

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

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Abstract

N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide (NBS2ABT) was synthesized by the condensation of 2-aminobenzothiazole and 2-nitrobenzenesulphonylchloride under reflux. Five metal complexes of Cu(I), Ni(II), Mn(II), Co(II) and Zn(II) of the ligands were synthesized. The compounds were characterized using magnetic susceptibility measurements, mass spectrometry, elemental microanalysis, UV/VIS spectrophotometry, infra red, ¹H 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 *E. coli*, *Proteus* species, *P. aeruginosa* and *S. aureus* which were isolated from dogs. The culture species were *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and the fungi, *C. krusei* (ATCC 6258) and *C. albicans* (ATCC 90028). 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. All the compounds showed varying activities against the cultured typed bacteria and fungi used. The Lethal Concentration (LC₅₀) ranged from 5.00±0.86-618.90±30.8 ppm. These are within the permissible concentrations.

Key words: N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide, Metal complexes, Antimicrobial, Ligand

1. INTRODUCTION

This is part of the series of work we have done on syntheses of thiazole derivatives and their metal complexes. Literatures have shown that thiazoles have antituberculous, antibacterial, antifungal properties⁽¹⁻⁵⁾. The success of sulfapyridine in combating bacterial infections, particularly pneumococcal infections has led to the preparation and study of other heterocyclic derivatives of sulfonamide⁽⁶⁾. 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 ligands⁽⁷⁾. It is known that metal chelates of ligands with sulphur or nitrogen donor atoms have interesting physicochemical properties as well as physiological activities⁽⁸⁻⁹⁾. In an era of decreasing microbial susceptibilities to current available antimicrobials, there is a pressing need to develop new agents and therapeutic strategies for the treatment of infectious diseases⁽¹⁰⁾.

This work is aimed at synthesizing N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide and its Cu(I), Ni(II), Mn(II), Co(II) and Zn(II) complexes, characterizing them, and investigating how their structural differences affects antimicrobial activities when compared with conventional sulfonamides.

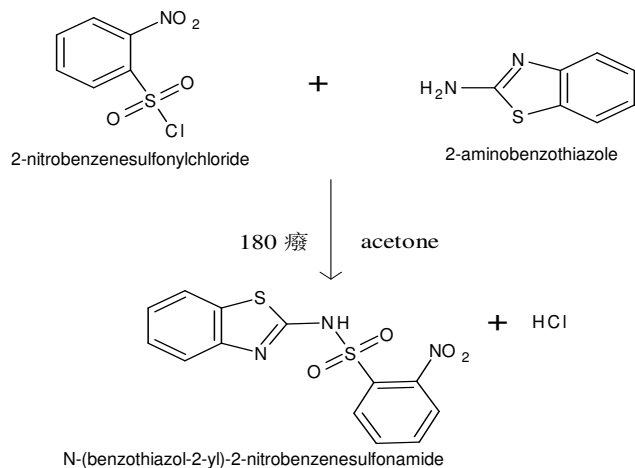
2. EXPERIMENTAL

2.1 Reagents and apparatus

The ligand, N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide (NBS2ABT) and the metal complexes were prepared based on the procedure earlier reported by Obasi *et al.*⁽¹¹⁾. All reagents were of analytical grade and were used as supplied except otherwise stated. UV-Visible spectra were obtained on UV-2550 UV-VIS Spectrophotometer, (SHIMADZU). FTIR spectra 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 400 MHz whereas the carbon-13 spectra were observed at about 200 MHz. Elemental analysis was done using LECO-CHNS 932 microanalysis apparatus, the mass spectrometric analysis was done using Thermo Finnigan LCQ DUO machine by electrospray ion trap method, and the magnetic susceptibility of the complexes were determined using Sherwood Scientific Magnetic Susceptibility Balance, Mk1 Model (Cambridge, UK), all at the Department of Pure and Applied Chemistry, University of Strathclyde, Scotland, UK.

