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The Potential Antifungal and Antibacterial of 5-Chloro-1-Methyl-4-Nitroimidazole

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ABSTRACT

Imidazole is a nitrogen-containing heterocyclic ring that possesses biological and pharmaceutical importance. The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine, and nucleic acid, while the nitroimidazole and its derivatives possess an extensive spectrum of biological activities such as antibacterial, anticancer, antitubercular, antifungal, analgesic, and anti-HIV activities. This paper aims to evaluate the *in vitro* antifungal and antibacterial activities of 5-chloro-1-methyl-4-nitroimidazole. The synthesis of this compound was carried out via a three-step reaction such as aminolysis, chlorination and nitration. From the FT-IR, ^{1}H NMR, and ^{13}C NMR spectra of the obtained compound, the absorption bands, signals, and coupling constant conform to the proposed compound. Furthermore, when tested for its in-vitro antifungal and antibacterial activities, the derivative showed excellent antifungal activity against a clinical strain of *Candida albicans and Aspergillus niger* at a minimum bactericidal concentration of 2.5 mg/mL, while the bacterial isolate of *Pseudomonas aeruginosa* and *Klebsiella. pneumonie* was at 4.0 mg/mL.

Keywords: Imidazole, synthesis, characterization, antibacterial, antifungal.

INTRODUCTION

Over the years, microbial infections were frequently fatal, with sulfa-drugs being the initial attempt at combating pathogens (Ranpariya and Tarpara, 2023). But in 1928, at St. Mary's Hospital, London, Alexander Fleming's discovery of *Penicillium notatum* inhibiting *Staphylococcus aureus* marked the beginning of the antibiotic era and revolutionizing the treatment of infectious diseases caused by isolates (Baran et al., 2023). Antibiotics act through various mechanisms that include inhibiting of cell wall biosynthesis, disrupting cell membranes, and interfering with nucleic acid or protein synthesis (Murugaiyan et al., 2022). More also, the overuse and misuse of these available antibiotics have led to the emergence of multidrug-resistant microbes, posing a significant global health challenge (Anderson et al., 2023). In addressing this, studies have shown that heterocycles with a diverse range of biological activities, such as antibacterial and antifungal properties, have the potential to enhance innate immunity and inhibit the growth of these microbes (Tang et al., 2023). Notably, heterocycles like quinoline, benzimidazoles, imidazolines, imidazolidines, and imidazoles possess these capabilities owing to their structural features and electron-rich nature (Sethiyaa et al., 2021).

Imidazole is the trivial name for 1,3-azole, while the earlier given names were glyoxaline and imidazole. It is a five-membererd ring heterocycle with two-heteroatoms. Imidazole is a colorless solid that is highly soluble in water and other polar solvents due to its amphoteric properties, displaying both weak acid and basic characteristics (Siwach and Verma, 2021.) It exists in two tautomeric forms with the hydrogen atom taking position between the two nitrogen atoms. The synthesis and reactivity of imidazoles have been extensively studied, highlighting their importance in pharmaceutical science (Serdaliyeva et al., 2022). Overall, imidazole plays a crucial role in the development of new pharmacological agents, showcasing their potential in treating

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various diseases and contributing significantly to advancements in medicinal chemistry. Imidazole is indeed prevalent in significant biomolecules like biotin, histidine, histamine, and pilocarpine alkaloids (Tripathi, and Malviya, 2023; Yu and Neborak, 2022).

Nitroimidazole derivatives have shown promise in various biological activities, including anti-cancer, anti-inflammatory and anti-microbial properties by interacting with enzymes, proteins, and receptors effectively (Alghamdi, 2021). For example, Owolabi *et al* 2019, described the synthesis, tentative characterization and antimicrobial activities of 1-ethyl-2-methyl-4-nitroimidazole-5-thiol and its derivatives. The derivatives revealed a moderate antifungal activity against a fungal strain, *C. albicans* when compared to a standard drug, penicillin.

