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Syntheses, Characterization and Antimicrobial Screening of *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide and Its Cu(I), Ni(II), Mn(II), Co(II) and Zn(II) Complexes

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N-(Benzothiazol-2-yl)-4-methylbenzenesulphonamide (PTS2ABT) was synthesized by the condensation of 2-aminobenzothiazole and 4-methylbenzenesulphonylchloride in acetone at 160 ºC. The resulting crude precipitates were recrystallized in acetone/ethanol mixture. Five metal complexes of copper(I), nickel(II), manganese(II), cobalt(II) and zinc(II) of PTS2ABT were synthesized. The compounds were characterized using magnetic susceptibility measurements, UV/visible spectrophotometry, elemental microanalysis, infra red, ^{1}H NMR 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*, 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, 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, PTS2ABT was very active against it. The lethal concentration (LC_{50}) ranged from 12.16 ± 1.3 to 495.30 ± 86.81 ppm. These are within the permissible concentrations.

Key Words: *N*-(Benzothiazol-2-yl)-4-methylbenzenesulphonamide, 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 systems[1]. Thiazole are known to posses antitubercular[2], hypotensive and hypothermic[3] activities. It is known that metal chelates of ligands with sulphur or nitrogen donor atoms have interesting physicochemical properties as well as physiological activities[4,5]. 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].

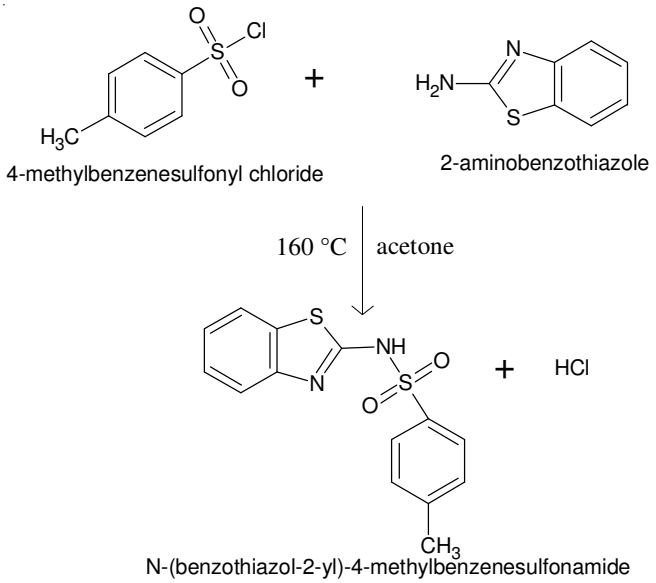
Mercury(II) and copper(II) complexes of 6-methyl-2-aminobezothiazole have equally been discovered to show high activity against *Aspergillus niger, Alternaria alternate, Curvularia plunata* and *Penicillium fumculorus*[7]. Furthermore, mercury(II) complex of sulfathiazole has been discovered to show high antibacterial activity against *Escherichia coli*[8], which causes sepsis and also diarrhea in humans. Obasi *et al.*[9] 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.*[10] 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. 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

The ligand, *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide (PTS2ABT) was prepared based on our modified method from that by Sprague *et al.*[11]. All the 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). ^{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.