2.2 Synthesis of *N*-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide(NBS2ABT)

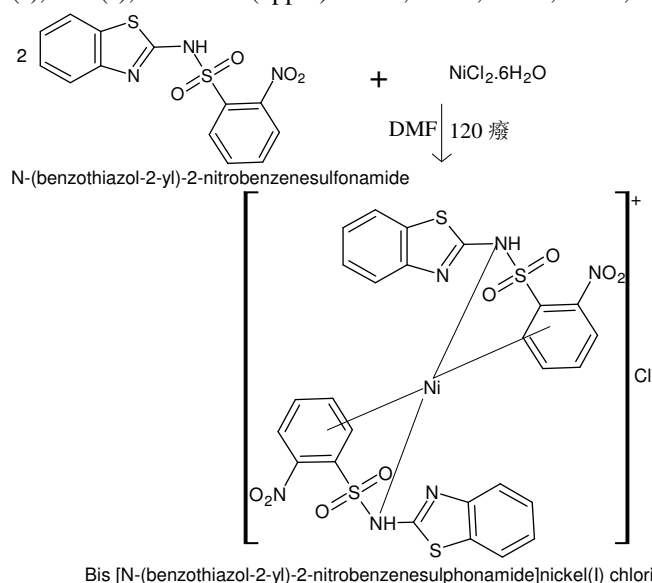
The procedure for the synthesis was as reported by Obasi *et al.*⁽¹¹⁾. A yellow precipitate was formed and was recrystallised in absolute ethanol. UV (λ_{max} nm) (DMSO), (ϵ):195.2, (1.15×10^3), 370.0 (6.10×10^4); IR (ν cm^{-1}): 3450 (br), 2853(s), 2734 (sh), 1655(sh) 1627 (sh), 1543 (s), 1461 (s), 1378 (s), 1217 (m), 846 (w), 746 (m), 645 (w); ^1H NMR (δ ppm): 7.51(4H, m), 8.12 (4H,m), 9.62 (1H,s); ^{13}C NMR (δ ppm): 168.4, 147.9, 136.8, 134.7, 134.5, 133.0, 129.8, 128.1, 125.7, 124.9, 123.6, 113.9.



Scheme 1: Synthesis of NBS2ABT

2.3 Synthesis of Bis[*N*-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide] nickel(I) chloride (Ni(I)NBS2ABT)

The procedure for the synthesis was as reported by Obasi *et al.*⁽¹¹⁾. A light yellow needle-like solid was formed. This was filtered and dried in a stream of air and stored in desiccator. UV (λ_{max} nm) (DMSO), (ϵ):364.5 ($27\ 440\ \text{cm}^{-1}$), (24.1×10^3), 371.0 ($26\ 950\ \text{cm}^{-1}$) (181×10^4), 734.5 ($13\ 620\ \text{cm}^{-1}$) (18.2×10^5); IR (ν cm^{-1}): 3430 (br), 2924 (s), 2854 (s), 1601 (s), 1534 (s), 1459 (s), 1380 (s), 1333 (s), 1246 (s), 958 (s), 837 (s), 685 (s), 663 (w), 345 (s), 350 (s); ^{13}C NMR (δ ppm): 167.3, 136.5, 127.6, 125.4, 124.0, 113.1.

