SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL ACTIVITIES OF SOME NOVEL IMIDAZOLE DERIVATIVES

BY

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UNIVERSITY OF BENIN,

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MARCH, 2024

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A RESEARCH PROJECT REPORT IN THE DEPARTMENT OF CHEMISTRY, SUBMITTED TO SCHOOL OF POST GRADUATE STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DOCTORATE DEGREE IN ORGANIC SYNTHESIS

CHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY

MARCH, 2024

CERTIFICATION

We certify that this research work was carried out by **Eriamiatoe Imuetinyan** in the Department of Chemistry, University of Benin.

Eriamiatoe Imuetinyan **Project Student**	**Date**
Prof B.J Owolabi **Project Supervisor**	**Date**
Dr. O. Iyekowa **Co-Project Supervisor**	**Date**
Prof J.U Iyasele **Head of Department**	**Date**

DEDICATION

This project is dedicated to Almighty God and to my beloved Husband Mr. Eriamiatoe Teddy and my sweet Mother Mrs. Helen Osiomwan.

ACKNOWLEDGEMENT

With a profound gratitude, I thank God Almighty for giving me life, wisdom, knowledge, protection and provision in order to carry out this research work.

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ABSTRACT

Heterocyclic compounds are very widely distributed in nature and very abundant in plant and animal products. They are included in many biochemical materials essential for life like nucleic acids (nucleotides), sugars and their derivatives, vitamin C and also, most members of vitamin B group (vitamin B6- pyridoxine). They are also found in application of diverse field such as agriculture, pharmaceutical and manufacturing industries. Researches have shown that heterocyclic nuclei give high chemotherapeutic values such as anti-malaria, anti-diabetics, anticancer and also act as a remedy for the development of novel drugs.

Imidazole containing moiety occupied a unique position in organic compounds. It is a five-membered nitrogenous heterocyclic moiety that has three carbons, two nitrogens, four hydrogen atoms, and two double bonds having general molecular formula of $C_3H_4N_2$. It is also known as 1,3-diazole because of the nitrogen atoms present at the first and third positions (non–adjacent position) of the ring, one nitrogen bear a hydrogen atom as the pyridine structure, and the other is called pyrrole type nitrogen and position four and five are equivalent. It formed the basis of many therapeutic natural products such as histamine, purine, histidine among others

The research work focuses on the synthesis, characterization and antimicrobial activities of the derivatives of some new imidazole derivatives. Wallach synthesis was used to synthesize 1-methyl-5-chloro-imidazole which was nitrated to give 1-methyl-4-nitro-5-chloroimidazole. H_2S gas was bubble into the solution 1-methyl-4-nitro-5-chloroimidazole in the presence of sodium ethoxide which gave 62% yield of 1-methyl-4-nitroimidazole-5-thiol (compound 4). Compound 4 was coupled with benzoylchloride and phenylethylbromide to produce compound 4B and 4A respectively. 1-methyl-4-nitroimidazole-5-thiol underwent oxidative chlorination to yield an unstable intermediate of 1-methyl-4-nitroimidazole-5–sulfonyl chloride. All attempt to stabilized 1-methyl-4-nitroimidazole-5–sulfonyl chloride failed. With the sulfonyl chloride at position 5 on imidazole ring, it was then coupled with proline, proline methylester, biphenylhydroxide, 2-aminophenol and N- ethylaniline which yielded 1-methyl-4-nitroimidazole-5-sulphonylproline,1-methyl-4-nitroimidazole-5-sulphonylprolinemethylester, 1-methyl-4-nitroimidazole-5-sulphonyl(2-hydroxylbiphenyl), 1-methyl-4-nitroimidazole-5-sulphonyl(2-aminophenol),1-methyl-4-nitroimidazole-5-sulphonyl(N-ethylaniline).

The 4-nitro-2-methyl-1-H-ethanolyimidazole was chlorosulfonated with thionyl chloride, and then coupled with aniline and 2,4 dichloroaniline to give 1-chloroethane-2-[thioacylaniline-4-nitroimidazole and 1-chloroethane-2-[thioacyl-(2,4-dichloroaniline)]-4-nitroimidazole respectively. The synthesized compounds were purified and confirmed using thin layer chromatography (TLC)

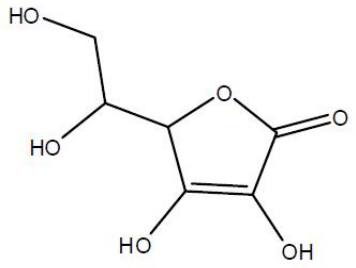
and column chromatography, FT-IR, ^{1}HNMR and ^{13}CNMR spectral and were evaluated for Anti-bacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis* and Anti-fungal activity against *Candida albicans* and *Aspergillus niger*.

CHAPTER ONE

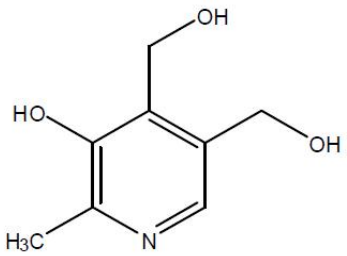
1.0 INTRODUCTION

A cyclic structure having at least one hetero atom in the ring is called a heterocyclic compound. Nitrogen, oxygen, and sulphur are the most common heteroatoms (Tripathi, 2008). To be regarded as a heterocycle, apart from the heteroatom, the ring must be stable having conjugated double bonds and exhibiting aromatic character (Owolabi and Olarinoye, 2008).

In nature, heterocyclic compounds are widely distributed and necessary for life (vijay *et al*., 2007). They are involved in the synthesis of many biological substances that are essential to life, including sugars, vitamin C [1], nucleic acids, and five- or six-membered rings (furan and pyran, respectively). Most members of vitamin B group possess heterocyclic ring containing nitrogen, as in vitamin B6 (pyridoxine) [2], which is a derivative of pyridine, essential in amino acid metabolism (Achson, 2009).



[1]



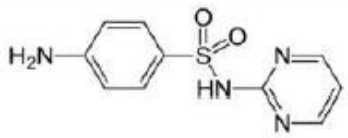
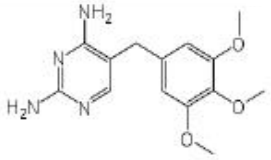
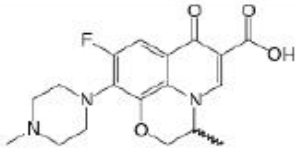
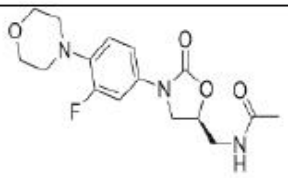
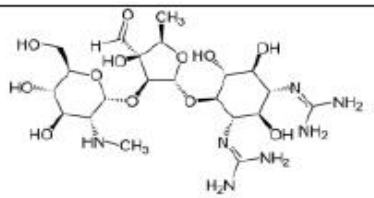
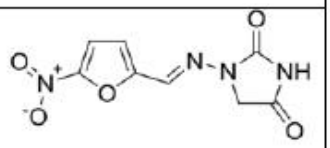
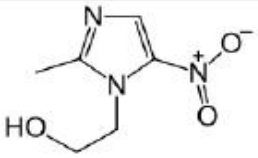
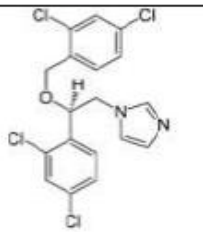
[2]

The biological activity of compounds is mainly dependent on their molecular structures (Al-Shihry, 2005). Heterocyclic compounds are acquiring more importance in recent years as these can be found in a large number of compounds which display biological activities (Padmavathi *et al*., 2007). Heterocyclic compounds, particularly five and six membered heterocyclic compounds, have attracted the attention of pharmaceutical community over the years due to their therapeutic values (Shinde *et al*., 2003).

Heterocyclic compounds make up a large number of natural antibiotics. Heterocyclic chemistry is one of the primary areas of study in organic chemistry.. Of all published organic

chemistry literature, published research on heterocyclic synthesis accounted for around 60 % in 1998 but nowadays the fraction is much larger considering that novel heterocyclic compounds are published in different fields such as pharmaceuticals, materials and others (Ahmed *et al.,*2007). Many benzo-fused heterocyclic ring systems, including benzoxazole, benzimidazole, indole, and benzthiazole, have been studied and shown to have notable pharmacological properties (Bazgir *et al*., 2008). Heterocyclic compounds have occupied a significant role in drug industry. Heterocyclic moieties like thiadiazole, oxazole, isoxazole, oxadiazole, imidazole, benzimidazole, thiazole, triazole, benzoxazole, benzothiazole and other compounds containing one or more of these heterocyclic rings or their derivatives bring a lot of attention in the last two decades because of their wide pharmaceutical and non-pharmaceutical uses such as antimicrobial, antiviral, anti-inflammatory agents, pesticidal, dyes, lubricants and analytical reagents (Bazgir *et al*., 2008). Analysis of drugs in late development stages or in the market shows that 68% of them are heterocycles (Dahiya *et al*., 2008) Therefore, it is not surprising that during past decades, compounds bearing heterocyclic nuclei have received much attention due to their chemotherapeutic value in the development of novel antimicrobials and anthelmintics (Ansari and Lal, 2009).

Table 1.1: Heterocyclic Ring-Based Structures of a Few Chemotherapeutic Agents

	
Sulfadiazine	Trimethoprim
	
Ofloxacin	Linezolid
	
Streptomycin	Nitrofurantoin
	
Metronidazole	Miconazole

The imidazole is a five membered heterocyclic structure which has shown high therapeutic properties on related drugs and has encouraged Medicinal and Organic Chemists to produce a novel of imidazole nucleus with Chemical therapy medicines.

Several imidazole derivatives have been employed in various processes to yield an extensive array of heterocycles. Later, in the last three decades, many scientists have synthesized various imidazole heterocyclic precursors containing active hydrogen atom on nitrogen and evaluated in terms of their pharmacological activity (Feldman and Wesley, 2018).

The potential for enhancing several clinical medication dispositions has increased with imidazole medicines (Katritzky et al, 2019). Medicinal properties of imidazole include anti-cancer,

anti-aging agents, anticoagulants, anti-inflammatory, antibacterial, antifungal, antiviral, anti-`tubercular, anti-diabetic and antimalarial (Katritzky et al, 2019). There have been reports that imidazole and its derivatives are used to treat a variety of illnesses. Significant advancements in chemotherapeutic drugs of exceptional importance in medicine, biology, and pharmacy have been sparked by the creation of the potent and sophisticated imidazole.

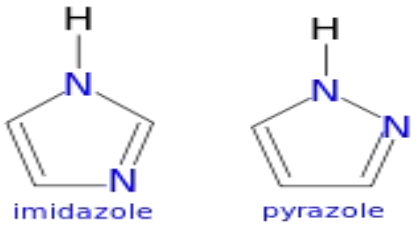
1.1 AZOLE

An azole is a class of five-membered nitrogen heterocyclic ring compounds containing at least one other non-carbon atom of either nitrogen, sulphur or oxygen (Eicher and Hauptmann, 2003). Azole parent compounds are aromatic, with one lone pair of electrons from each heteroatom in the ring and two double bonds.

1.1.1 Classes of Azole

The azoles include:

- One nitrogen atom and any other heteroatoms e.g. pyrrole.
- Two or more nitrogen atoms e.g. pyrazole, imidazole, triazole, tetrazole, etc.
- one nitrogen atom and one oxygen atom e.g. oxazole and isoxazole.
- one nitrogen atom and one sulphur atom e.g thiazole and isothiazole.



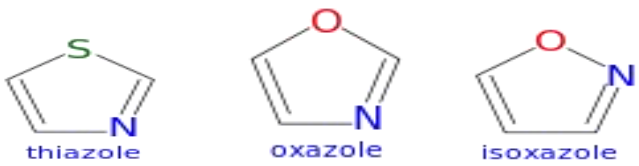


Fig. 1.1: Structure of Some Azoles.

1.1.2 Antimicrobial Action of Azole

Two clinically useful azole families, the imidazoles and the triazoles, have good antimicrobial activities (Inijing et al, 2016). The azole antifungal drugs (except for abafungin) inhibit the enzyme, lanosterol 1,4-demethylase; the enzyme necessary to convert lanosterol to ergosterol (De Kruijff and Demel, 2017). The inhibition of fungi growth in fungal membrane functions and structure can be caused by the depletion of ergosterol in fungal membrane. Although, fewer binding sites are accessible for Amphotericin B (AmB) as a result of the membrane's depletion, therefore, is not possible to utilise AmB and azoles together in therapy.

The first azole that could be administered orally to treat systemic fungal infections was ketoconazole, an imidazole. Although, it is effective against several types of fungi but its main confrontational effect is liver toxicity (Ruge *et al*., 2005) which can be fatal. Even when treatment is discontinued, organ damage continues to grow. Ketoconazole has an antagonistic effect on other medications.

Fluconazole (a triazole) can be used to treatment fungi infections affecting the mouth, vagina, skin tissues and nails. Fluconazole is not hepatotoxic at normal dosage levels and side-effects are usually mild (De Kruijff and Demel, 2017).

1.2 IMIDAZOLE

The general formula for imidazole is C3H4N2. It is an aromatic heterocyclic molecule. The solid is colourless and dissolves in water to produce a solution that is slightly alkaline(Alan R. Katritzky, 1984).It belongs to the alkaloid and diazole classes.

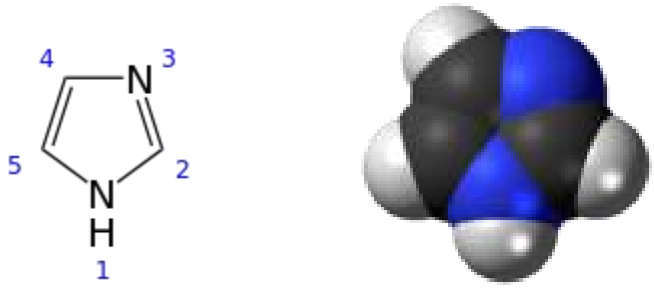


Fig. 1.2: Skeletal Formula and Space-Filling Model of Imidazole

DNA, histidine, and histamine are examples of vital biological substances that contain the imidazole ring structure. When fused to a pyrimidine ring, it forms purine, which is the most widely occurring nitrogen-containing heterocycle in nature (Rosemeyer, 2004).

1.2.1 Structure of Imidazole

The proton on either of the two nitrogen atoms made imidazole's two equivalent tautomeric structures, and it is a planar 5-membered ring. The imidazole structure exhibit aromatic characteristics because of the presence of a sextet of π – elections which consist of a pair of electrons from the protonated nitrogen atom and one from each of the remaining four atoms of the ring (sanchita *et al*., 2010) and more so, three pairs of π electrons can be found in the π cloud, and each carbon in the ring possesses a p-orbital perpendicular to the ring.. The lone-pair of electrons on N-1 are part of the cloud as they are in a p-orbital while the lone pair on N-3 are not part of the cloud as they in an sp^2 orbital perpendicular to the p-orbitals and thus providing a point of attack for protons and other electrophiles (Theresa, 2010).

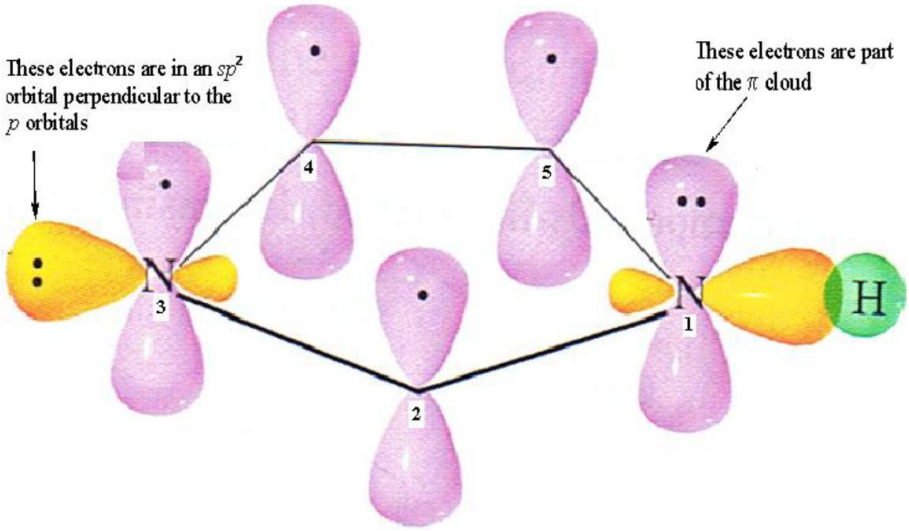


Fig. 1.3: Orbital Structure of Imidazole (Bruice, 2007 and Thomas *et al*., 2006)

Bhatnagar *et al*., (2011) also reported the resonance structure of imidazole as shown below.

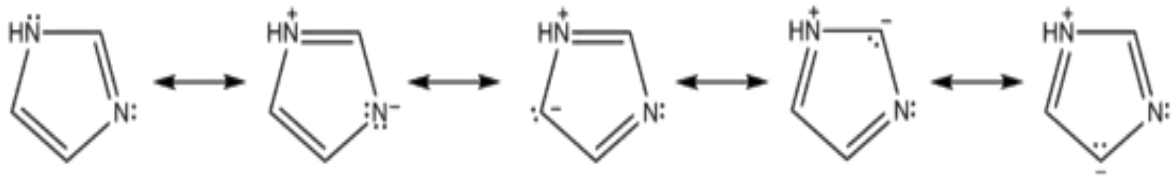
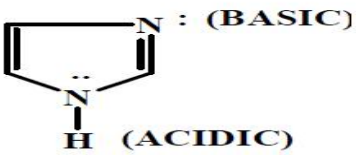


Fig. 1.4: Resonance Structure of Imidazole

1.2.2 Reactivity of Imidazole

Imidazole have properties similar to pyrrole and pyridine. The electrophilic reagent would attack the unshared electron pair on N-3 but not that on the pyrrole nitrogen since it is the part of the aromatic sextet (Bhatnagar, 2003). While the imidazole ring is rather susceptible to electrophilic attack on an annular carbon, it is much less likely to become involved in nucleophilic substitution reaction unless there is a strongly electron withdrawing substituents elsewhere in the ring (Bhatnagar *et al*., 2011). Without this activation, C-2 is vulnerable to nucleophilic attack, particularly when it comes to benzimidazoles, which have a fused benzene ring that allows for enough electron withdrawal to support a range of nucleophilic substitution reactions.

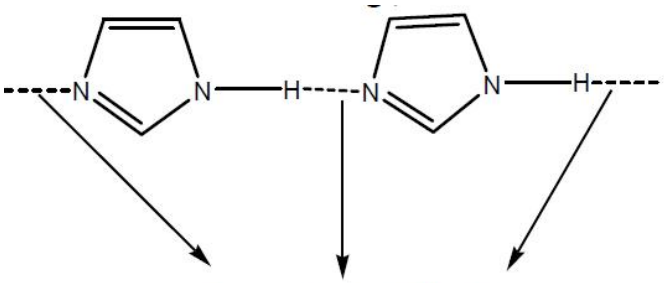


Imidazole and benzimidazole's overall reactivity is dependent on a resonance structure where the dipolar donors play a limited role. These predict electrophilic attack in imidazole at N-3 or any ring carbon atom, nucleophilic attack at C-2 or C-4 and also the amphoteric nature of the molecule (Kartaraski, 2018).

1.2.3 Physical Properties of Imidazole

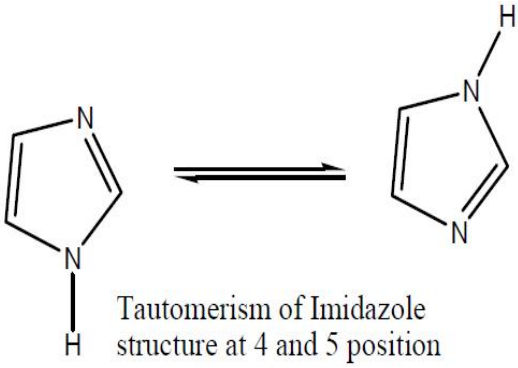
Some of the physical properties of imidazole are given below.

1. It is a colourless liquid having a high boiling point of 256 °C than all other 5- membered heterocyclic compounds due to the intermolecular hydrogen bonding where there is linear association of molecule (Jain and Sharma, 2005).



Intermolecular H-bonding

2. It has a melting point between the range of 89 - 910°C.
3. It has molar mass of 68.077g/mol.
4. Imidazole is white or pale yellow solid in appearance.
5. It has a chemical formula of $C_3H_4N_2$ and density of 1.23g/cm^3.
6. Its solubility in water give mildly alkaline solution.
7. It possesses tautomeric structure since positions 4 and 5 are equivalent.

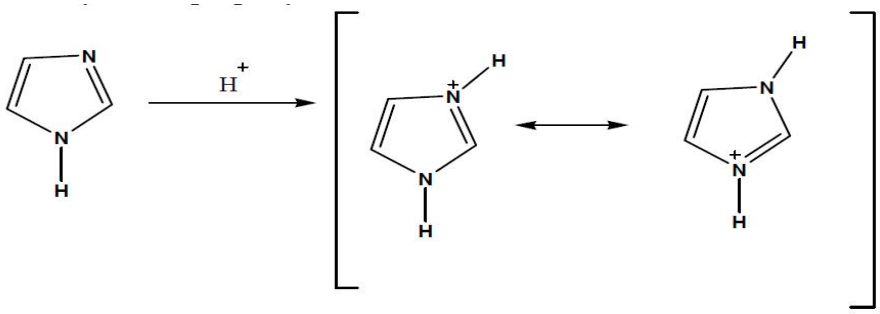


Tautomerism of Imidazole structure at 4 and 5 position

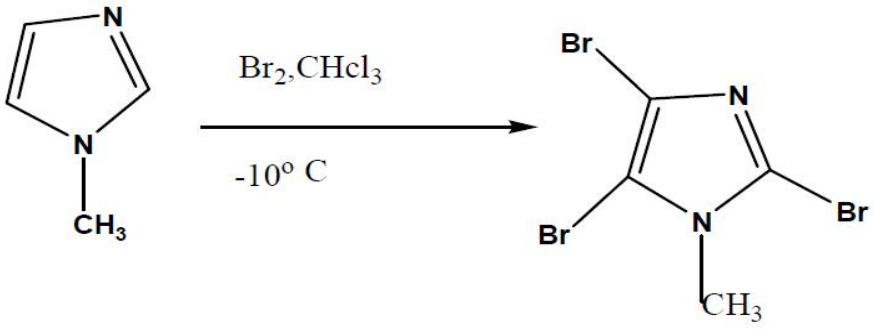
8. Its spectroscopic parameters are λmax of 207nm, I.R. = 1550, 1492, 1451 (cm-1),ζ = 2.30, 2.86 (Jain and Sharma, 2005).

1.2.4 Chemical Properties of Imidazole

1. **Reactions with Acids:** The base imidazole is monoacidic. It reacts with acids to generate crystalline salts. It also possesses weakly acidic property (Bansal, 2006).



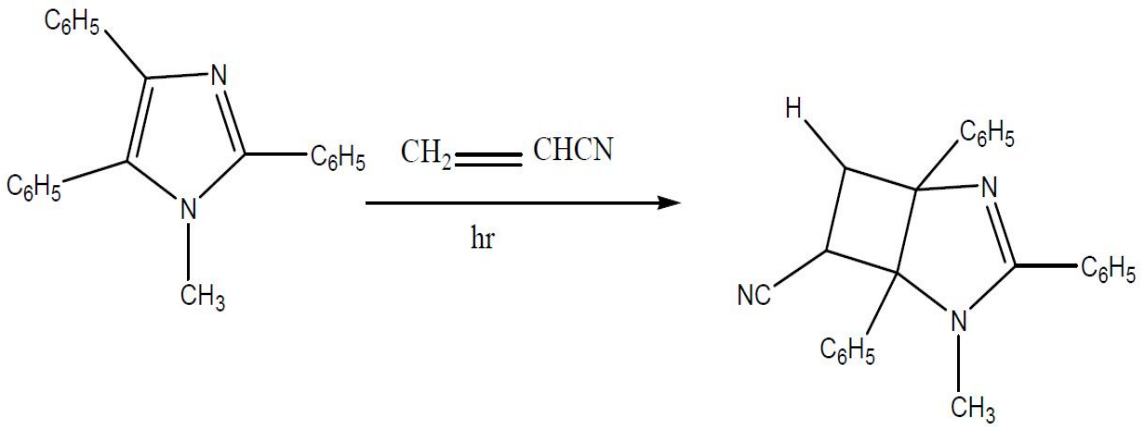
2. **Halogenation:** the halogenation of imidazole is complex and also depends on the substrate, reagents and reaction conditions (sharma, 2015). Bromination yields 2,4,5-tribromo derivative. Iodination takes place in alkaline conditions to give 2,4,5-triodoimidazole (Bansal, 2006).



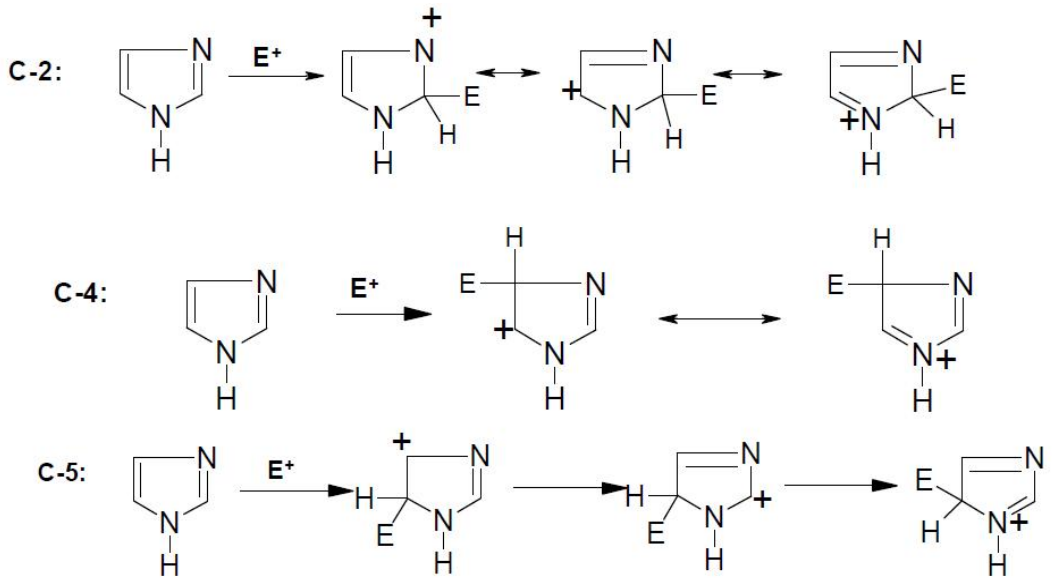
2,4,5-tribromo -1-methylimidazole

3. **Reaction with Oxidizing and Reducing Agents:** Imidazole is resistant to auto-oxidation in the presence of chromic acid, but it becomes unstable when potassium permanganate is present.. However, imidazole readily opens the ring to form oxamide with H_2O_2 (Kartaraski, 2018). Imidazole derivative can be obtained from oxygen reactions in the presence of a sensitizer (single oxygen)

4. **Cyclo-addition Reactions:** Under photochemical circumstances, the imidazole ring's carbon-carbon bond can produce an addition, as in the case of the reaction between imidazole and acrylonitrile and as shown below.



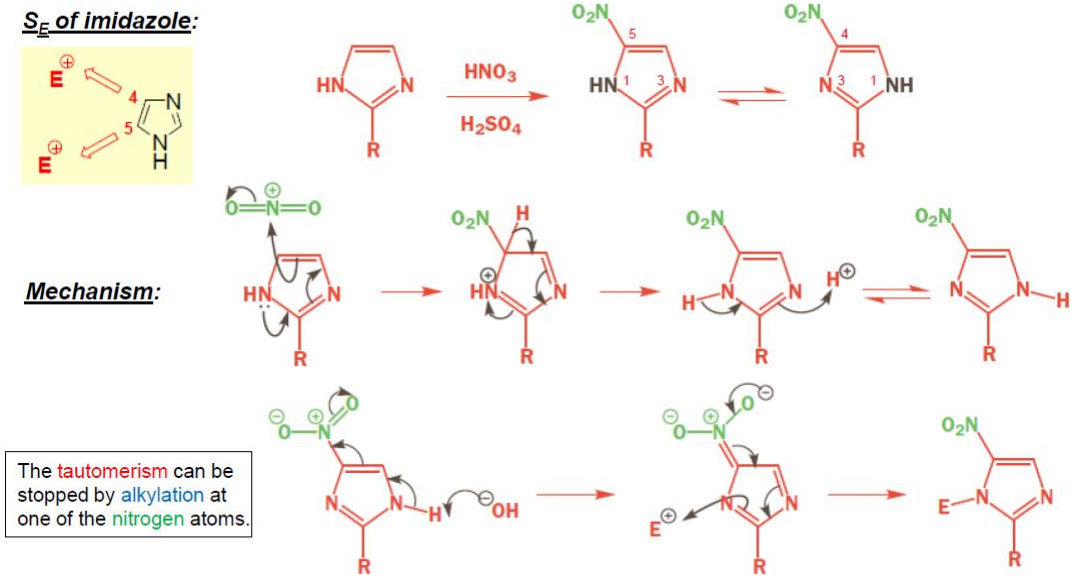
6. Imidazole possess increased reactivity towards electrophilic attack than pyrazole or thiazole, furan and thiophene. From the following resonance structure of the intermediate ion, it is evident that the attack takes place at the 4th and 5th position in imidazole ring (Bhatnagar *et al*., 2011).



Scheme 1.1: Mechanism of the reactivity of imidazole.

1.2.5 Reactivity of Imidazole

1. C4-substituted imidazoles easily undergo nitration producing the equilibrating mixture of tautomers as shown below:

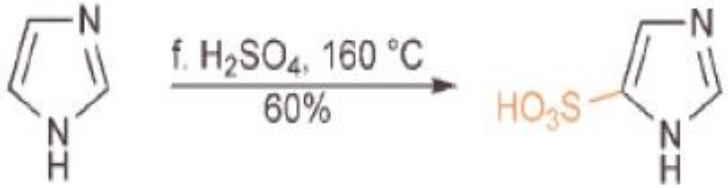


Scheme 1.2: Mechanism of nitration on imidazole

2. **Halogenation Reaction:** Imidazole is brominated with ease at all free nuclei positions including C - 2. Substitution generally occurs first at C-2 but proceeds further yielding 2,4,5 - tribromoimidazole as end - product.

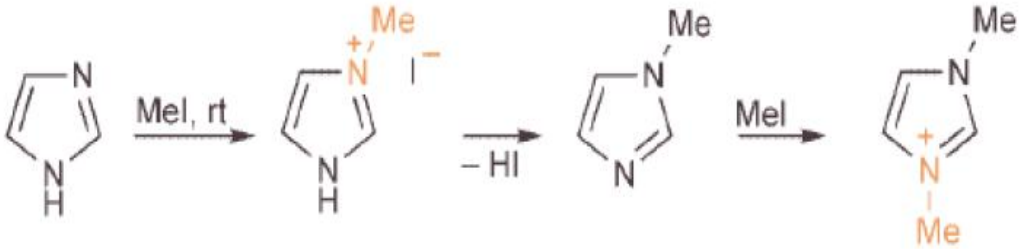
Scheme 1.3: Halogenation of imidazole

3. **Sulfonation Reaction:** Imidazole can be sulfonated with concentrated sulphuric acid at elevated temperature at C - 5 position.



Scheme 1.4: Sulfonation of imidazole

4. **Quaternisation:** Imidazole is easily quaternised (N - alkylated) in the imine nitrogen with alkyl halides (RX). The intermediate is a protonated N - alkylimidazole loses its hydrogen to unreacted imidazole thereby act as a base. The subsequent reaction is obtained from the mixture of imidazolium, 1 - alkyl and 1,3-dialkyl-imidazolium salts.



