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

Synthesis of some sulfa drug derivatives as antibacterial agents

A Thesis

Submitted to the College of Science/Al-Nahrain University as a Partial Fulfillment of the Requirements for the Degree of M.Sc. in Chemistry

By

Anmar Hameed Jabbir

B.Sc. Chemistry/College of Science/Alkufa University(2012)

Supervised by

Assist. Prof. Dr. salman A. Ahmed

2016 **1437**

Supervisor Certification

I certify that this thesis entitled **"Synthesis of some sulfa drug derivatives as antibacterial agents"** was prepared by **"Anmar Hameed Jabbir"** under my supervision at the College of Science/ Al-Nahrain University as a partial requirements for the Degree of Master of Science in Chemistry.

Assistant Professor

Dr. Salman A. Ahmed

In view of the available recommendation, I forward this thesis for debate by the Examining Committee.

Assistant Professor

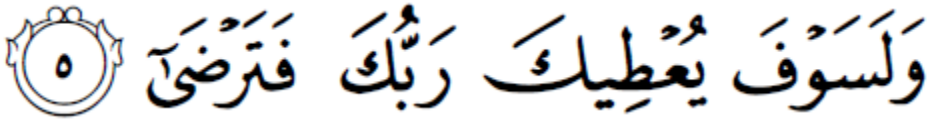
Dr. Nasreen R. Jber

Head of Department Chemistry

College of science

Al Nahrain university

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ



صَدَقَ اللهُ العلي العَظِيم

سورة الضحى

TO MY FAMILY AND TO EVERYONE WHO HAVE HELPED ME IN PUTTING THIS WORK

Acknowledgements

I wish to express my great thanks to ***ALLAH*** *for uncountable gifts and for helping me to present this thesis. It is a pleasure to express my sincere thanks and appreciation to my supervisor, Assistant Prof.* ***Dr. Salman A. Ahmed*** *for suggesting the subject of this thesis and supervision of my work.*

I would like to thank my ***father, mother, brothers, sisters*** *and all* ***friends specially Ali N. Hassan*** *for their supporting during the course of the study and work.*

The preparation and analysis of the organic compounds would have been much more difficult without the help of ***Dr.Nisreen R. Jber, Dr.Jawad AL-musawy, Dr.Kareem Sabah and Mrs.Rana A. Mohsien*** my great thank to them.

Thanks to the staff of the chemistry department, college of science, AL-Nahrain University.

Also, I am very grateful to all the people who helped me during my work.

Anmar

Table of Contents

Symbols and Abbreviations

AIDS	Acquired Immunodeficiecy Syndrome
ATP	Adenosine Tri Phosphate
C	Cytosine
C	Celsius
CA	Carbonic Anhydrase
Ca	Calcium
CF	Cystic Fibrosis
cGMP	Cyclic Guanosine Mono Phosphate
cm^{-1}	1/Centimetre
CNS	Central Nervous System
DMSO	Di Methyl Sulfoxide
DNA	Deoxyribo Nucleic Acid
FTIR	Fourier Transform Infra-Red
G	Guanine
HIV	Human Immunodeficiency Virus
Hrs.	Hours
λ_{max}	Lambda Max
Mg	Magnesium
mg	Milligram
ml	Milli Liter
mm	Millimetre
PABA	Para Amino Benzoic Acid
PH	Logarithm of the reciprocal of the hydrogen ion activity
Phe	Phenyl alanine
Pro	Proline
RNA	Ribonucleic Acid
THF	Tetra Hydro Folic Acid
Tyr	Tyrosine

um	Micrometre
UV-VIS	Ultra violet-Visible

Abstract

This research is divided into two parts:

Part one:

This part involved in the synthesis of some sulfa drugs which play an important role in the medicinal chemistry because of their antibacterial activity.

The synthesized compounds:

1.p-Amino-N-(2-methylphenyl)benzene Sulfonamides.

2.p-Amino-N-(2-methoxyphenyl)benzene Sulfonamides.

3. p-Amino-N-(4-chlorophenyl)benzene Sulfonamides.

4. 4-(Morpholinosulfonyl) aniline.

5. 4-(Piperidin-1-yl sulfonyl) aniline.

Sulfa drug compounds were identified by using elemental analysis, FTIR spectroscopy, UV-Visible spectroscopy and by measuring their melting points.

Part two:

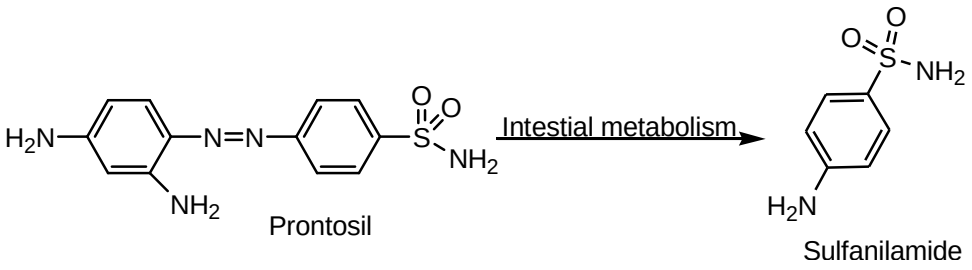
This part involved the activity of the synthesized sulfa compounds on different pathogenic bacteria(staphylococcus aureus and pseudomonas aeruginosa) in vitro by using well diffusion method, different concentrations of sulfa drugs were prepared. Some compounds showed an inhibitory effect on the growth of the two bacteria and the others showed no inhibition activity.

CHAPTER ONE

INTRODUCTION

1.1. Sulfa drugs:

Sulfonamides were first synthesized by Gelmo et al.[1] in 1908 while doing research into azo dyes. Directly following this work, Hoerlien et al. discovered dyes containing the sulfanylgroup that had affinity for proteins of silk and wool. This led to the discovery by Eisenberg in 1913, that chrysolidine, one of the azo dyes studied, had pronounced bactericidal actionin vitro. However, until 1932 the therapeutic properties of sulfonamides were not recognized. The German scientists Domagk et al. found that prontosil had pronounced in vivo antibacterial activity. They observed that mice with streptococcal septicemia could be cured with prontosil. Domagk also discovered that prontosil was rapidly reduced in the cell to sulphanilamide and that it was in fact the sulphanilamide and not the prontosil, which was the actual antibiotic.[2]



Scheme(1.1): In vivo metabolism of prontosil[3]

Many different sulfonamides were synthesized during the late 1930's A great number of those were discovered to possess considerable antibacterial activity for a variety of streptococci and pneumonococci bacteria. Several sulfa pyrimidines which were introduced in 1941[4] were found to possess considerable antibacterial activity as well as lower toxicity than previous sulfonamides. This advancement led to many new sulfonamides being synthesized. Today there are over 5000 sulfa drugs in existence but only 33 of those have been introduced for general medical use[5].The use of sulphonamides as drugs dates back to the beginning of

the twentieth century, when the discovery of the medicinal use of sulphonamides and their derivatives was a milestone in the history of chemotherapy. It represented the first investigation o f synthetic organic molecules as potential drugs to fight infection carried in the bloodstream.(6)

Sulfonamides are an important class of synthetic bacteriostatic antibiotics still used today for the treatment of bacterial infections and those caused by other microorganisms. They are also known as sulfa drugs and were the main source of therapy against bacterial infections before the introduction of penicillin in 1941. Although sulfonamides have for the most part been replaced by other agents, they still maintain considerable action in certain types of infection, for example in the urinary tract, eye and ear, and bronchitis (7). Sulfonamides are compounds, which have a general structure represented by (Fig 1.1). In this structure, R may be alkyl, aryl or hetero aryl etc.(8)

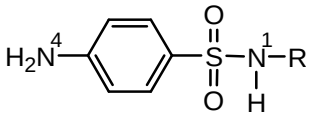
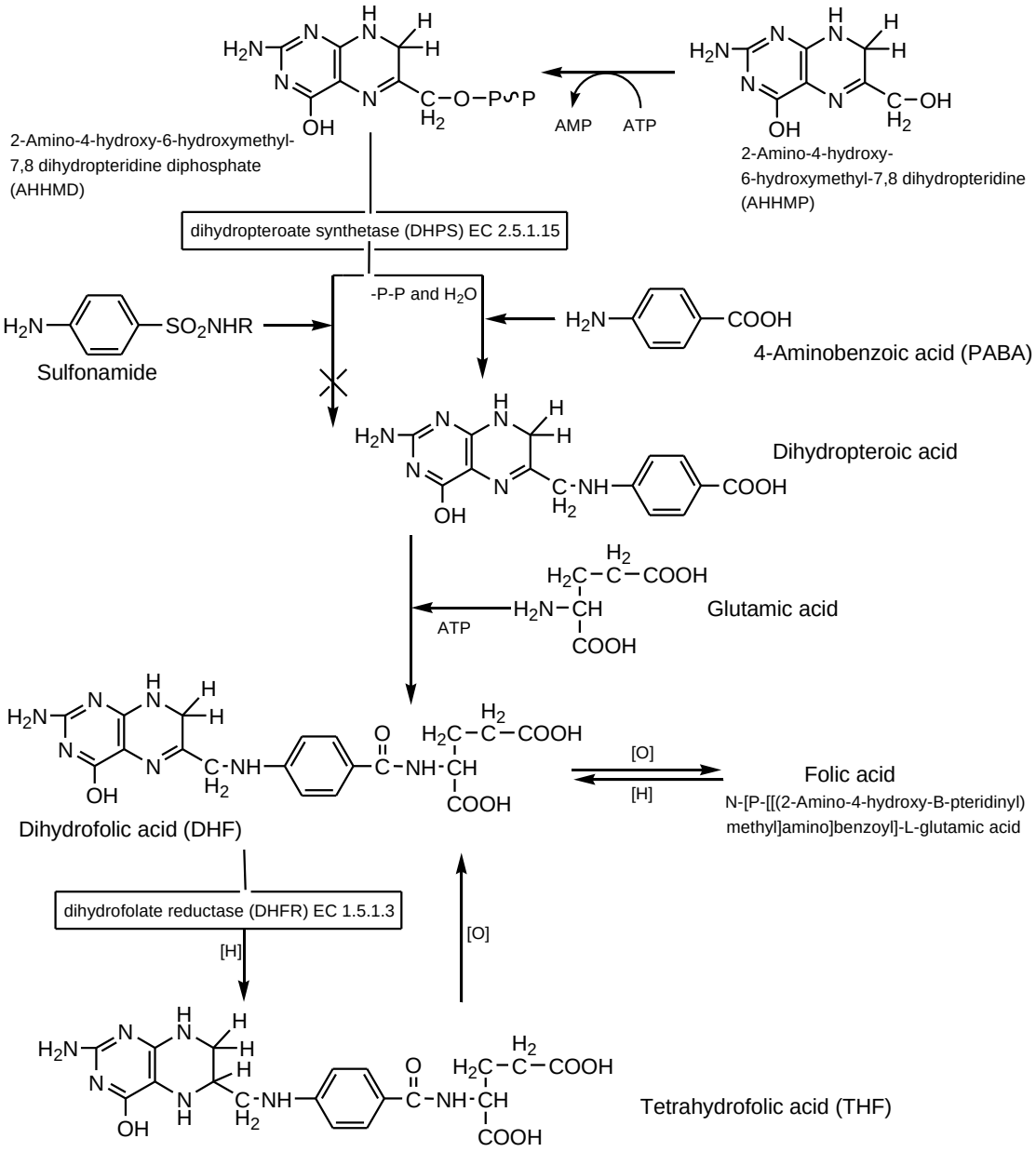


Figure (1.1): Chemical structure of sulfonamides

1.2. Action of sulfa drugs:

The activity of the sulfa drugs has been extensively studied and can be explained in the following manner. Sulfonamides are typically administered in doses that are bacteriostatic, meaning they prevent or limit bacterial multiplication. Sulfonamides achieve this bacteriostatic action by inhibiting the synthesis of folic acid in bacteria. Bacteria synthesize their own folic acid using endogenous compounds and enzymes , Endogenous compounds are those that occur naturally in the biological system, Specifically, sulfonamides inhibit the enzyme

dihydropteroate synthetase, an enzyme that catalyzes the conversion of p- aminobenzoic acid (PABA) and dihydropteroate diphosphate to dihydropteroic acid, a precursor to folic acid and DNA. Sulfonamides compete with PABA for the "active site" in the dihydropteroate synthtase enzyme, and are considered to be "competitive inhibitors" of this enzyme. The structural similarity of the sulfonamides to PABA "tricks" the enzyme into binding with the drug (sulfonamide) instead of the endogenous compound (PABA). The displacement of the PABA by the sulfonamide leads to the formation of a "false" metabolite in the folic acid synthesis, which cannot continue through the synthetic sequence.[9,10,)]



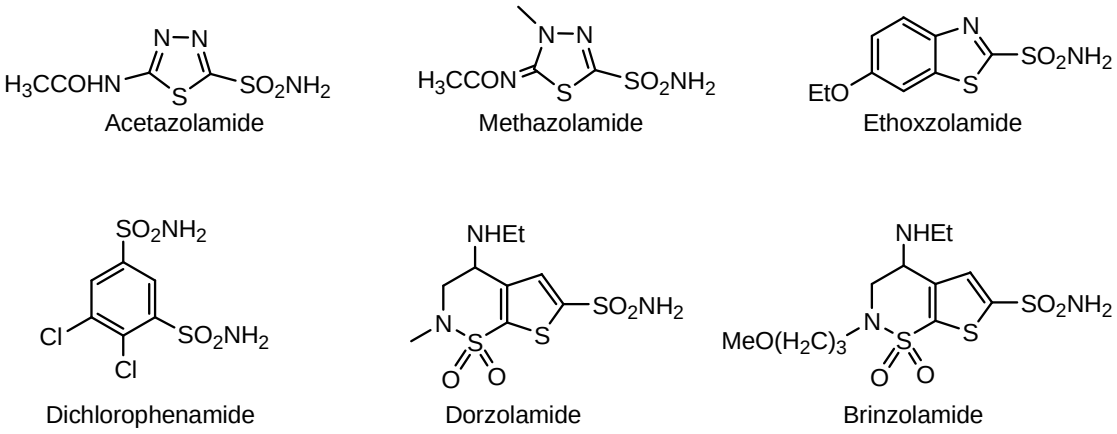
Scheme (1.2): Mechanism of sulfonamides action [(11)]

1.3. Sulfa drugs as therapeutic agents:

1.3.1. Sulfonamides with anti-carbonic anhydrase activity:

Sulfonamides are also known to inhibit the enzyme carbonic anhydrase (CA), an enzyme present in red blood cells and kidneys that catalyses the hydration of carbon dioxide and the dehydration of bicarbonate at physiological pH, ($CO_2+H_2O \rightarrow HCO3^-+H^+$).[(12)]The

formation of bicarbonate is essential as it is involved in the carboxylation step of key metabolic pathways in gluconeogenesis, lipogenesis, ureagenesis, as well as the biosynthesis of amino acids and pyrimidines.[13] CA controls the release of CO2 from the body via its transfer from tissue to blood and blood to the lungs. CA is also responsible for the secretion of electrolytes in tissues and organs as well as homeostasis, and because of its ubiquitous nature has been the target for inhibitors in the clinical treatment of a variety of diseases.[14]The discovery that sulfonamides inhibit CA has led to them being used for more than 50 years as agents to reduce blood pressure, in the treatment of conditions such as heart failure, glaucoma, epilepsy, and now potentially cancer. Acetazolamide**,** methazolamide, ethoxzolamide and dichlorophenamide**,** are four such sulfonamides that have been in clinical use as systemic CA inhibitors since the 1950's (Figure1.2). In addition, dorzolamide**,** and brinzolamide**,** have been launched as topically acting antiglaucoma pharmacological agents since the 1990's (Fig. 1.2).[14,15]



Figure(1.2): Some important Sulfonamides used as carbonic anhydrase inhibitors.

1.3.2. Sulfonamides with anticancer activity:

Chloroquinoxaline is one such sulfanilamide derivative that has progress to Phase II clinical trials (Figure1.3).[16] Chloroquinoxaline inhibited the formation of solid tumors in breast, lung, ovarian, and skin cancer, and whilst its mode of action is unclear, it is understood that folate metabolism remains unaffected, hence deviating from the usual distinctive nature of sulfonamide action. However, the lack of success during phase II clinical trials in nonsmall cell lung cancer meant its development as a clinical drug candidate was suspended.[7]

In 1992 Yoshino et al. discovered sulfonamide displays weak in vitro activity in tumor-bearing mice.[17]This encouraging result led to the design of derivatives of sulfonamide with the aim of finding improved anti-tumor activity. Thus, Yoshino et al. reported sulfonamide E7010as a suitable candidate which displayed pleasing levels of tumor growth inhibition against 26 human tumor cell lines (Figure 1.3). In rodent colon tumor models it was possible to reach a 60-99% inhibition of tumor growth. E7010 has been clinically assessed in phase I and II trials, and is currently being developed as an anti-tumor agent.[18]

Ueda et al. in 1995 developed a second generation sulfonamide ER-34410 based on the structure of E7010 that exhibited double the potency of the parent compound (Figure 1.3). It was effective in vitro against human tumor cell lines, and could be administered at a lower dose compared with E7010.[19]More recently, studies by Ozawa et al. have disclosed a series of N-7 indolyl benzene sulfonamides of which E7070is revealed to be the most potent anti-tumor sulfonamide known (Figure1.3). E7070is effective in vitro and in vivo against human colon carcinoma, and exhibited tumor growth suppression. This was evident in

its ability to exert complete tumor regression in lung and colon carcinomas.[20] E7070more commonly known as indisulam is currently in phase II clinical trials, and is awaiting approval to be used as an anticancer drug.[21]

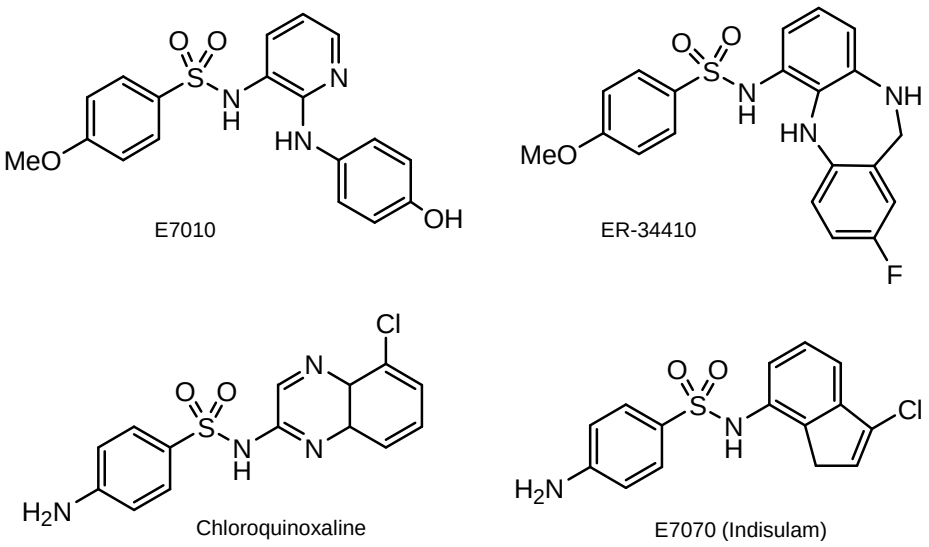


Figure (1.3): Some important sulfonamides used asanticancer agents.

1.3.3. COX inhibition by sulfonamides:

Since 1999 sulfonamides have been used extensively as selective COX-II inhibitors. COX enzymes are responsible for catalysing the conversion of arachidonic acid to prostaglandins, which in turn is responsible for many biological functions such as platelet aggregation, normal renal function, and vasodilation, but most notably the perception of pain.[22,23] The COX family is further subdivided into COX-I and COX-II, of which COX-II is the key contributor in induced inflammatory responses. Hence, ever since COX-II was identified in 1991, it has become a target for inhibition as a means to control pain resulting from rheumatoid and osteoarthritis. This has led to the discovery of aryl sulfonamides celecoxib and more recently vadecoxibin 2002 as selective

COX-II inhibitors (Figure1.4).[23]Both are used clinically in the management of pain and inflammation, and as selective COX-II inhibitors they do not exhibit the side effects displayed through non-selective COX inhibition most commonly seen with non steroidal anti-inflammatory drugs (NSAIDs) such as aspirin.[24]

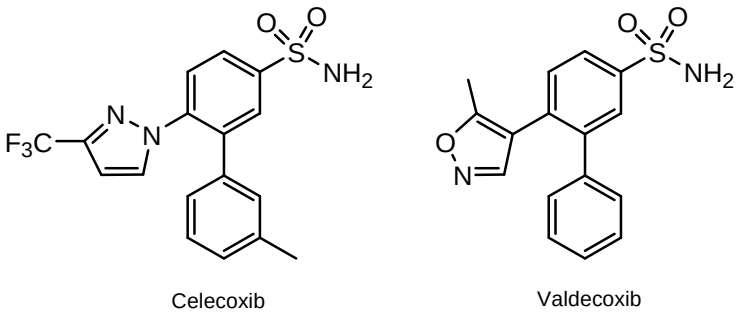


Figure (1.4) Some important sulfonamides used asCOX inhibitors.

