Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Science
Department of Chemistry

Preparation of urea derivatives and application of these compounds as urease inhibitors

A

thesis

submitted to the

College of Sciences

Al-Nahrain University as a partial fulfillment

of the requirements for the Degree of M. Sc in

Chemistry

By:

Noor Riyadh Hasan

(*B.Sc.* **2003**)

2006 *1427*

Supervisor Certification

We certify that this thesis was prepared under our supervision at the Department of Chemistry, College of Science, and Al-Nahrain University as partial requirements for the **Degree of Master of Science in Chemistry.**

Signature:
Supervisor: Dr. Selman Ali Ahmed
Title: Assist. Prof.
Date:

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

Signature:
Name: Assist. Prof.Dr. Afaf Al- Derzi
Head of the Department of Chemistry, College of Science, Al-Nahrain University.

Examining Committees Certification

We, the Examining Committee, certify that we read this thesis and have examined the student **(Noor Riyadh Hasan)**, in its contents and that, in our opinion; it is adequate as a thesis for the Degree of Master of Science, in Chemistry.

Chairman

Signature:
Name: Dr.Isam F.Al-Jomaily
Title: Prof.
Date:

Member

Signature:
Name: Dr.Hameed M.Jasim
Title: Assist.Prof.
Date:

Supervisor

Signature:
Name: Dr.Selman A.Ahmed
Title: Assist.Prof.
Date:

Member

Signature:
Name: Dr.Suaad M.Al-Majidi
Title: Instructor
Date:

Approved for the College of Committee of Graduate Studies.

Signature:
Name: **Assist.Prof.Dr. LAITH ABDUL AZIZ AL-ANI**
Address: Dean of the College of Science Al-Nahrain University
Date:

Acknowledgement

First and foremost, for the One God who 's ever blessings are showering my path… Thanks God for his favors of being what I am…

For his generous assistance in this thesis, I would like to acknowledge Dr. Selman Ali Ahmed … who always inspires me…

I would like to acknowledge the Genetic engineering Institute for the great help and supply me with needed materials .I can 't really express my deep gratitude and appreciation to Dr.Isam F. Al Jomaily – head of the Genetic engineering institute –the real scientist and the one who adds to me many things that really mean a lot…I don 't know how to thank you and I hope that I can follow your steps to add something to my field.

And also I would like to thank the Central laboratory of the ministry of Health for the help in the diagnosis of the bacteria used in the thesis.

My deep gratitude to my father and mother for the gift of their life and youth to raise me and their support to help me …for their making me drink from spring of virtues…Simply for making me a human being…

Lastly I can not fully express my gratitude to the exceptional man, dear brother and close friend, Hasan…Thank you hoping that you will be a great surgeon one day…

Abstract

The current study involves the preparation of number of urea derivatives from reaction of urea or thiourea with aromatic amines as in compounds:

"**Phenylthiourea, N-phenylurea, N-(2-methoxyphenyl) urea, N-(4-aminophenyl) urea, N-(2-methylphenyl) urea, N-(2-methoxyphenyl) thiourea, N-[4- (amino carbonyl) amino phenyl] urea, N-[4-(amino carbonyl) amino phenyl] acetamide, N-pyrazin-2-ylurea, N-pyridin-2-ylurea, N,N´-bis(3-bromophenyl)urea, N,N´-bis(2-methoxyphenyl)urea ".**

In addition to the preparation of three compounds considered as Schiff bases that were prepared from reaction of thiosemicarbazide with aldehyde or aromatic ketone as in the compounds:

"(**N-(4-hydroxybenzylidene) hydrazinecarbothioamide, 2-hydroxybenzaldehydethiosemicarbazone, 1-(4-hydroxyphenyl) ethan-1-onethiosemicarbazone)"**

The identification of the prepared compounds was done using spectroscopy (FTIR) and purities of the compounds were determined using TLC which was pure and melting points for these compounds were determined in addition to biological activities

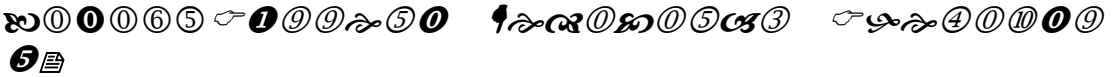
against *Proteus mirabilis* were estimated using disc diffusion method around the plate where antibacterial activities estimated.

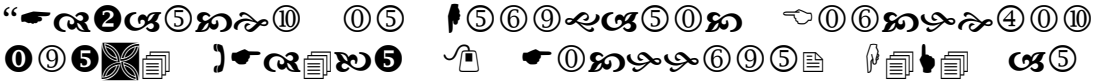
Some of the prepared compounds effects on urease activity were studied and showed good effect in urease inhibition. Some compounds show competitive inhibition and others non-competitive type.

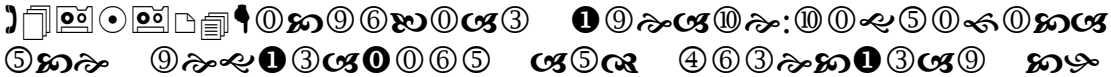
And lastly V_{max}, K_i, K_m, i % values of the enzymes were calculated using line weaver Burck equation.

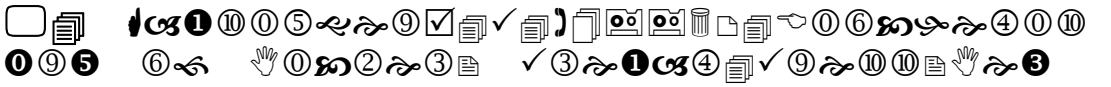
Contents

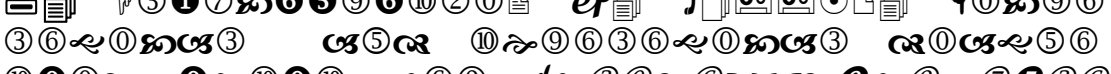
List of the table











Reference

[illegible]

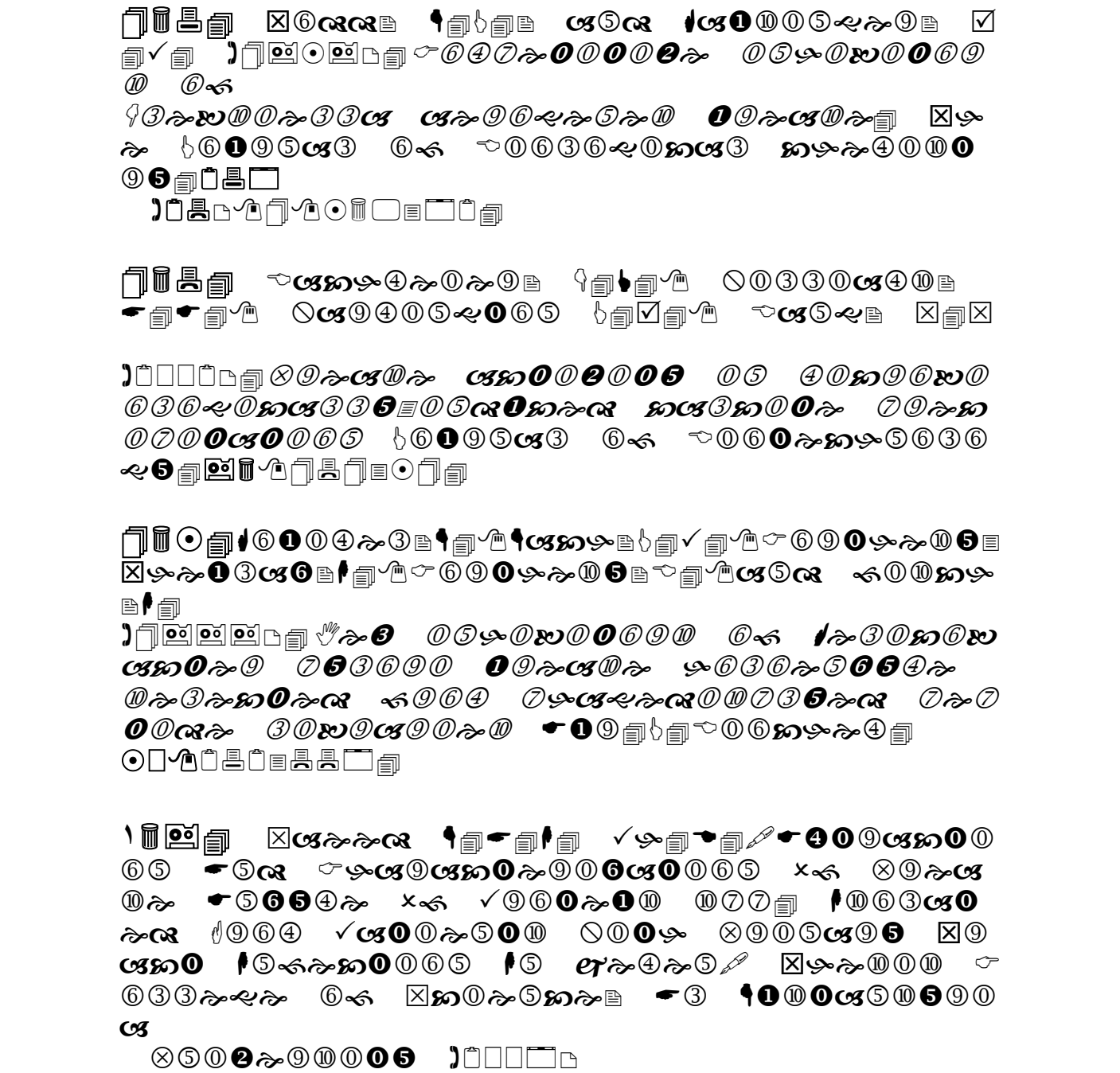
[illegible]

[illegible]

[illegible]

[illegible]

[illegible]
٣٢١ - ٣٢٦



Chapter One
Introduction

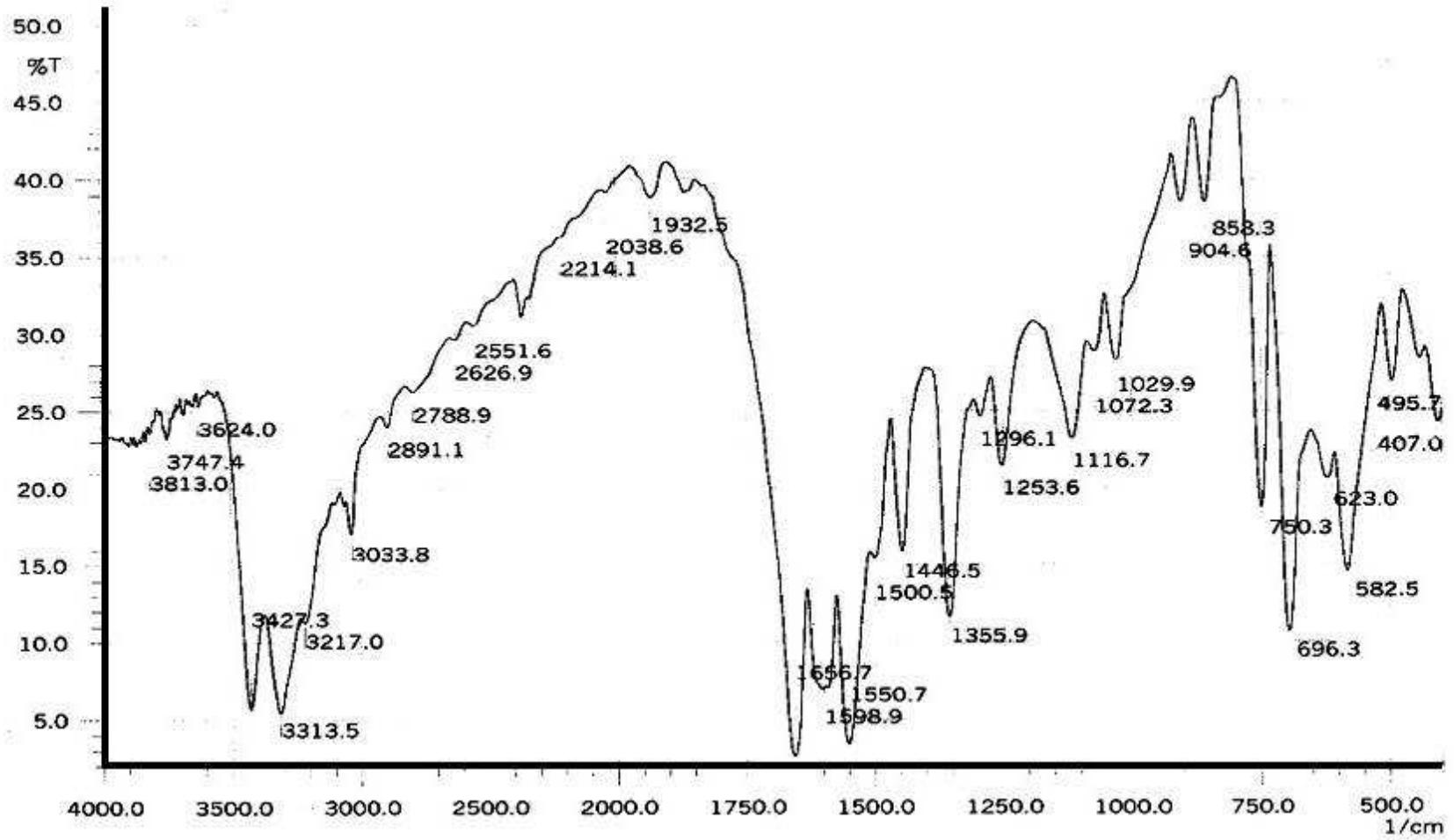
Chapter Two

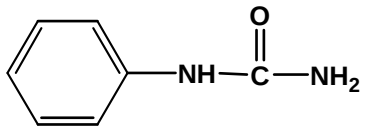
Materials & Methods

Chapter three

Results & Discussion

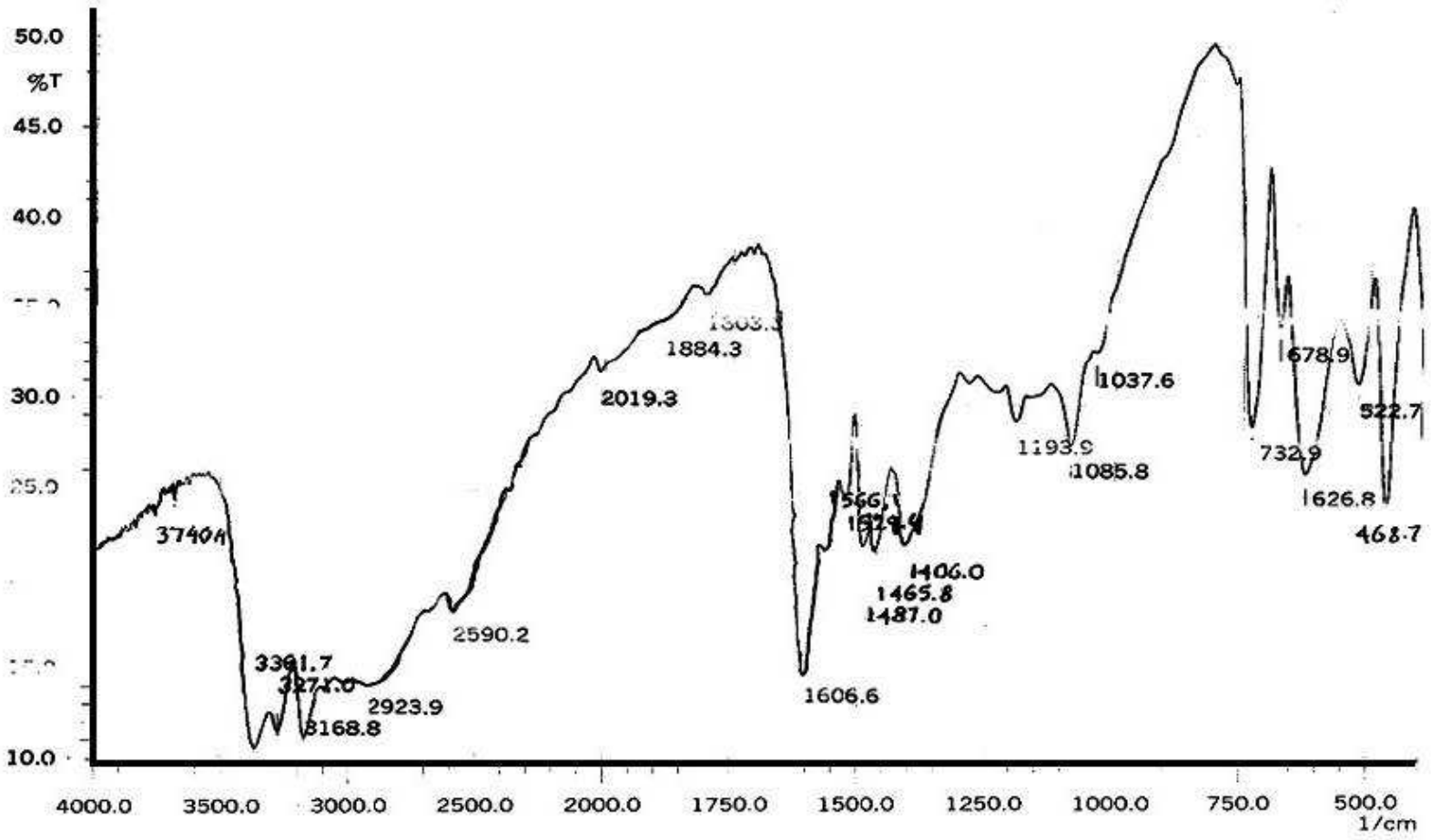
Schemes

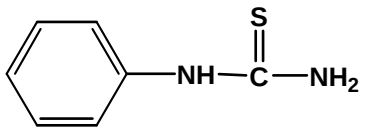




yield % = 52.17

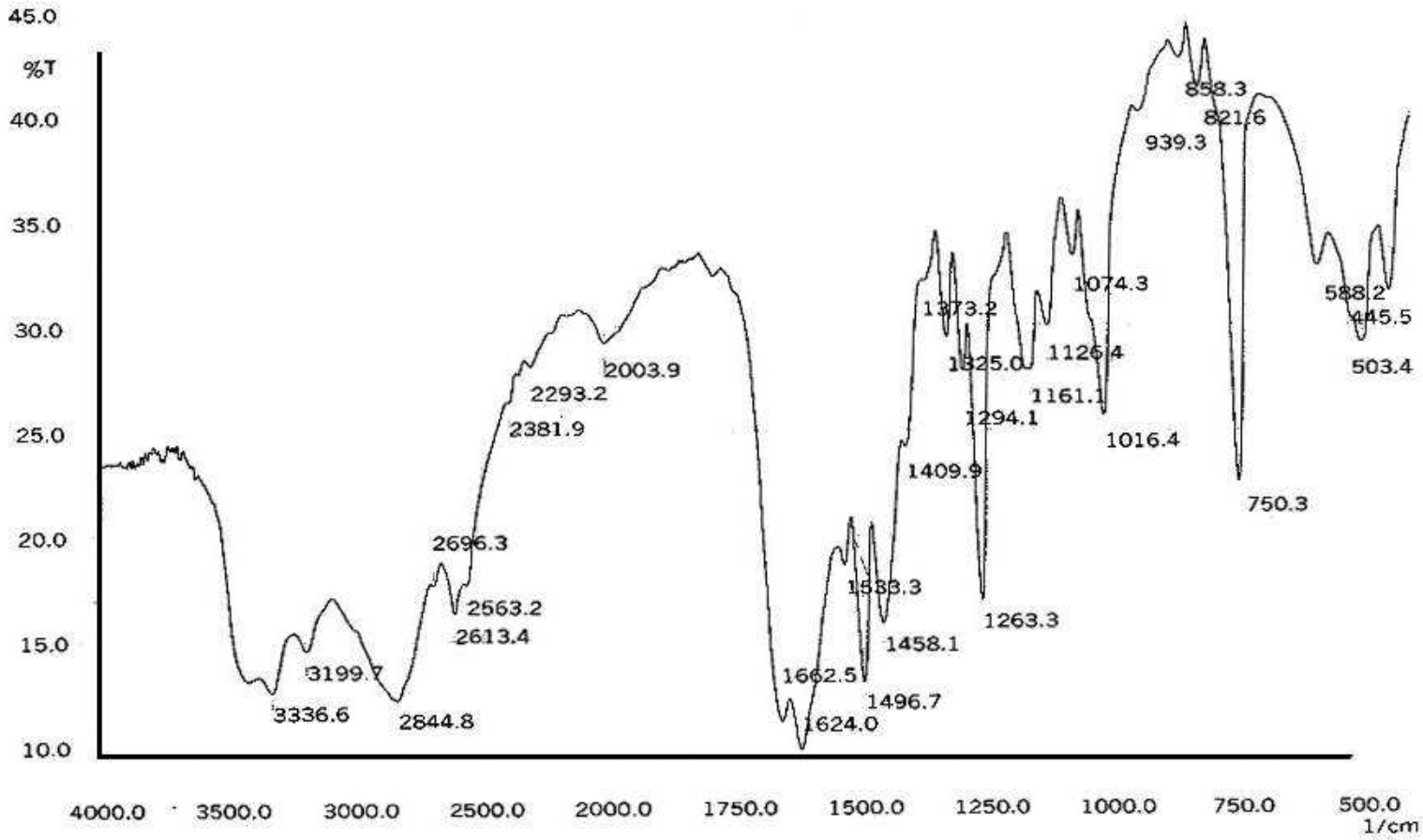
Figure (1): structure, yield %and F.T.IR spectrum of. N-phenyl urea

