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Ministry of Higher Education
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AL-Nahrain University
College of Science
Department of Chemistry

Preparation of some Schiff bases as Urease inhibitors and study their effect on *Proteus mirabilis* bacteria

A Thesis

Submitted to the College of Science at Al-Nahrain University as a Partial Fulfillment of the requirements for the Degree of Master of Science in Chemistry (Biochemistry)

BY

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وزارة التعليم العالي و البحث العلمي
جامعة النهرين/كلية العلوم
قسم الكيمياء

تحضير بعض قواعد شف كمثبطات لانزيم اليوريز ودراسة تأثيرها على بكتيريا المتقلبات *Proteus M.*

أطروحة مقدمة إلى
كلية العلوم / جامعة النهرين
وهي جزء من متطلبات نيل درجة ماجستير علوم
في الكيمياء (الكيمياء الحياتية)

من قِبـــــــل
احمد حسين علي التميمي
بكالوريوس علوم كيمياء (جامعة النهرين 2006)

ربيع الأول 1431هـ أذار 2010 م

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وَقُلْ رَبِّ زِدْنِي عِلْمًا

صَدَقَ اللَّهُ الْعَظِيمُ

سورة طه
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الإهداء

إلى الذي وهب لي عمره و عطفه
وكان لي أباً و صديقاً...

أبي...

إلى من حملتني وهنا على وهن
وسهرت الليالي على راحتي...

أمي...

إلى من اشد بهم أزري في الحياة...

أخوتي الأعزاء...

إلى رفاق الدرب الطويل...

أصدقائي الأوفياء...

إليكم جميعاً اهدي هذا الجهد المتواضع

احمد / 2010

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Ahmed
2010

Supervisors Certification

We certify that this thesis was prepared by the graduate student **Ahmed Hussein Ali Al-Timemy** under our supervision at (Al-Nahrain University) as partial requirements for the degree of **Master of Science in Chemistry (Biochemistry).**

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Title: Assistant Professor
Date: / /2010

In view of the available recommendations, I forward thesis for debate by the examining committee.

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Committee Certification

We certify that we have read this thesis and as examination committee examined the students in its content and that in our opinion it is adequate with ( ) standing as a thesis for the degree of **Master of Science in Chemistry (Biochemistry).**

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Title: **Assistant Professor**
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Symbols and Abbreviations

Abs.	Absorbance
µg	Microgram
Aliph.	Aliphatic
Arom.	Aromatic
Conc.	concentration
D.W.	Distilled Water
F.T.I.R.	Fourier Transform Infra Red
G.F.R.	Glomerular Filtration Rate
G.U.E.	General Urine Exam
G-6-P-DH	Glucose-6-Phosphate-dehydrogenase
gm	gram
H.K.	Hexokinase
hr.	hour
I	Intermediate
Ibs.	Libras(pound/ $Inch^2$)
L	Liter
LDC	Lysine decarboxylase
LPS	Lipopolysaccharide
M	Molarity
M.H.A.	Mueller Hinton agar
Max.	Maximum
mg	milligram
min.	minutes
Min.	Minimum
mL	milliliter
mm.	Millimeter

MIC	Minimum Inhibition Concentration
mRNA	messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogenase
No.	number
ODC	Orinthine decarboxylase
ONPG	Orthonitrophenol galactoside
P	probability
P.	*Proteus*
PABA	Para-Amino Benzoic Acid
PMX	Polymyxine
ppt.	precipitate
p-sub.	Para-substitution
R	Resistant
R.B.S.	Random Blood Sugar
r.p.m.	round per minute
RBC	Red Blood Cell
RNA	Ribonucleic acid
S	Sensitivity
S.Cr.	Serum Creatinine
S.D.	Standard Deviation
sol.	Solution
sps.	Species
ß	Beta
T.L.C.	Thin Layer Chromatography
TDA	Tryptophane deaminase
tRNA	transfer ribonucleic acid
U.A.	Uric Acid
U.T.I.	Urinary Tract Infection
V	Volume
W.H.O.	World Health Organization

Summary

The current study involves preparation number of cysteine derivatives by the reaction of cysteine with different aromatic aldehydes forming the following Schiff bases:

"2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid, 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid, 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid and 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid".

The new prepared derivatives were identified by using spectroscopy (FTIR) and measuring their melting points.

The biological activities against *Proteus mirabilis* were estimated using well-diffusion method around the plate then the antibacterial activities were estimated.

The effects of the prepared compounds on urease activity were studied. The results showed good effect in urease inhibition. All the prepared compounds showed competitive inhibition since the value V_{max} was the same and Km increased.

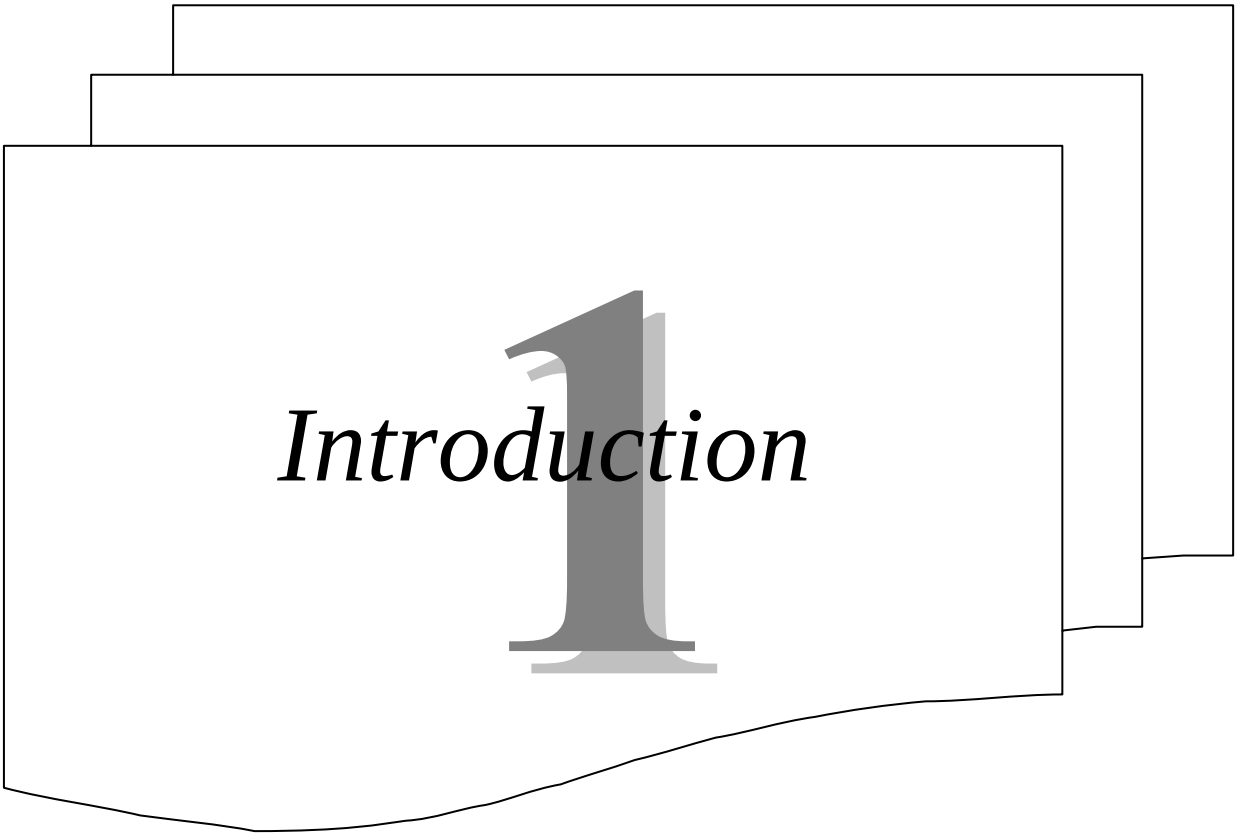
Finaly, V_{max}, K_i, K_m and i % values of the enzyme was calculated using line weaver Bruk equation.

It is noticed from enzymatic and biological studies that Schiff bases compounds are strong inhibitors of the urease enzyme where

the(2-(4-(dimethylamino)benzylideneamino)--3mercaptopropanoic acid) inhibition force was (Ki=0.0043 M) which is the highest inhibition force comparing with the other prepared compounds. The percentage of inhibition (%i) was equal to (96.77%).

Contents

Chapter One



1.1 Introduction

Urinary tract infections (U.T.Is) represent one of the most frequently registered cases of malady daily in the Iraqi hospitals. It is as such in other countries all over the world. These infections are caused by different pathogens like viruses, chlamydia and bacteria. *Proteus mirabilis* stands as the important bacteria that cause (U.T.I). But it can be one of the opportunistic pathogens when it is carried to the urinary tract to cause U.T.I. which is considered one of the leading causes for renal stone formations [1, 2].

1.2 Urease (urea amido hydrolase):

Urease (urea amido hydrolase, EC3.5.1.5) catalyzes the hydrolysis of urea into ammonia and carbamate. Carbamate spontaneously decomposes to yield ammonia and carbonic acid. It has been shown that jack bean urease, which typifies the plant ureases, is a nickel-containing homooligomeric enzyme exhibiting a high degree of specificity to urea [1, 2]. Bacterial ureases, such as the *Klebsiella aerogenes* enzyme whose stereo structure has recently been determined, are also nickel-containing enzymes but their quaternary structures are distinct from that of the jack bean enzyme [3 - 5]; most known bacterial and plant ureases show an optimum pH for enzyme activity at neutral and slightly alkaline conditions as shown in the figure (1-1) [6].

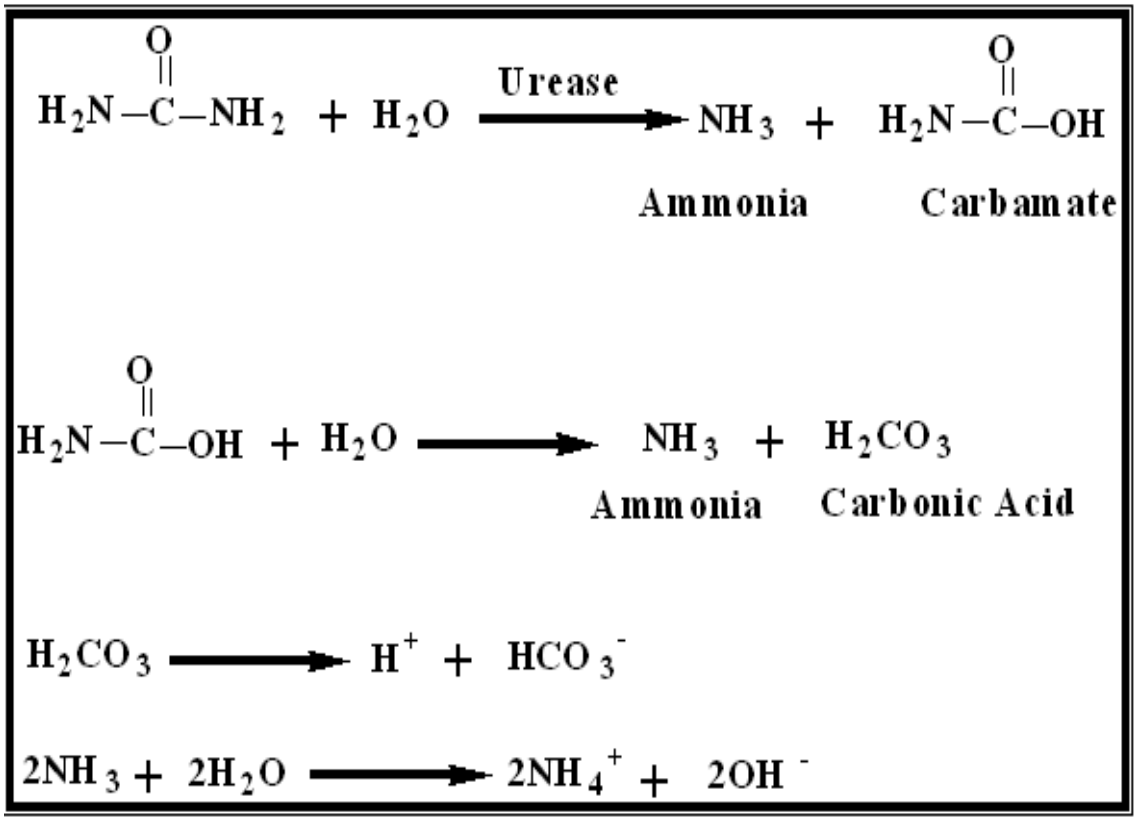


Figure (1-1): Equation of urea hydrolysis to evolve ammonia and carbamate molecule and carbonic acid [6]

After equilibrium between ammonia and water occurs which results in ammonium hydroxide that rapidly increases pH [2, 7 - 9]. Musculus (1876) is considered to be the first that studied the bacterial Urease after that Miquel (1890) diagnosed the production of Urease in many microorganisims including bacteria [5, 10-17]. Urease is produced by pathogenic or nonpathogenic bacteria [18, 19]. urease is very important as it causes disease and its considered a virulence factor in many bacteria , it has been shown the production of such enzyme in about (100) types of bacteria [8].

As the infection with *proteus, pseudomonas, staphylococcus, corynibacterium, morganella, micrococcus and klebsiella* cause urinary tract infection (UTI) and urolithiasis.

