JIMMA UNIVERSITY **COLLEGE OF NATURAL SCIENCES** SCHOOL OF GRADUATE STUDIES **DEPARTMENT OF CHEMISTRY**



SYNTHESIS AND CHARACTERIZATION OF 1H-IMIDAZO [5, 6-F][1, 10] PHENANTHROLINE-2(3H)-THIONE AND ITS Ni (II) AND Cu (II) **COMPLEXES AND THEIR ANTIBCTERIAL ACTIVITY**

BY: **RADIET ANBESSIE**

A THESIS SUBMITTED TO SCHOOL OF GRADUTE STUDIES OF JIMMA UNIVERSITY IN PARTIAL FULFILMENT THE **REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY (INORGANIC CHEMISTRY)**

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DECLARATION

I declare that the work described in this thesis is my original work under the supervision of my advisors, Mr. Gebru Gebretsadik (M.Sc., PhD candidate) and Mr. Tilahun Wubalem (M.Sc.) at the Department of Chemistry, Jimma University for the degree of M.Sc. in Inorganic Chemistry.

I also declare that the substance of this thesis has neither been submitted elsewhere nor is being currently submitted for any other degree.

I further declare that the thesis embodies the results of my own research or advanced studies and that it has been composed by me. Where appropriate I had made acknowledgment to the work of others.

Name: Radiet Anbessie

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A THESIS SUBMITTED TO SCHOOL OF GRADUTE STUDIES OF JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY (INORGANIC CHEMISTRY)

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Advisors			
Head, Department of Chemistry	y		

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ABBREVIATIONS AND ACRONYMS

AR	Analytical Reagent		
DMSO	Dimethyl Sulphoxide		
DNA	Deoxyribonucleic Acid		
IR	Infrared		
PD	1, 10-Phenanthroline-5, 6- dione		
phen	1, 10-Phenanthroline		
phendione	1, 10-Phenanthroline-5, 6- dione		
TLC	Thin Layer Chromatography		
TMS	Tetra Methyl Silane		
TU	Thiourea		
UV-Vis	Ultraviolet-Visible		
M.p	Melting Point		
L	Ligand		
δ	Chemical Shift		
MHA	Mueller-Hinton Agar		
ppm	Parts Per Million		
¹ H NMR	Proton Nuclear Magnetic Resonance		
¹³ C NMR	¹³ C Nuclear Magnetic Resonance		
1, 10-PDTU	1H- Imidazo [5, 6-f][1, 10] Phenanthroline-2(3H)-Thione		

ABSTRACT

Heterocyclic compounds are compounds that have a broad spectrum of biological activities. 1,10-phenanthroline derivatives have attracted considerable interest from both synthetic and medicinal chemists. The aim of this study was to synthesize, characterize and evaluate antibacterial activity of 1H-imidazo [5, 6-f][1, 10] phenanthroline-2(3H)-thione and its Ni (II) and Cu (II) complexes. 1H-imidazo [5, 6-f][1, 10] phenanthroline-2(3H)-thione was synthesized by direct reaction of 1, 10- phenanthroline-5.6-dione (PD) and thiourea (TU). The complexes of Ni (II) and Cu (II) with this ligand were prepared by direct reactions of the chloride salts of the metals and ligand (1:2) mole ratios in ethanol. The structures of synthesized ligand and metal complexes were characterized and confirmed by elemental analyses (C, H, N and S), FT-IR, ¹H-NMR, ¹³C-NMR, Atomic absorption spectroscopy, UV-Vis spectra and molar conductivity measurement. The data from the elemental analysis suggest a good agreement between the calculated and the experimental values for the elements and the IR studies revealed that ligand acted as bidentate through nitrogen atoms in synthesized complexes. The metal complexes were isolated in good yield and are thermally stable. The molar conductivity measurements of the complexes indicates that their electrolytic nature and geometry structure. On the basis of spectral studies an octahedral geometry for Ni (II) complex and distorted octahedral geometry for Cu (II) complex. A comparative antibacterial study test was performed using the disk diffusion method against on two gram positive bacterial (Staphylococcus aureus (ATCC 25923), Bacillus subtillus (ATCC 6633) and two gram negative bacteria (Escherichia coli (ATCC 35218) and pseudomonas aeruginosa (ATCC 27853). Ni (II) complex shows higher bioactivity than Gentamicin against Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 27853) while Cu (II) complex is more bioactive than Ni (II) complex against Bacillus subtilis. The Cu (II) and Ni (II) complexes exhibit higher antibacterial activities than the free ligand.

Keywords: Antibacterial activity, complexes, 1, 10-Phenanthroline, Thiourea.

1. INTRODUCTION

1.1 Background of the Study

Heterocyclic chemistry is a very important branch of organic chemistry accounting for about two third of organic compounds. Heterocyclic compounds are compounds that contain heteroatoms such as sulfur, oxygen or nitrogen, as part of the ring in addition to carbon; may either simple aromatic rings or non-aromatic rings. Some examples are pyridine (C_3H_5N), pyrimidine ($C_4H_4N_2$) and dioxane ($C_4H_8O_2$). Hetero-atoms play significant role in the cyclic part of heterocyclic compounds due to their unshared electrons pairs and electro negativity difference between heteroatom and carbon [1]. They have a wide range of applications including as antiviral, anticancer, antibacterial, antifungal, antioxidant, as corrosion inhibitor, and as also serving as additives with a variety of other functional groups [2]. A heterocyclic compound with donor group plays in turn an important role in coordination chemistry. Ring-fused heterocycles which contain more than one nitrogen atom are key structures in a large variety of biochemical processes. For example, purine, pteridine and flavin as well as their metal complexes play an important role in many enzymatic reactions. The nitrogen containing heterocyclics are of great important molecular systems [3].

Heterocyclic compounds are widely used as their structures can be easily manipulated to the intended functional modification. Such variations may differences in acidity or basicity, susceptibility to attack by electrophiles or nucleophiles and polarity which are the direct consequences of variation in electronic distribution across the molecular frame. Among nitrogen heterocycles, 1,10-phenanthroline and its derivatives represent an important class of organic molecules that have attracted considerable interest from both synthetic and medicinal chemists, due to the presence of the 1, 10-phenanthroline ring system as a structural pattern in several natural biologically important products [4].

1,10-Phenanthroline and its derivatives such as 5,6-diamino-1,10-phenanthroline(I), 1,10-phenanthroline-5,6-dione(II), 2,9-dimethyl-1,10-phenanthroline-5,6-dione(III) and 1,10-phenanthroline-5,6-dioxim(IV) (Figure 1) play important roles as molecular scaffoldings for

supramolecular-assemblies, building blocks for the synthesis of metallo-dendrimers, thin films of luminescent complexes and ligands of synthesis of ring opening metathesis polymerization (ROMP) monomer [5]. 1, 10- phenanthroline is a typical chelating ligand for transition metals and its metal complexes are useful as a photo-sensitizer, electroluminescent materials and catalysts for organic synthesis [6]. Another importance of 1, 10- phenanthroline complexe is the capacity to absorb visible light and thereby to access to excited states by means of metal-to-ligand charge transfer (MLCT) process and are able to transform solar energy to a form of usable energy. The electron promoted to a ligand orbital in the excited state can be injected into the conducting band of the electrode oxide, starting in this way the conversion of light into electricity [7].

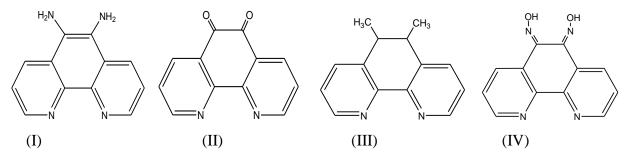


Figure 1.Some example of derivatives of 1, 10-phenanthroline

Thiourea $(CS(NH_2)_2)$ is an organic compound that consists of carbon, nitrogen, sulfur and hydrogen atoms and was synthesized in late 19^{th} century, Since then search for new thiourea derivatives with potent biological activities has intensified [8]. Thiourea is very important for the structural modifications to synthesize new derivatives because it is basically occurs in two tautomeric forms and has three functional groups. Thiourea and its derivative molecules are known to be versatile polydentate ligands as they have several electron-donor centers; viz nitrogen and sulfur that provide a various binding possibilities. In addition, in thiourea nitrogen assigned as hard while sulphur as soft bases that enable thiourea to coordinate both with hard and soft acids. Thiourea may coordinate as neutral ligands, monoanions or as dianions. A numbers of heterocyclic compounds containing nitrogen and sulfur showed a wide variety of biological activity. Thiourea and its derivatives as well as their corresponding metal complexes have various biological properties such as antibacterial, anticancer, antifungal, antimalarial and antituberculosis that make them widely used in many field especially pharmaceutical industries [9]. Thus, thiourea derivatives are a very versatile ligand which able to coordinate to range of metal centers. Besides that, they also readily form supramolecular structures via hydrogen bonding [10].

Synthesis of nitrogen containing heterocyclic compounds and their complex formation with metal ions has parallel development with respect to their coordination chemistry. The study on the bonding modes of potentially ligands provides various synthetic routes for synthesizing magnetic materials, catalysts and biological model compounds. Ligands containing both nitrogen and oxygen exhibit versatile coordination chemistry and are capable of forming polymeric and molecular metal complexes having fascinating structural and magnetic properties. There is a great deal of interest in the synthesis and characterization of transition metal complexes of heterocyclic compounds. This is due to their wide application in different fields such as antibacterial, antifungal and their binding ability to transition metal ions [11].

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including new kind of infectious diseases and the increasing number of medicinal drug resistant pathogens. In spite of a large number of antibiotics and chemotherapeutic drugs 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 antibacterial agent. Therefore there is a real perceived need for the discovery of new compounds endowed with antibacterial activities. The aim of this study was to synthesize, characterize and evaluate antibacterial activity of thiourea derivative of 1, 10-phenanthroline novel ligand (figure 2) and its Nickel (II) and Copper (II) complexes.

