

SYNTHESIS AND STUDY OF HETEROCYCLIC COMPOUNDS AS POSSIBLE ANTI-MICROBIAL AGENTS

**THESIS SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR THE
PH.D. (SCIENCE) DEGREE IN
ORGANIC CHEMISTRY**

SUBMITTED BY

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UNDER THE GUIDANCE OF

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MUMBAI – 400 012**

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**SYNTHESIS AND STUDY OF HETEROCYCLIC
COMPOUNDS AS POSSIBLE ANTI-MICROBIAL
AGENTS**

A Thesis submitted
To the
University of Mumbai
For the
Ph. D.(Science) Degree
In Organic Chemistry

Submitted By
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STATEMENT BY THE CANDIDATE

As required by the University Ordinance 770, I wish to state that the work embodied in this thesis titled “Synthesis And Study Of Heterocyclic Compounds As Possible Anti-Microbial Agents” forms my own contribution to the research work carried out under the guidance of Dr. Anil S. Bobade, at the Haffkine Institute For Training, Research & Testing. This work has not been submitted for any other degree of this or any other University. Whenever reference has been made to previous works of others, it has been clearly indicated as such and included in the Bibliography.

Mr. Ashish Lawoo Asrondkar

Certified by,

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Assistant Director,
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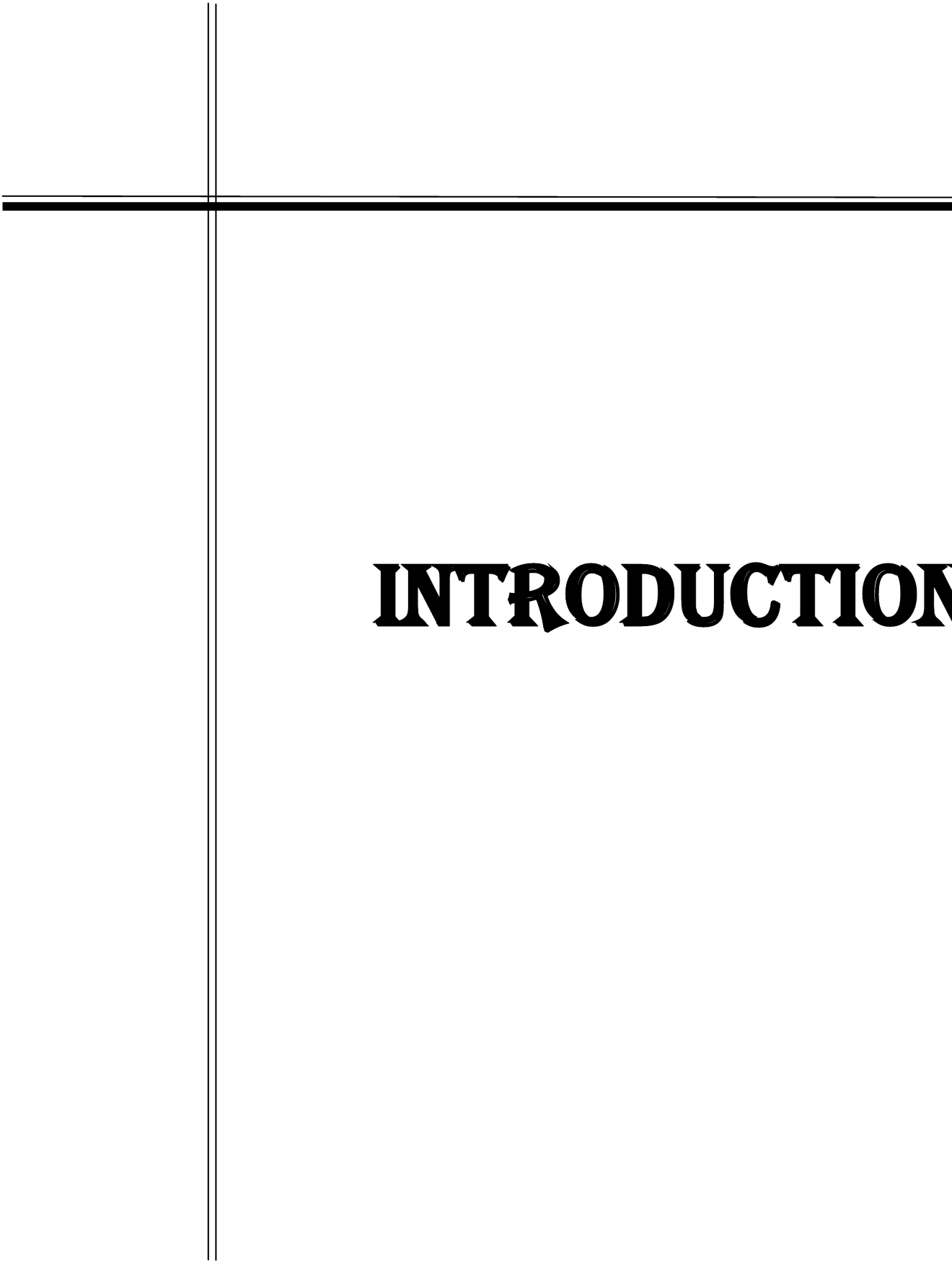
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LIST OF CHROMATOGRAM

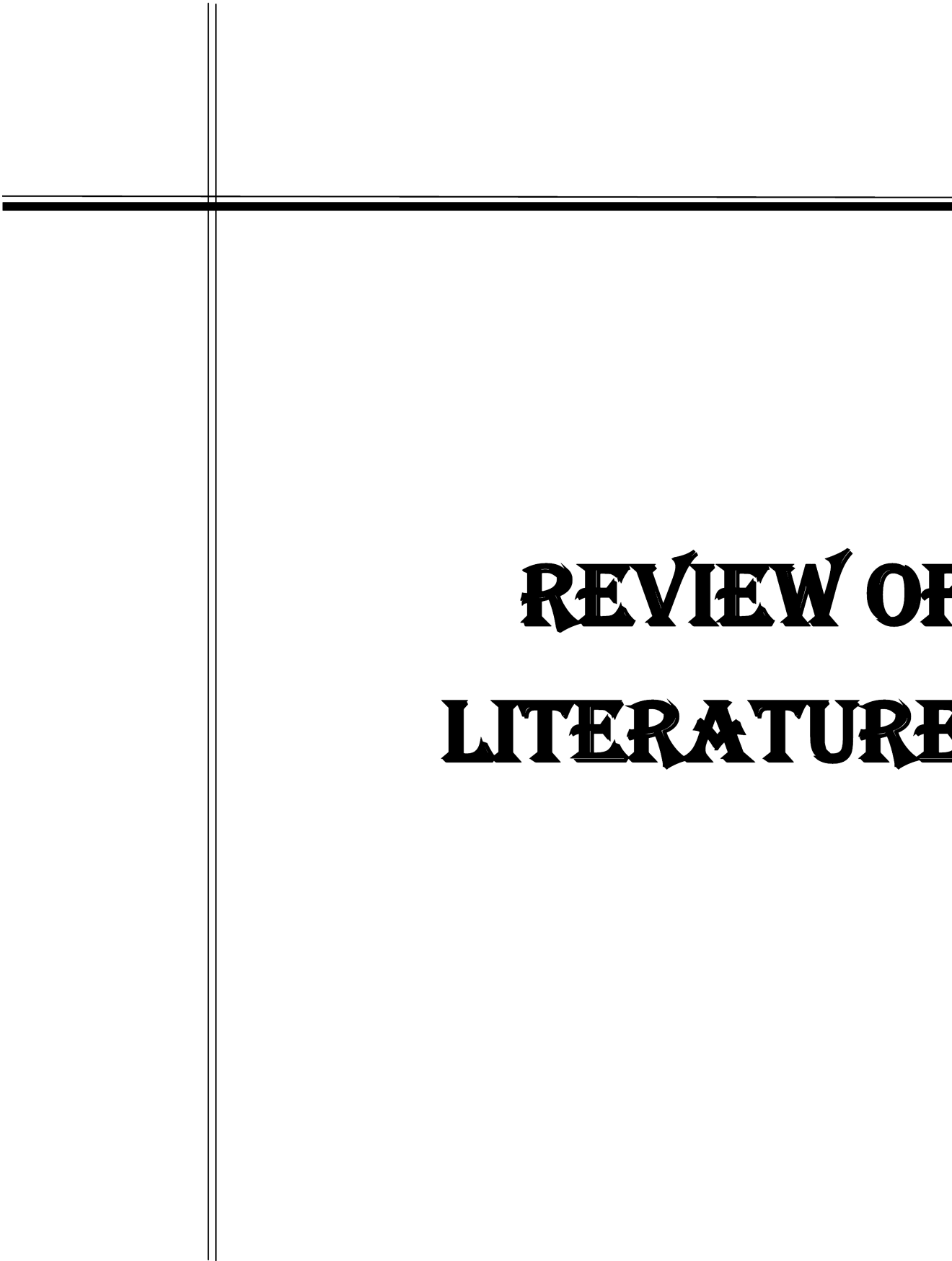
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List of Abbreviations

G	Gram
mL	Milliliter
M	Molar
μg	Microgram
Mg	Milligram
μL	Microliter
%	Percentage
Min	Minute
Hr	Hour
EC50	Effective Concentration
g/mol	Gram per mole
μg/ml	Microgram per milliliter
ml/min	Milliliter per minute
μl/ml	Microliter per milliliter
°C	Degree Celsius
m/z	Mass to charge ratio
Mol.Wt.	Molecular weight
EQUIPMENTS AND INSTRUMENTS	
TLC	Thin layer chromatography
IR	Infrared
FTIR	Fourier transform infrared spectroscopy
UV	Ultraviolet
HPLC	High performance liquid chromatography
GCMS	Gas chromatography mass spectrometry
NMR	Nuclear Magnetic Resonance spectroscopy
CHEMICALS AND MATERIALS	
NH ₂ NH ₂	Hydrazine hydrate
NaCl	Sodium chloride
KBr	Potassium chloride
NaH ₂ PO ₄	Sodium dihydrogen phosphate
Na ₂ HPO ₄	Disodium hydrogen phosphate
DFS	Diclofenac sodium
DPPH	2,2-Diphenyl-1-picrylhydrazyl
K ₂ CO ₃	Potassium carbonate
CS ₂	Carbon disulphide
KOH	Potassium hydroxide
HCl	Hydrochloric acid
POCl ₃	Phosphoryl oxychloride
DMF	Dimethyl formamide
NaHCO ₃	Sodium Bicarbonate
DMSO	Dimethyl sulphoxide
BHT	Butylated Hydrogen Toluene

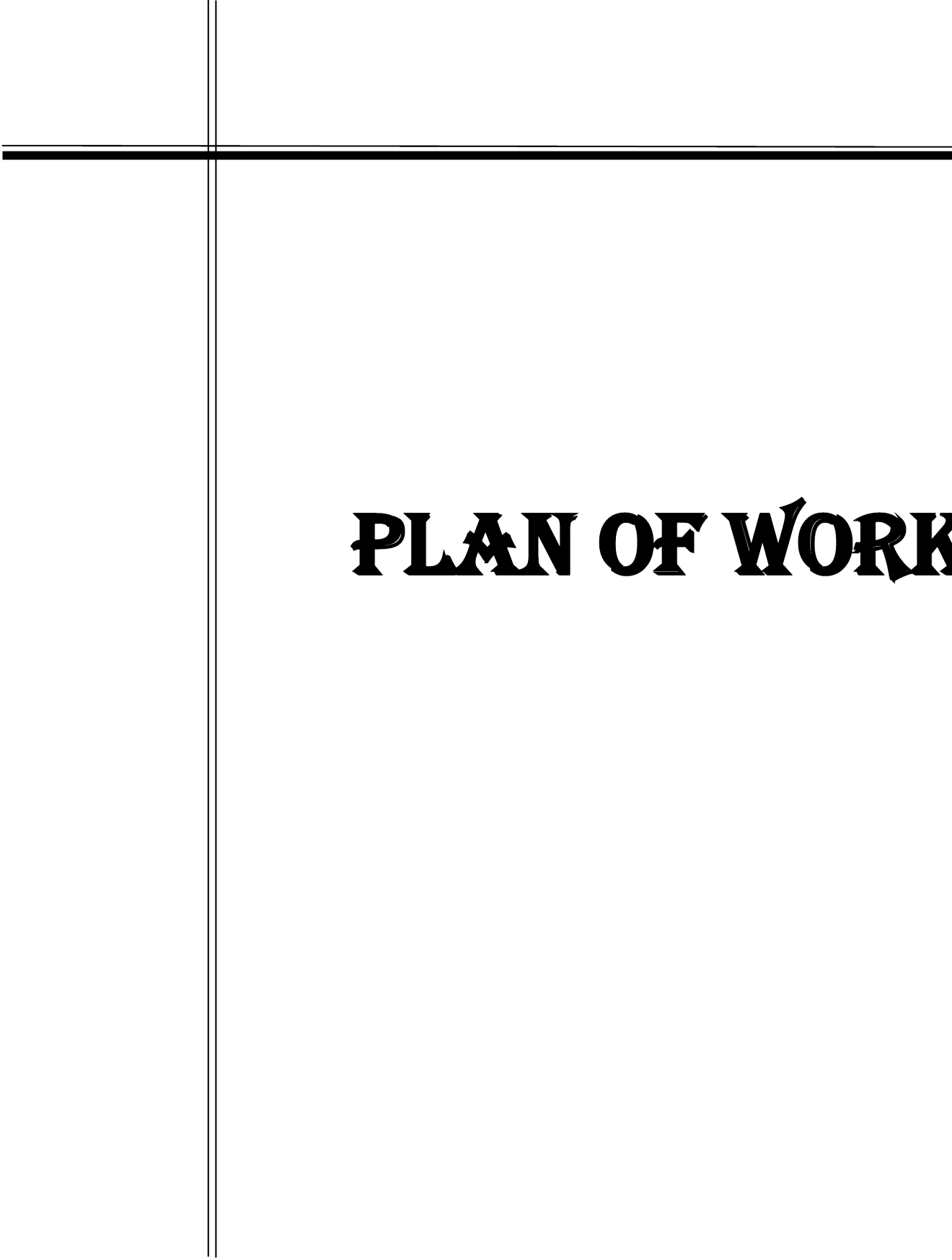


INTRODUCTION

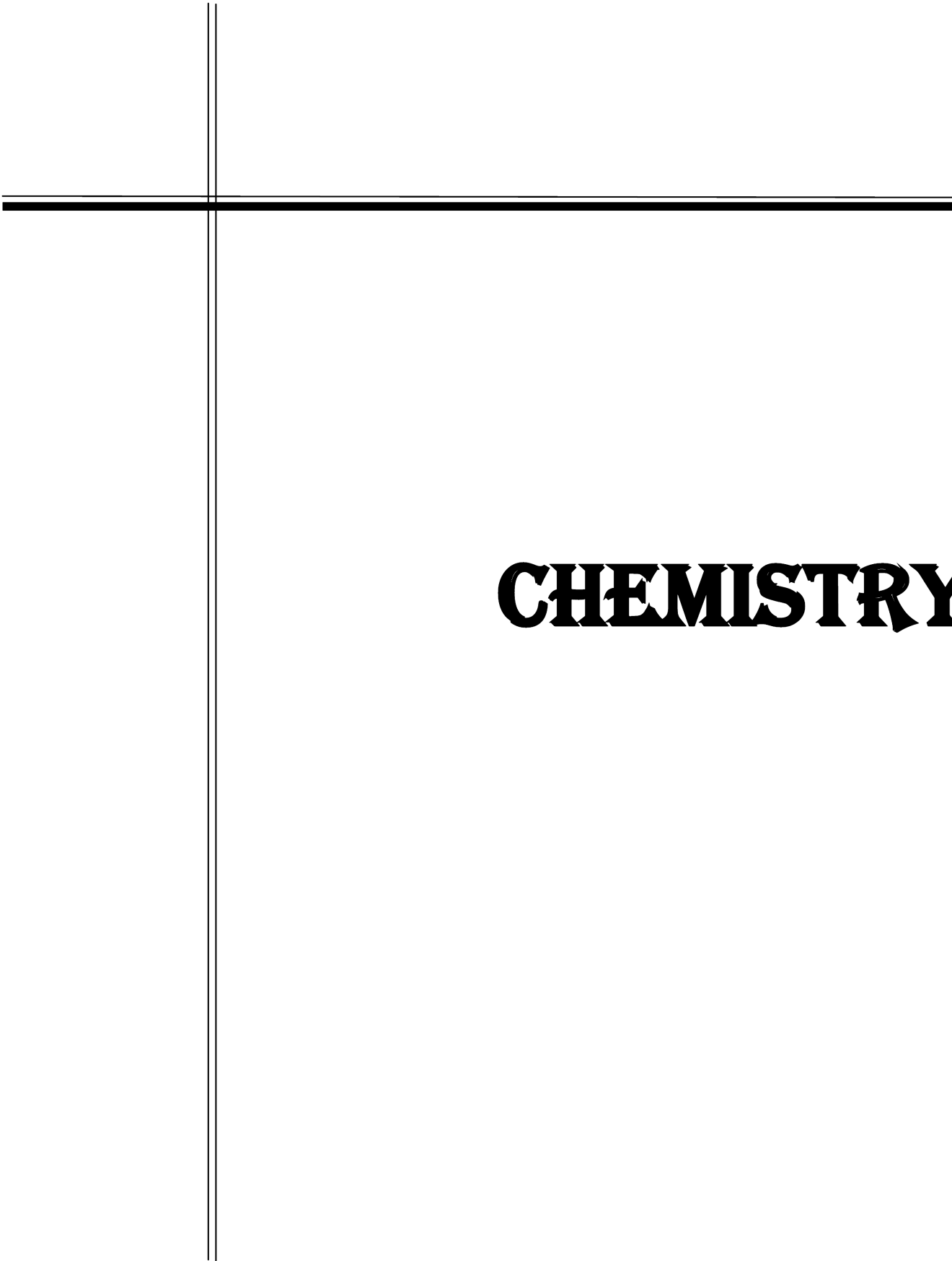


REVIEW OF LITERATURE

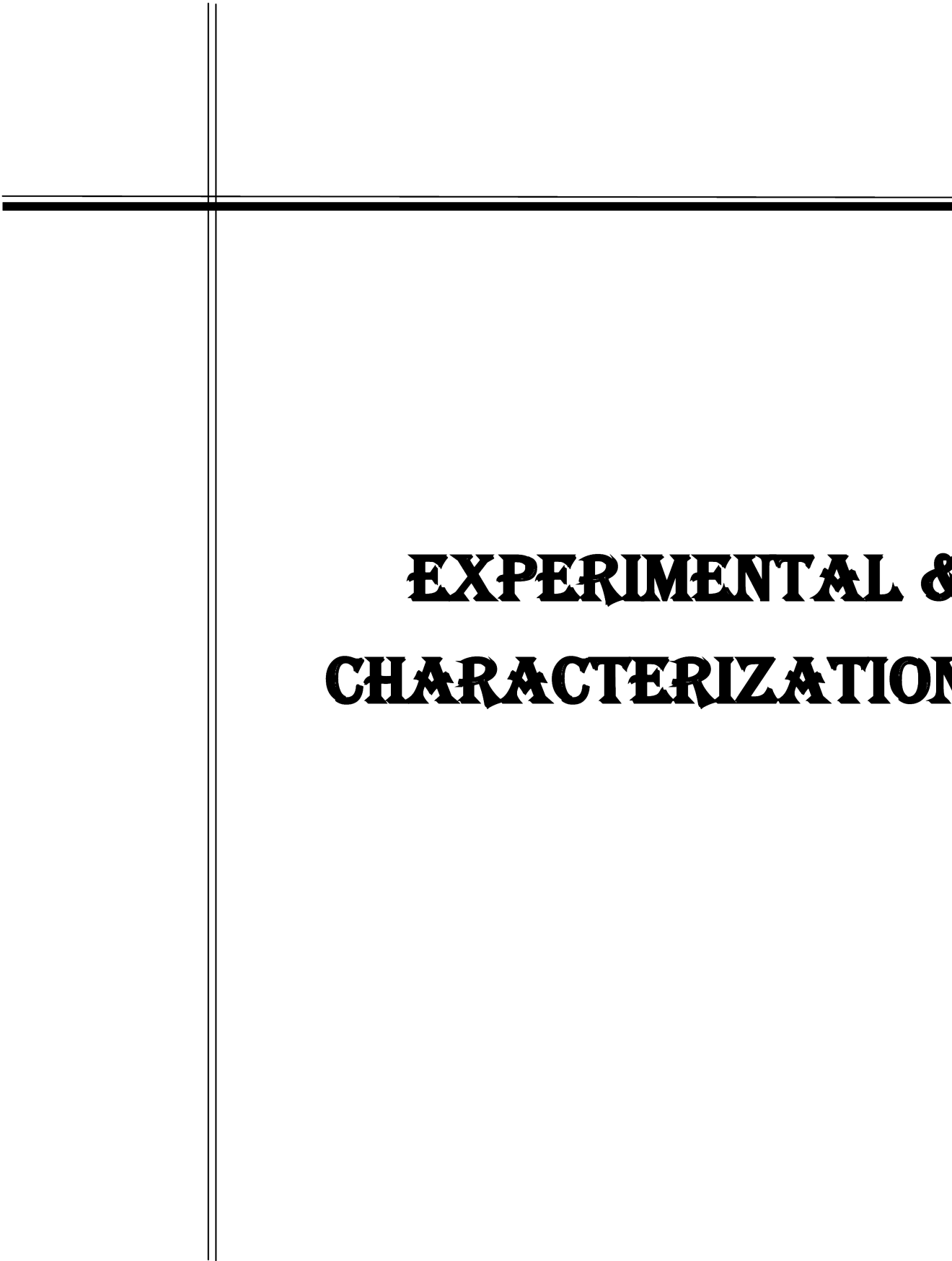
Dedicated to my
Aai- Pappa
& Swara, Stuti

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PLAN OF WORK



CHEMISTRY

A decorative crosshair consisting of a vertical line and a horizontal line, both composed of two parallel lines, intersecting in the upper left quadrant of the page.

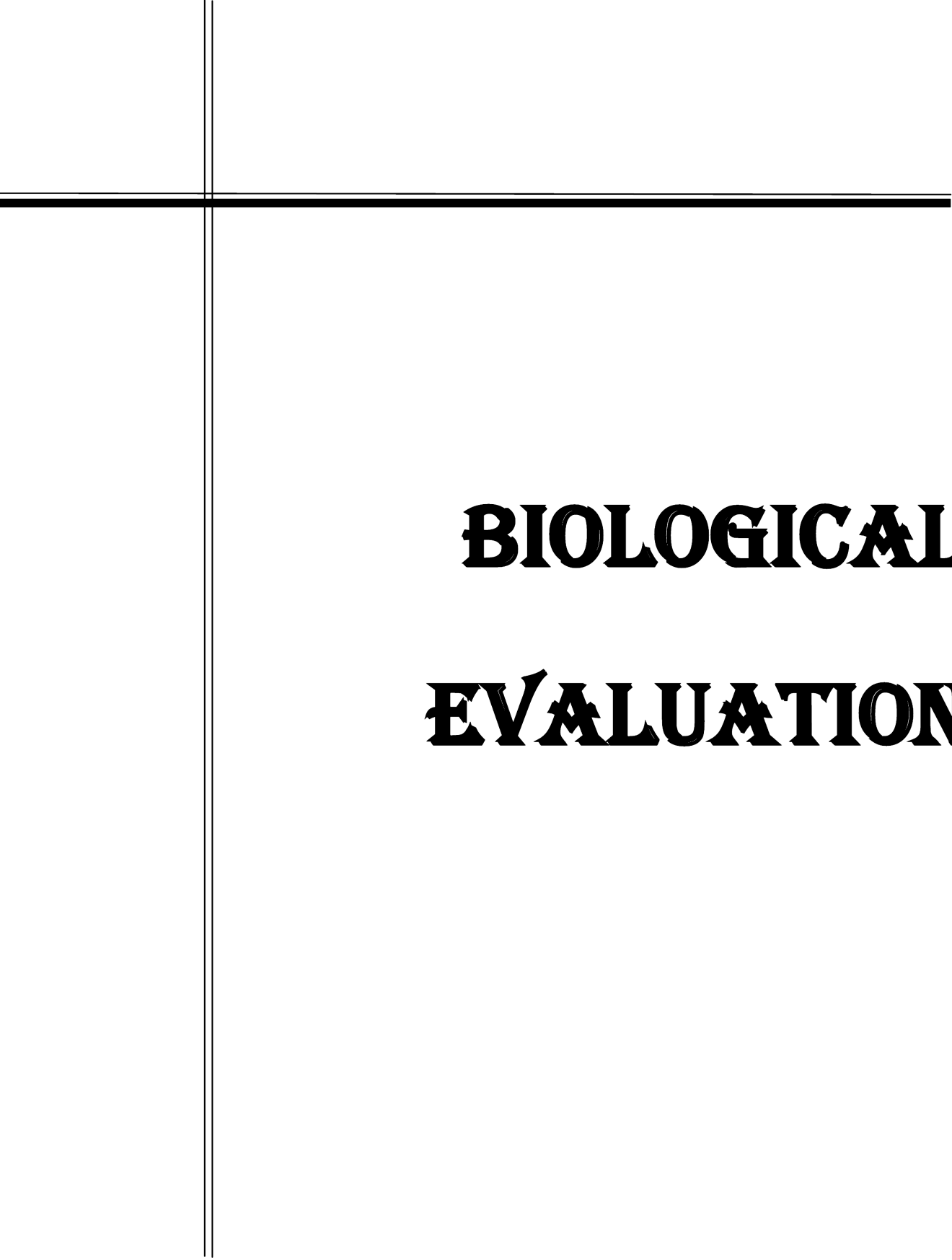
EXPERIMENTAL & CHARACTERIZATION

**SYNTHESIS OF 2-{[(6- SUBSTITUTED
PHENYL [1,2,4]TRIAZOLO[3,4-*B*]
[1,3,4] THIADIAZOL-3-YL) METHYL]
SULFANYL}-1,3-BENZOTHAZOLE**

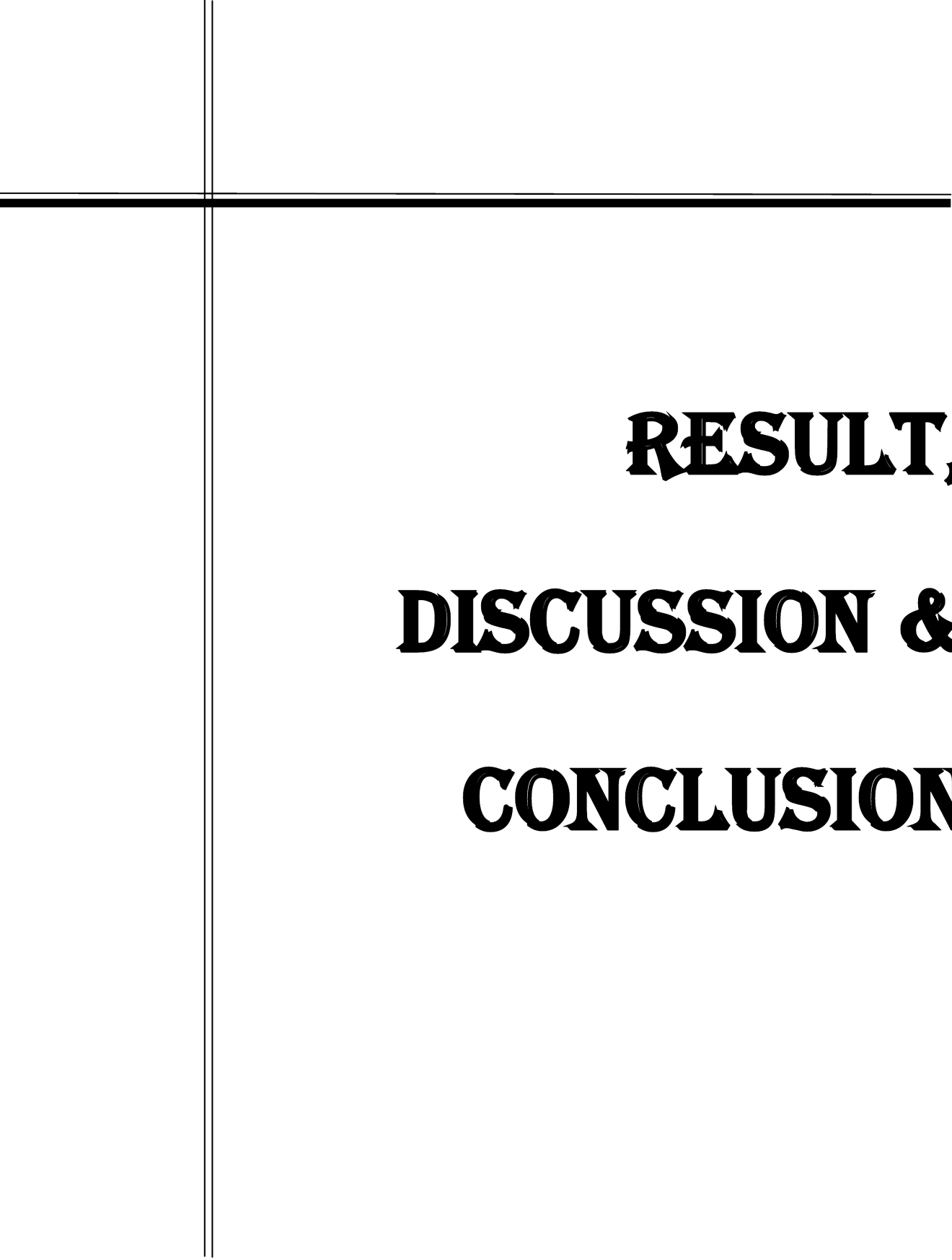
**SYNTHESIS OF *N*[(6,7-DIMETHYL
[1,2,4] TRIAZOLO [3,4-*B*] [1,3,4]
THIADIAZEPIN-3-YL) METHYL] -
SUBSTITUTED 1,3-BENZOTRIAZOL-2-
AMINE**

**SYNTHESIS OF S-{5-[(1,3-
BENZOTHAZOL-2-YLSULFANYL)
METHYL] -1,3,4-OXADIAZOL -2-YL}
SUBSTITUTED
BENZENECARBOTHIOATE**

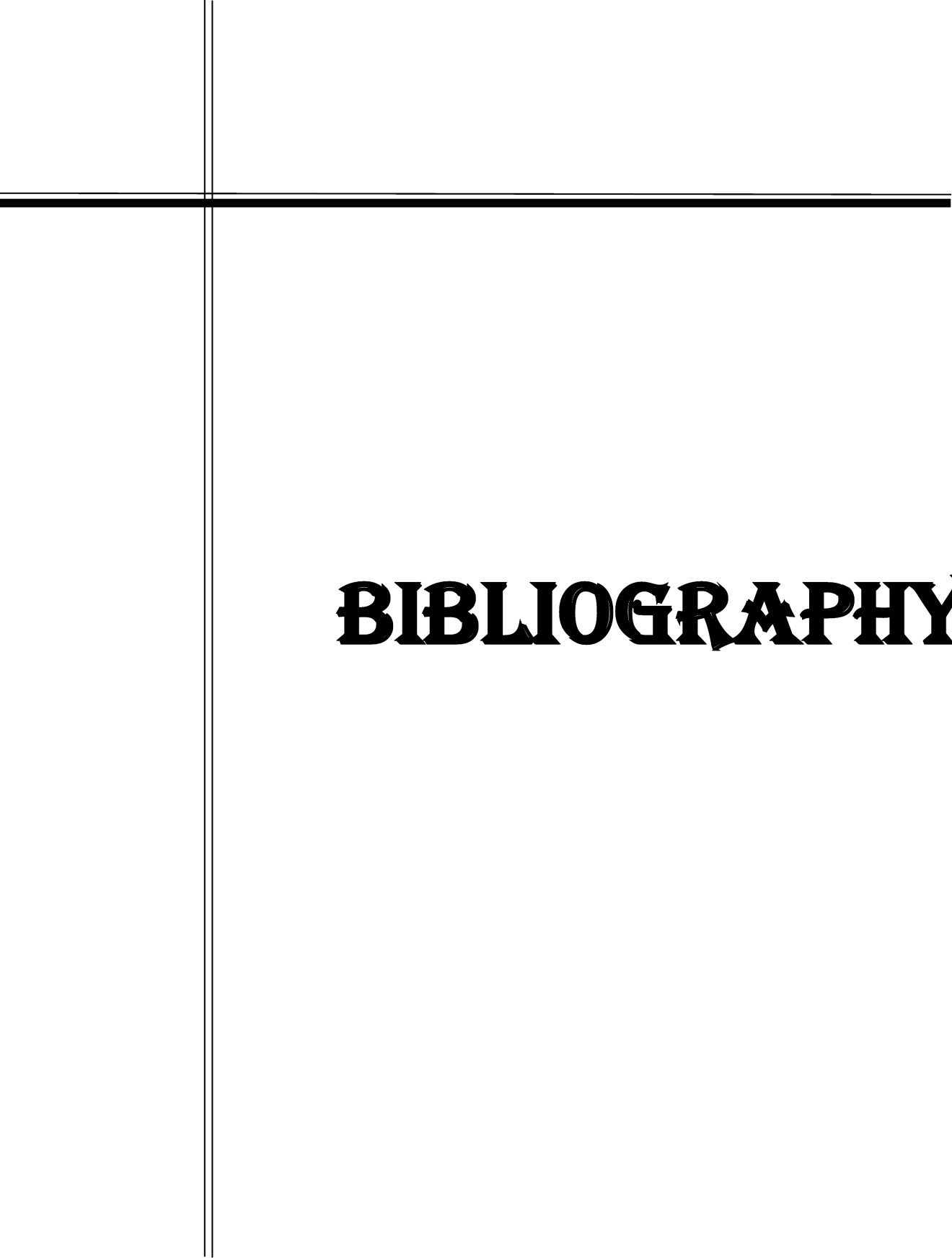
**SYNTHESIS OF 4-{[(*E*)-
(SUBSTITUTED-4-OXO-4*H*-CHROMEN
-3-*YL*)METHYLYDENE]AMINO}-*N*-
(QUINOXALIN-2-*YL*) BENZENE
SULFONAMIDE**



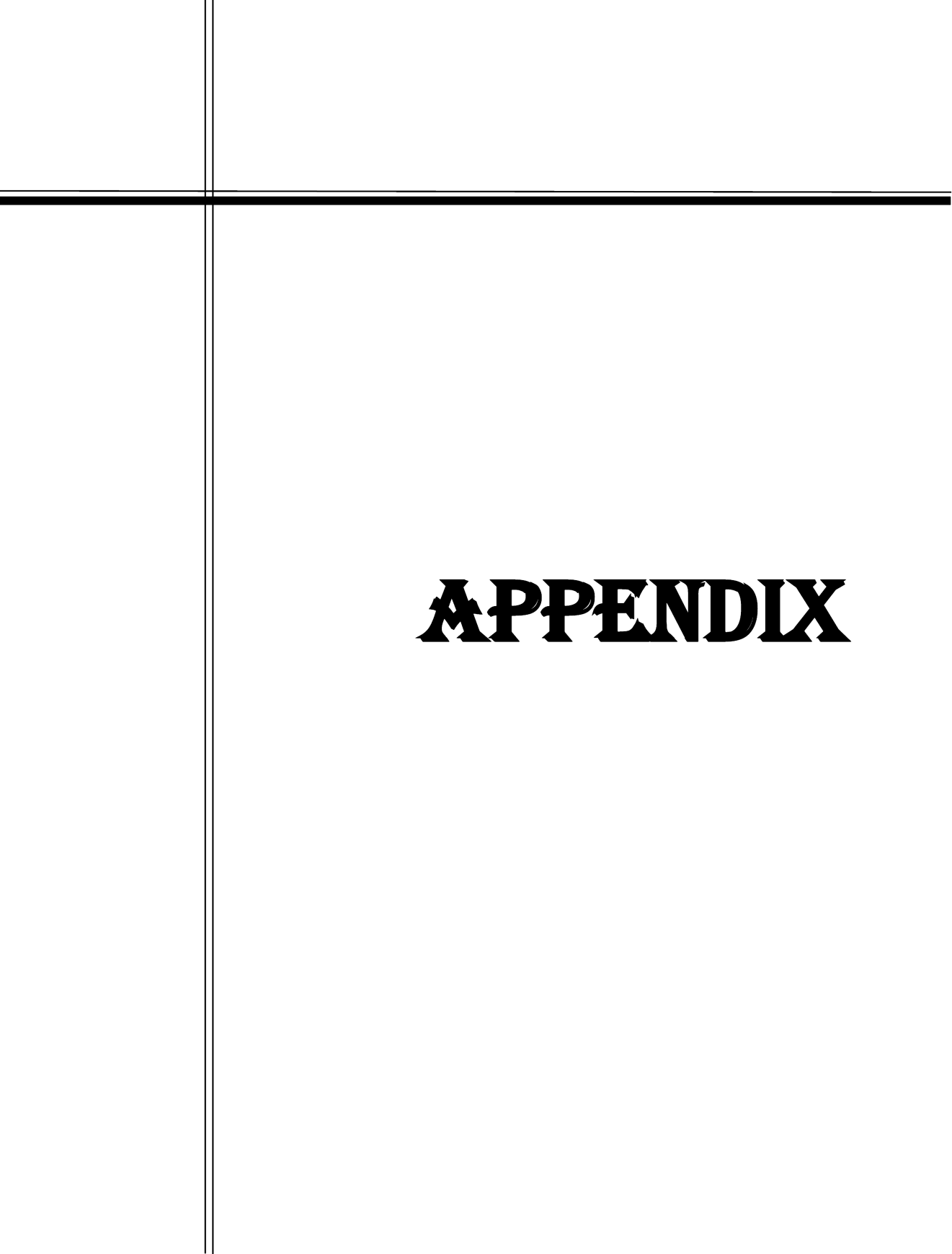
BIOLOGICAL EVALUATION



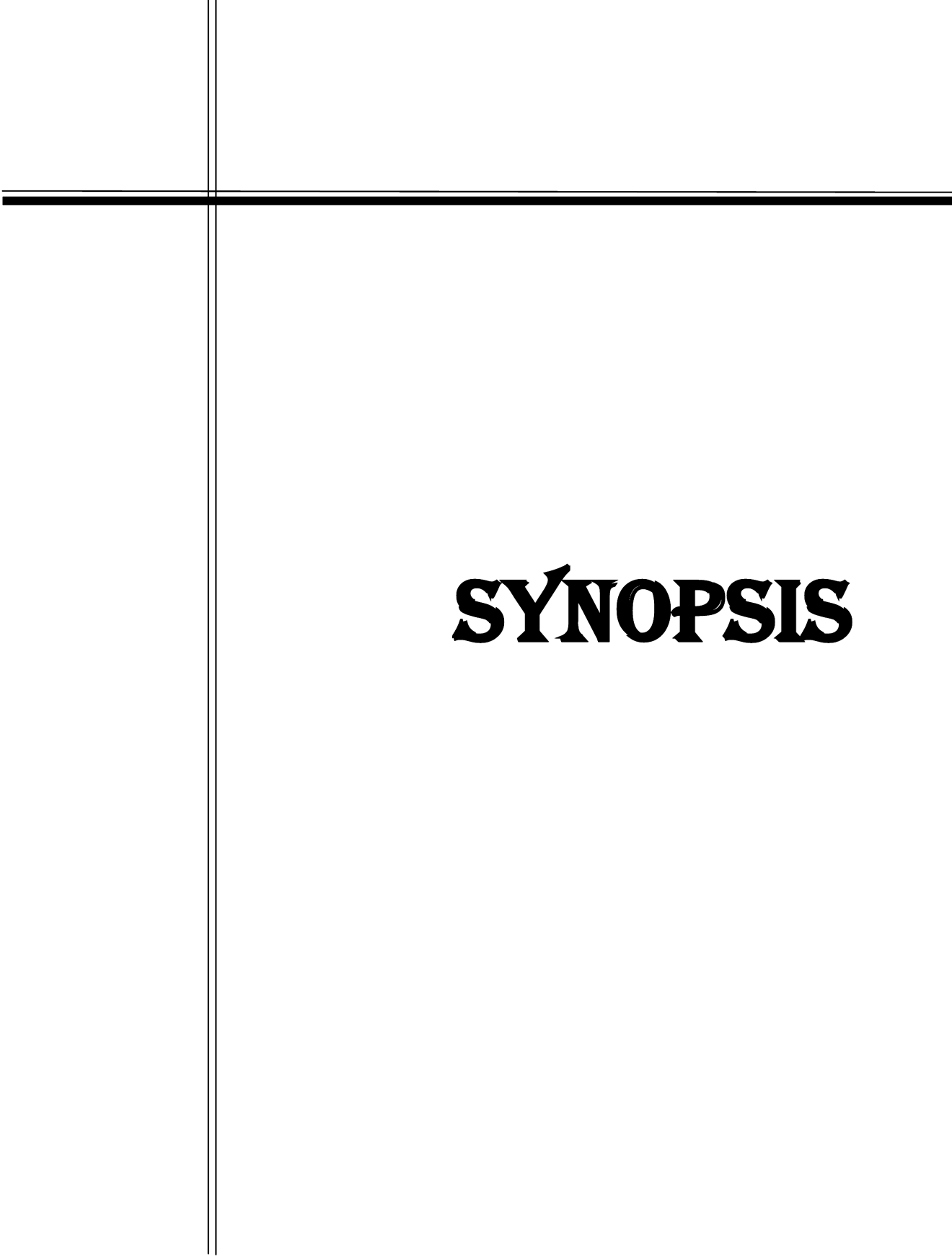
RESULT, DISCUSSION & CONCLUSION



BIBLIOGRAPHY



APPENDIX



SYNOPSIS

INTRODUCTION

The History of Chemotherapy:

- Paul Ehrlich and Sahachiro Hata developed Salvarsan(Arsphenamine) against syphilis in 1910: The concept of chemotherapy to treat microbial diseases was born.
- Sulfa drugs (sulfanilamide) discovered in 1932 against Gram+ bacteria
- 1928: **Fleming** discovered penicillin
- 1940: Howard **Florey** and Ernst **Chain** performed first clinical trials of penicillin

Mechanisms of antibacterial action:

There are four main mechanisms by which antibacterial agents act.

- **Inhibition of cell metabolism**

Antibacterial agents which inhibit cell metabolism are called antimetabolites. These compounds inhibit the metabolism of a microorganism, but not the metabolism of the host. They do this by inhibiting an enzyme-catalysed reaction which is present in the bacterial cell, but not in animal cells. The best known examples of antibacterial agents acting in this way are the sulfonamides.

- **Inhibition of bacterial cell wall synthesis**

Inhibition of cell wall synthesis leads to bacterial cell lysis (bursting) and death. Agents operating in this way include penicillins and cephalosporins. Since animal cells do not have a cell wall, they are unaffected by such agents.

- **Interactions with the plasma membrane**

Some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell. Polymyxins and tyrothricin operate in this way.

- **Disruption of protein synthesis**

Disruption of protein synthesis means that essential enzymes required for the cell's survival can no longer be made. Agents which disrupt protein synthesis include the rifamycins, aminoglycosides, tetracyclines, and chloramphenicol.

- **Inhibition of nucleic acid transcription and replication**

Inhibition of nucleic acid function prevents cell division and/or the synthesis of essential enzymes. Agents acting in this way include nalidixic acid and proflavin.

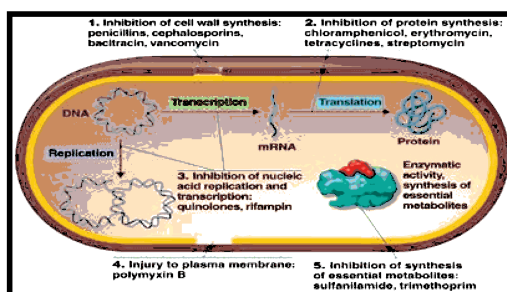


Fig.1: Mechanisms of antibacterial action

History of the Development of Antimicrobial Agents

Looking back on the history of human diseases, infectious diseases have accounted for a very large proportion of diseases as a whole. It was not until the latter half of the 19th century that microorganisms were found to be responsible for a variety of infectious diseases that had been plaguing humanity from ancient days. Accordingly, Chemotherapy aimed at the causative organisms was developed as the main therapeutic strategy. The first antimicrobial agent in the world was salvarsan, a remedy for syphilis that was synthesized by Ehrlich in 1910. In 1935, sulfonamides were developed by Domagk and other researchers. These drugs were synthetic compounds and had limitations in terms of safety and efficacy. In 1928, Fleming discovered penicillin. He found that the growth of *Staphylococcus aureus* was inhibited in a zone surrounding a contaminated blue mold (a fungus from the *Penicillium* genus) in culture dishes, leading to the finding that a microorganism would produce substances that could inhibit the growth of other microorganisms.

The antibiotic was named penicillin, and it came into clinical use in the 1940s. Penicillin, which is an outstanding agent in terms of safety and efficacy, led in the era of antimicrobial chemotherapy by saving the lives of many wounded soldiers during World War II. During the subsequent two decades, new classes of antimicrobial agents were developed one after another, leading to a golden age of antimicrobial chemotherapy. In 1944, streptomycin, an amino glycoside antibiotic, was obtained from the soil bacterium *Streptomyces griseus*. Thereafter, chloramphenicol, tetracycline, acrolide, and glycopeptides (e.g., vancomycin) were discovered from soil bacteria. The synthesized antimicrobial agent nalidixic acid, a quinolone antimicrobial drug, was obtained in 1962. Improvements in each class of antimicrobial agents continued to achieve a broader antimicrobial spectrum and higher antimicrobial activity.

Kinds of Antimicrobial Agents and their Primary Modes of Action

Table 1 : Summary of the classes of antibiotics and their properties including their biological source and mode of action

Chemical class	Examples	Biological source	Spectrum (effective against)	Mode of action
Beta-lactams (penicillins and cephalosporins)	Penicillin G, Cephalothin	Penicillium notatum and Cephalosporium species	Gram-positive bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Semisynthetic penicillin	Ampicillin, Amoxycillin		Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Clavulanic Acid	Clavamox is clavulanic acid plus amoxycillin	Streptomyces clavuligerus	Gram-positive and Gram-negative bacteria	"Suicide" inhibitor of beta-lactamases
Monobactams	Aztreonam	Chromobacter violaceum	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Carboxypenems	Imipenem	Streptomyces cattleya	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Aminoglycosides	Streptomycin	Streptomyces griseus	Gram-positive and Gram-negative bacteria	Inhibit translation (protein synthesis)
	Gentamicin	Micromonospora species	Gram-positive and Gram-negative bacteria esp. Pseudomonas	Inhibit translation (protein synthesis)
Glycopeptides	Vancomycin	Streptomyces orientales	Gram-positive bacteria, esp. Staphylococcus aureus	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly

Part I: Introduction & Review of Literature

Lincomycins	Clindamycin	Streptomyces lincolnensis	Gram-positive and Gram-negative bacteria esp. anaerobic Bacteroides	Inhibits translation (protein synthesis)
Macrolides	Erythromycin	Streptomyces erythreus	Gram-positive bacteria, Gram-negative bacteria not enterics, Neisseria, Legionella, Mycoplasma	Inhibits translation (protein synthesis)
Polypeptides	Polymyxin	Bacillus polymyxa	Gram-negative bacteria	Damages cytoplasmic membranes
	Bacitracin	Bacillus subtilis	Gram-positive bacteria	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly
Polyenes	Amphotericin	Streptomyces nodosus	Fungi	Inactivate membranes containing sterols
	Nystatin	Streptomyces noursei	Fungi (Candida)	Inactivate membranes containing sterols
Rifamycins	Rifampicin	Streptomyces mediterranei	Gram-positive and Gram-negative bacteria, Mycobacterium tuberculosis	Inhibits transcription (bacterial RNA polymerase)
Tetracyclines	Tetracycline	Streptomyces species	Gram-positive and Gram-negative bacteria, Rickettsias	Inhibit translation (protein synthesis)
Semisynthetic tetracycline	Doxycycline		Gram-positive and Gram-negative bacteria, Rickettsias Ehrlichia, Borrelia	Inhibit translation (protein synthesis)
Chloramphenico	Chloramphenico	Streptomyces venezuelae	Gram-positive and Gram-negative bacteria	Inhibits translation (protein synthesis)

The table above is a summary of the classes of antibiotics and their properties including their biological source and mode of action.

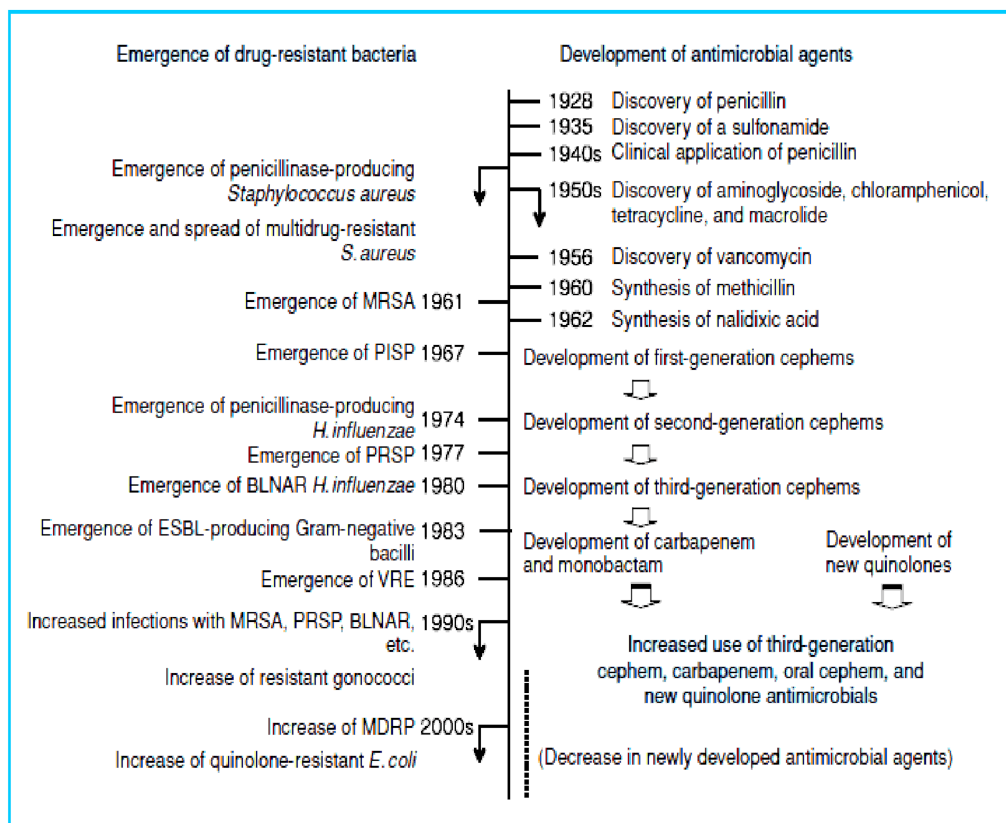
Antimicrobial drugs have caused a dramatic change not only of the treatment of infectious diseases but of a fate of mankind. Antimicrobial chemotherapy made remarkable advances, resulting in the overly optimistic view that infectious diseases would be conquered in the near future.

However, in reality, emerging and re-emerging infectious diseases have left us facing a countercharge from infections. Infections with drug resistant organisms remain an important problem in clinical practice that is difficult to solve. If an improper antimicrobial agent appears to be chosen for the treatment of infection with drug-resistant microorganisms, the therapy may not achieve beneficial effect, and moreover, may lead to a worse prognosis. In addition, in a situation where multi drug-resistant organisms have spread widely, there may be quite a limited choice of agents for antimicrobial therapy.

At present, fewer brand new antimicrobial agents are coming onto the market. Considering this situation together with the increasing awareness of drug safety, we are now facing a situation of severely limited options among antimicrobial agents.

This paper provides an outline of the history of antimicrobial agents, and thereafter describes resistant organisms that have emerged in response to antimicrobial agents and discusses practical clues to prevent resistant microorganisms.

Table 2: Trend of development of Anti-microbial agent & emergence of drug resistant bacterial



Elements of Chemotherapy

I. Principles of Antimicrobial Therapy

A. Terminology of Chemotherapy

- Chemotherapeutic drug: Any chemical used in the prevention or treatment of disease.
- Prophylaxis: preventative measures.
- Antibiotics: substances produced by the natural metabolic processes of microorganisms which can inhibit or destroy other microorganisms.
- Synthetic drugs: Man-made compounds with antimicrobial properties.
- Broad/Narrow spectrum: Agents which are effective against a wide/limited range of microorganisms.

B. Drug, Microbe, Host—Basic Interactions

The ideal chemotherapeutic drug should destroy the infectious agent without harming the host's cells. Therefore, the characteristics of the drug, microbe and host must all be taken into consideration when planning a chemotherapeutic regime.

C. Origins of Antimicrobial Drugs

- Some bacteria and fungi are able to inhibit the growth of other microorganisms in the same habitat by producing antibiotics.
e.g. Streptomyces, Bacillus (Bacteria), Penicillium, Cephalosporium (Fungus)

II. Characteristic Interaction Between Drug and Microbe

Primary effect of antimicrobial drugs is to disrupt the cell processes of microbes or to inactivate viruses, usually by inhibiting the function of enzymes or destroying cellular structures.

- Selectively toxic: drugs which produce adverse effects on microbial cells without simultaneously damaging the host cell.

A. Mechanisms of Drug Action

- Microbicidal drugs kill microorganisms by inflicting damage on specific cellular targets required for living (eg. cell wall, ribosomes).
- Microbistatic drugs interfere with cell division and thereby slow or stop cell reproduction, allowing the host defenses time to destroy the infectious agent. There are five basic mechanisms of drug action.
 1. Antimicrobial drugs that affect the Bacterial cell wall and prevent the cell from lysing. Penicillins and cephalosporins inhibit peptidase enzymes responsible for cell wall production.
 2. Antimicrobial drugs that disrupt cell membrane function
 - Disrupts selective permeability of the cell membrane. Can kill cells which are not actively dividing. Most tend to be somewhat toxic to mammalian cells.
 - **Amphotericin B**- Somewhat selective for fungal membranes.
 3. Antimicrobial drugs that affect nucleic acid synthesis
 - **Sulfonamides**- Act as metabolic analogs which competitively inhibit nucleotide synthesizing enzymes. (Sulfa drugs are selectively toxic because mammalian cells do not possess this enzyme system.)
 - **Chloroquine**- Binds and cross links the double helix, preventing DNA replication.
 4. Antimicrobial drugs that block translation. Drug reacts with the ribosome-mRNA complex. Can cause damage to eukaryotic mitochondria, which contain prokaryotic-like ribosomes.

- Aminoglycosides (streptomycin, gentamicin) attach to 30S subunit.
 - Tetracycline blocks attachment of t RNA to A site.
5. **Antimicrobial Drugs** that affect metabolic pathways. Some drugs act as competitive inhibitors, meaning that they can fit into the active site of enzymes (tying up the enzyme without making a product), blocking the correct substrate from entering the active site. Sulfonamide and trimethoprim (sulfa drugs) work by inhibiting enzymes needed to synthesize nucleotides and amino acids.

III. Survey of Major Antimicrobial Drug Groups

A. Antibacterial drugs

1. **Penicillins:** Penicillin G and Penicillin V are most important. Drug of choice for most sensitive gram-positive cocci (streptococci, pneumococci) as well as some gram negative cocci and spirochetes. Narrow spectrum. Semisynthetic penicillins (ampicillin, carbenicillin, amoxicillin) have broader spectra and can be used to treat some gram-negative enteric rods.
2. **Cephalosporins:** Broad spectrum, resistant to penicillinases, cause fewer allergic reactions than penicillins.
3. **Aminoglycosides:** Broad spectrum drugs isolated from *Streptomyces* and *Micromonospora*. These drugs inhibit protein synthesis by binding to the 30 S ribosomal subunit. Widely used for infection by gram (-) negative rods (*Escherichia coli*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*) eg. Streptomycin, Gentamicin, Tobramycin, amikacin
4. **Tetracyclines:** Broad spectrum which bind to prokaryotic ribosomes and stop protein synthesis. Side effects (GI distress and deposition in the bones) must be taken into account.
5. **Chloramphenicol:** Very potent, broad spectrum antibiotic. Prevents peptide bond formation during protein synthesis. Extreme toxicity to mammalian systems (fatal aplastic anemia) limits its use to life threatening infections with no other suitable treatment.
6. **Macrolide Antibiotics** (Erythromycin, Clindamycin, Vancomycin)
 - **Erythromycin:** Broad spectrum, low toxicity, blocks translation by attaching to the ribosome.

- **Clindamycin:** Broad spectrum, more active than erythromycin. Serious gastrointestinal side effects limit its use.
 - **Vancomycin:** Narrow spectrum antibiotic used to treat staphylococcal infections when penicillins are contraindicated. Extreme toxicity limits its use to life threatening conditions.
7. **Ketolides (Ketek):** A class of drug with a structure similar to macrolides. Used for respiratory tract infections thought to be caused by drug resistant bacteria such as *Streptococcus pneumoniae*.
8. **Rifampin:** Isolated from *Streptomyces*. Narrow spectrum antibiotic primarily used to treat mycobacterial infections (ie. tuberculosis, leprosy).
9. **Bacillus Antibiotics:**
- Bacitracin: Narrow spectrum antibiotic produced by *Bacillus subtilis*. Found in antibiotic ointment (ie. Neosporin).
 - Polymyxin: Isolated from *Bacillus polymyxa*. Narrow spectrum. Also found in antibiotic ointments.
10. **New classes of Antibiotics:** Drugs with unique mechanisms of action. Their use is generally restricted so as to lessen the chance of bacteria developing resistance.
- Fosfomycin: Inhibits cell wall synthesis.
 - Synercid: Binds to 50S ribosome. Blocks translation.
 - Daptomycin: Disrupts membrane function.

11. Synthetic Antibacterial Drugs

- **Isoniazid:** Prescribed for *Mycobacterium tuberculosis*.
- **Oxazolidinones: (Zyvox):** Inhibits the initiation of protein synthesis. Used to treat MRSA and VRE infections.
- **Fluoroquinolones:** (Ciprofloxacin, Norfloxacin, Sparfloxacin, levofloxacin). High potency, broad spectrum drugs. Use is limited to slow development of resistance.

B. Agents to treat Fungal infections

Being eukaryotic, Fungi are generally resistant to most antibacterial agents and furthermore, drugs that do adversely affect fungi are often harmful to human cells.

- Polyenes: Attack membranes causing loss of selective permeability (Amphotericin B). Used both topically and systemically.

- Griseofulvin: Especially useful for fungal infections of epidermis, hair and nails, such as athlete's foot. Drug is concentrated in these tissues. Nephrotoxicity limits its use.
- Azoles (Ketoconazole, fluconazole, clotrimazole, miconazole): Used both orally and topically to fight fungal infections.
- Flucytosine: Cytosine analog sometimes used in combination with amphotericin B.

C. Antiparasitic Chemotherapy

- Antimalarial drugs: Quinine (naturally produced), along with the synthetic produced chloroquine and primaquine are used to treat malaria. Several drugs are helpful because there are several stages in the life cycle of Plasmodia as well as several species of the parasite.
- Metronidazole (Flagyl) is widely used for several protozoan infections

D. Anthelmintic drug therapy

- Flukes, tapeworms and roundworms are much larger than other parasites and also have a metabolism much more similar to their human hosts. Consequently, eradication is difficult. Successful drugs immobilize, disintegrate or inhibit the metabolism of the worms. Side effect can include severe cramps.e.g. Mebendazole, thiabendazole, pyrantel, niclosamide, praziquantel, ivermectin

E. Antiviral chemotherapeutic agents

Traditionally fought with vaccination, yet virus epidemics are still common (AIDS, genital warts, measles).Antiviral compounds halt viral multiplication by:

- Barring penetration of the virus into the host cell Fuzeon (HIV) Tamiflu, Relenza (Influenza)
- Blocking transcription or translation of viral molecules
- Preventing the maturation of viral particles
- Nucleotide analogs: Compete with nucleotides for incorporation into viral DNA. Once incorporation has occurred, DNA synthesis is blocked.
 - ✓ Acyclovir, valacyclovir, famcyclovir, gancyclovir
 - ✓ Azidothymidine (AZT, HIV infection)
 - ✓ Protease inhibitors, prevent assembly of viral particles
 - Saquinavir
 - Ritonavir

IV. The Acquisition of Drug Resistance:

Adaptive response in which microorganisms become able to tolerate an amount of drug that would ordinarily be inhibitory. Drug resistance develops when chromosomal mutations or transfer of extra chromosomal DNA from a resistant species to a sensitive one. Resistance factors (R factors) are transferred via **conjugation, transformation or transduction**. R factors are naturally present in some microorganisms before they have been exposed to the drug.

A. Specific mechanisms of drug resistance

1) Drug inactivation mechanisms: Microbes may produce enzymes that permanently alter drug structure. Decreased permeability to a drug: Microbe develops methods for removing (or preventing entrance) of a drug. eg. Beta-lactamases (penicillinase, cephalosporinase) disrupt the ring structures of some penicillins and cephalosporins.

