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Al-Qadisiyha University
College of Pharmacy

Synthesis, Characterization and Biological Activity of benzothiazole Complexes

A Research

Submitted to the College of pharmacy Al-Qadisiya University in Partial Fulfillment of Requirements of B.Sc. Degree of Science in pharmaceutical

By

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Abstract:

The work includes the synthesis of new ligand derived from 12-mercapto benzothiazole ,1,2-phenylene diamine and Diacetyl monoxime in ethanol as medium of the reaction .

The complexes were prepared by using of Cu (II), Cd (II), and Hg (II) salts with the prepared ligand in the same procedure methods. The metal ions Cu (II), Cd(II) and Hg(II) were coordinated with the prepared ligand in (1:1), (M:L) ratio.

The spectroscopic studies (FTIR, UV-Vis.) and melting points were used to characterized the ligand and its complexes.

The biological activity tests showed the ability of the prepared ligand and its complexes to inhibit growth of *Staphylococcus aureus and Escherichia Coli*. at prepared concentration. Most of the prepared complexes effective against both types of bacteria in varying degree, with the high activity for Hg(II) complexes.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَتَعَـالَى اللَّــهُ الْمَلِـكُ الْحَـقّ ۞ وَلا

تَعْجَـلْ بِـالْقُرْآنِ مِـنْ قَبْـلِ أَنْ يُقْضَـى

إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْماً

صدق الله العلي العظيم

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INTRODUCTION

[1-1] Heterocyclic compounds [1-4]

Introduction

Heterocyclic chemistry is a very important branch of organic chemistry. A cyclic organic compound containing all carbon atoms in ring formation is referred to as a *carbocyclic compound.* If at least one atom other than carbon forms a part of the ring system then it is designated as a *heterocyclic* compound Nitrogen, oxygen and sulfur are the most common heteroatoms but heterocyclic rings containing other hetero atoms are also widely known.

Heterocyclic compounds may be classified into *aliphatic* and *aromatic.* The aliphatic heterocycles are the cyclic analogues of amines, ethers, thio ethers, amides, etc. Their properties are particularly influenced by the presence of strain in the ring. These compounds generally consist of small (3- and 4-membered) and common (5 to 7 membered) ring systems.

The aromatic heterocyclic compounds, in contrast, are those which have a heteroatom in the ring and behave in a manner similar to benzene in some of their properties.

Furthermore, these compounds also comply with the general rule proposed by Huckel [(4n+2), where n= no of rings]. Besides the vast distribution of heterocycles in natural products, they are also the major components of biological molecules such as DNA and RNA. DNA is without doubt the most important macromolecule of life. Nucleotides, the building blocks of our genes are derivatives of pyrimidine and purine ring structures. Chlorophyll and heme, the oxygen carriers in plants and animals respectively are derivatives of large porphyrin rings.

Heterocycles are an important class of compounds, making up more than half of all known organic compounds. Heterocycles are present in a wide variety of drugs, most vitamins, many natural products, biomolecules, and biologically active compounds, including antitumor, antibiotic, anti-inflammatory, antidepressant, antimalarial, anti-HIV, antimicrobial, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal, and insecticidal agents.

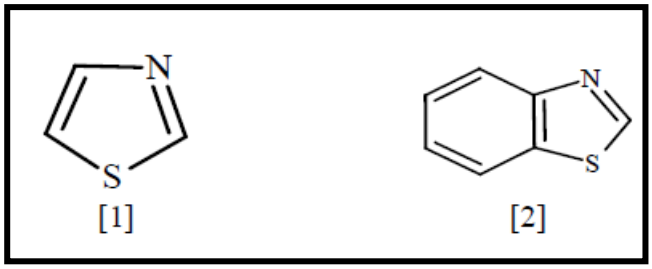
Also, they have been frequently found as a key structural unit in synthetic pharmaceuticals and agrochemicals. Most of the heterocycles possess important applications in materials science such as dyestuff, fluorescent sensor, brightening agents, information storage, plastics, and analytical reagents.

Most of the sugars and their derivatives, including vitamin C, for instance, exist in the form of five-membered (furan) or six-membered (pyran) rings containing one oxygen atom. Most member of vitamin B group possess heterocyclic ring containing nitrogen. One example is vitamin B6 (pyridoxine), which is a derivative of pyridine, essential in amino acid metabolism.

[1-2] Benzothiazole[5-7]

Thiazole (1) is structurally related to thiophene and pyridine, but in most of its properties it resembles to the latter. Thiazole was first described by Hantzsch and Waber in 1887. Popp confirmed its structure in 1889.The numbering in thiazole starts from the sulphur atom. Structure (2) is benzothizole. The basic structure of benzothiazole consist of benzene ring fused with 4, 5 position of thiazole. The two rings together constitute the basic nucleus 1, 3-benzothiazle.

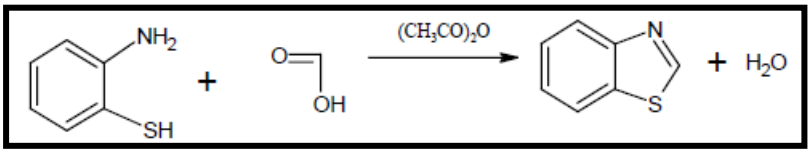
Benzothiazole is weak base, having varied biological activities and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug discovery.



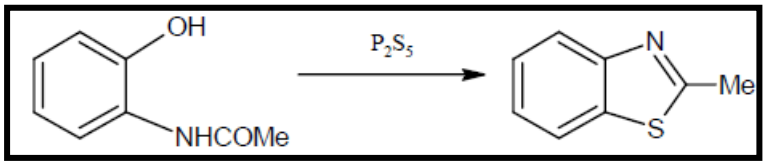
[1] [2]

Benzothiazole moites are part of compounds showing numerous biological activities such as antimicrobial anticancer , anthelmintic , anti-diabetic activities.

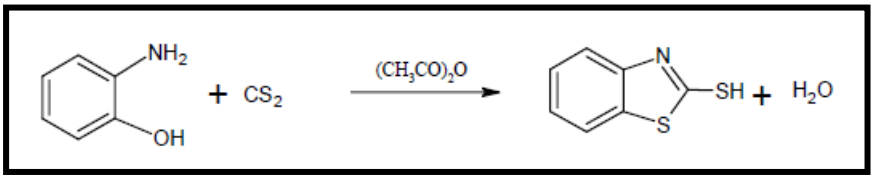
They have also found application in industry as anti-oxidants. Various benzothiazoles such as 2-aryl benzothiazole received much attention due to unique structure and its uses as radioactive amyloid imagining agents , and anticancer agents. Benzothiazole may be prepared by action of acid anhydrides(or) chlorides on o-aminophenol and formic acid in presence of acetic anhydride.



