



Faculty of Sciences

Department of Chemical and Forensic Sciences

**Synthesis and Characterization of Mixed Ligand Transition
Metal Complexes and their Antibacterial Properties Against
Drug-resistant Bacteria**

By

JAMES THOMAS PAPISO MATSHWELE

Student ID number: 17100004

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**Supervisor(s): Dr Sebusi Odisitse, Dr Ofentse Mazimba and
Prof. Florence Nareetsile**

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Transition metal complexes are widely studied in medicinal chemistry, this is because of their interesting biological activity **against** various human diseases. This project is aimed at the synthesis of mixed ligand complexes of copper, cobalt and nickel using solvent reaction and mechanochemical methods. The four ligands being 4-chloromethyl pyridine **4PY**, and four synthesized ligands pyridin-4-ylmethyl 4-nitrobenzoate **L1**, 4-(pyridine-4-ylmethoxy)aniline **L2**, pyridin-4-ylmethyl 4-aminobenzoate **L3**, and 4-((4-nitrophenoxy) methyl)pyridine **L4** were successfully synthesized using **a** solvent reaction method **which** all followed the S_N2 mechanism. Their characterization was done **using** proton NMR, mass spectrometry, elemental analysis and Single crystal X-Ray diffraction (SC-XRD). The cobalt, nickel and copper pyridyl complexes were synthesized **in** considerable yields while characterization techniques confirmed their successful synthesis. The complexes were codenamed as metal and the ligand (**ML**), compounds of **4PY** were synthesized using the conventional solvent assisted methods but produced lower and less purer products than the green mechanochemical synthesis. The electronic spectra together with the magnetic susceptibility studies showed that the cobalt metal complexes were d^7 tetrahedral complexes, while the nickel were d^8 tetrahedral, with **NiL1** showing a partial d^8 octahedral geometry, and the copper complexes were d^9 tetrahedral complexes. The cobalt complexes showed better bioactivity towards the Gram-negative bacteria, especially towards *K. pneumoniae*. Most active compounds against Multidrug Resistant-*K. pneumoniae* were bacteriostatic while all the compounds except **CoL3** and **CoL4** were bacteriostatic against *K. pneumoniae*. The Alkaline phosphatase (AKP) release assay of **Co4PY-H**, **Co4PY**, **CoL2**, **CoL3** showed that they disrupted the cell membrane integrity of *K. pneumoniae*, additionally, **CoL3** disrupted the cell membrane of MRSA. Although more active towards the Gram-positive bacteria, the nickel complexes were less effective than the control silver sulfadiazine (**AgSD**). The active nickel complexes should have been more active than the control drug **AgSD**, according to molecular docking scores against the *S. aureus* protein PBP2a, the *K. pneumoniae* OmpK36, and the DHPS protein, but their activity was quenched by their solubility problems. The copper complexes were more effective against Gram-positive bacteria, with **CuL4** performing best,

notably against MRSA and *S. aureus*, and **4PY** complexes being the least effective. Additionally, it was shown that the **CuL4** and **Cu4PY-H** disrupted the *S. aureus* cell membrane more effectively than the **AgSD** control.

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Dedication

Magdalene Jessie Matshuele and Moletsi Michael Matshuele

Mum and Dad, to all the love you give me. To the nurturing you gave me from birth to now as a grown man, I dedicate this achievement to you. This could have not been possible without you believing in me. I love you always.

List of Abbreviations

CT: Charge Transfer bands

WHO: World Health Organization

mecA: Virulent bacterial gene that allows antibiotic resistance to beta-lactam based drugs

MRSA: Methicillin-resistant *Staphylococcus aureus*

MDR: Multidrug resistant

FT-IR- Fourier Transform Infrared Red spectroscopy

UV/Vis: Ultraviolet/Visible light spectroscopy

CD: Circular Dichroism spectroscopy

NMR: Nuclear Magnetic Resonance spectroscopy

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

SEM: Scanning Electron Microscopy

LPS: Lipopolysaccharide

SN₂: Substitution, nucleophilic, bimolecular reaction

SN₁: Substitution, nucleophilic, unimolecular reaction

PBMC: Peripheral blood mononuclear cell

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye

PBS: Phosphate buffered saline

PBP: Penicillin Binding Protein

MIC: Minimum Inhibitory Concentration

K. pneumoniae: *Klebsiella pneumoniae*

MDR-*K. pneumoniae*: Multi-drug *Klebsiella pneumoniae*

Omp: Outer membrane Protein

HRESI-MS: High resolution electrospray ionization mass spectrometry

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List of Publications

The work of this thesis has been featured in the following publications:

1. **Matshwele, James TP**, Florence Nareetsile, Masego Tebogo, Ofentse Mazimba, Fortunate P. Masita, Lebogang Julius, Mosimanegape Jongman, and Sebusi Odisitse. "Physicochemical properties, crystal structures, antibacterial properties and in silico characterization of cobalt and copper metal complexes and their acido complex crystals with 4-chloromethylpyridine." *Polyhedron* 226 (2022): 116085.
2. Nareetsile, F. M., **Matshwele, J. T.**, & Odisitse, S. (2022). Metallo-Drugs as Promising Antibacterial Agents and Their Modes of Action. *Journal of Medicinal and Chemical Sciences*, 5(6), 1109-1131
3. **Matshwele, James TP**, Mosimanegape Jongman, Moses O. Koobotse, Ofentse Mazimba, Daphne Mapolelo, David O. Nkwe, Florence Nareetsile, and Sebusi Odisitse. "Synthesis, characterization of nitro or amino substituted pyridyl ligands bridged by an ester or ether bond, and their antibacterial assessment against drug resistant bacteria." *Results in Chemistry* (2022): 100401.
4. **Matshwele, James TP**, Sebusi Odisitse, Ofentse Mazimba, Florence Nareetsile, Lebogang G. Julius, and Dalitso Keitumetse. "Crystal Structure of Pyridin-4-ylmethyl 4-Nitrobenzoate, $C_{13}H_{10}N_2O_4$." *X-ray Structure Analysis Online* 38 (2022): 7-8
5. **James T. P. Matshwele**, Sebusi Odisitse, Ofentse Mazimba, Florence Nareetsile, Lebogang G. Julius, and Dalitso Keitumetse, "Synthesis and Crystal Structure of Pyridin-4-ylmethyl 4-aminobenzoate, $C_{13}H_{12}N_2O_2$ " (*Springer-Crystallography Reports*)
6. **Matshwele, J. T.**, Jongman, M., Demissie, T. B., Koobotse, M. O., Mazimba, O., Mapolelo, D., ... & Odisitse, S. (2023). Reduction of Bacterial Folic Acid Production and Cell Membrane Disruption of *Klebsiella pneumoniae* by Two Amino Substituted Pyridyl Compounds: An Experimental and In Silico Approach. *Chemistry Africa*, 1-11

Manuscript Drafts

7. Synthesis, Characterization and Antibacterial Properties of Pyridyl-based Tetrahedral Cobalt Complexes Against Drug-resistant Bacteria
8. Antibacterial Activity of Pyridyl-Based Nickel Complexes Against Drug-Resistant Bacteria
9. Synthesis and Characterization of Copper Complexes with Antibacterial Properties Against Drug Resistant Bacteria

1.0 Introduction

1.0.1. Bacteria and their Cellular Structure

Bacteria are microscopic organisms of a single cell nature which includes pathogenic and non-pathogenic species [1]. Bacteria are classified as either Gram-positive or Gram-negative, which explains their outer membranes structures. Gram-positive bacteria have a cell wall while Gram-negative bacteria do not, this is observed through a technique called Gram staining [2]. Figure 1 shows the organelles found in both the Gram-positive and Gram-negative bacteria. The Gram-positive bacteria have a larger peptidoglycan layer which makes up the cell wall, while the Gram-negative bacteria have a smaller peptidoglycan layer. However, Gram-negative bacteria also have a lipopolysaccharide plasma membrane, and their membranes are generally challenging to access as compared to the Gram-positive cell walls. Hence, they use a porin/efflux pump to accept essentials into the cell and remove waste out of the cell [3]. Some bacteria have a virulent polysaccharide membrane called a capsule that helps bacteria from phagocytosis by other organisms or human phagocytic cells [2], [3].

The bacterial cell also has a gel matrix called a cytoplasm where all the cellular processes take place, which is composed of enzymes, water, nutrients, gases, and wastes. The cytoplasm contains all the important cellular organelles and macromolecules such as ribosomes, plasmids, and the chromosome [2]. The bacterial cell also has a cytoplasmic membrane which is made up of proteins and a phospholipid membrane that regulates the flow of materials going in and out of the cell. Since bacteria only have one chromosome their double stranded DNA is found in a membrane-less region called a nucleoid. Most bacteria also have pili which assists them in attachment to surfaces. Pili are hairlike growths from the outer membrane of the bacterial cell, while some bacteria have hairlike structures that aid bacteria in locomotion called flagella [3]. Lastly, all bacteria have ribosomes. These are factories that translate the bacterial genetic code to produce amino acids which are the building blocks of proteins [3]. .

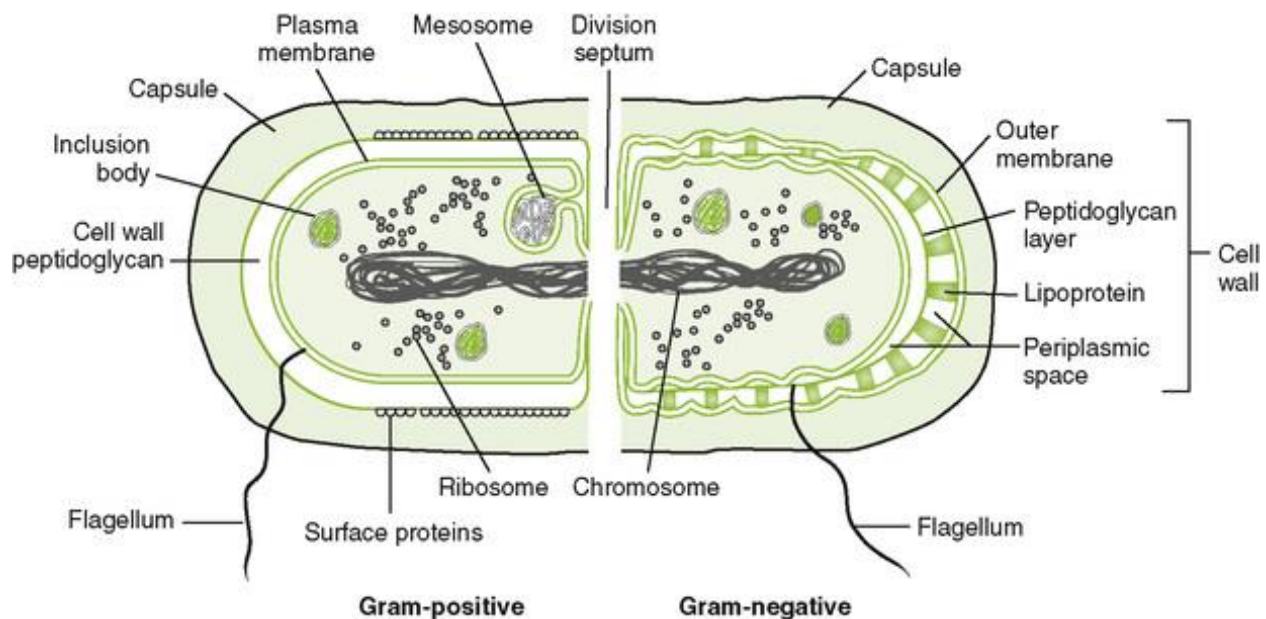


Figure 1: The structure of the Gram-positive and Gram-negative bacterial cell structure and organelles [4].

1.0.1.1. Antibacterial Classes and their Mode of Action

An ideal drug/antibiotic drug is one that has toxicity towards bacterial cells and no or less activity towards animal cells. Bacteria have many unique targets for antimicrobials (see Figure 2), and each antibacterial class has a unique mode of action against the survival of the bacterial cell. There are antibacterial drugs that mechanically disrupt the integrity of the cell membrane, which spills out the cell organelles and thus killing the bacteria [5]. The other antibacterial drugs inhibit the synthesis of macromolecules which stops the bacteria from growing or surviving, these drugs are the cell wall growth inhibitors [6]–[8], metabolic pathway synthesis inhibitors [9], [10], ribosome function inhibitors and DNA synthesis inhibitors [11], [12].

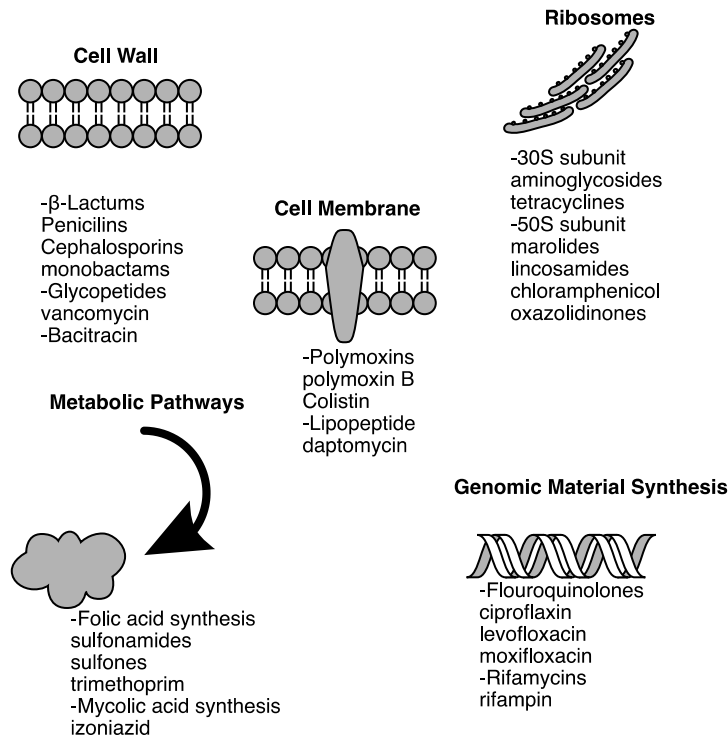


Figure 2: Antibiotic classes and their targets in the bacterial cell.

Different classes of antibacterial drugs affect bacterial cells in many ways as observed in Figure 2. Some affect the bacterial cells mechanically by physically disrupting structures of the bacterial cell, such as the cell membrane disrupting drugs. Otherwise, some antibacterial drugs affect the bacteria by inhibiting biosynthesis of some macromolecules in the survival and growth of the bacterial cells. Table 1 shows the different antibacterial drug classes, their modes of action and biological targets.

Table 1: Antibacterial Drug Classes their Targets and Modes of Actions

Mode of Action	Target	Drug Class	References
Inhibit cell wall biosynthesis	Penicillin-binding proteins	β -lactams: penicillins, cephalosporins, monobactams, carbapenems,	[6]–[8]
	Peptidoglycan subunits	Glycopeptides	[5], [13], [14]
	Peptidoglycan subunit transport	Bacitracin	
Membrane disruption	Lipopolysaccharide, inner and outer membranes	Polymyxin B, colistin, daptomycin	[5], [14], [15]
Antimetabolites	Folic acid synthesis enzyme	Sulfonamides, trimethoprim	[9], [10], [16]
Inhibit biosynthesis of proteins	30S ribosomal subunit	Aminoglycosides, tetracyclines	[12], [17]
	50S ribosomal subunit	Macrolides, lincosamides, chloramphenicol, oxazolidinones	
Inhibit nucleic acid synthesis	RNA	Rifamycin	[18]–[22]
	DNA	Fluoroquinolones	

1.0.1.2. Antibiotic Resistance

Antimicrobial drug resistance is a process of bacterial species losing susceptibility to an antibiotic and this bacterial defense mechanism occurs when there is a mutation in the bacteria which results in the reduction or total elimination of drug efficacy. The WHO states that the misuse and overuse of antibiotics, a lack of access to clean water, sanitation, and hygiene (WASH) for humans and animals, inadequate infection and disease prevention and control in hospitals and farms, a lack of access to high-quality, reasonably priced medications, vaccines, and diagnostics, a lack of awareness and knowledge, and a lack of legal enforcement are the main causes of antimicrobial resistance [23]. Also, according to WHO (2014) antibiotic resistance has become one of the world's greatest public health issues and WHO states that in the last 25 years there have been no new anti-biotics developed. However, there has been an increase in antibiotic resistance while the conventional effective antibiotics are decreasing in efficacy [24]. Bacteria may resist antibiotics by two important ways, this is through reducing the concentration of the antibiotic

before it reaches the target or by improving the bacterial organelles the antibiotic works on so as to avoid cell death. The illustration in Figure 3 explains this phenomenon.

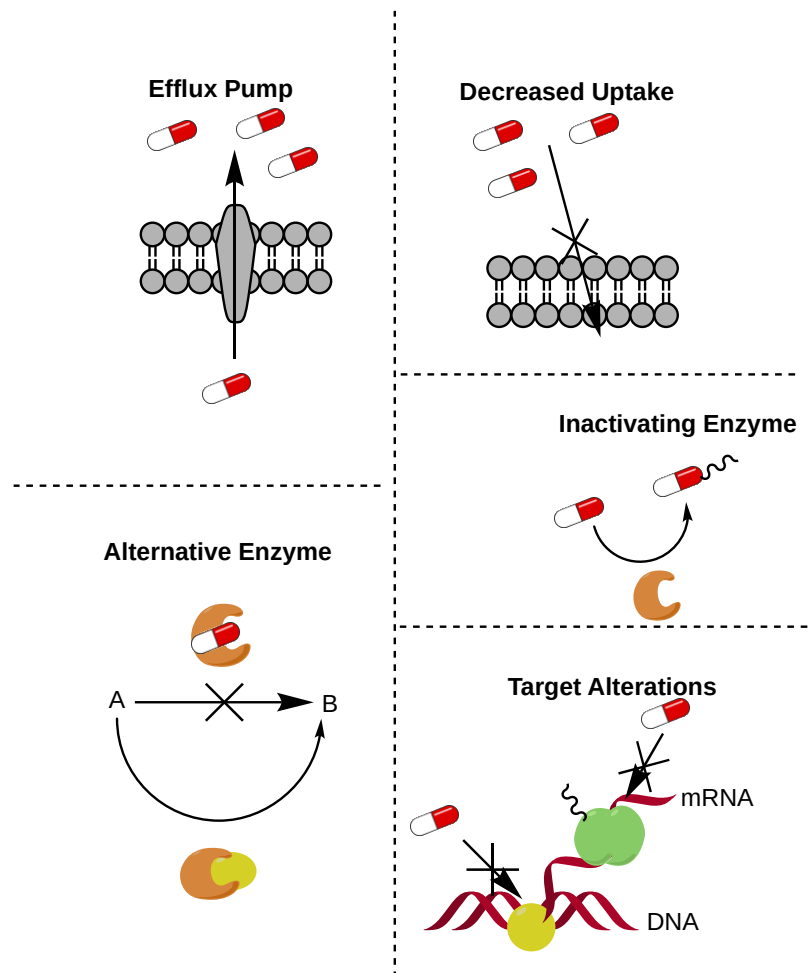


Figure 3: Bacterial antibiotic resistance

In their race to avoid antibiotics, some bacteria have been reported to decrease permeability of their membrane which makes it difficult for the antibiotics to enter the cell and attack at the target [25]. Gram-negative bacteria have an efflux pump, which is vital for sending out important information from the bacterium and even essential nutrients and waste materials. However, in terms of resistance this pump releases some antibiotics before they reach their target [26]. Some bacteria genetically encode for enzymes that can destroy antibiotics before they reach the target in the cell. An important example is the beta-lactamase producing bacterial species, which destroy most of the beta-lactam containing antibiotics such as penicillin [27]. Bacteria are also reported to change chemical groups on their surface to alter the binding **target** of antibiotics in

the cell. Some bacteria can genetically encode for production of alternative target molecules of the antibiotics. An example is *Staphylococcus aureus* (*S. aureus*), this organism through its virulent *mecA* gene genetically encode for a new penicillin binding protein. This protein has low affinity to beta-lactam antibiotics, thus the cell wall synthesis of this bacterial species is continued, and this is the basis in *MRSA* resistance [28].

1.0.1.3. Multi-Drug-*Klebsiella pneumoniae*

Klebsiella pneumoniae is a non-locomotive Gram-negative and lactose fermenting bacterium of the family Enterobacteriaceae, which is both an opportunistic pathogen and commensal organism in the human body. This bacteria species is normal flora of the human body, and its fellow *Klebsiella* genus species are known to cause nosocomial infections, this happens when the bacteria are aspirated to the lungs of any animal [29]. The continuous consumption and the use of low-quality antibiotics is hypothesized to have caused these species' multi-drug resistance. Current research speculates the antibiotic resistance virulence genes of these species to be originating from plasmids [30]. There are various genes which encode for these resistant factors which can be spread through horizontal transfer, an example is those that are beta lactamase resistant. The ability of *Klebsiella* species to produce a broad and extended spectrum beta-lactamases enzyme (EBSL) gives them resistance to almost all beta-lactam antibiotics with an exception to carbapenems. These species also show some drug resistance towards aminoglycosides, tetracyclines, trimethoprim fluoroquinolones and chloramphenicol [31].

1.0.1.4. Methicillin Resistant *Staphylococcus aureus*

Staphylococcus aureus bacteria are Gram-positive non-locomotive bacterial species that are facultative anaerobic and belong to the family *Staphylococcaceae*. These bacteria are also members of the normal microbiota of the body [32]. Even though these bacteria are mostly commensal, they are still able to cause skin infections, respiratory problems and even food poisoning. This occurs when the non-pathogenic strains gain virulence by producing virulent factors such as toxins and expression of cell surface proteins that inactivate antibodies [32]. Because of natural selection *MRSA* emerged, this is when the normal *S. aureus* developed genes that encoded for different proteins that had reduced or no affinity to beta-lactams thus having

resistance against all beta-lactam inhibiting drugs [33]. In detail, the *S. aureus* resistance is produced because of chromosomal and plasmid mediated beta-lactamases. This resistance towards methicillin-based antibiotics is caused by the *mecA* gene, which is part of the mobile genomic element in staph chromosomes. Gene expression of the *mecA* produces a low affinity penicillin binding protein (*PBP2a*). When the high affinity *PBP* is changed, the conventional beta-lactam inhibiting antibiotics will not be able to inhibit cell wall synthesis, thus the resistance is acquired [33].

1.0.2. Drug Design and Choice of Ligand and Central Metal

Drug design or the rational drug design is an inventive process of making a drug that can target a specific site or multiple sites in a disease [34]. Drug design is most of the time reliant on the target biomolecules. Medicinal chemists together with biologists study the biological target and design drugs to be able to specifically interact with biomolecules. These biomolecules are often proteins and genomic material, because of this due research process, rational drug design is also called **reverse pharmacology** [35]. Drug design mostly involves the design and synthesis of small organic molecules, in that regard it may often be called **ligand design**. However, in this research the organic molecules will be coordinated to various metals to produce metal based potential drugs. The drug design process is often but not fully reliant on computational methods, and this technique was coined computer-aided drug design [36], [37]. The opposite of rational drug design is called **forward pharmacology** or **classical pharmacology**. This type of drug discovery relies on tests of natural products and small molecules to any disease without any knowledge of how they may affect the target disease, basically on a trial-and-error basis [38]. This method of drug discovery has been the basis for a lot of discovered drugs, especially that it does not involve a lot of research on the hypothesis. The modes of action or targets of these drugs are studied after it has been observed that the potential drugs cause a phenotypic change [38]. Ligand design depends on a lot of factors in terms of disease inhibition as per the biomolecules of the target. Small molecules are often designed to affect the target biomolecules in various ways, the following list explain ways in which these molecules interact with target biomolecules.

a. Agonists

This is a therapeutic agent that binds to a biological receptor to activate it resulting in a biological response. In therapeutic agonism biological receptors may be activated by different agonistic therapies. These can be neurotransmitters and hormones that are termed endogenous agonists or the chemical drug exogenous agonists. There are other agonists that fall under physiological agonists, this type of agonists induces the same biological responses without binding to the same receptor [39].

b. Antagonists

Antagonistic drugs are the opposite of agonists because they reduce the biological response by blocking or bonding to a biological receptor. The pharmacological inference is that this class of therapies have good affinity to their respective biological receptors but do not have any phenotypic change inducing activity. So, the mode of action of antagonists results in dampened activity from the agonistic or true binder of the biological receptor unlike the activating agonists. These are usually called blockers due to their function; an example of antagonistic drugs is the group of beta-blockers [40].

c. Inverse agonists

Inverse agonists have affinity to a biological receptor and can induce a phenotypic response. However, these agonists produce a different or opposite phenotypic response from the biological receptor. Example of these include the ion channel blocker muscimol, an inverse agonist to the *GABA_A* receptor [41].

d. Modulators

These types of therapies often called selective receptor modulators are defined as those that can function as both agonist and antagonist depending on the desired biological receptor response. These selective receptor modulators affect specific tissues for a biological response and thus may also be called selective tissue drugs. However, the only approved drug of this kind is a selective estrogen modulator [42].

e. Enzyme activators and inhibitors

Enzyme activators are a type of therapies that bind to enzyme because they are enzyme specific, and in turn increasing the activity of the enzymes. In contrast enzyme inhibitors are those that decrease the activity of enzymes. In this activity, the inhibitors bind to the enzymes at the active site thus blocking or reducing the enzymatic activity [43], [44].

f. Ion channel openers and blockers

Ion channel openers are those therapeutic agents that facilitates the opening of ion channels. On the contrary ion channel blockers prevent the opening of the ion channels. These processes can be done using different chemicals such as ions and other organic chemicals [45], [46].

g. Absorption, Distribution, Metabolism, And Excretion (ADME)

Another important step in the drug design process is the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analysis of potential drugs. This helps in understanding the interaction of drugs with the host and the target biomolecules. This can be studied using biological assays or computer aided calculations. This stage studies how the drug will be administered to the body, and how the body adsorbs it. Potential drugs could be administered as liquids intravenously, as a syrup, a pill or even a cream. Furthermore, the potential drugs metabolic pathways will be explored to assess the drugs fate in the body. Scientists at this stage also study the excretion of the potential drugs which focuses on where and how the drug is finally excreted. The most promising candidate drugs may be modified to enhance their properties during these studies. This stage also studies the toxicity of the potential drugs. The results from this stage will then be submitted to relevant authorities to decide whether to take them on to clinical trials [47].

1.0.2.1. Potential Structure-activity Relationship of the Chosen Functional Groups

To achieve a functioning drug, certain functional groups are designed on the potential drug. In this work the pyridine derivatives of 4-picolyl will be coordinated to different metals and tested for their antibacterial properties. The functional groups will be chosen carefully to design ligands and metal complexes that may hypothetically induce antibacterial activity. Pyridine derivatives

and compounds with pyridine moieties have important properties which make them great candidates for drugs. These properties include their stability, hydrogen bonding properties, small molecular weights and basicity [48]. Pyridine and its derivatives possess lipophilicity which allows for cell access. This is because the Lipopolysaccharide layer (LPS) of bacterial cells is made up of fats. The hydrophobic nature of these ligands facilitates the pyridine derivative entering the cell as they would interact well with the LPS [49]. These derivatives also have intercalative properties enhanced by their planarity; intercalation is a physical binding process where a compound fits itself between planar base pairs of double stranded genomic material [50], [51]. Functional groups found in potential drugs are important choices as they may affect the efficacy, cytotoxicity and genotoxicity. In that regard, the choice of the functional groups was based on their biological importance. Figure 4 shows the proposed ligands for this work, which contain the amine, nitro, chloride, ether, and an ester pharmacophore.

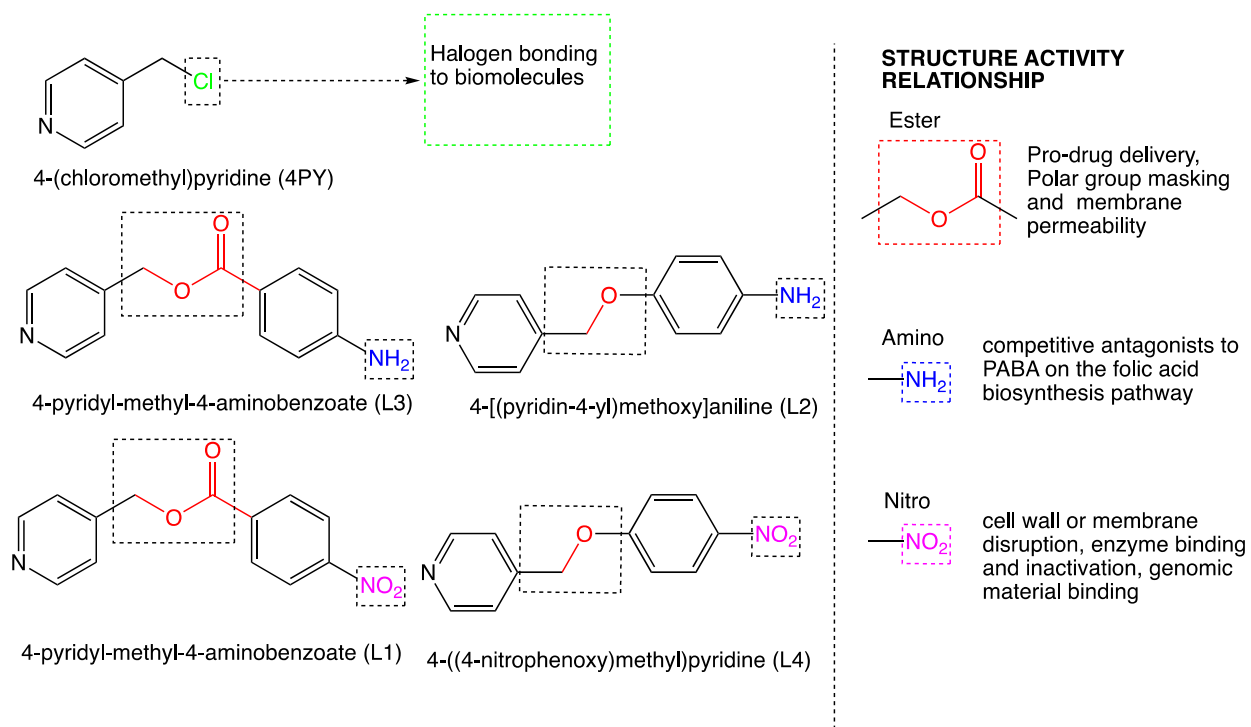


Figure 4: The proposed ligands, 4-chloromethyl pyridine (**4PY**), 4-pyridyl-methyl-4-aminobenzoate (**L1**), 4-pyridyl-methyl-4-nitrobenzoate (**L3**), 4-[(pyridin-4-yl)methoxy]aniline and (**L2**) 4-((4-nitrophenoxy)methyl)pyridine (**L4**).

Various drugs and experimental pharmaceuticals have substituted the chloride moiety in their formulas. According to published research, there may be halogen bonding in biological systems, which implies that halogen atoms might be a key element in experimental medications. In halogen bonding, halides act as Lewis acids and therefore get attracted to any electron donating groups around the chlorine bearing molecule [52]–[54]. This means that the chloride bearing ligand will hypothetically have affinity to electron rich donor atoms in biomolecules such as oxygen, sulphur, nitrogen and phosphorus. Scientists discovered the affinity of binding from two classes of inhibitors of human cathepsin *L* and *MEK1* kinase. Their affinity improved greatly with the introduction of halogens (bromine, chlorine, and iodine) to the aryl ring as substituents [55]. Basically, halogen substituents increase the potential of interaction with biomolecules as they possess halogen bonding capabilities. Having a chlorinated experimental drug also improves the chances of drug-membrane permeability because of the polarization of the halogen bond in the main molecule [56].

Another important moiety found in drugs is the ester functional group, it is known to assist drugs in their biological activity. The ester group assistance stems from various chemical properties of this functional group, such as their ability to be hydrolysed by esterase enzymes. This property makes it possible for these ester group containing drugs to act as prodrugs and thus deliver the actual active group to the target biomolecule [57], [58]. Furthermore, ester groups are used to mask highly polar functional groups such as alcohols, phenols, and carboxylic acids, this ability assists the experimental drug to become permeable to the cell membranes [58], [59]. The amine group is important as it is found in the precursor of folic acid, 4-aminobenzoic acid/para-aminobenzoic acid (PABA). Folic acid is an important nutrient in bacteria, it is used to produce nucleic acids and proteins [60]. The ligands **L2** and **L3** with their amine groups are hypothesized to act as competitive antagonists to PABA on the folic acid biosynthesis pathway at the Dihydropteroate synthase (DHPS) enzyme. This antagonism is hypothesized to be due to their affinity to the biocatalyst because of their possession of the amine group. If that becomes the case, then the pathway will not proceed to form the other precursor of folic acid dihydropteroic acid but will instead form another product which will halt the folic acid biosynthesis pathway.

This means that folic acid synthesis will be affected therefore altering the production of proteins, nucleic acids, and the growth of the bacterial cell. Figure 5 depicts this phenomenon.

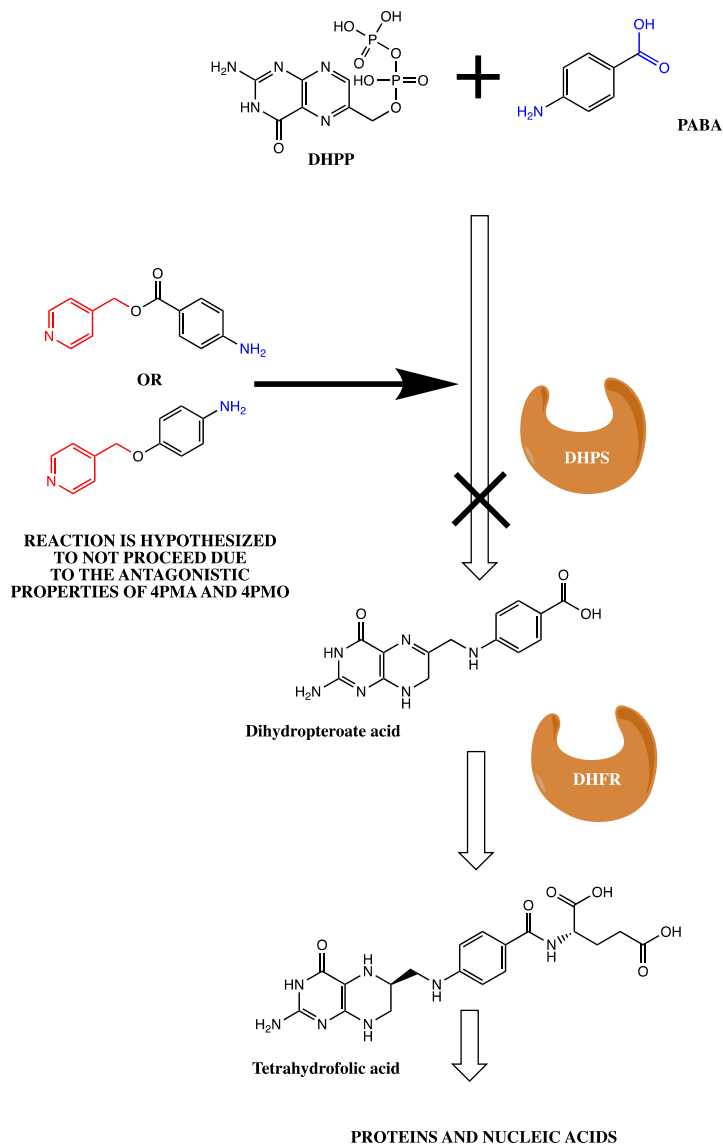


Figure 5: Hypothesis of **L2** and **L3** inhibiting the biosynthesis of folic acid at the Dihydropteroate synthase (DHPS) enzyme section of the folic acid pathway, therefore inhibiting the growth of the bacterial organisms. NB.: Dihydrofolate reductase (DHFR), para-aminobenzoic acid (PABA), Dihydropteroate diphosphate (DHPP).

Furthermore, the **L1** and **L4** ligands have a nitro group, compounds bearing the nitro group have mixed reactions in literature. These compounds sometimes bear toxicity issues, but also have some important antibacterial properties. The mode of action of the nitro pharmacophore

involves the disruption of the cell membrane. This disruption is observed through the reduction of the nitro pharmacophore by nitro-reductase enzymes, resulting in the production of reactive oxygen species. These reactive oxygen species are formed from the nitro functional group. These reducing agents include the flavin nucleotides, and nicotinamide adenine dinucleotide [61]. The drug bank website has an array of different nitro-containing antibacterials with various modes of actions, these include cell wall or membrane disruption, enzyme binding and inactivation, genomic material binding compounds and so many more [62]. This pharmacophore is ideal to compare with the amino group, this study will reveal the efficacy of the two groups.

1.0.2.2. Potential Structure-activity relationship of the Chosen Metal Centres

After the design of the appropriate ligands, the appropriate central metals are selected. The choice for the central metal was based on the available literature on metals antibacterial activity. Table 2 summarizes some of the possible modes of actions of metal complexes against bacteria as described in literature. However, it should be noted that there are less to no literature on the modes of action of some metal based compounds against bacteria. Literature mostly includes *in vitro* screening assays of the antibacterial activity of these metals. Furthermore, the next section will describe transition metal chemistry in medicine then further give examples of these metals as antibacterial agents.

Table 2: Some Examples of Metal Centre Biological Targets

Metal Centre	Biological Target or Mode of Action	Reference
Cobalt	No data	
Copper	Damage to the cell membranes, oxidative damage by producing reactive oxygen species, DNA damage, Enzyme inhibition, DNA intercalation	[63]–[66]
Nickel	No data	

1.0.3. Coordination Chemistry in Medicine

Coordination compounds have been widely researched for their potential application in medicine and drug discovery [67]–[72]. Their physical and chemical properties due to the coordinated metallic centre may in many cases potentiate their efficacy as potential drugs. These properties include; their variable oxidation states, ligand exchange kinetics, genomic binding properties and protein binding properties [73]–[76]. The alkylating agent *cis*-diamminedichloridoplatinum(II) (cisplatin) is an excellent example of a metallodrug. This platinum complex has been used as an antitumor agent since the 1970s [77]. The properties listed above are traits this complex has. This complex specifically works by binding to the DNA of the cancerous cell. Firstly, the complex enters the cell through the membrane. Thereafter, with its good ligand exchange kinetics, cisplatin substitutes one of the chlorides with a water molecule which enables the complex to now bind to a single nitrogen on the DNA nucleobase. This is followed by the second chloride being replaced by another water molecule which enables further binding to another nucleobase by cisplatin. This characteristic binding of cisplatin distorts the DNA molecule thus preventing effective repair mechanisms to the DNA which further results in apoptosis of the cancerous cell. This whole process is based on the physical and chemical properties of the transition metal compound [78]. Ever since the success of cisplatin many transition metal chemists have explored other metal centres for various diseases. However, in this study the focus will be on the antibacterial activity of cobalt, nickel, and copper complexes with mixed ligands. This will be done through the design of compounds with these metals as the central metal. To achieve their potential various physical inorganic chemistry methods will be employed to understand their solid state, physical and chemical properties. Nevertheless, their medicinal properties will then be tested using various biological methods. The literature that will follow from section 1.5.3.2 gives a brief overview of complexes of the above-mentioned metal centres in various medicinal applications. The solid-state chemistry of the test compounds is worth understanding before applying them as potential drugs.

1.0.3.1. Solid-state chemistry of potential drugs

Solid-state chemistry is known as the study of the synthesis, structure, and properties of materials in the solid state. Various solid-state properties of synthesized complexes are examined primarily for their application as potential drugs and other materials. These include studies that assess polymorphism, complex interaction with solvents in solution (solvate) and salt formation. These are important properties to understand as they have an impact on solubility and stability of potential drug candidates. These two properties are amongst most important properties that are essential to the successful development of drugs as they may affect the bioavailability of the drug hence reducing or increasing efficacy in drug design [79], [80]. These studies also help in understanding the physical and chemical properties of synthetic compounds in terms of their half-life, shelf life, temperature dependant transformations and solid-state reactions or interactions.

1.0.3.2. Silver Compounds as Antimicrobial Agents

According to Alexander, the Greeks have been documented to have used Silver to treat ulcers and to stimulate wound healing. He also states that silver has been used over millennia as one of the most effective antimicrobial agents to treat and prevent infections [81]. An example of a silver antibiotic in use is the ionic (4-Amino-N-2-pyrimidinyl-benzene-sulfonamidato-NN,01)-silver or simply silver sulfadiazine shown in Figure 6. This drug is used to prevent infections in severe burns, it works by the slow release of the antibacterial silver ions which are reported to disrupt the thiol function in the bacterial cell wall [24].

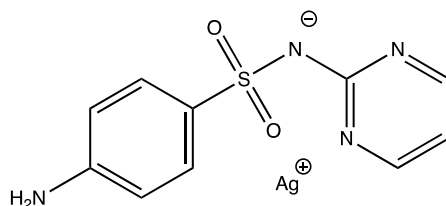


Figure 6: Silver anti-bacterial organo salt

Cardoso *et al* reported the synthesis and characterization of camphorimine complexes of silver with the general formula $[\text{Ag}(\text{NO}_3)^Y\text{L}]$ where ^YL is Camphor di-imine ligands and Y being either 1,

2 and 3. These were reported to be fully characterized by spectroscopical and analytical techniques. One of their compounds $[\text{Ag}(\text{NO}_3)(\text{OC}_{10}\text{H}_{14}\text{NC}_6\text{H}_4\text{NC}_{10}\text{H}_{14}\text{O})]$ showed that it forms as a coordination polymer which arranges through sequential $\text{Ag}(\text{NO}_3)$ units that are bridged by the bi-camphor ligand. This was realized using single crystal X-ray diffraction (SC-XRD) and an ORTEP drawing of the structure is shown in Figure 7. The disk diffusion method and Minimal Inhibitory Concentrations (MIC) assays were used to study the antimicrobial properties of the new complexes. The characterized compounds were tested against Gram-positive and Gram-negative bacteria; *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Burkholderia contaminan*. Their compound showed the lowest MIC for *S. aureus*, *E. coli*, *B. contaminans*, and *P. aeruginosa*. The MIC data showed that their compounds were bactericidal [82].

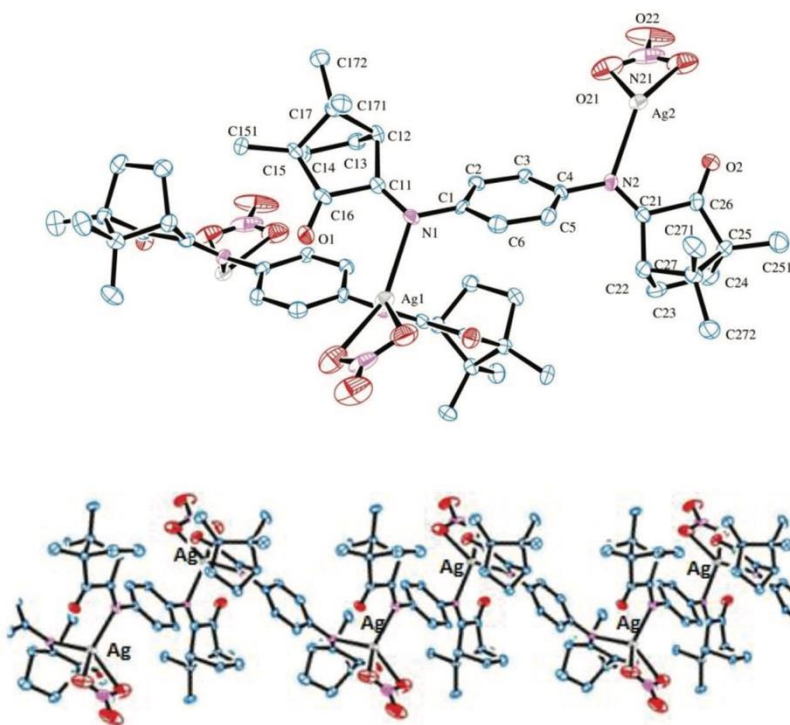


Figure 7: ORTEP drawing of the polymeric AgNO_3 bi-camphor linked complex [82].

Viganor *et al* synthesized a water-soluble silver complex shown in Figure 8 $[\text{Ag}_2(\text{phen})_3(\text{udda})]$ (where uddaH_2 = undecanedioic acid). They tested their complex against both bacterial and mammalian cells. The complex showed antibacterial activity against three bacteria *E. coli*, *S. aureus* and *P. aeruginosa*. The complex was seen to be toxic to the bacterial organism *E. coli*

where it had an IC_{50} of 9.54 μ M, while for *S. aureus* and *P. aeruginosa* the complex also had some potency with activity of $IC_{50} = 14.18 \mu$ M and IC_{50} was 32.47 μ M respectively. As for the mammalian cell, the complex was tested on the breast cancer cell line MCF-7 and ovarian cell line SKOV-3. The complex continued to showed potency for these cells as with the bacterial organisms. Furthermore, they studied the DNA binding properties of the complex and found out that it had some excellent intercalative DNA binding capabilities for the bacterial DNA, and these were greater than that of ethidium bromide. Fortunately, the complex did not show any DNA damage in mammalian cells [83].

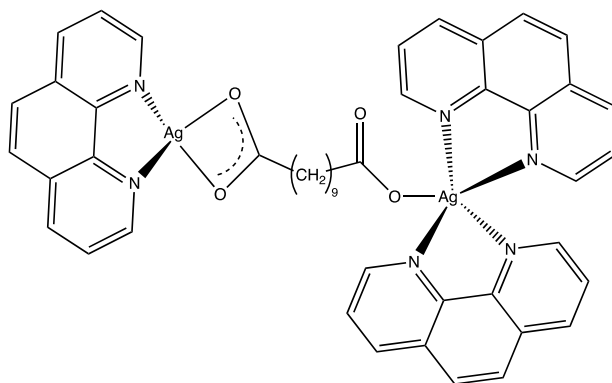


Figure 8: Water soluble silver complex with antibacterial activity [83].

Pöthig *et al* described complexes termed silver pillarplexes and reported their potent antimicrobial, antifungal activity cytotoxic activity. Their pillarplexes showed some moderate cytotoxicity on human HepG2 cells and good antimicrobial activity. They assumed the potency to be due to the low stability of the silver pillarplexes. [84]. Streciwilk *et al* described the synthesis and application of complexes of p-benzyl-substituted NHC–silver(I) acetate compounds derived from 4,5-di-p-diisopropylphenyl- or 4,5-di-p-chlorophenyl-1H-imidazole. The corresponding silver(I) compounds which they coined **4b**, **4e** and **4g** were thus characterised by SC-XRD and are shown in Figure 9.

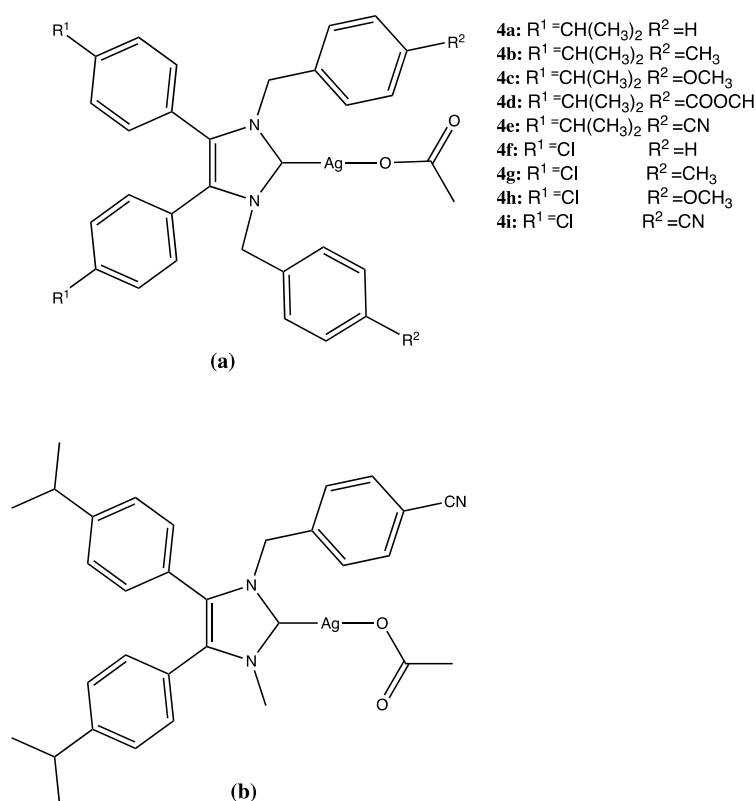


Figure 9:(a) shows symmetrically substituted NHC-silver(I) acetate complexes 4a-i. While (b) shows the un-symmetrically substituted NHC-silver(I) acetate complex 5c [85].

The complexes and their ligand's antibiotic properties against the Gram-negative bacteria *E. coli* and the Gram-positive bacteria *S. aureus* were studied using the disc diffusion assay. The imidazolium halide compounds had some antibiotic activity towards these organisms. While Imidazolium halide precursor compounds were seen to have weak zones of inhibition of the range of 1–5 mm. The NHC–silver(I) acetate derivatives similarly had weak to medium zones of inhibition in the range 2–6 mm. Furthermore, they saw their NHC–silver(I) acetate complexes showing cytotoxic properties towards the breast cancer cell line MCF-7. This is because they produced IC_{50} values of 4.7 (± 0.5), 2.1 (± 0.8), 3.4 (± 0.6), 15 (± 2), 25 (± 7), 24 (± 3), 14 (± 3), 21 (± 5) and 50 (± 2) μM , respectively. Moreover, the same NHC–silver(I) complexes were also observed to have cytotoxicity against the renal cancer cell-line Caki-1 with IC_{50} values of 8.7 (± 1.5), 4.6 (± 0.6), 5.5 (± 0.6), 34 (± 1), 42 (± 4), 44 (± 3), 29 (± 1), 100 (± 2) and 140 (± 10) μM , respectively. Their other un-symmetrically substituted compound codenamed 5c showed IC_{50} values of 24 (± 3) μM against MCF-7 and 44 (± 3) μM against Caki-1 [85].

1.0.3.3. Ruthenium Compounds as Antimicrobial Agents

There has been a growing interest in finding potential ruthenium-based drugs for various diseases due to the biological properties of ruthenium. Researchers have been exploring these bioactive properties of ruthenium complexes as potential antimicrobial, anticancer and antiviral agents [86]–[91]. Even though ruthenium does not have any specific biochemical role, it has been observed to show low toxicity in biological systems [89]. There is currently no known conventional ruthenium based antimicrobial drugs, but there has been increasing research interest in that field. Li *et al* (2016) synthesized some asymmetric dinuclear ruthenium (II) polypyridyl complexes that are shown in Figure 10. Their study reported these complexes to have potent antibacterial properties [91] and revealed that one of the ruthenium centers in the diatomic complex was inert while the other ruthenium center was labile. The ruthenium centers were coordinated and linked by the ligand *bis*[4(4'-methyl-2,2'-bipyridyl)]-1,n-alkane]. These complexes had antibacterial properties towards the bacterial organisms *S. aureus*, MRSA, *E. coli* (*E. coli*) and *P. aeruginosa* [91]. Li *et al* (2016) also reported that the diatomic ruthenium complexes were generally less toxic towards eukaryotic cells. These complexes were generally better active against prokaryotic cells opposed to eukaryotic cells. Genomic material binding studies with the normal eukaryotic cells suggested that toxicity towards these was due to their affinity to the eukaryotic cell genomic material [91].

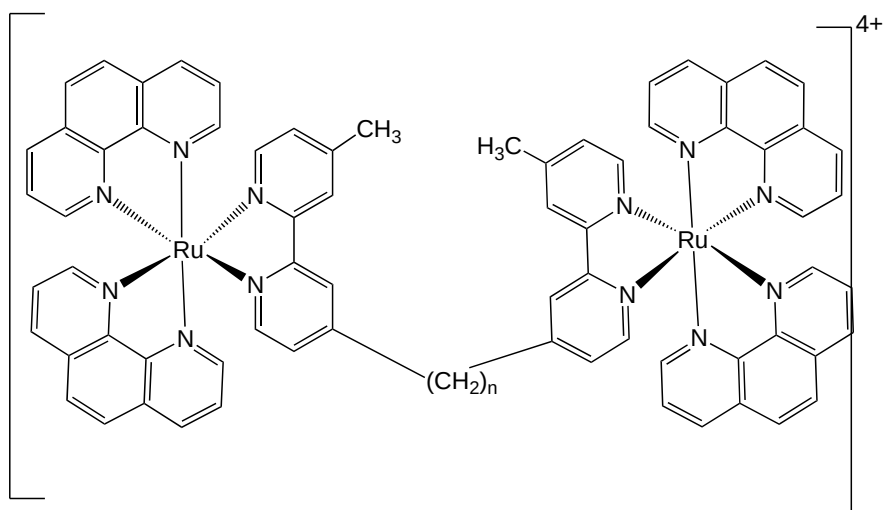


Figure 10: Asymmetric dinuclear ruthenium (II) polypyridyl complexes [91].

Patel *et al* reported some interesting complexes of ruthenium (II/III) coordinated to fluoroquinolones shown in Figure 11 [92]. They tested their DNA binding properties, DNA cleavage properties and also antibacterial properties [92]. Their results indicated that the ruthenium coordinated fluoroquinolones were more active against bacteria than the free fluoroquinolones. This observation suggested that the antibacterial activity of fluoroquinolones was activated by the introduction of the ruthenium centre. Patel and his associates further realized that the ruthenium (III) complexes had better antibacterial activity than those of the ruthenium (II). The complexes also partially intercalated DNA as shown by the UV/Vis DNA binding experiments. DNA cleaving studies of the ruthenium complexes using gel electrophoresis suggested that they have DNA cleaving properties as compared to the free fluoroquinolones. Antibacterial studies also displayed the complexes to have low toxicity towards bacterial cells. However, they realized that with increased concentration of the complexes there was increased antibacterial activity. They suggested the toxicity towards these cells to be because of the lipophilicity of the compounds, meaning that the more lipophilic the compound the easier it is to enter the bacterial outer LPS membrane and the host lipid bilayer/cell membrane [92].

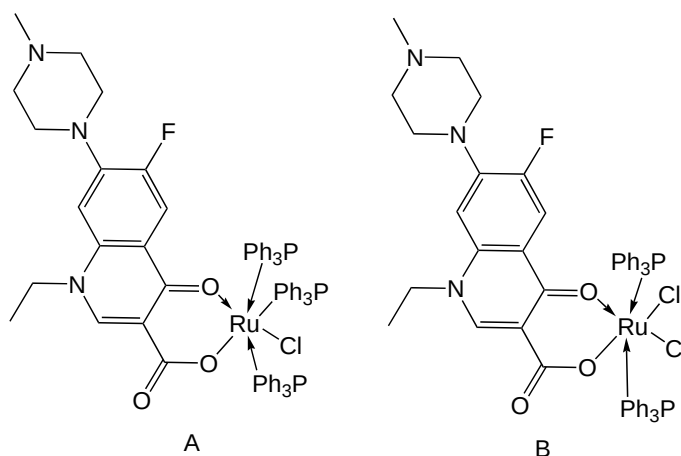


Figure 11: Ruthenium (II/III) coordinated to fluoroquinolones [92].

Sun *et al* described a ruthenium (II) mixed ligand polypyridyl complex (see Figure 12) and further observed antibacterial activity against *S. aureus* and *Micrococcus tetragenus* [93]. Sun and associates noticed the complex to be efficiently taken up by the bacterial cell, this was achieved through cellular uptake studies and laser confocal microscopy. Scanning electron microscopy was

also used to assess cell wall damage of the bacterial cell, it was identified that the cell membrane of the bacteria was damaged by the ruthenium complex. This cell wall damage together with gel electrophoretic studies assumed the complex to work by effective membrane permeability and genomic material nucleation as the gel electrophoretic studies showed the disappearance of genomic material [93].

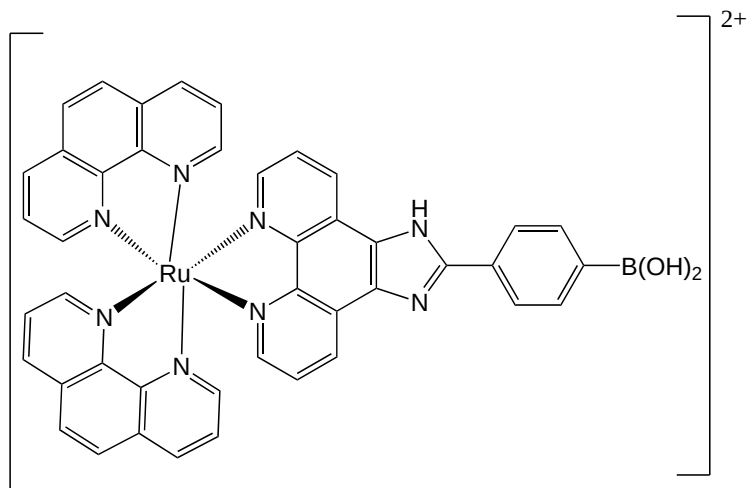


Figure 12: Ruthenium (II) mixed ligand polypyridyl complex [93].

Some ruthenium (II) bipyridyl complexes were observed to show activity towards some drug resistant bacteria [94]. The complexes in the particular study had a varied aromatic moiety bound to a diazo bidentate nitrogen ligand as seen in Figure 13 where the substituents are labelled under Ar in Figure 13. The complex bearing the heptoxy chain had more activity towards MRSA as opposed to the positive control. It was also discovered that the hexoxy bearing ligand had similar antibacterial activity towards MRSA. However, the activity was not as potent as compared to the heptoxy chain bearing ligand, as seen by the smaller zones of inhibition to the hexoxy bearing ligand. The data in this research indicated that the MIC results corroborated well with the disc diffusion assay, proving that the heptoxy chain bearing complex was more potent than the hexoxy chain bearing ligand by having a smaller MIC. This means the heptoxy complex is more active at lower concentrations as opposed to the hexoxy ligand [94].

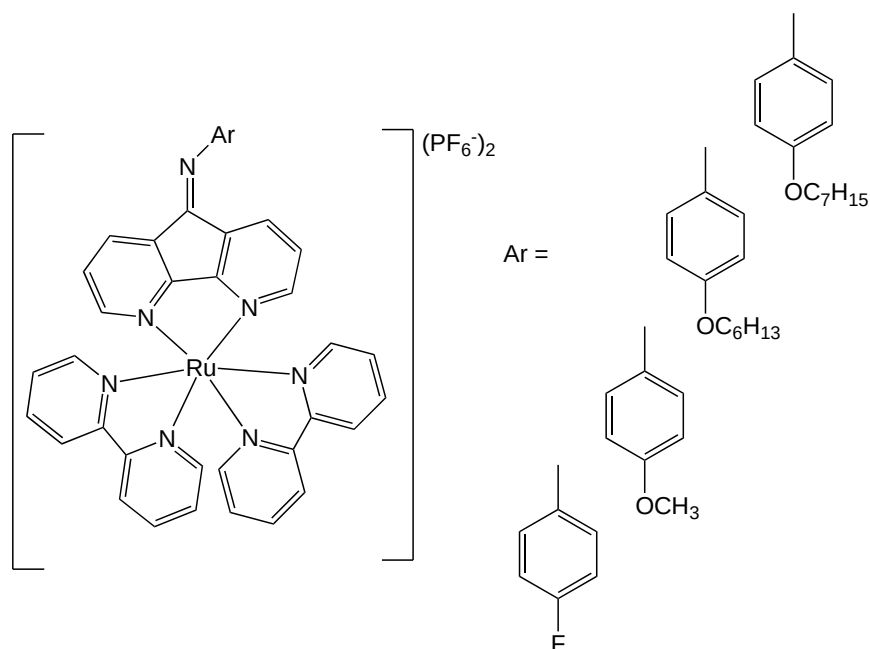


Figure 13: Ruthenium (II) complexes of various N-phenyl substituted 4,5 diazofluorenes [94].

1.0.3.4. Cobalt Compounds as Antimicrobial Agents

A survey of the literature indicated that cobalt medicinal chemistry research is not as common as that of platinum and ruthenium. Even though that is the case, this metal possesses some useful properties that may be favourable in drug design. One of these important properties include the accessibility of the metal's two oxidation states under physiological conditions (Co (II/III)). This means that under hypoxic conditions Co (III) pro-drugs may be reduced to the more active Co (II) [95]. Co (III) is known to be kinetically inert, but it is also known to undergo ligand exchange reactions similar to those of Pt (II) and Ru (III). These reactions include aquation, which may allow these cobalt complexes to be activated through aquation mechanism [96]. Cobalt metal is also an essential trace element in the human body. Therefore, it is relatively less toxic. In that case this allows the design and development of cobalt metal drugs with lower potential of toxicity on host cells. Furthermore, the bioavailability of cobalt in the human body may allow for drugs of this metal to use bioavailable cobalt transport mechanisms, to exploit this trait for improved drug delivery. Transport mechanisms such as those for the movement of cobalt containing molecules like cobalamin [96].

Several cobalt complexes of various structural nature have shown to have bioactivity, such as the dilapacholate diaquo cobalt (II) di-dimethylformamide complex which was detected through SC-XRD. The complex was seen to crystallize in the monoclinic space group $P21/n$ with 2 molecules per unit cell. Furthermore, the structure displayed a crystallographic inversion center with the Co (II) ion. This was also observed in a distorted octahedral environment of this ion on a D_{2h} local symmetry, where it is coordinated to two lapacholate anions that bind as bidentate ligands through carbonyl and phenolic oxygen atoms. This complexes' crystal structure is shown Figure 14. Finally, this complex showed potential bioactivity at a concentration of 25 ppm. This metal complex showed biological activity against *S. aureus* strains [97].

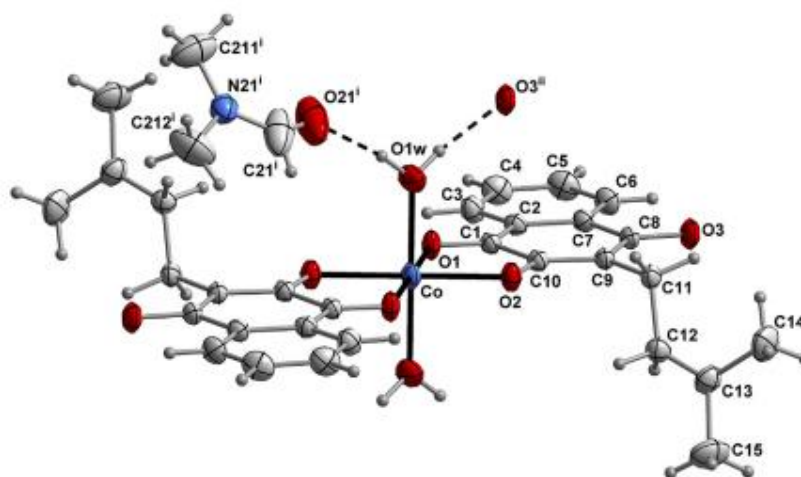


Figure 14: The molar ellipsoids of the antibacterial dilapacholate diaquo cobalt (II) di-dimethylformamide [97].

Vamsikrishn *et al*/ synthesized some new bivalent metal complexes of $M = \text{Cu(II)}, \text{Ni(II)}, \text{Co(II)}$ with the formula $M(\text{L1})_2$ and $M(\text{L2})_2$ displayed in Figure 15. The ligands used where $\text{L1} = 2-((\text{benzo [d] thiazol-6-ylimino)methyl})-4\text{-bromophenol}$, $\text{L2} = 1-((\text{benzo [d] thiazol-6-ylimino)methyl})\text{naphthalen-2-ol}$. They characterized their complexes through elemental analysis, SEM, Mass spectrometry, proton and carbon NMR, electronic spectra, FTIR, ESR and magnetic susceptibility measurements. Their characterization data indicated that their complexes had a square planar geometry. Their biological properties, regarding DNA binding properties were investigated using electronic absorption spectroscopy, fluorescence, and viscosity

measurements. The complexes were shown to intercalate **into** calf thymus DNA. Gel electrophoresis was used to study DNA cleavage efficacy of these complexes. Gel electrophoresis studies done in the presence of H_2O_2 and UV light showed that all the complexes to possessed nuclease activity. Their *in vitro* antibacterial activities against Gram-positive (*Bacillus subtilis*) and Gram-negative bacteria (*E. coli*, *Pseudomonas putida*, *K. pneumonia*.) indicated that the complexes have potent bactericidal activity compared to the free ligands [98].

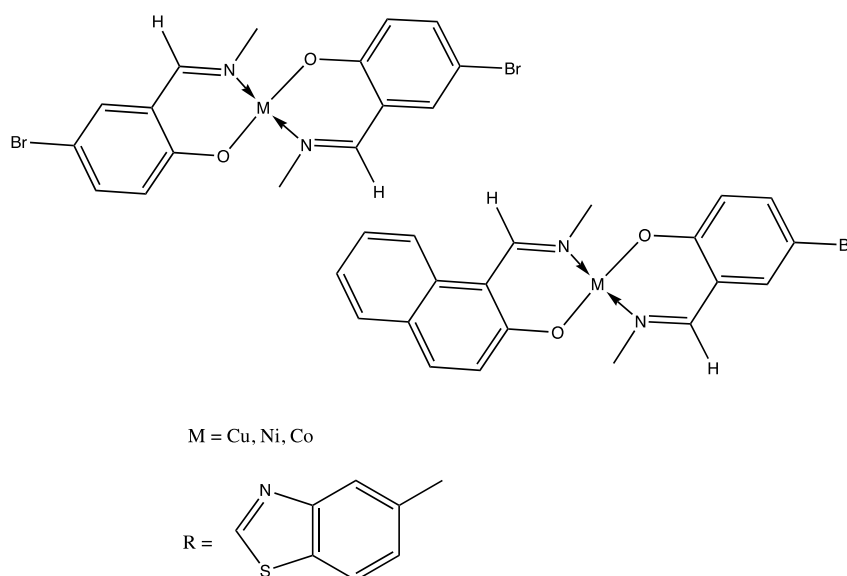


Figure 15: Square planar Complexes of Cu, Ni, and Co with DNA binding and antibacterial properties [98].

Miodragovic *et al* described a crystal structure of a new Co (III) complex shown in Figure 16. Cobalt was coordinated with the antiulcer drug famotidine and ethylenediamine. These scientists claimed at the time of their publication that this complex was the second described crystal structure with the ulcer drug famotidine [99]. They also noticed their complex to possess a different mode of complexation with famotidine as compared to other metal complexes. The drug is complexed as a tetradentate ligand using the nitrogen and sulfur donor atoms from guanidine at position N6, thiazole at position N4, thioether at position S2 and the terminal N3 atom. The other binding positions are the two NH_2 groups being N3H_2 and N6H_2 which are deprotonated to allow the drug to coordinate as a dianion. The asymmetric unit of this complex

includes a chloride anion and a water molecule to stabilize the stoichiometry of the complex. Furthermore, they realized that the complex structure had a lot of atoms which could be hydrogen acceptors or donors in hydrogen bonding. In that regard, their crystal structure revealed dominance of hydrogen bonding. The complex also displayed some interesting noncovalent bonds within the famotidine anion ($\text{CH}\cdots\pi$ and $\text{NH}\cdots\pi$). These were seen as stabilizing factors to the crystal structure. They also recognized stacked $\pi\cdots\pi$ interactions of neighboring complex cations [99]. Lastly, the complexes' antibacterial and antifungal properties together with the famotidine ligand were studied against *E. coli*, *S. aureus* and *Micrococcus lysodeikticus*. For the fungi, *Aspergillus niger* and *Candida albicans* were investigated and it was discovered that the coordinated famotidine cobalt complex had better activity as compared to the free famotidine ligand [99].

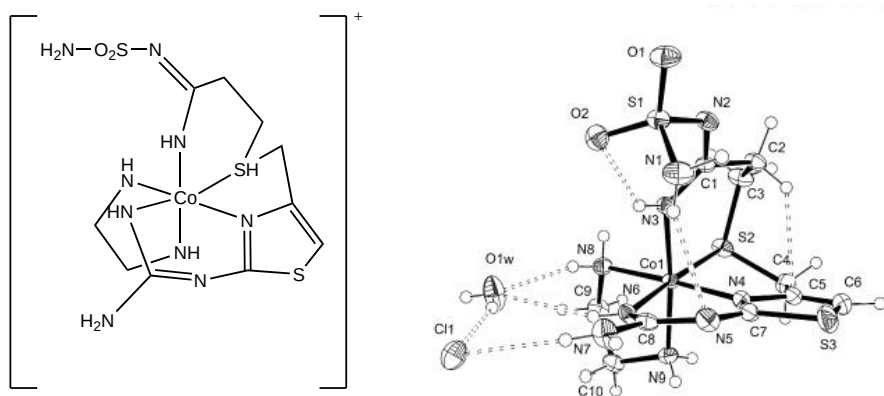


Figure 16: The Co (III) complex with the ulcer drug famotidine and its crystal structure molar ellipsoids showing hydrogen bonds [99].

Cohan *et al* synthesized cobalt complexes that showed some antibacterial properties against *E. coli*, *S. aureus*, and *P. aeruginosa*. The complexes were formed using a variety of benzothiazole-derived ligands. They prepared these by reacting 2-acetamidobenzaldehyde with the following: 2-amino-4-methoxy-, 2-amino-4-chloro-, 2-amino-6-nitro- and 2-amino-6-methylsulfonylbenzothiazole. These congeners were thus coordinated to cobalt to produce Co (II) complexes with octahedral geometry on the central metal atom while the ligands were observed to act as tridentate ligands [100].

1.0.3.5. Copper Compounds as Antimicrobial Agents

Copper is an essential trace metal in the human body, which helps in various bodily functions such as the production of red blood cells with iron, and optimised metabolic processes. This metal may exist in at least two oxidation states (I and II). However, copper mostly exists as Cu (II) in the human body. Copper can accept electrons, and this is vital in oxidative reduction processes in the disposal of free radicals from the body [101]. Copper is also a medically functional metal, and it has been applied in medicine in ancient times. There has always been folklore belief of copper jewellery helping against rheumatoid arthritis [102]. However, there are some examples where copper has shown some medicinal properties. An example of these are the copper complexes described by Nleonu *et al*, who synthesized ciprofloxacin complexes of cobalt, copper, iron and zinc (II) ions. After preparation the complexes were characterized by using physicochemical and spectroscopic studies. Furthermore, they examined the complexes for their antibacterial properties. Their antibacterial experiments showed better efficacy with the ciprofloxacin as complexes as compared to free ligands against *S. aureus* [103].

Khalil *et al* reported six derivatives of ciprofloxacin and their complexes of copper shown in Figure 17. The study elucidated the structures of these complexes using spectroscopic methods such as electronic spectra, FTIR, proton NMR, mass spectrometry, and Electron paramagnetic resonance (EPR). Furthermore, they examined these compounds for their antibacterial properties against Gram-negative and Gram-positive bacteria. The structural elucidation data revealed that the ligands were bound as bidentate ligands while the metal centres complexed using the pyridone carbonyl donor groups and through the carboxylate oxygen donor atoms. The ligand and complex crystal fields were thus assumed to be *tetragonally-distorted-octahedral* for all the complexes. The antibacterial properties of the Cu (II) complexes with the ciprofloxacin derivatives presented higher antibacterial properties towards both Gram-positive and Gram-negative bacterial organisms as compared to the free ciprofloxacin antibiotic drug. Interestingly, Khalil *et al* (2020) quantified the three-dimensional structure-activity relationship (3D-QSAR) by using 30 antibiotic conventional compounds of the quinolone class. Computational studies such as Density functional theory (DFT) calculations were used to understand the optimized geometrical structures using the hybrid functional B3LYP method and 6-311G(d,p) basis set. The 3D-QSAR

calculations showed eight optimum parameters that gave good predictive modulation ($R^2 = 0.996$, $F = 12.004$, $\sigma = 0.426$). Finally, *in silico* molecular docking was studied on the derivatives which showed that there were two types of interactions between the *E. coli* and the compounds, and these were hydrogen bonding and Van der Waals interactions, with also inhibition at the docked site [104].

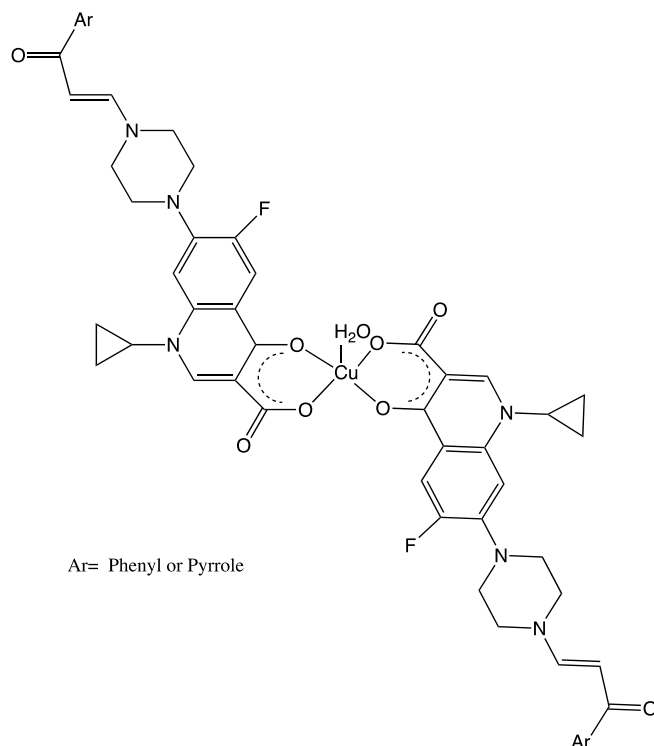
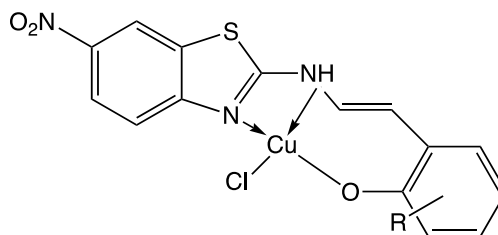


Figure 17: Copper antibacterial ciprofloxacin complex [104].

