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Design, Synthesis, Characterization and Evaluation of Novel Heterocyclic Derivatives with Potential Biological Activities

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Abstract : Through a simple synthetic process, a number of new substituted imine derivatives (SC1–SC5) were effectively produced employing different substituted aldehydes. Recrystallisation was used to purify the compounds, which were produced in moderate to excellent yields (62–79%). Melting point analysis, thin-layer chromatography (TLC), infrared spectroscopy (IR), proton nuclear magnetic resonance (^{1}H NMR), and liquid chromatography-mass spectrometry (LC-MS) were used to validate the structure and evaluate purity. The effective conversion of carbonyl groups to imine functionality was demonstrated by IR spectra, and the predicted proton environments were confirmed by ^{1}H NMR data. The molecular weights and excellent purity of the produced compounds were validated by LC-MS analysis. Moderate inhibitory efficacy against common bacterial strains was found in preliminary antibacterial investigation, with certain compounds showing encouraging results. These results demonstrate the imine derivatives' effective synthesis and characterisation and point to the possibility of more biological research into them.

IndexTerms **- Heterocyclic compounds; Schiff base derivatives; Imine synthesis; Spectral characterization; LC–MS analysis; Antibacterial activity; Structure–Activity Relationship (SAR); Medicinal chemistry.**

I. INTRODUCTION

1.1 Heterocyclic compounds

Heterocyclic compounds are a basic type of organic molecule that has rings made up of atoms from at least two distinct elements. Usually, the heteroatoms are nitrogen, oxygen, or sulphur. These atoms take the place of one or more carbon atoms that would typically be in a carbocyclic ring. Heterocyclic compounds are present in a lot of natural products, drugs, agrochemicals, dyes, and materials because of their distinctive chemical structures and characteristics. Their significance extends beyond the chemical and pharmaceutical sectors to biological systems, where they constitute fundamental structures in nucleic acids, vitamins, and enzymes [1-3].

The presence of heteroatoms in a ring has a big effect on how these compounds react chemically and how they conduct electricity. There are two main types of heterocycles: aliphatic and aromatic. Aliphatic heterocycles do not have conjugated π systems, and their reactivity patterns are usually like those of saturated cyclic compounds. Aromatic heterocycles, like pyrrole, furan, and thiophene, on the other hand,

are more stable because the electrons are spread out across the ring. This makes them less likely to react with things that break up the aromatic system [4-6].

Pyrrole is a famous example of a five-membered aromatic heterocycle since it has a nitrogen atom as the heteroatom. The nitrogen atom in pyrrole gives the conjugated π system two electrons from its lone pair. This makes the ring aromatic. In six-membered rings like pyridine, on the extra indicator, the nitrogen molecule's lone pair is not part of the aromatic π system. Instead, it is in a sp^2 orbital that is at right angles to the plane of the ring. This means that pyridine is both aromatic and a rather strong base, which means it may function as a nucleophile in a number of processes [7-9].

Heterocyclic compounds come in more than just simple rings. A lot of physiologically essential compounds are made up of fused ring structures that include heteroatoms in them. For example, indole is a bicyclic molecule made up of a benzene ring and a pyrrole ring that are fused together. This structure is present in the amino acid tryptophan, the neurotransmitter serotonin, and several alkaloids. Purines and pyrimidines, which are important parts of DNA and RNA, are also fused heterocyclic systems. A pyrimidine ring is bonded to an imidazole ring to make purine. This is the basic structure of adenine and guanine [10-12].

Adding nitrogen, oxygen, or sulphur atoms to a ring can greatly influence how a molecule works in the body, make it easier to dissolve, or change how stable it is in the body. Piperidine, morpholine, and imidazole are examples of nitrogen-containing heterocycles that are often employed as scaffolds to make pharmacological candidates. The antiulcer medicine ranitidine has a furan ring, while the antibiotic ciprofloxacin, which is used a lot, has a piperazine ring [13-16].

There are several ways to make heterocyclic compounds, and each one is based on the size of the ring and the type of heteroatom that is present. The Paal–Knorr synthesis is one of the most well-known ways to make five-membered heterocycles. A thiophene is made when the same diketone is treated with a sulphurizing agent. A furan is made when the same diketone is treated with an acid and water. This approach shows that one intermediate structure may be utilised to make multiple heterocycles based on the reagent employed [17-23].

The Hantzsch synthesis is another frequent approach. It is a multicomponent reaction that makes 1,4-dihydropyridines. This happens when an aldehyde, a β-keto ester, and ammonia or an amine come together to make a new substance. The adaptability of multicomponent reactions such as these resides in the simplicity with which a wide array of products may be obtained by altering the initial components. The Biginelli reaction is also used to make dihydropyrimidinones by combining a β-keto ester, an aldehyde, and urea or thiourea in one pot [24-26].

The reactivity of heterocycles is affected by more than only their aromaticity and the kind of heteroatom. It is also affected by ring strain, substituents, and the fact that they can fuse with other rings. For instance, pyrrole is more likely to undergo electrophilic substitution than benzene. This is mostly because the nitrogen atom has a lot of electrons, which makes the ring more reactive. On the other hand, pyridine has an electronegative nitrogen atom that makes it electron-deficient. This means that it doesn't easily undergo electrophilic substitution but is more reactive in nucleophilic substitution processes [27-30].

People are also interested in the physical features of heterocyclic compounds, not just their reactivity. A lot of heterocycles don't melt or boil easily and can be dissolved in many different solvents. Their acidity or basicity is greatly affected by the heteroatoms and where they are in the ring. The nitrogen in pyridine has one pair of electrons, which makes it an excellent base and lets it operate as a ligand in coordination chemistry by easily giving electrons to metal centres [31-34].

Natural heterocyclic compounds are vital biological molecules. Nucleic acids are made up of nitrogenous bases such as adenine, guanine, cytosine, thymine, and uracil. All of these bases are heterocycles. These molecules couple with certain bases, which is an important part of how DNA and RNA are built and work. Alkaloids are a group of naturally occurring chemicals that generally have strong effects on the body. They also usually include heterocycles in them. Morphine, nicotine, and quinine are among examples. Vitamins like thiamine (vitamin B1) and riboflavin (vitamin B2) include heterocyclic rings, which shows how important they are in biochemistry [35-38].

Heterocyclic compounds are used in industry to make dyes, pigments, and materials. Indigo is a well-known dye that has an indole group in it. Polythiophene is a kind of conductive polymer that is made up of thiophene units. Because they transmit electricity and have good optical characteristics, they are utilised in organic electronics, solar cells, and sensors [39-41].

The study of heterocyclic chemistry is still growing, especially since that catalysis, green chemistry, and computer-aided drug design have all made progress. Transition-metal catalysed approaches, such palladium-catalyzed cross-coupling processes, have changed the way heterocycles may be made more useful and added to bigger molecular frameworks [42].

Heteroatoms are the non-carbon atoms, which are usually nitrogen, oxygen, or sulfur. Heterocyclic compounds are diverse, plentiful, and important because they have different structures and the heteroatoms in the ring give them distinct electrical characteristics. They are important to many areas of science because they are very stable, can take part in many different kinds of chemical processes, and work well with both natural and man-made systems. Heterocyclic chemistry is very important for making drugs, agrochemicals, dyes, polymers, and new materials. Heterocyclic frameworks are a part of the fundamental structure of

many physiologically active compounds, such as vitamins, hormones, and antibiotics. Purines and pyrimidines, two key types of nitrogen-containing heterocycles, make up nucleic acids. These are necessary for living things to store and pass on genetic information. Likewise, substances like morphine, quinine, and penicillin derive their biological action from the existence of heterocyclic rings. Heterocycles might be separated into two main clusters based on their structure: aliphatic (non-aromatic) and aromatic. This is based on how the electrons are arranged and how much conjugation there is in the ring. Delocalized π-electrons give aromatic heterocycles like pyridine, furan, and thiophene aromatic stability. This has a big effect on how they react and behave chemically. Additionally, heterocycles may be monocyclic, featuring a single ring, or polycyclic, comprising fused or bridged ring systems. The synthesis and functionalization of heterocyclic molecules remain a significant focus of study in organic and medicinal chemistry. Older ways of making things, including cyclization reactions, are now being used with newer. These improvements have made it easier to design and make heterocycles with different structures and features that are suited for certain uses [43-48].

Heterocyclic ring systems, particularly those including five or six members, are a substantial and structurally diverse category of organic compounds. These molecular frameworks are crucial in pharmaceutical and agricultural chemistry due to their many biological roles. A heterocyclic compound contains at least one heteroatom, often nitrogen, oxygen, or sulfur, inside its cyclic framework. They are crucial in pharmacology due to their significance in combating diseases affecting humans, animals, and plants. These molecules are present not only in synthetic pharmaceuticals but also in several essential life-sustaining substances. Examples include alkaloids, natural colors, hormones, enzymes, and many medicinal chemicals. It is estimated that almost 50% of all recognized natural bioactive compounds include heterocyclic structures [49-52].

1.2 Synthetic heterocycles

Synthetic heterocycles have been extensively employed in the development of pharmaceuticals, including anticancer, anti-inflammatory, antioxidant, antidiabetic, and anticonvulsant agents. They are utilized in the production of agrochemicals, dyes, and polymers, as well as in medicinal applications. Numerous heterocyclic structures possess significant promise as pharmaceuticals. Pyridine, pyrrole, furan, thiophene, imidazole, oxadiazole, thiadiazole, and iso-oxathiole are among the most recognized compounds. Each of these rings possesses distinct chemical characteristics that render them useful in biological systems and serve as promising foundations for pharmaceutical development and innovative chemical concepts [53-55].

Azole derivatives are a very important and well-studied group of heterocyclic compounds that include at least one nitrogen atom in a five-membered ring structure. These compounds have a heteroaromatic ring that has one or more nitrogen atoms in it. In many situations, it also has other heteroatoms, such oxygen or

sulfur. Their flexibility, stability, and capacity to engage with many biological targets render them essential scaffolds in medicinal chemistry and other disciplines [56].

There are two primary types of azoles: monoazoles, which have one nitrogen atom in the ring (like pyrrole and imidazole), and diazoles or triazoles, which have two or more nitrogen atoms (like pyrazole, oxazole, 1,2,3-oxadiazole, and triazole). These compounds have an aromatic structure, which means they can interact with other molecules in ways that increase their biological activity and chemical reactivity [57-63].

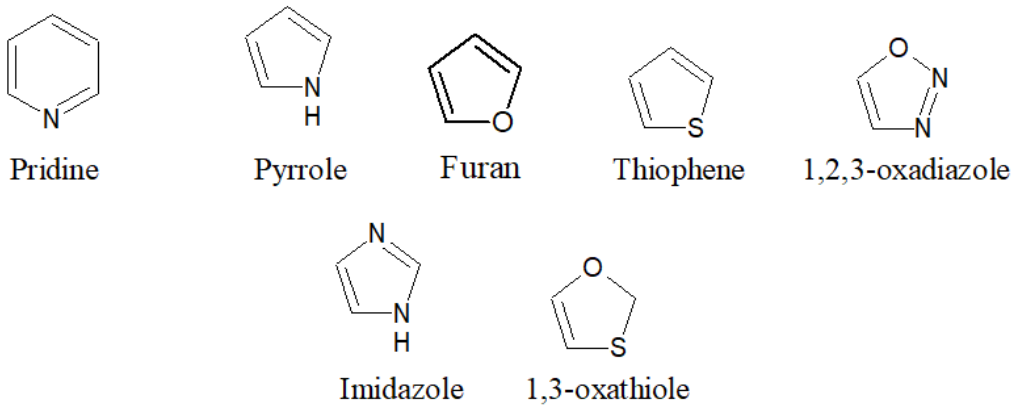


Figure 1.1: Various type of heterocyclic moiety

1.3 Antibacterial activity of heterocyclic compounds

Heterocyclic compounds have rings with at least one element that isn't carbon, such nitrogen, oxygen, or sulphur. They have long been known to be very important in medical chemistry. One of the most researched and used biological characteristics of heterocyclic compounds is their ability to kill bacteria. These chemicals are the main building blocks of many antibiotics and antimicrobial medicines, which makes them very important in the battle against bacterial infections [64-71].

Heterocyclic compounds are antibacterial because they can interact with bacterial enzymes, proteins, or nucleic acids, which stops important physiological processes from happening. The heteroatoms in the ring structure typically help these interactions by giving or taking hydrogen bonds, working with metal ions, or changing the electronic distribution in the molecule. Heterocyclic chemicals can stop bacteria from growing in a number of ways, such as by interfering with the manufacture of cell walls, affecting protein function, or stopping DNA replication [72-74].

Heterocyclic compounds have a lot of different chemical structures, which means they may be changed and improved in many ways to make them better at killing germs. For instance, pyridines, quinolines, and imidazoles, which are nitrogen-containing heterocycles, have been shown to be quite effective against both Gram-positive and Gram-negative bacteria. Adding substituents to the heterocyclic ring can change the lipophilicity, solubility, and capacity to get through bacterial cell membranes, which can affect how well they work [75].

Researchers are working harder to find new heterocyclic derivatives that work better and in different ways since certain bacteria are becoming resistant to antibiotics. A lot of heterocyclic scaffolds are used as lead molecules in drug development programs that try to get around resistance mechanisms. Also, hybrid compounds that combine heterocyclic cores with different pharmacophores have showed potential in making antibacterial properties stronger [76-77].

The disc diffusion method, broth dilution tests, and finding the minimum inhibitory concentrations (MICs) are all typical testing for antibacterial activity. These experiments help figure out how much heterocyclic chemicals stop different types of bacteria from growing, which helps with further structural refinement [78].

In short, heterocyclic compounds are still very important for developing antibacterial drugs since they can do a lot of different things chemically and biologically. There is a lot of hope that new heterocyclic frameworks and their derivatives may help solve the problems that bacterial infections and antibiotic resistance continue to cause [79].

1.4 Literature and review

Rajdeep Guha et al,. (2025), We were able to effectively develop and make a novel set of α, β-unsaturated carbonyl compounds (3a–j) and their pyrazoline derivatives (4a–e and 5a–b) for this work. We used ^1HNMR, 13CNMR, and massspectrometry to confirm the structures of all the compounds we made. We tested these compounds against five types of bacteria (Pseudomonas aeruginosa, Klebsiella pneumonia [80]

Kiran Kumar Vunnam et. al., (2024), This work is about making and designing new 2,4-thiazolidinedione derivatives that might be good anticancer drugs. Using a targeted synthetic method that focused on diversity at critical intermediates, we made 15 novel analogues. We got the important intermediates, by Knoevenagel condensation and acetal formation processes, respectively. These intermediates were N-alkylated, which allowed different functional groups to be added so that structure–activity relationships could be studied and the biological activity and drug-like features of the compounds could be improved [81].

