

**JIMMA UNIVERSITY**  
**SCHOOL OF GRADUATE STUDIES**  
**DEPARTMENT OF CHEMISTRY**



**SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF**  
**Cu(II) AND Hg(II) COMPLEXES WITH A SCHIFF BASE LIGAND**  
**SYNTHESIZED FROM DIETHYL-2-AMINO-4-METHYLTHIOPHENE-**  
**3,5-DICARBOXYLATE AND 2,6-DIAMINOPYRIDINE**

**Kassahun Desalegn**

**October 2014**  
**JIMMA, ETHIOPIA**

**SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF  
Cu(II) AND Hg(II) COMPLEXES WITH A SCHIFF BASE LIGAND  
SYNTHESIZED FROM DIETHYL-2-AMINO-4-METHYLTHIOPHENE-  
3,5-DICARBOXYLATE AND 2,6-DIAMINOPYRIDINE**

**By: Kassahun Desalegn Gebremariam**

**Advisor: Dr. Mohammed Fakruddin Ali Ahammed**

**Co-advisor: Mr. Gezahegn Faye**

## Acknowledgements

First of all my heartfelt gratitude goes to God who by his absolute love and protection I could exist and be able to perform this work.

Next, I would like to thank my advisor Dr. Mohammed Fakruddin Ali Ahammed for his valuable scientific comment and guidance starting from the beginning. My deepest gratitude also goes to Mr. Gezahegn Faye whose genuine help was with me throughout this work.

I gratefully acknowledge Department of Chemistry, Jimma University for its unreserved help and encouragement by providing necessary materials for my research.

I would also like to express my gratitude to Addis Ababa University for running NMR, IR and conductivity experiments of for the synthesized samples.

I am extremely thankful to the *Issera Woreda* (*Dawuro Zone*, SNNPR) for its sponsorship of my M. Sc. study.

Last but not the least I wish to thank my family and friends for their moral support, consistent encouragement and valuable advice.

## Contents

Acknowledgements.....	i
Table of contents.....	<b>Error! Bookmark not defined.ii</b>
List of Figures .....	vii
List of abbreviations and symbols .....	v
Abstract.....	vi
1. INTRODUCTION .....	1
1.1. Objective of the study .....	2
1.1.1. General objective .....	2
1.2.2. Specific objectives .....	2
1.2. Statement of the problem.....	2
1.3. Significance of the study.....	2
2. LITRATURE REVIEW.....	4
2.1. Heterocyclic compounds.....	4
2.1.1. Sulfur- nitrogen Heterocyclic compounds .....	4
2.2. The Chemistry of Copper (II) .....	5
2.2.1. Biological properties of copper.....	7
2.3. Mercury and its compounds.....	8
2.4. Chemistry of Schiff base.....	8
2.4.1. Chemistry and Biological Importance of Schiff bases.....	9
2.4.3. Metal complexes contain Schiff base.....	10
3. MATERIALS AND METHODS.....	11
3.1. Chemicals and Reagents .....	11
3.2. Instruments and Experimental Conditions .....	11
3.3. Synthesis of ligand.....	11
3.4. Synthesis of Cu (II) and Hg (II) Complexes .....	12
3.5. Bioactivity activity testing .....	13
4. RESULTS AND DISCUSSION .....	14
4.1. Color, Melting Point and Solubility Tests .....	14
4.2. Infrared spectra .....	15
4.3. NMR Spectra .....	17

4.4. Conductivity Measurement .....	20
4.5. Electronic spectra.....	20
4.6. Antibacterial Activity testing .....	21
5. CONCLUSIONS AND RECOMMENDATIONS .....	22
REFERENCES .....	23

## List of tables

<b>Table 1:</b> Oxidation states and stereochemistry of copper complexes.....	6
<b>Table 2:</b> Recognized copper-dependent enzymes and their biochemical function .....	7
<b>Table 3:</b> Physical characteristics and analytical data of ligand and complexes .....	24
<b>Table 4:</b> The solubility of ligand and metal complexes in different solvents.....	14
<b>Table 5:</b> IR spectral bands of ligands and its metal complexes.....	15
<b>Table 6:</b> Conductivity values of complexes .....	20
<b>Table 7:</b> Antibacterial activities of the ligand and its metal complexes .....	21

<b>List of figures</b>	<b>page</b>
<i>Figure 1: IR spectra of Ligand (Schiff base)</i> .....	16
<i>Figure 2: IR spectra of copper(II) complex</i> .....	16
<i>Figure 3: IR spectra of mercury(II) complex</i> .....	17
<i>Figure 4: <sup>1</sup>H NMR spectra of ligand</i> .....	18
<i>Figure 5: <sup>13</sup>C NMR spectra of ligand</i> .....	19
<i>Figure 6: DEPT-135 NMR spectra of ligand</i> .....	19

## List of abbreviations and symbols

**IR** – infrared

**UV-Vis** – ultraviolet visible

**DMSO** – dimethyl sulfoxide

**M** – Metal

**L** – Ligand

**DAMTDDP**-diethyl-2-amino-4-methyl thiophene-3, 5-dicarboxylate 2, 6-diamino pyridine

**M.p** – melting point

**g** – gram

**TLC**– thin layer chromatography

**MHA** - Mueller-Hinton Agar

**IZ** - inhibition zone

**<sup>1</sup>H NMR**- Proton Nuclear Magnetic Resonance

**<sup>13</sup>C NMR** -<sup>13</sup>C Nuclear Magnetic Resonance

**nm** -Nanometer ( $10^{-9}$ m)



## Abstract

*Ethyl-4-amino-5-(((6-aminopyridin-2-yl)imino)(ethoxy)methyl)-2-methylthiophene-3-carboxylate* was synthesized by the condensation of diethyl-2-amino-4-methylthiophene-3, 5-dicarboxylate and 2,6-diaminopyridine. The complex of Cu(II) and Hg(II) with this ligand was prepared by refluxing the mixture of the solutions of the sulphate salts of the metals and ligand in methanol. The resulting compounds were characterized using NMR, IR UV-Vis and molar conductance. The metal ions in complex are tricoordinated to the ligand through pyridine nitrogen, azomethine nitrogen and thiophene sulfur. The high conductance values of the complexes indicate that all the complexes were electrolytes. The antibacterial test indicates that the ligand was active against *Staphylococcus aureus* while it is inactive against *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*. The  $\text{Cu}^{2+}$  and  $\text{Hg}^{2+}$  complexes of the ligand exhibit higher antibacterial activities than the free ligand.

**Keywords:** Ligand, diethyl-2-amino-4-methylthiophene -3,5-dicarboxylate, 2,6-dipyridine, Cu(II) complex, Hg(II) complex, antibacterial Activity

## 1. INTRODUCTION

Schiff bases have been studied as a class of ligands [1, 2] and are known to coordinate with metal ions through the azomethine nitrogen. [3]. Schiff base ligands and their metal complexes have recently attracted more attentions [4, 5] because of their biological, biochemical, analytical, antimicrobial, anticancer, antibacterial, antifungal and antitumor activity. The synthesis of transition metal complexes with Schiff base ligand are studied due to sensitivity, selectivity and synthetic flexibility towards metal atoms [6]. The development of new biologically active binuclear Schiff base metal complexes with identical legating environments has undergone an inspiring growth and significant attention in recent years to study the inorganic perspectives of these metal centers for small molecule activation in biological process. Heterocyclic compounds such as pyridine and thiophene related molecules are good ligands due to the presence of one or more ring nitrogen and sulfur atoms with a localized pair of electrons. The application potential has led to the formation of series of novel Schiff base compounds with a wide range of reactivity and stability, physical, chemical and biological properties [7].

