"SYNTHESIS AND EVALUATION OF SUBSTITUTED NAPTHOXAZINETHIONES & NAPTHOXAZINONES DERIVATIVES"

A THESIS SUBMITTED TO

NIRMA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

BY

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UNDER THE GUIDANCE OF

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APRIL 2011

CERTIFICATE

This is to certify that ***Mr.Kamlesh Shah*** *has prepared his thesis entitled* ***"Synthesis & Evaluation of Substituted Napthoxazinethiones & Napthoxazinones derivatives ",*** *in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmaceutical chemistry, Institute of Pharmacy, Nirma University.*

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Date : 30th APRIL 2011

DECLARATION

I declare that the thesis ***"Synthesis & Evaluation of Substituted Napthoxazinethiones & Napthoxazinones derivatives "****, has been prepared by me under the guidance of Dr.Manjunath Ghate, I/c Director, and Mr. Kuntal Manna, Assistant Professor, Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.*

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ACKNOWLEDGEMENT

"Skill you need.

To reach your goal and

To keep you ever on the roll

Professionalism calls for skills

Service demands dedication

Both skills and commitment are needed

As you climb life's ladder

Don't give up mid way there still a long way

Before you reach your goal"

Man is an inexhaustible spring of potential .The endowments of intelligence and the resultant creativity, accords him the epithet "Crest jewel" of creation. Creativity proffers him the opportunity to bask in the delight of the divine effulgence within him. This further leads him to the wondrous discovery and realization that the world around him is a glorified reflection of the same effulgence.

"When god made hands, He gave us the ability to work"

Success also has different meanings for each of us.

"All successful people have one thing in common, they have faith in themselves when we believe we can succeed our entire self help us find ways of doing it."

To gratefully and appreciatively acknowledge the blessings of the Almighty whose very existence has helped me sail smoothly so far ,I bow my head before him and thank

him form the core of my heart for being there every time and anytime I needed him.

But, I was not alone in this praiseworthy journey of success: with deep gratitude and humbleness I would like to express my indebtedness and reverence. I express my profound and sincere thanks to **Dr.Manjunath Ghate** and Head, Dept. of Pharmaceutical chemistry whose able guidance and supervision led me to carryout this work successfully. Without whom this work could not be the same.

With deep expression of devotion, regards and pleasure I thank **Mr.Kuntal Manna** for his unremitting supervision, priceless guidance and unvarying encouragement and his helping hand throughout my work.

I would like to express my humble and whole hearted thanks to **Dr.Anuradha Gajjar**, **Ms. Jignasha Savjani** for her back-up, active shore up and able direction at every step.

My jovial gratitude is due to **Dr.Hardik Bhatt, Mr. Vivek Vyas** For being immeasurably considerate, non-formal and open in offering priceless and timely own advices during my dissertation work.

Thanks to **Divy, Raja, Krish, Monu, Mudra, RVD, Vinchi, Vinny , Hasmin** for giving me such a pleasant time when working together with them since I knew them and creating such a great friendship at the Lab, at the food court and many places in between.

It is pleasure to mention: **Dhruv, Ratnesh, Mrudul, Naeem, Rakesh, Kartik, Manish, Mikul, Ranger,**

Samir, Gladiator 1(Pratik), Gladiator 2(Vishal), Ujju, Op, Rakesh for sharing various thoughts.

Thank to my juniors Sunil, Bhavesh, Pavan, Umashanker, Chirag, Krishna, Yesha, Ujala, Mansi, Kinjal, Harshang, Ahmed, Paresh, Chetan, Meghavi for helping me and to all my Seniors Anuj, Vikas, Keshan, Naishad, Mitesh ,Anuj, Devang, Nikunj, Sumit, Mukesh for their guidance.

I thanks my CS friends Alex, Foster, Mark ,J.D, Bhargav, Parth, Vinit, Maulik, Dev D, Nirav, Dhama, Soni, Praveen, HL, Evo, Midi, Big B,Dead man, Jigney ,Eagle ,Animal for being with me every time I needed them.

I sincerely thanks to Dr. P. Lalitha, for library facilities during my work also to Surendrabhai & Rajubhai, who provided me books & Journals whenever needed. I also great thankful to Jignesh bhai Who Provide us Each facility with lots of support in our work, I also thanked to Satej bhai, Shreyansh bhai, Rohit bhai , Dipesh bhai, Bipin bhai.

Where would I be without my family? My parents deserve special mention for their inseparable support and prayers. Last but not the least we should not overlook, what lies behind our success, it is strength of my parents who had scarified their present to secure future for us.

My Father, Mr. Indraraj Shah in the first place is the person who put the fundament my learning character, showing me the joy of intellectual pursuit ever since I was a child. Thank you Dad for being there, for your care and the special and true understanding, we share. My Mother, Mrs. Nirmala Shah is the one who sincerely raised me with her

caring and gently love. Thank you mom for words not said when words can't mend and also the thoughtful care and love that you lend. I would like thank My Brother ***Mahesh Shah*** *for being the special brother & My Sister* ***Jayshree & Neeta*** *, you are more so endless family love, thank you for screaming for shouting to get through for all of those times when I need you. Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.*

It is my immense pleasure to wind up my thesis with these beautiful lines of Robert Frost - "Woods are lovely, dark and deep but I have promises to keep miles to go ……before I sleep"

Thank you

Kamlesh Shah

DEDICATED

TO

MY PARENTS

INDEX

LIST OF TABLES

LIST OF FIGURES

CHAPTER-1

INTRODUCTION

1.1 Origin and early evolution

Bacteria are a large domain of single-celled, prokaryote microorganisms typically a few micrometres in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals. [1] Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste,[2] water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water; in all, there are approximately five nonillion (5×10^{30}) bacteria on Earth,[3] forming a biomass on Earth, which exceeds that of all plants and animals.[4] Bacteria are vital in recycling nutrients, with many steps in nutrient cycles depending on these organisms, such as the fixation of nitrogen from the atmosphere and putrefaction. However, most bacteria have not been characterised, and only about half of the phyla of bacteria have species that can be grown in the laboratory.[5] The study of bacteria is known as bacteriology, a branch of microbiology.

There are approximately ten times as many bacterial cells in the human flora as there are human cells in the body, with large numbers of bacteria on the skin and as gut flora.[6] The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and a few are beneficial.. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people a year, mostly in sub-Saharan Africa.[7] In developed countries, antibiotics are used to treat bacterial infections and in agriculture, so antibiotic resistance is becoming common. In industry, bacteria are important insewage treatment, the production of cheese and yogurt through fermentation, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.[8]

Once regarded as plants constituting the Class Schizomycetes, bacteria are now classified as prokaryotes. Unlike cells of animals and other eukaryotes, bacterial cells do not contain a nucleus and rarely harbour membrane-bound organelles. Although the term *bacteria*traditionally included all prokaryotes, the scientific classification changed after the discovery in the 1990s that prokaryotes consist of two

very different groups of organisms that evolved independently from an ancient common ancestor. These evolutionary domains are called Bacteria and Archaea.[9]

The ancestors of modern bacteria were single-celled microorganisms that were the first forms of life to appear on Earth, about 4 billion years ago. For about 3 billion years, all organisms were microscopic, and bacteria and archaea were the dominant forms of life.[10][11] Although bacterial fossils exist, such as stromatolites, their lack of distinctive morphology prevents them from being used to examine the history of bacterial evolution, or to date the time of origin of a particular bacterial species. However, gene sequences can be used to reconstruct the bacterial phylogeny, and these studies indicate that bacteria diverged first from the archaeal/eukaryotic lineage.[12]

Bacteria were also involved in the second great evolutionary divergence, that of the archaea and eukaryotes. Here, eukaryotes resulted from ancient bacteria entering into endosymbioticassociations with the ancestors of eukaryotic cells, which were themselves possibly related to the Archaea.[13][14] This involved the engulfment by proto-eukaryotic cells of alpha-proteobacterial symbionts to form either mitochondria or hydrogenosomes, which are still found in all known Eukarya (sometimes in highly reduced form, e.g. in ancient "amitochondrial" protozoa). Later on, some eukaryotes that already contained mitochondria also engulfed cyanobacterial-like organisms. This led to the formation of chloroplasts in algae and plants. There are also some algae that originated from even later endosymbiotic events. Here, eukaryotes engulfed a eukaryotic algae that developed into a "second-generation" plastid.[15][16] This is known as secondary endosymbiosis.

1.2 Bacterial cellular morphologies

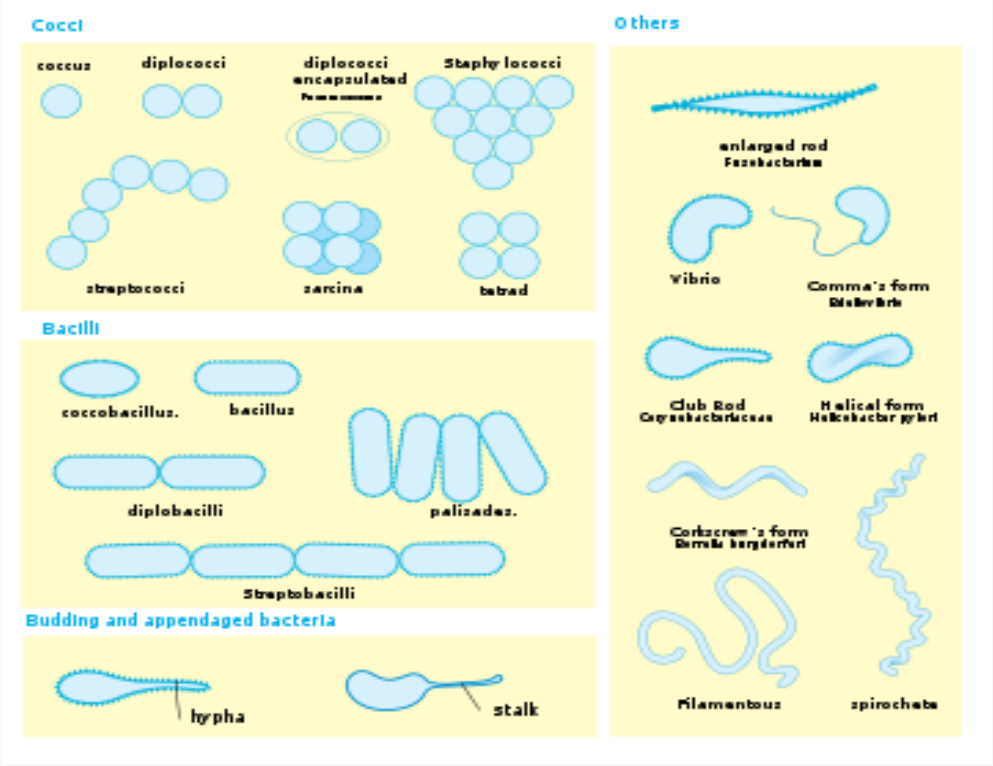


Figure 1.1 Bacteria display many cell morphologies and arrangements

Bacteria display a wide diversity of shapes and sizes, called *morphologies*. Bacterial cells are about one tenth the size of eukaryotic cells and are typically 0.5–5.0 micrometres in length. However, a few species–for example *Thiomargarita namibiensis* and *Epulopiscium fishelsoni*–are up to half a millimetre long and are visible to the unaided eye.[33] Among the smallest bacteria are members of the genus *Mycoplasma*, which measure only 0.3 micrometres, as small as the largest viruses.[17] Some bacteria may be even smaller, but these ultramicrobacteria are not well-studied.[18]

Most bacterial species are either spherical, called cocci (*sing.* coccus, from Greek *κόκκος-kókkos*, grain, seed) or rod-shaped, called bacilli (*sing.* bacillus, from Latin *baculus*, stick). Elongation is associated with swimming.[19] Some rod-shaped bacteria, called vibrio, are slightly curved or comma-shaped; others, can be spiral-shaped, called spirilla, or tightly coiled, called spirochaetes. A small number of species even have tetrahedral or cuboidal shapes.[20] More recently, bacteria were discovered deep under the Earth's crust that grow as long rods with a star-shaped cross-section.

The large surface area to volume ratio of this morphology may give these bacteria an advantage in nutrient-poor environments.[21] This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton, and is important because it can influence the ability of bacteria to acquire nutrients, attach to surfaces, swim through liquids and escape predators.[22][23]

Many bacterial species exist simply as single cells, others associate in characteristic patterns.Bacteria can also be elongated to form filaments, for example the Actinobacteria. Filamentous bacteria are often surrounded by a sheath that contains many individual cells. Certain types, such as species of the genus *Nocardia*, even form complex, branched filaments, similar in appearance to fungal mycelia.[24]

Bacteria often attach to surfaces and form dense aggregations called biofilms or bacterial mats. These films can range from a few micrometers in thickness to up to half a meter in depth, and may contain multiple species of bacteria, protists and archaea. Bacteria living in biofilms display a complex arrangement of cells and extracellular components, forming secondary structures such as microcolonies, through which there are networks of channels to enable better diffusion of nutrients.[25][26]In natural environments, such as soil or the surfaces of plants, the majority of bacteria are bound to surfaces in biofilms.[27] Biofilms are also important in medicine, as these structures are often present during chronic bacterial infections or in infections of implanted medical devices, and bacteria protected within biofilms are much harder to kill than individual isolated bacteria.[28]

Even more complex morphological changes are sometimes possible. For example, when starved of amino acids, Myxobacteria detect surrounding cells in a process known as quorum sensing, migrate towards each other, and aggregate to form fruiting bodies up to 500 micrometres long and containing approximately 100,000 bacterial cells.[29] In these fruiting bodies, the bacteria perform separate tasks; this type of cooperation is a simple type of multicellular organisation. For example, about one in 10 cells migrate to the top of these fruiting bodies and differentiate into a specialised dormant state called myxospores, which are more resistant to drying and other adverse environmental conditions than are ordinary cells.[30]

1.3 Classification on the Basis of Shapes

Bacteria are usually classified on the basis of their shapes. Broadly, they can be divided into:

- Rod-shaped bacteria (Bacilli)
- Sphere-shaped bacteria (Cocci)
- Spiral-shaped bacteria (Spirilla)

Classification on the Basis of Gram Strain

This classification is based on the results of Gram Staining Method, in which an agent is used to bind to the cell wall of the bacteria.

- Gram-positive
- Gram-negative

Classification on the Basis of Oxygen Requirement

This classification is based on the requirement of oxygen for the survival of the bacterium.

- Aerobic (Need Oxygen)
- Anaerobic (Do not need Oxygen)

Classification on the Basis of Growth and Reproduction

This classification is based on the growth and reproduction aspects of bacteria.

- Autotrophic Bacteria (Obtain carob and/or sugar from sunlight or chemical reactions)
- Heterotrophic Bacteria (Obtain carob and/or sugar from the environment)[31]

1.4 Classification of Antimicrobial Agents

1.4.1 Antibiotics that inhibit bacterial cell wall synthesis – This is the most common mechanism of antibiotic activity.

1. Beta-Lactam Antibiotics

a. Most cell wall active antibiotics are classified as beta-lactam antibiotics, because they share a common beta-lactam ring structure, a chain of 10-65 disaccharide residues consisting of alternating molecules of N acetylglucosamine and N-acetylmuramic acid. Chains then cross-linked with peptide bridges that create a rigid mesh for bacteria.

b. Specific enzymes (transpeptidases, carboxypeptidases, endopeptidases) catalyze building of chains and cross-links. The enzymes are called penicillin-binding proteins (PBPs); they bind beta-lactam antibiotics. Binding of antibiotic bind to the PBPs in growing bacterial cell wall inhibits synthesis of peptidoglycan, causing bacterial cell death. Beta-lactam antibiotics are bactericidal agents.

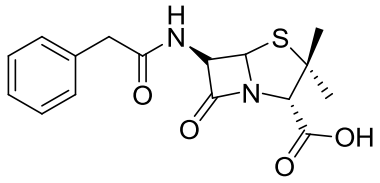
c. Penicillins

1. Effective antibiotics, low toxicity to humans.

2. Structure: Organic acid with beta-lactam ring.

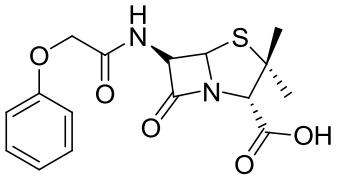
3. Obtained from cultures of mold, Penicillium chrysogenum, which produces 6-aminopenicillanic acid. Biochemical modification of this intermediate yields derivatives with decreased acid lability and increased absorption in G. I. tract, resistance to destruction by penicillinase, or a broad spectrum of activity that includes gram–negative bacteria.

4. **Penicillin G** is incompletely absorbed because it is inactivated by gastric acid. Used as an intravenous drug to treat infections caused by a limited number of susceptible organisms.



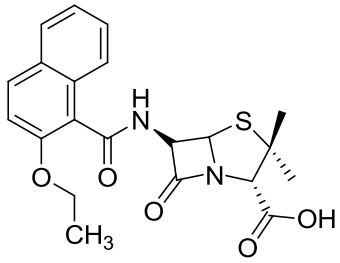
Penicillin G

5. **Penicillin V** is more resistant to acid and is the preferred oral form to treat susceptible bacteria.



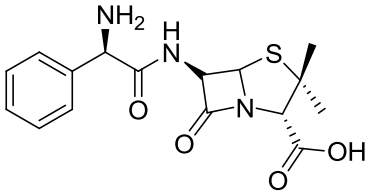
Penicillin V

6. **Nafcillin** and **Oxacillin**, are penicillinase-resistant penicillins, used to treat infections caused by susceptible staphylococci.



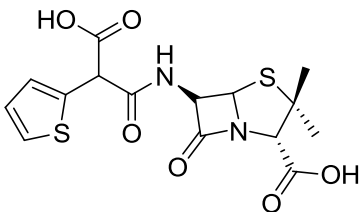
Nafcillin

7. **Ampicillin** was first extended-spectrum penicillin. Its activity was limited to Escherichia and Proteus species.

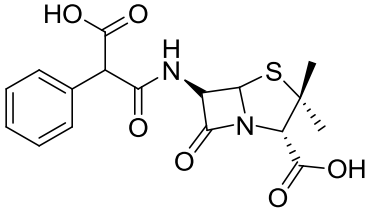


Ampicillin

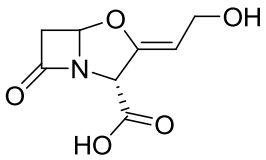
8. Other extended-spectrum penicillins (carbenicillin, ticarcillin, piperacillin) effective against a broader range of gram-negative bacteria, including Klebsiella, Enterobacter, Pseudomonas.



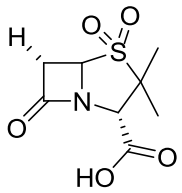
Carbenicillin



Ticarcillin



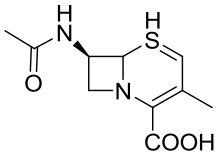
Clavulanic Acid



Sulbactam

d. Cephalasporins and **Cephamycins**

1. **Cephalosporins** are beta-lactam antibiotics derived from 7-aminocephalosporanic acid, isolated from the mold Cephalosporium.



Cephalosporin

2. **Cephamycins** are closely related to cephalosporins. They contain oxygen in place of sulfur in the dihydrothiazine ring, rendering them more stable to betalactamase hydrolysis.

3. **Cephalosporins/cephamycins** have same mechanism of action as penicillins but a wider anti- bacterial spectrum, resistance to beta-lactamases, improved pharmacokinetic properties.

4. Biochemical modifications in the basic antibiotic molecule resulted in antibiotics with improved activity and pharmacokinetic properties

a. Activity of narrow-spectrum first generation antibiotics is restricted to E. coli,

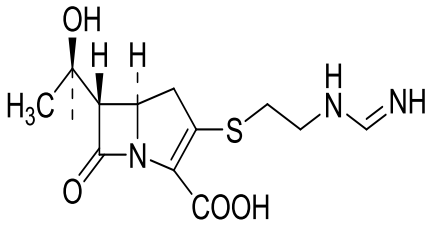
Klebsiella, Proteus mirabilis, and oxacillin-susceptible gram-positive cocci.

b. Expanded-spectrum, second-generation antibiotics are also active against Haemophilus influenzae, Enterobacter, Citrobacter, Serratia, Bacteriodes fragilis.

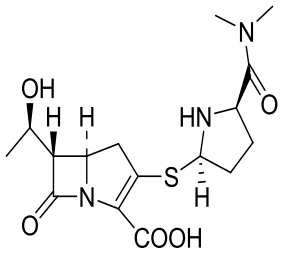
c. Broad-spectrum third-generation antibiotics and expanded spectrum, fourth-generation antibiotics are active against most Enterobacteriaceae and Pseudomonas aeruginosa. Expanded-spectrum antibiotics are more stable against beta-lactamases.

d. Other Beta-Lactam Antibiotics

1. **Carbapenems (Imipenem, meropenem)** – broadspectrum antibiotics active against virtually all groups of organisms with few exceptions.

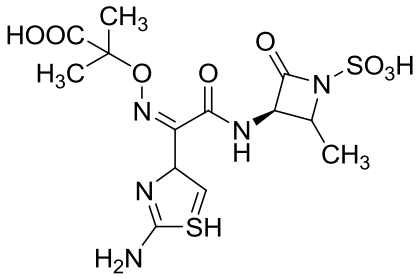


Imipenem



Meropenem

2. **Monobactams (Aztreonam)** – narrow-spectrum antibiotics active only against aerobic, gramnegative bacteria.



