# THE SYNTHESIS AND CHARACTERIZATION OF COPPER(II) COMPLEXES CONTAINING THIOSEMICARBAZONE AND SEMICARBAZONE LIGANDS DERIVED FROM FERROCENE AND PYRIDYL FRAGMENTS

MASTER OF SCIENCE

BY

DANIEL SHIPWIISHO LIKIUS

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This work was carried out from October 2007 to September 2008 in the Inorganic Chemistry Laboratory, in the department of Chemistry and biochemistry at the University of Namibia under the supervision of Prof. Enos Kiremire.

**ABSTRACT**

The synthesis and characterization, spectroscopic, and biological studies of 2-acetylferrocenyl-4-phenylthiosemicarbazone, 2-acetylferrocenyl-4-methylthiosemicarbazone, 2-acetylpyridine-2-thiophenecarboxylsemicarbazone and 2-acetylferrocenyl-2-thiophenecarboxylsemicarbazone with copper(II) chloride are reported here. The ligands were synthesized by condensation reaction of appropriate carbonyl and amine compounds. The reactions were carried out in the presence of acetic acid. The complexes were characterized by means of Elemental Analysis (EA), Fourier Transform Infrared (FT-IR) spectroscopy, Nuclear Magnetic Resonance ($^{1}$HNMR and $^{13}$CNMR) spectroscopy. The metal complexes and their corresponding ligands were tested against malaria parasites. It was found that the copper complexes synthesized are more biologically active then their corresponding ligands.

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**LIST OFABBREVIATION AND SYBOLS**

| | |
|---|---|
| $CDCl_3$ | deuteriochloroform (chloroform-d) |
| $^{13}$C-NMR | carbon-13 nuclear magnetic resonance spectroscopy |
| DMF | dimethylformamide |
| DMSO | dimethylsulphoxide |
| EA | elemental analysis |
| FP-2 | fulcipal-2 enzyme |
| FT-IR | fourier transform-infrared spectroscopy |
| ITC | isatin-ß-thiosemicarbazone |
| 1H-NMR | proton nuclear magnetic resonance spectroscopy |
| nM | nonamolar |
| PfCRT | chloroquine resistance transporter |
| Pgh1 | P-glycoprotein-like protein |
| PL | pyridoxal |
| PLP | pyridoxal phosphate |
| TMS | tetramethylsilane |
| *W-2* | Chloroquine resistant |

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**DECLARATIONS**

I, Daniel Shipwiisho Likius, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.



……. Date……..

Daniel Shipwiisho Likius

**To my later grandmother, Mukwamalanga Letisia Andreas Kashinduka (1943-2008)**

## PUBLICATIONS

Published papers:

1. E.M.R. Kiremire, L.S. Daniel, K. Chibale, H. Kambafwile and P.J.Rosenthal: Inducement of antimalarial biologicasl activity in some S-based ligands by Zn(II) ions. Biosciences, Biotechnology research, Asia , Vol5 (1), 121-123 (2008)

2. E.M.R. Kiremire, L.S. Daniel, H. Muashekele, K. Chibale and H. Kambafwile: Further investigation into phenomental paramagnetic shift influence of Cu(II) ion on ferrocene-thiosemicarbazone-based bimetallic complexes. Material Science Research Journal, Vol. 4, 263-267 2007).

3. E.M.R. Kiremire, L.S. Daniel, H. Muashekele, K. Chibale and H. Kambafwile: Phenomenal paramagnetic shifts in certain ferrocene thio-based copper(II) metal complexes. Oriental Journal of Chemistry, Vol. 23 (3), 785-791 (2007)

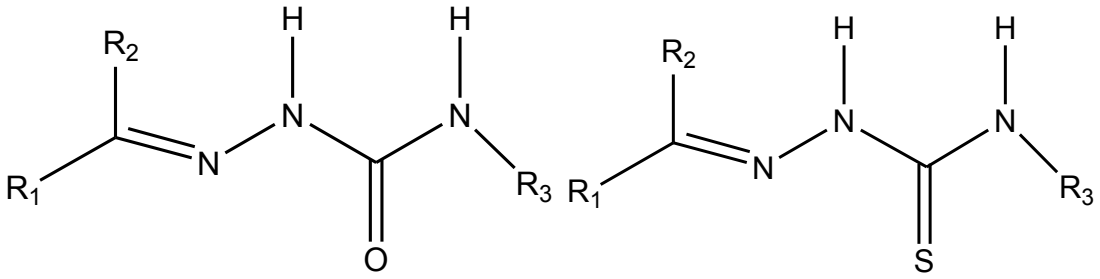
4. E.M.R. Kiremire, L.S. Daniel, H. Muashekele, K. Chibale and H. Kambafwile: The crystal structure of a new anti-malarial bis{3-[1-(2-pyidyl)ethylidene]hydrazinecarbodithioato}Cadmium(II) complex, Oriental Journal Of Chemistry. Vol. 23 (2), 415-422 (2007).

# CHAPTER 1: INTRODUCTION

## 1.1 General introduction

Medicinal application of metals can be traced back almost 500 years (Molavi, 2003). But the lack of experience by traditional medical chemists and pharmacologists in dealing with biologically active metal complexes, poses a substantial activation energy barrier to identifying active metal complexes and shepherding them to the clinic (Fujii *et al.* 2005). This factor retards the development of organometallic as well as of metal- based pharmaceuticals. However, it provides enterprising transitional metal chemists with opportunities to pioneer the development of new drugs (Gómez-Bosquet *et al.*, 1998).

The synthesis and structural investigation of thiosemicarbazones and their metal complexes are of considerable attention because of their potentially beneficial pharmacological properties and the wide variation in their modes of bonding and stereochemistry (Vinod *et al.,* 2006). Thiosemicarbazones that are most widely studied are the sulphur and nitrogen-based ligands (Marina *et al.,* 2007). Besides, thiosemicarbazones have emerged as important sulphur containing ligands in the last two decades (Chen-Jie *et al.,* 1999; Sampath *et al.,* 2003; Martin *et al.*, 2007; Tudor *et al.,* 2007). Semicarbazones and thiosemicarbazone (Fig. 1) as well as their metal complexes have been the subject of great interest of many researchers for a number of years (Martin *et al.,* 1997; Chen-Jie *et al.,* 1999; Sampath *et. al.,* 2003; Leovac *et al.,* 2005, Vinod *et. al.,* 2006, Marina *et al.,* 2007; Tudor *et. al.,* 2007). On the other hand, isothiosemicarbazides and isothiosemicarbazones were the subject of monographs (Soumitra *et al.,* 1998; Leovac *et al.,* 2005).




(a) Semicarbozone (b) Thiosemicarbazone

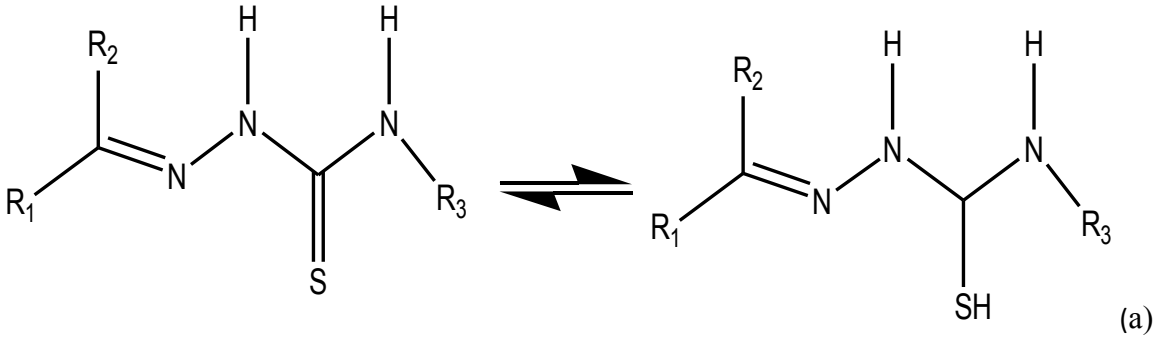
**Figure 1.** General structures of (a) semicarbazone and (b) thiosemicarbazone ligands. Where $R_{1,2\ or\ 3}$ can be alkyl, aryl or H.

### 1.2 The importance of metal complexes

The significance of these compounds, apart from their diverse chemical and structural characteristics, stems not only from their potential but also from their proven application as biologically active molecules with a wide spectrum of activities (Leovac *et al.,* 2005; Vinod *et al.,* 2006; Marina *et al.,* 2007; Tudor *et al.,* 2007). This is especially related to thiosemicarbazones and their metal complexes, for which the relationship between structure and biological activities has been covered by West *et al.* (1993) and Leovac *et al*. (2005). Furthermore, many semicarbazones and thiosemicarbazones form stable colored metal complexes, some of which have been proposed as analytical reagents (Martin *et al.,* 2007).

### 1.3 The coordination mode of the metal complexes

The coordination chemistry of semicarbazone and thiosemicarbazone appears to be very interesting from the point of view of both the number of metals forming complexes with them and the diversity of the ligand systems themselves which include macrocyclic systems (Lima *et al.,* 1999; Zahid *et al.,* 2005). Different metal complexes synthesized from organic thio-based ligands are described in the literature (Jose *et al*., 2002; Bakir *et al* 2005; Zahid *et al.,* 2005). Many of these were prepared for physiological studies and their structures were not fully discussed (Soumitra *et al.,* 1998; Martin *et al.,* 2007). Thiosemicarbazone can exist in several tautomeric forms, but the most interesting ones are those shown in figure 2. Such molecular tautomerization can give rise to different binding modes. The molecules can act as tridentate (involving N, S and part of $R_1$ derivative atoms) in thione form or bidentate (involving N and S atoms) ligand in the thiol form.



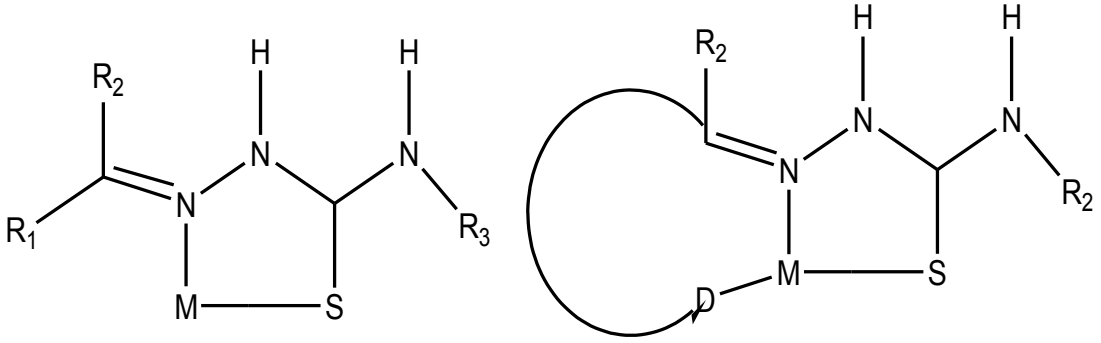

(a)

Thione form (b) Thiol form

**Figure 2.** Most important tautomeric forms of thiosemicarbazones

In the solid state, these thiosemicarbazones exist in the thione form (Fig. 2(a)); in solution, however, they are known to tautomerize into the thiol form, fig 2(b), (Basuli *et al.,* 1997 and 1998). Complexation usually takes place via dissociation of the acidic proton, resulting in the

formation of a five-membered chelate ring (fig 3 (a)). When an additional donor site D is incorporated in such ligands, via one or two intervening atoms, D, N, S tricoordination (tridentate) usually takes place (fig. 3(b)). Metal complexes displaying such coordination modes have been observed (Basuli *et al.,* 1997 and 1998).




(a) Bidentate coordination (b) Tridentate coordination

**Figure 3:** General coordination modes of thiosemicarbazone

### 1.4 Biological studies of thiosemicarbazone metal complexes

Thiosemicarbazones and their metal complexes have a wide range of biological properties. Because of this, a large number of organic and metal-organic ligands derived from thiosemicarbazones have been the subject of intense structural and medicinal studies. Some thiosemicarbazones have been found to be antibacterial (Cocco *et al.,* 1990; Cardia *et al.,* 2000; Kasuga *et al.,* 2001; Rodriquiz-Arguelles *et al.,* 2005), antifungal (Offiong and Martelli, 1993 and 1994; Chohan, 2003), antitumor (Ainstcough *et al.,* 1998; Sau *et al,.* 2003); antiarthritic (Missbash *et al.* 1996), antiamebic (Bharti *et al.,* 2003); antiviral (Varadinova *et al.,* 2001), antimalaria (Walcourt *et al.,* 2004) and more significantly some have been found to possess anti-HIV activity (Mishra *et al.,* 2002; Genova *et al.,* 2004; Bal *et al.,* 2005).

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Acetylferrocenyl thiosemicarbazone and its metal complexes.

### 2.1.1 The synthesis of acetylferrocenyl-4-phenylthiosemicarbazone ($HL^1$) and acetylferrocenyl-4-methylthiosemicarbazone ($HL^2$)

Information on the use of thiosemicarbazide for the preparation of ferrocenyl-containing heterocyclic systems (fig.4) are lacking in the literature (Zahid *et al.,* 2005). However, the metal complexes with thiosemicarbazones of ferrocenecarbaldehyde, acetylferrocene, and 1,1-diacetylferrocene have been reported (Jose *et al.*, 2002). Some thiosemicarbazone ligands reported have been synthesized by using equimolar quantities of each anisaldehyde, 4-

chlorobenzaldehyde, 4-fluorobenzaldehyde and vanillin in ethanol with an ethanolic solution of 4-phenyl thiosemicarbazide or 4-nitrophenyl-thiosemicarbazide (Belicchi *et al.,* 1992). The reaction mixtures were refluxed on a water bath for 1 hour. As precipitation appeared, the reaction mixture was allowed to further reflux while stirring for 2 hours. The precipitate formed was separated out, filtered off, washed several times with water, recrystallized from ethanol, and finally dried in vacuo over fused calcium chloride (Belicchi *et al.,* 1992).

Mishra *et al*. (2002) prepared a salicylaldehyde 4-phenylthiosemicarbazone ligand by addition of a methanolic solution containing an appropriate aldehyde to a methanolic solution of 4-phenylthiosemicarbazide. The reaction was then stirred at room temperature for 2-3 hours. The white needle-like products were collected by filtration and dried over KOH. Methanol was dried using magnesium turnings and iodine followed by distillation. The yield obtained was ……..???

Furthermore, Zahid *et al* (2005) reported the preparation of a 1,1'-diacetylferrocene- derived thiosemicarbazone ligand. In this case, the solution of 1-1'-diacetylferrocene in ethanol was added to a stirred hot ethanol solution of thiosemicarbazide. The mixture was refluxed for 4 hours. After allowing the solution to cool at room temperature, the solvent was evaporated to give a dark orange solid product. Removal of the solvent gave an orange crystalline solid which was recrystallized from hot dichloromethane.

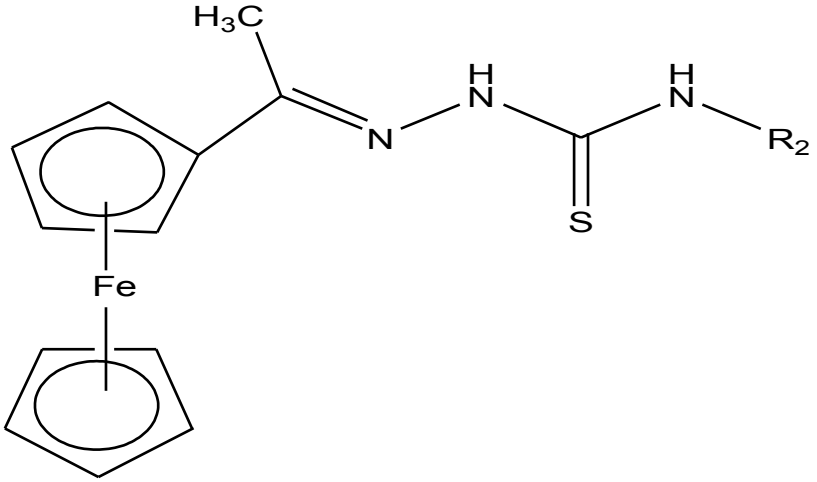



**Figure 4.** Structures of acetylferrocenyl-4-phenylthiosemicarbazone ($HL^1$; $R_2$ = phenyl (Ph)) and acetylferrocenyl 4-methylthiosemicarbazone ($HL^2$; $R_2$ = methyl (Me)).

Konstantinovic *et al.* (2007) reported the preparation of the isatin-ß-thiosemicarbazone (ITC) ligand by dissolving isatin-2,3-indolinedione and thiosemicarbazide in a 1:1 molar ratio in ethanol. The mixture was refluxed in a water bath for 1 hour. After cooling to room temperature, the yellow microcrystalline solid was separated, washed with ethanol, followed by diethyl ether and dried over silica gel. The yield was 56% (Konstantinovic *et al.,* 2007),

### 2.1.2 The synthesis and characterization of metal complexes containing $HL^1$ and $HL^2$.

Reports are available on the structures of thiosemicarbazones containing metal ions. The FT-IR spectra showed that $HL^1$ and $HL^2$ act as neutral or monobasic bidentate ligand, coordinating the transition metals through either thione- or thiol-sulphur and azomethime-N atoms (Zahid *et al.,* 2005)**.**

According to Bakir *et al*. (2005), both metal complexes of $HL^1$ and $HL^2$ were prepared by refluxing a mixture of a metal salt with the ligand dissolved in ethanol. An alternative method was used by Jose *et al*. (2002) and Elzahamy *et al,* (2008). In this case, sodium acetate was added to the copper complex with a thiosemicarbazone- containing ferrocenyl group ( Zahid *et al.,* 2005). Jose *et al.* (2002) also prepared copper complexes with monoacetylferrocene thiosemicarbazone. Youngjin *et al.* (1999) conducted studies on transitional metal complexes with 1-acetylferrocene-4-phenylthiosemicarbazone. Jose *et al. (*2005) further reported a series of six ferrocene derivatives containing a semicarbazone or thiosemicarbazone side chain that were investigated by cyclic voltammetry measurements. More recently, Kiremire *et al,* (2007) studied the phenomenal paramagnetic shift in certain ferrocene thio-bsased copper (II) complexes. It was found that wereas the Cu (II) paramagnetic influence may shift or even block the $^1$HNMR signals of the ligand in the complex; it completely inhibited the appearance of $^{13}$CNMR signals in the complexes studies. Furthermore, some literature on the synthesis and characterization of thiosemicarbazone ligands containing ferrocene and their metal complexes using $^1$HNMR, $^{13}$CNMR and FT-IR spectroscopy (Gomez-Bosquet *et al.,* 1998; Soumitra *et al.,* 1998; Bakir *et al.,* 2005) has been published.

### 2.1.3 The biological activities of metal complexes containing $HL^1$ and $HL^2$

The biological activities of acetylferrocenyl thiosemicarbazone metal complexes have been reported (Soumitra *et al.,* 1998). Acetylferrocenyl thiosemicarbazone metal complexes are able to suppress the proliferation of normal or transformed tumour cells (Frankline, 2004). It was discovered that numerous acetylferrocenyl thiosemicarbazone metal complexes inhibit the development of diverse experimental animals' tumour (eg. Ehrlichascites tumour, sarcoma 180, B16 melanoma and colon 38 carcinoma) and the growth of human carcinomas (Sulekh *et al.,* 2003). Certain ferrocenium compounds were especially cytostatically found to be effective against human colarectal carcinomas (Sulekh *et al.,* 2003; Frankline, 2004; Agarwal *et al*., 2005; Costa-Ferreira *et al.,* 2006).