2) Decreased permeability or increased clearing of drug from the cell. E.g. Some plasmids code for proteins which are able to pump multiple drugs from the cell (i.e. MDR pumps).

3) Change of drug receptor: The cellular structure which is normally targeted by a certain drug can be altered so that it is no longer recognizable.

eg. erythromycin and clindamycin resistance is associated with alteration of the 50S ribosomal binding site.

4) Changes in metabolic patterns: Microbes can develop alternative metabolic pathways or enzymes. eg. sulfonamide resistance results when microbes develop novel patterns of folic acid synthesis.

B. Natural selection and drug resistance

Any population of microbes will most likely contain a few members with resistance to a certain drug but as long as the drug is not present in the environment, there is no selective advantage and the number of resistant forms will remain low. When the population is exposed to the drug, sensitive microbes will be killed and all members of the population will now be resistant.

V. Characteristics of the Host/Drug Reaction

Although absolute selective toxicity is the goal of chemotherapy, this is rarely achievable. Approximately 5% of people on a chemotherapeutic regimen experience serious side effects. Side effect can be grouped into one of three categories.

A. Toxicity to organs

- **Liver:** Responsible for detoxifying foreign chemicals in the blood, therefore can easily be damaged by a drug or its metabolic products.
- **Kidney:** Filters blood to remove waste products. Some drugs (nephrotoxic) interfere with this process by irritating the nephron tubules.
- **Heart/bone marrow:** Often seen with drugs given to fight parasitic infections. Irregular heartbeat, depression of hematopoiesis (commonly seen with chloramphenicol)
- **Nerves:** Primarily aminoglycosides. Often affect the vestibulocochlear nerve, leading to dizziness, deafness, motor or sensory disturbances.
- **Skin/hard tissues:** Photodermatitis. Deposition in hard bones and teeth. Both seen with tetracycline.

B. Allergic reactions

- The drug or a by-product of its metabolism can act as an antigen. Penicillins and sulfonamides account for most antimicrobial allergies.

C. Suppression and alteration of the microbiota by antimicrobics

- Broad spectrum antibiotics can disrupt the ecological balance of the body, leading to superinfection (rare resistant species that now have a chance to overgrow due to a lack of competition)
e.g. Use of Cephalosporins can change the pH of the vagina by killing lactobacilli, leading to superinfection by *Candida albicans*.
e.g. Overuse of broad spectrum antibiotics (tetracycline etc.) can lead to antibiotic associated colitis as *Clostridium difficile* overgrows the normal microbiota of the GI tract.

VI. Considerations in Selecting an Antimicrobial Drug**A. Identifying the agent:**

- Initial findings (gram stain etc.) allow treatment to be started (empirical methods-based on past experience) eg. *Streptococcus pyogenes* responds well to penicillin
- Epidemiological statistics may be used to predict the most likely infectious agent eg. *Haemophilus influenza* is the leading cause of meningitis in children

B. Testing for drug susceptibility of microorganisms

- Testing is especially important with those species commonly exhibiting resistance.e.g. *Staphylococcus sp.*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*
- Kirby-Bauer technique: Allow many drugs to be tested simultaneously. Less effective for anaerobic, fastidious or slow growing organisms.
- Tube dilution tests are more sensitive and allow the determination of the minimum inhibitory concentration (MIC).
- E-test allows MIC to be determined while retaining the advantages of Kirby-Bauer.

C. Applying the results of drug susceptibility testing

- Patient's response to the drug of choice (*in vivo* effects) must be compared to laboratory effects (*in vitro*).
 - ✓ Drug inaccessible to infectious agent
 - ✓ Some resistant cells in the culture
 - ✓ Infection is the result of more than one pathogen

D. Medical considerations in anti-infective chemotherapy

- Narrowest spectrum drug which is effective is usually the best choice
- Therapeutic index (TI): Measure of the selective toxicity of a drug. High ratios are better than low ratios.

$$TI = \frac{\text{Toxic dose}}{MIC}$$

Patient history will preclude the use of some drugs because of allergies, organ damage or possible synergistic effects.

E. Antibiotic associated problems

- A large percentage of prescriptions are useless because the infective agent is a virus.
- A Shotgun approach to antimicrobial therapy (using a broad spectrum drug when a narrow spectrum would suffice) is often used, even though this can lead to superinfection and toxicity problems.
- Drugs are often prescribed without culture or susceptibility testing even when it is clearly warranted.
- Overuse of antibiotics has led to increased numbers of resistant species.
- Antibiotics used in animal feed have led to resistant microbes within the animal population.

Antibiotics and Antimicrobial Agents

I. Substance classified as an antibiotics

- ✓ It is a product of metabolism
- ✓ It is a synthetic products produced as a structural analogue of a naturally occurring antibiotics
- ✓ It antagonizes the growth or survival of microorganisms
- ✓ It is effective in low concentration

II. To be an effective chemotherapeutic agent:

The agent must-

- kill or inhibit the microbe causing the infection
- be relatively harmless for human tissue by showing selective toxicity

Ideal Qualities of a Chemotherapeutic Agent

1. It should destroy or inhibit many kinds of pathogens be BROAD SPECTRUM (effective against many different species)
2. should inhibit microbes in such a way as to prevent the development of antibiotic resistant forms of pathogens
3. should not produce undesirable side effects in the patient allergic reactions, nerve damage, kidney or GI irritation
4. It shouldn't eliminate normal flora
5. If taken orally it shouldn't be inhibited by stomach acid; if given by injections, it shouldn't be inactivated by binding to blood protein
6. it should be highly soluble in body fluids
7. it must be able to reach high enough concentrations in tissues or blood in the patient to kill or inhibit pathogens.

From 10 to 12 years and \$125 million may be required to take a successful drug from discovery to its use to treat and protect patients. In recent years the pharmaceutical industry has directed serious attention toward the discovery and development of antifungal agents. The purpose of this review is to present an overview of the azole antifungal drugs that have been developed and are in use, as well as the azole agents that are under development and evaluation for use in the chemotherapy of both systemic and superficial fungal diseases.

Brief Overview of Antifungal Chemotherapy And Drug Development

Fungi were recognized as pathogens prior to bacteria, and antifungal chemotherapy was successfully attempted before antibacterial chemotherapy. However, the development of effective antifungal agents has been disappointingly slow. Thrush, an oral manifestation of *Candida albicans* infection, was recorded in 1665 as a fatal disease ^[1]. In 1835, Basside Lodi determined that the causative agent of muscardine, i.e. silkworm disease, was a fungus that multiplied within and on the insect. Bassi is considered to be the founder of the concept of parasitic infection with his research on the pathogenesis of the etiologic agent of silkworm disease, *Beauveria bassiana* ^[2].

Thus, the first organism scientifically established to cause disease was a fungus. The treatment of sporotrichosis with potassium iodide in 1903 is believed to be among the first reports of antifungal chemotherapy ^[3]. In spite of this early beginning, the discovery of the first major antifungal drug, nystatin, was not until 1949 ^[4]. Azole antifungal drugs, the subject of this review, were not introduced for clinical use until 1969. In comparison to antibacterial agents, the field of antifungal chemotherapy has not progressed rapidly. Until recently, infections caused by fungi were not considered to be a major problem. Fungal diseases are not reportable; thus, documentation of their incidence and trends in this field is difficult ^[5]. However, with the increase in the number of patients compromised by human immunodeficiency virus, cancer chemotherapy, organ transplants, and long-term antimicrobial therapy, the incidence of opportunistic fungal infections is increasing. One analysis of data from 1970 to 1976 revealed increases of 158 and 78% in aspergillosis and cryptococcosis, respectively ^[6]. A more recent study revealed that the incidences of certain opportunistic or hospital-acquired fungal infections or both almost doubled from 1976 to the period of 1980 to 1982 ^[7]. The human immunodeficiency virus (acquired immunodeficiency syndrome) patient population also has greatly added to the incidence of opportunistic fungal infections ^[8]. Fungi are eucaryotic and thus are biochemically similar to the human host. This similarity makes the development of a drug that is effective against the invading fungus, but safe for the host, an arduous and lengthy study in basic and clinical research.

• Objective Of Basic Research In The Field Of Antimicrobial Chemotherapy:

The objective of basic research in the field of antimicrobial chemotherapy is to rationally design, synthesize, and/or isolate novel chemical entities that can be used to treat disease effectively and safely.

- ✓ For antifungal agents: directing research to specific fungal target is one logical approach. This multidisciplinary task involves Chemistry, Biochemistry, molecular Biology, Pharmacology, Immunology, Microbiology, endocrinology, Enzymology as well as other scientific discipline. Once the product candidate is identified, research is conducted on the safety, Bioavailability, formulation and drug delivery system for the potential drug.
- ✓ Following successful completion of these basic and developmental research steps clinical research ensues.

Table 3: Phase of development of Pharmaceutical products.

Basic research	Discovery of compound, including interaction of chemistry, biochemistry, microbiology, pharmacology, and enzymology
Pharmacology	All stages of preclinical investigations, including in vitro and in vivo (animal models) assays, safety assessment in animal models, pharmacology, and formulation studies
Clinical trials	
Phase I	Clinical pharmacology: first tolerance and pharmacokinetics tests in normal, healthy volunteers
Phase II	Efficacy and safety tests in limited number of patients with the target disease
Phase III	Controlled clinical trials to determine efficacy and safety in large numbers of patients with the target disease; data are used for registration of the compound with government agency for marketing approval
Launched (marketed)	Marketing started in one or more countries
Phase IV Studies	Surveillance after approval of drug; based on government request
Phase V studies	Studies to seek additional marketing claims, new formulations, and combinations

The drug progresses through rigorous tests in humans to determine efficacy and safety. Beginning with phase I, i.e., clinical pharmacology, initial tolerance tests are conducted on small numbers of normal, healthy adults. If the product is determined to be safe, it then progresses to tests on larger numbers of patients with the target disease (phases II and III) to determine efficacy and continued safety. After regulatory approval (the Food and Drug Administration in the United States or an international equivalent), surveillance of the drug continues based on government request (phase IV). The phase V stage in clinical research involves further marketing claims and new formulations and combination

HISTORY OF ANTIFUNGAL AZOLES

The first report of antifungal activity of an azole compound, benzimidazole, was in 1944 by Woolley ^[9], who was studying biotin deficiency in animals and microbes. He noted the structural similarity of biotin and purines to benzimidazole, but the biological effects of Benzimidazole were not reversed by biotin, whereas they were reversed by the purines adenine and guanine.

Since mycotic diseases were of minimal interest in 1944, Woolley's initial discovery was largely ignored, although his data were confirmed in 1949 (M. C. Goldsworthy and S. J. Gertler, Chem. Abstr. 43:6351, 1949). Thirty years later, Vanden Bossche observed that phenethylimidazole, another azole moiety with antifungal activity, inhibited the uptake of purines in yeast form *Candida* spp. by interference at the cell membrane ^[10].

In 1952, Jerchel et al. ^[11] revived Woolley's work and reported that certain substituted benzimidazole compounds had significant antifungal activity. This publication encouraged other investigators to screen this group of chemicals in search of a clinically useful antifungal agent. The breakthrough came in 1958 to 1959 when chlormidazole, a chlorobenzyl imidazole, was developed and studied in clinical trials ^[12].

Chlormidazole was sold as a 5% topical cream, the first azole derivative developed and marketed as an antifungal drug. With the introduction of chlormidazole, interest in the antifungal activity of azole compounds began to increase. For example, after the introduction of thiabendazole, a thiazolyl- benzimidazole, in 1961 by Merck Sharp & Dohme for use as a broad-spectrum antihelminthic drug, Robinson et al. ^[13] tested the compound for antifungal activity *in vitro*.

It was effective against many dermatophytes and *Aspergillus* species, but since its activity against yeast-like fungi was minimal, the compound was not developed as an antifungal agent. Similarly, mebendazole, a benzoyl-benzimidazole developed by Janssen Pharmaceutica (Beerse, Belgium) in 1973 as a broad-spectrum antihelminthic agent, was shown to have antifungal activity ^[14].

Despite the fact that the antifungal activity of these two compounds was not pursued, the data supported the concept that two azole compounds had potential as antifungal drugs for human use. In the late 1960s, three compounds from two different laboratories were introduced in the literature; these drugs firmly established azoles as antifungal agents. Clotrimazole, developed by Bayer AG (Wuppertal, Federal Republic of Germany), and miconazole and econazole, developed by Janssen Pharmaceutica, were introduced within months of each other.

This era of azole antifungal compounds was so new and competitive that the less descriptive report of clotrimazole antifungal activity ^[15] was published 3 years prior to the more detailed description of the chemical synthesis ^[16].

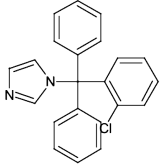
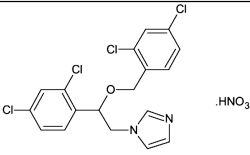
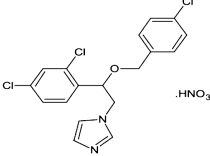
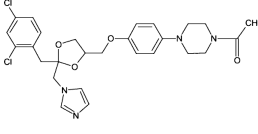
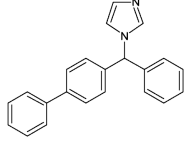
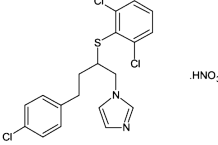
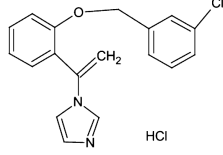
These three imidazoles continue to be used today for treatment of fungal infections, demonstrating the success of these early discoveries. Unfortunately, their use also reveals the slow evolution of the azole class of antifungal drugs during the past two decades. Although progress with this group of antifungal agents has been slow, several clinically useful compounds have been developed, and many, which appear promising, are presently under development and clinical evaluation.

A few imidazole compounds (ketoconazole, in particular) represent major advances in antifungal chemotherapy. New triazole derivatives, e.g., fluconazole and itraconazole, appear to be less toxic and more active than ketoconazole ^[17]. These and several other antifungal azole derivatives are discussed in this review.

Antifungal imidazoles available for Clinical use

The imidazoles represent one of the two major classes of antifungal azole derivatives. Many of these drugs are limited in their clinical use by their spectrum of activity, potency, solubility, or side effects, but the imidazole group has contributed significantly to the therapy of both superficial and systemic mycotic infections. Chemical structures, marketed name(s), formulations, route of administration, and clinical uses are listed in Table 4.

TABLE 4. Generic& trade (marketing) names, chemical structures, formulations, indications of antifungal imidazoles and Terconazole, a triazole derivative, currently available for clinical use

Generic name	Trade name	Chemical structure	Formulation	Indication
Clotrimazole	Canesten Mycelex Mycelex- G Lotrimin Gyne-Lotrimin		1% cream & Vaginal tablets 10- mg oral troches	Superficial fungal infections, including dermatomycoses, tinea versicolor, and cutaneous an vaginal candidiasis.
Miconazole	Monistat Monistat-Derm Monistat I.V.		2%cream & lotion,200-mg vaginal suppositories,10 -mg/ml sterile solution for i.v. use	Systemic fungal infections, including coccidioidomycosis, candidiasis, cryptococcosis, pseudallescheriosis, & chronic mucocutaneous candidiasis. Superficial fungal infections
Econazole	Spectazole Pevaryl		1% topical & vaginal creams 1% soln, spray, and powder	Superficial fungal infections, including dermatomycoses, tinea versicolor, and cutaneous and vaginal candidiasis.
Ketoconazole	Nizoral Fungarol Fungarest Orifungal		200-mg tablets for oral use 2% cream and solution	Systemic fungal infections, including blastomycosis, certain forms of coccidioidomycosis & histoplasmosis, chronic mucocutaneous candidiasis, paracoccidioidmcosis, and pseudallescheriosis, Superficial fungal infections
Bifonazole	Mycospor Mycosporan		1% cream and solution	Superficial fungal infections, including dermatomycoses, tinea versicolor, and cutaneous candidiasis
Butoconazole	Femstat		2% vaginal cream 100-mg vaginal ovules	Vaginal candidiasis
Croconazole	Pilzcin			Superficial fungal infections, including dermatomycoses, tinea versicolor, and cutaneous candidiasis

I. Clotrimazole:

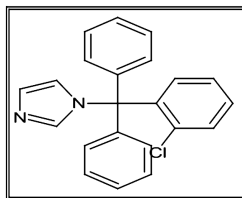


Fig.2: Structure of Clotrimazole

Clotrimazole was among the first of the imidazole antifungal drugs developed. It was synthesized in 1969 by chemists at Bayer AG, although the synthesis was not reported until 1972. Clotrimazole has well-established in vitro activity against isolates of dermatophytes, pathogenic yeasts, and filamentous and dimorphic fungi, as well as some gram-positive bacteria.

The drug is useful in the treatment of dermatophytic infections, superficial fungal infections, i.e., tinea versicolor, and various *Candida* infections, including oral thrush and vaginal candidiasis. Since its effectiveness has been proven, clotrimazole has been used as a control drug in many clinical trials of the newer azole derivatives. The in vitro activity of clotrimazole against most systemic pathogens is comparable to that of amphotericin B.

Clotrimazole is more active than griseofulvin and nystatin against dermatophytes as well. Its broad spectrum and potent activity have been confirmed in many studies. Very short contact time is required for clotrimazole to effect its antifungal activity, and as with the other imidazoles, the suggested mode of action appears to be disturbance of the fungal cell membrane.

II. Miconazole:

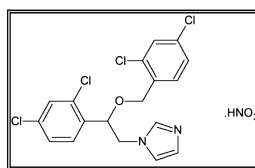


Fig.3: Structure of Miconazole

Miconazole Fig.3 is intended for the treatment of serious systemic fungal infections like Candidiasis, Cryptococcosis, Petriellidiasis and Paracoccididomycosis. The adverse reactions include thrombophlebitis, pruritis, fever and G.I. disturbances.

Miconazole is solubilize with polyethylene glycol and castor oil to facilitate adequate intestinal absorption.

Antifungal Triazoles available for Clinical use and under Development

Antifungal triazole derivatives presently under development represent a much needed advance in the field of antifungal chemotherapy. They are the second major chemical group of antifungal azole derivatives. In general, the triazole group appears to have a broader spectrum of antifungal activity and reduced toxicity when compared with the imidazole antifungal drugs. To date, terconazole is the only triazole marketed for clinical use against fungal infections, and its uses are limited.

The two leading triazole candidates under development are **fluconazole** and **itraconazole**. Both of these compounds are active orally and appear to have broader spectra of activity and less toxicity than ketoconazole.

III. Fluconazole

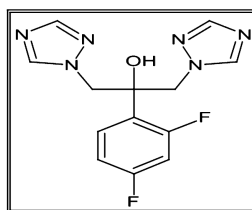


Fig. 4: Structure Of Fluconazole

It is a water-soluble bis-triazole with broad-spectrum antifungal properties that is suitable for both oral and intravenous administration as the free base.

IV. Itraconazole

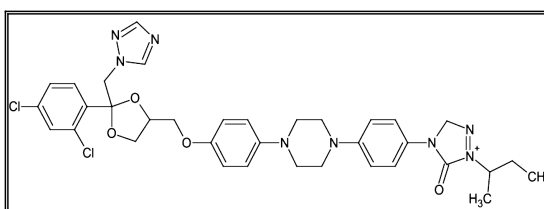
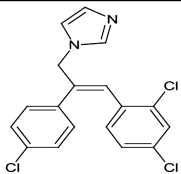
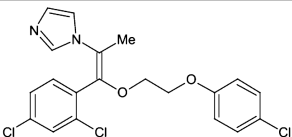
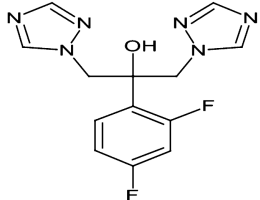
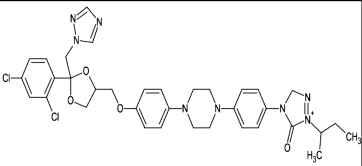


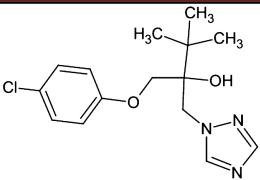
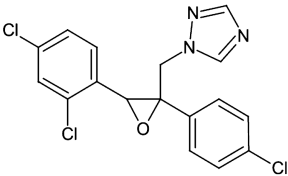
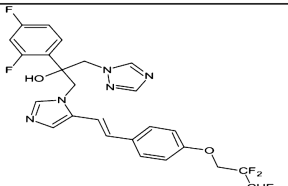
Fig.5: Structure Of Itraconazole

Itraconazole is an orally active, broad-spectrum antifungal agent that has become an important alternative to ketoconazole. The primary indications for itraconazole are for the treatment of systemic fungal infection including blastomycosis, histoplasmosis (including patients infected with HIV virus) and sporotrichosis.

Other triazoles reviewed in this section are in earlier phases of development; therefore, it is difficult to estimate their potential value as antifungal drugs at this time. Chemical structures, proposed routes of administration, and proposed clinical uses of these compounds are listed in Table 3.

TABLE 5. Generic name, developer, chemical structure, proposed route(s) of administration, and proposed clinical uses of imidazole and triazole antifungal derivatives currently under development

Generic name (developer)	Chemical structure	Proposed route(s) of administration	Proposed clinical uses
Imidazoles Aliconazoles (knoll)		Topical	Superficial fungal infections, including dermatomycoses, tinea versicolor, and cutaneous and vaginal candidiasis
Omoconazole (Siegfried AG)		Topical	Superficial fungal infections, including dermatomycoses, tinea versicolor, and cutaneous and vaginal candidiasis
Triazoles Fluconazole (Pfizer U.K.)		Oral, Topical	Superficial & systemic fungal infections, including tinea versicolor, dermatomycoses, and cutaneous, vaginal and systemic candidiasis; special indication for fungal meningitis.
Itraconazole (Janssen)		Oral Topical	Superficial & systemic fungal infections, including tinea versicolor, dermatomycoses, and cutaneous, vaginal and systemic candidiasis & systemic aspergillosis, sporotrichosis, & chromomycosis

Vibunazole (Bayer AG)		Oral Topical	Superficial & systemic fungal infections, including tinea versicolor, dermatomycoses, and cutaneous, vaginal and systemic candidiasis & systemic aspergillosis
Alteconazole (Knoll)		Oral Topical	Superficial & systemic mycoses
ICI 195,739 (Imperial Chemical Industries)		Oral Topical	Superficial & systemic mycoses

Proposed modes of action of antifungal Azole derivatives

The elucidation of the mode(s) and site(s) of action of antifungal azole derivatives has been and continues to be an active and rapidly progressing area of investigation. At present, the specific ways in which the antifungal imidazoles and triazoles affect fungal cells have not been characterized. There is a growing body of evidence that the primary mode of action of these antifungal drugs is the inhibition of ergosterol biosynthesis in the cytoplasmic membrane of fungi, resulting in the accumulation of lanosterol or other sterol intermediates ^[18].

Vanden Bossche and co-workers ^[19] and other investigators have demonstrated with a number of antifungal azole derivatives that these compounds affect cytochrome P-450 involved in the 14a-demethylation of lanosterol.

Fluconazole and itraconazole are more selective for the fungal cytochrome P-450 isozymes than is ketoconazole; thus, mammalian physiology is less seriously disturbed by the newer triazoles as compared to the imidazole ketoconazole.

Inhibition of ergosterol biosynthesis leads to disorganization of the fungal plasma membrane. Several studies have demonstrated that plasma membrane physiology and function are adversely affected by relatively low concentrations of azole derivatives that lead to changes in permeability and transport function ^[20]. Membrane damage subsequently leads to metabolic imbalances that result in the inhibition of fungal cell growth or eventual death of the cell or both. This primary antifungal activity of inhibition of cell membrane biosynthesis tends to be a fungistatic rather than a fungicidal action.

In general, antifungal azole derivatives require some host immune response to complete the elimination of the invading fungus. This fungistatic activity is one reason why most azole derivatives require long terms of therapy for treatment of the more severe systemic infections and do not work well in immuno compromised patients. Less well understood are the secondary effects of the antifungal azole derivatives. Varied test conditions by different groups of investigators have produced confusing data concerning the fungistatic versus fungicidal activity in this class of antifungal drugs ^[21].

Sud and Feingold ^[22] demonstrated that both miconazole and clotrimazole exerted direct physicochemical cell membrane damage on yeast cells that was a lethal event; interestingly, ketoconazole did not demonstrate lethal activity in this assay.

Studies by Cope ^[23] and Beggs ^[24] both demonstrated direct damage to fungal cell membranes as measured by the release of potassium ions from cells of *C. albicans* and *C. parapsilosis* exposed to high concentrations of miconazole. Bifonazole also has been reported to exert direct cell membrane damage to cells of *C. albicans* and *Torulopsis glabrata* ^[25].

Antifungal azole derivatives also may affect fungal respiration. Sud and Feingold ^[26] discovered that the fungistatic potentials of miconazole and clotrimazole were abrogated in reduced oxygen tensions. Miconazole also has been shown to adversely affect cytochrome and peroxidase activity in cells of *C. albicans* and *Saccharomyces cerevisiae* ^[27].

Ketoconazole blocks the transport of electrons in the respiratory chain of *C. albicans* ^[28]. The data published to date suggest that there are at least two distinct mechanisms of azole derivative antifungal action ^[29]. The first mechanism is physicochemical: this is dependent on the growth phase of the fungus, requires high concentrations of drug, is a fungicidal event, and is not common to all azole derivatives. The second mechanism is metabolic: this activity occurs at lower concentrations of drug, is a fungistatic event, and is generally common to all azole derivatives.

Clinically, most antifungal azole derivatives are fungistatic drugs; reports of fungicidal activity are largely from in vitro studies that do not correlate well with clinical reality. In conclusion, much remains to be learned about the mode(s) and site(s) of action of the antifungal azole derivatives. Serious questions presently are under investigation by a number of research groups. One subject that requires additional investigation is the emergence of azole-resistant fungal isolates ^[30]. The mechanisms that lead to this resistance must be elucidated. Definitive answers should be forthcoming.

This overview puts into perspective many of the antifungal azole derivatives that have been marketed for clinical use and those that presently are under development and evaluation. There is no question that new, safer, and more effective antifungal drugs are needed to satisfy the increasing needs of patients afflicted with fungal infections.

Ketoconazole has been the standard for a number of years among the azole derivatives, but the Triazoles, Fluconazole and Itraconazole may be superior antifungal compounds in that they are more potent and less toxic and have a broader spectrum of activity. These two compounds may represent the next major step forward in the development of more potent antifungal drugs. A moderate selection of topically active antifungal azole derivatives also is available for use, and new ones are presently being developed and evaluated.

In essence, we cannot continue to rely on antifungal drugs developed years or decades ago; we must continue to search for newer, safer, more effective chemotherapeutic agents. Some of the compounds described in this overview may contribute to the slow, but steady evolution of improved antifungal drugs.

PLAN OF WORK

In drug design, the aim must be thorough investigation of well chosen or designed compounds taking into consideration the electronic effect, hydrophobic effect or steric effect. The most widely used mode of approach so far has been the use of some drug of known structure as a model from which other congeners or homologues and analogues are synthesized.

The present work in this thesis has been planned keeping in mind the various chemical structures of existing antibacterial agents and antifungal agents and done the molecular modification of group of moiety approach implies elimination, introduction or substitution of certain moieties or group in the biologically active molecule.

➤ **Aim of the work**

To Synthesise and Study of Heterocyclic Compounds as Possible Anti-microbial agents.

Objectives of the Research:

1. To determine the most appropriate methodologies for the synthesis of new heterocyclic derivatives with selected active biological moieties.
2. To evaluate and characterize derivatives with the help of analytical methods like Thin layer chromatography (TLC), Infrared spectroscopy (IR), High performance liquid chromatography (HPLC) and Gas chromatography-Mass spectrometry (GC-MS).
3. To evaluate this compound for their biological activity.
In vitro Anti-microbial, Anti-fungal, Anti-inflammatory, Anti-oxidant activity.

III-A] - Synthesis of 6-ethoxy-2-{[(6- Substituted phenyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazol-3-yl) methyl] sulfanyl}-1,3-benzothiazole

Among the various types of known heterocyclic ring system, benzothiazole derivatives plays vital role in biological field such as antitubercular, anti-allergic, anti-inflammatory and fungicidal activity^[31-34]. 2-Amino-6-substituted benzothiazole are known to exhibit excellent antibacterial activity. According to Mayer's hypothesis, "A morphological relationship between bacteria and fungi may be parallel by a bio-chemical relationship". i.e. substance act as antifungal agents are likely to exhibit antitubercular activity also.

The importance of 1,2,4-triazole moiety as a major pharamacophoric requirement to exhibit enhance antifungal activity is well manifested by its inevitable presence in most recent antifungal drugs, viz. Flucanazole^[35] (Fig.6).

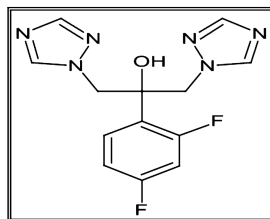


Fig.6: Structure of Flucanazole

The presence of Oxygen and nitrogen in the heterocyclic system has attracted the attention of medicinal chemist because the diverse biological activities and profound efficacy. The present review highlights the use of benzothiazole moiety as a template for development of newer therapeutic agents. Biological properties of the nucleus include anticancer, antioxidant, anti-inflammatory, and antimicrobial & the brief information available on the triazole derivatives clearly indicates their therapeutic importance. Prospective titled compounds (Fig.7) can be designed and synthesized for a variety of biological activities.

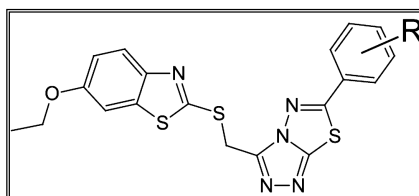


Fig.7: Structure Of 6-ethoxy-2-{[(6- Substituted phenyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazol-3-yl) methyl] sulfanyl}-1,3-benzothiazole

II-B] Synthesis of *N*-[(6,7-dimethyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazepin-3-yl) methyl] -substituted 1,3-benzothiazol-2-amine

The progress achieved in the synthesis of heterocyclic compounds with biological potential is due to improvement of methodological study of tested substances too. Thiazepinones, benzothiazepines and other thiazepine derivatives are moieties whose biological activity has received much attention ^[36-41]. However [1, 3, 4,] thiadiazepine are relatively unknown heterocycles and may be due to absence of adequate method of synthesis no pharmacological tests have been published.

Triazoles are the class of heterocyclic compounds ^[42] which are under study since many a years. Its diversity in showing the pharmacological activities is mind blowingly identified well by the medicinal chemists. Triazole, with many a compounds as incorporating with other heterocyclic nucleus, hydrazides^[43], substituted triazoles^[44] incorporated with antibiotics ^[45] are some of great uses which fascinates the chemists to continue research on it and find out more hidden potentials of this nucleus. From the above literature findings, it was decided to synthesize following compounds (Fig.8) and be test for their potency against bacterial and fungal infection.

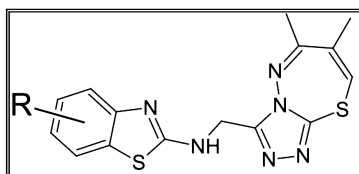


Fig.8: Structure Of *N*-[(6, 7-dimethyl [1, 2, 4] triazolo [3,4-*b*] [1,3,4] thiadiazepin-3-yl) methyl] -substituted 1,3-benzothiazol-2-amine

III-C] Synthesis of *S*-{5-[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl] methyl} -1,3,4-oxadiazol -2-yl} substituted benzenecarbothioate

2,5-Disubstituted 1,3,4-Oxadiazole constitute a unique class of Nitrogen and Oxygen containing five membered heterocycles. Oxadiazole ^[46] derivatives have been found to exhibit diverse biological activities such as antibacterial, fungicidal, insecticidal, anti-inflammatory and anti-HIV. Thus taking into consideration the above all observations, the synthesis of the derivatives of benzothiazole with 1,3,4-oxadiazole react with acid chloride moiety were synthesize.

Recently Hosam Saad et,al ^[47]; have reported 2-aryl-5-substituted-1,3,4-Oxadiazoles show anti bacterial activity (Fig.9).

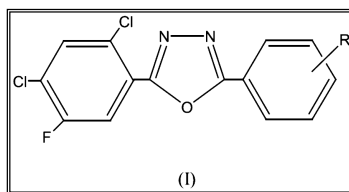


Fig.9: 2-aryl-5-substituted-1,3,4-Oxadiazoles

Hence taking into considerations the above facts it was planned to synthesize the series of compound having combination of 2,5-Disubstituted Oxadiazole with different acid chlorides so as to study their anti bacterial & anti fungal properties. The title compound (Fig.10) has following structure.

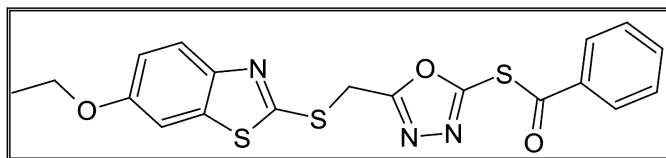


Fig.10: Structure Of *S*-{5-[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl] methyl} -1,3,4-oxadiazol -2-yl} substituted benzenecarbothioate

III-D] Synthesis of 4-{[(*E*)-(6-methyl-4-oxo-4*H*-chromen-3-yl)methylidene]amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide

Sulphonamide were the first ,systemic chemotherapeutic agents employed to prevent & cure bacterial infections. They have wide range of antibacterial activity against both Gram +ve & Gram –ve bacteria .The important members of this class include sulfanilamide (Fig.11), sulphamethoxazole(Fig.12), sulfisoxazole(Fig.13)

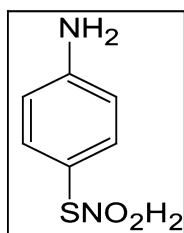


Fig.11: sulfanilamide

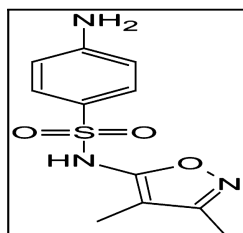


Fig.12: sulphamethoxazole

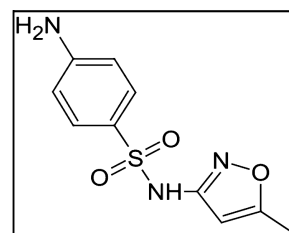


Fig.13: sulfisoxazole

In the above mentioned drugs, the sulfonamide moiety is considered to be very essential for their activity against bacterial infections. Several quinoxaline-2-carboxylic acid derivatives show inhibiting effect on the growth of staphylococcus aureus bacteria^[48], anti-tuberculosis activity^[49]. The quinoxaline skeleton is a relatively unexploited heterocyclic compound in drug discovery^[50].

During the last twenty years, the study of the biological activities of chromene derivatives has been the aim of many scientists^[51]. Recently, the anticoagulant, antibacterial, anti-helminthic, hypothermal and vasodilatory properties of chromene has been reviewed^[52]. Fused chromenes are interesting due to their significant antibacterial^[53] and novobiocin^[54] activities.

Taking the lead from these known facts, it was proposed to synthesized various biologically active compounds by incorporating sulfonamide & quinoxaline moiety into the chromene (Fig.14)and check for biological activity.

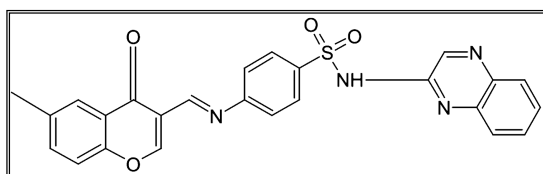


Fig.14: Structure Of 4-{[(*E*)-(6-methyl-4-oxo-4*H*-chromen-3-yl) methylidene] amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide

➤ **Analytical Characterization of title compounds:**

The structures of the newly synthesized compound were analyzed by physical method by Melting point & with the help of analytical methods (IR, HPLC and GC-MS).

- **Spectroscopic technique**

Infrared spectroscopy (IR)

Nuclear Magnetic Resonance spectroscopy (NMR)

- **Chromatographic techniques**

- ✓ Thin layer chromatography (TLC)
- ✓ Gas chromatography - mass spectrometry (GC-MS)
- ✓ High performance liquid chromatography (HPLC)

➤ **Biological Activity of title compounds:**

Different heterocyclic derivatives, derived from different biologically important moieties, were prepared and were screened for following biological evaluations:

- **Anti Bacterial Screening:** By tube dilution method
- **Anti Fungal Screening:** By tube dilution method
- **Anti-oxidant assay:** By DPPH assay
- **Anti-inflammatory assay:** *In vitro* HRBC membrane lysosomal assay

CHEMISTRY

- 1. Chemistry of Benzothiazole**
- 2. Chemistry of Hydrazide**
- 3. Chemistry of Triazole**
- 4. Chemistry of Acid chlorides**
- 5. Chemistry of Schiff base**
- 6. Chemistry of Amide**
- 7. Chemistry of 1,3,4-oxadiazole**

I. Benzothiazole

Benzothiazole is a privileged bicyclic ring system. It contains a benzene ring fused to a thiazole ring. The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like- antimicrobial, antitubercular, antitumour, antimalarial, anticonvulsant, anthelmintic, analgesic and anti-inflammatory activity. In addition, the benzothiazole ring is present in various marine or terrestrial natural compounds, which have useful biological activities. Due to their importance in pharmaceutical utilities, the synthesis of various benzothiazole derivatives is of considerable interests.

- **Characteristics of nucleus:**

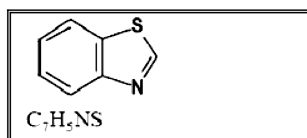


Fig.15: Structure of Benzothiazole

Physical and Chemical Properties:-

- | | |
|--------------------|--|
| ▪ Chemical Name | :- Benzothiazole |
| ▪ Description | :- Yellow liquid with unpleasant odor |
| ▪ Boiling Point | :- 231°C |
| ▪ Melting Point | :- 2°C |
| ▪ Flash Point | :- >110°C |
| ▪ Density | :- 1.2460 g/cm ³ at 20°C |
| ▪ Solubility | :- Slightly soluble in water; very soluble in Ethanol diethyl ether and carbon disulfide; soluble in acetone |
| ▪ Refractive Index | :- 1.6379 at 20°C |
| ▪ Vapor Pressure | :- 0.07 mm Hg at 20°C |

Synthesis of 2-amino benzothiazole (Reported methods)

1. Hofmann Method:-

1-Mercapto-benzothiazol was first obtained by A. W. Hofmann in an attempt to prepare the disulphydryl derivative of thiocarbanilide by the action of carbon disulfide on o-aminophenol. He obtained the same substance by the action of sodium hydro- sulfide on chlorophenyl mustard oil (1-chloro-benzothiazole). The product thus obtained, after recrystallization from alcohol, melted at 1790C and was easily oxidized to a disulfide melting at 1800C. Hofmann also noted formation of 2- aniline benzothiazole from the reaction of 2-aminothiophenol and phenyl isothiocyanate, ^[55]

2. Sulfuric acid as a catalyst:-

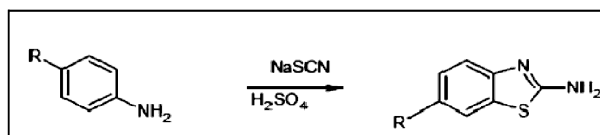


Fig. 16: Scheme for the synthesis of benzothiazole by using sulfuric acid as a catalyst

In this method sodium thiocyanate and cyclize p-substituted aniline were used into 2-amino-6-substituted benzothiazole in the presence of sulfuric acid which act as a catalyst ^[56]

3. Benzene as a catalyst:-

In this method of synthesis the cyclizations of isothiocyanates to 2-aminobenzothiazole in presence of benzene. ^[57]

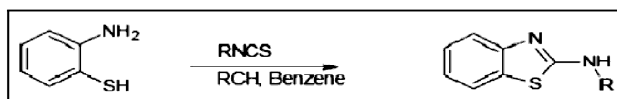


Fig.17: Scheme for the synthesis of benzothiazole by using benzene as a catalyst

4. Lawesson's reagent:-

In this method the synthesis of benzothiazole in which o-anisidine was first reacted with p-nitrobenzoyl chloride to form N-2'-methoxyphenyl-4-nitrobenzamide. The amide was then converted to the thiobenzamide using Lawesson's reagent (2,4-bis(4-

methoxyphenyl)-1,3-dithia- 2,4-diphosphetane- 2,4 disulphide) which is a useful thiation reagent to replace the carbonyl oxygen atoms of ketone, amides and esters by sulphur. In the presence of potassium ferricyanide, it cyclized to the 2-(4'- nitrophenyl)-benzothiazole^[58]

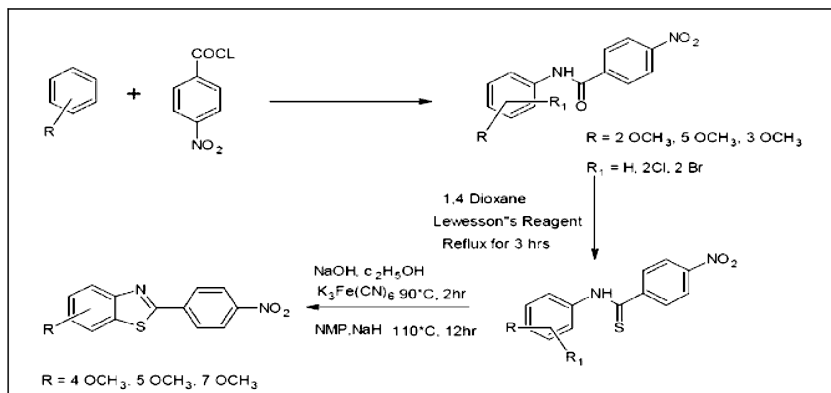


Fig.18: Scheme for the synthesis of benzothiazole by using Lawesson's reagent

5. Bromine as catalyst:-

Recently several methods reported which utilize bromine as catalyst. Basically cyclization with bromine achieved by oxidation of aniline, substituted aniline and arylthiourea in acid or chloroform with alkali thiocyanate. Hegerschoff in early 1900s synthesis 2-aminobenzothiazole and found that an aryl thiourea can be cyclized with liquid bromine in chloroform to form a 2-aminobenzothiazoles. (Hegerschoff 1901). Here the 2-amino-6-methylmercapto benzothiazole was prepared by oxidation of 4-methylmercaptophenylthiourea with bromine as a catalyst.^[59]

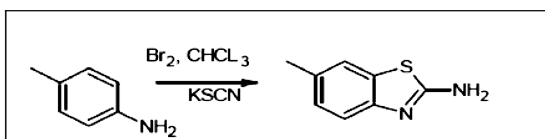


Fig.19: Scheme for the synthesis of benzothiazole by using bromine as a catalyst

6. Benzyl trimethyl ammonium tribromide as catalyst:-

In this method Benzyl trimethyl ammonium (PhCH₂NMe₃Br₃), is an electrophilic bromine source for the conversion of substituted aryl thioureas to 2- aminobenzothiazoles under mild conditions in a variety of solvents with good yields. One of the key benefits for this reagent when compared with molecular bromine in ease of addition and handling,

which minimizes the risk of forming brominated side products. They have extended the use of this reagent to a direct, one-pot synthesis of 2-aminobenzothiazoles from either aryl isothiocyanate and anilines or tetra butyl ammonium thiocyanate and anilines.^[60]

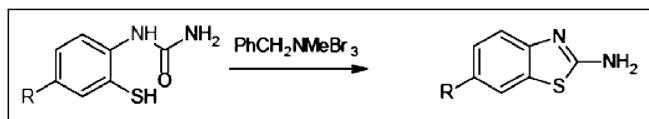


Fig.20: Scheme for the synthesis of benzothiazole by using Benzyl trimethyl ammonium tribromide as catalyst

7. Appel's salt:-

In this method benzothiazole nucleus was synthesized through cyclisation which involve reaction of 2-Bromo-4-nitroaniline with 4,5-dichloro-1,2,3-dithiazol-1-ium chloride, also referred to as Appel's salt, to form (Z)-2-bromo-N-(4-chloro- 5H-1,2,3-dithiazol-5-ylidene)-4-nitroanilin. This intermediate could be cyclized to the desired benzothiazole (Chi *et al.*, 2009).^[61]

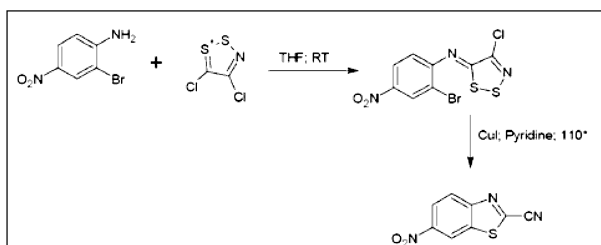


Fig.21: Scheme for the synthesis of benzothiazole by using Appel's salt

Method A:- One-pot reaction of appropriate anilines with thiocyanogen generated from bromine and alkaline thiocyanate in acetic acid medium led to formation of the desired product [XIV] in good to moderate yields.

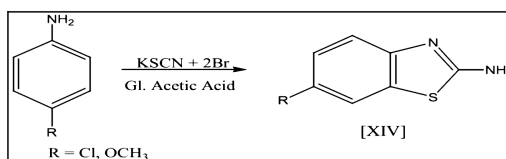


Fig. 22: Scheme for synthesis of benzothiazole using bromine and alkaline thiocyanate in acid medium

Method B: In this method cyclization of phenylthiourea [XV] with bromine in chloroform leads to the formation of 2-amino benzothiazole [XVI].

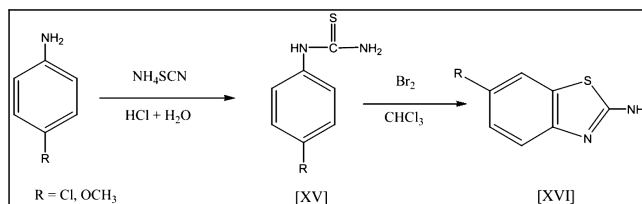


Fig.23: Cyclization of phenylthiourea with Bromine

In the present study, the raw materials *viz.* 2-amino substituted benzothiazoles [XVII] are synthesized by thiocyanating substituted anilines with KSCN and bromine in acetic acid at cold conditions, according to the literature methods ^[62] .

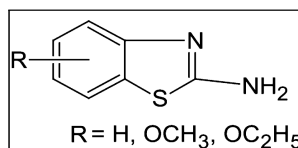
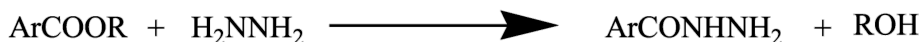


Fig. 24: Substituted Benzothiazoles

Chemistry of Hydrazone

Acid hydrazides are obtained by the reaction of methyl or ethyl esters with hydrazine hydrate.



The general procedures followed for the synthesis of acid hydrazides in the present work is analogous to that reported by Vogel^[63].

Chemistry of Dithiocarbamate salts

- i. Busch and starke^[64] obtained crystalline potassium salt of 2-benzylidithio carbazinic acid (2) by stirring together benzhydrazide (1), carbon disulphide and a solution of KOH in absolute alcohol for 2-3 hours at 35-40°C and then for 12-18 hrs at room temperature. Later Hoggarth synthesized the potassium salts of aryl dithiocarbamate using the same procedures^[65].



Fig. 25: Preparation of potassium salt of 2-benzylidithio carbazinic acid

- ii. Reid et al prepared the potassium salt [4] by stirring together KOH, CS₂ and aryl hydrazide in absolute alcohol for 12-16 hours and the product was washed with dry ether^[66].

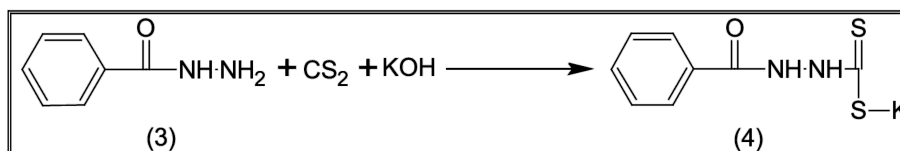


Fig. 26: Preparation of potassium salt with KOH, CS₂ and aryl hydrazide

In present investigation, the method employed for the synthesis of potassium salts of substituted aryl dithiocarbamate is that of Ried et al.; the advantage of this method is the better yield obtained. However the reaction mixture was stirred for 4-5 hours only, as it was seen that the yields obtained after stirring for 4-5 hours or more were the same.

CHEMISTRY OF TRIAZOLE

Methods of preparation of 4-Amino triazole and its derivatives:

1. 4-Amino triazole (7) has been prepared^[67] by the reaction of ethyl formate (5) with hydrazine (6) to give formhydrazide, which was heated at 150 to 200° C to effect self condensation to give (7).

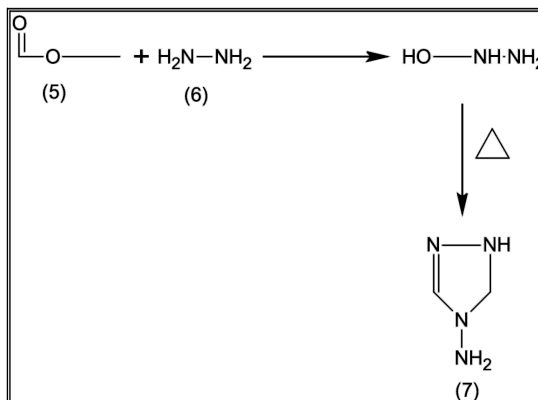


Fig. 27: Preparation of 4-amino triazole

Similar interaction of formic acid and hydrazine gave good yield of 4-Amino triazole (8) [68,69]

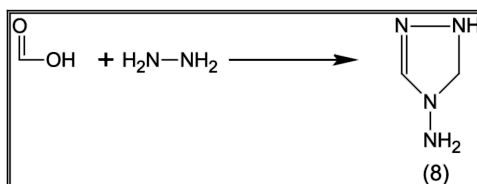


Fig. 28: Preparation of 4-amino triazole

2. Other carboxylic acids and their functional derivatives have also been used to prepared alkyl and substituted alkyl -4-Amino triazoles, e.g. acetic acid and hydrazine hydrate at high temperature give 3,5-dimethyl-4-Amino triazole (9)^[70]

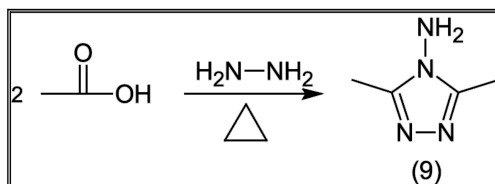


Fig. 29: Preparation of 3,5-dimethyl-4-Amino triazole

3. Similar reaction have been reported for the preparation of 4-Amino-3,5-bis (amino alkyl) triazole (10) ^[71,72], which is prepared by the reaction of glycine with hydrazine to give (10).

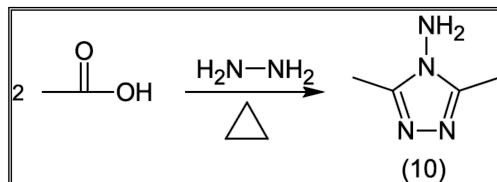


Fig. 30: Preparation of 4-Amino-3,5-bis (amino alkyl) triazole

4. Sulfur and sulfur containing compounds catalyze the formation of 4-Amino triazoles e.g. 4-Amino triazole (14) substituted at 3,5-position with different substituents was prepared by the reaction of equimolar amount of acetonitrile (11) and propionitrile (12) with a mixture of hydrazine (13) and sulfur ^[73].

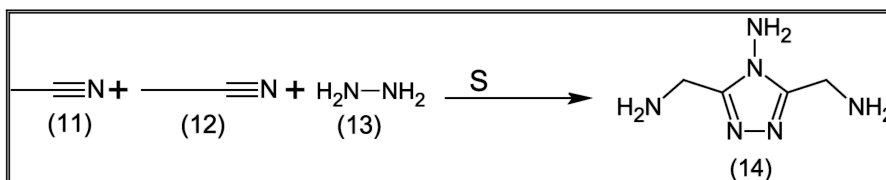


Fig. 31: Preparation of substituted 4-Amino-triazole

5. The preparation of 4-Amino-3,5-diaryl triazoles (18) are usually effected by the acidic rearrangement of 1,2-dihydro tetrazines (17), which are obtained by the reaction of equimolar amount of aryl nitriles (15) and hydrazine (16) in refluxing benzene or toluene ^[74].

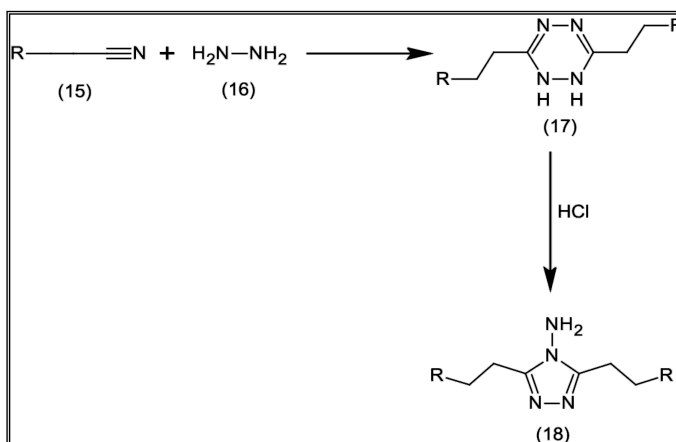


Fig. 32: Preparation of substituted 4-Amino-3,5-diaryl triazoles

6. Perfluoro alkyl triazole (21) are prepared by the various methods e.g. perfluoro acetonitrile (19) with hydrazine (20) in a sealed tube at room temperature gave (21) ^[75]

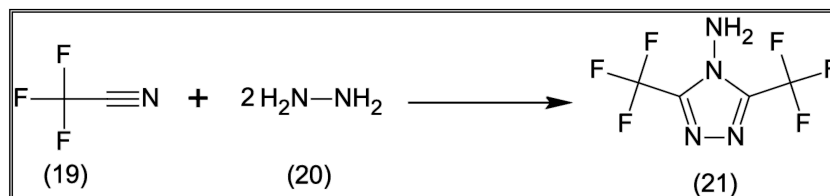


Fig. 33: Preparation of Perfluoro alkyl triazole

7. 4-amino -3,5-diphenyl triazoles (24) are also formed by the decomposition of other hetero aromatic ring systems e.g. reaction of pyrimidine (22) with hydrazine. Also the thermolysis of 5-phenyl tetrazole (23) in various solvents ^[76] gives (24).

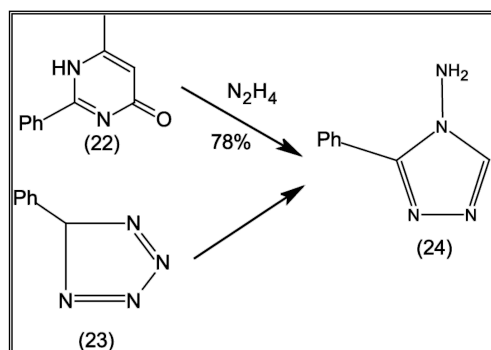


Fig. 34: Preparation of 4-amino -3,5-diphenyl triazoles

Methods of preparation of 3-(substituted phenyl)-4-amino-5-mercapto-1,2,4-triazole:

1. One of the methods for synthesis of triazole is Hoggarth synthesis ^[77]. This methodology involves the condensation of carboxylic acid hydrazides with carbon disulphide and potassium hydroxide to yield the potassium dithiocarbazate (26) which after s-alkylation with methyl iodide, undergo ring closure with an excess of hydrazine, to produce the amino thiols (29) in good yield.

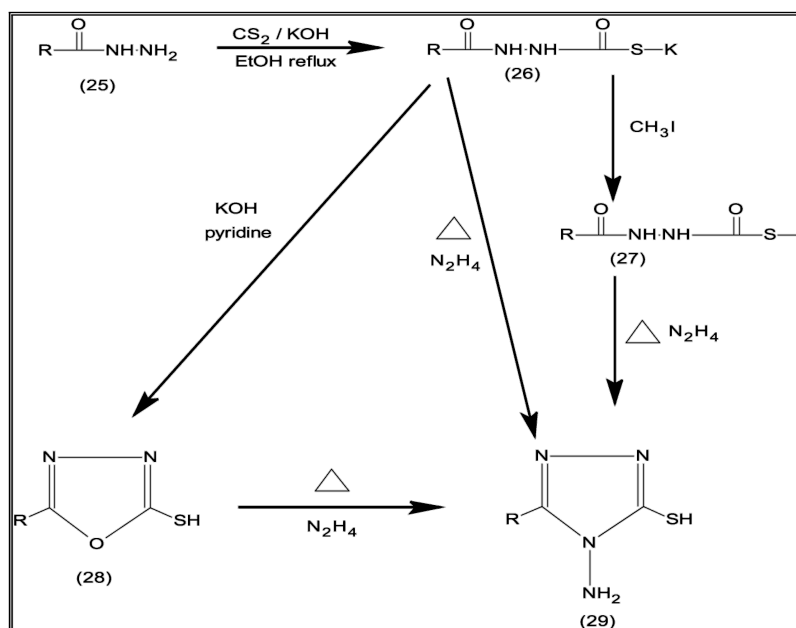


Fig. 35: Preparation of 3-(substituted phenyl)-4-amino-5-mercapto-1,2,4-triazole

- More recently, Heindel and Reid have reported the direct conversion of the carbazate salt by the reaction with excess of hydrazine, this avoids 5-methylation, resulting in the higher yield and a shorter reaction time^[78].
- Alternatively, oxadiazoles (28) can be converted to triazoles (29) by the reaction with hydrazine.

It has also been reported that the same carboxylic acid react with thiocarbo-hydrazide to afford directly the 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (32). It has also been found that the reaction of thiocarbohydrazide with carboxylic acids can be conveniently applied to both aliphatic and aromatic acid derivatives^[79,80].

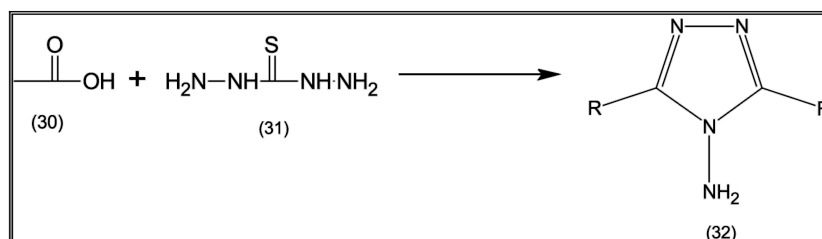


Fig. 36: Preparation of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles

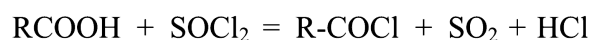
Chemistry of Acid chlorides

Acid chlorides are used primarily as intermediates in the synthesis. They are fairly reactive compounds and are usually liquids. Acid chlorides are obtained from acids through the replacement of the hydroxyl group with chloride. This exchange may be accomplished by the use of a number of inorganic chlorine compounds, among these thionyl chloride, phosphorous pentachloride, sulphuryl chloride and phosgene gas are most important.

Phosphorous pentachloride is a highly reactive compound and its use offers a general method for the preparation of acid chlorides. The products of the reaction are phosphorous oxychloride and hydrogen chloride, in addition to the acid chloride.

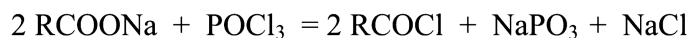


The use of thionyl chloride^[81] for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction results in the formation of sulphur dioxide and hydrogen chloride in addition to the acid chloride and the isolation of the chloride are for this reason a simple matter.

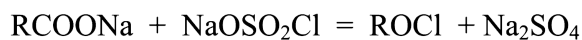


The thionyl chloride must be pure as impure product has been known to cause a somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid by crystallization.

Phosphorous oxy-chloride reacts with the sodium salt of carboxylic acid to give acid chloride. This method is of importance with acids which undergo decomposition with other chlorides of phosphorous.



The reaction of phosgene^[82] with carboxylic acids and p-toluene sulphonylchloride or of sodium chlorosulphonate with salts of carboxylic acids is also used for the preparation of acid chlorides.



The acid chlorides are obtained readily through the reaction of sulphuryl chlorides with anhydrous salts of organic acids. The reagents are well mixed and subjected to distillation.

In the present work some acid chlorides were prepared by known methods using corresponding benzoic acids and the some commercially available acid chlorides were used from Sigma-Aldrich Chemicals.

➤ **Reaction mechanism:**

Acids and their salts are the only common starting materials of acid chlorides and several reagents are utilized as a source of nucleophilic chloride. All the reagents from reactive intermediate so, that the leaving group in the substitution is not hydroxyl group.

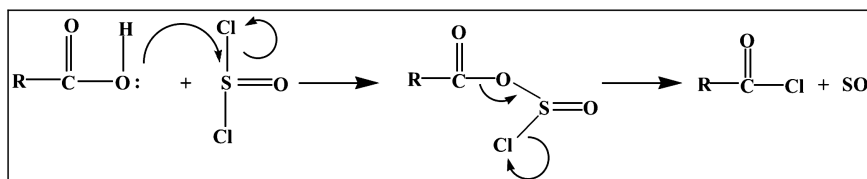


Fig. 37: Reaction mechanism of acid chloride

Chemistry of Schiff bases

A Schiff base (or azomethine), named after Hugo Schiff, is a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group—but not hydrogen.