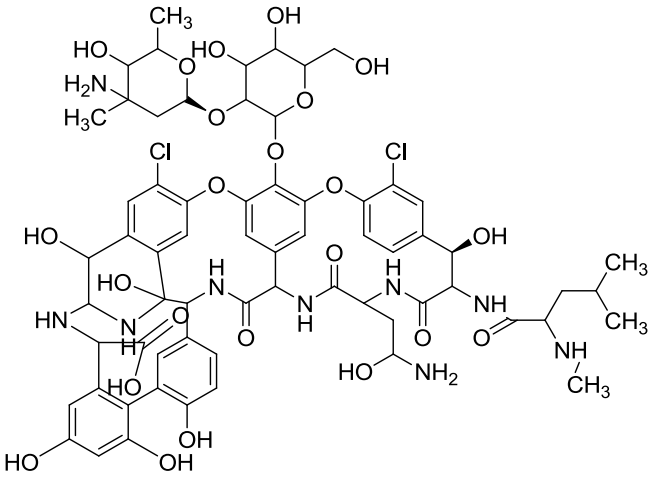
Aztreonam

2. **Glycopeptides - Vancomycin**

a. Vancomycin obtained from Streptomyces orientalis, is a complex glycopeptide that disrupts cell wall pe- peptidoglycan synthesis in growing gram(+) bacteria

b. Vancomycin interacts with D-alanine-D-alanine ter- mini of pentapeptide side chains, which interferes with formation of bridges between peptidoglycan chains.

c. Vancomycin is used to manage infections caused by oxacillin-resistant staphylococci and other gram-positive bacteria resistant to beta-lactam antibiotics.



Vancomycin

3. **Polypeptides**

a. Bacitracin isolated from Bacillus licheniformis, is a mixture of polypeptides used in topically to treat skin infections caused by gram(+) bacteria(staphy- lococcus/group A streptococcus).

1. Inhibits cell wall synthesis; interferes with dephosphorylation and recycling of lipid carrier responsible for moving peptidoglycan precursors through cytoplasmic membrane to cell wall.

b. Polymyxins

1. Cyclic polypeptides from Bacillus pollymyxa.

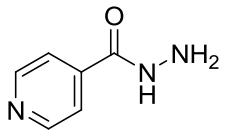
2. Insert into bacterial membranes by interacting with lipolysaccharides and the phospholilpids in outer membrane, displacing Mg(+2) and Ca(+2), disorganizing membrane structure, producing increased cell permeability, cell death.

3. Polymyxin B and E (colistin) can cause serious nephrotoxicity. Their use is limited to the external treatment of localized infections such as external otitis, eye infections, and skin infections. Most active against gram-negative bacilli.

c. Isoniazid, Ethionamide, Ethambutol, Cycloserine

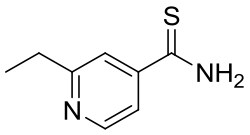
1. Cell wall-active antibiotics used to treat myco- bacterial infections.

2. **Isoniazid** is bactericidal against actively replicating mycobacteria; disrupts synthesis of mycolic acid used to build cell walls.



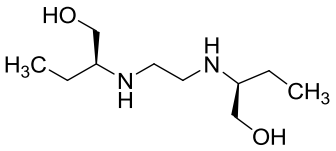
Isoniazid

3. **Ethionamide** also blocks mycolic acid synthesis.



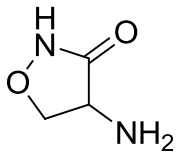
Ethionamide

4. **Ethambutol** interfes with synthesis of arabinogalactan in the cell wall; antimycobacterial.



Ethambutol

5. **Cycloserine** inhibits D-alanine-D-alanine synthetase and alanine racemase, which catalyze cell wall synthesis.



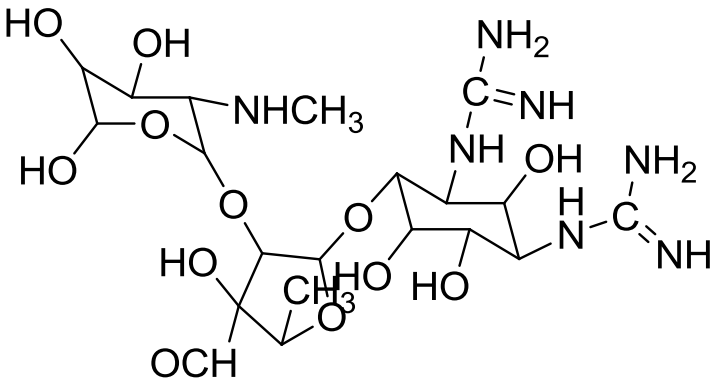
Cycloserine

1.4.2 Antibiotics That Bind to Bacterial Ribosomes and Inhibit Protein Synthesis - This is the second largest class of antibiotics. They act by inhibiting protein synthesis. Members of this class include:

1. **Aminoglycosides**

a. Consist of amino sugars linked through glycosidic bonds to an aminocyclitol ring.

b. Examples are **streptomycin, neomycin, kanamycin,** and **tobramycin.** Originally isolated from sreptomyces species. Gentamicin and sisomicin were isolated from Micromonospora species.



Streptomycin

c. Amkiacin and **Netilmicin** are synthetic derivatives of **Kanamycin** and **Sisomicin,** respectively.

d. These antibiotics pass through the bacterial outer membrane (in gram-negative bacteria), cell wall, and cytoplasmic membrane to the cytoplasm, where they inhibit bacterial protein synthesis by irreversibly binding to 30S ribosomal proteins.

1. Causes production of aberrant proteins as the result of misreading of the mRNA

2. Also interrupts protein synthesis by causing premature release of ribosome from mRNA.

e. Aminoglycosides are bactericidal because they bind irreversibly to ribosomes and are commonly used to treat serious infections caused by many gram (-) bacilli, some gram (+)-positive organisms.

f. Anaerobes, streptococci, and enterocci are resistant to aminoglycosides. To treat these organisms, need co-administration of an aminoglycoside with an inhibitor of cell wall synthesis(e. g., penicillin ampicillin, vancomyhcin).

g. Most commonly used antibiotics in this class are **Genatmicin** and **Tobramycin,** which both have broad spectrum of activity. **Netilmicin** is less ototoxic, but has less antibacterial activity. All three Aminoglycosides are used to treat systemic infections caused by susceptible gram (-) bacteria, such as Enterobac- teriaceae and Pseudomonas species.

h. Amikacin is used to treat infections caused by gram (-) bacteria that are resistant to other aminoglycosides. Streptomycin used for treatment of tuberculosis, tularemia, and streptococcal or enterococcal infections (in combination with a penicillin).

i. Resistance to the antibacterial action of aminoglycosides can develop by

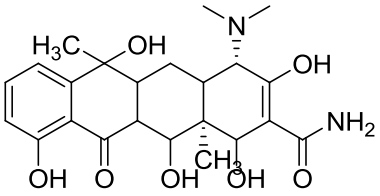
1. Mutation of the ribosomal binding site (uncommon, except in genus Enterococcus).
2. Decreased uptake of antibiotic into bacterial cells (Ex: Pseudomonas and anaerobic bacteria).

3. Enzymatic modification of antibiotic, by phosphorylation, adenylation, and acetylation of its amino and hydroxyl groups.

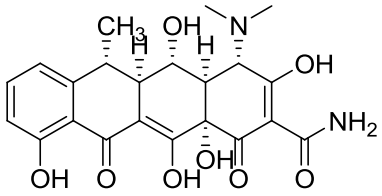
2. **Tetracyclines**

a. Broad-spectrum bacteriostiatic antibiotics, inhibit protein synthesis in bacteria by binding reversibly to 30S ribosomal subunits, blocking binding of amino- acyl-transfer RNA (tRNA) to 30S ribosome-mRNA.

b. Tetracycline, Doxycycline, and **Minocycline** are effective in treatment of infections caused by Chlamydia, Mycoplasm, and other selected gram-positive and gram-negative bacteria.



Tetracycline



Doxycycline

c. All **Tetracyclines** have similar spectrum of activity; difference among them is pharmacokinetic properties.

d. Resistance to **Tetracyclines** occurs from decreased penetration of antibiotic into bacterial cells, active efflux of antibiotic out of cell, alteration of ribosomal target site, or enzymatic modification of antibiotic

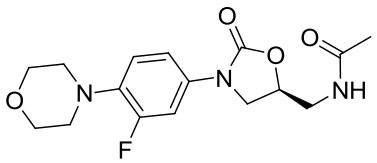
e. Mutations in chromosomal gene encoding the outer membrane porin protein, OmpF, can lead to low-level resistance to tetracyclines, and other antibiotics (beta-lactams, quinolones, chloramphenicol).

3. **Oxazolidnones**

a. Narrow-spectrum class of antibiotics that block initiation of protein synthesis by interfering with formation of initiation complex at 30S ribosomal subunit.

b. Because of specificity of this mechanism, cross resistance with other protein inhibitors does not occur.

c. Representative of this class is linezolid, which is toxic to all staphylococci, streptococci, and enterococci (including strains resistant to penicillins, vancomycin, and the aminoglycosides); particularly reserved to treat multidrug-resistant enterococci.



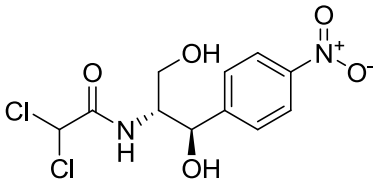
Linezolid

4. **Chloramphenicol**

a. Broad antibacterial spectrum similar to that of tetracycline; drug of choice for treating typhoid fever.

b. Interferes with bacterial protein synthesis, also disrupts protein synthesis in human bone marrow cells and can produce asplastic anemia.

c. Exerts bacteriostatic effects by binding reversibly to the peptidyl transferase component of the 50S ribosomal subunit, blocking peptide elongation.

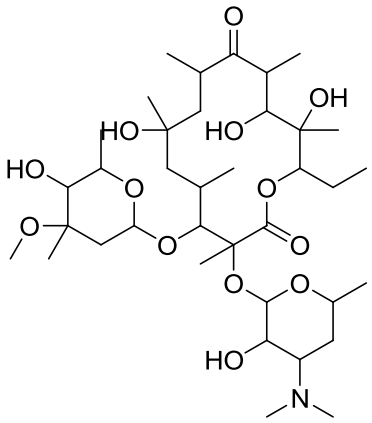


Chloramphenicol

5. **Macrolides**

a. Macrocyclic lactone ring bound to two sugars, desosamine and cladinose.

b. Erythromycin, derived from strep. erythreus, is the model macrolide antibiotic.



Erythromycin

c. Modification of macrolide structure led to development of newer agents (azithromycin and clarithromycin), which are used to treat mycobacterial infections such as myco-bacterium avium complex.

d. Macrolides: broad–spectrum bacteriostatic antibiotics used to treat pulmonary infections caused by Mycopasma, Legionella, Chlamydia species, infections caused by Campylobacter species and grampositive bacteria in patients allergic to penicillin.

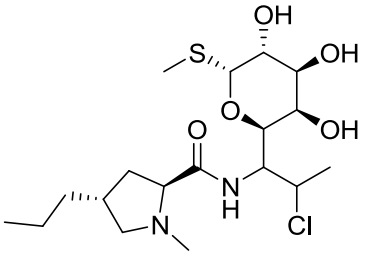
e. Exert their effects by reversible binding to the 50S ribosome, which blocks polypeptide elongation.

f. Resistance to macrolides comes from methylation of 23S ribosomal RNA, which prevents binding by the antibiotic. Destruction of lactone ring by erythromycin esterase or active efflux of the antibiotic from bacterial cell also causes resistance.

6. **Clindamycin**

a. In family of Lincosamide antibiotics; derivatives of Lincomycin, isolated from Strep. lincolnensis.

b. Blocks protein elongation by binding to 50S ribosome. Inihibits peptidyl transferase by interfering with binding of amino acid-acyl-tRNA complex.



Clindamycin

7. **Streptogramins**

a. Cyclic peptides produced by Streptomyces species.

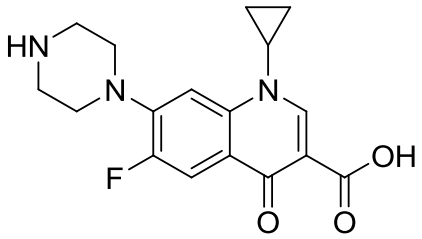
b. Ex: Quinupristin-Dalfopristin.Dalfopristin binds to the 50S ribosomal subunit and induces a conformation change that facilities binding of Quinupristin. Dalfopristin prevents peptide chain elongation, and quinupristin initiates premature release of peptide chains from the ribosome. This drug combination is active against staphylococci, streptococci, and E. faecium. This antibiotic combination is restricted to treating vancomycin-resistant E. faecium.

1.4.3 Antibiotics that Inhibit bacterial DNA synthesis or RNA synthesis.

1. **Quinolones**

a. Synthetic chemotherapeutic agents that inhibit bacterial DNA gyrases or topoisomerase, which are required for DNA replication, recombination, and repair. Bind to a complex of DNA and DNA gyrase. 11DNA gyrase consists of two apha and two beta subunits. Quinolones bind to alpha subunits.

b. Nalidixic acid used to treat urinary tract infections caused by a variety of gram-negative bacteria; resistance developed rapidly, causing it to fall out of use. Replaced by newer, more active quinones, such as ciprofloxacin, levofloxacin,and gatofoxacin, made by modifying two ring quinolone nucleus. These also bind to DNA gyrase.



Ciprofloxacin

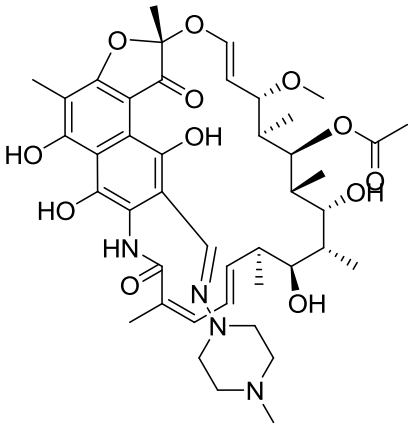
c. Newer members have strong activity against grampositive and gramnegative bacteria. Resistance develops rapidly in Pseudomonas, oxacillin-resistant staphylocci, and enterococci.

d. Alteration of alpha subunit of DNA gyrase is principal mechanism of bacterial resistance; decreased drug uptake has also been observed stemming from changes in porin proteins in bacterial surface. Both resistance mechanisms are chromosomally mediated

2. **Rifampin and Rifabutin**

a. Rifampin derivative of **Rifamycin B** binds to DNA-dependent RNA polymerase, inhibits initiation of RNA synthesis.

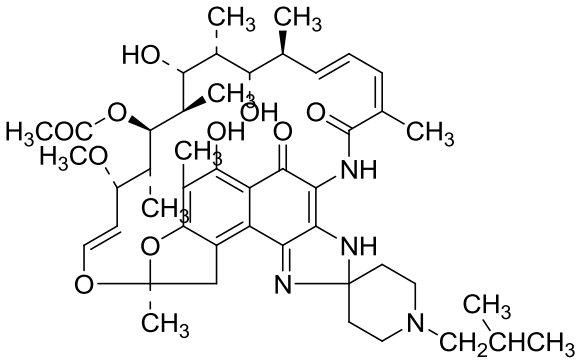
b. **Rifampin** is bactericidal for Mycobacterium tuberculosis and active against aerobic gram-positive cocci, including staphylococci and streptococci.



Rifampin

c. Resistance can develop rapidly, so rifampin is usually combined with one or more other effective antibiotics. Rifampin resistance in gram-positive bacteria results from mutation in the chromosomal gene that encodes the beta subunit of RNA polymerase. Gram-negative bacteria are intrinsically resistant to rifampin because of decreased uptake.

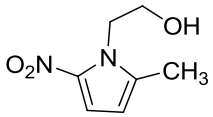
d. Rifabutin, a **Rifamycin** derivative, has a similar mode and spectrum of activity, is active against M. avium.



Rifabutin

3. **Metronidazole**

a. Originally introduced as oral agent for treating Trichomomas vaginitis. Also effective in treating amebiasis, giardiasis, and serious anaerobic bacterial infections, including those caused by B. fragilis.



Metronidazole

b. Antimicrobial properties due to reduction of nitro group by bacterial nitroreductase, producing cytotoxic compounds that cause DNA strand breaks.

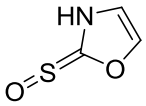
c. Resistance results from decreased uptake of anti-biotic or elimination of cytotoxic compounds before they interact with host DNA.

4. **Antimetabolites**

1. **Sulfonamides** are anti-metabolites that compete with p-aminobenzoic acid, preventing synthesis of folic acid required by certain micro-organisms. Mammalian organisms do not synthesize folic acid(required as a vitamin), so sulfonamides do not interfere with mammalian cell metabolism.

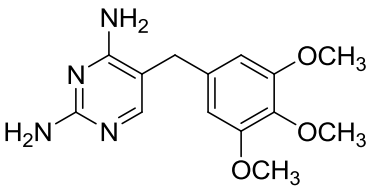
a. Sulfonamides effective against a broad range of gram positive and gram negative organisms(Nocardia/Chlamydia)

b. Short-acting **Sulfonamides(Sulfisoxazole)** used to treat acute urinary tract infections (E. coli).



Sulfisoxazole

2. **Trimethoprim** interferes with folic acid metabolism by inhi- biting bacterial dihydrofolate reductase; prevents conversion of dihydrofolate to tetrahydrofolate, which blocks formation of thymidine, some purines, methionine, and glycine.



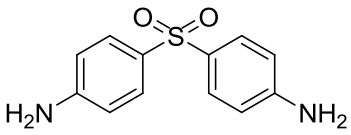
Trimethorprim

3. **Trimethoprim + Sulfamethoxazole** produces synergistic combination active at two steps in folic acid synthesis.

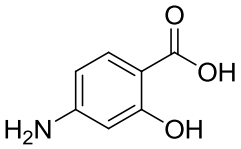
a. Trimethoprim-Sulfamethoxazole is effective against large variety of gram positive and gram-negative microorganisms and drug of choice for treating acute and chronic urinary tract infections.

b. This drug combination effective to treat infections caused by Pneumocystis carinii, bacterial infections of lower respiratory tract, otitis media, gonorrhea.

4. **Dapsone** and **P–Aminosalicylic Acid** are antifolates used to treat mycobacterial infections.



Dapsone



PAS

5. Resistance to these antibiotics can come from:

a. Permeability barriers, in Pseudomonas

b. Decreased affinity of DHFR for trimethoprim.

c. Utilization of exogenous thymidine in enterococci, makes enterocci intrinsically resistant.[32],[33],[34],[35],[36]

1.5 Mechanism of action, Spectrum of Activity,Resistance

1.5.1 Antimicrobials That Bind To The 30s Ribosomal Subunit

1.Aminoglycosides (bactericidal)

Streptomycin, Kanamycin, Gentamicin, Tobramycin, Amikacin, Netilmicin And Neomycin (Topical)

a. Mode of action :- The aminoglycosides irreversibly bind to the 30S ribosome and freeze the 30S initiation complex (30S-mRNA-tRNA), so that no further initiation can occur. The aminoglycosides also slow down protein synthesis that has already initiated and induce misreading of the mRNA.

b. Spectrum of Activity :- Aminoglycosides are active against many gram-negative and some gram-positive bacteria. They are not useful for anaerobic bacteria, since oxygen is required for uptake of the antibiotic, or for intracellular bacteria.

c. Resistance :- Resistance to these antibiotics is common

d. Synergy :- The aminoglycosides synergize with β-lactam antibiotics such as the penicillins. The β-lactams inhibit cell wall synthesis and thereby increase the permeability of the bacterium to the aminoglycosides.

2.Tetracyclines (bacteriostatic)
Tetracycline, minocycline and doxycycline

a. Mode of action:-The tetracyclines reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.

b. Spectrum of activity:- These are broad spectrum antibiotics and are useful against intracellular bacteria

c. Resistance:- Resistance to these antibiotics is common

d. Adverse effects :-Destruction of normal intestinal flora often occurs, resulting in increased secondary infections. There can also be staining and impairment of the structure of bone and teeth

3. Spectinomycin (bacteriostatic)

a. Mode of action :- **Spectinomycin** reversibly interferes with mRNA interaction with the 30S ribosome. It is structurally similar to aminoglycosides but does not cause misreading of mRNA

b. Spectrum of activity:- **Spectinomycin** is used in the treatment of penicillin-resistant *Neisseria gonorrhoeae*

c. Resistance :-This is rare in *Neisseria gonorrhoeae*

1.5.2 Antimicrobials That Bind To The 50s Ribosomal Subunit

1. Chloramphenicol, lincomycin, clindamycin (bacteriostatic)

a. Mode of action :-These antimicrobials bind to the 50S ribosome and inhibit peptidyl transferase activity.

b. Spectrum of activity

- **Chloramphenicol** - Broad range
- **Lincomycin** and **Clindamycin** - Restricted range

c. Resistance :-Resistance to these antibiotics is common

d. Adverse effects :-Chloramphenicol is toxic (bone marrow suppression) but it is used in the treatment of bacterial meningitis.

2. Macrolides (bacteriostatic) - **Erythromycin** (also azithromycin, clarithromycin)

a. Mode of action :- The macrolides inhibit translocation of the peptidyl tRNA from the A to the P site on the ribosome by binding to the 50S ribosomal 23S RNA.

b. Spectrum of activity :- Gram positive bacteria, *Mycoplasma*, *Legionella*

c. Resistance:- Resistance to these antibiotics is common.

