SYNTHESIS, CHARACTERIZATION AND *IN VITRO* BIOLOGICAL STUDIES OF SOME METAL(II) COMPLEXES OF RIFAMPICIN, CLOFAZIMINE AND OF SCHIFF BASES DERIVED FROM DAPSONE

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MAY, 2016

DECLARATION

I hereby declare that this work is the product of my research efforts, undertaken under the supervision of PROF. HABU NUHU ALIYU and has not been presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this thesis and the subsequent write-up of this thesis by Emmanuel Chukwudi Ozoro (SPS/10/PCH/00006) were carried out under my supervision.

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APPROVAL PAGE

This thesis has been examined and approved for the award of the degree of Ph.D in Inorganic Chemistry.

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Ozoro, Emmanuel C.

DEDICATION

I dedicate this Thesis to my mother, Late Mrs. Cecilia Ezenagum Ozoro (Nee Ugulu), a shining light in my life. I miss you so much mama. May God bless your sweet and gentle soul, amen.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline Phosphate
ATNR	Amine Terminated Liquid Natural Rubber
AZM-SA	Zinc Complex derived from Salicylaldehyde and Acetazolamide
BB	Mid-Boderline Lepromatous
BL	Boderline Lepromatous
BT	Boderline Tuberculoid
CAM	Clarithromycin
CLOF	Clofazimine
DAP-ACA	Dapsone Schiff Base synthesized from Acetylacetone
DAP-SAL	Dapsone Schiff Base synthesized from Salicylaldehyde
DLL	Diffuse Lepromatous Leprosy
DMF	Dimethyl Formamide
DMSO	Dimethyl sulfoxide
EAC	Ehrlich Ascites Carcinoma
ENL	Erythema Nodosome Leprosome
FTIR	Fourier Transform Infrared
HIV	Human Immuno Virus
HNMR	Proton Nuclear Magnetic Resonance
IR	Infrared
ITSC	Indoxyl thiosemicarbazone
LD50	Lethal Dose Affecting 50% of Sample Population
LL	Lepromatous

LVFX	Levoflaxicin
MB	Multibacillary Leprosy
MDT	Multidrug Therapy
M(DAP-ACA)	Metal(II) Complex Derived from Dapsone Schiff base with Acetylacetone
M(DAP-SAL)	Metal(II) Complex Derived from Dapsone Schiff base with Salicylaldehyde
[M(RIF)]	Metal(II) Complex Derived from Rifampicin
[M(CLOF)]	Metal(II) Complex Derived from Clofazimine
NMR	Nuclear Magnetic Resonance
PABA	Para- aminobutyric acid
PB	Paucibacillary Leprosy
PCP	Pneumocystis Pneumonia
PDA	Potato Dextrose Agar
RBC	Red Blood Cell
RIF	Rifampicin
RML	Robert Microlit Laboratories
SPFX	Sparfloxicin
TEM	Transmission Electron Microscopy
UV	Ultraviolet
WBC	White Blood Cell
WHO	World Health Organisation
XRD	X-ray Diffraction
XPS	X-ray Photoelectron Spectroscopy

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ABSTRACT

Two Schiff bases have been synthesized from the drug dapsone (4,4 diaminodiphenyl sulphone) by its chemical interaction with ethanolic solution of salicylaldehyde and methanolic solution of acetylacetone respectively and the complexes of Cu(II), Mn(II), Fe(II), Ni(II), Co(II) and Zn(II) derived from these Schiff bases have been synthesized and studied. Also, the complexes of Cu(II), Mn(II), Fe(II), Ni(II), Co(II) and Zn(II) derived from the drug substances rifampicin and clofazimine respectively have been synthesized and studied. The metal(II) complexes were characterized on the basis of solubility, magnetic measurements, infra-red (IR) spectral studies, molar conductance measurements, decomposition temperature determinations, determination of percent water of crystallization and elemental analyses while the synthesized Schiff bases were studied based on solubility, melting point determination, infra-red (IR) spectral studies and elemental analyses. The prepared Schiff bases and metal(II) complexes are coloured, stable and non-hygroscopic compounds. Based on the results obtained from the characterization of the synthesized compounds, it was found that they are respectively soluble in most organic solvents but insoluble in distilled water except the metal(II) complexes derived from (DAP-SAL) and (DAP-ACA) respectively, rifampicin and its metal(II) complexes. These showed slight solubility in distilled water. The decomposition temperatures of all the metal(II) complexes was within the range 205°C to >250°C. The gram magnetic moment of each of the metal(II) complexes was determined from which their effective magnetic moments were also determined. It was found that the compounds are paramagnetic, except for the diamagnetic zinc(II) complexes. The Infra-red spectral data of the ligands when compared to those of their respective metal(II) complexes showed that coordination of the metal ion to the ligand was via the azomethine nitrogen and the phenolic –(OH) of the Schiff base in the case of (DAP-SAL) and (DAP-ACA) metal(II) complexes, while coordination of the metal ion to rifampicin was via the phenolic –(OH), azomethine nitrogen and carbonyl oxygen of the ligand. For clofazimine metal(II) complexes, coordination of the metal ion was via the azomethine and diazine nitrogen atoms of the ligand and chloride ions from the metal salt. The thermogravimetric analysis data show the presence of water of crystallization in all the complexes. The relatively low molar conductance values of the metal(II) complexes (9-20 ohm^{-1} cm^2 mol^{-1}) indicate their non-ionic nature. Elemental analyses determination show the metal:ligand (M:L) ratio for all the metal(II) complexes to be (1:1). Antibacterial and antifungal activities of the drugs, synthesized Schiff bases and metal(II) complexes were evaluated using disc diffusion method. The antibacterial assay was carried out on three pathogenic bacteria, E*scheria coli, Staphylococcus aureus* and S*almonella typhi,* while the fungal isolates used were As*perigillus flavus*, A*sperigillus niger* and *Asperigillus fumigatos*. The results showed that both synthesized ligands and complexes are not only effective on both bacterial and fungal isolates but some of them showed even higher activity when compared to the parent drugs (dapsone, rifampicin and clofazimine) which were used as controls. Toxicity tests carried out on all the synthesized compounds show them to be non-toxic.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Schiff Bases

Schiff bases are condensation products of primary amines and carbonyl compounds and were discovered by a German Chemist Nobel Prize winner, Hugo Schiff in 1864 (Ashraf *et al.,* 2011). The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R', where R and R' are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. Schiff bases are also known as anils, imines or azomethines (Cohen, 1975). Several studies showed that the presence of a lone pair of electrons in sp^2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance. The availability of different types of amines and carbonyl compounds enabled the synthesis of Schiff bases with diverse structural features. The bonding ability of the ligands depends on the nature of atoms that act as coordination site, their electronegativity and steric factors (Kostova and Luciano, 2013).

By virtue of the presence of lone pair of electrons on the nitrogen atom, electron donating character of the double bond and the electronegativity of nitrogen, N of the azomethine group (>C=N) acts as a good donor site and Schiff base as an active ligand. The formation of chelates gives extra stability to the complexes especially when the ring is five or six membered. Hence the presence of a functional group with replaceable hydrogen atom near to >C=N will be additional factor of stability. To prevent them from rapidly decomposing or polymerising, an aryl group must be bonded to the nitrogen or to the carbon of the >C=N double bond (Kostova and Luciano, 2013).

Schiff bases are versatile ligands which coordinate to metal ions via azomethine nitrogen. The common Schiff bases are crystalline solids which are feebly basic but at least some form insoluble salts with strong acids.

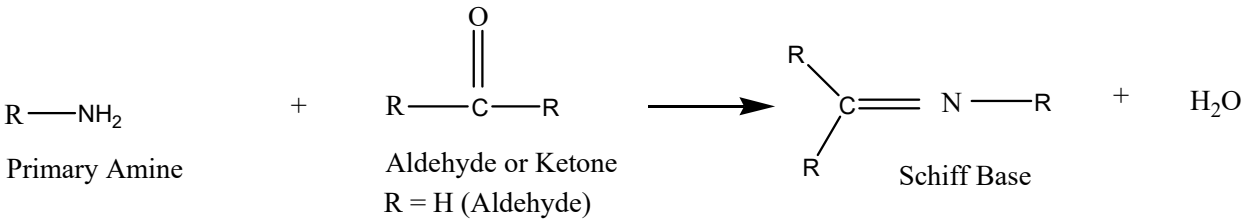
A Schiff base behaves as a flexi-dentate ligand and can co-ordinate through the oxygen (O) atom of the de-protonated phenolic group and the nitrogen (N) atom of azomethine group (Worku *et al.,* 2002). Flexi-dentate ligands are the poly-dentate ligands that do not use all its donor atoms to get coordinated to the metal ion. This means they use different numbers of donor atoms in different complexes. Aromatic aldehydes especially with an effective conjugation system form stable Schiff bases, whereas aliphatic aldehydes are unstable and readily polymerize (Paquette and Benjamin, 1968). Schiff base ligands with aldehydes are formed more readily than with ketones (carbonyl carbon) (Xavier and Srividhya, 2014).

1.1.1 Preparation of Schiff Base

The first preparation of imines was reported in the 19th century by Hugo Schiff (1864). Since then a variety of methods for the synthesis of imines have been described (Zheng-Yi *et al.,* 2000). The classical synthesis reported by Schiff involves the condensation of a carbonyl compound with an amine under azeotropic distillation but it does not follow simple nucleophilic addition, but gives an unstable addition compound called carbinolamine. The compound thus obtained is unstable and loses water molecule. The dehydration step during formation of Schiff base is actually the rate determining step and the reaction is catalyzed by acid or base (Stehlik, 2009).

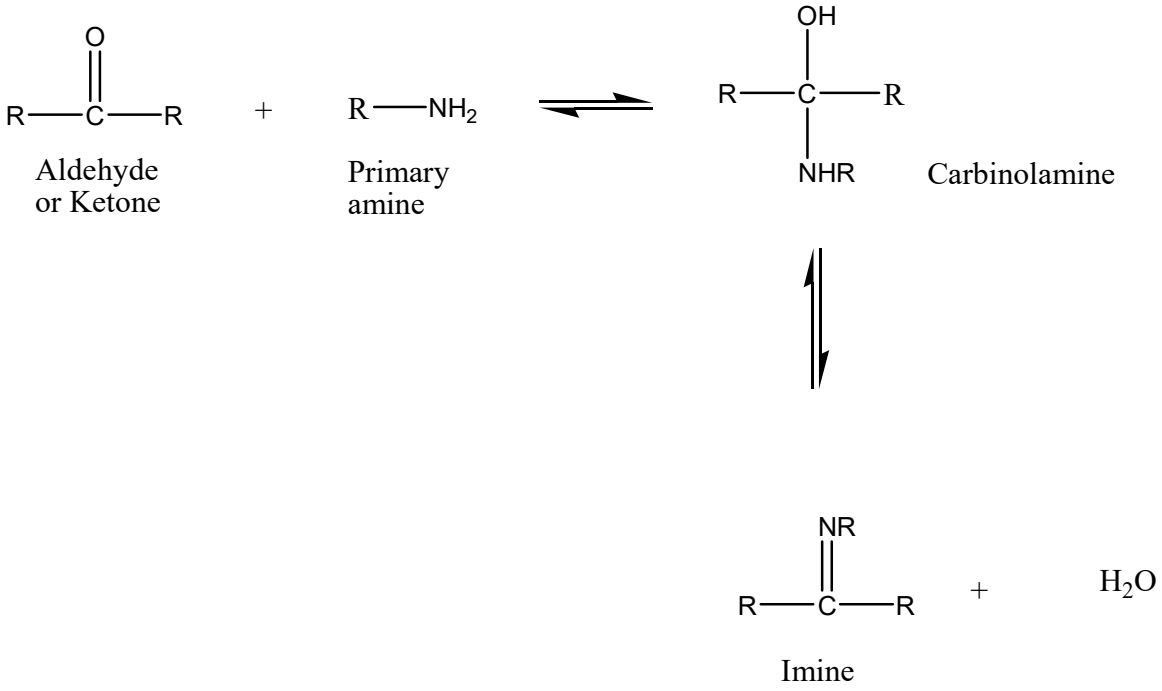
The removal of product or separation of water from the reaction mixture assists the formation of the product (Clements *et al.,* 2004). Thus molecular sieves are sometimes used to completely remove water formed in the system (Taguchi and Westheimer, 1971). The aqueous acids or bases may hydrolyze Schiff bases towards their respective aldehydes or ketones and amines as well. In this regard, high concentration of acid is not needed due to basic character of amines which hinder the formation of carbinolamine and equilibrium is shifted towards left side because protonated amine does not act as nucleophile (Schiff, 1864). This is the reason that mildly acidic pH are quite good for the formation of Schiff bases. Moreover, bases can also catalyze dehydration of carbinolamines. Chakraborti *et al* (2004), however, demonstrated that the efficiency of these methods is dependent on the use of highly electrophilic

carbonyl compounds and strongly nucleophilic amines. The reaction scheme for the formation of Schiff base is shown in schemes 1.1 and 1.2 below.



Where R may be an alkyl or aryl group

Scheme 1.1: Formation of Schiff Base



Scheme 1.2: Formation of Carbinolamine Intermediate

In real sense, the formation of Schiff bases is a combination of two types of reactions i.e. addition and elimination. Schiff bases can undergo hydrolysis on silica gel and due to this reason, purification of Schiff bases by chromatography is not recommended (Zollner, 1989). Many Schiff bases can be hydrolysed back to their aldehydes or ketones and amines by aqueous acid or base (Solankee and Thakor, 2006). The general formula of azomethine group is the most common structural feature of Schiff bases.

1.1.2 Denticity and Basicity of Schiff Base

The Schiff base ligands are classified according to the number of donor atoms and are named as monodentate, bidentate, and polydentate ligands (tridentate, tetradentate etc). Schiff bases primarily possess nitrogen donor atom. In general the donor nature of the ligand depends both on the type of aldehyde/ketone used and the nature of primary amine/diamine. The basicity of the Schiff base also plays a key role in the formation and stabilization of the complexes. The –OH group when present in the Schiff base can induce tautomerism in the compound, which leads to a compound with different structures. A large number of Schiff base compounds show keto-enol tautomerism. Also the deprotonation of alcoholic and phenolic groups are favoured due to the stabilization of various oxidation states of different metal ions (Kumar and Ravikant, 2014).

1.2 Leprosy

Leprosy, also known as Hansen's disease is a chronic infectious disease that primarily affects the skin, the peripheral nerves, the upper respiratory tract and the eyes (Sasaki *et al.,* 2001). It is often referred to as 'the oldest disease known to man' (Browne, 1985). The earliest written records describing leprosy come from India and date back to about 600 BC. The earliest remains of people confirmed to be affected with leprosy stem from Egyptian excavations which disclosed leprous skulls buried in the second century B.C. The causative agent is a microaerophillic acid fast bacterium*, Mycobacterium leprae*, first identified in 1873 by the Norwegian physician, Gerhard Henrik Armauer Hansen (Irgens, 1973). The term leprosy originates from the Latin word *lepros*, meaning defilement. Although documented since antiquity, leprosy remains endemic in some developing parts of the world (Ishii *et al.,* 2000) and despite its early discovery, leprosy is even today a disease which is not well understood. The inability to culture *M. leprae* is an important reason (Meina, 2004).

Mycobacterium leprae is thought to have originated in East Africa and spread across the globe through migratory trends, reaching the western world within the last 500 years (Monot *et al.,* 2005). In

2012, The World Health Organization recorded a prevalence of approximately 180,000 cases (WHO, 2013). Through eradication efforts, the total number of cases worldwide has decreased, yet the number of new cases each year has remained consistent (Scollard *et al.,* 2006).

Although much about the transmission of *Mycobacterium leprae* is unknown, prolonged contact with an infected person increases an individual's chance of becoming infected. Armadillos can harbor the bacteria but are not seen as a threat to human contraction of the disease. In addition, insects could be possible carriers of *Mycobacterium leprae* but this is unclear. In humans, the bacteria are thought to be passed through the skin and nasal mucosa (WHO, 2013). A study by Job (1989) has demonstrated that large numbers of bacteria can be found on the skin of infected persons, providing a possible means of transmission. Mortality is difficult to measure with leprosy, as the infection is not the immediate cause of death in many cases (WHO, 2013).

Mycobacterium leprae cannot be easily cultured in the laboratory and so much is unknown about the infectious dose, incubation and transmission of the disease (Scollard *et al.,* 2006). With a doubling time of 14 days, *Mycobacterium leprae* has the longest doubling time of any studied bacteria (Cole *et al.,* 2001). The World Health Organization states that *Mycobacterium leprae* has an incubation period of an average of 5 years (WHO, 2013). Humans and armadillos are currently the only known reservoirs of the bacteria, with infected humans accounting for up to 7 billion organisms per gram of tissue (WHO, 2013). *Mycobacterium leprae* mostly lives in the extremities of facial region within macrophages and Schwann cells of the peripheral nervous system (Cole *et al.,* 2001). *Mycobacterium leprae* survives and replicates in macrophages, dividing approximately 100 organisms per cell (Hagge *et al.,* 2002). The bacteria prevent phagosome and lysosome fusion to avoid degradation (Sibley *et al.,* 1987).

The classification of leprosy is based upon two basic criteria: the clinical manifestations and the results of skin smears. In the classification based on skin smears, patients showing negative smears at all sites are grouped as Paucibacillary leprosy (PB), while those showing positive smears at any site are

grouped as having Multibacillary leprosy (MB). Clinical manifestations of leprosy can be divided into two major categories: Type 1 and Type 2 reactions. Type 1 leprosy includes Boderline lepromatous (BL), Mid-boderline (BB) and Boderline tuberculoid (BT) (Ridley and Jopling, 1986). The symptoms include lesions that are red and hardened, fluid accumulation in peripheral nerves, sensory loss and neuropathy. Type 2 reactions (Erythema nodosum leprosum) affect multibacillary patients (Lepromatous or LL and borderline lepromatous). In contrast to Type 1 reactions, symptoms develop suddenly, subside after a couple of weeks, and may reoccur many times over a period of months. Tender, red lesions erupt on the face, extremities and trunk. These patients may develop inflammation of the eyes leading to blindness and other complications. General muscle inflammation, joint inflammation and orchitis may occur (Scollard *et al.,* 2006).

For many years, the care of leprosy patients was mired in ignorance, prejudice and denial. No treatment for the disease existed at the time and patients had little or no hope of a cure. In 1941, Guy Faget, the medical officer in charge of the U.S National Leprosarium administered promine to a number of volunteers (Faget *et al*., 1943). Promine is a drug of the sulphone group which has been shown to give some protection to guinea-pigs infected with human tuberculosis bacilli (Feldman *et al*., 1942). This brought about a great improvement in the condition of the patients and effective treatment of leprosy became a reality.

Establishing leprosy infection is difficult. A study which employed serological testing indicated leprosy infection to be far more common than leprosy disease (Van Beers, 1998), which confirms suggestions from the 1980s (Fine, 1982; Noordeen, 1985). In recent years, considerable progress has been made in the development of diagnostic tools for leprosy infection, including skin and serological tests and molecular polymerase chain reaction tests (Van Beers, 1998). Still, it is not possible to predict who will develop leprosy disease and who will not. Genetic factors appear to influence resistance to leprosy

infection and to development and expression of disease (Naafs *et al.,* 2001), but the importance of these factors is unclear.

Dapsone was synthesized in 1908 by Fromm and Wittman (Fromm and Wittmann, 1908). Promine and other similar derivatives were the first sulphones to be used because the parent compound, dapsone was too toxic. It was not until 1947 that dapsone was administered in leprosy (Cochrane *et al.,* 1949; Lowe, 1950). The current standard treatment for leprosy is a multi-drug treatment (MDT) which consists of corticosteroids and antimicrobials (Noordeen, 1991). For paucibacillary leprosy, rifampicin and dapsone are used for a period of six months (WHO, 1982), while rifampicin, clofazimine and dapsone are used in multibacillary leprosy. The recommended duration of treatment for MB leprosy was gradually shortened from at least two years (WHO, 1982), to 24 months (WHO, 1994), to 12 months (WHO, 2002). Rifampicin, ofloxacin and minocycline can be combined in single lesion paucibacillary leprosy. Oral prednisolone can be used in secondary complications such as neuropathy and eye problems. Minocycline or ofloxacin can be used in the event of a rifampicin allergy, resistance, or presence of a disease antagonistic to rifampicin. Other chemotherapeutic agents like levofloxacin (LVFX), sparfloxacin (SPFX) and clarithromycin (CAM) are also effective against *Mycobacterium leprae* (Sugita *et al.,* 1996; Ishii *et al*., 1997; WHO, 1998). Multi drug therapy (MDT) is a key element of the leprosy treatment and elimination strategy because monotherapy in leprosy largely results in the development of resistance to the drug used.

Globally, about 212,000 more people were affected by leprosy in 2015. Out of this figure, 60 percent occurred in India. The other high burden countries are Brazil and Indonesia. The data also indicated that 8.9 percent of the new cases of leprosy involved children, while 6.7 percent had visible deformities. With 2,892 new cases, Nigeria has been ranked 3rd among African countries with the highest burden of leprosy (Obinna and Olawale, 2017). Over the last 10 years, according to the Global Health Observatory Data Repository, Nigeria recorded a total of 43,179 cases. The Data Repository which looked

at reported cases country by country, showed that other Africa countries with the highest and 2nd highest burden of leprosy respectively are Democratic Republic of the Congo, DRC, with 4, 237 cases, and Ethiopia, 3,970.Over the past decade, DRC had 62,845 cases while Ethiopia recorded 47,304 cases (WHO, 2015)

On January 29, 2017 at the commemoration of the 64th Anniversary of World Leprosy Day, with the theme: "Zero Disability Among Children Affected by Leprosy" the Minister of Health, Professor Isaac Adewole said that leprosy still posed a challenge due to the pockets of high endemicity in Jigawa, Kano, Kaduna, Kebbi, Bauchi, Taraba, Niger, Kogi, Ebonyi, Abia, Cross River, Edo, Osun, Ogun and Lagos States. (WHO, 2017). In 1998, after the introduction of the Multi- Drug Therapy (MDT), Nigeria achieved WHO'S elimination target of less than one case per 10,000 population at the national level, saying lateness in presenting cases at the health care facilities made matters worse (Obinna and Olawale, 2017).