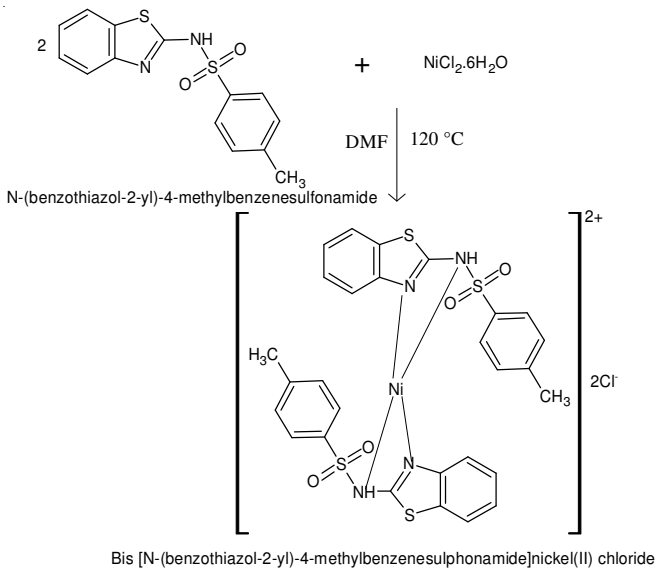
Synthesis of *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide (PTS2ABT): To a solution of 2-aminobenzothiazole (6.0 g; 40 mmol) in acetone (25 mL) was added a solution of 4-methylbenzenesulphonylchloride (7.7 g; 41 mmol) in acetone (25 mL) with stirring. The mixture was refluxed for 1 h at 160 ºC. During the refluxing, a white precipitate was formed. The precipitate was recrystallized in acetone/ethanol mixture (**Scheme-I**).



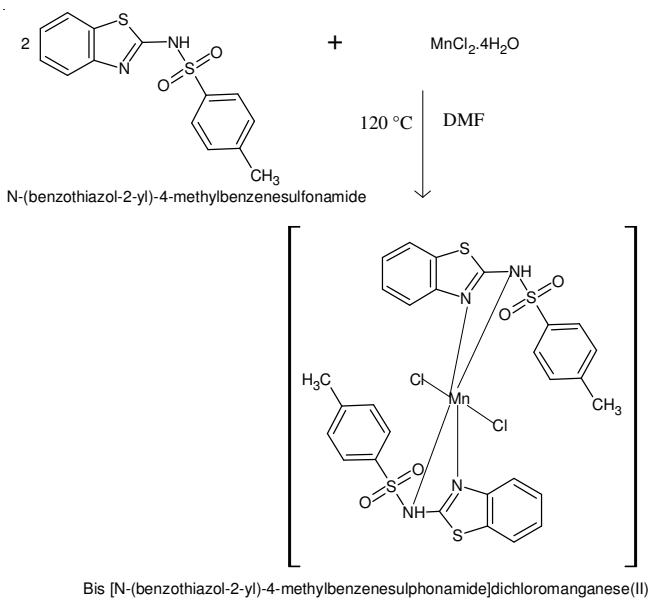
Scheme-I: Synthesis of *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] nickel(II) chloride (Ni(II)PTS 2ABT): To a solution of *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide (0.61 g; 2.00 mmol) in DMF (10 mL) was added aqueous solution of nickel(II) chloride hexahydrate (0.30 g; 1 mmol). This was refluxed for 0.5 h at 120 ºC during which a white precipitate was formed. This was filtered and dried in a stream of air and stored in desicator **(Scheme-II**).

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] dichloromanganese(II) (Mn(II)PTS 2ABT): To a solution of *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide (0.61 g; 2 mmol) in DMF (10 mL) was added aqueous solution of manganese(II) chloride tetrahydrate (0.25 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 desicator (**Scheme-III**).



Scheme-II: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] nickel(II) chloride (Ni(II)PTS2ABT)

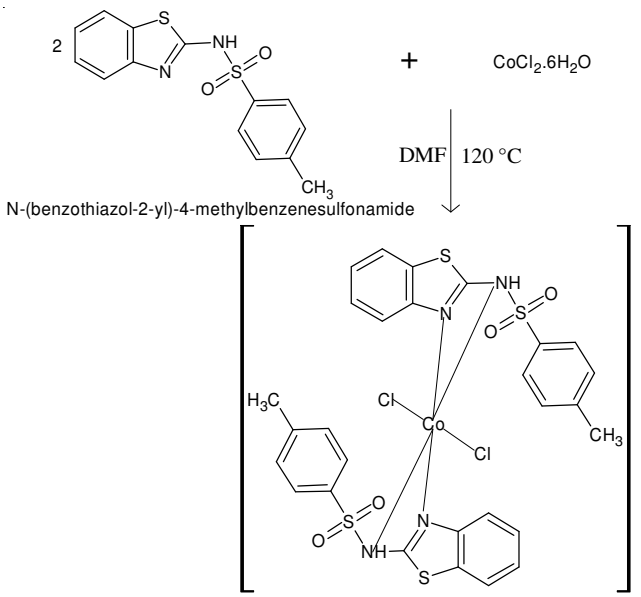


Scheme-III: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] dichloromanganese(II) (Mn(II)PTS2ABT)