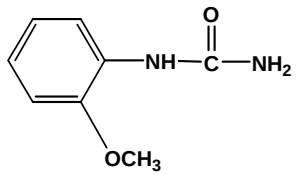




yield %=75

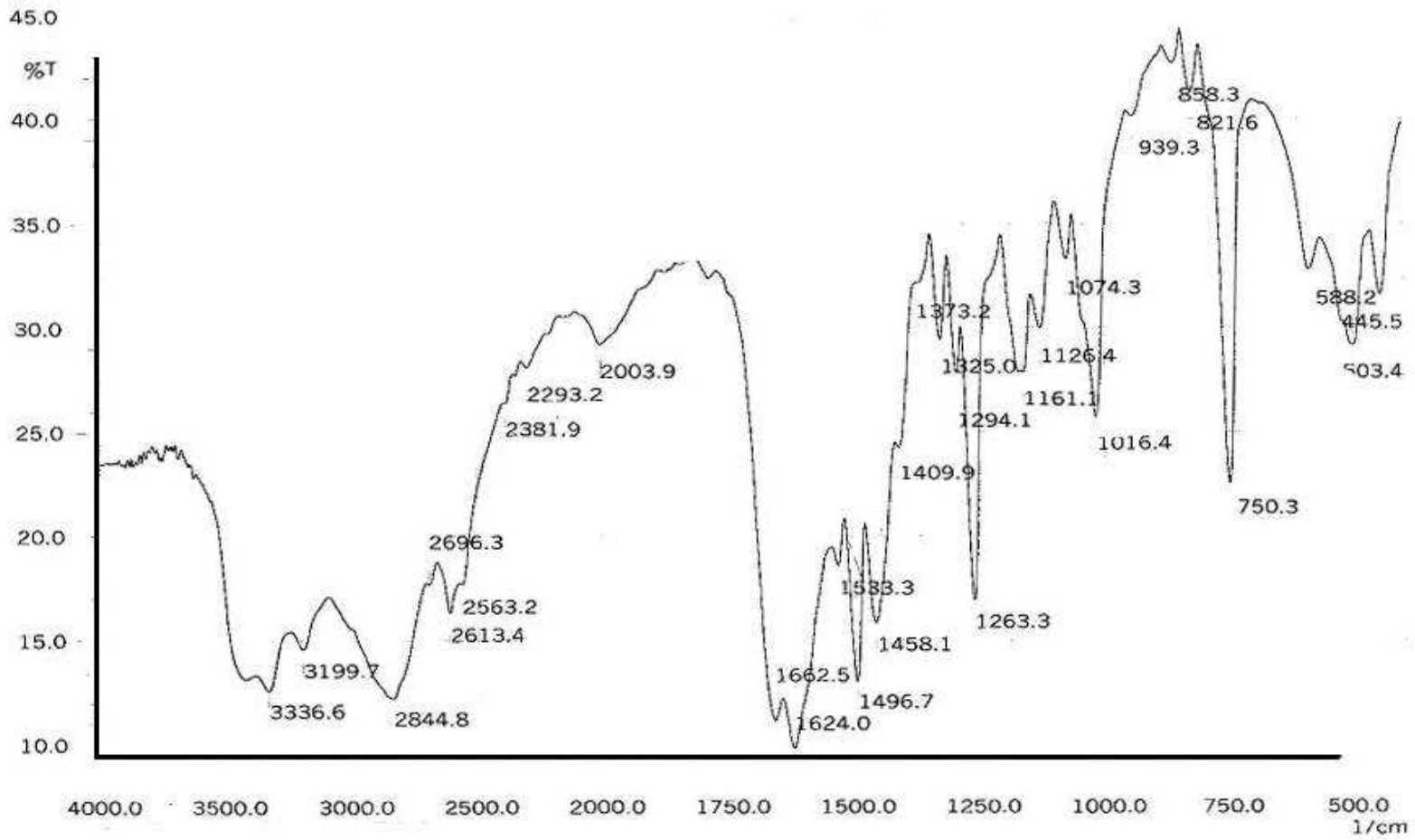
Figure (2): structure, yield % and F.T.IR spectrum of N-phenylthiourea

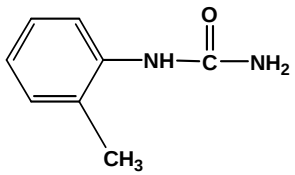




yield %=60.71

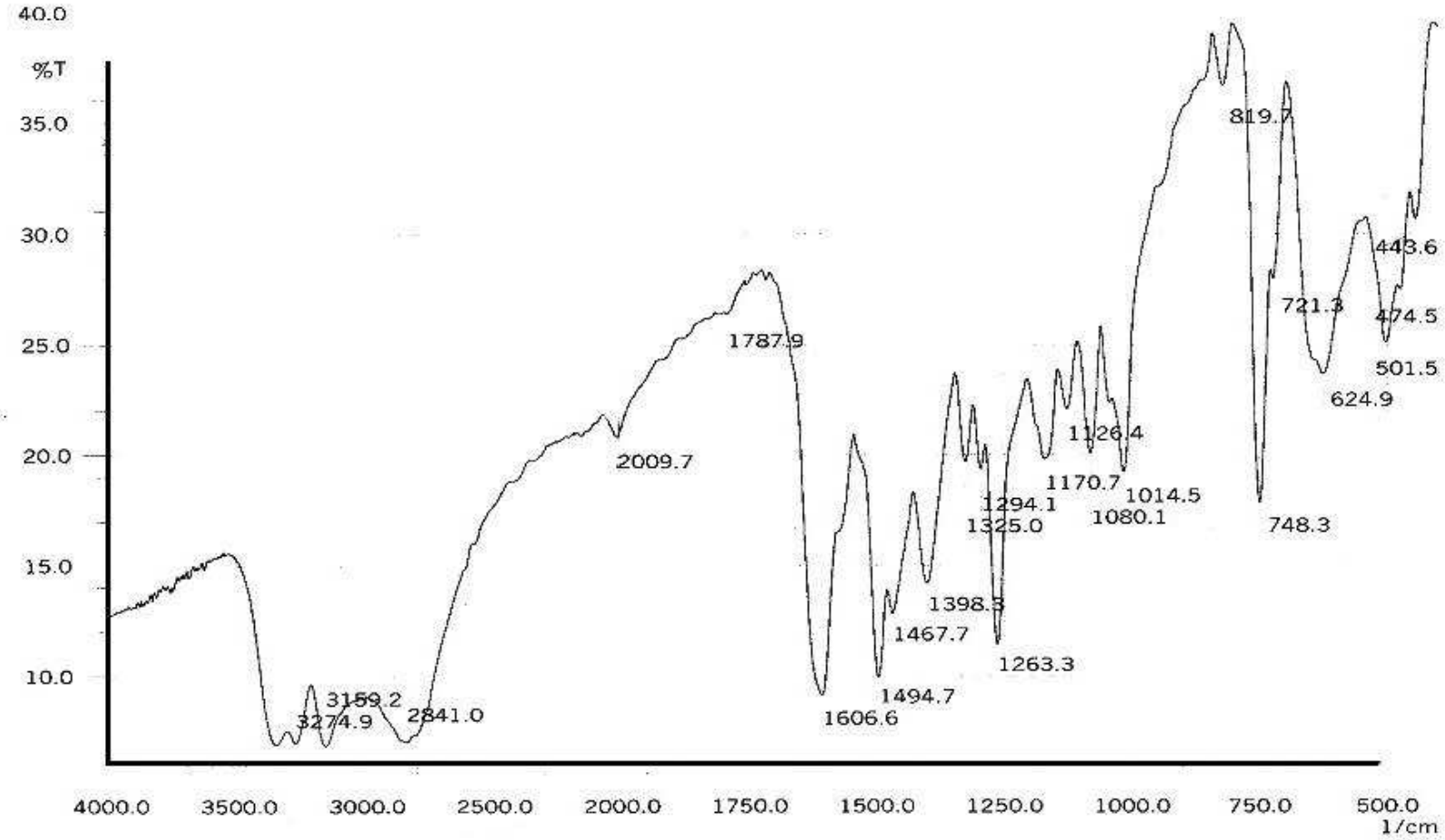
Figure (3): structure, yield %, and F.T.IR spectrum of N-(2-methoxyphenyl)urea

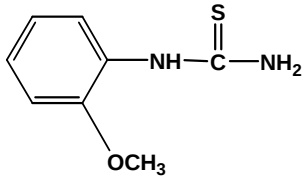




yield %=84

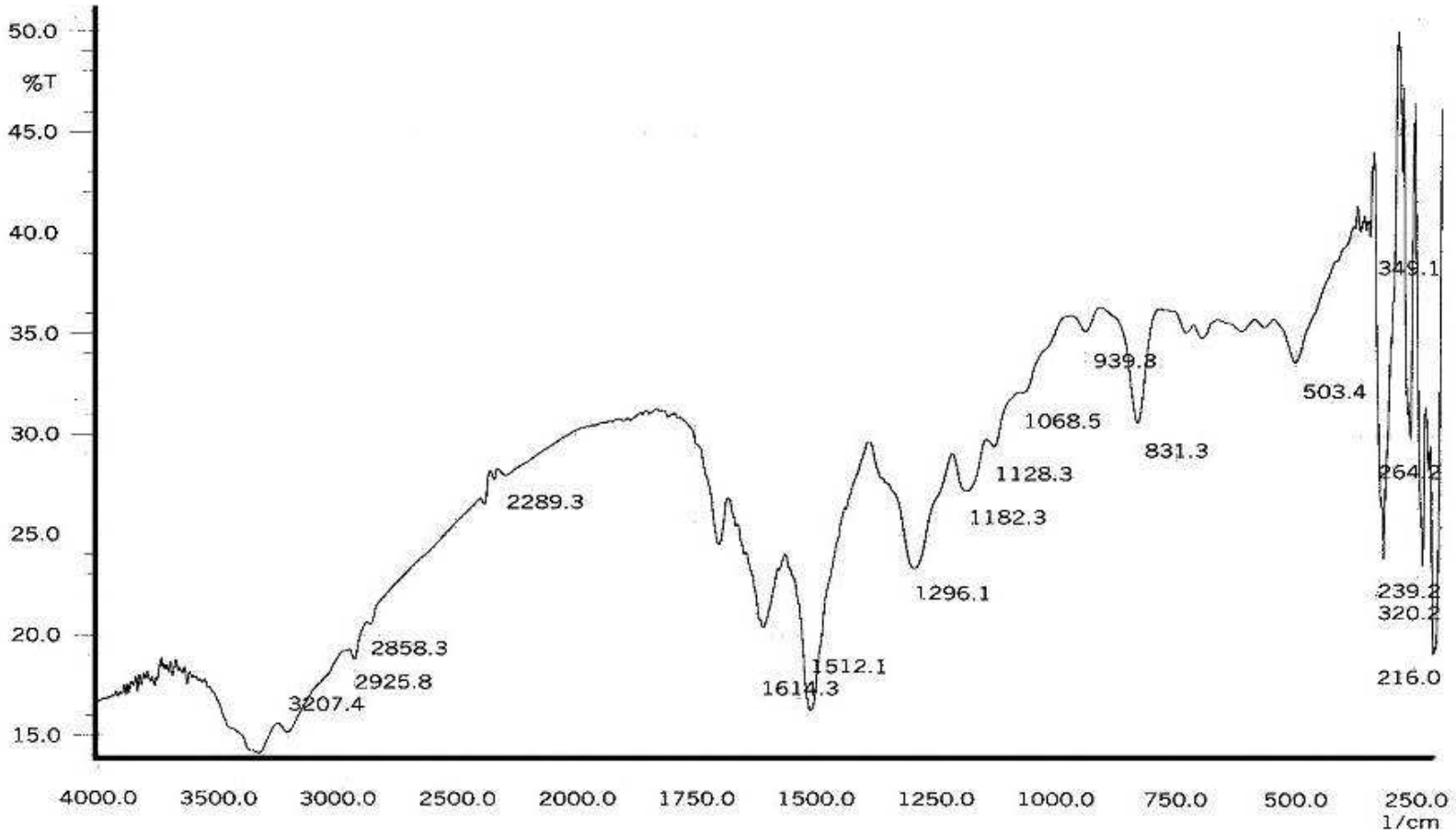
Figure (5): structure, yield % and F.T.IR spectrum of N-(2-methylphenyl)urea

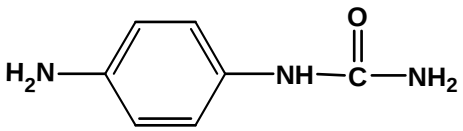




yield %=62.5

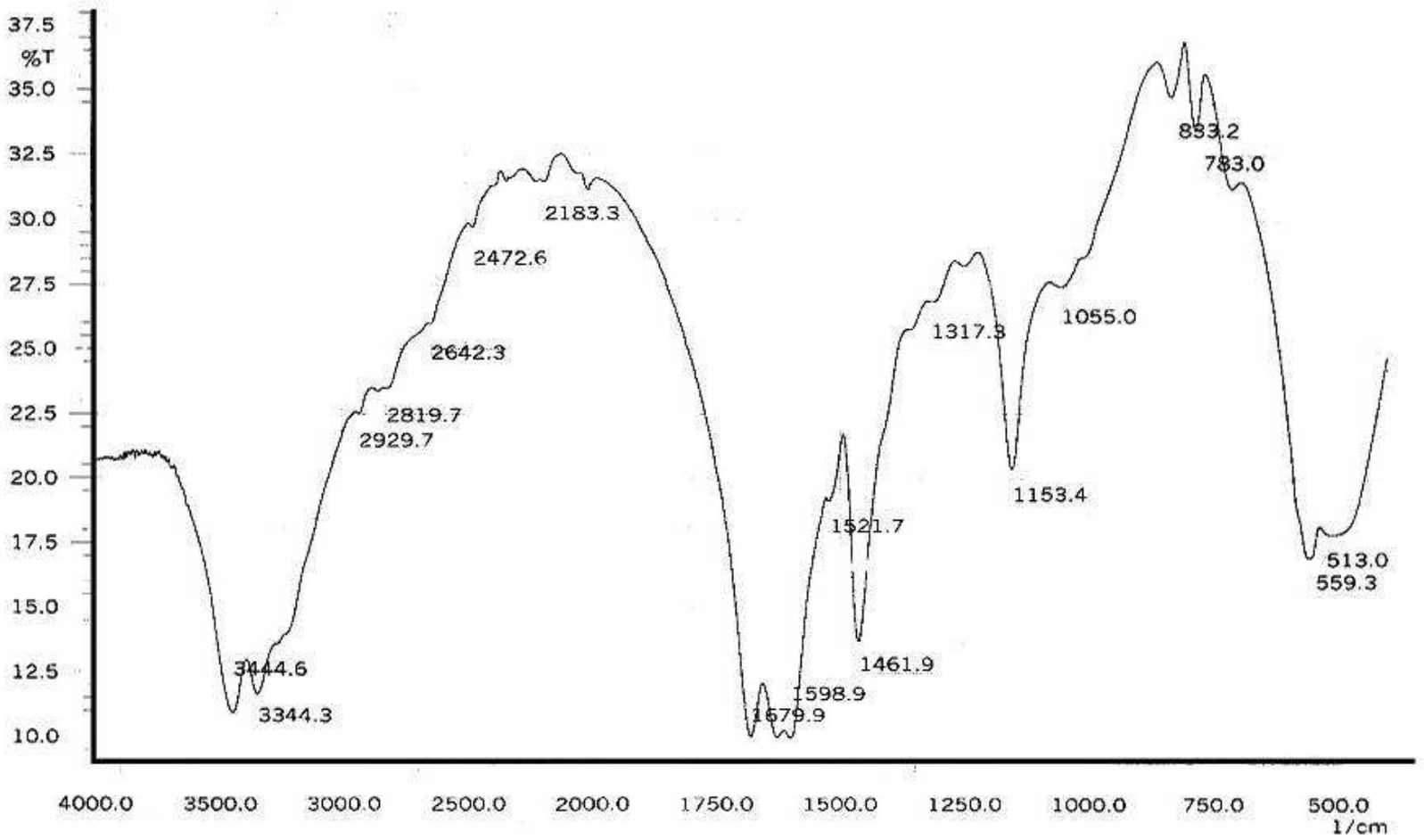
Figure (6): structure, yield % and F.T.IR spectrum of N-(2-methoxyphenyl)thiourea

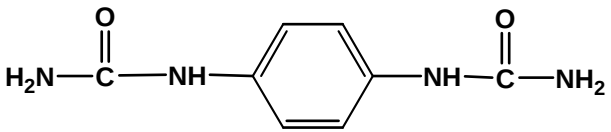




yield %=76

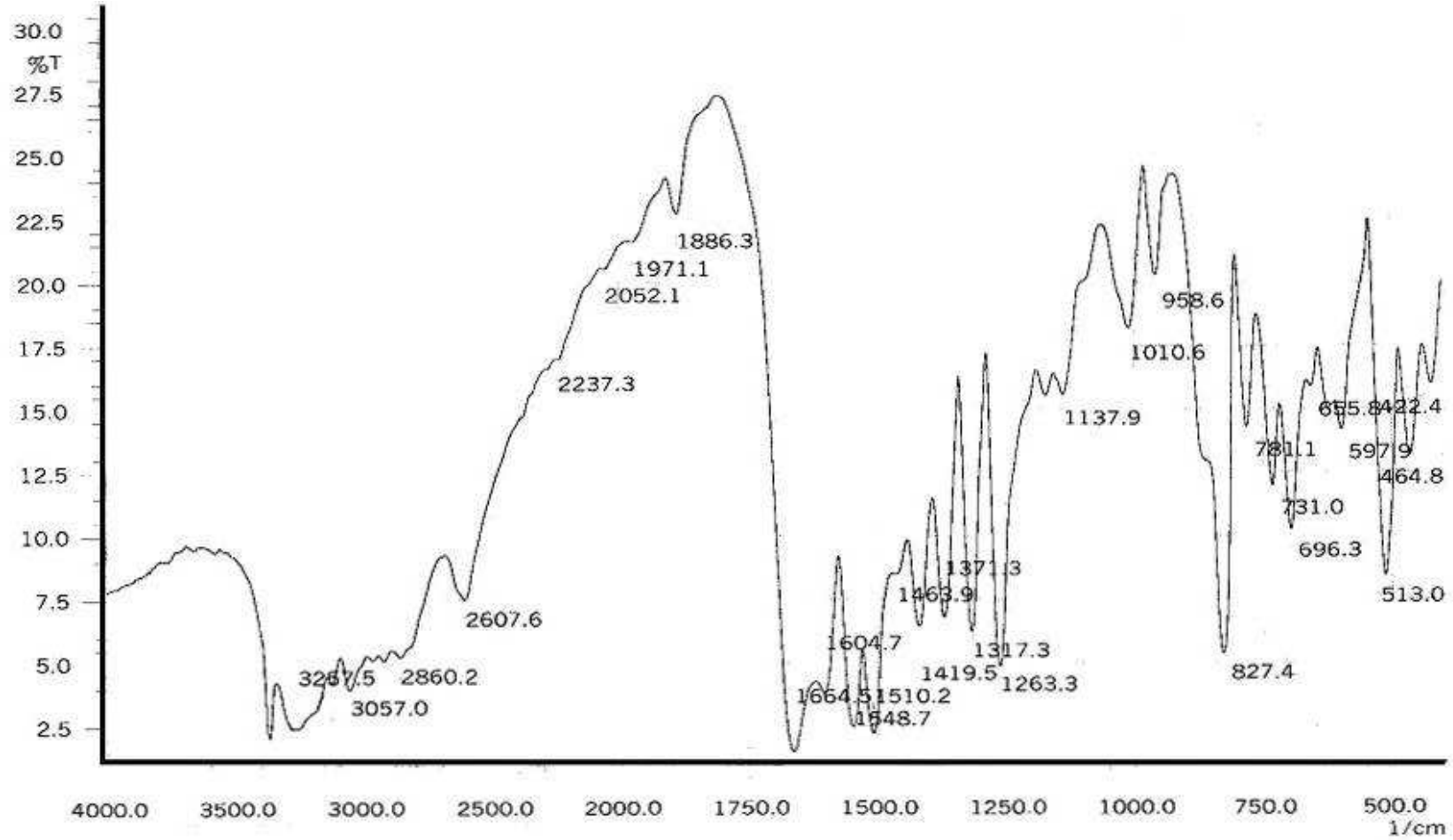
Figure (4): structure, yield %and F.T.IR spectrum of N-(4-aminophenyl)urea

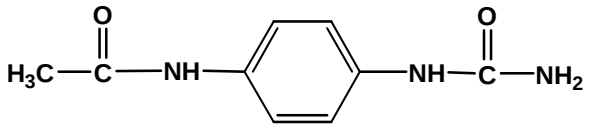




yield %=69.77

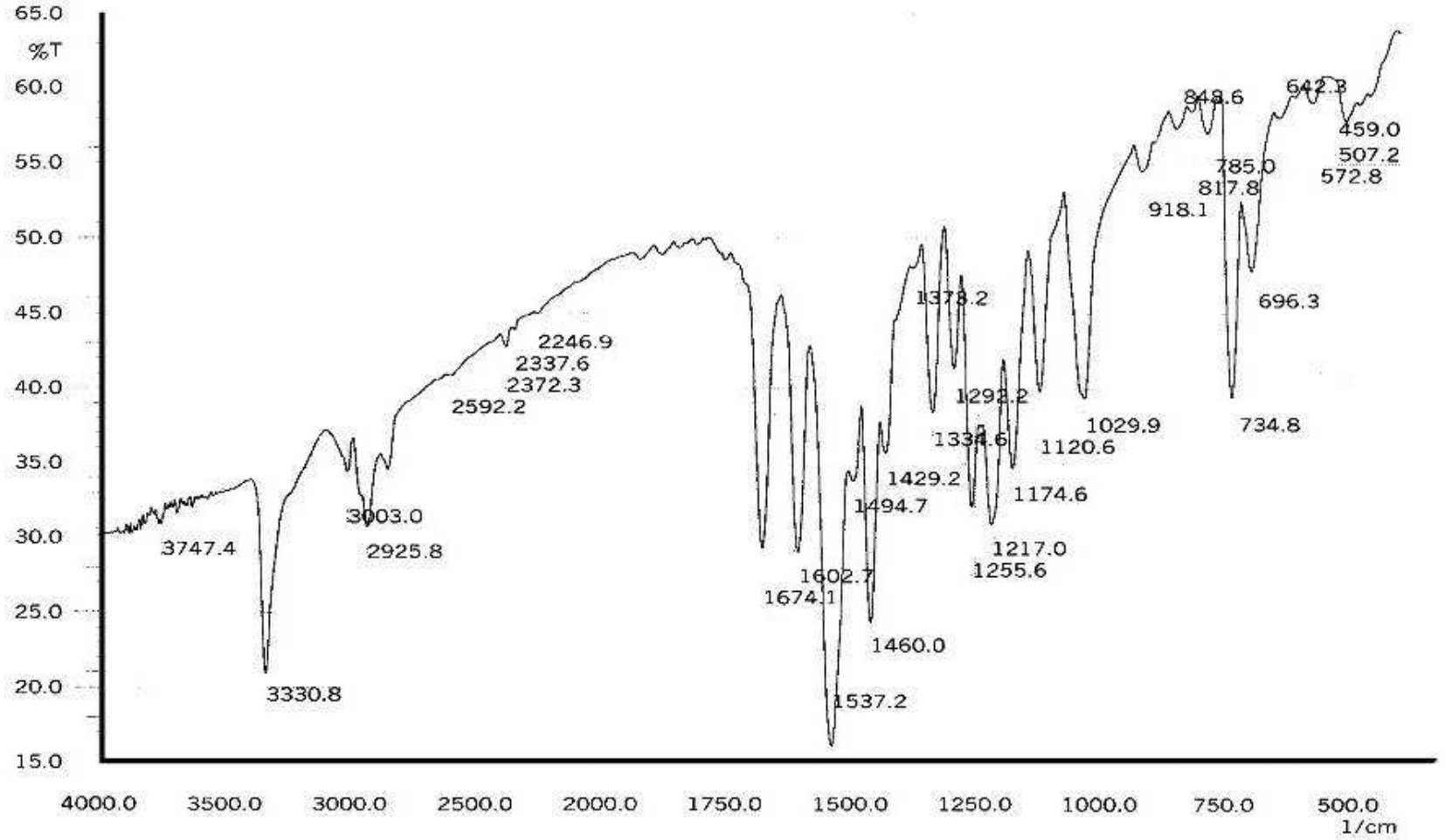
Figure (7): structure, yield %and F.T.IR spectrum of N-[4- (amino carbonyl)amino phenyl]urea

