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SYNTHESIS OF NEW HETEROCYCLIC COMPOUNDS AS POSSIBLE ANTIMICROBIAL AGENTS

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Submitted in partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

DEPARTMENT OF PHARMACY

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT,

SOLAN, HIMACHAL PRADESH INDIA

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CERTIFICATE

This is to certify that the work titled **"SYNTHESIS OF NEW HETEROCYCLIC COMPOUNDS AS POSSIBLE ANTIMICROBIAL AGENTS"** submitted by **Sonali Suman (081775)** and **Vishakha Pandey (081774)** in partial fulfillment for the award of the degree of **Bachelor of Pharmacy** of Jaypee University of Information Technology, Waknaghat is a record of bonafide research work carried out by them under my supervision and guidance. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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Date: 27th May 2012

ACKNOWLEDGMENT

A single flower cannot make a garland or a single star cannot make the beautiful shiny sky at the night. A project work can never be outcome of a single individual's talent or efforts.

As we conclude our project, we have many people to thank; for all the help, guidance and support they lent us throughout the course of our work.

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Last but not the least, we are grateful to our **Parents, friends** and **well wishers** who have been the pillars of our strength.

Sonali Suman.
Sonali Suman

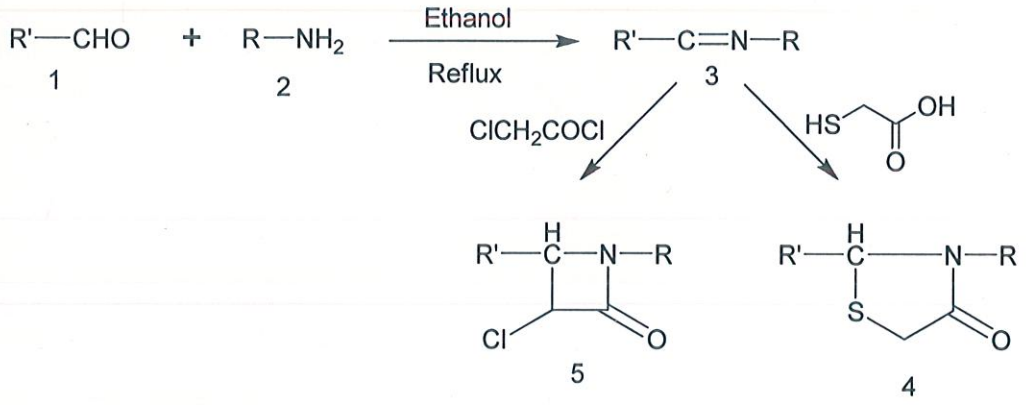
Vishakha..
Vishakha Pandey

Date: 27th May, 2012

SUMMARY

Anti microbial drugs have caused a variety of drastic change not only in the treatment of infection but also in improvement in the human life. However, in reality, emerging and re-emerging infectious disease have lead scientists to discover and design new drugs with higher potency, less side effects and a broader spectrum of action with reduced toxicity.

With this objective, we designed the strategy to synthesize chemical compounds as possible antimicrobial agent using imines. Imines (compounds with double bond between carbon and nitrogen) were prepared using aldehyde and amine under laboratory conditions as specified.
Using these synthesized imines, further thiazolidiones were prepared. In selected thiazolidiones an attempt to introduce a beta lactam ring were made to obtain beta lactam ring bearing compounds. The products were characterized on the basis of appearance (color, physical state), melting point and TLC monitoring (R_f value). Recrystallisation of products was performed to obtain pure compounds. Microbial studies was performed (antibiotic susceptibility testing by broth microdilution method) to study the antimicrobial activity of the prepared compounds.



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PBP -penicillin binding proteins

NAM-N-acetyl muramic acid

NAG- N-acetyl glucosamine

R_f– Retention factor

NT- not taken

MIC- Minimum Inhibhitory Concentration

LB-Luria Berti

ELISA- Enzyme Linked Immuno Sorbent Assay

CFU- Colony Forming Unit

CHAPTER 1

1.1 Introduction

Antimicrobial drugs have caused a dramatic change not only of the treatment of infectious diseases but of a fate of mankind. Antimicrobial chemotherapy made remarkable advances, resulting in the overly optimistic view that infectious diseases would be conquered in the near future. However, in reality, emerging and re-emerging infectious diseases have left us facing a countercharge from infections. Infections with drug resistant organisms remain an important problem in clinical practice that is difficult to solve. If an improper antimicrobial agent happens to be chosen for the treatment of infection with drug-resistant microorganisms, the therapy may not achieve beneficial effect,[1] and further, may lead to a worse prognosis. In addition, in a situation where multidrug-resistant organisms have spread widely, there may be quite a limited choice of agents for antimicrobial therapy. At present, fewer brand new antimicrobial agents are coming onto the market.[2] Considering this situation together with the increasing awareness of drug safety, we are now facing a situation of severely limited options among antimicrobial agents.

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans[3].

A substance is classified as antibiotic if:

1. It is a product of metabolism (although it may be duplicated or even have been anticipated by chemical synthesis)
2. It is a synthetic product produced as a structural analog of a naturally occurring antibiotic.
3. It antagonizes the growth and/or the survival of one or more species of microorganisms.
4. It is effective in low concentrations.

1.2 History

Looking back on the history of human diseases, infectious diseases have accounted for very large proportion of diseases as a whole. It was not until the latter half of the 19th century that microorganisms were found to be responsible for a variety of infectious diseases that had been plaguing humanity from ancient days. Accordingly, chemotherapy aimed at the causative organisms was developed as the main therapeutic strategy. The first antimicrobial agent in the world was salvarsan, a remedy for syphilis that was synthesized by Ehrlich in 1910.[4] In 1935, sulfonamides were developed by Domagk and other researchers. These

drugs were synthetic compounds and had limitations in terms of safety and efficacy. In 1928, Fleming discovered penicillin. He found that the growth of *Staphylococcus aureus* was inhibited in a zone surrounding a contaminated blue mold (a fungus from the Penicillium genus) in culture dishes, leading to the finding that a microorganism would produce substances that could inhibit the growth of other microorganisms. The antibiotic was named penicillin,and it came into clinical use in the 1940s. Penicillin, which is an outstanding agent in terms of safety and efficacy, led in the era of antimicrobial chemotherapy by saving the lives of many wounded soldiers during World War II. During the subsequent two decades, new classes of antimicrobial agents were developed one after another, leading to a golden age of antimicrobial chemotherapy. In 1944, streptomycin, an aminoglycoside antibiotic, was obtained from the soil bacterium *Streptomyces griseus*. Thereafter, chloramphenicol, tetracycline, macrolide, and glycopeptide (e.g., vancomycin) were discovered from soil bacteria. The synthesized antimicrobial agent nalidixic acid, a quinolone antimicrobial drug, was obtained in 1962. Improvements in each class of antimicrobial agents continued to achieve a broader antimicrobial spectrum and higher antimicrobial activity.

The β-lactam antibiotics include penicillin, cephalosporin, carbapenems, and monobactam. Penicillin were originally effective for Gram positive organisms such as *S. aureus*. Later, to address penicillin-resistant *S. aureus* which produces the penicillin-hydrolysing enzyme penicillinase, methicillin was developed. On the other hand, attempts to expand the antimicrobial spectrum yielded ampicillin, which is also effective for Gram-negative *Enterobacteriaceae*, and piperacillin, which is effective even for *Pseudomonas aeruginosa*. Cephems were developed in the 1960s, and came into widespread use. Cephems are classified into several generations according to their anti microbial spectra.

Carbapenem is an antibiotic class including panipenem, imipenem, and meropenem. These drugs are effective not only for Gram-positive and Gram-negative bacteria but also anaerobes, and their antimicrobial activity is strong. The monobactam antibiotic aztreonam exerts an antimicrobial effect only on Gram-negative bacteria. Continuing improvements have been made for antimicrobial agents in various aspects in addition to the antimicrobial spectrum and activity. The drugs have been developed to achieve better pharmacodynamics including the absorption of oral drugs, concentration in the blood, and distribution to the inflammatory focus. In addition, as antimicrobial chemotherapy has been established and matured, more importance has been attached to the drug safety. Antimicrobial agents that are associated with serious side effects have been replaced by other safer drugs. Quinolone antimicrobials represent an example of drugs with improved pharmacodynamics and safety.

Nalidixic acid, the first drug of this class, was active only against Gram-negative bacteria, and its use was limited to urinary tract infections because it achieves only low blood concentrations and poor tissue distribution, and was metabolized rapidly in the human body.

1.3 Classification of antibiotics

Based on Spectrum of activity:

- Broad spectrum- has the ability to antagonize the growth of a large number of pathogens.
  Ex. Tetracycline and Chloramphenicol
- Narrow spectrum – has higher degree of specificity in antagonizing growth of pathogens.
  Ex. Bacitracin and Nystatin

Based on chemical structure:

- Beta-Lactam Antibiotics: Penicillin, cephalosporin, monobactam, carbapenems.
- Sulfonamides and related drugs: Sulfadiazine, Sulfones- Dapsone (DDS).
- Quinolones: Nalidixic acid, Norfloxacin.
- Tetracycline: Doxycycline.
- Amino glycoside: Streptomycin, Gentamicin, Neomycin.
- Macrolide antibiotic : Erythromycin, Clarithromycin.
- Glycopeptide antibiotic : Vancomycin

Based on Mechanism of action:

Site of Action	Antibiotic	Process Interrupted	Type of Activity
Cell wall	Bacitracin	Mucopeptide synthesis	Bactericidal
			Bactericidal
	Cephalosporin	Cell wall cross linking	Bactericidal
	Cycloserine	Cell wall peptide synthesis	Bactericidal
			Bactericidal
	Penicillin	Cell wall cross-linking	
	Vancomycin	Mucopeptide synthesis	
Cell membrane	Amphoteracin B	Membrane function	Fungicidal
	Nystatin	Membrane function	Fungicidal
	Polymixin	Membrane integrity	Bactericidal
Ribosomes			
50s subunit	Chloramphenicol	Protein synthesis	Bacteriostatic
	Erythromycin	Protein synthesis	Bacteriostatic
	Lincomycin	Protein synthesis	Bacteriostatic
30s subunit	Aminoglycosides	Protein synthesis and fidelity	Bactericidal
Nucleic acids	Actinomycin	DNA and mRNA synthesis	Pancidal
	Griseofulvin		Fungicidal
DNA and/or RNA	Mitomycin C	DNA and mRNA synthesis	Pancidal
	Rifampicin	DNA synthesis mRNA synthesis	Bactericidal

Table 1.1: Classification on basis of mode of mode of action

Based on Type of action:

- Bactericidal drug – kill bacteria within its spectrum of activity.
  E.g. Penicillin, Cephalosporin, Rifampin, Metronidazole.
- Bacteriostatic drug – inhibit bacterial growth.
  E.g. Sulfonamide, Tetracycline, Chloramphenicol.

Based on Type of organism against which primarily active:

- Antibacterial: Penicillin, Aminoglycoside, Erythromycin.
- Antifungal: Ketoconazole, Griseofulvin.
- Antiviral: Acyclovir, Amantadine, Zidovudine.
- Antiprotozoal: Chloroquine, Metronidazole.
- Antihelmintic: Mebendazole, Niclosamide.

1.4 Resistance: Problem that arises with the use of AMAs

Resistance: It refers to unresponsiveness of a microorganism to an antimicrobial agent, and is a common phenomenon of tolerance seen in higher organisms.

Intrinsic Resistance Some microbes have always been resistant to certain AMAs (anti microbial agents). They lack the metabolic process or the target site which is affected by the particular drug.

e.g. Gram-negative bacteria resistant to vancomycin.

Acquired Resistance Bacteria which are ordinarily susceptible to antimicrobial agents acquire resistance due to mutation of resident gene or by acquisition of new genes.