Benzothiazoles are also formed by action of phosphorus pentasulfide on o-acylaminophenoles.



2-mercaptobenzothiazole is vulkanisation accelerator it may be prepared as follows.



[1-3] IR Spectroscopy study[8]

The IR spectrum of the compound showed absorption peak at 3344cm-1, 3025cm-1, 1630cm-1, 690cm-1 due to stretching of υ(N-H), υ (C-H), υ (C=N), υ (C-S).

[1-4] Biological activity[9]

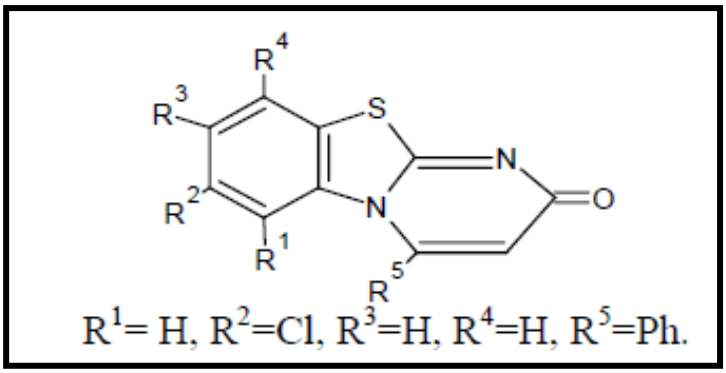
The chemistry and biological study of heterocyclic compounds has been an interesting field for a long time in medicinal chemistry. Benzothiazole derivatives are an important class of compounds, which is becoming increasingly important due to their broad spectrum of biological activities .

Literature survey shows that many Benzothiazole derivatives are known to exhibit pharmacological activities such as antitumor and antiviral , anti proliferative, anticancer , antimicrobial , antibacterial , anthelmintic , antidiabetic , anti-Inflammatory , antimalarial , antifungal etc.

Microbes are causative agents for various types of disease like pneumonia, ameobiasis, typhoid, malaria, common cough and cold various infections and some severe diseases like tuberculosis, influenza, syphilis, and AIDS as well.

Various approaches were made to check the role of benzothiazole moiety as antimicrobial agent from the discovery of molecule to the present scenario.

Gupta S *et al* reported synthesis of series of pyrimido [2, 1-b] benzothiazoles by conjugation addition to imino nitrogen of 2-aminobenzothiazoles to alkyne *β*-carbon atom of acytylenic acid followed by ring closure and synthesized compounds are studied for antimicrobial activity against *E. coli* and *Enterobacter* as test organisms at conc 100µg per disc using vancomycine and meropenam as standard drug.

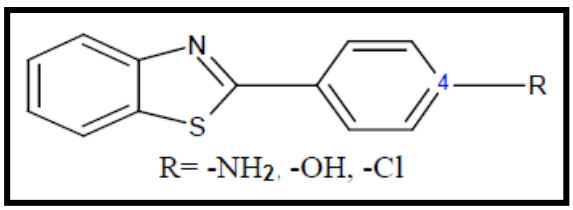


R^1= H, R^2=Cl, R^3=H, R^4=H, R^5=Ph.

[1-5] Benzothiazoles compounds[10,11]

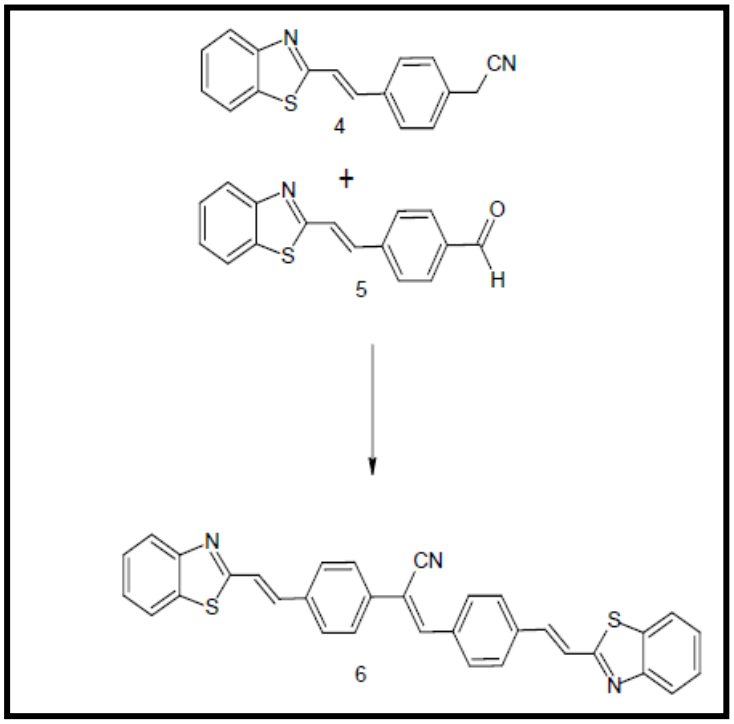
There are many compounds contain benzothiazole ring .

Presence of hydrophobic moieties in molecule is conductive for cytotoxic activity of benzothiazole derivatives against cancer cell lines. The amino, hydroxyl, and chloro group containing benzothiazole shows better anticancer activity.



R= -NH_2, -OH, -Cl

They explored synthesis of benzothiazole based organic nano-particles. The elaboration of conjugated system was performed by reacting equimolar quantities of 4 and 5 in dry THF and ter-butyl alcohol at 50ºC while a small amount of terbutylammonium hydroxide was slowly dropped in mixture



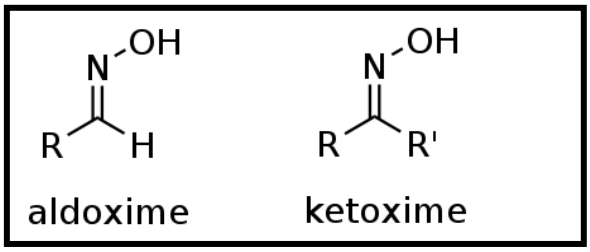
[1-6]Imine-Oxime[12,13]

An oxime is a chemical compound belonging to the imines, with the general formula $R^1R^2C{=}N\,O\,H$, where R^1 is an organic side-chain and R^2may be hydrogen, forming an aldoxime, or another organic group, forming a ketoxime. O-substituted oximes form a closely related family of compounds. Amidoximes are oximes of amides with general structure RC(=NOH)(NRR').