Kolate *et al* prepared Cu (II) complexes made from two hydrazone ligands, 2-[(6-Nitro-benzothiazole-2-yl)-hydrazonomethyl]-4,6-dichloro-phenol (BHD5) and 2-[(6-Nitro-benzothiazole-2-yl)-hydrazonomethyl]-4-chloro-phenol (BH5C) that are shown in Figure 18. They characterized their compounds using spectroscopic techniques, elemental analysis, proton NMR, mass spectrometry, molar conductance and thermogravimetry. They formed their copper complexes on a stoichiometry of 1:1 metal to ligand ratio. The ligand was observed to bond as a tridentate ligand towards the metal ion using the NNO donor atoms. Furthermore, through their spectroscopic characterization, they realized that the complexes have a tetrahedral geometry. The compounds were further investigated for potential antimicrobial properties towards Gram-

positive bacteria *Bacillus subtilis*, Gram-negative bacteria *Salmonella typhi* and two fungi, *Candida tropicalis* and *Kluyveromyces marxianus*. The studies revealed that the complexes had better antimicrobial activity as opposed to their free ligands. Furthermore, the DPPH antioxidant assay also showed that the copper complexes had much better biological activity as compared to the free ligand [105].



R = 3,5 Cl₂ and 5Cl

Figure 18: Antimicrobial copper complexes with hydrazone derivative ligands [105].

Mandal *et al* reported three mononuclear Cu (II) complexes of the formula CuL_n and one Ni (II) complex of the formula NiL_n. The ligands used were H₂L₁ = 3,3'-{(methylazanediy)-bis-[(propane-3,1-diyl)-azanylylidene-methanylylidene]}-bis-(4-bromophenol), H₂L₂ = 2,2'-[1,2-phenylene-bis-(azanylylidene-methanylylidene)]-bis-(4-bromophenol) and H₂L₃ = 3,3'-{(methylazanediy)-bis-[(propane-3,1-diyl)-azanylylidene-methanylylidene]}-bis-(4,6-dibromophenol). The molar displacement ellipsoids of the Cu (II) complexes together with the Ni (II) are shown in Figure 19. The complexes were prepared using the metal salts copper perchlorate for the complexes made with ligand 1 to 3 while nickel perchlorate with ligand 4. All these complexes were made on a stoichiometry of 1:1 of metal to ligand. Furthermore, the complexes were characterized using some physical and chemical methods such as SC-XRD. From these structural studies they were able to realize that the complexes of ligands 1, 3 and 4 had a distorted trigonal bipyramidal geometry. However, the complex of ligand 2 adopted a distorted square planar geometry. Assessments of these ligands and their corresponding Ni (II) and Cu (II) complexes for antibacterial properties against two Gram-positive bacteria *S. aureus* *B. subtilis* and two Gram-negative *P. aeruginosa* *E. coli* showed that the complexes had much better activity as compared to their free ligands. They also found that the complexes exhibited an increase in bactericidal

properties with an increase in dosage. Unfortunately, their data revealed that the complexes did not fare well with the conventional medicines, as these medicines had better activity than the new complexes [106].

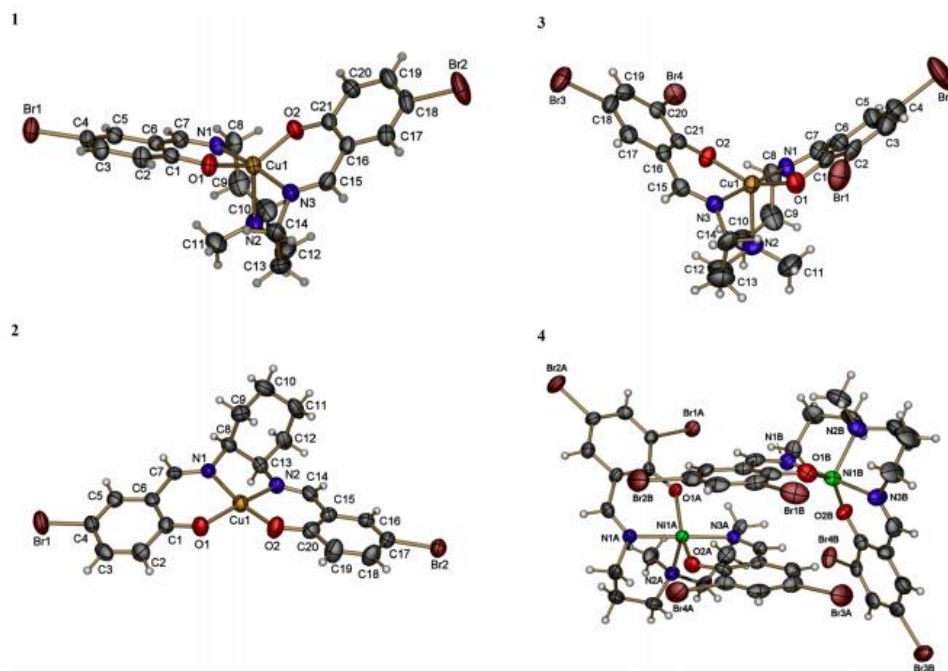


Figure 19: Displacement ellipsoids of Cu (II) complexes and Ni (II) complex with ligands 1-4 [106].

1.0.3.6. Nickel Compounds as Antimicrobial Agents

Nickel metal is an important transition metal that may exist in the +2 oxidation state in aqueous solutions [107]. This transition metal has been observed to possess some important biological properties. Nickel in the human body biologically interacts with biological iron to assist in oxygen transport in hemoglobin. Nickel also stimulates metabolic process in the body and is also an important cofactor in several plants and animal enzyme [108] in the metabolism of sugars but additionally in genetic code transmission [109]. Researchers have surveyed the idea of using nickel-based compounds as potential drugs for various diseases including infectious diseases. For example, Subramanian and Sakunthala (2013) prepared some new complexes of Cu (II), Zn (II), Ni (II) and Mn (II) with a new Schiff base ligand. The ligand was observed to be a tetradentate

ligand in its binding to the metal centres. Finally, they assayed the complexes for their antibacterial properties towards one Gram-positive and two Gram negative bacteria *S. aureus*, *K. pneumoniae* and *E. coli*. The conventional antibiotic streptomycin was used as a control standard while the ligand and the complexes were the test studies. From their data, the zones of inhibition of the synthesized complexes showed that they have higher activity than the free ligand and the antibiotic standard. They prepared the tetradentate Schiff base complexes by reacting the Schiff base ligand on the stoichiometry of metal salt in 2:1 mole ratio. The ligands were derived from 2-hydroxy-1-naphthaldehyde and 5-amino-1-naphthol. Finally, the ligand and the complexes of Cu (II), Zn (II), Ni (II) and Mn (II) ions were characterized by elemental analysis, FTIR and UV/Vis and conductivity measurements, which all showed their successful preparation [110].

Mondelli *et al* described the synthesis, SC-XRD structural analysis, voltametric analysis and the antibacterial activity of some nickel sulfonamide based complexes; $[\text{Ni}(\text{sulfisoxazole})_2(\text{H}_2\text{O})_4] \cdot 2\text{H}_2\text{O}$ and $[\text{Ni}(\text{sulfapyridine})_2]$ shown in Figure 20. SC-XRD analysis of $[\text{Ni}(\text{sulfisoxazole})_2(\text{H}_2\text{O})_4] \cdot 2\text{H}_2\text{O}$ showed that it crystallized in a monoclinic system with a space group of $C2/c$. This showed the central nickel ion to possess a slight distorted octahedral geometry. The nickel center complexed with two sulfisoxazole molecules using the donor nitrogen atoms, and the structure indicated four water molecule co-ligands to the sulfonamides ligands. However, the second complex $[\text{Ni}(\text{sulfapyridine})_2]$ crystallized in the orthorhombic Bravais lattice system with a space group $Pnab$. Interestingly, this other complex also had a distorted octahedral geometry on the nickel ion. The donor atoms that coordinated the metal center were the two-aryl amine nitrogen atoms from two sulfonamides ligands. The ligands bonded as multidentate ligands because of the monodentate moiety groups, the other two bidentate moieties from the four nitrogen atoms in the sulfonamide ligand and lastly the other two from the heterocyclic nitrogen atoms. Voltametric experiments from their study showed irreversible peaks at 1040 and 1070 mV which were attributed to Ni(II)/Ni(III) redox process. Finally, they tested the antibacterial properties of these two complexes. $[\text{Ni}(\text{sulfapyridine})_2]$ had less activity against *E. coli* and *S. aureus* than the free ligand. They assumed this to be due to the poor solubility of the complex and the strong covalent bonds which attribute to bigger molecular

weights that may affect permeability of the compound into the bacterial cell. While the other complex $[\text{Ni}(\text{sulfisoxazole})_2(\text{H}_2\text{O})_4] \cdot 2\text{H}_2\text{O}$ showed similar activity to its ligand sulfisoxazole against *S. aureus* and *E. coli* [111].

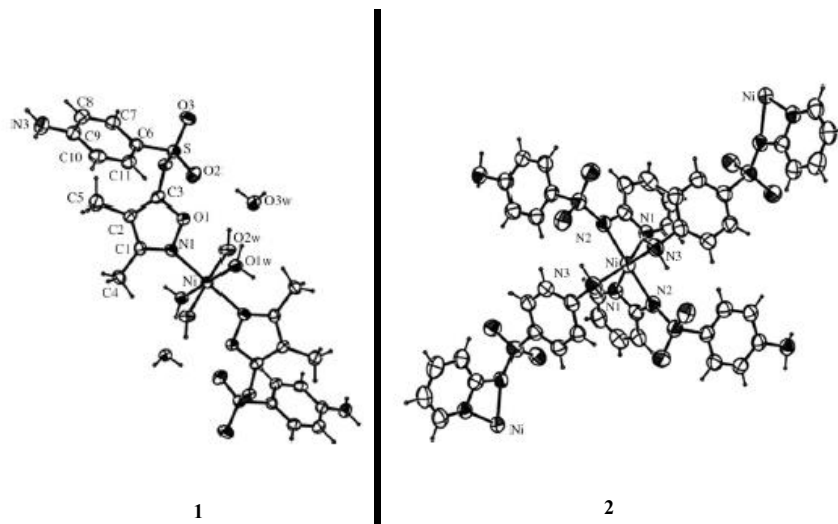


Figure 20: The molar ellipsoids of the antibacterial $[\text{Ni}(\text{sulfisoxazole})_2(\text{H}_2\text{O})_4] \cdot 2\text{H}_2\text{O}$ and $[\text{Ni}(\text{sulfapyridine})_2]$ [111].

Joseph *et al* prepared some antibacterial copper and nickel salicylidene thiosemicarbazone (SALTSC) and 5-bromosalicylidene thiosemicarbazone (5-Br SALTSC) Schiff based complexes. The Schiff base ligands were used to prepare the Cu (II) and Ni (II) complexes namely CuSALTSC, 5-BrCuSALTSC, NiSALTSC, and 5-BrNiSALTSC. Furthermore, their antibacterial activity against the Gram-positive *S. aureus* and the Gram-negative *E. coli* was tested. The 5-BrCuSALTSC was active against both the *S. aureus* and against *E. coli* bacteria at working concentrations of 100 mg/disk and 150 mg/disk respectively. The nickel complex 5-BrNiSALTSC was only active against *E. coli* at a working concentration of 150 mg/disk [112].

Three trinuclear Ni (II) complexes namely $[\text{Ni}_3(\text{abb})_3(\text{H}_2\text{O})_3(\mu\text{-ttc})](\text{ClO}_4)_3$, $[\text{Ni}_3(\text{tebb})_3(\text{H}_2\text{O})_3(\mu\text{-ttc})](\text{ClO}_4)_3 \cdot \text{H}_2\text{O}$, and $[\text{Ni}_3(\text{pmdien})_3(\mu\text{-ttc})](\text{ClO}_4)_3$ (where abb = 1-(1H-benzimidazol-2-yl)-N-(1H-benzimidazol-2-ylmethyl)methan-amine, ttcH₃ = trithiocyanuric acid, tebb = 2-[2-[2-(1H-benzimidazol-2-yl)ethylsulfanyl]ethyl]-1H-benzimidazole, and pmdien = N,N,N',N'',N''-

pentamethyldiethylenetriamine) were synthesized, characterized and examined for antibacterial and cytotoxic properties. The three complexes depicted as crystal structures in Figure 21 showed some antibacterial properties towards *S. aureus* and *E. coli*. However, there was better activity towards Gram-negative bacteria than the Gram-positive bacteria. The abb ligand together with its complex had the best antibacterial activity. Due to the $[\text{Ni}_3(\text{pmdien})_3(\mu\text{-ttc})](\text{ClO}_4)_3$ having better bioactivity, they further tested for its cytotoxic activity together with its ligand ttcNa_3 on the cancer cell lines G-361, HOS, K-562, and MCF7. They detected cytotoxic properties of this complex against the cancer cell lines. However, the ligand ttcNa_3 did not show any cytotoxic activity. They further studied the electrochemical redox behavior of the three trinuclear Ni (II) complexes which was studied using cyclic voltammetry, this study revealed that the nickel was in the +2-oxidation state for all the complexes and showed the presence of ttc [113].

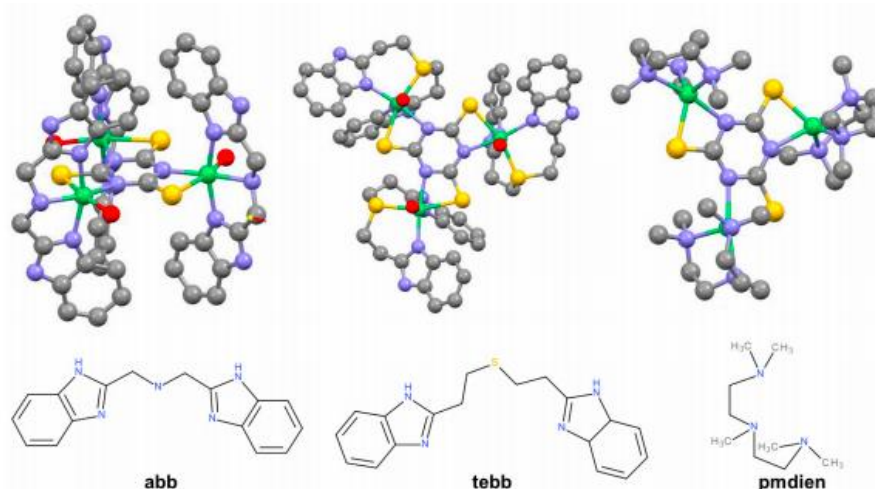


Figure 21: The trinuclear Ni (II) complexes $[\text{Ni}_3(\text{abb})_3(\text{H}_2\text{O})_3(\mu\text{-ttc})](\text{ClO}_4)_3$, $[\text{Ni}_3(\text{tebb})_3(\text{H}_2\text{O})_3(\mu\text{-ttc})](\text{ClO}_4)_3 \cdot \text{H}_2\text{O}$, and $[\text{Ni}_3(\text{pmdien})_3(\mu\text{-ttc})](\text{ClO}_4)_3$ [113].

Islam *et al* (2015) reported a simple yet potentially important nickel metal complex. The complex was synthesized using nickel chloride salt and pyridine lignd. This gave a complex of the stoichiometry 1:2 nickel to pyridine with the molecular formula $[\text{Ni}(\text{C}_5\text{H}_5\text{N})_2\text{Cl}_2]$. They characterized the complex using elemental analyses, electronic spectra, FTIR, proton NMR, carbon NMR spectra, magnetic susceptibility, and molar conductivity measurements. All the data showed the successful synthesis of this complex, with the conductivity measurements

indicating that the complex was a nonelectrolyte. Findings by Islam *et al* (2015) show the complex existed as either *cis* or *trans*, FTIR and carbon NMR showed this phenomenon as shown in Figure 22. Furthermore, the study tested both the pyridine ligand and Ni (II) complex for antimicrobial properties against *Salmonella typhi*, *Shigella dysenteriae*, *E. coli*, *Bacillus cereus*, and phytopathogenic fungi *Macrophomina phaseolina*, *Alternaria alternata*, *Fusarium equiseti*, *Colletotrichums corcolei*, and *Botryodiplodia theobromae*. The results for these assays proved that the complex had better activity towards the bacteria as opposed to the free pyridine ligand. The results also indicated more activity against *E. coli* by the complex. As for the fungi, the data displays that the complex inhibited of mycelial growth. Specifically, there was more activity of the ligand and the complex on the inhibition of growth of *Macrophomina phaseolina* as compared to the other fungi [114].

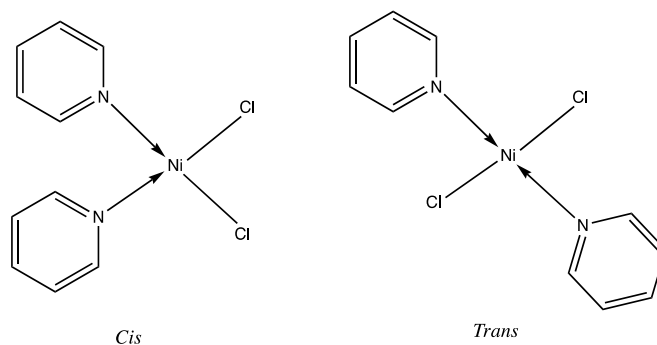


Figure 22: The Ni (II) complex existing as both cis and trans isomers [114].

1.0.3.7. Palladium Compounds as Antimicrobial Agents

Palladium has been known to have great potential in medicine because it belongs to the platinum group of metals, and it is a known antagonistic metal in the human body. It is generally a less toxic metal as seen through various animal model studies [115]. In that case, literature was surveyed to assess the extent of palladium use as a potential antimicrobial agent. New Pd (II) complexes with 2-pyrral amino acid Schiff ligands (see Figure 23) were synthesized and elucidated. Their antibacterial properties activity against six bacterial species being the Gram-positive *S. aureus*, MRSA, *Staphylococcus epidermidis* and *Streptococcus pyogenes* and the Gram-negative *P. aeruginosa* and *K. pneumoniae* using the disc diffusion assay and MIC method. The Pd (II) complexes had antibacterial properties against the bacteria with the 2-pyrral-l-histidine

palladium(II) complex and the 2-pyrrol-1-tryptophan palladium(II) complex displaying the best antibacterial properties. The compounds were all characterized using proton NMR, FTIR, Electronic spectra, elemental analysis and conductivity measurements [116].

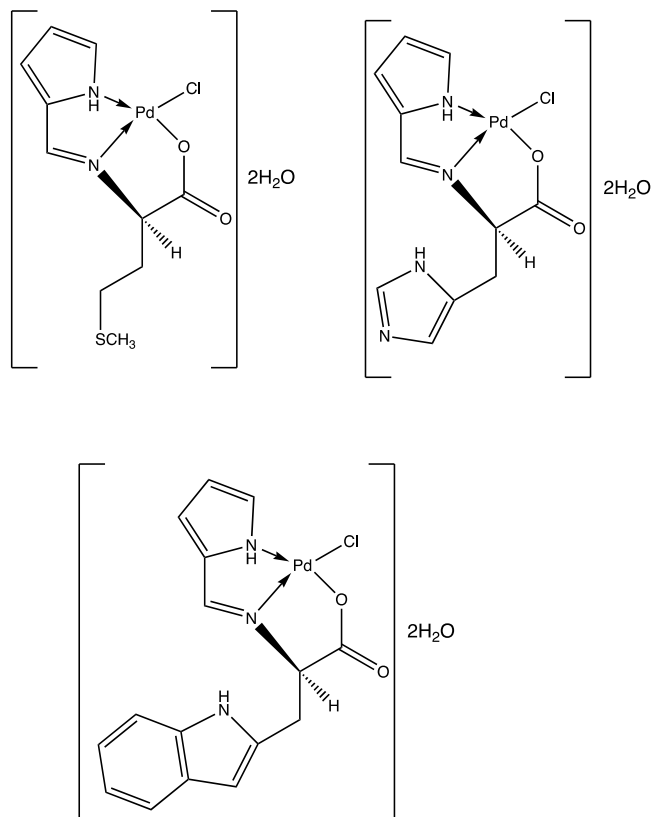


Figure 23: The antibacterial Pd (II) complexes with 2-pyrrol amino acid Schiff ligands [116].

Zalevskaya *et al* reported terpene-derived chiral palladium complexes and their antimicrobial properties against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *Acinetobacter baumannii*, *E. coli*, *Candida albicans*, and *Cryptococcus neoformans*. They discovered that all the palladium complexes had good antifungal activities. However, the palladacycles which have a palladium–carbon bond demonstrated activity towards the Gram-positive *S. aureus*. They discovered all the complexes to have poor activity towards the Gram-negative bacteria [117]. Three palladium complexes of the formulars $[\text{PdL}_4]2\text{Cl}$, PdL_2Cl_2 and $[\text{PdL}_4]2\text{Cl} \cdot 4\text{H}_2\text{O} \cdot \text{L}$ (see Figure 24) were synthesized and characterized by elemental analysis and spectral techniques. The ligand listed as L represented the imidazo[1,2- α]pyridine ligand. The other two complexes $[\text{PdL}_4]2\text{Cl}$ and $[\text{PdL}_4]2\text{Cl} \cdot 4\text{H}_2\text{O} \cdot \text{L}$ managed to form crystals and so were also characterized by SC-XRD. Cellular

viability using MTT reduction assay was done on three human ovarian tumour cells and three colorectal tumour cells. The complex PdL_2Cl_2 had moderate anticancer activity by demonstrating lower resistance against ovarian cancer cells as compared to cisplatin. PdL_2Cl_2 complex also displayed anticancer potential in all the colorectal tumour cells. However, the other complexes did not have any anticancer properties, but rather showed some antibacterial properties towards *S. aureus* [118].

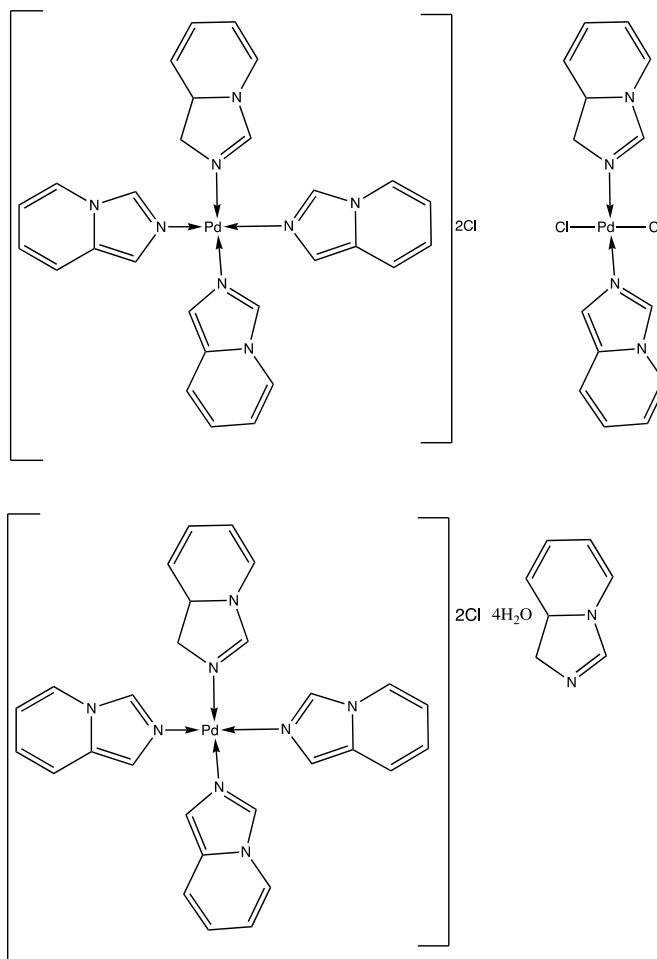


Figure 24: The anticancer and antibacterial three palladium complexes $[\text{PdL}_4]2\text{Cl}$, PdL_2Cl_2 and $[\text{PdL}_4]2\text{Cl} \cdot (4\text{H}_2\text{O}) \cdot \text{L}$ [118].

Another set of three Pd (II) complexes shown in Figure 25 were synthesized with the ofloxacin (OFL) drug, glycine and alanine amino acids (AA) as mixed chelate ligands. The compounds were characterized by elemental analysis, electronic spectra, molar conductivity, FTIR, proton and carbon and NMR, XRD, SEM, EDX and thermal analyses. The characterization experiments

showed that the molar ratio of the complexes was as follows 1:2 [Pd(OFL)₂]Cl₂ and 1:1:1 as [Pd(OFL)(AA)]Cl. Moreover, spectroscopic data indicated the OFL ligand as a bidentate ligand which coordinates using the nitrogen atoms of the piperazine ring. Finally, these complexes antibacterial activity was studied against *K. pneumoniae*, *E. coli*, *S. aureus*, and *Staphylococcus epidermidis*. They determined that their new complexes indicated better potential as compared to the standard drugs ceftriaxone or gentamycin against all the four micro-organisms. They attributed the antimicrobial properties of the palladium complexes to chelation. In principle, this means that the donor atoms in the ligands may be partially sharing the positive charges from metal. Thus, increasing the possible π -electron delocalization over the metal complex, which may increase the lipophilic character of the metal complex. This then favors the metal complexes permeation into the cell wall and cell membrane [119].

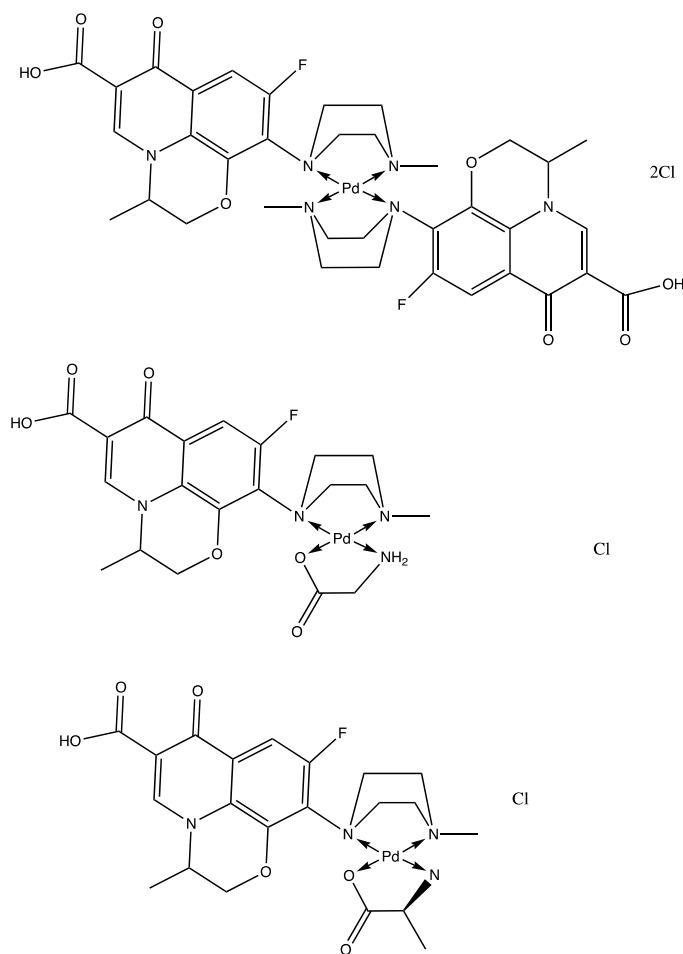


Figure 25: Palladium (II) complexes with ofloxacin (OFL) drug and glycine and alanine amino acids (AA) [119].

1.0.3.8. Rhodium Compounds as Antimicrobial Agents

Investigation of rhodium complexes in medicine is in its infancy, but due to rhodium belonging to the platinum group metals, there have been some studies on its potential medicinal benefits. An example of these properties is seen with research by Lapasam *et al* (2020) where they synthesized complexes of the ligands 4-phenyl-1-(pyridin-4yl)methylene thiosemicarbazide and 4-phenyl-1-(pyridin-4yl)ethylidene thiosemicarbazide. They coordinated these ligands to ruthenium, rhodium and iridium metals. The compounds were thus fully elucidated using FT-IR, electronic spectra, proton and carbon NMR, and elemental analysis. Additionally, they evaluated antibacterial properties of the complexes towards one Gram-positive bacteria *S. aureus* and two Gram-negative bacteria *E. coli* and *K. pneumoniae*. The data indicated that Cp* rhodium complexes had good antibacterial properties for all the three bacterial strains. However, the other complexes of Cp* with iridium and *p*-cymene ruthenium displayed moderated activity towards the three bacterial strains. Unfortunately, the complexes had a lower activity compared to the conventional reference drugs [120].

Another group of nine arene metal precursor complexes (see Figure 26) were coordinated to thiosemicarbazone ligands by Shadap *et al*. These complexes displayed antibacterial properties towards Gram-positive *S. aureus* and gram-negative *E. coli* and *K. pneumoniae*. The scientists realized that the thiosemicarbazone ligands increased the potency of the complexes towards all the bacterial strains. The activity indicated that some of their rhodium complexes displayed better activity than the other metal complexes. The synthesized complexes included some cationic mono- and binuclear complexes with the three nitrogen and sulfur bonding heteroatoms. Their ruthenium complexes demonstrated mononuclear bidentate complexes, while the rhodium and iridium complexes possessed dinuclear bridging coordination through the sulfur and imine nitrogen atoms. They characterized all these complexes using spectroscopic techniques [121].

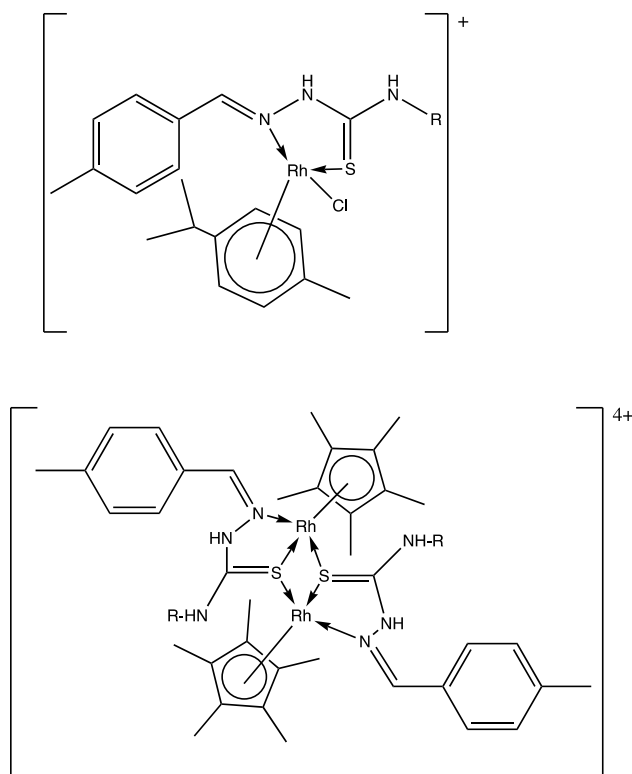


Figure 26: Examples of the antibacterial thiosemicarbazone metal complexes with rhodium central metal [121].

Yang *et al* elucidated a rhodium complex which interfered with iron-transport **in** the bacterial species *S. pneumoniae*. The complex was prepared as a dirhodium (II) complex with the formula Rh_2Ac_4 shown in Figure 27. The rhodium complex showed potent antibacterial properties towards *S. pneumoniae* with little cytotoxicity towards the host cells *in vitro*. They studied the antibacterial mode of action of this complex using Isobaric tags for relative and absolute quantitation (iTRAQ-based proteomics) together with cellular and biochemical assays. The bioassays indicated that Rh_2Ac_4 affected the following in the bacteria: six molecular functional groups, metal ion binding and twelve metabolic pathways. They also realized that this complex decreased expression levels of proteins associated with haem metabolism. Additionally, the assays showed that Rh_2Ac_4 was probably taken up by *S. pneumoniae* through the PiuABCD haem uptake system, because the rhodium complex is structurally like the iron haem complex. Therefore, it may as well be competing with iron haem complex thus decreasing iron

uptake through the PiuABCD haem uptake system. Thus, they concluded this as the property of the complex that brings about antibacterial activity towards *S. pneumoniae* [122].

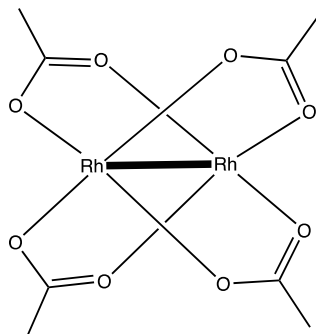


Figure 27: The antibacterial Rh_2Ac_4 complex that interferes with iron uptake [122].

Although platinum, ruthenium, and palladium compounds have been extensively studied and utilized in chemotherapy, this work explores the potential of alternative transition metal complexes, specifically cobalt, nickel, and copper, as antibacterial agents. While the aforementioned metals have shown promise in medicinal applications, the use of cobalt, nickel, and copper complexes in antibacterial research offers distinct advantages such as their unique electronic and structural properties, potential for novel ligand design, and potential cost-effectiveness. By investigating the antibacterial activity of these alternative transition metal complexes against *S. aureus* and *K. pneumoniae*, including their drug-resistant strains, this study aims to contribute to the expanding repertoire of antibacterial agents and provide insights into the therapeutic potential of these less explored metal complexes.

1.0.4. Problem Statement

Bacteria have been gaining resistance to conventional antibiotics over the years. The world health organization (WHO) through reviewing a lot of peer reviewed researches around the world has observed drug resistant bacteria to be a growing public health issue. WHO cites that there has been no new antibiotics developed for over 25 years while antibiotic resistance **in bacteria** is still growing. In that regard, this work seeks to employ synthetic medicinal chemistry and drug discovery to develop a new class of metal based antibacterial drugs. This research seeks to design

and synthesize various metal complexes to earmark specific or multiple targets within the bacterial species MDR-*K. pneumoniae* and MR-*S. aureus*.

1.0.5. Aims and Objectives

Aim

The aim of this work was to synthesize, characterize mixed ligand complexes of Co, Ni, and Cu then evaluate their biological properties against drug-resistant bacterial species and their non-drug-resistant bacterial counterparts.

Objectives

1. To synthesize and characterize 4-pyridyl-methyl-4-nitrobenzoate (**L1**), 4-[(pyridin-4-yl)methoxy]aniline (**L2**), 4-pyridyl-methyl-4-aminobenzoate (**L3**), and 4-((4-nitrophenoxy)methyl) pyridine (**L4**) ligands using Proton Nuclear Magnetic Resonance Spectroscopy (^1H -NMR), Electrospray Ionization- Mass Spectrometry (ESI-MS), Fourier Transform Infrared Spectroscopy (FTIR), and Elemental Analysis (EA).
2. To synthesize and characterize complexes of cobalt, nickel, and copper, with **L1**, **L2**, **L3**, **L4** and **4PY**, using Electrospray Ionization- Mass Spectrometry (ESI-MS), Fourier Transform Infrared Spectroscopy (FTIR), and Elemental Analysis (EA).
3. To characterize and study the physicochemical properties of the complexes using UV/Vis, magnetic susceptibility studies, SC-XRD, TGA and DSC.
4. To screen the compounds against prokaryotic cells; *K. pneumoniae*, *S. aureus*, MDR-*K. pneumoniae* and MRSA using MIC assay and Time Kill assay.
5. To study the possible mode of action of the active compounds by studying; cell wall/membrane damage using the Alkaline phosphatase (AKP) activity, and folic acid (FA) biosynthesis inhibition using FA ELISA assay.
6. To study the biomolecular binding capabilities of the most active compounds using molecular docking studies.

Methods

2.1. Experimental Section

This chapter focuses on the methods of synthesis of the ligands and their corresponding metal complexes, their characterization, and solid and solution state chemistry studies. Furthermore, this chapter shows the methods used to evaluate the biological studies of the synthesized compounds against non-drug resistant and resistant bacterial species.

2.0.1. Materials and Instruments

2.0.1.1. Chemicals

Chemical Name and Purity	Supplier
Methanol AR	Skylabs
Potassium Carbonate AR	Advanced Technocry INC
p-nitrobenzoic acid 98%	Fluka Chemica
p-nitrophenol 98%	Sigma-Aldrich
Dichloromethane AR	Rochelle Chemicals
p-aminobenzoic acid 98%	Skylabs
Sodium hydroxide AR	Skylabs
Dimethylsulfoxide	Sigma-Aldrich
Acetonitrile LC/MS Grade	Riedel-de Haen
Ethanol 96%	Skylabs
Copper (II)chloride dihydrate	Skylabs
4-(chloromethyl)pyridine hydrochloride	Sigma-Aldrich
p-aminophenol	Sigma-Aldrich
Cobalt (II) chloride hexahydrate 99.9%	Sigma-Aldrich
Nickel (II) chloride hexahydrate 99.9%	Sigma-Aldrich
Dimethyl formamide CP	Skylabs

2.0.1.2. Instruments

Nuclear Magnetic Resonance Spectroscopy

¹H-NMR and spectra were acquired in 5 mm NMR tubes at 298 or 310 K by an Agilent 600 MHz spectrometer (manufacturer: Agilent Technologies USA) in the range 0-12 ppm. Solvents used were deuterated chloroform with TMS as the reference material. All data processing was carried out using MestReNova-9.0.1-13254.

Fourier Transform Infra-Red Spectroscopy

The neat film Infrared spectroscopy of the ligand intermediate, ligands and the complexes were recorded in the 4000-500nm and 500-200nm region using PerkinElmer System 2000FTIR spectrometer (manufacturer: Perkin Elmer USA)

Magnetic susceptibility

The magnetic susceptibility data was obtained using the Gouy method and the parameters specified in the discussion (Baddar, Hilal and Sugden 1949).

Ultra-Violet/Visible Spectroscopy

UV-Visible absorption spectra were recorded in one centimeter path length quartz cells at room temperature, using a Shimadzu UV-31-1 PC scanning spectrophotometer (manufacturer: Shimadzu Corp Japan)

High-resolution Electro-Spray Ionisation-Mass Spectrometry Acquisition parameters

A 10 µL of the sample was injected into the Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Dionex, Sunnyvale, California, USA) and run through a loop for one minute at 50% Solvent A consisting of 0.1 % formic acid in H₂O (v/v) and 50% solvent B consisting of 0.1 % formic acid in Acetonitrile (v/v) at a flow rate of 0.3 mL/min. Mass spectra were recorded on a Bruker Compact Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The data was processed on MestReNova version 9.0.1-13254.

Elemental Analyzer

Elemental analyses (CHNO) were performed with a model 240 PerkinElmer elemental analyser (PerkinElmer, USA).

Single Crystal XRD

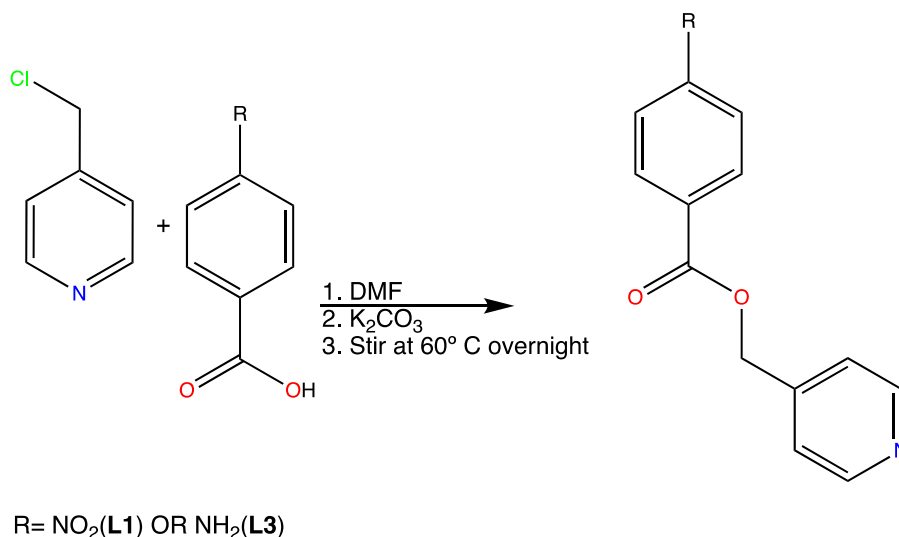
Suitable crystals were selected and placed on a needle and placed on the goniometer of a Bruker D8-Quest CCD diffractometer (Bruker, USA). The crystals were kept at room temperature during data collection. The structures were solved by using the SHELXT structure solution program using Intrinsic phasing and refined with the SHELXL refinement package using Least Squares minimization on the Bruker APEX 3 software and visualization was done using the CSD Mercury software.

Thermal Instruments

Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) were carried out on a simultaneous thermal analyzer NETZSCH STA 449 F3 Jupiter® (NETZSCH, Germany). Their data was obtained in the temperature range of 30 to 800 °C at a heating rate of 10 °C/min under nitrogen gas.

2.0.2. Synthesis of the Ligands and their Corresponding Metal Complexes

2.0.2.1. Synthesis of pyridin-4-ylmethyl 4-nitrobenzoate and pyridin-4-ylmethyl 4-aminobenzoate



Scheme 1: Proposed route for the synthesis of the ligands L1 and L3.

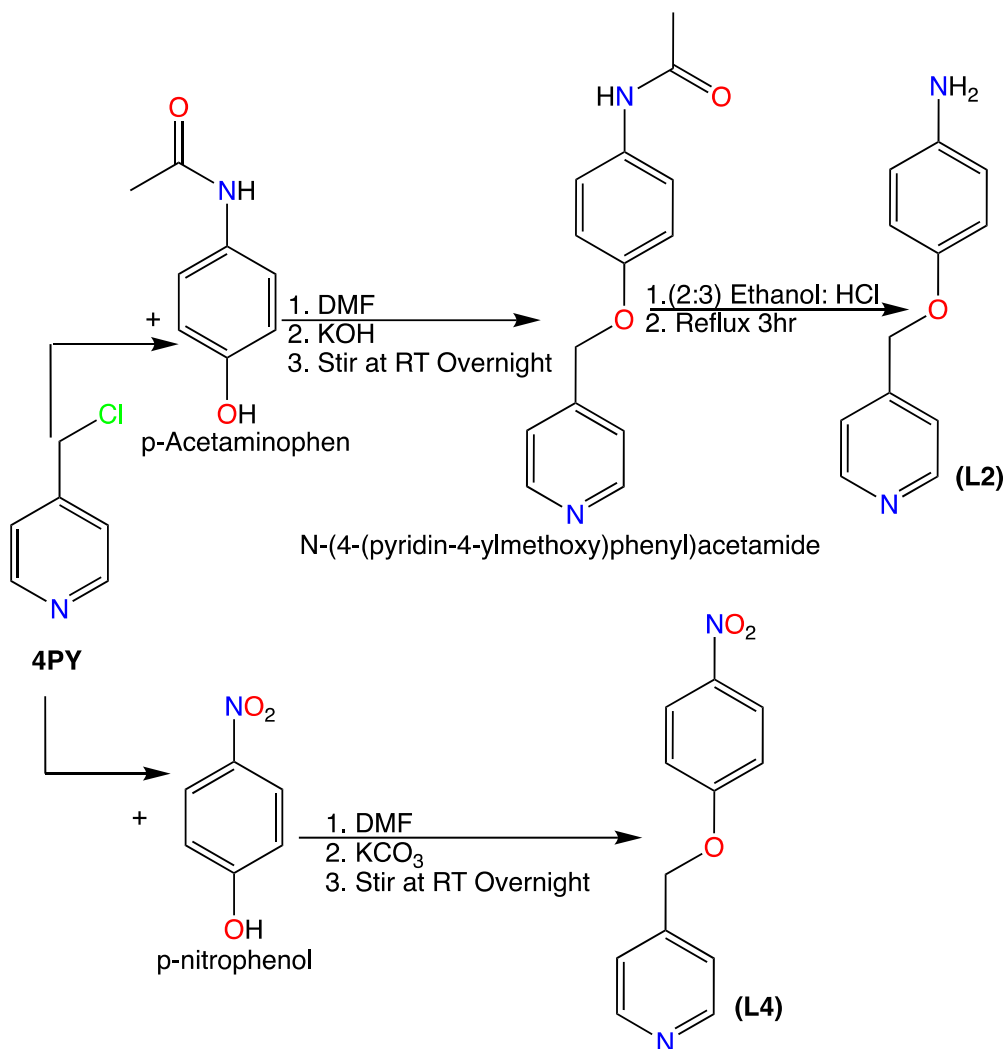
2.0.2.1 (a) Synthesis of pyridin-4-ylmethyl 4-nitrobenzoate (L1)

4-nitrobenzoic acid (2.80 g, 16.8 mmol) was mixed with 25 mL of dimethyl formamide. Potassium carbonate (2.40 g, 17.4 mmol) was then added to the resulting mixture before refluxing, and the mixture was stirred for 30 min with a dry calcium chloride drying tube connected to the reaction vessel. The resulting white precipitate was then mixed with 4-chloromethyl pyridine (3.00 g, 18.3 mmol) dissolved in 25 mL dimethylformamide, quickly covered with a dry calcium chloride drying tube. The mixture was then refluxed overnight (24 hr) at 70 °C. Afterwards, the maroon precipitate obtained was cooled and neutralized with 10 mL of 10% sodium hydrogen carbonate, 40 mL distilled water and filtered under suction. It was washed again with 30 mL of cold distilled water, then the off-white solid obtained was air dried before being taken for analysis. The product was then recrystallized by dissolving in minimal methanol and golden rhombohedral crystals were obtained. (4.30 g, Yield 91%)

2.0.2.1 (b) Synthesis of pyridin-4-ylmethyl 4-aminobenzoate (L3)

4-aminobenzoic acid (2.80 g, 12.3 mmol) was dissolved in 25 ml of dimethylformamide and then mixed with potassium carbonate (1.70 g, 12.3 mmol). The mixture was refluxed and stirred for 30 minutes with an anhydrous calcium chloride head connected to it. The resulting white precipitate formed was then mixed with 4-picolyl chloride hydrochloride (2.00 g, 12.3 mmol) dissolved in 25 ml dimethylformamide and then quickly covered again with a calcium chloride drying tube. The contents were then heated overnight at 70 °C. The maroon precipitate formed was cooled followed by neutralization with 2 ml of 10 % sodium hydrogen carbonate. 35 ml of distilled water was added, then the product was extracted three times with 50 ml portions of dichloromethane, thereafter the golden organic layer was then air dried. Suitable golden crystals were obtained after the solvent had evaporated and were analyzed. (4.60 g, Yield 96%).

2.0.2.2. Synthesis of the ligands 4-(pyridin-4-ylmethoxy) aniline and 4-((4-nitrophenoxy) methyl) pyridine



Scheme 2: Proposed preparation route for the ligands L2 and L4.

2.0.2.2 (a) Synthesis of 4-(pyridin-4-ylmethoxy)aniline (L2)

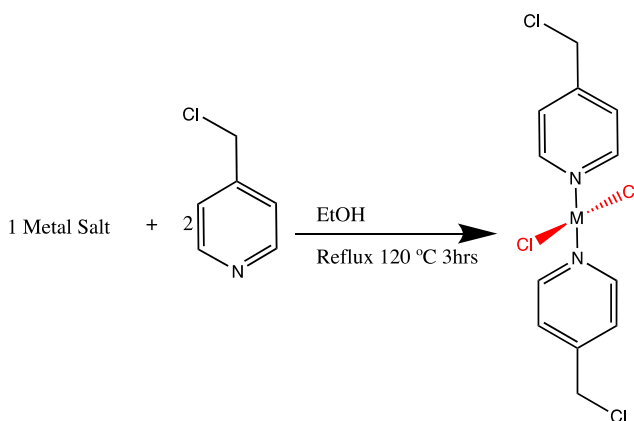
p-Acetaminophen (2.00 g, 13.2 mmol), potassium hydroxide (1.86 g, 33.1 mmol) and dimethylformamide, DMF (20 ml) were charged into a 250 ml round bottom flask, thereafter 4-chloromethylpyridine hydrochloride (2.17 g, 13.2 mmol) dissolved in 10 ml DMF was added. The reaction mixture was stirred at room temperature for 4 hr. After this, water was added slowly to the mixture and the resulting white crystalline material was then filtered. To acquire the amine group, the acetamide moiety was hydrolyzed. The hydrolysis was done in 20 mL ethanol and 30

mL concentrated hydrochloric acid. The refluxing was maintained for 3 hr after which the reaction mixture was allowed to cool and neutralized to pH 7 using sodium hydrogen carbonate and the reaction mass was allowed to stand overnight to give the title compound which was filtered and weighed (2.00 g, yield 76.4%).

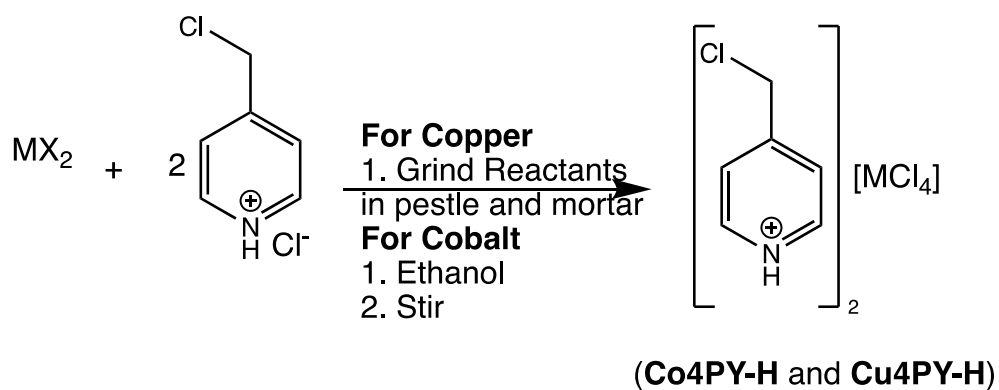
2.0.2.1 (b) Synthesis of 4-((4-nitrophenoxy) methyl)pyridine (L4)

4-nitrophenol (1.50 g, 10.7 mmol), potassium carbonate (5.60 g, 40.5 mmol) and dimethylformamide (20 ml) were charged into a 250 ml round bottom flask, thereafter 4-chloromethylpyridine hydrochloride dissolved (1.77 g, 10.7 mmol) in 10 ml DMF was added. The reaction proceeded at room temperature for 4 hr. After this, water was added to the mixture and white crystalline precipitate formed. The crystalline material was then filtered to acquire the compound. (2.00 g, 81 %).

2.0.2.3. Synthesis of the Metal complexes of Complexes with 4-chloromethyl pyridine

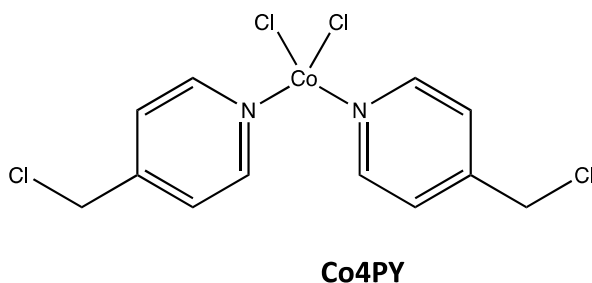


Scheme 3: Synthesis of various complexes with 4-chloromethylpyridine, the red bonds indicate that some complexes have a 4-coordinate geometry.



Scheme 4: Synthesis of the ionic compounds **Cu4PY-H** and **Co4PY-H**

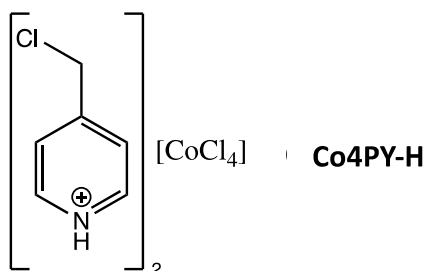
2.0.2.3 (a) Synthesis of dichloro-bis-4-picolyl cobalt (II)



Structure 1: Chemical structure of dichloro-bis-4-picolyl cobalt (II) (**Co4PY**)

A blue solution of cobalt (II) chloride hexahydrate (1.87 g, 0.00784 mol) dissolved in 10 ml of methanol was mixed with 4-chloromethylpyridine solution (2.00 g, 0.0157 mol) dissolved in 10 ml of methanol. The color of the mixture remained blue. The mixture was then stirred and refluxed for about 2 hr. After refluxing, the mixture was gently heated to reduce the solvent and as the solvent decreased, a blue powder started to form. After cooling the mixture to room temperature, the blue powdered solid was removed by filtration under vacuum, and it immediately turned pink as the solvent dried. The powder was further washed with dichloromethane until a colorless filtrate was observed. The filtrate was kept in a cold room and the crystals started to form. The compound obtained was blue crystals which immediately turned purplish-pink, and a suitable quality crystal was chosen for single-crystal X-ray diffraction studies (2.29 g, yield 76.3%).

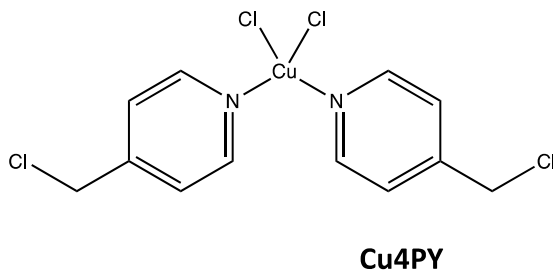
2.0.2.3 (b) Synthesis of 4-chloromethylpyridinium tetrachlorocobaltate (II)



Structure 2: Chemical structure of 4-chloromethylpyridinium tetrachlorocobaltate (II) (**Co4PY-H**)

4-chloromethyl pyridine hydrochloride (0.50 g, 0.0030 mol) was dissolved in 10 mL of methanol in a 50 mL round bottom flask and a yellow solution was formed. Then (0.36 g, 0.0015 mol) of cobalt (II) chloride hexahydrate was also dissolved in 3 mL of methanol in a 25 mL flask forming a blue solution. The solutions were thus mixed with continuous stirring and the color of the mixture became deep blue. The reaction mixture was further stirred for 3 hr at room temperature. After complete stirring the mixture was left in a fume hood for slow evaporation of the solvent. As the solvent evaporated a powder was formed which was filtered and washed with 30 mL methanol. The filtrate was stored in a cold room for crystallization. The compound obtained was bright-blue crystals of suitable quality for single-crystal X-ray diffraction studies (0.42 g, yield 61.2%).

2.0.2.3 (c) Synthesis of dichlorobis-4-picolyl copper (II)

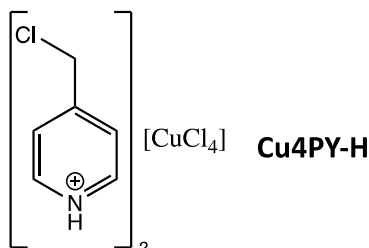


Structure 3: Chemical structure of dichloro-bis-4-picolyl copper (II) (**Cu4PY**)

The complex was prepared by the reaction of copper (II) chloride dihydrate (1.34 g, 0.00784 mol) dissolved in 3 mL of ethanol with 4-chloromethylpyridine solution (2.00 g, 0.0157 mol) in dichloromethane. The mixture immediately formed a blue precipitate. The mixture was left to cool under room temperature and after cooling the light blue powder was removed by filtration

under vacuum and washed with ethanol until a colorless filtrate was observed. The blue precipitate was recrystallized with distilled water to form blue needle like crystals of suitable quality for single-crystal XRD analysis (2.42 g, yield 79.4%).

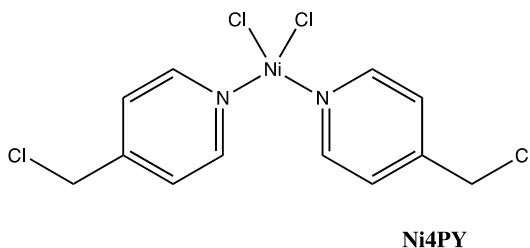
2.0.2.3 (d) Synthesis of 4-chloromethylpyridinium tetrachlorocuprate (II)



Structure 4: Chemical structure of 4-chloromethylpyridinium tetrachlorocuprate (II) (**Cu4PY-H**)

Copper (II) chloride (0.20 g, 0.0012 mol) and 4-chloromethylpyridine hydrochloride (0.38 g, 0.0023 mol) were ground in a pestle and mortar until a uniform yellow colored powder was obtained. Thereafter, the compound was dissolved in methanol to give a yellow solution which was left overnight. The solution formed bright-yellow crystals of suitable quality for single-crystal X-ray diffraction studies (0.37 g, yield 67.5%).

2.0.2.3 (e) Synthesis of dichloro-bis-4-picolylnickel (II)



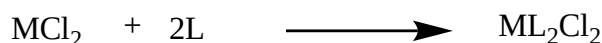
Structure 5: Chemical structure of dichloro-bis-4-picolylnickel (II) (**Ni4PY**)

Nickel (II) chloride hexahydrate (1.87 g, 0.00784 mol) was dissolved in 10 mL ethanol. The solution was greenish in color and to this solution (2.00 g, 0.0157 mol) 4-chloromethylpyridine solution was added drop wise with continuous stirring. The color of the mixture remained green, and the reaction mixture was further stirred and refluxed for 4 hr. The reaction mixture was then stirred for 24 hr under room temperature to ensure the complete formation of the metal complex. During this period a light green colored precipitate started to form. Then the light yellow colored powdered complex was separated by vacuum filtration. After filtration the

complex was washed with ethanol. The complex was left to dry in a fume hood. Attempts to form crystals for single-crystal X-ray diffraction studies yielded no proper crystals for such analysis. (1.41 g, yield 47.1%).

2.0.2.4. Green Synthesis of Metal Complexes with the Ether and Ester Ligands

A mole ratio of 1 metal salt: 2 ligand was used to produce metal (II) complexes using a mortar and pestle, the contents were ground for around 15-60 minutes.

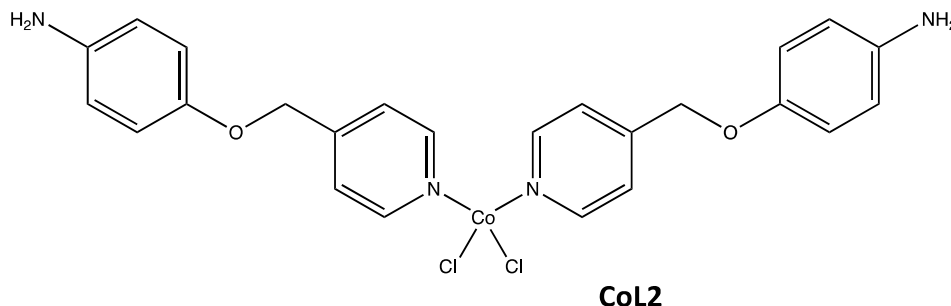


M= Co, Ni, Cu

L= **L1**, **L2**, **L3** and **L4**

Scheme 5: General preparation of metal (II) complexes with **L1** - **L4**

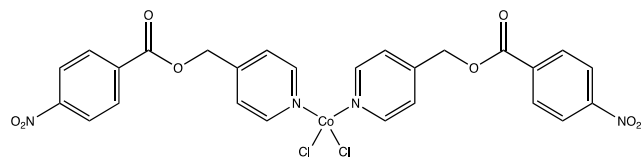
2.1.2.4 (a) Synthesis of dichoro-bis-(4-[(pyridin-4-yl)methoxy]aniline) cobalt(II)



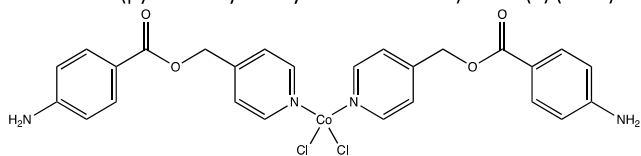
Structure 6: Chemical structure of dichoro-bis-(4-[(pyridin-4-yl)methoxy]aniline) cobalt(II) (**CoL2**)

(0.40 g, 2 mmol) of 4-[(pyridin-4-yl)methoxy]aniline and 1 mmol (0.24 g, 1 mmol) of cobalt(II) chloride hexahydrate were weighed into agate mortar which has been washed and dried before use. The reactants were ground together for 30 minutes to ensure complete reaction between the metal salt and the ligand to afford a blue solid product that was characterized as **CoL2** (0.43 g, yield, 80.2%)

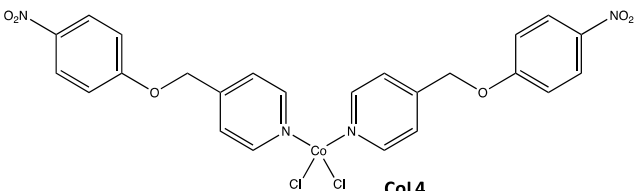
The other complexes were synthesized using a similar mechanochemical method as that of CoL2. The structures of these complexes are provided **in Structure 7**. Detailed information regarding their physical properties, characterization, and biological application can be found in Chapter 3.



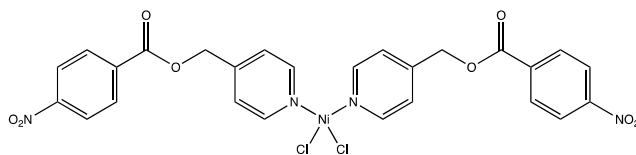
Col1
dichloro-bis-(pyridine-4-ylmethyl-4nitrobenzoate)cobalt (II) (**Col1**)



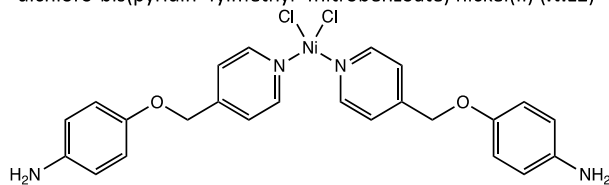
Col3
dichloro-bis(pyridine-4ylmethyl-4aminobenzoate) cobalt (II) (**Col3**).



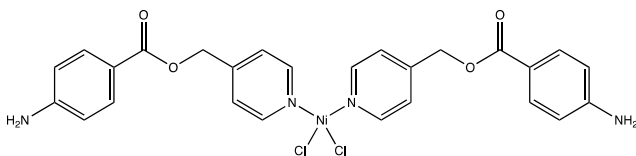
Col4
dichoro-bis-4-((4-nitrophenoxy)methyl)pyridine cobalt(II) (**Col4**)



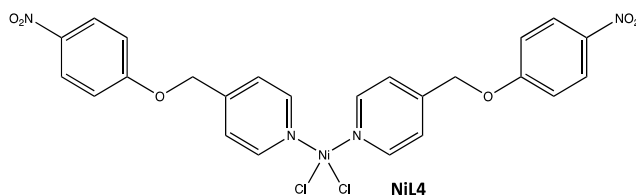
Nil1
dichloro-bis(pyridine-4ylmethyl-4nitrobenzoate) nickel(II) (**Nil1**)



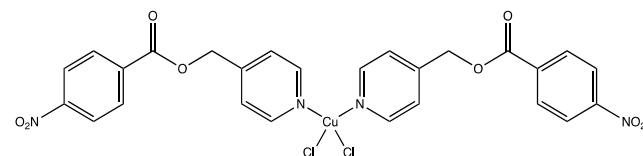
Nil2
dichloro-bis-4-[(pyridine-4-yl)methoxy]aniline nickel(II) (**Nil2**)



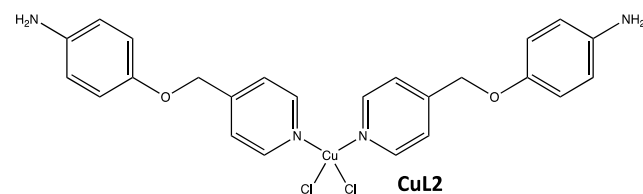
Nil3
dichloro-bis(pyridine-4ylmethyl-4aminobenzoate) nickel (II) (**Nil3**)



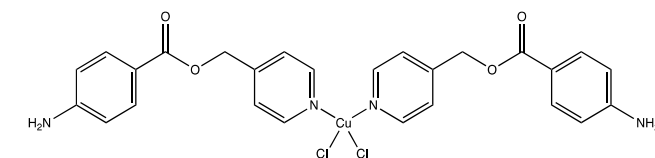
Nil4
dichoro-bis-4-((4-nitrophenoxy)methyl)pyridine nickel (II) (**Nil4**)



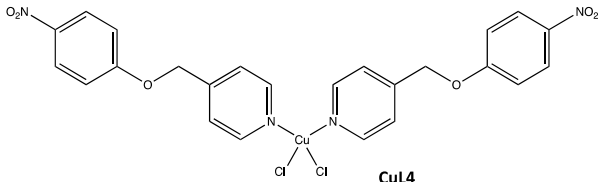
Cul1
dichloro-bis(pyridine-4ylmethyl-4nitrobenzoate) copper(II) (**Cul1**)



Cul2
dichoro-bis-4-[(pyridine-4-yl)methoxy]aniline copper(II) (**Cul2**)



Cul3
dichloro-bis(pyridine-4yl-methyl-4aminobenzoate) copper (II) (**Cul3**)



Cul4
dichloro-bis-4-((4-nitrophenoxy)methyl)pyridine copper (II) (**Cul4**)

Structure 7: The synthesized cobalt, nickel and copper complexes with the ligands.

2.0.3. Bioactivity studies

2.0.3.1. Minimum Inhibition Concentration Assay Screening

The *in vitro* antibacterial activity of the compounds was tested by the broth microdilution method against the bacterial strains *K. pneumoneiae*, MDR-*K. pneumoniae*, *S. aureus*, and MRSA. Eight concentrations of each of the test compounds were serially diluted: (0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625) mM. Bacterial inocula, with an incubation time not over 6 h, were adjusted to the 0.5 McFarland standard and further diluted down to 5×10^5 CFU/mL using Mueller Hinton Broth (MHB). For the determination of the MIC, MHB was used for serial dilutions to make up a final volume of 100 μ l in 96-well plates, and 100 μ l of bacterial solution was added to each solution. The bioassays experiment was analyzed in triplicate using a 96 well microplate reader. Then finally the assay was analyzed, graphed, and statistically evaluated using GraphPad prism.

Equation 1: Normalizing the bacterial growth experiment

$$percentage\ viability = \left(\frac{OD\ treatment}{OD\ compound} \right) \times 100$$

Where:

OD treatment = The absorbance of the growth of treated bacteria

OD compound = The absorbance of the free compounds

2.0.3.2. Time Kill Kinetics Assay

Following a previously used procedure with slight modifications [123], the bacteria with the best performing compounds (those with MIC 0.3 mM) were evaluated *in vitro* against the bacterial species *K. pneumoniae*, MDR-*K. pneumoniae*, *S. aureus*, and MRSA. In brief, the time kill experiment was carried out utilizing the compounds at their MIC and optical density (OD) measurements to determine if they were bacteriostatic or bactericidal. The bacterial isolates were grown to McFarlan standard. Thereafter the broth cultures were diluted to 5×10^5 CFU/ml using nutrient broth. The compounds were dissolved and loaded in a microplate with nutrient

broth. The diluted bacterial isolates were added to the wells and incubated at 37 °C. Then the plates were run on a plate reader at time intervals of 0, 1, 2, 3, 4, 5, 6 and 24 hr. A control test was performed for the organisms without the compounds. The procedure was performed in triplicate and a graph of the $\log_{10}$ %viability at the active MIC was plotted against time using Graphpad Prism.

2.0.3.3. Determination of Folic Acid Biosynthesis Inhibition through ELISA Assay

The determination of the inhibition of folic acid biosynthesis by the active compounds was studied by an Enzyme Linked Immunosorbent Assay (ELISA) (G-Bioscience, IT7955). following with amendment a previously described method [124]. Briefly, the bacterial isolates were grown to McFarlan standard and the cultures were diluted to 5×10^5 CFU/ml using nutrient broth (NA). Thereafter, 250 μ L of 2XMIC of the compounds was loaded in 2 ml sample tubes containing 250 μ L of inoculum in NA, therefore making 1XMIC of the test compounds. The treated bacterial isolates together with the vehicle control were incubated at 37 °C for 24 hr. Thereafter, the analysis of the extracellular folates was done following the manufacturer's protocol. The procedure was performed in duplicate and the data was treated using Microsoft Excel and Graphpad Prism. The concentrations in pg/ml were interpolated from the FA standard curve in Figure 28.

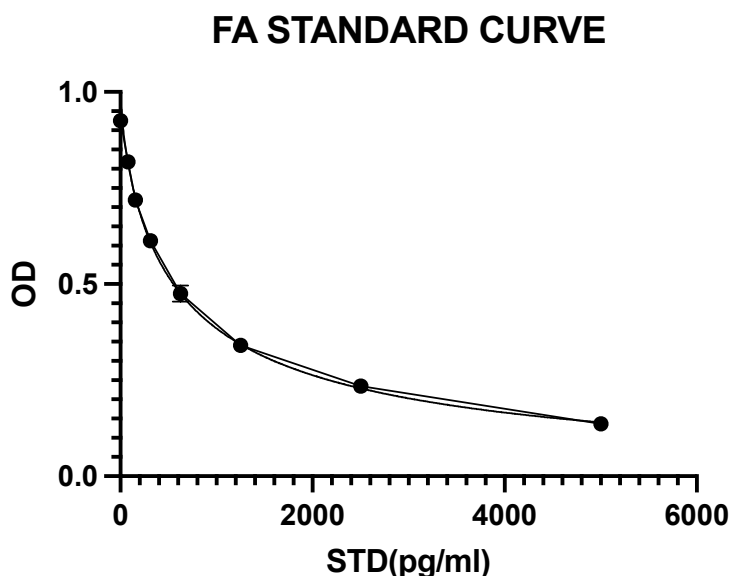


Figure 28: FA standard curve used to interpolate concentrations of folates

2.0.3.4. Cell Wall/Membrane Damage Alkaline Phosphatase Assay

Alkaline phosphatase (AKP) activity was used to assess the integrity of the outer membranes of the test organisms. AKP activity was measured using a commercial AKP kit (abbexa abx 096002). The method to study this activity was amended from [125]. Briefly, the bacterial isolates were grown to McFarlan standard and the cultures were diluted to 5×10^5 CFU/ml using nutrient broth (NA). Then, 250 μ L of 2XMIC of the compounds was loaded in 2 ml sample tubes containing 250 μ L of innoculum in NA, therefore making 1XMIC of the test compounds. The treated bacterial isolates together with the vehicle control were incubated at 37 °C for 2 hr. Finally, the AKP analysis was done following the kit manufacturer's protocol.

2.0.3.5. Molecular Docking Studies

The molecular geometries of all the compounds were optimized by density functional theory (DFT) calculations, using the B3LYP functional [126]–[128] together with the 6-31G(d,) and LANDZ for basis sets and the Gaussian 16 program package [129]. The optimized geometries were converted to PDB files using the *Gaussview* software. The molecular docking studies of the compounds and the positive control were performed using the AutoDock 4.2.6 software [130] against the active sites of the proteins Dihydropteroate Synthase (DHPS) (PDB:3tye) and outer membrane porin protein OmpK36 of *K. pneumoniae* (PDB:6rd3). The co-crystallized substrates and water molecules were removed from the receptors using the MGL 1.5.6 software [131]. After cleaning the proteins, only polar hydrogens were added together with the Kollman charges. The grid box for docking against DHPS was constructed using 50, 50, and 50 pointing in the x, y, and z dimensions, respectively, with a grid point spacing of 0.375 Å. The center grid box was set at -76.869, 85.278, and 94.784 Å for the x, y, and z centers, respectively. Whereas for docking against *K. pneumoniae*, a grid box was constructed using 126, 126, and 126 in the x, y, and z dimensions with grid-center values of 1.018, 2.207 and 18.640 Å for the x, y, and z centers, respectively. The total number of genetic algorithms in both cases was set at 100, which generated one hundred different conformations for each.

Results and Discussions

This section is a four-part chapter which fully describes the preparation, structural elucidation, and properties of the synthesized compounds. Furthermore, the antibacterial properties of the ligands, the cobalt, nickel, and copper complexes are discussed.

3.0 Synthesis and Characterization of Amino and Nitro based Pyridyl Ligands

This sub section focuses on the synthesis and the characterization of four pyridine-based ligands. The four ligands are from two classes, the ester and the ether bridged ligands.

3.0.1 O-Alkylation of the Amino and Nitro Ester Ligands

The two ester compounds were prepared through the o-alkylation of the carboxylic oxygen in both *p*-nitrobenzoic acid and *p*-aminobenzoic acid. Since *p*-chloromethyl pyridine possesses a primary alkyl halide, the reaction was designed to favour S_N2 mechanism which was achieved by using a polar aprotic solvent dimethylformamide. Since potassium carbonate is a weak base, the *p*-nitrobenzoic acid or aminobenzoic acid were stirred with the base to deprotonate the acid prior to adding the *p*-chloromethyl pyridine. To ensure complete alkylation, the reaction was stirred at 70 °C overnight. The compound **L1** showed an impurity of the *p*-nitrobenzoic acid through TLC which was alleviated by limiting *p*-nitrobenzoic acid and adding the base in a little excess to accommodate the acidic *p*-chloromethyl pyridine hydrochloride. This amendment proved to reduce the impurity of the compound where there was an observation of golden crystals of **L1**. Conversely, **L3** was obtained as a pure yellow oily substance that easily crystalized when mixed with methanol and then air dried. The compounds **L1** and **L3** were both formed at high yields of 94.1 and 96% respectively.

