Design and Synthesis of Thiadiazole-Derived Compounds as Potential Antimicrobial Agents

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By

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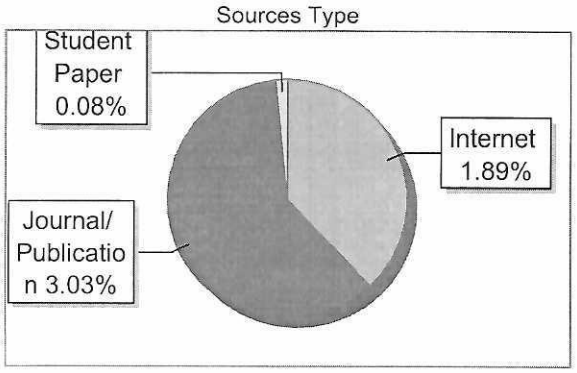
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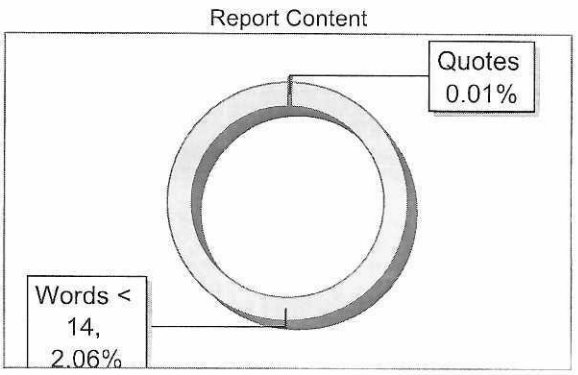
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Date: **(Hridesh Singh Chauhan)**

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LIST OF ABBREVIATIONS

S. No.	Abb.	FullForm
1	AMR	Antimicrobial Resistance
2	WHO	World Health Organization
3	MRSA	Methicillin-Resistant Staphylococcus aureus
4	VRE	Vancomycin-Resistant Enterococci
5	MDR-TB	Multi-Drug-Resistant Tuberculosis
6	MDR	Multi-Drug Resistant
7	CDC	Centers for Disease Control and Prevention
8	SERCaP	Single-Exposure Radical Cure and Prophylaxis
9	COF	Covalent Organic Framework
10	BET	Bromodomain and Extra-Terminal domain
11	PROTAC	Proteolysis-Targeting Chimera
12	BD	Bromodomain
13	SAR	Structure–Activity Relationship
14	QSAR	Quantitative Structure–Activity Relationship
15	ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
16	AChE	Acetylcholinesterase
17	BuChE	Butyrylcholinesterase
18	hERG	Human Ether-à-go-go-Related Gene
19	DFT	Density Functional Theory
20	HOMO	Highest Occupied Molecular Orbital
21	LUMO	Lowest Unoccupied Molecular Orbital
22	TD-DFT	Time-Dependent Density Functional Theory
23	DPPC	1,2-Dipalmitoyl-sn-Glycero-3-Phosphatidylcholine
24	MIC	Minimum Inhibitory Concentration
25	IC_{50}	Half Maximal Inhibitory Concentration
26	EPR	Electron Paramagnetic Resonance
27	FTIR	Fourier Transform Infrared Spectroscopy
28	NMR	Nuclear Magnetic Resonance
29	UV-Vis	Ultraviolet–Visible Spectroscopy
30	PC	Principal Component
31	DNA	Deoxyribonucleic Acid
32	COX-2	Cyclooxygenase-2
33	NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
34	TB	Tuberculosis
35	HSV	Herpes Simplex Virus

ABSTRACT

Aim and Objective: The global rise of antimicrobial resistance (AMR) has drastically reduced the efficacy of conventional antibiotics, creating an urgent need for novel therapeutic molecules capable of targeting resistant pathogens. The primary aim of this research is to design, synthesize, and evaluate a new series of thiadiazole derivatives as potential antimicrobial agents. Specific objectives include: (1) analyzing the structure–activity relationship (SAR) of 1,3,4-thiadiazole scaffolds; (2) synthesizing structurally diverse derivatives through rational molecular modification; (3) evaluating their antibacterial activity against selected drug-resistant strains; and (4) utilizing computational approaches such as molecular docking, QSAR modeling, and ADMET prediction to identify compounds with optimal biological and physicochemical properties. This work intends to provide promising lead molecules for overcoming microbial resistance and contribute to medicinal chemistry efforts in next-generation antimicrobial drug discovery.

Methodology: The study adopted an integrated experimental and computational strategy. Novel thiadiazole derivatives were synthesized using standard cyclization and condensation reactions, incorporating functional groups such as halogens, amino, methoxy, hydroxyl, and aromatic substituents to enhance antimicrobial potency. Reaction conditions were optimized for yield, purity, and regioselective substitution. The synthesized compounds were characterized using IR, NMR, UV-Vis spectroscopy, and elemental analysis. Antimicrobial activity was assessed against Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*), using standard assays to determine inhibitory potential. SAR studies were conducted to correlate biological activity with chemical modifications at different positions of the thiadiazole ring. Computational methodologies complemented the experimental work. Molecular docking was performed to evaluate binding affinity to microbial targets such as DNA gyrase, topoisomerase, and folate pathway enzymes. QSAR modeling was used to predict potency based on physicochemical descriptors, while ADMET profiling assessed solubility, permeability, metabolic stability, and toxicity. The combined data enabled the identification of high-priority lead compounds.

Results and Discussion: The synthesized thiadiazole derivatives displayed significant antimicrobial activity, validating the potential of the 1,3,4-thiadiazole core as a promising scaffold. Halogen substitution at the C-5 position enhanced lipophilicity and improved membrane permeability, resulting in higher antibacterial activity. Amino and methoxy groups at C-2 increased hydrogen-bonding interactions with microbial enzymes, strengthening inhibitory effects. Compounds with aromatic and heterocyclic side chains demonstrated better penetration through bacterial membranes and higher target selectivity. Docking studies revealed strong interactions with key microbial enzymes, with several compounds exhibiting high affinity for DNA gyrase and dihydrofolate reductase, suggesting dual mechanisms of enzyme inhibition. Some derivatives also showed membrane-disruptive potential, supporting a multifaceted antimicrobial mechanism. QSAR analysis confirmed the importance of electron-withdrawing groups in enhancing potency, while ADMET predictions indicated favorable absorption characteristics and low predicted toxicity for selected lead molecules. Biological testing showed noticeable activity against *S. aureus*, *E. coli* with some compounds performing comparably to standard antimicrobial drugs. The study highlights that strategic structural modifications substantially improve antimicrobial properties, offering a pathway for further optimization.

Conclusion: This research demonstrates that thiadiazole derivatives possess strong potential as next-generation antimicrobial agents capable of addressing the growing threat of AMR. Structural modifications, supported by SAR insights and computational modeling, led to the development of potent derivatives with enhanced biological activity, favorable drug-like properties, and selective microbial targeting. The findings provide valuable lead structures for future drug development and open avenues for designing thiadiazole-based therapeutics aimed at multidrug-resistant infections. Further preclinical evaluation and formulation studies are recommended to advance these compounds toward clinical applicability.

Keywords: Thiadiazole derivatives; Antimicrobial resistance; Drug discovery; SAR; Molecular docking; ADMET.

INTRODUCTION

1.1. Background of the Study

One of the biggest issues confronting public health today is the growing threat of antimicrobial resistance (AMR). When bacteria, fungi, viruses, and parasites change in a way that makes the medications used to treat them ineffective, this is known as antimicrobial resistance (AMR) **(Walsh et al., 2023)**. Many currently available antibiotics, antivirals, and antifungals might become outdated as a result of this phenomenon, which would have major ramifications for the treatment of illnesses that were previously curable. An extensive review of AMR, the pressing need for novel antimicrobial drugs, and the possibility of thiadiazole derivatives as viable options for fighting resistant infections are all covered in this part **(Khamitova *et al.*, 2024).**

1.1.1. Antimicrobial Resistance (AMR)

A- Global Impact of AMR on Healthcare and Public Health

One of the greatest problems in public health today is the worldwide spread of bacteria and other viruses that are resistant to antibiotics. Antimicrobial resistance (AMR) happens when bacteria, fungi, viruses, and parasites adapt to become unresponsive to the treatments that were once successful against them **(Walsh et al., 2023).** Many currently available antibiotics, antivirals, and antifungals are at risk of becoming obsolete as a result of this phenomena, which has major consequences for the management of diseases that were previously manageable. An extensive review of antimicrobial resistance (AMR), the critical shortage of effective antimicrobials, and the possible use of thiadiazole derivatives in the fight against resistant bacteria and viruses are all covered in this part. Modern medicine's triumphs are in jeopardy due to the rise of bacteria and other microbes that are resistant to antibiotics. Antimicrobial resistance (AMR) is now responsible for over 700,000 deaths annually, and if current trends persist, that figure is expected to rise significantly in the coming decades. Worldwide, antimicrobial resistance (AMR) is predicted to cause an extra10 million fatalities annually by 2050,

making it the top cause of mortality worldwide, even more so than cancer **(Ferri et al., 2017).**

Surgery, chemotherapy, and newborn care are just a few of the sectors hit hard by the proliferation of antibiotic-resistant bacteria. Pneumonia, UTIs, and sepsis are examples of illnesses that were formerly manageable with antibiotics but are now more challenging and expensive to treat. It is becoming more difficult for healthcare professionals to deliver effective therapies due to the increasing resistance to first-line antibiotics such penicillins, cephalosporins, and fluoroquinolones **(Bartoletti et al., 2016).**

The fast international transmission of infections with acquired resistance is another consequence of the free flow of people, animals, and products throughout the world. Antibiotic resistance is worsened in underdeveloped nations due to poor treatment standards, abuse of antibiotics, and limited access to high-quality medications **(Beltran-Alcrudo et al., 2019).**

B- Existing Antibiotic Resistance is on the Rise

An alarming amount of bacterial resistance to currently available antibiotics has been observed. A growing number of bacteria, among others, MRSA, VRE, and MDR-TB, are becoming increasingly resistant to antibiotics, which is a major cause for worry. There is a growing problem with bacteria developing resistance to carbapenems and other extended-spectrum beta-lactam antibiotics, which means that there are fewer alternatives for treating severe infections **(Li et al., 2022).**

Multiple pathways contribute to antibiotic resistance, including as microbial gene acquisition. By changing the antibiotic's target location, removing the drug from the cell, or degrading it with enzymes, these modifications allow infections to survive while antibiotics are present **(Normark and Normark, 2002).**

Human and animal abuse and misuse of antibiotics is a major contributor to the fast rate of resistance development. Antibiotic overuse for viral infections or failure to finish a recommended course of treatment are examples of inappropriate prescriptions that add to the selection pressure that causes

resistance. The broad usage of antibiotics as growth promoters in cattle and agriculture also contributes to the acceleration of resistance **(Rahman et al., 2022).**

C- Economic Burden of AMR on Healthcare Systems Worldwide

In addition to posing a serious financial risk to healthcare systems across the world, AMR undermines the effectiveness of treatments. The entire cost of healthcare is driven up by factors such as longer hospital stays, more intense treatments, and the use of more expensive antibiotics. Approximately $20 billion is spent each year by the US healthcare system on antibiotic-resistant diseases, according to a CDC research **(Aijaz et al., 2023)**, and an extra $35 billion in lost productivity as a result of sickness.

The problem is far worse in nations with low incomes. Higher death rates and longer hospital stays are the result of a combination of factors, including inadequate infection control, inaccurate diagnosis, and restricted access to new drugs. The growing tide of drug-resistant illnesses is putting a strain on healthcare systems worldwide, especially in low-income nations **(Alara and Alara, 2024).**

D- Need for New Antimicrobial Agents

The Urgent Requirement for Novel, Effective Drugs to Combat Resistant Pathogens

The increasing antimicrobial resistance (AMR) issue has heightened the critical need for new antimicrobial drugs. The introduction of new antibiotics has been severely delayed in recent years, despite the fact that several such drugs have been created in the past. According to Salaam et al. (2023), there is a "dry pipeline" for antibiotic development due to a host of issues, such as the high cost of research and development, the complexity of drug discovery, and the quick rate of resistance.

Various obstacles hinder the progress of new antibiotic development:

1. **Scientific complexity**: Identifying new drug targets and developing effective agents requires sophisticated biological and chemical knowledge.

2. **Regulatory hurdles**: Getting new antibiotics approved for use involves rigorous testing for safety, efficacy, and resistance profiling, which can take many years.

3. **Limited financial incentives**: The high cost of developing new drugs and the relatively low return on investment in the case of antibiotics (due to their targeted use and resistance development) make pharmaceutical companies less motivated to invest in antibiotic research **(Gai *et al.*, 2019).**

Challenges in Discovering New Antimicrobial Agents

With resistance on the rise, the old methods of drug discovery are no longer cutting it. Antibiotics were originally found by screening natural products or by making little changes to already existing compounds to make them more effective. The quick development of resistance, however, has diminished the efficacy of most of these strategies. Some of the difficulties in finding new antibiotics are:

- **Limited chemical diversity**: The pharmaceutical industry has already explored many chemical classes for antimicrobial activity, leaving fewer unexplored options.

- **Resistance to novel compounds**: Even newly discovered antibiotics may quickly become resistant, as bacteria evolve rapidly.

- **Complexity of microbial pathogens**: The diversity of microbial pathogens and their ability to adapt to new treatments requires a more targeted and innovative approach to drug discovery **(Wright, 2007).**

1.1.2. Thiadiazole Derivatives as Promising Antimicrobials

1.1.2.1. Introduction to the Thiadiazole Ring as a Bioactive Scaffold

Thiadiazole derivatives are one group of chemicals that has recently received a lot of interest because of its potential. The sulphur and nitrogen atoms in the thiadiazole ring give it distinctive chemical and biological characteristics. It is a five-membered heterocyclic structure. A suitable scaffold for drug research,

the thiadiazole ring has been demonstrated to exhibit antibacterial, antiviral, anti-inflammatory, and anticancer properties **(Ahmad et al., 2024).**

The pharmacokinetic, selectivity, and potency of thiadiazole derivatives can be improved by a variety of chemical changes, which has piqued their interest. Thiadiazole rings may have their biological activity drastically changed by adding different substituents at different places on the ring. This opens the door for the development of tailored molecules with improved antibacterial capabilities **(Jain *et al.*, 2013).**

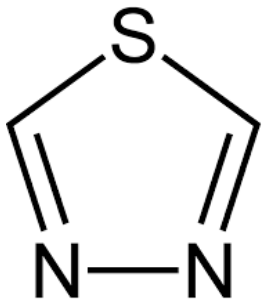


Figure 1.1: Structure of 1,3,4-Thiadiazole Ring (Hu *et al.*, 2014).

1.1.2.2. Historical Use of Thiadiazole Derivatives in Medicinal Chemistry

The antibacterial, antifungal, and anti-inflammatory characteristics of thiadiazole derivatives were initially studied in the early days of medicinal chemistry. Modern drug resistance problems have increased the importance of thiadiazole-based medicines, which in their initial generation demonstrated encouraging action against a number of infections **(Dawood and Farghaly, 2017).**

The possibility of using thiadiazole derivatives to treat malaria and TB was first recognised in the 1970s. Numerous tests conducted thereafter confirmed these chemicals broad-spectrum antibacterial efficacy. There is hope for the treatment of drug-resistant diseases thanks to the introduction of substituted thiadiazoles, which have increased action against resistant microorganisms **(Arshad *et al.*, 2022).**

1.1.2.3. Previous Research Highlighting Their Antimicrobial Potential

Thiadiazole derivatives have been the subject of a great deal of research about their antibacterial properties. According to studies, thiadiazoles may kill not just bacteria but also fungus, viruses, and Gram^{+ive} and Gram^{-ive} microorganisms. Ibraheem et al. (2018) found that 1,3,4-thiadiazole derivatives effectively combat healthcare-associated infections caused by Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans.

In order to learn more about the structural changes that maximise antibiotic action, several SAR investigations have been carried out. Thiadiazole derivatives may be made far more effective by adding substituents such aromatic rings, alkyl groups, or halogens, according to the research. They are promising prospects for future research because of the little toxicity and great efficacy shown in both in vitro and in vivo experiments **(Zaki *et al.*, 2023).**

1.2. What is Thiadiazole ?

Thiadiazole, a heterocyclic molecule with antibacterial, anticancer, anti-inflammatory, and antifungal properties, has become an important building block in medicinal chemistry. Among the several thiadiazole derivatives, 1,3,4-thiadiazole stands out due to its structural stability and its amenability to structural modifications that improve its biological activities. With an eye on their possible use in antimicrobial medication development, this section will go over the chemical composition, methods of synthesis, and characteristics of thiadiazole derivatives **(Atmaram and Roopan, 2022).**

1.2.1. Chemical Structure

The five-membered heterocyclic molecule known as the 1,3,4-thiadiazole ring consists of two carbon atoms, two hydrogen atoms, one sulfur atom, and one nitrogen atom. How reactive and stable the chemical is depends on where the sulphur and nitrogen atoms are located in the ring structure. In its most fundamental form, 1,3,4-thiadiazole looks like this:

- **Position 1**: Sulphur (S)
- **Position 3 and 4**: Nitrogen (N)

- **Position 2 and 5**: Carbon (C)

The ring becomes aromatic and somewhat stable as a result of this arrangement's planar structure. The ring's capacity to interact with biological targets is largely due to its electron-withdrawing character, which is augmented by the highly electronegative nitrogen and sulphur atoms **(Hemanth et al., 2022).**

It is common practice to add functional groups to the thiadiazole ring at the 2-position in order to increase the compound's lipophilicity and its ability to cross bacterial membranes. Adding hydroxyl (OH), amino (NH_2), methoxy (OCH_3), or carboxyl (COOH) as a 5-position substitute increases the compound's bioavailability and activity. These changes have the potential to greatly affect the toxicity, selectivity, and antibacterial effectiveness of thiadiazole derivatives **(Ungureanu *et al.*, 2024).**

1.2.2. Functional Group Modifications and Their Role in Enhancing Antimicrobial Activity

The 1,3,4-thiadiazole ring is structurally flexible, allowing for the introduction of numerous functional groups that might boost antibacterial action. Among the most typical changes are:

1. Substitution at Position 2: The 1,3,4-thiadiazole ring is structurally flexible, allowing for the introduction of numerous functional groups that might boost antibacterial action. Among the most typical changes are: An increase in the compound's lipophilicity and its capacity to penetrate bacterial cell membranes may be achieved by adding halogens, alkyl groups, or aryl groups at this location. To increase the antibacterial efficacy, elements like fluorine, chlorine, and bromine are often employed to impart electron-withdrawing characteristics **(Glyn RJ, Pattison, 2021).**

2. Substitution at Position 5: Hydroxyl (OH), amine (NH_2), or carboxyl (COOH) functional groups are often introduced at the 5-position. The compound's solubility and polarity may be altered by these groups, enhancing its interaction with biological targets such microbial receptors and enzymes **(Wasserman *et al.*, 1986).**

3. Addition of Heteroatoms: Adding oxygen or nitrogen atoms to different spots on the thiadiazole ring may increase the molecule's hydrogen bonding potential, which in turn makes it better at interacting with proteins and enzymes produced by microbes **(Pakulski and Pinkowicz, 2021).**

1.2.3. Synthesis Methods

The five-membered ring is usually formed during the synthesis of thiadiazole derivatives by means of cyclization processes, condensation reactions, and green chemistry approaches, among other important synthetic pathways.