The name "oxime" is derived from "oximide" (i.e., oxy- + amide). According to the German organic chemist Victor Meyer (1848–1897) – who, with Alois Janny, synthesized the first oximes – an "oximide" was an organic compound containing the group (=N-OH) attached to a carbon atom.

However, in 1882, Meyer and Janny succeeded in synthesizing methylglyoxime ($CH_3C(=NOH)CH(=NOH)$), which they named (acetoximic acid).

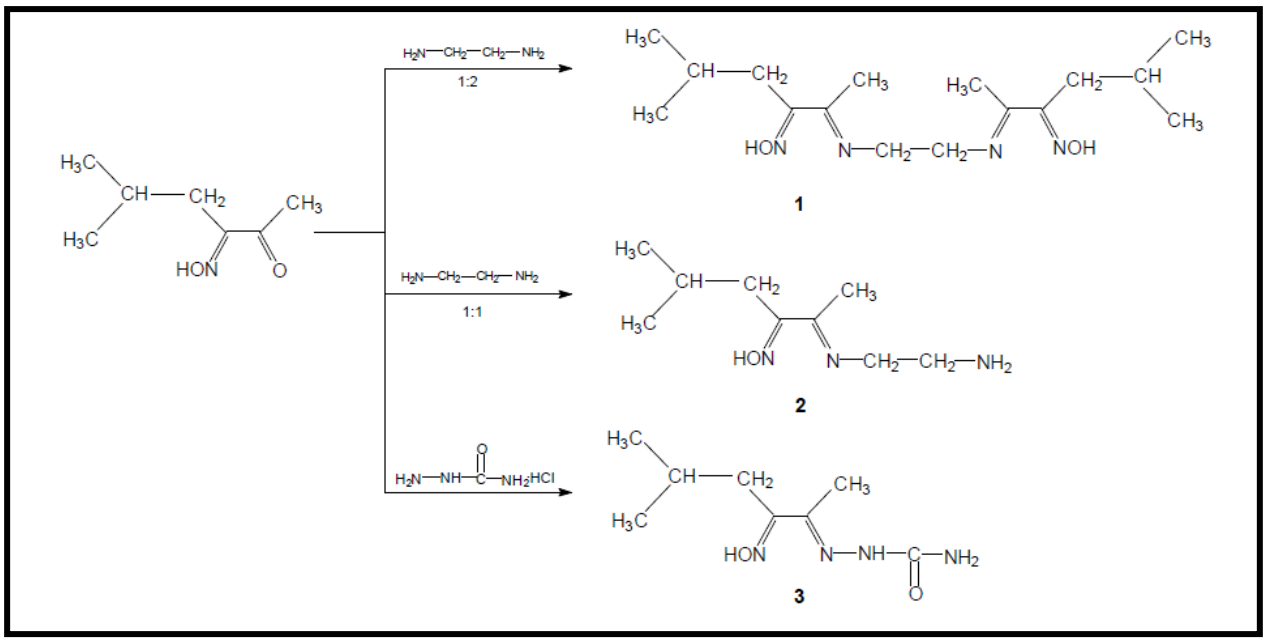
Subsequently, they synthesized 2-propanone, oxime ($(CH_3)_2C=NOH$), which they named "Acetoxim" (acetoxime), in analogy with Acetoximsäure.

Oximes 

have three characteristic bands in the infrared spectrum, at wavenumbers 3600 (O-H), 1665 (C=N) and 945 (N-O).

[1-7]Imine-Oxime compounds[14]

The imines with amino substituents groups were synthesized. It was shown that derivatives of NH_2 / NH containing compounds at the carbonyl group of 3-hydroxyimino-5-methyl-2-hexanone are formed as a result of these reactions. All compounds were exhibited toward various strains of microbes to study their biological activity.

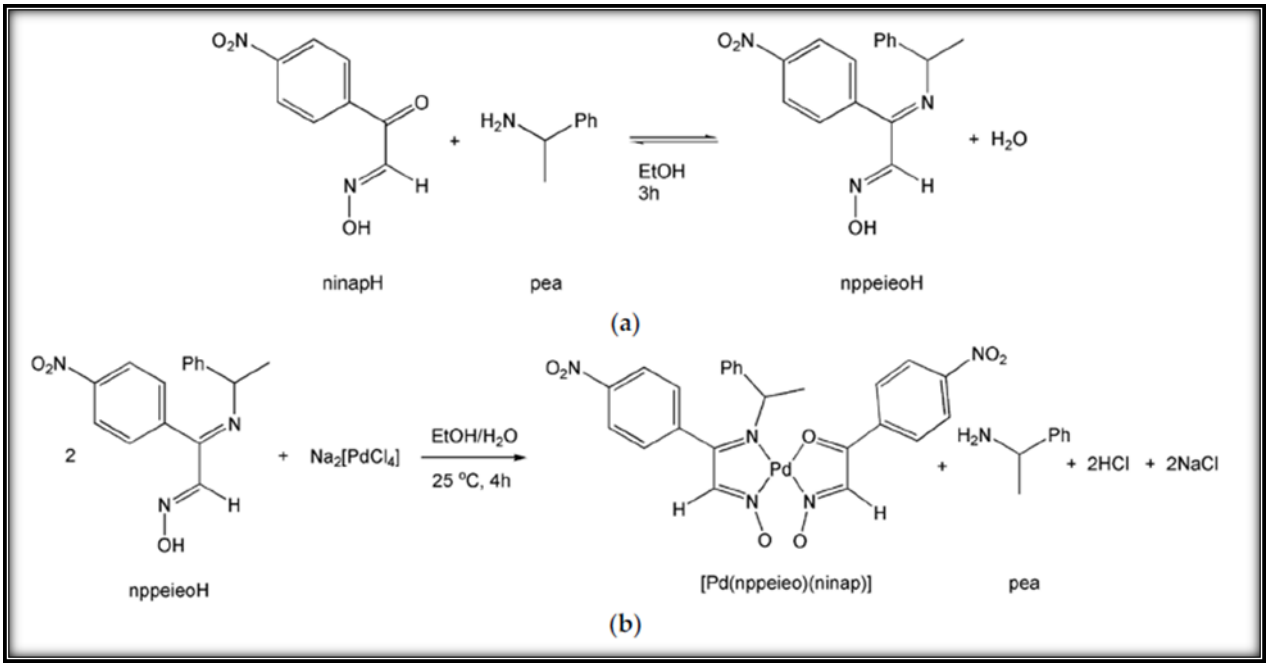


[1-8] Imino – oxime and benzothiazole complexes[15,16]