1.3.4. Sulfonamides as HIV inhibitors:

Sulfonamides also possess activity against HIV proteases. HIV protease consists of a homodimer with aspartyl active sites (Asp25 and Asp125), which have the ability to cleave difficult bonds, such as Tyr-Pro and Phe-Pro. Thus far, several HIV protease inhibitors are clinically available, and are often used in conjunction with reverse transcriptase inhibitors to deliver the multi-drug treatment known as the Highly Active Anti- Retroviral Therapy (HAART). It was found that nonpeptidic protease inhibitors display higher bioavailability as well as slower excretion rate compared to the conventional peptide-base protease inhibitors. Among those protease inhibitors Amprenavir and Tipranavir are sulfonamide derived drugs.[25,26]

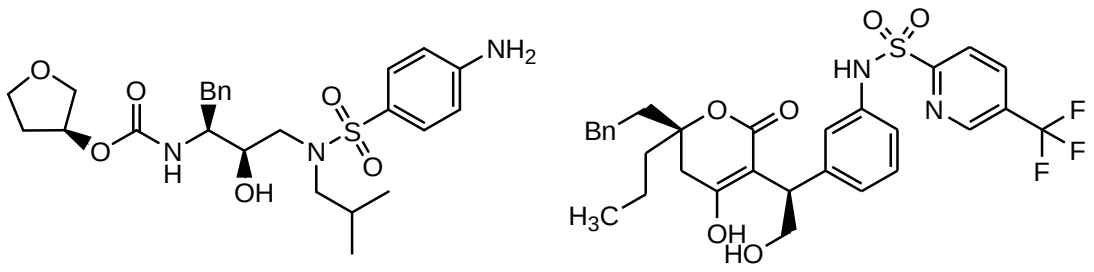
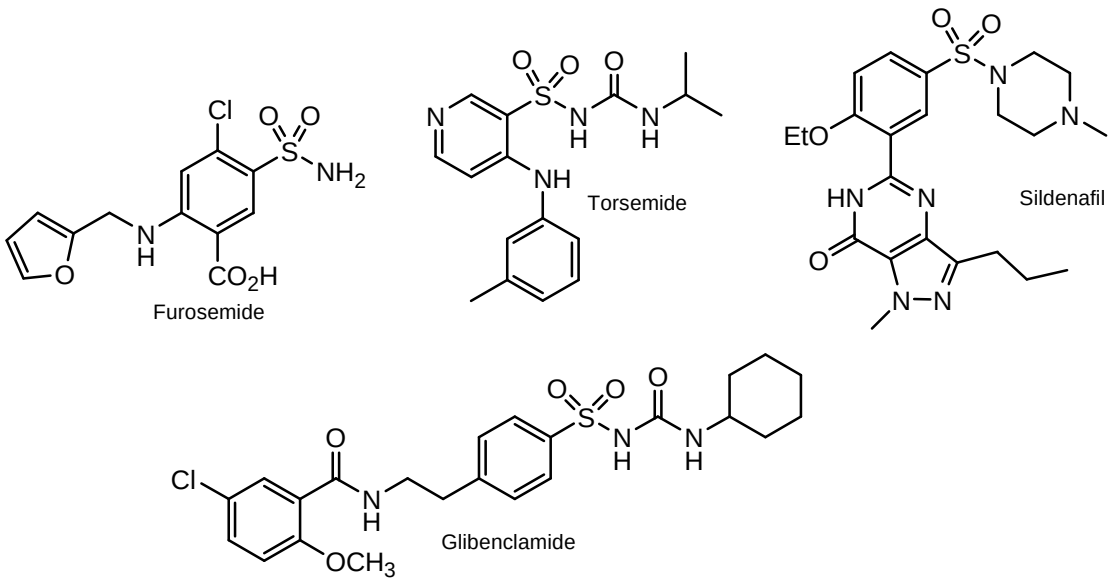


Figure (1.5) Some important sulfonamides used asHIV inhibitors.

1.3.5. Other applications for sulfonamides:

Several other sulfonamides have been clinically accepted and are currently employed in the treatment of various diseases (Figure 1.6). Furosemide and torsemideare two such sulfonamides that have found value as diuretics which relieve hypertension in patients with chronic systolic heart failure,[27,28] whilst glibenclamide is a sulfonamide that is prescribed for treatment of type II diabetes. Glibenclamideis a potent and selective ATP-sensitive potassium ion channel blocker, which applies a glucose lowering effect by stimulating calcium influx and thus insulin production in theB-cells of the pancreas.[29]Finally amongst the most topical of current sulfonamide drugs is sildenafil. Sildenafil (Viagra) is used for the treatment of erectile dysfunction. Erection is caused by binding of nitric oxide NO (released from the brain) to guanylate cyclase, causing the build-up of cyclic guanosine monophosphate (cGMP) resulting in smooth muscle relaxation and increase blood flow to the male organ. Viagra works by inhibiting phosphodiesterase-5 which is responsible for metabolizing cGMP, resulting in prolonged erection.[30]



Figure(1.6) : sulfonamides used for several diseases.

1.4. Pathogenic bacteria:

The pathogenesis of bacterial infection includes initiation of the infectious process and the mechanisms that lead to development of signs and symptoms of disease Characteristics of bacteria that are pathogens include transmissibility, adherence to host cell invasion of host cells and tissues. Toxigenicity and ability to evade the host's immune system. Many infections caused by bacteria that are commonly considered to be pathogens are in apparent or asymptomatic. Disease occurs if the bacteria or immunologic reactions to their presence cause sufficient harm to the person.[31]

1.4.1. The Staphylococcus genus:

The genus Staphylococcusis composed of Gram-positive bacteria with diameters of 0.5-1.5 μm, characterized by individual cocci that divide in more than one plane to form grape-like clusters.[32] These bacteria are non-motile, nonspore forming facultative anaerobes, featuring a complex nutritional requirement for growth[33,34], a low G+C

content of DNA, a tolerance to high concentrations of salt and resistance to heat.[35] *S.* aureus the most pathogenic specie of the genus Staphylococcus, being implicated in both community-acquired and nosocomial infections. It often asymptomatically colonizes the skin and mucous membranes of healthy individuals, in particular the anterior nares. In effect, it has been estimated that about 20-30 % of the population are permanently colonized by this bacterium, while other 30 % are transient carriers.[36] This colonization represents an increased risk of infection by providing a reservoir from which bacteria are introduced when the host defense is compromised. Due to the importance of S.aureus infections and the increasing prevalence of antibiotic-resistant strains, this bacterium has become the most studied staphylococcal species.[37]

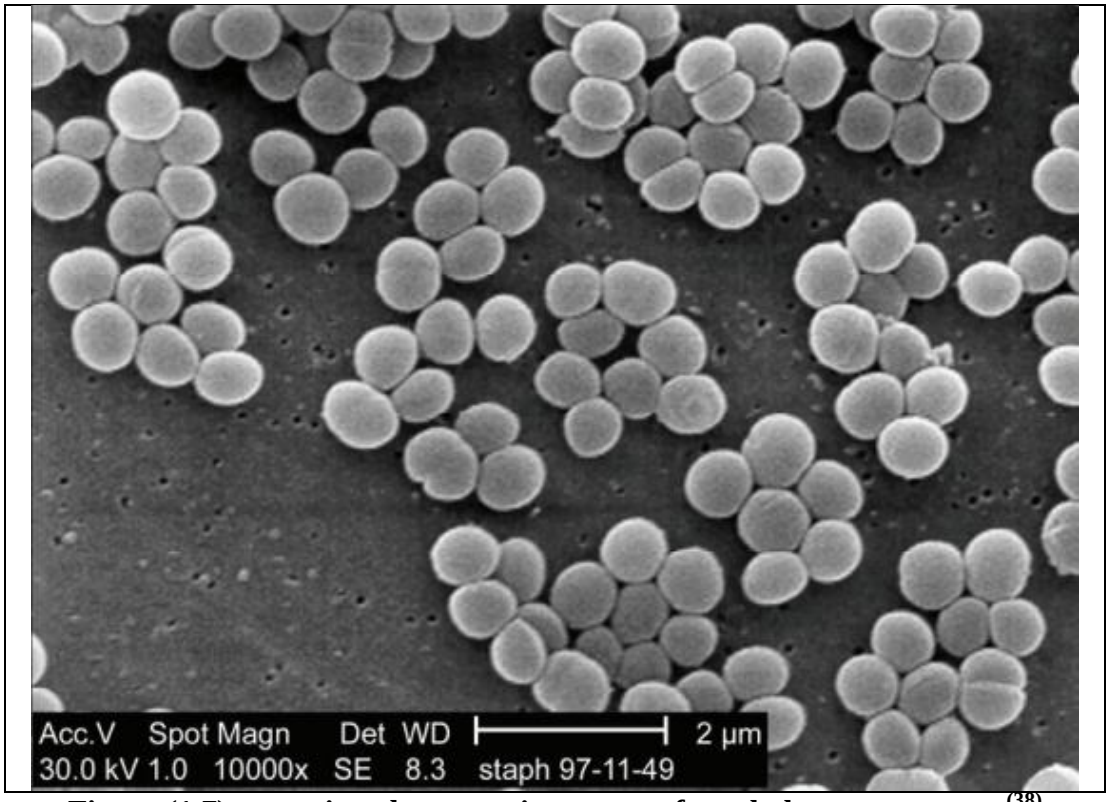


Figure (1.7): scanning electron microscopy of staphylococcus aureus.[38]

S.aureus is a clinically significant classical pathogen that causes a range of community acquired diseases and is also a leading nosocomial

pathogen. The diseases that are caused by s.aureus can be divided into several categories[39]:

1. toxin mediate diseases: food poisoning, scalded skin syndrome and toxic shock syndrome (TSS).
2. infection of the skin and soft tissue: furuncles, boils, cellulitis and impetigo.
3. infection of deep site : bone, joins, hear valves, spleen and liver.
4. infection of the lungs and urinary tract: pneumonia and urinary infection due to catheterization.

1.4.2. Pseudomonasaeruginosa:

Pseudomonas aerugionosa (P.aruginosa) is a non-fermentative, aerobic Gram negative rod, measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Almost all strains are motile by means of a single polar flagellum. It normally lives in moist environments, and uses a wide range of organic compounds for growth, thus giving it an exceptional ability to colonize ecological niches where nutrients are limited, from water and soil to plant and animal tissues.

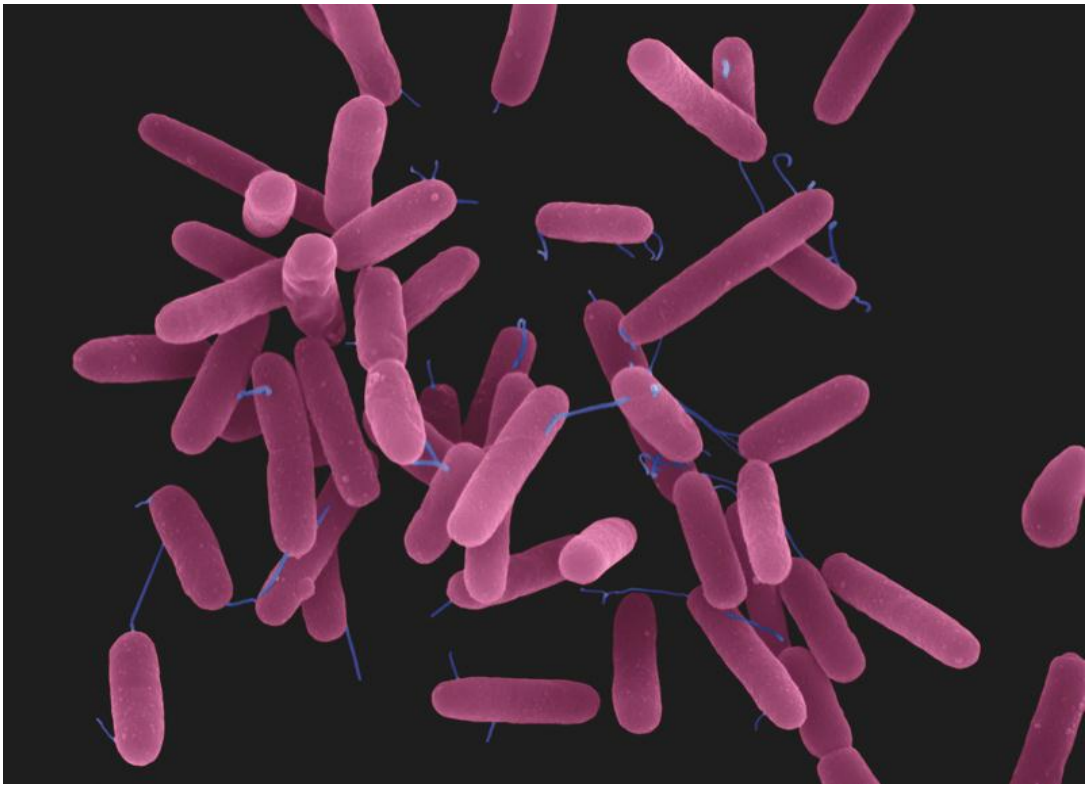


Figure (1.8): Pseudomonasaeruginosa.[42]

Typical biochemical features of P. aeruginosa isolates are: positive oxidase test, growth at 42 °C, hydrolysis of arginine and gelatine, and nitrate reduction. P.aeruginosa strains produce two types of soluble pigments, pyoverdin and pyocyanin.[40] Pseudomonas aeruginosa has been found to cause a variety of infections in clinical practice besides chronic CF lung infection, including common acute septicemia from burn or surgical wound infection, urinary tract infection, corneal ulceration (from wearing contact lenses), endocarditis (caused by intravenous drug use, etc.), and pneumonia (from use of ventilator and endotracheal tube).[41]

1.5.Biological activity:

1.5.1.Mechanism of antimicrobial action:

It should always be remembered that drugs are seldom the sole solution of cure but act together with the natural defenses of the body. The mechanism of action of most antimicrobial drugs is not completely understood. However, these mechanisms of action can be placed under four headings.[43,44] Figure (1.9) show the overall mechanism.

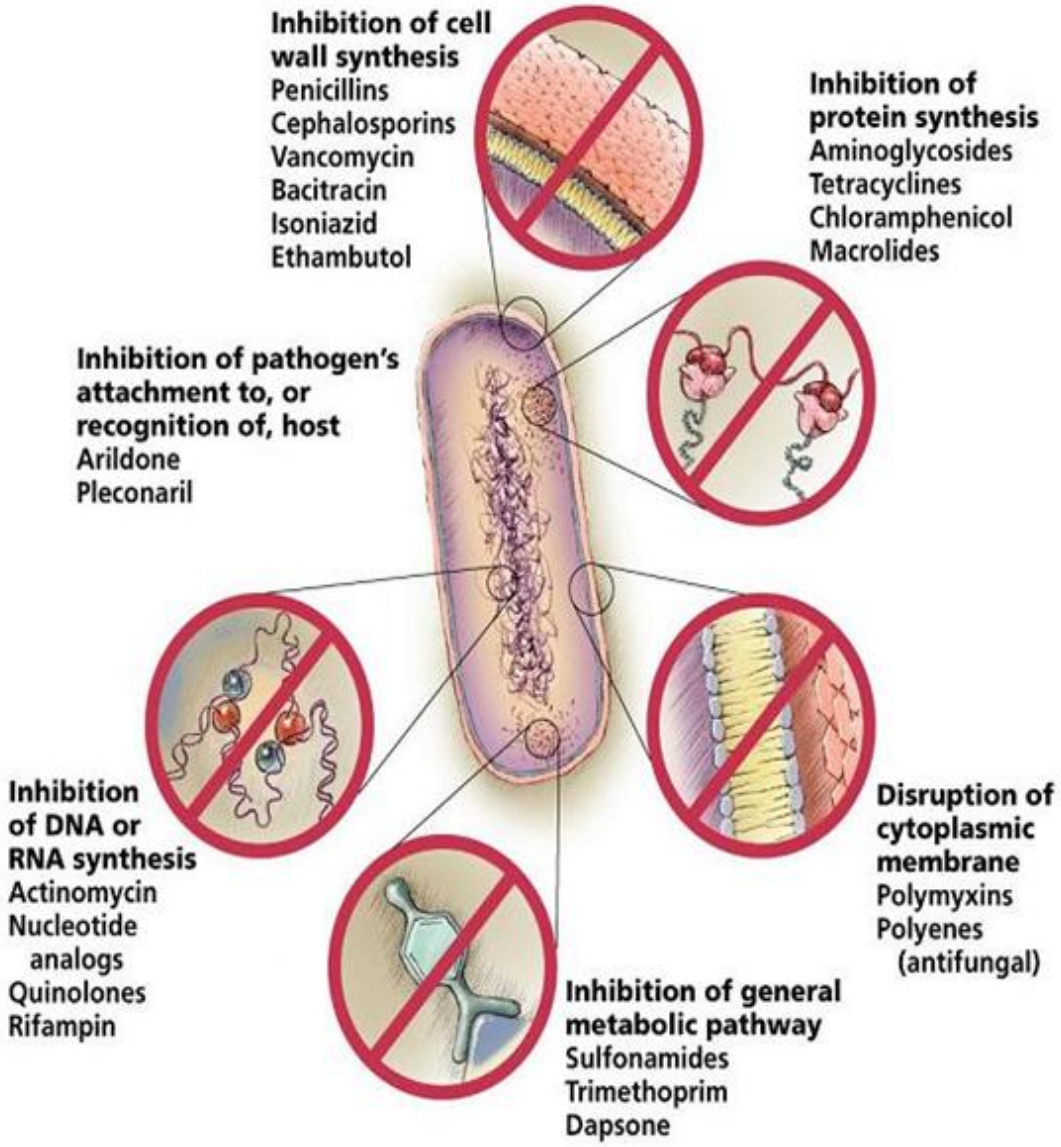


Figure (1.9): Mechanism of antimicrobial action.[43]

1.5.1.1.Inhibition of cell wall synthesis:

Bacteria possess a rigid outer layer of the cell wall. which maintain the shape of the microorganism and provide protection against much lower osmotic pressure of the environment (the internal pressure is three to five times greater in gram-positive than in gram-negative bacteria)[45,46], as in Figure(1.10).

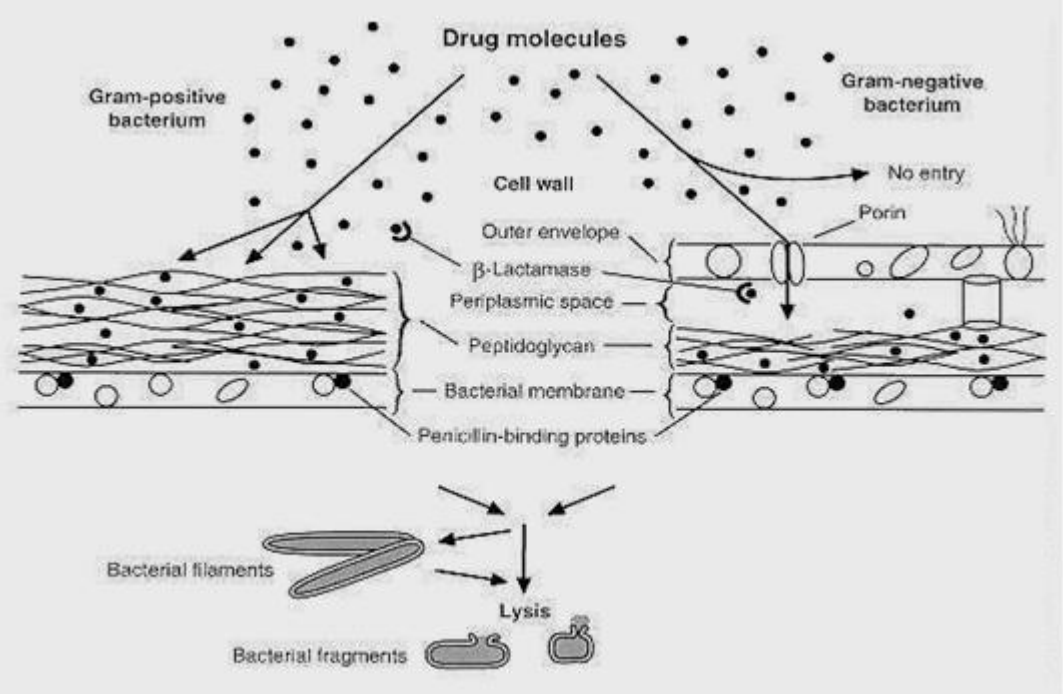


Figure (1.10): Outer wall of Gram-positive and Gram-negative species.[46]

Structurally, bacteria resemble primitive plants in that the cellular contents are surrounded by an inner peptidoglycan cell wall in addition to an inner plasma membrane and, in Gram-negative bacteria, an outer lipid bilayer. Specific antibacterial interfere with the synthesis of the cell wall, weakening the peptidoglycan scaffold within the bacterial wall so that the structural integrity eventually fails. Since mammalian cells have a plasma membrane but lack the peptidoglycan wall structure, this class of antibacterial selectively targets the bacteria with no significant negative effect on the cells of the mammalian host[45].

Bacterial multiplication involves a breakdown and an extension of the wall, interference with these processes, prevents the organism from resisting osmotic pressures, so that it bursts. As these cells of higher organisms, e.g. human, these 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 (bacitracin, cephalosporins, cycloserine, penicillins, and vancomycin)[47].

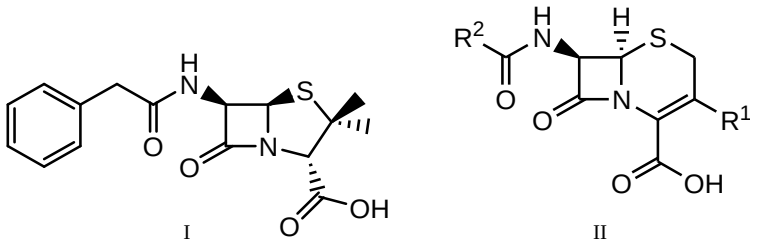


Figure (1.11):structures 0f penicillins (I) and cephalosporins (II).