All these bacteria produce the enzyme that has principal role in urolithiasis by increasing the pH from 5 to 9 causing the mineral salts to precipitate in mucous material [20] which is produced by the bacteria and enters in its cellular structures and acts as nevus around which salts are precipitated to form stones. It also has been found that bacterial cells inside renal stones in patients treated with antibiotics [3]

1.3 Cellular localization of Urease:

Urease is extracellular enzyme [21, 22] and most studies show that urease concentrates in yeasts and cytoplasmic parts of most bacterial strains, [2, 8, and 23] as enzyme is detected in the cytoplasm of 22 bacteria [24]. Electronic microscope shows the conjugation of the inner membrane *Staphylococcus* and in the periplasm and outer membrane of *Proteus mirabilis* [2] , while *Helicobacter pylori* is the only bacteria that has enzyme in cytoplasm and on cell membrane ; because of the ability of the bacterial outer membrane to adsorb the enzyme that is released from adjacent cell autolysis [25].

1.4 Environmental Urease:

Urease activity is widely distributed in soil and aquatic environments, where it plays an essential role in nitrogen metabolism [26]. For example, degradative processes involving protein and nucleotide turnover require urease activity.

More importantly, effective urea fertilization requires controlled ureolysis to enhance efficiency and minimize crop damage.

1.5 Urease regulation of bacteria:

Urease is either induced or constitutive according to bacteria and depends on environmental factors; some studies pointed to that enzyme production is associated with nitrogen balance.

Ammonia or nitrogen rich compounds like urea inhibit its production and it is inhibited when nitrogen quantity in environment is limited [2, 6, 27].

There are environmental factors that affect the urease production like pH, urea concentration, glucose or ammonia initiated in *Providencia* and *Proteus* while *Klebsiella* regulates enzyme production in response to nitrogenous compound like ammonia, glutamine, and lysine [8].

In *Streptococcus salivarius*, enzyme production is regulated by surrounding pH; as low pH of this bacteria produce urease to increase pH of the media [28].

In *Bordetella bronchioseptica* urease production is regulated thermally [28]. While urease production in *Pseudomonas aeruginosa* is regulated by the nitrogen regulation from ammonia and the following enzymes:
Glutamine synthetase, NADP-dependent Glutamate dehydrogenase and glutamate synthetase [29 , 30]. As bacteria prefers nitrogen metabolism to ammonia with glutamine or glutamate according to the following equations in figure (1-2): [24]

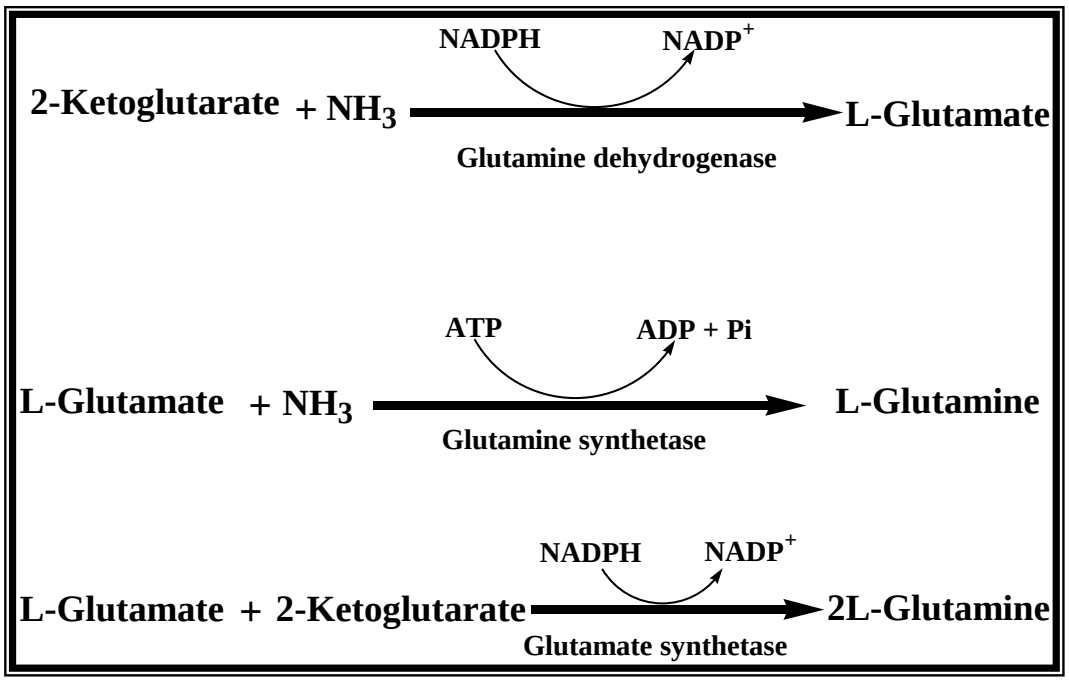


Figure (1-2): Nitrogen metabolism to glutamine or glutamate

Other studies show that control system of nitrogen in bacteria is controlled by a complicated sequence which initiates the production of RNA polymerase which identifies promoters concerning with nitrogen control gene [(2)].

1.6 Urease mechanism:[(31)]

Historically the earliest Ni-containing enzyme to be described was urease from jack bean meal, which was crystallized by James Sumner in 1926. However, analytical techniques did not allow urease to be recognized as a Ni-containing enzyme until 50 years later.

Urease plays a key role in nitrogen metabolism in plants and microbes whereas land dwelling animals excrete urea as the end product of their nitrogen metabolism; clearly, they do not produce urease. The active site (Figure 1-3) contains two Ni ions, ~3.5 A apart which are

bridged by a carbamylated lysine residue. Both Ni ions are coordinated by two His nitrogen atoms, oxygen from the bridging carbamyl group and oxygen from bound water.

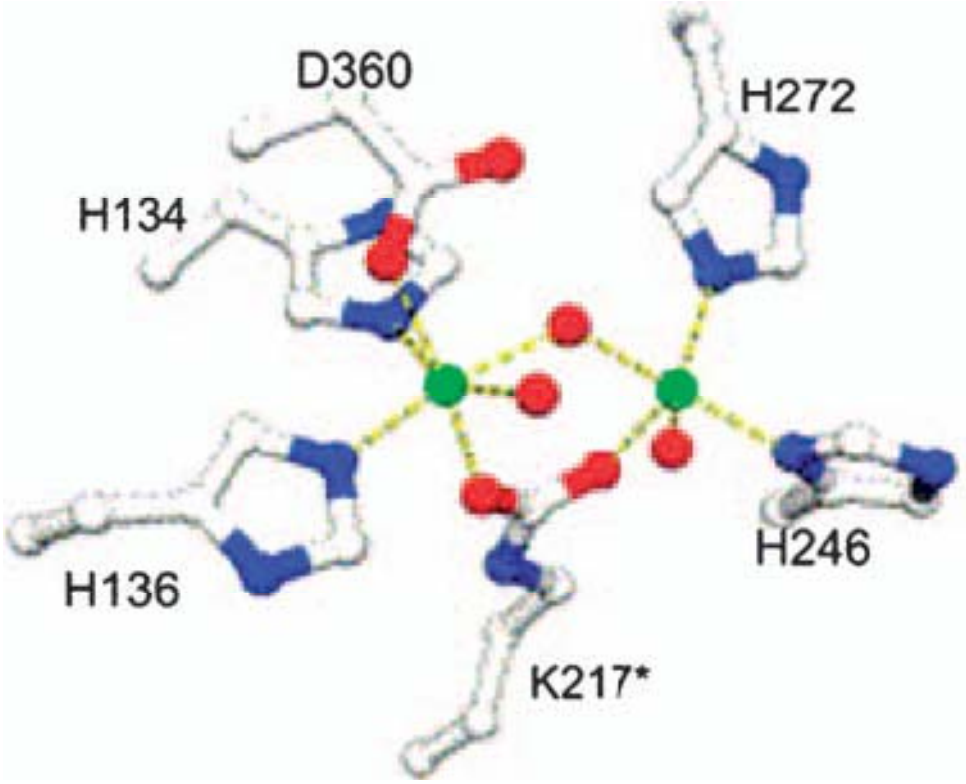


Figure (1-3): Dinuclear Ni active site of urease. Ni atoms are shown in green, metal-bound waters as red spheres; the carbamylated Lys is K217*.

One of the Ni atoms in addition has an oxygen ligand from an Asp residue. CO2 is required for formation of the carbamylated Lys bridge between the two Ni atoms, and mutation of this Lys results in loss of activity. A proposed reaction mechanism is presented in Figure (1-4).

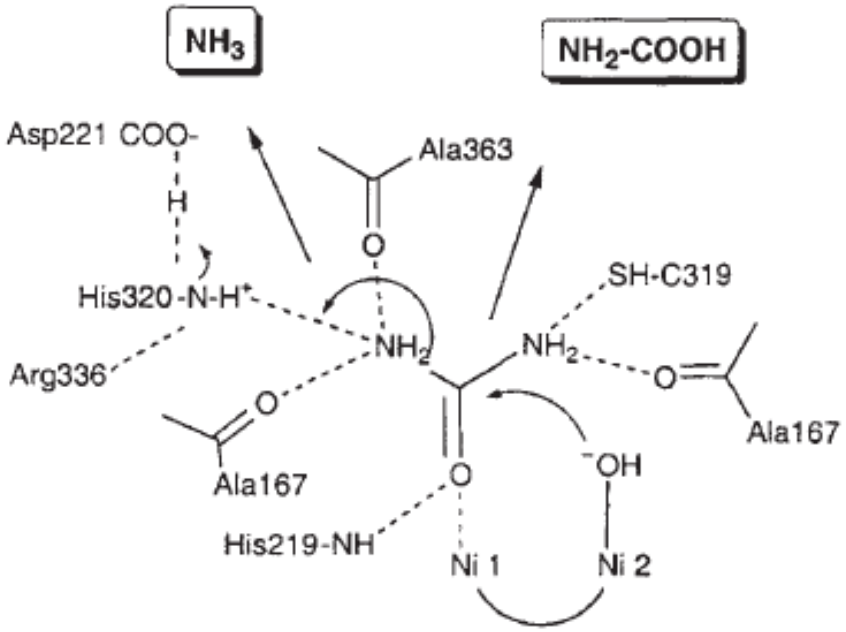


Figure (1-4): Reaction mechanism of urease [31]. Ni 1 binds urea and acts as a Lewis acid to polarise the carbonyl group, making its carbon more electrophilic, while Ni2 facilitates deprotonation of a bound water molecule to generate a nucleophilic hydroxyl species.

The large kinetic barrier to urea hydrolysis is presumed to be lowered by:

(i) Coordination of the carbonyl group of urea to Ni 1, making the carbonyl more electrophilic,

(ii) Binding of water to Ni2 to generate an activated hydroxyl species and

(iii) Hydrogen bonding interactions of all four of the protons of urea with electrophilic groups of the protein. Attack of the metal-activated hydroxyl would generate a tetrahedral intermediate. Protonation of this intermediate would eliminate ammonia, leaving carbamate bridged between the two Ni atoms. Dissociation of carbamate from the dimetallic site would be followed by spontaneous hydrolysis to carbonate and a

second molecule of ammonia, with a protonated His residue acting as a general acid to promote ammonia release.

1.7 Urea and Ammonium Ion Transport:

Measurement of whole-cell urease activity involves four processes: entry of urea into the cell, hydrolysis, exit of ammonia, and quantitation of the released ammonia or measurement of the pH increase as illustrated in figure (1-5). Membranes are often assumed to be freely permeable to urea and ammonia in these assays; however, this assumption is invalid. For example, urease activity measured in cell extracts greatly exceeded the activity of intact cells for *Bacillus pasteltuii* [32 - 34] and *Providetnia rettgeri* [35]. Ureolytic rates for several other microorganisms [36] appeared to be unaffected by the state of the cell; however, only very high (83 mM) levels of urea were examined. We find that ureolytic rates for intact microorganisms are generally inaccurate at low urea concentrations. The lack of free diffusion for substrate and product means that kinetic values for urease can only be obtained for permeabilized cells. In addition to sonication or other disruptive methods, permeabilization of cells can also be achieved by addition of detergents such as 0.1% hexadecyltrimethylammonium bromide [33, 37].

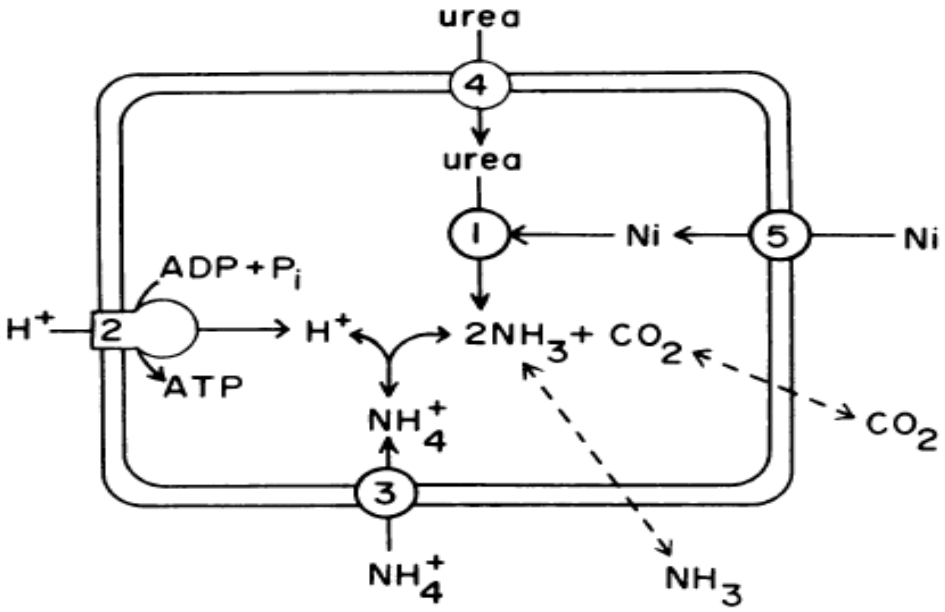


Figure (1-5): Transport processes related to microbial ureolysis [32].