3.0.1.2 Purity, Physical Properties and Solubility Studies of the Ester Ligands

The ligands were both soluble in most organic solvents with partial solubility in water as shown in Table 3.

Table 3: Physical Properties of **L1** and **L3**

Ligand	Appearance	Melting Point °C	Solubility	%Yield
L1	Gold crystals	145	Methanol, Ethanol r, DMF, DCM, Ethyl acetate, acetone	94.1
L3	Yellow crystalline solid	135	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone	96.0

L1 was synthesized using an excess of potassium carbonate, which had an impact on the purity of the ligand. Empirical formula calculations revealed that potassium carbonate might have been present as an impurity at 0.2 moles. This information was used to normalize the obtained elemental analysis data with the calculated values, as demonstrated in Table 4. In contrast to **L1**, **L3** exhibited no impurities and approached a level of purity close to 100%.

Table 4: Elemental Analysis and Purity of **L1** and **L3**

CALCULATED (FREE CHN)				FOUND			PURITY	
Ligand	N	C	H	N	C	H	IMPURITIES (mol)	% PURITY
L1	9.61 (10.85)	55.38 (60.47)	3.46 (3.90)	9.81	55.20	3.55	0.2 K ₂ CO ₃	90
L3	12.27 (12.27)	68.41 (68.41)	5.30 (5.30)	12.44	68.37	5.35	-	100

3.0.1.3 FTIR Characterization of the Ester Ligands

The vibrational spectra of the two ligands showed that all the functional groups were observed. **L1** spectrum shows a peak at 3052-3108 cm⁻¹ assigned to the aromatic (C-H) stretch. Also, the (C=C) aromatic stretch was assigned to peak appearing at 1517.9 cm⁻¹. The presence of the ester group was confirmed by the stretching frequency at 1721.4 cm⁻¹. Moreover, the aromatic nitro group was also observed at 1323-1405.8 cm⁻¹ as well as the C-N pyridinyl peak at 1435.9 cm⁻¹. As

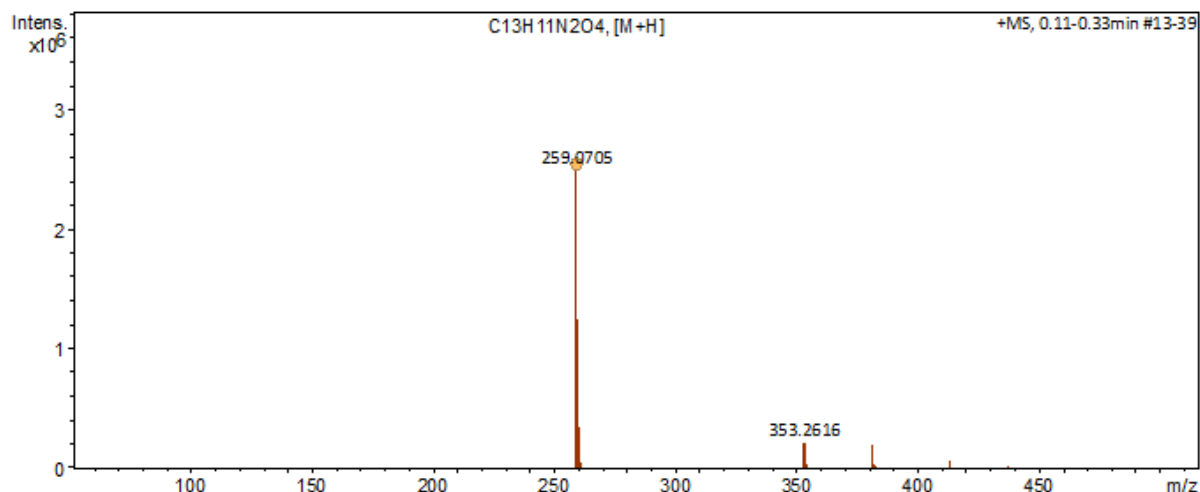
for the amine analogue **L3**, all the necessary functional groups were also observed. The aromatic (C-H) was assigned to 3182.6 cm^{-1} while the (C=C) aromatic stretch was assigned to the 1646.4 cm^{-1} peak. The ester group was observed by the 1682.3 cm^{-1} peak. Furthermore, the aromatic amine group was observed at $3308.7\text{--}3402.5\text{ cm}^{-1}$. Finally, the (Ar-N) pyridinyl peak was observed at 1439.8 cm^{-1} . It was also discovered that some of the similar functional group vibrations from the two ligands had different frequencies, with the most prominent being the ester band. The deviation of the ester band in the spectrum of **L3** was inferred to be due to the substituent effects of the electron donating amine as opposed to the nitro group in **L1** [132]. The summary of the FTIR vibrational bands for the ligand are shown in Table 5 while all the spectra are curated under appendix 5.1.

Table 5: Vibrational Frequency Bands of **L1** and **L3**.

Molecular motion	L1 (cm^{-1})	L3 (cm^{-1})
C=C	1517.9	1520.8
O=C-O ester	1721.4	1682.3
Ar-C-H	3052-3108	3182.6
Ar-NH ₂	-	3308.7-3402.5
Ar-NO ₂	1323-1405.8	-
Ar-C=N	1435.9	1439.8
Aryl -O stretch	1270	1265.2

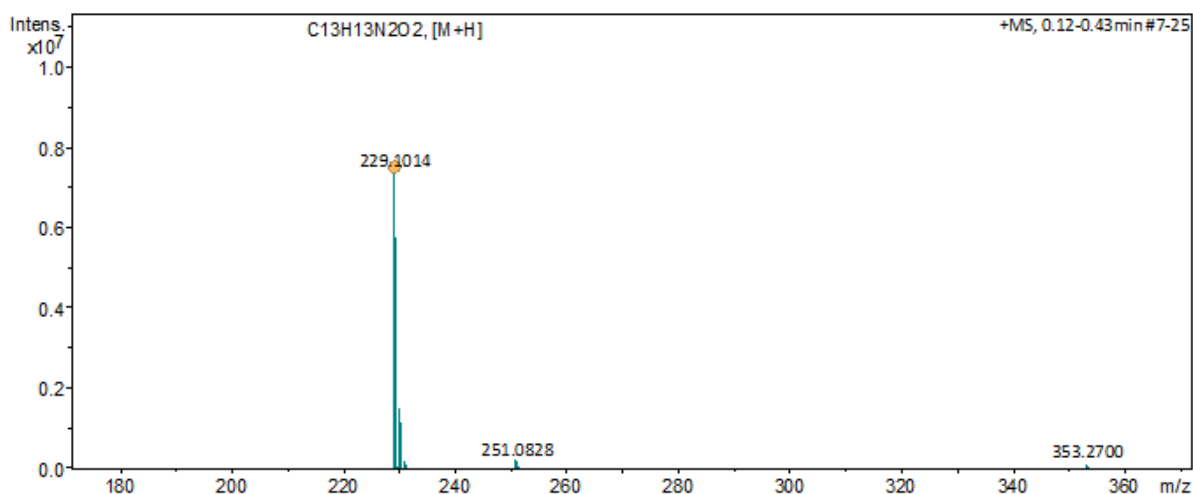
3.0.1.4 Mass Spectrometric Characterization of the Ester Ligands

High resolution mass spectrometry was used to determine and corroborate the structures of the ligands. The spectrum 1 shows the successful synthesis of **L1** and the data shows a molecular ion peak of the protonated ligand, $[\text{M}+\text{H}]^+$ after being run on a positive ion mode. The molecular mass of the ligand was 258.06 g/mol compared to the protonated 259.0705 m/z .



Spectrum 1: Mass spectrum of **L1**.

The **L3** ligand also displayed similar mass spectrometry (see spectrum 2), the ligand molecular ion peak was also protonated. The spectrum did not show many fragmentations, but the prominent molecular ion peak of 229.1014 m/z that corroborated with the un-protonated molecular mass of 228.09 g/mol.

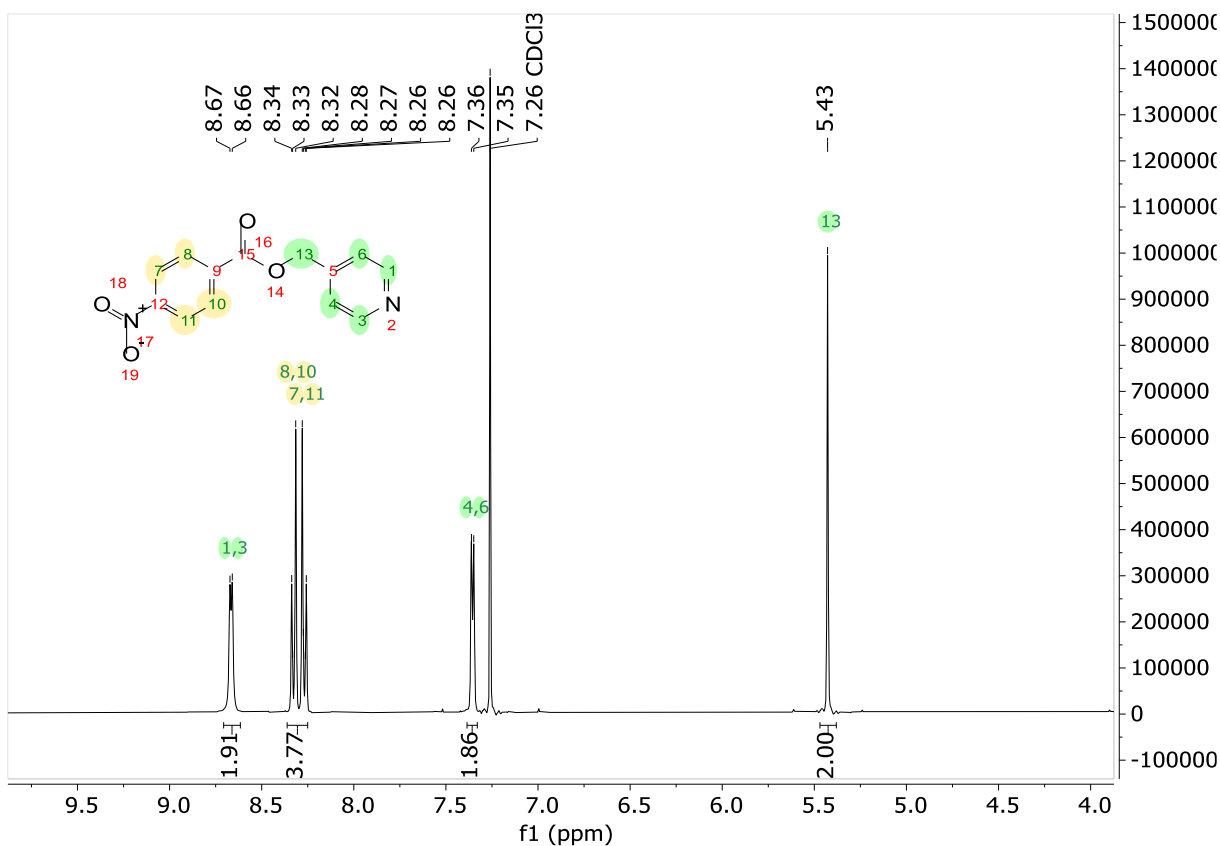


Spectrum 2: Mass spectrum of **L3**.

3.0.1.5 Proton NMR Characterization of the Ester Ligands.

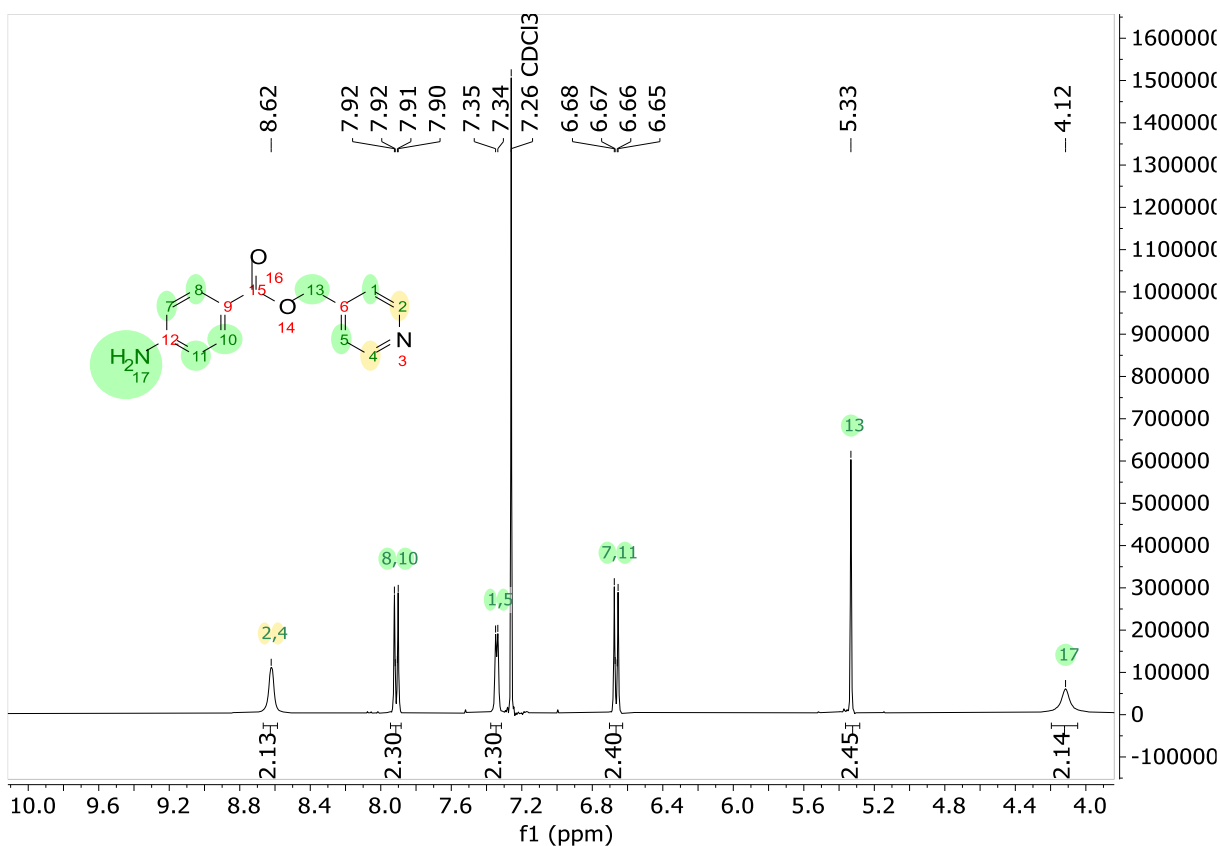
Nuclear magnetic resonance spectroscopy was employed to elucidate the structure of the ligands. The proton NMR spectra of the ligands **L1** and **L3** were clean and completely showed the chemical shifts or chemistry of the protons available in the ligands. In the proton spectrum 3 of

the nitro ester ligand **L1**, a diagnostic oxygenated methyl singlet signal was observed at 5.43 ppm. This 4-picolinic arm's methyl group exhibited a chemical shift more downfield than for ordinary methyl protons due to the deshielding nature of the ester moiety. Furthermore, the pyridyl protons that are *ortho* to the methyl substituent were observed at 7.36 ppm. These were followed by the doublet of a doublet on the aromatic ring bearing the nitro group which was observed at 8.26-8.34 ppm and coupling constant of $J = 8, 4$ Hz, which was indicative of *para*-substituted aromatic ring. Finally, the largely deshielded pyridyl protons that are on the carbon atoms bound to the pyridyl nitrogen were observed at 8.67 ppm. The spectrum's integration also shows that indeed the compound was successfully synthesized, this was observed with the number of protons per peak and the total number of protons in the spectrum. Spectrum 3 also shows the assigned chemical shifts of **L1** together with the integration of these chemical shifts.



Spectrum 3: Proton NMR spectrum of **L1**. ^1H NMR (400 MHz, Acetone- d_6) δ 8.67 (m, 2H, J), 8.34 (dd, 2H, $J = 8, 4$ Hz), 8.26 (dd, 2H, $J = 8, 4$ Hz), 7.36 (m, 2H), 5.43 (s, 2H).

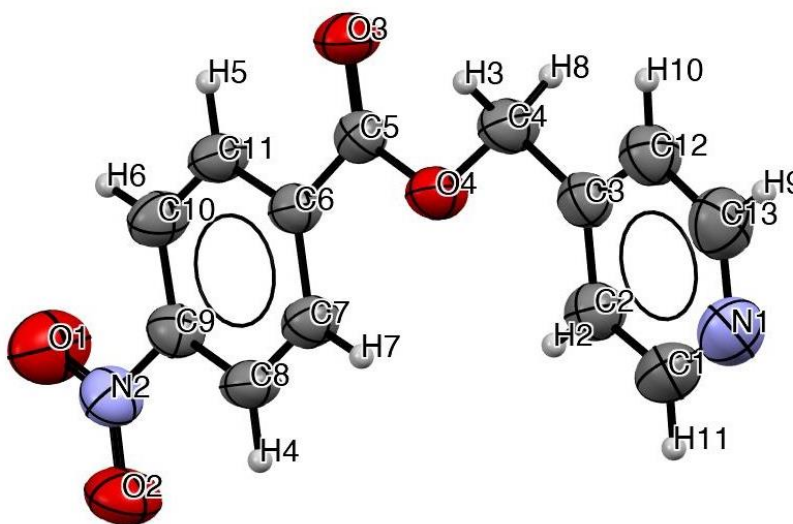
As for the structure of the analogue **L3** which was the amino ester ligand, similar properties of the proton NMR signals were observed. However, there was an observed nudge at 4.12 ppm which was assigned to the amino group protons. Similarly, to **L1** there was a diagnostic methyl shift at 5.33 ppm with a singlet multiplicity. Furthermore, the aromatic chemical shifts were also assigned as observed on Spectrum 4. The more deshielded protons, such as the methyl diagnostic protons which were closer to the pyridyl nitrogen and ester bridge were observed shifted upfield (5.33 ppm) on the spectrum. Similar chemical environments of the protons in the aromatic ring bearing the amino group did not split as a doublet of a doublet like in **L1**. This shows that the two molecules do not have the same geometry, which may be caused by the two different nitrogen substituents.



Spectrum 4: Proton NMR of **L3**. ¹H NMR (400 MHz, Acetone-d₆) δ 8.62 (brs, 2H), 7.92(d, 2H, *J* = 8 Hz), 7.34 (d, 2H, *J* = 4 Hz), 6.71 – 6.63 (dd, 2H, *J* = 8, 4 Hz), 5.33 (s, 2H), 4.12 (brs, 2H).

3.0.1.6 Single-Crystal XRD of the Ester Ligands

The structure of pyridin-4-ylmethyl 4-nitrobenzoate **L1** was determined by single crystal X-ray diffraction methods. The compound crystallized in a monoclinic system, $P2_1/n$. The crystal solution and refinement details are outline in appendix 5.2. The structure shows that indeed the compound was successfully synthesized. Structure 8 shows the labelled molecular structure of the compound as determined by SC-XRD.



Structure 8: Labelled ORTEP at 50% probability of **L1**.

The crystal structure of the reported molecule's analogue 2-pyridyl 4-nitrobenzoate has been reported before [133]. The key distinction of the analogue is the absence of a methyl group connecting the ester and pyridyl groups. The compound was obtained as rhombohedral-shaped golden crystals. The crystal characteristics of the molecules were comparable, with the bond angles and lengths of the reported molecule falling within the reported range and resembling those of the analogue. Both compounds crystallized in the monoclinic crystal system. The reported molecule exhibits a transformation under the $P2_1/n$ space group, while the analogue also undergoes a transformation under the $P2_1/c$ space group. All the aromatic C-C bonds measured were in the expected ranges of about ~ 1.4 Å which resulted in correctly shaped aromatic rings. The C-N pyridyl bonds (C1---N1---C13) were also in acceptable bond lengths of ~ 1.3 Å. The ester bonds (C4---O4) were also in range at ~ 1.4 Å while the double bond (C5=O3) of

the ester was shorter than the single bond as expected at 1.204 Å. The nitro bonds were observed at the expected measurements of (N2---O1) 1.223 Å and (N2---O2) 1.218 Å. All the bonds were within the acceptable ranges when compared to Literature values [134]. The selected bond lengths are summarized in Table 6.

Table 6: Bond lengths of **L1**.

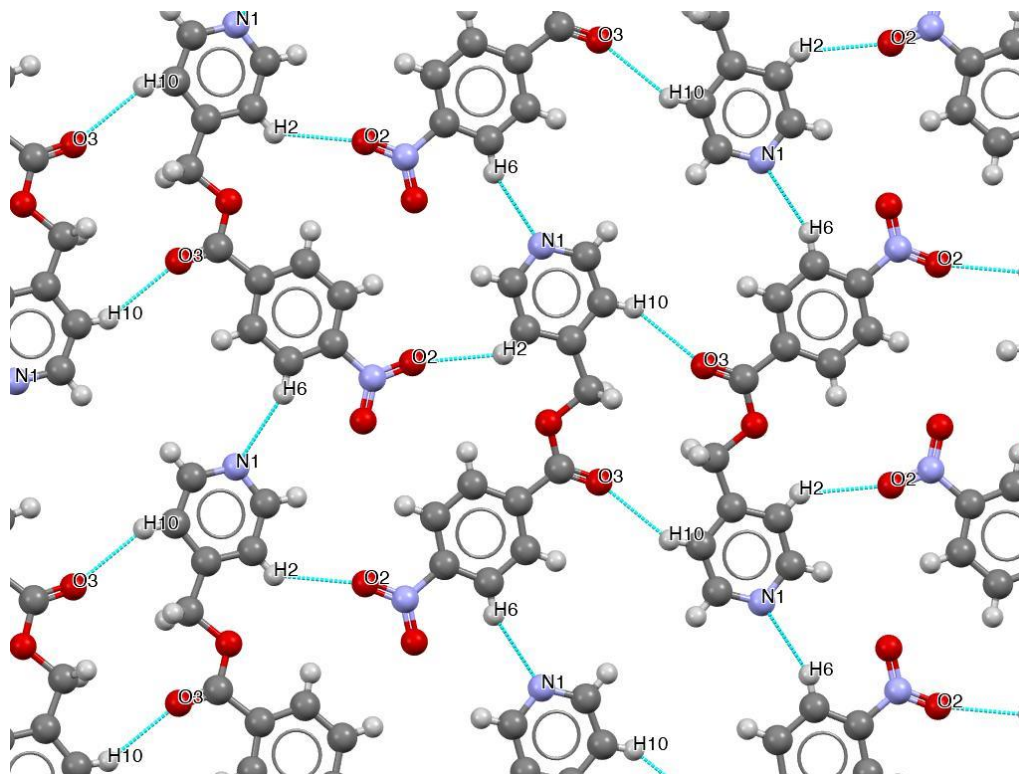
Atom	Atom	Length/Å
C1	N1	1.324(4)
C4	O4	1.443(3)
C5	O3	1.204(3)
C5	O4	1.344(3)
C9	N2	1.474(3)
C13	N1	1.329(4)
N2	O1	1.223(3)
N2	O2	1.218(3)

As shown in the structure 8 of **L1**, the molecule's non hydrogen atoms and conjugated π system exhibits planarity with a benzene ring substituted with a nitro group and an ester group on the *para* position. The torsion angles in and especially the bonds C1-C4-O4-C5 177.8(2), C6-C5-O4-C4 175.0° (2), C7-C8-C9-N2 179.8° (2), C8-C9-N2-O1-176.5° (2), C10-C9-N2-O2-176.8° (2), C12-C1-C4-O4-170.3° (2), N2-C9-C10-C11 180.0° (2), O3-C5-C6-C7 -178.4° (2) and O4-C5-C6-C11 -176.4° (2) define this planarity.

Table 7: Selected Torsion angles of **L1**

Bond	Angle/°
C1-C4-O4-C5	177.8(2)
C6-C5-O4-C4	175.0(2)
C7-C8-C9-N2	179.8(2)
C8-C9-N2-O1	-176.5(2)
C10-C9-N2-O2	-176.8(2)
C12-C1-C4-O4	-170.3(2)
C7-C8-C9-N2	179.8(2)
O3-C5-C6-C7	-178.4(2)
O4-C5-C6-C11	-176.4(2)

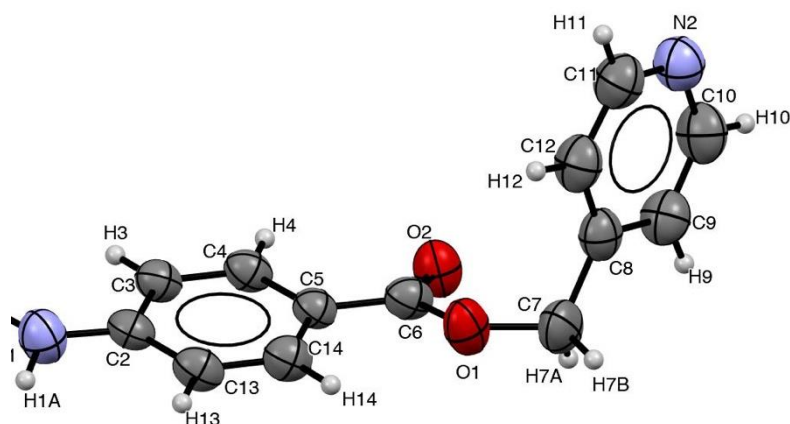
In the expanded unit cell, several contacts were observed, including N1---H6 (2.530 Å), H2---O2 (2.553 Å), and H10---O3 (2.563 Å). These contacts reveal that the expanded structure maintains planarity (**Structure 9 of L1**). Furthermore, these contacts can be classified as weak hydrogen bonding interactions, as indicated by their lengths and nature. The hydrogen bonding interactions exhibit the same symmetry, with coordinates described by the x, y, and z directions. This symmetry suggests a consistent pattern of hydrogen bonding throughout the structure. Regarding the packing arrangement, the structure is packed in a planar manner along the c-axis. This implies that the layers of molecules in the crystal stack on top of each other, forming a planar arrangement perpendicular to the c-axis. Based on the observed contacts and their lengths, the hydrogen bonding interactions in this structure are classified as weak.



Structure 9: Hydrogen bonding contacts of **L1** as observed along C-axis.

The structure of pyridin-4-ylmethyl 4-aminobenzoate **L3** was also determined by single crystal X-ray diffraction. Contrary to **L1**, **L3** crystallized in the orthorhombic system, and transforms under the $Pna2_1$ space group. The crystal solution and refinement details are outlined in appendix 5.2.

The confirmed structure shows that indeed the compound was successfully synthesized. Structure 10 shows the labelled molecular structure of the compound as determined by SC-XRD.



Structure 10: Labelled ORTEP at 50% probability of **L3**.

All the bonds were observed in the expected ranges. Firstly, the aromatic C-C bonds measured were in the expected ranges of about ~ 1.4 Å which resulted in correctly shaped aromatic rings. The pyridyl bond at (C10---N2---C11) was also acceptable at ~ 1.3 Å [134]. The ester bond was also acceptable (C6---O1) at ~ 1.2 Å. Just as observed in **L1**, the double bond (C6=O2) of the ester was shorter than the single bond at 1.222 Å. The amine moiety nitrogen (C2-N1) bond was also observed at the expected measurement of 1.344 Å [134]. The selected bond lengths are summarized in Table 8.

Table 8: Selected Bond lengths of **L3**

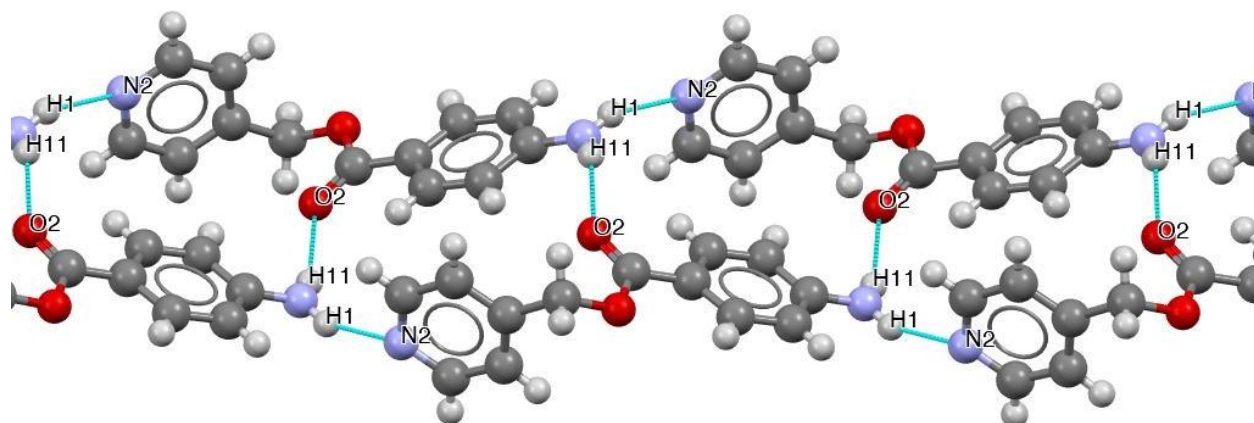
Atom	Atom	Length/Å
O1	C6	1.355(5)
O1	C7	1.442(7)
N1	C2	1.350(7)
O2	C6	1.222(6)
N2	C10	1.336(7)
N2	C11	1.326(7)
C2	C3	1.407(6)
C2	C13	1.397(7)

Contrary to **L1**, **L3** was not planar as observed in structures 20. Table 9 shows the selected torsion angles of **L3**. The molecular angles O1-C7-C8-C9 -143.6 °(5) and O1-C7-C8-C12 37.9 °(7) indicate where the molecule's planarity was lost. Thus, this shows the planarity of the molecule was lost on the bonds from the ester moiety through to the pyridyl ring. The aniline moiety was on the same plane with the ester moiety. This is suspected to be due to the free rotating sp^3 bond of the methyl group C7. Even though this is the case, **L1** was completely planar. The only difference between the two molecules was the nitro and the amine moieties. The planarity of **L1** is suspected to be due to the strong electron withdrawing ability of the nitro moiety as opposed to the electron donating ability of the amino group.

Table 9: Selected Torsion Angles of **L3**

Bond	Angle/°	Bond	Angle/°
O1-C7-C8-C9	-143.6(5)	C6-C5-C14-C13	-178.2(4)
O1-C7-C8-C12	37.9(7)	C7-O1-C6-C5	174.0(4)
N1-C2-COL1-COL4	177.5(4)	C7-C8-C9-C10	180.0(5)
N1-C2-C13-C14	-177.1(5)	C7-C8-C12-C11	179.6(5)
COL4-C5-C6-O1	171.1(4)	C14-C5-C6-O2	167.5(5)
C6-O1-C7-C8	70.4(6)		

In the crystal structure, significant contacts of the type N2---H1 (2.259 Å) and O2---H11 (2.213 Å) were observed, indicating the presence of hydrogen bonding interactions. These contacts play a crucial role in influencing the overall structural arrangement of the expanded structure. The strength of these hydrogen bonds can be categorized as weak interactions, as reflected by their relatively longer bond lengths. However, despite their weak nature, these hydrogen bonds contribute to the overall stability and organization of the crystal structure. Regarding the packing arrangement, the crystal structure exhibits a ladder-like packing motif along the c-axis (Structure 11). This implies that the molecules align in a stacked manner, forming layers that extend parallel to the c-axis direction. The ladder-like packing arrangement creates an intricate network of intermolecular interactions, which further reinforces the structural integrity of the expanded crystal.



Structure 11: Hydrogen bonding contacts of **L3** as observed along C-axis.

3.0.2 O-Alkylation of the Amino and Nitro Ether Ligands

The ligand, **L2** was synthesized through the o-alkylation of *p*-acetaminophen with *p*-chloromethyl pyridine. The use of *p*-acetaminophen was due to the competitive nucleophilicity of the oxygen and nitrogen moieties in *p*-aminophenol. Thus, the amine group in *p*-aminophenol was protected by the acetamide moiety by forming *p*-acetaminophen. The alkylation was proposed to follow an *SN2* reaction mechanism, this was because of the use potassium hydroxide which is a strong base, a primary alkyl-halide *p*-chloromethyl pyridine, and the use of a deprotonated *p*-acetaminophen ion which is a strong nucleophile. Slight excess of potassium hydroxide was used to deprotonate the hydroxyl group in *p*- acetaminophen. Thereafter, the nucleophilic oxygen attacks the carbocation on the *p*-chloromethyl pyridine. After the alkylation, the deprotection was archived by the acid hydrolysis of the amide group to amine group. As for **L4**, the same procedure for the preparation of **L2** was followed. Because **L4** is a nitro analogue of **L2**, there was no need for the protection of this group and the reaction was done using *p*-nitrophenol. **L2** was recovered as a brown powder with a yield of 76.4% while **L4** was recovered as a grey solid at a good yield of 81.0%.

3.0.2.1 Purity, Physical Properties and Solubility Studies of the Ether Ligands

The ligands were both soluble in most organic solvents. Also, **L2** solubility in water was observed upon heating. However, after a short time it started precipitating out. Table 10 shows the solubility, and physical properties, of the two ligands.

Table 10: Physical Properties of **L2** and **L4**

Ligand	Appearance	Melting Point °C	Solubility	%Yield
L2	Brown powder	125	Methanol, Ethanol, DMF, DCM, Ethyl acetate, acetone	76.4
L4	White powder	147	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone	81.0

In the case of ligands **L2** and **L4**, a careful examination of the elemental analytic data revealed the presence of impurities originating from the starting materials or the base used during the reaction. Specifically, **L2** exhibited an impurity of p-Acetaminophen, which was successfully rectified by prolonging the reaction time. On the other hand, for **L4**, the ligand was subjected to a thorough washing process with hot distilled water to eliminate the impurity arising from the potassium carbonate base. Despite the initial presence of these impurities, it is noteworthy that the elemental analytic data was adjusted accordingly, considering the contribution of these impurities. Consequently, these measures ensured the successful synthesis of both ligands, thereby affirming the purity and integrity of the final compounds. Table 11 shows the found and the calculated empirical formulae of the two ligands.

Table 11: Elemental Analysis and Purity of **L2** and **L4**

Ligand	CALCULATED (FREE CHN)			FOUND			PURITY	
	N	C	H	N	C	H	IMPURITIES (mol)	% PURITY
L2	12.60 (13.99)	69.51 (71.98)	6.03 (6.04)	12.81	69.34	5.97	0.55 p- Acetaminophen	71
L4	8.22 (12.17)	45.11 (62.61)	2.96 (4.38)	8.78	45.07	3.14	0.8 K ₂ CO ₃	68

3.0.2.2 FTIR Characterization of the Ether Ligands

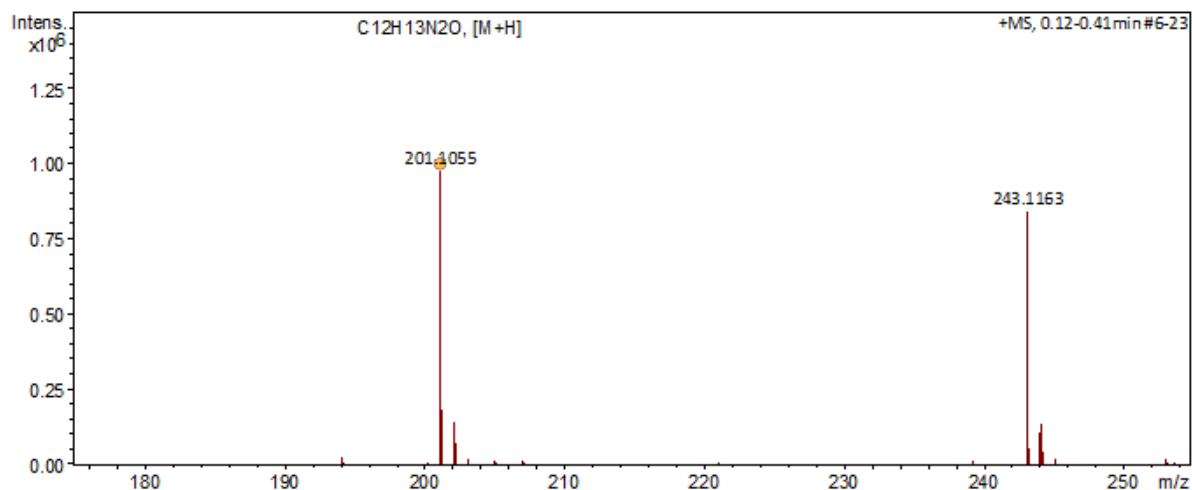
The following expected functional groups were observed in these compound's FTIR data: the aromatic (C-H) vibration at frequencies 3082 cm^{-1} ; the aromatic (C=C) vibrations at frequencies 1507.1 cm^{-1} , the (A-N) vibrations at frequencies 1463.2 cm^{-1} , and the (C-O) vibration, which was a sharp and strong peak at 1232.1 cm^{-1} . The primary (N-H) vibration stretches at $3297.6\text{--}3363.2\text{ cm}^{-1}$ which indeed shows that the hydrolyses did take place. These functional groups indicated the formation of **L2** ligand. The **L4** ligand also showed the important functional groups from its spectra, the aromatic (C-H) vibration was observed at frequencies 3083.5 cm^{-1} ; the aromatic (C=C) vibrations at frequencies 1502.9 cm^{-1} , the (A-N) vibrations at frequencies 1459.5 cm^{-1} , and the (C-O) vibration, which was a sharp and strong peak at 1265.9 cm^{-1} . The nitro vibration band was also observed at $1341\text{--}1445\text{ cm}^{-1}$. These compounds summarized FTIR frequencies are shown in Table 12.

Table 12: Vibrational Frequency Bands of **L2** and **L4**.

Molecular motion	L2 (cm^{-1})	L4 (cm^{-1})
C=C	1507.1	1502.9
C-O-O ether	1094.6-1050.9	1179.2-1031.4
Ar-C-H	3082.0	3083.5
Ar-NH ₂	3297.6-3363.2	-
Ar-NO ₂	-	1341-1445
Ar-C=N	1463.2	1459.5
Aryl -O stretch	1232.1	1265.9

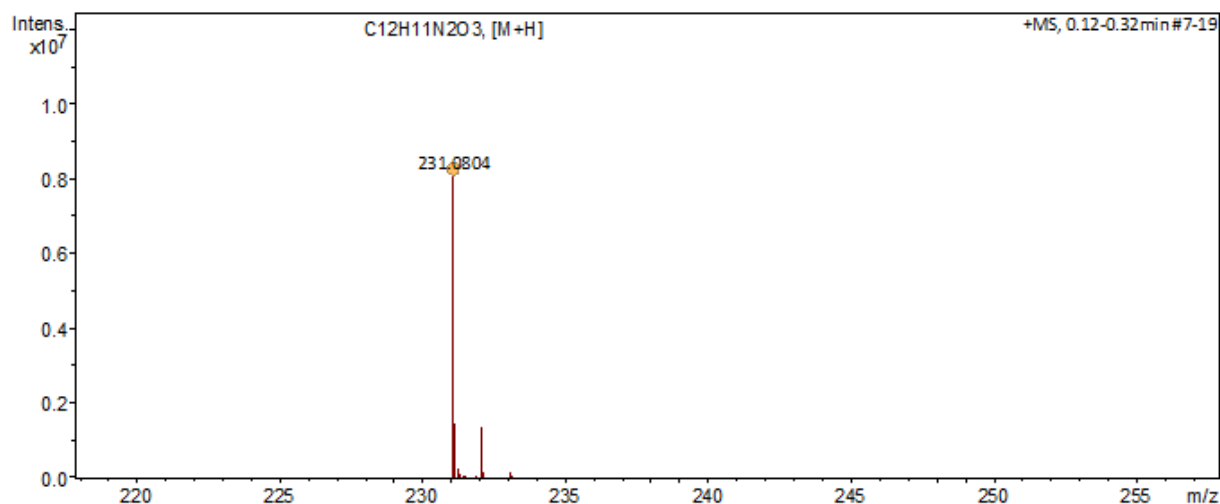
3.0.2.3 Mass Spectrometric Characterization of the Ether Ligands

The high-resolution electrospray ionization mass spectrometry of the ether ligands confirmed their successful synthesis as shown on spectrum 5 and 6. The spectrum of **L2** shows that the molecular ion peak was a protonated fragment, with a mass of 201.1055 m/z compared to the molar mass of 200.09 g/mol .



Spectrum 5: Mass spectrum of **L2**.

Similarly, to **L2**, the mass spectrum of **L4** indicated that the molecular ion fragment was protonated, with a mass to charge ratio of 231.0804 m/z. The spectrum did not show complicated fragmentation and corroborated well with a molecular mass of 230.07 g/mol.

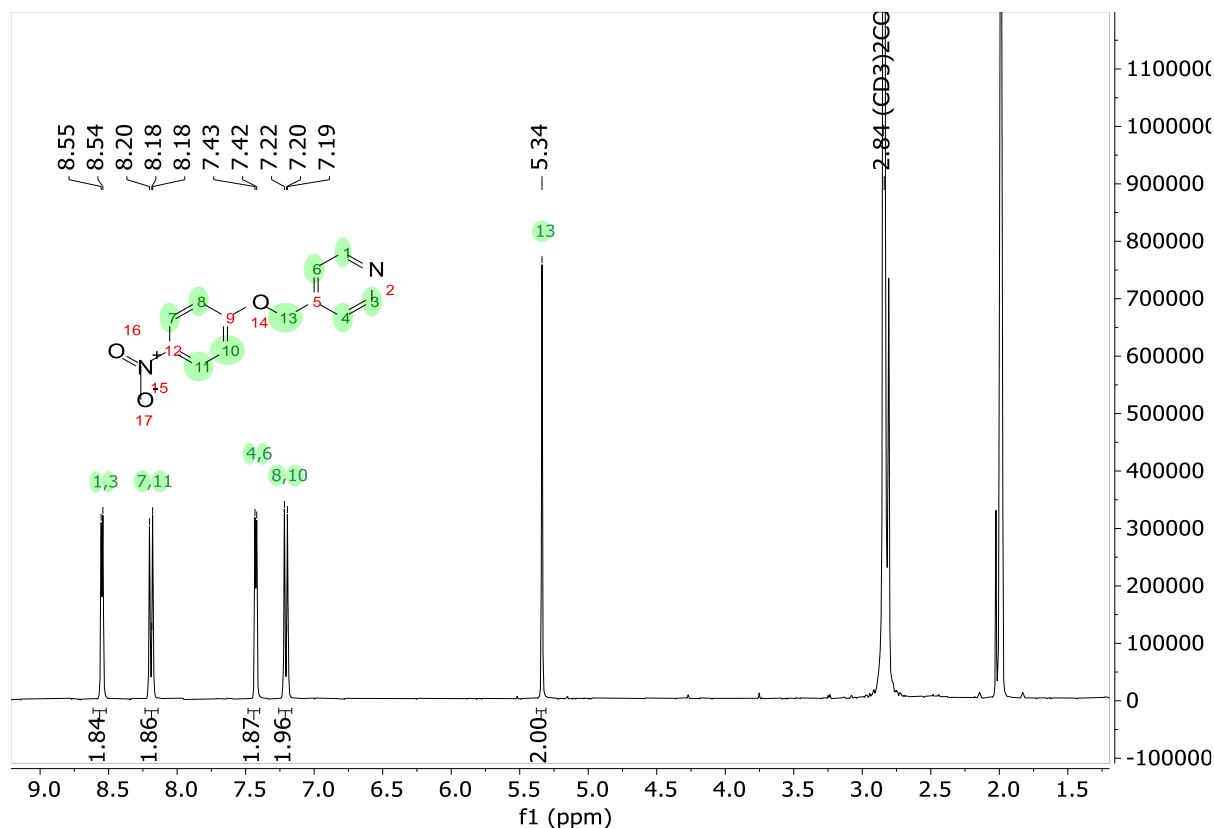


Spectrum 6: Mass spectrum of **L4**.

3.0.2.4 Proton NMR Characterization of the Ether Ligands

The proton NMR spectra of the nitro and amino ether analogues **L2** and **L4** also showed that the compounds were successfully synthesized. On the spectra of the amino ligand **L4**, the diagnostic methylene singlet peak was observed at 5.34 ppm, this peak was assigned to the picolinic arm that bridges the nitrophenolate to the pyridyl moiety. Aromatic protons from the nitrophenolate

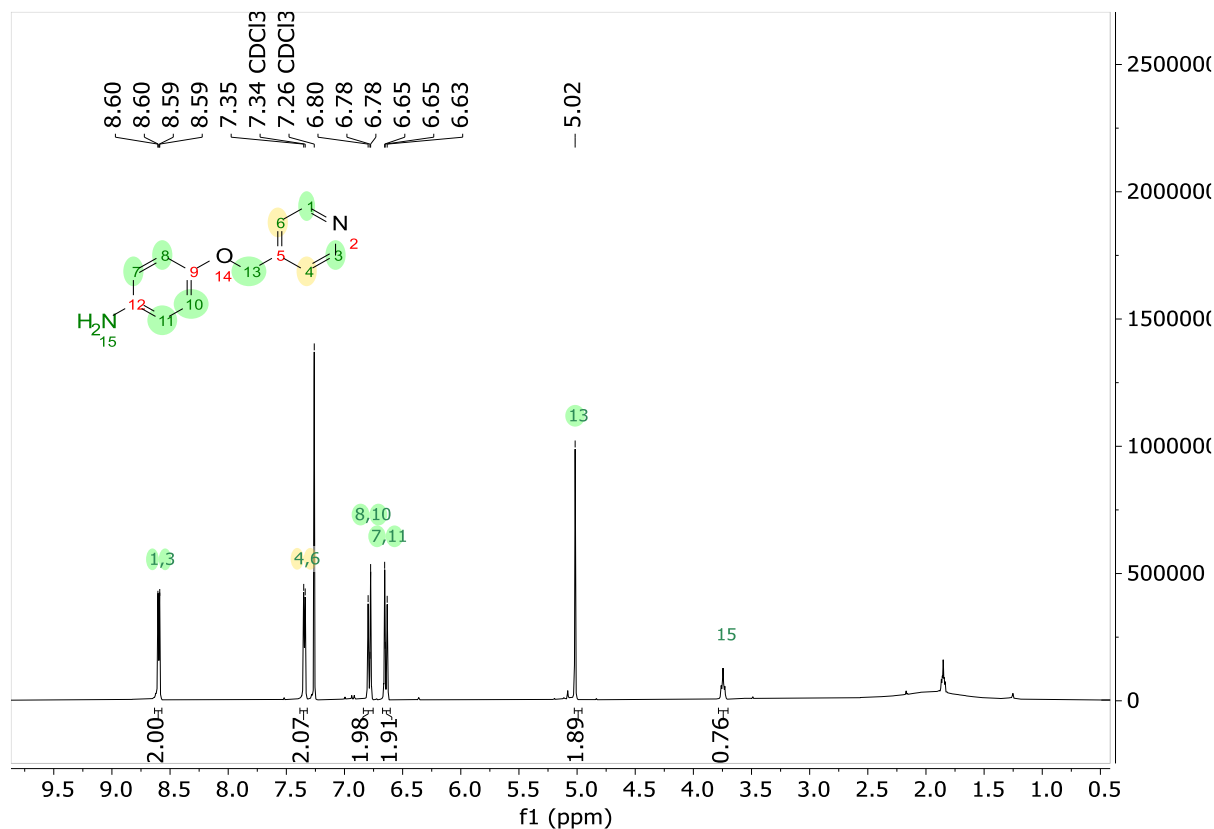
moiety were observed upfield of the pyridyl protons. The Nitro-substituted aromatic ring protons labelled **8** and **10** and **7** and **11** were the aromatic protons at 7.20 ppm and 8.18 ppm respectively, while the pyridyl ring aromatic protons were assigned to be **4**, **6** at 7.42 ppm and **1**, **3** at 8.55 ppm. Spectrum 7 shows the assignment of peaks and the integration of peaks which show the successful synthesis of **L4**.



Spectrum 7: Proton NMR spectrum of **L4**. ¹H NMR (400 MHz, Acetone-d₆) δ 8.55 (d, 2H, *J* = 4 Hz), 8.18 (d, 2H, *J* = 8 Hz), 7.43 (d, 2H, *J* = 4 Hz), 7.20 (d, 2H, *J* = 8, 4 Hz), 5.34 (s, 2H).

Similarly, the amino ether analogue **L2** diagnostic singlet peak was seen at 5.02 ppm. Also, the aminophenolate amine peak was observed at 3.74 ppm. Contrary to **L4** the aromatic protons showed that **L2** has a different geometry which suggests the same effect observed in the nitrogen-based substituents of **L1** and **L3**. However, in the ether ligands the nitro analogue did not have the aromatic doublet of a doublet instead it was found in **L2** at 6.63 – 6.68 ppm. This doublet was followed by the more deshielded aromatic protons in the pyridyl ring ranging from

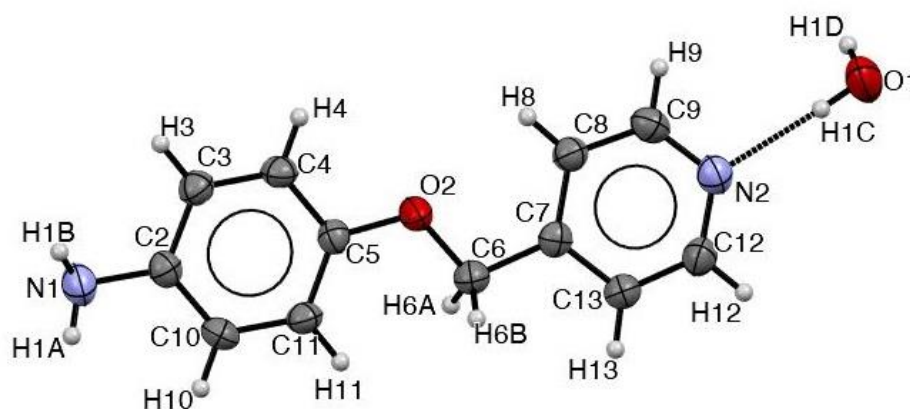
7.34 – 7.35 ppm for the protons **4**, **6** while the protons **1**, **3** on the carbon atoms directly attached to the pyridyl nitrogen were the downfield at 8.59 – 8.60 ppm. Spectrum 8 shows the spectrum and the assigned peaks to each proton.



Spectrum 8: Proton NMR spectrum of **L2**. ^1H NMR (400 MHz, CDCl_3) δ 8.60 (dd, $J = 5.1, 1.3$ Hz, 2H), 7.35 (d, 2H, $J = 4$ Hz), 6.78 (d, 2H, $J = 8$ Hz), 6.65 (d, 2H, $J = 8$ Hz), 5.02 (s, 2H), 3.74 (brs, 2H)

3.0.1.7 Single-Crystal XRD of the Ether Ligands

The structure of **L2** was determined by single crystal X-ray diffraction methods. **L2** crystallizes in an orthorhombic system and transformed under a $P2_12_12_1$ space group. The crystal solution and refinement details are outline in appendix 5.2. The crystal data shows that there was one water molecule present in the asymmetric unit which may have appeared during crystallization. Structure 12 shows the labelled molecular structure of the compound as determined by SC-XRD.



Structure 12: ORTEP structure of **L2** at 50% probability.

All bonds were within the predicted values, the ether bonds (C6—O1—C5) at around 1.4 Å were also suitable. The anticipated reading of 1.4 Å also revealed the presence of the amine moiety's nitrogen (C2—N1) bond. All bonds were compared to values from the Literature [135]. Table 13 shows the chosen bond lengths in summary.

Table 13: Selected Bond lengths of **L2**

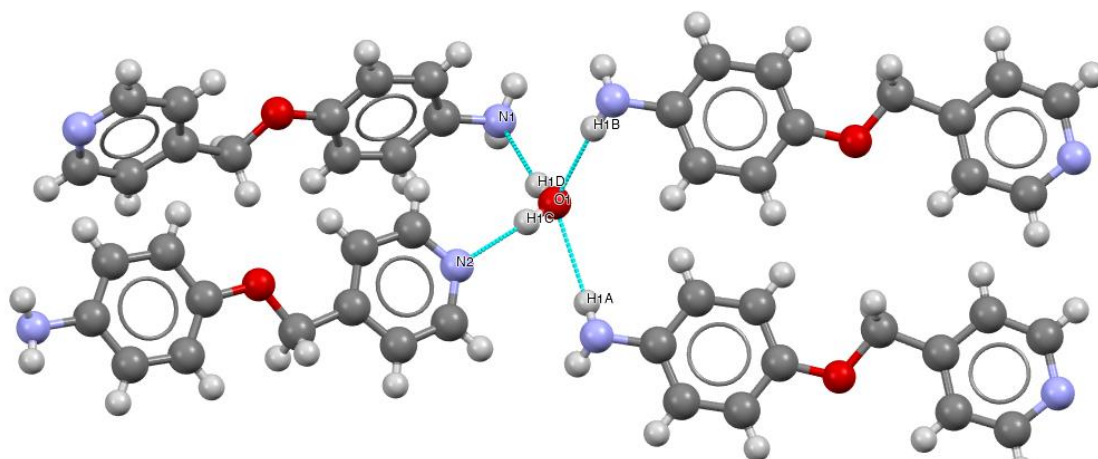
Atom	Atom	Length/Å
O1	C5	1.386(3)
O1	C6	1.413(3)
N2	C9	1.330(3)
N2	C12	1.334(4)
N1	C2	1.414(3)

Structure 12 confirms the planarity of **L2**. Table 14 presents the selected bond angles of **L2**, revealing the presence of a V-shaped ether bond with a measured angle of C5—O1—C6 at 117.4°(2) [135], which falls within the acceptable range. Additionally, Table 14 illustrates the characteristic angles observed in the aromatic rings, providing further insight into the molecular structure of **L2** [135].

Table 14: Selected bond angles of **L2**

Atom	Atom	Atom	Angle/°
C5	O1	C6	117.4(2)
C9	N2	C12	116.5(2)
C1	C2	N1	120.4(2)
C10	C2	N1	121.2(2)
O1	C5	C4	115.7(2)
C11	C5	O1	124.7(2)
O1	C6	C7	109.2(2)
N2	C9	C8	124.1(3)
N2	C12	C13	123.7(2)
	C5	C4	119.5(2)

The crystallographic analysis of **L2** revealed the presence of weak intermolecular and intramolecular bonds. The crystal structure displayed a four-molecule architecture, facilitated by water of crystallization, wherein three molecules interacted with the amino group and one molecule with the pyridyl nitrogen (Structure 13). The presence of water in the crystal structure had a significant impact on the intramolecular supramolecular chemistry of the molecule, specifically involving the amine group, pyridyl nitrogen, and ether oxygen. The presence of water molecules in the crystal structure alters the intramolecular supramolecular interactions, influencing the arrangement and connectivity of functional groups within the molecule. In the absence of water of crystallization, it was anticipated that intramolecular supramolecular interactions such as amino-ether and amino-pyridyl nitrogen would be more pronounced. Analysis of the hydrogen bond data revealed the following interactions: N1-H1A with a distance of 0.866 Å and an angle of 141.14°, forming a hydrogen bond with O1 and N1-H1B with a distance of 0.866 Å and an angle of 172.02°, forming a hydrogen bond with O1. These hydrogen bonding interactions demonstrate the ability of the amine group to act as a hydrogen bond donor and O1 as an acceptor in the supramolecular assembly. Overall, the crystallographic data and hydrogen bond analysis provide insights into the supramolecular chemistry of **L2**.



Structure 13: Hydrogen bonding contacts of **L2**

3.0.3 Antibacterial Studies of the Ligands

3.0.3.1 Bacterial Minimum Inhibition Concentration of the **Ligands**

The synthesized compounds were tested for their preliminary antibacterial properties, to establish their potential as lead compounds against drug resistant bacteria. MIC assay was done to determine the compounds minimum concentration needed to show bioactivity from the compounds. Silver sulfadiazine (**AgSD**) was used as a control because it is an approved antibiotic [136]. The **AgSD** showed better activity across all the organisms but the compounds **L2** and **L3** were more potent than **AgSD** towards *K. pneumoniae*. This drug is similar to **L2** and **L3** in terms of the amino pharmacophore in sulfadiazine. It is both a competitive inhibitor of bacterial PABA (through sulfadiazine) and affects both cell walls and cell membranes of bacteria (through the silver (I) ion [136]. The compounds displayed better activity towards the Gram-negative bacteria as compared to the Gram-positives (Table 15). This activity was interesting because Gram-negative bacteria can create resistance via modifying the hydrophobic characteristics of the outer membrane, as well as mutations in porins and other variables. Gram-positive bacteria lack this layer, making Gram-negative bacteria more antibiotic-resistant than Gram-positive bacteria [137]. The difference in the effective concentrations towards the non-drug resistant and resistant *K. pneumoniae* strains were not entirely significant especially for **L1**, **L4** and **4PY**. Conversely, it is important to note that the nitro compounds **L1** and **L4** showed less bioactivity, while the amino derivatives **L2** and **L3** were more bioactive against *K. pneumoniae*. The observed bioactivity of

the amino derivatives **L2** and **L3** corroborates with previous findings of a combination Trimethoprim–Sulfamethoxazole therapy against *K. pneumoniae*, which established amino based compounds for use against bacteria[138]. This indicates that the nitro and amino moieties were the important pharmacophores instead of the ether or ester in *K. pneumoniae*. The amino group may play an important role as a competitive antagonist to DHPS than PABA in the production of folic acid, resulting in inhibition of the growth and survival of the bacteria [60]. On the contrary, the nitro moiety has many potential modes of action, such as disruption of cell wall or membrane, binding and inactivation of enzymes and binding genomic material [139]. Interestingly, the combination (Trimethoprim–Sulfamethoxazole) was reported *in vitro* in various clinical isolates of *S. aureus*, and it was found to be potent as opposed to the ester and ether bridged compounds [140], [141]. Finally, the commercially available **4PY** compound is precipitated by hydrochloric acid (HCl) which reduces the pH of the media during growth. Consequently, this acid may have been responsible for the bioactivity in *K. pneumoniae* and not the compound itself, while the proliferation of *S. aureus* is tolerant to lower pH media as opposed to *K. pneumoniae* [141].

Table 15: MIC of the Ligands

Compound↓	Microbe→	MIC (mM)			
		MDR-K.			
		<i>K. pneumoniae</i>	<i>pneumoniae</i>	<i>S. aureus</i>	MRSA
4PY		0.19 ± 0.006	0.22 ± 0.003	0.57 ± 0.011	0.45 ± 0.04
L1		0.23 ± 0.03	0.23 ± 0.0022	0.52 ± 0.007	0.43 ± 0.03
L2		0.16 ± 0.02	0.24 ± 0.0008	0.37 ± 0.07	0.44 ± 0.007
L3		0.16 ± 0.02	0.23 ± 0.0005	0.37 ± 0.03	0.54 ± 0.05
L4		0.26 ± 0.05	0.22 ± 0.0008	0.51 ± 0.02	0.45 ± 0.05
AgSD		0.18±0.002	0.13±0.02	0.13±0.002	0.26±0.06
TREND		L2=L3>4PY>L1>L4	L4=4PY>L3=L1>L2	L2=L3>L4>L1>4PY	L1>L2>4PY=L4>L3

3.0.3.2 Time Kill Kinetics

Compounds with MIC of less than 0.3 mM were further characterised by determining their time kill kinetics profiles with the different organisms. Time kill assays measures the effects on a population of bacteria over a period following exposure to an antimicrobial agent. Considering

the time kill kinetics of the active compounds on the *K. pneumoniae* strain (Figure 29), the amino based compounds **L2** and **L3** were observed to be growing exponentially for the first 6 hr but stopped after 24 hr at their MIC. This led to the conclusion that they were bacteriostatic. The nitro-based counterparts **L1** and **L4** initially showed a bactericidal trend at their MIC for 5 hr, but after 24 hr, we observed an exponential growth which signified bacteriostatic activity. The observed trends of **L1** and **L4** were consistent with conventional drugs bearing a nitro substituent which are associated with cell membrane disruption as a mode of action [62]. Amine-containing drugs have previously been shown to inhibit bacterial growth by competitive inhibition of DHPS, an enzyme involved in folic acid biosynthetic pathway [60], [136]. Because of the presence of the amino substituent in **L2** and **L3**, the bacteriostatic effects were suspected to be due to the antagonism of the folic acid biosynthesis pathway, thus slowing the reproduction of the bacteria. Comparatively, the effects observed for **L2** and **L3** were similar to bacteriostatic effects of the control drug, **AgSD**, on *K. pneumoniae* after a 4-hour exposure to the drug. Of note, the control drug **AgSD** is known to be bactericidal due to the presence of the silver (I) ion, while the free sulfadiazine moiety is bacteriostatic [136].

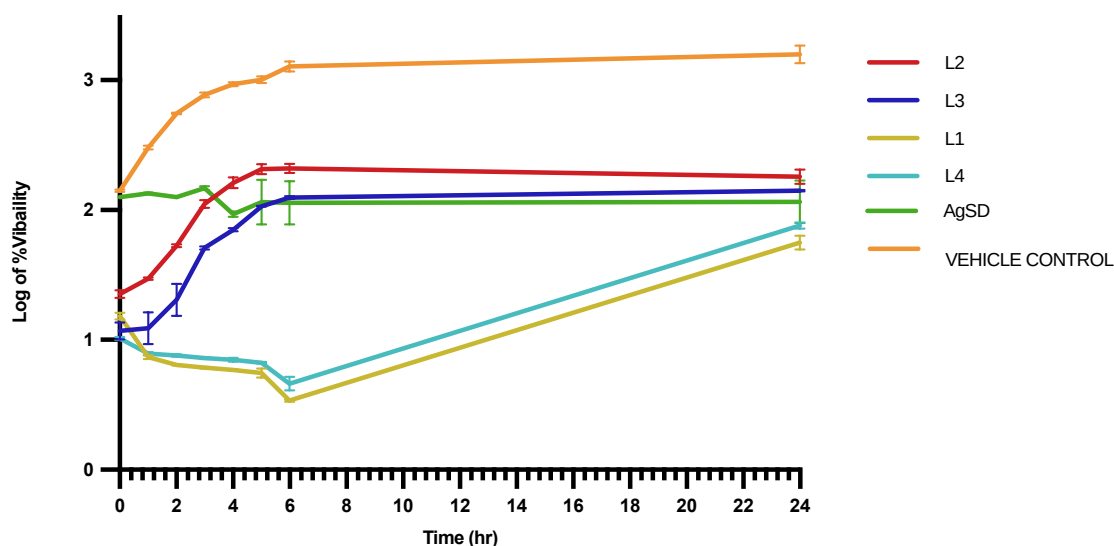


Figure 29: Time kill kinetics of the ligands against *K. pneumoniae*.

The time kill study of the active compounds on MDR-*K. pneumoniae* shown in Figure 30 were different compared to the non-drug resistant strain. The assay showed that all the active compounds were bactericidal against MDR-*K. pneumoniae* at their measured MIC, unlike against *K. pneumoniae* where **L2** and **L3** were bacteriostatic. During the experiment, the activity of all the compounds started at 1 hour, showing that the compounds had strong bactericidal effects towards MDR- *K. pneumoniae*. Furthermore, this observation suggests that these compounds may have a different mode of action towards the drug resistant strain as opposed to the non-drug resistant strain. Even though the amino based compounds showed bacteriostatic activity towards the non-drug resistant strain, amino based combination therapy (Trimethoprim–sulfamethoxazole) have previously been shown to be bactericidal when tested on clinical isolates of *K. pneumoniae* [138]. The kinetics of the control drug **AgSD** towards MDR- *K. pneumoniae* showed that it was bactericidal just as the other compounds. Similarly to *K. pneumoniae*, the bactericidal activity started at 4 hr of the experiment. This bactericidal observation against MDR- *K. pneumoniae* suggests that indeed the bioactivities of **L2** and **L3** were similar to **AgSD**. In conclusion, more indepth studies are required to verify the mode of action of the compounds tested herein.

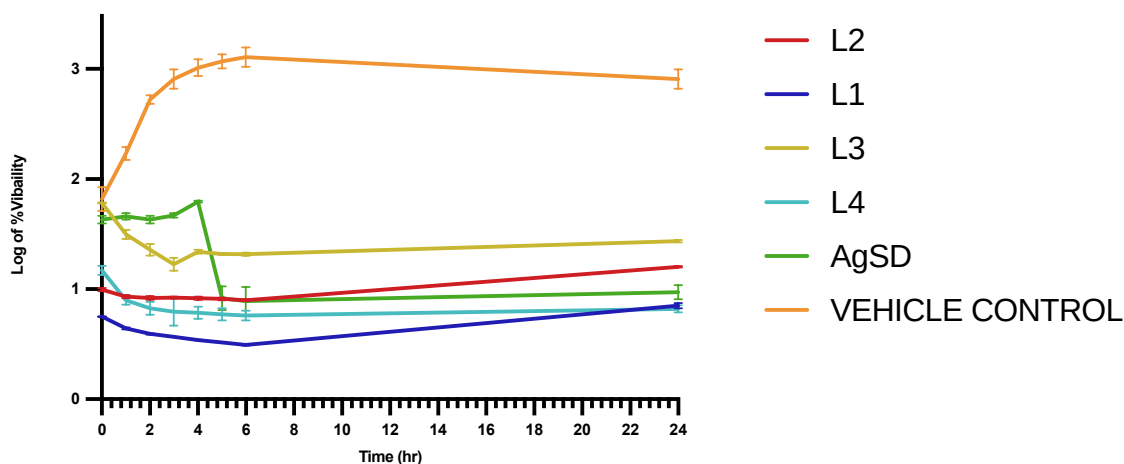


Figure 30: Time kill kinetics of the ligands against MDR-*K. pneumoniae*.

3.0.3.3 Folic Acid Production Studies of the L2 and L3

Folic acid production in bacteria plays a crucial role as it serves as a vital nutrient utilized for the synthesis of nucleic acids and proteins, essential components for the optimal functioning of bacterial cell [60]. In that regard, an ELISA assay was performed to assess the production of folic acid from both the treated and untreated *K. pneumoniae*. The assay was performed after a 24-hour incubation period of the bacteria separately treated with the active compounds **L3**, **L2** and the control drug **AgSD**. We observed that all bacteria separately treated with the active compounds and the control drug showed some production of folic acid. Figure 31 shows that **L3** resulted in less production of folic acid followed by **L2** and then **AgSD**. This can be attributed to compounds **L2** and **L3** functioning as competitive inhibitors of bacterial PABA which is a substrate of the enzyme dihydropteroate synthetase. Like PABA, **L3** and **L2** have the amino group which in case of PABA have been shown to bind to DHPS. Therefore, the efficacy of **L3** and **L2** may have resulted from preventing *K. pneumoniae* from producing folic acid through competitive inhibition. Notably, the presence of an amino group in the sulfonamide moiety of the control drug **AgSD** did not seem to have an effect of being a competitive inhibitor of bacterial PABA as in **L3** and **L2**. This could be attributed to the observations that **AgSD**'s primary mode of action involves disrupting the cellular membrane of the target bacteria rather than that of competitive inhibition [136]. As shown in Figure 31, **L3** was more effective at reducing the production of folate, followed by **L2** and lastly by **AgSD**. These findings suggest that the two compounds' amino group and structural similarity to PABA played a role in their inhibition of biosynthesis of folates. This is consistent with both the traditional sulphonamides and some recent findings that indicated that the presence of the amino group inhibited the biosynthesis of folic acid [60], [142]–[144].

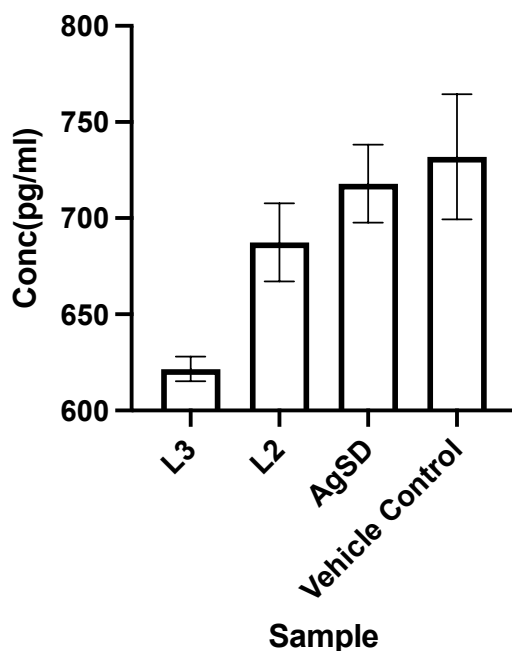


Figure 31: FA production by *K. pneumoniae* after treatment with **AgSD**, **L2** and **L3**

3.0.3.4 Cell Wall/Membrane Disruption Studies of the active Ligands by AKP Assay

The inner bacterial membrane acts as a permeability barrier, allowing the passage of small ions that help sustain some enzymatic activities, maintain normal metabolism, and promote cell membrane function [145], [146]. Thus, one of the modes of action of antibacterial agents is through the disruption of cell membrane which could result in the leakage of electrolytes [147]. Bacterial intracellular enzyme AKP is located between the cell wall and membrane [148], therefore extracellular AKP activity should not be detected in bacteria with intact cell walls and membranes. In comparison to vehicle control (2.21×10^{-5} U/g), AKP activity significantly increased when *K. pneumoniae* was treated with **AgSD** (6.06×10^{-5} U/g) (Figure 32), suggesting that the main mechanism of action of the control drug **AgSD** was via disruption of the cell membranes. Although the active compounds **L2** (3.91×10^{-5} U/g) and **L3** (3.45×10^{-5} U/g) induced less membrane disruption than **AgSD**, they also demonstrated a significant amount of AKP release above that of the vehicle control (2.21×10^{-5} U/g). These findings provide evidence that the disruption of the cell membrane of *K. pneumoniae* may be a secondary mechanism of action for these compounds. Questin, a compound with similar functional groups such as the ether,

carbonyl, and aromatic rings found in **L2** and **L3**, demonstrated similar activity of AKP release after a bacterial membrane was ruptured [149].

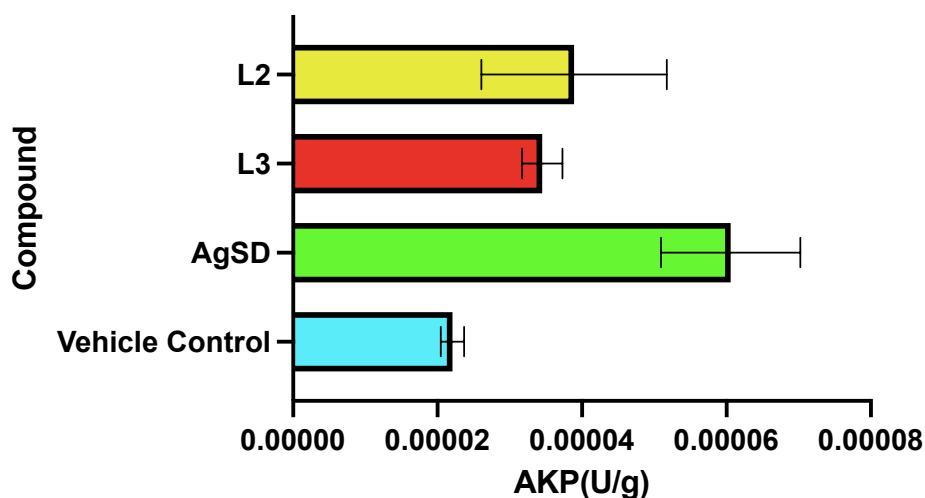


Figure 32: AKP Production *K. pneumoniae* after treatment with **AgSD**, **L2** and **L3**

3.0.3.5 Molecular Docking Studies of the active Ligands

In silico candidate drug screening benefits greatly from the use of molecular docking and it is becoming more significant in rational drug design. A computer process called docking looks for a ligand (compound in this case) that will match the protein's binding site both energetically and geometrically. In other terms, it is the study of how two or more molecules fit together, such as a protein and a ligand [150], [151]. The Gram-negative *K. pneumoniae* possesses of a cell membrane that serves a variety of important tasks, including acting as a diffusion barrier to external solutes and interacting with the bacterial environment [152]. Phospholipids, lipopolysaccharide, and outer membrane proteins (OMPs) make up the bilayer that constitutes this membrane. The porin family of OMPs, which makes up a majority of OMPs, creates water-filled channels in the outer membrane that allow the transport of tiny hydrophilic solutes. Porins can be categorized into two classes; the nonspecific porins (such as OmpC and OmpF), which allow for the diffusion of tiny polar molecules (600 Da), and specialized porins such as LamB, which support the diffusion of substrates [153]. The molecular docking studies in this work was focused on the β -lactam antibiotic resistant OmpK36 (PDB: 6rd3) of *K. pneumoniae* to appreciate the binding process through which all substances interacted with the essential amino acids of

this bacterial protein [154]. The basis for the choice of 6rd3 was due to the drug resistant nature of the protein towards drugs that target the cell membrane, also due to the acceptable crystal parameters of the structure. Table 16 shows the molecular docking scores of the compounds **L2**, **L3** and the control drug **AgSD** towards OmpK36, whereas Figures 33, 34 and 35 show the 3D and 2D pictorial representations of the binding interactions of **L2**, **L3** and the control drug **AgSD**. This report provides the first report on the molecular docking of the active compounds and the control drug **AgSD**. The control drug **AgSD** showed hydrogen bonding with the amino acid residue Arg37 and hydrophobic interactions with Lys116, Tyr35, and Glu110. Furthermore, **AgSD** presented van der Waals interactions with Phe111, Gln61, Asp18 and Leu20. This site was different from those of **L2** and **L3**, where **L2** showed hydrogen bonding with the amino acid residues Asp12 and Glu2, also hydrophobic interactions with Lys10 and Lys41. Moreover, **AgSD** showed Van der Waals interactions with the residues Tyr53, Glu43, Gly42 and Gln55. The results for the control drug showed that the structure where silver is coordinated to both nitrogen and oxygen agree well with the experimental results. The visualization of the interactions showed that **AgSD** has hydrogen bonding with the amino acid residue Tyr99, Glu110, and Gly112, and hydrophobic interactions with Arg75, Asp106 and Arg125. Furthermore, **AgSD** presented van der Waals interactions with Tyr95, Phe111, Gly113, and Ser118. **L1** was also active with interactions of the type; hydrogen bonding with the amino acid residues Asn120 and Ser331, also, hydrophobic interactions with Ile330 and Arg244. **L3** was also active on a different site, with interactions of the type; hydrogen bonding with the amino acid residues Asn120 and Ser331 and hydrophobic interactions with Ile330 and Arg244. Furthermore, **L3** showed Van der Waals interactions with the residues Gly117, Ala242, Tyr116 and Asp114. The binding energies of these compounds towards OmpK36 were -6.27, -5.52 and -5.27 kcal/mol for **AgSD**, **L3** and **L2** respectively, showing that the control was the best followed by **L3** and lastly **L2**. Ultimately, because the molecular docking study was done on the interactions of the outer membrane, it was observed that the data corroborated well with that of the AKP release activity where the bioactivity trend was observed to be as follows; **AgSD>L3>L2**.