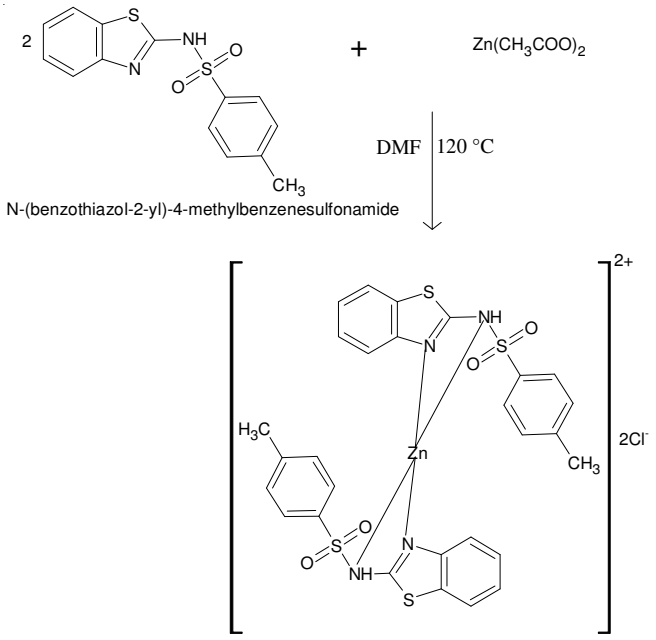
Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide]dichlorocobalt(II) (Co(II) PTS2ABT): To a solution of *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide (0.61 g; 2.00 mmol) in DMF (10 mL) was added aqueous solution of cobalt(II) chloride hexahydrate (0.30 g; 1 mmol). This was refluxed for 0.5 h at 120 ºC. On refluxing a blue crystalline solid was formed. This was filtered and dried in a stream of air and stored in desicator (**Scheme-IV**).

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzene-sulphonamide]zinc(II) chloride (Zn(II)PTS2ABT): To a solution of *N*-(benzothiazol-2-yl)-4-methylbenzene-

sulphonamide (0.61 g; 2 mmol) in DMF (10 mL) was added aqueous solution of zinc(II) acetate dihydrate (0.26 g; 1 mmol). This was refluxed for 0.5 h at 120 ºC during, which a white precipitate was formed. This was filtered and dried in a stream of air and stored in desicator (**Scheme-V**).

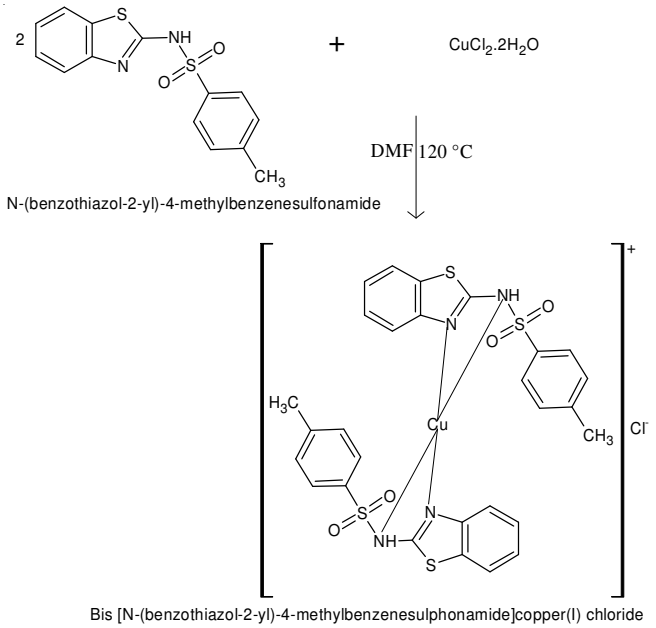


Scheme-IV: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] dichlorocobalt(II) (Co(II)PTS2ABT)



