Organometallic improvement of some tuberculosis drugs

By

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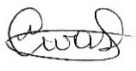
Declaration

I hereby **declare** that the thesis **"Organometallic improvement of some tuberculosis drugs"** submitted to the University of the Western Cape is my work, and that all sources I have used or quoted were indicated and acknowledged by complete references accurately reported.

Phelisa Cwasi **March 2022**

Signature: ……  ……..

Dedication

This work is dedicated to myself, Phelisa Cwasi. For taking a leap of faith, working hard and not giving up.

Acknowledgments

Psalm 46 vs 10

“Be still, and know that I am God” with those words I would like to thank the Almighty God for carrying me throughout this journey.

I would like to express my deepest gratitude to my supervisor Prof Martin O. Onani, this endeavour would not have been possible without your invaluable supervision, continuous support and feedbacks that helped me think critically as a scientific researcher, thereby propelling me to greater heights.

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To my mother, Thozama Cwasi, thank you for reminding me of my capabilities every time I was weary. Being a single parent is twice the work but you made it look easy, I admire your strength and resilience. I am proud to be your daughter.

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Abstract

Metal-based drugs are preferred motifs that serve as major pharmacophores in bioactive compounds for a variety of diseases, including as tuberculosis (TB). Heterocyclic, Schiff bases, aliphatic amines and other ligands are among the many potential scaffolds in drug design. To contribute to the development of these drugs, we discovered the metal-based organometallic complexes that could potentially be effective against tuberculosis, which is a common bacterial infectious disease that is caused by the bacillus mycobacterium tuberculosis (*Mtb*).

As the demand for new compounds with desired antibacterial properties has grown over the past few decades, interest in Schiff bases has also significantly increased. Thus, in this study, five Schiff base ligands namely {**HL^1** = (Z)-4-chloro-2-((phenylimino)methyl)phenol, **HL^2** = (Z)-4-chloro-2-((o-tolylimino)methyl)phenol, **HL^3** = (Z)-4-chloro-2-((p-tolylimino)methyl)phenol, **HL^4** = (Z)-4-chloro-2-(((2,4-dimethylphenyl)limino)methyl)phenol and **HL^5** = (Z)-4-chloro-2-(((2,6-dimethylphenyl)limino)methyl)phenol} were synthesized and fully characterized and complexed with $CuCl_2$ for the synthesis of copper (II) salicylaldimine compounds.

The ligands were successfully synthesized and obtained as stable intensely yellow-orange solids in good yields ranging from 70% to 89%. The ligands were insoluble in water, but soluble most common organic solvents such as methanol, chloroform and dichloromethane. The synthesized Schiff base ligands were characterized using standard spectroscopic techniques (FT-IR, UV-Vis), melting point, elemental analysis and SC-XRD. The ligands were subsequently used to prepare their corresponding Cu(II) complexes. The complexes were characterized by FTIR spectroscopy, UV-Vis spectroscopy, Cyclic Voltammetry, Elemental analysis and X-ray crystallography. The evaluation of the in-vitro antimicrobial activity against two bacteria strains namely; *SA* and *E.Coli* was done using the agar well diffusion method.

List of abbreviations and symbols

%T	Percentage of transmission
°C	degrees Celsius
^{13}C NMR	Carbon 13 nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
B. subtilis	Bacillus subtilis
C. Albicans	Candida albicans
$CDCl_3$	Deuterated chloroform
Cu	Copper
CV	Cyclic voltammetry
d	doublet
$E_{1/2}$	Half-wave potentials
E.A	Elemental analysis
E. Coli	Escherichia Coli
Ep	Peak potentials
et al.	et alia
FTIR	Fourier Transform Infrared
g	gram
Ipa	Anodic current

Ipc	Cathodic current
KBr	Potassium bromide
K. pneumonia	Klebsiella pneumonia
L	litre
LMCT	Ligand-to-metal charge-transfer
mg	milligrams
mL	millilitre
Mol	mole
mM	millimolar
M. kansasii	Mycobacterium kansasii
M. tuberculosis	Mycobacterium tuberculosis
NMR	Nuclear Magnetic Resonance
OH	Hydroxyl group
P. aeruginosa	Pseudomonas aeruginosa
ppm	parts per million
S. aureus	Staphylococcus aureus
S. epidermidis	Staphylococcus epidermidis
TMS	Tetramethylsilane
UV-Vis	Ultra-violet visible

Publications and conferences

The initial finding of this work was presented as virtual oral PowerPoint presentation entitled "*New Schiff base compounds of 5-chlorosalicylaldehyde and their coinage metal complexes.*" at the Virtual Conference on Chemistry and its Applications (VCCA-2022), theme of the virtual conference was "Resilience and Sustainable Research through Basic Sciences".

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Chapter 1

1.1 Introduction

Dr. Brian W. Pfennig describes organometallic chemistry as "the chemistry of compounds that have at least one metal-carbon bond (other than cyanide)" in his book Principles of Inorganic Chemistry. Coordination complexes from inorganic chemistry and synthetic techniques from organic chemistry are combined in organometallic chemistry. Organometallic chemistry has been interpreted in many different ways today, including bio-organometallic chemistry and catalytic chemistry. Organometallic chemistry is now widely used in the modern world, from the production of polymers, plastics, and gasoline to the creation of electronic circuits and solar panels as well as development of medical drugs[1].

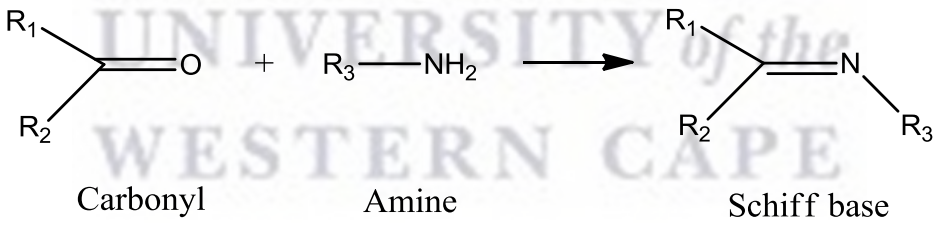
Organometallic chemistry developed out of an exchange between organic and inorganic chemistry. By combining a metal centre and an organic fragment into one molecule, the properties of both components were discovered to be significantly modified. The main-group elements from the *s* and *p* blocks of the periodic table (group 1-2 and 13-18) were the first examples to be discovered. The development of transition metal organometallic chemistry has impacted organic chemistry in a different way. Transition metal organometallics are generally catalysts whereas main-group organometallics are typically stoichiometric reagents. These catalysts improve selectivity for known reactions, but they also pave the way for the establishment of new synthetic pathways that may be used for complex molecule synthesis. Catalysts are only required in small quantities, often at a 1 mol% in relation to the reactants, but sometimes even at 1 part per million. They help promote green chemistry by preventing the waste production linked to main-group reagents[2-5].

Organometallic chemistry is a field of interdisciplinary science that is expanding at a rapid pace. The goal of organometallics chemistry research is to create transition metal complexes

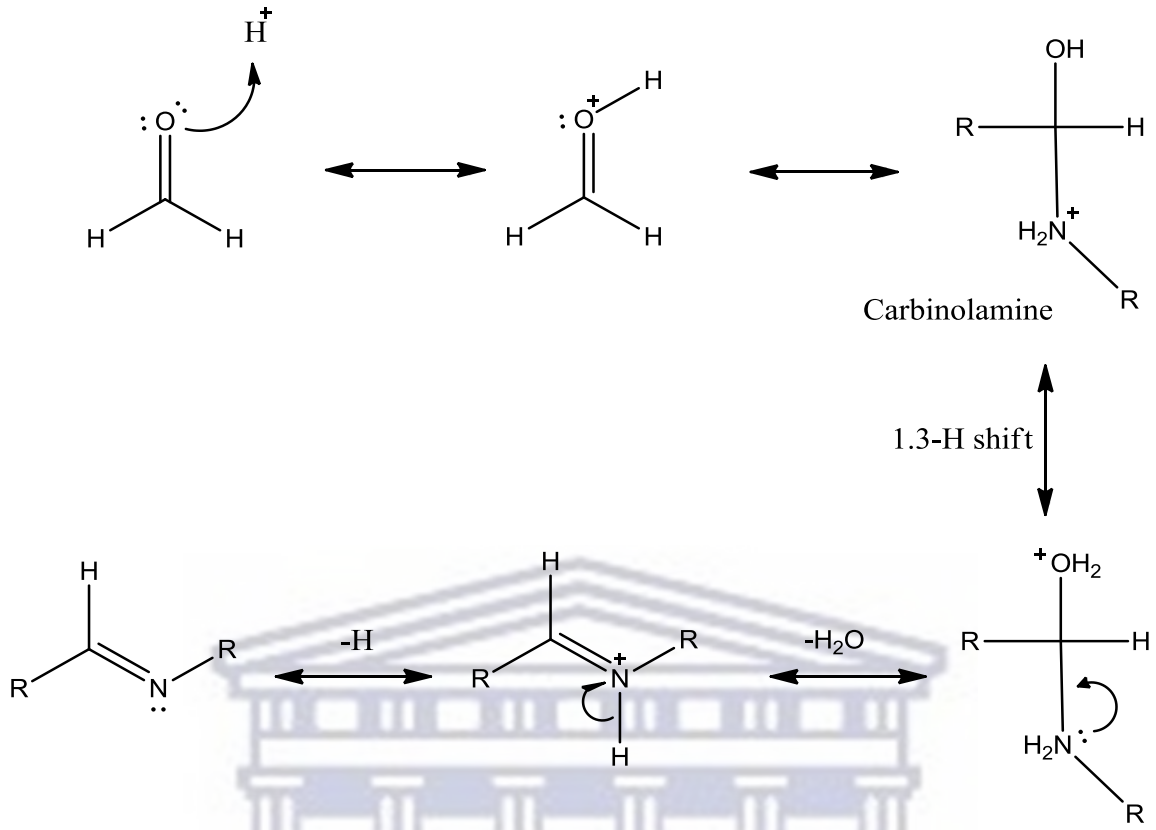
with new chemical properties, often by designing and incorporating new ancillary ligands. The most success part of organometallic chemistry is homogeneous catalysis. Over the past few years more scientists have been interested in the area of homogeneous catalysis by transition metal complexes, in order to address issues in catalysis, biological imaging and the development of novel materials[6,7].

1.2 Schiff bases

A Schiff base is a nitrogen analogue of an aldehyde or ketone in which the C=O group is replaced by C=N-R group, as displayed in Scheme 1.1 and 1.2. It is often formed by the condensation of an aldehyde or ketone with a primary amine and were first reported by Hugo Schiff in 1864[8,9]. The typical Schiff bases are crystalline solids that are weakly basic, some of which form insoluble salts with strong acids. Today, Schiff bases are used as ligands to synthesize metal complexes with a series of various structures or as intermediates in the synthesis of amino acids[10,11]. Schiff bases are also an important intermediate in several enzymatic reactions[12].



Scheme 1.1: General procedure for Schiff bases formation



Scheme 1.2: General mechanism of Schiff base formation, also one of the possible pathways for the condensation reaction of aldehydes with amines.

Due to their significant role in analytical chemistry, organic synthesis, metal refining, electroplating, and photography, Schiff base complexes have garnered a lot of interest[13]. The properties of the complexes depend on the type of metal ion. Schiff bases can act as potential binding sites for compounds with biological activity. Different transition and inner transition metals form complexes with bi, tri and tetra dentate Schiff bases containing nitrogen and oxygen donor atoms[14,15]. According to several studies, the condensation of salicylaldehyde with various primary amines and their metal complexes demonstrated potential antibacterial activity. The chelation or complexation of Schiff bases increases their antibacterial potency when tested against bacterial strain[16-19].

As the demand for new compound formulations with desired functions has grown over the past few decades, interest in Schiff bases has also significantly increased. The carbon-nitrogen

double bond in the structure of Schiff bases gives them excellent properties. Stereoisomers can change into one another because the carbon-nitrogen double bond in Schiff bases rotates more easily than the carbon-carbon double bond. The reason behind this is that the azomethine bond becomes polarized because nitrogen has a greater electronegative charge than carbon, see Figure 1.1 below [20].

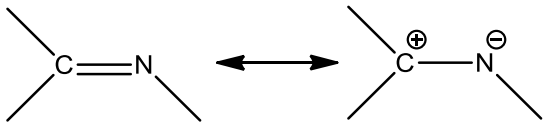


Figure 1.1: Polarization of the azomethine bond

The electron-rich centers and azomethine bond of Schiff bases allows them to be used as corrosion inhibitors for different metal-electrolyte systems because they adsorb and form a corrosion-mitigating surface film. The azomethine bond has π-acceptor properties which offers strong bonding with metallic ions [21-23].

In addition to the azomethine bond, numerous scaffolds, aromatic and substituted aromatic compounds, the presence of electron-rich species like nitrogen (N), oxygen (O), and sulfur (S) have all been found to structurally enhance the flexibility and beneficial properties of Schiff base compounds[24]. For instance, the occurrence of O- H···N and O···H-N type hydrogen bonds that undergo excited state tautomerization between enol-imine and ketoenamine, makes Schiff bases formed from salicylaldehyde derivatives with -OH groups in ortho and para positions to be of interest[25]. Additionally, the presence of nucleophile groups is crucial for chelating metal ions, which have numerous uses in the development of new drugs, catalysis, and chromatography[26].

Schiff bases have a variety of structural variations and are particularly helpful to the scientific community. The imine properties of Schiff bases have drawn the attention of several scientists worldwide due to their numerous applications. Schiff bases have attracted interest in chemistry

as dye and chelating agents, biology as antimicrobial agents, and in medicine as a model for the developing antibiotics[27]. It has been widely publicized that imines Schiff bases are effective antibacterial[28], antifungal[29], antiviral, anti-inflammatory[30], antimalarial, antiproliferative[31], and antioxidant agents[32]

1.3. Metal-based compounds in medicinal chemistry

Rosenberg's research team first reported the anticancer properties of the metal-based complex *cis*-diaminedichloroplatinum(II) over 50 years ago[33]. Since then, the vast spectrum of pharmacological properties of metal-based complexes have attracted considerable attention, including their antibacterial[34-37], anticonvulsant[38,39], anti-Alzheimer's disease[40,41], anti-diabetic[42,43], anti-inflammatory[44,45], anti-proliferative[46,47], and antitubercular activity[48,49].

Metals are important elements that are crucial to human homeostasis[37]. Transition metal complexes offer new possibilities for drug discovery that are not found for small organic molecules. Transition metal ions have a wide range of coordination geometries and oxidation states, which enables the design of several distinct complexes with either labile or inert coordinate bonds[50]. By carefully selecting the oxidation state and set of ligands, the kinetic and thermodynamic properties of metal complexes for therapeutic applications can be modulated[51-53]. The selection of suitable ligands enables the controlling of crucial physico-chemical properties such reactivity and/or substitutional inertness, hydrolytic stability, demetallization kinetics, and hydrophilicity/lipophilicity[54].

In this context, metals can serve as inert scaffolds that can assemble a group of suitable ligands into distinctive, recise and predictable 3D-shape. These metal compounds may have stereo-electronic complementarity with specific pharmalogical targets[55]. However, direct covalent binding of a labile metal complex to cellular macromolecules may results in biological effects.

This is the case with the most well-known metal-based drug, cisplatin (*cis*-[$PtCl_2(NH_3)_2$]), an anti-tumor agent[56,57]. Therefore, one of the pillars suggested by the WHO to reduce the disease in the coming years is the search for new drugs. Metal complex-based drug design approaches have shown promising results as results they have drawn attention in the past few years.

1.4 Copper (II) metal complexes

1.4.1 Brief history of copper, its uses and properties

Copper (Cu)**,** is a reddish, ductile transition metal of Group 11 (Ib) of the periodic table with atomic number 29. It is a remarkably effective heat and electrical conductor. In nature, copper can be found in its free metallic state as displayed in Figure 1.2. This native copper was first utilosed (c. 8000 BCE) as a substitute for stone by Neolithic (New Stone Age) humans. Native copper can be located as a primary mineral in basaltic lavas and also as reduced from copper compounds, such as chlorides, sulfides, carbonates and arsenides. Chalcocite, chalcopyrite, bornite, cuprite, malachite, and azurite are only a few of the minerals that include copper in combination.

Figure 1.2: A distorted crystal mass picture of copper with typical tarnish green stains. (From the Keweenaw Peninsula of Michigan by Hershel Friedman)

It is one of the most ductile metals, not particularly hard or strong. Strength and hardness are significantly increased by cold-working because of the elongated crystals of the same face-

centred cubic structure that is present in the softer annealed copper. Common gases including oxygen, nitrogen, carbon dioxide, and sulfur dioxide are soluble in molten copper and have a significant impact on the solidified metal's mechanical and electrical properties. In terms of thermal and electrical conductivity, the pure metal comes in second only to silver. Copper-63 (69.15 %) and copper-65 (6.5 %) are the two stable isotopes that make up natural copper (30.85 %)

Copper is universally involved in biological systems for a variety of processes including mitochondrial respiration, regulation of haemoglobin levels, and embryonic development[58]. As a trace element or a component of various exogenous compounds (such as aspirin, 3,5-diisopropylsalicyclic acid, copper complexes of anthranilic acid, and carboxylic acid, among others), it has significant biochemical activity in humans. It is an essential cofactor for numerous enzymes, including cytochrome C oxidase, ceruloplasmin, tyrosinase, albumin, as well as many biomolecules and nucleic acids[59,60].

Copper has historically been used as a disinfectant for drinking water, burns, sores, headaches, and itching. Hippocrates of Kos, the Greek physician commonly known as the "father of medicine", described copper as a curing agent for leg ulcers. For the treatment of venereal diseases, Celsus *et al*. documented the use of copper and different copper composites. When cholera epidemics broke out in Paris, it was discovered that the workers in copper mining had a strong immunological defense against the disease. This was the first disclosure concerning the role of copper and copper compounds in the immune system[61,62]. According to the French physician Luton, arthritic patients can benefit from using copper acetate, or salt of copper, both internally and externally to treat their condition. In 1895, the pharmacological effects of copper compounds were discovered in the treatment of cholera, chronic diarrhoea, and dysentery.

Additionally, it was shown that copper compounds deliberately speed up the healing of wounds and ulcers. It was demonstrated that the quantity of copper radioisotopes has a significant impact on radiotherapy and imaging applications[63]. Due to the potential and allure of copper and its complexes for the development of drugs in the medical sciences, researchers have focused more on studying the behind chemistry copper. However, the majority of copper's inorganic salts are toxic and can form a variety of compounds with different oxidation states, such as (I), (II), and (III). The biological activity of these is most stable in state (II), but states (I and III) are less stable while building complexes of 4, 5, and 6 coordinated species[64].

Again, the mode of action of copper is completely different from its organometallic equivalent as a drug because copper/copper ions alone cannot operate as a drug; however, it is more potent when paired with some organic moiety. New emerging study fields like nanoscience and nanotechnology are adding new information to utilizing copper and its complexes in drug delivery systems[65, 66].

1.4.2 The use of copper in medicinal studies

It is an important trace element for most organisms in all kingdoms. In humans, copper plays role as a cofactor for numerous enzymes, such as Cu/Zn-superoxide dismutase, cytochrome *c* oxidase, tyrosinase, ceruloplasmin and other proteins, crucial for respiration, iron transport and metabolism, cell growth, hemostasis. With the progress in medical sciences, copper has gained a lot of attention. The number of publications concerning copper and its compounds for potential medical applications has reached tens of thousands. Coordination chemistry of copper is well-studied and "straightforward" in comparison to many other elements. Administration of copper in a form of organometallic complexes can be done in order to selectively deliver copper ions or radionuclides to diseased tissues, or to modify pharmacokinetics and/or pharmacodynamics of ligands[67].

Hostýnek *et al.*[68] found that metallic copper can indeed penetrate skin, after being oxidized on air. Anti-inflammatory effect of Cu can be linked with modulation of prostaglandin synthesis[69-71], interleukin IL-2 expression[72], neutralization of reactive oxygen radicals by Cu/Zn-superoxide dismutase and other. Though copper deficiency is known to impair immunity, the exact mechanism is unclear[73].

In the past decade, several authors reported copper(II) complexes with potential anti-inflammatory properties. For treatment of rheumatoid arthritis, chelating agents that can facilitate transport of Cu(II) ions to sites of inflammation were researched[74].

Jackson *et al.*[75] attempted to design linear polyamine ligands that can mobilize copper in organism. The complexes cannot be too stable, because they would be quickly excreted with urine in unchanged form. Ligands formed neutral complexes only above pH 7.0 and were too labile for systemic administration, but still could be used to facilitate dermal absorption of copper. Complexes of those ligands, due to additional nitrogen atom were significantly more stable (~2 log units). Simulations showed that Cu complexes of the ligands are stable in blood plasma, and effectively mobilize copper ions without affecting significantly other metal ions levels[76]. Odisitse *et al.*[77,78] also reported dermally absorbed complexes of copper with ligands. The compounds showed approximately 24 h biological half-life which is desired for potential anti-inflammatory drugs.

Copper, both in metallic form and in many chemical compounds possess antimicrobial activity, which was already used by ancients. Cupric ions exhibit non-specific biocidal activity, although weaker than silver. Copper-silver electrolytic ionization systems are used in many hospitals to decrease number of *Legionella* residing in hot water pipes. Metals and alloys used in orthopedic implants can be doped with copper ions, in order to reduce risk of infection after prosthetic surgery. The trade-off is reduced to some extent corrosion resistance of the resulting

materials, but still on a reasonable level[79]. Due to non-specific toxicity, for the use of copper as an antibacterial therapeutic, the metal should be administered in a form of complex compounds, rather than simple inorganic salts. Nature of chelating agent, however, plays very important role, as there can be no simple correlation between antibacterial activity and complex stability[80].

Many various Cu(II) complexes with different ligands were reported to possess antibacterial and antifungal activity[81-83]. Singh *et al.*[84] utilized an approach to use ligands which already have antimicrobial activity and enhance it by complexation with copper. Antihypertensive drug pindolol, when complexed with Cu (complex stability constant log β = 11.28 in water-dioxan 40:60 at 25 °C), exhibits notable antimicrobial activity towards some bacterial and fungal strains[85]. Water soluble, polymeric complex shows good antimicrobial activity and is also capable of binding DNA[86].

1.4.3 The use of copper in biological systems

In humans, copper exhibits significant biochemical activity as either an essential trace metal or as a component of different exogenously administered compounds. In its first role it is bound to ceruloplasmin, albumin, and other proteins, while in its second function it is coupled to ligands of different sorts and forms complexes that interact with biomolecules, primarily proteins and nucleic acids. Numerous studies have shown how copper plays a variety of roles in biological systems. Particularly, to describe the involvement of copper in human diseases from a medicinal-chemical[87] and biochemical[88] view, concentrating on the molecular physiology of Cu transport[89].

Copper homeostasis[90], its relationship to iron metabolism[91], and its role in biological processes relevant to human physiology and pathology[92,93] are among the topics that receive a lot of attention in current research. While many of the functions that have been proposed to explain

the homeostasis of inorganic non-complexed copper in humans have been described, only a limited number of review studies have concentrated on the multiple biochemical processes that may be directly related to the use of copper complexes in medicine.

1.4.4 Properties of Copper (II) metal complexes

Among the transition metals, copper is an important and much researched metal because, after iron and zinc, it is the 3rd most abundant metallic element in the human body[94]. It is essential for the growth, development, and maintenance of bones, the brain, connective tissues, the heart, and many other bodily organs. It also plays a critical role in the activity of several enzymes that catalyze a wide range of reactions. Copper has a remarkable ability of forming complexes or chelates with a variety of organic substances. Copper Schiff base complexes are widely used in the food business, the dye industry, fungicidal, analytical chemistry, anti-inflammable activity, catalysis, agrochemical and anti-radical. In biological systems such complexes help with transportation and absorption. Also many physiological processes depend heavily on copper presence[95].

Cu(II) complexes are of great interest to inorganic chemists because they are commonly air and moisture stable, have informative and simple spectroscopic signatures[94-97], and perform effectively under solution reactions immediately after mixing[94]. In comparison to other transition ions, the Cu(II) complexes exhibit more stereochemical flexibility by adopting a larger variety of coordination geometries. This is because the d^9 electronic configuration of Cu(II) ion is Jahn-Teller active and one unpaired electron occupying the d-orbital[98,99]. Due to the d^9 configuration, Cu(II) complexes exhibit distorted octahedral and tetrahedral symmetries. Usually, the distortion is interpreted as an axial elongation in relation with the complex geometric flexibility. Therefore, most Cu(II) complexes have square planar or square pyramidal geometries with weakly bonded ligands in the axial position (s), but some have

trigonal bipyramidal geometry[100]. These complexes are important in the medical field for treating many diseases including cancer[101].

Since their publication[102], several Cu(II) complexes have been shown to exhibit a variety of biological properties with potential therapeutic uses. The practical use of these complexes depends on the right combination of their kinetic and thermodynamic properties, number of coordinated ligands, oxidation state of the central atom and the type of coordination polyhedron they form. In recent years, complexes containing Schiff bases synthesized from salicylaldehyde and different amino acids have drawn the most attention when it comes to Cu(II) complexes. These Cu(II) complexes have been studied due to the antimicrobial[103-107] and radio-protective properties that were discovered[108]. The vast amount of information available for their bioinorganic properties and mode of action in numerous biological systems combined with new opportunities from the technologies of medical chemistry, is creating an exciting invention for the development of new highly active drugs with minimized side effects. This could significantly advance the current clinical research and practice.

1.5 Tuberculosis as a global health issue

Bacteria are single cell prokaryotic microorganisms that carry their genetic material in double-stranded circular DNA molecule. Some bacteria species have small circular DNA plasmids. Their ribosomes are found in the cell cytoplasm, and all species except Mycoplasma have both cell membrane and a complex cell wall (see Figure 1.2). Typically, binary fission is how bacteria multiply. Under proper conditions, some bacteria have the ability to divide and multiply at a faster rate.

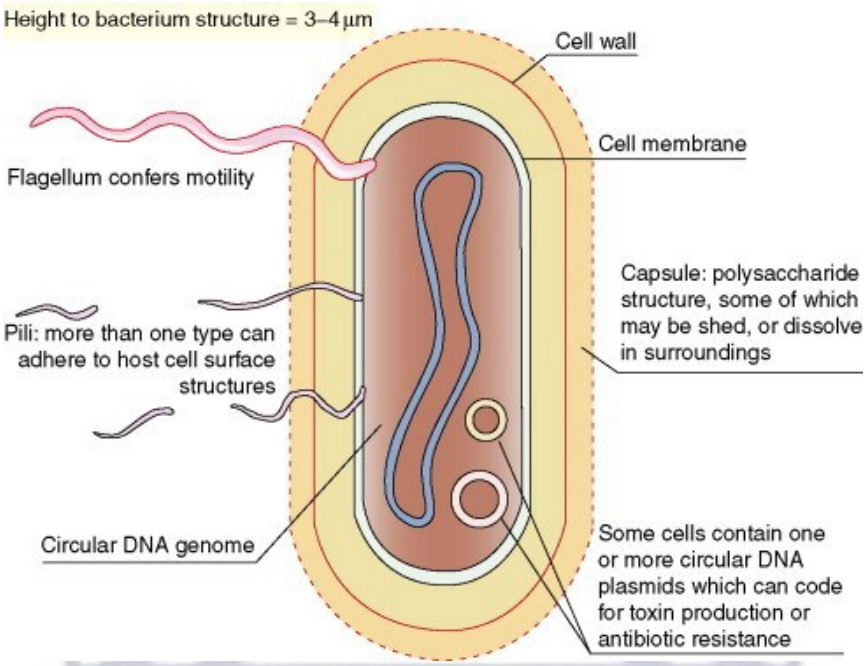


Figure 1.3: Structure of a bacterium[109].

However, some bacteria are infectious and can spread diseases to plants, animaly and human beings[110]. Typical bacterial infections include tooth decay, pneumonia, diabetes, chronic inflammation, bloodstream infections (sepsis), sexually transmitted diseases (gonorrhoea), and lung infections (tuberculosis). Tuberculosis is one of the most common bacterial infectious diseases that causes death worldwide[111-114].

The bacillus Mycobacterium tuberculosis is the source of the infectious disease tuberculosis (TB). Robert Koch made the discovery of the tubercle bacillus in 1882. Since then, tuberculosis has been known as a chronic granulomatous infectious disease. Infection spreads via respiratory route, when people with active TB spit, talk, sneeze, or cough, releasing droplets containing *M. Tuberculosis* bacilli into the air. People close by may become infected by inhaling the live bacteria. It usually affects the lungs in 80% of cases with symptoms like chest pain, coughing, shortness of breath, fever, weight loss, and night sweats.

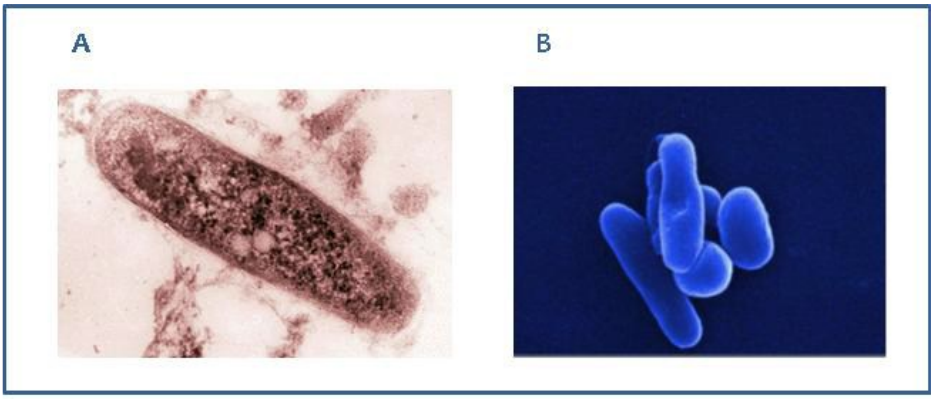


Figure 1.4: Morphological variations in *M. tuberculosis*. (A). Thin section transmission electron micrograph of *Mtb*. (B). Scanning electron microscope shows shape variation in *Mtb* at exponential phase of growth[115].

If an individual with active disease is not treated on time, they are most likely to typically infect 10 to 15 people on average, annually. TB, is liable for 2.5% of the global burden of disease and is the frequent cause of death in most young women, claiming more lives than the combined causes of maternal mortality. Due to their underdeveloped and deteriorating immune systems, babies and the elderly are more vulnerable; yet some people develop active TB disease. HIV co-infection and poverty have made it challenging to combat the disease.[116-117].

The primary issue with HIV-TB co-infection is that HIV interferes with the immune system, activating the LTBI and accelerating the progression of the disease. Additionally, TB also speeds up the progression and effects of HIV. On a global scale of death causes, it is now holding the seventh place. Therefore, understanding TB disease, its diagnosis, and therapy is crucial for management and outcomes. Even if TB services are accessible, what happens next depends on the health staff's response. It is necessary to address the information gaps surrounding care seeking and the inappropriate actions of care providers while interacting with probable TB cases to improve case notification[118].

The main issue in South Africa is the inadequacy of TB control tools for identifying isoniazid resistance, the first acquired resistance mutation, since the available molecular diagnosis tools only evaluate rifampicin resistance. In South Africa, instances of drug-resistant strains can be traced as far back as the 1980s (XDR) and late 1950s (MDR)[119]. According to a study done in the province of KwaZulu-Natal by Gandhi *et al.*[120], the F15/ LAM4/KZN strain type predominated among MDR TB and XDR TB cases, resulting in nosocomial transmission and increased mortality rates[121,122].

1.5.2 Tuberculosis in South Africa

In Africa, the COVID-19 epidemic is unfolding against a backdrop of the long-running TB and HIV epidemics. For both diseases, South Africa is one of the most critically impacted countries in the world, ranking the fourth highest rate of HIV-TB co-infection. Despite having 0.7% of the world's population, it also liable for about 20% (7.7 ~ 7.9 million people) of the global burden of HIV. Since 2010, South Africa has steadily improved its ability to manage both diseases.