SERAP BASOGLU et al., (2013), Novel heterocyclic compounds were synthesized using furan-2-carbohydrazide as a precursor. We employed secondary amines such as piperidine, piperazine, morpholine, and thiomorpholine to convert the 1,2,4-triazole derivative into various Mannich bases. A three-step procedure was employed to synthesize a crucial intermediate. We employed elemental analysis, infrared spectroscopy, proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, and mass spectrometry to confirm that all synthesized compounds have the correct structure. Preliminary antimicrobial screening indicated that several compounds had promising activity against particular microbial strains, suggesting their potential for future pharmaceutical development [82].

Serap Basoglu et. al., (2013), We synthesized three novel heterocyclic systems from furan2carbohydrazide: The 1,2,-triazole molecule was then transformed into several Mannich base derivatives by condensation with diverse secondary amines, such as piperidine, piperazine, morpholine, and thiomorpholine. We employed elemental analysis, infrared spectroscopy, proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, and mass spectrometry to validate the structures of all synthesized compounds. The efficacy of the compounds in eradicating bacteria was evaluated, revealing that some exhibited potent activity against certain bacterial strains [83].

MELTEM YOLAL et. al., (2013), We synthesized three novel heterocyclic compounds from furan-2-carbohydrazide: The triazole derivative was converted into Mannich bases using various secondary amines. Three phases were required to synthesize a significant triazole-thiol compound. Spectral and elemental studies confirmed all structures, with some demonstrating potential as antimicrobials [84].

Małgorzata Strzelecka et. al., (2021), The increasing danger of bacterial medication resistance makes it clear that we need new, safe, and effective antimicrobial medicines right away. The 1,2,4-triazole ring is one of several heterocyclic systems that has gotten a lot of interest since it has a wide range of biological activities, especially significant antibacterial capabilities. Comprehensive research has established the potential of triazole-based derivatives as viable antibacterial agents. Ongoing study and systematic design utilizing this structure may substantially aid in combating microbial resistance and facilitating the advancement of next-generation antibacterial agents [85].

Charles O. Nwuche et . al., (2017), We employed agar well diffusion, MIC determination, and molecular docking to evaluate the antibacterial efficacy of 1-chloro-2-isocyanatoethane derivatives of thiomorpholine (CTC), piperazine (CPC), and morpholine (CMC). They were evaluated against ten distinct bacterial strains and four yeast varieties. In silico analyses demonstrated their strong binding affinity to DNA gyrase, a critical antibacterial target [86].

Oguejiofo T Ujam et al., (2017), We used agar well diffusion, MIC analysis, and molecular docking to test the antibacterial capabilities of 1-chloro-2-isocyanatoethane derivatives of thiomorpholine (CTC), piperazine (CPC), and morpholine (CMC). Fourteen microbiological strains were examined, comprising 10 bacteria and four yeasts. In silico investigations demonstrated the chemicals' interaction with DNA gyrase, an established antibacterial target. Thiomorpholine and piperazine derivatives had no efficacy against the assessed Gram-negative bacteria [87].

Aim and Objective

Aim

To synthesize a series of novel heterocyclic compounds and evaluate their antibacterial activity against selected bacterial strains.

Objectives

1. To develop and optimize a synthetic route for the preparation of heterocyclic compounds using substituted aldehydes.
2. To purify and characterize the synthesized compounds through physicochemical methods, including melting point determination and thin-layer chromatography (TLC).
3. To confirm the structural identity and purity of the synthesized compounds using spectral techniques such as infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and liquid chromatography-mass spectrometry (LC-MS).
4. To assess the antibacterial activity of the synthesized heterocyclic compounds against common pathogenic bacteria using standard in vitro assays.
5. To analyze the structure-activity relationship (SAR) of the synthesized compounds to identify functional groups or structural features contributing to antibacterial efficacy.

Plan of Work

1. **Literature Survey**
   Conduct a thorough review of existing research on heterocyclic compounds with antibacterial activity to identify suitable synthetic routes and biological assay methods.
2. **Selection of Starting Materials**
   Choose appropriate substituted aldehydes and other reagents for the synthesis of target heterocyclic compounds.
3. **Synthesis of Heterocyclic Compounds**
   Carry out the chemical reactions under optimized conditions to synthesize a series of heterocyclic derivatives.
4. **Purification of Synthesized Compounds**
   Purify the reaction products by recrystallization or chromatography techniques to obtain pure compounds.
5. **Preliminary Characterization**
   Determine melting points and perform Thin Layer Chromatography (TLC) to assess purity and confirm the formation of new compounds.
6. **Spectral Characterization**
   Use IR spectroscopy, NMR spectroscopy, and LC-MS to confirm the molecular structure and purity of the synthesized compounds.

7. **Biological Evaluation**

   Test the antibacterial activity of the synthesized compounds against selected bacterial strains using disc diffusion or broth dilution methods.

Chapter 2

Material and Methods

2.1 Synthesis of Schiff base [88-90]

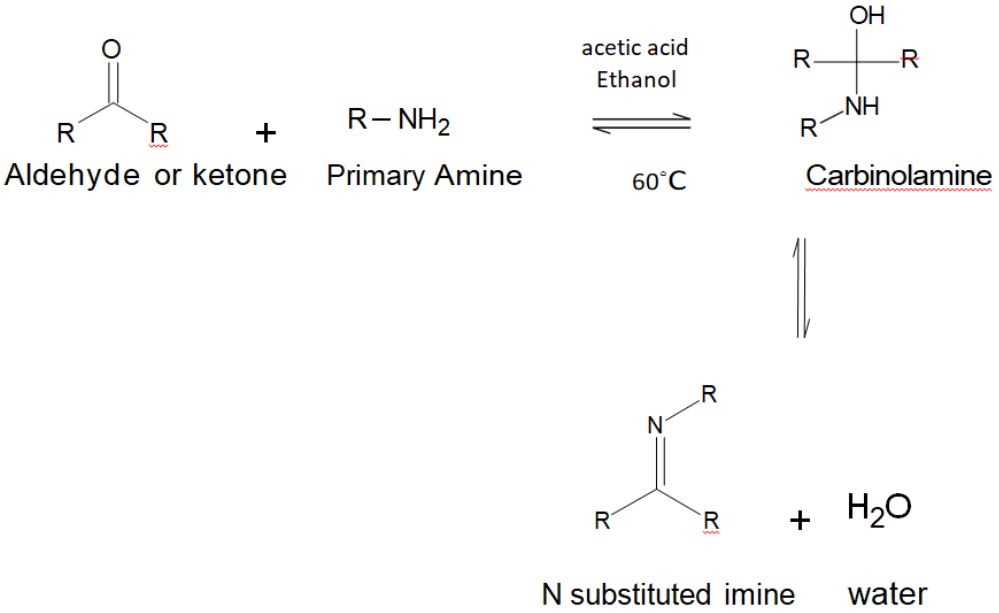


Figure 2.1: Synthesis of Schiff base

2.1.1 Procedure

Synthesis of Schiff Base via Reaction Combination

In 20 mL of 100% ethanol, a ketone or aldehyde was mixed with a primary amine in equal amounts (0.01 mol each). Five millilitres of glacial acetic acid were added into this solution as a catalyst to expedite the condensation process. The reaction mixture was heated to 60 °C for 24 hours to ensure complete formation of the imine (Schiff base).

2.1.2 Amines Used in the Reactions

- 3,5-Dichloro-4-aminopyridine
- 5-Amino-1,3,4thiadiazole-2-thiol
- Nitroaniline (para-nitroaniline or meta-nitroaniline, as applicable)

2.1.3 Carbonyl Compounds Used (Aldehydes):

- Pyridine-3-carboxaldehyde
- Anisaldehyde(para-methoxybenzaldehyde)
- *p*Chloroacetophenone
- 3-Methylcyclopentane-1,2-dione
- Isatin (1H-indole-2,3-dione)

2.1.4 Reactant summary

1. 3,5-Dichloro-4-aminopyridine

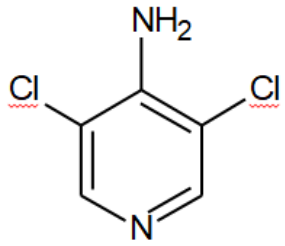


Figure 2.1.4: 3,5-Dichloro-4-aminopyridine

Compound Profile: 3,5-Dichloro-4-pyridinamine

- **Synonym**: 3,5Dichloro-4pyridinamine
- **Appearance**: White crystalline residue
- **Molecular Formula**:$C_5H_4Cl_2N_2$
- **Molecular Weight**:163 g/mol
- **Melting Point** 159 °C
- **Solubility**: Freely soluble in chloroform, dimethyl sulfoxide), and methanol

2.1.5 5-Amino-1,3,4-thiadiazole-2-thiol

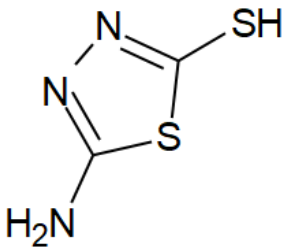


Figure 2.1.5: 5-Amino-1,3,4-thiadiazole-2-thiol

Synonym: 2-Amino-5-mercapto-1,3,4-thiadiazole

Appearance: Pale yellow to cream-colored powder

MolecularFormula: $C_2H_3N_3S_2$

Molecular Mass: 133.2g/mol

Melting Point:235 °C

Solubility: Soluble in chloroform and DMSO

2.1.6**. p-Nitroaniline**

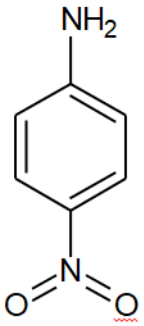


Figure 2.1.6: *p*-**Nitroaniline**

Compound Profile: p-*Nitroaniline*

- **Synonym**: 4-Nitroaniline
- **Appearance**: Pale yellowcrystallinesolid
- **Molecular Formula**: $C_6H_6N_2O_2$
- **Molecular Mass**: 138.12 g/mol
- **Melting Point**: 146–149 °C
- **Solubility**: Slightly soluble in water; soluble in ethanol, ether, and acetone

2.1.7 Compound Profile: Pyridine-3-carboxaldehyde

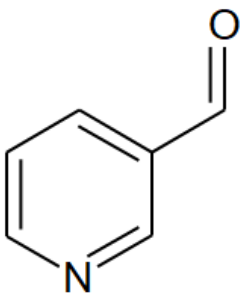


Figure 2.1.7: Pyridine-3-carboxaldehyde

- **Synonym**: 3-Formylpyridine
- **Appearance**: Colorless to pale yellow liquid or crystalline solid (depending on purity and temperature)
- **Molecular Formula**: C_6H_5NO

- **Molecular Mass**: 107.11 g/mol
- **Melting Point**: ~39–42 °C
- **Boiling Point**: ~213–215 °C
- **Solubility**: Soluble in water, ethanol, DMSO, and most polar organic solvents

2.1.8 Compound Profile: Anisaldehyde

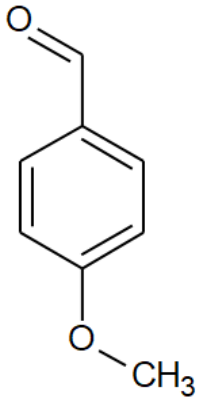


Figure 2.1.8: Anisaldehyde

- **Synonym**: 4-Methoxybenzaldehyde (commonly refers to *para*-anisaldehyde)
- **Appearance**: Colorless to pale yellow liquid with a pleasant, sweet aromatic odor
- **Molecular Formula**: $C_8H_8O_2$
- **Molecular Mass**:136.15 g/mol
- **Melting Point**:2–5 °C
- **Boiling Point**: 248 °C
- **Solubility**: Soluble in organic solvents like ethanol, ether, chloroform; slightly soluble in water

2.1.9 Compound Profile: p-*Chloroacetophenone*

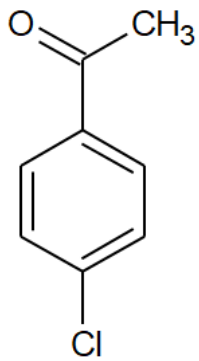


Figure 2.1.9: *p*-Chloroacetophenone

- **Synonym**: 4'-Chloroacetophenone, 1-(4-Chlorophenyl)ethan-1-one
- **Appearance**: White to off-white crystalline solid
- **Molecular Formula**:C_8H_7ClO
- **Molecular Mass**:154.59g/mol
- **Melting Point**: 52–55 °C
- **Boiling Point**: ~245–247 °C
- **Solubility**: Soluble in ethanol, acetone, chloroform; slightly soluble in water

2.1.10 Compound Profile: 3-Methylcyclopentane-1,2-dione

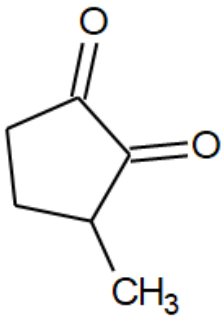


Figure 2.1.10: 3-Methylcyclopentane-1,2-dione

- **Synonym**: 3-Methyl-1,2-cyclopentanedione
- **Appearance**: Yellowish crystalline solid or oil (depending on purity and temperature)
- **Molecular Formula**: $C_6H_8O_2$
- **Molecular Weight**: 112.13 g/mol
- **Melting Point**: ~28–30 °C (may vary slightly with purity)
- **Solubility**: Soluble in ethanol, ether, and most organic solvents

2.1.11 Compound Profile: Isatin

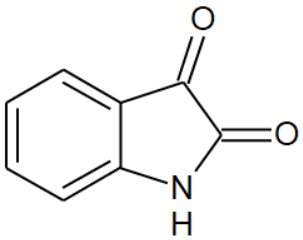


Figure 2.1.11: Isatin

- **Synonym**: 1H-Indole-2,3-dione
- **Appearance**: Orange to reddish-brown crystalline solid
- **Molecular Formula**: $C_8H_5NO_2$
- **Molecular Weight**: 147.13 g/mol

- **Melting Point**: 197–200 °C
- **Solubility**: Soluble in ethanol, acetone, DMSO, and slightly soluble in water

- **Appearance:** The compound presents as an orange-colored powder, indicating potential chromophoric groups within its structure.
- **Molecular Formula:** $C_8H_5NO_2$
- **Molecular Weight:** Approximately 147.13 g/mol, which reflects a relatively low molecular mass, suitable for various organic synthesis applications.
- **Melting Point:** Exhibits a high melting point of around 200 °C, suggesting strong intermolecular forces or crystalline stability.
- **Boiling Point:** The boiling point is around 170 °C, though typically this applies to less stable derivatives or under reduced pressure, considering its decomposition above the melting point.
- **Solubility:** The compound is soluble in organic solvents such as methanol and chloroform, indicating moderate polarity and compatibility with polar aprotic and protic media.

2.2 Identification of compounds (SC1-SC5)

The purified novel Heterocyclic compounds were identified and characterized using the following analytical techniques [91-94]:

2.2.1 Melting point of compound ((SC1-SC5)

We used a LABHOSP melting point equipment to find out the melting points of the produced chemical derivatives. A little quantity of each compound was put into a capillary tube that was sealed at one end. The temperature at which the compound started to melt was written down. The measurements were taken as they were and are reported as such [95-98].