1.5.1.2.Inhibition of cell membrane function:

Biologic membranes are composed basically of lipid, protein, and lipoprotein. The cytoplasmic membrane acts as a diffusion barrier for water, ions, nutrients, and transport systems. Most workers now believe that membranes are a lipid matrix with globular proteins randomly distributed to penetrate through the lipid bilayer.[48] A number of antimicrobial agents can cause disorganization of the membrane. These agents can be divided into cationic, anionic, and neutral agents. The best known compounds are polymyxin B and colistemethate (polymyxin E). These high-molecular-weight octapeptides inhibit Gram-negative bacteria that have negatively charged lipids at the surface. Since the activity of the polymyxins is antagonized by Mg^{2+} and Ca^{2+}, they probably competitively displace Mg^{2+} or Ca^{2+} from the negatively charged phosphate groups on membrane lipids. Basically, polymyxins disorganize membrane permeability so that nucleic acids and cations leak out and the cell dies.

The polymyxins are of virtually no use as systemic agents since they bind to various ligands in body tissues and are potent toxins for the kidney and nervous system. Gramicidins are also membrane-active antibiotics that appear to act by producing aqueous pores in the membranes. They also are used only topically.(49)

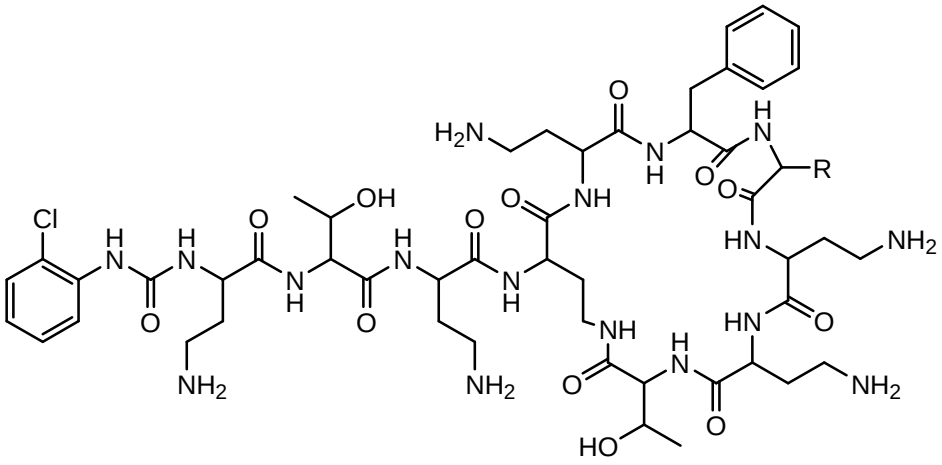


Figure (1.12): Structure of polymyxin (B).

1.5.1.3.Inhibition of proteins synthesis:

It is established that (Chloramphenicol, Tetracycline, Erythromycin, lincomycin and aminoglycoside) drugs can inhibit proteins synthesis in bacteria. The precise mechanism of action is not fully establishing for these drugs.(50)Bacteria have 70S ribosomes. The subunit of each type of ribosome is different in chemical composition, and their functional specification are sufficiently different, therefore antimicrobial drugs can inhibit proteins synthesis in bacterial ribosome by inhibition of transcription and translation of genetic material without having a major effect on mammalian ribosome.(51)

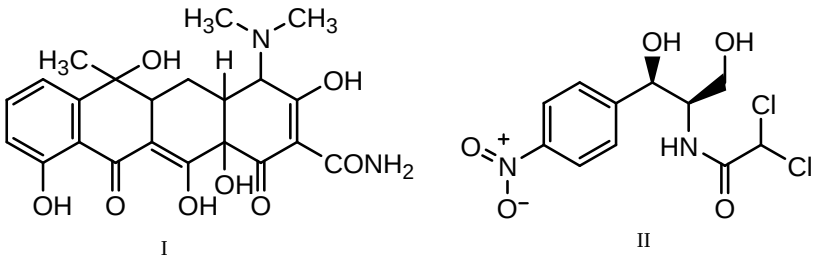


Figure (1.13): Structures of Tetracycline (I) and Chloramphenicol (II).

1.5.1.4.Inhibition of nucleic acid synthesis:

A nucleic acid inhibitor is a type of antibacterial that acts by inhibiting the production of nucleic acids. There are two major classes: DNA inhibitors and RNA inhibitors.

1. DNA inhibitors such as Quinolones are a key group of antibiotics that interfere with DNA synthesis by inhibiting topoisomerase, most frequently topoisomerase II (DNA gyrase), an enzyme involved in DNA replication. DNA gyrase relaxes super coiled DNA molecules and initiates transient breakages and rejoins phosphodiester bonds in superhelical turns of closed-circular DNA. This allows the DNA strand to be replicated by DNA or RNA polymerases. The fluoroquinolones, second-generation quinolones that include levofloxacin, norfloxacin, and ciprofloxacin, are active against both Gram-negative and Gram-positive bacteria.[52]

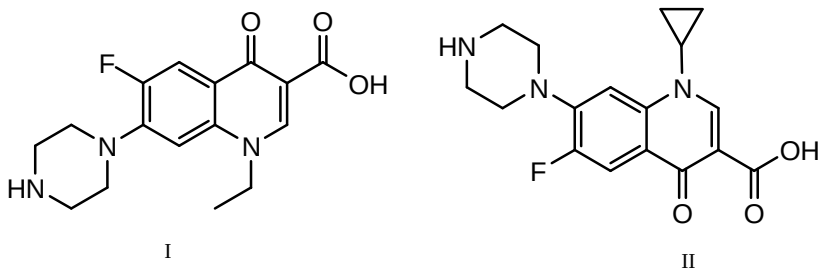


Figure (1.14): Structures of norfloxacin (I) and Ciprofloxacin (II).

2. RNA inhibitors such as Rifampicin blocks initiation of RNA synthesis by specifically inhibiting bacterial RNA polymerase. It does not interact with mammalian RNA polymerases, making it specific for Gram-positive bacteria and some Gram-negative bacteria. Doxorubicin and actinomycin D, are not specific for bacteria and interfere with both bacterial and mammalian systems.[53]

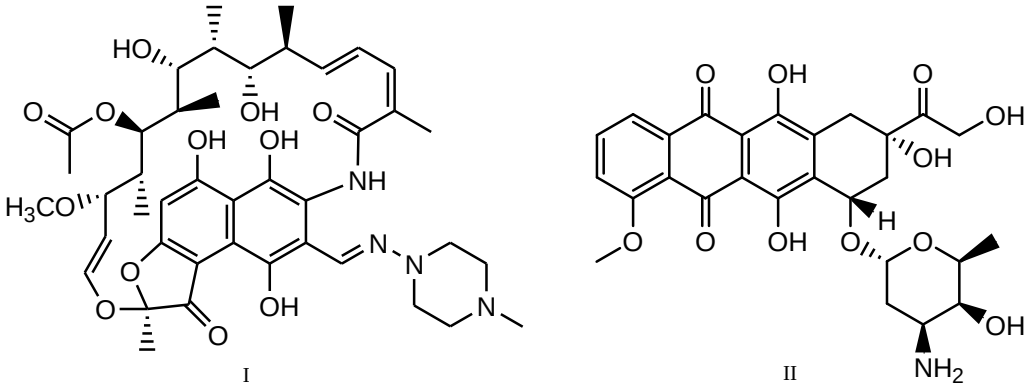


Figure (1.15): Structures of Rifampicin (I) and Doxorubicin (II).

1.5.2.Spectrum of antibacterial activity and Resistance:

Sulfonamides inhibit (bacteriostatic) gram-positive and gram-negative bacteria, Nocardia, Chlamydia trachomatis and some protozoa. Enteric bacteria such as *E.*coli, Klebsiella, Salmonella, Shigella and Enterobacterare also inhibited. Resistance to sulfonamides may develop when bacterial mutations result: (i) in PABA overproduction, (ii) in a folic acid synthesizing enzyme protein that has low affinity for sulfonamides and (iii) from a loss of cell permeability to sulfonamides.[6,54]

1.5.3.Pharmacokinetics:[6,55]

Sulfonamides can be classified into three major groups: oral absorbable agents, oral non absorbable agents and topical. oral absorbable agents may be further classified as short, medium, or long acting sulfonamides. Sulfonamides are absorbed from the stomach and small intestine and widely distributed to tissues, including the CNS. Sulfonamides and inactivated metabolites are excreted by the kidney mainly through glomerular filtration.

1.5.3.1.Oral absorbable agents:

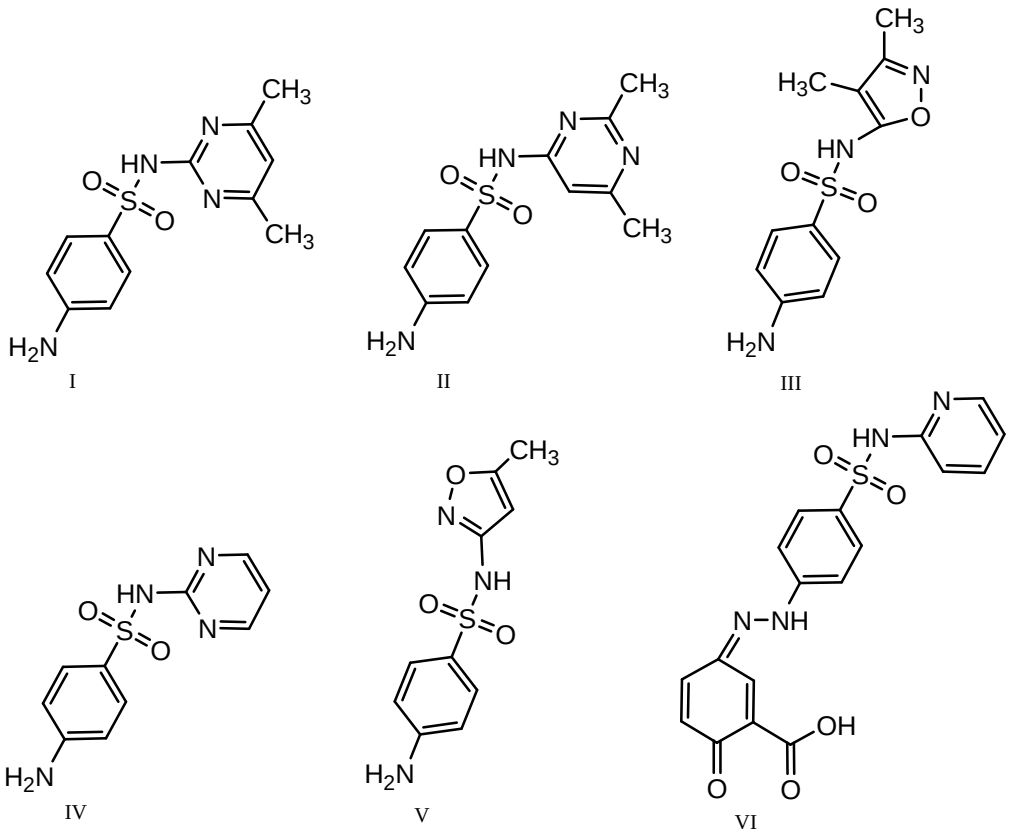
I. *Short Acting***:** sulfonamides with a half-life less than 10 hours. (e.g. sulfamethazole, sulfisoxazole and sulfanilamide have been used for the treatment of urinary tract infections).

II. *Intermediate Acting***:** Sulfonamides with a half-life between 10- 24 hours. (e.g. sulfamethoxazole and sulfadiazine have been used for various infections especially active against invasive aspergillosis in AIDS patients).

III. *Long Acting***:** Sulfonamides with a half-life longer than 24 hours. (e.g. Sulfadimethoxine and Sulfadioxine have been used for the treatment of ulceration colitis).

1.5.3.2.Oral non-absorbable agents: e.g. sulfasalazine. Sulfasalazine is reserved for the treatment of chronic inflammatory bowel disease.

1.5.3.3.Topical agents: e.g. sodium sulfacetamide, silver sulfadiazine. The former is effective treatment for bacterial conjunctivitis while the latter is preferred for prevention of infection of burn wounds.



Figure(1.15) :Some important sulfonamides as antibacterial Sulfamethazine(I), Sulfaisodimidine(II), Sulfaisoxazole(III), Sulfadiazine(IV), Sulfamethoxazole(V) and Sulfasalazine(VI).

1.5.4.Adverse effects of sulfa drugs:

The toxic effects which may arise during sulfonamide therapy are: crystalluria, acute hemolytic anemia, agranulocytosis, a plasticanemia, hypersensitivity reactions, gastrointestinal upsets including anorexia, nausea and vomiting, headache, dizziness, mental depression, and other signs of central nervous system involvement. Hepatitis is a rare but potentially dangerous complication of sulfonamide therapy.[6]

1.6.Aim of The Work:

1. Synthesis of some of sulfa drug compounds.
2. Characterization using: Elemental analysis, FTIR and UV-visible spectroscopy of the prepared compounds was important for my study.
3. Study the biological activity of the synthesized compounds against two types of bacteria:

I. Staphylococcus aureus (Gram positive).

II. Pseudomonas aeruginosa (Gram negative).

CHAPTER TWO

EXPEREMENTAL

1.2.Chemicals:

All of the reagents and starting materials used in the present work were of reagent grade and were used without further purifications unless otherwise noted. Table (2-1) shows all the utilized chemicals in the experimental course of the thesis.

Table (2-1) Chemicals and their manufacturers

No.	Compound	Formula	Company
1	Acetanilide	C_8H_9NO	Merck
2	Chlorosulfonic acid	$HClSO_3$	Fluka
3	p-Chloroaniline	C_6H_6NCl	Merck
4	DMSO	CH_3SOCH_3	Fluka
5	Di Ethyl ether	$C_4H_{10}O$	Fluka
6	Hydrocloric acid	HCL	BDH
7	Morpholine	C_4H_9NO	Ferak
8	o-Methyl aniline	C_7H_9N	BDH
9	o-Methoxy aniline	C_7H_9NO	BDH
10	Piperidine	$C_5H_{11}N$	BDH
11	Potassium bromide	KBr	BDH
12	Sodium bicarbonat	$NaHCO_3$	Fluka
13	Sulfamethoxazol	$C_{10}H_{11}N_3O_3S$	Bilim pharmaceuticals

2.2.Cultures Media:

The cultures media used in this work were listed in Table (2-2)

Table (2-2) Cultures media with the supplied company and origin purpose

NO.	Media	Company supplied from	Using
1	Mueller Hinton Agar (MHA)	Mumbai (India)	For determination of susceptibility of microorganism to antimicrobial agents.
2	Nutrient Broth (NB)	Mumbai (India)	For activation the microorganism before testing.
3	Blood Agar (BA)	Mumbai (India)	For isolation and cultivation of many fastidious pathogenic microorganism.

2.3. Instruments:

2.3.1. Melting point

The melting points were determined on electro thermal capillary apparatus, *Gallenkamp*, England.

2.3.2. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra in the range (400-4000) cm^{-1} cut were recorded as KBr disc on FT-IR.8300 Shimadzu Spectrophotometer. (Ibn- Sena/Baghdad University) .

2.3.3. Ultraviolet-Visible Spectroscopy (UV-Vis)

The UV-visible spectra were measured using Shimadzu UV-Vis. 160 A-Ultra-violet Spectrophotometer in the range (200-500) nm. (AL-Nahrain University).

2.3.4. Hot plate with magnetic stirrer

Gallenkamp / England

2.3.5. Elemantal analysis (CHNS)

Elemental C H N S analysis were carried out on a Fison EA 1108 analyzer (Ibn- Sena/ Baghdad University).

2.3.6. Autoclave

Sturdy SA- 300 VL (Taiwan). AL-Nahrain University/College of Applied Biotechnology.

2.3.7. Incubator

Memmert (Germany). AL-Nahrain University/College of Applied Biotechnology.

2.3.8.Oven

Memert/ Germany

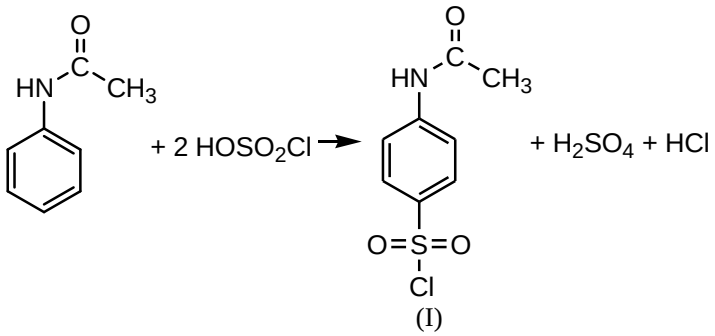
2.3.9.Balance

TP Series/ Chinese

2.4.Synthesis Steps Of The Compounds (IIIa, IIIb, IIIc, IIId and IIIe):

2.4.1.Synthesis Of p-Acetamidobenzenesulfonyl Chloride (I):

Equip a 250 ml flask and cooled in ice bath, Place (2.5 g. 0.018 moles) of dry acetanilide in the flask. Excess of chlorosulfonic acid(6.25ml, 0.093 moles) was added in small portions and shake the flask from time to time to ensure thorough mixing. When the addition has been made, under stirring, heat the reaction mixture on a water bath(50-60C) for 1 hour in order to complete the reaction. Allow the flask to cool to room temperature and slowly pour the contents, with vigorous stirring, into a beaker containing 100g. of crushed ice, a milky-white precipitate is formed. This suspension was filtered off, washed with a little cold water and air dried to give crude product, which was used immediately in the next step without further purification (M.P 148C).[56]

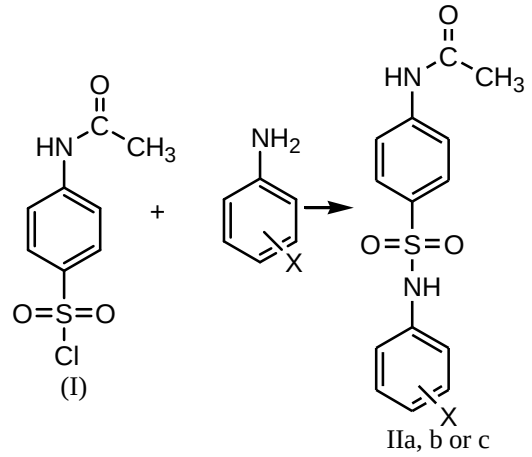


Scheme(2.1): Synthesis Of p-Acetamidobenzenesulfonyl Chloride(I)

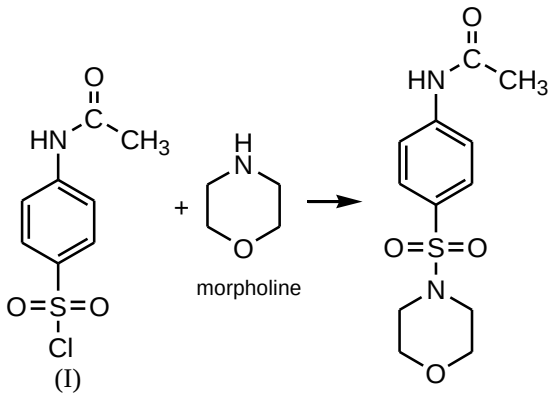
2.4.2.Synthesis Of p-Acetamido-N-(2-methylphenyl)benzene Sulfonamides(II_a):

To crude p-acetamidobenzenesulfonyl chloride dissolved in diethyl ether (30 ml) was added dropwise o-methyl aniline (2.5 ml, 0.023moles). Under stirring it took one hour for the reaction to complete. After the completion of the reaction, the precipitate was collected by filtration and air dried. The same procedure was repeated with 0.023moles of each

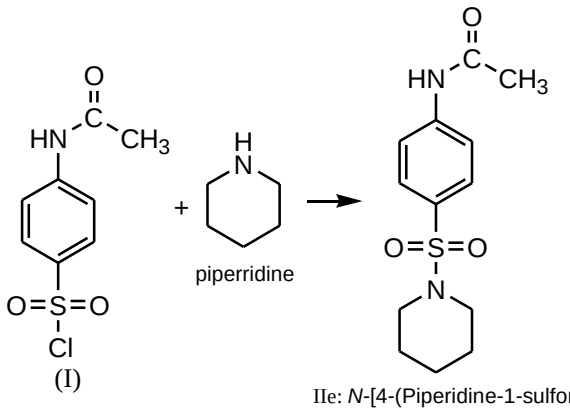
amine (2.56 ml o-methoxy aniline, 2.93g p-chloro aniline, 2.26 ml Piperidine, 2ml morpholine)[57]



IIa: x= *O*-CH_3, p-acetamido-N-(2-methylphenyl)benzenesulfonamide
IIb: x= *O*-OCH_3, p-acetamido-N-(2-methoxyphenyl)benzenesulfonamide
IIc: x= *P*-Cl, p-acetamido-N-(4-chlorophenyl)benzenesulfonamide



IId: *N*-[4-(Morpholine-4-sulfonyl)-phenyl]-acetamide

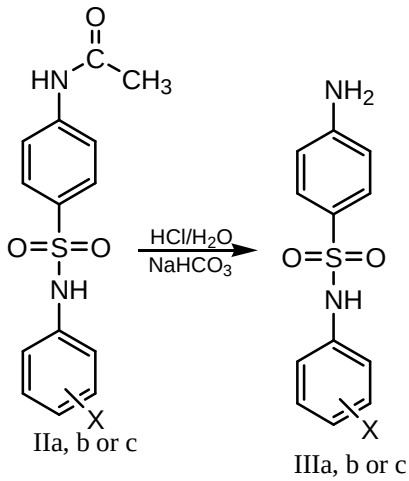


IIe: *N*-[4-(Piperidine-1-sulfonyl)-phenyl]-acetamide

Scheme(2.2): Synthesis Of (II_a, II_b, II_c, II_d, II_e) compounds.