Mycobacterium leprae can only survive in extremely selective environments as a result of reductive evolution. The bacteria still infect hundreds of thousands of people a year worldwide, but with the help of antibiotics the number has decreased. New antibiotic resistant strains are appearing, and stronger cocktails of drugs containing multiple antibiotics are now used to treat the disease. The multidrug cocktail currently used consists of the antibiotics dapsone, rifampicin, and clofazimine. It is possible that if the correct combination of minerals, vitamins, and amino acids is found that when added to a media in the correct environment for *Mycobacterium leprae* it could allow us to study the bacteria more and make drug research more effective. Even though 90% of those that come into contact with *Mycobacterium leprae* do not develop an infection, there are those that do show a wide spectrum of severity of symptoms as a result of a few key genes. If an infection is not detected early, there will be residual damage done to the peripheral nervous system of the infected individual because the lipids on the cell wall of the bacteria cause de-myelination of Schwann cells. Interestingly, the leprosy disease can sometimes be self-limiting and cure itself independently of drug treatment (WHO, 2013)

Mycobacterium leprae was the only known cause of leprosy until recently, when a new mycobacterium, *Mycobacterium lepromatosis,* was found to be the cause of diffuse lepromatous leprosy (DLL), a unique form of leprosy endemic in Mexico and the Caribbean (Han, 2008). Like *M. leprae, M. lepromatosis* has not been cultivated on artificial media. The discovery of this new species may provide an explanation for the clinical and geographical variability of leprosy.

1.3 Dapsone

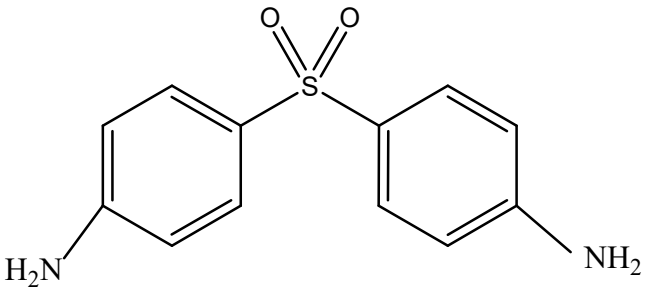


Fig. 1.3. Structure of Dapsone

Dapsone (4,4'-diamino diphenylsulfone: $C_{12}H_{12}N_2O_2S$: molar mass 248.302 $gmol^{-1}$) is widely used to treat a number of disorders. It is an antileprotic drug (Williams and Lemke, 2005; Rastogi, 1991). It is a pharmaceutical drug most commonly used in combination with rifampicin and clofazimine as multidrug therapy (MDT) for the treatment of leprosy infections as directed by the World Health Organization, W.H.O. (Shepherd, 1982) and as a sulphone analogue which has been proved to be a powerful antimicrobial agent (Lednicer, 1980). It is an odourless white to creamy-white crystalline powder with a slightly bitter taste and nearly water insoluble, though soluble in alcohol and very weakly basic. It has a melting point of 175-180°C, a pH of 6.98 and it is stable under ordinary conditions (Dapsone-Tech., 2013). Despite the lack of solubility, the drug is efficiently absorbed from the gastrointestinal tract. The mechanism of action of dapsone is probably similar to that of the sulphonamide which involves inhibition of the folic acid synthesis in susceptible organisms.

Dapsone is a very potent chelating agent and its effective coordination to metal ion may have significant biological implication (Ajibola, 1999). Binding sites of dapsone involves the amino nitrogen and sulphoxide oxygen (Tella and Obaleye, 2009). Structurally, dapsone has two amino groups (Fig. 1.3), therefore it can react with aldehyde or ketone to form Schiff bases, which are known to show bioactivity. Several derivatives of dapsone have been prepared in an attempt to increase its activity (Tella and Obaleye, 2009). The chemical modification of dapsone derivatives continue to be pursued with the intention of finding newer agents useful for the treatment of resistant strains of leprae (Dhople, 1991).

Dapsone is referred to as a 'sulpha drug'. Sulpha drugs are a group of compounds used for eliminating a wide range of infections in human and other animal systems. They possess SO_2NH moiety (Fig. 1.3), which is an important toxophoric functional group (Jain *et al.*, 2002). It has been reported that the biological activity of sulphur containing ligand increases on complexation (Biot *et al*., 1999). Metal complexes of sulpha drugs have been reported in literature with appreciable biological activities (Ajibola, 1999). Metal complexations of Schiff base derivatives of sulpha drugs were found to be further successful against some given bacterial strains (Zhou *et al*., 2000).

Sulphonamides with varying chemical, pharmacological and antibacterial properties are produced by attaching substituents to the amido group (SO_2NHR) or the amino group ($-NH_2$) of the sulphanilamide nucleus. Most sulphonamides are white, odourless, bitter, crystalline powder but are relatively insoluble in water. They are more soluble in alkali than in acid. They inhibit the incorporation of para - aminobutyric acid into folic acid (Brown, 1962; Long and Bliss, 1939) thus inhibiting DNA synthesis. Sulpha drugs are broad-spectrum agents, active against gram-positive and gram-negative bacteria and some protozoa (*Toxoplasma gondii* and *Plasmodia*).

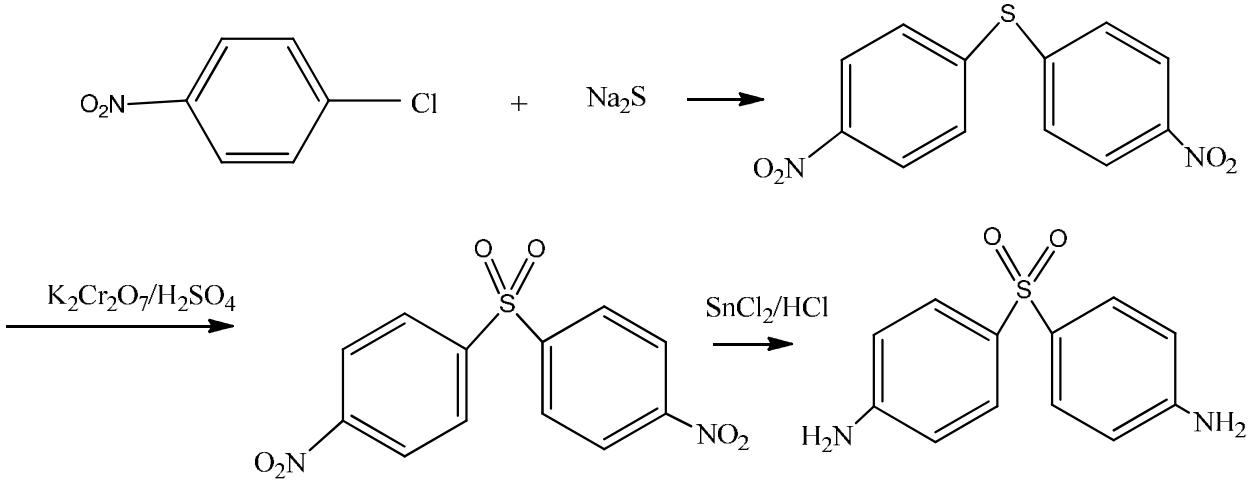
In addition to the treatment of leprae, dapsone is also used with pyrimenthamine in the treatment of malaria and has been effective in suppressing symptoms of malaria due to chloroquine resistant parasites *Plasmodium falciparum* (Colin, 1999). It is a second-line treatment for prophylaxis (prevention) against

pneumocystis pneumonia (PCP) caused by *Pneumocystis jirovecii* in HIV patients in whom CD4 counts are below 200 and in dermatology, dapsone has been found helpful in the treatment of numerous non-infectious inflammatory, autoimmune and bullous diseases (Wolf and Orni, 2001).

The use of dapsone is associated with certain side-effects including hepatitis, cholestatic, jaundice, hemolysis and methemoglobinemia. Other side effects include nausea, headache, insomnia, phycosis, peripheral neuropathy, coma and rash (Burkhart and Burkhart, 2009).

1.3.1 Synthesis of Dapsone

An effective method for synthesizing dapsone is obtained by condensing 4-chloronitrobenzene and 4-chloroacetanilide in the presence of sodium sulfide and obtaining the parasubstituted-diphenyl-sulfide intermediate which is subsequently oxidized by $KMnO_4/K_2Cr_2O_7$. The oxidized intermediate obtained is deacetylated and reduced for hydrogenation in presence of Raney-Nickel catalyst, known catalyst for this type of reaction (Chauhan, *et al.,* 1986).



Scheme 1.3: Synthesis of dapsone

A new process for synthesizing 4,4′-diamino-diphenylsulfone, resulting from a combination of the essential reactions of condensation, oxidation and reduction has been developed. It takes into consideration the industrial need to operate in mild conditions and with easily available and easy to handle reagents. This

synthetic process has been found to be more favourable in terms of purity and yield of dapsone. This new process for the synthesis of 4,4′-diamino-diphenylsulfone is characterized by the following steps:

i) condensation reaction of 4-aminothiophenol and 4-chloronitrobenzene with the subsequent formation of 4- (4′-nitrophenylsulfanyl)-phenylamine

ii) oxidation of the thioether formed in the previous step with an oxidising system formed by Na_2WO_4 and H_2O_2 into the corresponding sulfone

iii) reduction of 4-nitro-4′-aminoacetyldiphenylsulfone by hydrogenation in the presence of a catalyst obtaining the 4-4′-diaminodiphenylsulfone (Villa *et al.,* 2008)

1.4 Clofazimine

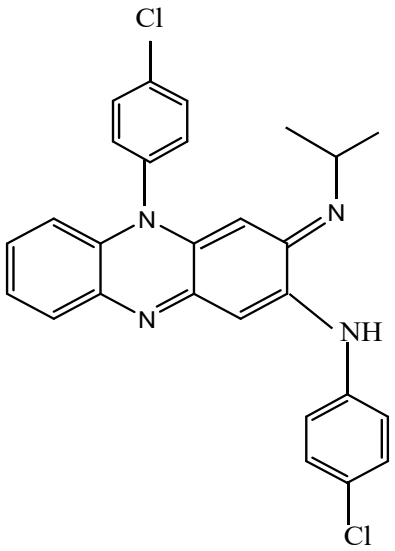


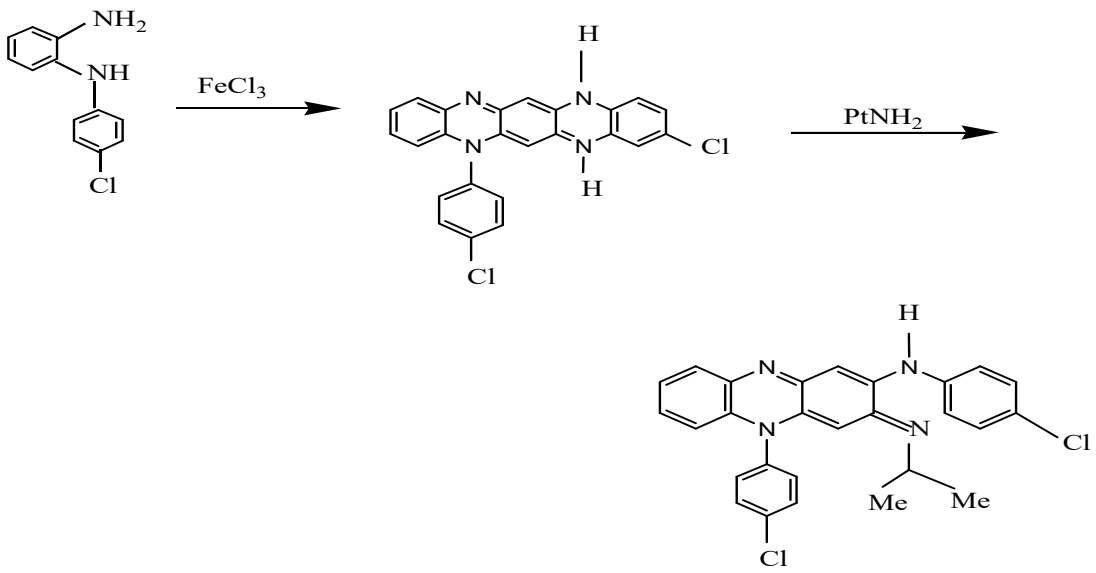
Fig. 1.4. Structure of Clofazimine

Clofazimine (N,5-bis(4-chlorophenyl)-3-(propan-2-ylimino-3,5-dihydrophenazine-2-amine)): $C_{27}H_{22}Cl_2N_4$: (molar mass 473.4gmol^{-1}), also called Lamprene is a fat-soluble riminophenazine dye used in combination with rifampicin and dapsone as a multidrug therapy (MDT) for the treatment of lepromatous leprosy, dapsone-resistant leprosy and lepromatous leprosy complicated by erythema nodosum leprosome. It is readily soluble in benzene, soluble in chloroform, poorly soluble in acetone and

in acetyl acetate, sparingly soluble in methanol and in ethanol and virtually insoluble in water. It has a melting point of 210-212°C and a pKa of 8.51 (Drugbank, 2014). It has been used in combination with other antimycobacterial drugs to treat *Mycobacterium avium* infections in AIDS patients and *Mycobacterium avium paratuberculosis* infection in Crohn's disease patients. Clofazimine also has marked anti-inflammatory effect and is given to control the leprosy reaction, erythema nodosum leprosom (ENL), (Drugbank, 2014). Structurally, clofazimine (Fig. 1.4) has an azomethine group (>C=N-), therefore functions as a Schiff base. Furthermore, it has been known that when Schiff bases form complexes with certain metals, their bioactivity is enhanced, hence it is expected that transition metal complexes of clofazimine will show higher bioactivity than the parent drug.

1.4.1 Synthesis of Clofazimine

Clofazimine is synthesized by oxidizing 2-(p-chloroanilino) aniline using a solution of iron (III) chloride in water. This leads to the formation of 2-(p-chloroanilino)-5-(p-cholophenyl)-3,5-dihydro-3-imino phenazine. Upon reacting this with a primary amine, in particular isopropylamine, the hydrogen atom in the imine region of the molecule is formally replaced with an alkyl group of the introduced amino group (in this case with an isopropyl group), forming the desired drug, clofazimine (Barry *et al*., 1957; Belton *et al.,* 1960; O' Sullivan *et al.* 1958). This procedure is illustrated in scheme 1.4.



Scheme 1.4: Synthesis of Clofazimine

1.5 Rifampicin

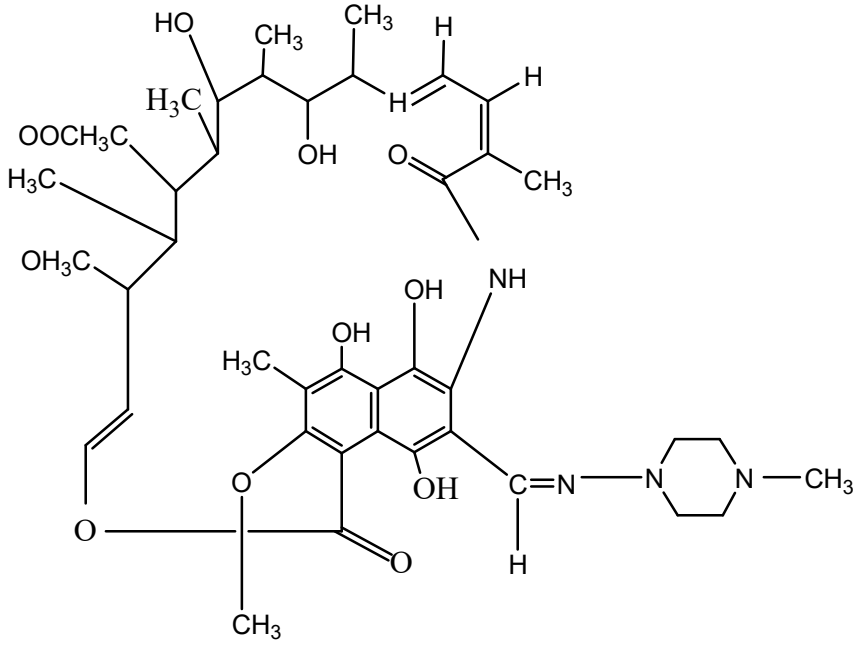


Fig. 1.5. Structure of Rifampicin

Rifampicin (Formula: $C_{43}H_{58}N_4O_{12}$: Molar mass 822.96gmol^{-1}), also called Rimactane is a bactericidal antibiotic drug of the rifamycin group. It is an orange-red or brown powder with a melting

point of 183-188°C, a pH of 7.38 and a pKa (in water) of 1.7 (Gallo and Radaell, 1976). It is soluble in DMSO, DMF, methanol, chloroform, ethyl acetate and acetone. It is slightly soluble in water at 25°C (Gallo and Radaell, 1976; Martindale, 1996). It is typically used to treat *Mycobacterium* infections including tuberculosis and leprosy. It is also an anti-hyperglycemic, anti-epileptic, neuropsychiatry therapeutics and anti-bacterial (Niemi *et al*., 2003). Rifampicin is used in combination with limipenem or salbactum in the treatment of Marine Pneumonia (Maria *et al*., 2006) and the complex of rifampicin with cyclodextrin is used as anti-tubercular drug. Rifampicin inhibits the action of bile acid. Synthesis may be a protective mechanism against drug and bile acid induced chiertasis (Tiangang *et al*., 2004). With multidrug therapy used as the standard treatment for leprosy disease, rifampicin is always used in combination with dapsone and clofazimine to avoid eliciting drug resistance. Structurally, rifampicin, like clofazimine functions as a Schiff base due to the presence of the azomethine (-C=N-) group in its molecule (Fig. 1.5) hence it can undergo chelation with transition metal ions to form complexes with bioactivity expected to be higher than that of the parent compound.

1.5.1 Synthesis of Rifampicin

At present, only three recent processes for the manufacture of rifampicin are known. Formerly, rifampicin was prepared by mildly oxidising a Mannich base of rifamycin SV and then mildly reducing the mixture thus obtained to give 3-formylrifamycin SV which is then reacted with 1-amino-4-methylpiperazine to form rifampicin.

In one of the recent processes, rifampicin is prepared by reacting rifamycin S with formaldehyde and with a primary aliphatic amine or with a condensation product thereof in the presence of manganese dioxide and then treating the reaction mixture with about 2 equivalents of 1-amino-4-methylpiperazine (Macielag, 2009).

In another process, rifampicin is obtained by reacting rifamycin S with an N-bis-alkoxymethyl-amine or an N-bis-hydroxymethyl-amine to give a well-defined intermediate compound, namely a 1,3-

oxazino rifamycin which reacts in distinctly basic medium with 1-amino-4-methylpiperazine to give rifampicin (Macielag, 2009).

In a more recent process for the preparation of rifampicin, rifamycin S is reacted with a 1,3,5-trisubstituted hexahydro-1,3,5-triazine in an aprotic dipolar solvent and optionally in the presence of formaldehyde, the reaction preferably being carried out without modifying the pH of the medium and preferably in the presence of certain acid substances, using controlled time and temperature conditions. 1-amino-4-methylpiperazine is then added directly to the reaction mixture, while keeping the pH value in the range of from 5 to 7, and then isolating the rifampicin formed (Macielag, 2009).

1.6 Application of Schiff Bases in Industries

1.6.1 Corrosion Inhibition

An interesting application of Schiff bases in industries is their use as an effective corrosion inhibitor, which is based on their ability to spontaneously form a monolayer on the surface to be protected (Ingold, 1986). Many commercial inhibitors include aldehydes or amines, but presumably due to the >C=N bond, the Schiff bases function more efficiently in many cases (Calderon *et al*., 2001). The principal interaction between the inhibitor and the metal surface is chemisorptions (Allen *et al.,* 2000). The inhibitor molecule should have centers capable of forming bonds with the metal surface by electron transfer. In such cases the metal acts as an electrophile and the inhibitor acts as a Lewis base (Hedstrom *et al*., 1992). Nucleophilic centers, such as oxygen and nitrogen atoms, of the protective compound have free electron pairs which are readily available for sharing (Anderson *et al*., 1993). Together with the atoms of the benzene rings they create multiple absorption sites for the inhibitor thus enabling stable monolayer formation.

1.6.2 Modern Technologies

Photo and thermochromic properties of Schiff bases as well as their biological activity make them applicable in modern technology. Among others, they are used in optical computers, to measure and

control the intensity of the radiation, in imaging systems, as well as in the molecular memory storage, as organic materials in reversible optical memories and photo detectors in biological systems (Tanaka et al., 2010; Pistolis et al., 1996). Due to photochromic properties, Schiff base containing compounds could behave as photostabilizers, dyes for solar collectors, solar filters. They are also exerted in optical sound recording technology (Tanaka *et al.,* 2010).

Among others, worthy of interest in the properties associated with Schiff bases include: properties of liquid crystal (Mocanu *et al*., 2010), chelating ability (Issa *et al.,* 1998), thermal stability (Atta *et al.,* 2006), optical nonlinearity (Jia *et al.,* 2011) and the ability to create the structure of a new type of molecular conductors using electrical properties to proton transfer (Amany and Ibrahim, 1992). Because of its thermal stability Schiff bases can be used as stationery phase in gas chromatography (Attah *et al.,* 2006*)*. The optical nonlinearity of these compounds allows us to use them as electronic materials, opto-electronic (in optical switches) and photonic components (Jia *et al*., 2011). Imine derivatives can be exerted to obtain conductive polymers.

Schiff bases as an electrical conductor possess a variety of uses. They are used as catalysts in photoelectrochemical processes, electrode materials and micro-electronic equipment, organic batteries or electrochromic display device (graphical output devices) (Kumar *et al*., 2009). Due to the presence of the imine group, the electron cloud of the aromatic ring and electronegative nitrogen, oxygen and sulfur atoms in the Schiff bases molecules, they effectively prevent corrosion of mild steel, copper, aluminium and zinc in acidic medium (Emregul *et al.,* 2006).

1.6.3 Applications of Schiff Bases as Chemical Intermediates

Schiff bases may also act as a group of organic intermediates, which are very often used in synthesis and chemical analysis. They are exerted in the production of pharmaceutical and agrochemical products. In the reaction with hydrogen cyanide, Schiff bases may form α-amino acid precursors (Strecker Synthesis). Also, chiral Schiff bases are used as initial substrates for the asymmetric synthesis of

α -amino acids, and as catalysts in asymmetric synthesis (Brodowska and Lodyga-Chruscinska, 2014). Furthermore, the imines obtained by the condensation reaction of arylamines and carbonyl compounds form a group of intermediates used in the preparation of important compounds (arenediazonium nitrates, N-arylarene carboxamides, the appropriate amines and cyanamides, β-lactams) (Ashraf *et al.,* 2011). Otherwise, Schiff bases are precursors of reactions of polycyclic derivatives of quinoline and isoquinoline by oxidative ring closure under the influence of ultraviolet light (Brodowska and Lodyga-Chruscinska, 2014). They are also used for the preparation of acyclic and macrocyclic compounds, such as cryptats, coronates and podates (Tanaka *et al.,* 2010). These compounds lead to the formation of Ruhemann's purple (reaction between an amino acid and ninhydrin), which allows to detect and assist in the identification of fingerprints (Brodowska and Lodyga-Chruscinska, 2014).