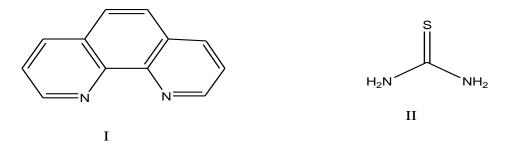


Figure 2. Structure of 1, 10-phenanthroline (I) and Thiourea (II)

1. 2. Statement of the Problem

There are various disease caused by pathogens which are harmful to human beings. To kill this microbes a lots of anti-microbial substances have been invented from various metal-ligand complexes. But through time to time elapse the ability of these complexes as anti- microbial activeness becomes reduced because of rapidly resistive adaptation of microbes. Therefore, to solve this challenge it requires valuable research for new product within a time to develop differently special anti-microbial chemicals. As a consequence, many researchers have pointed that heterocyclic ligands including 1, 10-phenanthroline and its derivatives have an excellent anti-microbial activity [4, 11]. However, no research work has been done on ligand synthesis from derivative of 1, 10-phenanthroline and thiourea and its Ni (II) and Cu (II) complexes as antibacterial activity. Hence, this research work was focused on synthesis, characterization and antibacterial activity of thiourea derivative of 1, 10-phenanthroline ligand and its Ni (II) and Cu (II) complexes.

1.3. Objectives of the Study

1.3.1. General objective

The main objective of this study was to synthesize, characterize and evaluate antibacterial activity of 1H-imidazo [5, 6-f][1, 10] phenanthroline-2(3H)-thione ligand and its Ni (II) and Cu (II) complexes

1.3.2 Specific objectives:

- To synthesize1H-imidazo [5, 6 -f] [1, 10] phenanthroline-2(3H)-thione ligand and its Ni (II) and Cu (II) complexes.
- To characterize 1H-imidazo [5, 6-f] [1, 10] phenanthroline-2(3H)-thione ligand and its Ni (II) and Cu (II) complexes.
- To evaluate antibacterial activity of the 1H-imidazo [5, 6-f][1, 10] phenanthroline-2(3H)thione ligand and its Ni (II) and Cu (II) complexes.

1.4. Significance of the Study

Transition metal complexes with O, N, and S donor ligands have gained so much attention in recent years due to their structural chemistry and wide biological activities. However, the emergency of new antibacterial resistant pathogens limits the use of antibacterial drugs. Therefore, the proposed ligand, 1H-imidazo [5, 6-f] [1, 10] phenanthroline-2(3H)-thione (1,10-PDTU) and its Nickel (II) and Cupper (II) complexes may provide the following significance

- 1. The synthesized ligand and its complexes may be potential input for developing a drug in the future.
- 2. The newly synthesized structure of the complexes may help to enhance knowledge on the structure of complexes.
- 3. The newly synthesized ligand and its complexes would serve as baseline for the next research development by other researchers.

2. REVIEW OF RELATED LITERATURE

2.1. Chemistry of 1, 10-Phenanthroline (phen) and its derivatives

In 1898, Blau synthesized 1, 10-phenanthroline through fusing two pyridine rings together with central benzene ring. Then, 1, 10-Phenanthroline has got special interest from researchers due to its structural and chemical properties; viz: planarity, aromaticity, basicity, and chelating ability makes 1, 10-phenanthroline versatile starting materials in the area of materials science and in many fields of chemistry [12]. 1, 10-phenanthroline is extensively investigated as a neutral N-N chelating ligand as well as analytical reagent. Substituted 1, 10- phenanthroline is used to enhance the chelating capacity. Substitution can be introduced on the heterocyclic as well as the non-heterocyclic regions through suitable reaction paths while appropriately judging the electronic, steric and chelating factors. The choice of substitution and derivation can have great influence on the chelating abilities [13]. Several of 1, 10-phenanthrolines derivatives such as 1, 10-phenanthroline-5,6-dione, 5, 6-dimethyl-1, 10- phenanthroline and 5, 6-diamino-1,10phenanthroline play an important roles for supramolecular assemblies because they can provide bidentate N-donor sites for chelating with metal ions to form bridge ligands [14]. 5, 6-diamino-1, 10-phenanthroline is particularly important in that it can either directly bridge two metal centers or be condensed with a variety of O-quinones to form additional derivatives [15]. 1, 10phenanthroline-5, 6-dione is a versatile molecule with applications in organic and biological chemistry, and in the synthesis of materials showing optical and electrical properties [16, 17]. It is of particular interest; since it has two nucleophilic centers (nitrogen and oxygen lone pairs) in a molecule. The presence of these heteroatoms creates not only the basic properties in the Lewis sense but also, because of the resonance conjugation, make it possible to alter the electron density in different parts of the molecule, especially by the reaction of an external electrophile with the unshared pairs of electrons of the heteroatom [18, 19].

2.2. Metal Complexes of 1, 10-Phenanthroline and its derivatives.

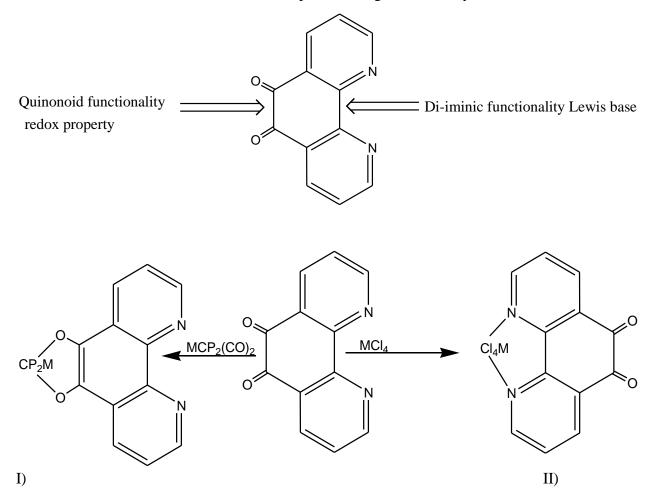
Transition metals containing organic group are structurally known to possess potential applications in the areas of biological, clinical, analytical, catalytic, microbial, insecticidal, antibiotics, growth factors, food additive, tumor inhibitor, cell division etc. This may either due

to the unused coordination sites in the metal and ligand systems, or selective oxidation state of the metal ions in the coordination sphere [20]. The dinuclear and polynuclear transition metal complexes are attracting much interest for many reasons [21]. Metal complexes bearing electro active ligands with two or more accessible oxidation states exhibit unique electronic structures resulting from the combination of the oxidation states of the metal and ligands [22]. One especial important and desirable class of derivatives are those which retain the dual symmetry of this ligand and thereby avoid some of the stereochemical problems associated with their tris chelated metal complexes, namely formation of merisomers and facisomers. 5, 6-Diamino- 1, 10phenanthroline is particularly important in that it can either directly bridge two metal centers or be condensed with a variety of ortho-quinones to form addition derivatives [15].

Several transition metals form stable complex salts with 1, 10- phenanthroline having a general form M (phen)₃X₂. YH₂O. The coordinating ability of 1, 10-phenanthroline is possessed by a number of its simple derivatives [23]. One of the present used ligand, 1, 10-phenanthroline-5, 6dione which is a fairly distinct electronic character from 1, 10- phenanthroline due to its two carbonyl groups which are expected to influence the properties of the complex significantly. On the other hand another ligand 5,6-dimethyl-1, 10- phenanthroline, possesses is- π -electronic system with 1, 10- phenanthroline and is considered to give some information on effects by the σ donation of the methyl groups [24]. The focus is on complexes of 1,10-phenanthroline-5, 6-dione because this ligand has the ability to form stable complexes with a wide variety of metal ions and carries an o-quinone moiety with pH dependent electro activity. Metal complexes of this ligand potentially allow for the variation and control of redox properties over a wide range as well as the fine tuning of potentials through pH changes [25].

1,10-Phenanthroline-5, 6-dione can be prepared starting from an already existing phenanthroline. The diketone functionality can also be easily transformed into other chelating groups such as diamine or dioxime. Moreover it is also a versatile organic link that can form bridges through amine condensation or a combination of coordination and condensation. 1, 10-Phenanthroline-5,6-dione has a good coordinating properties due to the presence of two coordinating functionalities in the same molecule (the quinonoid and diimine). Moreover the presence of two types of basic centers, nitrogen, and oxygen atoms, both sp² hybridized, contributes to make this

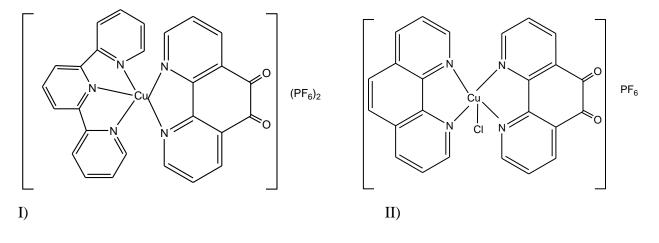
molecule an ideal system to study the different coordinating abilities of the two sets of donor atoms [26]. When it coordinates through the oxygen atoms, the entire complex, I, may be used as 'phenanthroline equivalent' in reactions with Lewis acids. On the other hand, nitrogen-bound complexes of 1, 10- phenanthroline 5, 6- dione, II, may be used as a 'quinone equivalent' ligand in reactions with compounds containing metal in a low oxidation state as shown in scheme 1. In both cases the result is the formation of complexes of higher nuclearity [27].