Table 16: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands and their complexes against OmpK36 from *K. pneumoniae* (PDB: 6rd3)

Compound	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
AgSD	-6.27	25.55 μ M	Arg37	Lys116,Tyr35, Glu110	Phe111,Gln61, Asp18,Leu20
AgSD ⁺	-5.70	65.97 μ M	Tyr99, Glu110, Gly112	Arg75,Asp106, Arg125	Tyr95,Phe111, Gly113, Ser118
L2	-5.27	136.23 μ M	Asp12,Glu2	Lys10,Lys41	Tyr53,Glu43, Gly42,Gln55
L3	-5.52	89.35 μ M	Asn120,Ser331	Ile330,Arg244	Gly117,Ala242, Tyr116,Asp114

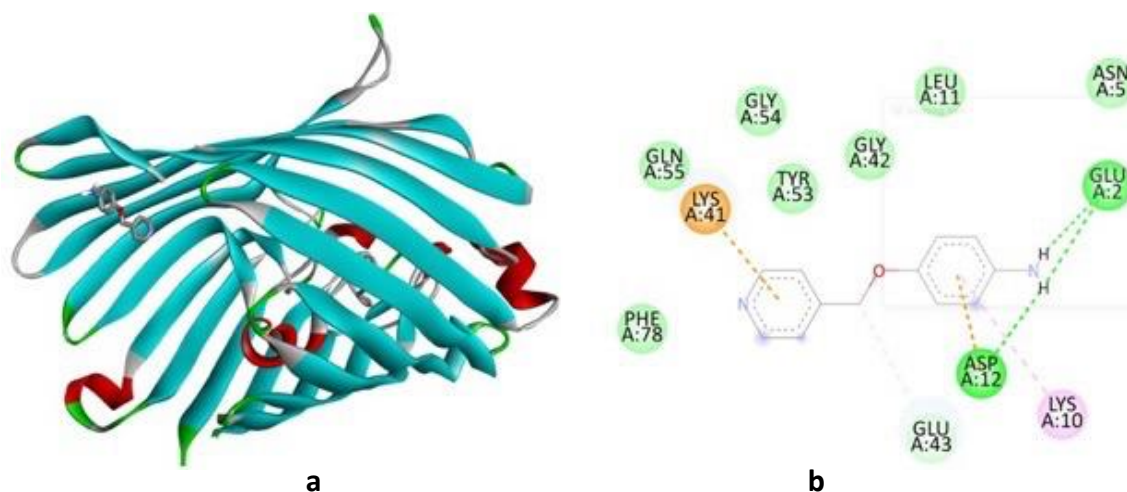


Figure 33: The binding interactions of **L2** against OmpK36 from *K. pneumoniae* (PDB: 6rd3) a) shows the binding site, b) shows the interacting amino acid residues.

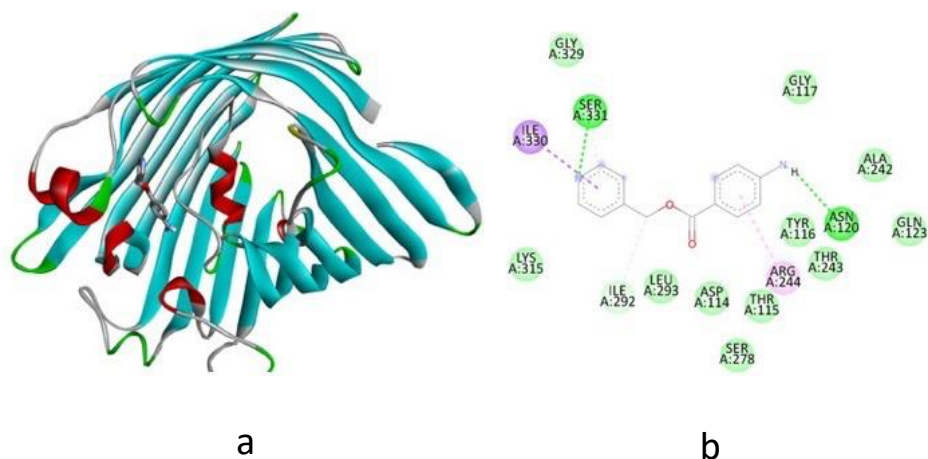


Figure 34: The binding interactions of **L3** against OmpK36 from *K. pneumoniae* (PDB: 6rd3) a) shows the binding site, b) shows the interacting amino acid residues.

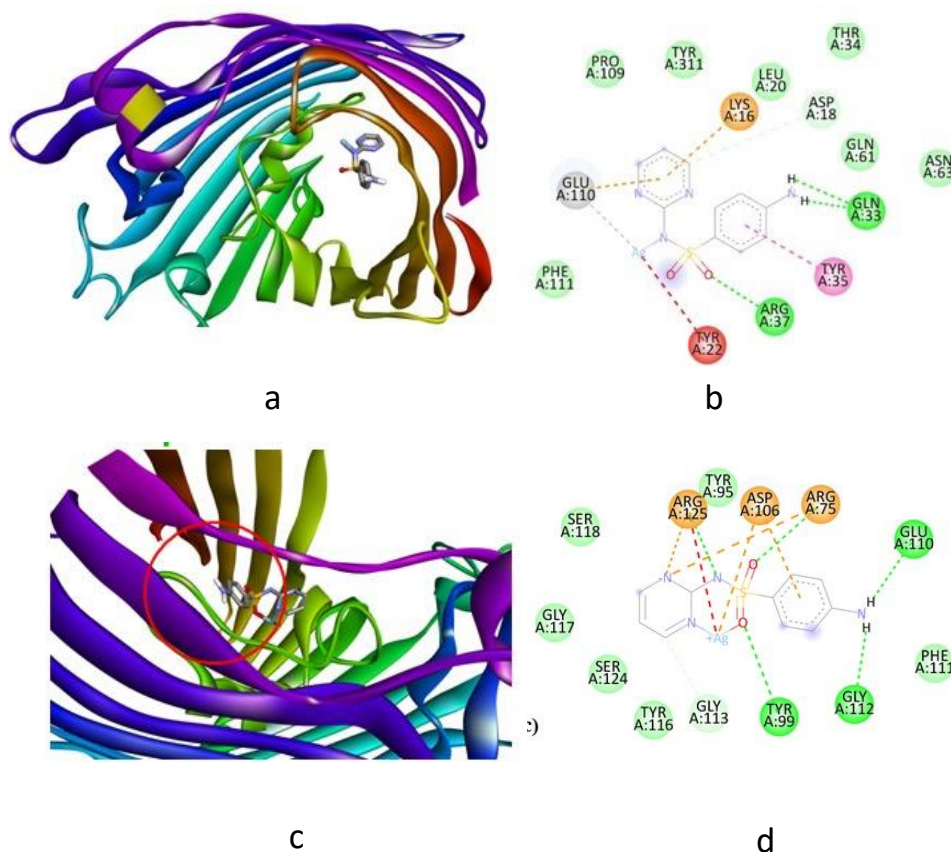


Figure 35: The binding interactions of **AgSD** against OmpK36 from *K. pneumoniae* (PDB: 6rd3) a) shows the binding site, b) shows the interacting amino acid residues and **AgSD*** c) shows the binding site, d) shows the interacting amino acid residues.

Dihydropteroate synthase (DHPS) is a key enzyme only found in bacteria and not eukaryotes which aid in folic acid biosynthesis by catalyzing the condensation of 6-hydroxymethyl-7,8-dihydropteridine pyrophosphate with *para*-aminobenzoic acid (PABA) to form 7,8-dihydropteroate. Folic acid is required for the synthesis of nucleic acids and therefore it is a target for sulfonamide antibiotics [155]. The choice for the DHPS protein 3tye was used because it was bound to a sulfonamide antibacterial and proved to be a better protein in comparison for PABA antagonism by the synthesized compounds. Molecular docking of the compounds towards the enzyme DHPS was done on the PABA binding pocket to understand if they have competitive antagonistic abilities to PABA. According to the binding energies, the compounds including the control had better binding scores than PABA, proving their antagonistic abilities to PABA. However, contrary to the folic acid ELISA, the control **AgSD** bound only to nitrogen had a better binding score than the compounds **L2** and **L3**. Even though their binding energies were within a similar range between – 6 to – 6,7 kcal/mol. Similar to the experimental results, **L1** showed better binding affinity (-6.37 kcal/mol) compared to the control **AgSD** bound to both nitrogen and oxygen in the sulphonamide ligand (6.15 kcal/mol). On the other hand, the binding affinity of **L2** was found to be slightly lower than that of the control drug. The interaction with the PABA binding pocket of the DHPS enzyme requires the three amino acid residues Lys220, Ser218 and Phe189. A common finding for all sulphur [156] drugs is that benzene-sulfonamide moieties occupy this PABA binding pocket [157]. An analysis of the amino acid binding residues shows that for hydrogen bonding **L3**, **AgSD** and PABA all bound to Arg254. While the control also bound to Thr67 like in PABA, and **L3** bound to Asp184 but PABA interacted with the similar Asp101. The compound's hydrophobic interactions show that the control **AgSD** and **L3** were similar in binding towards Arg68 and Met145, while **L2** did not interact with similar amino acid residues. However, **L3** interacted with Phe189, while the control **AgSD** bound to Lys220 through van der Waals amino acid residue interactions just like in PABA. Moreover, **L3** and **L2** did not bind to any amino acid residues PABA interacted with by van der Waals interactions. Conclusively, we report that **L3** and the control **AgSD** may be binding to the PABA binding pocket of DHPS. Our results on the binding properties of these compounds are similar to previous findings of analogous pharmacophores interacting with the PABA binding pocket of this enzyme DHPS [158]. The molecular docking

scores of the compounds and their amino acid residue interactions are summarized in Table 17 with their 3D and 2D pictorial representations shown in Figures 36 through 39.

Table 17: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands against Dihydropteroate Synthase DHPS (PDB: 3tye)

Compounds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
L2	-6.03	37.77 μ M	Tyr103,Trp123	Phe71,Lys73	Ser66,Thr67, Ala72,Ala190
L3	-6.37	21.53 μ M	Asp184,Arg254	Arg68,Pro69, Met145,Phe189	Thr67,Ile122, Ile143,Gly216
AgSD	-6.65	13.28 μ M	Thr67,Asn120, Arg254	Met23,Arg68, Met145,Asp184	Pro69,Lys220
AgSD*	-6.15	24.81 μ M	Thr67	Phe71,Lys73, Tyr103,Trp123	Ser66,Ile122, Asn147,Phe189
PABA	-3.80	1650 μ M	Thr67,Asp101, Gly216,Arg254	Ile122,Phe189	Arg68,Asn120, Asp184,Lys220

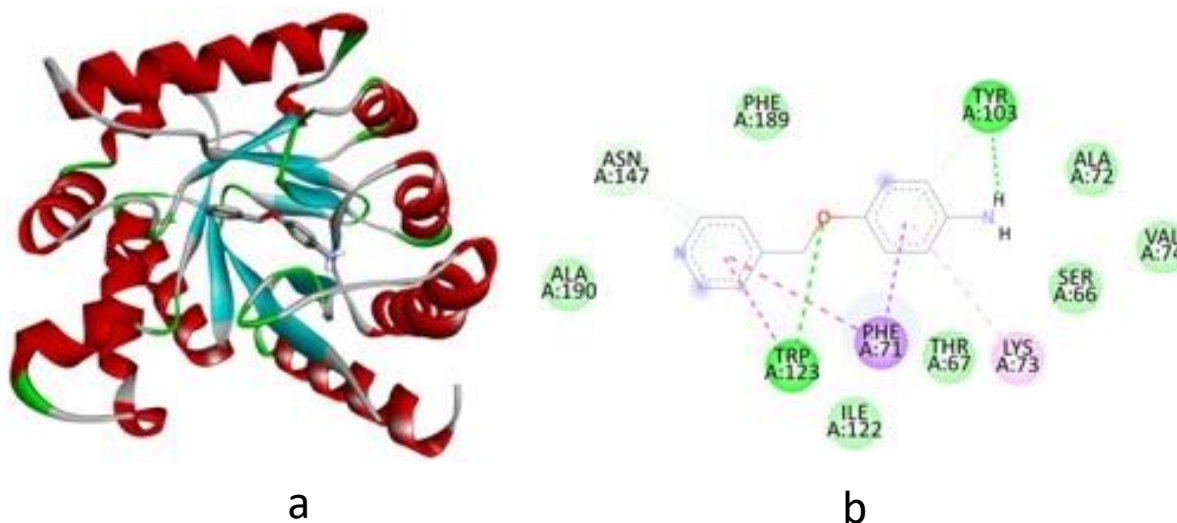
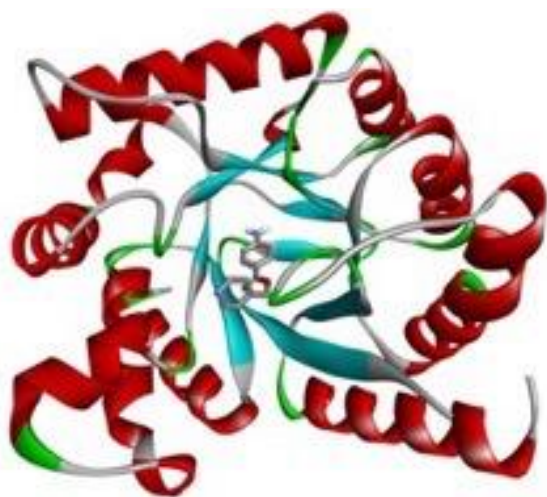
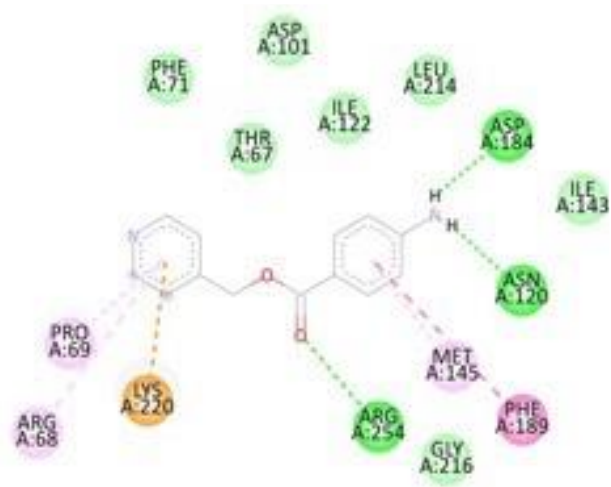


Figure 36: The binding interactions of **L2** against Dihydropteroate Synthase DHPS (PDB: 3tye) a) shows the binding site, b) shows the interacting amino acid residues.



a



b

Figure 37: The binding interactions of **L3** against Dihydropteroate Synthase DHPS (PDB: 3tye) a) shows the binding site, b) shows the interacting amino acid residues.

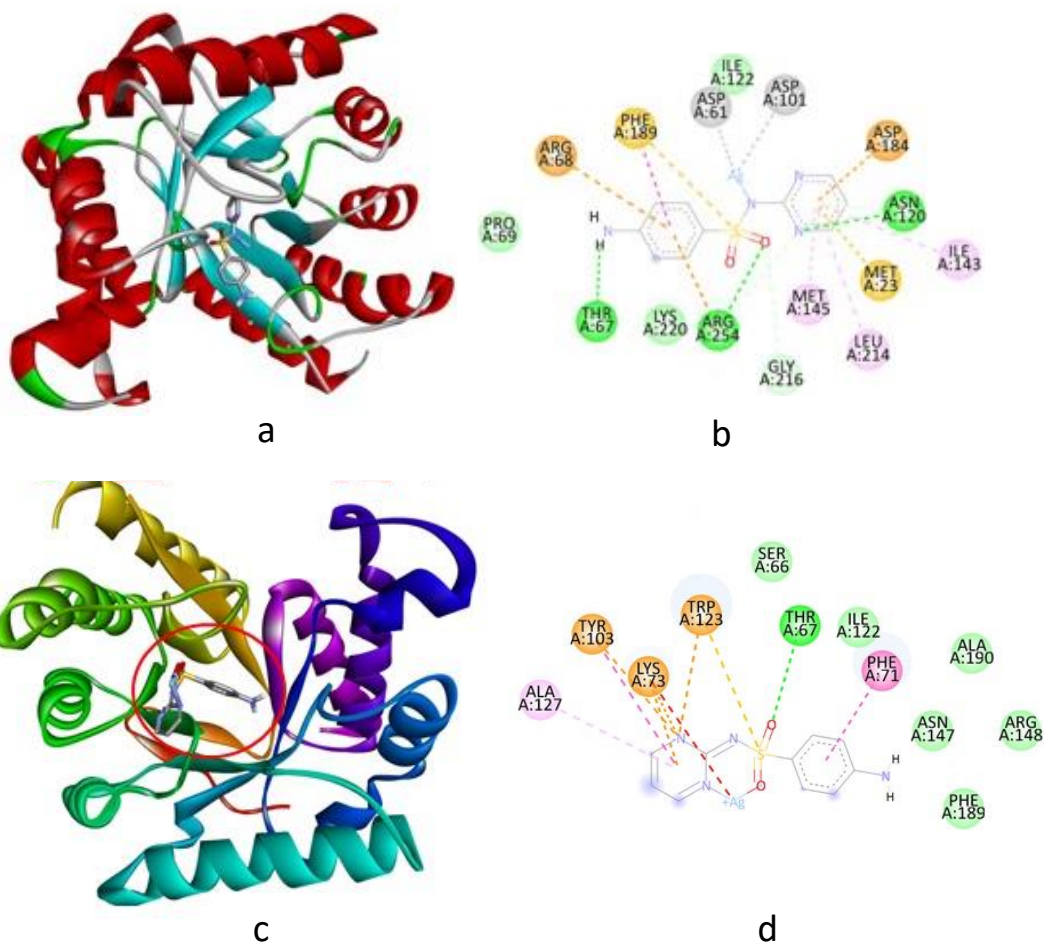


Figure 38: The binding interactions of **AgSD** against Dihydropteroate Synthase DHPS (PDB: 3tye) a) shows the binding site, b) shows the interacting amino acid residues and **AgSD*** c) shows the binding site, d) shows the interacting amino acid residues.

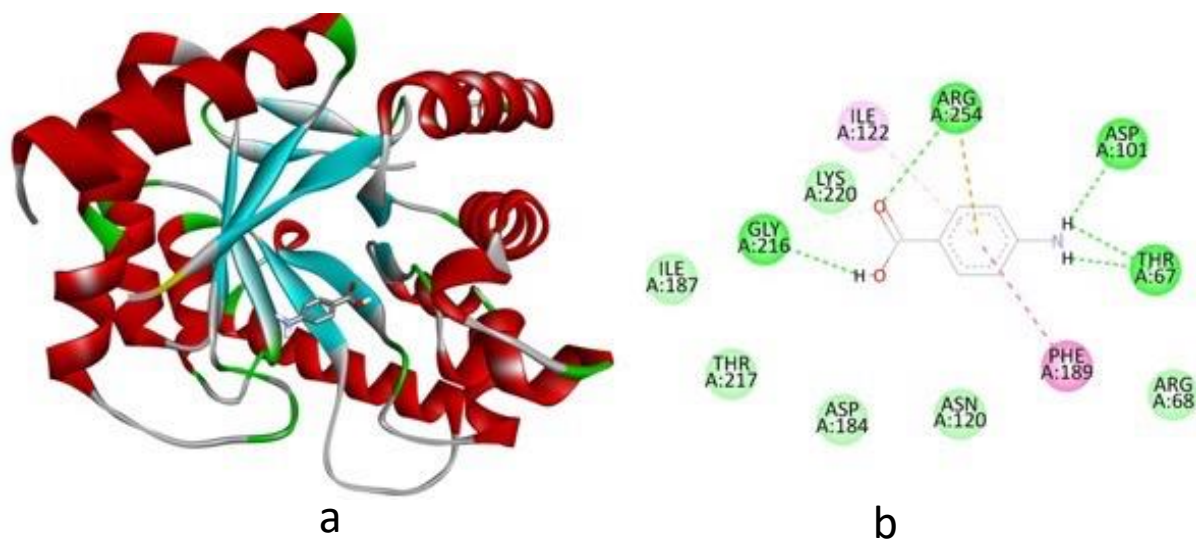


Figure 39: The binding interactions of PABA against Dihydropteroate Synthase DHPS (PDB: 3tye) a) shows the binding site, b) shows the interacting amino acid residues.

3.0.4 Conclusions

Compounds **L1-L4** of a pyridyl moiety bridged by an ether or ester groups and derivatized by either a nitro or amino substituent were synthesized, characterized, and assessed for their potential antibacterial properties. This study was done to evaluate potential lead compounds in the fight against antibiotic resistance. The compounds were more active towards Gram-negative bacteria (i.e., *K. pneumoniae* and MDR- *K. pneumoniae*) than Gram-positives (i.e., *S. aureus* ATCC 25931 and MRSA) with **L2** and **L3** being more potent against *K. pneumoniae* than **AgSD**. Furthermore, the time kill kinetics of the Gram-negative bacteria showed converse results, that is, for the drug resistant bacteria all the compounds were observed to be bactericidal. However, for the non-drug resistant bacteria, the amino group compounds **L2**, **L3** and the control **AgSD** were observed to be bacteriostatic while the nitro group compounds **L1** and **L4** were bactericidal. Furthermore, the active compounds **L2** and **L3** had multiple modes of action. It was observed that **L3** was better at reducing the production of folic acid through an ELISA, while molecular docking towards the enzyme DHPS revealed that all the compounds had competitive antagonism against PABA towards DHPS. AKP release activity revealed that **AgSD** was better at disrupting the cellular membrane followed by the compounds **L2** and **L3** that showed activity above that of the vehicle control. The molecular docking experiments with the porin protein OmpK36 revealed that the

compounds did not interact with the protein at the same positions. Furthermore, the binding energies of these compounds agreed with the AKP release data. Finally, the reported potent concentrations of the compounds qualify them as good candidates or lead compounds for the development of new antimicrobials. Future studies include studying these compounds' toxicity towards normal mammalian cells, their folic acid biosynthesis inhibition, as well as cell membrane disruption against the *K. pneumoniae* strains, to determine their possible mode of action.

3.1 Synthesis, Characterization and Antibacterial Properties of Cobalt Mixed Ligand Complexes

3.1.1 Synthesis of the Cobalt Complexes

The Co (II) complexes of **4PY**, **L1**, **L2**, **L3** and **L4** were successfully synthesized by reaction of metal salts with the ligands. The yields, physical properties such as appearance, colour, melting points and solubility tests data is shown in Table 18. Complexes of **4PY** were synthesized using the conventional solvent reactions while the complexes of **L1**, **L2**, **L3** and **L4** were done through a greener technology method of co-grinding. The method of grinding produces in most cases 80.2-87.99 % yields as shown in Table 18 within a shorter time as compared to 50-60 % by classical solution phase method which usually takes 3-4 hr for the reaction to complete.

3.1.2 Purity, Solubility and Physical Properties of the Cobalt Complexes

The cobalt complexes were synthesized in high yields; however, the solvent reaction compounds were formed at yields lower than 80%. All the complexes were seen to have solubility towards highly polar solvents such as dimethylformamide and dimethyl sulfoxide. The acido complex crystal of cobalt **Co4PY-H** was blue in both the solution and on the solid state. Otherwise, the complex **Co4PY** was blue in the solution and immediately turns pink when the solvent dries. This colour change was due to the existence of geometrical isomer of the cobalt complex [159]. It was suggested that the pink coloured complex acquires the *cis* geometry whereas the blue-coloured complex acquires the *trans* geometry. This colour change also suggests the solvent complex interaction, *trans* geometry is stable in solution and under higher temperatures whereas *cis* geometry is more stable in the absence of the solvent. When heating the pink compound to higher temperatures above 100 °C the colour change from pink to blue was observed. The melting point of the cobalt complex and the acido complex crystal were determined and are recorded in Table 18. The acido complex crystal of cobalt was blue in both the solution and in the solid state. Contrariwise, the complex was blue on the solution and immediately turned pink when the solvent dried.

The cobalt complex **Co4PY** exhibited a higher melting point compared to its respective acido complex crystal **Co4PY-H**, which is consistent with the findings reported in the literature [160].

This observation can be attributed to the presence of strong covalent bonds formed between the central metal and the ligand in the metal complex. In contrast, the acido complex crystal was expected to have a lower melting point due to the absence of strong bonds between the metal atom and the ligand. Instead, they are held together by weak intermolecular forces arising from halogen bonding interactions. Furthermore, the data on halogen bonding in the cobalt acid complex from section 3.1.7 supports the notion that weak intermolecular forces contribute to a lower boiling point in the acido complex. The measured bond lengths and angles (as shown below in section 3.1.7 Table 22) provide evidence of the halogen bonding interactions. These measurements further validate the presence of weak halogen bonding forces that contribute to the lower boiling point observed in the acido complex.

The cobalt complexes were successfully synthesized using different ligands and preparation methods. **CoL1**, obtained from co-grinding, formed a blue fine powder with a yield of 76% and a melting point of 211 °C. It exhibited solubility only in dimethylformamide and dimethyl sulfoxide, suggesting high polarity. Similarly, **CoL2**, prepared through mechanochemical synthesis, showed a good yield of 80.2% and high polarity, readily dissolving in highly polar solvents. This complex also displayed the highest melting point among the cobalt complexes, which can be attributed to potential hydrogen bonding involving the amino groups. Another cobalt complex, **CoL3**, exhibited a yield of 83%. Unlike the previous complexes, **CoL3** showed solubility in alcohols and partial solubility in ethyl acetate, acetonitrile, and water at room temperature. Its high melting point of 220 °C was influenced by the presence of both the amino group and the ester group. Lastly, **CoL4**, achieved a high yield of 87%, similar to the other mechanochemically formed complexes. In summary, the cobalt complexes exhibited different physical properties, such as solubility and melting points, which can be attributed to variations in the ligand structures employed.

Table 18: Physical Properties and Solubility of Cobalt Complexes

Complex	Appearance	Melting Point °C	Solubility	%Yield
Co4PY	Violet crystalline solid	147	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone, water	79.4
Co4PY-H	Blue crystalline solid	126	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone, water	62.1
CoL1	Violet powder	211	DMSO, DMF	76.1
CoL2	Blue powder	241	Methanol Δ , ethanol Δ , DMF, acetonitrile Δ , water Δ	80.2
CoL3	Blue Powder	220	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone, water	83.0
CoL4	Green powder	Above 250	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone, water	87.0

Δ : Soluble with heat

In terms of the purity of the synthesized complexes, elemental analysis was used to measure the elemental composition of the complexes. Furthermore, this data was also used to calculate the percentage purity of these compounds. From the data, it was observed that the ionic complex **Co4PY-H** was completely pure. However, all the other complexes showed an impurity of the cobalt chloride salt used in the reaction. Of these compounds, the less pure complexes were **CoL3** and **CoL4**. As for **CoL3** the complex was also found to have 3.7 moles of water, which means that recalculating the purity would change it to 71 % with only 2 moles of the cobalt chloride salt. But, **CoL4** had only 1.35 moles of water, this means that it had the worst purity of the complexes. To purify these, the reactions were done for longer and the products were then recrystallized from hot methanol before downstream analyses. The elemental analysis and purity of the complexes are summarized in Table 19.

Table 19: Elemental Analysis and Purity of Cobalt Complexes

Complex	CALCULATED (FREE CHN)			FOUND			PURITY	
	N	C	H	N	C	H	IMPURITIES (mol)	% PURITY
Co4PY	7.62 (7.49)	39.23 (38.53)	3.29 (4.85)	7.53	39.32	3.28	0.85 4PY	78
Co4PY-H	6.15 (6.15)	31.62 (31.62)	2.65 (2.65)	5.83	31.10	2.91	100% PURE	100
CoL1	7.34 (8.67)	40.92 (48.32)	2.64 (3.12)	7.02	40.95	2.87	0.90 CoCl ₂	85
CoL2	10.74 (10.57)	55.28 (54.36)	4.64 (4.56)	10.21	55.76	4.87	0.20 L2	93
CoL3	6.14 (9.56)	34.22 (53.26)	3.47 (4.13)	6.01	33.65	2.90	2 CoCl ₂ 3.70 H ₂ O	74
CoL4	6.41 (9.49)	32.97 (48.84)	2.62 (3.42)	6.31	32.78	2.40	2 CoCl ₂ 1.35 H ₂ O	68

3.1.3 FTIR Characterization of the Cobalt Complexes

To study the binding mode of the ligand to the metal in the complexes, the vibrational spectral data of the complexes was compared with those of the free ligand to determine the atoms that may have been involved in coordination. The vibrational frequencies of the selected peaks are recorded in Table 12 and the spectra of the ligand and the complexes are shown in Appendix 5.1. In the spectrum of **Co4PY-H** the Ar-N stretch was observed at 1415.4 cm⁻¹ showing no change as compared to the free ligand frequencies which suggest that the nitrogen atom of the pyridine was not involved in the bonding. However, in **Co4PY-H** this nitrogen was bound to hydrogen and a peak associated with this N-H stretch was found at 3370 cm⁻¹. This peak was not found on the spectrum of the complex **Co4PY**. From the spectrum of the cobalt complex **Co4PY**, the Ar-C=N absorption bands appeared at 1437 cm⁻¹ which was a shift to lower frequencies (the ligand Ar-C=N band was at 1480.5 cm⁻¹) which suggested the involvement of the nitrogen of the ligand in bonding to the metal. As for **CoL1**, all the important functional groups present in the ligand were observed in the spectrum of the complex. For instance, the aromatic C-H stretch was observed at 3046.3 cm⁻¹. Also, the pyridine nitrogen of the ligand was observed at 1425.9 cm⁻¹. Similarly,

CoL3 showed all the functional groups of the ligands in its FTIR spectrum. The most important being the Ar-NH₂ at 3299.6-3467.5 cm⁻¹. Also, the prominent strong ester shifts at 1645.6 cm⁻¹. The donor pyridyl shift was observed at 1432.4 cm⁻¹. **CoL2** also showed all the important functional groups of its ligand, the ether group was assigned to the 1044.2 cm⁻¹. While the Ar-NH₂ was observed at 3232.5-3291.8 cm⁻¹. Then its binding donor atom Ar-C=N was assigned to 1448 cm⁻¹. The final ether based complex **CoL4** showed similar bands as compared to **CoL2**. However, they differed only with the nitro and amino groups. The Ar-NO₂ of **CoL4** was assigned to 1341 to 1390 cm⁻¹. While the ether band was given the prominent shift 1063.6-1029 cm⁻¹. Then finally, the donor Ar-C=N was observed at 1445 cm⁻¹. The complexes show a shift into lower vibrational frequency of the ligand band at 1459.5 cm⁻¹ for the bonds that were affected by coordination of the metal. This was attributed to the reduction or increment of electron density around those bonds. The decrease of electron density will result in the decrease of vibrational frequency as per Hooke's law. A summary of the assigned vibrational frequencies for the cobalt complexes is shown in Table 20.

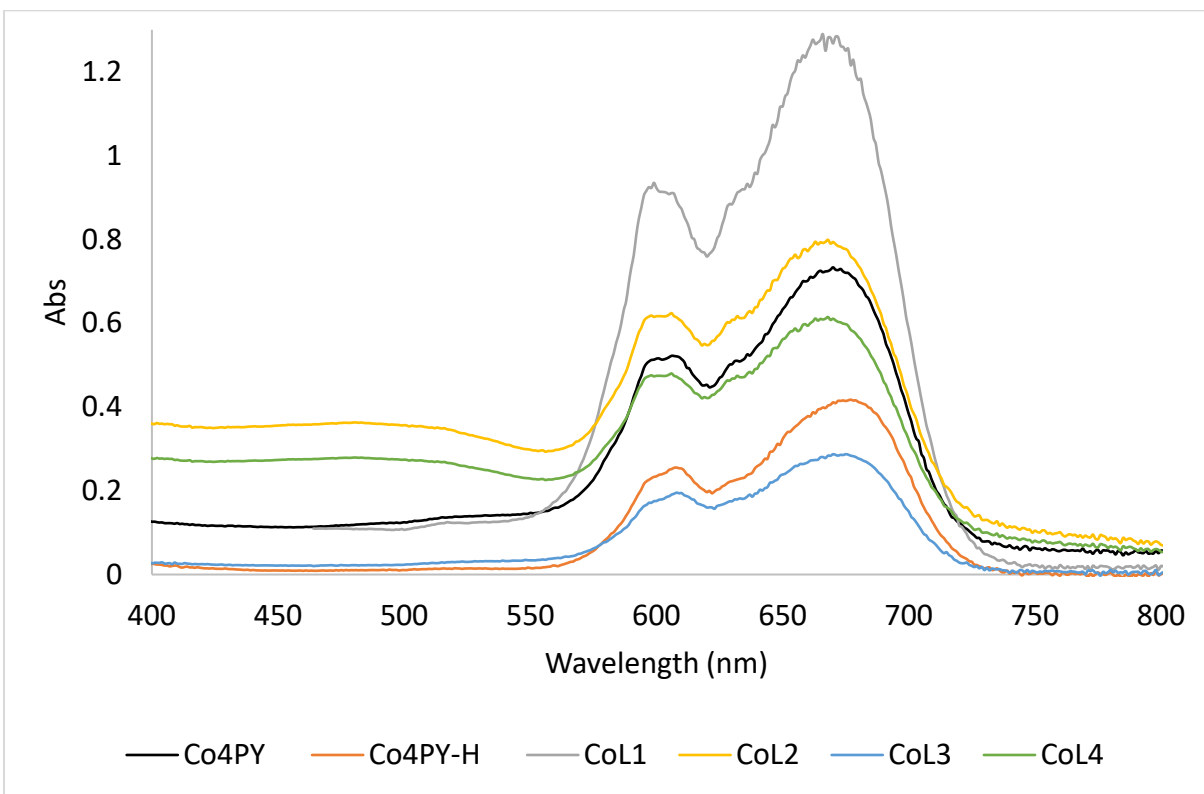
Table 20: Selected Vibrational Frequency Bands of Cobalt Complexes

Molecular motion	Co4PY (cm ⁻¹)	Co4PY-H (cm ⁻¹)	CoL1 (cm ⁻¹)	CoL2 (cm ⁻¹)	CoL3 (cm ⁻¹)	CoL4 (cm ⁻¹)
C=C	1502.1	1502.1	1525.7	1512.4	1500.2	1594.6
C-O-O ether	-	-	-	1044.2	-	1063.6-1029.0
O=C-O ester	-	-	1729.1	-	1645.6	-
Ar-C-H	3077.0	3041.8	3046.4	3151.3	3206.0	3173.4
Ar-NH ₂ /N-H	-	3370.0	-	3232.5-3291.8	3299.6-3467.5	-
Ar-NO ₂	-	-	1348.9-1425.5	-	-	1341.0-1390.0
Ar-C=N	1415.6	1415.6	1425.9	1450.0	1432.4	1445.0
Aryl-O stretch	-	-	1278.2	1240.8		1263.0

2.0.4. Electronic and Magnetic Properties of the Cobalt Complexes

Electronic spectra and magnetic measurements were performed on the synthesized complexes to understand the optical and magnetic properties of the complexes. Table 21 shows the summary of magnetic susceptibilities and the electronic transitions of the complexes. From the magnetic measurements the experimental effective magnetic moment for the ionic compound

Co4PY-H and the complex **Co4PY** were found to be 3.92 BM and 3.97 BM respectively and this resembles a d^7 Co (II) tetrahedral complex. This was because the experimental values were very close to the expected value of a spin only magnetic moment of a d^7 tetrahedral complex which is 3.87 BM which indicate that the t_2 orbital was symmetrically filled with three unpaired electrons. The UV-Visible spectra of cobalt complex and its acido complex crystal are shown in spectrum 9 and the peak assignments are shown in Table 13. The compound **Co4PY-H** spectra show three absorption bands at 336, 608 and 677 nm. The absorption bands below 350 nm were assigned to charge transfer transitions and ligand transitions. These transitions were of high energy and hence appear at shorter wavelengths. The band at 336 nm was assigned to $\pi - \pi^*$ transition from the aromatic system. Both the cobalt complex and the acido complex crystal show two bands at 609 and 675 nm and these bands were assigned to the $d-d$ transitions. The first absorption band was assigned to the transition, $^4A_2 \rightarrow ^4T_2$ whereas the second absorption band was assigned to the transition $^4A_2 \rightarrow ^4T_1$. According to cobalt (II) tetrahedral complex, three spin-allowed bands were expected but only two bands were observed.



Spectrum 9: Electronic Spectra of cobalt complexes

The cobalt complex **CoL2** had an effective magnetic moment of 5.21 BM which suggests an octahedral d^7 complex and very much close to the calculated value 5.20 BM. However, according to the elemental analysis data and mass spectrometry this complex possessed a tetrahedron geometry. Thus, it was concluded that this was a tetrahedrally coordinated cobalt(II) that had unique magnetic properties that may have come from the position the ligands and their functional groups which may have led to a small energy gap and higher magnetic susceptibility, consistent with Literature reports [161]. This cobalt (II) complex exhibited two prominent bands at 675 and 609 nm and a weak band at 484 nm on spectrum 9. According to a tetrahedral d^7 crystal field, which was observed on a d^3 Tanabe Sugano diagram and the following transitions were assigned $^4A_2 \rightarrow ^4T_2(F)$, $^4A_2 \rightarrow ^4T_1(F)$ and $^4A_2 \rightarrow ^4T_1(P)$ respectively. Furthermore, **CoL1** shared similar properties with **CoL2**, it was also concluded to be tetrahedrally coordinated, it had two visible spin allowed transitions 600 and 701 nm. However, bands below 350 nm were assigned as ligand transitions and charge transfer transitions. The ligand to metal charge transfer transitions occurred in the UV region, as it is the norm for most transition complexes [162]. The oxidation state and the number of unpaired electrons of **CoL1** complex were determined, the effective magnetic moment of **CoL1** complex was found to be 5.272 BM. This was consistent with a complex having a calculated electron spin of 1.5 in a d^7 crystal field. A d^3 Tanabe Sugano diagram shows 3 transitions, which also confirms the 2 transition bands observed in the UV-Vis spectra for cobalt complex shown in spectrum 9. The other band may have been buried within the high energy charge transfer bands or the ligand transitions. Also, the magnetic moment with orbital contribution was calculated to be 5.2 BM which was in line with that of the effective magnetic moment. Therefore, the complex was assumed to be a d^7 tetrahedral cobalt complex. The **CoL4** and **CoL3** complexes were also observed to be d^7 tetrahedral just as in **CoL2**. They both showed two energetically observable $d-d$ transitions and their effective magnetic moments were also consistent with a d^7 tetrahedral cobalt complex. The complexes **CoL1**, **CoL2**, **CoL3**, and **CoL4** all showed that conversely to the **4PY** complexes they had a magnetic moment with orbital contribution which is consistent with a high effective energy barrier of an electron spin of 1.5 in a tetrahedrally coordinated complex [161], section 3.1.7 confirms their tetrahedral structure with

the **CoL3** complex crystal structure. A summary of all the cobalt complexes electronic transitions and effective and calculated magnetic moments are shown in Table 21.

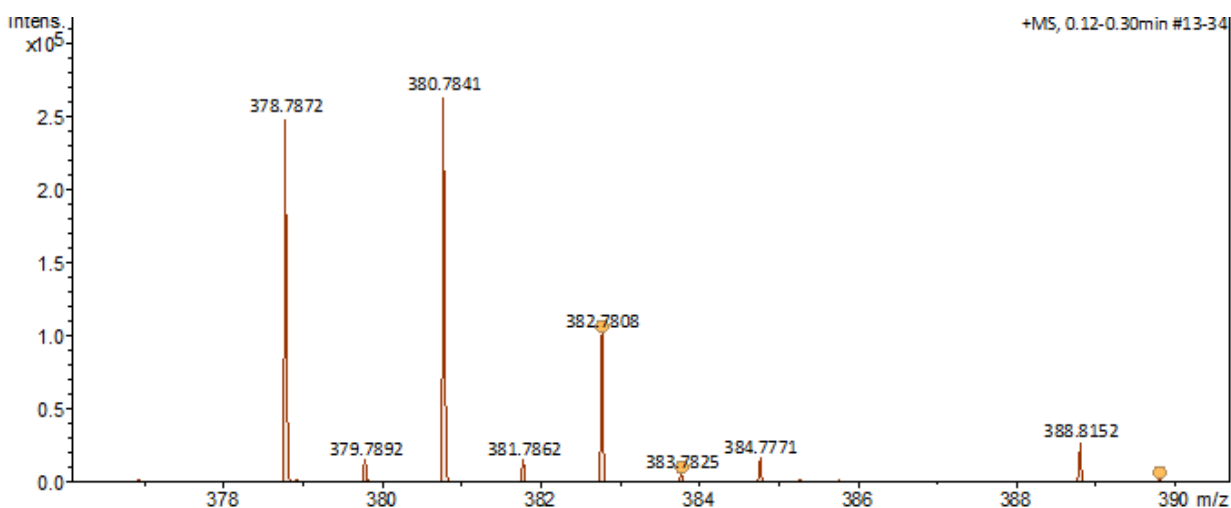
Table 21: Electronic Transitions and Magnetic Susceptibility of Cobalt Complexes

Complex	Electronic Transition		Magnetic Moments (BM)			
	(nm)		Transition Type	μ_{cal}	μ_{eff}	Crystal Field
Co4PY	π - π^* and CT	<350		3.87	3.97	d^7 Tetrahedral
	$d-d$	673 609	$^4A_2 \rightarrow ^4T_2(F)$, $^4A_2 \rightarrow ^4T_1(F)$			
Co4PY-H	π - π^* and CT	<350		3.87	3.92	d^7 Tetrahedral
	$d-d$	489 675 609	$^4A_2 \rightarrow ^4T_1(P)$ $^4A_2 \rightarrow ^4T_2(F)$, $^4A_2 \rightarrow ^4T_1(F)$			
CoL1	π - π^* and CT	<350		5.20	5.27	d^7 Tetrahedral
	$d-d$	600 701	$^4A_2 \rightarrow ^4T_2(F)$, $^4A_2 \rightarrow ^4T_1(F)$			
CoL2	π - π^* and CT	<350		5.20	5.21	d^7 Tetrahedral
	$d-d$	602 667	$^4A_2 \rightarrow ^4T_2(F)$, $^4A_2 \rightarrow ^4T_1(F)$			
CoL3	π - π^* and CT	<350		5.20	5.50	d^7 Tetrahedral
	$d-d$	610 675	$^4A_2 \rightarrow ^4T_2(F)$, $^4A_2 \rightarrow ^4T_1(F)$			
CoL4	π - π^* and CT	<350		5.20	5.50	d^7 Tetrahedral
	$d-d$	612 672	$^4A_2 \rightarrow ^4T_2(F)$, $^4A_2 \rightarrow ^4T_1(F)$			

2.0.5. Mass Spectrometric Characterization of the Cobalt Complexes

HRESI-MS was employed to confirm the structures of the cobalt complexes, with the mass spectra providing valuable insights into their composition. In this discussion, the mass spectrum of **Co4PY** will be used as an example to illustrate the typical characteristics observed in the

complex spectra. The mass spectrum of **Co4PY** (Spectrum 10) demonstrated the successful synthesis of the complex. The molecular ion peak appeared at 382.7808 m/z, corresponding to the molecular mass of the complex (382.91 g/mol). However, in the spectrum of Co4PY-H, the acido complex, the molecular ion peak was not observed. Instead, the complex transformed into a datively bound coordination compound, resulting in the appearance of the [M-2Cl] peak at 382.7808 m/z, aligning with the mass of **Co4PY**. Moving on to **CoL1**, its mass spectrum exhibited a molecular ion peak [M] with reduced intensity, which could be attributed to the poor solubility of the complex. Nevertheless, the peak at 645.9791 m/z corresponded to the molecular mass of 646.03 g/mol, confirming the presence of the desired complex. In the mass spectrum of **CoL2**, several adducts, including acetonitrile (used as a solvent) and the proton, were observed alongside the molecular ion fragment. The molecular ion [M+2CN+H]⁺ appeared at 614.0641 m/z, consistent with the molecular mass of 613.12 g/mol. Additional fragmentation may have resulted from transformations and the formation of larger mass-to-charge ratio adducts. The mass spectrum of **CoL3** provided further confirmation of the complex's structure where the molecular ion adducts [M+H] was observed at 586.0345 m/z, corresponding to the experimental findings of the unprotonated complex's mass of 585.05 g/mol. Lastly, the mass spectrum of **CoL4** depicted the complex's structure despite numerous observed transformations and adduct formations beyond the assigned molecular ion peak. The molecular ion peak was assigned to the [M+K] adduct, appearing at 627.9952 m/z and coinciding with the molar mass of 627.97 g/mol when potassium was included. Overall, the mass spectra of the cobalt complexes provided confirmation of their structures and allowed for the identification of specific adducts and fragments.



Spectrum 10: Mass spectrum of **Co4PY**

3.1.6 Thermal Studies of the Cobalt Complexes

This section presents the results of thermal and calorimetric analyses conducted on a series of cobalt complexes. The aim of this study was to gain insights into the thermal properties and decomposition behavior of these complexes. Appendix 5.3 contains the detailed findings, and for clarity and ease of comparison, the thermal spectra of **Co4PY** are only displayed. The thermogram of **Co4PY** exhibited four distinct mass losses. Initially, an 8.0% mass loss was attributed to the elimination of absorbed water, while a subsequent 5.8% loss had no specific explanation. The most significant mass loss, accounting for 67.31% of the total mass, corresponded to the detachment of two coordinated **4PY** ligands, in line with the calculated 66.33% loss. Additionally, a mass loss of 18.83% aligned with the calculated 18.57% loss of two chloride ligands, indicating complete decomposition. The differential scanning calorimetry (DSC) profile of **Co4PY** revealed an endothermic peak at 148.2 °C, which coincided with the melting point and simultaneous loss of the two ligands observed in the thermogram. Another endothermic peak at 137.4 °C correlated with the loss of unreacted **4PY** ligand impurities, followed by an exothermic peak associated with water molecule loss. It is noteworthy that **Co4PY** exhibited a higher melting temperature compared to the copper complex but had a greater number of mass losses. The thermal profiles of the cobalt acido complex crystal **Co4PY-H** displayed three distinct mass losses. The initial mass loss of 21.27% was attributed to the detachment of two chloride ligands from the anionic cobaltate center, which closely matched the calculated value of 15.6%. Subsequently, a mass loss

of 26% indicated the loss of one protonated 4PY ligand, in agreement with the calculated value of 27%. The final mass loss of 20% signified the decomposition of the central entity and the loss of the last two chloride ligands, which was calculated to be 16.56%. Notably, this compound exhibited behavior similar to the copper acido complex crystal, as it transformed into the complex form. The observed melting point endothermic peak at 344 °C aligned with the cobalt complex's DSC profile, which measured the melting point at 315 °C. In summary, the thermal and calorimetric analyses provided valuable insights into the thermal properties and decomposition behavior of the cobalt complexes. The thermograms and DSC profiles allowed for the characterization of mass losses and transformations occurring during heating, enabling a comprehensive understanding of the stability and decomposition processes.

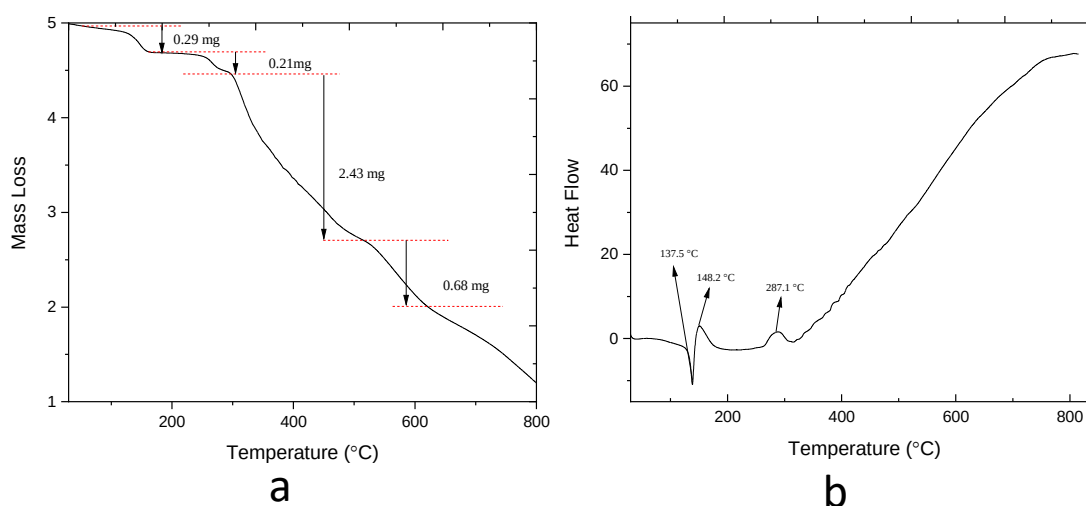
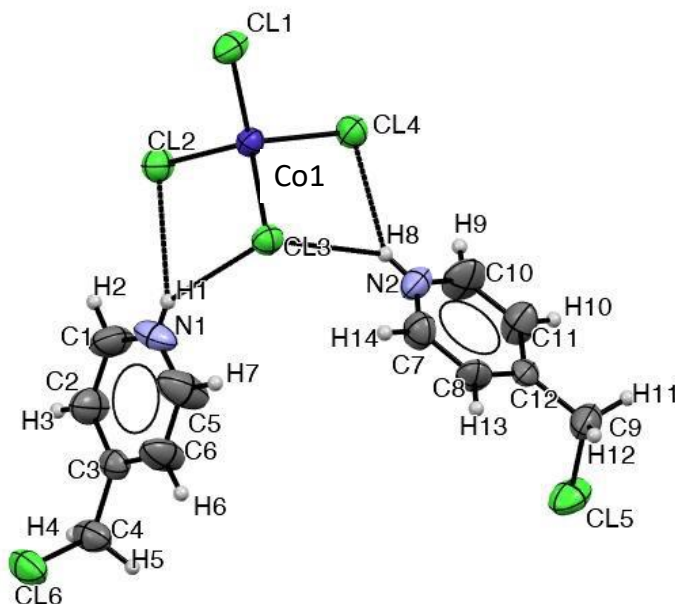


Figure 40: a) Thermogram and b) DSC profile of **Co4PY**

3.1.7 Single-Crystal XRD Analysis of the Crystalline Cobalt Complexes

The molecular structure of the complexes with crystals were determined using the single X-ray diffraction. SC-XRD is a non-destructive analytical technique which is used to provide detailed information about the internal lattice of crystalline substances. It provides information about the unit cell dimensions, bond lengths and bond angles. Furthermore, the information about the type of the lattice structure of the crystalline material and the space group of the crystal structure can be gathered by this technique. According to the crystal data obtained, the acido complex crystal Co4PY structure is monoclinic with a primitive lattice centering of a $P2_1/c$ space group. The crystal

refinement data of this structure and others are shown in Appendix 5.2. The molecular structure of the acido complex crystal compound with the atomic numbering scheme is shown in Structure 14.



Structure 14: The ORTEP structure of **Co4PY** AT 50% probability with atom numbering.

The crystal structure of **Co4PY-H** reveals a fascinating arrangement comprising two $[4PY-H]^+$ cations connected to the anion through halogen bonding. In this configuration, the halogen atoms form bonds with the metal of the central anion. These vital halogen bonds, represented by dotted lines between the chloride atoms coordinated to the metal and the cationic hydrogen atoms bonded to the nitrogen, play a pivotal role in shaping the overall structure. Notably, the central anion adopts a distorted tetrahedral conformation as a result. This disordered tetrahedron, identified as $[CoCl_4]^{2-}$, is positioned between the cations, ensuring charge balance and yielding a neutral molecule with a distinctive V-shaped conformation. Analyzing the bond lengths and angles within the molecule provides valuable insights into its structural characteristics. Tables 22 and 23 present the selected bond lengths and angles for detailed examination. Remarkably, the cobalt atom forms halogen bonds with the ligands, which significantly contribute to the complex's stability and structural arrangement. The halogen bonding interactions involve cationic hydrogen atoms and chloride atoms, exhibiting distances

ranging from 2.6 to 2.8 Å. It is worth noting that similar halogen bonding patterns have been observed in the copper acido complex crystal. Furthermore, the bond angles within the complex display variations spanning from 131 to 143°, further elucidating the intricate structural features of the **Co4PY-H** crystal. Moreover, the examination of Co-Cl bond angles provides evidence of a distorted tetrahedral configuration within the cobaltate center. The angles between the cobalt atom (Co1) and the surrounding chloride atoms (Cl1, Cl2, Cl3, and Cl4) reveal irregularities indicative of the disordered nature of the tetrahedron.

Table 22: Hydrogen bond geometry

D-H	d(D-H)	d(H...A)Å	<DHA (Angle/°)	d(D...A)Å
N1-H1...Cl2	0.718	2.832	137.57	3.397
N1-H1...Cl3	0.718	2.744	138.85	3.318
N2-H8...Cl4	0.816	2.601	143.33	3.291
N2-H8...Cl3	0.816	2.746	131.41	3.342

Symmetry codes: [x, y, z]

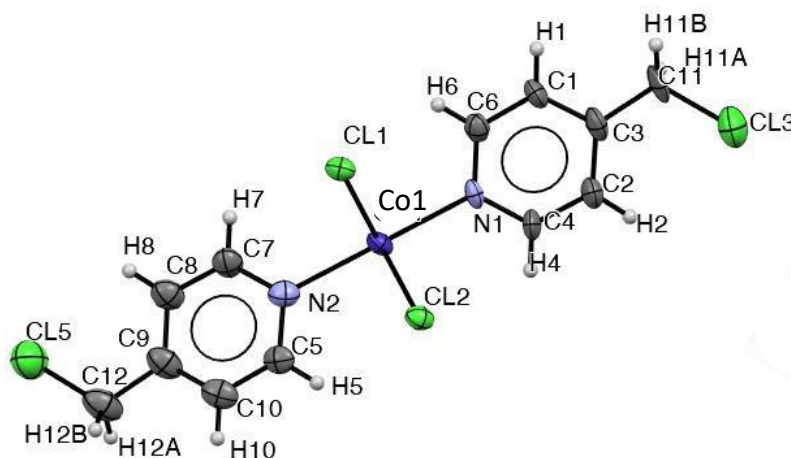
The bond lengths between Co1 and Cl2, Cl3, Cl4 atoms which are coordinated to the metal are longer than the bond Co1---Cl1 which is not bound through weak hydrogen bonds to the ligand (Table 23). The bond length of C-Cl3 which is connected to both ligands is the longest bond. The optimized structure of the compound seems different, and this is a similar observation to the acido complex crystal of copper, because the crystal structure depends on the interacting molecules in the crystal lattice while the optimized structure only relies on the free cation and anion to come up with the structure.

The bond angles between Co and two Cl which are connected to the same hydrogen atom are smaller than the bond angles between Cl atoms which are not connected to same hydrogen atom. This may suggest why there is a distortion in the tetrahedron of the metallic anion. Calculating τ for each bond angle, the distortion parameter (τ) of the tetrahedron in the cobalt complex crystal ranges from approximately 1.14 to 1.28. These values indicate a significant deviation from a perfect tetrahedral geometry, confirming the distorted nature of the tetrahedron in the cobaltate center. The selected bond angles of this structure are shown in Table 23.

Table 23: Selected bond angles for cobalt to chloride bonds of **Co4PY-H**

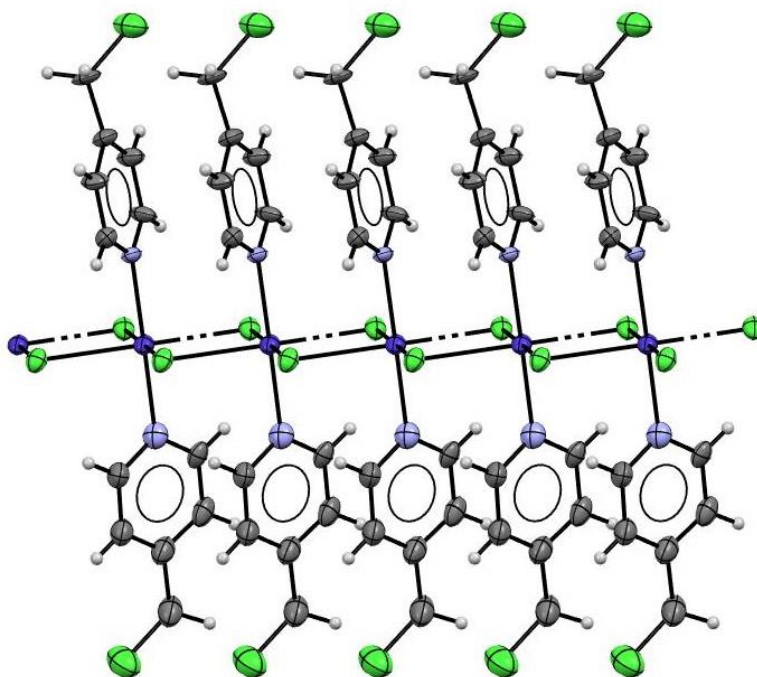
Atom	Atom	Atom	Angle/°
Cl2	Co1	Cl4	112.29(5)
Cl2	Co1	Cl3	106.86(5)
Cl4	Co1	Cl3	103.51(5)
Cl1	Co1	Cl2	111.37(5)
Cl1	Co1	Cl4	111.37(5)
Cl1	Co1	Cl3	111.09(5)

Co4PY crystal data obtained showed that the cobalt complex structure is a low symmetry triclinic crystal system with a primitive lattice centering of a *P*-1 space group. The data was not well resolved as the crystal quality was poor despite numerous attempts to grow good quality crystals of the complex. The final R-factor after solving and refining the structure was 19.10% which still suggested that the crystals were still of poor quality. The molecular structure of this compound with the atomic numbered scheme is shown in Structure 15.

**Structure 15:** The numbered ORTEP structure of **Co4PY** at 50% probability

The selected bond lengths of **Co4PY** are listed in Table 24. The bond lengths of the central metal to the ligands were observed to be well within the expected values and the Co1-Cl1 and Co1-Cl1', were observed around 2.5 Å. The angles indicate a distorted tetrahedral geometry around the central cobalt atom (Co1). The angles between the chlorine atoms (Cl1, Cl2) and the cobalt atom are approximately 83.42(18) degrees, which deviate from the ideal tetrahedral angle of 109.5

degrees. The angles involving the nitrogen atoms (N1, N2) and the cobalt atom are close to 90 degrees, indicating nearly perpendicular orientations. The calculated value of tau ($\tau \approx 19.65$ degrees) indicates a significant distortion from ideal tetrahedral geometry. This confirms that the molecule has a distorted tetrahedral conformation around the cobalt atom. Furthermore, the structure was polymeric [159] hence the Co1 bonds with Cl1' and Cl2' with a sort of seesaw structure even though the angles differ slightly from those in a seesaw geometry. This also corroborates with the observation that *trans* cobalt tetrahedral complexes are polymeric and pink or violet in color. This is also why the acido complex crystal of cobalt was observed to be blue because it had an overall stereochemistry of *cis*. However, as observed in Structure 16 of the expanded polymeric structure, the geometry at the central metal showed an octahedron due to polymerization. The Co-N bonds also showed that they were within the expected values as they all measured around 2.1 Å.



Structure 16: The polymeric ORTEP structure at 50% probability of the cobalt complex **Co4PY**

Table 24: Selected and calculated bond Lengths of **Co4PY**

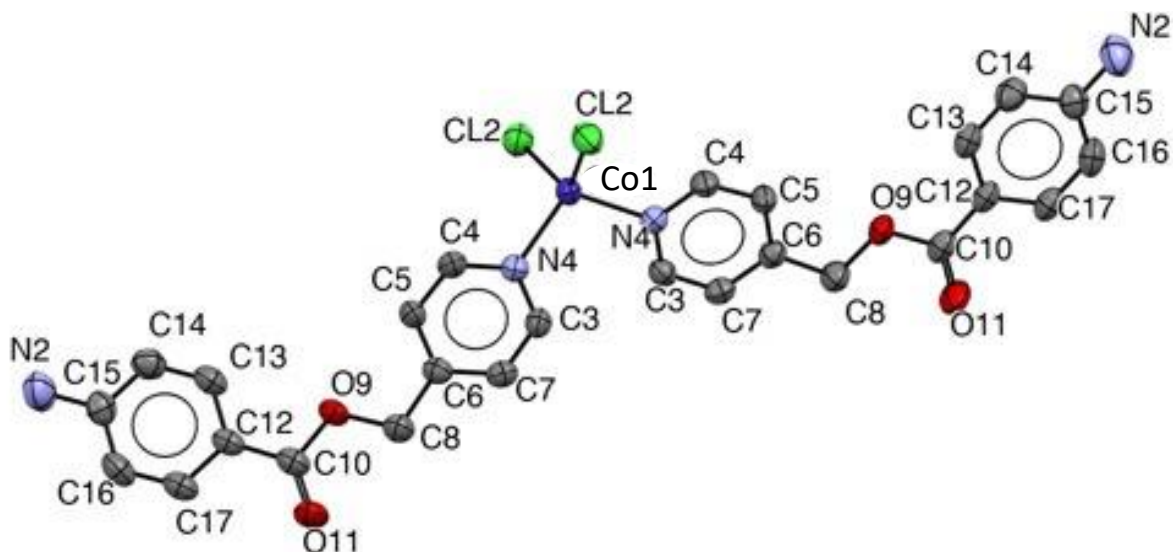
Atom	Atom	Length/Å
Co1	Cl1	2.533(6)
Co1	Cl2	2.546(6)
Co1	N2	2.17(2)
Co1	N1	2.136(17)
Co1	Cl1'	2.503(6)
Co1	Cl2'	2.490(6)

From the structure, there are two 4PY ligands and each ligand is bound to the cobalt center through the nitrogen atom of the pyridine and the ligands are separated at around 180° angles. The important selected bond angles of **Co4PY** are listed in Table 25. The expanded polymeric structure of the complex shows that the central metal atom is also bonded to two chloride ligands, this is the Cl1'-Co-Cl1, Cl2'-Co-Cl1 and N1-Co-N2 with a separation of around 179.1°. The other important angle is of the polymeric chloride ligands with respect to the central metal and the nitrogen donor atoms (Cl-Co-Cl3 and N-Co-Cl). These bond angles measured around 90°, with the least value being 83.42°.

Table 25: Selected bond angles of the **trans-Co4PY**

Atom	Atom	Atom	Angle/°
Cl1	Co1	Cl2	83.42(18)
N2	Co1	Cl1	89.4(6)
N2	Co1	Cl2	89.8(5)
N1	Co1	Cl1	89.9(5)
N1	Co1	Cl2	89.7(5)
N1	Co1	N2	179.3(8)

The obtained **CoL3** crystal data showed that this complex was a high symmetry monoclinic crystal system with a primitive lattice centering of a *I2/a* space group. The molecular structure of this compound with the atomic numbered scheme is shown in Structure 17.

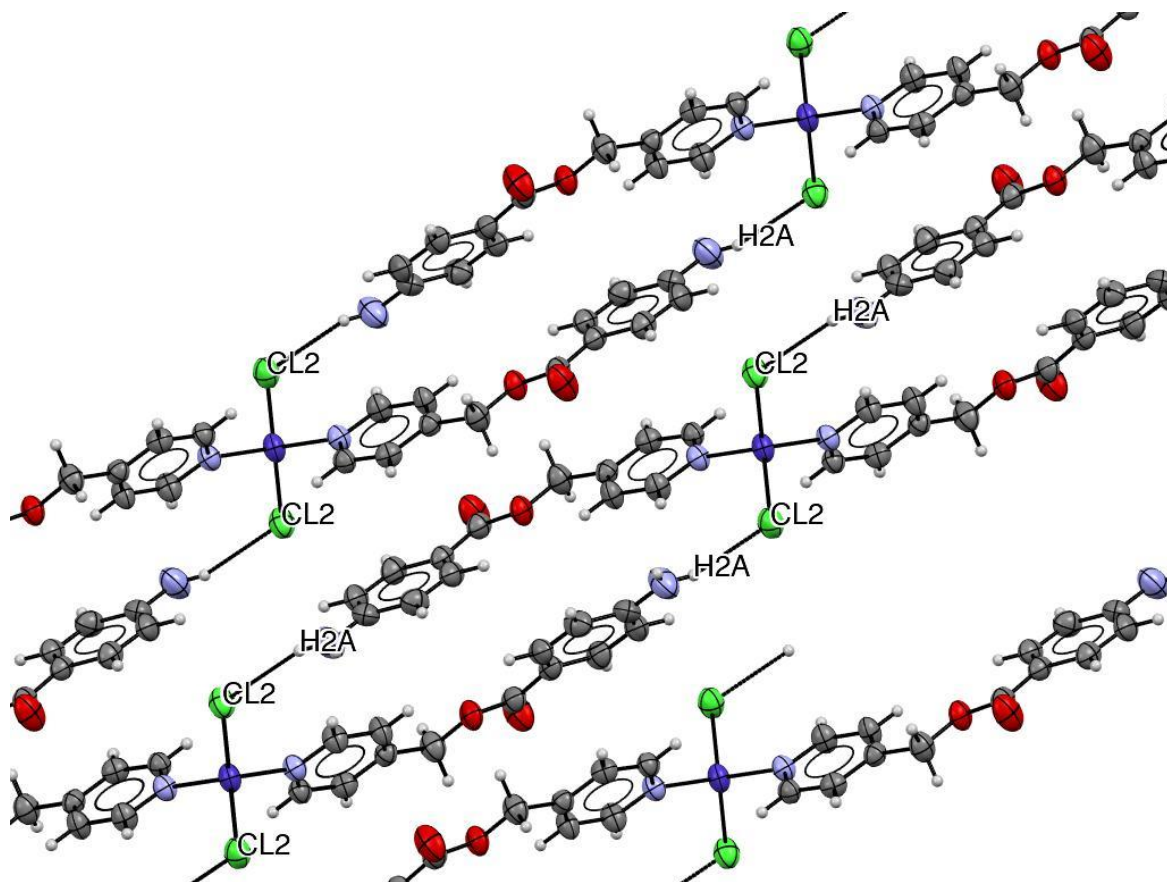


Structure 17: The numbered ORTEP structure of **CoL3** at 50% probability, hydrogen atoms removed for clarity.

The **CoL3** molecule displayed intermolecular hydrogen bonding-supramolecular chemistry in the crystal structure (Table 26). The interactions were as follows; the N3-H3...Cl2, the C8-H8B...O11 and finally the N2-H2A...Cl in interactions. They all had different hydrogen bonding symmetry codes, and the Structure 28 shows these interactions at the b-axis. Their bond lengths were around 3.3 Å except for the N3... H3-Cl2 which was longer at 3.6 Å.

Table 26: Hydrogen bond geometry

D-H	d(D-H)	d(H...A)Å	<DHA (Angle/°)	d(D...A)Å	Symmetry Codes
N3-H3...Cl2	0.930	2.810	138.31	3.559	[x, y-1, z]
C8-H8B...O11	0.970	2.498	131.75	3.266	[-x+1/2, -y-1/2, -z+3/2]
N2-H2A...Cl2	0.860	2.532	162.09	3.361	[x-1/2, y-1/2, z+1/2]



Structure 18: The supramolecular ORTEP structure of **CoL3** at 50% probability

The selected bond lengths of **CoL3** are shown in Table 27; the bond lengths between the central metal and the ligands were found to be well within the expected ranges, with the Co1-Cl2 bond length being 2.2389 Å. The complex adopted a *cis* configuration, this corroborated with the previously mentioned observation under the physical characteristics, which states that the blue color of the crystals in this structure suggested a *cis* geometry. The Co1-N4 bonds, which measured 2.036 Å, likewise demonstrated that they were within the predicted range of values.

Table 27: Selected and calculated bond lengths for **CoL3**

Atom	Atom	Length/Å
Co1	Cl2	2.2389(10)
Co1	Cl2 ¹	2.2389(10)
Co1	N4	2.036(3)
Co1	N4 ¹	2.036(3)

There were two **L3** ligands in the structure, and each ligand is bound to the cobalt center via the nitrogen atom of the pyridine. The ligands were separated at approximately 109.49° angles, indicating a *cis* geometry. Table 28 shows the important selected bond angles of **CoL3**, which indicated that the central metal atom was also bonded to two chloride ligands, the Cl2'-Co-Cl2, with a separation of around 117.16°, also suggesting a *cis* geometry.

Table 28: Selected bond angles for cobalt to chloride bonds of **CoL3**

Atom	Atom	Atom	Angle/°
Cl2	Co1	Cl2 ¹	117.16(6)
N4	Co1	Cl2 ¹	105.52(8)
N4 ¹	Co1	Cl2	105.52(8)
N4 ¹	Co1	Cl2 ¹	109.49(8)
N4	Co1	Cl2	109.49(8)
N4	Co1	N4 ¹	109.57(15)

3.1.8 Antibacterial Studies of the Cobalt Complexes

3.1.8.1 Bacterial Minimum Inhibition Concentration of the Cobalt Complexes

The cobalt complexes **Co4PY**, **Co4PY-H**, **CoL1**, **CoL2**, **CoL3** and **CoL4** were assessed for their possible antibacterial properties against, *K. pneumoniae*, *S. aureus* and their drug resistant counterparts. The MIC screening revealed that the cobalt compounds had more activity towards the Gram-negative bacteria with the exception of **CoL3** and **CoL1** which showed some activity towards MRSA but were less active towards MDR- *K. pneumoniae* (Figure 46 and Table 29).

Table 29: MIC of Cobalt Complexes

<i>Compound</i> ↓	<i>Microbe</i> →	<i>MIC (mM)</i>			
		<i>MDR-K. pneumoniae</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>MRSA</i>
<i>AgSD</i>		0.13±0.02	0.18±0.002	0.13±0.002	0.26±0.06
<i>Co4PY</i>		0.33±0.040	0.12±0.003	0.47±0.030	0.48±0.022
<i>Co4PY-H</i>		0.33±0.040	0.10±0.006	0.41±0.030	0.38±0.012
<i>CoL2</i>		0.26±0.040	0.13±0.01	0.37±0.008	0.41±0.013
<i>CoL1</i>		0.28±0.001	0.28±0.0003	0.27±0.011	0.30±0.009
<i>CoL4</i>		0.23±0.014	0.17±0.005	0.27±0.010	0.29±0.040
<i>CoL3</i>		0.29±0.014	0.29±0.04	0.34±0.120	0.28±0.140
<i>TREND</i>		CoL4>CoL2>CoL1>CoL3>Co4PY= Co4PY-H	Co4PY-H=Co4PY>CoL2>CoL4>CoL1>CoL3	CoL4=CoL1>CoL3>CoL2>Co4PY-H> Co4PY	CoL3>CoL4>CoL1>Co4PY-H>CoL2> Co4PY

Cobalt complexes have not been thoroughly investigated for their antibacterial properties, especially their possible modes of action and biomolecular interactions [163], [164]. However, competition between cobalt and iron in several metabolic processes including Fe–S cluster assembly, the formation of free radicals and the reduction of the free thiol pool, has been proposed as the explanation for the toxicity of cobalt ions in another Gram-negative bacteria, *E. coli* [165], [166]. Considering their potency in comparison to the control drug silver sulfadiazine

(AgSD), most of the compounds were less active than the control for the drug resistant bacteria. However, the cobalt complexes were more potent against *K. pneumoniae* than AgSD.