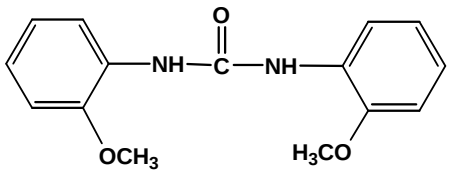




yield %=90.62

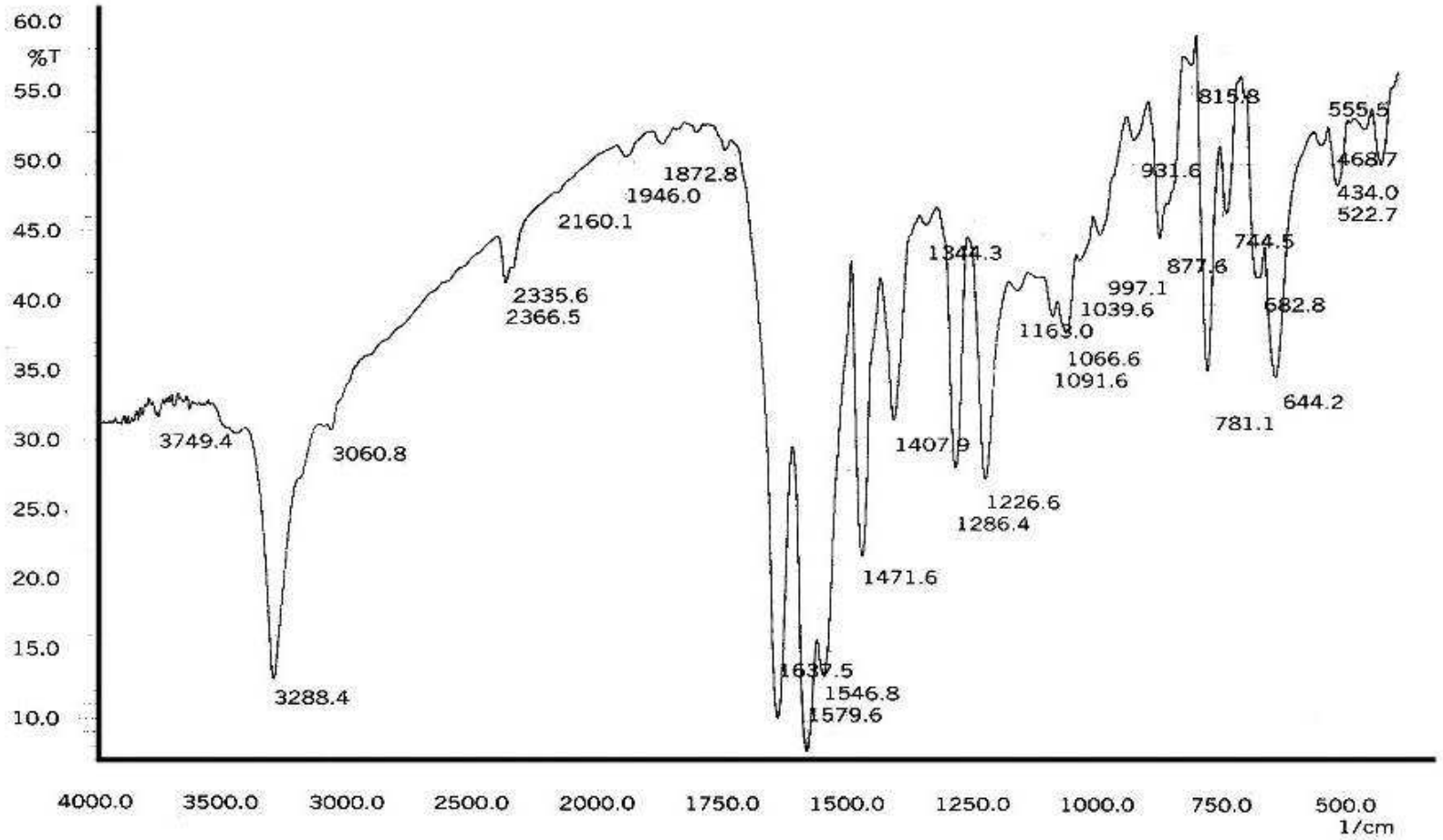
Figure (8): structure, yield % and F.T.IR spectrum of N-[4-(amino carbonyl)amino phenyl]acetamide

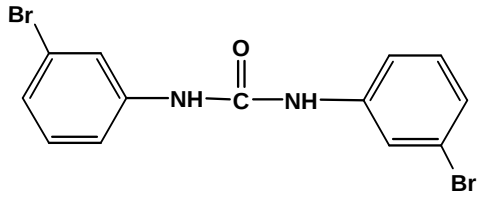




yield %=86.67

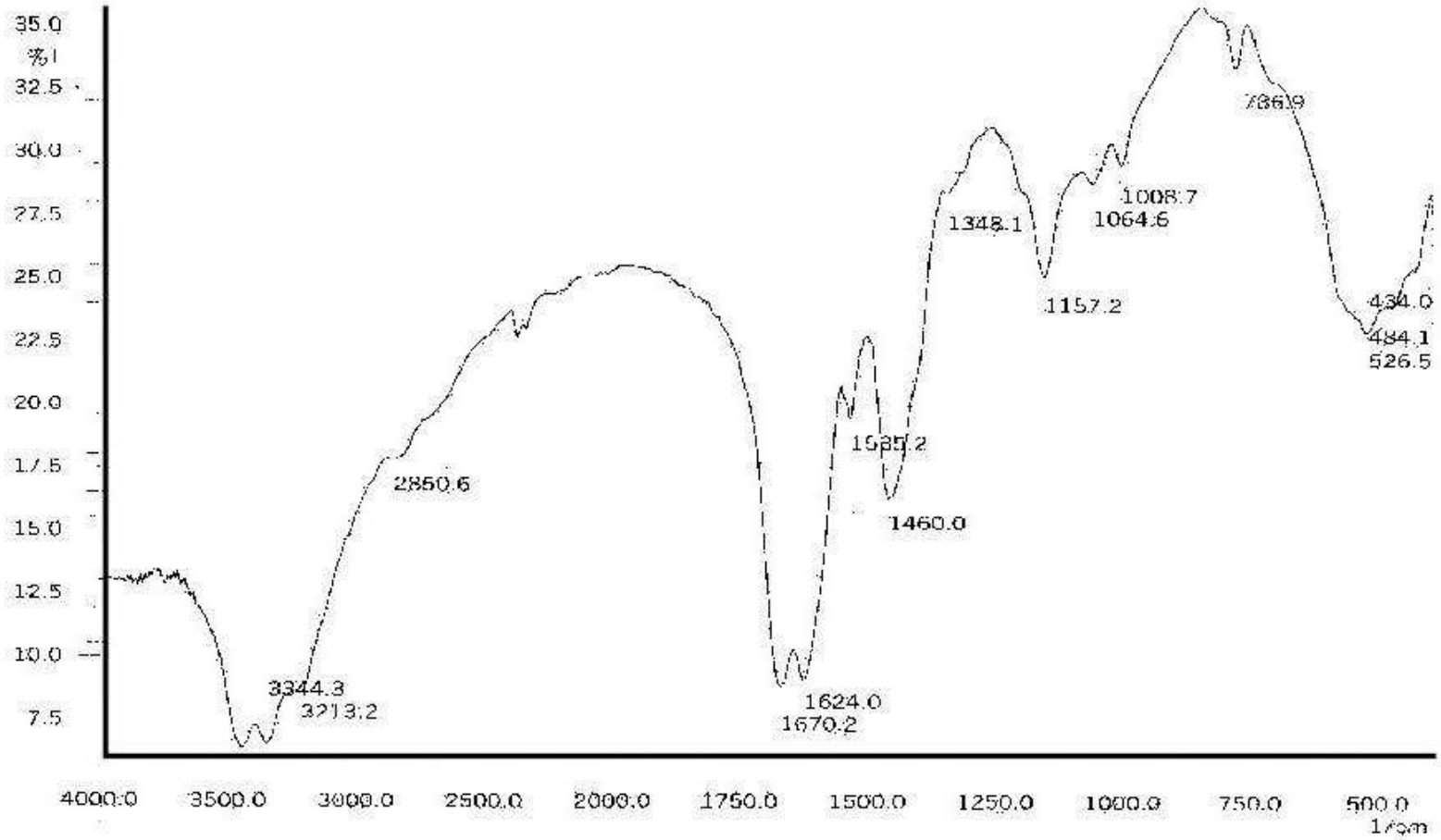
Figure (12): structure, yield %and F.T.IR spectrum of N,N´-bis(2-methoxyphenyl)urea

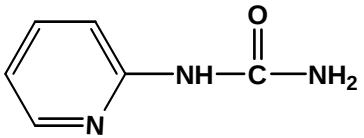




yield %=93.3

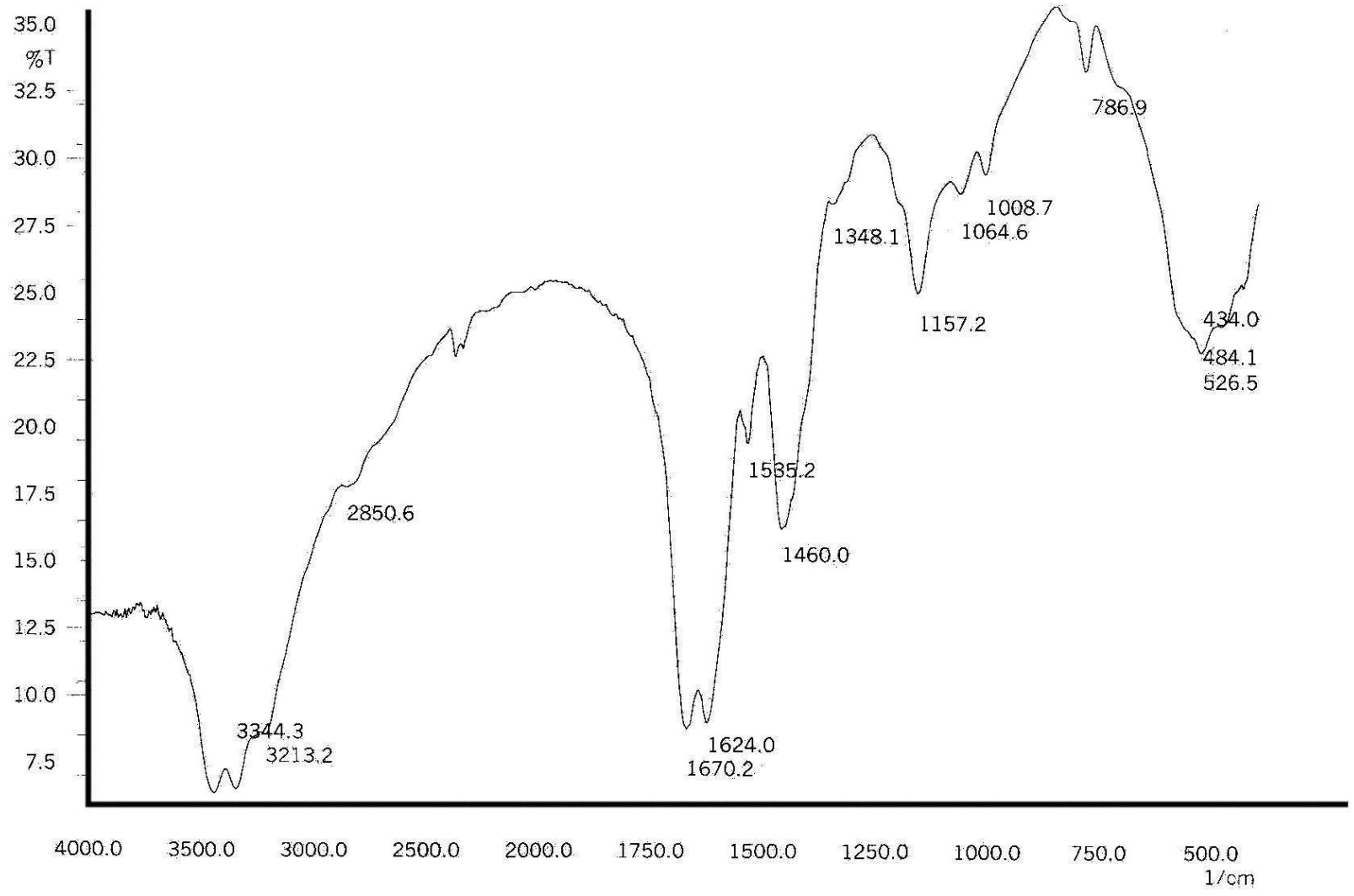
Figure (11): structure, yield % and F.T.IR spectrum of N,N´-bis(3-bromophenyl)urea

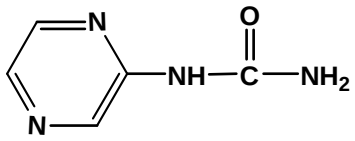




yield %=87.59

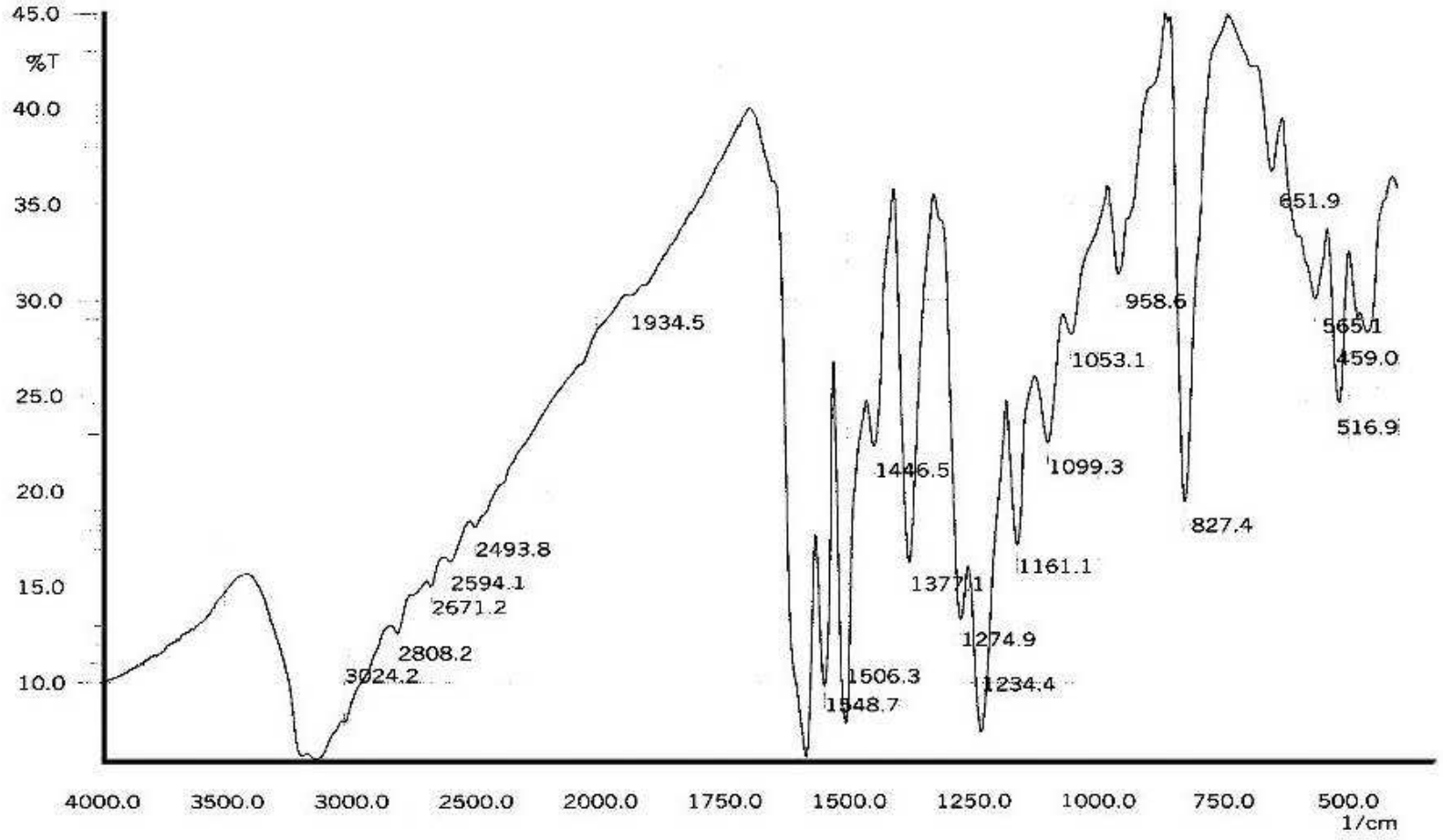
Figure (10): structure, yield % and F.T.IR spectrum of N-pyridin-2-ylurea

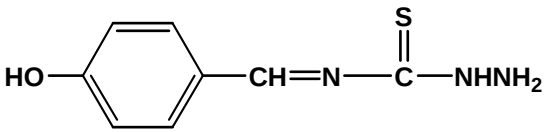




yield %=86.9

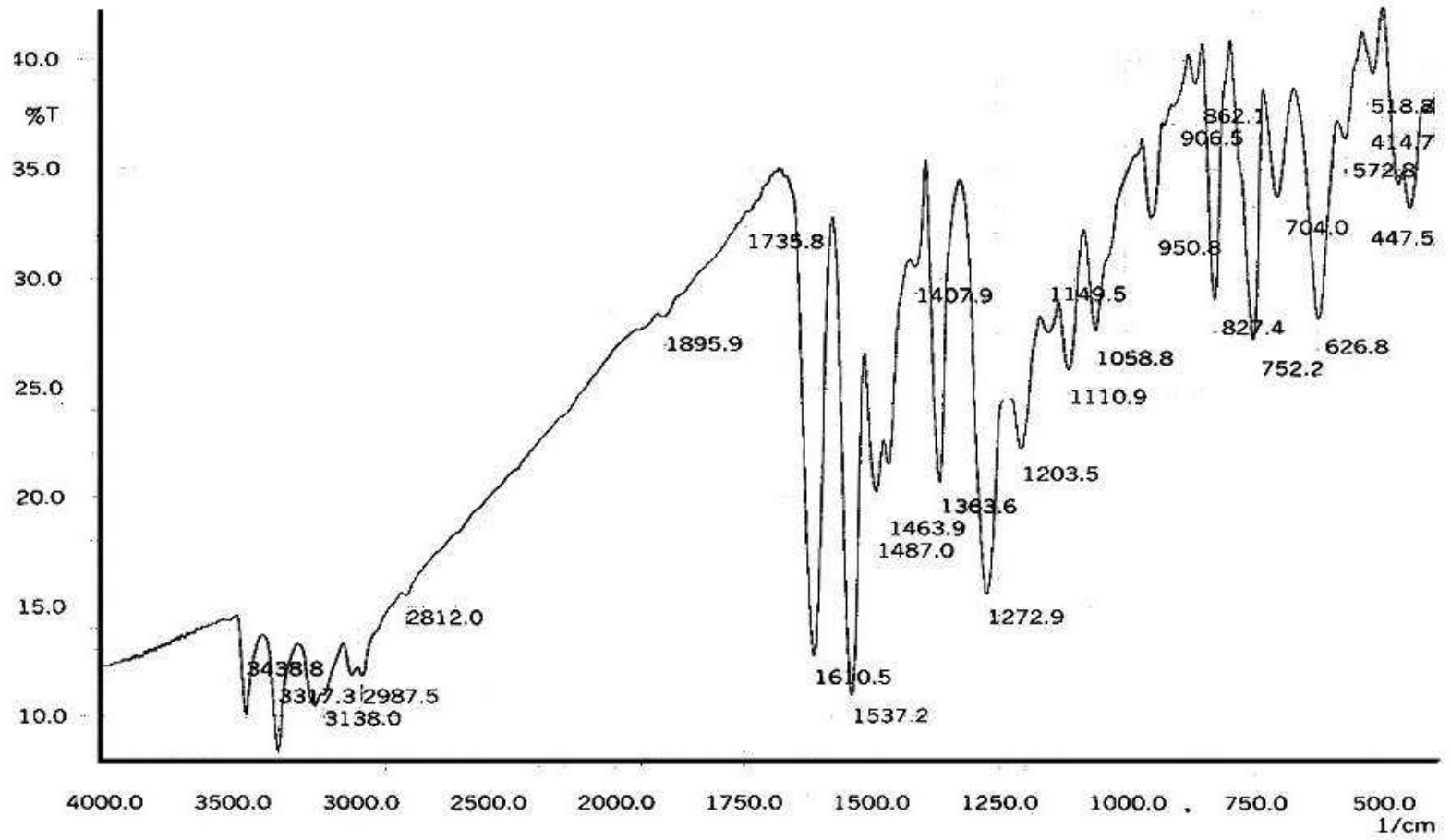
Figure (9): structure, yield %and F.T.IR spectrum of N-pyrazin-2-ylurea