1. Cyclization Reactions:

- The cyclization step is the main stay in the synthesis of 1,3,4-thiadiazole derivatives. In most cases, a thiadiazole ring is formed when thiosemicarbazides or hydrazines combine with appropriate carbonyl compounds. The production of substituted thiadiazoles results from the interaction of thiosemicarbazide with α, β-unsaturated carbonyl compounds.
- Cyclization relies on sulphurization and aromatisation, two critical procedures that guarantee good yield and stabilise the ring structure **(Kumar *et al.*, 2024).**

2. Condensation Reactions:

- Thiosemicarbazides may be converted to thiadiazole derivatives by reacting with carbonyl compounds in condensation processes, which take place in moderate acidic or basic environments. Derivatives with various functional groups may also be successfully generated using this procedure.
- Condensation of hydrazine derivatives with thioketones or thiol compounds, which contain sulphur, might result in the production of thiadiazole derivatives, for instance **(Mustafa *et al.*, 2004).**

3. Green Chemistry Methods:

- A more sustainable way to synthesise thiadiazoles has been made possible by the advent of green chemistry technologies. These techniques lessen the need on hazardous chemicals, solvents, and high-energy reactions.
- One environmentally friendly approach that has recently grown in favour is microwave-assisted synthesis, which uses less energy while increasing reaction speeds and yield. There is also an effort to lessen the environmental effect by investigating solvent-free environments and biocatalysts. **(Rubab *et al.*, 2022).**

1.2.4. Challenges and Advancements in Synthesis

While there are a number of well-established techniques for synthesising thiadiazole derivatives, there are still obstacles, particularly when it comes to:

- **Yield optimization**: Low yields mayoccur in certain processes because of unstable intermediates or competing side reactions. Improved catalysts and reaction conditions arethe primaryareas of interest for researchers looking to circumvent this **(Taylor *et al.*, 2023).**

- **Functional group selectivity**: Because nitrogen and sulphur atoms are reactive, it may be difficult to achieve selective replacement of functional groups at certain places on the thiadiazole ring. In order to guarantee accurate alterations, researchers are concentrating on creating synthetic pathways that are more regioselective **(Li *et al.*, 2013).**

- **Scalability**: The intricacy of some reactions makes it challenging to scale up laboratory synthesis to industrial manufacturing. The scalability of existing methods is being studied in light of recent developments in automated synthesis platforms and high-throughput screening **(Wohlgemuth *et al.*, 2015).**

The intricacy of some reactions makes it challenging to scale up laboratory synthesis to industrial manufacturing. The scalability of existing methods is being studied in light of recent developments in automated synthesis platforms

and high-throughput screening. Improving yields and selectivity by reaction condition optimisation has been a primary focus of recent developments. For instance, there have been notable advancements in reaction speed and product purity using microwave-assisted synthesis, and efforts are underway to create solvent-free procedures that minimise the use of hazardous solvents **(Isbrandt *et al.*, 2019).**

1.2.5. Properties

The physical and chemical characteristics of thiadiazole derivatives make them promising candidates for use in drug design, especially in the creation of antibacterial medicines.

1. Aromaticity: The 1,3,4-thiadiazole ring's aromaticity aids in its stability and rigidity, which are critical for preserving the molecule's structural integrity. The thiadiazole derivatives binding affinity is improved due to their aromatic character, which also allows for π-stacking interactions with enzymes or microbial receptors **(Naeem *et al.*, 2025).**

2. Polarity: Thiadiazoles are more soluble in polar solvents and may interact more easily with biological macromolecules including enzymes, proteins, and nucleic acids because of the ring polarity that is enhanced by the inclusion of sulphur and nitrogen atoms **(Gupta *et al.*, 1999).**

3. Lipophilicity: It is possible to improve membrane penetration, especially in bacterial cells, by modifying lipophilicity by substituting at various places on the thiadiazole ring. The compound's lipophilicity may be altered by adding halogens, alkyl groups, or hydroxyl groups; this changes the compound's bioavailability and target specificity **(Zakharova *et al.*, 2020).**

4. Biodegradability: An essential factor in medication design is biodegradability, and thiadiazole compounds often exhibit this property. Their safety for medical treatments is guaranteed by their capacity to break down in to non-toxic metabolites **(Rashdan *et al.*, 2019).**

5. Electrophilic Nature: One way this molecule might impede bacterial growth is by forming covalent connections with nucleophilic groups in

microbial enzymes. This is made possible by the electrophilic character of the thiadiazole ring, which is caused by the electron-withdrawing sulphur and nitrogen atoms **(Hazarika *et al.*, 2025).**

One way this molecule might impede bacterial growth is by forming covalent connections with nucleophilic groups in microbial enzymes. This is made possible by the electrophilic character of the thiadiazole ring, which is caused by the electron-withdrawing sulphur and nitrogen atoms. The properties of thiadiazole derivatives make them very desirable for use in drug design, which make them ideal for combating antibiotic resistance. To effectively combat antibiotic-resistant bacteria, it is essential to be able to alter the structure in order to increase the drug's selectivity, efficacy, and safety profile **(Cascioferro *et al.*, 2020).**

1.3. Historical Perspective of Thiadiazole Derivatives

The use of thiadiazole derivatives as antibacterial agents is an area of medicinal chemistry that has a rich and dynamic history. Research on thiadiazoles has grown substantially since their discovery, with a growing emphasis on the wide range of biological functions they exhibit. Theincreasing importance of thiadiazole derivatives in antibacterial, anti-inflammatory, and antifungal treatments is traced in this section, which examines their origins and evolution through time **(Babalola *et al.*, 2024).**

1.3.1. Early Discovery

Thiadiazole derivatives were first brought to the attention of medicinal chemists in the early 20th century, when studies on heterocyclic compounds for pharmacological purposes were initiated. The stability, aromaticity, and potential for functionalisation of the 1,3,4-thiadiazole ring structure made it an attractive scaffold that might be used to increase its biological activity **(Chandurwala et al., 2025).**

The primary goal of the early research on thiadiazole derivatives conducted in the 1930s and 1940s was to determine their potential antibacterial effects. Several thiadiazole compounds showed encouraging action against several bacterial pathogens, including those that cause gastrointestinal illnesses and

respiratory infections, according to the research. These preliminary findings sparked interest in thiadiazoles as potential antimicrobial medicines due to the widespread notion that they may effectively fight common bacterial diseases **(Albino et al., 2020).**

The thiosemicarbazide-derived thiadiazole compounds were among the first to draw interest due to their antibacterial and antifungal capabilities. Even though these chemicals weren't employed much in clinical settings back then, they showed that thiadiazole derivatives may be useful in therapy. Additional study into their structure-activity correlations (SAR) and optimisation for medicinal reasons is necessary, since these discoveries lay the groundwork for future studies **(El-Masry *et al.*, 2022).**

1.3.2. Development Over Time

Thiadiazoles' antibacterial action was only the beginning of their medicinal promise, which grew as the century went on. The primary goal of research in the 1950s and 1960s was to identify the biological effects of thiadiazole compounds and how they worked. The first SAR research found that by changing the functional groups connected to the thiadiazole ring, the antibacterial effectiveness and selectivity against certain microbes may be greatly improved **(Iorkula et al., 2025).**

Thiadiazole derivatives have a wide range of desirable properties that make them promising candidates for use in drug design. These compounds have begun to target a growing number of microorganisms, including fungi, protozoan parasites, and both Gram-positive and Gram-negative bacteria. Because of this, thiadiazoles became quite popular as a possible therapy for malaria, TB, and other severe infectious disorders. The antibacterial characteristics of some compounds were enhanced by their ability to inhibit microbial enzymes that are involved in cell wall production **(Jadhav et al., 2021).**

Finding that thiadiazole compounds have anti-inflammatory and anticancer effects was a major step forward in their development in the 1970s and 1980s. To treat inflammatory illnesses and autoimmune diseases, researchers found

that certain thiadiazole derivatives might modify the immune response and block inflammatory pathways. According to Latosińska and Latosińska (2011), these substances shown potential in inhibiting prostaglandin production and cyclooxygenase enzymes, two key players in the inflammatory process.

Thiadiazoles' antifungal effects were also studied more extensively at this time. The development of systemic fungal infections, particularly in people with impaired immune systems, prompted scientists to look into antifungal medicines based on thiadiazole. Among the many fungal pathogens that these chemicals were effective against was Candida albicans, the most common cause of yeast infections **(Mohamed *et al.*, 2022).**

1.3.3. Modern Research Focus: Antimicrobial, Anti-inflammatory, and Antifungal Properties

A lot of people started paying attention to thiadiazole derivatives while they were looking for new drugs in the 90s and 2000s. More and more, scientists saw their promise in meeting a variety of medicinal requirements. The emergence of bacteria that are resistant to several drugs prompted researchers to reevaluate the use of thiadiazoles as an antibacterial, particularly in the context of drug-resistance infections **(Castro et al., 2006).**

Thiadiazoles have not only shown promise against bacterial infections but also fungal infections (e.g., Aspergillus and Candida species) in recent research. Górecki et al. (2025) notes that this has rekindled research into developing better thiadiazole derivatives to combat healthcare-associated illnesses.

Recent studies have focused on developing more selective and bioavailable thiadiazole compounds. Thanks to developments in computational chemistry and molecular modelling, thiadiazole-based medications with enhanced antibacterial action and reduced toxicity may now be more precisely designed. Promzeleva et al. (2018) found that these chemicals may interfere with important microbial activities like DNA replication, protein synthesis, and cell membrane integrity.

In addition, researchers are looking at ways to prevent resistance and improve treatment effectiveness using combination medicines that include thiadiazole

derivatives and other antimicrobial drugs. In order to combat the problems caused by antibiotic-resistant microorganisms, a potential strategy is the combination of thiadiazoles with other medications **(Bhati, 2004).**

1.4. Biological Activity of Thiadiazole Derivatives

The biological activity of thiadiazole derivatives, especially their antibacterial capabilities, has been the subject of much research. Antibacterial, anticancer, anti-inflammatory, and antiviral actions are only a few of the many biological effects shown by these chemicals. In this part, we will give you the rundown on how thiadiazole derivatives work as antimicrobials, what other biological effects they have, and how they work **(Matysiak, 2015).**

1.4.1. Antimicrobial Activity

It is well-known that thiadiazole compounds have broad-spectrum antibacterial action, therefore they work against many different kinds of germs, fungus, and viruses. Particularly in the setting of antimicrobial resistance (AMR), their capacity to target both bacterial and fungal infections has made them appealing contenders in the battle against infectious illnesses **(El-Saghier *et al.*, 2024).**

1.4.2. Effective Against a Wide Variety of Bacteria, Both Positive and Negative Gram-Stamped

Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, and methicillin-resistant Staphylococcus aureus (MRSA) are among the many Gram-positive bacteria that have been shown to be vulnerable to thiadiazoles. These bacteria cause skin infections, lung infections, and sepsis; what makes them even more difficult to treat is that they are resistant to standard antibiotics **(Rivera and Boucher, 2011).**

The Gram-negative bacteria, including Pseudomonas aeruginosa, Salmonella spp., and Escherichia coli, may be inhibited by thiadiazole derivatives as well. Diseases of the digestive system, urinary tract, and skin may all be brought on by these microbes. As many antibiotics fail to pass through Gram-negative bacteria's outer membrane, thiadiazoles have shown to be an exception **(Akram et al., 2019).**

Systemic fungal infections, especially in immunocompromised individuals, may be caused by fungi such as Candida albicans, Aspergillus niger, and Cryptococcus neoformans; thiadiazoles have shown strong effectiveness against these pathogens **(Alwan *et al.*, 2015).**

1.4.3. Mechanisms of Action

There are three main ways in which thiadiazole derivatives exert their antibacterial effects:

1. Enzyme Inhibition:

- Thiadiazole compounds suppress key enzymes in DNA replication, protein synthesis, and cell wall formation, which are processes that microbes rely on. Some of these chemicals have been shown to block enzymes involved in DNA replication and supercoiling, such as topoisomerase and DNA gyrase. Thiadiazoles are effective in stopping infections because they impede bacterial proliferation by blocking certain enzymes.
- Furthermore, certain thiadiazoles have the added benefit of inhibiting beta-lactamase, which is an enzyme that resistant bacteria use to break down beta-lactam antibiotics. This means that the medications' effectiveness may be restored **(Zou *et al.*, 2025).**

2. Membrane Disruption:

- Thiadiazole derivatives also compromise the bacterial cell membrane's integrity. Because of this rupture, ions, amino acids, and nucleic acids essential components of cells—leak out, and cells die. Particularly for Gram-negative bacteria—which have an extra outer membrane that blocks many antimicrobial agents—thiadiazoles' capacity to engage with the lipid bilayer and disrupt membrane potential is crucial **(Fu *et al.*, 2025).**

3. Targeting Folic Acid Metabolism:

Antimetabolites are a property of some thiadiazole derivatives that allow them to inhibit bacterial folic acid metabolism. Inhibiting enzymes such as

dihydrofolate reductase or dihydropteroate synthase, thiadiazoles prevent the production of amino acids and nucleotides, which are necessary for the development and replication of bacteria. Inhibiting the folic acid production route is another function of sulfonamides, and this one is rather similar **(Fernández-Villa *et al.*, 2019).**

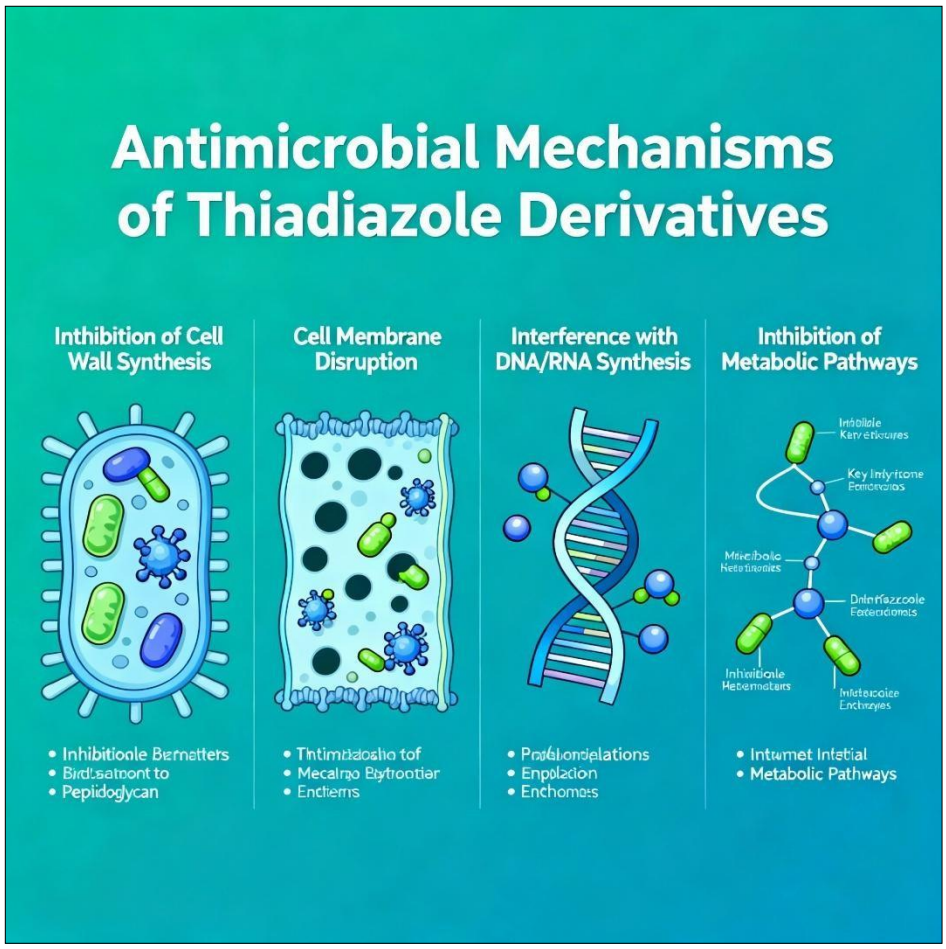


Figure 1.2: Mechanisms of Antimicrobial Action of Thiadiazole Derivatives (Kharb *et al.*, 2011).

1.4.4. Additional Biological Activities

In addition to its antibacterial capabilities, thiadiazole derivatives have shown promising results in several fields of medicinal research, including the fight against cancer, inflammation, and viruses **(Podila *et al.*, 2024).**

A-Anticancer Activity

Research has shown that thiadiazole derivatives may inhibit the growth of cancer cells by triggering a process known as apoptosis, or programmed cell death. These chemicals aim at many critical pathways that regulate cell proliferation, tumour growth, and cell cycle control. Thiadiazoles are promising new cancer treatments because they block these pathways, hence reducing cancer cell proliferation and metastasis **(Janowska et al., 2022).**

Thiadiazoles are effective against cancer in part because they block the PI3K/Akt/mTOR pathway, a network crucial for controlling cell proliferation and survival. The MAPK/ERK pathway is an important regulator of cancer cell invasion and migration, and thiadiazoles affect it as well. Thiadiazoles' ability to inhibit tumour development and decrease cancer cells' metastatic potential is due to their targeting of these pathways.

Furthermore, it has been shown that certain thiadiazole derivatives modulate angiogenesis-related gene expression. Angiogenesis is the process by which tumours get oxygen and nutrients via the formation of new blood vessels. Thiadiazoles are able to starve tumours and stop their growth because they block angiogenesis **(Gawande *et al.*, 2025).**

B- Anti-inflammatory Activity

Asthma, inflammatory bowel disease, rheumatoid arthritis, and other inflammatory illnesses may be amenable to thiadiazole derivatives because of their anti-inflammatory characteristics. Their ability to block the production of prostaglandins and inflammatory cytokines is the main mechanism by which they exert their anti-inflammatory actions **(Mehta et al., 2019).**

As COX-2 inhibitors, several thiadiazoles prevent the body from making prostaglandins, the molecules that mediate pain and inflammation. Thiadiazoles alleviate inflammation-related discomfort, oedema, and fever by blocking COX-2. In addition, the anti-inflammatory effects of some thiadiazoles are enhanced because they block NF-κB signalling, an important regulator of the immune response.

