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SYNTHESES AND ANTIMICROBIAL EVALUATION OF BROMINATED IMIDAZOLE AND ITS CO (II), CU(II), MN(II), NI(II), AND ZN(II) COMPLEXES

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Abstract:-
2, 4, 5-tribromoimidazole (tbi) was synthesized and its structure was elucidated using spectroscopic methods. its co(ii), cu(ii), mn(ii), ni(ii), and zn(ii) complexes were synthesized and their antimicrobial activities were evaluated against escherichia coli, bacillus cereus strain cf7, bacillus thuringensis strain eb151, pseudomonas aeruginosa strain 335k55, pseudomonas aeruginosa strain pg1, and candida albicans. these compounds were all inactive to both strains of pseudomonas spp. antimicrobial activities of these compounds is found to be in the order tbi > cu[tbi] > cr[tbi] > ni[tbi] > mn[tbi] > zn[tbi] > co[tbi]. the activity of tbi was reduced upon complexation by all the metal ions used.

Keyword:-
2,4,5-Tribromoimidazole, microbial sensitivity, bioactive metal complexes, Imidazole

INTRODUCTION

The chemistry of Imidazole and its derivatives has gained much interest in recent years due to their wide spectrum of pharmacological and biological activities. The imidazole ring is present in many bioactive compounds which show a high level of bioactivies such as antimicrobial [1-6], anticancer [7-9], analgesic [10-11], anti-HIV [12], and anti-inflammatory [13]. Substituted imidazoles alsoexhibit insecticidal [14], parasiticidal [15], and agrochemical [16] activities. However, there is scarce report in literature regarding the synthesis and antimicrobial activities of metal complexes of tribromoimidazole. Based on this, we have planned the syntheses and antimicrobial evaluation of some metal complexes of tribromoimidazole.

Materials and Methods

All the chemicals used in this study are of analytical grade and from reliable vendors.

Synthesis of 2, 4, 5-Tribromoimidazole

Synthesis of 2, 4, 5-tribromoimidazole was performed using the procedure by Stensio *et. al* (17). Imidazole (2.72 g, 0.04 mol) and anhydrous sodium acetate (40 g) were added to anhydrous acetic acid (360 ml) and stirred for 10 minutes. Bromine (19.2 g, 0.12 mol) in anhydrous acetic acid (40 ml) was added dropwise to the solution of Imidazole/sodium acetate being stirred for over 20 minutes. Sodium acetate (10 g) was added and stirring continued for 200 minutes. The acetic acid was evaporated in vacuo at 50 ^{o}C to almost dryness leaving a cream precipitate. The precipitate was washed with water (500 ml) six times and was filtered and dried. The resulting white powder weighed 16.26 g making up a yield of 74.18 %.

Complexation of 2, 4, 5-Tribromoimidazole

Complexation was performed using the same procedure for all the metal complexes. The metal salt (1 mmole) was dissolved in 2 ml of distilled water and added dropwise to 2 mmoles of 2,4,5-tribromoimidazole being stirred in 10 ml of acetone for over thirty minutes. The resulting coloured solutions were left to stand until the coloured complexes precipitated. The precipitates were washed with distilled water, dried and recrystallized from ethanol.

Characterization of compounds

2, 4,5-Tribromoimidazole and its metal complexes were characterized by FT-IR, GCMS and NMR spectroscopy. FT-IR spectra were obtained using BRUKER FT-IR spectrometer ALPHA II with a range of 4000-440cm^{-1}. GCMS spectra were obtained using Agilent Technologies single quadrupole GCMS 5977B GC/MSD. NMR spectrum was obtained using BRUKER 400 (400MHz for both ^{1}H and ^{13}C).

ANTIMICROBIAL TOXICITY TESTS

Collection and Identification of Microbes

Pure clinical grade microbial isolates of *Escherichia coli*, *Bacillus cereus* strain CF7, *Bacillus thuringensis* strain EB151, *Pseudomonas aeruginosa* strain 335K55, *Pseudomonas aeruginosa* strain PG1 and *Candida albicans* were obtained from the department of microbiology of the University of Port Harcourt, Choba, Nigeria. The organisms were resuscitated using the appropriate media. Nutrient Agar was used for the resuscitation of *Escherichia coli, Bacillus cereus and Bacillus thuringensis.* Cetrimide Agar was used for *Pseudomonas spp* while Potato Dextrose Agar was used for *Candida albicans*. All six microorganisms were re-identified using the standard methods described by Cowan and Steel [18]. They were subcultured on nutrient agar slants and stored at 40^{o} C until required for the study.