Metal ions play a vital role in a vast number of widely differing biological processes and depending on their concentration, they may either contribute towards the health of the organism or cause toxicity [8, 9]. Several metal chelate are known to possess antibacterial, fungicidal, antiviral and anticancer activity. In several cases, the metal chelate has been found to be more antimicrobial than the chelating agent's themselves [10]. Recently, great attention has been oriented towards heterocyclic systems containing nitrogen, oxygen or sulfur. Such class of compounds has wide applications as pharmaceutical drugs, biological activities, as anti HIV, anticancer, efficient plant protection and as analytical reagents for trace and ultra-trace heavy metal determination and pre concentration in aqueous media [11]. In this work an attempt has been made to synthesize and characterize Cu(II), and Hg(II) complexes of a Schiff base ligand derived from diethyl-2-amino-4-methyl thiophene-3,5-dicarboxylate with 2, 6-diamino pyridine.

## **1.1. Objective of the study**

### **1.1.1. General objective**

The aim of this study is to synthesize metal complexes with Schiff base ligand and to evaluate their antibacterial activities.

### **1.1.2. Specific objectives**

- ❖ To synthesize and characterize a Schiff base from diethyl-2-amino-4-methyl thiophene-3,5-dicarboxylate and 2, 6-diaminopyridine
- ❖ To prepare and characterize Cu(II) and Hg(II) complexes of the Schiff base synthesized.
- ❖ To study the antibacterial activities of ligand and metal complexes.

## **1.2 Statement of the problem**

. Due to the stereospecific synthesis of Schiff bases as ligands, their chemical as well as morphological properties can be modified with respect to the desired application. By means of functionalizing the electronic properties of these complexes, bioactivity can be enhanced.

Heterocyclic ligands offer several advantages over other common ligands such as their efficient electron donation, higher stability and functionality. Therefore, a new ligand diethyl-2-amino-4-methyl thiophene-3,5-dicarboxylate-2,6-diamino pyridine (DAMTDDP) and its Cu(II) and Hg(II) was synthesized. Thus, the present work describes:

- the process of the synthesis of DAMTDDP and its metal complexes.
- the biological activities of the ligand and its complexes.

## **1.3 Significance of the study**

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutic available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new

classes of metal complexes of antimicrobial agents.. Metal ion plays an important role in biology which has lead to the development of huge number of metal complexes with diverse therapeutic activity. Cisplatin, carboplatin and oxaliplatin are the well-known metal-based drugs widely used in treatment of cancer. Besides these complexes other metal complexes have shown promising results in the treatment of diseases like diabetes, rheumatoid arthritis, inflammatory and cardiovascular diseases etc.

The outcome of this research could be used as an initial step to develop a new metal-Schiff base complex which could have better antimicrobial activity.

## **2. LITRATURE REVIEW**

### **2.1. Heterocyclic Compounds**

Heterocyclic compounds are organic compounds which contain a ring structure and atoms in addition to carbon, such as sulfur, oxygen or nitrogen, as part of the ring. They may be either simple aromatic rings or non-aromatic. The presence of hetero-atom results in significant changes in the cyclic molecular structure due to the availability of unshared pairs of electrons and the difference in electro negativity between heteroatom and carbon.

#### **2.1.1. Sulfur- Nitrogen Heterocyclic Compounds**

The family of sulfur–nitrogen heterocyclic includes highly stable aromatic compounds that display physicochemical properties with relevance in the design of new materials Nitrogen and Sulfur aromatic heterocyclic compounds are formally derived from aromatic carbon cycles with a heteroatom taking the place of a ring carbon atom or a complete CH=CH group. The presence of hetero-atom results in significant changes in the cyclic molecular structure due to the availability of unshared pairs of electrons and the difference in electro negativity between heteroatom and carbon. Therefore, nitrogen and sulfur heterocyclic compounds display physicochemical characteristics and reactivity quite different from the parent aromatic hydrocarbons. On the other hand, the presence of many nitrogen and sulfur atoms in a ring is normally associated with instability and difficulties in the synthesis.

Thiophene and thiophene derivatives are sulfur heterocycles. They occur in coal tar distillates. The discovery of thiophene in coal tar benzene provides one of the classic anecdotes of organic chemistry. Thiophene was discovered as a contaminant in benzene. It was observed that isatin (1Hindole-2,3-Dione) forms a blue dye if it is mixed with sulfuric acid and crude benzene. Victor Meyer was able to isolate the substance responsible for this reaction. The compound was found to be a heterocyclic compound. Thiophene is from the Greek word, ‘‘theion’’ for sulfur and another Greek word ‘‘phaino’’ which means shining. The simple thiophenes are stable liquids which closely resemble the corresponding benzene derivatives in boiling point and even in smell. They occur in coal tar distillates, and the discovery of thiophene in coal tar benzene provides one of the classic anecdotes of organic chemistry. Thiophene structure can be found in certain natural products and is also incorporated in several pharmacologically active compounds.

Thiophene has a structure that is analogous to structure of pyrrole, and due to  $\pi$ -electron cloud and it behaves like benzene. Thiophene is considered as aromatic, although theoretical calculations suggest that the degree of aromaticity is less than that of benzene. The participation of the lone electron pairs on sulfur in the delocalized  $\pi$ -electron system is significant. Thiophenes are used as synthetic intermediates, taking advantage of the susceptibility of the carbon atoms adjacent to sulphur toward electrophilic reactions. It is considered to be aromatic and “ $\pi$ -excessive”. This is because the five  $sp^2$  – hybridized atoms may sustain a  $6\pi$ -electron system. Each carbon atom contributes one p-hybridized electron to the system and the lone pair provides the remaining two electrons [12].

Thiophene is the sulphur analogue of furan, and its structure is very similar. However, thiophene has a higher degree of stabilization energy than furan, and is more aromatic in character as is borne out by its chemical reactions. There are several possible reasons for this: - one is that sulphur has a larger bonding radius than oxygen which allows the C-C bond angles to widen a little, thus allowing some gain in stability by losing a little angle-compression strain, a second reason is that trivalent sulfur tolerates a positive charge better than oxygen. The sulfur atom carries an electron pair in  $sp^2$  hybrid orbital in the plane of the ring.

Five membered heterocyclic such as thiophene and pyrrole have widely been used as building blocks for the design of well-defined linear  $\pi$  conjugated oligomers and polymers [13]. In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications.

## 2.2. The Chemistry of Copper (II) Complexes

The copper (II) complexes have  $d^9$  electron configuration. This configuration of copper is characteristic of Jahn-Teller distortion for regular octahedral or tetrahedral symmetry, which has an effect on its stereochemistry. A typical distortion for 6-coordinate is an elongation along one fourfold axis so that there is a planar array of four short Cu-L bonds and two trans long ones. The Jahn-Teller effect is most often encountered in octahedral complexes of the transition metals. The phenomenon is very common in six-coordinate copper (II) complexes. The  $d^9$  electronic configuration of this ion gives three electrons in the two degenerate  $e_g$  orbitals, leading to a doubly degenerate electronic ground state. Such complexes distort along one of the molecular

fourfold axes (always labeled the  $z$  axis), which has the effect of removing the orbital and electronic degeneracies and lowering the overall energy. The distortion normally takes the form of elongating the bonds to the ligands lying along the  $z$  axis, but occasionally occurs as a shortening of these bonds instead (the Jahn–Teller theorem does not predict the direction of the distortion, only the presence of an unstable geometry). When such an elongation occurs, the effect is to lower the electrostatic repulsion between the electron-pair on the Lewis basic ligand and any electrons in orbitals with a  $z$  component, thus lowering the energy of the complex. If the undistorted complex would be expected to have an inversion centre A great majority of Cu(II) compounds are blue or green in color because of a single broad absorption band in the region of 600 – 900 nm which is due to spin allowed transition  ${}^2T_{2g} \leftarrow {}^2E_g$ . Considerable distortion in octahedral symmetry is observed in Cu(II) complexes. The magnetic moments of mononuclear Cu(II) complexes are generally in the range of 1.75 - 2.20 BM, regardless of stereochemistry and independent of temperature except at extremely low temperatures.