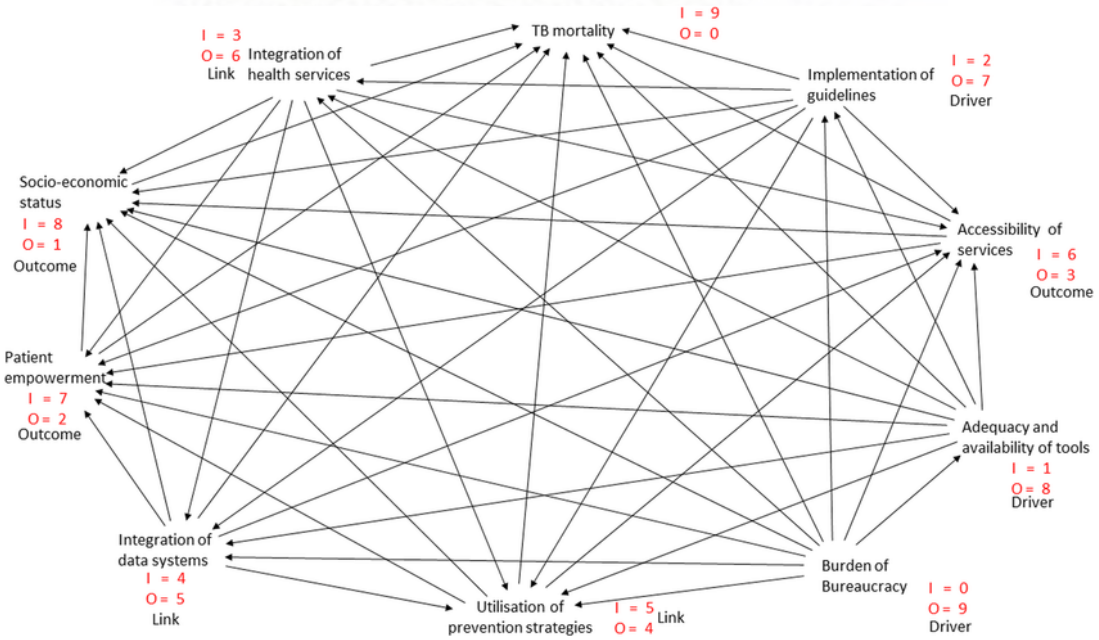


Figure 1.5: Interrelationship diagraph of key variables underlying TB mortality in South Africa, depicting drivers, links and outcomes. I: in; O: out; TB: tuberculosis[123].

In South Africa, TB is still one of the top causes of death. Annually, it causes more deaths than the COVID-19 pandemic had so far. The key variables that lead to high rates of mortality in South Africa with their driving factors, links and outcomes are displayed in Figure 1.4. The South African government launched a nationwide lockdown on March 27, 2020, by then the number of cases reported were 402, and the number of cases was doubling every two days. This was shortly after instituting COVID-19 mitigating measures, such as prohibiting air travel and closing schools. This severe measure, which aimed to stop viral transmission by limiting people's interactions and movement, had several unforeseen effects on the delivery of healthcare services for other prevalent conditions, particularly the prevention and treatment of HIV and TB[124].

An exploratory study was conducted by Lieve Vanleeuw *et al.* using qualitative interviews to focus on the lived experiences and perceptions of people with TB during the COVID-19 pandemic in South Africa, notably in the Western Cape. The study investigated how TB patients dealt with the COVID-19 pandemic and the resulting socio-economic effects, as well how it affected their health, income, and access to social support. At a health facility is Cape Town, 15 TB patients were interviewed, and data was analysed thematically. Findings showed TB patients to be the most vulnerable due to the high exposure and sensitivity to the COVID-19 shock, and their ability to cope was also impaired. In many households, the loss of income led to both an increase in food insecurity and a decreased capacity to provide for others. The lack of social support forced most vulnerable people to survive without food, which had a negative impact on their ability to continue treatment.

The time when the study was conducted, the Western Cape Province had a high COVID-19 and TB burden. In 2018 and 2017, the province had the second-highest number of confirmed drug-sensitive TB (DS-TB) cases and the third-highest number of confirmed drug-resistant TB (DR-TB) cases among the nine provinces in South Africa. The Cape Town Metropolitan

Municipality, which houses the study site, had the most confirmed DS-TB patients and the second-most confirmed MDR-TB cases of all 52 South African districts in 2018 and 2017, respectively[125]. The Western Cape recorded cumulative cases by 20 March 2021, the third-highest number nationwide at the time of the study [126].

The study therefore indicated that TB patients faced increased vulnerability due to the consequences of the COVID-19 response and proved that TB persists being a serious public health concern. Therefore, there is an urgent need for the development of new potent drug therapies aimed at combating resistant *Mtb* strains and which can also be co-administered with HIV antiretroviral drugs.

1.6 Problem statement

Diseases caused by bacteria have been a huge problem to manage before the human civilisation and still continue being a major global problem to date. The antimicrobial drugs that have been used so far to manage the diseases give many setbacks, including non-selectivity and bacterial development of resistance. Thus, there is a need for further research of potential antibacterial drugs. The challenges are to create drug candidates with a broad spectrum of antibacterial activities, particularly those that are effective in both gram-negative and gram-positive bacteria, and for them to be effective at low dosage concentrations.

Schiff bases have attracted a lot of attention in recent years because their desirable qualities, such as efficacy against bacteria and fungi[127-129]. Additionally, they have been widely used in metal coordination which is applied in various biochemical substrates[130-132]. Copper ions have been proven in literature that they form strong bonds with N-donor ligands due to their strong back bonding[133-136]. Bio-essential and Cu(II) complexes have been considered to have the highest activity in a variety of applications among transition metal complexes[137-139].

Surprisingly, despite the attention about biological properties of copper, not a single copper-based antibacterial drug has been approved and documented[140].

Thus, the aforesaid reasons mentioned are the motive for the synthesis of Cu(II) Schiff base complexes in this study. The positions of the substituent(s) on the aromatic ring of ligands were different so as to determine if they have any effect on antimicrobial activity of the complexes. Also, for the treatment of tuberculosis, there is still a gap in research of potential drugs developed from the first line drugs using the Schiff base condensation reaction. Therefore, in this study the synthesized Cu(II) Schiff base complexes and attempted to synthesize ligands of ofloxacin with pyrazinamide the with isoniazid, complex the ligands with $CuCl_2$, Ag_2O and $AuClPPh_3$.

1.7 Aims and objectives.

The primary aims of the project were:

1. To synthesize, characterize and investigate biological activities of copper (II) salicylaldehyde Schiff base complexes.
2. To investigate the potency of new compounds by which are a combination of either ofloxacin with pyrazinamide or isoniazid via Schiff base reaction.

The primary aims of the project were achieved by following the specific objectives of the study, which were to:

1. Synthesize Schiff base ligands using aniline derivatives with 5-chlosalicyladehyde.
2. Determine the yield, melting point and solubility of the ligands.
3. Characterize the ligands in the order: FTIR, UV-Vis, ^{1}H NMR, ^{13}C NMR, elemental analysis.
4. Synthesize Cu(II) Schiff base complexes using $CuCl_2$ metal salt.
5. Determine the yield, melting point and solubility of the complexes.

6. Characterize the Cu(II) complexes: FTIR, UV-Vis, CV and evaluate their antimicrobial activities using agar well diffusion and MIC.

1.8 Outline of thesis

Chapter 1

This chapter introduces organometallics chemistry and Schiff base compounds in presence of coordination chemistry. A brief explanation about the basicity character of Schiff bases, the potential uses of Schiff bases and medicinal chemistry of their complexes. Since copper is the metal used for the formation of complexes in this study, in this chapter a brief history and biological revolution of copper is explained. Additionally, copper(II) metal complexes properties which were obtained from previous studies were explained. This chapter also contains general information about bacteria roles, tuberculosis, the global effects, and the statistics specifically in South Africa. Followed by the problem statement which is the motivation for the study, aims and objectives to justify the logic behind the study are stated.

Chapter 2

This chapter is a review of the studies previously done to tackle the challenges of biological problems in disease management. It covers an overview chemistry nature of salicylaldehyde ligands and their corresponding complexes with their antimicrobial activity based on previous studies. Subsequently, the study rationale is stated.

Chapter 3

This chapter contains general experimental methods and materials used to synthesize and characterize the Schiff base ligands and complexes, namely, melting point, FTIR spectroscopy,

^{1}H and ^{13}C NMR, UV-Vis spectroscopy, elemental analysis, and cyclic voltammetry. Also, the methods and materials used for the biological studies of the complexes.

Chapter 4

This chapter reports results and discussions on the synthesis and characterisation of all synthesised compounds. The conclusion is drawn based on the results obtained in correlation to the structures, comparing them to previous successful studies of similar study. Followed by the discussion of the results obtained from biological studies of the tested compounds.

Chapter 5

This chapter summarizes the results obtained and draws conclusion based on study rationale. The chapter ends with future work and recommendations, which are clearly stated for study continuation.

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Chapter 2

Literature review

2.1 Introduction

Since ancient time, mankind has been searching for novel, effective original compounds to treat diseases [1]. Harvey noted that although natural products have been historically known to be a rich source of therapeutic molecules, their lack of accessibility and supply, complex chemistry, and inherently slow processing have led to minimal use of natural products over the years in industry [2]. Developments in the synthesis field are expected to continue as long as synthetic compounds hold the upper hand in addressing the demand of the highly competitive pharmaceutical industry to adapt to the current state-of-the-art advances in science and technology [1,3,4].

The most popular elective metal-based anticancer drug on the market is the platinum drug cisplatin, which was developed clinically in 1971 and licensed by the Food and Drug Administration (FDA) in late 1978 [5,6]. Since cisplatin and its analogs have been so effective at treating cancer, there has been a lot of research done in the past few decades to find alternative metal-based chemotherapeutic drugs [7,8]. The rationale for these studies is justified by the possibility that metal centres other than platinum might provide new opportunities for the development of clinically effective drugs [9]. Furthermore, there is an urgent need to discover and characterize new drugs with novel mechanisms of action, enhanced activity, improved selectivity and bioavailability to combat the serious problem of multidrug resistance in the treatment of bacterial infections. In view of this, salicylaldehyde Schiff bases and their corresponding metal complexes with potentially biological activities and coordination chemistry are attractive candidates for consideration.

2.2 Biological application of approved drugs

The discovery of antibiotics marked a significant turning point in pharmaceutical therapy by opening up new avenues the treatment of diseases. Drug development is a time-consuming and expensive process that requires evaluative biological studies before a drug candidate can be approved for commercialization. It starts with fundamental synthetic and evaluative (in vitro to in vivo) research in the academic field and pharmaceutical industry. The process normally takes time and requires a huge amount of funding, however, the pathogenic bacterial development and the diseases it causes are usually complex and fast-paced. This makes it challenging to effectively manage such diseases and increases the demand for new drugs that are potent with minimum side effects. Table 2.1 below shows a few of the antibacterial FDA approved drugs in the years 2017-2022 [10].

Table 2.1: Antibacterial drugs approved by the FDA from 2017 to 2019

Drug	Indication	Company (year)
Fetroja (cefiderocol)	Antibacterial drug for treatment of patients 18 years of age or older with complicated urinary tract infections (cUTI), including pylelonehritis caused by susceptible gram-negative microorganisms: *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae* complex.	Shionogi & Co., Ltd. (14 November, 2019)
Delafloxacin (Baxdela)	A fluoroquinolone for treatment of acute bacterial skin and skin structure infections, in adults. It is active against Gram-positive pathogens (*Staph. aureus, Staph. haemolyticus, Strep. agalactiae Strep. anginosus group and Enterococcus faecalis*) and some Gram-negative bacteria (*Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa*)	Melinta Therapeutics (19 June, 2017)
Eravacycline dihydrochloride (Xerava)	A tetracycline class antibacterial indicated for the treatment of complicated intra-abdominal infections in patients 18 years of age and older.	Tetraphase Pharmaceuticals, Inc. (27 August, 2018)
Xenleta (lefamulin)	A first-in-class, semi-synthetic pleuromutilin antibiotic for the treatment of community-acquired bacterial pneumonia (CABP).	Nabriva Therapeutics plc. (19 August, 2019)
Pretomanid (PA)	A nitroimidazooxazine antimycobacterial indicated for use in combination with bedaquiline and linezolid (the BPaL regimen) for the treatment of adults with pulmonary extensively drug resistant (XDR), treatment-intolerant or nonresponsive multidrug-resistant (MDR) tuberculosis (TB).	TB Alliance (14 August, 2019)
Fetroja (cefiderocol)	A siderophore cephalosporin indicated for the treatment of complicated urinary tract infections (cUTI), hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia (HABP/VABP) caused by susceptible Gram-negative microorganisms.	Shionogi Inc. (14 November, 2019)

2.2.1 Schiff bases as antibacterial agents

In the past decades, there has been growing interest on the binding ability of micromolecules such as Schiff bases to DNA. Coordination chemistry is enriched by Schiff bases since they have attracted most attention over the years as their metal complexes are mostly researched coordination compounds [11]. The significance of Schiff bases as biochemical and analytical reagents is also being documented frequently [12].

Several new compounds have been developed as a result of numerous reports on Schiff bases, some of which have biological significance. The HC=N linkage of Schiff bases, which is important for their antibacterial, antifungal, antioxidant, anticancer, and diuretic effects, made them to be referred as "fortunate ligands" due to the ease with which they are designed and prepared [12 - 14]. Schiff bases with different donor atoms such as N,O, and S have broad biological applications and they draw most attention due to their stability and ability to bind to metal ions [15 - 17].

Developing antibiotics against gram-positive and gram-negative bacteria such as *Staphylococcus epidermidis (S.E), Staphylococcus aureus (S.A), Pseudomonas aeruginosa (P.A), and Klebsiella pneumonia (K.P)* was challenging but their discovery paved the way for successful modern science achievements by reducing infectious bacterial populations [18]. Khan *et al* reported the side effects of the most widely used antibiotics for bacterial infections which are amoxicillin, chloramphenicol, norfloxacin, ciprofloxacin. They cause neurological changes brought on by the interaction between the drug and the central nervous system [19]. Thus, it is necessary that more research is done for new alternative antibiotics or drugs for bacterial infections that have relatively less side effects.

2.2.2 Schiff bases antibacterial agents in use.

Schiff bases derived from heterocyclic rings offer several benefits in the development of new compounds with biologically active characteristics. The most common heteroatoms found in heterocyclic compounds are usually nitrogen, oxygen, and sulphur, which is advantageous in their use as antimicrobial agents in biochemical pathways. Among the different types of heterocycle molecules that are under study [20,21], compounds that contain nitrogen such as benzimidazole and its derivatives represent important chromophores groups that possess desired biological properties [22,23]. According to Bansal *et al*, benzimidazole is a bicyclic heterocycle with a wide range of pharmacological activities [24]. The importance of benzimidazole nuclei as pharmacophores has attracted attention in drug discovery research [25].

Triazole and pyridine have been reported to be potent due to the presence of their multifunctional groups, which contributes to their diverse range of activities[26,27]. Consequently, the triazole derivatives are used for a wide range of biological applications because of their biochemical significance in the stability of protein binding. Holland-Nell *et al*. showed and described protein-triazole linkage can increase the stability of disulphide-linked peptides [28]. Hence, they are also used as pesticides approved drugs. The improvement of the peptide structure is significant for the development of new antibacterial potential compounds.

Triazole compounds have been studied for their potential as antibacterial agents because of their antibacterial properties. Angajala *et al*. studied triazole derivatives and the effect of their substituents on antibacterial activities [29]. In general, the antibacterial activity of triazole compounds with electron-withdrawing groups, such as NO_2 or Cl, showed good antibacterial activity than triazole compounds with electron-donating groups as substituents [30]. Triazole compounds with electron-withdrawing group substituted in the *meta* position showed average activity, contrarily, benzyl or phenyl rings, see Figure 2.1, substituted in the *para* position

showed high bactericidal activity. Furthermore, it was found that triazole compounds showed high activity towards gram-positive bacteria, specifically, *Methicillin-resistant Staphylococcus aureus* (MRSA) bacteria [29].

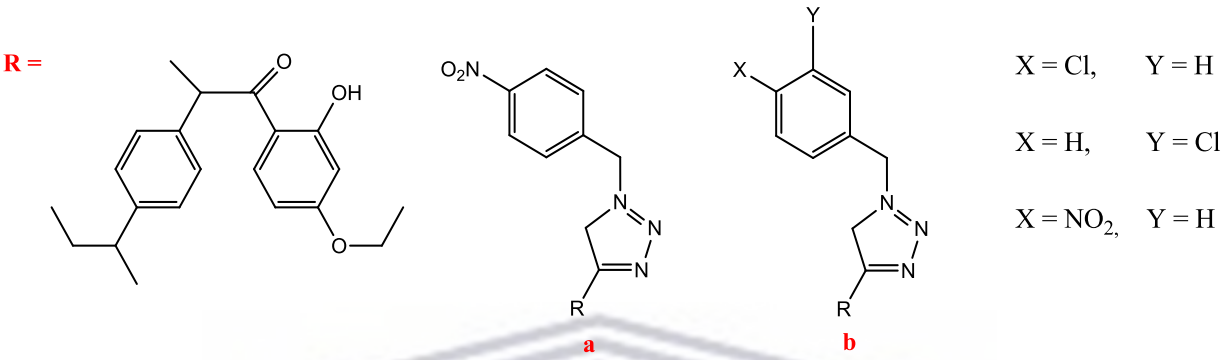


Figure 2.1: (R) primary backbone, (a) and (b) triazole antibacterial active compounds

Singh *et al*. studied Schiff bases complexes of zinc, cobalt, copper and nickel containing the triazole moiety [31]. The metal complexes showed a wide spectrum of antibacterial activity against *Bacillus Subtilis*, *S. aureus*, *Pseudomonas aeruginosa* and *E. coli*. The activity of the complexes against the bacteria tested was then explained based on the reduced polarity of the metal centre. In comparison to other metal complexes, Zn(II) complexes exhibited high activity with wide inhibition zones against the bacteria tested [31]. Comparatively, complex shown in Figure 2.2a with single bidentate ligand exhibited better activity compared to complex displayed in Figure 2.2b. Based on the results obtained, it was then concluded that there are no significant biological effects from the introduction of methyl substitution.

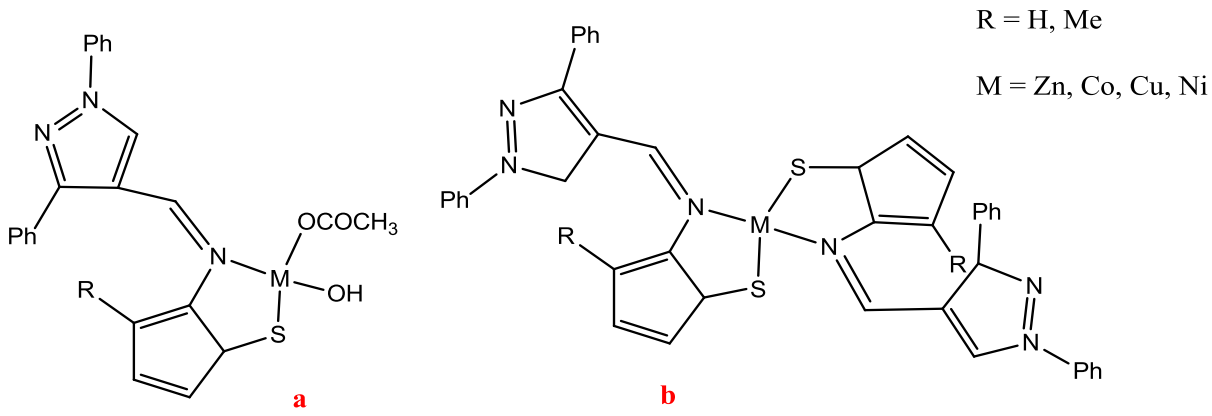


Figure 2.2: Schiff base complexes with triazole moiety of zinc, cobalt, copper and nickel as metal centres

2.3 Schiff base ligands classes, chelation, and their biological functions

Generally, Schiff bases are classified into four classes namely; bidentate (1), tridentate (2), tetradentate (3) or polydentate (4,5) ligands as displayed in Figure 2.3, which are capable of forming stable complexes with transition metal ions. The resulting Schiff bases can act as mixed-donor ligands and take part in bi-, tri-, tetra-, and higher coordination modes if they also contain additional functional groups like -OH, -NH_2, or –SH. As a result, the pharmacological activity of an organic scaffold that is pharmacologically active is altered by the choice of metals. Literature indicates that the nature of metal affects the pharmacological activity of compounds [32].

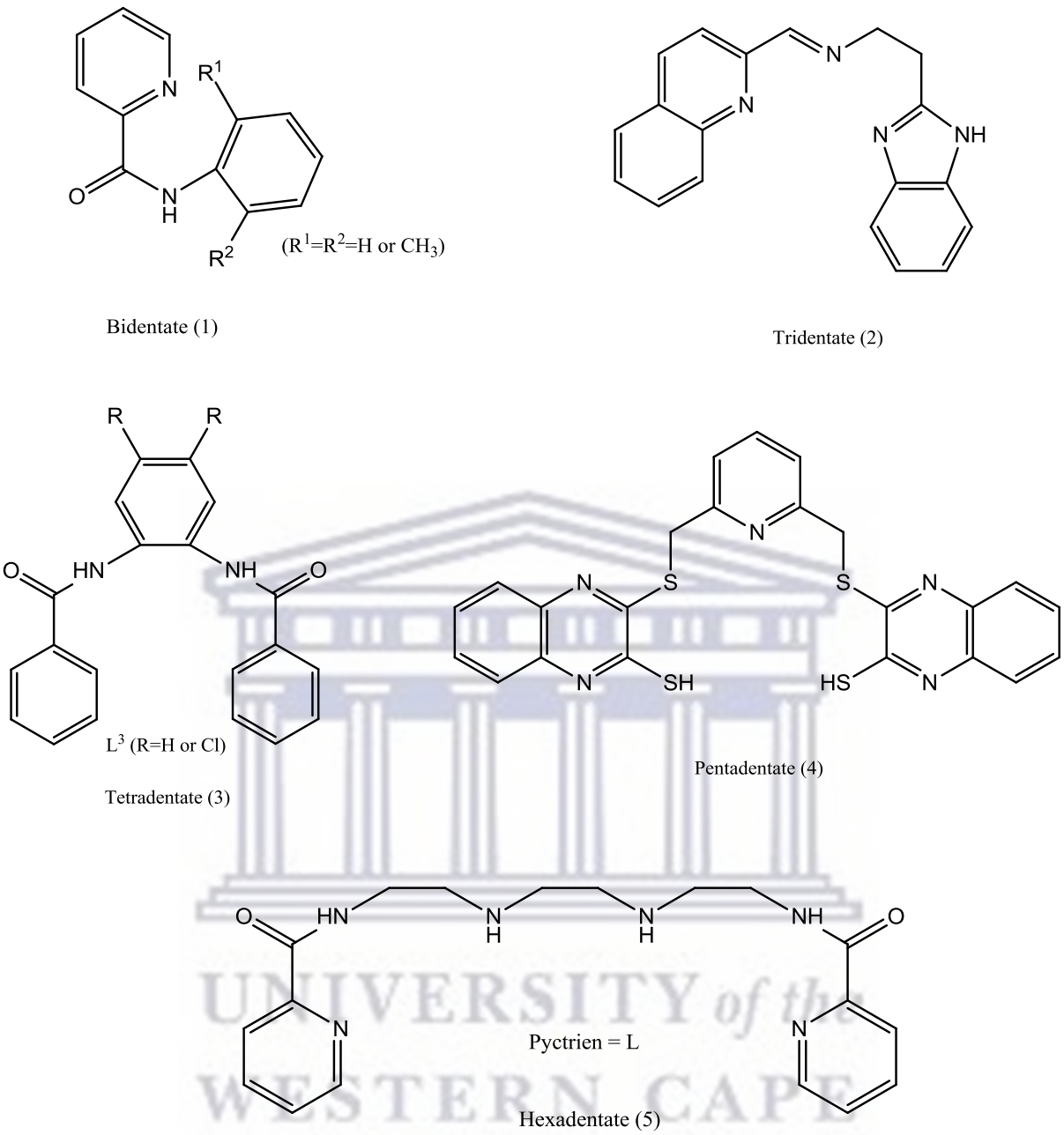


Figure 2.3: Schiff base ligands classes

Chelation and its relationship with various biological functions provides a foundation for innovative therapeutic methodologies that can be used to combat the global problem of bacterial and fungal resistance. The best way to make the organic moiety more lipophilic is

through metal chelation. In fact, ligands may enhance their bioactivity profiles by coordination, whereas some inactive ligands may acquire pharmacological characteristics. As a result, they have developed into a significant class of structure-selective binding agents for nucleic acids. The chelating structure of Schiff bases is one of the many reasons why they are in demand, and they are versatile since they can be easily prepared, are moderate electron donors with controllable electronic and steric effects. Their derivatives are one of the few classes of biologically active agents that have been thoroughly studied for the development of new potent agents that could be used by chemists for the synthesis purposes [33]. These ligands have drawn a lot of attention because of their diverse range of applications.

2.4 Significant Nature of salicylaldehyde Schiff base Ligands

Salicylaldehyde (Figure 2.4) is a key precursor to several chelating agents, some of which have significant economic value. It is a common highly functionalized arene that has frequently been used as a precursor to other chemicals. In Schiff base reactions, condensation of salicylaldehyde with amines results in chelating ligands such as salen when condensing with ethylenediamine and salicylaldoxime from condensing with hydroxylamine. Salicylaldehyde aldol condensation with diethyl malonate produces heterocycle coumarin derivative [34,35].

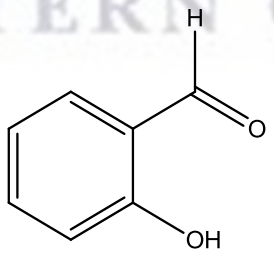


Figure 2.4: Salicylaldehyde molecular structure

Salicylaldehyde and its derivatives are used as carbonyl precursors for the synthesis of a wide range of Schiff bases. A few examples are their reactions with monoamine, diamines that have two primary amino groups, diamines that have one primary and one tertiary amino group of

which in this case it leads to tridentate (NNO) Schiff bases [36-38]. Additional coordinating groups attached to salicylaldehyde enhance the denticity of the resultant Schiff bases, their versatility and capacity to produce polynuclear complexes. An illustration of such a salicylaldehyde derivative is 3-methoxysalicylaldehyde (o-vanilin) (Figure 2.5), which was commonly used for the synthesis compartmental ligands [39,40].

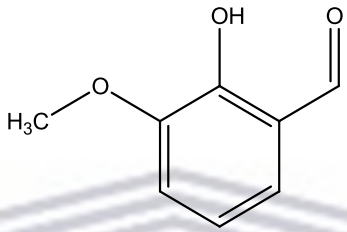


Figure 2.5: 3-methoxysalicylaldehyde, a salicylaldehyde derivative

2.4.1 Schiff bases containing a salicylaldehyde moiety.

Substituted salicylaldehyde Schiff bases are widely known antimicrobial agents existing in "free" form or as ligands used for metal complexes[41-44]. In Figure 2.6, a display of the rational design of novel of Schiff bases of sulfadiazine with salicylaldehyde is shown. Schiff bases of different sulphonamides have been reported to have similar biological effects [41,45,46]. Ameen *et al*. reported that contrimoxazole, sulfamethoxazike and sulfadiazine showed activity against Mycobacterium tuberculosis and non-tuberculosis mycobacteria after administration at clinically approved concentrations [47,48]. El-Baradie investigated the antibacterial activity of sulfadiazine Schiff base with unsubstituted salicylaldehyde and found that it had inhibitory activity for both gram-positive and gram-negative bacteria [49]. However, when Chohan *et al.* used 5-bromosalicylaldehyde the Schiff bases exhibited a wide range of antibacterial and antifungal properties [50].

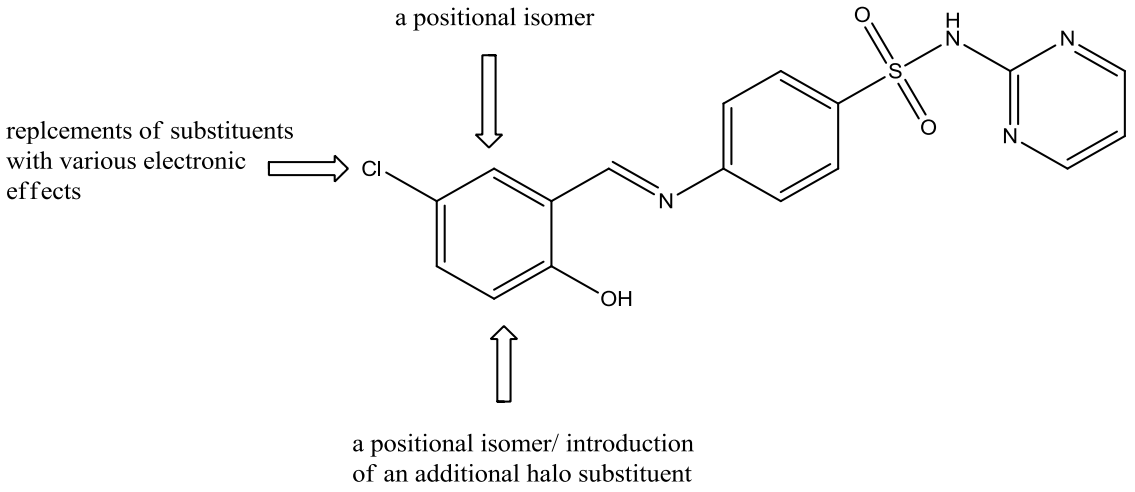


Figure 2.6: A rational design of novel of Schiff bases of sulfadiazine with salicylaldehyde.

Krátký *et al*. designed and prepared derivatives of sulfonamides based on using Schiff bases derived from 5-chlorosalicylaldehyde and amides from 5-chlorosalicylic acid. The compounds were all evaluated in vitro their antimicrobial activity against eight bacterial strains namely, *Methicillin-resistant S. aureus* (MRSA), *S. aureus*, *Enterrococcus sp*., *S. epidermis*, *K. pneumonia*, *E. coli*, *P. aeruginosa* and ESBL-positive *K. pneumonia*. The sulfonamides Schiff bases exhibited activity towards gram-positive bacterial strains *S. aureus* and MRSA. The MIC values of the bacterial strains were 31.25-500 µmol/L for *S. aureus* and 15.62-250 µmol/L for MRSA. It was interestingly observed that the activities of derivatives were slightly better on the MRSA strain than on methicillin-sensitive one. Based on the MIC values, *S. aureus* appeared to be the most bactericidal in comparison to the activity of other bacteria strains. It was also observed that *M. kansasii*, both collection and clinical isolated types, was the most susceptible species (MIC ≥1 µmol/L). The four gram-negative strains namely, *E. coli*, *P. aeruginosa* and two strains of *K. pneumoniae*, exhibited complete insensitivity with MIC values ≥500 µmol/L. In general, the addition of 5-chlosalicylaldehyde and 5-chlorosalicylic acid scaffolds enhanced antibacterial activity [51].

Shi *et al*. synthesized series of Schiff bases of 5-chlorosalicylaldehyde with different primary amines, namely, 2-ethylfuran, ethylpyridine, methylcyclopentane, 1-ethyl-4-fluorobenzene, ethylcyclohexane, 1-propylpiperazine, 4-bty. lmorpholine, 1-benzyl-4-methylpiperidine. The study mainly focused on the structure-activity relationships. All the prepared Schiff base compounds were tested for antibacterial activity against gram positive bacteria strains; S. *aureus, B. subtilis,* and gram-negative bacteria strains; *E. coli* and *pseudomonas* fluorescence. It was observed that the antibacterial activity of the compounds decreased with increased complexity of the cyclohexyl-like subset. The results obtained also implied that the presence of heteroatoms such as oxygen and nitrogen was beneficial for the activity of the compounds. One of the compounds with cyclopentyl ring had high activity in comparison with others which indicated that basic, smaller alicyclic ring compounds have the potential to exhibit high antibacterial activity [52].