Scheme 1.5: Quaternisation of imidazole

5. **Deprotonation:** Imidazole (pkA 14.5) is a highly strong acid than pyrrole (pkA 17.5). and therefore can be deprotonated easily. One suitable method is the use of dry Na/K – salt

obtained from an evaporated aqueous alkaline solution or NaH/DMF. The stable, delocalized imidazolyl anion can be subsequently alkylated, acylated or sulfonylated on nitrogen atom.

Scheme 1.6: Deprotonation of imidazole

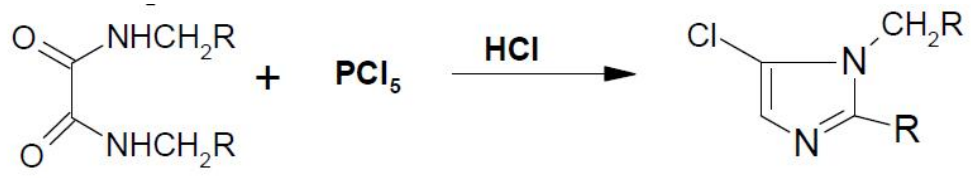
1.2.6 Imidazole Synthesis

Although, imidazole was synthesized from several imidazole derivatives as early as the 1840s, Heinrich Debus was the first chemist to do it in 1858. His synthesis used glyoxal and formaldehyde in ammonia to form imidazole (Debus, 1858), but his short-comings was that the synthesized imidazole was relatively low yields. Although it is still use in obtaining C - substituted imidazole.

Imidazole and its derivatives have been synthesised using a variety of techniques by merely changing the functional groups of the reactants. Other approaches include Debus synthesis, Radiszewski synthesis, dehydrogenation of imidazolines, from alpha halo ketones, Wallach synthesis, from aminonitrile and aldehyde and Marckwald synthesis (Bhatnagar *et al*., 2011).

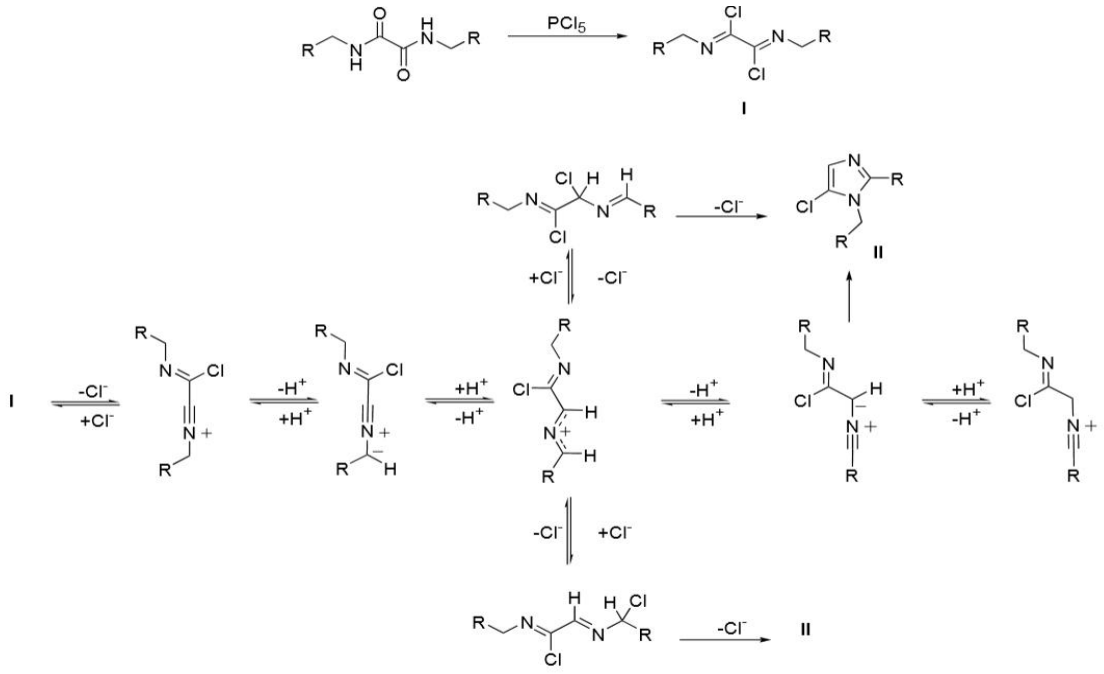
1.2.6.1 Wallach Synthesis

Wallach reported that when N,N-dimethyloxamide is treated with phosphorus pentachloride, a chloride containing compound is obtained which on reduction with hydroiodic acid give N-methylimidazole (Anshul *et al*.,2012). Under the same condition, N,N-dimethyloxamide is converted to a chlorine compound which on reduction gives 1-ethyl-2-methylimidazole (Wallach and Schuelze, 2011)



N,N-dimethyloxamide

Scheme 1.7: Reaction of N,N-dimethyloxamide with phosphorus penta-chloride



Benincori, T.; Brenna, E.; Sannicolo, F. *J. Chem Soc. Perk. Trans. I* **1993**, 675-679.

Scheme 1.8: Proposed Mechanism of Wallach Synthesis

1.2.7 Pharmacological and Chemotherapeutic Activities of Imidazole

Imidazoles belong to the class of azole antifungals which includes ketoconazole, metronidazole, decarbazone, miconazole, clotrimazole among others. Imidazole derivatives have a wide range of biological activities (Robertson *et al*., 2015). Literature survey revealed that imidazole and its derivatives are reported to have analgesic and anti-inflammatory activities (Suzuki *et al*., 2015), cardiovascular activity (Robertson *et al*., 2015), anti-neoplastic activity (Johnson *et al*., 2012), anti-fungal activity (Brewer *et al*., 2015), enzyme inhibition activity (Nathanson, 2019), anti-anthelmintic (Lunt *et al*., 2012), anti-filarial agent, anti-viral activity and anti-ulcer activity.

Furthermore, they can act as anti-bacterial agent. Along with metronidazole, other nitroimidazoles (misonidazole, metrazole and clotrimazole) are important anti-cancer drugs (Bhatnagar *et al*., 2011). Better imidazole derivatives with more effectiveness and fewer side effect are continued discovered in this contemporary scientific searches.

1.2.7.1 Ketoconazole

Ketoconazole is a synthetic, imidazole antifungal medication used primarily to treat fungal infections (Roosi, 2013). Ketoconazole is sold commercially as a tablet for oral administration and in a variety of formulation for topical administration such as creams and shampoos (Roosi, 2013).

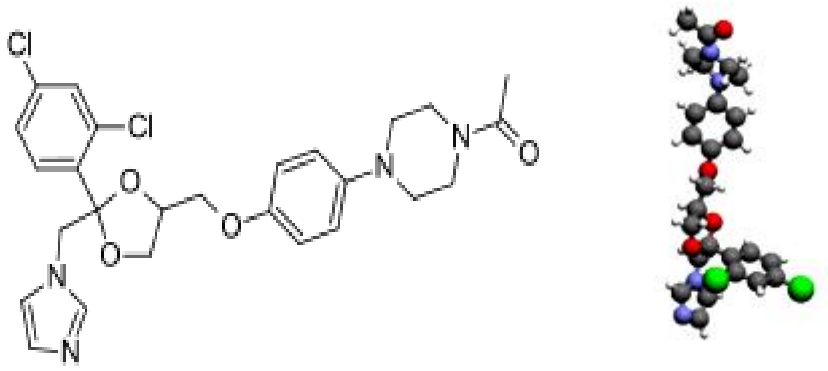


Fig. 1.5: Skeletal Formula and Space-Filling Model of Ketoconazole

As an antifungal, ketoconazole is structurally similar to imidazole, and interfere with the fungal synthesis of ergosterol, a constituent of fungal cell membranes, as well as certain enzymes (Loose *et al*., 2007). As with all azole antifungal agents, ketoconazole works principally by inhibiting the enzymes cytochrome P450 1, 4-alpha-demethylase (P450 14DM) (Loose *et al*., 2007). This enzyme participates in the route of sterol biosynthesis, which converts lanosterol to ergosterol.

Ketoconazole is best absorbed at extremely acidic levels, it is delivered orally; hence, the drug's absorption will be lowered by antacids or other factors that induce decreased stomach acid levels. Absorption can be increased by taking it with an acidic beverage, such as cola (Chin *et al*., 2008). Ketoconazole tends to accumulate in fatty tissues because it is highly lipophilic.

1.2.7.2 Clotrimazole

Clotrimazole is an antifungal medication commonly used in the treatment of fungal infections (of both humans and other animals) such as vaginal infections, oral thrust and ringworm (American Society of Health-System Pharmacists, Inc, 2014). It is also used to treat athlete's foot and jock itch (American Society of Health-System Pharmacists, Inc, 2014). It is on the World Health Organization's list of Essential Medicine, a list of the most important medication needed in a basic health system (WHO Model List of Essential Medicines, 2013).

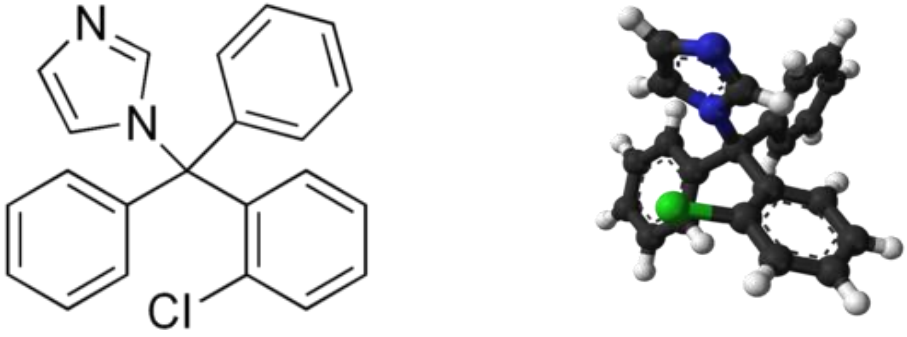


Fig. 1.6: Skeletal Formula and Space Model of Clotrimazole

Clotrimazole attaches itself to phospholipids in the cell membrane of candida or fungal cells to produce fatal amounts of those cells. This alters the fungal cell wall's permeability, which stops the synthesis of ergosterol and other sterols required for the formation of cell membranes. This leads to the cell's death via loss of intracellular elements (Lexicomp Online, 2014 and DrugBank, 2014).

In addition to its use as an antifungal, there is promising research on using clotrimazole against other diseases such as sickle cell anaemia, malaria, beriberi, tineapedis, Chagas disease and cancer (McNaughton – Smith GA Burns *et al*., 2008)

1.2.7.3 Tioconazole

Tioconazole is an antifungal medication of the imidazole class used to treat infections caused by a fungus or yeast. Ointments made with tioconazole can be used to treat women's vaginal yeast infections, ringworm, jock itch, athlete's foot, and sun fungus (Clissold and Heel, 2012).

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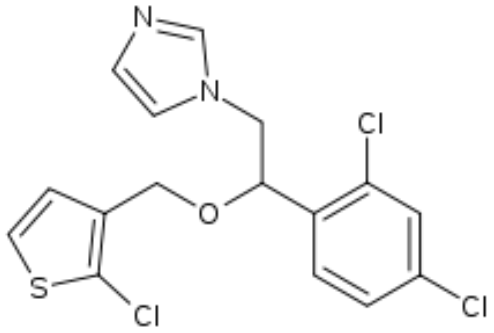


Fig. 1.7: Skeletal Formula of Tioconazole

In comparative studies, it was at least as effective as alternative imidazole antifungal drugs, and in a few trials, significantly greater efficacy has been reported for tioconazole, compared with clotrimazole, miconazole, econazole and systemic ketoconazole (Clissold and Heel, 2012).

By interacting with 1, 4-demethylase, a cytochrome P-450 enzyme that transforms lanosterol into ergosterol—an key component of the yeast membrane—tioconazole suppresses the synthesis of ergosterol, which increases cellular permeability. Furthermore, ticonazole may hinder the production of triglycerides, phospholipids, endogenous respiration, the transformation of yeast into mycelial forms, purine uptake, and the transport of calcium and potassium ions through the Gardos channel ion transport pathway by interfering with membrane phospholipids (www.druglib.com/activeingredient/tioconazole/).

1.2.7.4 Metronidazole

Metronidazole is an antibiotic and antiprotozoal which is marketed under the brand name Flagyl among others (The American Society of Health – System Pharmacists). For over 45 years it has been used as an antimicrobial agent in clinical medicine. To our knowledge, the first report on the effect of metronidazole for the management of anaerobic infections was published in 1962 by Shinn (Shinn, 1962). Later, it was introduced for the management of *Clostridium difficile* infection and is still recommended as an alternative to vancomycin for treatment of this infection (Shinn, 1962).

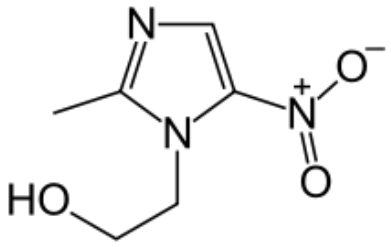


Fig. 1.8: Skeletal Formula of Metronidazole

Metronidazole has demonstrated strong activity against gram-positive and gram-negative anaerobic bacteria, including B. fragilis and C. difficile. Metronidazole has good pharmacokinetic and pharmacodynamic qualities and can be applied topically, intravenously, orally, and vaginally. It is primarily used to treat bacterial vaginosis, pelvic inflammatory disease (along with other antibacterials like ceftriaxone), pseudomembranous colitis, aspiration pneumonia, rosacea (topical), fungating wounds (topical), intra-abdominal infections, lung abscess, periodontitis, amoebiasis, giardiasis, trichomoniasis and infections caused by susceptible anaerobic organisms such as *Bacteroides, Fusobacterium Clostridium, Peptostreptococcus* and *Prevotella* species (Rossi, 2013).

Metronidazole is effective against a variety of protozoa and bacteria. Drug-resistant cells lack drug activation, whereas it enters the cell as a pro-drug by passive diffusion and is activated in the cytoplasm of the bacterium or certain organelles in the protozoa (Rossi, 2013).. The metronidazole molecule is converted to a short-lived nitroso free radical by intracellular reduction, which includes the transfer of an electron to nitro group of the drug (Diniz *et al*., 2000). The actual mechanism of action has not yet been fully elucidated but includes the inhibition of DNA synthesis and DNA damage by oxidation, causing single-strand and double-strand breaks that lead to DNA degradation and cell death (Land and Johnson, 2005).. The activated reduced metronidazole molecule builds nonspecifically to bacterial DNA, inactivating the organism's DNA and enzymes and leading to a high level of DNA breakage, with immediate action of the drug but no cell lysis (Land and Johnson, 2005).

1.2.7.5 Nitroimidazole

The nitroimidazole has wide range of biological effects both in human and animal therapeutics. The term, nitroimidazoles, also refers to class of antibiotics that share similar chemical structure (Edwards, 2009).

In view of organic chemist, nitroimidazole antibiotics can be classified based on the location of the nitro functional group (Retrieved from Handwiki, 2022). Position 4 and 5-nitroimidazole are equivalent from the perspective of drugs since these tautomers readily interconvert. Drugs of the 5-nitroimidazole includes metronidazole, tinidazole, dimetridazole, secnidazole and ornidazole (Retrieved from Handwiki, 2022). These drugs have useful for treating human and animal diseases. In addition, 5-nitroimidazole antibiotics (e.g

metronidazole) have been used to combat anaerobic bacterial and parasitic infections (Mital, 2009).

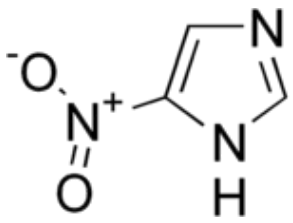


Fig. 1.9: Skeletal Formula of Nitroimidazole

The mechanism of biological action of nitroimidazoles commonly used to treat infection by anaerobic bacteria depends on the reduction of the nitro group producing intermediate species which interact with DNA, oxidizing it and resulting in strand breaking and double – helix destabilization (Edwards, 2009). The extent of strand breaking depends on the A + T content of the DNA (Edwards, 2010).

A growing number of therapeutic uses are made possible by the nitroimidazoles' diverse biological characteristics. Searching for relationships between the nitroimidazoles' chemical and biological characteristics may provide significant light on the compounds' mode of action. A nitroimidazole's ability to reduce and its effectiveness as a cytotoxin, radiation sensitizer, or mutagen

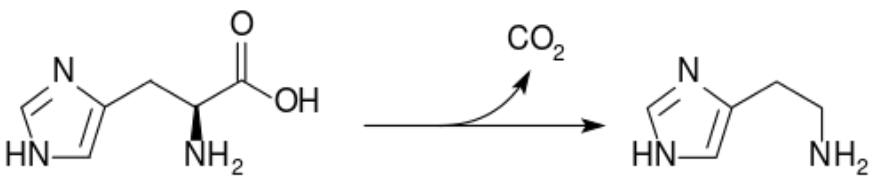
are correlated. The apparent obligatory rule of nitro group reduction for biological activity explains why 5-nitroimidazole exhibit selective toxicity for anaerobic micro-organisms (Goldman, 2005).

Other effects of such drugs may include the possible inhibition of DNA repair mechanisms which exacerbate DNA damage (Edwards., 2009). Inhibition of activity of nitroimidazoles may be caused by aminothiol radical scavengers and radioprotectors normally present in the cell or by the presence of other organisms in the environment (that is the vagina) capable of inactivating the drugs (Edwards., 2009).

5-nitroimidazole are mainly used against amoebiasis, giardiasis, trichomoniasis and anaerobic bacterial infections (Nair and Nagarajan, 1983). 4-nitroimidazoles are much less active than the corresponding 5-nitro isomers (Cosar *et al*., 1966). Numerous 5-nitroimidazole structure-activity correlations have been investigated, and thorough reviews of their chemistry and pharmacology are available. An antibacterial agent is secnidazole, also known as 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole. The widely used 5-nitroimidazoles, metronidazole, and tinidazole share structural similarities with secnidazole. These medications appear to be especially useful in treating trichomoniasis, amoebiasis, giardiasis, and bacterial vaginosis. They have a similar spectrum of effectiveness against anaerobic microorganisms.

1.2.7.6 Imidazole in Nature

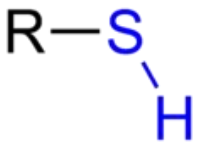
Imidazole is a constituent of numerous important biological substances. The most prevalent amino acid with an imidazole side chain is histidine. Histidine is an essential component of many proteins and enzymes and is essential to the structure and binding properties of haemoglobin. Imidazole-based histidine compounds play a very important role in intracellular buffering (Hochachka and Somero, 2002). Histidine can be decarboxylated to histamine, which is also a common biological compound. The relationship between histidine and histamine are shown below.



Theophylline, which is present in tea leaves and coffee beans, contains the compound imidazole, which activates the central nervous system (www.wikipedia.com/ imidazole). Additionally, Verma et al. (2013) noted that the purines, histamine, and biotin, as well as the amino acid histidine, vitamin B12, a component of DNA base structure, and purines, histamine, and biotin, all have imidazole nuclei as their primary structural component. Additionally, it can be found in the structures of numerous synthetic or natural medicinal compounds.

1.3 THIOLS

A thiol is an organosulfur molecule that has a carbon-bonded sulphydryl (-C-SH or R-SH) group, where R is an alkyl or other organic substituent (Retrieved from www.wikipedia.com/thiols). Thiols are the alcohol equivalent of sulphur, which means that in an alcohol, sulphur takes the place of oxygen in the hydroxyl group. Both thiol and sulphydryl gr A thiol is an organosulfur molecule in organic chemistry that has a carbon-bonded sulphydryl (-C-SH or R-SH) group, where R is an alkyl or other organic substituent. Thiols are the equivalent of sulphur in alcohols, meaning that sulphur replaces oxygen in the hydroxyl group of an alcohol. Both thiol and sulphydryl groups are terms used to describe the "-SH" functional group itself (Retrieved from www.wikipedia.com/thiols).oups are terms used to describe the "-SH" functional group itself (Retrieved from www.wikipedia.com/thiols).



There are a lot of thiols that smell strongly of rotten eggs or garlic. To help detect natural gas, which has no smell when it is pure, thiols are employed as odourants. The "smell of natural gas" is actually the odour of the thiol that is being used as an odourant. Thiols are sometimes referred to as mercaptans (Cremlyn, 1996). The term, mercaptan**,** was introduced in 1832 by William Christopher Zeise and is derived from the Latin words, mercurium captāns, (capturing mercury) because the thiolate group bonds very strongly with mercury compounds(Zeise, 1834).

1.3.1 Structure and Bonding

Similar linkage exists between alcohols and thiols. The normal length of a C-S bond is 180 pico meters, which is approximately 40 pico meters longer than that of a typical C-O bond because sulphur is a bigger element than oxygen (retrieved from www.wikipedia.com/thiols). Whereas the C-O-H group's angles are wider, the C-S-H group's angles are closer to 90 degrees. Whereas van der Waals interactions between the highly polarizable divalent sulphur centres function as the primary cohesive force, hydrogen bonds between individual thiol groups in solids or liquids are weak. An S-H bond is less polar than a hydroxyl group because there is less of an electronegativity gap between sulphur and hydrogen than there is between oxygen and hydrogen. According to www.wikipedia.com/thiols, thiols have a smaller dipole moment than the matching alcohol.

1.3.2 Physical Properties

The following are some of the physical properties of thiols.

1. **Odour:** The smell of many thiols is strongly reminiscent of garlic. Thiols have strong, unpleasant odours, especially those with low molecular weight. The spray of skunks consists mainly of low molecular weight thiols and their derivatives (Andersen and Bernstein, 1978). 10 parts per billion of these thiols-containing compounds are detectable by the human nose. Human perspiration contains (R)/(S)-3-methyl-3-sulphanylhexan-1-ol (MSH), which has an onion-like, fruity smell and may be detected at 2 parts per billion. Women emit significantly more MSH than men (Troccaz *et al*., 2009) (Methylthio) methanethiol ($MeSCH_2SH$; MTMT) is a strong-smelling volatile thiol, also detectable at parts per billion levels, found in male mouse urine. Copper has been shown to be required by a specific mouse olfactory receptor, MOR244-3, which is highly responsive to MTMT as well as to various other thiols and related compounds (Duan *et al*., 2012). A human olfactory receptor, OR2T11, has been identified which, in the presence of copper, is highly responsive to the gas odourants, ethanethiol and *t*-butyl mercaptan , as well as other low molecular weight thiols, including allyl mercaptan found in human garlic breath, and the strong-smelling cyclic sulphide thietane (Retrieved from https://www.chemistryworld.com/news/copper-key-to-our-sensitivity-to-rotten-

eggs-foul-smell/1017492.article). Thiols is also responsible for a class of wine flaws that result from the unintentional reaction of sulphur and yeast, as well as the "skunky" smell of beer that has been exposed to UV light.

Although, not all thiols have unpleasant odours, For example, furan-2-ylmethanethiol contributes to the aroma of roasted coffee, whereas grapefruit mercaptan, a monoterpernoid thiol, is responsible for the characteristic scent of grapefruit. The effect of the latter compound is present only at low concentrations. The pure mercaptan has an unpleasant odour.

2. **Boiling Points and Solubility:** The hydrogen bonds that bind thiols together are not as strong to that of alcohols and water molecules. As a result, compared to alcohols of comparable molecular weight, they have lower boiling temperatures and are less soluble in water and other polar solvents. For this reason also, thiols and corresponding thioether functional group isomers have similar solubility characteristics and boiling points, whereas the same is not true of alcohols and their corresponding isomeric ethers (Retrieved from www.wikipedia.com/thiols).

1.3.3 Preparation of Thiols

i) Reaction of methanol and hydrogen sulphide: Methane-thiol is produced industrially through the reaction of methanol and hydrogen sulphide. This reactions are done in the presence of acidic catalysts

$$CH_3OH + H_2S \rightarrow CH_3SH + H_2O$$

.

ii) The addition of hydrogen sulphide to alkenes is the second main pathway to thiols. Typically, UV radiation or acid catalysts are used to facilitate these reactions. Utilising sodium hydrogen sulphide and the appropriate organic halide, halide displacement has also been used (John, 1997).

$$RX + NaSH \rightarrow RSH + NaX \quad (X = Cl, Br, I)$$

iii) S-alkylation of thiourea

Thioglycolic acid is made from chloroacetic acid using this process. The direct reaction of a halogenoalkane with sodium hydrosulfide is generally inefficient on the normal laboratory scale because of the competing production of thioethers. Rather, an S-alkylation of thiourea converts alkyl halides to thiols.. This multistep, one-pot process proceeds via the intermediacy of the isothiouronium salt, which is hydrolyzed in a separate step (Speziale,1963).

$CH_3CH_2Br + SC(NH_2)_2 \rightarrow [CH_3CH_2SC(NH_2)_2]Br$

$[CH_3CH_2SC(NH_2)_2]Br + NaOH \rightarrow CH_3CH_2SH + OC(NH_2)_2 + NaBr$

The thiourea route works well with primary halides, especially activated ones. The preparation of both secondary and tertiary thiols is quite difficult. Secondary thiols can be prepared from dithioketals. A similar two-step procedure entails hydrolyzing the thiosulfate after it has been alkylated to produce the thiosulfonate, or "Bunte salt" One thioglycolic acid synthesis serves as an example of the procedure.

$ClCH_2CO_2H + Na_2S_2O_3 \rightarrow Na[O_3S_2CH_2CO_2H] + NaCl$

$Na[O_3S_2CH_2CO_2H] + H_2O \rightarrow HSCH_2CO_2H + NaHSO_4$

Organolithium compounds and Grignard reagents react with sulphur to give the thiolates, which are readily hydrolyzed(Jones and Moodie,1990).

$RLi + S \rightarrow RSLi$

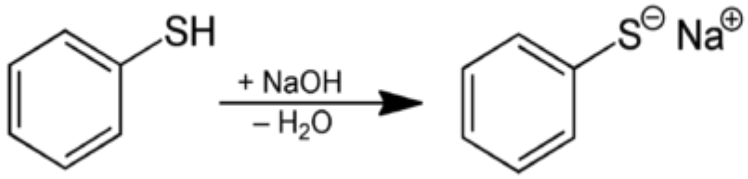
$RSLi + HCl \rightarrow RSH + LiCl$

Phenols can be converted to the thiophenols via rearrangement of their O-aryl dialkylthiocarbamates. Many thiols are prepared by reductive dealkylation of thioethers, especially benzyl derivatives and thioacetals (Ernest *et al*., 1993).

1.3.4 Reactions of Thiols

Thiols are different from alcohols in reactivity, the thiols are easily oxidized than the alcohols and the thiolates are better nucleophiles than some alkoxides. Thiols acan form thiolates, thioester and thioacetals.

1. **S-alkylation:** Thioethers are easily produced by alkylating the conjugate bases of thiols. RSH + R′Br + b → RSR′ + [Hb]Br (b = base)
2. **Acidity:** Compared to alcohols, thiols are more acidic and the conjugate base of a thiol is thiolate for example pKa for butanethiol is 10.5, while pKa for butanol is 15. Whereas phenol's pKa is 10, that of thiophenol is 6. A highly acidic thiol is pentafluorothiophenol (C_6F_5SH) with a pK_a of 2.68. Thus, thiols can also be treated with alkali metal hydroxides to yield thiolates (Retrieved from www.wikipedia.com/thiols).



Synthesis of thiophenolate from thiophenol

3. **Redox:** Thiols are easily oxidized by reagent such as iodine in the presence of base to give an organic disulphide (R–S–S–R).

$2\ R–SH + I_2 \rightarrow R–S–S–R + 2\ HI$

Oxidation by stronger reagents such as sodium hypochlorite or hydrogen peroxide can yield sulfonic acids (RSO_3H).

$R–SH + 3\ H_2O_2 \rightarrow RSO_3H + 3\ H_2O$

Oxidation can also be effected by oxygen in the presence of catalysts (Akhmadulina *et al*., 1993)

$2\ R–SH + ½\ O_2 \rightarrow RS–SR + H_2O$

Thiols participate in thiol-disulfide exchange as shown below.

RS–SR + 2 R′SH → 2 RSH + R′S–SR′

This reaction is important in nature.

4. **Metal ion Complexation:** With metal ions, thiolates behave as ligands to form transition metal thiolate complexes. The term, mercaptan, is derived from the Latin, *mercurium captans*, (capturing mercury) because the thiolate group bonds so strongly with mercury compounds.

According to hard/soft acid/base (HSAB) theory, sulphur is a relatively soft (polarizable) atom. This explains the tendency of thiols to bind to soft elements/ions such as mercury, lead, or cadmium. The stability of metal thiolates parallels that of the corresponding sulfide minerals.

1.3.5 Biological Importance of Thiols

1. **Cysteine and Cystine:** Cystine unit with disulphide bond (–S–S–) is obtained for an oxidation reaction when the thiol groups of two cysteine residues (as in monomers or constituent units) are brought close to each other in the course of protein folding. More so, if cysteines are part of the same peptide chain the disulphide bonds can contribute to protein's tertiary structure and quaternary structure, and the covalent bonds between different peptide chains are fairly strong. A physical manifestation of cysteine-cystine equilibrium is provided by hair straightening technologies (Reece, 2011).

Sulphydryl groups can form a non colvent bond with the enzyme's substrate if present in the active site of an enzyme and also contribute to covalent catalytic activity in catalytic triads and this can cause protein deformity, inactivity, and heavy metal poisoning.

2. Cofactors: cofactors have thiols units they are non-protein-based helper molecules. The thiol Coenzyme A produces a thioester during the biosynthesis and degradation of fatty acids and related long-chain hydrocarbons. For instance, the biosynthesis of methane arises from the reaction facilitated by coenzyme M, 2-mercaptoethyl sulphonic acid. Thiolates, forms strong complexes with many metal ions and its stability is equivalents to that of the corresponding sulphide minerals.

3. **In Skunks:** Skunks' low molecular weight thiols can produce offensive odours, which serve to ward off predators like wolves and humans. But since skunks cannot smell the thiols, owls can feast on them (Retrieved from www.wikipedia.com/thiols).

1.4 AIMS AND OBJECTIVES OF THE RESEARCH

From studies, numerous compounds containing heterocyclic residues are known to posse different pharmacological activities. Imidazole, a five-membered heterocyclic compound happens to be one of these heterocyclic residues. Because of its high therapeutic properties, synthetic and medicinal chemists have developed keen interest in the synthesis of compounds containing the imidazole moiety.

The aims of the research are to synthesize, characterise and determine the antimicrobial activity of some derivatives of imidazole.

For these aims to be achieved, the following objectives were set:

(i) synthesis of N, N – diethyloxamide,

(ii) synthesis of 5-chloro -1-methyl-4-nitro imidazole,

(iii) synthesis of 5-chloro-1- -methyl-4-nitroimidazole,

(iv) synthesis 1-chloroethane-2-[thioacyl-(2,4-dichloroaniline)]-4-nitroimidazole

(v) synthesis 1-chloroethane-2-[thioacylaniline-4-nitroimidazole

(vi) synthesis of 1-methyl-4-nitroimidazole-5-thiol

(vii) synthesis of 1 -methyl-4-nitroimidazole-5-sulphonylchloride

(viii) synthesis of 1 -methyl-4-nitroimidazole-5-sulphonylethylaniline,

(ix) synthesis of 1-methyl-4-nitroimidazole-5-benzoylsulphide,

(x) synthesis of [1-methyl-4-nitroimidazole-5-(2-phenylethyl)sulphide],

(xi) synthesis of 1 -methyl-4-nitroimidazole-5-sulphonyl(2-aminephenol),

(xii) synthesis of 1 -methyl-4-nitroimidazole-5-sulphonyl(2-hydroxylbiphenyl),

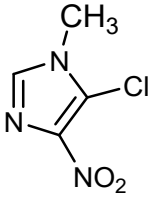
(xiii) determination of the melting point of synthesized compounds,

(xiv) FT-IR analysis of synthesized compounds,

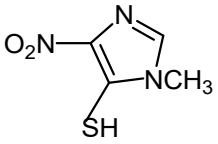
(xv) NMR analysis of synthesized compounds

(xvi) Determination of antimicrobial activity of synthesized compounds.

