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Syntheses, Characterization and Antimicrobial Screening of *N*-(Benzothiazol-2-yl)-2,5-Dichlorobenzenesulphonamide and Its Cu(I), Ni(II), Mn(II), Co(III) and Zn(II) Complexes

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N-(Benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide (DCBS2ABT) was synthesized by the condensation (by refluxing) of 2-aminobenzothiazole and 2,5-dichlorobenzenesulphonylchloride in acetone at 230 ºC. The resulting crude precipitates were recrystallized in absolute ethanol. Five metal complexes of copper(I), nickel(II), manganese(II), cobalt(III) and zinc(II) of the ligand were synthesized. The compounds were characterized using magnetic susceptibility measurements, UV/visible spectrophotometry, elemental microanalysis, infrared, ^{1}H and ^{13}C 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 aeroginosa* and *Staphylococcus aureus* 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 performed both *in vitro* and *in vivo*. Thus the inhibition zone diameter, the minimum inhibitory concentration and the lethal and effective concentrations (LC_{50} and EC_{50}) 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 the ligand, DCBS2ABT and all the metal complexes synthesized were very active against it. The lethal concentration (LC_{50}) ranged from 81.00 ± 6.8-256.30 ± 36.7 ppm. These are within the permissible concentrations.

Key Words: *N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide, Metal complexes, Antimicrobial.

INTRODUCTION

The search for potent antiinfective agents occupies an important position in science with the upsurge in disease diversity and declining sensitivity of the implicated organisms to available agents[1,2]. Interest in the coordination chemistry of thiazole and its derivatives with metal ions has risen due to the important role they play in biological systems[3]. Thiazole are known to posses antitubercular[4], hypotensive and hypothermic[5] activities. 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 animals[6].

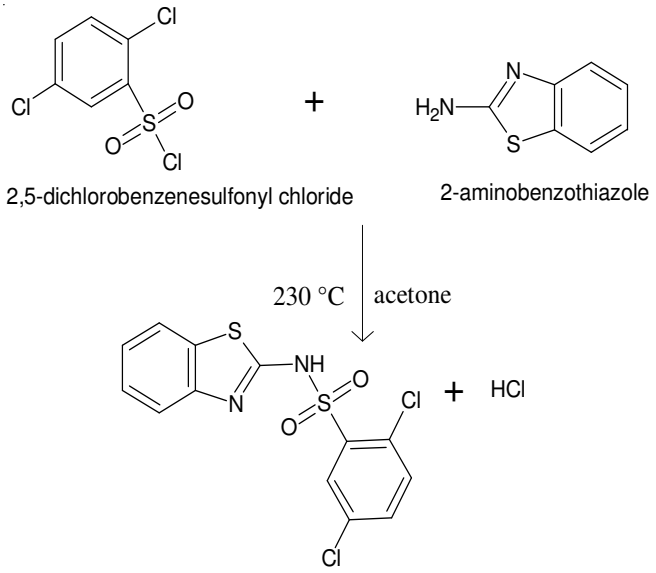
Mercury(II) and copper(II) complexes of 6-methyl-2-aminobenzothiazole have equally been discovered to show high activity against *Aspergillus niger*, *Alternaria alternate*, *Curvularia plunata* and *Penicillium fumculorus*[7]. Obasi, *et al.*[8] worked on some sulfonyl derivatives of 2-aminothiazole and the results obtained showed that the compounds were significantly active against *Staphylococcus aureaus* and *Escherichia coli*. Some novel *N*-(benzothiazol-2-yl)ethanamides were also synthesized and characterized by Obasi, *et al.*[8] 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 tested[9]. The present work is aimed at synthesizing new derivative of 2-aminobenzothiazole and its metal(II) complexes, characterizing them and investigating how their sstructural differences affects antimicrobial activities when compared with conventional sulfonamides.

EXPERIMENTAL

The ligand, *N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide (DCBS2ABT) 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 its metal

complexes were obtained on UV-2550 UV-VISIBLE Spectrophotometer, (SHIMADZU). FTIR spectra of the compounds were run as Nujol mulls on FTIR-84005 FTIR Spectrophotometer, (SHIMADZU). ^{13}C and ^{1}H NMR spectra were recorded on Bruker-BioSpin 500 MHz NMR spectrometer (UK) using DMSO and $CDCl_3$ as solvents respectively. The proton NMR peaks were observed at 400 MHz whereas the carbon-13 spectra were observed at about 200 MHz. Elemental analysis was done using LECO-CHNS 932 microanalysis apparatus and the magnetic susceptibility of the complexes were determined using Sherwood scientific magnetic susceptibility balance, Mk1 model (Cambridge, UK) both at department of Pure and Applied Chemistry, University of Strathclyde, Scotland, UK.