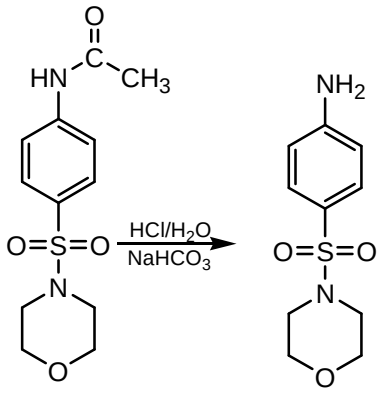
2.4.3.Synthesis Of p-Amino-N-(2-methylphenyl)benzene Sulfonamides (IIIa):

p-Acetamido-N-(2-methylphenyl)benzenesulfonamide (II_a) from the previous step is placed in a 50 mL round-bottom flask equipped with a magnetic stir bar. Dilute hydrochloric acid (5 ml of conc. HCl in 10ml of water) is added to the flask. The flask is fitted with a condenser and heated at reflux with constant stirring for (30-45) minutes, after which it is allowed to cool to room temperature. After cooling, the reaction mixture is neutralized by slow addition of a 10% NaHCO3 solution with stirring until it tests slightly alkaline to pH paper. A precipitate may have begun to form during neutralization. Cool the beaker in an ice bath to complete the precipitation of product. It may be necessary to gently scratch the inside bottom of the beaker to induce crystallization. The product is collected by filtration, washed with a small amount of ice cold water and air dried. The same procedure was repeated with (II_b, II_c, II_d, II_e) compounds.[56, 58]

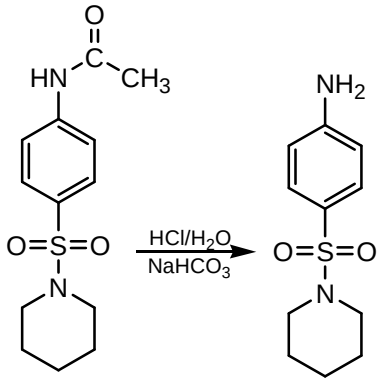


IIa, b or c IIIa, b or c

IIIa: x= *O*-CH_3, p-amido-N-(2-methylphenyl)benzenesulfonamide
IIIb: x= *O*-OCH_3, p-amido-N-(2-methoxyphenyl)benzenesulfonamide
IIIc: x= *P*-Cl, p-amido-N-(4-chlorophenyl)benzenesulfonamide



IId IIId: 4-(Morpholinosulfonyl) aniline



IIe IIIe: 4-(Piperidin-1-yl sulfonyl) aniline

Scheme(2.3): Synthesis Of (IIIa, IIIb, IIIc, IIId and IIIe) compounds.

Table (3-1)Physical properties of synthesized compounds

Colour	M.P.(°C)	Yield (%)	M. Wt.	formula	Elemental analysis theoretical (Experimental)			
					C	H	N	S
White	185-187	86	262.33	$C_{13}H_{14}N_2SO_2$	59.52(59.41)	5.38(5.40)	10.68(10.70)	12.22(11.99)
Pale white	204-206	80	278.33	$C_{13}H_{14}N_2SO_3$	56.10(55.98)	5.07(4.96)	10.06(9.88)	11.52(11.22)
Yellowish white	196-198	52	282.75	$C_{12}H_{11}N_2SO_2Cl$	50.97(50.88)	3.92(3.46)	12.54(12.55)	11.35(11.50)
White	174-176	71	242.29	$C_{10}H_{14}N_2SO_3$	49.57(49.34)	5.82(5.56)	11.56(11.71)	13.23(12.96)
White	160-162	78	240.32	$C_{11}H_{16}N_2SO_2$	54.98(55.20)	6.71(6.88)	11.66(11.36)	13.34(13.44)

2.5.Biological activity:

2.5.1.Antimicrobial activity:

In this study, the synthesized compounds were evaluated for their in vitro antimicrobial activity against the pathogenic bacteria, two bacterial species were used: one of them gram positive bacteria which was Staphylococcus aureus, the other gram negative bacteria which was pseudomonas aeruginosa. these microorganisms were obtained from College of Applied Biotechnology / AL.Nahrain University.

2.5.2.Bacterial Sensitivity Test of the Prepared Compounds:

Well diffusion method was used to determine the inhibiting power of the prepared compounds against the pathogenic bacteria[59,60]. Sulfamethoxazole was used to compare the power of inhibition.

2.5.3.Well-Diffusion Method:

This method depends upon the variation in concentration of the prepared compounds. A series of concentrations were made from the already prepared compounds with the range from (50, 25, 12, 6, and 3 mg/ml). Sulfamethoxazole was used as a reference antibiotic drugs and DMSO was used as a solvent for these compounds.

The following steps were done to measure the biological activity:

2.5.3.1. Nutrient broth and Mueller Hinton preparation:

Nutrient broth and Mueller Hinton agar were prepared by dissolving 28 gm for each liter of distilled water[61]. Sterilization was achieved by autoclaving under pressure of (1.5 atm) and temperature of (115C^{o}) for (15-20 minutes). The medium was cooled to 50C^{o}. Then put it in plate

(20 mL) Mueller Hinton for each plate and left in room temperature to dry so that the plantation medium will be solid, a semi-solid gelatinous layer was generated[(62)].

2.5.3.2.Activation of microorganisms :

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 hrs. at 37°C. The loop was sterilized by a flame before using so that the planted bacteria were not contaminated.

2.5.3.3.Antibacterial evaluation:

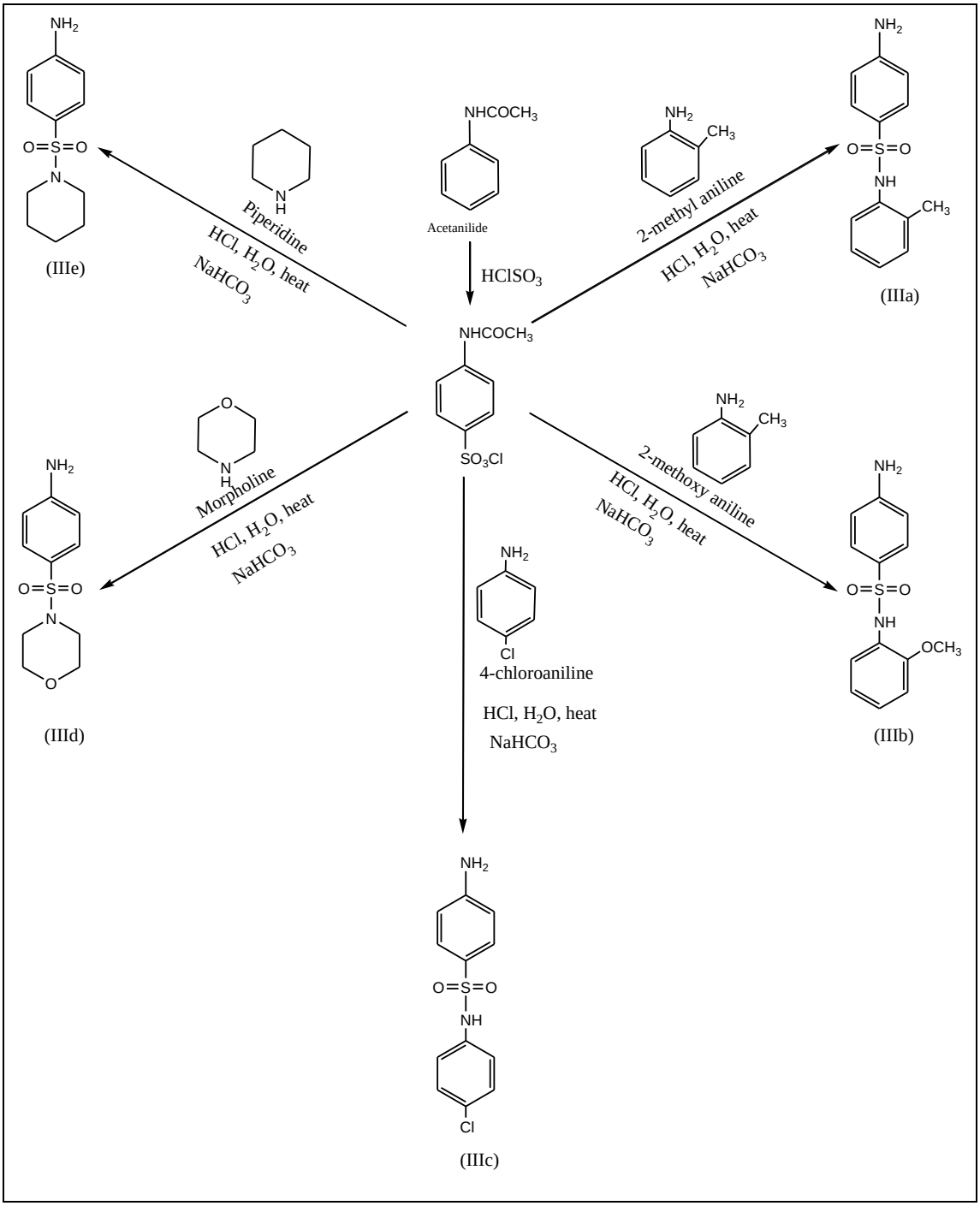
Medium Inoculated bacteria suspension were diluted by 1/100 by using normal-saline solution with concentration of (0.85%) to prevent crowded growth. (0.1 mL) of bacteria diluted suspension was transported to each plate and spread by using sterilized cotton spreader on test medium surface. The Plates were left for (15-20) min. at 37°C to make absorption. The plates of Mueller Hinton agar were inoculated by S.aureus and P.aeruginosa. In each medium six pores were made by the use of a sterile dry rod with a diameter of 5 mm, these pores were made with equal distance between each other, then the solutions of different concentrations of the prepared compounds were added using fixed amount of 0.1 mL from each concentration in one pore and DMSO as control, to see the effect of solvent for each type of bacteria. These plates were incubated at $37C^0$ for 24 hrs. After the incubation, the inhibition zone was measured for each pore using a ruler in a millimeter (mm). The zone of inhibition is defined as the translucent area which surrounds the disc including the diameter of the disc that lacks bacterial growth. The bacteria was considered sensitive, mean-sensitive or resistant depending on inhibition zone.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1. Chemistry:

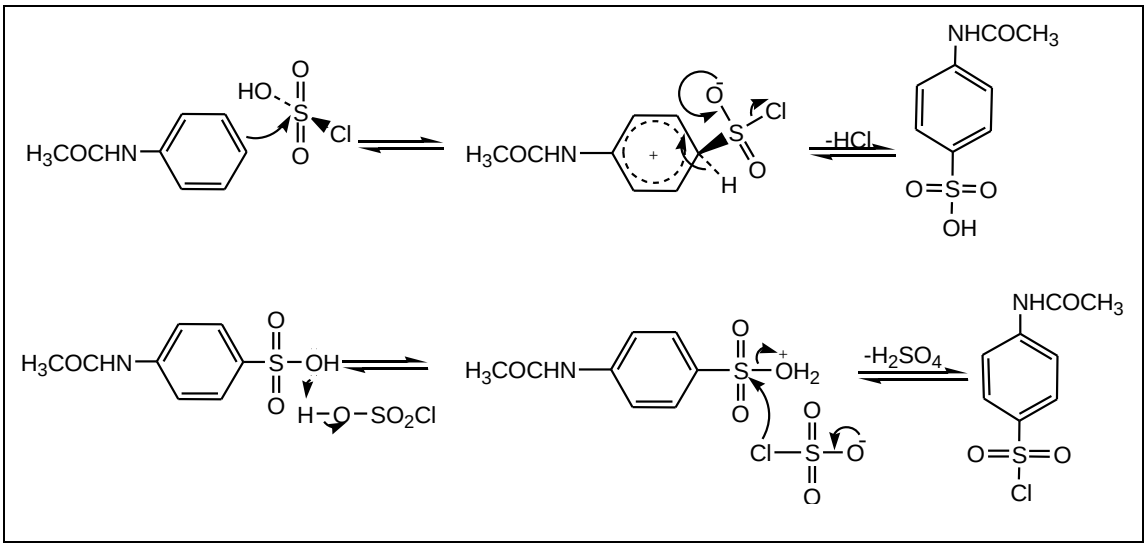
Acetanilide was choosen as a starting material based on its amino group which is already protected by acetyl and later on can be deprotected to get the free amino group again for further synthesis when the desired sulfa compounds could obtained. Acetanilide was converted to its corresponding sulfonyl chloride by chlorosulfonation by the direct reaction of chlorosulfonic acid with it[56]. The suggested amino compounds for preparation of sulfa derivatives are verified the from aromatic with either activated and deactivated group or aliphatic residue like piperidine and morpholine. These products were prepared by the condensation of precursor (I) with the amines, i.e., (2-methylaniline, 2-methoxyaniline, 4-chloroaniline, morpholine and piperdine) to furnish the 4-acetamido-N substituted benzenesulfonamides (IIa, IIb, IIc, IId and IIe) respectively. The acetyl group was removedto afford the4-amino-N-substituted benzenesulfonamides (IIIa,IIIb,IIIc,IIId and IIIe). The synthesis routes of the compounds (IIIa,IIIb,IIIc,IIId and IIIe) are shown in scheme (3.1).



Scheme (3.1):The chemical steps for synthesis of compounds (IIIa, IIIb, IIIc, IIId and IIIe)

3.1.1.Synthesis of p-Acetamidobenzenesulfonyl Chloride (I):

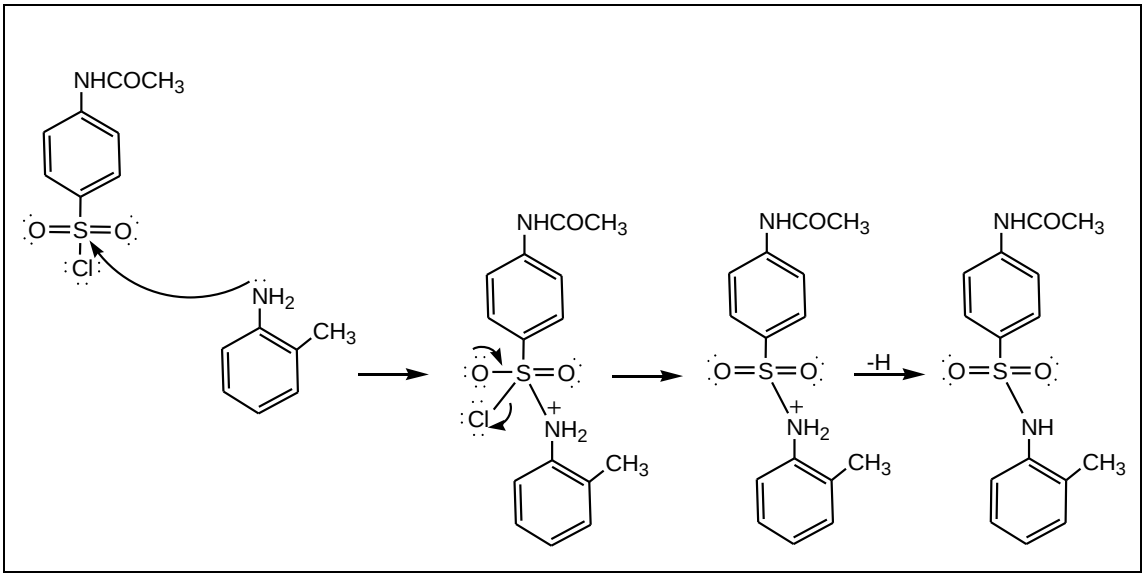
p-Acetamidobenzenesulfonyl Chloride was prepared through the reaction of acetanilide with chlorosulfonic acid, the product (**I**) was showed identical melting point (148 ^{o}C). The suggested mechanism for chlorosulfanation can be illustrated in scheme (3.2), the aromatic π-electorns can attack the partially positive charge sulfur atom. At the same time the chloride will leave to form the corresponding acid. The second molecule of chlorosulfonic acid will attach its chloride to the aromatic sulfonic acid to get the more stable sulfuric acid and converted the aromatic acid to its corresponding chloride[63].



Scheme (3.2): mechanism for synthesis of compound (I)

3.1.2. Synthesis of p-Acetamido-N-(2-methylphenyl)benzene Sulfonamides(II_a) and (II_b,II_c, II_d,II_e) Compounds:

The crude p-acetamidobenzenesulfonyl chloride reacts with o-Methyl aniline to produce the p-Acetamido-N-(2-methylphenyl) benzene sulfonamide (IIa) . The others were prepared in the same manner and used in the next step without further purification. The reaction mechanism could be followed the illustrated scheme (3.3)[63].

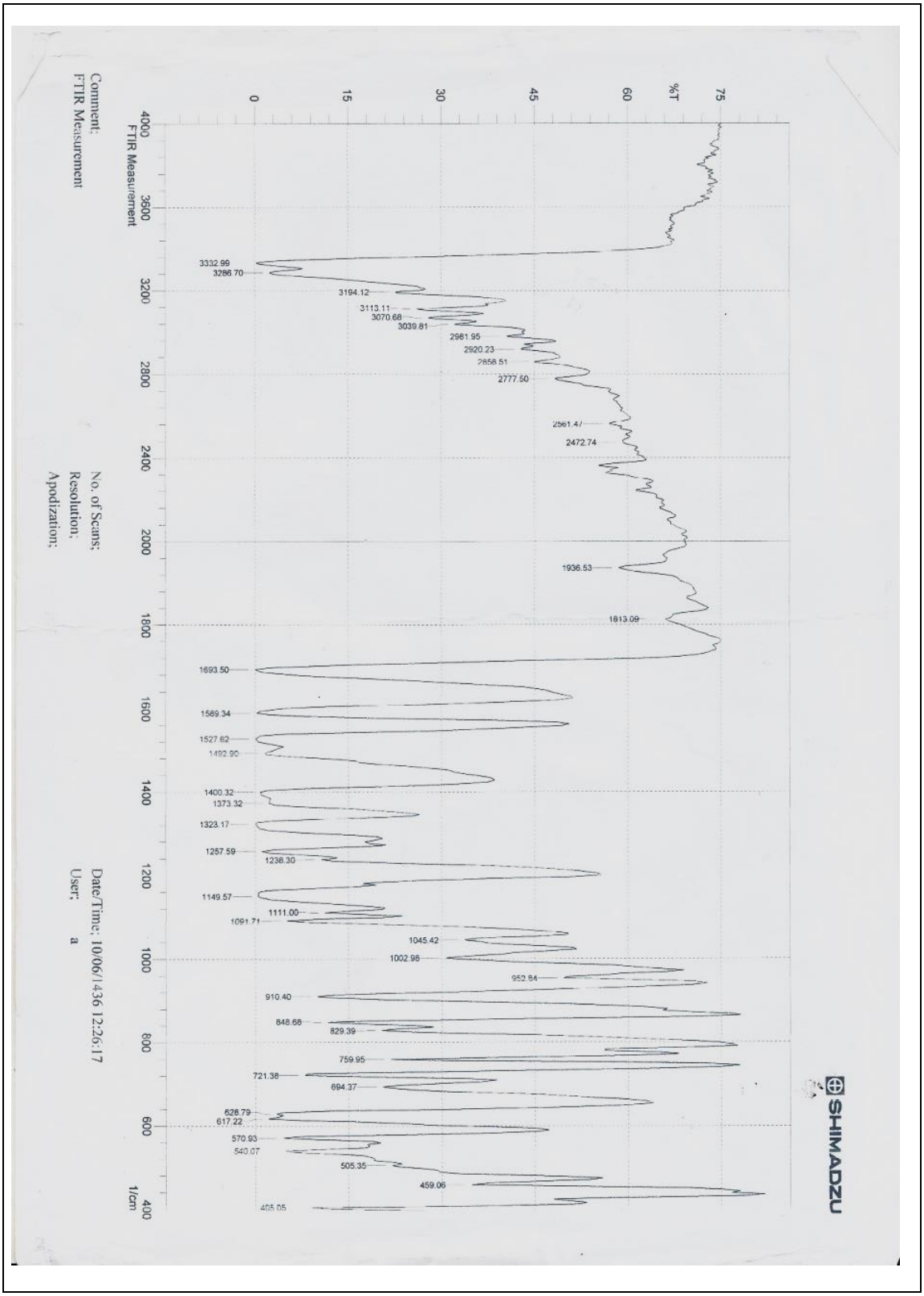


Scheme (3.3): mechanismReaction for preparation of (II_a, II_b,II_c, II_d, and II_e) Compounds.