Scheme-V: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] zinc(II) chloride (Zn(II)PTS2ABT)

Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] copper(I) chloride (Cu(I)PTS 2ABT): To a solution of *N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide (0.61 g; 2 mmol) in DMF (10 mL) was added aqueous solution of copper(II) chloride dihydrate (0.22 g; 1.00 mmol). This was refluxed for 0.5 h at 120 ºC during which a brown precipitate was formed. This was filtered and dried in a stream of air and stored in desicator (**Scheme-VI**).



Scheme-VI: Synthesis of *bis*-[*N*-(benzothiazol-2-yl)-4-methylbenzenesulphonamide] copper(I) chloride (Cu(I)PTS2ABT)

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 ligand, PTS2ABT and its metal complexes *e.g.*, Ni(II)PTS2ABT, Mn(II)PTS2ABT, Co(II)PTS2ABT, Zn(II)PTS2ABT, Cu(I)PTS2ABT against these multi-resistant bacteria were determined using the agar well diffusion method as described by Chah *et al*[12]. 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 (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 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 around the wells. The

TABLE-1
PHYSICAL PROPERTIES OF THE LIGAND, PTS2ABT AND OF ITS METAL COMPLEXES, THE MAGNETIC PROPERTIES OF THE COMPLEXES AND ELEMENTAL MICROANALYSIS OF THE LIGAND

S. No	Samples	m.p. (ºC)	Colour	Texture	m.w.	μ_{eff} (BM)	Properties
1	PTS2ABT	144-146	White	Crystalline	304.00	-	-
2	Ni(II)PTS2ABT	201-203	White	Crystalline	666.69	4.06	Paramagnetic
3	Mn(II)PTS2ABT	203-205	White	Crystalline	733.94	2.50	Paramagnetic
4	Co(II)PTS2ABT	204-206	Blue	Crystalline	737.93	2.77	Paramagnetic
5	Zn(II)PTS2ABT	200-202	White	Amorphous	673.39	1.07	Diamagnetic
6	Cu(I)PTS2ABT	199-201	Brown	Crystalline	707.05	1.34	Diamagnetic

Elemental microanalysis (%) of the ligand, PTS2ABT

	C		H		N	
	Calc.	Found	Calc.	Found	Calc.	Found
PTS2ABT	55.26	49.26	3.95	3.95	9.21	11.27

test was performed in triplicates, mean inhibitory zone diameter was recorded to the nearest whole millimetre.

The minimum inhibitory concentrations of the test compounds were determined using the agar dilution method as described by Ojo *et al*[13]. 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[14]. *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, PTS2ABT and its metal complexes are represented in **Schemes I-VI**.

Physical properties of the compounds and elemental microanalysis of the ligand: Table-1 shows some physical properties of both ligand and its complexes. The melting point of the ligand, PTS2ABT is 144-146 ºC while those of the complexes range from 199-206 ºC. The ligand and its metal complexes are white except the cobalt and copper complexes, which are blue and brown respectively. The ligand, PTS2ABT and its complexes are crystalline except the zinc complex which is amorphous.

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 large extent with the experimental result. The % carbon for PTS2ABT calculated (55.26) and that found (49.26), was an indication that the compound may have little amount of some solvents used in the synthesis and recrystallization.

Electronic spectra: The electronic transition result of the compounds synthesized are recorded (Table-2). Two bands were observed for the ligand at 285.6 nm and 370.0 nm. They are due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions.

Nickel complex of the ligand: 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[15,16]. Thus three bands were observed for Ni(II) complex of PTS2ABT (13, 620 cm^{-1}, 20, 490 cm^{-1} and 24, 040 cm^{-1}) and the transitions are assigned as follows; $\nu_1 = {}^3T_2\,(F) \leftarrow {}^3T_1\,(F)$; $\nu_2 = {}^3A_2\,(F) \leftarrow {}^3T_1\,(F)$ and $\nu_3 = {}^3T_1\,(P) \leftarrow {}^3T_1\,(F)$ transitions.