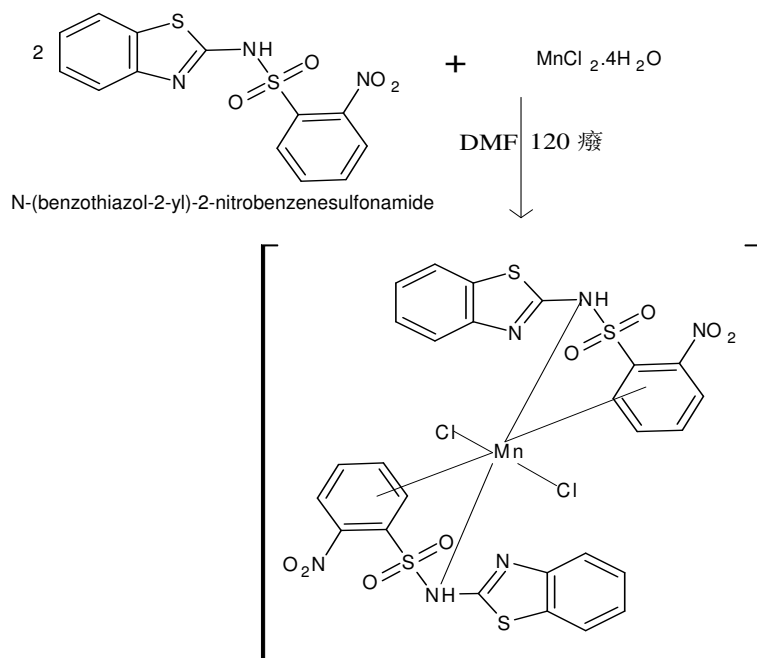


Scheme 2: Synthesis of Ni(I)NBS2ABT

2.4 Synthesis of Bis[*N*-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide]dichloromanganese(II) (Mn(II)NBS2ABT)

The procedure for the synthesis was as reported by Obasi *et al.*⁽¹¹⁾. A yellow needle-like solid was formed. This was filtered and dried in a stream of air and stored in desiccator. UV (λ_{max} nm) (DMSO), (ϵ):321.5 ($31\ 100\ \text{cm}^{-1}$),

(18.5×10^3), 328.5 ($30\ 440\ \text{cm}^{-1}$) (189×10^4), 734.0 ($13\ 620\ \text{cm}^{-1}$) (18.7×10^5); IR ($\nu\ \text{cm}^{-1}$): 3410 (br), 2954 (s), 2854 (s), 1605 (s), 1545 (s), 1462 (s), 1335 (s), 1254 (s), 955 (s), 836 (s), 683 (s), 587 (w), 395 (s), 360 (s); ^{13}C NMR (δppm): 170.1, 147.7, 139.4, 134.7, 134.6, 127.9, 124.8, 124.4, 123.3, 113.9.



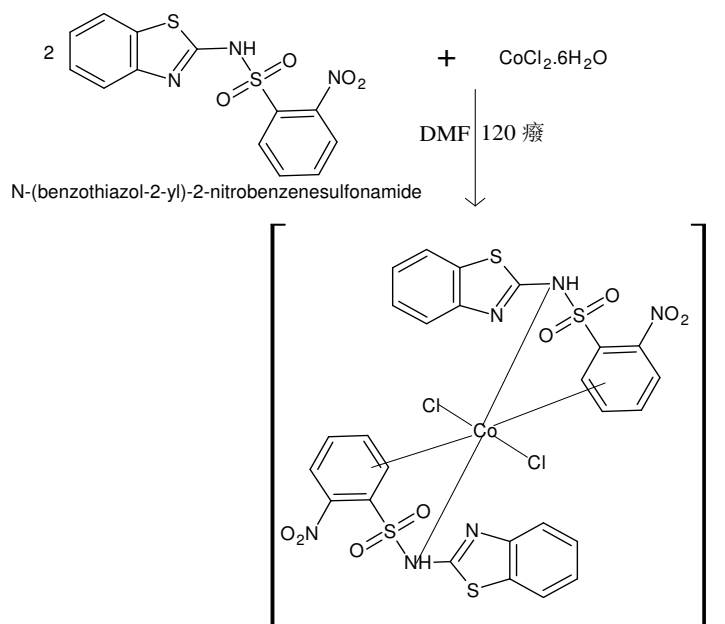
Bis [N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide]dichloromanganese(II)
Scheme 3: Synthesis of Mn(II)NBS2ABT