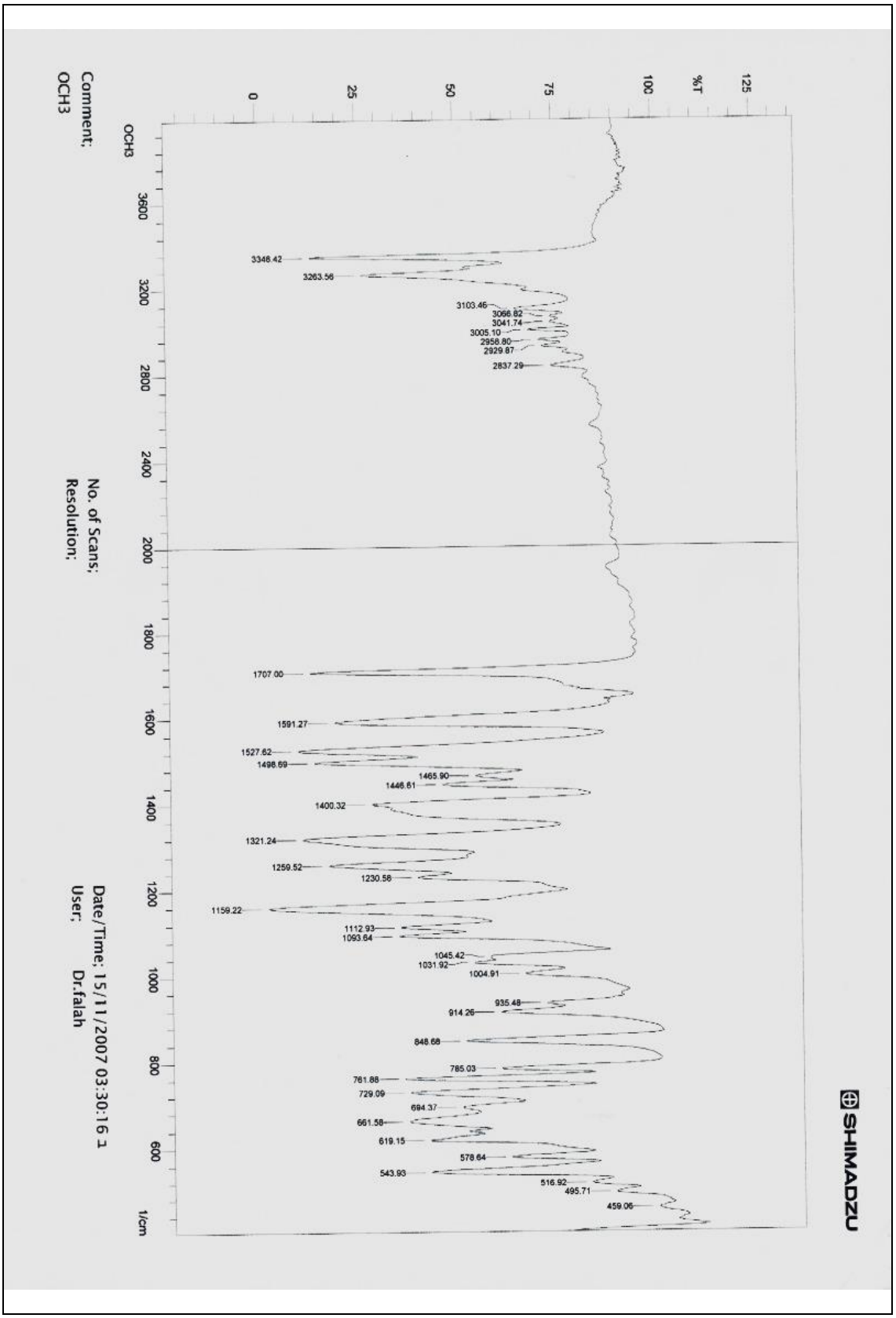
Compound (IIa) was showed a medium peak at 3332 cm^{-1} which assigned to the NH bond of sulfonamide. In addition the peak at 3286 cm^{-1} due to the stretching vibration of NH amide group. Peaks at 2981 and 2920 cm^{-1} could be attributed to the asymmetric and symmetric stretching vibration of CH_3 of methyl group, while the peaks at 1400 and 1373 cm^{-1} can be assigned to the bending vibration of CH_3 of methyl group. The spectrum showed a peak at 1693 cm^{-1} which assigned to the carbonyl stretching vibration. Stretching vibration of SO_2 was identified by the peak at 1323 and 1149 cm^{-1} (Fig 3.1).

Compound (IIb) was showed a medium peak at 3484 cm^{-1} which assigned to the NH bond of sulfonamide. In addition the peak at 3263 cm-1 due to the stretching vibration of NH amide group. The peaks at 2958 and 2929 cm^{-1} could be attributed to the asymmetric and symmetric stretching vibration of CH_3 of methoxy group, while the peaks at 1446 and 1400 cm-1 can be assigned to the bending vibration of CH_3 of methoxy group. The spectrum showed a peak at 1707 cm^{-1} which assigned to the carbonyl stretching vibration. he stretching vibration of SO_2 was identified by the peaks at 1321 and 1159 cm-1. Moreover, the

absorption at 1112 cm^{-1} can be assigned to the C-O bond of methoxy group (Fig 3.3).

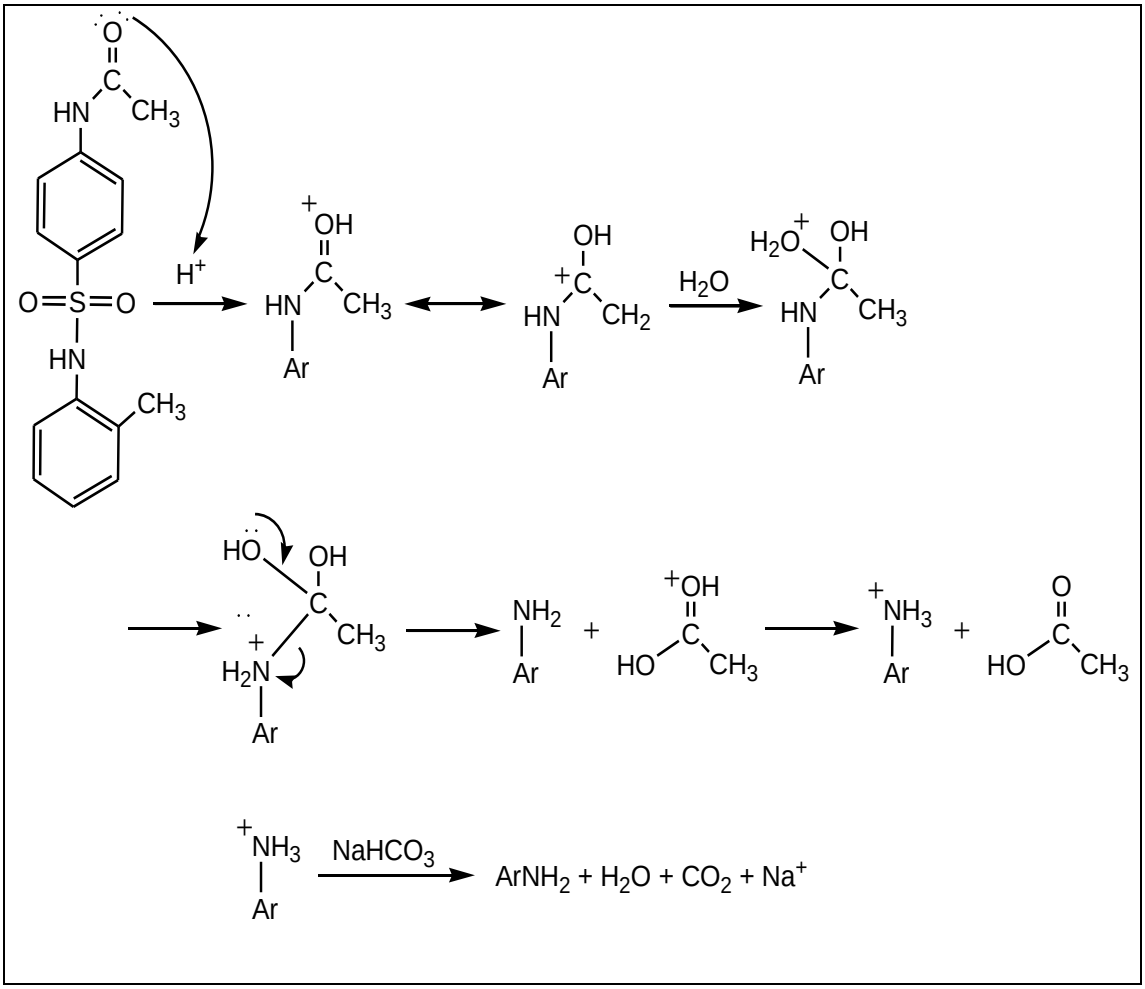


Figure(3.1): FTIR spectrum of p-Acetamido-N-(2-methylphenyl)benzene Sulfonamide(II_a)



Figure(3.2): FTIR spectrum of p-Acetamido-N-(2-methoxyphenyl)benzene Sulfonamide(II_b)

The crude products of step 2 were deprotected by acidic medium to produce the corresponding free amino compounds. For example, p-Acetamido-N-(2-methylphenyl)benzenesulfonamide(II_a) was treated with HCl in water for hydrolysis to produce the final product (IIIa). The pure compounds was obtain after recrystallization from ethanol. All compounds have been characteristized by FTIR, UV-Vis and CHNS. The reaction mechanism was illustrated in scheme (3.4)[64].



Scheme (3.4): mechanism synthesis for preparation of (IIIa) and (IIIb, IIIc, IIId, IIIe) Compounds.

3.2.Characterization of the prepared compounds by Infra-Red spectroscopy:

The infra-red spectrophotometer technique was used to characterize the prepared compounds through the assignment of stretching vibration bands. The FTIR spectra were shown the characteristic peaks of compounds as follow:

3.2.1.p-Amino-N-(2-methylphenyl)benzene Sulfonamides(IIIa):

This compound(IIIa)was showed the medium two peaks at 3444 and 3423 cm^{-1} due to the asymmetric and symmetric stretching vibration of NH_2 group while 3321 cm^{-1} of the NH of sulfonamide. The carbonyl of amido was disappear in the spectrum that mean the deprotection was done. The peaks at 2902 and 2864 cm^{-1} can be attributed to the asymmetric and symmetric stretching vibration of CH_3 group, while the peaks at 1465 and 1398 cm^{-1} can be assigned to the bending vibration of CH_3 group. The stretching vibration of SO_2 was identified by the peak at 1315 and 1149 cm^{-1}.

3.2.2.p-Amino-N-(2-methoxyphenyl)benzene Sulfonamides(IIIb)

This compound (IIIb) was showed the medium peak at 3484 cm^{-1} which assigned to the NH bond of sulfonamide. In addition the two peaks at 3367 and 3313 cm-1 due to the asymmetric and symmetric stretching vibration of NH_2 group. The spectrum showed no carbonyl absorption at 1681 cm^{-1}, i.e., the deprotection was done. The peaks at 2978 and2935cm^{-1} can be attributed to the asymmetric and symmetric stretching vibration of CH_3 of methoxy group, while the peaks at 1465 and 1396 cm^{-1} can be assigned to the bending vibration of CH_3 of methoxy. The stretching vibration of SO_2 was identified by the peak at

1323 and 1157 cm^{-1}. Moreover, the absorption at 1111 cm^{-1} can be assigned to the C-O bond of methoxy group.

3.2.3.p-Amino-N-(4-chlorophenyl)benzene Sulfonamides(IIIc):

This compound (IIIc) was showed the medium peak at 3484 cm^{-1} which assigned to the NH bond of sulfonamide. In addition the two peaks at 3383 and 3228 cm-1 due to the asymmetric and symmetric stretching vibration of NH_2 group. The spectrum showed no carbonyl absorption at 1681 cm^{-1}, i.e., the deprotection was done.The stretching vibration of SO_2 was identified by the peak at 1323 and 1157 cm-1. Moreover, the medium absorption at 640 cm^{-1} can be assigned to the C-Cl bond.

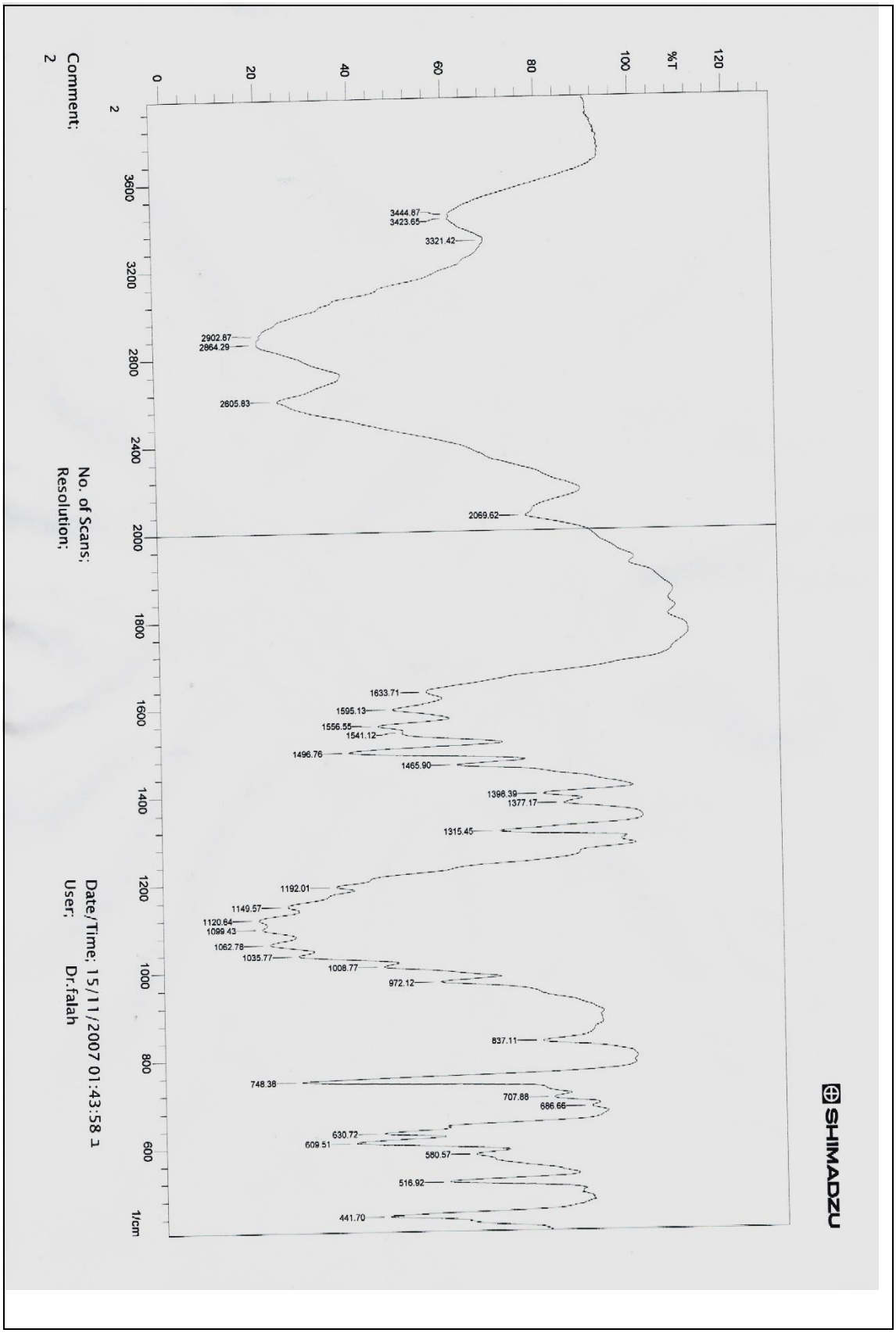
3.2.4. 4-(Morpholinosulfonyl) aniline (IIId):

This compound (IIId) was showed the medium two peaks at 3309 and 3263 cm-1 due to the asymmetric and symmetric stretching vibration of NH_2 group. The carbonyl of amido was disappear in the spectrum that mean the deprotection was done. The peaks at 2935 and 2888cm^{-1} can be attributed to the asymmetric and symmetric stretching vibration of $CH_2$of the morpholine group, while the peaks at 1404 and 1359 cm-1 can be assigned to the bending vibration of CH_2 group. The stretching vibration of SO_2 was identified by the peak at 1315and 1165 cm-1. In addition the peak at 1053 cm-1 can be assigned to the C-O group.

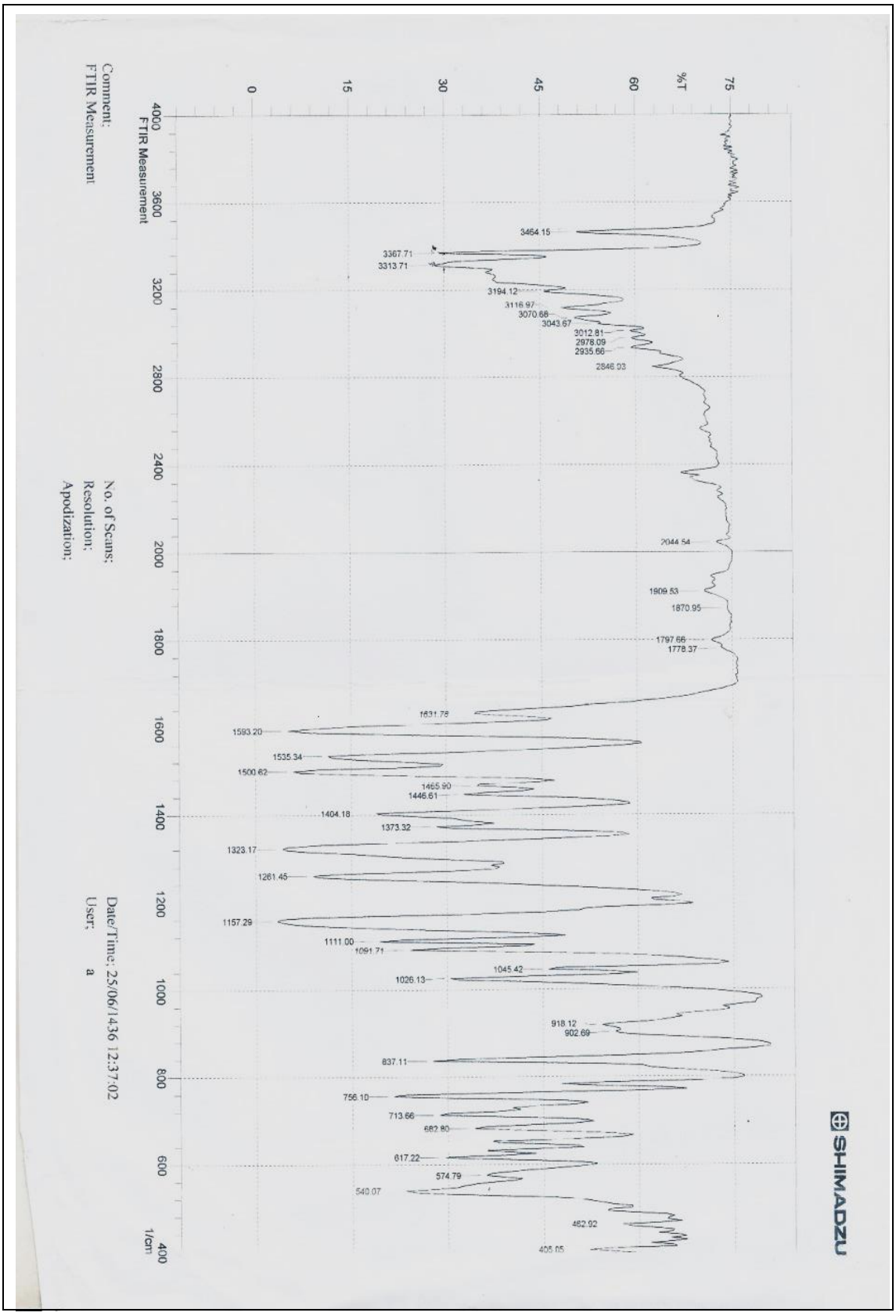
3.2.5. 4-(Piperidin-1-yl sulfonyl) aniline(IIIe):

This compound (IIIe) was showed the medium two peaks at 3294 and 3259 cm-1 due to the asymmetric and symmetric stretching vibration of NH_2 group. The carbonyl of amido was disappear in the spectrum that mean the deprotection was done. The peaks at 2927 and 2854cm^{-1} can be

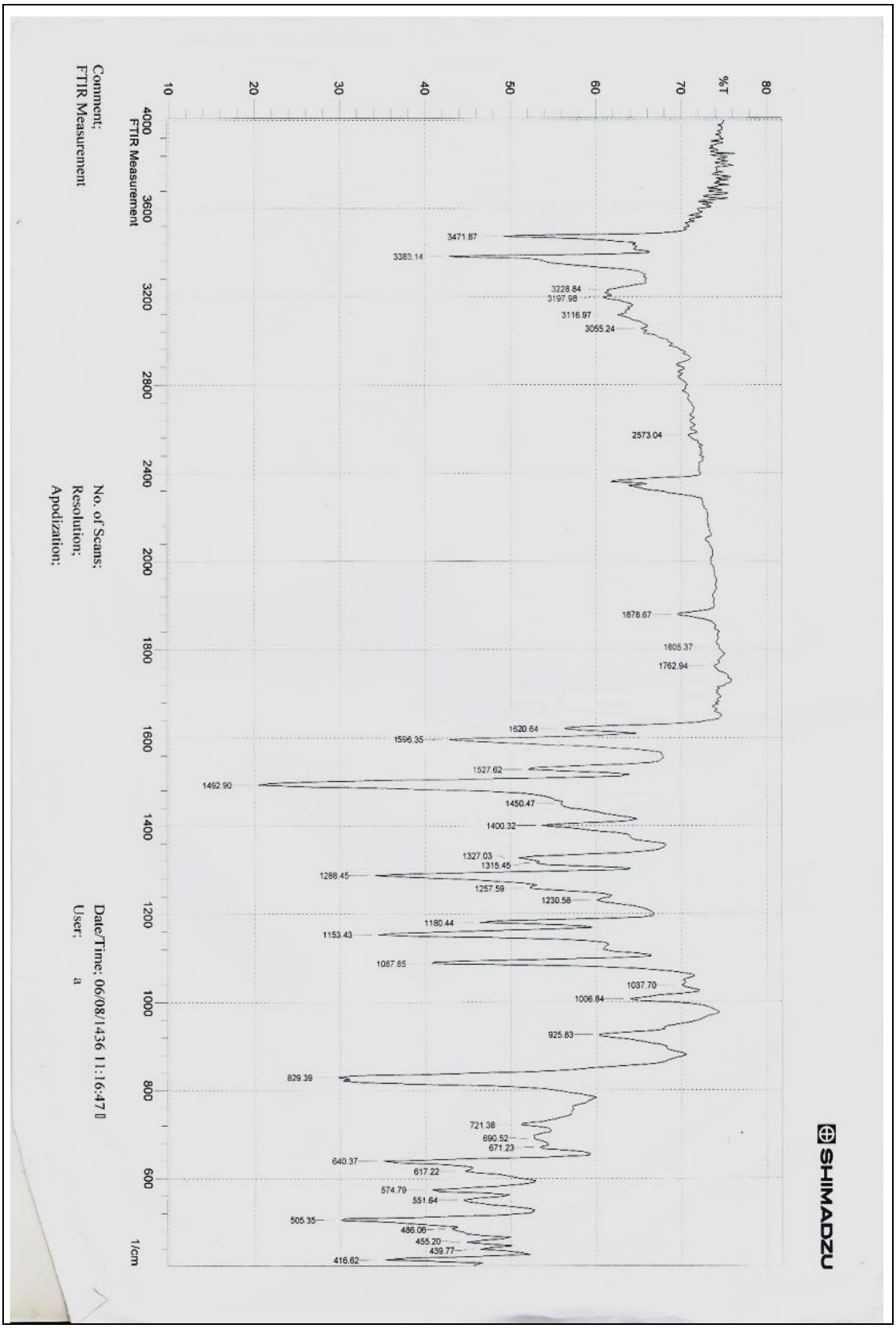
attributed to the asymmetric and symmetric stretching vibration of $CH_2$of the morpholine group, while the peaks at 1435 and 1359 cm^{-1} can be assigned to the bending vibration of CH_2group.The stretching vibration of SO_2 was identified by the peak at 1323 and 1161 cm^{-1}.