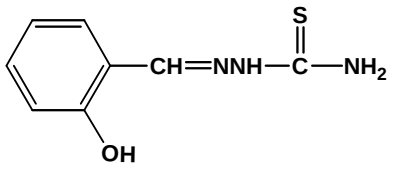




yield %=80.95

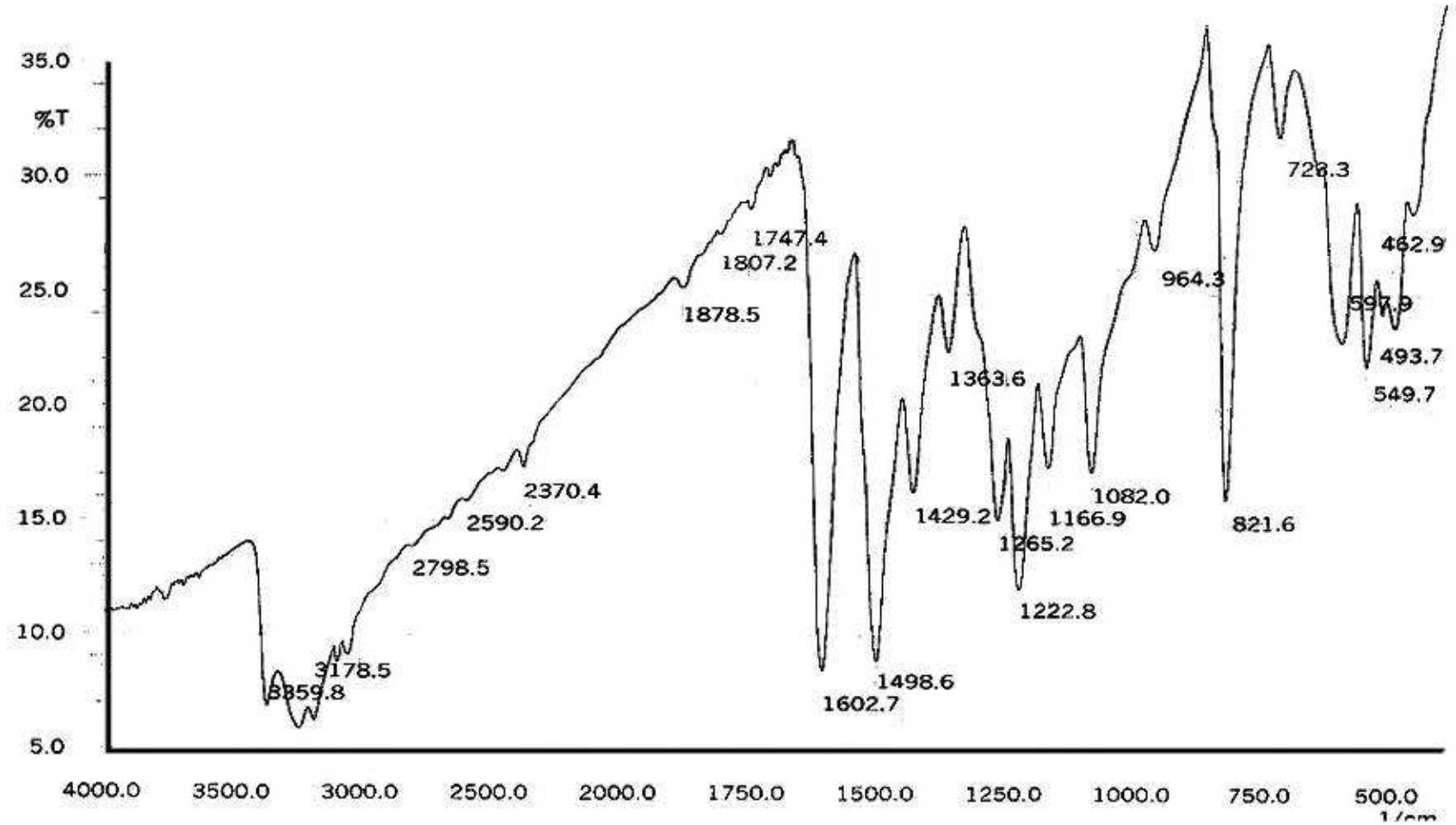
Figure (13.1): structure, yield % and F.T.IR spectrum of N-(4-hydroxybenzylidene)hydrazinecarbothioamide

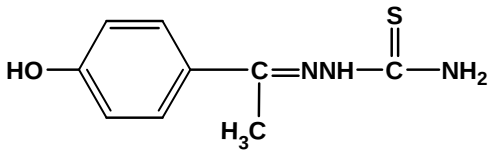




yield %=90.5

Figure (13.2): structure, yield %and F.T.IR spectrum of 2-hydroxybenzaldehydethiosemicarbazone





yield %=69.56

Figure (13.3): structure, yield %and F.T.IR spectrum of 1-(4-hydroxyphenyl)ethan-1-onethiosemicarbazone

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

تحضير مشتقات اليوريا ودراسة تطبيقاتها على إنزيم اليوريز

رسالة
مقدمة إلى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
نور رياض حسن
بكالوريوس ٢٠٠٣ (جامعة النهرين)

١٤٢٧ هـ ٢٠٠٦م

بسم الله الرحمن الرحيم

قَالَ رَبِّ اشْرَحْ لِي صَدْرِي(٢٥)وَيَسِّرْ لِي
أَمْرِي(٢٦)وَاحْلُلْ عُقْدَةً مِنْ
لِسَانِي(٢٧)يَفْقَهُوا قَوْلِي(٢٨)

(صدق الله العظيم)

الإهداء

إلى من يرى الناس فيَّجهودهما...

أبي وأمي إلى

نبع الاخوة ووفاء الصديق...

أخي

إلى عزيزة قلبي ورفيقه طفولتي...

أختي

إلى كل من ساعد في اخراج هذا البحث المتواضع...

زملائي وأحبابي

نور.....

الخلاصة

تضمنت الدراسة الحالية تحضير عدد من مشتقات اليوريا من تفاعل اليوريا او الثايويوريا مع امينات حلقية. كما في المركبات:

(phenylthiourea, N-phenyl urea, N-(2-methoxyphenyl) urea, N-(4-aminophenyl) urea, N-(2-methylphenyl) urea, N-(2-methoxyphenyl) thiourea, N-[4- (amino carbonyl) amino phenyl] urea, N-[4-(amino carbonyl)amino phenyl]acetamide, N-pyrazin-2-ylurea, N- pyridin-2-ylurea, N,N´-bis(3-bromophenyl)urea, N,N´-bis(2- methoxyphenyl) urea).

بالاضافة الى تحضير ثلاث مركبات تعتبر من قواعد شف التي حضرت من تفاعل (thiosemi carbazide) مع الديهايد وكيتون حلقي كما في المركبات:

(N-(4-hydroxybenzylidene) hydrazinecarbothioamide, 2-hydroxybenzaldehydethiosemicarbazone, 1-(4-hydroxyphenyl) ethan-1-onethiosemicarbazone)

تم تشخيص المركبات المحضرة بواسطة الطريقة الطيفية،(طيف الاشعة تحت الحمراءFTIR) وتم التاكد من نقاوة المركبات المحضرة بواسطة كروموتوغرافيا الطبقة الرقيقة(TLC).وكانت نقاوة المركبات جيدة وقيست درجة الانصهارلهذه المركبات.

كمـا و تـم قيـاس الفعاليـة المضـادة لبكتريـا (*proteus mirabilis*) اتجـاه المركبـات المحضـرة بطريقـة الانتشـار حـول القـرص فـي الطبـق، حيـث لـوحظ لهذه المركبات فعالية مضادة للبكتريا(Antibacterial activity).

كما تم دراسة تاثير بعض من المركبات المحضرة على نشاط انزيم اليوريز البكتيري واظهرت تاثير جيد في تثبيط نشاط الانزيم

،حيث اظهرت بعض المركبات تثبيط من النوع التنافسي والبعض الاخر من النوع الغير تنافسي.

ومن ثم تمت دراسه حركيات الانزيم واستخرجت قيم كل من K_i، $V_{max.}$، K_m، i%.حيث استخدمت معادلة الخط المستقيم لاستخراج هذه القيم (Line weaver Burck equation).

Studies on enzyme inhibition remain an important area of pharmaceutical research since these studies have led to the discoveries of drugs useful in a variety of physiological conditions. The enzyme inhibitors can interact with enzymes and block their activity towards natural substrates. Urease inhibitors have recently attracted much attention as potential new anti-ulcer drugs and in the treatment of urolithiasis. Ironically, urease was the first enzyme crystallized but its mechanism of action is still largely misunderstood. Inhibitors of urease can be broadly classified into two categories: **(1)** active site directed (substrate-like), **(2)** mechanism-based directed. Urease, due to its high substrate (urea) specificity, can only bind to a few inhibitors with a similar binding mode as urea. Several non-covalent interactions including hydrogen bonds and hydrophobic contacts stabilize the enzyme-inhibitor complex**.** Many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers. [(1)]

1:1 Urease (Urea amido hydrolase) :

Is one of the enzymes belongs to the group (hydrolases) which has classification number (E.C.3.5.1.5), and this enzyme contains nickel ion to induce urea hydrolysis to evolve ammonia and carbamate molecule and carbonic acid according to the equation in figure (1-1):

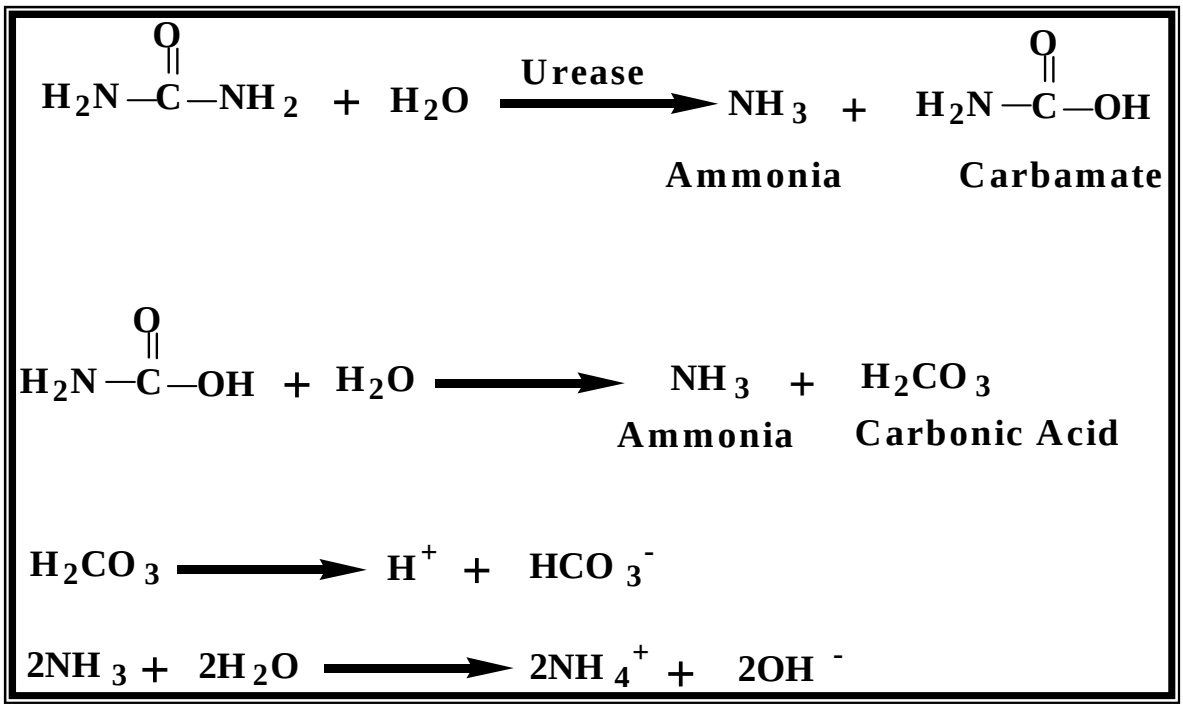


Figure (1-1): Equation of urea hydrolysis to evolve ammonia and carbamate molecule and carbonic acid

After equilibrium between ammonia and water occurs which results in ammonium hydroxide that rapidly increases pH [2,3,4,5]. Musculus (1876)is considered to be the first that studied the bacterial urease after that Miquel (1890) diagnosed the production of urease in many microorganisms including bacteria [6,7,8,9,10,11,12,13,14].Urease is produced by pathogenic or nonpathogenic bacteria [15,16]. Urease is very important as it helps causing disease and it is considered a virulence factor in many bacteria, it has been shown the production of such enzyme in about 100 types of bacteria [4].

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

All these bacteria produce this enzyme that has principal role in urolithiasis by increasing the pH from 5 to 9 causing the mineral salts to precipitate in mucous material [17] which is produced by the bacteria and enters in its cellular structures and acts as naivus 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].

Helicobacter pylori is considered as the main cause of gastritis, and the virulence factor of this bacteria is the production of urease to protect it from the acidic media of the stomach and also it causes an increase in ammonium hydroxide that produces erosion of the gastric mucosa colonized by the bacteria[18].

Urease produced by *ureaplasma urelyticum* leads to acute pulmonary infections as it causes erosion of the bronchial epithelium [19].

The urease produced by *streptococcus salivarius* that colonizes the buccal cavity and alters unhealthy conditions lead to teeth cavities [20].

Urease enzyme is also found in soil because of the organisms the live in soil and have role in nitrogen metabolism and soil fertilizations [21, 22].

1:2 Cellular Localization of Urease:

Urease is extracellular enzyme [23, 24] and most studies show that urease concentrates in yeasts and cytoplasmic parts of most bacterial strains [3, 4, 25] as enzyme is detected in the cytoplasm of 22 bacteria [26]. Electronic microscope shows the conjugation of the inner membrane to *staphylococcus* and in the periplasm and outer membrane of *Proteus mirabilis* [3].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[27].

1:3 Urease Regulations in 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 initiated when nitrogen quantity in environment is limited [3, 19, 28].

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 ,lysine.[4]

In *streptococcus salivarius*, enzyme production is regulated by surrounding pH; as low pH of this bacteria produce urease to increase pH of the media. In *Bordetella bronchioseptica* urease production is regulated thermally [29].While urease production in *Pseudomonas aeruoginosa* is regulated by the nitrogen regulation from ammonia and the following enzymes:

Glutamine synthetase, NADP-dependant Glutamate dehydrogenase and glutamate synthetase [30, 31]. As bacteria prefers nitrogen metabolism to ammonia with glutamine or glutamate according to the following equations in figure (1-2): [26]

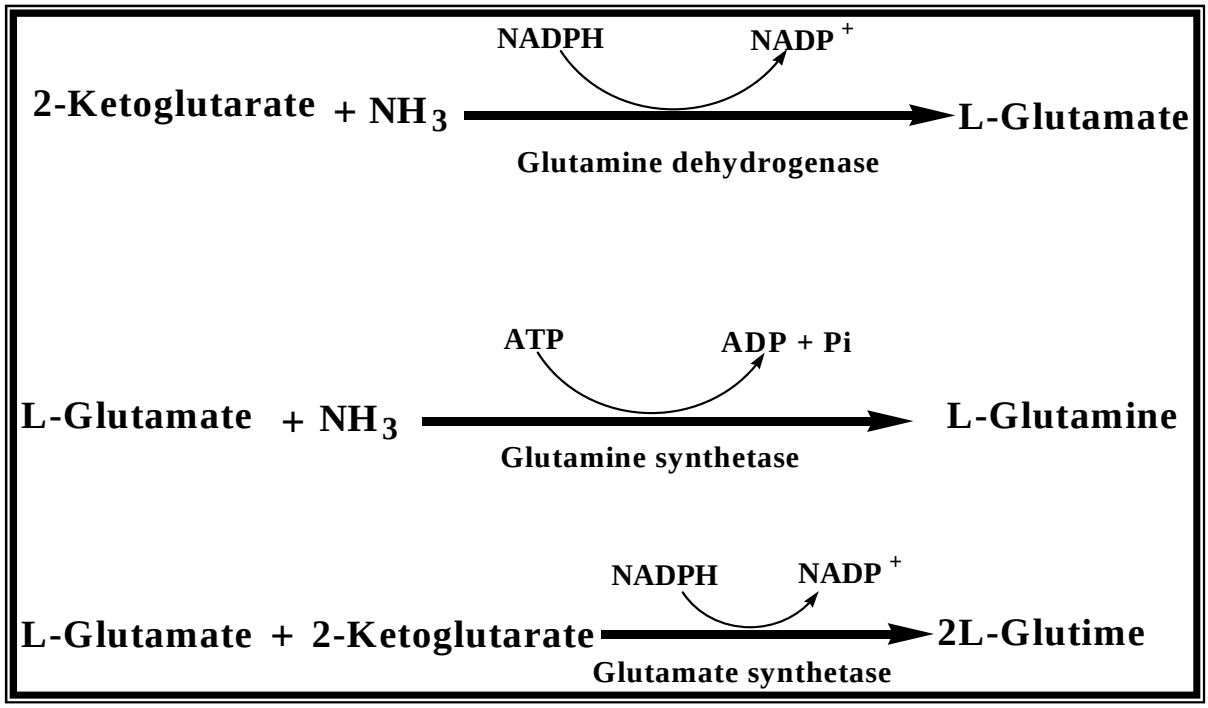


Figure (1-2): Nitrogen metabolism to glutamine or glutamate

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 [3].

1:4 Urease Mechanisms:

It is possible to demonstrate urease mechanism as assumed by Zerner and is defined as Zerner Model in the active site [32].

The figure (1-3) shows the presumed mechanism for the action of urease [19].

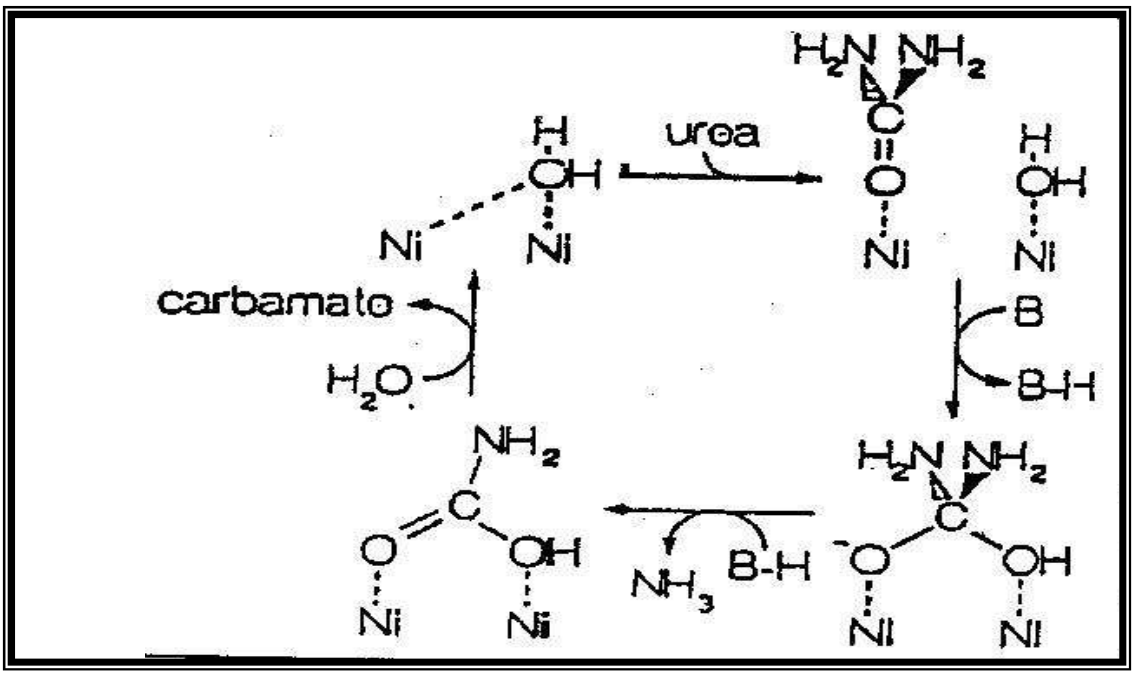


Figure (1-3): The presumed mechanism for the action of urease of Zerner model [19].

It is thought to be association between nickel ions with water molecule or nickel ions with hydroxide.

Mechanism of urease action depends on different nickel ions .One of the steps depends on connection and stimulation of urea molecule, and the other step of water molecule stimulation [33].

Study was done on the crystal form of urease structure and it was found that urea binds covalently with first nickel ion that substitute water ion through covalent bond of oxygen atom of urea to first nickel ion with the assistance of histidine. The following figure (1-4) shows nickel role in urease action [34].

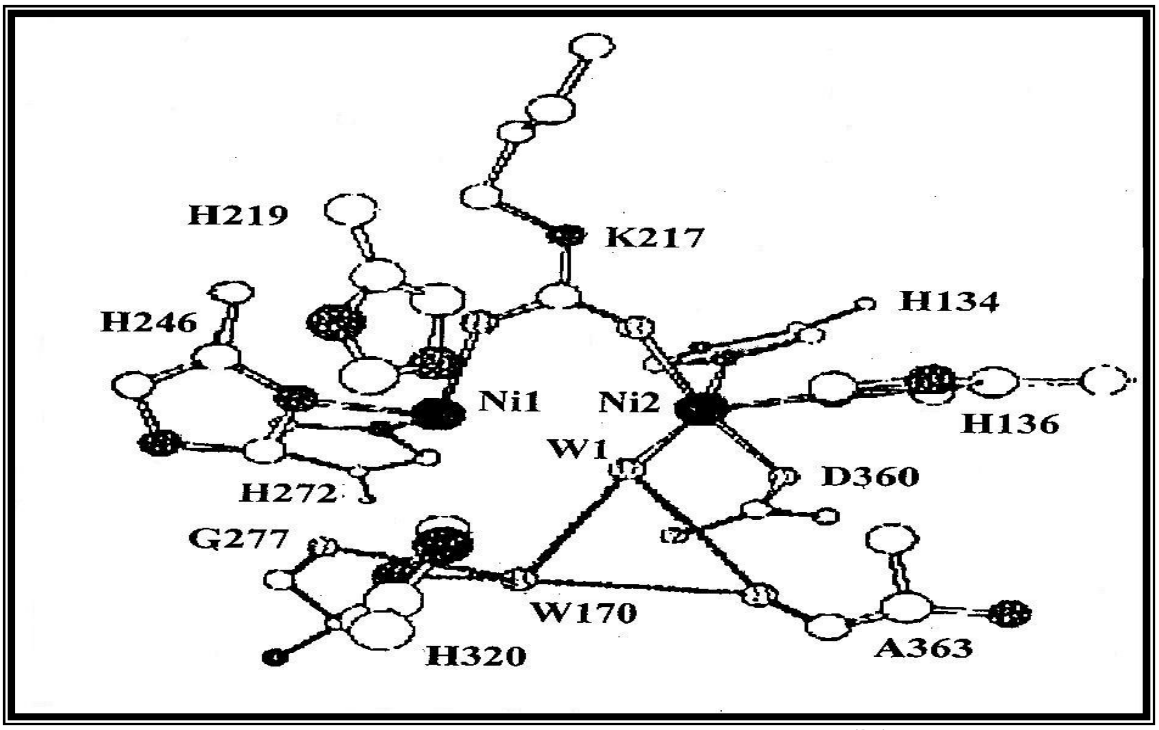


Figure (1-4): Nickel role in urease action [34]

There is a region at the active site (β-Histidne, 320) that works on stimulation of water molecule conjugated with the metallic ion and a state of resonance occur by having nitrogen for the positive and the presence of negative charge on hydroxyl group conjugated with metal, then these two metallic sites conjugate when attack hydroxyl that covalently binds with nickel ion [2, 19, 35].