Manganese complex of the ligand: Three bands were observed for the Mn(II) complex synthesized (13, 630 cm^{-1}, 20, 410 cm^{-1} and 31, 750 cm^{-1}), the transitions are assigned: $\nu_1 = {}^2T_{1g}\,(H) \leftarrow {}^2A_{1g}$ and $\nu_2 = {}^2E_g\,(H) \leftarrow {}^2A_{1g}$ transitions of octahedral geometry.

Cobalt complex of the ligand: 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 (11460, 13620 cm^{-1} and 32150 cm^{-1}), it is assigned: $\nu_1 = {}^2T_{2g}(H) \leftarrow {}^2T_{1g}(H)$; $\nu_2 = {}^2A_{2g}(H) \leftarrow {}^2T_{1g}(H)$ and ν_3 = LMCT transitions.

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.

Cu(I) complexes of the ligand: Two bands were observed for the Cu(I) complex of the ligand synthesized (13620 cm^{-1} and 33000 cm^{-1}). Based on the fact that the Cu(II) complex

TABLE-2
UV/VISIBLE SPECTRAL RESULT OF PTS2ABT AND ITS COMPLEXES

Samples	λ_{max} (nm)	ν_1 (cm^{-1})	ν_2 (cm^{-1})	ν_3 (cm^{-1})	$10^{-3} \in_1$	$10^{-4} \in_2$	$10^{-5} \in_3$	Assignments
PTS2ABT	285.6				3.04		4.68	$\pi \rightarrow \pi^*$
	370.0							$n \rightarrow \pi^*$
Ni(II)PTS2ABT	734.0	13 620	20 490	24 040	13.0	1.37	0.15	$\nu_1 = {}^3T_2$ (F) $\leftarrow {}^3T_1$ (F)
	488.0							$\nu_2 = {}^3A_2$ (F) $\leftarrow {}^3T_1$ (F)
	416.0							$\nu_3 = {}^3T_1$ (P) $\leftarrow {}^3T_1$ (F)
Mn(II)PTS2ABT	733.5	13 630	20 410	31 750	13.6	1.47	15.5	$\nu_1 = {}^2T_{1g}$(H) $\leftarrow {}^2A_{1g}$
	490.0							$\nu_2 = {}^2E_g$ (H) $\leftarrow {}^2A_{1g}$
	315.0							
Co(II)PTS2ABT	872.5	11 460	13 620	32 150	22.1	2.18	14.9	$\nu_1 = {}^2T_{2g}$(H) $\leftarrow {}^2T_{1g}$(H)
	734.0							$\nu_2 = {}^2A_{2g}$(H) $\leftarrow {}^2T_{1g}$(H)
	311.0							ν_3 = LMCT
Zn(II)PTS2ABT	299.5	33 390	34 070	34 700	1683	167	16.7	MLCT
	293.5							
	288.0							
Cu(I)PTS2ABT	734.5	13 620		33 000	15.4		16.4	MLCT
	303.0							

TABLE-3
IR SPECTRA OF THE PTS2ABT AND OF ITS COMPLEXES IN cm^{-1}

PTS2ABT	Ni(II) PTS2ABT	Mn(II) PTS2ABT	Co(II) PTS2ABT	Zn(II) PTS2ABT	Cu(I) PTS2ABT	Assignments
1590 m	1555 s	1550 s	1555 s	1555 s	1558 s	C=N stretching vibration
1553 s	1464 s	1463 s	1465 s	1464 s	1464 s	of benzothiazole ring
1312 s	1377 s	1378 s	1315 s	1378 s	1377 s	SO_2 stretching vibration
1300 m	1315 s	1316 s	1275 m	1316 s	1315 s	
959 sh	960 s	960 s	960 s	960 s	960 s	C-H bending vibration in
717 sh	813 m	875 m	813 m	835 m	835 m	substituted benzene ring
584 s	673 s	673 s	673 s	679 s	673 s	C-S-C stretching
			590 s			vibration of thiazole ring
	390 s	351 s	384 s	347 s	375 s	M-N and M-Cl stretching
	350 s				350 m	vibrations

was reduced to Cu(I) in this synthesis, there are no *d-d* transitions[17]. 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 metal complexes are also shown in Table-2.