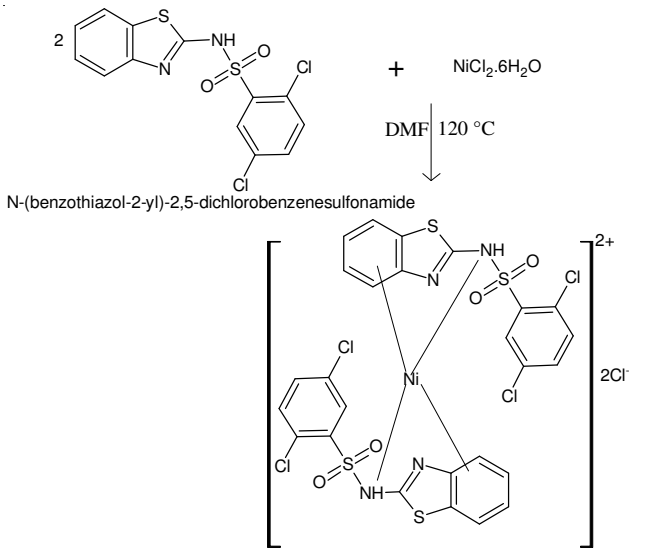
Synthesis of *N*-(benzothiazol-2-yl)-2, 5-dichlorobenzenesulphonamide (DCBS2ABT): To a solution of 2-aminobenzothiazole (3.0 g; 20 mmol) in acetone (15 mL) was added a solution of 2,5-dichlorobenzenesulphonylchloride (5.0 g; 20 mmol) in acetone (20 mL) with stirring. The mixture was refluxed for 1 h at 230 ºC. A milkish white precipitate was formed on refluxing and was recrystallized in absolute ethanol (yield *ca.* 79 %) (**Scheme-I**).



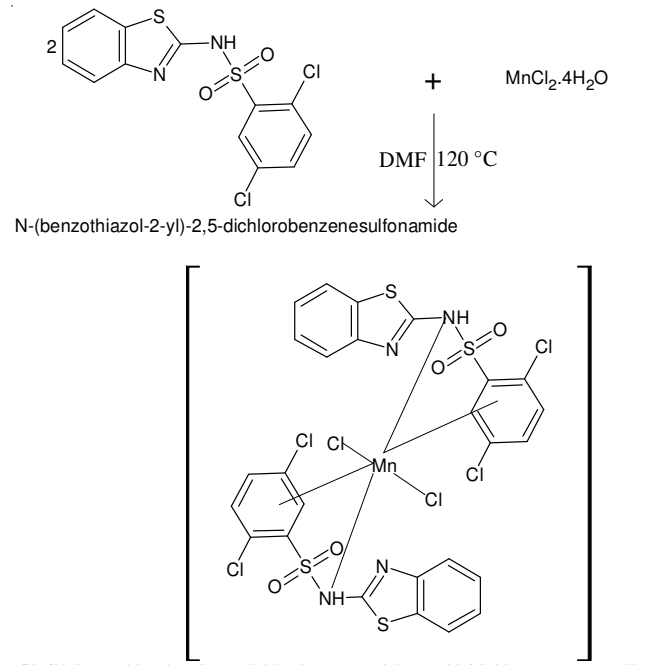
Scheme-I: Synthesis of *N*-(benzothiazol-2-yl)-2, 5-dichlorobenzenesulphonamide (DCBS2ABT)

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide] nickel(II) chloride (Ni(II)DCBS2ABT): To a solution of N-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide (0.72 g; 2.00 mmol) in DMF (10 mL) was added aqueous solution of nickel(II) chloride hexahydrate (0.28 g; 1.00 mmol). This was refluxed for 0.5 h at 120 ºC during which a white amorphous solid was formed. This was filtered and dried in a stream of air and stored in desiccator (**Scheme-II**).

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide] dichloromanganese(II) (Mn(II)DCBS2ABT): To a solution of *N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide (0.72 g; 2.00 mmol) in DMF (10 mL) was added aqueous solution of manganese(II) chloride tetrahydrate (0.20 g; 1 mmol). This was refluxed for 0.5 h at 120 ºC during which a white amorphous solid was formed. This was filtered and dried in a stream of air and stored in desiccator (**Scheme-III**).

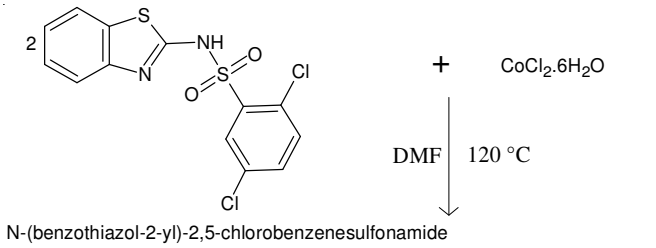


Scheme-II: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2, 5-dichlorobenzenesulphonamide]nickel(II) chloride (Ni(II)DCBS2ABT)

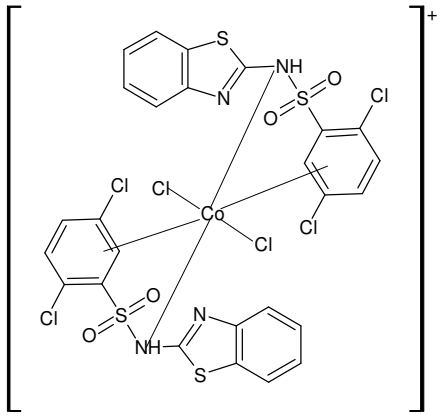


Scheme-III: Synthesis of bis-[*N*-(benzothiazol-2-yl)-2,5- dichlorobenzenesulphonamide]dichloromanganese(II) (Mn(II)DCBS2ABT)

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide] dichlorocobalt(III) ion (Co(III)DCBS2ABT): To a solution of *N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide (0.72 g; 2 mmol) in DMF (10 mL) was added aqueous solution of cobalt(II) chloride hexahydrate (0.24 g; 1 mmol). This was refluxed for 0.5 h at 120 ºC during which a blue powdery solid was formed. This was filtered and dried in a stream of air and stored in desiccator (**Scheme-IV**).



