SYNTHESIS, CHARACTERIZATION AND SCREENING OF SELECTED AMINE COMPLEXES OF THE ORGANOMETALLIC MOIETY [(η^5-C_5H_5)(CO)(PPh_3)Fe] FOR ANTIBACTERIAL ACTIVITY

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I56/CE/22223/2010

A Thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science (Chemistry) in the School of Pure and Applied Sciences of Kenyatta University.

October 2016
DECLARATION

I declare that this research thesis is my original work and has not been presented for a degree award in any other university.

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Approval by supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

I dedicated this thesis to my loving wife Wambui Mburu and my dear son Liam Levi Mburu for their endless love, support and encouragement in this challenging journey of academic pursuit.
ACKNOWLEDGEMENT

Most of all and in all honor I thank Almighty God. In total gratitude, acknowledge the following personalities who motivated, encouraged, gave me inspiration and sacrificed themselves to help me achieve this high education degree.

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God bless you all.
# LIST OF ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CP</td>
<td>Cyclopentadienyl</td>
</tr>
<tr>
<td>CO</td>
<td>Carbonyl</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EMB</td>
<td>Ethambutol</td>
</tr>
<tr>
<td>INH</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform Infra-red</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimum Bacterial Concentration</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>Multi-Drug Resistant Tuberculosis</td>
</tr>
<tr>
<td>XDR-TB</td>
<td>Extensively drug resistant Tuberculosis</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PPh$_3$</td>
<td>Triphenylphosphine</td>
</tr>
<tr>
<td>PZA</td>
<td>Pyrazinamide</td>
</tr>
<tr>
<td>RIF</td>
<td>Rifampin</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>HTMA</td>
<td>1,3,5,7-tetraazaadamantane</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4-diazabicyclo[2.2.2]octane</td>
</tr>
<tr>
<td>Conc.</td>
<td>Concentration</td>
</tr>
<tr>
<td>Bact’</td>
<td>Bacteria</td>
</tr>
<tr>
<td>GM</td>
<td>Gentamicin (positive control)</td>
</tr>
<tr>
<td>MHA</td>
<td>Mueller-Hinton agar</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

DECLARATION ........................................................................................................... ii

DEDICATION ............................................................................................................... iii

ACKNOWLEDGEMENT ............................................................................................ iv

LIST OF ABBREVIATIONS AND ACRONYMNS ....................................................... v

TABLE OF CONTENTS ........................................................................................... vi

LIST OF TABLES ...................................................................................................... viii

LIST OF FIGURES ................................................................................................... ix

ABSTRACT ............................................................................................................... x

CHAPTER ONE ......................................................................................................... 1

INTRODUCTION ....................................................................................................... 1

1.1 Background ....................................................................................................... 1

1.2 Statement of the Problem .................................................................................. 5

1.3 Hypotheses of the study .................................................................................... 6

1.4 Objectives of the study ..................................................................................... 6

1.4.1 General Objective ......................................................................................... 6

1.4.2 Specific Objective ......................................................................................... 7

1.4.3 Significance of the study ............................................................................. 7

CHAPTER TWO ......................................................................................................... 8

LITERATURE REVIEW ............................................................................................. 8

2.1 Transition metal complexes in medicine ............................................................ 8

2.2 Amine, carbonyl and PPh₃ ligands .................................................................... 12

2.3 Amine complexes containing \( [(η5-C₅H₅)(CO)₂Fe] \) ........................................... 16

2.4 Complexes containing the prochiral moiety \( [(η5-C₅H₅)(CO)(PPh₃)Fe] \) ........... 17

2.5 Determination of antibacterial activity ............................................................... 19

2.5.1 Disk diffusion ............................................................................................. 19

2.5.2 Agar dilution .............................................................................................. 20

2.5.3 Broth microdilution .................................................................................... 20

CHAPTER THREE ................................................................................................... 22
MATERIAL AND METHODS .................................................................................................................. 22
3.1 General .......................................................................................................................................... 22
3.2 Preparation of (η5-C3H5)Fe(CO)2I ............................................................................................... 23
3.3 Preparation of (η5-C3H5)Fe(CO)(PPh3)I .................................................................................... 24
3.4 Preparation of [(η5-C3H5)Fe(CO)(PPh3)(THF)]BF4 .................................................................... 25
3.4.1 Reaction of [(η5-C3H5)Fe(CO)(PPh3)(THF)]BF4 with 1,2-diaminoethane .................. 25
3.4.2 Reaction of [(η5-C3H5)Fe(CO)(PPh3)(THF)]BF4 with 1,3-diaminopropane ............. 26
3.4.3 Reaction of [(η5-C3H5)Fe(CO)(PPh3)(THF)]BF4 with 1,4-diaminobenzene ............ 27
3.5 Evaluation of antibacterial activity .............................................................................................. 28
3.5.1 Zones of inhibition ................................................................................................................. 28
3.5.2 Evaluation of minimum inhibitory concentrations (MICs) ............................................... 29
3.5.3 Evaluation of minimum bactericidal concentrations (MBCs) ........................................ 29
CHAPTER FOUR ................................................................................................................................. 30
RESULTS AND DISCUSSION ............................................................................................................... 30
4.1 Introduction .................................................................................................................................... 30
4.2 Synthesis of [(η5-C3H5)Fe(CO)(PPh3)(THF)]BF4 ....................................................................... 30
4.3 The α, ω-diaminoalkanes of [(η5-C3H5)Fe(CO)(PPh3)(THF)]BF4 complexes .................. 30
4.4 The paraphenelenediamine complex of [(η5-C3H5)Fe(CO)(PPh3)(THF)]BF4 ............... 33
4.5 Evaluation of antibacterial activity .............................................................................................. 35
4.5.1 Disc diffusion assay .............................................................................................................. 35
4.5.2 Minimum inhibitory concentrations ..................................................................................... 41
4.5.3 Determination of minimum bactericidal concentration (MBC) ....................................... 42
CONCLUSIONS AND RECOMMENDATIONS .................................................................................... 44
5.1 Introduction .................................................................................................................................... 44
5.2 Conclusions .................................................................................................................................. 44
5.3 Recommendations ....................................................................................................................... 45
REFERENCES ...................................................................................................................................... 46
APPENDICES ....................................................................................................................................... 55
6.1 Appendix 1: Colour of the complexes during precipitation ....................................................... 55
6.2 Appendix 2: Zones of inhibition evaluation for positive control ............................................ 56
6.3 Appendix 3: Evaluation of MBCs .............................................................................................. 57
### LIST OF TABLES

Table 3.1: Sensitivity Evaluation Table .................................................................28

Table 4.1: Mean Zones of Inhibition (mm) for complex-1........................................37

Table 4.2: Mean Zones of Inhibition (mm) for complex-2........................................39

Table 4.3: Mean Zones of Inhibition (mm) for complex-3.........................................40

Table 4.4: Significant MICs for the three complexes.................................................41

Table 4.5: Significant MBCs for the three complexes .................................................43
LIST OF FIGURES

FIGURE 1.1: Structures of first line TB drugs (A) Pyrazinamide (B) Isoniazid (C) Ethambutol (D) Rifampin ................................................. 5

FIGURE 2.1: Tamoxifen (A) and its ferrocene analogues, Ferrocifens (B)
(N = 2 – 5, 8) .................................................................................................................. 11

FIGURE 2.2: (A) Chloroquine and (B) the Ferrocene analogue, ferroquine .... 11

FIGURE 2.3: Structures of ruthenium complexes showing anticancer properties .............................................................................................................. 12

FIGURE 2.4: Gold (I) complexes .............................................................................................................................. 15

FIGURE 2.5: Structures of several Au (I) and Au(III) complexes containing triphenylphosphine and having anticancer activity ................. 16

FIGURE 4.1 Equation for the reparation of bridged diaminoalkane complexes ................................................................................................................. 31

FIGURE 4.2 General structure of the diaminoalkane complexes (n = 1 or 2) .................................................................................................................... 33

FIGURE 4.3 Ligand bridged complex of [{(H5-C3H3)(CO)(PPh3)Fe}2L](BF4)2 (L=1,4-diaminobenzene) ................................................................. 33

FIGURE 4.4 Zones of inhibition under different concentrations of complex-1 ... 35

FIGURE 4.5 Complex-1 against (A) P. aeruginosa (B) S. aureus (C) B. subtilis (D) E. coli ........................................................................................................... 36

FIGURE 4.6 Complex-2 against (A) P. aeruginosa (B) S. aureus (C) B. subtilis (D) E. coli ................................................................................................. 38

FIGURE 4.7 Complex-3 against (A) P. aeruginosa (B) S. aureus (C) B. subtilis (D) E. coli ................................................................................................. 40
ABSTRACT

The emergence of bacterial resistance to existing antibiotics and other drugs is a worldwide problem. New classes of antimicrobial compounds with complete new mode of action are therefore urgently needed to control the rise of the multidrug resistant pathogens. The objective of this study was to prepare, and characterize half-sandwich organometallic compounds of iron containing amine ligands with similar backbones as drug molecules used in the treatment of TB and to test their biological activities against selected bacteria. Half-sandwich organometallic complexes of the type \[
\left[ (\eta^5-C_5H_5)\text{Fe}(\text{CO})(\text{PPh}_3) \right]_2 \mu-(L) \] \(X_2\) (\(L = 1,2\)-diaminooethane, 1,3-diaminopropane and 1,4-diaminobenzene, \(X = \) univalent counter-anion such as BF\(_4^{-}\)) were synthesized by reacting two equivalents of \([ (\eta^5-C_5H_5)\text{Fe}(\text{CO})(\text{PPh}_3)(\text{THF}) ]\text{BF}_4^{\text{-}}\) with \(L\) in dichloromethane. The complexes were isolated by precipitation using hexane as air-stable compounds which were then recrystallized and characterized by FTIR and NMR, spectroscopy, melting point determinations and elemental analysis. The characterized compounds were then subjected to in vitro bioassays to determine antibacterial activities against selected bacteria by agar disc diffusion method against Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and Staphylococcus aureus with gentamicin as the positive control and 0.1% DMSO as the negative control. The organometallic complexes were found to be potent antibacterial agents. Their minimum inhibitory concentrations were determined through broth micro-dilution technique and found to inhibit the growth of the bacteria used when at as low concentrations as 6.25mg/ml for L = 1,2-diaminoethane, and 1,3-diaminopropane complexes. They indicated that the complexes (ethylenediamine and propylenediamine complexes), which mimicked ethambutol, were more active against all the bacteria used for this study. The paraphenelenediamine complex was found to have little activity against the bacteria used in this study.
CHAPTER ONE

INTRODUCTION

1.1 Background

The development of bacterial resistance to known antibiotics and other drugs used for treatment of other diseases caused by other microbes is a challenge in the whole world. The bacterial resistance is highly common in hospitals due to the heavy use of the antibiotics (Leeb, 2004). The antimicrobial resistance generated in hospitals ends up spreading outside these facilities because bacteria are able to share their resistance genes. This has resulted to emergence of difficult-to-treat forms of infectious diseases caused by drug-resistant pathogens on increasing rate. A good example is the extensively drug-resistant tuberculosis (XDR-TB). The XDR-TB is resistant the fundamental four first line anti-TB drugs. It is a rare type of multidrug-resistance tuberculosis (MDR-TB). The MDR-TB and XDR-TB take substantially longer period of time to treat than ordinary (drug-susceptible) TB. The second-line anti-TB drugs used for treatment of the two forms of TB are more expensive and have more side-effects compared with the first-line drugs used for the drug-susceptible TB (WHO, 2014).