There are evidences that point for the presence of a system for urea transplant depends on the energy in bacteria *Pseudomonas aeruginosa* ,there is a transplant protein specialized in the cytoplasmic membrane for transport of urea and stimulate ATP synthetase as in the hydrolysis of urea; the pH increases and it results from it a gradual concentration of protons that stimulates the ATP synthetase for energy production, and there is a specialized protein for the transport of ammonium ion ,

ammonia and carbon dioxide diffuse from the membrane with no need of media in some organisms, there is also a specialized protein depend on energy in presence of magnesium ion to transport nickel ion for the production of effective urease [3,36]. The figure (1-5) shows urea, ammonium ion and nickel transport system in bacteria [3].

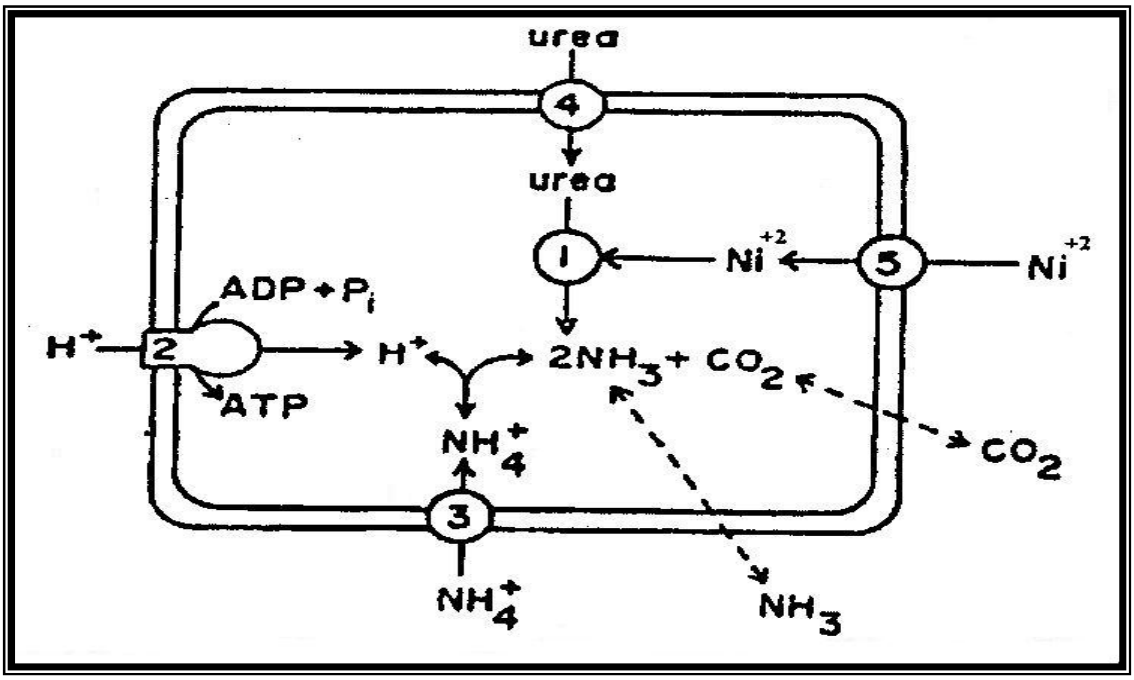


Figure (1-5): Urea, NH_4^+ and nickel transport system in bacteria [3]

1:5 Enzyme specificity:

This term is used to express the specificity of enzyme to stimulate one reaction or a limited number of reactions [37].these enzymes are considered as catalyst with high specificity, there are great differences between specificity of enzymes and it divided enzymes into:

1. High specificity which is specific for one material.

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

Urease is considered to be high-specific enzyme towards urea. There is another category towards divisions [38].

(a) Low specificity: And also called (Bond specificity) as enzymes (peptides, phosphates, and esterases) 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 [39].

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 [40].

1:6 Urease Importances for Different Organisms:

1:6:1 Animals:

For ruminants, they can utilize urea presence in blood to form proteins in nitrogen cycle that involves urea transport from blood to stomach then hydrated to ammonia and carbon dioxide by bacterial urease presence in stomach then hydrated to ammonia and carbon dioxide by bacterial urease presence in stomach, and ammonia is used as a source of nitrogen by microbes to form protein, then digested and absorb microbial proteins in alimentary canal. [41]

1:6:2 Microorganisms:

Urease enzyme is considered as virulence factor for one kind of bacteria [6, 42, 43].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[44,45,46].

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. [80, 82]

1:7 Proteus Bacteria

1:7:1 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 [47]. These bacteria are present widely in nature and play a very important role in organic substances degradation and were isolated from soil, water, home [48]. 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 can not. [49]

These bacteria can utilize urea by urease and liberate ammonia, CO_2 which elevate pH of the media to basic limit. [50, 51]

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 enzyme production and swarming [52].These bacteria spread among patients in hospital as nosocomial infection leading to difficult treated infection [56] and may be lethal in (15-80%) [57].

As patients with *proteus* is considered as source of infection to human himself (autoinfection) and others [58, 59].

1:7:2 Clinical Significance of Proteus:

Proteus is considered as opportunistic microorganism that belongs to enterobacteriaceae for its effect on health of human and animals [59, 60].as it causes gastrointestinal infection and genitourinary infection and its clinical significance comes after *E.coli, Klebsiella pneumoni* as they cause UTI[61,62]. It has been isolated from Urinary tract, wounds, burns, skin, eyes, ears, nose, and larynx and from alimentary tract [63].*Proteus* causes UTI in elderly patients [61]as it constitutes 15 % from bacterial infection of the urinary system [62,64,65]in addition to other surgical operations[66].*Proteus* also causes nosocomial infections [67]. 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. [68]. 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 [69].Catheterization is considered as main source for urinary tract infection [68, 70]. Infection with catheters constitutes about 12% of the urinary tract infection, In addition to other instruments [71].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 [72].

While Chane found that 70.4% of the infection sources are tools and contaminated instruments with *Proteus* [73].*Proteus mirabilis* constitutes about 70-90 % of the infection by Proteus [74].

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 [75].

Urinary system is exposed to many kinds of pathological bacteria that cause autoinfection and nosocomial infection like *E.coli, Klebsiella, Enterobacter,Serratia* species, *Pseudomonas aeruoginosa ,Staphylococcus saprophyticus ,S.epidermidis* , Candida ,*Enterococcus faecalis* in addition to *Proteus* species .[96]

Ankler [76,77] 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 [96].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[3,78].Its thought that *Proteus* has important role in rheumatoid arthritis as antibodies for the bacteria were found in those patients [79,80,81].

1:7:3 Virulence factors:

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

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 [82, 83, and 84].

2. Falgella and Swarming motility [19, 85]

3. Hemolysin:

Proteus bacteria can secrete hemolysin that destructs RBC and is one of factors that help in invasion [86].

4. Outer Membrane Proteins (OMPs) :[87, 88]

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 [89].

6. Capsule polysaccharide (CPS):

The capsule of *Proteus* bacteria have relation with their pathogenicity because of its ability to collect metallic magnesium ions as result of presence of some acids like uric acid, pyruvic acid or phosphate groups that result in urolithiasis [80, 90].

7. Natural resistance to polymyxine:

Proteus bacteria resist cyclic antibiotics of (PMX) kind because of connection of positive charge present in their structure with negative charge of lipid A region [91, 92].

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 [93, 94].

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) [3,95].

10. Protease production:

Proteus can resist the immune system by production of protease that split peptide chains present in immunological activities that permit the establishment of infection [95].

The urinary tract infection is common in community and hospitals [98] .In most cases infection is chronic and recurrent and 20% in chronic cases infection stops after therapy [99]. There are two kinds of infection is relapse and reinfection. In the first condition, the cause of infection is same of previous infection not like the second case.

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 [100]. Or lymphatogenous route from lymphatics [100], urinary system is infected with many infections leading to

disturbances of renal infections some of lead to end stage renal disease [100,101,102]. 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 [103], or the disease occur as mutation in alleles like renal coloboma syndrome while bladder is affected by benign or malignant lesion [103].

1:8 Chemotherapy of Infections:

Infection is major category of human disease and skilled management of antimicrobial drugs is of the first importance. The term chemotherapy is used for the drug treatment of parasitic infections in which parasites (viruses, bacteria, protozoa, fungi, and worms) are destroyed or removed without injuring the host. The use of the term to cover all the drug or synthetic drug therapy needlessly removes a distinction which is convenient to the clinician and has the sanction of long usage- By convention the term also used to include therapy of cancer. [104]

1:8:A History of Chemotherapy:

Many substances that we now know to posses therapeutic efficacy were first used in distant past. The ancient Greeks used male fern, and the Aztecs chenopodium, as intestinal antihelminthics.The ancient Hindus treated leprosy with chaulmoogra for hundreds of years mould have been applied to wounds, but despite the introduction of mercury as a treatment for syphilis (16th century) and the use of cinchona bark against malaria (17th century) the history of modern rational chemotherapy did not begin until

Ehrlich developed the idea from his observation that aniline dyes selectively stained bacteria in tissue microscopic preparations and could selectively kill them. [105,106]

1:8: B Classifications of antimicrobial drugs:

Antimicrobial agents may be classified according to the type of organism against which they are active and in this book follow the categories:

1. Antibacterial drugs
2. Antiviral drugs
3. Antifungal drugs
4. Antiprotozoal drugs
5. Antihelminthic drugs.

1:8: C How antimicrobials act:

It should always be remembered that drugs are seldom the sole instruments of cure but act together with the natural defenses of the body.
In general microorganisms are inhibited or killed by cell wall damage or cell wall synthesis inhibition or impairment of cytoplasmic membranes permeability or altering physical and chemical structures of protein of these microorganisms or through impairment of cellular enzymatic activities or protein and DNA synthesis inhibition [124].And also the bacterial resistance for the chemical compounds may be due to the presence of natural resistance or resistance resulted from genetic mutations or due to plasmid resistance or due to absence of suitable transporter for the compound and thus can not reach the target in the cell [125] or due to thick cell wall as it contains high lipid in the wall. Antimicrobials act at different sites in the target organism as follows:

1:8: C: 1 The cell wall:

This gives the bacterium its characteristic shape and provides protection against the much lower osmotic pressure of the environment. Bacterial multiplication involves breakdown and extension of the wall; interference with these processes prevents the organism from resisting osmotic pressures, so that it bursts. As these cells of higher, e.g. human, organisms do not possess this type of wall, drugs, which act here, may be especially selective, obviously the drugs are effective only against growing cells. They include: penicillins, cephalosporins, vancomycin, bacitracin, cycloserine. [107].

1:8: C: 2 The cytoplasmic membrane:

Inside the cell wall is the site of most of the microbial cell's biochemical activity. Drugs that interfere with its function include: polyenes (nystatin, amphotericin), azoles (fluconazole, itraconazole, minconazole) polymixins (colistin, poly-myxin B). [106,107]

1:8: C: 3 Protein syntheses:

Drugs that interfere at various points with the build-up of peptide chains on the ribosomes of the organism include: chloramphenicol, erythromycin, fusidicacid, tetracyclines, aminoglycosides, quinupristindalfopristin, linezolid.

1:8: C: 4 Nucleic acid metabolism:

Drugs may interfere directly with microbial DNA or its replication or repair, e.g. quinolones, metronidazole, or with RNA, e.g. fifampicin indirectly on nucleic acid synthesis, e.g. sulphonamides, trimethoprim.

1:8: D Use of antimicrobial drugs:

***Selection of antimicrobial drugs:**

The general rule is that selection of antimicrobials should be based on identification of the microbe and sensitivity tests. All appropriate specimens (blood, pus, urine, sputum, cerebrospinal fluid) must therefore be taken for examination before administering any antimicrobial. this process inevitably takes time and therapy at least more serious infections must usually started on the basis of the " best guess " with the worldwide rise in prevalence of multiply-resistant bacteria in the last decade knowledge of local antimicrobial resistance rates is an essential prerequisite to guide the choice of local " best guess " or empirical Antimicrobial therapy. Knowledge of the likely pathogens (and their current local susceptibility rates to antimicrobials) in the clinical situation thus cephalexin may be a reasonable first choice for lower urinary tract infection and benzylpenicillin for meningitis in the adult (Meningococcal or pneumococcal).

***Rapid diagnostic tests:**

Use of tests in this type is about to undergo a revolution with the widespread introduction of affordable , sensitive and specific nucleic acid detection assays (especially those based on the polymerase chain reaction – PCR).

*** Route of administration:**

Parentral therapy (which may be IM. or IV) is preferred for therapy of serious infections because high therapeutic concentrations are achieved reliably and rapidly. Initial parentral therapy should be switched to the oral route whenever possible. [105,106,108]

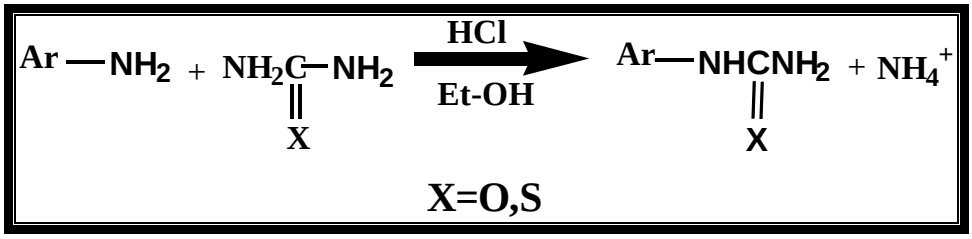
1.9 Aim of the work:

1. Preparation of the compounds (urea analogues and Schiff bases) which are considered as urease inhibitors with antimicrobial activity to treat urolithiasis in UTI (Urinary Tract Infection) caused by *Proteus mirabilis.*

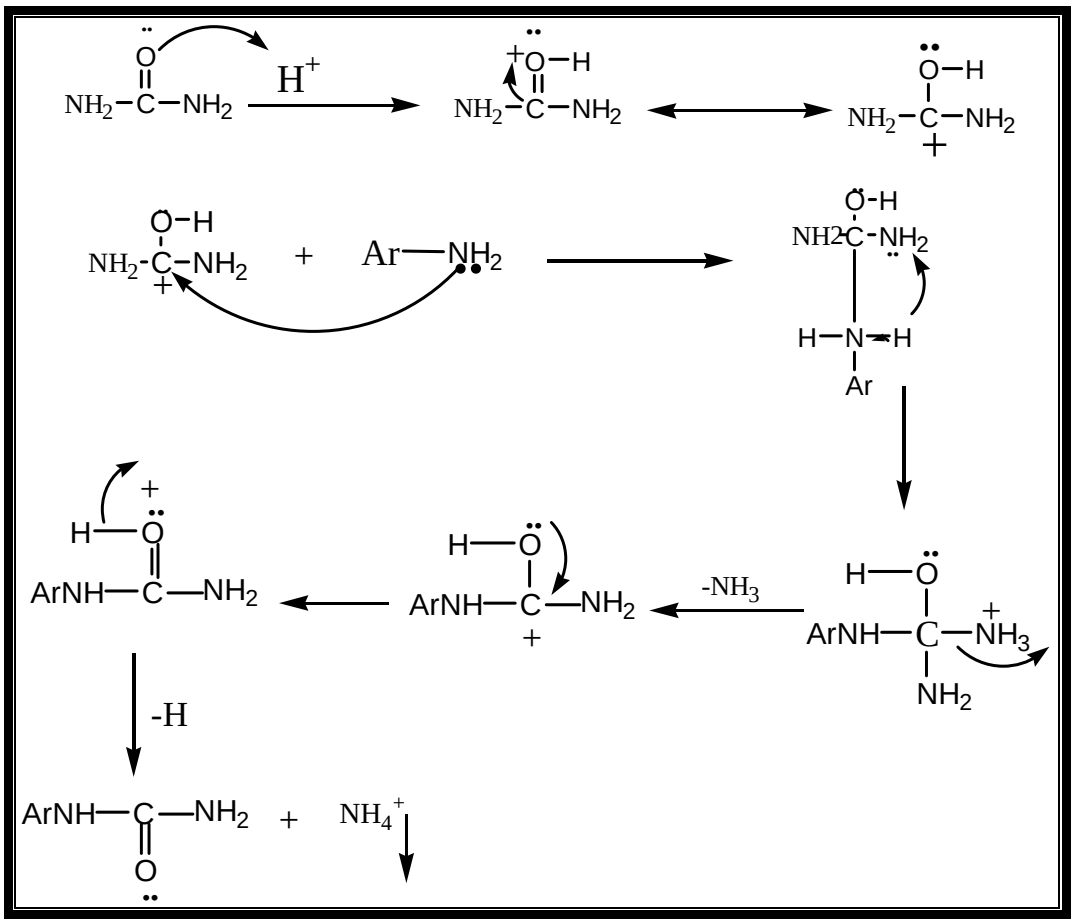
2. Examination of Biological and enzymatic activities of these compounds.

3.1.1 Urea derivatives preparations:

Compounds (1-12) which are urea derivatives or analogues; according to table (3-1). They were prepared from the reaction of urea or thiourea with aromatic amines in absolute ethanol solvent with presence of concentrated HCl as a catalyst according to the following equation:



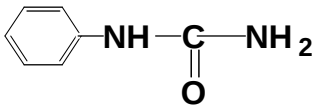
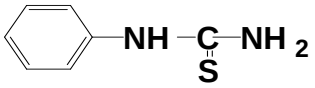
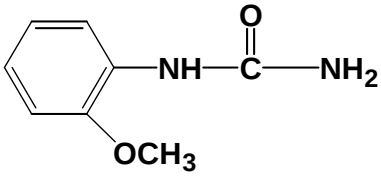
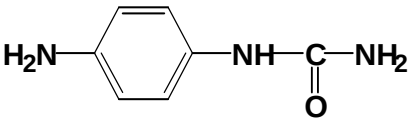
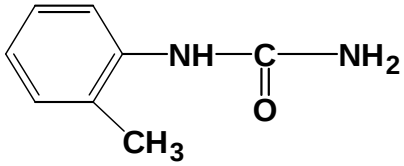
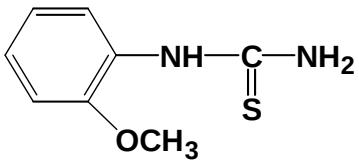
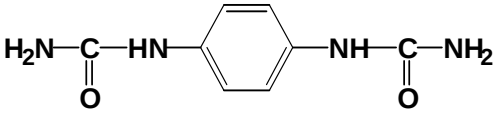
This reaction is considered as electrophilic nucleophilic reaction. Where amines represent the nucleophil and urea or thiourea represents the electrophil. The catalyst, concentrated HCl , increases the reaction rate through urea salts formation which are considered as strong electrophil that forms the amines, strong nueclophil attacks urea salts to form urea derivatives or urea substituted compounds that release NH_4^+ as demonstrated through the following suggestive mechanism :



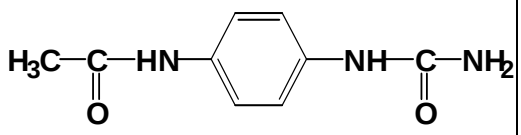
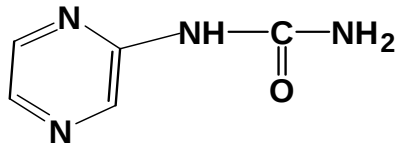
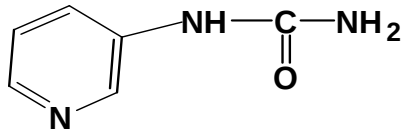
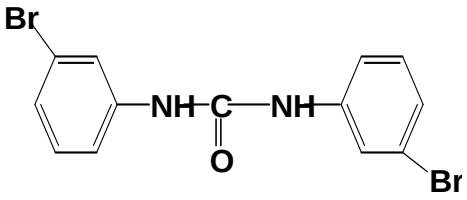
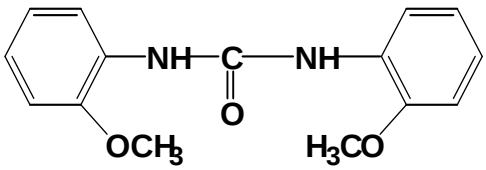
TLC (thin layer chromatography) technique was used to examine the formation and purity of the compounds by using a suitable mixture as eluent. FTIR was also used for examination.

Table (3-1) shows the physical properties of compounds (1-12).

Table (3-1): The physical properties of urea derivatives compounds.

No.	Structure of compounds	Chemical formal	M.wt.	Melting point(m.p.)	color
1.		$C_7H_8N_2O$	136.15	170-172	white
2.		$C_7H_8N_2S$	152.22	178-180	white
3.		$C_8H_{10}N_2O_2$	166.18	216-218	violet
4.		$C_7H_9N_3O$	151.17	265-267	Dark brown
5.		$C_8H_{10}N_2O$	150.18	193-195	violet
6.		$C_8H_{10}N_2OS$	182.24	222-224	Grey
7.		$C_8H_{10}N_4O_2$	194.19	>350	Deep violet

Continued table (3-1): The physical properties of urea derivatives compounds.