Scheme 1. Metal binding sites of 1, 10- phenanthroline-5, 6- dione [27]

Hence, 1,10- phenanthroline-5, 6- dione ligates to metals not only through *N*-*N* chelate but also with a free *O*-quinoid group or an *O*-*O* chelate with two free diiminic nitrogen atoms but also a bridging mode with two different coordination sites. The redox reaction of metal-phendione complexes are regulated by proton concentration in addition to the coordination modes of phendione to metals [28]

The five-coordinated copper (II) complexes comprised of both molecules of 1, 10-phenanthroline (phen) or the related ligands and one monodentate co-ligands have been attracting great interest for their diverse stereo and physicochemical properties. It is well known that these copper (II) complexes take either trigonal bipyramidal or square-pyramidal geometry, depending on the ligands, co-ligands, and counter ions (Figure 3). In addition, various structure-sensitive spectral and redox behaviors are recognized for such copper complexes [5]



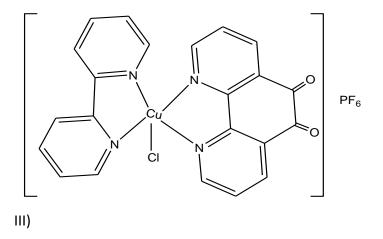
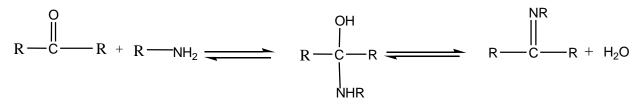


Figure 3. I. $[Cu(tpy)(phen-dione)](PF_6)_2$; II. $[Cu(phen)(phen-dione)Cl]PF_6$; III. $[Cu(bpy)(phen-dione)Cl]PF_6$.

2.3. Schiff Bases

A Schiff base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group. It is usually formed by condensation of an aldehyde or ketone with a primary amine; Where R may be an alkyl or an aryl group (Scheme 2). Schiff bases that contain aryl substituents readily synthesized as they are substantially more stable while those which contain alkyl substituents are relatively unstable [29]. The formation of a Schiff base from an aldehydes or ketones is a reversible reaction and generally takes place under acid or base catalysis, or upon heating.



Scheme 2. Condensation of Schiff's bases (29)

Schiff bases have often been used as chelating ligands in the field of coordination chemistry and their metal complexes are of great interest for many years. It is well known that N,O and S atoms play a key role in the coordination of metals at the active sites of numerous metallobiomolecules. Schiff base metal complexes have been widely studied because they have industrial, antifungal, antibacterial, anticancer and herbicidal applications [30]. Chelating ligands containing N, S and O 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. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities [31].

2.4. Chemistry of Nickel (II) and Copper (II) Complexes

2.4.1. Chemistry of Nickel (II) Complex

The main structural types of Ni (II) complexes are octahedral, tetrahedral and square-planar. The tetrahedral Ni (II) complexes with ${}^{3}T_{1}$ (F) ground state generally exhibit four transitions: ${}^{3}T_{1} \rightarrow {}^{3}A_{2}$, ${}^{3}T_{1} \rightarrow {}^{1}E$, ${}^{3}T_{1} \rightarrow {}^{3}T_{1}$ (P) and ${}^{3}T_{2} \rightarrow {}^{1}T_{1}$. The band ${}^{3}T_{1}$ (F) $\rightarrow {}^{3}T_{1}$ (P) is a strong band with high intensity compared with others. It occurs in the visible region (~15,000 cm⁻¹) and is relatively strong compared to the corresponding ${}^{3}A_{2} \rightarrow {}^{3}T_{1}$ transition in octahedral complexes.

Octahedral Ni (II) complexes have ${}^{3}A_{2g}$ ground state. This type of complexes is expected to have three spin –allowed transitions ${}^{3}A_{2}g \rightarrow {}^{3}T_{2}g$, ${}^{3}A_{2}g \rightarrow {}^{3}T_{1}g$ (P) and ${}^{3}A_{2}g \rightarrow {}^{3}T_{1}g$ (F) in the ranges of 7000-13000, 11000- 20000 and 19000-27000 cm^{-1,} respectively. Two spin forbidden transitions are also possible; ${}^{3}A_{2}g \rightarrow {}^{1}Eg$ and ${}^{3}A_{2}g \rightarrow {}^{1}T_{2}g$. Octahedral Ni (II) complexes have two unpaired electrons and depending on the orbital angular momentum contribution they possess magnetic moments ranging from 2.9 to 3.4 BM. For the vast majority of four coordinated Ni (II) complexes containing strong field ligands, square planar geometry is preferred. Square planar complexes of Ni (II) are thus invariably diamagnetic. They are frequently red, yellow or brown owing to the presence of the absorption band of medium intensity in the range 450-600 nm. Square planar Ni (II) complexes don't have any absorption band below 10000 cm⁻¹, due to large crystal field splitting. Hence they can be clearly distinguished from octahedral and tetrahedral complexes [32, 33].

2.4.2. Chemistry of Copper (II) Complex

Copper (II) complexes (d^9); characterized by Jahn-Teller distortion in a regular octahedral symmetry that affects its stereochemistry. The d^9 gives three electrons in the two degenerate eg orbitals leading to asymmetric in doubly degenerate electronic ground state. Such complexes distort along one of the molecular four fold 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 [34, 35].

The distortion in the limit results square planar arrangement about the copper which can often be induced on the copper by using large organic ligand that blocks the approach of the last two long bond forming ligands. The electronic spectra of Cu (II) ion in its complex could be explained based on Jahn Teller effect. In this case the E_g and T_{2g} states of the octahedral Cu (II) ion split

under the influence of tetragonal distortion. The distortion creates three transitions ${}^{2}B_{1g} \rightarrow {}^{2}A_{1g}$, ${}^{2}B_{1g} \rightarrow {}^{2}B_{2g}$ and ${}^{2}B_{1g} \rightarrow {}^{2}E_{g}$, which are unresolved in the spectra due to their closer energies. Copper (II) complexes are generally blue or green color. The origin of the color is due to the maximum of four electronic transitions; d-d transitions; charge transfer transitions (both metal to ligand and ligand to metal), and internal ligand transitions. Such transitions occur between the ground state and the excited states of the crystal field levels [36].

2.5. Applications of 1, 10-Phenanthroline and its Metal Complexes

1, 10-phenanthroline and its derivatives are frequently used in many processes involving metal complexes as ligands for catalysis, or as stabilizing agents for nanoparticle synthesis. 1, 10phenanthroline have been used as important heterocyclic ligands for a large number of metal complexes that play an important role in a variety of important technological and medicinal applications; for example, promising applications in the field of electroluminescent materials, organic light-emitting devices (OLED), organic semiconductors, or as chemical nucleases and therapeutic agents [3]. There is a vast amount of literature on numerous bioactive complexes, and the number of studies on the interactions of heterocyclic ligands with metal ions demonstrating important chemical and biological properties has been developing. In particular, investigation of the interactions between metal complexes derived from 1 ,10-phenanthroline, as well as derivatives of modified 1,10-phenanthroline moieties, and DNA is an area of great interest, due to the high selectivity that 1,10 phenanthroline compounds have demonstrated at the intercalation sites of the DNA molecule. For example, [Cu (phen)₂]⁺² [37]. Transition metal complexes drugs represent a novel group of antimicrobial agents with potential therapeutic applications. Investigations into Cu, Ag, and Mn complexes of 1, 10-phenanthroline as anticancer and antimicrobial agents have been carried out. Moreover, the addition of 1, 10phenanthroline to a metal complex has been antibacterial agents shown to enhance the anticancer and antimicrobial activity with some complexes demonstrating values comparable to or better than that of standard drugs [38].

2.6. Applications of 1, 10-Phenanthroline-5, 6-dione and its Metal Complexes

1, 10-phenanthroline-5, 6-dione is a versatile molecule with applications in organic and biological chemistry, and in the synthesis of materials showing interesting optical or electrical

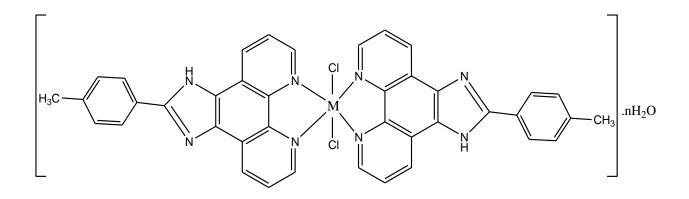
properties. In its metal-free state and when coordinated to metal ions, is considered to have many interesting biological properties, such as anticancer and antimicrobial actions. Transition metal complexes have attracted attentions of inorganic, metallo-organic as well as bio-inorganic chemists because of their extensive applications in wide ranging areas from material to biological sciences. Metal complexes of 1, 10-Phenanthroline-5, 6-dione, are particularly attractive species for developing new diagnostic and therapeutic agents that can recognize and cleave DNA.

In recent years, researchers have synthesized several PD-based compounds, including $[Ag(PD)_2]$ ClO_4 , $[Ag(PD)_2]^+$, $[Cu(PD)_3](ClO_4)_2.4H_2O$, $[Cu(PD)_3]^{2+}$ in an attempt to generate new complexes with improved antimicrobial activity and reduced toxicity to different cell lineage. $[Cu(PD)_3]^{2+}$ was found to be active against the multi-resistant, filamentous fungus while $[Ag(PD)_2]^+$ exhibited better activity against the yeast, *Candida albicans*. Moreover, the addition of 1, 10-Phenanthroline-5, 6-dione to a metal ion has been shown to enhance biological activity [39].