5-chloro-1-methyl-4-nitroimidazole itself is a derivative of imidazole as well as nitroimidazole. This derivative is commercially available but to the best of our knowledge, there is no report of its antifungal and antibacterial properties on these selected isolates such as *Candida albicans Aspergillus niger, Staphylococcus aureus, Pseudomonas aeruginosa Escherichia coli, Klebsiella pneumonia and Bacillus subtilis*, despite having chloro and nitro groups in its structure. We aim to fill this gap and also show its spectroscopic characteristics. Therefore, this article presents the potential antifungal and antibacterial of 5-chloro-1-methyl-4-nitroimidazole.

MATERIAL AND METHODS

2.1 Material

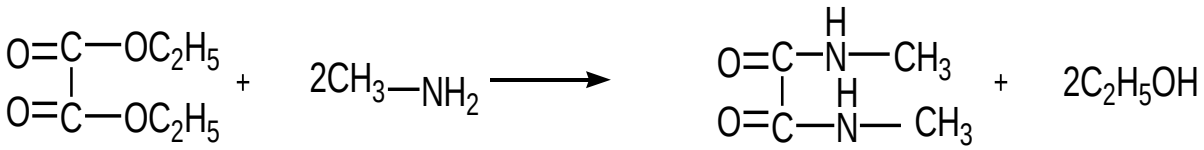
Analytical grade reagents and solvents were procured from Sigma Aldrich, Germany. JHD, England., BDH, England., Qualikems, India and Merck and Flow Inc, Poland.

Equipment: Hot plate, vacuum pump, muffles furnace, desiccator, and heating bath. Others are; Column Chromatography, Electrothermal melting point apparatus CAT No. 1A6304, England, Thin layer chromatography (TLC), Wincom XMTE-205 water-bath (HH-600) was used for concentration of the synthesized compounds. Electronic weighing balance (Model Nel 1994, Ohaus Corporation, USA) was used for weighing.

Antimicrobial Activities: The microorganisms used for this study were procured from University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The synthesized compound was screened for their antimicrobial activities against a gram positive bacteria (*S.aureus*) and gram negative *(E.coli, K.pneumae, B. subtilis, P. aeroginousa)* and antifungi activities against *A.niger* and *C.albicans*

2.2 Methods

2.2.1 Synthesis of *N, N* - Diethyloxamide (Compound 1): This was prepared according to the following reported procedure.



SCHEME 2.2.1

10 mL of diethyloxalate was poured into a dropping funnel. 11 mL of methylamine was measured and poured into a round bottom flask. The diethyloxalate was allowed to drop gently into the round bottom flask containing the methylamine. The round bottom flask was immersed in ice bath since the methylamine in the flask is volatile. The reaction was continuously stirred using a magnetic stirrer until all the diethyloxalate in the dropping funnel has been used up. During the process of stirring, crystals of *N, N*-dimethyloxamide (compound 1) were formed. The crystals of compound 1 were filtered, washed with distilled water and air-dried. The weight of compound 1 obtained was 68.9 g (59.4% yield).

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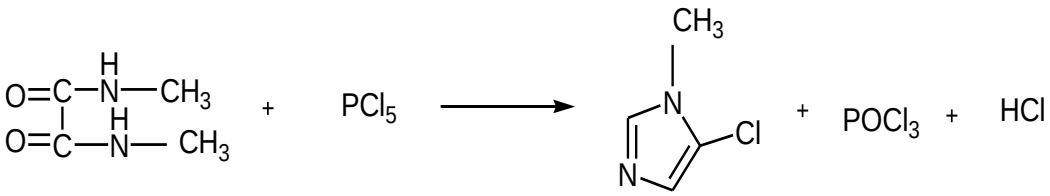
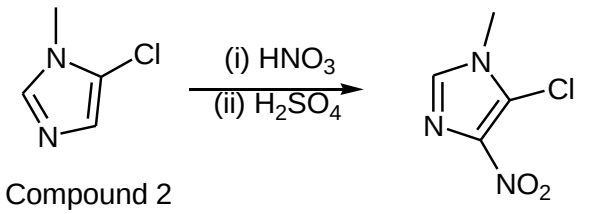
2.2.2 Synthesis of 5-chloro-1-methylimidazole (Compound 2): This was prepared according to the following reported procedure.