Urease (protein 1) is a cytoplasmic protein; nevertheless, several membrane-bound proteins may be indirectly associated with urea degradation in certain microorganisms [38, 39]. In the proposed model, ureolysis generates at net rise in intracellular pH and the resulting proton gradient is used to derive a proton-dependent ATP synthase (protein 2). It is unclear how ammonium ion is removed from the cell in such a model. Ammonium ion transport systems (protein 3) are known to occur in numerous microbes [40], however, these transporter's function in uptake of ammonium ion and are energy dependent. Ammonia and carbon dioxide can readily diffuse through the cell membrane, and no transporters are present for these molecules. In contrast, energy dependent urea transporters (protein 4) have been found in several microorganisms [41 - 45] even though urea is able to diffuse through membranes. Finally, energy-dependent nickel transport (protein 5), often via the magnesium transporter, is necessary to generate active, nickel-containing urease [46]. ADP. Adenosine diphosphate; Pi, inorganic phosphate.

1.8 Enzyme specificity:

This term is used to express the specificity of enzyme to stimulate one reaction or a limited number of reactions [47]. These enzymes are considered as catalyst with high specificity, there are great differences in specificity of different enzymes that divided enzymes into:

1. High specificity which is specific for one material.

2. Low specificity that is used for more than one substrate.

There is another category towards divisions [48].

(a) Low specificity: And also called (Bond specificity) as enzymes (peptidase, phosphatase, and esterasase) make these enzymes on long run as the basic substances that is supplied with chemical bond like: peptide bond, phosphate bond and carboxylate ester bond respectively.

(b) Group specificity: Like hexokinase which stimulates phosphorylation of many sugars.

(c) Absolute or near absolute specificity: Group of enzymes that stimulate reactions towards one substance or double of substances like urease that stimulates one direction reaction towards urea and towards similar analogue in a slow reaction.

Urease enzyme, that is produced from plants, bacteria and yeasts, is a good example of high specific enzyme towards urea [49].

Despite the trials done to find urea substitutes as a basic substance but there was no success as it was found that enzyme hydrolyses urea but inactive with methyl urea [50].

1.9 Urease Importances for Different Organisms:

1.9.1 Animals:

Microbial ureases play an important role in the nitrogen metabolism of ruminants such as cattle, sheep, and other animals that contain a forestomach [51]. Substantial amounts of animal-derived urea are recycled to the rumen, where ureolytic activity releases ammonia, the major source of nitrogen for most ruminal bacteria [52]. The microbial biomass generated is then utilized as a nutriment by the ruminant.

The concept of a ruminant nitrogen cycle is illustrated in figure (1- 6). Exogenous urea can be used to supplement nitrogen deficient feedstocks to enhance the quality of the ruminant diet. Urea hydrolysis also occurs in the intestinal tract of monogastric species such as humans, pigs, rats, cats, mice, and rabbits [53], but nitrogen cycling is quantitatively less important in these organisms compared with ruminants.

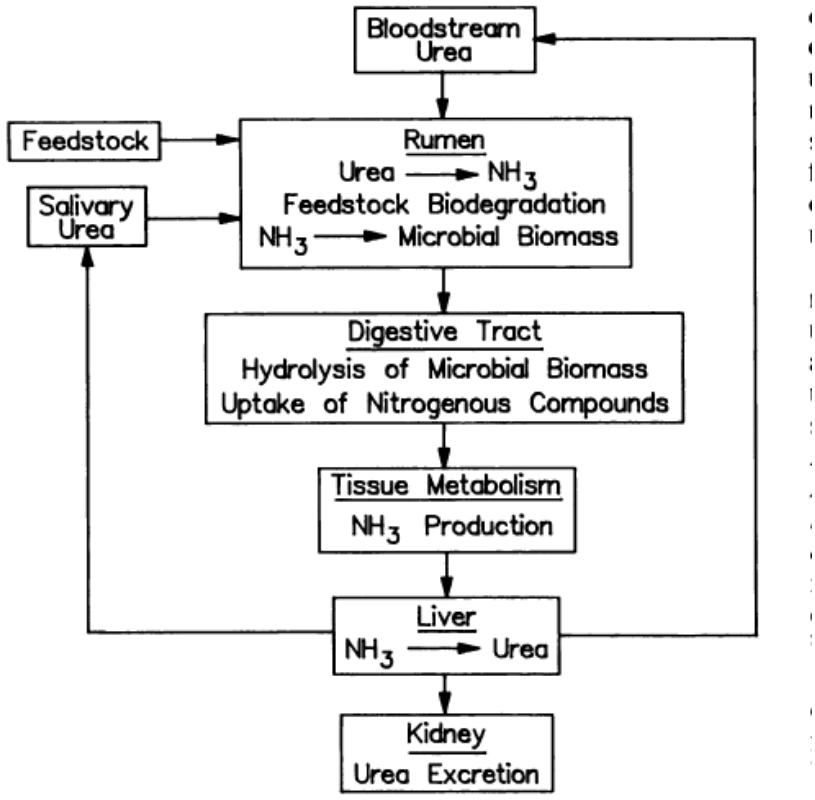


Figure (1-6): Nitrogen cycling system in ruminants [53].

A significant proportion of the nitrogen requirement of ruminal microorganisms is met by urea, which diffuses into the rumen from the bloodstream or enters with saliva. Urea is hydrolyzed by microbial ureases, and the product ammonia is taken up by the ruminal microorganisms.

Microbial biomass is subsequently degraded by the ruminant digestive system to supply nitrogen-containing compounds to the animal. Tissue metabolism releases ammonia, which is transformed into urea by the liver.

1.9.2 Microorganisms:

Urease enzyme is considered as virulence factor for one kind of bacteria [10, 54, 55].Many studies show that urease enzyme plays a role in alimentary canal and pathogenesis (that enables the bacteria in acidic media) causing dangerous diseases like stomach cancer[56 - 58].

Proteus mirabilis is considered as coccobacilli that causes urinary tract infection and these bacteria have many virulence factors that aid in causing infections like flagella, urease, LPS in addition to protease enzyme that play important role in bacteria. [59, 60]

1.10 Schiff bases:

Schiff bases derived from aromatic amines and aromatic aldehydes have a wide variety of applications in many fields, *e.g.*, biological, inorganic and analytical chemistry [61]. Application of many new analytical devices requires the presence of organic reagents as essential compounds of the measuring system. They are used, *e.g.*, in optical and

electrochemical sensors, as well as in various chromatographic methods, to enable detection of enhance selectivity and sensitivity [62].

Among the organic reagents actually used, Schiff bases possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties [63].

Schiff bases are widely applicable in analytical determination, using reactions of condensation of primary amines and carbonyl compounds in which the azomethine bond is formed (determination of compounds with an amino or carbonyl group); using complex formation reactions (determination of amines, carbonyl compounds and metal ions); or solvent (pH of solvent polarity indicators).

Unfortunately, most Schiff bases are chemically unstable and show a tendency to be involved in various equilibria, like tautomeric inter conversions, hydrolysis, or formation of ionized species [64]. Therefore, successful application of Schiff bases requires a careful study of their characteristics.

1.11 *Proteus* Bacteria:

1.11.1 History:

Proteus is a Greek word that Hauser (1885) called the bacteria he discovered and means "continuous changing shape" for its ability to move fast and change the colony shape. He mentioned two strains of these bacteria: *mirabilis, vulgaris.* He described colony of these bacteria as circular colony that change to irregular colony with amoeba like extension [65]. These bacteria are present widely in nature and play a very important role in organic substances degradation and were isolated from soil, water, home [66]. The physiological properties of these bacteria were

studied and differentiate the possibilities between these bacteria to use and ferment maltose where one strain can ferment maltose while others cannot. [67]

These bacteria can utilize urea by urease and liberate ammonia and CO2 which elevate pH of the media to basic limit. [68, 69]. This property is specific to *protues* which makes *protues* move fast and in addition to that the sea wave appearance on solid agar or what is called swarming, this can distinguish it from *Morganella* by producing H_2S gas and lipase enzyme in addition to urease production and swarming [70].These bacteria spread among patients in hospital as nosocomial infection leading to difficult treated infection [71] and may be lethal in (15-80%) [72].

As patients with *Proteus* is considered as source of infection to human himself (autoinfection) and others [73, 74].

1.11.2 Clinical Significance of *Proteus*:

Proteus is considered as apportunistic microorganism that belongs to enterobacteriaceae for its effect on health of human and animals [74, 75].as it causes gastrointestinal infection and genitourinary infection and its clinical significance comes after *E.coli*, *Klebsiella pneumoni* as they cause UTI[76,77]. It has been isolated from Urinary tract, wounds, burns, skin, eyes, ears, nose, and larynx and from alimentary tract [78].*Proteus* causes UTI in elderly patients [76]as it constitutes 15 % from bacterial infection of the urinary system, [77,79,80]in addition to other surgical operations[81].*Proteus* also causes nosocomial infections [82]. Before about 50 years, it was clear that ways of prevention of such infections inside hospital will be considered as great difficulty because of emerging resistant-strains of bacteria. [83]. Spread of infection is either endogenous

that is present on the skin, intestine and pulmonary systems from patients in hospital or exogenous by medical staff, tools, like catheterization [84].Catheterization is considered as main source for urinary tract infection [83, 85]. Infection with catheters constitutes about 12% of the urinary tract infection, In addition to other instruments [86].It was shown that catheter carries (2-4) %of the risk of infection and the source is the patients himself, i.e., it's endogenous and shows that *Proteus mirabilis* is the most common kind of infectious organisms [87].

While Chane found that 70.4% of the infection sources are tools and contaminated instruments with *Proteus* [88].*Proteus mirabilis* constitutes about 70-90 % of the infection by Proteus [89].

While *Proteus vulgaris* causes about 25% then other strains constitute (5-8) %, and the urinary system is considered of the most common sites that is infected with bacteria and especially in women as it causes (10-20) % of infection [90].

Urinary system is exposed to many kinds of pathological bacteria that cause autoinfection and nosocomial infection like *E.coli, Klebsiella, Enterobacter,Serratia* species, *Pseudomonas aeruginosa ,Staphylococcus saprophyticus ,S.epidermidis* , Candida ,*Enterococcus faecalis* in addition to *Proteus* species .[91]

Ankler [92,93] shows that urinary system infections caused by *Proteus* occur by the movement of these bacteria upward through its ability to move fast by flagella and this is called (ascending infections), and the infection may be complicated by pyelonephritis. and in some cases these bacteria may spread in the blood stream causing septicemia [91].These bacteria have many virulence factors that increase their percentage of infection; as its presence in urine, rapid growth rate, and fast movement

in addition to many factors that increase the health problems especially in elderly and causes infections, renal stones, bladder stones, renal failure and urinary tract obstruction[2,94].Its thought that *Proteus* has important role in rheumatoid arthritis as antibodies for the bacteria were found in those patients [63,95,96].

1.11.3 Virulence factors: [63]

Proteus bacteria have many virulence factors that cause destruction of hosts due to the ability of secreting many enzymes and toxins in addition to have certain surface structures increasing their virulence. Those factors are:

1. Fimbria and Adherence Ability:

These bacteria can adhere to uroepithelial cells and presence of fimbriae in large numbers on bacterial surface cause severe infection leading to pyelonephritis (inflammation of kidney parenchyma) due to bacteruria [10, 63, and 64].

2. Flagella and Swarming motility: [27, 59]

3. Hemolysin:

Proteus spp. can secrete hemolysin that destructs RBC and is one of factors that help in invasion [58].

4. Outer Membrane Proteins (OMPs) :[74, 75]

5. LPS-O-Antigen endoxine:

This is one of gram negative virulence factor that help in activities of bacterial antibodies present in serum like phagocytes [76].

6. Capsule polysaccharide (CPS):

The capsule of *Proteus* spp. have relation with their pathogenicity because of its ability to collect metallic magnesium ions as a result of the presence of some acids like uric acid, pyruvic acid or phosphate groups that result in urolithiasis [63, 91].

7. Natural resistance to polymyxine:

Proteus spp. resist cyclic antibiotics of (PMX) kind because of connection of positive charge present in their structure with negative charge of lipid A region [68, 69].

8. Invasiveness:

Infection occurs after *Proteus* penetration of the host that cause secretion of a protein helps them to change their shape to small rods with fast movements and multiplication inside host [87, 88].

9. Urease Production:

The *Proteus* spp. bacteria have the ability to increase the pH of urine by secreting of urease that splits urea to ammonia and carbon dioxide that precipitate magnesium ion and calcium ion that are present in urine and then result in urolithiasis (stone formation) [2, 90].

10. Protease production:

Proteus spp. can resist the immune system by production of protease that split peptide chains present in immunological activities that permit the establishment of infection [90].

The urinary tract infection is common in community and hospitals [73] .In most cases infection is chronic and recurrent and 20% in chronic cases infection stops after therapy [74].