2.7. Biological Importance of Metal Complexes

Metal ions are generally positively charged and act as electrophiles, seeking the possibility of sharing electron pairs with other atoms so that a bond or charge-charge interaction can be formed. Ligands are atoms or groups of atoms that are bonded to the metal ion. They are usually neutral or negatively charged and they donate electron density to the metal ion. The coordination number of a metal ion, that is, the number of ligand atoms bound to it is viewed in terms of concentric spheres; the inner sphere containing those atoms in contact with the metal ion, the second sphere containing those in contact with the inner sphere ligand atoms. The number of atoms in these spheres depends on the size of the metal ion and the sizes of the ligand atoms [40]. Metal complexes provide better opportunities to use as therapeutic agents. The results found showed that the metal complexes were found to be more active than the ligand. The lipophilicity is increased through the formation of chelates and drug action is also increased due to effective permeability of the drug into the site of action. Metal ion bond with ligands in some process to oxidize and reduce in biological systems [41].

The antibiotic activity of divalent transition metals (Co, Ni and Cu) complexes of 2-*p*-tolyl-1Himidazo[4,5-f][1,10] phenanthroline have been done against nine bacteria strains (*A. hydrophila* (ATCC 7966), *S. aureus* (ATCC 29213), *K. pneumoniae* (ATCC 21541), *P. aeruginosa* (ATCC 27853), S. marcescens (ATCC 21074), *E. aerogenes* (ATCC 5402), *B. subtilis* (ATCC 6633), *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29212)) in 2013. The screening results showed that the ligand was found to exhibit significant antibacterial activity against all the bacteria tested. Ni (II) complex showed good antibacterial activity against *E. coli* and *K. pneumoniae*, respectively. Cu (II) complex also displayed good antibacterial activity against the tested bacteria. However, no effect was observed against *P. aeruginosa and E. coli* by both the ligand and the complexes. Further, antibacterial activity Co (II) complex was found to be significant effect against all bacteria. In contrast no effect was observed against *P. aeruginosa* [42].

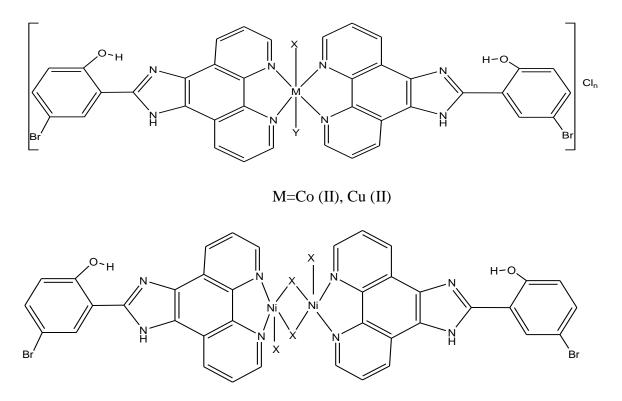


M=Co (II), n=1, Ni (II), Cu (II), n=2

Figure 4. Metal complexes of 2-*p*-tolyl-1H-imidazo [4, 5-f] [1,10]phenanthroline and their reported biological activity.

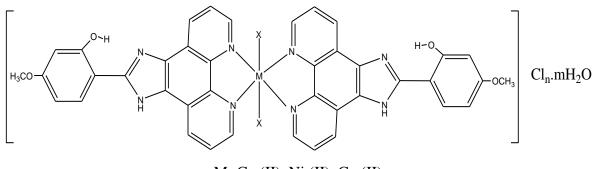
The Co (II), Ni (II) and Cu (II) complexes with imidazole-based ligands L1-L2 (4-bromo-2-(1H imidazo[4,5-f][1,10] phenanthroline-2-yl) phenol (L1)) and 2-(1H-imidazo[4,5f][1,10] phenanthroline-2-yl)-5-methoxyphenol (L2) were investigated in 2013. The in-vitro antimicrobial activities of metal complexes were evaluated against nine bacteria strains using agar method. The ligands displayed weak antibacterial activity against *B. subtilis*. However, good activity was observed against others bacteria. Cu (II) complexes displayed good antibacterial activity against all bacteria except for *B. subtilis*. Co (II) complexes exhibited activity against *S*.

aureus, B. subtilis, A. hydrophila, K. pneumoniae, E. aerogenes and E.coli. However, no activity was observed against *S. marcescens, E. coli* and *P. aeruginosa.* Additionally, Ni (II) complexes exhibited weak effect against to all bacteria tested [43].



 CuL^1 , X&Y=Cl, n=0; CoL^1 , X=Cl, Y=H₂O, n=1; NiL¹, X=Cl.

Figure 5. CuL^1 , CoL^1 and NiL^1 complexes and their reported biological effects.



M=Co (II), Ni (II), Cu (II)

 CuL^2 , X=Cl, n & m=0 ; CoL^2 , X= H₂O, n=0, m=1 ; NiL², X= H₂O, n=2, m=1

Figure 6.CuL², CoL^2 and NiL^2 complexes and their reported biological effects.

Zn (II) complexes of imidazole [4, 5-f] 1, 10-phenanthroline derivatives ligands have been synthesized in 2017. The in-vitro antimicrobial activities of metal complexes were evaluated against three Gram negative strains *E. coli*, *P. vulgaris*, *K. pneumonia*, and two Gram positive strains *S. aurous*, *B. subtilis* using agar method. The results of antimicrobial activity of ligands and their metal complexes are found to be comparable with standard drugs (tetracycline). The result revealed that almost all the synthesized compounds possess good antibacterial activity [13].

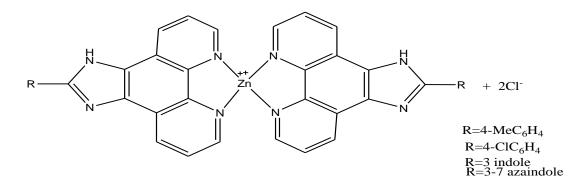


Figure 7. Zn (II) complexes of imidazole [4, 5-f] 1, 10-phenanthroline derivatives and their reported biological effects.

Cu (II), Co (II), Ni (II) ,Cr (II), Mn (II), Fe (II) and Zn (II) complexes with phen imidazole derivatives, which are 4-methyl (1Himidazo[5,6-f][1,10]phenanthroline), 2-(1H-imidazo[5,6 f][1,10]phenanthroline-2-yl)-5-methoxyphenol, (2,4,6-Tirmethoxy)imidazo[5,6-f]1,10-phenanthroline, 4-Fluro imidazo [5,6-f]1,10-phenanthroline, and [4-Hydroxy phenyl]imidazo [5,6-f]1,10-phenanthroline were investigated in 2014. The anti-bacterial activity test results showed that the ligands and their metal complexes have good antibacterial activity against the all selected bacterial strains. In this sense, researcher considers that all the ligands and their metal complexes might be effective as antibacterial agents [14].

In general, as shown in the above survey of related literature, there are a number of works done related to 1, 10-phenanthroline, 1, 10-phenanthroline-5,6- dione, ligands and their metal complexes. But to the best of researcher knowledge, there are no work done on the 1H-imidazo [5, 6-f][1, 10] phenanthroline-2(3H)-thione ligand and its Cu (II) and Ni (II) metal complexes.

3. MATERIALS AND METHODS

3.1. Chemicals and Solvents

All the chemicals and solvents used in this study were analytical reagent grade (AR) and used without further purification. Copper chloride (Nice Lab reagent Ltd), Nickel chloride (Nice Lab reagent Ltd), 1,10-phenanthroline (Nice indicator), thiourea (LOBA Chemie PVT.LTD.), potassium bromide (KBr), sodium hydroxide (NaOH),conc. Nitric acid (HNO₃), conc. Sulpheric acid (H₂SO₄), anhydrous magnesium sulphate (MgSO₄) (Finkem), silver Nitrate (Kiran light laboratories), Dichloromethane (Blulux Laboratory(p) Ltd), dimethyl sulfoxide (DMSO)(Blulux Laboratory(p) Ltd). Other solvents such as chloroform, ethanol, petroleum ether and methanol were purchased from LOBA Chemie Ltd Company. The bacterial strains were obtained from department of Biology Jimma University.

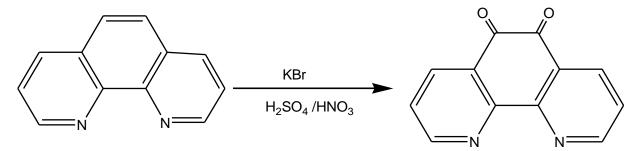
3.2. Apparatus and Instruments

Beakers, boiling flask, Rotary evaporator, pH meter, refrigerator, ,measuring cylinders, analytical digital balance, separator funnel, reflux condenser, heating mantel, acid burette, spatula, wash bottle, volumetric flasks, magnetic stirrer, magnetic bar, cuvette, filter paper (whatman No.1) and oven. NMR spectra of ligand were recorded using BRUKER 400 MHz (¹HNMR) and 100.06 MHz (¹³C-NMR) ultrashielded NMR spectrometer and TMS as internal standard. The infrared spectra were recorded on Infrared spectrophotometer in the range of 4000 cm⁻¹ - 400 cm⁻¹ in KBr medium. The percentage of C, H, N and S in the ligand and complexes was estimated by using Flash FA 1112 Elemental analyzer and that of the metal (Cu and Ni) quantity in the complexes by AAS. Electronic absorption spectra were measured on 6705 UV–Vis, JENWAY Spectrometer in the range of 200-800 nm regions in DMSO at room temperature. The melting point and molar conductivity of the synthesized compounds were determined using Griffin melting point apparatus and HQ 40d (multi) conductivity meter respectively. TLC was used to follow reaction progress/completion and purity of the products.