1.6.4 Medical and Biological Applications of Schiff Bases

Schiff bases have been used as medicinal compounds for centuries and form the basis for many common drugs such as morphine (analgesic), captopril (treatment of hypertension), vicristine (cancer therapy), rifampicin and clofazimine (leprosy treatment) and many others. They have also been found to possess pharmacological activities such as antimalarial (Li *et al.,* 2003), anticancer (Villar *et al.,* 2004), antifungal (Pandey *et al.,* 2003), antitubercular (Bhat *et al*., 2005), anti-inflammatory, antimicrobial (Wadher *et al.,* 2009; Venugopal and Jayashree, 2008) and antiviral (Karthikeyan *et al.,* 2006) etc.

1.6.4.1 Antibacterial Activity

Mortality increase caused by infectious diseases is directly related to the bacteria that have multiple resistances to antibiotics. The development of new antibacterial drugs enriched by innovatory and more effective mechanisms of action is clearly an urgent medical need (Rice, 2006). Schiff bases are identified as promising antibacterial agents. For example, N-(Salicylidene)-2-hydroxyaniline is active against *Mycobacterium tuberculosis* (Silva-da *et al.,* 2011), clofazimine and rifampicin are active against *Mycobacterium laprae.*

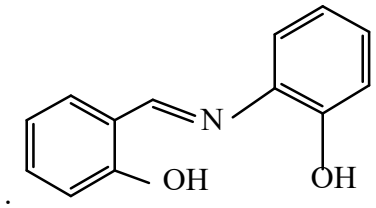


Fig. 1.6.3 : N-(Salicylidene)-2-hydroxyaniline

Schiff bases containing 2, 4-dichloro-5-fluorophenyl moieties also take part in effective inhibition of bacterial growth (Yang *et al.,* 2012). On the other hand, the compounds obtained from furylglyoxal and p-toluidene show antibacterial activity against: *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis* and *Proteus vulgaris*. Isatin derived Schiff bases present anti-HIV and antibacterial activity (Brodowska and Lodyga-Chruscinska, 2014). Other Schiff bases derivatives, which possess antibacterial activity are benzimidazole, thiazole, pyridine, glucosamine, pyrazolone, hydrazide, thiazolidiones, indole, thiosemicarbazone, p-fluorobenzaldehyde (Kumar *et al.,* 2009).

1.6.4.2 Antifungal Activity

Fungal infections usually are not only limited to the contamination of surface tissues. Recently, there was a considerable increase in the incidence of systemic fungal infections, which are potentially life-threatening (Sundriyal *et al.,* 2006). Exploration and development of more effective antifungal agents is necessity, and the individual Schiff bases are considered to be promising antifungal medicines (Rehman *et al.,* 2004). Some of them, such as imine derivatives of quinazolinones possess antifungal properties against *Candida albicans*, *Trichophyton rubrum*, *T. mentagrophytes*, *Aspergillus niger* and *Microsporum gypseum* (Rehman *et al.,* 2004). Schiff bases formed between furan or furylglycoxal with various amines exhibit antifungal activity against *Helminthosporium gramineum* causing leaf stripe in barley, *Syncephalostrum racemosus* contributing to fruit rot in tomato and *Colletotrichum capsici* causing anthracnose in chillies (Kumar *et al.,* 2009).

Schiff bases obtained by the synthesis of o-aminobenzoic acid and β-keto esters have found biocidal use against *S. epidermidis*, *E. coli*, *B. cinerea* and *A. niger* (Kalaivani *et al.,* 2012). By contrast, Schiff bases of isatin derivatives are used in the destruction of protozoa and parasites (Pandeya *et al.,* 1999).

1.6.4.3 Antiviral Activity

The use of vaccines may lead to the eradication of pathogens known viruses, such as smallpox, poliomyelitis (polio) etc. Although there are many therapeutic ways to work against viral infections, currently available antiviral agents are not fully effective, which is likely to cause a high rate of mutation of viruses and the possibility of side effects. Salicylaldehyde Schiff bases derived from 1-amino-3-hydroxyguanidine tosylate are good material for the design of new antiviral agents (Silva-da *et al.,* 2011). Isatin Schiff base ligands are marked by antiviral activity, and this fact is very useful in the treatment of HIV (Pandeya *et al.,* 1999). In addition, it was also found that these compounds have anticonvulsant activity and may be included in the anti-epileptic drugs (Sridhar *et al.,* 2002). Gossypol derivatives also present high antiviral activity. Increasingly, gossypol, often used in medical therapy is replaced by its derivatives, because of their much lower toxicity (Przybylski *et al.,* 2005). Schiff bases have obtained acceptable results for Cucumber mosaic virus, whose effectiveness was estimated at 74.7% (Kumar *et al.,* 2009).

1.6.4.4 Antimalarial Activity

Malaria is a disease which when neglected causes serious health problems. Human malaria is largely caused by four species of the genus Plasmodium (*P. falciparum*, *P. vivax, P. ovale* and *P. malariae*) (Brodowska and Lodyga-Chruscinska, 2014). The search for new drugs, vaccines and insecticides for the prevention or treatment of this disease is a priority. Schiff bases are interesting compounds, which could be part of antimalarial drugs. For example, the compound with such effect is

Ancistrocladidine which is a secondary metabolite produced by plants of the family *Ancistrocladaceae* and *Dioncophyllaceae,* and presenting an imine group in a molecular chain (Silva-da *et al.,* 2011).

Cryptolepine, valid indolchinoline alkaloid, isolated from African plant Cryptolepis sanguinolenta, also used in the treatment of malaria, is the product of multi-stage reaction, in which Schiff base is involved (Dutta *et al.,* 2006).

1.6.4.5 Anticancer Activity

Some Schiff bases have a high antitumor activity. Imine derivatives of N-hydroxy-N'-aminoguanidine block ribonucleotide reductase in tumor cells, so that they are used in the treatment of leukemia (Jesmine *et al.,* 2010).

Schiff bases of PDH [N-(1-phenyl-2-hydroxy-2-phenyl ethylidine)- 2′,4′-dinitrophenyl hydrazine], PHP [N-(1-phenyl-2-hydroxy-2- phenyl ethylidine)-2′-hydroxy phenyl imine] and HHP [N-(2-hydroxy benzylidine)-2′-hydroxy phenyl imine] reduce the average tumor weight (reduction in tumor growth increases with increasing dose) and decrease the growth of cancer cells in mice EAC cells. In addition, they have ability to rebuild depleted haematological parameters, such as hemoglobin, red blood cells (RBC) and white blood cells (WBC) towards the right content. They also show protective effect on hematopoietic system (Ozaslan *et al.,* 2011).

1.6.4.6 Antileprosy Activity

Clofazimine, dapsone and rifampicin are well known antibiotics. Antibiotics, also called antibacterials, are a type of antimicrobial drugs used in the treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria.

Dapsone belongs to a group of drugs known as sulphones. It works by decreasing inflammation and stopping the growth of bacteria (Lednicer, 1980). However, it is better known for its antibacterial activity against *Mycobacterium leprae* but is found to have reduced potency over the years due to secondary resistance of *M. leprae.*

Rifampicin is a rifamycin antibiotic used to prevent and treat tuberculosis and other infections while clofazimine is mostly used in combination with other antibiotics to treat various infections. Dapsone, clofazimine and rifampicin constitute the Multi Drug Therapy (MDT) for treating leprosy infections. This combination of drugs is considered to be more effective than using only the individual drugs (World Health Organization, 1998). However, the duration of treatment using the Multi Drug Therapy of dapsone, rifampicin and clofazimine extends to about 24-36 months in some cases and this leads to poor patient compliance which in most cases brings about incomplete cure of the disease (Meina, 2004). As such, there is need for research work so that these drugs are modified so as to make them more effective hence reducing the time duration for curing bacterial infections including leprosy. Rifampicin and clofazimine contain the azomethine group, and as such function as Schiff base ligands while dapsone is an amine and so can undergo condensation with a carbonyl compound under appropriate experimental conditions to yield a Schiff base (an imine). Intensive research on compounds containing azomethine group may result in more effective drugs. It is well known that coordination of Schiff bases with transition metals enhance the activity of such drugs.

1.7 Aim and Objectives of the Research

1.7.1 Aim of the Research

The aim of this research is to synthesize and characterize two Schiff bases of dapsone and some divalent metal complexes of the synthesized Schiff bases and also to synthesize and characterize some divalent metal complexes of rifampicin and clofazimine.

1.7.2 Objectives of the Research

The objectives of this study are the;

(i) synthesis of Schiff base derived from dapsone by reacting dapsone and salicylaldehyde,

(ii) synthesis of divalent metal complexes of the Schiff base synthesized in (i) above using each of the following metal chloride; Mn(II), Co(II), Ni(II), Fe(II), Cu(II) and Zn(II),

(iii) synthesis of Schiff base derived from dapsone by reacting dapsone and acetyl acetone,

(iv) synthesis of divalent metal complexes of the Schiff base synthesized in (iii) above using each of the following metal chloride; Mn(II), Co(II), Ni(II), Fe(II), Cu(II) and Zn(II),

(v) synthesis of divalent metal complexes of rifampicin using each of the following metal chloride; Mn(II), Co(II), Ni(II), Fe(II), Cu(II) and Zn(II) and the

(vi) synthesis of divalent metal complexes of clofazimine using each of the following metal chloride; Mn(II), Co(II), Ni(II), Fe(II), Cu(II) and Zn(II). Also,

(vii) characterization of all the synthesized Schiff bases and divalent metal complexes by IR, solubility test, elemental analysis, magnetic susceptibility, melting point, decomposition temperature and molar conductance measurements,

(viii) antimicrobial screening of all the synthesized divalent metal complexes, and the

(ix) toxicity tests on all the divalent metal complexes synthesized from the various ligands.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Transition Metal Complexes

Transition metal complexes are cationic, neutral or anionic species in which a transition metal is coordinated by ligands (Cox, 2005). Transition metals have varying utility and interesting chemistry. They are very necessary for life. Coordination compounds are important due to their roles in chemical and biological systems in various ways. Such compounds are extensively studied in chemistry. The interest in transition metal complexes is rapidly increasing as evidenced by the very large number of reports appearing annually (Brodowska and Lodyga-Chruscinska, 2014). So much interest in imines can be explained by the fact that they are widely distributed in many biological systems and are used in organic synthesis and chemical catalysis, medicine, pharmacy and chemical analysis, as well as new technologies (Upadhyay *et al.,* 2008; Anant and Devjani, 2011; Jesmine *et al.,* 2010; Sundriyal *et al.,* 2006; Barry *et al.,* 1957; Bately and Graddon, 1968; Boreham and Chiswell, 1977; Ahmed and Aktar, 1983; Anderson *et al.,* 1993). This also explains why the Schiff bases and their transition metal complexes continue to be of interest even after over hundred years of study.

2.2 Historical Background

Various aspects of Schiff base complexes of transition metal ions have been reviewed dating back to the 1960s and 1970s (Holm *et al.,* 1966; Yamada, 1966; Calligaris *et al.,* 1972; Hobday and Smith, 1972; Holm and O'Connor, 1971). As early as 1840, Ettling had isolated a dark-green crystalline product from the reaction of copper(II) acetate, salicylaldehyde and aqueous ammonia (Holm *et al.,* 1966). Other early workers include Delepine (Holm *et al.,*1966) who in 1899 prepared some complexes by reacting metal acetate, salicylaldehyde and primary amines in alcohol and confirmed the 2:1 stoichiometry earlier established by Schiff (Schiff, 1864).

The earliest systematic synthetic study of Schiff base complexes was embarked upon by Pfeiffer (1933) in 1931. He and his co-workers prepared a prodigious number and variety of complexes derived from Schiff bases of salicylaldehyde and pyrrole-2-aldehyde. In particular Pfeiffer (1933) showed that N, N'-bis (salicylidene) ethylenediiminatocobalt (II) was air-sensitive, changing from red to black in air. Dubsky and Sokol (Holm *et al.,* 1966) isolated N,N-bis (salicylidene) ethylenediiminatocopper(II) and nickel(II) and correctly formulated their structure. Combes (Holm *et al.,* 1966) isolated the first complex of β-ketoimine in 1889. A variety of complexes analogous to the β-ketoimine complexes were later prepared by Morgan and Main-Smith (Morgan and Smith, 1925).

Later work on Schiff base complexes of transition metal ions was directed towards understanding the stereochemistry and effect of coordination on the magnetic and electronic properties of metal ions. It has been reported that complexes of quadridentate salicylaldimines can assume a variety of stereochemical forms depending on the nature of the metal ion and the conformational behaviour of the chelate ring (Holm, 1960; Hariharan and Urbach, 1969; Hoyt and Everett, 1969). The conformational behaviour of the methylene chain also depends, in addition to the absolute configuration of the diamine and on the nature of the substituents in the carbon atoms of the diamine (Pasini and Gullotti, 1974). The ligand salicylaldimine and its metal complexes with bivalent metal ions are well known. Their structures change from the planar coordination of Schiff base to non-planar tetrahedral coordination depending on the magnitude of the coordination number n. Metal complexes of this type have been prepared for the series n = 2 to 10 for the bivalent cobalt (including n = 11, 12) (Holm *et al.,* 1966; Hariharan and Urbach, 1969; Weigold and West, 1967), nickel (including n = 11, 12), (Holm *et al.,* 1966; Holm and O'Connor, 1971; Holm, 1960; Hoyt and Everett, 1969; Mockler, 1972) and copper (Holm *et al.,* 1966; Holm and O'Connor, 1971; Bately and Graddon, 1968; Gruber *et al.,* 1968; Sinn and Harris, 1969). They have also been prepared for the series n=2 to 10 for manganese(II) (Lewis *et al.,* 1968; Boreham and Chiswell, 1977; Matsushita, 1973; Titus *et al.,* 1979) and zinc(II) (Holm *et al.,* 1966; Batley and Graddon, 1968) and attempts made to

correlate stereochemistry with the nature of the metal ions and length of the methylene chain or n = 2, showed that most divalent first-row transition metals are expected to form square-planar complexes. However, a decrease in ligand field strength has been reported for nickel(II), copper(II) and zinc(II) derivatives as n is increased from 2 to 4. This decrease corresponds to an increase in distortion from planarity with increase in the length of the methylene bridge. When the chain becomes longer (n > 6) steric constraints on complexes should decrease due to the increasing flexibility of the long alkyl chain. In this case, the environment about the metal atom should approach the preferred stereochemistry for the particular metal.

Nickel(II) complexes with coordination number (n) equals to 4 are diamagnetic and presumably planar (Holm, 1960). Similarly nickel(II) complexes of Schiff bases derived from pyrrole-2-carboxaldehyde and diamines having 2 to 5 methylene carbon atoms are diamagnetic and monomeric (Hoyt and Everett, 1969; Weber, 1967). These complexes are anhydrous solids but their diamagnetism, like that of N,N'-bis- (salicylidene)ethylenediiminatonickel(II) and other diamagnetic derivatives of nickel salicylaldimines, persist in solutions of non-coordinating solvents.

In the hope of inducing sufficient distortion from planar toward tetrahedral to reach the point of spin-multiplicity change (planar, S=0; tetrahedral, S=1), Hoyt and Everett (1969) synthesized nickel(II) complexes with n = 5 to 12. The magnetic moments of these complexes varied widely ranging from 1.1 B.M. (n = 6) to 3.08 B.M. (n = 12). These values fall within the intermediate range of the moments expected for low-spin and high-spin nickel(II) complexes but no tetrahedral species were evident from the spectra. For n = 11 and 12, diamagnetic derivatives were also isolated using excess ligands. This magnetic behaviour was explained in terms of a polymeric structure in which the observed partial paramagnetism of the complexes arose from the nickel(II) atoms existing in planar (diamagnetic) and octahedral

(paramagnetic) environments within the same polymeric unit. A similar phenomenon was observed by Mockler, (1972) for 5-chloro-2-hydroxybenzophenone analogues (n = 2 to 12).

Lever (1968) had earlier observed that the absence of a ligand field absorption band below 10000 cm^{-1} or 10 KiloKaiser (10.0 kK), due to large crystal field splitting, could be used to distinguish planar nickel(II) complexes from octahedral and tetrahedral symmetries. In those complexes thought to be octahedral, an additional band to that at about 16000 cm^{-1} (16.0 kk) was observed at about 9400 cm^{-1} (9.4 kK). Cobalt(II) is known to have a more pronounced tendency to form tetrahedral complexes than nickel(II) and copper(II) (Orgel, 1966). Despite this, N,N'bis(salicylidene)ethylenediiminato cobalt(II) was found to have a low-spin square-planar-structure. This peculiar situation was thought to have been imposed on the cobalt(II) ion by the steric requirement of the ligand (Orgel, 1966). However, as the methylene chain-length is increased, this steric hinderance is relaxed and the cobalt(II) may assume a preferred tetrahedral geometry to relieve the steric interactions in the Polymethylene bridge (Hariharan and Urbach, 1969; Weigold and West, 1967). Thus for n = 4 to 10 a pseudo-tetrahedral geometry was proposed (Hariharan and Urbach, 1969; Weigold and West, 1967). This configuration is more flattened in n = 3 and a high-spin flattened tetrahedral configuration and an octahedral configuration have been suggested for the anhydrous and hydrated forms respectively.

Later structural work (Delasi *et al.,* 1971) has, however, shown inactive form of N,N'-bis-(salicylidene)ethylenediiminato cobalt(II) to be dimeric. The peculiar property of this complex to add on, reversibly, molecular oxygen and carbon dioxide has attracted immense interest (Calvin and Markelev, 1946; Schaefer and Marsh, 1969; Bruckner *et al.,* 1969) and is still being actively investigated (Lancashire *et al.,* 1979; Cesarotti, 1974).

Calligaris *et al*., (1972) reviewed some structural work done on metal complexes of tetradentate Schiff base complexes, including the structure of the active monochloroform

adduct of Co(sal_2-en) (Schaefer and Marsh, 1969). This compound has a planar arrangement of the metal Schiff base with the chloroform molecules weakly hydrogen bonded to the phenolic oxygen atoms. The complex contains pairs of Co(sal_2-en) units 'centrosymmetrically arranged' (van der Waals distance 350 pm). The chloroform molecule can be easily lost and replaced by oxygen molecule. This might explain the oxygenation property of Co(sal_2-en).

Complexes of transition metal ions with multidentate organic ligands have been extensively studied because they have interesting spectral and magnetic properties, and also possess a diverse spectrum of biological activities (Angelique and Thomas, 1999; Richardson and Bernhardt, 1999; Buss *et al.,* 2004; Sommer *et al*., 1972; Shargi and Nasser, 2003). These complexes often possess remarkable and unique spectroscopic, photophysical and electrochemical properties which may be exploited in sensory and diagnostic applications and there have been a number of reviews on the utilisation of transition metal complexes as ion and molecular sensors (Beer, 1994; Veggel *et al*., 1994; Nabeshima, 1996; Canary and Gibb, 1997; Gray, 1995; Pecoraro *et al.,* 1997). Based on the widely diverse coordination environment of the transition metal complexes, and variation in the identities of the coordinating ligands, synthesis of such complexes with desired molecular geometry can be realized.

One of the main goals of present day inorganic coordination chemists and pharmaceutical investigation is the discovery and development of better drugs to fight diseases, and this has led to numerous studies on drug metal complexes. Structural modification of organic molecules has considerable biological relevance. The ability of a metal to combine with certain ligands makes them ideal for use in biological systems. A number of reviews on modification of the structure of the existing antimalarial drugs by incorporation of metals into their molecular structure appeared in literature. Notable among them are those of Spacu *et al*., (1968), Wasi *et al*., (1987), Hubel *et al*., (2000), Biot *et al*., (1999), Navarro *et al*., (2001), Sanchez-Delgado *et al*., (1993) and Tsangaris and Baxevanidis, (1974). Majority of these complexes were found to possess higher antimalarial activities than their parent drugs. The most

remarkable to date is the work carried out by Biot and co-workers (1999). They inserted ferrocene (organometallic compound) into molecular structure of some antimalarial drugs. There is strong evidence that significant structural change to the side chain either through altering its length or through the introduction of more structural motifs such as ferrocene circumvents chloroquine resistance since the parasite needs iron for its development inside the red blood cells.

In the series of studies on the modification of existing drugs, Tella and Obaleye (2010) reported the synthesis of some novel N-acetyl derivatives of sulpha drugs and their biological activities. They synthesized six acetyl derivatives of some sulpha drugs, namely, Sulfadoxine, Sulphadimidine, Sulphamethoxazole, Sulphdiazine, Dapsone and Sulphamethaxazole and in each case $–COCH_3$ group was introduced into the heterocyclic framework of the sulpha drugs. The derivatives were characterized by melting point, UV and IR spectroscopy, elemental analysis and thin layer chromatography. The compounds were screened against *Aspergillus niger, E. coli and Staphylococcus aureus.* Some of the derivatives were found to have higher activities than the parent drugs. Toxicological studies were carried out by investigating the effect of administration of the parent drugs and derivatives on Alkaline Phosphate (ALP) activities on serum, liver and kidney of albino rats. Overall, it was discovered that the derivatives exhibited lower toxicity on tested organs than the parent drugs.

Yamgar *et al.,* (2014) studied the synthesis and antimicrobial activity of novel Zn(II) metal complexes derived from three novel heterocyclic Schiff base ligands. The Schiff base ligands and metal complexes were characterized by spectroscopic techniques. According to the data obtained, an octahedral geometry for all the metal complexes was proposed. Antimicrobial activity of the Schiff base ligand and its metal complexes was studied against Gram negative bacteria: *E.coli* and *Pseudomonas fluorescens*, Gram positive bacteria: *Staphylococcus aureus*, and also against fungi: *C. albicans* and *A. niger*. Some of the metal complexes show significant antifungal activity (MIC < 0.2μg/mL). The "*in vitro*" data identified

$[Zn(NMAPIMHMC)_2]\cdot 2H_2O$, $[Zn(TMPIMP)_2]\cdot 2H_2O$, and $[Zn(HBABO)_2]\cdot 2H_2O$ as potential therapeutic antifungal agents against *C. albicans* and *A. niger*.

Wadher *et al.,* (2009) carried out the synthesis and biological evaluation of Schiff base of dapsone and their derivative as antimicrobial agents. In their study, a series of Schiff base and 2-azetidinones of 4, 4'-diaminodiphenylsulphone were synthesized. 4, 4' diaminodiphenylsulphone was condensed with various aromatic or heterocyclic aldehydes in ethanol in the presence of concentrated sulphuric acid as a catalyst to yield the Schiff base. These Schiff bases on treatment with chloroacetylchloride in the presence of triethylamine gave substituted 2-azetidinone. All the compounds synthesized were evaluated for their *in vitro* activity against several microbes.