SCHEME 2.2.2

15 g (0.129 mole) of *N*, *N* - dimethyloxamide and 45 g (0.234 mole) of PCl_5 were weighed out and poured into a conical flask. The flask was hand shaken for some few minutes. A brown colour of the product, 5-chloro-1-methylimidazole (compound 2) was obtained. The mixture was immersed in hot water for about 12 hours after which a deep brown compound was obtained. This was allowed to stand for 12 hours after which the by-products such as $POCl_3$ were distilled off using a vacuum distillation set up. A 10% (0.179 mole, 1.786 M) solution of NaOH was prepared and used to neutralise the medium containing the compound. Compound 2 was, then, extracted thrice from the medium using chloroform into a conical flask. The chloroform was distilled off from the imidazole compound using simple distillation method. The residue (a liquid) was the imidazole compound of interest. The volume of the imidazole compound was 22.5 mL.

2.2.3 Synthesis of 5-chloro-1-methyl-4-nitroimidazole (Compound 3): This was prepared according to the following reported procedure.



Compound 2 Compound 3

SCHEME 2.2.3

The volume of compound 2 (22.5 mL) obtained was poured into an evaporating dish. Three times the volume of compound 2 (67 mL) was the volume of concentrated HNO_3 added to compound 2 in the evaporating dish. The mixture was then evaporated almost to dryness using a water bath. After about 2 hours, 67 mL of concentrated H_2SO_4 was also added to the content in the evaporating dish. This was done in the cold with a continuous shaking for some few minutes. The resulting mixture was heated over a water bath for 1 hour to complete the nitration process. The solution was allowed to cool for some time before it was poured into crushed ice. This was shaken for some time and allowed to stand.

Thereafter, NaOH pellets were gradually added to the resulting solution. On the addition of NaOH pellets, crystals of 5-chloro-1-methyl-4-nitroimidazole (compound 3) begin to appear. The crystals were filtered, washed with distilled water and air-dried at room temperature. The compound was purified by recrystallization and the melting point was determined. The weight of compound 3 obtained was 21.42 g (51.94 %) and the melting point was 168 – 170°C.

GENERAL CONSIDERATIONS

IR spectra were recorded on a FTIR spectrophotometer. ^{1}H NMR spectra were recorded on 600 MHz spectrometer at 295 K in $CDCl_3$ chemical shifts (δ ppm) and coupling constants (Hz) are reported in standard fashion with reference to either internal standard tetramethylsilane (TMS) (δ= 0.00 ppm) or $CHCl_3$ (δ = 7.25 ppm). ^{13}C NMR spectra were recorded on 600 MHz spectrometer at 25 °C in $CDCl_3$; chemical shifts (δ ppm) are reported relative to $CHCl_3$ [δ = 77.00 ppm (central line of triplet)]. In the ^{1}HNMR, the following abbreviations

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were used throughout: s = singlet, d = doublet, t = triplet, q = quartet, qui = quintet, dd = doublet of doublets, m = multiplet and br s. = broad singlet.

RESULTS AND DISCUSSION

3.1 Thin Layer Chromatography

Table 1 displays the R_f values for both the intermediate and final compounds. Single round spots emerged upon exposing the chromatograms to iodine vapour, confirming the purity of the synthesized compounds and the conclusion of the reactions.

$$\text{Retention factor } (R_f) = \frac{\text{Distance moved by compound}}{\text{Distance moved by solvent}}$$

Table 1: Comparative R_f values of intermediate and final compound with the solvent used as their mobile phase.

Compounds	**R_f values**	**Solvent used as mobile phase**
Compound 1	0.83	$CHCl_3$ and C_2H_5OH (2:1)
Compound 3	0.65	$CHCl_3$ and C_2H_5OH (2:1)

Table 2: Zones of Inhibition of Bacterial and Fungal Isolates

Antibiotics	**Disc Potency (mg)**	**Zone of Inhibition (mm)**						
		S. aureus	***E. coli***	***B. subtilis***	***K. pneumonia***	***P. aeruginosa***	***A. niger***	***C. albicans***
Ciprofloxacin	0.01	32.00	NZ	33.00	26.00	38.00	0.00	0.00
Ketoconazole	25.00	0.00	0.00	0.00	0.00	0.00	9.00	7.00
Compound 3		30.00	25.00	25.00	20.00	24.00	25.00	30.00