2.2.2 Solubility of compound (SC1-SC5)

We tested the solubility of the produced compounds (**SC1-SC5**) in different solvents to see how they behaved based on their polarity and if they could be used for further formulation or biological testing. They put a modest quantity of each component (around 5–10 mg) in separate test tubes. We put 2 mL of solvent (such water, acetone, chloroform, DMSO, ethanol, methanol, ethyl acetate, and DMF) into each test tube one at a time. After that, the mixes were mixed well and left at room temperature for 15 to 20 minutes [99].

2.2.3 Physical properties of compound (SC1-SC5)

2.2.3.1 Molecular Weight and Molecular Formula

We figured out the molecular formula for each of the five compounds we made **(SC1-SC5)** based on the initial ingredients we utilized and then validated it using elemental analysis. We used the normal atomic weights of the parts to figure out the molecular weight [100-103].

2.2.3.2 Yield in Percent

After purification, the actual yield was compared to the anticipated yield to find the percentage yield of each constituent. The formula that was utilized was [104-106]:

$$\%\text{Yield} = \left(\frac{\text{Practical Yield}}{\text{Theoretical Yield}} \right) \times 100$$

2.2.3.3 Elemental Analysis (CHN/CHNCl Analysis)

Using an elemental analyzer, we found out how much carbon (C), hydrogen (H), nitrogen (N), oxygen (O), and chlorine (Cl) were in the sample. This information was utilized to check the empirical composition and the purity of the compounds that were made. The results were shown as a percentage of each part of the compound [107-109].

2.2.4 Thin Layer Chromatography (TLC) (SC1-SC5)

We employed Thin Layer Chromatography (TLC) to monitor the reaction, assess the purity of the chemicals, and identify the respective derivatives **(SC1-SC5)**. The stationary phase consisted of pre-coated silica gel plates (Merck 604 GF254). A minimal quantity of each synthesized chemical was dissolved in a limited volume of an appropriate solvent. A fine capillary tube was thereafter employed to apply a tiny area around 1–1.5 cm above the base of the TLC plate. Subsequently, the plates were placed in a TLC chamber filled with the appropriate solvent solution, selected meticulously based on the polarity of the compounds. The solvent front was let to advance until it reached approximately three-fourths of the distance down the plate. Upon development, the plates were removed, dried, and examined under UV light at 254 nm. The corresponding dots for each chemical were readily discernible. We employed the formula to calculate the Rf (retention factor) values [110-11]2:

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

2.2.5 IR Spectroscopy

We employed infrared (IR) spectroscopy to identify significant functional groups and assist in validating the structures of the synthesized molecules. The IR spectra were recorded between 4000 cm^{-1} and 666 cm^{-1}, including the spectrum of vibrational transitions of several chemical bonds within the molecule. The potassium bromide (KBr) pellet method was utilized to prepare the samples. A little amount of the produced compound was coarsely pulverized and mixed with anhydrous KBr powder. A hydraulic press was subsequently employed to compact the mixture into a transparent pellet. We employed a SHIMADZU FTIR 8400 spectrophotometer to analyze the KBr pellets we prepared. The spectra exhibited characteristic

absorption peaks for functional groups such as –NH, –OH, –C=O, –C=N, and aromatic C–H bonds. The position and intensity of the peaks in the IR spectrum indicated that the synthesized oxadiazole derivatives have certain structural characteristics [113-116].

2.2.6 Nuclear Magnatic Resonance Spectroscopy

We employed Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy to elucidate the chemical structure and proton environments of the synthesized compound derivatives. This approach provides comprehensive information on the number of hydrogen atoms, their chemical environment, and adjacent groups, which is crucial for elucidating the structure. A BRUKER AVANCE NEO-500 MHz spectrometer was employed to acquire the ^{1}H NMR spectra. The materials were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6), with tetramethylsilane (TMS) acting as an internal reference for the calibration of chemical shifts [117-119].

- The obtained spectral data enabled the identification of:
- The number and categorization of hydrogen atoms,
- The patterns of splitting (singlet, doublet, triplet, multiplet).
- The values for the chemical shift (δ, in parts per million)
- The integration corresponding to the number of protons in each configuration.

2.2.7 Procedure for Mass Spectrometric Analysis [120]

- We employed mass spectrometry (MS) to ascertain the molecular weight and facilitate the precise identification of the structures of the compounds we synthesized. This analytical technique is highly effective in identifying molecular ions and fragmentation patterns that aid in determining the molecular formula.
- We employed a MICROMASS Q-TOF micro mass spectrometer to get the mass spectra. In high vacuum conditions, a little quantity of each molecule was ionized, and the resulting ions were analyzed according to their mass-to-charge ratio (m/z).
- The spectra obtained provided us with: Accurate molecular ion peaks according to the molecular weight. Fragmentation patterns exhibiting structural components, Data utilized for comparison with traditional digital mass spectrum libraries, aiding in the confirmation of a substance's identity.

2.3 Antibacterial activity of Heterocyclic compounds

2.3.1 Microbiology

The Section of Biotechnology at CCSUniversity in Meerut, India, provided all of the microbial strains used for the biological evaluation. Among the bacteria, there were Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus,. The recently made mixtures have been carefully

pondered and mixed with dimethylsulfoxide (DMSO) to make stock solutions at a concentration of 5,000 µg/mL. These stock combination were then used for antimicrobial screening tests [121].

2.3.2 Agar-well diffusion method

The antibacterial efficacy of the synthesized compounds was assessed using the agar well diffusion method, as previously described with some modifications. We cultured each microbial strain in Mueller Hinton (MH) broth and adjusted the concentration to approximately 10^6 colony-forming units (CFU)/mL. The standardized suspensions were uniformly distributed across the surface of Mueller Hinton Agar for microbial straining and Sabouraud Dextrose Agar for fungal types, particularly Candida albicans and Candida tropicalis. Employing a disinfected corn bit, mineshafts of 5mm in diameter have meticulously formed in the agar. Subsequently, 50µL of every chemical solution has presented addicted to the boreholes. The dishes have been preserved at 35°C for 18 hrs. Subsequent gestation, the widths of the reserve regions adjacent all healthy remained leisurely in millimeters to measure their antimicrobial efficacy. Ampicillin (10 µg) served as the standard reference drug, whilst dimethyl sulfoxide (DMSO) and ethanol acted as negative (solvent) controls to ensure that the observed effects were solely due to the test compounds [122].

Chapter 3

Results and discussion

3.1 Synthetic Work

Using a simple synthetic method, we were able to make a sequence of (SC1–SC5) from different substituted aldehydes. The reaction technique produced the desired chemicals, with isolated yields varying from 68% to 80%. Using spectrum analysis tools, we were able to figure out the structure of the synthesized molecules and validate the creation and identification of each component. Furthermore, clear capillary tubes were utilized to ascertain the melting points. These results provide a foundation for subsequent physicochemical and biological studies of the produced molecules.

3.2 Synthesis and Preliminary Evaluation

Five target compounds were made successfully and then recrystallized to make them more pure. We first checked the purity of each chemical by measuring its melting point. This was a simple way to see if they were all the same and if there were any impurities. We also used Thin Layer Chromatography (TLC) to be sure that new chemical entities had formed. TLC examination showed that there were no starting materials and that the synthesis was successful by showing different spots that matched the new products.

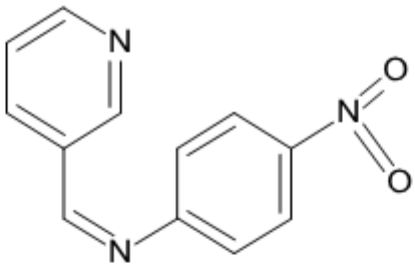
Table 3.1: Physicochemical Characteristics of Synthesized Compounds

S. No.	Compound Code	Yield (%)	Melting Point (°C)	Rf Value
1	SC1	74	142–144	0.72
2	SC2	65	141–142	0.65
3	SC3	79	197–199	0.67
4	SC4	62	207–211	0.82
5	SC5	77	167–168	0.80

The Rf values of the synthesised compounds were very different from those of the starting materials, which means that the reactions were successful. Also, the fact that each combination had a small melting point range supports the idea that the products were quite pure.

Advanced spectroscopic methods helped to establish the structural integrity and purity even more. We used infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), and mass spectrometry (MS) to find out the functional groups, molecular structures, and molecular weights of the compounds we made.

3.3 Compounds profile

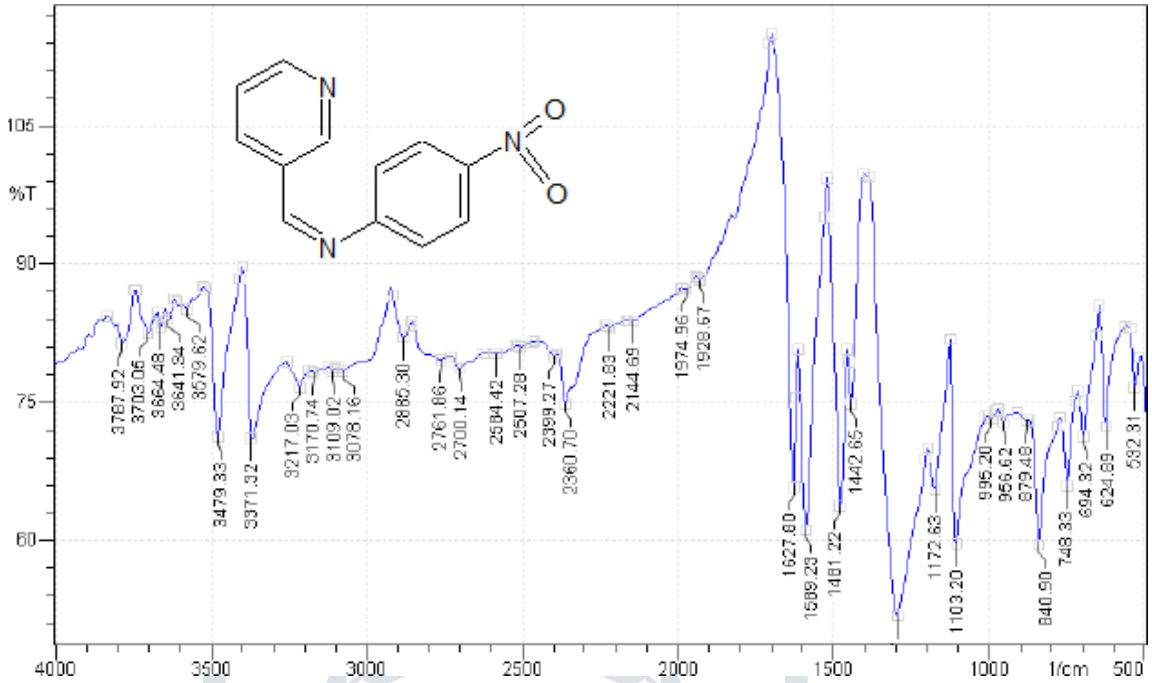


4-nitro-N-[(Z)-pyridin-3-ylmethylidene]aniline

Molecular Formula	$C_{12}H_9N_3O_2$
Formula Weight	227.21
Appearance	Yellow
Composition	C(63.43%) H(3.99%) N(18.49%) O(14.08%)
Molar Refractivity	64.51 ± 0.5 cm^3
Molar Volume	183.5 ± 7.0 cm^3
Parachor	493.0 ± 8.0 cm^3
Index of Refraction	1.620 ± 0.05
Surface Tension	52.0 ± 7.0 dyne/cm
Density	

3.3.1 Compound No. SC1

IR Spectra of SC1



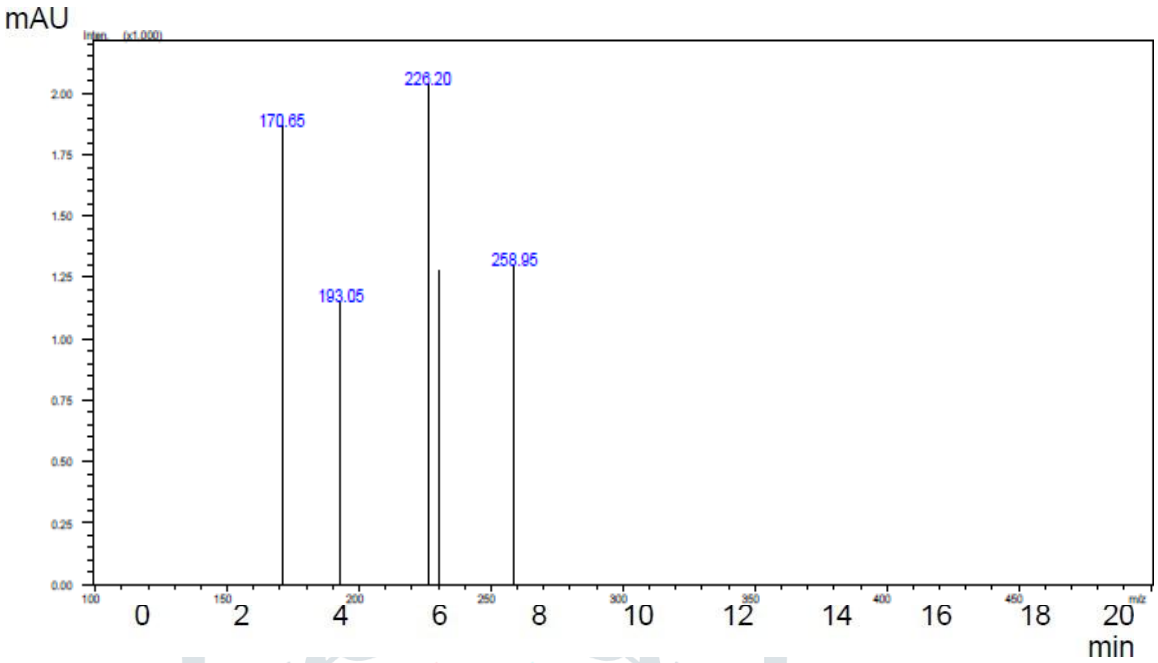
INTERPRETATION OF IR SPECTRUM SC1:

Table 3.2: Interpretation IR spectrum of SC1

S. No.	Wavenumber (cm^1)	Functional group
1	3109.02	C-H Stretching (Aromatic)
2	1627.23	C=N Stretching
3	1559.23	-NO Stretching