During the last few decades, the number of antibiotics discovered and ready for introduction into the market has greatly declined and has not been able to meet the challenges posed by ailments caused by resistant pathogens to the existing antibiotics (Nicolaou et al., 2009; Saxena and Gomber, 2010; Silver, 2011). New classes of antibiotics with totally new modes of action are now needed to fight the virulence of the multi-drug resistant disease causing microorganisms.
In recent years, metal containing compounds (organometallics and/or Werner-type coordination complexes) with proper rational design have attracted great attention for medicinal applications (Hillard et al., 2006; James and Liu, 2006; Hartinger and Dyson, 2009; Manosroi et al., 2010). This is due to the essential reactivity of metal centers and the likelihood of easy access to several geometries by ligand variation (James and Liu, 2006; Jaouen, 2006; Metzler-Nolte, 2006; Sawle et al., 2006; Metzler-Nolte, 2007; Stepnicka, 2008; Salmain and Metzler-Nolte, 2008; Strohfdlt and Tacke, 2008; David and Meggers, 2008; Hartinger and Dyson, 2009; Patra et al., 2010). The serendipitous discovery of cis-platin, [Pt(NH$_3$)$_2$Cl$_2$], as a potent anticancer agent in 1965, (Rosenber, et al., 1965; Rosenberg et al., 1969) made medicinal inorganic chemistry a major area of research.

Organometallic modification of an existing drug or drug like ligand is an attractive method to reverse the drug resistance problem. The strategy could indeed result to drugs with a metal specific mode of action, which is not available for the purely organic known molecules. Under this strategy one of the most widely studied examples in the field of medicinal organometallic chemistry is the antimalarial ferroquine. (Biot et al., 1997; Delhaes et al., 2002; Biot and Dive, 2010). This ferrocenyl derivative of chloroquine have similar activity to that of the purely organic chloroquine compound against *P. falciparum* strain (HB3 5CQS), but ten times more active against chloroquine resistant P.falciparum (Dd2) (Biot et al., 1997; Delhaes et al., 2002).

It is hard for microorganisms to acquire resistance against drugs with multiple targets, principally, if resistance is linked to the target modification. Silver 2003 has
reported silver based antimicrobials which have remained in use for a long time without resistance problem (Silver, 2003). Such drugs with multiple targets may be obtained with rational design of organometallics by structure modification of known drugs. There are substantial progresses in the development of organometallic pharmaceuticals as anticancer and anti-malarial compounds (Jaouen and Dyson, 2007; Peacock and Sadler, 2008; Hartinger and Dyson, 2009; Jaouen and Metzler-Nolte, 2010; Gasser and Metzler-Nolte 2011; Gasser and Metzler-Nolte, 2012;). In contrast, the development of organometallic compounds for use as antibacterial drugs is not greatly explored. Antibiotics with a metal specific mode of action may help reverse bacterial resistance, hence an important area of scientific research. (Eke and Abubakar, 2015).

In the case of tuberculosis (TB), all drugs that have been used are organic molecules, but there are reports of successful introduction of ferrocene into the structures of known organic molecules resulting in organometallic compounds with encouraging efficacy and activity (Razafimahefa et al., 2005; Ralambomanana et al., 2008). Some of the compounds studied by these groups were insoluble in water and organic solvents to allow, screening for bioactivity.

This study was motivated by the literature reports of the stability and water-solubility of various alkyl and heterocyclic amine complexes of the half-sandwich moiety \([\eta^5\text-C_5\text-R_5](\text{CO})_2\text{Fe}\) (R = H, CH$_3$) (M’thiruaine et al., 2011; M’thiruaine et al., 2012). These half sandwich complexes are reported to be soluble both in organic solvents and in water and can be recovered intact from water without significant loss. This is a necessary requirement for any drug molecule. Furthermore, IR, NMR,
and Mass spectrometer spectra have been recorded in water indicating that the compounds do not change once dissolved in water (M’thiruaine et al., 2012). These compounds are more likely to perform better than their ferrocene analogues since they are both lipophilic and sufficiently hydrophilic in addition to having the metal not fully sandwiched.

Presently, TB chemotherapy consists of a combination of the first line drugs, isoniazid (INH), pyrazinamide (PZA), rifampin (RIF), and ethambutol (EMB), (fig.1.1), given for a period of six months. Ethambutol has a very simple chemical structure, very low toxicity and a surprisingly modest efficacy against *M. tuberculosis* (Wilkinson et al., 1962). There are reports on the structural modification of EMB to improve its efficacy which have mainly focused on the diamine or diaminoolcohol (Sherpherd et al., 1966; Lee et al., 2003; Bogatcheva et al., 2006; Tripathi et al., 2006; Faugeroux et al., 2007; Yendapally and Lee, 2008). In this study the chiral carbon centres on ethambutol were replaced with chiral metal centres and the bioactivity of the resultant complexes studied. To achieve this ethylenediamine and related ligands were reacted with the chiral organoiron complex $[\eta^5$-C$_5$H$_5$(CO)(PPh$_3$)Fe(THF)] under appropriate conditions. The resultant compounds were chiral (metal-based chirality) and closely related to the drug ethambutol (EMB) but different from the ones referred to earlier.
Figure 1.1: Structures of first line TB drugs (a) Pyrazinamide (b) Isoniazid (c) Ethambutol (d) Rifampin

1.2 Statement of the Problem

Mycobacteria have developed resistance towards the first line anti-TB drugs and there is therefore need for new or re-engineered old drugs that can reverse the resistance. It is now established that ferrocene enhances activity and in some cases reverses resistance developed by disease causing pathogens when incorporated into known drug molecular structures or when it forms a part of certain organic ligands. Most of these compounds are however, more lipophilic than hydrophilic and the metal is less available to participate in biochemical transformations. It has also been reported that amine complexes of the half sandwich organoiron moiety [(η^5-C_5R_5)(CO)_2Fe] (R = H, CH_3) are soluble and stable in both water and organic
solvents. It is however, not known whether these compounds have any biological activity. Introduction of PPh₃ into the coordination sphere of the organoiron moiety, [(η⁵-C₅R₅)(CO)₂Fe] (R = H, CH₃), enhances stability and introduces metal-based chirality which is also a desirable property in most drug molecules. It is also not clear whether replacement of one CO ligand with the more lipophilic PPh₃ will have any effect on the solubility and stability of the target compounds in water and in organic solvents. It was, therefore, of interest to prepare and characterize complexes of the prochiral moiety [(η⁵-C₅H₅)(CO)(PPh₃)Fe] containing bidentate and monodentate amines and to determine their solubility and stability in water and their antibacterial activity.

1.3 Hypotheses of the study

i). Amine complexes of the organometallic moiety [(η⁵-C₅H₅)(CO)PPh₃Fe] are stable and can be isolated and characterized.

ii). Amine complexes of the organometallic moiety [(η⁵-C₅H₅)(CO)PPh₃Fe] are sufficiently hydrophilic and lipophilic to be tested for activity against specific bacterial strains.

1.4 Objectives of the study

1.4.1 General Objective

To prepare half sandwich organometallic complexes of selected amines and determine their activities against selected bacteria strains.
1.4.2 Specific Objective

i) To synthesize the starting complexes \([(\eta^5-C_5H_5)(CO)(PPh_3)Fe(THF)]BF_4\)

ii) To react \([(\eta^5-C_5H_5)(CO)(PPh_3)Fe(THF)]BF_4\) with ethylenediamine, propylenediamine, and 1,4-diaminobenzene and characterize isolated products using FTIR, NMR, elemental analysis.

iii) To determine the activity of the characterized amine complexes against selected bacteria.

1.4.3 Significance of the study

This research will provide information on the stability and solubility of the complexes \([(\eta^5-C_5H_5)(CO)(PPh_3)Fe(L)]BF_4\) (L = mono- or disubstituted alkyl amine). It will also provide information on the antibacterial activity of the isolated and characterized complexes and form a basis for further studies on antimycobacterial activity of those found to be active. If any antimycobacterial activity is found the particular complex may be subjected to further studies and possible development to a drug.
CHAPTER TWO

LITERATURE REVIEW

2.1 Transition metal complexes in medicine

Ongoing research in the medical field is focused at designing of pharmaceuticals which are potent against a wide range of disease causing pathogens with lesser side effects and able to overcome drug resistance (Patra and Metzler-Nolte, 2012; Eke and Abubakar, 2015).

Most compounds used as medicine are organic in nature. Little attention has been given to metalo-drugs as compared to organic compounds in the field of medicine and pharmaceuticals. However, many organic compounds used in medicine require traces of metal ions directly or indirectly for their proper functioning in the body. The well-being of our bodies is highly determined by the state of the metal ions and the complexes formed when they coordinate with biomolecules. Metals like Fe, Ca, Cu, Zn, Ni, and Mn are known to be essential in the body especially as metal cofactors for proper functioning of metalloenzymes in biochemical reactions in the body (Kwiatek, et al, 2002; Kastenholz, 2006; Kastenholz, 2007).