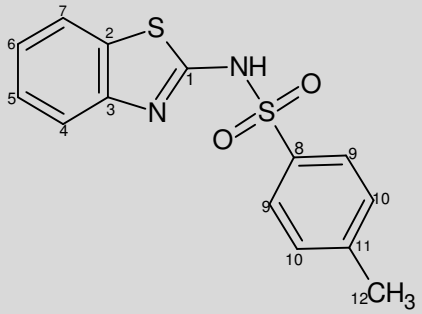
IR Spectra of the PT2ABT and of its metal complexes: Table-3 gives the main peaks of the ligand and metal complexes and presents a scheme for determining the mode of ligation of the ligand. The broad bands 3427, 3399, 3350, 3400, 3410 and 3400 cm^{-1} were assigned N-H stretching vibration for the ligand, PTS2ABT, its Ni(II), Mn(II) Co(II), Zn(II) and Cu(I) complexes reactively. It was also inferred that there was a coordination through the exocyclic N since there was a shift to lower frequencies in the ligand bands up to >17 cm^{-1} when compared with the complexes. As in the case of the ligand BS2ABT, two peaks observed around 2840-2968 cm^{-1} in all the compounds are assigned to C-H stretching vibration. The medium peaks observed between 1654-1603 cm^{-1} were assigned to C=C stretching vibration of aromatic ring. Two strong peaks each were observed between 1590-1463 cm^{-1}. These were assigned to C=N stretching vibration of benzothiazole ring. We also observed a reduction in the ligand up to >87 cm^{-1} compared to the complexes. It is inferred that C=N was involved in the coordination. Two medium to strong peaks observed between 1378-1275 cm^{-1} in the compounds are assigned SO_2 stretching vibration. The shoulder to strong peaks between 961-718 cm^{-1} were assigned C-H bending vibration of substituted benzene ring. The strong peaks between 584-680 cm^{-1} in both the ligand and the metal complexes were assigned C-S-C stretching vibration of benzothiazole ring. The strong peaks between 391-347 cm^{-1} in the metal complexes were assigned metal-nitrogen (M-N) and M-Cl stretching vibrations.

^{1}H and ^{13}C NMR spectral data: ^{1}H and ^{13}C NMR spectra data of the ligand and its complexes are shown on Tables 4 and 5 respectively. The ^{1}H NMR spectral results of PTS2ABT and its metal complexes are shown in Table-4, 6b. The peaks at 10.07 ppm (1H, s) for PTS2ABT, 13.17 ppm (1H, s) for Zn(II)PTS2ABT and Cu(I)PTS2ABT are assigned to N-H protons. The peaks at 7.11 ppm (4H, d) for PTS2ABT, 7.30 ppm (4H, m) for Zn(II)PTS2ABT and 7.35 ppm (4H, m) for Cu(I)PTS2ABT are assigned to phenyl protons. The peaks at 7.90 ppm (4H, t) for PTS2ABT, 7.79 ppm (2H, d) and 8.32 ppm (2H, d) for Zn(II)PTS2ABT and 7.76 ppm (4H, m) for Cu(I)PTS2ABT are assigned to benzothiazole protons. The peaks at 2.30 ppm (3H, s) for PTS2ABT, 3.36 ppm ((3H, s) for Zn(II)PTS2ABT and 3.34 ppm (3H, s) for Cu(I)PTS2ABT are assigned to CH_3- (methyl) protons. Some other peaks observed at 2.34 ppm and 2.35 ppm in Zn(II)PTS2ABT and 2.31 ppm and 2.35 ppm in Cu(I)PTS2ABT are due to interference of magnetic field from the metal ions in the complexes.