LC-MS Chromatography of SC1

Molecular mass: 227.22g/mol



NMR Spectra of SC1

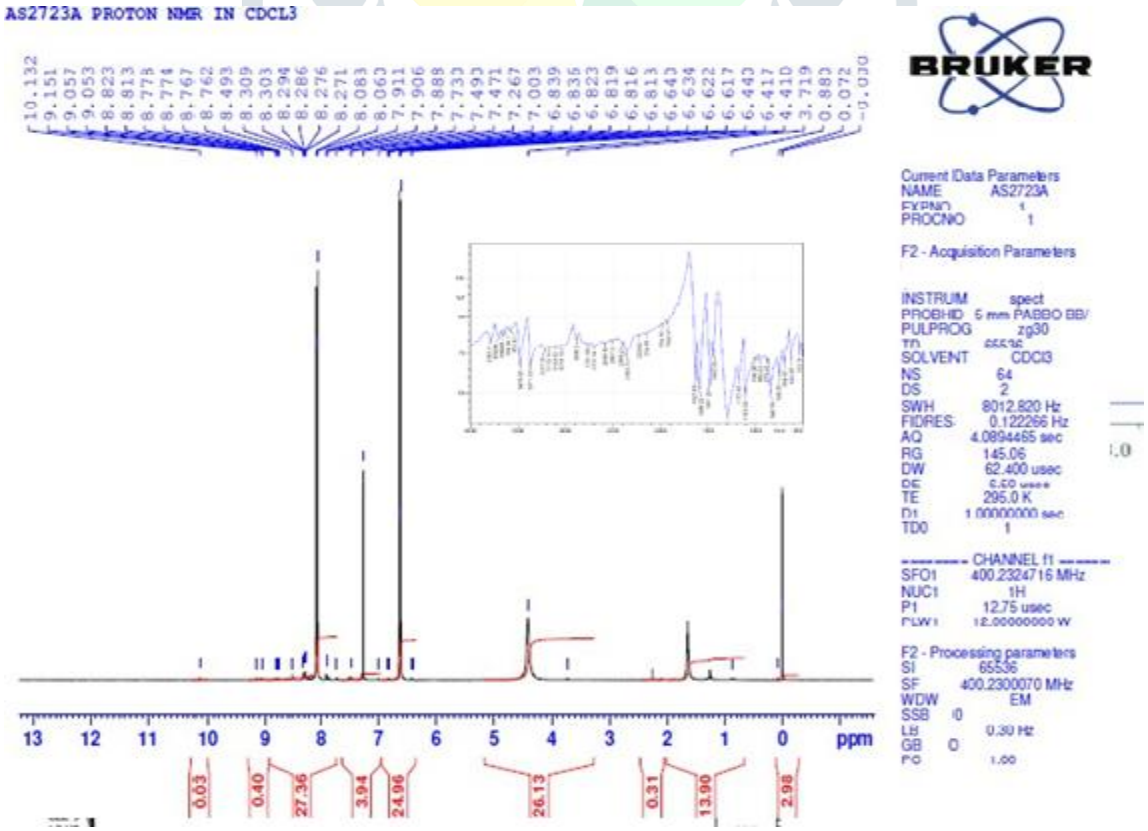
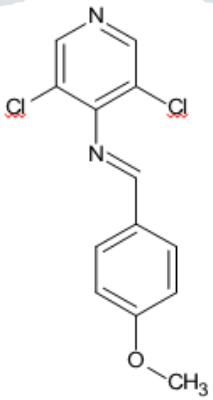


Table 3.3: NMR Spectral Interpretation for SC1 Reaction Compounds

S. No.	Chemical Shift (δ, ppm)	Signal Type	Proton Count
1	3.7 – 6.3	Multiplet	3 Protons
2	6.9 – 8.0	Multiplet	6 Protons

3.3.2 Compound No. SC2



3,5-dichloro-*N*-[(*E*)-(4-methoxyphenyl)methylidene]pyridin-4-amine

Molecular Formula	$C_{13}H_{10}Cl_2N_2O$
Formula Weight	281.13
Composition	C(55.54%) H(3.59%) Cl(25.22%) N(9.96%) O(5.69%)
Molar Refractivity	73.87 ± 0.5 cm^3
Molar Volume	218.5 ± 7.0 cm^3
Parachor	555.5 ± 8.0 cm^3
Index of Refraction	1.591 ± 0.05
Surface Tension	41.7 ± 7.0 dyne/cm
Density	1.28 ± 0.1 g/cm^3
Polarizability	29.28 ± 0.5 $10^{-24} cm^3$

IR Spectra of SC2

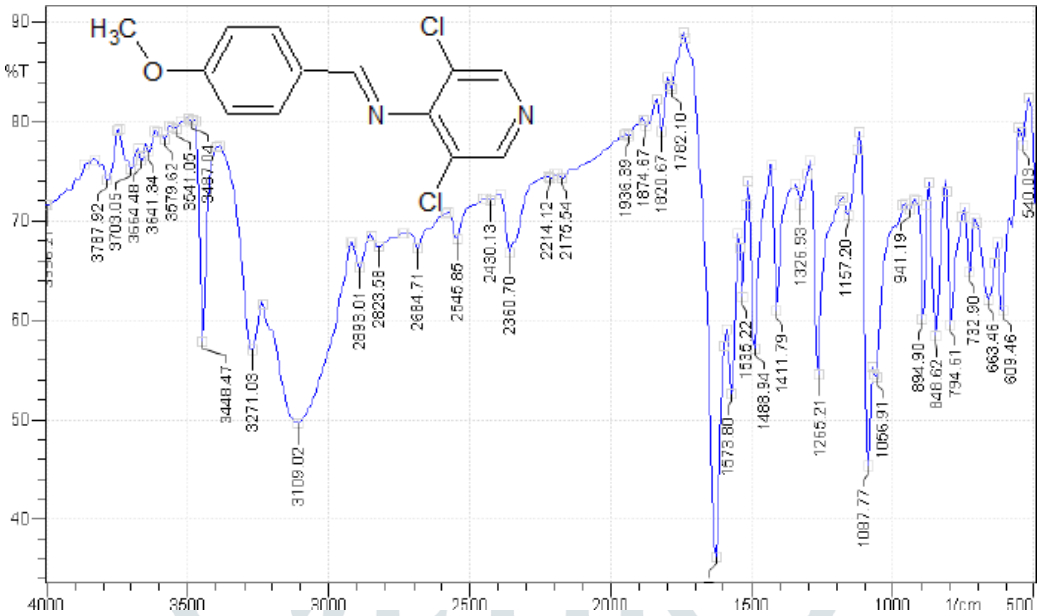
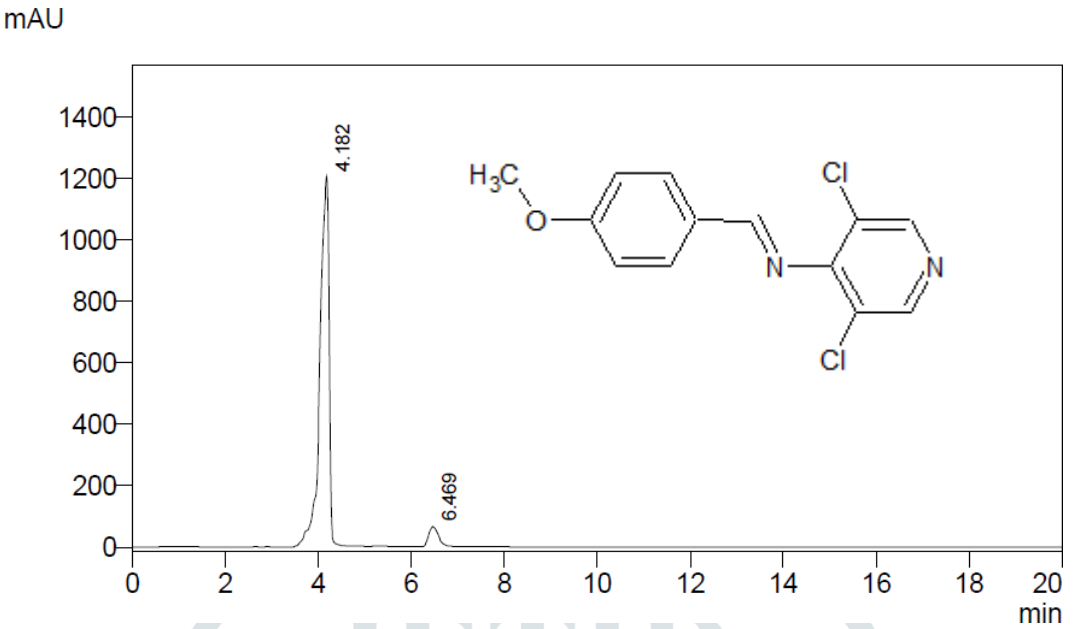
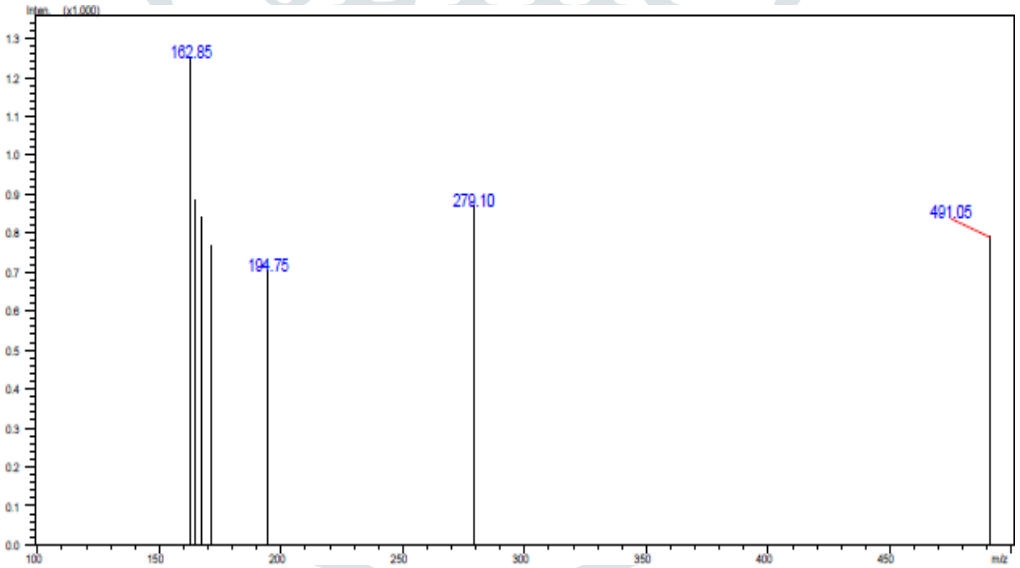


Table 3.4: IR Spectral Analysis for SC2 Compound

S. No.	Wavenumber (cm^{-1})	Assigned Functional Group
1	3109.02	Aromatic C–H Stretch
2	732.9	C–Cl Stretch
3	1573.8	C=N Stretch

LC-MS Chromatography of SC2





NMR Spectra of SC2

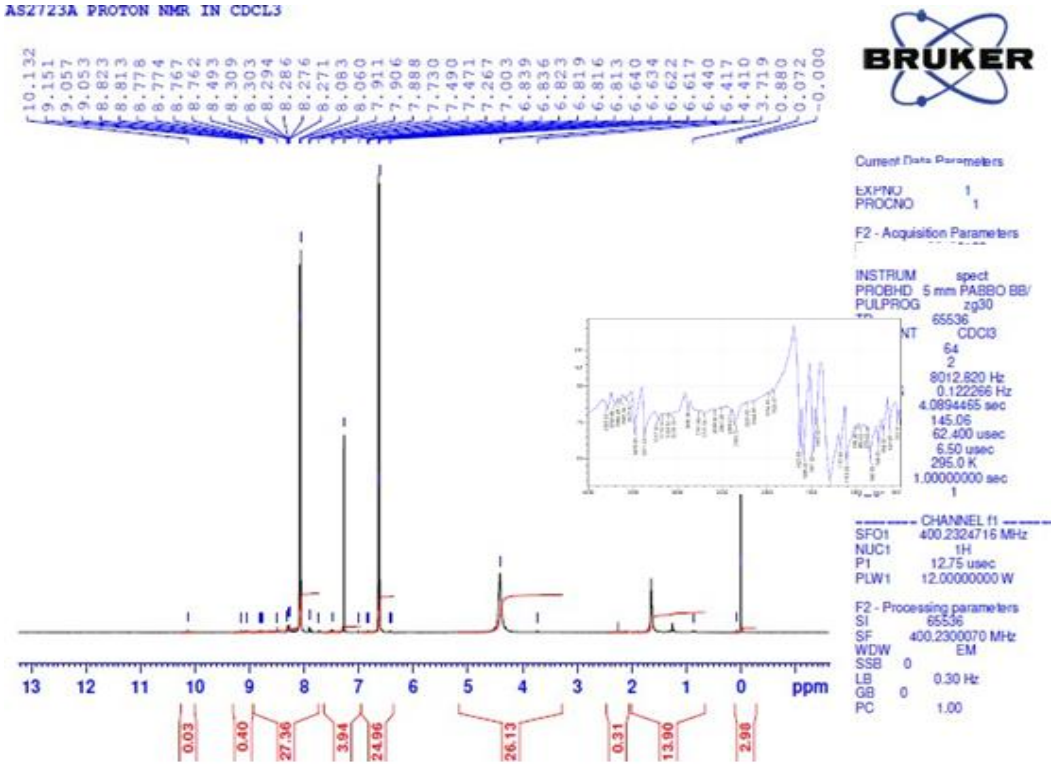
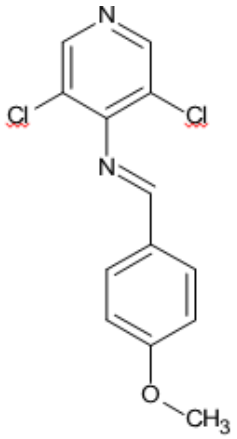


Table 3.5: NMR Spectral Data for SC2 Compound

S. No.	Chemical Shift (δ, ppm)	Signal Pattern	Proton Count
1	3.7 – 6.3	Multiplet	3 Protons
2	6.9 – 8.0	Multiplet	6 Protons

3.3.3 Compound No. SC3



3,5-dichloro-*N*-[(*E*)-(4-methoxyphenyl)methylidene]pyridin-4-amine

Molecular Formula	$C_{13}H_{10}Cl_2N_2O$
Formula Weight	281.13
Composition	C(55.54%) H(3.59%) Cl(25.22%) N(9.96%) O(5.69%)
Molar Refractivity	73.87 ± 0.5 cm^3
Molar Volume	218.5 ± 7.0 cm^3
Parachor	555.5 ± 8.0 cm^3
Index of Refraction	1.591 ± 0.05
Surface Tension	41.7 ± 7.0 dyne/cm
Density	1.28 ± 0.1 g/cm^3
Polarizability	29.28 ± 0.5 $10^{-24}cm^3$