2.4.2 Salicylaldehyde Schiff base complexes

Aldehydes have been used as ligands for quite a long period of time, and transition metal complexes synthesis with aldehyde groups are of significance importance, since the aldehyde group can undergo different types of chemical reactions [53]. An interesting example to use is salicylaldehyde because the hydrogen bonding between the hydroxyl group and the aldehyde prevents movement between the two groups [54]. Salicylaldehyde has been well known for forming strong intra-molecular hydrogen bonds that start between the hydroxyl of the phenolic group and the oxygen of the carbonyl group[55]. Studies have proven that coordination of substituted salicylaldehyde on a metal provides a wide spectrum of biological activities such as antimicrobial activity, DNA interaction, cytotoxicity, albumin binding and radical scavenging ability [56-66].

Athawale and Nerkar investigated the stability constants of complexes corresponding to a series of substituted salicylaldehyde Schiff bases and discovered electron-withdrawing substituents enhances the stability of complexes [67]. Sarì and Gürkan focused on the effect of electron-donating groups, they determined the stability constants using three salicylaldehyde ligands with the same metal ion and found that electron-donating groups increased the basicity of the donors, subsequently increasing the coordination of the ligands [68]. El-Sherif and Aljahdali noted that the order of the transition metal complexes order of stability constants was consistent with the alkalinity order of the ligands [69]. In comparison with other Schiff bases ligands, coordination compounds derived from salicylaldehyde are the most extensively researched compounds [70-72].

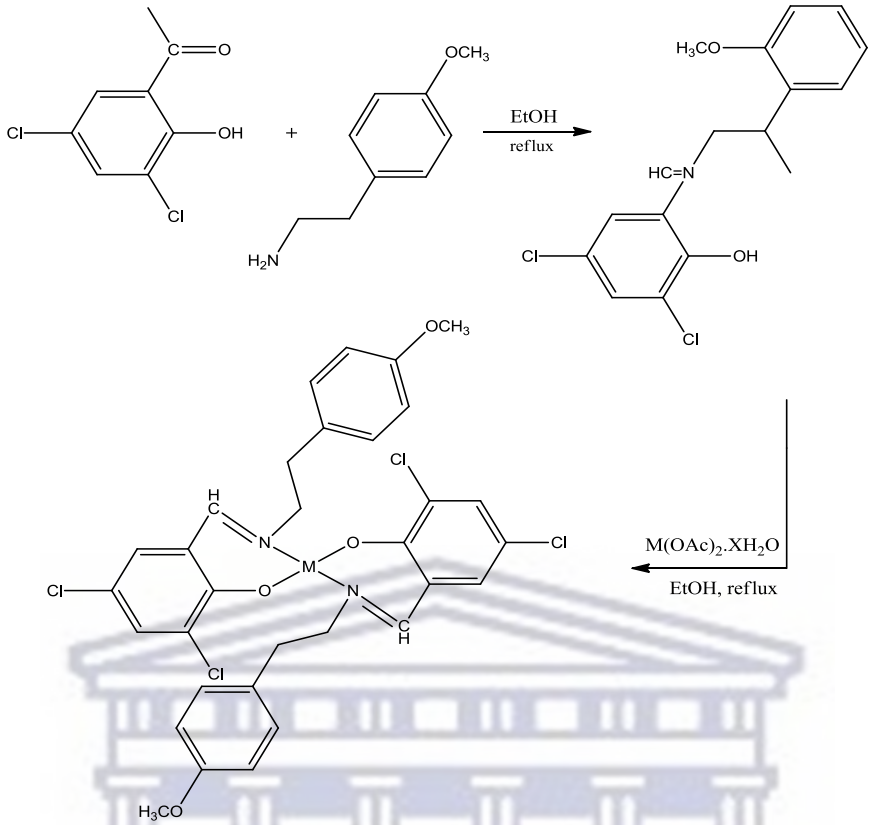
2.4.3 Studies done on copper containing complexes and their antibacterial activities.

Copper(II) Schiff base complexes are relatively stable and have received a lot of attention in research in the past decades [73]. Pitchunami *et al*. studies and reported the relative stability order of M(II) chalcone-based Schiff base complexes in theoretical order, Cu > Ni > Co > Zn. Hence, copper(II) complexes are suitable for biological use since they are less prone to break down to form toxic metabolites in harsh biological environment [74]. According to Pearson's coordination theory which is summarized in Table 2.2 below [75], copper(II) form stable complexes with salicylaldehyde Schiff bases that contain N-donor atoms.

Table 2.2: Classification of some metal ions and donor atoms according to Pearson's principle.

Hard Lewis acids	Borderline acids	Soft Lewis acids
H^+, Li^+, Na^+, K^+, Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Sc^{3+}, Ti^{4+}, Zr^{4+}, Cr^{3+}, Al^{3+}, Ga^{3+}, La^{3+}, Gd^{3+}, Co^{3+}, Fe^{3+}	Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Bi^{3+}, Rh^{3+}, Ir^{3+}	Cu^+, Au^+, Ag^+, Tl^+, Hg^+, Pd^{2+}, Cd^{2+}, Pt^{2+}, Hg^{2+}
Hard Lewis bases	Borderline bases	Soft Lewis bases
F^-, OH^-, H_2O, ROH, Cl^-, R_2O, $CH_3CO_2^-$, NH_3, RNH_2, NH_2NH_2, CO_3^{2-}, RO^-, NO_3^-, O_2^-, SO_4^{2-}, PO_4^{3-}, ClO_4^-	NO_2^-, Br^-, N_3^-, N_2, $C_6H_5NH_2$, pyridine, imidazole	RSH, RS^-, R_2S, S_2^-, CN^-, CO, I^-, R_3As, R_3P, C_6H_5, C_2H_4, RNC, H_2S, HS^-, H^-

Aggoun *et al*. [76] synthesized nickel(II) and copper(II) complexes derived from bidenate alkylamine (4-methoxyethylphenylamine) and dihalogenated salicylaldehyde Schiff base ligand as displayed in Scheme 2.1. The choice of metals was supported by literature reported by Pandey *et al*., they are of significant interest because of their structural adaptability and magnetic properties [77]. Furthermore, Ariyaeifar *et al*. also reported that nickel and copper play an important role in in several biological applications [78].

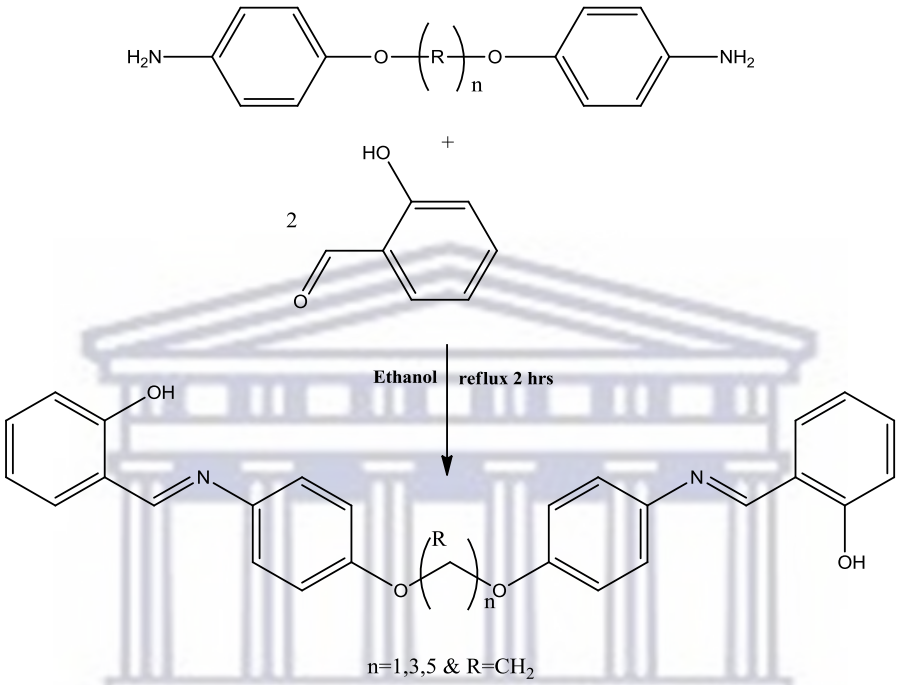


Scheme 2.1: Schematic synthesis process of the bidentate Schiff base ligand and its nickel and copper complexes

The synthesized Schiff base ligand and complexes were found to be stable at room temperature and fairly soluble in organic solvents. The IR spectrum of the ligand proved that the synthesis was successful by exhibiting an absorption peak around 1634 cm^{-1}. The ligand structural analysis showed that it acts a bidentate Schiff base, coordinating to the metal ions through the imine and hydroxyl group which led to a distorted square-planar geometry. The UV-Vis spectra showed a shifting of the electronic transitions when comparing the ligands and the complexes. The complexes spectra exhibited absorption bands at higher wavelengths which confirmed the coordination of the nitrogen and oxygen atoms to the metal ions.

Bushra *et al.* [79] synthesized tetradentate three new Schiff base ligands using salicylaldehyde with semi-aromatic diamine as shown in Scheme 2.2 below. The ligands were prepared by reduction using the corresponding dinitro-compounds which were further used to form Cu(II)

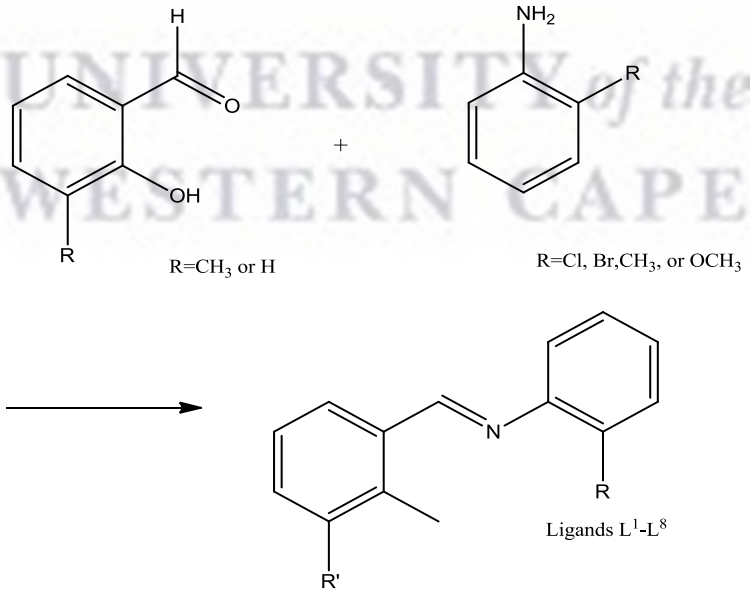
complexes. They used nitro compounds which are an important class of nitrogen derivatives, the nitro group is a combination of two equivalent resonance structures. Nitro compounds also have high dipole moments which results in low volatility than ketones of about the same molecular weight.



Scheme 2.2: Schematic synthesis of tetradentate Schiff base ligands of salicylaldehyde with semi-aromatic diamine

The compounds were then tested for their antibacterial activity against six bacteria strains, including, four gram-negative strains (*Salmonella typhimurium*, *Enterobacter aerogenes*, *Escherichia Coli*, *Bordetella bronchi septica*) and two gram-positive strains (*Staphylococcus aureus* and *Micrococcus luteus*). In all six bacteria strain none of the tested compounds had significant antibacterial activity. This may be attributed to the microbial cells impermeability which prevented the compounds from interacting with bacteria strains [80]. According to Sharma *et al*., substitution of nitro groups at the *ortho* position of the Schiff bases phenyl ring affects their antibacterial activity negatively and are inactive at high concentrations [81]. Aliyu and Sani proved that Schiff base Cu(II) complexes with aliphatic groups lacked antibacterial activity [82].

The beneficial properties of Schiff base ligands and complexes are not only influenced by the azomethine bond, but also by a variety of scaffolds, including aromatic and substituted aromatic compounds. Additionally, it has been reported that the presence of electron-rich species in the Schiff base molecule, such as nitrogen (N), oxygen (O) and sulphur (S), significantly enhances their applications [83, 84]. A great example is Schiff bases derived from salicylaldehyde derivatives, those with –OH group in the *ortho* and *para* positions are of significant interest because of the presence of O-H···N and O···H−N type hydrogen bonds that undergo excited state tautomerization that is between enol-imine and ketoenamine[85]. Sobola *et al*. [86] prepared copper(II) complexes of some *ortho*-substituted aniline Schiff base ligands (L1–L8). The compounds were then tested for their antibacterial and antifungal activity using four bacteria strains namely, *Escherichia Coli, Staphylococcus Aureus, Bacillus and Candida Albicans.* The Schiff base ligands were synthesized by condensation reaction as presented in Scheme 2.3 below. The copper salt used for Cu(II) Schiff base complexes was copper acetate monohydrate, $Cu(OAc)_2.H_2O$.



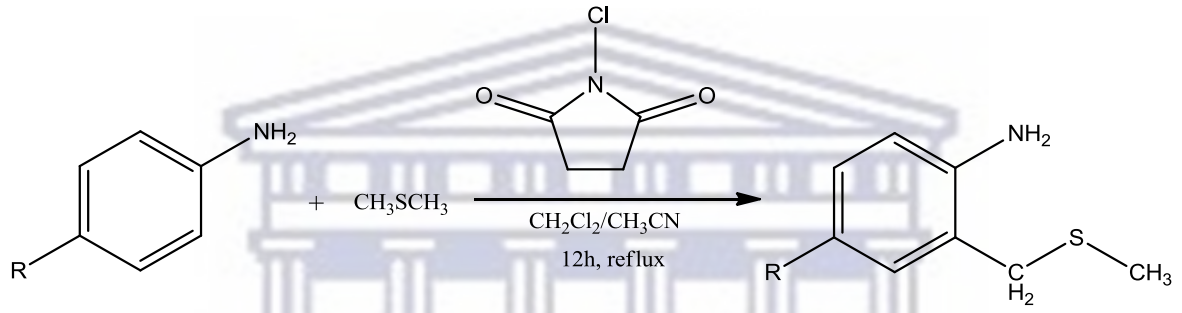
Scheme 2.3: Schematic synthesis of *ortho*-substituted aniline Schiff base ligands.

The compounds had strong antifungal activity efficacy against the tested organisms, however they were not as effective as penicillin. The salicylaldehyde based compounds were virtually inactive against the tested organisms, in contrast to the o-vanilin-based ligands which showed higher activity. This could be caused by the methoxy group substituted at the *ortho* position of the aldehyde moiety of Schiff base ligands. Additionally, ligands of toluidine and anisidine derivatives exhibited significant potency against some microorganisms. Thus, indicating that the electronic effects of the substituents have an effect on the Schiff bases activity since the chloro- and bromo- analogues only showed weak activity. However, according to Ansari *et al*. [87] there is high antimicrobial activity in a series of substituted benzimidazole Schiff base ligands for the o-chloro, o-methyl, o-methoxyl and p-amino analogues.

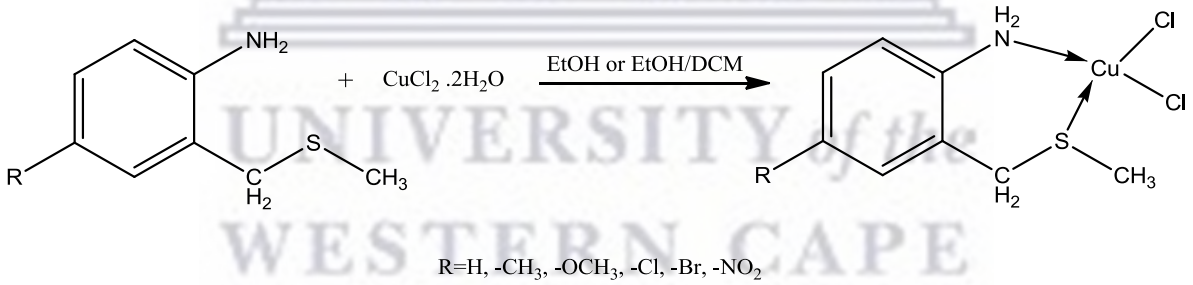
On chelation, the presence of the Cu(II) ions did not improve the antimicrobial activity of the ligands. In comparison to the Schiff base ligands, the copper complexes showed less activity. According to literature, it is believed that the lipophilicity of the metal ion is increased by chelation which in turn increases the antimicrobial activity of the metal complexes in comparison to the free ligands. Conversely, it has been shown that the free ligands do exhibit high activity in some cases; suggesting that activity is not exclusively dependent on the presence of the metal ions but rather a synergistic effect on a variety of factors. Therefore, one of the most important aspects to research more about in future is understanding the mechanisms of action of possible antimicrobial agents.

According to literature it is also believed that the type of substituent, electron-withdrawing group or electron-donating group, has an effect on the antibacterial activity of Schiff base compounds. To investigate this point further, Olalekan *et al*. [88] synthesized and characterized Copper(II) complexes of 2-(methylthiomethyl)anilines (Scheme 2.4). The choice of imine group comes from theory that alkylthioalkylated anilines are used as intermediates in the synthesis of several organic compounds [89-91] such as rubber, dyes, and herbicides [92]. The have

thioether sulphur and the aniline nitrogen in their moiety which allows them to act as coordinating ligands. Under mild reaction conditions, the sulphur and nitrogen in akylthioalkylated anilines reaction with metal ions results in stable complexes. In comparison to sulphonamide analogues, alkylthioalkylated aniline and their copper complexes have received less research in structural, spectroscopic, and biological areas. Temitope and co-workers reported the antimicrobial properties of Cu(II) complexes of 2-(methylthiomethyl)anilines compared to their corresponding ligands.



Scheme 2.4a: Schematic synthesis of 2-(methylthiomethyl)anilines Schiff bases

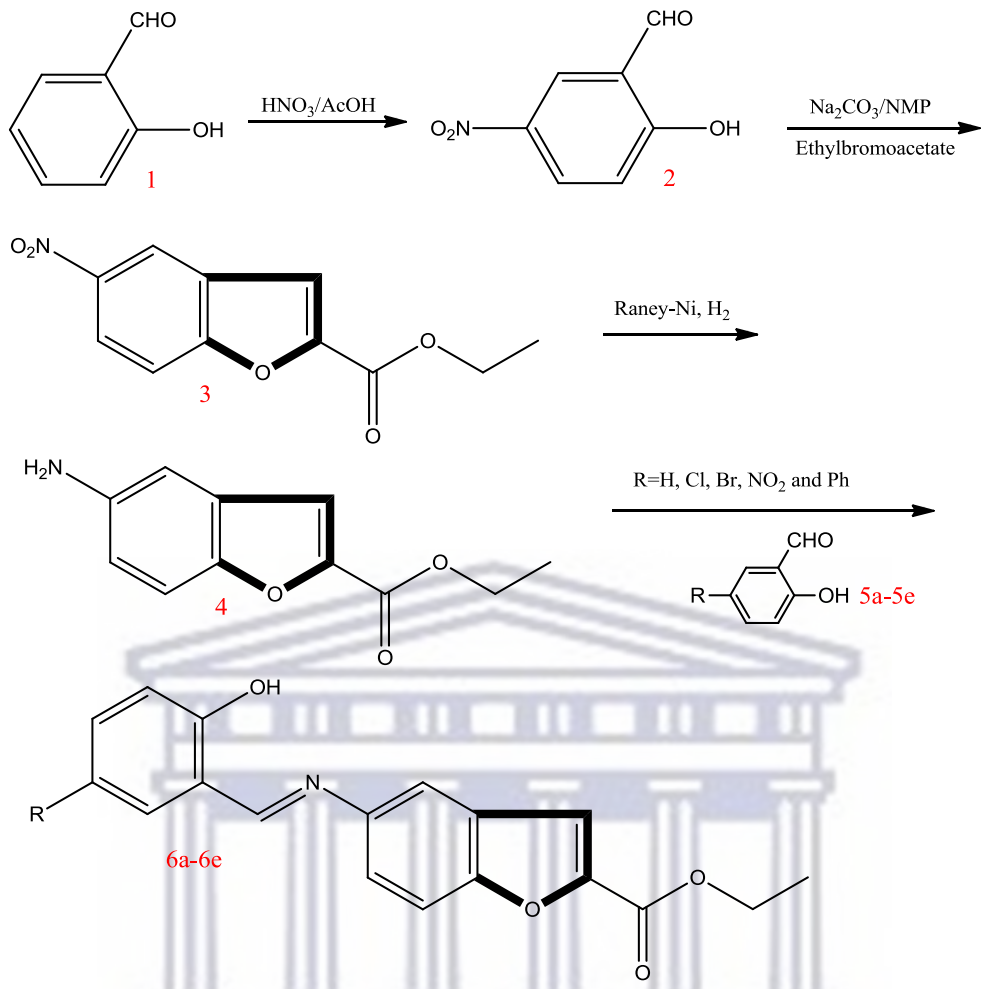


Scheme 2.4b: Schematic synthesis of Copper(II) complexes of 2-(methylthiomethyl)anilines

The ligands and complexes were tested for their *in vitro* antimicrobial activity against *E. Coli, B. Subtilis, S. Aureus and C. Albicans* using agar disc diffusion method. The diameter of zone of inhibition of the microbial growth by each compound was thereafter measured; the ranges were 7-8 mm for *E. coli ,* 7-18 mm for *B. subtilis,* 7-20 mm for *S. aureus and* 9-11 mm for *C. albicans*. In this study, the gram-positive bacteria were more susceptible to the test compounds than the gram-negative *E. coli* and the fungus *C. albicans*. The results revealed that the

inhibitory activity of the ligands increased after chelation to copper ion. The increased lipophilicity given to the complex by the copper ion could be the reason why the complexes have higher activity. It was observed that the ligands and complexes with electron donating groups were inhibiting microbial growth more effectively than those with electron withdrawing groups. The methoxy substituted complexes exhibited effective inhibitory activity against *S. Aureus and B. Subtilis* than methyl substituted complexes at the concentrated test.

Benzofuran derivatives show several biological properties including antimicrobial, anti-inflammatory, antifungal, antitumor and antiparasitic activities[93-98]. The vast spectrum of benzofuran scaffold biological properties has attracted much attention for them to be used for pharmacological applications. Additionally, substituted benzofurans are used in a number of fields in chemistry and agriculture, such as oxidants[99], fluorescent sensors[100], brightening agents, antioxidants and a variety of drugs[101]. In the work done by Nazirkar *et al*. [102], new benzofuran based Schiff base ligands (6a-6e) were synthesized and complexed with copper and zinc via sequential reactions starting with salicylaldehyde.



Scheme 2.5: Schematic preparation of the benzofuran based Schiff base ligands (6a-e).

The compounds *in vitro* anti-tubercular activity was evaluated using the Microplate Almar Blue assay method[103] against *M. tuberculosis* H37 Rv strain. All the synthesized Schiff base ligands and complexes had moderate to good activity in comparison with the standard drugs. Three compounds out of all of them showed inhibitory activity against *M. tuberculosis*. The compounds were then tested for their *in vitro* antibacterial activity against three bacteria strains, namely *S. Aureus, E. coli* and *B. Subtilis* using the agar disc diffusion method. From the diameter measurements of the inhibition zone, the complexes showed high activity compared to the Schiff base ligands. This could be explained by using Overtone's concept which states that compounds with lipophilic properties favours transfer through the lipid membrane of the

bacterial cell wall. Therefore, since complexes have increased lipophilicity, it enhances their antibacterial activity.

According to Ligand Field Theory (LFT), the positive charge on the central metal is minimized by the overlapping metal and ligand orbitals because it gains electrons from the Schiff base ligand donor groups[104,105]. As result of this delocalization of electrons, the metal complexes become more lipophilic. The enhanced lipophilic character allows them to travel across the bacterial cell wall easily and inhibit the bacteria enzymes[106]. The antibacterial activities of Cu(II) complexes were more effective than those of Zn(II) complexes. This may be related with Cu(II) stronger affinity for biomolecules which makes the complexes to be more permeable through cell membrane, hence they have stronger antibacterial activity than Zn(II)[107].

2.5 Rationale of the study

Schiff bases of substituted salicylaldehydes are well-known and have drawn much attention in the research world as antimicrobial agents. Salicylaldehyde Schiff bases have also been proven to have good chelating properties. The majority of research done on salicylaldehyde Schiff base complexes have been focusing on nitrogen and oxygen donor atoms and their backbone structures. It has been reported by several studies that copper ions have the ability to form strong bonds with N-donor ligands as they form strong back bonding[31,108,109]. In comparison with other transition metals copper has been ranked to be amongst the highest in various applications[110,111]. The shortfalls of antibacterial drugs and the aforementioned reasons have provided a valid justification of the synthesis of Schiff base compounds in this study. Additionally, the results from this study are preliminary work that will give guidance on the methodologies to follow for the improvement of tuberculosis first line drugs.

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Chapter 3

3.1 Experimental section

3.1.1 General remarks

All experimental manipulations were carefully carried out under inert nitrogen atmosphere using standard dual vacuum/nitrogen lines and Schlenk techniques unless stated otherwise. The solvents used were all dried. Methanol was dried by heating at reflux with magnesium turnings under nitrogen and kept over 3Å molecular sieve in tightly sealed round bottom flasks. Hexane and dichloromethane were dried over sodium wire and were kept in closed round bottom flasks in the fume hood. The chemicals were used as supplied so no further purification was done.

3.1.2 Instrumentation

Melting points were determined on a Stuart SMP10 melting point apparatus. Reaction progress and product mixtures were monitored by using Fourier Transform-Infrared (FTIR) spectroscopy on a Perkin-Elmer Spectrum two LiTa spectrometer in the 4000 – 400 cm^{-1} regions using KBr as background. The ^{1}H and ^{13}C NMR spectra were acquired on a 400 MHz Avance III HD Nanobay spectrometer (Bruker, Rheinstetten, Germany) equipped with a 5 mm BBO probe at 298 K using standard 1D and 2D NMR pulse sequences. Chloroform-d was used as the solvent with chemical shifts reported in ppm (δ) relative to TMS as internal standard. The cyclic voltammetry experiments were performed using PalmSens Ptrace 4.4 electrochemical workstation. Elemental analysis was conducted at the Central Analytical Facility (CAF) laboratory in Stellenbosch University.

3.1.3 Chemicals

Table 3.1 List of chemicals and suppliers

Chemical name	Chemical formula	Purity	Company
Aniline	CH_2Cl_2	99%	Sigma Aldrich
o-Toluidine	$CH_3C_6H_4NH_2$	≥99%	Sigma Aldrich
p-Toluidine	$CH_3C_6H_4NH_2$	99%	Sigma Aldrich
2,4-Dimethylaniline	$C_6H_3(CH_3)_2NH_2$	99%	Sigma Aldrich
2,6-Dimethyaniline	$C_6H_3(CH_3)_2NH_2$	≥99%	Sigma Aldrich
5-Chlorosalicylaldehyde	$ClC_6H_3(OH)CHO$	98%	Sigma Aldrich
Methanol	CH_3OH	99.9%	Sigma Aldrich
Dichloromethane	CH_2Cl_2	99%	Sigma Aldrich
Dimethyl sulfoxide	C_2H_6OS	99.5%	KIMIX
Triethylamine	$(CH_3)_3N$	≥99%	Sigma Aldrich
Chloroform-d	$CDCl_3$	99.8%	Sigma Aldrich
Magnesium sulphate anhydrous	$MgSO_4$	99.5%	KIMIX
Cu(II) chloride dihydrate	$Cl_2Cu.2H_2O$	≥99.0%	Sigma Aldrich

3.2 Characterization techniques

3.2.1 Fourier transfer infrared spectroscopy (FTIR)

The vibrations of the atoms within a molecule serve as the basis for the Fourier Transform Infrared spectroscopy (FTIR) technique as shown in Figure 3.1. The technique is used as a method to ascertain quantitatively and qualitatively the features of infrared-active molecules in samples of organic or inorganic solids, liquids, and gas samples. It is a quick and affordable technique for examining crystalline, microcrystalline, amorphous, or film-like solid samples[1]. An infrared (IR) spectrum is obtained by passing IR radiation through a sample and determining what fraction of the incident radiation is absorbed at each energy level. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule[2].

Additionally, chemical bonding will absorb varying intensities and frequencies in different environments. Therefore, IR spectroscopy entails collecting absorption data and analyzing it in the form of a spectrum; the frequencies at which IR radiation is absorbed (referred to as "peaks" or "signals") can be directly associated to bonds within the compound in question. Individual interatomic bonds may absorb at more than one IR frequency because they can vibrate in a variety of motions (stretching or bending). Although bending absorptions typically result in weaker peaks than stretching absorptions, they can nevertheless be useful for distinguishing between bonds of the same type (e.g., aromatic substitution).

The scale of samples analyzed ranges from microns to kilometers and sample preparation are relatively easy. When the right decisions are made regarding the IR source, the detecting method, and the accessories, the best IR spectra are obtained. The analyst must also be aware of the infrared spectrum region where the sample's distinctive functional groups are located[1,3]. In this study, the FTIR spectra were recorded as KBr pellets in the range 4000-400 cm^{-1} on a

PerkinElmer Spectrum 2000 FT-IR spectrophotometer for further elucidation of the structures and present functional groups in the Schiff base ligands (HL^1-HL^5) and complexes (C1-C5). The KBr pellets were made from grinding KBr using a mortar and pestle then using it as the background spectrum before running the compound spectrum.

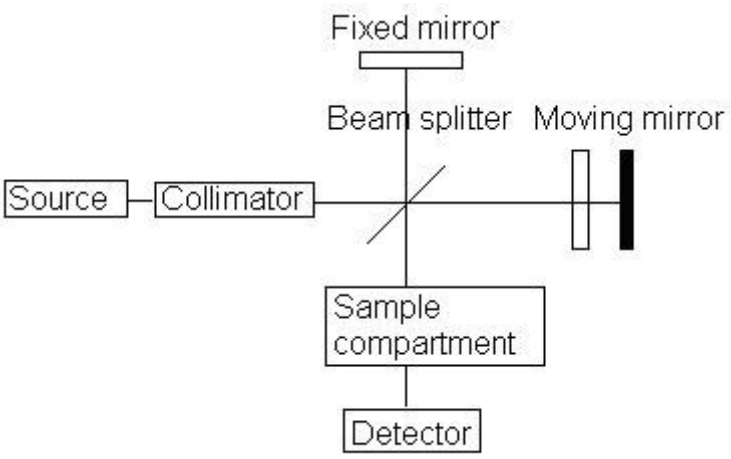

Figure 3.1: Diagram of a FTIR spectrometer[3].

3.2.2 Ultraviolet – Visible spectroscopy

Ultraviolet – visible spectroscopy is a technique that makes use of light in the visible and adjacent near ultraviolet (UV) ranges. It investigates how the transfer of electrons from π, or non-bonding orbitals alters the electronic energy levels in a molecule. Typically, it provides details on conjugated non-bonding electron systems, conjugated unsaturations, aromatic compounds, and π electron systems[4]. The UV region covers the range of 190 nm to 380 nm and the visible region ranges from 380 nm to 750 nm. The molecules go through electronic transitions at these wavelengths[5].

The energy diagram in Figure 3.2 highlights the possible transitions. The reference cuvette and the cuvette containing the sample are exposed to a sequence of light wavelengths coming from

the spectrometer that emits an energy matching the likely electronic transition within the molecule. In the ultraviolet and visible regions of the electromagnetic spectrum, a portion of the light energy is absorbed as an electron transition to a higher energy level or orbital. Although not specific enough for sample identification or isolation, the UV-Vis spectrum data or observed bands can be used as a benchmark against known compounds in a database[6]. The electronic absorption spectra of the compounds were carried out using 1:9 dilution ratio in DMSO at standard conditions and the results were recorded in the region 220 - 500 nm. For this study, the UV-Vis spectroscopy was used to determine the electronic transitions that correspond to the peaks of absorbance and prove that coordination occurred between the ligands and the metal ion. This in turn assisted in the determination of the compounds geometry.

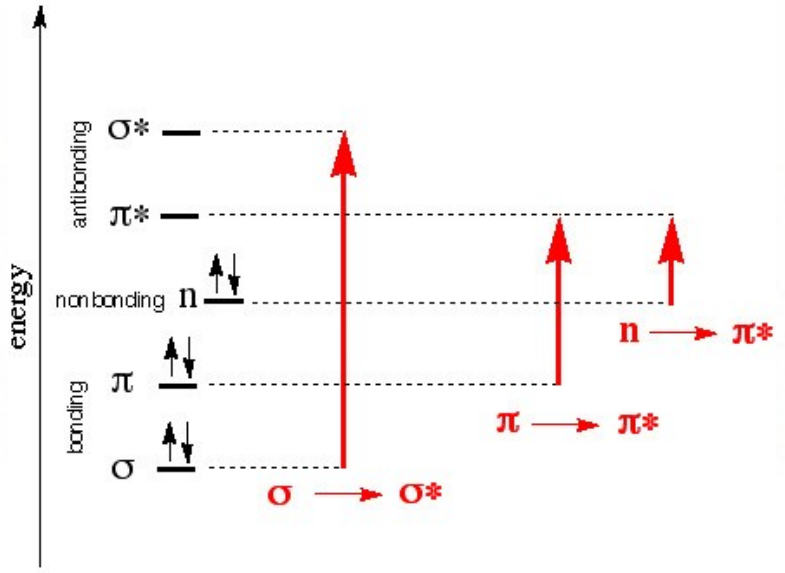

Figure 3.2: UV - Vis energy diagram[7].