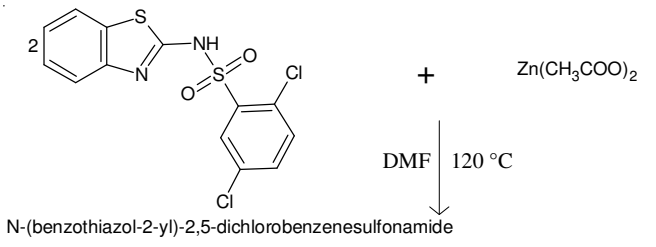
N-(benzothiazol-2-yl)-2,5-chlorobenzenesulfonamide



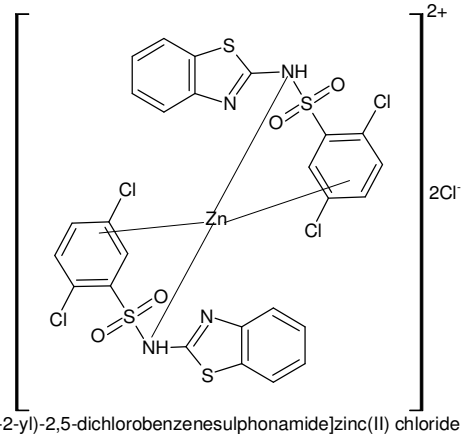
Bis [N-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide]dichlorocobalt(III) ion

Scheme-IV: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2, 5-dichlorobenzenesulphonamide]dichlorocobalt(III) ion (Co(III)DCBS2ABT)

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide] zinc(II) chloride (Zn(II)DCBS2ABT): To a solution of *N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide (0.72 g; 2.00 mmol) in DMF (10 mL) was added aqueous solution of zinc(II) acetate dihydrate (0.29 g; 1.00 mmol). This was refluxed for 0.5 h at 120 ºC during, which a white crystalline solid was formed. This was filtered and dried in a stream of air and stored in desiccator (**Scheme-V**).



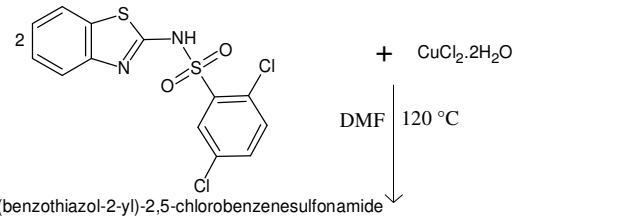
N-(benzothiazol-2-yl)-2,5-dichlorobenzenesulfonamide



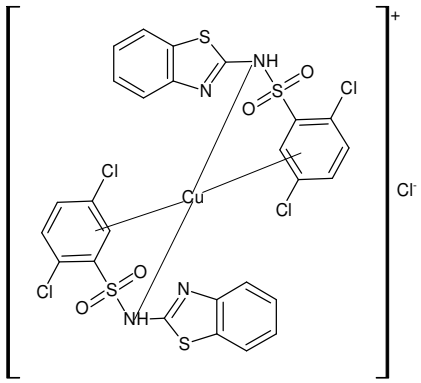
Bis [N-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide]zinc(II) chloride

Scheme-V: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2, 5-dichlorobenzenesulphonamide]zinc(II) chloride (Zn(II)DCBS2ABT)

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide] copper(I) chloride (Cu(I)DCBS2ABT): To a solution of *N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide (0.72 g; 2.00 mmol) in DMF (10 mL) was added aqueous solution of copper(II) chloride dihydrate (0.25 g; 1.00 mmol). This was refluxed for 0.5 h at 120 ºC during which a light green amorphous solid was formed. This was filtered and dried in a stream of air and stored in desiccator (**Scheme-VI**).



N-(benzothiazol-2-yl)-2,5-chlorobenzenesulfonamide



Bis [N-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide]copper(I) chloride

Scheme-VI: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-2, 5-dichlorobenzenesulphonamide]copper(I) chloride (Cu(I)DCBS2ABT)

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 aeroginosa* 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 (BDCP), International Centre for Ethnomedicine and Drug Development (IntaceEED), Nsukka, Nigeria.