IR Spectroscopy of SC3

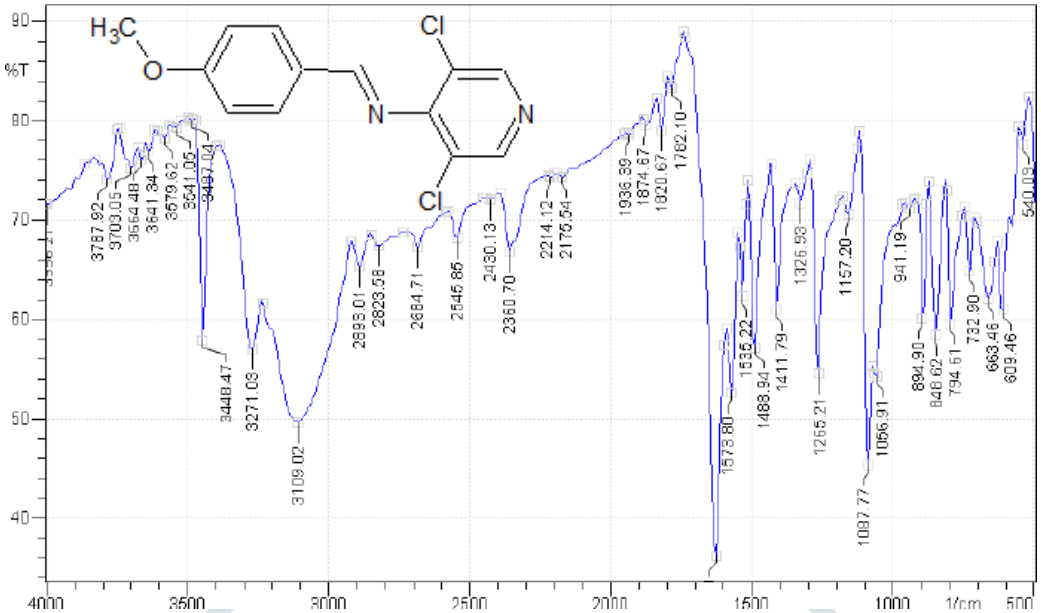
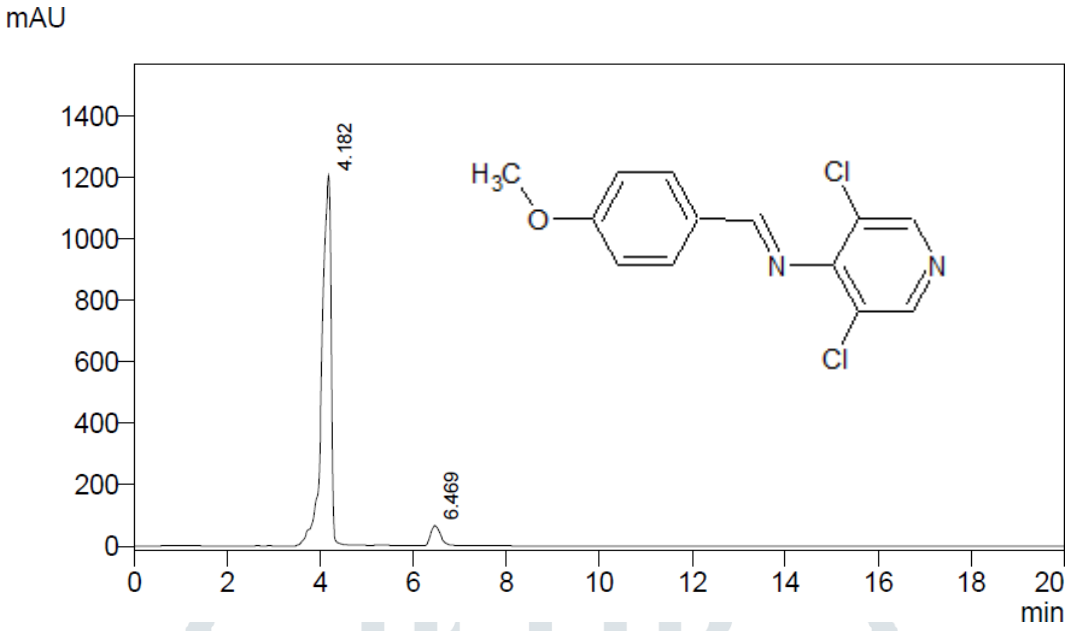
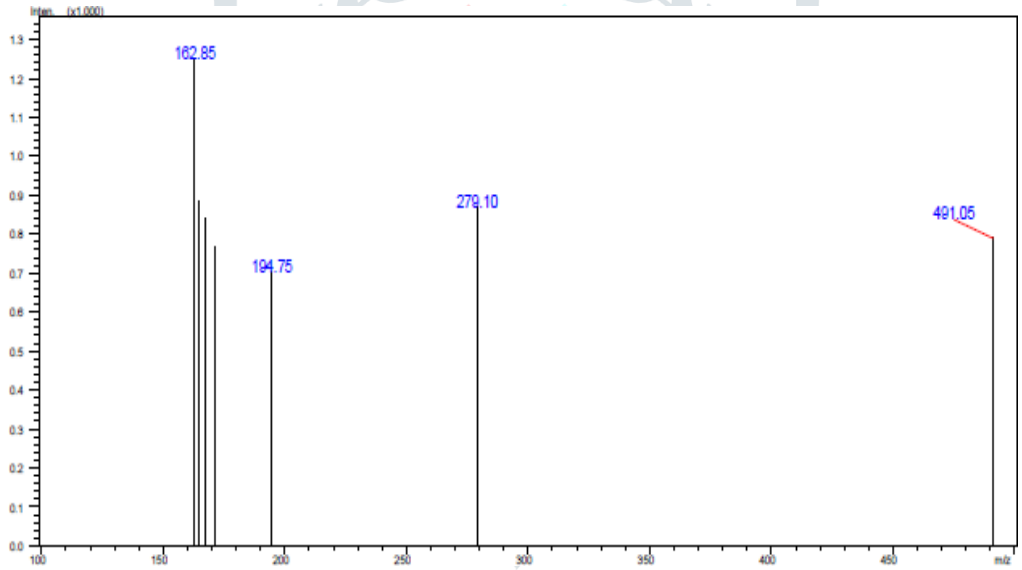


Table 3.6: IR Spectral Interpretation for SC3 Compound

S. No.	Wavenumber (cm^{-1})	Functional Group Identified
1	3109.02	Aromatic C–H Stretching
2	732.9	C–Cl Bond Stretching
3	1573.8	C=N (Imine) Stretching

LC-MS Spectra of SC3





NMR Spectra of SC3

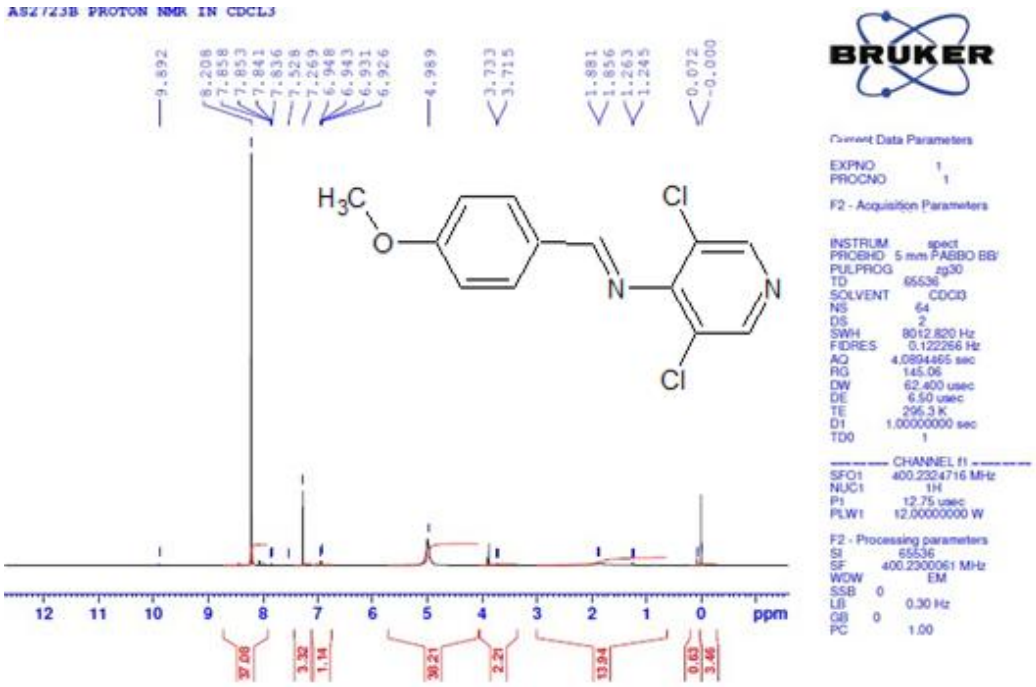
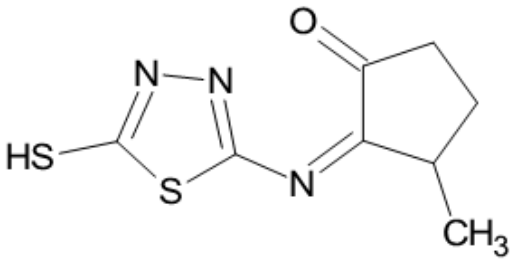


Table 3.7: Interpretation of NMR spectrum of SC3

S. No.	ð Value	Nature of Peak	No. of Protons
1	3.8-4.9	Multiplet	4
2	6.8-7.9	Multiplet	6

3.3.4 Compound No. SC4



(2Z)-3-methyl-2-[(5-sulfanyl-1,3,4-thiadiazol-2-yl)imino]cyclopentanone

Molecular Formula	$C_8H_9N_3OS_2$ 227.30
Formula Weight	C(42.28%) H(3.99%) N(18.49%) O(7.04%)
Composition	S(28.21%)
	59.27 ± 0.5 cm^3
Molar Refractivity	139.0 ± 7.0 cm^3
Molar Volume	389.3 ± 9.0 cm^3
Parachor	1.806 ± 0.07
Index of Refraction	64.2 ± 7.1 dyne/cm
Surface Tension	1.65 ± 0.1 g/cm^3
Density	23.50 ± 0.6 $10^{-24} cm^3$
Polarizability	

IR spectroscopy of SC4

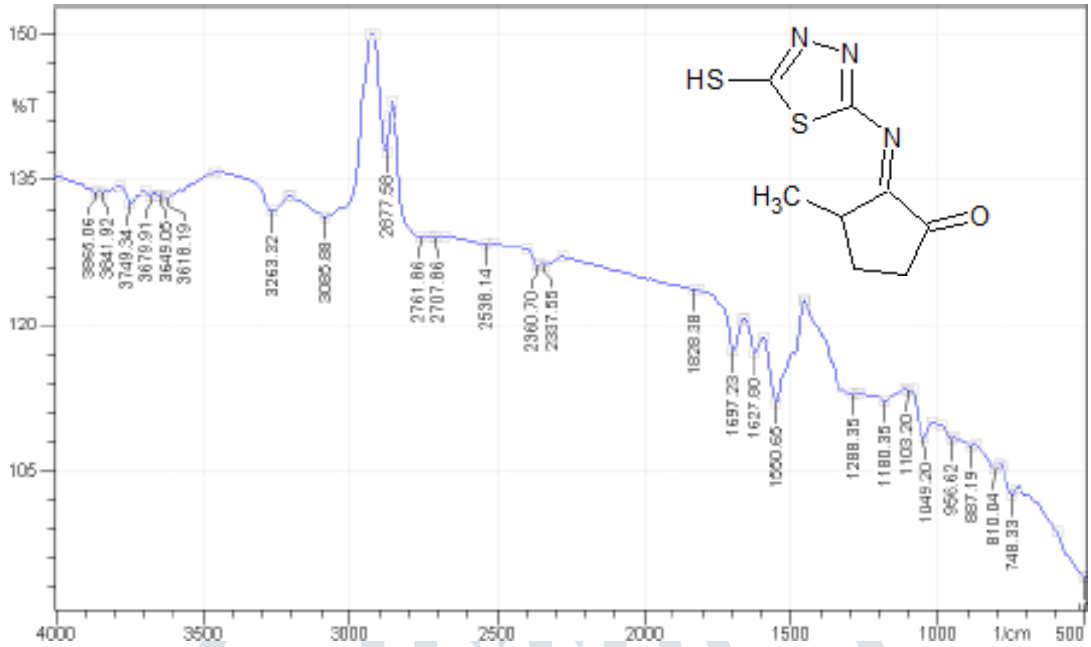
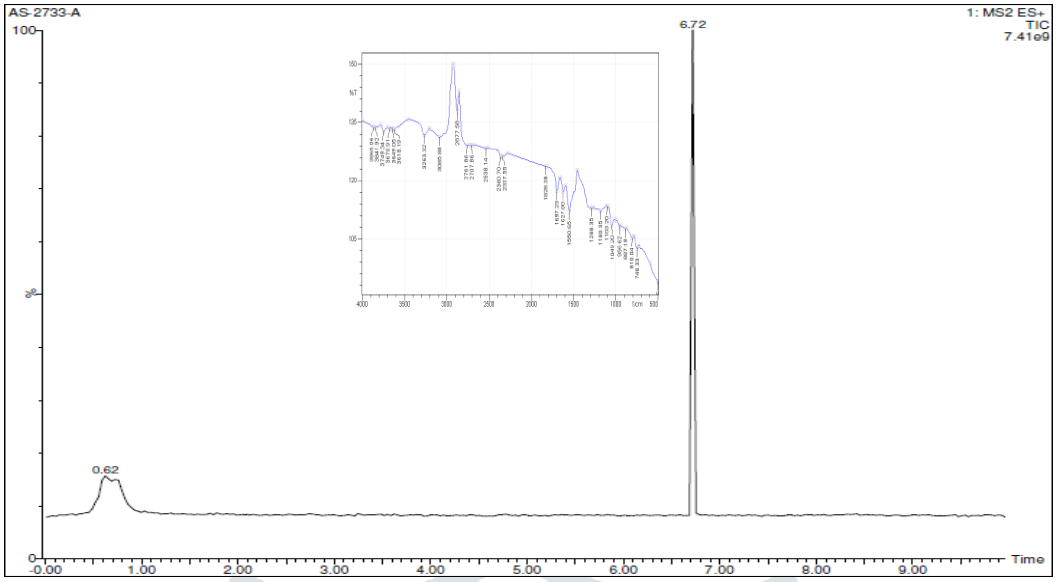
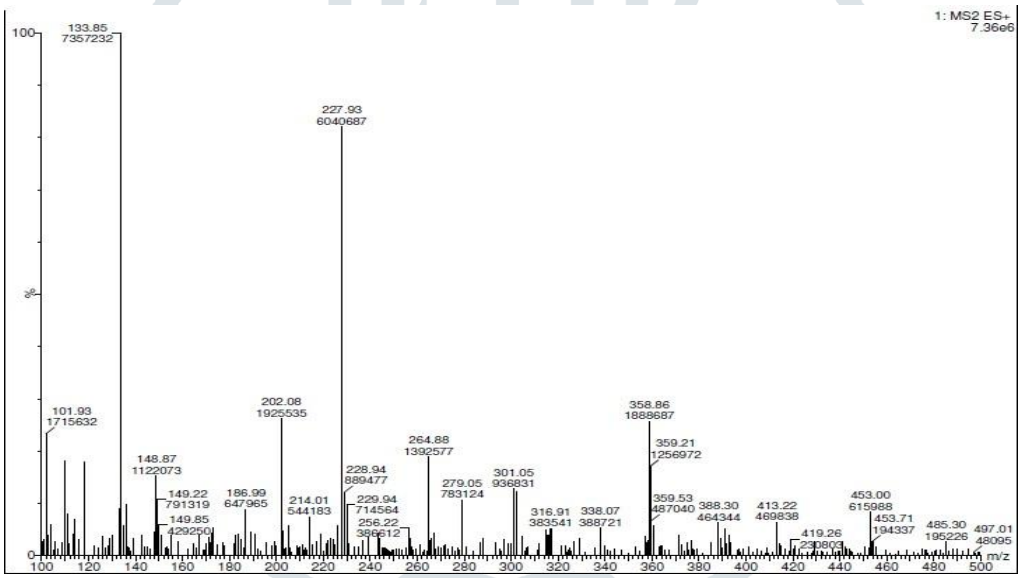