Pathogenic bacteria transport to the urinary tract via ascending route from the colon region via urethra and the surrounding tissue to the bladder, its presence in the kidney depends on the virulence factors of the bacteria and host factors. Bacteria might spread via hematogenous route from the blood as in cases of bacteremia [82]. Or lymphatogenous route from lymphatics [82], urinary system is infected with many infections leading to disturbances, some of renal infections (\chronic type) lead to end stage renal disease [83, 84, and 85]. Most diseases that affect the urinary tracts are caused by pathogens, fungi, viruses like pyelonephritis and Tuberculosis (T.B), the kidneys might be affected by metabolic disorders and hereditary nephritis, nephritic syndrome, lipoid nephrosis and congenital malformation and anomalies [86], or the disease occur as mutation in alleles like renal coloboma syndrome while bladder is affected by benign or malignant lesion [87].

1.12 Urinary tract infection (UTI):

U.T.I. is the microbial invasion of any tissue of the urinary tract, extending from the renal cortex to the urethral meatus [92]. About 95 % of the U.T.I. occurs by microorganisms include *E.coli* and other Entrobacteriaceae such as *Proteus* spp., *Staphylococcus sapraphyticus, Enterococci and Candida* and 5 % of the infection occurs via the hematogenous route, and may occur after bacteremia [92].

1.12.1 The risk-factors for U.T.I:

The most important factors on U.T.I., in terms of the location of infection and pathogenicity are (stones, diabetes mellitus, sex, age, pregnancy, catheterization process, sircumsition, renal failure, and congenital malformations of urinary tract [67, 89, 90, 92, 95, and 96]. These factors may facilitate the infection process by *Proteus* spp., and consequently U.T.I [97]. While congenital malformations are considered to be the main causes of urinary tract observation which leads to urinary stasis, and then increases the probability of U.T.I. [87, 88].The formation of stones inside the bladder and kidney is one of the characteristic features of *Proteus* spp. [87] which needs a surgical operation or other medical interventions [89].

1.13 Aim of the work:

This study was aimed to the preparation of cystiene derivative compounds (Schiff bases) which regarded as inhibitors of urease enzyme along with an antimicrobial activity.

Chapter Two

Experimental Part

2.1 Materials:

The following chemical substances are used in this study. They are listed in table (2-1)

Table (2-1): Chemical materials used in the current study

Substance	Supplier / origin
4-Bromobenzyldehyde	**Merck / Germany**
Cysteine	**BDH / England**
4-Chlorobenzyldehyde	**Merck / Germany**
4-Dimethylaminobenzyldehyde	**Merck / Germany**
1, 4-Dioxan	**Fluka / Switzerland**
Glacial acetic acid	**BDH / England**
4-Hydroxybenzyldehyde	**Merck / Germany**

2.2 Instruments:

The following instruments were used in the current study as shown in table (2-2).

Table (2-2): Instruments used in the current study

Instruments	Supplier / origin
Fourier Transform Infrared Spectrophotometer (FTIR)	The infrared spectra were recorded on Shimadzu 8300 Fourier Transform infrared spectrophotometer (FTIR) by using the (KBr) in the wave number range (500-4000)cm-1
Hot plate	Gallenkamp / England
Incubator	Gallenkamp / England
Magnetic Stirrer	Gallenkamp / England
Melting point apparatus	Melting points were measured using hot stage gallenkamp M.F.B 600.01 melting point apparatus was used to measure the melting point of all prepared compound
Oven	Memmert / Germany
Ultraviolet visible spectrophotometer(UV-visible)	The ultraviolet spectra were recorded on a Shimadzu UV-1650.PC UV-visible spectrophotometer
Water Bath	Gallenkamp / England

2.3 Media for the Culture:

The following media were used in the current study as shown in table (2-3).

Table (2-3): Media of the culture

Media	Supplier / origin
1.Nutiernt broth	Difco and oxoid / England
2.Nutierent agar	Oxoid / England

2.4 Isolates of the bacteria:

Proteus mirabilis was studied and the bacterial species was identified and supplied by Institute of Genetic Engineering / Baghdad University.

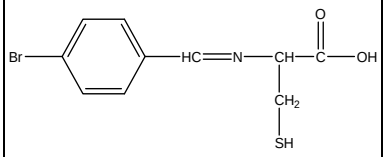
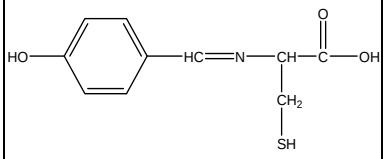
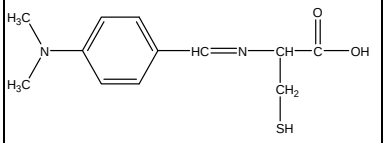
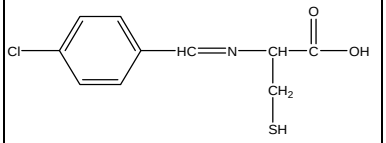
2.5 Preparation of Chemical Compounds (Schiff Bases):

A series of cysteine derivatives were prepared and their biological activities were examined.

Cysteine (0.002 mole, o.3 g) was dissolved in 7.5 ml of absolute ethanol in round bottom flask and (0.002 mole, 0.46 g) of 4-bromobenzyldehyde, (0.002 mole, 0.3 g) 4-hydroxybenzyldehyde, (0.002 mole, 0,3 g) 4-dimethylaminobenzyldehyde and (0.002 mole, 0.28 g) 4-chlorobenzyldehyde dissolved in 7.5 ml of the same solvent with 2 drops of glacial acetic acid then added to the round bottom flask, the mixture was refluxed for (1-2 hours), precipitates of different colors were formed. The product washed with absolute ethanol.

The physical propertites and melting point of the prepared compounds are shown in table (2-4).

Table (2-4): shows the name, structures, weight of prepared compounds, their melting point and color of precipitates.

Name of compounds	Chemical formula	Structure of Compounds	Weight of Schiff bases in (g) of 0.002 mole conc.	M. wt.	Melting point (^{0}C)	Color of resultant precipitate after reaction with absolute ethanol
2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid	$C_{10}H_{10}NO_2SBr$		0.46	288	240 - 242	White
2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid	$C_{10}H_{11}NO_3S$		0.3	225	269 - 271	Pinky
2-(4-(N, N-dimethylamino) benzylideneamino)-3-mercaptopropanoic acid	$C_{12}H_{16}N_2O_2S$		0.3	252	297 - 299	Yellow
2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid	$C_{10}H_{10}N0_2SCl$		0.28	244	249 - 251	Grey

2.6 Bacterial Sensitivity Test of the Prepared Compounds:

Well diffusion method was used to determine the inhibiting power of the prepared compounds against the isolated bacteria. [97]

2.6.1 Well- Diffusion Method:

This method depends upon the variation in concentration of the prepared compounds. A series of concentrations were made from the already prepared compounds with the range from (1, 10, 25 and 50 mg/ml). The following steps are done to measure the biological activity:

1. Nutrient Agar:

Nutrient agar was prepared by dissolving (8 g.) nutrient agar in (250 ml) distilled water and sterilized by autoclaving at 121˚C/ 1.5 atmosphere for 15 minutes. Then it was cooled to (40-45) ˚C. Then put it in plate by about (15-20 ml) nutrient agar for each plate. Then left at room temperature till it became solid. The plates were placed in incubator at 37˚C for 24 hours to ensure that plates are sterilized and get rid of excess humidity. The plates were kept in refrigerator till use.

2. Stimulation of Bacterial isolates:

A touch of colony was taken by a loop to the test tube that contains 5 ml of the sterilized nutrient broth; it was shaken well and incubated in incubator for 24 hours at 37˚C.

3. Bacterial Culture:

Serial dilutions of the stimulated bacteria (by 10 test tubes) were made using normal saline avoid overcrowding. The dilution of 10^{-8} CFU (Colony forming Unit) bacteria was taken indicates low growth. 100 μL of diluted for each plate was added .bacteria were spread; and the plate was rotated by 60 ° angle of each spread done. Plate was left for (15-20) minutes at 37°C to dry.

4. Well Distribution over the Plate

The plates of Mueller Hinton agar was inoculated by *Proteus* bacteria isolated , by taking a swab and put it in bacterial solution isolate and then spread it on a surface of medium by three ways (carpet streaking method) and left for 10-15 minutes . In each medium four pores were made by the use of a sterile dry rod with a diameter of 6 mm , these pores were made with equal spaces were left between pores and another , and also between pores and plate margin by use of a prepared diagram on a paper to be put under the dish then the solutions of different concentrations of the prepared compounds were added in an a fix amount of 0.5 mL from each concentration in one pore , and then these plates were incubated at 37^0C for 24 hr and the last step the inhibition diameter was measured for each pore using a ruler and these measured in a millimeter (mm). The zone of inhibition is defined as the translucent area which surrounds the disc including the diameter of the disc that lacks bacterial growth.

2.7 Determination of Urease Activity for *Proteus mirabilis* by Indophenols assay:

Reagents:

These reagents were prepared as they were mentioned in [109] .

a. Reagent A (Indophenol solution):

(5) Gram of phenol and (0.025) gram of sodium nitroprusside were dissolved in (500 ml) distilled water.

b. Reagent B (Hypochlorite solution):

(2.5) gram of sodium hydroxide and (4.2) ml of sodium hypochlorite were dissolved in (500) ml of distilled water.

c. Urea (stock solution):

(0.3) gram of urea was dissolved in 10 ml distilled water it was freshly prepared and used.

d. Buffer solution (Phosphate solution):

These reagents were prepared as they were mentioned in [110] (0.174) gram K_2HPO_4 and (0.0185) gram Na-EDTA of (1 mM) were mixed in volumetric flask and were dissolved in (50 ml) distilled water. The pH was fixed at 7.5 (7.5 is the optimum pH for the urease activity).

1. Measurement of Urease activity with inhibitors by Indophenols assay:

Different concentrations of inhibitors (0.05, 0.5, 0.1, 1, 5, 10, 20, 30, and 40) mM dissolved in (DMSO) were used with constant substrate concentration to know the rate of inhibition and MIC (Maximum Inhibitory Capacity).This method was done to determine the inhibitory concentration of urease.

2. Preparation of different substrate (urea) concentration:

The (1, 2.5, 5, 7.5, 10, 20, 30, 50 mM) concentrations were prepared from serial dilution of the 500 mM of stoke (0.3 gram urea in 10 ml distilled water) and used the concentration of the highest inhibition percentage of the previous step. This step is used to identify the kind of the inhibition and calculate K_m, V_{max} and K_i values by the Line weaver Burk equation.

3. Measurement the activity without the inhibitors: -

(This is Control)

(Different concentration of substrate)

Reagents	Sample µL	Blank µL
Buffer 7.5	215	240
Urea	25	-
Enzyme	10	10

It was incubated in water bath at 37˚C for (10 minutes).

Reagent A	5 ml	5 ml

It should be shaken well

Reagent B	5 ml	5ml

It should be shaken well

It was incubated in water bath at 37˚C for (20 minutes).Then it was read with absorbance at 625 nm by spectrophotometer. Then calculated the enzymatic activity and drew the relations between urea concentrations and enzyme activity.

b. The Method with inhibitor (different concentration of substrate):

Reagents	Sample µL	Blank µL
Buffer 7.5	205	205
Inhibitor	10	10
Urea	25	-
D.W	_	25
It was incubated in water bath at 37˚C for (10 minutes)		
Enzyme	10	10
It was incubated in water bath at 37˚C for (15 minutes)		
Reagent A	5 ml	5 ml
It should be shaken well		
Reagent B	5 ml	5 ml

It was incubated in water bath at 37˚C for (20minutes).Then it was read with absorbance at 625 nm by spectrophotometer. And calculate the enzymatic activity that draw the relations between Urea concentrations and enzyme activity.

2.8 Calculations:

1. From the linear Line weaver Bruk equation, calculated V_{max} and K_m [98]

$$\frac{1}{V} = \left(\frac{K_m}{V_{max}}\right) \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Where V=velocity of the enzyme (µmole/min/ml)

K_m=Michaelis constant for enzyme (mM)

S= substrate concentration (mM)

V_{max}= Maximum velocity for enzyme (µmole/min/ml)

2. Calculation K_i value for the competitive inhibitors by the following equation :[98]

$$K_{m\,app.} = K_m\left(1 + \frac{[I]}{K_i}\right)$$

Where $K_{m\,app.}$ = Michaelis constant for inhibitor (mM)

K_m=Michaelis constant for enzyme (mM)

[I]= Inhibitor concentration (mM)

K_i= Strength of inhibitor (mM)

3. Calculation according to the following equation: [98]

$$\% \ i = [\ 100 - a] \times 100$$

$$\% \ Recovery = 100 - \%inhibition = 100 - \% \ i$$

Where %i=degree of inhibition as a percent

a = relative activity as fraction $=V_i/V_。$

V_i for competitive inhibitor:

V_i : The velocity in the presence of inhibitor.

$$V_i = \frac{[S].V_{max}}{K_{m\ app.} + [s]}$$

V_o : The velocity in the absence of inhibitor.

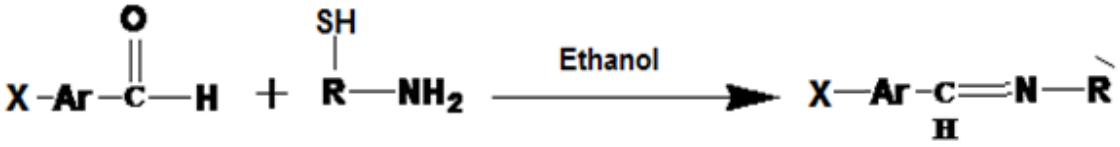
$$V_o = \frac{[S].V_{max}}{K_m + [S]_g}$$

Chapter Three

3 Results & Discussion

3.1 Preparation of Schiff bases compounds:

Due to the biological importance of the Schiff bases; four compounds of Schiff bases were prepared in addition to the compound (amino acid); to compare the biological activity through the reaction of aliphatic amino acid with aromatic aldehyde like *(p- N, N-* dimmethylaminobenzyldehyde, *p*-bromobenzyldehide, *p*-chlorobenzildehyde and *p*-hydroxybenzyldehyde) in an absolute ethanol of glacial acetic acid as catalyst, as in the following equation.



