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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL STUDY OF A SCHIFF BASE LIGAND DERIVED FROM 5-AMINO-1, 3, 4-THIADIAZOLE-2-THIOL AND 2, 6-DIAMINOPYRIDINE AND ITS CU(II) METAL COMPLEX

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JIMMA, ETHIOPIA

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BY:

SEGNI ASAFA

Advisors: **Mohammed Fakruddin Ali (PhD)**

Girma Selale (M. Sc.)

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List of abbreviations and symbols

MTIEPE- 1-(6-(1-((5-mercapto-1-3-thiodial-2-yl) imino) ethyl) pyridine-2-yl) ethanone

M. pt- Melting point

TLC – thin layer chromatography

Cu-L - Cu (II) complex of the Ligand

Λ_M - Molar conductance

TMS- Tetra methyl silane

SOD- superoxide dismutase

DAP-2, 6-diacetylpyridine

ATT- 5-amino1,3,4-thiadiazole-2- thiol

Abstract

The Schiff base 1-(6-(1-((5-mercapto-1-3-thiodial-2-yl) imino) ethyl) pyridine-2-yl) ethanone (MTIEPE) was synthesized by condensation of 2, 6-diacetylpyridine and 5-amno-1, 3, 4-thiadiazole -2-thiol in ethanol using microwave method of synthesis. Cu(II) complex of MTIEPE was synthesized by refluxing the solution of the $CuSO_4.5H_2O$ *and the ligand in the ethanol in 1:1 molar ratio and the progress of the reaction was monitored using TLC. The ligand and Cu(II) complex were characterized using IR,NMR, and molar conductance. From the observed data it was concluded that the formula of the Cu(II) complex of MTIEPE ligand is* $CuOSO_3$ *with octahedral geometry. Finally the antibacterial study of the Ligand MTIEPE and its copper (II) complex was carried out by disc diffusion method on selective bacteria. The complex showed greater antibacterial activity than the reference antibiotic, gentamycine, towards Staphylococcus aureus among the tested bacteria. Thus, with further investigation and exploitation this copper (II) complex can be used as antibacterial drugs for the treatment of infections caused by this bacterium.*

Key words. Thiadiazole, thiadiazole derivative, Cu (II) complex of thiadiazole derivative, antibacterial activity

1. INTRODUCTION

In this study metal complex derived from Schiff base (MTIEPE) from reagents 2, 6-diacetylpyridine and 5-amino-1, 3, 4-thiadiazole-2-thiol was synthesized and characterized. The coordination chemistry of Schiff bases derived from thiadiazole has received much attention and interest [1, 2]. Thiadiazole and its related compounds are very useful reagents in biological systems.

1.1. Metal complex

Chelating ligands in the field of coordination chemistry and their metal complexes are of great interest. It is well known that N, S and O atoms play a key role in the coordination of metals at the active sites of numerous metallobiomolecules. Chelating ligands containing O, N and S donor atoms show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions [3]. Medicinal applications of metals have played an important role in medicine since thousands of years. Many essential metal ions in our diets in varying quantities are essential, although its significance has been recently realized.

Metal complex provides better opportunities to use as therapeutic agents. The mode of action of metal complex on living organism is differing from non-metals. It shows great diversity in action. The lipophilicity of the drug is increased through the formation of chelates and drug action is increased due to effective permeability of the drug into the site of action.

1.1.1. The chemistry of copper

Copper is a transition metal in group 11 or IB, one of several elements found in 4^{th} row in the periodic table with symbol Cu, atomic number 29, and atomic mass: 63.546. Copper and its compounds have many important uses in modern society. Most electrical equipment has copper wiring and to make many alloys.

Copper is an essential trace element that is vital to the health of all living things. In humans, copper is essential for the proper functioning of organs and metabolic processes. The human body has complex homeostatic mechanisms which attempt to ensure a constant supply of available copper, while eliminating excess copper whenever this occurs. However, like all essential elements and nutrients, too much or too little nutritional ingestion of copper can result in a corresponding condition of copper excess or deficiency in the body, each of which has its own unique set of adverse health effects.

The chemical nature of copper is very important in determining its biological availability, both in the environment and in food. Some of the uses of copper come from its ability to control the growth of organisms. This occurs when copper is biologically available and at concentrations that are detrimental. As a result, copper is used in a range of cidal agents. For example, copper has been demonstrated to be an effective antibacterial, antiplaque agent in mouthwashes and toothpastes.

Few dietary components are more misunderstood than copper. Although copper is the third most abundant essential trace mineral in the body, after iron and zinc, most people consider it unimportant. Even worse, many people have actually taken steps to exclude it from their diets and dietary supplements, believing it to be nothing more than a cause of free radical reactions. This is surprising, because copper has been recognized as an essential nutrient since the 1920's [4]. In the past seventy years, much has been learned about the important biological roles of copper and the copper-dependent enzymes. In fact, copper is emerging as one of the most important minerals in our diet. While unbound, free copper does generate free radicals in vitro, the relevance of this in the body has been called more imaginary than real [5]. In fact, copper has an entirely different role in the body, being a component of two of our most important antioxidant enzymes, copper-zinc superoxide dismutase and ceruloplasmin [6, 7].

Unbound, free copper is not found in large quantities in the human body. Instead, almost all of the copper in our bodies is bound to transport proteins (ceruloplasmin and copper-albumin), storage proteins (metallothioneins), or copper containing enzymes. A substantial number of copper metalloenzymes have been found in the human body.

Most features of severe copper deficiency can be explained by a failure of one or more of these copper-dependent enzymes. For instance, depigmentation can be explained by a tyrosinase deficiency and the defects of collagen and elastin causing abnormalities in the connective tissue and vascular system can be explained by a lysyl oxidase deficiency. Unfortunately, most research into copper deficiency has focused on acute, severe deficiency. This is relatively rare in humans and animals on typical, varied diets. Marginal, chronic deficiency, however, is much more common [8].
As an example in lysyl oxidase is one of the most important and best understood roles of copper in the body [9, 10]. This is the main enzyme involved in the necessary cross-linking of connective tissue. Optimal functioning of lysyl oxidase ensures the proper cross linking of collagen and elastin, vital for the strength and flexibility of our connective tissue. A reduction in lysyl oxidase activity affects the integrity of numerous tissues, including our skin, bones, and blood vessels. In copper deficiency the level of lysyl oxidase isn't altered, but the activity of the enzyme can be reduced by more than fifty percent. Not surprisingly, some of the hallmarks of copper deficiency are connective tissue disorders, osteoporosis and blood vessel damage. Although most research utilizing copper complexes has been to determine anti-inflammatory activity, copper complexes have shown potential as a physiological approach to the treatment of numerous chronic diseases. This potential has been expanded to include, in addition to inflammatory diseases, gastrointestinal ulcers, cancers, carcinogenesis and diabetes. In these conditions much of the research interest has centered on finding that many copper complexes demonstrate superoxide dismutase activity [11]. Because of this, many of these compounds have been designated as superoxide dismutase -mimetic. One of the recent reviews on this topic of copper complexes is a good example of the breadth of research that has been published on this topic. Unfortunately, despite the tremendous promise that copper complexes have in many varied diseases and conditions, clinical interest in these compounds has been almost nonexistent.

Copper is slowly becoming less misunderstood, one can only hope that it will eventually be properly utilized in its potential for maintaining health and treating disease. The polynuclear copper(II) complexes have aroused extensive interest due to their importance in biological processes and in inorganic material science. The metal ion dependent oxidative DNA cleavage by Cu(II) complexes is of topical interest. In the presence of suitable chelating ligands, the d^9 electronic state of Cu(II) may easily accept an electron to produce stable d^{10} Cu(I) species. Such redox-active systems may achieve the decomposition of H_2O_2 to reactive oxygen species (ROS) which can oxidize DNA by multiple attacks at the sugars and nucleo bases [12].The coordination geometry around the Cu(II) ion plays a major role as well in the redox-mediated formation of reactive oxygen species. The activation of molecular oxygen by a mononuclear Cu(II) complex in the presence of DNA is expected to lead to the abstraction of a proton/hydrogen from the sugar backbone or from the bulk solvent.