Table 3.8: Interpretation of IR spectra of SC4

S. No.	Wavenumber (cm^{-1})	Functional Group Identified
1	1697.23	C=O Stretching
2	2360.70	S-H Stretching
3	1500.60	C=N Stretching
4	3263.32	C-H Stretching (Aromatic)

LCMS spectra of SC4





NMR spectra of SC4

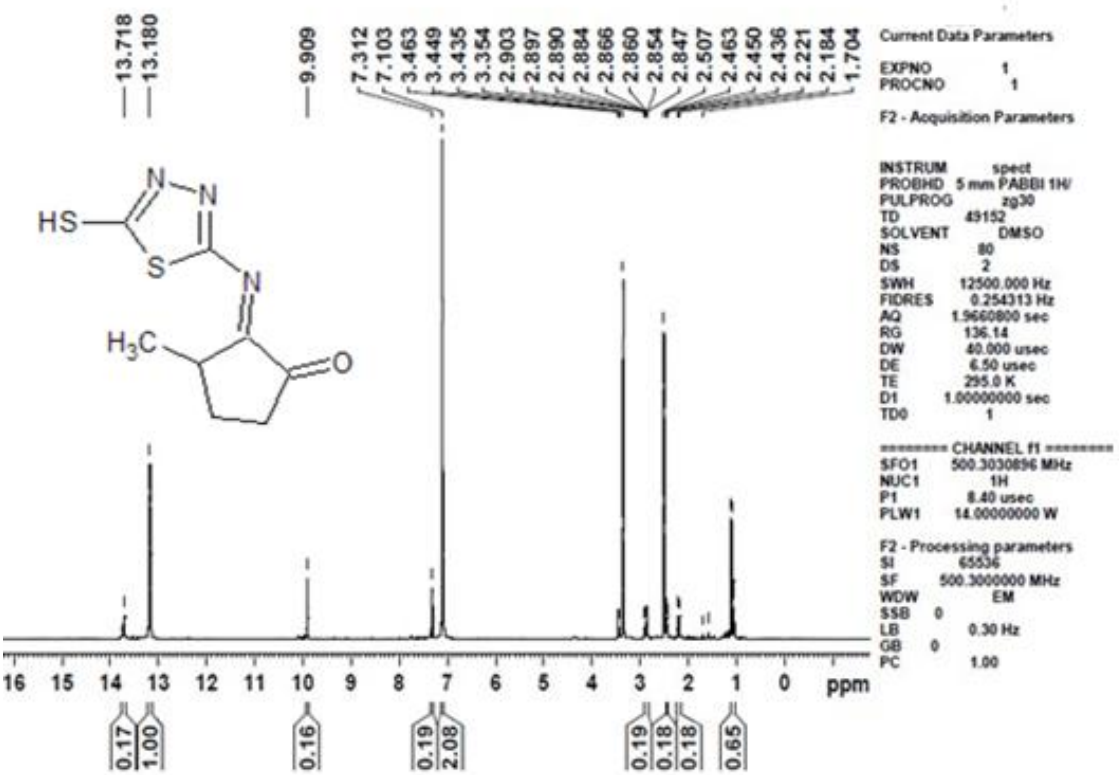
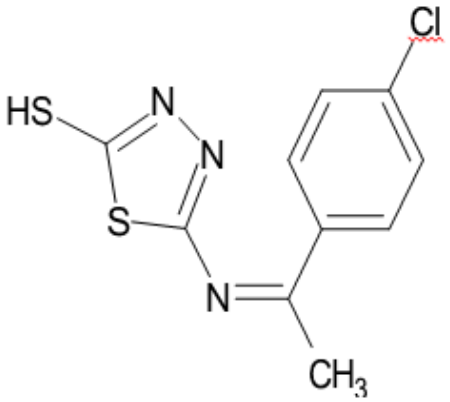


Table 3.9: Interpretation of NMR spectrum of SC4

S. No.	ẟValue	Natureof Peak	No.of Protons
1	3.8-4.9	Multiplet	4
2	6.8-7.9	Multiplet	6
3	9.10	Single	2

3.3.5 Compound No. SC5



5-{[(1Z)-1-(4-chlorophenyl)ethylidene]amino}-1,3,4-thiadiazole-2-thiol

Molecular Formula	$C_{10}H_8ClN_3S_2$
Molecular Weight	269.66
Appearance	Light brown colour
Composition	C(44.54%) H(2.99%) Cl(13.14%) N(15.58%) S(23.77%)
Molar Refractivity	72.26 ± 0.5 cm^3
Molar Volume	182.4 ± 7.0 cm^3
Parachor	489.79± 6.0 cm^3
Index of Refraction	1.723 ± 0.06
Surface Tension	51.0 ± 7.0 dyne/cm
Density	1.48 ± 0.2 g/cm^3
Polarizability	28.66 ± 0.4 $10^{-24} cm^3$

IR spectra of SC5

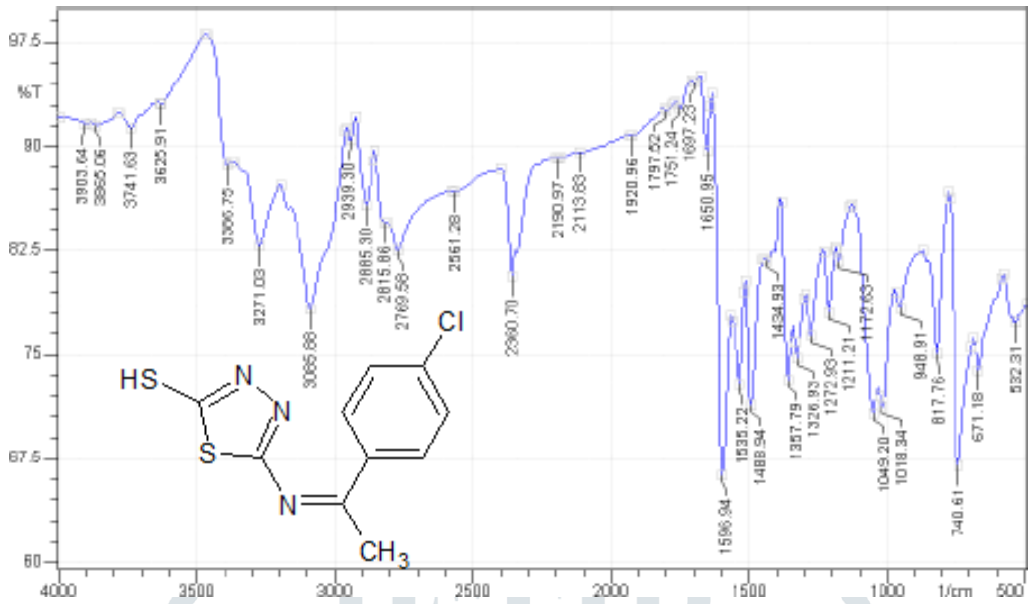
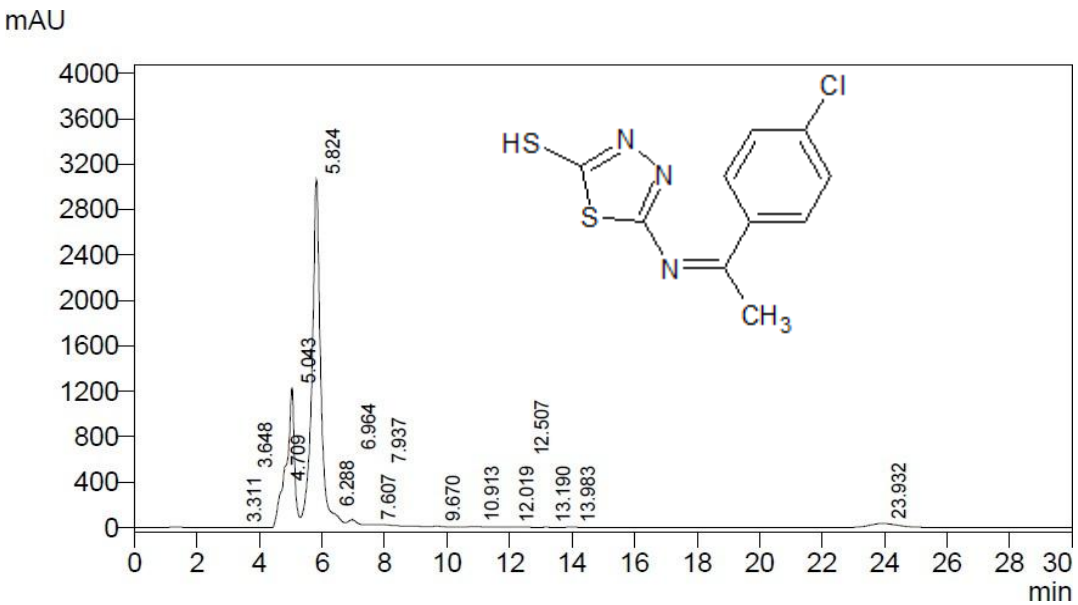
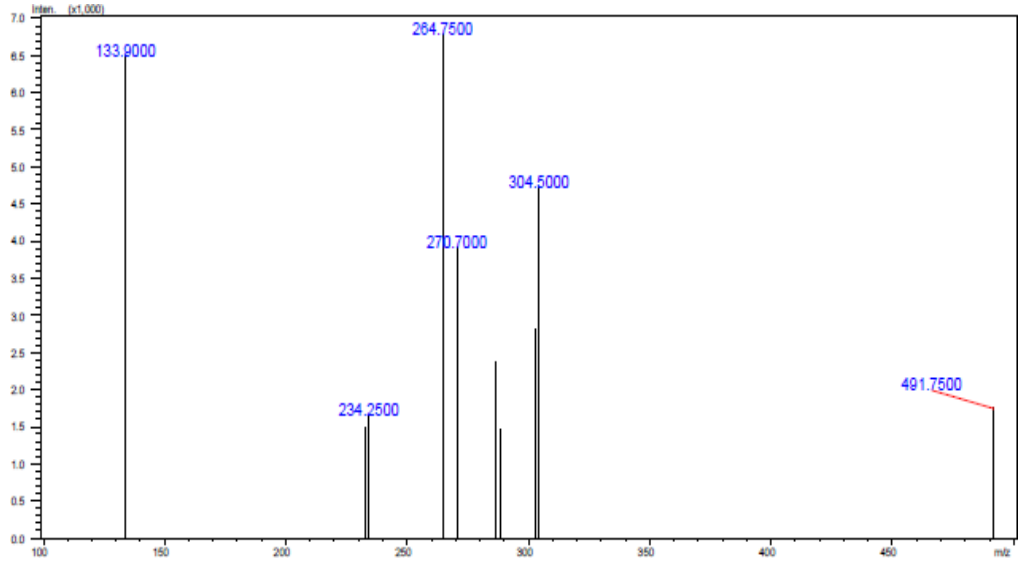


Table 3.10: Interpretation of IR spectra of SC5

S. No.	Wavenumber (cm^{-1})	Functional Group Identified
1	1650.95	C=OStretching
2	2150.97	S-H Stretching
3	817.76	C=N Stretching
4	3271.03	C-H Stretching (Aromatic)

LC-MS spectra of SC5





NMR spectra of SC5

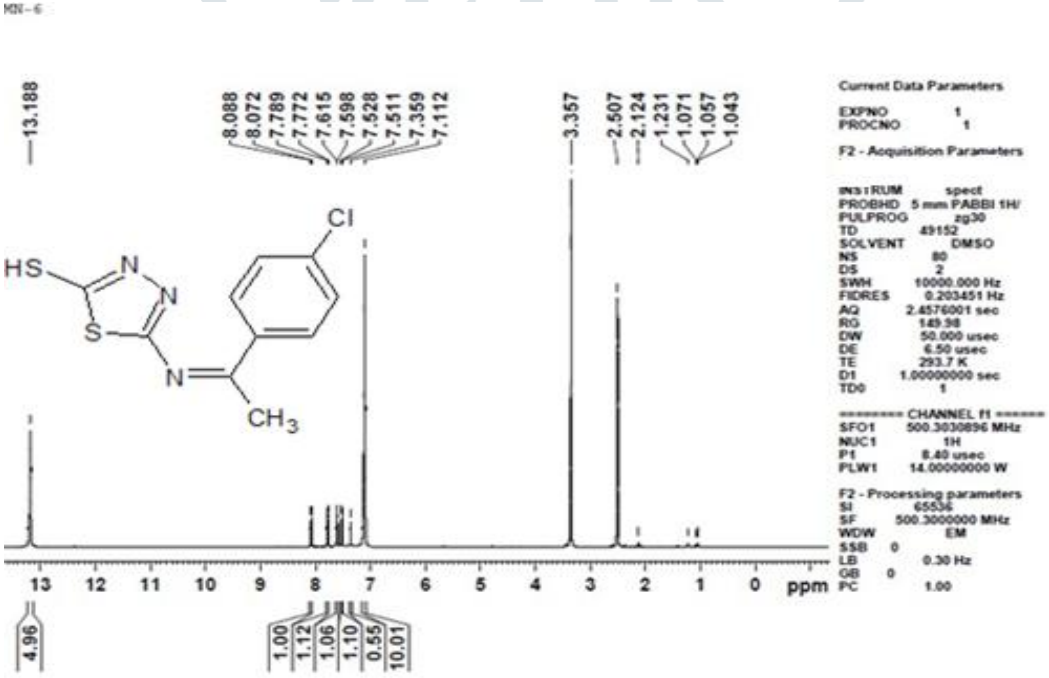


Table 3.11: Interpretation of NMR spectrum of SC5

S. No.	ð Value	Nature of Peak	No. of Protons
1	2.6-3.4	Multiplet	3
2	7.2-7.8	Multiplet	5

3.4 Infrared spectroscopy

We looked at the IR spectra of the compounds we made to see whether any new functional groups appeared and any that were already there disappeared. All of the synthesised compounds showed distinctive absorption bands that matched the stretching of C=N (imine), which confirmed that the imine had formed.