Recently, Kiremire *et al.* (2006) reported that the metal complexes containing a dithio-based ligand were subjected to biological tests on falcipain-2 (FP-2) and falcipain-3 (FP-3) cystein protease enzymes from malaria parasite *Plasmodium falciparum*. The complexes exhibited high biological activity. Other biological studies of acetylferrocenyl metal complexes that are in the literature include those by Bakir *et al*. (2005) and Soumitra *et al.* (1998) reported biological studies of thiosemicarbazone containing ferrocene and their metal complexes against gram bacteria, gram positive and gram negative bacterial species.

## 2.2 Acetylpyridine 2-thiophenecarboxyl-semicarbazone ($HL^3$) and its metal complexes

### 2.2.1 The synthesis of acetylpyridine 2-thiophenecarboxyl-semicarbazone ($HL^3$) and its metal complexes

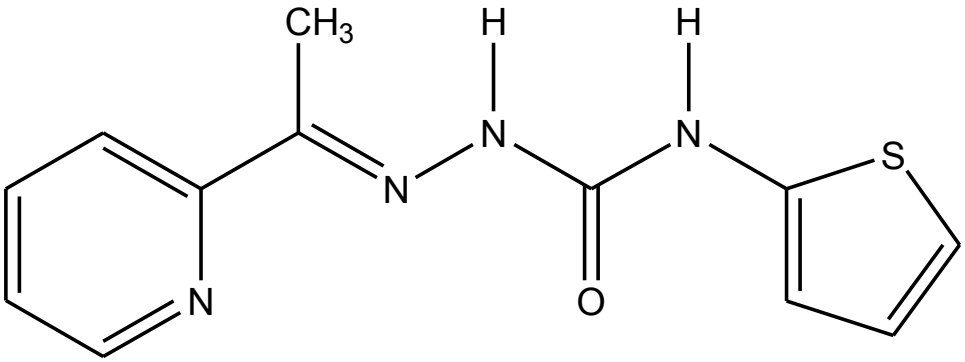



**Figure 5.** Structure of -Acetylpyridine 2-thiophenecarboxyl-semicarbazone ($HL^3$)

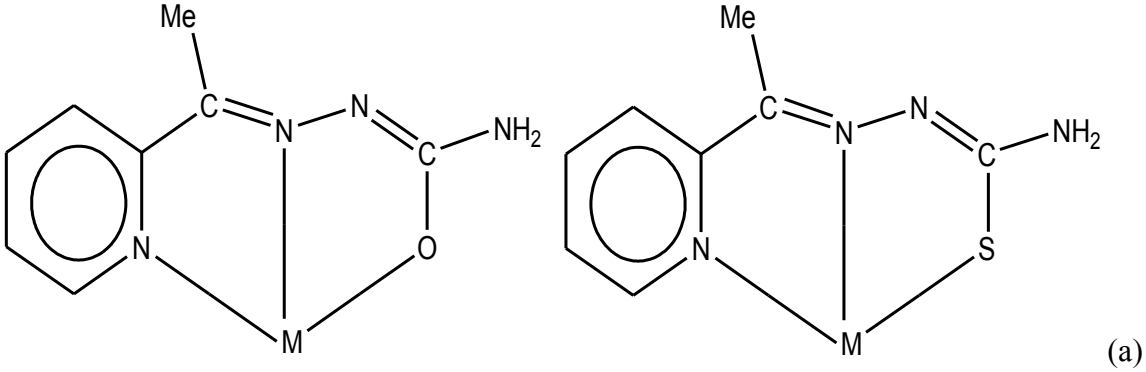
The study of acetylpyridine 2-thiophenecarboxyl-semicarbazone ($HL^3$), figure 5 and its metal complexes are totally lacking in the literature. However, a few studies of the related structures have been reported. Atalay and Akgemici (1998) reported the synthesis of 2-benzoylpyridine 4-phenyl-3-thiosemicarbazone. This ligand was synthesized by adding concentrated HCl to the solution of 2-benzoylpyridine and 4-phenyl-3-thiosemicarbazide in ethanol. The mixture was refluxed for 3 hours and then cooled to room temperature. The precipitated solid was filtered off and dissolved in hot water. The solution was neutralized with $Na_2CO_3$, the product filtered off, washed with hot water and then recrystallized with a water-ethanol (1:1) mixture. The yield was 57%.

The primary reason for to the synthesis of the complexes lies in the fact that these compounds can serve as models for studying a wide range of biological reactions which are catalyzed by enzymes in which pyridoxal phosphate (PLP) as the physiologically active form of pyridoxal, appears as an essential component (Leovac *et al.,* 2005). It has been shown that in the presence of metal ions, free pyridoxal can catalyze a variety of metabolic reactions, e.g. of amino acids (transmination, decarboxylation, racemization and carbon–carbon bond cleavage) in which PLP acts as a co-enzyme (Ainscough *et al.,* 1997). On the other hand, some recent pharmacological studies of Schiff bases of pyridoxal and aminoguanidine enabled Japanese authors to set up the hypothesis that this Schiff base is more effective than aminoguanidine which is known as a good inhibitor of the formation of advanced glycation end products, and is considered to be promising for the treatment of diabetic complications (Taguchi, 2002; West, 2004).

Like the majority of similar Schiff bases, pyridoxal semicarbazone and thiosemicarbazone are mainly obtained in good yield by the condensation reaction of aqueous or alcoholic solutions of pyridoxal and the corresponding semicarbazide derivative (De Sousa *et al.,* 2002). A deprotonated form of the neutral ligand can be obtained by adding a suitable base such as $CO_3^{2-}$ or $OAc^-$ (Belicchi *et al*., 1992).

### 2.2.2 The coordination mode of pyridoxal semicarbazone and thiosemicarbazones

The common coordination mode of these ligands is presented in fig. 6. It is reported that the coordination of ligand to copper is via an oxygen atom in the semicarbazone and via a sulphur atom in the thiosemicarbazone (Dauglas *et al.,* 1996; Lima *et al.,* 1999). The other coordination site is via pyridine nitrogen atom and leads to N-coordination. This results in tridentate coordination as shown in fig. 6 below (Lima *et al.,* 1999). Furthermore, according to Tudor *et al.* (2007), as well as Indrani *et al.* (2002), the geometry of the studied complexes are influenced by the nature of the ligand, which determines a variation of charge density at the coordination site and by the nature of the metal salts used in their preparation.




(a) Semicarbazone (b) Thiosemicarbazone

**Figure 6.** Coordination modes of deprotonated ligand forms of (a) pyridoxal semicarbazone- and (b) pyridoxal thiosemicarbazone.

### 2.2.3 The biological activities of pyridoxal semicarbazones and thiosemicarbazones

One of the main reasons why the literature concerning thiosemicarbazones is richer and more diverse than that of the semicarbazones is the higher biological activity of the former (John *et al.,* 1882). Since the pioneering work of Domagk in 1946 on the antituberculosis activity of *p*-acetamidobenzaldehyde thiosemicarbazone (trivial names Thiacetazone or Tibon), the number of papers concerning the pharmacological use of these compounds has dramatically increased, due to the wide spectrum of their biological activity found in recent years (De Sousa *et al.,* 2002).

Nowadays, it is known that thiosemicarbazones show antitumour, antiviral, antifungal, antibacterial and antimalarial activities (Kiremire *et al.,* 2007). The primary task of researchers

is to investigate new compounds in respect to their activity against tumours, viruses, fungi, bacteria or malaria. A correlation between the structure and biological activity has been established indicating that the tridentate NNS 2-(N)-heterocyclic thiosemicarbazones as most efficient as therapeutic agents (Bakir *et al.,* 2005). A new example of a potentially successful Fe (III) chelator in treating iron overload diseases is 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine®) which has recently entered clinical trials (Costa-Ferraira *et al*., 2006; Sulekh *et al.,* 2003). However, other compounds besides the pyridoxal thiosemicarbazone ligands have also been found to be potentially useful in pharmacological applications (Rodriquez-Arquelles *et al.,* 2005). It has repeatedly been shown that compared to the ligands, the thiosemicarbazone-based metal complexes (for example, Cu and Zn) are more efficient inhibitors of cancer cell growth (Bakir *et al.,* 2005).

As mentioned above, semicarbazones have rather limited biological activities spectrum (Omar *et al.,* 1984). They are mainly used as anticonvulsants, anti-protozoa agents, radioprotectors or radio-pharmaceuticals. There are also several reports on their anti-leukemia activity in mice, as well as on antimicrobial and pesticidal effects in plants (West, 2004; De Sousa, *et al.,* 2002). Among an ample variety of thiosemicarbazone derivatives and their metal complexes, only the examples of biological tests with pyridoxal-based ligands can be found in the literature ( Costa-ferreira *et al.,* 2006). One of the very promising applications of these compounds, such as pyridoxal aroylhydrazone is their use as iron(III) chelating ligands in treating diseases of iron overload (Ainscough *et al.,* 1998.). For this purpose, Offiong and Martelli (2006), after testing a number of compounds of this class, identified the most efficient molecule. They found 2-hydroxy-1-napthylaldehyde isonicotinoyl hydrazone was most effective in intracellular Fe chelation than desferrioxamine, the ligand of choice so far, and was capable of antiproliferative action in some tumors. A study of this and similar aroylhydrazone compounds *in vivo* is now in progress (Varodinova *et al.,* 2001). However, as far as the biological activities of ligands based on semicarbazone or thiosemicarbazone and their complexes are concerned, these are also mentioned in several reports (Costa-Ferraira *et al*., 2006; Sulekh *et al.,* 2003, Kiremire *et al.,* 2007, Soumitra *et al.,* 1998). Agarwal *et al*. (2005 a, b) made a major contribution to these studies with their *in vitro* investigations of the activities of copper(II) compounds.

John *et al,* (1982) reported on a series of 2-acetylpyridine thiosemicarbazone complexes that possess significant antimalarial activities. The molecular features that have been shown to be essential for antimalaria activities are the presence of a 2-pyridylalkylidene moiety or selenocarbonyl group (Vinod *et al.* 2006) (in contrast to a carbonyl group). These features would also be expected to promote effective transition-metal chelating properties (Soumitra *et al.,* 1998)**.** In addition, Zahid *et al.* (2005), has observed that the presence of certain bulky groups at position $N^4$ of the thiosemicarbazone moiety greatly enhances antimalarial activity.

Cardia *et. al.,* (2000), have reported a correlation between structure and anti-mycobacterium activity in a series of 2-acetylpyridine thiosemicarbazone. In many cases, by coordination to

different transition metals ions that can be found in biological systems, it is possible to obtain complexes that are more efficient drugs than the corresponding free ligands (Zahid *et al.,* 2005). Copper(II) complexes possess a wide range of biological activities and are among the most potent antiviral, antitumor and anti-inflammatory agents (Tudor *et al.,* 2007). For example, a copper(II) complex of 2-formylpyridine thiosemicarbazones has been shown to inhibit the RNA-depedent DNA polymerases and the transforming ability of Rous sarcoma virus (RSV) (Tudor *et al.,* 2007)**.**

In addition, copper(II) complexes of 2-acetylpyridine thiosemicarbazones possess strong antineoplastic activity against a number of transplantable tumors, spontaneous murine tumors and human tumors (Frankline, 2004)**.** The mechanism of their antitumor action is thought to involve either inhibition of the enzyme ribonucleotide reductase, an obligatory enzyme in a DNA synthesis (Ainstcough *et al.,* 1998), or creation of lesions in DNA strand (Frankline, 2004). The antifungal activity of Cu(II) complexes with 5-nitro-2-furfural thiosemicarbazone has been demonstrated (Offiong and Martelli, 1993; West *et al.,* 1993)**.** In addition, Agarwal *et al.*(2006), reported the synthesis, magnetospectral, antibacterial and antifungal properties of Cu(II) complexes of 4[N-(benzylidene)amino]-, 4[N-(4-methoxybenzylidene)amino]-, 4[N-(4-dimethylaminobenzylidene)amino]- thiosemicarbazone.

### 2.3. Malaria as a killer disease

Malaria is a major disease in developing countries, causing 1-2 million deaths in 2001 (WHO, 2002). This burdensome disease is caused by the parasite *Plasmodium falciparum* and the species *P. falciparum* is by itself responsible for the majority of deaths (Charlwood, 1997; Newlands, 1999). The parasite is transmitted by the female *Anopheles* mosquito through the liver and the blood of the mammalian host (see figure 7)**.** The symptoms occur at stage 6, the erythrocytic stage in figure 7 (Maitland *et al.,* 2003)**.**. There is a high concern with deaths of children due to severe malaria. Its syndromes are cerebral malaria and severe malaria both due to acidisis which leads to death within 24 hours after hospital admission (Maitland *et al.,* 2003). The most used antimalaria drug in the countries where the strains of the parasite are still sensitive to it is chloroquine (CQ) (Newlands, 1999; Kiremire *et. al.;* 2006, Bloland, 2001). Apart from other quinoline-based antimalarias, antifolates and antibiotrics, the thiosemicarbazones, semicarbazones and their synthetic derivatives are increasingly used to combat the malaria disease (Bloland, 2001, Maitland *et al.,* 2003).

Malaria is exceedingly prevalent in Africa (Newlands, 1999). In fact, malaria is one of the leading killer diseases in Africa (apart from the relatively more recent HIV/AIDS which is next in degree of magnitude in the Tropical Africa Region) (Kiremire *et al*., 2006). Despite the continued use of insecticides and pesticides to kill mosquitoes and various drugs to kill the protozoa, the rate at which malaria is killing

people is still increasing (Blumberg, 2006). In 1999, it was reported that 1.5 million children die of malaria annually in Africa, Latin America and South-East Asia (Newlands, 1999). In Namibia, the spread of malaria disease is very high in three regions; Caprivi, Kavango and Oshana (Newlands, 1999).

The eradication of malaria continues to be frustrated by the continued drug resistance of malaria (Newlands, 1999; Kiremire *et al.,* 2006). Therefore, there is a great need to continue to search for drugs, which are cheap and more effective.

A large number of collaborations are trying to develop a vaccine against malaria, as stated by the NIAID report (James and Miller, 2001). These researches target either the blood stage or liver stage of the parasite (Maitland and Marsh, 2003). As far as chemotherapy is concerned, the search goes on for (1) synthetic analogues of quinine and chloroquine, (2) artemisinin analogues, (3) febrifugin analogues, (4) inhibitors of fatty acid synthesis or of membrane synthesis (inhibitors of choline uptakes), and (5) proteases inhibitors (Woster, 2003; Wiesner *et al.,* 2003 ). Some of the current researches even benefit from results obtained in other therapeutic areas such as oeteoporosis and cancer (James and Miller, 2001).

Unfortunately there has been a spread of resistant strains of the parasite to the chloroquine in numerous regions such as South America, Africa and South-East Asia (James and Miller, 2001). The resistance is also an economic burden to these countries (Maitland *et al.,* 2003). Unlike resistance to antifolates (correlated with gene mutations), resistance to chloroquine may be due to a combination of a lower influx and a large efflux of the drug probably involving a chloroquine resistance transporter (PfCRT) and a P-glycoprotein-like protein (Pgh1) but this is not yet fully understood (Hyde, 2002). These facts demonstrate the need for a rapid detection of resistant strains to adapt a to the treatment and the development of new therapies (Bloland, 2001).

Biological activities of transition metal complexes are also well known (James and Miller, 2001). In this connection, it is of interest to examine the potentialities of the synthesis of heterocyclic ligands based on ferrocene, pyridine and thiosemicarbazide derivatives and to explore the possibilities of the use of these compounds as ligands for the preparation of transition metal complexes and test their biological activities against malaria. In this research, the preparation and characterization as well as the biological study of the complexes prepared was emphasized. The techniques employed in characterizing the complexes are Elemental analysis (EA), proton-NMR and infrared (IR) spectroscopy.

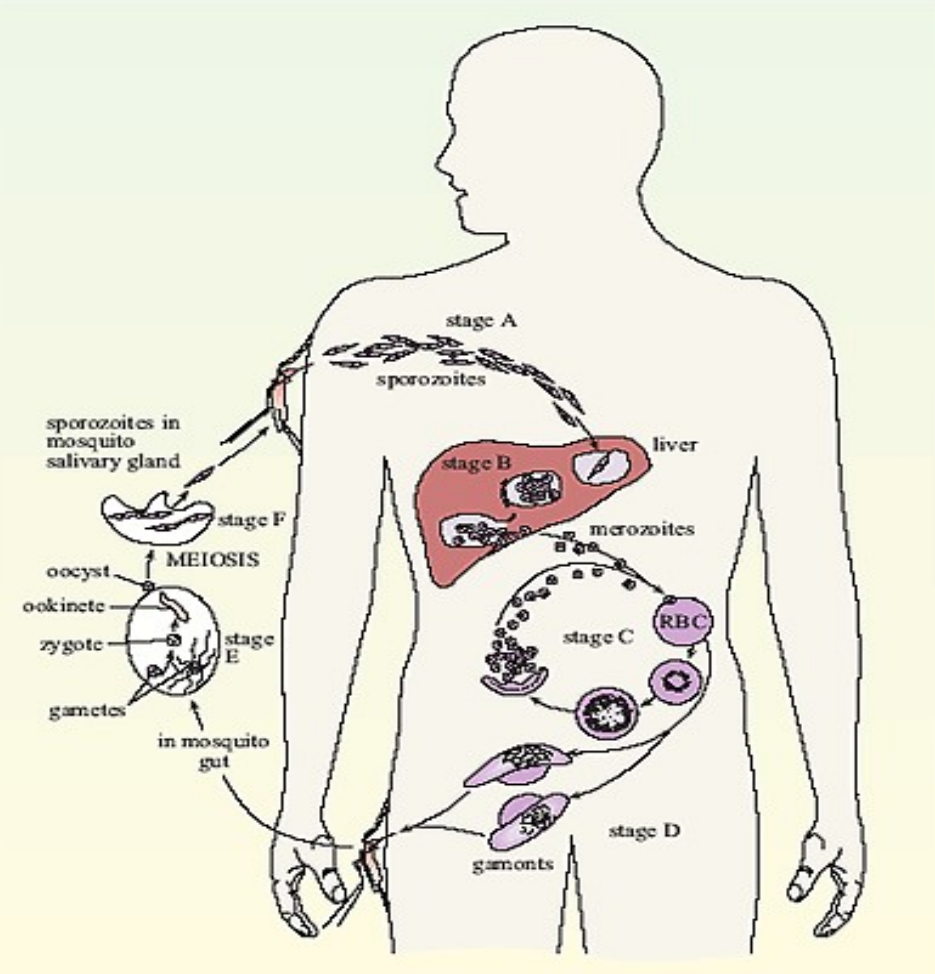



**Figure 7.** Schematic life cycle of the malaria parasite: from a bite of a female Anopheles mosquito to the liver-stage, through the erythrocyte-stage (blood stage) and back to the mosquito. (www.open2.net/.../worldaroundus/mosquitoes.html.2008)

**2.4 Statement of the problem**

Thiosemicarbazones that have been mostly characterized are sulphur and nitrogen containing compounds (Vinod *et al.,* 2006). Semicarbazone and thiosemicarbazone ligands and their metal complexes are of great interest because of their biological activity (Leovac *et al.* 2005, Vinod *et al.,* 2006, Marina *et al.,* 2007; Tudor *et al.,* 2007). Due to this, many compounds derived from semicarbazone and thiosemicarbazone have been the subject of most biological studies (West *et al.,* 1993). Some of the thiosemicarbazone and semicarbazone ligands have been found to be antibacterial, antifungal, antitumor, antarthric, antiamebic, antiviral, specific anti-HIV,

(Chohan, 2003; Varadinova *et al.,* 2001, Kasuga *et al.,* 2001; Cardia *et al.,* 2000; Cocco *et al.,* 1990 and very few possess antimalaria activity (Walcourt *et al.* 2004)

Limited literature information has been published on the synthesis and characterization of semicarbazone and thiosemicarbazone ligands containing ferrocene or pyridine fragments. Furthermore, nothing has been reported on the characterization of their copper complexes (Konstantinovic *et al.,* 2007). In several studies, the ligand and their copper complexes have been tested for their biological activities against bacterial, fungal, tumor and viral infections (Offiong and Martelli, 1994). However very little has been done on the test against malaria parasites. The test that was done by Rodriquiz-Arguelles *et al.* (2005), against bacteria shows that the ligands do not have biological activity, while their copper complexes have higher biological activity compared to other metal complexes.