X= Cl, Br, OH, $N(CH_3)_2$ $\overline{R}$ = $HOOCCHCH_2SH$ N: substituel hemiamin.

In the reaction, nucleophilic amino acid attacks the carbon of the carbonyl group of aldehyde to form N-substituted hemiaminals to yield stable compound as shown in figure (3-1):[99]

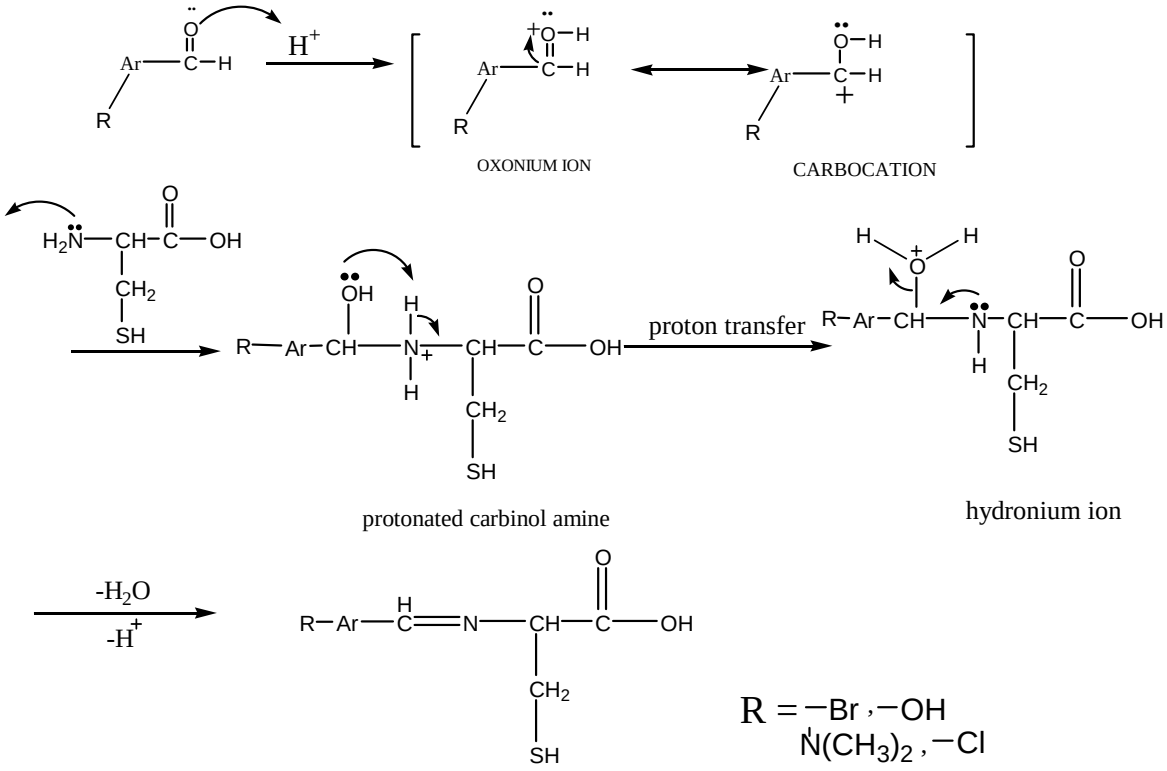


Figure (3.1): Mechanism of the preparation of Schiff compounds. [99]

3.2 Infra red Spectra for Schiff derivatives

The infrared spectrum of the compound (2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid) showed absorption band at (1627.8 cm^{-1}) which could be attributed to (C=N). This bond is characteristic to Schiff bases stretching vibration. *P*-substitution showed absorption band at (813.9 cm^{-1}) could be attributed to p-substitution bending vibration [100,101].

In compound 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid, (C=N) which is characteristic to Schiff bases, showed absorption band at (1625.9m^{-1}) could be attributed to (C=N) stretching vibration. *P*-substitution showed absorption band at (835 cm^{-1}) which could be attributed to *P*-substitution bending vibration [100,101,102].

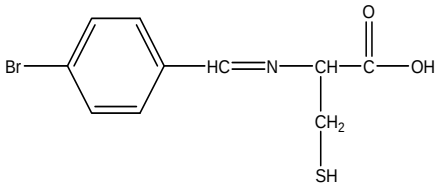
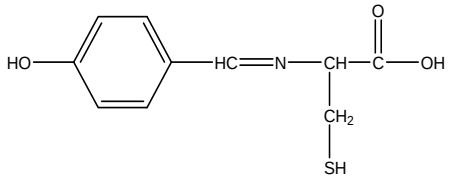
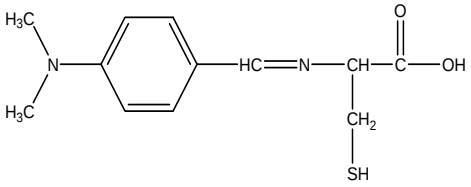
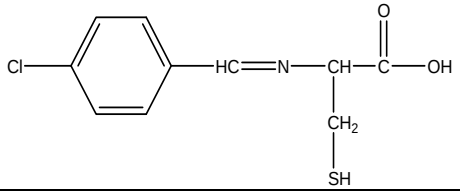
For the compound 2-(4-(N,N-dimethylamino)benzylideneamino)-3-mercaptopropanoic acid, the infrared spectrum showed absorption band at (1600 cm-1) . This band may be attributed to (C=N). P-substitution showed an absorption band at (817.8cm-1) which may be attributed to *p*-substitution bending vibration.

The Schiff bases were characterized by FT-IR spectra which showed disappearance of two bands in range [3250 and 3100 cm^{-1}] represent the symmetric and asymmetric stretching vibration for amine group in amino acid (Cystiene) respectively, disappearance of C=O absorption band of the aldehyde derivatives in range [1650-1700 cm^{-1}] and appearance of the stretching vibration of C=N band in range [1600-1627 cm^{-1}] indicate the formation of Schiff base. FT-IR spectra also showed appearance of stretching vibration band in range [2330-2362 cm^{-1}] belongs to SH group of

Cystiene.

FT-IR spectra of compounds are shown in figure (3-2, 3, 4 and 5). Table (3-2) showed the main FT-IR absorption bands for all synthesized Schiff bases and the other functional groups that found in their structures. The absorption bands for the Schiff bases are shown in table (3-1):

Table 3-1: Bands of the characteristic infrared absorptions for the Schiff bases in cm^{-1}

No.	Structure	ν O-H	ν C-H Ar.	ν C-H Al.	νC=O	νC=C Ar.	Additional bands
1		3442	3006	2923	1543	1463	ν C-Br 744
2		3512	3101	2950	1571	1517	ν O-H 3512
3		3442	3041	2806	1529	1489	ν C-N-C 1386
4		3446	3010	2923	1543	1469	ν C-Cl 750

3.3 Biological study Results

3.3.1 Biological Activity

Pathogenic microorganisms cause different kinds of diseases to human and animals. Discovery of chemotherapeutic agents played a very important role in controlling and preventing such diseases. Chemotherapeutic agents are isolated either from living organisms known as antibiotics like penicillin and tetracycline….etc, or they are chemical compounds prepared by chemists such as the sulpha drugs etc. [(103)] Microorganisms have the ability to develop resistance to these chemotherapeutic agents and such strains which are resistant causing major problem in treatment of microbial infections. For this reason searching for new antimicrobial agents is continuous process and great efforts have been employed to find new antibiotics or new compounds with good antimicrobial activity which might be suitable to be used as chemotherapeutic agents.

One microorganism were isolated and identified, to be used *in vitro* techniques, the microorganism was:

Proteus mirabilis, (gram negatives rods that are distinguished from other members of enterobacteriaceae by their ability to produce the enzyme phenyl alanine deaminase in addition to urease, causes UTI, [(104)] the technique was the (Well sensitivity test), this method involves the exposure of the zone of inhibition toward the diffusion of microorganism on agar plates. A standard (3mm) diameter of wells impregnated with a specified volume of the compound solution representing the (MIC), minimum inhibition zone, this

well was placed on an agar plate cultured by the test organism. The plates were incubated for (24 hr. at 37 °C). The zone of inhibition of bacterial growth around the well was observed. [97,105].

3.3.2. Inhibitory ability of prepared compounds against gram negative bacteria:

The prepared compounds showed inhibitory activity in a concentration of 10 mg/ml where they have the highest inhibitory ability for the compounds 2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid, 2-(4–hydroxybenzylideneamino)–3-mercaptopropanoic acid, 2-(4-(N,N-dimethylamino)benzylideneamino)-3-mercaptopropanoic acid and 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid against *Proteus mirabilis* ,where the inhibition zone diameter was (8-9)mm and the concentrations of some compounds show inhibition zone diameter between (5-7)mm .

Some concentrations show only small inhibition zone in (3) mm. The compounds 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid, 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid shown high inhibitory activities due to the fact that strong donating groups are found in their structures and the donating groups are [OH , $N(CH_3)_2$] as illustrated in figure (3-6, 7 and 8)

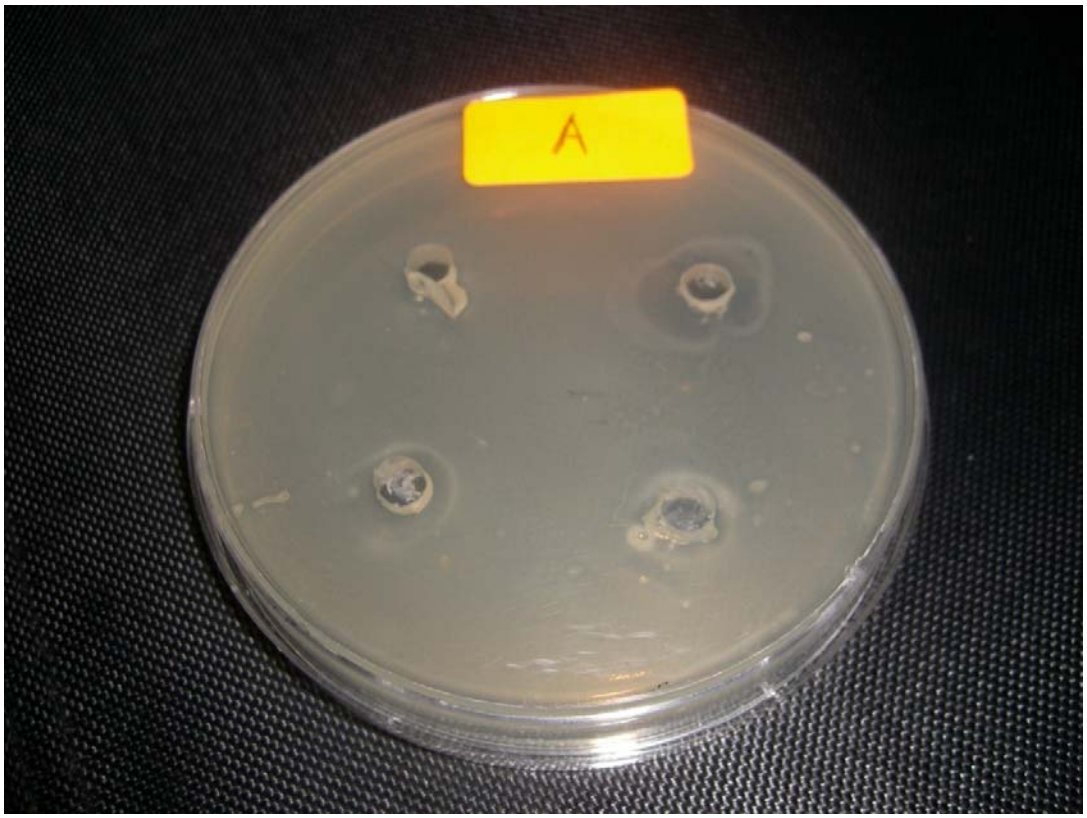


Figure (3-6): inhibition zone of 2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid