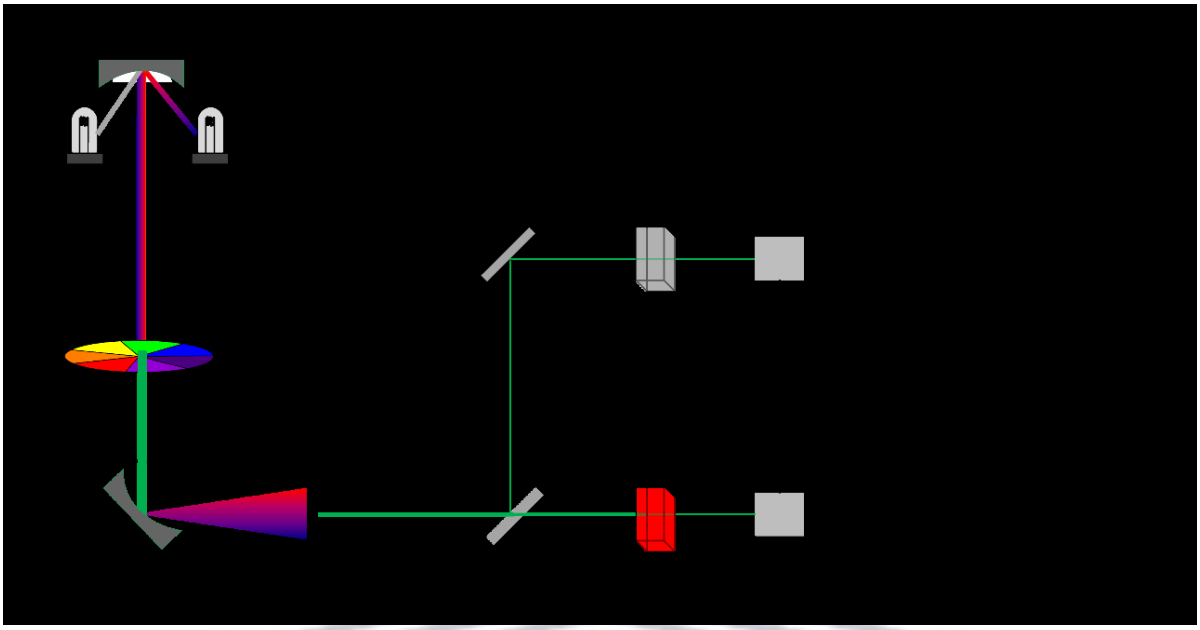

Figure 3.3: Diagram of a UV - VIS spectrometer[8].

3.2.3 Nuclear magnetic resonance (NMR)

Over the past five decades, nuclear magnetic resonance spectroscopy, or NMR as it is more generally known, has emerged as the method of choice for determining the structure of organic compounds. It is the only spectroscopic technique for which a complete analysis and interpretation of the entire spectrum is normally expected. NMR is non-destructive, and with modern instruments, good data may be obtained from samples weighing less than a milligram.

The ^{1}H NMR gives information about the chemical environment of a proton or group of protons relative to tetramethylsylane (TMS). The ^{13}C NMR analysis is based on the same principles as the ^{1}H NMR technique, but there is no splitting in the spectrum. The carbon environments or set of equivalent carbons in the compound correspond to the single peaks displayed in the ^{13}C NMR spectrum. The ligands were dissolved in deuterated chloroform at room temperature using trimethyl silane (TMS) as internal standard. The solvent was chosen due to its ability to readily dissolve all the synthesized ligands. The NMR assisted in understating the different

chemical environments, the chemical shift observed was used for assigning some structural features of the compounds. The shape and size of peaks were indicators of chemical structure too – integration.

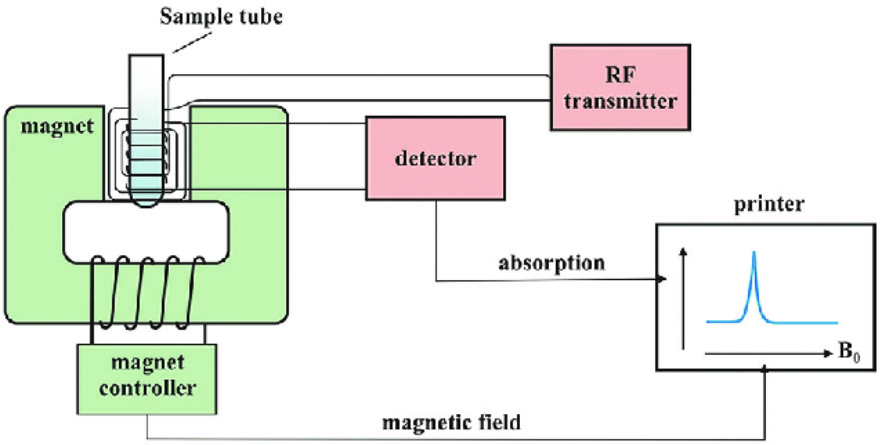

Figure 3.4: Diagram of NMR spectrometer[9].

3.2.4 Elemental analysis

The carbon, hydrogen, nitrogen, and sulfur analysis were carried out using the Server 1112 Series Elemental Analyzer at the University of Stellenbosch's central analytical laboratory. The process involves weighing the samples before loading them into tin cups and an automatic sampler. The tin cups, which are necessary for the proper combustion in the elemental analyzer, are transported through a tube and then flash-combusted at a temperature of 1800 °C.

The helium serves as the gas carrier while the gaseous combustion products N_2, NO_x, H_2O, SO_2, O_2 and CO_2 are transported through a copper oxide-filled column before passing through a Cu column where O_2 is converted to CuO and nitrogen oxides are reduced to elementary nitrogen. The remaining gases are passed via a temperature-programmed desorption column (TPD), where N_2 passes straight through, and the other gases are bound. Water is captured through a separate column. Using a programmed temperature increase in the column, the bound

gases are released individually. These gases pass via a thermal conductivity detector (TCD), which emits an electrical signal whose intensity is proportional to the concentration of carbon, hydrogen, nitrogen, and sulfur. For this study, elemental analysis was used to determine the precise quantity of elements present in the Schiff base ligands and complexes, which were carbon, hydrogen, and nitrogen, in percentage form.

3.2.5 Cyclic Voltammetry

All cyclic experiments of the compounds were performed using PalmSens Ptrace 4.4 electrochemical. Cyclic Voltammetry (CV) is an electrochemical technique that is widely used to study the reduction and oxidation processes of molecular species. It is also invaluable to study the thermodynamics of redox processes and the kinetics of electronic transfer reactions and determine the potential window of electrodes or devices. Voltammograms also give information about charge-transfer and mass-transport processes that occur at the working electrodes surfaces[10-14]. The redox characteristics of conductive and semi-conductive materials are investigated using cyclic voltammetry[15]. The fundamental principle of cyclic voltammetry is the measurement of the resulting current when a linear potential is applied to an electrode between potential limits.

In cyclic voltammetry, a three-electrode system which includes a working electrode, a reference electrode and a counter electrode. The scan rate results from the rate at which potential changes with time. During the experiment, a supporting electrolyte is used to prevent migration of charges and products. The working electrode potential is scanned linearly and a potentiostat measures the current flowing from the applied potential. The resulting current potential plot is known as the cyclic voltammogram, which is shown in Figure 3.5.

In this study, CV was conducted as a characterization technique to evaluate the redox behaviour of Cu(II) Schiff base complexes and the effects of scan rate on current. The

experiment was conducted using DMF containing 0.3 M of TBAP as a supporting electrolyte. The solutions used were all purged using inert gas, nitrogen. This was done because oxygen has a relatively low reduction potential, it must be removed from the solution so that it does not interfere with the measurement. Prior to each experiment, the glassy carbon electrode was properly cleaned by polishing it using alumina powder and sonicated for 10 min in de-ionized water.

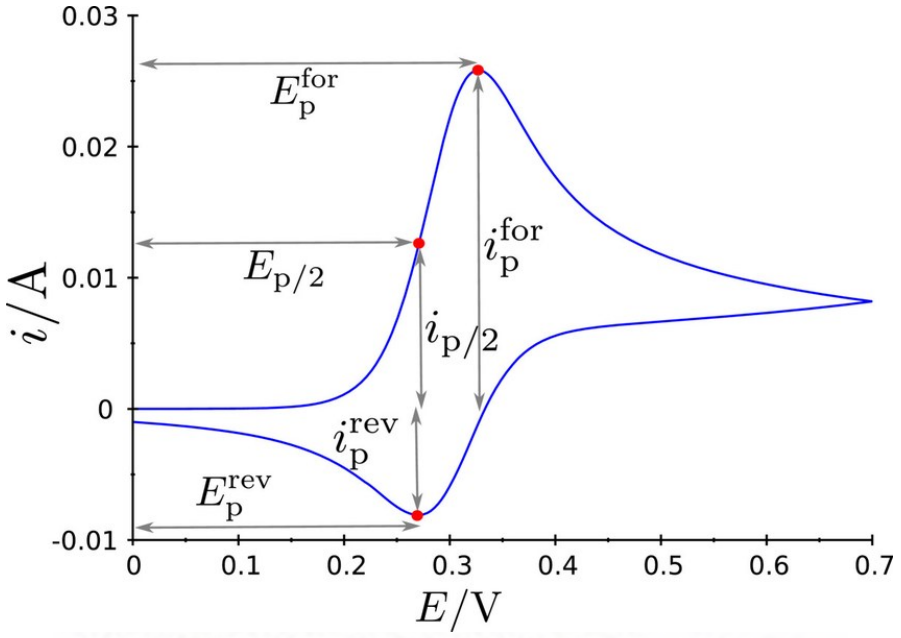

Figure 3.5: A typical diagram of cyclic voltammogram[16].

3.3 Methodology

3.3.1 In-laboratory standard procedure

All the chemical reactions were performed at the Inorganic research labs (Department of Chemistry, UWC) following all the safety regulations. All waste was disposed following the laboratory disposal protocol. The safety regulations and the disposal protocol followed were communicated by the occupational safety and health officer from the risk and compliance department, UWC.

3.3.2 Cleaning of glassware

All the glassware was thoroughly washed with soap and warm water, followed with 70% ethanol, and then rinsed with acetone and placed in the oven to dry before use.

3.3.3 Synthesis of Schiff base ligands using aniline derivatives with 5-chlorosalicylaldehyde

3.3.3.1 HL^1 - (Z)-4-chloro-2-((phenylimino)methyl)phenol

The synthesis followed a 1:1 ratio. In a Schlenk tube, 5-salicylaldehyde (1000mg, 0.64 mmol) dissolved in 30 ml dry methanol was added to aniline (59.00mg, 0.64 mmol) also dissolved in 30 ml dry methanol, dropwise. Upon addition of aniline solution, the colour changed from clear solution to a light-yellow solution. To the light-yellow solution, magnesium sulphate (50.00 mg) was then added which changed the colour to bright yellow. The solution was stirred under nitrogen while refluxing for 4 hours. Colour change was be observed and recorded. Cannula filtration was done to separate magnesium sulphate from the solution prior to stirring it under reduced pressure using a vacuum pump. The bright yellow solid product was then recrystallized in a 1:3 ratio of hexane/dichloromethane, submerged in acetone with dry ice for approximately 30 min to 1 hour, then removed the excess of the solvents before being dried under high vacuum pump. The bright orange product obtained with crystals texture was then weighed on a scale to determine mass. Yield: 114.4 mg (76.3%) M.P (100-108°C). IR data (KBr, v/cm^{-1}): v(OH) 3389.52, v(HC=N) 1614.30, v(C=C) 1562.12, v(C-O) 1274.52. 1H NMR data ($CDCl_3$, ppm): δ 6.91 (d, 1H), 7.1 (d, 1H), 7.20 (t, 1H), 7.24 (d, 1H), 7.36 (t, 1H), 7.37 (s, 1H), 8.49 (s, 1H, HC=N). ^{13}C NMR data ($CDCl_3$, ppm): 118.95 (1-C), 120.00 (2-C), 121.19 (3-C), 123.67 (C-Cl), 127.37 (5-C), 129.52 (6-C), 131.22 (7-C), 133.60 (8-C), 147.98 (9-C), 159.70 (OH), 161.20 (HC=N). E.A: C 67.39, H 4.35, N 6.05 Found: 66.96, H 3.72, N 5.92.

3.3.3.2 HL^2 - (Z)-4-chloro-2-((o-tolylimino)methyl)phenol

The synthesis followed a 1:1 ratio, following the procedure used to synthesize ligand HL^1. 5-salicylaldehyde (1000mg, 0.64 mmol) and o-toluidine (68.00 mg, 0.64 mmol) were used as starting materials. An orange yellow product was obtained and it was treated as in ligand HL^1 in section 3.5.3.1. Yield: 132.4 mg (82.7%) M.P (80-88 °C). IR data (KBr, ν/cm^{-1}): ν(OH) 3389.52, ν(HC=N) 1614.30, ν(C=C) 1562.12, ν(C-O) 1274.52. 1H NMR data ($CDCl_3$, ppm): δ 2.32 (s, 3H, CH_3), 6.88 (d, 1H), 6.90 (d, 1H), 7.12 (d, 1H), 7.15 (d, 1H), 7.22 (d, 1H), 7.24 (d, 1H), 7.28 (s, 1H), 8.49 (s, 1H, HC=N). ^{13}C NMR data ($CDCl_3$, ppm): 17.18 (CH_3), 118.6 (2-C), 119.9 (3-C), 121.2 (C-Cl), 122.8 (5-C), 127.00 (6-C), 127.21 (7-C), 130.10 (8-C), 130.60 (9-C), 132.90 (10-C), 137.20 (11-C), 145.90 (12-C), 159.40 (OH), 161.00 (HC=N). E.A: C 68.44, H 4.92, N 5.70. Found: C 67.48, H 3.82, N 5.20.

3.3.3.3 HL^3 - (Z)-4-chloro-2-((p-tolylimino)methyl)phenol

The synthesis followed a 1:1 ratio, following the procedure used to synthesize ligand HL^1. 5-salicylaldehyde (1000mg, 0.64 mmol) and p-toluidine (68.00 mg, 0.64 mmol) were used as starting materials. A light orange product was obtained and it was treated as in ligand HL^1 in section 3.5.3.1. Yield: 112.5 mg (70.3%) M.P (79-86 °C). IR data (KBr, ν/cm^{-1}): ν(OH) 3389.52, ν(HC=N) 1614.30, ν(C=C) 1562.12, ν(C-O) 1274.52. 1H NMR data ($CDCl_3$, ppm): δ 2.33 (s, 3H, CH_3), 7.00 (d, 1H), 7.20 (d, 1H), 7.23 (d, 1H), 7.25 (d, 1H), 7.30 (s, 1H), 8.45 (s, 1H, HC=N). ^{13}C NMR data ($CDCl_3$, ppm): 21.10 (CH_3), 117.10 (2-C), 118.40 (3-C), 120.20 (4-C), 123.90 (C-Cl), 130.60 (6-C), 131.80 (7-C), 133.10 (8-C), 136.80 (9-C), 147.10 (10-C), 158.91 (OH), 160.90 (HC=N). E.A: C 68.44, H 4.92, N 5.70. Found: C 67.40, H 4.87, N 5.61.

3.3.3.4 HL^4 - (Z)-4-chloro-2-(((2,4-dimethylphenyl)limino)methyl) phenol

The synthesis followed a 1:1 ratio, following the procedure used to synthesize ligand HL^1. 5-salicylaldehyde (1000mg, 0.64 mmol) and 2,4-dimethylaniline (77.00 mg, 0.64 mmol) were

used as starting materials. A deep yellow product was obtained and it was treated as in ligand HL^1 in section 3.5.3.1. Yield: 143.4 mg (84.4%) M.P (76-84 °C). IR data (KBr, *v/cm^{-1}*): *v*(OH) 3389.52, *v*(HC=N) 1614.30, *v*(C=C) 1562.12, *v*(C-O) 1274.52. 1H NMR data ($CDCl_3$, ppm): δ 2.30 (s, 3H, CH_3), 6.90 (d, 1H), 6.95 (d, 1H), 7.00 (d, 1H), 7.19 (s, 1H), 7.24 (d, 1H), 7.30 (s, 1H), 8.44 (s, 1H, HC=N). 13C NMR data ($CDCl_3$, ppm): 18.16 (CH_3), 21.02 (CH_3), 117.24 (3-C), 118.75 (4-C), 120.20 (5-C), 123.56 (C-Cl), 127.64 (7-C), 131.01 (8-C), 131.63 (9-C), 132.56 (10-C), 137.34 (11-C), 144.32 (12-C), 158.80 (OH), 159.50 (HC=N). E.A: C 69.36, H 5.43, N 5.39. Found: 69.47, H 5.51, N 5.31.

3.3.3.5 HL^5 - (Z)-4-chloro-2-(((2,6-dimethylphenyl)limino)methyl) phenol

The synthesis followed a 1:1 ratio, following the procedure used to synthesize ligand HL^1. 5-salicylaldehyde (1000mg, 0.64 mmol) and 2,6-dimethylaniline (77.00 mg, 0.64 mmol) were used as starting materials. A light yellow product was obtained and it was treated as in ligand HL^1 in section 3.5.3.1. Yield: 142.9 mg (84.1%) M.P (78-88 °C). IR data (KBr, *v/cm^{-1}*): *v*(OH) 3389.52, *v*(HC=N) 1614.30, *v*(C=C) 1562.12, *v*(C-O) 1274.52. 1H NMR data ($CDCl_3$, ppm): δ 2.12 (s, 3H, CH_3), 6.95 (d, 1H), 7.03 (d, 1H), 7.23 (d, 1H), 7.26 (t, 1H), 7.28 (s, 1H), 8.20 (s, 1H, HC=N). 13C NMR data ($CDCl_3$, ppm): 18.47 (CH_3), 118.60 (2-C), 119.49 (3-C), 123.74 (C-Cl), 125.28 (5-C), 128.41 (6-C), 128.66 (7-C), 131.19 (8-C), 133.01 (9-C), 147.73 (10-C), 159.80 (OH), 165.62 (HC=N). E.A: C 69.36, H 5.43, N 5.39. Found: C 68.98, H 5.30, N 5.42.

3.3.4 Synthesis of salicylaldehyde Cu(II) Schiff base complexes

3.3.4.1 C1: (Z)-4-chloro-2-((phenylimino)methyl)phenol with Cu(II) chloride

The synthesis followed a 1:1 ratio. Ligand HL^1 (100mg, 0.430 mmol) was stirred at room temperature with Et_3N (100 μL, 400 μmol) in methanol (10 mL) for 1 hour under nitrogen to deprotonate HL^1. A solution of $CuCl_2.2H_2O$ (100mg, 0.400 mmol) was subsequently added dropwise into the solution and the reaction mixture was stirred overnight. Thereafter

complexation, the solvent volume was then reduced under vacuum to ~ 3mL and the complex was precipitated with cold diethyl ether. The precipitate was filtered and washed with copious amount of diethyl ether (3 times using ~30 mL) and kept under reduced pressure for several hours until it was completely dry. The colour of the obtained product was fern green with a mass of 132.8 mg (66.38%). MP (162-165 °C). IR data (KBr, v/cm^{-1}): v(HC=N) 1603.11, v(C=C) 1452.92, v(C-O) 1320.34, v(Cu-O) 567.64, v(Cu-N) 451.45. E.A: C 59.49, H 3.46, N 5.34. Found: C 58.35, H 3.37, N 5.21.

3.3.4.2 C2: (Z)-4-chloro-2-((o-tolylimino)methyl)phenol with Cu(II) chloride

The complex was prepared analogously to C1. A solution of $CuCl_2.2H_2O$ (100mg, 0.400 mmol) and HL^2 (100mg, 0.410 mmol) were used as starting materials. A brownish green product was obtained and it was treated as in C1. Yield: 158.0 mg (71.83%). MP (177-179 °C). IR data (KBr, v/cm^{-1}): v(HC=N) 1603.25, v(C=C) 1456.49, v(C-O) 1319.89, v(Cu-O) 538.47, v(Cu-N) 431.16. E.A: C 60.82, H 4.01, N 5.07. Found: C 59.35, H 3.88, N 4.96.

3.3.4.3 C3: (Z)-4-chloro-2-((p-tolylimino)methyl)phenol with Cu(II) chloride

The complex was prepared analogously to C1. A solution of $CuCl_2.2H_2O$ (100mg, 0.400 mmol) and HL^3 (100mg, 0.410 mmol) were used as starting materials. A myrtle green product was obtained and it was treated as in C1. Yield: 166.2 mg (75.54%). MP (181-186 °C). IR data (KBr, v/cm^{-1}): v(HC=N) 16093.74, v(C=C) 1462.99, v(C-O) 1321.71, v(Cu-O) 526.68, v(Cu-N) 424.28. E.A: C 60.82, H 4.01, N 5.07. Found: C 59.70, H 3.83, N 4.87.

3.3.4.4 C4: (Z)-4-chloro-2-(((2,4-dimethylphenyl)limino)methyl) phenol with Cu(II) chloride

The complex was prepared analogously to C1. A solution of $CuCl_2.2H_2O$ (100mg, 0.400 mmol) and HL^4 (100mg, 0.390 mmol) were used as starting materials. An olive green product was obtained and it was treated as in C1. Yield: 177.1 mg (77.01%). MP (215-217 °C). IR data

(KBr, *v/cm*$^{-1}$): *v*(HC=N) 1601.63, *v*(C=C) 1457.52, *v*(C-O) 1319.88, *v*(Cu-O) 514.39, *v*(Cu-N) 457.96. E.A: C 62.02, H 4.51, N 4.82. Found: C 61.71, H 3.97, N 4.53.

3.3.4.5 C5: (Z)-4-chloro-2-(((2,6-dimethylphenyl)limino)methyl) phenol with Cu(II) chloride

The complex was prepared analogously to C1. A solution of $CuCl_2.2H_2O$ (100mg, 0.400 mmol) and HL^5 (100mg, 0.390 mmol) were used as starting materials. A phthalo green product was obtained and it was treated as in C1. Yield: 187.9 mg (81.70%). MP (211-213 °C). IR data (KBr, *v/cm*$^{-1}$): *v*(HC=N) 1604.82, *v*(C=C) 1461.32, *v*(C-O) 1323.17, *v*(Cu-O) 511.80, *v*(Cu-N) 458.62. E.A: C 62.02, H 4.51, N 4.82. Found: C 61.11, H 3.61, N 4.41.

3.4 Biological activity of the complexes

The bioactivity of the complexes was assessed following the biosafety level 2 guidelines at the Biolabels Node research labs (Department of Biotechnology, UWC). The biological waste was disposed according to the laboratory's disposal protocol outlined in their standard operating procedures.

3.4.1 Antimicrobial activity of the complexes

The Antibacterial activity of the complexes was evaluated on four bacterial strains of *E.Coli, P. Aeruginosa, S. Aureus* and *MRSA* using agar well diffusion method as described[17].

3.4.1.1 Bacterial culture

A single colony was picked from an agar plate containing each of the selected bacterial strains and transferred into a 2 mL tube that contains Muller Hinton broth. The cultures were incubated at 37℃ with shaking at 250 rpm for 2 hours.

3.4.1.2. Agar Well Diffusion Method

A cotton swab was dipped into the bacterial suspension and spread over the entire surface area of the agar. The $CuCl_2$ metal salt and the complexes were tested against the bacteria at two concentrations, 5mg/ml and 10mg/ml, using DMSO for the stock solution of both concentrations. Then 50 µL of the positive control (10 mg/L ciprofloxacin), negative control (DMSO) and stock solution were deposited into the wells that had been punctured and clearly labelled outside the plate. The plates were then incubated at 37℃ for 24 hours after treatment. The zones of inhibitions were observed and measured around each disc using vanier calliper.

3.5 References

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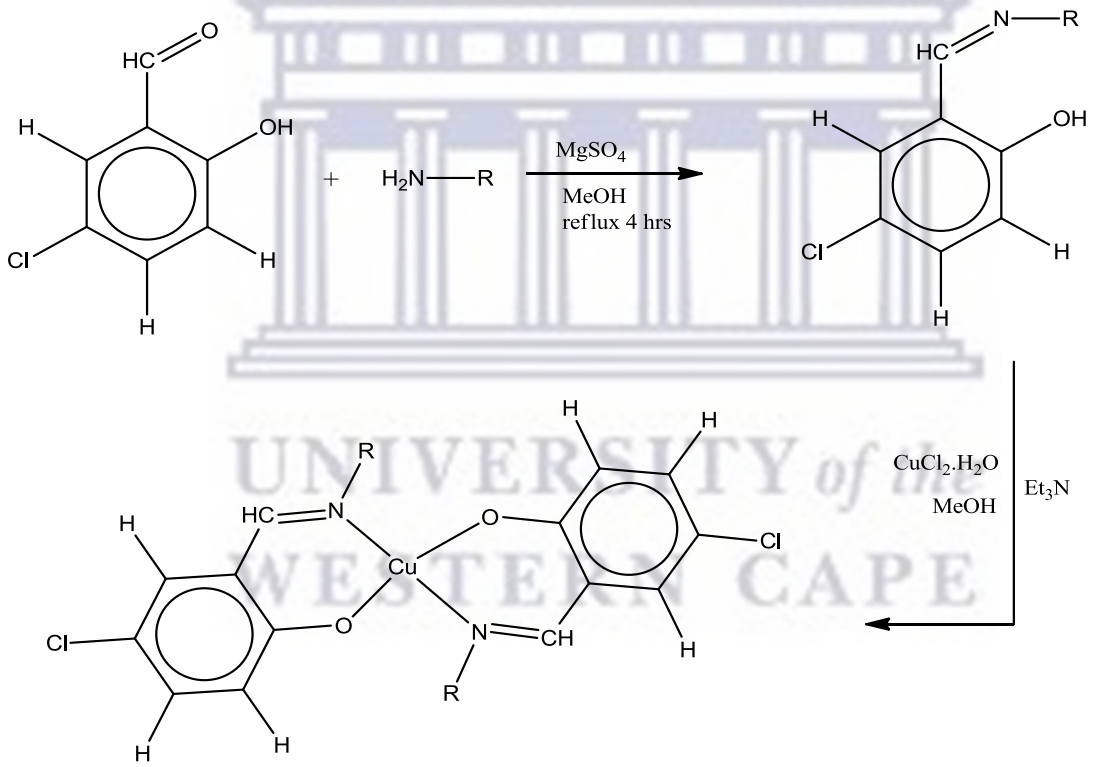
Chapter 4

Results and discussion

4.1 Introduction

Five Schiff base ligands were prepared from the reaction of 5-chlorosalicylaldehyde with aniline derivatives (aniline, 2-metyhylaniline, 4-methylaniline, 2,4-dimethylaniline, 2,6-dimethylaniline) in a 1:1 mole ratio. These ligands have substituents with different electronic effects, which may modify its physical and chemical properties. The method used was based on literature[1]. The general equation for the reaction is shown in Scheme 4.1.



R = Aniline, 2-Methylaniline, 4-Methylaniline, 2,4-Methylaniline, 2,6-Methylaniline

Scheme 4.1: General reaction for the preparation of Schiff base ligands and Cu(II) complexes.

The chemical and structural formulas of these ligands were confirmed by FTIR spectroscopy, UV-Vis spectroscopy, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. Each of these Schiff bases were then reacted with Cu(II) salt which is $CuCl_2$ according to the literature method[2]. This

metal ion was chosen because it has been shown in literature[3] that the inhibitory activity of the ligands increases after chelation to copper ion. It has also been reported in the study done by Nazirkar *et al*.[4] that Cu(II) has stronger affinity for biomolecules which makes the complexes to be more permeable through cell membrane, hence they have stronger antibacterial activity[5].

The chemical and structural formulas of these complexes were proposed from FTIR spectroscopy and elemental analyses. Additionally, the crystal structure was determined by single-crystal X-ray crystallography. Finally, the electronic and electrochemical properties of these complexes were respectively probed by UV-Vis spectroscopy and cyclic voltammetry.

4.2 Physical properties of the prepared Schiff base ligands compounds

According to the 1996 edition of Vogel's Textbook the prepared compounds were obtained in good yields (>60%). The elemental analysis (C.H.N) results were in good agreement with the theoretical values calculated, which indicated the identities and purities of the compounds. The ligands were obtained as solids in the form of crystals, whereas the complexes were in powder form. This is the common nature in which Schiff base ligands and complexes are usually obtained[6]. The ligands were obtained in the colour yellow, and the complexes were in shades of green. The yellow colour of ligands is caused by the hyposochromic shift in the ultraviolet absorption spectra which is a result of extension conjugation[7].

The complexes were obtained in the colour green, which can be explained based on the nature of the ligand. The salicylaldehyde Schiff base ligands synthesized are weak field ligands which causes the complexes to absorb lower-energy light hence the green colour. The colour of the compounds are similar to the ones reported in the study done by Ahmed *et al*. for the synthesis of Zn(II) complex with tridentate (NNO Donor) Schiff Base Ligand[8]. Therefore, it can be concluded that the Schiff base ligands coordinated to the metal centre of complexes have an influence in the colour of complexes.

4.2.1 Melting point

The melting point of the Cu(II) Schiff base complexes were higher than of the ligands, which is an indication that they are more stable than ligands. Consequently, it is also an indication that Schiff base complexes have higher thermal stability. From the results obtained, it was observed that the melting point of the ligand depends upon the attached group to the nitrogen atom of *H*C=N. The group attached to the ligand has an influence on molecular packing, subsequently the size of the whole ligand[9].

4.2.2 Solubility

One of the main tasks of this project was to determine solubility of ligands in different solvents. In order to find efficient ways to develop new drugs, it is important to have a model that can predict compound properties that do not fulfil specific requirements for drug like compounds. One of these compound properties is solubility. Solubility can be described in different ways such as intrinsic solubility, which refers to the substance's neutral state, and apparent solubility, which may be measured for compounds that are ionizable at a certain pH. The compounds were completely soluble in common organic solvents at room temperature in ethanol, chloroform, DCM, and DMSO. The polar character of the synthesized compounds contributed to their solubility in a common polar solvent. However, a few of them required heating and thus were regarded as poor in solubility rather than insoluble. The compounds were soluble in ethanol because ethanol's structure enables for the dissolving into polar, non-polar and hydrophilic and hydrophobic compounds.

DMSO, is commonly used in the pharmaceutical industry and is recognized as the most potent readily accessible organic solvent. Balakin *et al*. reported that the solvent has high dielectric constant which causes it to have solubilizing ability, but that can also be linked to stereochemistry since the molecular structure of DMSO is a trigonal pyramid with a lone pair

on top[10]. One of the properties of DMSO that makes the compounds to completely dissolve is that it is a polar aprotic solvent that is miscible in a variety of organic solvents as well as water.

4.3 FTIR spectroscopy

The IR spectrum of HL^1 and C1 is given in Figure 4.3. The other IR spectra of the Schiff base ligands their Cu(II) complexes are given in Appendix 1.