Major Mechanisms used by bacteria to resist the action of antimicrobial agent[5]:

- Inactivation of the compound by production of inactivating compounds.
- Alteration or overproduction of the antibacterial target through mutation of the target protein gene
- Acquisition of new gene that encodes a drug insensitive target
- Decrease permeability of the cell wall envelope to the agent
- Active efflux of the compound from the periplasm or interior of the cell

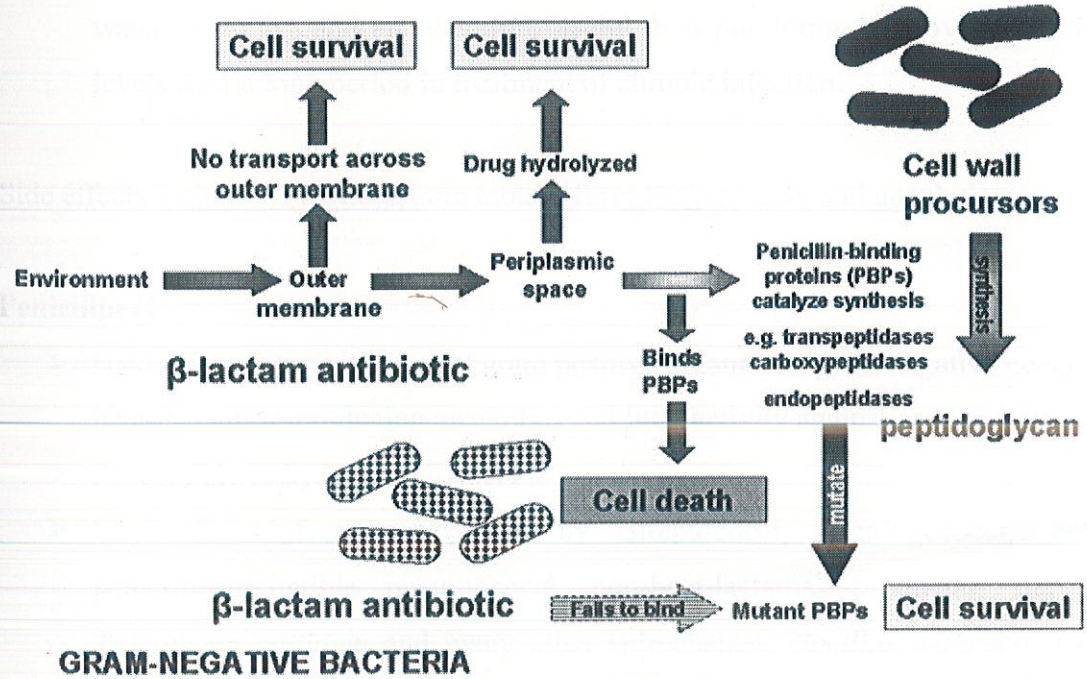


Fig 1.1: Method of resistance development in Gram Negative bacteria

1.5 Beta-Lactam compounds

These are the antibiotics having a β-Lactam ring. It includes Penicillin, Cephalosporin, Monobactam and Carbapenems[6].

1.5.1 Penicillin

- Discovered more than 70years ago by Alexander Fleming (1929)
- Ten years later, unlimited quantities of **Penicillin G** were available for clinical use
- Classified as beta lactams because of unique four membered lactam ring
- Principal limitations of Penicillin G is its instability in acidic pH.
- Susceptible to destruction by beta-lactamase (penicillinase).
- Inactive against Gram negative bacilli.

Properties:

1) The crystalline penicillin must be protected from moisture, but when kept dry, the salt will remain stable for years without refrigeration.
2) The free acid is not suitable for oral and parenteral administration.
3) Sodium and potassium salts of most penicillin are soluble in water and are readily absorbed orally and parenterally.
4) Salts of penicillin with organic bases, such as benzathine and procaine have limited water solubility and are therefore useful as depot forms to provide effective blood levels over a long period in treatment of chronic infection.

Side effects include GI distress, sore mouth, furry tongue, rash, and anaphylaxis.

Penicillin G

- Have greatest activity against gram positive organisms, gram negative cocci, and non beta-lactamase-producing anaerobes and little activity against gram negative rods.
- Susceptible to hydrolysis by beta lactamases.
- Used for infections caused by streptococci, meningococci, enterococci, peniciliinsusceptible pneumococci, non-beta-lactamase-producing staphyslococci, *Treponema pallidum* and many other spirocheates, *Bacillus anthracis, Clostridium* species, actinomyces and other gram positive rods and non-beta-lactamase-producing gram negative anaerobic organisms.

- Used to treat enterococcal endocarditis, streptococcal pharyngitis, syphilis.

Antistaphylococcal penicillins (methicillin , nafcillin, oxacillin, cloxacillin, dicloxacillin)

- Resistant to staphylococcal beta lactamases
- Active against staphylococci and streptococci
- Inactive against enterococci, anaerobic bacteria and gram negative cocci and rods
- Used to treat serious systemic staphylococcal infections.

Extended-spectrum penicillins (ampicillin, amoxicillin **and antipseudomonal penicillins)**

- Retain antibacterial spectrum of penicillin
- Improved activity against gram negative organisms
- Used to treat urinary tract infections, sinusitis, otitis, and lower respiratory tract
- infections.

Mechanism of Action

- Penicillins inhibit bacterial growth by interfering with a specific step in bacterial cell wall synthesis.
- Beta lactam **antibiotics** bind to PBPs on the cell wall.
- This inhibits the transpeptidation reaction, peptidoglycan synthesis is blocked and the cell dies.

Beta-lactam Resistance

It develops due to four general mechanisms:

1. inactivation of **antibiotics** by beta lactamase
2. modification of target PBPs
3. impaired penetration of drug to target PBPs
4. presence of an efflux pump

Destruction of drug by **β-lactamase** of gram-negative bacteria, which is confined in its periplasm , between the inner & outer membranes, while in gram-positive bacteria secrete their β-lactamase into the surrounding medium.

- Alteration of PBP targets so that the PBP's have a markedly reduced affinity to the drug.

- Coupling, in gram-negative bacteria, of a decrease in outer-membrane permeability with rapid efflux of the antibiotic from the periplasm to the cell exterior there is a mutation of gene encoding outer-membrane protein channels called porins which decrease the entry of B-lactam into the cell, while additional protein form channels that actively pump B-lactams out of the cell.
- Strategy: combine B-lactam with an inhibitor (clauvalanic acid, sulbactam & tazobactam) that avidly binds the inactivating enzyme, preventing the attack on the antibiotics.

Because many strains of the gram-negative bacteria have become resistant due to plasmid related production of beta-lactamase, new formulae were made containing the antibiotic together with a beta-lactamase inhibitor. Two combinations are available, both for oral and parenteral administration:

- ampicillin + sulbactam
- Co-amoxiclav: amoxicillin + clavulanic acid (Augmentin by GlaxoSmithKline)

1.5.2 Cephalosporins

- similar to penicillins but more stable to many bacterial β-lactamases and therefore have a broader spectrum of activity
- not active against enterococci and *Listeria monocytogenes*
- first generation cepahalosporins have better activity against gram-positive organisms
- the later generations have improved activity against gram-negative aerobic organisms.

Side effects include cross-sensitivity to a person who is allergic to penicillin, GI effects, and nephrotoxicity.

First Generation Cephalosporins

- include cefadroxil, cefazolin, cephalexin, cephalothin, cephapirin and cephadrine.
- Active against gram-positive cocci, including pneumococci, streptococci, and staphylococci.
- Not active against methicillin-resistant strains of staphylococci.
- Active against *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis.*
- Activity against *P. aeruginosa,* indole-positive proteus, enterobacter, *Serratia*

marcescens, citrobacter and acinetobacter is poor.

- Used for the treatment of urinary tract infections, minor staphylococcal lesions, minor polymicrobial infections such as cellulites or soft tissue abscess and surgical prophylaxis.

Second Generation Cephalosporins

- Include cefaclor, cefamandole, cefonicid, cefuroxime, cefprozil, loracarbef,
- ceforanide, cephamycins, cefotoxitin, cefmetazole and cefotetan.
- Active against anaerobes, gram negative microbes, *Klebsiella*, *H. influenzae.*
- Used to treat sinusitis, otitis, lower respiratory tract infections, peritonitis, diverticulitis, community-acquired pneumonia and meningitis.

Third Generation Cephalosporins

- Include cefoperazone, cefotaxime, ceftizoxime, ceftriaxone, cefixime, cefpodoxime, proxetil, ceftibuten and moxolactam.
- Active against gram negative bacteria, citrobacter, *Serratia marcescens*, and *P. aeruginosa.*
- Used to treat meningitis, *H. influenza* infection, and gonorhea.

Fourth Generation Cephalosposins

- Includes cefepime which is more resistant to hydrolysis by beta-lactamases.
- Has good activity against *P. aeruginosa*, *Enterobacteriaceae*, *S. aureus* and *S. pneumoniae.*
- Used to treat meningitis.

Other Inhibitors of Cell Wall Synthesis

- Includes **Vancomycin** antibiotic produced by *Streptococcus orientalis.*
- Inhibits cell wall synthesis by inhibiting transglycosylase, preventing further elongation of peptidoglycan and cross linking. The peptidoglycan weakens and cell becomes susceptible to lysis.
- Bactericidal for gram positive bacteria, pathogenic staphylococci, *E. faecium* and *E. faecalis*.
- Poorly absorbed from intestinal tract and administered orally only to treat enterocolitis caused by *Clostridium difficile*.

- Mostly administered intravenously to treat sepsis or endocarditis caused by methicillin-resistant staphylococci.

Adverse effects of vancomycin involve fever, chills, exanthema, and phlebitis at the site of infusion. Reversible leukopenia, thrombocytopenia, or eosinophilia may develop as well. Flushing due to histamine release ("red man syndrome") and/or hypotension frequently occur after rapid intravenous administration. Renal failure and hearing loss are the most fearing sequellae of treatment with vancomycin.

- **Bacitracin** obtained from the Tracy strain of *Bacillus subtilis* in 1943 and inhibits cell
- wall synthesis by interfering with dephosphorylation in cycling of the lipid carrier that
- transfers peptidoglycan subunits to the growing cell wall.
- highly nephrotoxic and administered as topical application.
- Used for the suppression of mixed bacterial flora in surface lesions of the skin, in
- wounds, or on mucous membranes.

1.5.3 Beta- Lactam: Mode of Action

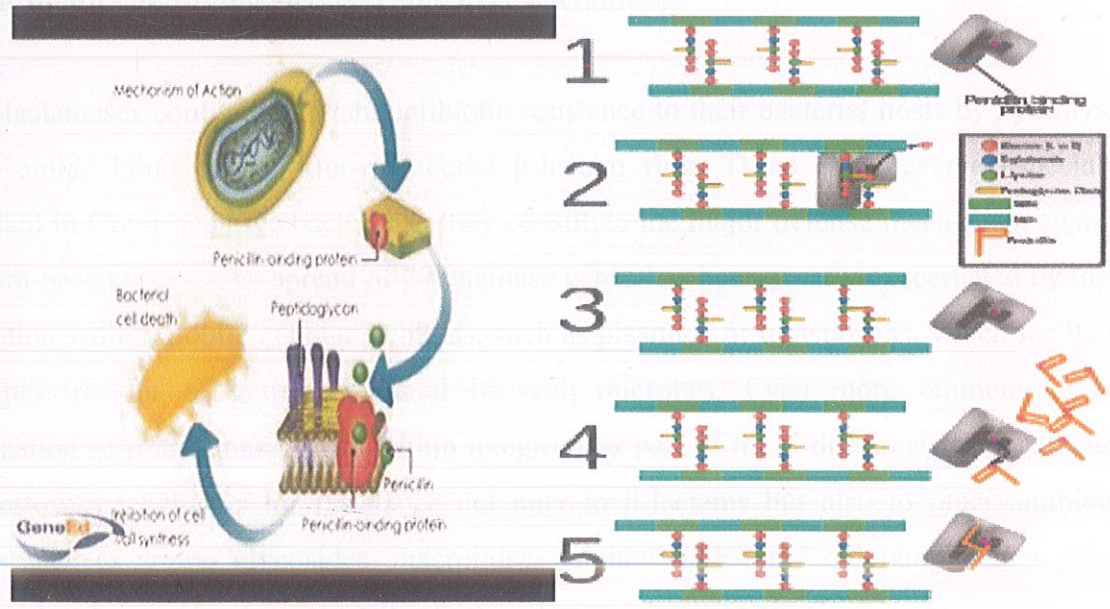


Fig 1.2: Mode of action of Beta Lactam

Bacteria of all species rely on a heavily cross-linked peptidoglycan layer (cell wall) for the preservation of cell shape and rigidity. This cell wall is comprised of a basic repeating unit of an alternating disaccharide —NAG and NAM. The latter sugar in this disaccharide is

modified by a characteristic pentapeptide. This varies amongst the Gram-negative and Gram-positive species, but always terminates in two D-alanine residues. The individual peptidoglycan units are produced inside the cell, but their final cross-linking is catalyzed outside the cytoplasmic membrane by a group of membrane anchored bacterial enzymes known as the cell-wall transpeptidases. In this cross-linking reaction, a peptide bond is formed between the penultimate D-alanine on one chain and the free amino end of a diamino pimelic acid(Gram-negative) or an L-lysine (Gram-positive) residue on the other chain. The linkage is formed with the penultimate D-alanine, causing the terminal D-alanine to be cleaved in the process. Transpeptidase enzymes utilize an active site serine and perform their catalytic cycle by way of an acylation/deacylation pathway. b-lactam antibiotics efficiently inhibit the bacterial transpeptidases, therefore these enzymes are often termed penicillin binding proteins or PBPs.They are able to do this owing to the stereochemical similarity of the b-lactam moiety with the D-alanine–D-alanine substrate. In the presence of the antibiotic, the transpeptidases form a lethal covalent penicilloyl–enzyme complex that serves to block the normal transpeptidation reaction. This results in weakly cross-linked peptidoglycan, which makes the growing bacteria highly susceptible to cell lysis and death.

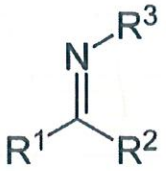
1.6 Antibiotic-modifying enzymes: the beta -lactamases

The β-lactamases confer significant antibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the four-membered β-lactam ring. These enzymes are especially important in Gram-negative bacteria as they constitute the major defense mechanism against β-lactam-based drugs. The spread of β-lactamase genes has been greatly exacerbated by their integration within mobile genetic elements, such as plasmids or transposons, which facilitate the rapid transfer of genetic material between microbes. Even more ominous is the organization of β-lactamase genes within integrons as part of multi-drug resistance cassettes that bestow mechanisms for resistance not only to β-lactams but also to other antibiotic classes such as amino glycosides, macrolides, sulphonamides and chloramphenicol .Once expressed, β-lactamases are secreted into the periplasmic space (in Gram-negative bacteria), bound to the cytoplasmic membrane, or excreted (in Gram positive bacteria).