The hemoglobin, chlorophyll and Coenzyme B12, are some of the examples where metal ions play a major role in the biological systems. It is approximated that 0.03% of the human body by weight is composed of metals or metal ions. Low metal ion concentrations may be harmful for the body health. There are reports that low concentrations Cd, Cr, Ti, V, Cu, Se and Zn have been cited in the cancerous parts of the kidney compared to the non-cancerous parts (Kwiatek, 2002). Some biomolecules having atoms like N, O, S and P etc. may form coordination bonds
with metal ions in specific conditions to form new compounds. Biological properties of these ligands as well as metal moieties in the body are highly affected by Chelation and in many cases it causes synergistic effects of both metal ions and ligand which can be positive or negative to the well-being of the body. (Singh and Bharti, 2009).

A growing interest on metal complexes as pharmaceuticals for use as diagnostic/chemotherapeutic agents has been witnessed in medicinal chemistry, a field initially dominated by organic compounds and natural products (Rafique et al., 2010). Transition metal complexes (inorganic coordination and organometallic) have played a critical role in the advancement of modern chemotherapy. They have been used for treatment of many ailments such as anticancer metal complexes, anti-diabetic agents, antimanic agents, anti-inflammatory agents, antifungal agents, anti-hypertensive agents, antibacterial agents, vitamin metal complexes and antimalarial (Nakai et al., 2005; Singh and Bharti 2009). For instance, as anticancer agents, platinum drugs (Cisplatin or carboplatin) are most commonly used in treatment of cancer for 50% of all cancer chemotherapies for the past over 30 years.

Complexes of platinum, ruthenium as well as lanthanum and gallium have undergone preclinical and clinical studies. However, their severe toxicity and side effects remains a great challenge. There are ongoing efforts worldwide aiming to identify novel metal-based substances with less side effects and new modes of action. The focus has been in varying the nature and types of ligands used (Top et al., 2001; Top et al., 2003; Allardyce et al., 2005).
Organometallic complexes i.e. transition metal complexes where there is at least one metal-carbon bond are fairly new in this application but are quite promising (Top et al., 2001; Top et al., 2003; Allardyce et al., 2005). Exploits in bioorganometallic chemistry in the past three decades have renewed hope in the design of drugs that will combat ailments, restore potency by overcoming drug resistance which have bedeviled well-known organic drugs, and of course, tackling the issue of toxicity (Eke and Abubakar, 2015).

Compared to organic compounds, organometallic compounds are attractive because they have a great structural range, more diverse stereochemistry and by rational ligand design, provide control over major kinetic properties. They are also kinetically stable and relatively soluble in organic solvents and usually the metal atoms is in low oxidation state. (Hartinger and Dyson, 2009; Gasser et al., 2011). Because of these crucial differences compared to “classical metal complexes”, organometallic compounds offer many opportunities in the development of new classes of medicinal agents, potentially with new metal specific modes of action. Most of the organometallic compounds in use as medicine and under clinical tests have been obtained by introducing an organometallic moiety to an already known organic drug structure or drug like ligands (Nguyen et al., 2007).

Nguyen and co-workers demonstrated that the cytotoxicity of tamoxifen can be improved by attaching a ferrocene to the parent organic molecule tamoxifen (Nguyen et al., 2007). Replacing tamoxifen’s phenyl ring with ferrocene (Fig. 2.1 A and B) resulted in compounds (Ferrocifens) with strong anticancer effects on both
hormone dependant (Era+) and hormone independent breast cancer cells (Top et al., 2001; Top et al., 2003; Jaouen et al., 2004; Vessi'eres et al., 2006).

Figure 2.1: Tamoxifen (a) and its ferrocene analogues, Ferrocifens (b) (n = 2 – 5, 8)

The ferrocenyl derivative of chloroquine (Fig. 2.2) has similar activity to that of the organic compound against the chloroquine sensitive P. falciparum strain (HB3 5CQS), while comparing the activity of ferroquine and chloroquine against chloroquine resistant P. falciparum (Dd2) showed that the ferroquine was ten times more active than the chloroquine (Biot et al., 1997; Delhaes et al., 2002).

Figure 2.2: (a) Chloroquine and (b) The Ferrocene analogue, ferroquine

Ruthenium complexes with the metal in the oxidation state 2+ or 3+ have displayed anticancer activity, especially against metastatic cancers (Clarke, 2003). The Ru(III) complex Natrans [Ru(Im)(Me2SO)Cl4] (NAMI) and (ImH)trans
[Ru(Im)(Me₂SO)Cl₄] (NAMI-A) its analogues are currently in a clinical trial. Several Ru(II) arene compounds of the type \([\text{Ru}^{II}(\eta^6\text{arene})(en)X]^+\) (\(X = \text{Cl or I, arene} = p\text{-cumene or biphenyl, en = ethylenediamine or N-ethylethlenediamine,}\) (Figure 2.3) were demonstrated to inhibit the proliferation of human ovarian cancer cells (Morris et al., 2001).

![Figure 2.3](image)

Figure 2.3: Structures of ruthenium complexes showing anticancer properties.

Other well-known organometallic compounds used in medicine include, the arsenic (As) anticancer agent arsenic trioxide, the orally active gold (Au) anti-rheumatoid agent auranofin, selenium (Se) anti-inflammatory agents ebselen, anti-manic depressive agent lithium carbonate, anti-ulcer agents scrafate and polaprezinc (Lippard and Beng, 1994; Singh and Bharti, 2009). There are very many other examples of organometallic compounds already in clinical tests to overcome the problem resistance of disease causing pathogens and adverse side effects.

2.2 Amine, carbonyl and \(\text{PPh}_3\) ligands

These ligands are best known for their applications in industrial catalysis and very little for medicinal or biological roles when coordinated to metals. Carbon and nitrogen on the other hand are found in most of the organic drug structures
(Kaufmann and Krise, 2007) and proteins in biological systems. The amine group is found in many molecules found in living organisms or products obtained from them which are developed in the course of growth of the living organism. The antimicrobial activities of complexes of nitrogen-containing ligands have been studied widely in the recent past. For example, the diaminoalkanes (Tripathi et al., 2005; Vergara et al., 2009), ferrocenyl diamine (Razafimahefa et al., 2005) and ferrocenyl diaminoalcohol (Ralambomanana et al., 2008) complexes have shown activity against *Mycobacterium tuberculosis* H37Rv. Some like ferrocene derivative of tamoxifen have shown anti-cancer effects on both hormone dependant (Era+) and hormone independent breast cancer cells (Top et al., 2001; Top et al., 2003; Jaouen et al., 2004; Vessières et al., 2006).

The carbonyl, CO, is an important ligand due to its ability to accept electrons into π*-molecular orbitals. This characteristic stabilizes transition metal elements in low oxidation states, an important property of transition metal based compounds used as drugs. Metal carbonyls have attracted a lot of interest as prospective CO-releasing molecules. The metal carbonyls such as η⁴-(4-bromo-6-methyl-2-pyrone)tricarbonyliron (0) (Sawle et al., 2006), the tricarbonyldichlororuthenium (II) dimer, dimanganesedecacarbonyl and tricarbonylchloro(glycinato)ruthenium (II) (Motterlini et al., 2002) have been shown to improve vasodilatory, cardioprotective and anti-inflammatory activities. CO delivered through organometallic CO-releasing molecules has been shown to cause rapid death of disease causing bacteria such as *Escherichia Coli* and *Staphylococcus Aureus* (Nobre et al., 2007; Davidge et al., 2009). The increased activity of CO against
anaerobic bacteria has been attributed to preferential binding of CO to the iron(II) of the hemoglobin protein (Nobre et al., 2007).

Metal carbonyl compounds have characteristic $\nu$CO bands appearing in the 1800-2200 cm$^{-1}$ regions in infrared (IR) where few other functional groups absorb. Based on this, the interaction of iron carbonyl complexes with the nitrogen-containing ligands can easily be followed by IR spectroscopy during research and the development of the organometallic chemistry, (M’thiruaine et al., 2012).

Triphenylphosphine, PPh$_3$ is extensively applied in the preparation of organic and organometallic compounds. It coordinates well to most transition metals. It has many applications such as in homogenous catalysis. For example, Walter Reppel used [NiBr$_2$PPh$_3$] for the synthesis of acrylate esters from alkynes, carbon monoxide and alcohols. Hydroformylation catalyst, RhH(PPh$_3$)(CO), by Wilkinson is much studied (Reppe and Schweckendiek, 1984). Being a strongly binding ligand it does not easily de-coordinate from the metal complex. It imparts chirality to the metal center in addition to conferring stability to the complexes. There are very few reports of use Triphenylphosphine in drug synthesis. However, Krˇikavova´ and coworkers have reported a series of gold(I) complexes (Figure 2.4) involving Triphenylphosphine (PPh$_3$) and one N-donor ligand derived from deprotonated mono- or disubstituted hypoxanthine and their in vitro evaluations of anticancer and anti-inflammatory activities with some of the complexes showing higher activity against the employed cancer cells, as compared with cisplatin (Krˇikavova et al., 2014).
In addition, other phosphines-containing Gold complexes have been shown to be active as antitumor agents (McKeage et al., 2002). Tetrahedral Au(I) complexes with 1,2-bis (diphenylphosphino)ethane and 1,2-bis(dipyridylphosphino)ethane ligands (Figure 2.5a) display a wide range of anticancer activity in vivo against some cisplatin resistant cell by altering mitochondrial functions and inhibiting protein synthesis. This is in contrast to cisplatin which targets the DNA. Water soluble tetrakis ((tris(hydroxymethyl))phosphine) gold(I) complex (Figure 2.5b) was reported to be active against several cancer cell lines. An Au (I) complex having both monophosphine and diphosphine ligands (Figure 2.5c) have been reported to be highly active against several tumour cell lines. An in vitro cytotoxicity study of, [Au(bipy)-(OH)₂]PF₆ and [Au(bipy⁻⁻⁻(H)(OH))PF₆ (Figure 2.5d) demonstrated a Low cisplatin cross resistance (Marcon et al., 2002) was observed. The two complexes are quite stable under physiological conditions, with [Au(bipy⁻⁻⁻(H)(OH))[PF₆] being resistant to sodium ascorbate reduction. Mechanistically their primary target for their antitumor activity is not the DNA (Christiana and Stephen, 2003).
2.3 Amine complexes containing \([\eta^5\text-C_5\text{H}_5](\text{CO})_2\text{Fe}\) 

Iron(II) amine complexes incorporating π- acid ligands, such as CO in conjunction with pentadienyl ligands such as cyclopentadienyl and pentamethylcyclopentadienyl have been reported (M'thiruane et al., 2010; M’thiruane et al., 2012). The iron dimer \([\text{CpFe(CO)}_2]_2\) is also known to react with amines such as ethylamine, butylamine, cyclohexylamine, piperidine and morpholine, as well as bidentate ligands such as 1,10 phenanthroline and 2,2 bipyridyl to give monosubstituted \([\eta^5\text-C_5\text{H}_5])_2\text{Fe}_2(\text{CO})_3\text{L}\) (L = amine), while the reaction of the iodo complex \([\text{Cp}_2\text{Fe}_2(\text{CO})_3\text{I}]\) with these amines results in the ionic complex \([\eta^5-\text-C_5\text{H}_5])_2\text{Fe}_2(\text{CO})_3\text{L}\) (L = amine).
C₅H₅(CO)₂FeL⁺ (Tripathi et al., 1976). The cationic complexes [(η⁵-C₅H₅)(CO)₂Fe(NHR₂)]PF₆ (R = Me, Et, SiMe₃) have also been synthesized by treating [(η⁵-C₅H₅)(CO)₂FeCl] with NHR₂. The cyclopentadienyliron dicarbonyl N-heterocyclic complexes mostly reported include those of pyridine (Schumann et al., 1991), pyrrole (Martin et al., 1996; Martin and Hanks, 1997; Powell et al., 1997) and imidazole (Nesrneyanov et al., 1977).