Oxidation states and chemistry of copper [13]

Oxidation state	Coordination number	Geometry
Cu(I), d^{10}	2	Linear
	3	Planar
	4	Tetrahedral
	4	Distorted Planar
	5	Square Planar
	6	Octahedral
Cu(II), d^{9}	3	Trigonal Planar
	4	Tetrahedral(Distorted)
	4	Square
	6	Distorted Octahedral
	5	Trigonal bipyramidal
	6	Octahedral
	7	Pentagonal bipyramidal
	8	Distorted dodecahedron
Cu^{III},d^{8}	4	Square
	6	Octahedral

1.1.2. Copper complex

Metal complexes have been extensively utilized in clinics for centuries and have attracted numerous inorganic chemists to analyze them, with the main focus being medical applications. Copper, an essential trace element with an oxidative nature and bioessential activity in human metabolism, does not exist in an ionic form in biological systems. Thus, measurement of copper in the body is evaluated in the form of complexes with organic compounds. Schiff bases are a critical class of compounds in medical chemistry that have demonstrated significant chemotherapeutic and antibacterial application. Schiff base Cu(II) complexes revealed great potential for antiproliferative, antibacterial, and gastro protective activity[14,15,16].

Copper(II) complexes are attractive since Cu(II) is known to play a significant role in naturally occurring biological systems as well as a pharmacological agent. Copper is a biologically relevant element and many enzymes that depend 115 on copper for their activity have been identified. The metabolic conversions catalyzed by most of these enzymes are oxidative. Because of their biological relevance a large number of copper(II) complexes have been synthesized with different perspectives[16,17].

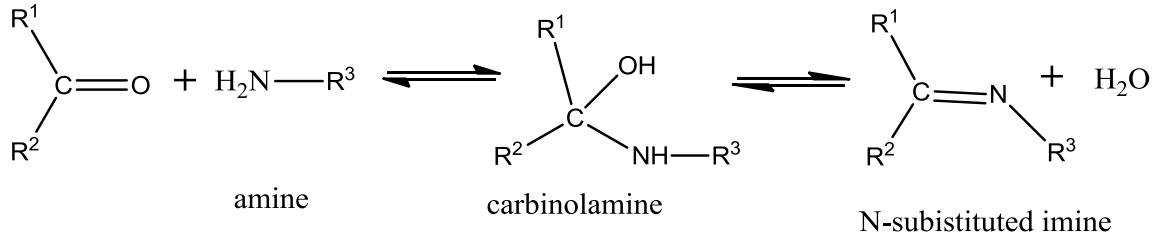
1.2. Ligand

Ligand is an ion or a molecule can donate a lone pair of electron(s) to a central metal atom/ion and forms a coordination complex. Ligands are viewed as Lewis bases. The bonding between metal and ligand generally involves formal donation of one or more of the ligand's electron pairs. The nature of metal-ligand bonding can range from covalent to ionic. Furthermore, the metal-ligand bond order can range from one to three.

Metal and metalloids are bound to ligands virtually in all circumstances, although gaseous naked metal ions can be generated in high vacuum. Ligands in a complex dictate the reactivity of the central atom, including ligand substitution rates, the reactivity of the ligands themselves and redox reactions. Ligand selection is a critical consideration in many practical areas, including bioinorganic and medicinal chemistry, homogeneous catalysis and environmental chemistry [2].

1.2.1. Schiff Bases

The condensation of an aldehyde or ketone with primary amine leads to the formation of an imine called Schiff base [18, 19]. They have the general formula RN=CR' where the R and R' are alkyl, aryl, cycloalkyl or heterocyclic groups. Schiff bases of aliphatic aldehydes are relatively unstable and are readily polymerizable while those of aromatic aldehydes, having an effective conjugation system, are more stable.



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

Schiff base can be synthesized usually by microwave heating and refluxing the mixture of aldehyde/ketone and the amine solution.)

A microwave is a form of electromagnetic energy, which falls at the lower end of the electromagnetic spectrum. Recently, microwave heating has emerged as a powerful technique to promote a variety of chemical reactions [20, 21].Microwave synthesis is attractive in offering reduced pollution, low cost, saving time and offer high yields together with simplicity in processing and handling [22].The recent introduction of single-mode technology assures safe and reproducible experimental procedures and microwave synthesis has gained acceptance and popularity among the synthetic chemist community.

The application of microwave irradiation to organic synthesis has been the focus of considerable attention in recent years and is becoming an increasingly popular technology [23].

1.2.2. Metal complexes of Schiff Bases

Metal complex is a chemical species consisting of a central atom or ion bonded to surrounding molecules or ions. The central atom of a coordination complex commonly is a metal cation. Various ligands or complexing agents may surround the central atom of a coordination complex.

Metal complexes or coordination complexes consist of a metal atom or ion bonded to a surrounding array of molecules or anions, which are in turn known as ligands or complexing agents. The atom within a ligand that is bonded to the central atom or ion is called the donor atom. A typical complex is bound to several donor atoms, which can be different or same.

A transition metal is one, which forms one or more stable ions, which have incompletely filled d-orbital(s). Based on the definition outlined above, scandium and zinc do not count as transition metals, because: Scandium has the electronic structure [Ar] $3d^1 4s^2$. When it forms ions, it always loses the 3 outer electrons and ends up with an argon structure. The Sc^{3+} ion has no d electrons and so does not meet the definition. Zinc has the electronic structure [Ar] $3d^{10} 4s^2$. When it forms ions, it always loses the two 4s electrons to give a 2+ ion with the electronic structure [Ar] $3d^{10}$. The zinc ion has full d levels and does not meet the definition either.

By contrast, copper, [Ar] $3d^{10} 4s^1$ forms two ions. In the Cu^+ ion the electronic structure is [Ar] $3d^{10}$. However, the more common Cu^{2+} ion has the structure [Ar] $3d^9$. Copper is definitely a transition metal because the Cu^{2+} ion has an incomplete d level. Variable oxidation state (number): One of the key features of transition metal chemistry is the wide range of oxidation states (oxidation numbers) that the metals can show.

It would be wrong, though, to give the impression that only transition metals can have variable oxidation states. For example, elements like sulfur or nitrogen or chlorine have a very wide range of oxidation states in their compounds - and these obviously are not transition metals.

However, this variability is less common in metals apart from the transition elements. Of the familiar metals from the main groups of the Periodic Table, only lead and tin show variable oxidation state to any extent.

Most of the 3d transition metal ions exhibit vital roles in biological systems. They are called metalloproteins. Metal ions, which are centers for enzymatic activity, determine the geometry of active sites and act as biological redox facilitators. As a consequence of their partially filled d orbitals, transition metals exhibit variable oxidation states and a rich variety of coordination geometries and ligand spheres [24].

1.2.3. Applications of metal complexes of Schiff bases

1.2.3..1. Application of Metal complexes of Schiff Bases as Medicines Many Schiff bases are known to be medicinally important and used to design medicinal compounds [25]. It has been demonstrated that the biological activity of Schiff bases either increase or decrease upon chelation with metal ion. Cu(II) complex of and 8-formyl-7-hydroxy-4-methyl coumarin show potent antibacterial activity against Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and Salmonella typhi and antifungal activities against Aspergillus niger, Aspergillus flavus and Cladosporium. The Cr (III), Fe (III) and Co (III) complexes formed form tetradentate (ONNO) Schiff base ligands, 1,4-bis[3-(2-hydroxy-1-naphthaldimine) propyl]piperazine and 1,8-bis[3-(2-hydroxy-1-naphthaldimine)-p-menthane, show moderate antimicrobial activity compared to standard antibiotics. The antibacterial activity of the tridentate Schiff base, formed by condensation of 2-amino-3-carboxyethyl-4,5-dimethylthiophene with salicylaldehyde, was found to increase on chelation with transition metal ions[26].