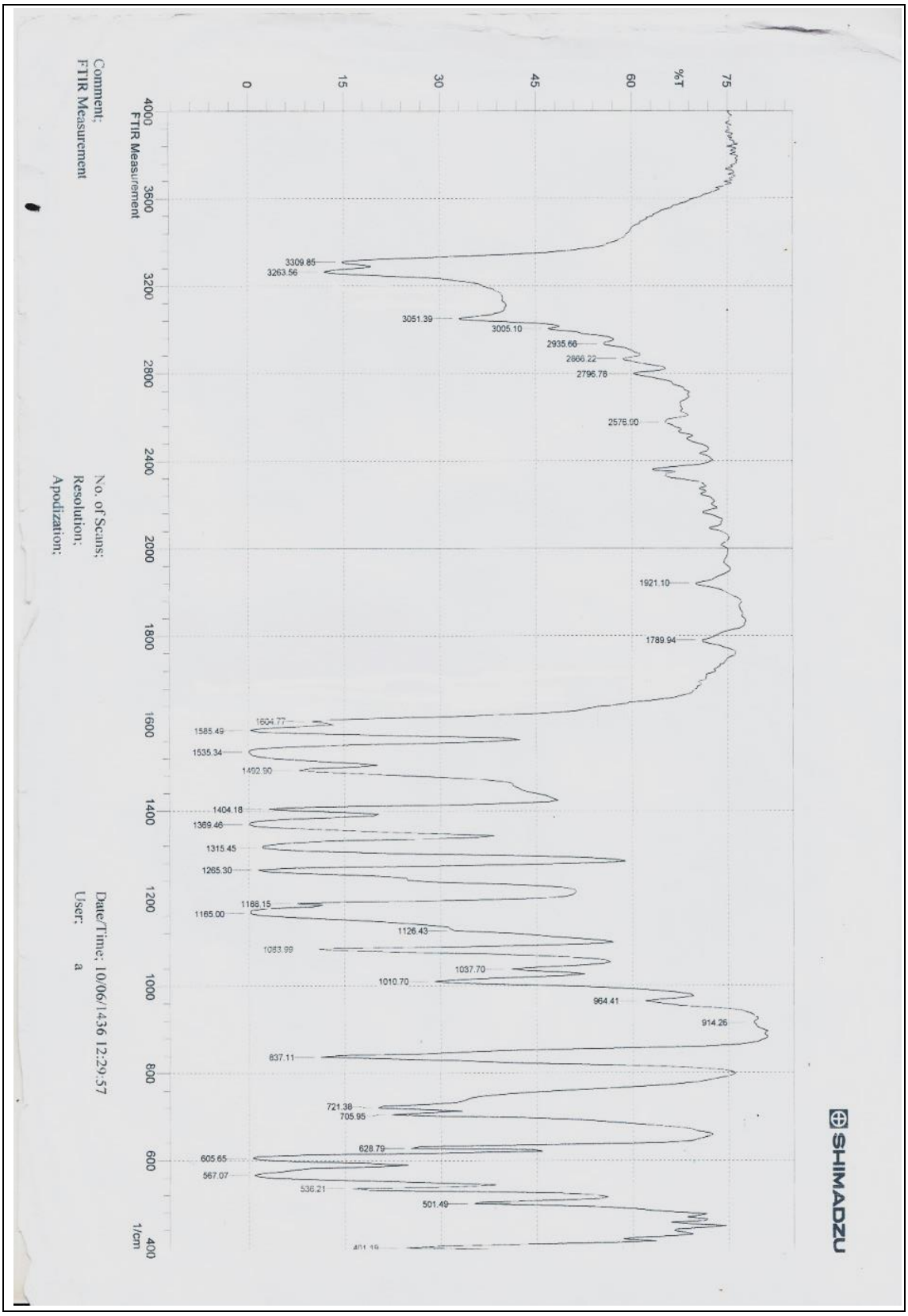
Figure(3.3): FTIR spectrum of compound (IIIa)



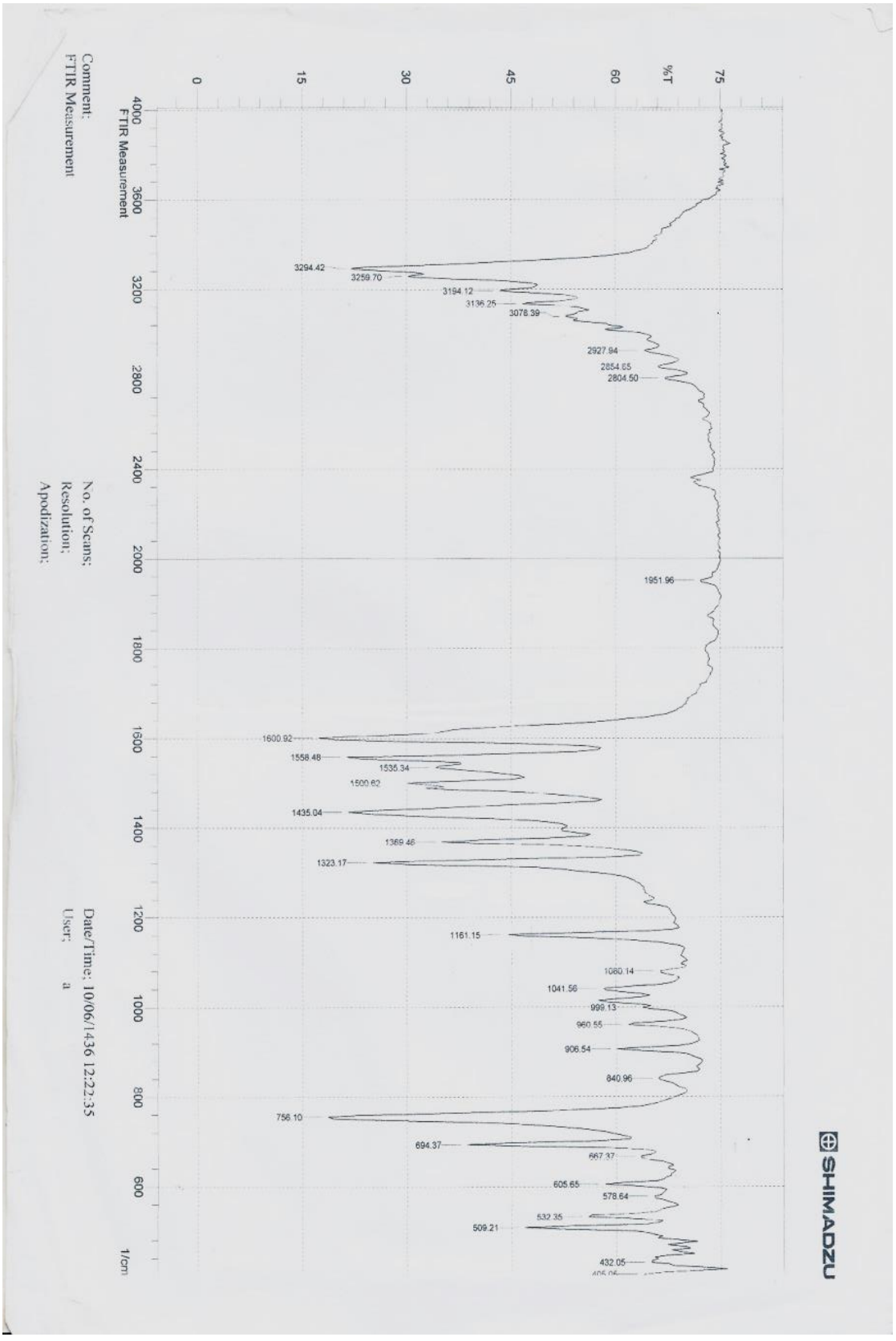
Figure(3.4): FTIR spectrum of compound (IIIb)



Figure(3.5): FTIR spectrum of compound (IIIc)



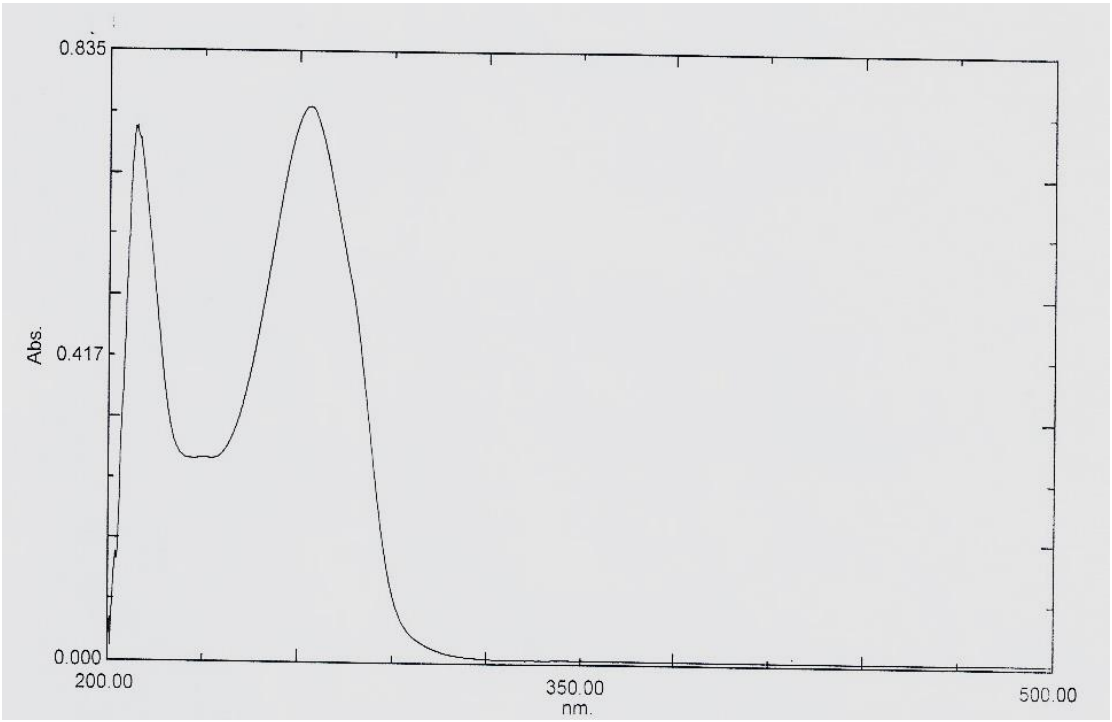
Figure(3.6): FTIR spectrum of compound (IIId)



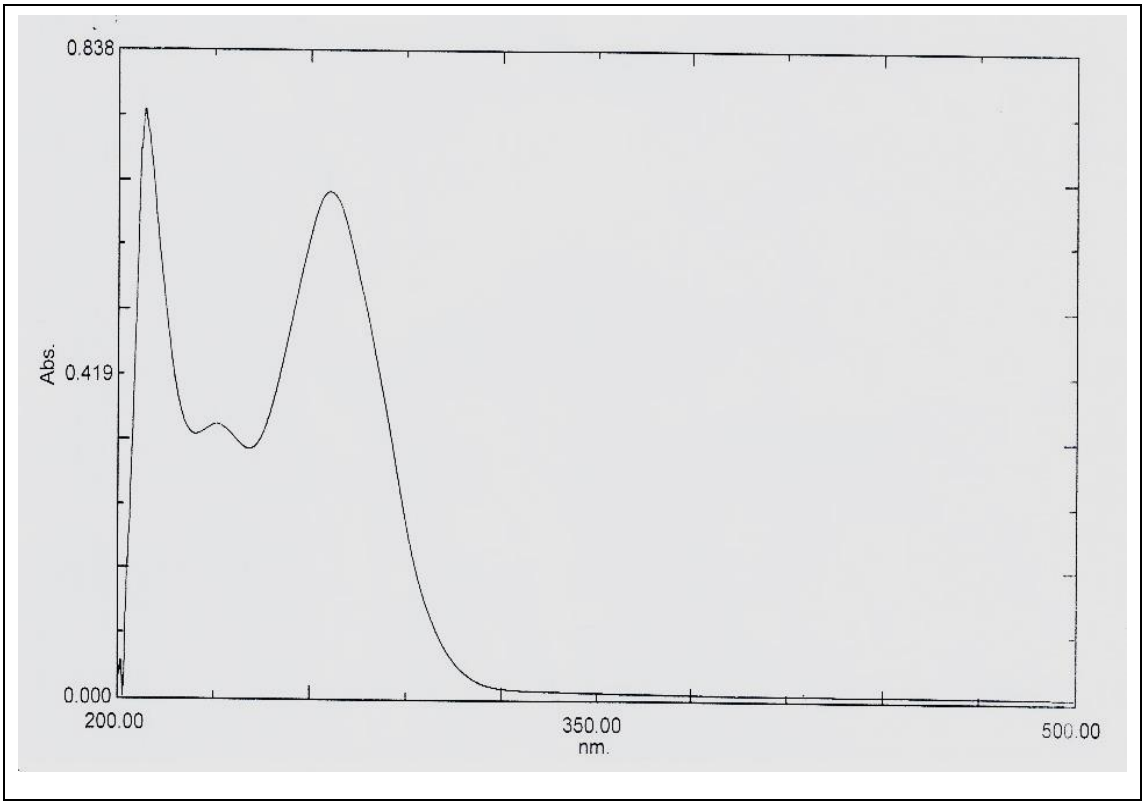
Figure(3.7): FTIR spectrum of compound (IIIe)

3.3.Characterization of prepared compounds by Ultraviolet-Visible spectroscopy:

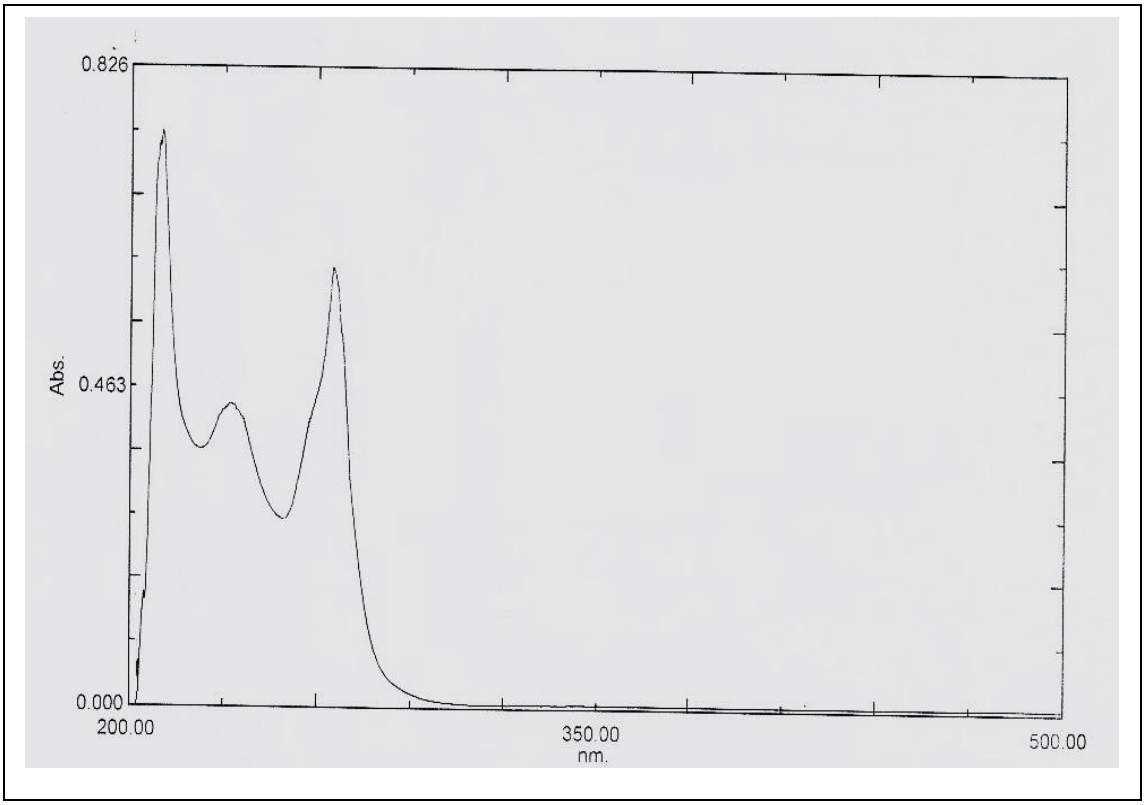
Spectral band (λ_{max}) values from the recorded UV-Vis spectra are given in Table (3.1). UV-Vis spectra of the synthesized compounds were recorded using DMSO as solvent in range of 200-500 nm. The characteristic UV bands with λ_{max} around 230 and 270 nm were indicative of the presence of benzene chromophore and sulfonamides moiety .The UV-Visible spectra of synthesized compounds were shown in Figures (3.8) –(3.12):



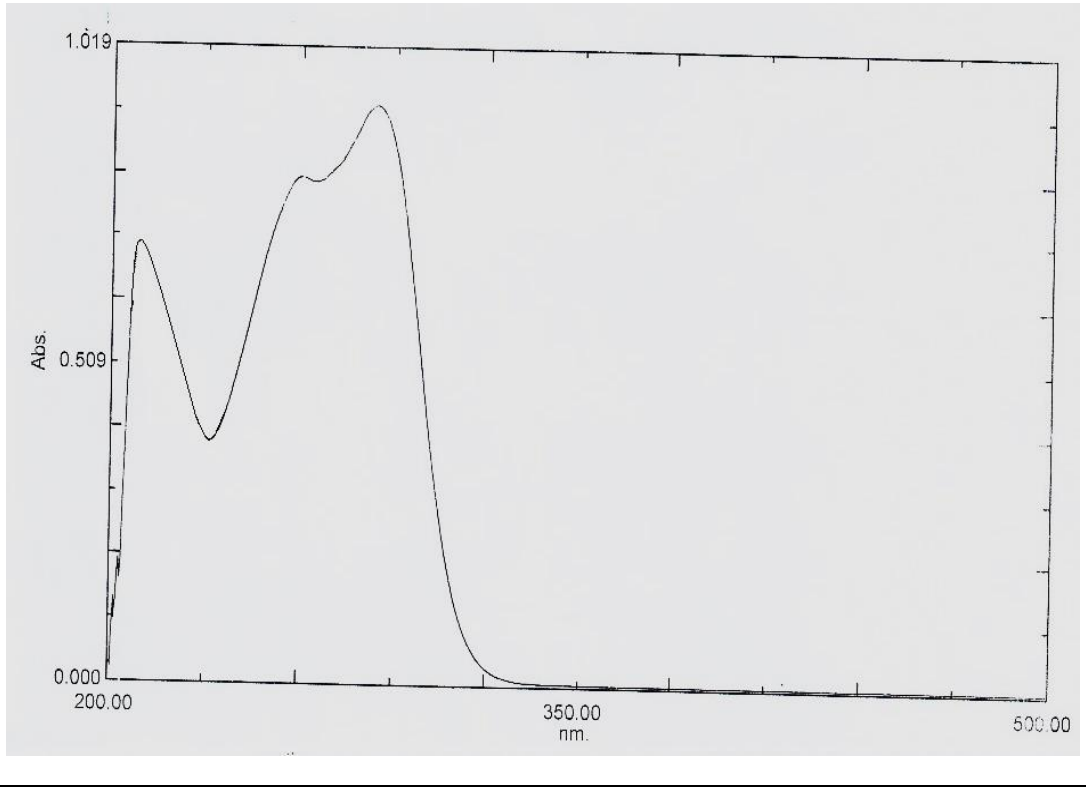
Figure(3.8): The ultraviolet-visible spectrum for the (IIIa) in DMSO solvent.