Antimicrobial Activities of synthesized compounds

The Agar well diffusion method was method was used to evaluate the antimicrobial toxicity level of these compounds [19]. All the equipment used were sterilized by washing and autoclaved for 15minutes before use. Five concentrations (25, 50, 100, 150, and 200 mg/ml) of each of the synthesized compounds were made by dissolving the equivalent weight in 2 ml of 30 % dimethyl sulphoxide and stored

Results and Discussion

Table 1: Physical appearance and Forms of the Compounds

NAME	COLOUR	FORM
$Cu[TBI]_2$	Orange	Powder
$Ni[TBI]_2$	White	Crystals
$Zn[TBI]_2$	White	Crystals
$Mn[TBI]_2$	Cream	Flakes
$Co[TBI]_2$	Pink	Crystals
TBI	White	Powder

In a refrigerator till required for further use [2021]. Mueller Hilton Agar (38 g) was dissolved in 1000 ml of distilled water and homogenized. Homogenized MHA (20 ml) were bottled and autoclaved and were poured into sterile Petri dishes containing 2 ml of the microorganism (1 X 10^8 cfu) and swirled gently for homogeneity. All the Petri dishes were incubated at 37 ^oC for 24 hours to allow the microorganisms grow. Five holes were made using a cup borer (r = 2mm) on each Petri dish with respect to the five concentrations. 0.2 ml each of the various concentrations of the synthesized compounds was then administered into the labelled holes on the Petri dishes and the Petri dishes were incubated for 24 hours at 37 ^oC to allow for possible inhibition. The diameter of inhibition was measured in triplicates and the mean values reported as the zone of inhibition by the test compounds.

Minimum Inhibitory Concentration

Minimum Inhibitory Concentrations of the test compounds were determined by preparing and administering low concentrations of the synthesized compounds and administered to the organisms. Serial dilution of 25mg/ml was done by successively reducing the strength of the compounds by 50%. Concentrations (mg/ml) of each compound were made as follows: 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, 0.10, 0.05, 0.03, and 0.01. Each concentration was administered on each of the microorganisms in duplicates and the mean values recorded. Minimum inhibitory concentration is taken as the lowest concentration at which there was a visible inhibition.

Table 2: Infrared Absorption Bands for TBI and it Metal Complexes

Name	C-Br	C-N	N-H	C=C	C=N
$Cu[TBI]_2$	659 m	1285 m	3069 w	1643 w	1526 m
$Ni[TBI]_2$	659 m	1286 m	3067 w	1656 w	1525 m
$Zn[TBI]_2$	659 m	1298 m	3067 w	1664 m	1526 m
$Mn[TBI]_2$	659 m	1297 m	3066 w	1675 m	1525 m
$Co[TBI]_2$	659 m	1285 m	3067 w	1677 w	1525 m
TBI	661 m	1298 m	3068 m	1685 w	1528 m

It can be seen from Table 2 that the absorption values for the five functional groups in the ligand-2, 4,5-Tribromoimidazole were shifted upon addition of metal complexes. The band of *the* N-H bond in the ligand has a medium intensity whereas the same bond has weak intensities in all the metal complexes suggesting that the N-H bond is the coordination point.