No.	Structure of compounds	Chemical formal	M.wt.	Melting point(m.p.)	color
8.		$C_9H_{11}N_3O_2$	193.2	>350	Brown
9.		$C_5H_6N_4O$	138.13	290-292	brown
10.		$C_6H_7N_3O$	137.14	230-232	brown
11.		$C_{13}H_{10}N_2OBr_2$	370.04	>350	Grey
12.		$C_{15}H_{16}N_2O_3$	272.3	>300	Brown

3.1.2 Infrared spectra for urea derivatives compound:

The FTIR for compound (N-phenyl urea) as shown in table (3-2) absorption band of carbonyl of amide group at (1657 cm^{-1}) could be attributed to (C=O) stretching vibration. (C=C) aromatic group showed absorption band at (1598.9cm^{-1}) could be attributed to (C=O) aromatic stretching vibration absorption band of (-NH_2) group at (3427.3-3313 cm^{-1}) could be attributed to (-NH_2) stretching vibration [115,116,117]. The FTIR for compound (N-phenylthiourea), absorption band of thioamide group was at (1193.9 cm^{-1}) could be attributed to (C=S) stretching vibration. (-NH_2) group absorption band was at (3361.7-3271 cm^{-1}) could be attributed to (-NH_2) stretching vibration. The absorption band of (C=C) aromatic group at (1606.6 cm^{-1}) could be attributed to (C=C) aromatic stretching vibration. Absorption band of (C-H) aromatic was at (3168.8 cm^{-1}) could be attributed to (C-H) aromatic stretching vibration [115,116,118]. In FTIR for compound (N-(2-methoxyphenyl) urea), absorption band of carbonyl of amide group at (1662.5 cm^{-1}) could be attributed to (C=O) stretching vibration. o-substitution absorption band peak was shown at (750.3 cm^{-1}) could be attributed to o-substitution bending vibration. Amino group absorption band peak was shown at (3336.6-3199 cm^{-1})

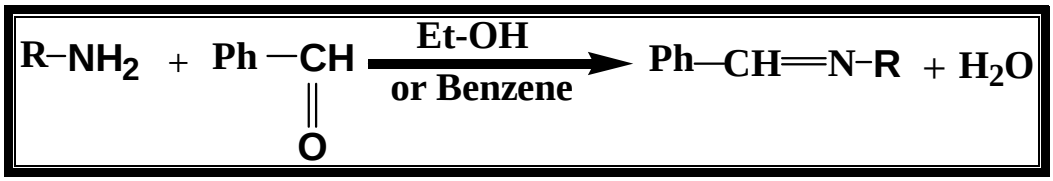
Could be attributed to (-NH_2) stretching vibration. In FTIR for compound (N-(2-methylphenyl) urea) as shown in table (3-2); absorption band of carbonyl of amide at (1662 cm^{-1}) could be attributed to (C=O) stretching vibration, (-NH_2) group showed absorption band at (3444.6-3336.6 cm^{-1}) could be attributed to (-NH_2) stretching vibration. m-substitution showed peak absorption band at (750.3cm^{-1}) could be attributed to m- substitution bending vibration [115,116,117,118]. FTIR for compound (N-(2-methoxyphenyl) thiourea) as shown in table (3-2); the absorption band of the thioamide group was at (1606 cm^{-1}) could be attributed to (C=S) stretching vibration. (-NH_2) group showed peak absorption band at (3274.9-3350 cm^{-1}) could be attributed to (-NH_2) stretching vibration , o- substitution showed peak absorption band at (748.3 cm^{-1}) could be attributed to o- substitution bending vibration [115,116,117] .The FTIR for compound (N-(4-aminophenyl)urea) as shown in table (3-2); carbonyl of amide group showed absorption band at (1680 cm^{-1}) could be attributed to (C=O) stretching vibration. p-substitution showed peak absorption band at (831.3 cm^{-1}) could be attributed to p- substitution bending vibration. The absorption band of the (C=C) aromatic group appeared at (1614 cm^{-1}) could be attributed to (C=C) stretching vibration. In the FTIR for compound (N-[4- (amino carbonyl) amino phenyl] urea) as shown in table (3-2); there is symmetrical structure where (-NH_2) showed peak absorption

Band at (3344.3-3444.6 cm^{-1}) could be attributed to (-NH_2) stretching vibration. Carbonyl of amide group showed absorption band at (1679.9cm^{-1}) could be attributed to (C=O) stretching vibration. P-substitution showed peak absorption band at (833.2 cm^{-1}) which could be attributed to p-substitution bending vibration [116,118]. In compound (N-[4-(amino carbonyl) amino phenyl] acetamide) as shown in table (3-2); carbonyl of amide group showed absorption band at (1664.5 cm^{-1}) could be attributed to (C=O) stretching vibration. Absorption band of the (-NH_2) group was at (3257-3465.8 cm^{-1}) could be attributed to (-NH_2) stretching vibration, p-substitution showed peak absorption band at (827.4 cm^{-1}) which could be attributed to p-substitution bending vibration [116,118]. The FTIR for compound (N, N´-bis (2-methoxyphenyl) urea) as shown in table (3-2); the absorption band of one peak (-NH) group at (3330.8 cm^{-1}) could be attributed to (-NH) stretching vibration. The absorption band of carbonyl of amide group at (1674 cm^{-1}) could be attributed to (C=O) stretching vibration, o- substitution showed peak absorption band at (734.8 cm^{-1}) which could be attributed to o- substitution bending vibration. In compound (N, N´-bis (3-bromophenyl) urea) as shown in table (3-2), the absorption band of one peak (-NH) group at (3288 cm^{-1}) which could be attributed to (-NH) stretching vibration. The absorption band of carbonyl of amide group at (1637.5 cm^{-1}) could be attributed to (C=O) stretching

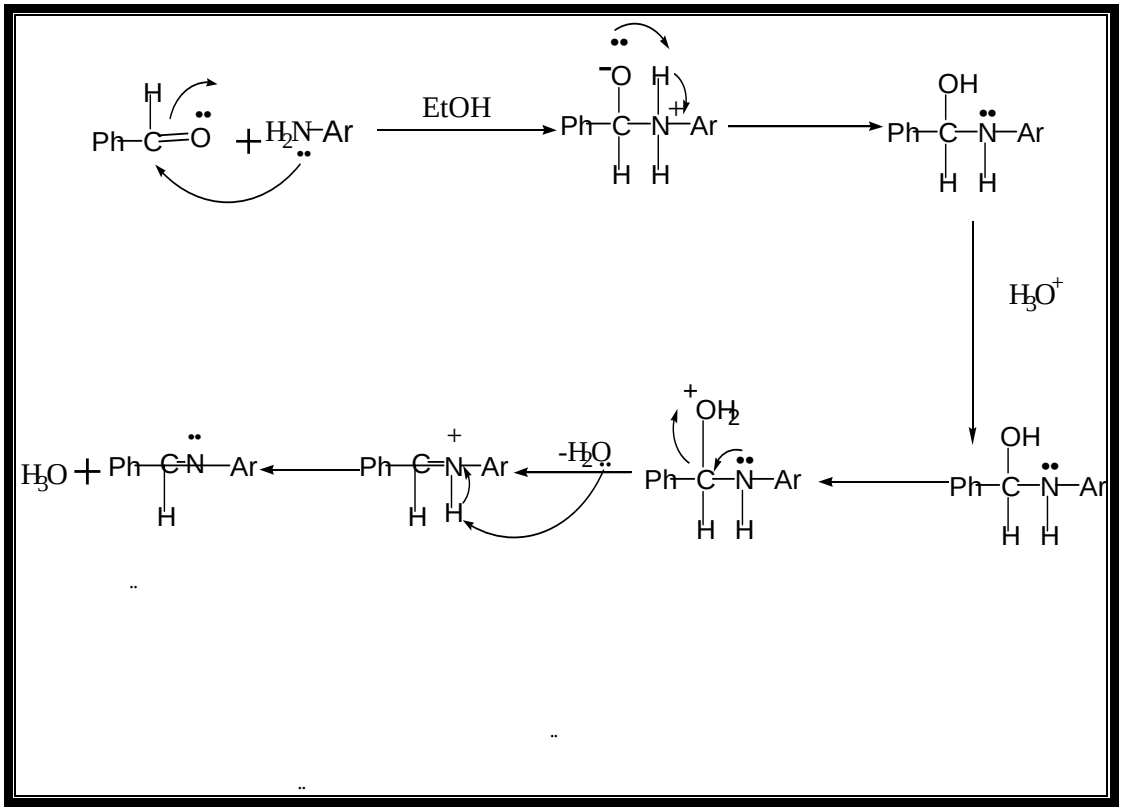
Vibration. m-substitution showed peak absorption band at (781 cm^{-1}) which could be attributed to m-substitution bending vibration [115,116,118]. In the FTIR for compound (N-pyridin-2-ylurea); the absorption band of carbonyl of amide group at (1670 cm^{-1}) which could be attributed to (C=O) stretching vibration. The absorption band of the ($-NH_2$) group was at (3344-3436.9 cm^{-1}) which could be attributed to ($-NH_2$) stretching vibration. In the FTIR for compound (N-pyrazin-2-ylurea); the absorption band of carbonyl of amide group at (1670 cm^{-1}) could be attributed to (C=O) stretching vibration. The absorption band of the ($-NH_2$) group was at (3344-3450 cm^{-1}) which could be attributed to ($-NH_2$) stretching vibration. The following table (3-2) shows the absorption band for the above prepared compounds [115,116,117,118].

3.2.1 Preparation of Schiff bases compounds:

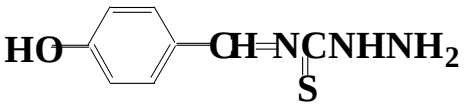
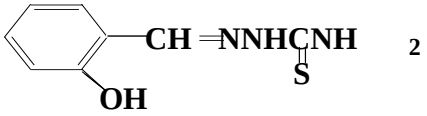
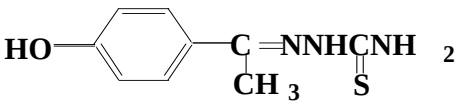
Due to the biological importance of the Schiff bases; three compounds of Schiff bases were prepared in addition to the previous compounds (urea derivative); to compare the biological activity through the reaction of aliphatic amine with aromatic aldehyde like (p-hydroxybenzyldehyde, salicildehyde) and aromatic ketone like (4-hydroxyacetophenone) in an absolute of glacial acetic acid as catalyst, as in the following equation:



In the reaction, nucleophilic amine attacks the carbon of the carbonyl group of aldehyde or ketone to form N-substituted hemiaminals that loses water molecule to yield stable compound as shown below in this mechanism:[119]



Table(٣-٣) : The physical properties of Schiff bases:

No.	Structure of compounds	Chemical formula	M.wt.	Melting point(m.p.)	color
13.1		$C_8H_9N_3OS$	195.24	295-297	Pale orange
13.2		$C_8H_9N_3OS$	195.24	298-300	yellow
13.3		$C_9H_{11}N_3OS$	209.27	>300	white

3.2.2 Infrared spectra of the Schiff bases:

The infrared spectra of the compound (N-(4-hydroxybenzylidene) hydrazinecarbothioamide) showed absorption band at (1610 cm^{-1}) which could be attributed to (C=N) this bond is characteristic to Schiff bases stretching vibration. p-substitution showed absorption band at (827. 4 cm^{-1}) could be attributed to p-substitution bending vibration [116,120]. The infrared spectra of the compound (2-hydroxybenzaldehydethiosemicarbazone) showed absorption band at (3438-3317 cm^{-1}) which could be attributed to ($-NH_2$) of thioamide stretching vibration. o-substitution showed peak absorption band at (752.2 cm^{-1}) could be attributed to o-substitution bending vibration. (C=N) group, characteristic of Schiff bases, showed peak absorption band at (1610.5cm^{-1}) which could be attributed to (C=N) stretching vibration [121]. In compound (1-(4-hydroxyphenyl) ethan-1-onethiosemicarbazone), (C=N) which is characteristic to Schiff bases, showed peak absorption band at (1602 cm^{-1}) could be attributed to (C=N) stretching vibration. p-substitution showed peak absorption band at (821.6 cm^{-1}) which could be attributed to p-substitution bending vibration [116,120,121]. The table (3-4) shows the absorption band of the Schiff bases:

الجدول للاطياف شف بيس

The important signs that ensure the formation of Schiff bases are:

1. Disappearance of the absorption band of aldehyde carbonyl group (C=O) that usually appears at (1695-1720 cm^{-1}).

2. Disappearance of the absorption band of (C-H) aldehyde group that usually appears at (2650-2850 cm^{-1}) that forms two bands of equal intensities.

3. Appearance of absorption band of (C=N), which is a characteristic feature of Schiff base formation.

3.3 Results of the biological studies:

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. [122] 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 (Disc sensitivity test), this method involves the exposure of the zone of inhibition toward the diffusion of microorganism on agar plates. A standard (5mm) diameter sterilized filter paper disc impregnated with a specified volume of the compound solution representing the (MIC), minimum inhibition zone, this disc 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 disc was observed. [110,123]

3.3.2 Inhibitory ability of prepared compound against gram negative bacteria

The prepared compounds show inhibitory activity in a concentration of 10 mg/ml where they have the highest inhibitory ability for the compound (N-(2-methoxyphenyl) urea, N-(4-aminophenyl) urea, N-(2-methylphenyl) urea, N-(2-methoxyphenyl) thiourea, N-pyrazin-2-ylurea, N-(4-hydroxybenzylidene) hydrazinecarbothioamide,

2-hydroxybenzaldehydethiosemicarbazone,1-(4-hydroxyphenyl)ethan-1-onethiosemicarbazone against *Proteus mirabilis* ,where the inhibition zone diameter was (20-30)mm and the concentrations of some compounds show inhibition zone diameter between (8-18)mm, where for N-[4- (amino carbonyl)amino phenyl]urea there was no inhibition zone, i.e., it has no inhibitory activity in the above mentioned concentrations.

Some concentrations show only small inhibition zone between (2-7) mm. The compounds (N-(2-methoxyphenyl)urea,N-(4-aminophenyl)urea, N-(2-methylphenyl)urea,N-(2-methoxyphenyl)thiourea,N-pyrazin-2-ylurea) with high inhibitory activities are due to the fact that strong donating groups are found in their structures and the donating groups are (NH_2, OCH_3,CH_3).The compounds

(N-(4-hydroxybenzylidene)hydrazinecarbothioamide,2-hydroxybenzaldehydethiosemicarbazone,1-(4-hydroxyphenyl)ethan-1-onethiosemicarbazone) 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 (OH) donating group that increases the activity of these compounds against bacteria. The following table (3-5) shows the inhibition zone diameters of the prepared compounds against *Proteus mirabilis.*

Table (3-5): The inhibition zone diameter (mm) for *Proteus mirabilis*

NO.	50 mg/ml	25 mg/ml	10 mg/ml	1 mg/ml	0.1 mg/ml
1.	6	7.5	10	12	0
2.	9	12	6	0	0
3.	0	18	25	5	0
4.	14	10	28	1.5	2
5.	28	17	30	7	0
6.	23	15	26	0	0
7.	0	0	0	0	0
8.	0	0	4	0	0
9.	18	16	13	9	3
10.	20	18	5	0	0
11.	16	6	4	0	0
12.	14	2	2.5	0	0
13.1	18	17	28	10	6
13.2	8	10	26	0	0
13.3	16	14	30	13	3

3.4 Urease Inhibitors:

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

These compounds are:

(N-[4-(aminocarbonyl)aminophenyl]acetamide);(N-[2-methoxyphenyl]urea;(N-[4-hydroxybenzylidene]hydrazinecarbothioamide .(2_hydroxybenzaldehydethiosemicarbazone);(N-pyridin-2-ylurea);(1-[4-hydroxyphenyl]ethan-1-onethiosemicarbazone);(N-[4-aminophenyl]urea.

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. (126)

To compare between the inhibition strength for each inhibitor, we made a constant concentration for all inhibitors; both competitive and non-competitive 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 shows that (N-pyridin-2-yl urea) is considered as urease inhibitor, where its K_i value=0.022 M and (V_{max} value=526.316 µmole/min/ml) and (K_{mapp}value =1.8mM), and this inhibitor was non-competitive type as V_{max} value for inhibitor differ from V_{max} for enzyme. While K_m value was equal for both enzyme and inhibitor, therefore this kind of inhibitor is considered as non-competitive inhibitor.[126]

While for (N-[4-(amino carbonyl) amino phenyl] acetamide) the inhibitory strength (K_i=0.03M), this is considered as competitive inhibition type, where values of V_{max} for both enzyme and inhibitor were the same (V_{max} value=1000) µmole/min/ml, K_m value 3mM.

(N-[2-methoxyphenyl] thiourea) is considered as enzyme inhibitor with inhibitory strength (0.0181 M) which is of non-competitive type Where K_m for the enzyme and inhibitor is the same ;(Km =0.08 Mm) while (V_{max} value for inhibitor =476.19 µmole/min/ml.)[126].

(1-[4-hydroxyphenyl] ethan-1-onethiosemicarbazone) is considered as Schiff base, where inhibitory strength (K_i=0.00504 M) and this is considered as competitive inhibitor where V_{max} for enzyme and inhibitor is the same (V_{max} =1000 µmole/min/ml) While Michaelis constant ($K_{m\ app.}$ = 8.93 mM). (N-[4-hydroxybenzylidene] hydrazinecarbothioamide) is urease enzyme competitive inhibitor where the (inhibitory strength=0.014 M) and

This inhibition is of competitive type as V_{max} for enzyme and inhibitor is the same and equals (1000 µmole/min/ml) while constant (K_m=4.31 mM). (2_hydroxybenzaldehydethiosemicarbazone) is considered as competitive urease inhibitor where the inhibitory strength (Ki=0.0138 M) and (V_{max} value =1000 µmole/min/ml), while (K_m=4.4 mM). [126]

(N-[4-aminophenyl] urea is considered as urea derivative which is considered as urease inhibitor where inhibitory strength (K_i=0.015 M) of competitive inhibitor type where (V_{max}=1000 µmole/min/ml) and (K_m=4.2 mM). (N-pyrazin-2-yl urea) is considered as urease inhibitor where (K_i =0.0257M) and shows competitive type of inhibition where (K_m value=3.2mM) and (V_{max}= 1000 µmole/min/ml).

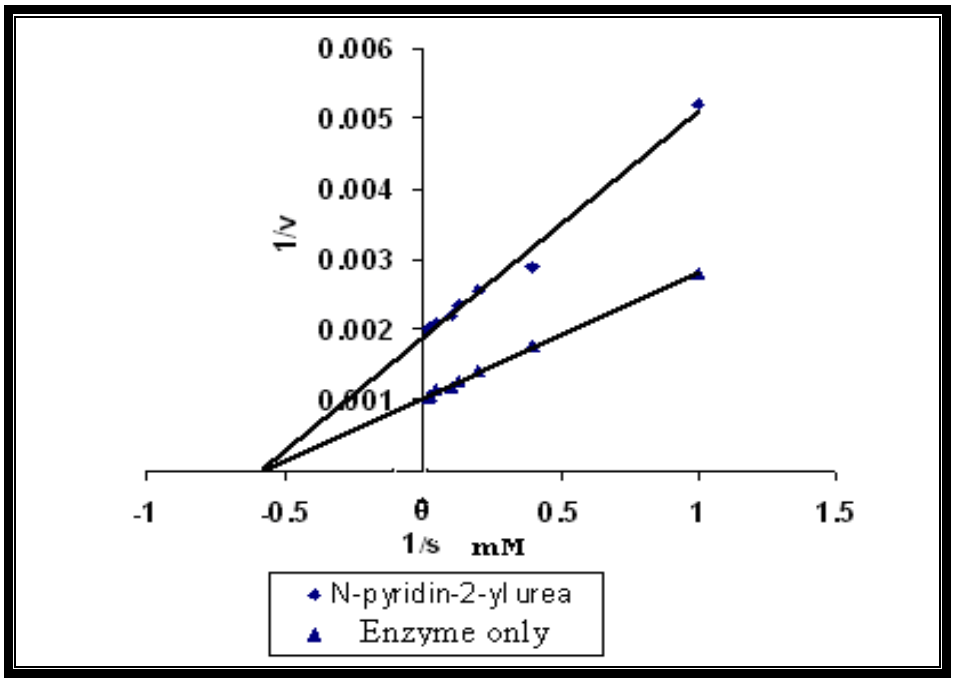


Figure (1): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of N-pyridin-2-yl urea

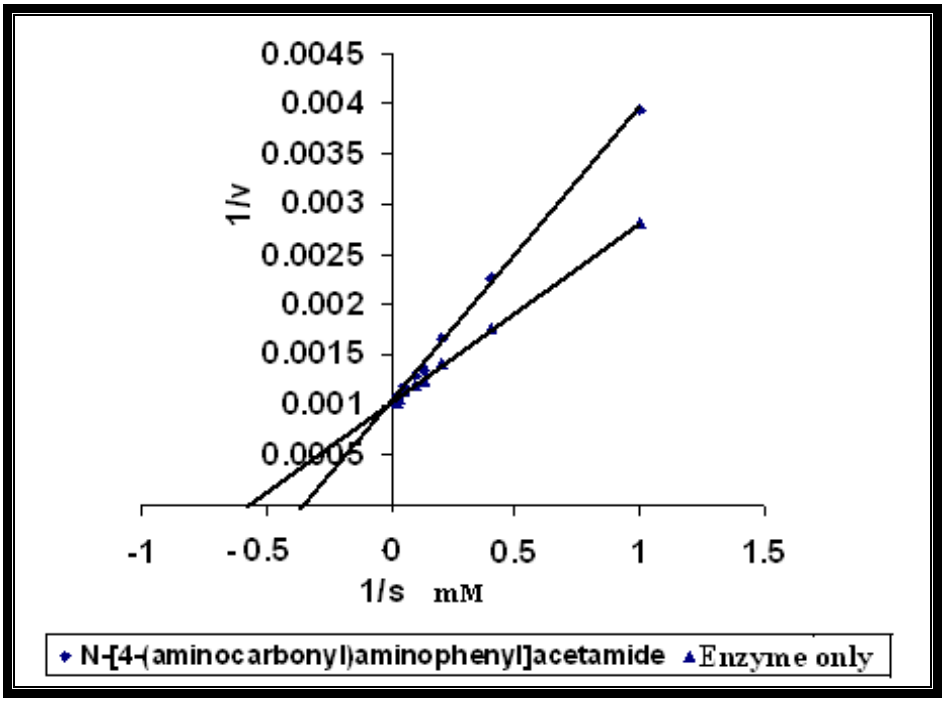


Figure (2): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of N-[4-(amino carbonyl) amino phenyl] acetamide

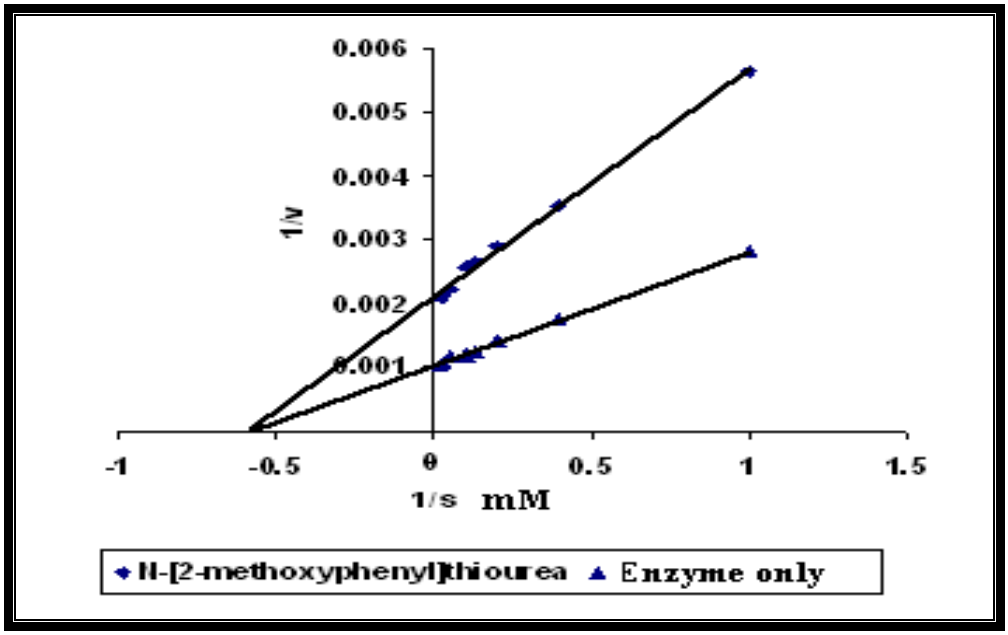


Figure (3): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of N-[2-methoxyphenyl] thiourea