Malaria is a major disease in developing countries (Newlands, 1999). The disease is known to be caused by the parasite, *Plasmodium falciparum* (Charlwood, 1997). Although chloroquine is widely used an antimalaria drug, strains of the parasite are still resistant to it (Newsland, 1999; Kiremire *et al.,* 2006). Pharmaceutical industries have been experiencing a number of problems related to the spread of malaria resistant strains against the chloroquine treatment (Charlwood, 1997). In recent years the industries have turned to semicarbazone and thiosemicarbazone containing complexes with the aim of finding more effective and cheaper pharmaceutically drugs (Bloland, 2001). Although many semicarbazone and thiosemicarbazone complexes that are biologically active have been known for a long time, the mechanisms of their biological activities are not fully understood (Belicchi *et al.* 2007).

**2.5 Aims of the study**

This research will focused on three aspects namely, the synthesis of new semicarbazone and thiosemicarbazone complexes with pyridine and ferrocene derivatives using a modified method than those reported in the literatures. The complexes were characterized using elemental analysis (EA), proton NMR, carbon-13 NMR, and IR specroscopy. Finally, their biological activities against the malaria parasite were tested. The antimalaria activity was evaluated against *falcipain-2* and *falcipain-3* of the malaria parasite. Furthermore, a test was done against cystein protease enzyme, *falcipain-2* of the malaria parasite (*in vitro)* and against chloroquine resistant strain *W-2 (in vitro)*.

**Objectives of the research project:**

0. To synthesizes metal complexes from acetylferrocenyl-thiosemicarbazone ($HL^1$ and $HL^2$), acetylpyrimidyl-thiosemicarbazone ($HL^3$) and acetylferrocenyl-semicarbazone ($HL^4$) ligands (see figure 8-11).
1. To analyze and identify the structures of the synthesized metal complexes by combining different spectroscopic techniques such elemental analysis (EA), proton-NMR, and infrared (IR) spectroscopy.
2. To test the biological activities of metal complexes against cystein protease enzyme, *falcipain-2* of the malaria parasite (*in vitro)* and against chloroquine resistant strain *W-2 (in vitro)*.

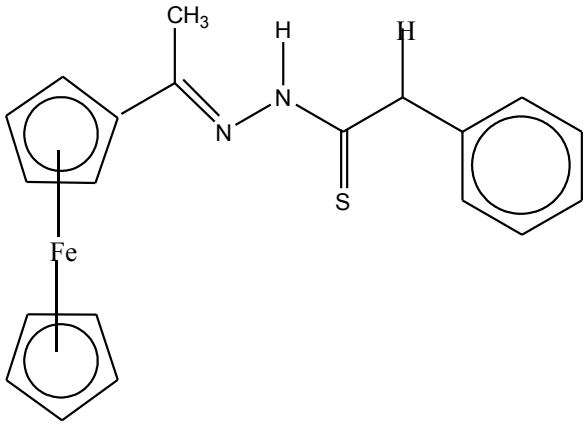



**Figure. 8** Ligand $HL^1$

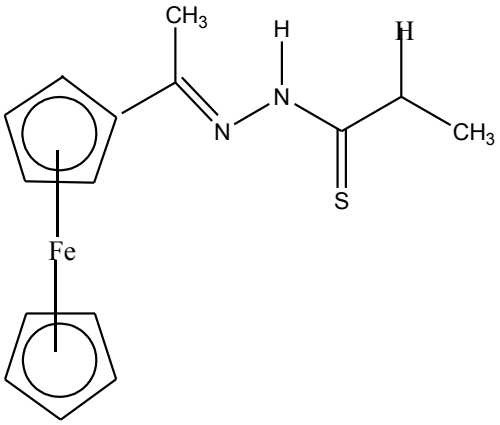



**Figure. 9:** Ligand ($HL^2$)

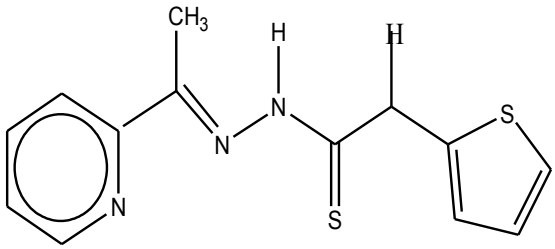



**Figure: 10** Ligand $HL^3$

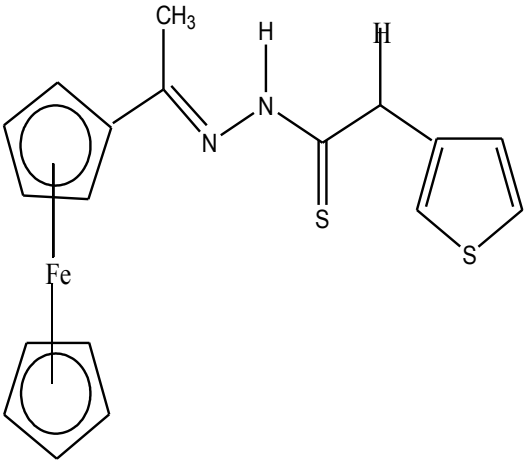



**Figure:11** Ligand $HL^4$

## CHAPTER 3: MATERIALS AND METHODS

### 3.1 Substances used in this study

All the starting chemicals and solvents **(Table 1)** were of reagent grade and were purchased from Aldrich Chemical Co. and were used without further purification**.**

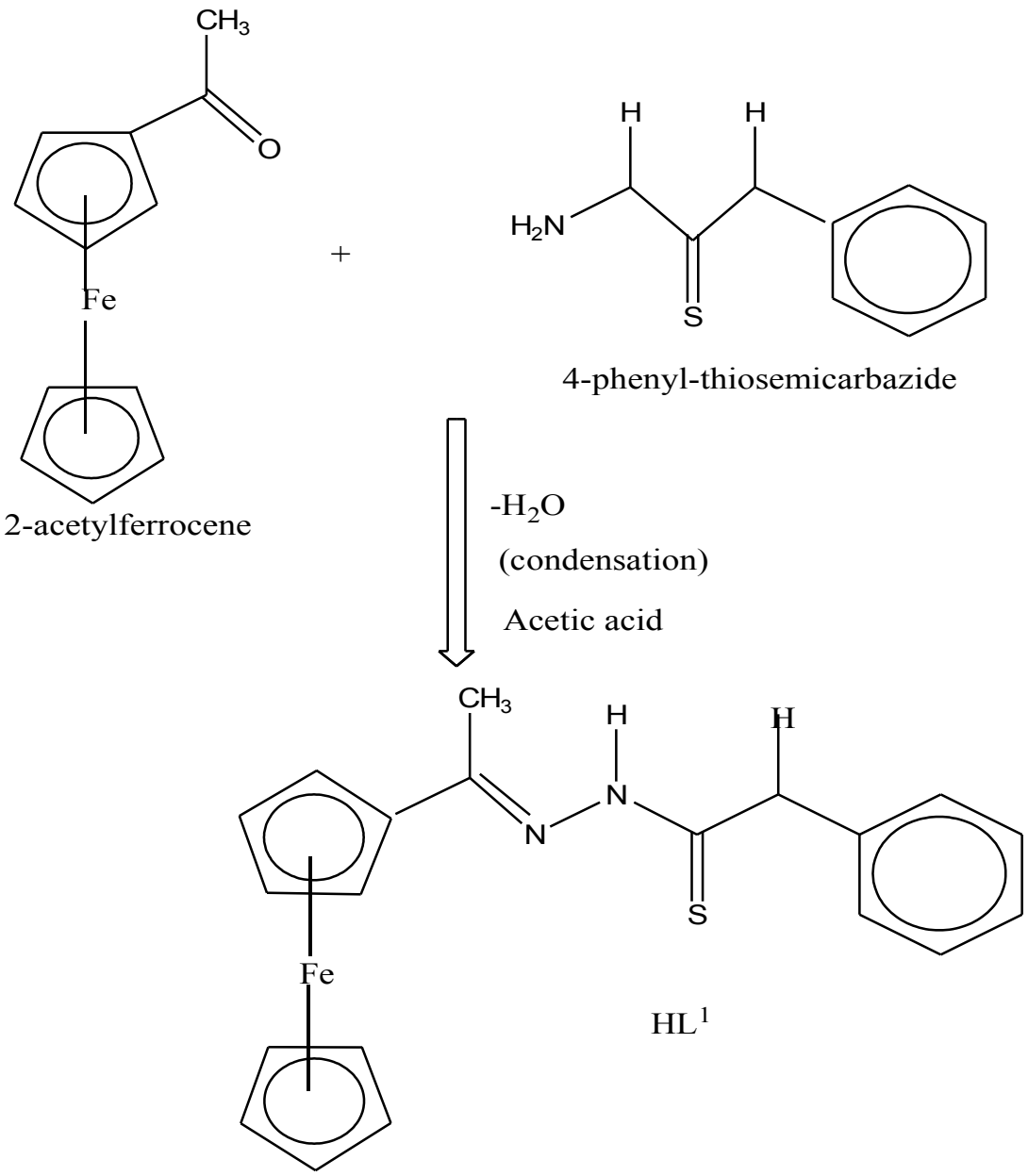
**Table 1.** Characterization of main starting materials used in this study.

| Starting compounds | Molecular formula | Molecular mass g/mol |
|---|---|---|
| 2-thiophenecarboxylic acid hydrazide, 98% | $C_5H_6N_2OS$ | 142.18 |
| 4-phenyl-3-thiosemicarbazide,99% | $C_7H_9N_3S$ | 167.23 |
| 2-acetylferrocene, 99% | $C_{12}H_{12}FeO$ | 228.07 |
| 2-acetylpyridine, 98% | $C_7H_7NO$ | 121.05 |
| 4-methyl-3-thiosemicarbazide, 99% | $C_2H_7N_3S$ | 105.04 |
| Copper(II) chloride hexahydrate | $CuCl_2.6H_2O$ | 270.32 |

### 3.2 The synthesis of the acetylferrocenyl thiosemicarbazone ligands

#### 3.2.1 The Synthesis of acetylferrocenyl-4-phenylthiosemicarbazone ($HL^1$) ligand

The acetylferrocene-phenylthiosemicarbazone ligand, $HL^1$ was prepared by using equimolar quantities of $8.7^{X}10^{-3}$ mole of 4-phenylthiosemicarbazide (1.46 g, 99%) and acetylferrocene (2.00 g) in 140 mL methanol. The reaction mixture was then refluxed for 3 hours at 70 °C. Acetic acid (2.5 mL) was added drop-wise at the beginning of reflux. After refluxing, the reaction mixture was transferred into a refrigerator at a temperature of 5°C for 24 hours. The brown precipitate was filtered off, washed with 20 mL of methanol, and then with 10 mL of ether and dried on a vacuum pump for 40 minutes. The percentage yield of unrecrystallized ligand was 56%. The schematic of the synthesis of $HL^1$ ligand is shown in scheme 1.

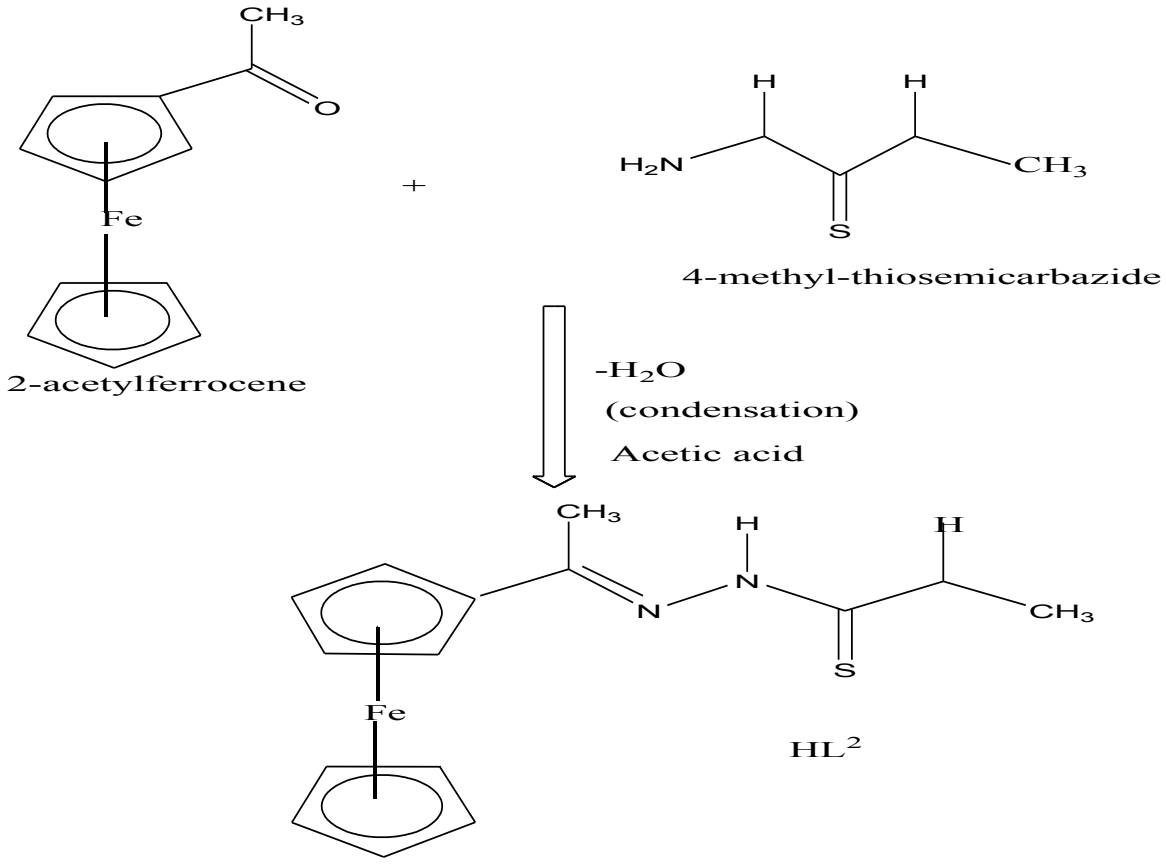



**Scheme 1.** Synthesis of $HL^1$ ligand

**3.2.2 The synthesis of acetylferrocene-4-methylthiosemicarbazone $HL^2$ ligand**

The acetylferrocene-4-methyl-thiosemicarbazone ligand, $HL^2$ was prepared by using equimolar quantities of $9.0X10^{-3}$ mole of each 4-methylthiosemicarbazide (0.95 g, 99%) and acetylferrocene (2.00 g) in 280 mL methanol. The reaction mixture was then refluxed for 3 hours. At the beginning of the reflux, 5 mL of acetic acid was added drop-wise. After refluxing, the reaction was transfer into a refrigerator at a temperature of 5°C for 24 hours. The brown precipitate was filtered off, washed with 15 ml of methanol, 10 ml of ether and dried on a vacuum pump for 40 minutes. The precipitate was weight and the percentage yield calculated.

Percentage yield of unrecrystallized ligand was 65%. The schematic of the synthesis of $HL^2$ ligand is shown in scheme 2.