The antibacterial and antifungal activities of the ligand, DCBS2ABT and its metal complexes *e.g.*, Ni(II)DCBS2ABT, Mn(II)DCBS2ABT, Co(III)DCBS2ABT, Zn(II)DCBS2ABT, Cu(I)DCBS2ABT 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 h 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 solubilized in 20 % v/v dimethyl sulfoxide and 0.05 mL of

TABLE-1
PHYSICAL PROPERTIES OF THE LIGAND, DCS2ABT AND OF ITS METAL COMPLEXES, THE MAGNETIC PROPERTIES OF THE COMPLEXES AND ELEMENTAL MICROANALYSIS OF THE LIGAND

S. No.	Samples	m.p. (°C)	Colour	Texture	Mw	μ_{eff} (BM)	No. of Electrons	Properties
1	DCBS2ABT	260-262	Milky	Powdery	359.00	-	-	-
2	Ni(II)DCBS2ABT	251-253	White	Amorphous	776.69	1.51	0	Diamagnetic
3	Mn(II)DCBS2ABT	249-251	White	Amorphous	843.94	2.05	1	Paramagnetic
4	Co(III)DCBS2ABT	255-257	Blue	Powdery	847.93	0.68	0	Diamagnetic
5	Zn(II)DCBS2ABT	257-259	White	Crystalline	783.39	0.68	0	Diamagnetic
6	Cu(I)DCBS2ABT	262-264	Light green	Amorphous	817.05	0.52	0	Diamagnetic

Elemental microanalysis of the ligand, DCBS2ABT

	C (%)		H (%)		N (%)	
	Calc.	Found	Calc.	Found	Calc.	Found
DCBS2ABT	43.45	42.97	2.23	2.25	7.80	7.42

TABLE-2
UV/VISIBLE SPECTRAL RESULT OF DCBS2ABT AND ITS COMPLEXES

Samples	λ_{max} (nm)	ν_1 (cm^{-1})	ν_2 (cm^{-1})	ν_3 (cm^{-1})	$10^{-3} \in_1$	$10^{-4} \in_2$	$10^{-5} \in_3$	Assignment
DCBS2ABT	216.4; 370.0				1.63	2.50		$\pi \rightarrow \pi^*$; $n \rightarrow \pi^*$
Ni(II)DCBS2ABT	734.0; 310.0	13 620	32 260		32.9	191		$\nu_1 = {}^1T_2$ (F) $\leftarrow {}^1T_1$ (F)
Mn(II)DCBS2ABT	310.0; 288.5; 273.5	32 260	34 660	36 560	2083	211	21.1	$\nu_1 = {}^2T_{1g}$(H) $\leftarrow {}^2A_{1g}$ $\nu_2 = {}^2E_g$ (H) $\leftarrow {}^2A_{1g}$
Co(III)DCBS2ABT	490.5; 418.5; 311.0	32 260	34 660	36 560	34.7	4.03	17.7	$\nu_1 = {}^1T_{2g}$(I) $\leftarrow {}^1T_{1g}$(I) $\nu_2 = {}^1A_{2g}$(I) $\leftarrow {}^1T_{1g}$(I) ν_3 = LMCT
Zn(II)DCBS2ABT	310.5; 300.5; 281.5	33 110	33 300	35 500	1180	131	12.6	MLCT
Cu(I)DCBS2ABT	317.5; 311.5	31 500	32 100		1890	184		MLCT

Legend: LMCT = Ligand-Metal Charge Transfer Transition; MLCT = Metal-Ligand Charge Transfer Transition

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 ºC for 24 h while the fungi were incubated at 30 ºC for 48 h 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 inhibitory zone diameter 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 ºC, 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 ºC to check for sterility. Overnight nutrient broth cultures of the test bacteria were adjusted to contain approximately 10^8 cfu/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 ºC for 24 h 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 compounds[13]. *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) 48 h-shrimp nauplii in 1 mL 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 ºC) for 24 h after which the survivors in each well were counted. The results were analyzed using Finney Probit Analysis (MS-DOS-Computer-Program) to determine the LC_{50} 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, DCBS2ABT and its metal complexes are represented in **Schemes I-VI**.

Table-1 shows some physical properties of both ligand and its metal complexes. The melting point of the ligand, DCBS2ABT is 260-262 ºC while those of the complexes range from 249-264 ºC. The ligand has a milky colour while the nickel, manganese and zinc complexes are white. The cobalt and the copper complexes are blue and light green respectively. The ligand, DCBS2ABT and its cobalt complexes are powdery while the nickel, manganese and copper complexes are amorphous. The zinc complex is however crystalline.

The result of the elemental microanalysis is recorded in Table-1. The amount of carbon, hydrogen and nitrogen of the ligand calculated theoretically correspond to a reasonable extent with the experimental result.

