzothiazole ring carbon (C1), peaks at the range of 132.9-136.8 ppm in the complexes and 129.8 ppm in the ligand are assigned benzothiazole ring carbon (C2). Peaks at 133.1 ppm the ligand and at the range of 142.5-142.7 ppm in the metal complexes are assigned benzothiazole ring carbon (C3). Peaks at 128.4 ppm in the ligand and the range of 129.7-133.0 ppm in the metal complexes are assigned benzothiazole ring carbon (C4). Peaks at 126.0 ppm in the ligand, and the range of 125.3-127.8 ppm in the metal complexes are assigned benzothiazole ring carbon (C5). Peaks at 125.0 ppm in the ligand, and the range of 124.2-126.4 ppm in the metal complexes are assigned benzothiazole ring carbon (C6). Peaks at the range of 126.4-129.7 ppm in the ligand and its metal complexes are assigned benzothiazole ring carbon (C7). Peaks at the range of 129.3-136.7 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C8). Peaks at the range of 125.3-129.7 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C9). Peaks at the range of 123.3-126.0 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C10). Peaks at the range of 113.4-124.0 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C11).

Magnetic Properties of the Complexes

The result of the magnetic properties of the complexes is shown in Table 1. The result gave an interesting data. It was generally observed that the metal complexes were of low spin. This is an indication that the ligand is a strong field and thus was able to cause pairing of the electrons. As expected the zinc complex investigated gave very small effective magnetic moment, μ_{eff} (0.67 BM). Therefore the zinc complex is diamagnetic, and have sp^3 hybridized geometry, thus tetrahedral structure. Ni(II)BS2ABT complex showed effective magnetic moment of 2.88 BM. This showed high spin configuration and correspond to two unpaired electrons, indicating paramagnetism. It has sp^3 hybridized geometry, thus tetrahedral structure. Co(II)BS2ABT showed effective magnetic moment of 2.28 BM. This is an indication of low spin paramagnetism corresponding to one unpaired electron. We proposed sp^3d^2 hybridization of octahedral geometry. The manganese complex investigated showed effective magnetic moment of 1.79 BM. This is indication of low spin paramagnetism corresponding to an unpaired electron. We proposed d^2sp^3 hybridization of octahedral geometry. The Cu(I) complex investigated showed diamagnetism, indicating no unpaired electrons in the metal d-orbitals. Since there is no possibility of electron pairing in the d-orbitals, we are presuming that the ligand may have induced reduction of the Cu^{2+} to Cu^+ . This is also confirmed by the dirty white colour of the complex formed. We also proposed sp^3 hybridization of tetrahedral geometry for all the Cu(I) complex investigated.

Antimicrobial Activity of the Ligand and of Its Metal Complexes

The antimicrobial activities of the ligand and of its metal complexes are recorded in Tables 6a and 6b.

Table 6a showed the activities against multi-resistant bacterial strains isolated under clinical conditions. The Inhibitory Zone Diameter (IZD) in mm and Minimum Inhibitory Concentration (MIC) in mg/ml of the compounds were determined. Two strains each of *E. Coli* (*E. Coli* strain 1 and *E. Coli* strain 15), and *Proteus* species (*Proteus* spp strains 25 and *Proteus* spp strains 26), *Pseudomonas aeruginosa* strains 34 and multi-resistant *Staphylococcus aureus* (SR) strain, all isolated from dogs at clinical conditions were used. Ciprofloxacin and trimethoprim-sulphamethoxazole were used as the standard drugs. We determined the MIC on the concentration range 0.125-10 mg/ml. We discarded

concentrations above 10 mg/ml. Based on this, only the ligand showed activity against the tested microbes- *E. Coli* Strain 15 and *Pseudomonas aeruginosa* strains 34 with MIC of 10 mg/ml and IZD of 10 mm. The complexes showed no detectable activity against the multi-resistant bacteria tested.

Table 6b showed activities of the compounds against Typed Strains (ATCC Cultures) microorganisms. The bacteria cultures used are *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). The fungi, *Candida krusei* (ATCC 6258) and *Candida albicans* (ATCC 90028) were also used. As with the multi-resistant bacteria Strains, the Inhibitory Zone Diameter (IZD) in mm and Minimum Inhibitory Concentration (MIC) in mg/ml of the compounds were determined. Ciprofloxacin, and trimethoprim-sulphamethoxazole were used as the antibacterial standard drugs while Fluconazole disk was used as antifungal standard drugs.