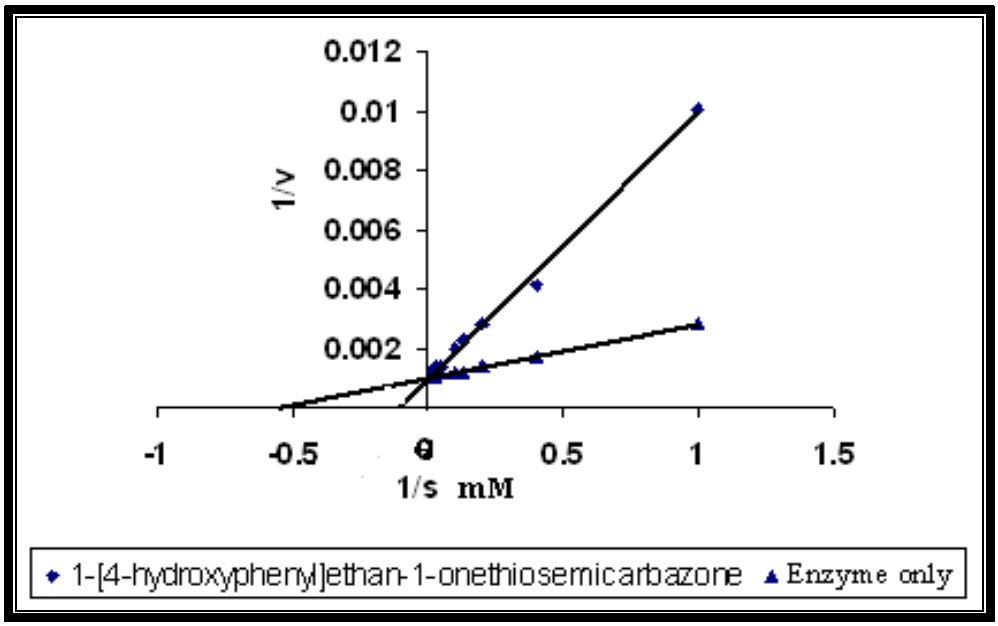


Figure (4): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of 1-[4-hydroxyphenyl] ethan-1-onethiosemicarbazone

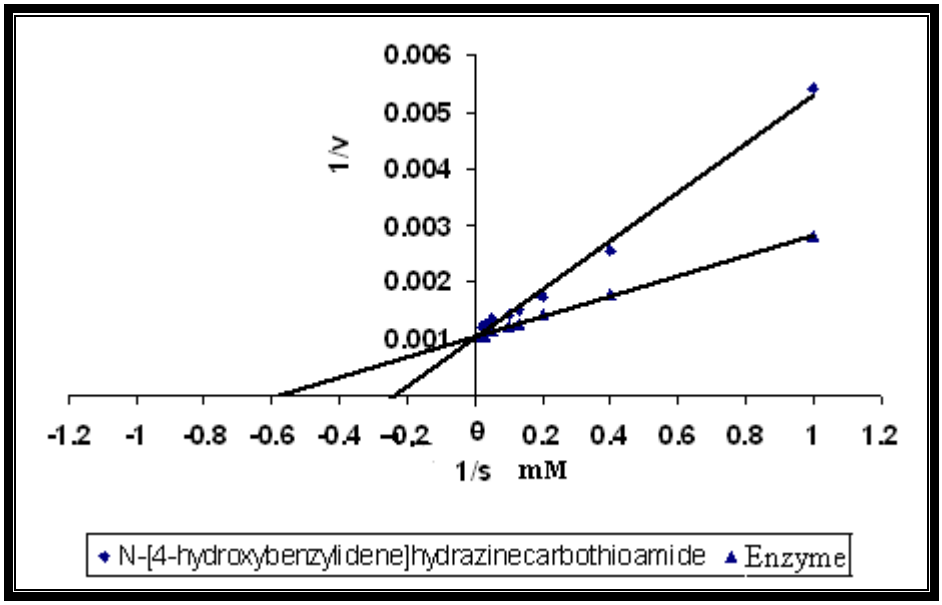


Figure (5): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of N-[4-hydroxybenzylidene] hydrazinecarbothioamide

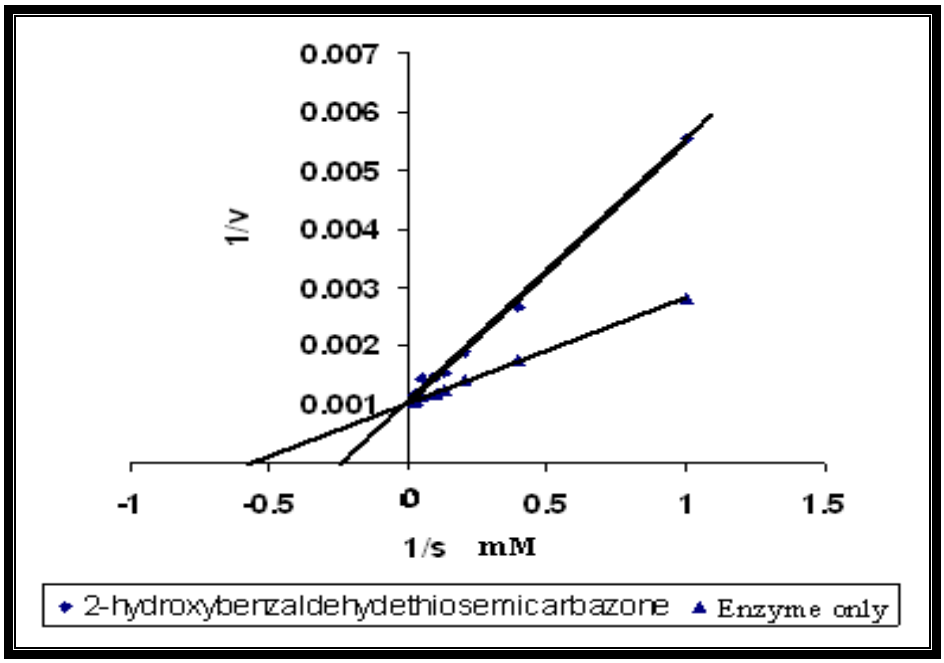


Figure (6): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of 2-hydroxybenzaldehydethiosemicarbazone

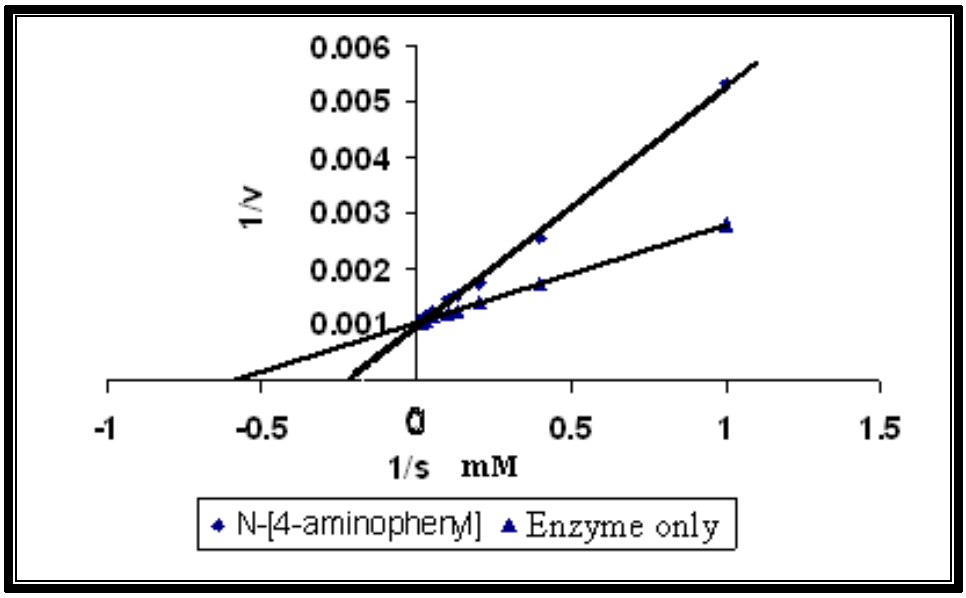


Figure (7): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of N-[4-aminophenyl]

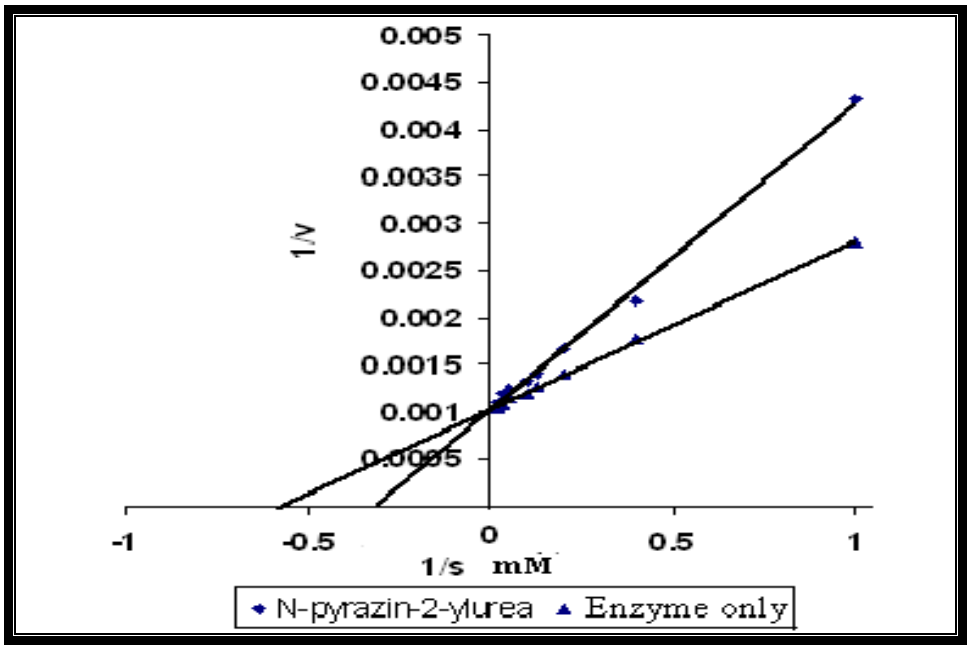


Figure (8): Relationship between urea concentration and urease activity by linear weaver Burck equation in presence and absence of N-pyrazin-2-ylurea

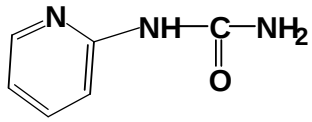
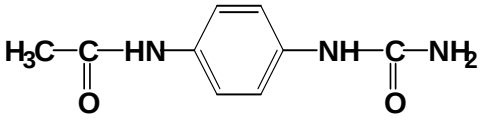
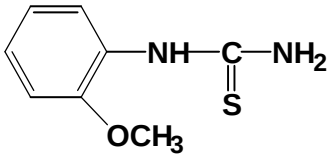
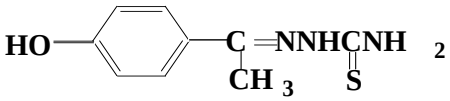
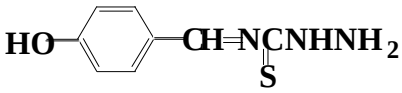
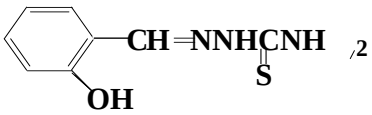
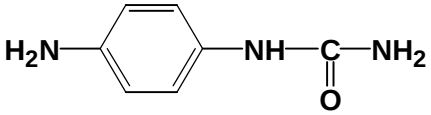
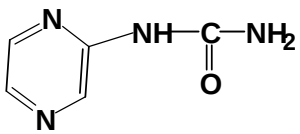
From this equation, V_{max} and K_m were calculated both for enzyme and the inhibitor. From these results, it was found that K_i value is inversely proportional with inhibitory strength, i.e., so when K_i value is high, we need high concentration of the inhibitor to have the same effect. (126)

It is noticed from the results that (1-[4-hydroxyphenyl] ethan-1-onethiosemicarbazone), a Schiff base, is considered as the strongest inhibitor used in this study as it's (K_i value =0.00504M) 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.

In addition to the donating group that is present in the compound structure with comparison with (N-[4-(amino carbonyl) amino phenyl] acetamide) which is the weakest inhibitor where K_i value is high in comparison with other inhibitors which is (0.03M).

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 (3-6) shows V_{max}, K_m and K_i for these compounds.

Table (3-6): Estimation of $V_{max, Km}$ and K_i for urease inhibitors and the type of inhibition

Structure of compounds	K_m (mM)	$V_{max.}$ (ml/unite)	K_i (M)	Type of inhibitor
Without inhibitor (Only enzyme)	1.8	1000	-	-
	1.8	526.316	0.022	Non-competitive
	3	1000	0.03	competitive
	1.8	476.19	0.0181	Non-competitive
	8.93	1000	0.00504	competitive
	4.310	1000	0.014	competitive
	4.4	1000	0.0138	competitive
	4.2	1000	0.015	competitive
	3.2	1000	0.0257	competitive

We also calculated the percentage of inhibition for each inhibitor (i %) as shown in chapter two. [126]

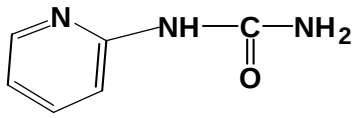
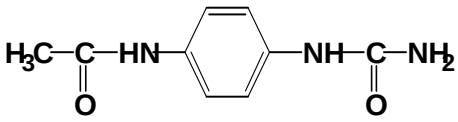
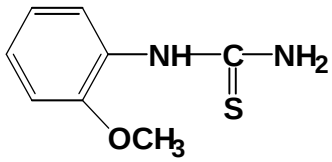
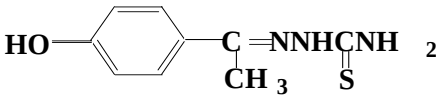
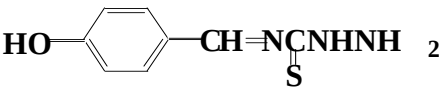
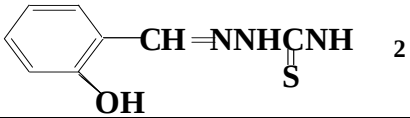
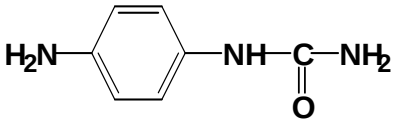
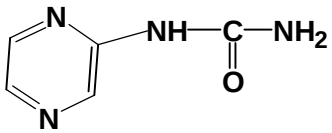
The concentration of inhibitor and substrate were constant to compare the inhibition percentage for each inhibitor. It is notice from calculation of the inhibition percentage, as shown in chapter two [126], that the highest percentage of inhibition was (95.9) when the:

(1-[4-hydroxyphenyl] ethan-1-onethiosemicarbazone) was used compare with other inhibitors. 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:

(N-[4-(aminocarbonyl) aminophenyl] acetamide).

The following table (3- 7) shows the percentage of the inhibition:

Table (3-7): Estimation of inhibition percentage of urease inhibitors

Structure of compounds	$V_\circ$ (initial velocity for enzyme) µmole/L/ min	V_i (initial velocity for inhibitor) µmole/L/m in	a (relative activity)	i% (percent of inhibitor)
	10.989	3.03	0.276	72.4
	10.989	3.983	0.362	63.8
	10.989	2.486	0.226	77.4
	10.989	0.451	0.041	95.9
	10.989	1.907	0.174	82.6
	10.989	1.852	0.168	83.2
	10.989	2.037	0.185	81.5
	10.989	3.503	0.319	68.1

Based on the studies of these scientists, it was noticed in this study that the compound (N-[4-(amino carbonyl) amino phenyl] acetamide), (N-[4-aminophenyl] urea, (N-pyrazin-2-yl urea) are urea derivatives and are urease enzyme inhibitors of the competitive type.

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 (1-[4-hydroxyphenyl] ethan-1-onethiosemicarbazone) has the best activity among these inhibitors. It was also noticed those urease inhibitors:

(N-[4-(aminocarbonyl) aminophenyl] acetamide);

(1-[4-hydroxyphenyl] ethan-1-onethiosemicarbazone);

(N-[4-hydroxybenzylidene] hydrazinecarbothioamide);

(2_hydroxybenzaldehydethiosemicarbazone);(N-[4-aminophenyl)urea;

(N-pyrazin-2-yl urea) were competitive inhibitors in which 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. It was noticed that the increase in urea concentration added to reaction media leads to increase in V_{max} and its return to the value before the addition of the inhibitor which is in turn causes an increase in K_m as in Table (3-6).

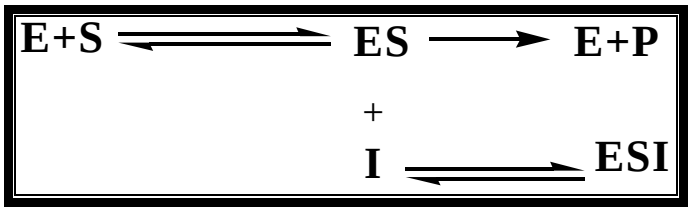
The V_{max} remains constant despite the increase in K_m and this is evidence that it's of competitive type. They compete with substrate (urea) to bind with active site of urease and when urea concentration is increased the inhibitor effect is reduced increasing the chance of substrate binding with enzyme,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

It was noticed that the increase in urea concentration added to reaction media leads to increase in V_{max}, and its return to its value before the addition of the inhibitor.

The increase in the K_m value does not mean that the (EI) 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 [126]:



The type of inhibition, competition between substrate and inhibitor occurs to bind with the same active site of enzyme. When enzyme binds with inhibitor, it presents the binding of substrate with enzyme. When there is high substrate concentration there is little chance for binding of the enzyme with the inhibitor causing a return of V_{max} of enzyme to the original value with the increase in the value of Michaelis constant (K_m)[127]

Study was done on urea derivatives and showed that some were of competitive type of inhibition and other of non-competitive type.

Scientists studied compounds that are considered as urea derivatives. Mobley [28] showed that thiourea is a urease (extracted from *Proteus mirabilis*) inhibitor.

Bennett & Wren [131]; Byers and Lopreore [132] showed that thiourea, urea derivative, is a competitive inhibitor of urease. Hydroxyurea has more inhibitory action that thiourea and also considered as urease inhibitor. It was mentioned by Mobley study [28], and in a study on urease extracted from *Proteus penneri*, Mobley [133] noticed that hydroxyurea was inhibitor of urea. Tanaka [130] noticed the high activity of hydroxyurea as inhibitors of urease from jack beans.

Tanka [128] noticed that hydroxyurea is an inhibitor with inhibition action on urease enzyme of jack beans. Uesato [134] showed that hydorxyurea is active inhibitor for urease enzyme whether it's of plants or microorganisms in origin and is of competitive inhibitor of urea.

Pope [135] showed that (acetohydroxamic acid) AHA is also urea derivative that is effective inhibitor of urease extracted from *Helicobacter pylori* and is competitive type of inhibition. Todd and Hausinger showed [136] that AHA is an effective urease inhibitor of *Pseudomonas* and shows competitive type of inhibition.

Mobley [28] showed that AHA is considered a powerful inhibitor of urease extracted from *Campylobacter pylori* .Bachmeier [137] showed and Mobley [19] that AHA is effective type. Saeed M. Awadth showed [139] that thiourea, hydroxyurea, AHA, are considered as inhibitors of urease extracted from *Proteus mirabilis* and are of competitive type.

It was noticed in this study that the compounds prepared as urea-derivatives in the (N-pyridin-2-yl urea) and (N-[2-methoxyphenyl] thiourea), the results showed that they are urease inhibitors and are of non-competitive type, i.e., they don't compete with substrate (urea) when binding with urease as each has special binding site on the enzyme.

It is noticed that the increase in urea (substrate) concentration to the media does not affect the activity of urease; its addition doesn't return the V_{max} to its original value before addition of inhibitors, as a result V_{max} remains low, and K_m constant. The result of decrease in V_{max} while K_m value remains constant is evidence it's of non-competitive type. Segel showed [127] that in this inhibition type there will be binding of the substrate and inhibitor at different sites on the enzyme, i.e., they bind independently. This leads to the formation of complex (ESI), i.e., the substrate and inhibitor binds with enzyme at the same time. The appearance of (ESI) is due to the presence of any amount of inhibitor in reaction media and its formation does not lead to the formation of product and the quantity of this enzyme remains unchanged.

The enzyme doesn't act with its maximum action which leads to reduction in V_{max} with presence of this kind of inhibition.

Mobley [133] and Houimel [138] showed the activity of acetohydroxamic acid (AHA) as an inhibitor of urease enzyme extracted from *Helico bactar pylori* and *Proteus pennere*.It is of non-competitive type of inhibition in binding with the active site of enzyme.

Urease is considered as the first enzyme that was prepared in a crystallized form but its action is still unknown clearly [128].

The roles of nickel ion and amino acids present at active site of urease are still unknown [129].

It is noticed from enzymatic and biological studies that Schiff bases compounds are strong inhibitors of the urease enzyme where the (1-[4-hydroxyphenyl] ethan-1-onethiosemicarbazone) inhibition force was (K_i=0.00504 M) which is the highest inhibition force for the prepared compounds. 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 (20-30) mm. In addition to the other Schiff bases compounds, which have a high inhibition ability where (2-hydroxybenzyldehydethiosemicarbazone) had K_i (0.014 M) and also the compound (2_hydroxybenzaldehydethiosemicarbazone) had inhibitory force (K_i=0.018M) results of the biological activity of these compounds were high on *Proteus mirabilis* with (10mg/ml) with inhibition zone diameter (20-30)mm. Compounds with strong donating group like (N-[2-methoxyphenyl]urea),(N-[4-aminophenyl]urea,(N[2-methylphenyl] urea, (N-[2-methoxy phenyl]thiourea),that contain donating group (OCH_3, NH_2,CH_3); 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.