In 2016 , the ligand and its complexes synthesized a new imine oxime, namely (4-nitro-phenyl)-(1-phenyl-ethylimino)-acetaldehyde oxime (nppeieoH). Spectroscopic and X-ray diffraction studies showed that nppeieoH is hydrolyzed in aqueous

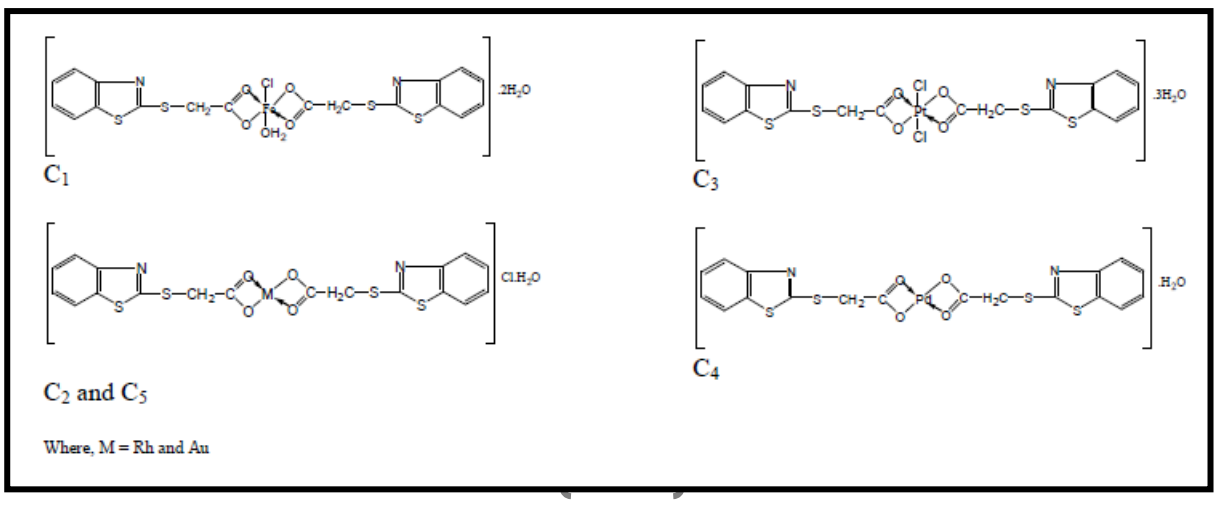
solution, forming nitro isonitroso acetophenone (ninap) and the hydrolysis product binds to Pd(II) to yield [Pd(nppeieo)(ninap)].

The vibrational and the electronic spectra of nppeieoH and its Pd(II) complex, have been synthesized and characterized by various techniques, including IR, NMR, UV-Vis, elemental analysis melting points , conductivity measurements.



New metal complexes of the ligand 2-thioacetic acid benzothiazole with the metal ions Fe(III), Rh(III), Pd(II), Pt(IV) and Au(III) were prepared in alcoholic medium.The prepared complexes were characterized by FTIR Spectroscopy, electronic spectroscopy, elemental analysis, ^{1}H-NMR, ^{13}CNMR and conductivity measurements. Molar ratio and continuous variation studies in solution gave comparable result with those obtained from solid state study.

From the spectral measurements, monomer structures for the complexes were proposed.



[1-9] Aim of the work

The aim of the work is summarized in following points :

1- Synthesis of the imine –oxime ligand derived from 2-mercaptobenzothiazole.

2- Synthesis of complexes of the ligand with [Cu(II), Cd(II) and Hg(II)] .

3- Characterization of the synthesized ligands and their complexes by using IR , UV-Vis. , melting points .

3- Evaluation of biological activity for the ligands and their complexes against selected types of bacteria which include [*Staphylococcus aureus* as gram positive] and [*Escherichia Coli* as gram negative] .

CHAPTER TWO

EXPERIMENTAL

[2-1] Chemicals

Table (2-1): shows the used chemicals in the experimental part .

Table (2-1): Chemicals and their Sources

Chemicals	Purity (%)	Supplied from
2-Mercaptobenzoxazole	99	Aldrich
1,2-Phenylene diamine	97	Aldrich
$CuCl_2.2H_2O$	98	Merck
$CdCl_2.2H_2O$	99	BDH
$HgCl_2$	99	Merck
Nutrient agar medium	-	BDH

[2-2] Physical Measurements

2.2.1 -Melting point

Melting points of ligands and their complexes were measured using Stuart SMP3 melting point apparatus .

2.2.2 -UV-Visible spectroscopy

The electronic spectra of ligands and their metal complexes have been recorded in the range of (200-1000) nm using (Shimadzu U.V-165PCS spectrophotometer) by using ethanol as solvent .

2.2.3 -Infra-red spectroscopy

Infra-red spectra have been recorded in the range (400-4000cm^{-1}) using KBr disk for the ligands and their complexes by using (Shimadzu FT-IR 8400S spectrophotometer).

2.2.4- Biological activity

Biological activity is evaluated by using Autoclave, Incubator and PH meter.

[2-3] Synthesis of the ligand

2.3.1- Synthesis of the compound (A)

A solution of 1,2-Phenylenediamine (0.01mole, 1.1 g) in (25ml) ethanol, a solution Butane-2,3-dione monoxime (0.01mole, 1 g) in ethanol (25ml) was added , and 5-6 drops of conc. HCl was added to this mixture and refluxed for (4 h). cold and the crystals obtained were separated by filtration and recrystallized from redistilled ethyl alcohol.

2.3.1- Synthesis of the ligand (L)

To a solution of 2-mercaptobenzothiazole (0.01mole, 1.7 g) in (25ml)ethanol, a solution of compound(A) (0.01mole, 1.9 g) in ethanol (25ml) was added. The mixture was refluxed for (4 h). The volume of the solvent was reduced under vacuum to the half of the original volume. On cooling, filtered off and washed with ethanol and recrystallized from absolute ethanol .

[2-4] Synthesis of the Complexes

The ligand (L) (0.001mol , 0.3gm) was dissolved in 20 ml ethanol in a 100 ml round-bottom flask. A solution of (0.001mol) of the metal chloride Cu(II) , Cd(II) and Hg(II), respectively in 20 ml ethanol was added dropwise , with continuous stirring at room temperature. Reflux for 1hr . The resulting precipitates were filtered off, washed with ethanol , dried and recrystallized from ethanol .