There are less negative effects associated with these chemicals, making them a promising alternative to conventional non-steroidal anti-inflammatory medicines (NSAIDs) **(Brune and Patrignani, 2015).**

C- Antiviral Activity

A number of thiadiazole compounds have shown promise as antiviral agents in recent research, namely against HSV, HIV, and hepatitis B. Thiadiazoles prevent viruses from replicating and maturing by blocking enzymes essential to their processes, such as viral proteases and reverse transcriptase. According to Farghaly et al. (2022), thiadiazoles are able to stop the virus from multiplying in host cells by striking these enzymes **(Farghaly *et al.*, 2022).**.

Furthermore, by reacting with the viral envelope or surface glycoproteins, some thiadiazoles prevent the virus from entering host cells. This stops the virus from infecting good cells, which means it can't spread as much. Thiadiazole derivatives are a possible new direction in antiviral therapy research because of their multi-targeting characteristics, which make them effective against a wide variety of viruses **(La Frazia *et al.*, 2013).**

1.5. Structure-Activity Relationship (SAR) of Thiadiazole Derivatives

1.5.1. Substituent Effects on Activity

Substituent type and location on the thiadiazole ring significantly affect the antibacterial activity of 1,3,4-thiadiazole derivatives. Researchers may improve the therapeutic effects of these compounds by altering the functional groups linked to the ring, which enhances their bioactivity, lipophilicity, and selectivity.

1. Electron-Withdrawing Groups (EWGs):

- At the C-5 position of the thiadiazole ring, it is usual to add halogens (such as chlorine or fluorine) and nitro or cyano groups (-NO_2 or -CN). The electrophilicity of the ring is increased by these electron-withdrawing substituents, which allows for greater interactions with nucleophilic groups in microbial enzymes or receptors.

- Adding halogens may make the chemical more lipophilic, which means it can cross bacterial cell membranes easier **(Pace *et al.*, 2005).**

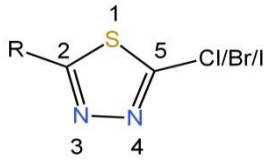


Figure1.3: Thiadiazole Derivative with Halogen at C-5 Position (Konstantinova *et al.*, 2014).

2. Electron-Donating Groups (EDGs): Methoxy (-OCH_3) and amino (-NH_2) groups, which are electron donors, affect the ring's electron density and are usually located at the C-2 or C-5 locations. The nucleophilicity of the molecule may be increased by these groups, which might lead to better binding to certain microbial targets. Additionally, EDGs influence the compound's solubility, which has the potential to enhance bioavailability **(Cardano *et al.*, 2025).**

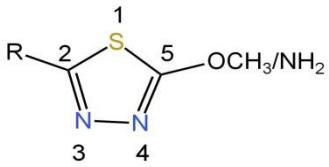


Figure 1.4: Thiadiazole Derivative with Amino/ methoxy Group at C-5 Position (Wang *et al.*, 2011).

3. Halogen Substituents: The addition of fluorine (F), chlorine (Cl), or bromine (Br) at the C-5 position improves the compound's lipophilicity and membrane permeability, making it more efficient in penetrating bacterial cells. By making the thiadiazole ring more electrophilic, these halogens boost the antibacterial activity by interacting with enzymes and proteins produced by microbes **(Zhou *et al.*, 2016).**

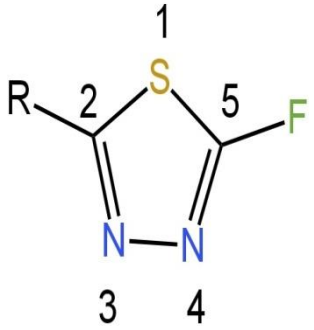


Figure 1.5: Thiadiazole Derivative with Fluorine at C-5 Position (Chowrasia *et al.*, 2017).

1.5.2. SAR of Thiadiazole Derivatives

An analysis of 1,3,4-thiadiazole derivatives' structure-activity relationship (SAR) shows that the addition of side chains and the placement of substituents on the ring greatly affect the compound's biological activity. These alterations may improve the antimicrobial's efficacy, selectivity, and solubility, according to important research **(Oruç *et al.*, 2004).**

1. Position of Substituents: The compound's action may be substantially altered by modifying the thiadiazole ring at the C-2, C-5, or C-4 locations. One way to improve a compound's interaction with microbial enzymes is to place amino ($-NH_2$), hydroxyl (-OH), or methoxy ($-OCH_3$) groups at the C-2 position. This increases hydrogen bonding **(Surov *et al.*, 2013).**

2. Side Chain Modifications: Adding heterocyclic, alkyl, or aryl side chains to a molecule at the C-2 or C-5 position may change its lipophilicity and make it more effective in penetrating microbial membranes. In addition to improving the compound's selectivity for certain infections, these side chains may facilitate its interaction with particular microbial targets, such as receptors, enzymes, or membrane proteins **(Gaba and Mohan., 2016).**

3. Functional Group Positioning: Hydroxyl (-OH), carboxyl (-COOH), and sulfhydryl (-SH) groups, among others, affect the molecule's three-dimensional structure depending on their placement. By strategically placing these groups, the compound's binding affinity and antibacterial activity may be

Enhanced via hydrogen bonding interactions with target enzymes or receptors **(Peng and Zhang, 2021).**

1.5.3. Key Studies Exploring the SAR of Thiadiazole Derivatives

The effect of thiadiazole ring alterations on antibacterial properties has been the subject of many SAR investigations. For example, one study found that halogenated thiadiazoles were more effective against both Gram-positive and Gram-negative bacteria than non-halogenated ones when it came to antibacterial activity **(Reddy et al., 2016).**

An additional note worthy research found that thiadiazole derivatives' antibacterial activity against Staphylococcus aureus was greatly increased by amino and methoxy groups at the C-2 position. They also observed that alkyl substitution at the C-4 position led to increased lipophilicity, which improved the compound's ability to penetrate bacterial membranes **(Muglu et al., 2022).**

In order to maximise antibacterial effectiveness while minimising toxicity and side effects, these investigations highlight the significance of choosing the right substituents at critical places on the thiadiazole ring.

1.6. Mechanism of Action of Thiadiazole Derivatives

The capacity of thiadiazole derivatives to target critical cellular processes has led to their emergence as powerful antibacterial agents. Inhibiting enzymes, disrupting membranes, and selectively targeting microbe receptors or enzymes are all parts of their complex action processes. Their ability to tackle drug-resistant infections and their broad-spectrum action are both enhanced by these methods **(Abumelha *et al.*, 2025).**

1.6.1. Inhibition of Microbial Enzymes

Inhibiting key microbial enzymes is one of the main ways that thiadiazole derivatives achieve their antibacterial actions. Enzymes are essential for many bacterial biological functions, including DNA replication, protein synthesis, and production of the cell wall. Evidence suggests that thiadiazoles interact with DNA replication enzymes such as DNA gyrase and topoisomerase. Enzymes like these play an essential role in DNA replication and cellular

division by ensuring that the DNA strands remain properly supercooiled. Thiadiazole derivatives kill bacteria by blocking certain enzymes that are involved in DNA replication **(Fisher and Mobashery, 2020).**

Folic acid metabolism is another target of some thiadiazole derivatives, which similarly block DNA gyrase and topoisomerase. The production of aminoacids and nucleotides—essential building blocks for bacterial growth—requires folic acid. An enzyme called dihydrofolate reductase is involved in the process of converting dihydrofolate to tetrahydrofolate, an essential building block for nucleotide synthesis; thiadiazoles have the ability to inhibit this enzyme. Like other antimicrobials, such as sulfonamides, thiadiazoles inhibit bacterial growth and cell division by interfering with this mechanism **(Hashem *et al.*, 2020).**

1.6.2. Disruption of Cell Membrane Integrity

One of the most important ways that thiadiazole derivatives kill microbes is by compromising the membrane integrity of their cells. The bacterial cell membrane is crucial for several reasons, including preserving the cell's structure, controlling the transfer of nutrients, and shielding the cell from outside influences. Thiadiazoles have the capacity to disrupt the bacterial cell wall's lipid bilayer, leading to an increase in permeability. According to Shia et al. (2024), this causes the loss of important cellular components such ions, proteins, amino acids, and nucleic acids, ultimately resulting in cell death.

Thiadiazole derivatives are very efficient against Gram-negative bacteria due to their propensity to rupture cell membranes. Gram-negative bacteria have an extra outer membrane that many antibiotics can not get through. Thiadiazoles are able to penetrate bacterial cell walls and interfere with their functions because of their lipophilicity, which is typically made even stronger by the addition of halogens or hydroxyl groups. Because it impacts the membranes of both bacteria and fungus, this process helps explain why thiadiazoles are effective against a wide variety of microbes **(Xie *et al.*, 2022).**

1.6.3. Selective Targeting

The capacity of thiadiazole derivatives to spare host cells as much harm as possible while targeting bacterial systems selectively is a major benefit. In order to make thiadiazole-based medicines safer for therapeutic usage, this selectivity is essential for decreasing their toxicity. To ensure that the molecule preferentially interacts with the microbial target, structural changes to the thiadiazole ring may boost its binding affinity to bacterial enzymes or receptors that are not present in human cells **(Varna et al., 2023).**

Bacterial DNA replication enzymes such as topoisomerase and gyrase are absent from human cells. Thiadiazole derivatives may be engineered to bind to these enzymes selectively. Because of its selectivity, the chemical may efficiently hinder bacterial development without affecting human biological processes. Further improvement of effectiveness and reduction of off-target effects may be achieved by adding halogens or hydroxyl groups to certain places on the thiadiazole ring, which increases the binding affinity of the molecule for microbial targets **(Nancy and Soam, 2022).**

Thiadiazoles alleviate a key issue in the search for novel antibiotics the potential for systemic toxicity and side effects by specifically targeting enzymes and receptors on microbes. Due to their selective action, thiadiazoles show great promise as potential treatments for many bacterial and fungal illnesses. This is particularly true in situations when conventional antibiotics have become ineffective because of resistance **(Kapoor *et al.*, 2025).**

1.7. Thiadiazole Derivatives in Drug Discovery

The wide-ranging antibacterial, anticancer, and anti-inflammatory effects of thiadiazole derivatives have made them attractive therapeutic targets. In order to maximise their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) qualities, there are obstacles to overcome concerning solubility, stability, and toxicity, not with standing their promise **(Mittal *et al.*, 2024).**

1.7.1. Current Role in Drug Development

Because of their effectiveness in targeting bacterial enzymes and membranes, thiadiazoles are becoming more popular as antimicrobial treatments in the battle against multidrug-resistant illnesses (such as MRSA and MDR-TB).

Through their ability to block cell growth pathways and induce apoptosis, they also show potential as anticancer medicines. Their anti-inflammatory and antiviral properties further increase their therapeutic utility, positioning them as promising candidates for combination treatments **(Krishna *et al.*, 2022).**

1.7.2. Challenges in Drug Design

1. Solubility: Thiadiazoles have a low affinity for water, which makes it difficult for the body to absorb them. To increase solubility, modifications such as hydroxyl or amino groups may be used.

2. Stability: The therapeutic potential might be diminished by chemical instability when subjected to different environments. It is possible to improve stability by using prodrug methods and protective groups.

3. Toxicology: Thiadiazole compounds should be engineered to specifically target microbial systems, reducing harm to human cells, in order to decrease off-target toxicity **(Liu *et al.*, 2022).**

1.7.3. Modifications Needed to Improve ADMET Properties

Thiadiazole derivatives may have their ADMET characteristics enhanced by making adjustments such

- Raising lipophilicity to improve bioavailability and penetration of cell membranes.
- Preventing early breakdown by enhancing metabolic stability.
- Increasing water solubility and optimising functional group placement to provide better renal excretion and decreased toxicity **(Kundu *et al.*, 2019).**

1.7.4. Potential for Overcoming Drug Resistance

In the fight against superbugs and other multidrug-resistant bacteria, thiadiazoles have shown great promise. They show promise in combating antibiotic-resistant infections by focusing on bacterial membranes, enzymes, and folic acid metabolism. Even against resistant diseases, their multi-targeting capabilities makes them effective, and combination therapy may further boost their efficacy **(Elshobary *et al.*, 2025).**

1.8. Significance of the Study

1.8.1. Contribution to Medicinal Chemistry

This research on thiadiazole derivatives as possible antibiotics is a huge step forward for medicinal chemistry. Researchers have shown that thiadiazoles, a family of heterocyclic chemicals, offer great potential as antimicrobials and anti-inflammatory agents, among many other medicinal applications. This study will provide fresh insights into the potential of thiadiazole derivatives as antibacterial and antifungal agents by synthesising and developing new compounds **(Rusu et al., 2023).** These compounds might be important alternatives to traditional antibiotics. As a result of this research, next-generation antimicrobial medications may be better able to combat illnesses caused by superbugs and germs that are resistant to several drugs. Thiadiazoles may be fine-tuned to target microbiological infections more effectively and with fewer side effects using structure-activity relationship (SAR) research **(Salah *et al.*, 2015).**

1.8.2. Addressing AMR (Antimicrobial Resistance)

Many antibiotics and antifungals may become useless due to antimicrobial resistance (AMR), which has become one of the most important global health concerns of the modern era. There is an immediate need for new types of antimicrobial medicines due to the increasing prevalence of multidrug-resistant (MDR) bacteria, which have rendered current therapies ineffective. As a possible solution to the issue of antimicrobial resistance (AMR), this research will investigate molecules derived from thiadiazole **(Coque et al., 2023**). These substances have shown antimicrobial properties against several microbes, including yeasts, bacteria (both Gram-positive and Gram-negative), and more. This study will contribute to the global effort to combat antibiotic resistance by bringing attention to drug-resistant bacterial species, such as MRSA and MDR-TB. The study will aid in the identification of compounds that may overcome resistance mechanisms by researching their mechanisms of action and structure-activity relationships (SAR). This will provide innovative leads for therapeutic development **(Terreni *et al.*, 2021).**

1.8.3. Advancements in Drug Discovery

By optimising and designing thiadiazole compounds using advanced computer modelling and in silico approaches, this work also contributes significantly to the progress of drug development. For the purpose of predicting the binding affinity and mechanism of action of the synthesised drugs, many methods will be used, including molecular docking, QSAR modelling, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) predictions **(Eissa et al., 2024).** Researchers will be able to enhance their chances of finding effective antimicrobial medicines while saving time and resources by using these computational methods to evaluate and optimise thiadiazole derivatives prior to experimental production. Modern drug discovery relies heavily on computational techniques for drug design, and this work will add to the growing body of evidence supporting the utility of in silico approaches to the creation of new therapies **(Celik *et al.*, 2022).**

1.9. Research Objectives

The goals of this research are as follows:

1. To develop and produce a range of thiadiazole derivatives that might be used as antimicrobials.
2. The second objective is to study the effect of different functional group modifications on the antibacterial activity of thiadiazole derivatives in order to optimise their structure-activity relationship (SAR).
3. To test the effectiveness of the synthesised thiadiazole derivatives against both Gram-positive and Gram-negative bacteria in terms of their antibacterial activities.
4. Using computational approaches like as molecular docking and QSAR modelling, we may anticipate the ADMET characteristics, binding affinity, and mechanism of action of the compounds that have been synthesised **(Tareq Hassan Khan, 2010).**

1.10. Research Questions

The following research issues are intended to be addressed by this study:

Q-1: What structural modifications to the thiadiazole ring lead to enhanced antimicrobial activity?

Studying the effect of various substituents on the antibacterial efficacy and selectivity of thiadiazole rings.

Q-2: How do thiadiazole derivatives interact with microbial targets at the molecular level?

Investigating how thiadiazole derivatives work by studying their binding affinities to various microbes and their enzymes and membrane proteins.

Q-3: Can computational studies reliably predict the antimicrobial efficacy of thiadiazole derivatives?

Predicting the activity and toxicity of the synthesised thiadiazole derivatives using molecular docking and QSAR models; then, conducting experimental tests to validate the results.

1.11. Scope of the Study

Experimental and computational methods are both included in this study:

1. Experimental:

- Developing and synthesising a library of thiadiazole derivatives that differ in the functional groups and substituents positioned on the thiadiazole ring.
- Biological testing to determine the effectiveness of antimicrobials against specific microbial strains.

2. Computational:

- Making use of molecular docking to foretell how well made thiadiazole derivatives would attach to germs.
- Quantitative Structure-Activity Relation (QSAR) modelling to investigate structure-antimicrobial activity, and ADMET predictions to evaluate drug-like characteristics.

3. Biological Testing: Paying special attention to important microbiological strains, including a variety of Gram-positive and Gram-negative bacteria, fungi, and viruses, such as Escherichia coli, Staphylococcus aureus, and Candida albicans.

1.12. Thesis Structure

The thesis will be structured as follows:

- **Chapter 1: Introduction**: An outline of the study aims, the function of thiadiazole derivatives in infection prevention, and the antimicrobial resistance (AMR) issue will be given in this chapter.
- **Chapter 2: Literature Review**: The present state of the art in antimicrobial agent research is summarised, with an emphasis on thiadiazole derivatives and their possible use in future medication development.
- **Chapter 3: Methodology**: This chapter will outline the experimental strategy for thiadiazole derivative production, the techniques used to assess their antibacterial activity biologically, and the computational investigations that were performed, such as molecular docking and QSAR modelling.
- **Chapter 4: Results and Discussion**: Described here are the structure-activity relationships (SAR), compared to already available antimicrobial medications, and an analysis of the antibacterial activity of the synthetic chemicals.
- **Chapter 5: Conclusion**: Results, future directions, and therapeutic uses of thiadiazole derivatives in the treatment of drug-resistant illnesses are the main points of this work.