1.5.3 Antimicrobials That Interfere With Elongation Factors

1. Fusidic acid (bacteriostatic)

a.Mode of action :- Fusidic acid binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

1.5.4 Inhibitors Of Nucleic Acid Synthesis And Function

The selectivity of these agents is a result of differences in prokaryotic and eukaryotic enzymes affected by the antimicrobial agent.

1.5.4.1 Inhibitors Of RNA Synthesis And Function

1. Rifampin, Rifamycin, Rifampicin (bactericidal)

a. Mode of action :- These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of RNA synthesis.

b. Spectrum of activity :- They are wide spectrum antibiotics but are used most commonly in the treatment of tuberculosis

c. Resistance:- Resistance to these antibiotic is common.

d. Combination therapy Since resistance is common, rifampin is usually used in combination therapy

1.5.4.2 Inhibitors Of DNA Synthesis And Function

1. Quinolones - Nalidixic Acid, Ciprofloxacin, Oxolinic Acid (bactericidal)

a. Mode of action :- These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

b. Spectrum of activity:- These antibiotics are active against Gram-positive cocci and are used in urinary tract infections

c. Resistance :- This is common for nalidixic acid and is developing for ciprofloxacin

1.5.5 Antimetabolite Antimicrobials

1.5.5.1 Inhibitors Of Folic acid Synthesis

The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesize their own folic acid. In contrast, mammalian cells use folic acid obtained from food.

1. Sulfonamides, sulfones (bacteriostatic)

a. Mode of action :- These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteric acid.

b. Spectrum of activity :- They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance:- Resistance to these antibiotics is common

d. Combination therapy :-The sulfonamides are used in combination with trimethoprim. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

2. Trimethoprim, Methotrexate, Pyrimethamine (bacteriostatic)

a. Mode of action :-These antimicrobials bind to **Dihydrofolate reductase** and inhibit formation of **Tetrahydrofolic acid**.

b. Spectrum of activity :-They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance:- Resistance to these antibiotics is common

d. Combination therapy :- These antimicrobials are used in combination with the Sulfonamides. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

1.5.6 Anti-Mycobacterial Agents

Anti-mycobacterial agents are generally used in combination with other antimicrobials since treatment is prolonged and resistance develops readily to individual agents.

1. Para-aminosalicylic acid (PAS) (bacteriostatic)

a. Mode of action:- This is similar to sulfonamides

b. Spectrum of activity :- PSA is specific for *Mycobacterium tuberculosis*

2. Dapsone (bacteriostatic)

a. Mode of action :- Similar to sulfonamides

b. Spectrum of activity :- Dapsone is used in treatment of leprosy

3. Isoniazid (INH) (bacteriostatic)

a. Mode of action :- **Isoniazid** inhibit synthesis of mycolic acids.

b. Spectrum of activity :- **INH** is used in treatment of tuberculosis

c. Resistance:- Resistance has developed

1.6 Antimicrobial Drug Resistance

1.6.1 Principles And Definitions

1. Clinical Resistance

Clinical resistance to an antimicrobial agent occurs when the MIC of the drug for a particular strain of bacteria exceeds that which is capable of being achieved with safety *in vivo*. Resistance to an antimicrobial can arise:

- By mutation in the gene that determines sensitivity/resistance to the agent
- By acquisition of extrachromosomal DNA (plasmid) carrying a resistance gene.

Resistance that appears after introduction of an antimicrobial agent into the environment usually results from a selective process, *i.e.* the antibiotic selects for survival of those strains possessing a resistance gene. Resistance can develop in a single step or it can result from the accumulation of multiple mutations.

2. Cross Resistance

Cross resistance implies that a single mechanism confers resistance to multiple antimicrobial agents while multiple resistance implies that multiple mechanisms are involved. Cross resistance is commonly seen with closely related antimicrobial agents while multiple resistance is seen with unrelated antimicrobial agents.[38]

1.6.2 Mechanisms Of Resistance

1. Altered permeability of the antimicrobial agent

Altered permeability may be due to the inability of the antimicrobial agent to enter the bacterial cell or alternatively to the active export of the agent from the cell.

2. Inactivation of the antimicrobial agent

Resistance is often the result of the production of an enzyme that is capable of inactivating the antimicrobial agent.

3. Altered target site

Resistance can arise due to alteration of the target site for the antimicrobial agent.

4. Replacement of a sensitive pathway

Resistance can result from the acquisition of a new enzyme to replace the sensitive one.[37],[38],[39]

1.7 Spectrum of activity(Table 1.1)

Narrow spectrum	Broad spectrum
Penicillin G	Tetracycline
Streptomycin	Chloramphenicol
Erythromycin	

1.8 Type of action(Table 1.2)

Primarily bacteriostatics	Primarily bacteriocidals
Sulfonamides	Penicillins, Aminoglycosides
Erythromycin	Cephalosporins,Cotrimoxazole
Tetracycline	Polypeptides, Nalidixic acid
Ethambutol	Ciprofloxacin, Pyrazinamide
Chloramphenicol	Metronidazole
Clindamycin	Rifampin
Linezolid	Isoniazid
	Vancomycin

1.9 Antibiotics are obtained from(Table 1.3)

Fungi:	**Bacteria**:	**Actinomycetes**
Penicillin	Bacitracin	Aminoglycosides
Cephalosporin	Tyrothricin	Tetracyclines
Griseofulvin	Aztreonam	Macrolide
	Colistin	Polyene
	Polymyxin B	Chloramphenicol

1.10 References

1. *Taxonomy Browser*. NCBI. Retrieved,**2008**
2. Fredrickson, J. K. et. al. Zachara JM, Balkwill DL,"Geomicrobiology of High-Level Nuclear Waste-Contaminated Vadose Sediments at the Hanford Site, Washington State".*Applied & Environmental Microbiology* **2004**,4230-4241
3. Whitman,ColemanDC,WiebeWJ"Prokaryotes:theunseenmajority".*Proceeding of the National Academy of Sciences of the United States of America*, **1998**,6578-83.
4. C.Michael Hogan,Sidney Draggan and C.J.Cleveland, Bacteria. *Encyclopedia of Earth*. National Council for Science and the Environment, Washington DC(2010)
5. Rappé MS, Giovannoni SJ."The uncultured microbial majority". *Annual Review of Microbiology **2003***,369–94
6. Sears CL "A dynamic partnership of gut flora". *Anaerobe* **2002**, 247–51.
7. Ishige T, Honda K, Shimizu S "Whole organism biocatalysis". *Current Opinion in Chemical Biology*, **2005**,174–80
8. Woese CR, Kandler O, Wheelis ML "Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya". *Proceedings of the National Academy of Sciences of the United States of America*,**1990**,4576–92
9. Schopf J "Disparate rates, differing fates: tempo and mode of evolution changed from the Precambrian to the Phanerozoic". *Proc Natl Acad Sci USA*,**1994,** 6735–42
10. DeLong E, Pace N "Environmental diversity of bacteria and archaea". *Syst Biol* ,**2001,**470–78
11. Brown JR, Doolittle WF "Archaea and the prokaryote-to-eukaryote transition".*Microbiology and Molecular Biology Reviews***, 1997,**456–502
12. Poole A, Penny D "Evaluating hypotheses for the origin of eukaryotes". *Bioessays*,**2007,**74–84
13. Dyall S, Brown M, Johnson P "Ancient invasions: from endosymbionts to organelles". *Science* **2004,** 253–70.

14. Lang B, Gray M, Burger G "Mitochondrial genome evolution and the origin of eukaryotes". *Annu Rev Genet*,***1999***,351–97
15. McFadden G "Endosymbiosis and evolution of the plant cell". *Curr Opin Plant Biol***,**513–92,**1999**
16. Robertson J, Gomersall M, Gill P "Mycoplasma hominis: growth, reproduction, and isolation of small viable cells". *J Bacteriol*, **1975,**1007–18,
17. Velimirov "Nanobacteria, Ultramicrobacteria and Starvation Forms: A Search for the Smallest Metabolizing Bacterium". *Microbes and Environments*,**2001**,67–77
18. Dusenbery, David B. *Living at Micro Scale*,Harvard University Press, Cambridge, **2009,**20-25
19. Fritz I, Strömpl C, Abraham W "Phylogenetic relationships of the genera Stella, Labrys and Angulomicrobium within the 'Alphaproteobacteria' and description of Angulomicrobium amanitiforme sp. nov". *Int J Syst Evol Microbiol*, **2004**,651–678
20. Wanger, G; Onstott, TC; Southam, G"Stars of the terrestrial deep subsurface: A novel `star-shaped' bacterial morphotype from a South African platinum mine". *Geobiology***,2008,**325–330
21. Cabeen M, Jacobs-Wagner C "Bacterial cell shape". *Nat Rev Microbiol*,**2005,**601–10
22. Young K "The selective value of bacterial shape". *Microbiol Mol Biol Rev*,**2006** 660–703
23. Douwes K, Schmalzbauer E, Linde H, Reisberger E, Fleischer K, Lehn N, Landthaler M, Vogt T "Branched filaments no fungus, ovoid bodies no bacteria: Two unusual cases of mycetoma". *J Am Acad Dermatol***,2003,**170–183
24. Donlan R "Biofilms: microbial life on surfaces". *Emerg Infect Dis*,**2002,**881–90
25. Branda S, Vik S, Friedman L, Kolter R "Biofilms: the matrix revisited". *Trends Microbiol*,**2005**,20-21
26. Davey M, O'toole G "Microbial biofilms: from ecology to molecular genetics".*Microbiol Mol Biol Rev*,**2000**,847–867

27. Donlan RM, Costerton JW "Biofilms: survival mechanisms of clinically relevant microorganisms". *Clin Microbiol Rev*,**2002**,167–193
28. Shimkets L "Intercellular signaling during fruiting-body development of Myxococcus xanthus". *Annu Rev Microbiol*,**1999**,525–549
29. Kaiser D "Signaling in myxobacteria". *Annu Rev Microbiol*,**2004**,75–98
30. Gitai "The new bacterial cell biology: moving parts and subcellular architecture",**2005**, 577–86
31. Shih YL, Rothfield L "The bacterial cytoskeleton". *Microbiology and Molecular Biology Reviews*,**2006,**729–54

32. John H. Block, JMB. Wilson and Gisvold's Textbook of Organic and Medicinal chemistry. In: John M. Beale, *J Ed. Antibacterial Antibiotics*. New York: Lippincott Williams& Wilkins Custemer service,**2004**,302-313
33. Parenti F and LanciniG,Rifamycins. In: O'Grady F and Lambert HP(eds) *Antibiotic and Chemotherapy: Anti-infective Agents and Their Use in Therapy*, **1997**, 453–459

34 O'Neill A, Oliva B, Storey C, Hoyle A, Fishwick C and Chopra I , RNA Polymerase inhibitors with activity against rifampicin-resistant mutants of Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy*,***2000***,3163–3166

35 Piddock LJV , Antibacterials – mechanisms of action. *Current Opinion in Microbiology*,**1998,** 502–508

36 Hancock REW and Chapple DS , Peptide antibiotics. *Antimicrobial Agents and Chemotherapy*,**1999,** 1317–1323

37 Kremer R, Baulard AR and Besra GS , Genetics of mycolic acid biosynthesis. In: Hatfull GF and JacobsWR(eds) *Molecular Genetics of Mycobacteria*,**2000** 173–190

38 Dax SL , *Antimicrobial Chemotherapeutic Agents.* London: Blackie Academic and Professional,**1997**

39. http://pathmicro.med.sc.edu/mayer/antibiot.htm

40 Zhang Y and Telenti A , Genetics of drug resistance in Mycobacterium tuberculosis. In: Hatfull GF and Jacobs WR (eds) *Molecular Genetics of Mycobacteria*, **2002**,235–254

CHAPTER-2

AIM OF PRESENT WORK

&

LITERATURE REVIEW

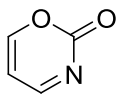
2.1 Aim of Present Work

The need for new antibacterial therapies and strategies is greater than it has been in a quarter of a century and despite considerable effort, little progress has been observed in the development of agents. After all, even the most stubborn of bacterial infections require no more than six months of treatment and the average course of therapy is under seven days. It has been reasoned that it makes more commercial sense to treat chronic conditions requiring years of therapy rather than curing diseases with short courses of treatment. Not only are infectious disease therapeutics the second largest source of revenue for pharmaceutical companies (behind cardiovascular drugs), with antibacterial drugs taking the lion's share, but there are no fewer than six branded products garnering over $1 billion annually, this despite fierce generic competition. No other area of therapeutic focus can boast this level of commercial, not to mention therapeutic, success. There is less argument against the need for new antibacterial agents and therapeutic strategies to avoid the emergence and dissemination of resistant bacteria.[1]In developing countries, periodic malnutrition, poor sanitation, the spread of HIV infection and, almost paradoxically, increasing population density, coupled with the ready availability of cheap, generic antibacterial (often of poor quality) are leading to a mushrooming of resistant bacterial strains. In sum, bacterial diseases in general and antibiotic-resistant bacterial infections specifically are increasing in both developing and developed countries. Ironically, even improved therapies for other diseases are contributing to an increase in infectious diseases for example, immune suppressive therapies in transplant patients, aggressive chemotherapeutic practices in oncology and, most recently, the use of tumour necrosis factor α antagonists in treating rheumatoid arthritis.[2].

Napthelene and 1,3 Oxazinone Moiety have number of various activities like Antimicrobial,Anticancer,Antiepileptic.

2.2 Literature Review

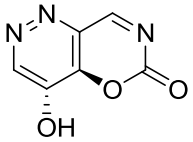
Oxazinone is an important 6-membered heterocyclic ring system which is present in many biologically important natural products like maystansine, maystanprine, maytanbutine and colubrinol. In addition, oxazinone derivatives are known to exhibit a variety of biological activities such as anti microbial, antiulcer, anticonvulsant, penetration enhancer, sedative, analgesic, vasodilator, hypertensive and antidepressant. 1,3-Oxazin-2-one **(1)** derivatives have also been used as key intermediates in the synthesis of several natural products.[3,4]



1,3 Oxazine-2 -one

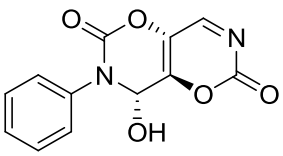
1

The 1,3-oxazine nucleus features prominently in many biologically important natural products and other bioactive molecules**(2,3)**



4-Hydroxy-6*H*-Pyridazio[3,4-e][1,3]oxazin-6-one

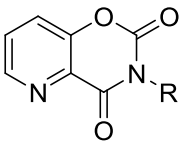
2[5]



(*S*)-4-hydroxy-3-phenyl-3,4-dihydro-[1,3]oxazino[6,5-*e*][1,3]oxazine-2,6-dione

3[3]

Due to their broad spectrum of biological activities, including analgesic, antipyretic, bacteriostatic, fungistatic and monoaminoxidase inhibitory activity, pyrido[2,3-e][1,3]oxazine-2,4-diones **(4,5,6)** are an interesting class of compounds for further structural modifications.[5],[6]



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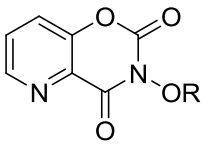

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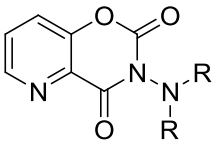

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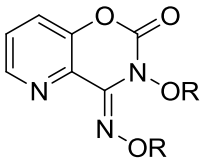
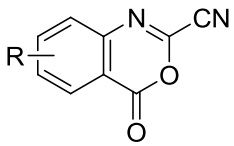


7

Novel 1, 3-benzoxazinones **(8)** has been synthesized by Besson et al. and their antibacterial in vitro activity of some derivatives was evaluated against gram -ve bacteria and gram +ve bacteria.[7],[8]



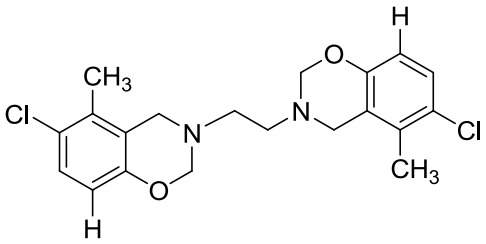
R=H,Cl

4-oxo-4*H*-benzo[*d*][1,3]oxazine-2-carbonitrile

8

A series of 1,2-bis (3,4-dihydrobenzo[e][1,3]oxazin-3(4H)-yl)ethane **(9)** derivatives was synthesized by Mathew et al. through an eco-friendly Mannich type

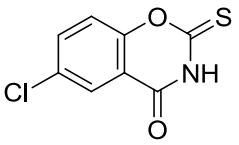
condensation–cyclization reaction of phenols or naphthols with formaldehyde and primary amines in water at ambient temperature. Preliminary in vitro antimicrobial activity of the synthesized compounds was assessed against six pathogenic fungi, two Gram-negative and two Gram-positive bacteria. Some of the screened compounds have shown significant in vitro antimicrobial effect.[9]



1,2-bis(6-chloro-5-methyl-2*H*-benzo[e][1,3]oxazin-3(4*H*)-yl)ethane

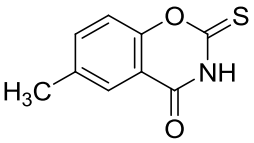
9

Dihydro-l,3-benzoxazine-2-tliione-4 **(10,11,12,13)** one or derivatives there of which have one to three substituents on the benzene ring, have fungicidal and bactericidal activities against microbes.[10]



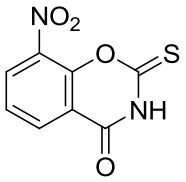
6-chloro-2-thioxo-2*H*-benzo[*e*][1,3]oxazin 4(3*H*)-one

10



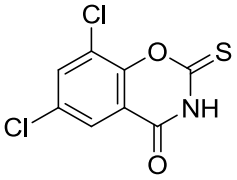
6-methyl-2-thioxo-2*H*-Benzo[e][1,3]oxazin-4(3*H*)-one

11`



8-nitro-2-thioxo-2*H*-Benzo[e][1,3]oxazin-4(3*H*)-one

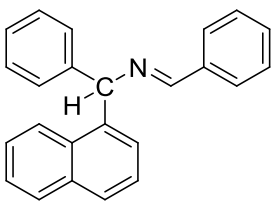
12



6,8-dichloro-thioxo-2*H*-Benzo[e][1,3]oxazin-4(3*H*)-one

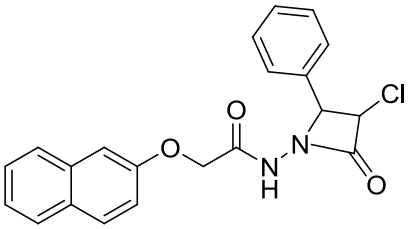
13

Naphthalene containing drugs are available, such as Naficillin**(14)**, Naftifine, Terbinafine**(15)**,Tolnaftate**(16)** etc. which play vital role in the control of microbial infection1. Naphthalene and its derivative have shown a large spectrum of antimicrobial activity .several research has been done and has proved β-naphthol as an excellent lead moiety for designing a synthetic derivative, which would posses good biologically activity.[11]



Naficillin

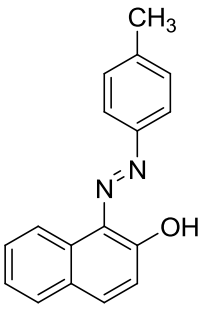
14



Terbinafine

15

Compounds of naphthalene have been reported to show a variety of biological activities including antimicrobial activities.[12]

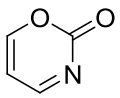


Tolnaftate

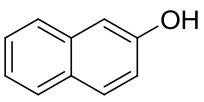
16[13]

There are 2 moiety which are responsible as anti microbial activity.