Mixed O and N donor ligands such as Schiff bases are of interest in that they provide examples of square-planar coordination and square-pyramidal coordination by dimerization [14, 15] and they have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical, anti-inflammable activity, antiradical activities and biological activities [16]. A considerable number of Schiff's base copper complexes have potential biological interest, being used as more or less successful models of biological compounds [17] and also important in the development of inorganic biochemistry, catalysis and optical materials [18].

**Table 1:** Oxidation states and stereochemistry of copper complexes

Oxidation state	Coordination number	Geometry
Cu(II), $d^9$	3	Trigonal planar
	4	Distorted tetrahedral
	4	Square planar
	5	Tringonal bipyramidal
	6	Octahedral

### 2.2.1. Copper in Biological systems

There has been much attention paid to copper because it is an essential element for life. It is associated with a number of copper-dependent enzymes that are in biological processes. The most important copper-dependent enzymes in mammals are listed in Table 2.

**Table 2:** Recognized copper-dependent enzymes and their biochemical function.

<b>Cu-dependent enzyme</b>	<b>Function</b>
Cytochrome C oxidase	Cellular utilization of oxygen
Superoxide dismutase	Disproportionation of superoxide
Tyrosinase (catechol oxidase)	Synthesis of dopa from tyrosine
Dopamine-B-hydroxylase	Synthesis of norepinephrine from dopamine
Alfa-Amidating enzyme	Modifies C-terminal ends of hypothalamic peptide hormones ending in glycine
Diamine oxidase	Inactivation of histamine and polyamines
Amine oxidase (extracellular)	Inactivation of histamine, tyramine, dopamine, serotonin
Peptidylglycine monooxygenase	Bioactivation of peptide hormones
Hephaestin	Ferroxidase, in trans-golgi of enterocytes; aids iron absorption homology to ceruloplasmin
$\beta$ -Amyloid precursor protein	Normal function currently unknown
S-Adenosylhomocysteine	Sulfur amino acid metabolism hydrolase
Angiogenin	Induction of blood vessel formation

Elevated copper levels in plasma can be important for the etiology of some illness [19]. For example, copper ions are closely involved in neurodegenerative disorders, especially in Parkinson's disease. Moreover, there has been interest in the medical uses of copper, in particular as a complexing ion of known biologically active ligands and drugs. Throughout the years of scientific research copper (II) complexes have been found to possess various activities such as antiulcer, antiamebic, antidiabetic, anticonvulsant, anti-inflammatory, antimicrobial and antitumor [20]. In particular, anti-inflammatory, anti-microbial and anti-cancer activity of copper



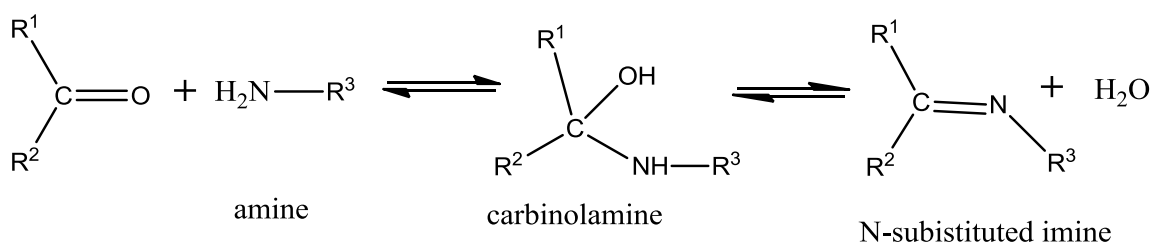
complexes has been studied. The mechanism by which Antimicrobial Copper kills bacteria is a complex by nature, but the effect is simple.

### 2.3. Mercury and its compounds

Mercury plays an important role in biological (example Hg(II) complexes of the type,  $[(L)M(SeCN)_2]$  (where L=Histidine or Glycine and M=  $Hg^{2+}$ ), for use as a potential antibacterial agents) and chemical processes. It also forms useful amalgams with many metals, which find various applications in diverse fields. Due to the numerous applications and the toxic nature of amalgams and mercury compounds, there is a need for simple and accurate analytical methods that allow for the rapid determination of mercury content in samples [21]. Mercury is one of the most toxic heavy metals on earth. The toxicity of mercury depends on its occurring forms; organo-mercurials such as methyl mercury are more toxic than elemental mercury and other inorganic mercury compounds [22]. Mercury containing ligands e.g., Hg(II) and Hg(I) are known to form stable complexes with class b metal ions, such as gold(I) and Se(II) because mercury is considered to be a soft Lewis base [23-25]. The Coordination chemistry of mercury (II) differs from most other transition metals due to its large size and  $d^{10}$  configuration. Its interference in biological systems, and its potential as a toxin or as a medicine, has required a better understanding of its coordinative properties [26-28].

### 2.4. Schiff Bases

A Schiff base is a nitrogen analog of an aldehydes or ketones in which the C=O group is replaced by C=N-R group [29]. It is usually formed by condensation of an aldehydes or ketones with a primary amine (Scheme 1).



Scheme 1. Formation of Schiff base from aldehyde or ketone in acid catalyzed dehydration

Where, R may be an alkyl or an aryl group. Schiff bases that contain aryl substituents are substantially more stable and more readily synthesized, while those which contain alkyl substituent are relatively unstable. Schiff bases of aliphatic aldehydes are relatively unstable and readily polymerizable while those of aromatic aldehydes having effective conjugation are more stable.

#### **2.4.1. Chemistry and Biological Importance of Schiff Bases**

Schiff bases have a large number of synthetic uses in organic chemistry. Acylation of Schiff bases by acid anhydrides, acid chlorides and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of the acylating agent to the carbon-nitrogen double bond. Reactions of this type have been put to good use in natural product synthesis. Schiff bases appear to be an important intermediate in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base. Stereochemical investigation carried out with the aid of molecular model showed that Schiff base formed between methyl glyoxal and the amino group of the lysine side chains of proteins can bent back in such a way towards the N atom of peptide groups that a charge transfer can occur between these groups and oxygen atoms of the Schiff bases. Transition metal complexes of such ligands are important enzyme models. The rapid development of these ligands resulted in an enhance research activity in the field of coordination chemistry leading to very interesting conclusions. Schiff bases are versatile ligands which coordinate to metal ions via azomethine nitrogen. These compounds and their metal complexes are very important as catalysts in various biological systems, polymers, dyes and medicinal and pharmaceutical fields.

In azomethine derivatives, the C=N linkage is essential for biological activity, several azomethines were reported to possess remarkable antibacterial, antifungal, anticancer and diuretic activities. Schiff-base complexes are considered to be among the most important stereochemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety. A considerable number of Schiff-base metal complexes are of potential biological interest, being used as more or less successful models of

biological compounds. Not only they have played a seminal role in the development of modern coordination chemistry, but they can also be found at key points in the development of inorganic biochemistry, catalysis and optical materials. Interaction of metal ions with N, O and S atoms from Schiff bases organic moieties have attracted much attention in recent years. This interaction provides an interesting series of ligands whose properties can be greatly modified by introducing different organic substituent's, thereby causing a variation in the ultimate donor properties. These Schiff base complexes have different geometries and properties. Schiff bases were also found to be biologically active and show excellent biological properties such as anti oxidant, antiviral, antibacterial, antifungal and many other properties.

#### **2.4.2. Metal Complexes of Schiff Bases**

Schiff base ligands containing O, S and N donor atoms play an important role in coordination chemistry. They are generally bidentate or tri- dentate ligands capable of forming very stable complexes with transition metals. Schiff bases appear to be an important intermediate in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. The Schiff bases have high affinity to chelate with the transition metal ions, hence are attracting attention due to potential applications in areas of catalysis, thermal, electrical, optical, magnetic, medicinal and pharmaceutical chemistry, antibacterial, anti-inflammatory and antipyretic antifungal and antitumor activity. Metal complexes are suitable to molecular materials, on the basis of electronic properties associated with the metal center [30-32]. A large number of Schiff bases with their binding ability forms stable and active Schiff base metal complexes.