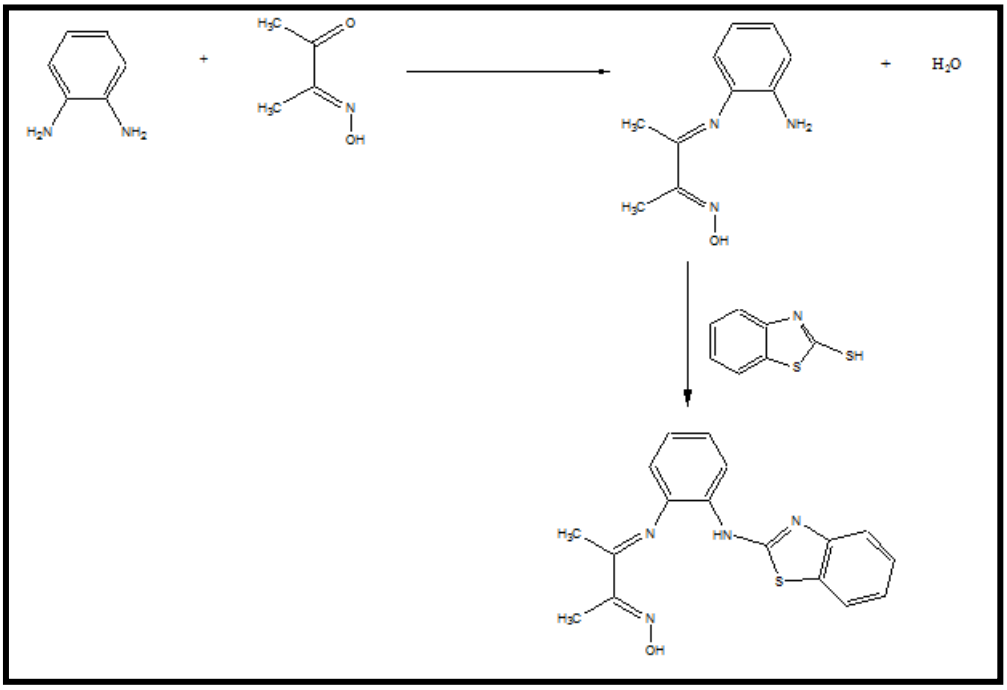


Figure (2-1) Preparation of the ligand

[2-5] Biological Activity

2.5.1 – Preparation of Agricultural Medium

Agricultural Medium was prepared, according to the providing company, by adding (28 gm) dry Nutrient agar to (1L) distilled water in volumetric flask with the adjustment of the (pH=7.4), then mixed and dissolved by heating, then the nozzle of volumetric flask was closed by cotton and sterilized with autoclave in 121°C for 20 minutes ; after that cooling, to the medium temperature of (45^{o}C), and poured into Petri dishes about (25ml)for a dish then left to cool and solidifies so that the medium became ready to transplant bacteria.

2.5.2 – Preparation of Solutions

The solutions of ligands and metal complexes under study were prepared with concentration (250 ppm) in ethanol as solvent.

2.5.3 – Treatment Method

The bacteria was spread out in the dishes and on the surface of Nutrient agar . In the Petri dish holes were put in a diameter (5mm) after hardening the medium by using a cork - borer, about 4 holes in dishes, taken (5ml) from these solutions on the dishes (inside the holes) then, these dishes were kept in the refrigerator for (4hr) to ensure better spread in the medium.

The Petri dishes were put in the incubator for (24hr) at 37 ℃. After that, the inhibition zone was measured for the compounds by the use of the millimeter ruler.

Table (2-2): Physical properties of the synthesized ligand and its complexes

No.	compounds	colour	M.P(^{o}C)	Yield(%)
1	L	Brown	123-125	81
2	[Cu(L)Cl_2]	Green	153-155	72
3	[Cd(L)] Cl_2	Light brown	171-173	67
4	[Hg(L)] Cl_2	Light brown	192-194	81

CHAPTER THREE

RESULTS

&

DISCUSSION

[3-1]Synthesis of the ligand(L)

The ligand (L) was prepared from reaction 1,2-phenylene diamine with dicetylmonoxime , then the product reacted with 2-mercapto benzothiazole by using reflux method .

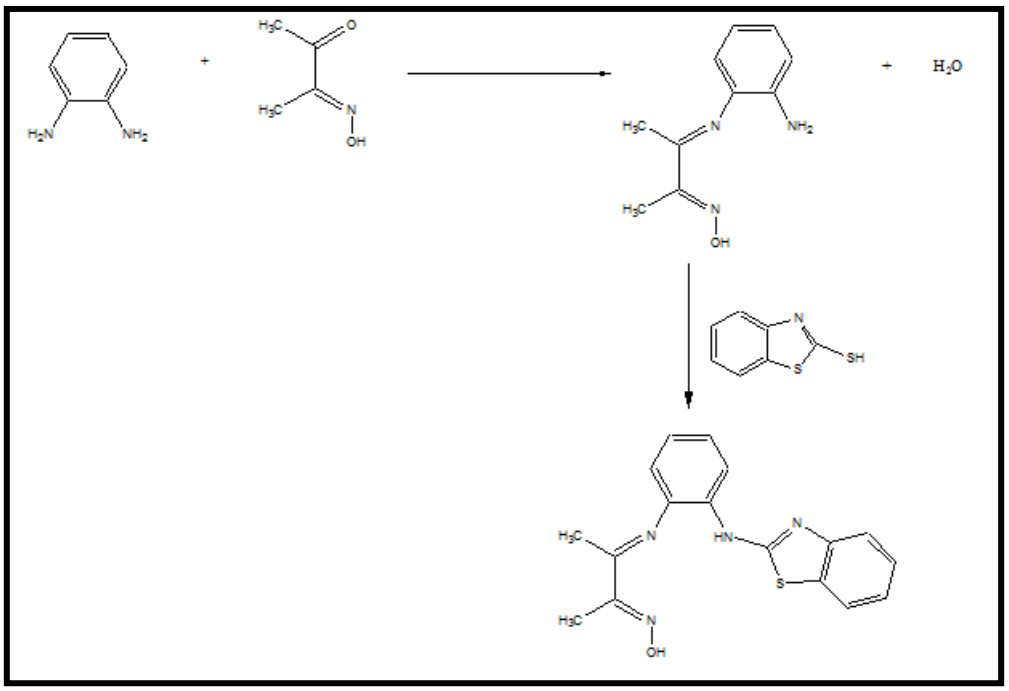


Figure (3–1) The synthesis of the ligand (L)

[3-2] The Infra-red Spectra of the ligand (L) and its complexes[17-19]

The FTIR spectrum of the free ligand (L), Figure (3-2) showed two bands at (3362 , 3184) cm^{-1} assigned for υ(O-H) and υ(N-H) groups, respectively, and the bands at (1682 and 1654) cm^{-1} , which due to υ(C=N) of the azomethine group for imine –oxime and imine group of benzothiazole ring , respectively. . The bands at (1578) and (3098)cm^{-1} were assigned to υ(C=C) and υ(C–H) aromatic, respectively. Also the spectrum shows band at (1032) cm^{-1} attributed to the υ(C-S).