Co (II), Ni (II), Cu (II) and Zn (II) complexes of the Schiff base derived from vanillin and DL-α-aminobutyric acid were also found to exhibit higher antibacterial activity compared to the free Schiff bases. Several mono and binuclear transition metal complexes of the Schiff base derived from phenylamino acetohydrazide and dibenzoylmethane, are more potent bactericides and fungicides than the ligand .Sharma and Piwnica-Worms reported Schiff base complexes that target hemozoin aggregation like the antimalarial drug, chloroquine.

Investigations on the interactions of DNA with transition metal complexes provide leads for rational drug design, as well as means for the development of sensitive chemical probes for DNA. These interactions would be either covalent or non-covalent. In covalent binding the labile part of the complexes is replaced by a nitrogen base of DNA. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove binding of cationic metal complexes along periphery of the DNA helix, the major or minor groove. Intercalation involves the partial insertion of aromatic heterocyclic rings between the DNA base pairs.

Gupta and co-workers [16] reported DNA binding properties of a series of transition metal complexes having potential NNO-tridentate donor Schiff bases derived from the condensation of 2,6-dibenzoyl-4-methylphenol with diamines.DNA binding studies of the cationic Ni(II) complex of the 5-triethyl ammonium methyl salicylideneortho-phenylendiimine ligand, shows that the metal complex strongly interacts with DNA [Ref]. Zn(II) and Cu(II) complexes of this Schiff base interact with native calf thymus DNA by groove or intercalating binding mode. The Co(II) and Ni(II) complexes of salicylaldehyde-2-phenylquinoline-4-carboylhydrazone interact with calf-thymus DNA via a groove binding mode.

Cu(II) forms stable complexes with nitrogen and oxygen donor ligands. Cu(II) complexes of nitrogen ligands are generally more stable than Cu(I) complexes [25]. The most common coordination number of Cu(II) is 4, 5 and 6 but regular geometry is rare, and the distinction between square-planar and distorted octahedral coordination is not easily made. 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.

Popova and Berova [12] reported that copper and its complexes are good for liver function, its level in blood and urine has influence in pregnancy disorders, nephritis hepatitis, leprosy, anemia and leukemia in children. Some Schiff bases possess simple harmonic generation activity. Amido-Schiff base form chelates with Cu(II) and act as a thrombin inhibitor [27].

Copper complex with isatin (1H-indole-2,3-dione) and its derivatives show a variety of biological effects, including inhibition of monoamine oxidase. Copper in trace quantities is required by all living organisms to maintain proper cellular functions. 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 $^{2}T_{2g} \_ {}^{2}E_{g}$. Considerable distortion in octahedral symmetry is observed in Cu(II) complexes [13]. 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 temperature.

1.2.3..2. Catalytic activities of Metal Complexes of Schiff Bases

Many copper (II) Schiff base complexes are known to be useful reagents for oxidative and hydrolytic cleavage of DNA. In addition to the biological properties, a large number of copper (II) Schiff base complexes have been used as catalysts in the aziridinationand cyclopropanation of olefins and in the per oxidative oxidation of phenol to dihydroxy benzenes in which they act as models for catalase enzymes [28].

1.3. Objective of the study

1.3.1. General Objective

The aim of this work is to synthesize, characterize and investigate antibacterial activity of Cu(II) complexes of Schiff bases.

1.3.2. Specific Objective

To prepare novel Schiff base (MTIEPE) from reagents, 2, 6-diacetylpyridine and 5-amino-1,3,4-thiadiazole-2-thiol at maintained at constant low temperature by microwave method.

To synthesis copper complex from Schiff base (MTIEPE) and copper sulphate salt.

To characterize the obtained ligand (MTIEPE) and its copper complex by using molar conductance, infrared spectroscopy and NMR spectroscopy.

To evaluate antibacterial activity of MTIEPE and its copper complex on selected bacteria: *Salmonella typhimurium*, *Staphylococcus aureus* and *E. coli*.

1.4. Statement of the Problem

Metal-Ligand complexes are well-known since they have various applications, for example they can be applied for chemotherapy, in solar cell as molecular wire, as catalysts in asymmetric organic synthesis and biocatalysts. These applications are based on their stereo electronic properties, chirality's and nature of metal and ligands. Vast application of macrocyclic ligand–complexes is recently well documented. Due to the stereo specific synthesis of metal–Schiff's base as ligand, 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 [14].Metal complexes were shown to possess superior biological properties, catalytic efficiency compared to classical inorganic compounds.

Thus, the present work attempted to investigate the way to:-

Synthesize Schiff base (MTIEPE).

Study the antibacterial activity and structural elucidation of Cu(II) complex with (MTIEPE) ligand.

1.5. Significance of the Study

Schiff base and its Cu(II) complex are of significant interest because of their biological activity including antibacterial activity. Cu(II) complex of this Schiff base may provide better opportunities to use as therapeutic agents. The lipophilicity of the drug is increased through the formation of chelates and drug action is increased due to effective permeability of the drug into the site of action. Therefore, the outcome of this research project may be useful in developing antibacterial drug.

2. REVIEW OF RELATED LITERATURE

Much attention has been devoted by bioinorganic as well as medicinal chemists to present the relationship between the metal ions and their complexes as antitumour and antibacterial agents [21, 22]. Several reviews showed that the coordination chemistry of many compounds greatly influence their biological action highlighting the catalytic function of metal in many biological processes. Metal complexes have shown many useful evidences in medicinal aspects.

The work reported in this paper describes the synthesis as well as the elucidation of the chemistry and structures of a new series of Cu (II) complexes of Schiff bases (MTIEPE*)* derived from reagents, 2,6-diacetylpyridine and 5-amino-1,3,4-thiadiazole-2-thiol. The Schiff base was synthesized from 1,3,4-thiadiazole and pyridine derivatives.

2.1. Thiadiazoles and Thiadiazole derivatives

Heterocyclic compounds such as thiazoles, thiadiazoles, indoles, oxadiazoles, benzisoxazoles and pyrroles have been successfully used as antibacterial, anticancer, antipyretic, schistosomicidal, hypoglycemic, antihypertensive, antitubercular, anti-inflammatory and anti-HIV agents. In addition, they have also been used in agriculture, plastics, polymers, dyes and textiles [22]. Hence, heterocyclic chemistry still continues to draw the attention of synthetic organic chemists and is of great scientific interest. A large number of organo-sulfur compounds occur in living and non-living objects. They belong to open chain, alicyclic, aromatic and heterocyclic types of compounds containing sulfur atom or atoms as a part of chain/ring or both in the structure [21].

Isolation, identification and applications of these organo-sulfur compounds lead to the fact that some of the compounds are useful in scientific, technical and industrial growth. During the last three decades organo-sulfur chemistry developed at a much faster pace than any other branches of chemistry[15].

The role of organic sulphides in rubber vulcanization, hair curling, muscle contraction, natural aromas, vitamins, hormones, antibiotics, radio-protective agents, dye stuffs, binding materials organic semiconducting materials [29] and organic light emitting diodes.

Thiadiazole is five-member heterocyclic compounds; particularly nitrogen and sulphur heterocyclic; thiadiazole have been successfully tested against several diseases and therefore received special attention in pharmaceutical and medicinal chemistry due to their diverse potential applications. Thiadiazoles and their derivatives can be considered as simple five member heterocyclic possessing one sulphur and two nitrogen atoms.

The thiadiazole exist in different isomeric forms such as 1,2,4-, 1,2,5-, 1,2,3- and 1,3,4-thiadiazoles (a-d) [30]. Thiadiazoles are numbered by designating heteroatoms as shown below.

Figure 1: Types of Thiadiazoles

The resistance of pathogens towards available drugs is rapidly becoming a major worldwide problem. The need to design new compounds to deal with this resistance has become one of the thrust areas of research today. Thiadiazoles continuously draws interest for development of newer drug moiety. Researchers have demonstrated a broad spectrum of biological properties of thiadiazole in both pharmaceutical and agrochemical fields [14].