1,3 oxazinone

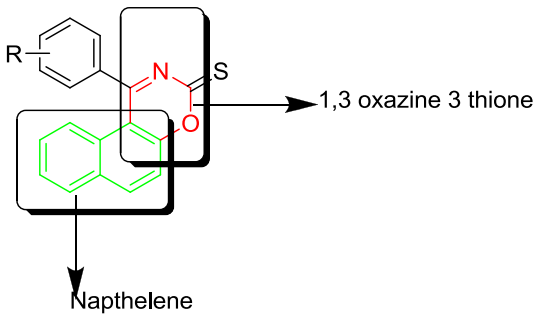


Napthelene



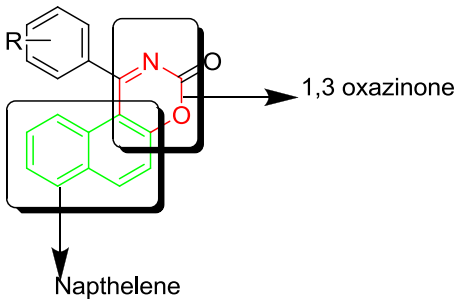
Design Compound

Series 1



1-phenyl-3*H*-naphtho[1,2-*e*][1,3]oxazine-3-thione

Series 2



1-phenyl-3*H*-naphtho[1,2-*e*][1,3]oxazin-3-one

2.3 References

1. Sahyoun NR, Lentzner H, Hoyert D, Robinson KN: Trends in causes of death among the elderly. *Aging Trends*, **2002**,1-10

2. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM: Tuberculosis associated with infliximab, a tumour necrosis factor alpha-neutralizing agent. *N Engl J Med*, 345,**2001**,1098-1104,

3.BrajB.Lohray, SundarababuBaskaran, B.Yadi Reddy, K.Srinivas Rao, Enantiospecific Synthesis of 6-Substituted N-Aryl-1,3-Oxazin-2-Ones, *Tetrahedron Letters 39*,**1998**,6555-6556

4 Lal Dhar S. Yadav, Vishnu P. Srivastava,Vijai K. Rai, Rajesh Patel ,Diversity oriented synthesis of fused-ring 1,3-oxazines from carbohydrates as biorenewable feedstocks, *Tetrahedron 64*, **2008**,4246-4253

5 Thomas Kurz, Synthesis of novel pyrido[2,3-e][1,3]oxazines, *Tetrahedron 61*,**2005** 3091–3096

6.Francois-Xavier Le Foulon, Emmanuelle Braud, Frederic Fabis, Jean-Charles Lancelot and Sylvain Rault , Synthesis and combinatorial approach of the reactivity of 6 and 7-arylthieno[3,2-d][1,3]oxazine-2,4-diones *Tetrahedron 59*,**2003,**10051–10057

7 Hua Cao, Huan-Feng Jiang, Chao-Rong Qi, Wen-Juan Yao, Huo-Ji Chen, Brønsted acid- promoted domino reactions: a novel one-pot three-component synthesis of 3,4,5-trisubstituted-3,6-dihydro-2H-1,3-oxazines,*TetrahedronLetters50,**2009*** 1209–1214

8 Fitzmaurice, R. J.; Etheridge, Z. C.; Jumel, E.; Woolfson, D. N.; Caddick, S. Chem. Commun,**2006** 4814 – 4816

9 Corey, E. J.; Cheng, X.-M. The Logic of Chemical Synthesis; John Wiley and Sons: New York, NY,**1989,**423

10 Hassan Sheibania, Mohammad Hossein Mossleminb, Soheila Behzadib, Mohammad Reza Islamia, Habibollah Foroughi, and Kazem Saidia,A simple and an efficient approach to the synthesis of a specific tautomer of 1,3-thiazinones and 1,3-oxazinones *ARKIVOC*,**2005,**88-96

11 abdolkarim zare, alireza hasaninejad, esmael rostami, ahmad reza moosavi-zare, nasrin pishahang, mehrnoosh roshankar, fatemeh khedri and maasoomeh khedri, An Efficient Solvent-Free Protocol for the Synthesis of 1-Amidoalkyl-2-naphthols using Silica-Supported Molybdatophosphoric Acid,*E-Journal of Chemistry*,**2010,**1162-1169

12 Yogesh Rokade1 and Nirmal Dongare, synthesis and antimicrobial activity of some azetidinone derivatives with the naphthol, *Rasayan j. Chem*,**2010,**641-645

13 V. Mkpenie, g. Ebong, i. B. Obot and b. Abasiekong, Evaluation of the Effect of Azo Group on the Biological Activity of 1-(4-Methylphenylazo)-2-naphthol *E-Journal of Chemistry*,**2008**,431-434

CHAPTER-3

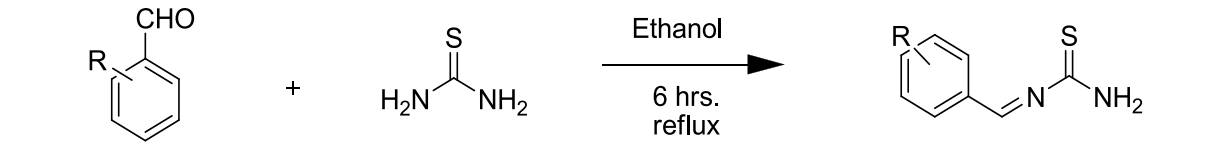
EXPERIMENTAL WORK -I

SYNTHESIS & CHARACTERIZATION OF NOVEL NAPTHOXAZINETHIONES &NAPTHOXAZINONES DERIVATIVES

3.1 Synthetic Scheme Used For The Development of Substituted Napthoxazinthiones derivatives with Thiourea(Series 1).

Step-1

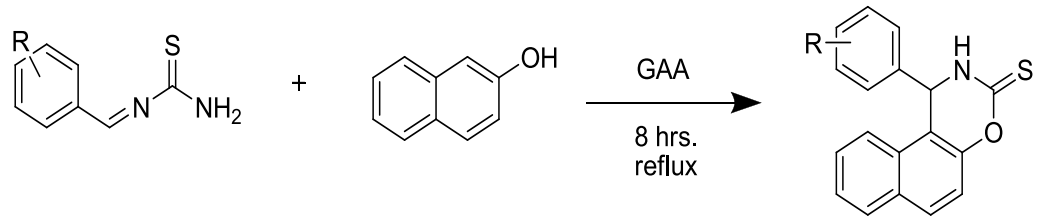
Substituted aldehyde in reaction with thiourea in the presence of ethanol gives Substituted benzylidenethiourea derivatives.



Substituted Aldehyde thiourea Substitutedbenzylidenethiourea

Step-2

Substitutedbenzylidenethiourea in reaction with β-napthol in the presence of glacial acetic acid gives substituted napthoxazinthiones derivatives.

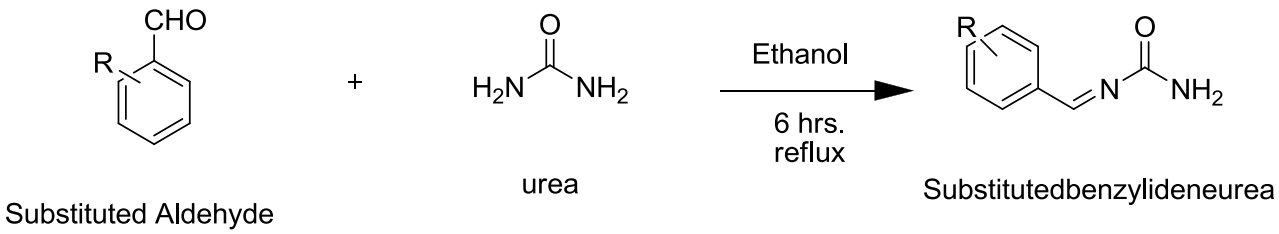


Substitutedbenzylidenethiourea naphthalen-2-ol Substituted 1-phenyl-1H-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-thione

Synthetic Scheme Used For The Development of Substituted Napthoxazinones derivatives with Urea(Series 2).

Step-1

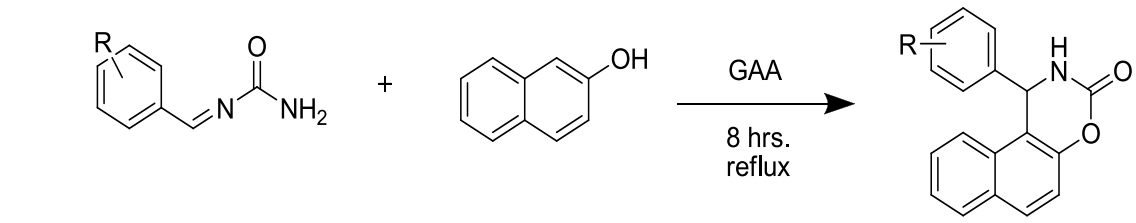
Substituted aldehyde in reaction with urea in the presence of ethanol gives Substituted benzylideneurea derivatives.



Substituted Aldehyde urea Substitutedbenzylideneurea

Step-2

Substitutedbenzylideneurea in reaction with β-napthol in the presence of glacial acetic acid gives substituted napthoxazinones derivatives.



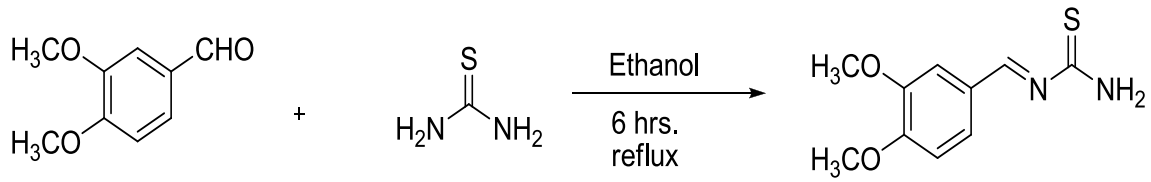
Substitutedbenzylideneurea naphthalen-2-ol

Substituted 1-phenyl-1H-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

3.2 Synthesis of Intermediates.

3.2.1 Synthesis of Substituted Benzylidenethiourea derivatives.

(A) Synthesis of 1-(3,4-dimethoxybenzylidene)thiourea



3,4-dimethoxybenzaldehyde thiourea (*E*)-1-(3,4-dimethoxybenzylidene)thiourea

Requirments:-

Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
3,4 Dimethoxy Benzaldehyde	166.17	0.83	0.005	1
Thiourea	76.12	0.38	0.005	1

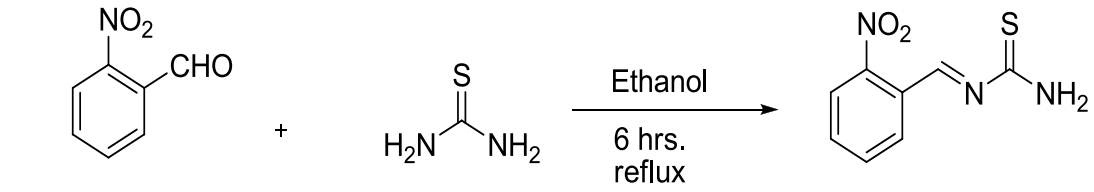
Procedure:- A mixture of Benzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Solvent system used for TLC : Hexane:Ethyl acetate (4:6)

Result:

Product	Result
Percentage Yield	72.22%
R_f	0.38
Melting Point	150-154 ^{0}C

(B) Synthesis of 1-(2-Nitrobenzylidene)thiourea



2-nitrobenzaldehyde thiourea (*E*)-1-(2-nitrobenzylidene)thiourea

Requirments:-

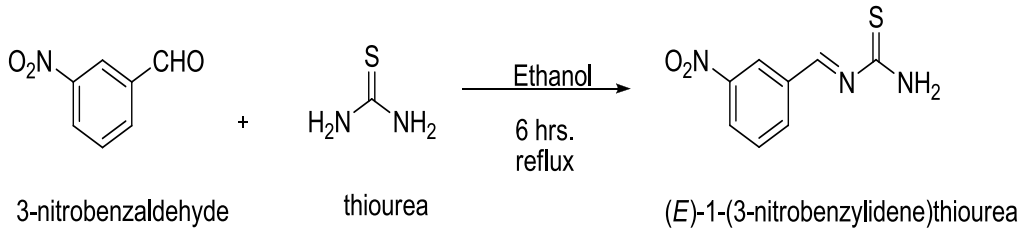
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
2-Nitro Benzaldehyde	151.12	0.76	0.005	1
Thiourea	76.12	0.38	0.005	1

Procedure:- A mixture of 2-Nitro Benzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	Result
Percentage Yield	67.42%
R_f	0.7
Melting Point	180-184 ^{0}C

(C) Synthesis of 1-(3-Nitrobenzylidene)thiourea



3-nitrobenzaldehyde thiourea (*E*)-1-(3-nitrobenzylidene)thiourea

Requirments:-

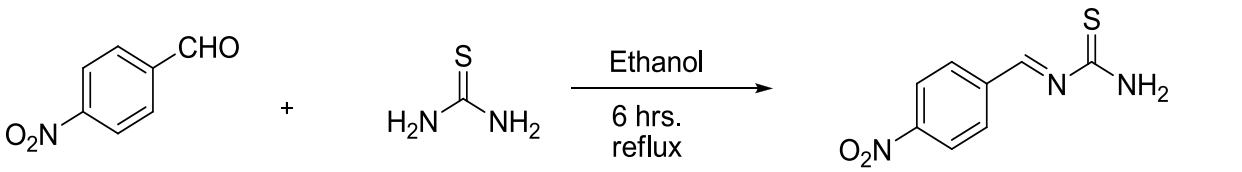
Reagents	**Mol.Weight(g/mol)**	**Quantity(g)**	**Mol**	**Mol Ratio**
3-Nitro Benzaldehyde	151.12	0.76	0.005	1
Thiourea	76.12	0.38	0.005	1

Procedure:- A mixture of 3-Nitrobenzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	**Result**
Percentage Yield	53.02%
R_f	0.42
Melting Point	170-174 ^{0}C

(D) Synthesis of 1-(4-Nitrobenzylidene)thiourea



4-nitrobenzaldehyde thiourea 1-(4-nitrobenzylidene)thiourea

Requirments:-

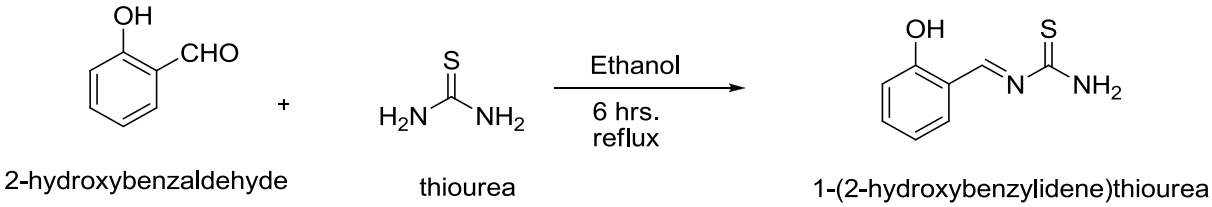
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
2-Hydroxy Benzaldehyde	122.12	0.61	0.005	1
Thiourea	76.12	0.38	0.005	1

Procedure:- A mixture of 4-NitroBenzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	Result
Percentage Yield	68.09%
R_f	0.58
Melting Point	210-212 ^{0}C

(E) Synthesis of 1-(2-Hydroxybenzylidene)thiourea



2-hydroxybenzaldehyde thiourea 1-(2-hydroxybenzylidene)thiourea

Requirments:-

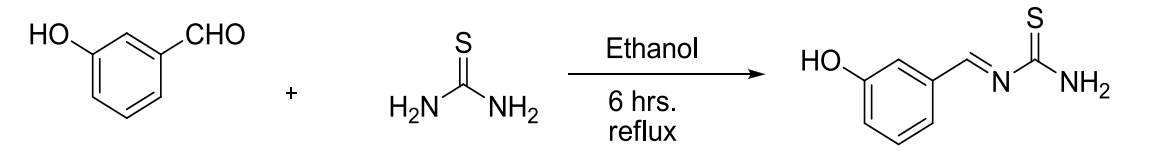
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
4-Nitro Benzaldehyde	151.12	0.76	0.005	1
Thiourea	76.12	0.38	0.005	1

Procedure:- A mixture of Benzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	Result
Percentage Yield	49.33%
R_f	0.31
Melting Point	182-187 ^{0}C

(F) Synthesis of 1-(3-Hydroxybenzylidene)thiourea



3-hydroxybenzaldehyde thiourea 1-(3-hydroxybenzylidene)thiourea

Requirments:-

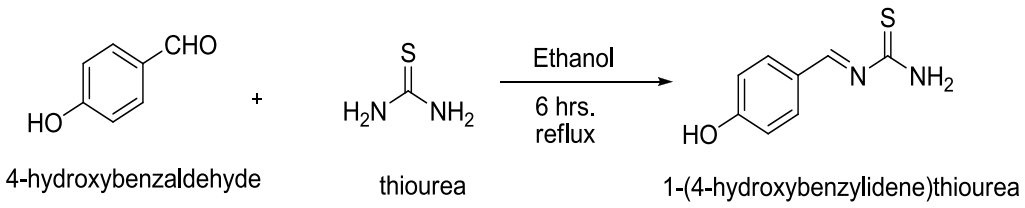
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
3-Hydroxy Benzaldehyde	122.12	0.61	0.005	1
Thiourea	76.12	0.38	0.005	1

Procedure:- A mixture of 3-Hydroxybenzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	Result
Percentage Yield	69.11%
R_f	0.45
Melting Point	194-197 ^{0}C

(G) Synthesis of 1-(4-Hydroxybenzylidene)thiourea



4-hydroxybenzaldehyde thiourea 1-(4-hydroxybenzylidene)thiourea

Requirments:-

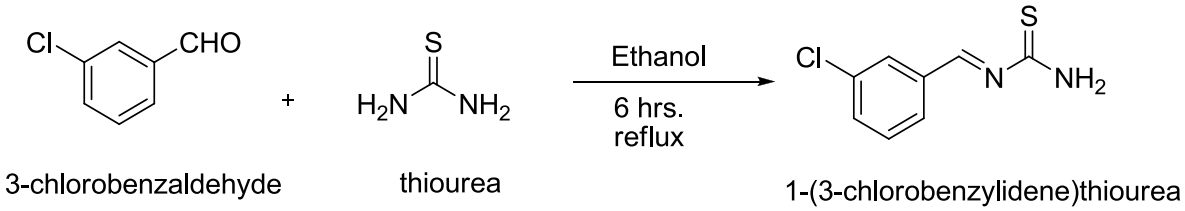
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
4-Hydroxy Benzaldehyde	122.12	0.61	0.005	1
Thiourea	76.12	0.38	0.005	1

Procedure:- A mixture of 4-Hydroxybenzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	Result
Percentage Yield	79.26%
R_f	0.77
Melting Point	124-127 ^{0}C

(H) Synthesis of 1-(3-Chlorobenzylidene)thiourea



3-chlorobenzaldehyde thiourea 1-(3-chlorobenzylidene)thiourea

Requirments:-

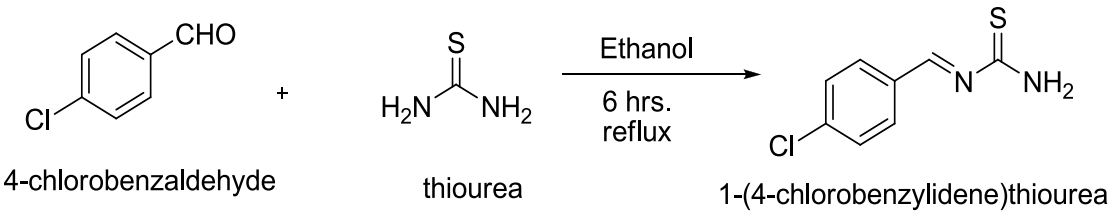
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
3-chloro Benzaldehyde	140.57	0.70	0.005	1
Thiourea	76.12	0.38	0.005	1

Procedure:- A mixture of 3-Chlorobenzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	Result
Percentage Yield	82.42%
R_f	0.52
Melting Point	200-205 ^{0}C

(I) Synthesis of 1-(4-Chlorobenzylidene)thiourea



4-chlorobenzaldehyde thiourea 1-(4-chlorobenzylidene)thiourea

Requirments:-

Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
4-chloro Benzaldehyde	140.57	0.70	0.005	1
Thiourea	76.12	0.38	0.005	1

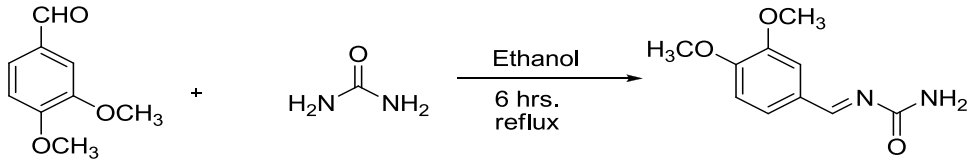
Procedure:- A mixture of 4-Chlorobenzaldehyde (0.005mol) & Thiourea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol

Result:

Product	Result
Percentage Yield	78.09%
R_f	0.58
Melting Point	215-219 ^{0}C

3.2.2 Synthesis of Substituted Benzylideneurea Derivatives

(A) Synthesis of 1-(3,4-dimethoxybenzylidene)urea



3,4 Dimethoxy Benzaldehyde Urea (*E*)-1-(3,4-dimethoxybenzylidene)urea

Requirments:-

Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
3,4 Dimethoxy Benzaldehyde	166.17	0.83	0.005	1
Urea	60.06	0.30	0.005	1

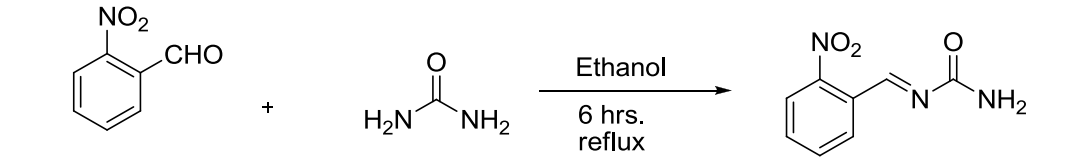
Procedure:- A mixture of 3,4 DimethoxyBenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Solvent system used for TLC : Hexane:Ethyl acetate (4:6)

Result:

Product	Result
Percentage Yield	78.44%
R_f	0.48
Melting Point	140-160 ^{0}C

(B)Synthesis of 1-(2-Nitrobenzylidene)urea



2-nitrobenzaldehyde urea (*E*)-1-(2-nitrobenzylidene)urea

Requirments:-

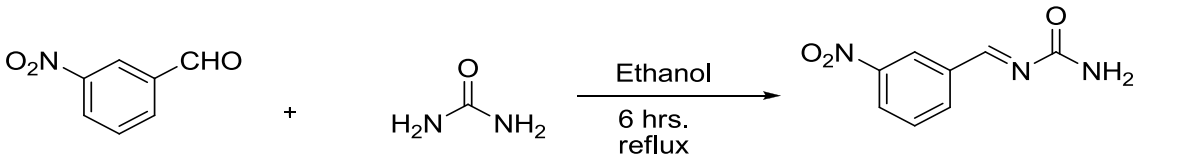
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
2-Nitro Benzaldehyde	151.12	0.76	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of 2-Nitrobenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:-