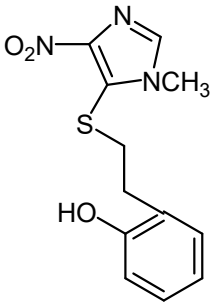
The following are the expected structure of the synthesized compounds with their IUPAC nomenclature:



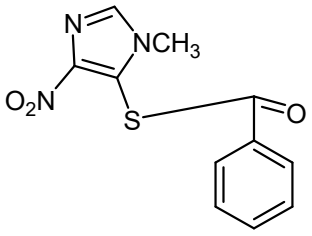
Compound 3 5-chloro -1-methyl-4-nitro imidazole



Compound 4 1-methyl-4-nitroimidazole-5-thiol

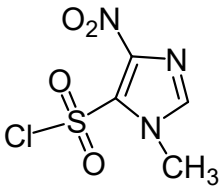


Compund 4A 1-methyl-4-nitroimidazole-5-(2-phenylethyl)

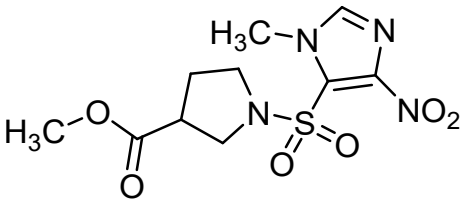


sulphide

Compound 4B: 1-methyl-4-nitroimidazole-5-benzoylsulphide

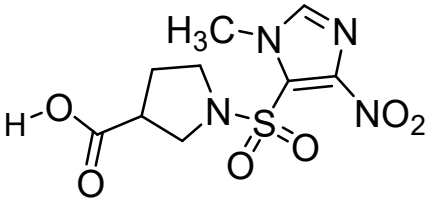


Compound 4C 1 -methyl-4-nitroimidazole-5-sulphonylchloride

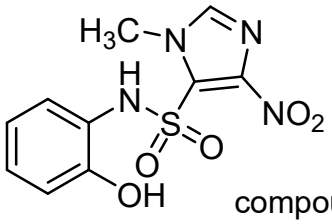


compound 4D 1 -methyl-4-nitroimidazole-5-

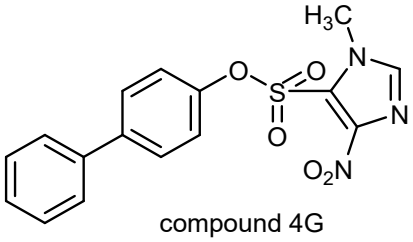
sulphonylprolinemethylester



(xvii) compound 4E 1-methyl-4-nitroimidazole-5-

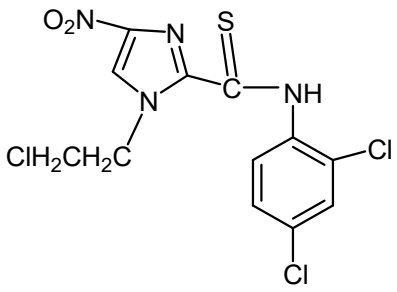


sulphonylproline compound 4F 1-methyl-4-nitroimidazole-5-sulphonyl(2-

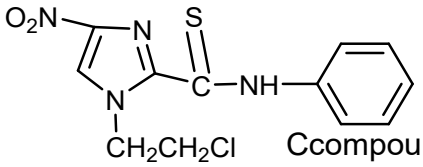


aminophenol) compound 4G 1-methyl-4-nitroimidazole-5-sulphonyl(2-

hydroxylbiphenyl),



Compound5A:1-chloroethane-2-[thioacyl-(2,4-dichloroaniline)]-4-nitroimidazole



Ccompound 5B 1-chloroethane-2-[thioacylaniline-4-nitroimidazole

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals and Reagents

All the chemicals and reagents used in this research work were of analytical grade and standard respectively. Distilled water was also used. The table below shows the specific chemicals and reagents as well as their sources.

Table 2.1: Chemicals and Reagents with their Sources

Chemical and Reagents	Source
Chloroform	JDH
Absolute Ethanol	JDH
Methanol	JDH
Concentrated hydrochloric acid (HCl)	BDH
Calcium Sulphate	LOBA
Sodium hydroxide (NaOH) Pellets	Kermel
Silica gel	LOBA
Potassium hydroxide (KOH) pellets	Kermel
Concentrated Sulphuric acid (H_2SO_4)	BDH
napthanol	JDH
Benzoyl chloride	JDH
Pyridine	BDH

Sodium metal	Kermel
Iron (II) sulphide (FeS)	BDH
Phosphorus pentachloride (PCl_5)	Sigma
Diethyloxalate	Kermel
Ethylamine	Kermel
Concentrated Nitric acid (HNO_3)	BDH
2,4 dichloroaniline	BDH
Ethylaniline	BDH
4-Chloroaniline	BDH
m-cresol	BDH
aniline	BDH
2,4 dinitrophenylhydrazine	BDH

2.1.2 Instruments and Equipment

The instruments used in this project are listed below.

Table 2.2: Instruments with their Sources

Instruments and Equipment	Source
Vacuum Pump	UNIBEN Chemistry Laboratory
Desiccator	UNIBEN Chemistry Laboratory
Fume Cupboard	UNIBEN Chemistry Laboratory
Electric Melting Point Apparatus	UNIBEN Chemistry Laboratory
Magnetic Stirrer	UNIBEN Chemistry Laboratory
FT-IR Spectrophotometer	Agilent Technologies, Ekiti
NMR	University of Kwa-zulu, Natal, South Africa.

2.2 METHODS OF CHARACTERISATION AND IDENTIFICATION

General methods were used for the identification of the synthesized compounds.

2.2.1 Thin Layer Chromatography (TLC)

To confirm the purity of the products and track the reaction's progress, the ascending TLC was performed on pre-coated silica gel GF254 (type 60) plates. Reacting with iodine vapour allowed for the detection of the end products..

2.2.2 Melting Point Determination

All of the melting points described in this research were determined using the Koffer Melting Point Apparatus. This was done using open glass capillaries.

2.2.3 Infrared Spectra (FT-IR) Analysis

The FT-IR spectra of the synthesized compounds were recorded on a buck IR M500 spectrophotometer in the range 4000 – 350cm^{-1} using KBr discs.

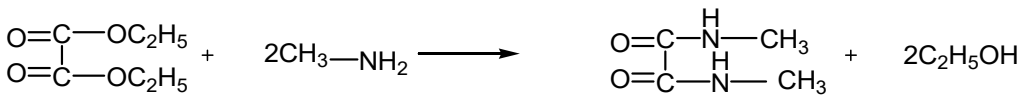
2.2.4 ^{1}H-NMR and ^{13}C-NMR Analysis

The ^{1}HNMR and Carbon-13 NMR of the synthesized compounds were obtained in University of Kwazulu Natal, South Africa. The solvent used are deuterated chloroform and deuterated ethanol analysis was carried out using 600Hz of ^{1}HNMR and ^{13}CNMR manufactured by Bruker BioSpin GmbH with TopSpin 3.6.4 program with Model ID as UUID '198daf5e-4822-11ee-872c-186024a20f75'.

2.3 CHEMICAL SYNTHESIS OF IMIDAZOLE DERIVATIVES

Schemes 2.1 to Scheme 2.6 illustrates the methods that were taken to synthesise the compounds. These procedures are detailed in sections 2.3.1 to 2.3.6.

2.3.1 Synthesis of N, N - Diethyloxamide (Compound 1)

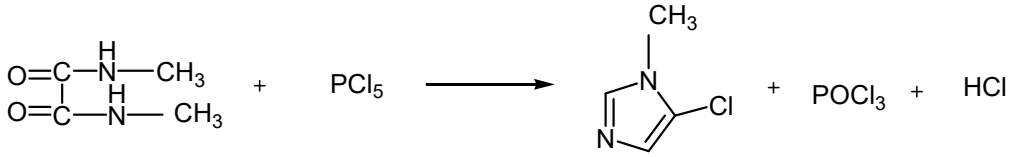


Scheme 2.1: N, N - Diethyloxamide (Compound 1)

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 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).

2.3.2 Synthesis of 5-chloro-1-methylimidazole (Compound 2)

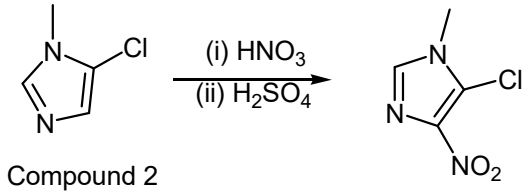


Scheme 2.2: 5-chloro-1-methylimidazole

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-ethyl-2-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 KOH 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 as shown in plate 1.

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.

2.3.3 Synthesis of 5-chloro-1-methyl-4-nitroimidazole (Compound 3)



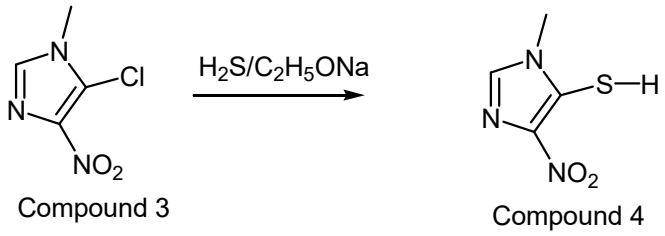
Compound 2 Compound 3

Scheme 2.3: 5-chloro-1-methyl-4-nitroimidazole

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.

2.3.4 Synthesis of 1 -methyl-4-nitroimidazole-5-thiol (Compound 4)



Compound 3 Compound 4

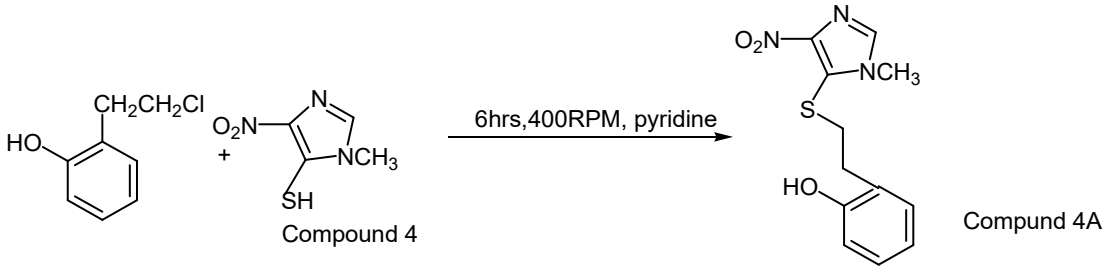
Scheme 2.4: 1 -methyl-4-nitroimidazole-5-thiol

3.1 g (0.019 mole) of the compound 3 was dissolved in cold solution of sodium (2 g or 0.086 mole) in absolute ethanol (40 ml). H_2S generated from the reaction between FeS and concentrated HCl was passed through the reaction mixture for 6 hours as shown in plate 2.

Then, the resulting solution was maintained at a temperature of 50°C for 12 hours. It was then refluxed for 4 hours. Thereafter, a small amount (10 ml) of distilled water was added to the resulting solution to dissolve any NaCl formed and then hydrolysed with concentrated HCl.

On hydrolysing with concentrated HCl, a carton colour/grey crystals of compound 4 was formed. The compound was filtered, washed with distilled water and air-dried. The product was weighed as 1.692 g (58.82 % yield) and the melting point was 115 - 117°C.

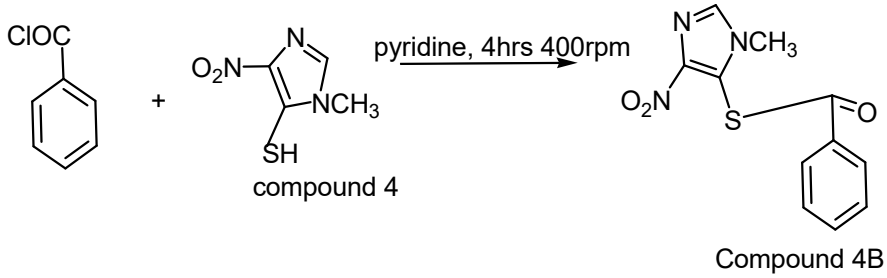
2.3.5 Synthesis of 1-methyl-4-nitroimidazole-5-{2-phenyl ethyl sulphide} (Compound 4A)



Scheme 2.5: methyl-4-nitroimidazole-5-{2-phenyl ethyl sulphide} (Compound 4A)

374 mg (0.002 mole) of compound 4 and 254 mg (0.254 ml) of 2-phenylethylbromide were weighed out and poured into a flat bottom flask. 30 cm^3 of pyridine was added to the flask as the reaction medium. The reaction then was stirred for 6 hours using a magnetic stirrer. The compound formed was extracted four times from the reaction medium using chloroform. The extracted compound was dried using $CaSO_4$ for 24 hours. The chloroform was distilled off using simple distillation method to obtain the new compound which was a very white needle-like crystalline solid. The compound was weighed as 313 mg (47.69 % yield) and the melting point was 210-212^{O}C.

2.3.6 Synthesis of 1--methyl-4-nitroimidazole-5-benzoylsulphide (Compound 4B)

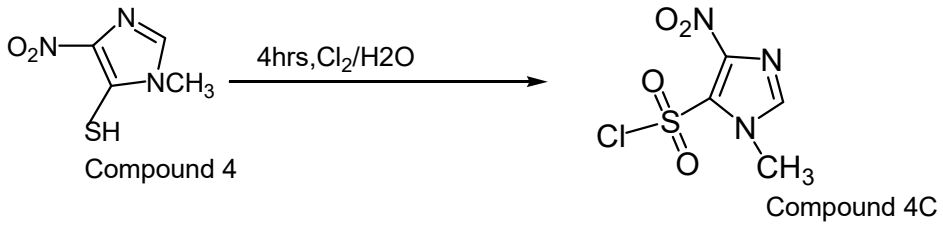


Scheme 2.6: 1--methyl-4-nitroimidazole-5-benzoylsulphide

374 mg (0.002 mole) of compound 4 was weighed out and poured into a flat bottom flask containing 10 ml of chloroform 157 mg (0.157 ml) of benzoyl chloride (benzoyl chloride) was also dissolved in 10 ml of chloroform. This was later poured into the flat bottom flask containing compound 4. 10 ml of pyridine was added to the flat bottom flask as the reaction medium. The reaction was then stirred for 2 hours using a magnetic stirrer. A cloudy solution was formed after stirring for 1 hour. The solution was poured into 20 ml of water containing drops of HCl and then

shaken for some few minutes. Chloroform was used to extract the compound formed from the reaction medium. This was done three times. The extracted compound was dried using $CaSO_4$ for 24 hours. The chloroform was distilled off using simple distillation set up to obtain the compound of interest. The crystals of the compound were brownish in colour with a melting point of 114 - 116°C. The compound was weighed as 346 mg (52.09 % yield).

2.3.7 Synthesis of 1-methyl-4-nitroimidazole-5-suphonylchloride (Compound 4C)

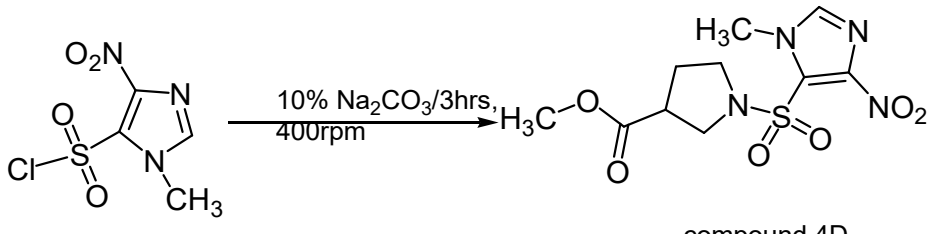


Compound 4 Compound 4C

Scheme 2.7: 1-methyl-4-nitroimidazole-5-suphonylchloride

Two grams of compound 4 was suspended in 80 ml of 2M HCl cooled in ice and stirred with an efficient magnetic stirrer. Chlorine gas was passed into it at a moderate rate for 4 h. The creamy white precipitate of compound 4C was filtered *in situ* using vaccum pump, washed well with chloroform and used immediately for coupling (1.42 g, 50.60 %)

2.3.8 Synthesis of 1-methyl-4-nitroimidazole-5-suphonylproline methyl ester (Compound 4D)

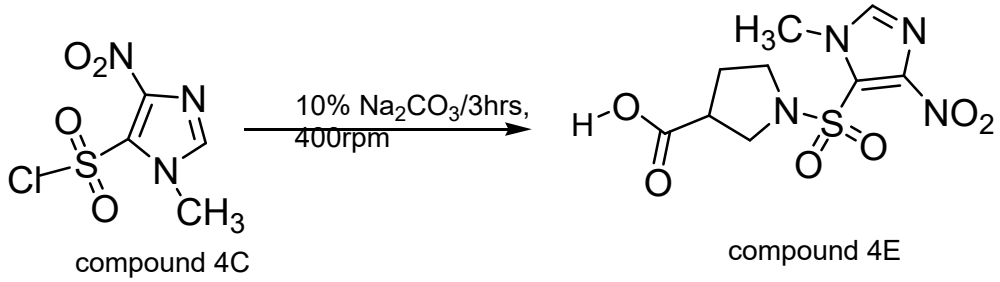


compound 4C compound 4D

Scheme 2.8: 1-methyl-4-nitroimidazole-5-suphonylproline methyl ester

The unrecrystallized compound 4C was slowly added to ice-salt cooled solution of proline methyl ester in 50 ml of 5 % Na_2CO_3 and it was stirred for 3 hrs. The reaction was extracted 3 times with $CHCl_3$, washed and allowed to dried (anh. $MgSO_4$). The oil product 450 mg was recrystallized from ethanol. The compound was weighed as 108 mg (53.82 % yield).

2.3.9 Synthesis of 1-methyl-4-nitroimidazole-5-suphonylproline (Compound 4E)



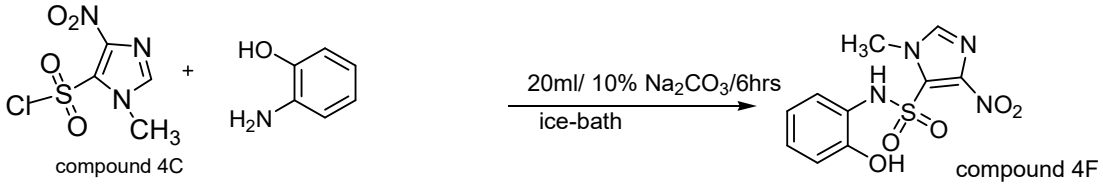
compound 4C

compound 4E

Scheme 2.9: 1-methyl-4-nitroimidazole-5-suphonylproline

The aqueous solution of the above experiment from compound 4C was neutralised with conc. HCl. A plate-like solid precipitate was filtered, washed well and air dried, weighing 950 mg with 67.69 % yield. recrystallized from water gave a pure product with a melting point 207- 209 ^{o}C.

2.4.0 Synthesis of 1-methyl-4-nitroimidazole-5-suphonylaminophenol (Compound 4F)



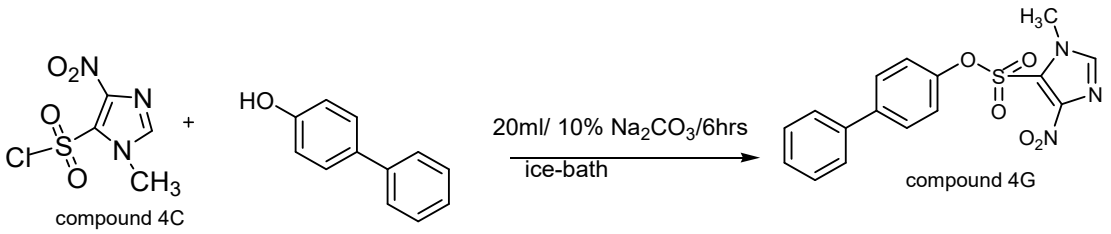
compound 4C

compound 4F

Scheme 2.10: 1-methyl-4-nitroimidazole-5-suphonylaminophenol

374 mg (0.002 mole) of compound 4C was weighed out and poured into ice-salt cooled solution of 20 ml of 10 % Na_2CO_3 and 157 mg of 2-aminophenol. The reaction was stirred for 6 hours using an efficient magnetic stirrer. A cloudy solution was formed after stirring for 1 hour. The solution was poured into 20ml of water containing drops of HCl and then shaken for some few minutes. The precipitate was filtered, washed and dried. The precipitate was filtered, washed, dried and recrystallized with ethanol. The crystals of the compound 4F were grey in colour with a melting point of 175°C - 177°C. The compound was weighed as 307 mg (62.09 % yield).

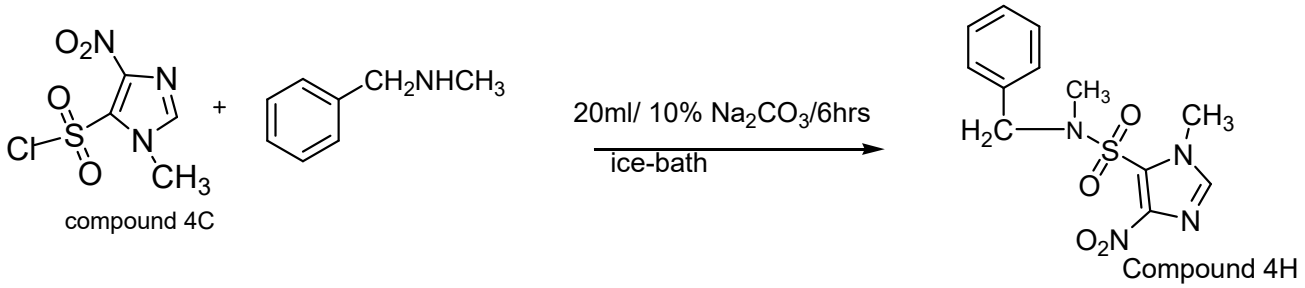
2.4.1 Synthesis of 1-methyl-4-nitroimidazole-5-suphonylhydroxylbiphenyl (Compound 4G)



Scheme 2.11: 1-methyl-4-nitroimidazole-5-suphonylhydroxylbiphenyl

374 mg (0.002 mole) of compound 4C was weighed out and poured into ice-salt cooled solution of 20 ml of 10 % Na_2CO_3 and 157 mg of 2-hydroxylbiphenyl. The reaction was stirred for 6 hours using an efficient magnetic stirrer. A cloudy solution was formed after stirring for 1 hour. The solution was poured into 20 ml of water containing drops of HCl and then shaken for some few minutes. Chloroform was used to extract the compound formed from the reaction medium. This was done three times. The extracted compound was dried using $CaSO_4$ for 24 hours. The chloroform was distilled off using simple distillation set up to obtain the compound of interest. of the compound were creamy-yellow in colour with a melting point of 92ºC – 94ºC. The compound was weighed as 281 mg (46.68 % yield).

2.4.2 Synthesis of 1-methyl-4-nitroimidazole-5-suphonyl-N-ethylaniline (Compound 4H)

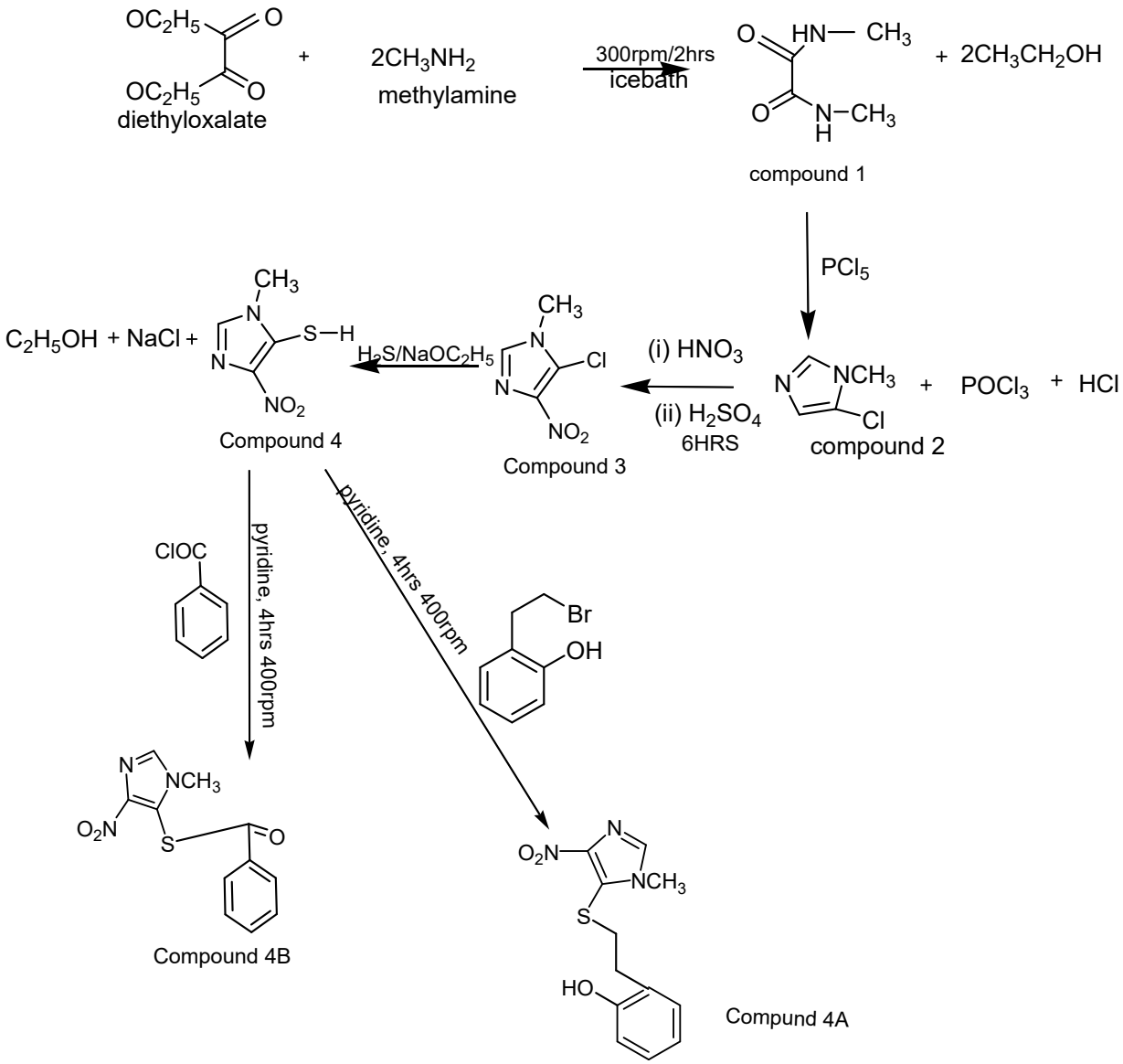


Scheme 2.12: 1-methyl-4-nitroimidazole-5-suphonyl-N-ethylaniline

374 mg (0.002 mole) of compound 4C was weighed out and poured into ice-salt cooled solution of 20 ml of 10% Na_2CO_3 and 157 mg of ethylaniline. The reaction was stirred for 6 hours using an efficient magnetic stirrer. A cloudy solution was formed after stirring for 1 hour. The solution was poured into 20 ml of water containing drops of HCl and then shaken for some few minutes. Chloroform was used to extract the compound formed from the reaction medium. This was done

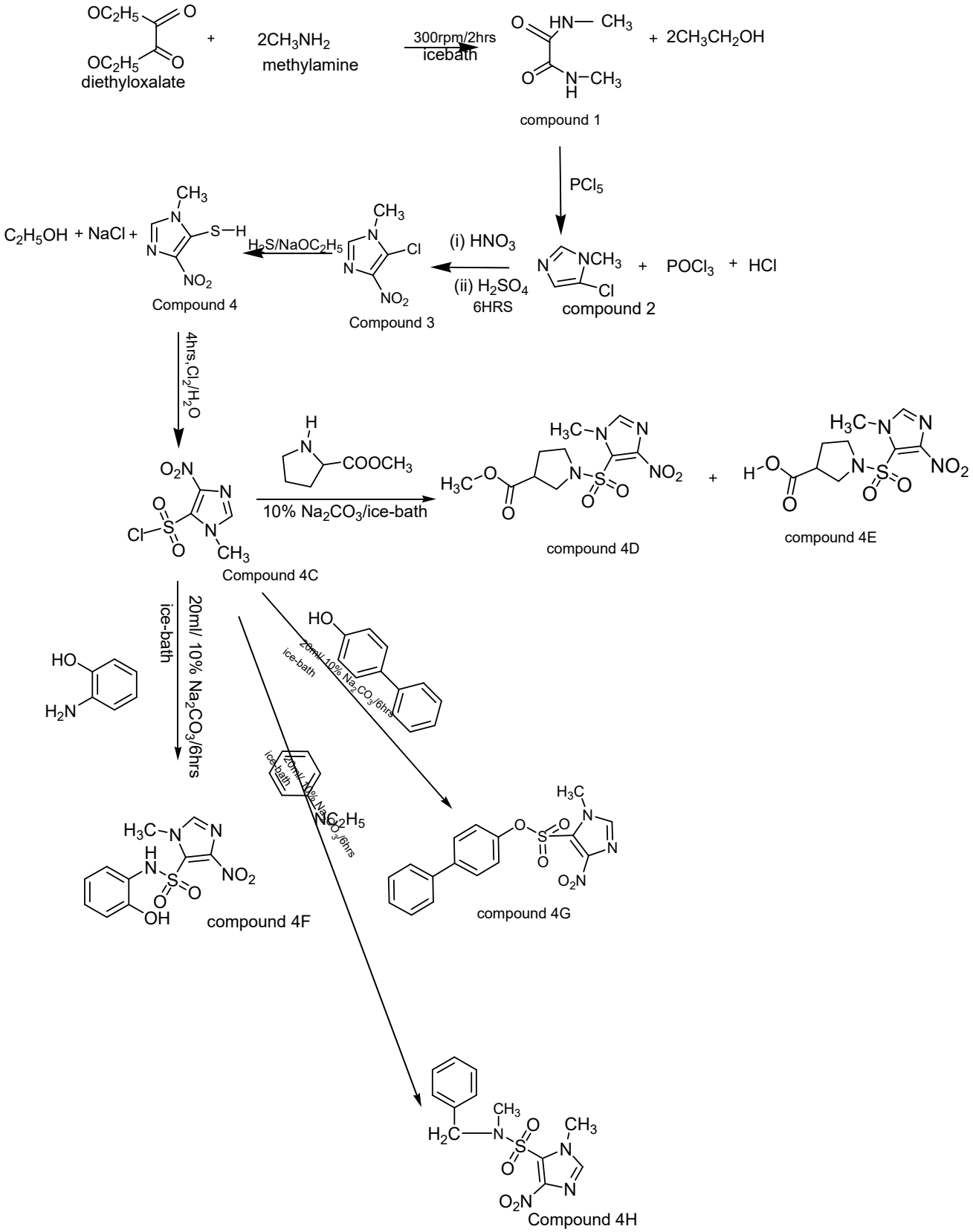
three times. The extracted compound was dried using $CaSO_4$ for 24 hours. The chloroform was distilled off using simple distillation set up to obtain the compound of interest. More so, a short column was done. The crystals of the compound were grey in colour and recrystallized with ethanol with a melting point of 172°C – 174°C. The compound was weighed as 263 mg (50.82 % yield).