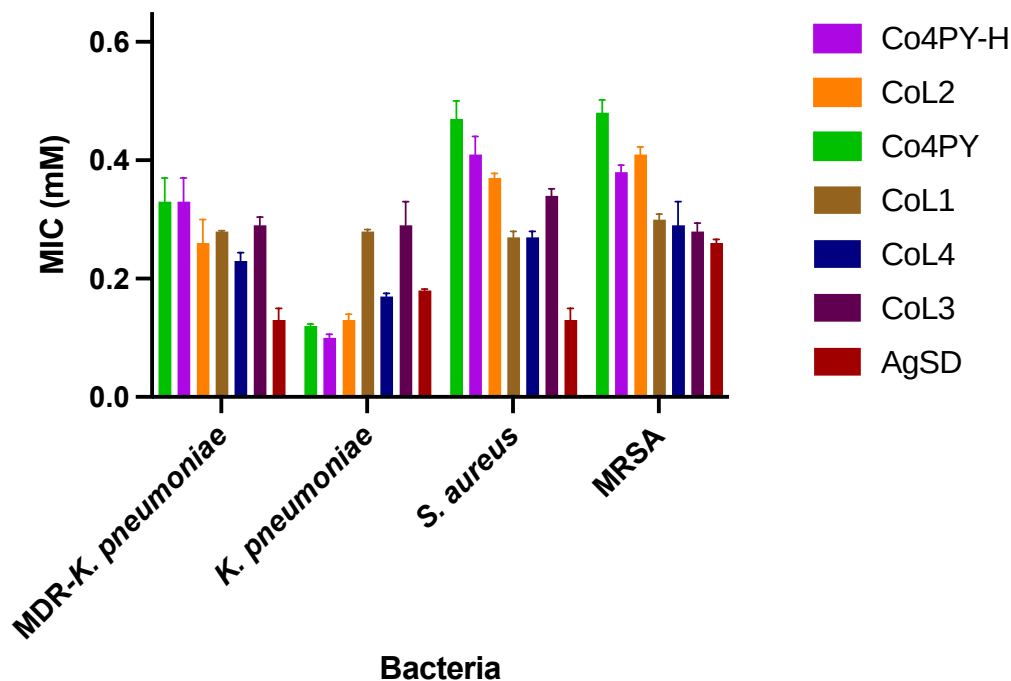


Figure 41: Summarized MIC values of cobalt complexes

3.1.8.1 Time Kill Kinetics of Cobalt Complexes

Time kill assay were conducted to determine bacteriostatic or bactericidal properties of the most active complexes. The study used the potent drugs against *K. pneumoniae* and MDR- *K. pneumoniae* at their MIC (Figure 47 and 48). The results revealed that, except for the control drug AgSD, which was bactericidal, the cobalt compounds were bacteriostatic at their MIC against MDR- *K. pneumoniae*. The compounds began with a bactericidal trend for 6 hr before switching to bacteriostatic behaviour after 24 hr of incubation, which was important to note.

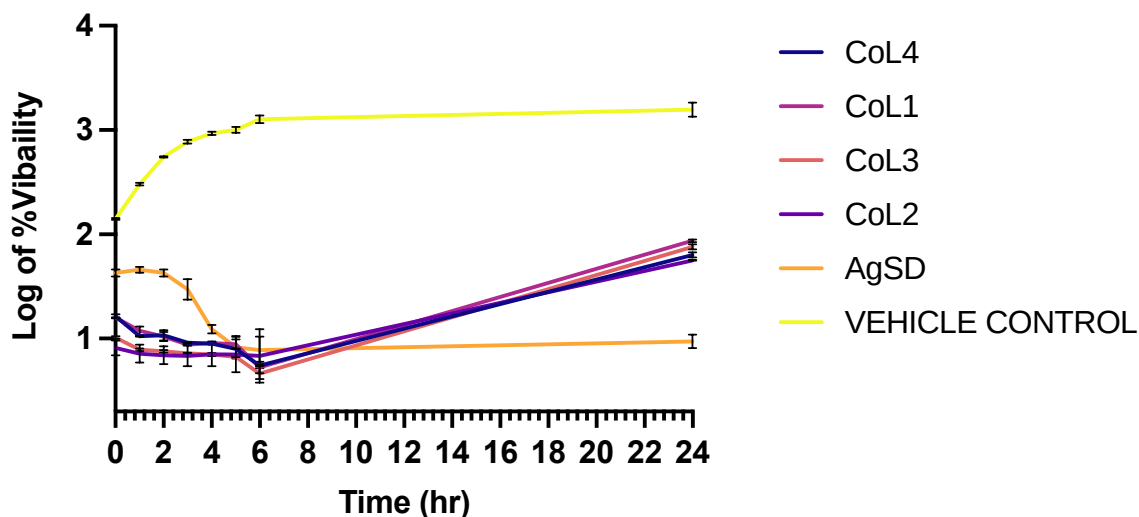


Figure 42: Time kill Kinetics of active cobalt complexes against MDR- *K. pneumoniae*

Additionally, the majority of the active compounds against *K. pneumoniae* were shown to be bacteriostatic (Figure 48); the sole exception was **Co4PY**, which did not initially exhibit a bactericidal trend like the other tested compounds. The only substances that were found to be bactericidal were **CoL4** and **CoL3**, with **CoL3** exhibiting the strongest bactericidal activity. In conclusion, the overall observation of the time kill kinetics suggests that the structural changes amongst the compounds had an impact on their activity. The results reported herein are preliminary, and require mode of action studies to confirm the activity and the method to which they are killing or stopping the growth of the bacteria.

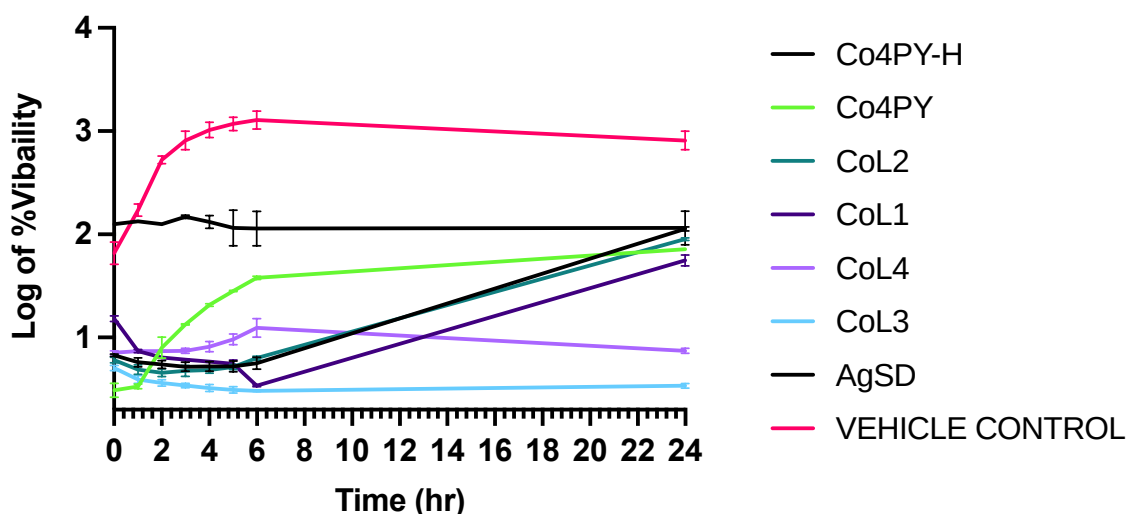


Figure 43: Time kill Kinetics of active cobalt complexes against *K. PNEUMONIAE*

3.1.8.1 Folic Acid Production Studies of the Cobalt Complexes

To measure the synthesis of folate from both the treated and untreated *K. pneumoniae*, an ELISA was carried out. The bacteria were incubated with the only active cobalt compound containing an amine group **CoL2**, together with the negative control drug **AgSD**. According to Figure 44, **CoL2** appears to be more efficient than the control **AgSD** in reducing folate production *k. pneumoniae*. This was understandable given that **AgSD** main mode of action is membrane disruption, and the presence of the sulfadiazine moiety in the control medication **AgSD**, which contains an amino group, allows it to be a competitive inhibitor of bacterial PABA in addition to its primary mode of action [136]. These findings suggest that the compound **CoL2** amino group may have contributed to the reduced production of folates. This is in line with previous research that showed the presence of the amino group impeded the biosynthesis of folic acid, as well as conventional sulphonamides [60], [142]–[144].

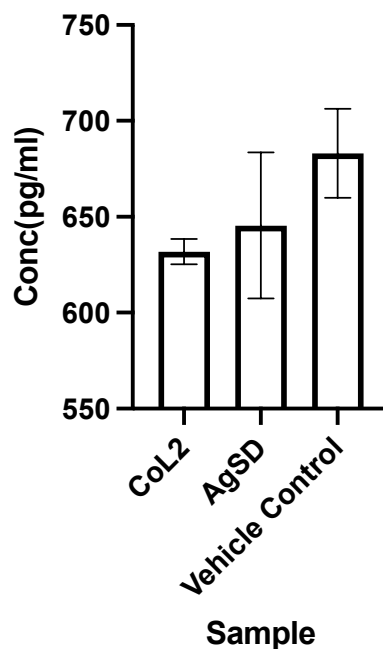


Figure 44: Folate production of *K. pneumoniae* after treatment with **CoL2**

Figure 45 shows that the compound **CoL3** reduced the production of folates in MRSA more effectively than the control **AgSD**. Additionally, **CoL3** has an amino group that might have contributed to the decreased folate production. The conclusions reached regarding the activity of **CoL2**, and the control drug may be applied to **CoL3**. This is because **CoL3** and **CoL2** share

functional groups other than the oxygen bridges in their ligands, which were formed by an ester in the case of **CoL3** and an ether in the case of **CoL2**.

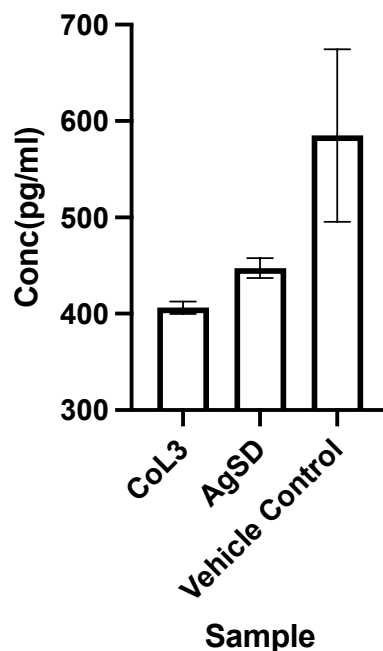


Figure 45: Folate production of MRSA after treatment with **CoL3**

3.1.8.1 Cell Wall/Membrane Disruption Studies of the active Cobalt Complexes by AKP Assay

The alkaline phosphatase (AKP) assay was used to investigate the damage of the bacteria's cell membrane to assess if the alteration of cell membrane permeability was one of the cobalt complexes' modes of action [147]. The AKP release properties of the compounds with high activity were investigated: **Co4PY-H**, **Co4PY**, **CoL2**, **CoL3**, and the control **AgSD**. According to the graph in Figure 46, **Co4PY-H**, **Co4PY**, and **CoL2** were tested against *K. pneumoniae*, and all the drugs produced more AKP than the vehicle control. The best performing compounds were in the order **CoL2>Co4PY-H=Co4PY>AgSD**. Most of the Literature with antibacterial cobalt complexes does not show their modes of action, literature comparison of this data was not possible. Conclusively, these findings suggest that cobalt core and the functional groups chloro, pyridyl, and ether contributed to membrane disruption as part of the modes of action of the cobalt complexes.

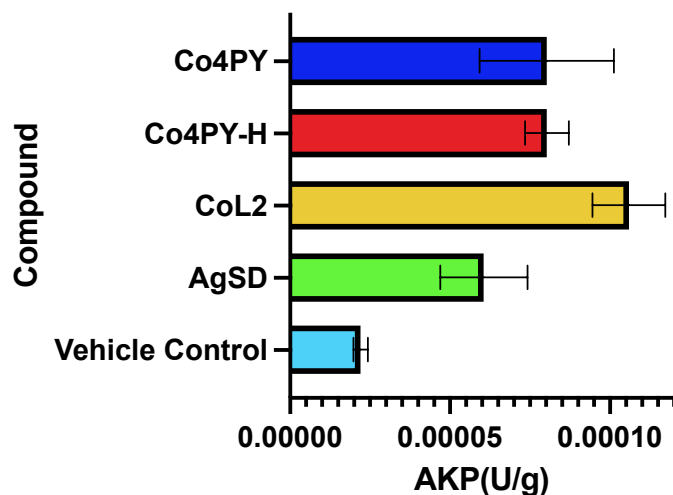


Figure 46: AKP Production of *K. pneumoniae* after treatment with active cobalt complexes

The most potent cobalt compound against MRSA, **CoL3**, was also shown to produce considerably higher AKP than the control medication **AgSD** and the vehicle control (Figure 47). This suggests that this compound may be more effective against MRSA than conventional **AgSD**. The only assay that proved contrary was the MIC, which showed that the control **AgSD** had a higher MIC than **CoL3**. Nonetheless, this assay revealed that the compound's mode of action against MRSA may include membrane disruption.

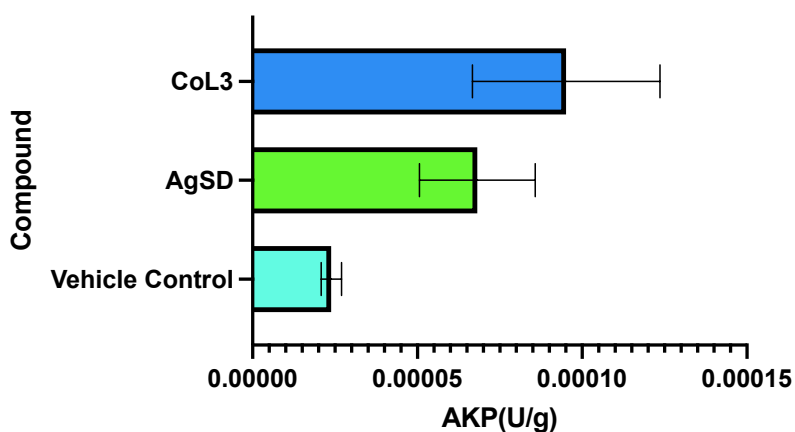


Figure 47: AKP production of MRSA after treatment with **CoL3**

3.1.8.1 Molecular Docking Studies of active Cobalt Complexes

The cell wall in bacteria develops a rigid framework of uniform thickness surrounding the cell and is responsible for the cell's distinctive shape (rod, coccus, or spiral). It is made up of a rigid peptidoglycan layer. Peptidoglycan, the primary building block of bacterial cell walls, is synthesized by membrane-associated penicillin-binding proteins (PBPs). These proteins were identified and given names based on how well they bound the β -lactam antibiotic penicillin [167]. Molecular docking studies were employed with the most active cobalt complex and the control drug towards MRSA, **CoL3** and **AgSD**, against the crystal structure of a PBP of MRSA namely PBP2a shown in Table 30 and Figures 48 and 49 [168]. It was discovered that a quinazoline antibiotic with similar pharmacophores, including aromatic rings, carbonyl, and carboxylic acid, bound to the PBP2a protein when specific residues (Lys406, Lys597, Ser598, Glu602, and Met641) in the protein active site changed their spatial arrangements, inferring interaction with the protein [168]. Similarly, from the molecular docking scores of this study, the binding energy of **CoL3** was found to be at an acceptable -8.38 kcal/mol and inhibition constant of 0.71 μ M in the active site. The molecule had the following interactions at the active site of the protein; hydrogen bonding with Thr165, hydrophobic interactions with Arg151, Arg241, Pro258, Val277, and Pro213, then finally Van der Waals interactions with Ser240, Gly257, Val256, and Tyr196. The only similar residues were Ser598 in the quinazoline antibiotic and at a similar residue Ser240 in **CoL3** which is where the active site is, indicating activity in a different position or arrangement in the active site of PBP2a.

Table 30: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands and their complexes against methicillin-resistant *S. aureus* (PDB: 4cjn)

Compounds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
CoL3	-8.39	0.71 μ M	Thr165	Arg151,Arg241, Pro258,Val277, Pro213	Ser240,Gly257, Val256, Tyr196
AgSD	-5.88	49.35 μ M	Gly271,Val277	Asp275,Ala276, His293	Tyr272, Lys273, Gln292,Glu294

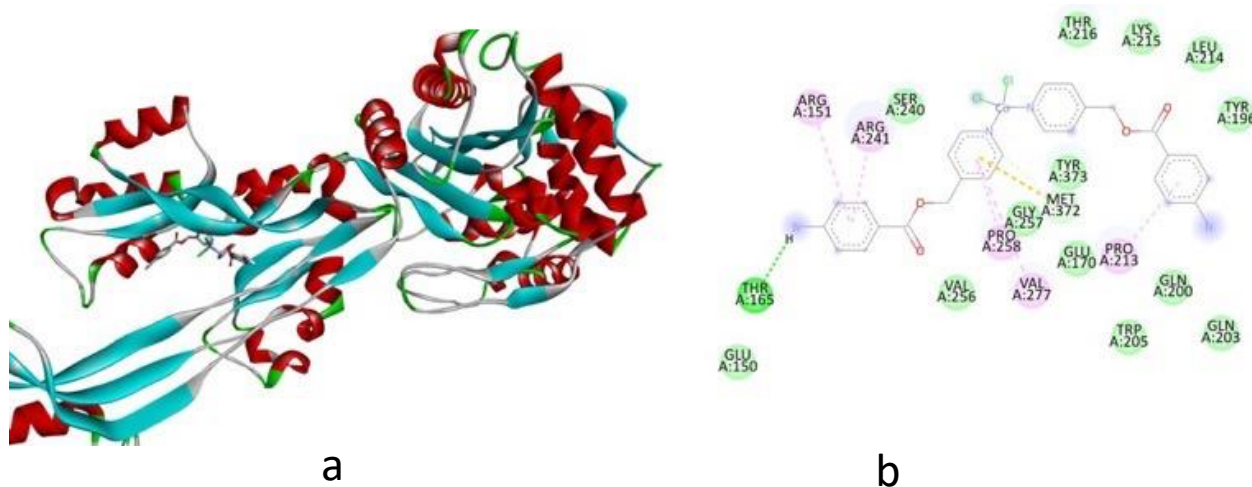


Figure 48: The binding interactions of **CoL3** against methicillin-resistant *S. aureus* (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

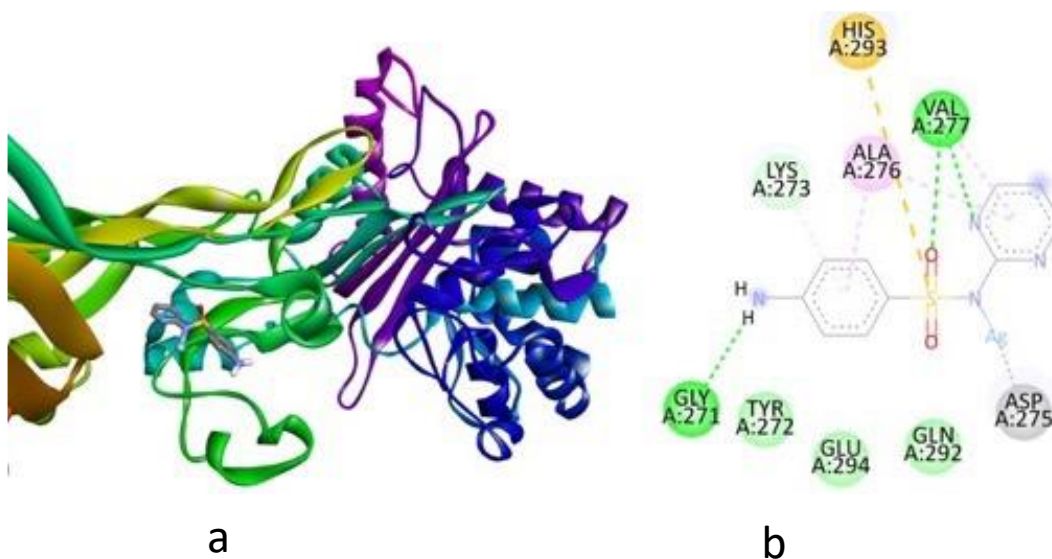


Figure 49: The binding interactions of **AgSD** against methicillin-resistant *S. aureus* (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

Molecular docking analysis was used to determine if the compounds exhibit competitive antagonistic capacities to PABA on the PABA binding pocket of the enzyme DHPS. From the data shown in Table 31, **CoL3** was the best performing due to its better binding energy of -8.34 kcal/mol, followed by **CoL2** with -7.74 kcal/mol, then the control **AgSD** with -6.65 kcal/mol and PABA being the worst with -3.80 kcal/mol. Since PABA was the substrate to DHPS, it interacts with the binding pocket of the enzyme. Thus from the calculations, PABA interacts through hydrogen bonding with the amino acid residues; Thr67, Asp101, Gly216, Arg254, the hydrophobic interactions Ile122, and Phe189 and finally through van der Waals interactions by Arg68, Asn120, Asp184, Lys220 (Figure 53). The three amino acid residues Lys220, Ser218 and Phe189 were necessary for the interaction with the PABA binding pocket of the DHPS enzyme. The presence of benzene-sulfonamide moieties in this PABA binding region is a characteristic of all sulfa drugs [157]. The best performing compound **CoL2** showed similar interacting amino acid residues being Thr67, Asp101, Arg219, lys220, Arg68, Phe189 (Figure 50). **CoL3** also displayed similar binding characteristics to PABA, this was observed with the amino acid interactions which were Arg68, lys220, Ile122, and Asp184 (Figure 51). The control drug **AgSD** showed better binding abilities to the protein DHPS when compared to PABA, the drug interacted with the following amino acid

residues also found with PABA, Thr67, Asn120, Arg254, Arg68, lys220, and Asp184 (see Figure 52). These findings proved that the two complexes **Col2** and **Col3** were the best at reducing the production of folate and thus qualify them as folic acid biosynthesis inhibitors. The findings about these compounds' binding capabilities are consistent with earlier observations regarding homologous pharmacophores interacting with the PABA binding site of this enzyme DHPS [158].

Table 31: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands against Dihydropteroate Synthase DHP (PDB: 3tye)

Compounds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
Col2	-7.74	2.11 μ M	Thr29,Thr67, Asp101	Arg68,Lys220, Val231	Asn27,His256, Phe189,Arg219
Col3	-8.34	0.78 μ M	Asn27,Pro69, Asp184	Arg68,Met145, Lys220,Arg234	Ile122,Ser218, Arg219,His256
AgSD	-6.65	13.28 μ M	Thr67,Asn120, Arg254	Met23,Arg68, Met145,Asp184	Pro69,Lys220
PABA	-3.80	1650 μ M	Thr67,Asp101, Gly216,Arg254	Ile122,Phe189	Arg68,Asn120, Asp184,Lys220

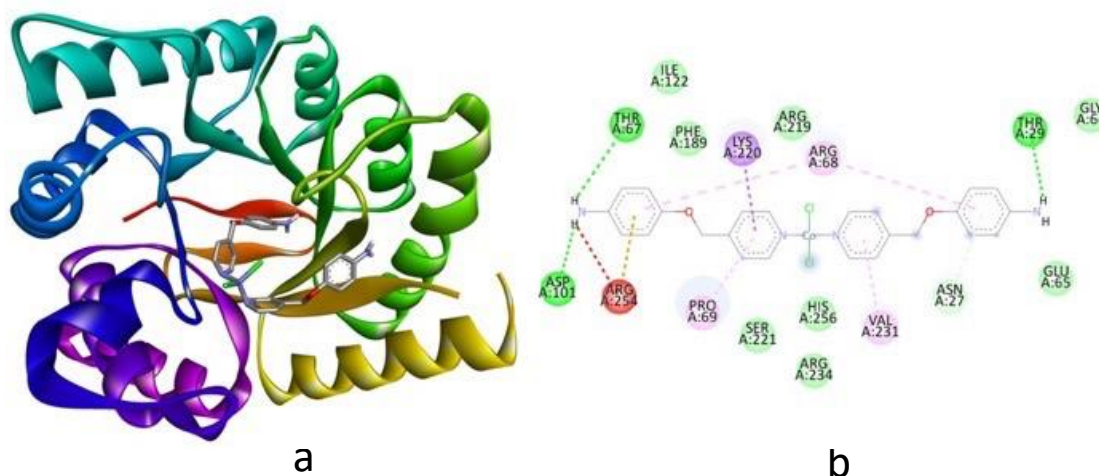


Figure 50: The binding interactions of **Col2** against Dihydropteroate Synthase DHPS (PDB: 3tye). a) shows the binding site, b) shows the interacting amino acid residues.

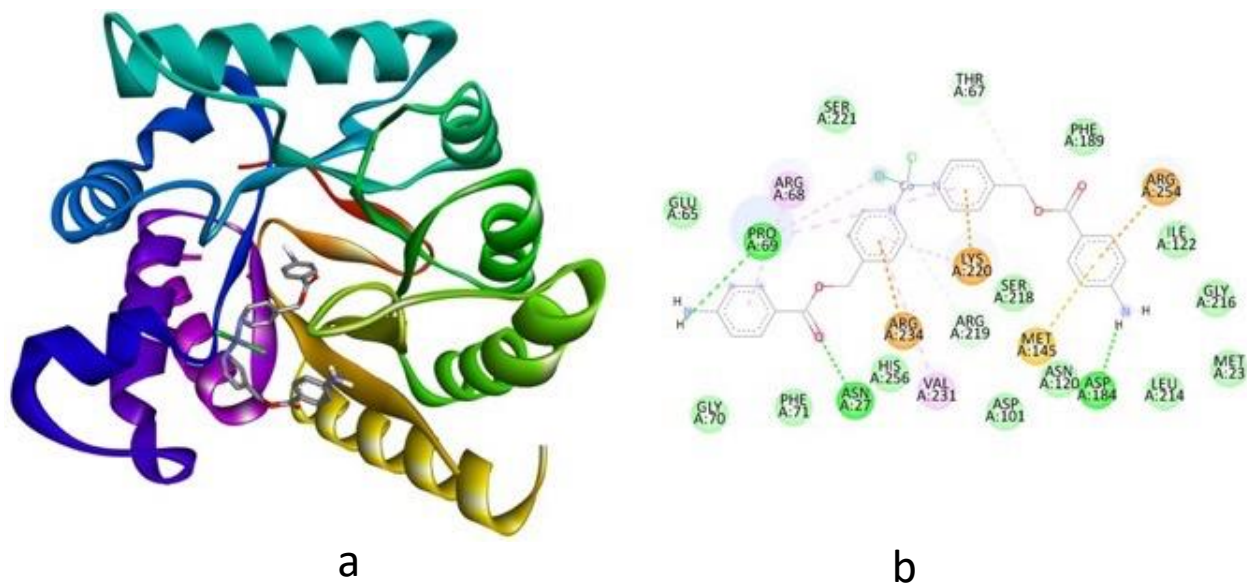


Figure 51: The binding interactions of **CoL3** against Dihydropteroate Synthase DHPS (PDB: 3tye). a) shows the binding site, b) shows the interacting amino acid residues.

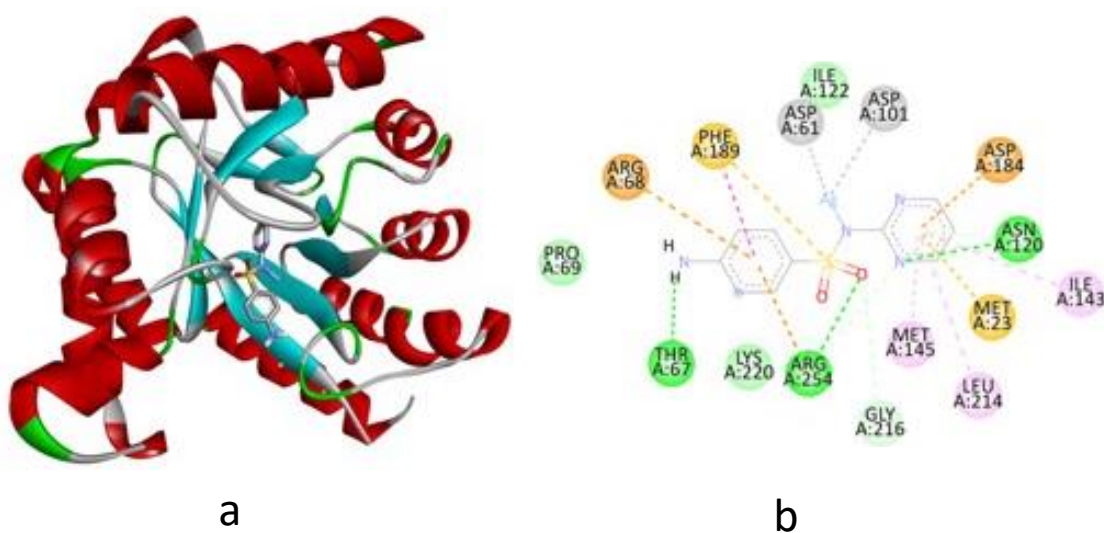


Figure 52: The binding interactions of **AgSD** against Dihydropteroate Synthase DHPS (PDB: 3tye). a) shows the binding site, b) shows the interacting amino acid residues.

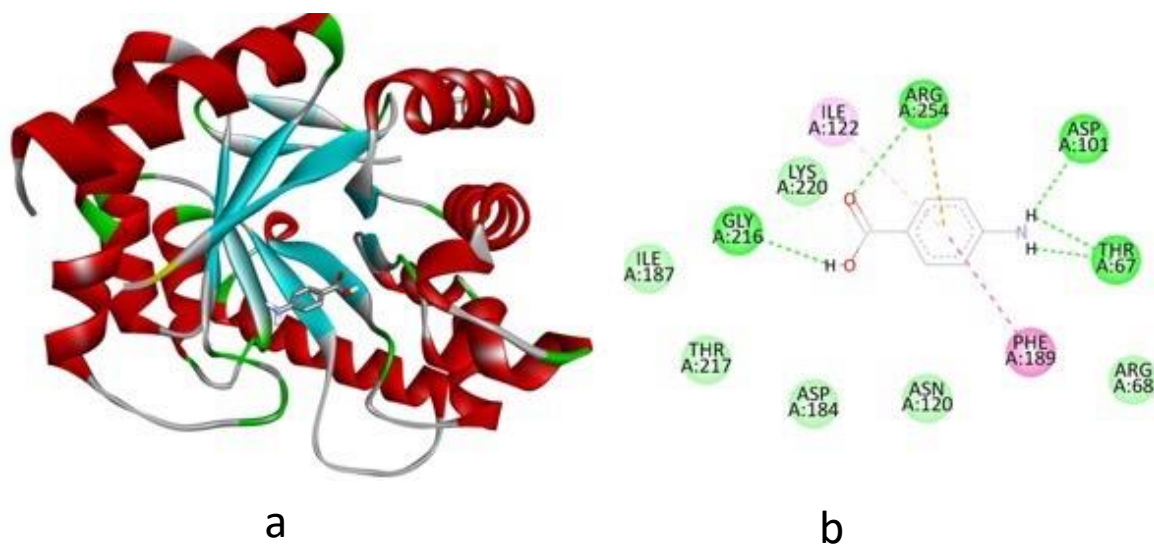


Figure 53: The binding interactions of PABA against Dihydropteroate Synthase DHPS (PDB: 3tye). a) shows the binding site, b) shows the interacting amino acid residues.

The β -lactam antibiotic resistant OmpK36 (PDB: 6rd3) of *K. pneumoniae* was studied using molecular docking to understand the binding process by which all drugs interacted with the essential amino acids of this bacterial protein [154]. The compounds were docked to the protein as is and not towards a particular binding pocket, and from this **CoL2** (-7.65 kcal/mol) was observed to be the best followed by **AgSD** (-6.27 kcal/mol) and lastly **Co4PY** (-5.84 kcal/mol). It was evident that **Co4PY** was binding at a different site compared to the other compounds based on the amino acid residue interactions, while **CoL2**, and **AgSD** had similar amino acid interactions with the following residues Glu110, Phe111 (see Figures 54 through 56). These findings were interesting as they were similar to what was found in the experimental AKP assay, where the trend was **CoL2**>**Co4PY**>**AgSD**. Finally, these findings still prove that cell membrane disruption is one of the active compounds' modes of action.

Table 32: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands and their complexes against OmpK36 from *K. pneumoniae* (PDB: 6rd3)

Cpds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
CoL2	-7.65	2.46 μ M	Asn120,Thr115, Glu110,Gly112	Ile330,Lys315	Arg244,Phe111, Thr243,Thr116
Co4PY	-5.84	52.51 μ M	-	Pro109,Arg270, Tyr311,Val341	Thr301,Tyr303, Lys16,Ley108
AgSD	-6.27	25.55 μ M	Arg37	Lys116,Tyr35, Glu110	Phe111,Gln61, Asp18,Leu20

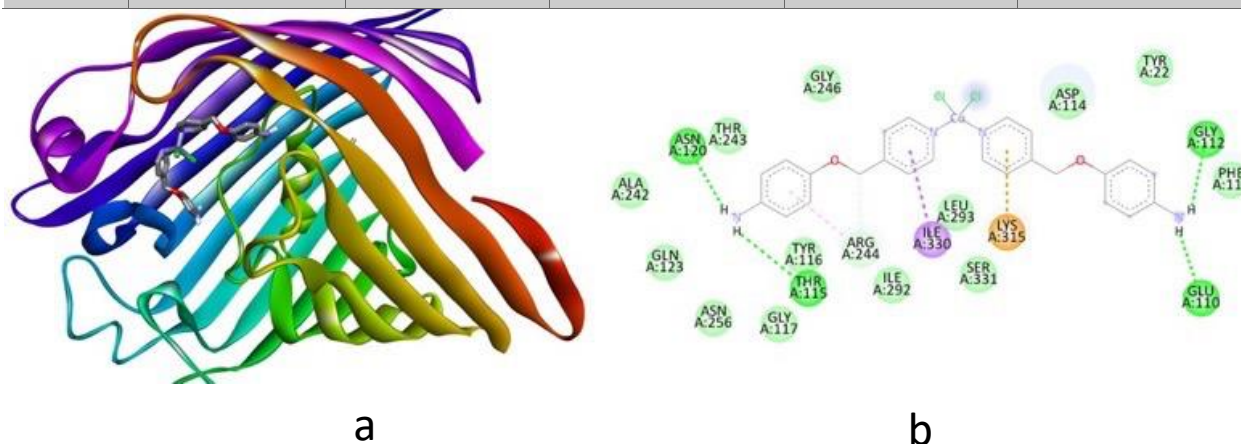


Figure 54: The binding interactions of **CoL2** against OmpK36 from *K. pneumoniae* (PDB: 6rd3). a) shows the binding site, b) shows the interacting amino acid residues.

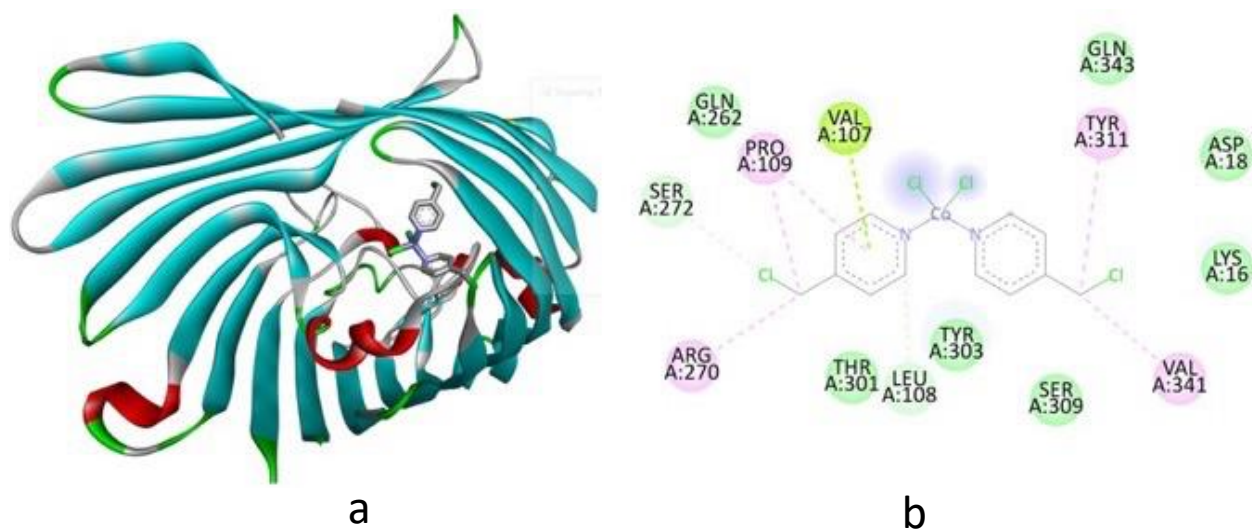


Figure 55: The binding interactions of **Co4PY** against OmpK36 from *K. pneumoniae* (PDB: 6rd3). a) shows the binding site, b) shows the interacting amino acid residues.

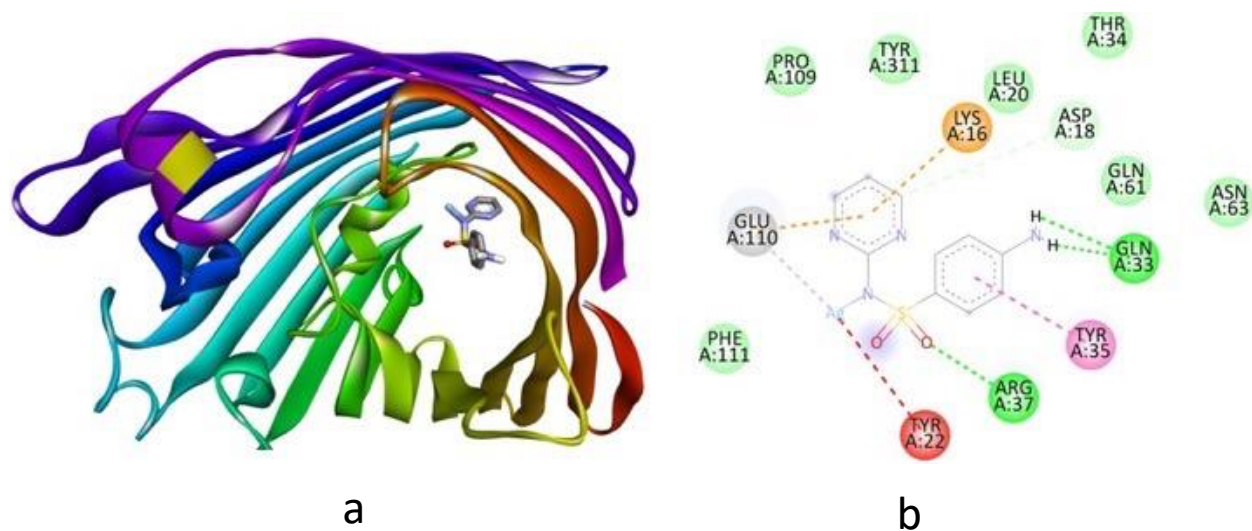


Figure 56: The binding interactions of **AgSD** against OmpK36 from *K. pneumoniae* (PDB: 6rd3). a) shows the binding site, b) shows the interacting amino acid residues.

3.1.9 Conclusions

Six complexes of cobalt were synthesized and characterized using various physicochemical techniques. The synthetic method used for most of the compounds was co-grinding, this technique proved to yield more product that is pure. Furthermore, thermal studies showed that the cobalt complexes were of good stability as observed by their transformations and high melting points, with **CoL4** being the most stable. The cobalt complexes HRESI-MS results demonstrated their effective synthesis and validated the complementary characterisation findings. Three complexes managed to form crystals, of these, two were of the **4PY** ligand while the other one was from the **L3** ligand. Their crystal data show their successful synthesis. However, **Co4PY** was observed to be a polymeric *trans* pseudo-octahedron, while its acido complex counterpart **Co4PY-H** was seen as a supramolecular *cis* tetrahedron with the ligand acting as the cation. **CoL3** conversely was a *cis* tetrahedron with high symmetry monoclinic crystal system with a primitive lattice centering of a *I2/a* space group. The cobalt complexes were observed to be more active towards the Gram-negative bacteria, particularly *K. pneumoniae*. According to the time kill kinetics, the most active compounds against MDR- *K. pneumoniae* were bacteriostatic. On contrary, all the compounds except **CoL3** and **CoL4** were bacteriostatic against *K. pneumoniae*. Further assays showed that **CoL2** and **CoL3** affected the folate biosynthesis of *K. pneumoniae* and MRSA respectively. While **Co4PY-H**, **Co4PY**, **CoL2**, **CoL3** disrupted the cell membrane integrity of *K. pneumoniae*, additionally, **CoL3** disrupted the cell membrane of MRSA. Finally, molecular docking calculations of these active complexes provided some evidence for their experimental modes of action.

3.2 Synthesis, Characterization and Antibacterial Properties of Nickel Mixed Ligand Complexes

3.2.1 Synthesis of the Nickel Complexes

The synthesis of nickel (II) complexes with **4PY**, **L1**, **L2**, **L3**, and **L4** was successfully achieved by reacting nickel chloride with the corresponding ligands. Similar to the cobalt complexes, the synthesis of **4PY** complexes employed a solvent-based reaction, while the complexes involving the synthesized ligands were prepared using a more environmentally friendly co-grinding method. The co-grinding method demonstrated higher yields ranging from 70% to 90%, as indicated in Table 33, and required less time compared to the classical solution phase method, which yielded 47.1% and took 3-4 hours for completion.

3.2.2 Purity, Solubility and Physical Properties of the Nickel Complexes

Most of the nickel complexes had poor solubility, they dissolved under heat in highly polar solvents. The **Ni4PY** complex was light green powder which was stable in dry air and under room temperature. The complex was insoluble in water and acetonitrile but partially soluble in other organic solvents such as methanol and ethanol and other solvents such as DMF and DMSO. This complex was found to have melting point of 264-272 °C. The **NiL1** complex was successfully synthesized to give a yellow powder. The percentage yield of the product was 71.8%. The melting point was determined to be 210 °C. **NiL1** complex also dissolved partially in DMF but was partially soluble in all other solvents. The complex **NiL3** complex was attained at an acceptable yield of 77% with a high melting point of 304.6 °C. The complex had better solubility than its analogue **NiL1** even though it became soluble on heating, this was proposed to be due to the amine moiety as opposed to nitro moiety in **NiL1**. **NiL2** had a good yield of 76.11% and a high melting point of 335.7 °C. The complex was a pale-yellow powder. This complex had the worst solubility compared to all the other complexes. The analogue **NiL4** also had poor solubility which may have been due to the nitro moiety. **NiL4** had a good yield of 78 % and melting point of 220 °C.

Table 33: Physical Properties of Nickel Complexes

Complex	Appearance	Melting Point (°C)	Solubility	%Yield
Ni4PY	Pale green powder	264.0	All partially Soluble: DMSO, Methanol Δ , ethanol Δ , DMF, acetonitrile Δ , water Δ	47.1
NiL1	Yellow powder	210.0	All partially Soluble: DMSO, Methanol Δ , ethanol Δ , DMF, acetonitrile Δ , water Δ	71.8
NiL2	Pale yellow powder	335.7	All partially Soluble: DMSO, Methanol Δ , ethanol Δ , DMF, acetonitrile Δ , water Δ	76.11
NiL3	Pale green powder	304.6	All partially Soluble: DMSO, Methanol Δ , ethanol Δ , DMF, acetonitrile Δ , water Δ	77.0
NiL4	Pale yellow Powder	220.0	All partially Soluble: DMSO, Methanol Δ , ethanol Δ , DMF, acetonitrile Δ , water Δ	78.0

Δ : Soluble with heat

The purity of the nickel complexes and their elemental analysis (Table 34) were studied using elemental analyser. From the data, the complexes were pure with percentage purities above 90 %. The other complexes had impurities of at least less than 0.5 moles of the nickel salt. Even though that was the case, the complexes were characterized.

Table 34: Elemental Analysis and Purity of Nickel Complexes

Complex	CALCULATED			FOUND			PURITY	
	N	C	H	N	C	H	IMPURITIES (mol)	% PURITY
Ni4PY	7.2 (7.28)	37.03 (37.46)	3.24 (3.14)	5.18	26.11	2.80	0.25 H ₂ O	98
NiL1	8.28 (8.67)	46.17 (48.34)	3.16 (3.12)	7.85	46.60	2.93	0.60 H ₂ O 0.15 NiCl ₂	96
NiL2	9.97 (10.57)	51.31 (54.38)	4.81 (4.56)	10.40	50.87	4.52	1.40 H ₂ O 0.05 NiCl ₂	94
NiL3	9.43 (9.56)	52.55 (53.28)	4.22 (4.13)	9.45	52.20	4.38	0.45 H ₂ O	98
NiL4	8.78 (9.50)	45.17 (48.86)	3.21 (3.42)	9.23	44.76	2.74	0.15 H ₂ O 0.35 NiCl ₂	92

3.2.3 FTIR Characterization of the Nickel Complexes

To study the binding mode of the ligands to the metal in the complexes, the FTIR spectra of the complexes were compared with those of the free ligands to determine the atoms that may have been involved in coordination. The FTIR frequencies of the chosen peaks were recorded in Table 35 and the spectra of the compounds are shown on Appendix 5.1 FTIR Spectra. From the spectrum of **Ni4PY**, it may be observed that the Ar-C=N vibrational frequency reduced which proves that the coordination was through the pyridyl nitrogen. As for the **NiL1** complex, all the important ligand functional groups were observed such as the ester group O=C-O at 1734.1 cm⁻¹, and the Ar-NO₂ peak at 1349-1427 cm⁻¹. Finally, the Ar-C=N observed at 1427 cm⁻¹ was observed as the coordinating donor atom as this shifted from the free ligand which was 1440 cm⁻¹. The amino analogue to **NiL1** the **NiL3** complex showed important ligand functional groups, these were the O=C-O at 1724.3 cm⁻¹, and the Ar-NH₂ at 3305-3402.3 cm⁻¹. This complex bonding atom was observed to be the Ar-C=N at 1427.4 cm⁻¹ which shifted from 1439 cm⁻¹ of the free ligand. The **NiL2** complex also showed important functional groups from its ligand **L2**, these include the ether C-O-C peak at 1042.8 cm⁻¹, the Ar-NH₂ peak at 3234.8-3285.6 cm⁻¹. Then the coordinating donor atom was observed to be the Ar-C=N which shifted from 1463.4 cm⁻¹ to 1451.9 cm⁻¹. The nitro analogue of this complex **NiL4** also showed the ligand **L4** important functional groups. These

are the C-O-C peak at 1037.6 cm⁻¹, the Ar-NO₂ peak at 1384.4-1424.9 cm⁻¹ and finally the bonding Ar-C=N observed at 1445.9 cm⁻¹. This peak shifted from the free ligand peak of 1459.5 cm⁻¹.

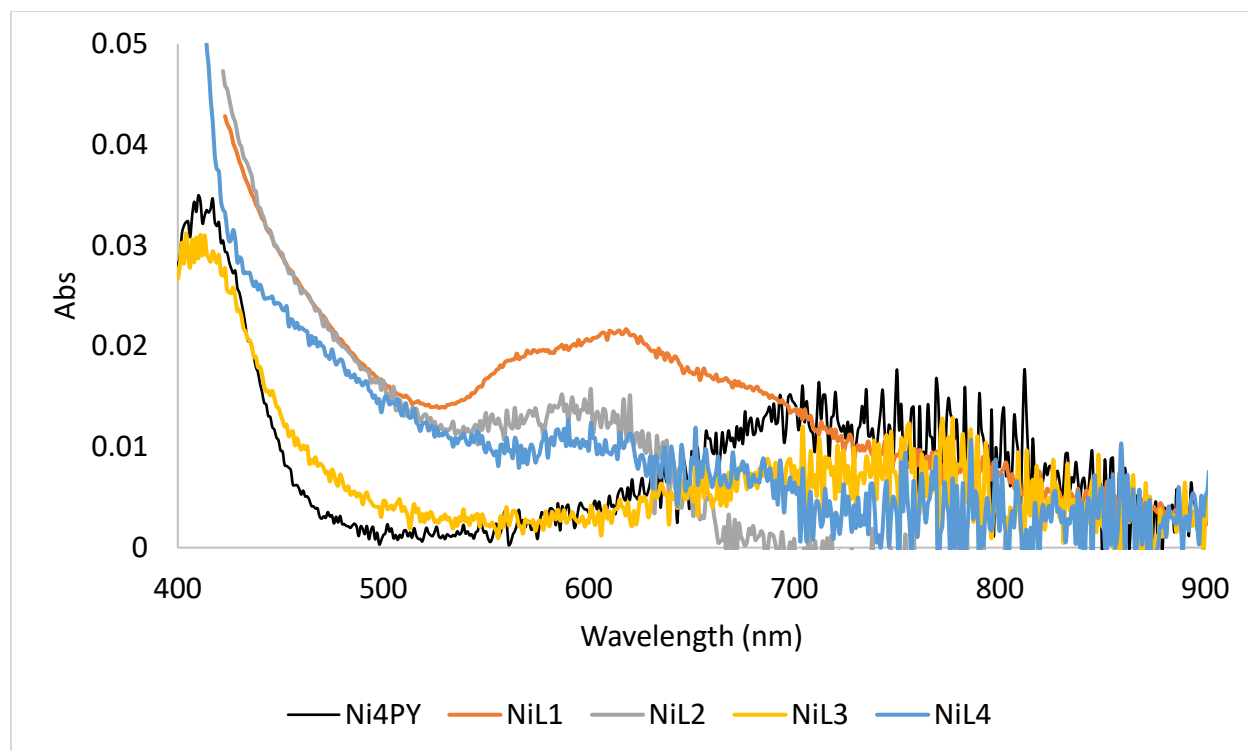
Table 35: Selected Vibrational Frequency Bands of Nickel Complexes

Molecular motion	Ni4PY (cm ⁻¹)	NiL1 (cm ⁻¹)	NiL2 (cm ⁻¹)	NiL3 (cm ⁻¹)	NiL4 (cm ⁻¹)
C=C	1508.3	1525.9	1510.6	1515.0	1502.9
C-O-C ether	-	-	1042.8	-	1037.6
O=C-O ester	-	1734.1	-	1724.3	-
Ar-C-H	3041.8	3048.5	3151.9	3151.3	3107.0
Ar-NH ₂	-	-	3234.8-3285.6	3305.4-3402.3	-
Ar-NO ₂	-	1349-1427	-	-	1384.4-1424.9
Ar-C=N	1434.0	1427.0	1451.9	1427.4	1445.9
Aryl-O stretch	-	1276.0	1240.8	1283.7	1263.0

3.2.4 Electronic and Magnetic Properties of the Nickel Complexes

The electronic spectrum 11 of the nickel complexes were done using harsh solvents such as hydrochloric acid because of their poor solubility. The effective magnetic moment of **Ni4PY** was 4.30 BM and it correspond to a high spin d^8 tetrahedral complex, and this suggests that nickel has only two unpaired electrons and it exist in +2 oxidation state. This value does not differ significantly from the expected spin only magnetic moment of a d^8 tetrahedral complex of 4.47 BM. This high magnetic susceptibility was due to orbital contribution as there were two unpaired electrons in the unsymmetrically filled t_2 orbital. For a high spin d^8 complex, three spin allowed absorptions bands were expected but only two absorption bands were found on the electronic spectrum of the nickel (II) complex. The two absorption bands at 446 and 750 nm were assigned to the $^3T_1(F) \rightarrow ^3T_2$ and $^3T_1(F) \rightarrow ^3A_2$ transitions. The spectrum for **NiL1** nickel complex showed two spin allowed $d-d$ transitions at 420 nm and at 624 nm while its effective magnetic moment for was found to be 2.67 BM but for a tetrahedral d^8 crystal field the calculated magnetic moment due to spin only is 2.83. This means that the complex was not tetrahedral but a pseudo-octahedral [169]. By measuring the magnetic susceptibility, the Jahn-Teller effect indicates that nickel (+2) tetrahedral complexes have a partial octahedral geometry. The Jahn-Teller effect occurs when a molecule or complex exhibits electronic states that are degenerate (or nearly degenerate) in a certain geometry. Distortion eliminates the degeneracy and reduces the energy

level. Nickel (+2) tetrahedral complexes feature a degenerate electronic state and partially full d-orbitals. This degeneracy is due to the tetrahedral d-orbital sets eg and t2g. A perfect tetrahedron has degenerate states because the energy of both sets is equal. To counteract the degeneracy's unstable nature, the complex undergoes a distortion as a result of the Jahn-Teller effect. Degenerate orbitals warp because of bonding at energy levels that vary depending on the surrounding environment. In order to break degeneracy and save energy, nickel (+2) tetrahedral complexes transform into partial octahedra. The distorted tetrahedron has an elongated or compressed shape along a bond axis. Bond lengths change due to elongation or compression, leading to symmetry breakdown and d-orbital degeneracy. By eliminating degeneracy and providing stability, the partial octahedral geometry completes the complex. Magnetic susceptibility tests often reveal the Jahn-Teller phenomena due to the influence of distortion on complex magnetic properties. Spin-only magnetic susceptibility applies to a d8 system with complete tetrahedral geometry. The magnetic susceptibility departs from spin-only when the Jahn-Teller distortion occurs and the complex takes on a partly octahedral structure, as a result of the splitting of degenerate electronic states. This was because this value was close to the calculated magnetic moment of a d^8 octahedral complex. A d^2 Tanabe Sugano diagram shows two transitions; ${}^3T_1(F) \rightarrow {}^3T_2(F)$ and also, ${}^3T_1 \rightarrow {}^3A_2(F)$, which also confirm the two absorption bands observed in the spectrum for the nickel complex. The electronic spectra and magnetic properties of **NiL2** were also studied, the calculated effective magnetic moment was found to be 4.78 BM which assumed a tetrahedral d^8 complex and close to the calculated value of 4.47 BM. The nickel (II) complex had two absorption bands centered at 420 and 607 nm, which could be tentatively assigned considering tetrahedral symmetry around the metal to ${}^3T_1(F) \rightarrow {}^3T_2(F)$ and ${}^3T_1(F) \rightarrow {}^3A_2(F)$ transitions respectively. The electronic spectrum of nickel complexes was not as smooth as other spectrums, and this was assumed to be due to the solubility issues encountered with these complexes.



Spectrum 11: Electronic Spectra of nickel complexes

The **NiL3** complex was also observed to be d^8 tetrahedral complex, its effective magnetic moment was 3.87 BM and the calculated magnetic moment was 4.47 BM. Even though there was a deviation, it shows that the complex was indeed a tetrahedral complex with orbital contribution in the t_2 orbitals as they are not filled. Furthermore, this complex electronic spectrum showed two visible $d-d$ transitions 417 nm and 747 nm which were assigned to ${}^3T_1(F) \rightarrow {}^3T_2(F)$ and ${}^3T_1(F) \rightarrow {}^3A_2$ transitions respectively. The **NiL4** complex also had two energetically visible $d-d$ transitions 420 nm and 607 nm which were assigned to ${}^3T_1(F) \rightarrow {}^3T_2(F)$ and ${}^3T_1(F) \rightarrow {}^3A_2$ transitions respectively. Together with this complex effective magnetic moment it was assigned to be a d^8 tetrahedral nickel complex. This was because its measured 4.67 BM compared to a calculated 4.47 BM. This showed that the complex also had orbital contribution in its t_2 orbitals. All the nickel complexes were assigned the oxidation state +2 and were all paramagnetic as they all had two unpaired electrons. **NiL1** was the only complex with a partial d^8 octahedral geometry while all the other complexes were d^8 tetrahedral complexes. The summarized transitions and magnetic susceptibility studies of the nickel complexes are shown in Table 36.

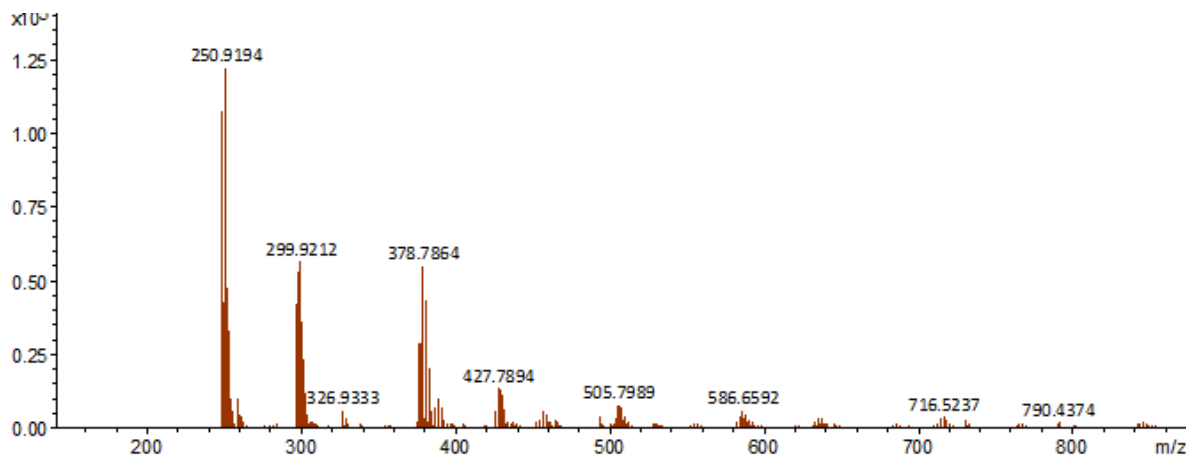
Table 36: Electronic Transitions and Magnetic Susceptibility of Nickel Complexes

Complex	Electronic Transition ((nm) [$\epsilon \times 10^4(\text{L mol}^{-1} \text{cm}^{-1})$])		Magnetic Moments (BM)			
			Transition Type	μ_{cal}	μ_{eff}	Crystal Field
Ni4PY	π - π^* and CT	<350		4.47(orbital contribution)	4.30	d^8 Tetrahedral
	d - d	446	$^3T_1(F) \rightarrow ^3T_2$			
		750	$^3T_1(F) \rightarrow ^3A_2$			
NiL1	π - π^* and CT	<350		2.83(spin only)	2.67	d^8 Tetrahedral or pseudo-octahedral
	d - d	420	$^3T_1(F) \rightarrow ^3T_2$			
		624	$^3T_1(F) \rightarrow ^3A_2$			
NiL2	π - π^* and CT	<350		4.47	4.78	d^8 Tetrahedral
	d - d	420	$^3T_1(F) \rightarrow ^3T_2$			
		607	$^3T_1(F) \rightarrow ^3A_2$			
NiL3	π - π^* and CT	<350		4.47	3.87	d^8 Tetrahedral
	d - d	417	$^3T_1(F) \rightarrow ^3T_2$			
		747	$^3T_1(F) \rightarrow ^3A_2$			
NiL4	π - π^* and CT	<350		4.47	4.67	d^8 Tetrahedral
	d - d	420	$^3T_1(F) \rightarrow ^3T_2$			
		607	$^3T_1(F) \rightarrow ^3A_2$			

3.2.5 Mass Spectrometric Characterization of the Nickel Complexes

In order to characterize the structures of the nickel complexes, the HRESI-MS was employed to corroborate previous characterization techniques. Unfortunately, the nickel complexes were poorly soluble, thus most of their spectra had to be magnified to see the molecular ion peak. The mass spectrum of **Ni4PY** complex is displayed (spectrum 12) as an example of the nickel complexes mass spectra in this research. The **Ni4PY** complex had a molecular mass of 381.91 g/mol ascribed to the 427.7894 m/z peak. The ascribed peak was due to the $[M+ACN+H]$ adduct, which validated the structure with a new mass of 426.97 g/mol when the adduct counterparts were added to the molecular mass. The **NiL1** spectrum corresponds to the adduct $[M+Na]$ with a mass of 667.8751 m/z and a molar mass plus sodium of 666.99 g/mol. The **NiL2** molecular ion peak was at 529.0484 m/z with the designated adduct $[M+H]$, which agreed with the molar mass and proton of 529.07 g/mol. Similarly, to **Ni4PY** the **NiL3** displayed the molecular ion

peak with the $[M+CN+H]$ adduct of 627.0715 m/z which confirmed the mass. The structure of **NiL3** was verified at 628.03 g/mol when the two adduct components were added to the molar mass. It was determined that the complex's poor solubility was the cause of the low intensity molecular ion peak $[M]$ that was visible in the **NiL4**. However, **NiL4** $[M]$ [revealed a molecular mass to charge ratio of 588.9942 m/z , which was validated to be 588.07 g/mol.



Spectrum 12: Mass spectrum of **Ni4PY**

3.2.6 Thermal Studies of the Nickel Complexes

Thermal techniques being TGA, and DSC were used to study the thermal stability of the complexes of nickel. The **Ni4PY** thermogram in Figure 62 reveals that the complex lost four components with comparable masses of approximately 2 mg, indicating that these components had similar molar masses. Their respective percentage losses were 25%, 22%, and 20% which was hypothesized to be the disappearance of the two **4PY** ligands and $NiCl_2$. The DSC profile shows that the melting point of this compound was observed at 210.6 °C, while beyond that the compound was gradually decomposing. The thermogram of the complex **NiL1** in showed four mass losses, where the first one at 2.56% was attributed to the loss of one water molecule that was calculated to be at 2.7%. The steep mass loss of 7.39 mg or 38.6% was attributed to the loss of the **L1** ligand which was calculated to be 38.9%. The final two mass losses were ambiguous; nonetheless they were assigned to two $-Cl$ ligands at the 1.43 mg mass or 7.5%, while the calculated was 10.7%. The last loss of 1.7 mg or 12.5% was assigned to the possible loss of $-N_2O$ with a calculated loss of 13.2%. The **NiL1** DSC profile showed two endothermic glass transition transformations at 76.6°C and 96.8°C before reaching the cold crystallization at 171.5°C and the

melting point at 225.4 °C and ultimately the decomposition beyond this. The **NiL2** thermogram showed two small mass losses, suggesting that the complex may have been the most stable. The first mass loss was 0.82 mg at 7.76% and was attributed to water with a calculated loss of 6.38%. The second and last mass loss was assigned to the potential mass loss of **-NH₂** from the ligands. As for the DSC profile, there was the endothermic glass transformations observed at 69.3 °C, and 306.7 °C, the crystallization exotherm was observed at 315.8 °C then finally the melting point at 335.7 °C and decomposition beyond this. Similarly, to its analogue **NiL1**, the thermogram of **NiL3** showed four distinct mass losses, the first two were very small. The mass loss at 0.71 mg at 8.21 % was proposed to be the loss of two water molecules with a calculated loss at 6%, while the second mass loss was given to the loss of the two chloride ligands from the complex at 10.4% compared to the calculated 11.4%. The last two mass losses were thus observed to be the loss of the two **L3** ligands at 36 and 42 % compared to a calculated 37.6%. Additionally, the DSC profile of **NiL3** showed variable transformations comparable to that of **NiL1**, the glass transition was seen at 87.6 °C and 121.8 °C while the cold crystallization was at 171.3 °C. The melting point of the complex was observed to be 304.6 °C with the decomposition beyond this temperature. The final thermal data was for **NiL4**. The complex showed high stability just as its analogue **NiL2**. This was based on the loss of only three components from the complex, where the first mass loss of 0.55 mg or 8.5% was allotted to the loss of the 0.35 moles of the NiCl₂ impurity observed under elemental analysis. Then the 2.68 mg or 41.4% molar mass was close to the calculated loss of one **L4** ligand at 37%. Then finally, the 7.88% or 0.51 mg was assigned to the loss of one of the **-N₂O** from the ligands. The DSC profile showed the endothermic glass transformation at 77.4 °C and the melting point at 243.1 °C.

Thermal techniques being TGA, and DSC were used to study the thermal stability of the complexes of nickel. The first thermogram of **Ni4PY** in Figure 57 shows that the complex lost four components of a similar mass of around 2 mg, suggesting that they had a comparable molar mass. Their percentage losses were 25, 22, and 20%. These were suggested to be the loss of two of the ligands **4PY** and NiCl₂. The DSC profile shows that the melting point of this compound was observed at 210.6 °C, while beyond that the compound decomposed.

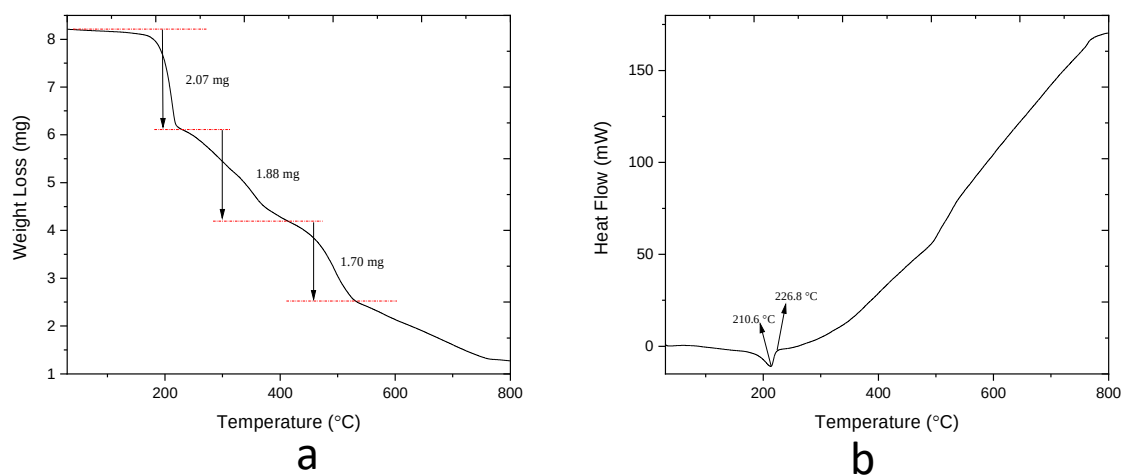


Figure 57: a) Thermogram and b) DSC profile of **Ni4PY**

3.2.7 Antibacterial Studies of the Nickel Complexes

3.2.7.1 Bacterial Minimum Inhibition Concentration of the Nickel Complexes

The antibacterial properties of the synthesized compounds were tested towards the four strains of bacteria, their ligand's antibacterial properties were previously discussed under Section 3.1, sub section 3.1.3. Figure 63 shows the summarized data in a bar graph, while Table 28 shows the mean MIC values together with their standard deviations. The control used was silver sulfadiazine (**AgSD**), this is an approved drug used to treat bacterial infections of burn wounds. The compound is a silver based ionic complex with sulfadiazine [136]. In general, the nickel complexes were less potent than the control **AgSD**. Nonetheless, the highest activity of these compounds were towards *S. aureus*. There was an observed trend on the Gram-positive bacteria where the nitro moiety complexes (**NiL1** and **NiL4**) were more active as opposed to the Gram-negative bacteria. The amino moiety complexes (**NiL2** and **NiL3**) showed no trend in activity towards the Gram-negative or the Gram-positive bacteria.

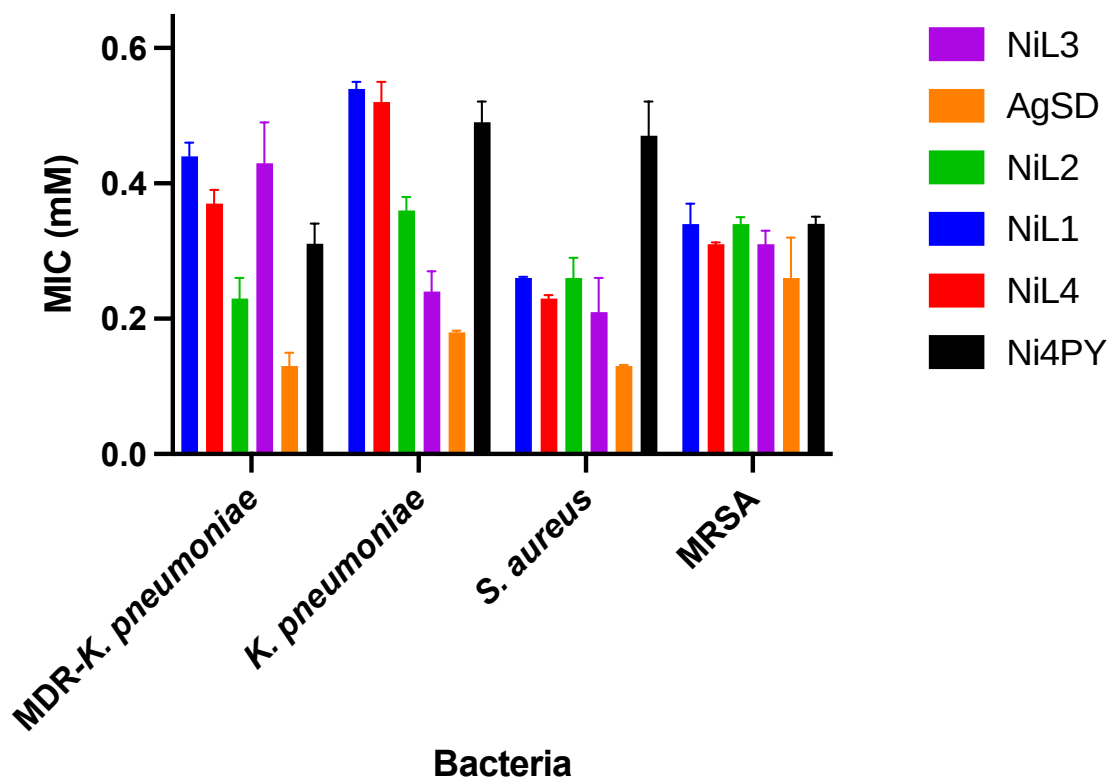


Figure 58: Summarized MIC values of nickel complexes

Additionally, the trend in activity towards these compounds per strain is summarized in Table 37. The trend suggests that **NiL3** was the best performing compound while **Ni4PY** was the worst. In comparison to the ligands in Section 3.1 sub section 3.1.3, there activity towards the Gram-negative bacteria showed that the complexes had higher MICs as compared to the free ligands. This was in exception of **L1** and **NiL1**, which may suggest that the introduction of the metal centre deactivates the activity of the free ligand or rather the access through the Gram-negative cell membrane played a role. Gram-negative bacteria use the porin or efflux pump to ingest essentials and excrete waste, and this may be small for most of these compounds to enter the cell, consequently reducing the activity of the complexes [170], [171]. On contrary, the activity of the compounds towards the Gram-positive bacteria showed an inverse relationship, whereby there was better activity from the complexes as opposed to the ligands, except for **L2** and **NiL2**. This enhanced activity could be explained by chelation theory and biological activity. Due to the partial sharing of the metal ion's positive charge with donor groups and the potential for p

electron delocalization throughout the whole chelate ring, chelation significantly reduces the polarity of the metal ion. In addition to lowering the polarity of the metal ion, chelation also makes the chelate more lipophilic. This favours the interaction of the metal ion with the cell walls and causes interference with regular cell functions [172], [173].

Table 37: MIC of Nickel Complexes

Compound ↓	Microbe →	MIC (mM)			
		<i>K. pneumoniae</i>	MDR- <i>K. pneumoniae</i>	<i>S. aureus</i>	MRSA
AgSD		0.18±0.002	0.13±0.02	0.13±0.002	0.26±0.06
Ni4PY		0.49±0.030	0.31±0.030	0.47±0.050	0.34±0.010
NiL2		0.36±0.020	0.23±0.030	0.26±0.030	0.34±0.010
NiL1		0.54±0.010	0.44±0.020	0.26±0.002	0.61±0.030
NiL4		0.52±0.030	0.37±0.020	0.23±0.005	0.31±0.003
NiL3		0.24±0.030	0.43±0.06	0.21±0.050	0.31±0.02
TREND		NiL3>NiL2>Ni4PY>NiL4 >NiL1	NiL2>Ni4PY>NiL4>NiL3 >NiL1	NiL3>NiL4>NiL2>NiL1> Ni4PY	NiL3>NiL4>NiL2>Ni4PY >NiL1

3.2.7.2 Time Kill Kinetics

The time kill kinetics were studied to distinguish between the bacteriostatic and the bactericidal properties of the nickel complexes. Even though all the compounds were less potent than the control, the study was done on compounds that had activity that was at least 0.3 mM or lower. Considering the time kill kinetics of the active compounds against *S. aureus* shown in Figure 59, all the compounds including the control **AgSD** were observed to be bacteriostatic towards *S. aureus*. This was because the trend of the growth of all the compounds including the control was not declining nor exponentially growing. The Log %Viability of the **AgSD** seemed to be higher compared to that of the other compounds with **NiL2** slightly more potent than the rest.