Malik *et al.,* (2010) carried out the synthesis, characterization and antimicrobial studies of Zn(II) complex of chemotherapeutic importance. The Zn(II) complex of Schiff base derived from salicyladehyde and acetazolamide was synthesized keeping in view that some metal complexes were found to be more potent than their parent drugs. The synthesized complex was characterized on the basis of elemental analysis, conductivity, magnetic measurements, particle size analysis, IR and NMR spectral studies. Comparative antibacterial behavior of Schiff base with their complex was also studied. The particle size analysis concluded that complexation of acetazolamide (pure drug) with Zn(II) via the formation of Schiff base can enhance the dissolution rate of the pure drug and thereby improve its bioavailability and has the potential to produce faster onset of action. The results of antibacterial activity concluded that the synthesized Zn(II) complex of AZM-SA has better antibacterial activity at different concentrations.

Aliyu and Ado (2011) carried out physicochemical studies on Mn(II) and Ni(II) complexes with Schiff base derived from 2-amino benzoic acid and salicylaldehyde. The complexes were prepared and characterized by gravimetry, potentiometry, molar conductance and infrared analyses. The potentiometric and spectrophotometric studies of the complex compounds revealed 1:1 metal to ligand ratio.

Aliyu and Yerima (2013) synthesized N-(4-nitrobenzylidene) naphthalene-1-ylamine Schiff base by the reaction of 1-naphthylamine and p-nitrobenzaldehyde in absolute ethanol medium. The Schiff base was reacted with Mn(II) and Cu(II) chlorides in an ethyl acetate medium and the resulting complexes were characterized by solubility, elemental analysis, IR and UV-visible spectroscopy and molar conductivity measurements. The synthesized complexes showed appreciable antibacterial and antifungal activities at high concentrations.

Panayot *et al.,* (1997) investigated the complexation of copper(II) with a new semisynthetic cynnamyl derivative of rifampicin in methanol solutions using UV-visible and EPR spectroscopy. They studied the process at different ligand-to-metal ratios (L:M from 100:1 to 1:10). In an excess of the ligand, two mononuclear complexes of the type CuL are formed with different modes of coordination. At equimolar concentrations of the reagents, another polynuclear complex with empirical formula $Cu(L)ClO_4$ was obtained and isolated in a solid phase. It was deduced that each Cu^{2+} in the complex is bound with two O-donors (0-1 and 0-8) from one ligand molecule and by O-4 and N-donors from another one.

Lawal *et al.,* (2014) prepared some transition metal complexes of nicotinamide and characterized them using melting point, conductivity measurement, infrared, electronic, ^{1}HNMR and atomic absorption spectroscopic methods. The antibacterial and antifungal studies of the metal complexes and the ligand have been evaluated against *Escherichia coli*, *Staphylococcus* and *Bacillus subtilis*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillum* species. It was found that nicotinamide formed stable metal complexes with these metal ions. The analysis of the spectroscopic data showed that nicotinamide acts as monodentate ligand, coordinating through the nitrogen atom of the pyridine ring. All the complexes exhibited 4- coordinate geometry. The results of the antimicrobial studies showed that the metal complexes have higher inhibitory activity than the original nicotinamide against the tested bacteria and fungi species.

2.3 Application of Transition Metal Complexes

The importance of Schiff base complexes for bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well recognized and reviewed (Kaul *et al.,* 1985; Anant and Devjani, 2011; Kumar *et al.,* 2009); Fakhari *et al.,* 2005; Park *et al.,* 2006; Ashraf *et al.,* 2011). A large number of Schiff bases and their complexes are of significant attention because of their biological activity including anti-tumor, antibacterial, fungicidal and anti-carcinogenic properties and catalytic activities (Ren *et al.,* 2002; Venkatachalam and Ramesh, 2007)

2.3.1 Application of Transition Metal Complexes in Industry

2.3.1.1 Dyes and Polymers

Chromium azomethine complexes, cobalt complex Schiff base (Itrat *et al.,* 2013) unsymmetrical complex 1:2 chromium (Anant and Devjani, 2011) dyes give fast colours to leathers, food packages, wools etc. Azo groups containing metal complexes (Kumar *et al.,* 2009) are used for dying cellulose polyester textiles. Some metal complexes are used to mass dye polyfibers (Kaul *et al.,* 1985). Cobalt complex (Anant and Devjani, 2011) of a Schiff base (salicylaldehyde with diamine) has excellent light resistance and storage ability and does not degrade even in acidic gases (CO_2). Novel tetradentate Schiff base acts as a chromogenic reagent for determination of Ni in some natural food samples (Fakhari *et al.,* 2005).

Photochemical degradation of natural rubber yield amine terminated liquid natural rubber (ATNR) when carried out in solution, in presence of ethylenediamine. ATNR on reaction with glyoxal yield poly Schiff base (Fakhari *et al.,* 2005), which improves aging resistance. Organocobalt complexes with tridentate Schiff base act as initiator of emulsion polymerization and co-polymerization of dienyl and vinyl monomers (Young and Cooper, 1983).

2.3.1.2 Catalysis

The Schiff base transition metal complexes are a family of attractive oxidation catalysts for a variety of organic substrates because of their cheap and easy synthesis and their chemical and thermal stability (Sheldon and Kochi, 1981). Important oxidation reactions include the transformation of alcohols to either the corresponding carbonyl compounds or carboxylic acids, the oxidation of sulfides to sulfoxides, alkenes to epoxides and diols, and the activation of hydrocarbons etc. The catalytic activities of the Mn(II), Fe(III), Co(II) and Cu(II) complexes are observed for their activity towards phenol hydroxylation reaction. All these complexes show good activity. The activities of these cobalt complexes are slightly lower than that of copper(II), iron(II) and manganese (II) analogues of the investigated Schiff bases (Van Wyka *et al.,* 2007). The major product of the reaction was found to be catechol (Park *et al.,* 2006). The cobalt(II) complex is found to be inactive, which may be due to the dimer formation which makes it unable to bind with the oxygen to form the intermediate. The copper complex was found to be the most active catalyst.

Co(II), Fe(III) and Ru(III) complexes of Schiff bases derived from hydroxybenzaldehyde are used in oxidation of cyclohexane into cyclohexanol and cyclohexanone in presence of hydrogen peroxide. The most efficient catalysts are the Fe(III) complexes which is unusual because, in general, the cobalt(II) complexes have high activity for alkane oxidation reactions (Vigato and Tamburini, 2004). Chromium-salen complexes are well known catalysts both in heterogeneous and homogeneous processes. Binucleating complexes of Fe(II), Co(II), Ni(II), and Zn(II) with Schiff bases neytralbis(iminopyridyl)benzene and monoanionicbis(iminopyridyl) phenolate act as catalysts in the oligomerisation of ethylene. New manganese(II) and manganese(III) complexes of substituted N,N'-bis(salicylidine)-1,2-diimino 2-methylene appear to be efficient models for peroxidase activity (Cozzi, 2004). New Copper(II) complexes of indoxylthiosemicarbazone (ITSC) show one pair of well defined reduction peaks at different potential in the forward scan, which represent the reduction of Cu^{2+} to Cu^{+} by

one electron process and subsequent oxidation of Cu^{+}. The quas-irreversible nature of the Cu^{2+}/Cu^{+} is due to inherent reducing tendency of thiosemicarbazone ligands. A wide variety of cobalt(II) complexes are known to bind dioxygen more or less reversibly and are therefore frequently studied as model compounds for natural oxygen carrier and for their use in O_2 storage, as well as in organic syntheses due to their catalytic properties under mild conditions (Granger *et al.,* 2005).

2.3.2 Medical Applications of Transition Metal Complexes

Imine complexes have a broad range of biological properties such as antitumor, antiviral, antifungal and antibacterial (Radecka-Paryzek *et al.,* 2007). They are also used in the treatment for diabetes and Acqiured Immune Deficiency Syndrome (AIDS). As biological models, they help in understanding the structure of biomolecules and biological processes occurring in living organisms. They participate, inter alia, in photosynthesis and oxygen transport in organisms. They are involved in the treatment of cancer drug resistance, and often tested as antimalarials. It also could be used for the immobilization of enzymes (Boghaei *et al.,* 2008; Prashanthi *et al.,* 2008).

2.3.2.1 Biological Activity

Schiff bases are characterized by an imine group –N=CH-, which helps to clarify the mechanism of transamination and racemization reaction in biological system. They exhibit antibacterial and antifungal effect in their biological properties (Golcu *et al.,* 2005). Metal-imine complexes have been widely investigated due to antitumor and herbicidal use. They can work as models for biologically important species (Ashraf *et al.,* 2011).

Microorganisms adsorb metal ions on their cell walls and as a result respiration processes of cells are disturbed and protein synthesis is blocked which is the requirement for further growth of organisms (Li *et al.,* 2003). The growth inhibition effects of metal ions are considerable. The only passage of lipid soluble material is favoured by the lipid membrane that surrounds the cell in accordance with the overtone's concept of cell permeability, as the antifungal activity is controlled by lipophillicity factor. The

overlap of ligand orbitals and the behavior of metal ions to share charge with the donor groups is reduced upon chelation. Besides this, the delocalization of π electrons over the whole ring is due to chelation and liphophilicity of complexes is enhanced. The proliferation of microorganisms is further restricted because the penetration of complexes in lipid membranes is facilitated by increased lipophilicity (Li *et al.,* 2003). The impermeability of microbial cells and differences in ribosomes of cells are the major reasons for variations in the effectiveness of different compounds against a variety of organisms. In most of the cases, ligands are less effective antifungal agents than their metal complexes.

Lanthanide Schiff base complexes were tested for antibacterial activity against *E.coli* and *B.subtilis* in which both ligand and metal complexes were active against the two microorganisms. All metal complexes synthesized, namely $[LaL_2(NO_3)_3]$, $[CeL_2(NO_3)_3]$, $[PrL_2(NO_3)_3]$, $[NdL_2(NO_3)_3]$, $[SmL_2(NO_3)_3]$, $[GdL_2(NO_3)_3]$, $[TbL_2(NO_3)_3]$, $[DyL_2(NO_3)_3]$ and $[ErL_2(NO_3)_3]$ are all highly active against the two bacteria. *E.coli* was found to be highly inhibited by complexes of praseodymium and erbium and moderate activity towards lanthanum and samarium. *B.subtilis* was found to be highly inhibited by cerium, praseodymium and erbium complexes and moderate activity with lanthanum complex. The ligand was active towards both *E.coli* and *B.subtilius* (Jayabalakrishnan *et al.,* 2002). The cerium(III) complexes are less active towards *Bacillus subtilis* and *Escherichia coli* in comparison to the free ligand, while the free ligand and the metal complexes showed higher and moderate antifungal activities. The binary cerium(III) complexes showed no effect towards *Alternaria alternata* and less activity towards *Syncephlastrum racemosum.* The formation of the polymeric Ce(IV) Schiff base complex is as a result of four salen units which are coordinated with one Ce(IV) ion. For steric reasons, it is likely that two salen units in neighbouring positions at the polymer backbone always take part in the complex formation. The model Ce(IV) does not indicate a reversible redox behavior in any of the investigated coordination polymer films (Jayabalakrishnan *et al.,* 2002).

2.3.2.2 Antibiotics Agents

Antibiotics are substances which, even at low concentrations, inhibit the growth and reproduction of bacteria and fungi. The treatment of infectious diseases would be inconceivable today without antibiotics (Koolman and Roehm, 2005).

Many metal complexes have powerful antimicrobial activities and some of them are already in the market. Behrens and Diaz (1986) synthesized the transition metal complexes of nalidixic acid. Nalidixic acid is used in the clinical treatment of urinary tract infections caused by Gram negative bacteria. The mode of coordination of the drug was investigated by spectroscopic studies. From the spectra data, nalidixic acid anion binds through the carboxylate group either as a chelate or as a bridge to give polymeric structure. Zupanicic *et al*., (2001) synthesized $[CfH_2]_2[ZnCl_4].2H_2O$ from Ciprofloxacin and $ZnCl_2$ in dilute HCl. The compound was shown to be ionic consisting of tetrachlorozincate (II) dianion and two protonated monoatomic ciprofloxacin molecules. The second one which is a complex $[Cu(Cf)(H_2O)_3]SO_4.\ 2H_2O$ was synthesized by Turel *et al*., (1999). The complex was prepared by direct reaction of copper sulphate pentahydrate with ciprofloxacin in distilled water. X-ray crystallographic studies showed that the ciprofloxacin atom is bonded to the metal through carbonyl oxygen and carboxylic oxygen atom. Water molecules also coordinated to the copper. Obaleye *et al*., (2001) synthesized and characterized metal(II) complexes of tetracycline in HCl. For Mn(II), Fe(II), Zn(II), Co(II) and Cd(II), the coordination of the metal to tetracycline is through one of the hydroxyl bands of tetracycline and oxygen of the carboxamide group and the proposed structures of the complexes were tetrahedral. For Ni(II) and Cu(II), the proposed structure of the complexes were still tetrahedral, but the coordination is via oxygen of the ν(C=O) and hydroxyl band of the drug. By using well-known antibiotics, Ogunniran *et al*., (2007) complexed ampicillin trihydrate, chloramphenicol and oxytetracycline with Ni(II), Fe(III) and Co(II) chloride salts. Thus, the three ligands acted as terdentate. The values of zone of inhibition for *E.coli, S. aureus* and *K.pneumonas* revealed enhanced antimicrobial activities upon complexation with metal salts.

2.4 Review of Dapsone and Rifampicin Complexes

The structure of known biologically active molecules can be modified to result in new biologically active metal coordinated complexes. The goal of such modification is to get a molecule that is improved in some way, such as potency, stability, reduced side effect or targeted delivery. The improvement is achieved without sacrificing the molecules' desirable properties. Some of these complexes are biologically more active than their parent ligands, making them promising candidates to join the league of metal based drugs already in the market. It should be known that traditional studies of organic drugs at a fundamental level are not complete without a parallel program on metal pharmacology. In general, because metals can undergo ligand exchanges, metal complexes are pro-drugs as ligand substitution can activate the metal complex toward binding to target molecules. It should be recognized that a metal complex is not just a metal, it is a metal ion plus its ligand. The metal ion plus the ligand determine the biological activity.

In an effort to modify the drug dapsone, Tella and Obaleye (2009) synthesized and characterized copper(II) complexes of 4, 4-diaminodiphenylsulphone and investigated their biological activities. Five complexes of copper(II) 4,4-diaminodiphenylsulphone were synthesized. Copper salts of counter ion (sulphate, nitrate, chloride) and different reaction media (solvents) namely; methanol, ethanol, acetone, ethyl acetate and distilled water were used for the synthesis. The complexes varied in colour and composition. The compounds were characterized by conductivity, IR, UV, NMR and mass spectroscopy. The ligand coordinated to the metal ions in a monodentate and bidentate manner. All the five complexes had tetrahedral configuration. The biological activities data showed that the complexes were more active against *Esherichia coli*, *Klebbsiella pneumoniae* and *Staphylococcus aureus* than the free ligand (4,4-diaminodiphenyl sulphone).

Salil *et al* (2009) carried out the complexation of zinc(II) and copper(II) salts with 4,4-diaminodiphenylsulphone (dapsone). Conductometric studies and elemental analysis suggested ML_2 stoichiometry. Molar conductance values indicated the non-electrolytic nature of the complexes. The

ligand was found to behave in a bidentate manner and formed co-ordinate bond through azomethine nitrogen. The structure assigned to the synthesized complexes was supported by infrared spectral studies.

Vijayalakshmi *et al* (2015) carried out studies on dapsone transition metal complexes in which Co(II), Ni(II) and Zn(II) metal complexes were synthesized from the drug dapsone. They also investigated the structural aspects of these complexes. The metal-ligand ratio was found to be 1:2 for all the complexes. The effective magnetic moment per metal atom for the Co(II) and Ni(II) complexes was found to be 4.62 BM and 3.2 BM respectively. Magnetic susceptibility measurements were irrelevant for diamagnetic zinc complex. All the metals appeared to form tetrahedral complexes with dapsone. The low molar conductance values of the complexes (17 mho for the cobalt(II) complex and 34 mho for the Ni(II) complex) indicated their non-ionic nature. From the IR spectra of these complexes, it was found that the sulphur atom in the S-O group of dapsone coordinated with the metal ion because the absorption bands due to S-O symmetric stretching at 1250 cm^{-1} and 1470 cm^{-1} in the free ligand were shifted to lower frequencies (1130 cm^{-1} and 1400 cm^{-1} in Ni(II) and Co(II) complexes respectively).

Chitra (2012) screened rifampicin for its activity towards *Mycobacteria*, *Ecoli* and *Streptocoli*. *Mycobacterium* bacteriological activity was also conducted with metal complexes of rifampicin using Cd(II) and V(II) salts. In each case, a 1:1 metal-rifampicin complex was obtained. The results of antimicrobial screening showed that the divalent metal complexes were more effective compared to the rifampicin.

Pati *et al* (2016) synthesized a novel anti-TB drug complex consisting of zinc and rifampicin (Zn-Rif) and encapsulated it into transferring-conjugated silver quantum dots (Zn-RIF-Tf-QD) to improve delivery in macrophages. Successful synthesis of Zn-Rif and encapsulated complex Zn-RIF-Tf-QD was confirmed by UV/Vis-spectroscopy, TEM, FTIR, photoluminescence, XRD, XPS and NMR. Activity assays showed that Zn-RIF-Tf-QD exhibited 10-fold higher antibacterial activity against *Mycobacterium smegmatis and Mycobacterium bovis* compared to Zn-Rif and Zn metal. He also found that treatment with

Zn-RIF-Tf-QD effectively killed mycobacteria residing inside macrophages without exhibiting cytotoxicity and genotoxicity. The obtained results demonstrated that Zn-RIF-Tf-QDs have great potentials as anti-TB drugs. The study shows an effort made towards improving chemotherapy of tuberculosis.

Sonar (2016) carried out a conductance study of the interaction between copper(II) and rifampicin in aqueous medium at 303K. The stability constant of the resulting 1:1 complex was also determined by conductactometry technique. The free energy change of the studied complex was evaluated at 303K using thermodynamic relations. The negative value of ΔG (-6140.40 $JK^{-1}Mole^{-1}$) showed that the complex formation procedure was spontaneous.

Rao *et al* (2015) synthesized and evaluated cyclodextrin complexes of rifampicin obtained by inclusion complexation of rifampicin using methyl β-cyclodextrin with the aim of improving the aqueous solubility, *in vitro* anti-tubercular activity and bioavailability of rifampicin. The compexes were prepared by different methods and were subjected to solubility and *in vitro* studies. It was found that *in vitro* anti-tubercular activity, bioavailability and stability in solution and solid state were enhanced in the complexes compared to rifampicin.

From the available literature, it can be deduced that extensive studies have not been carried out into the synthesis and characterization of Schiff base and metal complexes of dapsone and very few have been recorded on the synthesis and characterization of transition metal(II) complexes of rifampicin and clofazimine which are anti-leprosy drugs and function as Schiff bases because of the presence of azomethine nitrogen in their structures.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

In the preparation of the Schiff base and metal(II) Schiff base complexes, chemicals of analytical grade purity and distilled water were used throughout. Dapsone was obtained from Sam Pharmaceutical Ltd. Ilorin, Nigeria while clofazimine and rifampicin were obtained from Greenfield Pharmaceuticals, China.

All the glass wares used for the preparations were thoroughly washed with detergent and rinsed repeatedly with tap water. They were then soaked in a concentrated solution of nitric acid for about 2-3 hours after which they were rinsed 3-4 times with distilled water and dried in an oven at a temperature of about 110 °C.

All weighings were carried out using electric meter balance AB 54 at Bayero University, Kano. Mass susceptibility was determined using Sherwood Scientific MSB MK1 Magnetic Susceptibility Balance at Bayero University, Kano. Melting Points/Decomposition temperatures were determined using a digital WRS-IB Microprocessor Melting Point Apparatus. Infrared spectral analyses were recorded using FTIR-8400S Fourier Transform Infrared Spectrophotometer in the range 400-4000 cm^{-1} using KBr discs at National Research Institute for Chemical Technology, Zaria. Conductivity measurements were carried out using a George Kent Model 5003 portable conductivity measuring set. CHN Elemental Analyses were carried out at Robertson Microlit Laboratory (U.S.A) using Perkin-Elmer CHN 2400 and Metal Analyses were carried out also at Robertson Microlit Laboratory (U.S.A) using Shimadzu AA670 Atomic Absorption Spectro Photometer (AAS), after digestion of the synthesized compounds using concentrated nitric acid.

Three pathogenic bacteria viz: *Staphylococcus aureus, Escheria coli, Salmonella typhi and* three fungal isolates (*Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus)* were used for

antimicrobial testing at Bayero University, Kano. The microbes were obtained and identified at the Department of Microbiology, Bayero University, Kano. The microbes were originally sourced from Mallam Aminu Kano Teaching Hospital, Kano. Nutrient agar and potato dextrose sugar were used as bacteriological and fungal media respectively.

Toxicity studies were carried out at the animal husbandry section of Crescent International School, Badawa New-Layout, Kano, Nigeria.

3.2 Methods

3.2.1 Synthesis of Schiff Base Derived from Dapsone (4,4'-diaminodiphenyl sulphone and Salicylaldehyde.

For this preparation, dapsone (2.438g, 0.01mol) and salicylaldehyde (2.442g, 0.02mol) were respectively taken in 25cm³ absolute ethanol in separate beakers. Both solutions were mixed in a round bottom flask and refluxed for 1 hour. During heating, yellow crystals were formed which were filtered, washed several times with absolute ethanol, dried and weighed (Salil *et al.*, 2009).

3.2.2 Synthesis of (DAP-SAL) Metal(II) Complexes

For the preparation of DAP-SAL metal(II) complexes, DAP-SAL (2.763g, 0.006mol) solution was prepared in 60% acetone and mixed with 0.003mol solution of metal(II) chloride in a round bottom flask. The resulting solution was refluxed for four hours and kept for 48 hours after which coloured crystals appeared in the reaction mixture. The prepared complexes were filtered, washed with 60% acetone, dried and weighed (Salil *et al*., 2010).

3.2.3 Synthesis of Schiff Base derived from Dapsone (4,4'-diaminodiphenylsulphone) and Acetylacetone.

For this synthesis, dapsone (2.483g, 0.01mol) and acetylacetone (2.002g, 0.02mol) were taken in 25cm³ of methanol respectively. Both solutions were mixed in a round bottom flask and refluxed for about 2½ hours. During heating, very light yellow crystals were formed in the reaction mixture, and were obtained by

filtration. The crystals were washed with methanol, dried over phosphorus pentoxide and weighed (Salil *et al.*, 2009).