2.4.3 Scheme of Reactions from Compound 1 to Compounds 4, 4A and 4B



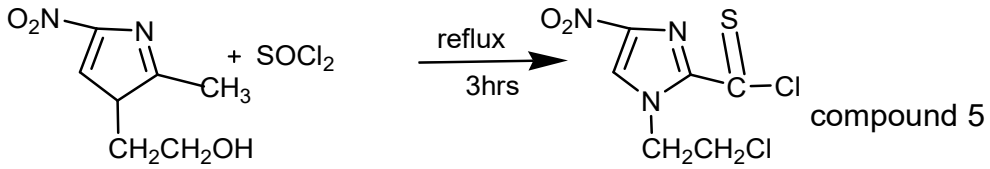
Scheme 2.13: Reactions from Compound 1 to Compounds 4, 4A and 4B

2.4.4 Scheme of Reactions from Compound 1 to Compounds 4, 4C to 4H



Scheme 2.14: Reactions from Compound 1 to Compounds 4, 4C to 4H

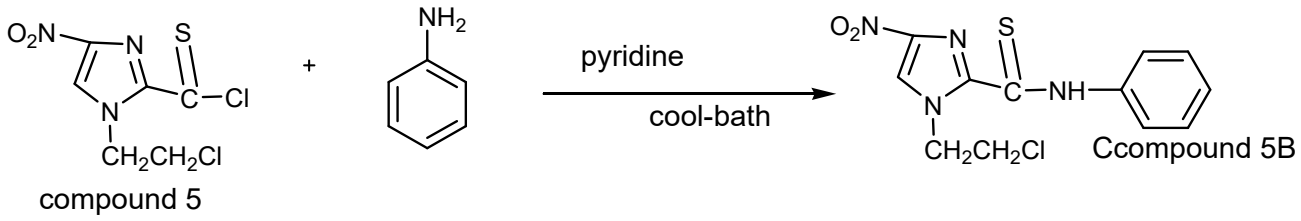
2.4.5 Synthesis of [H-(1-ethylchloride)-2-thioacyl-4-nitroimidazole] compound 5



Scheme 2.15: [H-(1-ethylchloride)-2-thioacyl-4-nitroimidazole]

4 g of metronidazole was weighed and 20 ml of freshly distilled thionyl chloride was added in to a 100 ml round bottom flask and was refluxed for 4 hrs. The excess thionyl chloride was distilled off with 40 ml chloroform using simple distillation set-up. The orange colour oil of compound 5 was formed.

2.4.6 Synthesis of [H-(1-ethylchloride)-2-thioacylaniline-4-nitroimidazole] Compound 5B



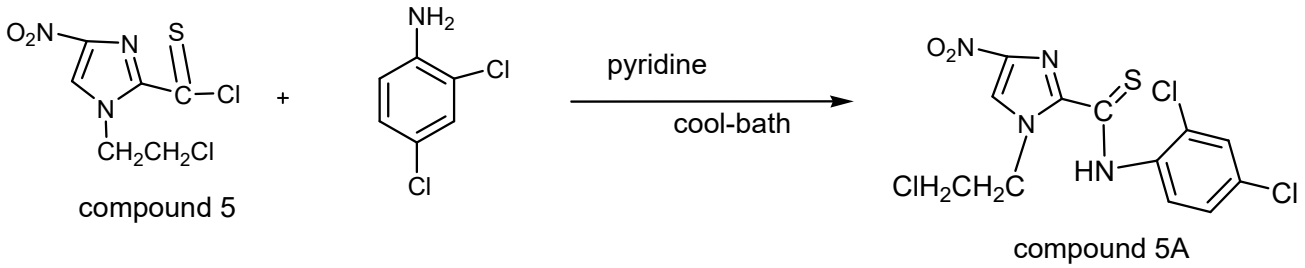
Scheme 2.16: [H-(1-ethylchloride)-2-thioacylaniline-4-nitroimidazole]

1 mole of compound 5 was dissolved in a solution of freshly distilled pyridine and 0.54 ml of aniline and was stirred for 4 hrs in cool-bath and then extracted with chloroform and allow to dried with $CaSO_4$ for 24 hrs.

The chloroform was distilled off using simple distillation set-up under vacuum. The resulting dark brown oil was recrystallized from hexane and ethanol and for further purification short column was carried out using ethylacetate and hexane (1:2).

2.4.7 Synthesis of [H-(1-ethylchloride)-2-thioacyl(2,4-dichoroaniline)-4-nitroimidazole]

Compound 5A

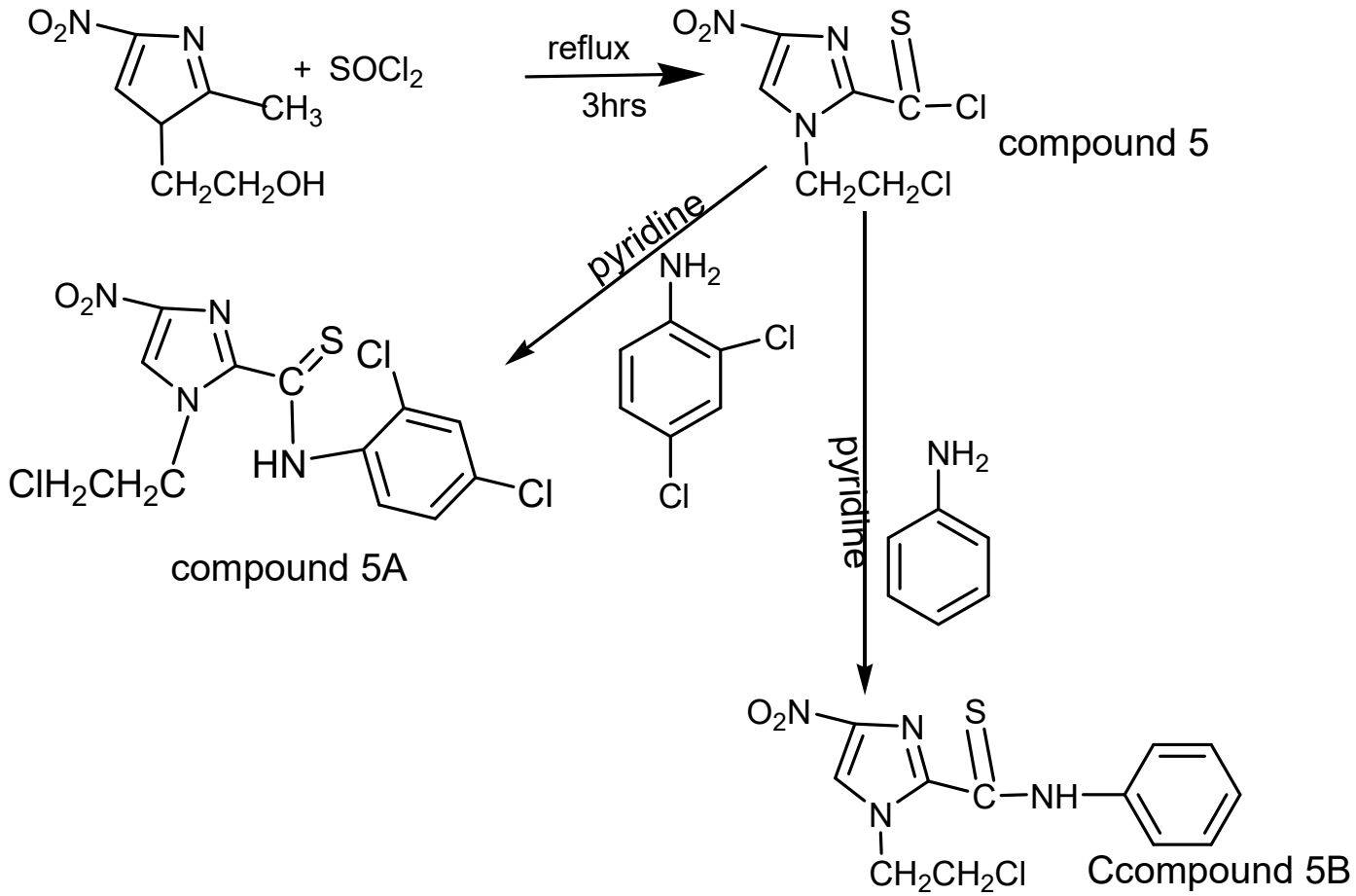


Scheme 2.17: [H-(1-ethylchloride)-2-thioacyl(2,4-dichoroaniline)-4-nitroimidazole]

1 mole of compound 5 was dissolved in a solution of freshly distilled pyridine and 0.75 g of dichloroaniline and was stirred for 4 hrs in cool-bath and then extracted with chloroform and allow to dried with $CaSO_4$ for 24 hrs.

The chloroform was distilled off using simple distillation set-up under vacuum. The resulting dark brown oil was recrystallized from hexane and ethanol and for further purification short column was carried out using ethylacetate and hexane (1:2).

2.4.8 Scheme of Reactions from Compound 5 to Compounds 5A and 5B



2.5 ANTIMICROBIAL ACTIVITY

The microorganisms employed in this study were procured from the University of Benin Teaching Hospital, Benin City. The synthesized compounds were, then, screened for their antibacterial activities *in-vitro* against gram-positive (*Staphylococcus aureus*), gram-negative (*Escherichia coli*, *Pseudomonas aeroginosa*, *B. subtilis* and *Klebsiella. pneumoniae*) and antifungal activities against *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans*

2.5.1 Media

Nutrient broth and nutrient agar, all product of Himedia Laboratory, Mumbai, India, were used for this study. The composition of the medium was beef extract, 3.0 g, peptone, 5 g, sodium chloride, 8.0 g, and agar, 15.0 g.

2.5.2 Antimicrobial Agents

Ciprofloxacin (10 µg/ml) and ketoconazole (25 mg/ml) are the antimicrobial agents that were used for the antimicrobial test.

2.5.3 Agar Well Diffusion Assay

Using the agar well diffusion technique, the compounds' antibacterial activity was ascertained. Each nutrient agar plate was seeded with 0.1 millilitres of an overnight culture of every organism. The sterile molten nutrient agar was seeded into each well using a sterile standard cork borer (6.0 mm in diameter) and left to set for 24 hours. A 0.2 ml (15 mg/ml) solution of the synthesised compounds was then applied to each well. The organism's plates were incubated at 37°C for 24 hours after which the diameter of the zones of inhibition was measured (Monica, 2006).

2.5.4 Determination of Minimum Inhibitory Concentration (MIC)

In order to prepare concentrations of 20 mg/ml, 10 mg/ml, 8 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1 mg/ml of synthesised compounds, the minimum inhibitory concentration (MIC) values of each compound were determined using two fold micro dilution. A drop of the organism's suspension,

which had previously been diluted to about 10^6 cfu/ml, was aseptically incorporated into molten nutrient agar and allowed to set. The plates were incubated at 37 ° C for 24 hours. The lowest concentrations preventing visible growth for each of the test organisms were recorded as the minimum inhibiting concentration.

The experiment was carried out in triplicate for the synthesized compounds concentration and the antimicrobial agents listed in section 2.4.3 were used as positive control while distilled water was used as the negative control against the organism's isolates (Humpries, 2021).

2.5.5 Determination of Minimum Bacterial Concentration (MBC)

Nutrient agar plates were divided into different section and labelled with different concentration on the base of the plates. These were used to plate out the contact of each minimum inhibitory concentration plate in the respective section of the plates. The plates were incubated aerobically for 18 – 24 hours at 37 ° C after which the minimum bacterial concentrations were recorded (Monica, 2006).

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Characterisation and Identification of the Synthesized Compounds

3.1.1.1 *Determination of Melting Points*

The final compounds' and their intermediates' synthesised melting points were discovered to deviate from the melting points of their constituent raw components. This is shown in Table 3.1

Table 3.1: Some Physical Properties of the Synthesized Compounds and their Intermediate

Properties	**Compounds 3**	**Compound 4**	**Compound 4A**	**Compound 4B**
Name	1-methyl-4-nitro-5-chloroimidazole	1-methyl-4-nitroimiazole-5-thiol	1-methyl-4-nitroimidazole-5-phenylethylsulphide	1-methyl-4-nitroimidazole-5-benzoylsulphide
Formula	$C_4H_4N_3O_2Cl$	$C_4H_5N_3O_2S$	$C_{12}H_{14}N_3O_3S$	$C_{11}H_9N_3O_4S$
Colour	Creamy white	Deep cream/Carton	Colourless	Light brown
Nature	Crystalline	Powdery	Crystalline	crystalline
State	Solid	Solid	Solid	Solid
Melting Point (°C)	168 – 170	115 - 117	210 – 212	117 – 119
Yield (%)	68.68	58.82	47.69	52.09
Molar mass ($gmol^{-1}$)	171.50	159.17	280	279
Properties	**Compounds 4C**	**Compound 4D**	**Compound 4E**	**Compound 4F**
Name	1-methyl-4-nitroimidazole-5-suphonylchloride	1-methyl-4-nitroimiazole-5-suphonyl prolinemethylester	1-methyl-4-nitroimidazole-5-suphonylproline	1-methyl-4-nitro-imidazole-5-suphonylaminophenyl
Formula	$C_4H_4N_3O_4ClS$	$C_{10}H_{13}N_4O_6S$	$C_9H_{12}N_4O_6S$	$C_{10}H_{12}N_4O_6S$
Colour	Creamy white	Brown	Brown	grey
Nature	Crystalline	oily	Plate-like solid	crystalline
State	Solid	Liquid	Solid	Solid
Melting Point (°C)	--	--	207-209	175
Yield (%)	50.60	53.82	67.69	62.09
Molar mass ($gmol^{-1}$)	225.61	318.31	305.28	298.28
Properties	**Compounds 4G**	**Compound 4H**	**Compound 5A**	**Compound 5B**
Name	1-methyl-4-nitroimidazole-5-suponylbiphenylhydroxyl	1-methyl-4-nitroimiazole-5-suphonyl-N-ethylaniline	[H-(1-ethylchloride)-2-thioacyl(2,4-dichoroaniline)-4-nitroimidazole	[H-(1-ethylchloride)-2-thioacylaniline-4-nitroimidazole
Formula	$C_{16}H_{13}N_3O_5S$	$C_{12}H_{14}N_3O_5S$	$C_{12}H_9N_4O_2SCl_3$	$C_{12}H_{11}N_3O_2SCl$
Colour	Creamy yellow	grey	Light brown	deep brown
Nature	Crystalline	Powdery	Oily	oily
State	Solid	Solid	Viscous	viscous
Melting Point (°C)	209-211	171-173	57-58	45-46
Yield (%)	46.68	50.82	54.69	56.09
Molar mass ($gmol^{-1}$)	359.00	312.33	379.50	296.5

3.1.1.2 Thin Layer Chromatography

Table 3.2 shows the R_F values of the intermediates and final compounds which showed single round spots appeared after exposing the chromatograms to iodine vapour indicating the purity of the synthesized compounds and the completion of the reactions.

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

Table 3.2: Comparative R_F Values of Intermediates and Final Compounds with the solvent used as their mobile phase

Compounds	R_F Values	Solvents Used (mobile phase)
Compound 1	0.91	$CHCl_3$ and C_2H_5OH (2:1)
Compound 3	0.83	$CHCl_3$ and C_2H_5OH (2:1)
Compound 4	0.14	$CHCl_3$ and EtOAc (1:2)
Compound 4A	0.30	$CHCl_3$ and EtOAc (1:2)
Compound 4B	0.23	$CHCl_3$ and C_2H_5OH (2:1)
Compound 4C	--	--
Compound 4D	0.60	C_6H_{14} and C_2H_5OH (2:1)
Compound 4E	0.43	C_6H_{14} and C_2H_5OH (2:1)
Compound 4F	0.53	C_6H_{14} and C_2H_5OH (4:1)
Compound 4G	0.77	C_6H_{14} and C_2H_5OH (3:1)
Compound 4H	0.86	C_6H_{14} and C_2H_5OH (2:1)
Compound 5	0.35	C_6H_{14} and C_2H_5OH (1:2)
Compound 5A	0.65	C_6H_{14} and C_2H_5OH (1:2)
Compound 5B	0.58	C_6H_{14} and C_2H_5OH (1:2)

3.1.1.3 Antimicrobial Activities of Synthesized Compounds

Table 3.3 to Table 3.8 show the results of the antibacterial and antifungal activities of the synthesized compounds (compounds 3, 4, 4A, 4B, 4D, 4E, 4F, 4G, 4H, 5A, 5B).

Table 3.3: Reference Antibiotic Sensitivity Test 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

Table 3.3a: Antibacterial Activity of Synthesized Compounds at 20 mg/ml (IZD)

Compounds	Zone of Inhibition (mm)				
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Bacillus subtilis***
Compound 3	30.00	25.00	24.00	20.00	25.00
Compound 4D	20.00	16.00	20.00	16.00	18.00
Compound 4E	0.00	0.00	0.00	9.00	10.00
Compound 4F	9.00	0.00	10.00	0.00	9.00
Compound 4H	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin	30.00	0.00	22.00	0.00	33.00

Table 3.3b: Antibacterial Activity of Synthesized Compounds at 10mg/ml, 5mg/ml and 1 mg/ml (IZD)

	Microbes	Compound 4 **10 mg/ml**	Compound 5B **10 mg/ml**	Compound 4A **8 mg/ml**	Compound 4B **5 mg/ml**	Compound 5A **1 mg/ml**	ciprofloxacin **10 mg/ml, 8 mg/ml, 5 mg/ml, 1mg/ml**
Bacterial Isolates	*E. coli*	12.00	15.00	0.00	0.00	0.00	0.00
	S. aureus	0.00	16.00	0.00	12.00	0.00	30.00
	B. subtilis	10.00	9.00	0.00	0.00	0.00	33.00
	P. aeruginosa	0.00	9.00	0.00	9.00	0.00	22.00
	k. pneumoniae	0.00	15.00	0.00	9.00	0.00	0.00
Fungal Isolates	*A. niger*	0.00	15.00	15.00	18.00	0.00	0.00
	C. albicans	0.00	0.00	0.00	24.00	0.00	0.00

Table 3.3c: Antifungal Activity of Synthesized Compounds at 20 mg/ml

Compounds	Zone of inhibition (mm)	
	Aspergillus niger	***Candida albicans***
Compound 4D	28.00	0.00
Compound 4E	0.00	0.00
Compound 4H	15.00	0.00
Compound 4F	20.00	19.00
Ketoconazole	20.00	25.00
Control (tween-80 30%)	–	–

Table 3.3d: Antifungal Activity of Synthesized at 10 mg/ml

compounds	Zone of inhibition (mm) on fungi isolates	
	Aspergillus niger	***Candida albicans***
Compound 4@ 10 mg/ml	0.00	0.00
Compound 5B @ 10 mg/ml	22.00	20.00
Compound 3@ 10 mg/ml	25.00	20.00
Compound 4A @ 8 mg/ml	15.00	0.00
Compound 4B @ 5 mg/ml	18.00	24.00
Compound 5A @ 5 mg/ml	0.00	0.00
Ketoconazole	20.00	25.00
Control (Tween-80 30%)	–	–

Table 3.4a: 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

Table 3.4b: 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 3.5a: Antimicrobial Activity of Compound 4 (MIC)

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

NG = No Growth, G = Growth

Table 3.5b: Antimicrobial Activity of Compound 4 (MBC)

	microbes	Control (30% Tween-80)	2.5 mg/ml	1.25 mg/ml
Bacterial Isolates	*E. coli*	-	G	G
	S. aureus	-	-	-
	B. subtilis	-	G	G
	P. aeruginosa	-	-	-
	k. pneumoniae	-	-	-
Fungal Isolates	*A. niger*	-	--	--
	C. albicans	-	--	--

NG = No Growth, G = Growth

Table 3.6a: Antimicrobial Activity of Compound 4A (MIC)

	microbes	Control (50% Tween-80)	2.5 mg/ml	1.25 mg/ml
Bacterial Isolates	*E. coli*	-	0.00	0.00
	S. aureus	-	0.00	0.00
	B. subtilis	-	0.00	0.00
	P. aeruginosa	-	0.00	0.00
	k. pneumoniae	-	0.00	0.00
Fungal Isolates	*A. niger*	-	NG	--
	C. albicans	-	--	--

NG = No Growth, G = Growth

Table 3.6b: Antimicrobial Activity of Compound 4A (MBC)

	microbes	Control (30% Tween-80)	2.5 mg/ml	1.25 mg/ml
Bacterial Isolates	*E. coli*	-	--	--
	S. aureus	-	--	-
	B. subtilis	-	--	-
	P. aeruginosa	-	--	-
	k. pneumoniae	-	--	-
Fungal Isolates	*A. niger*	-	G	G
	C. albicans	-	-	-

NG = No Growth, G = Growth

Table 3.7a: Antimicrobial Activity of Compound 4B (MIC)

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

NG = No Growth, G = Growth

Table 3.7b: Antimicrobial Activity of Compound 4B (MBC)

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

NG = No Growth, G = Growth

Table 3.8a: Antimicrobial Activity of Compound 4D (MIC)

	microbes	Control (30% Tween-80)	15 mg/ml	7.5 mg/ml	3.75 mg/ml
Bacterial Isolates	*E. coli*	-	G	G	G
	S. aureus	-	NG	NG	G
	B. subtilis	-	NG	NG	G
	P. aeruginosa	-	NG	NG	G
	k. pneumoniae	-	NG	NG	G
Fungal Isolates	*A. niger*	-	--	--	
	C. albicans	-	NG	G	

NG = No Growth, G = Growth

Table 3.8b: Antimicrobial Activity of Compound 4D (MBC)

	microbes	Control (50% Tween-80)	15 mg/ml	7.5 mg/ml	3.75 mg/ml
Bacterial Isolates	*E. coli*	-	NG	G	G
	S. aureus	-	NG	NG	G
	B. subtilis	-	NG	NG	G
	P. aeruginosa	-	NG	NG	G
	k. pneumoniae	-	NG	G	G
Fungal Isolates	*A. niger*	-	-	G	G
	C. albicans	-		NG	G

NG = No Growth, G = Growth

Table 3.9: Antimicrobial Activity of Compound 4E (MIC)

	microbes	Control (30% Tween-80)	15 mg/ml	7.5 mg/ml	3.75 mg/ml
Bacterial Isolates	*E. coli*	-	--	--	--
	S. aureus	-	--	--	--
	B. subtilis	-	G	G	G
	P. aeruginosa	-	--	--	--
	k. pneumoniae	-	G	G	G
Fungal Isolates	*A. niger*	-	--	--	--
	C. albicans	-	--	--	--

NG = No Growth, G = Growth

Table 3.10: Antimicrobial Activity of Compound 4F (MIC)

	microbes	Control (30% Tween-80)	15 mg/ml	7.5 mg/ml	3.75 mg/ml
Bacterial Isolates	*E. coli*	-	-	-	-
	S. aureus	-	G	G	G
	B. subtilis	-	G	G	G
	P. aeruginosa	-	G	G	G
	k. pneumoniae	-	--	-	-
Fungal Isolates	*A. niger*	-	G	G	G
	C. albicans	-	NG	NG	G

NG = No Growth, G = Growth

Table 3.11: Antimicrobial Activity of Compound 4H (MIC)

	Microbes	Control (30% Tween-	15 mg/ml	7.5 mg/ml	3.75 mg/ml

		80)			
Bacterial Isolates	*E. coli*	-	-	-	-
	S. aureus	-	-	-	-
	B. subtilis	-	-	-	-
	P. aeruginosa	-	-	-	-
	k. pneumoniae	-	--	-	-
Fungal Isolates	*A. niger*	-	G	G	G
	C. albicans	-	--	--	--

NG = No Growth, G = Growth

Table 3.12a: Antimicrobial Activity of Compound 5B (MIC)

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

NG = No Growth, G = Growth

Table 3.12b: Antimicrobial Activity of Compound 5B (MBC)

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

NG = No Growth, G = Growth

3.1.1.4 IR and NMR Analysis of Synthesized Compounds

Table 3.13: Characteristic IR Bands of Compound 3

Serial No.	Functional Group (Vibration Mode)	Frequency (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

^{1}HNMR Fig 2.0: (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 2.0a:** (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).

Table 3.14: Characteristic IR Bands of Compound 4

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	Sp^2 C - H stretch	3000
2.	C - N stretch	1100
3.	C - S stretch	803.96

Table 3.15: Characteristic IR Bands of Compound 4A

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	sp^3 C - H stretch	2918, 2851
2.	Phenolic OH ring	1602, 1494
3.	Aromatic C-H bend	1910
4.	Phenolic C-O	1080
5.	Monosubstituted benzene	697, 706.54

^{1}HNMR Fig 2.2 : (600 MHz, $CDCl_3$, ppm): δ 3.04 (t, 2H, methylene-C), δ 3.47 (t, 2H, S-methylene), δ 3.71 (s, 3H, N-CH_3), δ 4.82 (s, 1H, O-H), δ 6.79-7.06 (m, 4H, Protons of the phenyl ring), δ 7.26 (Solvent signal), δ 7.49 (s, 1H, N-methine). **^{13}C NMR Fig 2.2a :** δ 30.99 (s, S-CH_2), δ 34.04 (s, C-CH_2), δ 34.35 (s, N-CH_3), δ 76.28 (Solvent signal), δ 132.27 (s, C-S), δ 138.69 (s, C=N), δ 150.76 (s, C-NO_2), δ 154.50 (s, C-OH).

Table 3.16: Characteristic IR Bands of Compound 4B

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	sp^3 C - H stretch	2918, 2851
2.	Aromatic C-NO_2, Stretch	1328.34
3.	Aromatic C-H bend	1910
4.	Monosubstituted benzene	697, 706.54
5.	C-S Stretch	1079.5

^{1}HNMR Fig 2.3 : (600 MHz, $CDCl_3$, ppm): δ 3.79 (solvent signal), δ 3.79 (s, 3H, N-CH_3), δ 6.50 (s, 1H, N=C-H of the imidazole ring), δ 7.71-7.749 (m, 5H, methine on the benzoyl ring). **^{13}C NMR Fig 2.3a:** (600 MHz, $CDCl_3$, ppm): δ 34.35 (N-CH_3), δ 129.6-134.9 (s, carbons of the benzoyl ring), δ 138.24 (s, C-S), δ 138.69 (s, N=C-N), δ 144.93 (s, C-NO_2), δ 180.29 (s, C=O).

Table 3.17: Characteristic IR Bands of Compound 4D

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	sp^3 C - H stretch	2950.18, 2879.05
2.	C=O Stretch, ester	1723.50
3.	S=O Stretch, sulfonamide	1350.61
4.	C-N Stretch, amine	1138.20
5.	C=C Stretch, conjugated alkene	1600.32

Table 3.18: Characteristic IR Bands of Compound 4E

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	sp^3 C - H stretch	2927.50, 2850.1
2.	C-N Stretch, amine	1200.8
3.	C=C Stretch, cyclic alkene	1594.18
4.	S=O Stretch, sulfoxide	1050
5.	C-NO_2, Stretch	1500
6.	C-O, Stretch, acid	1442.63

Table 3.19: Characteristic IR Bands of Compound 4F

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	O – H, stretch	3198.41, 3125.58
2.	N-H Stretch, Aromatic amine	3496.52
3.	S=O, sulfonamide	1335
4.	CH_3-N, Deformation	1440.31
5.	disubstituted benzene	652, 775.06
6.	C-H Stretch	2701.35

^{1}HNMR Fig 2.5 : (600 MHz, C_2D_5OD, ppm): δ 1.20 (CH_3 of the solvent), δ 3.65 (CH_2 of the solvent), δ 7.50 (C-H of the imidazole), δ 9.68 (s, 1H, N-H), δ 3.76(O-H), δ 3.74(N-CH_3), δ 6.38-6.67 (m, 4H, C-H of the benzene ring). **^{13}C NMR Fig2.5a :** (600 MHz, C_2D_5OD, ppm): δ 15.84 (CH_3 of the solvent). δ 57.45 (CH_2 of the solvent), δ 34.35 (N-CH_3), δ 148.97 (C-OH), δ 138.65 (C-H of the benzene ring), δ 143.50 (C-NO_2) δ 139.32 (C-SO_2).

Table 3.20: Characteristic IR Bands of Compound 4G

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	sp^3 C - H stretch	2950
2.	S=O Stretch, sulphonates	1357.55
3.	CH_3-N deformation	1421.38
4.	C-H bend, aromatic	1654.09
5.	Monosubstituted benzene	650

^{1}HNMR Fig 2.6 : (600 MHz, C_2H_5OD, ppm): δ 1.21 (CH_3 of the solvent), δ 3.61 (CH_2 of the solvent), δ 7.49 (C-H of the imidazole), δ 3.78(N-CH_3), δ 7.49-7.81 (m, 10H, C-H of the benzene ring). **^{13}C NMR Fig2.6a :** (600 MHz, C_2D_5OD, ppm): δ 15.84 (CH_3 of the solvent). δ 57.45 (CH_2 of the solvent), δ 34.35 (N-CH_3), δ 138.69(C=N), δ 127.29-149.73 (C-H of the benzene ring), δ 159.64 (C-NO_2) δ 130.14 (C-SO_2), δ 149.73 (C-O).

Table 3.21: Characteristic IR Bands of Compound 4H

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	sp^3 C - H stretch	2927
2.	C-N Stretch	1150
3.	C-N Stretch, aromatic amine	1300.28
4.	C-H bend, aromatic	2000
5.	Monosubstituted benzene	618.83

^{1}HNMR Fig 2.7 : (600 MHz, C_2D_5OD, ppm): δ 1.20 (CH_3 of the solvent), δ 2.85 (t, 2H, benzyl methylene), δ 3.11 (t, 2H, CH_2-NH), δ 3.65 (CH_2 of the solvent), δ 3.77 (s, 3H, N-CH_3), δ 4.61 (s, 1H, N-H), δ 7.14-7.25 (m, 5H, C-H of the phenyl ring), δ 7.75 (s, 1H, C-H of the Imidazole ring). **^{13}C NMR Fig 2.7a:** δ 154.71 (s, C-NO_2), δ 147 (s, C-SO_2), δ (s, C=N), δ 139. 08 (s, C-CH_2-CH_2), δ 57.45 (s, CH_2 of the solvent), δ 45.35 (s, C-NH), δ 35.66 (s, N-CH_3), δ 15.84 (s, CH_3 of the solvent).

Table 3.22: Characteristic IR Bands of Compound 5A

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	sp^3 C - H stretch	2918, 2851
2.	Aromatic ring	1602, 1494
3.	CH_2 bend	1453
4.	CH_3 bend	1375
5.	Monosubstituted benzene	697, 760

^{1}HNMR Fig 2.8 : (300 MHz, $CDCl_3$, ppm): δ 3.80 (t, 2H, methylene bonded to chlorine), δ 4.23 (t, 2H, N-CH_2), δ 7.26 (solvent signal), δ 7.27-7.29 (d, 1H, methine of the benzene ring), δ 7.43 (s, 1H, methine between the C-Cl's of the benzene ring), δ 7.73 (s, 1H, methine of the imidazole ring), δ 10.02 (s, 1H, N-H attached to the benzene ring). **^{13}C NMR Fig 2.8a :** (300 MHz, $CDCl_3$, ppm): δ 42.85 (s, methylene carbon bonded to Cl), δ 50.97 (s, methane bonded to N of the imidazole), δ 76. 98 (solvent signal), δ 126.19-131.00 (s, carbons of the phenyl ring), δ 132. 96 (s, C=N), δ 134.83 (C-N-H), δ 139.31 (s, methine carbon of the imidazole ring), δ 158.46 (s, C-NO_2), δ 184. 22 (s, C=S).

Table 3.23: Characteristic IR Bands of Compound 5B

Serial No.	Functional Group (Vibration Mode)	Frequency (cm^{-1})
1.	N-H Stretch, aromatic amide	3500.64
2.	C-N Stretch, aromatic	850.43
3.	C-Cl stretch, monochloroalkanes	687.15
4.	C=S stretch	1278.5
5.	C-H Stretch	2398.36

^{1}HNMR Fig 2.9 : (600 MHz, $CDCl_3$, ppm): δ 3.72 (t, 2H, C-methylene), δ 4.86 (t, 2H, N-methylene), δ 7.26 (solvent signal), δ 9.56 (s, 1H, N-H), δ 8.73 (m, C-H), δ 7.16-7.36 (m, methine in the phenyl ring). **^{13}C NMR Fig 2.9a :** δ 42.85 (s, Cl-methine), δ 51.73 (s, N-methine), δ 76.98 (solvent signal), δ 144.57 (s, N-N-C), δ 146.71 (s, C-NO_2), δ 175.96 (s, C=S). δ 138.01 (s, C-N-H).

3.2 DISCUSSION

3.2.1 IR and NMR Analysis

Table 3.13 shows the characteristic IR bands of compound 3. The 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 2.0**: (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 2.0a:** (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.