2.5 Synthesis of Bis[N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide]dichlorocobalt(II) (Co(II)NBS2ABT)

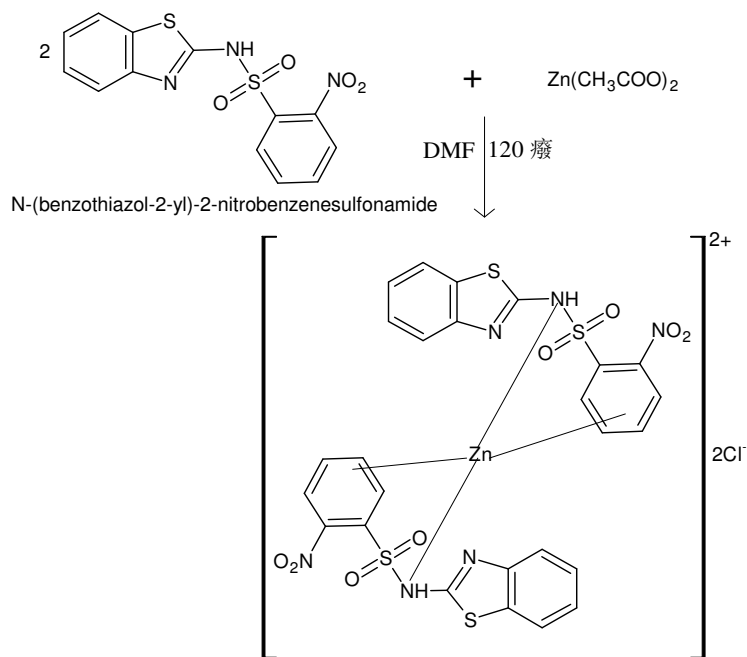
The procedure for the synthesis was as reported by Obasi *et al.*⁽¹¹⁾. A light blue needle-like solid was formed. This was filtered and dried in a stream of air and stored in desiccator. UV (λ_{max} nm) (DMSO), (ϵ):321.5 ($31\ 100\ \text{cm}^{-1}$), (186×10^3), 328.5 ($30\ 440\ \text{cm}^{-1}$) (19.0×10^4), 734.0 ($13\ 620\ \text{cm}^{-1}$) (18.8×10^5); IR ($\nu\ \text{cm}^{-1}$): 3430 (br), 2923 (s), 2854 (s), 1601 (s), 1534 (s), 1465 (s), 1380 (s), 1334 (s), 1246 (s), 958 (s), 837 (s), 663 (s), 581 (w), 392 (s), 350 (s); ^{13}C NMR (δppm): 168.3, 147.7, 136.7, 134.5, 134.4, 132.9, 129.6, 128.0, 125.6, 124.8, 123.4, 113.8.

2.6 Synthesis of Bis[N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide]zinc(II) chloride (Zn(II)NBS2ABT)

The procedure for the synthesis was as reported by Obasi *et al.*⁽¹¹⁾. A light yellow crystalline solid was formed. This was filtered and dried in a stream of air and stored in desiccator. UV (λ_{max} nm) (DMSO), (ϵ):340.0 ($29\ 410\ \text{cm}^{-1}$), (14.7×10^3), 344.0 ($29\ 060\ \text{cm}^{-1}$) (147×10^4), 348.5 ($28\ 700\ \text{cm}^{-1}$) (1450×10^5); IR ($\nu\ \text{cm}^{-1}$): 3362 (br), 2924 (s), 2854 (s), 1610 (s), 1540 (s), 1466 (s), 1374 (s), 1310 (s), 1275 (w), 947 (s), 836 (s), 684 (s), 615 (w), 345 (s), 355 (s); ^1H NMR (δppm): 7.41(4H, m), 7.85(2H,d), 7.93(2H,d), 13.43(1H,s); ^{13}C NMR (δppm): 168.3, 147.7, 136.9, 134.5, 134.4, 132.9, 130.0, 129.7, 125.7, 124.8, 123.4, 113.9.