Compounds having thiadiazole nucleus have wide spectrum of pharmacological activities [23] such as antimicrobial, antitubercular, antileishmanial, anti-inflammatory, analgesic, CNS depressant, anticonvulsant, anticancer, antioxidant, antidiabetic, molluscicidal, antihypertensive, diuretic, analgesic properties [20].

The growing patent literature from the sixties demonstrate that the 1,3,4-thiadiazole and its derivatives have received much attention. This is primarily due to large number of uses of 1,3,4-thiadiazoles in the most diverse areas, for examples, dyestuffs industry, photography and corrosion inhibitors [24].

Numerous 1,3,4-thiadizoles have been synthesized and reported as bactericides[25], fungicide, insecticides, herbicides, flower control agent, herbicides anti-inflammatory, tranquilizing agent and hypoglycemic activity. Recently thiadiazolyl-2-propanol amine derivatives have been prepared and showed some activity toward blood pressure and heart rate.

On the other hand, Schiff bases are well known to possess promising biological activities. In view of the above observations it was considered worthwhile to synthesize some 1, 3, 4-thiadiazoile Schiff-base derivatives starting from 2-amino-5-mercapto-1, 3, 4-thiadizole compound, in view of the fact that a number of these compounds possessing biological activity.

2. 2. Pyridine

Pyridine was first isolated and characterized by Anderson in 1846. It was obtained from bone oil and from coal tar. The cyclic nature of pyridine was recognized by Korner and Dewar in 1869 [31]. Pyridine is a heterocyclic organic compound with the chemical formula C_5H_5N. It is structurally related to benzene, with one CH group replaced by a nitrogen atom. It is used as a precursor to agrochemicals and pharmaceuticals and is also an important solvent and reagent [26].

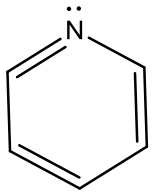


Figure 2. Pyridine

Pyridine is miscible with water and virtually all organic solvents. Most chemical properties of pyridine are typical of a heteroaromatic compound [32]. In organic reactions, pyridine behaves both as a tertiary amine, undergoing protonation, alkylation, acylation, and N-oxidation at the nitrogen atom, and as an aromatic compound, undergoing nucleophilic substitutions. Because of the electronegative nitrogen in the pyridine ring, the molecule is relatively electron deficient.

It, therefore, enters less readily electrophilic aromatic substitution reactions, which are characteristic of benzene derivatives. However, unlike benzene and its derivatives, pyridine is more prone to nucleophilic substitution and metalation of the ring by strong organometallic bases. The reactivity of pyridine can be distinguished for three chemical groups. With electrophiles, electrophilic substitution takes place where pyridine expresses aromatic properties.

With nucleophiles, pyridine reacts via its 2^{nd} and 4^{th} carbon atoms and thus behaves similar to imines and carbonyls. The reaction with many Lewis acids results in the addition to the nitrogen atom of pyridine, which is similar to the reactivity of tertiary amines. Pyridine can act as Lewis base, donating its pair of electron to a Lewis acid as in the sulfur trioxide pyridine complex.

Pyridine derivatives of different heterocyclic nucleus have shown potent pharmacological proprieties like antifungal, antibacterial [33], antimicrobial and insecticidal. In this connection, great attention has recently been paid for the synthesis of pyridine.

Pyridine itself is a relatively weak ligand in forming complexes with transition metal ions. For example, it forms a 1:1 complexes with Ni((II), and Cu(II) [10]. Picolinic acid, which is a substituted derivative of pyridine, forms strong complexes due to the chelate effect; 2,2'-bipyridine and 1,10-phenanthroline, which can also be viewed as substituted derivatives of pyridine, also form strong complexes, such as in Ferroin, which can be used as an redox indicator in the quantitative analysis of iron. Pyridine has a conjugated system of six π-electrons that are delocalized over the ring. The molecule is planar and, thus, follows the 3 Hückel criteria for aromatic systems.

In contrast to benzene, the electron density is not evenly distributed over the ring, reflecting the negative inductive effect of the nitrogen atom. For this reason, pyridine has a dipole moment and a weaker resonant stabilization than benzene (resonance energy 117 $kJ \cdot mol^{-1}$ in pyridine versus 150 $kJ \cdot mol^{-1}$ in benzene). The electron localization in pyridine is also reflected in the shorter C–N ring bond (137 pm for the C–N bond in pyridine versus. 139 pm for C–C bond in benzene), whereas the carbon–carbon bonds in the pyridine ring have the same 139 pm length as in benzene.

2.2.1. 2, 6-diacetylpyridine

A 2, 6-diacetylpyridine, also called pyridine-2,6-diactyl, is a pyridine derivative with formula $C_4H_3N(COCH_3)_2$. It is a white solid that is primarily used as a precursor to ligands in coordination chemistry. The synthesis of 2, 6-diacetylpyridine begins with oxidation of the methyl groups in 2, 6-lutidine to form dipicolinic acid. This process has been well established with potassium permanganate and selenium dioxide. The same process is catalyzed enzymatically. The diketone can be formed from the diester of picolinic acid groups through a Claisen condensation. The resulting adduct can be decarboxylated to give diacetylpyridine [34].

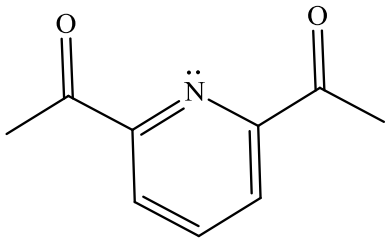


Figure 3. 2, 6-diacetylpyridine

2.3. The Chemistry of Schiff Bases

Schiff bases are generally bidentate or tridentate ligands capable of forming very stable complexes with transition metals. Schiff bases have number of applications. Some are used as liquid crystals. In organic synthesis, Schiff base reactions are useful in making carbon-nitrogen bonds. 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 [7].

Schiff bases are important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to presence of imine (N=CH-). A large number of Schiff bases and their complexes are of significant interest and attention because of their biological activity including anti-tumor, antibacterial, fungicidal, anti-carcinogenic and catalytic activity [32, 35].

Heterocyclic amines have been widely used for the synthesis of new Schiff's bases. Azoles, thiadiazole and their derivatives continue to draw the attention of synthetic organic and inorganic chemists due to the large group of compounds possessing a wide spectrum of uses. Heterocyclic compounds possessing the 1,3,4-thiadiazole ring system shows antifungal, bacteriostatic as well as antihelmintic effects. 1, 3, 4-thiadiazoles are very interesting compounds due to their important applications in many pharmaceutical, biological and analytical field.

Metal complexes of Schiff bases prepared by direct interaction of the Schiff base with the metal salts which involves the direct synthesis of the Schiff base without using or in the absence of the metal ion and followed by addition of the metal ion as salt solution for the synthesis of the complex. The second method for the preparation of metal complexes of Schiff bases is a template condensation of an aldehyde (ketone), primary amine and metal salts which involves based on the use of the metal ion to direct the reaction towards the desired ligand product or aiding its isolation.

2.3.1. Schiff Bases and their Applications

Schiff bases have a wide variety of applications in many fields; biological, inorganic and analytical chemistry. Application of many new analytical devices requires the presence of organic reagents as essential compounds of the measuring system. They are used in optical and electrochemical sensors, as well as in various chromatographic methods, to enable detection of enhance selectivity and sensitivity. Among the organic reagents actually used, Schiff bases possess excellent characteristics [9], structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties.

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 [18, 19]. 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. A considerable number of Schiff base metal complexes are of potential biological interest, being used as more or less successful models of biological compounds [9]. 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 substituents, 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.

In recent years there has been considerable attention focused on the chemistry of metal complexes of Schiff bases containing nitrogen and oxygen. Schiff base ligands and their metal complexes have been found to have a variety of applications in many fields including biology materials synthesis, photochemistry, magnetism medical imaging and industrial use as catalysts etc.