3.3. Synthesis Procedures

3.3.1. Preparation of 1, 10-Phenanthroline-5, 6-dione (PD)

1, 10-phenanthroline-5, 6-dione (PD) was prepared the following procedure reported in Calderazzo, *et al.* [26]. In a three-necked flask containing solid mixture of ice cold 1, 10-phenanthroline (10.0 g , 50.5 mmol) and KBr (10 g , 84 mmol), ice cold mixture of 96 % H₂SO₄ (90 mL) and 68 % HNO₃ (45 mL) was added drop wise from dropping funnel with stirring at 0°C (addition time 20 min) (Scheme 3). The resulting red to orange suspension was allowed to warm up to room temperature and then refluxed for 10 h at 150 °C. The reaction was monitored with TLC until the starting materials completely reacted. The yellow suspension was cooled at room temperature and poured on to ice water. The pH of the mixture was carefully raised to 6 by addition of about 150 ml 30 % NaOH and extracted with CH₂Cl₂. The extract was dried over MgSO₄, the solvent was removed by rotary evaporator and the product was dried in oven at 80 °C. Finally, product was recrystallized from hot methanol to give a yellow crystalline solid. Yield: 50.47% M.P. (°C): 258-260



Scheme 3. The synthesis of 1, 10-phenanthroline-5, 6-dione [26].

3.3.2. Synthesis of 1H-imidazo [5, 6-f][1, 10] phenanthroline-2(3H)-thione (1, 10- PDTU) The ligand was synthesized by condensing the 1, 10-phenanthroline-5, 6- dione (PD) and thiourea (TU) (Scheme 4). Equimolar concetration of 1, 10-phenanthroline-5, 6- dione (4.29g, 0.0204 mol) in hot methanol (40 mL) was prepared. Separately, thiourea (1.55 g, 0.0204 mol) dissolved in hot methanol (40 mL). The white thiourea solution was added drop wise to the yellow 1, 10-phenanthroline-5, 6- dione solution while stirring continuously with a magnetic stirrer immediately turned in to light yellow color precipitate indicating the formation of new compound. The reaction mixture was refluxed for 10 h at 70 $^{\circ}$ C. The progress of the reaction

was monitored by careful observation and using TLC test. After 10 h refluxing, the reaction product gave a single spot; thus the precipitate was filtered and washed successively with petroleum ether to remove impurities. The product was dried in oven at 80 $^{\circ}$ C [44]. Yield: 68.5% M.P. ($^{\circ}$ C): 264-267.

3.3.3. Synthesis of Ni (II) complex

NiCl₂.6H₂O (0.38 g, 1.6 mmol) was dissolved in 40 mL of ethanol in a 250 mL two-necked flask and in a beaker the ligand (0.81g, 3.2 mmol) was dissolved in 40 mL of hot ethanol. The orange color of ligand solution was added slowly to the green nickel solution while stirring continuously. The light green color of nickel (II) solution immediately turned to dark green color indicating the formation of new compound. The mixture was refluxed for 11 h at 60 0 C and the reaction progress was followed by carefully observation and TLC. After 11 h refluxing brown precipitate and single spot was observed. The brown precipitate was then filtered and washed several times with petroleum ether and chloroform to remove impurities and finally the resulting complex was dried in an oven at 80 0 C [45]. Yield: 36.45% M.P. (0 C): 313-314

3.3.4. Synthesis of Cu (II) complex

A solution of CuCl₂.2H₂O (0.34g, 2 mmol) was dissolved in 20 mL of ethanol and (1.01 g, 4 mmol) of the ligand in 40 mL of hot ethanol was prepared separately. Then, the solution of ligand was added to the copper solution with stirring continuously. The deep green color of the original copper (II) solution immediately changed into deep red color indicating the formation of new compound. The mixture was refluxed for 12 h at 60 ^oC and the reaction progress was monitored by carefully observation and using TLC test. After 12 h refluxing green precipitate and single spot was observed. The green precipitate was then filtered, washed with petroleum ether and chloroform, and finally dried in oven at 80 ^oC [46, 42]. Yield: 82.33% M.P.(^oC):289-291.

3.4. Test of the Purity of the Products

The purity of the synthesized ligand and its complexes were checked by using thin layer chromatography (TLC) conducted on silica coated aluminum plates as stationary phase and ethanol as a mobile phase. A single spot in all samples indicates that the samples are pure. Also their purity was checked on melting point.

3.5. Physicochemical Methods of Characterization

Fourier Transform Infrared (FT-IR) spectra recorded in 4000-400 cm-1 were used to identify the presence of functional groups and to receive valuable information regarding the coordinating sites of the ligand in the metal complexes as KBr discs on a perkin Elmer. Nuclear Magnetic Resonance (NMR) spectra were recorded in DMSO on Bruker Ultra shield 400 MHz spectrometer for ¹H and 100.06MHz for ¹³C ; the chemical shift values were reported in parts per million (ppm) relative to TMS as internal standard to elucidate the structure of the ligand. The electronic spectra of the ligand and complexes in solution were run in the range of 200-800 nm on 6705 UV/Vis spectrophotometer (jenway). Electronic spectra used to identify and determine energy of d-d electronic transitions and electronic charge transfers.

The percentage composition of C, H, N and S for ligand and complexes were run on EA1112 Flash CHNS-analyzer at condition of carrier gas flow rate of 120 mL/min, reference flow rate 100 mL/min, oxygen flow rate 250 mL/min, furnace temperature of 900 ^oC and oven temperature of 75 ^oC. The metal quantity in the complexes were estimated by ZEF nit 700p (anaytikjenu)-Flame atomic absorption spectroscopy. Molar conductivities of complexes in DMSO were recorded at room temperature using HQ 40d (multi) conductivity meter to know the electrolytic nature and geometry structure of the complexes.

In melting point determination, solid melts when it undergoes a phase change from solid to liquid. It can be used not only to identify a compound but also in qualitative assess of purity of a compound. Melting point of synthesized ligand and complexes were determined using Griffin melting point apparatus by inserting sample in capillary tube with 2 cm height in temperature increment 2 ^OC per min. Characterization works such as ¹H and ¹³C NMR, FT-IR, AAS and elemental analysis were done in AAU while UV-Vis, and conductivity measurements were done in JU.

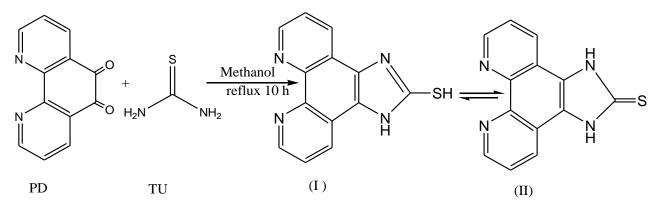
3.6. Antibacterial Activity

The antibacterial activity studies were conducted using Agar disc diffusion method as described by Shaik, A.K. *et al* [14, 47]. The *in-vitro* antibacterial screening effects of the new synthesized compounds were tested against four bacterial strain *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218), *Bacillus subtillus* (ATCC 6633) and *pseudomonas aeruginosa* (ATCC 27853) by disc diffusion method using Muller Hinton agar (MHA) as the medium. They were all obtained from Department of Biology in Microbiology laboratory, Jimma University. The discs measuring 6 mm in diameter were prepared from whatman No.1 filter paper sterilized by dry heat at 121 °C for 1 h. The sample solution of 100 mg/mL each test compounds was prepared by dissolving the compounds in DMSO and the solutions was loaded on the wall of the culture and in culture incubated at 37 °C for 24 h for the bacterial culture and the efficiency was evidenced by the zone of inhibition appeared as a clear area around the disc. The diameter of such zones of inhibition was measured using a meter ruler, and the mean value for each organism was recorded and expressed in millimeters. Gentamycin and DMSO were used as a positive control and negative control respectively.

4. RESULTS AND DISCUSSION

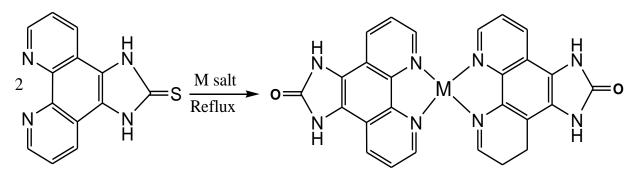
4.1. Synthesized Ligand and Complexes

Combination of thiourea with 1, 10-phenanthroline-5, 6- dione under reflux afforded orange precipitate indicating the formation of new substance. Reported literatures suggested that the reaction of thiourea with 1, 2- diketone could result compounds of the form I to II (Scheme 4) [48, 49, 50]. However, in this work IR spectrum of the free ligand shows a broad band at 3232 cm⁻¹ which arises from the v(N-H) stretching. The result of DEPT also shows the presence of three CH-groups which would be true only when the ligand is symmetrical that in turn suggests that compound (II) is the major product in this reaction condition.



Scheme 4. Synthesis of 1H-imidazo [5, 6-f][1, 10] phenanthroline-2(3H)-thione

Reacting the ligand with hydrated metal chlorides was expected only to undergo M-L coordination but the result of elemental analysis of the M-L complexes provides that sulfur constituents of the complexes is zero which suggests that the ligand has undergone desulphurization simultaneously with coordination [51, 62].



Scheme 5. Formation of M (II) complex, Where M = Ni and Cu

4.2. Physical Properties of Ligand, 1, 10-PDTU and its Complexes

The newly prepared ligand and complexes are colored solids, stable at room temperature and possess high melting point (> 264 °C). They are partially soluble in methanol, ethanol, water and completely soluble in DMSO but insoluble in petroleum ether and chloroform which indicates their polar nature. The remaining physical characteristics are summarized in Table-1.