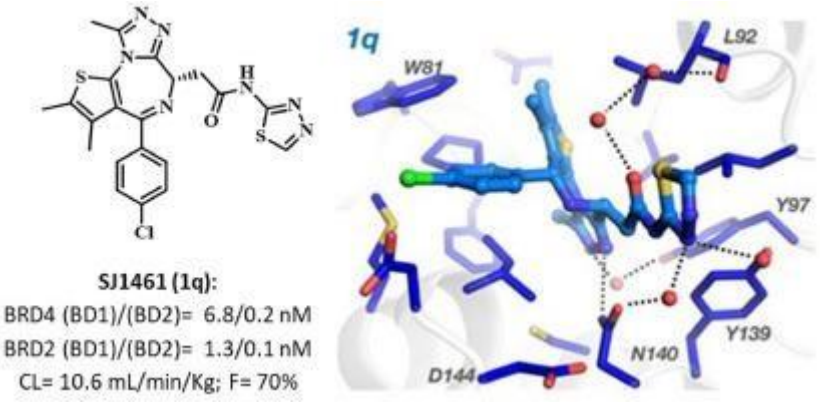
REVIEW OF LITERATURE

In 2023, Ji *et al.* addressed the formidable challenge of converting covalent organic frameworks (COFs) into practical macroscopic forms. Their groundbreaking method resulted in the development of NNS-VCOF, a new macroscopic ultralight aerogel generated from thiadiazole. A tritopic aromatic aldehyde derivative and 2,5-dimethyl-1,3,4-thiadiazole were combined in an acid-catalyzed aldol condensation to produce this extraordinary compound. The intrinsic flame retardancy of NNS-VCOF aerogel is due to its three-dimensional sponge-like structure and unique nitrogen heterocyclic framework., rapidly self-extinguishes after ignition, and exceptional mechanical properties, Thanks to its superhydrophilicity and suitable optical qualities, this material displayed effective photocatalytic hydrogen evolution capabilities in powdered form, in addition to being an extraordinary macroscopic substance **(Ji *et al.*, 2023).**

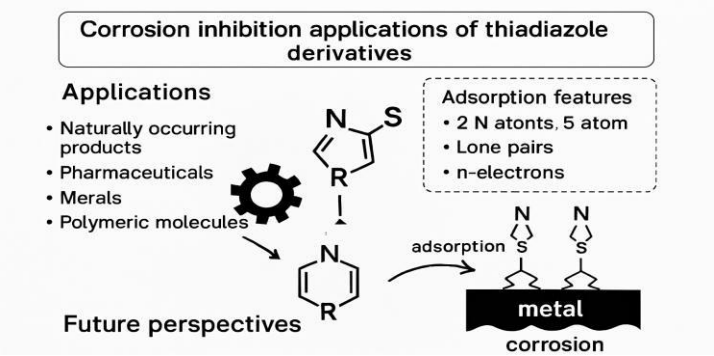


In 2023, Koravovic *et al*. reported a significant advancement in the field of bromodomain and extraterminal (BET) protein degradation using a CLICK chemistry-based PROTAC. New heterocyclic amides generated from JQ1 were synthesised according to their study, which was influenced by the X-ray structure of this PROTAC attached to BRD2 (BD2). Potent BET inhibitors with better overall profiles than JQ1 and birabresib were identified as a consequence of this research effort. One remarkable chemical, 1q (identified as SJ1461), which has a structure derived from thiadiazole, showed amazing

affinity for BRD4 and BRD2, and it was very effective against a panel of medulloblastoma and acute leukaemia cell lines. Increased binding affinity was explained by polar interactions with the AZ/BC loops, particularly involving Asn140 and Tyr 139, which were shown by further structural studies, suchas a co-crystallization study of 1q with BRD4-BD1. The pharmacokinetic studies of this family of compounds also showed that the heterocyclic amide group improved drug-like characteristics. The results of this investigation led to the identification of 1q (SJ1461), an orally accessible BET inhibitor that is both powerful and promising as a potential lead candidate for further research into BET protein degradation **(Koravovic *et al.*, 2023).**



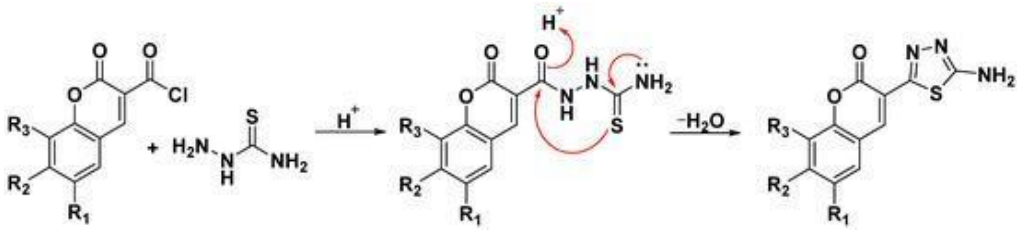
In 2023, Liu *et al*. focused on thiadiazole compounds. Because of their distinct nitrogen and sulphur atom structures, these compounds have shown high adsorption on metal surfaces. As a result, they are both eco-friendly and effective inhibitors in a variety of water conditions. Salts and polymeric compounds based on thiadiazole were also mentioned as having corrosion-inhibiting potential. Their performance is affected by structural factors such as alkyl chains, π-bonds, and extra heteroatoms. In order to understand how they work, there view included both computational and experimental investigations. The essay wrapped off by suggesting possible directions for further studies in this field **(Liu *et al*., 2023).**



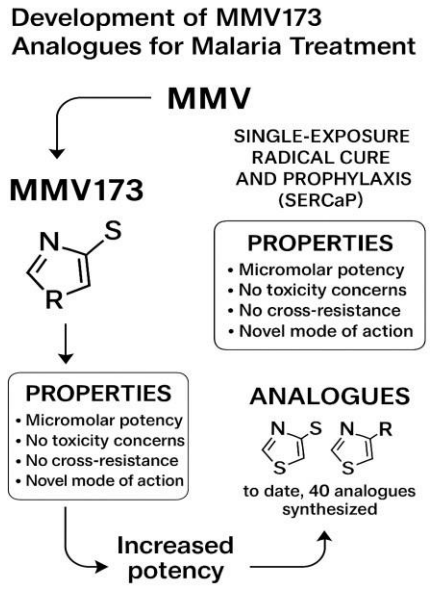
In 2022, RADHA *et al.* synthesized and characterized a novel thiadiazole-based Schiff base ligand and its metal complexes (Co, Ni, Cu, Zn). Through the use of several methods, their study demonstrated that the majority of complexes exhibited octahedral geometry, with the exception of Zn(II), which had a tetrahedral configuration. Through the use of B3LYP functional, the ligand and Zn(II) complex were perfected. Theoretical characteristics and fluorescence capabilities of the ligand were also evaluated in the research. In addition, the antibacterial activity of the metal complexes was shown to be better than that of the ligand. For example, complexes involving copper and iron showed promise against breast cancer (MCF-7) cell lines and in vitro α-amylase inhibition **(Radha and Prabakaran, 2022).**

In 2022, Karcz *et al*. created a new class of compounds called coumarin-thiadiazole hybrids by substituting coumarin-3-carboxylic acids. The compounds were studied in great detail using XRD crystallography and other spectroscopic methods. Similar to popular coumarin-based fluorescence standards, a number of these new compounds displayed strong visible-spectrum fluorescence. The antineurodegenerative agent potential of the compounds was assessed by testing their fluorescence characteristics and their capacity to inhibit butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE). Additionally, we tested the compounds against pathogenic bacteria and fungi to see if they had any antibacterial properties. While the findings were satisfactory, we were disappointed that only a small number of compounds demonstrated moderate AChE and BuChE activity when compared to market standards. On the other hand, they failed miserably in a battery of biochemical tests measuring activity and toxicity. It is worth mentioning that a few of these hybrid compounds exhibited remarkable emission, which might

Lead to their use as imaging agents or fluorescence standards **(Karcz *et al.*, 2022).**



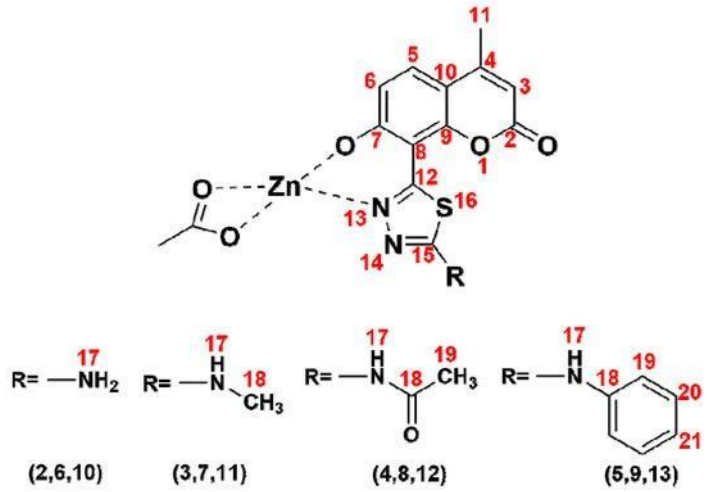
In 2022, Ahmad *et al.* highlighted the significant work of MMV (Medicines for Malaria Venture) in its mission to alleviate the burden of malaria in endemic regions. In their quest to create a malaria treatment that works both as a cure and a preventative measure, they came up with MMV173, a thiadiazole-based molecule. There were no harm concerns linked with MMV173, which showed micromolar efficacy against blood stage malaria infection (Pf3D7-IC50). The compound's unique method of action and lack of cross-resistance are noteworthy. It was also soluble and killed at amodest pace. But problems with its high rat and human metabolism and substantial hERG channel activation surfaced. Careful planning and synthesis of a series of analogues meant to improve potency while reducing worries about metabolic and hERG channel activity allowed us to tackle these difficulties. So far, structural changes to MMV173 have resulted in the synthesis of forty analogues. The analogues were two to three times as effective as MMV173, although the problem of high metabolism is still there **(Ahmad *et al.*, 2022).**



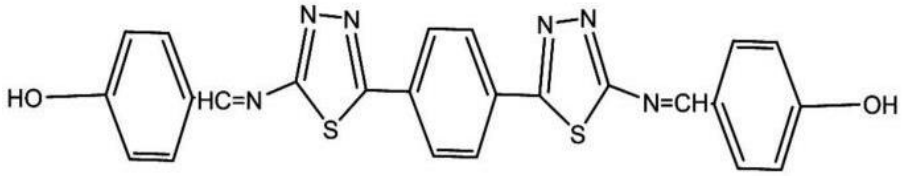
In 2021, Obakachi *et al.* emphasized the ongoing challenge of cancer as a leading cause of mortality, underscoring the pressing need for novel and more effective anticancer treatments. As a potential route for developing novel anticancer drugs, they highlighted the current upsurge in interest in small compounds with five-membered heterocyclic groups. In this setting, the 1,3,4-thiadiazole stood out as a scaffold having several uses in medicinal chemistry. The 1,3,4-thiadiazole derivatives have the ability to bind to biological targets with little toxicity and great selectivity due to their distinctive mesoionic ring structure, which enables them to readily cross cellular membranes. Reviewing the literature on derivatives incorporating the 1,3,4-thiadiazole motif, the authors sought to synthesise recent advances in synthetic methodologies, structural-activity relationships (SAR), and anticancer characteristics. The goal was to emphasise these compounds' potential as effective anticancer agents **(Obakachi *et al.*, 2021).**

In 2021, Karcz *et al.* synthesised and characterized coumarin-thiadiazole hybrids and their corresponding Cu(II) and Zn(II) complexes utilizing spectroscopic methods as part of a research. The findings showed that all of the coumarin-thiadiazole hybrids acted as bidentate chelators for Cu(II) and Zn(II) ions. The proportion of ligand to metal in the isolated complexes varied with the metal at the center. The ligand-to-metal ratio in Zn(II) complexes was often 1:1, whereas in Cu(II) complexes it was typically 2:1. The chemicals

were then tested as possible antibacterial agents. But when compared to drugs sold in stores, their antibacterial activity was much lower. As all substances showed moderate acetylcholinesterase (AChE) inhibitory activity, their potential as antineurodegenerative medicines was evaluated with more optimism **(Karcz *et al.*, 2021).**

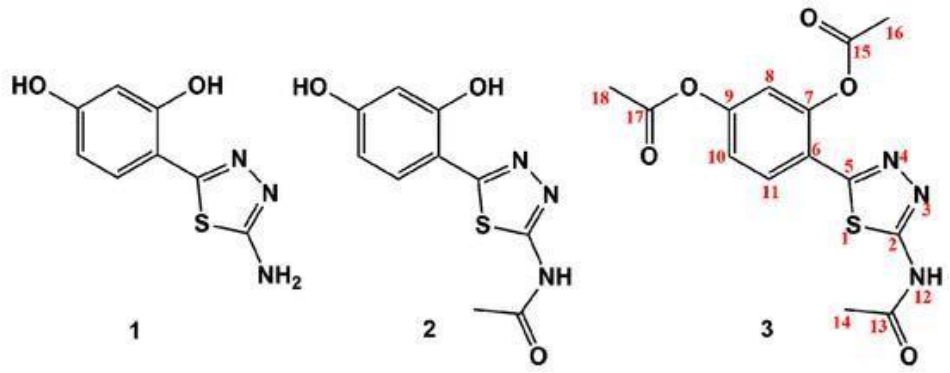


In 2021, Saeed *et al.* addressed the escalating need for novel cancer treatments by exploring new medicinal compounds. The cytotoxicity of this novel chemical was examined against breast cancer (MCF-7) cells at two different dosages (250 and 300 mg/mL). An evaluation of the compound's potential anticancer effects was conducted via the use of DNA fragmentation, acridine orange/propidium iodide staining, and cell line viability. The cytotoxic impact on MCF-7 breast cancer cells was shown by the results of the cell viability experiments. The interaction between the new chemical and the Vero and MCF-7 cell lines did not seem to cause DNA fragmentation. This Schiff base molecule inhibited the multiplication of breast cancer cells (MCF-7) without triggering apoptosis, demonstrating clear anti-cancer action. These results suggested that the chemical may have therapeutic use in the treatment of breast cancer **(Saeed *et al.*, 2021).**

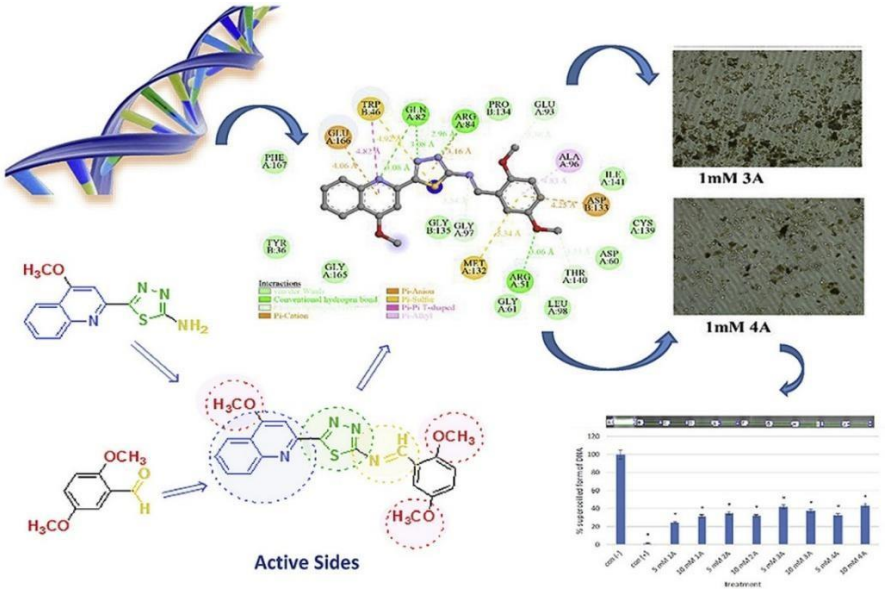


4,4'-((1*E*,1'*E*)-((1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl))bis(azaneylylidene))bis(methaneylylidene))diphenol

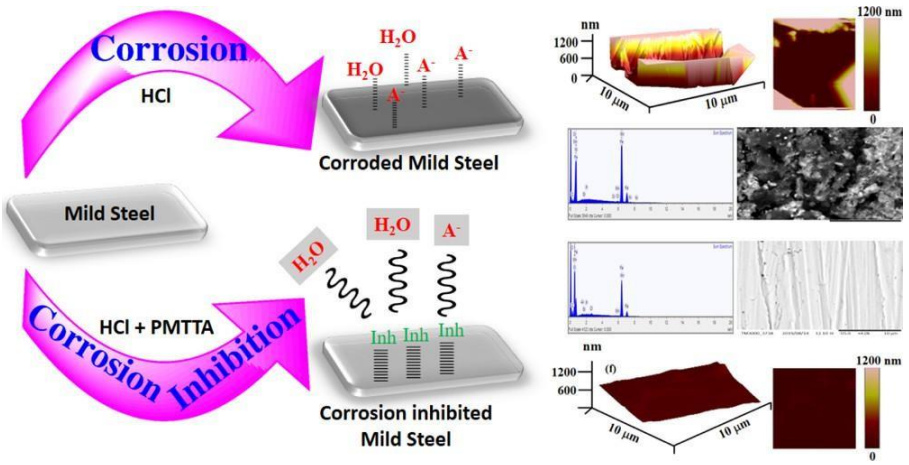
In 2020, Karcz *et al.* synthesized three novel 1,3,4-thiadiazole derivatives and formed complexes with Cu(II) and Zn(II) ions. A battery of spectroscopic methods were used to characterise the compounds and complexes in great detail. The findings demonstrated the presence of dihydrate complexes with distinct chelation patterns. The results of the antibacterial tests were mild, and there was also some slight antioxidant action. The thiadiazole derivatives showed a potent synergistic antibacterial action against Staphylococcus aureus when mixed with the antibiotic kanamycin. The inclusion of kanamycin resulted in a lowered minimum inhibitory concentration (MIC) for the most active derivative. In addition, a number of the compounds amplified kanamycin's antimicrobial activity. Antioxidant activity was much lowerin the most active thiadiazole derivative than in TROLOX **(Karcz *et al.*, 2020).**



In 2020, Gur *et al.* investigated the possible medicinal uses of Schiff bases generated from 5-substituted-1,3,4-thiadiazole-2-amine. After synthesizing and creating these compounds, they used FTIR, 1H NMR, and elemental analysis to validate their structures. Several biological characteristics were examined in the research. The DNA was significantly protected against oxidative Fenton mixtures by compounds 3A and 4A. Compounds 2B and 1A also showed potent antibacterial action against S. epidermidis. The PC-3 and MDA-MB-231 cancer cell lines were shown to be cytotoxic when compound 3A was tested, with IC50 values of 370.7 μM and 505.1 μM, respectively. Molecular docking research looked at their potential anti-cancer and anti-bacterial effects. Additional research into compound 3A's synergistic effects with chemotherapy medications could lead to a more efficient treatment approach that causes less harm to healthy cells **(Gur *et al.*, 2020).**