### 3. EXPERIMENTAL

#### 3.1. Chemicals and Reagents

All the chemicals and solvents used for the synthesis were analytical reagent grade obtained from Sigma Aldrich and used without further purification.

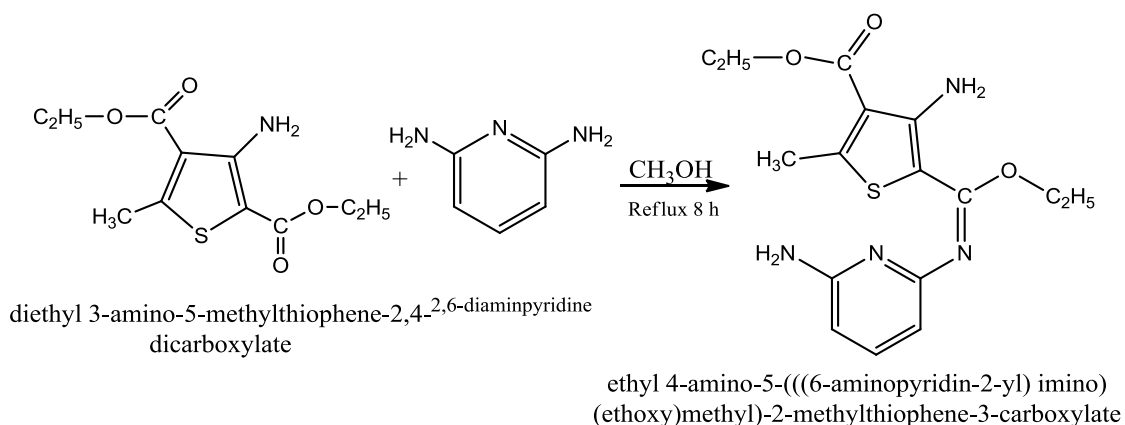
Diethyl-2-amino-4-methylthiophene-3,5-dicarboxylate, 2,6-diamino pyridine, methanol, ethanol, hexane, acetone, petroleum ether, ethyl acetate, calcium chloride, sodium acetate, copper sulphate, mercuric sulphate, dimethyl sulfoxide (DMSO).

#### 3.2. Instruments and Experimental Conditions

Infrared (IR) spectra were recorded using a Perkin Elmer FT-IR Spectrum BX spectrometer in the range 4000 - 400  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR analyses were carried using BRUCKER 400 ultra shielded NMR Instrument. Results were expressed in parts per million down fielded from tetramethylsilane as internal reference, in deuterated chloroform. UV-Vis spectroscopic studies were done in the range 200 - 800 nm using Spectronic Genesys 2PC spectrometer. The molar conductivity of the complexes in DMSO was recorded at room temperature using EC 214 Bench conductivity meter. The melting points of ligand and metal complexes were measured melting point measured using the Mel-Temp II Lab Device.

#### 3.3. Synthesis of Ligand

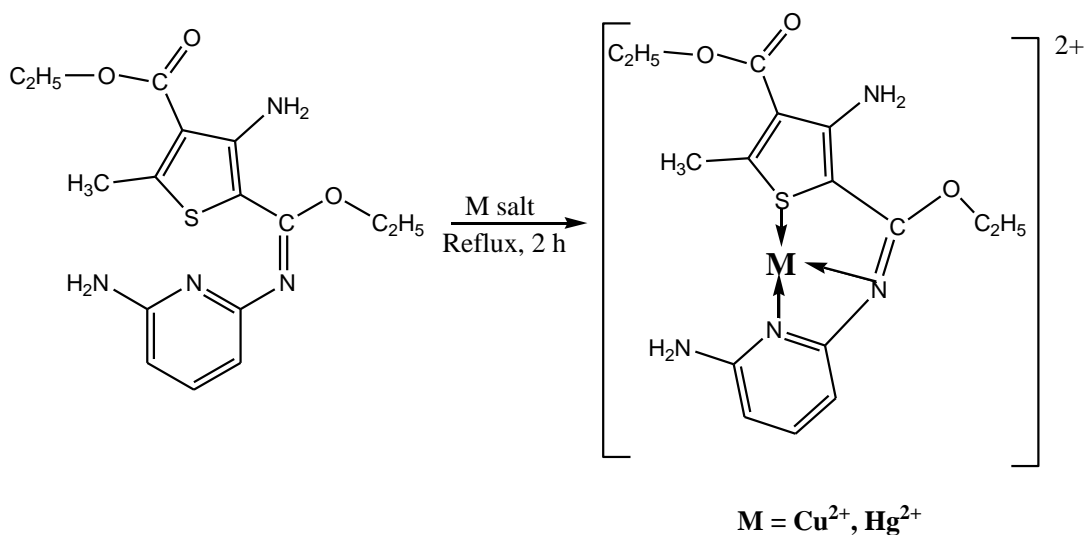
The ligand was synthesized by condensing the diethyl-4-methylthiophene-3,5-dicarboxylate and 2,6-diamino pyridine (Scheme 1). Equimolar amounts of diethyl-2-amino-4-methylthiophene-3,5-dicarboxylate (0.004 mol) and 2,6-diamino pyridine (0.004 mol.) were dissolved in methanol (25 ml each) and then mixed. The mixture was refluxed for 8 h. TLC examination suggested the complete conversion of the starting materials to the ligand. On cooling, deep red crystalline precipitates were formed. The product was filtered, washed with methanol and dried in a desiccator. The reaction of the between diethyl-2-amino-4-methylthiophene-3,5-dicarboxylate and 2,6-diamino pyridine is shown below (Scheme 1).



Scheme 1: Synthesis of the ligand

### 3.4. Synthesis of Cu(II) and Hg(II) Complexes

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{HgSO}_4$  were separately dissolved in hot methanol (20 ml) each was mixed with a hot methanol (20 ml) solution of ligand. The reaction mixtures were refluxed on a water bath for 2 h at  $50^\circ\text{C}$ . On cooling the reaction mixture at room temperature the colored (dark for Copper and white for Mercury) complexes precipitated out. The complexes were filtered and recrystallized at  $4^\circ\text{C}$  in refrigerator. The obtained precipitates were washed with methanol and dried in desiccators over calcium chloride.



Scheme 2: Synthesis of the complexes

### 3.5. Bioactivity activity testing

Antimicrobial tests were carried out on four species of bacteria namely: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. The microbial isolates were maintained on an agar slant at 4°C in the laboratory. The strains were sub-cultured on fresh agar plate, 24 h prior to any antibacterial test.

**Sensitivity Test:** Sample of ligand and complex were diluted in sterilized distilled water at 100 mg/ml. One milligram of test compound was placed on a sterilized filter paper disc and allowed to dry. Reference antibiotic (RA), Gentamicin, was also prepared in the same manner and 10 µg placed on a sterilized filter paper disc and dried, prior to testing.

**Diffusion Tests:** *In vitro* antibacterial activity of the ligand and complex were evaluated using disc-diffusion method. Mueller-Hinton Agar was employed as microbial growth medium. The antibacterial diffusion tests were carried out as described by Berghe and Vlietink [46] using a cell suspension of about  $1.5 \times 10^6$  CFU/mL obtained from the McFarland Turbidity Standard number 0.5. Mueller-Hinton (MH) agar was poured (to a height of 5 mm) in to sterile 9 cm diameter Petri dishes and allowed to solidify. The solid MH agar was inoculated with bacteria strains using a platinum wire loop which had been previously sterilized by heating it red hot in a flame, cooled and then used for the application. The dishes were allowed to dry for 10 min at 37°C in an incubator. Sterilized forceps were used for the application of the paper discs containing the test compounds on previously inoculated MH agar dishes, with that of the RA placed at the centre. The plates were kept for 30 min at ambient temperature to allow for pre diffusion, and then incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of growth inhibition zone (IZ) in mm around the discs. Three replicas were performed for each sample and mean values of the growth inhibition zone were calculated.