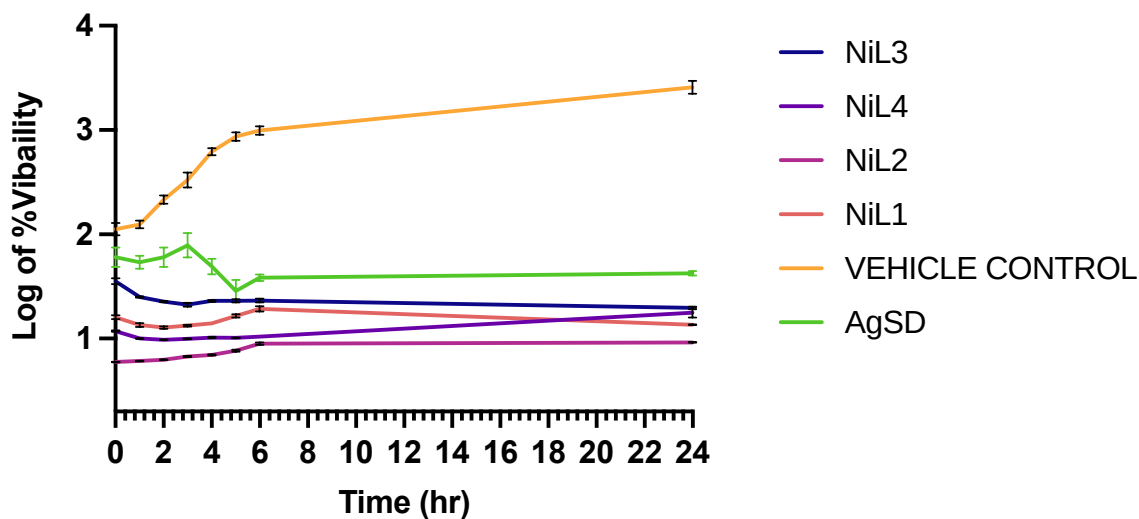


Figure 59: Time kill Kinetics of active nickel complexes against *S. aureus*

Only **NiL3** and **NiL4** showed some potency towards MRSA, similarly to *S. aureus*, both these complexes and the control **AgSD** were bacteriostatic (Figure 60). The nitro-ether complex **NiL4** showed a bactericidal trend (<5 hr), but later grew exponentially towards a bacteriostatic trend. This bioactivity was different to that of the non-drug resistant bacteria in Figure 31, this may be attributed to the drug resistant nature of this strain.

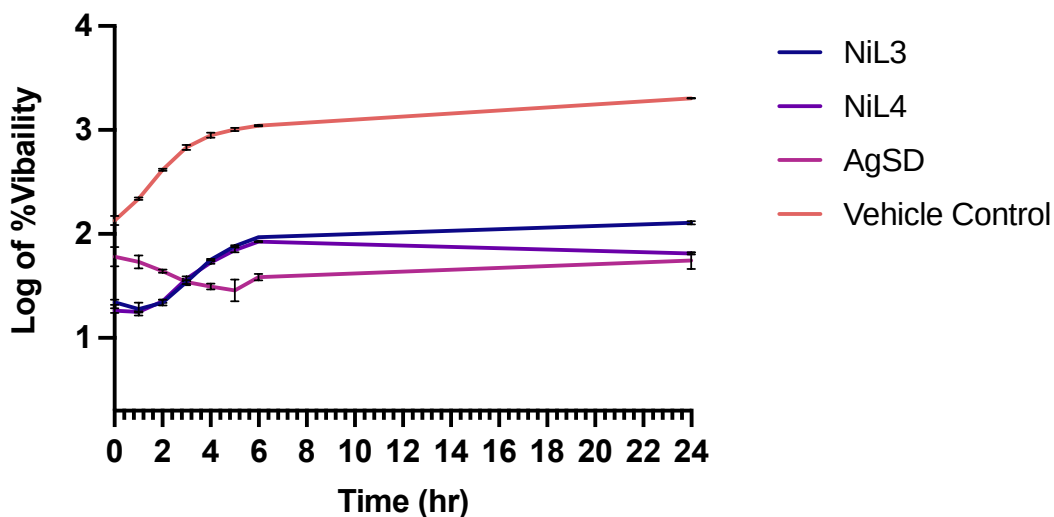


Figure 60: Time kill Kinetics of active nickel complexes against MRSA

The ester-amino complex **NiL3** was the only compound that showed some potency towards the non-drug resistant *K. pneumoniae* and thus its time kill kinetics were studied towards this

bacteria (Figure 61). From Section 3.1 sub section 3.1.3, the free ligand showed a bacteriostatic trend, which was still the case when coordinated to nickel. However, the complex **NiL3** displayed a strong bactericidal trend for the first 6 hr of the experiment but then grew exponentially after 24 hr of the experiment. This may be attributed to the fact that the complex had a higher MIC of 0.24 mM which was different from 0.16 mM of the free ligand.

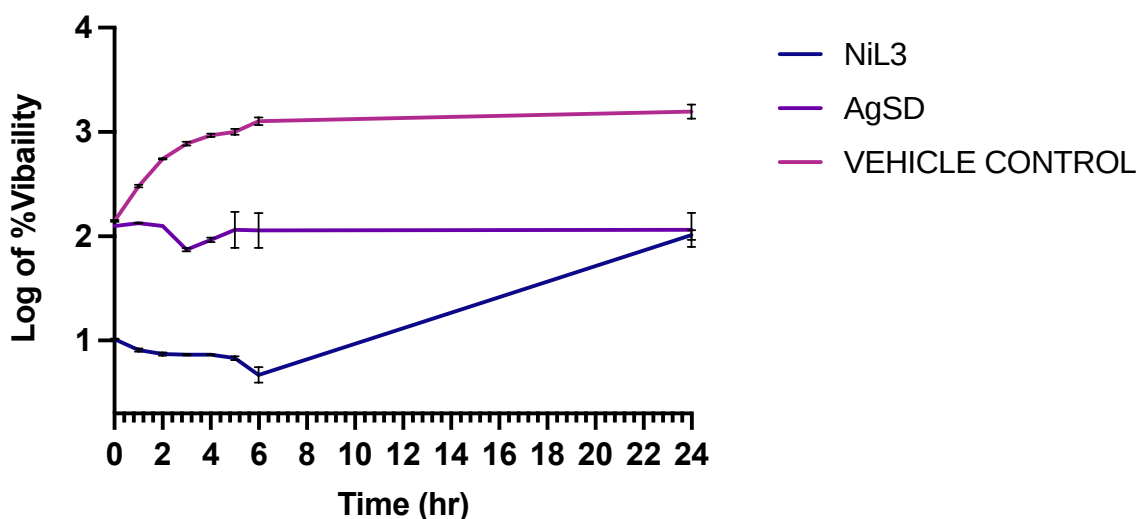


Figure 61: Time kill Kinetics of active nickel complexes against *K. pneumoniae*

NiL2 was the only compound that showed some potency towards MDR- *K. pneumoniae*. This compound had a comparable MIC to that of the free ligand and was the only exception from the other compounds which had better activity against Gram-negative bacteria. **NiL2** showed bacteriostatic activity (Figure 62), however, this activity started to show at 3 hr of the experiment. After, the 3 hr the bactericidal trend started to look similar to that of the control **AgSD**. According to results in Section 3.1 sub section 3.1.3, the ether-amino ligand **L2** also showed a bacteriostatic trend. This suggests that ligand and the complex may have similar antibacterial properties towards the drug resistant organism MDR- *K. pneumoniae*.

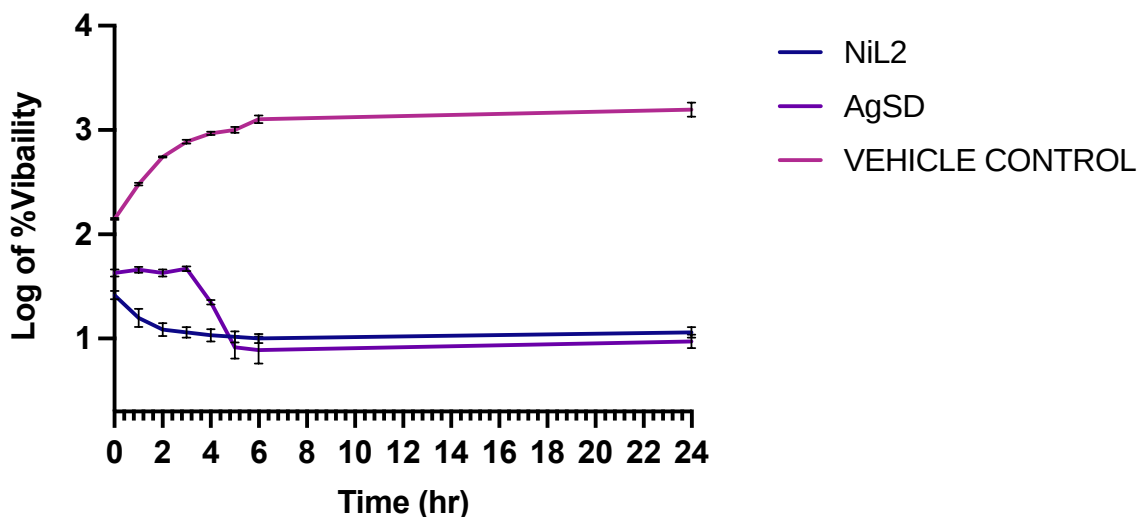


Figure 62: Time kill Kinetics of active nickel complexes against MDR- *K. pneumoniae*

Due to the low potency of the nickel complexes in comparison to the control **AgSD** and even their respective ligands, the mode of action studies was not conducted, and the study ended with only the time kill kinetics.

3.2.7.3 Molecular Docking Studies of active Nickel Complexes

NiL3, **NiL4** and **AgSD**, the most active nickel complexes and the control drug against *S. aureus*, were used in molecular docking investigations against the crystal structure of PBP2a, a PBP of MRSA, which is depicted in Table 38 and Figures 62 to 64 [168]. The docking data reveal that, **NiL4** (-9.59 kcal/mol) was better at binding to PBP2a followed by **NiL3** (-9.17 kcal/mol), while the control drug **AgSD** performed far below the two compounds with a binding energy of -5.88 kcal/mol. The active site of the PBP2a protein contains the amino acids Lys406, Lys597, Ser598, Glu602, and Met641. These residues allowed a quinazoline antibiotic with related pharmacophores, such as aromatic rings, carbonyl, and carboxylic acid, to bind to the PBP2a protein active site [168]. The complex **NiL4** interacted with the following similar amino acid residues Lys148, Ser149 and Met372 while **NiL3** interacted with Glu239, and Ser240. The control drug **AgSD** interacted with two similar amino acids in the active site, the Lys273, and Glu294. The data was dissimilar to the experimental MIC data where the control was significantly better at 0.13 mM, while the complexes had higher MICs at 0.21 mM and 0.23 mM for **NiL3** and **NiL1**

respectively. This deviation may have been caused by the poor solubility of these complexes, where the true MIC of the complexes may have been masked.

Table 38: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands and their complexes against methicillin-resistant *S. aureus* (PDB: 4cjn)

Compounds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
Nil3	-9.17	0.190 μ m	Glu239,Pro213, Thr216	Leu214,Val256, Val277	Arg151,Arg241, Tyr196,Ser240
Nil4	-9.59	0.094 μ M	Lys148,Ser149, Arg241,Asn260	Pro258,Val277, Met372	Gly257,His293, Val256,Thr216
AgSD	-5.88	49.35 μ M	Gly271,Val277	Asp275,Ala276, His293	Tyr272, Lys273, Gln292,Glu294

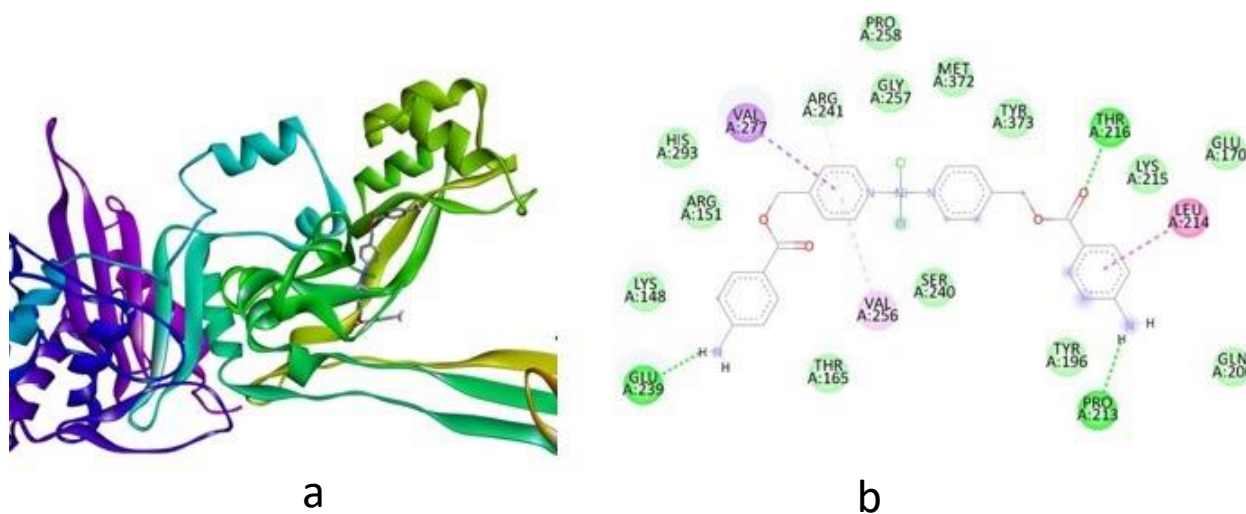


Figure 63: The binding interactions of **Nil3** against methicillin-resistant *S. aureus* (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

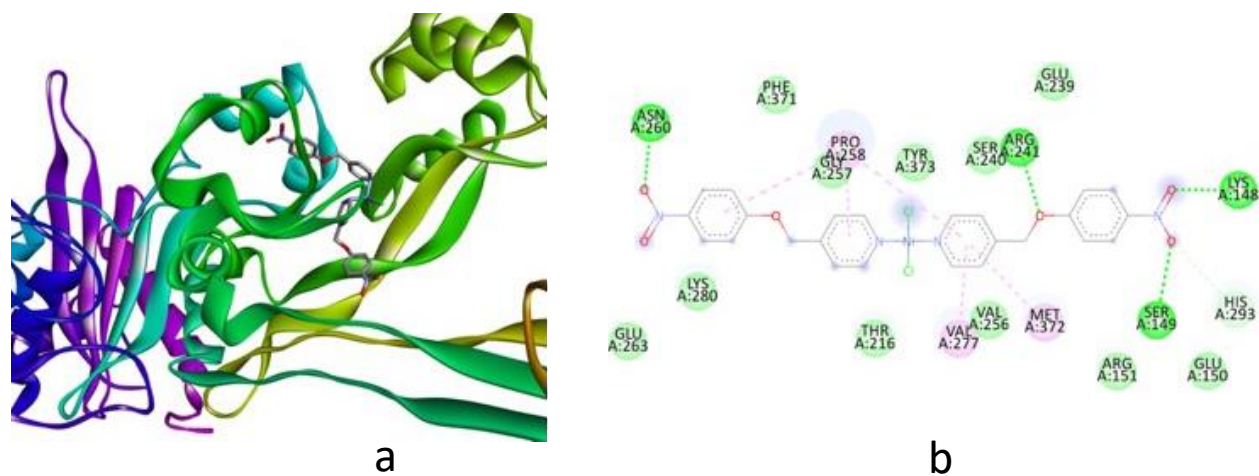


Figure 64: The binding interactions of **NiL4** against methicillin-resistant *S. aureus* (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

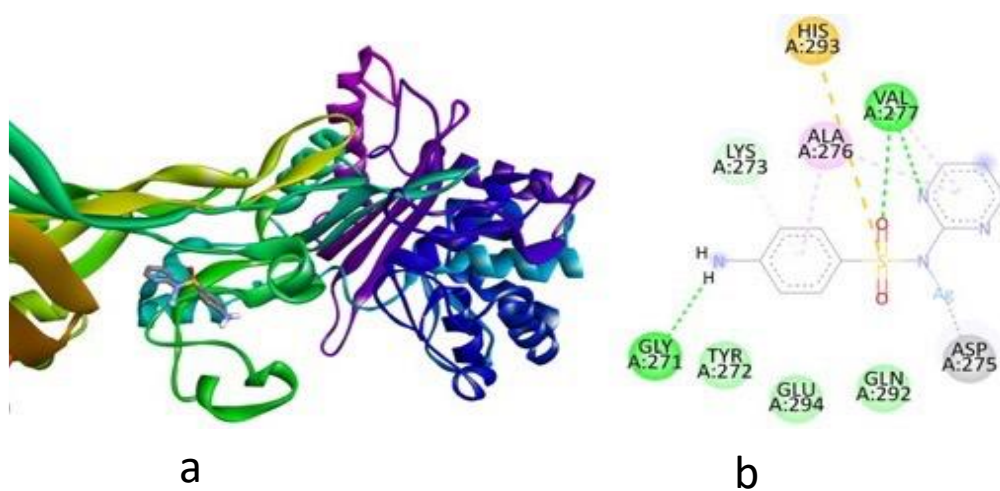


Figure 65: The binding interactions of **AgSD** against methicillin-resistant *S. aureus* (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

The most active nickel complexes were docked onto the PABA binding pocket of the enzyme DHPS to see if they had any competitive antagonistic properties toward PABA. Even though **NiL3** was performing well towards *K. pneumoniae*, *S. aureus* and MRSA in the MIC assay, the control drug **AgSD** was still significantly more active. However, according to the molecular docking studies **NiL3** (see Table 39 and Figures 66 to 68) was a better PABA antagonist towards binding

with DHPS. This was observed by the binding energy where **NiL3** bound at -8.61 kcal/mol followed by **AgSD** at -6.65 kcal/mol and PABA at -3.80 kcal/mol. It was evident that **NiL3** interacted with amino acid residues of the binding pocket of DHPS, these includes the Lys220, Asp184, Asn120, Arg68, Thr67 and Asn101. The control drug **AgSD** was also binding with DHPS at the PABA binding pocket as indicated by the amino acid residues Thr67, Asn120, Arg254, Arg68, Asp184 and Lys220 which were also found in the interactions of PABA with DHPS. The converse results of the MIC and molecular docking studies still confirm that the solubility issues of the nickel complexes affected their bioactivity, and should they have dissolved better, their MIC would have been lower.

Table 39: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands against Dihydropteroate Synthase DHPS (PDB: 3tye)

Compounds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
NiL3	-8.61	0.49 μ M	Thr29,Asn120, Asp184,Arg254	Arg68,Ile122, Met145,Lys220, Val231	Asn27,Thr67, Pro69,Asp101
AgSD	-6.65	13.28 μ M	Thr67,Asn120, Arg254	Met23,Arg68, Met145,Asp184	Pro69,Lys220
PABA	-3.80	1650.00 μ M	Thr67,Asp101, Gly216,Arg254	Ile122,Phe189	Arg68,Asn120, Asp184,Lys220

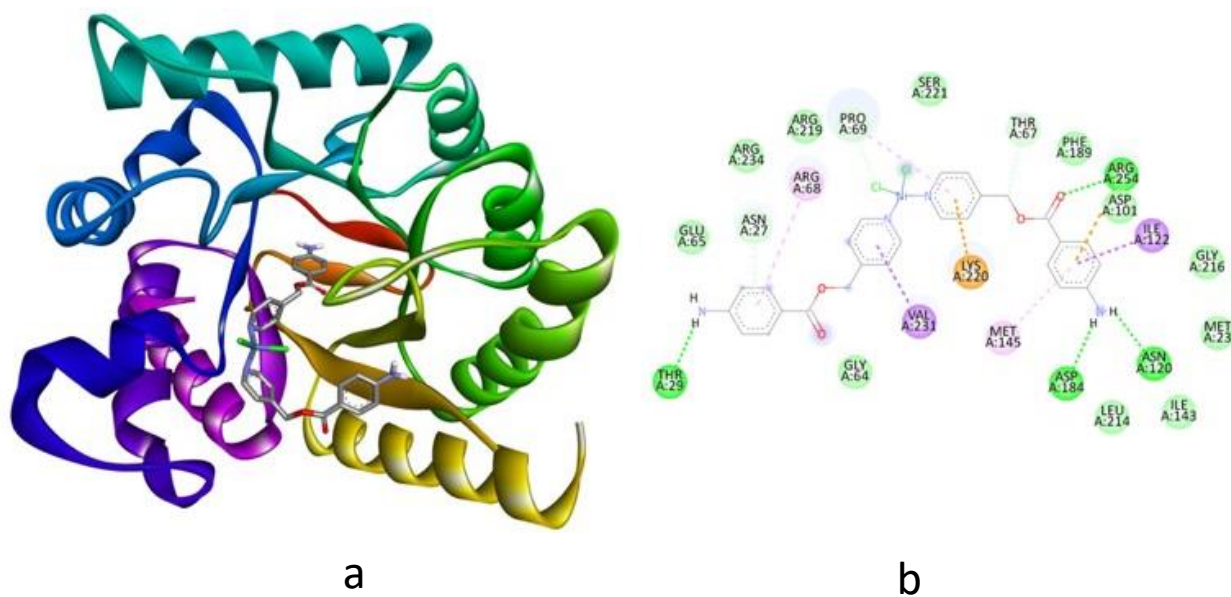


Figure 66: The binding interactions of **NiL3** against Dihydropteroate Synthase DHPS (PDB: 3tye). a) shows the binding site, b) shows the interacting amino acid residues.

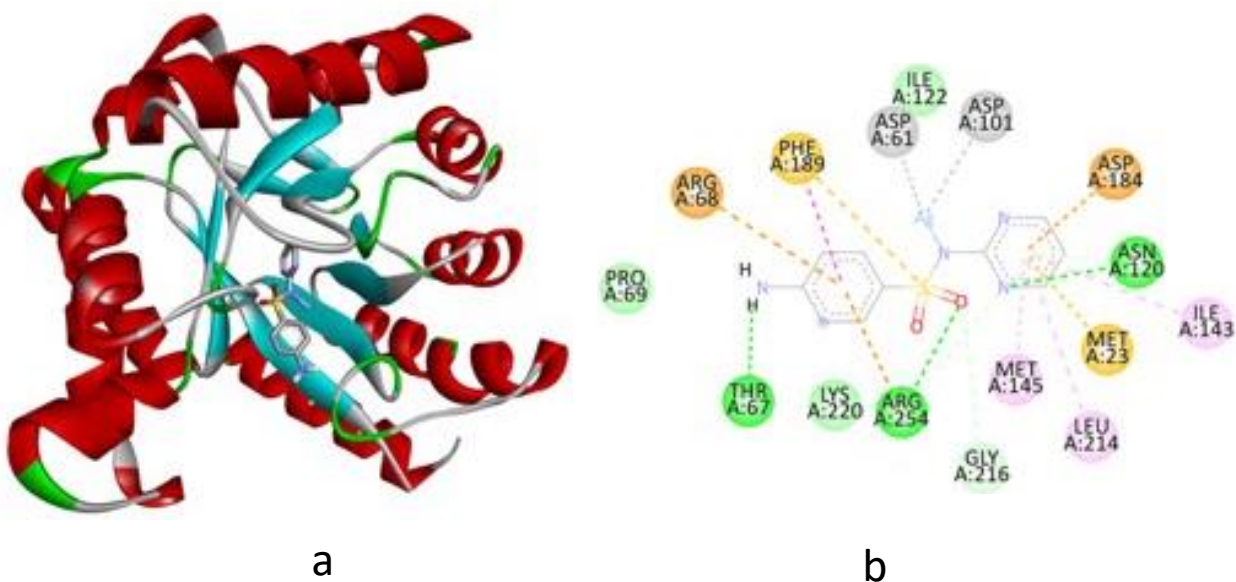


Figure 67: The binding interactions of **AgSD** against Dihydropteroate Synthase DHPS (PDB: 3tye). a) shows the binding site, b) shows the interacting amino acid residues.

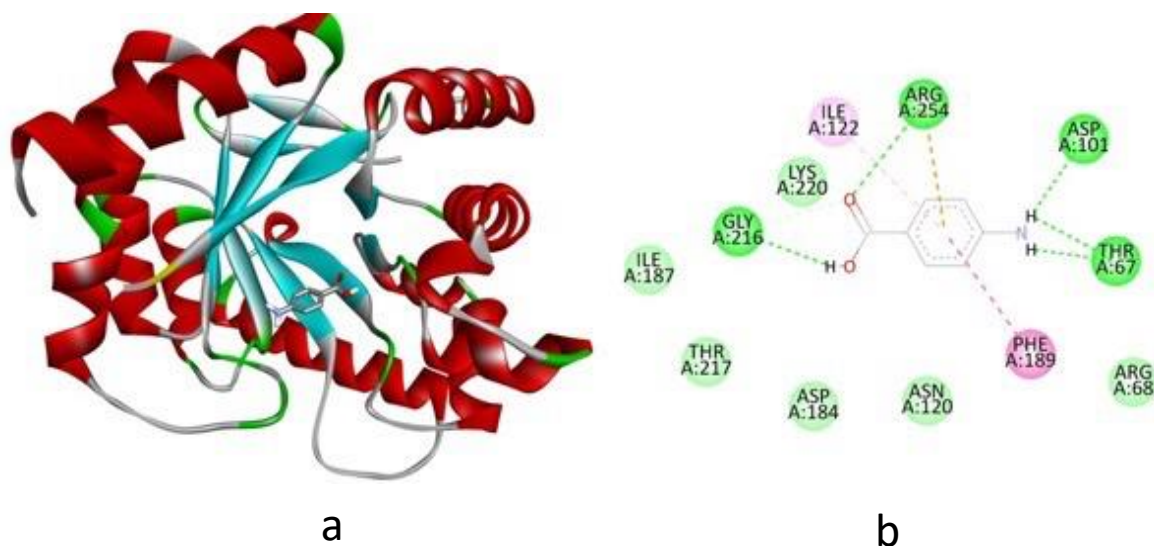


Figure 68: The binding interactions of PABA against Dihydropteroate Synthase DHPS (PDB: 3tye). a) shows the binding site, b) shows the interacting amino acid residues.

The molecular docking method was used to study the β -lactam antibiotic resistant OmpK36 (PDB: 6rd3) of *K. pneumoniae* to comprehend the way the active complexes interacted with the crucial amino acid residues of this bacterial protein (Table 40) [154]. The active complex **NiL3** bound to the protein better than the control at -7.20 kcal/mol compared to the -6.27 kcal/mol of **AgSD**. The two compounds were observed to be interacting with the protein at similar binding sites, they both interacted with the Glu110 and Phe111 amino acid residues (see Figures 69 and 70). Also, some residues that were not so different such as the Arg, Tyr, and Asp. These findings confirm that cell membrane disruption was a possible mode of action of the **NiL3** compound.

Table 40: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands and their complexes against OmpK36 from *K. pneumoniae* (PDB: 6rd3)

Compounds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
Nil3	-7.20	5.25 μ M	Arg75,Tyr95, Glu110,Phe111, Asp119,Arg125	Trp73,Tyr116	Asp106,Gly112, Gly113,Ser118
AgSD	-6.27	25.55 μ M	Arg37	Lys116,Tyr35, Glu110	Phe111,Gln61, Asp18,Leu20

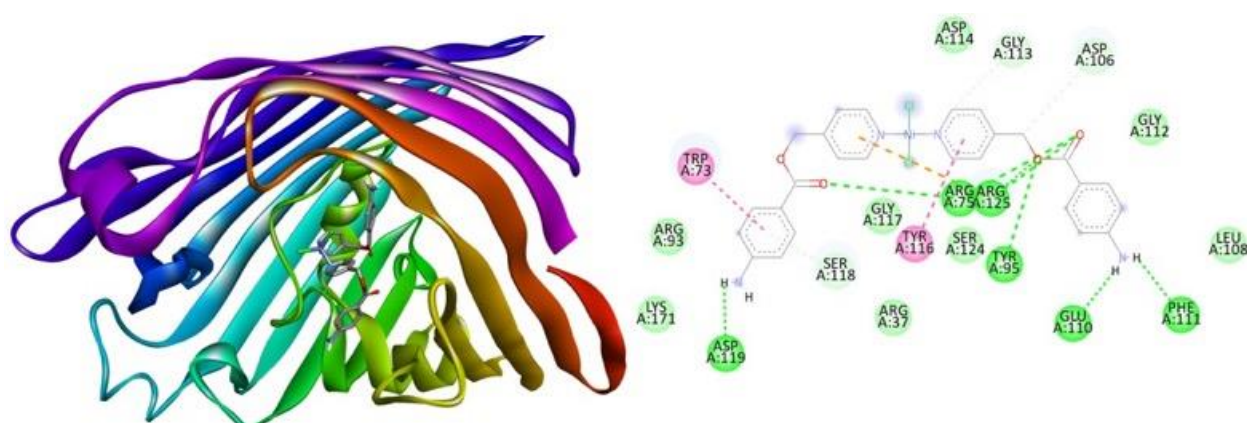


Figure 69: The binding interactions of **Nil3** against OmpK36 from *K. pneumoniae* (PDB: 6rd3)

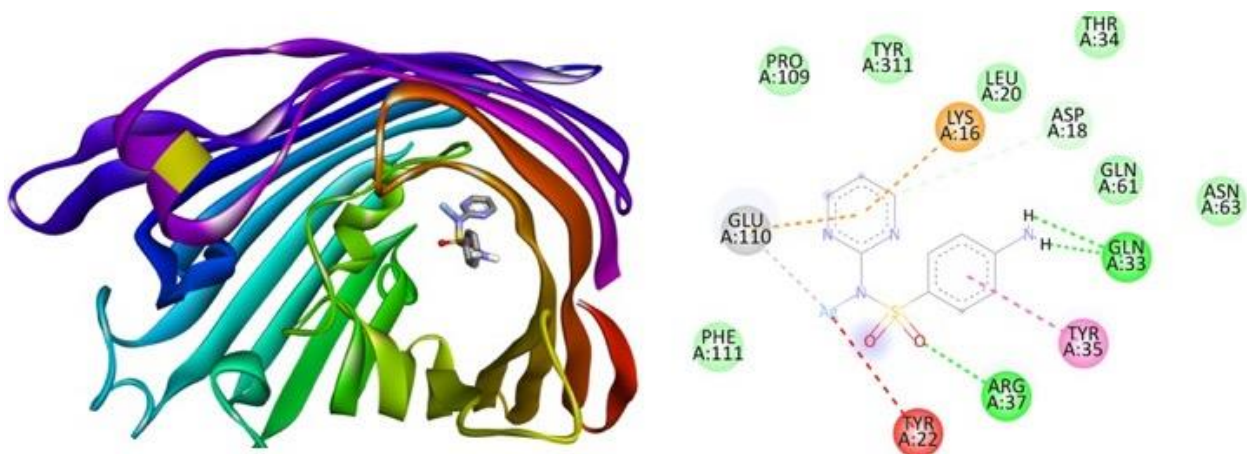


Figure 70: The binding interactions of **AgSD** against OmpK36 from *K. pneumoniae* (PDB: 6rd3)

3.2.8 Conclusions

Five complexes of nickel were synthesized and characterized using various characterization techniques. From the five, **Ni4PY** was synthesized using the conventional solvent reaction, while the rest of the complexes were synthesized through a green mechanochemical method. The green method gave higher yields and purer products when compared to **Ni4PY**. The nickel complexes were challenging to dissolve, which made other tests on them close to futile and made it difficult to form any crystals. Nevertheless, they were observed to be d^8 tetrahedral complexes through electronic spectra and shown to be highly stable from the thermal studies. The HRESI-MS data of the nickel complexes exhibited their successful synthesis and corroborated the complimentary structural characterization data. The nickel complexes were less potent than the control **AgSD**, however, showing more activity towards the Gram-negative bacteria. Regarding the time kill kinetics, all the compounds including the control **AgSD** were observed to be bacteriostatic towards *S. aureus*. The complexes **NiL3** and **NiL4** and the control **AgSD** were bacteriostatic towards MRSA, similarly to *S. aureus*. The molecular docking scores against the *S. aureus* protein PBP2a, the *K. pneumoniae* OmpK36, and the DHPS protein show that the active nickel complexes should have been more active than even the control drug **AgSD**, but, due to solubility issues their activity was reduced.

3.3 Synthesis, Characterization and Antibacterial Properties of Copper Mixed Ligand Complexes

3.3.1 Synthesis of the Copper Complexes

The ligands **4PY**, **L1**, **L2**, **L3** and **L4** were reacted with copper chloride to synthesize copper (II) complexes. Similar to the cobalt and nickel complexes, the synthesis of **4PY** complexes employed solvent-based reactions, while a greener co-grinding method was employed for the synthesis of the complexes with the other ligands. The co-grinding method demonstrated higher yields of 80-90% (Table 41) in a shorter reaction time compared to the classical solution phase method, which typically took 3-4 hours to complete.

3.3.2 Purity, Solubility and Physical Properties of the Copper Complexes

The copper compounds were all blue with the exception of **CuL2** and **Cu4PY-H** which were black and yellow respectively. The copper complex **Cu4PY** was blue colored microcrystals and powder whereas its corresponding acido complex crystals were yellow. All the complexes and the **Cu4PY-H** were stable in dry air and moist air and under room temperature. The complex **Cu4PY** was found to have a higher melting point than the acido complex crystal **Cu4PY-H** and this agrees with the Literature [174] because complexes consist of a strong covalent bond between a metal and the ligand. The acido complex crystal **Cu4PY-H** had a low melting point of 175 °C which was attributed to the weak hydrogen bonding that holds the ligand and the metal together. The melting points of all the copper complexes are shown in Table 42. The other copper complexes showed a melting point in the range of 170 to 250 °C. All the complexes were isolated at favorable yields (62-88%), especially those synthesized mechanochemically. Furthermore, all these compounds had similar solubility, however, the compounds of **4PY** were more soluble than the rest. Their solubility was poor in most organic solvents except alcohols but was poor in water. The summarized physical and solubility properties of these complexes is shown in Table 42.

Table 41: Physical Properties of Copper Complexes

Complex	Appearance	Melting Point (°C)	Solubility	%Yield
Cu4PY	Blue powder	260	Methanol, ethanol, DMF, and DMSO	79.4
Cu4PY-H	yellow crystalline solid	175	Methanol, ethanol, DMF, DMSO, acetone, water	62.1
CuL1	Green powder	215	DMSO, DMF, Methanol Δ , Ethanol Δ .	81.2
CuL2	Black powder	195	Methanol Δ , ethanol Δ , DMF, acetonitrile Δ , water Δ	87.9
CuL3	Pale green Powder	176	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone, water	87.0
CuL4	Brown powder	Above 250	Methanol, ethanol, DMF, DCM, Ethyl acetate, acetone, water	85.0

Δ : Soluble with heat

Analysis of the purity and elemental composition of the synthesized compounds was done using elemental analysis. From this data, it was observed that the compounds were successfully synthesized. However, the copper complex **Cu4PY** showed an impurity of 0.6 mol of the starting material copper (II) chloride, while the acido complex crystal **Cu4PY-H** had 0.5 mol water. The other complexes were also found to be highly pure with little impurities of the starting materials. The ligand was found to be an impurity in **CuL2** and CuCl_2 in **CuL1** while others only showed water as an impurity. Therefore, elemental analysis shows that these complexes were successfully synthesized. Table 42 shows the calculated and observed carbon, nitrogen, and hydrogen empirical composition of the two compounds.

Table 42: Elemental Analysis and Purity of Copper Complexes

Complex	CALCULATED			FOUND			PURITY	
	N	C	H	N	C	H	IMPURITIES (mol)	% PURITY
Cu4PY	5.18 (6.08)	26.63 (31.30)	2.23 (2.63)	5.15	26.76	2.29	0.60 CuCl ₂	83
Cu4PY-H	5.94 (6.08)	30.57 (31.30)	3.21 (2.63)	5.78	30.19	2.81	0.50 H ₂ O	97
CuL1	7.59 (8.61)	42.3 (47.98)	2.73 (3.10)	7.35	42.49	2.73	0.65 CuCl ₂	88
CuL2	10.54 (10.47)	54.22 (53.89)	4.55 (4.52)	11.08	53.95	4.58	0.05 C ₁₂ H ₁₂ N ₂ O	98
CuL3	8.65 (9.48)	48.21 (52.84)	4.13 (4.09)	8.38	47.77	4.26	3.15 H ₂ O	91
CuL4	9.24 (9.42)	47.52 (48.46)	3.52 (3.39)	9.65	47.04	3.01	0.65 H ₂ O	98

3.3.3 FTIR Characterization Copper Complexes

The FTIR spectra of the copper complexes and those of the free ligands were compared to analyse the ligands' binding modes to the metal in the complexes and to identify coordination-related atoms. The FTIR frequencies of the selected peaks are listed in Table 43 and the compound spectra are displayed in Appendix 5.1. In terms of the complexes of **4PY**, the free ligand showed an absorption band at 1629.2-1589 cm⁻¹ that was assigned to the Ar=N aromatic stretch, while 1403-1279.2 cm⁻¹ was assigned the Ar-N stretch. The same peaks were observed at negligible differences to the copper acido complex crystal **Cu4PY-H**. However, the copper complex **Cu4PY** showed a significant increase in the Ar-N vibrations at 1661.3-1562.8 cm⁻¹ showing that indeed there was coordination towards the aromatic nitrogen. Furthermore, another important band of choice was the N-H from the pyridyl nitrogen, this was observed at 3370.3 cm⁻¹ on the free ligand **4PY**, while for **Cu4PY-H** it was shifted to 3379.9 cm⁻¹ showing that indeed there was a cationic pyridyl group in the acido complex due to the bound hydrogen. But, for the complex **Cu4PY**, there was no N-H stretch observed as there was a coordinate bond between the aromatic nitrogen with the metal as opposed to the acido complex crystal's pyridyl nitrogen. As for the **CuL1**

complex, the important functional groups found in the ligand were observed, this includes the ester group O=C-O at 1736.2 cm^{-1} , and the Ar-NO₂ peak at 1348.6-1410.6 cm^{-1} . Furthermore, the Ar-N observed at 1427 cm^{-1} was observed as the coordinating donor atom as this reduced from the free ligand which was 1440 cm^{-1} . The amino analogue of **CuL1** the **CuL3** complex also showed some of the important ligand functional groups. This includes the O=C-O at 1718 cm^{-1} , while the Ar-NH₂ was at 3205.4-3305.4 cm^{-1} . The coordinating donor atom was observed to be within the Ar-N at 1427.4 cm^{-1} where in the free ligand it was at 1439.8 cm^{-1} . The **CuL2** complex showed the important ligand functional groups from **L2**, these bands include the ether C-O-C peak at 1047.3 cm^{-1} , the Ar-NH₂ peak at 3209-3321.4 cm^{-1} . The coordinating donor atom was observed to be the Ar-N where in the ligand it was at 1463.4 cm^{-1} to 1432 cm^{-1} in the complex. Finally, the nitro analogue, **CuL4** also showed some of the important ligand **L4** functional groups. These are the C-O-C peak at 1033.2 cm^{-1} , while the Ar-NO₂ peak was observed at 1341-1390.29 cm^{-1} and finally the bonding Ar-N observed at 1448 cm^{-1} .

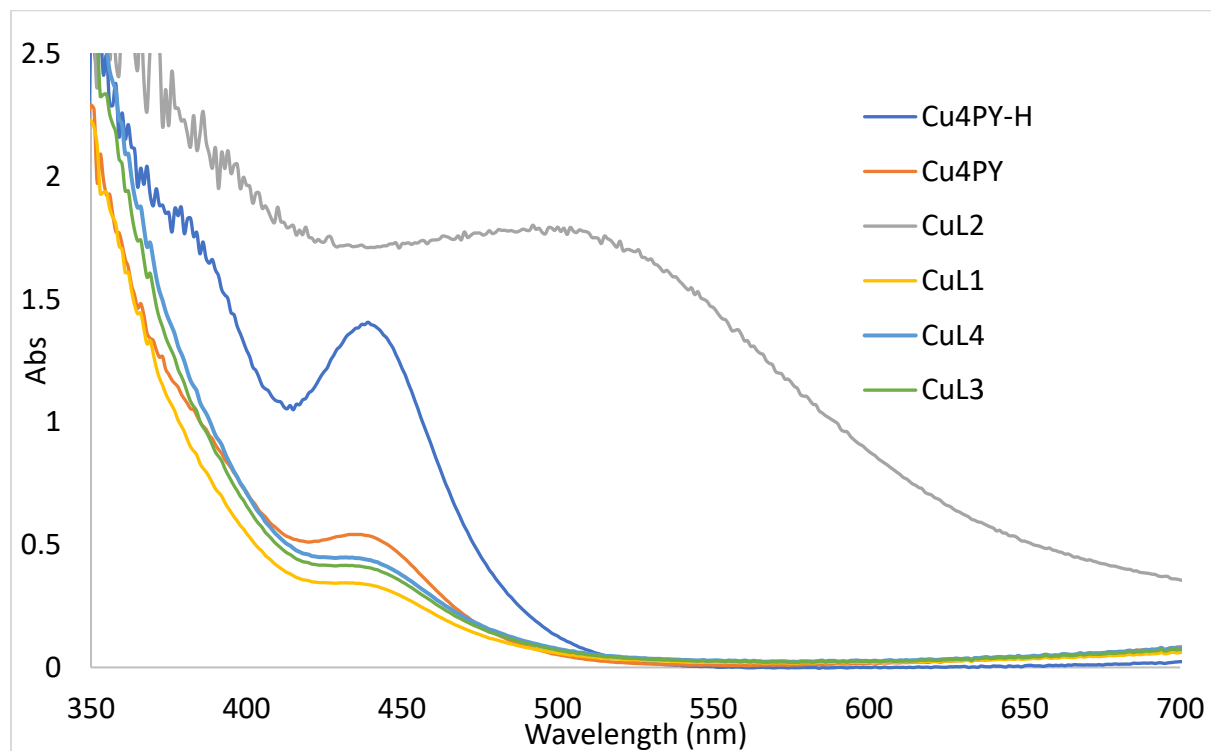
Table 43: Selected Vibrational Frequency Bands of Copper Complexes

Molecular motion	Cu4PY (cm ⁻¹)	Cu4PY-H (cm ⁻¹)	CuL1 (cm ⁻¹)	CuL2 (cm ⁻¹)	CuL3 (cm ⁻¹)	CuL4 (cm ⁻¹)
C=C	1504.0	1504.3	1525.7	1512.8	1499.3	1508.7
C-O-O ether	-	-	-	1047.3	-	1033.2
O=C-O ester	-	-	1736.2	-	1718.0	-
Ar-C-H	3041.8	3051.8	3113.6	3078.0	2971.0	3167.6
Ar-NH ₂	-	-	-	3209-3321.4	3205.4-3305.4	-
Ar-NO ₂	-	-	1348.6-1410.6	-	-	1341-1390.2
Ar-N	1428.8	1405.2	1427.0	1432.6	1430.6	1448.0
Aryl-O stretch	-	-	1276.0	1245.3	1274.3	1265.9
Ar-NH	-	3379.9	-	-	-	-

3.3.4 Electronic and Magnetic Properties of the Copper Complexes

The electronic and the magnetic properties of each pair of compounds of copper and cobalt proved to be similar. This was of note as this proves the importance of the soft material acido complex in terms of their optical and magnetic properties. From this study, the effective magnetic moment of **Cu4PY-H** and **Cu4PY** were 2.22 BM and 2.16 BM respectively, these correspond to a

d^9 tetrahedral complex [175]. These values were observed to be greater than the expected spin only magnetic moment of a d^9 tetrahedral complex 1.73 BM, this deviation from the expected value can be accounted by the orbital contribution as the t_2 orbital is unsymmetrically filled and hence there is a possibility that the electron can move within the degenerate t_2 orbitals. After the observation of similar magnetic properties of the two complexes, it was further observed that they had similar optical properties where they both had a single absorption band on the electronic spectra at 440 nm shown in Spectrum 13. This was also supportive of the magnetic properties to exhibit that the compounds were both d^9 tetrahedral compounds. Finally, according to the Tanabe Sugano diagram of a d^1 Cu(II) tetrahedral complex this absorption band was expected and thus assigned to the $d-d$ transition $^2T_2 \rightarrow ^2E$. It should be noted that the molar absorptivity of the acido complex was observed to be higher than that of the complex, it was $27.94 \text{ L mol}^{-1} \text{ cm}^{-1}$ from a concentration of 0.05 M while the complex had a weaker band observed at $10.68 \text{ L mol}^{-1} \text{ cm}^{-1}$ from a concentration of 0.05 M. This observation shows that the acido complex crystal had better optical properties than the conventional complex.



Spectrum 13: Combined UV/Vis spectra of the copper complexes

The magnetic susceptibility of **CuL2** was found to be 2.16 which suggested a tetrahedral d^9 complex, this value was close enough to the spin only magnetic moment of 1.73 BM (Table 44). The spectrum of copper complex showed a large shoulder at 511 nm (Spectrum 13), and this was the only copper complex with a red shifted $\lambda_{\max}$. However, this complex complies with a tetrahedral d^9 configuration thus the band was assigned to the $^4T_2 \rightarrow ^4E_2$ transition. **CuL1** also had only one absorption band of the $d-d$ transitions at 438 nm. The effective magnetic moment for this copper (II) complex was found to be 2.241 BM, suggesting a tetrahedral d^9 crystal field. A d^1 Tanabe Sugano diagram showed that only one transition was expected similarly to the other copper complexes, hence the $\lambda_{\max}$ band was also assigned to the $^4T_2 \rightarrow ^4E_2$. The same was observed for **CuL3** and **CuL4** where there was one observable transition at 439 and 440 nm respectively, they were also found to have magnetic moments of 2.13 and 2.14 BM respectively. These finding also suggested that these complexes are tetrahedral d^9 complexes just as the other copper complexes.

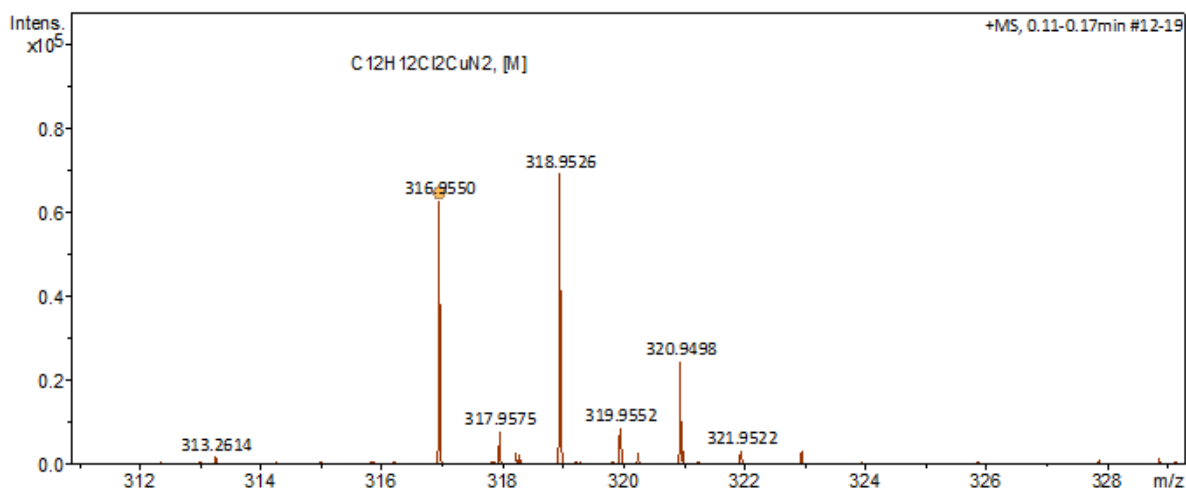
Table 44: Electronic Transitions and Magnetic Susceptibility of Copper Complexes

Complex	Electronic Transition		Magnetic Moments (BM)			
	(nm)		Transition Type	μ_{cal}	μ_{eff}	Crystal Field
Cu4PY	π - π^* and CT	<350		1.73	2.22	d^9 Tetrahedral
	d - d	436	$^2T_2 \rightarrow ^2E$			
Cu4PY-H	π - π^* and CT	<350		1.73	2.16	d^9 Tetrahedral
	d - d	436	$^2T_2 \rightarrow ^2E$			
CuL1	π - π^* and CT	<350		1.73	2.24	d^9 Tetrahedral
	d - d	438	$^2T_2 \rightarrow ^2E$			
CuL2	π - π^* and CT	<350		1.73	2.16	d^9 Tetrahedral
	d - d	511	$^2T_2 \rightarrow ^2E$			
CuL3	π - π^* and CT	<350		1.73	2.13	d^9 Tetrahedral
	d - d	439	$^2T_2 \rightarrow ^2E$			
CuL4	π - π^* and CT	<350		1.72	2.14	d^7 Tetrahedral
	d - d	440	$^2T_2 \rightarrow ^2E$			

3.3.5 Mass Spectrometric Characterization of the Copper Complexes

The high-resolution ESI-MS technique was used to confirm the structures of the copper complexes. The Spectrum 14 of **Cu4PY** indicated the typical spectra observed for the copper complexes, the data shows that the compound lost its chlorides by displaying a molecular ion peak 316.9550 m/z of [M-2Cl], which corresponded to the molar mass of 316.004 g/mol. **Cu4PY-H** also demonstrated that it had been successfully synthesized, although as shown with previous methods, this acid complex eventually changes into the complex form. This was shown with a similar activity that resulted in the complex releasing two chloride ligands and simplifying to an ion with a mass to charge ratio of 316.9544 m/z, confirming the molar mass of 316.004 g/mol. The high-resolution ESI-MS technique was used to confirm the structures of the copper complexes. The assigned molecular ion peak of **CuL1** was [M+K] at 686.9673 m/z, which

correlated to a molar mass of 687.9631 g/mol. There was also a de-halogenated fragment [M-2Cl] at 579.0347 m/z, which agreed with the molar mass minus the two chlorides at 578.94 g/mol. The **CuL2** complex mass spectrum revealed a molecular ion fragment with two adducts, acetonitrile, which was utilized to dissolve the complex, and sodium ion. Despite this, the molecular ion adducts [M+ACN+Na] at 595.2218 m/z corresponded to the molecular 596.0757 g/mol. The structure of **CuL3** was verified with a mass to charge ratio of 612.0431 m/z, which was assigned the molecular ion adduct [M+Na]. The spectrum was confirmed by the experimental results of 612.04 g/mol. The **CuL4** assigned molecular ion peak was [M+K] at 630.9700 m/z, corresponding to a molar mass of 631.9731 g/mol. There was also a de-halogenated fragment [M-2Cl] at 523.0479 m/z, which coincided with the molar mass of 522.1094 g/mol minus the two chlorides.



Spectrum 14: Mass spectrum of **Cu4PY**

3.3.6 Thermal Studies of the Copper Complexes

The **Cu4PY** complex and the acido complex **Cu4PY-H** had distinctive colors, the **Cu4PY** complex was blue colored microcrystalline powder while **Cu4PY-H** was a yellow crystalline solid. Both the complex and the acido complex were observed to be stable in dry air and moist air under room temperature and pressure. However, overtime the acido complex transforms to the complex as seen by the color changing to blue. Considering the thermogram of the copper complex **Cu4PY** in Figure 71 as a typical thermogram, there were two noticeable mass losses. The first mass at

0.74 mg, which translates to 9.7% was inferred to be for two water molecules that may have been absorbed during storage. The second mass loss at 2.69 mg or 35.3% was assigned to the loss of the ligand 4PY where in calculation this loss was 33%. As for the DSC profile, the transition observed at the endothermic peak 174 °C, was attributed to the loss of the water as observed on the thermogram, while the following exothermic peak around 178 °C, was deduced to be the crystallization of the complex. The melting point of this compound was assigned to the endothermic peak at 260 °C because at this temperature the thermogram shows the loss of the ligand of which after this the compound started to decompose.

CuL1 exhibited four distinct mass losses, the first loss of 2.7% (0.41 mg) was attributed to the removal of water that had accumulated during storage, while the second loss of 76% corresponded closely to the calculated 77% for two **L1** ligands. The final mass loss of 12% was attributed to the detachment of two chloride ligands from the central copper atom. The DSC profile showed a glass transformation at 97.6 °C, a small crystallization exotherm at 108.7 °C, and a melting point at 218.7 °C, followed by decomposition. **CuL2** exhibited three mass losses: the first loss of 2.2% (0.39 mg) and calculated 3.0% were ascribed to the removal of water accumulated during storage, while the loss of 52.4% (9.37 mg) and calculated 37.5% indicated the loss of fragmented **L2**. The DSC profile displayed a glass transformation at 165.4 °C, a crystallization exotherm at 273.4 °C, and a melting point at 280.3 °C, followed by decomposition. **CuL3** showed three mass losses: the first loss of 6.4% (0.67 mg), matching the calculated 6% loss, was attributed to the removal of two water molecules. The second mass loss of 40% (4.19 mg), close to the calculated 39% loss, was assigned to the loss of **L2** ligand. The final mass loss of 15% (1.61 mg) was suggested to be the detachment of two chloride ligands from the central copper atom, calculated as 12%. The DSC profile revealed a glass transition at 97.6 °C, crystallization at 124.8 °C, and melting at 154.1 °C, followed by decomposition. **CuL4** demonstrated better stability, similarly to **CuL2**. It displayed two mass losses: the first loss of 5% (0.33 mg), in line with the calculated 5.7% loss, represented the removal of two moles of water from storage. The second mass loss of 39.6% (2.62 mg) closely matched the calculated 37% loss for one **L4** ligand. The DSC profile indicated an endothermic glass transformation at 156.2 °C, a slight exothermic transformation at 192.6 °C, and a melting point at 230.8 °C.

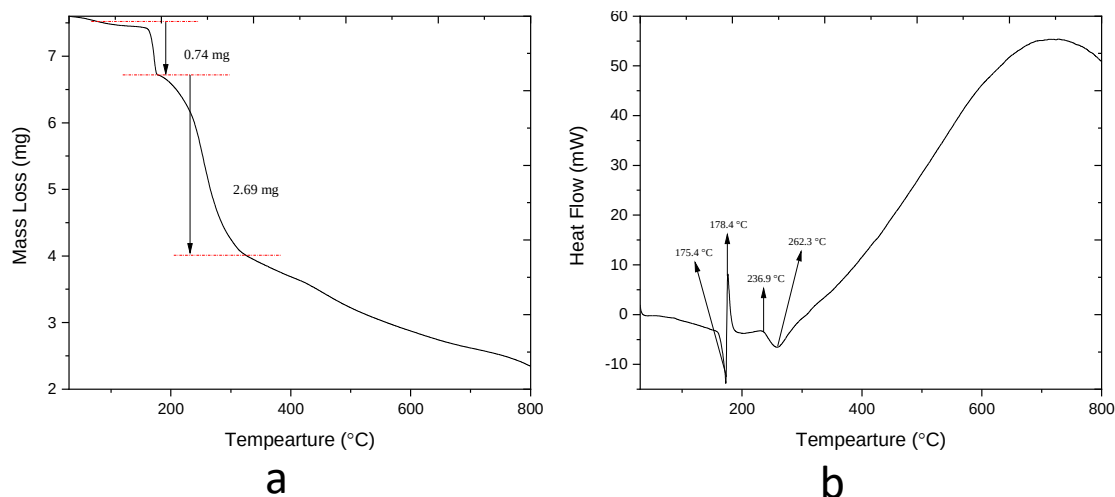
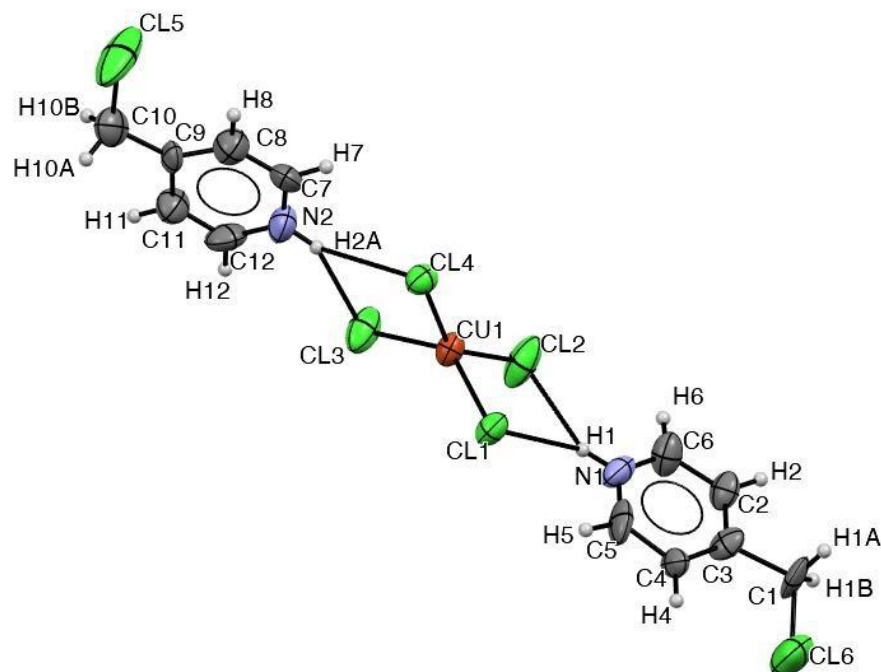


Figure 71: a) Thermogram and b) DSC profile of **Cu4PY**

3.3.7 Single-Crystal XRD Analysis of the Crystalline Copper Complexes

According to the crystal refinement data, the copper acido complex crystallized in the monoclinic *Ia* space group. The crystal refinement data of the structures are shown in Appendix 5.2. The ionic complex **Cu4PY-H** showed four molecules in the unit cell, while the complex **Cu4PY** only had two. Many attempts to grow good quality crystals of the copper complex were attempted but failed. However, the acquired data was used to describe the structure. The molecular structure of these compounds are shown in Structure 19 and 20.



Structure 19: The ORTEP structure of **Cu4PY-H** at 50% probability.

The crystal structure analysis of the acido complex (**Cu4PY-H**) reveals interesting features and bonding arrangements. Table 46 presents selected bond lengths, showing the Cu-Cl bonds in the range of 2.225 to 2.256 Å. The structure adopted a *trans* stereochemistry with respect to the spatial arrangement of the [4PY-H]⁺ cation. Notably, two cations of [4PY-H]⁺ are weakly bound through bifurcate hydrogen bonding from four chloride ligands, as shown in Table 45. The hydrogen bonds between the nitrogen atoms (N1 and N2) and chlorine atoms (Cl1-Cl4) exhibit lengths ranging from 2.38 to 2.77 Å, forming angles between 127.8° and 148.7°. The distorted square planar coordination of the [CuCl₄]²⁻ anion is situated between the two cations, effectively balancing the charge on the central anion and rendering the whole molecule neutral. The bond angles between adjacent chloride ligands are observed to be greater than 90°, indicating a slight distortion from a perfectly square planar geometry. Furthermore, a perfect square planar geometry has $\tau = 0$ radians but in this data the least value was 0.161 radians. In this case, it was observed that there was non-zero tau values for the bond angles, indicating a deviation from a perfectly square planar geometry. Detailed bond angle measurements are presented in Table 47, where angles between Cu1-Cl1-Cl4 and Cu1-Cl2-Cl4 are approximately 97.6° and 141.5°.

respectively. Similarly, angles involving Cl3 display values around 95.8° and 139.8°, while the Cl3-Cu1-Cl2 angle is approximately 96.8°.

Table 45: Hydrogen bond geometry of **Cu4PY-H**

D-H	d(D-H)	d(H...A)Å	<DHA (Angle/°)	d(D...A)Å
N1-H1...Cl1	0.86	2.77	127.8	3.363
N1-H1...Cl2	0.86	2.38	148.7	3.151
N2-H2A...Cl4	0.86	2.65	133.2	3.298
N2-H2A...Cl3	0.86	2.47	144.6	3.211

Symmetry codes: [x, y, z]

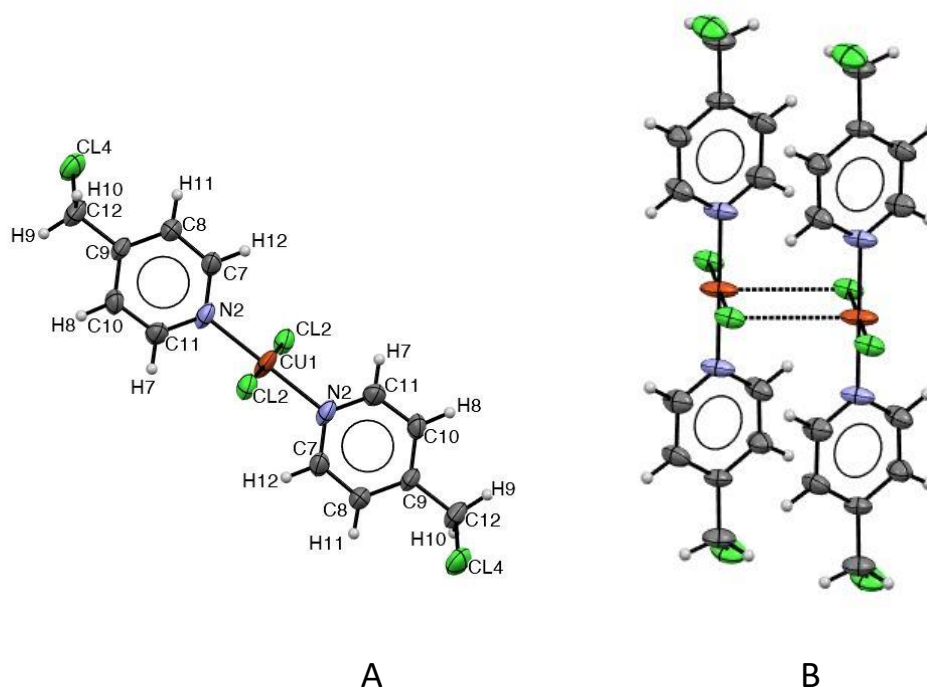
Table 46: Selected and calculated bond lengths for **Cu4PY-H**

Atom	Atom	Length/Å
Cu1	Cl1	2.252(4)
Cu1	Cl4	2.256(4)
Cu1	Cl2	2.250(5)
Cu01	Cl3	2.225(5)

Table 47: Selected and calculated bond angles of the distorted square planar of [CuCl₄]²⁻ in **Cu4PY-H**

Atom	Atom	Atom	Angle/°
Cl1	Cu1	Cl4	97.61(6)
Cl2	Cu1	Cl1	95.81(17)
Cl2	Cu1	Cl4	141.5(2)
Cl3	Cu1	Cl1	139.8(2)
Cl3	Cu1	Cl4	95.84(17)
Cl3	Cu1	Cl2	96.77(8)

As opposed to the data obtained for the acido complex crystal, the complex **Cu4PY** crystalized in a triclinic crystal system with a primitive lattice centering under a *P1* space group. The molecular structure of this compound with the atomic numbered scheme in Structure 30.



Structure 20: Part A shows the numbered ORTEP structure of **Cu4PY** at 50% probability, while B shows the expanded short contact showing Cu-Cl (neighbour) bond that shows a pseudo-octahedron coordination polyhedron in **Cu4PY**

The structure of **Cu4PY** features two **4PY** ligands in the molecule, each bound to the copper metal through a nitrogen atom of the pyridine moiety, with an angular separation of 178.1° . Additionally, the copper metal is coordinated to two chloride atoms, which are also separated by an angle of 178.6° . These angles contribute to a slightly distorted square planar geometry, deviating from the ideal square planar arrangement. Moreover, the ligands in the structure adopt a trans configuration with respect to each other. Analyzing the bond lengths in Table 48, we observe that the Cu-Cl bond lengths in **Cu4PY** and the acido complex are both approximately 2.3 Å. This suggests a similar bonding environment for copper in both complexes. Table 50 provides the selected bond angles in **Cu4PY**. The bond angles of Cl2-Cu1-Cl3 and N2-Cu1-Cl3 are measured to be 178.6° and 89.6° , respectively. Similarly, the angles N2-Cu1-Cl2 and N2-Cu1-N1 are found to be 90.8° and 178.1° , respectively. The angles N1-Cu1-Cl3 and N1-Cu1-Cl2 are approximately 90.0° and 89.6° , respectively. These angles contribute to the distorted square planar geometry

observed in the structure. The calculated value of tau (τ) for **Cu4PY** is approximately 35.95 which indicates a deviation from the ideal tetrahedral geometry. It suggests that the bond angles in **Cu4PY** are not perfectly 109.5°, confirming a distortion in the tetrahedral geometry of the complex. Structure 20B reveals a short contact Cu-Cl bond with a length of 3.176 Å, which is longer than the Cu-Cl2 bond length (2.292 Å). This suggests that the short contact bond may not contribute to the coordination polyhedron but rather to a dimeric structure of the complex. These findings support the presence of a tetrahedral polyhedron. Conversely, the cobalt complex **Co4PY** displays Co-Cl bonds similar to those in the extended polymeric structure, contrasting with the copper complex. This discrepancy suggests a pseudo-octahedral coordination polyhedron for **Co4PY**.

Table 48: Selected and calculated bond Lengths of **Cu4PY**

Atom	Atom	Length/Å
Cu1	Cl3	2.305(8)
Cu1	Cl2	2.283(8)
Cu1	N2	2.00(2)
Cu1	N1	2.01(2)

Table 49: Selected bond angles of **Cu4PY**

Atom	Atom	Atom	Angle/°
Cl2	Cu1	Cl3	178.6(4)
N2	Cu1	Cl3	89.6(8)
N2	Cu1	Cl2	90.8(8)
N2	Cu1	N1	178.1(17)
N1	Cu1	Cl3	90.0(6)
N1	Cu1	Cl2	89.6(7)

3.3.8 Antibacterial Studies of the Copper Complexes

3.3.8.1 Bacterial Minimum Inhibition Concentration of the Copper Complexes

The antibacterial properties of the copper complexes were studied to determine the active compounds, furthermore, their mode of action was assessed. Section 3.1 subsection 3.1.3 discusses the ligands' antibacterial properties. Figure 72 depicts the summarized grouped data in

a bar graph, while Table 50 displays the mean MIC values and standard deviations. Generally, the copper complexes showed higher bioactivity towards the Gram-positive bacteria, with the complexes of **4PY** being the least active. The complex **CuL4** was the best performing across the board with exceptionally potent results observed against MRSA and a *S. aureus*. There was no observed trend with the difference in oxygen bridges, together with the nitrogen moieties.

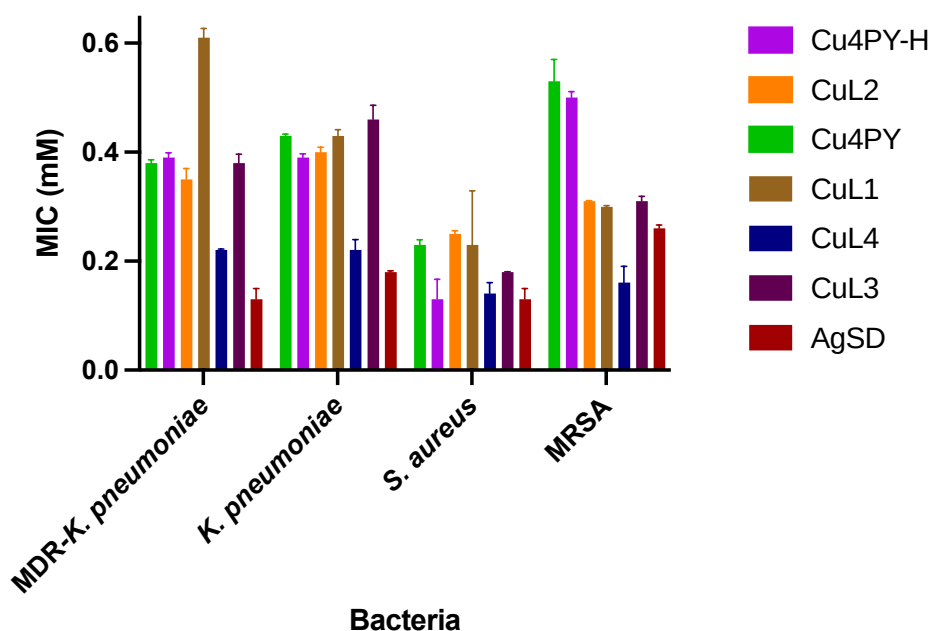


Figure 72: Summarized MIC values of copper complexes

The ligands MIC data explicitly showed that their activity was only towards the Gram-negative strains, while most of the copper complexes only showed potency towards the Gram-positives. However, **CuL4** was also bioactive towards the Gram-negative strains and was even comparable with the ligands. The reason copper complexes demonstrated to have a considerable inhibitory potential for Gram-positive bacteria was because copper is physically and chemically stable and binds amine and carboxyl groups more readily on the surface of Gram-positive bacteria. These properties make copper more effective in physiological situations and have more antibacterial activity against Gram-positive bacteria [176][177], [178]. Silver sulfadiazine (**AgSD**), a silver-based ionic complex with sulfadiazine, was used as a control. Comparing the control **AgSD** and the new compounds showed that **Cu4PY-H** and **CuL4** were potent against *S. aureus*, while only **CuL4** was more potent against MRSA. Finally, the acido complex of copper to **Cu4PY-H** and its conventional

complex **Cu4PY** had similar activity towards all bacteria with exception to *S. aureus*. In *S. aureus* the acido complex crystal of copper had higher activity as opposed to the complex.

Table 50: MIC of Copper Complexes

Compound ↓	Microbe →	MIC (mM)			
		<i>K. pneumoniae</i>	MDR- <i>K. pneumoniae</i>	<i>S. aureus</i>	MRSA
Cu4PY		0.43±0.003	0.38±0.006	0.23±0.009	0.53±0.04
Cu4PY-H		0.39±0.009	0.43±0.007	0.13±0.0364	0.50±0.011
CuL2		0.40±0.009	0.35±0.02	0.25±0.006	0.31±0.0012
CuL1		0.43±0.017	0.61±0.011	0.23±0.099	0.30±0.002
CuL4		0.22±0.019	0.25±0.002	0.14±0.02	0.16±0.03
CuL3		0.46±0.026	0.38±0.016	0.18±0.0005	0.31±0.009
AgSD		0.18±0.002	0.13±0.02	0.13±0.002	0.26±0.06
Trend		CuL4>Cu4PY-H>CuL2>CuL1=Cu4PY>CuL3	CuL4>CuL2>Cu4PY=CuL3>Cu4PY>CuL1	Cu4PY>CuL4>CuL3>Cu4PY=CuL1>CuL2	CuL4>CuL2>CuL2=CuL1>u4PY-H>u4PY

3.3.8.2 Time Kill Kinetics

A time kill analysis determined if the bioactive compounds were bacteriostatic (prevents bacteria from multiplying without necessarily killing them) or bactericidal (kills bacteria) following the successful screening of the compounds. The copper compounds used in the study against MRSA and *S. aureus* were especially potent and were used at their minimum inhibitory concentrations (MIC) (Figure 73 and 74). The results indicated that copper compounds were bacteriostatic at their MIC because they showed an exponential growth after 24 hr.

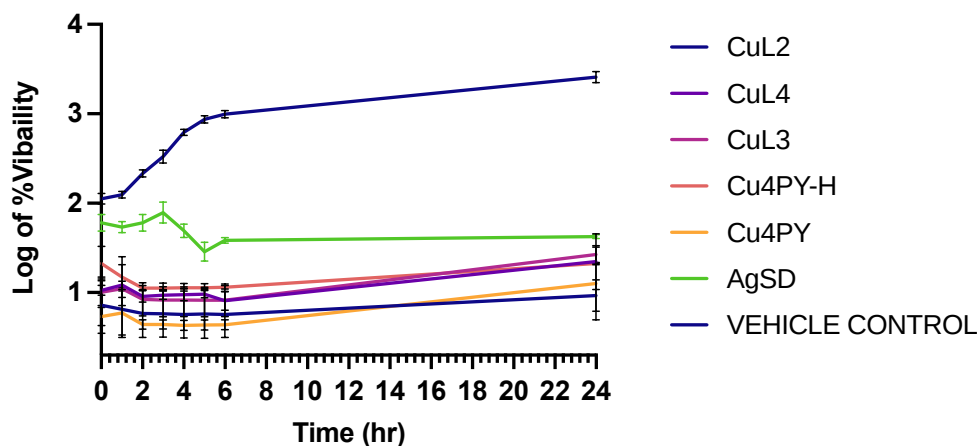


Figure 73: Time kill Kinetics of active copper complexes against *S. aureus*

The time kill kinetics of the control **AgSD** showed a bacteriostatic trend in both *S. aureus* and MRSA, the growth was even similar in Log %Viability (Figure 88 and 89). Interestingly, the bioactivity of **AgSD** was showing stronger bacteriostatic trend as opposed to the compounds which were closer to a bactericidal trend in growth.

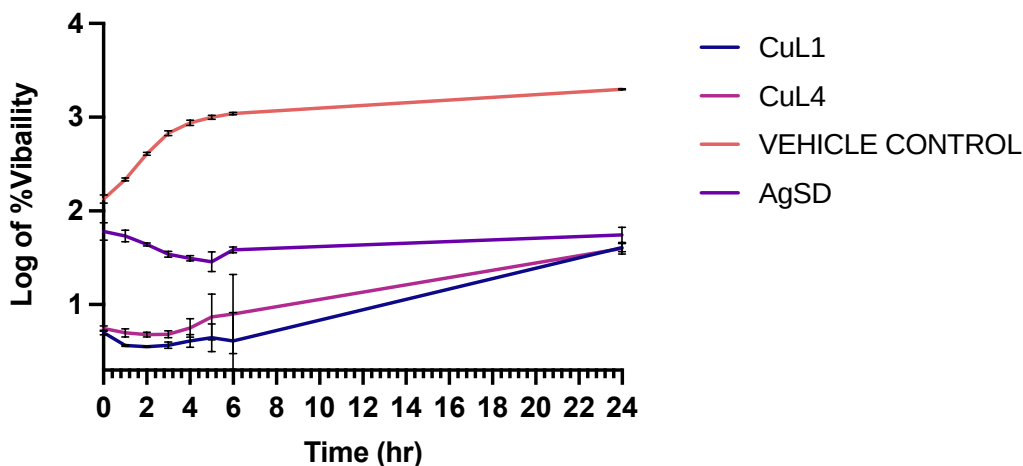


Figure 74: Time kill Kinetics of active copper complexes against MRSA

3.3.8.3 Cell Wall/Membrane Disruption Studies Of The Active Copper Complexes by AKP Assay

The bacterial cell membrane damage was examined using the alkaline phosphatase (AKP) assay, the intracellular enzyme AKP is found in bacterial cell walls and membranes [148]. In general, AKP activity in the extracellular environment cannot be detected in bacteria with intact cell walls and

membranes [145], [146]. Therefore, altering cell membrane permeability is one of the antibacterial mechanisms that could lead to electrolyte leakage [147]. In finding the modes of action of the active copper compounds against *S. aureus*, the assay showed that the nitro group complex **CuL4** may be the best performing complex followed by **Cu4PY-H** and **AgSD** (Figure 75). However, the error bars do show that the activity of the other compounds exceed the mean average of **CuL4**. But also, the upper error bar of **CuL4** still showed prominence to the other compounds. This was an interesting observation as the control **AgSD** drug's mode of action is cell wall/membrane disruption. The activity of the **CuL4** complex may have also been attributed to, the nitro group as it is known to be reduced by the nitro-reductases enzymes thus producing reactive oxygen species that affect cell membranes [61].