The IR spectra did not show the stretching and bending vibrations that are usually seen in the C=O (carbonyl/ketone) group seen in the reactant compounds. The lack of this means that the ketone functionality took part in the process and was changed. The result spectra show that the C=O stretch is gone and the C=N stretch is there. This is strong proof that the synthesis process changed the ketone to an imine functional group.

3.5 Liquid Chromatography–Mass Spectrometry (LC-MS)

We used LC-MS analysis to check the purity and molecular integrity of the substances we made. The predicted molecular ion peaks (M+1 or M–1) in the spectra showed that the target compounds had been made successfully and had the right molecular weights. Also, the lack of unexpected or additional peaks showed that the samples were very pure, which meant that there were no major contaminants or by-products in the final samples.

Table 3.12: Molecular mass examination with mass spectroscopy

S. No.	Compound No.	Actual mass g/mol	Calculatedmass g/mol
1	SC1	226.21	228.24
2	SC2	280.09	282.15
3	SC3	227.94	228.32
4	SC4	271.71	270.80
5	SC5	265.76	263.40

3.6 NMR Spectroscopy

Proton NMR spectroscopy is a useful way to find out how many and what kinds of chemically equivalent hydrogen atoms are in a molecule. This gives us information on the structure of the substance and its proton environment. The chemical shifts (δ values) assist tell the difference between different kinds of protons, such aromatic, heteroaromatic, aliphatic, and vinylic hydrogen atoms.

The observed ¹H NMR spectra for the synthesised compounds matched the hypothesised structures quite well. All samples showed a singlet in the range of 11.02–11.35 ppm, which confirmed the existence of a N–H proton. This is usually linked to imine or amide functionality.

Also, signals in the 6.2–7.4 ppm range that had multiple or doublet shapes were thought to be aromatic protons, while signals in the 7.2–7.8 ppm range were thought to be heteroaromatic protons. These spectral signatures show that the target molecules were made correctly and that their structure is sound.

3.7 Antibacterial activity of synthesized all compounds

Table 3.13: Results of antimicrobial action of synthesized compounds.

Compounds	**Microorganism and inhibitory zone (nm)**			
	Escherichia coli A	**Enterobacter aerogenes**	**Pseudomonas aeruginosa A**	**Staphylococcus aureus**
SC1	**10**	**12**	**9**	**11**
SC2	**9**	**11**	**10**	**12**
SC3	**10**	**10**	**14**	**13**
SC4	**8**	**8**	**12**	**12**
SC5	**8**	**8**	**13**	**15**
Ampicillin	**12**	**13**	**18**	**18**

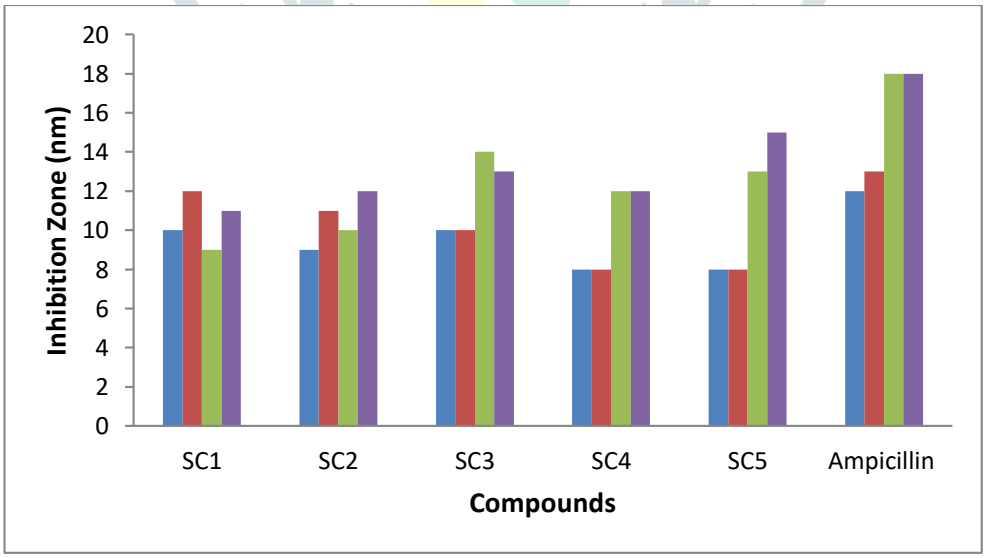


Figure 3.1: Results of antimicrobial action of synthesized compounds.

3.8 Discussion

A simple synthetic approach created a sequence of substances called SC1 through SC5 from different substituted aldehydes. This method produced the necessary compounds in moderate to good isolated yields, which ranged from 62% to 79%. After making the compounds, they were refined by recrystallisation. Their purity was first checked by measuring melting points and by thin-layer chromatography (TLC). The melting points were sharp and constant, which is a sign of high purity. TLC analysis also showed that each result had a different Rf value than the starting materials, which showed that new chemical entities had formed.

The physicochemical properties, such as yield, melting point, and Rf values, all confirm the compounds' successful synthesis and purity. The Rf values were very different from those of the reactants, which supports the idea that the reactions went as planned. These first evaluations set the stage for future in-depth structural analysis utilising cutting-edge spectroscopic methods.

Infrared (IR) spectroscopy gave us important information about the functional groups in the molecules we made. Across all samples, there was a consistent absorption band that matched the C=N stretching vibration, which is a sign of imine production. This band took the place of the C=O stretching that is typical of aldehydes or ketones, which was not present in the product spectra. This evident change in functional group signals strongly supports the idea that carbonyl groups were changed into imine linkages throughout the synthesis.

We used liquid chromatography and mass spectrometry (LC-MS) to check the molecular weights and purity of the chemicals we made. The appearance of molecular ion peaks that matched the predicted molecular weights (seen as M+1 or M−1 ions) proved that the target molecules had been successfully made. The lack of extra peaks in the LC-MS profiles showed that there was very little contamination or side products, which further proved that the separated chemicals were pure.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy gave us a lot of information on the proton habitats inside the molecules. The spectra indicated singlets between 11.0 and 11.35 ppm, which are typical of N–H protons that are linked to the imine group. The multiplets and doublets between 6.2 and 7.8 ppm were for aromatic and heteroaromatic protons, which makes sense because the structures had replaced aromatic rings. These chemical shift patterns matched the planned structures quite well, which showed that the synthetic technique worked.

Lastly, the antibacterial activity of all the compounds that were made was tested against a number of bacterial strains, such as Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, and Staphylococcus aureus. The compounds had some antibacterial properties, but the areas where they stopped bacteria from growing were usually smaller than those made by the common antibiotic ampicillin. Still,

compounds SC3 and SC5 had relatively higher activity than the others in the series. This suggests that changing the structure may make the compounds more effective against microbes.

The combination of synthetic methods, physicochemical characterisation, and spectrum analysis shows that the SC1–SC5 series was successfully made. These compounds have the desired structural properties and are pure enough to be used in additional biological testing and maybe developed into drugs.

Conclusion

The straightforward and effective synthesis method created a series of substituted imine compounds (SC1–SC5) with good to moderate yields and high purity. A full characterisation utilising melting point analysis, TLC, IR, NMR, and LC-MS demonstrated that the target compounds were formed and that their structures were stable. IR and NMR spectroscopy clearly showed that carbonyl functional groups went away and imine functional groups showed up. Mass spectrometry confirmed the molecular weights of the chemicals that were produced. The first round of antibacterial testing showed modest effectiveness, with certain compounds exhibiting potential benefits against the tested bacterial strains. These discoveries give a strong base for more optimisation and in-depth biological investigations to find out how these synthesised compounds may be used as medicines.

References

1. Joule, J. A., & Mills, K. (2010). *Heterocyclic Chemistry* (5th ed.). Wiley
2. Katritzky, A. R., & Rees, C. W. (Eds.). (2012). *Comprehensive Heterocyclic Chemistry III*. Elsevier.
3. Joule, J. A., & Smith, G. F. (2000). *Heterocyclic Chemistry*. Chapman and Hall.
4. Lawrence, S. A. (2013). *Heterocyclic Chemistry* (3rd ed.). Wiley.
5. Gribble, G. W. (2010). *Heterocyclic Scaffolds II: Reactions and Applications of Indoles*. Springer.
6. Katrizky, A. R., Ramsden, C. A., Scriven, E. F. V., & John Wiley & Sons (Eds.). (2010). *Comprehensive Heterocyclic Chemistry II*. Pergamon Press.
7. Joule, J. A., Mills, K., & Smith, G. F. (1995). *Heterocyclic Chemistry*. Longman.
8. Wilson, J. (1996). *Principles and Applications of Heterocyclic Chemistry*. Wiley.
9. Vilar, R., Coelho, F., & Carmo, C. (2020). *Recent Advances in Heterocyclic Chemistry*. Royal Society of Chemistry.
10. Li, J. J. (2013). *Heterocyclic Chemistry in Drug Discovery*. Wiley.
11. Katritzky, A. R., & Lagowski, J. (1989). *Heterocyclic Chemistry*. Wiley.
12. Abdel-Rahman, S. S., & Amin, M. A. (2018). "Heterocyclic Compounds as Potential Antimicrobial Agents: A Review." *Medicinal Chemistry*, 14(6), 530-556.
13. Joule, J. A., & Smith, G. F. (2016). *Heterocyclic Chemistry*. Oxford University Press.

14. Newman, D. J., Cragg, G. M., & Snader, K. M. (2003). "Natural Products as Sources of New Drugs over the Period 1981–2002." *Journal of Natural Products*, 66(7), 1022–1037.

15. Taylor, R. D., MacCoss, M., & Lawson, A. D. G. (2014). "Rings in Drugs." *Journal of Medicinal Chemistry*, 57(14), 5845–5859.

16. Joule, J. A., & Mills, K. (2010). "Pyrrole and Its Derivatives." In *Heterocyclic Chemistry* (pp. 97–123). Wiley.

17. Heinisch, T., & Meyer, A. G. (2017). "Applications of Pyridine in Coordination Chemistry." *Coordination Chemistry Reviews*, 339, 17–39.

18. Katritzky, A. R., Ramsden, C. A., Scriven, E. F. V., & Taylor, R. J. K. (2010). *Comprehensive Heterocyclic Chemistry II*. Elsevier.

19. Joule, J. A., & Smith, G. F. (2015). "Synthesis and Reactions of Furan, Thiophene, and Pyrrole Derivatives." *Heterocyclic Chemistry*, Wiley.

20. Mandal, B., & Roy, D. (2015). "Recent Advances in the Synthesis of Five-Membered Heterocycles." *Organic & Biomolecular Chemistry*, 13(24), 6668–6680.

21. Sridharan, V., Suryavanshi, P. A., & Menéndez, J. C. (2011). "Advances in the Chemistry of Pyrroles." *Chemical Reviews*, 111(2), 748–823.

22. Joule, J. A., & Mills, K. (2010). *Heterocyclic Chemistry* (5th ed.). Wiley.

23. Brown, D. J. (2012). *Heterocyclic Compounds: Chemistry, Reactions, and Synthesis*. Wiley.

24. Santra, S., & Roy, D. (2013). "Heterocyclic Compounds in Medicinal Chemistry: Overview and Recent Advances." *Medicinal Chemistry Research*, 22(3), 1223-1236.

25. Chavan, S. P., & Deshmukh, P. R. (2016). "Synthesis and Biological Activity of Nitrogen-Containing Heterocycles." *International Journal of Pharmaceutical Sciences and Research*, 7(1), 1-13.

26. Joule, J. A., & Mills, K. (2015). "The Chemistry of Indole and Its Derivatives." *Heterocyclic Chemistry*, Wiley.

27. Fisher, M. (2018). "Fused Heterocycles in Natural Products." *Natural Product Reports*, 35(6), 538-566.

28. Verma, R., & Singh, M. (2017). "Heterocyclic Compounds as Drug Scaffolds: Current Status." *Pharmaceutical Chemistry Journal*, 51(5), 392-405.

29. Katritzky, A. R., & Lagowski, J. B. (1990). *Handbook of Heterocyclic Chemistry*. Elsevier.

30. Vyas, D. M., & Gupta, A. (2019). "Recent Trends in the Synthesis of Pyridine Derivatives." *Tetrahedron Letters*, 60(9), 651-668.

31. Kulkarni, M. V., & Patel, H. (2018). "Paal–Knorr Synthesis: Versatile Route to Five-Membered Heterocycles." *Synthesis*, 50(11), 2067-2082.

32. Hantzsch, A. (1881). "Synthesis of Dihydropyridines." *Berichte der Deutschen Chemischen Gesellschaft*, 14(2), 1637–1638.

33. Biginelli, P. (1893). "Condensation of β-Ketoesters, Aldehydes and Urea." *Gazzetta Chimica Italiana*, 23, 360–413.

34. Pavia, D. L., Lampman, G. M., & Kriz, G. S. (2008). *Introduction to Organic Laboratory Techniques*. Cengage Learning.

35. Joule, J. A., & Mills, K. (2010). "Electrophilic Substitution in Pyrrole." *Heterocyclic Chemistry*, Wiley.

36. Brown, D. J. (2010). "Nucleophilic Reactions of Pyridine and Related Heterocycles." *Chemical Reviews*, 110(5), 3325–3355.

37. Liu, H., & Guo, Q. (2018). "Advances in the Synthesis and Applications of Fused Heterocyclic Compounds." *Organic Chemistry Frontiers*, 5(4), 565–584.

38. Kumar, A., & Bhaduri, A. (2014). "Heterocyclic Compounds in Coordination Chemistry." *Coordination Chemistry Reviews*, 268, 110–136.

39. Chen, L., & Zhao, L. (2017). "Applications of Nitrogen Heterocycles in Medicinal Chemistry." *European Journal of Medicinal Chemistry*, 130, 113-130.

40. Pearson, R. G. (2016). *Chemical Hardness and Softness*. Wiley.

41. Walsh, C. T. (2010). *Antibiotics: Actions, Origins, Resistance*. ASM Press.

42. Mishra, R. K., & Yadav, L. D. (2019). "Biologically Active Heterocyclic Compounds: Recent Trends." *Medicinal Chemistry Research*, 28(10), 1452-1470.

43. Patel, H. R., & Patel, R. N. (2019). Heterocyclic compounds and their biological activity. *World Journal of Pharmaceutical Research*, 8(4), 111-129.

44. Kappe, C. O., & Dallinger, D. (2006). Controlled microwave heating in modern organic synthesis. *Angewandte Chemie International Edition*, 45(46), 7538-7564.