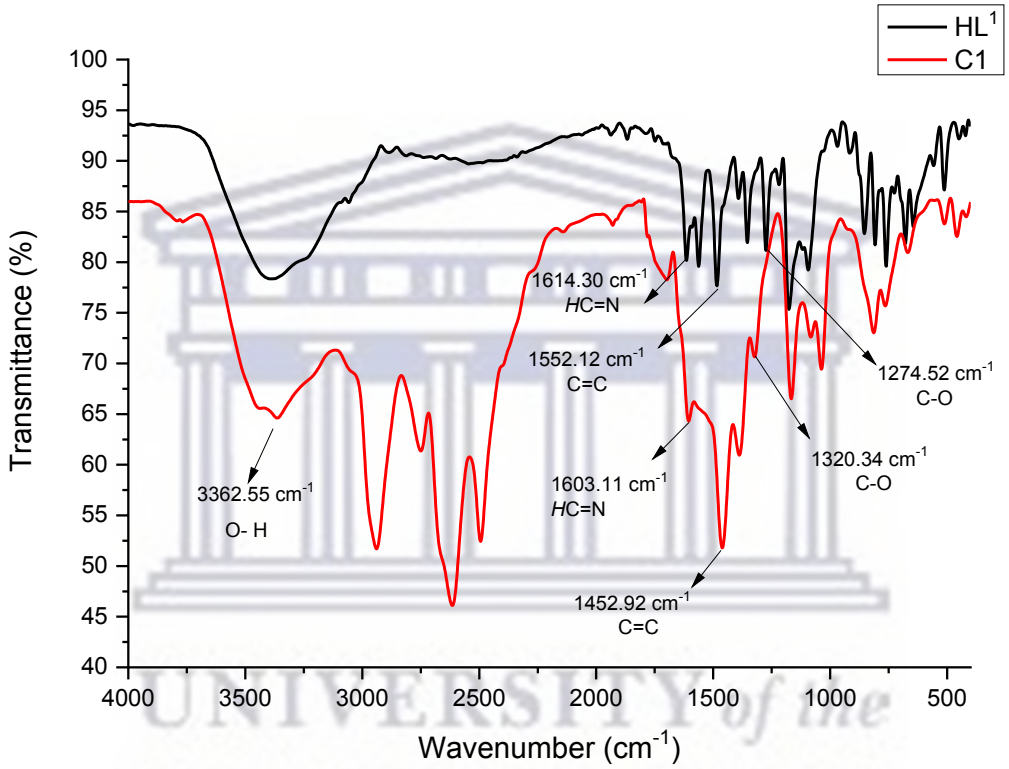


Figure 4.1: FTIR spectrum of HL^1 - (Z)-4-chloro-2-((phenylimino)methyl)phenol and its Cu(II) complex.

The IR spectra showed all the desired functional groups of the Schiff base ligands and their complexes. The IR spectra of the Schiff base ligands revealed the most characteristic absorption bands *ν*(HC=N), *ν*(OH), *ν*(C-O) and *ν*(C=C). The spectra showed a strong band in the region 1610.39 – 1623.19 cm^{-1} which is characteristic of azomethine (stretching frequency (*H*C=N) group, indicating the formation of the Schiff base. The azomethine group (*H*C=N) stretching frequency was in agreement with literature. The range is similar to that of ligands

synthesized by Sobola *et al*.[11] and Bushra *et al*.[12]. The spectra also exhibited a broad band of stretching frequency of OH group at 3337.90 – 3391.94 cm^{-1}, which is closely related to the results recorded by Bhushan Nazirkar *et al*. The broadness of the OH group peak was due to a strong intramolecular hydrogen bonding between the imine nitrogen and the hydroxyl group. Additionally, the phenolic C-O group of the ligands appeared as a strong band at 1278.70-1274.52 cm^{-1}, which was confirmed based on the findings by Bushra *et al.* and Sobola *et al.* The C=C sharp peak was noted to appear in the range 1569.76-1561.21 cm^{-1}.

The IR spectra of the Schiff base Cu(II) complexes, however, the functional groups peaks shifted and new groups were discovered. The complexes *ν*(*H*C=N) stretching frequency appeared at the range 1601.06-1610.06 cm^{-1}, which shifted to a lower wavelength number as compared to the ligand. This shifting is a result of the chelation of the imine nitrogen group to the metal atom[13-16]. The broad *ν*(OH) band of the ligand phenolic group disappeared in complexes spectra, indicating deprotonation of the phenolic group[17]. This finding was further supported by C-O stretching bands blue-shifting to the region 1389.29-1319.88 cm^{-1} respectively, proving that the phenolic oxygen atom was involved in the coordination sphere[18-20].

The complexes, however, showed broader new peaks that were assigned to coordinated water in the region 3371.58-3339.96 cm^{-1}. The Schiff base ligand mode of coordination was further supported by the presence of the two additional bands that appeared in the complexes spectra at 567.64-511.80 cm^{-1} and 458.62-424.28 cm^{-1} attributed to the *ν*(Cu-O) and *ν*(Cu-N) stretches[19,21,22]. A peak around 1596 cm^{-1} was observed by Samposion *et al*. in their copper complex spectrum, which was attributed to the *ν*(C=C) group. Similarly, in this study the complexes spectra exhibited a peak in the range 1462.99-1452.92 cm^{-1} which belongs to the *ν*(C=C) group[23,24].

4.4 UV-Vis spectroscopy

The electronic absorption spectra of the compounds were carried out using 1:9 dilution ratio in DMSO at standard conditions and the results were recorded in the region 220 - 500 nm. The electronic spectra of HL^1 and C1 is given in Figure 4.4 and Figure 4.5, the other spectra of ligands (HL^4-HL^5) and complexes (C2-C5) can be found in Appendix 1.

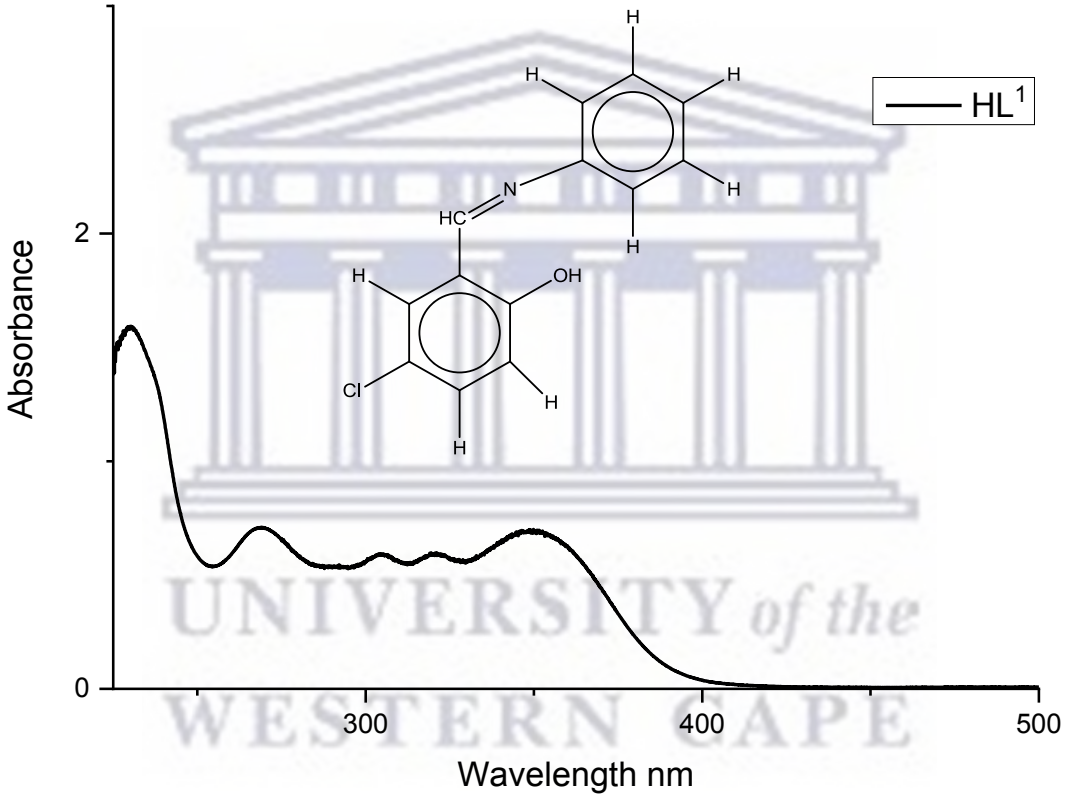


Figure 4.2: UV-Vis spectrum of HL^1 - (Z)-4-chloro-2-((phenylimino)methyl)phenol

Table 4.1: UV-Vis data of the Schiff base ligands

Ligands	Electronic transitions		
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	λmax
HL^1	269.02	350.45	231.20
HL^2	265.51	350.93	230.77
HL^3	266.32	350.86	231.81
HL^4	235.71	284.36	-----
HL^5	236.24	283.58	-----

The ligands HL^1-HL^3 exhibited two bands at λ = 265.51 - 269.02 nm and λ = 350.45 - 350.86 nm, which were associated with transitions $\pi \rightarrow \pi^*$ of the aromatic ring and $n \rightarrow \pi^*$ of the azomethine group, respectively. The ligands also showed absorption bands at λ = 230.77 - 231.81 nm has electronic transition of Schiff base azomethine group. For the ligands HL^4 and HL^5, the transition band $\pi \rightarrow \pi^*$ of the aromatic ring was around λ = 235 nm and λ = 284 nm for the $n \rightarrow \pi^*$ band transition of the azomethine group. These values are in agreement with other Schiff base ligands transitions reported in literature by Bushra *et al.*[12]. Hussain *et al* also reported similar absorption bands in his study about Schiff bases of sulfamethoxazole[25].

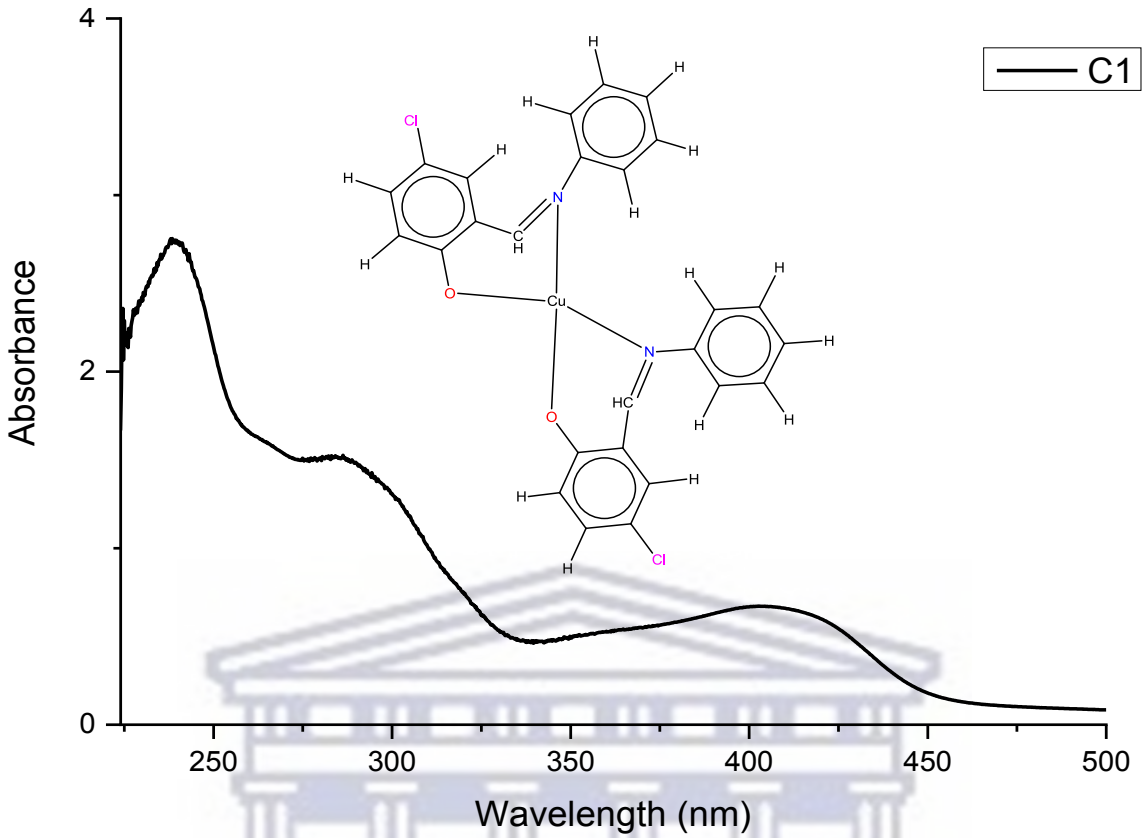


Figure 4.3: UV-Vis spectrum of C1

The prepared complexes exhibited similar results; the bands were different from those of ligands. The complexes spectra exhibited two absorption bands in the UV region between at 272.47-269.05 nm and 306.25-312.50 nm. Studies showed the assignment of the observed intense high-energy band are attributed to the $\pi \rightarrow \pi^*$ intra-ligand charge transfer (ILCT) transition,[26,27] and the $n - \pi^*$ ligand-to-metal charge transfer (LMCT) transition[28]. When compared to their respective ligands, all the complexes showed a shift in the transition's values for all absorption bands. The complexes shifted to lower wavelengths in the regions assigned to $n - \pi^*$ transition of the azomethine functional group. This shift demonstrated that nitrogen atoms donated lone pairs to the metal for coordination, and therefore successful synthesis of the complexes. Bartylzel[29] reported similar results, they obtained bands around 280 nm and 363-381 nm, respectively. The bands in complexes were more intense in comparison with the Schiff base ligands, Shafaatian *at el.*[30] and Grivani *et al.*[31] described this behaviour as a result

of extensive conjugation of π electrons in the complexes which is an indication of the anionic character of the Schiff bases and the chelation of the ligands to Cu (II) ions.

4.5 NMR spectroscopy studies

NMR was used for the structural elucidation of the ligands, ^{1}H and ^{13}C were the two experiments employed to provide information about the ligands characteristics and to further confirm if the Schiff base framework was successful. The ligands were dissolved in deuterated chloroform at room temperature using trimethyl silane (TMS) as internal standard. The solvent was chosen due to its ability to readily dissolve all the synthesized ligands.

4.5.1 ^{1}H NMR

A typical ^{1}H NMR spectrum of the Schiff base ligands (HL^{1}) is given in Figure 4.6, the other ligands spectra are given in Appendix 1. Analysis of the ligands spectra indicated that the Schiff base condensation reaction was successful. This was confirmed by an observed proton signal at 8.19 – 8.50 ppm (singlet) due to the hydrogen atom in the azomethine (***H***C=N) moiety. The ligands contained no traces of unreacted aldehyde (C**H**O) protons, which could be confirmed by the absence of signals in the region 9.50 – 10.5 ppm. The region of the azomethine functional group is in conformity with studies reported by Bushra *et al*.[12] where they observed the sharp signal at the region 8.2-8.3 ppm. The imine protons appeared downfield because of the decreased electron density in moiety which deshields the protons. The protons thus feel a stronger magnetic field and a higher frequency is needed for resonance; thus they are deshielded and absorb downfield. However, the methyl group protons appear as a singlet(s) within 2.10 - 2.40 ppm, since it is an electron donating group there is an increased electron density, resulting in lower frequency needed to achieve resonance hence lower chemical shift. Aromatic protons appear as multiplets in the range of 6.57–7.60 ppm and are in good agreement with the integral areas.

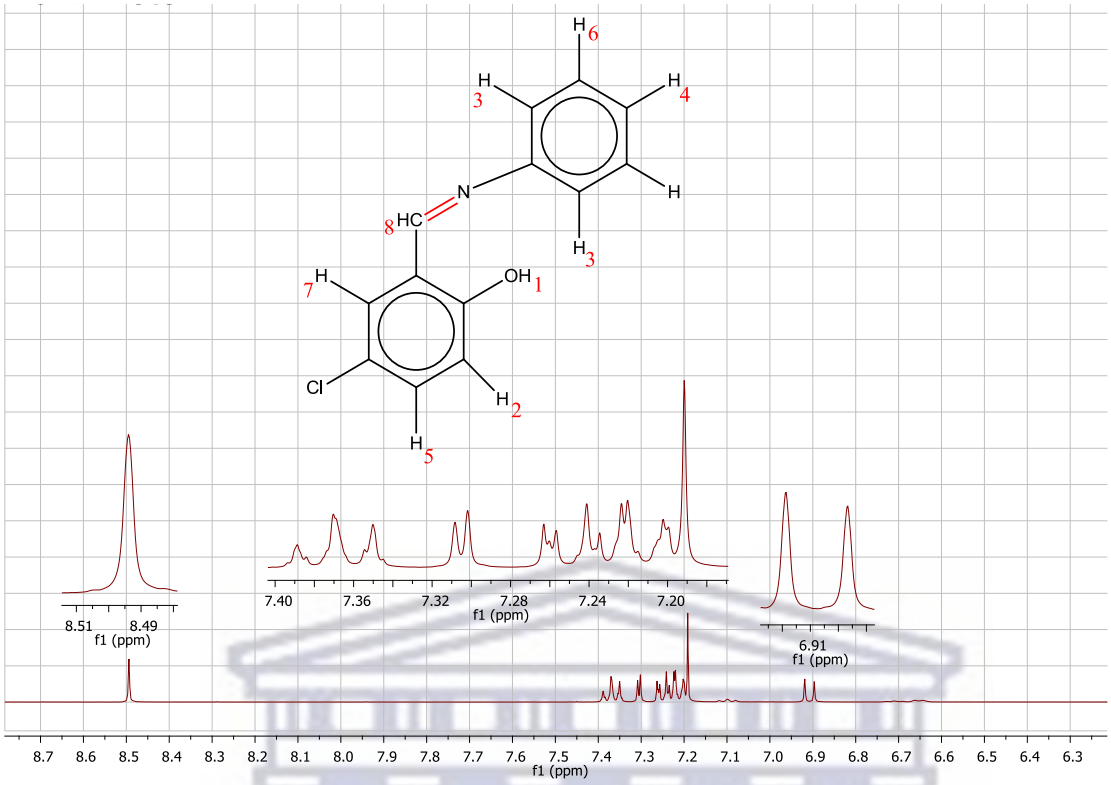


Figure 4.4: ^{1}H NMR of HL^{1}

4.5.2 ^{13}C NMR

The ^{13}C NMR spectra of ligands HL^{1}-HL^{5} are displayed below. The allocation of the carbon peak values was done according to literature functional group chemical shifts values. Sobola *et al.*[11] reported in the literature their observations of the imine functional group of the prepared ortho-substituted aniline Schiff base ligands around 163.51-160.88 ppm which further proved the Schiff base synthesis was successful. This was similar to the results obtained in this study. The signals for the azomethine carbons appeared around 156.60-160.00 ppm. In the work done by Gokulnath *et al.* for the synthesis of Schiff base ligands derived from salicylaldehyde with 4-amino benzoic acid, the ligands ^{13}C NMR spectra exhibited phenolic carbon signals in the region 165.29 - 167.26 ppm[32]. Similarly, the phenolic carbon signals for this study were observed at 161.10-165.70 ppm. The aromatic carbons were in the range 115.18-147.88 ppm as displayed in Figure 4.5.

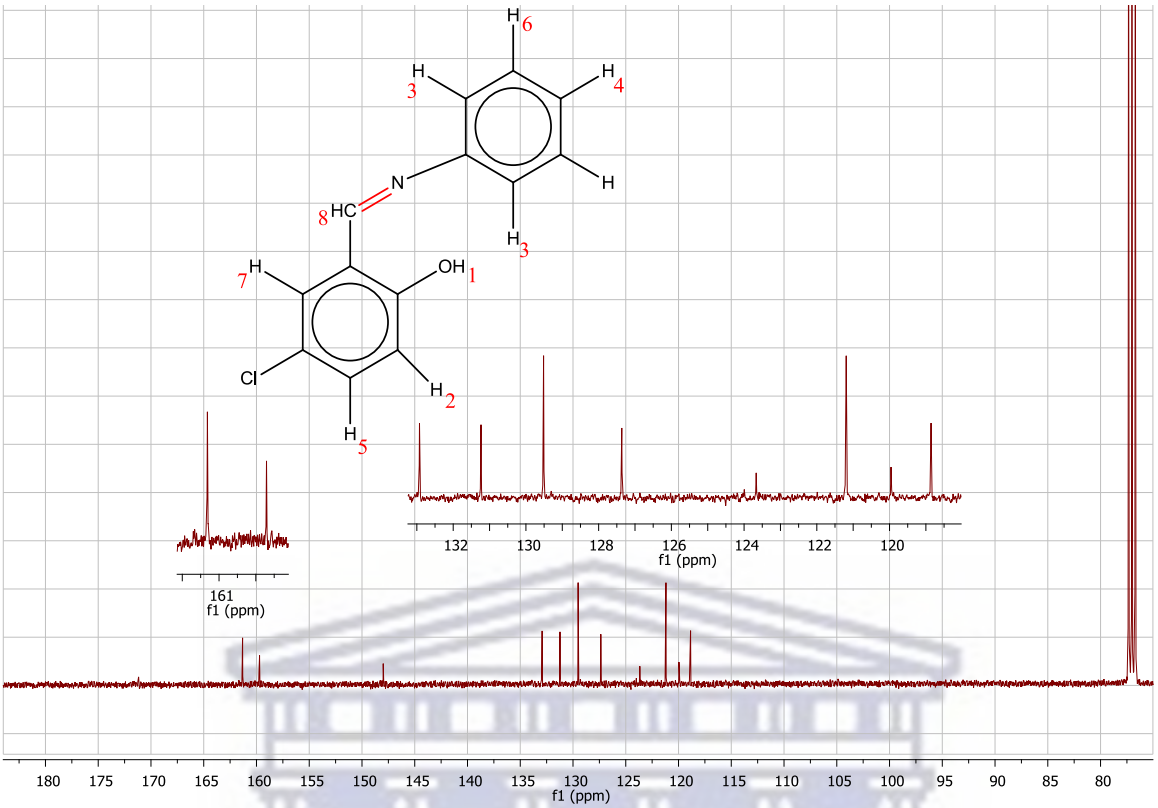


Figure 4.5: ^{13}C NMR of HL^{1}

4.6 Electrochemical studies

The electrochemical behaviour of the complexes was evaluated by cycling the potential between -2.0 and 2.5 V at 50 and 100 mV/s as shown in Figure 4.6.

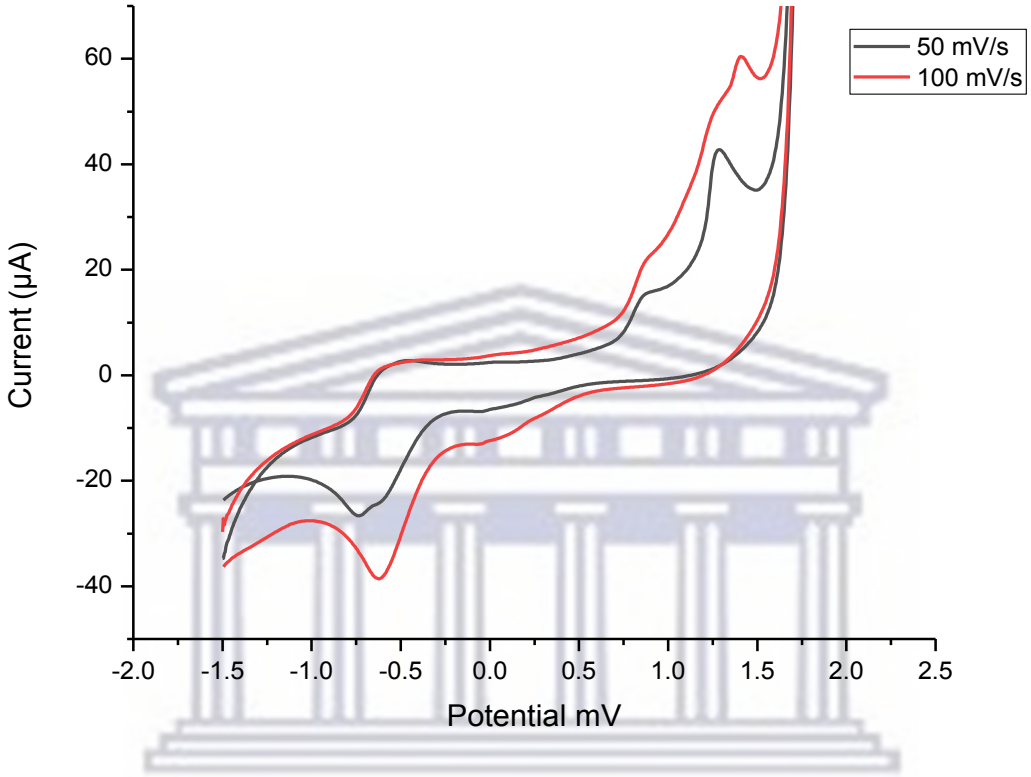


Figure 4.6: Cyclic voltammogram of C1 at scan rate of 50 and 100 mV/s.

Copper(II) is considered as one of the biologically important chemical species that serves as a co-factor in metalloproteins and metalloenzymes. Its redox activity may be crucial for maintaining its biological functions in interaction with various biomolecules, notably those that act as electron donor atoms at various pH levels. The present study reports the electrochemical redox behaviour of Cu(II), in Table 4.2 below are the redox current and potential values of the copper complexes.

Table 4.2: The oxidation and reduction peak current and potential values of the Cu(II) complexes

Scan rate mV/s	Anodic peak current Ipa (μA)	Cathodic peak current Ipc (μA)	Anodic peak potential Epa (mV)	Cathodic peak potential Epc (mV)	Formal potential E◦ (mV)	Ipa/Ipc
Cu(II)HL[1]						
50	42.8	26.6	1.29	0.74	0.55	1.61
100	60.4	38.5	1.41	0.61	0.80	1.57
Cu(II)HL[2]						
50	10.2	25.7	0.86	0.80	0.06	0.40
100	20.9	40.3	0.89	0.61	0.28	0.52
Cu(II)HL[3]						
50	51.7	91.3	0.92	0.66	0.26	0.57
100	18.6	42.0	0.89	0.61	0.28	0.44
Cu(II)HL[4]						
50	10.5	26.3	0.88	0.80	0.08	0.40
100	23.1	42.3	0.91	0.62	0.29	0.55
Cu(II)HL[5]						
50	11.4	27.0	0.87	0.80	0.07	0.42
100	24.0	43.1	0.90	0.63	0.27	0.56

In Table 4.3 above, it is noticeable that the cathodic peaks exhibit higher current response and shifted to lower potential values, but anodic peaks show lower values and shifted to higher potential values. That is, the potential values of the anodic peaks increased with increasing scan rate, whereas the potential at the cathodic peaks decreased with increasing scan rate[33,34]. This observation was also based on how the voltagramms deviate from a diffusion-controlled polarogram shaped curve to nearly diffusion-controlled peak- shaped curve as the scan rate was increased. The electrochemistry of the complexes showed one reversible redox couple

representing good rate capability, better capacitive behaviour of the electrode material good diffusion-controlled electron mobility[35].

Higher scan rate results in a higher number of redox reactions due to the presence of the electroactive species at the electrode's (working electrode) surface. The cyclic voltammetric curves exhibited redox couple at potentials in the range of 0.86-1,41 (anodic) and 0.61-0.80 (cathodic) which can be attributed to the reduction of the metal centre[36,37]. From the Table 4.3 above, the voltammograms also showed a directly proportional relationship between the scan rate and current. It has been reported in by Sohel Rana *et al*. in literature that an increase in scan rate increases the current number of time scanning in the system per second. When interaction between the metal and ligand increases, more complexes are formed which means there are more electrons transferred hence the current increases[38].

4.7 Single X-ray crystallography

The Schiff base compounds were unequivocally characterized using X-ray diffraction studies to elucidate the effects of substituents, the nature of the metal centre on conformation, the conjugation bond degree, and the molecular interactions in the crystals. The Schiff base ligands were yellowish shiny crystals and the Cu(II) complexes were green-brownish crystals. The molecular structures of HL^1 and C1 are depicted in Figure 4.10 and Figure 4.11, the molecular structures of HL^4, HL^5, C2, C3 are given in Appendix 1.

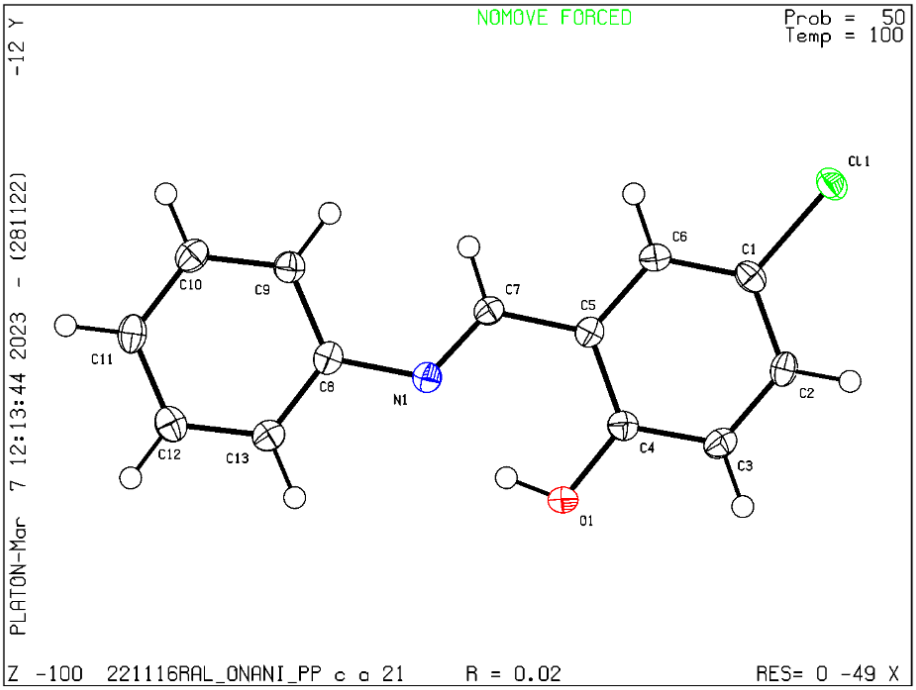


Figure 4.7: X-ray crystal structure of HL[1].

Table 4.3: Crystallographic data and refinement for the Schiff base ligands

Crystallographic data	HL^1	HL^4	HL^5
Empirical formula	$C_{13}H_{10}ClNO$	$C_{30}H_{28}Cl_2N_2O_2$	$C_{15}H_{14}ClNO$
Formula weight	231.67	519.44	259.72
Temperature (K)	100	100	100
Crystal system	Orthorhombic	Monoclinic	Orthorhombic
Space group	$Pca2_1$	$P2_1/n$	$P2_12_12_1$
a (Å)	12.1794 (2)	4.6658(1)	3.8881(1)
b (Å)	4.4837 (1)	19.5745(4)	15.9434(4)
c (Å)	19.2644 (3)	13.7512(2)	19.9126(4)
V (Å^3)	1052.01 (3)	1248.79(4)	1234.37(5)
Z	4	2	4
$F_{(000)}$	480.0	544.0	544.0
μ ($mm^{-1)}$	3.00	2.584	2.617
D_x (Mg m^{-3})	1.463	1.381	1.398
Cu *K*α radiation, λ (Å)	1.54184	1.54184	1.54184
θ max (°)	71.279	70.964	71.085
θ min (°)	4.6	3.944	3.544
h	14	5	4
k	5	23	19
l	23	16	24
Npar	150	166	166
Measured reflections	10798	11576	13155
Independent reflections	1999	2338	2328
Reflections with I>2σ(I)	1987	2075	2235
Final R indices [I > 2σ(I)]	R_1 = 0.0213 wR_2 = 0.0577	R_1 = 0.0385 wR_2 = 0.1009	R_1 = 0.0336 wR_2 = 0.0949
Goodness-of-fit on F^2 (S)	1.063	1.087	1.069

The Schiff base condensation reaction between 5-chlorosalicylaldehyde and three amine derivatives namely, aniline, 2,4-dimethylaniline and 2,6-dimethylaniline was proven to be successful by the single x-ray crystallography by depicting the desired molecular structures of the ligands. The Schiff base ligand were crystallised as orthorhombic, monoclinic at different space groups, P*ca*2_1, P2_1/*n*, and P$2_12_12_1$.

Table 4.4: Selected lengths and bond angles for the Schiff base ligands.