Product	Result
Percentage Yield	64.44%
R_f	0.38
Melting Point	162-164 ^{O}C

(C) Synthesis of 1-(3-Nitrobenzylidene)urea



3-nitrobenzaldehyde urea (*E*)-1-(3-nitrobenzylidene)urea

Requirements:-

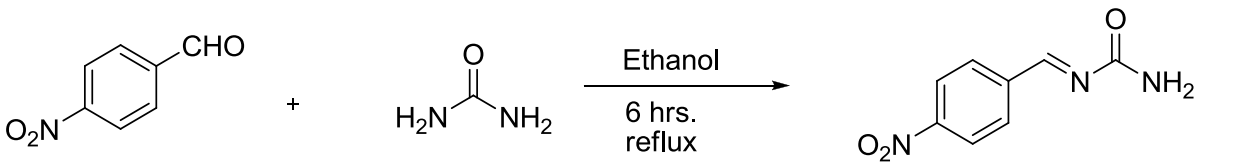
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
3-Nitro Benzaldehyde	151.12	0.76	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of 3-Nitrobenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:-

Product	Result
Percentage Yield	61.09%
R_f	0.62
Melting Point	177-179 ^{O}C

(D) Synthesis of 1-(4-Nitrobenzylidene)urea



4-nitrobenzaldehyde urea 1-(4-nitrobenzylidene)urea

Requirements:-

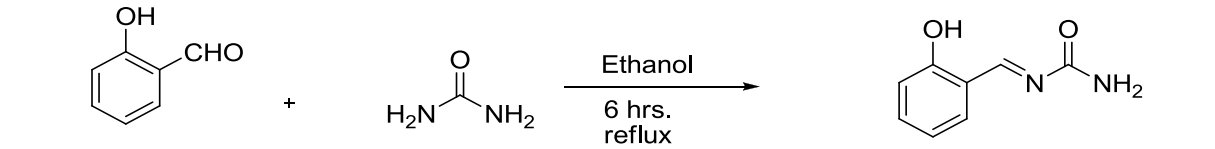
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
4-Nitro Benzaldehyde	151.12	0.76	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of 4-Nitrobenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:-

Product	Result
Percentage Yield	71.20%
R_f	0.54
Melting Point	184-188 ^{O}C

(E) Synthesis of 1-(2-Hydroxybenzylidene)urea



2-hydroxybenzaldehyde urea 1-(2-hydroxybenzylidene)urea

Requirements:-

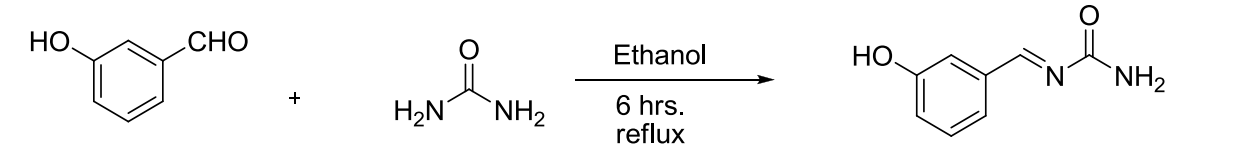
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
Salisaldehyde	122.12	0.61	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of Salisaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:-

Product	Result
Percentage Yield	67.91%
R_f	0.8
Melting Point	190-194 ^{O}C

(F) Synthesis of 1-(3-Hydroxybenzylidene)urea



3-hydroxybenzaldehyde urea 1-(3-hydroxybenzylidene)urea

Requirements:-

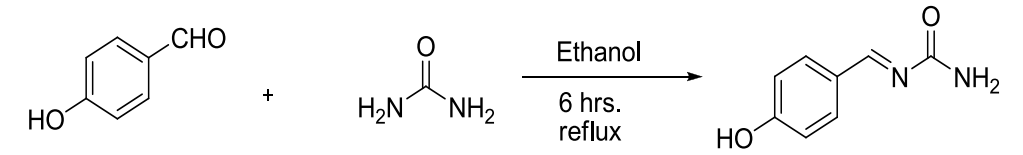
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
3-Hydroxy Benzaldehyde	122.12	0.61	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of 3-Hydroxybenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:-

Product	Result
Percentage Yield	60.23%
R_f	0.37
Melting Point	152-157 ^{O}C

(G) Synthesis of 1-(4-Hydroxybenzylidene)urea



4-hydroxybenzaldehyde urea 1-(4-hydroxybenzylidene)urea

Requirements:-

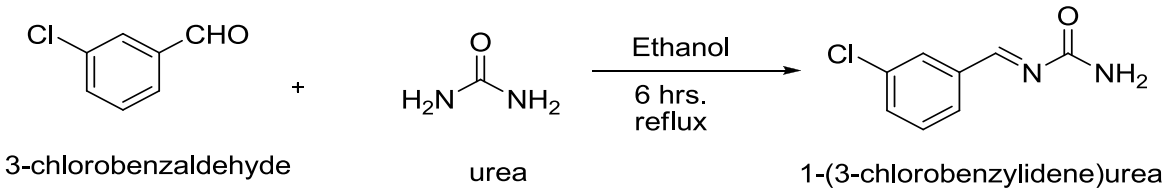
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
4-Hydroxy Benzaldehyde	122.12	0.61	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of 4-Hydroxybenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:-

Product	Result
Percentage Yield	74.13%
R_f	0.75
Melting Point	179-183 ^{O}C

(H) Synthesis of 1-(3-Chlorobenzylidene)urea



3-chlorobenzaldehyde urea 1-(3-chlorobenzylidene)urea

Requirements:-

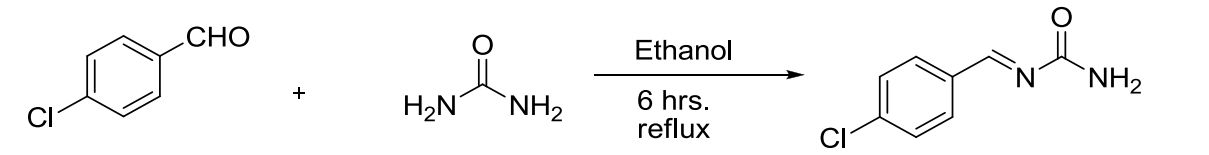
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
3-Chloro Benzaldehyde	140.57	0.70	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of 3-Chlorobenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:-

Product	Result
Percentage Yield	89.42%
R_f	0.49
Melting Point	169-173 ^{O}C

(I) Synthesis of 1-(4-Chlorobenzylidene)urea



4-chlorobenzaldehyde urea 1-(4-chlorobenzylidene)urea

Requirements:-

Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
4-Chloro Benzaldehyde	140.57	0.70	0.005	1
Urea	60.06	0.30	0.005	1

Procedure:- A mixture of 4-chlorobenzaldehyde (0.005mol) & Urea (0.005) Was dissolved in 40 ml Ethanol. Than the mixture was refluxed for 6 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

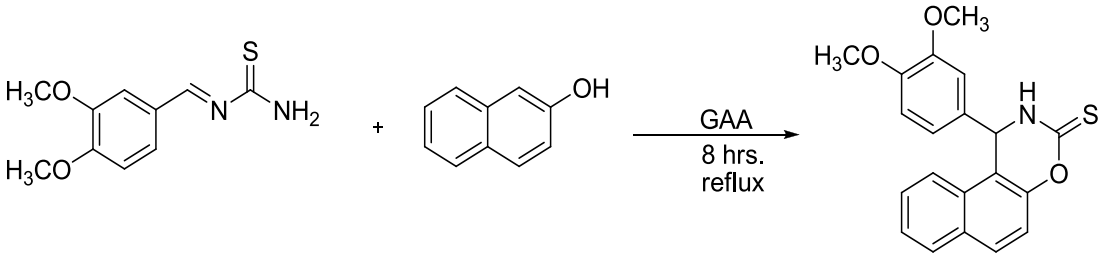
Result:-

Product	Result
Percentage Yield	78.88%
R_f	0.65
Melting Point	204-208 ^{O}C

3.3 Synthesis Of Target Compounds

3.3.1 Synthesis of Substituted Napthoxazinthiones derivatives

(A) Synthesis of 1-(3,4-dimethoxyphenyl)-1*H*-naphtho[1,2-e][1,3]oxazine3(2*H*)-thione(KIS 1)



(1-(3,4-dimethoxybenzylidene)thiourea naphthalen-2-ol 1-(3,4-dimethoxyphenyl)-1H-naphtho[1,2-e][1,3]oxazine-3(2H)-thione

Requirments:-

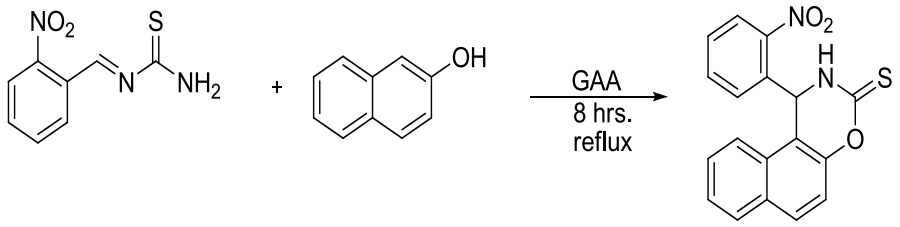
Reagents	Mol.Weight (g/mol)	Quantity(g)	Mol	Mol Ratio
(1-(3,4dimethoxybenzylidene)thiourea	224.28	0.56	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:- A mixture of (1-(3,4dimethoxybenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	62.25%
R_f	0.43
Melting Point	200-205 ^{0}C

(B) Synthesis of 1-(2-nitrophenyl)-1*H*-naphtho [1,2 e][1,3]oxazine3(2*H*)-thione(KIS 2)



1-(2-nitrobenzylidene)thiourea naphthalen-2-ol 1-(2-nitrophenyl)-1*H*-naphtho[1,2-e][1,3]oxazine-3(2*H*)-thione

Requirments:-

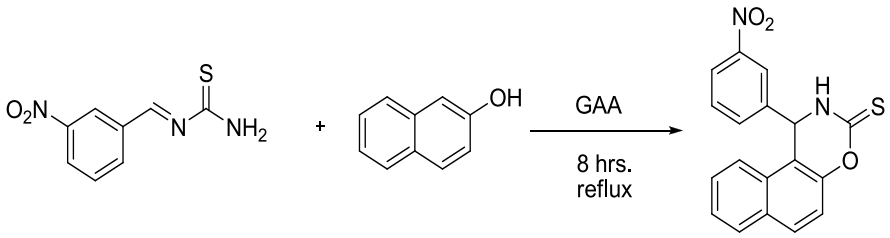
Reagents	Mol.Weight (g/mol)	Quantity(g)	Mol	Mol Ratio
1-(2nitrobenzylidene)thiourea	209.23	0.52	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure :- A mixture of (1-(2 Nitrobenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	57.31%
R_f	0.43
Melting Point	190-193 ^{0}C

(C) Synthesis of 1-(3-nitrophenyl)-1*H*-naphtho [1,2e][1,3]oxazine3(2*H*)-thione(KIS 3)



1-(3-nitrobenzylidene)thiourea naphthalen-2-ol 1-(3-nitrophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazine-3(2*H*)-thione

Requirments:-

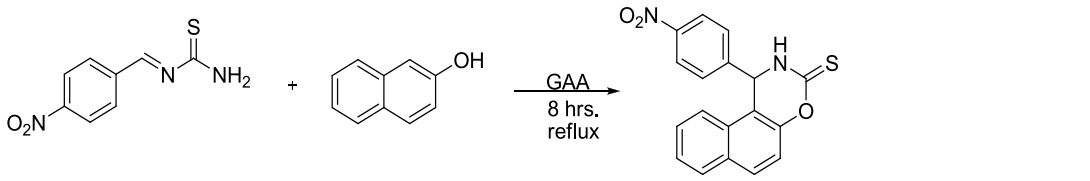
Reagents	Mol.Weight (g/mol)	Quantity(g)	Mol	Mol Ratio
1-(3nitrobenzylidene)thiourea	209.23	0.52	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:- A mixture of (1-(3 Nitrobenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	50.11%
R_f	0.53
Melting Point	180-183 ^{0}C

(D)Synthesis of 1-(4-nitrophenyl)-1*H*-naphtho [1,2-e][1,3]oxazine3(2*H*)-thione(KIS 4)



1-(4-nitrobenzylidene)thiourea naphthalen-2-ol 1-(4-nitrophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazine-3(2*H*)-thione

Requirments:-

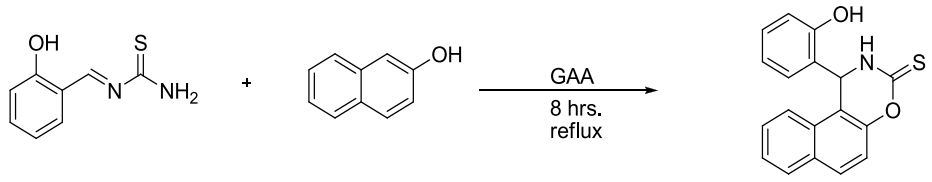
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(4 nitrobenzylidene)thiourea	209.23	0.52	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:- A mixture of (1-(4 Nitrobenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	64.11%
R_f	0.43
Melting Point	218-222 ^{0}C

(E) Synthesis of 1-(2-Hydroxyphenyl)-1*H*-naphtho [1,2-e][1,3]oxazine3(2*H*)-thione(KIS 5)



1-(2-hydroxybenzylidene)thiourea naphthalen-2-ol 1-(2-hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazine-3(2*H*)-thione

Requirments:-

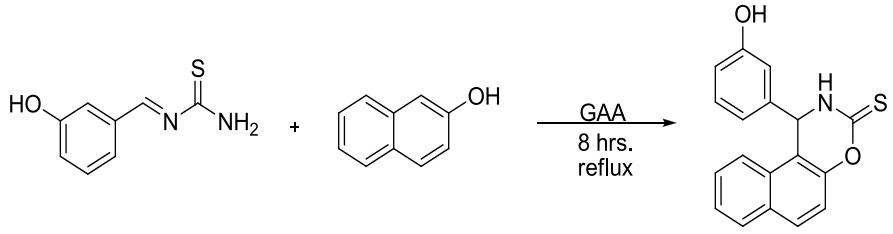
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(2-hydroxybenzylidene)thiourea	180.23	0.45	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:-A mixture of (1-(2-Hydroxybenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	75.11%
R_f	0.39
Melting Point	188-192 ^{0}C

(F)Synthesis of 1-(3-Hydroxyphenyl)-1*H*-naphtho [1,2-e][1,3]oxazine3(2*H*)-thione (KIS 6)



1-(3-hydroxybenzylidene)thiourea naphthalen-2-ol 1-(3-hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazine-3(2*H*)-thione

Requirments:-

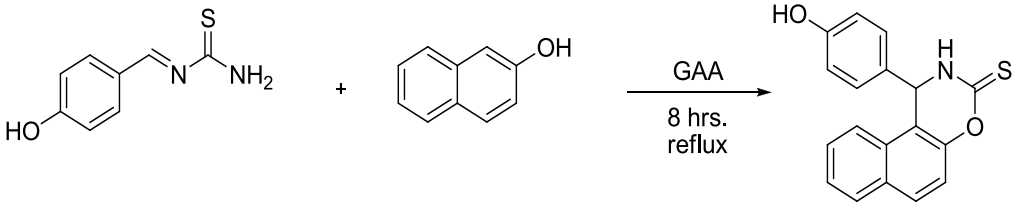
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(3-hydroxybenzylidene)thiourea	180.23	0.45	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:-A mixture of (1-(3-Hydroxybenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	65.68%
R_f	0.37
Melting Point	205-207 ^{0}C

(G)Synthesis of 1-(4-Hydroxyphenyl)-1*H*-naphtho [1,2-e][1,3]oxazine3(2*H*)-thione(KIS 7)



1-(4-hydroxybenzylidene)thiourea naphthalen-2-ol 1-(4-hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazine-3(2*H*)-thione

Requirments:-

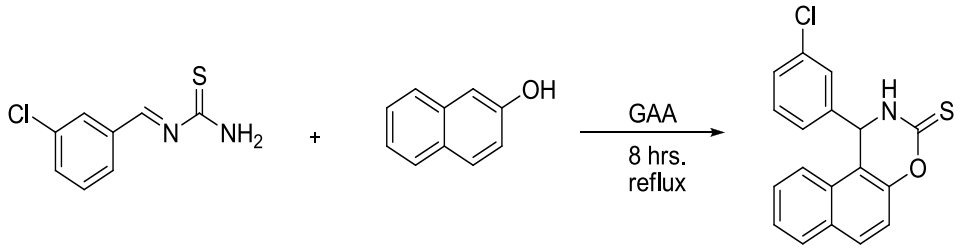
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(4-hydroxybenzylidene)thiourea	180.23	0.45	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:- A mixture of (1-(4-Hydroxybenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	74.15%
R_f	0.64
Melting Point	230-234 ^{0}C

(H)Synthesis of 1-(3 chloro phenyl)-1*H*-naphtho [1,2-e][1,3]oxazine3(2*H*)-thione(KIS 8)



1-(3-chlorobenzylidene)thiourea(*E*)-1-(3-chlorobenzylidene)thiourea

naphthalen-2-ol

1-(3-chlorophenyl)-1*H*-naphtho[1,2-e][1,3]oxazine-3(2*H*)-thione

Requirments:-

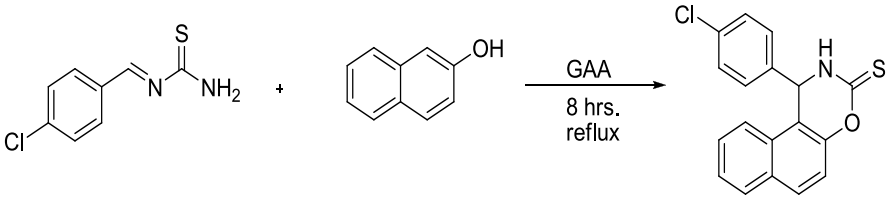
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(3-chlorobenzylidene)thiourea	198.67	0.49	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:A mixture of (1-(3-Chlorobenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	81.09%
R_f	0.6
Melting Point	201-205 ^{0}C

(I)Synthesis of 1-(4- chloro phenyl)-1*H*-naphtho [1,2-e][1,3]oxazine3(2*H*)-thione (KIS 9)



1-(4-chlorobenzylidene)thiourea naphthalen-2-ol 1-(4-chlorophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazine-3(2*H*)-thione

Requirments:-

Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(4-chlorobenzylidene)thiourea	198.67	0.49	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

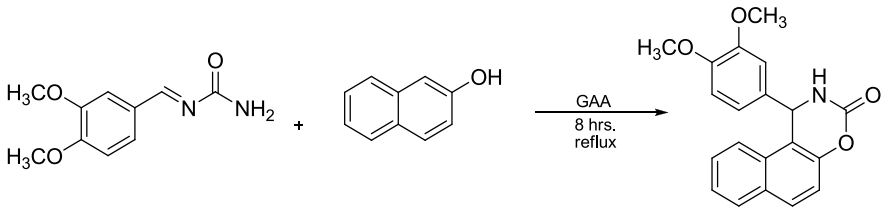
Procedure:-A mixture of (1-(4-Chlorobenzylidene)thiourea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	84.74%
R_f	0.5
Melting Point	232-235 ^{0}C

3.3.2 Synthetis of Substituted Napthoxazinones derivatives

(A) Synthesis of 1-(3,4-dimethoxy phenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one (KIS 10)



1-(3,4-dimethoxybenzylidene)urea naphthalen-2-ol 1-(3,4-dimethoxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

Requirments:-

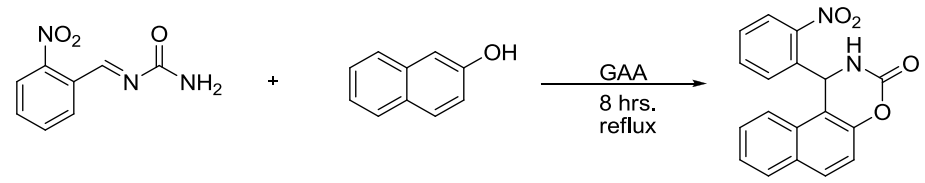
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(3,4-dimethoxybenzyledene)urea	208.21	0.52	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure:A mixture of (1-(3,4dimethoxybenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	64.74%
R_f	0.32
Melting Point	172-175 ^{0}C

(B)Synthesis of 1-(2-nitro phenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one(KIS 11)



1-(2-nitrobenzylidene)urea naphthalen-2-ol 1-(2-nitrophenyl)-1*H*-naphtho[1,2-e][1,3]oxazin-3(2*H*)-one

