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EVALUATION OF THE MICROBIAL TOXICITIES OF 4, 5-DICHLOROIMIDAZOLE AND ITS MN (II), NI (II), AND ZN (II) COMPLEXES

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Abstract:-
4, 5-Dichloroimidazole (4,5-DCI) was synthesized, recrystallized from water and characterized using spectroscopic methods. It was screened for its 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 compound. Their sensitivities were increased appreciably by the Ni^{2+} and Mn^{2+} complexes of 4,5-dichloroimidazole and reduced by its Zn^{2+} complex. The sensitivities of these microorganisms towards these compounds were higher than their sensitivities towards Levofloxacin – a reference antibacterial.

Keywords:- *4, 5-Dichloroimidazole, Antimicrobial activity, bioactive metal complexes, Imidazole, Microbial sensitivity, Microbial Toxicity*

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 antiinflammatory [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 4,5-dichloroimidazole and its metal complexes.

Materials and Methods

All the chemicals used in this study were of analytic grade and did not require further purification.

Syntheses of 4, 5-Dichloroimdazole and its 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.

Complexation of 4,5-Dichloroimidazole

Complexation was done using the same procedure for all metal salts. The metal salts (1 mmole) salt in 2 ml of distilled water and added dropwise to 2 mmoles of 4,5dichloroimidazole 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

4,5-Dichloroimidazole 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 4000440cm^{-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 for the compounds

Collection and Identification of Microbes

Pure clinical grade microbial isolates of *Escherichia coli*, *Bacillus cereus* strain CF7, *Bacillus thuringensis* strain B151, *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 [19]. 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 [20]. 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 [21-22]. 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.

Minimum Inhibitory Concentration

Minimum Inhibitory Concentrations of the test items were determined by preparing and administering low concentrations of the synthesized compounds and administered to the organisms. Serial dilution of 25 mg/ml was done by 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.

Results and Discussion

The structure of 4, 5-dichrloroimidazole was supported by the molecular weight of 137 amu from its mass spectra. Its fragmentation pattern and infrared spectra also supported the possible arrangements of the atoms. The fragmentation pattern is shown in Table 1 below.

Table 1: Fragmentation Pattern of 4,5-Dichloroimidazole

Peak	possible fragmentation pattern
41	$[N_2CH]$
94	$[C_2Cl_2]^+$
109	$[C_2NCl_2]$
122	$[C_3NHCl_2]$
137	$[C_3N_2H_2Cl_2]$

The physical properties of the complexes and dichloroimidazole and its metal complexes are the frequencies of infrared absorption of 4, 5- shown in Table 2

Table 2: Colour and IR Data of 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							

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.

Antimicrobial Activity of4, 5-Dichloroimidazole and its Metal Complexes

The compounds were assayed for their antimicrobial activities against five microorganisms: Escherichia coli, Bacillus cereusstrain CF7, Bacillus thuringensisstrain EB151, Pseudomonas aeruginosastrain 335K55, and Pseudomonas aeruginosastrain PG1. Levofloxacin was used as a reference antimicrobial.The diameter of inhibition at 200mg/ml is shown in Table 3.The MIC values are shown in Table 4

Table 3: Zone of Inhibition by 4,5-DCI and its Metal Complexes at 200mg/ml

Compound 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)***
$Ni[DCI]_2$	23.00	21.00	25.00	26.00	24.00
$Zn[DCI]_2$	12.00	14.00	21.00	24.00	16.00
$Mn[DCI]_2$	18.00	18.00	24.00	22.00	26.00
DCI	16.00	18.00	22.00	23.00	22.00
Levofloxacin	14.00	13.50	18.50	18.20	17.00

Table 4: Minimum Inhibitory Concentrations of 4,5-DCI and its Metal Complexes

Compound	***Pseudomonas aeruginosa strain PG1 (mg/ml)***	***Pseudomonas aeruginosa strain 335K55 (mg/ml)***	***Escherichia coli (mg/ml)***	***Bacillus cereus strain CF7 (mg/ml)***	***Bacillus thuringensis strain EB151 (mg/ml)***
$Ni[DCI]_2$	12.50	6.25	0.05	0.10	0.10
Zn[DCI]2	3.13	6.25	0.20	0.20	0.03
Mn[DCI]2	0.20	0.10	0.39	0.05	0.20
DCI	1.56	0.78	0.39	0.20	0.05
Levofloxacin	6.25	0.78	1.56	1.56	0.78

On the basis of the diameters of inhibition, the microorganisms were found to be more sensitive to 4, 5-DCI than the reference antimicrobial. The sensitivities of these microorganisms to 4,5Dichloroimidazole were increased by the metal ions Ni^{2+} and Mn^{2+}. Zn^{2+} complex is however appreciably active but the sensitivity shown by the microorganisms were less than those observed for 4, 5-DCI.

On the basis of minimum inhibitory concentrations, Mn[DCI] is the most effective against *Pseudomonas auruginosa* PG1 and 335k55, and *Bacillus cereus* CF7. Ni [DCI] is the most active against *Escherichia coli* while Zn [DCI] is the most active against *Bacillus thuringensis* EB151.

Conclusion

This study has shown that the sensitivity of microorganisms to bioactive substances can be increased 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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