Interactions of the cation [(η⁵-C₅H₅)(CO)₂Fe]⁺ with various nitrile and pyridine derivatives have been studied by Schumann and co-authors (Schumann et al., 1991). They found that oxidative cleavage of [(η⁵-C₅H₅) Fe (CO)₂]₂ by [(η⁵-C₅H₅)₂Fe]BF₄ in the presence of excess L (L = monosubstituted nitrile or pyridine) yields cationic complexes [(η⁵-C₅H₅) (CO)₂FeL]BF₄. However, when the oxidation was done in the presence of the strong nitrogen donor, NH₆R₃-n (n= 0-3; R= CH₃, C₆H₅) all CO groups and C₅H₅ were eliminated (Schumann et al., 1991). The aniline complex [Cp(CO)₂Fe(NH₂Ph)]⁺ is also known and has been obtained from the reaction between [Cp(CO)₂Fe(THF)]⁺ and aniline (Reger and Coleman, 1977).

2.4 Complexes containing the prochiral moiety [(η⁵-C₅H₅)(CO)(PPh₃)Fe]

There have been few reports on the amine complexes of the prochiral [(η⁵-C₅H₅)(CO)(PPh₃)Fe] moiety. The fragment [(η⁵-C₅H₅)(CO)(PPh₃)Fe] is an effective chiral auxiliary for a range of reactions attached to acyl ligands (Bashiardes and Davies, 1988). It has been established that the fragment [(η⁵-C₅H₅)(CO)(PPh₃)Fe] is an important prochiral auxiliary for asymmetric organic synthesis (Davies et al., 1987; Davies, 1988, Davies, 1990). The iron chiral auxiliary induces high stereoselectivity in the reactions of a great range of attached ligands. For example,
deprotonation of the acyl ligand in \( ((\eta^5-C_5H_5)Fe(CO)(PPh_3)COCH_2R) \) produces the E enolate (Fe trans to R) which undergoes highly stereoselective alkylation reactions. The enolates need a strong base (butyllithium) and very low temperature (-78°C) (Baird, et al. 1984; Davies and Maberly, 1985). As opposed to the methoxycarbene salt \( ((\eta^5-C_5H_5)Fe(CO)(PPh_3)\{C(OMe)-(CH_2R)\}BF_4 \) may be deprotonated at moderate temperature with a mild base such as methoxide to form the corresponding Z-enol ether (Fe cis to R). Alkylation of this complex (Fe cis to R) is highly stereo selective and demethylation of the therefore formed methoxycarbene cation with iodine forms the corresponding elaborated acyl complex (Steidle and Diener, 2011). The two sequences described above are stereo complementaly given the same face selectivity induced by the iron chiral auxiliary.

Davies G, and co-workers have done the conformational analysis on the chiral complex \( ((\eta^5-C_5H_5)Fe(CO)_2(PPh_3)COMe) \) and two fluorinated derivatives \( [\text{CpFe(CO)}_2(PPh_3)PC_{6}H_{3}F_{2}COMe] \) and \( [\text{CpFe(CO)}_2[P(C_{6}F_{5})ph_2COMe] \) to characterize the dynamic processes of the PPh_3 ligand (Sawle et al., 2005). The phosphine complex \( ((\eta^5-C_5H_5)Fe(CO)(PPh_3)(Et_2O)][BAr_4] \) (Ar' = 3,5-(CF_3)_2C_6H_4) has been synthesized by protonation of \( [((\eta^5-C_5H_5)Fe(CO)(PPh_3)CH_3] \) using H(OEt_2)Bar' at -80°C (Shvo and Hazun, 1975).

There have been very few reports on amine complexes of the moiety \( ((\eta^5-C_5H_5)Fe(CO)(PPh_3)] \) in which amino alkane is coordinated to the metal via nitrogen. The only closest complexes in the literature are those containing the \( \eta^1-N\)-imidato(1-) ligands (Allardyce et al., 2005). There have been no studies on the bioactivity of these class of complexes nor the amine complexes of the \( (\eta^5-C_5H_5)(CO)_2Fe^{2+} \)
moiety. This study proposes to test the antibacterial activity of the isolated compounds against selected bacteria.

2.5 Determination of antibacterial activity

Agar dilution, broth microdilution, and disk diffusion may be used to determine the antibacterial activity. They are standard methods used for measuring the *in vitro* activity of compounds against microbes (Jiang, 2011). Antimicrobial susceptibility tests are influenced by, the microorganism tested and the degree of solubility of the test compounds (Valgas et al., 2007). The most applied susceptibility test method is the disk diffusion (Kim and Kim, 2007; Mayachiew et al., 2010).

2.5.1 Disk diffusion

Bioactivity of a big number of antimicrobial compounds can be investigated using this method concurrently. The bacterial to be tested is inoculated onto the whole surface of a Mueller Hinton agar plate with sterile cotton tipped swab to form a uniform distribution. Paper disks (6mm diameter) soaked in the test compound solution is placed on the same surface using a sterilised pair of forceps. The plates are incubated anaerobically and zones measured using a ruler. The bigger the diameter the more active the compound against that microbe. The greatest demerit is the inability to produce the minimum concentration value and the difficulty to carry the susceptibility tests involving demanding and slow growing microbes (Wilkins and Thiel, 1973; Dickert *et al.*, 1981) Furthermore, both diffusion rate of the test compound and the growth of the growth of the lawn target species are sensitive to physical and chemical factors like temperature, pH and water activity. Moreover, in contrast to substances used in clinical settings as antimicrobials, there lacks
standards methods of interpreting zones of inhibition to support natural and new compounds antimicrobial susceptibility testing. Disk diffusion method consumes a lot of time and it is also labor intensive.

2.5.2 Agar dilution

This is a quantitative bioactivity testing method because minimum inhibitory concentration values are obtained. Two fold serial dilutions of the compound being tested are made in MHA medium and then bacterial suspensions are inoculated on the MHA using a cathra replicator with 1 mm pins. This method allows simultaneous test of the susceptibility of a number of bacteria in one plate. The method also allows test of the fastidious organisms because the agar with supplements is able to adequately support growth of bacteria. The method is not commonly used because it is both time demanding and labor intensive (Jiang, 2009; Klancnik et al., 2010).

2.5.3 Broth microdilution

It is quantitative method commonly used in clinical laboratories. Susceptibility panel in 96 well microtiter plates contains different concentrations of the test compound. Standardized numbers of bacteria are the inoculated into microtiter wells and incubated for 24 hours at 35 °c. The Minimum Inhibitory Concentration values are observed as the lowest concentrations where there is no growth of the test microbes. This method is widely used, allowing simultaneous testing of many antimicrobials with easy especially when microtiter trays used are commercially prepared. Compared with the agar methods, this method is not labor intensive. The demerits associated with this method include lack of or poor growth of many anaerobic microbes and inconsistency in results for fastidious anaerobes resulting from too
much exposure to oxygen during the preparations (Jiang, 2009; Klancnik et al., 2010). The bacterial strains selected for this study were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. 
CHAPTER THREE
MATERIAL AND METHODS

3.1 General
All operations were conducted under inert atmosphere (dry nitrogen) using standard Schlenk line techniques. Nitrogen gas was dried by bubbling concentrated sulphuric acid and passing through columns of phosphorus (V) oxide supported on pumice stones. Reagent grade tetrahydrofuran (THF) and diethyl ether were dried by distilling from sodium benzophenone and stored over sodium wire. Acetone and hexane were distilled from anhydrous CaCl₂ and used immediately when required. Dichloromethane was distilled from phosphorus pentoxide and used immediately. The other chemical reagents were obtained from the suppliers shown in parentheses: 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobenzene, silver tetrafluoroborate, dicarbonylcyclopentadienyliron(II) dimer (Aldrich), iodine (Merck), sodium metal, anhydrous sodium sulphate, sodium thiosulphate, petroleum ether (60-80°C), anhydrous calcium chloride and toluene (Unilab) were used as supplied.

Melting points were determined using Gallenkamp (sanyo) melting point apparatus. Infrared spectra were recorded using an ATR Perkin–Elmer Spectrum 100 spectrophotometer between 4000 and 400 cm⁻¹, in the solid state. NMR spectra were recorded on Bruker topspin 400 and 600 MHz spectrometers. The deuterated solvents CDCl₃ (Aldrich, 99.8%) and DMSO (Merck, 99%), were used as purchased. Solutions for NMR spectroscopy were prepared under nitrogen using nitrogen-saturated solvents. The precursor, [(η⁵-C₅H₅)Fe(CO)₂]₂, was used as purchased. The precursors (η⁵-C₅H₅)Fe(CO)I, (η⁵-C₅H₅)Fe(CO)(PPh₃)I and [(η⁵-C₅H₅)Fe(CO)]
(PPh₃)(THF)BF₄, were prepared by the literature methods (Aris and Brown., 1974; Reger and Coleman, 1977, Munyeza., et al., 2007).