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3. EXPERIMENTAL

3.1. Chemicals and Reagents

Reagents 5-amino-1, 3,4–thiadiazole-2- thiol, 2,6-diacetyl pyridine, copper sulphate ($CuSO_4.5H_2O$) (dehydrate), conc. HCl and solvent like methanol, ethanol, petroleum ether, ether,, ethyl acetate, DMSO were of analytical grade obtained from Sigma Aldrich and used without further purification.

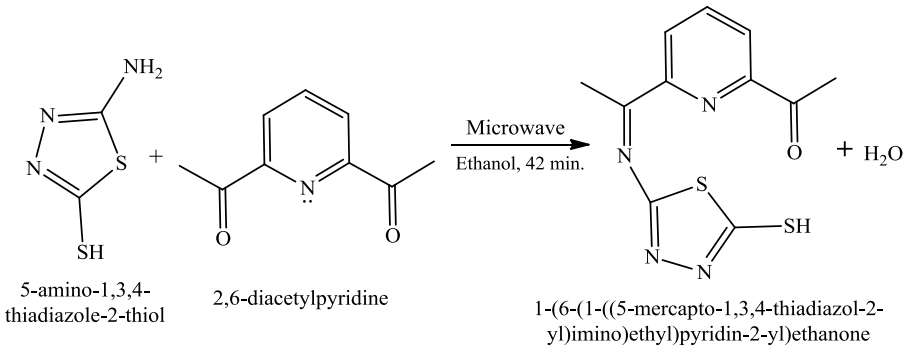
3.2. Instruments

The purity of the synthesized compounds was tested by TLC. Infrared (IR) spectra were recorded using a Perkin Elmer FT-IR Spectrum BX spectrometer in the range 4000 – 400 cm^{-1} with samples prepared as KBr pellets. NMR spectra were recorded using BRUKER 400 MHZ spectrometer in DMSO solvent for ligand. The melting point or decomposition temperature was determined using Digital melting point apparatus. UV-vis spectroscopic studies were done in the range 200 - 800 nm. The molar conductivity of the complexes in DMSO was recorded at room temperature using EC 214 Bench conductivity meter.

3.3. Synthesis of Ligand

A mixture of 5-amino-1,3,4-thiadiazole-2-thiol in ethanol (0.5 g), of the 2, 6-diacetylpyridine (0.612 g) and of concentrated HCl (2 drops) was kept inside a microwave oven operating at 800 W and low temperature. And the progress of the reaction was monitored using TLC eluting solvent a mixture of petroleum ether and ethyl acetate in 8:2 ratios respectively. On checking the progress of the reaction after about fifteen minutes, the TLC showed two spots and the reaction mixture again kept inside a microwave oven for about 27 minutes. After 42 minutes the colour of the reaction mixture was converted from white to light yellow colour. After completion of the reaction the product was poured into ethanol and then allowed to cool to room temperature for 3 days.

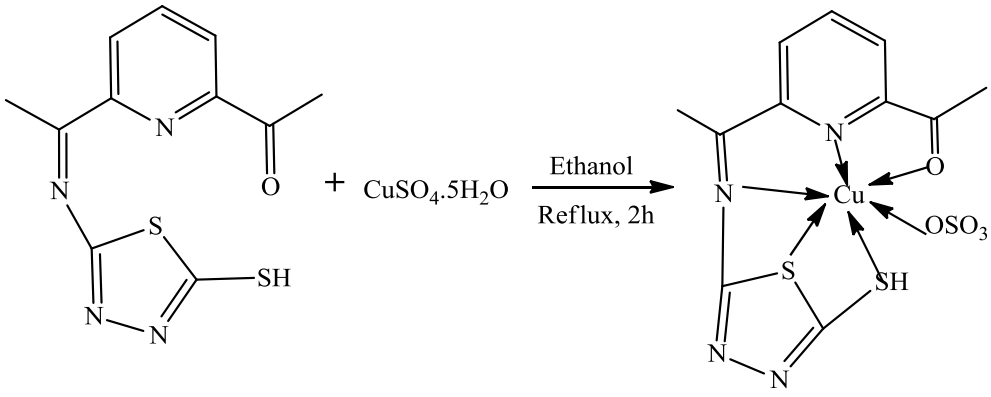
The resulting solid was recrystallized from hot ethanol. The melting point of the dried product was measured.



Scheme 1: Preparation of the ligand

3.4. Synthesis of Cu(II) Complex of the Ligand

The ligand (0.2 g) was dissolved in hot ethanol (5 mL) and was slowly added to a solution of $CuSO_4.5H_2O$ in hot ethanol (10 mL) with a continuous stirring. The reaction mixture was refluxed for 2 hours. The green colored precipitates was obtained after cooling at room temperature, filtered off, washed with ethanol and then with petroleum ether, finally dried at room temperature.



Scheme 2: Reaction of the ligand with Cu(II) ion

3.5. Antibacterial Study of MTIEPE and its Cu(II) Complex

Inhibitory activity of ligand, Cu(II) complex of the ligand and standard drugs against different bacteria were investigated by Disc Diffusion Method. All disks and materials were sterilized in an autoclave before experiments. A 10^{-2} M of solution of ligand (MTIEPE) and its Cu(II) complex were prepared in DMSO.

The Muller Hinton agar solution was spreaded on the petridish disk by using cotton. The petridish disk was divided into equal four parts. The standard drug was placed at the center of petridish disk and, DMSO, ligand and its metal complex solution were placed on the separated four parts of disk and placed in an incubator for 24 h. Finally inhibition zones of standard drug, DMSO, ligand and complexes were measured.

4. RESULTS AND DISCUSSION

Some of the important physical characteristics of the ligand (MTIEPE) and its Cu(II) complex are listed in Table 1.

Table 1. Analytical and physical data for the ligand and its complex

Compound	Colour	M.pt(^{o}C)	Yield(%)	physical appearance
Ligand (MTIEPE)	brown	195 – 197	74.82	Powder
Cu(II)- MTIEPE Complex	Green	276 – 277	71.24	Powder

Melting point of Cu(II) complex synthesized is almost similar with the one which was prepared previously [36, 37].

4.1. TLC Test

The purity of the synthesized Schiff bases and its copper complex was checked on TLC plates and the spot was visualized under ultraviolet light. For this purpose, the compound was dissolved in ethanol. Ethanol was used as eluting agent. Also their purity was checked on melting point.

4.2. Molar Conductance Measurements

The specific conductance of the copper complex was determined by using JENWAY 4330 conductivity and pH meter in 10^{-3} M solution in DMSO as solvents at room temperature. The molar conductance of the complex is derived from the specific conductance by the following relation.

$$\Lambda_M = k \times 1000 / C,$$

Where Λ_M = molar conductance, k = specific conductance C = concentration,

.

The specific conductance k = 139.4 (μS/cm) was measured by dipping the tip of the pH meter into the solution of copper complex. By using the above relation, the molar conductance is calculated as 139.4 Scm^2mole^{-1}. From this it can be concluded that Cu(II) complex with the ligand is an electrolyte.

Furthermore, it indicates the bonding of the sulphate anions to Cu(II) metal complex. As such, the Cu(II) complex has one inner sphere sulphate corresponding with the $[CuLOSO_3]$ for octahedral geometry, which is confirmed by other data.

Table 2. Conductivity data of Cu-L complex

Complex	Solvent	Molar conductance (Λ_M) Scm^2mole^{-1}	Type
Cu-L complex	DMSO	139.4	Electrolyte

4.3. IR Spectra Analysis

The functional group on synthesized ligand and copper complex were established through IR spectroscopic data. In this technique, the molecule in question is exposed to infrared photons. Functional groups absorb infrared photons of characteristic energies. Then a plot of photon energy versus intensity of absorption is obtained and called the infrared spectrum. Therefore, IR spectroscopy allows us to deduce the functional groups that are present and absent in a molecule.