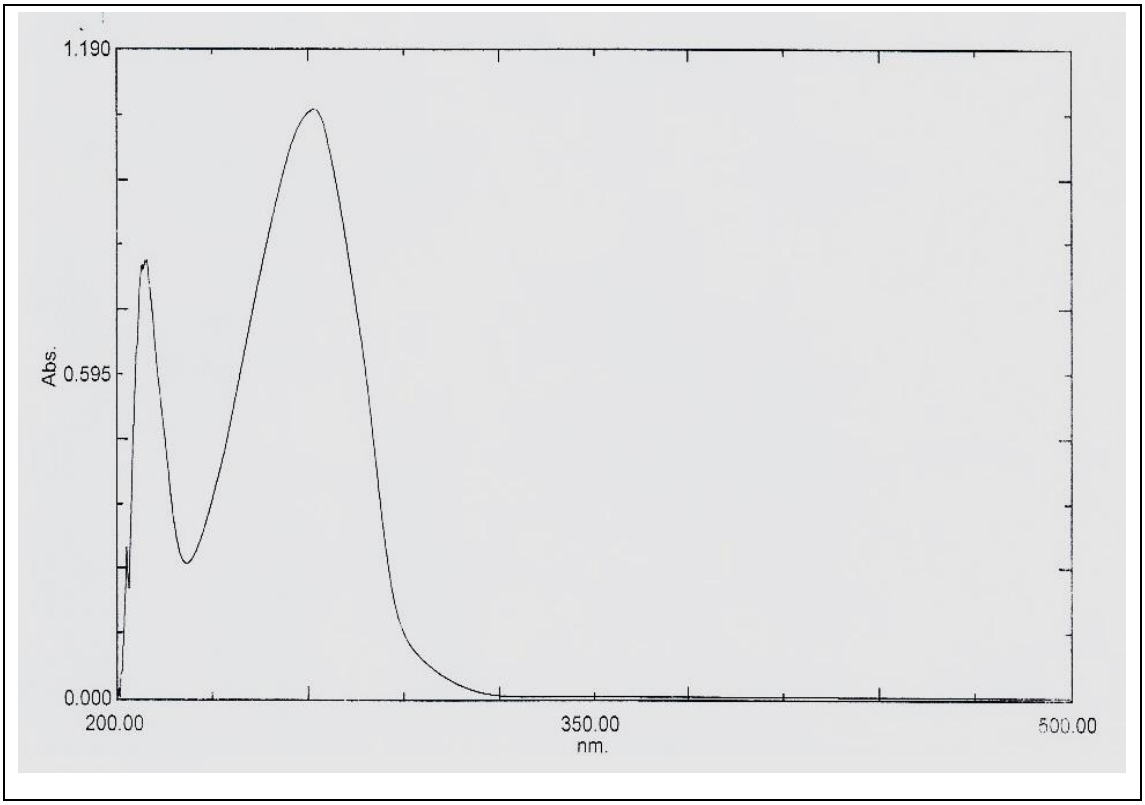
Figure(3.9): The ultraviolet-visible spectrum for the (IIIb) in DMSO solvent.



Figure(3.10): The ultraviolet-visible spectrum for the (IIIc) in DMSO solvent.



Figure(3.11): The ultraviolet-visible spectrum for the (IIId) in DMSO solvent.



Figure(3.12): The ultraviolet-visible spectrum for the (IIIe) in DMSO solvent.

Table (3.1) electronic spectra of the prepared compounds.

Comps.	Absorption Bands(nm)	Assignment
IIIa	208 233 263	π-π* π-π* n-π*
IIIb	208 231 266	π-π* π-π* n-π*
IIIc	210 237 265	π-π* π-π* n-π*
IIId	208 260 283	π-π* π-π* n-π*
IIIe	209 263	π-π* n-π*

3.4.Biological study Results:

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 sulfa drugs. 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[(65)].Two

microorganisms were isolated and identified to be used in vitro techniques, this microorganisms were:

Staphylococcus aurues (gram positive) and pseudomonas aerginosa (gram negative), the technique was the (Well sensitivity test), this method involves the exposure of the zone of inhibition toward the diffusion of microorganism on agar plates. A standard (5mm) diameter of wells impregnated with a specified volume of the compound solution, this well was placed on an agar plate cultured by the test organism. The plates were incubated for (24 hrs. at 37 °C). The zone of inhibition of bacterial growth around the well was observed.[66]

3.4.1.Inhibition ability of prepared compounds against gram positive bacteria:

The inhibition zones caused by the various compounds on the staphylococcus aureus bacteria were examined.(50,25,12,6 and 3 mg/ml concentrations for all of these compounds) and results were listed in Table (3.2) and Figures (3.13) –(3.17).

Table (3.2): The inhibition zones in mm for compounds (IIIa, IIIb, IIIc, IIId, IIIe and Antibiotic)

Compounds	Concentrations mg/ml	Inhibition Zones in mm
IIIa	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIb	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIc	50	17
	25	14
	12	8
	6	No Inhibition
	3	No Inhibition
IIId	50	16
	25	10
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition
IIIe	50	10
	25	No Inhibition
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition
Sulfamethoxazole as Antibiotic Control	50	Synergistic
	25	Same
	12	Same
	6	Same
	3	Same
DMSO as Solvent	—	No Inhibition

Values of the inhibition zones, including diameter of the well (5 mm).

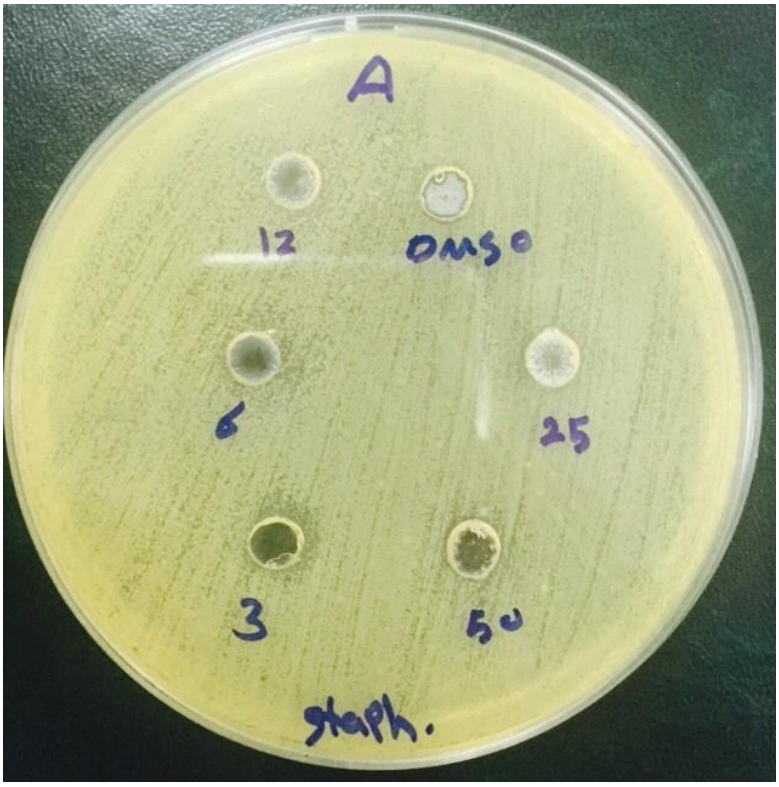


Figure (3.13): The effect of compound (IIIa) and DMSO on staphylococcusaureus

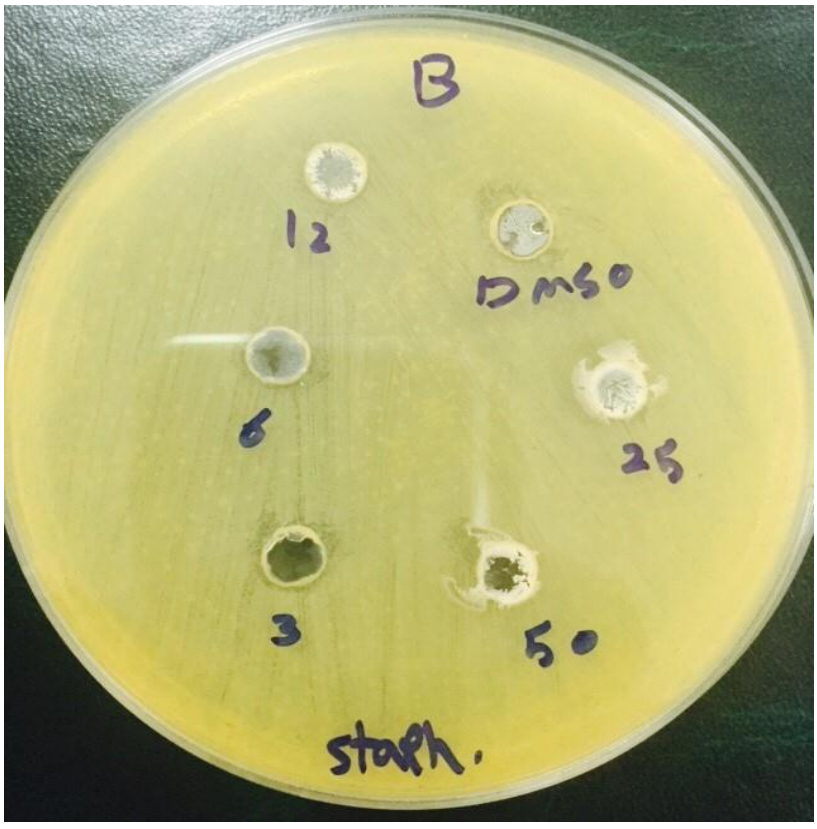


Figure (3.14): The effect of compound (IIIb) and DMSO on staphylococcusaureus

Figure (3.15): The effect of compound (IIIc) and DMSO on staphylococcusaureus

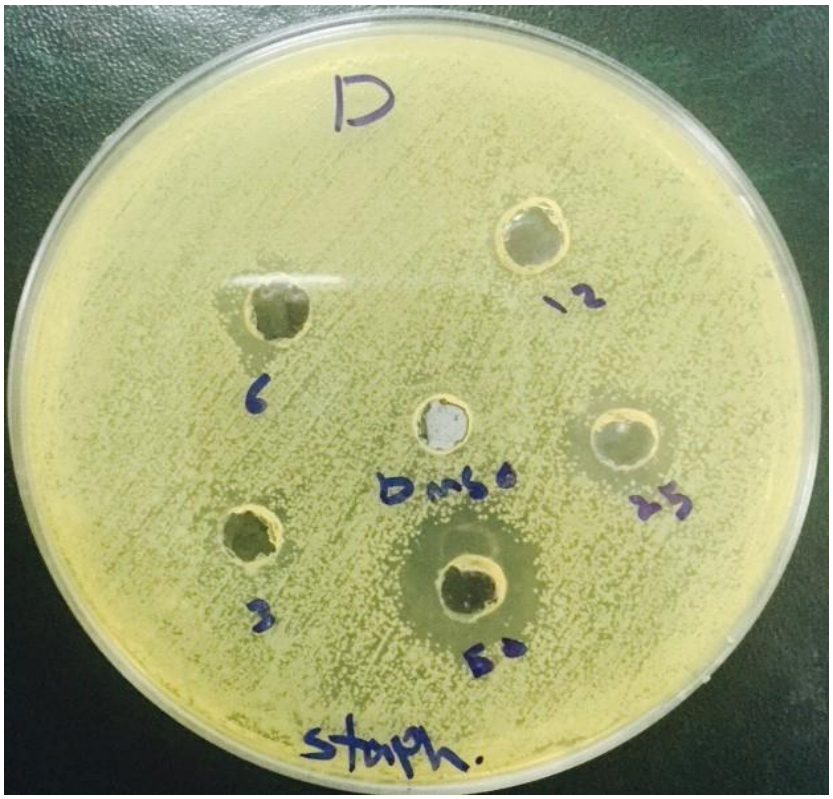


Figure (3.16): The effect of compound (IIId) and DMSO on staphylococcusaureus

Figure (3.17): The effect of compound (IIIe) and DMSO on staphylococcusaureus

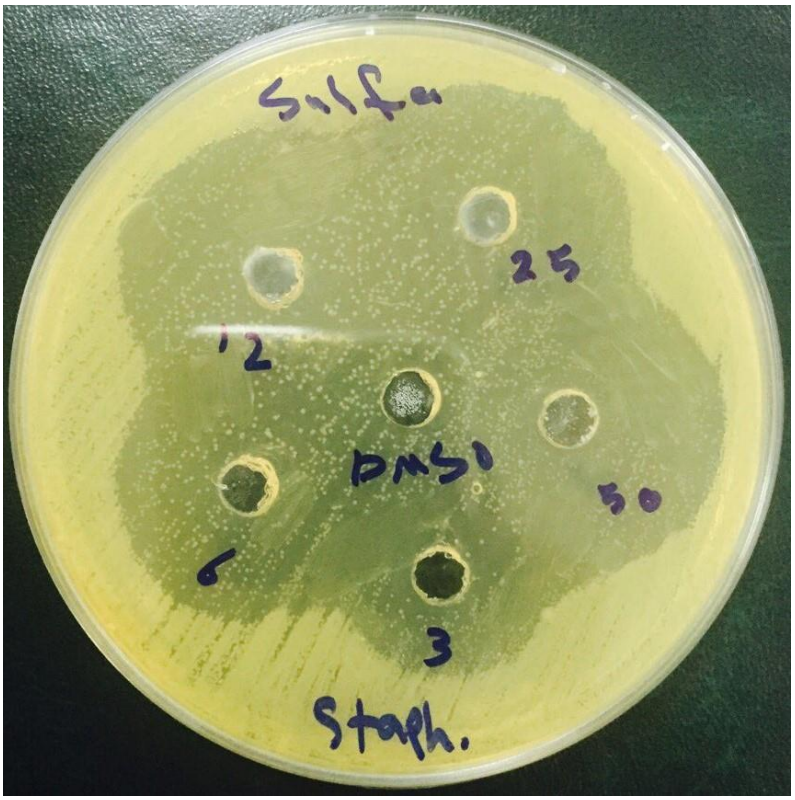


Figure (3.18): The effect of sulfamethoxazole and DMSO on staphylococcus aureus

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

The inhibition zones caused by the various compounds on the pseudomonas aeurginosa bacteria were examined. (50,25,12,6 and 3 mg/ml concentrations for all of these compounds). The results were listed in Table (3.3) and Figures (3.19) –(3.23).

Table (3.3): The inhibition zones in mm for compounds (IIIa, IIIb, IIIc, IIId, IIIe and Antibiotic)

Compounds	Concentrations mg/ml	Inhibition Zones in mm
IIIa	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIb	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
IIIc	50	13
	25	No Inhibition
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition

Compounds	Concentrations mg/ml	Inhibition Zones in mm
IIId	50	16
	25	No Inhibition
	12	No Inhibition
	6	No Inhibition
	3	No Inhibition
IIIe	50	No Inhibition
	25	Same
	12	Same
	6	Same
	3	Same
Sulfamethoxazole as Antibiotic Control	50	Synergistic
	25	Same
	12	Same
	6	Same
	3	Same
DMSO as Solvent	—	No Inhibition

Values of the inhibition zones, including diameter of the well (5 mm).