Table 3.14 shows the characteristic IR bands of compound 4. The IR spectrum showed absorption bands at 3000cm^{-1} (sp^3 C – H stretch), 1100 cm^{-1} (C-N, Stretch), 803.98 cm^{-1} (C-S, Stretch). In addition, the NMR result showed ^{1}HNMR Fig 2.0: (600 MHz, MeOD ppm): δ 4.96 (s, solvent signal), δ 3.48 (s, 3H, N-CH_3), δ 7.49 (s, 1H, N=C-H of the imidazole ring), δ 3.35 (s, 1H, C-S-H stretch). ^{13}C NMR Fig 2.0a: (600MHz, MeOD 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 also correspond to the expected compound 4.

Table 3.15 shows the characteristic IR bands of compound 4A. The IR spectrum showed absorption bands at 2918cm^{-1}, 2851cm^{-1},(sp^3 C – H stretch),1602cm^{-1}, 1494 cm^{-1} (phenolic- OH ring stretch), 1606cm^{-1}(C = N stretch), 1910 cm^{-1} (aromatic C-H bend). Furthermore, the result of NMR analysis showed that the ^{1}HNMR Fig 2.2: (600 MHz, $CDCl_3$, ppm): δ 3.04 (t, 2H, methylene-C), δ 3.47 (t, 2H, S-methylene), δ 3.71 (s, 3H, N-CH_3), δ 4.82 (s, 1H, O-H), δ 6.79-7.06 (m, 4H, Protons of the phenyl ring), δ 7.26 (Solvent signal), δ 7.49 (s, 1H, N-methine). ^{13}C NMR Fig 2.2A: δ 30.99 (s, S-CH_2), δ 34.04 (s, C-CH_2), δ 34.35 (s, N-CH_3), δ 76.28 (Solvent signal), δ 132.27 (s, C-S), δ 138.69 (s, C=N), δ 150.76 (s, C-NO_2), δ 154.50 (s, C-OH) and this correspond to the expected compound 4A.

Table 3.16 shows the characteristic IR bands of compound 4B. The IR spectrum showed absorption bands at 2918cm^{-1}, 2851cm^{-1},(sp^3 C – H stretch),1328.24 cm^{-1} (C- NO_2 stretch of the imidazole ring), 1079.5cm^{-1}(C – S stretch), 1910 cm^{-1} (aromatic C-H bend), 697cm^{-1}, 706.24cm^{-1} (monosubstituted benzene) . Furthermore, the result of NMR analysis showed ^{1}HNMR Fig 2.3 (600 MHz, $CDCl_3$, ppm): δ 3.79 (solvent signal), δ 3.79 (s, 3H, N-CH_3), δ 6.50 (s, 1H, N=C-H of the imidazole ring), δ 7.71-7.749 (m, 5H, methine on the benzoyl ring). ^{13}C NMR Fig 2.3A: (600 MHz, $CDCl_3$, ppm): δ 34.35 (N-CH_3), δ 129.6-134.9 (s, carbons of the benzoyl ring), δ 138.24 (s, C-S), δ 138.69 (s, N=C-N), δ 144.93 (s, C-NO_2), δ 180.29 (s, C=O) and this corresponds to the expected structure of compound 4B.

Table 3.17 shows the characteristic IR bands of compound 4D. The IR spectrum showed absorption bands at 2950cm^{-1}, 2879.05cm^{-1},(sp^3 C – H stretch),1723.50 cm^{-1} (C=O stretch of the ester), 1350.6cm^{-1}(S=O stretch of the sulfonamide), 1138.20 cm^{-1} (C-N stretch of amine), 1600.32cm^{-1} (C=C of the conjugated alkene).

Table 3.18 shows the characteristic IR bands of compound 4E. The IR spectrum showed absorption bands at 2927.50cm^{-1}, 2850.1cm^{-1},(sp^3 C – H stretch),1200.8 cm^{-1} (C- N stretch of the amine), 1594.18cm^{-1}(C=C stretch of a cyclic alkene), 1050 cm^{-1} (S=O stretch), 1500cm^{-1} (C-NO_2 stretch), 1442.63cm^{-1}(C-O stretch of an acid).

Table 3.19 shows the characteristic IR bands of compound 4F. The IR spectrum showed absorption bands at 3198.41cm^{-1}, 3125.58cm^{-1},(O–H stretch of the intermolecular of phenol), 3496.53 cm^{-1} (N-H stretch of the aromatic amine), 1335.0cm^{-1}(S=O stretch of the sulfonamide), 1440.31 cm^{-1} (N-CH_3 deformation), 657cm^{-1}, 775.24cm^{-1} (disubstituted benzene), 2701.35 cm^{-1} (C-H stretch). Furthermore, the result of NMR analysis showed ^{1}HNMR Fig 2.5:(600 MHz, C_2D_5OD, ppm): δ 1.20 (CH_3 of the solvent), δ 3.65 (CH_2 of the solvent), δ 7.50 (C-H of the imidazole), δ 9.68 (s, 1H, N-H), δ 3.76(O-H), δ 3.74(N-CH_3), δ 6.38-6.67 (m, 4H, C-H of the benzene ring). **^{13}C NMR Fig 2.5A:** (600 MHz, C_2D_5OD, ppm):

δ 15.84 (CH_3 of the solvent). δ 57.45 (CH_2 of the solvent), δ 34.35 (N-CH_3), δ 148.97 (C-OH), δ 138.65 (C-H of the benzene ring), δ 143.50 (C-NO_2) δ 139.32 (C-SO_2).and this corresponds to the expected structure of compound 4F.

Table 3.20 shows the characteristic IR bands of compound 4G. The IR spectrum showed absorption bands at 2950cm^{-1}(Sp^3–C-H stretch), 1357.53 cm^{-1} (S=O stretch, sulfonamide), 1421.38 cm^{-1} (N-CH_3 deformation), 650cm^{-1} (monosubstituted benzene), 1654.09cm^{-1} (C-H bend of aromatic). Furthermore, the result of NMR analysis showed **^{1}HNMR Fig 2.6:** (600 MHz, C_2D_5OD, ppm): δ 7.81 (C-H of the imidazole), δ 1.21 (CH_3 solvent signal), δ 3.61(CH_2 of the solvent), δ 7.49- δ 7.81 (s, C-H, biphenyl ring), δ 3.78 (s, 3H, N-CH_3 in the imidazole ring)**. ^{13}C NMR Fig 2.6A:** (600 MHz, C_2D_5OD, ppm): δ159.64 (C-NO_2), δ 130.14 (C-SO_2), δ 126.96- 138.69 (C-H of the benzene ring), δ 15.84 (CH_3 of the solvent signal), δ 57.45(CH_2 of the solvent signal), δ 149.73(C- O of the benzyl), δ 34.35 (N-CH_3) and this corresponds to the expected structure of compound 4G.

Table 3.21 shows the characteristic IR bands of compound 4H. The IR spectrum showed absorption bands at 2927cm^{-1}(Sp^3–C-H stretch), 1150cm^{-1} (C-N stretch, alkene), 1300.28 cm^{-1} (C-N stretch of aromatic amine), 618.85cm^{-1} (monosubstituted benzene), 2000cm^{-1} (C-H bend of aromatic). Furthermore, the result of NMR analysis showed **^{1}HNMR Fig 2.7:** (600 MHz, C_2D_5OD, ppm): δ 7.81 (C-H of the imidazole), δ 1.21 (CH_3 solvent signal), δ 3.61(CH_2 of the solvent), δ 7.49- δ 7.81 (s, C-H, biphenyl ring), δ 3.78 (s, 3H, N-CH_3 in the imidazole ring)**.** (300 MHz, C_2D_5OD, ppm): δ 1.20 (CH_3 of the solvent), δ 2.85 (t, 2H, benzyl methylene), δ 3.11 (t, 2H, CH_2-NH), δ 3.65 (CH_2 of the solvent), δ 3.77 (s, 3H, N-CH_3), δ 4.61 (s, 1H, N-H), δ 7.14-7.25 (m, 5H, C-H of the phenyl ring), δ 7.75 (s, 1H, C-H of the Imidazole ring). **^{13}C NMR 2.7A:** δ 154.71 (s, C-NO_2), δ 147 (s, C-SO_2), δ (s, C=N), δ 139. 08 (s, C-CH_2-CH_2), δ 57.45 (s, CH_2 of the solvent), δ 45.35 (s, C-NH), δ 35.66 (s, N-CH_3), δ 15.84 (s, CH_3 of the solvent) and this corresponds to the expected structure of compound 4H.

Table 3.22 shows the characteristic IR bands of compound 5A. The IR spectrum showed absorption bands at 2918cm^{-1}(Sp^3–C-H stretch), 1153cm^{-1} (CH_2 bend), 1602cm^{-1} (aromatic ring), 697cm^{-1} (monosubstituted benzene), 1375cm^{-1} (Sp^3 C-H bend). Furthermore, the result of NMR analysis showed **^{1}HNMR Fig 2.8** (600 MHz, $CDCl_3$, ppm): δ 3.80 (t, 2H, methylene bonded to chlorine), δ 4.23 (t, 2H, N-CH_2), δ 7.26 (solvent signal), δ 7.27-7.29 (d, 1H, methine of the benzene ring), δ 7.43 (s, 1H, methine between the C-Cl's of the benzene ring), δ 7.73 (s, 1H, methine of the imidazole ring), δ 10.02 (s, 1H, N-H attached to the benzene ring). **^{13}C NMR Fig 2.8A**: (600 MHz, $CDCl_3$, ppm): δ 42.85 (s, methylene carbon bonded to Cl), δ 50.97 (s, methine bonded to N of the imidazole), δ 76. 98 (solvent signal), δ 126.19-131.00 (s, carbons of the phenyl ring), δ 132. 96 (s, C=N), δ 134.83 (C-N-H), δ 139.31 (s, methine carbon of the imidazole ring), δ 158.46 (s, C-NO_2), δ 184. 22 (s, C=S) and this corresponds to the expected structure of compound 5A.

Table 3.23 shows the characteristic IR bands of compound 5B. The IR spectrum showed absorption bands at 3500.64cm^{-1}(N-H stretch, aromatic amide), 850.43cm^{-1} (C-N stretch, aromatic), 687.15cm^{-1} (C-Cl stretch monochloroalkane), 1278.5cm^{-1} (C=S stretch), 2398.36cm^{-1} (C-H stretch). Furthermore, the result of NMR analysis showed **^{1}HNMR Fig 2.9**: (600 MHz, $CDCl_3$, ppm): δ 3.72 (t, 2H, C-methylene), δ 4.86 (t, 2H, N-methylene), δ 7.26 (solvent signal), δ 9.56 (s, 1H, N-H), δ 8.73 (m, C-H), δ 7.16-7.36 (m, methine in the phenyl ring). **^{13}C NMR Fig 2.9A**: δ 42.85 (s, Cl-methine), δ 51.73 (s, N-methine), δ 76.98 (solvent signal), δ 144.57 (s, N-N-C), δ 146.71 (s, C-NO_2), δ 175.96 (s, C=S). δ 138.01 (s, C-N-H) and this corresponds to the expected structure of compound 5B.

3.2.2 Antimicrobial Activity

Antimicrobial activity of the synthesised compounds was tested. From 20 mg/ml to 1 mg/ml, the compounds' activity against the tested microorganisms decreased as their

concentration was reduced. This indicates that the activity of the compounds is dose-dependent.

3.2.2.1 Antibacterial Activity

3.2.2.1.1 Zone of Inhibition

Table 3.3 to Table 3.3d represents the zone of inhibition for both standard drugs and the synthesised compounds 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 3.3 showed 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

From table 3.3a and Table 3.3b represent the zone of inhibition for all synthesised compounds at their different concentration and this show the activities of the synthesised compounds on the different bacteria isolates.

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 or inhibition for *E. coli* (25.00 mm) than the standard drug (ciprofloxacin).

Compound 4D at the 20 mg/ml concentration shows an activity with a zone of inhibition for *S. aureus* (20.00 mm), *P. aeruginosa* (20.00 mm), *K. pneumonia* (16.00 mm), *B. subtilis* (18.00 mm), *E. coli* (16.00 mm). the compound 4D activity is as 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* and *K. pneumonia*. The compound 4D was able to show higher and better activity or inhibition for *K. pneumonia* (16.00 mm) and *E. coli* (16.00 mm) than the standard drug (ciprofloxacin) and synthesised compound 3.

Compound 4E at the 20 mg/ml concentration shows no activity or any zone of inhibition for *S. aureus*, *P. aeruginosa* and *E. coli* but has activity for *K. pneumonia* (9.00 mm), *B. subtilis* (10.00 mm). The Compound 4E activity shows that it cannot be used as a broad spectrum bacterial activity as compared to the standard drugs.

Compound 4F at the 20 mg/ml concentration has a zone of inhibition for *S. aureus* (9.00 mm), *P. aeruginosa* (10.00 mm), and *B. subtilis* (9.00 mm) but has no inhibition for *E. coli* and *K. pneumonia.* Compound 4F has activity that is low compared to the standard drug 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* and *K. pneumonia.*

Compound 4H at the 20 mg/ml concentration has no zone of inhibition for all selected bacterial isolates.

From the Table 3.3b, Compound 4 at the 10 mg/ml concentration has no activity or any zone of inhibition for *S. aureus*, *P. aeroginosa*, *K. pneumonia* and but has activity *B. subtilis* (10.00 mm) and *E. coli* (12.00 mm) and the compound 4 has better activity for *E. coli* as compared to the standard drug (ciprofloxacin) which has a better activity at same concentration for *S. aureus* (30.00 mm), *P. aeroginosa* (22.00 mm), *B. subtilis* (33.00 mm) but has no inhibition for *E. coli* and *K. pneumonia.*

Compound 5B at the 10 mg/ml concentration has activity with a zone of inhibition for *S. aureus* (16.00 mm), *P. aeroginosa* (9.00 mm), *K. pneumonia* (15.00 mm), *B. subtilis* (9.00 mm) and *E. coli* (15.00 mm) and this show that compound 5B can be used as broad spectrum antibacterial.

Compound 4A at the 8 mg/ml concentration has no activity or any zone of inhibition for all the selected bacterial isolates used in this research.

Compound 5A at the 1 mg/ml concentration has no activity or any zone of inhibition for all the selected bacterial isolates used in this research.

Compound 4B at the 5 mg/ml concentration has activity with a zone of inhibition for *S. aureus* (12.00 mm), *P. aeroginosa* (9.00 mm), *K. pneumonia* (9.00 mm), but has no activity for *B. subtilis* and *E. coli* the compound 4B has better activity for *K. pneumonia* as compared to the standard drug ciprofloxacin which has no activity for *K. pneumonia.*

3.2.2.1.2 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.

Table 3.4a, 3.5a, 3.6a, 3.7a, 3.9, 3.10 and 3.12a showed the MIC for Compound 3, 4, 4A, 4B, 4D, 4E, 4F and Compound 5B respectively.

For compound 3, the MIC values are presented in Table 3.4a. 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.

For compound 4, the MIC values are presented in Table 3.5a. The Minimum Inhibitory Concentration (MIC) of compound 4 against *E. coli* and *B. subtilis* had an observable growth at concentration of 5 mg/ml and 2.5 mg/ml. This suggests that compound 4 do not effectively hindered the growth of these isolates at these concentrations. In other words, at the 5 mg/ml and 2.5 mg/ml concentration, the synthesized compound exhibited inactivity against these particular isolates.

For compound 4A, MIC was not conducted because it shows no activity for the bacterial isolate selected for this research and it is indicated at table 3.6a.

For compound 4B, the MIC values are presented in Table 3.7a. The Minimum Inhibitory Concentration (MIC) of compound 4B against *P. aureus, P. aeruginosa, E. coli, K. pneumoniae* and *B. subtilis* had no observable growth at concentration of 2.5 mg/ml and 1.25 mg/ml. This suggests that compound 4B effectively hindered the growth of these isolates at these concentrations.

For compound 4D, the MIC values are presented in Table 3.8a. The Minimum Inhibitory Concentration (MIC) of compound 4D against *S. aureus, P. aeruginosa, K. pneumoniae* and *B. subtilis* had no observable growth at concentration of 15 mg/ml, 7.5 mg/ml and 3.75 mg/ml. This suggests that compound 4D effectively hindered the growth of these isolates at these concentrations. However, at these concentrations, the MIC indicated growth for *E. coli.* In other words, the synthesized compound exhibited inactivity against E. coli. More also, when the concentration was reduced to 3.75 mg/ml the MIC indicated growth for all bacterial isolates used. This indicated that compound 4D is dose dependent.

For compound 4E, the MIC values are presented in Table 3.9. The Minimum Inhibitory Concentration (MIC) of compound 4E against *P. aeruginosa,* and *B. subtilis* had observable growth at concentration of 15 mg/ml, 7.5 mg/ml and 3.75 mg/ml. This suggests

that compound 4E cannot effectively hinder the growth of these isolates at these concentrations.

For compound 4F, the MIC values are presented in Table 3.10. The Minimum Inhibitory Concentration (MIC) of compound 4F against *S. aureus, P. aeruginosa,* and *B. subtilis* had an observable growth at concentration of 15 mg/ml, 7.5 mg/ml and 3.75 mg/ml. This suggests that compound 4F also cannot effectively hinder the growth of these isolates at these concentrations.

For compound 5A, MIC was not conducted because it shows no activity for the bacterial isolate selected for this research and it is indicated at table 3.11.

Lastly, for compound 5B, the MIC values are presented in Table 3.12a. The Minimum Inhibitory Concentration (MIC) of compound 5B against *S. aureus, K. pneumonia* and *E. coli* had no observable growth at concentration of 5 mg/ml and 2.5 mg/ml. This suggests that compound 5B effectively hindered the growth of these isolates at these concentrations. However, there was an observable growth against *Bacillus subtilis* at the concentration 5 mg/ml and 2.5 mg/ml, which indicated that the synthesized compound 5B is inactive against *B. subtilis.* More also, at the concentration of 5 mg/ml the compound 5B was able to inhibit *P. aeruginosa* but when the concentration reduced to 2.5 mg/ml, the MIC indicated growth *P. aeruginosa.* In other words, at the 2.5 mg/ml concentration, the synthesized compound exhibited inactivity against *P. aeruginosa*. It can be suggested that Compound 5B can be dose-dependent.

3.2.2.1.3 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentrations (MBC) for all the tested synthesized compounds were observed at different concentration. This can be seen from the results obtained in Tables 3.4b, 3.5b, 3.7b 3.8b. and 3.12b. Therefore, it can be inferred that each synthesized compounds has their lowest concentration required to kill any of the tested organisms.

For compound 3, the MBC values are presented in Table 3.4b. The Minimum bactericidal Concentration (MBC) of compound 3 against *S. aureus* and *B. subtilis* shows an observable growth which indicated that compound 3 cannot eradicate these particular isolates but can only inhibit at the concentration of 8 mg/ml, 5 mg/ml, 4 mg/ml, 2.5 mg/ml and 1.5 mg/ml. However, the compound 3 can kill *P. aeruginosa,* and *K. pneumonia* at 8 mg/ml, 5 mg/ml and 4 mg/ml concentrations as it has no observable growth at concentration of 8 mg/ml, 5 mg/ml and 4 mg/ml. This suggests that compound 3 can effectively eradicate the growth of these isolates at these concentrations. However, when the concentration reduced to 2.5 mg/ml and 1.5 mg/l, the MBC indicated growth showing it can only hinder the growth for *E. coli, P. aeruginosa* and *K. pneumonia* but cannot eradicate the isolates. More also, for *E. coli,* the compound 3 can only kill the *E. coli* at 8 mg/ml and 5 mg/ml.

For compound 4, the MBC values are presented in Table 3.5b. The Minimum Bactericidal Concentration (MBC) of compound 4 against all tested bacterial isolates had an observable growth at concentration of 2.5 mg/ml and 1.25 mg/ml. This suggests that compound 4 do not effectively hindered the growth of these isolates at these concentrations. In other words, at the 5 mg/ml and 2.5 mg/ml concentration, the synthesized compound exhibited inactivity against these particular isolates.

For compound 4A, MBC was not conducted because it shows no activity for the bacterial isolate selected for this research and it is indicated at table 3.6b.

For compound 4B, the MBC values are presented in Table 3.7b. The Minimum Bactericidal Concentration (MBC) of compound 4B against *S. aureus, P. aeruginosa, E. coli, K. pneumonia* and *B. subtilis* had no observable growth at concentration of 2.5 mg/ml, this indicated that at this concentration compound 4B can effectively eradicate the bacterial isolates but at 1.25 mg/ml the compound 4B cannot eradicate the bacterial but can hinder the growth of the isolates.

For compound 4D, the MBC values are presented in Table 3.8b. The Minimum Inhibitory Concentration (MBC) of compound 4D against *S. aureus, B. subtilis* and *P. aeruginosa* had no observable growth at 15 mg/ml and 7.5 mg/ml which indicated that compound 4D eradicated the isolates at that concentration, however, for *K. pneumonia* and *E. coli,* the compound 4D eradicate at 15 mg/ml but lower than that concentration it cannot kill the isolates it can only inhibit the growth of the organisms. This suggests that compound 4D effectively hindered the growth of these isolates at these concentrations and also dose-dependent.

Lastly, for compound 5B, the MBC values are presented in Table 3.12b. The Minimum Bactericidal Concentration (MBC) of compound 5B against *K. pneumonia* and *E. coli* had no observable growth at concentration of 5 mg/ml and 2.5 mg/ml. This suggests that compound 5B effectively eradicate the growth of these isolates at these concentrations. However, there was an observable growth against *P. aeruginosa* and *S. aureus* at the concentration 5 mg/ml and 2.5 mg/ml, which indicated that the synthesized compound 5B cannot eradicate these isolates at this concentration but can only, inhibit the isolates.

3.2.2.2 Antifungal Activity

3.2.2.2.1 Zone of Inhibition

Table 3.3c and Table 3.3d represents the zone of inhibition for standard drug and the synthesised compounds respectively which was measured in millimetre at 20 mg/ml and 10 mg/ml concentrations respectively. The zone of inhibition was observed after 24hrs and their result was compared to that of the standard drug which was Ketoconazole.

The table 3.3c showed the zone of inhibition for the tested standard drug ketoconazole and the synthesised compound for fungi isolates. The ketoconazole has it highest zone of inhibition at the concentration of 20 mg/ml against *C. albicans* with 25.00 mm and *A. niger* with 20.00 mm.

Compound 4D at the 20 mg/ml concentration shows an activity with a zone of inhibition against *A. niger* with 28.00 mm and has no activity against *C. albicans* (0.00mm). The compound 4D activity has an excellent activity over the standard drug ketoconazole against *A. niger.*

Compound 4E at the 20 mg/ml concentration shows no activity or any zone of inhibition for *C. albicans* and *A. niger*. The Compound 4E activity shows that it cannot be used to inhibit *C. albicans* and *A. niger* as compared to the standard drugs and compound 4D.

Compound 4F at the 20 mg/ml concentration has a zone of inhibition for *C. albicans* (19.00 mm) and *A. niger* (20.00 mm). the compound 4F has same activity with the standard drug (ketoconazole) against *A. niger* (20.00 mm) but has a moderately lower activity as compared to ketoconazole against *C. albicans* (19.00 mm).

Compound 4H at the 20 mg/ml concentration has a no zone of inhibition for *C. albicans* but has an activity with the zone of inhibition of 15.00 mm for *A. niger* and this is very low as compared to the standard drugs (ketoconozoa) against *A. niger.*

Table 3.3d shows the zone of inhibition for fungi isolates at 10 mg/ml, 8 mg/ml and 5 mg/ml with comparison to the standard drug ketoconazole.

Compound 3 at 10 mg/ml concentration has a zone of inhibition for *A. niger* (25.00 mm) and *C. albicans* (20.00 mm). The compound 3 was able to show higher and better activity or inhibition for *A. niger* (25.00 mm) than the standard drug (ketoconazole).

Compound 4 at 10 mg/ml concentration has no activity or any zone of inhibition for *A. niger* and *C. albicans.*

Compound 5B at 10 mg/ml concentration has activity with a zone of inhibition for *A. niger (22.00 mm) and C. albicans (20.00 mm)* and this show that compound 5B has high activity against *A. niger* (22.00 mm) when compared to ketoconazole but has a lower activity against *C. albicans* (20.00 mm).

Compound 4A at the 8 mg/ml concentration has no activity or any zone of inhibition for *C. albicans* but shows a lower activity against *A. niger* (15.00 mm) when compared to ketoconazole.

Compound 5A at 5 mg/ml concentration has no activity or any zone of inhibition for all the selected fungus isolates used in this research.

Compound 4B at 5 mg/ml concentration has activity with a zone of inhibition for *A. niger* (18.00 mm) *and C. albicans* (24.00 mm). The compound 4B has less activity as compared to the standard drug ketoconazole.

3.2.2.2.2 Minimum Inhibitory Concentration (MIC)

The following Table 3.4a, table 3.5a, table 3.6a, table 3.7a, table 3.10, table 3.11 and table 3.12a shows the MIC for the following compound 3, compound 4, compound 4A, and compound 4B, compound 4D, compound 4F, compound 4H and Compound 5B respectively.

For compound 3, the MIC values are presented in Table 3.4a. 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/ml. 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.

For compound 4, the MIC values are presented in Table 3.5a. The Minimum Inhibitory Concentration (MIC) of compound 4 against *A. niger* and *C. albicans* had observable growth at concentration of 5 mg/ml and 2.5 mg/ml. This suggests that compound 4 do not effectively hindered the growth of these isolates at these concentrations. In other words, at the 5 mg/ml and 2.5 mg/ml concentration, the synthesized compound exhibited inactivity against these particular isolates.

For compound 4A, The Minimum Inhibitory Concentration (MIC) of compound 4A against *A. niger* and *C. albicans* had no observable growth at concentration 2.5 mg/ml. This suggests that compound 4A effectively hindered the growth of these isolates at this

concentration. However, The Minimum Inhibitory Concentration (MIC) of compound 4A is 2.5 mg/ml.

For compound 4B, the MIC values are presented in Table 3.7a. The Minimum Inhibitory Concentration (MIC) of compound 4B against *C. albicans* had no observable growth at concentration of 2.5 mg/ml and 1.25 mg/ml. This suggests that compound 4B effectively hindered the growth of these isolates at these concentrations. Therefore, the Minimum Inhibitory Concentration (MIC) of compound 4B is 1.25 mg/ml. however, against *A. niger* there was an observable growth at concentration of 2.5 mg/ml and 1.255 mg/ml. This suggests that compound 4B do not effectively hindered the growth of *A. niger* at these concentrations.

For compound 4D, the MIC values are presented in Table 3.8a. The Minimum Inhibitory Concentration (MIC) of compound 4D against *C. albicans* had no observable growth at concentration of 15 mg/ml. This suggests that compound 4D effectively hindered the growth of these isolates at this concentration. However, at 7.5 mg/ml, the MIC indicated growth. In other words, the Minimum Inhibitory Concentration (MIC) of compound 4D could be 10 mg/ml.

For compound 4F, the MIC values are presented in Table 3.10. The Minimum Inhibitory Concentration (MIC) of compound 4F against *C. albicans* had no observable growth at concentration of 15 mg/ml and 7.5 mg/ml. This suggests that compound 4F effectively hindered the growth of this isolate at these concentrations. However, at 3.75 mg/ml, the MIC indicated growth. In other words, the Minimum Inhibitory Concentration (MIC) of compound 4F is 7.5 mg/ml.

For compound 4H, the MIC values are presented in Table 3.10. The Minimum Inhibitory Concentration (MIC) of compound 4H against *A. niger* and *C. albicans* had observable growth at concentration of 15 mg/ml. 7.5 mg/ml and 3.75 mg/ml. This suggests

that compound 4H do not effectively hindered the growth of these isolates at these concentrations.

Lastly, for compound 5B, the MIC values are presented in Table 3.12a. The Minimum Inhibitory Concentration (MIC) of compound 5B against *C. albicans* had no observable growth at concentration of 5 mg/ml and 2.5 mg/ml. This suggests that compound 5B effectively hindered the growth of this fungus isolate at these concentrations. However, there was an observable growth against *A. niger* at the concentration 5 mg/ml and 2.5 mg/ml which indicated that the synthesized compound 5B is inactive against *A. niger.* Therefore, the Minimum Inhibitory Concentration (MIC) of compound 5B is 2.5 mg/ml against *C. albicans*

3.2.2.2.3 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentrations (MBC) for all the tested synthesized compounds were observed at different concentration. This can be seen from the results obtained in Tables 3.4b, 3.5b, 3.7b 3.8b and 3.12b. Therefore, it can be inferred that each synthesized compounds has their lowest concentration required to kill any of the tested organisms.

For compound 3, the MBC values are presented in Table 3.4b. The Minimum bactericidal Concentration (MBC) of compound 3 against *A. niger and C. albicans* shows no observable growth which indicated that compound 3 can kill 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.

For compound 4B, the MBC values are presented in Table 3.7b. The Minimum bactericidal Concentration (MBC) of compound 4B against *A. niger* and *C. albicans* had an observable growth at concentration of 2.5 mg/ml, and 1.25 mg/ml, the compound 4B cannot eradicate the fungi but can hindered the growth of the isolates.

For compound 4D, the MBC values are presented in Table 3.8b. The Minimum bactericidal Concentration (MBC) of compound 4D against *C. albicans* had no observable

growth at 15 mg/ml and 7.5 mg/ml which indicated that compound 4D eradicated the isolates at that concentration but at 5 mg/ml the compound 4D cannot eradicate the fungus but can only inhibit it, however, for *A. niger,* the compound 4D eradicate at 15 mg/ml but lower than that concentration it cannot eradicate the isolates it can only inhibit the growth of the organisms. This suggests that compound 4D effectively hindered the growth of these isolates at these concentrations and also dose-dependent.

For compound 4F, the MBC values are presented in Table 3.8b. The Minimum bactericidal Concentration (MBC) of compound 4F against *C. albicans* had no observable growth at 10 mg/ml and 7.5 mg/ml which indicated that compound 4D eradicate *C. albicans* at that concentration but at 5 mg/ml the compound 4F cannot eradicate the fungus but can only inhibit it.

Lastly, for compound 5B, the MBC values are presented in Table 3.12b. The Minimum Bactericidal Concentration (MBC) of compound 5B against *C. albicans* had no observable growth at concentration of 5 mg/ml. However, there was an observable growth at 2.5 mg/ml, which indicated that the synthesized compound 5B cannot lethal *C. albicans* at this concentration but can only, inhibit the isolates.

3.3 CONCLUSION

In this research work, we report the synthesis of N, N – dimethyloxamide. This was made to react with PCl_5 to yield 5-chloro-1-methylimidazole (compound 2). Compound 2 was then nitrated to yield 5-chloro-1-methyl-4-nitroimidazole (compound 3). The "chloro group" in compound 3 was then replaced with a " thiol group" to form compound 4 (1-methyl-4-nitroimidazole-5-thiol). Compound 4 was then used as intermediate to synthesize other new substituted imidazole derivatives and these include compound 4A and compound 4B.

However, Compound 4 was allowed to undergo oxidative chlorination to obtain an unstable intermediate 1-methyl-4-nitroimidazole-5-sulphonylchloride (Compound 4C) and all attempt to stabilized it proved abortive. Compound 4C was used to synthesise other new substituted imidazole derivatives and that to include Compound 4D, Compound 4E, Compound 4F, Compound 4G and Compound 4H.

More also, we report also that metronidazole (compound 5) was made to react with $SOCl_2$ to yield an intermediate that was immediately coupled with 2,4 dichloroaniline and aniline that resulted to other substituted imidazole derivatives and these include compound 5A and compound 5B.

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. Most of the synthesized compounds revealed better activity against most of the tested organisms.

Compound 3 (1-methyl-4-nitro-5-chloroimidazole) 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. albicans* than compounds 4 and 4A. Compounds 3, 4B, 4D, 4E and 5B showed a better antibacterial activity against *K. pneumonia*

and also, compound 4, 5B and 4D showed a better antibacterial activity against *E. coli* than the standard, ciprofloxacin.