Requirments:-

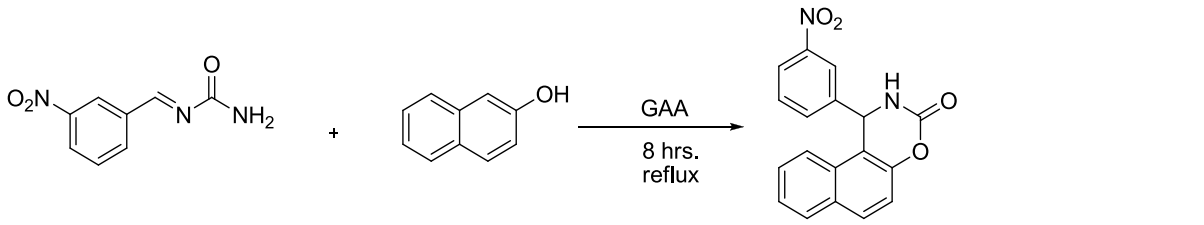
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(2-nitrobenzyledene)urea	193.16	0.48	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure. A mixture of (1-(2-Nitrobenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	48.74%
R_f	0.31
Melting Point	194-198 ^{0}C

(C)Synthesis of 1-(3-nitro phenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one(KIS 12)



1-(3-nitrobenzylidene)urea naphthalen-2-ol 1-(3-nitrophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

Requirments:-

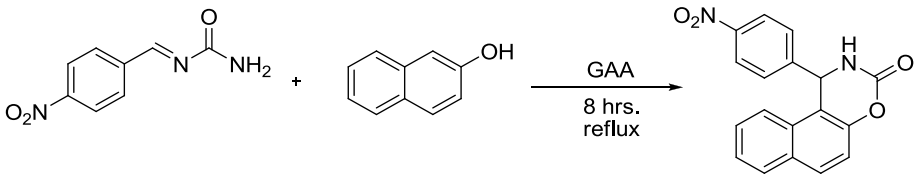
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(3-nitrobenzyledene)urea	193.16	0.48	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure-A mixture of (1-(3-Nitrobenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	67.76%
R_f	0.41
Melting Point	234-238 ^{0}C

(D)Synthesis of 1-(4-nitro phenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one (KIS 13)



1-(4-nitrobenzylidene)urea naphthalen-2-ol 1-(4-nitrophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

Requirments:-

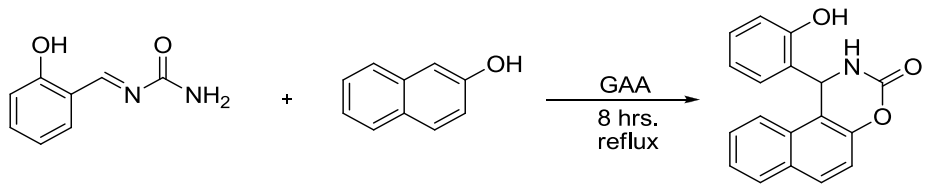
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(4-nitrobenzyledene)urea	193.16	0.48	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure. A mixture of (1-(4-Nitrobenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	74.01%
R_f	0.79
Melting Point	215-219 ^{0}C

(E)Synthesis of 1-(2-hydroxyphenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one (KIS 14)



1-(2-hydroxybenzylidene)urea naphthalen-2-ol 1-(2-hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

Requirments:-

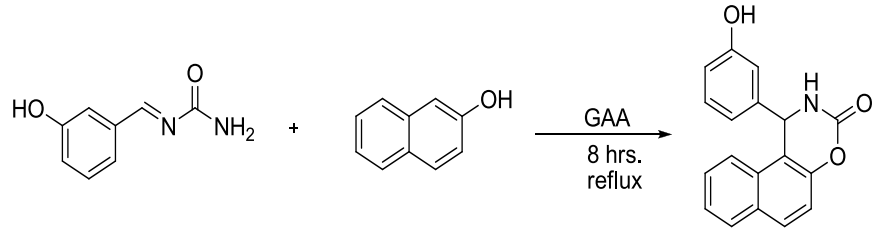
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(2-hydroxybenzyledene)urea	164.16	0.41	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure. A mixture of (1-(2-Hydroxybenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	57.18%
R_f	0.44
Melting Point	165-169 ^{0}C

(F)Synthesis of 1-(3-hydroxyphenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one (KIS 15)



1-(3-hydroxybenzylidene)urea naphthalen-2-ol 1-(3-hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

Requirments:-

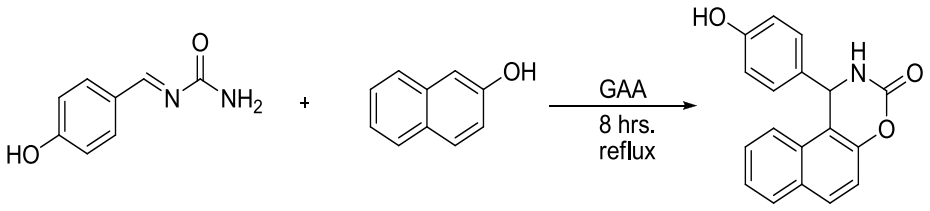
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(3-hydroxybenzyledene)urea	164.16	0.41	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure. A mixture of (1-(3-Hydroxybenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	49.30%
R_f	0.63
Melting Point	176-179 ^{0}C

(G)Synthesis of 1-(4-hydroxyphenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one (KIS 16)



1-(4-hydroxybenzylidene)urea naphthalen-2-ol 1-(4-hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

Requirments:-

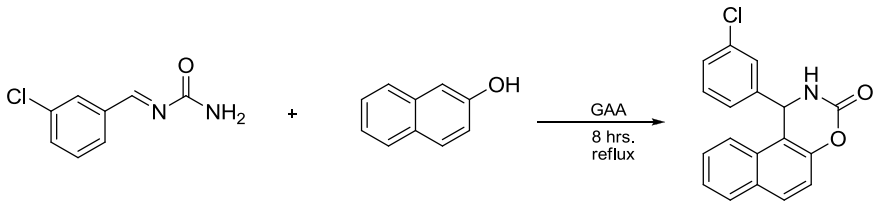
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(4-hydroxybenzyledene)urea	164.16	0.41	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure. A mixture of (1-(4-Hydroxybenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	73.49%
R_f	0.52
Melting Point	207-211 ^{0}C

(H)Synthesis of 1-(3-chlorophenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one (KIS 17)



1-(3-chlorobenzylidene)urea naphthalen-2-ol 1-(3-chlorophenyl)-1*H*-naphtho[1,2-e][1,3]oxazin-3(2*H*)-one

Requirments:-

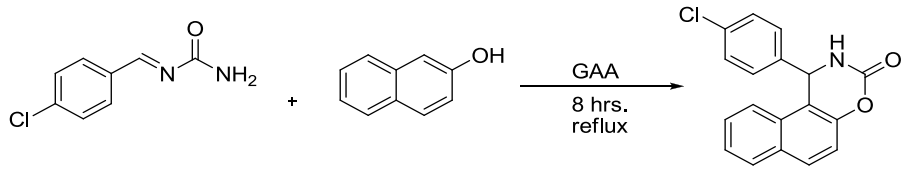
Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(3-chlorobenzyledene)urea	182.61	0.46	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure. A mixture of (1-(3-Chlorobenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

Product	Result
Percentage Yield	83.50%
R_f	0.61
Melting Point	241-245 ^{0}C

(I)Synthesis of 1-(4-chlorophenyl)-1H-naphtho [1,2-e][1,3]oxazin-3(2H)-one (KIS 18)



1-(4-chlorobenzylidene)urea naphthalen-2-ol 1-(4-chlorophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

Requirments:-

Reagents	Mol.Weight(g/mol)	Quantity(g)	Mol	Mol Ratio
1-(4-chlorobenzyledene)urea	182.61	0.46	0.0025	1
Napthalen-2-ol	144.17	0.72	0.0050	2

Procedure. A mixture of (1-(4-Chlorobenzylidene)urea (0.005mol) & Napthalen-2-ol (0.005) Was dissolved in 40 ml GAA. Than the mixture was refluxed for 8 hrs. Reaction was monitored by precoated TLC. After completion of reaction solution was poured into ice cold water and kept for some time. The crude solid was filter . The product was re-crystallized from ethanol.

Result:

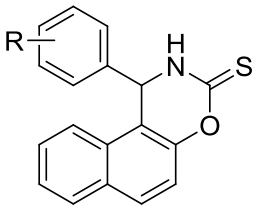
Product	Result
Percentage Yield	79.43%
R_f	0.62
Melting Point	178-181 ^{0}C

3.4 Result and Discussion

Napthoxazinthiones & Napthoxazinones derivatives were successfully synthesized by reacting substituted aldehyde with urea by heating on reflux to yield benzylidene thiourea and benzylidene urea which on futher treatment with naphthalene-2-ol result in the formation of novel Napthoxazinthiones & Napthoxazinones derivatives with a percentage yield of 50-85% and 48-84% respectively. A series of total 18 compounds were synthesized having Napthoxazinethiones & Napthoxazinones moiety and were giving the peaks at **1013 cm^{-1}**(C-O str-),**3593 cm^{-1}**(O-H str), **3245 cm^{-1}**(N-H str), **3160 cm^{-1}**(C-H str) ,**1080 cm^{-1}(**C-N str), **1580 cm^{-1}** (N-H bend), **1517 cm^{-1}** (C=Cstr.(aro) were common in **KIS 6** and **KIS 15**. A sharp peak was observed at **1200 cm^{-1}**(C=S) and **1723 cm^{-1}** (C=O) in **KIS 6** and **KIS 15** respectively. The mass spectra performed by electronic ionization shows the molecular ion peak at **308.8** and **290.8** for **KIS 6** and **KIS 15** which were also the base peaks for the respective molecule.

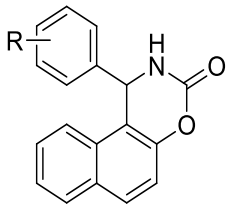
The structures of the novel synthesized compounds of series Napthoxazinethiones & Napthoxazinones were elucidated by IR and MASS spectroscopic tools. The summery of code and Functional Group(R) of the synthesized compounds shown in Table 3.1and Table 3.2

Series 1



Substituted 1-phenyl-1H-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-thione

Series 2



Substituted 1-phenyl-1H-naphtho[1,2-*e*][1,3]oxazin-3(2*H*)-one

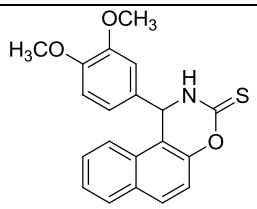
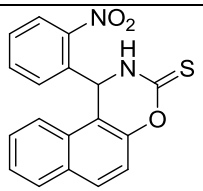
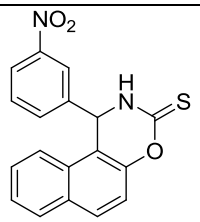
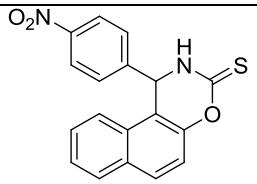
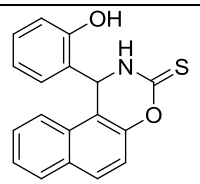
Table 3.1

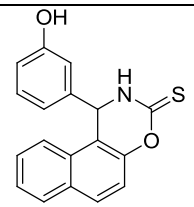
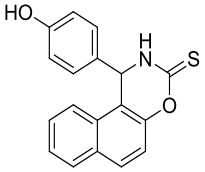
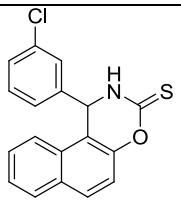
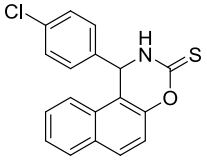
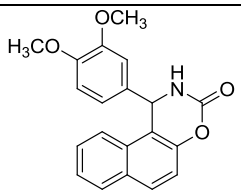
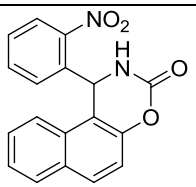
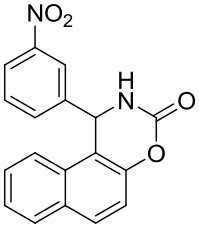
Code of Compound	**R**
KIS 1	-3,4 OCH_3
KIS 2	-2 NO_2
KIS 3	-3 NO_2
KIS 4	-4 NO_2
KIS 5	-2 OH
KIS 6	-3 OH
KIS 7	-4 OH
KIS 8	-3 Cl
KIS 9	-4 Cl

Table 3.2

Code of Compound	**R**
KIS 10	-3,4 OCH_3
KIS 11	-2 NO_2
KIS 12	-3 NO_2
KIS 13	-4 NO_2
KIS 14	-2 OH
KIS 15	-3 OH
KIS 16	-4 OH
KIS 17	-3 Cl
KIS 18	-4 Cl

Table 3.3 Summary of The Physical Data of the Novel compounds

Compound Code	Structure	Chemical formula	% Yield	R_f Value Hexane:Ethyl acetate (4:6)	Melting Point(^{0}C)
KIS 1		$C_{20}H_{17}NO_3S$	62.25	0.43	200-205
KIS 2		$C_{18}H_{12}N_2O_3S$	57.31	0.49	190-193
KIS 3		$C_{18}H_{12}N_2O_3S$	50.11	0.53	180-183
KIS 4		$C_{18}H_{12}N_2O_3S$	64.11	0.43	218-222
KIS 5		$C_{18}H_{13}NO_2S$	75.11	0.39	188-192

KIS 6		$C_{18}H_{13}NO_2S$	65.68	0.37	205-207
KIS 7		$C_{18}H_{13}NO_2S$	74.15	0.64	230-234
KIS 8		$C_{18}H_{12}ClNOS$	81.09	0.6	201-205
KIS 9		$C_{18}H_{12}ClNOS$	84.74	0.5	232-235
KIS 10		$C_{20}H_{17}NO_4$	64.74	0.32	172-175
KIS 11		$C_{18}H_{12}N_2O_4$	48.74	0.31	194-198
KIS 12		$C_{18}H_{12}N_2O_4$	67.76	0.41	234-238

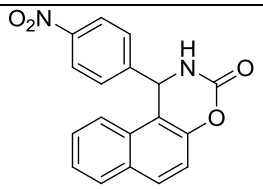
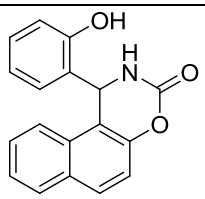
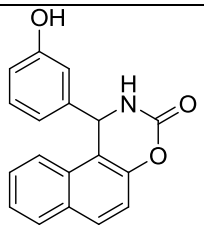
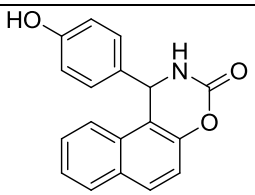
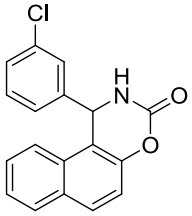
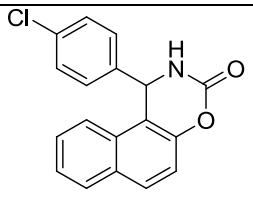
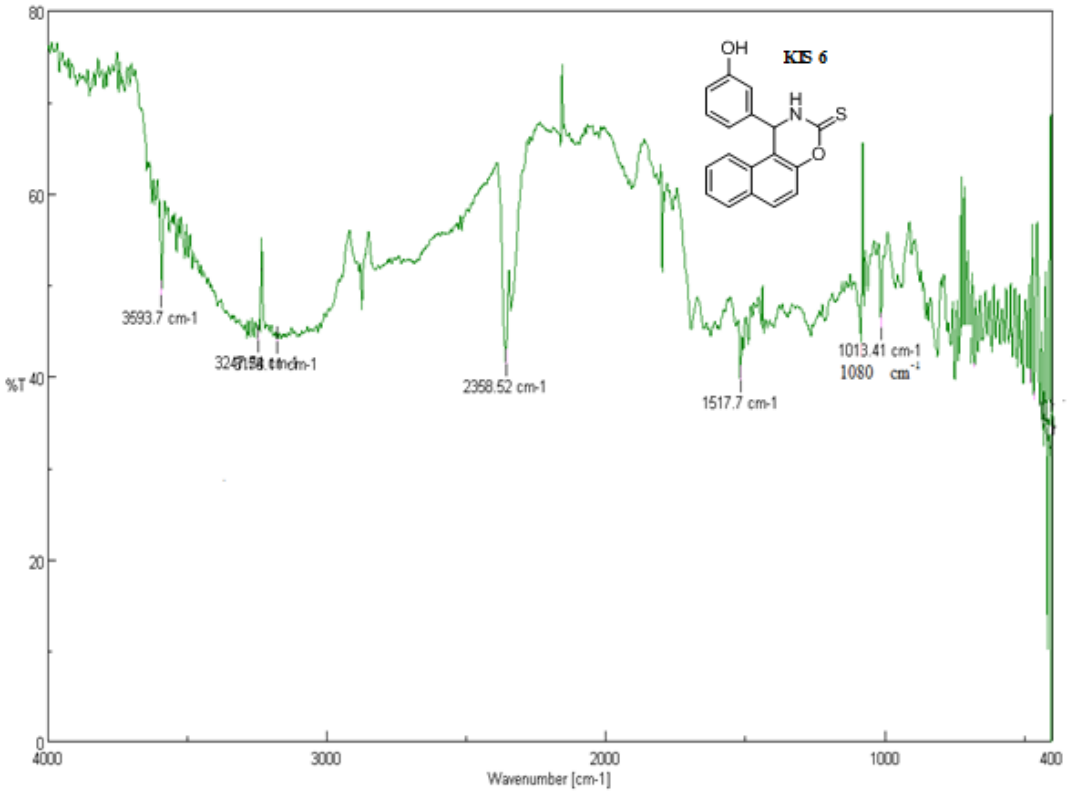
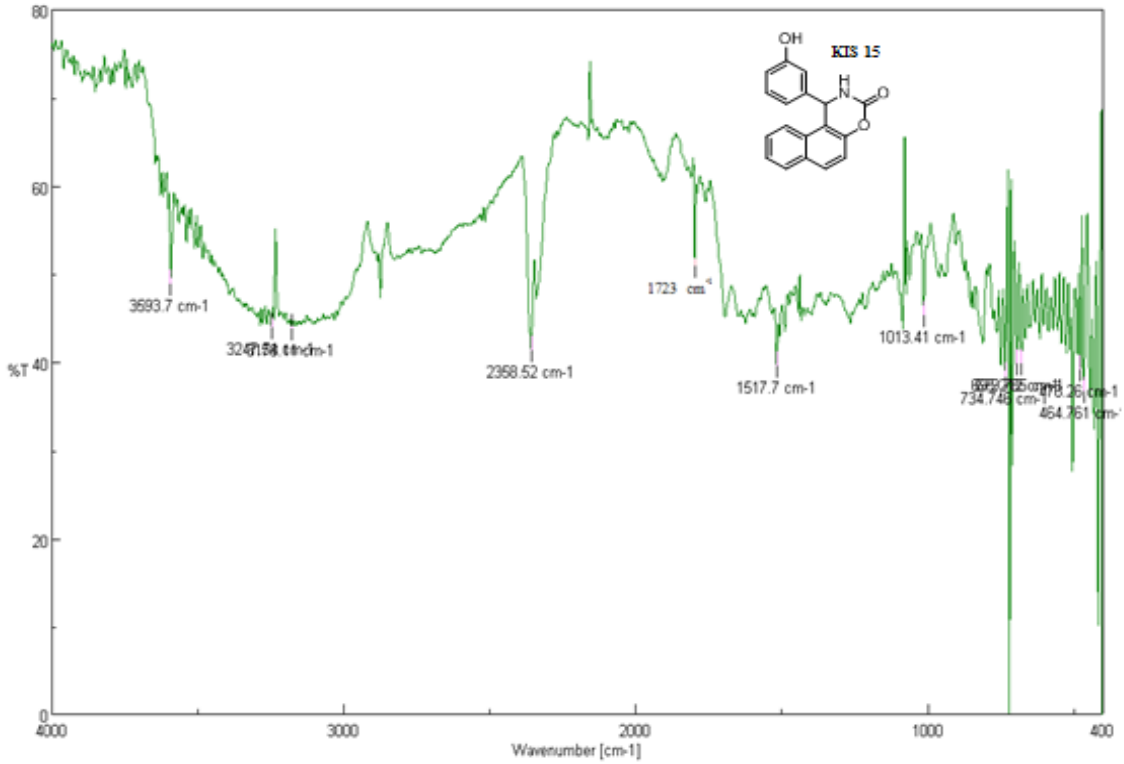
KIS 13		$C_{18}H_{12}N_2O_4$	74.01	0.79	215-219
KIS 14		$C_{18}H_{13}NO_3$	57.18	0.44	165-169
KIS 15		$C_{18}H_{13}NO_3$	49.30	0.63	176-179
KIS 16		$C_{18}H_{13}NO_3$	73.49	0.52	207-211
KIS 17		$C_{18}H_{12}ClNO_2$	83.50	0.61	241-245
KIS 18		$C_{18}H_{12}ClNO_2$	79.43	0.62	178-181