3.2.4 Synthesis of (DAP-ACA)SB Metal(II) Complexes

To synthesize DAP-ACA metal(II) complexes, DAP-ACA (0.833g, 0.002mol) was dissolved in 20cm^3 of methanol. The solution was heated in a round bottom flask at 60°C for complete dissolution of the ligand. 0.001mol of the metal(II) chloride in 10cm^3 of methanol was added to the above ligand solution. The mixture was refluxed for 2hrs and cooled to room temperature. A coloured microcrystalline precipitate of the complex was obtained after one week. The product was separated at room temperature, washed with a mixture of methanol and water in the ratio 3:1 by volume (methanol : water) and dried at room temperature (Tella and Obaleye, 2009).

3.2.5 Synthesis of Metal (II) Complexes of Rifampicin

To synthesize rifampicin metal(II) complexes, rifampicin (2.47g, 0.003mol) was dissolved in 50cm³ ethanol (absolute) and 0.003mol of a metal(II) chloride was dissolved in 50cm³ distilled water (1:1 molar ratio). The solutions were mixed together in a round bottom flask and refluxed for 6 hours over a water bath. A concentrated solution of the metal(II) complex was obtained which was allowed to cool, where upon it precipitated and was filtered and washed with ethanol. The complex obtained was recrystallized from an ethanol-water mixture in the ratio 4:1 by volume respectively, dried at room temperature and weighed (Chitra, 2012).

3.2.6 Synthesis of Metal (II) Complexes of Clofazimine

In this preparation, clofazimine (1.42 g, 0.003mol) was dissolved in 50cm^3 of chloroform. The solution was heated to 65˚C for complete dissolution of the solid. 0.003mole of the required metal (II) chloride in 20 cm^3 methanol was then added. The resulting solution was refluxed for 18 hours in a round bottom flask and cooled to room temperature. A coloured precipitate formed after one week and was

separated by filtration and washed with methanol. The complex was recrystallized from a methanol-water mixture in the ratio of 4:1 respectively, dried at room temperature and weighed (Chitra, 2012).

3.3 Characterization Techniques

3.3.1 Solubility of Ligands/Metal (II) Complexes

The solubility of each of the metal(II) complexes and Schiff bases was tested using common organic solvents and distilled water. 20 mg of each of the test substance was taken and dissolved into 4 cm^3 corresponding solvent in a test tube, shaken thoroughly and the solubility was checked (Saha *et al.,* 2009).

3.3.2 Estimation of Percentage Water of Crystallization in the Complexes

To estimate the percentage water of crystallization, 0.2 g of each of the metal(II) complexes was taken in a previously weighed empty porcelain crucible and placed in an oven at 110 ^{0}C. The complex compounds were each removed and weighed periodically until constant weight was obtained (Moeller, 1980). The percentage composition of water of crystallization in each of the complexes was calculated as follows;

$$\frac{\text{Loss in weight}}{\text{Weight complex taken}} \times 100\%$$

3.3.3 Magnetic Susceptibility Measurements

The gram magnetic susceptibility of each of the metal(II) complexes was measured using the MSB1 Magnetic Susceptibility Balance. The instrument was switched on and made ready for use. The complex compound to be measured was dried and ground to a powdery form. The RANGE knob of the machine was turned to x1 scale and the reading on the balance was set to 000 by gently turning the ZERO knob. The weight of a dry empty sample tube was determined using a weighing balance, after which the weighed sample tube was carefully placed into the tube guide of the machine and the corresponding reading (R_o) was taken. The dry complex compound was packed into the tube to a depth of between 2.5

and 3.5 cm. The base of the tube was tapped gently on the bench to ensure uniform packing. The outside of this sample tube was then cleaned with a dry tissue paper and the tube placed in the tube guide of the machine. After about 30 seconds a fairly constant reading was obtained and recorded (R). The tube was removed from the tube guide of the machine and the base of the sample tube was gently tapped on the bench. The reading was once more recorded. This process was repeated until a constant value for R was obtained. The packed sample tube was accurately weighed using a balance and the length, l, of the sample in cm was measured, ensuring the top of the sample in the tube was level. Finally, the sample tube was carefully washed out with chloroform, rinsed with water and lastly with acetone before placing it in the oven to dry.

The gram magnetic susceptibility for the measured sample was calculated using;

$$\chi_g = \frac{C_{Bal}\,(l)\,(R - R_o)}{10^9 m}$$

Where;

l = sample length (cm)

m = sample mass (g)

R = reading for tube plus sample (g)

R_0 = reading for empty tube (g)

C_{Bal} = balance calibration constant (equal to 1) (Lancashire, 2010).

3.3.4 Toxicity Test of the Metal (II) Complexes

Toxicity test examines the toxic effects when a chemical is absorbed into the body, via mouth, skin, and lungs. The most common test of acute (short-term) toxicity is the LD50 test. Many different substances are tested in this way, including all drugs, agricultural chemicals, chemical cleaners, and some cosmetics.

LD50 is a test that implies that if we administer any dose of a drug to a group of animals for the estimation of the therapeutic effectiveness of that drug, and about 50% of animals in the group die, the implication is that the particular dose of drug is a lethal dose (LD50). The smaller the LD50 value, the more toxic a chemical is (Marina, 2005). The LD50 toxicity tests are meant to provide information about adverse effects of substances after a single dose, symptoms of overdose and the human lethal dose, selection of doses for the more prolonged animal tests and hazard classification of industrial chemicals.

Adult *Rattus norvegicus* (laboratory rats) from both sexes with mean body weight of (225±25 g) were obtained from the animal husbandry section of Crescent International School Kano, Nigeria and used to measure the acute toxicity of the ligands and their metal(II) complexes. Eight rats were used to test the toxicity of each synthesized compound, making a total of 56 rats for each group of ligand and its metal(II) complexes. The experiments were performed over a period of about nine weeks.

Each of the metal(II) complexes/ligand used for the experiment was dissolved in $2cm^3$ dimethylformamide (DMF) and prepared in seven different concentrations: 0.4 $g/2cm^3$ 0.5 $g/2cm^3$, 0.6 $g/2cm^3$, 0.7 $g/2cm^3$, 0.8 $g/2cm^3$, 0.9 $g/2cm^3$ and 1.0 $g/2cm^3$ and given orally. A group of animals drenched with same volume of solvent was used as a control.

The dose- effect data were deduced using LANGMUIR equation:

$$E = \frac{CE\max}{C50 + C}$$

Where;

E_{max} is the maximal effect produced on the rats

C50 refers to the dose which kills 50% of the animals

E refers to the required effect and C to the dose which produces that effect.

Using least square-nonlinear model, E_{max} and C50 can be generated.

Deaths were calculated after 24 hours periods of administering complexes/ligands on the animals.

Each animal was used only once. For ethical reasons all animals were sacrificed at the end of the study.

3.4 Antibacterial and Antifungal Activity Test

3.4.1 Test Organisms

The bacterial species *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* and fungal species *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatos* were used as test organisms and they were maintained on Muller Hilton Agar (bacterial) and Potato Dextrose Agar (fungi) solutions.

3.4.2 Assay of Antimicrobial Activity

Disc diffusion assay was carried out to evaluate the antimicrobial activity of the synthesized metal(II) complexes and ligands. The plates were incubated at 37°C for 24-48 hours (NCCLS, 2008) during which activity was evidenced by the presence of zones of inhibition surrounding the disc. Antibacterial and antifungal activity was expressed as mean diameter of inhibition zones (mm) produced when compared to controls (Yeamin *et al.,* 2003; Ramon *et al.,* 2003).

3.4.3 Media

Muller Hilton Agar media and Potato Dextrose Agar (PDA) were used and were prepared in distilled water. Agar medium was accurately weighed and suspended in $100cm^3$ of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely.

3.4.3.1 Preparation of Stock Solution

The ligands and complexes were dissolved separately in Dimethyl Sulphoxide (DMSO) to obtain three different concentrations (15 μ g, 30 μ g and 60 μ g) per disc.

3.4.3.2 Preparation of Turbidity Standard

Barium sulphate (1%w/v) standard suspension was used as turbidity standard. The prepared turbid solution was transferred into a test tube as the standard for comparism (Cheesbrough, 2005).

3.4.3.3 Standardization of Inoculum

Using inoculation loop, enough material from an overnight culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard (NCCLS, 2008).

3.4.3.4 Preparation of Sensitivity Discs

Whatman No.1 filter paper discs (6mm in diameter) were punched out with the aid of paper punch and placed in Bijour bottles. They were then sterilized by autoclaving at 121°C for 15 minutes. The discs were allowed to cool.

3.4.3.5 Disc Diffusion Test

Standard inocula of the isolates were swabbed on to the surface of the prepared and solidified Muller Hilton Agar in separate Petri-dishes. The prepared discs of the extracts and the standard were placed onto the surface of the inoculated media at intervals. The plates were incubated at 37°C for 24 hours before observation and measurement of the zones of inhibition (NCCLS, 2008; Syed *et al*., 2010).

3.5 Other Characterization Methods

3.5.1 Elemental Analysis

Elemental analysis was carried out to determine the approximate percentage weights of C, H and N (CHN elemental analysis) in each of the ligands and metal complexes so as to determine their approximate molecular formula. The analysis was carried out using the Perkin-Elmer CHN 2400 Elemental Analyser based on the classical Pregl-Dumas method where samples are combusted in a pure oxygen environment, with the resultant combustion gases measured in an automated fashion. Another operating gas used during this process is helium which serves as a carrier gas. About 20 mg of each of the samples for analysis was encapsulated in an aluminium vial and inserted automatically into the Combustion Zone of the analyser using a single-sample auto injector. In the presence of excess oxygen and combustion reagents, the

samples were combusted completely and reduced to the elemental gases CO_2, H_2O and N_2. All results are computed automatically and are given as percent weight of each element.

3.5.2. Metal Analysis

Metal analysis was carried out to determine the percentage weight of each of the metal elements in the complexes so that the approximate ligand to metal ratio (M : L) can be determined. For metal analysis, 0.2 g of each metal complex was placed in a 100 cm^3 beaker containing
25 cm^3 water. Concentrated nitric acid (5 cm^3) was added and the resulting mixture stirred and then heated to dryness. The beaker and its content were allowed to cool to room temperature. About 25 cm^3 distilled water was then added and the mixture was stirred and filtered. The filtrate, which contained the metal ion was collected and analysed for percentage weight of metal ion in the synthesized complexes using Perkin-Elmer Model 460 Atomic Absorption Spectrophotometer.

3.5.3 Molar Conductance

Molar conductance is the conductivity of an electrolyte solution divided by the molar concentration of the electrolyte and so measures the efficiency with which a given electrolyte conducts electricity in solution. The molar conductance of each of the metal(II) complexes was determined using a George Kent Model 5003 portable machine. A 0.02 $moldm^{-3}$ solution of KCl was prepared in a beaker using deionised water. The electrode of the conductivity set was washed several times with the freshly prepared KCl solution. The conductivity of the freshly prepared KCl solution was then measured at 25 °*C* from which the cell constant of the electrode was calculated. Molar conductivity measurements were carried out on 1 x 10^{-3} $moldm^{-3}$ solution of each of the metal complexes in Dimethyl Formamide (DMF) at 25°C.

3.5.4 Infrared Measurements

Infrared absorption spectra of the ligands and metal(II) complexes were recorded on a FTIR spectrophotometer in the 400-4000cm^{-1} range using potassium bromide (KBr) discs so as to determine the identity of each of the synthesized compounds by determining coordinating atoms and the relative strength

of bonds. Each of the sample and KBr were ground to reduce the particle size to less than 5mm in diameter. This was necessary as larger particles scatter the infrared beam and cause a slope base line of spectrum. A small amount of powder sample (about 1-2% of the KBr amount) was taken and mixed with the KBr powder. The die set was assembled and the ground powder mixture added to it. The die together with the powder were put into a Handi Press and pressed for about 2 minutes to form a thin and transparent pellet which was then analysed using the infrared analyser.

CHAPTER FOUR
4.0 RESULTS AND DISCUSSION

4.1 Results

The results of the colours, percentage yields, melting points of the Schiff bases, decomposition temperatures and molar conductance of the transition metal(II) complexes are presented in the following tables (4.1- 4.4) below:

Table 4.1: Colour, Percentage Yield, Melting Point, Decomposition Temperature and Molar Conductance of (DAP-SAL)SB and Metal(II) Complexes

Ligand / Complex	Colour	Yield (%)	M.pt ($^{\circ}C$)	D. Temp. ($^{\circ}C$)	M. Conductance ($ohm^{-1}cm^{2}mol^{-1}$)
(DAP-SAL)SB	Yellow	89.6	211.3	-	-
[Mn(DAP-SAL)]. H_2O	Light pink	81.0	-	>250.0	13.00
[Cu(DAP-SAL)]. H_2O	Blue	75.2	-	>250.0	16.00
[Fe(DAP-SAL)]. H_2O	Light green	62.4	-	243.2	9.00
[Co(DAP-SAL)]. H_2O	Pink	61.7	-	>250.0	10.00
[Zn(DAP-SAL)]. H_2O	White	53.5	-	239.0	13.00
[Ni(DAP-SAL)]. H_2O	Yellow	83.9	-	220.2	12.00

KEY:

(DAP-SAL)SB = Schiff base derived from dapsone and salicylaldehyde

[M(DAP-SAL)]. X H_2O = Metal(II) complex derived from DAP-SAL

Table 4.2: Colour, Percentage Yield, Melting Point, Decomposition Temperature and Molar Conductance of (DAP-ACA)SB and Metal(II) Complexes

Ligand / Complex	Colour	Yield (%)	M.pt ($^{\circ}C$)	D. Temp. ($^{\circ}C$)	M. Conductance ($ohm^{-1}cm^2mol^{-1}$)
(DAP-ACA)SB	Pale Yellow	85.2	199.0	-	-
[Mn(DAP-ACA)]. $2H_2O$	Light pink	63.7	-	>250.0	20.00
[Cu(DAP-ACA)]. $6H_2O$	Bluish green	70.4	-	220.2	10.00
[Fe(DAP-ACA)]. H_2O	Light brown	51.3	-	230.4	11.00
[Co(DAP-ACA)]. H_2O	Pale yellow	70.7	-	220.0	12.00
[Zn(DAP-ACA)]. $5H_2O$	Pale yellow	82.6	-	222.0	9.00
[Ni(DAP-ACA)]. $5H_2O$	Light brown	84.2	-	228.5	15.00

KEY:

(DAP-ACA)SB = Schiff base derived from Dapsone and Acetylacetone

[M(DAP-ACA)] . X H_2O = Metal(II) Complex derived from (DAP-ACA)SB.

Table 4.3: Colour, Percentage Yield, Melting Point, Decomposition Temperature and Molar Conductance of Rifampicin Metal(II) Complexes

Ligand / Complex	Colour	Yield (%)	M.pt (°C)	D. Temp. (°C)	M. Conductance ($ohm^{-1}cm^2mol^{-1}$)
Rifampicin	Reddish brown	-	188.3	-	-
[Mn(RIF)].2H_2O	Light orange	50.9	-	205.3	14.00
[Cu(RIF)]. 2H_2O	Brown	68.2	-	217.6	9.00
[Fe(RIF)].2H_2O	Deep brown	65.0	-	221.0	12.00
[Co(RIF)].H_2O	Grey	50.9	-	217.2	11.00
[Zn(RIF)].H_2O	Off-white	72.2	-	210.8	11.00
[Ni(RIF)].2H_2O	Red	65.4	-	217.6	10.00

KEY:

[M(RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.4: Colour, Percentage Yield, Melting Point, Decomposition Temperature and Molar Conductance of Clofazimine Metal(II) Complexes

Ligand / Complex	Colour	Yield (%)	M.pt (°C)	D. Temp. (°C)	M. Conductance ($ohm^{-1}cm^2mol^{-1}$)
Clofazimine	Deep Orange	-	198.0	-	-
[Mn(CLOF)Cl_2]. H_2O	Orange	58.6	-	216.2	18.00
[Cu(CLOF)Cl_2].H_2O	Brown	61.1	-	219.5	16.00
[Fe(CLOF)Cl_2]. H_2O	Dark green	78.4	-	227.2	13.00
[Co(CLOF)Cl_2]. H_2O	Dark brown	58.8	-	212.2	11.00
[Ni(CLOF)Cl_2].H_2O	Light brown	60.7	-	216.0	11.00
[Zn(CLOF)Cl_2].H_2O	Violet	60.2	-	220.2	13.00

KEY:

[M(CLOF)Cl_2] . XH_2O = Metal(II) complexes derived from Clofazimine.

The results of the elemental analyses determination for the synthesized Schiff bases and metal(II) complexes and percentage water of crystallization in (DAP-SAL) metal(II) complexes are represented in the following tables (4.5-4.8) below:

Table 4.5: Elemental Analysis of (DAP-SAL) Schiff Base and its Metal(II) Complexes and Percentage water of crystallization in (DAP-SAL) Metal(II) Complexes.

Schiff Base/Complex	Elemental Analysis Found (Calculated) %				Water of Cryst.%
	C	H	N	Metal	
(DAP-SAL)SB	67.7 (67.81)	4.9 (4.38)	6.2 (6.08)	-	-
[Cu(DAP-SAL)] . H_2O	59.6 (59.82)	3.9 (3.48)	5.5 (5.37)	11.9 (12.17)	2.38
[Fe(DAP-SAL)]. H_2O	60.9 (60.72)	3.8 (3.53)	5.8 (5.45)	10.7 (10.86)	2.15
[Ni(DAP-SAL)]. H_2O	60.5 (60.38)	3.8 (3.51)	5.7 (5.42)	11.1 (11.35)	3.54
[Mn(DAP-SAL)]. H_2O	60.2 (60.82)	3.7 (3.53)	5.8 (5.46)	10.7 (10.70)	3.25
[Co(DAP-SAL)]. H_2O	60.6 (59.21)	3.8 (3.44)	5.7 (5.31)	11.1 (13.07)	2.00
[Zn(DAP-SAL)]. H_2O	59.6 (59.61)	3.9 (3.46)	5.6 (5.35)	12.1 (12.48)	2.34

KEY:

[M(DAP-SAL)] . XH_2O = Metal(II) complex derived from Dapsone-Salicylaldehyde Schiff base.

Table 4.6: Elemental Analysis of (DAP-ACA) Schiff Base and its Metal(II) Complexes and Percentage water of crystallization in (DAP-ACA) Metal(II) Complexes.

Schiff Base/Complex	Elemental Analysis Found (Calculated) %				Water of Cryst.%
	C	H	N	Metal	
(DAP-ACA)SB	63.9 (63.44)	6.1 (5.81)	7.0 (6.73)	-	-
[Mn(DAP-ACA)].2H_2O	56.5 (56.29)	5.0 (4.72)	6.3 (5.97)	11.4 (11.70)	6.2
[Cu(DAP-ACA)].6H_2O	55.4 (55.28)	4.9 (4.64)	6.2 (5.86)	13.0 (13.29)	16.9
[Fe(DAP-ACA)].H_2O	56.4 (56.18)	4.9 (4.71)	6.3 (5.96)	12.4 (11.87)	3.6
[Co(DAP -ACA)].H_2O	55.8 (54.66)	4.7 (4.59)	6.1 (5.79)	12.1 (14.26)	3.9
[Ni(DAP-ACA)].5H_2O	55.9 (55.84)	4.8 (4.69)	6.2 (5.92)	12.7 (12.40)	14.8
[Zn(DAP-ACA)].5H_2O	55.2 (55.07)	4.9 (4.62)	6.2 (5.84)	13.9 (13.62)	14.5

KEY:

(DAP-ACA)SB = Schiff Base derived from Dapsone and Acetylacetone

[M(DAP-ACA)]. XH_2O = Metal(II) Complex derived from (DAP-ACA)SB.

Table 4.7: Elemental Analysis of (Rifampicin and its Metal(II) Complexes and Percentage water of crystallization in Rifampicin Metal(II) Complexes.

Schiff Base/Complex	Elemental Analysis Found (Calculated) %				Water of Cryst.%
	C	H	N	Metal	
Rifampicin	62.2 (61.86)	7.5 (7.00)	7.1 (6.71)	-	-
[Cu(RIF)]. $2H_2O$	57.9 (57.61)	6.7 (6.30)	6.6 (6.25)	6.8 (7.09)	3.15
[Mn(RIF)]. $2H_2O$	58.4 (58.17)	6.6 (6.36)	6.6 (6.31)	5.9 (6.19)	4.25
[Ni(RIF)]. $2H_2O$	58.2 (57.92)	6.6 (6.33)	6.5 (6.28)	6.3 (6.58)	3.55
[Fe(RIF)]. $2H_2O$	58.2 (58.11)	6.5 (6.35)	6.4 (6.30)	6.6 (6.28)	3.11
[Co(RIF)]. H_2O	58.4 (57.27)	6.7 (6.26)	6.7 (6.21)	6.8 (7.64)	2.30
[Zn(RIF)]. H_2O	58.7 (57.49)	6.5 (6.28)	6.8 (6.24)	7.2 (7.28)	2.00

KEY:

[M(RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.8: Elemental Analysis of Clofazimine and its Metal(II) Complexes and Percentage water of crystallization in Clofazimine Metal(II) Complexes.