1.7 Imine

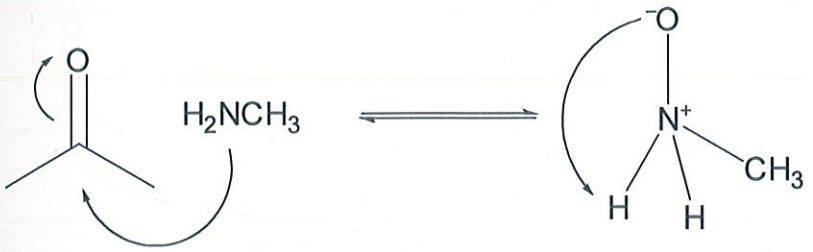
An **imine** is a functional group or chemical compound containing a carbon–nitrogen double bond, with the nitrogen attached to a hydrogen atom (H) or an organic group. If this group

is *not* a hydrogen atom, then the compound is known as a Schiff base. The carbon has two additional single bonds. Schiff Base gives good antimicrobial activity and pharmacological applications and it can be prepared by the acid catalyzed reaction of amines & ketones or aldehydes. It gives a good fungicidal activity.

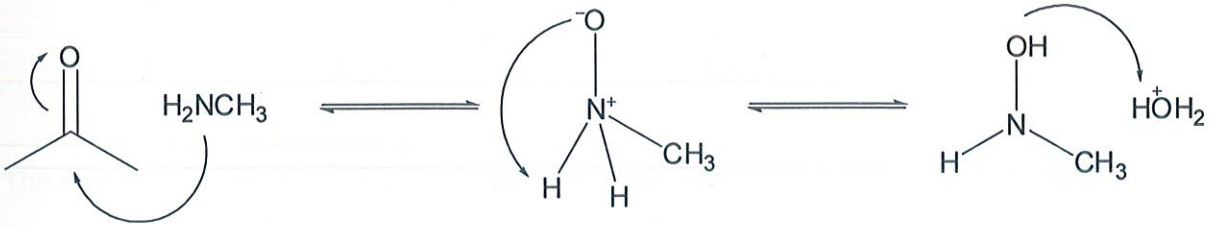


Schiff bases, named for Hugo Schiff, are formed when any primary amine reacts with an aldehyde or a ketones under specific conditions. They are organic compounds with the general formula RR′C=NR″, where R and R′ represent hydrogen, an alkyl or an aryl and R″ is an alkyl or aryl; in the latter case, Schiff bases are also called anils.

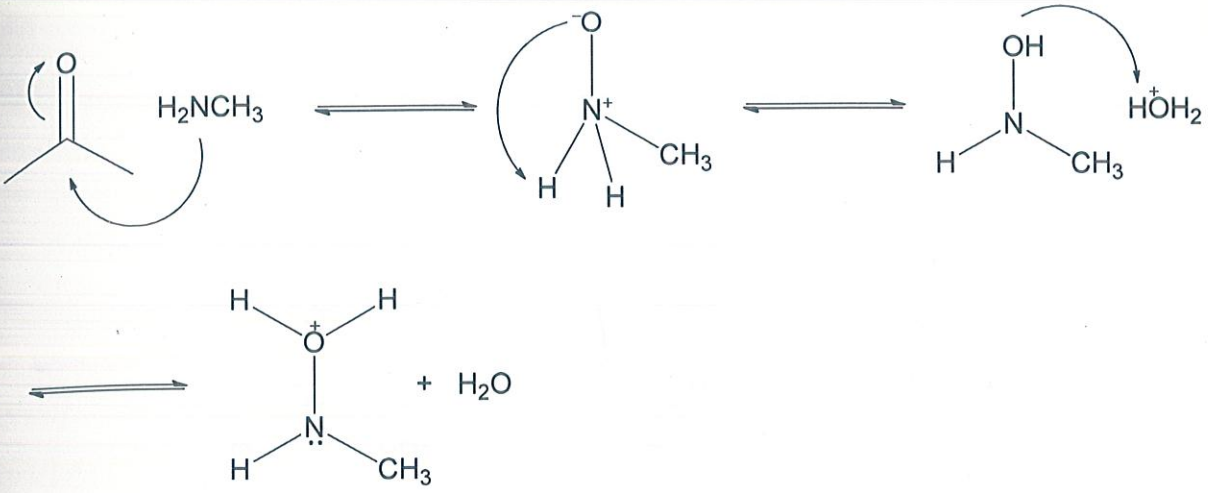
Mechanism of Imine Formation



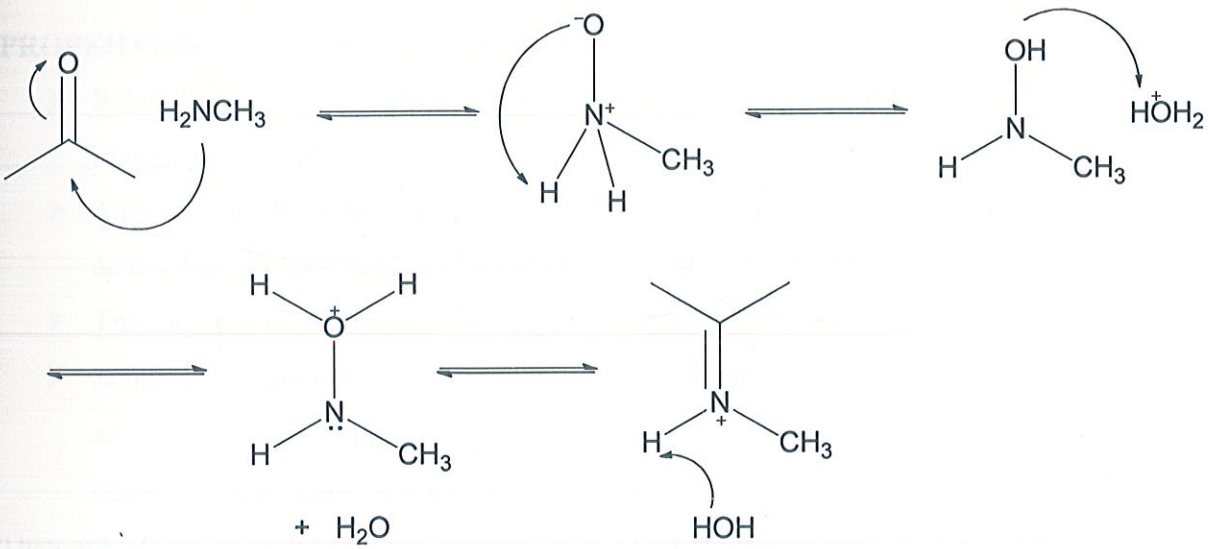
An amine is a stronger nucleophile than an alcohol, so it can add directly to a ketone. This is an acid catalyzed reaction, though. The acid comes in later.



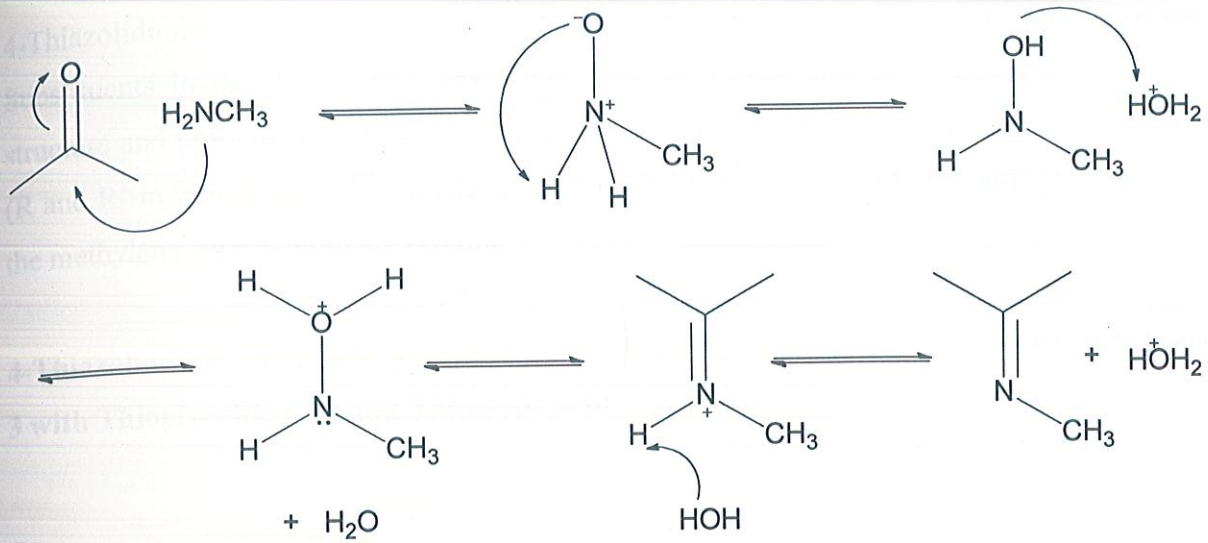
Proton transfer from the nitrogen to the negatively charged oxygen gives a neutral molecule. This is an aminol. This is directly analogoues to the hemiacetal, although the mechanism is slightly different. Formation of the hemiacetal requires an acid catalyst, while the aminol does not. From here on the mechanism will be very similar to acetal formation.



At this point the acid catalyst becomes involved. In order to remove the OH, it must be protonated to give a better leaving group-water.



The lone pair on the amine comes down to push out water. This gives protonated imine.



Finally, water accepts the proton from the iminium ion. This is the final step. The acid catalyst is regenerated and a molecule of water is made as well as the imine. [7]

PROPERTIES

- Schiff bases are crystalline or oily substances that are insoluble in water and soluble in organic solvents.
- They are weak bases, forming salts with acids in an anhydrous medium; in aqueous acid solutions they undergo hydrolysis to yield an amine and aldehyde.
- The majority of Schiff bases are stable in alkaline solutions.
- Schiff bases undergo hydrogenation to give secondary amines (RR 'CH-NHR") and add on many compounds containing mobile hydrogen, such as β-dicarbonyl compounds, ketones, and imines.

They are produced mainly by the condensation of aldehydes or ketones with primary amines. The reaction was first completed by H. Schiff in 1864 (hence the name of the compounds). Schiff bases are valuable intermediate products of organic synthesis, for example, in the preparation of secondary amines and various heterocyclic compounds. The Schiff bases known as azomethine dyes are used for dyeing acetate and synthetic fibers; they are also used in color photography to reduce the photosensitivity of photographic emulsions.

1.8 Thiazolidione

Thiazolidione, a saturated form of thiazoles with carbonyl group on fourth carbon, has been considered as a moiety of choice as it possesses a broad spectrum of pharmacological activities against several targets.[8]

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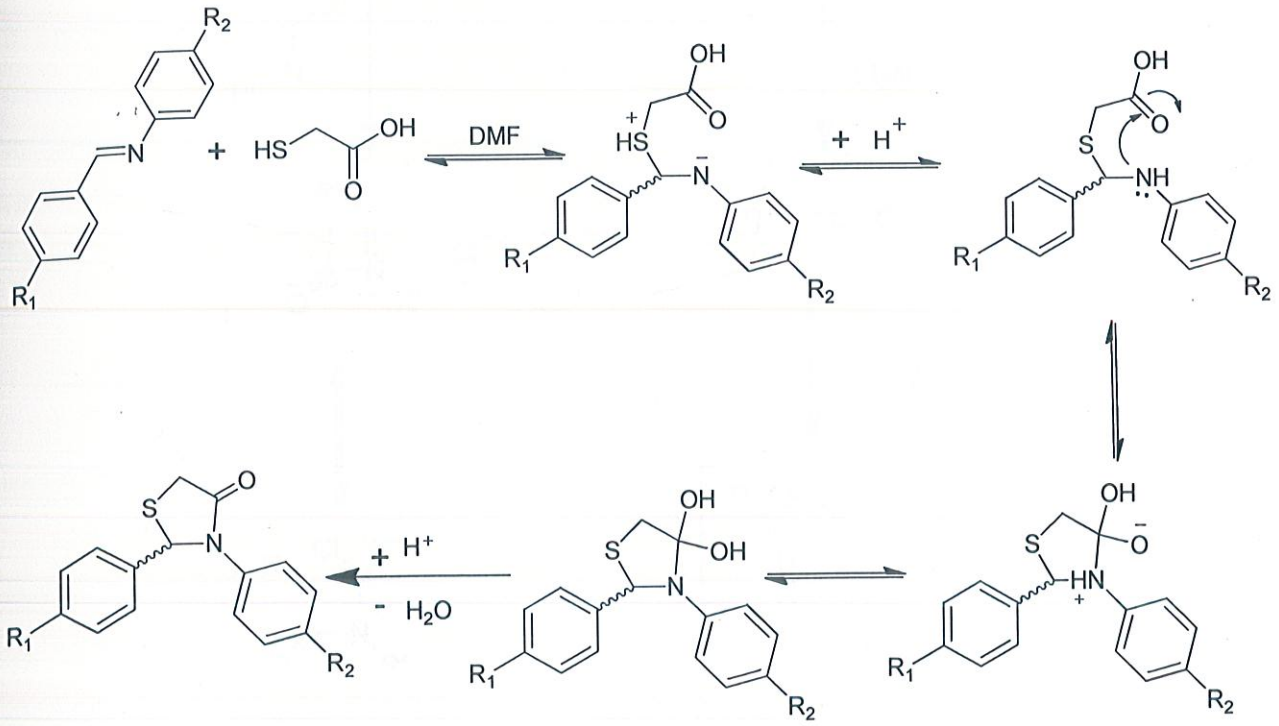
4-Thiazolidiones are derivatives of thiazolidine with a carbonyl group at the 4-position. Substituents in the 2-, 3-, and 5-positions may be varied, but the greatest difference in structure and properties is exerted by the group attached to the carbon atom in the 2-position (R and R' in 2 or X in 3). [9] Variations in the substituents attached to the nitrogen atom and the methylene carbon atom are possible.

