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SYNTHESES AND EVALUATION OF THE ANTIMICROBIAL ACTIVITIES OF HALOGENATED IMIDAZOLES AND THEIR MN(II), NI(II), AND ZN(II) COMPLEXES

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Abstract:-

4, 5-Dichloroimidazole (4, 5-DCI) and 2, 4,5-Tribromoimidazole were synthesized, recrystallized from the appropriate solvents and characterized using spectroscopic methods. They were screened for their antimicrobial activities against five bacterial strains namely: Escherichia coli, Bacillus cereus strain CF7, Bacillus thuringensis strain EB151, Pseudomonas aeruginosa strain 335K55, and Pseudomonas aeruginosa strain PG1. These microorganisms were very sensitive to the compounds. Their sensitivities were increased appreciably by the Ni^{2+} and Mn^{2+} complexes of 4, 5-dichloroimidazole and reduced by its Zn^{2+} complex. Their sensitivities towards 2, 4, 5-tribromoimdazole were either greatly reduced or totally lost upon complexation. The differences in these sensitivities are attributed majorly to the halogens as the metal ions are common to both ligands.

Keywords: - *4, 5-Dichloroimidazole, 2, 4, 5-Tribromoimidazole, Antimicrobial activity, Bioactive metal complexes, Microbial sensitivity*

INTRODUCTION

Imidazole is a five-membered heterocyclic compound with two nitrogen atoms making up the ring. Its compounds have a wide spectrum of activities against many organisms. Derivatives of imidazole have also been discovered to possess extensive bioactive properties as antimicrobial [1-8], anti-HIV [9], anticancer [10-12], analgesic [13-14], and anti-inflammatory [15] agents. Halogenated imidazoles are not left out in the bioactive substances containing the imidazole moiety. The bioactivities of a great number of substances have been enhanced by complexing with bioactive metals [16-17]. This study takes a look at the sensitivity of some bacteria towards two halogenated imidazoles and their metal complexes.

Materials and Methods

All the chemicals used in this study are of analytic grade and did not require further purification. The microorganisms used in this study were of clinical grade and were appropriately re-identified.

Syntheses of the ligands and their Metal Complexes

4, 5-Dichloroimidazole

4, 5-Dichloroimidazole was synthesized using the procedure of Lutz and Delorenzo [18] with some modifications. Sodium hydroxide (4.72 g, 0.12 moles) was added to a stirred solution of sodium hypochlorite (3.5 % v/v, 500 ml) and stirred till it dissolved completely. Imidazole (8 g, 0.12 moles) was added at room temperature to the stirred solution which turned yellow with the temperature rising to 44^{o}C and a pH of 11. It was allowed to stand for five minutes after which concentrated hydrochloric acid (40 ml) was added till a cream coloured precipitate appeared (pH=6). The precipitate was washed, dried and recrystallized from water to obtain yellow colour crystals. The product obtained after recrystallization had a mass of 6.38g, corresponding to a yield of 49.34% with a melting point range of 180-181^{o}C.

2, 4, 5-Tribromoimidazole

Synthesis of 2, 4, 5-tribromoimidazole was performed using the procedure by Stensio *ET. Al* (19). 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 the ligands

Complexation was done using the same procedure for all metal salts. The metal salts (1 mmole) in 2 ml of distilled water and added dropwise to 2 mmoles of the ligand 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

The ligands and their 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 Agilent technologies NMRvnmrs400 (400MHz for both ^{1}H and ^{13}C).

Antimicrobial Toxicity Tests for the compounds

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, and *Pseudomonas aeruginosa* strain PG were obtained from the department of microbiology of the University of Port Harcourt, Choba, Nigeria. These 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*. All five organisms were re-identified using the standard methods described by Cowan and Steel [20]. 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 [21]. All the equipment used were sterilized by washing and autoclaving for 15 minutes 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 in a refrigerator till required for further use [22-23]. Mueller Hilton Agar (38 g) was dissolved in 1000 ml of distilled water, homogenized and 20 ml each was poured into spice bottles 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 ^{o}C for 24 hours to allow the

microorganisms grow. Five holes were made using a cup borer (r = 2 mm) 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 ^{o}C 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. The physical properties and the frequencies of infrared absorption of DCI and its metal complexes are shown in Table1. The remarkable differences in the absorption value of N-H bond in 4,5-dichloroimidazole and its complexes suggests that the nitrogen atom is the coordination centre with the metals.