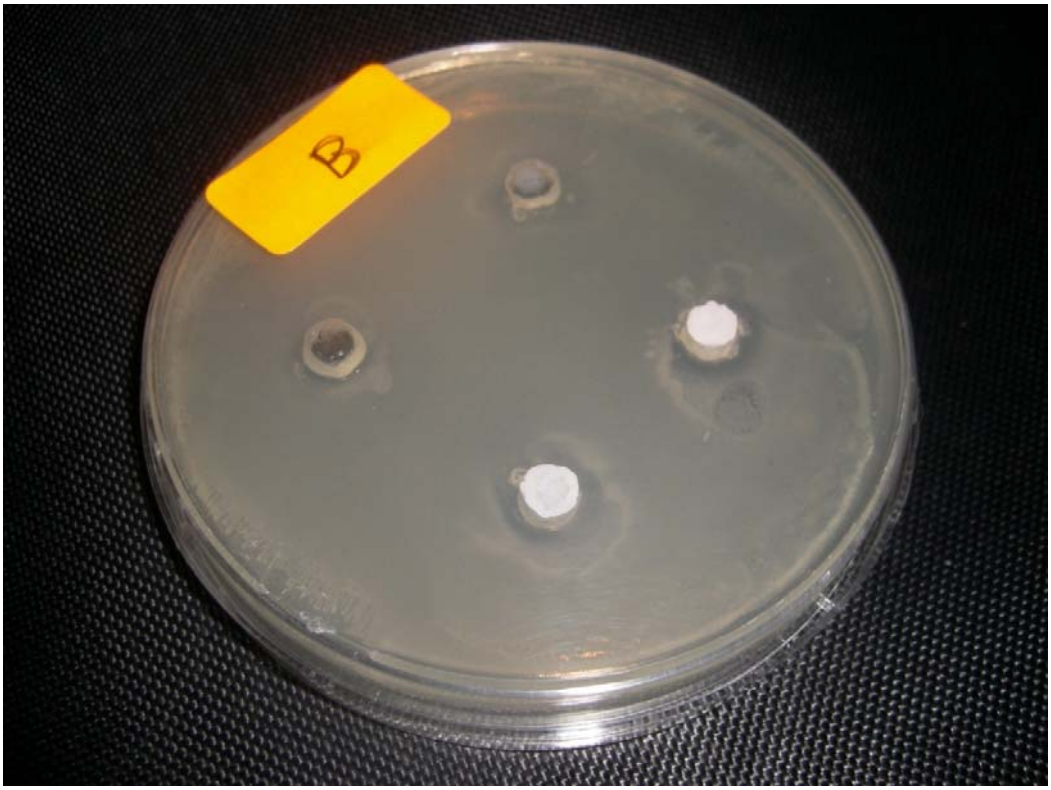


Figure (3-7): Inhibition zone of 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic

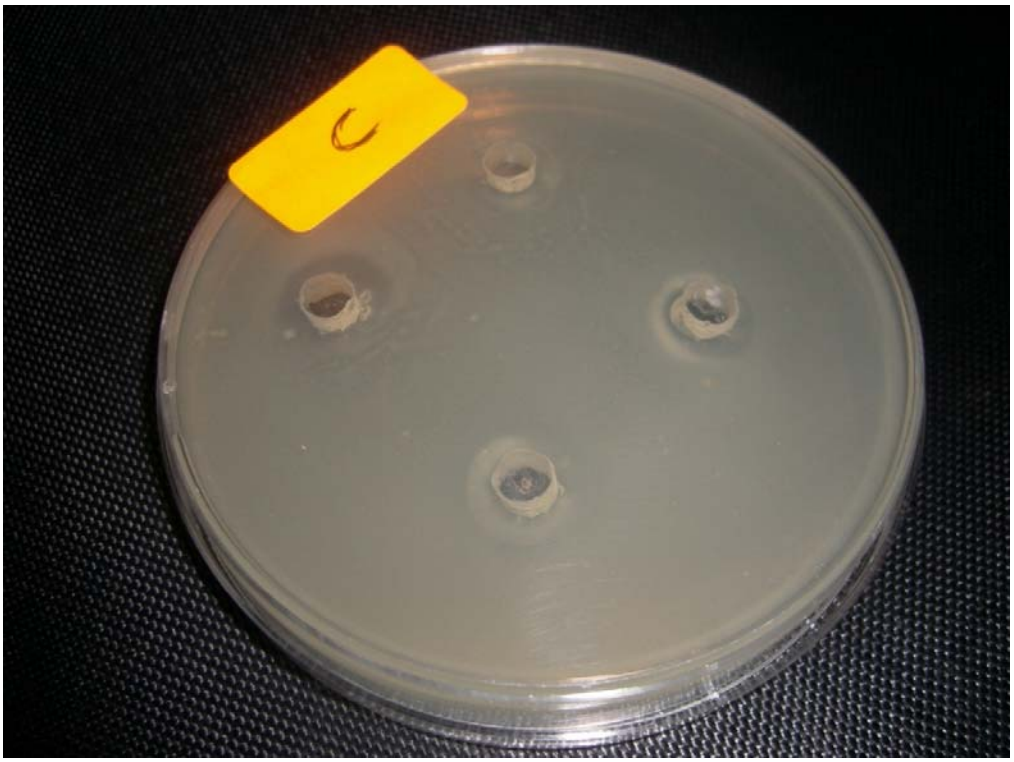


Figure (3-8): Inhibition zone of 2-(4-(N, N-dimethylamino)benzylideneamino)-3-mercaptopropanoic acid

The compounds 2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid, 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid, 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid and2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid are considered as Schiff bases and their antibacterial effects are high because (C=N) group is found in their structures which is peculiar to the Schiff bases. In addition to the presence of (Br, Cl) withdrawing group that less than the activity of these compounds against bacteria. The following table (3-2) shows the inhibition zone diameters of the prepared compounds against *Proteus mirabilis.*

Table (3.2): Effect of four Schiff base compounds against growth of *Proteus mirabilis* expressed by inhibition zones:

Schiff base compound No.	**Concentration (mg /ml)**			
	1	**10**	**25**	**50**
1- Br	3	9	4	3
2- OH	5	7	8	6
3- N(CH3)2	5	6	7	6
4- Cl	4	8	6	3

1: 2- (4-Bromobenzylideneamino) – 3 – mercaptopropanoic acid.

2: 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid.

3: 2-(4-(N,N-dimethylamino)benzylideneamino)-3-mercaptopropanoic acid

4: 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid

3.4 Urease Inhibitors:

Study was performed to test the effects of four chemical compounds chosen from previously prepared compounds as in table (2-1) on purified urease enzyme activity extracted from *Proteus mirabilis* bacteria.

All the prepared compounds in this study are Shciff bases. These are:

1- 2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid
2- 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid
3- 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid
4- 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid

The results of inhibition study showed that all the prepared compounds have inhibition effects on urease and they differ in their degree of urease inhibition. We also calculated the value of K_i and i % for each inhibitor and specified the kind of inhibitor whether competitive or non-competitive by estimation of K_m and V_{max} for each enzyme and inhibitors.[98]

To compare between the inhibition strength for each inhibitor, we made a constant concentration for all inhibitors to deduce the K_i and this is measured with molarity as shown in the calculations in chapter two.[126]

The results of this study show that (2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid) is considered as urease inhibitor, where its Ki value=0.03 M and (Vmax value=909 µmole/min/ml) and (Kmappvalue =2.5mM), and this inhibitor was competitive type as Vmax value for inhibitor is the same for the Vmax for enzyme as shown in figure (3-9).

For (2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid) the inhibitory strength (Ki=0.01M), this is considered as competitive inhibition type, where values of Vmax for both enzyme and inhibitor were the same (Vmax value = 909) µmole/min/ml, Km value 4.3 mM as shown in figure (3-10).

The third compound (2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid) is considered as enzyme inhibitor with inhibitory strength (0.0043 M) which is of competitive type because V_{max} is the same for the enzyme and the inhibitor (Vmax value = 909 µmole/min/ml) whereas the value of Km was equal to 8.33 Mm as showniunfigure (3-11).

The fourth compound (2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid) is considered as enzyme inhbitor, where inhibitory strength (Ki=0.0136 M) and this is considered as competitive inhibitor where Vmax for enzyme and inhibitor is the same (Vmax =909 µmole/min/ml) While Michaelis constant (Km app. = 3.7 mM) as shown in figure (3-12).

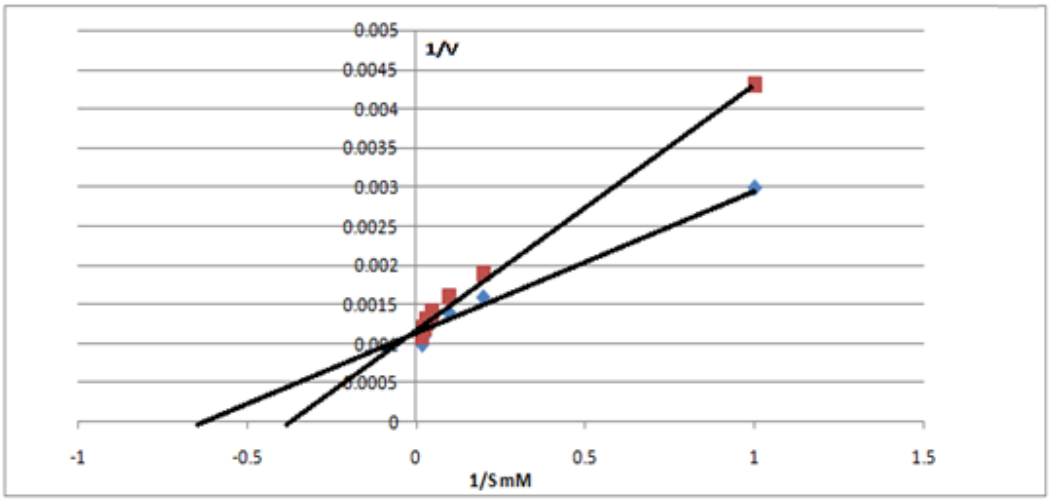


Figure (3-9). The relationship between concentration of urea and the activity of urease drawn by linear weaver Bruk equation with the presence and absence of (0.002 mole) 2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid

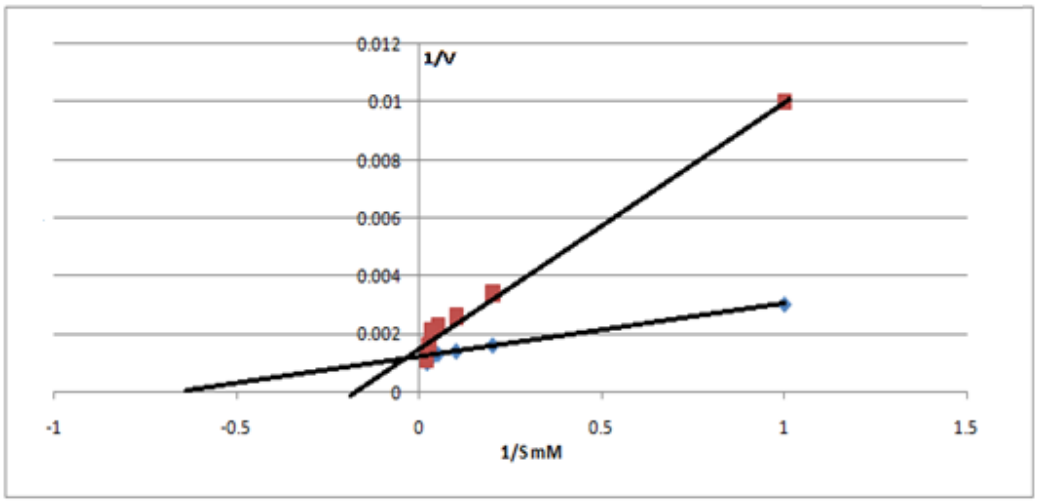


Figure (3-10). The relationship between concentration of urea and the activity of urease drawn by linear weaver Bruk equation with the presence and absence of (0.002 mole) 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid

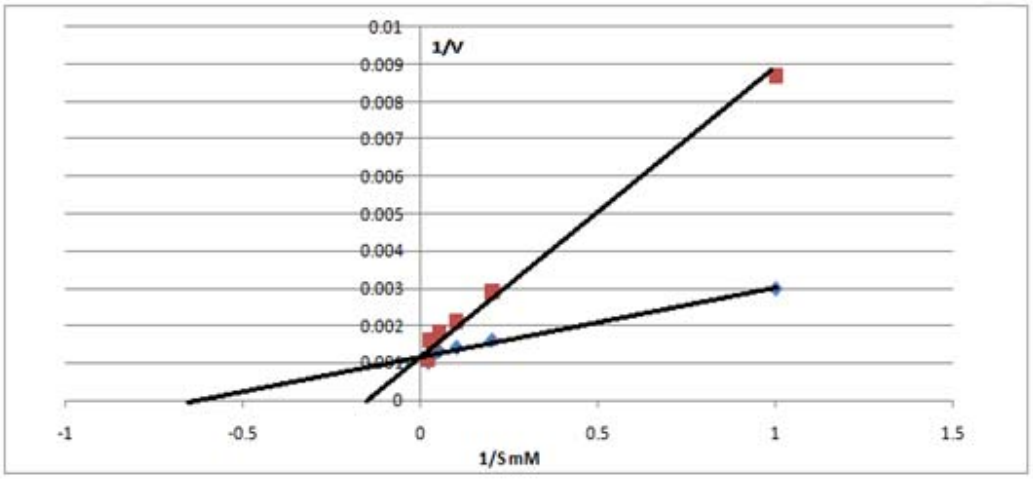


Figure (3-11). The relationship between concentration of urea and the activity of urease drawn by linear weaver Bruk equation with the presence and absence of (0.002 mole) 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid

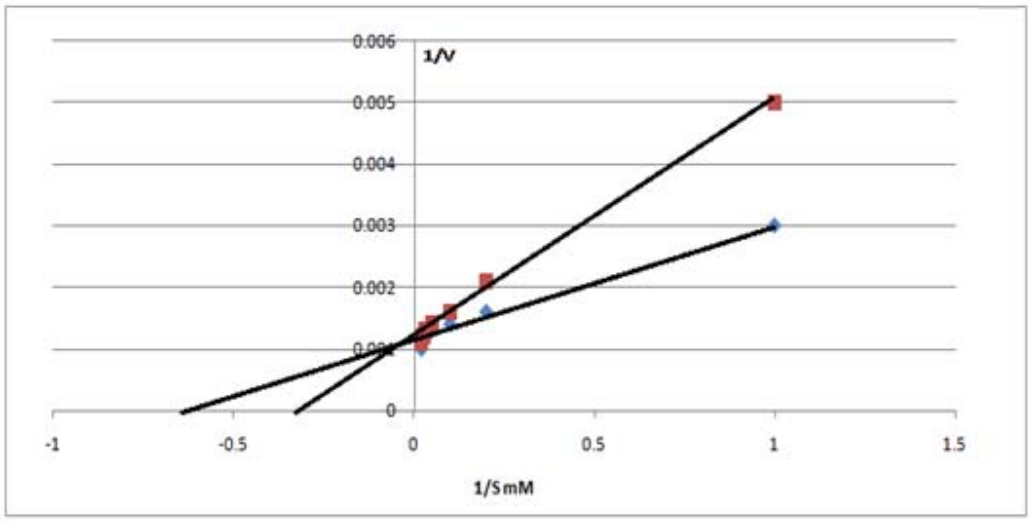


Figure (3-12). The relationship between concentration of urea and the activity of urease drawn by linear weaver Bruk equation with the presence and absence of (0.002 mole) 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid

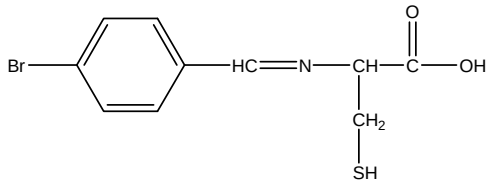
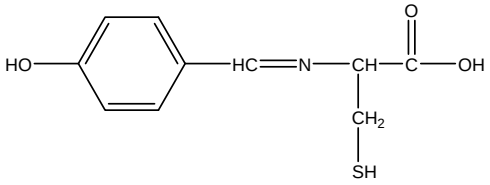
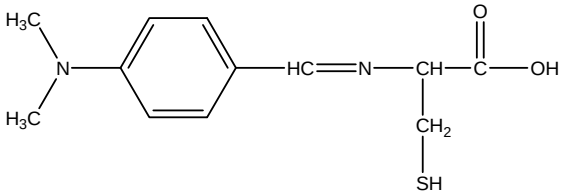
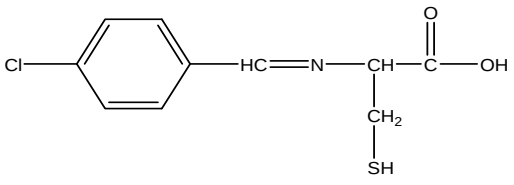
From this equation, V_{max} and K_m were calculated both for enzyme and the inhibitor. From these results, it was found that K_i value is inversely proportional with inhibitory strength, i.e., so when K_i value is high, we need high concentration of the inhibitor to have the same effect. (126)

It is noticed from the results that (2-(4-(N,N-dimethylamino)benzylideneamino)-3-mercaptopropanoic acid), a Schiff base, is considered as the strongest inhibitor used in this study as it's (Ki value =0.0043M) and this is because of (C=N) bond presence in its structure which is characteristic of Schiff base and has high activity on enzyme itself. The presence of functional group on the phenyl ring (di-methyl amine) acts as a donating group that form a strong inhibitor

In comparison with (2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid) which is the weakest inhibitor where Ki value is high in comparison with other inhibitors which is (0.03M). This is because the functional group on the phenyl ring is considered as a withdrawing group.

It was notice from study that donating group increases the inhibitory ability that is present in the chemical structure of the enzyme. The following table (3-3) shows V_{max}, K_m and K_i for these compounds.

Table (3-3): Estimation of $V_{max, Km}$ and K_i for urease inhibitors and the type of inhibition

No.	Structure	V_{max} (mM)	K_m (ml/unit)	K_i (M)	Inhibition type
	Enzyme only without inhbitor	**909**	**1.50**	**___**	**____**
1		**909**	**2.50**	**0.0300**	**Competitive**
2		**909**	**4.30**	**0.0100**	**Competitive**
3		**909**	**8.33**	**0.0043**	**Competitive**
4		**909**	**3.70**	**0.0136**	**Competitive**

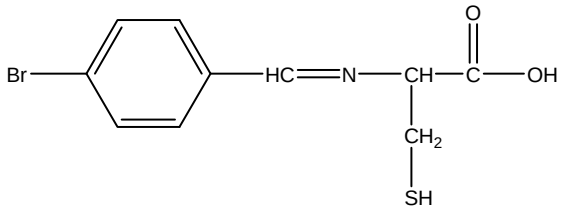
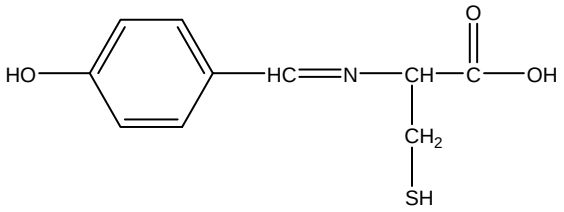
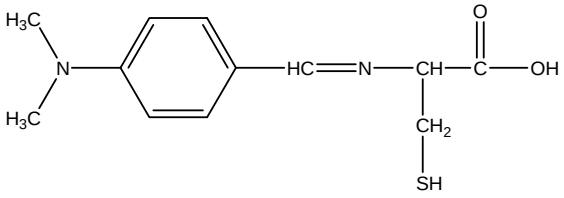
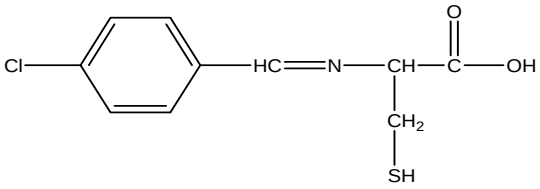
We also calculated the percentage of inhibition for each inhibitor (i %) as shown in chapter two. [98]

The concentration of inhibitor and substrate were constant to compare the inhibition percentage for each inhibitor. It is notice from calculation of the inhibition percentage, as shown in chapter two [98], that the highest percentage of inhibition was (96.77) when the:

(2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid)was used compare with other inhibitors at the same concentration. As it is considered as Schiff base with high activity due to the presence of (C=N) characteristic of Schiff base. The least inhibition percentage was (63.8) by:

(2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid). The following table (3- 4) shows the percentage of the inhibition:

Table (3-4): Estimation of inhibition percentage of urease inhibitors

No.	Structure	V_i µmole/L /min	V_o µmole/ L/min	a (relative activity)	%i (percentage of inhibition
1		4.34	11.96	0.362	63.8
2		1.4	11.96	0.117	88.23
3		038	11.96	0.0322	96.77
4		9.16	11.96	0.165	83.4

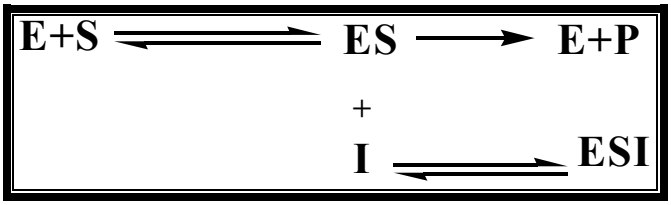
From the results of the study, it has been found that all compounds used were urease inhibitors extracted from Proteus mirabilis bacteria. It was noticed that (2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid) has the best activity among these inhibitors. It was also noticed those urease inhibitors 2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid, 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid, 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid and 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid were competitive inhibitors in which they compete with substrate (urea) to bind with active site of urease.

The V_{max} remains constant despite the increase in K_m and this is evidence that it's of competitive type. They compete with substrate (urea) to bind with active site of urease and when urea concentration is increased the % inhibitor effect is reduced increasing the chance of substrate binding with enzyme, and the enzyme regains its activity which has been lost [98].

The increase in the K_m value does not mean that the (EIS) complex has a lower affinity for the substrate.

$$K_{m\ app.} > K_m$$

Competitive inhibition increases K_m and substrate concentration to reach V_{max} but the V_{max} is constant and unchanged. Therefore in the presence of competitive inhibitor a much greater substrate concentration is required to attain any fraction of V_{max}. K_m is inversely proportional with affinity. This formula shows competitive inhibition [98]:



Urease is considered as the first enzyme that was prepared in a crystallized form but its action is still unknown clearly [106]. The roles of nickel ion and amino acids present at active site of urease are still unknown [107].

It is noticed from enzymatic and biological studies that Schiff bases compounds are strong inhibitors of the urease enzyme where the (2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid) inhibition force was (Ki=0.0043 M) which is the highest inhibition force for the prepared compounds(%i= 96.77%). The biological activity for this compound was tested by *Proteus mirabilis* and was noticed to have the highest inhibition at (10mg/ml) where the inhibition zone diameter between (8-9) mm. In addition to the other Schiff bases compounds, which have a high inhibition ability where (2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid) had Ki (0.01 M) and also the compound (2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid) had inhibitory force (Ki=0.0136M) results of the biological activity of these compounds were high on Proteus mirabilis with (10mg/ml) with inhibition zone diameter 8 mm. Compounds with strong donating group like (2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid),that contain donating group (OH); then the inhibition forces for these compounds were relatively high in comparison to the

biological activity, it was noticed that inhibition zone for these compounds were high in diameter. The biggest diameter of inhibition zone was at (10mg/ml) concentration of inhibitor.

The diameter was 9 mm. From this comparison of these results, it was noticed that there is a relationship between the biological and enzyme activities. It was also noticed that inhibition force for the compounds contain (C=N) group characteristic for the Schiff base have a high enzyme activity with high inhibition percentage.

The presence of the donating groups was an auxiliary factor for increasing the inhibition force and diameter of inhibition. These compounds are strong inhibitory compounds. It was noticed that (2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid) compounds were the less compounds of inhibition. The compound (2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid) was weak inhibitor in comparison with other compounds studied in this study. The inhibition force for this compound was weak (Ki=0.03M) where it is highest Ki value for the studied compounds and had the least inhibition force.

The study of urease inhibition has medical, environmental, agricultural importance. Urease is considered a virulent factor for pathogenic bacteria lead to many infections like urolithiasis, UTI, pyelonephritis, gastric ulcers….etc [27,108].

The development in discovery and the importance of urease inhibition leads to reduction in pollution and help to increase the uptake of nitrogen by plants and treatment of the infection caused by urea splitting organisms

[107].Urease inhibitors are considered as effective treatment because they inhibit urolithiasis caused by microorganisms that produce urease when infecting the urinary tracts [28].

Conclusions:

1. From the results of this study, it is found that the prepared urease inhibitors (Schiff bases) which are 2-(4-bromobenzylideneamino)-3-mercaptopropanoic , 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid, 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid and 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid are active compounds due to the presence of C=N group and had a competitive inhibitors for urease which compete the substrate for binding to the active site of urease.

2. It is noticed that 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic has the highest inhibitory effect against urease.

K_i=0.0043 M; i=96.77%; V_{max}= 909 unit/ml; K_m=8.33 mM and is competitive type of inhibition. While N2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid has the lowest inhibitory effect with (K_i=0.03 M, Vmax 909 unit/ml, K_m 2.5 mM, i %=63.8 %).

3. All the prepared compounds have biological activity as antibacterial agents, 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid gave the highest biological activity .

Recommendations for Future Work:

1. Preparation of new compounds with different functional groups (Cl, Br and OH) and study of their biological and enzymatic activities.
2. Study of the mechanism of action of these compounds on the enzyme through X-ray study.
3. Carry out experiments of enzyme inhibition *in vivo* on experimental animals with histological and physiological studies.

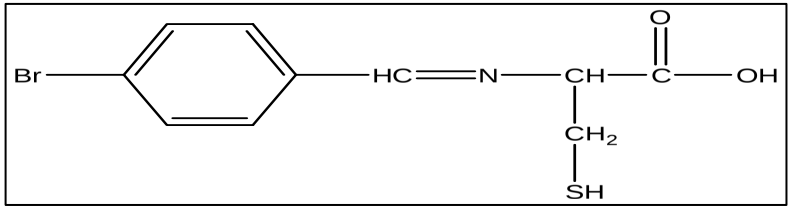
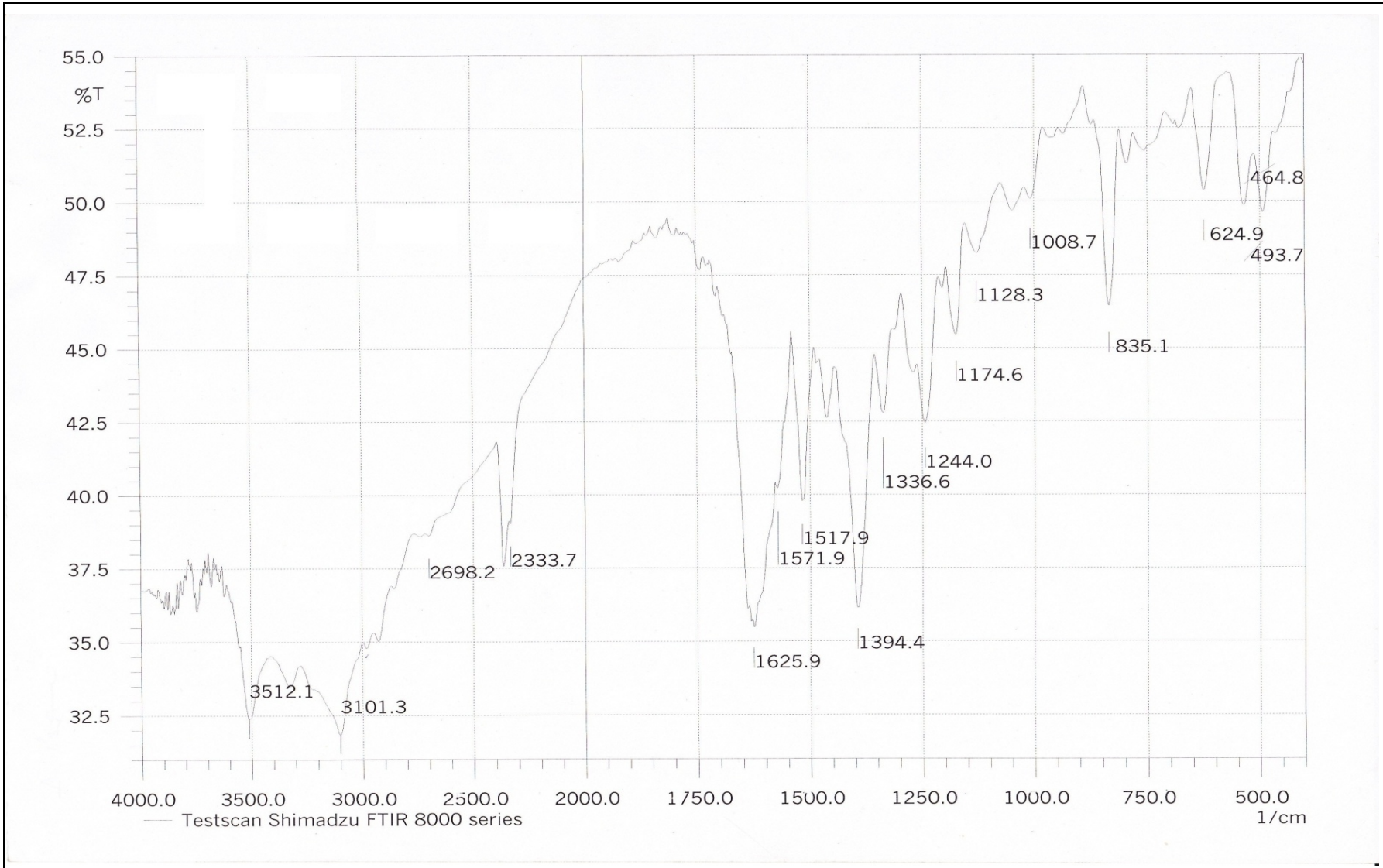
$C_{10}H_{10}NO_2SBr$	