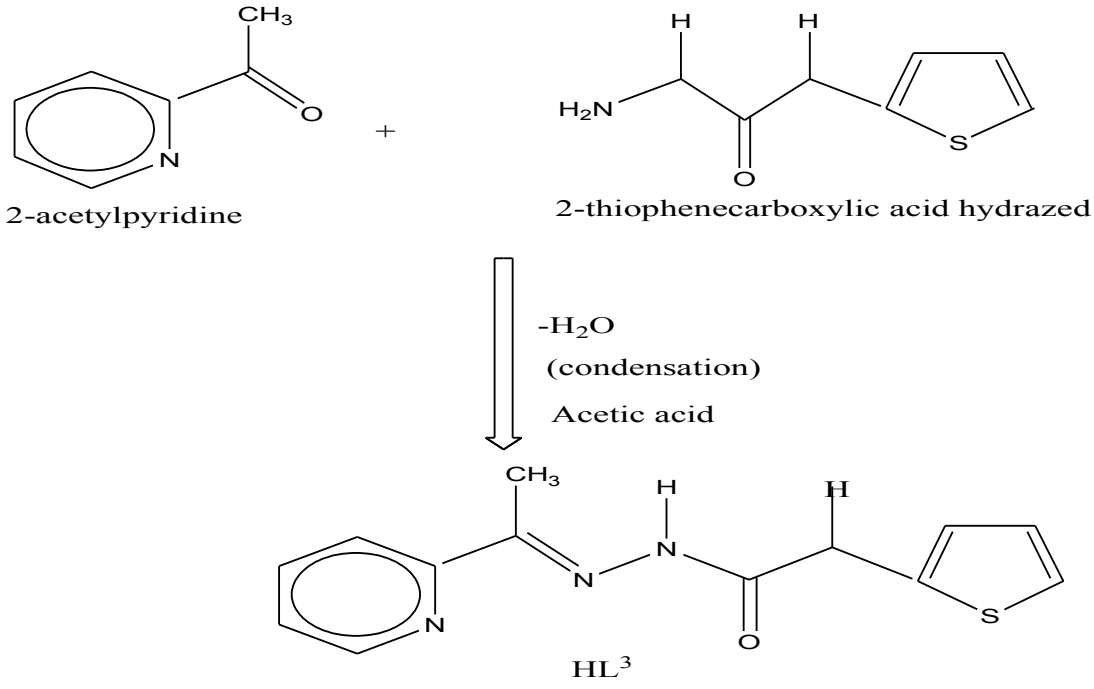



**Scheme 2.** Synthesis of $HL^2$ ligand

### 3.3 The synthesis of acetylpyridine-2-thiophenecarboxyl-semicarbazone ($HL^3$) and acetylferrocene-2-thiophenecarboxyl semicarbazone ($HL^4$) ligands

#### 3.3.1 The synthesis of acetylpyridine-2-thiophenecarboxyl-semicarbazone ligands ($HL^3$)

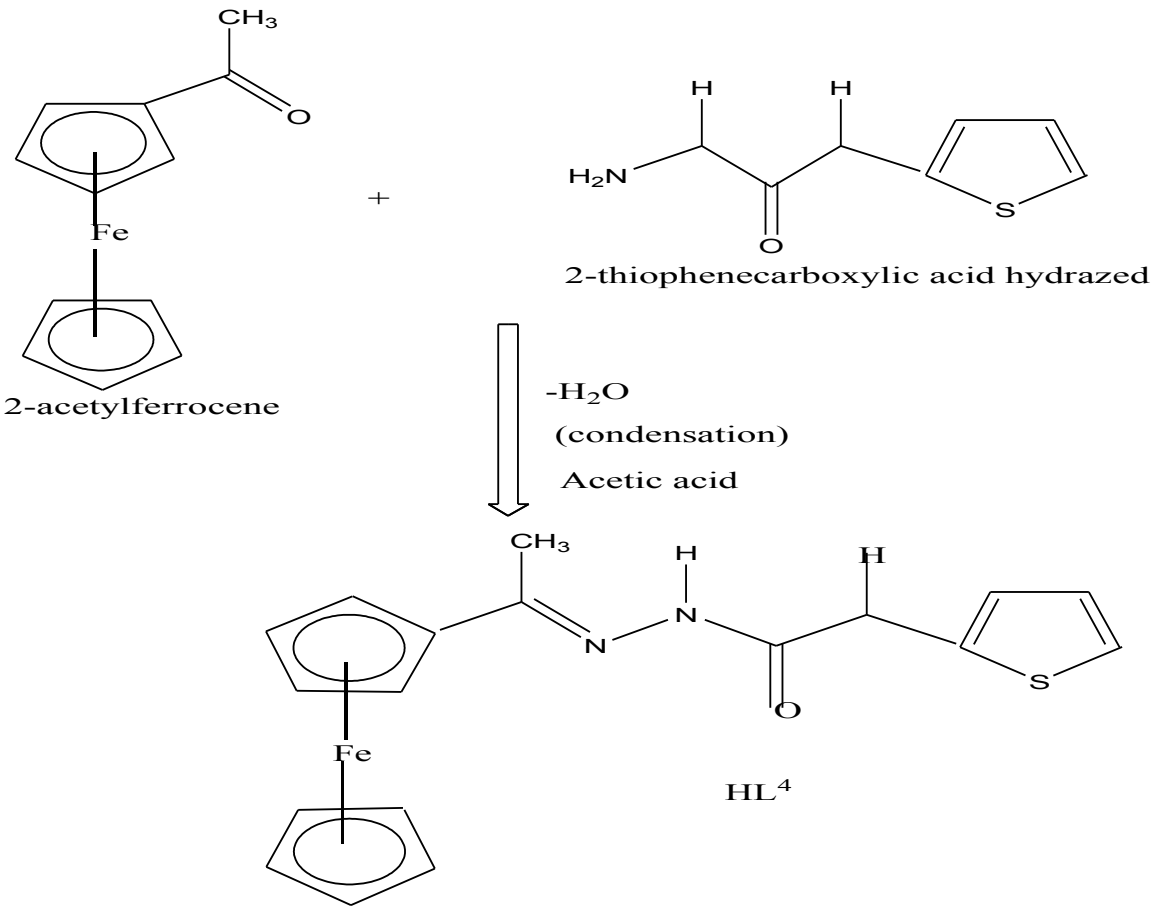
The pyridoxal semicarbazone ligand, $HL^3$ was prepared by adding equimolar quantities of 0.025 mole of 2-thiophenecarboxylic hydrazide (3.52 g, 99%) and acetylferrocene (3.00 g) in 200 mL ethanol. The reaction mixture was then refluxed for 3 hours. Acetic acid (1.5 mL) was added drop-wise. After refluxing, the reaction was transferred into a refrigerator at temperature of 5°C for 24 hours. White crystals of product were filtered off, washed with 10 mL of ethanol, followed by 10 mL of acetone, and then by 5 mL of ether and dried on a vacuum pump for 40 minutes. Percentage yield of unrecrystallized ligand was 73%. The schematic of the synthesis of $HL^3$ ligand is shown in scheme 3.




**Scheme 3:** Reaction of the synthesis of $HL^3$

### 3.3.2 The synthesis of acetylferrocene-2-thiophenecarboxyl-semicarbazone ($HL^4$) ligand

The acetylferrocene-2-thiophenecarboxyl-semicarbazone ligand, $HL^4$ was prepared by using equimolar quantities of $7.0\times10^{-3}$ mole of 2-thiophenecarboxylic acid hydrazide (1.00 g, 99%) and acetylferrocene (1.60 g) in 100 mL ethanol. The reaction mixture was then refluxed for 3 hours. At the beginning of refluxing, 3 mL of acetic acid were added drop-wise. After refluxing, the mixture was transfered into a refrigerator at temperature of 5°C for 24 hours. The dark brown crystals of product were filtered off, washed with 10 mL of acetone, then again with 10 mL of ether and dried on a vacuum pump for 40 minutes. Percentage yield of unrecrystallized ligand was 53%. The schematic of the synthesis of $HL^4$ ligand is shown in scheme 4.




**Scheme 4:** Reaction of the synthesis of $HL^4$

**3.4 The synthesis of copper (II) complexes**

**3.4.1 The synthesis of thiosemicarbazone complexes of the copper(II) metal.**

The copper(II) complexes of $HL^1$ and $HL^2$ were prepared according to the following procedure. A solution of the copper(II) chloride hexahydrate (0.10 g, 0.001mol) in water (50 mL) was added dropwise while stirring the solution of the ligand $HL^1$ (0.44 g, 0.002 mol) in acetone (150 mL). The precipitate was separated by filtration using micro-filter paper (0.45 µm), washed several times with ethanol, followed by 20 mL of diethyl ether and dried on a vacuum pump. The copper(II) complex of $HL^2$ (0.63 g, 0.002 mol) in 50 mL acetone was similarly synthesized using the same procedure.

**3.4.2 The synthesis of semicarbazone complexes of the copper(II) metal.**

The copper (II) complexes of $HL^3$ and $HL^4$ were prepared according to the following procedure. A solution of the copper(II) chloride hexahydrate (2.0 g, 0.01 mol) in water (100 mL) was added dropwise while stirring to a solution of the ligand $HL^3$ (2.87 g, 0.01 mol) in DMSO (50 mL). The precipitation was separated by filtration, washed several times with ethanol, 20 mL of diethyl ether and dried on a vacuum pump. The copper(II) complex of $HL^4$ ( $CuCl_2 .2H_2O$, 3.15 g, 0.001 mol and $HL^4$ in DMSO, 80 mL) was similarly synthesized using the same procdure.

### 3.5 The purification of ligands and metal complexes for analysis

All ligands and complexes were purified by dissolving them in a hot solution of methanol, filtering the hot solution on sintered crucibles whose pores were of size 0.45 μm and allowing the solution to evaporate to one-third (1/3) of the initial volume. Precipitates were formed in two to three days. Acetone and dimethylsulphoxide (instead of using methanol) were used to purify the $HL^3$ and $HL^4$ compound respectively. The precipitates were formed immediately. They were then sent to the University of Cape Town (South Africa), for elemental analysis, proton-NMR, carbon-13NMR and Fourier Transform-IR spectroscopy.

### 3.6 The biological studies of the synthesized ligands and their copper(II) complexes

The samples of all the ligands and copper complexes synthesised were sent to the University of California, San Francisco, USA for testing the biological activities against malaria. The antimalaria activity was evaluated against *falcipain-2* and *falcipain-3* of the malaria parasite. The test was done *in vitro* against cystein protease enzymes, *falcipain-2* and *falcipain-3* of the malaria parasite and against chloroquine resistant strain *W-2*.

# CHAPTER 4: RESULTS AND DISCUSSION

### 4.1. The physical properties of the compounds synthesized

The analytical and physical data for the compounds synthesized are shown in Table 2. The reaction of 4-phenyl-3-thiosemicarbazide, 4-methyl-3-thiosemicarbazide and 2-thiophenecarboxylic acid hydzide with acetylferrocene in methanolic solution yielded the corresponding thiosemicarbazone ligands ($HL^1$, $HL^2$ and $HL^4$ respectively). The reaction of 2-acetylpyridine with 2-thiophenecarboxylic acid hydzide in ethanolic solution also yielded the corresponding semicarbazone $HL^3$ with molecular formula $C_{12}H_{11}N_3SO$ (Mw 245.32 g/mol). All the ligands formed were brown in colour, except $HL^3$ which is white.

The ligands ($HL^1$, $HL^2$, $HL^3$ and $LH^4$) were prepared by refluxing the appropriate amount of an ethanolic solution of 2-acetylferrocene with the corresponding thiosemicarbazide (for $HL^1$ and $HL^2$) and semicarbazide (for $HL^4$), in 1:1 molar ratios in the presence of acetic acid. The structures of the synthesized ligands were established with the help of their elemental analysis, IR and NMR spectroscopy (Table 2, 3 and 4) respectively. The elemental analyses data agree well with the proposed formulae for the ligands (figs 7, 8, 9 and 10). This is because the theoretically calculated percentage values are in close agreement with the experimental values obtained from elemental analysis. The reactions of acetylferrocene–4-phenyl thiosemicarbazone ($HL^1$), acetylferrocene-4-methylthiosemicarbazone ($HL^2$) and acetylferrocene-4-thiophenesemicarbasone ($HL^4$) with copper(II) chloride hexahydrate affords the corresponding cyclometallated copper complexes, in which one hydrogen atom has been lost from the aza hydrogen site via a hydrogen chloride elimination reaction (Fig. 11) **(**Dongwon *et al.,*1996).

**Table 2.** The physicochemical characteristics of synthesized ligands and their copper(II) complexes.

| **Complex** | **Formula** | **Molar mass g/mol** | **colour** | **Yield %** | **Elemental analysis, % found (% calculated)** | | |
|---|---|---|---|---|---|---|---|
| | | | | | **C** | **H** | **N** |
| **$HL^1$** | $C_{19}H_{19}N_3SFe$ | 377.02 | orange | 56 | 60.49 (60.46) | 5.04 (5.19) | 11.14 (11.03) |
| **$CuL^1Cl_2$** | $C_{19}H_{18}CuN_3SCl_2$ | 510.35 | green | 73 | 51.61 (51.34) | 4.30 (6.03) | 9.32 (9.46) |
| **$HL^2$** | $C_{14}H_{17}N_3FeS$ | 315.25 | brown | 65 | 53.37 (53.51) | 5.40 (5.17) | 13.34 (12.70) |
| **$CuL^2Cl_2$** | $C_{14}H_{16}N_3CuSCl_2$ | 323.87 | dark brown | 54 | 40.70 (40.32) | 3.88 (3.93) | 10.18 (9.63) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **$HL^3$** | $C_{12}H_{11}N_3SO$ | 245.32 | white | 73 | 58.78 (58.92) | 4.49 (4.47) | 17.14 (17.06) |
| **$CuL^3_2$** | $(C_{12}H_{10}N_3OS)_2Cu$ | 345.07 | green | 45 | 41.99 (41.36) | 2.92 (2.85) | 12.25 (11.87) |
| **$HL^4$** | $FeC_{17}H_{16}N_2SO$ | 352.26 | brown | 53 | 57.99 (58.02 | 4.55 (4.19) | 7.96 (7.28) |
| **$CuL^4_2$** | $(FeC_{17}H_{15}N_2SO)_2$ Cu | 471.17 | red | 43 | 57.91 (53.30) | 4.15 (3.96) | 7.19 (7.32) |

Solubility test of all the ligands and complexes was done in order to determine the suitable solvents that could be utilized for recrystallization and other spectroscopic measurements. Table 3 shows that all compounds synthesized, none of them is soluble in water. $HL^1$ and $HL^2$ are very soluble in common organic polar solvents, such as ethanol, methanol, chloroform, acetone and ether, while $HL^3$ and $HL^4$ are poorly soluble in these solvents at room temperature. $HL^3$ is partially soluble in chroloform and highly soluble in dimethylsulphoxide (DMSO) and dimethylformamide (DMF). $HL^4$ is insoluble in ethanol and ether but soluble in other solvents listed in the table. The interaction of these ligands with $CuCl_2$ in a 1:1 molar ratio in methanol (except with ligand $LH^3$ where DMSO was used as a solvent) yielded a stable solid copper(II) complexes. The copper(II) complexes that are derived from $HL^1$ and $HL^2$ are green ($CuL^1Cl_2$ and $CuL^2Cl_2$ respectively), while $CuL^3_2$ and $CuL^4_2$ complexes are brown and red respectively. The copper(II) complexes made from $HL^1$ and $HL^2$ are not soluble in common polar solvent such as ethanol and methanol while the copper(II) complexes synthesized from $LH^3$ are only soluble in DMSO and DMF. The copper complex derived from $HL^4$ ligand is soluble in all organic solvents shown in the table 3.

The solubility test showed that the solid compounds are all air and moisture stable, and are intensely colored amorphous solids, which decompose without melting. These compounds can be stored for months without any significant changes in their composition. The color of aqueous solutions did not change no matter how long the compound remained in the solution, indicating that this thiosemicarbazones do not decompose in organic solvents.

**Table 3.** The solubility tests of the $HL^1$, $HL^2$, $HL^3$ and $HL^4$ and their copper(II) complexes

| | **Solvents** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **compoud** | **$H_2O$** | **EtOH** | **MeOH** | **$(CH3)_2CO$** | **$Et_2O$** | **$CHCl_3$** | **DMSO** | **DMF** |
| $HL^1$ | - | + | ++ | +++ | ++ | ++++ | ++++ | ++++ |
| $CuL^1Cl_2$ | - | - | - | ++ | - | ++++ | ++++ | ++++ |
| $HL^2$ | - | - | + | +++ | - | ++++ | ++++ | ++++ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $CuL^2Cl_2$ | - | - | - | + | - | + | +++ | +++ |
| $HL^3$ | - | - | - | - | - | + | +++ | ++++ |
| $CuL^3_2$ | - | - | - | - | - | - | +++ | +++ |
| $HL^4$ | - | - | - | + | - | ++ | +++ | ++ |
| $CuL^4_2$ | - | ++ | +++ | +++ | + | ++++ | ++++ | ++++ |

**Key:** **-** insoluble, + partially soluble, ++ soluble, +++ very soluble, ++++ very very soluble

The resulting complexes, green for $CuL^1Cl_2$ and $CuL^2Cl_2$, dark brown for $CuL^3_2$ and red complex for $CuL^4_2$, were isolated as air stable microcrystalline solids in high yield (table 2). These compounds are virtually insoluble in common organic solvents but soluble in DMF and DMSO (table 2).The complexes synthesized are monometallic centred compounds as revealed by the results of partial elemental analysis which are in good agreement with assigned formulations as shown in table 2.

**4.2 The Fourier Transform-Infrared spectra**

The IR spectra of $HL^1$, $HL^2$, $HL^3$ and $HL^4$ are shown in the appendix $A_1$-$A_4$ and the data extracted are given in Table 4.

**Table 4:** Infrared absorption frequencies ($cm^{-1}$) of $HL^1$, $HL^2$, $HL^3$ and $HL^4$.

| **$HL^1$** | **$HL^2$** | **$HL^3$** | **$HL^4$** |
|---|---|---|---|
| 3372m 3323s 3099s 3018s 3013w 2983m 2929s 2855m 1595m 1523m 1498m 1467m 1446m 1414w 1358w 1284m 1258w 1235w 1183m 1123m 1107m 1078sh 1041m 1026w 1002s 983sh 532sh 410m | 3346s 3177m 3101m 3061m 3031m, 3019m, 3008m 2927m 1632, 1580w 1567sh 1516sh 1492w 141m4 1378m 1338m 1248m 1125 1158m 1097m 1045m 103w 2011s 993w 532w 410m | 3413s 3264m 3084m 3033m 3020w 3015s 2927s 2855w 1599w 1547sh 1567sh 1516w 1497s 1491m 1440s 1318m 1222s 1211w 1231w 1219s 1081w 1065w 1002m 953w | 3413s 03349s 3101m 3099m 3033w 3014sh 3019m 2927m 2855s 1759w 1683sh 1638s 1601sh 1528w 1492w 1399m 1370m 1277w 1223s 1214sh 1222m 1219w 1108w 1031s 1002s 954w 410s |

*s*=strong; *m*=medium; *w*= weak; *sh*=shoulder

The significant IR bands of the ligands, their metal complexes and their assignments are given in Table 5. The chief IR bands of the ligands were identified on the basis of data of similar

compounds (Agarwal *et al.,* 2005 b). The IR spectra of the ligands are almost identical in the region of 1002-1550 $cm^{-1}$ to those of their corresponding copper complexes. This is because the molecular functional groups in both the ligands and the corresponding complexes are identical.

**Table 5.** Analyzed infrared absorption frequencies ($cm^{-1}$) of ligands and their copper(II) complexes.

| **Assign-ments** | **$LH^1$** | **$CuL^1Cl_2$** | **$LH^2$** | **$CuL^2Cl_2$** | **$LH^3$** | **$CuL^3{}_2$** | **$LH^4$** | **$CuL^4{}_2$** |
|---|---|---|---|---|---|---|---|---|
| ***ν*(C-H)** | 3018 | 3021 | 3019 | 3020 | 3020 | 3019 | 3020 | 3019 |
| **Ferrocenyl group** | 3099, 1498,410 | 3084, 1497, 410 | 3101, 1492, 410 | 3101, 1491, 410 | - | - | 3099 1492 410 | 3099 1492 410 |
| ***ν*(C=$N^{1`}$)** | 1595 | 1599 | 1580 | 1599 | 1595 | 1601 | 1638 | 1639 |
| ***ν*($N^{1`}$-$N^{2`}$)** | 1002 | 1002 | 1011 | 1034 | 1002 | 1002 | 1001 | 1001 |
| ***ν*($N^{2`}$-H)** | 3323 | - | 3346 | - | 3394 | - | 3349 | 3349 |
| ***ν*($N^{4`}$-H)** | 3413 | 3413 | 3412 | 3413 | 3413 | 3413 | 3413 | 3413 |
| ***ν*($C^{3`}$=S)** | 1414 | - | - | - | 1413 | - | 1414 | - |
| ***ν*($C^{3`}$=O)** | - | - | 1515 | | | | 1516 | 1516 |
| ***ν*($C^{3`}$=$N^2$** | - | 1568 | - | 1528 | - | 1528 | - | - |
| ***ν*($C^{3`}$-O)** | - | - | - | 1210 | - | - | - | 1208 |
| ***ν*(Cu-Cl)** | - | 1187 | - | 1168 | - | 1185 | - | 1187 |

The spectra of all synthesized compounds show no broad band in the range 2500-3300 $cm^{-1}$ assigned to *ν*(OH), suggesting the absence of water molecules ((Jose *et. al.,* 2002)). Furthermore, all ligands showed the absence of a band at ~1715 $cm^{-1}$ due to the characteristic carbonyl *ν*(C=O) stretching vibration of the respective starting acetylferrocene and acetylpyridine (Sharp, 2005). Instead, the appearance of a new band at 1599 $cm^{-1}$ is assigned to the *ν* C=$N^{1`}$(see Figure 9) double bond vibration arising from the condensation reaction to form the proposed ligands (Agarwal *et al.,* 2005 b)**.** The absorption frequency (*ν*) is given by the equation:

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

where *k* is the spring constant for the bond, and $\mu$ is the reduced mass of the two atoms in the chemical bond.