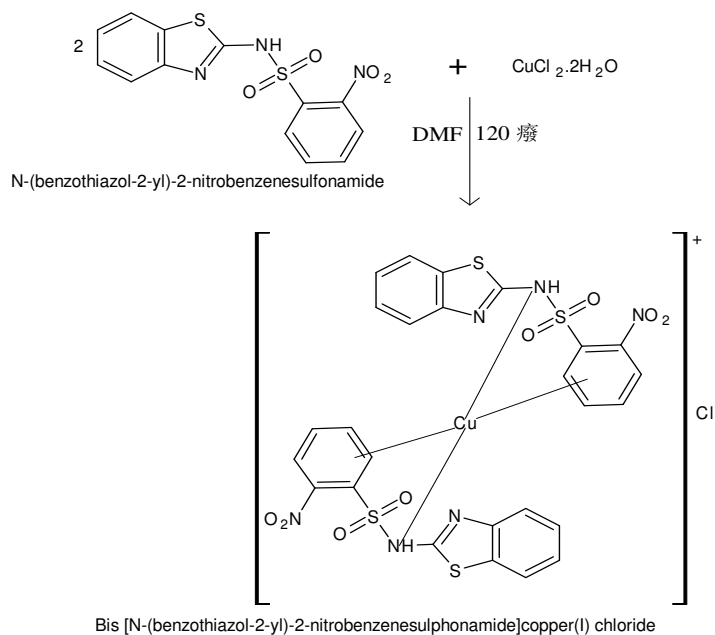
Bis [N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide] dichloro cobalt(II) complex
 Scheme 4: Synthesis of Co(II)NBS2ABT



Bis [N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide]zinc(II) chloride
 Scheme 5: Synthesis of Zn(II)NBS2ABT

2.7 Synthesis of Bis[N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide] copper(I) chloride (Cu(I)NBS2ABT)

The procedure for the synthesis was as reported by Obasi *et al.*⁽¹¹⁾. A brownish powdery solid was formed. This was filtered and dried in a stream of air and stored in desiccator. UV (λ_{max} nm) (DMSO), (ϵ):340.0 ($29\ 410\ \text{cm}^{-1}$), (14.7×10^3), 344.0 ($29\ 000\ \text{cm}^{-1}$) (146×10^4); IR ($\nu\ \text{cm}^{-1}$): 3400 (br), 2924 (s), 2854 (s), 1601 (s), 1534 (s), 1465 (s), 1380 (s), 1334 (s), 1256 (s), 957 (s), 837 (s), 663 (s), 350 (s), 325 (s); ^1H NMR (δ ppm): 7.44(4H, d), 8.15(2H,d), 13.56(1H,s); ^{13}C NMR (δ ppm): 168.3, 147.7, 136.9, 134.5, 134.4, 132.9, 129.7, 128.0, 125.7, 124.8, 123.4, 113.9.



Scheme 6: Synthesis of Cu(I)NBS2ABT

2.8 Antimicrobial properties

2.8.1 *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 as reported in our earlier publications⁽¹¹⁾. The Typed strains were obtained from Bioresources Development and Conservation Program (BDPC), International Centre for Ethnomedicine and Drug Development (IntaceEED), Nsukka, Nigeria.

The antibacterial and antifungal activities of the ligand, NBS2ABT, and its complexes against these multi-resistant bacteria were determined using the agar well diffusion method as described by Chah *et al*⁽¹²⁾.

The minimum inhibitory concentrations (MICs) of the test compounds were determined using the agar dilution method as described by Ojo *et al*⁽¹³⁾.

2.8.2 *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. 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.

3 RESULTS AND DISCUSSION

4 Physical Properties of the Compounds and Elemental microanalysis of the ligand

Table 1 shows some physical properties of both ligand and its complexes. 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. Because ion spray mass spectrometer was used, the mass of NBS2ABT was observed as 358 m/z, the excess mass of 23 m/z was due to sodium ion present in the mass spectrometer chamber.

5 Electronic Spectra

Two bands were observed for the ligand. They are due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions.

Three bands were observed for Ni(II) complex of NBS2ABT, and the transitions are assigned as follows;

$$\nu_1 = {}^3T_2(F) \leftarrow {}^3A_2(F)$$

$$\nu_2 = {}^3T_1(F) \leftarrow {}^3A_2(F)$$

$v_3 = {}^3T_1(P) \leftarrow {}^3A_2(F)$ transitions.