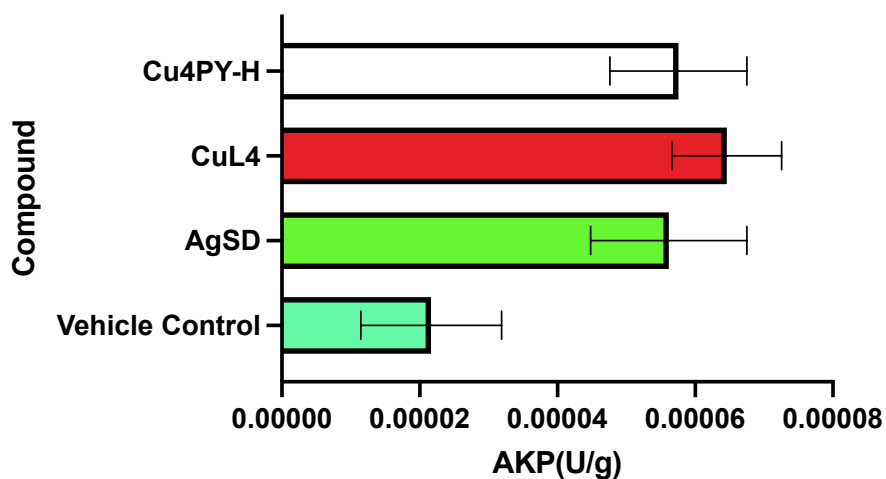


Figure 75: AKP production by *S. aureus* after treatment with copper compounds

Conversely, **Cu4PY-H** was not as active in MRSA, but **CuL4** activity was similar in MRSA as in *S. aureus*. Therefore, **CuL4** was also tested for its membrane disruption activity (Figure 76). **CuL4** remained active in AKP release activity, similar to the control, just as observed in *S. aureus*. It was determined that one of the compounds' mechanisms of action was cell wall/membrane breakdown since the compounds caused *S. aureus* and MRSA to release more AKP than the vehicle control did.

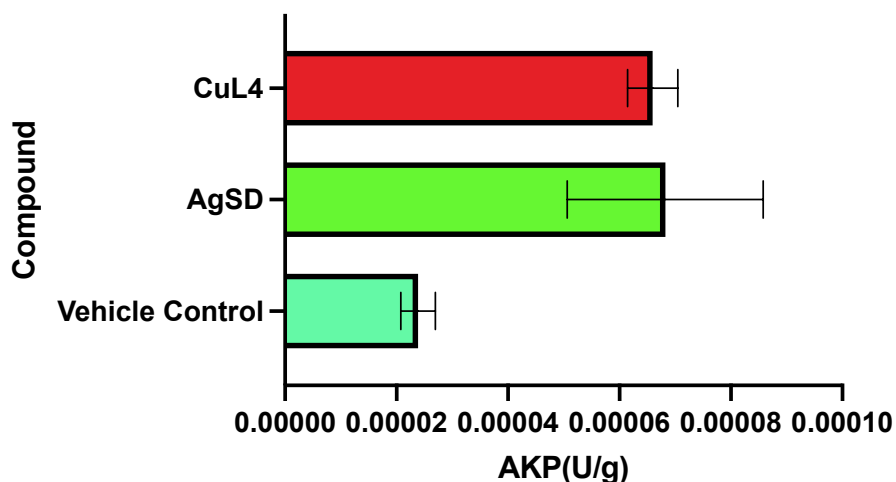


Figure 76: AKP release by MRSA after treatment with **CuL4**

3.3.8.4 Molecular Docking Studies of active Copper complexes

CuL4, **Cu4PY**, and **AgSD**, the most active copper complexes and the control drug against MRSA, were used in molecular docking experiments against the crystal structure of a PBP of MRSA called PBP2a, which is depicted in Table 51 and Figures 76 to 78 [168]. The protein active site contains the amino acid residues Lys406, Lys597, Ser598, Glu602 and Met641. A quinazoline antibiotic with pharmacophores comparable to those of the complexes bound to the PBP2a protein at the protein active site was compared to the active complexes [168]. The **CuL4** compound with a binding energy of -11.02 kcal/mol was the best followed by the control **AgSD** at -5.88 kcal/mol while **Cu4PY** was the least performing at -5.78 kcal/mol. With regards to the AKP release study, for MRSA **CuL4** (6.6×10^{-5} U/g) and the control **AgSD** (6.8×10^{-5} U/g) seemed to behave similarly, while with *S. aureus* **CuL4** (6.5×10^{-5} U/g) was significantly higher than the control **AgSD** (5.6×10^{-5} U/g) and **Cu4PY** (5.7×10^{-5} U/g) which behaved similarly to each other. Conclusively, the molecular docking data also confirm that cell membrane disruption was one of the modes of action of the active complexes against their susceptible bacteria.

Table 51: Molecular docking scores and the corresponding prominent residual amino acid interactions of the ligands and their complexes against methicillin-resistant *S. aureus* (PDB: 4cjn)

Cpds	Binding energy (kcal/mol)	Inhibition constant (K_i)	H-bonding with	π -Sigma/ π -Alkyl	van der Waals
CuL4	-11.02	0.01 μ M	Lys322,Lys273, Asp295	Ala276,His293	Ser149,Gln292,Lys289, Glu294,Val277,
Cu4PY	-5.78	58.27 μ M	-	Arg151,Arg241, Val277,Met372	Glu150,Thr165, His293Ser240
AgSD	-5.88	49.35 μ M	Gly271,Val277	Asp275,Ala276, His293	Tyr272, Lys273, Gln292,Glu294

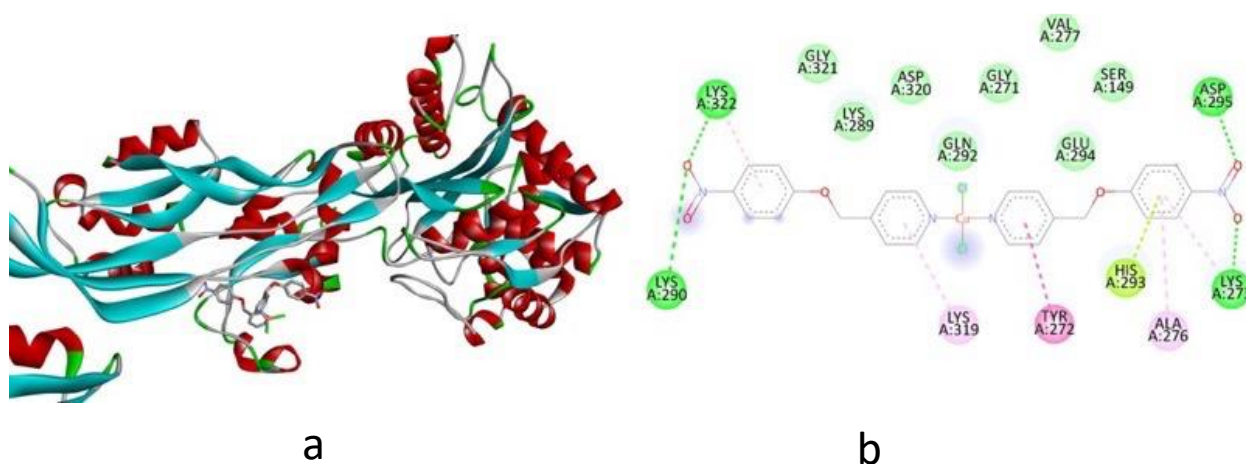


Figure 77: The binding interactions of **CuL4** against MRSA (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

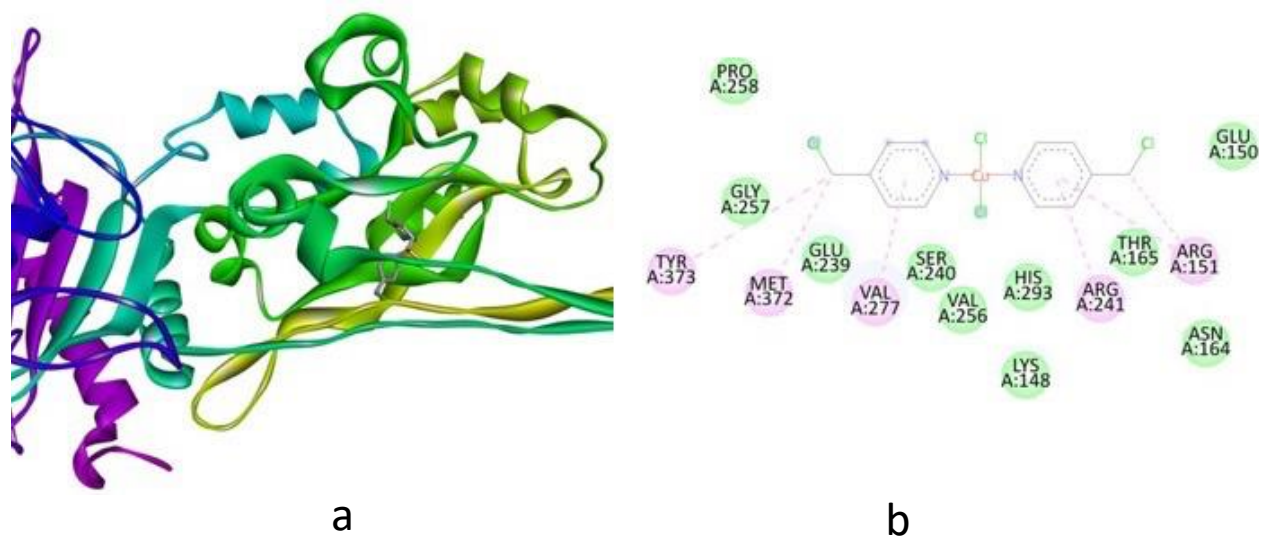


Figure 78: The binding interactions of **Cu4PY** against MRSA (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

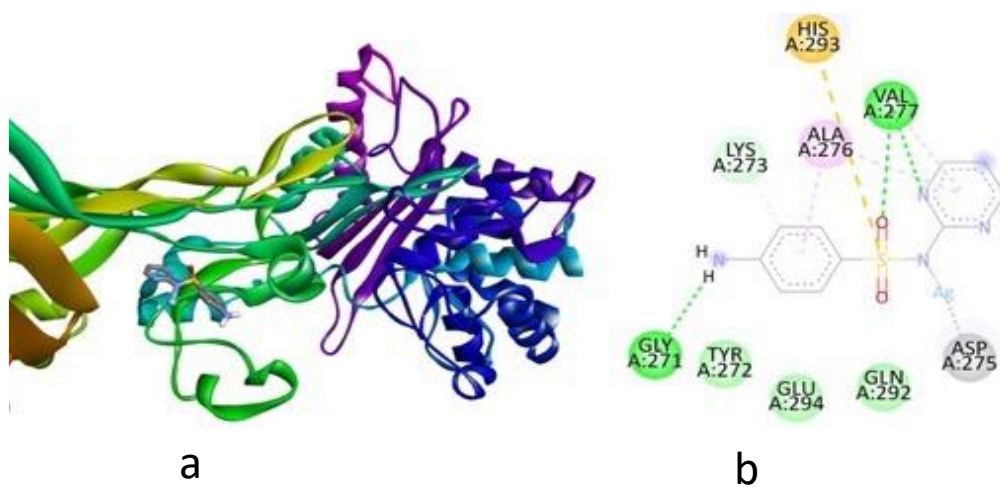


Figure 79: The binding interactions of **AgSD** against MRSA (PDB: 4cjn). a) shows the binding site, b) shows the interacting amino acid residues.

3.3.9 Conclusions

Complexes of copper with five pyridyl ligands were synthesized and characterized using physical and spectroscopic techniques. Two of the complexes made with **4PY** ligand were prepared using

the solvents, while the rest were prepared through the green solid state grinding method. The ground complexes were purer and had higher yields when compared to the conventional solvent assisted reactions. These complexes were observed to have good solubility, however, with polar solvents. They all showed good thermal stability especially those of the ether complexes **CuL2** and **CuL4**. The HRESI-MS data of all the copper complexes corroborated other complimentary techniques and confirmed the successful synthesis of the complexes. Due to their solubility in mostly polar solvents, forming good quality crystals proved to be a challenge, nevertheless, **Cu4PY** and its acido-complex counterpart managed to crystallize. Their Single crystal data showed that these complexes are structurally different. The **Cu4PY** complex was a *trans* dimeric molecule, while the acido-complex was a *trans* supramolecule with cationic complex cores of copper of the type $[MCl_4]^{2-}$. The copper complexes were more active towards the Gram-positive bacteria, with the complexes of **4PY** being the least active while **CuL4** was the best performing especially towards MRSA and a *S. aureus*. From the time kill kinetics, all the active complexes were bacteriostatic against MRSA and *S. aureus*. Furthermore, the **CuL4** and **Cu4PY-H** were observed to disrupt the *S. aureus* cell membrane better than the control **AgSD** while **CuL4** disrupted the MRSA cell membrane similarly to the control **AgSD**. Finally, molecular docking scores confirm the cell membrane disruption results, where **CuL4** was seen to outperform the other active compounds.

4.0 Comparative Discussions

The synthesis, characterization, and antibacterial studies of four ligands and the commercial **4PY** together with their complexes of cobalt, nickel and copper were performed. The ligands were synthesized and gave favourable yields following a proposed S_N1 mechanism. The compound **L1** and **L3** together with their crystal structures have not been described before, while **L2** and **L4** have been synthesized before in various patents as precursors to other compounds. The other characterization techniques showed the successful synthesis of these ligands, where in FTIR all the important functional groups were observed. Additionally, their proton NMR showed the important chemical shifts of the available protons in the ligands, together with their elemental analysis that showed their successful synthesis and high purity.

The complexes of **4PY** were synthesized using the conventional solvent method while the other complexes were synthesized using the solid state mechanochemical methods. The grinding method proved to form purer products in higher yields. The cobalt and copper complexes with **4PY** formed different complexes, these were the datively bound complexes and acido-complexes of the type $[MCl_4]^{2-}$. Their thermal studies showed that these complexes transform to their datively bound counterparts with increase in temperature, while other complexes of the synthesized ligands were observed to be of good thermal stability, especially those of **L2** and **L4**. The nickel complexes were observed to be less soluble when compared to those of cobalt and copper, and also thermally decomposed at higher temperatures than the rest. Electronic spectra and magnetic susceptibility of the copper complexes indicated that all the copper complexes were similar chemically where they all displayed a similar electronic spectra and magnetic susceptibility of around 2 BM. While on the contrary, the cobalt complexes of **4PY** showed lower magnetic susceptibility when compared to the other cobalt complexes, especially for **Co4PY-H** that was of the $[MCl_4]^{2-}$ form and **Co4PY** showing a pseudo-octahedron through single crystal data. The nickel complexes showed that they were all d^7 tetrahedral complexes with their electronic spectra however, magnetic susceptibility showed that **NiL1** might be a pseudo-octahedral complex based on the acquired magnetic data.

The antibacterial MIC data showed that the ligands had better activity towards the Gram-negative bacteria as shown in Figure 80. From the data in Figure 80 and 81, the bioactivity of the compounds per bacteria was as follows:

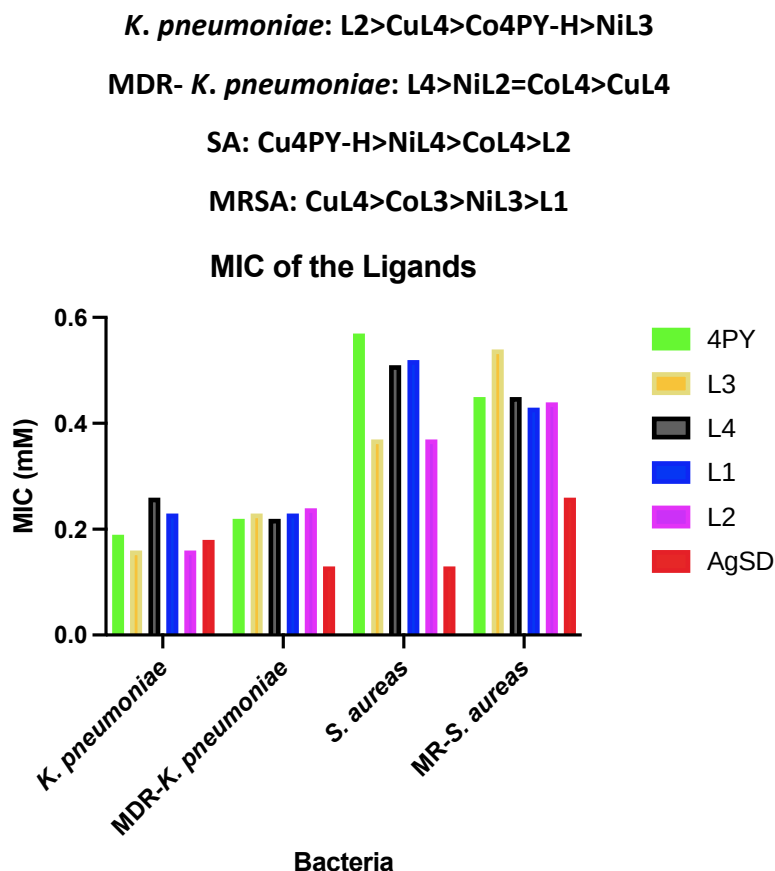


Figure 80: Summarized MIC of the ligands

The MIC data of all the compounds indicated that the highest activity was observed from the cobalt complexes and the free ligands, followed by the copper and nickel complexes. Analysis of the data per ligand shows that the activity varied due to the difference of the coordinated ligand. According to complexes of **L1**, activity was observed towards the Gram-negative bacteria with the cobalt metal complexes and the free ligand being potent, while copper and nickel showed slightly better activity towards Gram-positive bacteria. The same trend was observed with **L2**, **L3**, **L4**, and **4PY** complexes.

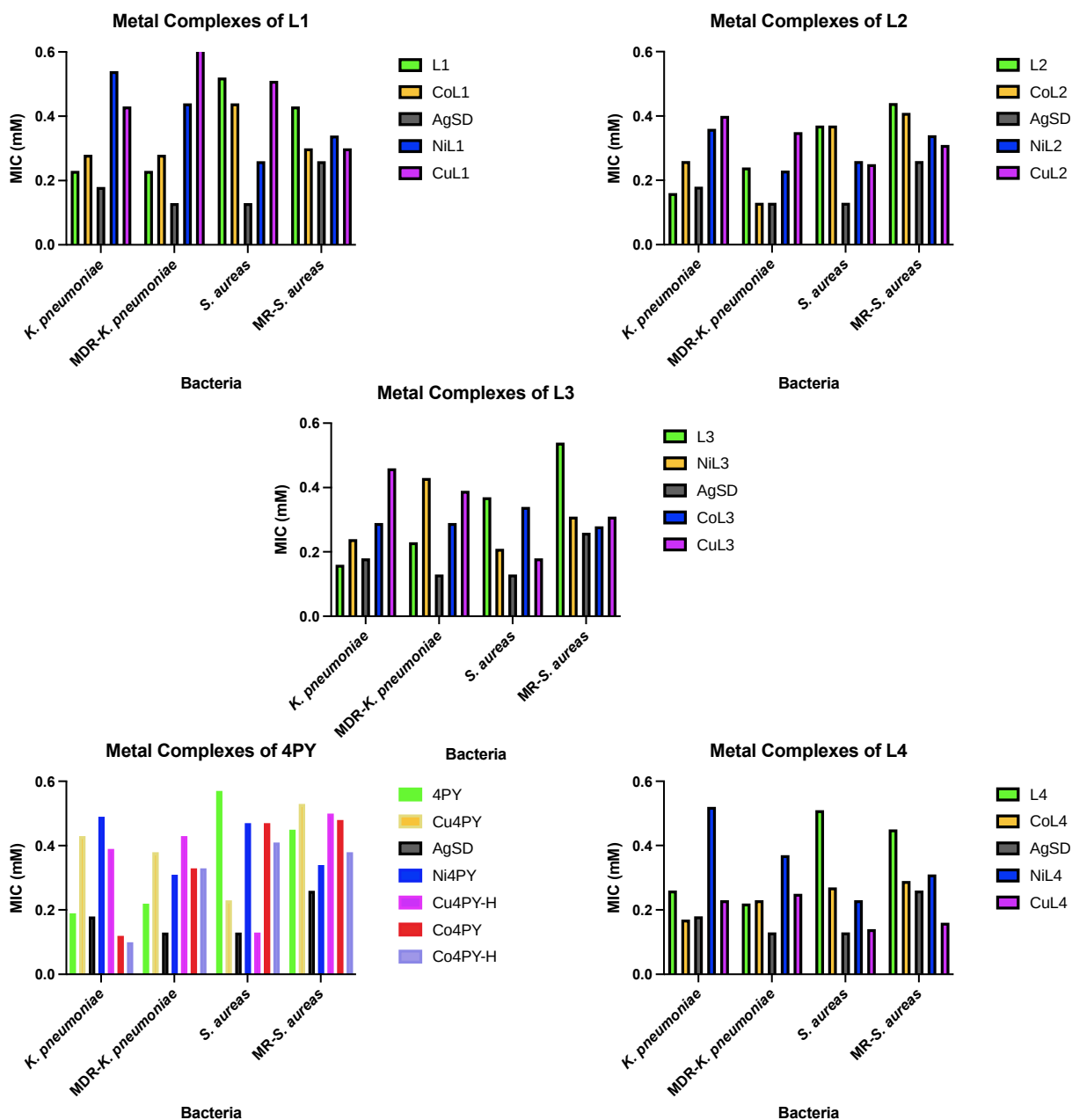


Figure 81: Summarized MIC data according to ligands and their complexes

Following the MIC assay, a time kill kinetic study showed in Figure 82 displays a summary of the active complexes and ligands against the four bacterial strains. As evident from these graphs and MIC results, MRSA was the least susceptible bacteria, and the nickel complexes displayed a clear bacteriostatic trend similar to that of the control drug **AgSD**. While on contrary, the copper complexes started off with a bactericidal trend that changed to an exponential growth after 6 hr

in the experiment. The *S. aureus* time kill results showed that all the active complexes intensely slowed the growth of the bacteria, this was evident with the observed bacteriostatic trend that was stronger than that of the control drug **AgSD**. Interestingly, the bacteria that was suspected to be the least susceptible, MDR- *K. pneumoniae*, had **NiL2** behaving similarly to the control drug **AgSD** by killing the bacteria from the beginning of the experiment. While all the other active compounds showed a bacteriostatic trend. Finally, the time kill kinetics of *K. pneumoniae* showed that it was the most susceptible bacteria, especially towards the cobalt complexes and the free ligands. From these active compounds only **CoL2** and **CoL3** were observed as bactericidal agents while all the others behaved in a bacteriostatic manner that was stronger than the control drug **AgSD**. Conclusively, it was observed that the nickel and copper complexes were more active towards the Gram-positive bacteria, while the cobalt complexes together with the free ligands displayed more activity towards the Gram-negative bacteria which was consistent with the MIC results.

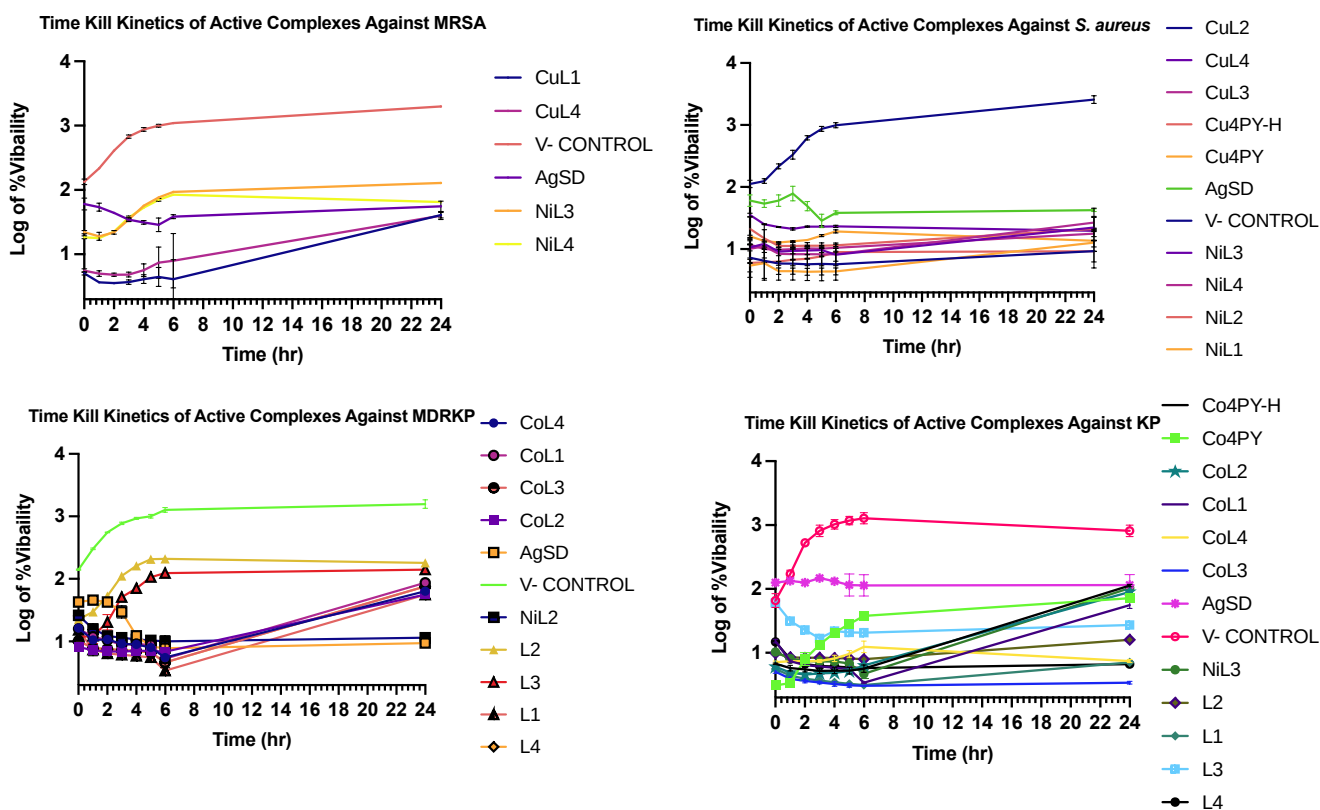


Figure 82: Summarized Time Kill kinetic study results

After the time kill kinetic assay, the AKP release assay was done to determine which active compounds (those with MIC less or equal to the control **AgSD**) disrupted the cell membrane of the susceptible bacteria. From the results displayed in Figure 83, it was observed that all the active complexes and ligands disrupted the cell membranes by displaying more AKP release well over the vehicle control. Considering the data on MRSA, the **CoL3** complex showed exceptional results that were even better than the control **AgSD**, while **CuL4** and the control were similar in AKP release. Conversely, in *S. aureus* **CuL4** was better than **Cu4PY-H** complex and **AgSD** in releasing AKP. In terms of *K. pneumoniae*, all compounds produced more AKP than the vehicle control. However, the best performing compounds in terms of AKP release were **CoL2** followed by **Co4PY-H** and **Co4PY** and the control **AgSD**. This shows that these compounds' mechanisms of action may include membrane disruption.

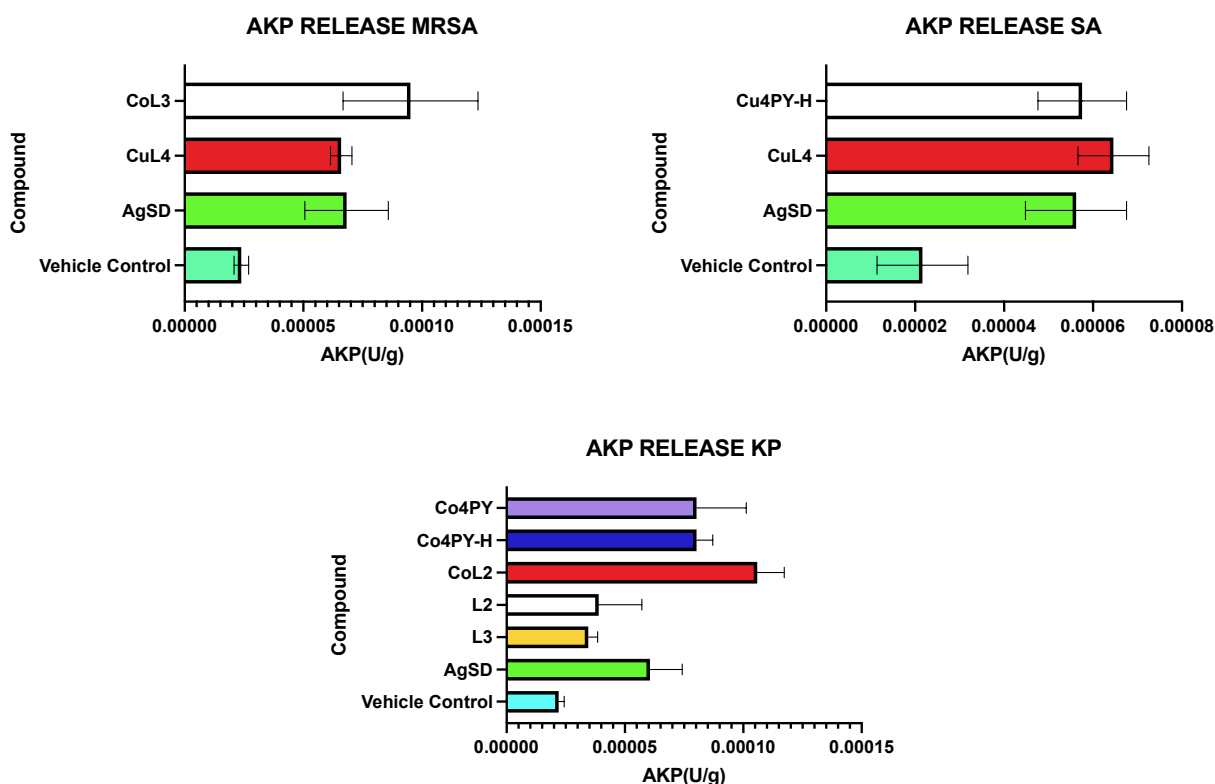


Figure 83: Summarized AKP release results

A folate production assay was done in order to assess the competitive antagonism of the amino containing compounds towards PABA (see Figure 84). The summarized folate production

inhibition assay results, and from these results only **CoL2** and **CoL3** were the only ones qualified to be tested for this, and the ligands **L2** and **L3** which were all potent against *K. pneumoniae* and MRSA. In terms of the *K. pneumoniae* results, **L3** and **CoL2** showed more inhibition as there was less folate produced. It may also be inferred that upon complexation with cobalt **L2** is enabled, this is observed by the increased activity with the inclusion of the cobalt centre. Furthermore, the same can be said by **CoL3** where the free ligand had less activity towards MRSA but upon coordination with a cobalt centre there was some observed activity. However, the folate production was not completely dissimilar to that of the control even though the assay still showed that folate production inhibition was one of the modes of action of **CoL3**.

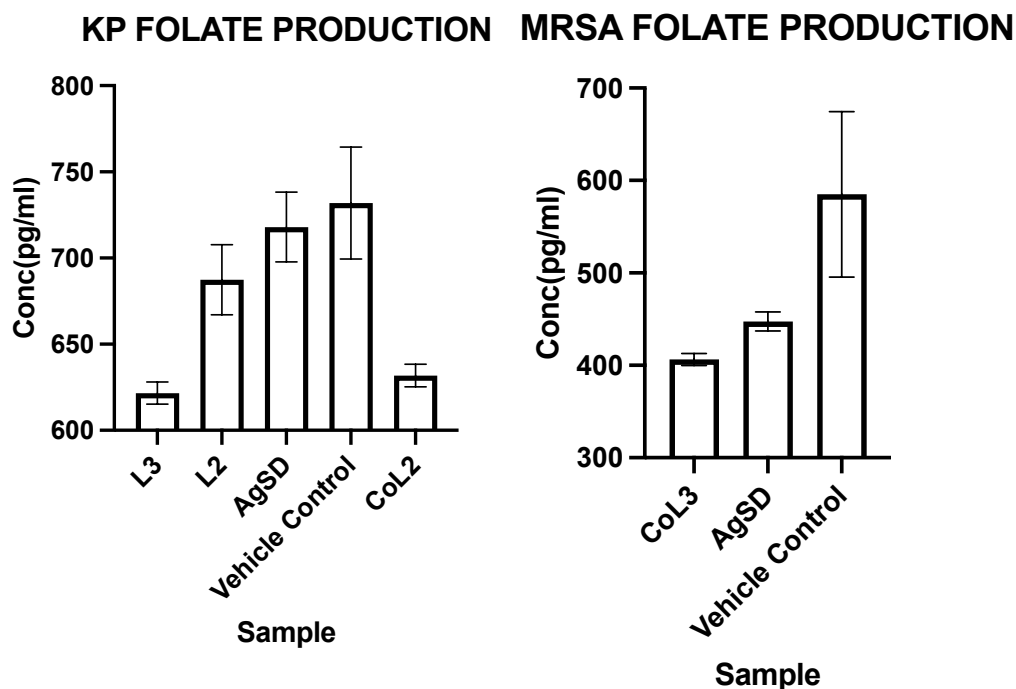


Figure 84: Summarized folate inhibition results

Figure 85 summarizes the protein binding investigations; the active compounds were docked to PBP2a for *S. aureus*, OmPK36 for *K. pneumoniae*, and DHPS to assess PABA antagonism. The results of the molecular docking studies validated the experimental data and also verified the results of the mechanisms of action assay. However, the nickel complexes were observed to be functioning well *in silico*, but their experimental activity was suppressed due to their low solubility. The data also demonstrate that the active complexes outperformed the control drug

in the most cases. According to the data, the active compounds tested against proteins showed the following trends:

PBP2a: CuL4>NiL4>NiL3>CoL3>AgSD>Cu4PY

OmPk36: CoL2>NiL3>AgSD>Co4PY>L3>L2

DHPS: NiL3>CoL3>CoL2>L3>AgSD>L2>PABA

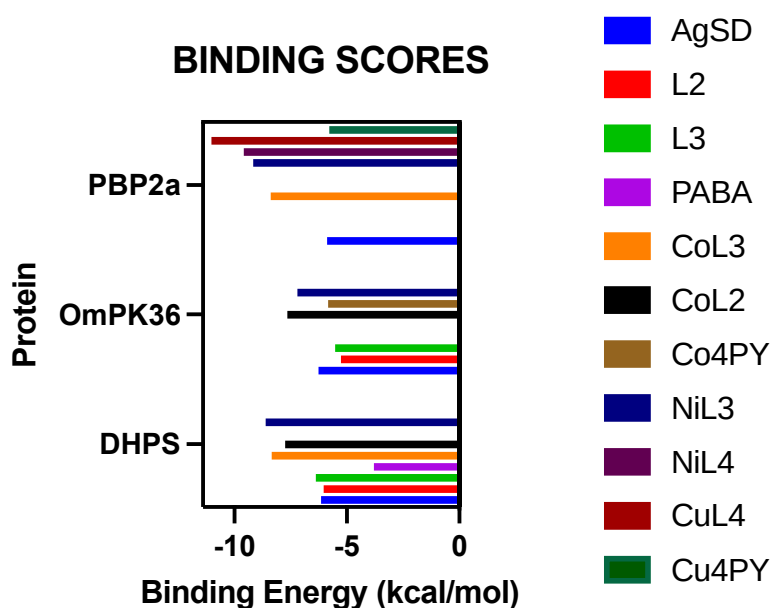


Figure 85: Summarized binding scores for all the compounds and their respective proteins

4.1 Conclusions

The synthesis and characterization of four pyridyl ligands, 4-chloromethyl pyridine and their corresponding cobalt, nickel and copper complexes is herein reported. The ligands were all produced at favourable yields above 70% and were successfully synthesized as per the characterization data. The complexes were all successfully synthesized and characterized, however, not all the complexes formed suitable crystals for SC-XRD analysis. The nickel complexes were the least soluble complexes and had no suitable crystal. The solution prepared complexes were of lower yields as compared to their mechanochemically prepared counterparts. Thereafter synthesis, the antibacterial properties through MIC and Time-Kill kinetics were done, followed by the mode of action studies using folic acid ELISA assay, alkaline phosphatase assay

and molecular docking studies. The ligands were observed to have higher activity towards the Gram-negative *K. pneumoniae* bacteria and the most active killed bacteria by affecting folic acid production and cell membrane disruption. The complexes showed that the cobalt complexes were performed higher than the rest of the compounds, with the most active cobalt complexes also having multiple modes of action. The copper and nickel complexes on contrary showed higher activity to the Gram-positive *S. aureus* bacteria, and multiple modes of action.

4.2 Recommendations and Future Work

The following recommendations and future work are proposed to enhance this research:

- a. Synthesis of the complexes in solution to compare their properties.
- b. More physicochemical studies to be done, such as Cyclic voltammetry, Electro paramagnetic Spectroscopy, chemical kinetics, hydrolysis and even powder XRD so as to fully understand their physicochemical properties.
- c. SC-XRD characterization of the complexes that failed to crystallize.
- d. Modification of the ligands to enhance activity.
- d. More biological assays to fully confirm their modes of action, and to also assess their pharmacokinetics.

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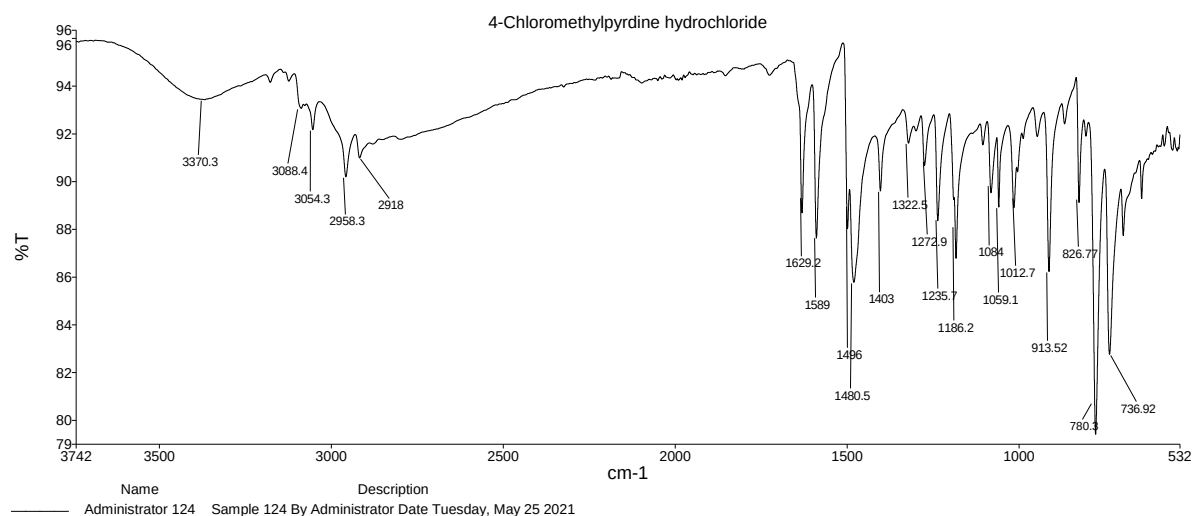
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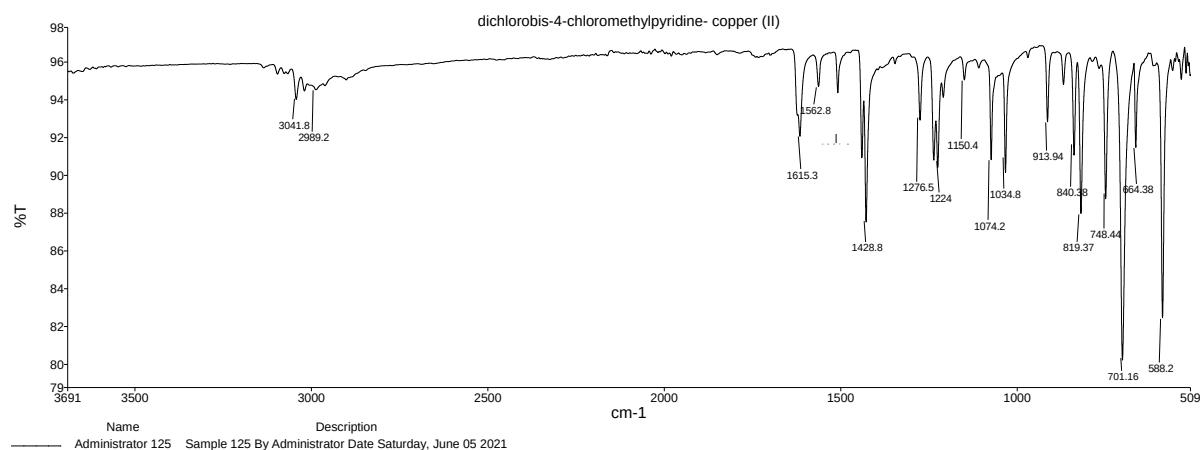
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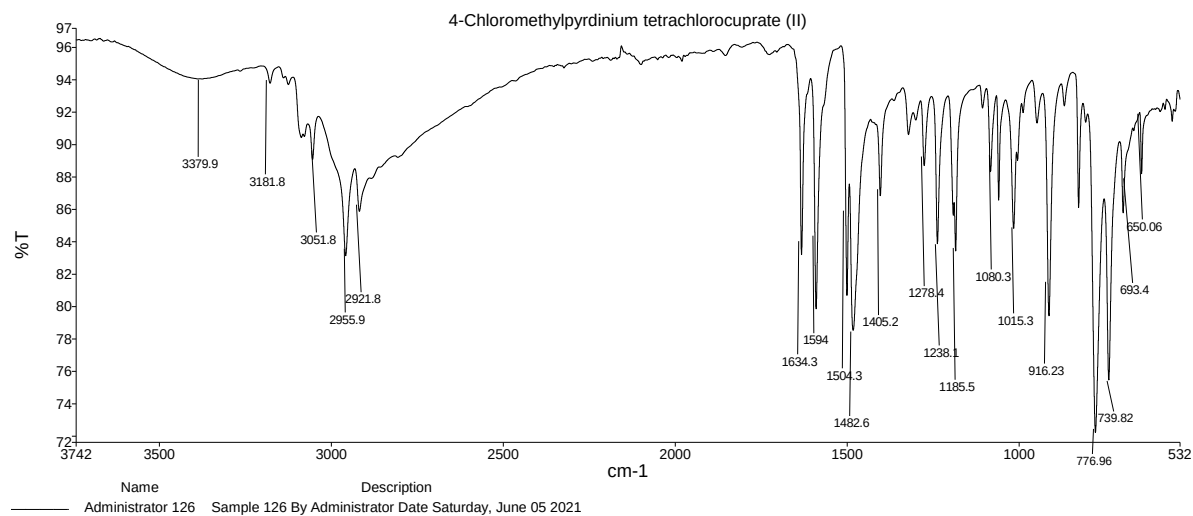
5.0. FTIR Spectra