4-Thiazolidiones have been prepared by the reaction of various substituted Schiff bases 3 with Thioglycollic acid and Thiolactic acid.

Mechanism Of Thiazolidin-4-ones Formation

[10]



Thiazolidione are well known for their versatile pharmacological activities such as antimicrobial,' antihyperglycemic, anti\histaminic, antitubercular, antifungal, anticonvulsant, aldose reductase inhibitor, analgesic, diuretic, hypolipidemic etc.

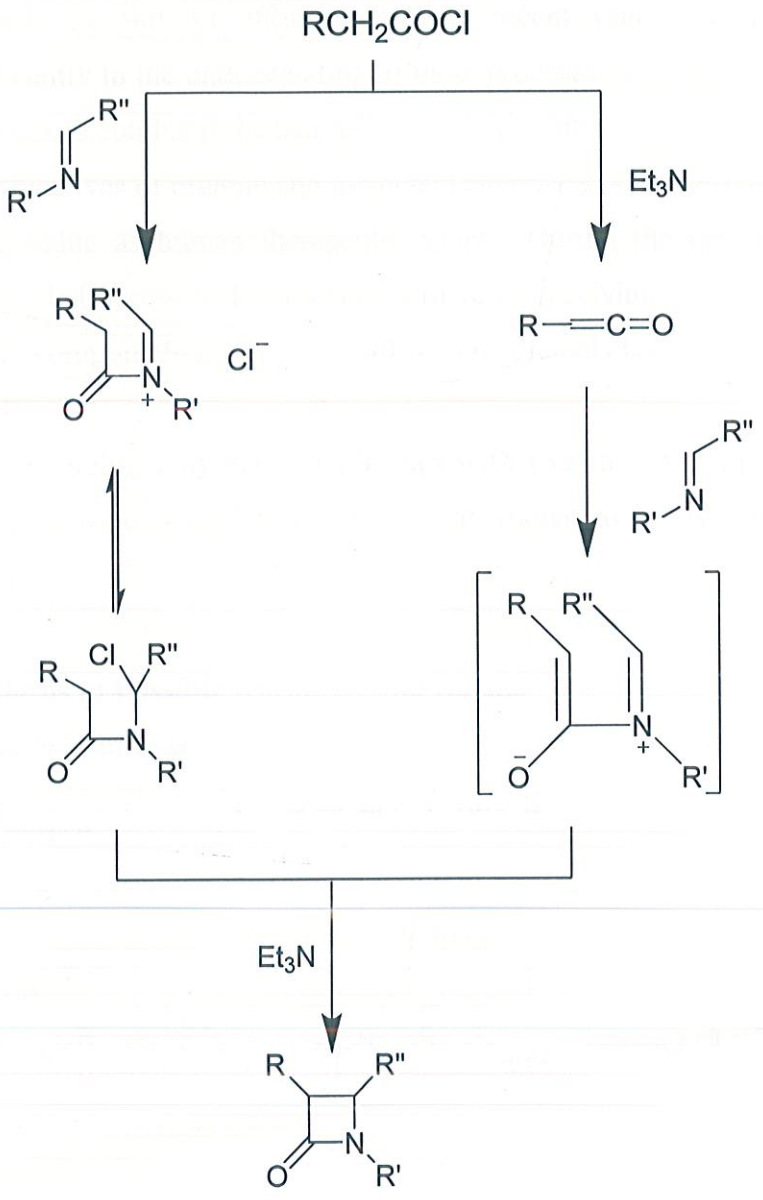
Thiazolidione have been the subject of extensive research because of their deep involvement in the regulation of different physiological processes. Thiazolidiones such as troglitazone, pioglitazone, and rosiglitazone are potent reducers of plasma glucose level in

vivo.Thiazolidinediones are also potential cancer chemopreventive agents against colon, breast, tongue, and gastric carcinogenesis.

Bacterial resistance to the antibiotics and antimicrobial agents is a big blow to humanity and continual search for newer chemotherapeutic agents is the only way to fortify against this awful threat. Thus, in this study, a strategy has been planned to synthesize novel thiazolidiones and screening for their antimicrobial activity as per standard methodology by the use of bacterial strains like *S.aureus*, *E.coli*, *B.subtilis* etc

1.9 Mechanism of β-Lactam Formation

[11]



CHAPTER 2

2.1 Objective

Recent years have witnessed a substantial increase in our understanding of the mechanisms responsible for β-lactam resistance. The structures of the molecular determinants of resistance — particularly in complex with antibiotics or inhibitors — are poised not only to explain resistance, but also to inspire novel methods of combating it. The β-lactam class of antibiotics has proven itself to be invaluable in the treatment of bacterial infections.

Bacterial resistance to β-lactam antibiotics can be achieved by any of three strategies: the production of β-lactam-hydrolyzing β-lactamase enzymes, the utilization of β-lactam-insensitive cell wall transpeptidases, and the active expulsion of β-lactam molecules from Gram-negative cells by way of efflux pumps. In recent years, structural biology has contributed significantly to the understanding of these processes and should prove invaluable in the design of drugs to combat β -lactam resistance in the future.

One of the main objectives of organic and medicinal chemistry is the design, and synthesis of molecules having value as human therapeutic agents. During the past decade, synthesis chemistry has provided access to heterocyclic structures receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry.

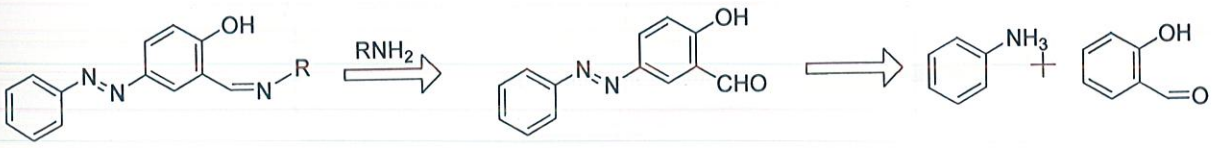
There are numerous biologically active molecules with five member rings, containing two hetero atoms. Thiazolidine is an important scaffold known to be associated with several biological activities.

Design and Synthesis of Possible Antimicrobial Agents

a. Synthesis of imines

3 1 2

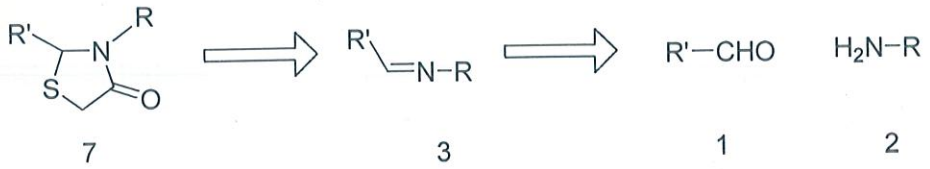
Scheme1



6 5 2 4

Scheme 2

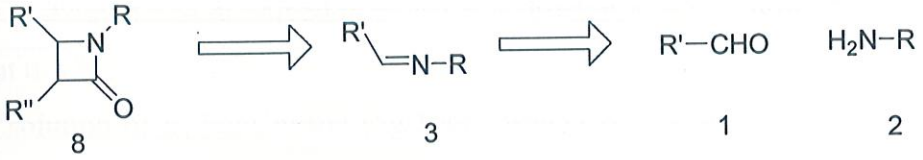
b.Synthesis of 4-thiazolidiones derivatives



7 3 1 2

Scheme 3

c.Synthesis of β-lactam derivatives



8 3 1 2

Scheme 4

2.2 Strategy

We planned to synthesize various derivatives of β-lactam and 4-thiazolidione derivatives. These compounds could be synthesized from imines, obtained from aldehyde and amine.

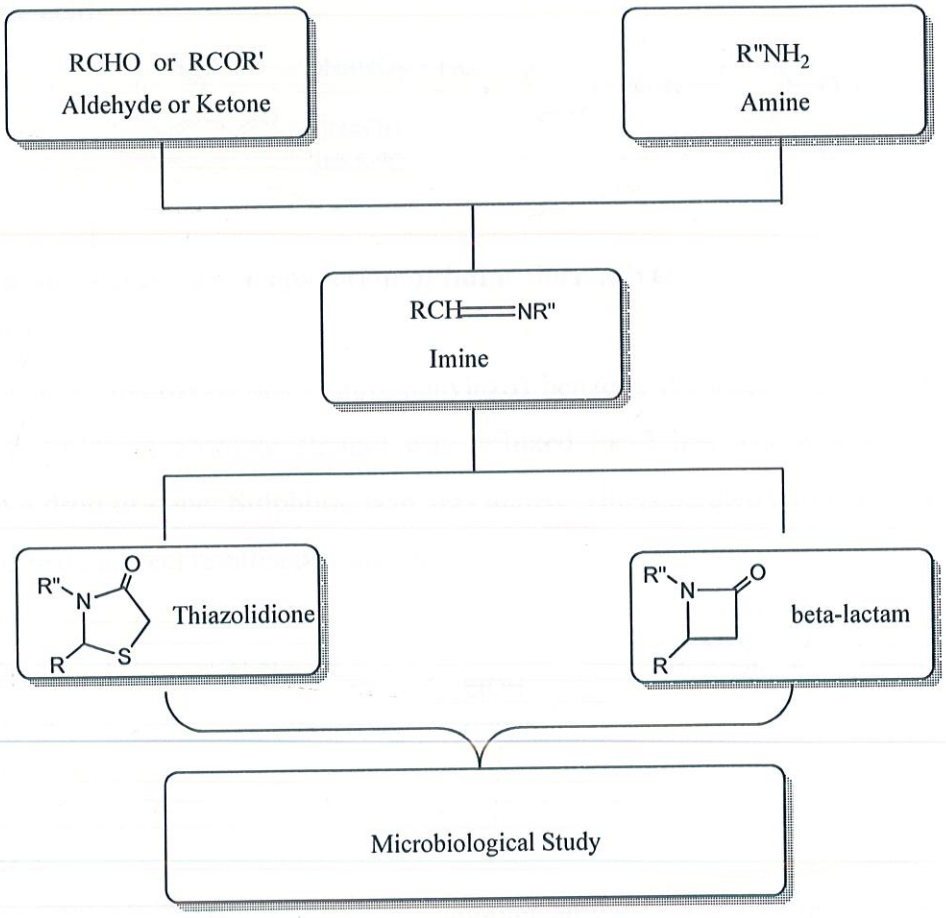


Fig 2.1 Chart showing Scheme of study

CHAPTER 3

3.1 Preparation of imines

3 1 2

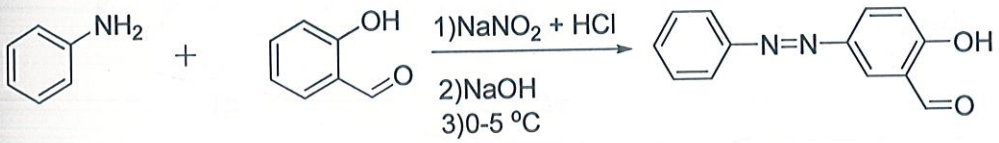
3.2 Experimental

[A] Preparation of Azodye

Aniline (3.72 mL) was dissolved in aqueous hydrochloric acid (28mL, 6N) and mechanically stirred at 0-5 °C.

A cold solution of sodium nitrite (5g/10mL water) was added drop wise to the constantly stirred reaction mixture. The diazotized solution was immediately added in small portions to salicylaldehyde (5ml dissolved in 40 ml, 6N NaOH), with constant stirring at 0-5c. the stirring was continued for 4hrs.

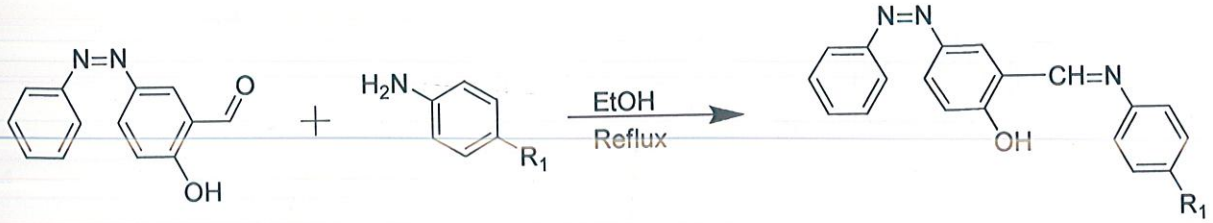
The solid obtained was filtered under suction washed with cold water and recrystallised from glacial acetic acid.