Compounds 3, 4D and 5B revealed a good to an excellent and better antifungal activity against *A. niger* than the standard, ketoconazoa. However, a moderately low to low activity was exhibited by the test compounds against *S. aureus, B. subtilis, E. coli*, *P. aeruginosa, K. pneumonia*, *A. niger and C. albicans* when compared to that of the standard. In addition, compound 5A showed no activity against any of microbial isolates.

3.4 CONTRIBUTION TO KNOWLEDGE

The research work has generated new data that will be useful for further research on 1-methyl-4-nitroimidazole-5-thiol, 1-methyl-4-nitroimidazole-5-sulphonylproline,1-chloroethane-2-[thioacyl chloride]-4-nitroimidazole and their derivatives. Through the research work, we have been able to synthesize some imidazole derivatives that have been able to show some degree of antimicrobial activity and therefore can be incorporated into drugs and antimicrobial formulations. Compound 4D and 5B, for example, can be used to medications to treat conditions brought on by *Escherichia coli*, *Candida albicans, Aspergillus niger*, and *Klebsiella pneumonia.* In addition, the research has also shown that the synthesized compounds are potent against *Klebsiella pneumonia* and therefore could be used in the treatment of diseases caused by *Klebsiella pneumonia.*

3.5 RECOMMENDATIONS

The following recommendations are made with regards to the synthesized compounds (compounds 3, 4, 4A, 4B, 4D, 4F, 4G, 4H, 5A, 5B).

1. The synthesized compounds should be studied for their antiviral and anti-inflammatory activities.
2. Antimicrobial studies should be carried out against other bacteria and fungi.
3. Full characterisation of the synthesized compounds should also be carried out.
4. Compound 3, Compound 5 and Compound 4C could be used in synthesizing other thioimidazole compounds, thioacylimidazole and suphonamideimidazole which could likely show greater antimicrobial activities and hence incorporated into drugs and antimicrobial formulations.
5. Compound 4D and Compound 5B should be incorporated into drugs in the treatment of ailments or diseases caused by *Klebsiella pneumonia*, *Escherichia coli* and *Aspergillus niger*.
6. Compounds 3, 4B and 4E should be incorporated into drugs used in the treatment of diseases caused by *Klebsiella pneumonia*.
7. Compounds 3, 4D and 5B should also be incorporated into drugs used in the treatment of diseases caused by *Aspergillus niger*.

3.6 LIMITATIONS

Full characterisation of the new imidazole compounds could not be carried out due to none availability of analytical instruments such as Mass Spectrometer for the characterisation of the synthesized compounds. However, further work will focus on these areas.

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APPENDIX

CALCULATION OF THE PERCENTAGE YIELD OF COMPOUND 3

Molecular weight of compound 2 = 116.50g

Molecular weight of compound 3 = 171.50g

From the equation in scheme 2.3

1 mole of compound 2 ≡ 1 mole of compound 3

⇒ 116.5g of compound 2 ≡ 171.50g of compound 3

∴ 22.5g of compound 2 $\equiv \frac{22.5g\ x\ 171.5g}{116.5g}$ of compound 3

= 33.12g of compound 3

∴22.5g of compound 2 ≡ 33.120g of compound 3

Theoretical yield = 33.120g

Actual yield = 22.75g

% yield = $\frac{actual\ yield}{thoeretical\ yield}\ x\ \frac{100\%}{1}$

$= \frac{22.75g}{33.12g}\ x \frac{100\%}{1} = 68.68\%$

∴ Percentage yield of compound 3 is 68.68%

CALCULATION OF THE PERCENTAGE YIELD OF COMPOUND 4

Molecular weight of compound 3 = 171.50g

Molecular weight of compound 4 = 159.17g

From the equation in scheme 2.4

1 mole of compound 3 ≡ 1 mole of compound 4

⇒ 171.50g of compound 3 ≡ 159.17g of compound 4

$$3.1g\ of\ compound\ 3 \equiv \left(\frac{3.1g\ x\ 159.17g}{171.50g}\right) of\ compound\ 4$$

=2.877g of compound 4

$$3.1g\ of\ compound\ 3\ \equiv 2.877g\ of\ compound\ 4$$

∴ 3.1g of compound 3 ≡ 2.877g of compound 4

Theoretical yield = 2.877g

Actual yield = 1.69g

$$\%\ yield = \frac{actual\ yield}{thoeretical\ yield}\ x\frac{100\%}{1}$$

$$= \frac{1.69g}{2.877g}\ x\frac{100\%}{1}$$

$$= 58.82\%$$

∴ Percentage yield of compound 4 is 58.82%

CALCULATION OF THE PERCENTAGE YIELD OF COMPOUND 4A

Molecular weight of compound 4 = 159.17g

Molecular weight of compound 4A = 280g

From the equation in scheme 2.5

1 mole of compound 4 ≡ 1 mole of compound 4A

⇒ 159.17g of compound 4 ≡ 280g of compound 4A

∴ 0.374g of compound 4 ≡ $\frac{0.374g \times 280g}{159.17g}$ of compound 4A

= 0.658g of compound 4A

∴0.374g of compound 4 ≡ 0.658g of compound 4A

Theoretical yield =0.658g

Actual yield = 0.314g

% yield = $\frac{actual\ yield}{thoeretical\ yield}\ x\ \frac{100\%}{1}$

= $\frac{0.314g\ x\ 100\%}{0.658g}$

= 47.69%

∴ Percentage yield of compound 4A is 47.69%

CALCULATION OF THE PERCENTAGE YIELD OF COMPOUND 4B

Molecular weight of compound 4 = 159.17g

Molecular weight of compound 4B = 279g

From the equation in scheme 2.6

1 mole of compound 4 ≡ 1 mole of compound 4B

⇒ 159.17g of compound 4 ≡ 279g of compound 4B

∴ 0.374g of compound 4 ≡ $\frac{0.374\ x\ 279g}{159.17g}$ of compound 4B

= 0.655g of compound_4B

∴0.374g of compound_4 ≡ 0.655g of compound_4B

Theoretical yield = 0.655g

Actual yield = 0.341g

% yield = $\frac{actual\ yield}{thoeretical\ yield}\ x\ \frac{100\%}{1}$

$$= \frac{0.341g \times 100}{0.655g}\%$$

$$= 52.09\%$$

∴ Percentage yield of compound_4B is 5.09%

Created at: 10:19 05/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-02-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/Imue-B/Sample-02/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 2 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/Imue-B/sample-02#
Date: 05/05/2023

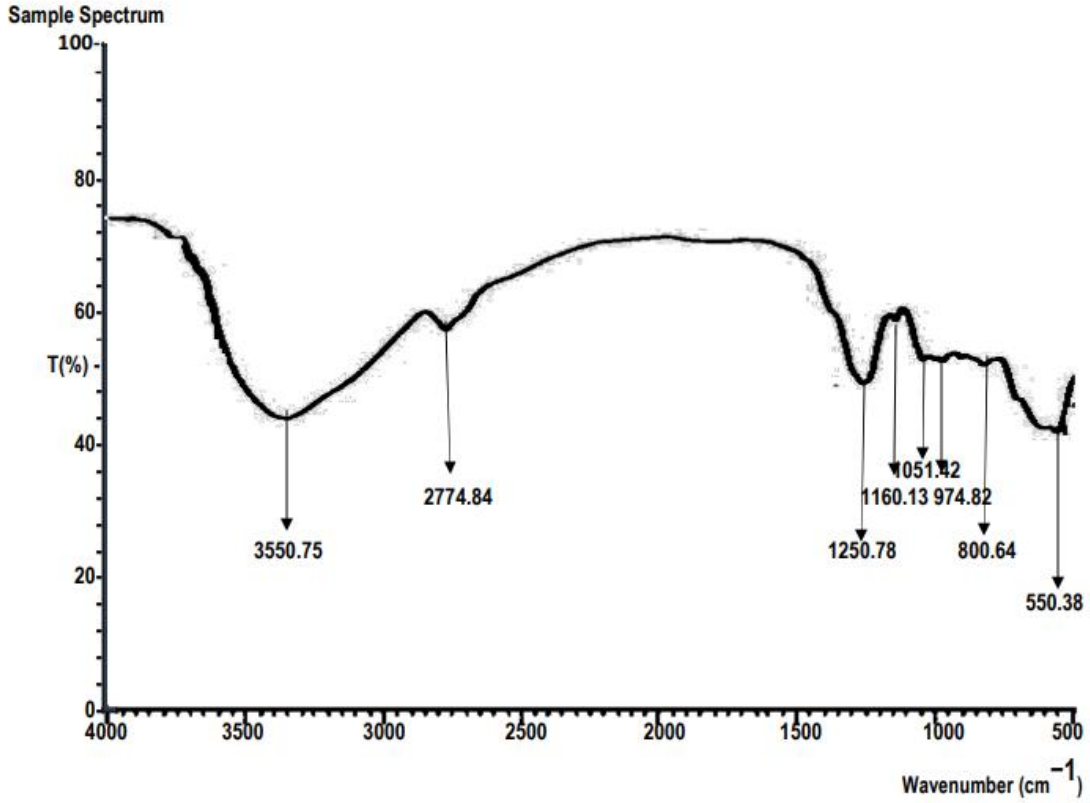

Fig A: FT-IR of Compound_3

File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-20-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/Imue-T/Sample-20/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 20 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/Imue-T/sample-20#
Date: 05/05/2023

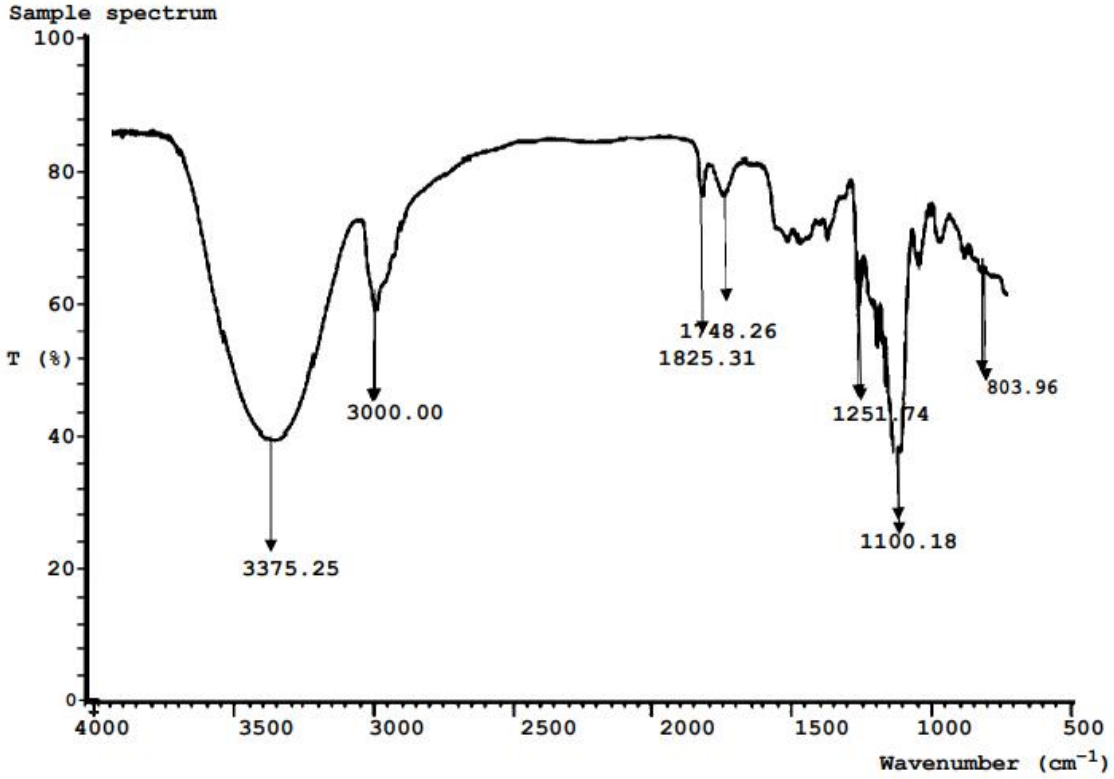

FIG B: FT-IR OF COMPOUND_4

Created at: 14:56 07/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-48-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/imue-25/Sample-48/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #:48 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/imue-25/sample-48#

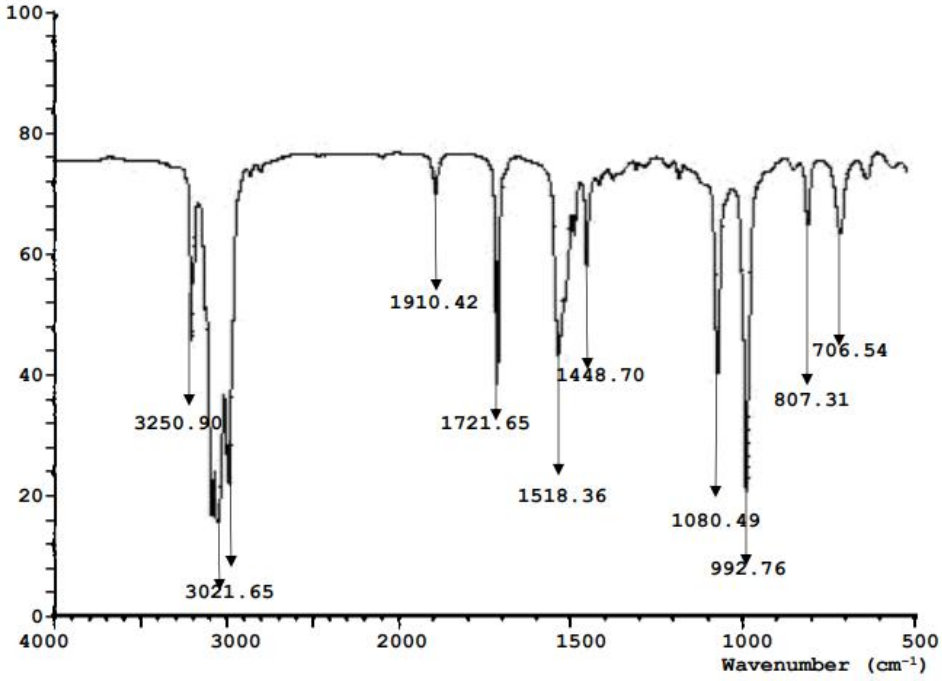

FIG C: FT-IR OF COMPOUND_4A

Created at: 12:07 07/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-34-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/imue-11/Sample-34/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 34 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/imue-11/sample-34#

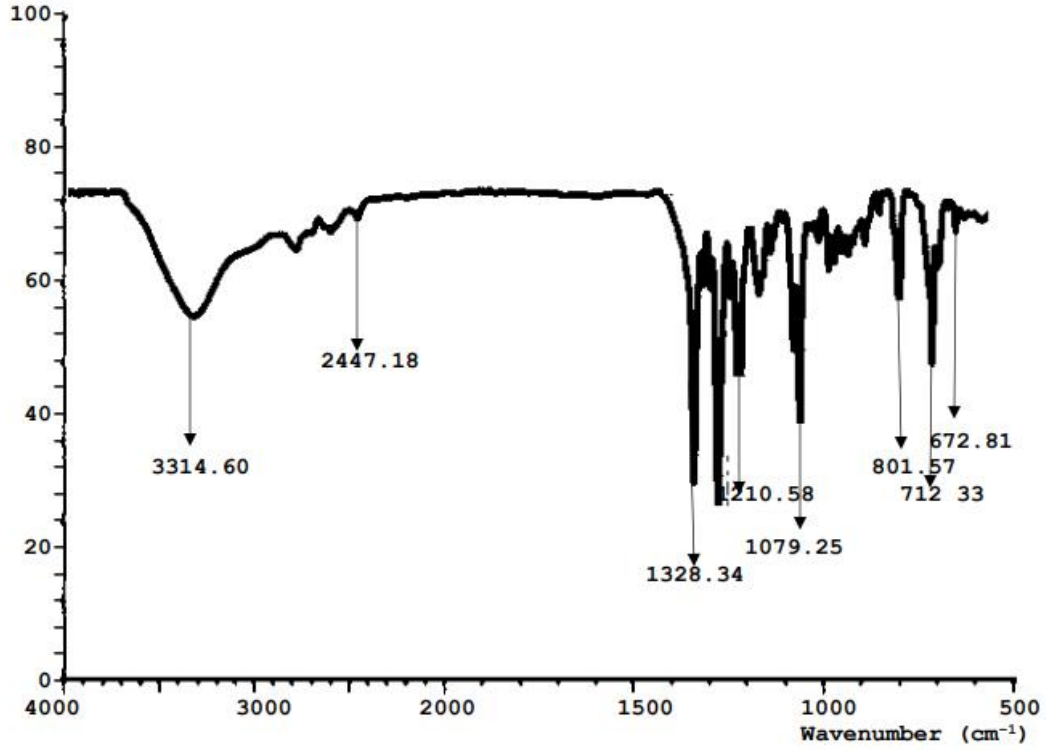

FIG C: FT-IR OF COMPOUND 4B

Created at: 14:09 05/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-21-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/Imue-U/Sample-21/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 21 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/Imue-U/sample-21#
Date: 05/05/2023

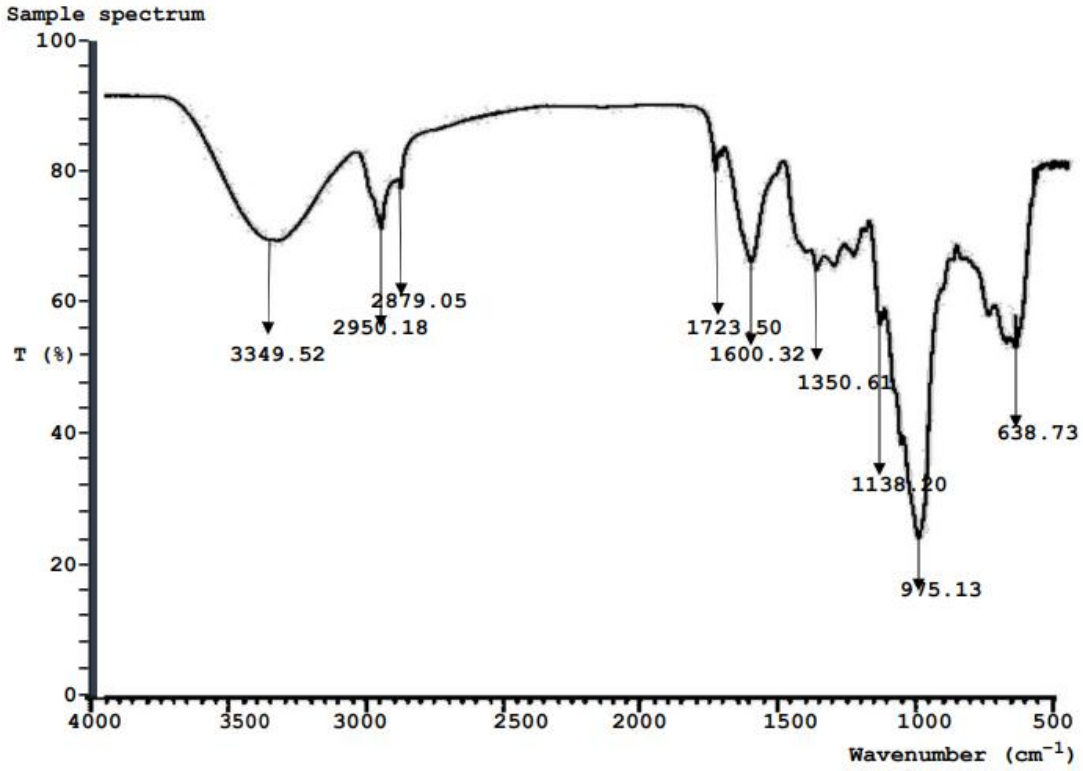

FIG D: FT-IR OF COMPOUND 4D

Created at: 10:47 05/May/2023
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Sample 1D: AURL/FTIR/Imue-E/Sample-05/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 5 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/Imue-E/sample-05#
Date: 05/05/2023

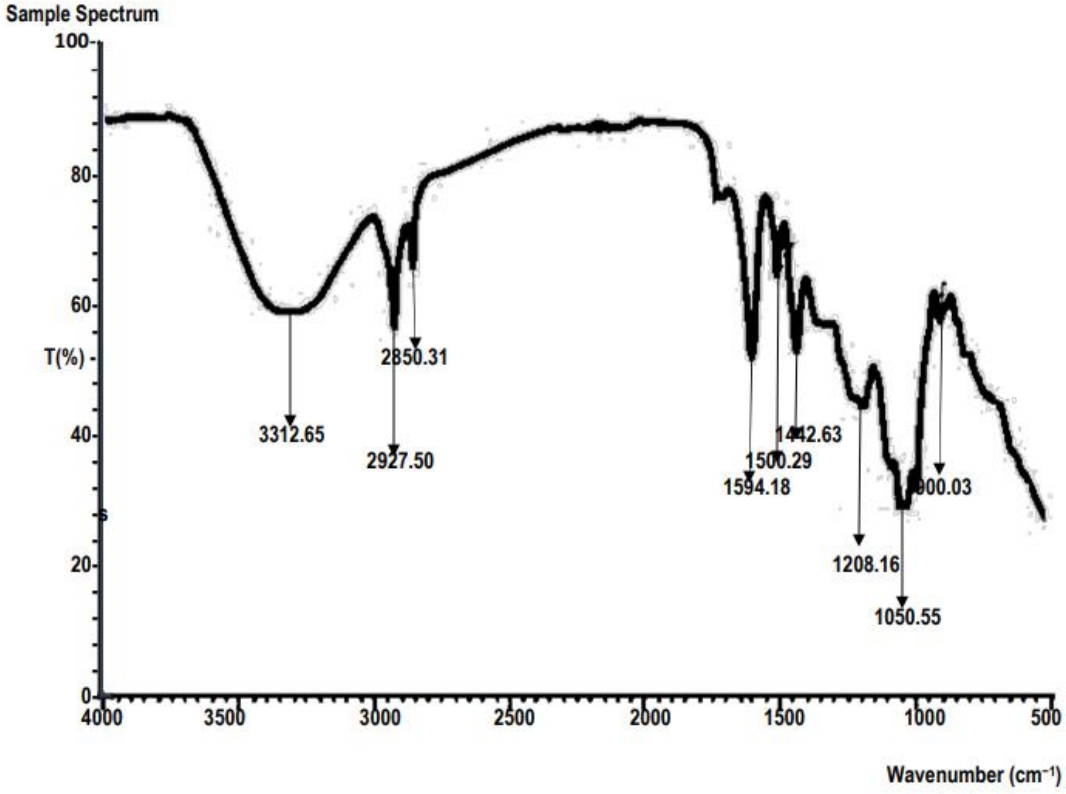

FIG E: FT-IR OF COMPOUND 4E

Created at: 13:45 05/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-19-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/Imue-S/Sample-19/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 19 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/Imue-S/sample-19#
Date: 05/05/2023

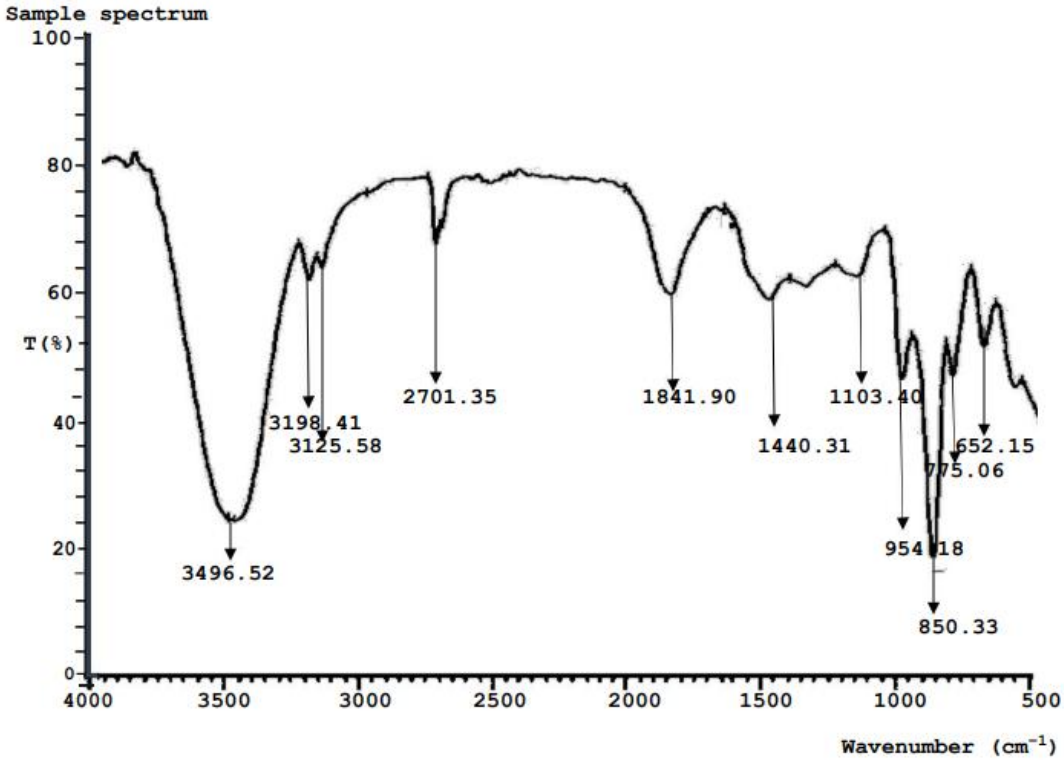

FIG F: FT-IR OF COMPOUND 4F

Created at: 12:46 05/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-14-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/Imue-N/Sample-14/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 14 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/Imue-N/sample-14#
Date: 05/05/2023

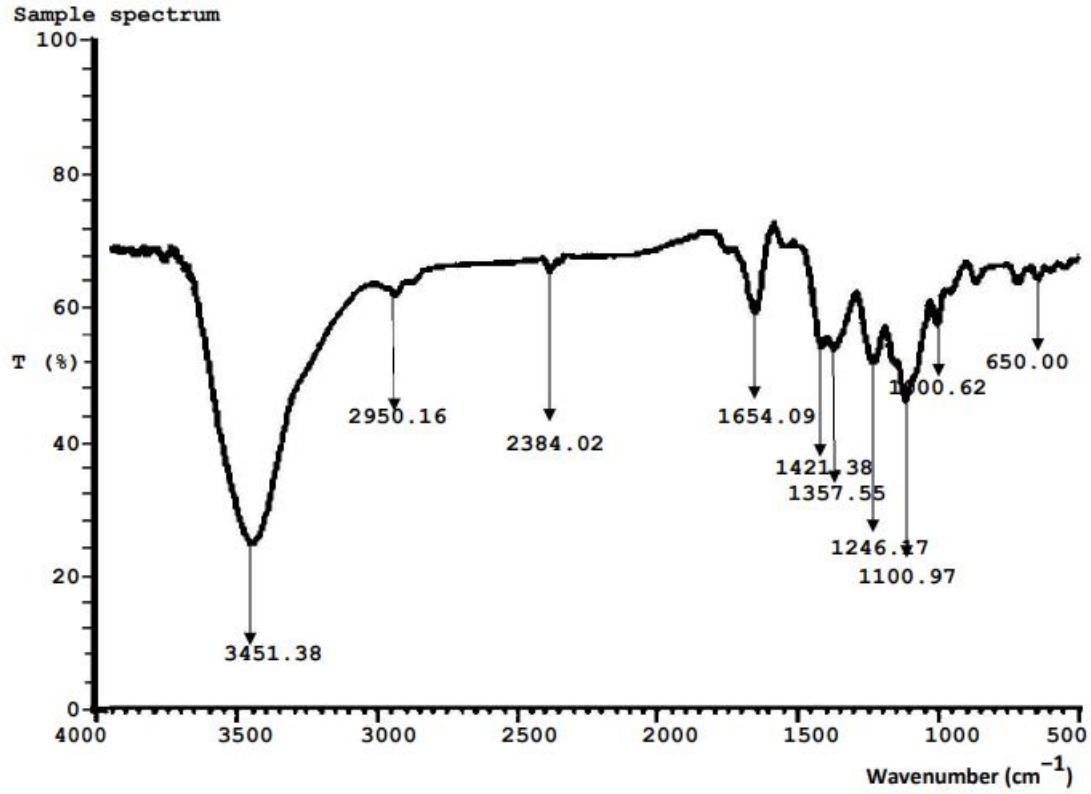

FIG G: FT-IR OF COMPOUND 4G

Created at: 10:28 05/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-03-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/Imue-C/Sample-03/10md11.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 3 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/Imue-C/sample-03#
Date: 05/05/2023

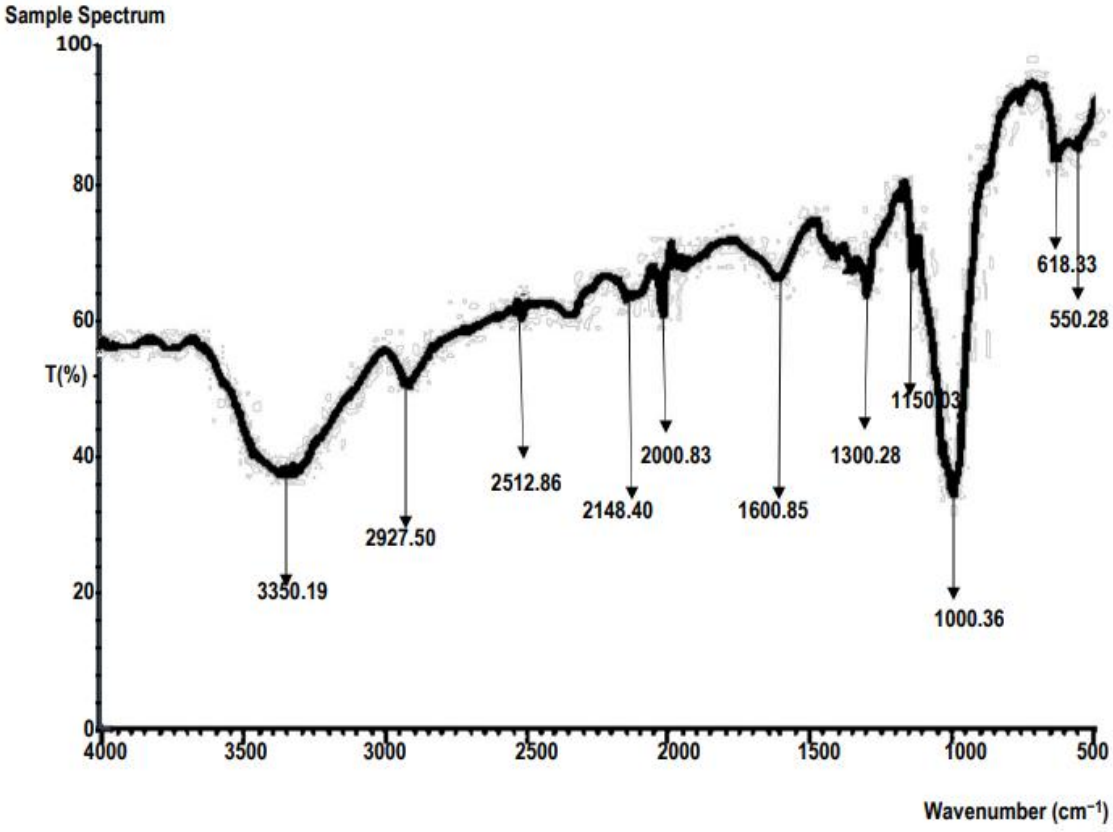

FIG H: FT-IR OF COMPOUND 4H

Created at: 12:54 07/May/2023
File Location: C\Program Files\Shmadzu\Microlab PC\Result\Sample-38-2023-05-30T09-40a2r
Sample 1D: AURL/FTIR/imue-15/Sample-38/10mdl1.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 38 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/imue-15/sample-38#