TABLE-4
^{1}H NMR SPECTRA OF THE PTS2ABT AND OF ITS COMPLEXES IN ppm

PTS2ABT	Ni(II)PTS2ABT	Mn(II)PTS2ABT	Co(II)PTS2ABT	Zn(II)PTS2ABT	Cu(I)PTS2ABT	Assignments
10.07(1H, s)	-	-	-	13.17(1H, s)	13.17(1H, s)	N-H protons
7.11 (4H, d)	-	-	-	7.30(4H, d)	7.35(4H, d)	Phenyl protons
7.90 (4H, t)	-	-	-	8.32(2H, d) 7.79(2H, d)	7.76(4H, m)	Benzothiazole protons
2.30 (3H, s)	-	-	-	3.36(3H, s)	3.34(3H, s)	H_3 C- (methyl) protons

TABLE-5
^{13}C NMR SPECTRA OF THE PTS2ABT AND OF ITS COMPLEXES IN ppm



PTS2ABT	Ni(II)PTS2ABT	Mn(II)PTS2ABT	Co(II)PTS2ABT	Zn(II)PTS2ABT	Cu(I)PTS2ABT	Assignments
130.1	126.4	125.3	126.4	126.4	-	Benzothiazole carbon (C6)
130.5	130.1	127.8	130.1	128.6	125.3	Benzothiazole carbon (C7)
137.9	136.8	136.5	136.8	136.8	127.8	Phenyl carbon (C8)
129.1	125.3	124.2	125.3	125.3	-	Phenyl carbon (C9)
127.9	124.2	123.3	124.2	124.2	123.3	Phenyl carbon (C10)
123.4	123.3	113.3	123.3	123.3	113.3	Phenyl carbon (C11)
22.0	21.5	21.5	21.5	21.5	21.5	Methyl carbon (C12)

The complexes, Ni(II)PTS2ABT, Mn(II)PTS2ABT and Co(II)PTS2ABT are paramagnetic and as such the spectra were not included since they made little or no sense.

Peaks at 167.5 ppm in the ligand, 143.2 ppm for the copper complex and 167.4 ppm in the rest of the other metal complexes are assigned benzothiazole ring carbon (C1), peaks at the 139.7 ppm in the complexes except the copper complex which showed at 130.1 ppm and 139.8 ppm in the ligand are assigned benzothiazole ring carbon (C2). Peaks at 143.3 ppm in the ligand, 139.7 ppm in the copper complex and at 143.2 ppm in the other metal complexes are assigned benzothiazole ring carbon (C3). Peaks at 136.9 ppm in the ligand and the range of 126.4-130.1 ppm in the metal complexes are assigned benzothiazole ring carbon (C4). Peaks at 130.3 ppm in the ligand and the range of 124.2-127.8 ppm in the metal complexes are assigned benzothiazole ring carbon (C5). Peaks at 130.1 ppm in the ligand and the range of 125.3-126.4 ppm in the metal complexes are assigned benzothiazole ring carbon (C6). Peaks at the range of 125.3-130.5 ppm in the ligand and its metal complexes are assigned benzothiazole ring carbon (C7). Peaks at the range of 127.8-137.9 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C8). Peaks at the range of 124.2-129.1 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C9). Peaks at the range of 123.3-127.9 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C10). Peaks at the range of 113.3-123.4 ppm in the ligand and its metal complexes are assigned phenyl ring carbon (C11). More still, peaks at the range of 21.5-22.0 ppm are assigned methyl carbon (C12) in the ligand, PTS2ABT and its metal complexes.