### 3.2 Preparation of \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2\text{l}\)

The complex \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2\text{l}\) (4) was prepared from \([\((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2\text{l}\)]_2\) and I₂ in dichloromethane as described in literature (King and Stone, 1963). A clean dry Schlenk tube containing a magnetic bar and fitted with a condenser was flushed with dry nitrogen for 3 minutes. Iodine (5g, 19.7 mmol), \([\((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2\text{l}\)]_2\) (500 mg, 1.41 mmol), and dry CH₂Cl₂ (15 ml) were added. The mixture was stirred at 75-80°C on an oil bath for 30 min, after which the reaction mixtures was allowed to cool to room temperature. Sodium thiosulfate solution (1.5 g in 12 ml of distilled water) was added to the mixture and shaken to remove any excess iodine. The black dichloromethane layer was transferred by canula into a small beaker and dried with anhydrous sodium sulfate.

The mixture was filtered into a round-bottomed flask (50 ml). The solvent was reduced using rotatory evaporator to about 3 ml. A small volume of petroleum ether (60-80 °C) added to precipitate a dark blue product. The crystals were isolated by vacuum filtration, washed with small portions of petroleum ether, and allowed to air dry. FTIR (solid state): ν (CO) 2034, 1976 cm⁻¹ agrees with literature reports (Munyaneza et al. 2007). Yield 5.1g, 85%, mp. 114-116°C (dec). This product was of sufficient purity as confirmed by the melting points similar to what is in literature (Munyaneza et al. 2007).
3.3 Preparation of \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}(\text{PPh}_3)\text{I}\)

The complex \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}(\text{PPh}_3)\text{I}\) was prepared from \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2\text{I}\) and Triphenylphosphine in refluxing toluene as described in literature (Munyeza A. et al., 2007). 75 mL of toluene was purged with N\(_2\) for at least 30 min. A clean dry Schlenk tube containing a magnetic bar and fitted with a reflux condenser was charged with 50 mL of the N\(_2\)-purged toluene solution of 3.0g, 9.8596 mmol of the \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2\text{I}\) and one equivalent of Triphenylphosphine. This mixture was refluxed at 110\(^\circ\)C on an oil bath until the original brown color changed to a deep green solution (at least 2 h). The hot solution was filtered under reduced pressure through a Buchner funnel yielding a solid product \([((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2\text{PPh}_3)\text{I}\) and a filtrate non-salt product \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}(\text{PPh}_3)\text{I}\). The non-salt product was the complex of interest in this study. The salt product \([((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}_2(\text{PPh}_3)\text{I}\) insoluble in toluene formed in a very small percentage 0.3g, 6% as expected from literature (Munyeza et al., 2007).

The solvent was removed from the filtrate containing \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}(\text{PPh}_3)\text{I}\) with a rotary evaporator at room temperature to obtain a green residue. The residue was re-dissolved in a small volume (15-25mL) of methylene chloride and the solution filtered with a Buchner funnel. About 5 mL of heptane was added to the filtrate to precipitate \((\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)}(\text{PPh}_3)\text{I}\) and the solvent was slowly evaporated under reduced pressure. The resulting crystals were washed in a filter funnel with two 10 mL portions of petroleum ether and dried in a vacuum desiccator, yield 1.28g 80%. This product showed a strong FTIR (solid state) band \(\nu (\text{CO})\) at 1951 cm\(^{-1}\) which agrees well with literature reports for the same compound (Aris and Brown, 1974; Munyeza et al., 2007;).
3.4 Preparation of $[(\eta^5-C_5H_5)Fe(CO)(PPh_3)(THF)]BF_4$

The complex $[(\eta^5-C_5H_5)Fe(CO)(PPh_3)(THF)]BF_4$ was prepared as described by Reger and Coleman (Reger and Coleman, 1977) from $(\eta^5-C_5H_5)Fe(CO)(PPh_3)I$ and AgBF$_4$ in dry, degased tetrahydrofuran. AgBF$_4$ (0.40g, 2.05mmol) was placed in a clean dry Schlenk tube containing a magnetic stirrer bar and dried for six hours under reduced pressure at 80°C. The AgBF$_4$ was allowed to cool under nitrogen and $(\eta^5-C_5H_5)Fe(CO)(PPh_3)I$ (1.0 g, 1.855 mmol) was added followed by freshly distilled tetrahydrofuran (13ml) under dry nitrogen. The mixture was stirred for 15 min. The solvent was evaporated, and the residue extracted with dichloromethane (25ml) and filtered using a cannula. The filtrate was concentrated to 10 ml, 20 ml of hexane added to precipitate green crystals. The green crystals were washed with 20ml hexane and dried under reduced pressure; yield 0.89g, 84%. This product showed a strong FTIR (solid state) band, ν(CO) at 1985 cm$^{-1}$ as reported in the literature (Reger and Coleman, 1977) and was used without further purification.

3.4.1 Reaction of $[(\eta^5-C_5H_5)Fe(CO)(PPh_3)(THF)]BF_4$ with 1,2-diaminoethane

The 1,2-Diaminoethane (0.5ml, 0.75mmol) was added drop wise to a solution of $[(\eta^5-C_5H_5)Fe(CO)(PPh_3)(THF)]BF_4$ (0.845g, 1.48 mmol) in CH$_2$Cl$_2$ (15 ml) at room temperature in a Schlenk tube containing a magnetic bar and covered with alluminium foil. The mixture stirred rapidly for 20 min and then allowed to stand at room temperature for 8 h during which the green solution changed to a wine red solution and a brown precipitate. The solution was filtered through a cannula into a clean dry Schlenk tube under nitrogen. The filtrate was concentrated to 5ml, hexane (30ml) was added and the mixture cooled to -78°C in liquid nitrogen-dry ice mixture to precipitate a yellow solid. The mother liquor was removed through a cannula and
the solid dried under reduced pressure to give yellow microcrystalline solid (appendix 1). This was purified further by recrystallization from dichloromethane and excess hexane mixture. A similar observation was made when diethyl ether was used to precipitate the compound. Yield: 0.564 g, 72%. Calculated for C_{39}H_{46}B_{2}F_{8}Fe_{2}N_{2}P_{2}O_{2}, C, 54.46; H, 5.35; N, 3.23; Found C, 52.70; H, 5.09; N, 3.99; \textsuperscript{1}H NMR (400 MHz, DMSO): δ 8.29-7.27 (m, 30 H, Ph), 5.56 (s, 5H, Cp), 4.59 (s, 4H, NH2), 2.51 (s, 4H, CH2). \textsuperscript{13}C NMR (400 MHz, DMSO): δ 89.54 (Cp), 53.36 (CH2), 207.20 (CO) 133.94-129.19 (PPh3). FTIR (solid state): ν(CO) 2059.8, 1955.7 cm\textsuperscript{-1}; ν(NH) 3436.9, 3155.3 cm\textsuperscript{-1} M.P. 154°C.

3.4.2 Reaction of [(η⁵-C₅H₅)Fe(CO)(PPh₃)(THF)]BF₄ with 1,3-diaminopropane

The 1,3-diaminopropane (0.06ml, 0.72mmol) was added drop wise to a solution of [(η⁵-C₅H₅)Fe(CO)(PPh₃)(THF)]BF₄ (0.845g, 1.48 mmol) in CH₂Cl₂ (15 ml) at room temperature in a Schlenk tube containing a magnetic bar and covered with alluminium foil. The mixture was stirred rapidly for 20 minutes and then allowed to stand at room temperature for 8 hours during which the green solution changed to a wine red solution and a yellow precipitate. The solution was filtered through a canula into a clean dry Schlenk tube under nitrogen. The filtrate was concentrated to 5ml, hexane (30ml) was added and the mixture cooled to -78°C in a dry-ice acetone bath to precipitate a yellow solid. The mother liquor was removed through a canula and the solid dried under reduced pressure to give orange microcrystalline solid; yield: 0.555 g, 70%. This was purified further by recrystallization from a dichloromethane and excess hexane mixture. Calculated for C_{40}H_{48}B_{2}F_{8}Fe_{2}N_{2}P_{2}O_{2}, C,54.97; H, 5.49; N, 3.20; Found C, 52.97; H, 5.30; N, 2.99; \textsuperscript{1}H NMR (400 MHz, DMSO-d₆): δ 7.6407-6.99 (m, 30 H, Ph), 5.57 (s, 5H, Cp), 3.43 (s, 4H, NH2), 2.51
(m, 4H, αCH₂), 1.74 (t, 4H, βCH₂). 13C NMR (400 MHz, DMSO): δ 87.45 (Cp), 53.36 (αC), 39.14 (βC), 210.75 (CO), 133.17-130.08 (PPh₃). FTIR (solid state): ν(CO) 2059.8, 2013.5 cm⁻¹; ν(NH) 3452.5, 3259.5 cm⁻¹. M.P. 217 °C.