Schiff Base/Complex	Elemental Analysis Found (Calculated) %				Water of Cryst.%
	C	H	N	Metal	
Clofazimine	68.6 (68.46)	4.5 (4.68)	11.7 (11.88)	-	-
[Mn(CLOF)Cl_2]. H_2O	61.3 (61.59)	3.7 (3.83)	10.7 (10.69)	10.6 (10.43)	2.11
[Cu(CLOF)Cl_2]. H_2O	60.4 (60.60)	3.7 (3.77)	10.7 (10.51)	11.2 (11.87)	2.09
[Fe(CLOF)Cl_2]. H_2O	61.7 (61.48)	3.6 (3.82)	10.6 (10.67)	9.9 (10.59)	2.08
[Co(CLOF)Cl_2]. H_2O	59. 8(59.99)	3.9 (3.73)	10.3 (10.41)	13.2 (12.75)	2.08
[Ni(CLOF)Cl_2]. H_2O	60.9 (61.15)	3.5 (3.80)	10.7 (10.61)	11.3 (11.07)	2.12
[Zn(CLOF)Cl_2]. H_2O	60.5 (60.39)	3.6 (3.75)	10.8 (10.48)	12.7 (12.17)	2.08

KEY:

[M(CLOF)Cl_2]. XH_2O = Metal(II) complexes derived from Clofazimine

Table 4.9: Solubility of (DAP-ACA) and its Metal(II) Complexes in Common Solvents

Compound	Distilled water	Ethanol	Methanol	DMF	DMSO	Acetone	Chloroform	Ethyl acetate	Diethyl ether
(DAP-ACA)SB	IS	SS	S	S	SS	S	S	IS	S
[Mn(DAP-ACA)]. $2H_2O$	SS	SS	S	S	SS	S	S	SS	S
[Cu(DAP-ACA)]. $6H_2O$	SS	SS	S	S	SS	S	S	SS	S
[Fe(DAP-ACA)] . H_2O	SS	SS	S	S	SS	S	S	SS	S
[Co(DAP -ACA)]. H_2O	SS	SS	S	S	SS	S	S	SS	S
[Zn(DAP -ACA)]. $5H_2O$	SS	SS	S	S	SS	S	S	SS	S
[Ni(DAP-ACA)]. $5H_2O$	SS	SS	S	S	SS	S	S	SS	S

KEY:
(DAP-ACA)SB = Schiff Base derived from Dapsone and Acetylacetone

[M(DAP-ACA)]. XH_2O = Metal(II) Complex derived from (DAP-ACA)SB

Table 4.10: Solubility of (DAP-SAL) and its Metal(II) Complexes in Common Solvents

Compound	Distilled water	Ethanol	Methanol	DMF	DMSO	Acetone	Chloroform	Ethyl acetate	Diethyl ether
(DAP-SAL)SB	IS	SS	S	S	SS	S	S	IS	S
[Mn(DAP-SAL)]. H_2O	SS	SS	S	S	SS	S	S	SS	S
[Cu(DAP-SAL)]. H_2O	SS	SS	S	S	SS	S	S	SS	S
[Fe(DAP-SAL)]. H_2O	SS	SS	S	S	SS	S	S	SS	S
[Co(DAP-SAL)]. H_2O	SS	SS	S	S	SS	S	S	SS	S
[Zn(DAP-SAL)]. H_2O	SS	SS	S	S	SS	S	S	SS	S
[Ni(DAP-SAL)]. H_2O	SS	SS	S	S	SS	S	S	SS	S

KEY:
(DAP-ACA)SB = Schiff Base derived from Dapsone and Salicylaldehyde

[M(DAP-SAL)]. XH_2O = Metal(II) Complex derived from (DAP-SAL)SB

S - Soluble

IS - Insoluble

SS - Slightly Soluble

Table 4.11: Solubility of Rifampicin and its Metal(II) Complexes in Common Solvents

Compound	Distilled water	Ethanol	Methanol	DMF	DMSO	Acetone	Chloroform	Ethyl acetate	Diethyl ether
Rifampicin	SS	S	S	S	SS	S	S	IS	S
[Mn(RIF)]. $2H_2O$	SS	SS	S	S	S	S	S	SS	S
[Cu(RIF)]. $2H_2O$	SS	SS	S	S	S	S	S	SS	S
[Fe(RIF)]. $2H_2O$	SS	SS	S	S	S	S	S	SS	S
[Co(RIF)]. H_2O	SS	SS	S	S	S	S	S	SS	S
[Zn(RIF)]. H_2O	SS	SS	S	S	S	S	S	SS	S
[Ni(RIF)]. $2H_2O$	SS	SS	S	S	S	S	S	SS	S

KEY:

[M(RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.12: Solubility of Clofazimine and its Metal(II) Complexes in Common Solvents

Compound	Distilled water	Ethanol	Methanol	DMF	DMSO	Acetone	Chloroform	Ethyl acetate	Diethy ether
Clofazimine	IS	SS	SS	S	S	SS	S	SS	S
[Mn(CLOF)Cl_2]. H_2O	IS	SS	S	S	S	SS	S	SS	SS
[Cu(CLOF)Cl_2]. H_2O	IS	SS	S	S	S	SS	S	SS	SS
[Fe(CLOF)Cl_2]. H_2O	IS	SS	S	S	S	SS	S	SS	SS
[Co(CLOF)Cl_2]. H_2O	IS	SS	S	S	S	SS	S	SS	SS
[Ni(CLOF)Cl_2]. H_2O	IS	SS	S	S	S	SS	S	SS	SS
[Zn(CLOF)Cl_2]. H_2O	IS	SS	S	S	S	SS	S	SS	SS

KEY:

[M(CLOF)Cl_2]. XH_2O = Metal(II) complexes derived from Clofazimine

S - Soluble

IS - Insoluble

SS - Slightly Soluble

The results of the magnetic susceptibility measurements of the synthesized Schiff bases and transition metal(II) complexes are presented in tables 4.13 - 4.16 below:

4.13: Magnetic Measurement Values of (DAP-SAL) Metal(II) Complexes

Complex Compound	X_g (erg.G^{-2}.cm^{-3})	Magnetic Moment μ_{eff} (B.M.)	Number of unpaired electrons (n)	d configuration
[Mn(DAP-SAL)]. H_2O	3.0500 x 10^{-5}	6.2	5 (high spin)	d^5
[Cu(DAP-SAL)]. H_2O	2.8947 x 10^{-6}	2.1	1	d^9
Fe(DAP-SAL)]. H_2O	2.4729 x 10^{-5}	5.6	4 (high spin)	d^6
[Co(DAP-SAL)]. H_2O	1.2105 x 10^{-5}	4.0	3 (high spin)	d^7
[Ni(DAP-SAL)]. H_2O	9.970 x 10^{-6}	3.6	2	d^8
[Zn(DAP-SAL)]. H_2O	- 2.6087 x10^{-8}	0	Diamagnetic	d^{10}

KEY:

X_g = Gram Magnetic Susceptibility in (erg.G^{-2}.cm^{-3}).

[M(DAP-SAL)]. XH_2O = Metal(II) Complex derived from Dapsone-Salicylaldehyde Schiff base

Table 4.14: Magnetic Measurement Values of (DAP-ACA) Metal(II) Complexes

Complex Compound	X_g (erg.G^{-2}.cm^{-3})	Magnetic Moment μ_{eff} (B.M.)	Number of unpaired electrons (n)	d configuration
[Mn(DAP-ACA)]. 2H_2O	3.3026 x10^{-5}	6.2	5 (high spin)	d^5
[Cu(DAP-ACA)].6H_2O	1.4087 x 10^{-6}	1.5	1	d^9
[Fe(DAP-ACA)]. H_2O	2.6522 x 10^{-5}	5.6	4 (high spin)	d^6
[Co(DAP -ACA)]. H_2O	1.4002 x 10^{-5}	4.1	3 (high spin)	d^7
[Zn(DAP -ACA)]. 5H_2O	-2.3044 x 10^{-6}	0	Diamagnetic	d^{10}
[Ni(DAP-ACA)]. 5H_2O	1.2232 x 10^{-5}	3.8	2	d^8

KEY:

X_g = Gram Magnetic Susceptibility in (erg.G^{-2}.cm^{-3}).

[M(DAP-ACA)]. XH_2O = Metal(II) Complex derived from Dapsone-Acetylacetone Schiff base

Table 4.15: **Magnetic Measurement Values of Metal(II) Complexes of Rifampicin**

Complex Compound	X_g (erg.G^{-2}.cm^{-3})	Magnetic Moment μ_{eff} (B.M.)	Number of unpaired electrons (n)	d configuration
[Mn(RIF)]. $2H_2O$	1.6940×10^{-5}	6.2	5	d^5
[Cu(RIF)]. $2H_2O$	8.1231×10^{-7}	1.8	1	d^9
[Fe(RIF)]. $2H_2O$	1.3512×10^{-5}	5.5	4	d^6
[Co(RIF)]. H_2O	8.3256×10^{-6}	4.4	3	d^7
[Ni(RIF)]. $2H_2O$	6.2543×10^{-6}	3.9	2	d^8
[Zn(RIF)]. H_2O	-2.690×10^{-7}	0	Diamagnetic	d^{10}

KEY:
X_g = Gram Magnetic Susceptibility in (erg.G^{-2}.cm^{-3}).

[M(RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.16: **Magnetic Measurement Values of Metal(II) Complexes of Clofazimine**

Complex Compound	X_g (erg.G^{-2}.cm^{-3})	Magnetic Moment μ_{eff} (B.M.)	Number of unpaired electrons (n)	d configuration
[Mn(CLOF)Cl_2]. H_2O	3.124 x 10^{-5}	6.4	5	d^5
[Cu(CLOF)Cl_2]. H_2O	2.930 x 10^{-6}	2.2	1	d^9
[Fe(CLOF)Cl_2]. H_2O	2.532 x 10^{-5}	5.8	4	d^6
[Co(CLOF)Cl_2]. H_2O	1.411 x 10^{-5}	4.4	3	d^7
[Ni(CLOF)Cl_2]. H_2O	9.990 x 10^{-6}	3.7	2	d^8
[Zn(CLOF)Cl_2]. H_2O	-2.992 x 10^{-8}	0	Diamagnetic	d^{10}

KEY:

X_g = Gram Magnetic Susceptibility in (erg.G^{-2}.cm^{-3}).

[M(CLOF) Cl_2]. XH_2O = Metal(II) complexes derived from Clofazimine.

The results of the infrared spectral analyses determination for the synthesized Schiff bases and metal(II) complexes are represented in the following tables (4.17 – 4.20):

Table 4.17: Infrared Spectral Data of (DAP-SAL) Schiff Base and its Metal(II) Complexes

Compound	υ (O-H)	υ (O-H) (H_2O)	υ (C-O)	υ (C=N)	υ (M-O)	υ (M-N)
(DAP-SAL)SB	3383	-	1142	1600	-	-
[Mn(DAP-SAL)]. H_2O	-	3405	1126	1526	582	402
[Cu(DAP-SAL)]. H_2O	-	3402	1053	1525	547	430
[Fe(DAP-SAL)]. H_2O	-	3401	1032	1512	582	407
[Co(DAP-SAL)]. H_2O	-	3401	1036	1509	502	420
[Ni(DAP-SAL)]. H_2O	-	3403	1032	1565	554	393
[Zn(DAP-SAL)]. H_2O	-	3405	1030	1516	554	414

KEY:

(DAP-SAL)SB = Dapsone Schiff base derived from Salicylaldehyde

[M(DAP-SAL)]. X H_2O = Metal(II) complex derived from (DAP-SAL)

Table 4.18: Infrared Spectral Data of (DAP-ACA) Schiff Base and its Metal(II) Complexes

Compound	υ (O-H)	υ (O-H) (H_2O)	υ (C-O)	υ (C=N)	υ (M-O)	υ (M-N)
(DAP-ACA)SB	3383	-	1292	1630	-	-
[Mn(DAP-ACA)]. $2H_2O$	-	3401	1134	1522	579	395
[Cu(DAP-ACA)]. $6H_2O$	-	3403	1124	1522	568	415
[Fe(DAP-ACA)]. H_2O	-	3404	1138	1525	600	455
[Co(DAP -ACA)]. H_2O	-	3379	1128	1552	541	407
[Ni(DAP-ACA)]. $5H_2O$	-	3405	1142	1643	606	453
[Zn(DAP-ACA)]. $5H_2O$	-	3412	1144	1532	617	399

KEY:

(DAP-ACA)SB = Schiff Base derived from Dapsone and Acetylacetone

[M(DAP-ACA)]. XH_2O = Metal(II) Complex derived from (DAP-ACA).

Table 4.19: Infrared Spectral Data of Rifampicin and its Metal(II) Complexes

Compound	υ (O-H)	υ (O-H) (H_2O)	υ (C-O)	υ (C=O)	υ (C=N)	υ (M-O)	υ (M-N)
Rifampicin	3407	-	1149	1644	1544	-	-
[Mn(RIF)]. $2H_2O$	-	3405	1131	1648	1525	610	407
[Cu(RIF)]. $2H_2O$	-	3404	1024	1652	1527	603	395
[Fe(RIF)]. $2H_2O$	-	3404	1147	1651	1527	610	393
[Co(RIF)]. H_2O	-	3404	1021	1651	1528	666	399
[Ni(RIF)]. $2H_2O$	-	3405	1142	1656	1527	605	362
[Zn(RIF)]. H_2O	-	3406	1034	1656	1528	603	393

KEY:

[M(RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.20: Infrared Spectral Data of Clofazimine and its Metal(II) Complexes

Compound	υ (C=N)	υ $(C=N)_{Diazine\ ring}$	υ (M-N)
Clofazimine	1642	1421	-
$[Mn(CLOF)Cl_2].\ H_2O$	1520	1312	402
$[Cu(CLOF)Cl_2].\ H_2O$	1520	1431	396
$[Fe(CLOF)Cl_2].\ H_2O$	1515	1334	368
$[Co(CLOF)Cl_2].\ H_2O$	1520	1321	390
$[Ni(CLOF)Cl_2].\ H_2O$	1509	1319	421
$[Zn(CLOF)Cl_2].\ H_2O$	1516	1419	399

KEY:

$[M(CLOF)Cl_2].\ XH_2O$ = Metal(II) complexes derived from clofazimine

Table 4.21: Antibacterial Activities of (DAP-ACA) Schiff Base and its Metal(II) Complexes showing the Zones of Inhibition (mm) against Bacteria Pathogens

Compound	*E-coli*			*Staph. aureus*			*Salmonella Typhi*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)
Dapsone*	8	6	6	6	6	6	-	-	-
(DAP-ACA)SB-Ligand	9	7	-	11	9	8	12	9	7
[Mn(DAP-ACA)]. $2H_2O$	11	9	7	-	-	-	10	8	7
[Cu(DAP-ACA)].$6H_2O$	9	-	-	12	10	7	11	9	8
[Fe(DAP-ACA)]. H_2O	10	7	-	10	9	8	-	-	-
[Co(DAP-ACA)]. H_2O	16	14	10	13	10	9	-	-	-
[Ni(DAP-ACA)].$5H_2O$	13	11	9	12	-	-	13	12	8
[Zn(DAP-ACA)].$5H_2O$	11	8	7	12	9	8	10	9	8

KEY:

E-coli - Escheria coli

Staph. aureus - Staphylococcus aureus

mm - Millimetre of the diameter of zone of inhibition

(*) = Control Experiment

(-) = Not Measurable

(DAP-ACA)SB = Schiff Base derived from Dapsone and Acetylacetone

[M(DAP-ACA)]. XH_2O = Metal(II) Complex derived from (DAP-ACA)SB.

Table 4.22 : Antibacterial Activities of (DAP-SAL) Schiff Base and its Metal(II) Complexes showing the Zones of Inhibition (mm) against Bacteria Pathogens

Compound	*E-coli*			*Staph. aureus*			*Salmonella Typhi*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)
Dapsone*	7	6	6	7	6	6	-	-	-
(DAP-SAL)SB-Ligand	10	9	7	12	10	9	11	10	8
[Mn(DAP-SAL)]. H_2O	10	7	-	-	-	-	8	7	-
[Cu(DAP-SAL)]. H_2O	12	10	7	13	1	8	-	-	-
[Fe(DAP-SAL)]. H_2O	10	8	7	-	-	-	-	-	-
[Co(DAP-SAL)]. H_2O	16	14	10	13	10	9	-	-	-
[Ni(DAP-SAL)]. H_2O	-	-	-	9	8	7	11	9	7
[Zn(DAP-SAL)]. H_2O	14	12	9	15	12	10	11	9	7

KEY:

E-coli - *Escheria coli*

Staph. aureus - *Staphylococcus aureus*

(*) = Control Experiment

(-) = Not Measurable

mm - Millimetre of the diameter of zone of inhibition

(DAP-SAL)SB = Dapsone Schiff Base derived from salicylaldehyde

[M(DAP-SAL)]. XH_2O = Metal(II) complex derived from DAP-SAL

Table 4.23: Antibacterial Activities of Rifampicin and its Metal(II) Complexes showing the Zones of Inhibition (mm) against Bacteria Pathogens

Compound	*E-coli*			*Staph. aureus*			*Salmonella Typhi*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15(μg)
Rifampicin*	13	10	9	11	9	8	-	-	-
[Zn(RIF)]. H_2O	10	7	-	-	-	-	8	7	-
[Mn(RIF)]. $2H_2O$	-	-	-	10	-	-	11	9	8
[Cu(RIF)]. $2H_2O$	15	12	11	15	13	10	12	10	-
[Fe(RIF)]. $2H_2O$	13	10	8	14	10	8	12	11	9
[Co(RIF)]. H_2O	13	10	8	11	9	8	-	-	-
[Ni(RIF)]. $2H_2O$	12	11	8	11	8	7	-	-	-

KEY:

E-coli - *Escheria coli*

Staph. aureus - *Staphylococcus aureus*

(*) = Control Experiment

(-) = Not Measurable

mm - Millimetre of the diameter of zone of inhibition

[M(RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.24 : Antibacterial Activities of Clofazimine and its Metal(II) Complexes showing the Zones of Inhibition (mm) against Bacteria Pathogens

Compound	*E-coli*			*Staph. aureus*			*Salmonella Typhi*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)
Clofazimine*	9	7	-	12	10	7	11	9	8
$[Mn(CLOF)Cl_2].H_2O$	9	8	-	13	10	8	10	9	7
$[Cu(CLOF)Cl_2].H_2O$	10	8	7	14	11	-	11	9	8
$[Fe(CLOF)Cl_2].H_2O$	10	8	7	-	-	-	14	10	8
$[Co(CLOF)Cl_2].H_2O$	-	-	-	12	10	8	11	9	8
$[Ni(CLOF)Cl_2].H_2O$	9	8	7	12	10	7	-	-	-
$[Zn(CLOF)Cl_2].H_2O$	11	9	8	10	8	7	12	8	7

KEY:

E-coli - *Escheria coli*

Staph. aureus - *Staphylococcus aureus*

(*) = Control Experiment

(-) = Not Measurable

mm - Millimetre of the diameter of zone of inhibition

$[M(CLOF)Cl_2].XH_2O$ = Metal(II) complexes derived from Clofazimine

Table 4.25: Antifungal Activities of (DAP-SAL) Schiff Base and its Metal(II) Complexes showing the Zone of Inhibition (mm) against Pathogenic Fungi

Compound	*Asp.flv*			*Asp.niger*			*Asp.fmg*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)
Dapsone*	7	-	6	-	-	7	-	6	7
(DAP-SAL)SB-Ligand	-	-	-	12	10	9	11	10	8
[Mn(DAP-SAL)]. H_2O	12	10	9	14	12	10	-	-	-
[Cu(DAP-SAL)]. H_2O	9	8	7	11	9	8	-	-	-
[Fe(DAP-SAL)]. H_2O	11	8	-	9	8	7	11	9	8
[Co(DAP-SAL)]. H_2O	9	-	-	13	11	8	-	-	-
[Ni(DAP-SAL)]. H_2O	10	8	-	-	-	-	13	10	9
[Zn(DAP-SAL)]. H_2O	-	-	-	9	8	7	12	8	7

KEY:

Asp.flv – Asperigillus flavus

Asp.niger – Asperigillus niger

Asp.fng- Asperigillus fumigatos

(*) = Control Experiment

(-) = Not Measurable

mm - Millimetre of the diameter of zone of inhibition

(DAP-SAL)SB = Dapsone Schiff Base derived from salicylaldehyde

[M(DAP-SAL)]. XH_2O = Metal(II) complex derived from DAP-SAL

Table 4.26: Antifungal Activities of (DAP-ACA) Schiff Base and its Metal(II) Complexes showing the Zone of Inhibition (mm) against Pathogenic Fungi

Compound	*Asp.flv*			*Asp.niger*			*Asp.fmg*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)
Dapsone*	-	7	-	6	-	6	-	-	6
(DAP-ACA)SB-Ligand	13	11	10	11	9	-	-	-	-
[Mn(DAP-ACA)]. $2H_2O$	-	-	-	12	9	7	10	8	7
[Cu(DAP-ACA)].$6H_2O$	-	-	-	10	8	7	12	10	8
[Fe(DAP-ACA)]. H_2O	11	10	8	14	12	9	-	-	-
[Co(DAP-ACA)]. H_2O	12	11	9	-	-	-	14	10	9
[Ni(DAP-ACA)].$5H_2O$	13	10	9	11	-	-	13	12	8
[Zn(DAP-ACA)]. $5H_2O$	12	10	8	12	9	8	10	8	7

KEY:

Asp.flv – Asperigillus flavus

Asp.niger – Asperigillus niger

Asp.fng- Asperigillus fumigatos

(*) = Control Experiment

(-) = Not Measurable

mm - Millimetre of the diameter of zone of inhibition

(DAP-ACA)SB = Schiff Base derived from Dapsone and Acetylacetone

[M(DAP-ACA)]. XH_2O = Metal(II) Complex derived from (DAP-ACA)SB

Table 4.27: Antifungal Activities of Rifampicin and its Metal(II) Complexes showing the Zone of Inhibition (mm) against Pathogenic Fungi

Compound	*Asp.flv*			*Asp.niger*			*Asp.fmg*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)
Rifampicin*	10	9	8	12	10	7	-	-	-
[Zn(RIF)]. H_2O	10	7	-	-	-	-	8	7	-
[Mn(RIF)]. $2H_2O$	13	12	10	-	-	-	12	10	8
[Cu(RIF)]. $2H_2O$	-	-	-	11	8	7	-	-	-
[Fe(RIF)]. $2H_2O$	13	10	8	14	10	8	12	11	9
[Co(RIF)]. H_2O	9	8	7	10	9	8	-	-	-
[Ni(RIF)]. $2H_2O$	10	9	7	-	-	-	13	10	7

KEY:
Asp.flv – Asperigillus flavus

Asp.niger – Asperigillus niger

Asp.fng- Asperigillus fumigatos

(*) = Control Experiment

(-) = Not Measurable

mm - Millimetre of the diameter of zone of inhibition

[M(RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.28: Antifungal Activities of Clofazimine and its Metal(II) Complexes showing the Zone of Inhibition (mm) against Pathogenic Fungi

Compound	*Asp.flv*			*Asp.niger*			*Asp.fmg*		
	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)	60 (μg)	30 (μg)	15 (μg)
Clofazimine*	-	-	-	10	8	7	12	10	8
$[Mn(CLOF)Cl_2].H_2O$	12	8	7	-	-	-	10	8	7
$[Cu(CLOF)Cl_2].H_2O$	9	-	-	12	10	9	11	9	8
$[Fe(CLOF)Cl_2].H_2O$	12	11	8	9	8	7	-	-	-
$[Co(CLOF)Cl_2].H_2O$	-	-	-	10	7	-	9	8	7
$[Ni(CLOF)Cl_2].H_2O$	11	8	-	9	8	7	11	9	8
$[Zn(CLOF)Cl_2].H_2O$	-	-	-	10	9	8	9	7	-

KEY:

Asp.flv – Asperigillus flavus

Asp.niger – Asperigillus niger

Asp.fng- Asperigillus funigatos

(*) = Control Experiment

(-) = Not Measurable

mm - Millimetre of the diameter of zone of inhibition

$[M(CLOF)Cl_2].XH_2O$ = Metal(II) complexes derived from Clofazimine

Table 4.29: C50 Concentrations of (DAP-ACA) Schiff Base and its Metal(II) Complexes.