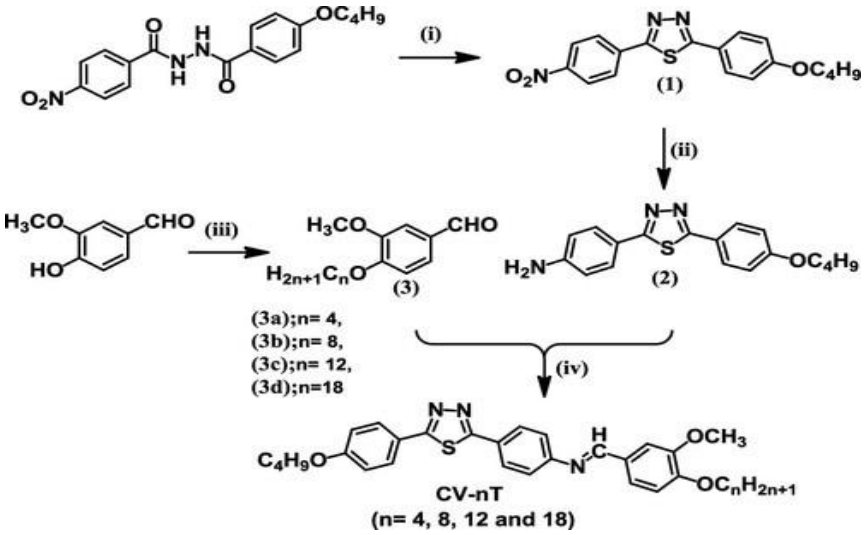


In 2020, Chugh *et al.* performed a study comparing the corrosion prevention capabilities of several bis-Schiff bases generated from thiadiazoles on mild steel in 1 M HCl. In order to assess the effectiveness of these bis-Schiff bases as corrosion inhibitors, their research used a range of electrochemical methodologies, including potentiodynamic polarisation and electrochemical impedance spectroscopy, in conjunction with weight loss trials. The best concentration for all inhibitors was determined to be 125 ppm, which resulted in the maximum inhibition effectiveness. Research on potentiodynamic polarisation revealed a mixed-type, mostly cathodic-inhibition character. To further understand the adsorption process, the Langmuir adsorption isotherm was used. The activation energy values changed when the inhibitor was added, suggesting that the inhibitor interacted with the metallic surface in a mixed fashion. The adsorption of bis-Schiff bases on the metal surface, affording protection against corrosion, was verified by further tests by scanning electron microscopy with energy-dispersive spectroscopy, X-ray diffraction, and X-ray photoelectron spectroscopy. The research also used molecular dynamics simulations and quantum chemistry computations to look at how the inhibitor's molecular structure relates to its inhibitory efficacy; all of the studies came to the same conclusion **(Chugh *et al.*, 2020).**



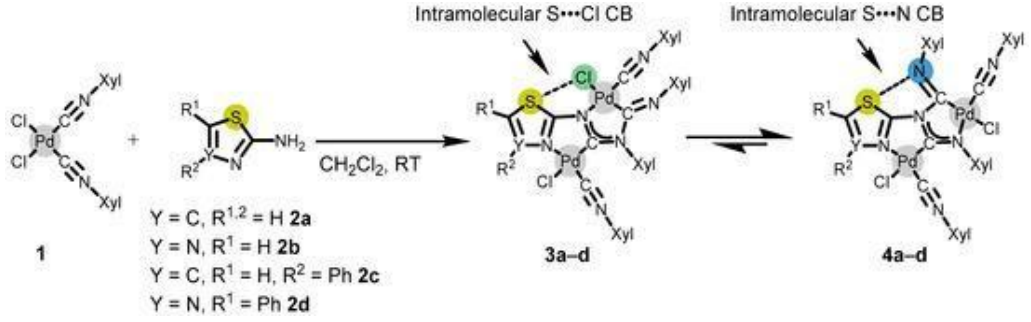
In 2019, Moghaddas developed a new process for making chemicals from thiadiazoles. For this method, the heat source was microwave radiation, and the raw materials were cyclodehydranate, carboxylic acids, and tiocarbono-hydrazide, all of which were supported bysilica-based dichlorophosphate.The method's many benefits included a lack of environmental contamination, a quick response time, great efficiency, and simplicity of implementation **(Moghaddas., 2019).**

In 2019, Saha *et al.* Studied the 1,3,4-thiadiazole core, novel short-core, hockey stick-shaped liquid crystalline molecules, and their thermotropic characteristics. These bent-core thiadiazole derivatives exhibited polar switching behaviour during the cybotactic nematic and smectic mesophases, according to the research. According to X-ray diffraction analysis, the four-ring molecules were able to spontaneously organise themselves in the nematic phase reason being, their outer phenyl ring had a lateral methoxy group. Potential molecular antiferromagnetic packing requiring a stronger electric field for switching was proposed as the explanation for the unusual temperature-dependent spontaneous polarisation following cooling. The compounds had an excellent quantum yield of around 0.39, and they emitted blue light at about 445 nm and showed high absorption at about 356 nm. There was also a notable Stokes shift, the size of which was affected by the solvent, as discovered in the study **(Saha *et al.*, 2019).**

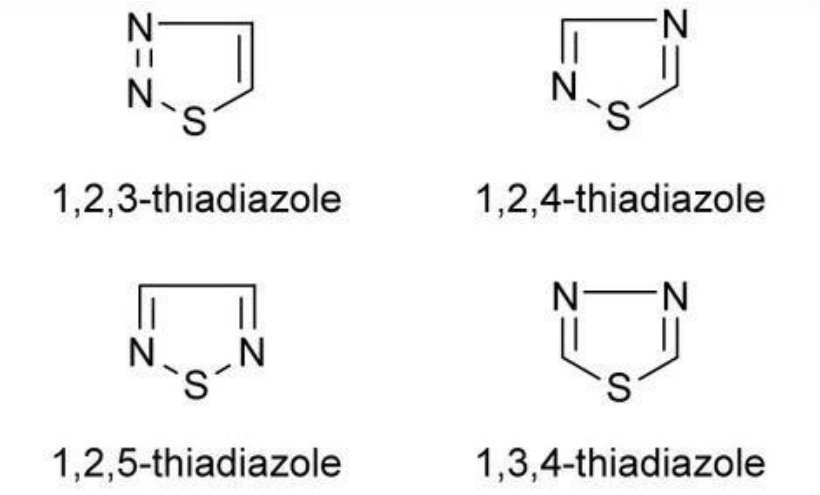


In 2019, Abo *et al.* created and characterised new bis-1,3,4-thiadiazole compounds. In order to evaluate the chemical and medicinal qualities of these substances, they used DFT calculations, UV-Vis and FTIR spectroscopy methods, and other analytical tools. Derivatives that were studied showed electrophilicity indices that were more than 2.5. In chloroform, the compounds mostly absorbed electromagnetic light at about 350 nm, with additional absorption shoulders that may have been caused by π–π* and/or n–π* electronic transitions. To better understand where electrophilic and nucleophilic reactions may take place, electrostatic potential maps were created to show areas with high and low electrostatic potential **(Abo *et al.*, 2019).**

In 2018, Mikherdov *et al.* created a binuclear combination of Pd II and cis-[PdCl2(CNXyl)2] by connecting it with 4-phenylthiazol-2-amine (3c). They used X-ray crystallography to deduce the complex's structure and HRESI+-MS and 1H NMR spectroscopy to characterize it. The structure has intra-/intermolecular bifurcated chalcogen bonding (BCB), which may take several forms depending on the substituents in the thiazaheterocyclic fragment, as shown by their study. Intermolecular chalcogen bonds were predicted to have contributed to the crystal packing by Hirshfeld surface analysis, which led to the formation of dimeric associates for 3c and other solid-states tructures. Both density-functional theory (DFT) and ab initio (AIM) studies verified the existence and favourable character of these BCB interactions **(Mikherdov *et al.*, 2018).**

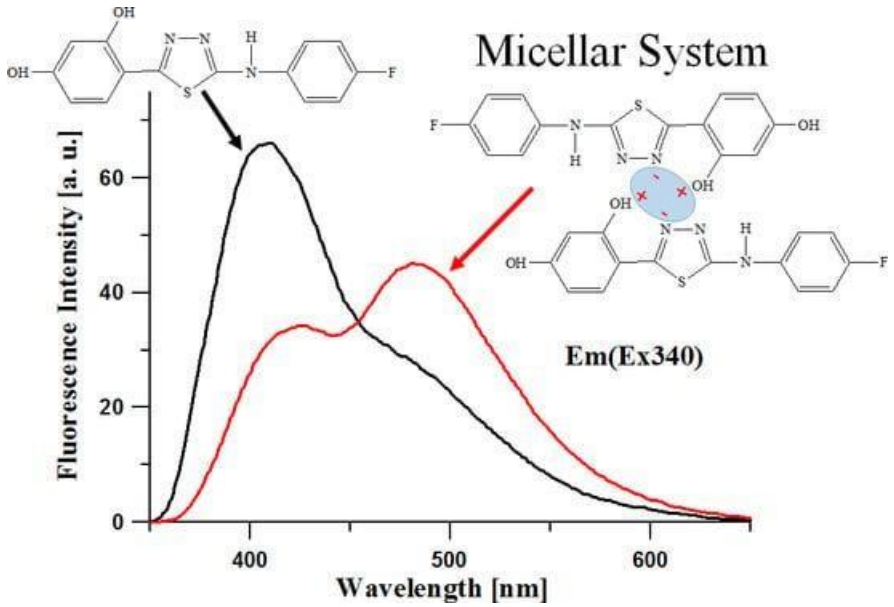


In 2018, Serban *et al.* discussed the urgent problem of bacteria and fungi developing resistance to antibiotics and the need of developing alternative chemicals to fight these diseases. When it came to creating antibacterial drugs, they highlighted the role of heterocyclic compounds, especially thiadiazoles. Thiadiazoles display a wide range of pharmacological actions and have a history of use in medicinal chemistry. A 2-amino-1,3,4-thiadiazole moiety has shown promise as a lead chemical for therapeutic development, and this study focused on the antibacterial characteristics of derivatives containing this moiety. A number of these compounds outperformed conventional antibiotics in terms of antibacterial efficacy. A promising building block for pharmacologically active derivatives in the future is the 2-amino-1,3,4-thiadiazole moiety, thanks to the derivatisation reactivity of the amine group **(Serban *et al.*, 2018).**



In 2018, Czernel *et al.* studied the 1,3,4-thiadiazole group chemical 2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole (FABT) using spectroscopy in a micellar system containing the non-ionic detergent Triton X-100. Dual fluorescence, which is affected by detergent and chemical

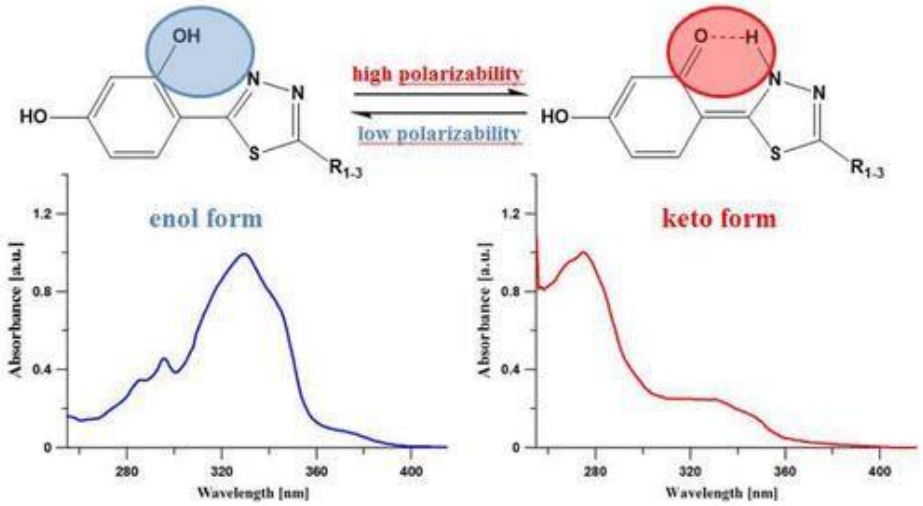
concentrations, was shown to exist in FABT. This dual fluorescence, never seen before, was found in an aqueous environment including methanol. The aggregation of FABT molecules in the micellar system was associated with the dual fluorescence, according to a number of spectroscopic methods, dynamic light scattering, and Principal Component Analysis. Within a narrow concentration range for detergents that produce these fluorescence effects, the research hints to the potential for charge transfer. These results show that this class of fluorophores has great potential as a probe for measuring fluorescence in biological material **(Czernel *et al.*, 2018).**



In 2017, Karcz *et al.* modeled a number of complexes with Zn(II), Cu(II), and Pd(II) ions using 4-(5-heptyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (L1). The structures of these compounds were characterized using a range of spectroscopic methods. There seems to be a 2:1 (L1:metal) stoichiometry in the L1-Zn(II) and L1-Pd(II) complexes, however in the L1-Cu(II) complex there are two ligands, an acetate ion and an L1 molecule. A deprotonated hydroxyl group on the resorcinyl group and a N atom on the thiadiazole heterocycle probably help L1 bind to metal ions **(Karcz *et al.*, 2017).**

In 2017, Matwijczuk *et al*. used a variety of spectroscopic techniques to characterise three novel 1,3,4-thiadiazole-derived compounds with biological activity. Their research using ultraviolet-visible light showed that the presence of keto and enol tautomers varied depending on the solvent, with the former

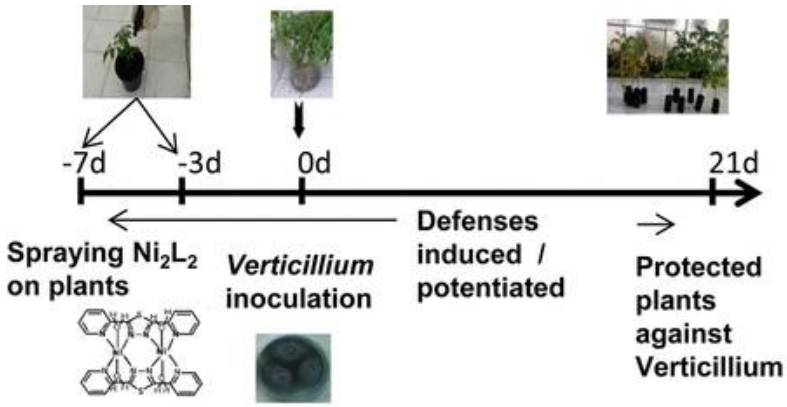
being more common in nonpolar solvents and the latter in more polar ones. Both tautomeric forms were detected in solution by certain spectra. Both temperature and solvent were important factors in determining the keto/enol equilibrium. FTIR spectroscopy further validated the equilibrium between ketones and enols. It was discovered that the polarisability of the solvent, not its dipole moment, was the determining factor in the solvent-induced tautomerism. This 1,3,4-thiadiazole analogues' tautomeric equilibrium was also significantly affected by the substituent in the molecule **(Matwijczuk *et al.*, 2017).**



In 2017, Raj *et al*. examined the relevance of 1,3,4-thiadiazole molecules to the pharmaceutical industry. In the process of synthesizing and evaluating several 1,3,4-thiadiazole derivatives, we looked for their antibacterial, antifungal, antioxidant, anticonvulsant, antidepressant, anxiolytic, antihypertensive, anticancer, and antifungal activities. The review shed light on the anticancer potential, medicinal chemistry, and structure-activity connections of these compounds, with a specific emphasis on their effects on human cell lines **(Raj *et al.*, 2017).**

In 2016, Zine *et al.* tested compounds showed protective properties against V. dahliae when sprayed over leaves at a dosage of 100µg mL-1. A synergistic impact was seen when thetransition metal Ni and theligand L were combined, leading to a 57% decrease in illness incidence. Furthermore, Ni2L2 demonstrated a 95% decrease in the foliar symptoms and vascular browning

index that are linked to Verticillium wilt. According to the research, L and Ni2L2 activate plant defence responses by inducing an oxidative burst, increasing total phenolic content, and improving peroxidase and polyphenol oxidase activity. These factors were associated with this protective potential. These results point to the possibility that L and Ni2L2 might activate plant defence systems in a new way **(Zine *et al.*, 2016).**

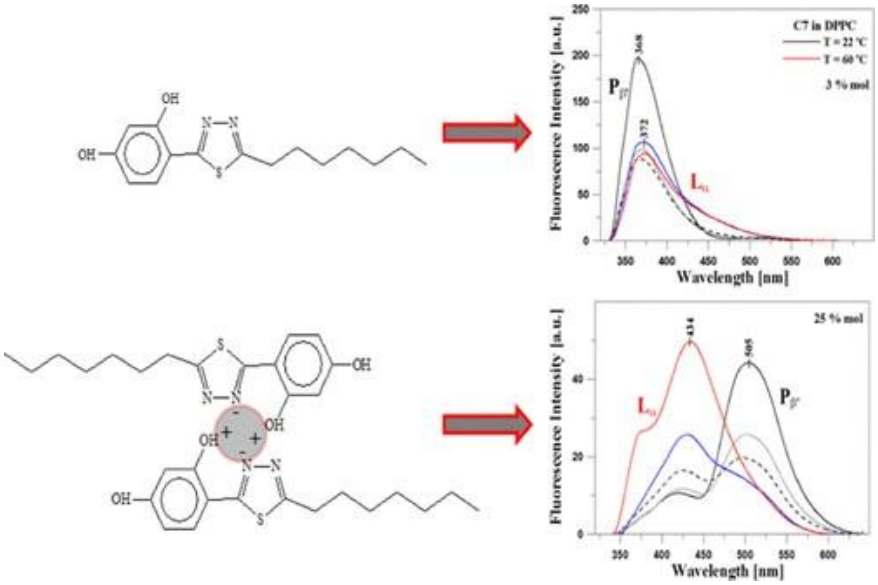


Protection afforded by Ni2L2 against Verticillium wilt of tomato

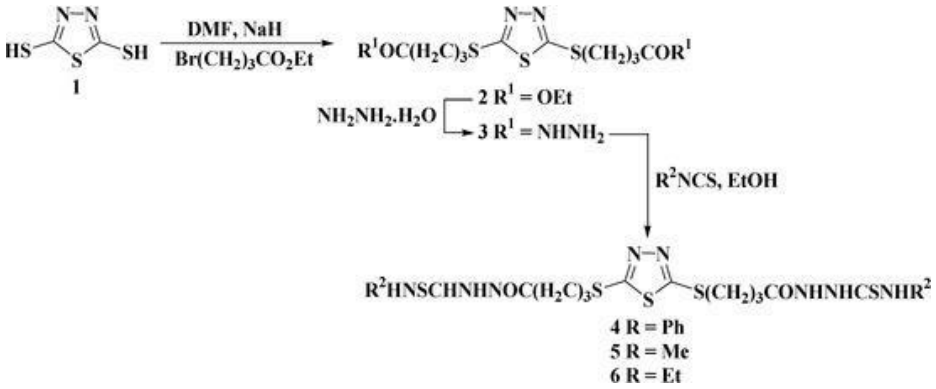
In 2016, Dwivedi *et al*. released an essayreviewing the literature on medicinal chemistry's use of thiadiazole derivatives. The varied pharmacological effects of these compounds have brought them to the forefront. They contain a three-heteroatom and a five-membered heterocyclic structure. The chemical and biological features of thiadiazole derivatives are discussed in the review, with an emphasis on their importance in the creation of novel medications. It gives the most latest information on this class of chemicals and its many pharmacological uses, as well as insights into different synthetic techniques and field breakthroughs **(Dwivedi *et al.*, 2016).**