As can be deduced from the equation, since the atomic mass of sulfur is bigger than that of carbon, the reduced mass of C=S is bigger than that of C=O, the absorption frequency of C=S will be lower than that of C=O.

Absorption bands occurring at *ca.* 3018-3021 $cm^{-1}$ for v(C-H) is for methyl and aromatic C-H fragments present in all compounds (Sharp, 2005). According to Zahid et al (2005), the characteristic ferrocene group bands appear at ca. 3084-3101, 1492-1498 and 410 $cm^{-1}$. $HL^3$ does not show bands at 3084-3101, 1492-1498 and 410 $cm^{-1}$ indicating the absence of ferrocenyl group as expected. All ligand bands at around *ca.*1595 $cm^{-1}$ for *v*(C=$N^1$), *ca.* 1002 $cm^{-1}$ for *v*($N^{1'}$-$N^{2'}$), *ca*. 3349, *ca*. 1038-1040 for *v*(C-N) and *ca*.3413 for *v*($N^{4'}$-H) confirmed the suggested structures **(**Agarwal *et al.,* 2005 b **)**.

In all copper(II) complexes, the band, *ca.* 1413 $cm^{-1}$ remains almost at the same position confirming that $N^{4'}$-H did not participate in chelation (Sharp, 2005). The band at *ca.* 3323-3349 for *v*($N^{2'}$-H) is absent in all complexes; however, the band at *ca.* 1595$cm^{-1}$ for v(C=$N^{1'}$) higher (*ca.* 1-9 $cm^{-1}$) in the complexes and indicates an interaction with the metal. It is known that if a metal coordinate bond is formed with a nitrogen atom already bonded to N atoms, a shift of the N-N stretching bands to higher frequencies occurs, probably due to the increase in the polarity of the N-N bond (Agarwal *et al.,* 2005 b ). The absorption bond that occur at *ca.* ~1515 $cm^{-1}$ is assignable to *v*(C=O) (Jose *et al.,* 2002) and only occurs in the semicarbazone compounds. The IR spectra, shows bands at ca. ~1414 $cm^{-1}$ is for *v*(C=S) and only occurs in the thiosemicarbazone compounds (Bakir, *et al*., 2005).

The IR spectra of the free ligands display a sharp band at ca 3413 $cm^{-1}$, assignable to the $N^{4'}$-H group mode which remains at almost the same positions in the metal complexes, suggesting that the group is not involved in chelation (Bakir, *et al*., 2005). In the IR spectra of the free ligands, bands at *ca*. 3323-3395 $cm^{-1}$ disappear in the corresponding copper(II) complexes, indicating possible deprotonation of the ligands upon complexation (Singh *et al.,* 2005).

The bands at 1580-1595 $cm^{-1}$ and 1638 $cm^{-1}$ in $HL^1$ and $HL^2$ respectively, due to *v*(C=$N^{1'}$), are shifted to higher wave numbers (10-20 $cm^{-1}$) in the copper(II) complexes, suggesting coordination through the azomethine nitrogen (Bakir, *et al*., 2005). The bands at 1413-1414 $cm^{-1}$ and 1515-1516 $cm^{-1}$ due to v(C=O) and v(C=S), respectively, disappear in the complexes, indicating coordination of oxygen or surfur to the central metal atoms and formation of Cu-O or Cu-S type bonding. In addition, new weak to strong intensity bands are observed in the far IR spectra of the complexes. The band at *ca* 360-365, 305-315 and 422 $cm^{-1}$ can be assigned to *v*(Cu-N), *v*(Cu-S) and *v*(Cu-O), respectively (Sharp, 2005). The appearance of these bands further supports bonding of the ligands to the metal through nitrogen, sulfur and oxygen.

The coordination via the $N^{1'}$atom shifts $\nu(N^{1'}\text{-}N^{2'})$ to higher wavenumbers (0-33 $cm^{-1}$) than in the free ligands. The $C{=}N^{1'}$ stretching vibration also shifts to higher wave numbers. This occurs regardless of whether the ligand coordinates or not and the magnitude of the shift is likewise uncorrelated with the coordination mode, probably because it is also influenced by the involvement of $N^{4'}$ in hydrogen bonds when it does not coordinate to the metal. Coordination does not significantly affect the ferrocene as the bands for ferrocene remain unchanged at 3084-3101, 1492-1498 and 410 $cm^{-1}$ (Agarwal *et al.,* 2005 a).

The disappearance of the band *ca.* 1038-1045 cm-1 for $\nu(C^{3'}\text{-}N^{2'})$ provided further evidence in support of the involvement of this nitrogen in coordination to the copper atoms (Sharp, 2005 ). Furthermore, a characteristic band at 1528-1568 $cm^{-1}$ due to $\nu(C^{3'}\text{-}N^{2'})$ in the spectra of all the complexes suggests formation of the double bond and deprotonation of the $N^{2'}$-H (Chattertee and Ghosh, 1998). The bands, at 1413-1414 and 1514-1516 $cm^{-1}$ assigned to v(C=S) and v(C=O) respectively, in the free ligands are either shifted, split, or weakened in all of the complexes indicating the participation of the S or O-atom in complex formation (Jose *et al.,* 2002). Moreover, in the far infrared region the band at~360 $cm^{-1}$ attributed to v(Cu-N) was observed for all the complexes (table 3), which was not found in the spectral of the free ligands. It however, suggested coordination of the $\nu$(M-N) to the metal atom (Sharp, 2005).

The spectra of all studied complexes show no broad band absorption in the range 3157-3500 $cm^{-1}$ assigned to v(OH), suggesting the absence of water molecules (Sharp, 2005). $CuL^3{}_2$ and $CuL^4{}_2$ complexes show significant shift to lower frequencies for the v(C-O) band at *ca.* 1210 and 1208 $cm^{-1}$respectively (Chattertee and Ghosh, 1998 ). Table 3 also contains information on the region *ca.* 1413-1414 $cm^{-1}$ where a tentative assignment for the C=S vibration can be made (Sharp, 2005).

**4.3. Proton and Carbon-13 NMR spectra**

The $^1$HNMR and 13CNMR spectra of the free ligands, $HL^1$, $HL^2$, $HL^3$ and $HL^4$ in DMSO solutions in fig. 12-16 with assignments were collected in Tables 6-8. The $^1$HNMR spectra for free ligands, $HL^1$, $HL^2$, $HL^3$ and $HL^4$ were analyzed and are found to give the same signals, with the same integration ratios as their corresponding copper(II) complexes, except for the absence of the $N^{4'}$-H signal at around 9.80-8.20 ppm (Belkic, 2008). Since there are no big differences between the peaks for ligands and their copper (II) complexes, the spectra of the complexes are not presented here.

Figure 12 shows that the three broad signals had the integration ratios 2: 2: 1 at position 7.70, 7.39 and 7.18 respectively. These peaks may be assigned to the protons at hydrogen number 3, 4 and 5 for phenyl (Ph) protons as shown in figure 12 (Berger and Sicker, 2008). Clearly the signal at 10.22 $cm^{-1}$ be assigned to the proton at hydrogen number 2` for $N^{2'}$-H, as also shown in figure 12 (Belkic, 2008). The signals at 4.91, 4.40 and 4.21 may be assigned to ferrocenyl

protons (Berger and Sicker, 2008). The peak at 2.49 is assigned to DMSO protons in the solvent (Bible, 2008).

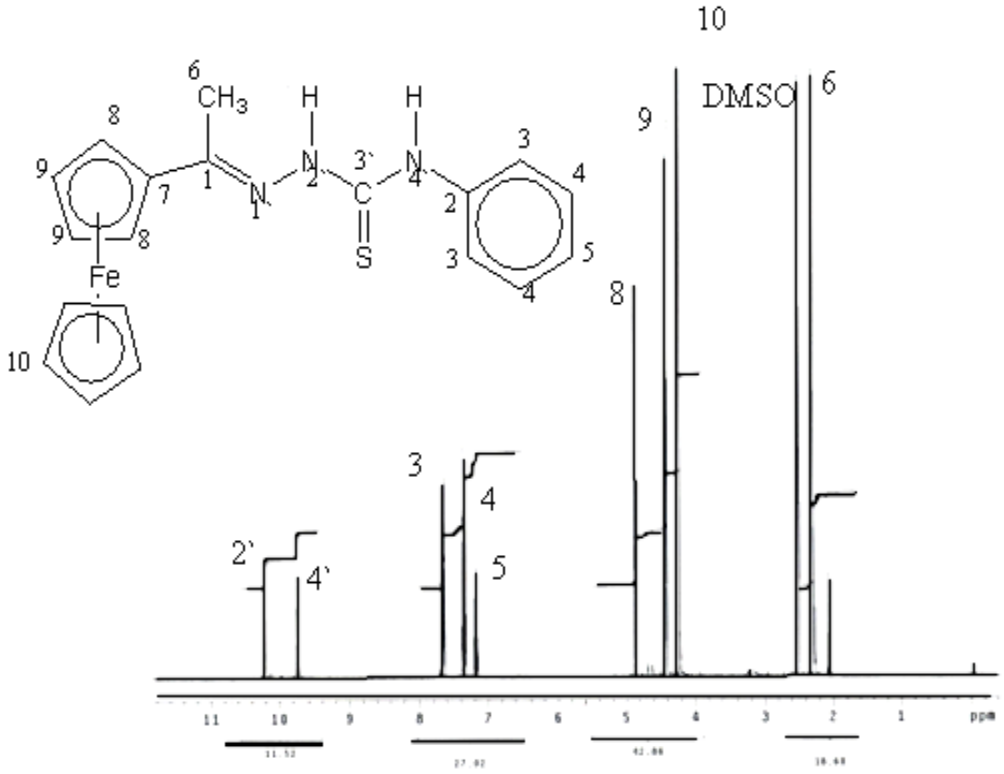



**Figure 12.** $^{1}$HNMR of ligand $HL^{1}$ with the assigned hydrogen numbers

The data reported along with the possible assignments in Table 6 shows that the free ligand, $HL^{1}$, displays all the expected signals at 2.28, 4.21-4.91, 9.75 and 10.22 ppm assigned to $CH_3$, ferrocenyl, $N^{4`}$-H and $N^{2`}$-H respectively (Belkic, 2008). In the spectra of their diamagnetic Cu (II) complexes (not shown), theses proton shifted slightly downfield by 0.1-0.3 ppm due to the increased conjugation and extension of the delocalized *p*-system of the thiosemicarbazine and semicarbazine group (Sharp, 2005).

**Table 6.** $^{1}$HNMR spectra of $LH^{1}$ in ppm (integration ratios) related to TMS.

| **$^{1}$HNMR of $HL^{1}$** | | | | | |
|---|---|---|---|---|---|
| **Peak position (integrat. ratio)** | **Hydrogen as assign in fig. 12** | **assignment** | **Peak position (integrat. ratio)** | **Hydrogen assignment in fig.12** | **assignment** |
| 10.22 (1) | 2` | $N^{2`}H$ | 4.91 (2) | 8 | ferrocenyl |
| 9.75 (1) | 4` | $N^{4`}H$ | 4.40 (2) | 9 | ferrocenyl |
| 7..70 (2) | 3 | Ph | 4.21 (5) | 10 | ferrocenyl |

| | | | | | |
|---|---|---|---|---|---|
| 7.39 (2) | 4 | Ph | 2.49 | - | DMSO |
| 7.18 (1) | 5 | Ph | 2.28 (3) | 6 | methyl |

$^{13}$CNMR spectra of the ligand $HL^1$ are presented in Table 7. The absorbtion at 199 ppm represents the carbon-1 (see assigned number from fig.12) (Case and Zuiderweg, 2008). The absorption at 184 ppm is assigned to carbon -3` that links to the sulfur atom (Agarwal and Prasad, 2005). absorbtion at 147, 128, 119 and 112 ppm are assigned to carbon in phenyl fragment while absorbtion at 79, 72,69 and 68 ppm are assigned to carbons that make up theferrocenyl fragment (Berger and Sicker, 2008). The absorbtion at 26 ppm is obviously for the methyl group carbon. The $^{13}$CNMR of the copper complex, $CuL^1Cl_2$, shows only one signal suggesting that ferrocenyl and phenyl rings carbons are broadened beyond recognition except of those for the methyl group (Frehlich, 2008). This happen due to paramagnetic effects (Kiremire, *et al.,* 2007). Paramagnetic shift is due to presence of an unpaired electrons on copper(II) ion. The magnetic moment of this electron interfere with the absorption of carbon signals by broadening them beyond recognition (Kiremire *et al.,* 2007).

**Table 7**: 13CNMR spectra for ligand $HL^1$ in ppm related to TMS.

| **$^{13}$CNMR of $HL^1$** | | | | | |
|---|---|---|---|---|---|
| **Peak position (ppm)** | **Assigned carbon # (fig.12)** | **Carbon assignment** | **Peak position (ppm)** | **Assigned carbon # (fig. 12)** | **Carbon assignment** |
| 199 | 1 | $C^1$=$N^{1`}$ | 79 | 7 | $C^7$-ferrocenyl |
| 184 | 3` | $C^{3`}$=S` | 72 | 8 | $C^8$-ferrocenyl |
| 147 | 2 | $C^2$-Ph | 69 | 10 | $C^{10}$-ferrocenyl |
| 128 | 3 | $C^3$-Ph | 68 | 9 | $C^9$-ferrocenyl |
| 119 | 5 | $C^5$-Ph | 29 | 6 | $C^6$-Methyl |
| 112 | 4 | $C^4$-Ph | | | |

The $^1$HNMR spectrum of $HL^2$ (See fig.13) showed absorption in the region 12 to 0 ppm (TMS). There are three signals upfield at 2.91, 2.34 and 2.28 ppm which may be assigned to the methyl protons (Berger and Sicker, 2008), DMSO protons and another methyl proton respectively (Case and Zuiderweg, 2008). The absorbtion at 4.90, 4.42 and 4.21 ppm by correlation with the absorption in $HL^1$ (fig. 12), may be assigned to ferrocenyl protons. The downfield peaks at 8.00 ppm are due to the imidazole protons (Agarwal and Prasad, 2005).

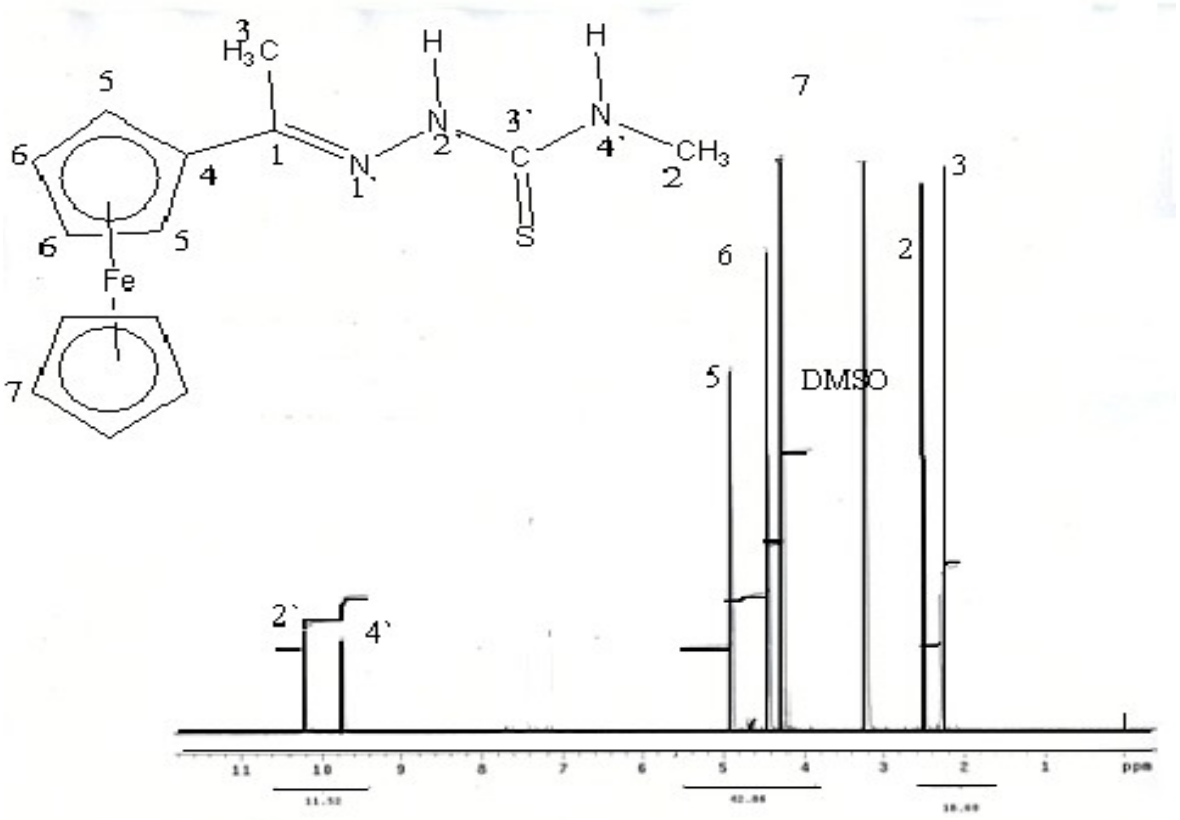



**Figure 13.** $^{1}$H NMR of ligand $HL^{2}$ with the assigned hydrogen numbers

In the $^{13}$CNMR spectra of $HL^{2}$, the ligands displays signals assigned to methyl, ferrocenyl, C=N, and C=S carbons, respectively (Belkic, 2008). The $^{13}$CNMR spectra of $HL^{2}$ are in keeping with their $^{1}$HNMR spectra (Table 7). $C^{1}$ can be seen to be slightly move shielded in the complexes than in the free ligands, which indicates a shift from the thione form $N^{2}$-$C^{3`}$=S towards the thiol form $N^{2}$=$C^{3`}$-S consequent upon coordination of the sulfur atom to the copper (Sharp, 2005). These signals appear downfield in comparison with the corresponding signals of the ligand indicating coordination with the central copper atom (Case and Zuiderweg, 2008). It was observed that DMSO did not have any coordinating effect on the spectra of the ligands or on its metal complexes (Kiremire *et al.,* 2007).