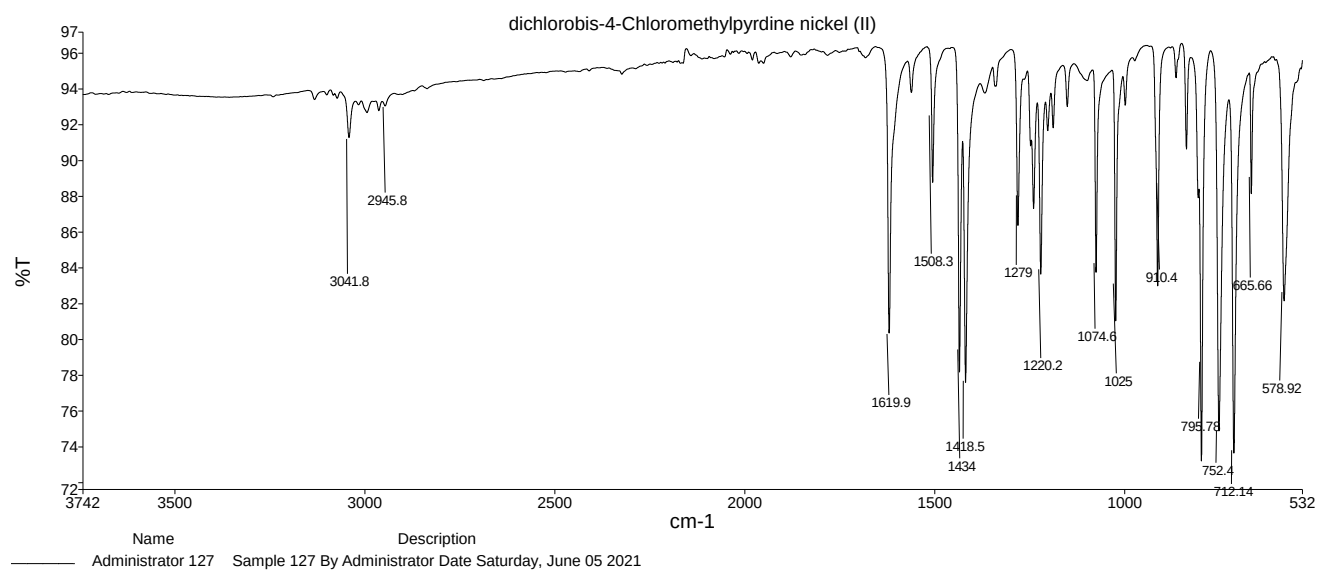
Spectrum 15: FTIR Spectrum of 4PY



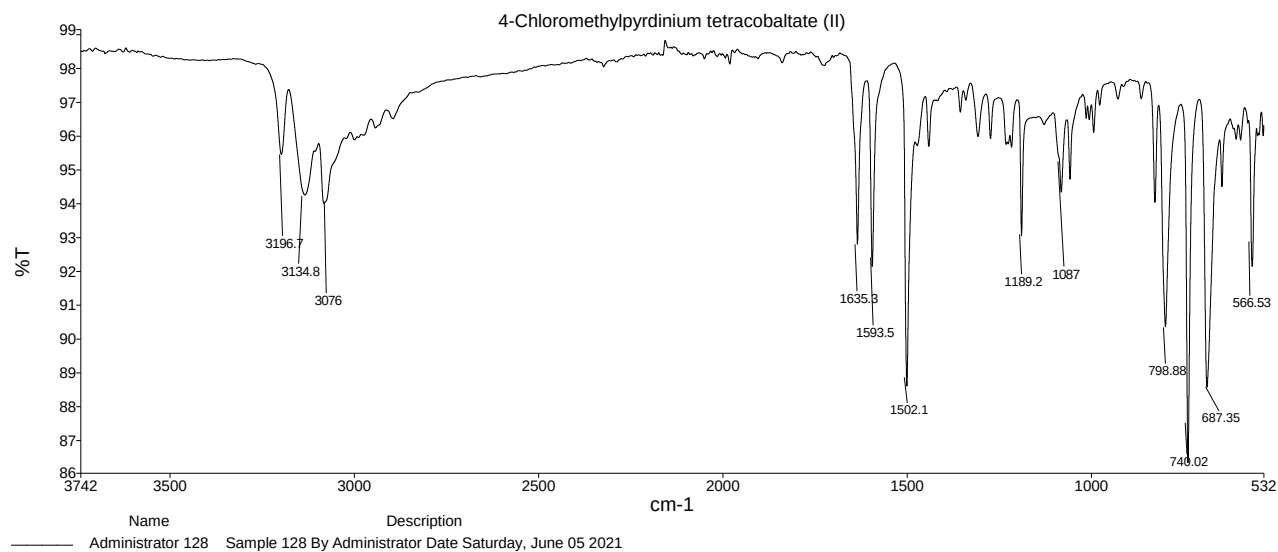
Spectrum 16: FTIR Spectrum of Cu4PY



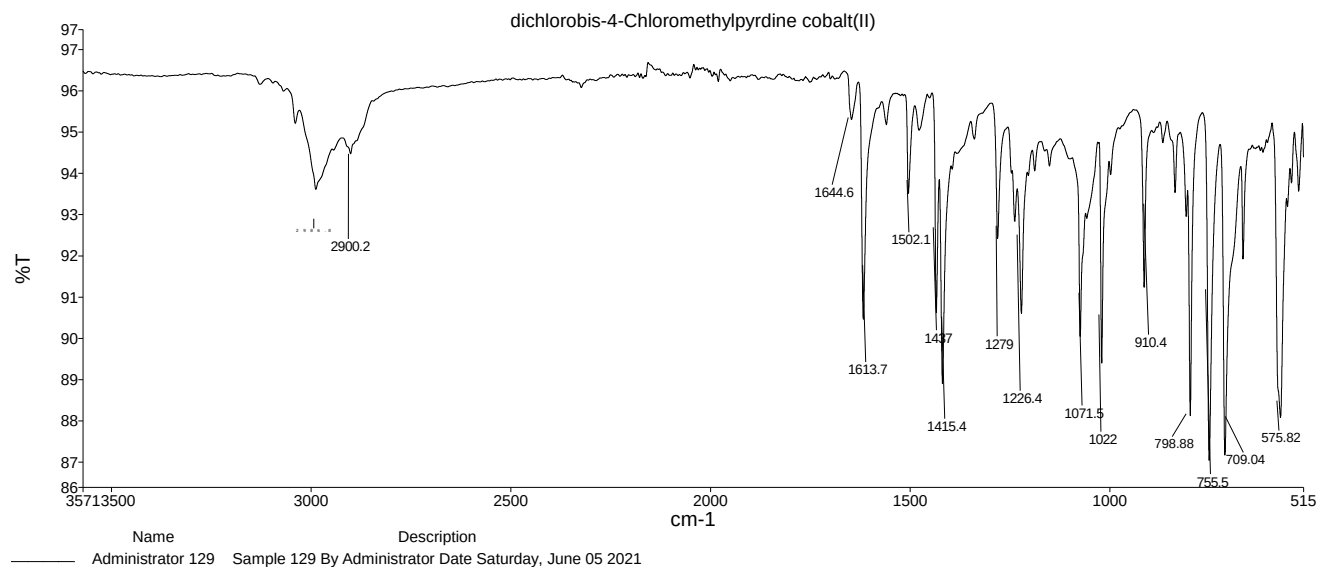
Spectrum 17: FTIR Spectrum of Cu4PY-H



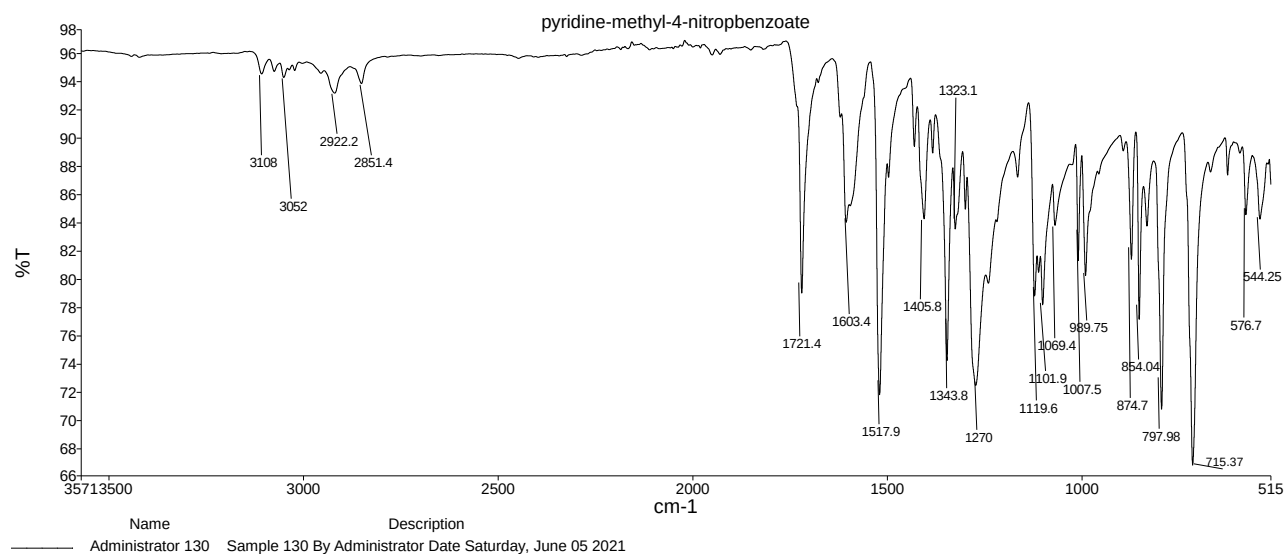
Spectrum 18: FTIR Spectrum of Ni4PY



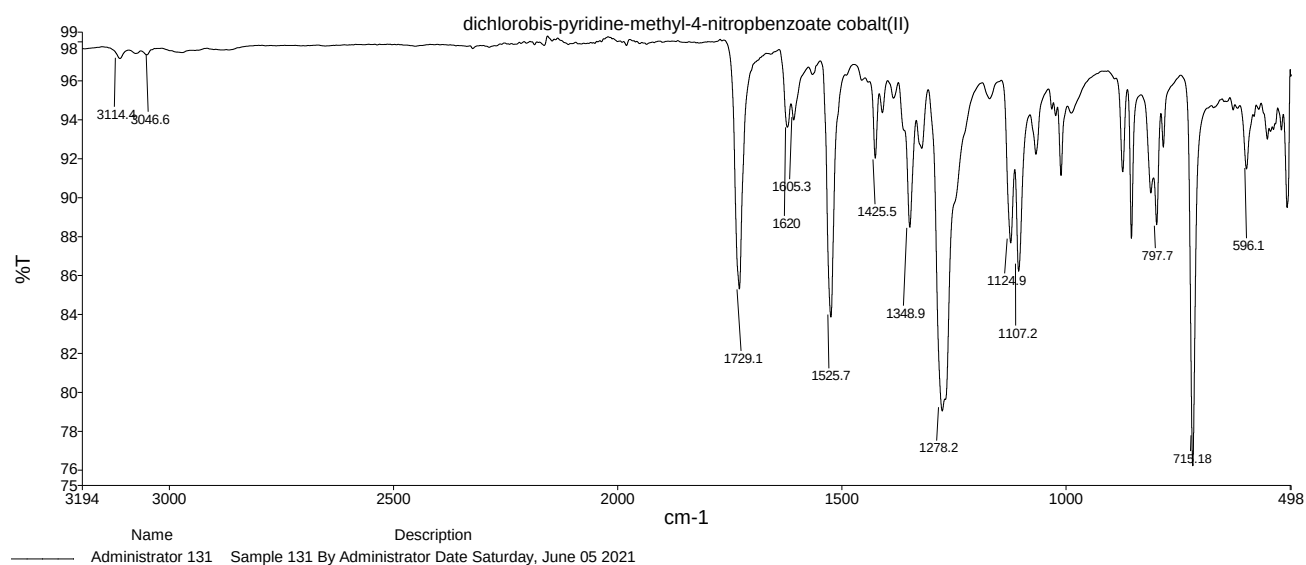
Spectrum 19: FTIR Spectrum of Co4PY-H



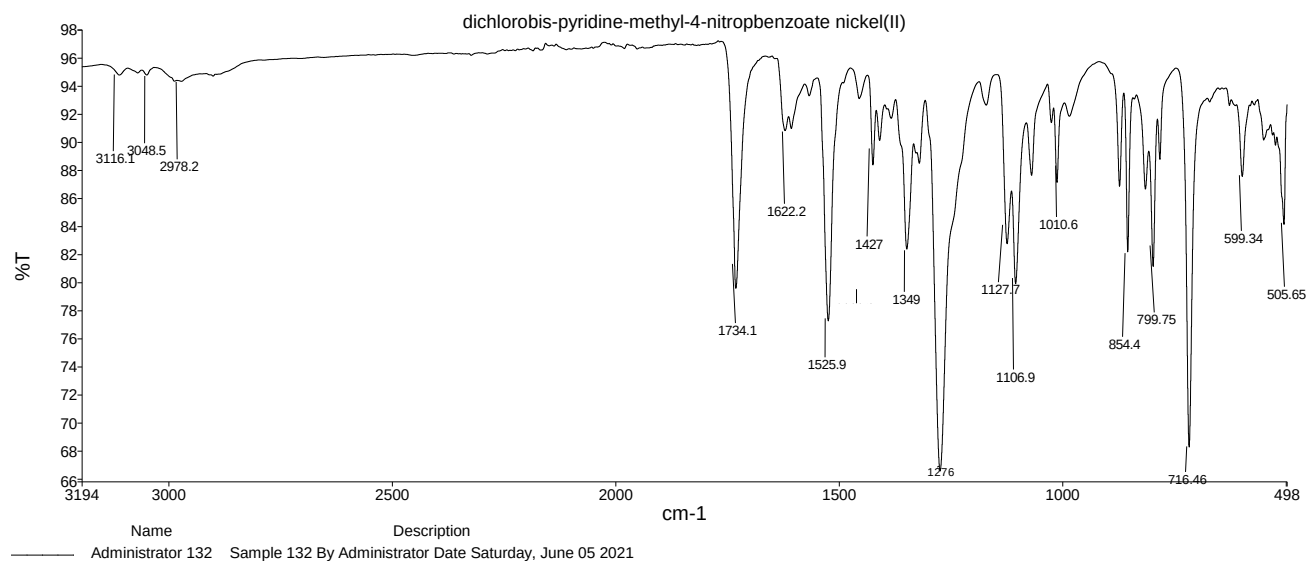
Spectrum 20: FTIR Spectrum Co4PY



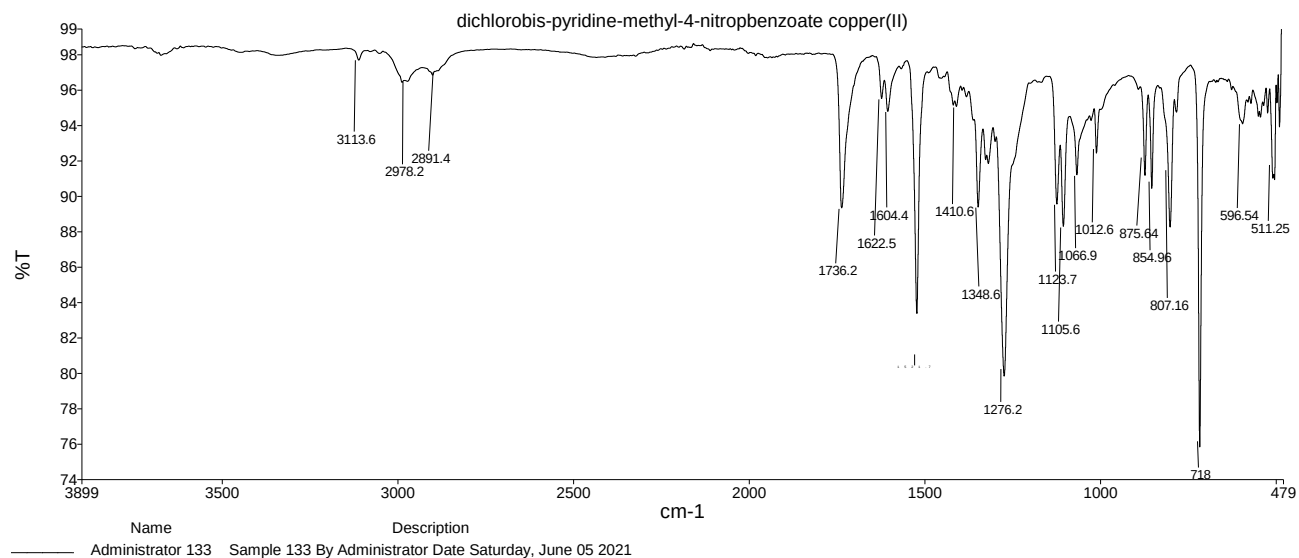
Spectrum 21: FTIR Spectrum of L1



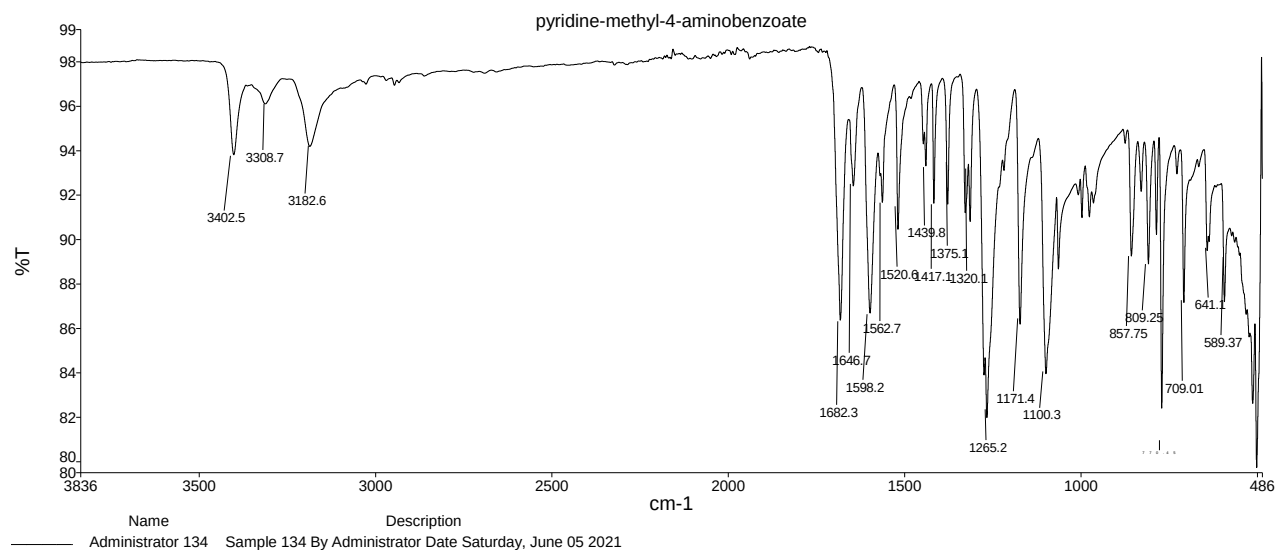
Spectrum 22: FTIR Spectrum of CoL1



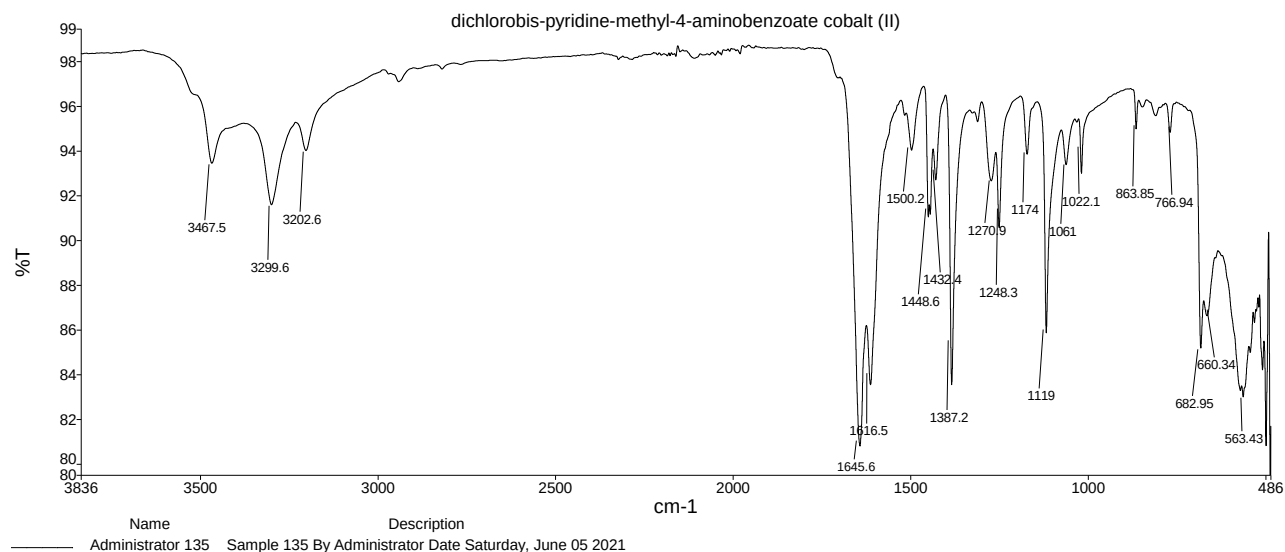
Spectrum 23: FTIR Spectrum of NiL1



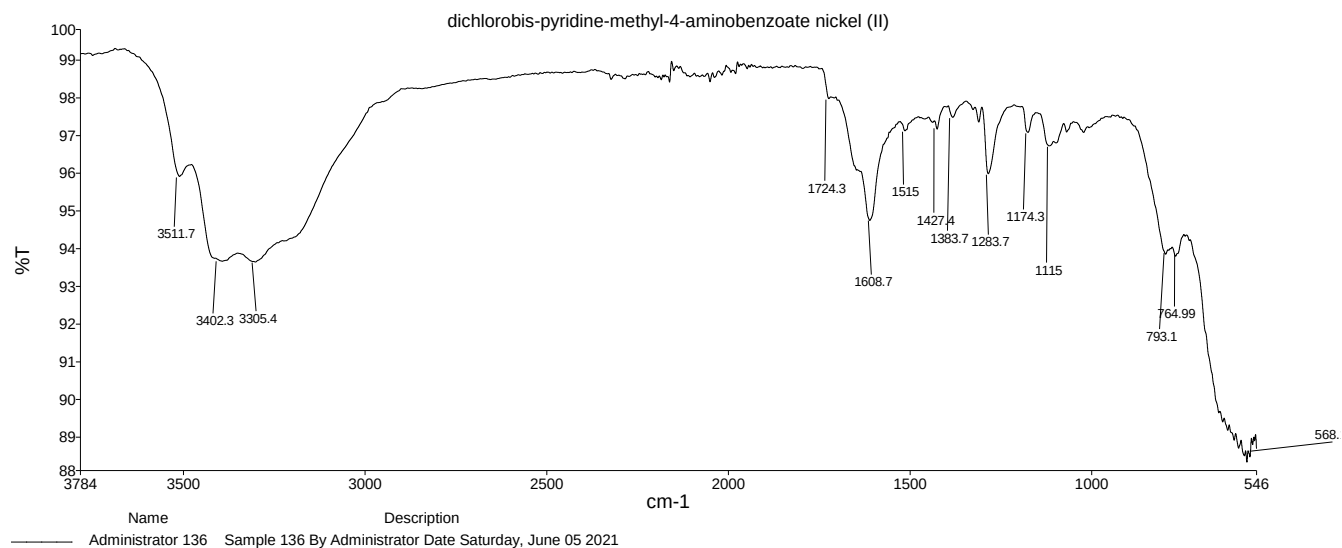
Spectrum 24: FTIR Spectrum of CuL1



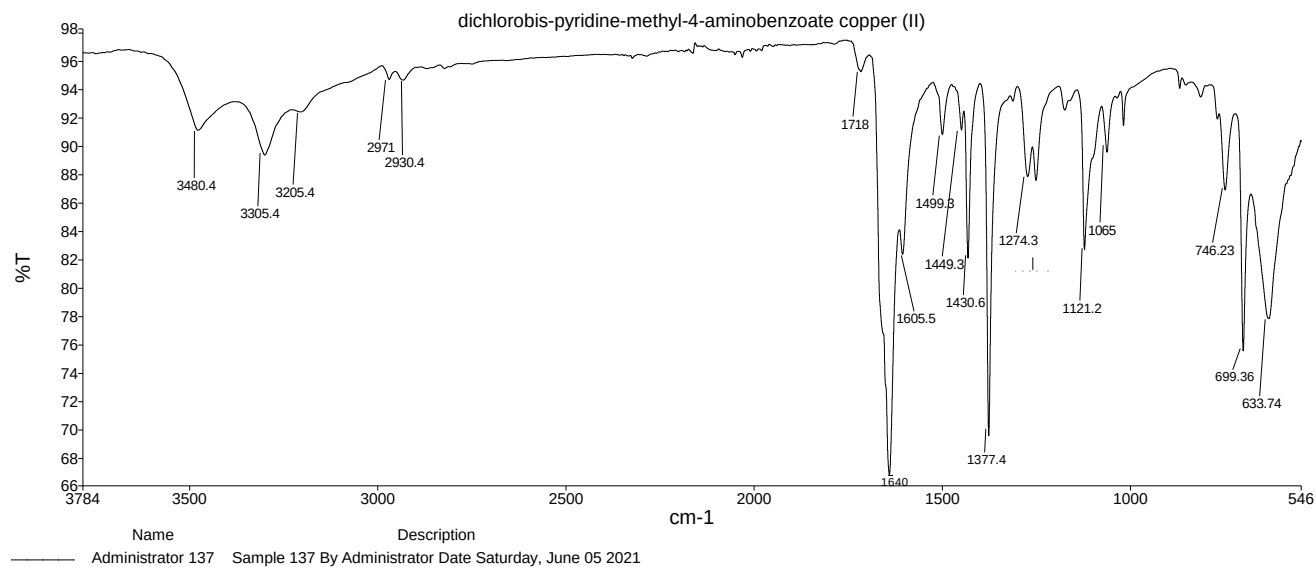
Spectrum 25: FTIR Spectrum L3



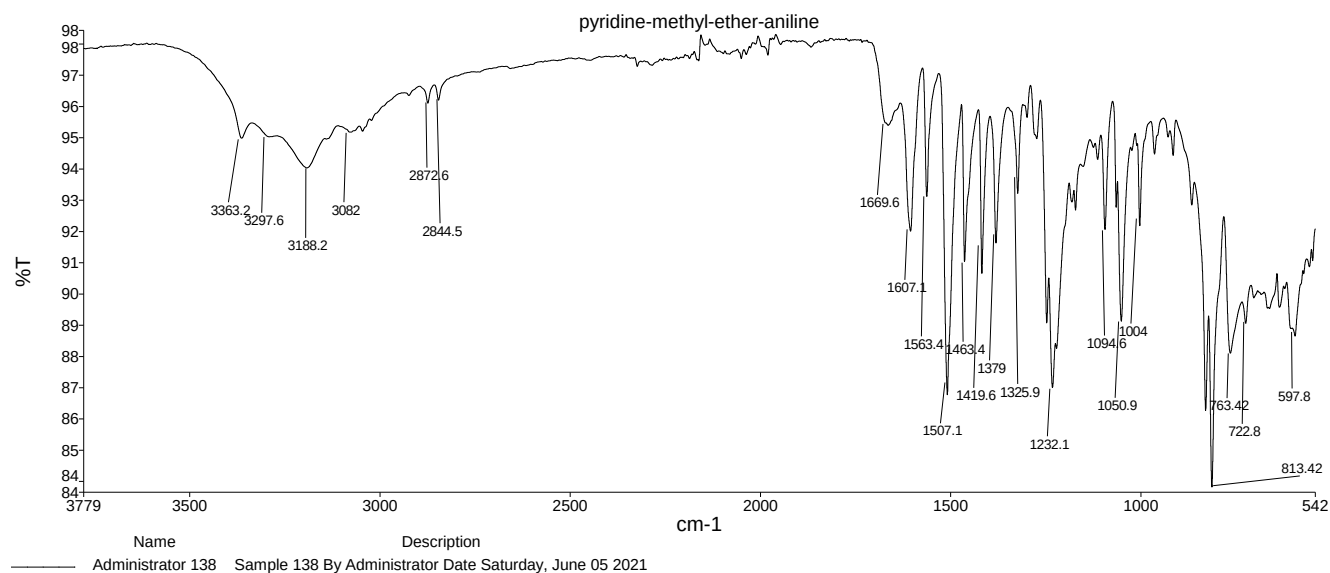
Spectrum 26: FTIR Spectrum of CoL3



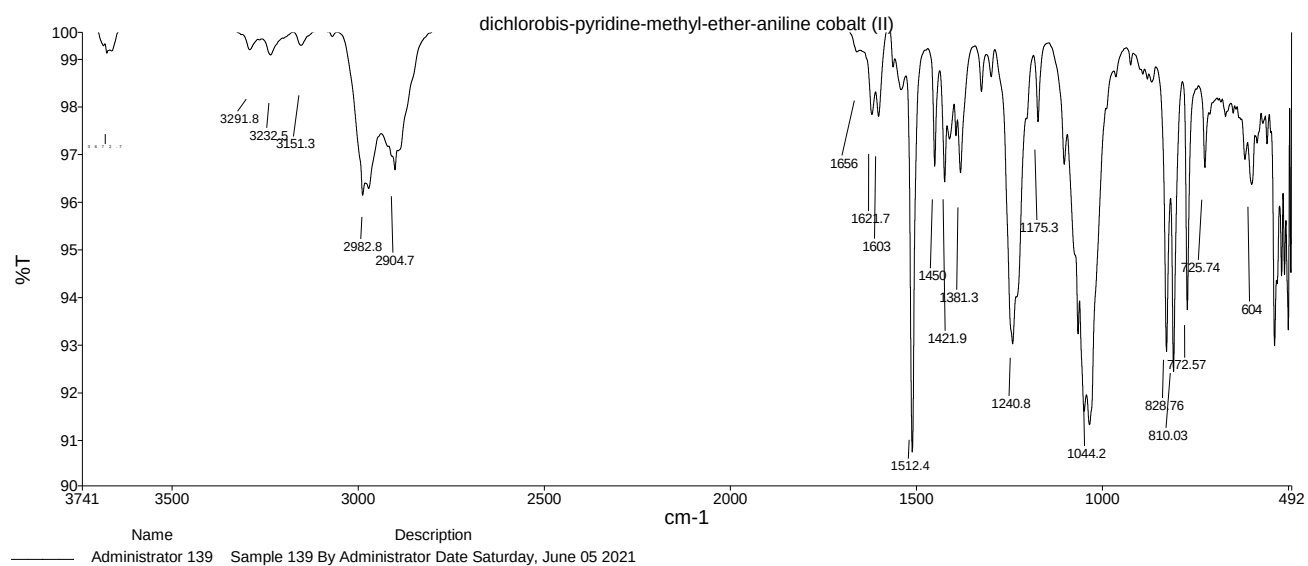
Spectrum 27: FTIR Spectrum of NiL3



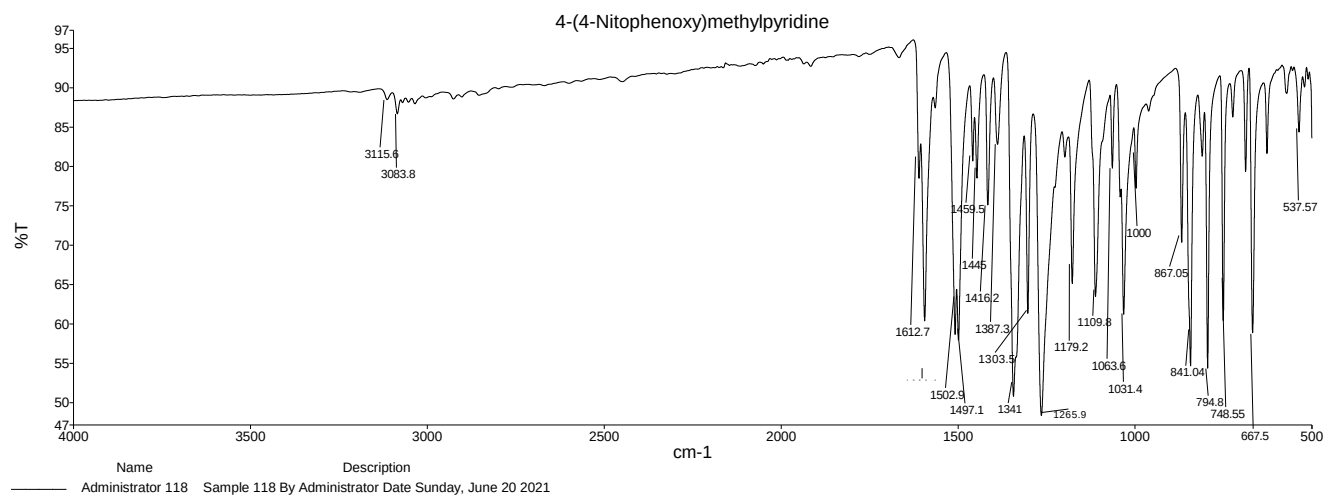
Spectrum 28: FTIR Spectrum CuL3



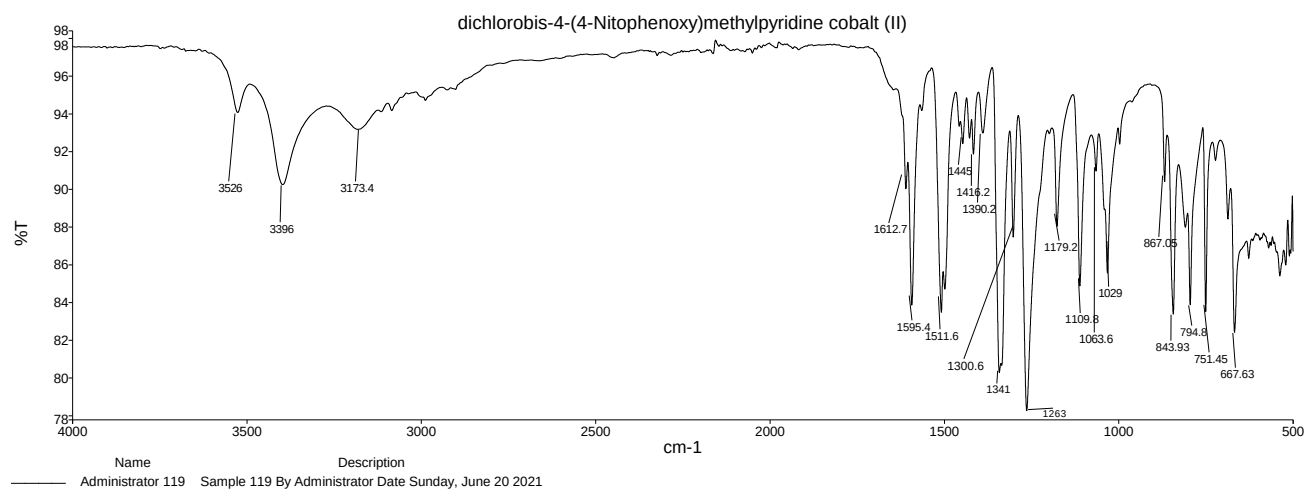
Spectrum 29: FTIR Spectrum L2



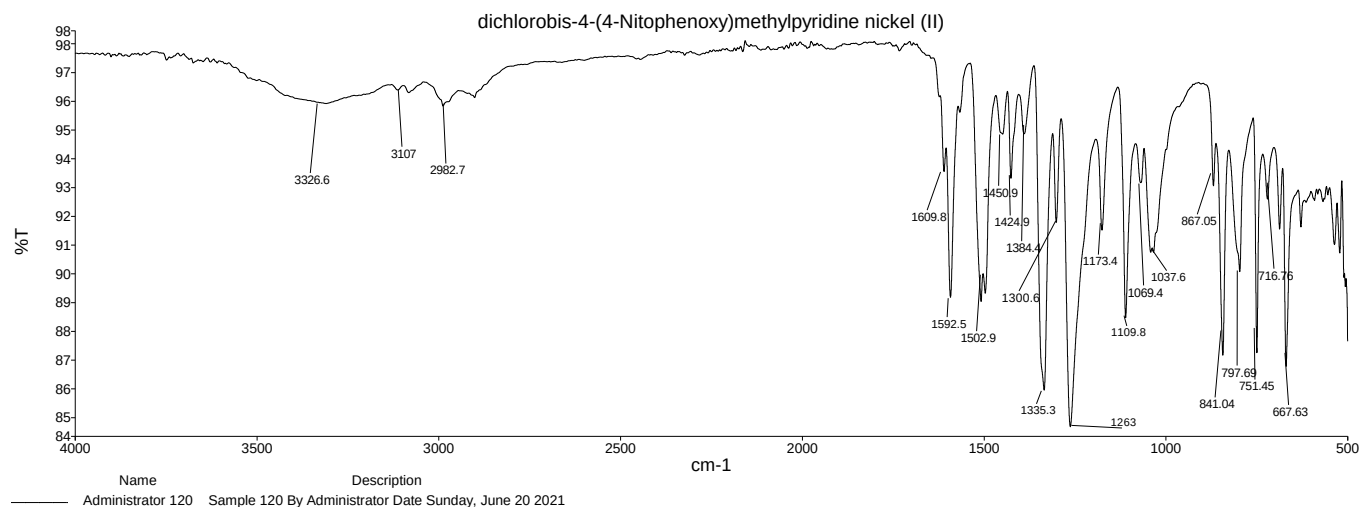
Spectrum 30: FTIR Spectrum CoL2



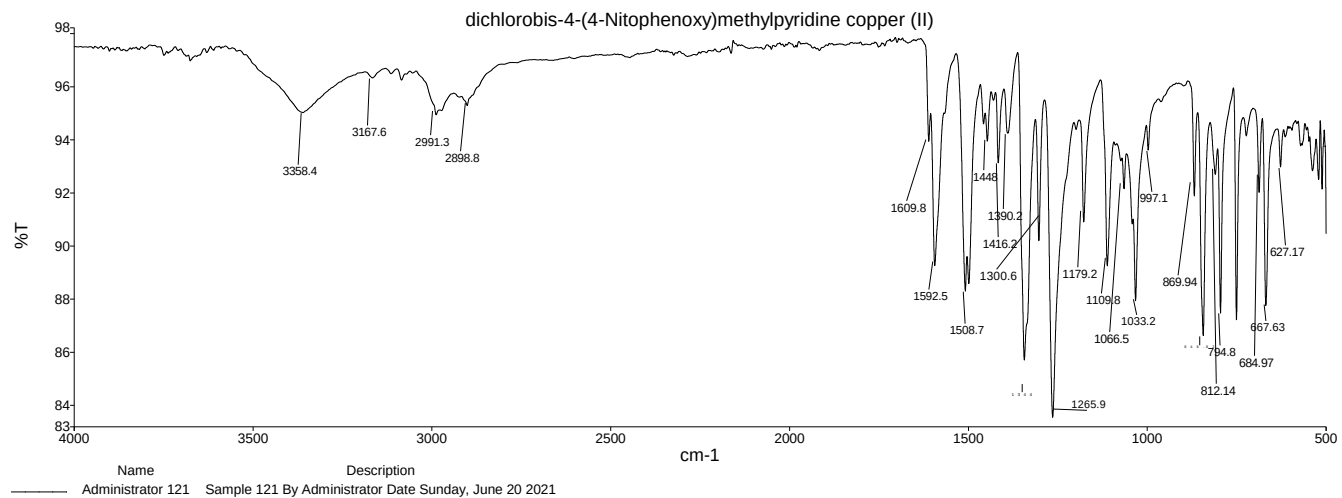
Spectrum 31: FTIR Spectrum of L4



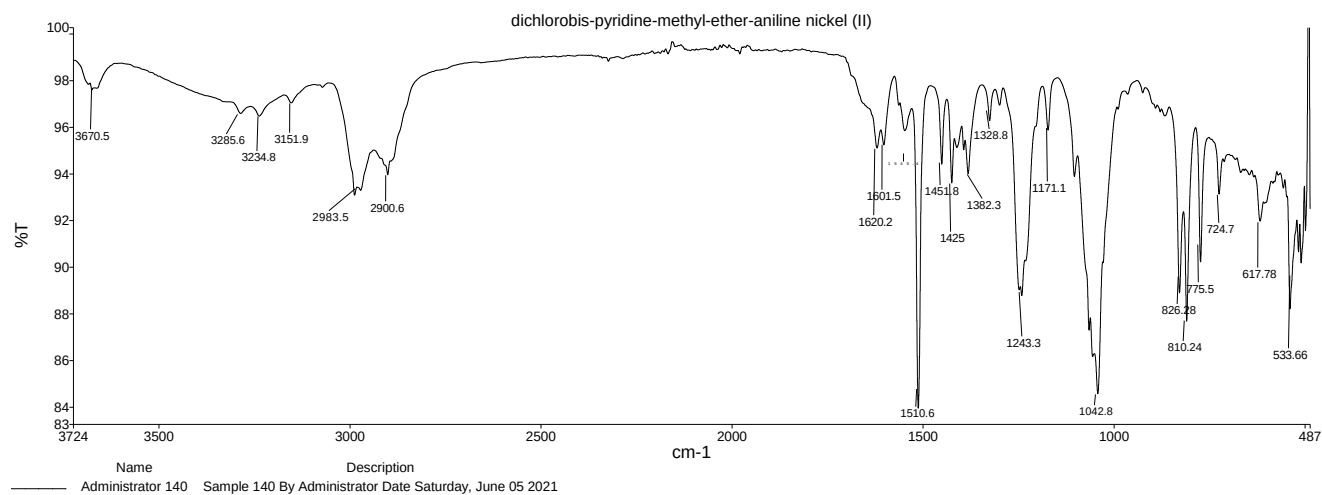
Spectrum 32: FTIR Spectrum CoL4



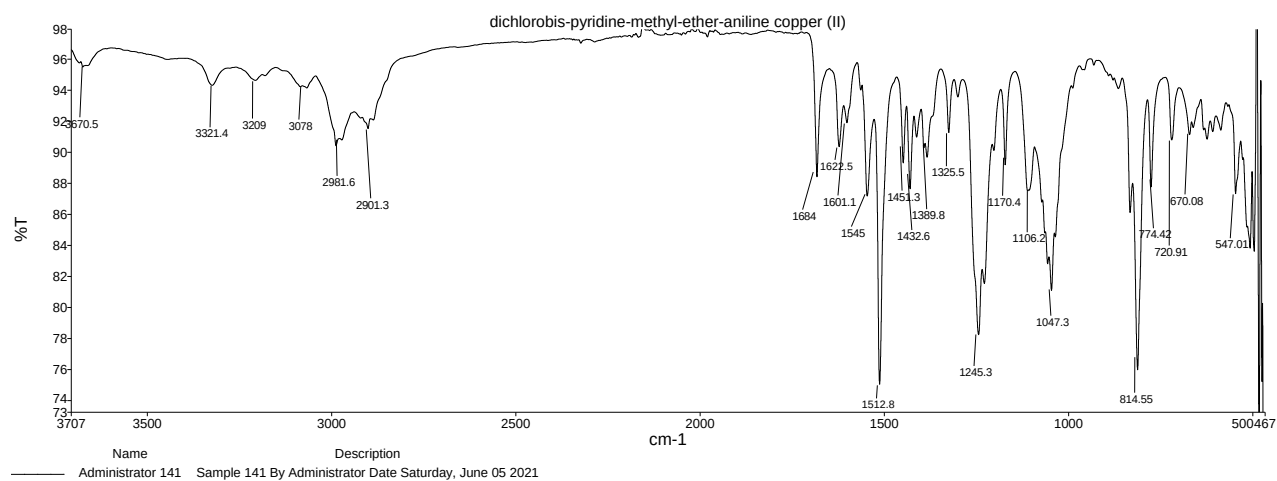
Spectrum 33: FTIR Spectrum NiL4



Spectrum 34: FTIR Spectrum CuL4



Spectrum 35: FTIR Spectrum of NiL2



Spectrum 36: FTIR Spectrum of CuL2

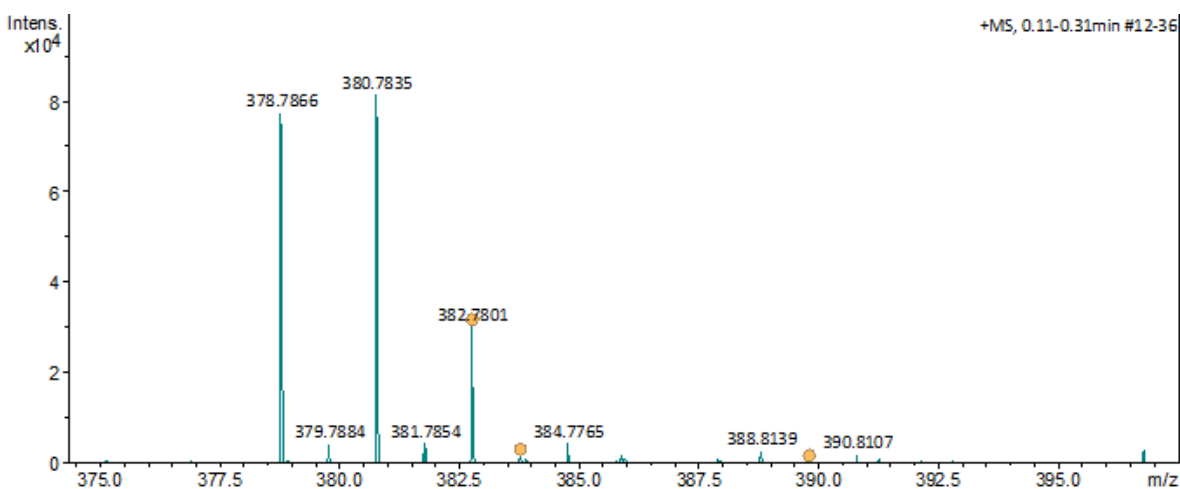
5.1. Crystallographic Refinement Details and Tables

Table 52: Crystal data and structure refinement of the Compounds

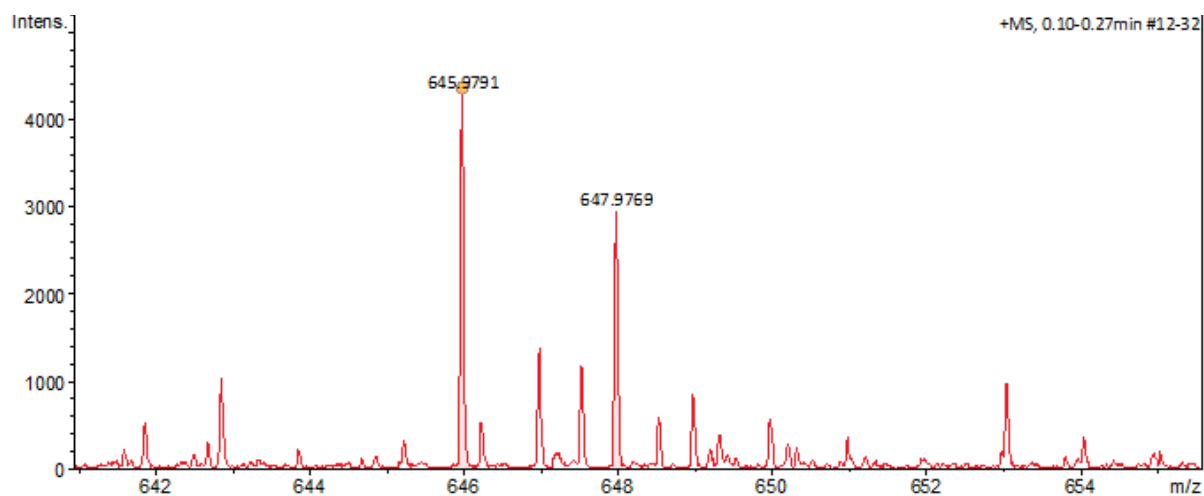
Molecular formula	L1	L2	L3	Cu4PY-H	Cu4PY	Co4PY-H	Co4PY	CoL3
Formula weight	258.23	218.25	228.25	462.49	389.58	457.88	384.97	586.32
Temperature/K	293(2)	296	296(2)	295	297.0	295	296	296
Crystal system	monoclinic	orthorhombic	orthorhombic	Monoclinic	Triclinic	Monoclinic	Triclinic	monoclinic
Space group	<i>P2₁/n</i>	<i>P2₁2₁2₁</i>	<i>Pna2₁</i>	<i>Ia</i>	<i>P-1</i>	<i>P2₁/c</i>	<i>P1</i>	<i>I2/a</i>
a/Å	9.981(2)	5.6503 (11)	11.1735 (16)	14.8120 (17)	3.9768 (7)	8.2464 (8)	3.7339 (6)	16.863(16)
b/Å	12.347 (3)	7.359 (2)	8.3548 (13)	8.6491 (6)	8.2136 (13)	15.4918 (15)	11.809 (2)	6.3088(6)
c/Å	10.161 (3)	27.326 (6)	12.3831 (17)	16.1440 (12)	11.889 (2)	14.5320 (14)	17.054 (3)	24.772(3)
α/°	90	90	90		70.105(8)		70.251 (11)	90
β/°	101.450 (9)	90	90	116.106 (4)	82.576 (10)	98.800 (4)	84.617 (11)	97.643 (3)
γ/°	90	90	90		84.937 (8)		82.839 (11)	90
Volume/Å³	1227.2 (5)	1136.2 (5)	1156.0 (3)	1857.2 (3)	361.71 (11)	1834.6 (3)	701.2 (2)	2611.7 (5)
Z	4	4	4	4	1	4	2	4
ρ_{calc}/cm³	1.398	1.276	1.311	1.654	1.788	1.658	1.823	1.491
μ/mm⁻¹	0.106	0.088	0.09	2.032	2.233	1.802	1.97	0.902
F(000)	536	464	480	924	195	916	386	1204
Crystal size/mm³	0.003 × 0.001 × 0.001	0.4 × 0.3 × 0.1	0.51×0.33 ×0.32	0.02 × 0.1 × 0.1	0.3 × 0.1 × 0.1	0.1 × 0.1 × 0.1	0.2 × 0.01 × 0.01	0.3 × 0.3 × 0.2
Radiation	MoKα (λ=0.7107)	MoKα (λ=0.71073)	MoKα (λ=0.71073)	MoKα (λ=0.71073)	MoKα (λ=0.71073)	MoKα (λ=0.71073)	MoKα (λ=0.71073)	MoKα (λ=0.71073)
2θ range for data collection/°	6.182 to 49.174	6.288 to 57.44	6.088 to 53.364	5.618 to 52.966	7.332 to 62.248	5.674 to 45.552	6.956 to 52.05	6.252 to 43.472
Index ranges	-11 ≤ h ≤ 11, -14 ≤ k ≤ 14, -11 ≤ l ≤ 11	-6 ≤ h ≤ 7, -9 ≤ k ≤ 9, -35 ≤ l ≤ 36	-13 ≤ h ≤ 14, -10 ≤ k ≤ 10, -15 ≤ l ≤ 14	-18 ≤ h ≤ 18, -10 ≤ k ≤ 10, -20 ≤ l ≤ 20	-5 ≤ h ≤ 5, -11 ≤ k ≤ 11, -15 ≤ l ≤ 17	-8 ≤ h ≤ 8, -16 ≤ k ≤ 16, -15 ≤ l ≤ 15	-4 ≤ h ≤ 4, -14 ≤ k ≤ 14, -20 ≤ l ≤ 20	-17 ≤ h ≤ 17, -6 ≤ k ≤ 6, -25 ≤ l ≤ 25
Reflections collected	11425	15282	5807	18416	5397	21350	13650	8464

Independent Reflections	2033 [R _{int} = 0.0678, R _{sigma} = 0.0429]	2925 [R _{int} = 0.0859, R _{sigma} = 0.0615]	2245 [R _{int} = 0.0571, R _{sigma} = 0.0623]	3794 [R _{int} = 0.0906, R _{sigma} = 0.0605]	2215 [R _{int} = 0.0, R _{sigma} = 0.1319]	2463 [R _{int} = 0.1, R _{sigma} = 0.0521]	2696 [R _{int} = 0.1, R _{sigma} = 0.0781]	1531 [R _{int} = 0.0587, R _{sigma} = 0.0391]
Data/restraints/parameters	2033/0/172	2925/0/150	2245/1/154	3794/2/190	2215/0/89	2463/0/198	2696/212/172	1531/0/168
Goodness-of-fit on F²	1.093	1.041	1.04	1.095	1.185	1.027	1.265	1.12
Final R indexes [I >= 2σ (I)]	R ₁ = 0.0425, wR ₂ = 0.1050	R ₁ = 0.0550, wR ₂ = 0.1285	R ₁ = 0.0489, wR ₂ = 0.1233	R ₁ = 0.0439, wR ₂ = 0.1123	R ₁ = 0.1453, wR ₂ = 0.3383	R ₁ = 0.0407, wR ₂ = 0.0888	R ₁ = 0.1910, wR ₂ = 0.4155	R ₁ = 0.0338, wR ₂ = 0.0772
Final R indexes [all data]	R ₁ = 0.0834, wR ₂ = 0.1393	R ₁ = 0.0730, wR ₂ = 0.1380	R ₁ = 0.0989, wR ₂ = 0.1760	R ₁ = 0.0744, wR ₂ = 0.1271	R ₁ = 0.2243, wR ₂ = 0.3920	R ₁ = 0.0567, wR ₂ = 0.0959	R ₁ = 0.2240, wR ₂ = 0.4319	R ₁ = 0.0422, wR ₂ = 0.0836
Largest diff. peak/hole / e Å⁻³	0.17/-0.18	0.24/-0.20	0.18/-0.20	0.60/-0.51	5.12/-0.70	0.33/-0.28	2.66/-3.03	0.22/-0.19
CCDC				2178504	2178505	2178506	2178507	

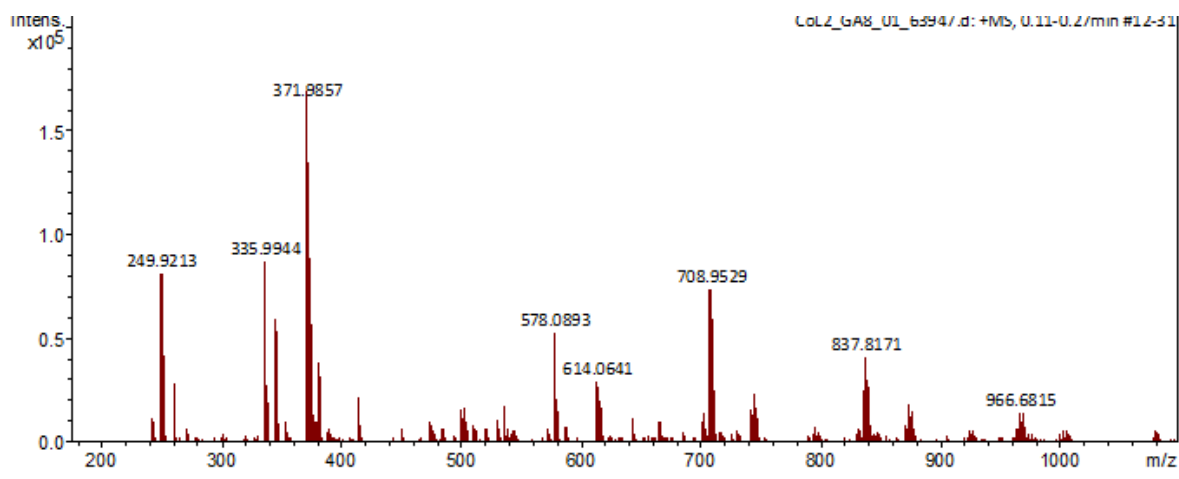
2.1. High Resolution ESI-Mass Spectra



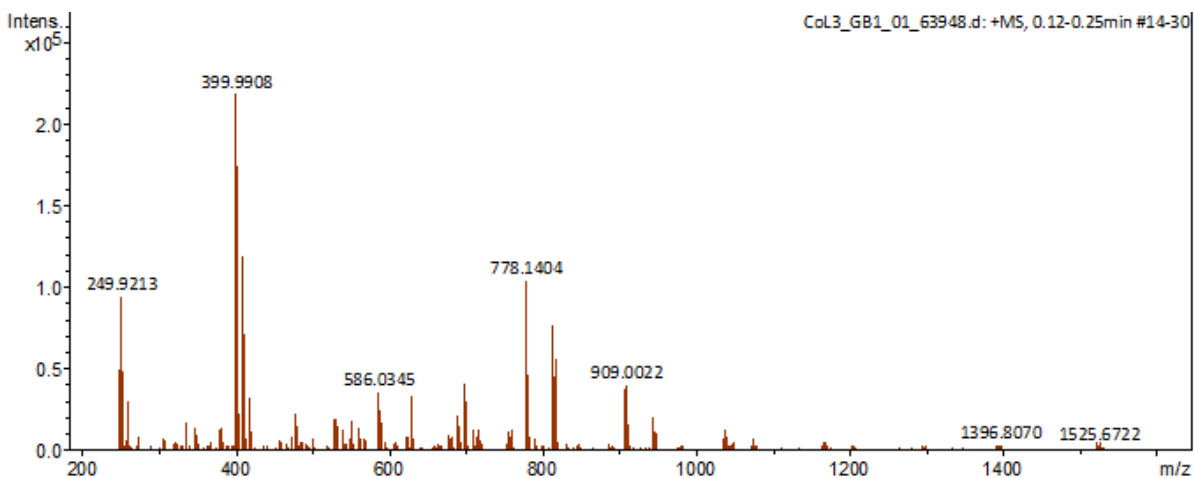
Spectrum 37: Mass spectrum of **Co4PY-H**.



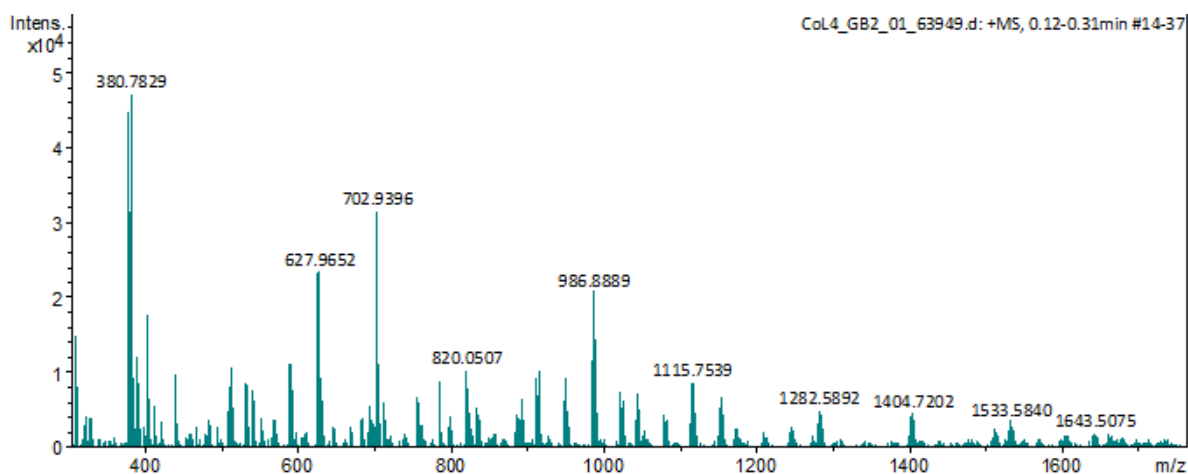
Spectrum 38: Mass spectrum of **CoL1**.



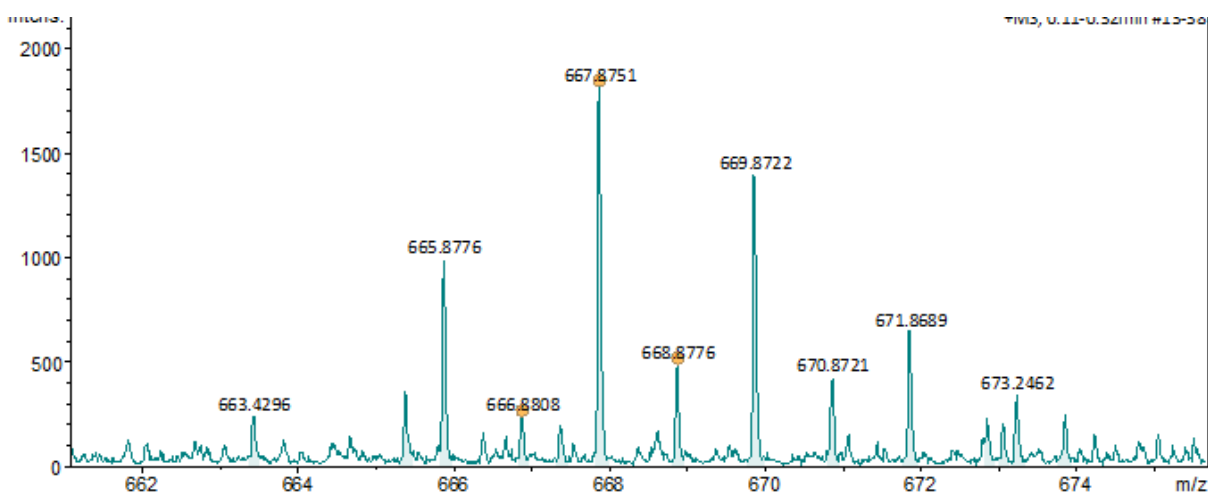
Spectrum 39: Mass spectrum of **CoL2**.



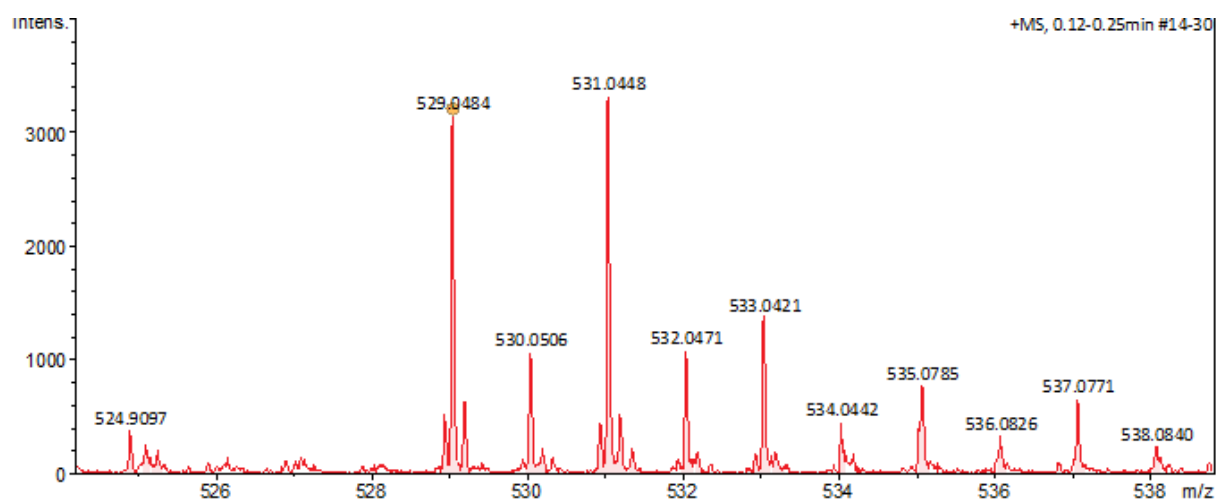
Spectrum 40: Mass spectrum of **CoL3**.



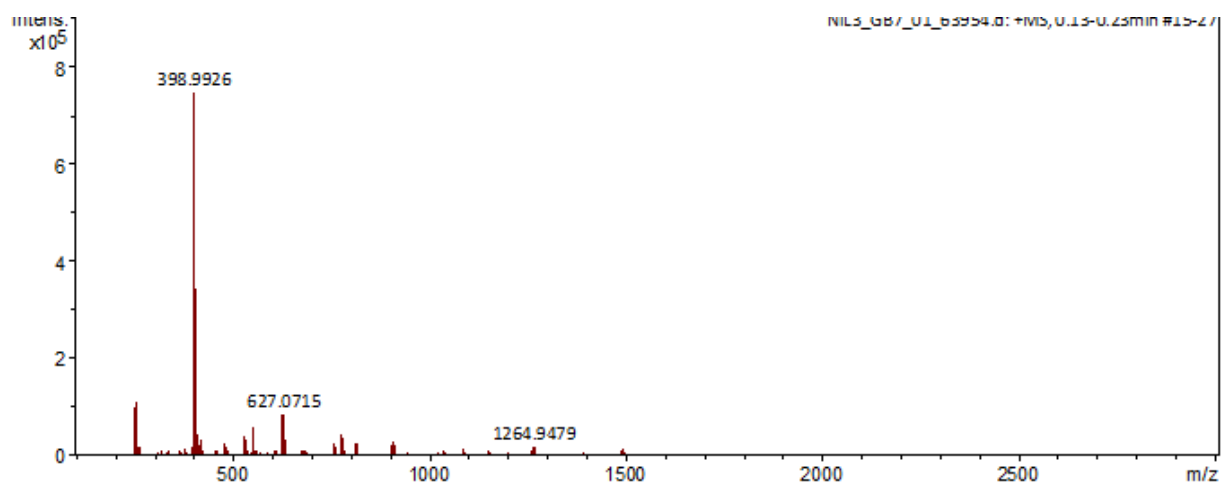
Spectrum 41: Mass spectrum of **CoL4**.



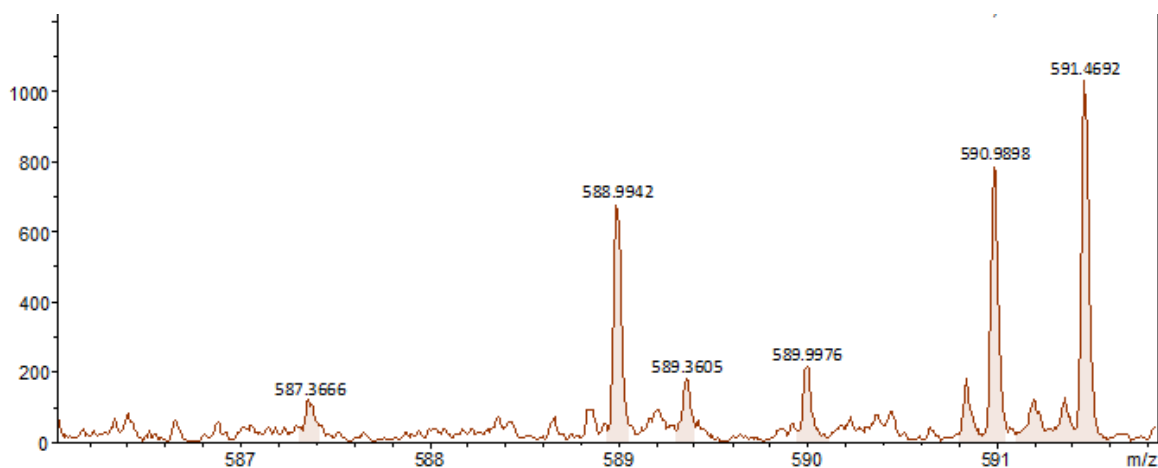
Spectrum 42: Mass spectrum of NiL1.



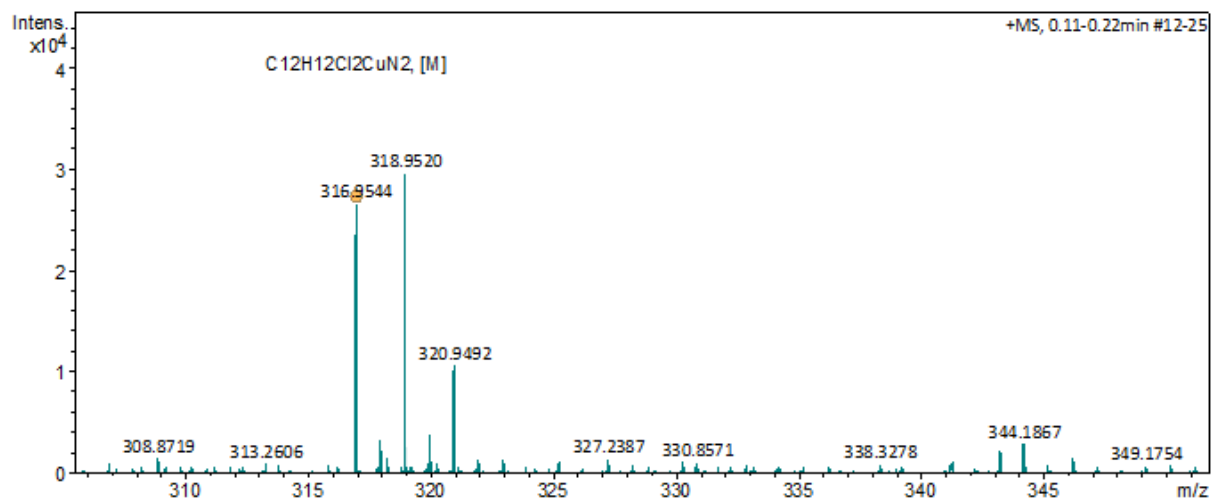
Spectrum 43: Mass spectrum of NiL2.



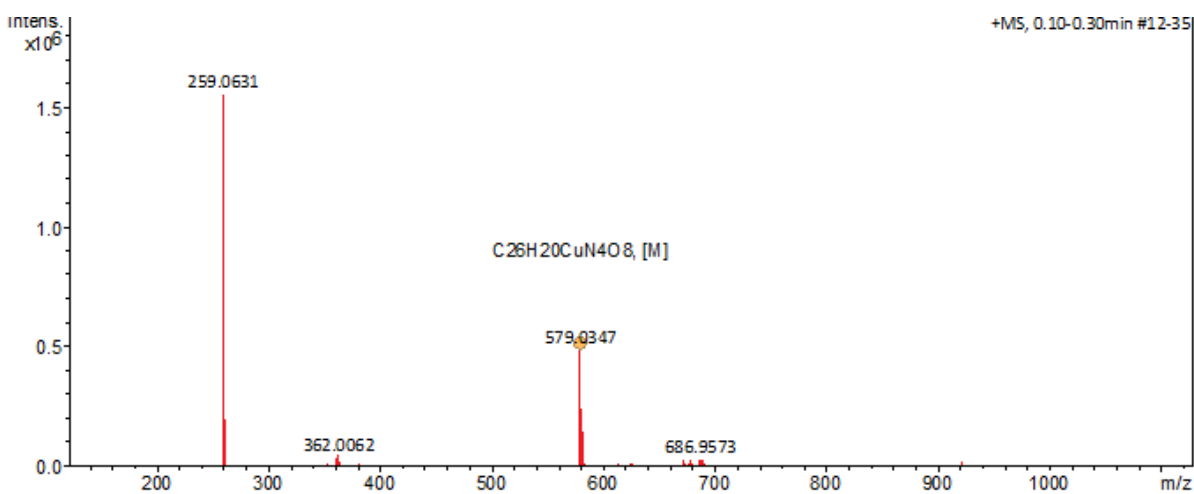
Spectrum 44: Mass spectrum of NiL3.



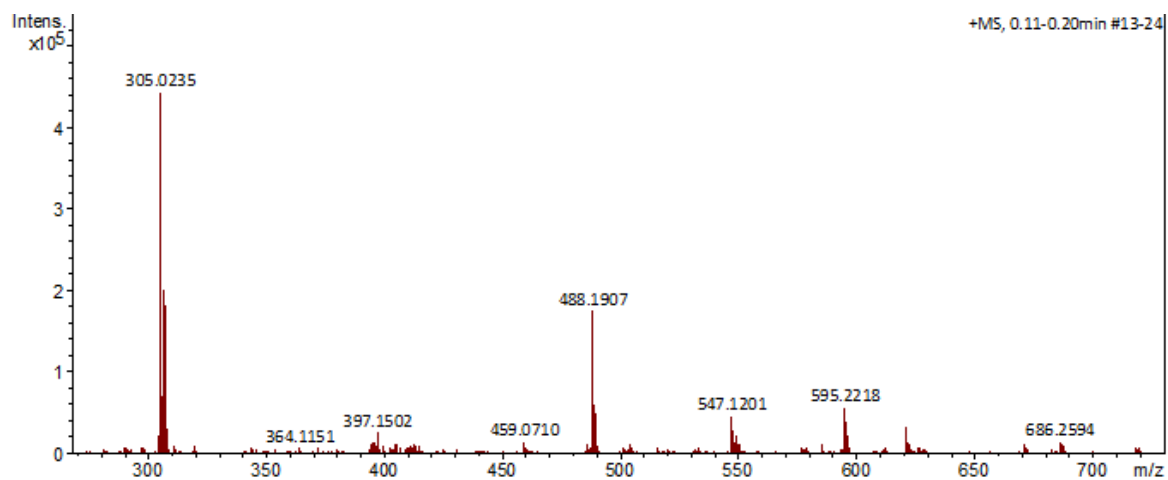
Spectrum 45: Mass spectrum of NiL4.



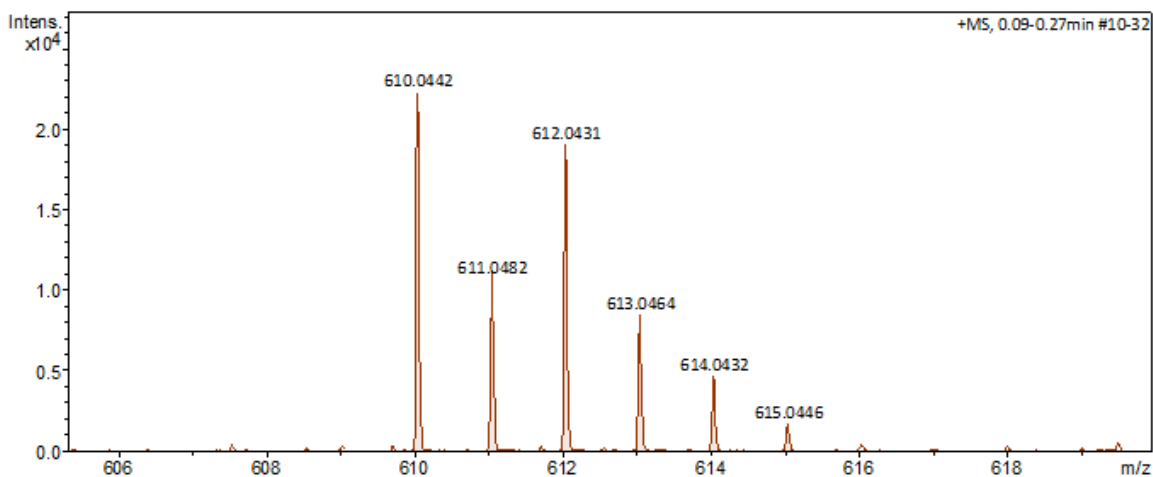
Spectrum 46: Mass spectrum of **Cu₄PY-H**.



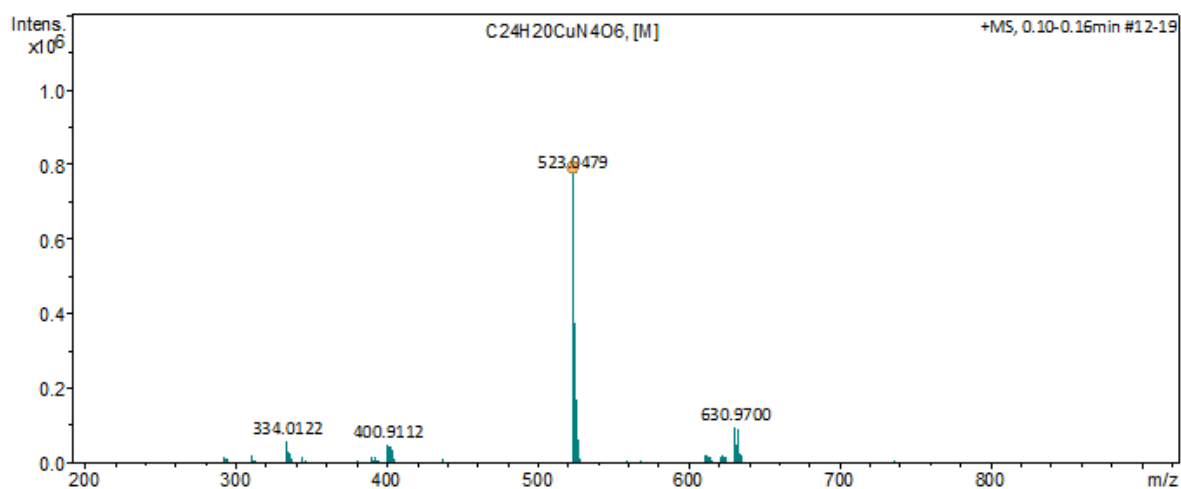
Spectrum 47: Mass spectrum of **CuL1**.



Spectrum 48: Mass spectrum of CuL2.



Spectrum 49: Mass spectrum of CuL3.



Spectrum 50: Mass spectrum of CuL4.

2.2. Thermograms and DSC Profiles

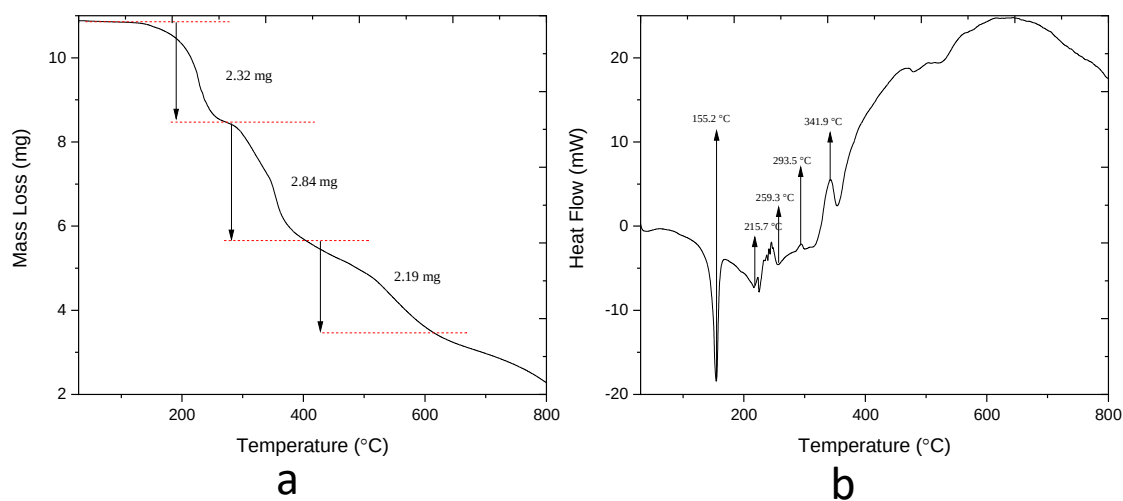


Figure 86: a) Thermogram and b) DSC profile of **Co₄PY-H**

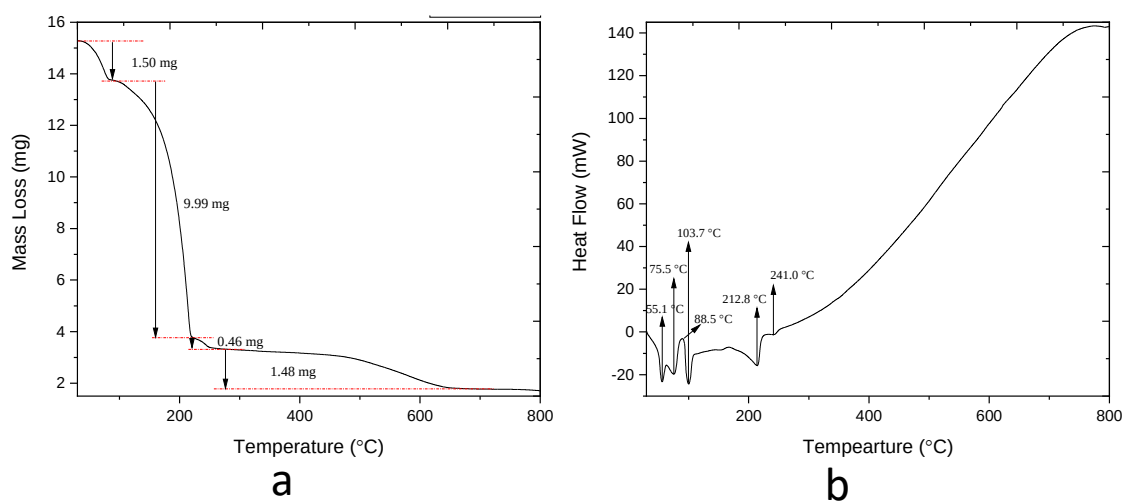
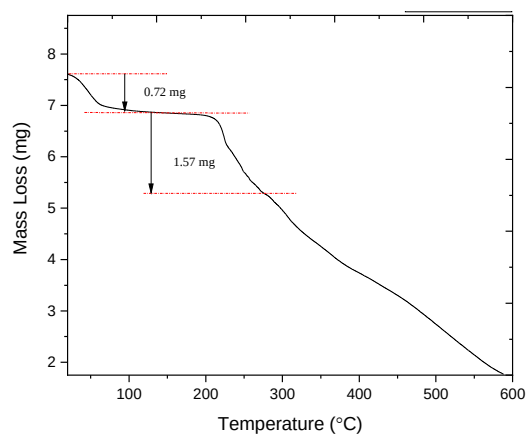
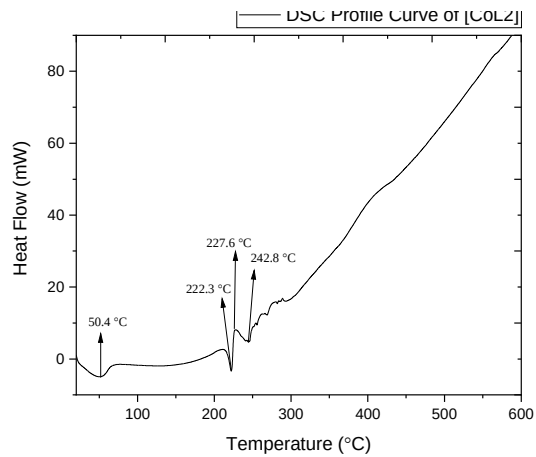


Figure 87: a) Thermogram and b) DSC profile of **CoL1**

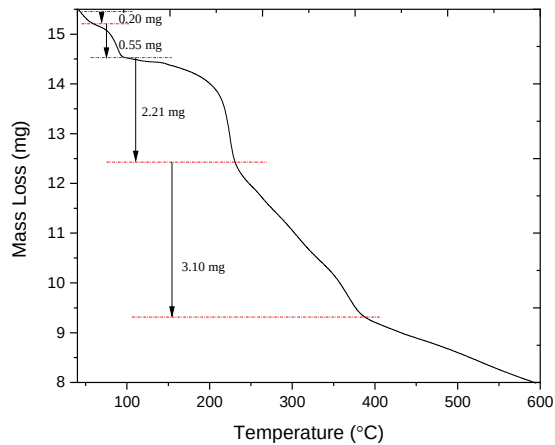


a

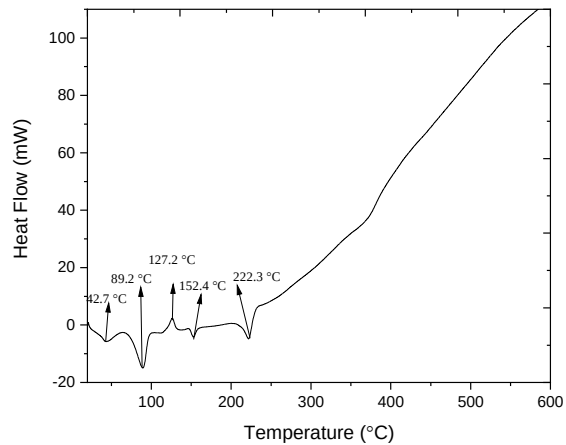


b

Figure 88: Thermogram and DSC profile of CoL2



a



b

Figure 89: Thermogram and DSC profile of CoL3

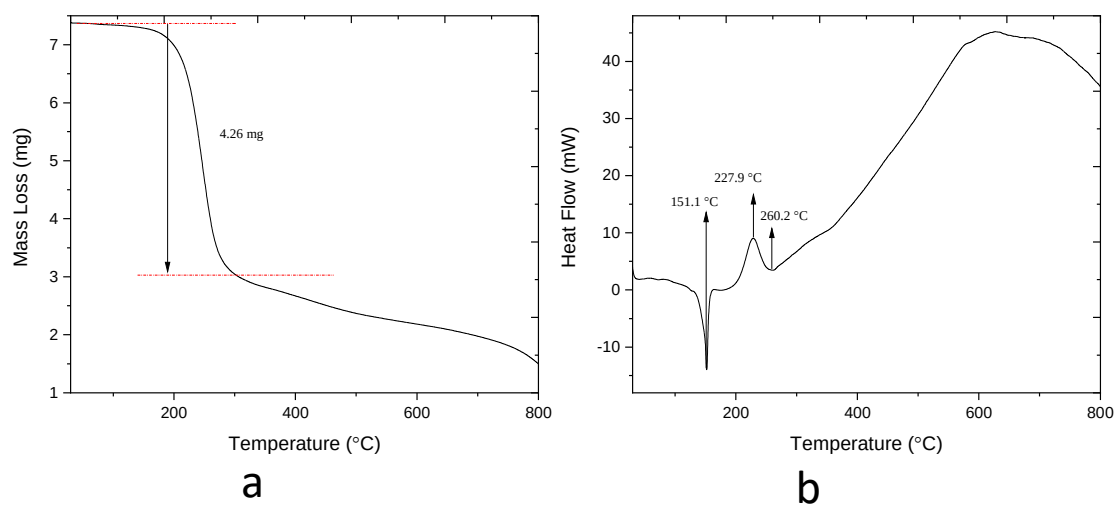


Figure 90: Thermogram and DSC profile of **CoL4**

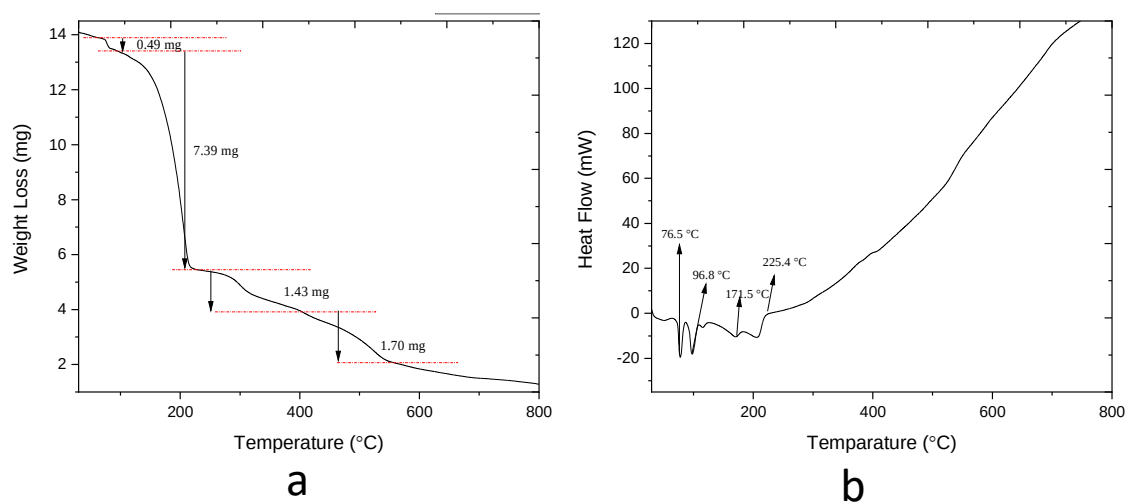


Figure 91: a) Thermogram and b) DSC profile of **NiL1**

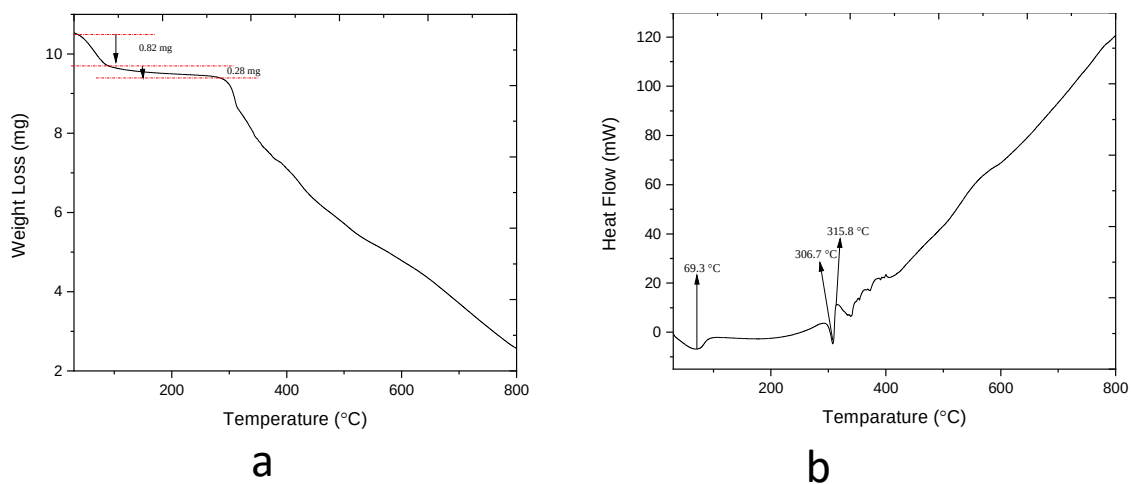


Figure 92: a) Thermogram and b) DSC profile of **NiL2**

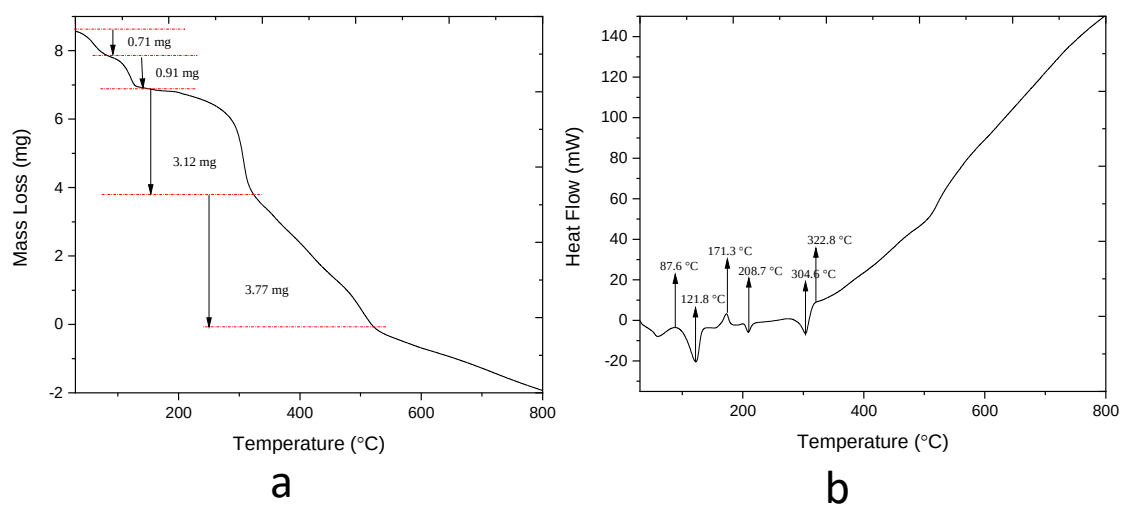


Figure 93: a) Thermogram and b) DSC profile of **NiL3**

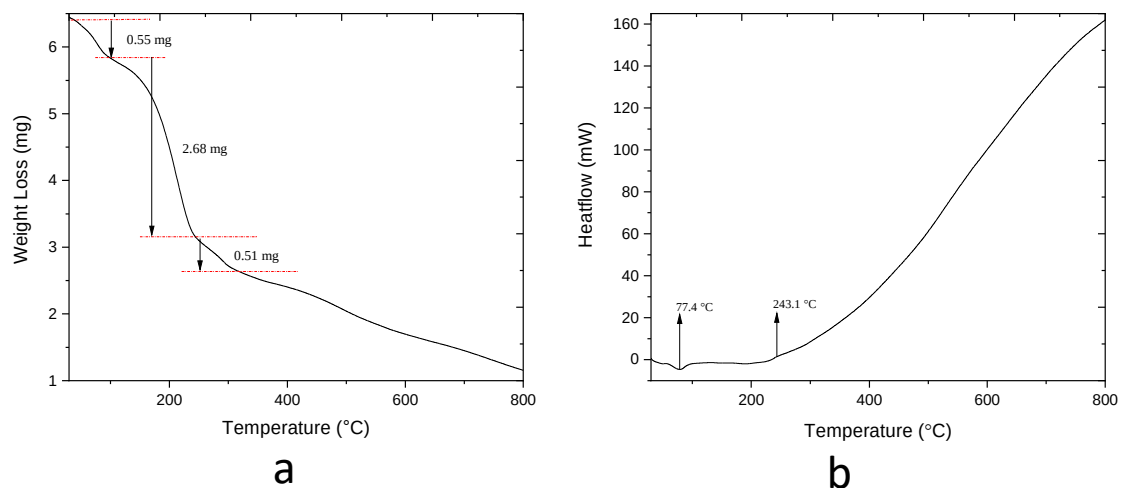


Figure 94: a) Thermogram and b) DSC profile of **NiL4**

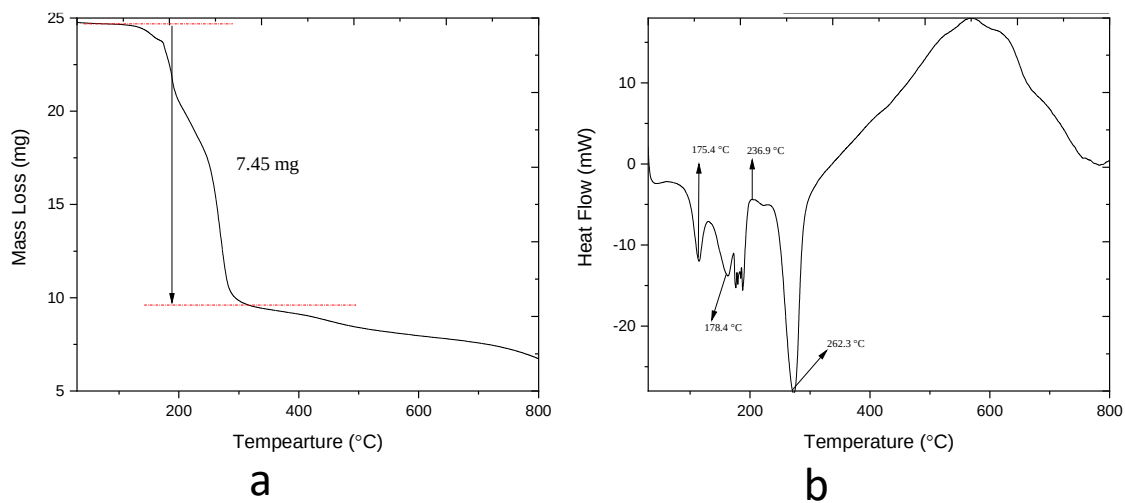


Figure 95: a) Thermogram and b) DSC profile of **Cu4PY-H**

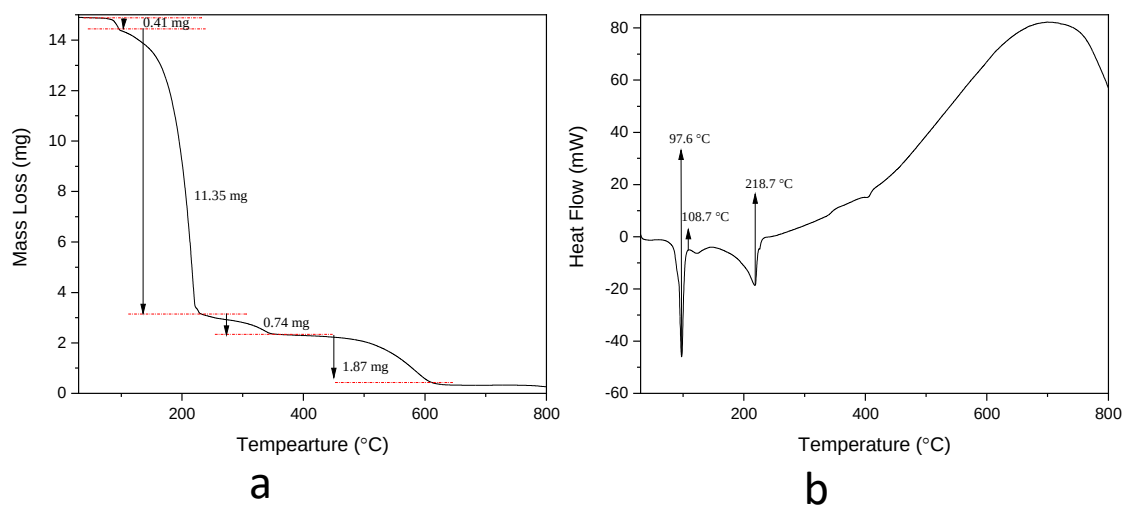


Figure 96: a) Thermogram and b) DSC profile of **CuL1**

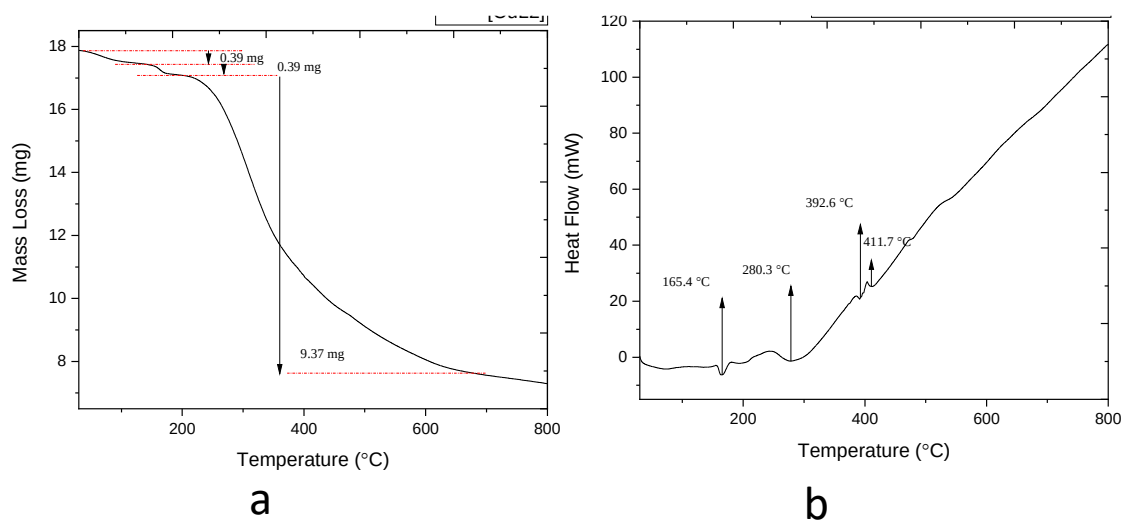


Figure 97: a) Thermogram and b) DSC profile of **CuL2**

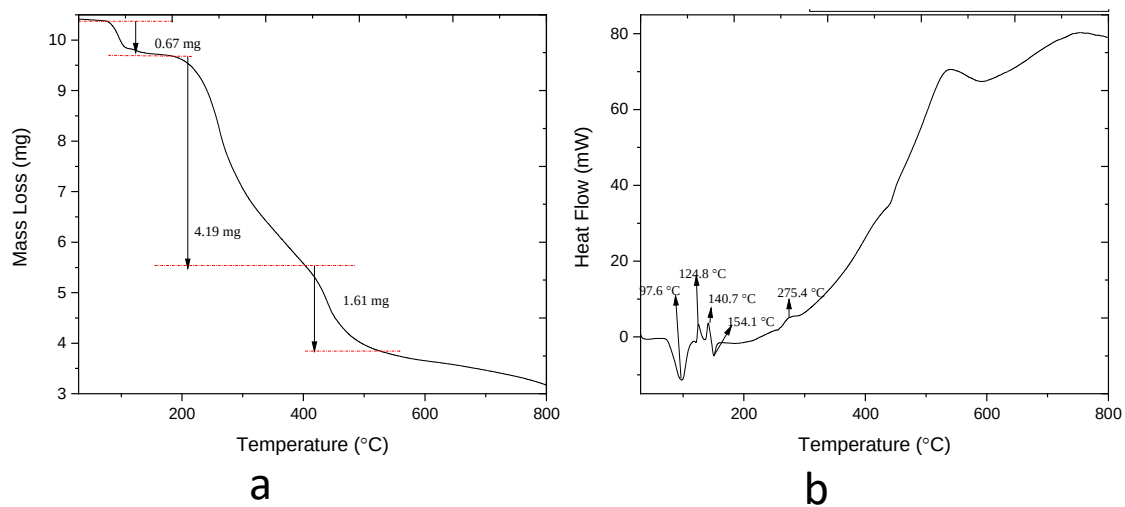


Figure 98: a) Thermogram and b) DSC profile of **CuL3**

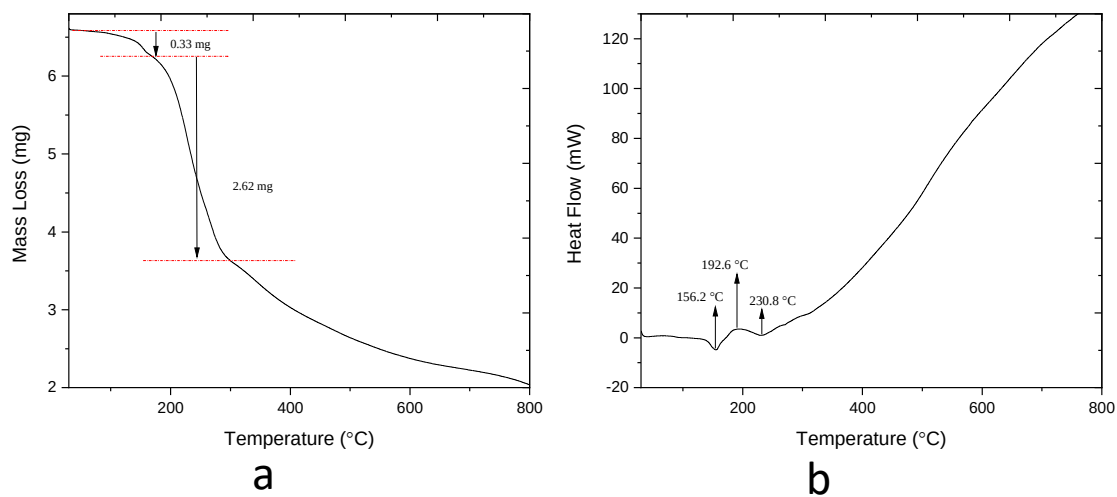


Figure 99: a) Thermogram and b) DSC profile of **CuL4**