Electronic spectra: The electronic transition result of the compounds synthesized are recorded in Table-2. Two bands

TABLE-3
IR SPECTRA OF THE DCBS2ABT AND OF ITS COMPLEXES IN cm^{-1}

DCBS2ABT	Ni(II)DCBS2ABT	Mn(II)DCBS2ABT	Co(III)DCBS2ABT	Zn(II)DCBS2ABT	Cu(I)DCBS2ABT	Assignments
3442 (br,w)	3421.83 (br)	3415.08 (br)	3350 (br)	3420 (br)	3400 (br)	N-H stretching vibration
2864(s)	2924.18 (s)	2924.18 (s)	2924.18 (s)	2923.22 (s)	2924.18 (s)	C-H stretching vibration
2750(sh)	2853.78 (s)	2853.78 (s)	2853.78 (s)	2853.78 (s)	2853.78 (s)	
1655.94(s)	1600.97 (s)	1606.76 (s)	1600.97 (s)	1600.97 (s)	1606.76 (s)	C=C stretching vibration of aromatic ring
1544.20(s)	1546 (s)	1545.03 (s)	1546 (s)	1549.86 (s)	1544.07 (s)	C=N stretching vibration of
1461.95(s)	1462.09 (s)	1462.09 (s)	1462.09(s)	1462.09 (s)	1462.09 (s)	benzothiazole ring
1335.25(s)	1375.29 (s)	1374.33 (s)	1375.29 (s)	1376.26 (s)	1374.33 (s)	SO_2 stretching vibration
	1334.78 (s)	1335.75 (s)	1334.78 (s)	1333.82 (s)	1334.78 (s)	
1285 (s)	1253.77 (s)	1253.77 (s)	1253.77 (s)	1253.77 (s)	1253.77 (s)	C-Cl stretching vibration
808.98 (s)	954.80 (s)	954.80 (s)	954.80 (s)	954.80 (s)	954.80 (s)	C-H bending vibration in
745.6 3(s)	836.17 (s)	836.17 (s)	836.17 (s)	835.21 (s)	835.21 (s)	substituted benzene ring
649.07 (s)	651.96 (s)	651.96 (s)	682.82 (s)	682 (s)	682.82 (s)	C-S-C stretching vibration
579.97 (s)	587.34 (s)	587.34 (s)	651.96 (s)	651.00 (s)	651.96 (s)	of thiazole ring
	350 (s)	382.88 (s)	375 (s)	375 (s)	374 (s)	M-N, M-Cl and M-(C=C)
	345 (s)	355 (s)	340 (s)	350 (s)	350 (s)	stretching vibrations

Legend: br= broad; m= medium; w= weak; s= strong; sh= shoulder

were observed for the ligand at 216.4 nm and 370.0 nm. They are due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions.

Nickel complex: For most octahedral and tetrahedral Ni(II) complexes, three bands are expected, however for some tetrahedral Ni(II) complexes like $[NiI_4]^{2-}$ two broad bands may be found[14,15]. Two bands were observed for Ni(II)DCBS2ABT (13, 620 cm^{-1} and 32, 260 cm^{-1}), they were assigned $\nu_1 = {}^1T_2$ (F) $\leftarrow {}^1T_1$(F) transition of square planar geometry.

Manganese complex: Three bands were observed for the Mn(II) complex synthesized (32 260 cm^{-1}, 34 660 cm^{-1} and 36 560 cm^{-1}), the transitions are assigned as follows:
$\nu_1 = {}^2T_{1g}$ (H) $\leftarrow {}^2A_{1g}$; $\nu_2 = {}^2Eg$ (H) $\leftarrow {}^2A_{lg}$ transitions of octahedral geometry.

Cobalt complex: In a cubic field, three spin-allowed transitions are anticipated because of the splitting of the free-ion ground ^4F term and the accompanying ^4P term. Of course it is essentially a 2-electron transition from $t_{2g}^5 e_g^2$ to $t_{2g}^3 e_g^4$. Three bands were observed for the cobalt complex (32, 260 cm^{-1}, 34, 660 cm^{-1} and 36, 560 cm^{-1}), it is assigned:

$$\nu_1 = {}^2T_{2g}(H) \leftarrow {}^2T_{1g}(H)$$
$$\nu_2 = 2A2g(H) \leftarrow {}^2T_{1g}(H)$$
$$\nu_3 = \text{LMCT transitions}$$

Zinc(II) complexes: 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.

Copper(I) complexes: Two bands were observed for the Cu(I) complex of the ligand synthesized (31, 500 cm^{-1} and 32, 100 cm^{-1}). Based on the fact that the Cu(II) complex was reduced to Cu(I) in this synthesis, there are no *d*←*d* transitions[16]. With this fact, coupled with the colour of the complex, it is 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.