Figure (3-2): Structure, Physical Properties and F.T.I.R. spectrum of 2-(4-bromobenzylideneamino)-3-mercaptopropanoic acid



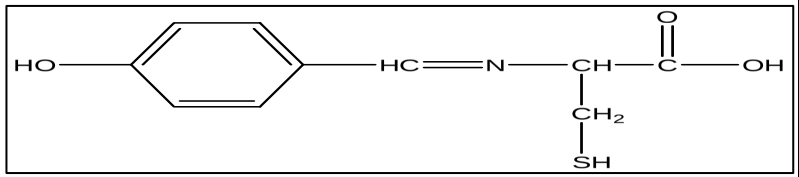
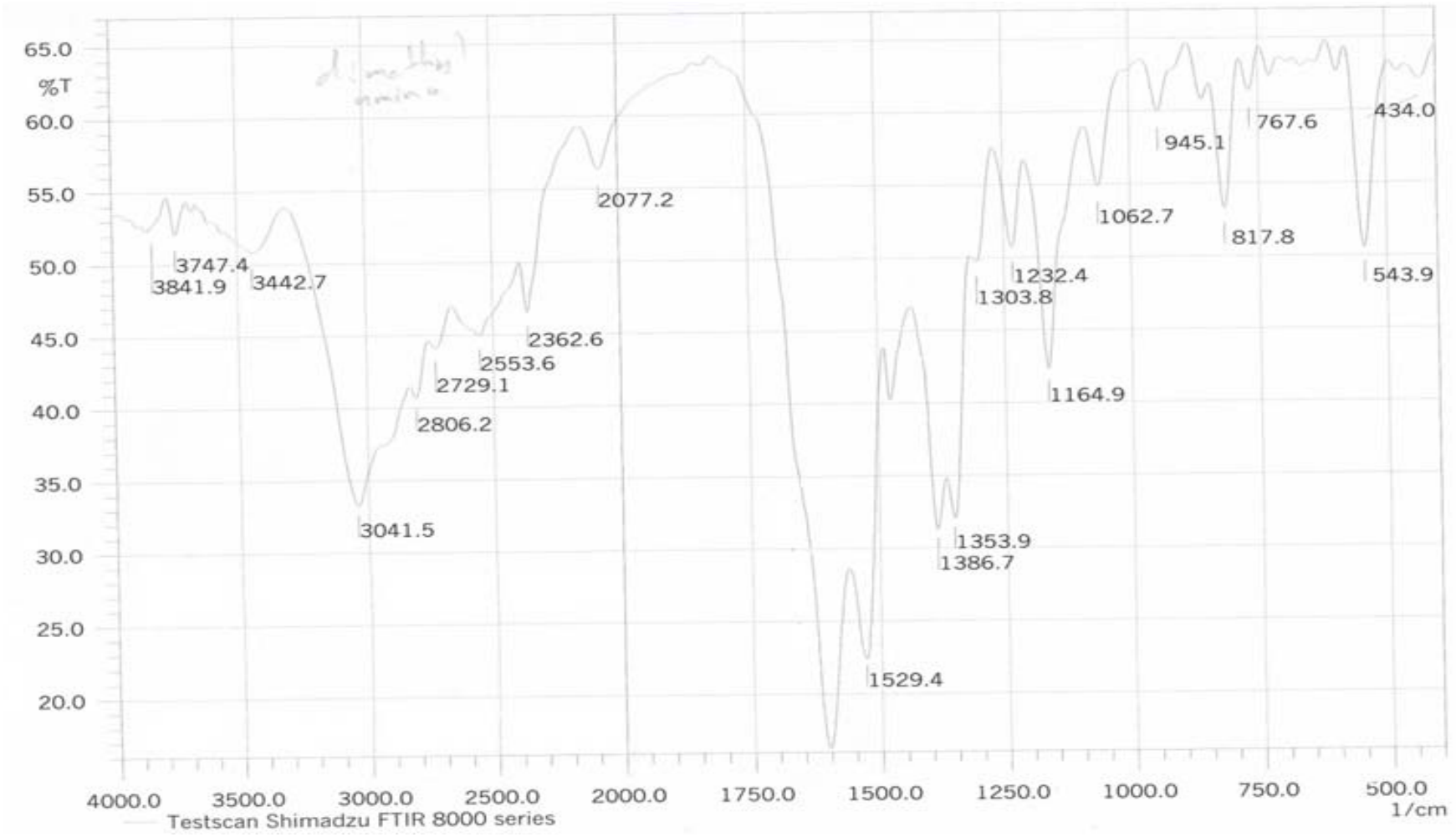
$C_{10}H_{11}NO_3S$	

Figure (3-3): Structure, Physical Properties and F.T.I.R. spectrum of 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid



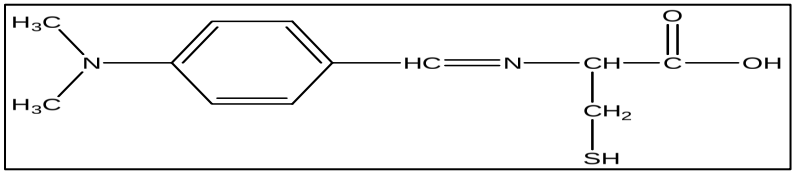
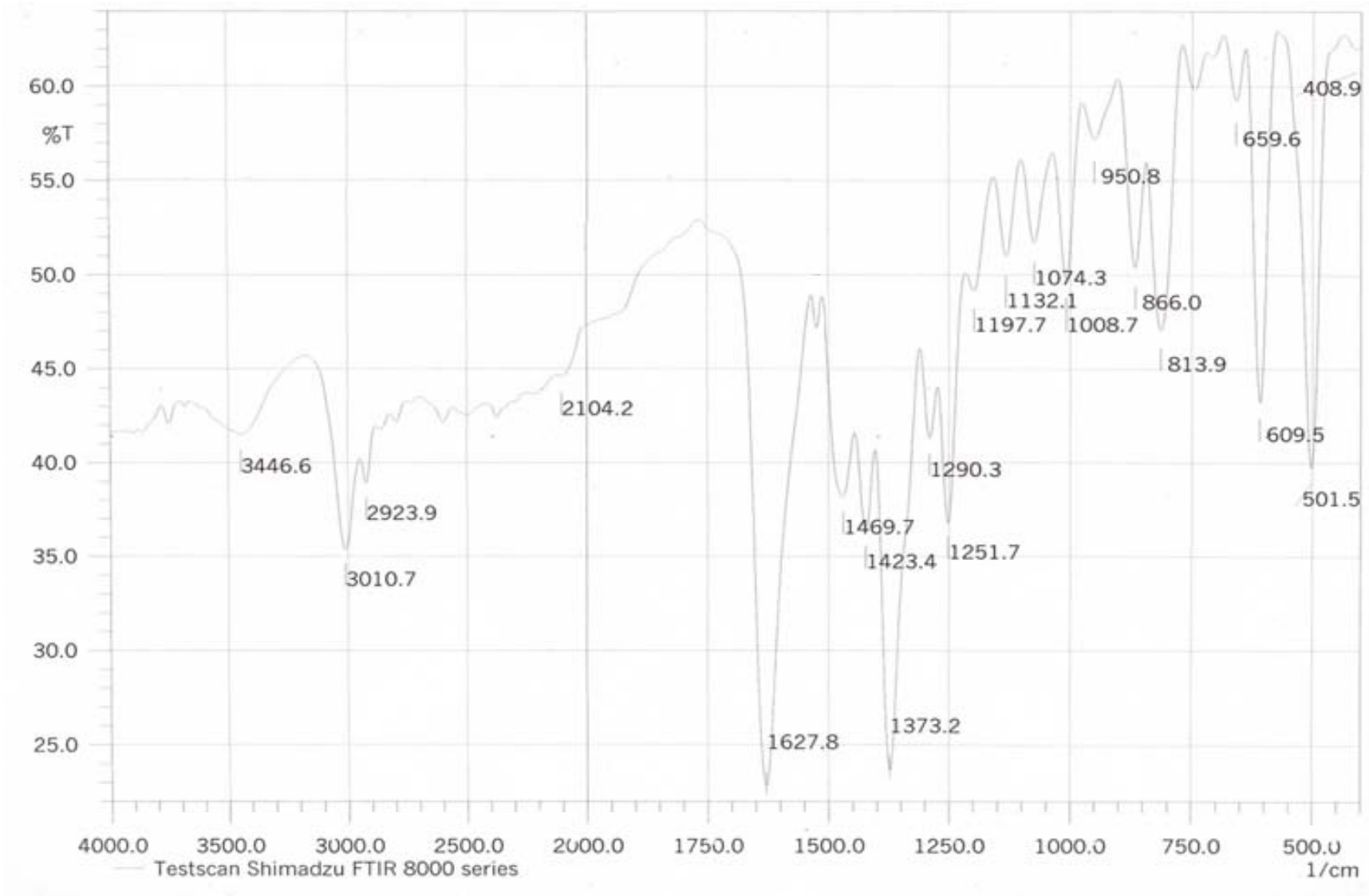
$C_{12}H_{16}N_2O_2S$	

Figure (3 -4) : Structure, Physical Properties and F.T.I.R. spectrum of 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid



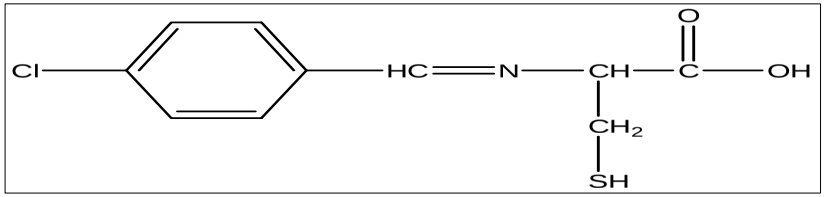
$C_{10}H_{10}N0_2SCl$	

Figure (3-5): Structure, Physical Properties and F.T.I.R. spectrum of 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid

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الخلاصة

اشتملت الدراسة الحالية على تحضير عدد من مشتقات السستين من خلال تفاعل الحامض الاميني السستين مع الالدهايدات الحلقية ينتج عنها:

"2-(4-bromobenzylideneamino)-3-mercaptopropanoic , 2-(4-hydroxybenzylideneamino)-3-mercaptopropanoic acid, 2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid and 2-(4-chlorobenzylideneamino)-3-mercaptopropanoic acid".

اعتبرت المركبات المحضرة اعلاه كقواعد شف وتم التعرف على تشخيصها عن طريق حساب درجات انصهارها وطيف الاشعة تحت الحمراء.

تم قياس الفعاليات البايولوجية ضد بكتيريا *Proteus mirabilis* بأستعمال طريقة انتشار الابار الصغيرة حول الصفيحة وايضا تم تقدير الفعاليات المضادة للبكتيريا للمركبات المحضرة.

تم دراسة تأثيرات المركبات المحضرة على انزيم اليوريز حيث اعطت تاثيرات جيدة على انزيم اليوريز. كافة المركبات المحضرة اعطت تثبيط من النوع التنافسي ذلك ان قيم ال V_{max} كانت متشابهة وقيم Km متزايدة.

اخيرا، تم حساب قيم V_{max}, K_i, K_m and i % للانزيم باستعمال linar weaver Burck equation .

من ملاحظة الفعالية البايولوجية والفعالية الانزيمية ،نستنتج ان مركبات ال Schiff bases المحضرة هي مثبطات قوية لانزيم اليوريز حيث ان المركب **2-(4-(dimethylamino)benzylideneamino)-3-mercaptopropanoic acid** اعطى اقوى قوة تثبيط (96.77 %) وان قيمة ثابت التثبيط كانت تساوي (Ki=0.0043 M) .