**Table 8.** $^{1}$HNMR spectra of $HL^{2}$ (see fig. 13) in ppm (integration ratios) related to TMS.

| **$^{1}$HNMR of $HL^{2}$(see Fig 13)** | | | | | |
|---|---|---|---|---|---|
| **Peak position (integrat. ratio)** | **Hydrogen as assign in fig. 13** | **assignment** | **Peak position (integrat. ratio)** | **Hydrogen as assign in fig. 13** | **assignment** |
| 10.23 (1) | 2` | $N^{2`}H$ | 4.21 (5) | 7 | ferrocenyl |
| 8.47 (1) | 4` | $N^{4`}H$ | 2.91 (3) | 2 | Methyl |

| | | | | | |
|---|---|---|---|---|---|
| 4.90 (2) | 5 | ferrocenyl | 2.34 (-) | - | DMSO |
| 4,42 (2) | 6 | ferrocenyl | 2.28 (3) | 3 | Methyl |

The $^{1}$HNMR spectrum of $HL^{3}$ (see fig 14) shows a absorption at 10.84 ppm due to the hydrogen atom of $N^{2`}$-H (table 8) (Ainscough *et al.,* 1998). The pyridine and thiophene rings protons were observed at ~8.90-7.12 ppm and 8.25-7.21 ppm respectively (Agarwal *et al.,* 2005(b)). These peaks form a broad singlet since they are in the same system because of conjugation. The peak at 2.45 ppm is for methyl group hydrogen numbered 6 in figure 14 (Case and Zuiderweg, 2008).

**Table 9.** $^{13}$CNMR spectra for ligand, $HL^{2}$ ( see fig. 13) in ppm related to TMS.

| **$^{13}$CNMR of $HL^{2}$(see fig. 13)** | | | | | |
|---|---|---|---|---|---|
| **Peak position (ppm)** | **Assigned carbon # (fig.13)** | **Carbon assignment** | **Peak position (ppm)** | **Assigned carbon # (fig.13)** | **Carbon assignment** |
| 201 | 1 | $C^{1}$=$N^{1`}$ | 68 | 7 | $C^{7}$- ferrocenyl |
| 181 | 3` | $C^{3`}$=O | 67 | 6 | $C^{6}$- ferrocenyl |
| 76 | 4 | $C^{4}$-ferrocenyl | 27 | 2 | $C^{2}$-methyl |
| 73 | 5 | $C^{5}$- ferrocenyl | 26 | 3 | $C^{3}$-methyl |

$^{13}$CNMR spectra in table 10 show a absorbtion at 156 ppm overlapping with carbon -1 and Carbon-3` respectively (carbons are assigned in fig. 14) (Kiremire *et. al.,* 2007). Absorbtion at position 139, 124, 128 and 122 ppm are assigned to pyridine carbon atoms and at 135, 124 and148 ppm for thiophene carbons (Sharp, 2005). Peaks at 40 is for methyl (Agarwal *et al.,* 2005(b).

The $^{1}$HNMR spectra of $HL^{4}$ showed absorbtion in the region 12 ppm to TMS (fig. 15). There are three signals at 4.91, 4.40 and 4.21 ppm in the ratio of 2:2:5 (Table 9). By correlation with the peaks in above ligands ($HL^{1}$ and $HL^{2}$), these peaks may be assigned to ferrocenyl protons (Bakir *et al.,* 2005). The downfield absorbtion at related to 7.40 ppm and at 8.10 ppm are for the thiophene protons (Sharp, 2005). Peaks at 2.45 and 2.28 ppm corresponded to DMSO and methyl protons (Atalay and Akgema, 1998).

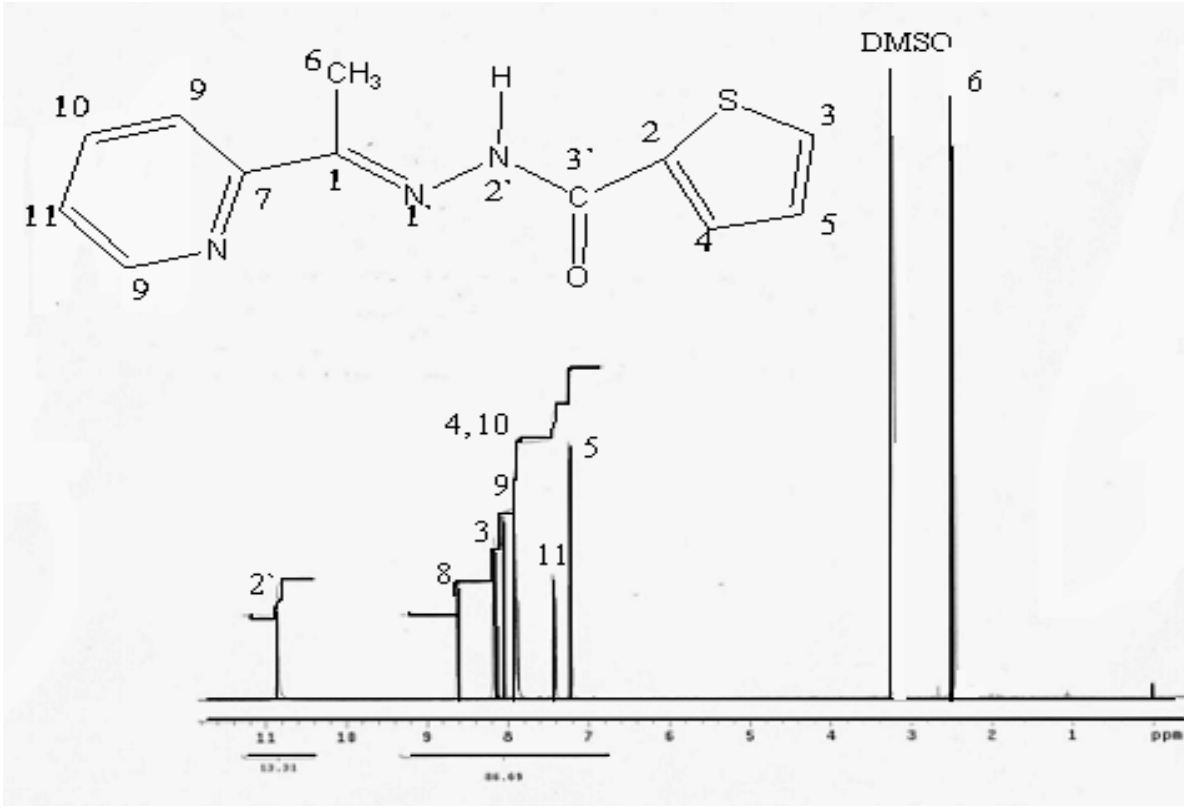



**Figure 14**. Structure of $HL^3$ (inset) showing the assigned hydrogen numbering

$^{13}$CNMR spectra data in table 8 at 201 and 181 ppm are for carbon -1 and Carbon-3` respectively (carbons are assigned in fig. 13) (Case and Zuiderweg, 2008). At 76, 73, 68 and 67 ppm are the absorptions that can be assigned to ferrocenyl carbon atoms (Abraham and Mobli, 2008). Absorption at 27 and 26 ppm are for methyl carbon atom (Bharti *et. al.,* 2003)

| **$^1$HNMR of $HL^3$(see fig. 14)** | | | | | |
|---|---|---|---|---|---|
| **Peak position (integrat. ratio)** | **Hydrogen as assign in fig. 14** | **assignment** | **Peak position (integrat. ratio)** | **Hydrogen as assign in fig. 14** | **assignment** |
| 10.84 (1) | 2` | $N^{2`}H$ | 8.12 (1) | 9 | pyridine |
| 8.18 (1) | 3 | thiophene | 7.90 (1) | 10 | pyridine |
| 7.85 (1) | 4 | thiophene | 7.47 (1) | 11 | pyridine |
| 7.26 (1) | 5 | thiophene | 2.48 (-) | - | DMSO |
| 8.73 (1) | 8 | pyridine | 2.45 (3) | 6 | $CH_3$ |

**Table 10:** $^1$HNMR spectra of $HL^3$ (see fig. 14) in ppm (integration ratios) related to TMS.

From figure 8-11 above, one can determine that, the free ligands ($HL^1$, $HL^2$, $HL^3$and $HL^4$) exhibit a singlet at 11.26-11.30 ppm and 11.40-11.55 ppm, due to the –NH proton (Sharp, 2005). The absence of this signal in the spectra of the complexes, suggests that this proton has been lost via thioenolization and ketoenolization of > C=S and > C=O groups and coordination of the sulfur and oxygen atom to the metal atom, respectively (Soumitra *et al.,* 1998). The complexes show resonances in the region 6.70-8.18 ppm attributable to aromatic protons, which appear almost in the same position as in the respective ligands (De Graaf, 2008). The $N^2$–H group gives a singlet at 3.40-3.48 ppm in the free ligands but disappear in the complexes. According to Soumitra *et al*.(1998), this shows that coordination induces a number of changes in the $^1$HNMR spectra of the ligands and the $N^2$–H group is deprotonated.

The $N^{4`}$-H gives a singlet at 9.75 ppm (Table 6) and at 8.47 ppm (Table 7) in the free ligands $HL^1$ and $HL^2$ as well as in the copper(II) complexes respectively. These also show that the –$N^{4`}$-H group is not taking part in the complexation (Sulekh *et al.,* 2003). The $^1$HNMR spectra of all the ligands except $HL^3$ and $HL^4$ show a single series of strong signals, suggesting that in these cases there is a single predominant species present (Jose *et al.,* 2002).

**Table 11:** $^{13}$CNMR for Ligand, $HL^3$ (see fig. 14) in ppm related to TMS.

| $^{13}$CNMR of HL$^{3}$(see Fig. 14) | | | | | |
|---|---|---|---|---|---|
| **Peak position (ppm)** | **Assigned carbon # (fig.14)** | **Carbon assignment** | **Peak position (ppm)** | **Assigned carbon # (fig.14)** | **Carbon assignment** |
| 156 | 1 and 3` | $C^{1}$=N and $C^{3`}$=O | 128 | 9 | $C^{9}$-pyridine |
| 148 | 2 and 7 | $C^{2}$-thiophene and $C^{7}$-pyridine | 124 | 5 and 11 | $C^{5}$-thiophene and $C^{11}$-pyridine |
| 139 | 8 | $C^{8}$-pyridine | 122 | 10 | $C^{10}$-pyridine |
| 135 | 4 | $C^{4}$-thiophene | 40 | 6 | $C^{6}$-Methyl |

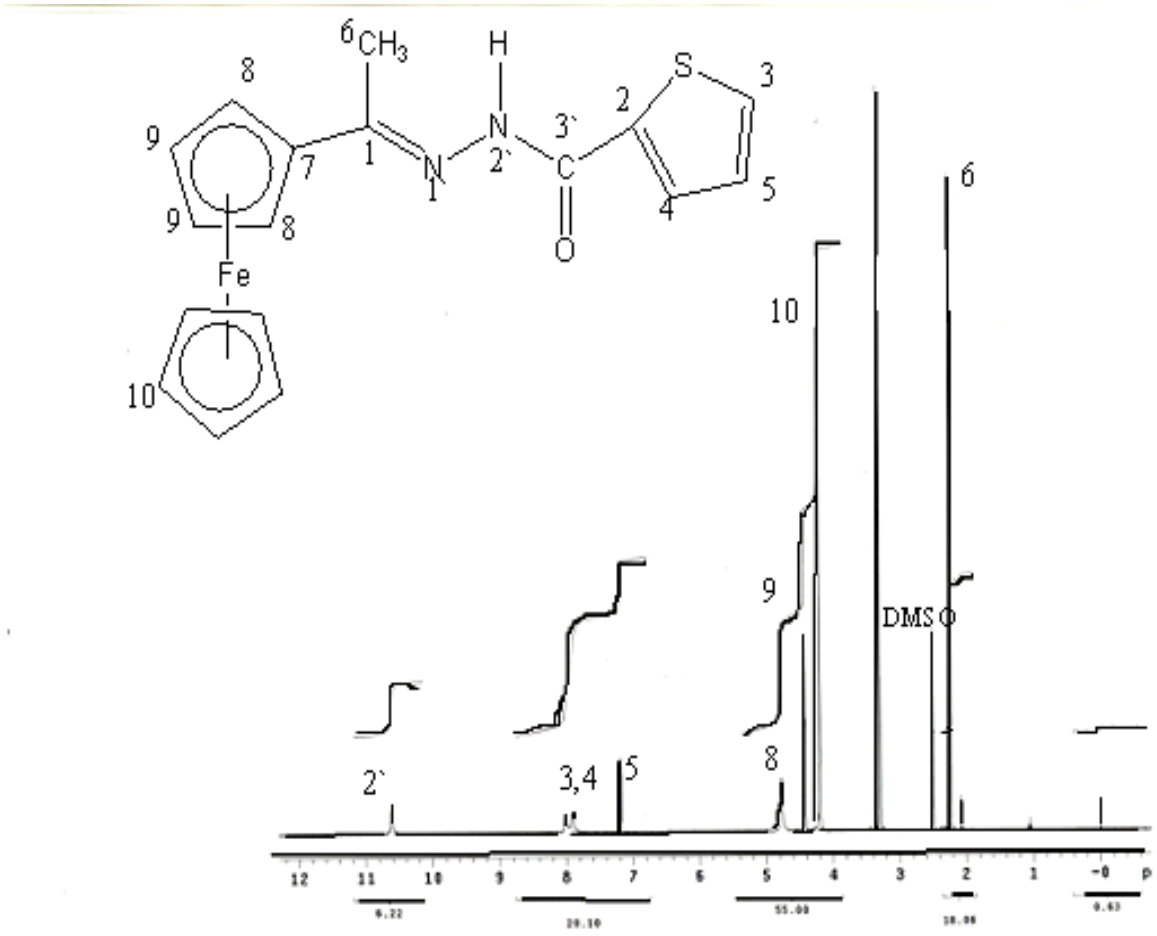



**Figure 15.** Structure of HL$^{4}$ showing the assigned hydrogen numbering

In $^{1}$H NMR spectra of complexes $CuL^{3}Cl_2$ and $CuL^{4}Cl_2$ (see table 10 and table 11 respectively), the signals for $N^{2`}$ protons disappeared and for the $N^{4`}H$ group are substantially shifted upfield (0.58-0.77 ppm) compared to those of the initial ligands. The behavior of two signals for $N^{2`}$-H and $N^{4`}$-H are additional supporting evidence for the proposed structures. The number of protons calculated from the intergration curves, and those obtained from the values of the expected CHN analyses are in agreement.

**Table 12.** $^{1}$HNMR spectra of the $HL^{4}$ (see fig 15) in ppm related to TMS.

| **$^{1}$HNMR of $HL^{4}$(see fig. 15)** | | | | | |
|---|---|---|---|---|---|
| **Peak position (integrat. ratio)** | **Hydrogen as assign in fig. 15** | **Possible fragment** | **Peak position (integrat. ratio)** | **Hydrogen as assign in fig. 15** | **Possible fragment** |
| 10.61 (1) | 2` | $N^{2`}H$ | 4.40 (2) | 9 | ferrocenyl |
| 8.01 (1) | 3 | thiophene | 4.21 (5) | 10 | ferrocenyl |
| 7.97 (1) | 4 | thiophene | 2.45 (-) | - | DMSO |
| 7.42 (1) | 5 | thiophene | 2.28 (3) | 6 | $CH_3$ |
| 4.91 (2) | 8 | ferrocenyl | - | - | - |

The $^{1}$HNMR AND $^{13}$CNMR spectra of the free ligands $HL^{3}$ and $HL^{4}$ and their copper (II) chelates were determined in DMSO-$d_6$. The $^{1}$HNMR spectra of the $HL^{4}$ shows a single series of strong signal, suggesting a cyclopentadienyl moieties of the ferrocenyl group (Simmons, 1978). The data reported along with the possible assignments in table 11 show that the free ligands display all the expected signals at 2.28, 4.21-4.91, 7.42-8.01 and 10.61 ppm assigned to $CH_3$, ferrocenyl, thiophene and $N^{2`}H$ respectively. In the spectra of their copper complexes, these protons shifted downfield by 0.1-10.1 ppm due to the increased conjugation and extension of the delocalized *p*-system of the semicarbazine chains into the cyclopentadienyl and pyridine ring system of $CuL^{4}Cl_2$ and $CuL^{4}Cl_2$ respectively.

**Table 13.** $^{13}$CNMR for Ligand, $HL^{4}$ (fig 15) in ppm related to TMS.

| **$^{13}$CNMR of $HL^{4}$** | | | | | |
|---|---|---|---|---|---|
| **Peak position (ppm)** | **Assigned carbon # (fig.15)** | **Carbon assignment** | **Peak position (ppm)** | **Assigned carbon # (fig.15)** | **Carbon assignment** |
| 201 | 3` | $C^{3`}$=O | 79 | 7 | $C^{7}$-ferrocenyl |
| 144 | 2 | $C^{2}$-thiophene | 72 | 8 | $C^{8}$-ferrocenyl |
| 133 | 3 | $C^{3}$-thiophene | 69 | 10 | $C^{10}$-ferrocenyl |
| 132 | 4 | $C^{4}$-thiophene | 26 | 6 | $C^{6}$-methyl |

| | | | | | |
|---|---|---|---|---|---|
| 128 | 5 and 9 | $C^5$-thiophene and $C^9$-ferrocenyl | - | - | - |

**4.4 Antimalarial studies**

The antimalarial activity of the copper(II) complexes synthesized together with the parent ligands has been screened against malaria parasite *Plasmodium falciparum*. The results (Table 14) exhibit that the complexes show moderate activity against the cysteine protease enzyme, *falcipain-2* (FP-2) and chloroquine-resistant (*W-2*) strain both from the malaria parasite *Plasmodium falciparum*. The activities are provided in nanomolar (nM) units which was evaluated based on strength ratio (S.R) for a better clarification. Compounds which have got a reading of 6000 nM or less are regarded as biologically active (Kambafwile, 2009).

**Table 14**. Antimalaria activity of synthesized ligands and their copper(II) complexes against FP-2.

| **Compounds tested** | **FP-2 average (nM)** | **FP-2 (SD)** | **Active / Not Active** |
|---|---|---|---|
| **Control** | 9.5 | 1 | A |
| **$CuL^1Cl_2$** | 473.65 | 24 | A |
| **$CuL^3_2$** | 4807 | 250 | A |
| **$HL^1$** | 5486.5 | 247 | A |
| **$HL^4$** | 56400 | 537 | A |
| **$CuL^4_2$** | 78775 | 615 | NA |
| **$HL^2$** | >100000 | - | NA |
| **$CuL^2Cl_2$** | >100000 | | NA |
| **$HL^3$** | >100000 | | NA |

*nM= nonamolar, SD= standard deviation, A= Biologically active, NA= biologically inactive.

The results of the biological activities of the ligands and their copper(II) complexes against malaria parasites are summarized in Table 14-15. The data indicate that none of the ligands, $HL^1$, $HL^2$, $HL^3$ and $HL^4$ showed biological activities with W-2 (table 15). However, $HL^1$ and $HL^4$ show biological activity with FP-2 (table 14). $HL^2$ and $HL^3$ showed no biological activities with either FP-2 or W-2.

**Table 15:** Antimalaria activity of synthesized ligands and their copper(II) complexes against W-2.

*nM= nonamolar, SD= standard deviation, A= biologically active, NA= biologically inactive.