Schiff bases are of the general formula $\mathbf{R_1R_2C=N-R_3}$, where $\mathbf{R_3}$ is an aryl or alkyl group that makes the Schiff base a stable imine. A Schiff base derived from aniline, where $\mathbf{R_3}$ is a phenyl or substituted phenyl, can be called an *anil*.

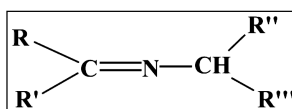


Fig. 38: General formula of Schiff base

Schiff bases are formed by the condensation of an amine and the carboxyl group of aldehydes.



Schiff ^[83] for the first time reported condensation of this kind and hence the condensation products are referred to as “Schiff bases”.

Schiff bases are characterized by the -N=CH- (imine) group which is important in elucidating the mechanism of transamination and racemisation reaction in biological systems^[84,85]. Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behavior studied ^[86].

Mechanistically, the formation of an imine involves two steps (Fig. 37). First, the amine nitrogen acts as a nucleophile, attacking the electrophilic carbonyl carbon of aldehydes or ketones. In the next step (Fig. 39), the nitrogen is deprotonated, and the electrons from this N-H bond push the oxygen off of the carbon, leaving a compound with a C=N double bond (an imine) and a water molecule displaced.

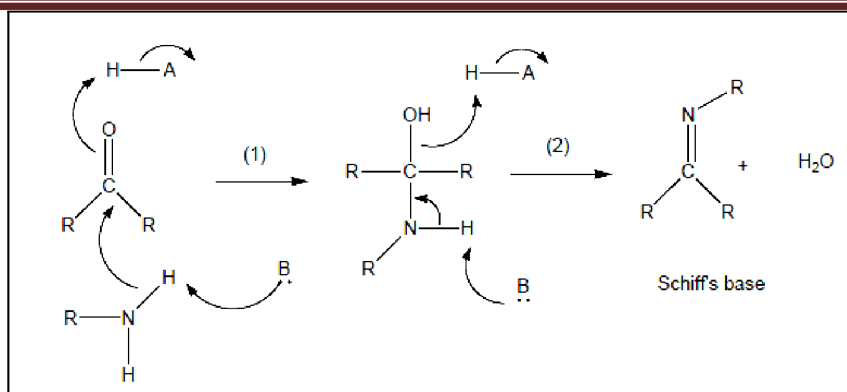


Fig. 39: Mechanism of Schiff's base (Imine) formation

The end result of this reaction (Fig. 40) is a compound in which the C=O double bond is replaced by a C=N double bond. This type of compound is known as an imine, or Schiff's base.

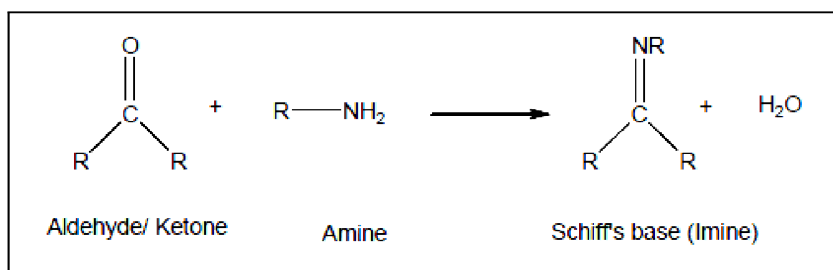


Fig. 40: Formation of Schiff's base

Pelton et al^[87] had shown the antifungal and antibacterial activity of some substituted salicylaldehydes. Schiff bases from different aldehydes have shown encouraging anti-tuberculosis activity *in vitro* in our laboratory^[88]. Also the presence of an azomethine linkage in compounds has been shown to decrease their toxicity considerably^[89]. Many procedures have been described in the literature for the synthesis of Schiff bases^[90-92].

The important methods are listed below:

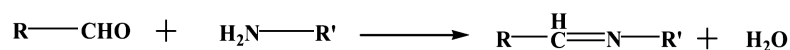
1. The simplest method appears to be the one in which equimolar quantities of an amine and aldehydes are condensed by boiling in methanol or ethanol.
2. In 1946, Tipson and Clapp^[93] obtained Schiff bases by refluxing the amine and the aldehydes in toluene with a few drops of piperidine. The water formed during the course of the reaction was removed by employing a Dean-stark trap.

3. In 1950, Hayes et al^[94] prepared Schiff bases by heating a mixture of an aldehydes and an amine in benzene and removing the water formed during the reaction using Dean-Stark apparatus.
4. In 1953, Giovambettista and Rabassa^[95] suggested the use of acetic acid as a catalyst for the synthesis of Schiff bases in good yield.
5. In 1971, the condensation of hydrazine and an aldehydes using glacial acetic acid as a solvent was described.

As the first method was found to give good yields it was followed initially. The addition of few drops of acetic acid was found to improve the yield. Later on, during the course of investigation the last method using acetic acid as a solvent was found to be the best for the following reasons.

1. Simple apparatus and no need of Dean Stark Trap.
2. Good Yield.
3. Time course of the reaction was reduced.

Reaction mechanism:



The amine and the aldehydes are heated in the presence of an acid. The acid protonated the carboxyl oxygen and promotes the attack of nucleophilic amine.

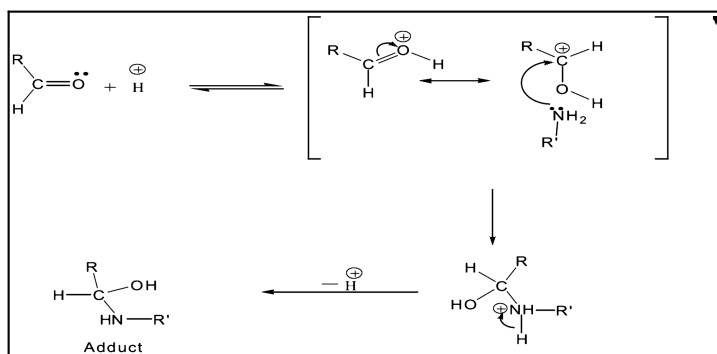


Fig. 41: Reaction mechanism of schiff base

The adduct thus formed undergoes a further reaction to eliminate water and form a carbon nitrogen double bond.

Chemistry of amides

Amides are derived from carboxylic acids. A carboxylic acid contains the –COOH group, and in an amide the –OH part of that group is replaced by an –NH₂ group. So amides contain the –CONH₂ group. (R-CO-NR'R'')

The amide linkage is one of the most fundamental and widespread chemical bonds in nature^[96]. Amides are common moieties in naturally occurring substances like proteins and peptides and are also found in various synthetic materials. This makes the amide functionality an important building block in synthetic chemistry and many methods for its synthesis were reported in the literature^[97-101]

Amide compounds have attracted much attention because of their extensive applications in the pharmaceutical industry; they have been generally used as a protective group in synthetic routes, an amide functional group is contained in many bioactive compounds or drugs, and amide modification is an important optimization methodology for new chemical entities in medicinal chemistry^[102-106] Furthermore, peptide bond, a special kind of amide bond in life science, constitutes the backbone of proteins.

The stable and polar amide functionality is an important unit among the organic molecules present in natural occurring materials. It is also found in many synthetic substances, an intermediate or as active pharmaceutical product or prodrugs^[107]. Due to its interest in organic synthesis the preparation of amides from the corresponding amines is an important and well known transformation. It has been noted recently that around 66% of all preliminary screening reactions in industrial medicinal chemistry laboratories involve amide formation^[108-110]

Since, amide bond linkage is ubiquitous and important core structure in pharmaceuticals, chemicals, as well as many natural products^[111-113] and hence it was thought to synthesize new amide linkage by condensation of formylated pyrazolyl benzothiazole and different substituted benzhydrazide.

CHEMISTRY OF 1,3,4 OXADIAZOLE

Oxadiazole is considered to be derived from furan by replacement of two methane (-CH=) group by two pyridine type nitrogen (-N=). There are four possible isomers of oxadiazole (1,2, 3, 4) depending on the position of nitrogen atom in the ring and are numbered as shown in Fig. 38 ^[114]

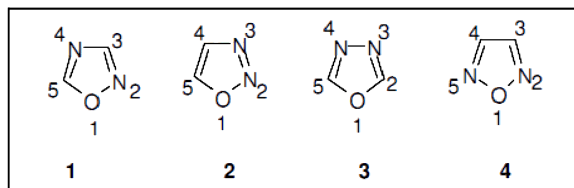


Fig.42: Isomers of Oxadiazole

Literature survey reveals that particularly 1, 3, 4-oxadiazole derivatives exhibit wide range of biological activities including anticancer ^[115] anti-inflammatory ^[116], fungicidal ^[117], herbicidal ^[118], pesticidal, analgesic ^[119], anticonvulsant ^[120-121], anti-HIV ^[122], antibacterial and plant growth regulator activities ^[123].

2.1 Chemistry of oxadiazole

Oxadiazole is a very weak base due to the inductive effect of the extra heteroatom ^[124]. There placement of two -CH= groups in furan by two pyridine type nitrogen (-N=) reduces aromaticity of resulting oxadiazole ring to such an extent that the oxadiazole ring exhibit character of conjugated diene. The electrophilic substitutions in oxadiazole ring are extremely difficult at the carbon atom because of the relatively low electron density on the carbon atom which can be attributed to electron withdrawal effect of the pyridine type nitrogen atom. However the attack of electrophiles occurs at nitrogen, if oxadiazole ring is substituted with electron-releasing groups. Oxadiazole ring is generally resistant to nucleophilic attack. Halogen-substituted oxadiazole, however, undergo nucleophilic substitution with replacement of halogen atom by nucleophiles. Oxadiazole undergo nucleophilic substitution similarly as occurring at an aliphatic sp² carbon atom ^[125]. The present manuscript specially emphasizes on chemistry, methods of synthesis and Reactivity of 1, 3, 4-oxadiazole and its derivatives.

2.2 Synthesis of 1, 3, 4-oxadiazole backbone

M.C Hosur ^[126] reported synthesis of 2-mercapto-5-aryl-1, 3, 4-oxadiazole (**6**) from the properly substituted acid hydrazide (**5**) in presence of CS₂/KOH. (**Fig.43**). This method is very popular since ease in workup and high yields are consistently observed. However, long reaction time is a limiting factor. Number of examples are cited in Literature employing this methodology for synthesis of 1, 3, 4-oxadiazole thione / thiol derivatives ^[127-130].

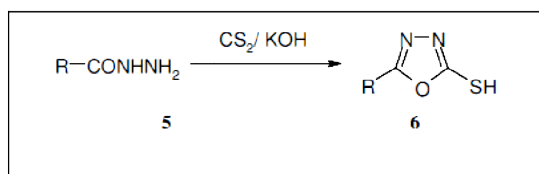


Fig. 43: synthesis of 2-mercapto-5-aryl-1, 3, 4-oxadiazole using carbon disulphide in alkaline media.

M.A.Elborai et al ^[131] reported synthesis of 2-amino-5-(2'-thienyl)-1, 3, 4-oxadiazole (**8**) by the condensation of 2-thienyl hydrazide (**7**) with CNBr (Fig.44). It is a convenient method of synthesis of amino-1, 3, 4-oxadiazole because of shorter reaction time. More reports are cited in literature, which employed this method to obtain the 1, 3, 4- amines. ^[132-133]

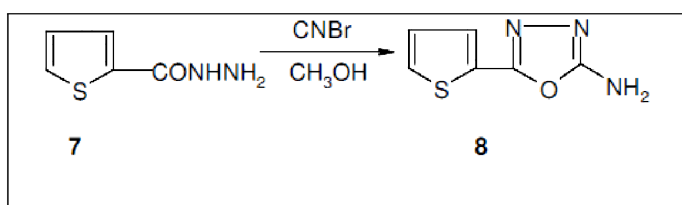


Fig.44: synthesis of 2-amino-5-(2'-thienyl)-1, 3, 4-oxadiazole

H. Singh and coworkers ^[134] reported synthesis of 2-arylmethyl-Δ²-1, 3, 4-oxadiazoline-5-ones (**10**) from acylurea ^[135] using bromine in NaOH (Fig.45).

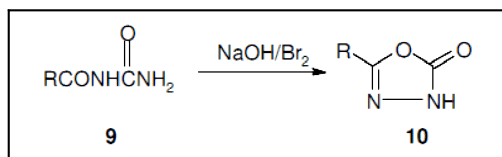


Fig.45: Synthesis of 1, 3, 4-oxadiazolone using bromine in alkaline media

D. H. Boschelli et al ^[136] synthesized substituted aryl type of (12) 1, 3, 4-oxadiazolone by reacting 1, 1'-carbonyldiimidazole (CDI) and triethylamine (TEA) in presence of tetrahydrofuran (THF) assolvent (Fig.46).

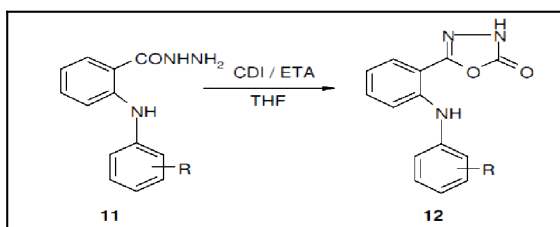


Fig.46: Synthesis of 1, 3, 4-oxadiazolone using carbonyl di-imidazole (CDI)

Formation of disubstituted-1, 3, 4-oxadiazole via cyclodehydrogenation using phosphorusoxychloride (POCl₃) has also been accounted in various literatures ^[137-140]. Condensation of various alkyl hydrazides (13) with substituted aromatic acids in presence of POCl₃ yielded respective 2-alkyl-5-aryl -1, 3, 4-oxadiazoles (14). (Fig.47).

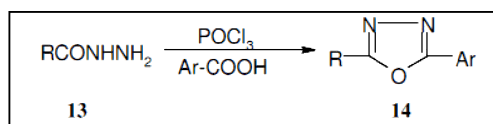


Fig.47: Synthesis of 2,5-disubstituted 1, 3, 4-oxadiazole by cyclodehydrogenation.

N. Sikder et al^[141] synthesized long chain bis-1, 3, 4-oxadiazoles (16), from the cyclization of N, N'-dinitrobenzoyl malonic or adipic dihydrazide (15) in presence of POCl₃. (Fig.48). Variety of disubstituted 1, 3, 4-oxadiazoles have been obtained by using POCl₃ as dehydrating agent.

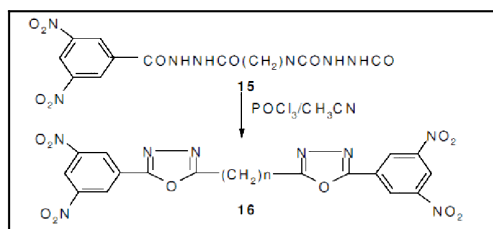


Fig.48: Synthesis of 1, 3, 4-oxadiazole from semicarbazide

Various oxidizing agents have been employed to obtain 1, 3, 4-oxadiazole derivatives fromhydrazides, semicarbazides, thiosemicarbazides and Schiff's bases. S. P.

Singh and group^[142] reported the synthesis 1, 3, 4-oxadiazole (17) through a one pot procedure (Fig.49) involving the reaction of acylhydrazide with aldehydes followed by in-situ cyclization using Chloramine T (p-CH₃-C₆H₄SO₂N--Cl Na⁺.3H₂O) as an oxidizing agent.

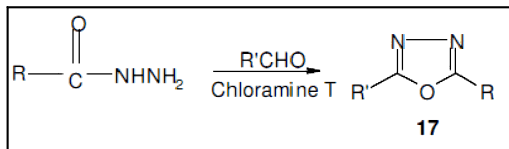


Fig. 49: Synthesis of 1, 3, 4-oxadiazole using Chloramine T

5-(1-p-chlorophenyl-5-methyl-1, 2, 3 - triazol-4-yl)-2-phenylamino- 1, 3, 4-oxadiazole (19) was synthesized from corresponding thiosemicarbazide (18) in presence of mercuric acetate as an oxidizing agent^[143] (Fig.50).

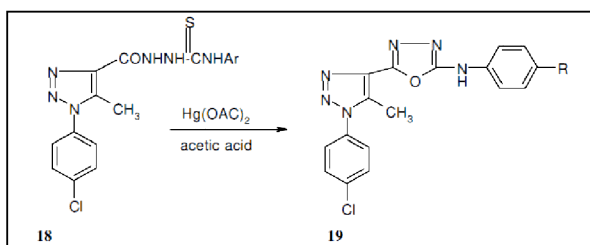


Fig.50: Synthesis of 1, 3, 4-oxadiazole using mercuric acetate

The Schiff's bases of 2-phenyl-3-semicarbazidoindole (22) on oxidative cyclodehydrogenation with ferric chloride in acetic acid gave substituted-3-(5'-phenyl-oxadiazolyl)-aminoindole (21) (Fig.51)^[144].

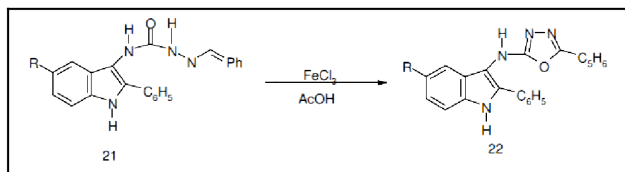


Fig.51: Synthesis of 1, 3, 4-oxadiazole analogues from Schiff's Bases using FeCl₃

The Schiff's bases of 1-(2, 4-dichlorobenzoyl)-4-phenyl thiosemicarbazide were oxidized by iodine at room temperature in presence of alkali to yield cyclised product (23) (Fig.52) ^[145]

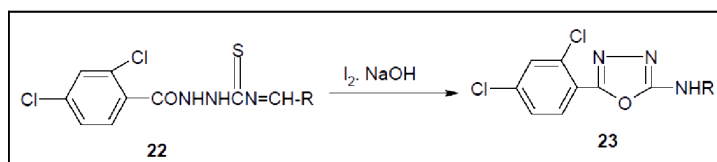


Fig.52: Synthesis of 1, 3, 4-oxadiazole analogues using iodine /potassium iodide

H. Saikochi and coworkers synthesized 5-substituted -2-2-(furyl)-1, 3, 4-oxadiazoles(24) using LTA as an effective oxidizing agent (Fig.53) . This method is widely employed for synthesis of 2, 5-disubstituted 1, 3, 4-oxadiazole because of ease in work up and higher yield. Utilization of hypervalent reagent is a green approach in organic synthesis.

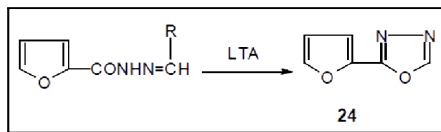


Fig.53: Synthesis of 1, 3, 4-oxadiazole analogues using lead tetra acetate

O. Parekh and colleagues ^[146] employed hypervalent iodine (IBD) for oxidation of Schiff's base to obtain 2, 5-disubstituted 1, 3, 4-oxadiazole (25) (Fig.54)



Fig.54: Synthesis of 1, 3, 4-oxadiazole analogues using hyper valent iodine

Traditionally organic reactions are heated using an external heat source and therefore transferred by conductance. This is comparatively slow and inefficient method for transferring energy into the system because it depends on thermal conductivity of the various material that must be penetrated and results in increasing the temperature of the reaction vessel being higher than that of the reaction mixture. By contrast microwave irradiation produces efficient internal heating by direct coupling of microwave energy

with polar molecules. Microwave assisted organic synthesis is mainly based on the efficient heating of materials by microwave dielectric heating effects. Microwave dielectric heating is dependent on the ability of a specific material to absorb microwave energy and convert it to heat ^[147,148]. In many instances significantly reduced reaction times, high yields and cleaner reaction profiles are being experienced, allowing for more rapid reaction optimization and library synthesis. Solvents with lower boiling points can be used under pressure (closed vessel condition) and be heated at temperatures considerably higher than their boiling point. Microwave heating allows direct ‘in core’ heating of the reaction mixture, which results in a faster and more even heating of the reaction mixture. M Kidwai and coworkers ^[149] reported synthesis of disubstituted 1, 3, 4-oxadiazole derivatives (27) using mixture of hydrazide, substituted aromatic acids and thionyl chloride under microwave irradiation (MWI) within 6-7 min. (Fig.55).

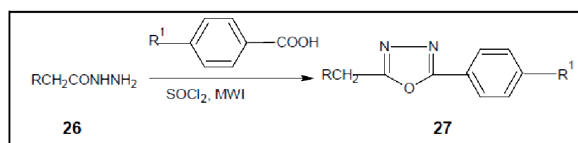


Fig.55: Synthesis of 1, 3, 4-oxadiazole analogues under Microwave

Similarly, Khalid M. Khan and his group ^[150] reported the microwave assisted synthesis of such diaryl-1, 3, 4-oxadiazoles (28). In this a number of commercially available hydrazides were treated with different carboxylic acids in the presence of phosphorus oxychloride to afford the desired products (Fig.56)

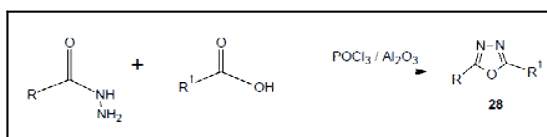


Fig.56: Synthesis of 1, 3, 4-oxadiazole analogues under Microwave via cyclodehydration

Reactions of 1, 3, 4-Oxadiazole

The substituted and unsubstituted 1, 3, 4-oxadiazole undergoes varieties of organic reactions. Due to very low π -electron density on the carbon atom, the attack of electrophile preferentially occurs at the position 3 with the formation of 1, 3, 4-oxadiazolium salts (29)(Fig.57).

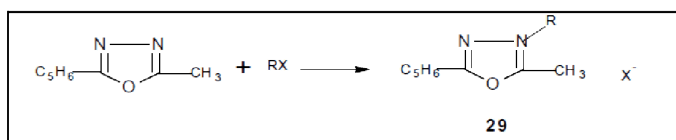


Fig.57: Attack of electrophiles (R) at 3rd position

However, the alkylation of 2-alkoxy-1, 3, 4-oxadiazole with alkyl halides produces labile oxadiazolium salt (30) which undergo O-dealkylation to provide 4-alkyl-oxadiazolin-5-ones (31) (Fig.58). 2, 5-Diphenyl-1, 3, 4-oxadiazole can be nitrated or sulfonated by conc. HNO_3 and oleum. This results in attack on phenyl ring itself.

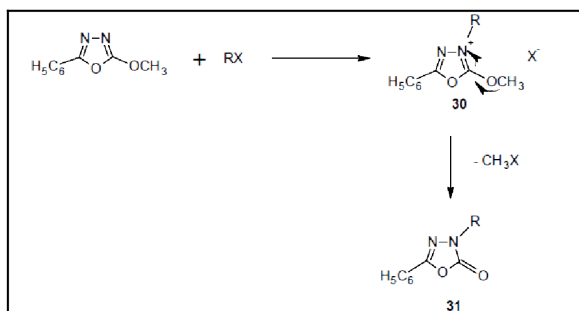


Fig.58: Mechanism of reaction with electrophiles

Reactions with nucleophiles are also reported. The carbon atoms in 1, 3, 4-oxadiazole ring are relatively with low π electron density and therefore attack of nucleophiles occurs at this carbon atom. The reaction proceeds either with nucleophilic substitution (32) or with ring cleavage (33)(Fig.59).

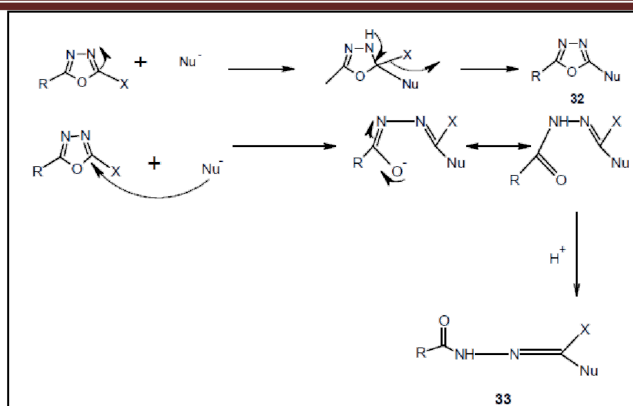


Fig.59:Nucleophilic substitutions and ring cleavage

1, 3, 4-oxadiazole substituted with halogen or sulfonyl group at the position-2 easily undergo nucleophilic substitution reaction. The reaction of 2-chloro-1, 3,4-oxadiazole with nucleophiles such as amines, thiourea or azide proceeds with the substitution of chloro group by nucleophile and result in the corresponding 2-substituted-1, 3, 4-oxadiazoles (34) (Fig.60).

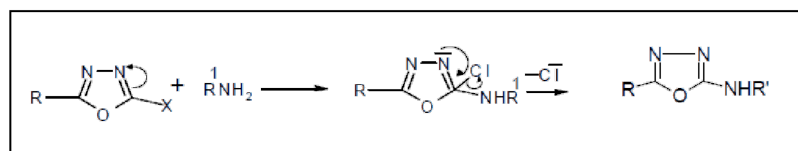


Fig.60:Reaction with amines

The reaction of alkyl or aryl- 1, 3, 4-oxadiazoles (35) with nucleophile involve the cleavage of 1, 3, 4-oxadiazole ring ending to the formation of hydrazine derivative which may recycle to form 1, 2, 4 –triazoles (36) (Fig.61).

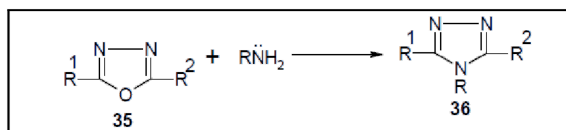


Fig.61: Formation of 1, 2, 4 –triazoles from 1, 3, 4-oxadiazole

Alkyl and aryl-1, 3, 4 -oxadiazole undergo acid or base catalyzed ring opening reaction in aqueous solution with formation of diacylhydrazines. 1, 3, 4 -oxadiazole is thermally stable and the stability of the ring is increased with the substitution of the aryl groups. However, 1, 3, 4-oxadiazolin-5-one undergo thermal and photochemical ring opening reaction with the loss of carbon dioxide to provide nitrilimines. It is well

documented that diaryl 1,3, 4-oxadiazole react with benzothiophene leading to formation of oxa-diazepine as a major product. But irradiation of this molecule with benzophenone as sensitizer results in (2+2)cyclo adduct product. 2, 5 substituted- 1, 3, 4- oxadiazole are greatly affected by strong acid or base. Cleavage of the ring is followed by hydrazine formation. In general aryl derivatives are less sensitive than alkyl substituted derivatives.

A special type of rearrangement known as ‘Dimroth rearrangement’ is exhibited by 1,3,4-oxadiazole having a substituent containing heteroatom like O,S,N at position 2 or 5 in presence of strong acid or strong alkali upon long duration heating ^[151]. If it is intended to carry out the reaction involving heteroatom at position 2 or 5 of 1, 3, 4 – oxadiazole, the reaction should be carried out at lower temperature (< 80 °C) after mixing the reagent at room temperature. Otherwise, possibility of Dimroth rearrangement always exists which may yield mixture of both.

3.0 EXPERIMENTAL & CHARACTERIZATION

Experimental section covers synthesis and characterization of title compounds with following chromatographic and spectroscopic techniques.

Identification & Characterization of prepared compounds were carried out by following procedure:

1. Thin Layer Chromatography
2. Chemical Test
3. Infra Red Spectroscopy
4. High Performance Liquid Chromatography [HPLC]
5. Gas Chromatography Mass spectroscopy [GC-MS]
6. Nuclear Magnetic Resonance Spectroscopy [NMR]
7. Melting Point Determination

3.1.0 Thin layer chromatography (TLC)

TLC was performed to confirm the completion of reaction and purity of product formed. TLC was carried out in three steps- spotting, development, and visualization.

3.1.1 Identification of development solvent

Identification of development solvent was carried out to find an ideal solvent in which all the derivatives are run and progress of reaction checked. Various solvents were tried from which we got the satisfactory results in ethyl acetate and n-hexane. Ethyl acetate: n-hexane (30:70 %v/v) proportions were used.

3.1.2 Procedure

The sample to be analyzed was dissolved in a methanol to produce a very dilute (about 1%) solution. spotting of the sample was done using capillary tube and transferred a small amount of this dilute solution to one end of a TLC plate, in this case a thin layer of

powdered silica gel that has been coated onto a plastic sheet was used. Then the TLC plate was placed into a shallow pool of a development solvent. Ethyl acetate and n-Hexane was used as mobile phase in the ratio of (30:70 %v/v). When the solvent has traveled almost to the top of the plate, the plate was removed, and the solvent allowed evaporating. Finally, the visualization of the spots was done under the UV light.

3.1.3 Chemical tests ^[152-153]

Chemical tests are generally performed for the confirmation of functional groups in the compounds but these tests are not that much confirmatory compared to IR and GC-MS, so that these tests were performed at initial stage only to confirm the presence of functional groups in the synthesized derivatives.

3.1.4 Infrared (IR) spectroscopy ^[154-155]

IR spectroscopy was carried out for the characterization and the identification of functional groups in the solid samples. Material containing sample must be transparent to the IR radiation. So, the salts like NaCl, KBr were used.

3.1.4.1 Sample preparation

A small amount of finely ground solid sample (0.5 or 1 mg) was intimately mixed with about 100 times its weight of powdered potassium bromide (KBr) (100 mg) in mortar-pestle. Then this mixture was filled in the IR solid sample disc and put in the IR cell and mixture was run as such.

3.1.5 High performance liquid chromatography (HPLC) ^[156]

HPLC was carried out to separate the components in a sample, to quantify each component and to determine the purity of the samples.

3.1.5.1 Solubility Studies

These studies were carried out to find an ideal solvent in which all the derivatives are completely soluble and stable. Various solvents were tried for checking solubility of derivatives. Derivatives are soluble methanol.

3.1.5.2 Selection of analytical wavelength

Accurately weighed and transferred about 10 mg of title compound into a 100 mL volumetric flask, added about 70 mL of mobile phase and shake to dissolve the sample completely. Diluted to volume with mobile phase and mixed well. The standard solution was further diluted with mobile phase to get concentration of 10µg/ml. Similarly, the sample solutions were prepared for the other derivatives. The above solutions were scanned between 200 to 400 nm by UV spectroscopy and absorption bands of the sample solutions were recorded. For analytical purposes the wavelength of the photodiode array detector was set at which it presents better reproducibility than the other UV-bands.

3.1.5.3 Sample preparation

Accurately weighed and transferred about 10 mg of title compounds into a 100 mL volumetric flask, added about 70 mL of mobile phase and shake to dissolve the material completely. Diluted to volume with mobile phase and mixed well. The standard solution was further diluted with mobile phase to get concentration of 10µg/ml. Similarly, the sample solutions were prepared for the other derivatives. The above solutions were transferred in the vials and run for the HPLC analysis.

3.1.5.4 Optimization of Chromatographic Conditions

Initially Methanol: Acetonitrile combination was tried in different proportions. Then Methanol: Water was tried in different proportions in order to determine the best conditions for the effective separation of 4-hydroxy coumarin derivatives. The Flow rate was set at 1 and 1.5 ml/min to give best system suitability test parameters.

3.1.6 Gas Chromatography-Mass Spectrometry (GC-MS) ^[157-159]

GC-MS was carried out to determine the elemental composition of derivatives and to elucidate the chemical structures of derivatives.

3.1.6.1 Sample preparation

Accurately weighed and transferred about 10 mg of title compound into a 100 mL volumetric flask, added about 70 mL of methanol and shake to dissolve the material completely. Diluted to volume with methanol and mixed well. The standard solution was

further diluted with methanol to get concentration of 10µg/ml. Similarly, the sample solutions were prepared for the other derivatives. The above solutions were transferred in the vials and run for the GC-MS analysis.

3.1.7 Melting Point Determination:

Melting point of the synthesized compounds is carried out by open capillary tube method. Melting point is valuable criteria of purity for as a pure crystals having definite & sharp melting point. The purity should not to be assumed but must be established by observation of any changes in the melting point when the compound is subjected to the purification by recrystallisation. The synthesized compound shows minute changes after recrystallisation. Melting points were recorded on a Thermo-nik Melting point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected.

Experimental part is further divided into following sections:

3.2.0 III-A] Synthesis of 2-[(6- Substituted phenyl [1,2,4] triazolo [3,4-*b*] [1,3,4]

thiadiazol-3-yl) methyl] sulfanyl}-1,3-benzothiazole

3.3.0 III-B] Synthesis of *N*-[(6,7-dimethyl [1,2,4] triazolo [3,4-*b*] [1,3,4]

thiadiazepin-3-yl)methyl] -substituted 1,3-benzothiazol-2-amine

3.4.0 III-C] Synthesis of *S*-{5-[(1,3-benzothiazol-2-yl)sulfanyl] methyl} -1,3,4-

oxadiazol-2-yl} substituted benzenecarbothioate

3.5.0 III-D] Synthesis of 4-[(*E*)-(substituted-4-oxo-4*H*-chromen-3-yl)

methylidene] amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide

3.2.0 III-A] Synthesis of 2-{[(6- Substituted phenyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazol-3-yl) methyl] sulfanyl}-1,3-benzothiazole

III-A includes following steps

3.2.1 III-A.1 Synthesis of ethyl [(6-ethoxy-1, 3-benzothiazol-2-yl)sulfanyl]acetate

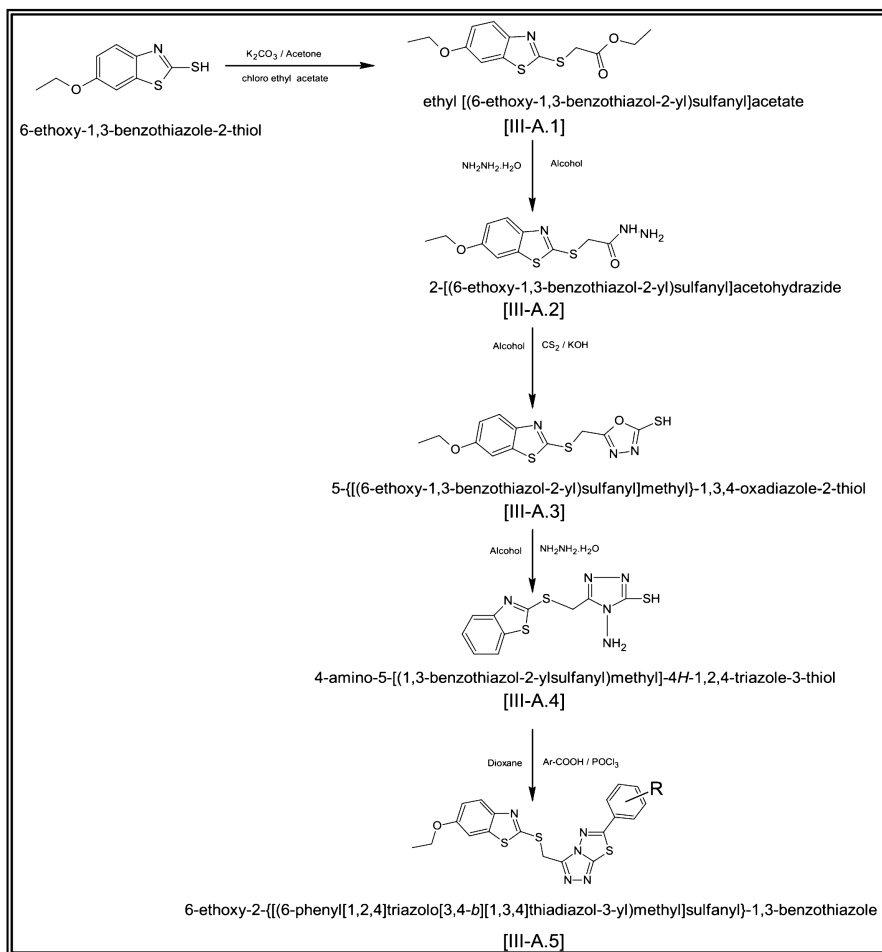
3.2.2 III-A.2 Synthesis of 2-[(6-ethoxy-1, 3-benzothiazol-2-yl) sulfany l] acetohydrazide

3.2.3 III-A.3 Synthesis of 5-{[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-Oxadiazole-2-thiol

3.2.4 III-A.4 Synthesis of 4-amino-5-{[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl] methyl}-4*H*-1,2,4-triazole-3-thio

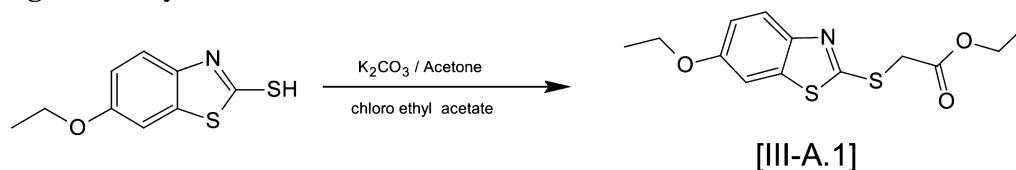
3.2.5 III-A.5 Synthesis of 6-ethoxy-2-{[(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]sulfanyl} -1,3-benzothiazole

Fig No 62: Schematic representation of III-A



3.2.1.0 III-A.1 synthesis of ethyl [(6-ethoxy-1, 3-benzothiazol-2-yl)sulfanyl]acetate^[160]

3.2.1.1 Fig No 63: Synthesis of III-A.1



3.2.1.2 Table No 6: Reaction Calculation table

Compound	Mol. Wt	Wt in gm/mL	Mole	Mole ratio
6-ethoxy-2-Mercapto Benzothiazole	211	2.11	0.01	1
ethyl chloroacetate	122	1.22	0.01	1
Potassium carbonate	138	0.69	0.005	0.5
Acetone	---	10	--	--

3.2.1.3 Procedure: -

6-ethoxy-2-Mercapto Benzothiazole was dissolved in acetone stirred for 30 mins and K_2CO_3 was added and reflux the reaction mixture for 24 hrs leading to formation of potassium salt of mercapto benzothiazole. After salt formation ethyl chloroacetate was added over the period of 15 mins. Reflux the reaction mixture till completion of reaction. Reaction was monitoring was done by TLC. Formed product was isolated by drowning in cold water. Clear solution was extracted with Diethyl ether 10mL three times to extract product from aqueous layer. Recrystallized the product from acetone.

3.2.1.4 Table no 7: Observation table

Melting point	85 ⁰ C	
Yield	70%	
Colour	Pale yellow	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

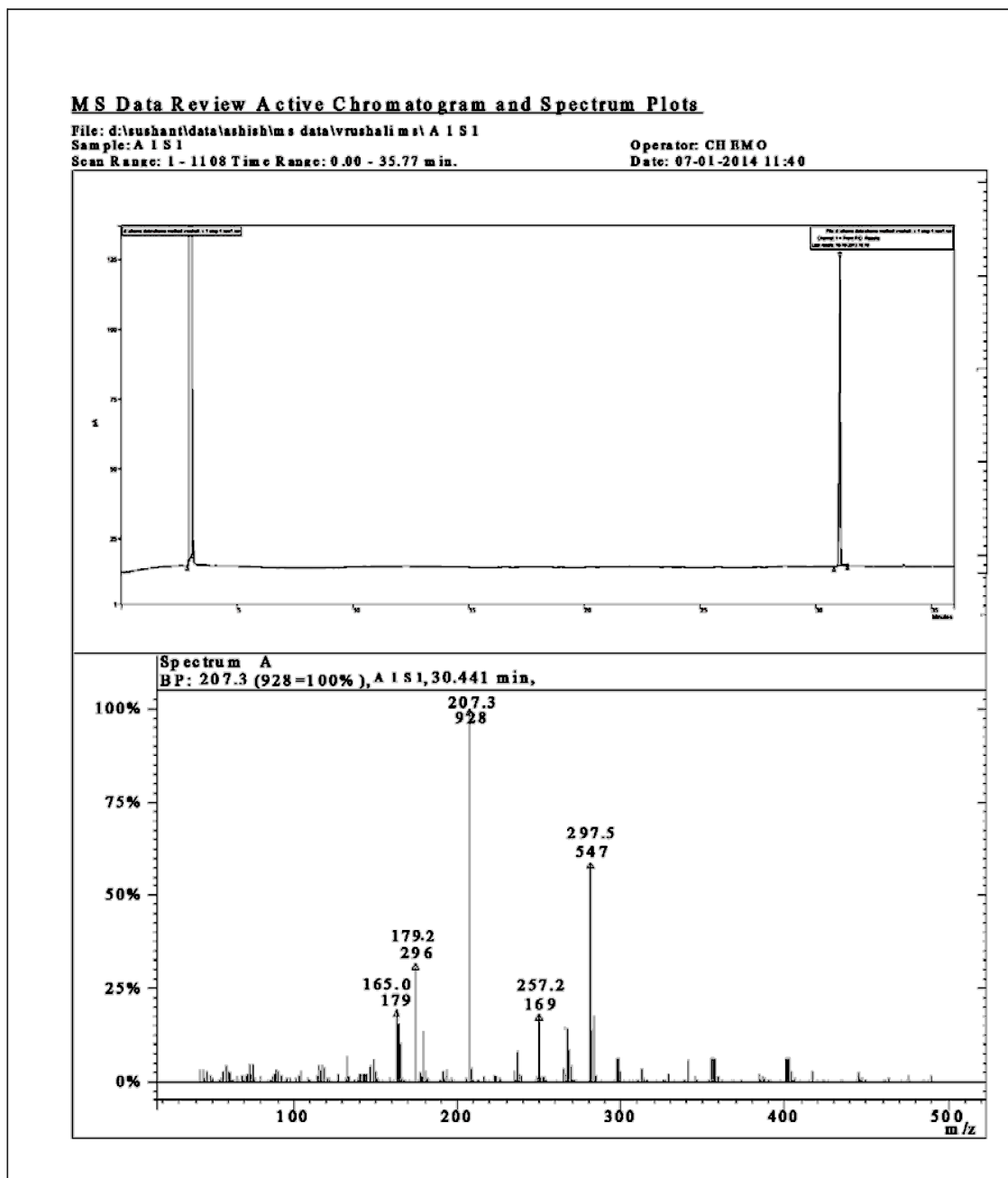
3.2.1.5 Table No 8: Chemical test - Ester confirmation test

Sr. no	Test	Observation	Inference
1.	Alkaline hydrolysis Test	Pink color disappeared on heating	Ester group confirmed.
2.	Hydroxamic acid test	Red violet color developed	Ester group confirmed.

3.2.1.6 Table No 9: GCMS method for III-A.1

GC conditions			
Column section	HP-5MS column (30m X 250μm X 0.25μm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	100	3
	10	120	3
	10	150	2
	10	180	2
	5	200	2
	5	220	3
	10	240	2
Injection mode	Split mode; split ratio 90:1		
Injection temperature	250°C	Carrier gas	Helium gas
Injection volume	1 μL	Detector temperature	280°C
Analysis time			
Run time	35 mins		
Retention Time	30.441mins		
MS condition			
Ion source temperature	280°C		
MS source	EI 70 Ev		
MS scan mode	EI Full scan m/z 40-500		

3.2.1.6. Spectrum 1: Mass spectra of III-A.1

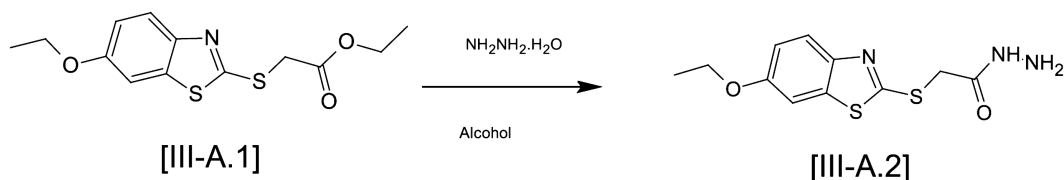


3.2.1.6 Table No 10: Mass spectral data of III-A.1

m/z values	Peak
297	Molecular ion peak
207	Base peak

3.2.2.0 III-A.2 Synthesis of 2-[(6-ethoxy-1, 3-benzothiazol-2-yl) sulfanyl] acetohydrazide ^[160-161]

3.2.2.1 Fig no 64: Synthesis of III-A.2



3.2.2.2 Table No 11: Reaction Calculation table

Compound	Mol. Wt.	Wt. in gm/mL	Mole	Mole ratio
III-A.1	297	2.97	0.01	1
NH ₂ NH ₂ .H ₂ O	68	1.36	0.02	2
Alcohol	--	10	--	--

3.2.2.3 Procedure: -

Crystallized product of III-A.1 was dissolved in alcohol reflux the reaction mixture. To this solution Hydrazine hydrate was added and refluxes the reaction mixture. Reaction was monitoring by TLC. After completion of reaction keep the reaction mixture on standing for overnight, needle shaped crystal formed. Filter the product and wash with water till pH 7-8. Recrystallized the product with Ethyl alcohol

3.2.2.4 Table No 12: Observation table

Melting point	173 ⁰ C	
Yield	60%	
Colour	White	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

3.2.2.5 Table No 13: Chemical test: Amide confirmation test

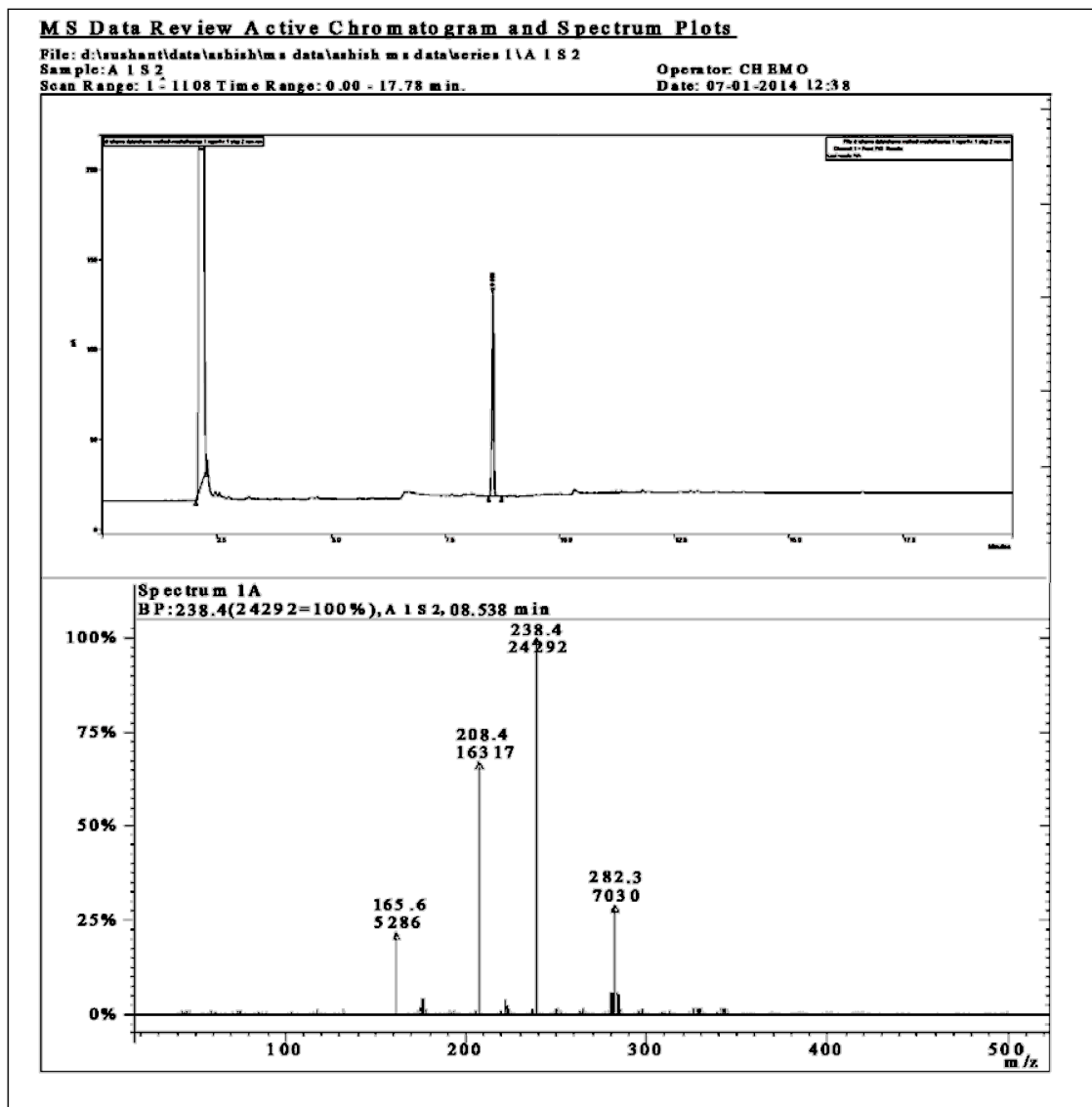
Sr.	Test	Observation	Inference
1.	Test for Amide	Effervescence due to evaluation of Nitrogen	Amide group Confirmed
2	Nitrous acid test	Effervescence due to evaluation of Nitrogen	Amide group confirmed.

3.2.2.6 Table No 14: GCMS method for III-A.2

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	280 ⁰ C	Detector temp	280 ⁰ C

MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time		17.78 mins
	Retention time		8.538 mins

3.2.2.7 Spectrum 2: Mass spectra of III-A.2

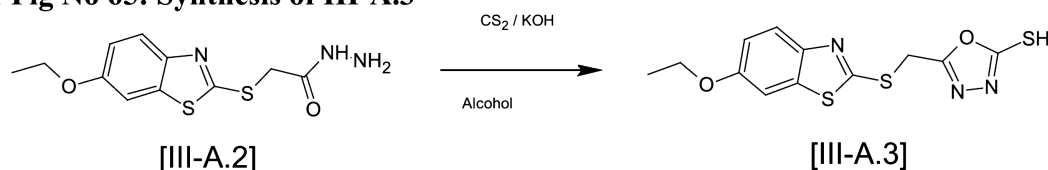


3.2.2.7 Table No 15: Mass spectral data of III-A.2

m/z values	Peak
282	Molecular ion peak
238	Base peak

3.2.3.0 III-A.3 Synthesis of 5-[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazole-2-thiol^[162]

3.2.3.1 Fig No 65: Synthesis of III-A.3



3.2.3.2 Table No 16: Reaction Calculation table

Compound	Mol. wt.	Wt. in gm/mL	Mole	Mole ratio
III-A.2	281	2.81	0.01	1
Carbon disulphide	76	0.76	0.01	1
Potassium hydroxide	56	0.56	0.01	1
Alcohol	--	15	--	--

3.2.3.3 Procedure: -

Crystallized product of III-A.2 was dissolved in alcohol to this KOH was added to potassium salt of III-A.2. Mixture of carbon disulfide in alcohol and KOH was prepared separately; this mixture was added to potassium salt of III-A.2. Reflux the reaction mixture, as reaction progress evolution of H₂S was started. After 8-10 hrs liberation of H₂S stopped. Further completion of reaction was monitored by TLC. The product was down in cold water, mixture was highly basic in nature is being neutralized by using dil. HCl to pH 7. Product was filtered wash with water.

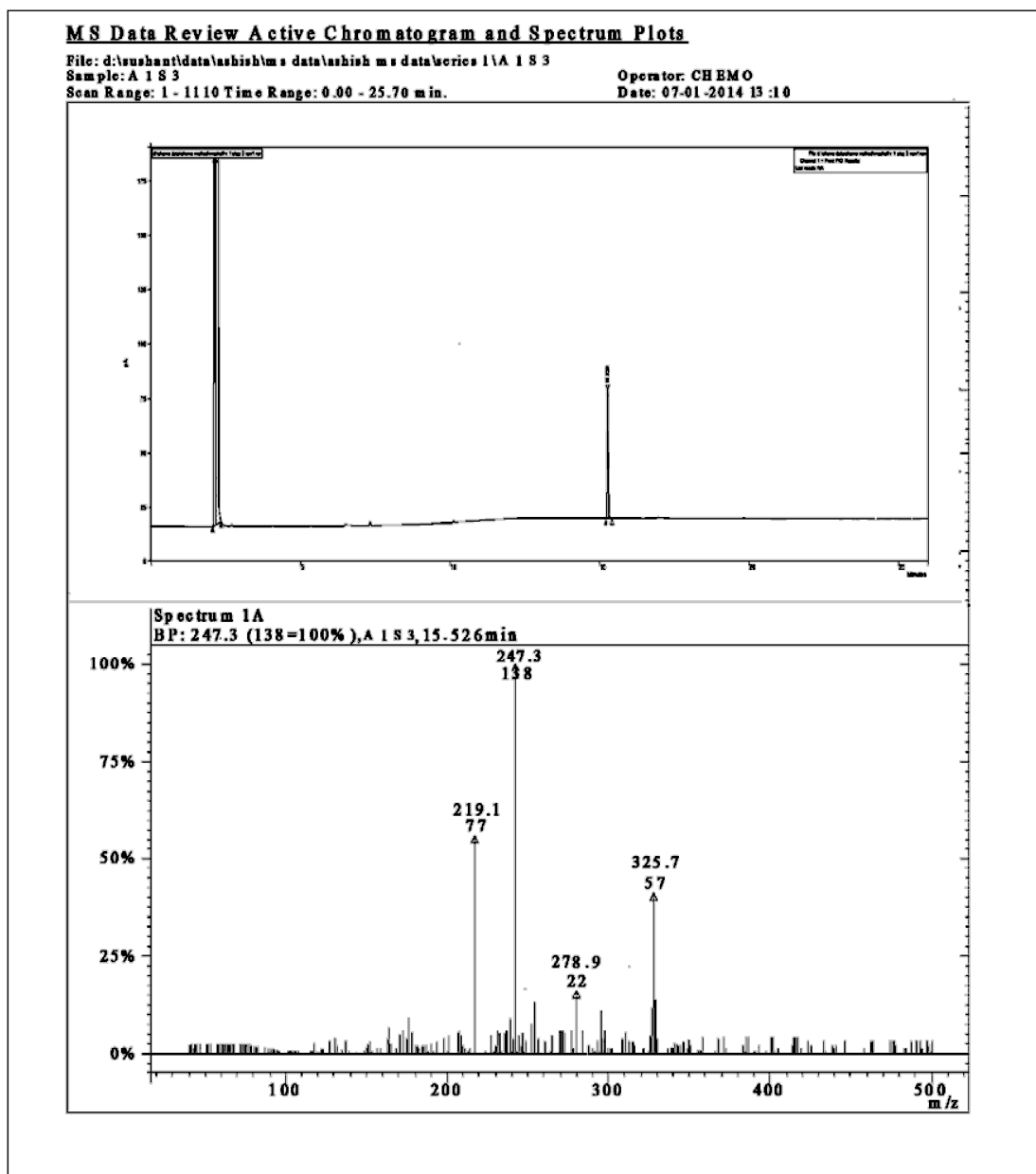
3.2.3.4 Table No 17: Observation table

Melting point	256 ⁰ C	
Yield	54%	
Colour	Pale yellow	
Nature	Amorphous	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

3.2.3.5 Table No 18: GCMS method for III-A.3

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (⁰ C/ min)	Temperature (⁰ C)	Hold time (min)
	0	40	1
	20	200	2
	20	300	9
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	320 ⁰ C	Detector temp	320 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time		25.70 mins
	Retention time		15.526 mins

3.2.3.6 Spectrum 3: Mass spectra of III-A.3

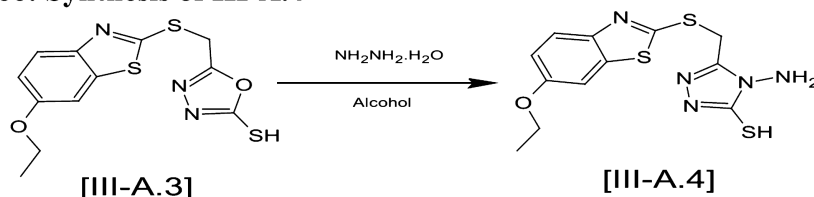


3.2.3.6 Table No 19: Mass spectral data of III-A.3

m/z values	Peak
326	Molecular ion peak
247	Base peak

3.2.4.0 III-A.4 Synthesis of 4-amino-5-[[[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl]-4H-1,2,4-triazole-3-thio]^[162]

3.2.4.1 Fig No 66: Synthesis of III-A.4



3.2.4.2 Table No 20: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-A.3	325	3.25	0.01	1
NH ₂ NH ₂ .H ₂ O	50	1.0	0.02	2
Alcohol	--	15	--	--

3.2.4.3 Procedure: -

Crystallized product of III-A.3 was dissolved in alcohol reflux the reaction mixture. To this solution Hydrazine hydrate was added and refluxes the reaction mixture for 8-10 hrs. Reaction was monitoring by TLC. The product was drowning in cold water, solid product was obtained. Product was filtered wash with water. Recrystallized the product with Ethyl alcohol.