Schiff base ligand	Bond length (Å)		Bond angle	
HL^1	Cl(1)-C(1)	1.746(2)	C(4)-O(1)-H(1)	108(2)
	O(1)-C(4)	1.350(3)	C(7)-N(1)-C(8)	121.7(2)
	O(1)-H(1)	0.86(4)	N(1)-C(7)-H(7)	119.6
	N(1)-C(7)	1.288(3)	N(1)-C(7)-C(5)	120.8(2)
	N(1)-C(8)	1.425(3)	O(1)-C(4)-C(5)	121.3(2)
			O(1)-C(4)-C(3)	118.8(2)
			Cl(1)-C(1)-C(6)	119.7(2)
			Cl(1)-C(1)-C(2)	119.0(2)
			N(1)-C(8)-C(13)	116.4(2)
			N(1)-C(8)-C(9)	124.2(2)
HL^4	Cl(1)-C(1)	1.747(2)	C(7)-N(1)-C(8)	123.66(2)
	O(1)-C(4)	1.343(3)	C(13)-C(8)-N(1)	124.40(2)
	N(1)-C(7)	1.284(3)	C(9)-C(8)-N(1)	116.30(2)
	N(1)-C(8)	1.412(3)	N(1)-C(7)-C(5)	120.69(2)
			O(1)-C(4)-C(3)	119.04(2)
			O(1)-C(4)-C(5)	121.39(2)
			C(6)-C(1)-Cl(1)	119.50(2)
			C(2)-C(1)-Cl(1)	119.36(2)
HL^5	Cl(1)-C(1)	1.748(2)	C(7)-N(1)-C(8)	122.2(2)
	O(1)-C(4)	1.348(3)	C(6)-C(1)-Cl(1)	119.24(2)
	N(1)-C(7)	1.281(3)	C(2)-C(1)-Cl(1)	119.50(2)
	N(1)-C(8)	1.426(3)	C(13)-C(8)-N(1)	123.4(2)
			C(9)-C(8)-N(1)	115.3(2)
			O(1)-C(4)-C(3)	118.8(2)
			O(1)-C(4)-C(5)	121.3(2)
			N(1)-C(7)-C(5)	121.3(2)

The structure determination of the ligands shows evidence for the existence of the azomethine group, which is a confirmation that the Schiff base framework was successfully achieved. In the crystal packing of the ligands, the structural data shows that the N(1)-C(8) bond length is

the range 1.412-1.425 Å while azomethine bond N(1)-C(7) is in the range 1.281-1.288 Å which are in good agreement with those reported by Eren *et al*. for the a new azo-azomethine ligand, 4-[(*E*)-phenyldiazenyl]-2-[(*E*)-{[4-(propan-2-yl)phenyl]imino}methyl]phenol (*HL*) and its Cu(II) complex, [CuL_2][39]. Al-Khathami *et al*. also reported similar results of pyridine and phenol ligands with azomethine bond length 1.273 Å [40]. The mean planes of O(1)-C(4) and Cl(1)-C(1) are in the ranges 1.343-1.350 Å and 1.746-1.748 Å to the central ring. The possibility of the attachment of chlorine and oxygen to the central ring is ruled out based on the angles around C1 and C4. The summation of the three angles around C1 are 358.44 Å for Cl(1)-C(1)-C(6) and 357.86 Å for C(2)-C(1)-Cl(1). The summation of the angles around C4 are 363.99 for O(1)-C(4)-C(5) and 356.64 for O(1)-C(4)-C(3). These values are close to the ideal value if 360 which is expected from the coplanar atoms C1, C3, C4, C5 and C6.

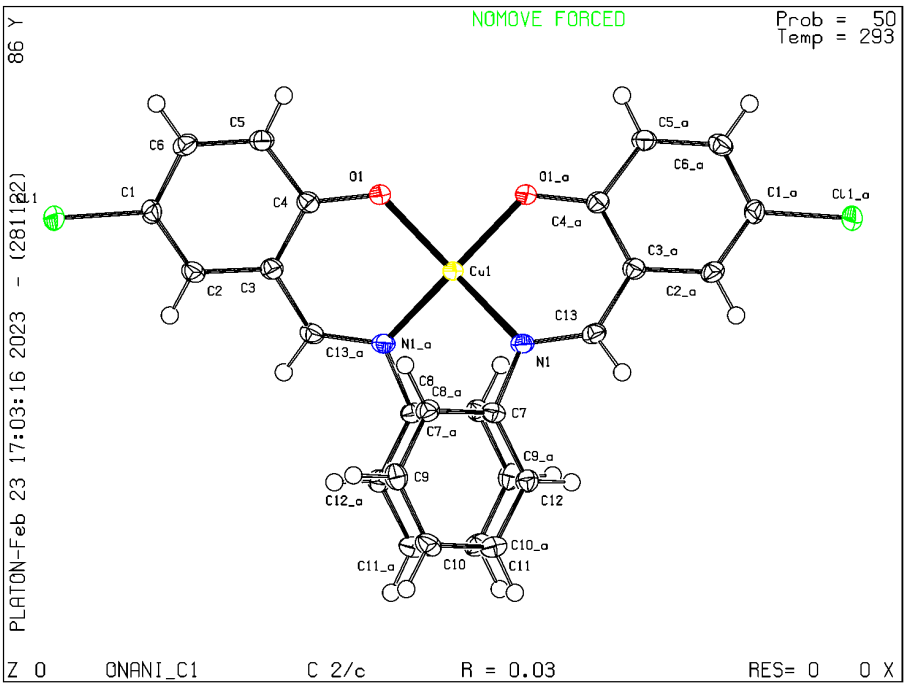


Figure 4.8: X-ray crystal structure of C1.

Table 4.5: Crystallographic data and refinement for the Schiff base Cu(II) complexes

Crystallographic data	C1	C2	C3
Empirical formula	$C_{13}H_9ClCuNO$	$C_{14}H_{11}ClCu_{0.5}0NO$	$C_{14}H_{11}ClCuNO$
Formula weight	294.20	276.46	308.23
Temperature (K)	293	100	100
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	C2/*c*	$P2_1/n$	$P2_1/c$
a (Å)	20.0297 (3)	9.8344 (2)	9.82202 (13)
b (Å)	9.28855 (12)	9.9193 (1)	10.54900 (12)
c (Å)	14.8261 (2)	12.8507 (2)	11.83716 (14)
β (°)	127.5888 (13)	108.326 (2)	102.0481 (12)
V (Å^3)	2185.74 (6)	1190.01 (4)	1199.46 (3)
Z	8	4	4
$F_{(000)}$	1184	566	624
μ ($mm^{-1)}$	4.89	3.60	4.49
D_x (Mg m^{-3})	1.788	1.543	1.707
Cu *K*α radiation, λ (Å)	1.54178	1.54184	1.54178
θ max (°)	70.913	71.1	71.1
θ min (°)	5.52	5.0	4.6
h	24	12	11
k	11	12	12
l	17	15	14
Measured reflections	19133	20971	21374
Independent reflections	2075	2252	2284
Reflections with I>2σ(I)	1978	2075	2190

Table 4.6a: Selected lengths and bond angles for C1.

Complex	Bond length (Å)		Bond angle	
C1	Cu(1)-N(1)	1.979	O(1)-Cu(1)-N(1)	93.78
	Cu(1)-O(1)	1.898	O(1)-Cu(1)-O(1)	87.73
	Cl(1)-C(1)	1.753(2)	N(1)-Cu(1)-O(1)	151.81
	N(1)-C(7)	1.436(2)	N(1)-Cu(1)-N(1)	97.86
	N(1)-C(13)	1.296(2)	Cl(1)-C(1)-C(2)	119.9(2)
			Cl(1)-C(1)-C(6)	119.2(2)
			Cu(1)-N(1)-C(7)	119
			Cu(1)-N(1)-C(13)	123.2
			C(7)-N(1)-C(13)	117.7(2)
			C(3)-C(13)-N(1)	126.5(2)
			N(1)-C(13)-H(13)	116.7
			Cu(1)-O(1)-C(4)	127
			N(1)-C(7)-C(12)	121.6(2)
			N(1)-C(7)-C(8)	118.1(2)

Table 4.6b: Selected lengths and bond angles for C2.

Complex	Bond length (Å)		Bond angle	
C2	N(1)-C(7)	1.295(3)	C(7)-N(1)-C(8)	115.5(2)
	N(1)-C(8)	1.462(3)	C(7)-N(1)-Cu(1)	123.9
	N(1)-Cu(1)	2.012	C(8)-N(1)-Cu(1)	120.3
	C(1)-Cl(1)	1.748(2)	C(6)-C(1)-Cl(1)	120.0(2)
	O(1)-Cu(1)	1.878	C(2)-C(1)-Cl(1)	118.9(2)
			N(1)-C(7)-C(5)	126.2(2)
			N(1)-C(7)-H(7)	116.9
			N(1)-C(8)-C(9)	119.5(2)
			N(1)-C(8)-C(13)	118.9(2)
			C(15)-O(1)-Cu(1)	130
			N(1)-Cu(1)-O(1)	92.12
			N(1)-Cu(1)-N(1)	180
			O(1)-Cu(1)-N(1)	87.88
			O(1)-Cu(1)-O(1)	180

Table 4.6c: Selected lengths and bond angles for C3.

Complex	Bond length (Å)		Bond angle	
C3	Cl(2)-C(11)	1.749(2)	C(7)-N(1)-C(1)	115.8(1)
	N(1)-C(7)	1.297(2)	C(7)-N(1)-Cu(1)	122.9
	N(1)-C(1)	1.440(2)	C(1)-N(1)-Cu(1)	120.5
	N(1)-Cu(1)	2.007	N(1)-C(7)-C(9)	126.5(1)
	O(1)-Cu(1)	1.888	N(1)-C(7)-H(1)	117(1)
			N(1)-C(1)-C(6)	119.9(1)
			N(1)-C(1)-C(2)	120.3(1)
			Cl(2)-C(11)-C(12)	119.5(1)
			Cl(2)-C(11)-C(10)	119.6(1)
			N(1)-Cu(1)-O(1)	91.24
			N(1)-Cu(1)-N(1)	180
			O(1)-Cu(1)-N(1)	88.76
			O(1)-Cu(1)-O(1)	180
			Cu(1)-N(1)-C(1)	120.5
			C(7)-N(1)-C(1)	115.8(1)
			N(1)-C(7)-H(1)	117(1)

The Jahn-Teller effect defines the coordination chemistry of Cu(II) which manifests as a tetragonally elongated octahedral configuration [41]. All the Cu(II) Schiff base complexes were crystallised as monoclinic at different space groups, C2/*c*, $P2_1/n$ and $P2_1/c$. The space group $P2_1/c$ has only one beta angle, which can be different from 90°, it is an angle between axes *a* and *c*. Contrarily, the beta angles differs strongly from 90° and the cell is more skewed and oblique in space group $P2_1/n$. The space group C2/*c* is a combination of the two primitive monoclinic groups P2/*c* and $P2_1/c$, when these two space groups are combined they generate C-centred lattice translations [42]. In all three cases, the molecule consists of a Cu(II) metal centre, salicylaldehyde moiety and aniline derivative. The Schiff base ligands are bound in a chelating bidentate mode to the Cu(II) ion via the nitrogen atoms of the azomethine group and oxygen atoms, respectively. The coordination geometry around the four-coordinated Cu(II) metal is a slightly distorted square planar with bond angles ranging from 87.88-180 Å, respectively. The angles of copper bonded to oxygen and nitrogen in C2 and C3 are precisely close, however, in the case of C1 the angles decreased.

This can be explained based on the molecular structures of C2 and C3, which contain methyl group as a substituent in the *ortho* and *para* position. According to the IUPAC stability constants database, copper(II) is a classic borderline electron-pair acceptor that forms strong complexes with borderline-soft ligands that contain nitrogen donor atoms, but it also has the ability to effectively bind to oxygen donor ligands. In all the compounds, Cu-O bond lengths were relatively shorter than Cu-N bond lengths, which is closely related to the work reported by Sixt *et al*. [43] and Lavaee *et al*. [44]. The atomic radius of oxygen is much smaller in comparison to the radius of nitrogen when binding to Cu(II) hence the Cu-O bond lengths are slightly shorter than Cu-N [45]. The carbon to nitrogen bond lengths of the ligands are in the range 1.296(2) - 1.297(2) Å, according to Hao *et al*. [46] and Duan *et al*. [47] these are typical values for Schiff base compounds double bond. The second carbon to nitrogen bond of 1.440 (2) – 1.462(3) Å is an indication of the single bond character to the aromatic ring of the Schiff base ligand.

4.8 Antimicrobial activity

A variety of variables, such as newly developing infectious diseases and an increase in the number of microbial pathogens that are multi-drug resistant, contribute to the fact that treating infectious diseases continues to be a significant and difficult challenge. Despite the number of antibiotics and chemotherapeutics that are now used in medicine, the development of both new and old antibiotic resistance over the past few decades has highlighted the critical need for new classes of antimicrobial drugs. There is a clear need for the discovery of new compounds with antimicrobial activity, possibly working through mechanisms that are separate from those of well-known antimicrobial agents because many clinical diseases are now resistant to the currently used classes of antimicrobial agents. Bioinorganic chemistry, which focuses on the use of metal complexes in biological systems, has opened a new horizon of research in coordination compounds.

The metal salt $CuCl_2$ and the Cu(II) Schiff base complexes were tested for their antimicrobial activity against *E. Coli* which is a gram-negative bacteria and *Staphylococcus Aureus*, a gram-positive bacteria using agar well diffusion method according to literature protocol. The $CuCl_2$ metal salt and the Cu(II) complexes showed inhibitory activity against *E.Coli* at both concentration and very weak activity against *Staphylococcus Aureus* as displayed in Figure 4.12. For this reason, the MIC values of the Cu(II) Schiff base complexes (C1-C5) were not further determined.

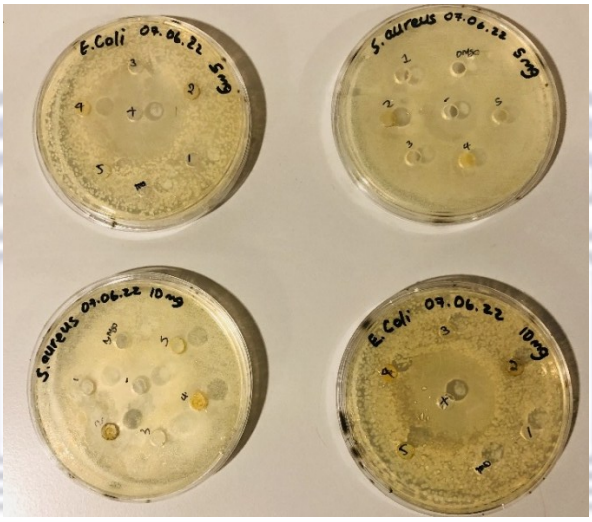


Figure 4.9: Antimicrobacterial activity of Cu(II) Schiff base complexes (C1-C5) against *E.Coli* and *Staphylococcus aureus*

The reason behind may be that it is highly likely that their ligands lose their coordination in solution medium, which then prevents their permeability into the cells resulting in poor activity. The complexes were expected to show high activity due the effect of the metal ions on the normal metabolic function of the cells. The expected high activity can be explained based on Overtone's concept of cell permeability Tweedy's Chelation theory[48-57]. The concept states that the lipid membrane surrounding the cell favours the passage of just the lipid-soluble materials, making liposolubility a key factor that regulates antibacterial and antifungal activity. Due to the overlap of the ligand orbital and partial sharing of the copper ion positive charge with donor

groups upon chelation, the polarity of the copper ion is reduced to a higher extent. Furthermore, this results in increased delocalization of π-electrons across the whole chelate ring and improves the lipophilicity of the complexes. The enhanced lipophilicity improves the ability of the complexes to penetrate through the lipid membranes and block the metal binding sites of the micro-organisms enzymes[58].

Other contributing factors that play a role in antibacterial activity of the complexes is the hydrophobic and hydrophilic properties. It has been proven in literature that the structural substituents of the coordinated ligands does affect the antibacterial potency of complexes[59-61]. The Schiff base ligands used to synthesize the Cu(II) complexes have methyl group(s) and/or a chlorine attached to the aromatic ring. Methyl groups are activating groups which means they donate electrons to the ring. Chlorine is a deactivating group which means it withdraws electrons from the ring. The complexes showed inhibitory activity towards the gram-negative bacteria, *E. Coli*, indicating that the complexes were partially hydrophobic which enables greater affinity towards the microbial bilayer membrane[62-64].

In most cases, gram-negative bacteria are usually responsible for health-related illnesses, for instance *E. Coli* bacterial strains have been reported to cause pneumonia, diarrhoea, urinary tract, food and blood poisoning[65]. The results obtained from this study indicate the structures that have the potential to be effective against bacteria, especially the gram-negative strains. Thus, these results open up for future development of compounds with biological potential as well as good direction for improved organometallic structural designs as structural models for antimicrobial agents.

4.9 References

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Chapter 5

5.1 Conclusion

The work done in this study was a preliminary work that will pave the way for the improvement of the first-line tuberculosis drugs. This thesis entailed the synthesis of five Schiff base ligands namely; **HL^1** = (Z)-4-chloro-2-((phenylimino)methyl)phenol, **HL^2** = (Z)-4-chloro-2-((o-tolylimino)methyl)phenol, **HL^3** = (Z)-4-chloro-2-((p-tolylimino)methyl)phenol, **HL^4** = (Z)-4-chloro-2-(((2,4-dimethylphenyl)limino)methyl)phenol and **HL^5** = (Z)-4-chloro-2-(((2,6-dimethylphenyl)limino)methyl)phenol} were synthesized and fully characterized. From the Schiff base ligands, Cu(II) complexes were successfully prepared by using $CuCl_2$ metal salt and evaluated in detail. The characterization techniques employed included FTIR, UV-Vis, ^{1}H NMR, ^{13}C NMR, CV, elemental analysis and SC-XRD. Furthermore, the synthesized Cu(II) complexes were evaluated for their antimicrobial potency against two bacteria strains, *E.Coli* and *S. Aureus*.

5.1.1 Synthesis summary

5.1.1.1 Characterization techniques results

All the Schiff base ligands and Cu (II) complexes were successfully prepared and obtained as solids in good yields 66-84%. The Schiff base ligands were synthesized using dry methanol and refluxed for 4 hours under nitrogen using $MgSO_4$ as the drying agent in a Schlenk line apparatus. The synthesis of the complexes was carried out at temperature under nitrogen, Et_3N was used to deprotonate the ligands and the product was precipitated in cold diethyl ether. This confirms that the first and fourth objectives were successfully accomplished. Furthermore, to successfully achieve the second and fifth objective the compounds were weighed on a scale to calculate yield%, melting point was determined using capillary tubes and a solubility test was performed. The compounds were found to be stable at room temperature and soluble in

common organic solvents. The Schiff base ligands were crystals and powder in texture, yellowish and orangish in colour, their melting point was in the range 76-100 °C. The Cu(II) Schiff base complexes were powder in texture, in shades of green and their melting point was in the range 162-217 °C. Elemental analysis further confirmed the success of the prepared Schiff base ligand and complexes.

The FTIR confirmed the successful preparation of the Schiff base ligands HL^1-HL^5 by the exhibition of the azomethine stretching frequency in the range of 1610.39 – 1623.19 cm^{-1}. However, in the IR spectra of complexes C1-C5 the azomethine group stretching frequency shifted to lower wavelength in the region 1601.06-1610.06 cm^{-1}, which proved that coordination via the nitrogen atom of the ligand took place. Additionally, the compounds exhibited two main peaks which were attributed to $\pi \rightarrow \pi^*$ intra-ligand charge transfer (ILCT) transition and the $n \rightarrow \pi^*$ of the ligand-to-metal charge transfer (LMCT) transition, and respectively. The ^1H and ^{13}C NMR spectra further confirmed that the Schiff base framework was successfully achieved by the presence of the proton single in the region 8.19-8.50 ppm and the carbon signals around 156.60-160.00 ppm. The ^1H NMR proved that the ligands contained no traces of unreacted aldehyde protons by the absence of signals in the region 9.50 – 10.5 ppm.

The Cu (II) Schiff base complexes were not characterized by NMR, due to the copper quadrupolar isotopes and paramagnetic nature of the Cu(II) ion, the signals in the spectra are very broad and make it almost impossible to be observed with a high-resolution NMR spectrometer. According to the data obtained from CV characterization, the potential values of the anodic peaks increased with increasing scan rate, whereas the potential at the cathodic peaks decreased with increasing scan rate which was in agreement with literature. The structural properties of the copper (II) complexes reported in this study showed the crystal structures

adopting square planar geometries around the central metal atom. Thus, the third and sixth objectives of the study were successfully achieved.

5.1.1.2 Antimicrobial studies

The $CuCl_2$ metal salt and the Cu (II) Schiff base complexes were tested for their antimicrobial studies against gram negative bacteria *E. Coli* and gram positive bacteria *S. aureus*. The metal salt and the complexes did not demonstrate wide-spectrum antimicrobial activity. Notably, the gram-negative bacteria was more susceptible to the tested complexes. The gram-negative bacteria are composed of an exterior lipid bilayer membrane rather than a cell wall. Which may explain why they were more susceptible, the complexes easily diffuse into the bacteria because of their strong affinity for the lipid outer membrane.

5.2 Recommendations and future work

Since the Cu (II) Schiff base complexes exhibited little to no activity for their antimicrobial activity. Stabilising the ligands may be employed first so that the complexes synthesized from them have high chances of killing the bacteria. To get a better understanding and design new compounds, it will be necessary to research further the mechanisms of action of the tested compounds. With knowledge that is provided by the results in this study, an attempt to synthesize copper centres with various counter ions (other than chlorine ions) of Cu (II) Schiff bases complexes in order to study their effect on structural and biological activities. Additionally, biologically active molecules with established antibacterial properties and modes of action can be appended with the Schiff base ligands. The complexes synthesized from the Schiff base ligands will then be able to precisely target the desired enzymes in the bacteria. This might enhance the activity of the complexes with less or no drawbacks. A cytotoxicity test will be done to check if they are harmful to mammalian cells.

The compounds can also be evaluated for other biological activities, such as anti-inflammatory, antifungal, anticancer and antioxidant. They have a huge potency not only in pharmacological tests but in catalysts too, so they can be used as catalysts in homogeneous reactions. The compounds can also be tested as catalysts in the polymerization of eco-friendly polymers with minor structural changes. Therefore, there is a lot of room to expand the scope of this study by evaluating these or similar complexes for different applications.

From the results of this study, we can now further investigate the potency of new compounds by which are a combination of either ofloxacin with pyrazinamide or isoniazid via Schiff base reaction. The compounds will further be reacted with compounds bearing therapeutic properties such as Ag_2O, $AuClPPh_3$ and $CuCl_2$ to further enhance their efficacy. The compounds will then be tested at the medical research council to evaluate their activities against mycobacterium tuberculosis. A cytotoxicity test will be performed using skin, colon, and lung cell lines.