Figure 3.1 Summary of IR Spectral Data of novel KIS 6 compound



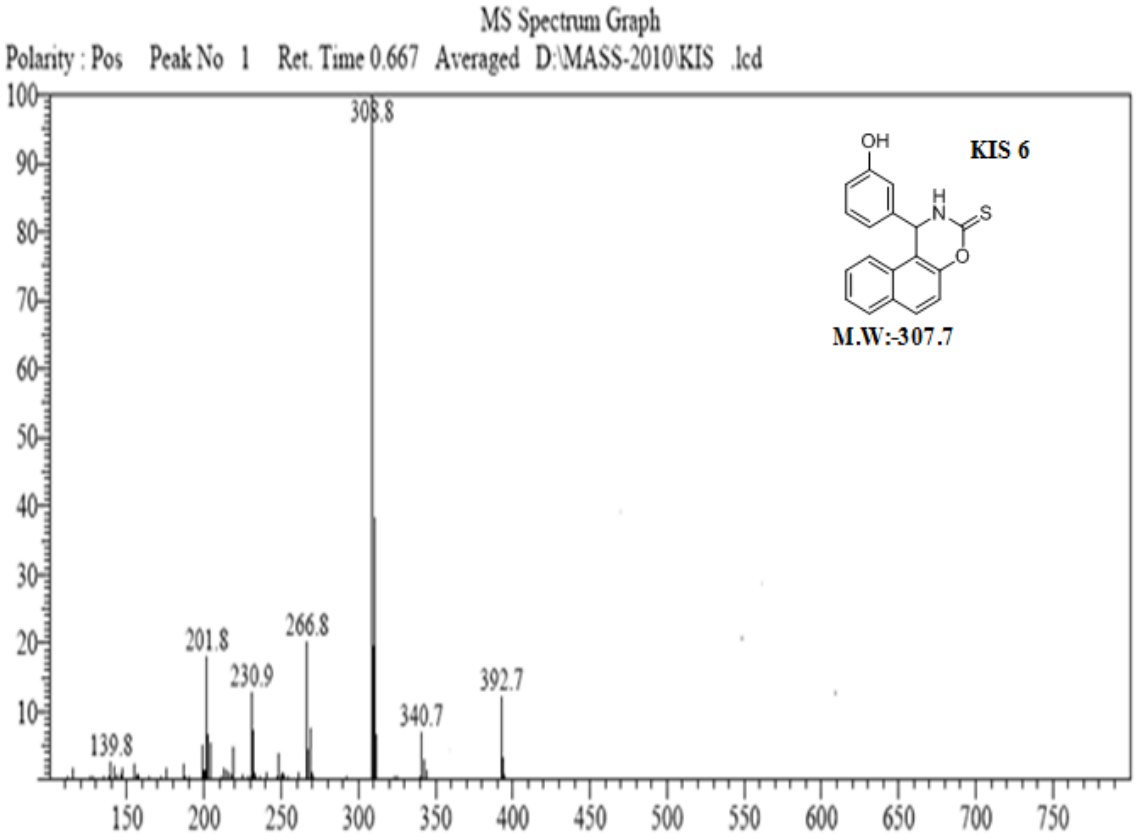
Group	Acquired frequency(cm^{-1})	Expected Frequency(cm^{-1})
C-O str	1013	1300-1000
O-H str	3593	3650-3600
N-H str	3245	3500-3100
C-H str	3160	3150-3050
C=S str	1200	1240-1000
C-N str	1080	1350-1000
N-H(bend)	1580	1640-1550
C=Cstr.(aro.)	1517	1475-1550

Figure 3.2 Summary of IR Spectral Data of novel KIS 15 compound



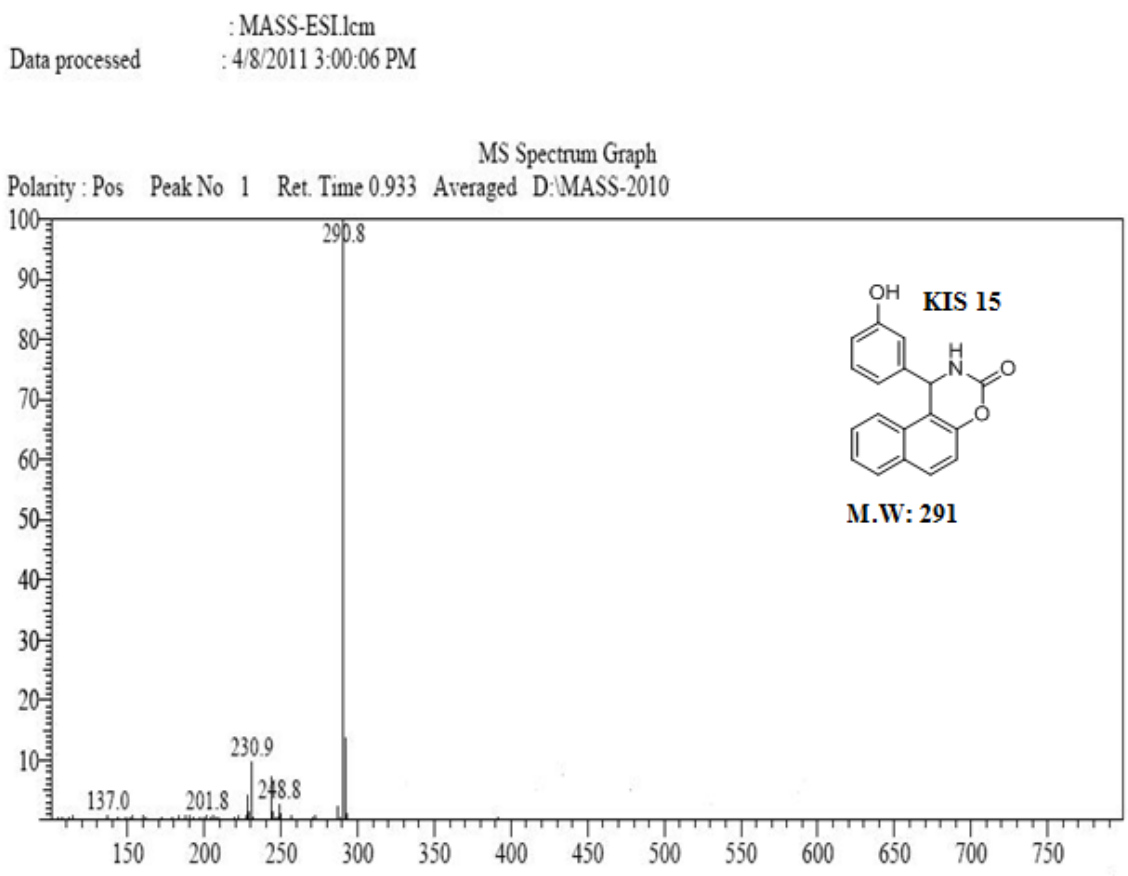
Group	Acquired frequency	Expected Frequency(cm^{-1})
C-O str	1013	1300-1000
O-H str	3593	3650-3600
N-H str	3245	3500-3100
C-H str	3160	3150-3050
C=O str	1723	1725-1705
C-N str	1080	1350-1000
N-H(bend)	1580	1640-1550

Figure 3.3 Mass Spectra of KIS 6



Mass(MS ES) : m/z =308.8 (M+,100%)

Figure 2.4 Mass Spectra of KIS 15



Mass(MS ES) : m/z =290.8 (M-,100%)

3.5 References

1. Sahyoun NR, Lentzner H, Hoyert D, Robinson KN: Trends in causes of death among the elderly. *Aging Trends*, **2002**,1-10

2. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM: Tuberculosis associated with infliximab, a tumour necrosis factor alpha-neutralizing agent. *N Engl J Med*, 345,**2001**,1098-1104,

3.BrajB.Lohray, SundarababuBaskaran, B.Yadi Reddy, K.Srinivas Rao, Enantiospecific Synthesis of 6-Substituted N-Aryl-1,3-Oxazin-2-Ones, *Tetrahedron Letters 39*,**1998**,6555-6556

4 Lal Dhar S. Yadav, Vishnu P. Srivastava,Vijai K. Rai, Rajesh Patel ,Diversity oriented synthesis of fused-ring 1,3-oxazines from carbohydrates as biorenewable feedstocks, *Tetrahedron 64*, **2008**,4246-4253

5 Thomas Kurz, Synthesis of novel pyrido[2,3-e][1,3]oxazines, *Tetrahedron 61*,**2005** 3091–3096

6.Francois-Xavier Le Foulon, Emmanuelle Braud, Frederic Fabis, Jean-Charles Lancelot and Sylvain Rault , Synthesis and combinatorial approach of the reactivity of 6 and 7-arylthieno[3,2-d][1,3]oxazine-2,4-diones *Tetrahedron 59*,**2003,**10051–10057

7 Hua Cao, Huan-Feng Jiang, Chao-Rong Qi, Wen-Juan Yao, Huo-Ji Chen, Brønsted acid- promoted domino reactions: a novel one-pot three-component synthesis of 3,4,5-trisubstituted-3,6-dihydro-2H-1,3-oxazines,*TetrahedronLetters50*,***2009*** 1209–1214

8 Fitzmaurice, R. J.; Etheridge, Z. C.; Jumel, E.; Woolfson, D. N.; Caddick, S. Chem. Commun,**2006** 4814 – 4816

9 Corey, E. J.; Cheng, X.-M. The Logic of Chemical Synthesis; John Wiley and Sons: New York, NY,**1989,**423

10 Hassan Sheibania, Mohammad Hossein Mossleminb, Soheila Behzadib, Mohammad Reza Islamia, Habibollah Foroughi, and Kazem Saidia,A simple and an efficient approach to the synthesis of a specific tautomer of 1,3-thiazinones and 1,3-oxazinones *ARKIVOC*,**2005,**88-96

CHAPTER-4

EXPERIMENT WORK-II

ANTIBACTERIAL EVALUATION OF NOVEL NAPTHOXAZINETHIONES &NAPTHOXAZINONES

4.1 Antibacterial Screening [1,2]

The microbiological assay is based upon a comparison of inhibition of growth of microorganisms by measured concentrations of test compounds with that produced by known concentration of a standard antibiotic. Two methods generally employed are turbidometric (tube-dilution) method and cup-plate method. In the turbidometric method inhibition of growth of microbial culture in a uniform dilution of antibiotic in a fluid medium is measured. It is compared with the synthesized compounds. Here the presence or absence of growth is measured. The cup plate method depends upon diffusion of antibiotic from a vertical cup through a solidified agar layer in a Petridish or plate to an extent such that growth of added micro-organisms is prevented entirely in a zone around the cup containing solution of the antibiotics. The cup-plate method is simple and measurement of inhibition of microorganisms is also easy. Here I have used this method for antibacterial screening of the test compounds.

4.1.1 Preparation of medium

Nutrient agar 2%
Peptone 1%
Beef extract 1%
Sodium chloride
0.5% Distilled water up to 100ml.

All the ingredients were weighed and added to water. This solution was heated on water bath for about one and half-hour till it became clear. This nutrient media was sterilized by autoclave.

4.1.2 Apparatus

All the apparatus like Petridishes, pipettes, glass rods, test-tubes etc. were properly wrapped with papers and sterilized in hot air oven for 1 hour at 180°c.

4.1.3 Culture

Bacterial strain used were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh and that are

1. Gram-positive bacteria

· *Staphylococcus aureus* (MTCC 737)

· *Bacillus subtilis* (MTCC 430)

2. Gram-negative bacteria

· *Escherichia coli* (MTCC 1687)

· *Pseudomonas aeruginosa* (MTCC 2642)

· *Klebsiella pneumoniae* (MTCC 109)

4.1.4 Preparation of Inoculum

In the aseptic condition from the working culture, small amount of culture was transferred to about 10-15 ml of sterile normal saline (0.9% NaCl solution). This solution was gently mixed and used for the antibacterial activity. About 0.5 ml of inoculum was added to the sterilized Petridish and melted agar was added, mixed gently and allowed to solidify. Wells were bored in the agar plate by borer and solution of the compounds was filled in the bore at a constant volume. The solution was allowed to diffuse for a period of 90 minutes. The Petridishes were then incubated at 37 °C for 24 hours after which zone of inhibition was measured.

4.1.5 Preparation of test solution

Specified quantity (10mg) of the compound was accurately weighed and dissolved in 100ml of DMSO and further dilution was made to get the concentration of 25µg/ml, 50µg/ml and 100µg/ml.

4.1.6 Preparation of standard solution

The standard drug ofloxacin was dissolved in appropriate quantity of DMSO to obtain the concentration of 50µg/ml and the zone of inhibition was measured.

4.1.7 Observations

Antibacterial activity of test compounds was recorded in terms of zone of inhibition (in millimeters) shown by each compound against various bacteria.

Table 4.1 Activity of synthesized compounds against *S.Aureus*

Compound code	Concentration (µg /1 ml)	Zone of Inhibition(mm)			
		E1*	E2*	E3*	Mean±SD*
KIS 1	50	10	11	10	10.33±0.57
	100	17	18	17	17.33±0.58
	200	24	25	24	24.33±0.58
KIS 2	50	9	8	9	8.67±0.58
	100	16	16	17	16.33±0.58
	200	23	22	23	22.67±0.58
KIS 3	50	9	8	9	8.67±0.58
	100	16	16	17	16.33±0.58
	200	23	24	23	23.33±0.58
KIS 4	50	10	10	10	10±0.58
	100	17	17	18	17.33±0.00
	200	23	24	24	23.67±0.58
KIS 5	50	10	9	10	9.67±0.58
	100	18	17	17	17.33±0.58
	200	23	22	22	22.33±0.58
KIS 6	50	16	16	17	16.33±0.58
	100	21	22	21	21.33±0.58
	200	27	26	27	26.67±0.58
KIS 7	50	10	11	11	10.67±0.58
	100	18	17	18	17.67±0.58
	200	22	22	21	21.67±0.58
KIS 8	50	14	14	14	14.00±0.58
	100	20	21	20	20.33±0.00
	200	25	26	25	25.33±0.58

KIS 9	50	12	13	12	12.33±0.58
	100	19	20	19	19.33±0.58
	200	24	25	24	24.33±0.58
KIS 10	50	9	10	10	9.67±0.58
	100	15	16	16	15.67±0.58
	200	22	21	22	21.67±0.58
KIS 11	50	11	12	11	11.33±0.58
	100	18	18	17	17.67±0.58
	200	25	26	25	25.33±0.58
KIS 12	50	9	8	9	8.67±0.58
	100	16	16	17	16.33±0.58
	200	23	24	23	23.33±0.58
KIS 13	50	8	8	9	8.33±0.58
	100	15	14	15	14.67±0.58
	200	20	20	21	20.33±0.58
KIS 14	50	11	10	10	10.33±0.58
	100	19	18	18	18.33±0.58
	200	24	24	23	23.67±0.58
KIS 15	50	14	15	16	15.00±0.58
	100	24	24	25	24.33±1.00
	200	28	27	27	27.33±0.58
KIS 16	50	9	10	10	9.67±0.58
	100	17	18	18	17.67±0.58
	200	21	21	22	21.33±0.58
KIS 17	50	17	17	16	16.67±0.58
	100	24	25	25	24.67±0.58
	200	27	28	27	27.33±0.58
KIS 18	50	13	12	13	12.67±0.58
	100	20	19	19	19.33±0.58
	200	25	25	24	24.67±0.58
STD	50	28	29	28	28.33±0.58

* (E1, E2, E3 represents data of 1st, 2nd and 3rd experiment respectively, SD: Standard Deviation)

Figure 4.1

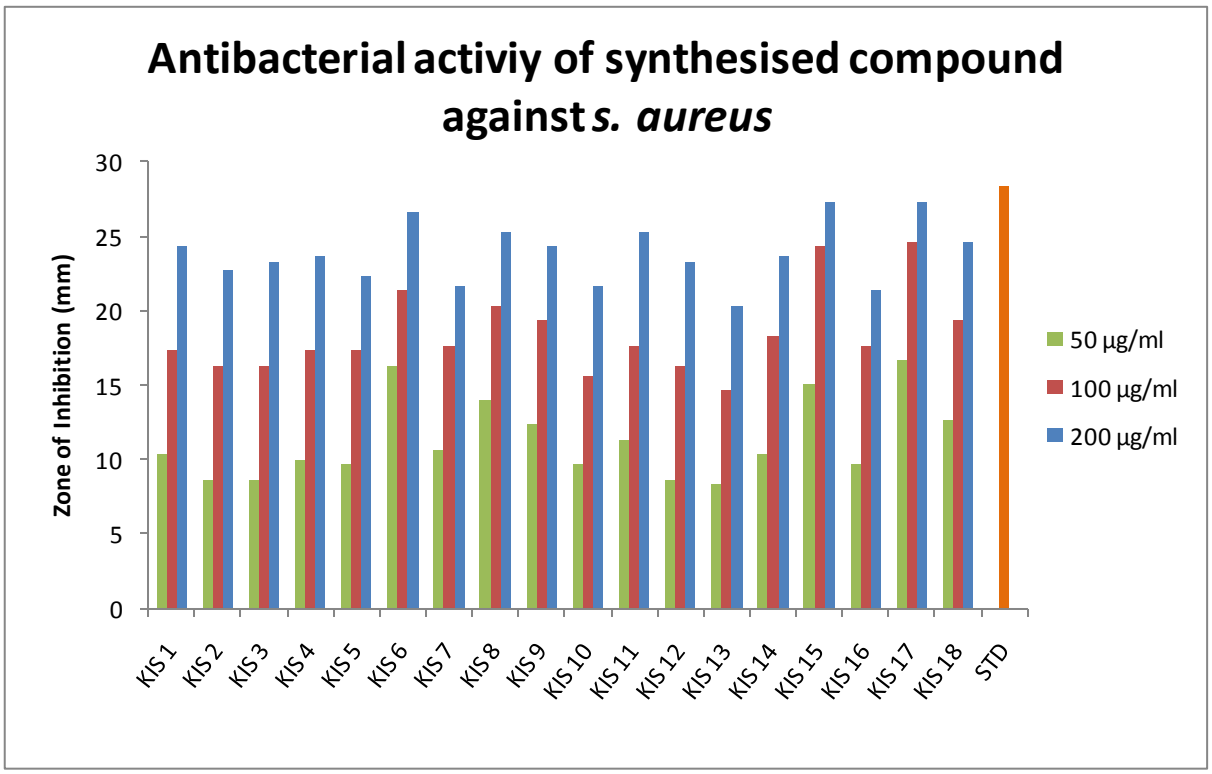

Antibacterial activiy of synthesised compound against s. aureus
Zone of Inhibition (mm)
30
25
20
15
10
5
0
KIS 1
KIS 2
KIS 3
KIS 4
KIS 5
KIS 6
KIS 7
KIS 8
KIS 9
KIS 10
KIS 11
KIS 12
KIS 13
KIS 14
KIS 15
KIS 16
KIS 17
KIS 18
STD
50 µg/ml
100 µg/ml
200 µg/ml

Table 4.2 Activity of synthesized compounds against *E.coli*

Compound code	Concentration (µg /1 ml)	Zone of Inhibition(mm)			
		E1*	E2*	E3*	Mean±SD*
KIS 1	50	9	9	10	9.33±0.58
	100	16	16	17	16.33±0.58
	200	24	23	23	23.33±0.58
KIS 2	50	11	10	11	10.66±0.58
	100	18	18	17	17.66±0.58
	200	25	24	25	24.66±0.58
KIS 3	50	8	8	9	8.33±0.58
	100	14	13	14	13.66±0.58
	200	21	22	21	21.33±0.58
KIS 4	50	8	8	9	8.33±0.58
	100	16	17	16	16.33±0.58
	200	23	24	24	23.66±0.58
KIS 5	50	9	10	9	9.33±0.58
	100	17	16	17	16.66±0.58
	200	21	22	22	21.66±0.58
KIS 6	50	17	17	16	16.66±0.58
	100	22	23	23	22.66±0.58
	200	28	27	27	27.33±0.58
KIS 7	50	9	10	10	9.66±0.58
	100	17	16	16	16.33±0.58
	200	21	21	22	21.33±0.58
KIS 8	50	13	11	13	12.33±0.58
	100	21	20	20	20.33±0.58
	200	25	26	25	25.33±0.58
KIS 9	50	11	12	13	12±1
	100	18	19	19	18.66±0.58
	200	23	24	24	23.66±0.58
KIS 10	50	10	10	10	10

	100	17	16	16	16.33±0.58
	200	23	23	22	22.66±0.58
KIS 11	50	13	12	13	12.66±0.58
	100	17	18	18	17.66±0.58
	200	24	24	25	24.33±0.58
KIS 12	50	10	10	11	10.33±0.58
	100	15	15	16	15.33±0.58
	200	24	23	24	23.66±0.58
KIS 13	50	9	8	8	8.33±0.58
	100	16	15	14	15±1
	200	21	21	20	20.66±0.58
KIS 14	50	10	11	11	10.66±0.58
	100	20	19	19	19.33±0.58
	200	23	24	24	23.66±0.58
KIS 15	50	16	17	16	16.33±0.58
	100	25	24	25	24.66±0.58
	200	27	26	28	27±1
KIS 16	50	8	8	9	8.33±0.58
	100	11	11	11	11
	200	23	24	23	23.33±0.58
KIS 17	50	16	17	16	16.33±0.58
	100	25	24	25	24.66±0.58
	200	28	27	28	27.66±0.58
KIS 18	50	14	13	13	13.33±0.58
	100	21	20	21	20.66±0.58
	200	26	25	26	25.66±0.58
STD	50	28	29	28	28.33±0.58

* (E1, E2, E3 represents data of 1st, 2nd and 3rd experiment respectively, SD: Standard Deviation)