3.2.4.4 Table No 21: Observation table

Melting point	220 ⁰ C	
Yield	76%	
Colour	White	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

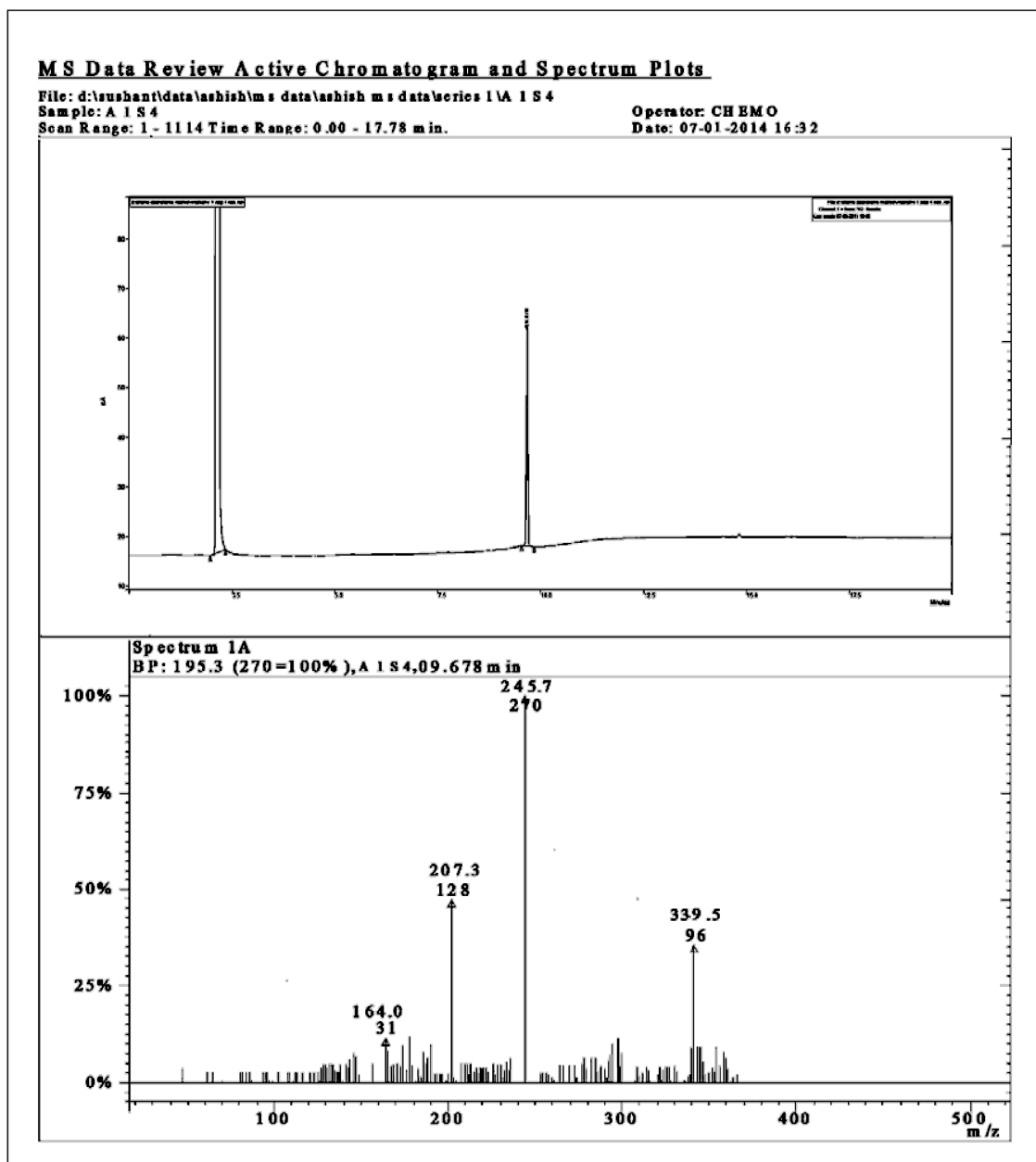
3.2.4.5 Table No 22: Chemical test: Primary amine

Test	Observation	Inference
Test for amine	Red dye observed	Primary amine confirmed

3.2.4.6 Table No 23: GCMS method for III-A.4

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	150	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	250 ⁰ C	Detector temp	280 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 Ev	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time		17.77 mins
	Sample elution time		9.678 mins

3.2.4.7 Spectrum 4: Mass spectra of III-A.4

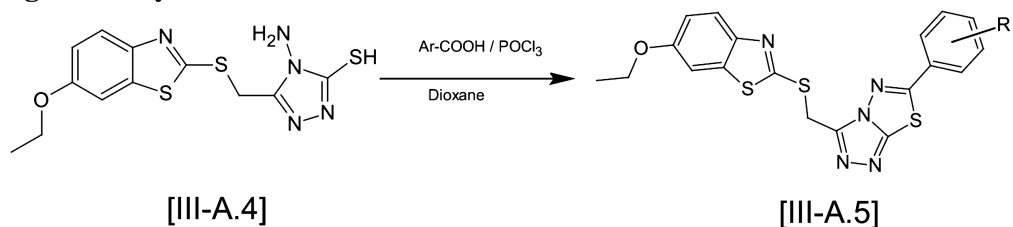


3.2.4.7 Table No 24: Mass spectral data of III-A.4

m/z values	Peak
339	Molecular ion peak
245	Base peak

3.2.5.0 III-A.5 Synthesis of 6-ethoxy-2-[(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl) methyl]sulfanyl} -1,3-benzothiazole^[163-64]

3.2.5.1 Fig No 67: synthesis of III-A.5



3.2.5.2 Table No 25: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-A.4	339	3.39	0.01	1
Benzoic acid	122	1.22	0.01	2
Phosphoryl oxychloride	153	1.53	0.01	1
Dioxane	--	15	--	--

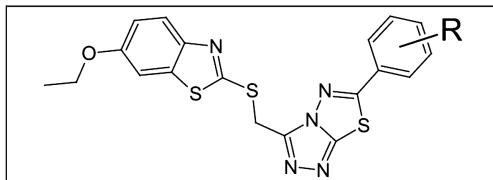
3.2.5.3 Procedure: -

Crystallized product of III-A.4 was dissolved in dioxane reflux the reaction mixture. To this solution previously prepared mixture of substituted benzoic acid and POCl_3 in dioxane was added and refluxes the reaction mixture for 6-8 hrs. Reaction was monitoring by TLC. The product was drown in cold water, solid product was obtained. Product was filtered, wash with water. Recrystallized the product in dioxane. Similarly other derivatives were prepared

3.2.5.4 Table No 26: Observation table

Melting point	228 ⁰ C	
Yield	76%	
Colour	Yellowish	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

3.2.5.4. Table No 27: Physical data of synthesized compounds

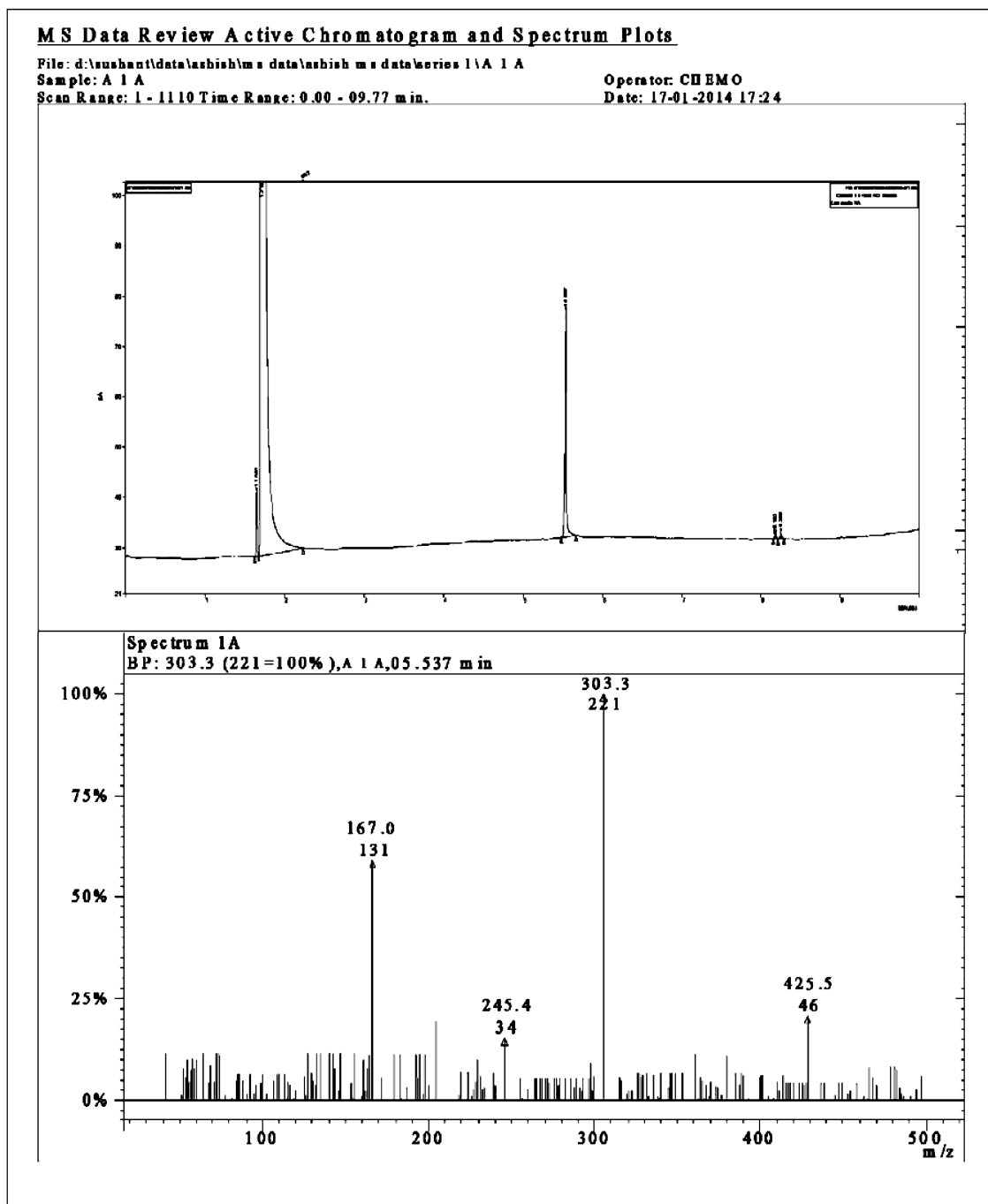


Sr. No	R	Code	Mol. wt.	Emp. For.	Colour	Solubility	MP (°C)	Yield (%)
1	-H	III-A.5a	425	C ₁₉ H ₁₅ N ₅ OS ₃	yellowish	DMSO	228	76
2	4-Cl	III-A.5b	459	C ₁₉ H ₁₄ ClN ₅ OS ₃	Dark brown	DMF	218	71
3	4-F	III-A.5c	443	C ₁₉ H ₁₄ FN ₅ OS ₃	Grayish	Methanol	206	68
4	4-OC ₂ H ₅	III-A.5d	469	C ₂₁ H ₁₉ N ₅ O ₂ S ₃	Yellowish	Acetone	230	81
5	4-OCH ₃	III-A.5e	455	C ₂₀ H ₁₇ N ₅ O ₂ S ₃	Light brown	methanol	218	66

3.2.5.5 Table No 28: GCMS method for III-A.5a-e

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ in)	Temperature (°C)	Hold time (min)
	0	200	0
	20	300	4.7
Injection mode	Split mode; split ratio 90:1		
Injection temperature	320°C	Carrier gas	Helium gas
Injection volume	1 µL	Detector Temperature	320°C
MS condition			
Ion source temperature	280°C	MS source	EI 70 Ev
MS scan mode	EI Full scan m/z 40-500		
Analysis time			
Run time	9.77 mins		
Retention Time III-A5a	5.537 mins		
Retention Time III-A5b	8.654 mins		
Retention Time III-A5c	6.206 mins		
Retention Time III-A5d	9.088 mins		
Retention Time III-A5e	6.183 mins		

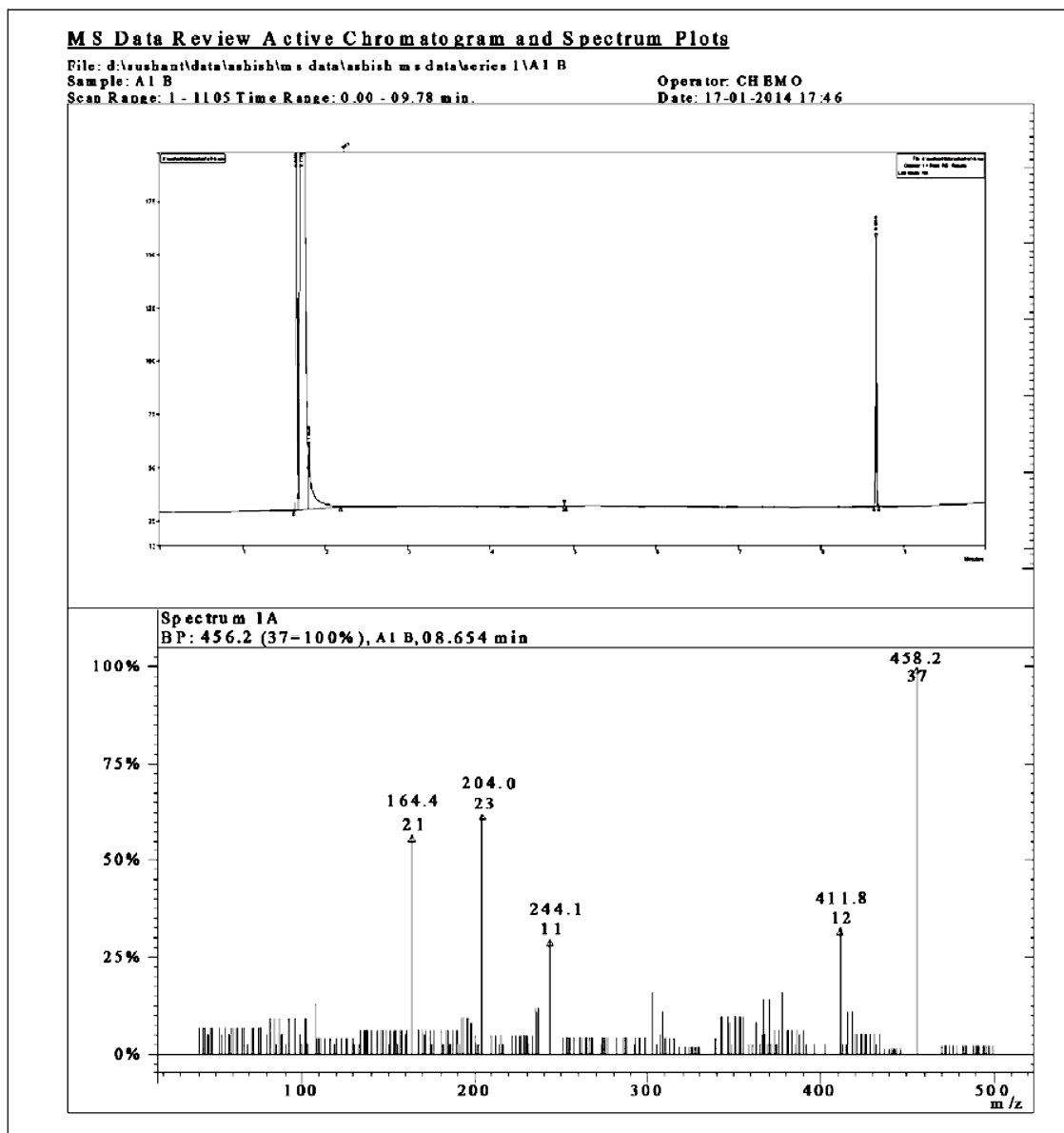
3.2.5.6.1 Spectrum 5: Mass spectra of III-A.5a



3.2.5.6.1 Table No 29: Mass spectral data of III-A.5a

m/z values	Peak
425	Molecular ion peak
303	Base peak

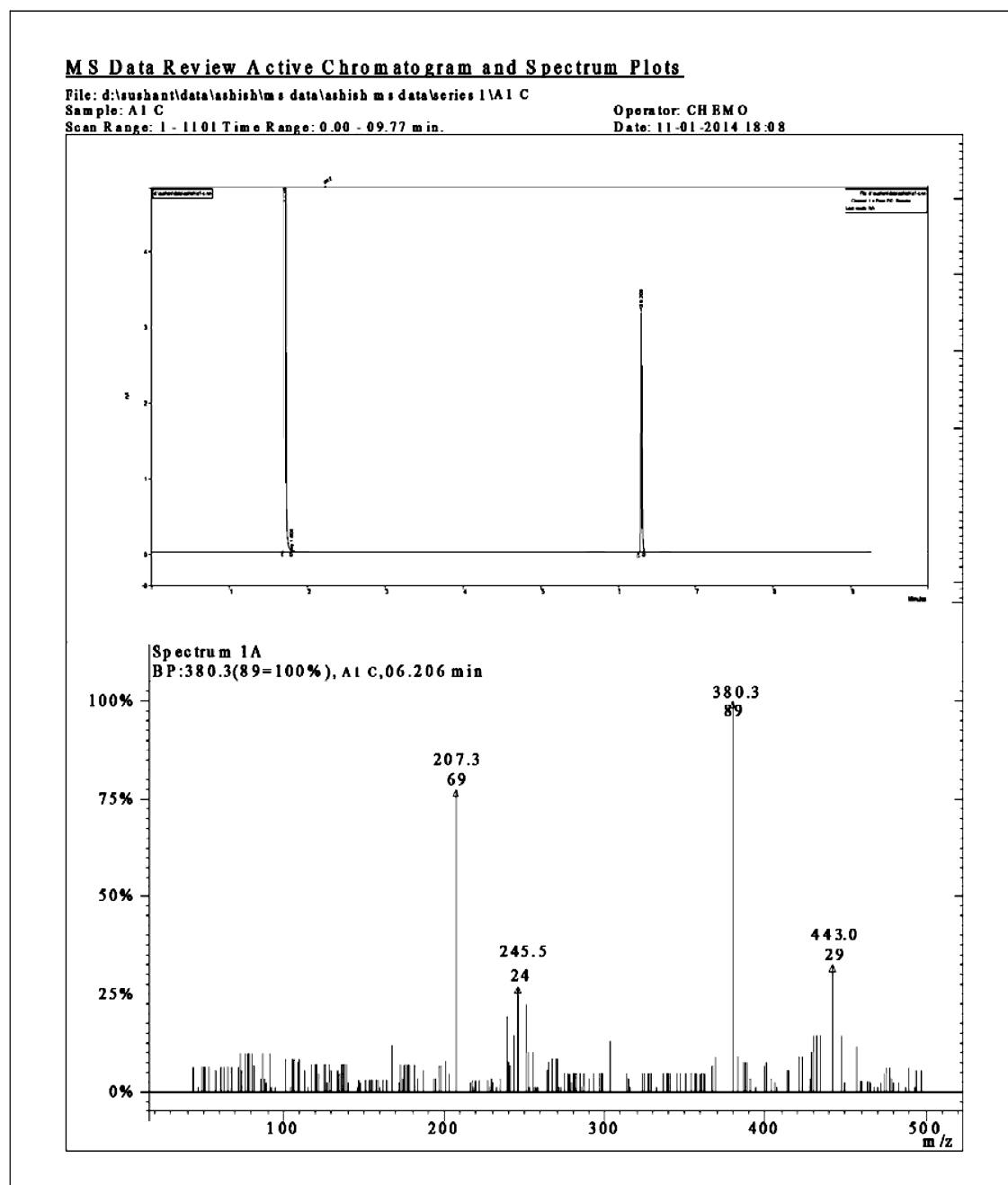
3.2.5.6.2 Spectrum 6: Mass spectra of III-A.5b



3.2.5.6.2 Table No 30: Mass spectral data of III-A.5b

m/z values	Peak
458	Molecular ion peak
458	Base peak

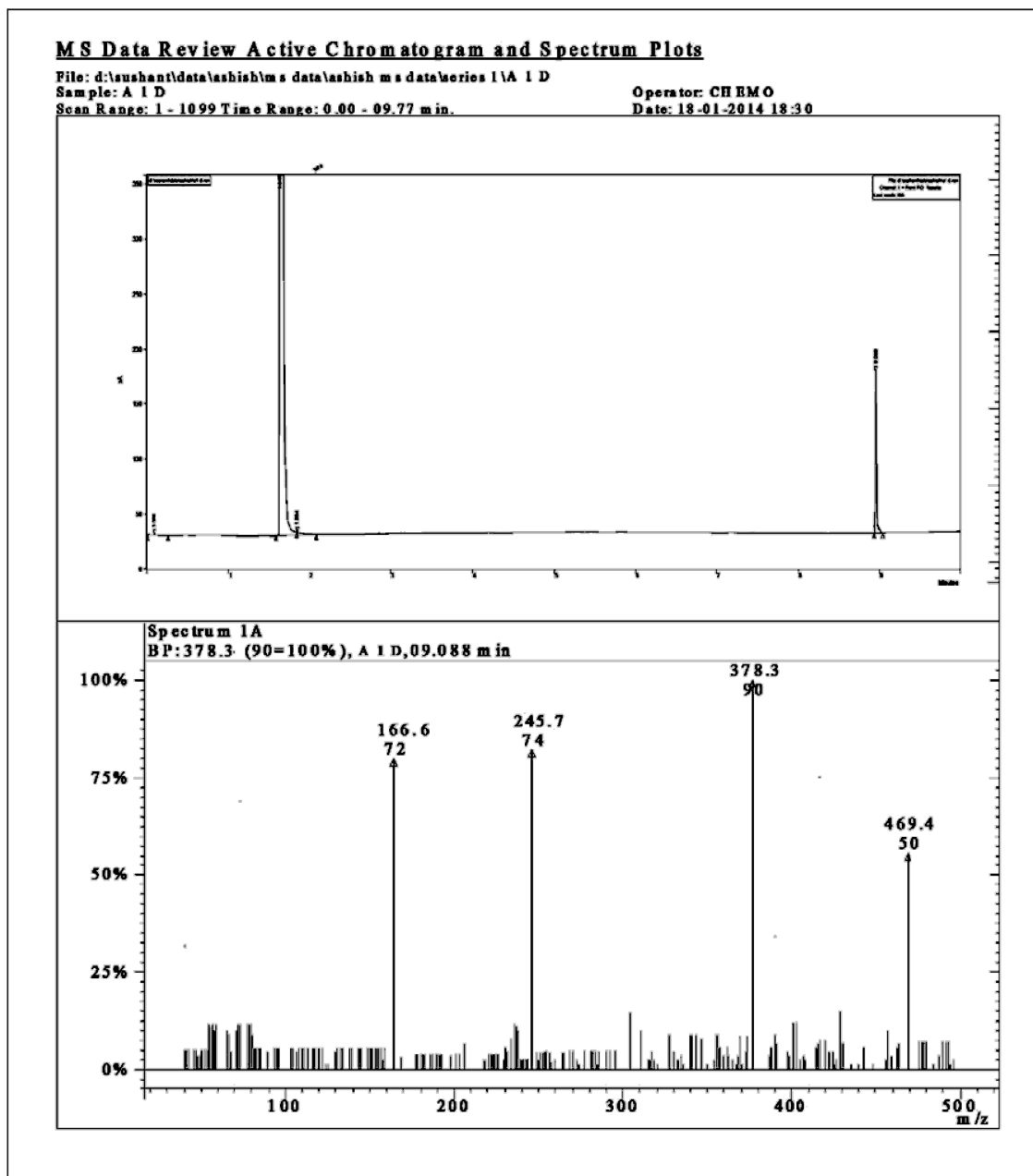
3.2.5.6.3 Spectrum 7: Mass spectra of III-A.5c



3.2.5.6.3 Table No 31: Mass spectral data of III-A.5c

m/z values	Peak
443	Molecular ion peak
380	Base peak

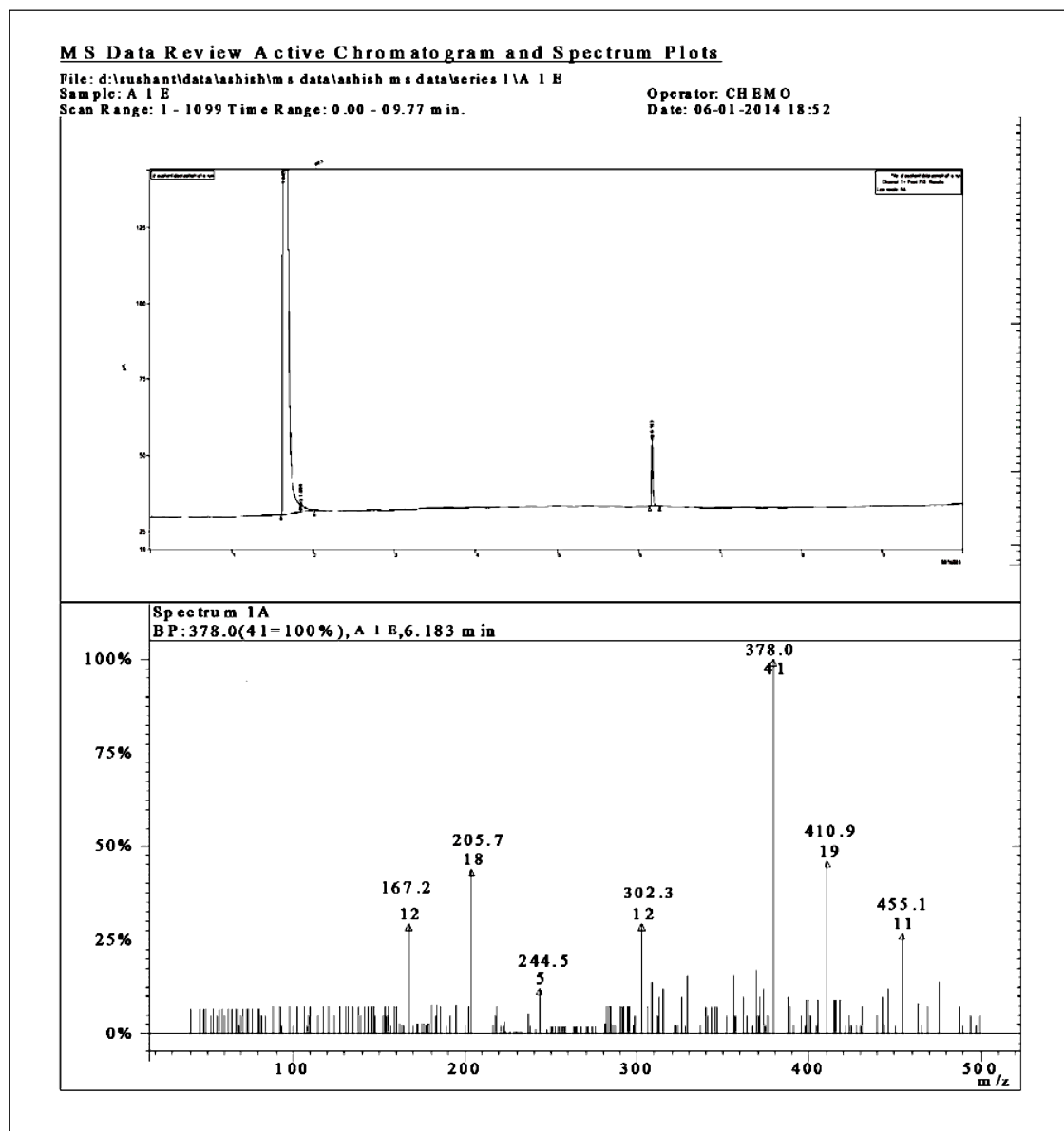
3.2.5.6.4 Spectrum 8: Mass spectra of III-A.5d



3.2.5.6.4 Table No 32: mass spectral data of III-A.5d

m/z values	Peak
469	Molecular ion peak
378	Base peak

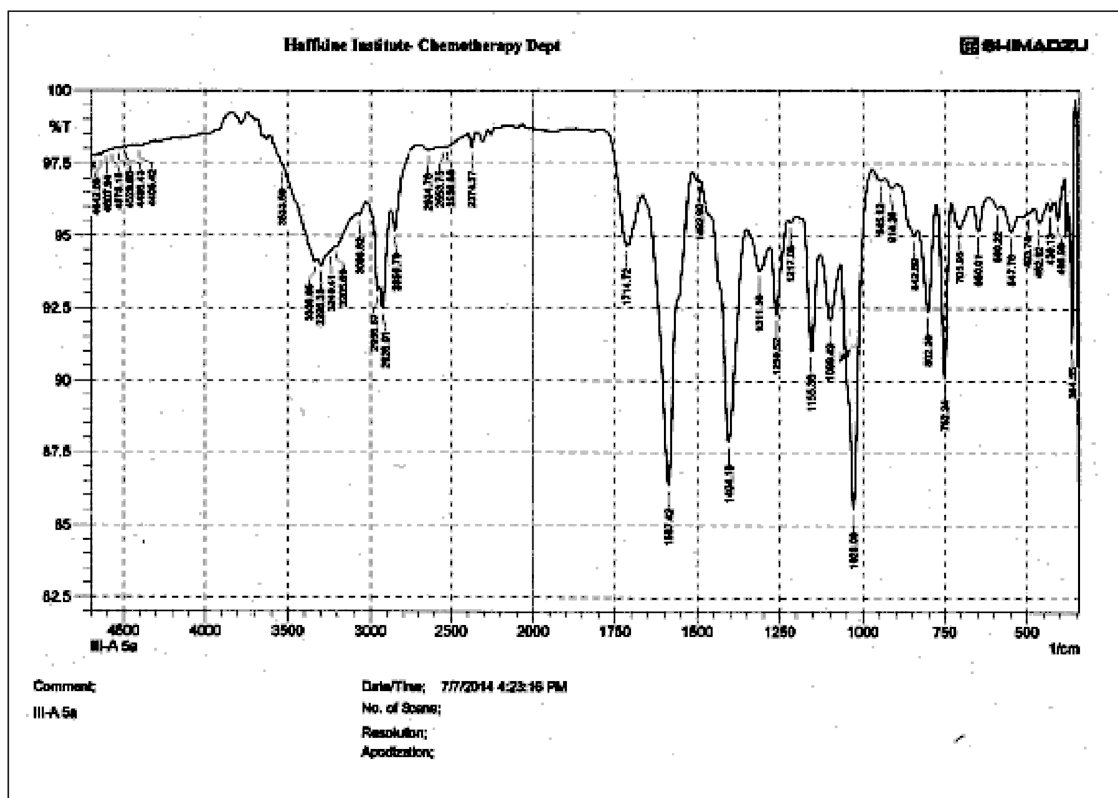
3.2.5.6.5 Spectrum 9: Mass spectra of III-A.5e



3.2.5.6.4 Table No 33: Mass spectral data of III-A.5e

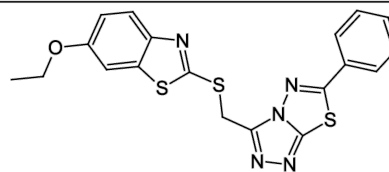
m/z values	Peak
455	Molecular ion peak
378	Base peak

3.2.5.7.1 Spectrum 10: IR spectra of III-A.5a

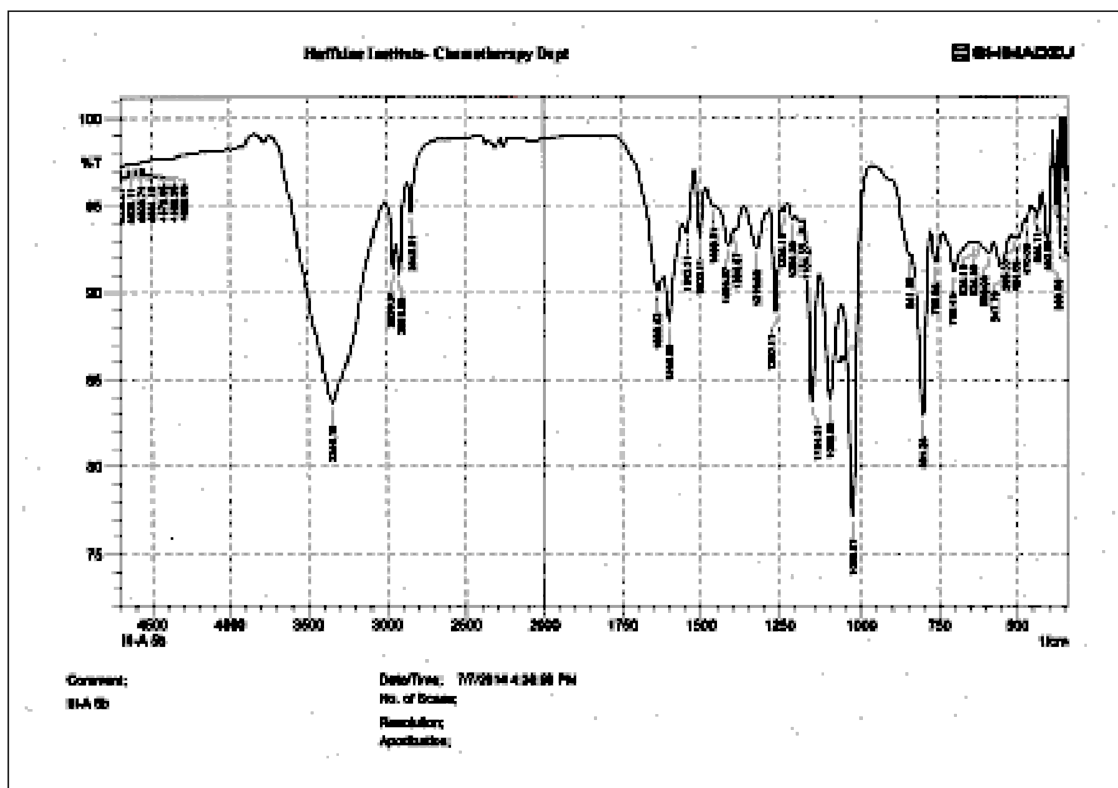


3.2.5.7.1 Table No 34: IR spectral data of III-A.5a

Functional Group	Peak (cm ⁻¹)	Standard IR Range
C-H stretching	3066	2850-3000
C=N stretching	1587	1615-1700
C-N bending	1492	1080-1360
C-S bending	1155	1030-1275

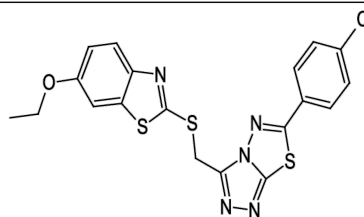


3.2.5.7.2 Spectrum 11: IR spectra of III-A.5b

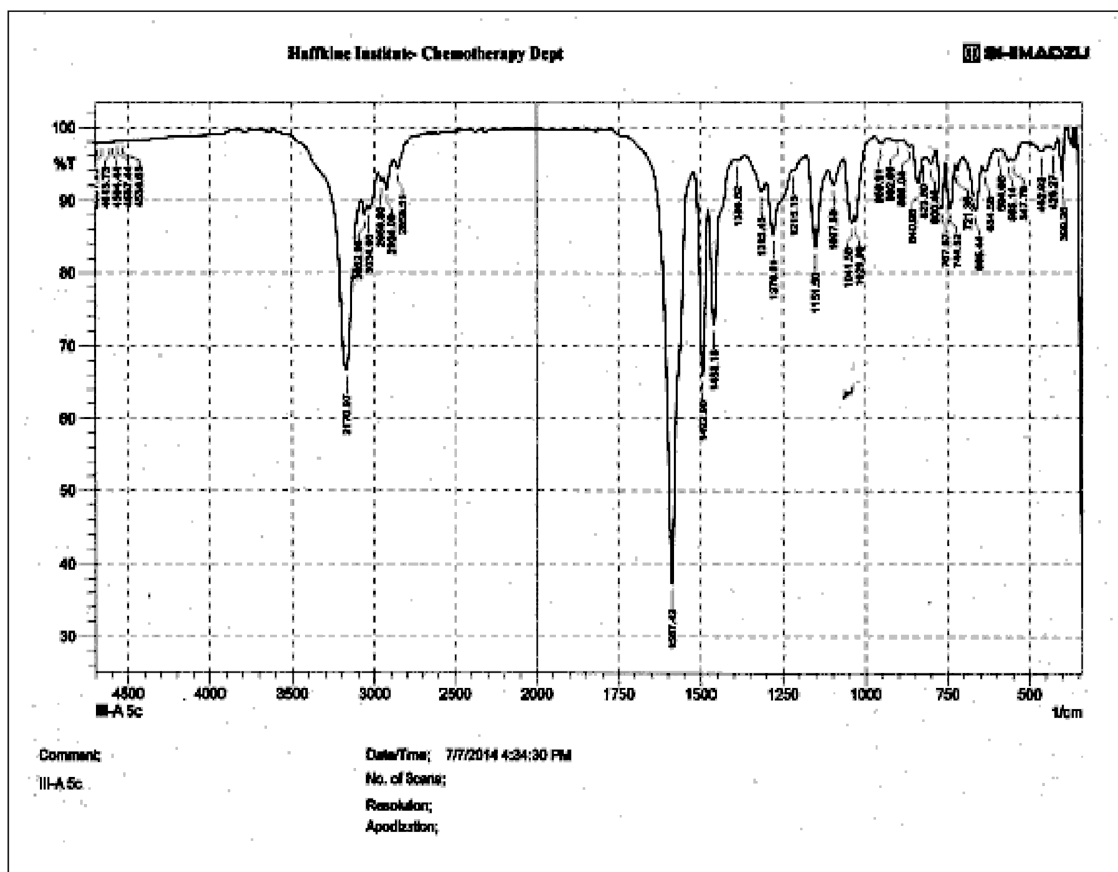


3.2.5.7.2 Table No 35: IR spectral data of III-A.5b

Functional Group	Peak (cm ⁻¹)	Standard IR Range
C=N stretching	1598	1615-1700
C-N bending	1456	1080-1360
C-S bending	1154	1030-1275
-Cl bending	759	700-800
C-H stretching	2956	2850-3000

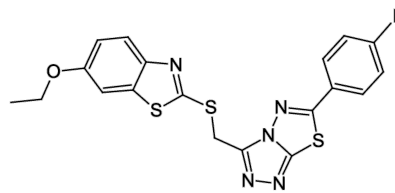


3.2.5.7.3 Spectrum 12: IR spectra of III-A.5c

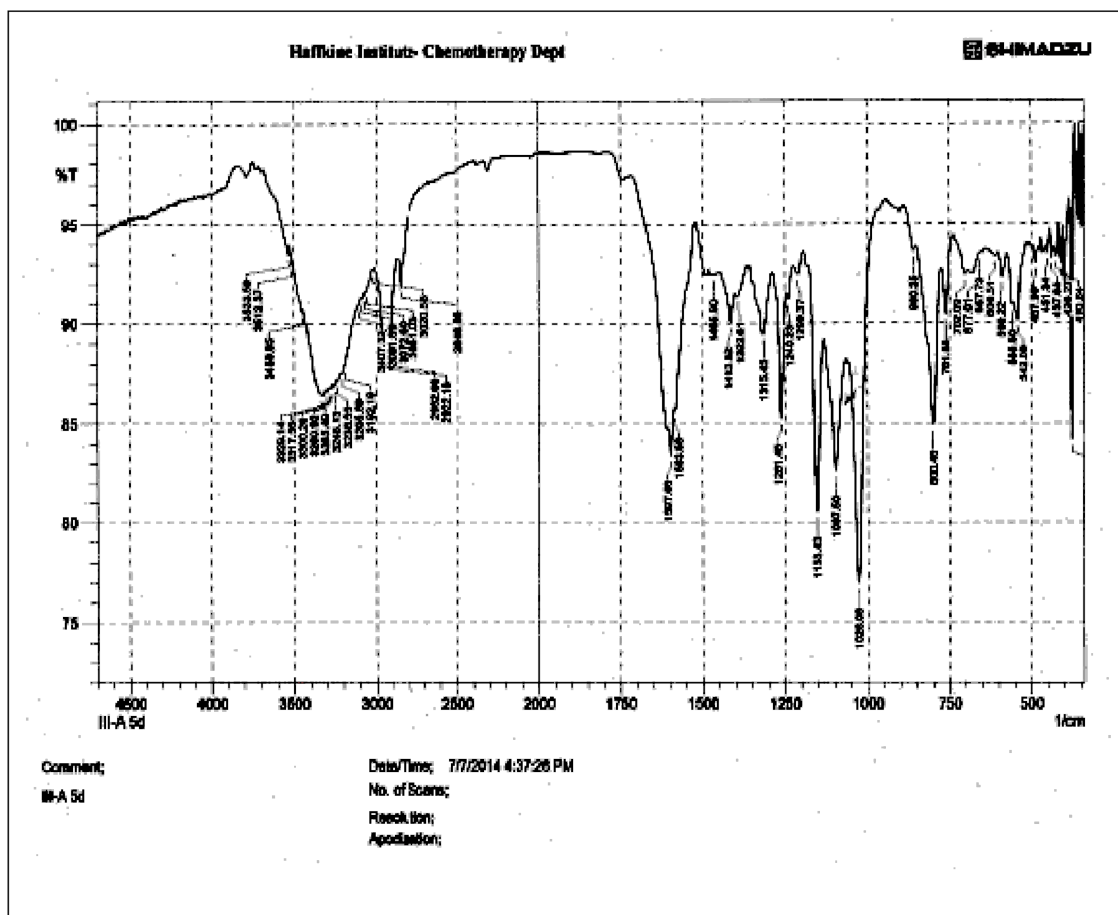


3.2.5.7.3 Table No 36: IR spectral data of III-A.5c

Functional Group	Peak (cm ⁻¹)	Standard IR Range
C-H stretching	3034	2850-3000
C=N stretching	1587	1615-1700
C-S bending	1151	1030-1275
C-N bending	1492	1080-1360
-F bending	1041	1000-1150



3.2.5.7.4 Spectrum 13: IR spectra of III-A.5d

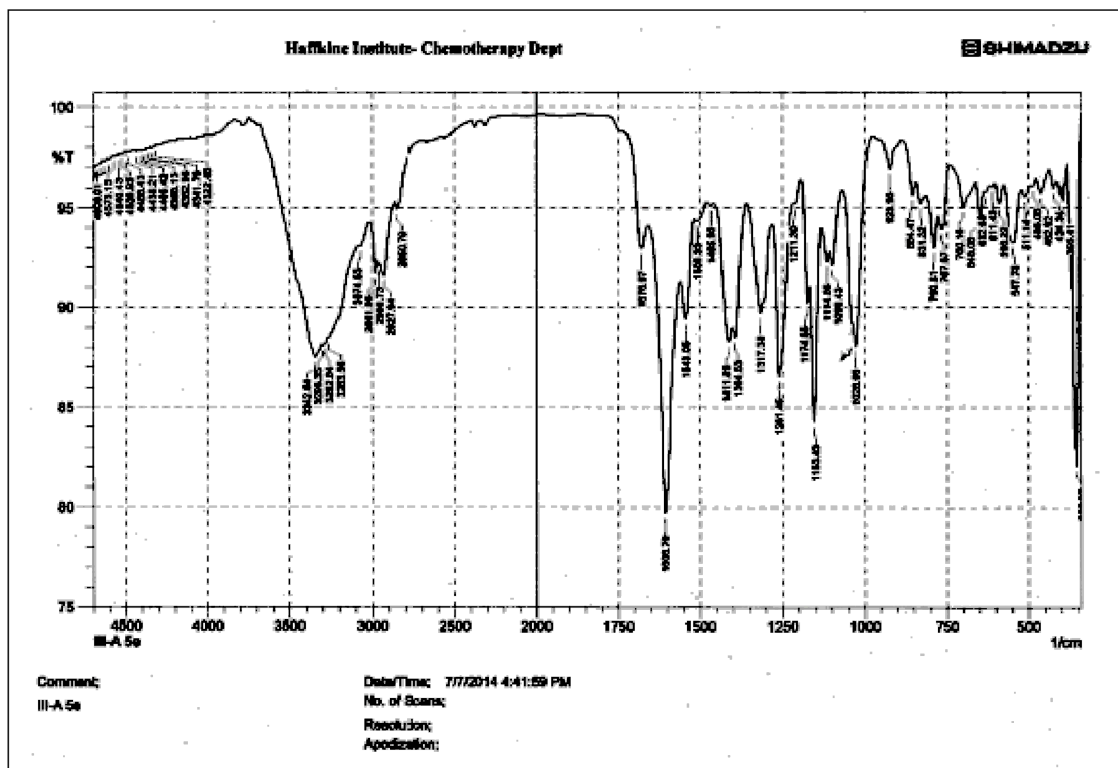


3.2.5.7.4 Table No 37: IR spectral data of III-A.5d

Functional Group	Peak (cm ⁻¹)	Standard IR Range
C-H stretching	3061	2850-3000
C=N stretching	1597	1615-1700
C-N bending	1465	1080-1360
C-S bending	1153	1030-1275
-O- bending	1097	1000-1320

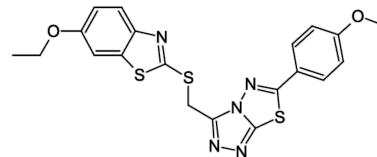
CCOC1=CC=C(C=C1)S2=NC3=NC(=N2)SC3S4=CC=CC=C4OC

3.2.5.7.5 Spectrum 14: IR spectra of III-A.5e

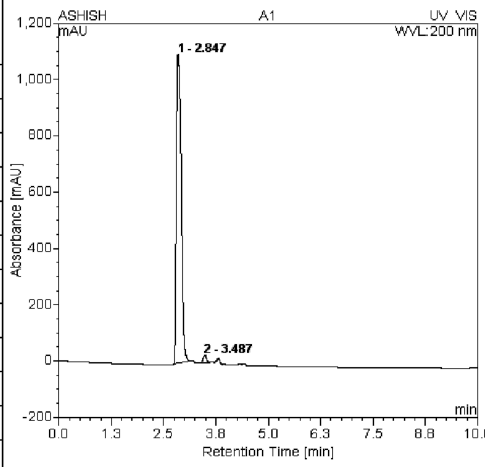


3.2.5.7.5 Table No 38: IR spectral data of III-A.5e

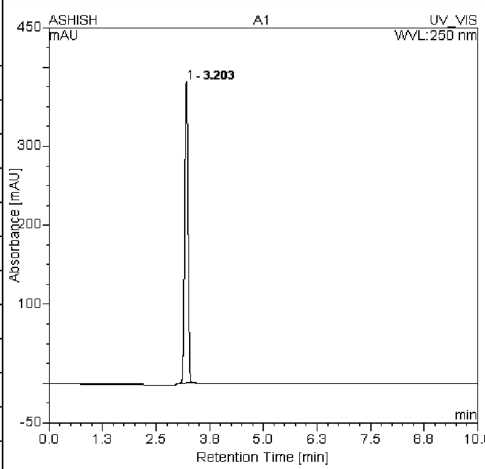
Functional Group	Peak (cm ⁻¹)	Standard IR Range
C-H stretching	3074	2850-3000
C=N stretching	1606	1615-1700
C-N bending	1465	1080-1360
C-S bending	1153	1030-1275
-O- bending	1028	1000-1320



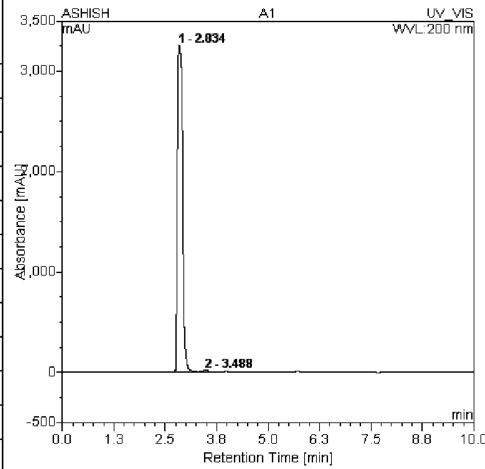
3.2.5.8.1 Chromatogram 1: HPLC data for III-A.5a

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	 ASHISH A1 UV_VIS WVL: 200 nm 1 - 2.847 2 - 3.487 Absorbance [mAU] Retention Time [min]
Mobile phase A	Acetonitrile (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 ^o C	
Wavelength	200 nm	
Sample concentration	10 ppm	
Retention time	2.85	
Peak Intensity	1098	
Theoretical Plates	2603	
Asymmetry	1.49	
Peak Width	0.201	

3.2.5.8.2 Chromatogram 2: HPLC data for III-A.5b

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	 ASHISH A1 UV_VIS WVL: 250 nm 1 - 3.203 Absorbance [mAU] Retention Time [min]
Mobile phase A	Acetonitrile (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 ^o C	
Wavelength	250 nm	
Sample concentration	10 ppm	
Retention Time	3.2	
Peak Intensity	2725	
Theoretical Plates	13037	
Asymmetry	1.13	
Peak Width	0.112	

3.2.5.8.3 Chromatogram 3: HPLC data for III-A.5c

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	 ASHISH A1 UV_VIS WVL: 200 nm 1 - 2.834 2 - 3.488 Absorbance [mAU] Retention Time [min]
Mobile phase A	Acetonitrile (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 ^o C	
Wavelength	200 nm	
Sample concentration	10 ppm	
Retention time	2.8 mins	
Peak Intensity	3018	
Theoretical plates	10916	
Asymmetry	1.10	
Peak Width	0.108	

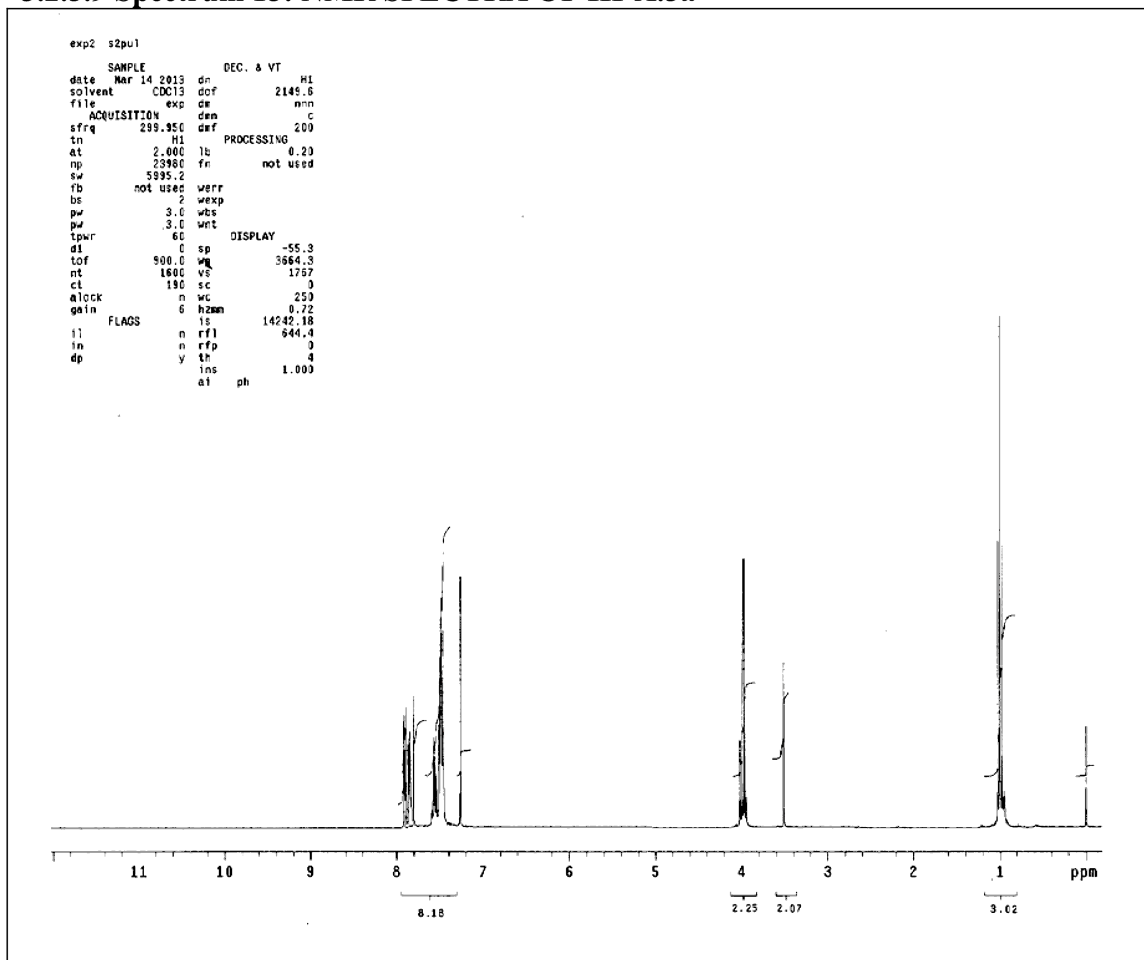
3.2.5.8.4 Chromatogram 4: HPLC data for III-A.5d

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Water (20%)	
Mobile phase A	Methanol (80%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	380 nm	
Sample concentration	10 ppm	
Retention Time	3.1mins	
Peak Intensity	1001	
Theoretical Plates	6911	
Asymmetry	1.31	
Peak Width	0.15	

3.2.5.8.5 Chromatogram 5: HPLC data for III-A.5e

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Water (20%)	
Mobile phase A	Methanol (80%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	250 nm	
Sample concentration	10 ppm	
Retention time	2.8 min	
Peak Intensity	1068	
Theoretical Plates	3806	
Asymmetry	1.33	
Peak Width	0.182	

3.2.5.9 Spectrum 15: NMR SPECTRA OF III-A.5a



3.2.5.9 Table No 39: NMR Spectral data of III-A.5a

δ values	H^1 NMR signal
3.5	s, 2H, CH_2
1.0	t, 3H, CH_3
4.0	q, 2H, CH_2
7-8	m, 8H, Ar-H

3.3.0 III-B] Synthesis of *N*-[(6,8-dimethyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazepin-3-yl) methyl] -substituted 1,3-benzothiazol-2-amine

III-B includes following steps

3.2.1 III-B.1 Synthesis of ethyl [(6-methoxy-1, 3-benzothiazol-2-yl)sulfanyl]acetate

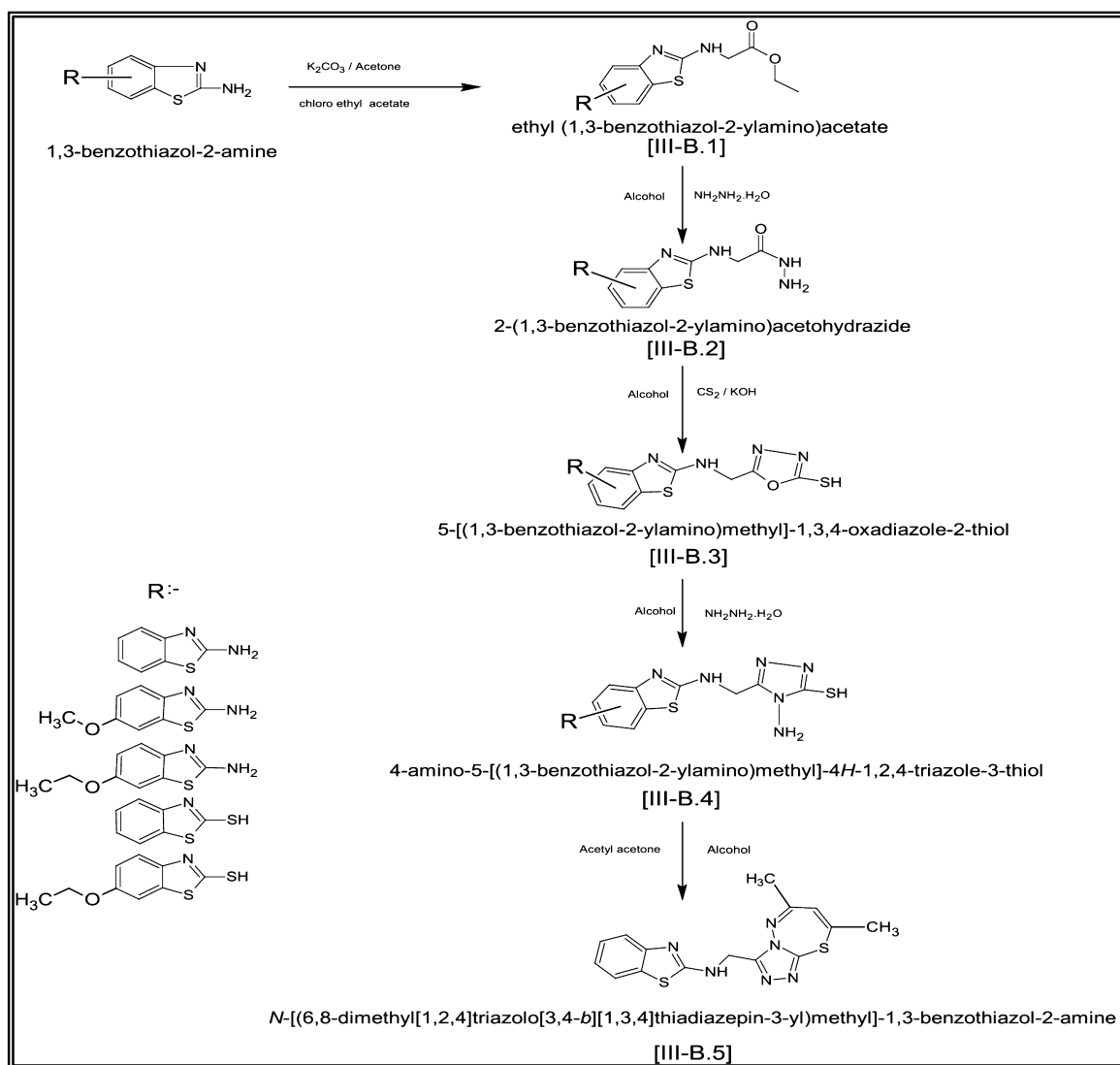
3.2.2 III-B.2 Synthesis of 2-[(6-methoxy-1, 3-benzothiazol-2-yl) sulfany l] acetohydrazide

3.2.3 III-B.3 Synthesis of 5-{[(6-methoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl} - 1,3,4-oxadiazole-2-thiol

3.2.4 III-B.4 Synthesis of 4-amino-5-{[(6-methoxy-1,3-benzothiazol-2-yl) sulfany l] methyl} -4*H*-1,2,4-triazole-3-thio

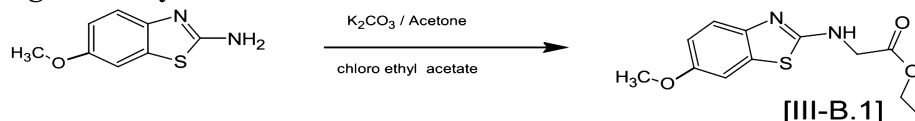
3.2.5 III-B.5 Synthesis of *N*-[(6,8-dimethyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazepin-3-yl) methyl] -6-methoxy 1,3-benzothiazol-2-amine

Fig No 68 Schematic representation of III-B



3.3.1.0 III-B.1 Synthesis of ethyl [(6-methoxy-1, 3-benzothiazol-2-yl) sulfanyl] acetate^[160]

3.3.1.1 Fig No 69: synthesis of III-B.1



3.3.1.2 Table No 40: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
6-methoxy-2-amino Benzothiazole	180	1.80	0.01	1
ethyl chloroacetate	122	1.22	0.01	1
Potassium carbonate	138	0.69	0.005	0.5
Acetone	--	10	--	--

3.3.1.3 Procedure: -

6-methoxy-2-amino Benzothiazole was dissolved in acetone stirred for 30 mins and K_2CO_3 was added and reflux the reaction mixture for 4 hrs leading to formation of potassium salt of amino benzothiazole. Once salt was formed ethyl chloroacetate was added over the period of 15 mins. Reflux the reaction mixture till completion of reaction. Reaction was monitoring was done by TLC. Formed product was isolated by down in cold water. Clear solution was extracted in Diethyl ether for three times to extract product from aqueous layer. Recrystallized the product with Acetone.