$CuL^1Cl_2$ exhibited most biological activity against FP-2 but was less active against *W-2*. $CuL^3{}_2$ and $CuL^4{}_2$ show pronounced growth inhibition against FP-2 while $CuL^2Cl_2$ is most active against W-2. $CuL^1Cl_2$ was determined to be of intermediate activily against FP-2 while $CuL^4{}_2$ showed no activity against *W-2*.

| **Compounds tested** | **W-2 average (nM)** | **W-2 (SD)** | **Active / Not Active** |
|---|---|---|---|
| **$CuL^2Cl_2$** | 669.9 | 6 | A |
| **Control** | 2482 | 35 | A |
| **$CuL^1Cl_2$** | 11485 | 1338 | NA |
| **$CuL^3{}_2$** | 14580 | 1329 | NA |
| **$HL^1$** | >20000 | | NA |
| **$HL^2$** | >20000 | | NA |
| **$HL^3$** | >20000 | | NA |
| **$HL^4$** | >20000 | | NA |
| **$CuL^4{}_2$** | >20000 | | NA |

The complexes are more active than their corresponding ligands (Table 14), which may be due to the chelation and the presence of sulfur atoms (Soumitra *et al.,* 1998). This could be taken as evidence that the introduction of copper(II) to the thiosemicarbazone increased the biological activities (Soumitra *et al.,* 1998). Generally, chelation/coordination reduces the polarity of the metal ion by partial sharing of its positive charge with donor groups and possibly enhances the pi-electron delocation within the whole chelate ring (Brown *et al.,* 1976). This process thus increases the lipophilic nature of the compound, which in turn, favors penetration through the membrane wall of the strain and suggests that one of the enzymes involved in haemoglobin degradation is inhibited (Sulekh *et al.,* 2003; Agarwal *et al*., 2005).

All the synthesized compounds have a hydrophilic core (O or S group) and a perimeter of hydrocarbons group (methyl or phenyl) which confers the lipophilic trait (Chary and Govil, 2008). The hydrophilic core enhances the solubility of the group and presumably facilitates transport across the membrance (Brown *et al.*, 1976). It is proposed that a change in the level of liphophilicity of the compound is reflected in a change in the level of biological activity

(Durham *et al.,* 1974). Substitution of a methyl group for the thiophenyl group on compound $CuL^2Cl_2$ greatly decreased the liphophilicity and the resulting compound $CuL^4_2$ decreased in biological activity (Soumitra *et al.,* 1998).

Differences in lipophilicity exist between compounds $CuL^1Cl_2$ and $CuL^2Cl_2$. These compounds have the same active thiosemicarbazone unit and same ferrocenyl derivative, but while compound $CuL^1Cl_2$ has the most lipophilic chain (pheny unit) this greatly increases its lipophilic potential. This results in $CuL^1Cl_2$ having more biological activity then $CuL^2Cl_2$ (Lewis and Shepherd 1970).

Compounds $CuL^1Cl_2$, $CuL^2Cl_2$ and $CuL^4_2$ have the same nucleus with different substituents on the $C^{3`}$ and/or on amino group, $N^{4`}$. The difference in activity may lie in the electronic and steric properties of the substituents (Lewis and Shepherd, 1970). The most active compound was the one with the phenyl substituent on $N^{4`}$ (compound $CuL^1Cl_2$).

The intermediate activity of compound $CuL^3_2$ may be due to the free pyridine ring, which could readily interact with membrane components, while in compound $CuL^4_2$ the lower level of activity may result from the unfavorable geometry of the thiophenyl group substituted on the $C^{3`}$ concomitant with the absence of the highly lipophilic hydrocarbon chain (Kizilcikli *et al.* 2007). When the thiophenyl group is replaced with a methyl group as in compound $HL^2$, there is a total loss of activity (Vinod *et al.,* 2006, Kizilcikli *et al.,* 2007). Furthermore, replacement of the thiocarbonyl group of both $HL^1$ and $HL^2$ to give a $HL^4$ (with C=O instead of C=S) which was devoid of antimalarial activity, provides an indication of the essentiality of the surfur atom in this class of compound (Chary and Govil, 2008).

### 4.5 Suggested structural formulae of the synthesized ligands and their copper(II) complexes.

From the elemental analysis and the spectral data, the structure of synthesized copper(II) complexes may be formulated as shown in figures 16, 17, 18 and 19. The structures assigned to these compounds are confirmed by the IR, $^1$HNMR and $^{13}$CNMR spectroscopic data and by analysis of the analogous structures available in the literature (Turdor *e. al.,* 2007).

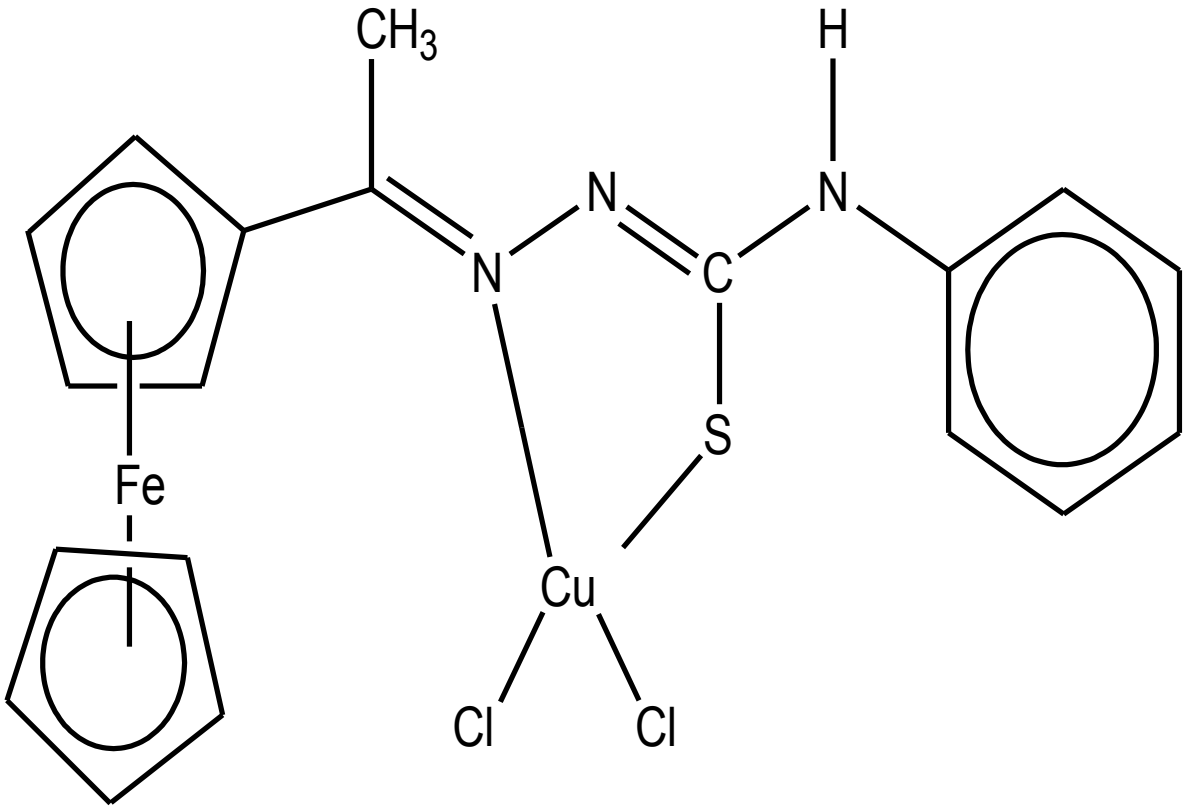



**Figure 16.** Structure of $CuL^{1}Cl_2$ complex

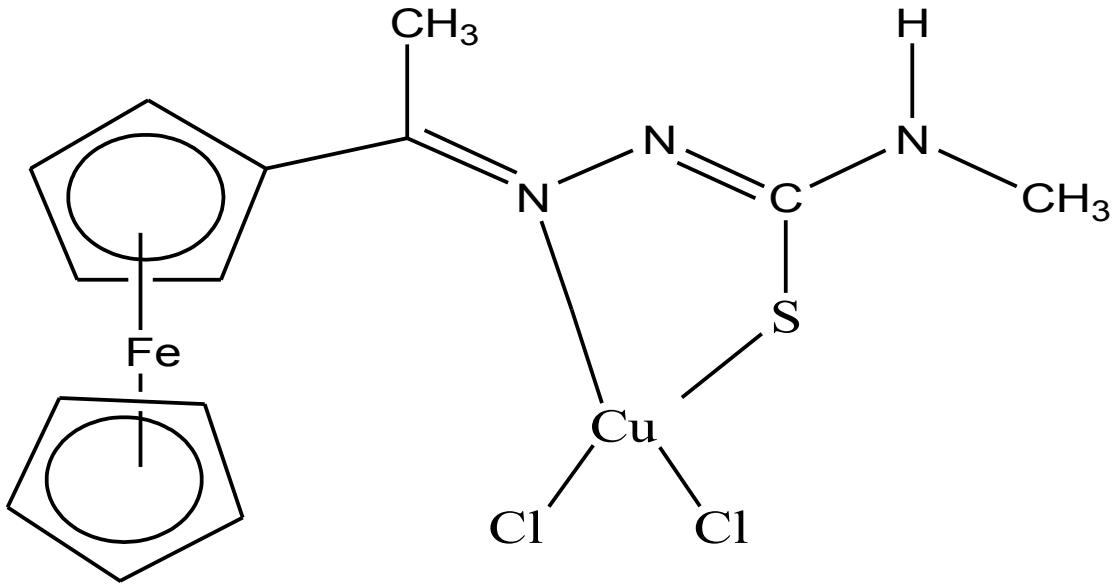



**Figure 17.** Structure of of $CuL^{2}Cl_2$ complex

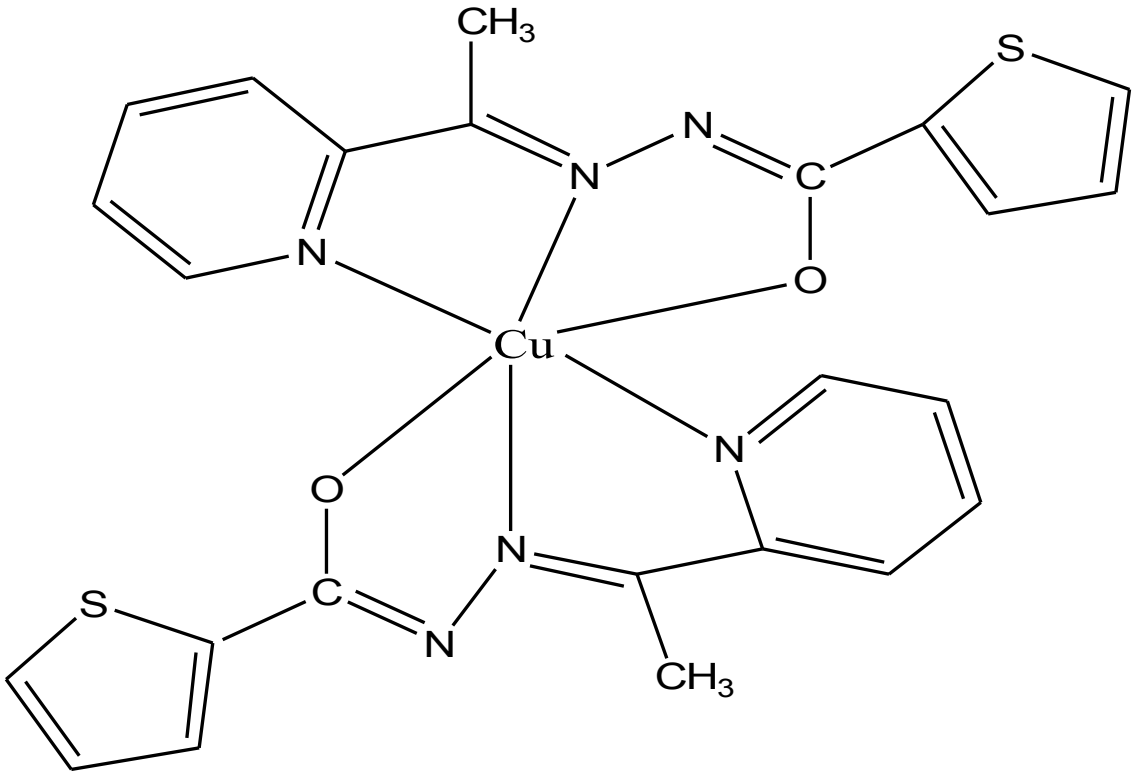



**Figure 18.** Structure of of $CuL^{3}_{2}$ complex

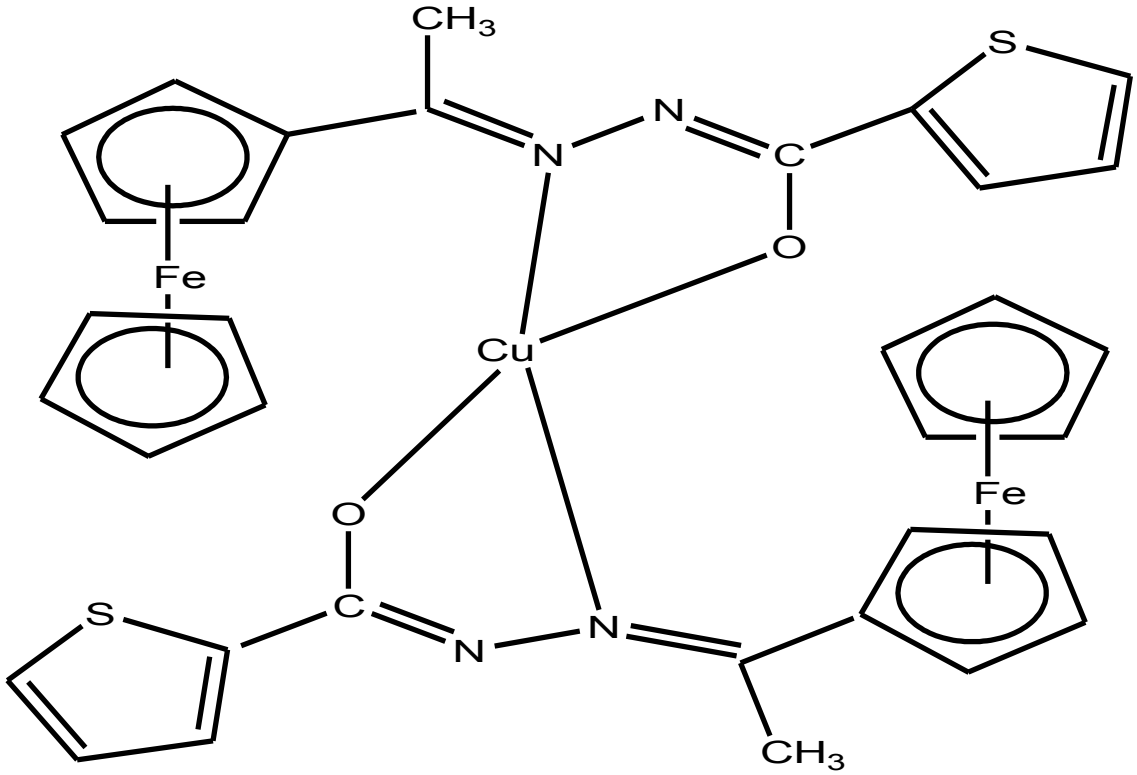



**Figure 19.** Structure of $CuL^{4}_{2}$ complex

## CHAPTER 5: CONCLUSION

In this study two thiosemicarbazone ligands and their copper complexes were prepared by modified methods, while two semicarbazone ligands and their copper complexes were prepared for the first time. The copper complexes of the ligands $HL^1$ and $HL^2$ reported in this work have not been traced in literature. Therefore these can be regarded as having been synthesized for the first time. All ligands and their complexes were characterized by EA, IR spectroscopy, $H^1$-NMR and $C^{13}$NMR spectroscopy. These are summarized in Table 16.

**Table 16.** The synthesized ligands and their corresponding complexes

| **Compound** | **Name** |
|---|---|
| **$HL^1$ (ligand)** | Acetylferrocenyl-4-phenylthiosemicarbazone |
| **$CuL^1Cl_2$ (complex)** | Copper(II) complex of $HL^1$ |
| **$HL^2$ (ligand)** | Acetylferrocenyl-4-methylthiosemicarbazone |
| **$CuL^2Cl_2$ (complex)** | Copper(II) complex of $HL^2$ |
| **$HL^3$(ligand)** | Acetylpyridine-2-thiophenecarboxyl-semicarbazone |
| **$CuL^3_2$ (complex)** | Copper(II) complex of $HL^3$ |
| **$HL^4$(ligand)** | acetylferrocenyl-2-thiophenecarboxyl-semicarbazone |
| **$CuL^4_2$ (complex)** | Copper(II) complex of $HL^4$ |

The first two ligands are thiosemicarbazone ligands while the last two ligands are semicarbazone ligands. The preparation of the copper(II) complexes were relatively straight forward (Agarwal *et al.,* (2005). Spectral together with literatures confirmed the elucidation of their structures. Elemental analysis (EA) served as a principal tool to distinguish complexes from their starting material. Infrared spectra for copper(II) complexes shows that the functional group stretching frequencies were significantly shifted to higher frequency relative to their values in the corresponding ligand. This indicates that the functional group is strengthened by complex formation (Turdor *et.al.,* 2007).

None of the ligands showed biological activity with *W-2*. However, $HL^1$ and $HL^4$ showed biological active with FP-2. $CuL^1Cl_2$ exhibited the most biological activity against FP-2 but showed the least biological activity against *W-2*. $CuL^3{}_2$ and $CuL^4{}_2$ showed pronounced growth inhibition against FP-2 while $CuL^2Cl_2$ is most active against *W-2*. The lipophilic nature of the $CuL^1Cl_2$ is important as it favours penetration through the membrane wall of the strain, resulting in one of the enzymes involved in haemoglobin degradation to be inhibited (Sulekh *et al.,* 2003). Furthermore, the hydrophilic core enhanced solubility of the group presumably facilitating transport across the membrance (Brown *et. al.,* 1976). This research confirms that a change in the level of liphophilicity of a compound is reflected in a change in level of its biological activity (Durham *et al.,* 1974).