3.4.3 Reaction of [(η⁵-C₅H₅)Fe(CO)(PPh₃)(THF)]BF₄ with 1,4-diaminobenzene

1,4-diaminobenzene (0.082g, 0.758 mmol) was added to a solution of [(η⁵-C₅H₅)Fe(CO)(PPh₃)(THF)]BF₄ (0.845g, 1.48 mmol) in CH₂Cl₂ (15 ml) at room temperature in Schlenk containing a magnetic bar stirrer and covered with aluminium foil, the mixture was stirred rapidly for 20 minutes. The mixture was then allowed to stand at room temperature for 8 hours during which the green solution changed forming a wine red solution and precipitated a maroon coloured solid. The solution was filtered through a canula into a clean dry Schlenk tube under nitrogen. The filtrate was concentrated to 5ml, hexane (30ml) was added and the mixture cooled to -78°C to precipitate a maroon solid. The mother liquor was removed through a canula and the solid dried under reduced pressure to give 0.605 g of a maroon microcrystalline solid (Yield = 74%). This was purified further by recrystallization from a dichloromethane-hexane mixture. A maroon microcrystalline solid was observed even when diethyl ether was used to precipitate the compound. Calculated for C₂₀H₁₈B₂F₂Fe₂N₂P₂O₂, C, 57.19; H, 5.41; N, 3.03; Found, C, 57.49; H, 3.15; N, 4.31. ¹H NMR (400 MHz, DMSO-d₆): 87.49-7.23 (m, 30 H, Ph), 7.63-7.52 (m 4H, Ph), 5.56 (s, 5H, Cp), 3.46 (s, 4H, NH₂). ¹³C NMR (400 MHz, DMSO): δ 133.98-129.39 (PPh₃), 67.49 (Cp), 129.3871 (pC), 130.63 (o/mC), 207.14 (CO). FTIR (solid state): ν(CO) 2051, 1997 cm⁻¹; ν(NH) 3307, 3280 cm⁻¹. M.P. 215°C.
3.5 Evaluation of antibacterial activity

3.5.1 Zones of inhibition

The antibacterial activity of the compounds was tested \textit{in vitro} by agar disc diffusion method. Mueller Hinton agar was prepared using the manufactures instructions for purposes of culturing the bacteria, which included: \textit{Pseudomonas aeruginosa}, \textit{Bacillus subtilis}, \textit{Escherichia coli} and \textit{Staphylococcus aureus}. A 0.5 McFarland standard was prepared by diluting a 24 hour bacterial culture using a normal saline salt solution (Wilkins and Thiel, 1973; Dickert and Braveny \textit{et al.}, 1981). 0.1 mL of the microbial suspension was inoculated into the Petri dishes. Filter paper discs (6 mm) were soaked into the test compound solution (made by dissolving 300 mg of the compound in 2 mL of 0.1% DMSO) and placed on the inoculated petridishes at reasonable distances. Discs soaked with DMSO and air-dried were used as negative controls. Gentamicin Standard was used as positive control. Gentamincine was used as a positive control because it is a broad spectrum antibiotic against both gram-positive and gram-negative bacteria. The plates were then be incubated at 37 °C for 24 hours. This was replicated three times for each test bacteria. Zones of inhibition were measured using a ruler (Wilkins and Thiel, 1973; Dickert \textit{et al.}, 1981). The bacterial response to the complexes was evaluated using the table below (Johnson and Case, 1995)

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>10 or less</td>
</tr>
<tr>
<td>Intermediate</td>
<td>11-15</td>
</tr>
<tr>
<td>Susceptible</td>
<td>16 or more</td>
</tr>
</tbody>
</table>
3.5.2 Evaluation of minimum inhibitory concentrations (MICs)

Broth micro-dilution technique was therefore employed to determine the minimum inhibitory concentrations using the 96 well micro-titer plates for each complex against the four bacterial strains used in this study. The wells were filled with 50 μl of nutrient broth. A solution of the test compound was prepared by taking 300 mg of the test compound and mixing it with 2 mL of DMSO (0.01% Dimethyl sulfoxide) for complete dissolution of the test compound. Then 50 μl of the test solution was allotted into the first well before doing successive dilutions by moving 50 μl of nutrient broth containing the test solution from the first well to the second well, and from the second well to the third well through the fourth well. Fifty microliters (50 μl) of the test isolate was then introduced into each well thus the final concentrations were 150 mg/ml, 100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.5625 mg/ml. One well (without test solution or standard antibiotic) was used as negative control of the growth of the bacteria in the medium whereas another well with 50 μl of antibiotic (Amoxicillin) was used as positive control. Incubation was done at 37 ºC for 24 hours (Irith et al., 2008; Jiang, 2011).

3.5.4 Evaluation of minimum bactericidal concentrations (MBCs)

For the determination of minimum bactericidal concentration (MBC), all wells as used in the determination of MICs above were used. Broths were sub-cultured on nutrient agar. The lowest concentration of the test compound that did not yield any colony on the nutrient agar after sub-culturing and incubating for 24 hours was taken as the MBC (Irith et al., 2008).
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The results of the characterization by FTIR, NMR, melting points and elemental analyses as well as in vitro bioassays against the Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and the Gram-positive *Bacillus subtilis*, and *Staphylococcus aureus* are presented and discussed in the sections that follow.

4.2 Synthesis of \([(\eta^5-C_5H_5)Fe(CO)(PPh_3)(THF)]BF_4\)

The complex is, insoluble in hexane, but very soluble in trichloromethane and dichloromethane. The FTIR spectrum of the complex, \([(\eta^5-C_5H_5)Fe(CO)(PPh_3)(THF)]BF_4\), shows a carbonyl absorption peak at 1987 cm\(^{-1}\) in agreement with literature (Reger and Coleman, 1977) compared to \((\eta^5-C_5H_5)Fe(CO)(PPh_3)I\) \(\nu(CO) 1942\) cm\(^{-1}\) (Munyeza et al., 2007). This suggests a weak synergic interaction between the iron center and the carbonyl due to reduced electron density on the metal center, because of the coordination of the THF. There is a progressive shift of the \(\nu CO\) band to higher energy as an increased amount of electron density is removed from the metal center, which reduces \(\pi\)-back-bonding. The complex \([(\eta^5-C_5H_5)Fe(CO)(PPh_3)(THF)]BF_4\) was used as the starting material in the subsequent reactions.

4.3 The \(\alpha, \omega\)-diaminoalkanes of \([(\eta^5-C_5H_5) Fe(CO)(PPh_3)(THF)]BF_4\) complexes

The poor electron donating nature of THF makes complex \([(\eta^5-C_5H_5) Fe(CO)(PPh_3)(THF)]BF_4\) very electrophilic and thus can react with a wide range of nucleophiles. The two \(\alpha, \omega\)-diaminoalkanes (1,2-diaminoethane and 1,3-
diaminopropane) ligands employed in this investigation reacted with two equivalents of the THF complex $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)(PPh}_3)(\text{THF})]\text{BF}_4$ at room temperature in dry, nitrogen-rich dichloromethane to afford symmetrically bridged bimetallic complexes **complex-1** $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)(PPh}_3)]_{2}\mu-(\text{NH}_2(\text{CH}_2)_2\text{NH}_2)(\text{BF}_4)_2$ and **complex-2** $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)(PPh}_3)]_{2}\mu-(\text{NH}_2(\text{CH}_2)_3\text{NH}_2)(\text{BF}_4)_2$ as shown in equation 1. The complexes were obtained as yellow or dirty yellow solids by precipitation from the diethyl ether or hexane. They were found to be soluble in dichloromethane, trichloromethane, water, acetone and dimethylsulfoxide but insoluble in hexane and diethyl ether, making them easily separated by filtration under nitrogen. They are stable in air but slowly decompose when in solution.

The diaminoalkane complexes have been characterized by FTIR, $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectroscopy and elemental analysis. Their FTIR spectra exhibited strong absorption bands in the $\nu(\text{CO})$ region in the range 1955.5–2059.8 cm$^{-1}$. This similar to those reported for related aminocarbene complexes $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe(CO)(PPh}_3)\{-=\text{C(NHR}_2\text{CHR}_1\text{)}\}]^{+}\text{BF}_4^{-}$ [$\text{R}_1=\text{H, Me or Pr}; \text{R}_2=\text{H, Me, CH(Me)Ph, CH}_2\text{CH}_2$ or CH$_2$CH$_2$OH] (Davies, *et al.*, 1998), other cationic CpFe(CO)$_2$ complexes (Lorenz *et al.*, 2001; Changamu *et al.*, 2007) and $[(\text{CpFe(CO)_2})_{2}\mu-(\text{NH}_2(\text{CH}_2)_n\text{NH}_2)](\text{BF}_4)_2$ ($n = 2$-6) (M’thuruaine *et al.*, 2010). The
positions of the ν(CO) absorption bands are at lower wavenumbers relative to those of [($\eta^5$-C$_5$H$_5$)Fe(CO)(PPh$_3$)(THF)]BF$_4$. This is in agreement with the increase in electron density on the iron center (caused by the coordinated aminoalkane group), which results in increased back-bonding to the carbonyl groups. This further weakens the C-O bond by pumping more electron density into the formally empty $\pi^*$ orbital and hence a lower ν(CO) absorption frequency. The $^1$H and $^{13}$C NMR data for complexes 1 and 2 were recorded in DMSO. The identification and assignments were made by comparing them with the $^1$H and $^{13}$C NMR of the bridged diamine complexes [{$($($\eta^5$-C$_5$H$_5$)Fe(CO)$_2$)$_2$µ-(NH$_2$(CH$_2$)$_n$NH$_2$)}($\text{BF}_4$)$_2$ ($n = 2$–$4$) (M’thiruane. et al., 2010) and by comparison with $^2$D NMR spectra data reported by Peng et al., for the bridged diphosphine complexes, [{$($($\eta^5$-C$_5$H$_5$) Fe(CO)$_2$)$_2$µ-(Ph$_2$P(CH$_2$)$_n$PPh$_2$)}$_2^{2+}$ ($n = 1$–$4$) (James et al., 2002). The proton NMR spectra of complexes 1 and 2 exhibited characteristic chemical shifts assignable to the cyclopentaadienyl protons at ca. 5.55 ppm, $^1$H and $^{13}$C NMR data suggested a symmetrical structure (Fig. 4), which is also evidence that the diaminoalkane bridges the two metal centers.

The $^1$H NMR spectrum of complex 1 shows chemical shifts assignable as follows: $\delta$ 8.29–7.27 (m, 30 H, Ph), 5.56 (s, 5H, Cp), 4.43 (s, 4H, NH$_2$), 2.51 (m, 4H, $\alpha$CH$_2$), 1.74 (t, 4H, $\beta$CH$_2$). The $^{13}$C NMR spectrum shows chemical shifts at 53.36, 89.54, 133.60 and 207.20 ppm corresponding to the two identical $\alpha$-carbons, five equivalent Cp carbons, PPh$_3$ phenyl rings carbons and two identical carbonyls, respectively. IR (solid state): ν(CO) 2059.8, 1955.7 cm$^{-1}$; ν(NH) 3436.9, 3155.3 cm$^{-1}$ M.P. 154°C.

The $^1$H NMR spectrum of complex 2 shows chemical shifts assignable as follows: $\delta$ 7.6407–6.99 (m, 30 H, Ph), 5.57 (s, 5H, Cp), 3.43 (s, 4H, NH$_2$), 2.51 (m, 4H, $\alpha$CH$_2$), 1.74 (t, 4H, $\beta$CH$_2$). $^{13}$C NMR spectrum shows chemical shifts at: $\delta$ 87.45 (Cp),
53.36 (αC), 39.14 (βC), 210.75 (CO), 133.17-130.08 (PPh₃). FTIR (solid state): ν(CO) 2059.8, 2013.5 cm⁻¹; ν(NH) 3452.5, 3259.5 cm⁻¹ M.P. 217 °C. The assignments were done by comparing the results with reported values for related compounds (M’Thiruine et al., 2011).