Figure 4.2

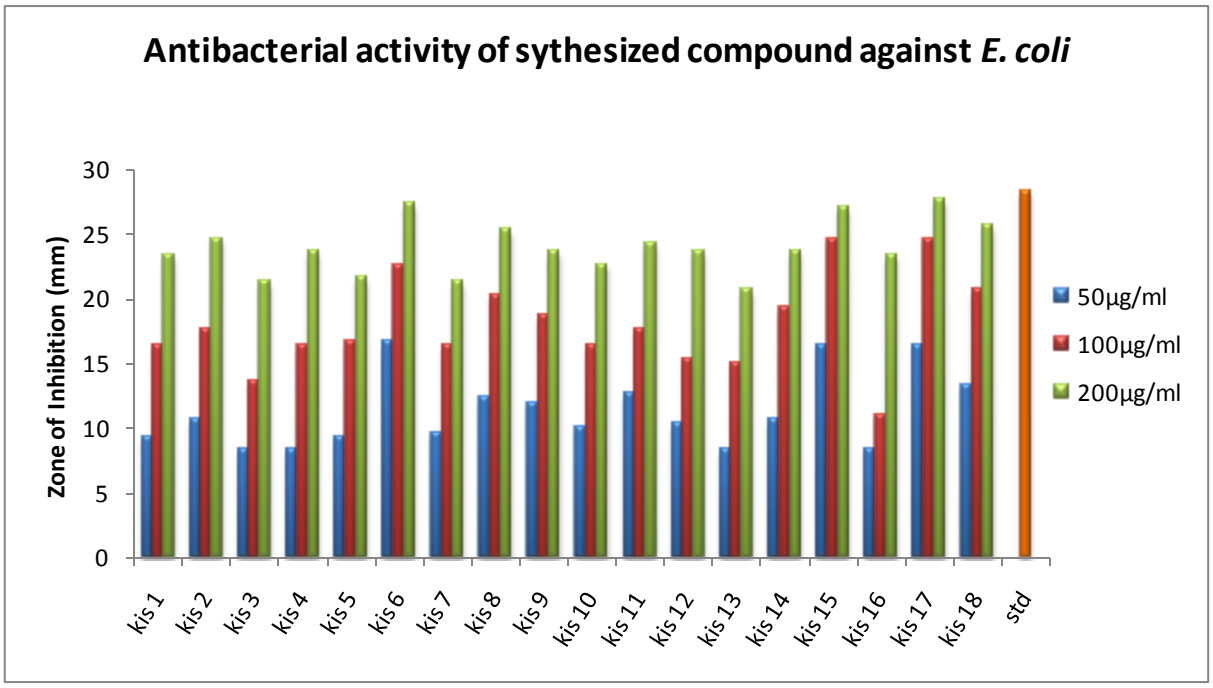

Antibacterial activity of sythesized compound against E. coli
Zone of Inhibition (mm)
30
25
20
15
10
5
0
kis 1
kis 2
kis 3
kis 4
kis 5
kis 6
kis 7
kis 8
kis 9
kis 10
kis 11
kis 12
kis 13
kis 14
kis 15
kis 16
kis 17
kis 18
std
50µg/ml
100µg/ml
200µg/ml

Table 4.3 Activity of synthesized compounds against *K. Pneumoniae*

Compound code	Concentration (µg /1 ml)	Zone of Inhibition(mm)			
		E1*	E2*	E3*	Mean±SD*
KIS 1	50	4	5	4	4.33±0.58
	100	9	8	9	8.66±0.58
	200	16	17	16	16.33±0.58
KIS 2	50	6	5	6	5.66±0.58
	100	11	10	11	10.66±0.58
	200	18	19	18	18.33±0.58
KIS 3	50	5	5	5	5
	100	9	8	9	8.66±0.58
	200	14	14	15	14.33±0.58
KIS 4	50	1	1	1	1
	100	5	5	5	5
	200	10	9	9	9.33±0.58
KIS 5	50	2	2	3	2.33±0.58
	100	5	4	5	4.66±0.58
	200	9	8	9	8.66±0.58
KIS 6	50	9	10	9	9.33±0.58
	100	15	16	15	15.33±0.58
	200	24	23	24	23.67±0.58
KIS 7	50	6	7	6	6.33±0.58
	100	10	10	11	10.33±0.58
	200	16	17	16	16.33±0.58
KIS 8	50	8	9	8	8.33±0.58
	100	14	13	14	13.66±0.58
	200	21	22	21	21.33±0.58
KIS 9	50	7	7	8	7.33±0.58
	100	12	11	11	11.33±0.58
	200	19	18	19	18.66±0.58

KIS 10	50	8	9	8	8.33±0.58
	100	15	15	16	15.33±0.58
	200	21	20	20	20.33±0.58
KIS 11	50	9	9	10	9.33±0.58
	100	13	12	13	12.66±0.58
	200	18	19	18	18.33±0.58
KIS 12	50	6	5	6	5.66±0.58
	100	11	12	11	11.33±0.58
	200	16	17	17	16.66±0.58
KIS 13	50	4	4	5	4.33±0.58
	100	8	9	8	8.33±0.58
	200	15	14	15	14.66±0.58
KIS 14	50	8	9	9	8.66±0.58
	100	13	14	14	13.66±0.58
	200	20	21	21	20.66±0.58
KIS 15	50	10	11	11	10.66±0.58
	100	17	18	17	17.33±0.58
	200	26	26	27	26.33±0.58
KIS 16	50	7	8	7	7.33±0.58
	100	10	11	10	10.33±0.58
	200	21	20	21	20.66±0.58
KIS 17	50	12	13	12	12.33±0.58
	100	23	24	23	23.33±0.58
	200	27	27	28	27.33±0.58
KIS 18	50	9	10	10	9.66±0.58
	100	14	14	15	14.33±0.58
	200	24	25	24	24.33±0.58
STD	50	27	28	27	27.33±0.58

* (E1, E2, E3 represents data of 1st, 2nd and 3rd experiment respectively, SD: Standard Deviation)

Figure 4.3

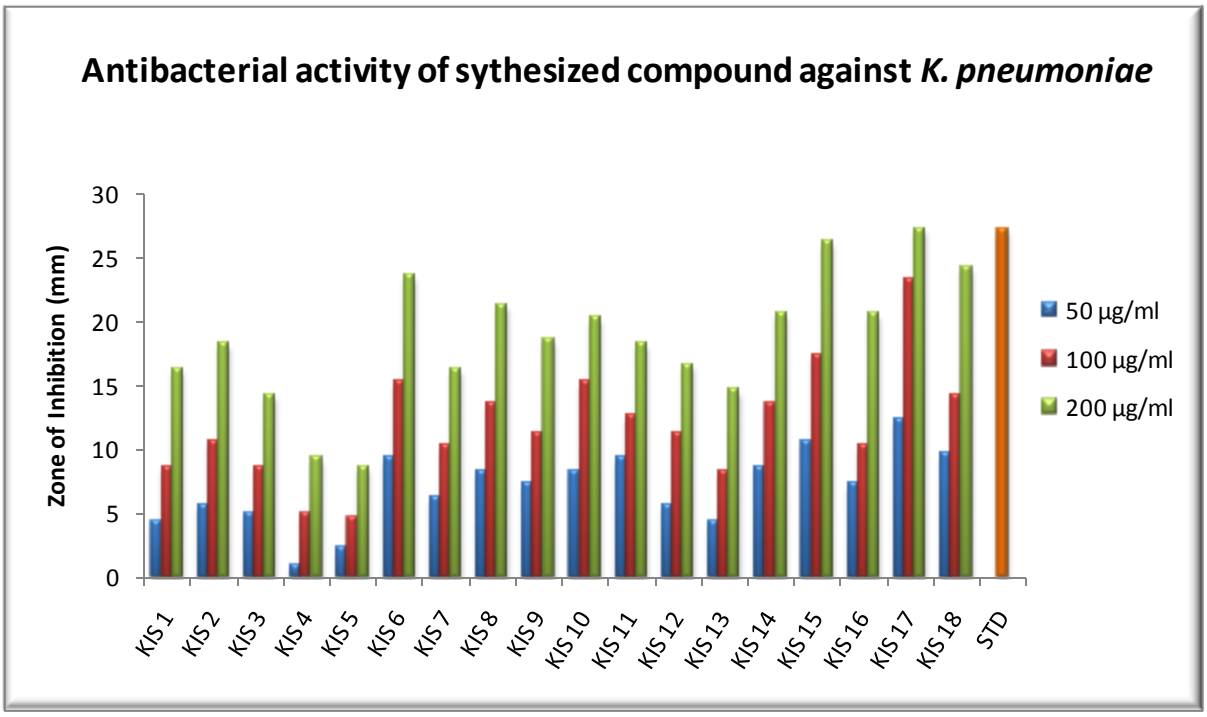

Antibacterial activity of sythesized compound against K. pneumoniae
Zone of Inhibition (mm)
30
25
20
15
10
5
0
KIS 1
KIS 2
KIS 3
KIS 4
KIS 5
KIS 6
KIS 7
KIS 8
KIS 9
KIS 10
KIS 11
KIS 12
KIS 13
KIS 14
KIS 15
KIS 16
KIS 17
KIS 18
STD
50 µg/ml
100 µg/ml
200 µg/ml

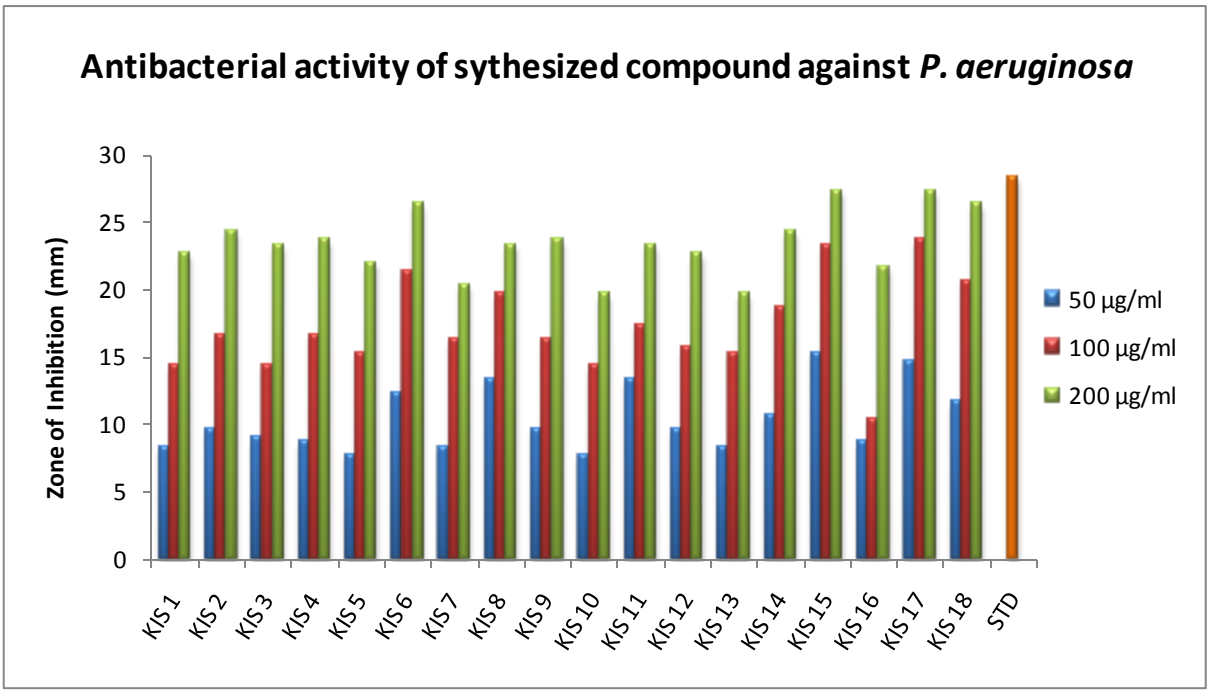
Table 4.4 Activity of synthesized compounds against *P.aeruginosa*

Compound code	Concentration (µg /1 ml)	Zone of Inhibition(mm)			
		E1*	E2*	E3*	Mean±SD*
KIS 1	50	8	9	8	8.33±0.58
	100	14	15	14	14.33±0.58
	200	23	23	22	22.66±0.58
KIS 2	50	10	10	9	9.66±0.58
	100	17	17	16	16.66±0.58
	200	25	24	24	24.33±0.58
KIS 3	50	9	9	9	9
	100	15	14	14	14.33±0.58
	200	24	23	23	23.33±0.58
KIS 4	50	9	8	9	8.66±0.58
	100	17	16	17	16.66±0.58
	200	24	23	24	23.66±0.58
KIS 5	50	8	8	7	7.66±0.58
	100	15	16	15	15.33±0.58
	200	22	22	22	22
KIS 6	50	13	12	12	12.33±0.58
	100	21	21	22	21.33±0.58
	200	26	27	26	26.33±0.58
KIS 7	50	9	8	8	8.33±0.58
	100	16	17	16	16.33±0.58
	200	20	21	20	20.33±0.58
KIS 8	50	13	14	13	13.33±0.58
	100	19	20	20	19.66±0.58
	200	24	23	23	23.33±0.58
KIS 9	50	10	9	10	9.66±0.58
	100	16	17	16	16.33±0.58
	200	24	23	24	23.66±0.58
KIS 10	50	8	7	8	7.66±0.58
	100	15	14	14	14.33±0.58
	200	20	19	20	19.66±0.58
KIS 11	50	13	14	13	13.33±0.58
	100	17	17	18	17.33±0.58
	200	23	24	23	23.33±0.58

KIS 12	50	9	10	10	9.66±0.58
	100	16	15	16	15.66±0.58
	200	23	23	22	22.66±0.58
KIS 13	50	8	8	9	8.33±0.58
	100	16	15	15	15.33±0.58
	200	20	19	20	19.66±0.58
KIS 14	50	10	11	11	10.66±0.58
	100	18	19	19	18.66±0.58
	200	25	24	24	24.33±0.58
KIS 15	50	16	15	15	15.33±0.58
	100	24	23	23	23.33±0.58
	200	27	27	28	27.33±0.58
KIS 16	50	9	8	9	8.66±0.58
	100	10	11	10	10.33±0.58
	200	22	22	21	21.66±0.58
KIS 17	50	15	14	15	14.66±0.58
	100	24	24	23	23.66±0.58
	200	27	28	27	27.33±0.58
KIS 18	50	12	11	12	11.66±0.58
	100	20	21	21	20.66±0.58
	200	27	26	26	26.33±0.58
STD	50	28	29	28	28.33±0.58

* (E1, E2, E3 represents data of 1st, 2nd and 3rd experiment respectively, SD: Standard Deviation)

Figure 4.4


Antibacterial activity of sythesized compound against P. aeruginosa
Zone of Inhibition (mm)
30
25
20
15
10
5
0
KIS 1
KIS 2
KIS 3
KIS 4
KIS 5
KIS 6
KIS 7
KIS 8
KIS 9
KIS 10
KIS 11
KIS 12
KIS 13
KIS 14
KIS 15
KIS 16
KIS 17
KIS 18
STD
50 µg/ml
100 µg/ml
200 µg/ml

4.2 Result and Discussion

The substituted Napthoxazinethiones and substituted Napthoxazinones were evaluated for their in vitro antibacterial activity by agar well diffusion technique using Agar media against 2 gram positive (Staphylococcus aureus (MTCC 737), Bacillus subtilis (MTCC 430)) and 3 gram-negative bacteria (Escherichia coli (MTCC 1687), Pseudomonas aeruginosa (MTCC 2642), Klebsiella pneumonia (MTCC 109) at different concentrations (50 µg/1 ml, 100 µg/1 ml, and 200 µg/1 ml). Ofloxacin (50µg/1) ml was used as standard drug. The bacterial strains were procured from IMTECH, Chandigarh.

Amongst all the compounds

KIS 6(1-(2-Hydroxyphenyl)-1*H*-naphtho[1,2-e][1,3]oxazine3(2*H*)-thione)

KIS 8(1-(3-chlorophenyl)-1*H*-naphtho[1,2-e][1,3]oxazine3(2*H*)-thione),

KIS 11(1-(2-nitrophenyl)-1*H*-naphtho [1,2-e][1,3]oxazin-3(2*H*)-one),

KIS15(1-(3-hydroxyphenyl)-1*H*-naphtho[1,2-e][1,3]oxazin-3(2*H*)-one),

KIS17(1-(3-chlorophenyl)-1*H*-naphtho[1,2-e][1,3]oxazin-3(2*H*)-one) active against all bacteria and shows greater activity.

KIS4(1-(4-Nitrophenyl)-1*H*-naphtho[1,2-e][1,3]oxazine3(2*H*)-thione),

KIS5(1-(2-Hydroxyphenyl)-1*H*-naphtho[1,2-e][1,3]oxazine3(2*H*)-thione)

shows less activity against K. Pneumoniae.

Remaining compounds shows moderate activity

4.3 References

1. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. *Clinical Microbiology and Infection* **2000**; 6:509-515.

2. H. Richardson, AHE-S, B. W. Senior. Agar Diffusion Method for the Assay of Colicins. *Applied Microbiology* **1968**:1468-1474.

CHAPTER-5

SUMMARY

5. 1 Summary

Bacteria causing serious hospital-acquired infections are becoming resistant to a larger number of available antimicrobials and the remaining therapies are less effective. Resistance to bacterial strain is becoming a challenge, which needs to be addressed immediately. There is a growing need to develop novel defence mechanisms and novel strategies to treat bacterial infections. Highly resistant isolates of such bacteria as *Staphylococcus aureus*, *Enterococci, Enterobacter*, and *Pseudomonas* are becoming more prevalent in hospitals yet there are few new drugs in development that target these pathogens. To successfully tackle antibacterial resistance, novel targets are only part of the challenges which also need to be addressed include new developments with gram-negative bacteria, predicting toxicology, selectivity, resistance, permeability and pharmacokinetics of new leads, as well as creating new models for optimization of leads and compounds. Soon after each antibacterial agent entered into clinical practice, resistance was reported in atleast one bacterial pathogen. Because the first antibiotics, excluding the synthetic sulfa drugs, were all identified or derived from natural products, resistance determinants had already accumulated in the environments from which these agents originated. Selection of resistant strains occurred so quickly for some bacteria-antibiotic combinations that clinical utility of the antibiotic was severely diminished within a 5-year time span. The first documented example of the rapid selection of a resistant population was the increase in penicillin resistance from < 8% to almost 60% in *Staphylococcus aureus* from 1945 to 1949. Intense investigation is being carried out on Pyrimidine compounds owing to their wide spectrum of activity. In the recent years many article shows that Napthlene and 1,3 oxazine containing compounds give good antibacterial activity. Prompted by these observations, as a part of present study aimed at developing new biologically active substituted Naphoxazinethiones and Napthoxazinones were synthesized. The target molecules were synthesized according to the steps reported in literature and all the reaction steps were optimized in context of present study. Intermediates, Benzyledenethiourea & Benzylideneurea were obtained by reaction of substituted aldehyde with Thiourea and Urea in ethanol. This further reaction with napthalene-2-ol to gave substituted Napthoxazinethione & Napthoxazinones respectively.

The structures of the synthesized compounds were established by MASS and IR spectroscopic techniques. The data of the physical characterization of the compounds are as shown in table 5.1

Table 5.1 Summary of The Physical Data of the Novel compounds

Compound Code	Chemical formula	% Yield	R_f Value	Melting Point(^{0}C)
KIS 1	$C_{20}H_{17}NO_3S$	62.25	0.43	200-205
KIS 2	$C_{18}H_{12}N_2O_3S$	57.31	0.49	190-193
KIS 3	$C_{18}H_{12}N_2O_3S$	50.11	0.53	180-183
KIS 4	$C_{18}H_{12}N_2O_3S$	64.11	0.43	218-222
KIS 5	$C_{18}H_{13}NO_2S$	75.11	0.39	188-192
KIS 6	$C_{18}H_{13}NO_2S$	65.68	0.37	205-207
KIS 7	$C_{18}H_{13}NO_2S$	74.15	0.64	230-234
KIS 8	$C_{18}H_{12}ClNOS$	81.09	0.6	201-205
KIS 9	$C_{18}H_{12}ClNOS$	84.74	0.5	232-235
KIS 10	$C_{20}H_{17}NO_4$	64.74	0.32	172-175
KIS 11	$C_{18}H_{12}N_2O_4$	48.74	0.31	194-198
KIS 12	$C_{18}H_{12}N_2O_4$	67.76	0.41	234-238
KIS 13	$C_{18}H_{12}N_2O_4$	74.01	0.79	215-219
KIS 14	$C_{18}H_{13}NO_3$	57.18	0.44	165-169
KIS 15	$C_{18}H_{13}NO_3$	49.30	0.63	176-179
KIS 16	$C_{18}H_{13}NO_3$	73.49	0.52	207-211
KIS 17	$C_{18}H_{12}ClNO_2$	83.50	0.61	241-245
KIS 18	$C_{18}H_{12}ClNO_2$	79.43	0.62	178-181

SUMMARY

The substituted Napthoxazinethiones and substituted Napthoxazinones were evaluated for their in vitro antibacterial activity by agar well diffusion technique using Agar media against 2 gram positive (Staphylococcus aureus (MTCC 737), Bacillus subtilis (MTCC 430)) and 3 gram-negative bacteria (Escherichia coli (MTCC 1687), Pseudomonas aeruginosa (MTCC 2642), Klebsiella pneumonia (MTCC 109) at different concentrations (50 µg/1 ml, 100 µg/1 ml, and 200 µg/1 ml). Ofloxacin (50µg/1) ml was used as standard drug. The bacterial strains were procured from IMTECH, Chandigarh.

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shows less activity against K. Pneumoniae.

Remaining compounds shows moderate activity