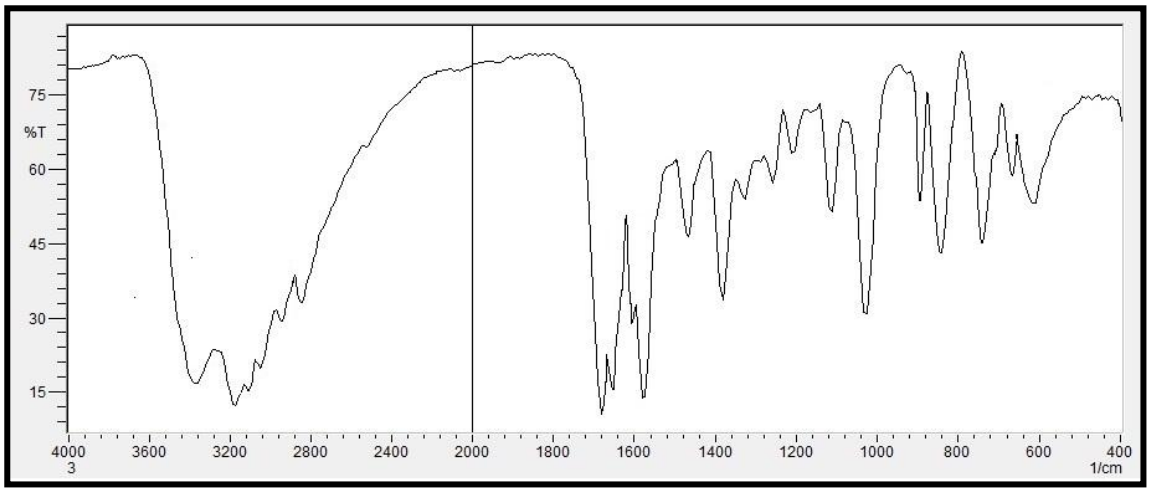
The band at (3184) cm^{-1}, due to the secondary amine group in the free ligand, was shifted to a lower wave number after complexation in the range (3163-3142)cm^{-1} for Cu(II), Cd(II)and Hg(II) complexes respectively , the shifted toward lower wave number due to coordinate nitrogen atom of amine group with metal ion . Also, the

band υ(C=N) of the azomethine group for imine –oxime and imine group of benzothiazole ring at(1682 and 1654) cm^{-1} , which shifted toward lower wavenumber at the range (1675-1602) cm^{-1}, this shifting indicated to occur coordination between metal ion and nitrogen atom of azomethine group.

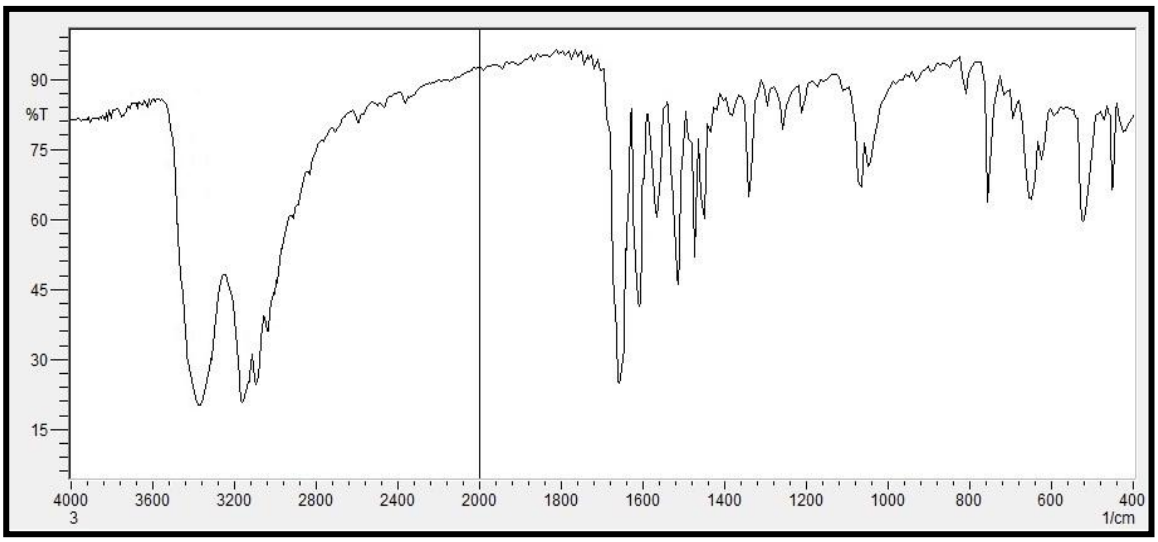
The new bands at the region (438-521)cm^{-1} are due to the υ(M–N) for Cu(II), Cd(II)and Hg(II) complexes, respectively . The FTIR data for free ligand and its complexes are shown in Table (3-2) .

Table (3-1): The infrared spectral bands for the ligand (L) and its synthesized complexes

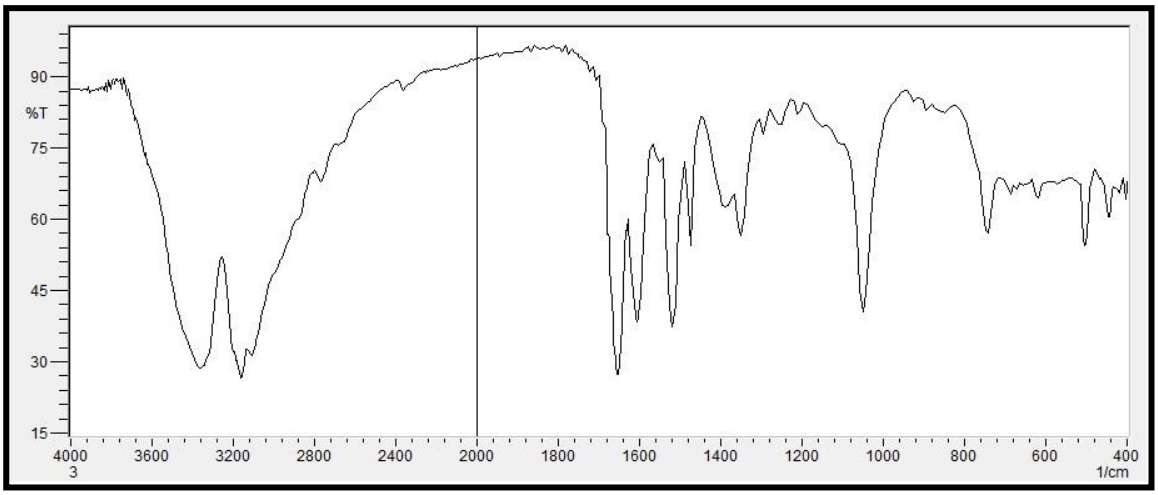
Symbol of compound	υ(O-H) υ(N-H)	υ(C=N)	υ(M–N)
L	3362 3184	1682 1654	-
$[Cu(L)Cl_2]$	3361 3142	1661 1612	442 521
$[Cd(L)]\ Cl_2$	3360 3163	1658 1602	441 502
$[Hg(L)]\ Cl_2$	3360 3148	1675 1641	438 475