[B] General procedure for preparation of imine derivatives

METHOD 1

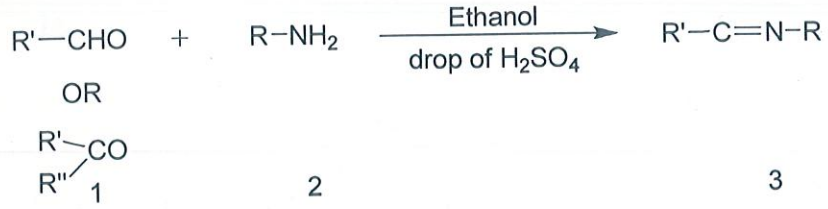
A mixture of 2- hydroxyl-5-(4'- nitrophenylazo) benzene carbaldehyde 1a(0.001 mol)and any another amine in absolute ethanol was refluxed for 2 hrs. The reaction mixture was cooled and a drop of conc. Sulphuric acid was added. The separated solid was filtered under suction, washed and recrystallised from ethanol.



METHOD 2

A mixture of Benzil (0.001 mol) and any another amine in absolute ethanol was refluxed. End point was determined by TLC. The reaction mixture was cooled and a drop of conc.

Sulphuric acid was added. The separated solid was filtered under suction, washed and recrystallised from ethanol.



3.3 Result and discussion

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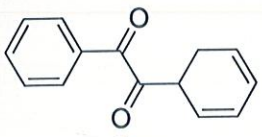
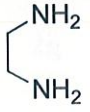
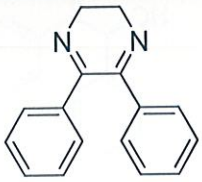
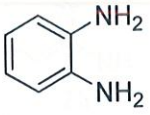
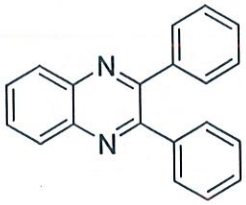
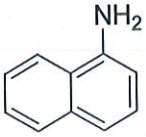
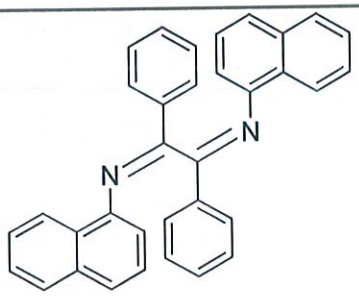
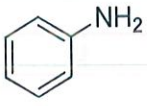
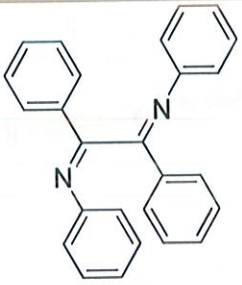
1.	 1a	 2a	 3a
2.	1a	 2b	 3b
3.	1a	 2c	 3c
4.	1a	 2d	 3d

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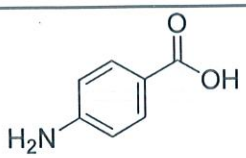

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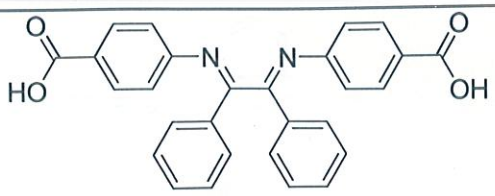

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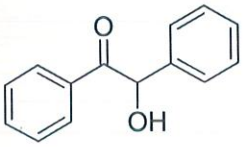

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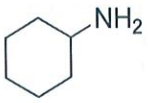

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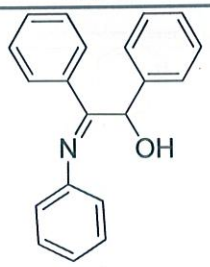

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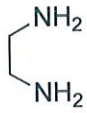

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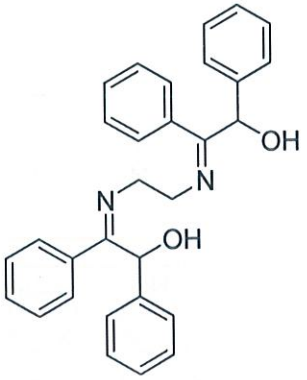

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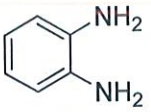

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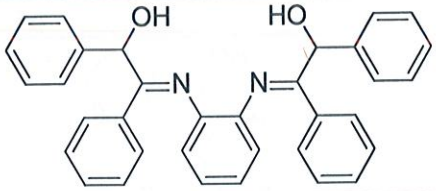

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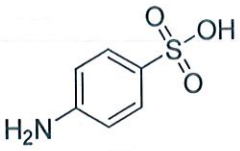

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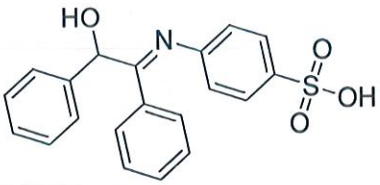

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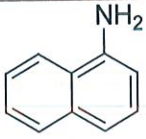

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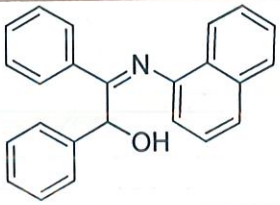

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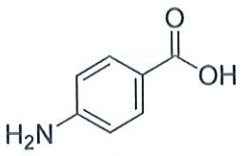
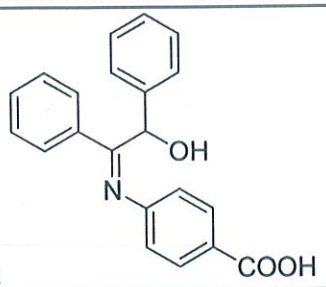
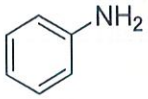
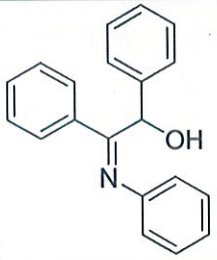
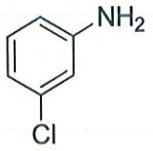
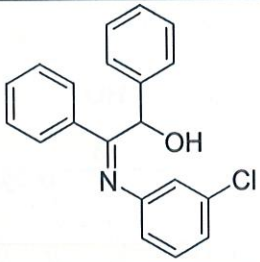
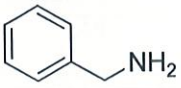
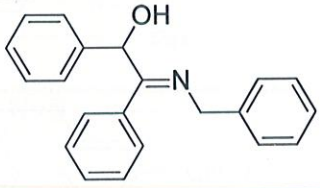
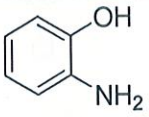
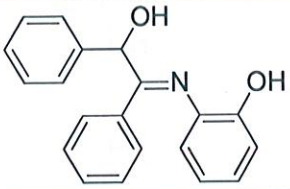
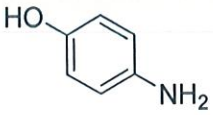
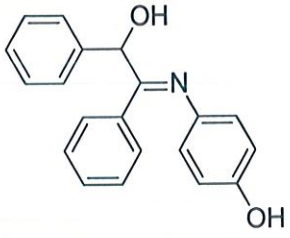
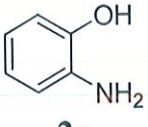
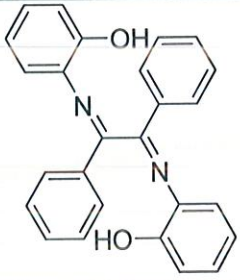
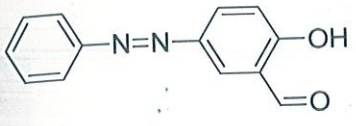
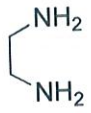
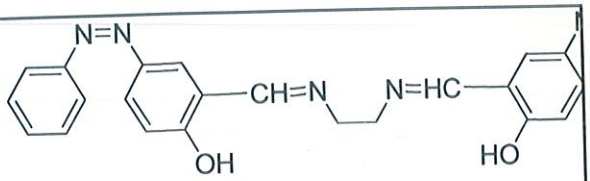
11.	1b	 2k	 3k
12.	1b	 2l	 3l
13.	1b	 2m	 3m
14.	1b	 2n	 3n
15.	1b	 2o	 3o
16.	1b	 2p	 3p
17.	1b	 2q	 3q

18.			
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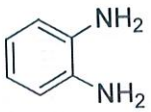
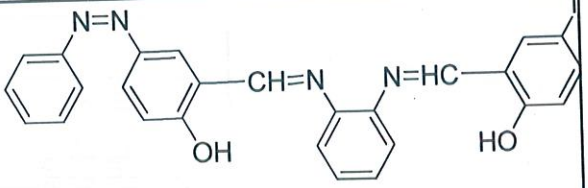
19.			
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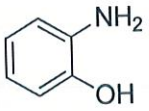
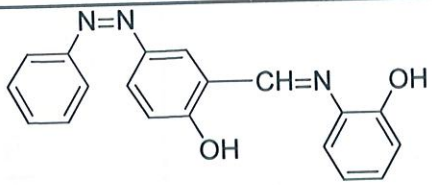
20.			
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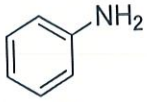
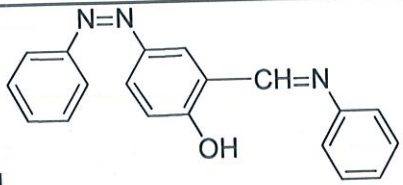
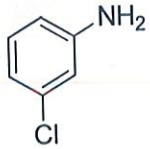
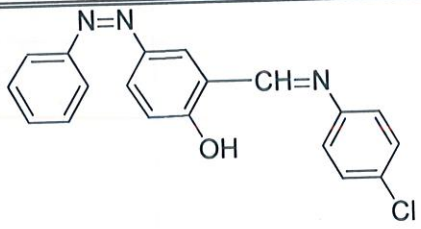
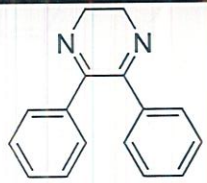
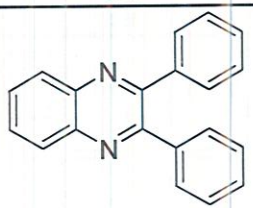
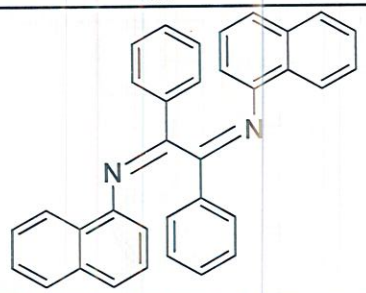
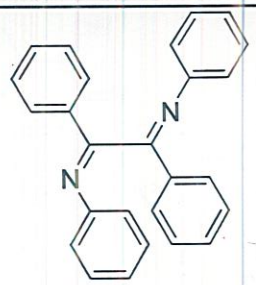
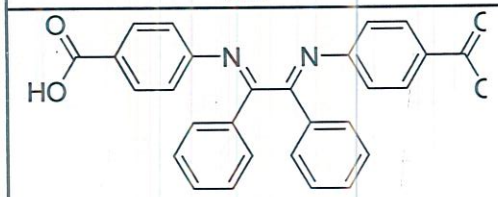
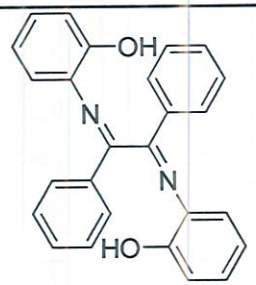
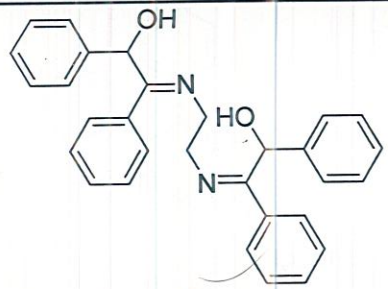
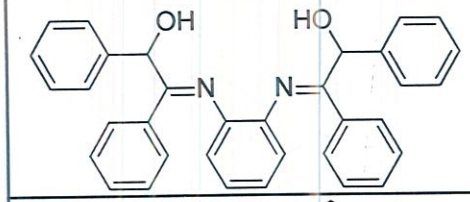
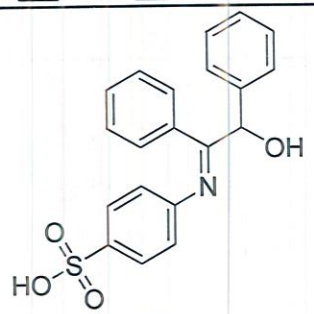
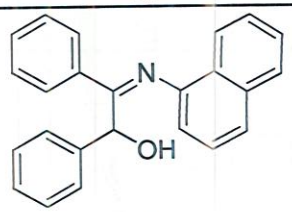
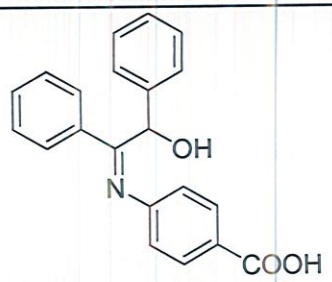
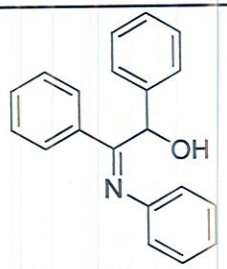
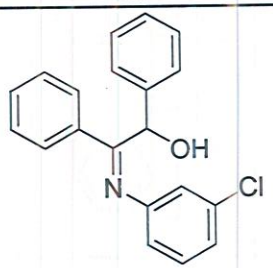
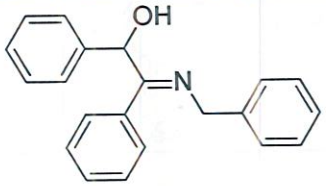
21.			
	5a	2u	6d
22.			
	5a	2v	6e