Results and Discussion

Table 1: Colour and IR Data of 4, 5-Dichloroimidazole (DCI) and Its Metal Complexes

Name	colour	Appearance	(C-Cl) cm^{-1}	(C-N) cm^{-1}	(N-H) cm^{-1}	(C=C) cm^{-1}	(C=N) cm^{-1}
$Ni[DCI]_2$	Purple	Powder	665 m	1314 m	3040 w	1629 w	1573 m
$Zn[DCI]_2$	Yellow	Crystals/Powder	662 m	1316 m	3108 m	1629 w	1554 m
$Mn[DCI]_2$	Grey	Flakes	665 m	1314 m	3042 m	1637 w	1537 m
DCI	Yellow	Crystals	665 m	1314 m	3128 w	1636 w	1573 m
Key: w = weak, m = medium							

Table 2 shows the physical properties and infra-red absorption of TBI and its metal complexes. The absorption peak of the N-Hn TBI was reduced from medium to weak suggesting that the imino nitrogen is involved in the coordination to the metal ions.

Table 2: Colour and IR Data of 2, 4,5-Tribromoimidazole (TBI) and Its Metal Complexes

Name	colour	Appearance	(C-Cl) cm^{-1}	(C-N) cm^{-1}	(N-H) cm^{-1}	(C=C) cm^{-1}	(C=N) cm^{-1}
$Ni[TBI]_2$	White	Crystals	659 m	1286 m	3067 w	1656 w	1525 m
$Zn[TBI]_2$	White	Crystals	659 m	1298 m	3067 w	1664 m	1526 m
$Mn[TBI]_2$	Cream	Flakes	659 m	1297 m	3066 w	1675 m	1525 m
TBI	White	Powder	661 m	1298 m	3068 m	1685 w	1528 m
Key: w = weak, m = medium							

Table 3 shows the result of the antimicrobial assay for DCI and its Metal Complexes. On the basis of the diameters of inhibition, the microorganisms were found to be sensitive to 4,5-DCI. The sensitivities of these microorganisms to 4,5-Dichloroimidazole were increased by the metal ions Ni2+and Mn2+. The Zn2+complex is however appreciably active but the sensitivities shown by the microorganisms were less than those observed for 4,5-DCI, Mn(II), and the Ni(II) complexes.

Table 3: Zone of Inhibition by 4,5-DCI and its Metal Complexes

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)*
	200	23.00	21.00	25.00	26.00	24.00
	100	17.00	12.00	20.00	21.00	17.00
$Ni[DCI]_2$	25	6.00	6.00	15.50	16.50	11.00
	200	12.00	14.00	21.00	24.00	16.00
	100	10.00	10.50	17.00	21.00	14.00
$Zn[DCI]_2$	25	6.00	6.00	11.00	15.00	10.00
	200	18.00	18.00	24.00	22.00	26.00
	100	14.00	11.00	20.00	20.00	18.00

Mn[DCI] $_2$	25	10.00	8.00	12.00	16.00	11.00
	200	16.00	18.00	22.00	23.00	22.00
DCI	100	10.00	11.00	14.00	17.00	18.00
	25	7.00	8.00	11.00	12.00	12.00

Table 4 shows the result of the antimicrobial assay of TBI and its complexes. The two Pseudomonas spp. We're not sensitive to any of the synthesized compounds. 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.

Table 4: Zone of Inhibition by 2, 4, 5-TBI and its Metal Complexes

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)*
	200	-	-	13	-	10
	100	-	-	10	-	5
Ni[TBI] $_2$	25	-	-	5	-	-
	200	-	-	16	-	-
	100	-	-	11.5	-	-
Zn[TBI] $_2$	25	-	-	7	-	-
	200	-	-	18	-	-
	100	-	-	11	-	-
Mn[TBI] $_2$	25	-	-	6.5	-	-
	200	-	-	22	12	23
	100	-	-	17	9.5	13
TBI	25	-	-	7	5.5	6

Conclusion

The values from the results obtained shows that chlorinated imidazoles are more effective as antimicrobial agents than the brominated imidazoles. This effects could be linked to the remarkable differences in the wavelengths of the absorptions of the major functional groups of both compounds.

This study has shown that the sensitivity of microorganisms to bioactive substances can be increased or decreased by the addition of bioactive metal ions. It has also shown that these compounds possess microbial toxicity even at very low concentrations, thus are potential antimicrobial agents.

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