3.3.1.4 Table No 41: Observation table

Melting point	119 ⁰ C	
Yield	70%	
Colour	Off white	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7:3)	

3.2.1.5 Table No 42: Chemical test: Ester confirmation test

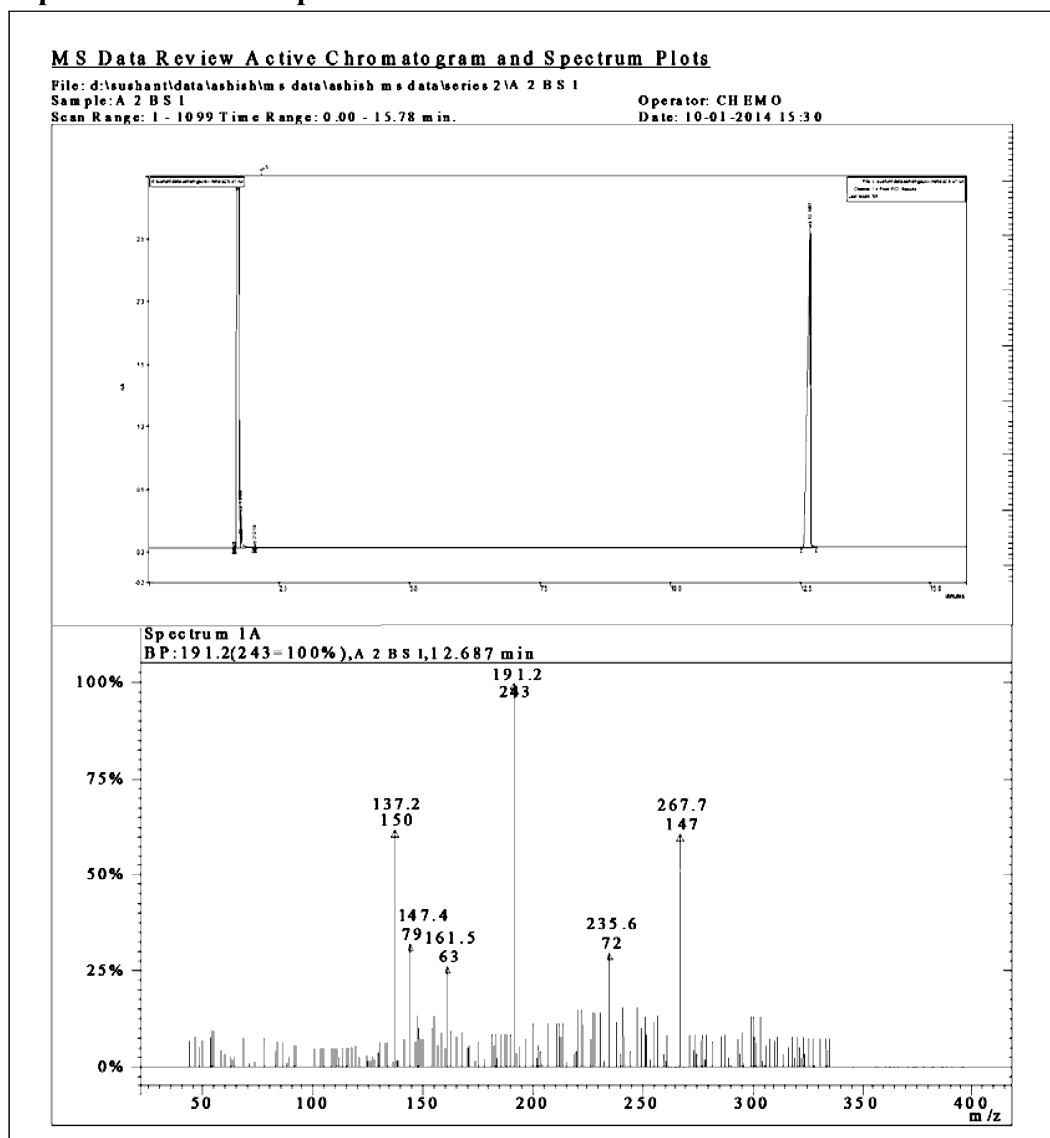
Sr. no	Test	Observation	Inference
1.	Alkaline hydrolysis Test	Pink color disappeared on heating	Ester group confirmed
2.	Hydroxamic acid test	Red violet color developed	Ester group confirmed

3.3.1.6 Table No 43: GCMS method for III-B.1

GC conditions			
Column section	HP-5MS column (30m X 250 μ m X 0.25 μ m)		
Oven temperature	Rate (⁰ C/ min)	Temperature (⁰ C)	Hold time (min)
	0	45	1
	20	300	2
Injection mode	Split mode; 90:1	Carrier gas	Helium gas

Injection temperature	300 ⁰ C	Detector temp	300 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time		15.77 mins
	Retention time		12.687 mins

3.3.1.7 Spectrum 16: Mass spectra of III-B.1

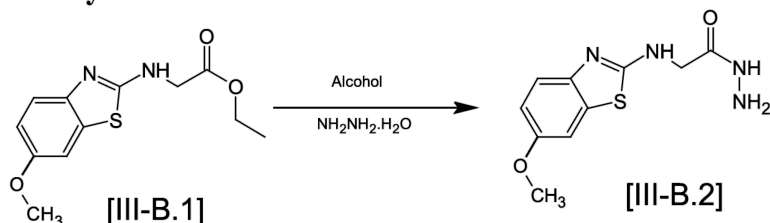


3.3.1.7 Table no 44: Mass spectral data of III-B.1

m/z values	Peak
267	Molecular ion peak
191	Base peak

3.3.2.0 III-B.2 Synthesis of 2-[(6-methoxy-1, 3-benzothiazol-2-yl) sulfanyl] acetohydrazide^[160-161]

3.3.2.1 Fig No 70: Synthesis of III-B.2



3.3.2.2 Table No 45: Reaction Calculation table:

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-B.1 product	266	2.66	0.01	1
NH ₂ NH ₂ . HCl	68	1.36	0.02	2
Alcohol	--	10	--	--

3.3.2.3 Procedure: -

Crystallized product of III-B.1 was dissolved in alcohol, reflux the reaction mixture till it forms clear solution. To this clear solution previously prepared mixture of Hydrazine hydrate and HCl was added and reflux the reaction mixture leading to formation of product. Reaction was monitored by TLC. After completion of reaction keep the reaction mixture on standing for overnight, needle shaped crystal formed. Filter the product and wash with water till pH 7-8. Recrystallized the product with Ethyl alcohol

3.3.2.4 Table No 46: Observation table

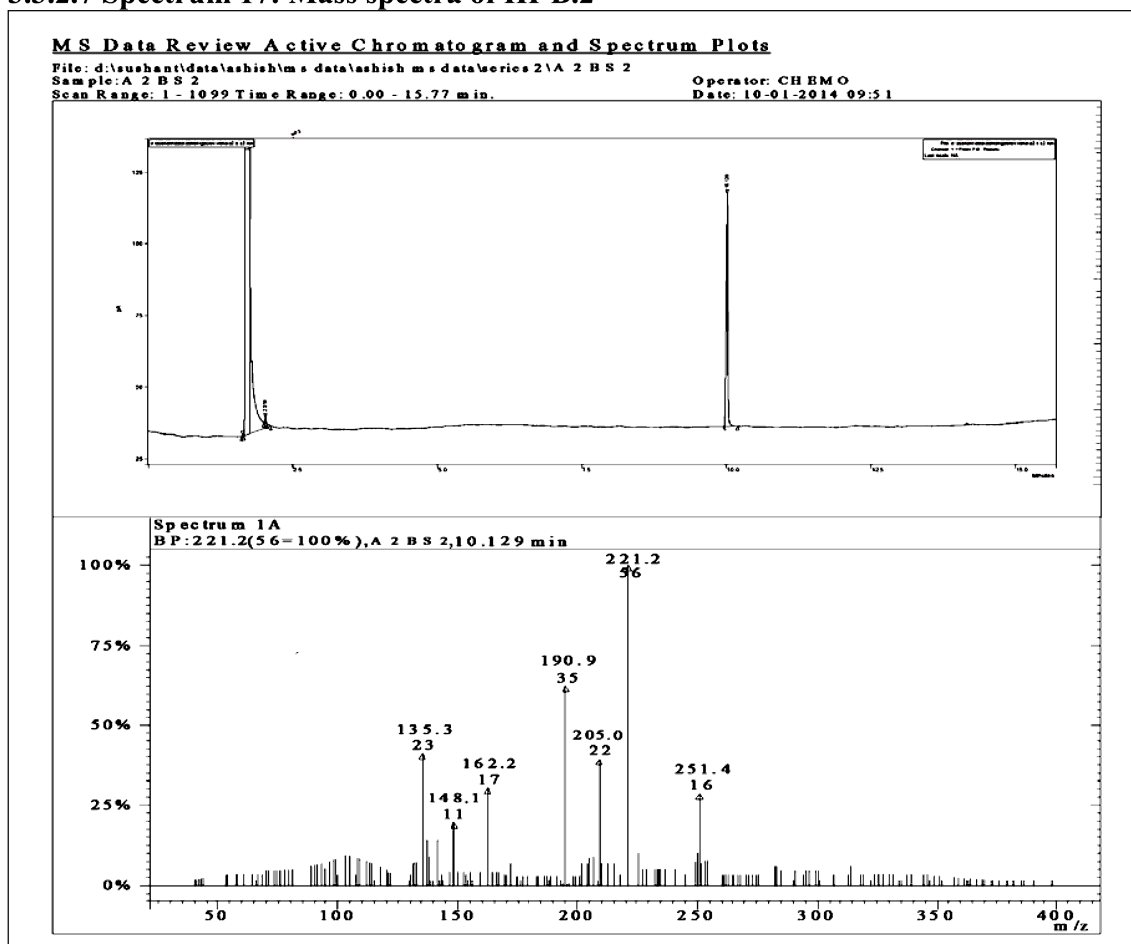
Melting point	168 ⁰ C	
Yield	60%	
Colour	White	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

3.3.2.5 Table No 47: Chemical test: Amide confirmation test

Sr. no	Test	Observation	Inference
1.	Test for Amide	Effervescence due to evaluation of Nitrogen	Amide group Confirmed
2	Nitrous acid test	Effervescence due to evaluation of Nitrogen	Amide group Confirmed

3.3.2.6 Table No 48: GCMS method for III-B.2

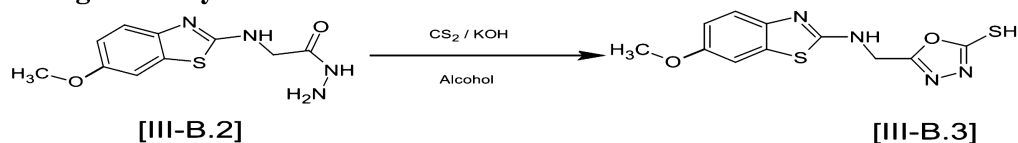
GC conditions			
Column section	HP-5MS column (30m X 250 μ m X 0.25 μ m)		
Oven temperature	Rate ($^{\circ}$ C/ min)	Temperature ($^{\circ}$ C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 $^{\circ}$ C	Detector temp	300 $^{\circ}$ C
MS condition			
Ion source temperature	280 $^{\circ}$ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time		15.77 mins
	Retention time		10.129 mins

3.3.2.7 Spectrum 17: Mass spectra of III-B.2**3.3.2.7 Table No 49: Mass spectral data of III-B.2**

m/z values	Peak
251	Molecular ion peak
221	Base peak

3.3.3.0 III-B.3 Synthesis of 5-[(6-methoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazole-2-thiol^[162]

3.3.3.1 Fig no 71: synthesis of III-B.3



3.3.3.2 Table No 50: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-B.2 product	252	2.52	0.01	1
Carbon disulphide	76	0.76	0.01	1
Potassium hydroxide	56	0.56	0.01	1
Alcohol	--	15	--	--

3.3.3.3 Procedure: -

Crystallized product of [III-B.2] was dissolved in alcohol to this KOH was added to potassium salt of [III-B.2]. Mixture of CS₂ in alcohol and KOH was prepared separately; this mixture was added to potassium salt of [III-B.2]. Reflux the reaction mixture, as reaction progress evolution of H₂S was started. After 8-10 hrs liberation of H₂S stopped. Further completion of reaction was monitored by TLC. The product was down in cold water, mixture was highly basic in nature is being neutralized by using dil. HCl to pH 7. Product was filtered wash with plenty of water to remove traces of impurities. Recrystallized the product with Ethyl alcohol

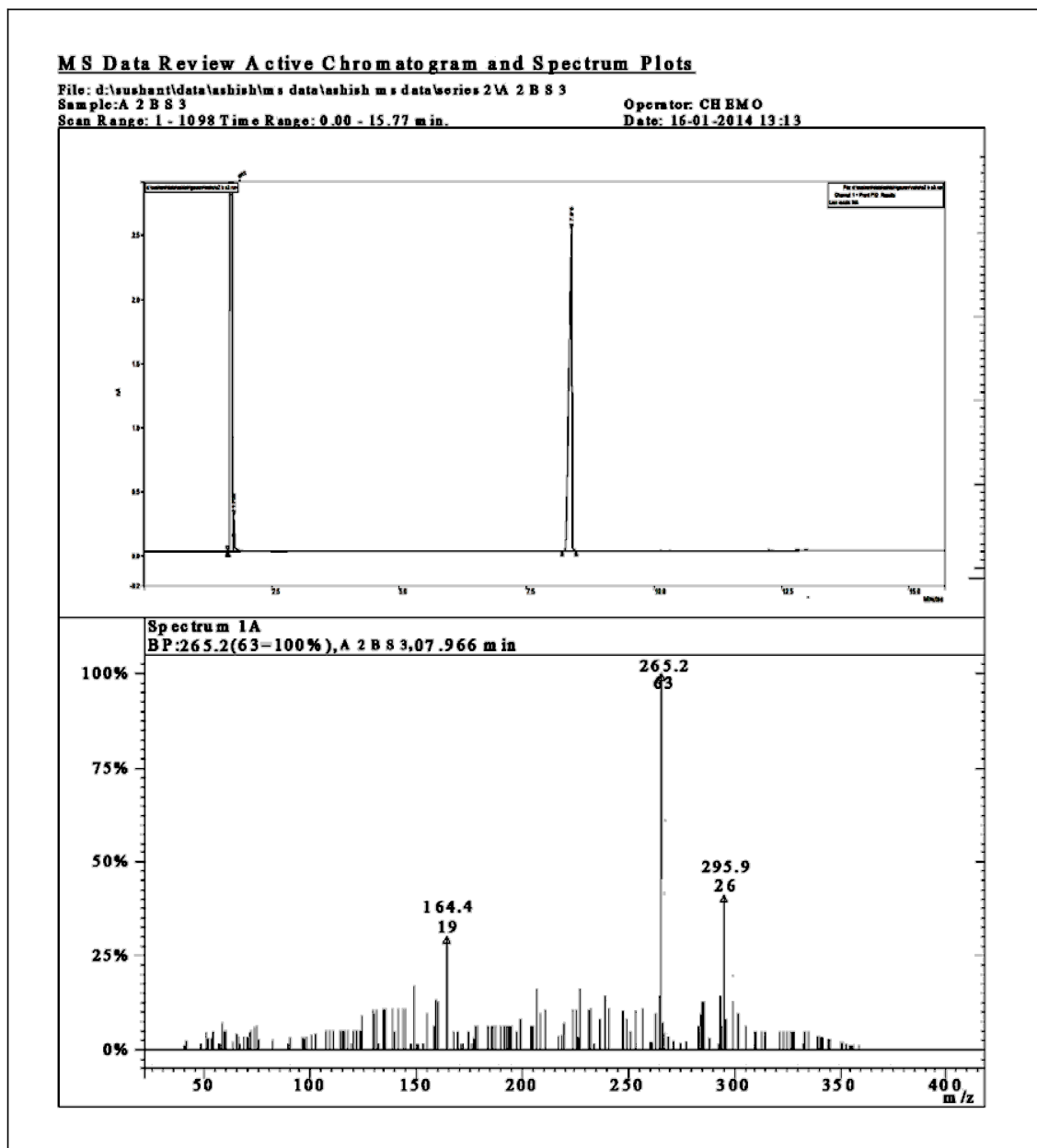
3.3.3.4 Table No 51: Observation table

Melting point	216 ⁰ C	
Yield	64%	
Colour	Pale yellow	
Nature	Amorphous	
TLC system	(Ethyl acetate: Hexane) (8:2)	

3.3.3.5 Table No 52: GCMS method for III-B.3

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (⁰ C/ min)	Temperature (⁰ C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 ⁰ C	Detector temp	300 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time	15.77 mins	
	Sample elution time	7.966 mins	

3.3.3.6 Spectrum 18: Mass spectra of III-B.3

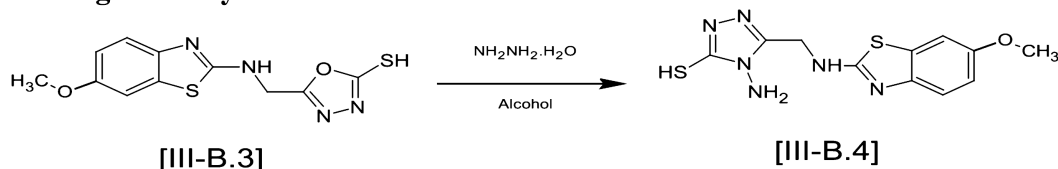


3.3.3.6 Table No 53: Mass spectral data of III-B.3

m/z values	Peak
295	Molecular ion peak
265	Base peak

3.3.4.0 III-B.4 Synthesis of 4-amino-5-[(6-methoxy-1,3-benzothiazol-2-yl) sulfanyl] methyl} -4*H*-1,2,4-triazole-3-thiol^[162,165]

3.3.4.1 Fig no 72: synthesis of III-B.4



3.3.4.2 Table No 54: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-B.3 product	295	2.95	0.01	1
Hydrazine hydrate	50	1	0.02	2
Alcohol	--	10	--	--

3.3.4.3 Procedure: -

Crystallized product of III-B.3 was dissolved in alcohol reflux the reaction. To this solution Hydrazine hydrate was added and refluxes the reaction mixture for 8-10 hrs. Reaction was monitoring by TLC. The product was drowning in of cold water, product was obtained. Product was filtered wash with water. Recrystallized the product with Ethyl alcohol.

3.3.4.4 Table No 55: Observation table

Melting point	187 ⁰ C	
Yield	71%	
Colour	yellowish white	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

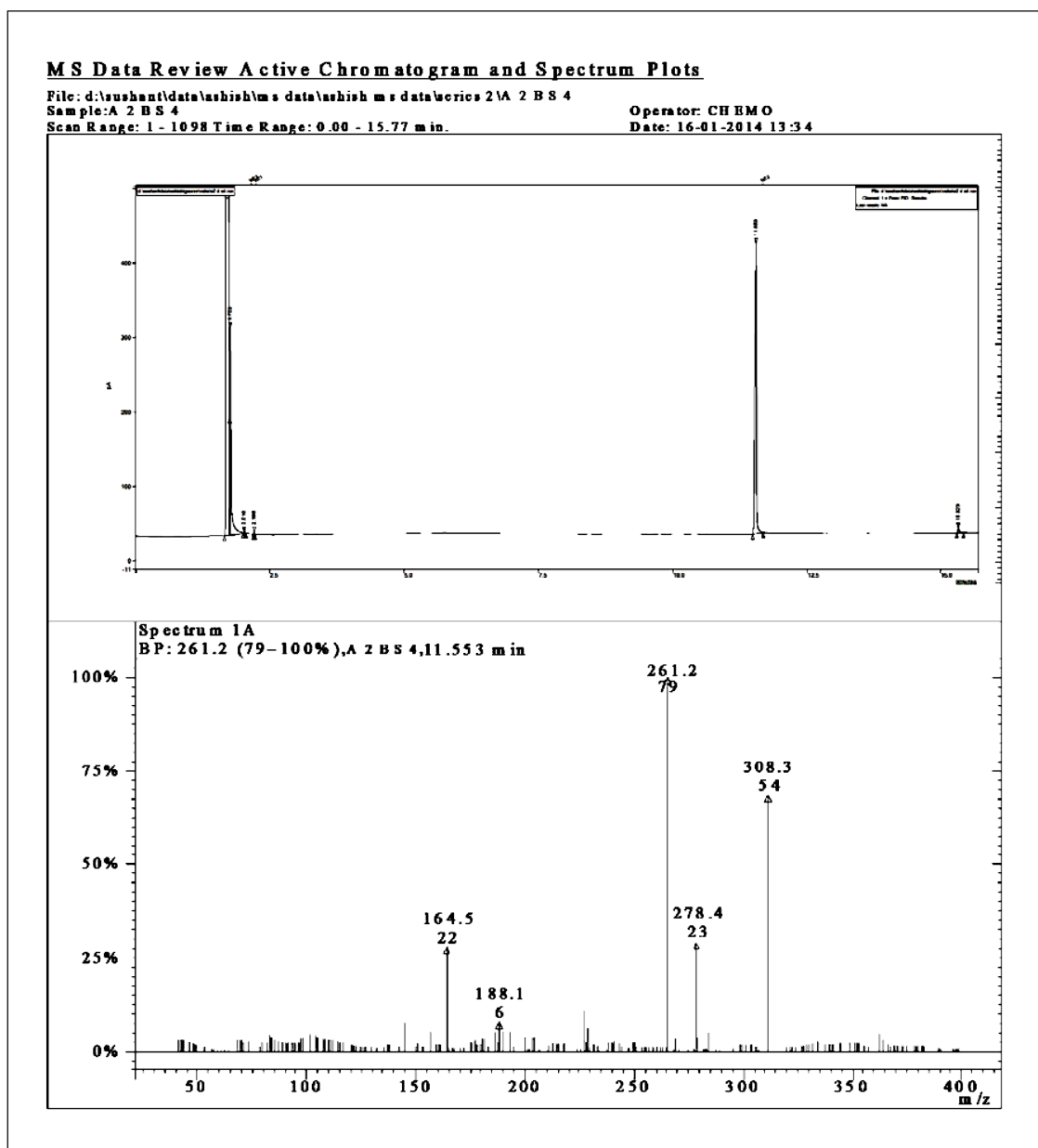
3.3.4.5 Table No 56: Chemical test: Primary Amine

Sr.	Test	Observation	Inference
1.	Test for amine	Red dye observed	Primary amine confirmed

3.3.4.6 Table No 57: GCMS method for III-B.4

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 ⁰ C	Detector temp	300 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time		15.77 mins
	Sample elution time		11.55 mins

3.3.4.7 Spectrum No 19: Mass spectra of III-B.4

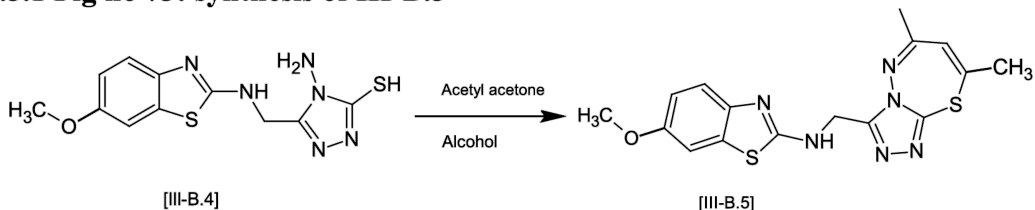


3.3.4.7 Table No 58: Mass spectral data of III-B.4

m/z values	Peak
308	Molecular ion peak
261	Base peak

3.3.5.0 III-B.5 Synthesis of 3-[(6-methoxy-1,3-benzothiazol-2-yl) sulfanyl]methyl}-6,8-dimethyl [1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazepine^[165]

3.3.5.1 Fig no 73: synthesis of III-B.5



3.3.5.2 Table No 59: Reaction Calculation table

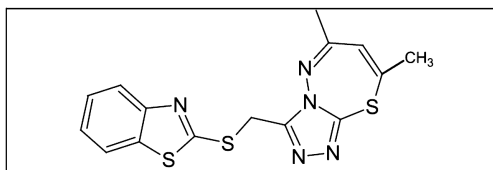
Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-B.4 product	308	0.308	0.001	1
Acetyl Acetone	100	0.1	0.001	1
Alcohol	--	15	--	--

3.3.5.3 Procedure: -

Crystallized III-B.5 product was dissolved in alcohol, reflux the reaction mixture till it forms clear solution. To this clear solution previously prepared mixture of Acetyl acetone in alcohol was added and refluxes the reaction mixture for 8-10 hrs leading to formation of product. Reaction was monitoring by TLC. The product was drowning in cold water, product was obtained. Product was filtered wash with water. Recrystallized the product with Ethyl alcohol. Similarly other derivatives were prepared.

3.3.5.4 Table No 60: Observation table

Melting point	248 ⁰ C	
Yield	54%	
Colour	Pale yellow	
Nature	Amorphous	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

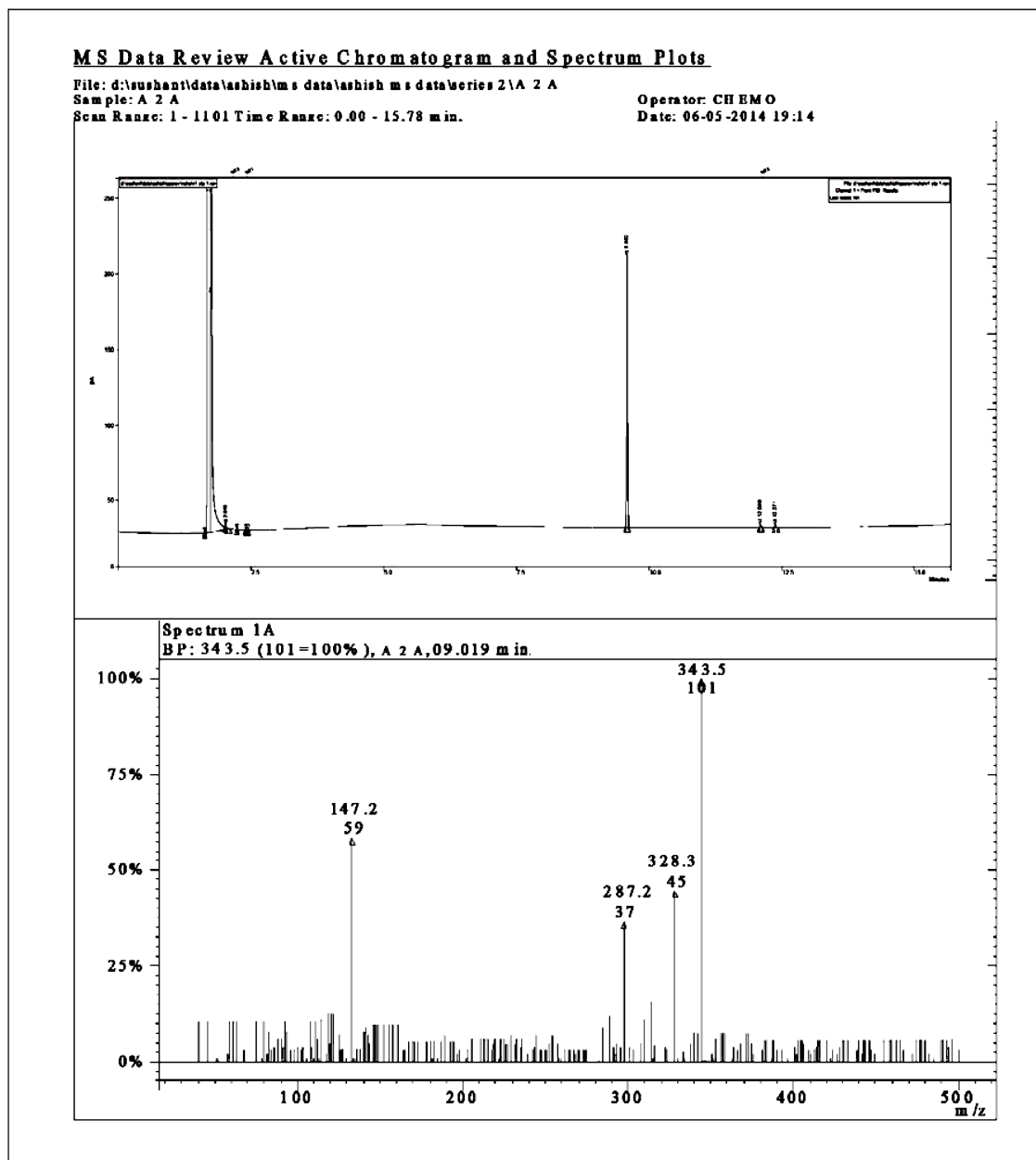
3.3.5.4. Table No 61: Physical data of synthesized compounds of III-B.5a-e

Sr. No.	R	Code	Mol. wt.	Emp. For.	Colour	Solubility	MP (°C)	Yield (%)
1	H	III-B.5a	342	C ₁₅ H ₁₄ N ₆ S ₂	Ochre	Ethyl alcohol	255	69
2	OCH ₃	III-B.5b	372	C ₁₆ H ₁₆ N ₆ OS ₂	Dark brown	Ethyl alcohol	248	73
3	OC ₂ H ₅	III-B.5c	386	C ₁₇ H ₁₈ N ₆ OS ₂	Grey	Acetone	215	58
4	H	III-B.5d	359	C ₁₅ H ₁₃ N ₅ S ₃	Pale yellow	Methanol	218	62
5	OC ₂ H ₅	III-B.5e	403	C ₁₇ H ₁₇ N ₅ OS ₃	Greenish	Methanol	203	54

3.3.5.5 Table No 62: GCMS method for III-B.5a

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	200	1
	25	300	10.7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	320°C	Detector temp	320°C
MS condition			
Ion source temperature	280°C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time		15.77 mins
	Retention Time III-B.5a		9.019 mins
	Retention Time III-B.5b		13.838 mins

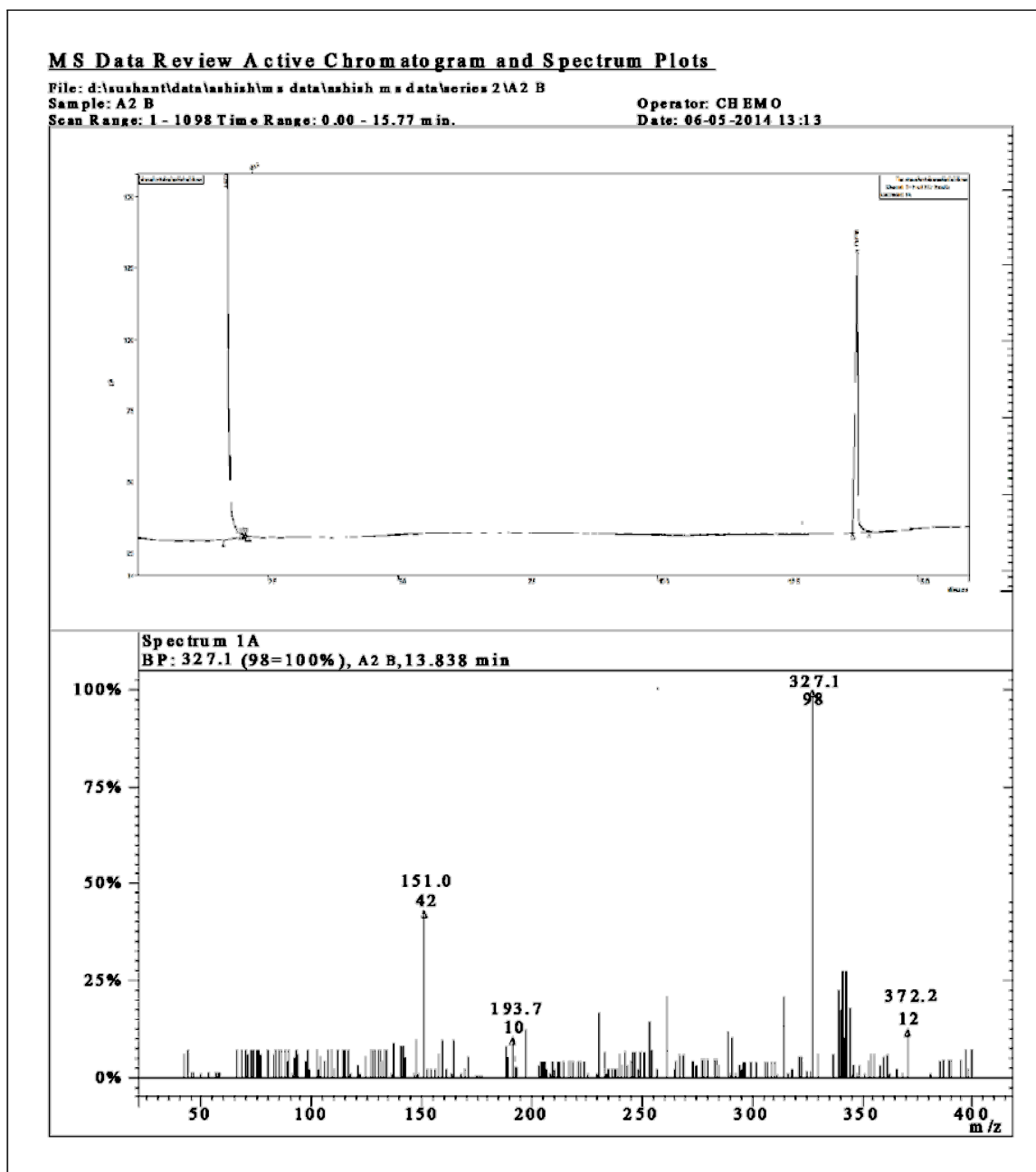
3.3.5.6.1 Spectrum 20: Mass spectra of III-B.5a



3.3.5.6.1 Table No 63: Mass spectral data of III-5a

m/z values	Peak
343	Molecular ion peak
343	Base peak

3.3.5.6.2 Spectrum 21: Mass spectra of III-B.5b

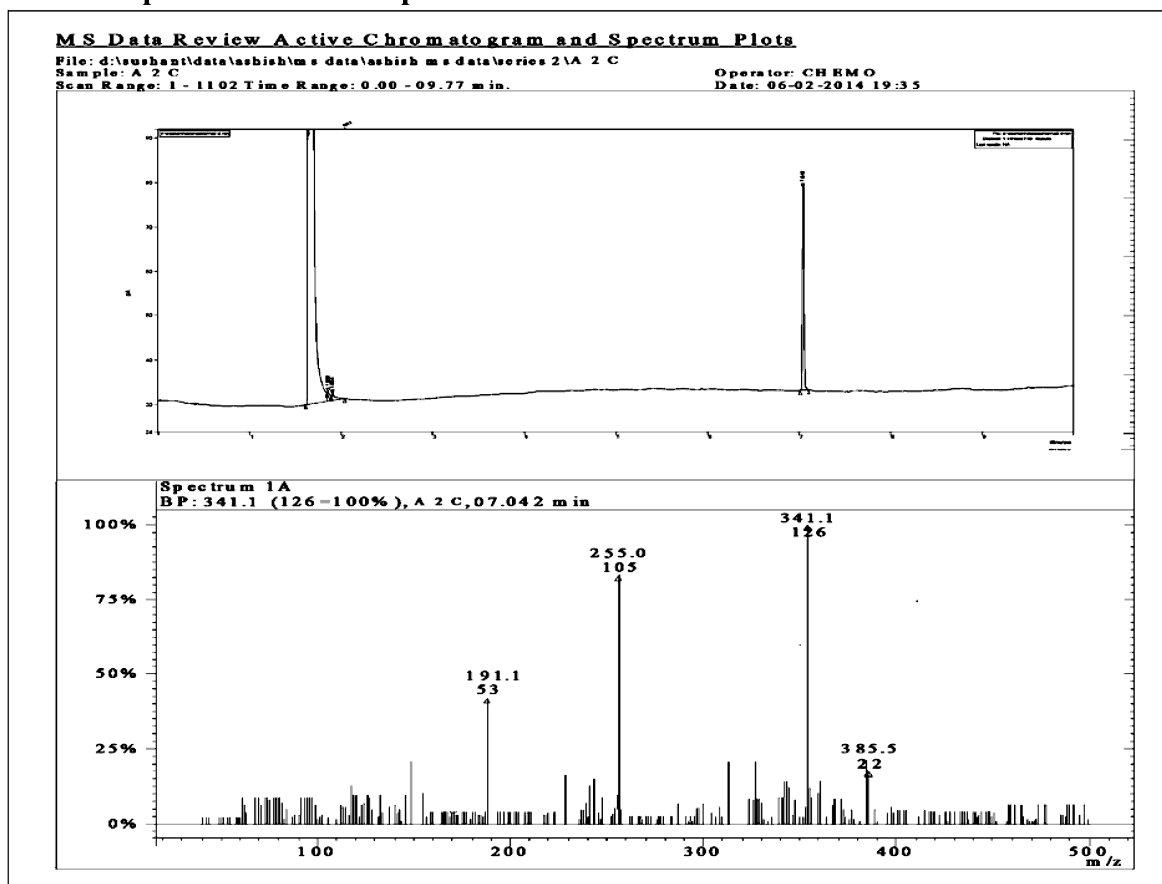


3.3.5.6.2 Table No 64: Mass spectral data of III-B.5b

m/z values	Peak
372	Molecular ion peak
327	Base peak

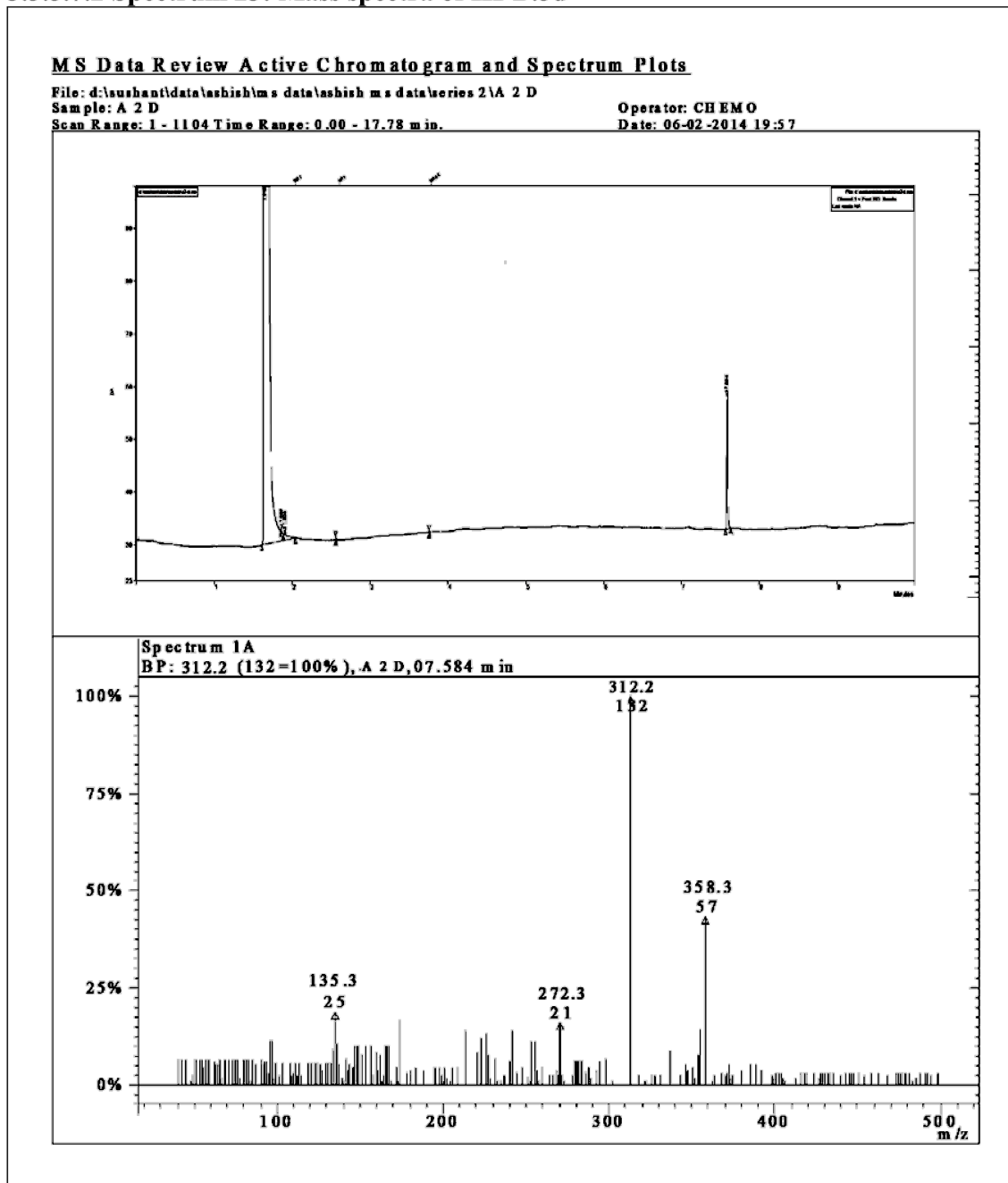
3.3.5.7 Table No 65: GCMS method for III-B.5c-e

GC conditions			
Column section	HP-5MS column (30m X 250 μ m X 0.25 μ m)		
Oven temperature	Rate ($^{\circ}$ C/ min)	Temperature ($^{\circ}$ C)	Hold time (min)
	0	200	0
	25	300	4.7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	320 $^{\circ}$ C	Detector temp	320 $^{\circ}$ C
MS condition			
Ion source temperature	280 $^{\circ}$ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time		9.77 mins
	Retention Time III-B.5c		7.042 mins
	Retention Time III-B.5d		7.584 mins
	Retention Time III-B.5e		6.535 mins

3.3.5.7.1 Spectrum 22: Mass spectra of III-B.5c**3.3.5.7.1 Table No 66: Mass spectral data of III-B.5c**

m/z values	Peak
385	Molecular ion peak
341	Base peak

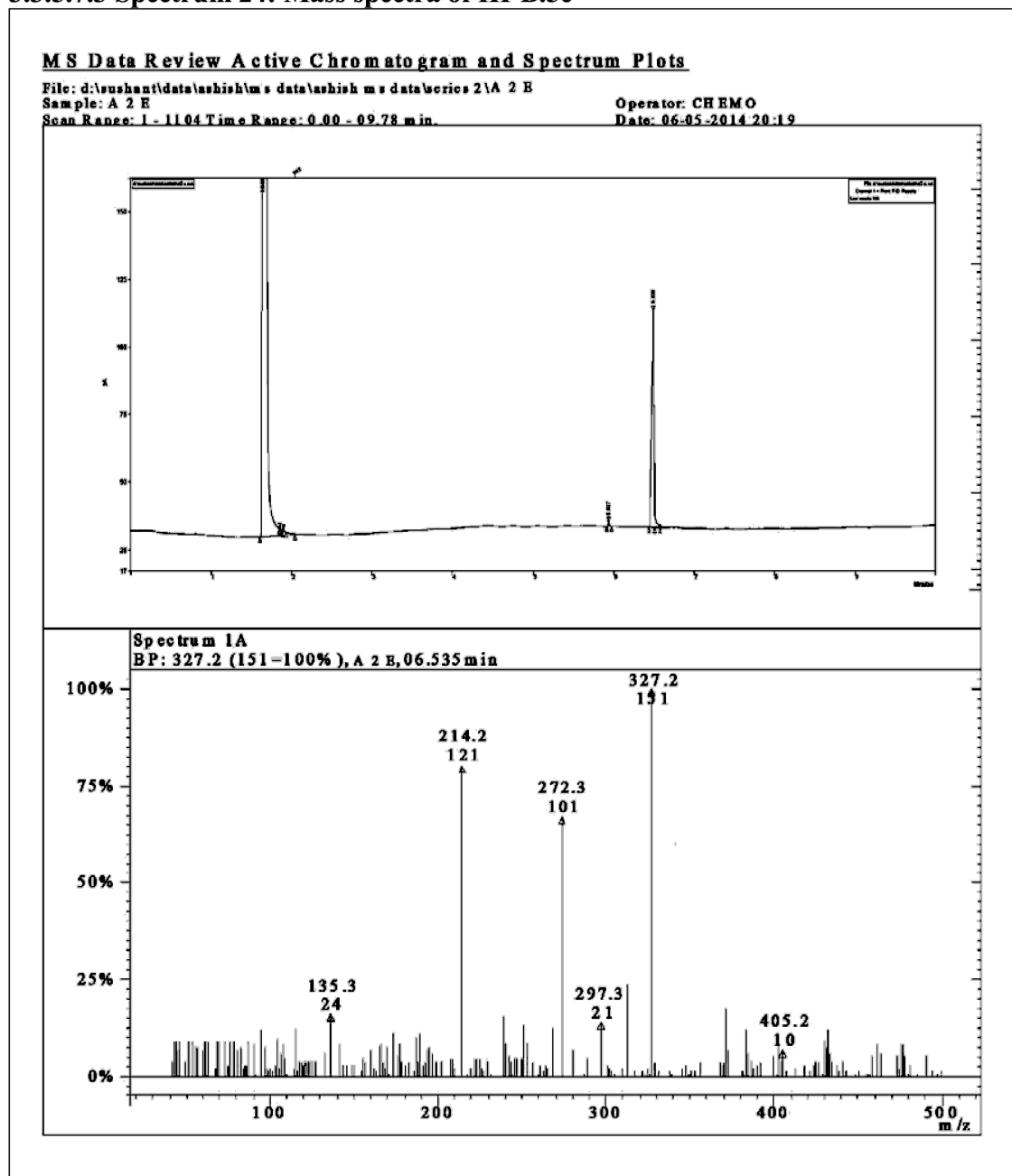
3.3.5.7.2 Spectrum 23: Mass spectra of III-B.5d



3.3.5.7.2 Table No 67: Mass spectral data of III-B.5d

m/z values	Peak
358	Molecular ion peak
312	Base peak

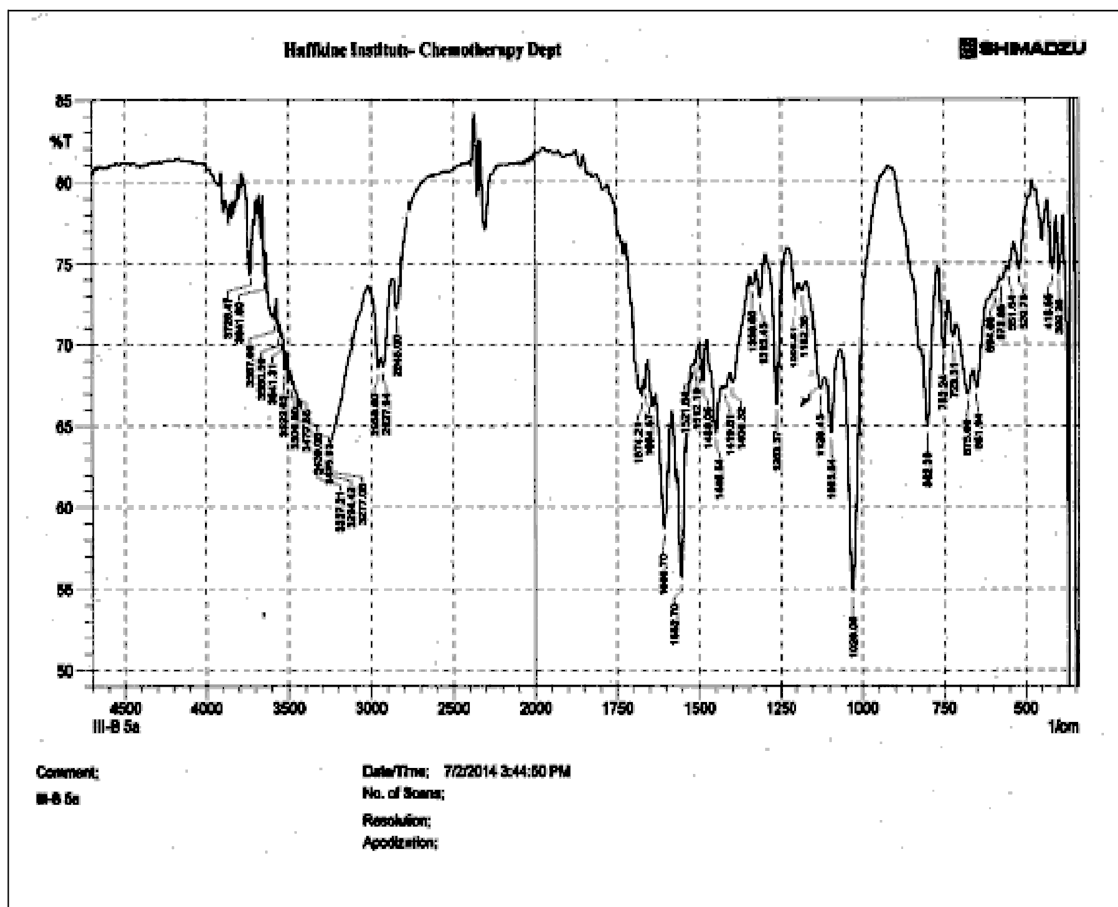
3.3.5.7.3 Spectrum 24: Mass spectra of III-B.5e



3.3.5.7.3 Table No 68: Mass spectral data of III-B.5e

m/z values	Peak
405	Molecular ion peak
327	Base peak

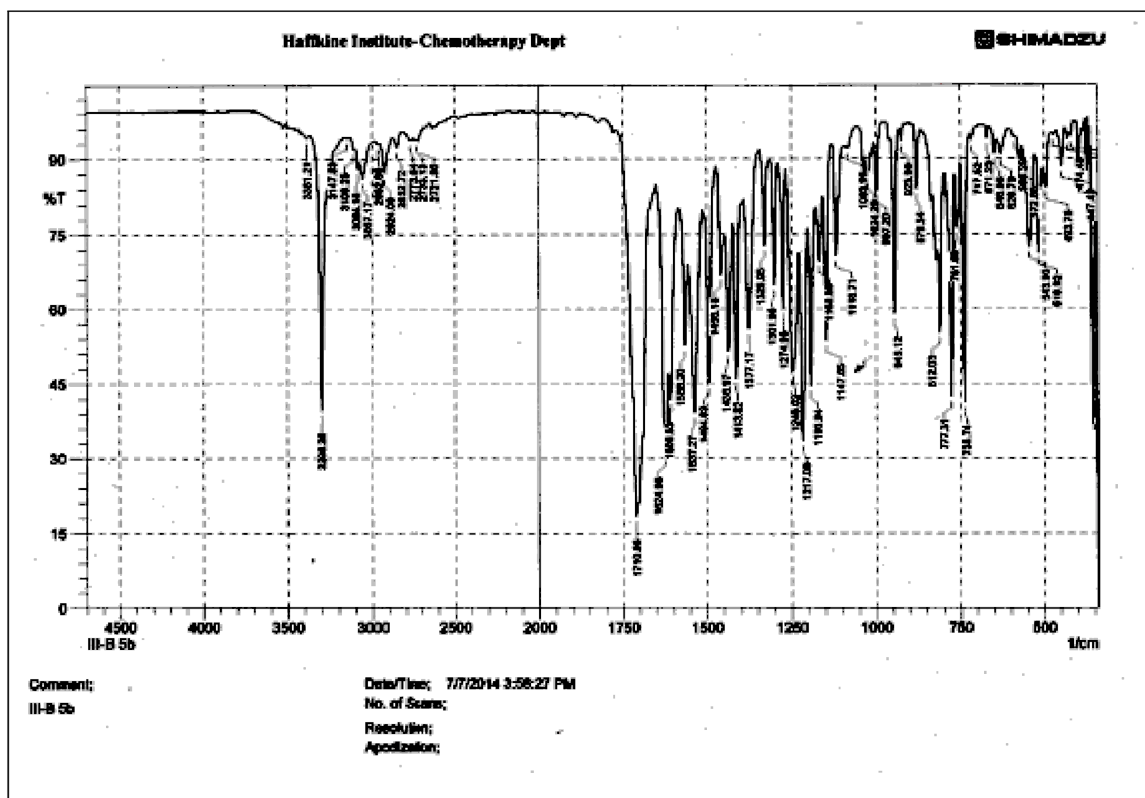
3.3.5.8.1 Spectrum 25: IR spectra of III-B.5a



3.3.5.8.1 Table No 69: IR spectral data of III-B.5a

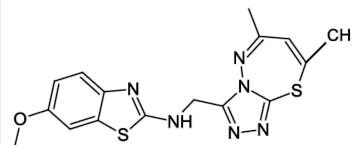
Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
N-H Sec. Amine stretching	3327	3300-3400
C-S bending	1263	1030-1275
-N= Tert. Amine bending	1205	1000-1250
C-H stretching	2958	2850-3000

3.3.5.8.2 Spectrum 26: IR spectra of III-B.5b

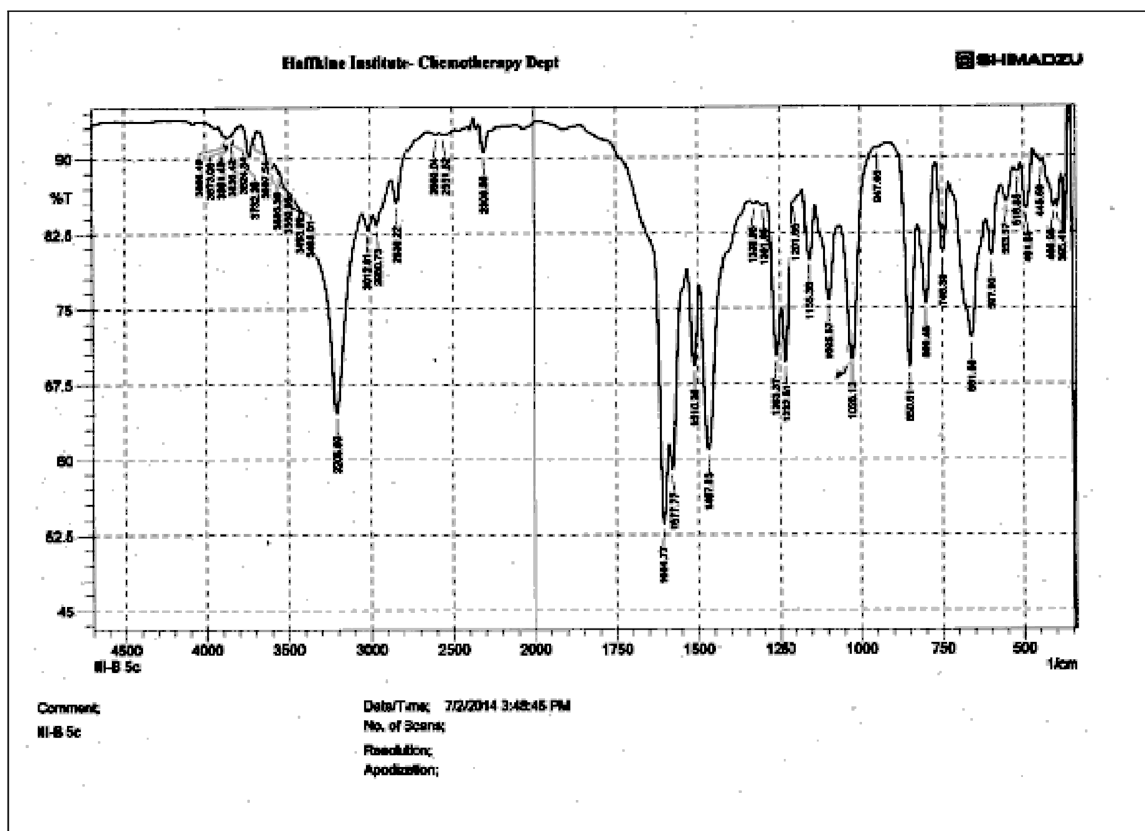


3.3.5.8.2 Table No 70: IR spectral data of III-B.5b

Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
N-H Sec. Amine stretching	3381	3300-3400
C-S bending	1217	1030-1275
-N= Tert. Amine bending	1193	1030-1250
-OCH ₃ bending	1118	1000-1320
C-H stretching	2924	2850-3000

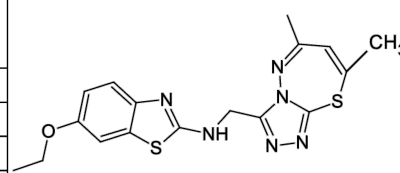


3.3.5.8.3 Spectrum 27: IR spectra of III-B.5c

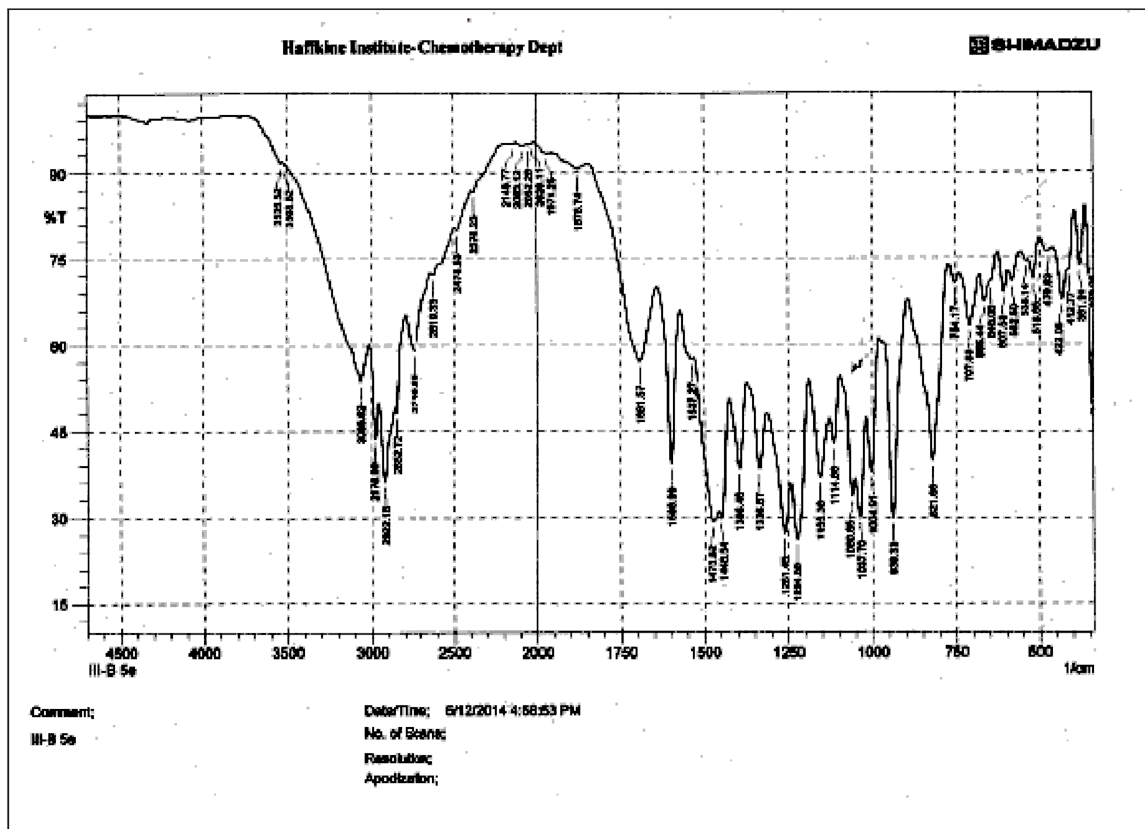


3.3.5.8.3 Table No 71: IR spectral data of III-B.5c

Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
C-S bending	1230	1030-1275
-N= Tert. Amine bending	1327	1000-1250
-OC ₂ H ₅ bending	1091	1000-1320
C-H stretching	2960	2850-3000

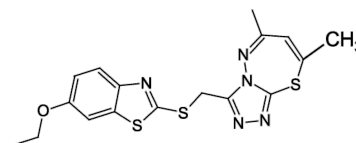


3.3.5.8.5 Spectrum 29: IR spectra of III-B.5e

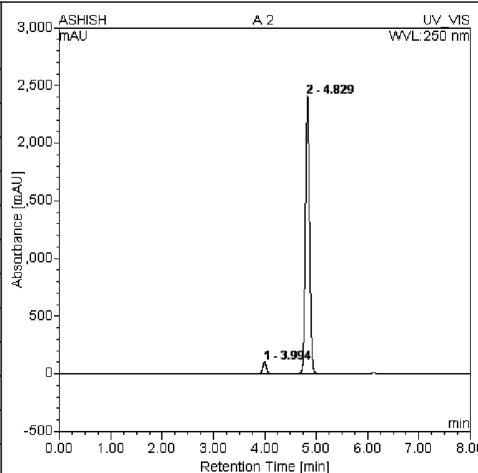


3.3.5.8.5 Table No 73: IR spectral data of III-5e

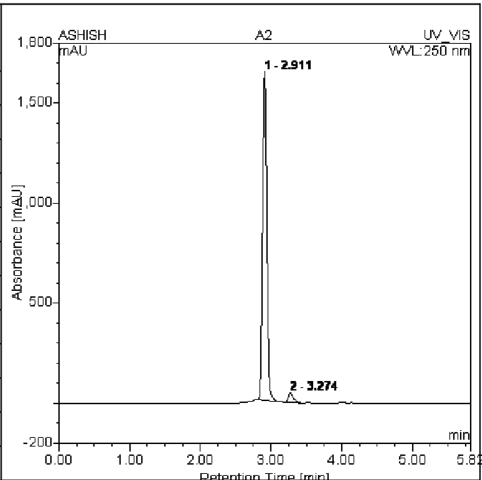
Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
-N= tert amine bending	1224	1000-1250
C-S bending	1155	1030-1275
C-H stretching	3066	2850-3000
-OC ₂ H ₅ bending	1114	1000-1320



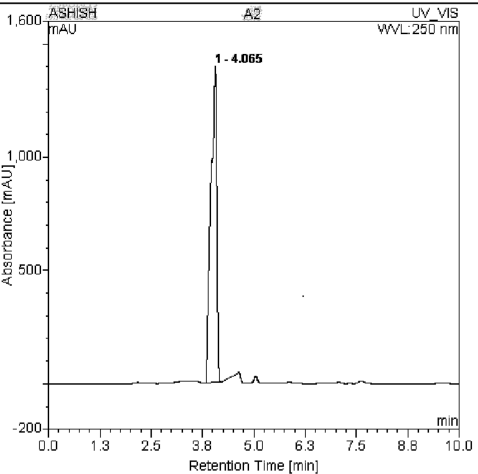
3.3.5.9.1 Chromatogram 6: HPLC data for III-B.5a

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Methanol (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	250 nm	
Sample concentration	10 ppm	
Retention Time	4.8 mins	
Peak Intensity	1133	
Theoretical plates	17003	
Asymmetry	1.08	
Peak Width	0.149	

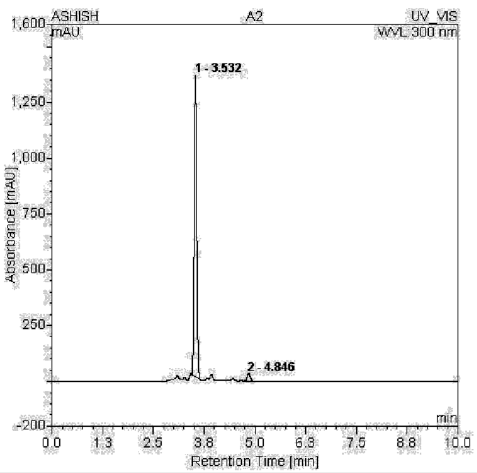
3.3.5.9.2 Chromatogram 7: HPLC data for III-B.5b

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Methanol (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	250 nm	
Sample concentration	10 ppm	
Retention Time	2.9 mins	
Peak Intensity	1641	
Theoretical Plates	10458	
Asymmetry	1.06	
Peak Width	0.113	

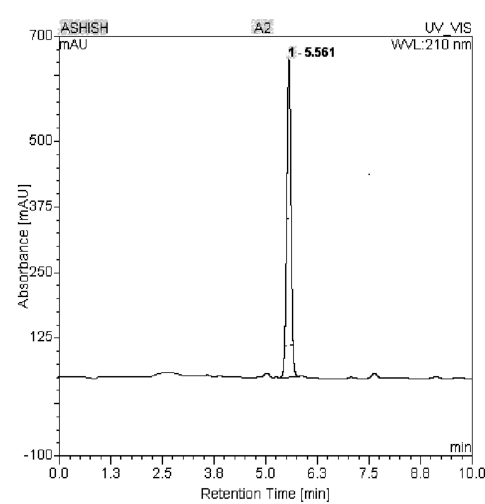
3.3.5.9.3 Chromatogram 8: HPLC data for III-B.5c

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Methanol (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	250nm	
Sample concentration	10 ppm	
Retention Time	4.065min.	
Peak Intensity	1396.541	
Theoretical Plates	2718	
Asymmetry	0.67	
Peak Width	0.209	