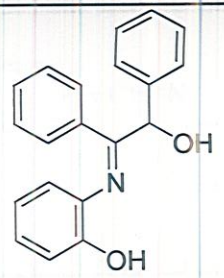
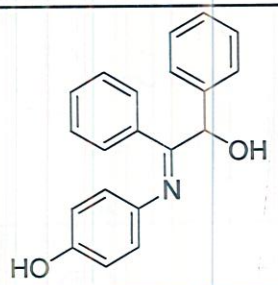
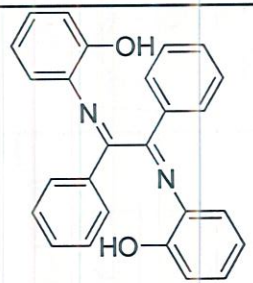
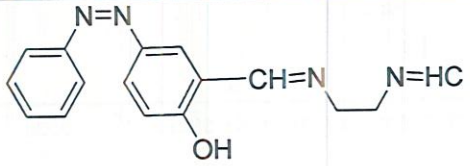
Table 3.1: Table of chemical structure of imine formed

	Physical State	Color	Melting Point	R_f	Percentage yield
 **3a**	SOLID CRYSTAL	SHINY YELLOW	172 °C -174 °C	0.7 (0.5% Ethyl acetate-Hexane)	87.09
 **3b**	SOLID CRYSTAL	SHINY LIGHT BROWN	121 °C -123 °C	1.3 (0.5% Ethyl acetate-Hexane)	90.00
 **3c**	SOLID PELLETS	BROWNISH YELLOW	98 °C	0.6 (0.5% Ethyl acetate-Hexane)	34.78

 **3d**	SOLID CRYSTAL	YELLOW	195°C	0.7 (0.5% Ethyl acetate-Hexane)	83.63
 **3e**	SOLID	BRIGHT YELLOW	225 °C -235 °C	1.3 (0.5% Ethyl acetate-Hexane)	65.84
 **3f**	SOLID	YELLOW	205 °C	0.6 (0.5% Ethyl acetate-Hexane)	67.00

 **3g**	CRYSTALLINE	BROWNISH YELLOW	115-118°C	0.7 (100% Hexane)	98.7
3h 	AMORPHOUS SOLID	GREENISH YELLOW	120-125°C	1.3 (15% Ethyl acetate-Hexane)	101.6
 **3i**	POWDER	SILVERISH WHITE SHINY	262-265°C	0.6 (5% Ethyl acetate-Hexane)	102.4
 **3j**	POWDER	REDDISH BROWN	120-125C	0.7 (100% Hexane)	102.6

 **3k**	AMORPHOUS	SHINY OFF WHITE	92-99°C	1.3 (5% Ethyl acetate-Hexane)	107.8
 **3l**	AMORPHOUS	SHINY CREAM	110-112°C	0.6 (0.5% Ethyl acetate-Hexane)	77.6
 **3m**	CRYSTALLINE	SHINY CREAM	122-125°C	0.7 (50% Ethyl acetate-Hexane)	72.5
 **3n**	AMORPHOUS	OFF WHITE	NT	0.7 (1% Ethyl actetate-Hexane)	30.4

 **3o**	AMORPHOUS	DIRTY GREY	108-110°C	1.2 (15% Ethyl acetate-Hexane)	52.4
 **3p**	AMORPHOUS	LIGHT BROWN	115-120°C	0.8 (0.5% Ethyl acetate-Hexane)	62.2
 **3q**	CRYSTALLINE	WHITE	102-105°C	0.7 (5% Ethyl acetate-Hexane)	32.6
 **6a**	SOLID PELLETS	BROWNISH YELLOW	235-240°C (decomposed)	0.7 (0.5% Ethyl acetate-Hexane)	73.90

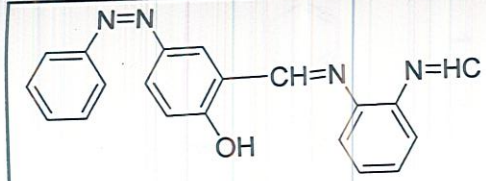
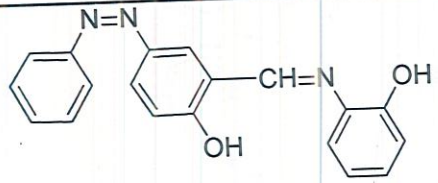
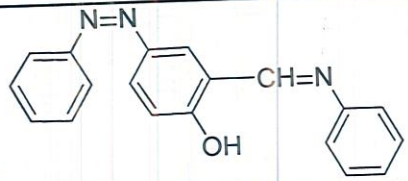
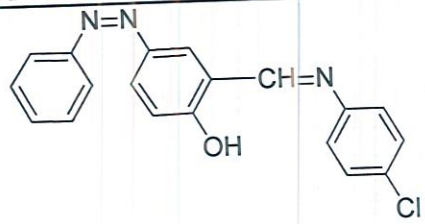
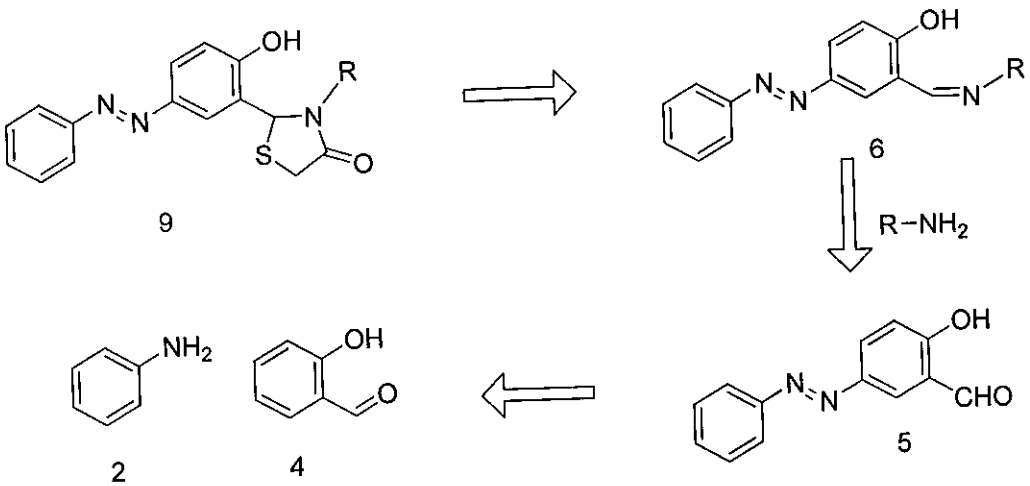
 **6b**	SOLID PELLETS	BROWNISH YELLOW	220-225°C (decomposed)	1.3 (0.5% Ethyl acetate-Hexane)	68.8
 **6c**	SOLID PELLETS	DARK PINK	152-158°C	0.6 (0.5% Ethyl acetate-Hexane)	67.9
 **6d**	CRYSTALLINE	LIGHT BROWN	182°C	0.6 (0.5% Ethyl acetate-Hexane)	72.4
 **6e**	CRYSTALLINE	DARK BROWN	178°C	1.2 (0.5% Ethyl acetate-Hexane)	72.3

Table 3.2: Table showing characteristics of prepared imines.

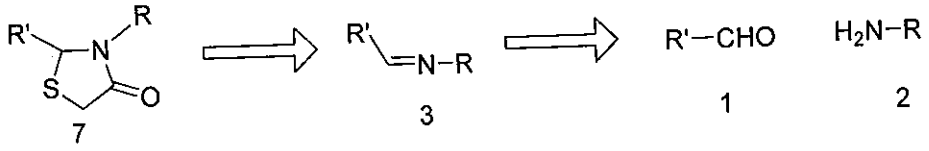
CHAPTER 4

4.1 Preparation of thiazolidiones

1)

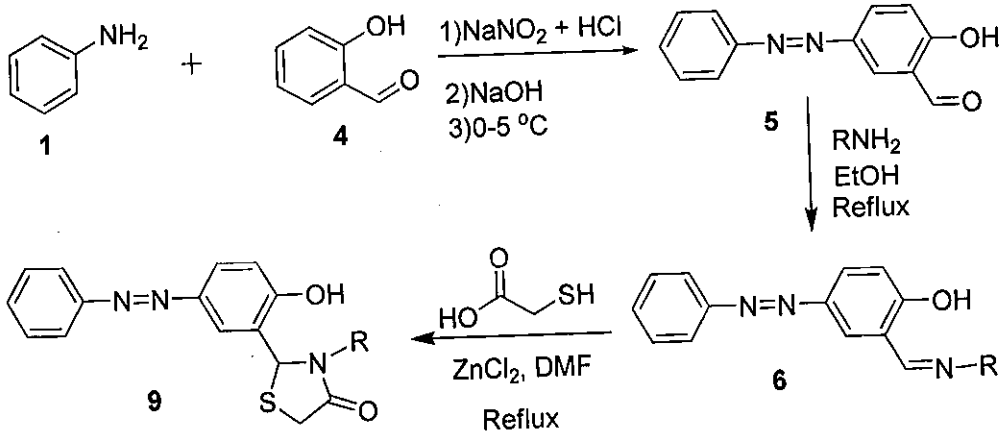


2)

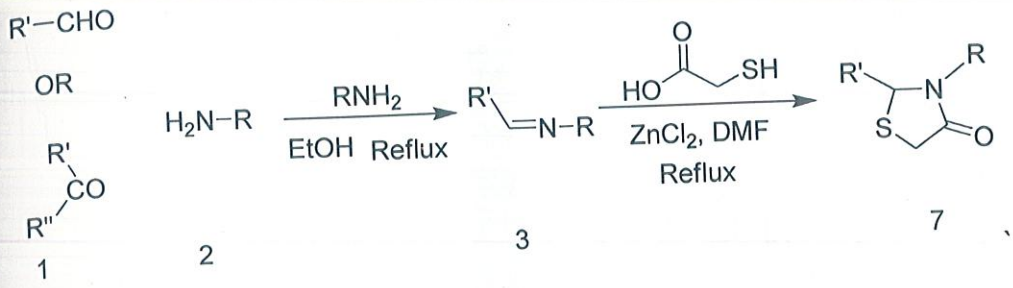


General procedure for thiazolidi-4-one derivatives

4.2 Experimental



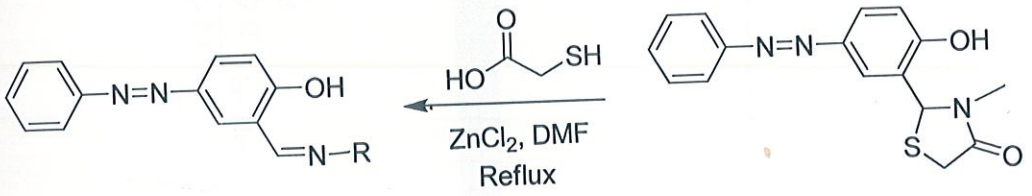
Scheme 5



Scheme 6

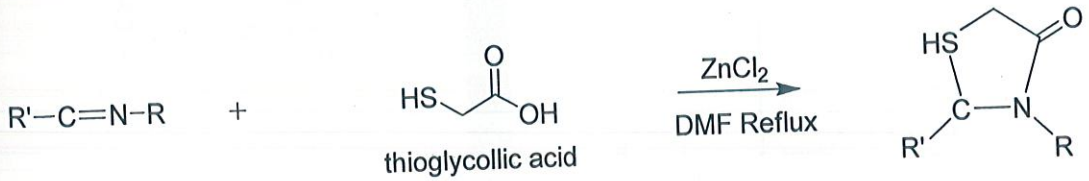
METHOD 1

The imines (1 equiv.) were dissolved in DMF (10 mL) and thioglycollic acid (1.1 eq.) was added followed by a pinch of zinc chloride. The reaction mixture was refluxed for 8-16 hours (TLC monitoring). The reaction mixture was cooled to RT then poured on crushed ice. A precipitate was formed. The precipitate was filtered off and washed with cold water; dried and ^{1}H NMR was recorded.