## 4. RESULTS AND DISCUSSION

### 4.1. Physical Properties of the Ligand and the Complexes

Color and melting point data of the ligand and complexes are presented in Tables 3 and 4. The synthesized metal complexes showed a sharp melting point of 216 °C and 186 °C for Cu and Hg complexes respectively, an indication that they are pure, and very different from the melting point of ligand was 80<sup>0</sup>C and starting reagents which have 95°C and 121.5°C for diethyl-4-methylthiophene-3,5-dicarboxylate and 2,6-diaminopyridine respectively. The color of the synthesized ligand is deep red while the color of the diethyl-4-methyl thiophene-3, 5-dicarboxylate and 2, 6-diamino pyridine is white and brown respectively. Thus, the obtained precipitate is different in color from the starting materials. The synthesized complexes are insoluble in petroleum ether and hexane but soluble in methanol, water and DMSO. The results obtained from solubility tests showed that the complexes are soluble in polar solvents, which indicates that the complexes are polar compounds.

**Table 3:** Physical characteristics data of Ligand and complexes

Compound	Color	Mol. Wt.	Yield(%)	mpt(°C)
Ligand (L)	Deep-red	348.43	61	80
L-Cu com.	Dark	598.43	56	216
L-Hg com.	White	645.08	58	189

**Table 4:** The solubility of ligand and metal complexes in different solvents

Solvent	Ligand	Copper complex	Mercury complex
Ethanol	soluble	partially soluble	partially soluble
Methanol	soluble	Soluble	Soluble
DMSO	soluble	Soluble	Soluble
Petroleum ether	insoluble	insoluble	Insoluble
Hexane	insoluble	insoluble	Insoluble

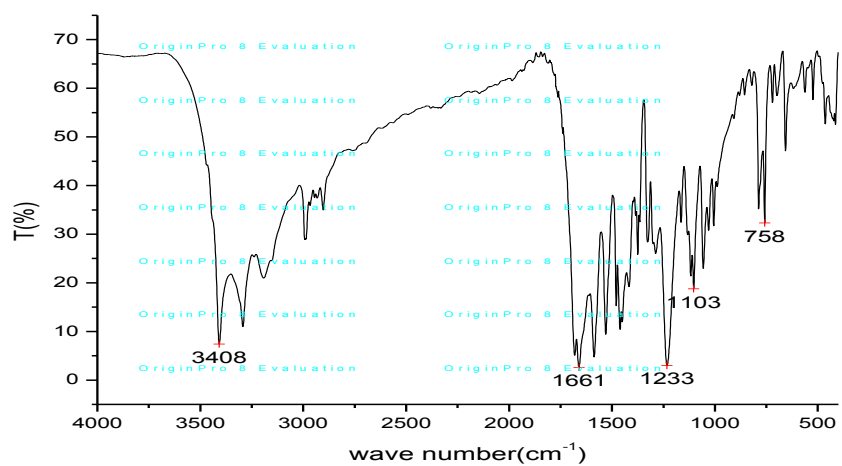
## 4.2. Infrared spectra

The IR spectroscopy can provide valuable information as to whether or not reaction has occurred. In order to study the binding mode of the ligand to the metal ion in the complex, the IR spectrum of the free ligand must be compared with the spectra of the metal complexes. Thus infrared spectra of the complexes taken in the region  $4000 - 500 \text{ cm}^{-1}$  were compared with that of the free ligand. Many signals in the IR spectrum of the ligand are also present in the spectrum of the complexes. There are some significant changes between the metal (II) complexes and free ligand. Two bands appeared around  $3400 \text{ cm}^{-1}$  and  $3300 \text{ cm}^{-1}$  are due to the free  $\text{NH}_2$  group present in ligand. The IR bands observed around  $1632 \text{ cm}^{-1}$ ,  $1616 \text{ cm}^{-1}$  and  $1560 \text{ cm}^{-1}$  of the ligand, copper complex and the mercury complex respectively are assigned to  $\nu\text{C}=\text{N}$  vibration. The azomethine band is shifted to lower frequency in metal complexes, suggesting that this group takes part in coordination [33]. The co-ordination of nitrogen to the metal atom would be expected to reduce the electron density on the azomethine link and thus cause a shift in the  $\text{C}=\text{N}$  band.

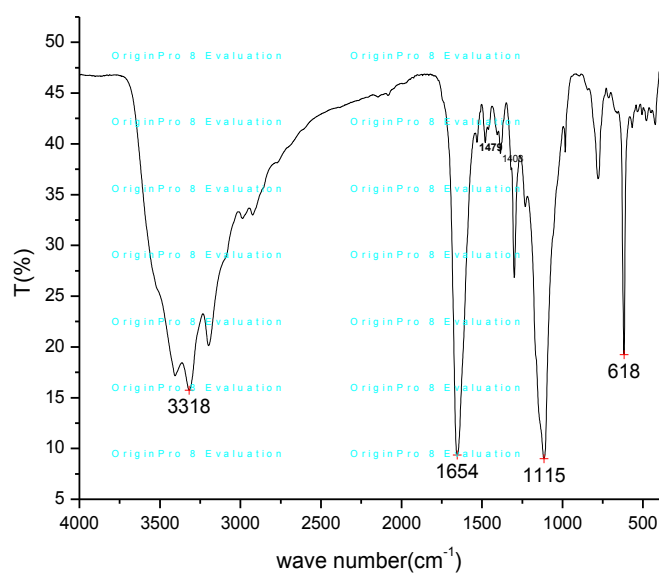
**Table 5.** IR Spectral Bands of Ligands and Its Metal Complexes

Compound	V(N-H)	V(C=N)	V(C=O)	C-S	M-N	M-S
Ligand	3408	1632	1661	758	-	-
$\text{Cu}^{2+}$ complex	3318	1616	1654	741	618	543(w)
$\text{Hg}^{2+}$ comp.	3409	1560	1656	780	622	541(w)