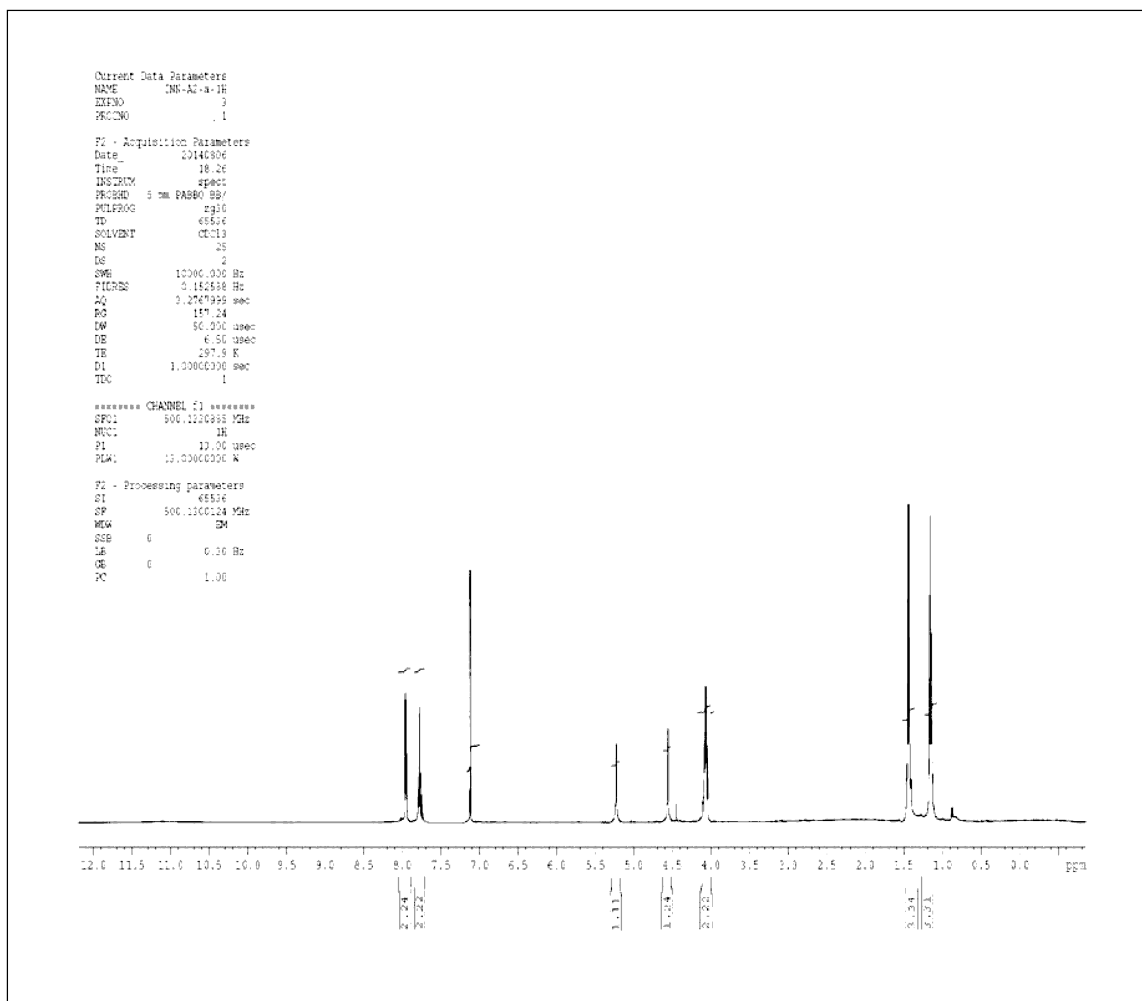
3.3.5.9.4 Chromatogram 9: HPLC data for III-B.5d

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Methanol (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	300 nm	
Sample concentration	10 ppm	
Retention Time	3.5 mins	
Peak Intensity	1133.729	
Theoretical Plates	7003	
Asymmetry	1.08	
Peak Width	0.149	

3.3.5.9.5 Chromatogram 10: HPLC data for III-B.5e

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Water (20%)	
Mobile phase A	Methanol (80%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	348 nm	
Sample concentration	10 ppm	
Retention Time	5.5 mins	
Peak Intensity	605.628	
Theoretical Plates	3770	
Asymmetry	1.00	
Peak Width	0.190	

3.3.5.10 Spectrum 30: NMR spectra of III-B.5a



3.3.5.10 Table No 74: NMR spectral data of III-B.5a

δ values	^1H NMR signal
4.5	s,1H, NH
4.0	s,2H, CH_2
1.0	s,3H, CH_3
1.0	s,3H, CH_3
5.3	s,2H, CH_2
7.5-8.0	m,4H, Ar-H

3.4.0 III-C] Synthesis of *S*-{5-[(1,3-benzothiazol-2-ylsulfanyl) methyl] -1,3,4-oxadiazol-2-yl} substituted benzenecarbothioate

III-C Includes following steps

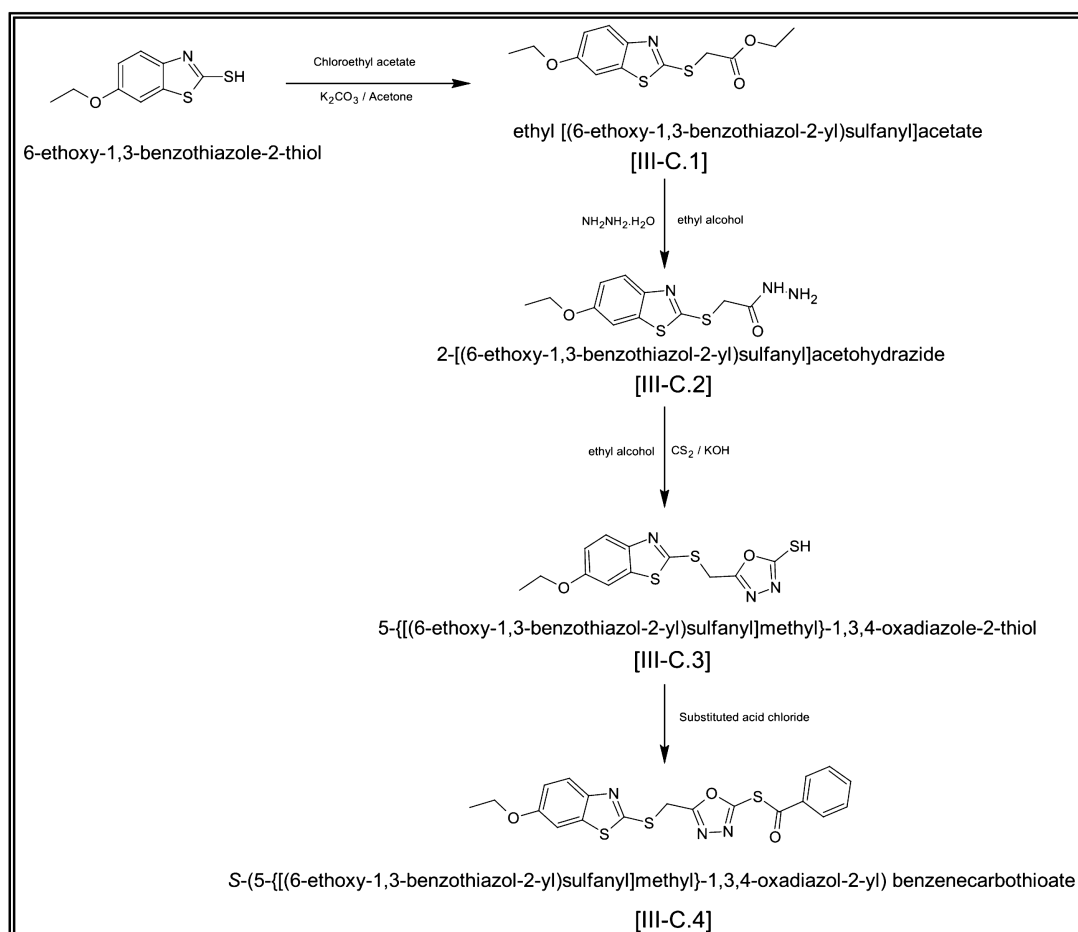
3.4.1 III-C.1 Synthesis of ethyl [(6-ethoxy-1, 3-benzothiazol-2-yl)sulfanyl]acetate

3.4.2 III-C.2 Synthesis of 2-[(6-ethoxy-1, 3-benzothiazol-2-yl) sulfany l] acetohydrazide

3.4.3 III-C.3 Synthesis of 5-{[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazole-2-thiol

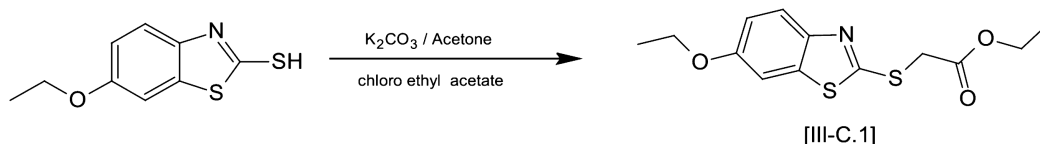
3.4.4 III-C.4 Synthesis of *S*-{5-[(1,3-benzothiazol-2-ylsulfanyl) methyl] -1,3,4-oxadiazol-2-yl} substituted benzenecarbothioate

Fig no 74: Schematic Representation III-C



3.4.1.0 III-C.1 synthesis of ethyl [(6-ethoxy-1, 3-benzothiazol-2-yl)sulfanyl]acetate^[160]

3.4.1.1 Fig no 75: synthesis of III-C.1



3.4.1.2 Table No 75: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
6-ethoxy-2-mercapto benzothiazole	211	2.11	0.01	1
ethyl chloroacetate	122	1.22	0.01	1
Potassium carbonate	138	0.69	0.005	0.5
Acetone	--	10	--	--

3.4.1.3 Procedure: -

6-ethoxy-2-mercapto benzothiazole was dissolved in acetone stirred for 30 mins and K_2CO_3 was added and reflux the reaction mixture leading to formation of potassium salt of mercapto benzothiazole. After salt formation ethyl chloroacetate was added over the period of 15 mins. Reflux the reaction mixture till completion of reaction. Reaction was monitoring was done by TLC. Formed crude product was isolated by down in cold water. Clear solution was extracted with Diethyl ether for three times to extract product from aqueous layer. Recrystallized the product with Ethyl alcohol

3.4.1.4 Table No 76: Observation table

Melting point	82 ⁰ C	
Yield	70%	
Colour	Cream	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

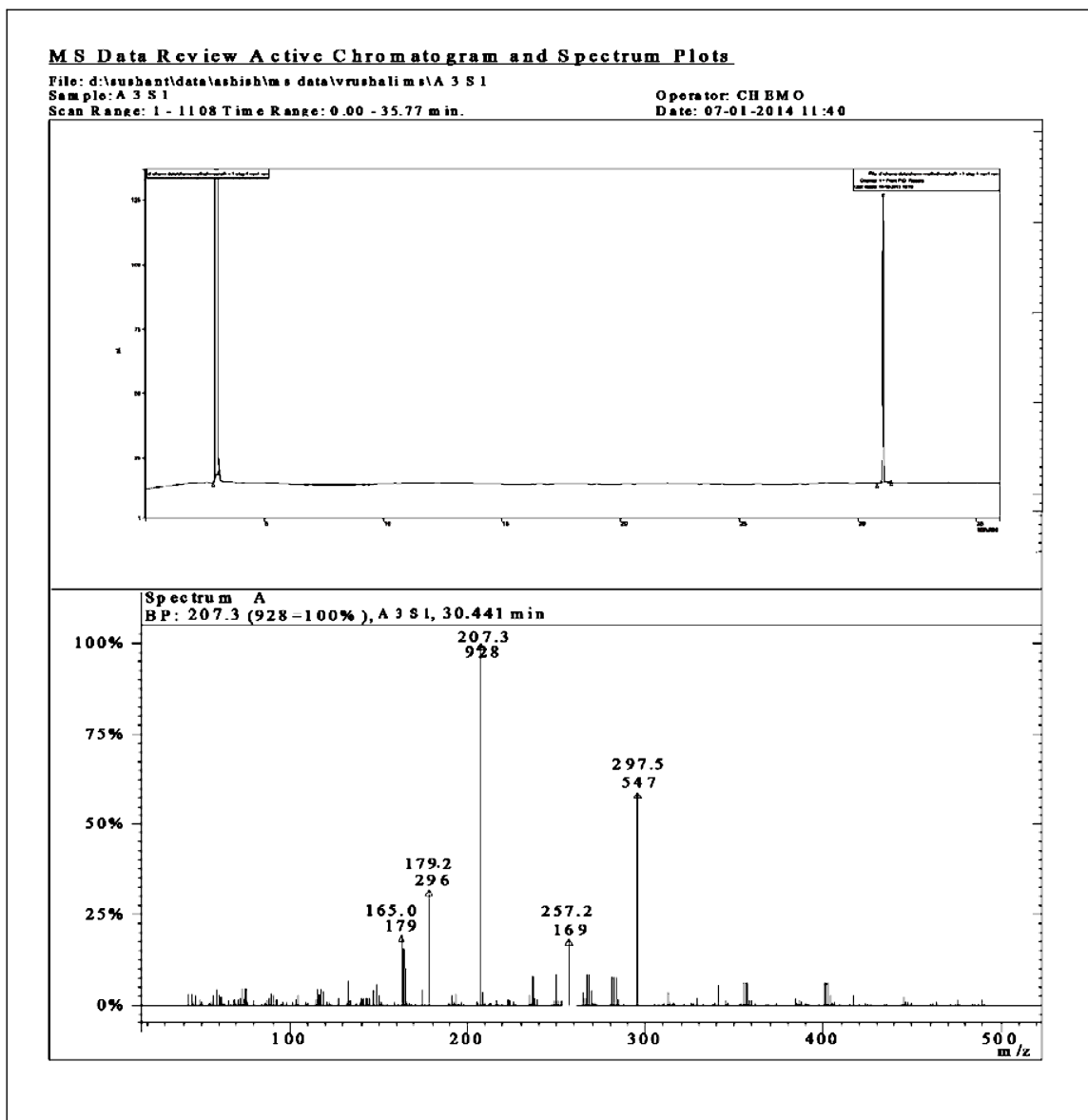
3.4.1.5 Table No 77: Chemical test: Ester confirmation test

Sr.no.	Test	Observation	Inference
1.	Alkaline hydrolysis Test	Pink color disappeared on heating	Ester group confirmed.
2.	Hydroxamic acid test	Red violet color developed	Ester group confirmed.

3.4.1.6 Table No 78: GCMS method for III-C.1

GC conditions			
Column section	HP-5MS column (30m X 250μm X 0.25μm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	100	3
	10	120	3
	10	150	2
	10	180	2
	5	200	2
	5	220	3
	10	240	2
Injection mode	Split mode; split ratio 90:1		
Injection temperature	250°C		
Injection volume	1 μL		
Carrier gas	Helium gas		
Detector temperature	280°C		
Analysis time			
Run time	35.77 mins		
Retention Time	30.041 mins		
MS condition			
Ion source temperature	280°C		
MS source	EI 70 Ev		
MS scan mode	EI Full scan m/z 40-500		

3.4.1.7 Spectrum 31: Mass spectra of III-C.1

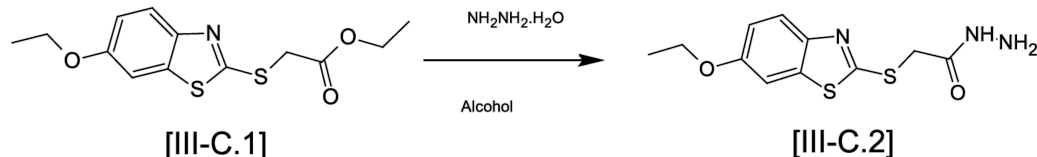


3.3.1.7 Table No 79: Mass spectral data of III-C.1

m/z values	Peak
297	Molecular ion peak
207	Base peak

3.4.2.0 III-C.2 Synthesis of 2-[(6-ethoxy-1, 3-benzothiazol-2-yl)sulfanyl] acetohydrazide^[160-161]

3.4.2.1 Fig no 76: synthesis of III-C.2



3.4.2.2 Table No 80: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-C.1 product	297	2.97	0.01	1
NH ₂ NH ₂ .HCl	68	1.36	0.02	2
Alcohol	--	10	--	--

3.4.2.3 Procedure: -

Crystallized product of III-C.1 product was dissolved in alcohol, reflux the reaction mixture till it forms clear solution. To this clear solution of Hydrazine hydrate was added and refluxes the reaction mixture for 8-10 hrs leading to formation of product. Reaction was monitored by TLC. After completion of reaction, keep the reaction mixture on standing for overnight, needle shaped crystal was formed. Filter the product and wash with water till pH 7-8. Recrystallized the product with Ethyl alcohol

3.4.2.4 Table No 81: Observation table

Melting point	174 ⁰ C	
Yield	60%	
Colour	White	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

3.4.2.5 Table No 82: Chemical test: Amide confirmation test

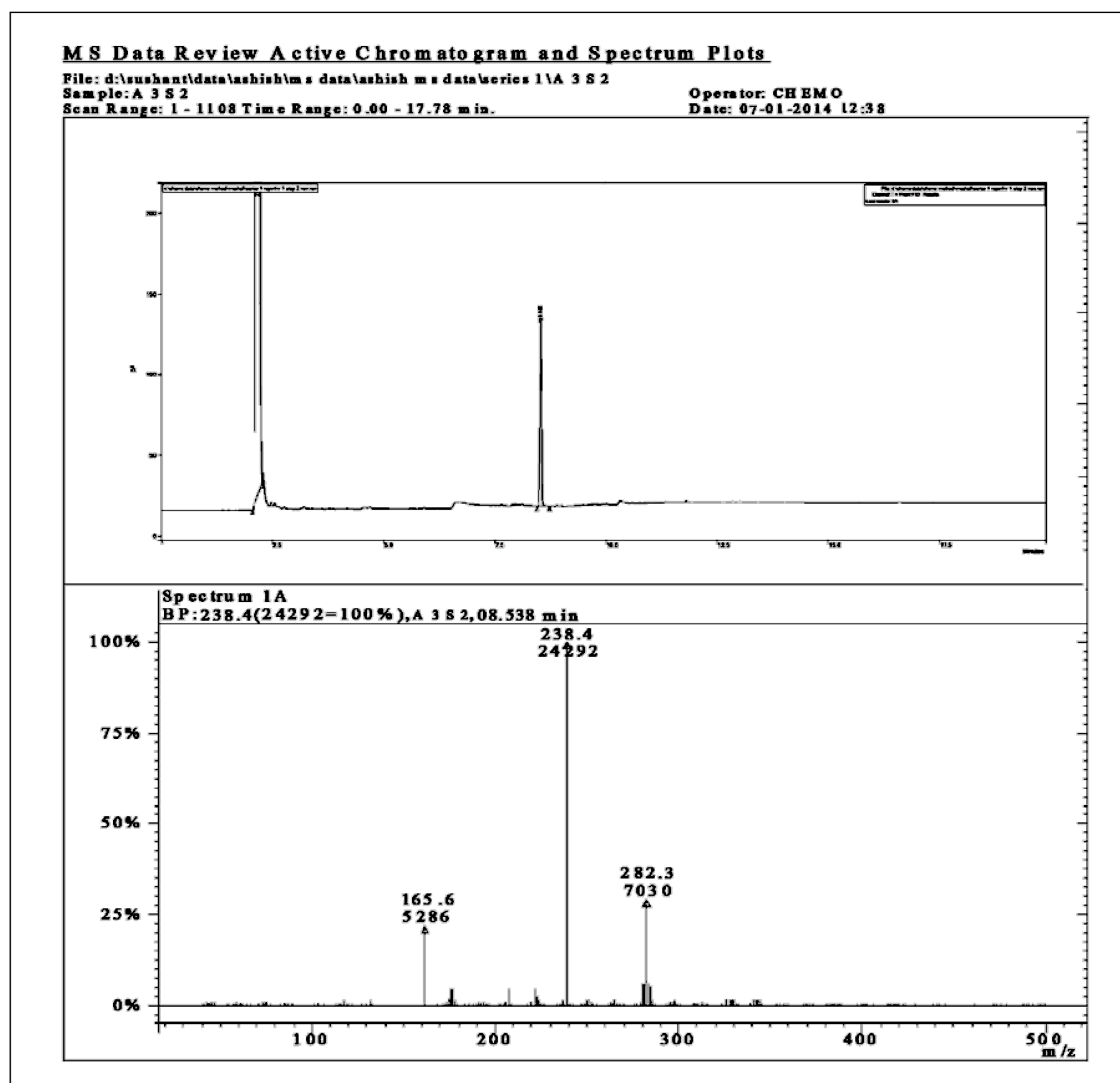
Sr. no.	Test	Observation	Inference
1.	Test for Amide	Effervescence due to evaluation of Nitrogen	Amide group confirmed
2	Nitrous acid test	Effervescence due to evaluation of Nitrogen	Amide group confirmed.

3.4.2.6 Table No 83: GCMS method for III-C.2

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas

Injection temperature	300 ⁰ C	Detector temp	300 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time		17.78 mins
	Sample elution time		18.53 mins

3.4.2.7 Spectrum 32: Mass spectra of III-C.2

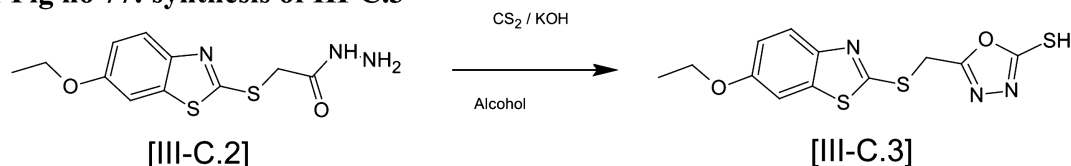


3.4.2.6 Table No 84: mass spectral data of III-C.2

m/z values	Peak
282	Molecular ion peak
238	Base peak

3.4.3.0 III-C.3 Synthesis of 5-[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]methyl}-1,3,4-oxadiazole-2-thiol^[162]

3.4.3.1 Fig no 77: synthesis of III-C.3



3.4.3.2 Table No 85: Reaction Calculation table:

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-C.2 product	281	2.81	0.01	1
Carbon disulphide	76	0.76	0.01	1
Potassium hydroxide	56	0.56	0.01	1
Alcohol	--	15	--	--

3.4.3.3 Procedure: -

Crystallized product of III-C.2 was dissolved in alcohol to this KOH was added to form potassium salt of III-C.2. To this mixture carbon disulfide was added. Reflux the reaction mixture, as reaction progress H₂S gas was evolved. After 8-10 hrs liberation of H₂S gas stopped. Further completion of reaction was monitored by TLC. The product was down in cold water, mixture was highly basic in nature, neutralized by using dil. HCl to pH 7. Product was filtered, wash with plenty of water to remove traces of impurities. Recrystallized the product with Ethyl alcohol

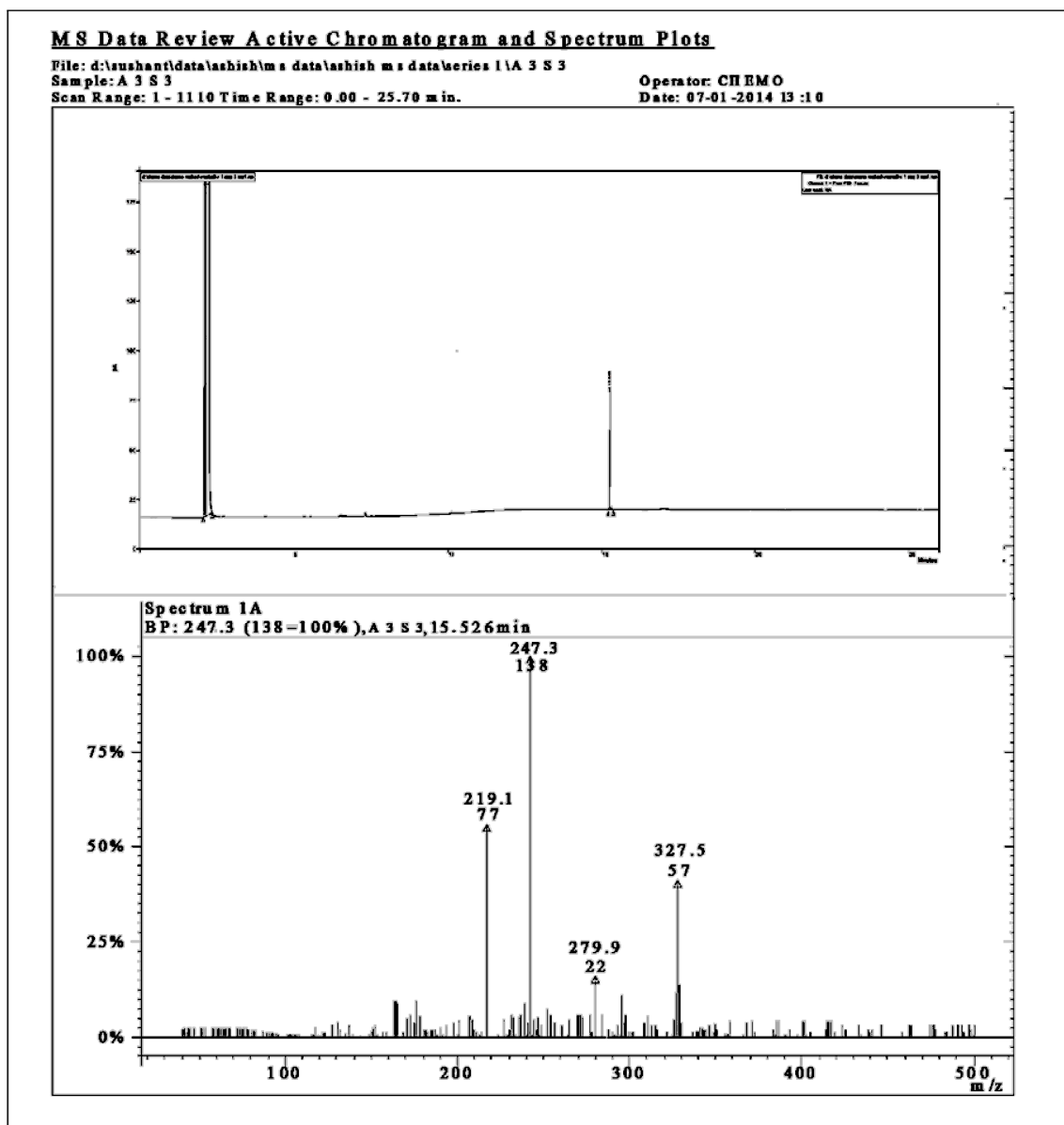
3.4.3.4 Table No 86: Observation table:

Melting point	257 ⁰ C	
Yield	54%	
Colour	Pale yellow	
Nature	Amorphous	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

3.4.3.5 Table No 87: GCMS method for III-C.3

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (°C/ min)	Temperature (°C)	Hold time (min)
	0	45	1
	20	200	2
	20	300	9
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 ⁰ C	Detector temp	300 ⁰ C
MS condition			
Ion source temperature	300 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time	15.77 mins	
	Sample elution time	9.906	

3.4.3.5 Spectrum 33: Mass spectra of III-C.3

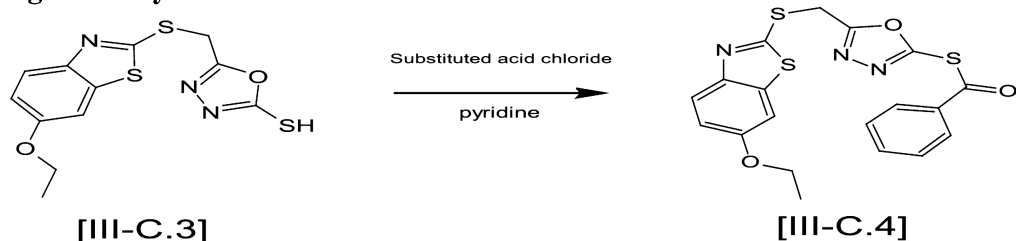


3.4.3.5 Table No 88: mass spectral data of III-B.3

m/z values	Peak
327	Molecular ion peak
247	Base peak

3.4.4.0 III-C.4 Synthesis of *S*-(5-[[6-ethoxy-1, 3-benzothiazol-2-yl] sulfanyl] methyl)-1,3,4-oxadiazol-2-yl benzenecarbothioate ^[166]

3.4.4.1 Fig no 78: synthesis of III-C.4



3.4.4.2 Table No 89: Reaction Calculation table

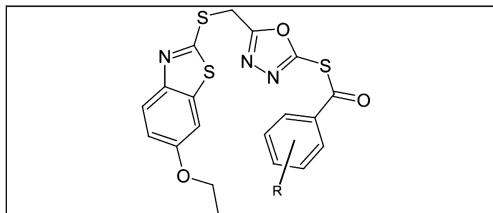
Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-C.3 product	326	0.326	0.001	1
Benzoyl chloride	140	0.140	0.001	1
Pyridine	--	15	--	--

3.4.4.3 Procedure: -

Crystallized product of III-C.3 was dissolved in pyridine to this benzoyl Chloride was added drop wise at room temperature, as it is exothermic reaction addition of benzoyl chloride was done slowly over the period of 30 mins., then stirred the reaction mixture at same temperature for next 30 mins slowly raised the temperature to reflux the reaction mixture. Completion of reaction was monitored by TLC. The product was down in cold water, solid appeared. Product was filtered, wash with plenty of water. Recrystallized the product with Ethyl alcohol. Similarly other derivatives were prepared.

3.4.4.4 Table No 90: Observation table

Melting point	153 ⁰ C	
Yield	80%	
Colour	Yellow	
Nature	Crystalline	
TLC system	(Ethyl acetate: Hexane) (7: 3)	

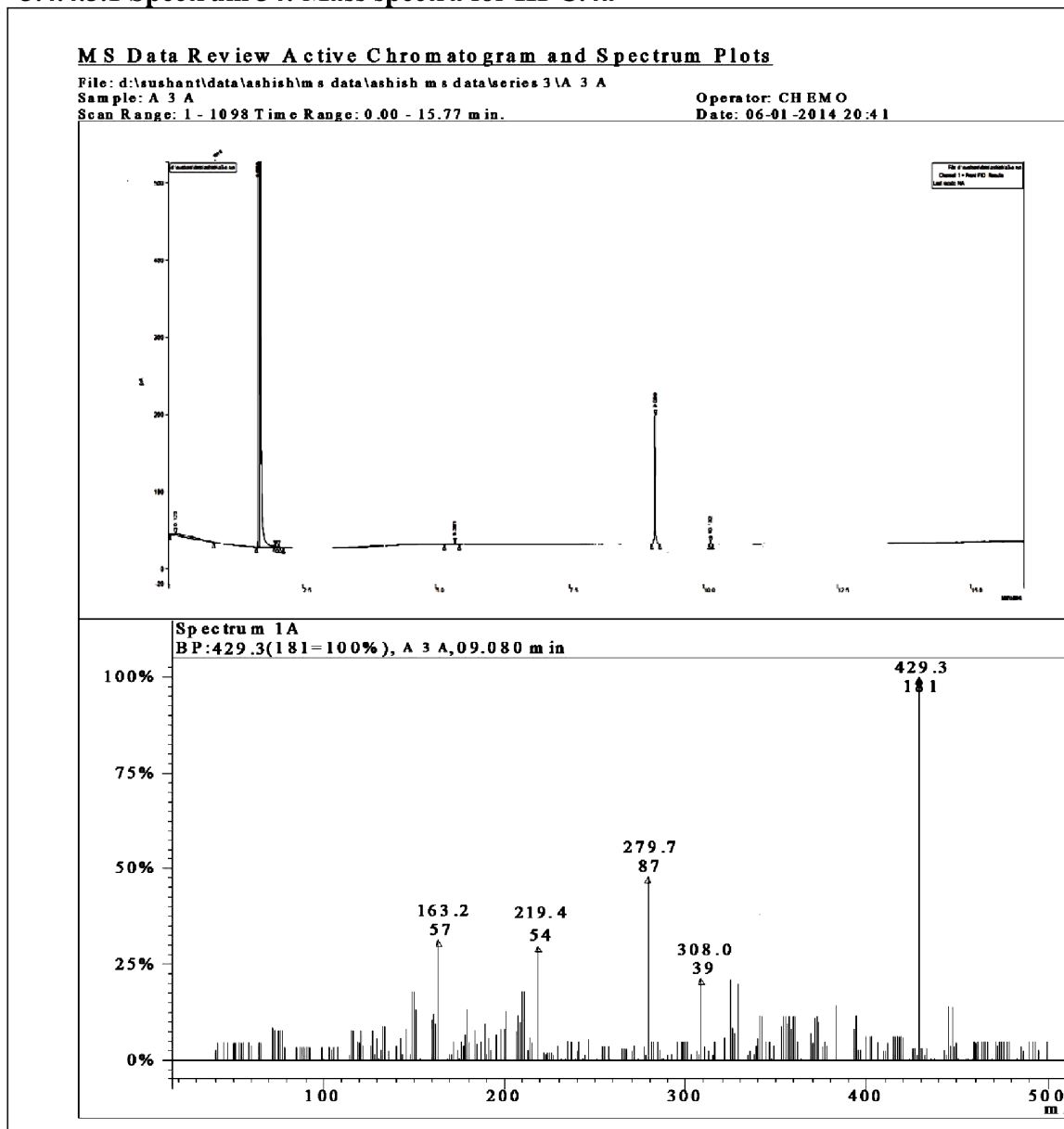
3.4.4.4. Table No 91: Physical data of synthesized compounds:

Sr. No	R	Code	Mol wt.	Emp. For.	Colour	Solubility	MP ($^{\circ}\text{C}$)	Yield (%)
1	-H	III-C.4a	429	$\text{C}_{19}\text{H}_{15}\text{N}_3\text{O}_3\text{S}_3$	Yellow	Ethyl alcohol	155	54
2	4-Cl	III-C.4b	445	$\text{C}_{19}\text{H}_{15}\text{N}_3\text{O}_4\text{S}_3$	Brown	Ethyl alcohol	140	58
3	4-OH	III-C.4c	463	$\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_4\text{S}_3$	Pale yellow	Acetone	154	62
4	4-OCH ₃	III-C.4d	459	$\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_4\text{S}_3$	Brown	Methanol	206	66
5	4-OC ₂ H ₅	III-C.4e	473	$\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_4\text{S}_3$	Light brown	Methanol	212	52

3.4.4.5 Table No 92: GCMS method for III-C.4a-e

GC conditions			
Column section	HP-5MS column (30m X 250 μm X 0.25 μm)		
Oven temperature	Rate ($^{\circ}\text{C}/\text{min}$)	Temperature ($^{\circ}\text{C}$)	Hold time (min)
	0	150	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	250 $^{\circ}\text{C}$	Detector temp	280 $^{\circ}\text{C}$
MS condition			
Ion source temperature	280 $^{\circ}\text{C}$		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time		15.77 mins
	Retention time III-C.4a		9.080 mins
	Retention time III-C.4b		13.838 mins
	Retention time III-C.4c		13.296 mins
	Retention time III-C.4d		8.400 mins
	Retention time III-C.4e		15.384 mins

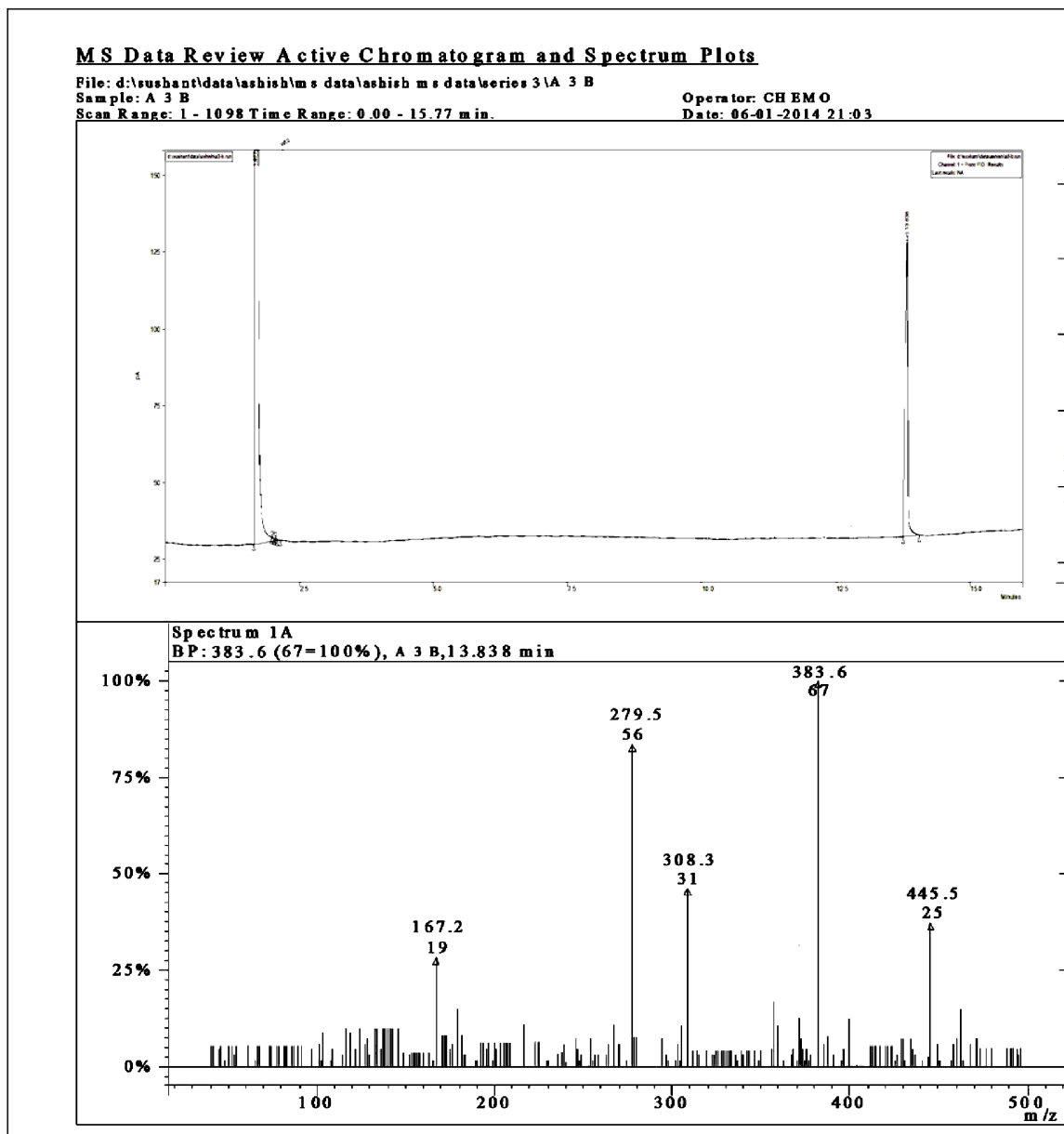
3.4.4.5.1 Spectrum 34: Mass spectra for III-C.4a



3.4.4.5.1 Table No 93: Mass spectral data for III-C.4a

m/z values	Peak
429	Molecular ion peak
429	Base peak

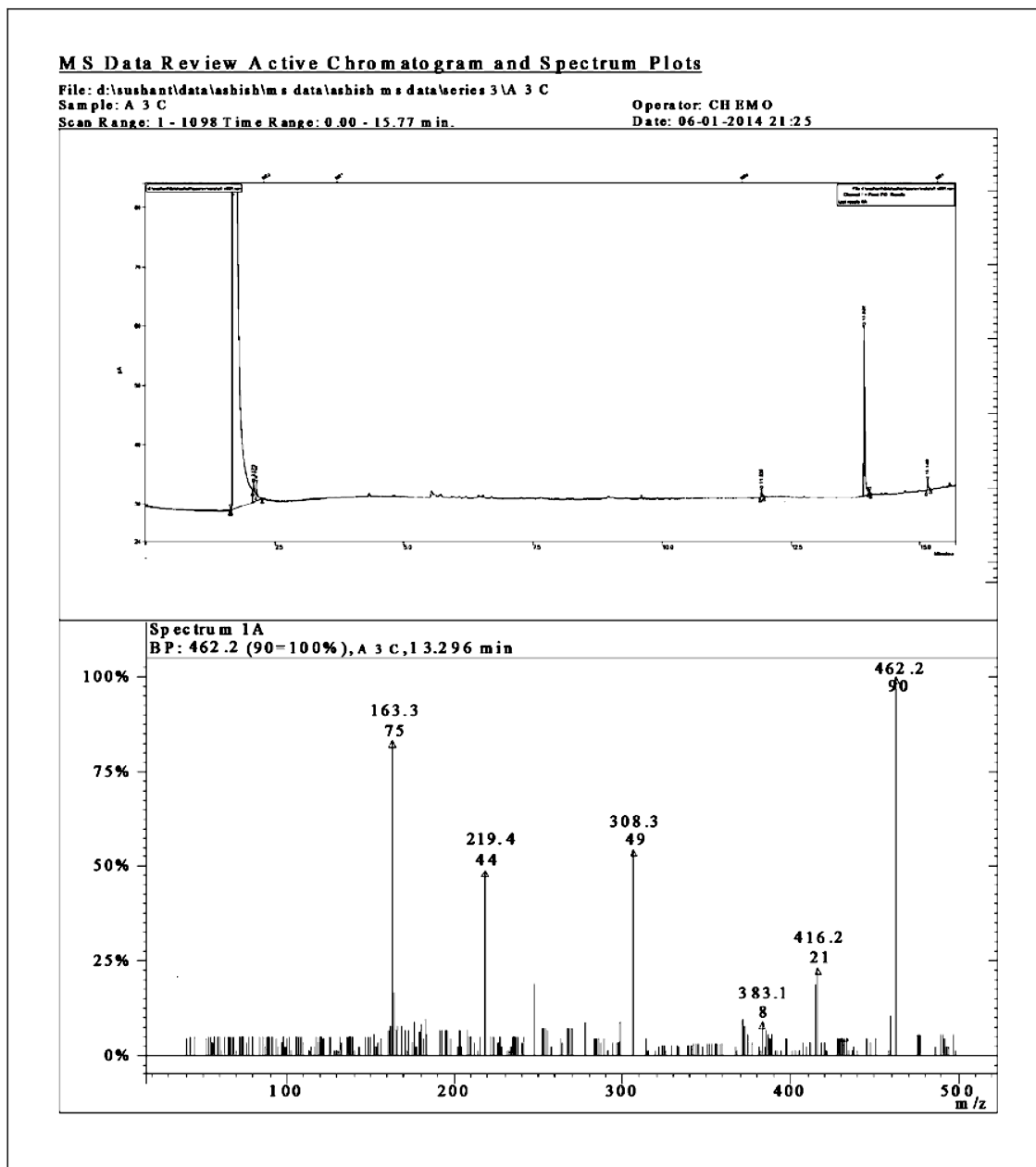
3.4.4.5.2 Spectrum 35: Mass spectra for III-C.4b



3.4.4.5.2 Table No 94: Mass spectral data for III-C.4b

m/z values	Peak
445	Molecular ion peak
383	Base peak

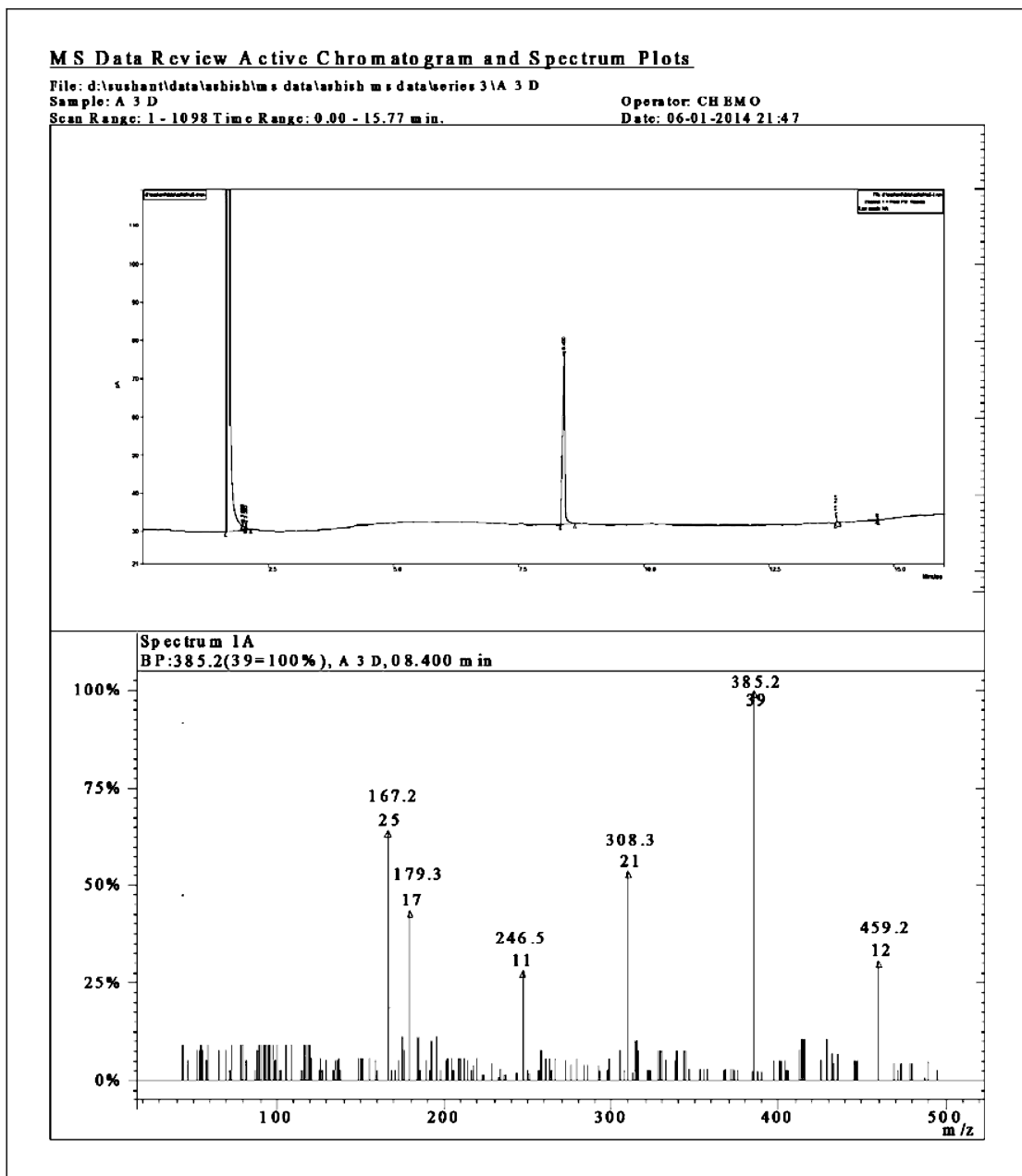
3.4.4.5.3 Spectrum 36: Mass spectra for III-C.4c



3.4.4.5.3 Table No 95: Mass spectral data of III-C.5c

m/z values	Peak
462	Molecular ion peak
362	Base peak

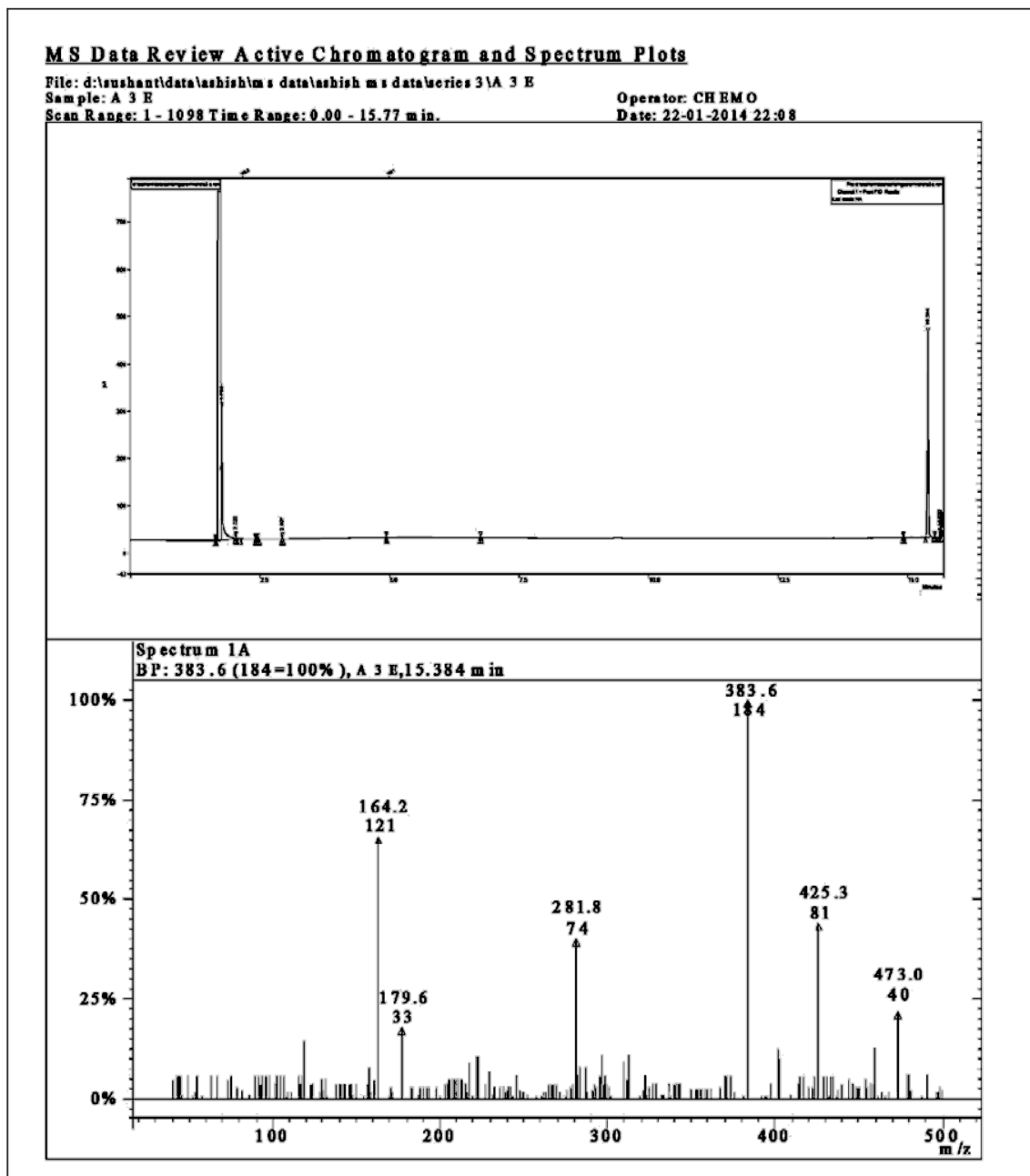
3.4.4.5.4 Spectrum 37: Mass spectra of III-C.4d



3.4.4.5.4 Table No 96: Mass spectral data of III-C.4d

m/z values	Peak
459	Molecular ion peak
385	Base peak

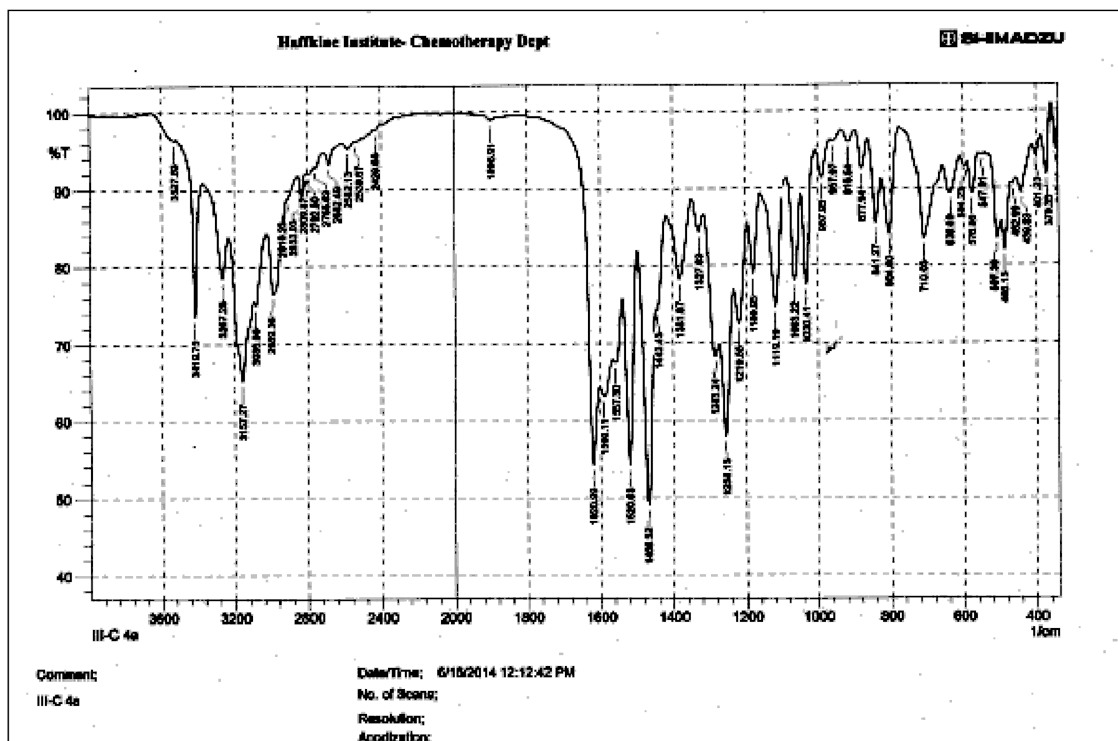
3.4.4.5.5 Spectrum 38: Mass spectra for III-C.4e



3.4.4.5.5 Table No 97: mass spectral data of III-C.4e

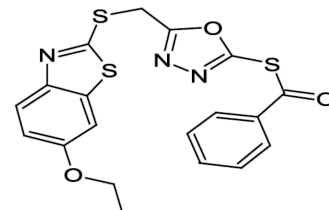
m/z values	Peak
473	Molecular ion peak
383	Base peak

3.4.4.6.1 Spectrum 39: IR spectra of III-C.4a

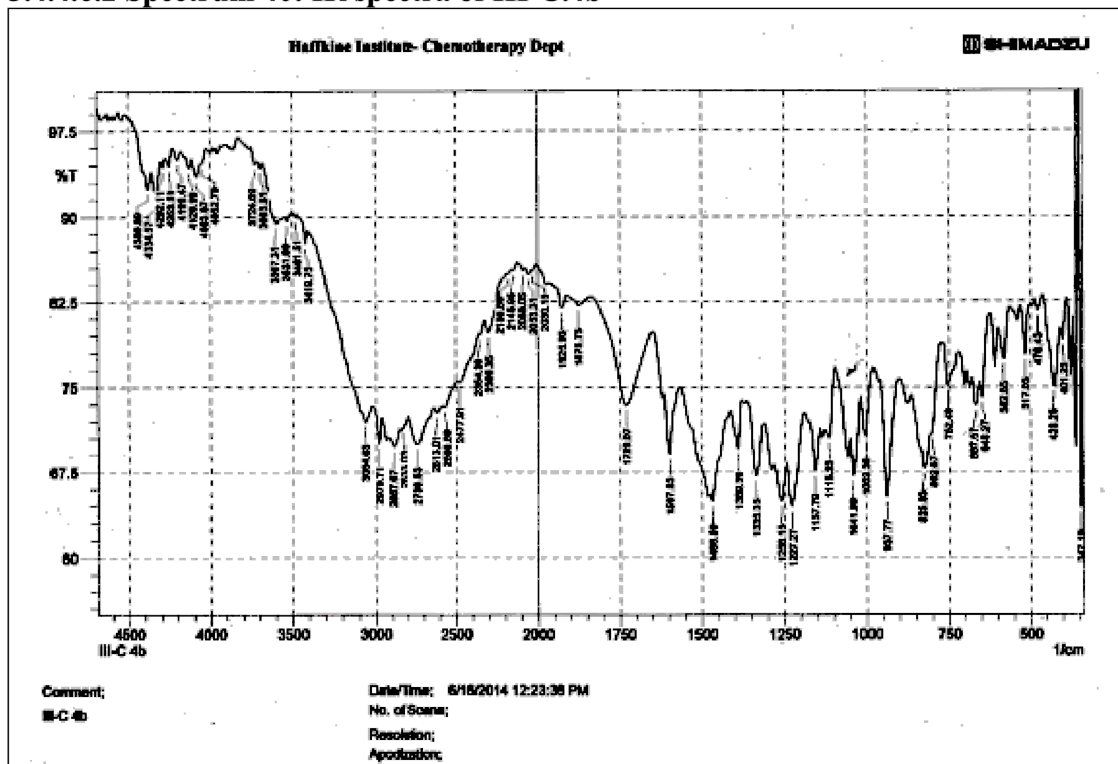


3.4.4.6.1 Table No 98: IR spectral data of III-C.4a

Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
-O- bending	1119	1000-1320
C-O bending	1219	1210-1320
=C-H & =CH ₂ bending	987	880-995
-N= tert. Amine bending	1258	1000-1275

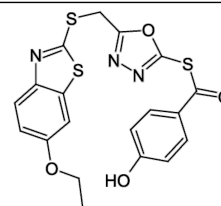


3.4.4.6.2 Spectrum 40: IR spectra of III-C.4b

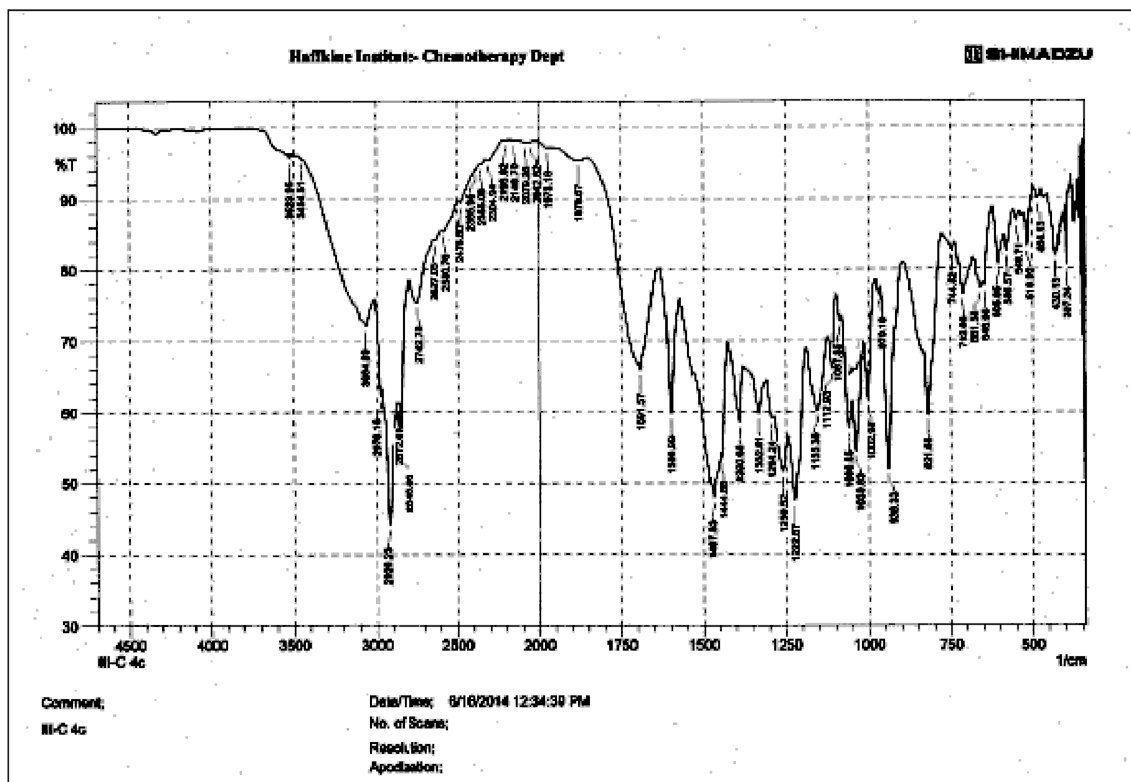


3.4.4.6.2 Table No 99: IR spectral data of III-C.4b

Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
-O- bending	1041	1000-1320
C-O bending	1258	1210-1320
=C-H & =CH ₂ bending	937	880-995
-OH stretching	3419	3200-3550



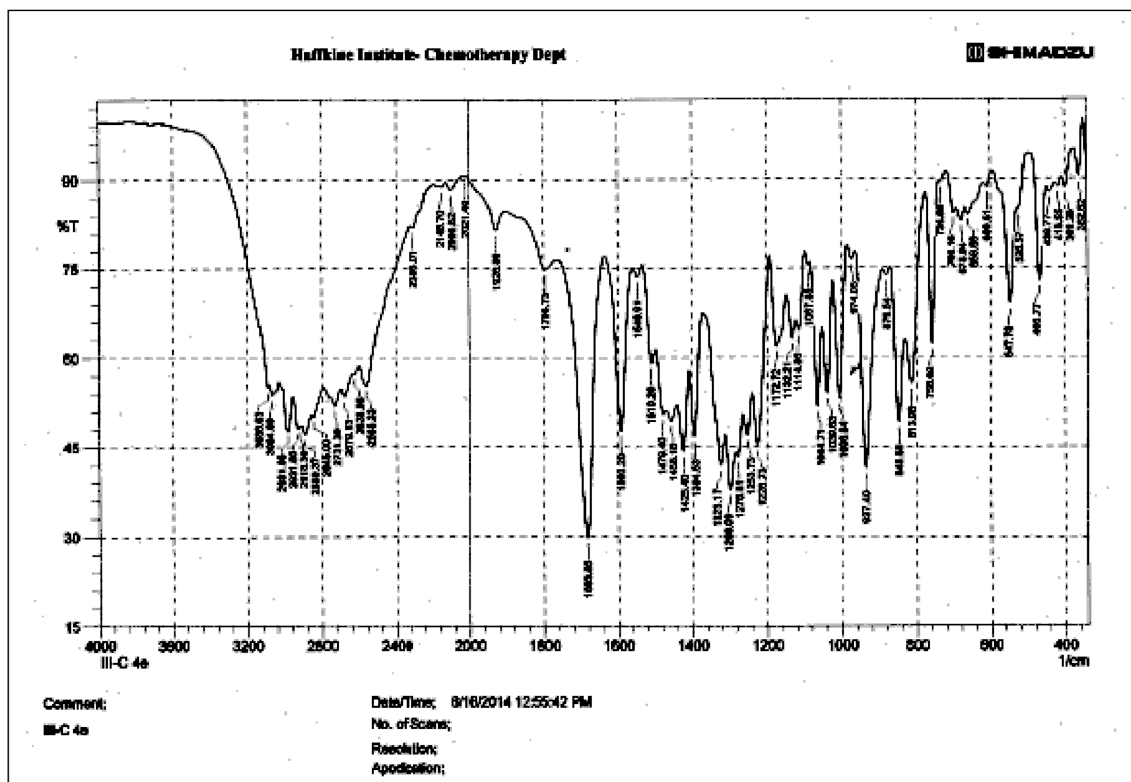
3.4.4.6.3 Spectrum 41: IR spectra of III-C.4c