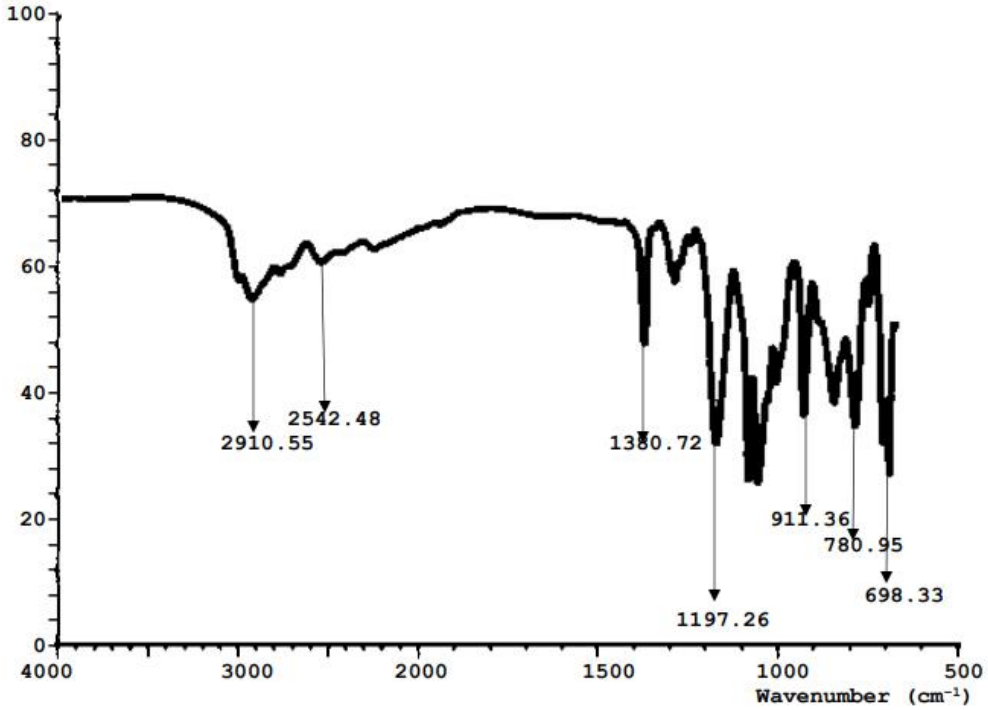

FIG I: FT-IR OF COMPOUND 5A

File Location: C\Program Files\Shmadzu\MicroLab PC\Result\Sample-39-2023-05-30T09-40
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Backgrounds scan time: 200 scans
Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 39 of 54
Save data: from 4000 cm^{-1} to 500 cm^{-1}
Client Name: Consults/FTIR/imue-16/sample-39#