Diameter was (20-30) 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 (N-[4-(amino carbonyl) amino phenyl] urea, N-[4-(amino carbonyl) amino phenyl] acetamide) compounds were the less compounds of inhibition. Compound (N-[4-(amino carbonyl) amino phenyl] acetamide) was weak inhibitor in comparison with other compounds studied in this study. The inhibition force for this compound was weak (K_i=0.03M) where it is highest K_i value for the studied compounds and had the least inhibition force. It was noticed from studying the biological activity of compound (N-[4- (amino carbonyl) amino phenyl] urea) that it had no biological activity at the studied concentrations. Compound (N-[4-(amino carbonyl) amino phenyl] acetamide) had a weak biological activity with inhibition zone of (2-7) mm at (10 mg/ml).

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 [19,130].

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

3.5. Conclusion:

1. It is noticed that Schiff bases are more active than urea analogues due to the presence of C=N group that has a role in their activity.

2. N-[4- (amino carbonyl) amino phenyl] urea doesn't have biological activity while 1-(4-hydroxyphenyl) ethan-1-onethiosemicarbazone has the highest biological activity among them.

3. It is noticed that 1-(4-hydroxyphenyl) ethan-1-onethiosemicarbazone has the highest inhibition force for the urease enzyme.
K_i=0.00504 M; i=95.9%; V_{max}= 1000 unit/ml; K_m=8.93 mM and is competitive type of inhibition. While N-[4-(amino carbonyl) amino phenyl] acetamide has the lowest inhibition force for the urease enzyme with (K_i=0.03 M, Vmax 1000 unit/ml, K_m 3 mM, i %=63.8 %).

4. From the results of this study, it is found that the urease inhibitors [N-[4-(amino carbonyl)aminophenyl]acetamide,1-(4-hydroxyphenyl)ethan-1-onethiosemicarbazone,N-(4-hydroxybenzylidene)hydrazinecarbothioamide,2-hydroxybenzaldehydethiosemicarbazone,N-(4-aminophenyl)urea,N-pyrazin-2-ylurea] are competitive type of inhibition which compete with substrate on the active site of urease. While the compounds (N-pyridin-2-ylurea, N-[2-methoxyphenylthiourea]) are non-competitive types of inhibition that do not compete with substrates on the active sites of enzymes.

3.6. Recommendation for future work:

1. Synthesis of new compounds with different groups from those synthesized in this study and studies their activities.

2. Study of the mechanism of action of these compounds on the enzyme through X-ray study.

3. Study of the toxicity of prepared compounds.

4. Carry out experiments of enzyme inhibition *in vivo* on experimental animals with histological and physiological studies.

2.1. Materials:

Substance	Supplier
2-Aminopyridine	Fluka (Switzerland)
3-Bromoaniline	BDH
4 – Hydroxylacetophenone	Fluka (Switzerland)
Aminopyrazine	BDH
Aniline hydrochloride	Merck (Germany)
Ansidine (m-methoxy anline)	BDH
Benzene	BDH
Dipotassium hydrogen phosphate(K_2HPO_4)	BDH
DMSO(Dimethylsulfoxide)	Merck (Germany)
Ethanol(Absolute)	BDH
Glacial acetic Acid	BDH (England)
Hydrocholoric Acid	Fluka(Switzerland)
Methanol	Fluka(Switzerland)
Na-EDTA	Fluka(Switzerland)
o-Toluidine	Fluka(Switzerland)
p- Acetoamidaniline	BDH (England)
Phenol	Merck (Germany)
p-Hydroxybenzaldehyde	Merck (Germany)
p-Phenyline diamine	BDH (England)
Salisaldehyde	BDH (England)
Sodium Hydroxide	Merck (Germany)
Sodium Hypochloride	BDH (England)
Sodium Nitroprusside	BDH
Thiosemicarbazide	BDH
Thiourea	BDH
Urea	Fluka (Switzerland)

2.2. Instruments:

Instruments	Supplier
Incubator	Gallenkamp(England)
Autoclave	Gallenkamp(England)
Water Bath	Gallenkamp (England)
Magnetic Stirrer	Gallenkamp (England)
pH-meter 330s	Jenway (UK)
Oven	Memmert (Germany)
Balance	Sertorius (Germany)
Vacuum Pump	Schuco.Inc (England)
Hot plate	Gallenkamp(England)
Fourier Transform Infrared Spectrophotometer (FTIR)	The infrared spectra were recorded on Shimadzu 8300 Fourier Transform infrared spectrophotometer (FTIR) by use the (KBr) in the wave number range (500-4000)cm^{-1}
Ultra Violet-visible spectrophotometer(UV-visible)	The ultra violate spectra were recorded on a Shimadzu UV-1650.PC UV-visible spectrophotometer.
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
Thin layer chromatography (TLC) was carried out using fertig fallen precoated sheets type poly gram silica gel and the plates were developed with iodine vapour.	

2.3 Culture media:

Media	Supplier
1.Nutiernt broth	Difco and oxoid(England)
2.Nutierent agar	Oxoid(England)

2.4 Bacterial isolates:

Proteus mirabilis was studied and the bacterial species was identified and supplied by central laboratory of Ministry of Health.

2.5 Experiments:

2.5. A: Preparation of chemical compounds:

A series of urea derivatives were prepared and their biological activities were examined. [109]

2.5. A.1. Preparation of N- phenyl urea:

(0.01 mole) of aniline hydrochloride and (0.01 mole) of urea were dissolved in (5ml) distilled water in round bottom flask and mixture was Refluxed for (2hours).The product was filtered and washed with cold distilled water. The product was recrystalised using water the purity was monitored by TLC, was used with chloroform and ethyl acetate in ratio (1:1) as eluent. Table (3-1) shows the m.p and physical properties in page (40).compound NO.1 and table for FTIR in page (46).

2.5. A.2. Preparation of N-phenylthiourea:

(0.01 mole) of aniline hydrochloride and (0.01 mole) of thiourea were dissolved in (8ml) of distilled water in round bottom flask and mixture was refluxed for (2hours).The precipitate was separated by cooling. The product was filtered and washed with cold distilled water. Table (3-1) shows the m.p and physical properties in page (40).compound NO.2 and table for FTIR in page (46).

2.5. A.3. Preparation of N-(2-methoxy phenyl) urea:

(0.01mole) of 2-anisidin and (0.01mole) of urea were mixed and dissolved in (10ml) absolute ethanol in round bottom flask. Two drops of concentrated HCl was added to solution. Mixture was refluxed for (10 hours), and then cooled in an ice bath to separate the product. Then mixture was filtered and washed with ethanol. The precipitate is violet in color. Table (3-1) shows the m.p and physical properties in page (40).compound NO.3 and table for FTIR in page (46).

2.5. A.4. Preparation of N-(4-amino phenyl) urea:

(0.01mole) of P-phenyline di amine and (0.01mole) of urea were mixed. Mixture was dissolved in (10ml) absolute ethanol and (1drop) of concentrated HCl was added in round bottom flask. The mixture was refluxed for (10hours).Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, recrystilazed by addition of distilled water the precipitate is (dark brown or deep-violet) in color. Table (3-1) shows the m.p and physical properties in page (40).compound NO.4 and table for FTIR in page (46).

2.5. A.5. Preparation of N-(2-methyl phenyl) urea:

(0.01 mole) of o-toludine and (0.03 mole) of urea were dissolved in (10 ml) of absolute ethanol in round bottom flask and two drops of concentrated HCl were added. Mixture was refluxed for (10 hours).The product was cooled in an Ice bath then filtered. And it was dried in an oven. The product was recrystalised from ethanol and gave violet crystals. Table (3-1) shows the m.p and physical properties in page (40).compound NO.5 and table for FTIR in page (46).

2.5. A.6. Preparation of N-(2-methoxyphenyl) thiourea:

(0.01 mole) of thiourea and (0.01 mole) of 2-ansidine were dissolved in (10 ml) of absolute ethanol in round bottom flask.Two drops of concentrated HCl were added. The mixture was refluxed for (10 hours).The product was recrystalised from ethanol to give grey crystals. Table (3-1) shows the m.p and physical properties in page (40).compound NO.6 and table for FTIR in page (46).

2.5. A.7.Preparation of N-[4-(aminocarbonyl)aminophenyl]urea:

(0.01 mole) of p-phenyline diamine and (0.01 mole) of urea were dissolved in (10 ml) of absolute ethanol in round bottom flask. Two drops of concentrated HCl were added. The mixture was refluxed for (10 hours).the mixture was cooled in an Ice bath and the precipitate was washed with ethanol to give deep violet crystal. Table (3-1) shows the m.p and physical properties in page (40).compound NO.7 and table for FTIR in page (46).

2.5. A.8.Preparation of N-[4-(aminocarbonyl)aminophenyl]acetamide :

(0.01) mole of p-acetoamideaniline and (0.01) mole of urea were mixed in round bottom flask. The mixture was dissolved in absolute ethanol. Two drops of concentrated HCl were added. Mixture was refluxed for (10 hours).then it was cooled in an ice bath. The precipitate was filtered and washed with ethanol to give brown crystals. Table (3-1) shows the m.p and physical properties in page (41).compound NO.8 and table for FTIR in page (47).

2.5. A.9 Preparation of N-pyrazin-2-yl urea:

(0.01 mole) of 2-aminopyrazine and (0.01 mole) of urea were mixed in round bottom flask. The mixture was dissolved in (10 ml) ethanol. Two drops of concentrated HCl was added. The mixture was refluxed for (12 hours).then cooled in an ice bath. It was filtered and washed with ethanol to give dark brown crystals. Table (3-1) shows the m.p and physical properties in page (41).compound NO.9 and table for FTIR in page (47).

2.5. A.10. Preparation of N-pyridin-2-ylurea:

(0.01 mole) of 2-aminopyridine and (0.01 mole) of urea were mixed in round bottom flask. The mixture was dissolved in (10 ml) absolute ethanol. Two drops of concentrated HCl was added. Mixture was refluxed for (10 hours).the cooled and recrystalised from ethanol. Table (3-1) shows the m.p and physical properties in page (41).compound NO.10 and table for FTIR in page (47).

2.5. A.11. Preparation of N, N`-bis(3-bromophenyl)urea:

(0.02 mole) of m-bromoaniline and (0.01 mole) of urea were dissolved in round bottom flask and dissolved in (10 ml) absolute ethanol. Two drops of concentrated HCl were added. The mixture was refluxed for (12 hours) to form the product. The mixture is cooled and filtered to give crystals. Table (3-1) shows the m.p and physical properties in page (41).compound NO.11 and table for FTIR in page (47).

2.5. A.12. Preparation of N, Ń-bis (2-methoxyphenyl) urea:

(0.02 mole) of 2-ansidin and (0.01 mole) of urea were mixed in round bottom flask and dissolved in (10 ml) absolute ethanol. Two drops of concentrated HCl were added. The mixture was refluxed for (12 hours).Ice bath was used to separate the product. Then it was filtered and precipitate was isolated. Table (3-1) shows the m.p and physical properties in page (41).compound NO.12 and table for FTIR in page (47).

2.5. A.13.PreparationsofSchiffbases [109]

2.5. A.13.1 Preparation of N-(4-hydroxybenzylidehydene) hydrazinecarbothioamide

(0.01 mole) of p-hydroxybenzaledeyde and (0.01 mole) of thiosemcarbazide were mixed in round bottom flask. Mixture was dissolved in (10 ml) of benzene. (1-2) drops of glacial acetic acid were added. Mixture was refluxed for (5 hours).Ice bath was used to separate the product. It was filtered and washed by benzene. Product is pale orange in color. Table (3-3) shows the m.p and physical properties in page (49).compound NO. (13.1) and table for FTIR in page (51).

2.5. A.13.2 Preparation of

2-hydroxybenzaldehydethiosemicarbazone:

(0.01 mole) of sulcildehyde and (0.01 mole) of thieosemcarbazid were mixed in round bottom flask and mixture were dissolved in (10 ml) absolute ethanol and (1 ml) of benzene. Mixture was refluxed for (5 hours).It was filtered. Recrystalised from ethanol to give yellow crystals. Table (3-3) shows the m.p and physical properties in page (49).compound NO. (13.2) and table for FTIR in page (51).

2.5. A.13.3 Preparation of 1-(4-hydroxyphenyl) ethan-1-onethiosemicarbazone:

(0.01mole) of p-hydroxyacetophenone and (0.01 mole) of thieosemcarbazid were mixed in round bottom flask. Mixture was dissolved in (10 ml) benzene. (1-2) drops of glacial acetic acid were added. The mixture was refluxed for (5 hours).then it was cooled and filtered. The product was recrystalised from ethanol to give white crystals. Purity was monitored by TLC, was used with chloroform and ethyl acetate in ratio (1:1) as eluent. Table (3-3) shows the m.p and physical properties in page (49).compound NO. (13.3) and table for FTIR in page (51).

2.5. B: Bacterial Sensitivity Test of the Prepared Compounds:

Disc diffusion method was used to determine the inhibiting power of the prepared compound against the isolated bacteria. [110]

١. Preparation of chemical compounds discs

The Following concentrations (0.1, 1, 10, 25, 50) mg/ml for each prepared compound (1-13.3) in Dimethylsulfoxide (DMSO) solvent. Then these compounds are filtered. For each above concentration 20 tablets disc from filter paper about 6 mm in diameter. Vials are mixed so the discs will be of equal concentration to that of the compounds. Then the discs were dried in Incubator.

2. Disc Diffusion Method:

The experiment was carried according to Bauer *et al* [(111)], modified by W.H.O [(112)] as follows:

a. Nutrient Agar:

Nutrient agar was prepared by dissolving the (8g.) nutrient agar in (250 ml) distilled water. Then Media was sterilized by autoclave at 121˚C, 1.5 atmospheres for 15 minutes. Then it was cooled to (40-45) ˚C. It was then put in plate by about (15-20) ml for each plate. It was then left at room temperature till it became solid. Then 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 then kept in refrigerator till needed.

b. 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.

c. Bacteria 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 slow 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.

d. Disc Spread over Plates:

Prepared discs (in step 1) were placed over the medium by forceps (with sterilized and pointed end) with compression of discs. Distances of (24 mm) were left between one disc and another; and 1 cm between the disc and the plate margin to avoid interference between zones of inhibition. Plates were marked according to concentrations. Plates were incubated by putting them inversely at 37˚C for 24 hours.

e. Measurement of Zone of Inhibition:

Zone of inhibition diameter was measured for each disc using a ruler.

NOTICE:

The Bacteria were considered either:

Sensitive (S); Inhibited (I); or Resistant (R) depending on diameter of zone of inhibition.

Zone of Inhibition:

Is the translucent area which surrounds the disc including the diameter of the disc that lacks bacterial growth.

2.5. C: Determination of Urease Activity for Proteus mirabilis by Indophenols assay:

1. Preparation of used solutions:

These reagents were prepared as they were mentioned in [113] .Indophenol reagent was prepared by:

1. Reagent A:

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

2. 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.

3. Urea:

(0.3) gram of urea was dissolved in 10 ml distilled water it was freshly prepared and used, it is the stocke solution.

4. Buffer solution (Phosphate solution):

These reagents were prepared as they were mentioned in [114] (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).

Enzyme Unit: The amount of enzyme which is needed to convert micromole (µmole) of urea to ammonia in one minute at temperature 37C°.

2. 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 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.

METHODS

Incubated in water bath at 37˚C for (10 minutes**).**

Enzyme	**10**	**—**	**10**
Buffer pH=7.5	**205**	**215**	**215**
Inhibitor	**10**	**10**	**—**
Urea	**25**	**25**	**25**

Incubated in water bath at 37˚C for (15 minutes)

Reagent A	**5 ml**	**5 ml**	**5 ml**

It should be well shaken.

Reagent B	**5 ml**	**5 ml**	**5 ml**

It should be well shaken.

Then it was incubated in water bath at 37˚C for (20 minutes).later on it was read with absorbance at 625 nm; enzyme activity was then calculated and inhibition percentage.

3. 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.

a. The method without the inhibitors: - (This is Control)

(Different concentration of substrate)

	Sample µL	Blank µL
Buffer 7.5	**215**	**225**
Urea	**٢٥**	**25 (maximum of concentration)**
Enzyme	**١٠**	**-**

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**	**٥ml**
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):

	Sample µL	Blank µL
Buffer 7.5	**205**	**215**
Inhibitor (Concentration of maximum inhibition)	**10**	**10**
Urea	**25**	**25 (Maximum concentration)**
It was incubated in water bath at 37˚C for (10 minutes)		
Enzyme	**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 and draw the relations between Urea concentrations and enzyme activity.

2.6. Calculation:

1. From the linear Line weaver Burck equation, calculated V_{max} and K_m [126]

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

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 the activity of enzyme by: [126]

Activity of enzyme = (O.D./13.8)×100000

O.D=optimum density (nm).

3. Calculation K_i value for the inhibitors both competitive and non-competitive by the following equation :[126]

$K_{m\ app.} = K_m (1+ [I]/K_i)$……. competitive inhibitor

$V_{max\ i} = V_{max} / (1+ [I]/K_i)$ ……non-competitive inhibitor

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

K_m=Michaelis constant for enzyme (mM)

[I]= Inhibitor concentration (mM)

K_i= Strength of inhibitor (mM)

4. Calculation inhibitor percentage according to the following equation: [126]

i%=100(1-a)

%i=degree of inhibition as a percent

a = relative activity as fraction =$V_i/V_\circ$

V_i and $V_\circ$ were calculated by:

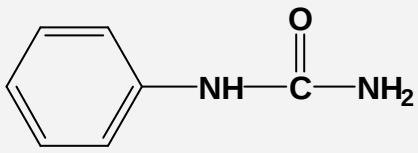
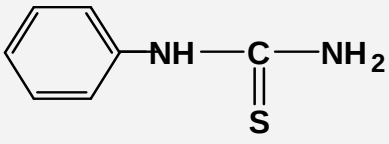
$V_{\circ}$=[S] V_{max} / K_m+[S]…….For enzyme initial velocity

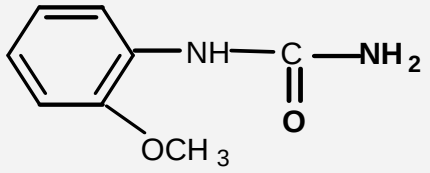
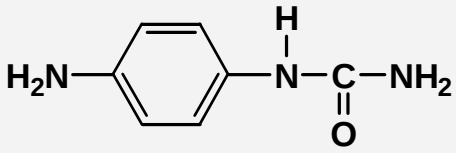
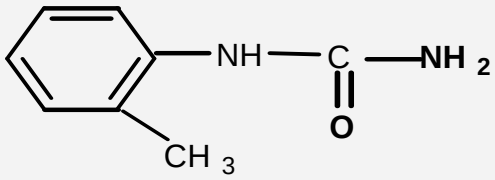
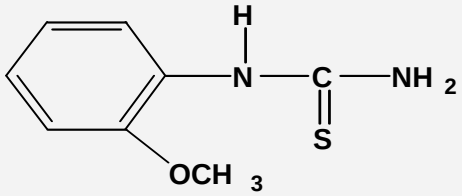
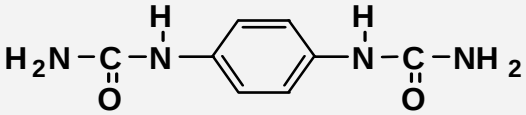
V_i=[S] V_{max}/ ([S] +K_m) (1+ [I] / K_i)……..initial velocity of non-competitive inhibitor

V_i=[S] V_{max} / K_m (1+ [I]/K_i) +[S]…….initial velocity of competitive inhibitor

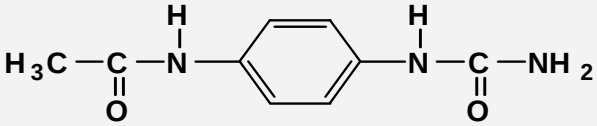
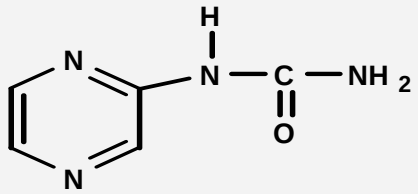
The following table (2-1) shows the Name and structures of prepared compounds

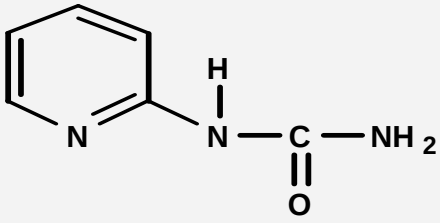
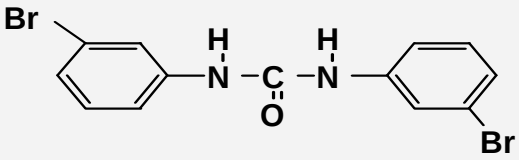
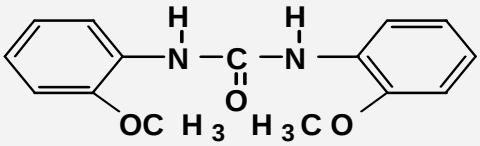
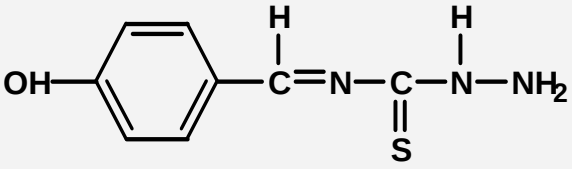
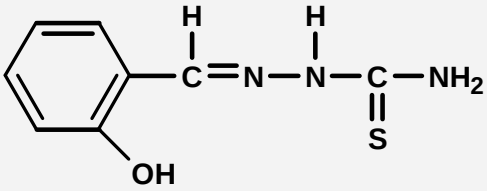
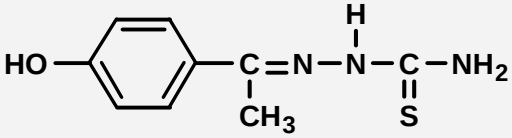
Table (2-1) shows the Name and structures of prepared compounds

No.	structure	The name of compounds
1.		N-phenylurea
2.		N-phenylthiourea

3.		**N-(2-methoxyphenyl)urea**
4.		**N-(4-aminophenyl)urea**
5.		**N-(2-methylphenyl)urea**
6.		**N-(2-methoxyphenyl)thiourea**
7.		**N-[4- (aminocarbonyl)aminophenyl]urea**

Continued table (2-1) shows the Name and structures of prepared compounds

No.	structure	The name of compounds
8.		**N-[4-(aminocarbonyl)aminophenyl]acetamide**
9.		**N-pyrazin-2-ylurea**

10.		**N-pyridin-2-ylurea**
11.		**N,N´-bis(3-bromophenyl)urea**
12.		**N,N´-bis(2-methoxyphenyl)urea**
13.1		**N-(4-hydroxybenzylidene)hydrazinecarbothioamide**
13.2		**2-hydroxybenzaldehydethiosemicarbazone**
13.3		**1-(4-hydroxyphenyl)ethan-1-onethiosemicarbazone**