Appendix 1

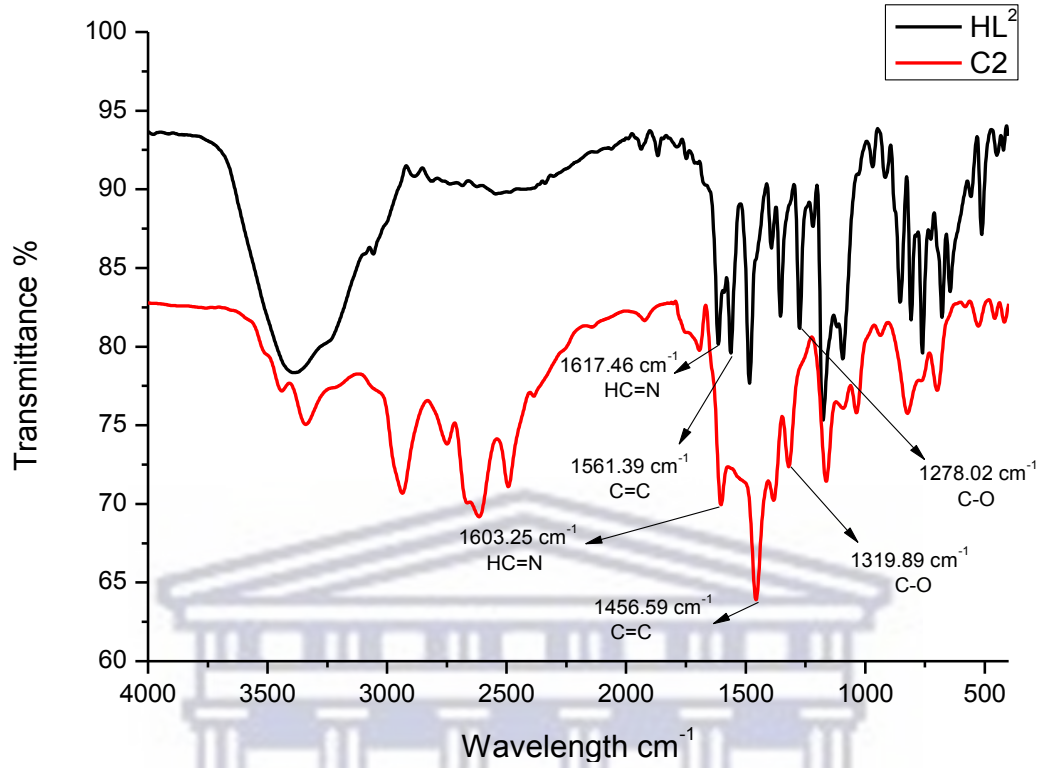

Figure A 1.1: FTIR spectrum of HL^2 and C2

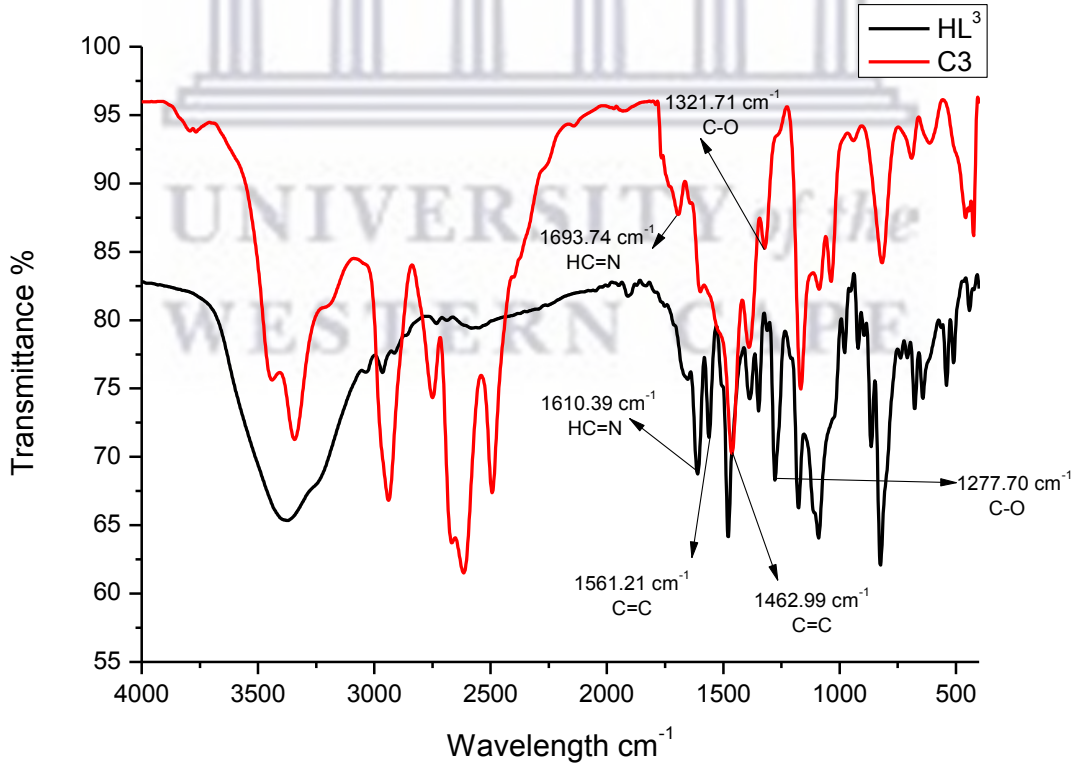

Figure A 1.2: FTIR spectrum of HL^3 and C3

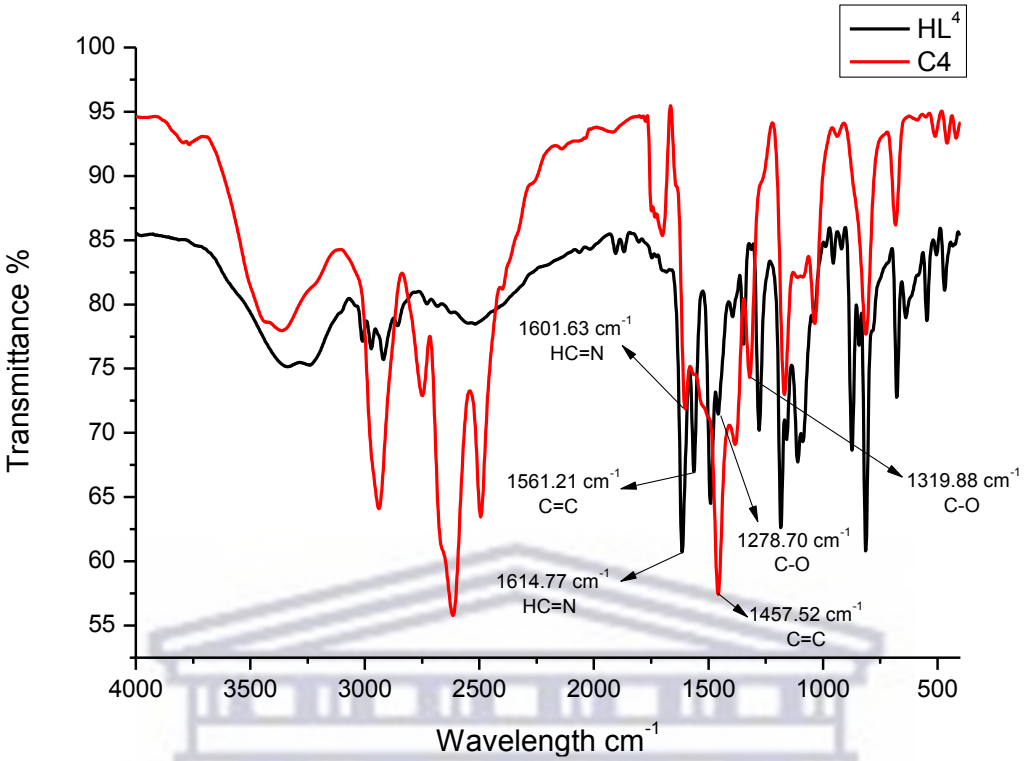

Figure A 1.3: FTIR spectrum of HL^4 and C4

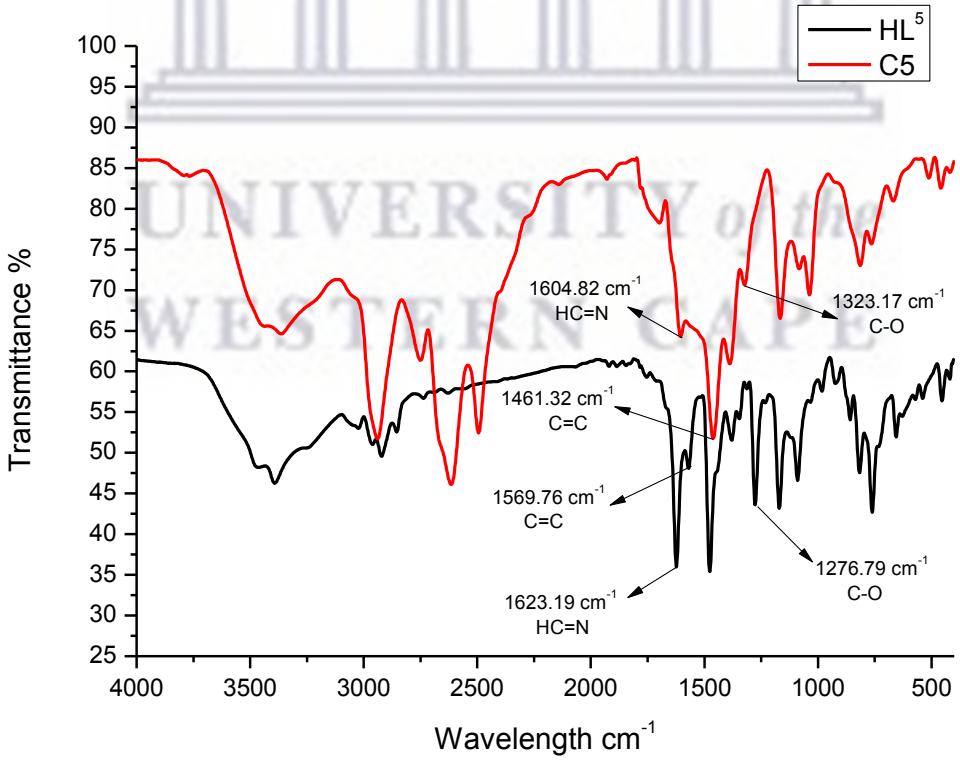

Figure A 1.4: FTIR spectrum of HL^5 and C5

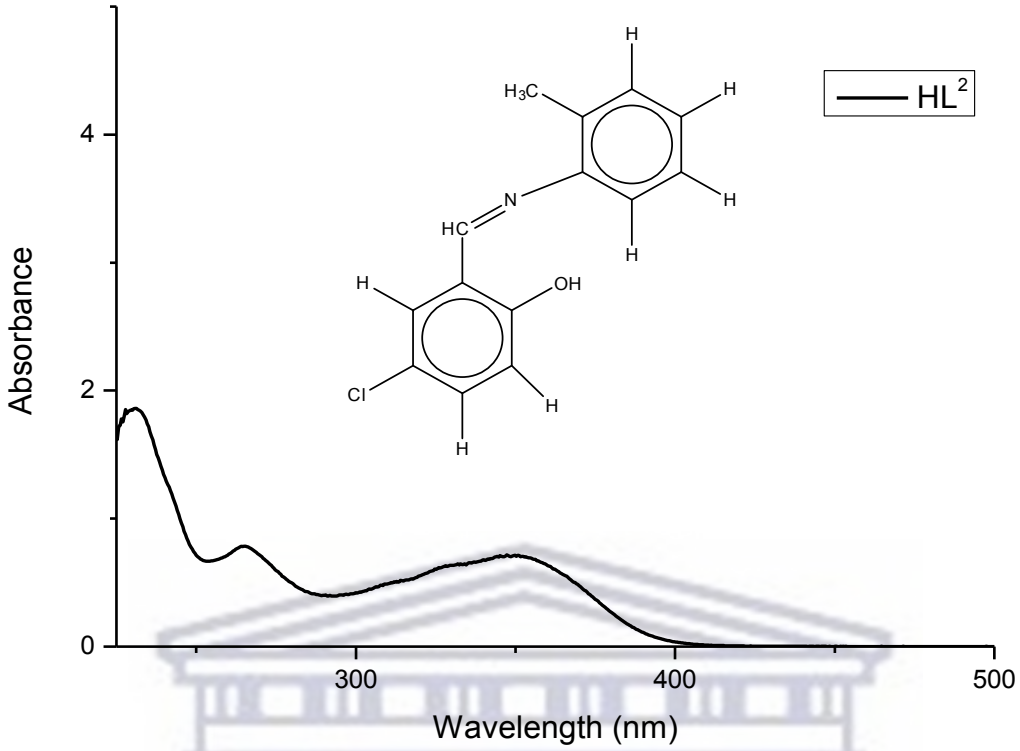

Figure A 1.5: UV-vis spectrum of HL^2

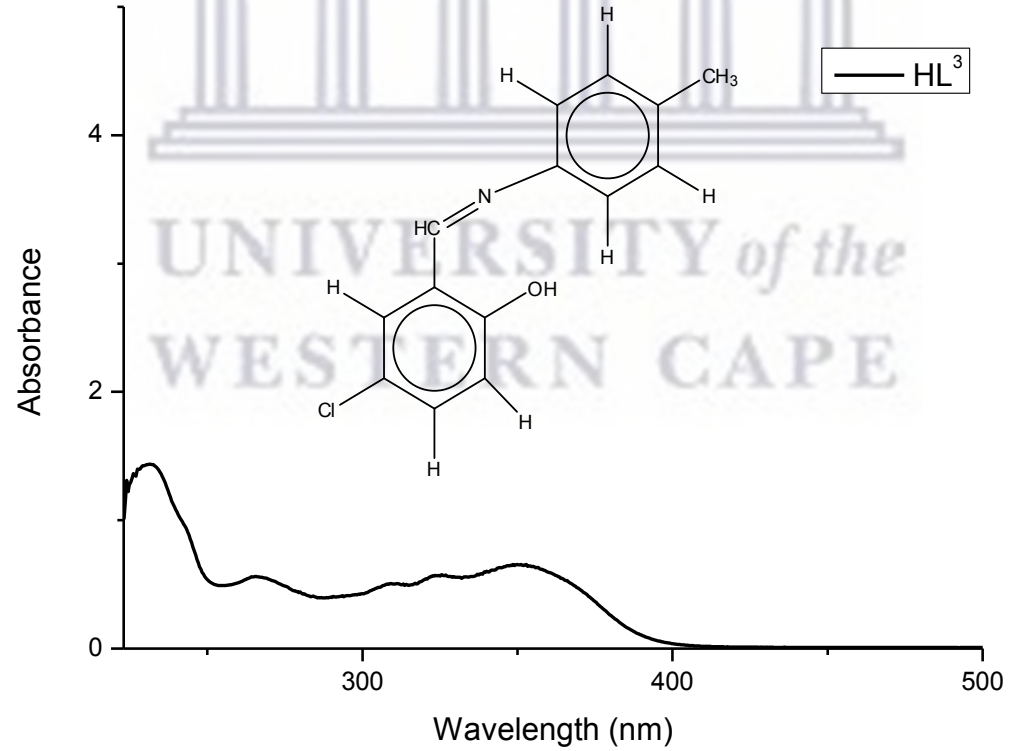

Figure A 1.6: UV-vis spectrum of HL^3

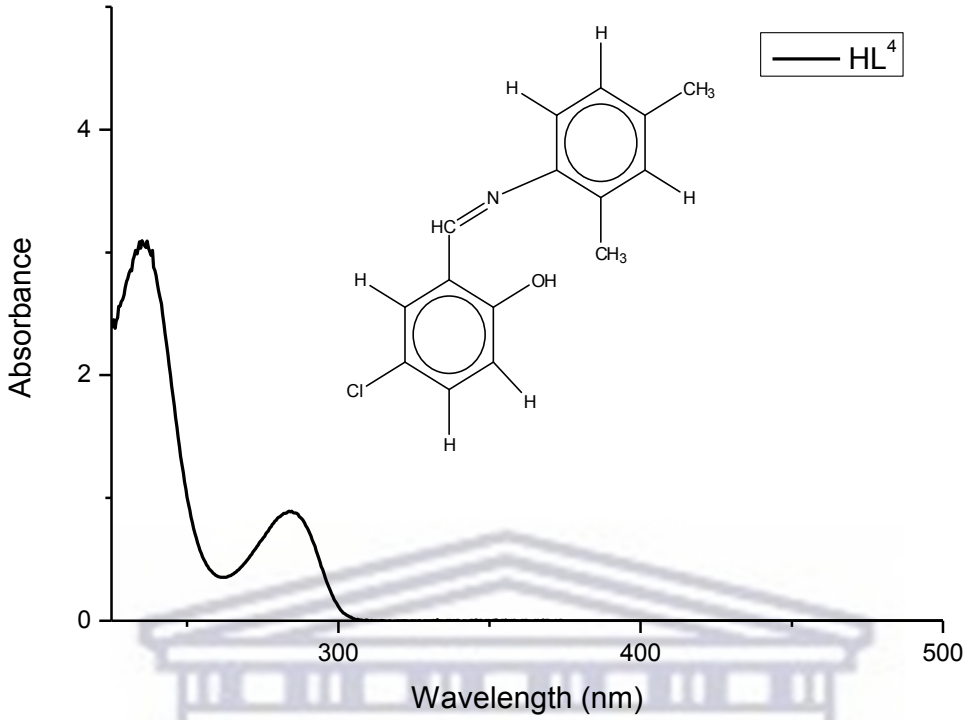

Figure A 1.7: UV-vis spectrum of HL^4

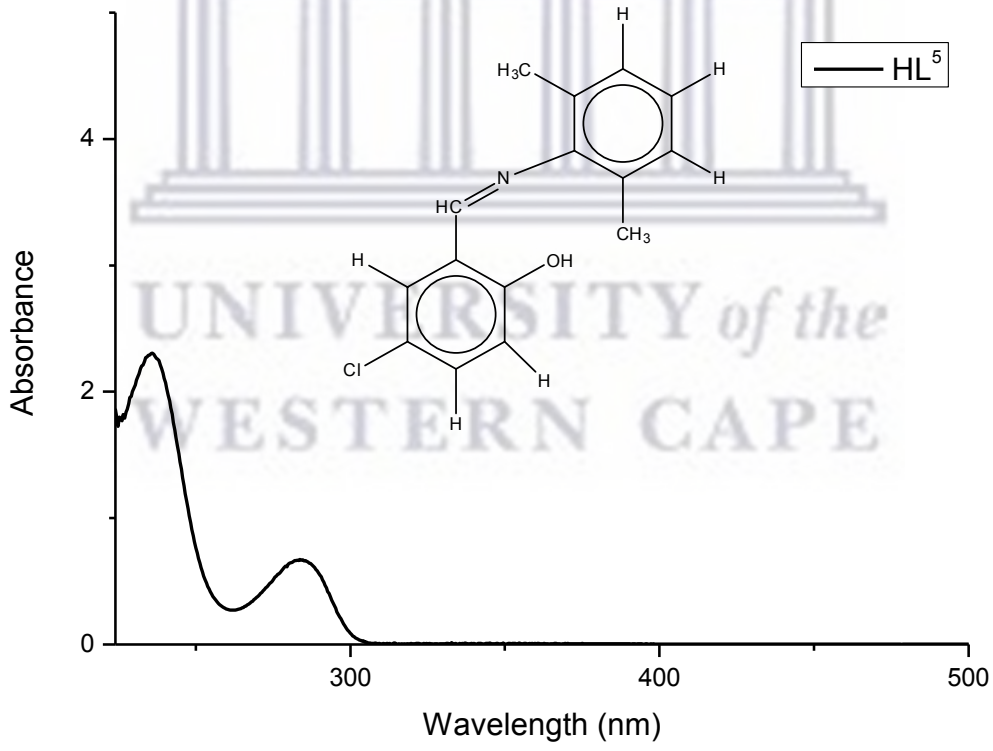

Figure A 1.8: UV-Vis spectrum of HL^5

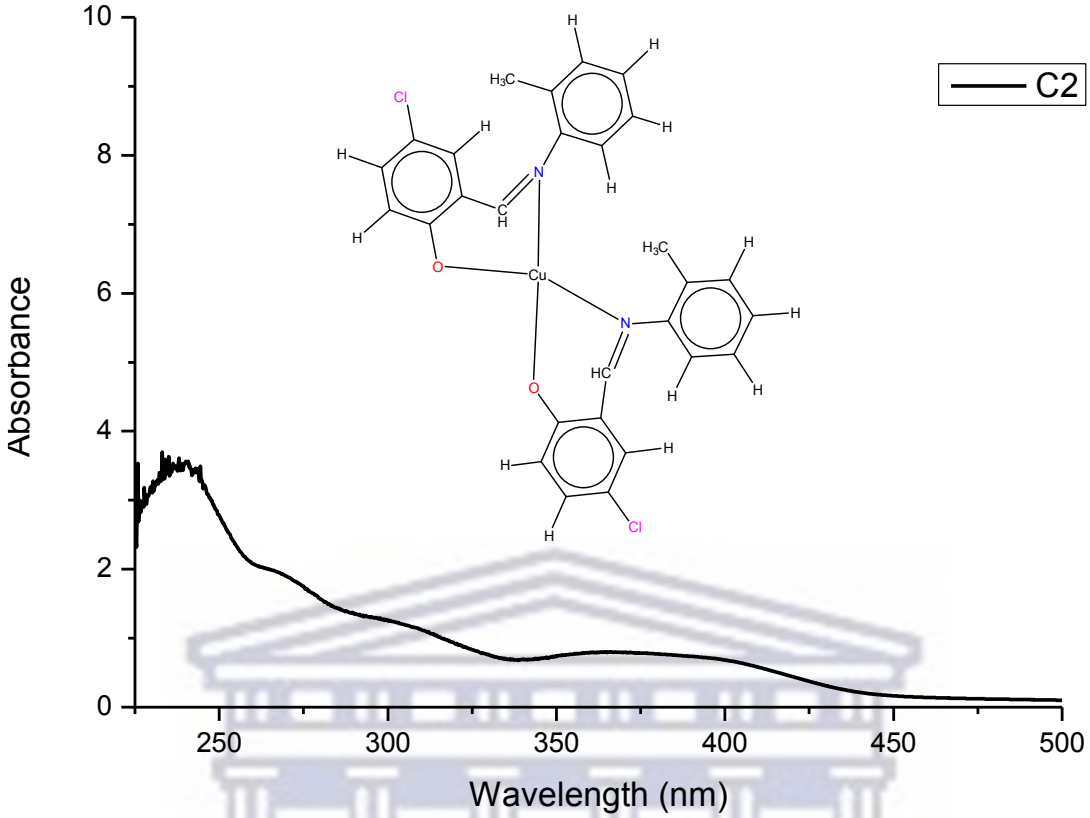

Figure A 1.9: UV-vis spectrum of C2

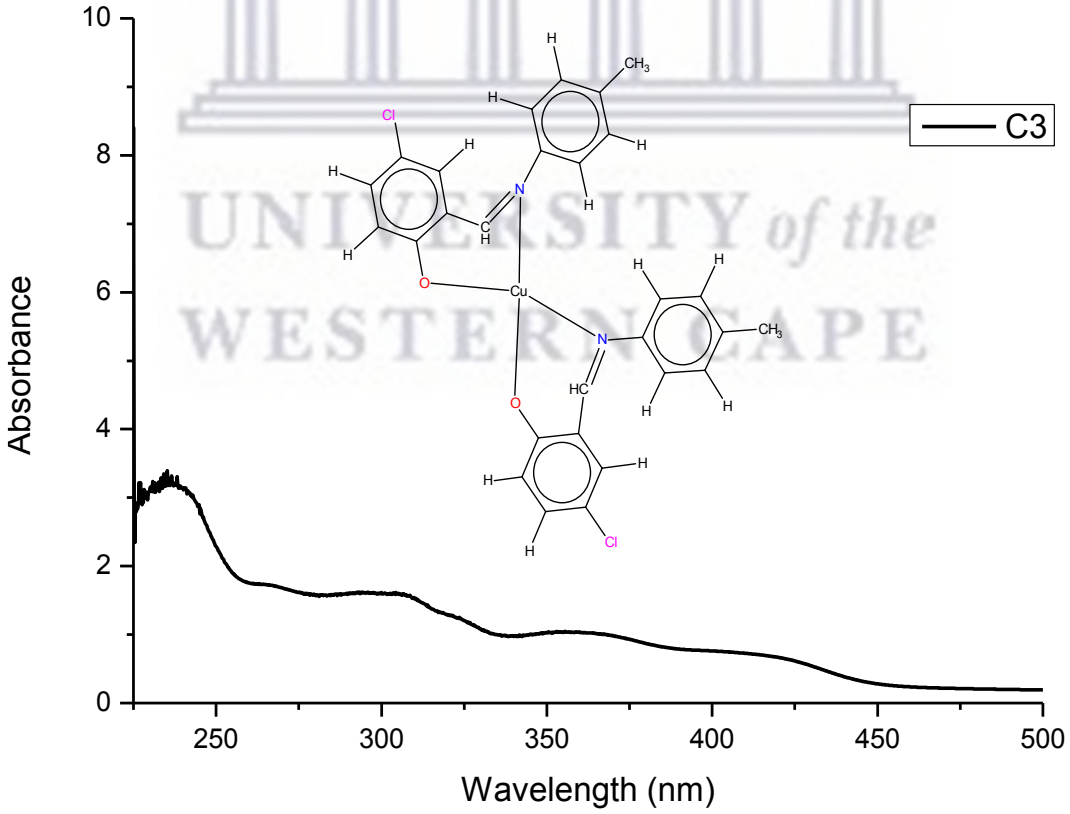

Figure A 1.10: UV-vis spectrum of C3

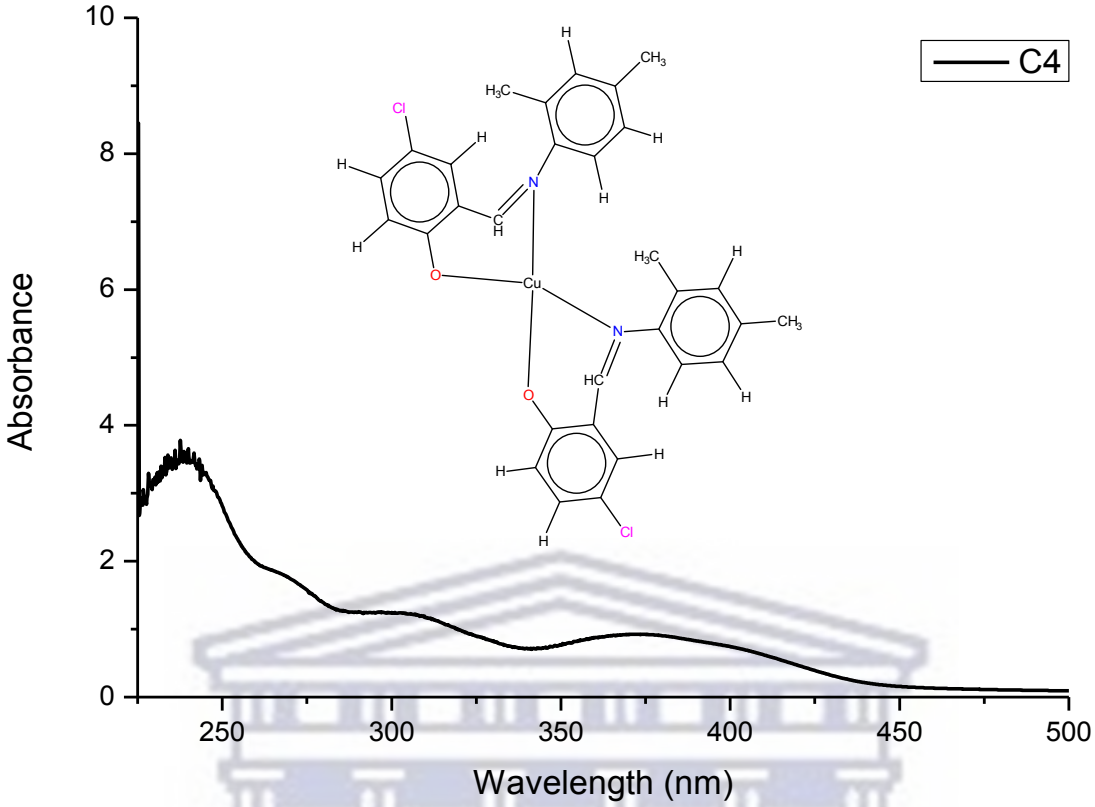

Figure A 1.11: UV-vis spectrum of C4

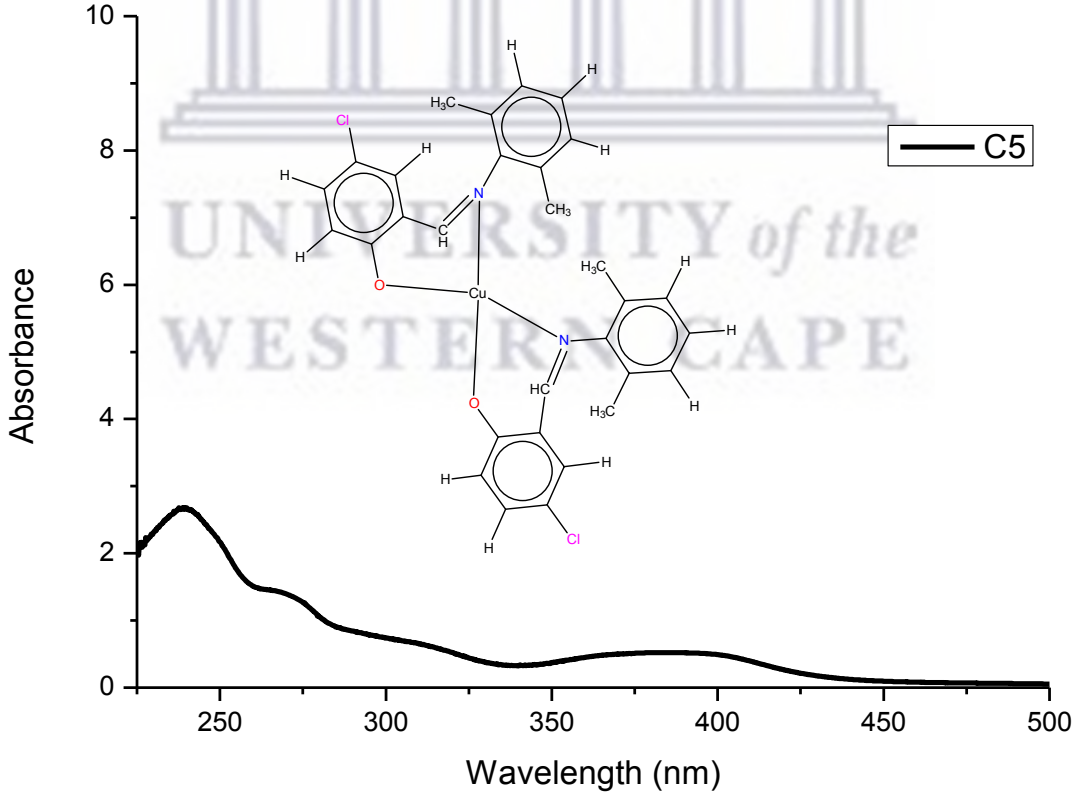

Figure A 1.12: UV-vis spectrum of C5

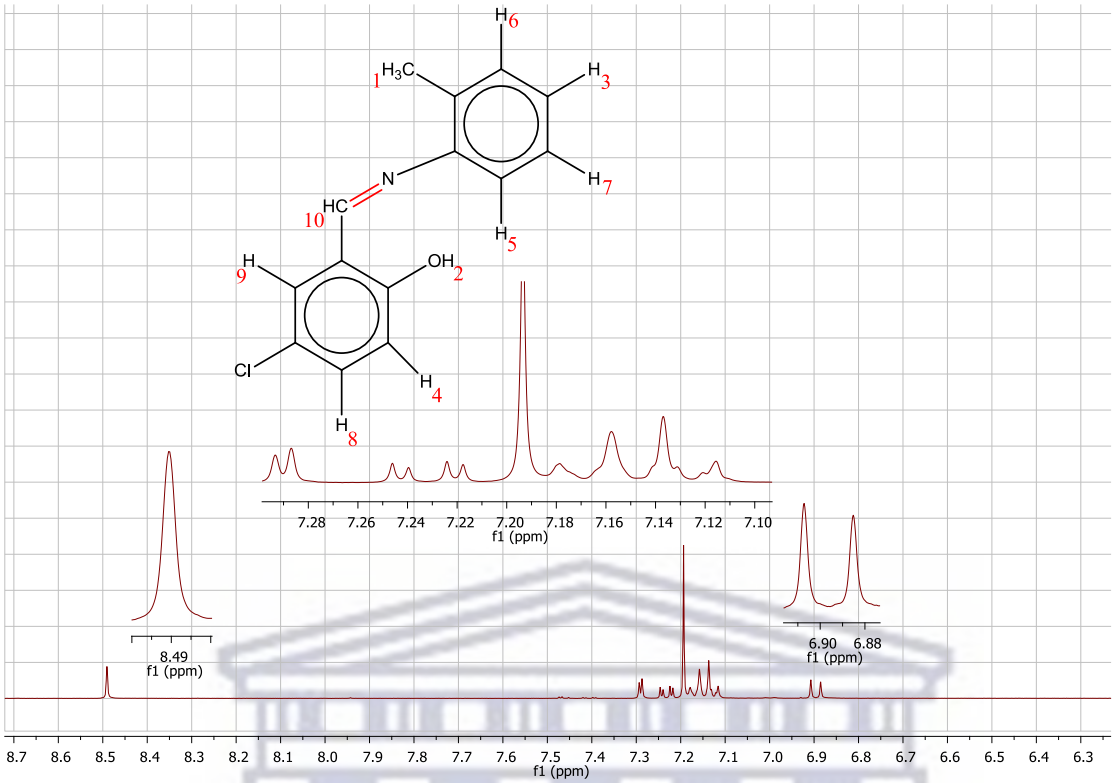

Figure A 1.13: ^{1}H NMR spectrum of HL^{2}

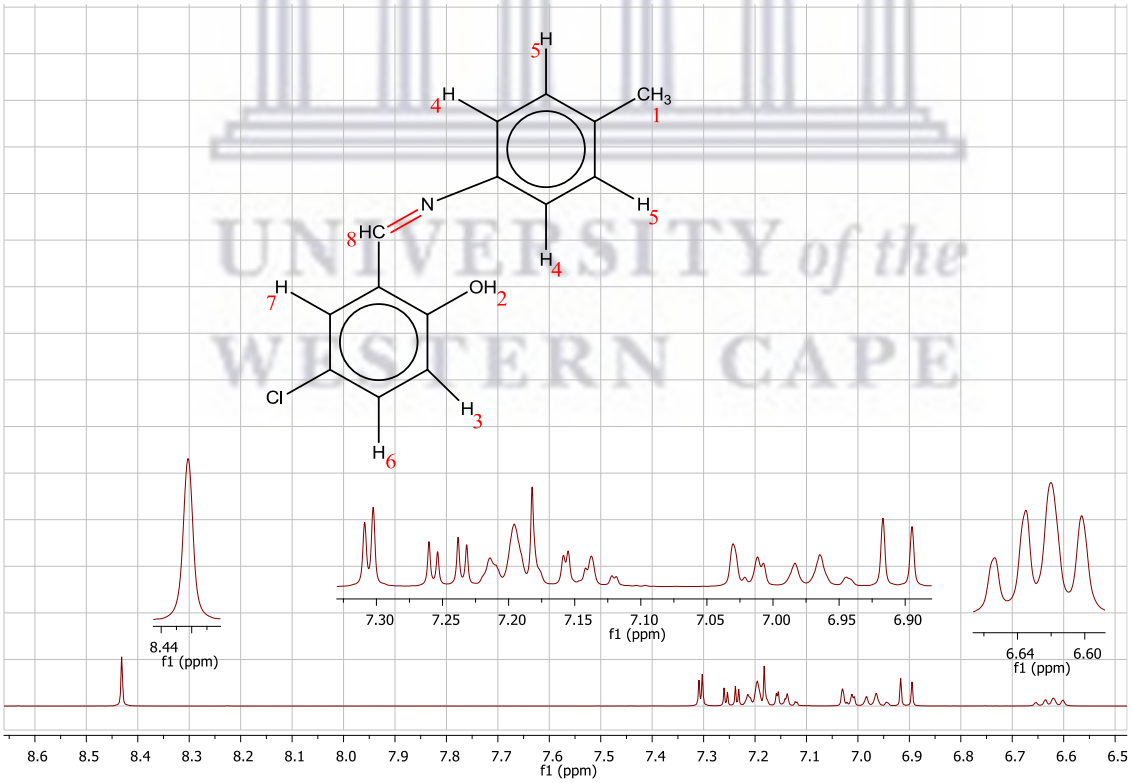

Figure A 1.14: ^{1}H NMR of HL^{3}

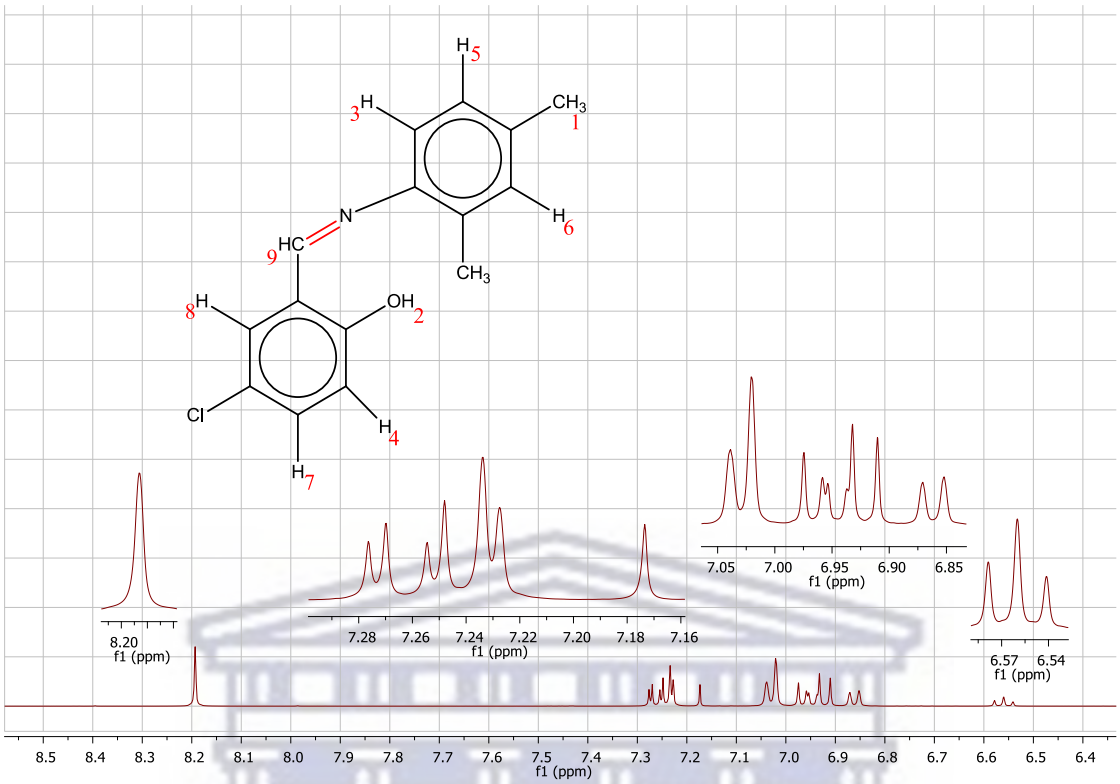

Figure A 1.15: ^{1}H NMR spectrum of HL^{4}