Compound	Conc.(g/2cm^3)	No. of Rats Used	No. of Deaths	% Death of Rats
(DAP-ACA)SB-Ligand	0.80	8	4	50.0
[Mn(DAP-ACA)].2H_2O	0.70	8	4	50.0
[Cu(DAP-ACA)]. 6H_2O	0.80	8	4	50.0
[Fe(DAP-ACA)]. H_2O	0.70	8	4	50.0
[Co(DAP -ACA)]. H_2O	0.70	8	4	50.0
[Zn(DAP -ACA)].5H_2O	0.80	8	4	50.0
[Ni(DAP-ACA)].5H_2O	0.80	8	4	50.0

KEY:

(DAP-ACA)SB = Schiff Base derived from Dapsone and Acetylacetone

[M(DAP-ACA)]. XH_2O = Metal(II) Complex derived from (DAP-ACA)SB.

Table 4.30: C50 Concentrations of (DAP-SAL) Schiff Base and its Metal(II) Complexes

Compound	Conc.(g/2cm^3)	No. of Rats Used	No. of Deaths	% Death of Rats
(DAP-SAL)SB-Ligand	0.70	8	4	50.0
[Cu(DAP-SAL)] . H_2O	0.80	8	4	50.0
[Fe(DAP-SAL)]. H_2O	0.80	8	4	50.0
[Ni(DAP-SAL)]. H_2O	0.80	8	4	50.0
[Mn(DAP-SAL)]. H_2O	0.70	8	4	50.0
[Co(DAP-SAL)]. H_2O	0.70	8	4	50.0
[Zn(DAP-SAL)]. H_2O	0.70	8	4	50.0

KEY:

(DAP-SAL)SB = Dapsone Schiff Base derived from salicylaldehyde

[M(DAP-SAL)]. XH_2O = Metal(II) complex derived from DAP-SAL

Table 4.35: C50 Concentrations of Rifampicin and its Metal(II) Complexes

Compound	Conc. (g/2cm^3)	No. of Rats Used	No. of Deaths	% Death of Rats
Rifampicin	0.60	8	4	50.0
[Cu(RIF)]. $2H_2O$	0.60	8	4	50.0
[Mn(RIF)]. $2H_2O$	0.70	8	4	50.0
[Ni(RIF)]. $2H_2O$	0.70	8	4	50.0
[Fe(RIF)]. $2H_2O$	0.70	8	4	50.0
[Co(RIF)]. H_2O	0.70	8	4	50.0
[Zn(RIF)]. H_2O	0.60	8	4	50.0

KEY:

[M (RIF)]. XH_2O = Metal(II) complexes derived from Rifampicin

Table 4.36: C50 Concentrations of Clofazimine and its Metal(II) Complexes

Compound	Conc. (g/2cm^3)	No. of Rats Used	No. of Deaths	% Death of Rats
Clofazimine	0.60	8	4	50.0
[Mn(CLOF)Cl_2]. H_2O	0.70	8	4	50.0
[Cu(CLOF)Cl_2]. H_2O	0.60	8	4	50.0
[Fe(CLOF)Cl_2]. H_2O	0.60	8	4	50.0
[Co(CLOF)Cl_2]. H_2O	0.70	8	4	50.0
[Ni(CLOF)Cl_2]. H_2O	0.70	8	4	50.0
[Zn(CLOF)Cl_2]. H_2O	0.80	8	4	50.0

KEY:

[M(CLOF)Cl_2]. XH_2O = Metal(II) complexes derived from Clofazimine

4.2 Discussion

The reaction between dapsone and salicylaldehyde in a molar ratio of 1:2 respectively, produced yellow crystalline solid of the Schiff base with a percentage yield of 89.6%. The interaction between the Schiff base and some metal(II) ions in acetone produced metal(II) complexes of the respective metal(II) ions having various colours with a percentage yield ranging between 62-84% (Table 4.1). The reaction between dapsone and acetylacetone in a similar ratio to that of the reaction between dapsone and salicylaldehyde produced pale yellow crystals of Schiff base with a percentage yield of 85.2%. Chemical interactions between the ligand solution and solutions of some metal(II) chloride salts produced respective metal(II) complexes of various colours with percentage yield ranging between 51-84% (Table 4.2). Rifampicin and clofazimine which are Schiff bases reacted respectively with various metal(II) chloride salts in the molar ratio 1:1 to produce corresponding metal(II) complexes having various colours with percentage yield in the range 51-72% (Tables 4.3 and 4.4). All the metal(II) complexes produced were non-hygroscopic crystals. They remained dry even when exposed to the air, showing that they do not absorb water to become sticky or moist. The fact that almost all of the synthesized metal(II) complexes are coloured can be attributed to ‘d-d’ orbital transitions of electrons between energy levels whose spacing corresponds to the wavelengths available in visible light (Rodgers, 1994). Since all the complexes suggest tetrahedral geometry, the degenerate d-orbitals are split into *e* and t_2 orbitals. The colours exhibited are intimately related to the magnitude of the spacing between the energy levels which depends on factors such as geometry of the complex, the nature of the ligands and the oxidation state of the central metal atom (Kotz and Purcell, 1987). It is expected that complexes that contain metal ions of d^{10} electron configuration are usually white, such as the case with [Zn(DAP-SAL)].H_2O and [Zn(RIF)].H_2O. This is because all the orbitals have paired electrons and so‘d-d’ transition does not take place (Rogers, 1994). However, in certain cases, the nature of the ligand has a strong influence on the splitting of the d-orbital. Even though the d^{10} configuration rules out ‘d-d’ transitions, the nature of the ligand can cause a large

energy separation of the d-orbitals (strong field ligands) while others can only cause a small splitting (weak field ligands). The respective colours of $[Zn(CLOF)Cl_2].H_2O$ (violet) and $[Zn(DAP\text{-}ACA)].5H_2O$ (pale yellow) can be attributed to the nature of the respective ligands or charge transfer transitions.

The melting points of the Schiff bases and decomposition temperatures of the metal(II) complexes recorded are in the range of 188-212°C and 196 - >250°C respectively (Table 4.1 - 4.4). These figures are relatively high and indicate that they are stable compounds (Ahmed and Akhtar, 1983) and not easily decomposed. The high decomposition temperatures of the metal(II) complexes suggest the 'chelating effect' of the respective ligands. Chelating ligands form more stable complexes than do an equivalent number of related monodentate ligands. This can be attributed to the fact that the enthalpy change ΔH for the formation of the complex is dependent on the nature of the ligand as well as the size of the ligand. Chelate effect is 'entropy-driven'. Chelate ligands are far less likely to be displaced by water molecules, even in cases where water is a better ligand for the metal than the particular donor in the chelate because binding water molecule causes an unfavourable decrease in entropy. Besides entropy favouring a polydentate ligand over an equivalent number of monodentate donors, macrocycles are already tied into rings so there are fewer degrees of conformation freedom to lose on coordination ('pre-organization' effect).

Analyses of the results obtained from the molar conductance measurements carried out on 10^{-3}M each of all the metal(II) complexes in DMF, are in the range 9-20 $ohm^{-1}cm^2mol^{-1}$ (Tables 4.1-4.4). These values are relatively low, indicating that the complexes are non-electrolytes (Burger, 1973; Cezar and Angela, 2000; Kettle, 1975; Sutton, 1968; Geary, 1971). The use of conductivity measurements enables the determination of the number of ions in solution. The low values obtained for the synthesized metal(II) complexes suggest that they occur in solution largely as neutral molecules (contain very few ions) since the conductance of a solution is a measure of the ability of that solution to carry a current. The transfer of

electricity through a solution results from the movement of ions. The fewer the ions in solution, it follows then that the lower the conductance of that solution.

The elemental analyses values shown in Tables 4.5 - 4.8 suggest a 1:1 (metal: ligand) ratio for all the metal(II) complexes. These values conform to the calculated values. Thus the composition of each metal(II) complex as determined from the CHN elemental analysis results of the respective ligand and metal(II) complex, percentage metal in each of the respective metal(II) complex and determined percentage water of crystallization of each metal(II) complex confirm a 1:1 metal:ligand ratio.

Analysis of the water of crystallization for (DAP-SAL) metal(II) complexes showed that they contain water molecules in the range 2.00 - 3.54% per molecule (Table 4.5). For the (DAP-ACA) metal(II) complexes, the percentage water of crystallization is higher (between 3.6 -16.9% per molecule) (Table 4.6). Rifampicin and clofazimine metal(II) complexes also contain water molecules within the range 2.00 - 4.25% per molecule (Tables 4.7 - 4.8)).

The solubility of each of the synthesized Schiff bases and metal(II) complexes was determined using distilled water and each of the following common organic solvents; ethanol, methanol, dimethylformamide (DMF), dimethylsulfoxide (DMSO), acetone, chloroform, ethyl acetate and diethyl ether. Solubility test is used to determine if a solute can dissolve in a solvent to form a homogenous solution. It is an important test in the characterization process because it reveals the solvents that the test substances are soluble in and so helps to determine solvents for analysis, such as molar conductance. It was observed that (DAP-ACA) and (DAP-SAL) Schiff bases were insoluble in water and also in ethyl acetate. Clofazimine was also insoluble in water but was slightly soluble in ethyl acetate. All the synthesized metal(II) complexes showed varying degrees of solubility in water and in the organic solvents. However, the metal(II) complexes of clofazimine were insoluble in water (Tables 4.9-4.12). Generally, transition metal complexes with organic ligands are organic molecules and are relatively non-polar (Jones and Fleming, 2010). As a result, they are soluble in organic solvents but not in polar solvents like water.

However, an investigation into the structure of rifampicin and its metal(II) complexes shows that like all the other synthesized compounds, they are neutral molecules (contain neither anionic or cationic species) but they contain a relatively high ratio of polar –(OH) groups compared to the other synthesized compounds. At molecular level, solubility is controlled by intermolecular forces. However, a simple empirical rule "like dissolves like" and it is based on the polarity of the systems, that is, polar molecules dissolve in polar solvents and non-polar molecules in non-polar solvents. If the predominant interactions are non-polar and hydrophobic for a given organic compound, the organic molecule will not be able to find complimentary interactions in water which is polar and hydrophilic. Therefore, the organic compound will prefer to stay aggregated and segregated from the water (Jones and Fleming, 2010).

The values of the Magnetic Moments for the (DAP-SAL) metal complexes all conformed to a tetrahedral geometry for the transition metal ions (Table 4.13). The values of the Magnetic Moments for all the (DAP-ACA) metal complexes conformed to a tetrahedral geometry (Table 4.14). The Ni(II), Fe(II) and Co(II) complexes of (DAP-ACA) Schiff base ligand all have magnetic moment values suggesting high spin complexes. Zn(II) complexes of all the Schiff base ligands have negative values for their gram magnetic susceptibility implying the absence of unpaired electrons. For the Rifampicin and Clofazimine metal complexes, $[Mn(RIF)].2H_2O$ and $[Mn(CLOF)Cl_2].H_2O$ with a magnetic moment of 6.2 and 6.4 BM respectively has 5 unpaired electrons each. $[Fe(RIF)].2H_2O$ with a magnetic moment of 5.5 BM and $[Co(RIF)].H_2O$ with a magnetic moment of 4.4 BM correspond to 4 and 3 unpaired electrons respectively. All these conform to high spin tetrahedral geometry (Figgis and Lewis, 1964). Similar results are obtained for the Clofazimine metal(II) complexes (Tables 4.15 and 4.16).

Infrared spectroscopy is widely used as a characterization technique for metal complexes. The basic theory involved is that the stretching modes of a ligand changes upon complexation due to weakening or strengthening of the bonds involved in the bond formation resulting in subsequent changes in the positions of the bands appearing in the infrared spectrum (Chandraleka and Chanramahon, 2014).

The infrared spectrum of (DAP-SAL) ligand shows a broad and strong absorption around 3383 cm^{-1} attributed to the phenolic υ (O-H) bond stretching accompanied by the alkane sp^3 C-H peak around 2930 cm^{-1}. In addition to these is the intense broad band at 1142 cm^{-1} due to the phenolic υ (C-O) (Table 4.17). However, the broad band around 3383 cm^{-1} in the ligand which is indicative of the phenolic υ (O-H) is absent in each of the DAP-SAL metal(II) complexes (Table 4.17). This indicates the deprotonation of the phenolic υ (O-H) on coordination with metal ion (Panda *et al,* 1996; Mishra and Pandey, 2005). Also, the υ (C-O) band around 1142 cm^{-1} in the ligand is shifted in the metal complexes to (1030-1126) cm^{-1} further suggesting bonding between the metal ion and oxygen of υ (C-O) (Raman *et al.,* 2004). A medium intensity band in (DAP-SAL) ligand spectrum at about 1600 cm^{-1} corresponding to υ (C=N) azomethine mode is shifted to lower frequencies in the metal(II) complexes (1509-1526) cm^{-1} (Table 4.17). This suggests the involvement of υ (C=N) in chelation (Nejati and Rezvani, 2003; Maurya, *et al.,* 2006). There are no prominent bands appearing in the 1650-1800 cm^{-1} region of the spectra of the metal complexes indicating participation of the azomethine nitrogen and phenolic oxygen atoms in coordination with metal ion (Bellamy, 1980). Some bands of weaker intensity in the metal(II) complexes at about (502-582) cm^{-1} and (393-430)cm^{-1} give inference about υ (M-O) and υ (M-N) bonding (Nakamoto *et al.,* 1970; Gohzalez-vergara *et al.,* 1982). The spectral bands in the metal complexes in the range 3401-3405 are assigned to υ (O-H) stretching vibrations indicating the presence of water of crystallization (Anacona and Toledo, 2001).

The infrared spectral patterns of (DAP-ACA) ligand and metal(II) complexes follow a similar pattern to that of (DAP-SAL) and its metal(II) complexes. A broad band appearing in the ligand spectrum around 3383 cm^{-1} is attributed to the phenolic υ (OH) and is noticeably absent in all the (DAP-ACA) metal(II) complexes indicating the deprotonation of phenolic (OH) and coordination of oxygen of the deprotonated -OH with metal ion (Table 4.18). A band around 1292 cm^{-1} in the ligand is shifted to lower

frequencies in the metal(II) complexes (1124-1144) cm^{-1} (Table 4.18) substantiating deprotonation. A band around 1630 cm^{-1} in the ligand corresponding to υ (C=N) azomethine group is shifted to lower frequencies (1493-1552) cm^{-1}. This indicates coordination through azomethine nitrogen (Shaker *et al*., 2010). Weak bands in the complexes at (541-617) cm^{-1} and (395-455) cm^{-1} indicate υ (M-O) and υ (M-N) respectively. The spectral bands in the metal(II) complexes in the range 3379-3412 cm^{1} are assigned to υ (O-H) stretching vibrations of water molecule. The infrared spectral information of (DAP-SAL) and (DAP-ACA) ligands and their respective metal(II) complexes supports the suggestion of coordination of the imino nitrogen and phenolic oxygen to the transition metal(II) ions during the formation of the respective metal(II) complexes.

A careful examination of the IR spectrum of rifampicin shows a strong and broad band at 3407 cm^{-1} which can be attributed to the phenolic υ (O-H). (Table 4.19). This band is absent in all the metal (II) complexes of rifampicin indicating deprotonation of the phenolic υ (O-H). Also, the υ (C-O) band in free rifampicin around 1149 cm^{-1} is shifted in the metal(II) complexes to between (1021-1147) cm^{-1} further supporting deprotonation and the involvement of υ (C-O) in coordination with metal(II) ion. The band at about 1544 cm^{-1} corresponding to the υ (C=N) stretching vibration in rifampicin is shifted to lower frequencies in the spectra of all the metal(II) complexes (1525-1528) cm^{-1} indicating the involvement of the azomethine nitrogen in coordination with the respective metal ions in the complexes (Shaker *et al.,* 2010). Rifampicin also exhibits a strong intensity band around 1644 cm^{-1} due to υ (C=O) and this has been shifted to higher frequencies (1648-1656) cm^{-1} in the spectra of the metal(II) complexes. This suggests the involvement of the carbonyl oxygen in the chelation (Chandra and Gupta, 2005). This higher side shift observed in the metal complexes can be attributed to the expected high mesomeric interactions in the complexes activated by the presence of the metal ion (Chandraleka and Chanramahon, 2014). Bands at (362-407) cm^{-1} and (603-666) cm^{-1} in the metal(II) complexes denote υ (M-N) and υ (M-O) bands

respectively and bands in the range (3404-3406) cm^{-1} in the metal(II) complexes can be assigned to υ (O-H) stretching vibrations indicating the presence of water of crystallization. The infrared spectra of rifampicin support the coordination of the azomethine nitrogen, the phenolic oxygen and the carbonyl oxygen in bond formation.

The infrared spectral studies of clofazimine and its synthesized metal(II) complexes have also been determined and summarized in Table 4.20. The strong broad band at 3406 cm^{-1} in the ligand is assigned to υ (N-H). This band is located within the υ (N-H) for a secondary amine (Coates, 2000) and it is not shifted in the spectra of the metal(II) complexes indicating that υ (N-H) is not involved in coordination with metal ion. An important band at 1517 cm^{-1} in the ligand attributed to υ (C=N) azomethine group is shifted in the metal(II) complexes and appears in the regions (1509-1521) cm^{-1}. This suggests the involvement of the nitrogen atom of the azomethine moiety in the coordination of the ligand to the metal ion (Shaker *et al.,* 2010). The vibrations which occur around 1421 cm^{-1} in clofazimine is attributed to the υ (C=N) of the diazine ring and is shifted in the metal complexes (1311-1418) cm^{-1} due to coordination via the nitrogen of the C=N in the diazine ring (Lawal *et al.,* 2014). The bands between (597-625) cm^{-1} in the infrared spectra of the metal (II) complexes can be assigned to υ (M-N) stretching band (Nakamoto *et al.,* 1970; Gonzalez- Vergara *et al.,* 1982). The infrared spectral information of clofazimine supports the coordination of the azomethine nitrogen and the nitrogen of υ (C=N) of the diazine ring

The results of the antibacterial and antifungal activities of the Schiff base ligands and their metal(II) complexes are presented in Tables 4.21-4.28. *Mycobacterium leprae* cannot be isolated and cultured in the laboratory (Wheeler, 2003; Scollard *et al.,* 2006). They can only survive in extremely selective environments as a result of reductive evolution. For this reason, direct *in-vitro* testing using leprae is not feasible in the laboratory. However, antimicrobial testing of the synthesized Schiff bases and metal(II) complexes derived from dapsone and metal(II) complexes derived from clofazimine and rifampicin can be carried out using other microbial strains with dapsone, clofazimine and rifampicin as

controls. In this way, the antimicrobial activities of the synthesized compounds can be tested against these already established antileprosy drugs and based on the results obtained, effective comparison can be made and useful conclusions drawn. The antibacterial activities tests of the synthesized compounds were carried out against three bacterial species *Escheria coli, Staphylococcus aureus and Salmonella typhi* while antifungal activities tests were carried out also using three fungal strains; *Asperigillus flavus, Asperigillus niger* and *Asperigillus fumigatos.* Dapsone, rifampicin and clofazimine were used as controls during the experiments. The zones of inhibition based upon size around each of the discs were measured and recorded (Tables 4.21 to 4.28). From the results obtained for (DAP-ACA) and its metal(II) complexes (Table 4.21), we can see that the ligand showed more activity when compared to dapsone and some of the metal(II) complexes showed even more activity than the ligand itself, especially [Co(DAP-ACA)].H_2O, [Ni(DAP-ACA)].$5H_2O$ and [Zn(DAP-ACA)].$5H_2O$. The results obtained for the antibacterial testing of (DAP-SAL) Schiff base and its metal(II) complexes (Table 4.22) showed a similar trend to that of (DAP-ACA) and its metal(II) complexes. It was observed that the (DAP-SAL) ligand showed a higher antibacterial activity compared to dapsone while [Co(DAP-SAL)].H_2O, [Zn(DAP-SAL)].H_2O and [Ni(DAP-SAL)].H_2O clearly showed higher antibacterial activities when compared to the Schiff base and dapsone respectively. The antibacterial activities of each of the rifampicin metal(II) complexes was also investigated (Table 4.23). Some of the complexes exhibited higher activity compared to rifampicin, especially [Cu(RIF)]$2H_2O$, [Co(RIF)].H_2O and [Fe(RIF)].H_2O A similar trend was obtained for clofazimine and its metal(II) complexes (Table 4.36) with [Cu(CLOF)Cl_2].H_2O, [Zn(CLOF)Cl_2].H_2O and [Fe(CLOF)Cl_2].H_2O showing marked higher activity than clofazimine. An investigation into the antifungal properties of the compounds showed that the majority of the compounds showed higher activity than their respective control compounds (Table 4.25 to 4.28).

Chelation may enhance or suppress the biochemical potential of bioactive organic species. The higher antimicrobial activity of metal complexes may be as a result of the effect of metal ions on the

normal cell membrane. Metal chelates bear polar and non-polar properties which makes them suitable for permeation into the cells and tissues. The change in hydrophilicity and lipophilicity probably leads to lower the solubility and permeability barriers of cells, which results in enhancing the bioavalaibility of chemotherapeutics on the one hand and potentiality at the other (Rehder, 2003).

In the present study to determine toxicity levels of the synthesized compounds, their respective C50 was determined by oral route and skin contact on *Rattus norvegicus* rats with body weight 225 ± 25g. The obtained data showed that the C50 of (DAP-ACA) Schiff base is about 0.80g/2cm^3 and within the range (0.70-0.80g/2cm^3) for (DAP-ACA) metal(II) complexes (Table 4.33). This indicates that the new dapsone derivatives synthesized from acetylacetone and the respective metal(II) chlorides have lower toxicity than dapsone itself with C50 of about 0.35g/2cm^3 for *Rattus norvegicus* rats within the body weight of 225 ± 25g (Hamed *et al.,* 2014). The C50 of (DAP-SAL) Schiff base and its metal(II) complexes showed a similar result to that of (DAP-ACA) and its metal(II) complexes with C50 ranging from 0.70-0.80g/2cm^3 (Table 4.34). The C50 of rifampicin and its respective metal(II) complexes is slightly lower than for the dapsone derivatives (0.60-0.70g/2cm^3) (Table 4.35). This indicates slightly higher toxicity when compared to the dapsone derivatives while the C50 of clofazimine and its metal(II) complexes are in the range (0.60-0.80g/2cm^3) which also indicates low toxicity. LD50 determinations for all the synthesized compounds was obtained in the range of 9000-11,000 mg/kg, which according to the Hodge and Sterner toxicity scale indicate practically non-toxic substances (Ahmed, 2015). We can safely conclude that all the synthesized compounds appear to be safe for consumption because they have low toxicity. Some chemicals can produce death in microgram doses and are commonly considered extremel0079 poisonous. Common examples are Butolin's toxin (0.00001mg/Kg) and Dioxine (0.001mg/Kg) (Wadher *et al*., 2009).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The experimental techniques, general characteristics and methods of preparing dapsone Schiff bases from aldehyde (salicylaldehyde) and ketone (acetylacetone) and their metal(II) complexes synthesized from Cu(II), Mn(II), Fe(II), Co(II), Ni(II) and Zn(II) have been studied. The experimental techniques, general characteristics and methods of preparing rifampicin and clofazimine metal (II) complexes have also been developed and studied. The metal - ligand ratio for all the metal(II) complexes from elemental analyses have been found to be (1:1). The magnetic behaviour of all the metal(II) complexes have been determined and were found to be paramagnetic, except for the diamagnetic zinc(II) metal complexes in which case magnetic measurement is not relevant. All the metal(II) ions tend to form tetrahedral complexes. The relatively low molar conductance values of the metal(II) complexes indicate their non-electrolytic nature. The Infra-red spectral data of the ligands when compared to those of their respective metal(II) complexes showed that coordination of the metal ion to the ligand is via the azomethine nitrogen and the phenolic –(OH) of the Schiff base in the case of (DAP-SAL) and (DAP-ACA) metal(II) complexes. While coordination of the metal ion to rifampicin is via the phenolic –(OH), azomethine nitrogen and carbonyl oxygen of the ligand. For clofazimine metal (II) complexes, coordination of the metal ion is via the azomethine nitrogen and diazine nitrogen atoms of the ligand. All the metal(II) complexes were found to be non-toxic and possessed antimicrobial effects, in some cases, higher than their Schiff base ligands.