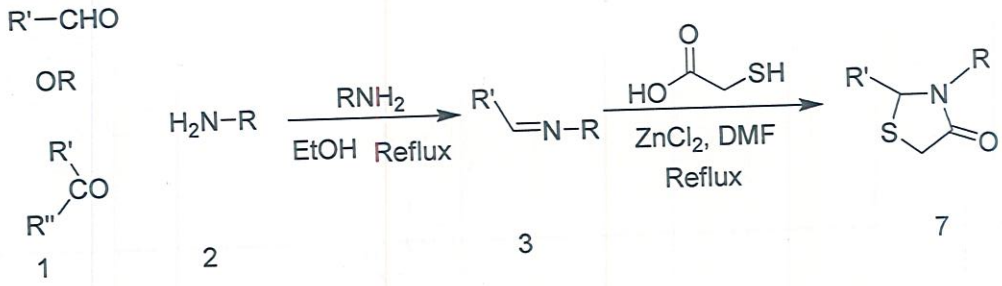


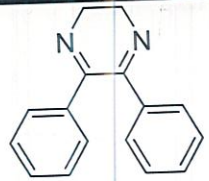
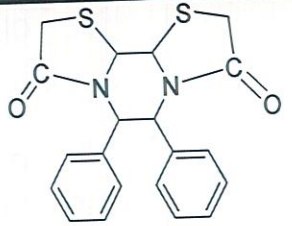
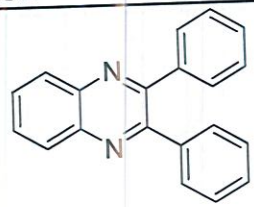
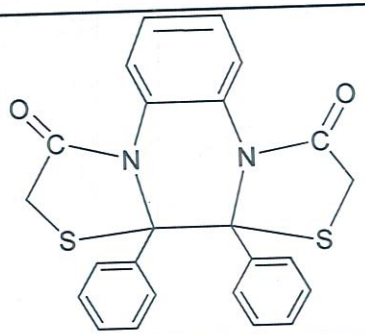
METHOD 2

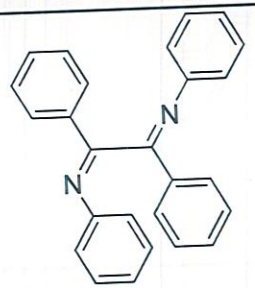
The imines (1 equiv.) were dissolved in DMF (10 mL) and thioglycollic acid (1.1 eq.) was added followed by a pinch of zinc chloride. The reaction mixture was refluxed for 8-16 hours (TLC monitoring). The reaction mixture was cooled to RT then poured on crushed ice. A precipitate was formed. The precipitate was filtered off and washed with cold water; dried and ^{1}H NMR was recorded[12].



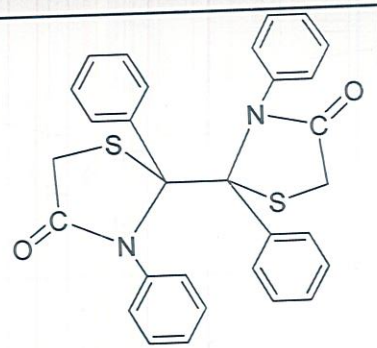
4.3 Result and discussion



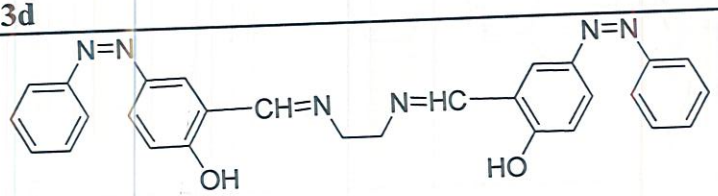
Imine (7)	4-thiazolidione derivative (8)
 3a	 7a
 3b	 7b



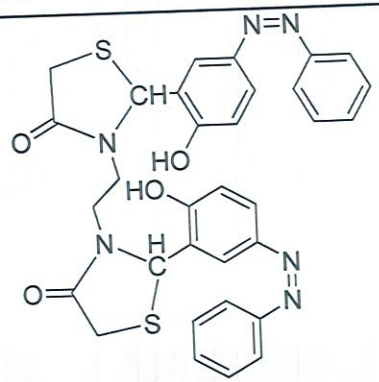
3d



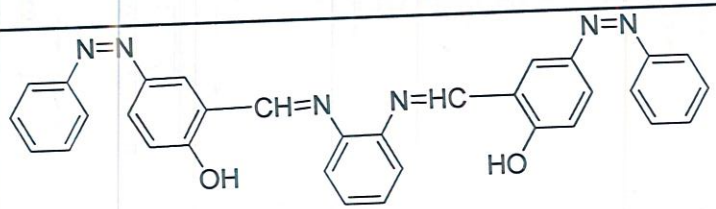
7c



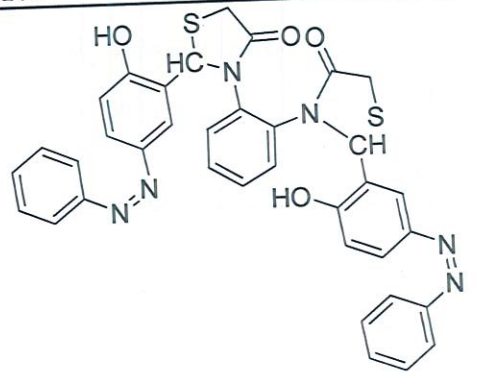
6a



10a



6b



10b

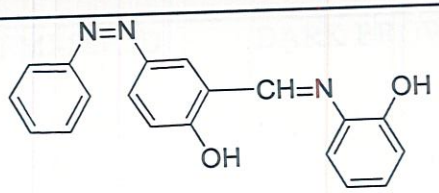
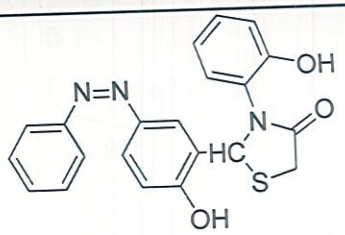
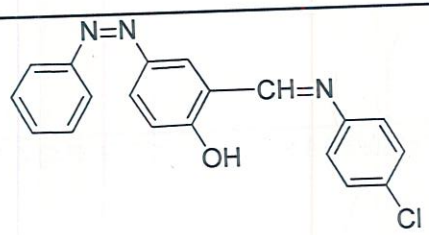
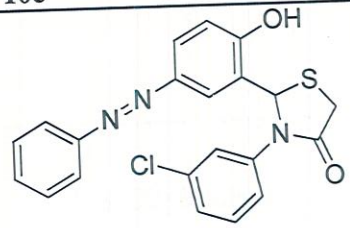
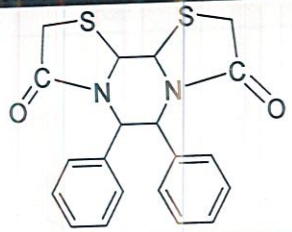
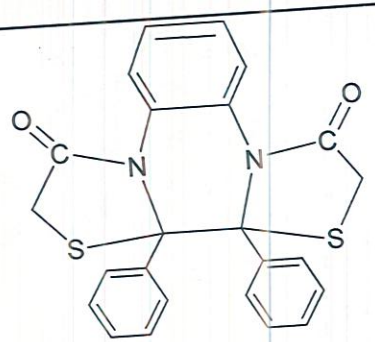
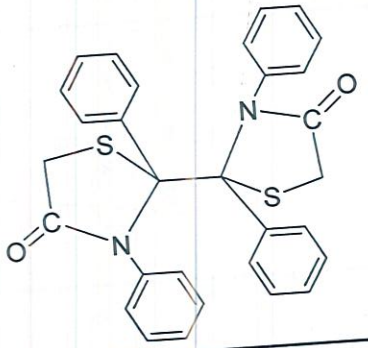
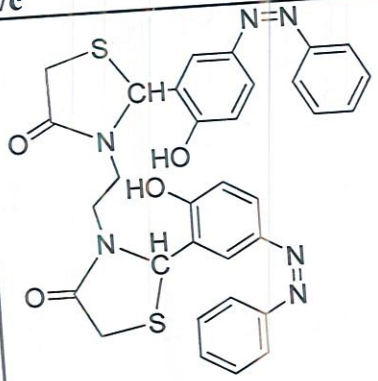
 **6c**	 **10c**
 **6e**	 **10d**

Table 4.1: Table of chemical structure of thiazolidinone formed.

4-thiazolidione derivative (8)	Physical State	Color	M.P	R_f	Percentage Yield
 **7a**	SOLID PELLETS	BROWNISH YELLOW	210-212°C	0.6 (5% Ethyl acetate-Hexane)	66..90

 **7b**	SEMI-SOLID	DARK BROWN	NT	0.7 (5% Ethyl acetate-Hexane)	73.90
 **7c**	SEMI-SOLID	ORANGISH	NT	0.7 (1% Ethyl actetate-Hexane)	32.6
 **9a**	AMORPHOUS	OFF WHITE	178-182^{0}C	0.7 (1% Ethyl actetate-Hexane)	27.4

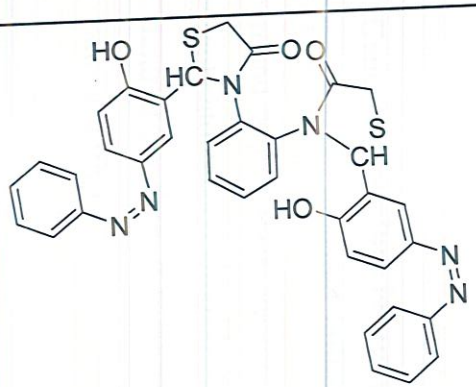
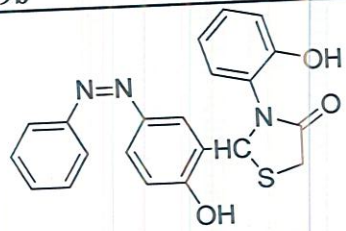
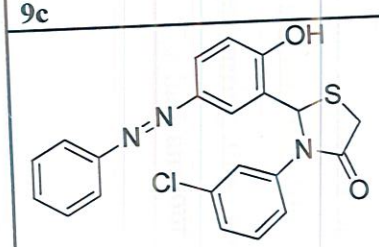
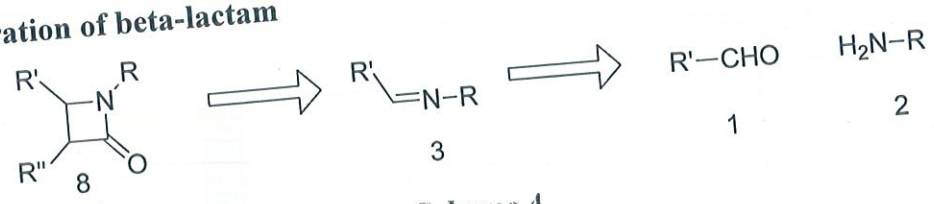
 **9b**	CRYSTALLINE	DIRTY GREY	127-130^{0}C	1.2 (15% Ethyl acetate-Hexane)	44.4
 **9c**	AMORPHOUS	LIGHT BROWN	115-120^{0}C	0.8 (5% Ethyl acetate-Hexane)	52.2
 **9d**	CRYSTALLINE	WHITE	102-105^{0}C	0.7 (5% Ethyl acetate-Hexane)	51.6

Table 4.2: Table showing characteristics of prepared thiazolidiones.

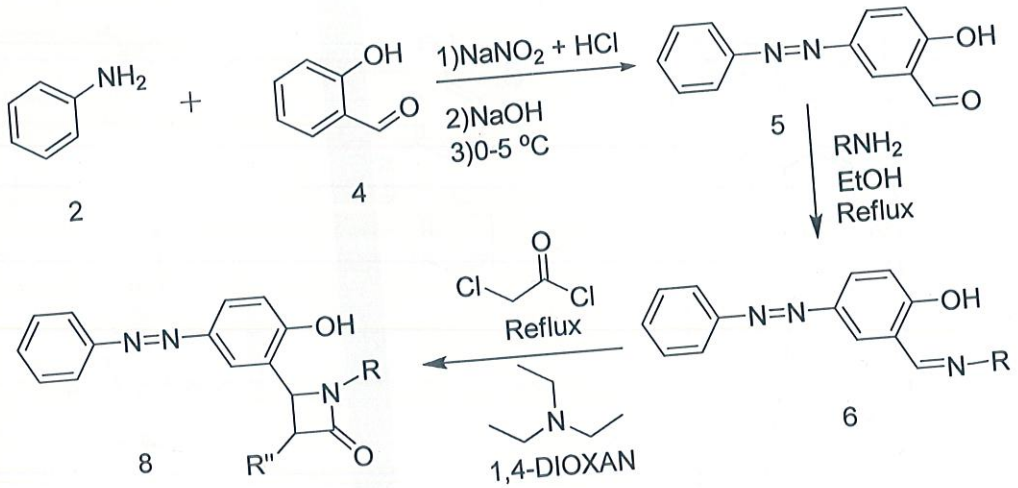
CHAPTER 5

5.1 Preparation of beta-lactam

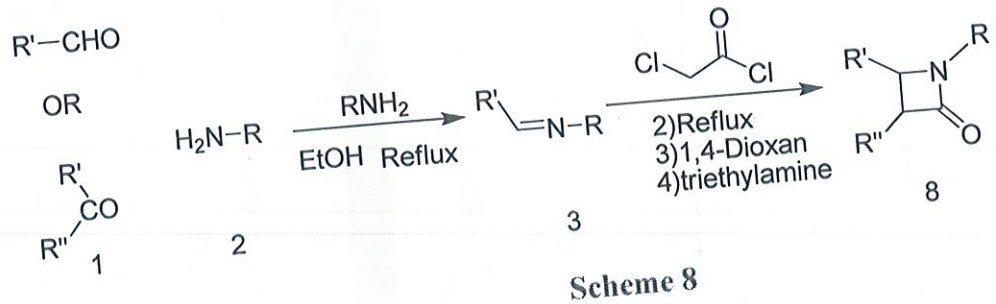


Scheme 4

5.2 Experimental



Scheme 7

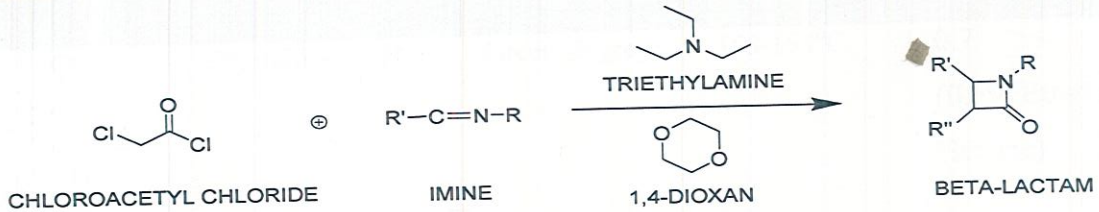


Scheme 8

General procedure for preparation of Beta-lactam

The already prepared imine (0.02mol) was taken and dissolved in 5-6ml of 1,4-dioxan. in round bottom flask. 0.02mol triethylamine was taken.into this 10ml of 1,4-dioxan and 0.02 mol chloroacetyl chloride were added with constant stirring .this mixture is heated for half an hour at 50c.then, it is cooled at room temperature for half an hour and after that heated for 8hrs at 110c.r