In 2016, Kluczyk *et al*. as a result of alterations to the DPPC phase transition, their fluorescence tests showed several emission bands. They learned the molecular architecture of these chemicals in DPPC multilayers by analysing their Fourier transform infrared spectra. The shorter alkyl substituent molecule had a stronger association with the membrane polar area, whereas the longer alkyl substituent compound interacted mostly with lipid hydrocarbon chains and primarily occupied the lipid polar head region. The structure of the chemical and the kind of alkyl substituent utilised had a major impact on these effects, which were thought to be caused by molecular aggregation. These

systems' aggregation model, which is readily applicable to pertinent biological material, was validated by changes in fluorescence lifetimes and long-wave emission **(Kluczyk *et al.*, 2016).**



In 2015, Rezki *et al.* a novel class of chemicals was created by attaching 2,5-disubstituted-1,3,4-thiadiazole to Schiff bases, 1,3,4-thiadiazole, 1,3,4-oxadiazole, and 1,2,4-triazole. They used a number of methods to characterise the chemicals. After synthesis, the compounds were tested for their ability to suppress cell development, kill bacteria and fungi, and show promise in other biological tests **(Rezki *et al.*, 2015).**



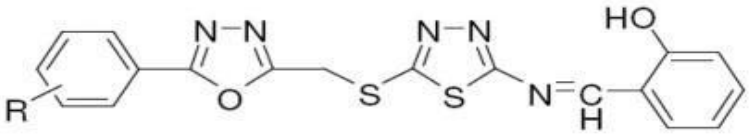
In 2015, Aouad *et al.* created a number of compounds, one of which was 4-amino-5-[(1,4,5-triphenylimidazol-2-yl)thiopropyl]-2H-1,2,4-triazole-3-thione (4), which was formed by alkylation and the addition of hydrazine hydrate to (1,4,5-triphenylimidazol-2-yl-thio)butyric acid hydrazide (3). Furthermore, they created 1,2,4-triazole-3-thiols (8-10), aminothiadiazoles (11-13), and 1-

[4-(1,4,5-triphenylimidazol-2-ylthio)butanoyl]-4-alkyl/arylthiosemicarbazides (5-7) by transforming them in an acidic environment. Further, by condensing compound 3 with different aromatic aldehydes, a series of Schiff bases (14-18) were produced. Preliminary evaluations of the compounds' antibacterial activity followed their characterisation by a battery of spectroscopic methods **(Aouad *et al.*, 2015).**

In 2015, chandra *et al*.condensed benzil and 5-amino-1,3,4-thiadiazole-2-thiol in a 1:2 ratio to produce benzil bis(5-amino-1,3,4-thiadiazole-2-thiol), a new tetradentate Schiff's base ligand. Using a battery of analytical tools, they defined the ligand. To create compounds containing N2S2 coordination spheres, this ligand coupled to Cu(II) and Ni(II) ions. Molecular modelling, elemental analysis, IR, electronic spectra, thermal analysis, EPR, molar conductance, and magnetic susceptibility were among the techniques used to examine the complexes. Aside from Ni(L)2, which acted as an electrolyte, none of the compounds displayed electrolytic characteristics. Different complexes have different coordination geometries. When tested against opportunistic infections, both the free ligand and its metal complexes were less effective than the metal complexes **(Chandra *et al.*, 2015).**

In 2014, zhang *et al.* created and produced a set of unique hybrid compounds with 1,3,4-oxadiazole and 1,3,4-thiadiazole as well as a Schiff base group. Afterwards, the anticancer activity of the compounds was evaluated in vitro using a CCK-8 assay on SMMC-7721, MCF-7, and A549 human tumor cell lines. Results from the bioassays demonstrated that the majority of the compounds had potent anticancer properties; in fact, a number of them outperformed the positive control, 5-fluorouracil (5-FU), across all of the tested cell lines. Compound 8d, with an IC50 value of 2.84 μM, showed the most inhibitory effect on SMMC-7721 cells. The anticancer activity against MCF-7 cells was remarkably strong when compounds 8k and 8n were tested, with IC50 values of 4.56 and 4.25 μM, respectively. Compound 8a showed a notable ability to inhibit cell proliferation in A549 cells, whereas compound 8n had a similar effect with IC50 values of 4.13 μM and 4.11 μM, respectively. These pharmacological findings highlight the role of 1,3,4-oxadiazole

substituents on the phenyl ring in regulating antiproliferative activity in different tumour cell lines **(Zhang *et al.*, 2014).**

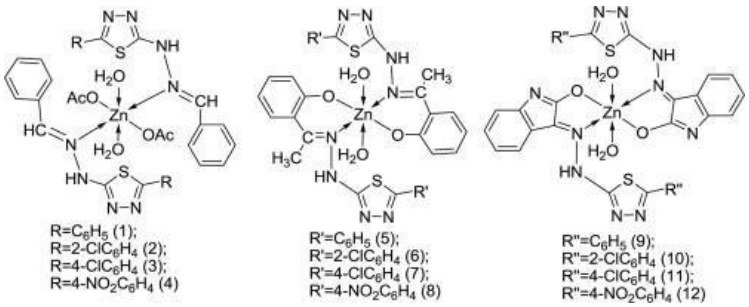


R= H, 4-F, 2-Cl, 4-Cl, 2-Br, 4-Br, 2-CH_3, 3-CH_3, 4-CH_3, 3-OCH_3, 4-OCH_3, 3,4,5-Tri-OCH_3, 3-NO_2, 4-NO_2

In 2014, Tandel *et al*. developed and tested a novel homologous series of Schiff's bases utilizing polarizing optical microscopy and differential scanning calorimetry; these bases were produced from substituted 2,3,4-thiadiazole. A series of thirteen homologues were synthesized by combining substituted 2-amino 1,3,4-thiadiazole with substituted 4-n-alkoxy benzoyloxy benzaldehydes. The compounds containing methyl to n-hexyl substituents exhibited only nematic phases, while those containing n-heptyl to n-octadecyl exhibited a combination of smectic and nematic phases. Looking at the association between alkoxy chain carbon count and transition temperatures, we found that the propensity for nematic-isotropic transition temperatures reduced and for smectic-nematic transition temperatures rose. The ester connection increased thermal stability during the smectic phase and decreased it during the nematic phase, as compared to other homologous series; this difference was statistically significant. The compounds were studied using a range of analytical techniques, such as infrared spectrum data, nuclear magnetic resonance, dynamic scanning calorimetry, and microscopic methods **(Tandel *et al.*, 2014).**

In 2013, Singh *et al.* combined the low molar conductance values and solubility in DMF and DMSO suggest that these complexes are not electrolytes. Based on the Schiff base ligands used, the complexes were classified according to their elemental analyses, it showed a stoichiometry of 1:2 between the metal and the ligand. Analytical techniques such as infrared spectroscopy and nuclear magnetic resonance (1H and 13C NMR) were used to describe the complexes. Based on measurements taken using a dynamic

Light scattering (DLS) analyser, the synthesized compounds had particle widths ranging from around 100 to 200 nm. In addition, antimicrobial and antifungal capabilities of Schiff bases and their complexes were tested against a variety of microbes; the findings showed that complexation enhanced antimicrobial activity **(Singh *et al.*, 2013).**



In 2013, Tehrani *et al*. created from 2-hydrazinyl-1,3,4-thiadiazole a number of new hydrazone derivatives using a one-pot synthesis method. The inhibitory effect of these compounds against arachidonic acid and ADP-induced platelet aggregation was tested. The derivatives with the strongest antiplatelet aggregation activity were compounds 3l, 3o, and 3p. The antimycobacterial activity of the derivatives was also tested; compounds 3g, 3k, 3p, and 3q showed high inhibitory effects against mycobacterial infections and were considered to have significant antimicrobial activity **(Tehrani *et al.*, 2013).**

In 2012, Pandey *et al.* created Schiff bases by combining several aromatic aldehydes with 2-amino-5-aryl-1,3,4-thiadiazole derivatives. Thiosemicarbazide, sodium acetate, and aromatic aldehydes were used in a process to generate the 1,3,4-thiadiazole derivatives. The Schiff bases' structures were confirmed by the infrared and 1H NMR spectra. Next, the substances were tested for their analgesic and anti-inflammatory properties in Swiss albino mice and Wistar albino rats, respectively. These chemicals were also very effective against the gram-positive Staphylococcus aureus and the gram-negative Escherichia coli bacteria **(Pandey *et al.*, 2012).**

In 2012, Li *et al.* Describes the production of a set of twelve hybrid compounds including a γ-substituted butenolide moiety and combining the 1,2,4-triazole scaffold with Schiff bases. A tandem asymmetric Michael addition/elimination process was a crucial step in the synthesis of these

chemicals. The in vitro antitumor activity of the chiral 1,2,4-triazole derivatives that were produced was subsequently assessed. One of the chiral compounds, 7l, showed the most potent anticancer activity against Hela cells, with an IC50 value of 1.8 μM, according to the research. The possibility of creating new anticancer drugs was raised by combining 1,2,4-triazole with γ-substituted butenolide groups **(Li *et al.*, 2012).**

In 2011, Malik *et al.* combined a Schiff base derived from 5-acetamido-1,3,4-thiadiazole-2-sulphonamide with a bidentate ligand to construct a class of ML2 transition metal complexes. A number of analytical methods were used to characterise the complexes. These included elemental analysis, IR spectroscopy, electronic, ESR, magnetic moment, and molar conductivity. Also, we analysed the sizes of the particles. According to the findings, the complexes did not include any electrolytes. Free energy changes and stability constants for the complexes were also computed. The complexes were found to possess an octahedral geometry according to spectral investigations and magnetic susceptibility tests. The fungicidal activity of the ligand and its metal complexes was tested against Aspergillus niger and A. flavus **(Malik *et al.*, 2011).**

In 2011, Mobinikhaledi *et al.* experimented with the synthesis of novel Schiff bases with the symbol 3a-l for a 1,3,4-thiadiazole group. The compounds in question were produced by subjecting 2-amino-5-mercapto-1,3,4-thiadiazole to acidic and phase transfer catalyst (PTC) conditions during a reaction with a range of aromatic aldehydes. Use of Fourier transform infrared and nuclear magnetic resonance spectroscopy in conjunction with elemental investigations allowed for the structural characterisation of these Schiff bases. We further tested these compounds' antimicrobial capabilities against two other bacterial strains: Staphylococcus aureus (RTCC, 1885) and Escherichia coli (ATCC, 35922) **(Mobinikhaledi *et al.*, 2011).**

In 2010, Solmaz investigated the effects of a Schiff base 5-((E)-4-phenylbuta-1,3-dienylideneamino)-1,3,4-thiadiazole-2-thiol (PDTT) on mild steel corrosion in a 0.5 M HCl (hydrochloric acid) solution. The study analysed the steel's surface using scanning electron microscopy and atomic force

microscopy, as well as electrochemical impedance spectroscopy, linear polarisation resistance, potentiodynamic polarisation, hydrogen gas evolution, and changes in open circuit potential over time. Mild steel corrosion was significantly inhibited in the acidic solution by the PDTT Schiff base, according to the findings. The adsorption of inhibitor molecules onto the steel surface was shown to be the culprit behind this inhibition. These molecules efficiently prevented active corrosion sites, resulting in an exceptionally high level of corrosion protection **(Solmaz., 2010).**

In 2010, Mathew *et al*. concentrated on the production of Schiff bases from 2-amino-1,3,4-thiadiazole compounds replaced with 5-phenyl. Thiosemicarbazide and a number of aryl carboxylic acids were subjected to a dehydrating agent, such as concentrated H2SO4, throughout the synthesis process in order to generate 5-phenyl substituted 2-amino-1,3,4-thiadiazole derivatives. Schiff bases were formed by further reacting these compounds with different types of aldehydes. The compounds' structures were verified using elemental analysis, 1H NMR, and infrared spectroscopy. All of the synthesised compounds were determined to comply to Lipinski's rule, which indicates strong bioactivity and bioavailability, as the research also evaluated the drug-like qualities of the compounds using Molinspiration cheminformatics software. The anthelmintic activity of these compounds was assessed using Pheretima posthuma as the testorganism. With the exception of compounds 3b, 3c, and 3d, the majority of the compounds demonstrated considerable activity when compared to the standard medication **(Mathew *et al.*, 2010).**

RESEARCH METHODOLOGY

3.1. Aims of the study

The research approach used to examine the antibacterial activity of 2,5-disubstituted-1,3,4-thiadiazole derivatives is outlined in this chapter. Developing, characterising, and testing these chemicals' biological effectiveness against therapeutically relevant bacterial strains is the major goal of this study. Creating a systematic method ensured the reliability, reproducibility, and correctness of the findings. The research methodology, including the study's design, sample selection, data collecting, and analytic procedures, are detailed in this chapter **(Barbosa and de Aguiar, 2019).**

3.2. Research Design

The study is based on a quantitative experimental design, with the primary steps being the synthesis and biological testing of chemicals.The primary goal of this research is to conduct a thorough assessment of the synthesised compounds' antibacterial activity by means of laboratory-based trials **(Goa *et al.*, 2022).**

Type of Study: In vitro testing is the main focus of this experimental investigation. A battery of antimicrobial tests was performed on the synthesised and characterised compounds to determine their efficacy against various bacterial strains **(Bouzina *et al.*, 2018).**

Experimental Setup: Benzoyl chlorides, salicylic hydrazine, and Lawesson's reagent were used in the synthesis to create 2,5-disubstituted-1,3,4-thiadiazole derivatives. Next, the antimicrobial activity was assessed by measuring the zone of inhibition and minimum inhibitory concentration (MIC) against a variety of bacterial species **(Gierczyk and Zalas, 2005).**

3.3. Population and Sample Selection

Strains of microorganisms and newly synthesised chemicals selected for their potential therapeutic use make up the research sample.

Synthesis of Compounds: Thirty 2,5-disubstituted-1,3,4-thiadiazole derivatives with various substitutions, including hydroxy (-OH), mercapto (-SH), and methoxy (-OCH_3), were synthesised in order to investigate various antibacterial properties **(Mallika *et al.*, 2025).**

Bacterial Strains: The following bacterial strains were tested to determine the antibacterial activity of the synthesised compounds:

- Staphylococcus aureus (Gram-positive)
- Escherichia coli (Gram-negative)
- Streptococcus mutans (Gram-positive)
- Staphylococcus epidermidis (Gram-positive)

These strains were chosen because of their importance in clinical infections and their tendency to show resistance to antibiotics **(Tavares *et al.*, 2020).**

3.4. Data Collection Methods

Chemical synthesis and biological testing were both used to collect data for this study.

Synthesis of Compounds: Synthesis of chemicals and biological tests were both used to gather data for this investigation. The compounds were created using the procedures outlined in Chapter 4 for conventional organic synthesis. Recrystallisation was used to purify compounds after synthesis, resulting in very pure products. These products were further studied using nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, and melting point measurement **(Tariq *et al.*, 2011).**

Characterization of Compounds:

- **Melting Point:** Melting point determination was used to verify the compounds' purity.
- **IR Spectroscopy:** Functional group identification was achieved by means of Fourier transform infrared spectroscopy applied to the molecules.

- **1H NMR Spectroscopy:** By using 1H NMR spectroscopy, the synthesised compounds' proton environment and structural integrity were validated.

- **Mass Spectrometry:** Mass spectrometry made sure that every chemical was pure and had a specific molecular weight **(Pavel *et al.*, 2012).**

Antimicrobial Testing:

- **Zone of Inhibition:** To determine the efficacy against bacteria, the agar diffusion method was used. After inoculating nutrient agar plates with the correct bacterial strains, 0.1 cc of the ready-made test solution was applied to each well. After incubation at 37°C for 24 to 48 hours, the millimeter-scale zone of inhibition was determined **(Athanassiadis *et al.*, 2009).**
- **MIC Testing:** Using the agar streak dilution technique, we were able to calculate the minimum inhibitory concentration (MIC) of each chemical. The procedure included reducing the compound's concentration in a series of steps until no bacterial growth was detected **(Wiegand *et al.*, 2008).**

Data Collection Tools:

- **UV-Visible Spectrophotometer:** Used for quantifying compounds by measuring their absorbance

- **Incubator:** For the purpose of measuring microbial growth, the plates are incubated at 37°C.

- **Standard Antibiotics:** The antimicrobial activity of the synthesised compounds was compared to that of ciprofloxacin, a reference drug **(Chalkley and Koornhof, 1985).**

3.5. Data Analysis Techniques

Statistical Analysis: Mean, standard deviation, and zone of inhibition measures were used to the results from antimicrobial tests as part of the descriptive statistics. A one-way analysis of variance (ANOVA) was used to

compare the antibacterial activity of various substances in the data. Post hoc tests like Tukey's HSD were used if significant differences were found **(Kowalski *et al.*, 2005).**

QSAR Analysis: A correlation between the chemical make-up of the substances and their ability to kill microbes was sought for by doing a Quantitative Structure-Activity Relationship (QSAR) study. For the analysis, the following methodologies were utilised **(Patel *et al.*, 2014).**

- Multivariate Linear Regression (MLR)
- Principal Component Regression (PCR)
- Partial Least Squares (PLS)

SoftwareUsed:

- GraphPad Prism for statistical analysis.
- Vlife MDS for QSAR analysis.

3.6. Limitations of the Study

This research, while comprehensive, has a few limitations:

Sample Size: The study involves 5 groups of synthesized compounds (6a-f to 10a-f), each consisting of 6 derivatives, totaling 30 compounds. The findings may not be applicable to a broader population due to the sample size's modest size. Stronger findings may be possible in future research if the sample size is increased and the demographics are better represented **(Amin *et al.*, 2013).**

In Vitro Testing: Clinical studies are necessary to confirm the antibacterial activity, which was demonstrated in vitro.