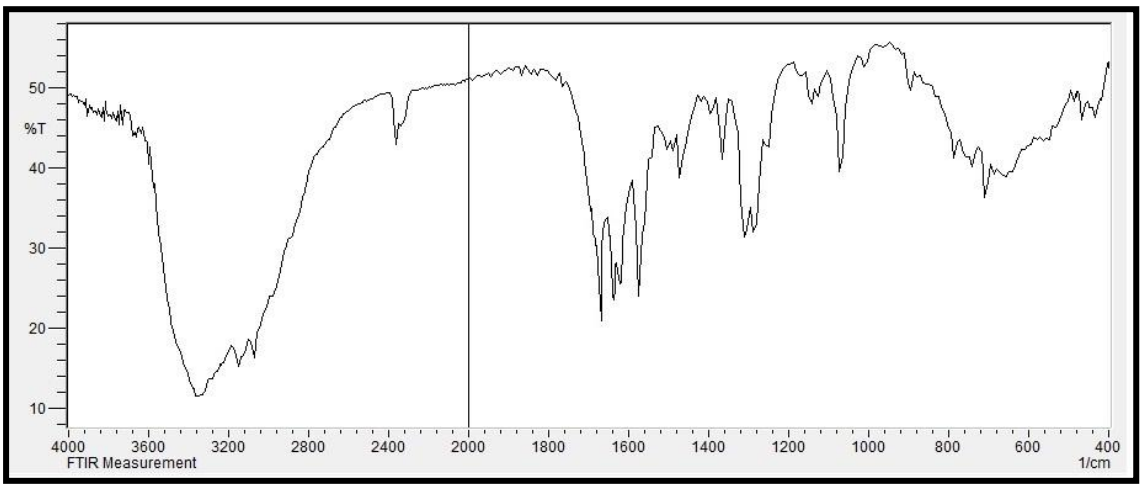
Figure(3-2): The FTIR spectrum of the ligand (L)



Figure(3-3): The FTIR spectrum of the complex [Cu(L)Cl_2]



Figure(3-4): The FTIR spectrum of the complex [Cd(L)]Cl_2



Figure(3-5): The FTIR spectrum of the complex [Hg(L)]Cl_2

[3-3] The Electronic Spectra[20-22]

The most coordination complexes are colored; their colures are different from one transition metal to another, and from ligand to other. This is an important indication to the occurrence of coordination. The difference of colour exhibit various absorption bands in intensity and in position, which is another indication for the coordination.

The electronic (d-d) transitions are due to metal ion occur at the region (380-1000 nm) and the electronic absorption is due to ligand in the ultra-violet at (200-380)nm .

The electronic spectrum of the ligand (L), Figure (3-6) , showed two peaks at (234 , 321) nm which are due to π-π* and n-π* transitions , respectively.

The electronic spectra of [Cu(II) , Cd(II) and Hg(II)] complexes, showed peaks at (334-405) nm , which due to charge transfer .

The complex of Cu(II), exhibited peak at (623) nm in the spectrum. (d-d) transition . The UV-Vis data for free ligand and its complexes are shown in Table (3-3) .

Table (3-2): The UV-Vis spectral peaks for the ligand (L) and its synthesized complexes

Symbol of compound	λ_{max}(nm)	transitions
L	234 321	π-π* n-π*
$[Cu(L)Cl_2]$	334 623	Charge transfer d-d transition
$[Cd(L)]\ Cl_2$	381	Charge transfer
$[Hg(L)]\ Cl_2$	405	Charge transfer

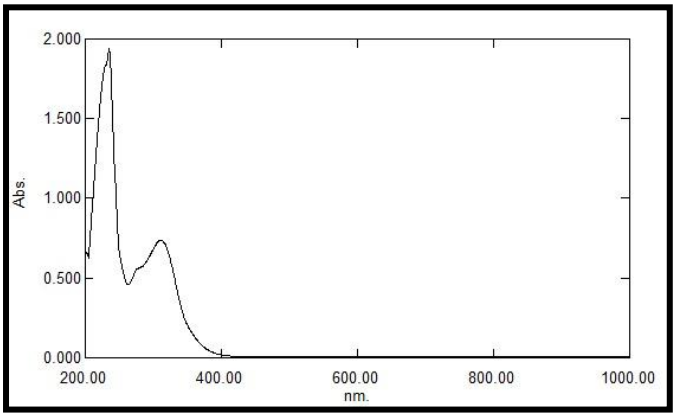


Figure (3-6) : The UV-Vis. spectrum of the ligand(L)