Like any other type of spectrum, an IR spectrum is a plot of energy (expressed as frequency or wavelength of photons) versus intensity of absorption or transmittance. Bond polarity and absorption intensity show correlation in IR spectra; less polar bonds cause weaker absorptions (smaller peaks) than more polar bonds.

The IR spectrum of the ligand is shown in Figure 4

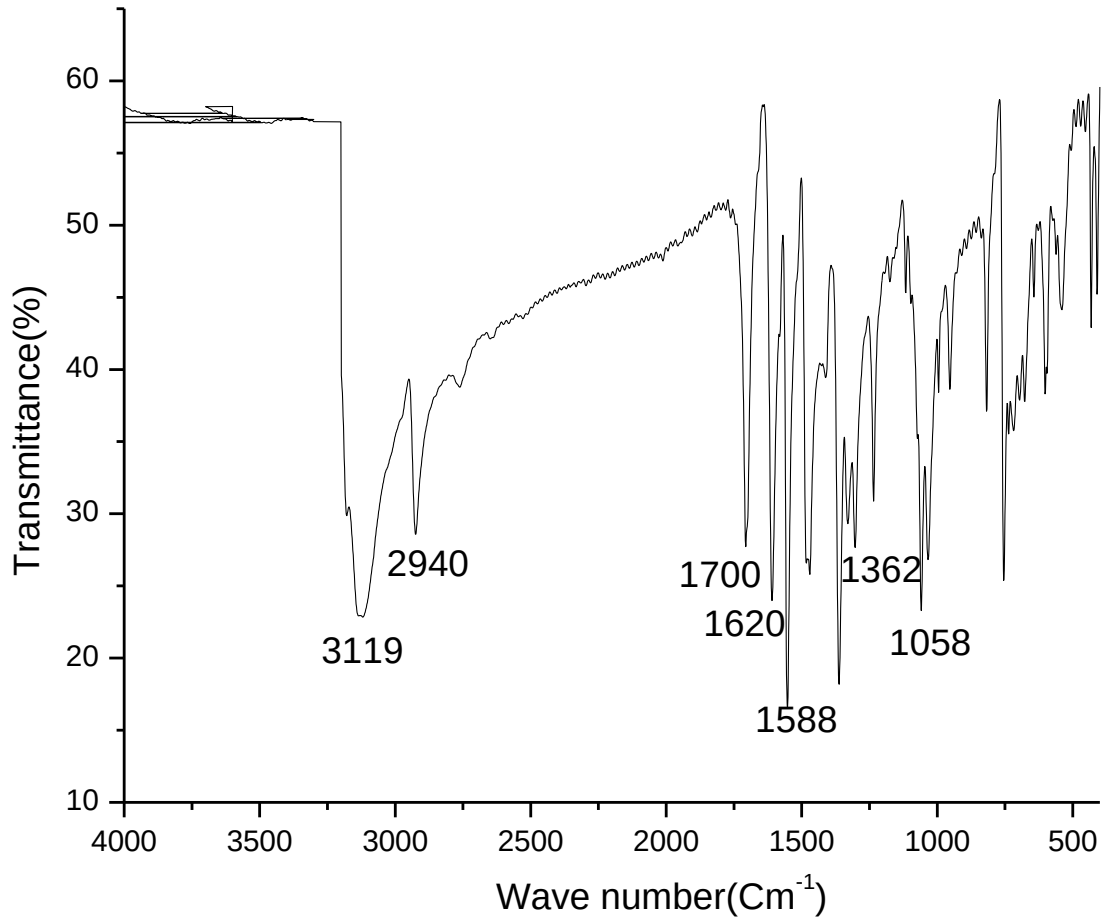


Fig 4. IR spectrum of the ligand

The IR spectrum confirms the formation of schiff base due to the peak located at 1620 cm^{-1} which indicates C=N stretching and disappearance of amide ($-NH_2$) group i.e. there is no peak above the 3119 cm^{-1}.

The C-H stretching mode of the methyl group is located at 2940 cm^{-1} which is supported by the band at 1362 Cm^{-1} which is assigned to CH_3 bending vibrations The Ar-H stretching mode of the pyridine ring is located at 3119 cm^{-1} [38]. The strong band at 1700 cm^{-1} correspond to C=O stretching vibration [39]. The C=C stretching mode of the pyridine ring is located at 1588 cm^{-1}. The band at 1058 cm^{-1} assigned to C-S stretching [38].

The below IR spectrum of the Cu(II) complex of the ligand is presented by Figure5.

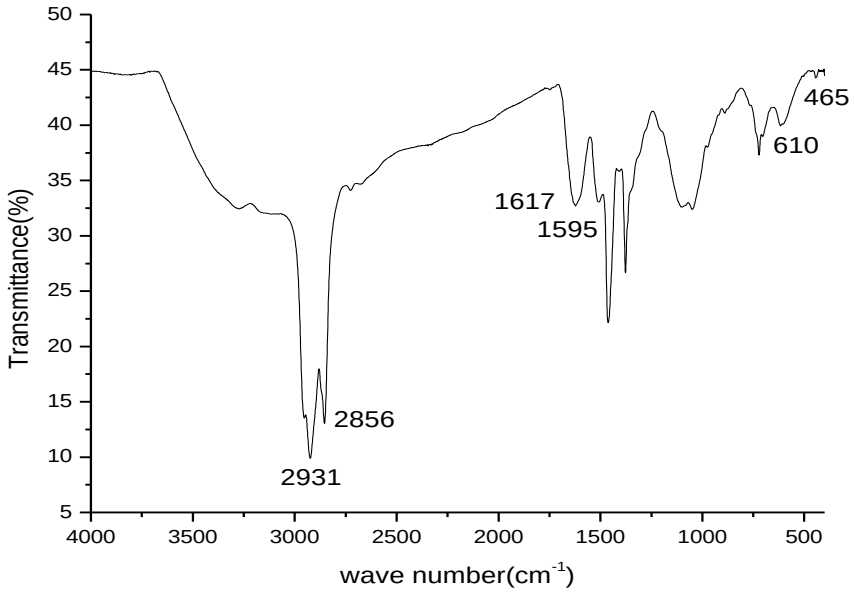


Figure 5. IR spectrum of Cu(II) complex of the ligand

Comparing the spectrum of the free ligand (MTIEPE) with its copper complex, a significant shift with reduced multiplicities of characteristics bands were observed that can be correlated with complex formation.

The shift the stretching frequencies of C=N to 1595 cm^{-1} and of C=O band to 1617 cm^{-1} in the complex indicates the involvement of N=C and C=O in complexation [40, 41]. . Bands observed at the regions (465) cm^{-1} and (610) cm^{-1} [42] respectively indicating coordination of metal with nitrogen (Cu–N), and metal with sulphur (Cu–S). This indicates that coordination has occurred through the nitrogen and sulphur atoms of (C═N) and (C–S–C) groups of the ligand [43-46].