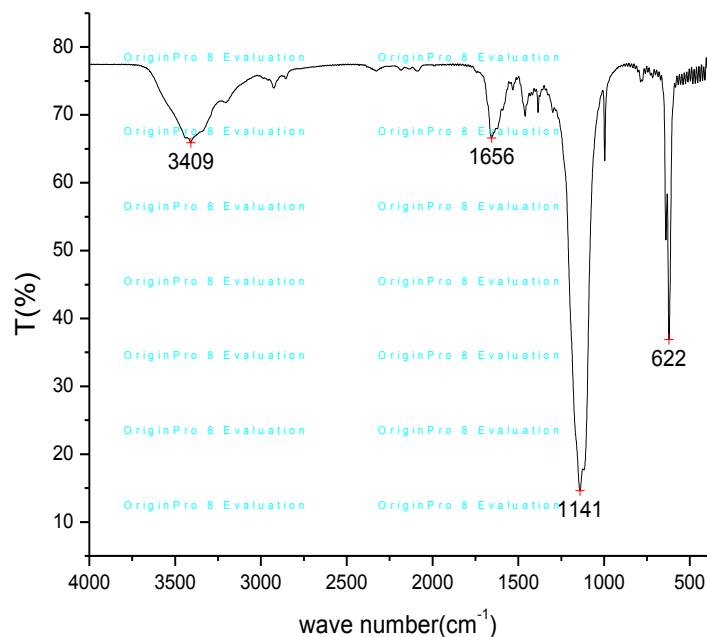




**Figure 1.** IR spectra of Ligand



**Figure 2:** IR spectra of copper (II) complex

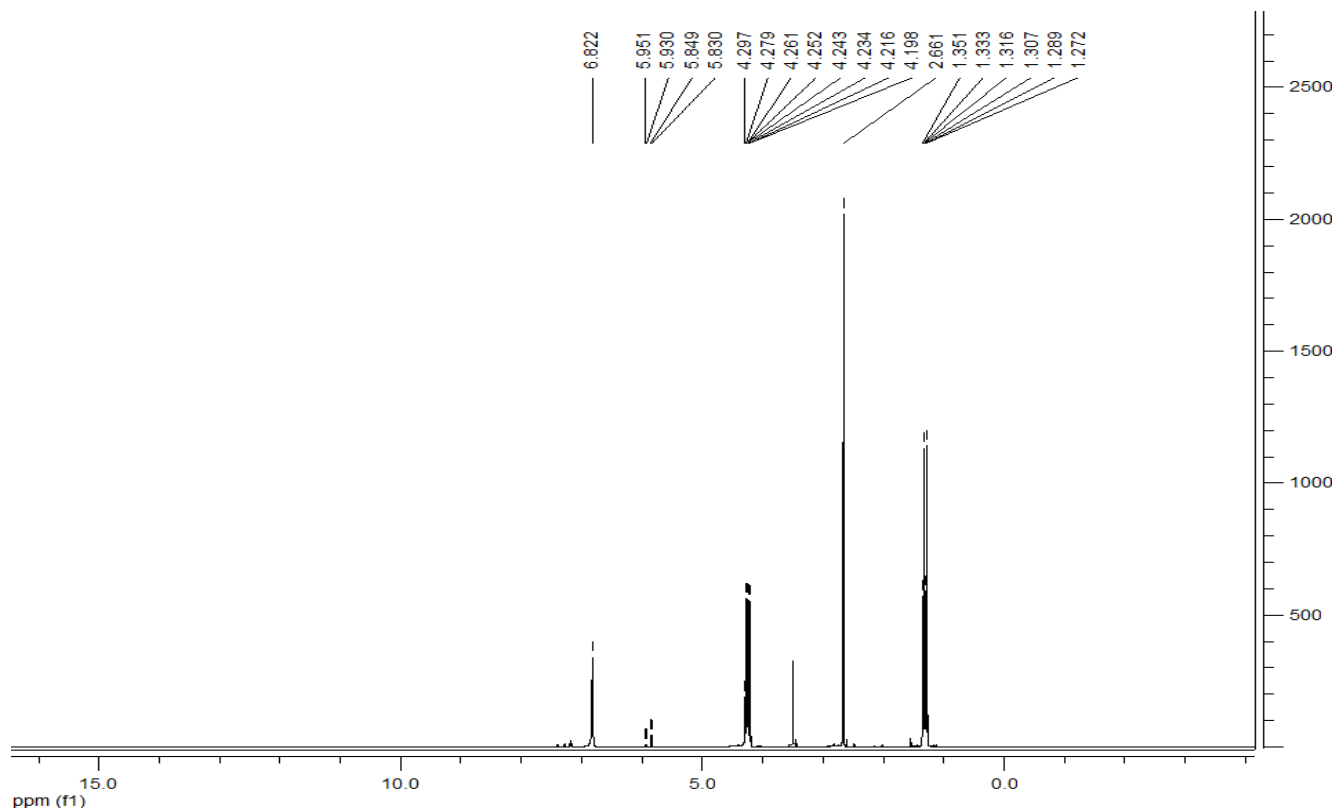


*Figure 3. IR-spectra of mercury (II) complex*

### 4.3 NMR Spectra

#### <sup>1</sup>H NMR

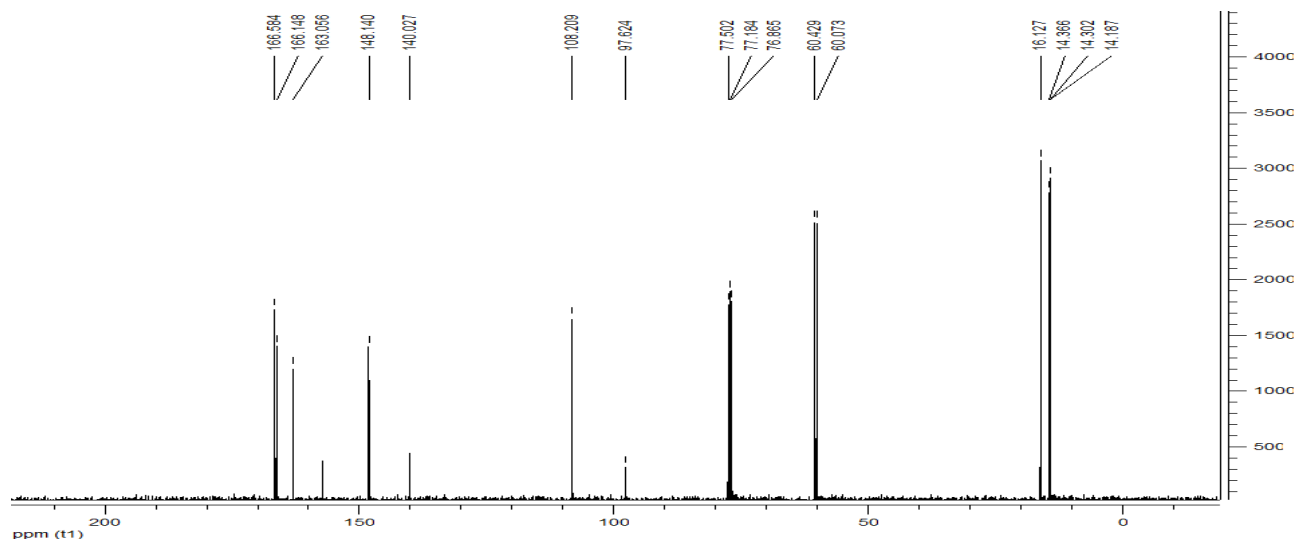
The structure of ligand was confirmed by <sup>1</sup>H NMR. The <sup>1</sup>H NMR spectrum of the ligand, recorded in deuterated chloroform (CDCl<sub>3</sub>) showed the following signals: The spectra of the ligand displayed a low field one proton singlet at ~ 6.8 ppm assignable to the amine proton (NH<sub>2</sub>) proton, CH<sub>2</sub>-CH<sub>3</sub> proton at 1.33 ppm (s, 6H), C-CH<sub>3</sub> proton at 2.2 ppm (singlet), -O-CH<sub>2</sub> at 4.3 ppm (s, 8H), 5.9 ppm are due to protons of aromatic ring.



**Figure 4:** <sup>1</sup>H NMR spectra ligand

### <sup>13</sup>C NMR spectra

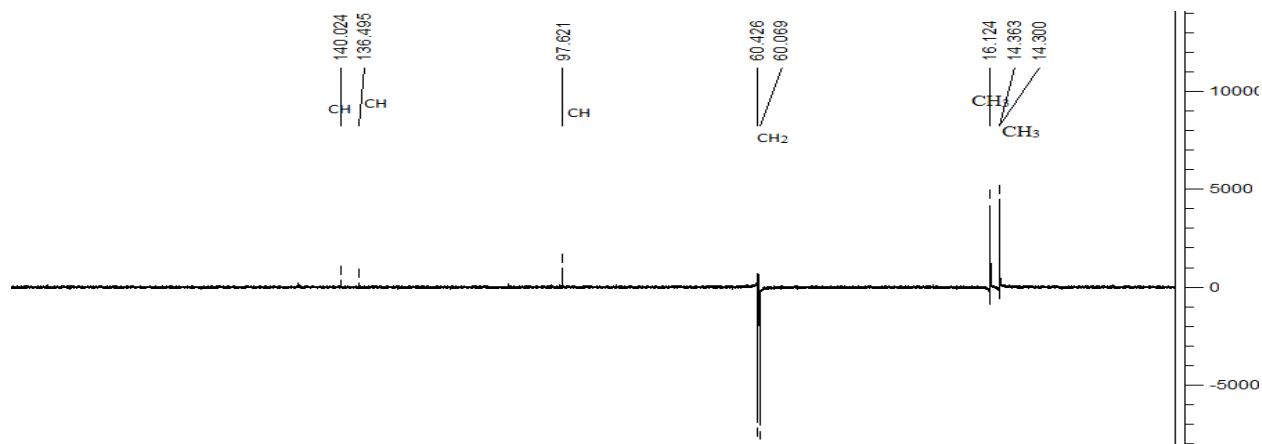
The <sup>13</sup>C NMR spectra of the Ligand are recorded in CDCl<sub>3</sub>. The azomethine carbon (C=N) gives a peak at 166 and 167 ppm, δ C-CH<sub>3</sub> at 14 and 15 ppm, δ C=O at 163 ppm, δ CH of benzene ring gives 77 and 78 ppm. δ CH<sub>2</sub> gives 98 -107 ppm, δ C-O at 60 ppm. So that, from these two spectra(<sup>1</sup>H and <sup>13</sup>C) it is concluded that the number of hydrogen and number of carbon of the ligand.