Three bands were observed for the Mn(II) complex synthesized, the transitions are assigned:

$v_1 = {}^2T_{1g}(H) \leftarrow {}^2A_{1g}$

$v_2 = {}^2E_g(H) \leftarrow {}^2A_{1g}$ transitions of octahedral geometry.

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, they are assigned:

$v_1 = {}^2T_{2g}(H) \leftarrow {}^2T_{1g}(H)$

$v_2 = {}^2A_{2g}(H) \leftarrow {}^2T_{1g}(H)$

$v_3 =$

LMCT transitions.

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.

Two bands were observed for the Cu(I) complex of the ligand synthesized. Based on the fact that the Cu(II) complex was reduced to Cu(I) in this synthesis, there are no $d \leftarrow d$ transitions¹⁵. 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.

6 IR Spectra of the NBS2ABT and of its complexes

The broad peaks observed at the range of 3362-3450 cm^{-1} in the compounds were assigned to N-H stretching vibration for the ligand NBS2ABT, its Ni(I) Mn(II), Co(II), Zn(II) and Cu(I) complexes respectively. We also observed that N-H was involved in the coordination because of the marked difference in the value of the ligand compared with the complexes. Two peaks each observed between 2734 cm^{-1} and 2954 cm^{-1} for the compounds were assigned to C-H stretching vibrations. Strong peaks observed at the range of 1601-1655 cm^{-1} in the ligand and the complexes were assigned to C=C stretching vibration of the aromatic ring. In this region there was a reduction in the stretching frequency of the ligand up to >45 cm^{-1} compared to the complexes. Thus an indication of coordination via the C=C bond. Two strong peaks each between 1461-1545 cm^{-1} in all the compounds were assigned to C=N stretching vibration of benzothiazole ring. Strong peaks between 1310-1380 cm^{-1} in all the compounds were assigned to SO_2 stretching vibration. The peaks at the range 1217-1275 cm^{-1} were assigned the NO_2 stretching vibration for the ligand and the complexes. However, strong peaks between 836-958 cm^{-1} in all the compounds were assigned C-H bending vibration of substituted benzene ring. Weak to strong peaks between 581-685 cm^{-1} in all the compounds were assigned to C-S-C stretching vibration of thiazole ring. The strong peaks observed for the metal complexes between 325-392 cm^{-1} were assigned M-N, M-(C=C) and M-Cl stretching vibrations.

7 ${}^1\text{H}$ and ${}^{13}\text{C}$ NMR Spectral Data

The peaks at 9.62 ppm (1H, s) in NBS2ABT, 13.43 ppm (1H, s) in Zn(II)NBS2ABT, and 13.56 ppm (1H, s) in Cu(I)NBS2ABT are assigned to N-H protons. The peaks at 7.51 ppm (4H, m) in NBS2ABT, 7.41 ppm (4H, m) in Zn(II)NBS2ABT, and 7.44 ppm (4H, d) in Cu(I)NBS2ABT are assigned to phenyl protons. The peaks at 8.12 ppm (4H, m) in NBS2ABT, 7.85 ppm (2H, d) and 7.93 ppm (2H, d) in Zn(II)NBS2ABT, and 8.15 ppm (2H, d) in Cu(I)NBS2ABT are assigned to benzothiazole protons. The singlet peaks observed at 3.36 ppm, 2.89 ppm and 2.50 ppm in Zn(II)NBS2ABT, and between 3.37 ppm- 2.50 ppm in Cu(I)NBS2ABT are due to metal ions contribution to the spectra in the complexes. The complexes, Ni(I)NBS2ABT, Mn(II)NBS2ABT and Co(II)NBS2ABT are paramagnetic and as such the spectra were not included since they made little or no sense. Peaks at 168.4 ppm in the ligand, and at the range of 170.1-130.0 ppm in the metal complexes are assigned benzothiazole ring carbons. Peaks at the range of 113.1-136.8 ppm in the ligand and its metal complexes are assigned phenyl ring carbon.