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} (1.07 BM). Therefore the zinc complex is diamagnetic and have sp^3 hybridized geometry, thus tetrahedral structure. Ni(II)PTS 2ABT complex showed effective magnetic moment of 4.06 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)PTS 2ABT showed effective magnetic moment of 2.77 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 2.50 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 brown 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
ANTIMICROBIAL ACTIVITY OF THE LIGAND AND OF THEIR METAL COMPLEXES AGAINST MULTI-RESISTANT BACTERIAL STRAINS ISOLATED UNDER CLINICAL CONDITIONS

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)
PTS2ABT	00	00	10	10	00	00	00	00	10	10	00	00
Ni(II)PTS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
Mn(II)PTS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
Co(II)PTS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
Zn(II)PTS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
Cu(I)PTS2ABT	00	00	00	00	00	00	00	00	00	00	00	00
Ciprofloxacin	00	0.05	00	0.05	00	0.05	25	0.05	27	0.05	00	0.05
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

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)
PTS2ABT	10	10	00	00	9	5	11	10	10	10
Ni(II)PTS2ABT	00	00	00	00	12	10	00	00	00	00
Mn(II)PTS2ABT	10	10	00	00	13	10	00	00	00	00
Co(II)PTS2ABT	11	10	00	00	00	00	00	00	00	00
Zn(II)PTS2ABT	10	10	00	00	00	00	00	00	00	00
Cu(I)PTS2ABT	9	10	00	00	12	10	00	00	00	00
Ciprofloxacin	25	0.005	18	0.005	17	0.005	-	-	-	-
Trimethoprim-sulphamethoxazole		0.025		0.025		0.025	-	-	-	-
Fluconazole disk	-	-	-	-	-	-	00	00	20	10

Table-6a showed the activities against multi-resistant bacterial strains isolated under clinical conditions. The inhibitory zone diameter in mm and minimum inhibitory concentration 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 (IZD) 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, PTS2ABT showed the highest of activity in that it was active against all of the typed strains used-both the bacteria except *Escherichia coli* (ATCC 25922) and the fungi with minimum inhibitory concentration of 5 mg/mL and inhibitory zone diameter of 9 mm against *Staphylococcus aureus* (ATCC 25923). Nickel and manganese complexes showed activity only against *Staphylococcus aureus* (ATCC 25923) with minimum inhibitory concentration of 10 mg/mL and inhibitory zone diameters of 12 and 13 mm respectively. None of the complexes of PTS2ABT was active against the fungi, *Candida krusei* (ATCC 6258) and *Candida albicans* (ATCC 90028) tested. 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 minimum inhibitory concentration 10 mg/mL and inhibitory zone diameter of 10 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 literature[18]. It is concluded 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, PTS2ABT wase 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)

SAMPLES	LC_{50} (ppm)	EC_{50} (ppm)
PTS2ABT	12.16 ± 1.3	1.2
Ni(II)PTS2ABT	316.70 ± 21.8	31.7
Mn(II)PTS2ABT	101.00 ± 18.2	10.1
Co(II)PTS2ABT	281.00 ± 18.0	28.1
Zn(II)PTS2ABT	318.00 ± 37.8	31.8
Cu(I)PTS2ABT	495.30 ± 86.81	49.5

The result showed that all the synthesized compounds showed high levels of bioactivity against 48 h-nauplii. PTS2ABT showed the highest bioactivity (12.16 ± 1.3 ppm) with EC_{50} of 1.2 ppm while Cu(I) PTS2ABT showed the lowest bioactivity (495.30 ± 86.81 ppm) with EC_{50} of 49.5 ppm. Comparing the complexes, the level of bioactivity is in the order Mn(II)PTS2ABT > Co(II)PTS2ABT > Ni(II)PTS2ABT > Zn(II)PTS2ABT > Cu(I)PTS2ABT.

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 (BSLT) 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 (CNS) depression.

Conclusion

N-(Benzothiazol-2-yl)-4-methylbenzenesulphonamide and its metal complexes were synthesized. The compounds were characterized using magnetic susceptibility, UV/visible spectrophotometer, elemental microanalysis, infra red, proton and ^{13}C NMR. The spectral analyses confirmed the structures of the compounds synthesized. The antimicrobial tests of the ligand and their 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)-4-methylbenzenesulphonamide, showed activities against multi-resistant *Escherichia coli* and *Pseudomonas aeroginosa*. 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, PTS2ABT 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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