![Diagram of complex structure](image1)

**Figure 4.2 General structure of the diaminoalkane complexes (n= 1or 2)**

### 4.4 The paraphenelenediamine complex of [(η⁵-C₅H₅) Fe(CO)(PPh₃)(THF)]BF₄.

Reaction of two equivalents of the THF complex, [(η⁵-C₅H₅) Fe(CO)(PPh₃)(THF)]BF₄, with 1,4-diaminobenzene led to the formation of a maroon solid found to be the dinuclear complex [{[(η⁵-C₅H₅)(CO)(PPh₃)Fe]₂L}](BF₄)₂ (L=1,4-diaminobenzene), **complex 3**

The complex was obtained in good yield (74%) by precipitation with diethyl ether or hexane. It is soluble in dichloromethane, trichloromethane, water, acetone and dimethylsulfoxide (DMSO) but insoluble in hexane and diethylether. It is stable in air but slowly decomposes when in solution.

![Diagram of complex structure](image2)

**Figure 4.3 Ligand bridged complex of [{[(η⁵-C₅H₅)(CO)(PPh₃)Fe]₂L}](BF₄)₂ (L=1,4-diaminobenzene)**
The complex was characterized by IR, $^1$H NMR and $^{13}$C NMR spectroscopy and elemental analysis. The FTIR spectra exhibited strong absorption bands in the $\nu$(CO) region in the range 1997 cm$^{-1}$ - 2051 cm$^{-1}$. Just like the alkyl amine complexes in this study, the positions of the $\nu$(CO) absorption bands were observed at lower wavenumbers relative to those of $[(\eta^5$-C$_5$H$_5$) Fe(CO)(PPh$_3$)(THF)]BF$_4$. This is in agreement with the increase in electron density on the iron center, which results in increased back bonding to the carbonyl groups. This further weakens the C-O bond by pumping more electron density into the formally empty $\pi^*$ orbital and hence a lower $\nu$(CO) absorption frequency. However, the absorption bands are at higher wave numbers relative to complex-2, which is attributed to the poor electron donating ability of benzene compared to methyl group. The $^1$H and $^{13}$C NMR data were recorded in DMSO. The identification and assignments were made by comparing them with the $^1$H and $^{13}$C NMR of the bridged diamine complexes $\{[(\eta^5$-C$_5$H$_5$) Fe(CO)$_2$]$_2$µ-(NH$_2$(CH$_2$)$_n$NH$_2$](BF$_4$)$_2$ (n = 2–4) (M’thiruane. et al., 2010) and by comparison with $^2$D NMR spectra data reported by Peng et al., 2001 for the bridged diphosphine complexes, $\{[(\eta^5$-C$_5$H$_5$) Fe(CO)$_2$]$_2$µ-(Ph$_2$P(CH$_2$)$_n$PPh$_2$]$^{2+}$ (n = 1–4) (James et al., 2002). The $^1$H NMR spectrum of complex-3 shows chemical shifts assigned as follows: $\delta$ 7.49-7.23 (m, 30 H, Ph), 7.62-7.52 (m 4H, Ph) 5.57 (s, 5H, Cp), 3.46 (s, 4H, NH$_2$). The $^{13}$C NMR spectrum shows five chemical shifts assigned as follows: $\delta$ 133.9784-129.3871 (PPh$_3$), 67.49 (Cp), 129.39 (pC), 130.63 (o/mC), 207.14 (CO). FTIR (solid state): $\nu$(CO) 2051, 1997 cm$^{-1}$; $\nu$(NH) 3307, 3280 cm$^{-1}$ and M.P. 215°C.
4.5 Evaluation of antibacterial activity

4.5.1 Disc diffusion assay

The results of disc diffusion test indicated that the three different complexes showed different degrees of growth inhibition, depending on bacterial strain and concentration of the test compound (Figure-4.3). The numbers represent various concentrations into which the paper discs were soaked in. Example, 1 represents concentration of 150mg/ml of solution and 0 is the negative control.

![Figure 4.4 Zones of inhibition under different concentrations of complex-1](image)

4.5.1.1 Antibacterial activity of \[\left[\left(\eta^5-C_5H_5\right)Fe(CO)(PPh_3)\right]_2\mu-(NH_2(CH_2)_2NH_2)(BF_4)_2\], (complex 1)

In 0.1% DMSO solution of the test compound 1, the highest activity was observed at the concentration of 150 mg/ml against \textit{P. aeruginosa} with zone of inhibition of (15 mm ), followed by \textit{S. aureus} with zone of inhibition of (12 mm ), \textit{B. subtilis} with zone of inhibition of (11 mm) and \textit{E. coli} with zone of inhibition of (10 mm) (Figure-4.4 a, b, c and d). The antibacterial activity of this test complex was also evident with concentrations as low as 3.125 mg/ml \textit{P. aeruginosa} with zone of inhibition of (9 mm) and \textit{S. aureus} with zone of inhibition of (8 mm) but \textit{B. subtilis}
and *E. coli* were not susceptible at these concentrations (Table 4.1). However, the antibiotic Gentamicin (positive controls) was more effective than the test complex with the diameter ranging 15 to 20 mm. A filter paper soaked in DMSO (negative control) did not show any bacteria inhibition.

Figure 4.5 complex-1 against (a) *P. aeruginosa* (b) *S. aureus* (c) *B. subtilis* (d) *E. coli*
4.5.1.2 Antibacterial activity of $\left\{\left\{\eta^5-C_5\text{H}_5\right\}\text{Fe(CO)}(\text{Pph}_3)\right\}_2\mu-(\text{NH}_2(\text{CH}_2)_3\text{NH}_2)\right\}(\text{BF}_4)_2$ (complex-2)

<table>
<thead>
<tr>
<th>Complex MIC conc’ (mg/ml )/Bacteria</th>
<th>B. subtilis</th>
<th>S.aureus</th>
<th>P.aeruginosa</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>11.33±0.17 I</td>
<td>12.00±0.00 I</td>
<td>15.00±0.58 S</td>
<td>11.00±0.00 I</td>
</tr>
<tr>
<td>100</td>
<td>11.00±0.58 I</td>
<td>11.50±0.29 I</td>
<td>14.67±0.33 S</td>
<td>9.33±0.33 R</td>
</tr>
<tr>
<td>75</td>
<td>9.33±0.33 R</td>
<td>11.00±0.00 I</td>
<td>14.00±0.58 S</td>
<td>9.00±0.17 R</td>
</tr>
<tr>
<td>50</td>
<td>9.00±0.00 R</td>
<td>10.33±0.73 I</td>
<td>13.33±0.33 I</td>
<td>8.83±0.17 R</td>
</tr>
<tr>
<td>25</td>
<td>8.00±0.00 R</td>
<td>9.33±0.17 R</td>
<td>11.33±0.33 I</td>
<td>7.50±0.33 R</td>
</tr>
<tr>
<td>12.5</td>
<td>6.83±0.16 R</td>
<td>9.17±0.17 R</td>
<td>10.00±0.00 R</td>
<td>7.33±0.33 R</td>
</tr>
<tr>
<td>6.25</td>
<td>-</td>
<td>8.33±0.17 R</td>
<td>9.33±0.33 R</td>
<td>7.00±0.00 R</td>
</tr>
<tr>
<td>3.125</td>
<td>-</td>
<td>8.00±0.00 R</td>
<td>9.00±0.00 R</td>
<td>-</td>
</tr>
<tr>
<td>1.5</td>
<td>-</td>
<td>7.00±0.00 R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin (positive control)</td>
<td>25.17±0.17 S</td>
<td>15.50±0.29 S</td>
<td>17.50±0.29 S</td>
<td>20.17±0.17 S</td>
</tr>
<tr>
<td>DMSO (negative control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(S) Sensitive, (I) Intermediate and (R) Resistant

Solution of test complex 2 in 0.1% DMSO, the highest activity was observed at the concentration of 150 mg/ml against $P$. aeruginosa with zone of inhibition of (16 mm), followed by $S$. aureus with zone of inhibition of (13 mm ), $B$. subtilis with zone of inhibition of (12 mm ) and $E$. coli with zone of inhibition of (10 mm ) (Figure-4.5 a, b, c and d). The antibacterial activity of this test complex 2 was also evident with concentrations as low as 3.125 mg/ml against $P$. aeruginosa with zone of inhibition of (10 mm) and $S$. aureus with zone of inhibition of (7mm) but $B$. subtilis and $E$. coli did not show susceptibility at these concentrations (Table 4.2). However, the antibiotic Gentamicin (positive controls) was more effective than the test complex 2 with the diameter ranging 15 to 25mm. A filter paper soaked in
DMSO (negative control) did not show any bacteria inhibition. The antibacterial activity of complex 2 also increased with increase in concentration. Complexes 1 and 2, showed almost equal antibacterial activity with complex 2 being just slightly higher.