Compound	Mol. Wt	Color	% yield	M.p (⁰ C)	Λ_{M}	Electrolytic
	(g/mol)				$(\Omega^{-1} \operatorname{cm}^2 \operatorname{mol}^{-1})$	nature
$Dione[C_{12}H_6N_2O_2]$	210	Yellow	50.47	258-260	-	-
$(L)[C_{13}H_8N_4S]$	252	Orange	68.5	264-267	-	-
$[NiC_{26}H_{20}N_8O_4] \ Cl_2$	637.693	Brown	36.45	313-314	119.5	Electrolyte
$[CuC_{26}H_{20}N_8O_4]Cl_2$	642.564	Green	82.33	289-291	139.6	Electrolyte

Table 1: Physical properties of newly synthesized ligand and complexes

4.3. Chloride test in the Complexes

The presence of chloride in the outer and inner coordination sphere of newly synthesized Ni (II) Complex and Cu (II) complex was tested. Addition of $AgNO_3$ (0.1 M) solution to aqueous solution of each complex afforded white precipitate confirming the presence of chloride in the outer coordination sphere [52]. The absence of chloride in the inner coordination sphere was proved using the same reagent however in this case each complex (30 mg) was digested in concentrated HNO₃ (2.5 mL) with heating till the formation of clear solution. Then, addition of AgNO₃ did not form white precipitate which ascertains the absence of chloride as ligand. The purpose of acid digestion was to break the coordination sphere.

4.4. Molar Conductivity of the Complexes

The conductivity measurements were carried out at room temperature by dissolving 20 mg of each complex in 30 mL of DMSO. Specific conductance (K) of the Ni (II) and Cu (II) complexes were obtained 119.5×10^{-6} and 139.6×10^{-6} S/cm respectively. The molar conductance (Λ_M) of the complexes were calculated using the relation $\Lambda_M = 1000$ K/C (where C is the molar concentration of the metal complex solution and K is specific conductance); the molar conductivity of the Ni

(II) Complex and Cu (II) complex were obtained 119.5 and 139.6 Ω^{-1} cm²mol⁻¹, respectively which suggests the complexes to be electrolytic nature in a 1:2. This supports the aforementioned evidence in the chloride test [53, 54].

4.5. Elemental Analysis: CHNS

Elemental analysis has been done to all of the ligand and complexes and the results are summarized in Table 2. The empirical formula of the ligand was proposed based on the percentage of carbon, hydrogen, nitrogen and sulfur. From the result, all the values obtained are in the range of accepted theoretical values. However, the percentage of sulfur in both complexes were observed zero which may be due to the oxidation of sulfur [55, 51]. The result of elemental analysis also suggests that both complexes to contain 1:2 metal to ligand ratio.

4.6. Estimation of Metal ions Content

The metal content in each complex was determined using atomic absorption spectroscopy (AAS). The organic parts of the complexes were completely eliminated before the estimation of the metals. A30 mg of each complex was placed in a clean and dry beaker, to which 10 mL portion of conc. HNO₃ was added and the content was heated gently in a hood until a few drop remain in the beaker. The 10 mL of additional conc.HNO₃ was added in the beaker and heated slowly until a few drop remained. The residue was dissolved and diluted using distilled water in a 50 mL flask. The solution was subjected to AAS study after appropriate dilution. Then, the metal percentage in the complexes was determined using the following relation.

$$%M = \frac{\text{Concentration (ppm)X Volume diluted X 100}}{\text{mass of sample taken X 1000}}$$

Based on the above relation, the percentage of Ni (II) and Cu (II) in their corresponding complex is estimated to be 8.78 and 10.32 respectively. The metal-ligand ratios in the complexes can be proposed based on the metal, C, H, N and S percentages.

The percentage of nickel in complex was determined to be 8.78 % (experimental value) while the theoretically calculated percentage composition of Ni in $[NiC_{26}H_{20}N_8O_4]$ Cl₂ complex is 9.20 %. In Cu (II) complex also percentage of Cu is found to be 10.32 % whereas theoretically calculated Cu in $[CuC_{26}H_{20}N_8SO_4]$ Cl₂ complex is 9.89 %. The small deviation of calculated and found

value of Ni in $[NiC_{26}H_{20}N_8O_4]$ Cl₂ and Cu in $[CuC_{26}H_{20}N_8SO_4]$ Cl₂ observed may be connected with the presence of impurities or instrumental error. Therefore, it is calculated that the metal to ligand ratio in both complexes are 1:2. Based on these data the chemical formula of the Ni (II) and Cu (II) complexes proposed to be $[NiC_{26}H_{20}N_8O_4]$ Cl₂ and $[CuC_{26}H_{20}N_8O_4]$ Cl₂ respectively.

Compounds	Found (Calculated) %				
	C%	H%	N%	S%	M%
	59.78	3.19	21.72	11.71	-
$[C_{13}H_8N_4S]$	(61.91)	(3.17)	(22.22)	(12.69)	-
	47.25	3.09	17.89		8.78
[NiC ₂₆ H ₂₀ N ₈ O ₄] Cl ₂	(48.93)	(3.14)	(17.56)	0	(9.20)
	48.05	3.01	16.82		10.32
$[CuC_{26}H_{20}N_8O_4]\ Cl_2$	(48.56)	(3.12)	(17.43)	0	(9.89)

Table 2: Elemental analysis of the prepared ligand and its complexes

4.7. NMR Spectral data of the Ligand, 1, 10-PDTU

4.7.1. ¹H -NMR spectrum of the 1, 10-PDTU

The structure of ligand was confirmed by ¹H NMR. The spectrum of ligand was possible to be observed four different aromatic protons in which peaks appearing in the range of (6.99-8.72) ppm due to different protons environments.

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From the ¹H-NMR spectra of ligand displayed chemical shifts at 8.72 ppm which is doublet peaks assigned to proton that was attached to the nitrogen atom. There are two equivalent aromatic protons of this value; H-2 and H-9.These proton signals appeared de-shielded due to the close proximity to the electronegative nitrogen atom. The doublet peak found at the chemical shifts of 8.25 ppm showed the equivalent environment of symmetric stretching of two protons (H-4, H-7) on aromatic ring that found between the middle of two nitrogen atoms. Another doublet of doublets peak at the chemical shift of 7.55 ppm referred to the two equivalent protons (H-3, H-8) far from nitrogen found in the hetreoaroamatic compounds and coupling of each protons on non-equivalent neighboring protons. The spectrum also showed long intense singlet

peak at the chemical shift of 6.99 ppm which was exhibited the equivalent environment of symmetric stretching of two (H-16, H-17) protons. This is also confirmed by the presence of a broad peak at 3433 cm⁻¹ in the IR spectrum. The integration values from the ¹H-NMR spectra for ligand agreed with the number of protons proposed for the respective structures. However, it can be clearly seen that there were unexpected peaks suggesting that there were solvent impurities remaining after purification. This is also evident from the ¹³CNMR spectra. The spectra shows lower chemical shift at (3.5) ppm is due to the impurities of ligand of proton methanol present and the chemical shift at (2.5) ppm due to solvent proton of DMSO [56]. The ¹H-NMR results are shown in Table3 and spectral data in (**Appendix Figure 1**).

4.7.2.¹³C NMR spectrum of 1, 10-PDTU

All the ¹³C NMR Spectra was presented in agreement with previous published literatures for ligand [57, 62].¹³C-NMR spectrum of the ligand indicates seven carbon resonances, out of which four of them with δ 182.2 ppm, 147.0 ppm, 132.5 ppm and 85.8 ppm are quaternary carbons and attributed to C-15, C-5, C-11, and C-12 respectively. The peak at δ 150.7 ppm, 136.6 ppm and 125.1 ppm are respectively due to C-2, C-4 and C-3 which are in agreement with the structure of the ligand. However, the peak at δ 39.1 ppm may be DMSO as impurity. The ¹³C-NMR results are shown in Table 3 and spectral data in (**Appendix Figure 2**).

4.7.3. DEPT 135 NMR spectrum of 1, 10-PDTU

The Distortionless Enhancement by Polarization Transfer (DEPT) method is an instrumental mode that provides a way to acquire the information of distinguishing among ¹³C signals for CH₃, CH₂, CH, and quaternary carbons. 135° pulse: carbon signals show different phases, signals for CH₃ and CH carbons give positive signals, quaternary carbons give no signal and signals for CH₂ carbons give negative signals. Hence, in the proposed ligand (**Appendix Figure 3**) contains only C-H group. There is no -CH₂ group in the compound because no any signal shown that exhibits negative phasing. The DEPT spectrum also shows three signals at 150.7 ppm, 136.6 ppm and 125.1 ppm for the C-H groups in the ligand and the signals at 182.2 ppm, 147.0 ppm, 132.5 ppm and 85.8 ppm disappeared which confirms that the four types of carbons are quaternary carbons. The assignment for resonance peaks of carbon atoms in the aromatic rings

were confirmed by DEPT135 NMR and all the signals matched with the proposed structure. The DEPT- 135 spectral data is summarized in Table 3 and spectral data in (**Appendix Figure 3**).

Types of C	¹³ C NMR data	DEPT data	Remarks	Types of	Chemical
	(δ/ppm)	(δ/ppm)		proton	shift (ppm)
C-2,C-9	150.7	150.7	C-H	H-2,H-9	8.72
C-5,C-6	147.0	-	С	H-4,H-7	8.25
C-4,C-7	136.6	136.6	С-Н	H-3,H-8	7.55
C-11,C-13	132.5	-	С	H-16,H-17	6.99
C-3,C-8	125.1	125.1	С-Н		
C-12,C-14	85.8	-	С		
C-15	182.2	-	С		

Table: 3.¹³C NMR (100.6MHz, DMSO), DEPT 135 and ¹H-NMR spectral data for ligand

C-Quaternary carbons

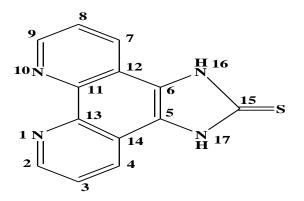


Figure 8. The structure of the synthesized ligand, 1, 10-PDTU

4.8. Infrared Spectra Studies of 1, 10-PDTU and Complexes

IR spectra give important information about the nature of the binding mode and functional group attached to the metal ion. In order to study the binding mode of the ligand to the metal in Cu (II) and Ni (II) complexes, the IR spectrum of the free ligand was compared with the spectrum of the complexes. The infrared bands of the characteristic groups of the ligand and its complexes, together with their assignments are listed in Table 4 and spectral data in (**Appendix Figure 4-6**).