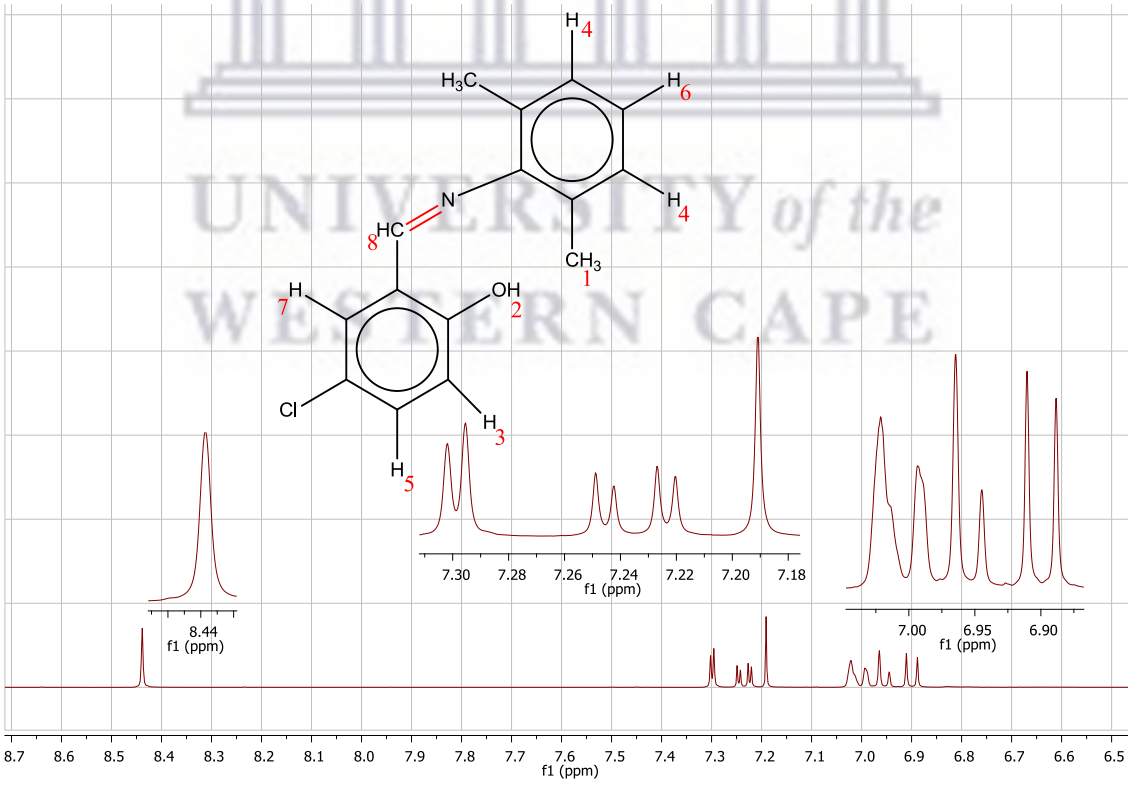

Figure A 1.16: ^{1}H NMR spectrum of HL^{5}

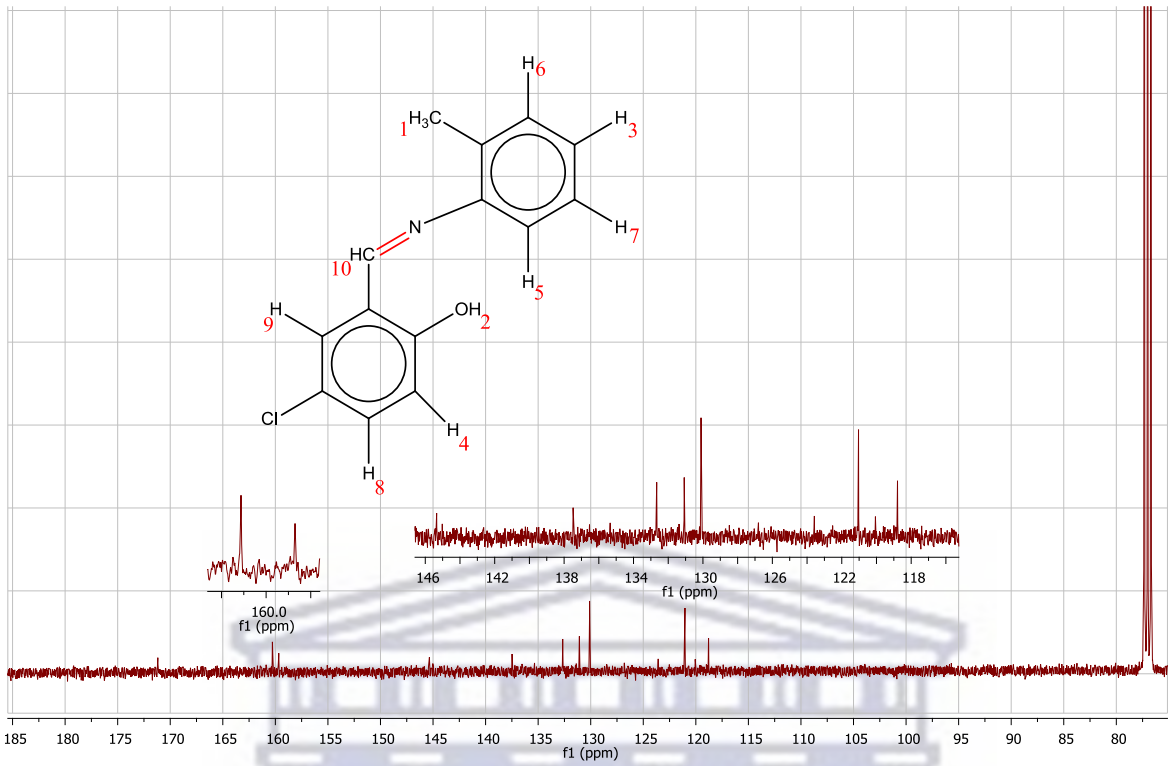

Figure A 1.17: ^{13}C spectrum of HL^2

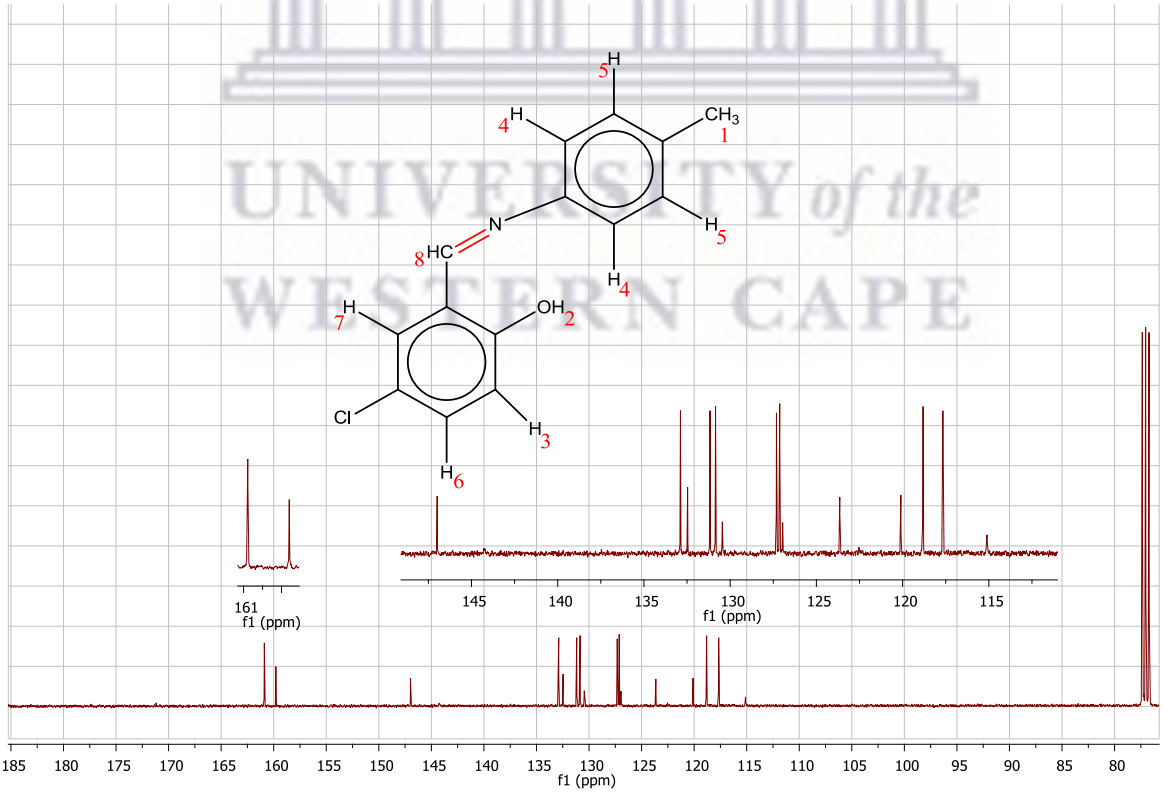

Figure A 1.18: ^{13}C NMR spectrum of HL^3

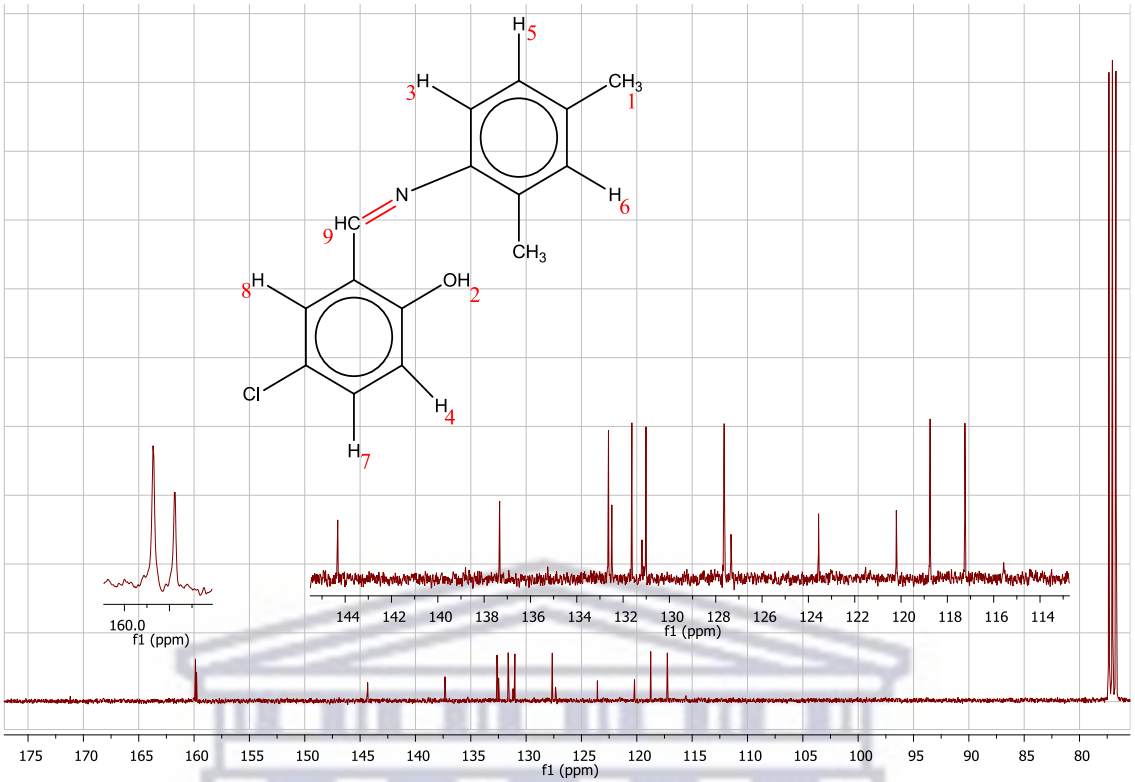

Figure A 1.19: ^{13}C NMR spectrum of HL^4

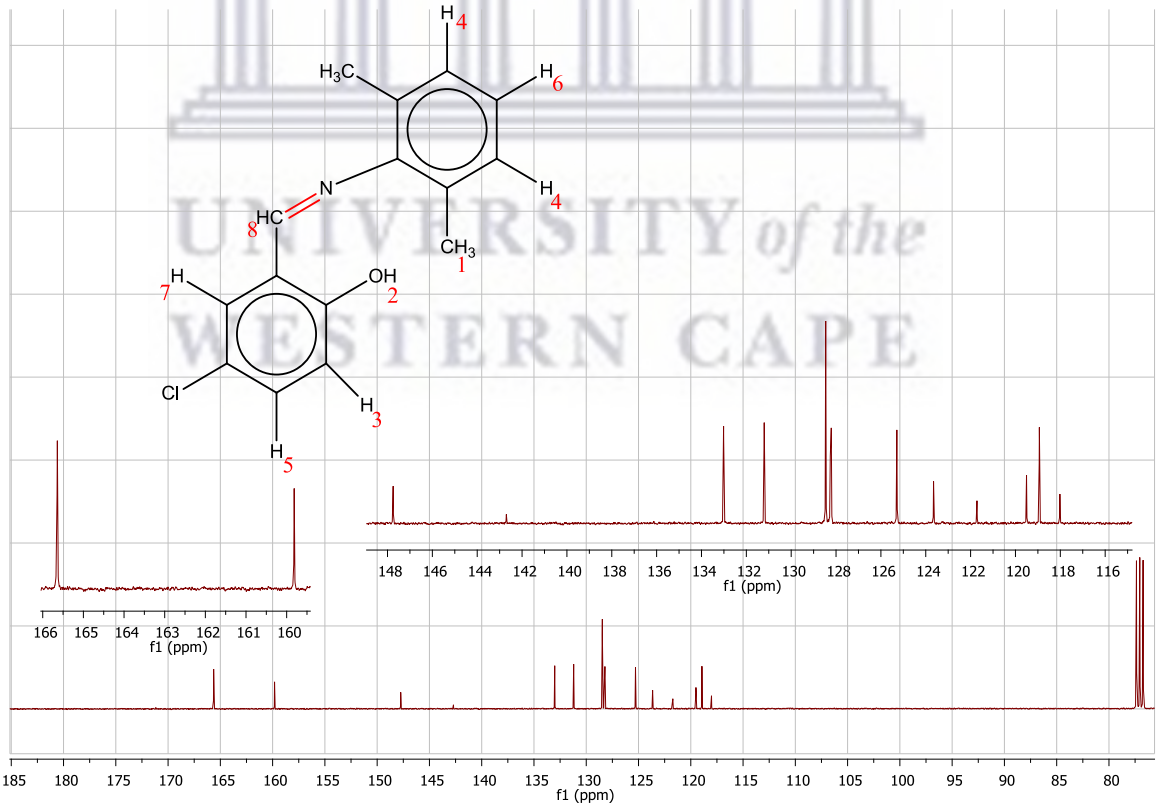

Figure A 1.20: ^{13}C NMR spectrum of HL^5

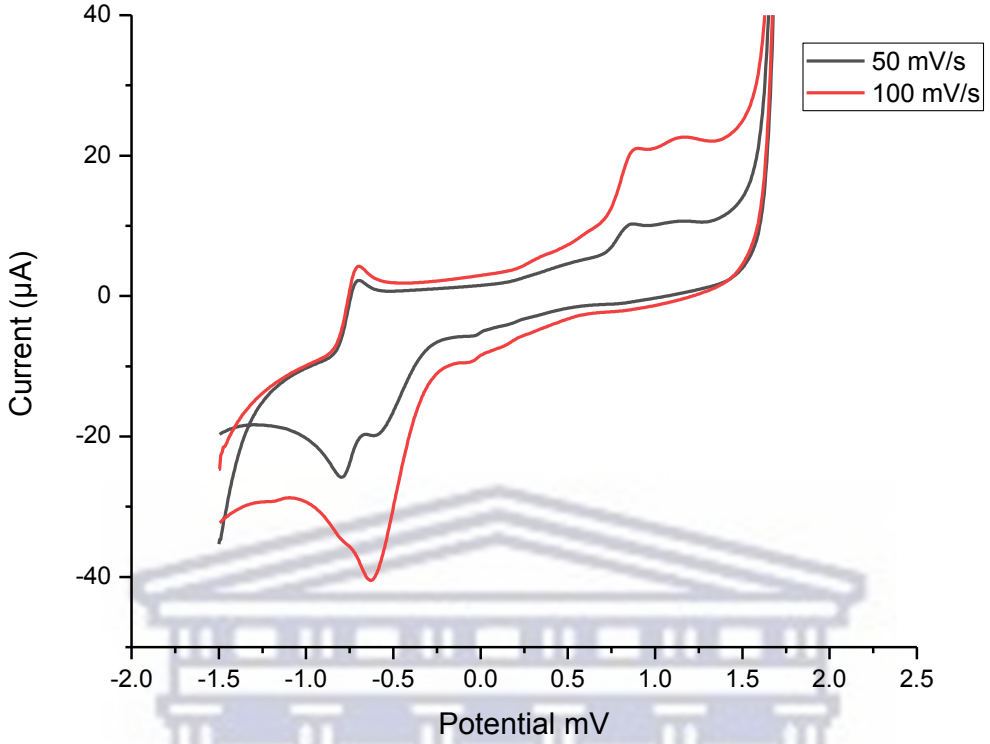

Figure A 1.21: Cyclic voltammogram of C2 at scan rate of 50 and 100 mV/s.

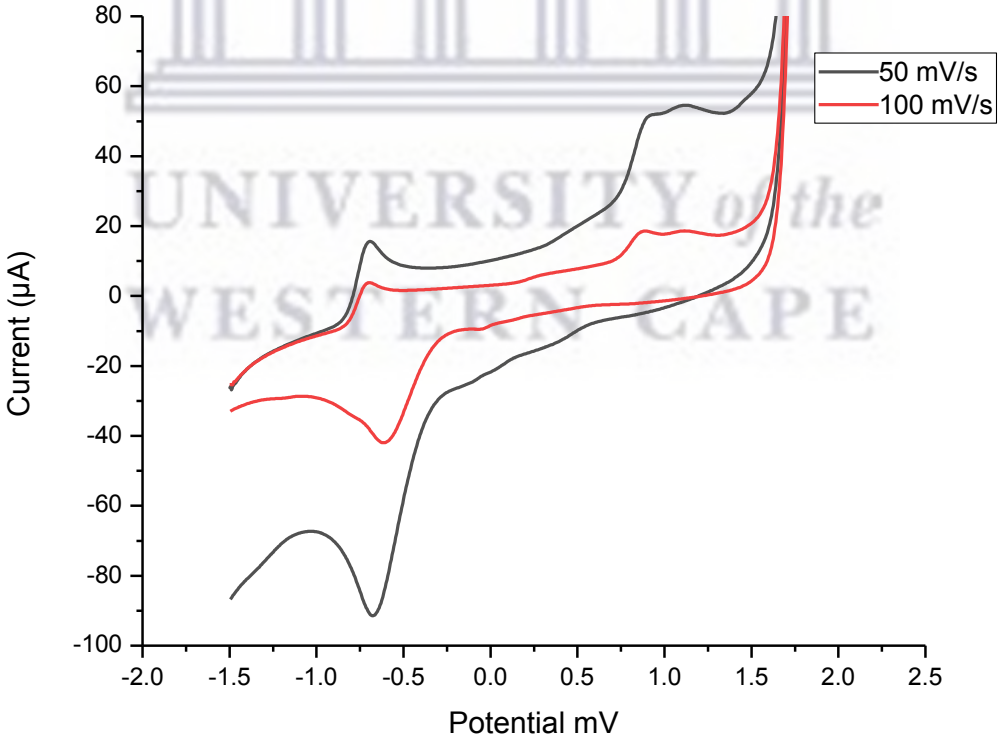

Figure A 1.22: Cyclic voltammogram of C3 at scan rate of 50 and 100 mV/s.

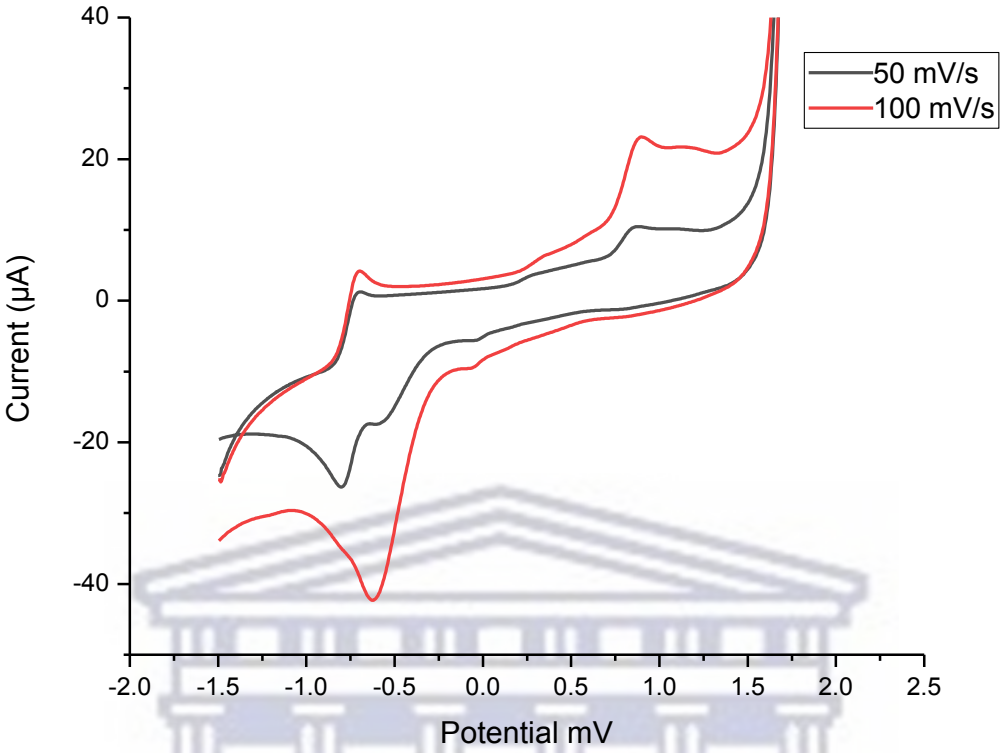

Figure A 1.23: Cyclic voltammogram of C4 at scan rate of 50 and 100 mV/s.

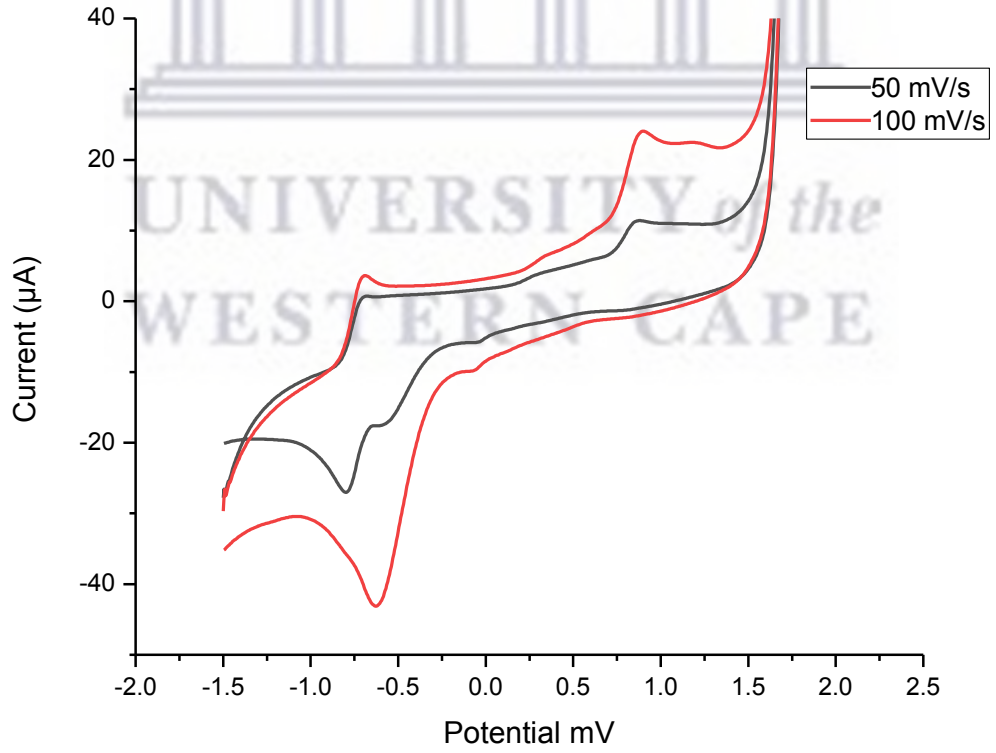

Figure A 1.24: Cyclic voltammogram of C5 at scan rate of 50 and 100 mV/s.

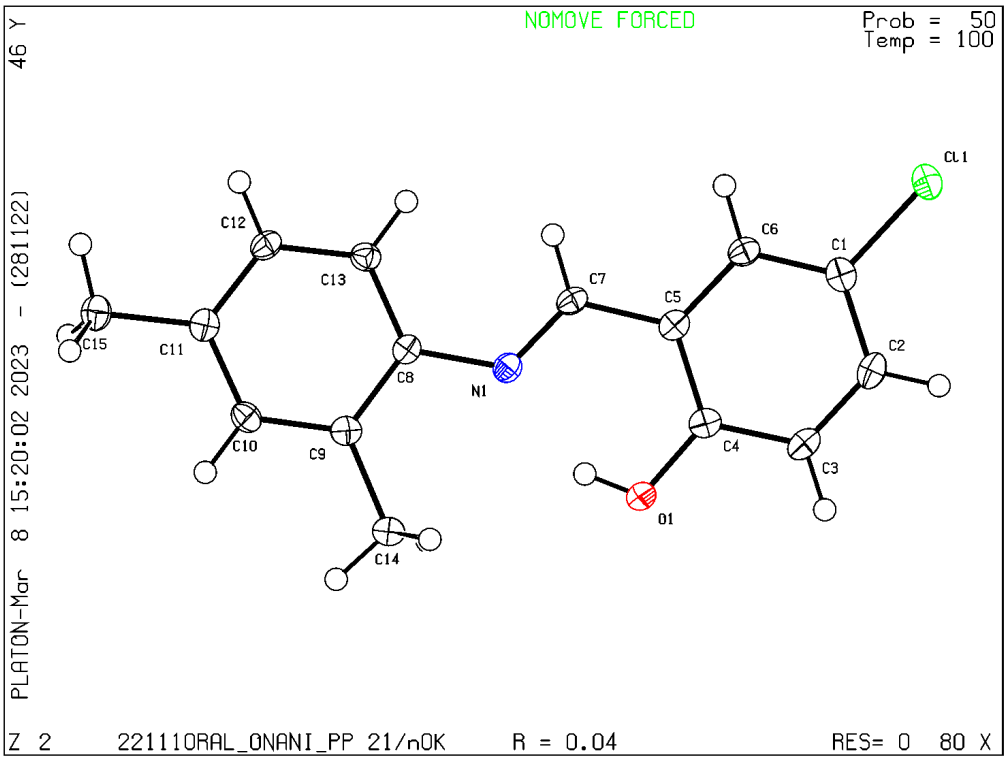

Figure A 1.25: X-ray crystal structure of HL[4]

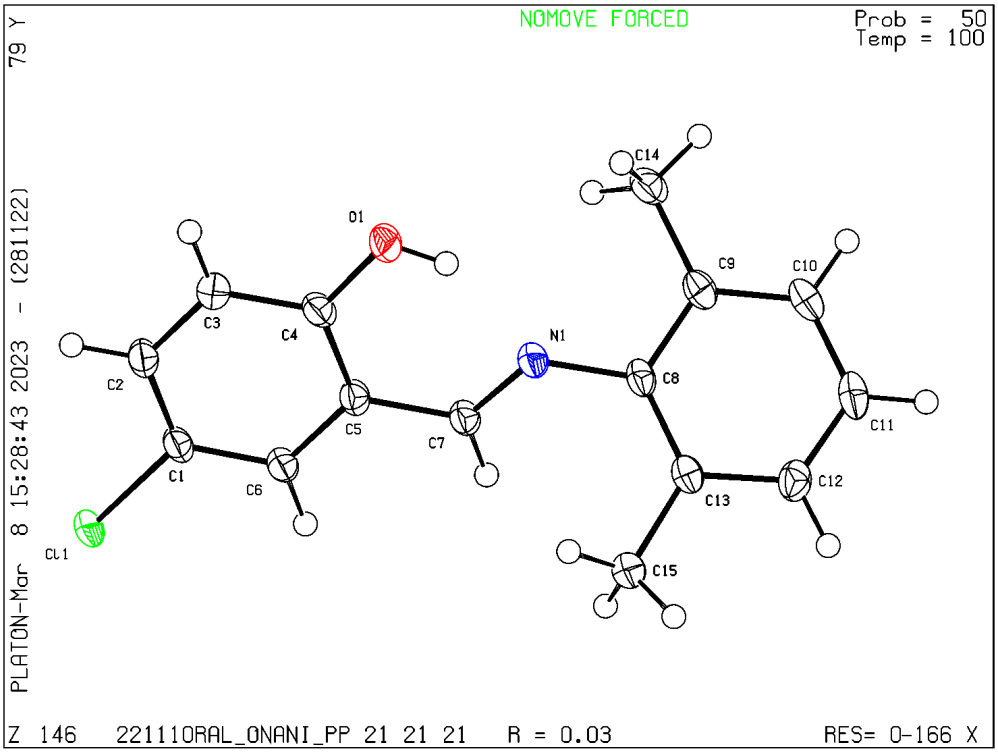

Figure A 1.26: X-ray crystal structure of HL[5]

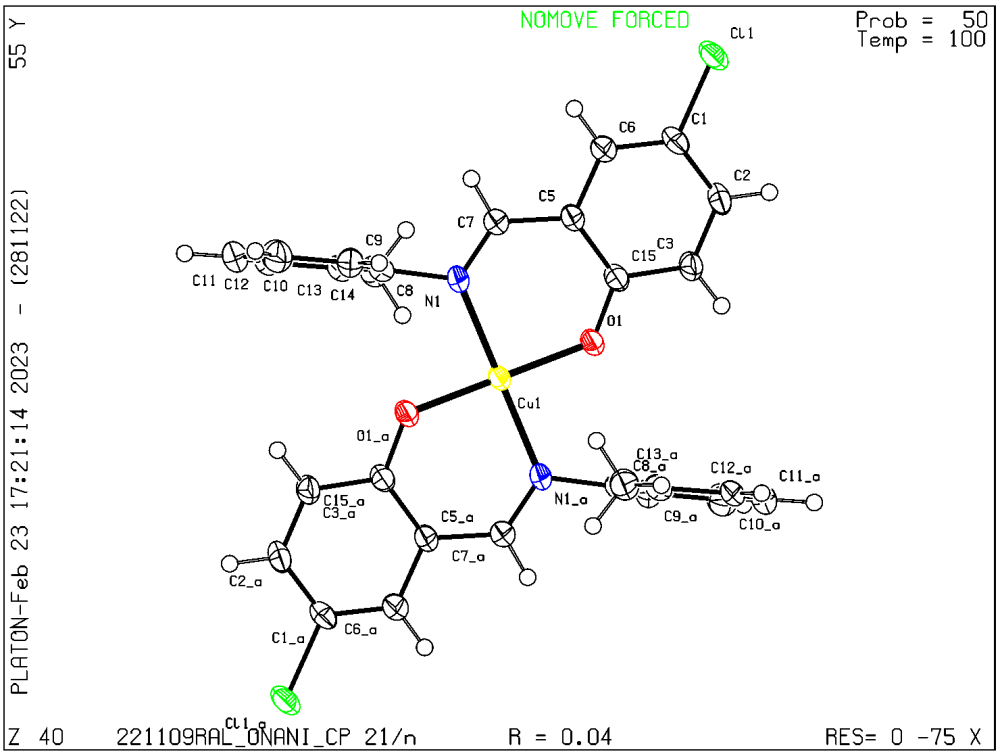

Figure A 1.27: X-ray crystal structure of C2.

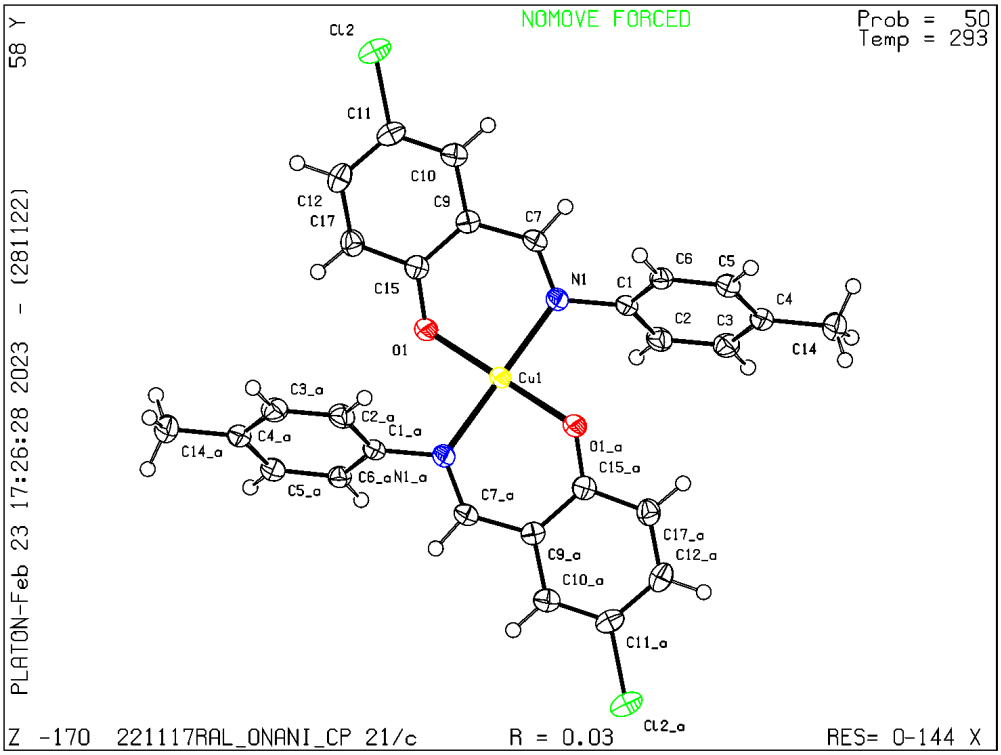

Figure A 1.28: X-ray crystal structure of C3