8 Magnetic Properties of the Complexes

The result of the magnetic properties of the complexes is shown in Table 1. 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} (1.53 BM). Therefore the zinc complex is diamagnetic, and has sp^3 hybridized geometry, thus tetrahedral structure. Ni(I)NBS2ABT complex showed effective magnetic moment of 1.84 BM. This showed high spin configuration and corresponds to an unpaired electron, indicating paramagnetism. We however concluded that there were some interactions between the ligand, NBS2ABT and the metal in the complex. We

presumed that the ligand, NBS2ABT may have reduced the Ni^{2+} to Ni^+ ion. We therefore proposed sp^3 hybridized geometry for the complex. $Co(II)NBS2ABT$ showed effective magnetic moment of 2.11 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 3.29 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 brown colour of the complex formed. We also proposed sp^3 hybridization of tetrahedral geometry for all the $Cu(I)$ complex investigated.

9 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 2 and 3.

Table 2 showed the activities against multi-resistant bacterial strains isolated under clinical conditions. 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), *P. aeruginosa* strains 34 and multi-resistant *S. aureus* (SR) strain, all isolated from dogs at clinical conditions were used.

Table 3 showed activities of the compounds against Typed Strains (ATCC Cultures) microorganisms. The bacteria cultures used are *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). The fungi, *C. krusei* (ATCC 6258) and *C. albicans* (ATCC 90028) were also used.

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, $Zn(II)NBS2ABT$ showed the highest of activity in that it was active against all the pathogens of the typed strains used-both the bacteria and fungi with MIC of 0.625 mg/ml and IZD of 10 mm against *S. aureus* (ATCC 25923) except *E. coli* (ATCC 25922) and *C. albicans* (ATCC 90028). This followed by the cobalt complex. All the compounds showed activity against *S. aureus* (ATCC 25923). None of the compounds were active against *P. aeruginosa* (ATCC 27853). All the compounds were active against *C. krusei* (ATCC 6258) except the cobalt complex. Only the ligand and its nickel complex were active against *C. albicans* (ATCC 90028) with MIC of 10 mg/ml and IZD of 10 mm. All the compounds synthesized did not show detectable activity against *E. coli* (ATCC 25922).

The result indicates that the zinc complex showed the highest activity against the pathogens. All the compounds except the cobalt complex were active against *C. krusei* (ATCC 6258). Fluconazole is primarily fungistatic but can be fungicidal against certain organisms in dose-dependent manner. Fluconazole was only active against the typed strain *C. albicans* (ATCC 90028) but not against *C. krusei* tested strains. This was confirmed from literature⁽¹⁶⁾. 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 *C. krusei* (ATCC 6258), we can say that the ligand, NBS2ABT and its complexes except the cobalt complex were more active than the fluconazole.

10 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}) are recorded in Table 4.

The result showed that all the synthesized compounds showed high levels of bioactivity against 48 h-nauplii. $Zn(II)NBS2ABT$ showed the highest bioactivity (5.00 ± 0.86 ppm) with EC_{50} of 0.5 ppm while $Mn(II)NBS2ABT$ showed the lowest bioactivity (618.90 ± 30.8 ppm) with EC_{50} of 61.9 ppm. Comparing the compounds, the level of bioactivity is in the order $Mn(II)NBS2ABT > NBS2ABT > Ni(I)NBS2ABT > Co(II)NBS2ABT > Cu(I)NBS2ABT > Zn(II)NBS2ABT$.

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 BSLT. The surviving nauphii were dull and inactive, which may be a sign of Central Nervous System (CNS) depression.

11 CONCLUSION

N-(benzothiazol-2-yl)-2-nitrobenzenesulphonamide and its metal complexes were synthesized. The compounds were characterized using magnetic susceptibility measurements, mass spectrometry, elemental microanalysis, UV/VIS spectrophotometry, infra red, proton and ^{13}C nmr spectroscopies. The spectral analyses confirmed the structures of the compounds synthesized. The antimicrobial tests of the ligands 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. 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 ligand, NBS2ABT and its complexes except the cobalt complex were more active than the fluconazole, and can be recommended for preclinical screening. The Lethal Concentrations (LC₅₀) were within the permissible concentrations.