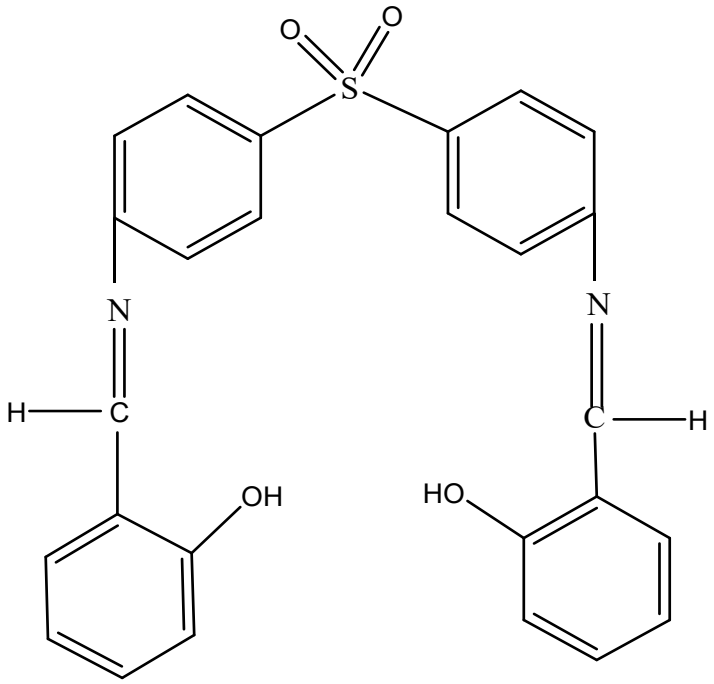


Fig. 5.1: Proposed Structure of (DAP-SAL) Ligand

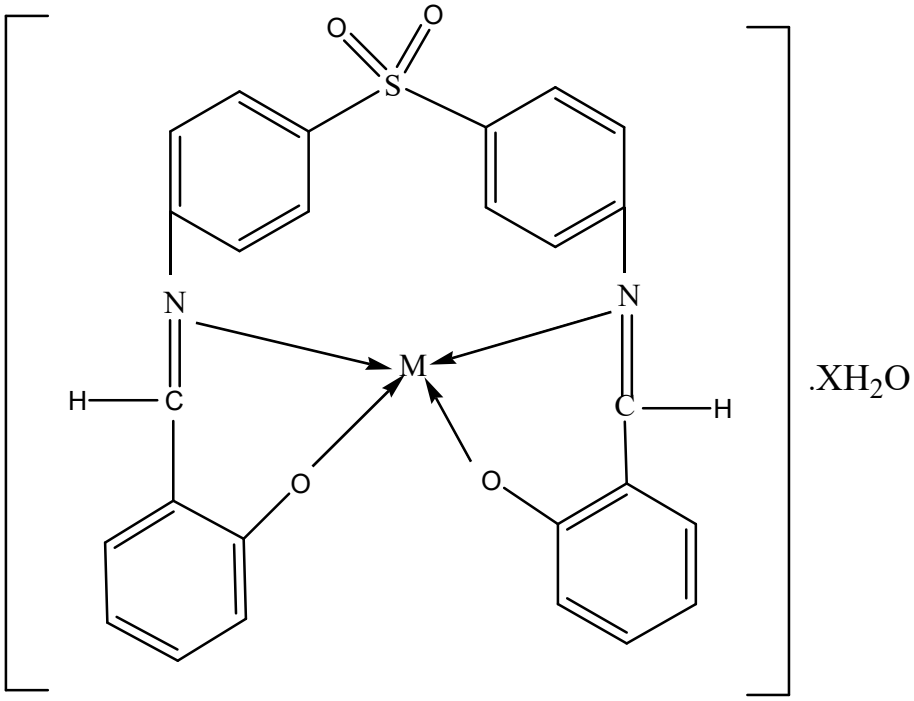


Fig. 5.2: Proposed Structure of (DAP-SAL) Metal (II) Complex
M = Mn, Ni, Co, Cu, Fe, Zn.

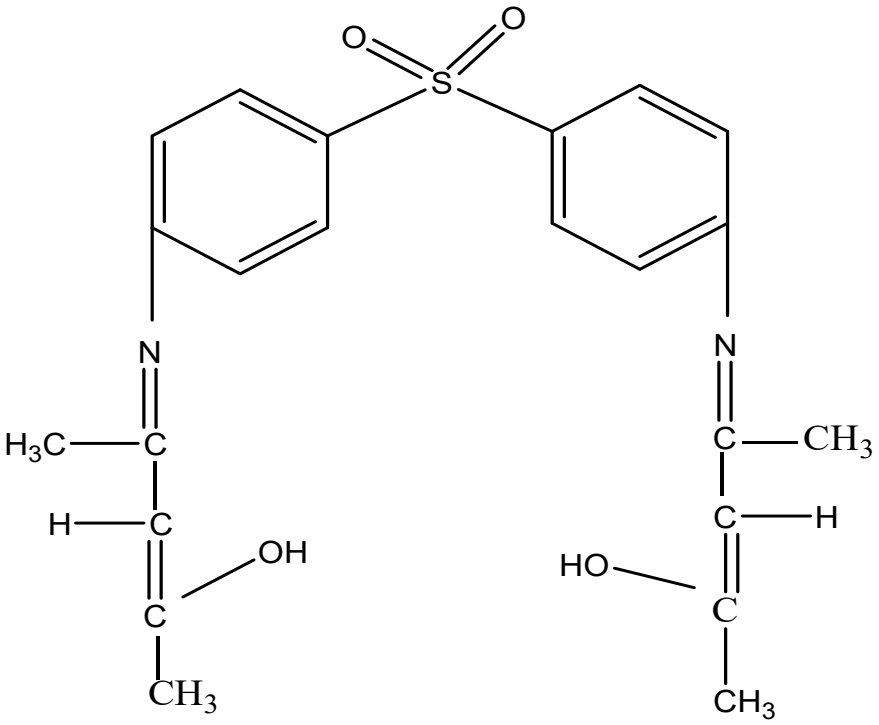


Fig. 5.3: Proposed Structure of (DAP-ACA) Ligand

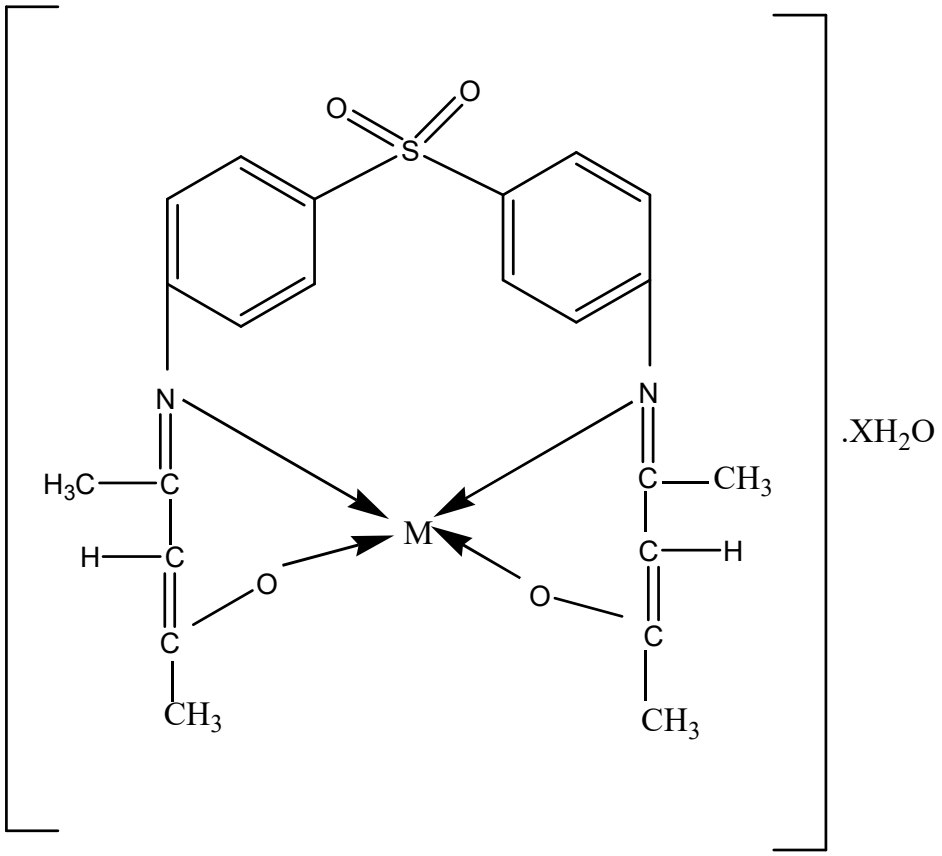
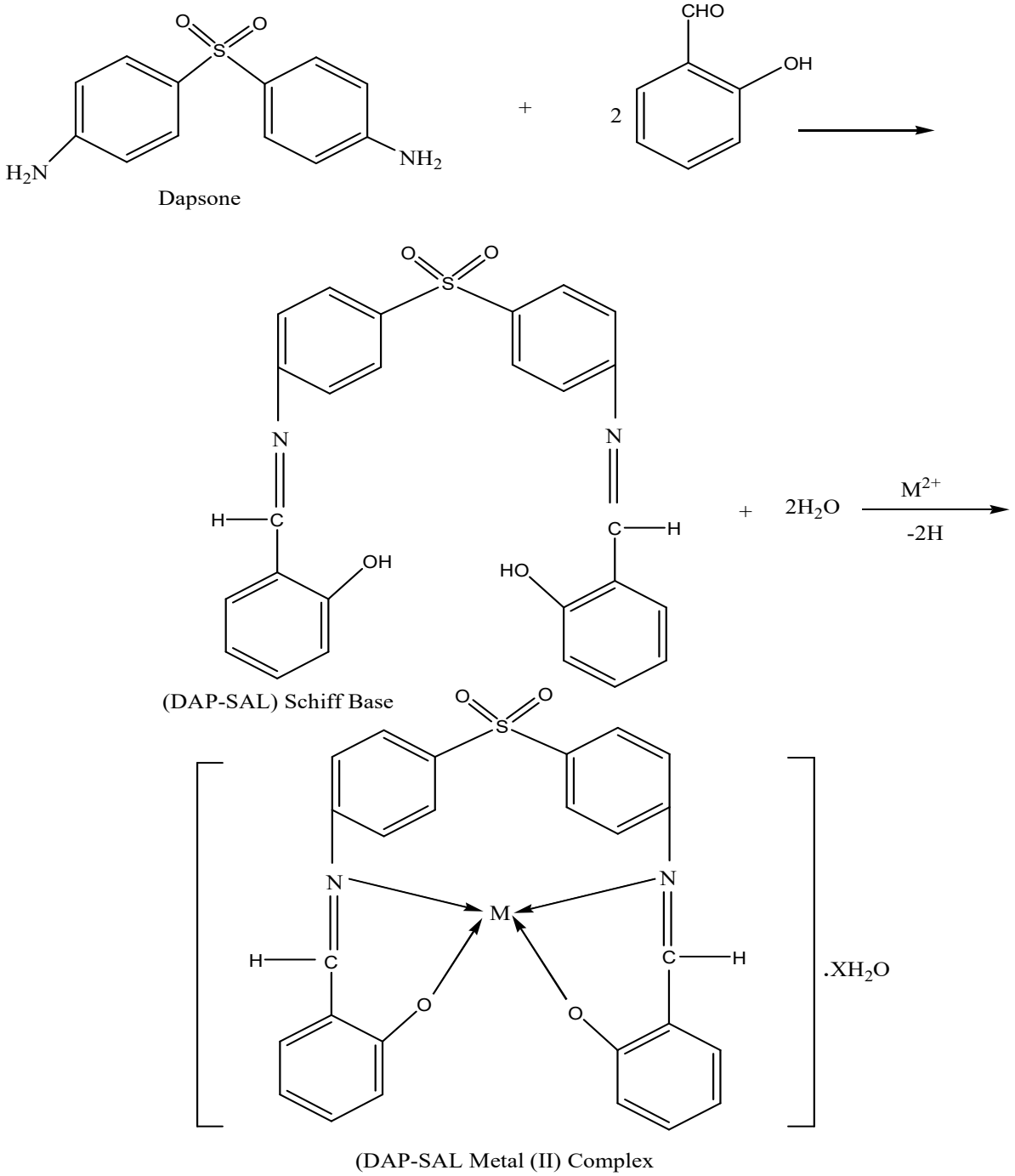
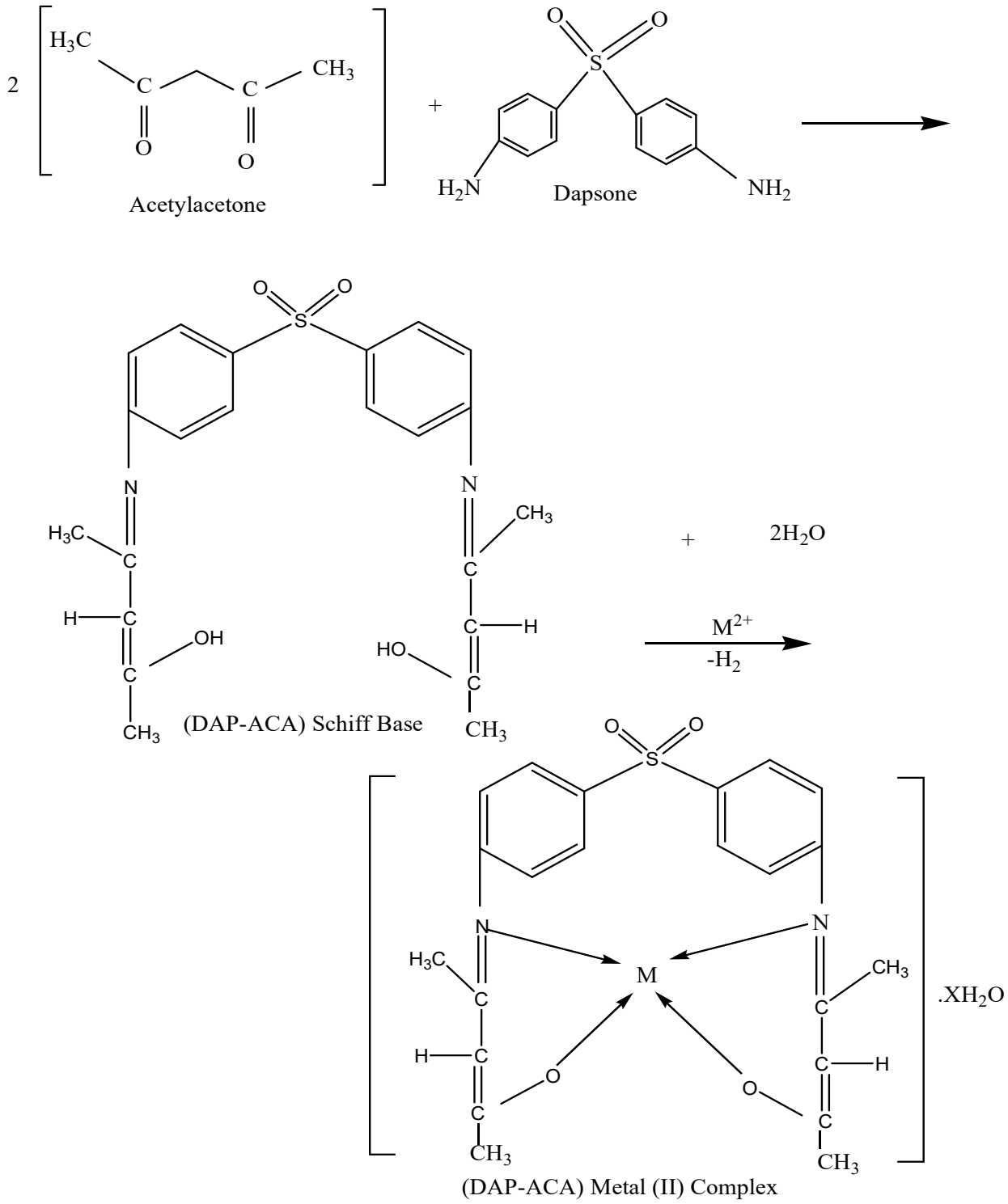


Fig. 5.4: Proposed Structure of (DAP-ACA) Metal (II) Complex
M= Mn, Cu, Ni, Co, Zn, Fe.



Scheme 5.1. Proposed Scheme for the Synthesis of (DAP-SAL) Schiff Base and Metal(II) Complex



Scheme 5.2: Proposed Scheme for the Synthesis of (DAP-ACA) Schiff Base and Metal(II) Complexes

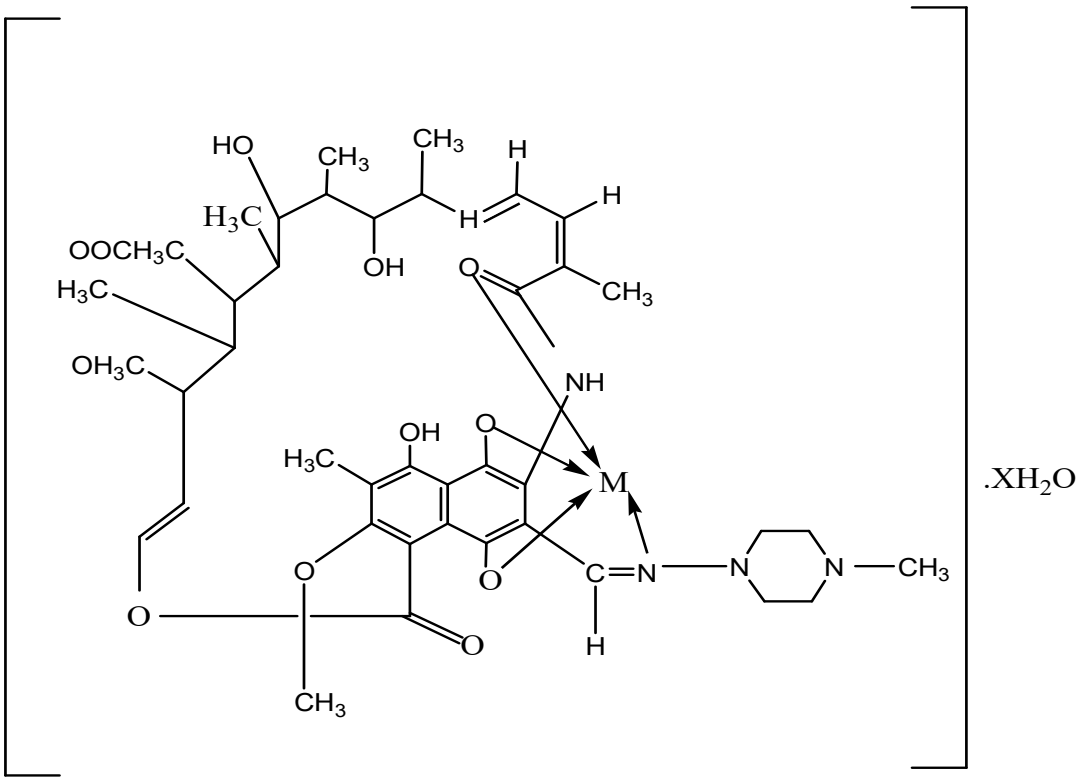


Fig. 5.5: Proposed Structure of Rifampicin Metal (II) Complex

Where M = Mn, Cu, Ni, Co, Zn Fe.

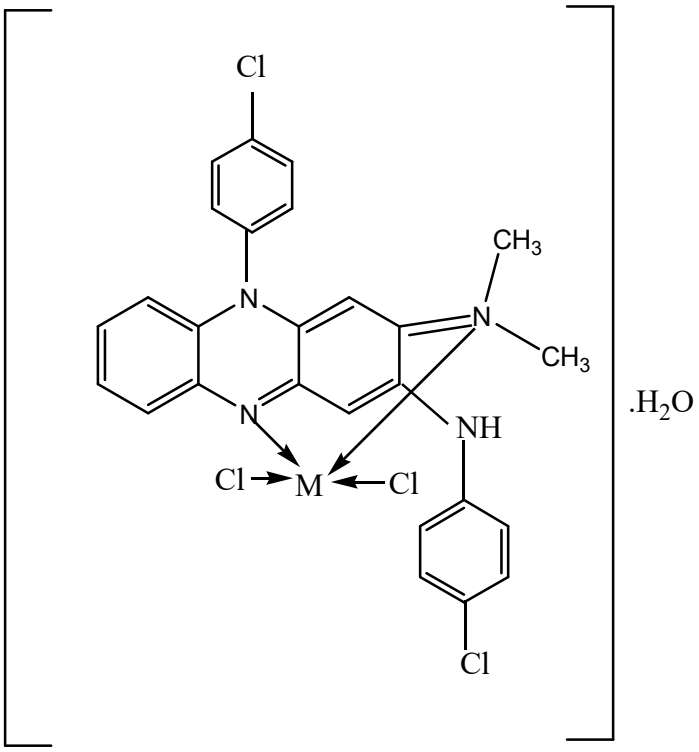


Fig. 5.6: Proposed Structure of Clofazimine Metal (II) Complex

Where M = Mn, Cu, Ni, Co, Zn Fe.

5.2 Recommendation

It is recommended that in order to ascertain the structures of the (DAP-SAL)SB and (DAP-ACA)SB ligands and all the metal (II) complexes synthesized further characterization involving the following methods should be carried out:

1) Proton NMR on the ligand.

2) X-ray diffraction analysis

3) ^{13}C-NMR

4) UV/Visible spectroscopy

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