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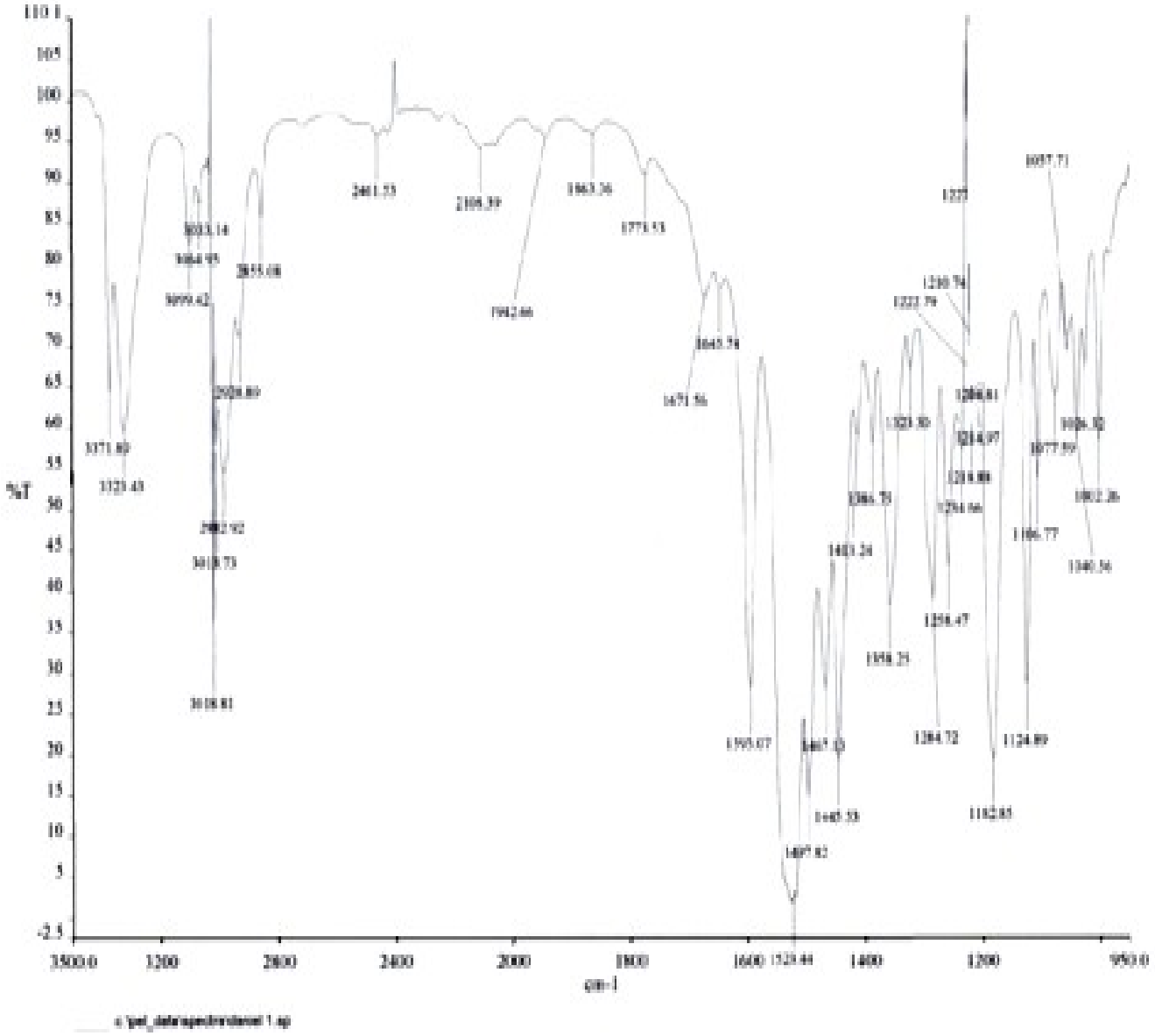
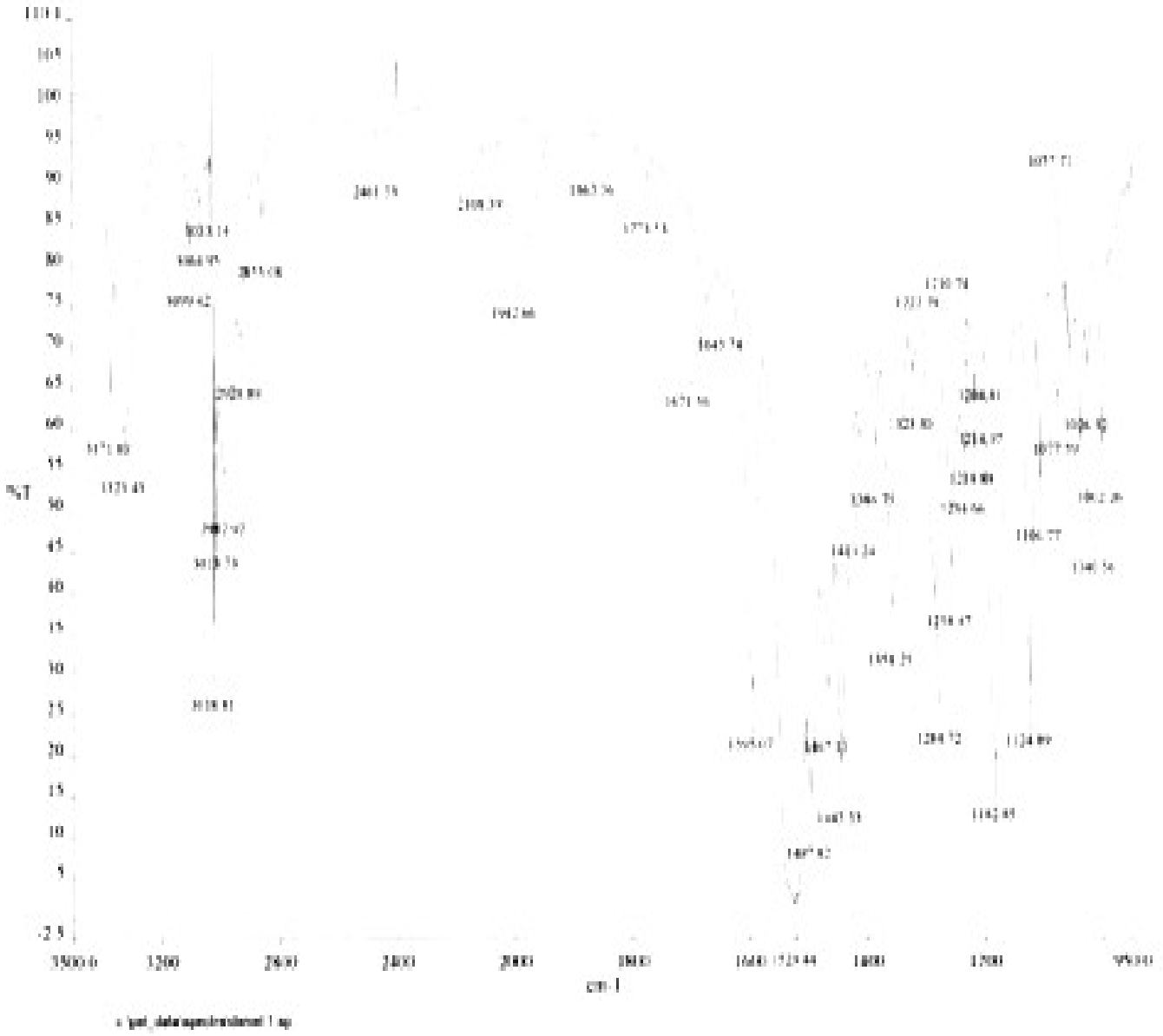
# APPENDICES

## APPENDIX A

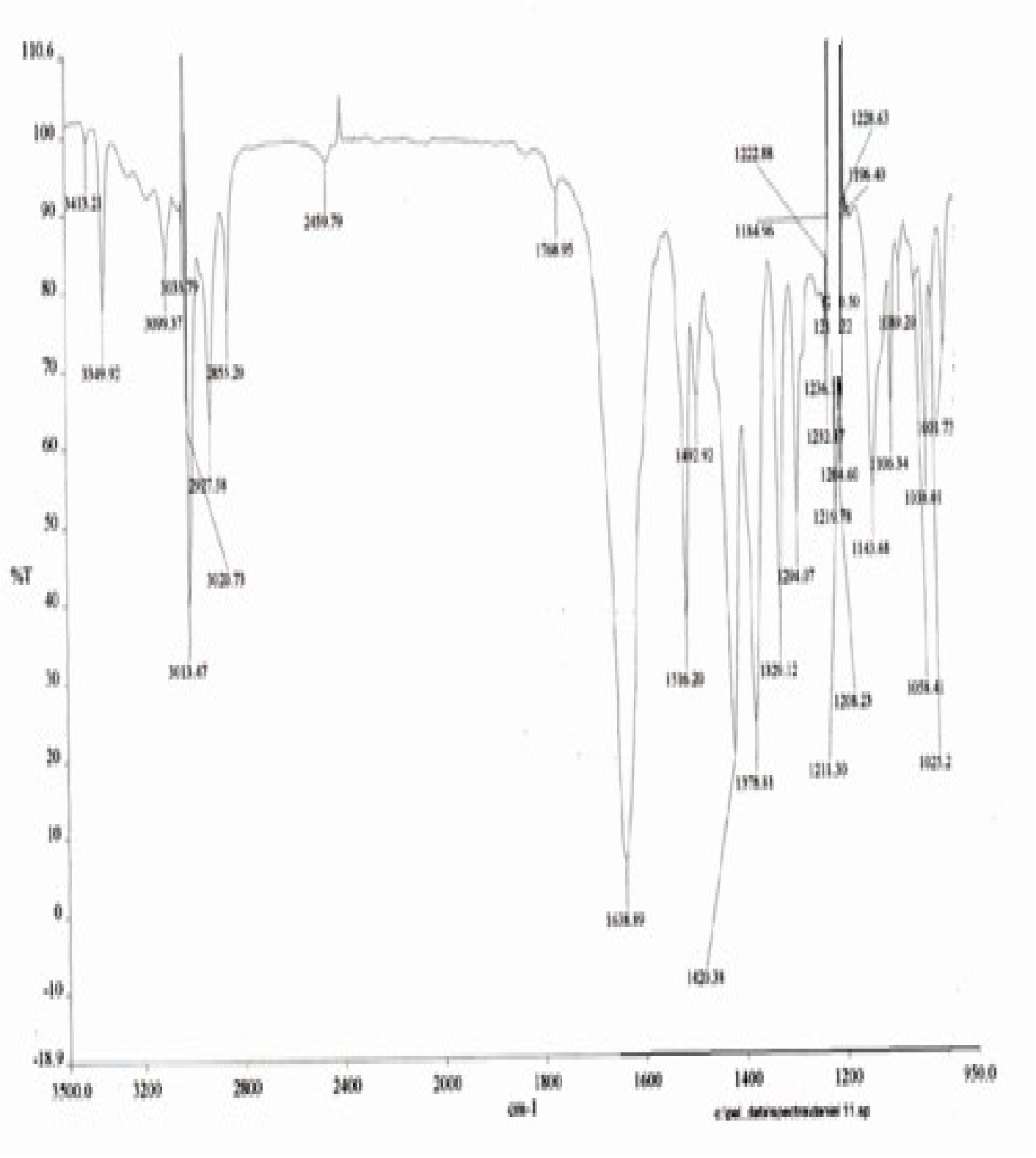
Appendix $A_1$: IR spectra of $LH^1$



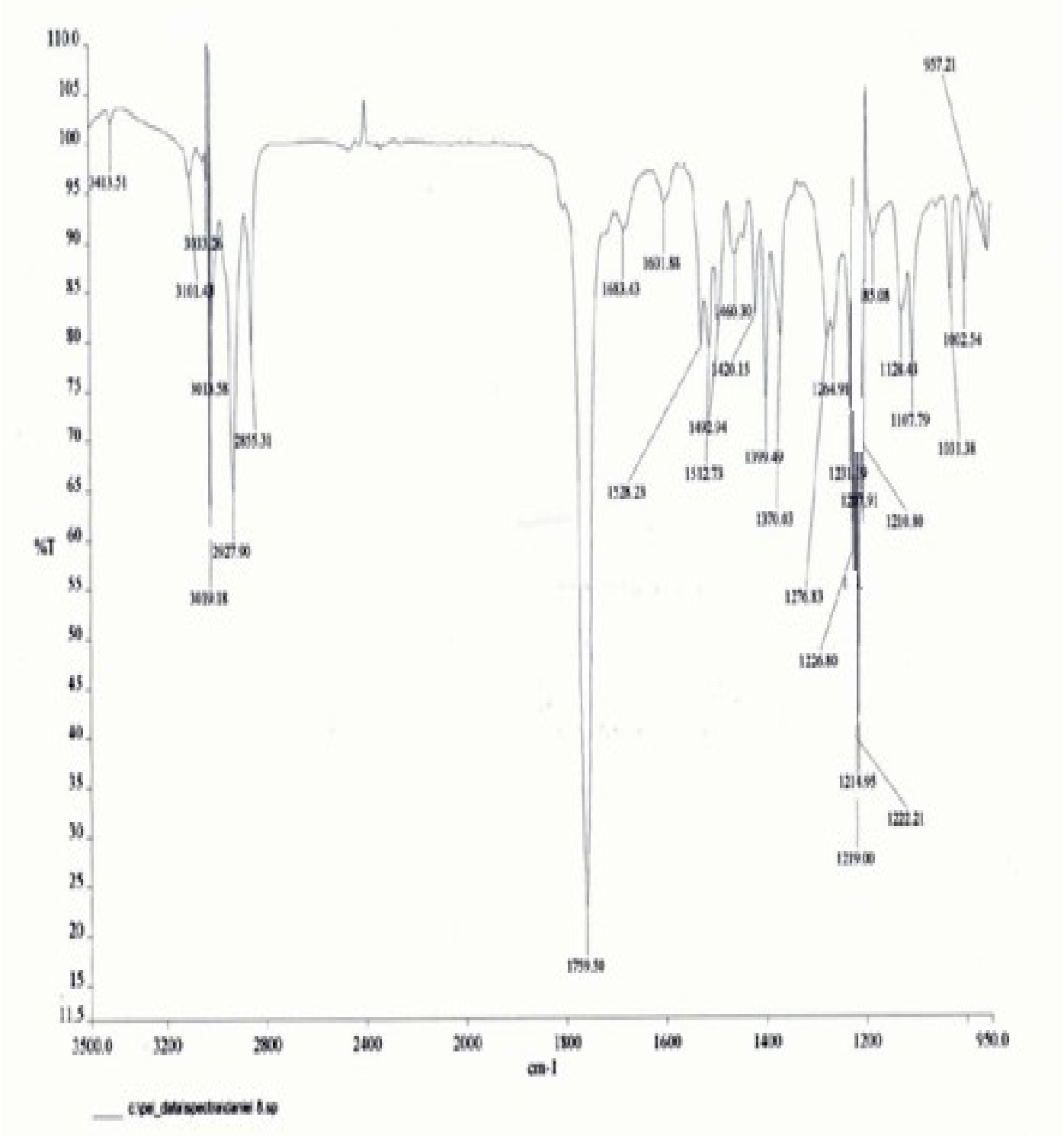

Appendix $A_2$: IR spectra of $LH^2$




Appendix $A_3$: IR spectra of $LH^3$




Appendix $A_4$: IR spectra of $LH^4$




### 5.3.2 APPENDIX B

Appendix $B_1$ Table 16, Table of characteristic proton NMR chemical shifts.

| *Type of proton* | *Type of compound* | *chemical shift range, ppm* |
|---|---|---|
| RC**H**$_3$ | 1° aliphatic | 0.9 |
| R$_2$C**H**$_2$ | 2° aliphatic | 1.3 |
| R$_3$C**H** | 3° aliphatic | 1.5 |
| C=C–**H** | vinylic | 4.6–5.9 |
| C=C–**H** | vinylic, conjugated | 5.5–7.5 |
| C≡C–**H** | acetylenic | 2–3 |
| Ar–**H** | aromatic | 6–8.5 |
| Ar–C–**H** | benzylic | 2.2–3 |
| C=C–C**H**$_3$ | allylic | 1.7 |
| **H**C–F | fluorides | 4–4.5 |
| **H**C–Cl | chlorides | 3–4 |
| **H**C–Br | bromides | 2.5–4 |
| **H**C–I | iodides | 2–4 |
| **H**C–OH | alcohols | 3.4–4 |
| **H**C–OR | ethers | 3.3–4 |
| RCOO–C**H** | esters | 3.7–4.1 |
| **H**C–COOR | esters | 2–2.2 |
| **H**C–COOH | acids | 2–2.6 |
| **H**C–C=O | carbonyl compounds | 2–2.7 |
| RC**H**O | aldehydic | 9–10 |
| RO**H** | hydroxylic | 2–4 |
| ArO**H** | phenolic | 4-12 |
| C=C–O**H** | enolic | 15–17 |
| RCOO**H** | carboxylic | 10–13.2 |
| RN**H**$_2$ | amino | 1–5 |
| RN**H**C(=O)R’ | amides | 5-8.5 |

(Sharp, 2005)

Appendix B$_2$ Table 17, Table of characteristic IR absorptions.*

| ***frequency, cm$^{-1}$*** | ***bond*** | ***functional group*** |
|---|---|---|
| 3640–3610 (s, sh) | O–H stretch, | free hydroxyl alcohols, phenols |

| | | |
|---|---|---|
| 3500–3200 (s,b) | O–H stretch, | H–bonded alcohols, phenols |
| 3400–3250 (m) | N–H stretch | 1°, 2° amines, amides |
| 3300–2500 (m) | O–H stretch | carboxylic acids |
| 3330–3270 (n, s) | –C≡C–H: C–H | stretch alkynes (terminal) |
| 3100–3000 (s) | C–H stretch | aromatics |
| 3100–3000 (m) | =C–H stretch | alkenes |
| 3000–2850 (m) | C–H stretch | alkanes |
| 2830–2695 (m) | H–C=O: C–H stretch | aldehydes |
| 2260–2210 (v) | C≡N stretch | nitriles |
| 2260–2100 (w) | –C≡C– stretch | alkynes |
| 1760–1665 (s) | C=O stretch | carbonyls (general) |
| 1760–1690 (s) | C=O stretch | carboxylic acids |
| 1750–1735 (s) | C=O stretch | esters, saturated aliphatic |
| 1740–1720 (s) | C=O stretch | aldehydes, saturated aliphatic |
| 1730–1715 (s) | C=O stretch | α, β–unsaturated esters |
| 1715 (s) | C=O stretch | ketones, saturated aliphatic |
| 1710–1685 (s) | C=O stretch | α, β–unsaturated aldehydes |
| 1685–1666 (s) | C=O stretch | α, β–unsaturated ketones |
| 1680–1640 (m) | –C=C– stretch | alkenes |
| 1650–1580 (m) | N–H bend | 1° amines |
| 1600–1585 (m) | C–C stretch | (in–ring) aromatics |
| 1550–1475 (s) | N–O asymmetric stretch | nitro compounds |
| 1500–1400 (m) | C–C stretch | (in–ring) aromatics |
| 1470–1450 (m) | C–H bend | alkanes |
| 1370–1350 (m) | C–H rock | alkanes |

| | | |
|---|---|---|
| 1360–1290 (m) | N–O symmetric stretch | nitro compounds |
| 1335–1250 (s) | C–N stretch | aromatic amines |
| 1320–1000 (s) | C–O stretch | alcohols, carboxylic acids, esters |
| 1300–1150 (m) | C–H wag (–CH2X) | alkyl halides |
| 1250–1020 (m) | C–N stretch | aliphatic amines |
| 1000–650 (s) | =C–H bend | alkenes |
| 950–910 (m) | O–H bend | carboxylic acids |
| 910–665 (s, b) | N–H wag | 1°, 2° amines |
| 900–675 (s) | C–H “oop” | aromatics |
| 850–550 (m) | C–Cl stretch | alkyl halides |
| 725–720 (m) | C–H rock | alkanes |
| 700–610 (b, s) | –C≡C–H: C–H bend | alkynes |
| 690–515 (m) | C–Br stretch | alkyl halides |

*m = medium, w = weak, s = strong, n = narrow, b = broad, s = sharp

(Frehlich, 2008)