Figure 4.6 complex-2 against (a) P. aeruginosa (b) S. aureus (c) B. subtilis (d) E. coli
Table 4.2 Zones of inhibition (mm) mean for complex-2

<table>
<thead>
<tr>
<th>Complex MIC conc’ (mg/ml) /Bacteria</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>12.33±0.17 I</td>
<td>12.50±0.28 I</td>
<td>15.33±0.33 S</td>
<td>11.00±0.29 I</td>
</tr>
<tr>
<td>100</td>
<td>11.33±0.17 I</td>
<td>12.33±0.17 I</td>
<td>15.17±0.17 S</td>
<td>10.00±0.00 R</td>
</tr>
<tr>
<td>75</td>
<td>11.00±0.00 I</td>
<td>12.00±0.00 I</td>
<td>14.00±0.58 S</td>
<td>9.33±0.37 R</td>
</tr>
<tr>
<td>50</td>
<td>10.33±0.33 R</td>
<td>11.67±0.33 I</td>
<td>13.33±0.33 I</td>
<td>9.00±0.00 R</td>
</tr>
<tr>
<td>25</td>
<td>10.17±0.17 R</td>
<td>10.50±0.26 R</td>
<td>13.33±0.17 I</td>
<td>8.00±0.00 R</td>
</tr>
<tr>
<td>12.5</td>
<td>9.33±0.17 R</td>
<td>10.00±0.00 R</td>
<td>13.00±0.00 I</td>
<td>6.67±0.17 R</td>
</tr>
<tr>
<td>6.25</td>
<td>-</td>
<td>8.33±0.33 R</td>
<td>12.00±0.00 I</td>
<td>-</td>
</tr>
<tr>
<td>3.125</td>
<td>-</td>
<td>7.67±0.17 R</td>
<td>10.33±0.33 R</td>
<td>-</td>
</tr>
<tr>
<td>1.5</td>
<td>-</td>
<td>7.00±0.00 R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin (positive control)</td>
<td>25.17±0.17 S</td>
<td>15.50±0.29 S</td>
<td>17.50±0.29 S</td>
<td>20.17±0.17 S</td>
</tr>
<tr>
<td>DMSO (negative control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(S) Sensitive, (I) Intermediate and ® Resistant

4.5.1.3 Antibacterial activity of of \([\{\eta^5-C_5H_5\}(CO)(PPh_3)Fe\}_2L\)](BF_4)_2 (L=1,4-diaminobenzene) (complex-3)

Complex- 3 did not show significant antibacterial activity even at high concentrations employed in this study. Maximum activity was observed at 150 mg/ml, but much lower diameters of zones of inhibition against S. aureus with zone of inhibition of ( 9 mm ), P. aeruginosa with zone of inhibition of ( 9 mm ), E. coli with zone of inhibition of ( 9 mm ) and B. subtilis with zone of inhibition of ( 8 mm ) (Table 4.3). No inhibition were shown for concentrations below 75mg/ml against S. aureus and below 100mg/ml against P. aeruginosa, B. subtilis and E coli for complex-3 (Figure-4.6 a, b, c and d).
Figure 4.7 complex-3 against (a) *P. aeruginosa* (b) *S. aureus* (c) *B. subtilis* (d) *E. coli*

Table 4.3: Zones of inhibition (mm) for complex 3

<table>
<thead>
<tr>
<th>Complex MIC conc’/Bacteria</th>
<th><em>B. subtilis</em></th>
<th><em>S. aureus</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>150 mg/ml</td>
<td>7.5±0.29 R</td>
<td>9.33±0.33 R</td>
<td>9.00±0.00 R</td>
<td>8.67±0.33 R</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>7.00±0.00 R</td>
<td>8.67±0.33 R</td>
<td>7.67±0.33 R</td>
<td>7.33±0.33 R</td>
</tr>
<tr>
<td>DMSO (negative control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin (positive control)</td>
<td>25.17±0.17 S</td>
<td>15.50±0.29 R</td>
<td>17.50±0.29 R</td>
<td>20.17±0.17 R</td>
</tr>
</tbody>
</table>

(S) Sensitive, (I) Intermediate and (R) Resistant
4.5.2 Minimum inhibitory concentrations

The three complexes showed strong antibacterial activity, with zones of inhibition of over 9 mm. The sensitivity testing from disc diffusion method showed that the three complexes exhibited clear zones of inhibition at concentration 150 mg/ml. Therefore, 150 mg/ml concentration was chosen as initial concentration in the determination of MIC for the three complexes. Using 96 well microtiter plates and undergoing serial dilutions, the highest concentration was 150mg/ml while the lowest concentration tested was 0.3906mg/ml of each complex against each of the four selected bacteria.

The tests showed MIC’s for complexes 1 and 2 against *S. aureus* and *P. aeruginosa* at concentration of the 6.25mg/ml whereas against *B. subtilis* and *E. coli* MIC was 12.5mg/ml for the two complexes.

**Complex 3** showed significant inhibition only at concentrations of 100mg/ml against the four bacteria in this study. The MIC for complex 3 was therefore 100mg/ml. The results are summarized in table 4.4 and can be seen to compare closely with what was observed with zones of inhibition.

<table>
<thead>
<tr>
<th>Complex /Bacteria</th>
<th><em>B. subtilis</em></th>
<th><em>S. aureus</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>12.5mg/ml</td>
<td>6.25mg/ml</td>
<td>6.25mg/ml</td>
<td>12.5mg/ml</td>
</tr>
<tr>
<td>Complex 2</td>
<td>12.5mg/ml</td>
<td>6.25mg/ml</td>
<td>6.25mg/ml</td>
<td>12.5mg/ml</td>
</tr>
<tr>
<td>Complex 3</td>
<td>100mg/ml</td>
<td>100mg/ml</td>
<td>100mg/ml</td>
<td>100mg/ml</td>
</tr>
</tbody>
</table>

Table 4.4: The significant MIC values for the three complexes against the bacteria used in this study
The results obtained indicated that \[\{[(\eta^5-C_5H_5)Fe(CO)PPh_3]}_2\mu-(NH_2(CH_2)_2NH_2)](BF_4)_2 \quad \text{(complex-1)} \] and \[\{[(\eta^5-C_5H_5)Fe(CO)PPh_3]}_2\mu-(NH_2(CH_2)_3NH_2)](BF_4)_2 \quad \text{(complex-2)} \] are more active than \[\{[(\eta^5-C_5H_5)(CO)PPh_3]}_2L)](BF_4)_2 \quad (L=1,4-diaminobenzene) \quad \text{(complex-3)} \] against all the bacterial strains used. Complexes 1 and 2 mimic ethambutol. A similar observation have been made with compounds having ferrocenyl groups having only two or three carbon atoms spacer between two amino functions (Ralambomanana et al 2008). The three compounds having shown above 9mm diameter of zones of inhibition, minimum batericidal concentrations were determined.

4.5.3 Determination of minimum bactericidal concentration (MBC)

For the determination of minimum bactericidal concentration (MBC), all wells broth whose concentrations were MIC and above were sub-cultured on nutrient agar. The lowest concentration of the test compound that did not yield any colony on the nutrient agar (Appendix-6.3) after sub-culturing and incubating for 24 hours was taken as the MBC for each compound on each bacterium (Table 4.5). There was no significant difference between MBC and MIC values. Just like zones of inhibition compound 1 and 2 with similar backbone as EMB were more active than complex - 3. The studies on antimicrobial activity indicate that, amongst other factors, constitution of organometallic compounds, the nature of the ligand in the complex and the strains of the microorganism have important influence on antimicrobial activity. The complexes showed to have activity to completely eliminate bacteria at certain concentrations, even though the zones of inhibition indicated moderate to resistant behavior to those concentrations. This may have been attributed to low
diffusion rates of the complexes in the auger, variation in divalent ions, thymidine or thymine and moisture content.

<table>
<thead>
<tr>
<th>Complex /Bacteria</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>25mg/ml</td>
<td>12.5mg/ml</td>
<td>12.5mg/ml</td>
<td>25mg/ml</td>
</tr>
<tr>
<td>Complex 2</td>
<td>25mg/ml</td>
<td>12.5mg/ml</td>
<td>12.5mg/ml</td>
<td>25mg/ml</td>
</tr>
<tr>
<td>Complex 3</td>
<td>100mg/ml</td>
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</table>
CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

The objective of this study was to determine the in vitro bioactivity of amine complexes of the half-sandwich organometallic moiety \(\{(\eta^5-C_5H_5)Fe(CO)(PPh_3)\}\) against selected bacteria. Accordingly then, three half-sandwich organometallic compounds of the type \(\{(\eta^5-C_5H_5)Fe(CO)(PPh_3)\}_2\mu-(L)\}X_2\ (L = 1,2-diaminoethane, 1,3-diaminopropane and 1,4-diaminobenzene, X = univalent counter-anion such as BF\(_4^-\)) were synthesized, characterized and their in vitro bioactivity determined.

5.2 Conclusions

i). The starting complex \(\{(\eta^5-C_5R_5)(CO)(PPh_3)Fe(THF)\}BF_4\) has been synthesized and obtained in excellent yields \(84\%\) and used for the synthesis of the other targeted complexes.

ii). The ligand bridged complexes, \(\{(\eta^5-C_5H_5)(CO)(PPh_3)Fe\}_2L\})\,(BF\(_4\))_2\, (L= ethylenediamine or propylenediamine or paraphenelenediamine), have been synthesized in good yields and characterized using FTIR and NMR specroscopy, melting point determination as well as elemental analysis.

iii). The bioactivities of the ligand bridged complexes, \(\{(\eta^5-C_5H_5)(CO)(PPh_3)Fe\}_2L\})\,(BF\(_4\))_2\, (L= ethylenediamine, propylenediamine or paraphenelenediamine) against Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and Staphylococcus aureus have evaluated with gentamicin as the positive control and DMSO as the negative control. The ethylenediamine and propylenediamine complexes were found to have good
activities while the paraphenelenediamine complex was found to have little activity against the selected bacteria.

5.3 Recommendations

The complexes prepared are stable and have been found to be active against the selected bacteria. Given that the anti-TB drugs are very specific in their activities, the following recommendations are made:

i). This results form the basis for further investigations to determine the activities of the compounds against *Mycobacterium tuberculosis*.

ii). The actual first line drug molecules used against TB, being amines, be coordinated to the half sandwich moiety \([(\eta^5-C_5H_5)(CO)(PPh_3)Fe]\) and the bioactivities of the resultant compounds against *Mycobacterium tuberculosis* determined.
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APPENDICES

6.1 Appendix 1: Colour of the complexes during precipitation

Complex 1
Complex 2
Complex 3

Complex-1. \[\{(\eta^5-C_5H_5)Fe(CO)(Pph_3)\}_2\mu-(NH_2(CH_2)_2NH_2)(BF_4)_2\]

Complex-2. and \[\{(\eta^5-C_5H_5)Fe(CO)(Pph_3)\}_2\mu-(NH_2(CH_2)_3NH_2)(BF_4)_2\]

Complex-3. of \[\{(\eta^5-C_5H_5)(CO)(PPh_3)Fe\}_2L\}(BF_4)_2 (L=1,4-diaminobenzene)\]
6.2 Appendix 2: Zones of inhibition evaluation for positive control

Positive control Gentamicin (against *P. aeruginosa* bacteria).
6.3 Appendix 3: Evaluation of MBCs

Complex- 3 (against \textit{S. aureus} bacteria).

Complex- 1 (against \textit{E. coli} bacteria).

Complex- 2 (against \textit{S. aureus} bacteria).