12 Acknowledgement

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Table 1: Physical properties of the ligand, NBS2ABT and of its metal Complexes, the Magnetic Properties of the Complexes, and Elemental microanalysis of the ligand

S/n	SAMPLES	Melting Point (°C)	Colour	Texture	Mw	μ_{eff} (BM)	No. of Electrons	Properties
1	NBS2ABT	210-212	Yellow	Powdery	358.00	-	-	-
2	Ni(I)NBS2ABT	193-195	Light yellow	Needle-like	764.19	1.84	1	Paramagnetic
3	Mn(II)NBS2ABT	187-189	Yellow	Needle-like	795.94	3.29	1	Paramagnetic
4	Co(II)NBS2ABT	185-187	Light blue	Needle-like	799.93	2.11	1	Paramagnetic
5	Zn(II)NBS2ABT	177-179	Light yellow	Crystalline	735.39	1.53	0	Diamagnetic
6	Cu(I)NBS2ABT	159-161	Light brown	Powdery	769.05	0.71	0	Diamagnetic
Elemental microanalysis of the ligand, NBS2ABT								
		%C		%H		%N		
		Calc.	Found	Calc.	Found	Calc.	Found	
NBS2ABT		46.58	44.98	2.69	3.07	12.54	12.66	

Table 2: Antimicrobial activity of the ligand and of its metal complexes against multi-resistant bacterial strains isolated under clinical conditions

Samples	Multi-resistant bacterial strains isolated from clinical conditions											
	<i>Escherichia coli</i> strains <i>E. Coli</i> Strain 1		<i>Escherichia coli</i> Strain 15		<i>Proteus</i> species strains <i>Proteus</i> spp strains 25		<i>Proteus</i> spp Strains 26		<i>Pseudomonas aeruginosa</i> strains 34		multi-resistant <i>Staphylococcus aureus</i> (SR) strain	
S/n	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	NBS2ABT	00	00	00	00	00	00	00	00	00	00	00
2	Ni(II)NBS2ABT	00	00	00	00	00	00	00	00	00	00	00
3	Mn(II)NBS2ABT	00	00	00	00	00	00	00	00	00	00	00
4	Co(II)NBS2ABT	00	00	00	00	00	00	00	00	00	00	00
5	Zn(II)NBS2ABT	00	00	00	00	00	00	00	00	00	00	00
6	Cu(II)NBS2ABT	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	00	0.05
8	Trimethoprim-sulphamethoxazole		0.025		0.025		0.025		0.025			0.025

Table 3: Antimicrobial activity of the compounds against Typed Strains (ATCC Cultures) microorganisms

S/n	Samples	Typed strains (ATCC Cultures)											
		<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)	IZD (mm)	MIC (mg/ml)
1	NBS2ABT	00	00	00	00	13	20	12	10	10	10	10	10
2	Ni(II)NBS2ABT	00	00	00	00	10	5	12	10	10	10	10	10
3	Mn(II)NBS2ABT	00	00	00	00	12	10	11	10	10	00	00	00
4	Co(II)NBS2ABT	00	00	00	00	12	20	00	00	00	00	00	00
5	Zn(II)NBS2ABT	10	10	00	00	10	0.625	12	10	10	00	00	00
6	Cu(II)NBS2ABT	10	10	00	00	12	10	10	10	00	00	00	00
7	Ciprofloxacin	25	0.005	18	0.005	17	0.005	-	-	-	-	-	-

Table 4: Lethal Concentration (LC₅₀) and Effective Concentration (EC₅₀) Results in ppm (Cytotoxic test)

S/N	SAMPLES	LC ₅₀ (ppm)	EC ₅₀ (ppm)
1	NBS2ABT	401.90±18.0	40.2
2	Ni(I)NBS2ABT	333.60±21.0	33.4
3	Mn(II)NBS2ABT	618.90±30.8	61.9
4	Co(II)NBS2ABT	164.56±40.18	16.5
5	Zn(II)NBS2ABT	5.00±0.86	0.5
6	Cu(I)NBS2ABT	19.66±2.44	2.0