Figure (3.19): The effect of compound (IIIa) and DMSO on pseudomonas aurginosa

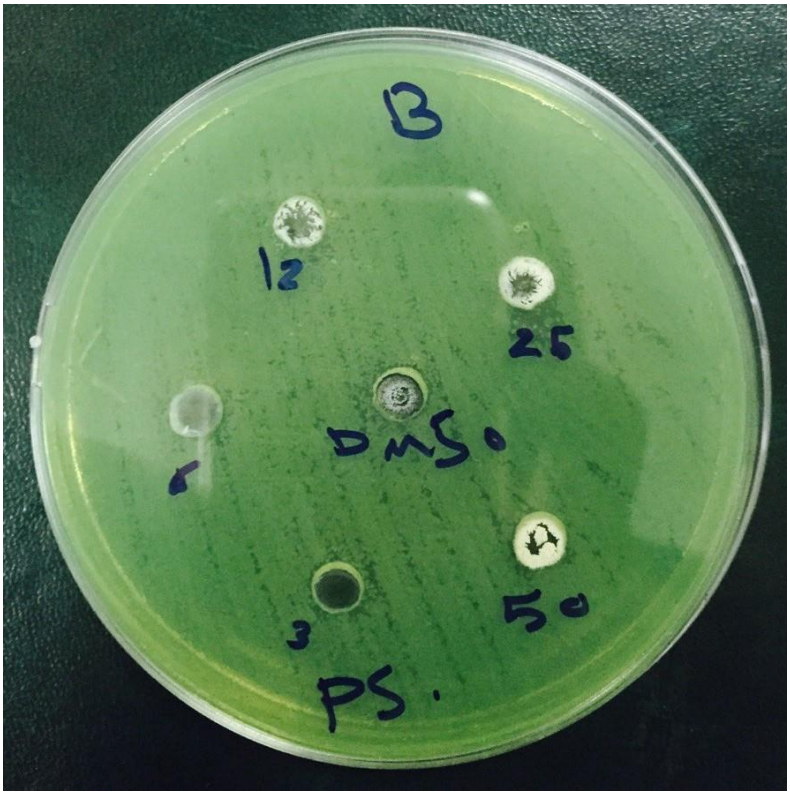


Figure (3.20): The effect of compound (IIIb) and DMSO on pseudomonas aurginosa

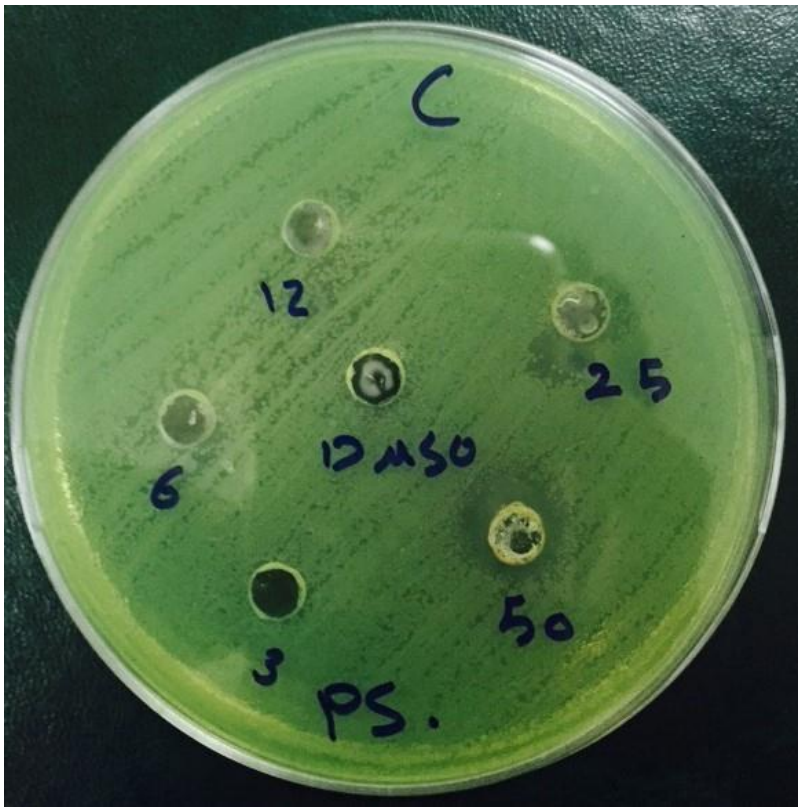


Figure (3.21): The effect of compound (IIIc) and DMSO on pseudomonas aurginosa

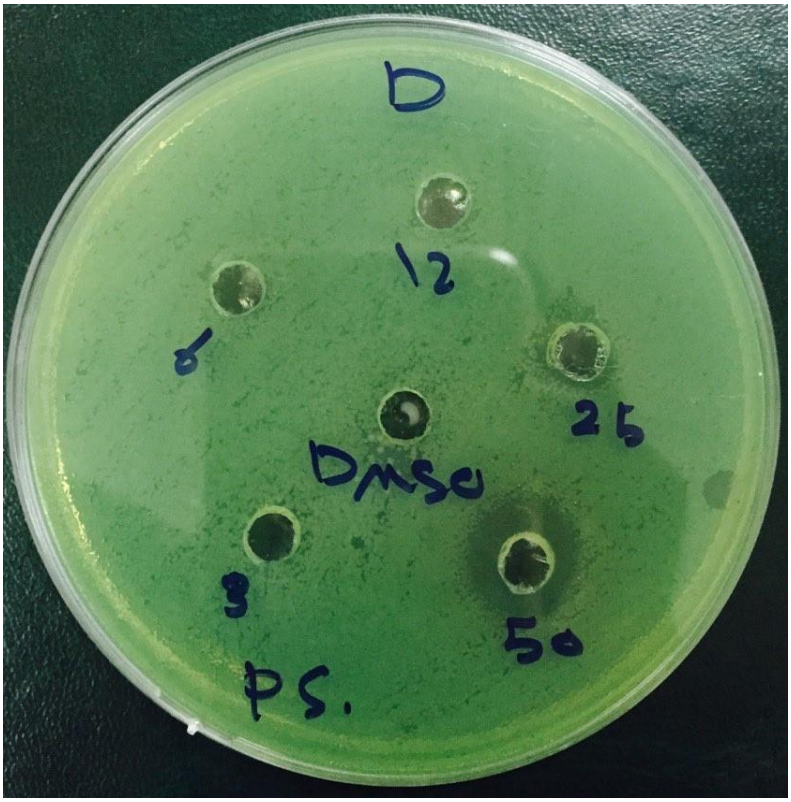


Figure (3.22): The effect of compound (IIId) and DMSO on pseudomonas aurginosa

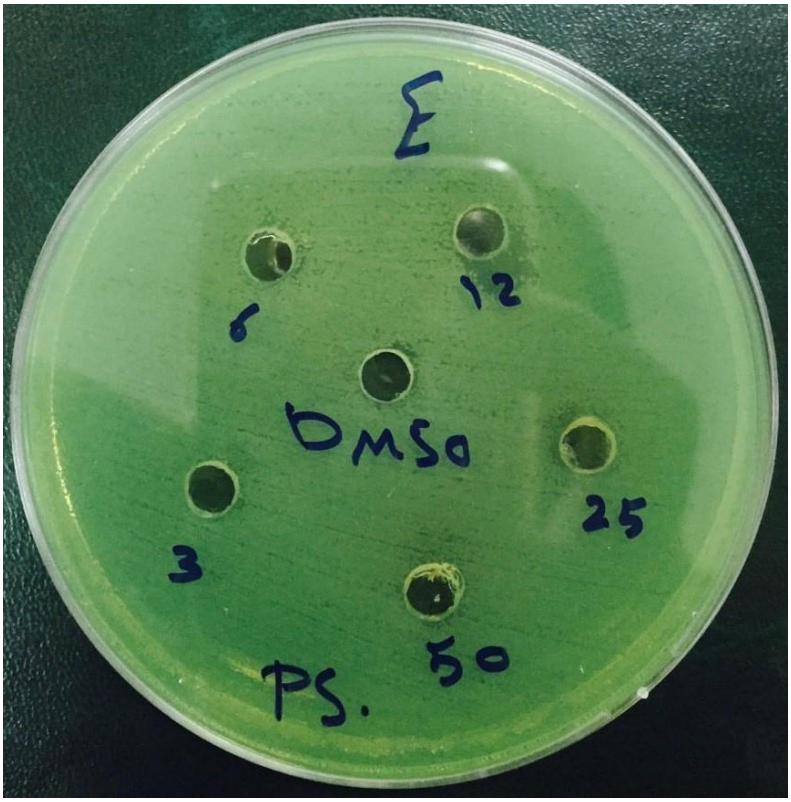


Figure (3.23): The effect of compound (IIIe) and DMSO on pseudomonas aurginosa

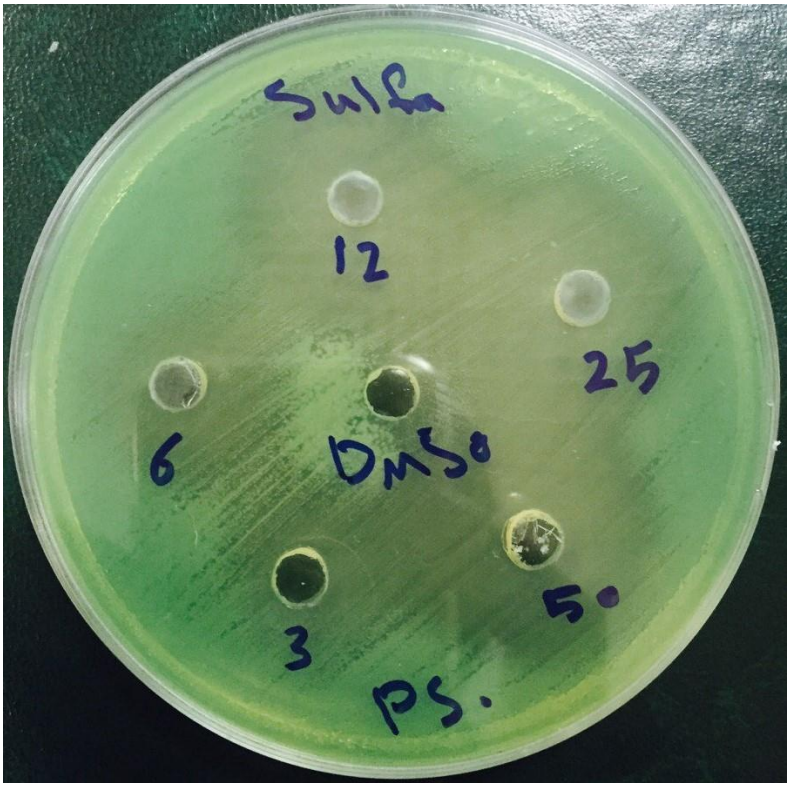


Figure (3.24): The effect of sulfamethoxazoleand DMSO on pseudomonas aeruginosa.

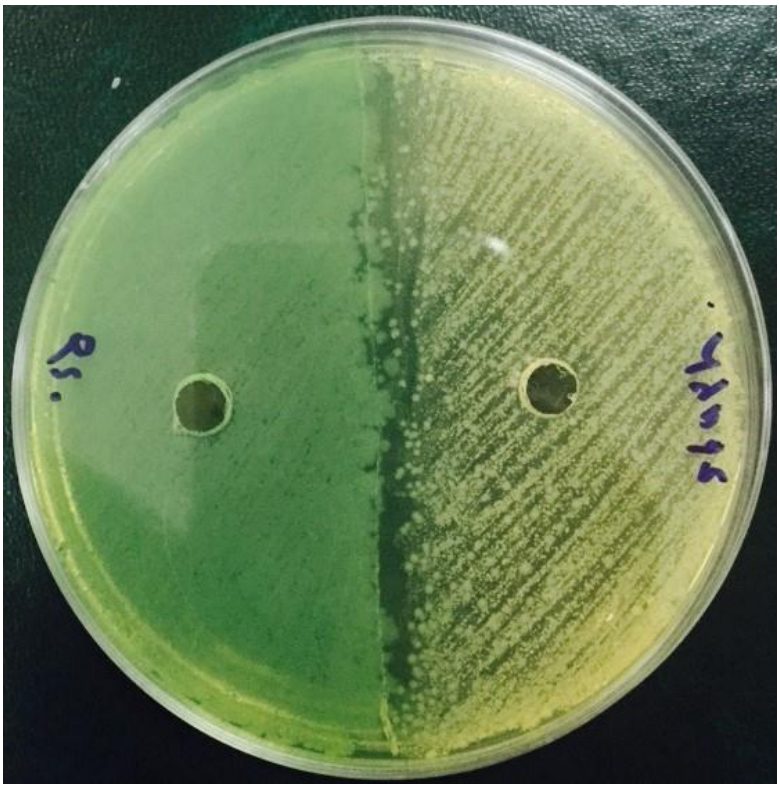


Figure (3.25): The effect of DMSO on staphylococcus aureus and pseudomonas aeruginosa

The prepared compounds (IIIa and IIIb) had no inhibiting action on growth of the used germs and, therefore, these compounds possessed no antibacterial activity against the previously mentioned types of gram positive and gram negative bacteria with all concentrations (resistance).The most interesting results were with compound (IIIc) which showed the best antibacterial activity on staphylococcus aureus with inhibition zones 17mm,14mm and 8mm at concentrations (50,25 and 12 mg/ml) respectively. On pseudomonas aeruginosa, the compound (IIIc) showed one inhibition zone 13mm at (50 mg/ml). The compound (IIId) showed two inhibition zones 16mm and 10mm at (50, 25 mg/ml) respectively on staphylococcus aureus, while compound (IIId) showed one inhibition zone 16mm at (50) mg/ml on pseudomonas aeruginosa. The compound (IIIe) have inhibition ability 9mm at (50) mg/ml on staphylococcus aureus, while on pseudomonas aeruginosa the inhibition zone was not observed (resistance).

3.6.Conclusions:

In this study, we carried out an antibacterial evaluation in vitro of a series of five synthetic sulfonamides (IIIa, IIIb, IIIc, IIId and IIIe), against gram positive and gram negative clinical strains isolated from patients presenting burns infections: Staphylococcus aureus and pseudomonas aeruginosa. From the results, antibacterial activity studies indicate that halo substituted sulfonamide (compound IIIc) was more active than the other members (IIIa,IIIb,IIId and IIIe).[(67)]The prepared compounds (IIIc,IIId and IIIe) showed inhibition activity as antibacterial agents less than sulfamethoxazole (the reference drug showed better biological activity than our compounds in all tested strains "synergistic effect" against staphylococcus aureus and pseudomonas aeruginosa).

3.6.Suggestion of the future work:

1- Evaluation of the cytoxicity of the prepared compounds.

2- Evaluation of the antibacterial activity of the synthesized compounds in vitro against different types of bacteria (Gram Positive and Gram Negative).

3-Preparation of new Schiff base derivatives via the synthesized compounds reaction with different aldehyde such as hydroxy aldehyde and halo aldehyde.

REFERENCES

References

1. Long P. and Bliss E., (1939), The clinical and Experimental use of sulfanilamide, Sulfa pyridine and Allied compounds, The macmillon Co., New York.

2. Michael T., (1997), Optimal analysis of sulfonamides from biological matrices using super critical fluids, Ph.D Thesis, Faculty of the Virginia polytechnic institute, Virginia university.

3. Patrick G.,(2001), An introduction to medicinal chemistry, 2nd Ed., OUP, England.

4. Sophian L., Piper D. and Schneller G., (1952), The Sulfa pyridines, A. colish, New York.

5. Cates L.,(1986), Sulfa drugs in Handbook of Chemotherapeutic Agents, CRC Press, BocaRaton.

6. Golzar G., (2005), Synthesis and Structural studies of metal complexes of Sulfa drugs, Ph.D.Thesis, Department of chemistry, Cardiff university.

7. Mok B., (2008), The synthesis of functionalized Sulfonamides, PH.D. Thesis, Chemistry Department, university college London.

8. Baran W., Adamek E., Ziemiansk J. And Sobczak A., (2011), Effects of the presence of Sulfonamides in the environment and their influence on human health, J. Hazardous materials, 196: 1-15.

9. Garcia M., Diaz S. and Barcelo D., (2009), Combining chemical analysis and ecotoxicity to determine environmental exposure and to assess risk from sulfonamids, Tr. Anal. chem., 75: 417-434.

10. Valderas M. Andi B., Barrow W., Paul F. and cook P., (2008), Examination of intrinsic sulfonamide resistance in Bacillus anthracis: a

novel assay for dihydropteroate synthase, Biochem. Biophys. Acta, 1780: 848-853.

11. Beale J., (2004), Textbook organic medicinal and pharmaceutical chemistry, 11th Ed. Lippincott Williams and wilkins, Baltimore.

12.Cecchi A., winum J. , Innocenti A. , Vullo D. ,Montreo J. , Scozzafava A. And Supuran C., (2005), Bioorg. Med. Chem. Lett. ,14 (23): 5775-5780.

13.Chieh-Chien Lee. , (2010), Synthetic Approaches To Biologically Active Sulfonamides, Ph.D. Thesis, Chemistry Department, University College London.

14.Vallo D. , Franchi M. , Gallori E., Pastorek J., Scozzafava A., Pastorekova S. And Supuran C., (2003), Bioorg. Med. Chem. Lett., 13: 1005-1009.

15.Supuran C., Scozzofava A., Menabouni L., Minicione F., Briganti F. And Minicione G., (2000), J.Med.Chem., 43: 4542-4551.

16.Fisherman S., Osborn L., Chun G., Plowman J., Smith A. And Christian M., (1993), Investigational New Drugs, 11th Ed.,

17.Yoshino H., Ueda N., Niijima J., Sngumi H., Kotake Y., Koyanagi N., Yoshimaysu K., Asada M. And Watanabe T., (1992), J.Med.Chem., 35: 2496-2497.

18.Yoshimatsu K., Yamaguchi A., Yoshino H., Koyanagi N. And Kitoh K., (1997), Cancer Research, 57: 3208-3213.

19.Casini A., Scozzafava A., Mastrolorenzo A. And Supuran T., (2002), Current Cancer Drug Targets, 55-75.

References

20.Ozawa Y., Sugi H., Owa T., Watanabe T., Koyanagi N., Yoshino H., Kitoh K. And Yoshimatso K., (2001), Eur. J. Cancer, 37: 2275-2282.

21.Dittrich C., Zandvleit S., Gueist M., Huitema D.. King A. And Wanders J., (2007), British J. Cancer, 96: 559-566.

22.Maier J., Schilling K., Schmidt R. And Geisslinger G., (2004), J. Biochem. Pharma., 67: 1469-1478.

23.Talley J., Brown L., Carter S., Graneto C., Koboldt M., Masferrer L., Perkins E., Rogers S. And Shaffer F., (2000), J. Med. Chem., 43: 775-777.

24.Ratish G., Javed K., Ahmad S., Bano S., Alam S., Pillai K., Singh S. And Bagchi V., (2009), Bioorg. Med. Chem. Lett., 19 (1): 255-258.

25.Schobert R., Stehle R. And Walter H., (2008), Tetrahedron, 64 (40): 9401-9407.

26.Turner R., (2002), Curr. Med. Chem., 1(2): 141-162.

27.Gottlieb S., Khata M., Wentworth D., Roffman D., Fisher M. And Karmer W., (1998), Am. J. Med., 104: 533-538.

28.Macfodyen J., Gorski C., Barter C. And Struthers Br., (2003), J. Ch. Pharmacol., 57: 622-631.

29.Abbink J., Pickers P., Lutterman A. And Tack C., (2002), J. Clinical Science, 102: 307-314.

30.Webb J., Freestone S., Allen J. And Muirhead J., (1999), 83(5A): 21C-28C.

31.Brooks F., Butel S. And Morse A., (2001), Medical Microbiology, 20th Ed., Lange Medical Books, New York.

References

32.Kloose E. And Bannerman L., (1994), Up Date On Clinical Significance Of Coagulase-Negative Stapylococci, Clinical Microbiology Reviews, 7(1): 117-140.

33.Plate K., Rosato E. And Wegrzyn G., (2009), Staphylococcus Aureus As An Infectious Agents, Acta Biochimica Polonica, 56 (4):597-612.

34.Wilkinson J. And Archer L., (1997), The Staphylococci in Human Diseases, Churchill Livingston, London.

35.Kloose E. And Lambe J., (1991), Manual Of Clinical Microbiology, ASM, Washington D.C.

36.Wertheim L., Melles C., Vos MC., Van W., Van A., Verbrugh A. And Nuowen L., (2005), The Role Of Nasal Carriage In Staphylococcus Aureus Infections, The Lance Infectious Diseases, 5 (12): 751-762.

37.Kluytmans J., Van A. And Verbrugh H., (1997), Nasal Carriage Of Staphylococcus Aureus: Epidemiology, Underlying Mechanisms And Associated Risks, Clinical Microbiology Reviews, 10 (10): 505-520.

38.Macay M., (2007), Activity Of Antibiotics Towards Extracellular And Intracellular Staphylococcus Aureus, Ph.D. Thesis, Faculty Of Medicine, Catholic University Of Louvain.

39.Clare M., (2010), Single Nucleotide Polymorphism (SNP)-Genotyping Of Community Acquired Methicillin Resistance Staphylococcus Aureus Including The Subtyping Of PVL Toxin Producers Using Real Time PCR, M.SC Thesis, Queensland University Of Technology.

40.Gilligan H., (1991), Microbiology OF Airway Diseases In Patients With Cystic Fibrosis, Clin. Microbiol Rev., 4: 35-51.

References

41.Lin Zing, (2004), Pseudomonas Aeruginosa Pathogenicity And Antibiotic Resistance, Ph.D. Thesis, University Of Florida.

42.Potera C., (2012), Common Bacterium Induces Histamine Production In Neutrophiles, Enviromental Health Perspectives, 120 (5): 190.

43.Michael A., Kohanski, Daniel J., Dwyer J. And James J., (2010),

How Antibiotics Kill Bacteria: From Targets To Networks, Nature Reviews Microbiology, 8: 423-435.

44.Schwalbe R., Steele-Moore L. And Goodwin A., (2007),

Antimicrobial Susceptibility Testing Protocols, New York.

45.Brewer N., Hellinger W., (1998), Antibiotics Tolerance Among Clinical Isolates Of Bacteria, New Engl. J. Med., 345: 1042-1053.

46.Bush K., (2012), Antimicrobial Agents Targeting Bacterial Cell Walls And Cell Membranes, Rev. Sci. Tech. Off. Int. Epiz., 31(1): 43-56.

47.Katzung B.,(1995), Basic And Clinical Pharmacology, 6th Ed., Appleton And Lange, New York.

48.Lambert T., (2012), Antibiotics That Effect The Ribosome, Rev. Sci. Tech. Off. Int. Epiz., 31(1): 57-64.

49.Strayer L., (2000), Biochemistry, 4th Ed., W.H. Freeman And Company, New York.

50.Katzl L. And Ashley W., (2005), Translation And Protein Synthesis: Macrolides, Chem. Rev., 105:499-528.

51.Mukhtar A. And Wright D., (2005), Streptogramins, Oxazolidinones And Other Inhibitors Of Bacterial Protien Synthesis, Chem. Rev., 105: 529-542.

References

52.Fletcher C., (1984), First Clinical Use Of Penicillin, British. Med. J., 289:1721-1723.

53.Rubin H., Tolkoff E. And Cortan S., (1986), Urinary Tract Infection, W.B. Saunders Co., Philadelphia.

54.Swedberg G., Fermer C. And Scold O., (1993), Point Mutations In The Dihydropteroate Synthesis Gene Causing Sulfonamide Resistance, Adv. Exp. Med,Biol., 338: 555-558.

55.Abraham D., (2003), Burgers Medicinal Chemistry And Drug Discovery, John Wiley And Sons, Hoboken.

56.Vogel A., (1956), Text book of Practical Organic Chemistry, 3rd ED., Longman Group, London.

57.Hadizadeh F., Moradi A., Naghibi G., Vojdani M., Behravan J And Ramezani M., (2007), Synthesis And Antitumor Activity of Substituted Succinamides Using a Potato Disc Tumor Induction Assay, International J. Biomedical Science, 60-64.

58.Pvia D., Lampman G., (2011), A Small Scale Approach to Organic Laboratory Techniques, 3rd ED., Cengage Learning.

59.Moumita B. And Thankamani V., (2013), Antimicrobial Activity Of Plant Mukia Maderasatana, Int. J. Pharm. Sci., 5(4): 199-202.

60.Sahun K., Ganguly S. And Kaushik A., (2014), Synthesis Of Some Novel Heterocyclic 1,2,4-Triazolo [3,4-B] [1,2,3] Thiadiazole Derivatives as Possible Antimicrobial Agents, J. App. Pharm. Sci., 4(2): 81-86.

61.Tilton R., Balows A., Hohnadel D. And Resis F.,(1992), Clinical Laboratory Medicine Mosby, Year book.

References

62.Cappuccino J. And Shermany N., (1987), Microbiology And Laboratory Manual, 2nd Ed., Benjamin/Cumming Publishing Inc., California.

63. Cremlyn R., (2002), Chlorosulfonic Acid: A versatile Reagent, British Library, UK.

64. Wuts P. And Greene T., (2006), Greene 's Protective Group In Organic Synthesis, 4th Ed., John Wiley And Sons, New Jersy.

65. Endimiani A., Luzzaro F. And Brigante G., (2005), Proteus Mirabilis Bloodstream Infections: Risk Factors And Treatment Outcome Related To The Expression Of Extended-Spectrum Beta-Lactamases, Journal Of Antimicrobial Agents Chemotherapy, 49(7):2598-2605.

66. Chaitanya S., Nagendrappa G And Vaidya P., (2010), Synthesis, Biological And Pharmacological Activities Of 2-Methyl-4Hpyrimido [2,1 - B][1,3]Benzothiazoles, J. Chem. Pharm. Res., 2(3): 206-213.

67. Badampudi S., Aluru R., Papammagari R. And Rao R., (2014), Synthesis, Characterization And Pharmacological Evaluation Of Certain Sulfonamide Containing Hetrocyclic Motifs, J.Pol.Ann.Med., 21(2): 75-81.

الخلاصـــــــــة

يقسم هذا البحث الى جزئين :

الجزء الأول:

يتضمن هذا الجزء تحضير البعض من أدوية السلفا التي تلعب دوراً هاما في الكيمياء الطبيه لفاعليتها التثبيطيه اتجاه البكتيريا.

وفيما يلي المركبات المحضره :

1.p-Amino-N-(2-methylphenyl)benzene Sulfonamides.

2.p-Amino-N-(2-methoxyphenyl)benzene Sulfonamides.

3. p-Amino-N-(4-chlorophenyl)benzene Sulfonamides.

4. 4-(Morpholinosulfonyl) aniline.

5. 4-(Piperidin-1-yl sulfonyl) aniline.

وتم تشخيص مركبات السلفا المحضره بواسطة التحليل الدقيق للعناصر والطرق الطيفيه (طيف الأشعه تحت الحمراء FTIR وطيف الأشعه فوق البنفسجيه Ultra violet) وتم قياس درجة الأنصهار لهذه المركبات المحضره.

الجزء الثاني:

درست فعالية مركبات السلفا المحضره على نوعين من البكتريا المرضيه (staphylococcus aureus and pseudomonas aurgenasa) خارج الجسم الحي وذلك بطريقة الأطباق بأستخدام تراكيز مختلفة من ادوية السلفا المحضره وأظهرت النتائج ان بعض المركبات تمتلك فعالية ضد هذه البكتريا بينما لم تظهر المركبات الأخرى أي فعاليه.

جمـــــــــــــــــــهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعـــــــــــــــة النهريـــــــن
كليـــــــــــــــة العـــــــلوم
قـــــــــــــــسم الكيميـــــــاء

تحضير بعض مشتقات السلفا ودراستها كعوامل تثبيط بكتيرية

رسالة

مقدمة الى كلية العلوم- جامعة النهرين

وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

من قبل

انمار حميد جابر

بكالوريوس علوم كيمياء/كلية العلوم/جامعة الكوفة

(2012)

المشرف

الاستاذ المساعد الدكتور سلمان علي احمد

1437 **2016**