Table 3: Antimicrobial Activity of Compound 3 (MIC)

	Microbes	**Control (30% Tween-80)**	**8 mg/mL**	**5 mg/mL**	**4 mg/mL**	**2.5 mg/mL**	**1.5 mg/mL**
Bacterial Isolates	*E. coli*	-	NG	NG	NG	G	G
	S. aureus	-	NG	NG	NG	NG	G
	B. subtilis	-	NG	NG	NG	NG	G
	P. aeruginosa	-	NG	NG	NG	G	G
	k. pneumoniae	-	NG	NG	NG	G	G
Fungal Isolates	*A. niger*	-	--	NG	--	NG	-
	C. albicans	-	--	NG	--	NG	-

NG = No Growth, G = Growth

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Table 4: Antimicrobial Activity of Compound 3 (MBC)

	Microbes	**Control (30% Tween-80)**	**8 mg/mL**	**5 mg/mL**	**4 mg/mL**	**2.5 mg/mL**	**1.5 mg/mL**	**1.25 mg/mL**
Bacterial Isolates	*E. coli*	-	NG	NG	G	G	G	
	S. aureus	-	G	G	G	G	G	
	B. subtilis	-	G	G	G	G	G	
	P. aeruginosa	-	NG	NG	NG	G	G	
	k. pneumoniae	-	NG	NG	NG	G	G	
Fungal Isolates	*A. niger*	-		NG	-	NG		G
	C. albicans	-		NG	-	NG		G

NG = No Growth, G = Growth

Table 5: Characteristic FT-IR Bands of Compound 3

Serial No.	**Functional Group (Vibration Mode)**	**Wave number (cm^{-1})**
1.	sp^3 N – CH_3 stretch	2774.84
2.	C = C bend	800.64
3.	C-N Stretch	1250.78
4.	C-Cl Stretch	550.38

Spectra Interpretation

^{1}HNMR Fig 1: (600 MHz, $CDCl_3$, ppm): δ 7.26 (s, solvent signal), δ 3.72 (s, 3H, N-CH_3), δ 7.49 (s, 1H, N=C-H of the imidazole ring). **^{13}C NMR Fig 2:** 600 MHz, $CDCl_3$, ppm): δ 142.11 (N-CH_3), δ 77.8 (s, solvent signal), δ 119.42 (s, C-Cl), δ 32.61 (s, C=N), δ 134.46 (s, C-NO_2).

3.2 Discussion

3.2.1 FT-IR and NMR Analysis

Table 5 shows the characteristic FT-IR bands of compound 3. The FT-IR spectrum displayed absorption bands at 2774.84cm^{-1}, 800.64cm^{-1} (C = C stretch), 1250.78cm^{-1} (C-N Stretch) and 550.38cm^{-1} (C – Cl stretch). The NMR result showed **^{1}HNMR Fig 1**: (600 MHz, $CDCl_3$, ppm): δ 7.26 (s, solvent signal), δ 3.72 (s, 3H, N-CH_3), δ 7.49 (s, 1H, N=C-H of the imidazole ring). **^{13}C NMR Fig 2:** (600 MHz, $CDCl_3$, ppm): δ 142.11 (N-CH_3), δ 77.8 (s, solvent signal), δ 119.42 (s, C-Cl), δ 32.61 (s, C=N), δ 134.46 (s, C-NO_2) and this correspond to the expected compound 3.

3.2.2 Antibacterial Activity

3.2.2.1. Zone of Inhibition

Table 2 represents the zone of inhibition for both standard drugs and the synthesised compound respectively which was measured in millimetre at different concentrations. After 24 hours, the zone of inhibition was seen, and the results were compared with those of the conventional medication, ciprofloxacin. The Table 2 showed

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the zone of inhibition for the tested standard drugs that include ciprofloxacin and ketoconazole for both the bacterial and fungi isolate respectively. The ciprofloxacin has highest zone of inhibition at the concentration of 10 ug/mL on the *P. aeruginosa* with 38.00 mm followed by *B. subtilis* with 33.00 mm, then *S. aureus* at 32.00 mm and *K. pneumonia* at 26.00 mm, but had no zone of inhibition for *E. coli.*