Table 3: Diameter of Inhibition Zone (Bacterial)

Compound	Conc (mg/ml)	*Pseudomonas aeruginosa strain PG1 (mm)*	*Pseudomonas aeruginosa strain 335K55 (mm)*	*Escherichia coli (mm)*	*Bacillus cereus strain CF7 (mm)*	*Bacillus thuringensis strain EB151 (mm)*
$Cu[TBI]_2$	200	-	-	16.00	-	14.00
	100	-	-	11.00	-	12.00
	25	-	-	6.00	-	8.00
$Ni[TBI]_2$	200	-	-	13.00	-	10.00
	100	-	-	10.00	-	5.00
	25	-	-	5.00	-	-
$Zn[TBI]_2$	200	-	-	16.00	-	-
	100	-	-	11.50	-	-
	25	-	-	7.00	-	-
$Mn[TBI]_2$	200	-	-	18.00	-	-
	100	-	-	11.00	-	-
	25	-	-	6.50	-	-
$Co[TBI]_2$	200	-	-	15.00	-	-
	100	-	-	9.00	-	-

	25	-	-	5.00	-	-
TBI	200	-	-	22.00	12.00	23.00
	100	-	-	17.00	9.50	13.00
	25	-	-	7.00	5.50	6.00
Levofloxacin	200	14.00	13.50	19.50	16.20	17.00
	100	10.20	9.00	15.50	10.20	13.00
	25	4.50	5.50	6.50	6.20	5.50

From Table 3, the two Pseudomonas spp. we're not sensitive to any of the synthesized compounds but were sensitive to the control. Escherichia coli, Bacillus cereusstrain CF7, and Bacillus thuringensisstrain EB151 were very sensitive to Tribromoimidazole while Escherichia coli andBacillus thuringensisstrain EB151 were sensitive to the Cu (II) and Ni (II) complexes. Escherichia coli showed good sensitivity to all the compounds. Upon complexation, the activity of the ligand was either reduced or completely lost. This reduction could be linked to the weak N-H peaks in the metal complexes. In comparison to the reference-levofloxacin, 2, 4,5-Tribromoimidazole had a wider zone of inhibition against E.coli and B.thuringensis and a lesser zone of inhibition against B.cereus. The sensitivities of the microorganisms toward the metal complexes thoughappreciable are less than that towards the reference.

Table 4:Diameter of Inhibition Zone (Fungal)

Compound	Conc (mg/ml)	*Candida Albicans*
Cr[TBI] 3	200	14.00
	100	9.00
	25	6.00
Cu[TBI]2	200	13.00
	100	10.00
	25	5.00
Ni[TBI] 2	200	-
	100	-
	25	-
Zn[TBI] 2	200	13.50
	100	10.00
	25	6.50
Mn[TBI] 2	200	13.00
	100	7.00
	25	5.50
Co[TBI] 2	200	14.00
	100	8.00
	25	5.00
TBI	200	14.00
	100	12.00
	25	8.00
Griseofulvin	200	18.50
	150	12.00
	25	7.20

From Table 4 above, the zone of inhibition by the ligand against the fungus was either slightly reduced or unchanged upon complexation with these metals. The ligand and its metal complexes are not as strong as the reference as an antifungal as the zones of inhibition by the synthesized compounds are all less than the zone of inhibition by the reference antifungal.

Table 5: Minimum Inhibitory Concentrations

Compound	*Escherichia coli (mg/ml)*	*Bacillus cereus strain CF7 (mg/ml)*	*Bacillus thuringensis strain EB151 (mg/ml)*	*Candida Albicans (mg/ml)*
Cu[TBI] 2	0.39	NA	3.13	0.39
Ni[TBI] 2	3.13	NA	>50	NA
Zn[TBI] 2	1.56	NA	NA	0.20
Mn[TBI] 2	0.78	NA	NA	1.56
Co[TBI] 2	6.25	NA	NA	3.13
TBI	0.78	3.13	1.56	0.10
Griseofulvin	0.39	0.78	0.39	0.20

Key: NA = Not Active

Table 5 shows the minimum inhibitory concentrations of the synthesized compounds and the reference antifungal agent. The MIC values against E.coli shows that the microorganism was sensitive to Cu (II), Mn(II), and the ligand at very low concentrations. B.thuringensis was sensitive to Cu (II), Zn (II), and TBI at low concentrations. This suggests that these compounds are potent antifungal agents

CONCLUSION

Metal complexes of 2, 4, 5-Tribromoimidazole have shown antimicrobial activities against some microbial strains. The sensitivities of the microorganisms to these complexes were less when compared to that of the ligand-2, 4,5-tribromoimidazole and this may be attributed to the weak N-H absorptions in the metal complexes. Complexation did not enhance the activities of the ligand, however, their values suggest that they are potent antimicrobial agents

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