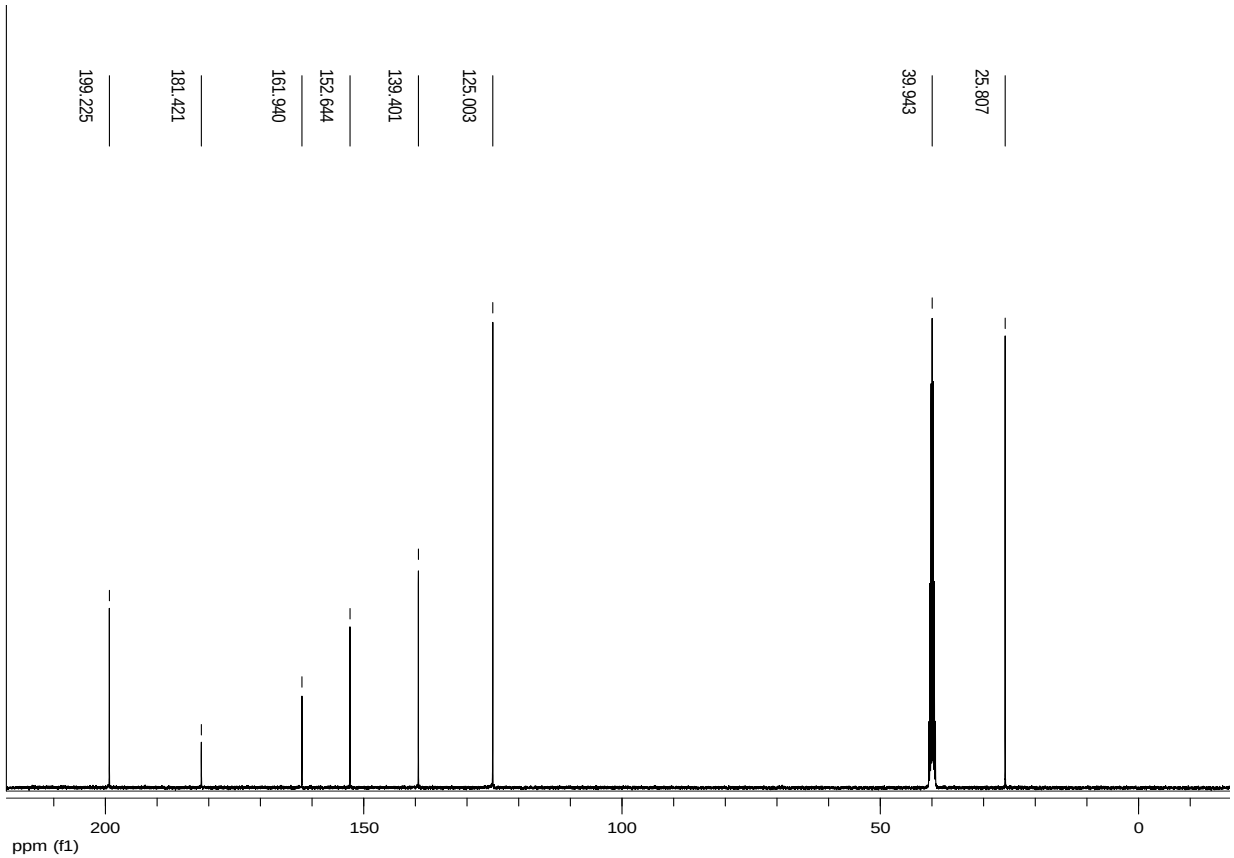
Table 3. Comparison of the IR spectra of the free ligand and the its Cu(II) complex

Compound	ν(C=N) cm^{-1}	ν(C=O) cm^{-1}	ν(C-H) cm^{-1} aromatic	ν(Cu-N) cm^{-1}	(M–S) cm^{-1}
Ligand(L)	1620	1700	3119	-	-
CuLO SO_3	1595	1617	2931	465	610

4.4. NMR Spectroscopic Analysis of the Schiff Base Ligand (MTIEPE)

Over the past fifty years, nuclear magnetic resonance spectroscopy, commonly referred to as NMR, has become the preeminent technique for determining the structure of organic compounds.

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure. Distortion less Enhancement by Polarization Transfer (DEPT) is a very useful method for determining the presence of primary, secondary and tertiary carbon atoms. The DEPT experiment differentiates between CH, CH_2 and CH_3 groups by variation of the selection angle parameter (the tip angle of the final ^1H pulse): 135° angle gives all CH and CH_3 in a phase opposite to CH_2, 90° angle gives only CH groups, the others being suppressed and 45° angle gives all carbons with attached protons (regardless of number) in phase. Signals from quaternary carbons and other carbons with no attached protons are always absent (due to the lack of attached protons).



Chemical shift (δ) TMS

Figure. 6. ^{13}C-NMR spectrum of the ligand

^{13}C-NMR Spectra of MTIEPE show that the presence of eight carbons in different chemical environment in the compound since only eight peaks were shown. ^{13}C-NMR shows carbon (RCH_3) (a) of this compound (Figure 7) at δ = 25.807 ppm which is up field due to its less steric effect and electronegative effect, (R_3CH) (e) at 39.943 ppm, (N=C) azomethine (imines) group at 152.644 ppm, (N=C-R) at 139.401 ppm and carbon(C=C- RH) (d) at 125.003 ppm in pyridine ring. As well as ^{13}C-NMR showed that carbon (N=C-S_2) (h) at 181.421 ppm due to the electro negativity of nitrogen and sulphur atoms and Carbon (N=C-SN) (g) at 161.940 ppm in thiadiazole ring. The carbonyl carbon (b) also deshielded and appeared at 199.225 ppm due to inductive effect, oxygen attracting electron from carbonyl carbon [47].

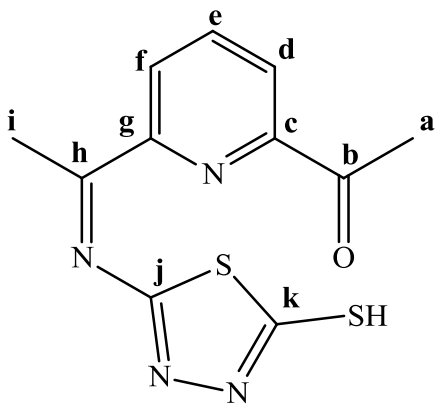


Figure 7. Assignment of ^{13}C NMR signals of the ligand

DEPT-135 spectroscopic analysis of the Schiff base (MTIEPE)