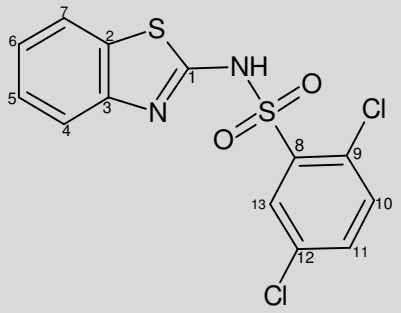
IR Spectra of the DCBS2ABT and its metal complexes: Table-3 gives the essential peaks of its metal and DCBS2ABT complexes and presents a scheme for determining the mode of ligation of the ligand. The broad peaks at 3442, 3422, 3415, 3350, 3420 and 3400 cm^{-1} were assigned to N-H stretching vibration of the ligand, DCBS2ABT, its Ni(II), Mn(II), Co(III), Zn(II) and Cu(I) complexes respectively. Because of the decrease in the stretching frequencies of the ligand up to 20 cm^{-1} when compared to the complexes, it is inferred that N-H was involved in the coordination. Two strong peaks observed between 2924-2750 cm^{-1} in all the compounds were assigned to C-H stretching vibration. The strong peaks between 1656-1601 cm^{-1} were assigned to C=C stretching vibration of aromatic ring in all the compounds. Since there are marked differences in the stretching frequencies of ligand compared to the complexes up to 50 cm^{-1}, we inferred that C=C was involved in the coordination. Two strong peaks each between 1544-1462 cm^{-1} in all the compounds were assigned C=N stretching vibration of benzothiazole ring. There was no significant difference between the values in the ligand and the complexes, so C=N was not involved in the coordination. Strong peaks between 1376-1334 cm^{-1} in the compounds were assigned to SO_2 stretching vibration. This did not take part in coordination since there is no significant difference in the values observed for the ligand and the complexes. Strong peaks at 1285 cm^{-1} for the ligand and 1254 cm^{-1} in all the complexes were assigned to C-Cl stretching vibration. However, two strong peaks each observed between 955-746 cm^{-1} in all the compounds were assigned to C-H bending vibration of substituted benzene ring. Also two strong peaks each observed between 683-587 cm^{-1} in all the compounds were assigned to C-S-C stretching vibration of thiazole ring. Strong peaks observed in the complexes between 383-345 cm^{-1} were assigned M-N, M- (C=C) and M-Cl stretching vibrations.

^{1}H and ^{13}C NMR spectral data: ^{1}H and ^{13}C NMR spectra data of DCBS2ABT and its metal complexes are shown on Tables 4 and 5 respectively. The peaks at 13.49 ppm (1H, s) for DCBS2ABT, 13.56 ppm (1H, s) for Ni(II)DCBS2ABT, 13.51 ppm (1H, s) for Co(III)DCBS2ABT and Cu(I)DCBS2ABT are assigned to N-H protons. Peaks at 7.84 ppm (2H, d) and 7.69 ppm (IH, m) in DCBS2ABT, 7.38 ppm (1H, m) and 7.43 ppm (2 H, m) in Ni(II)DCBS2ABT, 7.30 ppm (1H, m) and 7.71 ppm (2H, d) in Co(III)DCBS2ABT, 7.61 ppm (3H, m) in Zn(II)DCBS2ABT and 7.29 ppm (1H, m) and 7.71 ppm (2H, d) in Cu(I)DCBS2ABT are assigned phenyl protons. Peaks at

TABLE-4
^{1}H NMR SPECTRA (ppm) OF THE DCBS2ABT AND OF ITS COMPLEXES

DCBS2ABT	Ni(II)DCBS2ABT	Mn(II)DCBS2ABT	Co(III)DCBS2ABT	Zn(II)DCBS2ABT	Cu(I)DCBS2ABT	Assignments
13.49 (1H, s)	13.56 (1H, s)	-	13.51 (1H, s)	-	13.51 (1H, s)	N-H protons
7.69 (1H, m)	7.38 (1H, m)	-	7.30 (1H, m)	7.61 (3H, m)	7.29 (1H, m)	Phenyl protons
7.84 (2H, d)	7.43 (2H, d)		7.71 (2H, d)		7.71 (2H, d)	
8.04 (4H, d)	8.12 (4H, d)	-	8.05 (4H, d)	8.26 (4H, d)	8.05 (4H, d)	Benzothiazole protons

TABLE-5
^{13}C NMR SPECTRA (ppm) OF THE DCBS2ABT AND OF ITS COMPLEXES



DCB S2ABT	Ni(II)DCB S2ABT	Mn(II)DCB S2ABT	Co(III)DCB S2ABT	Zn(II)DCB S2ABT	Cu(I)DCB S2ABT	Assignments
168.5	168.3	168.4	168.5	-	-	Benzothiazole carbon (C1)
132.6	134.4	134.1	134.1	134.0	134.1	Benzothiazole carbon (C2)
134.2	134.6	134.2	134.2	133.8	134.2	Benzothiazole carbon (C3)
124.6	124.8	125.6	125.6	126.5	125.4	Benzothiazole carbon (C4)
113.8	123.4	123.5	123.5	123.2	123.5	Benzothiazole carbon (C5)
-	113.8	113.7	113.7	114.2	113.7	Benzothiazole carbon (C6)
123.6	124.6	124.5	124.5	124.2	124.5	Benzothiazole carbon (C7)
130.6	132.9	132.5	132.5	132.4	132.5	Phenyl carbon (C8)
141.2	147.7	141.1	141.1	141.4	141.1	Phenyl carbon (C9)
129.6	129.7	130.5	130.5	130.5	130.5	Phenyl carbon (C10)
128.0	128.0	129.5	129.5	129.5	129.5	Phenyl carbon (C11)
136.8	136.7	136.7	136.7	-	136.7	Phenyl carbon (C12)
125.7	125.6	127.9	127.9	-	127.9	Phenyl carbon (C13)