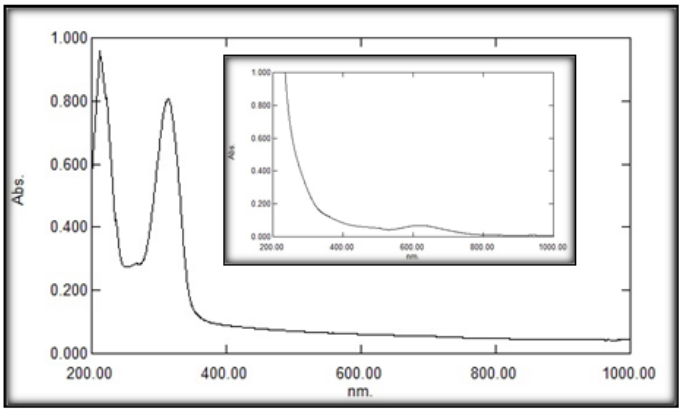


Figure (3-7) : The UV-Vis. spectrum of the complex [Cu(L)Cl]Cl

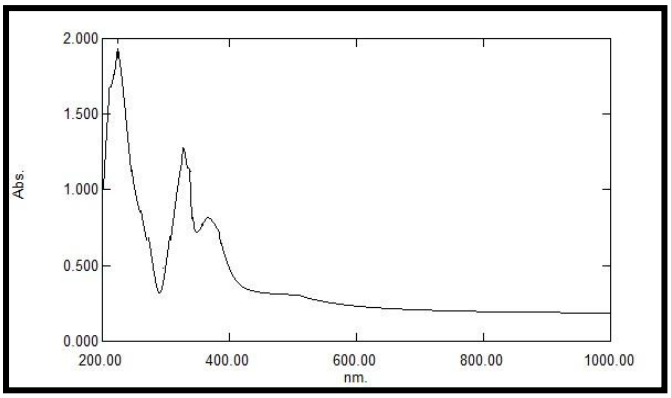


Figure (3-8) : The UV-Vis. spectrum of the complex [Cd(L)]Cl_2

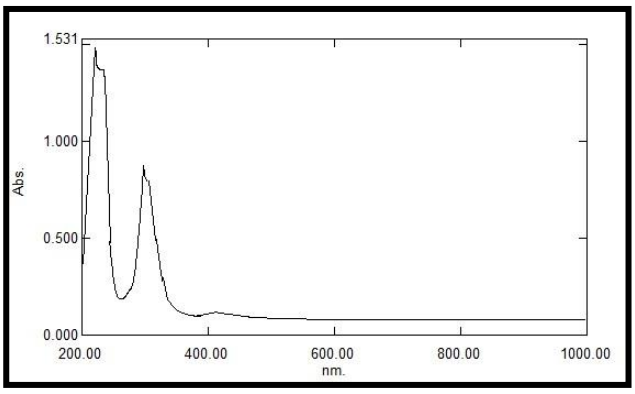


Figure (3-9) : The UV-Vis. spectrum of the complex [Hg(L)]Cl_2

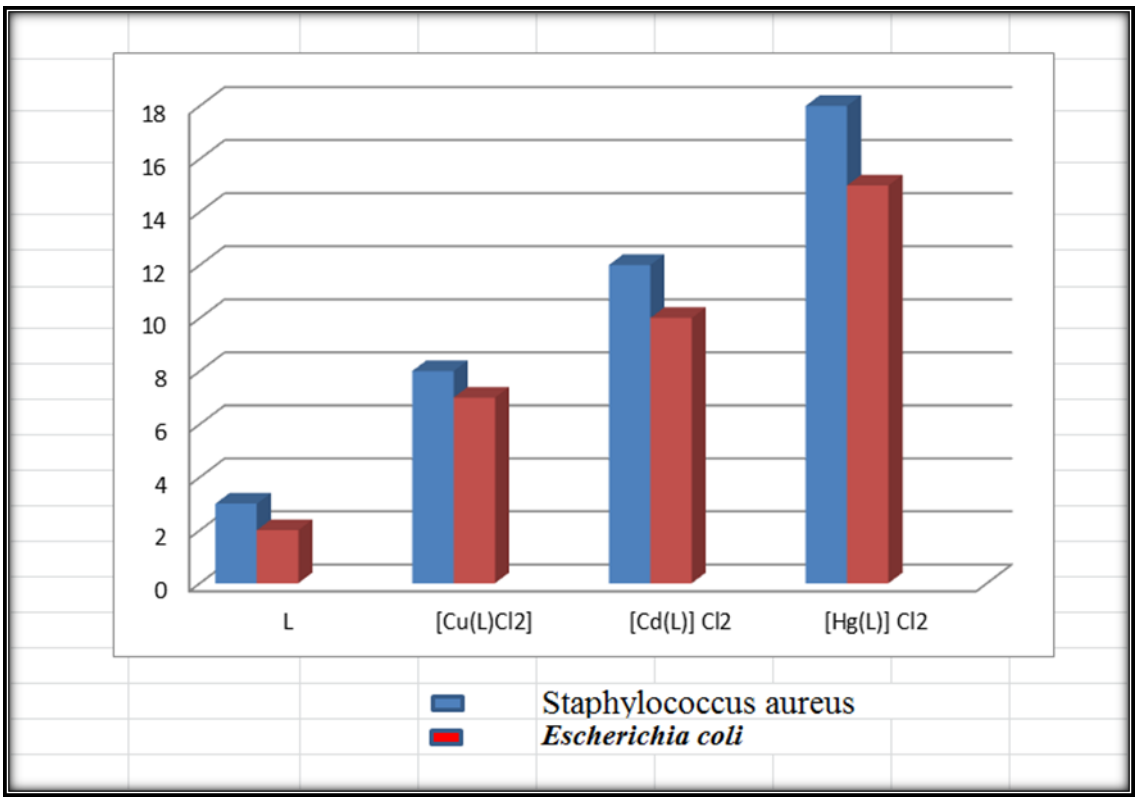
[3-4] Biological activity for the synthesized ligand and its complexes[23,24]

The biological activity of the synthesized ligand and its complexes were studied against selected types of bacteria which include [*Staphylococcus aureus* as gram positive] and [*Escherichia Coli* as gram negative] .They were cultivated in Nutrient agar medium in concentration (250ppm) by plate agar method to determine the inhibitory effect for these ligands and their complexes on the growth of these bacteria.

Table (3-3) showed the results of activity for the ligand and its complexes which were studied against bacteria. Most of the prepared complexes were observed to have ability to inhibition and to possibility of existence of two groups active in ligands structures ((=N-C-S and azomethine) at the same time .

Table(3-3):-Biological activity data (zone of inhibition in mm) of ligand (L)and its metal complexes

Compound	***Zone of Inhibition***	
	Staphylococcus aureus	***Escherichia coli***
L	3	2
$[Cu(L)Cl_2]$	8	7
$[Cd(L)]\ Cl_2$	12	10
$[Hg(L)]\ Cl_2$	18	15



[3-5] Conclusions

According to the results, the ligand (L) behaves as tetradentate ligand. The ligand (L) coordinates with metals ions through nitrogen atom of azomethine group in the complexes of Cu(II), Cd(II) and Hg(II).

The prepared ligands showed the ability to inhibition of growth toward *Staphylococcus aureus* and *Escherichia Coli*. at prepared concentration.

Most of the complexes prepared effectiveneces against both types of bacteria and varying degree, where the complex of Hg(II) with the ligand showed high activity toward types of these bacteria.

[3-6] Recommendations

1. Preparation of new ligands derivatives from 2-mercaptobenzothiazol.
2. Preparation of new complexes for these ligands with metal ions of second and third series, also, metal ions of lanthanides and actinides.

3. Characterization these ligands and their complexes by the elemental analysis, Infrared and electronic spectra, magnetic susceptibility measurements, flame atomic absorption spectroscopy and molar conductivity.

4.Evalution of biological activity for all ligand and their complexes.

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