Solubility Issues: During testing, the bioavailability of several compounds may have been affected because of their low solubility in DMSO **(Balakin *et al.*, 2022).**

MATERIALS AND METHODS

4.1. Chemical Analysis and Characterization Methods

The melting points of the newly synthesized compounds were measured using a standard melting point instrument to assess their purity. Functional groups including hydroxyl, mercapto, and methoxy were identified in the synthesised compounds using infrared (IR) spectral analysis employing a Fourier transform infrared spectrometer with the pressed pellet approach **(Dai *et al.*, 2023)**. Proton nuclear magnetic resonance (1H NMR) spectroscopy was used for study of the proton environment to confirm the chemical makeup of each material. The results of the infrared and ^{1}H nuclear magnetic resonance investigations validated the designated structures. The analytical reagents and chemicals used in this investigation were all purchased from Yarrow Chem Pvt Ltd. and were of AR (Analytical Reagent) grade. Merck and Lobachem were the main suppliers of the chemicals used in the research **(Hall and Sanders, 1980).**

4.2. Synthesis

Using substituted diacylhydrazine intermediates as a starting point, synthesising 2,5-disubstituted-1,3,4-thiadiazole derivatives, and last, forming Schiff base derivatives, a three-step procedure was used to synthesise the 2,5-disubstituted-1,3,4-thiadiazole derivatives. Here are the stages involved in the synthesising process: **(Shamroukh and Hegab, 2020).**

1. Synthesis of Substituted Diacylhydrazine (3a-f): The standard operating method calls for dissolving 1 mole of substituted salicylic hydrazine in 15 millilitres of toluene and then adding 0.96 g of methyl sulfonic acid to the mixture. The reaction was stirred for 10 minutes before adding 1 mole of substituted benzoyl chloride. The mixture was then permitted to stir for three hours. The diacylhydrazine derivatives were obtained by cooling the mixture with crushed ice after the reaction, filtering the result, washing it, and drying it **(Pandey *et al.*, 2016).**

2. Synthesis of 2, 5-Disubstituted-1, 3, 4-Thiadiazole Derivatives (4a-f): Synthesis of 4a–4f was accomplished by reacting 0.1 mol of diacylhydrazines with Lawesson's reagent while a sulphur source was present. The thiadiazole ring is formed in this reaction by first thionating the carbonyl group and then cyclizing it. The reaction was carried out by refluxing the mixture for three hours. After that, it was filtered, washed, dried, and chilled. Crushed ice was used to purify the mixture **(Mihailovic *et al.*, 2017).**

3. Synthesis of Schiff Base Derivatives of 1,3,4-Thiadiazole (6a-f,7a-f,8a-f, 9a-f, 10a-f): 30 millilitres of ethanol and a few drops of glacial acetic acid were added to 0.01 mole of 2,5-disubstituted-1,3,4-thiadiazole that had been transferred to a round-bottom flask (RBF). After dissolving the mixture by stirring, aromatic aldehydes wereadded onebyone. Five hours were thereafter spent refluxing the reaction at 70°C. Filtration, washing, drying, and cooling followed the stopping of the process. The pure Schiff base derivatives were obtained by recrystallisation using ethanol **(Elneairy *et al.*, 2022).**

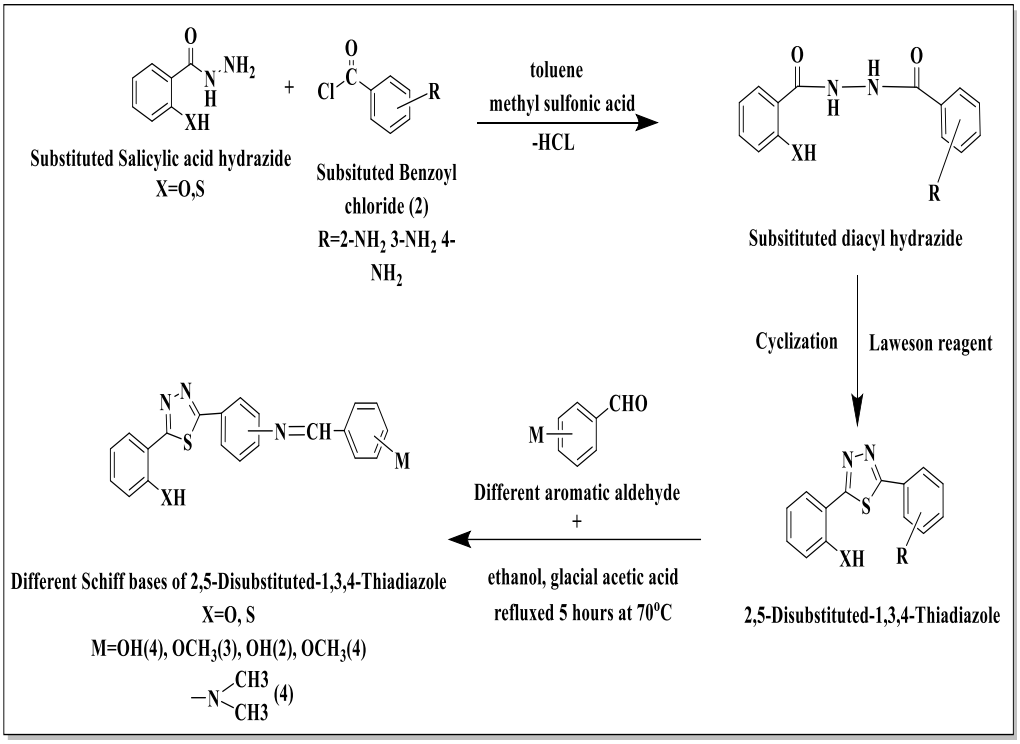


Figure 4.1: General Scheme for the synthesis of 2,5-disubstituted-1,3,4-thiadiazole (Mahmoud *et al.*, 2014).

4.3. Antimicrobial Activity

The synthesised derivatives were tested for their antibacterial properties using the cup plate method, a frequently used technique for microbiological testing:

1. Microbial Strains: The antibacterial activity was evaluated by testing it on nutrient agar medium with gram-positive Staphylococcus aureus, gram-negative Staphylococcus mutans, and E. coli bacteria. Every single bacterial strain was isolated using conventional microbial culture methods **(Fischer *et al.*, 2012).**

2. Preparation of Test Solution: By dissolving the synthesized compounds (5 mg each) in 5 ml of DMSO, the test solution was produced at a concentration of 1000 µg/ml. Every bacterial strain was tested using 0.1 ml of the test solution **(Chhillar *et al.*, 2006).**

3. Cup Plate Method: After streaking the nutrient agar plates with the bacterial cultures, sterile cork borer was used to create wells. One milliliter of the test solution was added to each well, and then the plates were maintained at 37 degrees Celsius for 48 hours. In order to determine the effectiveness of the antibiotic, the millimeter-sized zone of inhibition around each well was assessed **(Sivaramakrishnan and Razia, 2021).**

4. Minimum Inhibitory Concentration (MIC): Using the agar streak dilution technique, we were able to calculate the minimum inhibitory concentration (MIC) of the substances showed the highest activity. A 100 µg/ml stock solution was made by dissolving the synthesized compound in DMSO. A range of concentrations, from 1 to 10 µg/ml, were subsequently produced by serial dilution. Mixed with sterile nutrient agar medium, the test chemical dilutions were then placed onto petri plates and left to harden. The plates were then incubated at 37°C for one day after being covered with the bacterial suspension. The minimum inhibitory concentration (MIC) was defined as the concentration at which bacterial growth could not be detected **(Rodriguez-Tudela *et al.*, 2003).**

4.4. QSAR Study

To establish a connection between the antibacterial activity and the molecular structures of the produced derivatives, an investigation known as the Quantitative Structure-Activity Relationship (QSAR) was conducted. This research made use of a dataset that included 30 synthetic chemicals with established antibacterial properties:

1. Data Preparation: We used the pIC50 values, which are the logarithmic scale of IC50, as our dependent variable. There were two sets of compounds: one with twenty-four molecules for training and another with six molecules for testing. The distribution of biological activity in the test set was maintained during this division, resulting in a dependable model for prediction **(Armutlu *et al.*, 2008).**

2. Statistical Analysis: The Vlife MDS software was used on an Intel Core i3 processor-powered Dell Inspiron PC to conduct the QSAR study. In order to prevent invariable descriptors from impacting the findings, a total of 208 molecular descriptors were computed. The following procedures were implemented **(Khan *et al.*, 2024).**

- **Multivariate Linear Regression (MLR)**
- **Principal Component Regression (PCR)**
- **Partial Least Squares (PLS)**

3. Prediction Model: To determine how well the QSAR model predicted the MIC values for the test set chemicals, we compared them to the experimental MIC values. The reliability of the QSAR model was shown by the strong relationship between the anticipated and observed values **(Verissimo *et al.*, 2019).**

4.5. Method Development

To measure the 10e derivative, a UV-Visible spectrophotometric technique was created:

1. Solvent Selection: The drug's solvent of choice was ethanol, and a 10µg/ml concentration of the molecule was used to produce the working stock solution **(Truong *et al.*, 2019).**

2. Instrument and Procedure: The readings were recorded usinga Shimadzu 1800 UV-Visible twin beam spectrophotometer using quartz cuvettes with a 1 cm path length. Arange of wavelengths from 200 to 780 nm was used to scan the solution **(Rashid *et al.*, 2022).**

3. Detection Wavelengths: A scanning process from 200 to 800 nm was used to identify the best detection wavelengths for the working standard solution (10 µg/ml). Absorption maxima at 338 nm (UV technique), 266 nm (D1 method), and 236.5 nm (D3 method) were determined for more investigation **(Yang *et al.*, 2015).**

4. Validation: The concentration ranges for the standard solutions used to determine the LOD and LOQ for the UV and D3 methods were 5-30 µg/ml, and for the D1 method, it was 25-50 µg/ml. We drew the calibration curve, computed the regression equation, and found the correlation coefficient **(Nannetti *et al.*, 2017).**

RESULT AND DISCUSSION

5.1. Characterization of Synthesized Compounds

An innovative series of 2,5-disubstituted-1,3,4-thiadiazole derivatives was synthesised in this work. A wide range of biological and chemical characteristics were achieved by the 30 synthesised compounds that included aromatic rings functionalised with hydroxyl, mercapto, methoxy, or dimethylamine. The following substances have been synthesised:

- 5-(2-Hydroxyphenyl)-2-(N-benzylidene-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-benzylidene-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-benzylidene-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-benzylidene-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-benzylidene-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-benzylidene-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-methoxybenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-methoxybenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-methoxybenzylidene)-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-methoxybenzylidene)-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-methoxybenzylidene)-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-methoxybenzylidene)-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(2-hydroxybenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(2-hydroxybenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(2-hydroxybenzylidene)-3-aminophenyl)-1,3,4-thiadiazole

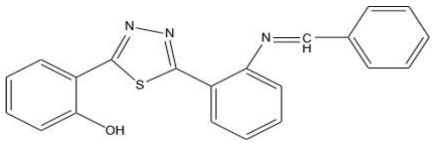
- 5-(2-Mercaptophenyl)-2-(N-(2-hydroxybenzylidene)-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(2-hydroxybenzylidene)-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(2-hydroxybenzylidene)-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-dimethylaminobenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-dimethylaminobenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-dimethylaminobenzylidene)-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-dimethylaminobenzylidene)-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-dimethylaminobenzylidene)-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-dimethylaminobenzylidene)-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Hydroxyphenyl)-2-(N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl)-1,3,4-thiadiazole
- 5-(2-Mercaptophenyl)-2-(N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl)-1,3,4-thiadiazole

A number of aromatic aldehydes, substituted salicylic hydrazides, methylsulfonic acid, various benzoylchlorides, and Lawesson's reagent were

all reactants in the synthesis. Mass spectrometry, infrared spectroscopy, and proton nuclear magnetic resonance (NMR) were used to analyze and confirm the chemical structures of all the synthesized substances. The results from these methods verified that the functional groups were properly integrated, and the compounds displayed the expected level of structural stability.

Spectra and molecular weights, together with all the other characterization data, are presented in Tables 4.1–4.30. Using these analytical techniques, we were able to verify that the synthetic process was precise and that the thiadiazole scaffold had the correct functional groups added to it.

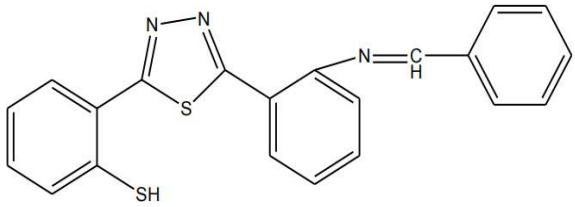
Table 5.1: Physico-analytical Properties of Compound 6a

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3OS$
Structure	
Molecular Weight	357 g/mol
Melting Point (°C)	130 -131
Physical Appearance	Light yellow
R_f Value	0.64 (Mobile phase : Benzene : Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield(w/w)	85%
Max Absorption (λ_{max})	366 nm
Combustion Analysis	C:70.57%, H:4.23%, N:11.76%, O:4.48%, S:8.97%
Chemical Name	5-(2-Hydroxyphenyl)-2-(N-benzylidene-2-aminophenyl)-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3627 (O-H stretch), 3018 (C-H stretch), 1531 (C=C stretch), 1618 (C=N stretch), 739 (o-disubstituted benzene)
- **^{1}HNMR (DMSO,ppm)**:10.83(1H,Ar-H), 8.65(1H,=CH), 8.25-7.23 (13H, Ar-H)
- **Mass Spectrum(m/z)**: 356 [M+]

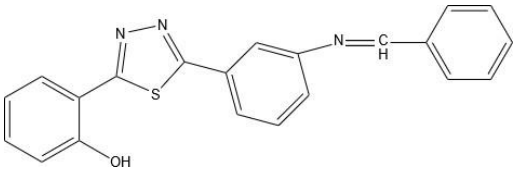
Table 5.2: Physico-analytical Properties of Compound 6b

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3S_2$
Structure	
Molecular Weight	373 g/mol
Melting Point(°C)	142 -143
Physical Appearance	Magenta red
R_fValue	0.32 (Mobile phase : Benzene:Acetone, 9:1)
Solubility	Soluble in DMSO, ethanol
Yield (w/w)	75%
Max Absorption (λ_{max})	264 nm
Combustion Analysis	C: 67.53%, H: 4.05%, N: 11.25%, S: 17.17%
Chemical Name	5-(2-Mercaptophenyl)-2-(N-benzylidene-2-aminophenyl)-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3052 (C-H stretch), 1532 (C=C stretch), 1638 (C=N stretch), 744 (o-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.66 (1H,Ar-CH), 8.18-7.45 (13H,Ar-H), 2.67 (1H, Ar-SH)
- **Mass Spectrum (m/z)**: 372 [M+]

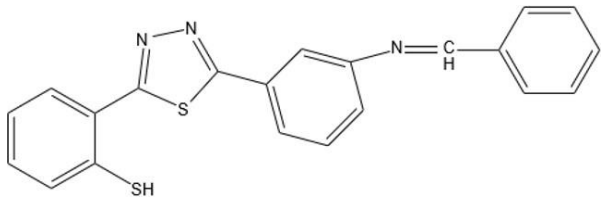
Table 5.3: Physico-analytical Properties of Compound 6c

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3OS$
Structure	
Molecular Weight	357 g/mol
Melting Point(°C)	156 -157
Physical Appearance	Cream color
R_f Value	0.63 (Mobilephase : Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	87%
Max Absorption (λ_{max})	366 nm
Combustion Analysis	C: 70.57%, H: 4.23%, N: 11.76%, O: 4.48%, S: 8.97%
Chemical Name	5-(2-Hydroxyphenyl)-2-(N-benzylidene-3-aminophenyl)-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3621 (O-H stretch), 3029 (C-H stretch), 1564 (C=C stretch), 1649 (C=N stretch), 693, 785 (m-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.88 (1H, Ar-OH), 8.82 (1H,=CH), 8.15-7.14 (13H, Ar-H)
- **Mass Spectrum (m/z)**: 356 [M+]

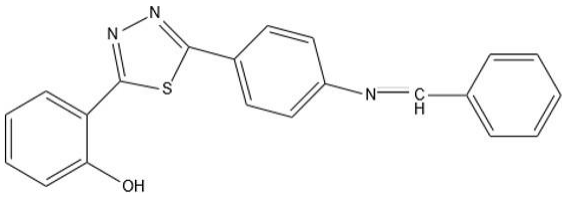
Table 5.4: Physico-analytical Properties of Compound 6d

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3S_2$
Structure	
Molecular Weight	373 g/mol
Melting Point(°C)	172 -173
Physical Appearance	Red colour
R_f Value	0.32 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	72%
Max Absorption (λ_{max})	256 nm
Combustion Analysis	C: 67.53%, H: 4.05%, N: 11.25%, S: 17.17%
Chemical Name	5-(2-Mercaptophenyl)-2-(N-benzylidene-3-aminophenyl)-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3078 (C-H stretch), 1537 (C=C stretch), 1654 (C=N stretch), 702, 792 (m-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.82 (1H, Ar-CH), 8.09-7.48 (13H, Ar-H), 2.22 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 372 [M+]

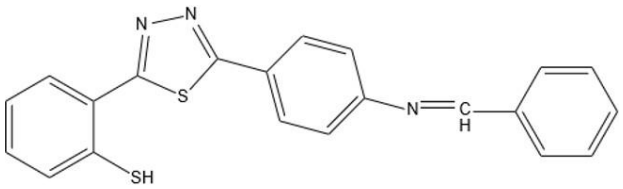
Table 5.5: Physico-analytical Properties of Compound 6e

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3OS$
Structure	
Molecular Weight	357 g/mol
Melting Point(°C)	185 -186
Physical Appearance	Pale yellow
R_f Value	0.64 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	82%
Max Absorption (λ_{max})	365 nm
Combustion Analysis	C: 70.57%, H: 4.23%, N: 11.76%, O: 4.48%, S: 8.97%
Chemical Name	5-(2-Hydroxyphenyl)-2-(N-benzylidene-4-aminophenyl)-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3632 (O-H stretch), 3065 (C-H stretch), 1584 (C=C stretch), 1651 (C=N stretch), 812 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.77 (1H, Ar-OH), 8.92 (1H,=CH), 8.14-7.16 (13H, Ar-H)
- **Mass Spectrum(m/z)**: 356 [M+]

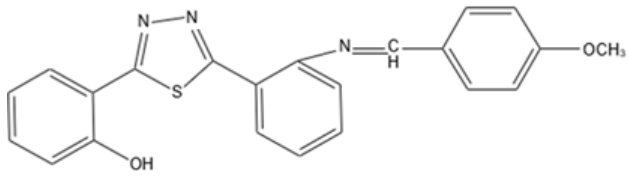
Table 5.6: Physico-analytical Properties of Compound 6f

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3S_2$
Structure	
Molecular Weight	373 g/mol
Melting Point(°C)	204 -205
Physical Appearance	Magenta red
R_f Value	0.31 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in DMSO
Yield (w/w)	71%
Max Absorption (λ_{max})	264 nm
Combustion Analysis	C: 67.53%, H: 4.05%, N: 11.25%, S: 17.17%
Chemical Name	5-(2-Mercaptophenyl)-2-(N-benzylidene-4-aminophenyl)-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3084 (C-H stretch), 1552 (C=C stretch), 1617 (C=N stretch), 823 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.94 (1H, Ar-CH), 8.18-7.45 (13H, Ar-H), 2.18 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 372 [M+]

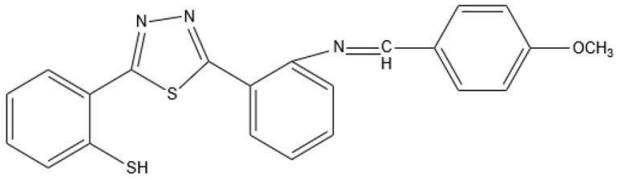
Table 5.7: Physico-analytical Properties of Compound 7a