3.4.4.6.3 Table No 100: IR spectral data of III-C.4c

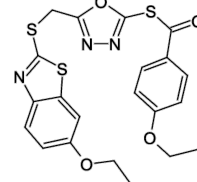
Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
-O- bending	1039	1000-1320
C-O bending	1259	1210-1320
=C-H & =CH ₂ bending	939	880-995
-Cl bending	713	700-800

3.4.4.6.5 Spectrum 43: IR spectra of III-C.4e

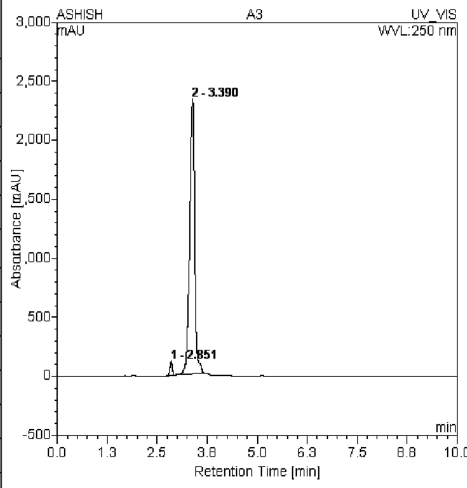


3.4.4.6.5 Table No 102: IR spectral data of III-C.4e

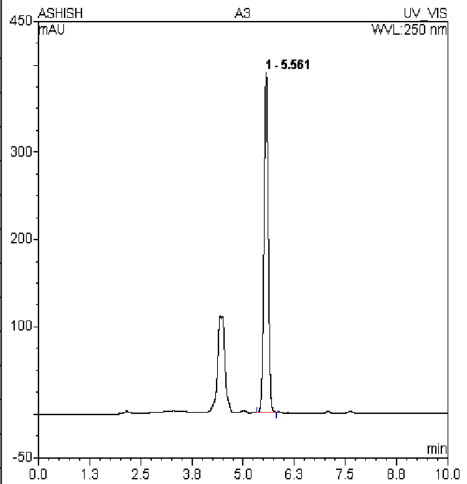
Functional Group	Peak (cm^{-1})	Standard IR Range (cm^{-1})
-O- bending	1064	1000-1320
C-O bending	1253	1210-1320
=C-H & =CH ₂ bending	937	880-995
C-S bending	1226	1030-1275



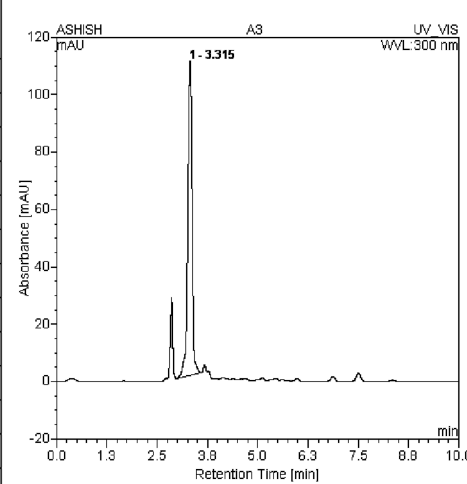
3.4.4.7.1 Chromatogram 11: HPLC data for III-C.4a

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Water (30%)	
Mobile phase B	Acetonitrile (70%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	250 nm	
Sample concentration	10 ppm	
Retention Time	3.3 mins	
Peak intensity	2330	
Theoretical Plates	4107	
Asymmetry	0.866	
Peak Width	0.201	

3.4.4.7.2 Chromatogram 12: HPLC data for III-C.4b

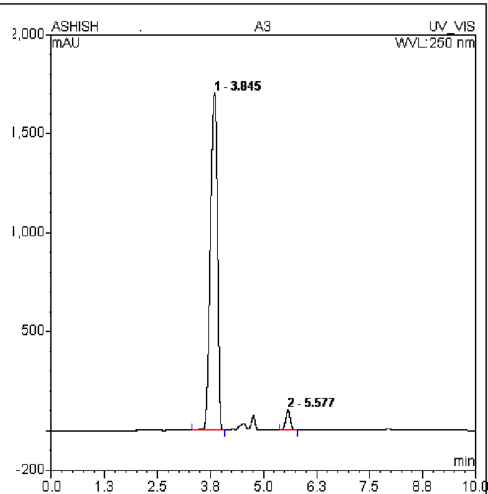
COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Water (30%)	
Mobile phase A	Acetonitrile (70%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	250 nm	
Sample concentration	10 ppm	
Retention Time	5.5	
Peak intensity	390	
Theoretical Plates	13782	
Asymmetry	0.99	
Peak Width	0.19	

3.4.4.7.3 Chromatogram 13: HPLC data for III-C.4c

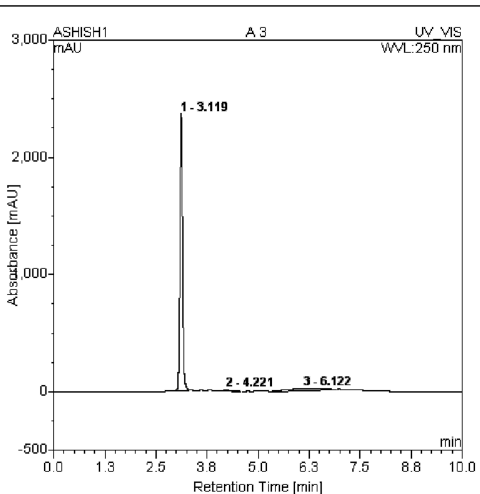
COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Water (30%)	
Mobile phase A	Acetonitrile (70%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	300 nm	
Sample concentration	10 ppm	
Retention Time	3.3mins	
Peak Intensity	109	
Theoretical Plates	4739	
Asymmetry	0.85	
Peak Width	0.18	

3.4.4.7.4 Chromatogram 14: HPLC data for III-C.4d

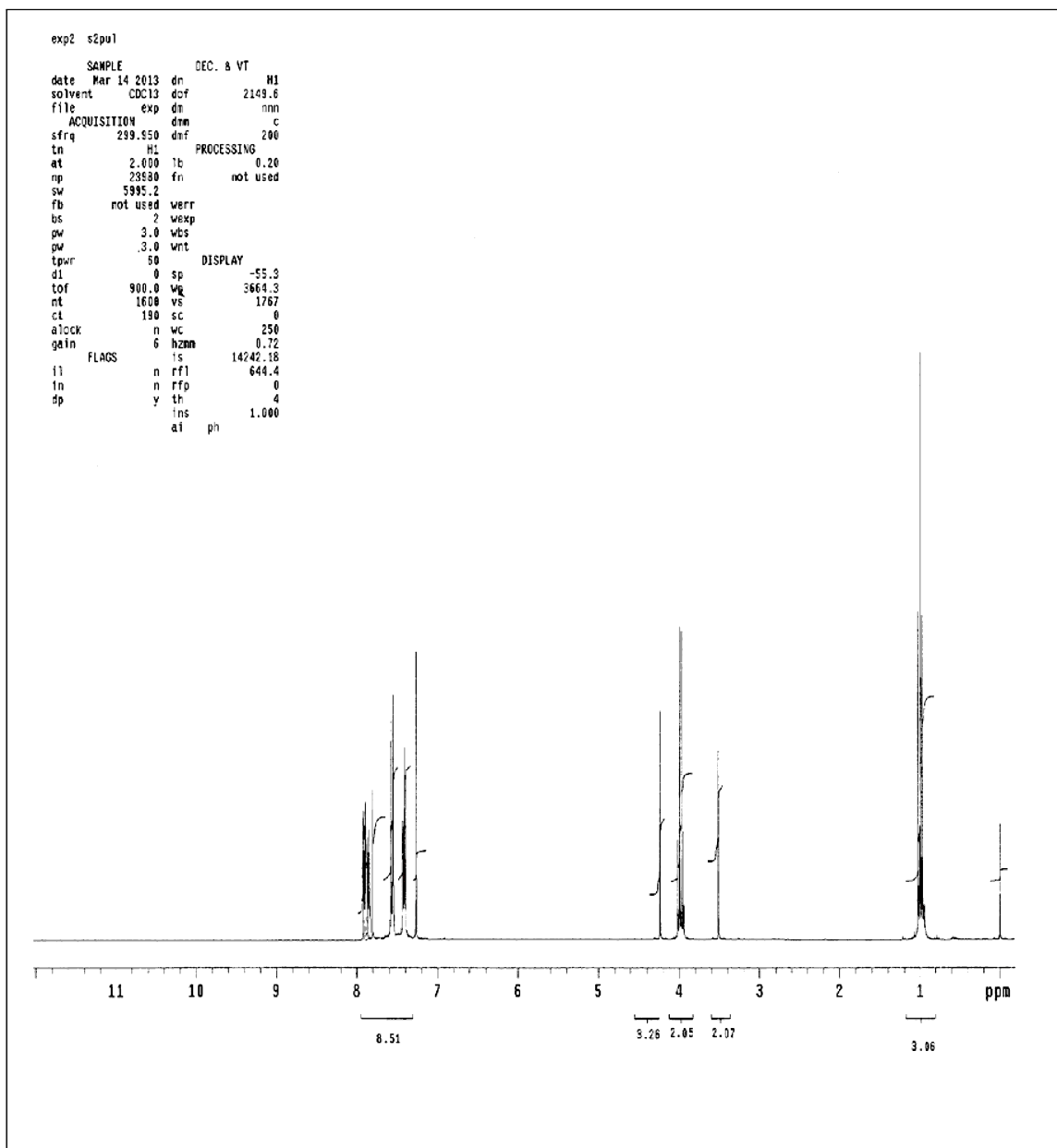
COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)
Mobile phase A	Water (30%)
Mobile phase A	Acetonitrile (70%)
Pump mode	Isocratic
Flow rate	1.0mL/min
Injection volume	10 μ L
Column temperature	25 $^{\circ}$ C
Wavelength	250 nm
Sample concentration	10 ppm
Retention time	3.85
Peak Intensity	1702
Theoretical Plates	2257
Asymmetry	0.84
Peak Width	0.302

**3.4.4.7.5 Chromatogram 15: HPLC data for III-C.4e**

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)
Mobile phase A	Water (20%)
Mobile phase A	Acetonitrile (80%)
Pump mode	Isocratic
Flow rate	1.0mL/min
Injection volume	10 μ L
Column temperature	25 $^{\circ}$ C
Wavelength	250 nm
Sample concentration	10 ppm
Retention Time	3.1mins
Peak Intensity	2364
Theoretical Plates	10591
Asymmetry	1.08
Peak width	0.121



3.4.4.8 Spectrum 44: NMR spectra of III-C.4d



3.4.4.8 Table No. 103: NMR spectral data of III-C.4d

δ values	^1H NMR signal
3.5	s, 2H, CH_2
1.0	t, 3H, CH_3
4.1	s, 3H, CH_3
4.0	q, 2H, CH_2
7-8	m, 7H, Ar-H

3.5.1.0 III-D.4 Synthesis of 4-[(*E*)-(substituted-4-oxo-4*H*-chromen-3-yl) methyldene]amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide

III-D includes following steps

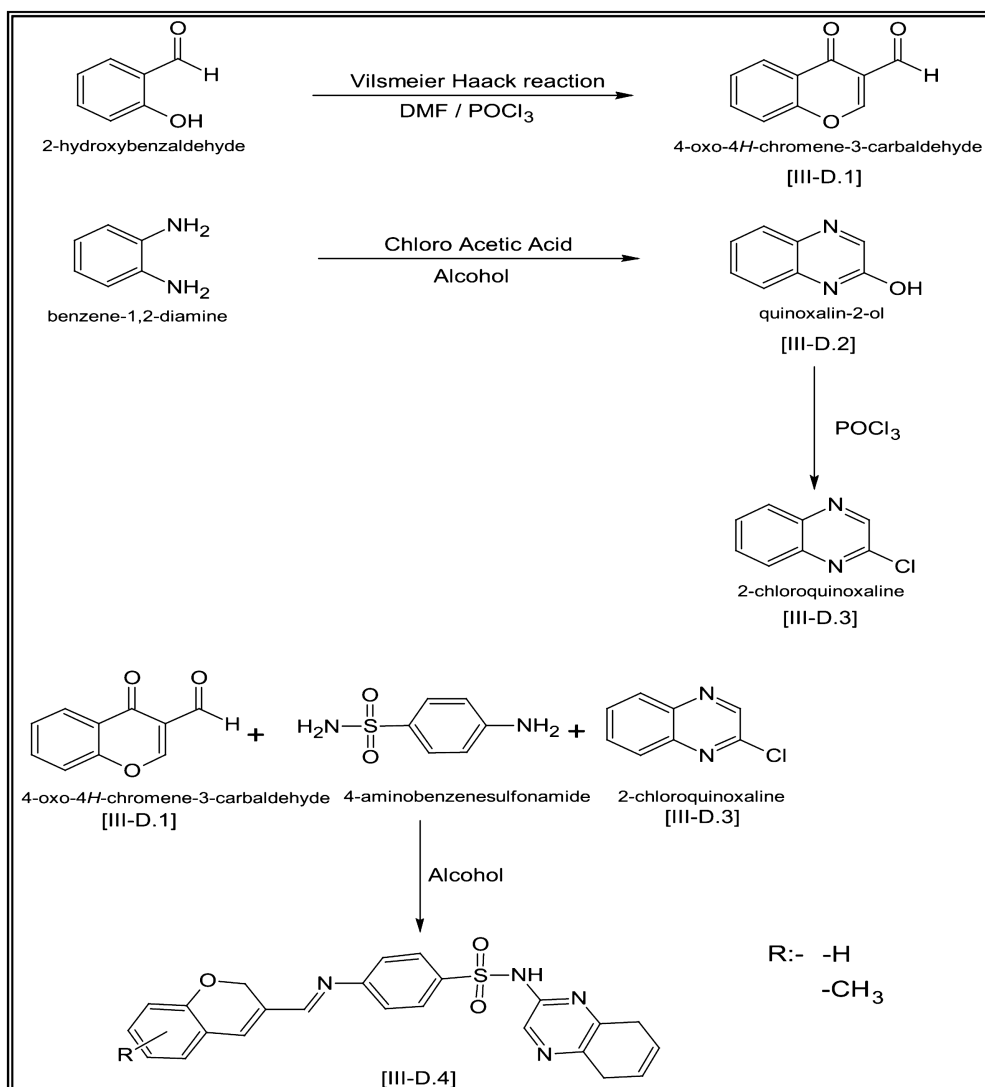
3.5.1 III-D.1 Synthesis of 4-oxo-4*H*-chromene-3-carbaldehyde

3.5.2 III-D.2 Synthesis of quinoxalin-2-ol

3.5.3 III-D.2 Synthesis of 2-chloroquinoxaline

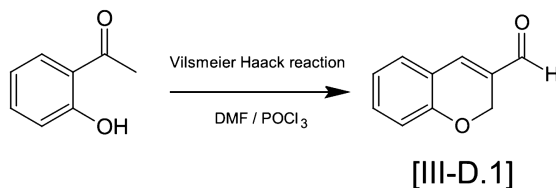
3.5.4 III-D.4 Synthesis of 4-[(*E*)-(6-methyl-4-oxo-4*H*-chromen-3-yl) methyldene] amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide

Fig no 79: Schematic Representation III-D



3.5.1.0. III-D.1 Synthesis of 4-oxo-4H-Chromene-3-Carbaldehyde^[167-68]

3.5.1.1 Fig no 80: Synthesis of III-D.1



3.5.1.2 Table No 104: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
2-OH acetophenone	122	1.22	0.01	1
Dimethyl formamide	--	10	--	--
Phosphoryl oxychloride	153	1.53	0.01	1

3.5.1.3 Procedure: -

2-Hydroxy acetophenone was dissolved in DMF at 0-5⁰C. To this solution DMF/POCl₃ was added and keep it for stirring till completion of reaction. Reaction was monitored by TLC. After completion of reaction neutralize the pH of reaction mixture. Filtered the product wash with water. Recrystallized the product by Ethyl alcohol. Similarly other derivatives were prepared.

3.5.1.4 Table No 105: Observation table

Melting point	153 ⁰ C	
Yield	48%	
Colour	White	
Nature	Crystalline	
TLC system	(THF: methanol) (8: 2)	

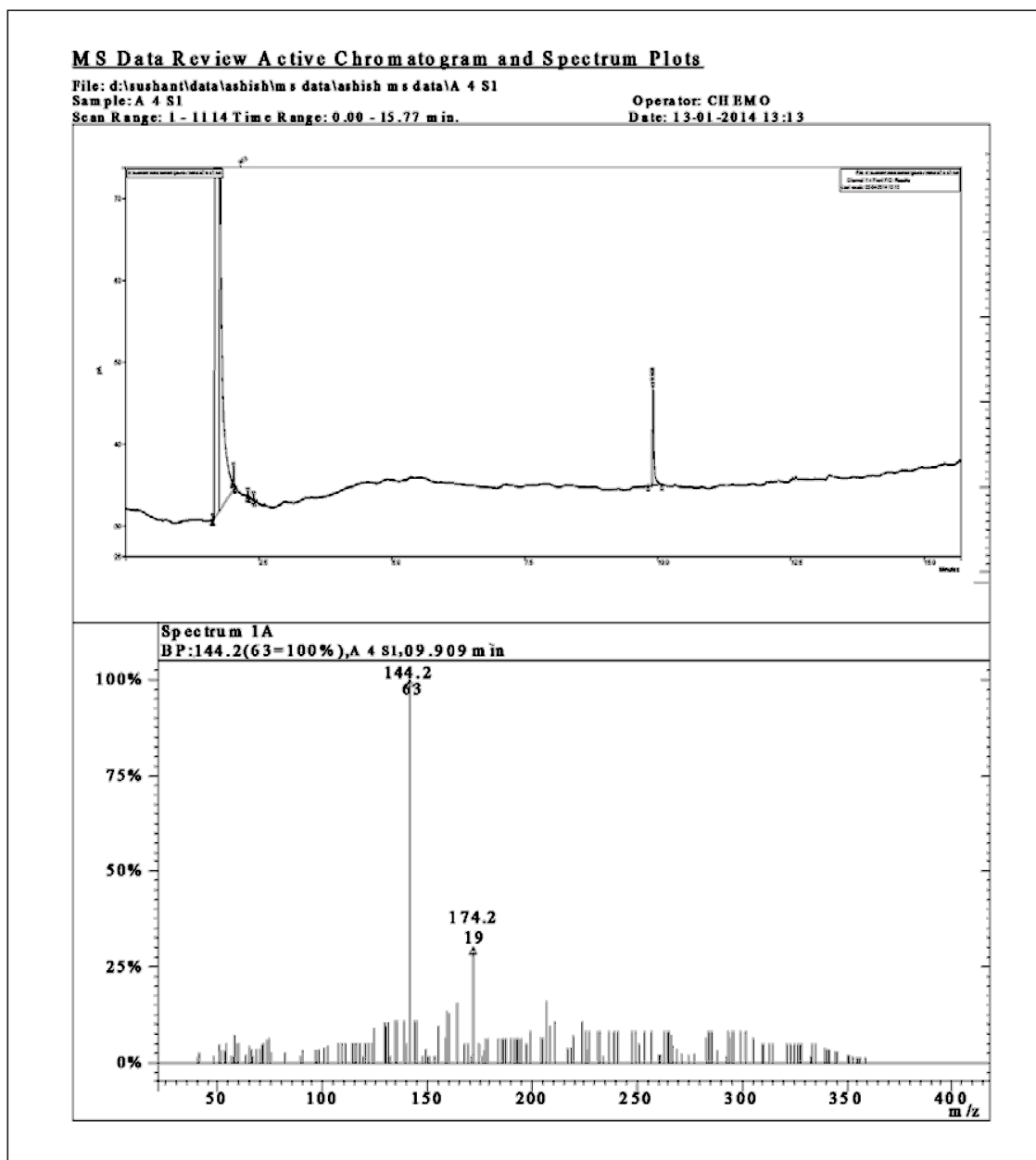
3.5.1.5 Table No 106: Chemical test: Aldehyde test

Sr. no.	Test	Observation	Inference
1.	Silver mirror test	Mirror observed	Aldehyde present

3.5.1.6 Table No 107: GCMS method for III-D.1

GC conditions			
Column section	HP-5MS column (30m X 250μm X 0.25μm)		
Oven temperature	Rate (⁰ C/ min)	Temperature (⁰ C)	Hold time (min)
	0	45	1
	20	300	2
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 ⁰ C	Detector temp	300 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time		15.77 mins
	Retention time		9.909 mins

3.5.1.7 Spectrum 45: MASS SPECTRA OF III-D.1

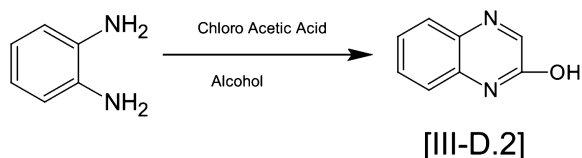


3.5.1.7 Table No 108: Mass spectral data of III-D.1

m/z values	Peak
174	Molecular ion peak
144	Base peak

3.5.2.0. III-D.2 Synthesis of quinoxalin-2-ol^[169]

3.5.2.1 Fig no 81: Synthesis of III-D.2



3.5.2.2 Procedure: -

Benzene-1,2-diamine was dissolved in alcohol, reflux the reaction mixture till it forms a clear solution. To this clear solution chloroacetic acid was added and reflux the reaction mixture. Reaction was monitored by TLC. After completion of reaction, neutralized the pH of reaction mixture. Filter the product and wash with water. Recrystallized the product by Ethyl alcohol.

3.5.2.3 Table No 109: Reaction Calculation table

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
Benzene-1,2-diamine	108	1.08	0.01	1
Chloroacetic acid	94	0.94	0.01	1
Alcohol	--	15	--	--

3.5.2.4 Table No 110: Observation table

Melting point	189 ⁰ C	
Yield	61%	
Colour	White	
Nature	Crystalline	
TLC system	(THF: Heptane) (8: 2)	

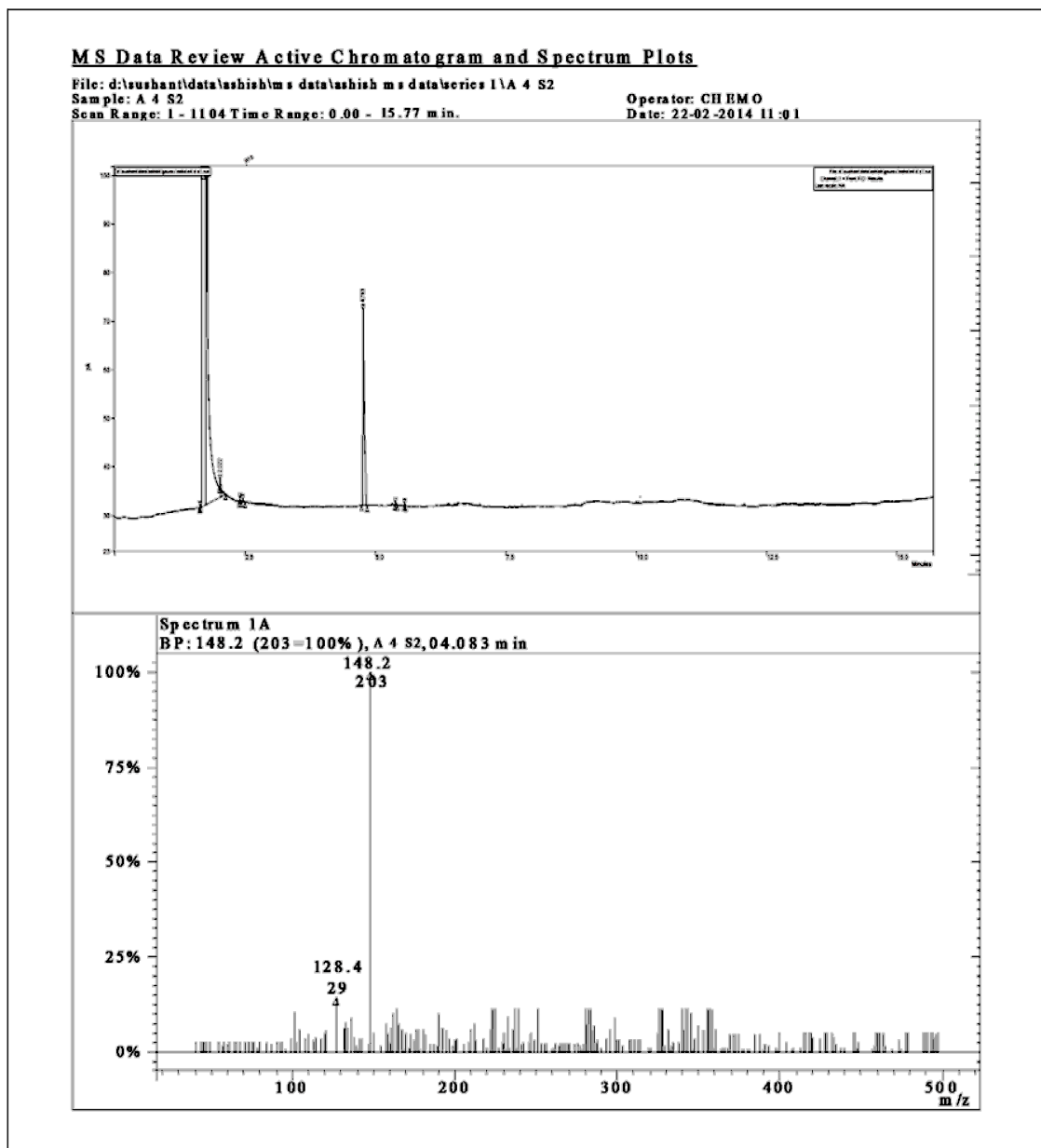
3.5.2.5 Table No 111: Chemical test: -OH group test

Sr. no.	Test	Observation	Inference
1.	Liebermann test	Red coloration	-OH present

3.5.2.6 Table No 112: GCMS method for III-D.2

GC conditions			
Column section	HP-5MS column (30m X 250µm X 0.25µm)		
Oven temperature	Rate (⁰ C/ min)	Temperature (⁰ C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 ⁰ C	Detector temp	280 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time		15.77 mins
	Sample elution time		4.083 mins

3.5.2.6 Spectrum 46: MASS SPECTRA OF III-D.2

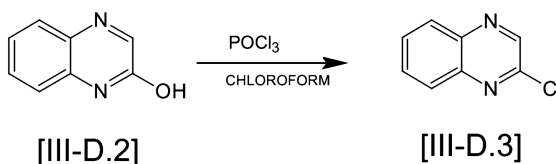


3.5.2.6 Table No 113: mass spectral data of III-D.2

m/z values	Peak
148	Molecular ion peak
148	Base peak

3.5.3.0. III-D.3 Synthesis of 2-chloroquinoxaline^[170]

3.5.3.1 Fig no 82: Synthesis of III-D.3



3.5.3.2 Procedure: -

Crystallized product of III-D.2 was dissolved in chloroform, reflux the reaction mixture till it forms clear solution. To this clear solution POCl_3 was added leading to the formation of product. Reaction was monitored by TLC. After completion of reaction neutralized the pH of reaction mixture. Recrystallized the product by chloroform.

3.5.3.3 Table No 114: Reaction Calculation table:

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
III-D.2	147	1.47	0.01	1
Phosphorus oxychloride	156	1.56	0.01	1
Chloroform	--	15	--	--

3.5.3.4 Table No 115: Observation table:

Melting point	214 ⁰ C	
Yield	55%	
Colour	Brown	
Nature	Amorphous	
TLC system	(THF: Heptane) (8: 2)	

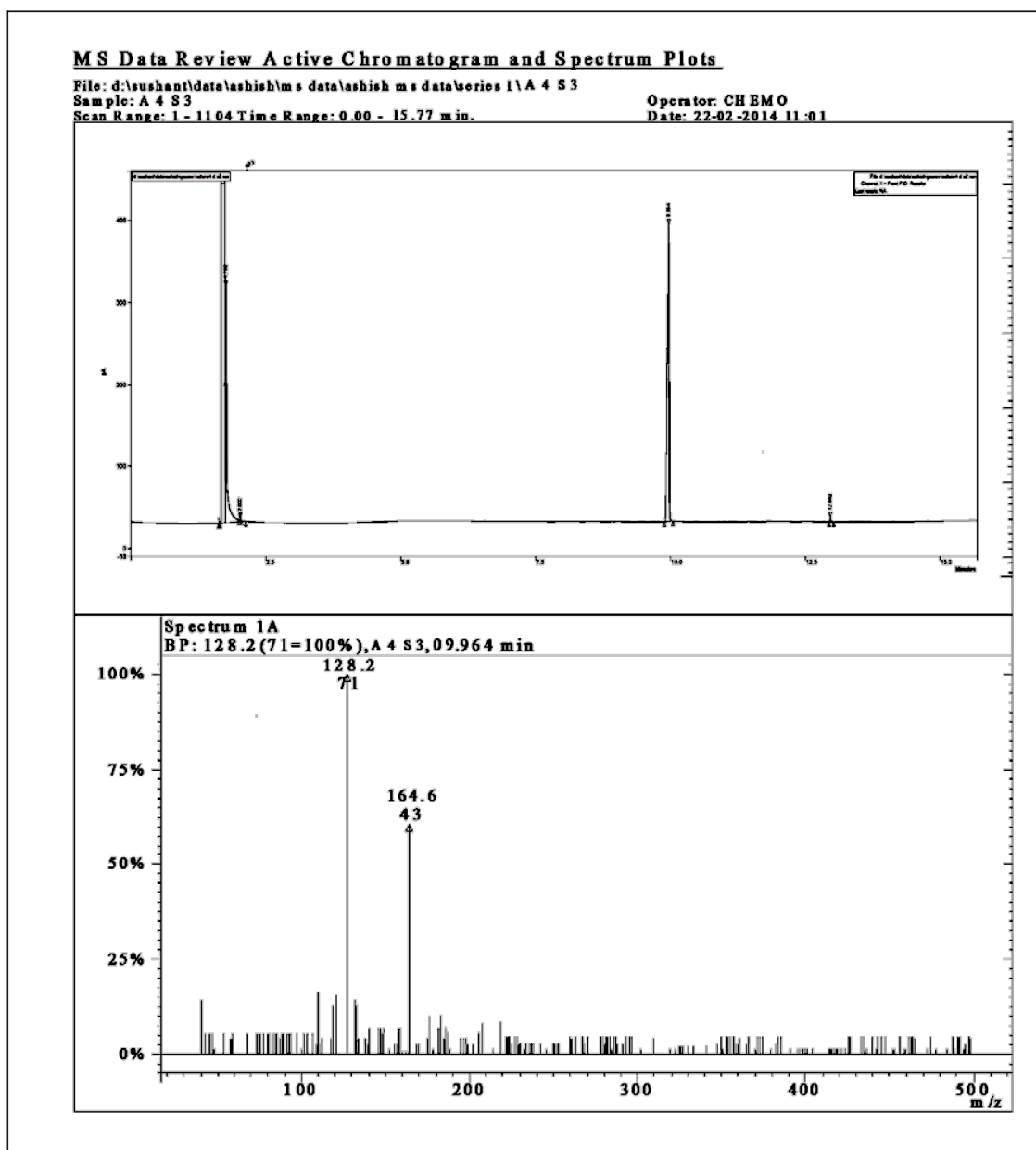
3.5.3.5 Table No 116: Chemical test: chlorine test

Sr. no.	Test	Observation	Inference
1.	Lassaignes test	Colourless layer	Chlorine present

3.5.3.6 Table No 117: GCMS method for III-D.3

GC conditions			
Column section	HP-5MS column (30m X 250 μ m X 0.25 μ m)		
Oven temperature	Rate (⁰ C/ min)	Temperature (⁰ C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 ⁰ C	Detector temp	300 ⁰ C
MS condition			
Ion source temperature	280 ⁰ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-500
Analysis time	Run time		15.77 mins
	Sample elution time		9.964 mins

3.5.3.6 Spectrum 47: MASS SPECTRA OF III-D 3

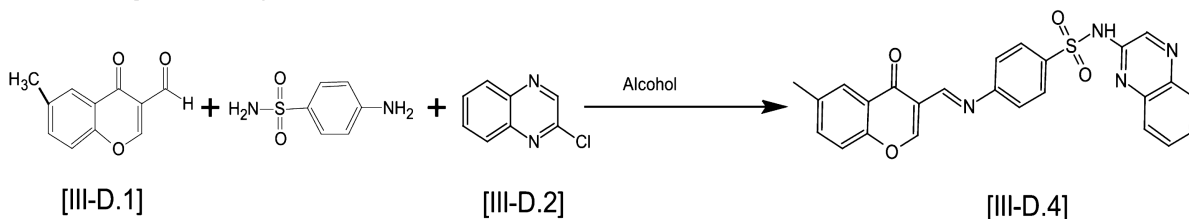


3.5.3.6 Table No 118: mass spectral data of III-D.3

m/z values	Peak
164	Molecular ion peak
128	Base peak

3.5.4.0 III-D.4 Synthesis of 4-[(*E*)-(6-methyl-4-oxo-4*H*-chromen-3-yl) methylidene] amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide^[171]

3.5.4.1 Fig no 83: Synthesis of III-D.4



3.5.4.2 Procedure: -

Crystallized product of III-D.1 was dissolved in alcohol reflux the reaction mixture till it forms clear solution. To this clear solution crystallized III-D.3 and sulphanilamide was added and reflux the reaction mixture. Reaction was monitored by TLC. After completion of reaction, evaporate the solvent and separate the product. Recrystallization was carried out by Ethyl alcohol.

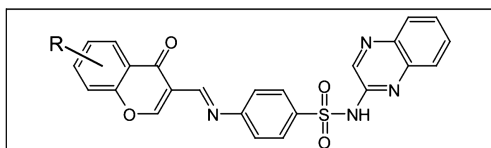
3.5.4.3 Table no 119: Reaction Calculation table:

Compound	Mol. wt.	Wt. gm/mL	Mole	Mole ratio
Step III product	164	1.64	0.01	1
Sulphanilamide	172	1.72	0.01	1
Step I product	174	1.74	0.01	1
Alcohol	--	20	--	--

3.5.4.4 Table No 120: Observation table:

Melting point	168 ⁰ C	
Yield	48%	
Colour	Pale yellow	
Nature	Crystalline	
TLC system	(THF: Heptane) (8: 2)	

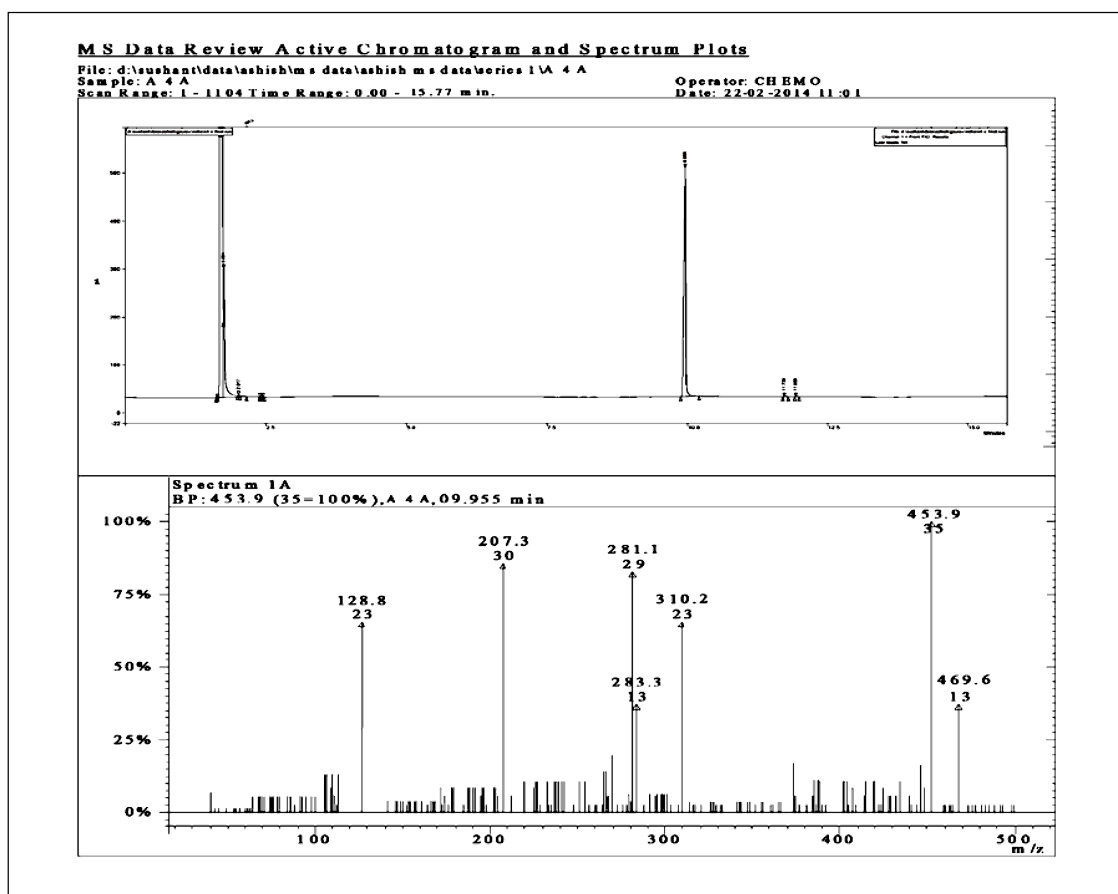
3.5.4.10 Table no 121: Physical data of synthesized compounds:



Sr. No	R	Code	Mol. wt.	Emp. For.	Colour	Solubility	MP (⁰ C)	Yield (%)
1	3-CH ₃	III-D.4a	470	C ₂₅ H ₁₈ N ₄ O ₄ S	Pale yellow	Methanol	168	55
2	-H	III-D.4b	456	C ₂₄ H ₁₆ N ₄ O ₄ S	Pale yellow	Methanol	153	48

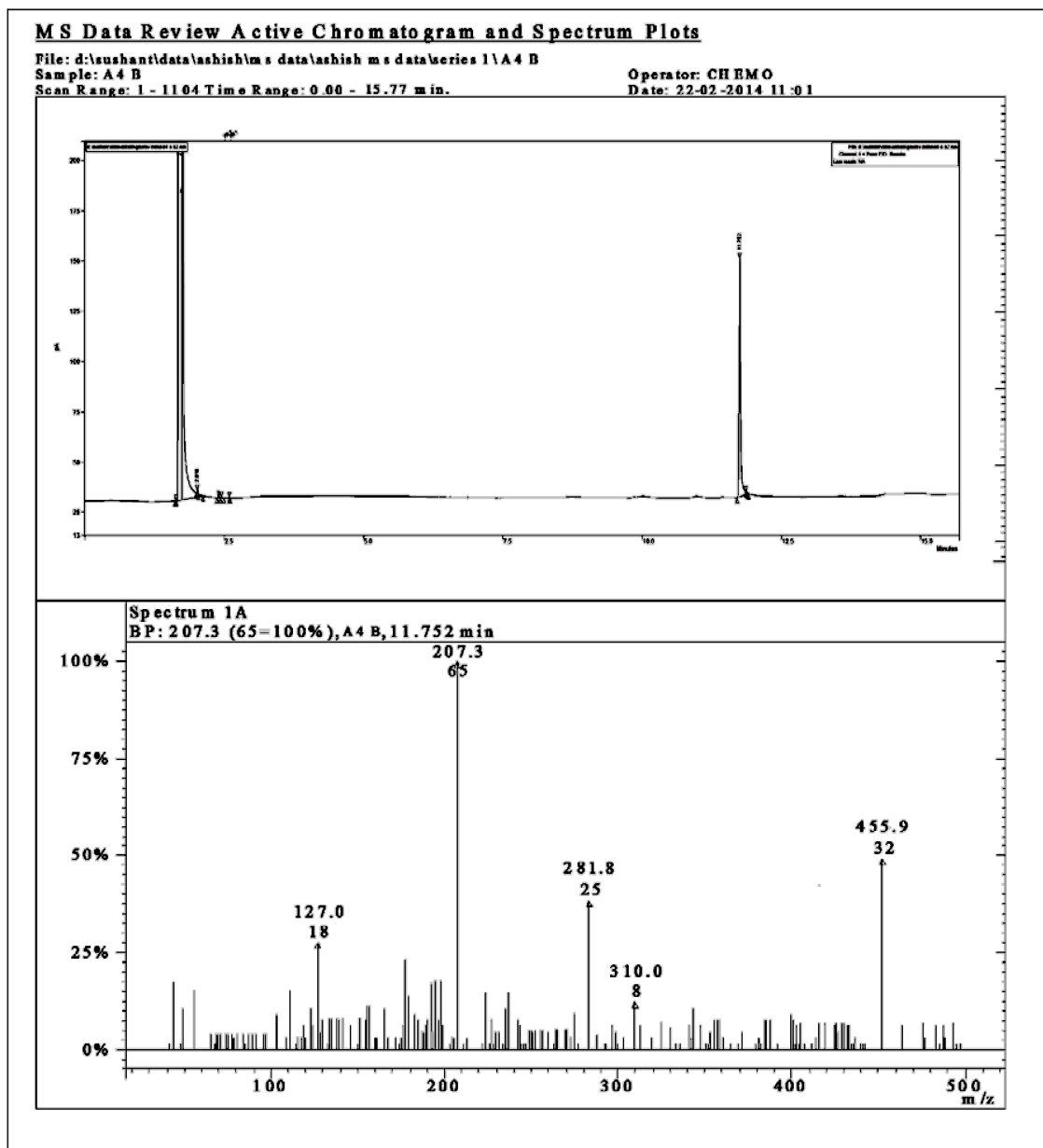
3.5.4.5 Table No 122: GCMS method for III-D.4a-b

GC conditions			
Column section	HP-5MS column (30m X 250 μ m X 0.25 μ m)		
Oven temperature	Rate ($^{\circ}$ C/ min)	Temperature ($^{\circ}$ C)	Hold time (min)
	0	145	1
	20	300	7
Injection mode	Split mode; 90:1	Carrier gas	Helium gas
Injection temperature	300 $^{\circ}$ C	Detector temp	300 $^{\circ}$ C
MS condition			
Ion source temperature	280 $^{\circ}$ C		
MS source	EI 70 eV	MS scan mode	EI Full scan m/z 40-400
Analysis time	Run time		15.77 mins
	Retention time III-D.4a		9.955 mins
	Retention time III-D.4b		11.752 mins

3.4.4.6.1 Spectrum 48: MASS SPECTRA OF III-D 4a**3.5.4.6.1 Table No 123: Mass spectral data of III-D.4a**

m/z values	Peak
469	Molecular ion peak
453	Base peak

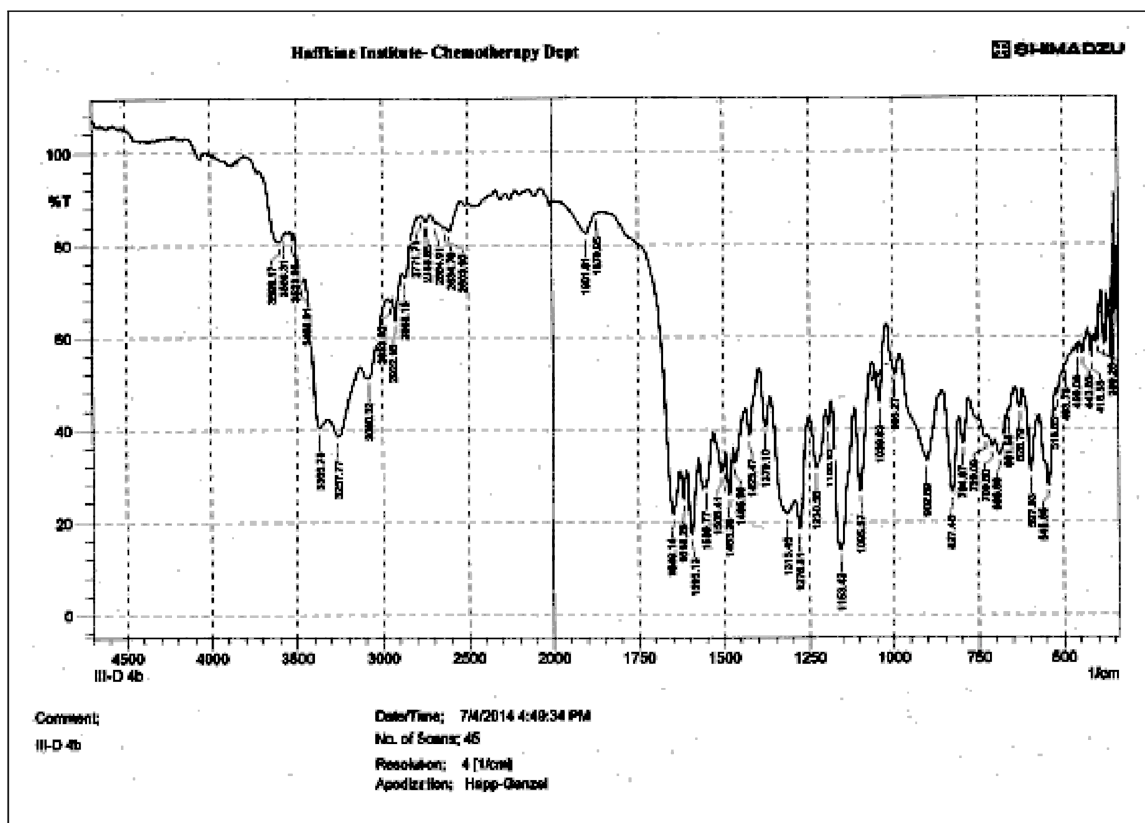
3.5.2.6.2 SPECTRUM 49: mass spectral data of III-D.4b



3.5.4.6.2 Table No 124: Mass spectral data of III-D.4b

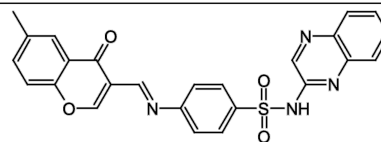
m/z values	Peak
455	Molecular ion peak
207	Base peak

3.5.4.7.1 Spectrum 49: IR spectra of III-D.4a

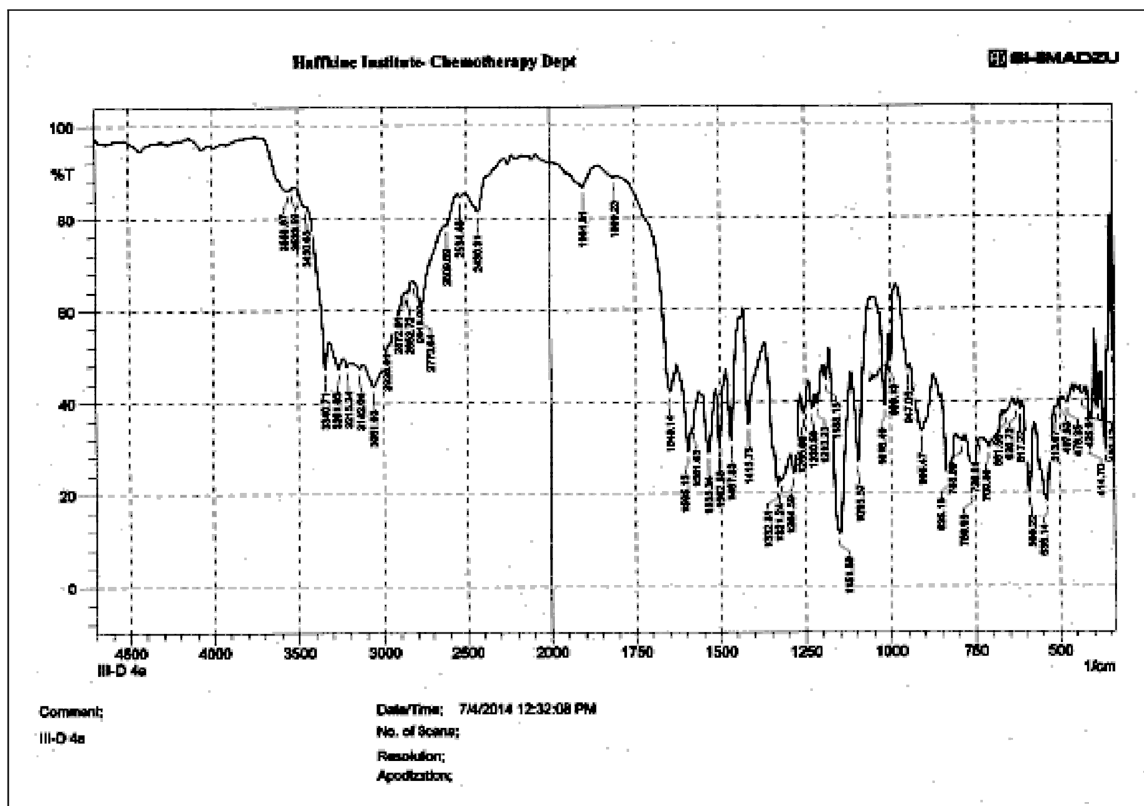


3.5.4.7.1 Table no 125: IR spectral data of III-D.4a

Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
N=H (Sec Amine) stretching	3365	3300-3400
S=O bending	1153	1155-1170
-O- bending	1095	1070-1150

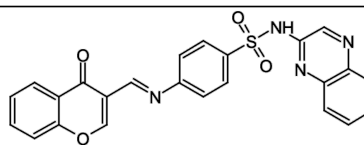


3.5.4.7.2 Spectrum 50: Fig: IR spectra of III-D.4b

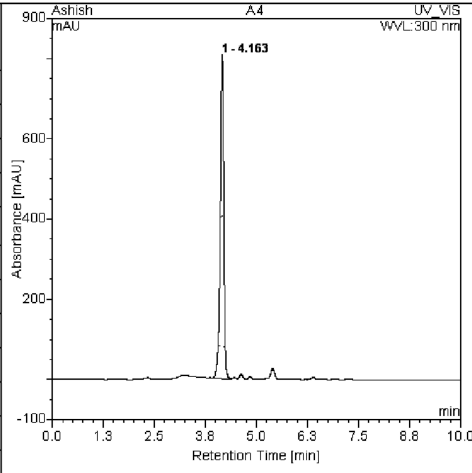


3.5.4.7.2 Table No 126: IR spectral data of III-D.4b

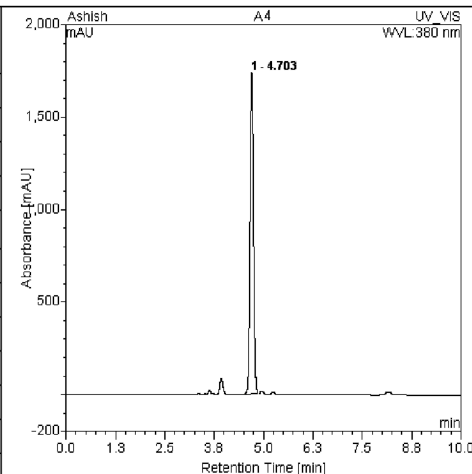
Functional Group	Peak (cm ⁻¹)	Standard IR Range (cm ⁻¹)
N-H (Sec Amine) stretching	3340	3300-3400
S=O bending	1151	1155-1170
-O- bending	1095	1070-1150



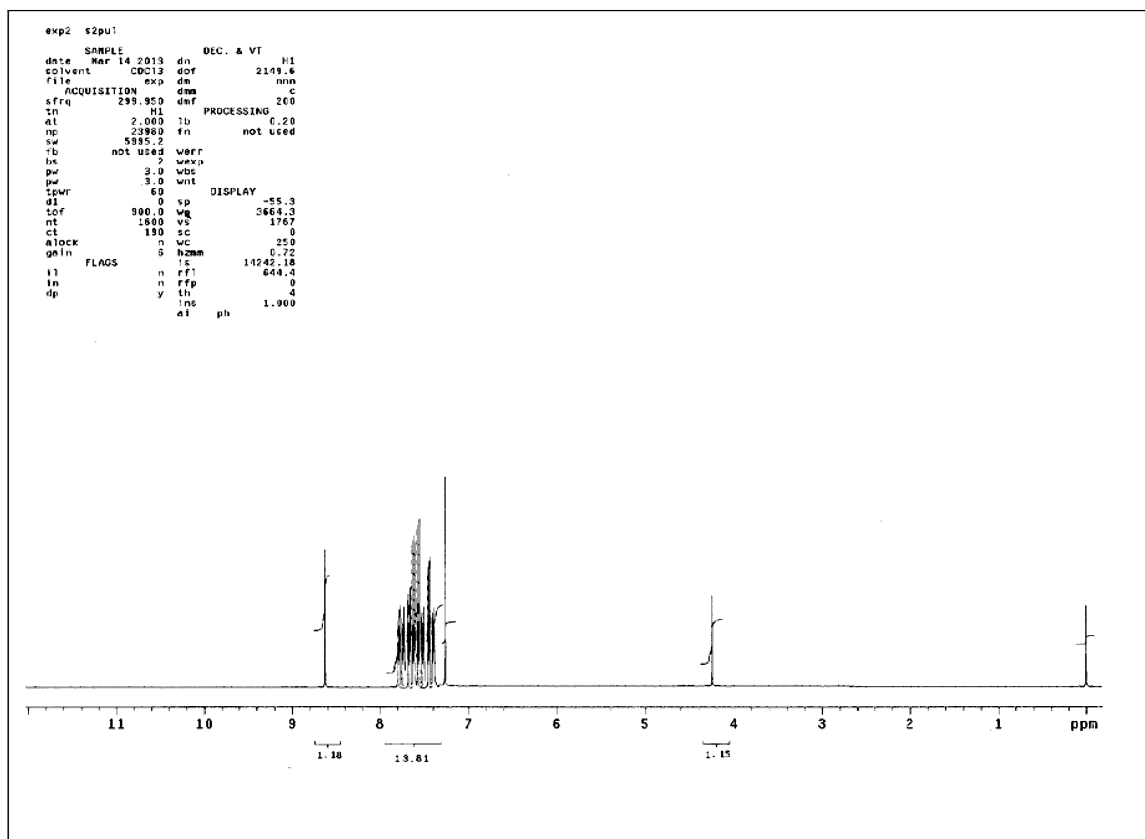
3.5.4.8.1 Chromatogram 16: HPLC data for III-D.4a

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Methanol (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	300 nm	
Sample concentration	10 ppm	
Retention Time	4.1 mins	
Peak intensity	806	
Theoretical Plates	13134	
Asymmetry	0.84	
Peak Width	0.147	

3.5.4.8.2 Chromatogram 17: HPLC data for III-D.4b

COLUMN	THERMO BDS C18 (300mm X 4.5 mm X 5 μ m)	
Mobile phase A	Methanol (100%)	
Pump mode	Isocratic	
Flow rate	1.0mL/min	
Injection volume	10 μ L	
Column temperature	25 $^{\circ}$ C	
Wavelength	380 nm	
Sample concentration	10 ppm	
Retention Time	4.7 mins	
Peak intensity	1737	
Theoretical Plates	13113	
Asymmetry	1.03	
Peak Width	0.16	

3.5.4.9 Spectrum No 52: NMR SPECTRA OF III-D.4a



3.5.4.9 Table No 127: NMR interpretation of III-D.4a

δ values	^1H NMR signal
4.2	s, 1H, NH
8.6	s, 1H, N=CH
7-8	m, 12H, Ar-H

4.0 BIOLOGICAL SCREENING

The development in chemotherapy of synthetic drugs totally depends upon the biological results of the chemotherapeutic agents, which ultimately give the guidelines to the research worker in the selection of agents for further clinical studies and set the pattern for future clinical studies and set the pattern of future drug evaluation.

Taking this into consideration the above fact, in our present work, different heterocyclic derivatives, derived from different biological important moieties, were prepared and were screened for their *in vitro* antibacterial activity against gram negative bacteria viz.

- *Escherichia coli* (ATCC 25992)
- *Salmonella typhi* (NCTC 786 MRSA)
- *Pseudomona. aeruginosa* (ATCC 15442)
- *Klebsiella aeruginosa*(Clinical isolate)
- *Vibrio Chlorae*

***In vitro* antifungal activity against:-**

- *Candida albican* (ATCC 10231)
- *Aspergillus niger* (ATCC 16404)

Details involved in the screening program is elaborated in the following section:

- **Drug Stock Solution:**

0.01 gm (10µg) of drug sample dissolved in 10ml Dimethyl Sulphoxide (DMSO) to give stock solution of 100µg/ml.

Serial dilutions of drug samples can be prepared as follows:

2 ml drug stock solution + 8 ml M.H. broth - 200 µg

1 ml drug stock solution + 9 ml M.H. broth - 100 µg

0.5 ml drug stock solution + 9.5 ml M.H. broth - 50 µg

0.4 ml drug stock solution + 9.6 ml M.H. broth - 40 µg

0.2 ml drug stock solution + 9.8 ml M.H. broth - 20 µg

0.1 ml drug stock solution + 9.9 ml M.H. broth - 10 µg

Controls:

1. Media Control: Sterilized medium was kept as a control so as to assure the sterility of the medium. If this control shows growth of any type then results are considered invalid.

2. Culture Control: It contains sterilized medium and culture of the organism. It should show growth in the medium, which assures optimum pH and sufficient nutrients to promote growth. If no growth is observed, then the medium is considered to be faulty and results are not reliable.

3. Solvent Control: It contains sterilized medium, solvent used to dissolve the drug and the suspended culture in it. Organism should show growth in the solvent used for preparation of the drug stock solution. If the growth is not observed, it can be concluded that solvent inhibits the growth of the organism and hence in that case results are considered invalid.

4. Turbidity Control: It contains medium and the drug stock solution. The turbidity is observed against the growth in different drug dilutions. It assures reliability against misinterpretation between growth and turbidity due to the drug.

5. Standard Control: It contains medium, organism culture and dilution of the standard drug stock solution corresponding to that of the drugs under test. The inhibition of growth was observed against that in standard.

4.1 ANTIBACTERIAL SCREENING:-

The test compounds have been subjected to *in vitro* screening against all Gram negative strains of bacteria using tube dilution technique ^[172]

Muller Hinton broth was used as a culture medium. DMSO was used as a solvent for the preparation of drug stock solution. Sterilized medium was dispensed in each borosilicate glass test tube (150+20mm). The drug solution was added in order to attain final drug concentrations as 200,100,50,20,10 µg/ml etc.

Inoculums of standard suspension (0.1ml of the test organism strain which contains 10⁶ bacilli/ml) was added. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of the growth of the organism. The lowest concentration,

which showed no visible growth, was taken as the Minimum Inhibitory Concentration (MIC).

- Trimethoprim (MIC 0.01 µg/ml) used as standard against bacterial stains.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*

4.1.1 *Escherichia coli* ^[173]

• Characteristics:

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod shaped, and are about 2.0 micrometers (µm) long and 0.25-1.0 µm in diameter, with a cell volume of 0.6–0.7 µm³. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Optimal growth of *E. coli* occurs at 37 °C but some laboratory strains can multiply at temperatures of up to 49 °C. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs.

• Diseases:

Abdominal pain - typically, the first symptom is severe abdominal cramping that comes on suddenly.

Diarrhea - a few hours after the sudden abdominal pain, the patient typically has watery diarrhea. A day later there may be bright red bloody stools, caused by sores in the intestines.

Vomiting - note that many patients who become ill may not vomit.

• Source of infection:

Ingesting contaminated water - even though tap water contains chlorine and has undergone ozone or ultraviolet treatment. Ingesting contaminated food - examples include ground beef, unpasteurized milk, or fresh vegetables.

4.1.2 *Salmonella typhi* ^[174]

• Characteristics:

This gram-negative enteric bacillus belongs to the family Enterobacteriaceae. It is a motile, facultative anaerobe that is susceptible to various antibiotics. Currently, 107 strains of this organism have been isolated, many containing varying metabolic characteristics. Identification can be attained by growth on MacConkey and EMB agars,

and the bacteria is strictly non-lactose fermenting. It also produces no gas when grown in TSI media, which is used to differentiate it from other Enterobacteriaceae.

• **Source of infection:**

The encounter of humans to *S. typhi* is made via fecal-oral route from infected individuals to healthy ones. The entry of this bacterial species into the human body is most commonly achieved by ingestion, with the importance of aerosol transmission unknown. Transmission of *S. typhi* has only been shown to occur by fecal-oral route, often from asymptomatic individuals.

• **Diseases:**

The two main diseases caused by these bacteria are:

- **Salmonellosis:** Caused by *Salmonella enteritidis* or *Salmonella typhimurium*. Since it is associated with animals, it is usually a food borne disease, spread through consumption of contaminated foods, especially eggs and poultry.
- **Typhoid Fever:** Typhoid fever is caused by *Salmonella typhi*. Also spread through ingestion of contaminated food or drink, this species is much less common.