**Figure 5:**  $^{13}\text{C}$  NMR spectra of the ligand

### DEPT-135 NMR spectra

The Distortionless Enhancement by Polarization Transfer (DEPT) method is an instrumental mode that provides a way to acquire the information of distinguishing among  $^{13}\text{C}$  signals for  $\text{CH}_3$ ,  $\text{CH}_2$ ,  $\text{CH}$ , and quaternary carbons.  $135^\circ$  pulse: carbon signals show different phases, signals for  $\text{CH}_3$  and  $\text{CH}$  carbons give positive signals, quaternary carbons give no signal and signals for  $\text{CH}_2$  carbons give negative signals. Hence, in the proposed ligand, three for  $\text{CH}_3$  and three for  $\text{CH}$  positive signals but two negative signals for  $\text{CH}_2$  group.



**Figure 6:** DEPT-135 NMR spectra of the ligand

#### 4.4. Conductivity Measurement

The measurement of molar conductance is an important tool that gives valuable information about the coordination number, the structure of the complexes and the nature of counter ions present in the complexes. It also helps us to confirm the electrolytic nature and thereby the numbers of ions present outside the coordination sphere. Conductivity of the complex was measured in dimethylsulfoxide (DMSO) at  $10^{-3}$  mol/L and room temperature. The molar conductance ( $\Lambda_M$ ) of a solution was calculated from the measured value of specific conductance;

$$\Lambda_M = 1000 \cdot K \cdot S / C$$

Where k: cell constant; S: conductivity of the solution in Siemens or the inverse of resistance in ohm ( $1S = 1 \text{ ohm}^{-1}$ ); C: dissolution concentration of complexes in mol/L [34].

**Table 6:** Conductivity of the complexes

Complex	Solvent	Molar Conductance ( $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ )	Nature of the complex
Cu(II) complex	DMSO	198.1	Electrolyte
Hg(II) complex	DMSO	245	Electrolyte

#### 4.5. Electronic spectra

The electronic absorption spectra are often very helpful in the evaluation of results furnished by other methods of structural investigation. The electronic spectra measurements were used for assigning the stereochemistries of metal ions in the complexes based on the positions and number of d-d transition peaks. The electronic absorption spectra of the ligand and complexes were recorded at room temperature using DMSO as a solvent. The ligand shows band around 420 nm to 440 nm were due to  $n \rightarrow \pi^*$  transition and also shows bands at 300 nm and 350 nm were due to  $\pi \rightarrow \pi^*$  transition. But copper (II) complex shows bands at 410 nm and 370 nm for  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transition respectively. The metal ion in the mercury (II) complex has  $d^{10}$  electronic configuration [35]. The electronic spectra of this complex shows charge transfer transition at 302 nm. So, the geometry of complex is trigonal planer.

#### 4.6. Antibacterial Activity Tests

The ligand and its metal complexes were screened against four human pathogenic bacteria viz. *Salmonella typhus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* bacterial strains to assess their potential as antibacterial agents by disc diffusion method [36, 37]. Activity was determined by measuring the diameter of the zones of inhibition (mm) and these are presented in Table 7. The results of the antibacterial activity of the compounds compared with standard drug (*Gentamicin*) have indicated that compounds were active but activity was lesser than the standard drug. It is found that the metal complexes have higher antibacterial activity than the free ligand. Cu(II) complex showed significant activity against *Staphylococcus Aureus* and *Salmonella typhi* bacterial strains with than Mercury complex. But Mercury complex showed more activity against *Pseudomonas aureginosa* and *Escherichia coli* bacterial strains than Copper complex. Antimicrobial activity of ligand was enhanced upon complexation which is due to the increase in lipo-solubility of the complex upon coordination with the ligand.

Hence, it is known that chelation tends to make the ligand to act as more powerful and potent bacterial agent [38 - 40]. A possible explanation for this increase in the activity upon chelation is that in a chelated complex, positive charge of the metal is partially shared with donor atoms present on ligands and there is an electron delocalization over the whole chelating ring. This, in turn, increases the lipid layers of the bacterial membranes). Generally, it is suggested that the chelated complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms [41, 42].

**Table 7:** Antibacterial Activities of the ligand and its metal complexes.

Compounds	Diameter of Zone of Inhibition in mm			
	<i>Staphylococcus Aureus</i>	<i>Salmonella typhi</i>	<i>Pseudomonas Aureginosa</i>	<i>Escherichia coli</i>
Ligand	8	-	-	-
Hg <sup>2+</sup> complex	10	9.5	14	16
Cu <sup>2+</sup> complex	12	17	12.5	15
Gentamicin	18	20	24	21
DMSO	-	-	-	-

(-) means no inhibition

## 5. CONCLUSIONS AND RECOMMENDATIONS

The ligand (*Ethyl-4-amino-5-(((6-aminopyridin-2-yl)imino)(ethoxy)methyl)-2-methylthiophene-3-carboxylate* ) was synthesized by the condensation of diethyl-2-amino-4-methyl thiophene-3, 5-dicarboxylate with 2, 6-diamino pyridine, and its complex with Cu(II) and Hg(II) were prepared and characterized. The structural characterizations of the synthesized compounds were characterized using the NMR, IR and UV spectral techniques. The antibacterial studies suggested that metal complexes showed significantly enhanced antimicrobial activity against bacterial strains in comparison to the free ligand. Present observations may serve as an initial step to further investigate and establish the structure of the complexes and their antimicrobial activities.

## REFERENCES

- [1]. Arora K., Sharma K.P., Complexes of Cobalt (II), Nickel (II) and Copper (II) with 3-Hydroxysalicyliden-p-aminoacetophenoneoxime. *Inorg. MetOrg. Chem.*, **2003**, 32, 913.
- [2]. Vigato, P. A., Tamburini, Functionalisation of silica gel with cyclic and acyclic Schiff bases and related d- and/or f-metal complexes, *Coord. Chem. Rev.*, **2004**, 248, 1717.
- [3]. Gupta Y.K., Agarwal S.C., Madnawat S.P. and Ram Narain. Synthesis, Characterization and Antimicrobial Studies of Some Transition Metal Complexes of Schiff Bases. **2012**, 2(4), 68-71.
- [4]. Mishra, Lallan and Sinha Ragini, Mononuclear and dinuclear ruthenium (III) polypyridyl complexes containing 2,6-bis(2-benzimidazolyl)-pyridine as co-Ligand: Synthesis, spectroscopic and redox activity, *Indian J. Chem., Sect. A*, **2000**, 29, 1131.
- [5]. Saritha P., Reddy B. Satyanarayan and Jayatyagaraju, Synthesis and structural studies on divalent transition metal complexes of 5-acetyl 2,4-dihydroxy acetophenone semicarbazone, *J. Indian Chem. Soc.*, **2006**, 83, 1204.
- [6]. Spinu C. and Kriza A. Co (II), Ni (II) and Cu (II) complexes of bidentate Schiff bases, *Acta Chim. Slov.*, **2000**, 47, 179.
- [7]. Maurya RC, Mishra DD, Jain S, Jaiswal M, Synthesis and characterization of some mixed ligand complexes of copper (II) and cobalt (II) with Schiff bases and heterocyclic organic compounds, *Synth. React. Inorg. Met-Org. Chem.*, **1993**, 23, 1335-1349.
- [8]. Roos J. T., Williams D. R., Synthesis and evaluation of several compounds with potential antiviral activity. *J. Inorg. Nucl. Chem.*, **1977**, 39,129-141.
- [9]. Cole A., Goodfield J., Williams D. R and Midley J. M. The complexation of transition series metal ion by Nalidixic acid, *Inorg. Chim. Acta.* **1984**, 92,91-97.
- [10]. Srivastava R. S., Synthesis, characterization and fungitoxicity of Bidentate High-Spin six Coordinate 3<sup>rd</sup> period metal complexes with N-(5-Phenyl-3,4-thiadiazol-2-yl)aceta benzamidines. *Inorg. Chim. Acta.*, **1981** 55,71-74.