8.04 ppm (4H, d) in DCBS2ABT, 8.12 ppm (4H, d) in Ni(II)DCBS2ABT, 8.05 ppm (4H, d) in both Co(III)DCBS 2ABT and Cu(I)DCBS2ABT, 8.26 ppm (4H, d) in Zn(II)DCB S2ABT are assigned to benzothiazole protons. However singlet peaks within 2.54 ppm-3.38 ppm in Ni(II)DCBS2ABT, within 1.91 ppm-3.42 ppm in Zn(II)DCBS2ABT, 2.50 ppm and 3.35 ppm in both Co(III)DCBS2ABT and Cu(I)DCBS2 ABT are due to the presence of the metal ion in the complexes. The spectra observed in Mn(II)DCBS2ABT, Co(III)DCBS2ABT and Cu(I)DCBS 2ABT showed almost the same splitting pattern, an indication of nearly the same effect in the magnetic field. Interestingly the peak indicating N-H proton completely disappeared in the complex Zn(II)DCBS 2ABT with quite a different splitting pattern from the other complexes in the same series. More still, with the disorganization of the splitting pattern in the phenyl moiety, it further confirms our prediction that the phenyl moiety was involved in the complex formation. The Mn(II) DCBS2ABT is paramagnetic and thus the spectral result was not included.

Generally the difference in shifts, splitting patterns, disappearance and disorganization of some peaks found in the ligand with respect to the complexes and the presence of the some peaks in the complexes that are not found in the ligand are indication of complex formation.

Peaks at 168.3-168.5 ppm in the ligand and the complexes except the copper and zinc complexes are assigned benzothiazole ring carbon (C1), peaks at the 132.6 ppm in the ligand and at the range of 134.0-134.4 in the complexes are assigned benzothiazole ring carbons (C2). Peaks at 134.2 ppm in the ligand, its manganese, cobalt and copper complexes and peaks at 134.6 ppm and 133.8 ppm in the nickel and zinc complexes respectively are assigned benzothiazole ring carbon (C3). Peaks at the range of 124.6-126.5 ppm in the ligand and in the metal complexes are assigned benzothiazole ring carbon (C4). Peaks at 113.8 ppm in the ligand and the range of 123.2-123.5 ppm in the metal complexes are assigned benzothiazole ring carbon (C5). Peaks at the range of 113.7-114.2 ppm in the metal complexes are assigned benzothiazole ring carbon (C6). Peaks at 123.6 ppm in the ligand and at the range of 124.2-124.6 ppm in the metal complexes are assigned benzothiazole ring carbon (C7). Peaks at 130.6 ppm in the ligand and at the range of 132.4-132.9 ppm in the metal complexes are assigned phenyl ring carbon (C8). Peaks in the range of 141.2-147.7 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C9). Peaks in the range of 129.6-130.5 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C10). Peaks at 128.0 ppm and 129.5 ppm in both the ligand and its metal complexes are

TABLE-6a
ANTIMICROBIAL ACTIVITY OF THE LIGAND AND OF ITS METAL COMPLEXES AGAINST MULTI-RESISTANT BACTERIAL STRAINS ISOLATED UNDER CLINICAL CONDITIONS

S. no.	Samples	Multi-resistant bacterial strains isolated from clinical conditions											
		Escherichia coli strains				*Proteus* species strains				*Pseudomonas aeroginosa* 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)
1	DCBS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
2	Ni(II)DCBS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
3	Mn(II)DCBS2ABT	00	00	00	00	00	00	11	10	00	00	00	00
4	Co(III)DCBS2ABT	00	00	00	00	00	00	12	10	00	00	00	00
5	Zn(II)DCBS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
6	Cu(I)DCBS2ABT	9	10	00	00	10	10	9	10	10	10	00	00
7	Ciprofloxacin	00	0.05	00	0.05	00	0.05	25	0.05	27	0.05	00	0.05
8	Trimethoprim-sulphamethoxazole		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

S. no.	Samples	Typed strains (ATCC Cultures)									
		Pseudomonas aeruginosa (ATCC 27853)		*Escherichia coli* (ATCC 25922)		*Staphylococcus aureus* (ATCC 25923)		*Candida krusei* (ATCC 6258)		*Candida albicans* (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	DCBS2ABT	10	10	00	00	11	10	12	10	10	10
2	Ni(II)DCBS2ABT	00	00	00	00	00	00	12	10	11	10
3	Mn(II)DCBS2ABT	00	00	00	00	00	00	12	10	11	10
4	Co(III)DCBS2ABT	00	00	00	00	00	00	12	10	11	10
5	Zn(II)DCBS2ABT	00	00	00	00	00	00	12	10	11	10
6	Cu(I)DCBS2ABT	12	10	13	10	10	10	14	10	10	10
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

assigned phenyl ring carbon (C11). More still, peaks at the range of 136.7-136.8 ppm are assigned phenyl carbon (C12) in the ligand, DCBS2ABT and its metal complexes. Peaks at the range of 125.6-127.9 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C13).