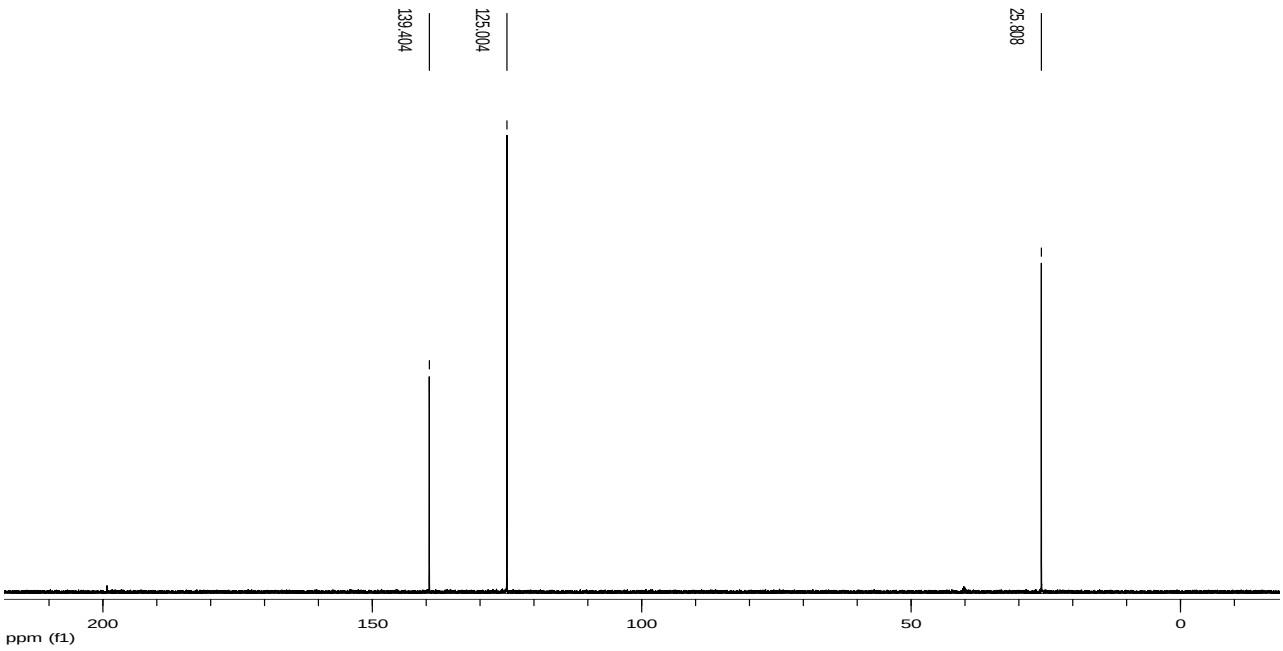
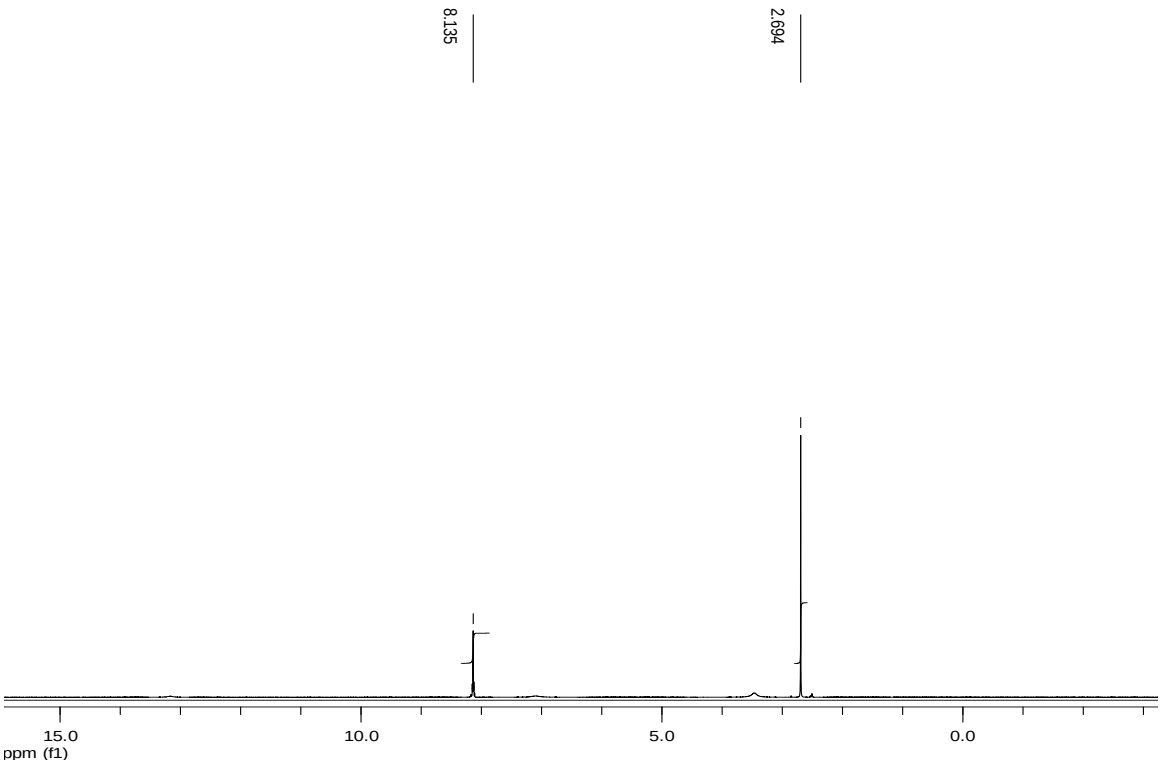


Figure 8. DEPT-135 Spectra of Ligand (MTIEPE)

From the above DEPT understand that the compound (Figure 6) contains only -CH and -CH_3.There is no -CH_2 group in the compound because no any peak shown that exhibits negative phasing (below the line).

The DEPT spectrum also shows three signals at δ 25.808,125.004, and 139.404 ppm for the $–CH_3$ group and two =C-H groups in the pyridine ring and the signals at 39.943, 152.644, 161.940, 181.421 and 199.225 ppm disappeared which confirms that the five types of carbons are quaternary carbons. Those non-proton-bearing carbons (b, c, g, h, j, k and l) are not seen in DEPT spectra because the technique relies on polarization transfer, that is, in this case, the transfer of proton magnetization onto the directly bound carbon. As well as the carbonyl, carbon does not appear in this spectrum because a short delay time was used (would need a long delay time and a larger number of scans). The signal shown at the 25.808 ppm nearer of the TMS shows $-CH_3$ group since it has no steric effect. The signal show at the 139.404 ppm far from the TMS shows -CH group at carbon d and f since they have similar steric effect. The signal shown at the 125.004 ppm shows -CH group at carbon (e) from proposed structure coded ligand (scheme 3).



Chemical shift (δ) TMS

Figure 8. ^1H NMR spectrum of ligand

The ^{1}H - NMR spectra of the Schiff base was recorded in DMSO (Fig 3) with standard TMS. For =CH proton pyridine ring, the ligand shows singlet in the region δ = 8.135 ppm due to unsaturated groups shift to downfield (left) when affecting nucleus is in the plane of the unsaturation. The ^{1}H -NMR signal at δ = 2.694 ppm sharp and singlet peak is due to $-CH_3$ proton [48, 49] since they have no unsaturation effect and electronegative effect.

4.6. Antibacterial Activity of Ligand and its Cu(II) Complex

The *in vitro* antibacterial activity of the DMSO, ligand and its metal complexes were tested against three different bacteria at a concentration of 10^{-2} M (Table 4). At this concentration the Cu(II) complex shows significant antimicrobial activity against the tested pathogens. The degree of inhibition varied with the nature of the compound. These concentrations of the ligand and complexes were used to get visible results. The highest zone of inhibition *i.e.* 25 and 30 mm were measured in Salmonella *typhimurium* and *Staphylococcus aureus* when treated with Cu (II) complex. The zone of inhibition is greatly affected by the thickness of the test agar layer. As the thickness increases, the zone of inhibition decreases. This can be attributed to the decrease of concentration of the ligand and its complexes per unit volume of the culture media. Another factor, which influences the inhibition zone, is inoculums size (concentration of the organism per unit volume). The diameter of the inhibition zone decreases with increase in the inoculums size.

Table 4: Antibacterial screening data of investigated ligand *(MTIEPE)*) and its Cu complex

Compound	Diameter of Inhibition Zone (mm)		
	Salmonella typhimurium	Staphylococcus aureus	E. coli
DMSO	-	-	-
Ligand(L)	20	-	20
Cu (L) complex	25	30	23
gentamycine	27	29	25

There is a significant reduction in the growth rate of microorganisms due to unfavorable culture media, low temperature and acidic pH. The activity test was conducted at an optimum temperature of 37 ^{o}C antibacterial activities. The synthesized compounds were investigated for their antimicrobial activity by agar diffusion method [50].

In case of DMSO control disc no zone of inhibition was which depicts the solvent has no effect on the tested bacterial species. Hence it can be concluded here that whole of the antimicrobial effect is due to the nature of the metal complexes and the ligand used. .

When compared with standard antibacterial drugs, the antimicrobial behavior of the Cu(II) complex showed momentous and identical biological properties [51]; even in Staphylococcus aurous showed more activity. This can be explained by the fact that the high antimicrobial activities and chelation of this metal ion complex, enhances the lipophylic character favoring its permeation through the lipid layer of cell membrane [7,8].

5. CONCLUSION AND RECOMMENDATION

A MTIEPE ligand was synthesized by microwave method from precursor 5-mercapto-1, 3, 4-thiadiazole-2-thiol with 2, 6-diactylpyridine in purified ethanol. The copper complex was synthesized by direct method. In direct method, ligand and Cu (II) ions were reacted. Based on conductivity, IR and NMR spectroscopy studies it is concluded that the ligand bonds to the metal ion through azomethine nitrogen, carbonyl oxygen (-C=O), pyridine nitrogen, sulphate ion and 1, 3, 4-thiadiazole sulfur atoms. The Cu(II) complex showed greater antibacterial activity than the reference antibiotic, gentamycin, towards Staphylococcus aureus. Thus, with further investigation Cu(II) complex of ligand could be used as antibacterial drugs for the treatment of infections caused by this bacterium.

Further investigation is required to completely establish the structure of the complex of Cu(II) ion with the synthesized ligand and its antibacterial activities for practical applications.

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