The MIC were determined majorly on the concentration range of 0.125-10 mg/ml. Based on this, all the compound synthesized showed activity against at least one of the tested microbes. We adjudged that as in the case of the activity against the multi-resistant bacteria, BS2ABT showed the highest of activity in that it was active against most of the typed strains used-both the bacteria and fungi with MIC of 2.5 mg/ml and IZD of 10 mm against *Staphylococcus aureus* (ATCC 25923), followed by the nickel complex with MIC of 5 mg/ml and IZD of 10 mm against *Staphylococcus aureus* (ATCC 25923). All the compounds showed activity against *Staphylococcus aureus* (ATCC 25923). None of the complexes of BS2ABT was active against *Pseudomonas aeruginosa* (ATCC 27853). All the compounds synthesized did not show activity against *Escherichia Coli* (ATCC 25922).

As with the case of the fungus, *Candida krusei* (ATCC 6258), the ligand synthesized was active against *Candida albicans* (ATCC 90028) with MIC 10 mg/ml and IZD of 11 mm. Like also the case with *C. Krusei*, none of the metal complexes showed activity against the *C. albicans* typed strain used, showing that the ligand has more active antifungal properties than the synthesized complexes. Fluconazole is primarily fungistatic but can be fungicidal against certain organisms in dose-dependent manner. Fluconazole was only active against the typed strain *Candida albicans* (ATCC 90028) but not against *C. Krusei* tested strains. This was confirmed from literature¹⁷. We can conclude that the compounds showed some degree of activity against the tested microorganisms which to a large extent can be compared with the standard drugs used. Since the standard antifungal drug used did not show activity against the *Candida krusei* (ATCC 6258), we can say that the ligand, BS2ABT was more active than the fluconazole.

Lethal Concentration (LC₅₀) and Effective Concentration (EC₅₀)

The result of the Cytotoxic tests viz; Lethal Concentration (LC₅₀) and Effective Concentration (EC₅₀) is recorded in Table 7.

The result showed that all the synthesized compounds showed high levels of bioactivity against 48 h-nauplii. BS2ABT showed the highest bioactivity (26.25±4.9 ppm) with EC₅₀ of 26 ppm while Co(II)BS2ABT showed the lowest bioactivity (1833.88±186.92 ppm) with EC₅₀ of 183.4 ppm. Comparing the complexes, the level of bioactivity is in the order Cu(I)BS2ABT > Ni(II)BS2ABT > Zn(II)BS2ABT > Mn(II)BS2ABT > Co(II)BS2ABT.

BSLT is a rapid, inexpensive and single bioassay for testing bioactivity of natural and synthetic products, which in most cases correlates reasonably well with cytotoxicity and antitumor properties of the products. The results of Brine Shrimps Lethality Test (BSLT) established that the ligand and the complexes are very potent bioactive compounds. EC₅₀ value for general bioactivity is approximately one tenth of the value is the LC₅₀ in BSLT. The surviving nauphii were dull and inactive, which may be a sign of Central Nervous System (CNS) depression.

Conclusion

N-(benzothiazol-2-yl)benzenesulphonamide and its metal complexes were synthesized. The compounds were characterized using magnetic susceptibility, UV/VIS spectrophotometer, infra red, proton and ^{13}C nmr. The spectral analyses confirmed the structures of the compounds synthesized. The antimicrobial tests of the ligand and its metal complexes were carried out on both multi-resistant bacterial and fungal strains isolated under clinical conditions and cultured species using agar-well diffusion method. The tests were both *in vitro* and *in vivo*. The antimicrobial activities of the compounds were compared with those of ciprofloxacin and trimethoprim-sulphamethoxazole as antibacterial agents and Fluconazole as an antifungal drug. N-(benzothiazol-2-yl)benzenesulphonamide, showed activities against multi-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. All the compounds showed varying activities against the cultured typed bacteria and fungi used. However, they were less active than the standard bacterial drugs used, and since the standard antifungal drug (fluconazole) used did not show activity against the *Candida krusei* (ATCC 6258), we can conclude that the compound, BS2ABT which showed activity against it was more active than the fluconazole and can be recommended for preclinical screening. The Lethal Concentrations (LC_{50}) were within the permissible concentrations.

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