5.3 Result and discussion



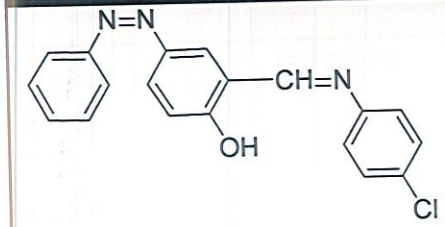
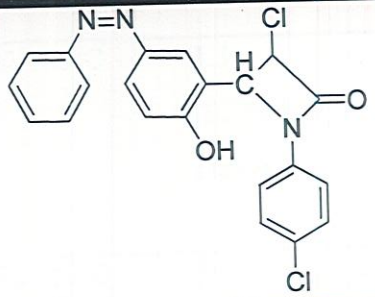
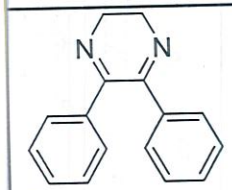
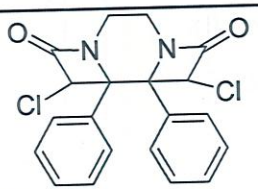
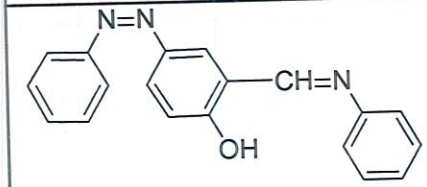
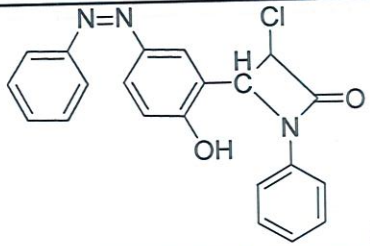
Imines	Beta-lactam
	
6d	8a
	
2a	8b
	
6d	8c

Table 5.1: Table of chemical structure of Beta-Lactam formed.

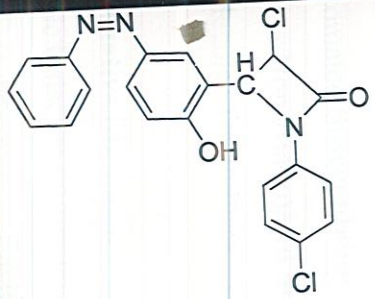
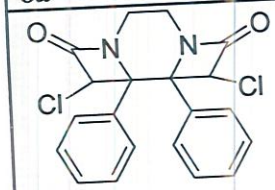
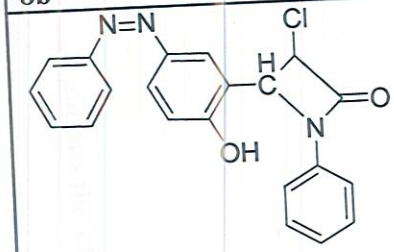
	PHYSICAL STATE	COLOR	M.P	R_f	PERCENTAGE YIELD
 **8a**	Crystalline solid	Greenish grey	155-158°C	0.7 (0.5% Ethyl acetate-Hexane)	62%
 **8b**	crystalline	Yellowish brown	182-185°C	1.1 (0.5% Ethyl acetate-Hexane)	78.6%
 **8c**	Crystalline solid	Black	178-180°C	0.4 (0.5% Ethyl acetate-Hexane)	66%

Table 5.2: Table showing characteristics of prepared Beta Lactam.

CHAPTER 6

Microbiological studies

6.1 Antibiotic Susceptibility testing

Species and strains of species of microorganisms have varying degree of susceptibility to different antibiotics. Furthermore the susceptibility of an organism to a given antibiotic may change, especially during treatment.[15]

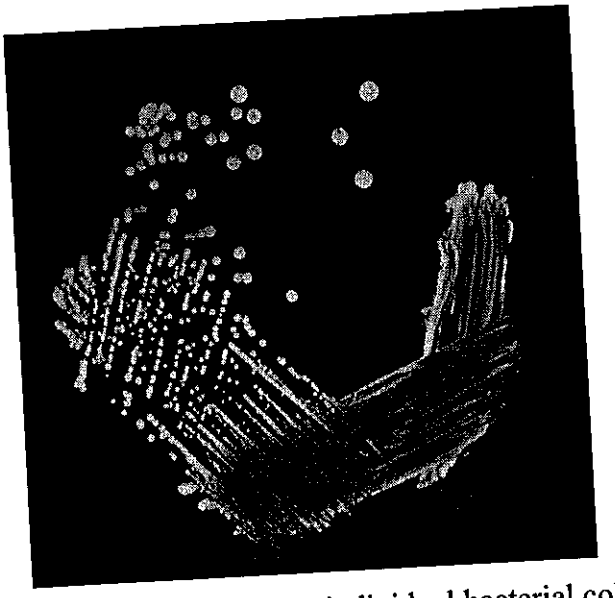


Fig 6.1: Streaking to isolate individual bacterial colony

Various methods of antibiotic susceptibility testing are:

1. Quantitative Methods
2. Qualitative Methods
3. Automated Susceptibility Tests
4. Newer Non-Automated Susceptibility Tests
5. Molecular Techniques

Quantitative Methods:

In these tests, the minimum amount of antibiotic that inhibits the visible growth of an isolate or MIC is determined. Bacterial isolate is subjected to various dilutions of antibiotics. The

highest dilution of antibiotic that has inhibited the growth of bacteria is considered as MIC. These tests can be performed on broth or agar.

1. Broth dilution methods
    a. Macrobroth dilution MIC tests
    b. Microbroth dilution MIC tests

2. Agar dilution methods

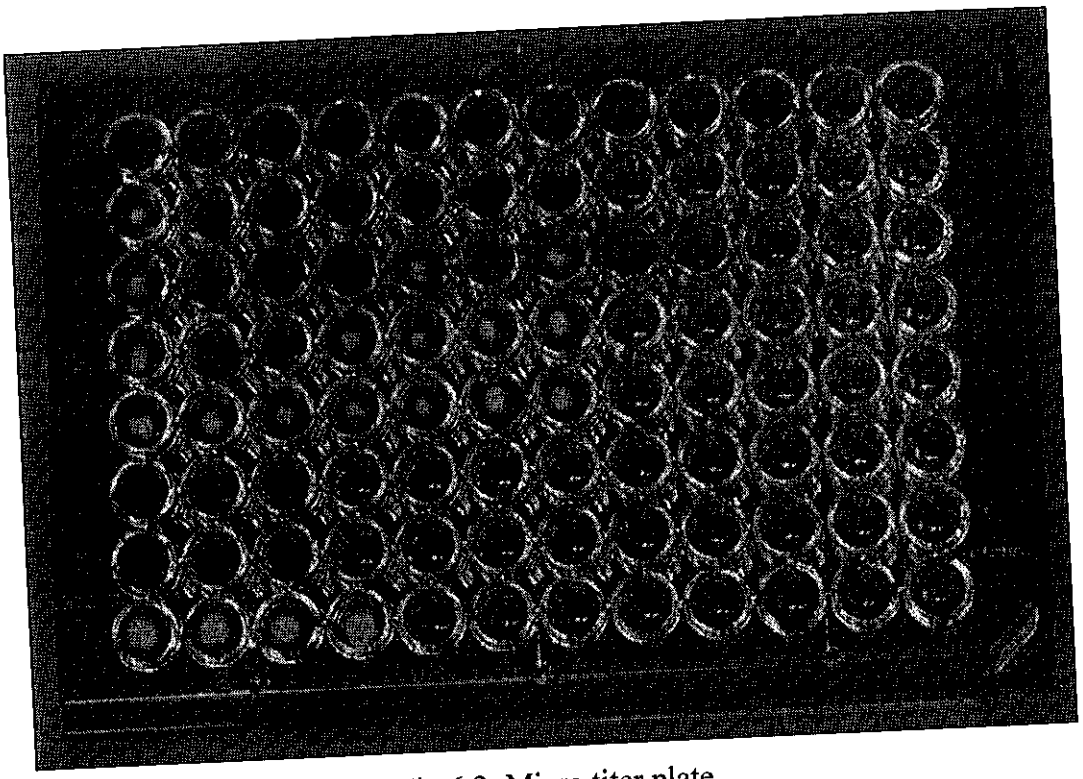


Fig 6.2: Micro titer plate

6.2 Procedure:

(i) LB agar media was prepared, autoclaved and incubated overnight at 37°C.

(ii) No growth was observed and media was poured into plates and incubated overnight at 37°C.

(iii) Streaking was done on the plates next day and incubated overnight at 37°C.

(iv) Single colony from the plate was taken on the loop and inoculated in media.

(v) Serial dilutions of the drug was prepared and pipette into wells of the microtitre plate.

(vi) The plate was scanned using ELISA reader and the data obtained were recorded and tabulated.

6.3 Result and discussion

The data obtained was not reproducible and inconclusive. Further verification of activity is needed to be done by CFU and broth dilution method and determination of MIC.

		1000 µg/µl	500 µg/µl	250 µg/µl	125 µg/µl	62.5 µg/µl	31.25 µg/µl	15.625 µg/µl	7.8125 µg/µl	3.90625 µg/µl	1.953125 µg/µl	0.9765625 µg/µl	DM(µg/µl)	M(µg/µl)	C(µg\µl)
0 hrs	**Row 1**	0.176	0.117	0.061	0.052	0.041	0.042	0.044	0.041	0.039	0.038	0.038	0.034	0.036	0.064
	Row 2	0.177	0.118	0.078	0.053	0.057	0.061	0.054	0.04	0.047	0.057	0.042	0.039	0.047	0.071
														0.045	0.042
5 hrs	**Row 1**	0.47	0.383	0.479	0.621	0.586	0.394	0.326	0.315	0.322	0.298	0.374	0.033	0.078	0.533
	Row 2	0.462	0.511	0.404	0.374	0.566	0.555	0.36	0.288	0.311	0.321	0.369	0.037	0.046	0.367
														0.046	0.325
11 hrs	**Row 1**	0.474	0.62	0.768	0.836	0.83	0.588	0.536	0.424	0.417	0.392	0.537	0.033	0.34	0.772
	Row 2	0.557	0.584	0.683	0.574	0.785	0.747	0.584	0.409	0.408	0.413	0.484	0.034	0.05	0.469
														0.046	0.417
17 hrs	**Row 1**	0.501	0.717	0.879	0.943	0.971	0.743	0.652	0.531	0.5	0.475	0.628	0.034	0.478	0.727
	Row 2	0.576	0.594	0.857	0.764	0.858	0.81	0.687	0.487	0.481	0.485	0.632	0.034	0.043	0.629
														0.062	0.529
24 hrs	**Row1**	0.437	0.601	0.962	1.013	1.013	0.854	0.965	0.707	0.663	0.59	0.727	0.032	0.666	0.99
	Row 2	0.52	0.756	0.954	0.979	0.961	0.938	0.892	0.648	0.588	0.546	0.922	0.033	0.048	0.624
														0.574	0.449

Table 6.1: Table showing data obtained for drug 8a from ELISA reader by using broth dilution method.

CONCLUSION

As discussed in strategy and work plan, imines were synthesized by condensation of equimolar amount of aldehyde and amines in ethanol. Thiazolidi-4-one derivatives were prepared by refluxing various amines with thioglycollic acid in presence of catalytic amount of zinc chloride. Reactions were monitored by thin layer chromatography. Purification was done either by recrystallization in suitable solvent or by triturating with suitable solvent. The chemical compounds were further taken for microbial testing by broth micro dilution method and data was recorded. These chemical compounds can be further modified and worked upon and can be tested for antimicrobial activity as potential antimicrobial agents.

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