The IR spectra of the free ligand show broad absorption band at 3433 cm⁻¹ which are due to amine v(N-H) stretching vibration. The strong broad band observed at the region of 3232 cm⁻¹ and 3378 cm⁻¹ of Ni (II) and Cu (II) complexes respectively due to the intermolecular hydrogen bonding as well as v(O-H) stretching vibration of the water molecules or ethanol alcohol bonded with metal ion as impurities [58]. However, the broadness and intensity of these bands was covered the band of amide v(N-H) in both complexes. The presence of v (N-H) in Ni (II) and Cu (II) complexes are further confirmed by the appearance of weak bands at 814 cm⁻¹ and 813 cm⁻¹, respectively. The band had been observed at 2972 and 2713 cm⁻¹ in the ligand spectrum due to v(C-H) in aromatic ring. This band also observed around 3074 cm⁻¹ in Cu (II) complex but in Ni (II) complex overlapped with v(O-H) band. The IR spectral band values of (C-H) bending in the aromatic rings are observed strong band at 731 cm⁻¹ in the free ligand. It also observed at 730cm⁻¹ ¹ and 725 cm⁻¹ respectively, in its corresponding Ni (II) and Cu (II) complexes [59, 60]. Comparing the spectra of the ligand and that of the complexes the v (C=N) (phenanthroline ring) group was observed strong band at 1617 cm⁻¹ in the free ligand and this band shifted in to 1626 cm⁻¹ and 1630 cm⁻¹ in Nickel (II) and Cu (II) complexes respectively; and this blue shift ascribed the participation of the v(C=N) (phenanthroline ring) group in coordination of the metal ion[43]. The strong absorption band at 1019 cm⁻¹ assigned to be v(C=S) thiocarbonyl group which disappear in the complexes implying the absence of v(C=S) group. In both complexes, the appearance of new strong band at 1698-1701 cm⁻¹ implies formation of v (C=O) as a consequence of C=S oxidation due to oxygen in air [61, 62, 55].

The IR spectra of the ligand show characteristic strong absorption band at 1422 cm⁻¹, due to the stretching of the (C=C) functional group. Strong absorption band at 1315 cm⁻¹ were also found, due to(C-N) group. In the IR spectra of the corresponding complexes, there were shifts in wave numbers for both groups. All these shifts can be explained by the fact that each nitrogen atom of the phenanthroline ligand donates a pair of electrons to the central nickel and copper metal ions. The complex formation of metal ions with ligand was further confirmed by the appearance of new weak intensity, nonligand bands in the 616 cm⁻¹ and 548 cm⁻¹ in the spectra of both complexes which are assigned to frequencies of v(Ni-N) and v(Cu-N)stretching vibrations respectively. This indicates that coordination has occurred through the nitrogen atoms of C=N

(phenanthroline ring) groups of the ligand [63,64]. Therefore, from the IR spectra, it is concluded that the ligand behaves as a neutral bidentate ligand coordinated to the metal ion.

Compound	v(N-H/	v(C-H)	v(C=0)	v(C=N)	v(C=C)	v(C-N)	v(C=S	(C-H)	v(M-N)
	O-H)	cm ⁻¹) cm ⁻¹	bending	cm ⁻¹				
	cm ⁻¹			(phen			cm ²	cm ⁻¹	
				ring)					
		2972-			1422s	1315s			-
$L[C_{13}H_8N_4S]$	3433b	2713w	-	1617s			1019s	731s	
[NiC ₂₆ H ₂₀ N ₈	3232s,b	-	1701w	1626s	1426s	1319w		730s	616w
O ₄]Cl ₂							-		
[CuC ₂₆ H ₂₀ N ₈	3378s,b	3074w	1698s	1630s	1430s	1301m		725s	548w
$O_4]Cl_2$							-		

Table: 4. Major IR spectral data of the ligand and its complexes

b=broad s=strong m=medium w=weak

4.9. Electronic Spectra of Prepared 1, 10-PDTU and its Complexes

Electronic spectra used to assign the stereochemistry of a metal complex based on the position and number of d-d transitions peaks [65]. The UV-Vis spectra of the ligand and the complexes were recorded in DMSO in the range of 200-800nm at room temperature.

The electronic spectra of the ligand (**Appendix Figure 7**) showed two strong bands at 260 nm (38461 cm⁻¹) and at 291 nm (34364 cm⁻¹). The band at 260 nm (38461cm⁻¹) may be assigned to $\pi \rightarrow \pi^*$ transition of the C=C group. This band also observed at 260 nm for Ni (II) and Cu (II) complexes, which shows that there is no any shift indicating that, no participation of this group in the coordination. While, the band observed at 291 nm (34364 cm⁻¹) may be attributed to $n \rightarrow \pi^*$ transitions of the C=N chromophores [66].

The electronic spectra of Ni (II) complex (**Appendix Figure 8**) show four major spectral bands at 260 nm (38461 cm⁻¹), 302 nm (33112cm⁻¹), 391 nm (25575 cm⁻¹) and at 495 nm (20202 cm⁻¹). The spectrum exhibits a band at 302 nm (33112 cm⁻¹), which is assigned to a charge transfer band [67]. From the expected three d-d transitions two of them are appeared in this complex. The

reflectance spectra of Ni (II) complex consist of the transition bands at 391 nm (25575 cm⁻¹) and at 495 nm (20202 cm⁻¹) assignable to the transitions ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g}$ (P) and ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g}$ (F) respectively, indicating an octahedral geometry around the Ni (II) ion [68, 69]. The third transition [${}^{3}A_{2g} \rightarrow {}^{3}T_{2g}$ (F)] band often tails into the ultraviolet region so covered by charge transition spectral band.

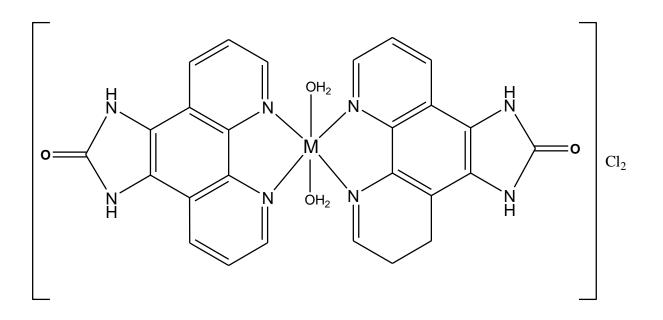
The electronic spectra of Cu(II) complex three bands were observed at 260 nm (38461cm⁻¹), 300 nm(33333 cm⁻¹), 342 nm(29240 cm⁻¹) and at 445 nm (22472 cm⁻¹) (**Appendix Figure 9**). The band observed at 300 nm (33333 cm⁻¹) and 342 nm (29240 cm⁻¹) may be assigned to charge transfer band. The d-d transition of the Cu (II) complex show a band at 445 nm (22472 cm⁻¹) attributed to ${}^{2}E_{g} \rightarrow {}^{2}T_{2}g$ transition, which is well-known behavior for distorted octahedral geometry of Cu (II) complex [70, 63]. Electronic spectra of the ligand and its metal complexes were summarized in Table 5 given below.

Compound	Wave	Wave No	Band Assignments	Proposed structure
	length(nm)	in cm-1		
Ligand (L)	260	38461	$\pi \rightarrow \pi^*(C=C)$	-
	291	34364	$n \rightarrow \pi^*(C=N)$	
	260	38461	$\pi \rightarrow \pi^*(C=C)$	Octahedral
	302	33112	СТ	structure
Ni(II)complex	391	25575	$^{3}A_{2}g \rightarrow ^{3}T_{1}g(P)$	
	495	20202	$^{3}A_{2}g \rightarrow ^{3}T_{1}g(F)$	
	260	38461	$\pi \rightarrow \pi^*(C=C)$	
Cu(II)complex	300	33333	СТ	Distorted
	342	29240	СТ	octahedral
	445	22472	$^{2}\text{Eg} \rightarrow ^{2}\text{T}_{2}\text{g}$	structure

Table 5. The electronic spectra of the ligand, Ni (II) and Cu (II) complexes.

4.10. Proposed Structure of the Complexes

Based upon inferences arrived from C, H, N, S and M analysis, molar conductivity, infrared, atomic absorption spectroscopy and electronic spectral studies the following structures of the complexes are proposed.



Where M=Ni (II) and Cu (II)

Figure 9. The proposed structure of Ni (II) and Cu (II) Complexes

4.11. Antibacterial Activity test

The newly synthesized ligand and its Ni(II) and Cu(II) complexes were screened against four bacterial strains namely; *Staphylococcus aureus* (ATCC 25923), *Bacillus subtillus* (ATCC 6633) (Gram positive) as well as *Escherichia coli* (ATCC 35218) and *pseudomonas aeruginosa* (ATCC 27853)(Gram negative)through disc diffusion method [71].The antibacterial activities of the compounds described measuring their inhibition zone (mm) in comparison with Gentamicin (standard drugs).The results of antibacterial activity of the ligand and its complexes are found to be comparable with standard drugs, presented in Table 6 and (**Appendix Figure 10-13**). The inhibition zone of metal complexes are higher than that of the ligand indicates that complexes exhibit higher anti-bacterial activities than the free ligand due to chelation which enhance lipophilicity so favors permeation into the bacterial membrane to cause the death of the organisms [72].