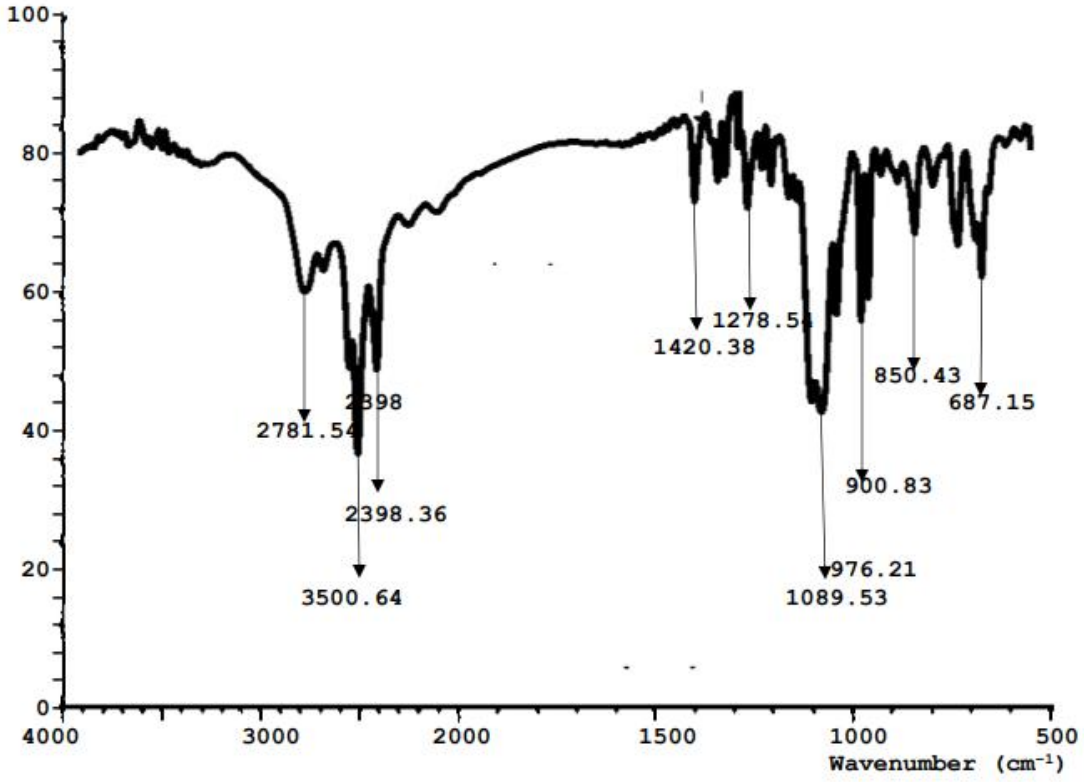

FIG J: FT-IR OF COMPOUND_5B

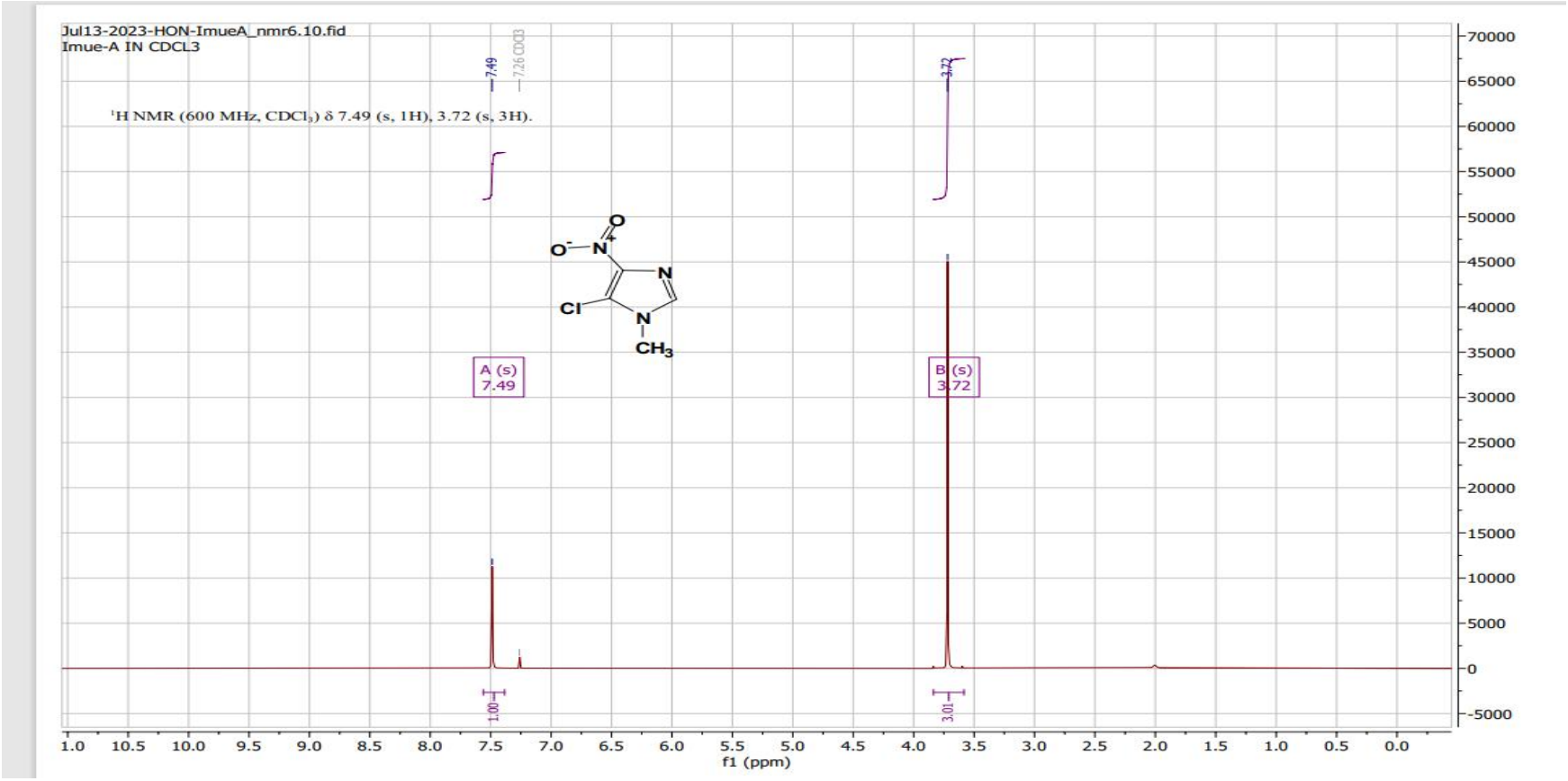

FIG 2.0: ^{1}HNMR OF COMPOUND_3

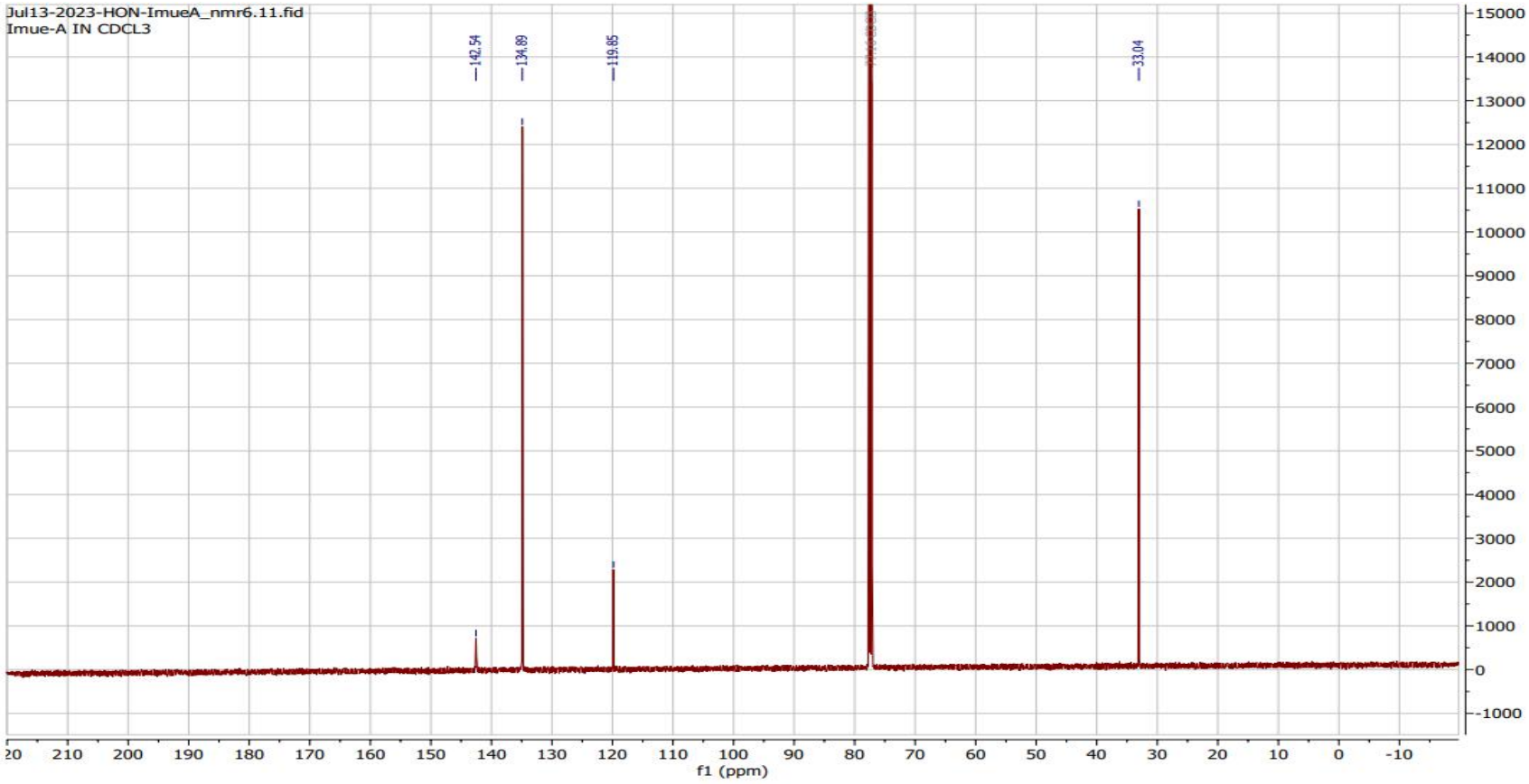

FIG 2.0A: ^{13}CNMR OF COMPOUND_3

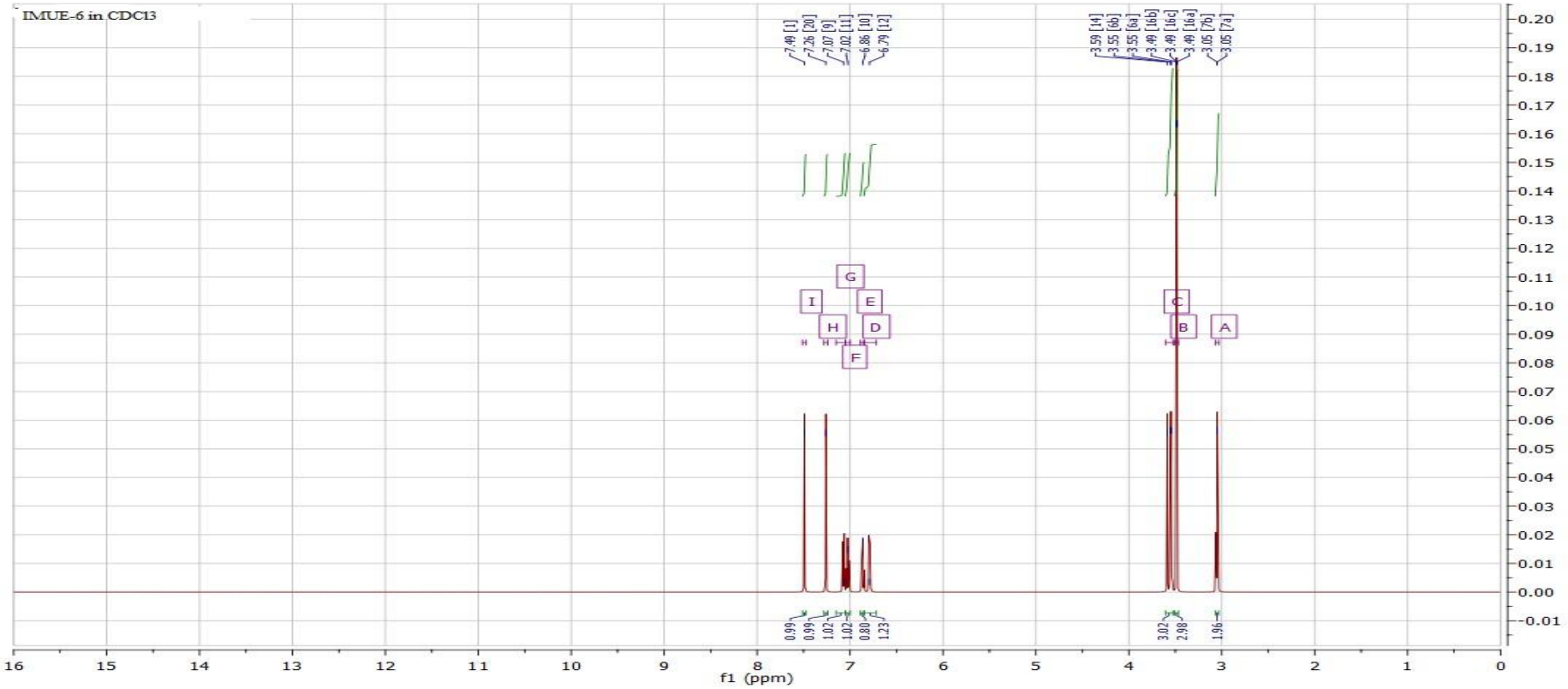

FIG 2.2: [1]HNMR OF COMPOUND_4A

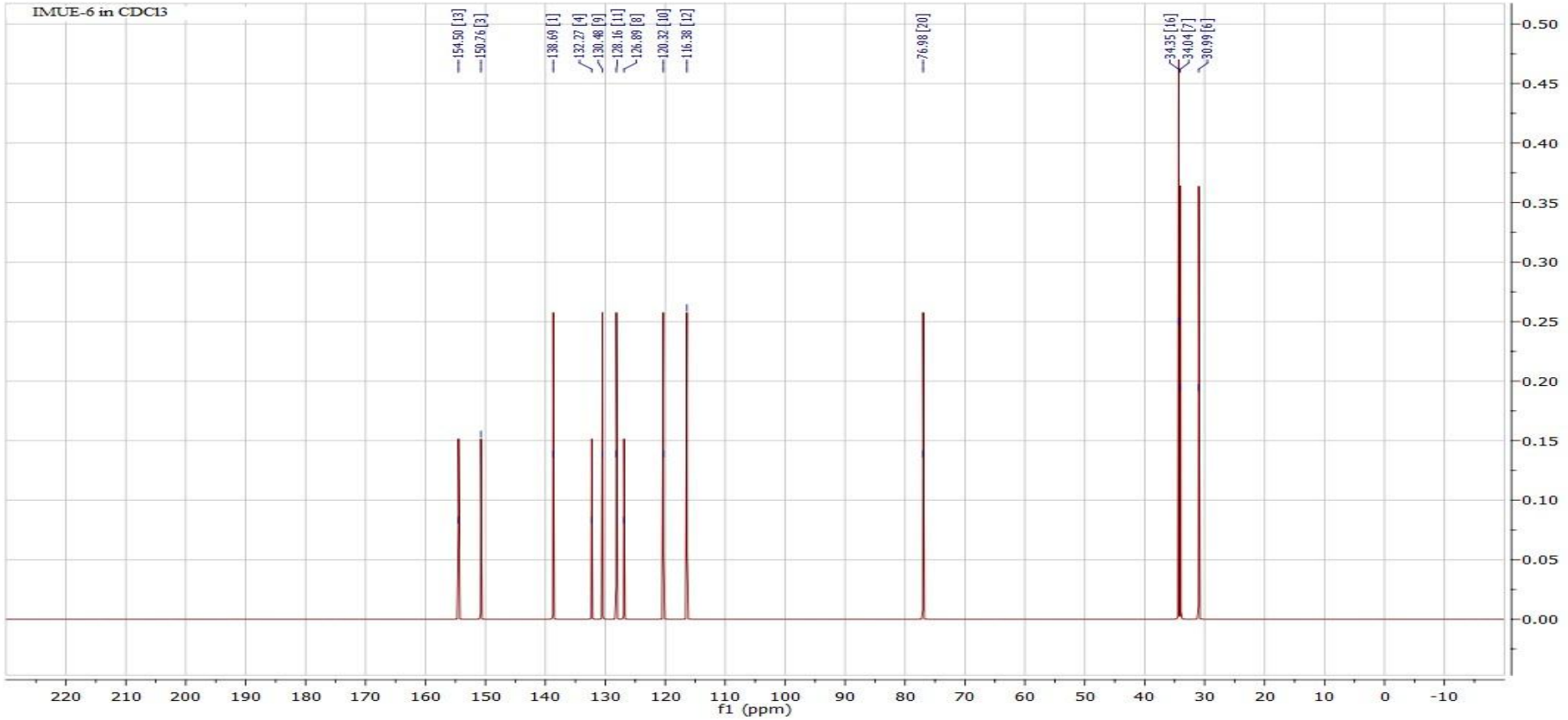

FIG 2.2A: ^{13}CNMR OF COMPOUND_4A

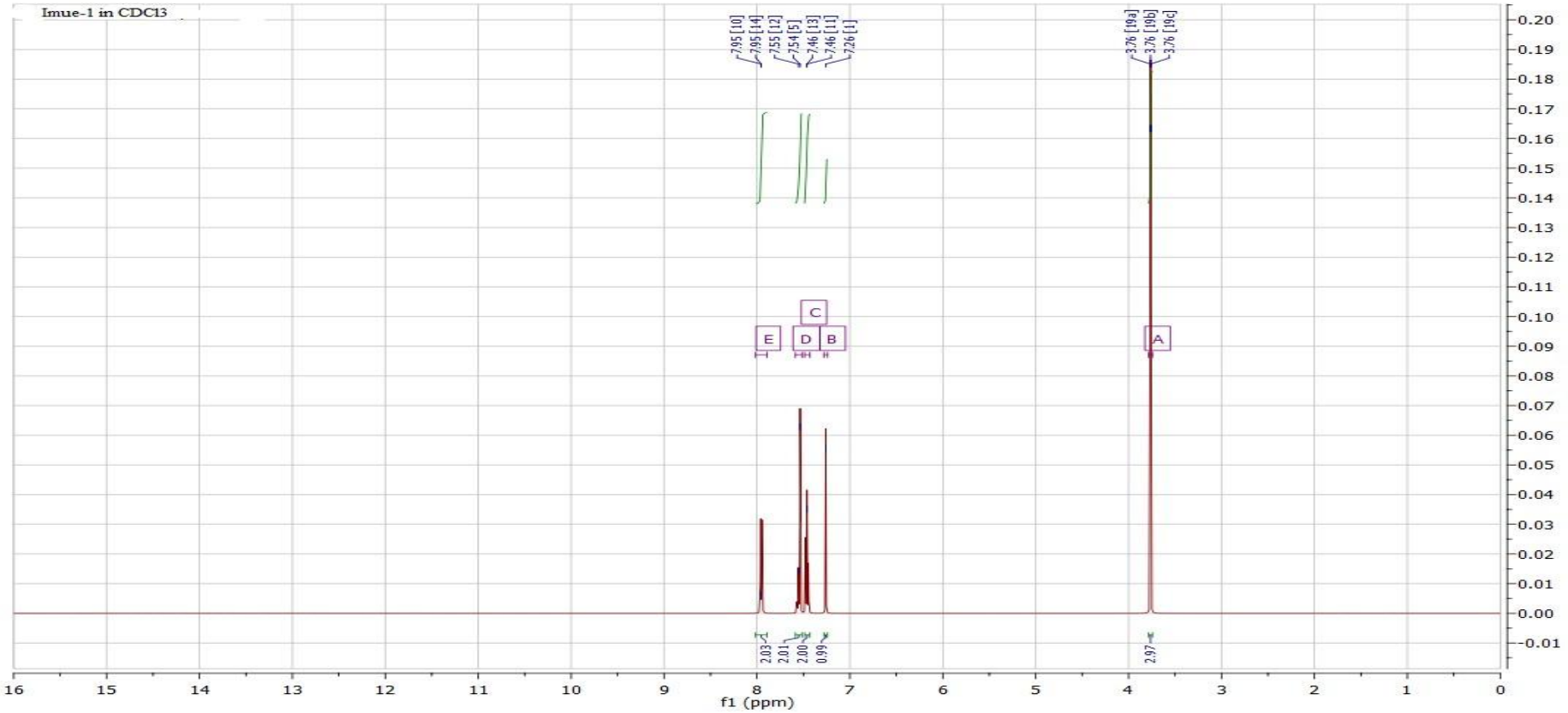

FIG 2.3: [1]HNMR OF COMPOUND_4B

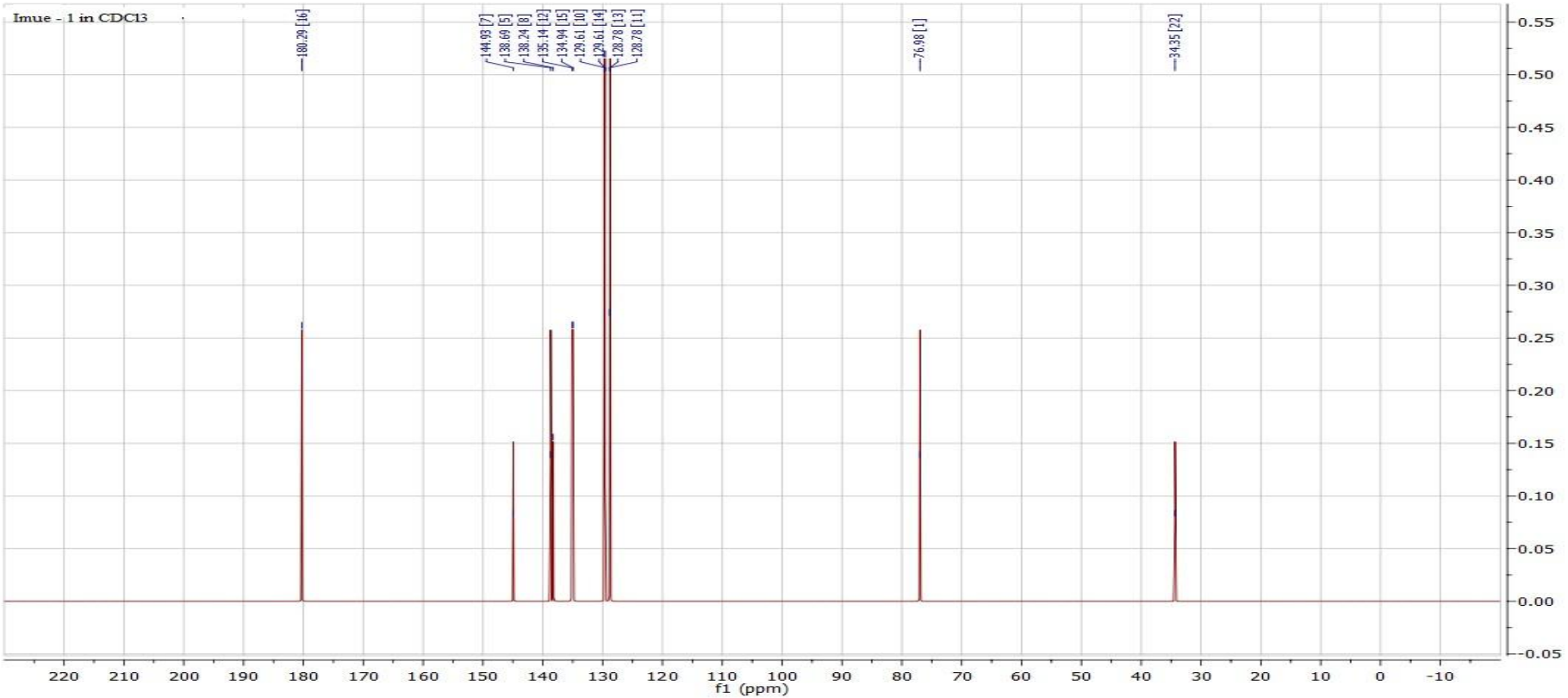

FIG 2.3A : ^{13}CNMR OF COMPOUND_4B

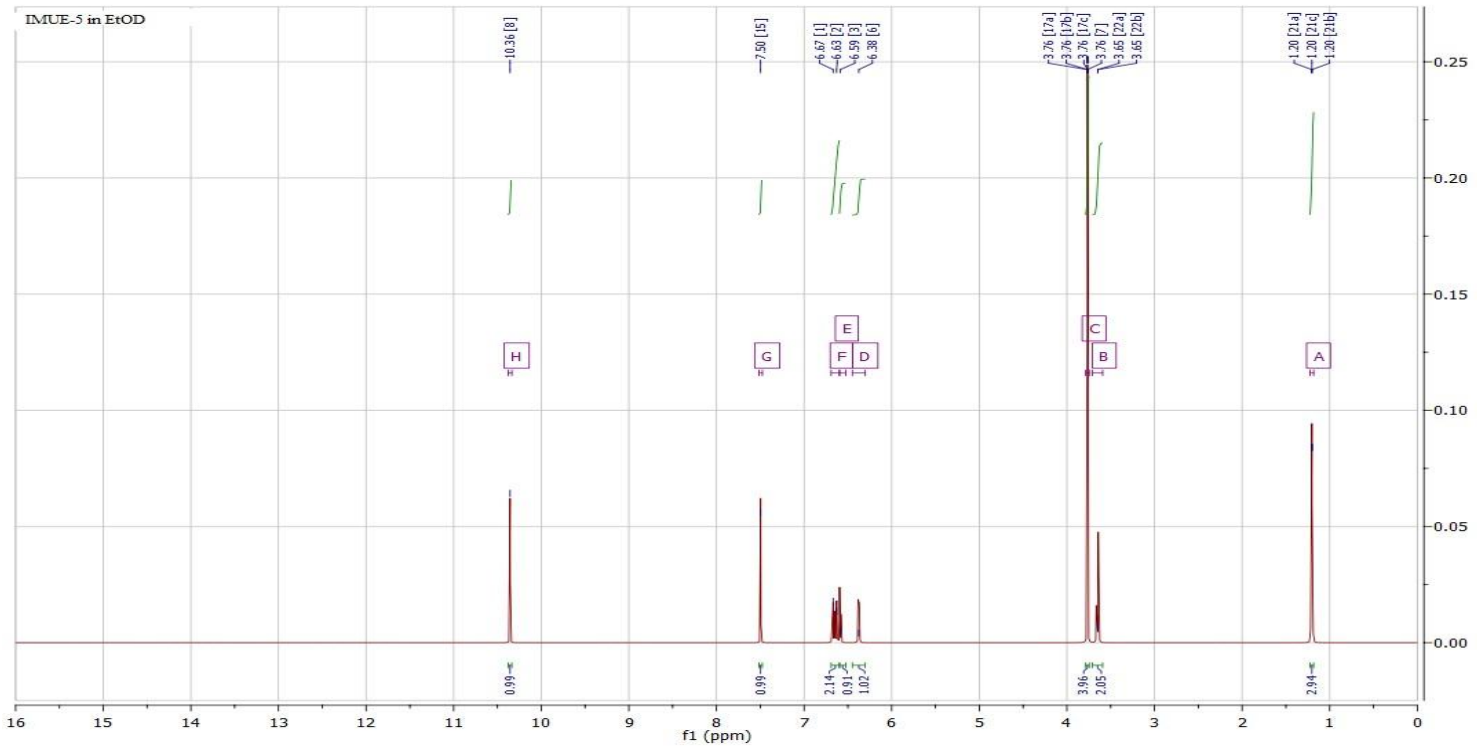

FIG 2.5: [1]HNMR OF COMPOUND_4F

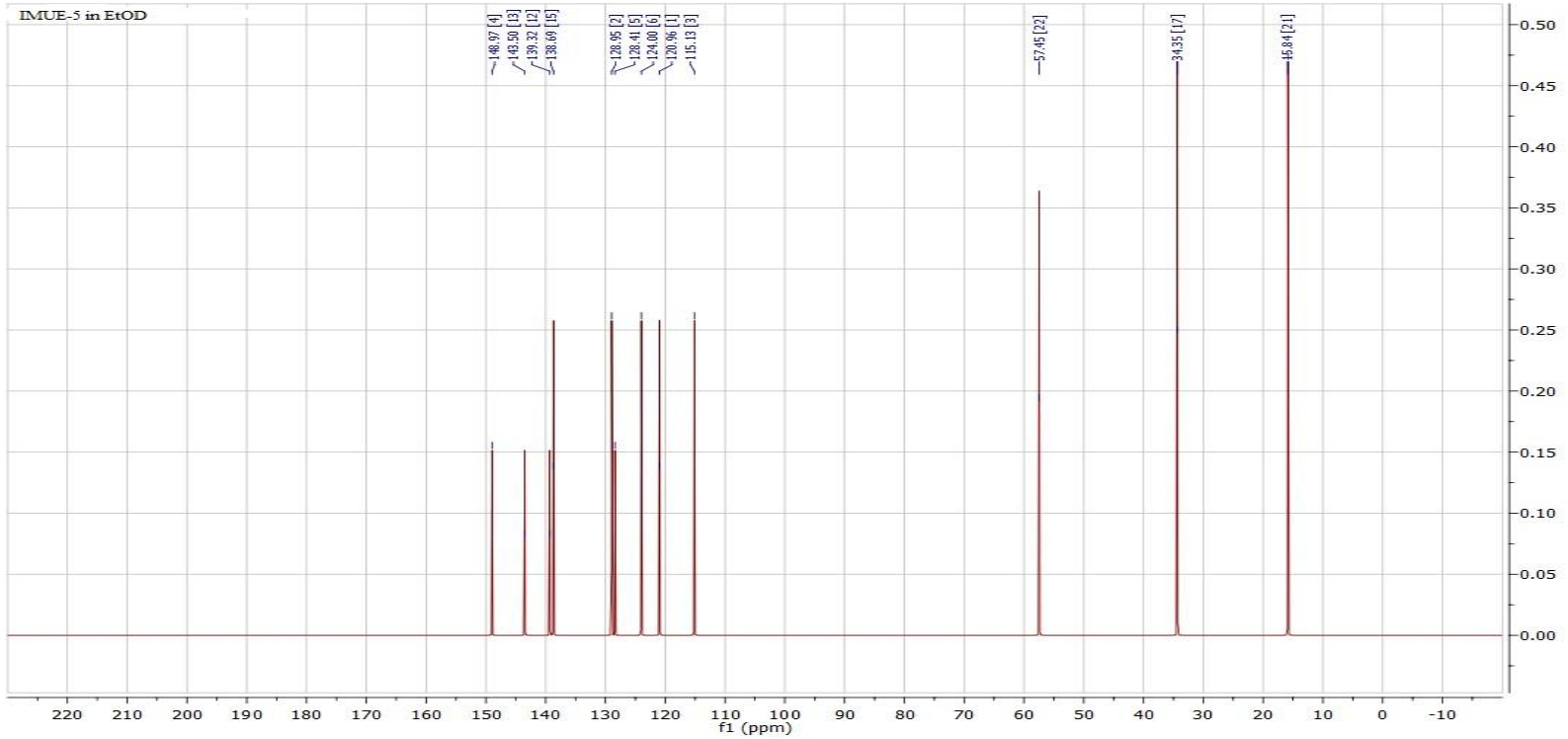

FIG 2.5A: ^{13}CNMR OF COMPOUND_4F

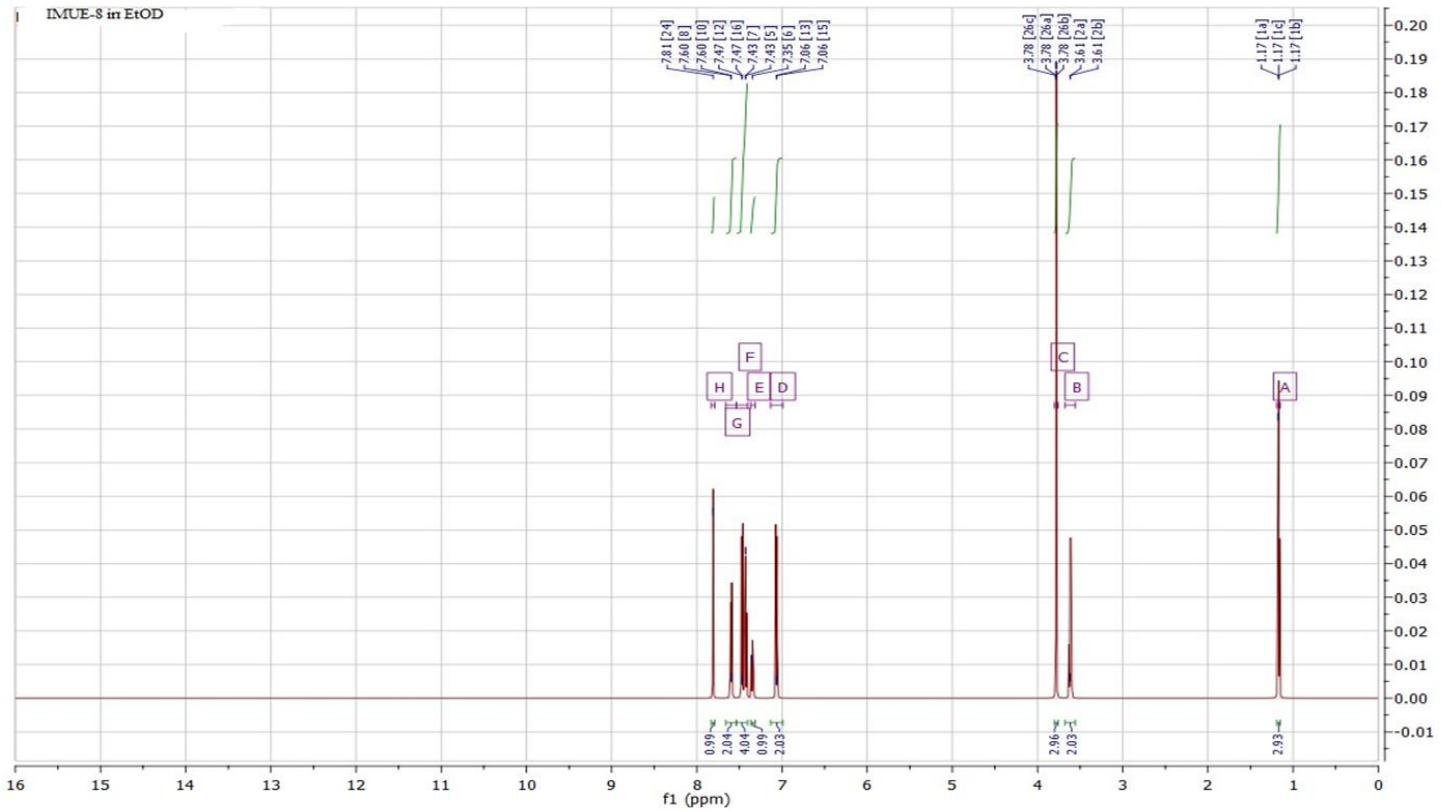

FIG 2.6: [1]HNMR OF COMPOUND_4G

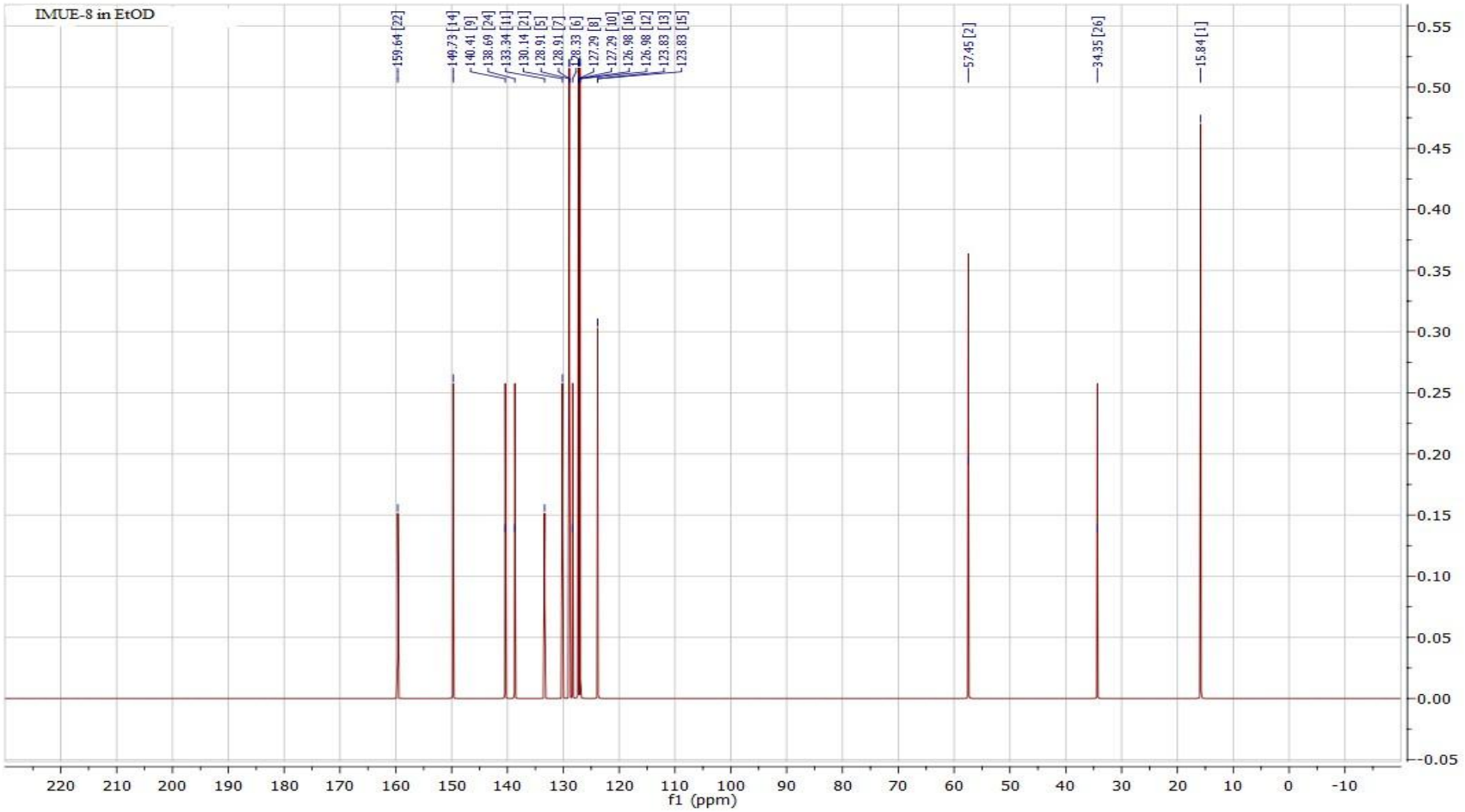

FIG 2.6A: ^{13}CNMR OF COMPOUND_4G

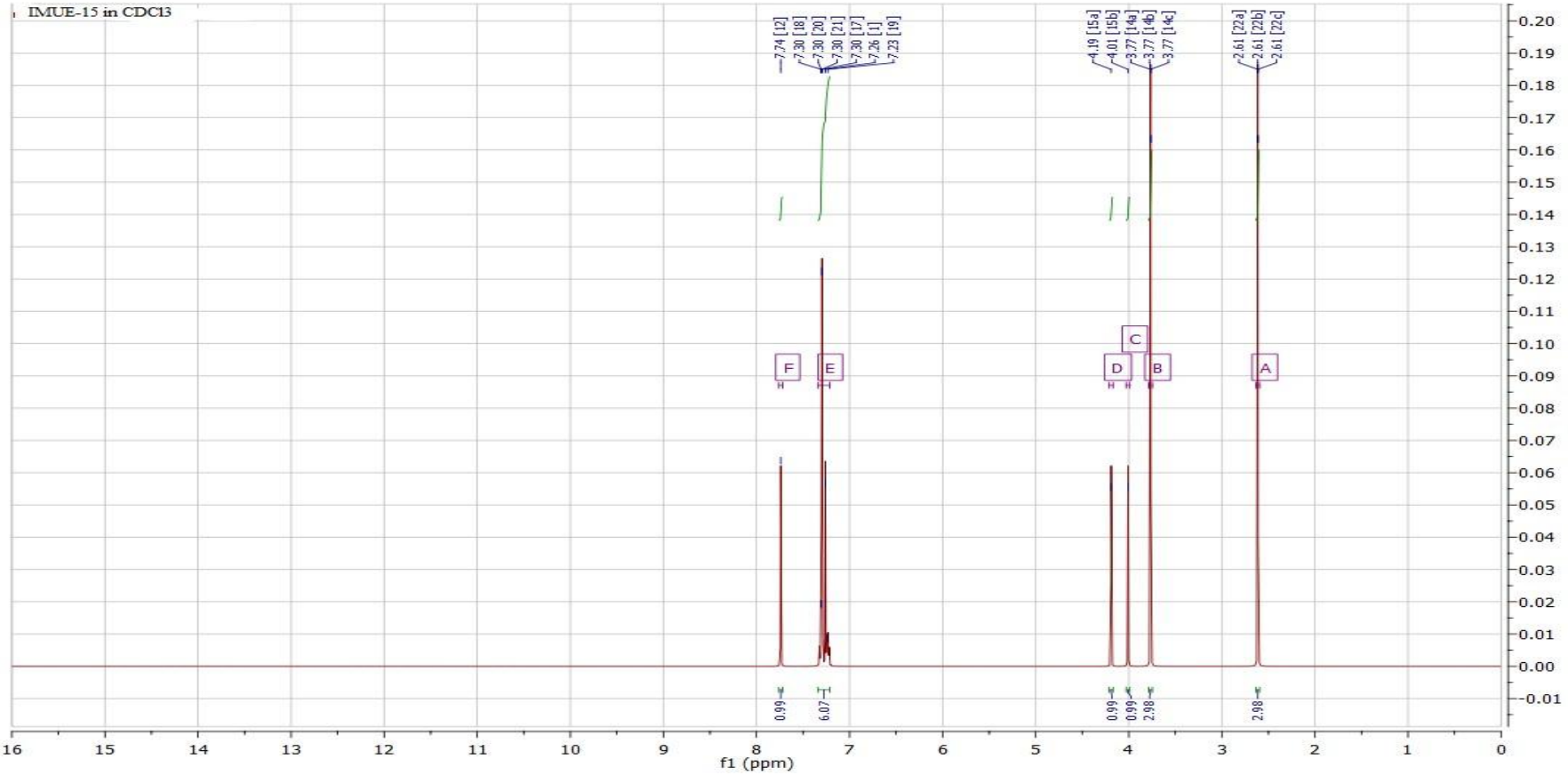

FIG 2.7: [1]HNMR OF COMPOUND_4H

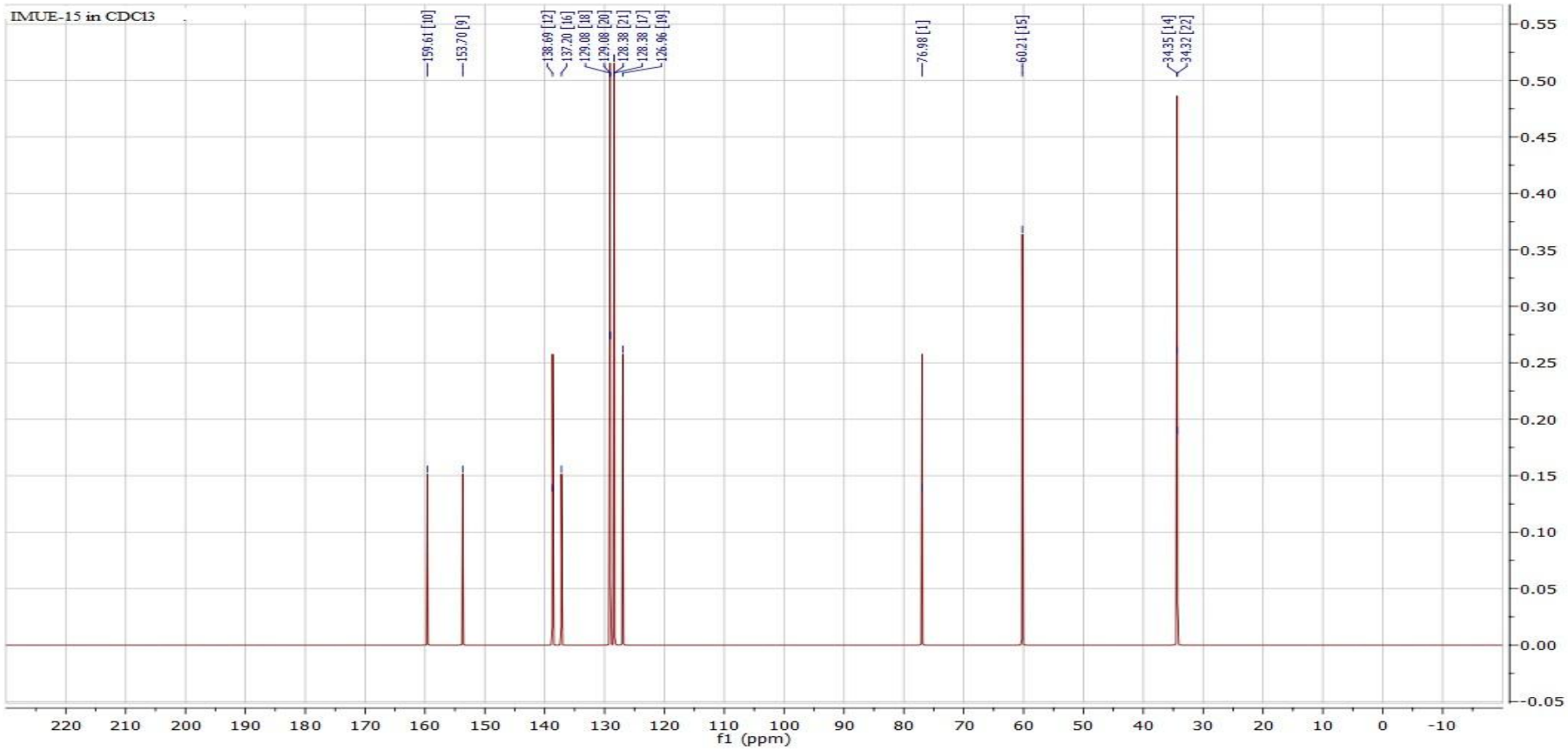

FIG 2.7A: ^{13}CNMR OF COMPOUND_4H

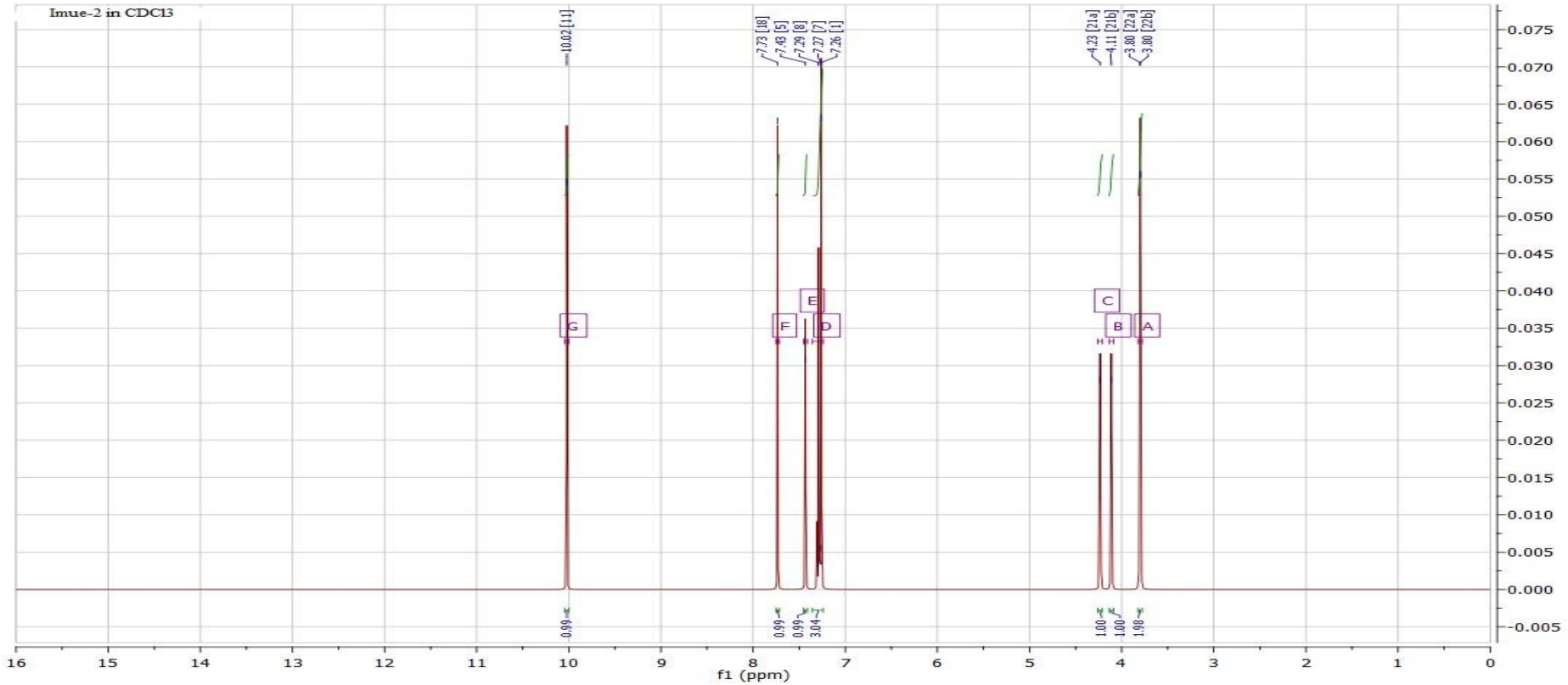

FIG 2.8: [1]HNMR OF COMPOUND_5A

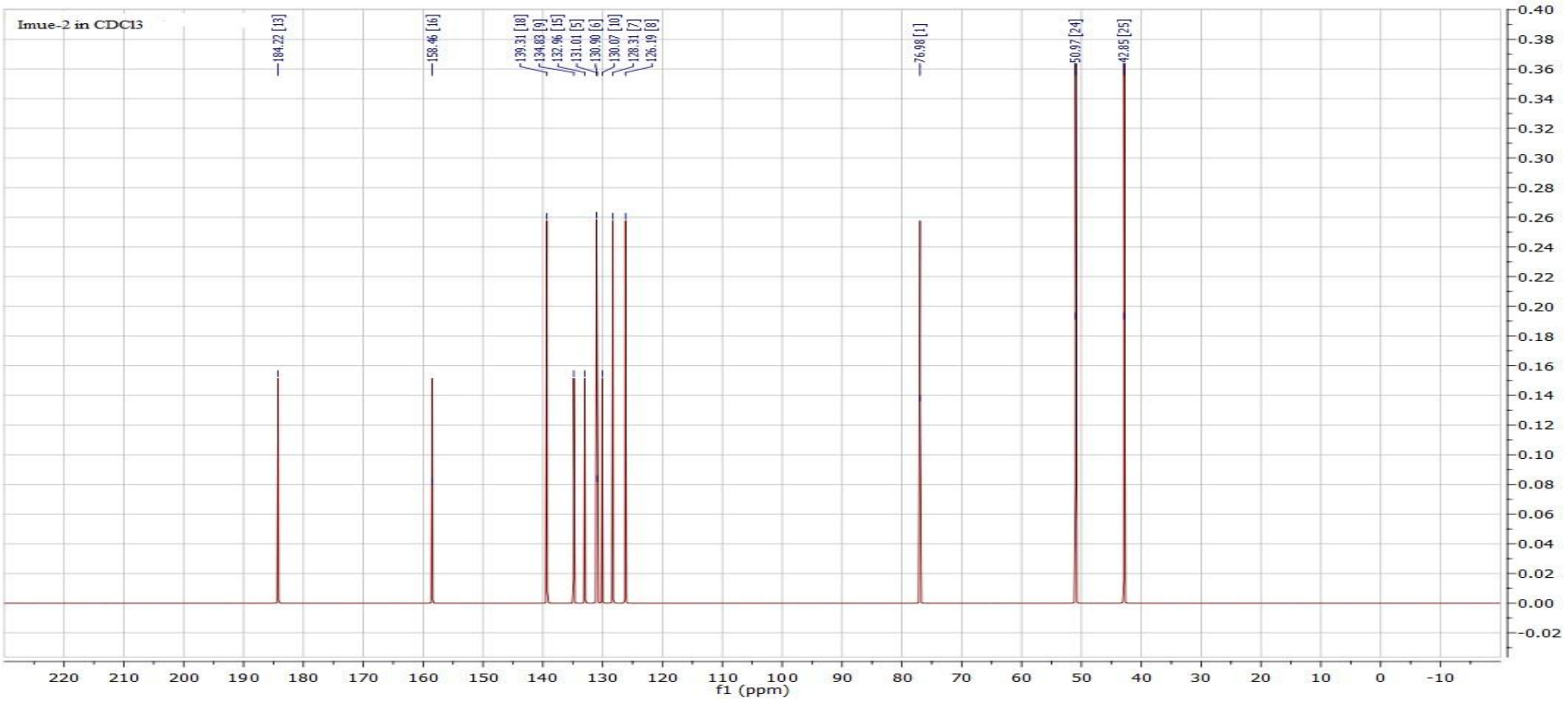

FIG 2.8A: ^{13}CNMR OF COMPOUND 5A

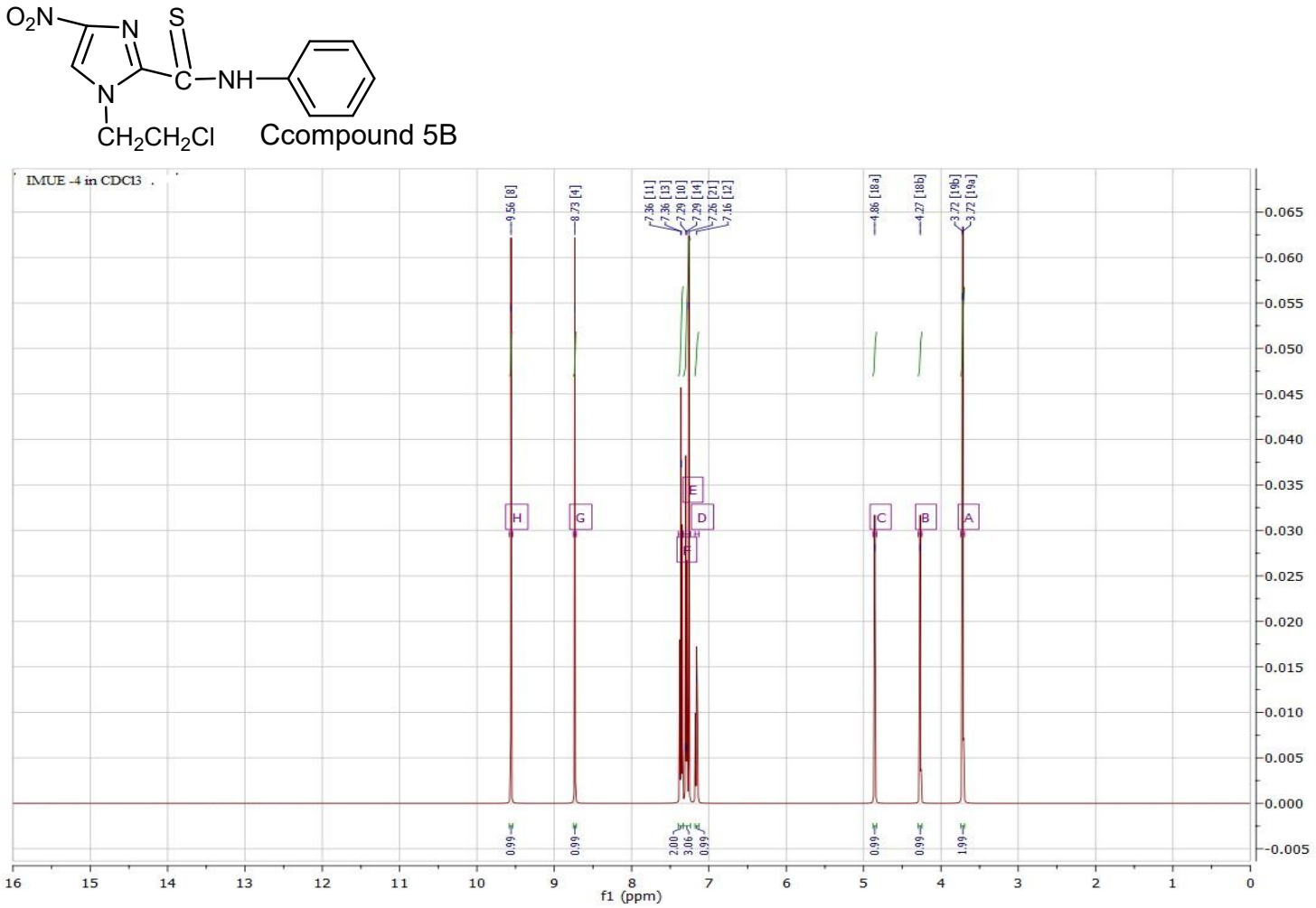

Fig 2.9: [1]HNMR OF COMPOUND_5B

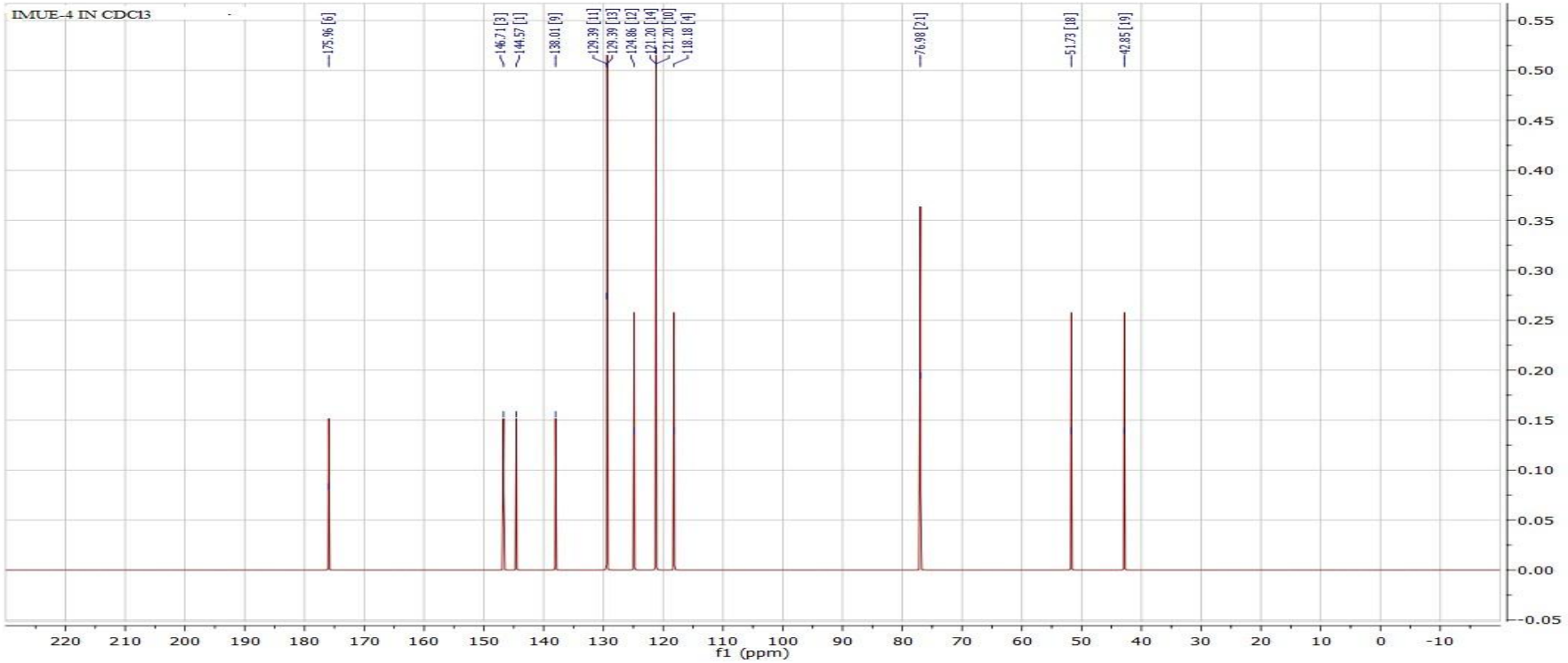

Fig 2.9: ^{13}C NMR OF COMPOUND_5B.

Plate 1: Extraction of Compound 2 using Chloroform

Plate 2: Passing H_2S through a Reaction Mixture of Compound 3 and Sodium ethoxide (C_2H_5ONa)

Plate 3: Vacuum Distillation Setup

Plate 4: Setup for Refluxing