4.1.3 *Pseudomonas aeruginosa*^[175]

• **Characteristics:**

Pseudomonas. Aeruginosa are shaped, aerobic gram with one or more polar flagella, providing motility. They are non-spore forming showing positive catalase test and positive oxidase test., the absence of gas formation from glucose, glucose is oxidized in oxidation/fermentation test using Hugh and Leifson O/F test, beta hemolytic (on blood agar), indole negative, methyl red negative, Voges-Proskauer test negative, and citrate positive. Other characteristics that tend to be associated with *Pseudomonas* species include secretion of pyoverdine, a fluorescent yellow-green siderophore.

• **Diseases:**

Endocarditic: Fever, murmur, and positive blood culture findings; peripheral stigmata such as Roth spots, Janeway lesions, Osler nodes, splinter hemorrhages, and splenomegaly

Pneumonia: Rales, rhonchi, fever, cyanosis, retractions, and hypoxia; occasionally shock; with cystic fibrosis, clubbing, increased anteroposterior (AP) diameter, and malnutrition

Eye infections: Lid edema, conjunctival erythema and chemosis, and severe mucopurulent discharge Bacteremia: Fever, tachypnea, and tachycardia; hypotension and shock; jaundice.

• **Source of infection:**

Serious *Pseudomonas* infections usually occur in people in the hospital and/or with weakened immune systems. Infections of the blood, pneumonia, and infections following surgery can lead to severe illness and death in these people. However, healthy people can also develop mild illnesses with *Pseudomonas aeruginosa*, especially after exposure to water. Ear infections, especially in children, and more generalized skin rashes may occur after exposure to inadequately chlorinated hot tubs or swimming pools.

4.1.4 *Klebsiella pneumoniae* ^[176]

• **Characteristics**

Klebsiella spp. are Gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, belonging to the family Enterobacteriaceae. These bacteria produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test. Members of the Enterobacteriaceae family are generally facultatively anaerobic, and range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length. *Klebsiella* spp. often occur in mucoid colonies.

• **Diseases:**

K. pneumoniae – a leading cause of community-acquired and nosocomial pneumonia and lung abscesses. Infection of the upper lobe is more common. Symptoms include: fevers, chills, and leukocytosis with red currant jelly-like sputum. Rare complications include lung infection involving necrosis and sloughing of the entire lobe. *K. pneumoniae* and *K. oxytoca* – cause community-acquired meningitis and brain abscesses. Clinical symptoms include: headaches, fever, altered consciousness, seizures, and septic shock.

• **Source of infection:**

It can be transmitted by inhalation of respiratory droplets or aspiration from the upper respiratory tract to the lungs. For urinary tract infection, it usually spreads from ascending spread of fecal flora. *Klebsiella pneumonia* generally affects people with underlying diseases, such as alcoholism, diabetes and chronic lung disease. Typically, *Klebsiella pneumonia* causes a severe, rapid-onset illness that often causes destruction in the lungs. Infected people get high fever, chills, flu-like symptoms and cough.

4.1.5 *Vibrio cholerae* ^[177]

- **Characteristics:**

Vibrio Cholerae can be described as gram-negative bacilli bacteria with a “comma” shaped body and a flagellum for movement. This organism can be identified through several different methods. Inoculation and incubation overnight can be done on a TCBS. After incubation a TCBS agar plate will show yellow colonies. *Vibrio Cholerae* can be detected by an immobilization test and a hanging drop preparation will show active motility. A gram stain test will show curved gram-negative bacilli and a catalase and oxidase test will show positive results. Biochemical reactions for *Vibrio Cholerae* are urease negative, indole positive test, Citrate positive, MR positive, and VP Negative.

- **Diseases:**

A bacterium called *Vibrio Cholerae* causes cholera infection. However, the deadly effects of the disease are the result of a potent toxin, called CTX, that the bacteria produce in the small intestine. CTX binds to the intestinal walls, where it interferes with the normal flow of sodium and chloride. This causes the body to secrete enormous amounts of water, leading to diarrhea and a rapid loss of fluids and salts. Symptoms are watery diarrhea, vomiting and leg cramps.

- **Source of infection:**

Contaminated water supplies are the main source of cholera infection, although raw shellfish, uncooked fruits and vegetables, and other foods also can harbor *Vibrio Cholerae*.

Cholera bacteria have two distinct life cycles one in the environment and one in humans. A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water.

4.2 ANTIFUNGAL SCREENING

For Antifungal Screening organisms used for the test were *Candida albicans* and *Aspergillus niger* by tube dilution method.

Sabraoud's medium was used as the growth media (pH=5.6). The sterile media was dispensed in a sterile borosilicate test tube. The drug solution was added in order to get the final drug concentrations of 200, 100, 50, 20, 10, 5, 2 µg/mL etc. The tubes were incubated at room temperature (28-30°C) in a dark place. *C. albicans* and *A. niger* were incubated for 7 days. The presence or absence of the growth was observed visually. The lowest concentration at which no growth was observed was taken as the end point (MIC). Miconazole (MIC= 6.25 µg/ml against *C. albicans* and *A. niger*) was used as a standard drug.

4.2.1 *Candida albicans* ^[178]

Candida is a genus of yeasts and is currently the most common cause of fungal infections worldwide. Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease. *Candida albicans* is currently the most commonly isolated species, and can cause infections (candidiasis or thrush) in humans and other animals.

- **Source of infection:**

Candidal infections commonly occur in warm moist body areas, such as underarms. Usually your skin effectively blocks yeast, but any breakdown or cuts in the skin may allow this organism to penetrate.

- **Disease:**

Candidiasis: In adults, oral yeast infections become more common with increased age. Adults also can have yeast infections around dentures, in skin folds under the breast and lower abdomen, nailbeds, and beneath other skin folds. Most of these candidal infections are superficial and clear up easily with treatment. Infections of the nail beds often require prolonged therapy. Yeast infections that return may be a sign of more serious diseases such as diabetes, leukemia, or AIDS.

4.2.2 *Aspergillus niger* ^[179]

A. niger is a fungus and one of the most common species of the genus *Aspergillus*. It is ubiquitous in soil and is commonly reported from indoor environments. Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins, It also produces the isoflavone orobol

- **Source of Infection:**

Aspergillus species can infect people and animals and begin to grow inside them, especially in the lungs. Usually only people with weakened immune systems are susceptible to infection by *Aspergillus*. A rare fungal infection that often causes a black mould to appear on some fruit and vegetables but may also infect humans through inhalation of fungal spores.

- **Disease:**

It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food Aspergillosis .The group of diseases caused by *Aspergillus* exposure is known as Aspergillosis. The main diseases of Aspergillosis are

- Allergic bronchopulmonary aspergillosis
- Acute invasive aspergillosis
- Disseminated invasive aspergillosis

Table No.:- 128 Anti-Microbial activity of synthesized compounds

Sr. No	Code	R	Anti-Microbial Activity (μ/ml) [MIC]						
			Bacterial strains					Fungal strains	
			<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>Kleb pneumoniae</i>	<i>Vibrio cholerae</i>	<i>C. albican</i>	<i>A. niger</i>
1	III-A.5a	-H	100	100	200	100	25	200	200
2	III-A.5b	4-Cl	200	100	100	100	200	200	200
3	III-A.5c	4-F	50	100	200	200	25	100	100
4	III-A.5d	4-OC ₂ H ₅	100	100	100	NA	200	100	200
5	III-A.5e	4-OCH ₃	200	200	200	NA	100	200	200
6	III-B.5a	-H	NA	200	200	100	100	200	200
7	III-B.5b	OCH ₃	200	200	200	200	50	200	100
8	III-B.5c	OC ₂ H ₅	100	100	200	50	200	NA	200
9	III-B.5d	H	200	200	200	NA	NA	100	200
10	III-B.5e	OC ₂ H ₅	100	100	100	100	50	100	NA
11	III-C.4a	-H	200	200	200	200	50	100	100
12	III-C.4b	4-Cl	200	200	200	200	200	100	100
13	III-C.4c	4-OH	200	200	100	100	50	100	200
14	III-C.4d	4-OCH ₃	100	100	100	100	25	200	100
15	III-C.4e	4-OC ₂ H ₅	200	200	200	200	25	200	200
16	III-D.4a	H	100	200	50	200	100	200	NA
17	III-D.4b	-CH ₃	200	200	100	50	200	NA	NA

- Ampicillin (MIC-0.04 μg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 μg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 μg/ml) as standard against *C. albicans* and *A. niger*.

Observation:

All compounds of showed good to moderate potency against all pathogenic stains shown in table

- In series I compound III-A.5a and III-A.5c shows remarkable activity at 25μg/mL and III-A.5c at 50μg/mL against bacterial strain *Vibrio Chlorae*.
- In series II compound III-B.5b and III-B.5d shows remarkable activity at 50μg/mL against bacterial strain *Vibrio Chlorae*. Compound III-B.5c shows remarkable activity at 50μg/mL against bacterial strain shows remarkable activity at 50μg/mL against bacterial strain *Vibrio Chlorae*.

- In series III compound III-C.4d, 4e shows remarkable activity at 25µg/mL against bacterial strain *Vibrio Chlorae* and III-C.4a, 4c at 50 µg/mL against bacterial strain *Vibrio Chlorae*.
- In series IV compound III-D.4a shows remarkable activity at 50µg/mL against bacterial stain *P. aeruginosa*, and compound III-D.4b shows activity at 50µg/mL against stain *kleb pneumoniae*.
- While rest of compound shows good to moderate activity against all anti-bacterial and anti-fungal stains

4.3 ANTI-OXIDANT ACTIVITY

Introduction:

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The free radical scavenging activity of antioxidants has been substantially investigated and reported in the literature by Miller and Rigelhof et.al. Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods.

A rapid, simple and inexpensive method to measure antioxidant activity of the compounds involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of compounds. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of compounds.

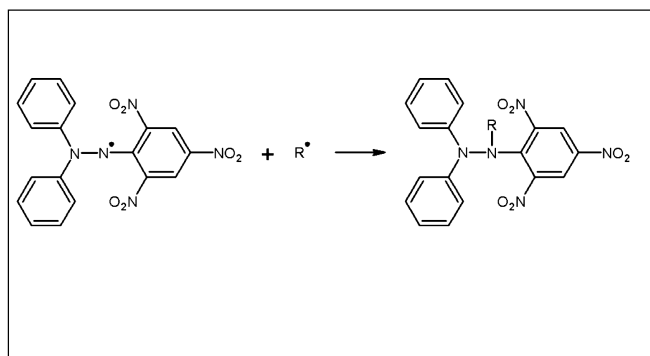
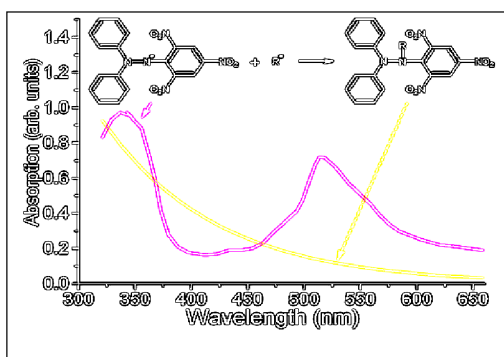


Fig no 84:-The structure of DPPH and its reduction by an antioxidant compound

Principle:

A simple method that has been developed to determine the antioxidant activity of the Test compounds utilizes the stable 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical.. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. Reaction mixture contained 100µm DPPH in methanol and different concentration of synthesized compounds viz: 100µg/ml. Absorbance at 517nm was determined after 30min at RT and the scavenging activity were seen. BHT was used as standard^[180]



$$\% \text{ inhibition} = \frac{\text{O.D of control} - \text{O.D of sample}}{\text{O.D of control}} \times 100$$

Requirements:

100% Methylated (diluent), Butylated Hydrogen Toluene (BHT-Standard), 0.0002% DPPH, Test tubes

Micro pipettes, U.V spectrophotometer

Reagents:

BHT(5mg/ml): 1mg of BHT in 2ml of 100% of methanol, 0.0002% of DPPH: - 0.0002g in 100ml of 100% of methanol

Test Sample: 5mg in 1ml of methanol (stock solution)

Protocol:**Table No.:- 129 Protocol for Standard BHT and Synthesized Compounds**

Serial No.	Working solution ($\mu\text{g/mL}$)	Methanol	Standard drug BHT/Test Comp. (μL)	DPPH (μL)
1	10	998	2	1800
2	20	996	4	1800
3	30	994	6	1800
4	40	992	8	1800
5	50	990	10	1800
6	60	988	12	1800
7	70	986	14	1800
8	80	984	16	1800
9	90	982	18	1800
10	100	980	20	1800
11	Blank	2000	—	—
12	Control	—	—	2000

Incubate all the tubes in dark for 30mins take absorbance at 517nm



Fig no 85: Negative control (tube no: 1), Positive control (tube no: 2)

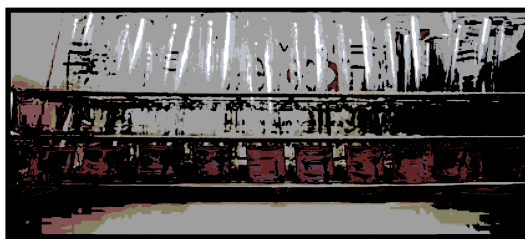


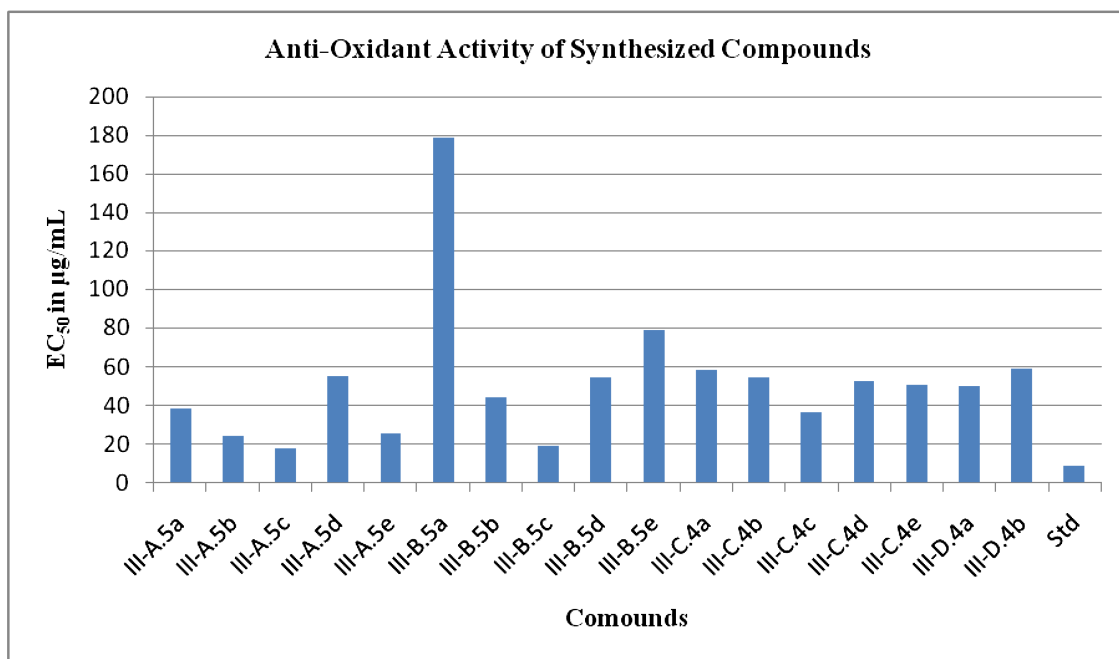
Fig no 86: Anti-oxidant assay tubes ranging from 10-100 $\mu\text{g/ml}$

Results:***Standard BHT:*****Table No.:- 130 Anti-oxidant activity of Standard BHT Drug**

Conc.	O.D at 515nm	% Inhibition
10	0.0257	66.53
20	0.0237	68.04
30	0.0223	70.26
40	0.0209	72.13
50	0.0193	74.26
60	0.0181	75.86
70	0.0168	77.60
80	0.0149	80.13
90	0.0141	81.20
100	0.0138	81.60
Blank	0.0453	
Positive control	0.0750	

Table no 131:- Anti-oxidant activity of Synthesized Compounds

Sr. no.	Code	R	IC ₅₀ ±SD
1	III-A.5a	-H	38.13±0.231
2	III-A.5b	4-Cl	23.71±1.333
3	III-A.5c	4-F	17.21±1.897
4	III-A.5d	4-OC ₂ H ₅	54.66±1.654
5	III-A.5e	4-OCH ₃	25.00±1.596
6	III-B.5a	-H	179±1.357
7	III-B.5b	OCH ₃	44.03±2.159
8	III-B.5c	OC ₂ H ₅	18.98±3.574
9	III-B.5d	H	54±0.319
10	III-B.5e	OC ₂ H ₅	79±1.357
11	III-C.4a	-H	58.23±2.364
12	III-C.4b	4-Cl	54.00±2.136
13	III-C.4c	4-OH	36.00±2.196
14	III-C.4d	4-OCH ₃	52.32±1.330
15	III-C.4e	4-OC ₂ H ₅	50.13±2.036
16	III-D.4a	H	49.99±1.39
17	III-D.4b	-CH ₃	59.00±1.26
18	Std		8.25±0.336

Graph 1: Anti-Oxidant activity of synthesized compounds**OBSERVATION:**

- In Radical scavenging activity done by DPPH method, standard drug BHT shows EC₅₀ at 8.25µg/mL
- All Synthesized compounds compared with standard, we observed that comp. III-A.5c shows remarkable activity at 17.21µg/mL
- Compound III-B.5c, III-A.5b shows EC₅₀ at 18.98 µg/mL, 23.71 µg/mL respectively
- While rest of compound shows good to moderate activity

4.4 ANTI-INFLAMMATORY TEST

Introduction:

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury and pain. Even though most of the synthetic anti-inflammatory drugs are available in the market, due to their well-known side effects, toxic effects and production cost, presently people are search for natural anti-inflammatory drugs without any adverse effects.

The anti-inflammatory activities of various compounds are assessed by *in vitro* HRBC membrane stabilization method. Blood are collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever's solution (Dextrose 2%, Sodium citrate 0.8%, Citric acid 0.05%, Sodium chloride 0.42% and Distilled water 100 ml) and centrifuged with Isosaline. To 1 ml of HRBC suspension equal volume of test drug in different concentrations are added. All the assay mixtures are incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution are estimated by using spectrophotometer at 560nm. The percentage of heamolysis was calculated.

Principle:

The lysosomal enzymes produced during the process of inflammation produces a variety of disorders. The extra cellular activity of these enzymes is said to be related to the acute of chronic inflammation. The non-steroidal drug (DFS in case) act either by inhibiting these lysomol enzymes or by stabilizing the lysosomal membrane (HRBC is similar to the lysosomal membrane)

In this case, the standard used was Diclofenac sodium (DFS) which acts by stabilizing the membrane. The hypo saline used causes the heamolysis of RBC, as the concentration of hypo saline increases this causes the membrane components to leak out. This is then measured spectrophotometrically at 560nm. Thus increases concentration of hypo saline, increases the O.D (as increase concentration of hypo saline increases the extent of heamolysis).

DFS stabilizes the membrane, thereby reducing the heamolysis .Thus with the increase in the components are prevented from leaking, Thus as the concentration of DFS increases, the O.D decreases thereby decreasing the effect of the hypo tonicity caused by hypo saline^[181]

$$\% \text{ Heamolysis} = (\text{Optical density of Control} - \text{Optical density of Test sample} / \text{Optical density of Control}) \times 100$$

The ability of the test compound to stabilize the membrane or to prevent hemolysis is compared with the standard.

MATERIALS AND METHODS:

The Human Red Blood Cell (HRBC) membrane stabilization method:

1. Fresh whole human blood (5mL) are collected and transferred to the centrifuged tubes containing Heparin or EDTA or Sodium citrate to prevent clotting.
2. The tubes are centrifuged at 3000 rpm for 10 min and are washed three times with equal volume of normal saline. The volume of the blood is measured and reconstituted as 10% v/v suspension with normal saline.
3. The reaction mixture consists of 1.0mL of test sample of different concentrations in normal saline and 0.5mL of 10% HRBC suspension, 1 ml of 0.2 M phosphate buffer, 1 mL hypo saline were incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 30 mins.
4. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm.
5. Diclofenac was used as standard and a control
6. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following formula.

Protocol

Step 1- RBC solution

- Alsever solution (3ml) + Blood (3ml).
- Centrifuge at 3000 rpm for 10min.
- Discard the supernatant and wash the pellet with iso-saline thrice.
- Dilute 1ml of pellet with 9ml of iso-saline i.e. 10% v/v.

Step 2- Sample solution

Concentration ($\mu\text{l/ml}$)	DFS (ml)	Isosaline (ml)	Total Volume (ml)
100	0.008	1.992	2
50	0.004	1.996	2
25	0.002	1.998	2
12.5	0.001	1.999	2
6.25	0.0005	1.9995	2

Step 3

Sample(ml)	Phosphate Buffer (ml)	Hypo saline (ml)	RBC solution (ml)
0.5	1	2	0.5
0.5	1	2	0.5
0.5	1	2	0.5
0.5	1	2	0.5
0.5	1	2	0.5

Step 4- Blank preparation

Isosaline (ml)	Phosphate buffer (ml)	Hypo saline (ml)	Isosaline (ml)
1.0	2.0	4.0	1.0

- Incubate all the tubes at 37°C for 30 min.
- Centrifuge at 3000 rpm 20min.
- Measure the O.D of the supernatant at 560 nm.

Alsever solution (50ml):

- Dextrose - 1gm
 - Sodium citrate - 0.4gm
 - Citric acid – 0.025gm
 - Sodium chloride - 0.21gm
- Dissolve in distilled water to make up the volume up to 50ml.

Phosphate buffer (anhydrous):

- A. 0.2 M of NaH_2PO_4
Dissolve 4.792gm of NaH_2PO_4 in 200ml of distilled water.
- B. 0.2 M of Na_2HPO_4

Dissolve 5.678gm of Na_2HPO_4 in 200ml of distilled water.

- C. Mix 32ml of 'A' with 168ml of 'B'.
- D. Take 150ml of 'C' adjust the pH to 7.5 and make up the volume to 200ml using distilled water

Hypo saline:

Dissolve 0.9gm of NaCl in 250ml of distilled water.

Iso saline:

Dissolve 2.125gm of NaCl in 250ml of distilled water

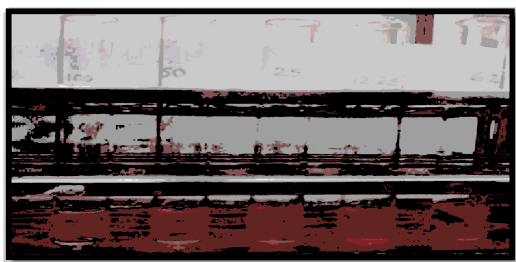


Fig no 86:- Anti-inflammatory assay tubes ranging conc. 6.25-100 $\mu\text{g/ml}$

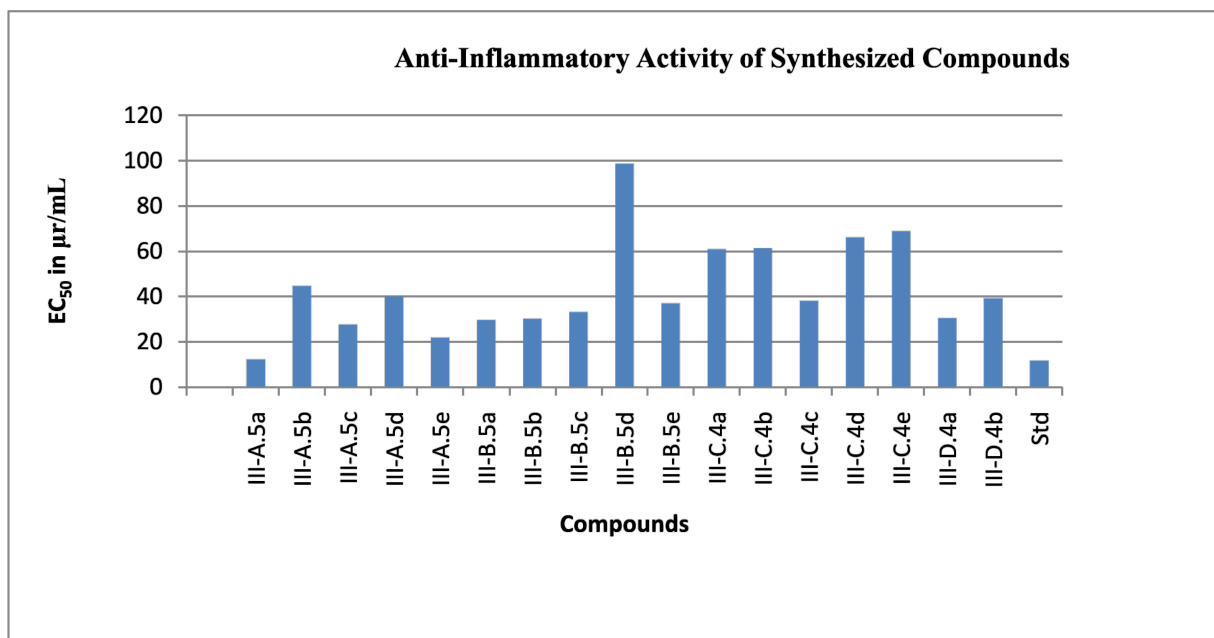
Result:

Table No.:-132Anti-inflammatory activity of Standard Drug Diclofenac

Concentration($\mu\text{g/ml}$)	O.D at 560nm	% heamolysis
6.25	0.0912	95.46
12.5	0.054	96.24
25	0.0455	96.21
50	0.0360	97.02
100	0.021	98.27

Table No. 133:- Anti-inflammatory activity of Synthesized Drug

Sr. no.	Code	R	IC ₅₀ ±SD
1	III-A.5a	-H	12.25±2.03
2	III-A.5b	4-Cl	44.67±1.369
3	III-A.5c	4-F	27.60±1.045
4	III-A.5d	4-OC ₂ H ₅	39.83±0.945
5	III-A.5e	4-OCH ₃	21.87±0.654
6	III-B.5a	-H	29.67±2.132
7	III-B.5b	OCH ₃	30.23±2.189
8	III-B.5c	OC ₂ H ₅	33.201±6.123
9	III-B.5d	H	98.74±1.350
10	III-B.5e	OC ₂ H ₅	37.01±4.13
11	III-C.4a	-H	61.02±1.123
12	III-C.4b	4-Cl	61.32±1.109
13	III-C.4c	4-OH	38.13±0.38
14	III-C.4d	4-OCH ₃	66.19±0.23
15	III-C.4e	4-OC ₂ H ₅	68.94±0.54
16	III-D.4a	H	30.46±1.951
17	III-D.4b	-CH ₃	39.21±1.753
18	Std		11.70±0.987

Graph 2: Anti-inflammatory activity of synthesized compounds

Observation

- In Anti-inflammatory activity done by Human Red Blood Cell (HRBC) membrane stabilization method, standard drug shows EC_{50} at 11.70 μ g/mL.
- Compound III-A.5a shows EC_{50} 12.25 μ g/mL
- Compound III-A.5e, III-A.5c, III-B.5a shows EC_{50} 21.87, 27.60, 29.67 μ g/mL respectively
- While rest compounds shows good to moderate activity

5.0 RESULT AND DISCUSSION

1. Discussion for compound III-A.5a-e

- The structure of newly synthesized compounds during this investigation has been established by their IR, NMR and Mass studies.
- The compound IIIA.1 has been synthesized by reacting mercapto benzothiazole with ethyl chloro acetate. The formation of III-A.1 has been confirmed by chemical test (ester test) and mass fragmentation pattern. The compound III-A.1 showed molecular ion peak at 297 m/z. and base peak at 207 m/z.
- The compound III-A.2 has been synthesized by reacting III-A.1 with hydrazine hydrate in alcohol. The formation of III-A.2 has been confirmed by chemical test (primary amine) and mass fragmentation pattern. The compound III-A.2 showed molecular ion peak 282 m/z and base peak at 238 m/z.
- The compound III-A.3 has been synthesized by reacting III-A.2 with carbon disulphide and potassium hydroxide in alcohol. The formation of III-A.3 has been confirmed by mass fragmentation pattern. The compound III-A.3 showed molecular ion peak 327 m/z and base peak at 247 m/z.
- The compound III-A.4 has been synthesized by reacting III-A.3 with hydrazine hydrate in alcohol. The formation of III-A.4 has been confirmed by chemical test (primary amine) and mass fragmentation pattern. The compound III-A.4 showed molecular ion peak 339 m/z and base peak at 294 m/z.
- The compound III-A.5a-e has been synthesized by reacting with III-A.4 with different benzoic acid and phosphoryl chloride in Dioxane. The formation III-A.5a-e has been characterized by IR spectrum. The compound III-A.5a-e showed characteristic peak at 3066 cm^{-1} due to C-H stretching, 1587 cm^{-1} due to C=N bending, 1492 cm^{-1} due to C-N stretching, 1155 cm^{-1} due to C-S stretching.
- Further the formation of III-A.5a-e confirmed by ^1H NMR spectrum. The compound III-A.5a-e showed δ value 1.0 (t,3H, CH_3), 3.5 (s,2H, CH_2), 4.0 (q,2H, CH_2), 7-8 (m,7H,Ar-H)
- The formation of III-A.5a-e confirmed by mass spectrum. The compound III-A.5a-e showed molecular ion peak at 428m/z, 456m/z, 443m/z, 469m/z, 455m/z respectively.
- The purity of compound III-A.5a-e has been confirmed by GC and HPLC.

2. Discussion for compound III-B.5a-e

- The structure of newly synthesized compounds during this investigation has been established by their IR, NMR and Mass studies.
- The compound III-B.1 has been synthesized by reacting amino benzothiazole with ethyl chloro acetate. The formation of III-B.1 has been confirmed by chemical test (ester test) and mass fragmentation pattern. The compound III-B.1 showed molecular ion peak at 267 m/z. and base peak at 191 m/z.
- The compound III-B.2 has been synthesized by reacting III-B.1 with hydrazine hydrate in alcohol. The formation of III-B.2 has been confirmed by chemical test (primary amine) and mass fragmentation pattern. The compound III-B.2 showed molecular ion peak 251 m/z and base peak at 221 m/z.

- The compound III-B.3 has been synthesized by reacting III-B.2 with carbon disulphide and potassium hydroxide in alcohol. The formation of III-B.3 has been confirmed by mass fragmentation pattern. The compound III-B.3 showed molecular ion peak 295 m/z and base peak at 265 m/z.
- The compound III-B.4 has been synthesized by reacting III-B.3 with hydrazine hydrate in alcohol. The formation of III-B.4 has been confirmed by chemical test (primary amine) and mass fragmentation pattern. The compound III-B.4 showed molecular ion peak 311 m/z and base peak at 261 m/z.
- The compound III-B.5a-e has been synthesized by reacting with III-B.4 with acetyl chloride in alcohol. The formation III-B.5a-e has been characterized by IR spectrum. The compound III-B.5a-e showed characteristic peak at 3066 cm^{-1} due to C-H stretching, 3327 cm^{-1} due to N-H stretching, 1338 cm^{-1} due to $\text{N}=\text{C}$ bending, 1263 cm^{-1} due to C-S stretching.
- Further the formation of III-B.5a-e confirmed by ^1H NMR spectrum. The compound III-B.5a-e showed δ value 1.0 (s,3H, CH_3), 4.0 (s,2H, CH_2), 5.3 (s,2H, CH_2), 4.5 (s,1H, NH), 7.5-8.0 (m,4H,Ar-H)
- The formation of III-B.5a-e confirmed by mass spectrum. The compound III-B.5a-e showed molecular ion peak at 343m/z, 372m/z, 385m/z, 358m/z, 405m/z respectively.
- The purity of compound III-B.5a-e has been confirmed by GC and HPLC.

3. Discussion for compound III-A.5a-e

- The structure of newly synthesized compounds during this investigation has been established by their IR, NMR and Mass studies.
- The compound III-C.1 has been synthesized by reacting mercapto benzothiazole with ethyl chloro acetate. The formation of III-C.1 has been confirmed by chemical test (ester test) and mass fragmentation pattern. The compound III-C.1 showed molecular ion peak at 297 m/z. and base peak at 207 m/z.
- The compound III-C.2 has been synthesized by reacting III-C.1 with hydrazine hydrate in alcohol. The formation of III-C.2 has been confirmed by chemical test (primary amine) and mass fragmentation pattern. The compound III-C.2 showed molecular ion peak 282 m/z and base peak at 238 m/z.
- The compound III-C.3 has been synthesized by reacting III-C.2 with carbon disulphide and potassium hydroxide in alcohol. The formation of III-C.3 has been confirmed by mass fragmentation pattern. The compound III-C.3 showed molecular ion peak 327 m/z and base peak at 247 m/z.
- The compound III-C.4a-e has been synthesized by reacting with III-C.3 with acetyl chloride in alcohol. The formation III-C.4a-e has been characterized by IR spectrum. The compound III-C.4a-e showed characteristic peak at 1394 cm^{-1} due to C-O-H stretching, 1261 cm^{-1} due to C-O stretching, 939 cm^{-1} due to C-H bending,
- Further the formation of III-C.4a-e confirmed by ^1H NMR spectrum. The compound III-C.4a-e showed δ value 3.5 (s,2H, CH_2), 1.0 (t,3H, CH_3), 4.1 (s,3H, CH_3), 4.0 (q,2H, CH_2), 7-8 (m,7H,Ar-H)
- The formation of III-C.4a-e confirmed by mass spectrum. The compound III-C.4a-e showed molecular ion peak at 429m/z, 445m/z, 462m/z, 459m/z, 473m/z respectively.
- The purity of compound III-C.4a-e has been confirmed by GC and HPLC.

4. Discussion for compound III-D.4a-b

- The structure of newly synthesized compounds during this investigation has been established by their IR, NMR and Mass studies.
- The compound III-D.1 has been synthesized by reacting hydroxy acetophenone with phosphoryl oxychloride in dimethyl formamide. The formation of III-D.1 has been confirmed by chemical test (aldehyde test) and mass fragmentation pattern. The compound III-D.1 showed molecular ion peak at 174 m/z. and base peak at 144 m/z.
- The compound III-D.2 has been synthesized by reacting phenyldiamine with chloroacetic in alcohol. The formation of III-D.2 has been confirmed by chemical test (hydroxyl group test) and mass fragmentation pattern. The compound III-D.2 showed molecular ion peak 148 m/z and base peak at 128 m/z.
- The compound III-D.3 has been synthesized by reacting III-D.2 with phosphoryl oxychloride in chloroform. The formation of III-D.3 has been confirmed by chemical test (chlorine test) has been confirmed by mass fragmentation pattern. The compound III-D.3 showed molecular ion peak 164 m/z and base peak at 128 m/z.
- The compound III-D.4a-e has been synthesized by reacting with III-D.3, III-D.1 and sulphonamide in alcohol. The formation III-D.4a-e has been characterized by IR spectrum. The compound III-D.4a-e showed characteristic peak at 3342 cm^{-1} due to N-H stretching, 1150 cm^{-1} due to S=O stretching, 1095 cm^{-1} due to -O-bending,
- Further the formation of III-D.4a-b confirmed by ^1H NMR spectrum. The compound III-D.4a-e showed δ value 8.6 (2H, N=CH), 4.2 (s,1H, NH), 7-8 (m, 12H, Ar-H)
- The formation of III-D.4a-e confirmed by mass spectrum. The compound III-D.4a-e showed molecular ion peak at 469m/z, 453m/z.
- The purity of compound III-D.4a-e has been confirmed by GC and HPLC.

CONCLUSION

- The main aim of the research work was to synthesize, purify and characterize the heterocyclic pharmacophores containing nitrogen and sulphur (heteroatom) as possible anti-microbial agents.
- It was interesting to note that the presence of halogen substitution at Para position of phenyl ring showed good antifungal activity against gram –ve bacterial strains
- Among halogen substitution fluoro was the most active substituent against gram –ve bacterial stains and it is not active against fungal strains.
- In Anti-Oxidant activity study, from observed data we conclude that, thiazole, Triazole, Thiadiazepine moieties and amide linkage with electron Withdrawing substituent's like Cl, F shows good radical scavenging activity.
- That means among all synthesized compounds with various heterocyclic moieties benzothiazole, Triazole, thiadiazepine, and sulphonamide moieties are suitable or give good result as for different biological activity and this work can be further evaluated.

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APPENDIX

Instruments and equipments

NAME OF INSTRUMENTS	COMPANY NAME	MODEL NO
GCMS	AGILENT TECHNOLOGIES	7890 GC and 220 Ion Trap MS
HPLC	THERMO SCIENTIFIC	ULTIMATE 3000 UHPLC (Dionex) System
IR	SHIMADZU	IRAffinity-1
UV	JASCO	JASCO V-500
UV Cabinet	EXPO	UV CABINATE C-19
Digital Balance	KERN	ABT-320-4M
Melting Point	CAMPBELL ELECTRONICS	Pmb-2 THERMONIK
Magnetic stirrer	REMI	1 MLH Magnetic Stirrer

SYNOPSIS
OF THE THESIS TO BE SUBMITTED TO
THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN THE FACULTY OF ORGANIC CHEMISTRY

TITLE OF THE PROPOSAL : Synthesis and study of Heterocyclic
Compound as possible Anti- Microbial
agents.

NAME OF THE CANDIDATE : Ashish Lawoo Suhasini Asrondkar

**QUALIFICATION OF THE
CANDIDATE** : M.Sc (Organic Chemistry)

**NAME AND DESIGNATION OF
THE RESEARCH SUPERVISOR** : Dr. Anil S. Bobade,
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**NUMBER AND DATE OF
REGISTRATION** : 39 / 14th JUNE 2012

DATE OF SUBMISSION : 07th May 2012

(Ashish Lawoo Asrondkar)
Signature of Candidate

(Dr. Anil S. Bobade)
Signature of Research Guide

Introduction

Two hundred years ago, the chemical science was an undivided field; around 1900, chemical science division into inorganic, organic and physical chemistry became necessary. The increase of factual material enforced a progressive segmentation into sub disciplines. Heterocycles form by far the largest of classical divisions of organic chemistry and are of immense importance biologically and industrially. The majority of pharmaceuticals and biologically active agrochemicals are heterocyclic while countless additives and modifiers used in industrial applications ranging from cosmetics reprography, information storage and plastics are heterocyclic in nature. One striking structural features inherent to heterocycles, which continue to be exploited to great advantage by the drug industry, lies in their ability to manifest substituents around a core scaffold in defined three dimensional representations. For more than a century, heterocycles have constituted one the largest areas of research in organic chemistry. They have contributed to the development of society from a biological and industrial point of view as well as to the understanding of life processes and to the efforts to improve the quality of life. Among the approximately 20 million chemical compounds identified by the end of the second millennium, more than two-thirds are fully or partially aromatic and approximately half are heterocyclic. The presence of heterocycles in all kinds of organic compounds of interest in electronics, biology, optics, pharmacology, material sciences and so on is very well known, between them, sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers through decades of historical development of organic synthesis¹. However, heterocycles with other heteroatoms such as oxygen², phosphorus³ and selenium⁴ also appears. Many natural drugs⁵⁻⁸ such as papaverine, theobromine, quinine, emetine, theophylline, atropine, procaine, codeine, reserpine and morphine are heterocycles. Almost all the compounds we know as synthetic drugs such as diazepam, chlorpromazine, Isoniazid, metronidazole, azidothymidine, barbiturates, antipyrine, captopril and methotrexate are also heterocycles. Some dyes (e.g. mauveine), luminophores, (e.g. acridine orange), pesticides (e.g. diazinon) and herbicides (e.g. paraquat) are also heterocyclic in nature. All these natural and synthetic heterocyclic compounds can and do participate in chemical reactions in the human body. Furthermore, all biological processes are chemical in nature. Such fundamental manifestations of life as the provision of energy, transmission of nerve impulses, sight, metabolism and the transfer of hereditary information are all based on chemical reactions involving the participation of many heterocyclic compounds, such as vitamins, enzymes, coenzymes, nucleic acids, ATP and serotonin⁹. Why does nature utilize heterocycles? The answer to this question is provided by the fact that heterocycles are able to get involved in an extraordinarily wide range of reaction types. Depending on the pH of the medium, they may behave as acids or bases, forming anions or cations. Some interact readily with electrophilic reagents, others with nucleophiles, yet others with both. Some are easily oxidized, but resist reduction, while others can be readily hydrogenated but are stable toward the action of oxidizing agents.

Synthetic heterocycles have widespread therapeutic uses such as antibacterial, antifungal, antimycobacterial, trypanocidal, anti-HIV activity, antileishmanial agents, genotoxic, antitubercular, antimalarial, herbicidal, analgesic, antiinflammatory, muscle relaxants, anticonvulsant, anticancer and lipid per oxidation inhibitor, hypnotics, antidepressant, antitumoral, anthelmintic and insecticidal agents¹⁰⁻¹⁶. There are larger number of synthetic heterocyclic compounds with other important applications such as fungicides, herbicides, anticorrosive agents, photo stabilizers, agrochemicals, dyestuff, copolymer, photographic developers, fluorescent whiteners, sensitizers, booster agent, antioxidant in rubber and flavouring agent¹⁷⁻²².

Introduction of a heteroatom into a cyclic compound imparts new properties. Heterocycles are chemically more flexible and better able to respond to the many demands of biochemical systems. The constantly accelerating rate of research and development in heterocyclic chemistry suggested that enormous numbers of heterocyclic systems are well known and this number is increasing very rapidly.

Sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers through decades of historical development of organic synthesis. The grounds of this interest were their biological activities and unique structures that led to several applications in different areas of pharmaceutical. Heterocyclic organic compounds such as Benzothiazole, Benzimidazole, triazole, Oxadiazole, coumarin and piperazine etc shows promising biological activities documented in this thesis. The present thesis is an attempt to analyze the various biological activities reported for heterocycles in the thesis with an update of recent research findings on these heterocyclic nuclei.

The thesis will consist of the following part:-

Part I :- Introduction

Part II:- Review of Literature & Plan of work

Part III :- Experimental & Characterization

Part III- Experimental & Characterization Section is further subdivided into following category-

III-A] Synthesis of 2-([(6- Substituted phenyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazol-3-yl) methyl] sulfanyl)-1,3-benzothiazole

III-B] Synthesis of *N*-[(6,7-dimethyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazepin-3-yl) methyl] -substituted 1,3-benzothiazol-2-amine

III-C] Synthesis of *S*-{5-[(1,3-benzothiazol-2-yl)sulfanyl] methyl} -1,3,4-oxadiazol -2-yl} substituted benzenecarbothioate

III-D] Synthesis of 4-([(*E*)-(6-methyl-4-oxo-4*H*-chromen-3-yl) methylidene] amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide

Part IV:- Biological Evaluation

Part V:- Result, Discussion & Conclusion

Part VI:- References

Part I :- Introduction

This part will give general Introduction about the brief details of Heterocyclic Moieties & their application in medicinal chemistry field, which is selected for the research work.

Part II:- Review of Literature & Plan of work

This part will cover salient aspects of the research project & highlights the aims & objectives in the synthesis work. It covers Chemistry of the main entities which is selected for the research with their probable mechanism.

Part III :- Experimental & Characterization

This part includes the synthesis work of the research projects with schematic representation of the series & their Chromatographic & spectroscopic characterization. This part will further sub-divided into following sections according to synthesis of titled series of compounds.

III-A] Synthesis of 2-[(6- Substituted phenyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazol-3-yl) methyl] sulfanyl]-1,3-benzothiazole

Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger compounds, usually bioactive structures. Its aromaticity makes it relatively stable; although, as a heterocycle, it has reactive sites, which allow for functionalization. Benzothiazole is a colorless, slightly viscous liquid with a melting point of 2°C and a boiling point of 227-228°C. The density of benzothiazole is 1.24 g/mL, and its molecular mass is 135.19 gmol⁻¹. Benzothiazole has no household use. It is used in industry and research.

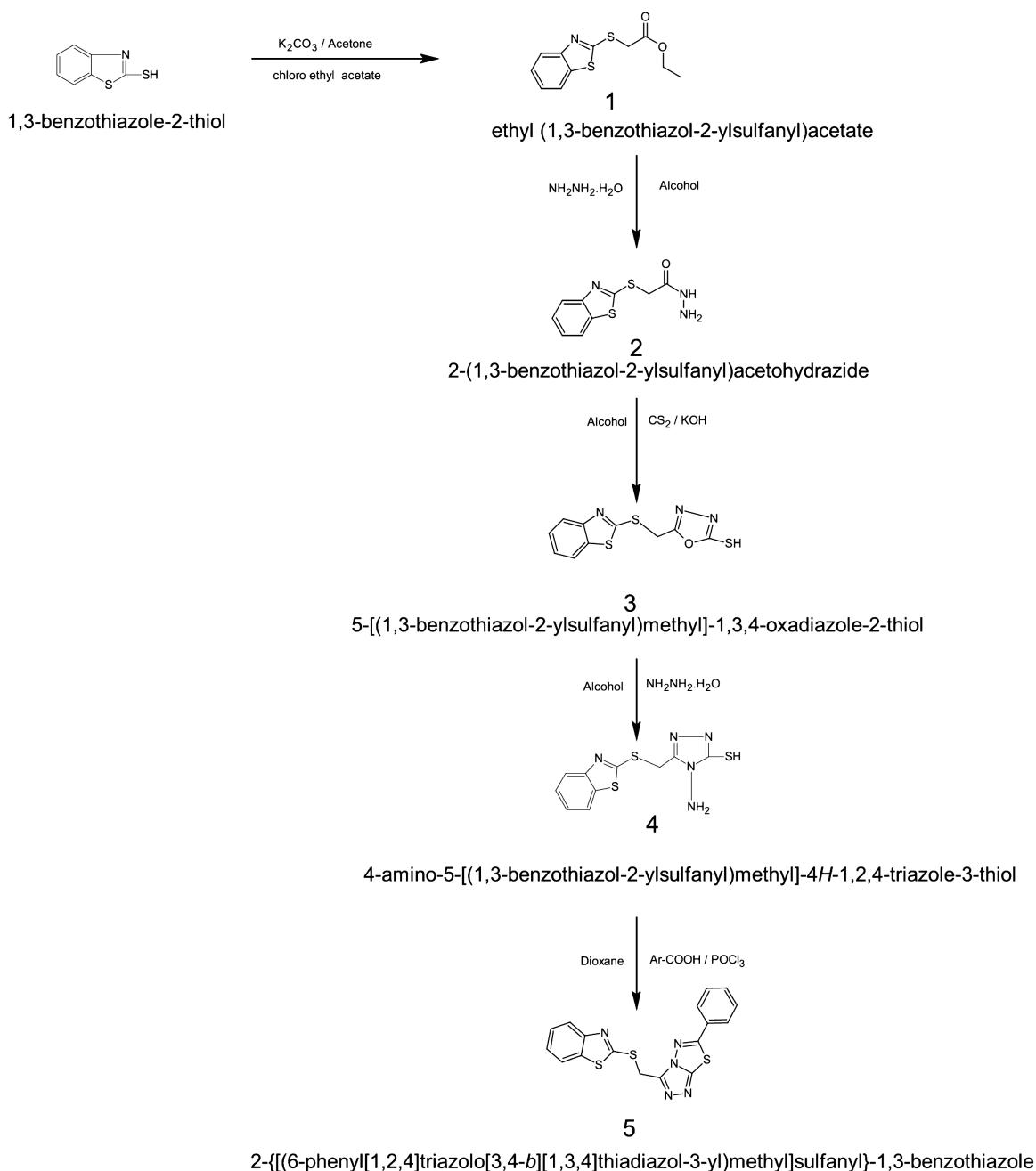
A large number of therapeutic agents are synthesized with the help of benzothiazole nucleus. During recent years there have been some interesting developments in the biological activities of benzothiazole derivatives. These compounds have special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities.²³

The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds. The success of the imidazole as an important moiety of number of medicinal agents led to the introduction of the triazoles. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen. The 1, 2, 4-triazole ring is a ubiquitous structural feature of many synthetic compounds with diversified therapeutic efficacy. A large volume of published literature over the last

few decades preludes a comprehensive review. The major activities exhibited by triazole derivatives are insecticidal, antibacterial, antiviral, sedative, hypnotic, anticonvulsant and anti-inflammatory action.²⁴

The present review highlights the use of benzothiazole moiety as a template for development of newer therapeutic agents. Biological properties of the nucleus include anticancer, antioxidant, anti-inflammatory, and antimicrobial & the brief information available on the triazole derivatives clearly indicates their therapeutic importance. Prospective titled compounds can be designed and synthesized for a variety of biological activities.

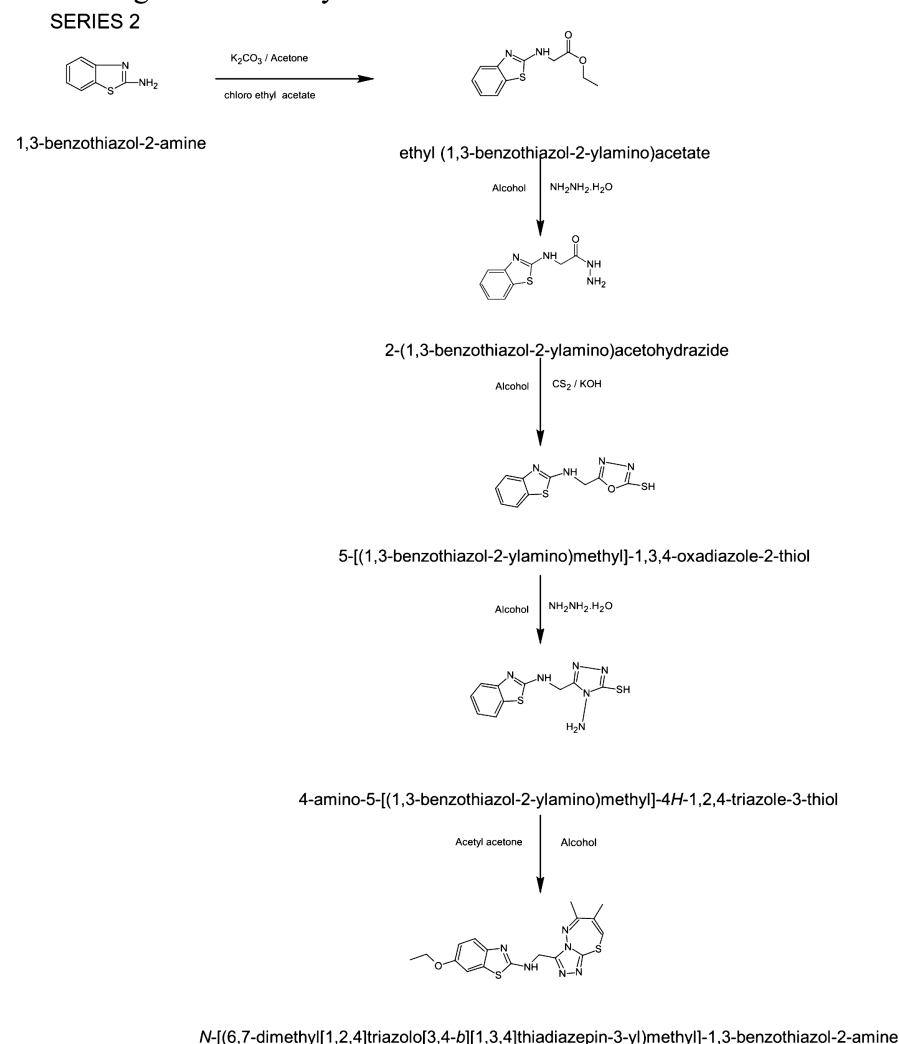
SERIES 1



III-B] Synthesis of *N*-[(6,7-dimethyl [1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazepin-3-yl) methyl] -substituted 1,3-benzothiazol-2-amine

Triazole units have attracted considerable attention in fields, such as medicinal and agrochemical research as well as in the material sciences due to their unique structure and properties.²⁵ Some of the modern-day drugs with triazole nucleus are as follows:

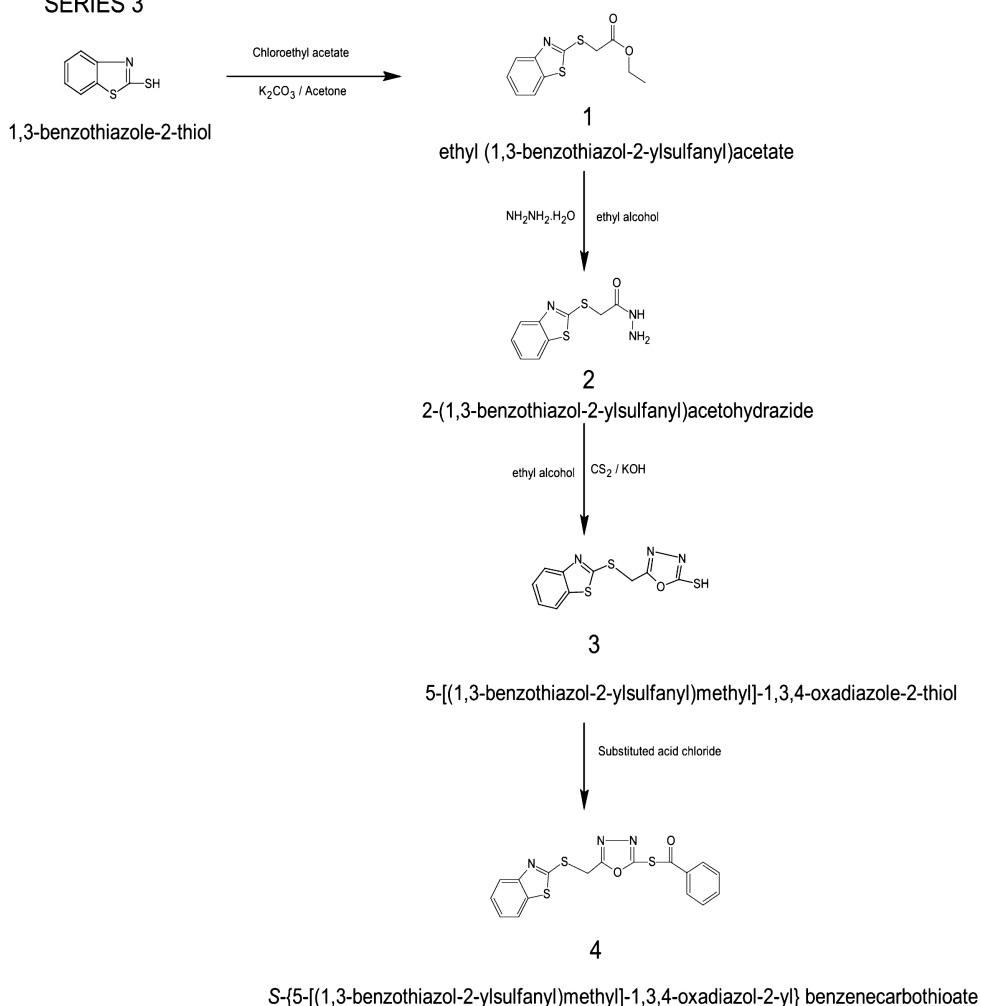
Fluconazole, Itraconazole, Terconazole, Posaconazole, Voriconazole (the powerful azoles antifungal agents), Ribavirin (antiviral agent). In addition to these important biological applications, mercapto- 1, 2, 4-triazoles are also of great utility in preparative organic chemistry,²⁶ viz, in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds, e.g. triazolo-thiadiazepine, triazolobenzothiazole. Thus from the present study I hope the synthetic feasibility and suitable insertion into many other structural frameworks will surely prompt the researchers to synthesize a huge number of compounds considering 1,2,4-triazole as an effective scaffold, which will not only be useful in modulating the existing efficacy in a better way, but also may explore certain targets which are yet to be touched.



III-C] Synthesis of *S*-{5-[(1,3-benzothiazol-2-ylsulfanyl) methyl] -1,3,4-oxadiazol -2-yl} substituted benzenecarbothioate

Oxadiazoles are five-membered heteroaromatic rings containing two carbons, two nitrogens, and one oxygen atom, and they exist in different regioisomeric forms. Oxadiazoles are frequently occurring motifs in drug like molecules, and they are often used with the intention of being bioisosteric replacements for ester and amide functionalities²⁷. The current study presents a synthesis of new series of a compounds which starts from benzothiazole as parent moiety incorporated with 1,3,4-oxadiazole. To facilitate the use of these heteroaromatic rings, novel synthetic routes broad spectrum of 1,3,4-oxadiazoles, under mild conditions, are described in the thesis.

SERIES 3



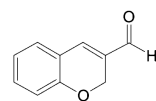
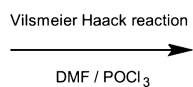
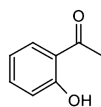
III-D] Synthesis of 4-[(*E*)-(6-methyl-4-oxo-4*H*-chromen-3-yl) methylidene] amino}-*N*-(quinoxalin-2-yl) benzene sulfonamide

The versatility of the quinoxalinones skeleton, in addition to its relative chemical simplicity and accessibility, makes these chemicals amongst the most promising sources of bioactive compounds. This has led to the discovery of a wide variety of compounds that are of high interest from the point of view of antimicrobial, antifungal, antiviral, anti-inflammatory, and antitumor effects among others. quinoxalinone derivatives that have been reported to show considerable pharmacological actions such as antimicrobial, anti-inflammatory, antidiabetic, antiviral, antitumor, and antitubercular activity²⁸.

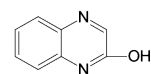
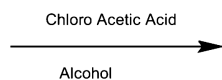
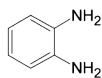
The benzofuran ring system itself is a common structure element that appears in a large number of medicinally important compounds²⁹. The benzofuran nucleus is widely distributed in natural products particularly among plant kingdom. In the chemistry of benzofurans in a large number of natural products has attracted due to their biological activities and their potential application as pharmacological agents³⁰.

It can act as an important tool for chemists to develop newer benzofuran based quinoxalinone derivatives that may prove to be better agents in terms of efficacy and safety.

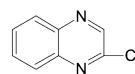
SERIES 4



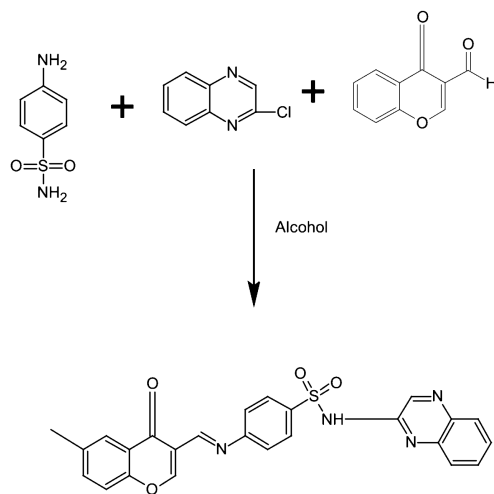
2H-chromene-3-carbaldehyde



quinoxalin-2-ol



2-chloroquinoxaline



The structures of the newly synthesized compound were analyzed by physical method by Melting point & with the help of analytical methods (IR, HPLC and GC-MS).

- **Spectroscopic technique**
Infrared spectroscopy (IR)
Nuclear Magnetic Resonance spectroscopy (NMR)
- **Chromatographic techniques**
Thin layer chromatography (TLC)
Gas chromatography - mass spectrometry (GC-MS)
High performance liquid chromatography (HPLC)

Part IV:- Biological Evaluation

This part covers the biological evaluation of synthesized compounds by suitable method. The development in chemotherapy of synthetic drugs totally depends upon the biological results of the chemotherapeutic agents, which ultimately give the guidelines to the research worker in the selection of agents for further clinical studies and set the pattern for future clinical studies and set the pattern of future drug evaluation.

Taking this into consideration the above fact, in our present work, different heterocyclic derivatives, derived from different biologically important moieties, were prepared and were screened for following biological evaluations:

1. Anti Bacterial Screening:-

The compounds reported in Series I, II III & IV have been subjected to antifungal and antibacterial screening *in vitro*. The *in vitro* antimicrobial screening was carried out by tube dilution method against various species of Gram Positive and Gram Negative bacteria viz. *Staphylococcus Aureus*. ATCC 3750 and *Salmonella Typhi*. NCTC786. The culture medium used was Mueller-Hinton Broth³¹.

2. Anti Fungal Screening:-

Some compounds reported in series in I, II, III and IV were also screened for their antifungal activity by the tube dilution technique³² against fungal strains viz. *Candida Albicans*.

In different screening methods, the standard drugs employed were *trimethoprim* as general anti-bacterial agent and *Miconazole* as an anti-fungal agent.

The results of the above antibacterial and antifungal screening discussed in the thesis.

3. Anti-oxidant assay

DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radical with red color absorbed at 517nm. If free radicals have been scavenged, DPPH will generate its color to yellow. This assay uses this character to show compounds free radical scavenging activity³³.

4. Anti-inflammatory assay

HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by the compounds to predict anti-inflammatory activity³⁴.

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