Ni (II) complex shows higher activity than Gentamicin against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218) and *pseudomonas aeruginosa* (ATCC 27853) while Cu (II) complex is more active than Ni (II) complex against *Bacillus subtillus* (ATCC 6633). This evidence implies Ni (II) complex can be potential candidate drug towards *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218) and *pseudomonas aeruginosa* (ATCC 27853) Table 6. The antibacterial activity in various bacteria

	Zone of inhibition in diameter (mm)						
Compound	Staphylococcus aureus(ATCC 25923)	Bacillus subtillus (ATCC 6633)	Escherichia coli (ATCC 35218)	pseudomonas aeruginosa (ATCC 27853)			
Ligand(L)	11	15	16	12			
Ni(II)Complex	21	22	23	24			
Cu(II)complex	17	24	19	16			
Gentamicin	20	24	21	22			
DMSO	Non active	Non active	Non active	Non active			

5. CONCULION AND RECOMMANDATION

5.1. Conclusion

A ligand, 1H-imidazo[5,6-f][1,10] phenanthroline-2(3H)-thione (1,10-PDTU) was synthesized by condensation reaction of 1, 10-phenanthroline-5,6-dione(PD) and thiourea (TU) and its complexes with Ni (II) and Cu (II) were synthesized and characterized using elemental analysis (C, H, N and S), IR, NMR, UV-Vis, AAS and conductivity measurement. Based on the elemental analysis, ¹H-NMR, ¹³C-NMR and DEPT data, the proposed structures of the ligand are depicted (Figure 8). The IR data show that the ligand coordinates through its nitrogen atoms of the C=N (phenanthroline ring) groups in bidentate mode. The high conductivity of the Cu (II) and Ni (II) complexes reveals their electrolytic nature and the existence of the chloride counter ion in the outer sphere. The analytical data showed that stoichiometry of both complexes to be 1:2 metals to ligand ratio and formulated as [NiC₂₆H₂₀N₈O₄] Cl₂ and [CuC₂₆H₂₀N₈O₄] Cl₂. On the basis of the combined analytical results, distorted octahedral geometry of Cu (II) and octahedral geometry Ni (II) complexes were achieved. Antibacterial activities of the prepared ligand and complexes have also been studied. From comparative study, both metal complexes showed significantly enhanced antibacterial activity against selected bacterial strains in comparison to the free ligand.

5.2. Recommendation

From the study result it has been concluded that the synthesized ligand exhibit bidentate nature and form stable complexes with promising antibacterial activity results. And researcher recommend that, the synthesize of other d-block transition metal complexes. In addition to this, further tests are needed to evaluate broad spectrum activities especially on nickel complex against other (additional) bacterial species to explore all possibilities to evaluate the potential of nickel complex in the development of pharmaceutical drugs.

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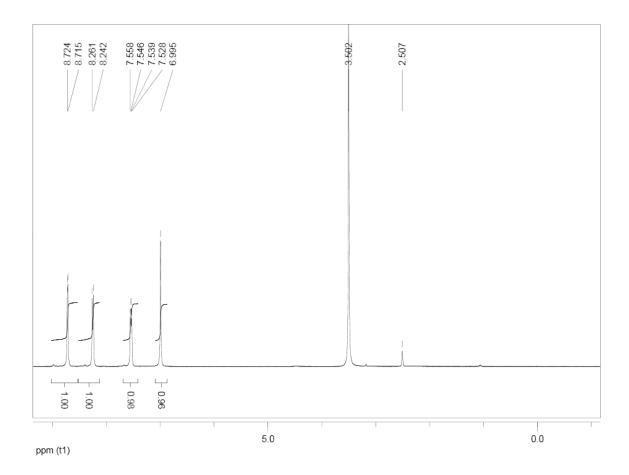
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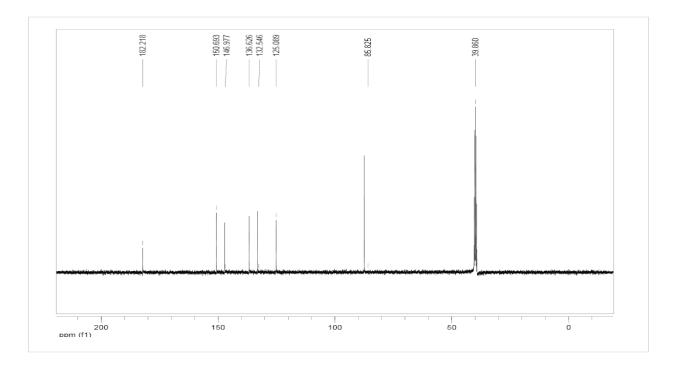
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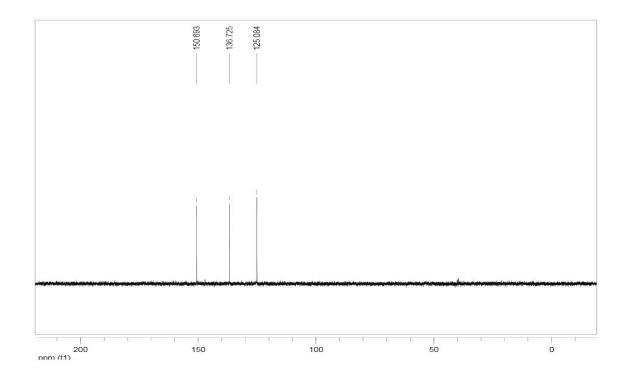
7. APPENDICES



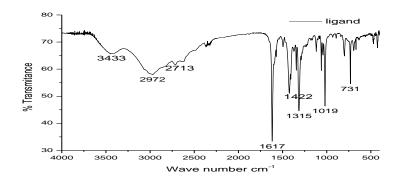
Appendix Figure 1. ¹HNMR spectrum of ligand, 1, 10-PDTU in DMSO



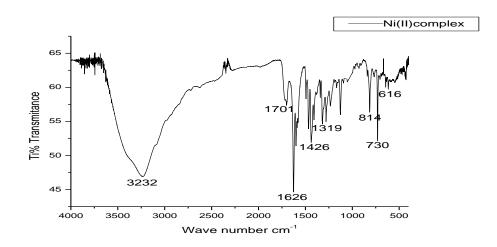
Appendix Figure 2. ¹³C NMR spectrum of ligand, 1, 10-PDTU in DMSO



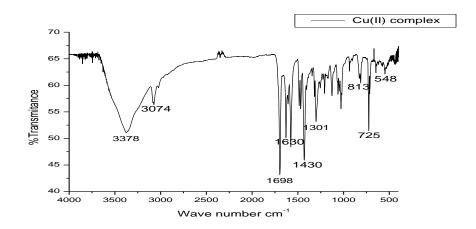
Appendix Figure 3. DEPT 135 NMR spectrum of ligand, 1,10-PDTU in DMSO



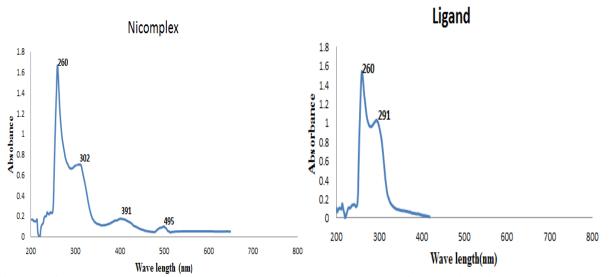
Appendix Figure 4. IR spectra of ligand, 1, 10-PDTU



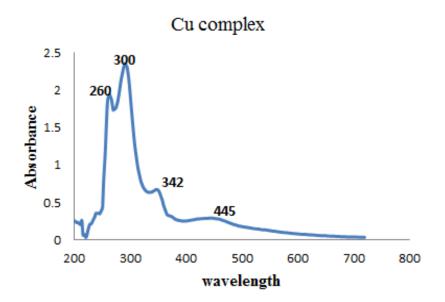
Appendix Figure 5. IR spectra of Ni (II) complex



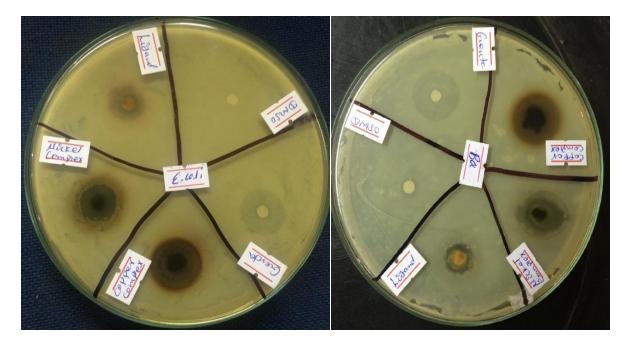
Appendix Figure 6. IR spectra of Cu (II) complex.



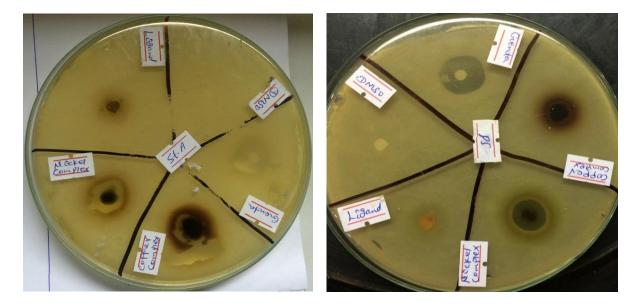
Appendix Figure 8. UV-visible spectra Ni (II) complex. Appendix Figure 7. UV-Visible spectra ligand



Appendix Figure 9. UV-Visible spectra Cu (II) complex



Appendix Figure 10. Inhibition of *Escherichia coli* **Appendix Figure 11.** Inhibition of *Bacillus subtilis*



Appendix Figure12. Inhibition of *staphylococcus aureus* **Appendix Figure 13.** Inhibition of *Pseu. Aeruginosa*