- [11]. Abdel-Rahman, R. M., Role of Uncondensed 1,2,4-triazine Compounds and Related Heterocyclic systems as Therapeutic Agents. Review Part XV, *Pharmazie*. **2001**, 56(1), 18-22.
- [12]. D. W. Young, *Heterocyclic Chemistry*, First Edition, London. **1975**, 49..
- [13]. M. S. Thomas, Complexes of thiophene derivatives as potential metallomesogens,**2006**, 1-30.
- [14]. M. V. Angelusiu, G. L. Almajan, D. C. Ilies, T. Rosu and M. Negoiu, Cu (II) complexes with nitrogen-oxygen donor Ligands: synthesis and biological activity. Chem. Bull. "Politehnica" Univ. (Timisoara,**2008**, 53,(67), 78-82.
- [15]. Thomas McPherson Brown, M. D. Treatment of rheumatoid disease. Walker Road, Fairview, **2000**.
- [16]. J. M. Gemi, C. Biles, J. B. Keiser, S. M. Poppe, S. M. Swaney, W. G. Tarapley, D. L. Romeso and Y. Yage, J. Med. Spectrophotometric and conductometric study of the complexation of *N*-salicylidene-2-aminophenol with Cu(II) in methanol and 1,4-dioxane binary solutions. Chem., **2004**, 43, 1034.
- [17]. K. S. Suslick and T. J. Reinert, J. Synthetic Applications of Ultrasound, *Modern Synthetic Methods*, Chem. Educ., **1986**, 62, 974.
- [18]. J. Tisato, F. Refosco and F. Bandoli, Application of Schiff bases and their metal complexes-A Review: Coord. Chem. Rev, **1994**, 135, 325.
- [19]. Arnal N.; Cristalli D. O., de Alaniz M. J. T., and Marra C. A., Clinical utility of copper, ceruloplasmin, and metallothionein plasma determinations in human neurodegenerative patients and their first-degree relatives, *Brain Res.*, **2010**, 1319, 118-130.
- [20]. Rivero-Muller A., De Vizcaya-Ruiz A.,; Plant N., Ruiz L.,and Dobrota M. Mixed chelate copper complex, Casiopeina IIglyR, binds and degrades nucleic acids: A mechanism of cytotoxicity. *Chem.-Biol. Interact.* **2007**,165, 189-199.

- [21]. P. Parameshwara, J. Karthikeyan, A.N. Shetty, P. Shetty, *J. Iranian Chem. Soc.*, **2006**, 3, 168.
- [22]. He Xiao-Ling, Wang Yong-Qiu, Ling. Ke-Qing, Preparation and Application of cdte Nanocrystals, *Talanta*, **2007**, 72, 747.
- [23]. W. Eikens, C. Kienitz, P.G. Jones, C. Thone, Synthesis and Characterization of Two New Copper (II) Complexes *J. Chem. Soc. Dalton Trans.*, **1994**, 83.
- [24]. S. Ahmad, A.A. Isab, A.R. Al-Arfaj, A.P. Arnold, Synthesis and Characterization of Two New Copper (II) Complexes *Polyhedron*. **2002**, 21, 2099.
- [25]. A.Isab Anvarhusein, I.M. Mohammed, Wazeer Fettouhi, Saeed Mohammed, Ashraf Ahmad Waqar, *Polyhedron*. **2006**, 25 2629.
- [26]. A. P. Arnold, K. S. Tan, D. L. Rabenstein, Novel mercury (II) complexes of Schiff base Ligands: Synthesis and spectral characterization, *Inorg. Chem.*, **1986**, 25, 2433.
- [27]. F.A. Devillanova, and G. Verani. Novel mercury (II) complexes of Schiff base Ligands: Synthesis and spectral characterization, *Transit. Met. Chem.*, **1977**, 2, 9.
- [28]. W. Levason, C. A. McAuli; *The Chemistry of Mercury*, McMillan, London, **1977**, pp
- [36]. Keppler B. K., Berger M. R., Klenner Th.,and Heim M. E., Metal complexes as antitumorAgents, *Adv. Drug. Res.*, **1990**, 19, 243.
- [29]. S. Annapoorani and C.N.Krishnan. Synthesis and Spectroscopic Studies of Trinuclear N4 Schiff Base Complexes. **2013**, 5(1), 180-185.
- [30]. Shukla, D, Gupta, L. K., and Chandra, S., Spectroscopic studies on chromium (III), Manganese (II), cobalt (II), nickel (II) and copper (II) complexes with hexadentate nitrogen–sulfur donor [N<sub>2</sub>S<sub>4</sub>] macrocyclic ligand. *Spectrochim. Acta.*, **2008**, 71A, 746–750.

- [31]. Spinu, C., Kriza, A., and Spinu L., Studies on N-[2-thienylmethylidene]-2-aminothiazole complexes of Fe (II), Co (II), Ni (II), Cu (II), Zn (II) and Cd (II), *Acta Chim. Slov.*, **2001**, 48, 257-264.
- [32]. Chandra, S., and Kumar, U., Spectral and magnetic studies on manganese (II), cobalt (II) and nickel (II) complexes with Schiff bases, *Spectrochim. Acta A.* **2005**, 61A, 219-227.
- [33]. A.P. Mishra, and M. Khare, Synthesis, characterization and biological studies of cobalt complexes with bidentate schiff bases, *J. Ind. Chem. Soc.*, **2000**, 77, 367.
- [34]. S. R. Aswale, P. R. Mandlik, S. S. Aswale and A. S. Aswar, Synthesis and characterization of Cr(III), Mn(III), Fe(III), Ti(III), VO(IV), Th(IV), Zr(IV) and UO<sub>2</sub> (VI) polychelates derived from bis-bidentate salicylaldimine Schiff base, *Indian J. Chem.* **2003**, 42A, 322-326.
- [35]. Lever A. B. P., *Inorganic Electronic Spectroscopy*, 2<sup>nd</sup> ed<sup>n</sup>, Elsevier, New York, **1984 pp.**
- [36]. National Committee for Clinical Laboratory Standards and Performance, antimicrobial susceptibility of Flavo-bacteria, compant, country, **1997 pp.**
- [37]. National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3, National Committee for Clinical Laboratory Standards, Villanova, **1993**.
- [38]. Ndahi N. P, Nasiru Y. P and Sand. be U K, Synthesis, characterization and antibacterial studies of some Schiff base complexes of Co (II), Ni (II) and Zn (II). *Asian J., Biochemical and Pharmaceutical Res.*, **2012**, 1(2), 2231-2560.
- [39]. Zahid H, C. and Maimoon F. J., Synthesis, characterization and biological evaluation of Co (II), Ni (II) and Zn (II) complexes with Cephadrine, Metal Based Drugs, **2000**, 7(5), 265-269.
- [40]. Raman N., Ravichandran S and Thangaraja C. Copper (II), nickel (II), and zinc (II) complexes of Schiff bases derived from benzyl-2,4 dinitrophenylhydrazone with aniline. *J.Chem.Sci.* **2004**, 116(4), 215-219.

- [41]. Franklin T.J., Snow G.A., Biochemistry of Antimicrobial Action, 2<sup>nd</sup> ed. Chapman and Hall, London. **1971**.
- [42]. Mehmet Soñnmez, Metin Celebi, and Ismet Berber,; Eur. J. Med. Chem., **2010**, 45 1935.