More also, the ketoconazole which the standard drugs for the fungus isolate exhibits the highest zone of inhibition of the concentration 25 ug/mL on the *A. niger* with 9.00 mm and *C. albicans* was with 7.00 mm. Compound 3 at the 20 mg/mL concentration has a zone of inhibition for *S. aureus* (30.00 mm), *P. aeruginosa* (24.00 mm), *K. pneumonia* (20.00 mm), *B. subtilis* (25.00 mm) and *E. coli* (25.00 mm). The compound 3 activity is moderately low as compared to the standard drugs used which is ciprofloxacin which has a better activity at same concentration for *S. aureus* (30.00 mm), *P. aeruginosa* (22.00 mm), *B. subtilis* (33.00 mm) but has no inhibition for *E. coli.* The compound 3 was able to show higher and better activity for *E. coli* (25.00 mm) than the standard drug, ciprofloxacin.

3.2.2.2 Minimum Inhibitory Concentration (MIC)

This is the lowest concentration of the compound to inhibit the growth of bacteria completely (Siddiqui et al., 2013). The minimum inhibitory concentrations (MIC) were determined following 24-48 hours of incubation for the synthesized compounds that showed activity against the selected bacterial and fungal strains.

For compound 3, the MIC values are presented in Table 3. The Minimum Inhibitory Concentration (MIC) of compound 3 against *S. aureus, P. aeruginosa, E. coli, K. pneumoniae* and *B. subtilis* had no observable growth at concentration of 8 mg/mL, 5 mg/mL and 4 mg/mL. This suggests that compound 3 effectively hindered the growth of these isolates at those concentrations. However, when the concentration was reduced to 2.5 mg/mL, the MIC indicated growth for *E. coli, P. aeruginosa* and *K. pneumonia.* Consequently, at the 2.5 mg/mL concentration, the synthesized compound exhibited inactivity against these particular isolates. When the concentration was reduced further to 1.5 mg/mL, the MIC indicated growth for all bacterial isolate used. This indicated that compound 3 is dose dependent.

The Minimum Inhibitory Concentration (MIC) of compound 3 against *A. niger* and *C. albicans* had no observable growth at concentration of 5 mg/mL and 2.5 mg/L. This suggests that compound 3 effectively hindered the growth of these isolates at these concentrations. However, The Minimum Inhibitory Concentration (MIC) of compound 3 is 2.5 mg/mL.

3.2.2.3 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentrations (MBC) for the tested synthesized compound were observed at different concentration. This can be seen from the results obtained in Tables 4. Therefore, it can be inferred that the synthesized compounds has their lowest concentration required to lethal any of the tested organisms.

For compound 3, the MBC values are presented in Table 4. The minimum bactericidal concentration (MBC) of compound 3 against *A. niger and C. albicans* shows no observable growth which indicated that compound 3 can lethal these particular isolates 5 mg/mL, 4 mg/mL, 2.5 mg/mL but at concentration of 1.25 mg/mL the compound 3 cannot eradicate the fungi isolate but can only inhibit.

CONCLUSION

In this research work, we report the synthesis of an imidazole derivative, 5-chloro-1-methylimidazole. First was the synthesis of *N, N* – dimethyloxamide. Second, the obtained product was made to react with PCl_5 to yield 5-chloro-1-methylimidazole (compound 2). Third, the compound 2 was then nitrated to yield 5-chloro-1-methyl-4-nitroimidazole (compound 3). The purity and characterisation of the synthesized compound were confirmed by determination of physical properties (melting points and R_f values), FT-IR spectroscopy, ^{13}CNMR and ^{1}HNMR. The synthesized compounds were evaluated for their antibacterial and antifungal against some species of bacteria and fungi. Compound 3 exhibited an antibacterial activity against all the tested bacterial species (*S. aureus, E. coli, P. aeruginosa, K. pneumonia* and *B. subtilis*) and antifungal activity against *A. niger* and *C.*

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albicans. Importantly, it showed a better antibacterial activity against *K. pneumonia* than the standard ciprofloxacin. Also, it revealed a good antifungal activity against *A. niger* than the standard, ketoconazoa.

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