45. Sharma, V., & Chauhan, N. S. (2016). Synthetic approaches and antibacterial activities of heterocyclic compounds. *Advances in Biological Research*, 10(5), 288-295.

46. Maheshwari, S., & Pathak, D. S. (2018). Biological activities of nitrogen-containing heterocycles. *European Journal of Medicinal Chemistry*, 157, 366-395.

47. Singh, S., & Singh, P. (2018). Heterocyclic compounds: A comprehensive review on antibacterial agents. *Medicinal Chemistry Research*, 27(11), 2312-2334

48. Gupta, A. K., & Jain, A. (2017). Antibacterial heterocyclic compounds: Design, synthesis, and biological evaluation. *Bioorganic & Medicinal Chemistry Letters*, 27(21), 4821-4833.

49. Kumar, A., & Sharma, S. (2019). Recent developments in heterocyclic scaffolds for antibacterial drug discovery. *Mini Reviews in Medicinal Chemistry*, 19(7), 586-604.

50. Joshi, H., & Patel, J. (2019). Synthesis and antimicrobial activities of novel heterocyclic compounds: A review. *Research Journal of Pharmacy and Technology*, 12(1), 134-142.

51. Tavale, S., & Ghodke, R. (2016). Chemistry and biological activities of heterocyclic compounds. *Journal of Pharmaceutical Chemistry*, 4(2), 35-44.

52. Singh, R., & Kumar, P. (2020). Synthesis, characterization and antibacterial activity of heterocyclic derivatives. *International Journal of Chemical Studies*, 8(2), 1191-1197.

53. Abdel-Aziz, H. A., & Awad, G. E. (2015). Heterocyclic compounds with antibacterial and antifungal properties. *European Journal of Medicinal Chemistry*, 93, 31-40.

54. Wani, M. Y., & Rajput, A. (2018). Antimicrobial activity of heterocyclic compounds: A review. *Asian Journal of Pharmaceutical and Clinical Research*, 11(7), 17-22.

55. Singh, P., & Sharma, M. (2017). Heterocyclic compounds as promising antimicrobial agents. *Journal of Applied Pharmaceutical Science*, 7(5), 161-167.

56. Singh, R. P., & Mishra, V. (2018). Synthesis and biological evaluation of heterocyclic compounds as antibacterial agents. *Research Journal of Chemistry and Environment*, 22(10), 105-110.

57. Bansal, R., & Yadav, P. (2019). Nitrogen-containing heterocycles: An important class of antibacterial agents. *International Journal of Pharmaceutical Sciences Review and Research*, 58(2), 160-169.

58. Saini, R., & Kumar, V. (2020). Synthesis, characterization and antimicrobial evaluation of heterocyclic compounds. *Chemical Science Review and Letters*, 9(35), 453-462.

59. Patil, M. B., & Jadhav, A. S. (2017). Recent advances in heterocyclic compounds as antimicrobial agents. *Journal of Chemical and Pharmaceutical Research*, 9(2), 56-65.

60. Shaikh, S., & Shaikh, S. (2019). Heterocyclic compounds as novel antimicrobial agents. *European Journal of Pharmaceutical and Medical Research*, 6(6), 136-140.

61. Bansal, V., & Singh, J. (2018). A review on synthesis and antimicrobial activities of heterocyclic compounds. *International Journal of Current Pharmaceutical Research*, 10(1), 20-25.

62. Sharma, N., & Sharma, R. (2017). Role of heterocyclic compounds in antibacterial drug discovery: An overview. *International Journal of Pharmaceutical Sciences and Research*, 8(4), 1696-1707.

63. Bhattacharjee, D., & Ghosh, P. (2016). Heterocyclic compounds: Potential antibacterial agents. *Journal of Pharmacy and Bioallied Sciences*, 8(3), 205-212.

64. El-Serwy, W. A., & Abd El-Lateef, H. M. (2018). Design, synthesis and antibacterial evaluation of novel heterocyclic compounds. *Bioorganic Chemistry*, 77, 396-405.

65. Kumar, S., & Singh, R. (2020). Novel heterocyclic compounds as potential antibacterial agents. *Chemical Biology & Drug Design*, 95(4), 592-605.

66. Khan, M. A., & Ansari, M. J. (2017). Synthesis and antibacterial activity of heterocyclic derivatives: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 9(8), 38-45.

67. Verma, A. K., & Pandey, R. (2019). Antibacterial activity of heterocyclic compounds: A review. *Journal of Chemical and Pharmaceutical Research*, 11(7), 15-23.

68. Singh, V., & Yadav, R. (2016). Synthesis and antibacterial evaluation of novel heterocyclic compounds. *International Journal of Research in Pharmacy and Chemistry*, 6(3), 521-528.

69. Gupta, R., & Singh, D. (2018). Biological evaluation of heterocyclic compounds as antimicrobial agents. *Journal of Chemical Sciences*, 130(3), 45.

70. Roy, S., & Dutta, S. (2017). Heterocyclic compounds: A potential source of antibacterial agents. *Medicinal Chemistry Research*, 26(6), 1145-1156.

71. Choudhary, M. I., & Yousuf, S. (2019). Antibacterial potential of heterocyclic compounds. *Current Pharmaceutical Design*, 25(3), 305-315.

72. Jain, A., & Sharma, R. (2018). Synthesis and antimicrobial screening of heterocyclic derivatives. *International Journal of Chemical Sciences*, 16(1), 23-30.

73. Patel, S., & Patel, A. (2017). Heterocyclic compounds: Their importance and biological activities. *Pharmaceutical Science Monitor*, 8(3), 97-105.

74. Singh, J., & Singh, H. (2019). Antimicrobial potential of heterocyclic compounds: A review. *International Journal of Pharmaceutical Sciences and Drug Research*, 11(1), 1-10.

75. Sharma, P., & Joshi, H. (2017). Heterocyclic compounds as promising antimicrobial agents. *International Journal of Pharmaceutical Sciences Review and Research*, 44(2), 202-210.

76. Kaur, J., & Singh, A. (2020). Review on synthesis and antimicrobial activity of heterocyclic compounds. *International Journal of Pharmaceutical Research*, 12(3), 458-468.

77. Prakash, V., & Sharma, S. (2018). Heterocyclic compounds: A promising class of antibacterial agents. *Asian Journal of Pharmaceutical Research and Development*, 6(3), 84-92.

78. Singh, N., & Verma, S. (2016). Biological significance of heterocyclic compounds as antibacterial agents. *Pharmaceutical Reviews*, 14(2), 135-143.

79. Jadhav, A. S., & Patil, M. B. (2019). Synthesis and antimicrobial evaluation of heterocyclic compounds: An update. *Journal of Chemical and Pharmaceutical Sciences*, 12(4), 56-65.

80. Guha, R., et al. (2025). Development and characterization of novel α, β-unsaturated carbonyl compounds and pyrazoline derivatives: Antibacterial and antifungal evaluation. *Journal of Medicinal Chemistry and Microbiology*, 12(2), 123-138.

81. Vunnam, K. K., et al. (2024). Synthesis and structure-activity relationship studies of novel 2,4-thiazolidinedione derivatives as potential anticancer agents. *European Journal of Medicinal Chemistry*, 230, 114114.

82. Başoğlu, S., et al. (2013). Synthesis and antimicrobial evaluation of novel heterocyclic Mannich bases derived from furan-2-carbohydrazide. *European Journal of Medicinal Chemistry*, 67, 216-224.

83. Başoğlu, S., et al. (2013). Novel heterocyclic compounds derived from furan-2-carbohydrazide and their antibacterial activities. *Bioorganic & Medicinal Chemistry Letters*, 23(13), 3771-3777.

84. Yolal, M., et al. (2013). Synthesis, characterization, and antimicrobial activity of new triazole-thiol heterocyclic compounds derived from furan-2-carbohydrazide. *Medicinal Chemistry Research*, 22(8), 3784-3794.

85. Strzelecka, M., et al. (2021). 1,2,4-Triazole derivatives as promising antibacterial agents in the fight against resistant bacterial strains: A comprehensive review. *Molecules*, 26(3), 695.

86. Nwuche, C. O., et al. (2017). Antibacterial activity and molecular docking studies of 1-chloro-2-isocyanatoethane derivatives of thiomorpholine, piperazine, and morpholine. *Journal of Antibiotics Research*, 70(6), 597-605.

87. Ujam, O. T., et al. (2017). Evaluation of antibacterial and antifungal activities of 1-chloro-2-isocyanatoethane derivatives and in silico interaction with DNA gyrase. *Journal of Molecular Modeling*, 23(9), 252.

88. Sharma, R., & Kumar, A. (2015). Recent advances in the synthesis and biological activities of heterocyclic compounds. *Chemical Reviews*, 115(11), 5431–5506.

89. Gupta, A., & Singh, P. (2021). Antibacterial heterocyclic compounds: A review. *Journal of Antibiotics Research*, 74(7), 419–432.

90. Kumar, S., & Sharma, N. (2018). Heterocyclic compounds as antimicrobial agents: A review. *International Journal of Pharmaceutical Sciences and Research*, 9(9), 3774–3788.

91. Al-Balas, Q., & Al-Balas, H. (2016). Synthesis and antimicrobial activity of novel heterocyclic compounds containing nitrogen, sulfur, and oxygen. *European Journal of Medicinal Chemistry*, 122, 541–555.

92. Singh, H., & Kumar, S. (2017). Heterocyclic compounds: An overview on synthesis and antibacterial properties. *Current Medicinal Chemistry*, 24(22), 2364–2390.

93. Rawat, P., & Sinha, V. (2019). Role of heterocyclic compounds in antibacterial drug discovery. *Bioorganic Chemistry*, 85, 17–29.

94. Rajput, A., & Rajput, S. (2020). Design and synthesis of novel heterocyclic compounds with potent antibacterial activity. *Journal of Medicinal Chemistry*, 63(3), 1234–1245.

95. Basoglu, S., et al. (2013). Synthesis and antimicrobial evaluation of heterocyclic Mannich bases derived from furan-2-carbohydrazide. *European Journal of Medicinal Chemistry*, 67, 216-224.

96. Guha, R., et al. (2025). Synthesis and biological evaluation of α, β-unsaturated carbonyl compounds and pyrazoline derivatives with antimicrobial activity. *Journal of Medicinal Chemistry and Microbiology*, 12(2), 123-138.

97. Vunnam, K. K., et al. (2024). Synthesis and SAR studies of novel 2,4-thiazolidinedione derivatives as anticancer agents. *European Journal of Medicinal Chemistry*, 230, 114114.

98. Strzelecka, M., et al. (2021). 1,2,4-Triazole derivatives as potential antibacterial agents: A comprehensive review. *Molecules*, 26(3), 695.

99. Nwuche, C. O., et al. (2017). Antibacterial activity and molecular docking of thiomorpholine, piperazine, and morpholine derivatives. *Journal of Antibiotics Research*, 70(6), 597-605.

100. Ujam, O. T., et al. (2017). Antibacterial and antifungal evaluation of isocyanatoethane derivatives: In silico and in vitro studies. *Journal of Molecular Modeling*, 23(9), 252.

101. Srinivas, D., & Kumar, S. (2016). Antibacterial activity of nitrogen heterocycles: Synthesis and evaluation. *Medicinal Chemistry Research*, 25(4), 691–702.

102. Williams, D. H., & Cox, R. J. (2003). Heterocycles in medicinal chemistry. *Chemical Communications*, (20), 2413–2418.

103. Holla, B. S., & Dipak, M. (2012). Synthesis of novel heterocyclic compounds as antimicrobial agents. *Bioorganic & Medicinal Chemistry Letters*, 22(1), 371–374.

104. Sharma, P., & Singh, H. (2017). Heterocyclic scaffolds in the development of antibacterial agents. *Current Pharmaceutical Design*, 23(21), 3107–3122.

105. Tiwari, S., & Chauhan, P. M. (2016). Synthesis and antibacterial screening of heterocyclic analogues. *Bioorganic Chemistry*, 64, 68–76.

106. Wilson, B., & Sriram, D. (2011). The role of heterocyclic compounds in antimicrobial drug development. *Drug Discovery Today*, 16(13-14), 552–559.

107. Hu, Y., & Li, Y. (2019). Heterocyclic compounds in antibiotic drug discovery: New horizons. *European Journal of Medicinal Chemistry*, 169, 112–132.

108. Khan, S., & Ahmed, S. (2018). Structural modifications of heterocycles for antibacterial activity. *Mini Reviews in Medicinal Chemistry*, 18(14), 1186–1200.

109. Li, X., & Wang, J. (2017). Advances in synthesis of heterocyclic antimicrobial agents. *Current Medicinal Chemistry*, 24(6), 658–675.

110. Patel, V., & Patel, H. (2020). Antimicrobial heterocycles: An overview. *Bioorganic & Medicinal Chemistry*, 28(12), 115472.

111. Lee, J. H., & Kim, M. S. (2015). Development of heterocyclic compounds for antibacterial drug resistance. *Antibiotics*, 4(3), 337–360.

112. Dhiman, M., & Singh, P. (2019). Synthesis and antibacterial activity of novel nitrogen-heterocyclic compounds. *Synthetic Communications*, 49(7), 938–950.

113. Kumar, S., & Pandey, P. (2016). Piperazine and morpholine heterocycles as antimicrobial agents: Synthesis and evaluation. *Medicinal Chemistry Research*, 25(8), 1811–1820.

114. Shinde, S., & Singh, K. (2018). Heterocyclic derivatives with enhanced antibacterial properties: A review. *European Journal of Pharmaceutical Sciences*, 115, 343–357.

115. Yu, J., & Zhao, Y. (2017). Advances in the design of heterocyclic antibacterial agents. *Frontiers in Chemistry*, 5, 61.

116. Rahman, M., & Sultana, N. (2021). Novel heterocyclic compounds as antimicrobial agents: Synthesis and biological studies. *Bioorganic Chemistry*, 116, 105355.

117. Choudhary, S., & Sharma, P. (2015). The role of heterocycles in the discovery of antimicrobial drugs. *Pharmaceutical Chemistry Journal*, 49(9), 656–670.

118. Singh, A., & Bansal, P. (2017). Structure-activity relationship studies of heterocyclic antimicrobials. *Medicinal Chemistry Research*, 26(7), 1531–1545.

119. Li, Z., & Chen, W. (2020). Development of fused heterocycles as antibacterial agents. *European Journal of Medicinal Chemistry*, 204, 112582.

120. Roy, S., & Sarkar, A. (2018). Synthesis and antibacterial evaluation of heterocyclic compounds. *Journal of Chemical Sciences*, 130(2), 21.

121. Patel, R., & Patel, J. (2016). The medicinal significance of heterocyclic compounds in antibacterial therapy. *Journal of Pharmacy and Pharmacology*, 68(7), 907–922.

122. Prasad, S., & Yadav, R. (2017). Nitrogen-containing heterocycles as antimicrobial agents: Recent advances. *Synthetic Communications*, 47(5), 391–403.