Magnetic properties of the metal complexes: The result of the magnetic properties of the present metal 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} (0.68 BM). Therefore the zinc complex is diamagnetic and has sp^3 hybridized geometry, thus tetrahedral structure. Ni(II)DCBS2ABT complex showed effective magnetic moment of 1.51 BM. This showed low spin configuration and correspond to no unpaired electrons, indicating diamagnetism. It is obvious that DCBS2ABT showed a strong field orientation and has caused the paring of the electrons. We therefore propose dsp^2 hybridization with square planar geometry. Co(III)DCBS2ABT showed effective magnetic moment of 0.68 BM. This is an indication of low spin diamagnetism corresponding to no unpaired electron. Thus we inferred that the ligand, DCBS2ABT may have induced oxidation in the metal ion. We proposed sp^3d^2 hybridization of octahedral geometry. The manganese complex investigated showed effective magnetic moment of 2.05 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 light green colour of the complex formed. We also proposed sp^3 hybridization of tetrahedral geometry for 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 aeroginosa* 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 minimum inhibitory concentration 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 aeroginosa* strains 34 with minimum inhibitory concentration of 10 mg/mL and inhibitory zone diameter 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), *Escherica 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 in mm and minimum inhibitory concentration 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 minimum inhibitory concentration 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, Cu(I)DCBS2ABT showed the highest activity in that it was active against all of the typed strains used-both the bacteria except *Escherichia coli* strain 15 and multi-resistant *Staphylococcus aureus* (SR) strain and all typed strains (ATCC Cultures) microorganisms. The ligand, DCBS2ABT and its zinc and nickel complexes did not show any activity against the typed strains bacteria tested. The manganese and cobalt complexes only showed activity against *Proteus* spp strains 26. The ligand, DCBS2ABT and all the complexes showed activity against the fungi strains, *Candida krusei* (ATCC 6258) and *Candida albicans* (ATCC 90028) tested with the copper complex showing the highest activity with minimum inhibitory concentration of 10 mg/mL and inhibitory zone diameters of 10 and 14 mm respectively. The nickel, manganese and cobalt complexes did not show any detectable activity against the bacteria typed strains (ATCC Cultures) used. The ligand was not active against *Escherichia coli* (ATCC 25922) used.

As with the case of the fungus, *Candida krusei* (ATCC 6258), the ligand and its complexes synthesized were active against *Candida albicans* (ATCC 90028) with the result showing that the copper complex has more active antifungal properties than the synthesized compounds. 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 literature[17]. 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 and its complexes were more active that the fluconazole.

Lethal concentration (LC_{50}) and effective concentration (EC_{50}): The result of the cytotoxic tests *viz*; Lethal concentration (LC_{50}) and effective concentration (EC_{50}) is recorded in Table-7.

TABLE-7
LETHAL CONCENTRATION (LC_{50}) AND EFFECTIVE CONCENTRATION (EC_{50}) RESULTS IN ppm (CYTOTOXIC TEST)

S. No.	SAMPLES	LC_{50} (ppm)	EC_{50} (ppm)
1	DCBS2ABT	158.80 ± 26.7	15.9
2	Ni(II)DCBS2ABT	81.00 ± 6.8	8.1
3	Mn(II)DCBS2ABT	128.00 ± 18.1	12.8
4	Co(III)DCBS2ABT	228.80 ± 17.0	22.9
5	Zn(II)DCBS2ABT	112.60 ± 26.8	11.3
6	Cu(I)DCBS2ABT	256.30 ± 36.7	25.6

The result showed that all the synthesized compounds showed high levels of bioactivity against 48 h-nauplii. Ni(II)DCBS2ABT showed the highest bioactivity (81.00 ± 6.8 ppm) with EC_{50} of 8.1 ppm while Cu(I)DCBS2ABT showed the lowest bioactivity (256.30 ± 36.7 ppm) with EC_{50} of 25.6 ppm. Comparing the compounds, the level of bioactivity is in the order Ni(II)DCBS2ABT > Zn(II)DCBS2ABT > Mn(II)DCBS2ABT > DCBS2ABT > Co(III)DCBS2ABT > Cu(I)DCBS2ABT.

Brine shrimps lethality test 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 established that the ligand and the complexes are very potent bioactive compounds. EC_{50} value for general bioactivity is approximately one tenth of the value is the LC_{50} in Brine shrimps lethality test. The surviving nauphii were dull and inactive, which may be a sign of central nervous system depression.

Conclusion

N-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide and its metal complexes were synthesized. The compounds were characterized using magnetic susceptibility, UV/VIS spectrophotometer, elemental microanalysis, infrared, 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. The copper complex of *N*-(benzothiazol-2-yl)-2,5-dichlorobenzenesulphonamide showed the highest activity in that it was active against all of the typed strains used-both the bacteria except *Escherichia coli* strain 15 and multi-resistant *Staphylococcus aureus* strain and all Typed strains (ATCC Cultures) microorganisms. The ligand, DCBS2ABT and its zinc and nickel complexes did not show any activity against the typed strains bacteria tested. All the other compounds synthesized 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 all the compounds synthesized were 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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