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_2S$
Structure	
Molecular Weight	387 g/mol
Melting Point(°C)	131 -132
Physical Appearance	Light yellow
R_f Value	0.62 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	81%
Max Absorption (λ_{max})	366 nm
Combustion Analysis	C: 68.20%, H: 4.42%, N: 10.85%, O: 8.26%, S: 8.28%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(4-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3618 (O-H stretch), 3032 (C-H stretch), 1569 (C=C stretch), 1648 (C=N stretch), 1230 (C-O stretch), 747 (o-disubstituted benzene), 821 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.65 (1H, Ar-OH), 8.61 (1H, Ar-CH), 8.01 - 7.13 (12H, Ar-H), 3.91 (3H, CH_3)
- **Mass Spectrum(m/z)**: 386 [M+]

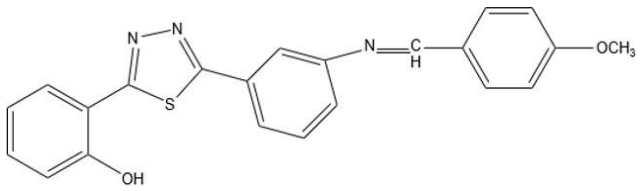
Table 5.8: Physico-analytical Properties of Compound 7b

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3OS_2$
Structure	
Molecular Weight	403 g/mol
Melting Point(°C)	145 -146
Physical Appearance	Magenta red
R_f Value	0.36 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	72%
Max Absorption (λ_{max})	264 nm
Combustion Analysis	C: 65.48%, H: 4.25%, N: 10.41%, O: 3.96%, S: 15.89%
ChemicalName	5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum(KBr, cm^{-1})**: 3067 (C-H stretch), 1526 (C=C stretch), 1624 (C=N stretch), 1265 (C-O stretch), 754 (o-disubstituted benzene), 816 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.62 (1H, Ar-CH), 8.15 - 7.14 (12H, Ar-H), 3.91 (3H, CH_3), 2.73 (1H, Ar-H)
- **Mass Spectrum (m/z)**: 402 [M+]

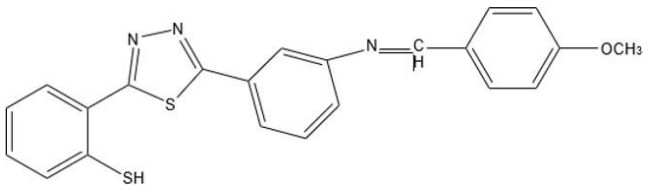
Table 5.9: Physico-analytical Properties of Compound 7c

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_2S$
Structure	
Molecular Weight	387 g/mol
Melting Point(°C)	146 -147
Physical Appearance	Pale yellow
R_f Value	0.61 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	79%
Max Absorption (λ_{max})	349 nm
Combustion Analysis	C: 68.20%, H: 4.42%, N: 10.85%, O: 8.26%, S: 8.28%
ChemicalName	5-(2-Hydroxyphenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3624 (O-H stretch), 3069 (C-H stretch), 1575 (C=C stretch), 1641 (C=N stretch), 1163 (C-O stretch), 753 (o-disubstituted benzene), 708, 769 (m-disubstituted benzene), 834 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.77 (1H, Ar-OH), 8.73 (1H, Ar-CH), 7.92 - 7.11 (12H, Ar-H), 3.86 (3H, CH_3)
- **Mass Spectrum(m/z)**: 386 [M+]

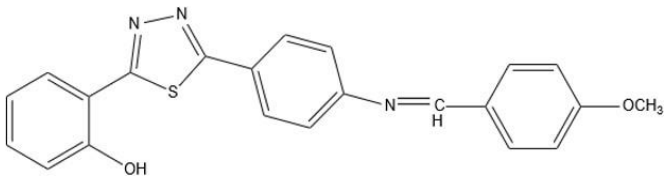
Table 5.10: Physico-analytical Properties of Compound 7d

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3OS_2$
Structure	
Molecular Weight	403 g/mol
Melting Point(°C)	186 -187
Physical Appearance	Magenta red
R_f Value	0.32 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in DMSO, Ethanol
Yield (w/w)	68%
Max Absorption (λ_{max})	265 nm
Combustion Analysis	C: 65.48%, H: 4.25%, N: 10.41%, O: 3.96%, S: 15.89%
ChemicalName	5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum(KBr,cm^{-1})**: The following compounds are involved in stretching: 3074(C-H), 1564(C=C), 1642(C=N), 1287(C-O),762(o-disubstitutedbenzene), 695, 782 (m-disubstitutedbenzene), 836 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.71 (1H,Ar-CH), 7.95 - 7.12 (12H,Ar-H), 3.86 (3H, CH_3), 2.13 (1H, Ar-SH)
- **Mass Spectrum (m/z)**: 402 [M+]

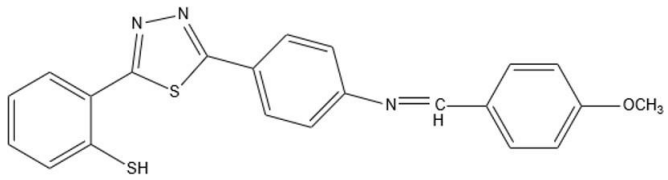
Table 5.11: Physico-analytical Properties of Compound 7e

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_2S$
Structure	
Molecular Weight	387 g/mol
Melting Point(°C)	192 -193
Physical Appearance	Light yellow
R_f Value	0.64 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	82%
Max Absorption (λ_{max})	336 nm
Combustion Analysis	C: 68.2%, H: 4.42%, N: 10.85%, O:8.26%, S: 8.28%
Chemica lName	5-(2-Hydroxyphenyl)-2-{N-(4-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr,cm^{-1})**: The following compounds are involved in stretching: 3641 (O-H), 3094(C-H), 1545(C=C), 1636(C=N), 1289 (C-O), 764 (o-disubstituted benzene), and 812 (p-disubstituted benzene).
- **^{1}H NMR (DMSO, ppm)**:10.68(1H, Ar-OH), 8.85(1H, Ar-CH), 7.94-7.13 (12H, Ar-H), 3.91 (1H, CH_3)
- **Mass Spectrum(m/z)**: 386 [M+]

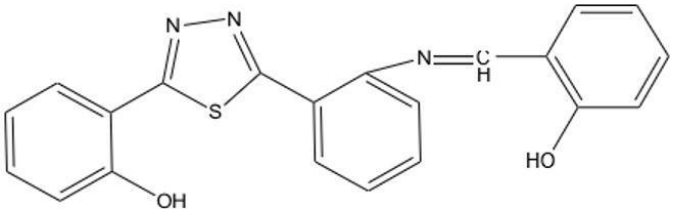
Table 5.12: Physico-analytical Properties of Compound 7f

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3OS_2$
Structure	
Molecular Weight	403 g/mol
Melting Point(°C)	202 -203
Physical Appearance	Magenta red
R_f Value	0.31 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol, DMSO
Yield (w/w)	68%
Max Absorption (λ_{max})	266 nm
Combustion Analysis	C: 65.48%, H: 4.25%, N: 10.41%, O: 3.96%, S: 15.89%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum(KBr, cm^{-1})**: 3089 (C-H stretch), 1575 (C=C stretch), 1648(C=N stretch), 1236 (C-O stretch), 738 (o-disubstituted benzene), 825 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.86 (1H,Ar-CH), 7.99 - 7.14 (12H,Ar-H), 3.91 (3H, CH_3), 2.32 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 402 [M+]

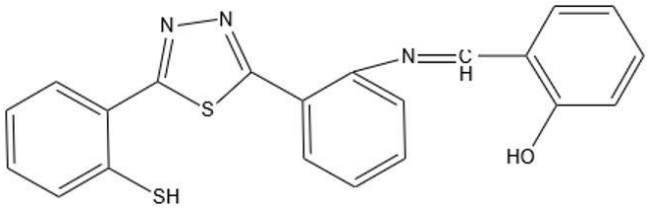
Table 5.13: Physico-analytical Properties of Compound 8a

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3O_2S$
Structure	
Molecular Weight	373 g/mol
Melting Point(°C)	170 -171
Physical Appearance	Pale yellow
R_f Value	0.68 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	75%
Max Absorption (λ_{max})	329 nm
Combustion Analysis	C: 67.54%, H: 4.05%, N: 11.25%, O: 8.57%, S: 8.59%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(2-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3608 (O-H stretch), 3012 (C-H stretch), 1564 (C=C stretch), 1618 (C=N stretch), 748 (o-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.63(1H, Ar-OH), 8.65(1H, Ar-CH), 8.17-7.05 (12H,Ar-H)
- **Mass Spectrum(m/z)**: 372 [M+]

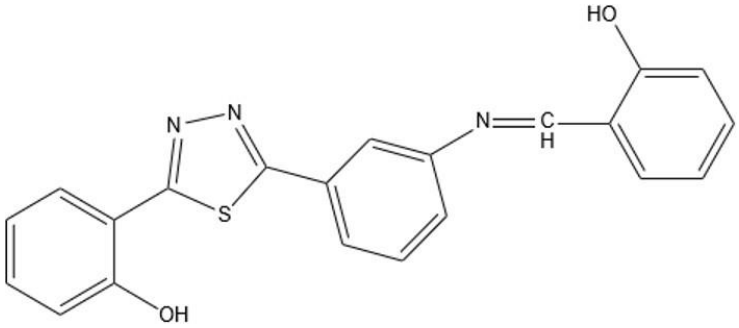
Table 5.14: Physico-analytical Properties of Compound 8b

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3OS_2$
Structure	
Molecular Weight	389 g/mol
Melting Point(°C)	182 -183
Physical Appearance	Red
R_f Value	0.28 (Mobilephase: Benzene: Acetone, 9:1)
Solubility	Soluble in DMSO, Ethanol
Yield (w/w)	71%
Max Absorption (λ_{max})	258 nm
Combustion Analysis	C:64.76%,H:3.88%,N:10.79%,O:4.11%,S:16.46%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(2-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3632 (O-H stretch), 3065 (C-H stretch), 1524 (C=C stretch), 1657 (C=N stretch), 749 (o-disubstituted benzene)
- **^{1}H NMR (DMSO,ppm)**: 10.52(1H, Ar-OH),8.66(1H, Ar-CH),8.15-7.05 (12H, Ar-H), 2.32 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 388 [M+]

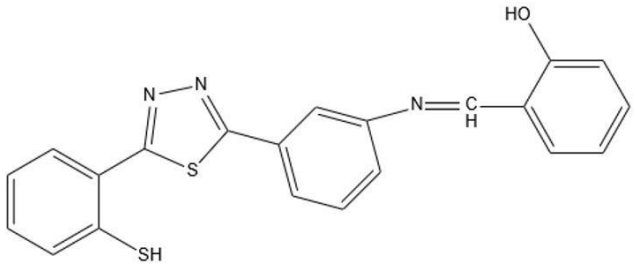
Table 5.15: Physico-analytical Properties of Compound 8c

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3O_2S$
Structure	
Molecular Weight	373 g/mol
Melting Point(°C)	189 -190
Physical Appearance	Light yellow
R_f Value	0.64 (Mobile phase: Benzene: Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	68%
Max Absorption (λ_{max})	338 nm
Combustion Analysis	C: 67.54%, H: 4.05%, N: 11.25%, O: 8.57%, S: 8.59%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(2-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3623 (O-H stretch), 3042 (C-H stretch), 1582 (C=C stretch), 1637 (C=N stretch), 739 (o-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.65(1H, Ar-OH), 8.79(1H, Ar-CH), 7.93-7.02 (12H,Ar-H)
- **Mass Spectrum(m/z)**: 372 [M+]

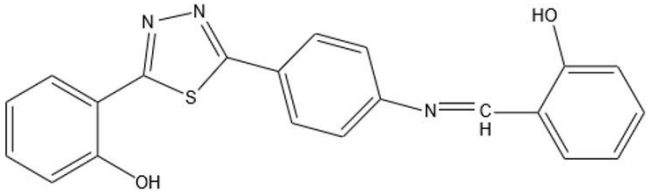
Table 5.16: Physico-analytical Properties of Compound 8d

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3OS_2$
Structure	
Molecular Weight	389 g/mol
Melting Point (°C)	176 -177
Physical Appearance	Magenta red
R_f Value	0.32 (Mobile phase: Benzene: Acetone, 9:1)
Solubility	Soluble in DMSO, Ethanol
Yield (w/w)	65%
Max Absorption (λ_{max})	266 nm
Combustion Analysis	C: 64.76%, H: 3.88%, N: 10.79%, O: 4.11%, S: 16.46%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(2-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: A few examples of stretching bonds include: 3641 (O-H), 3029 (C-H), 1574(C=C), 1643(C=N), 757(o-disubstituted benzene), 694, and 771 (m-disubstituted benzene).
- **^{1}H NMR (DMSO, ppm)**: 10.51(1H, Ar-OH), 8.77(1H, Ar-CH), 7.96-7.03 (12H, Ar-H), 2.31 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 388 [M+]

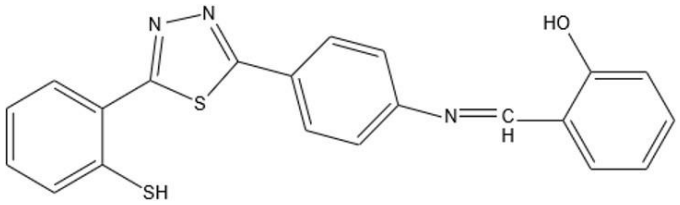
Table 5.17: Physico-analytical Properties of Compound 8e

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3O_2S$
Structure	
Molecular Weight	373 g/mol
Melting Point (°C)	203 -204
Physical Appearance	Light yellow
R_f Value	0.62 (Mobile phase: Benzene: Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	69%
Max Absorption (λ_{max})	346 nm
Combustion Analysis	C: 67.54%, H: 4.05%, N: 11.25%, O: 8.57%, S:8.59%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(2-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3637 (O-H stretch), 3081 (C-H stretch), 1541 (C=C stretch), 1636 (C=N stretch), 1289 (C-O stretch), 746 (o-disubstituted benzene), 816 (p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.61 (1H, Ar-OH), 8.90 (1H, Ar-CH), 8.03 - 7.05 (12H,Ar-H)
- **Mass Spectrum(m/z)**: 372 [M+]

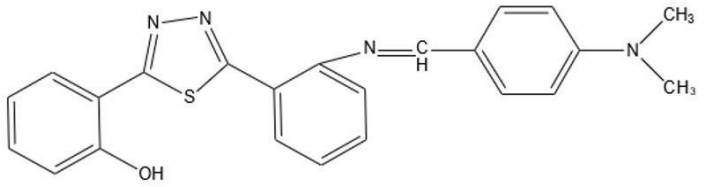
Table 5.18: Physico-analytical Properties of Compound 8f

Property	**Value**
Molecular Formula	$C_{21}H_{15}N_3OS_2$
Structure	
Molecular Weight	389 g/mol
Melting Point(°C)	209 -210
Physical Appearance	Magenta red
R_f Value	0.35 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol, DMSO
Yield (w/w)	68%
Max Absorption (λ_{max})	264 nm
Combustion Analysis	C: 64.76%, H: 3.88%, N: 10.79%, O: 4.11%, S: 16.46%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(2-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3638 (O-H stretch), 3081 (C-H stretch), 1542 (C=C stretch), 1628(C=Nstretch), 747(o-disubstitutedbenzene), 824(p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.49 (1H, Ar-OH), 8.91 (1H, Ar-CH), 7.97 - 7.05 (12H, Ar-H), 2.32 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 388 [M+]

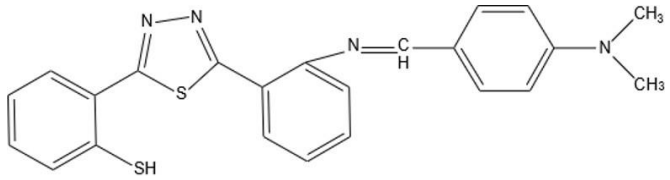
Table 5.19: Physico-analytical Properties of Compound 9a

Property	**Value**
Molecular Formula	$C_{23}H_{20}N_4OS$
Structure	
Molecular Weight	400 g/mol
Melting Point(°C)	176 -177
Physical Appearance	Cream color
R_f Value	0.64 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	71%
Max Absorption (λ_{max})	332 nm
Combustion Analysis	C:75.37%, H:5.8%, N:14.65%, O: 4.18%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3634 (O-H stretch), 3058 (C-H stretch), 1534 (C=C stretch), 1659(C=N stretch), 739(o-disubstituted benzene), 828(p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.57 (1H, Ar-OH), 8.62 (1H, Ar-CH), 8.03 - 6.75 (12H, Ar-H), 2.92 (6H, $2CH_3$)
- **Mass Spectrum (m/z)**: 399 [M+]

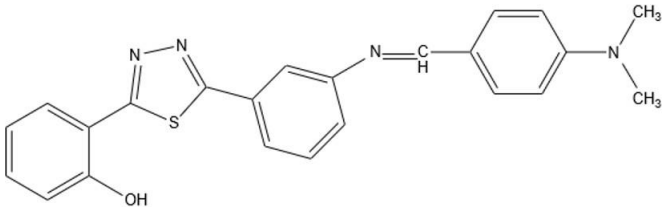
Table 5.20: Physico-analytical Properties of Compound 9b

Property	**Value**
Molecular Formula	$C_{23}H_{20}N_4S2$
Structure	
Molecular Weight	416 g/mol
Melting Point(°C)	171 -172
Physical Appearance	Magenta red
R_f Value	0.33 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	61%
Max Absorption (λ_{max})	264 nm
Combustion Analysis	C: 66.32%, H: 4.84%, N: 13.45%, O: 4.11%, S: 15.40%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3614 (O-H stretch), 3046 (C-H stretch), 1527 (C=C stretch), 1628(C=Nstretch), 763(o-disubstitutedbenzene), 806(p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.62 (1H,Ar-CH), 8.07 - 6.67 (12H,Ar-H), 2.92 (6H, $2CH_3$), 2.38 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 415 [M+]

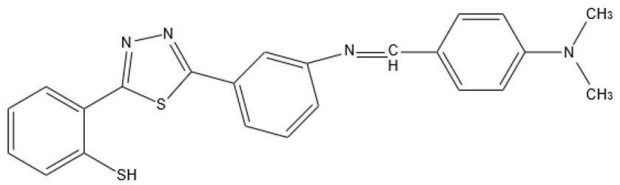
Table 5.21: Physico-analytical Properties of Compound 9c

Property	**Value**
Molecular Formula	$C_{23}H_{20}N_4OS$
Structure	
Molecular Weight	400 g/mol
Melting Point(°C)	195 -196
Physical Appearance	Light yellow
R_f Value	0.64 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	68%
Max Absorption (λ_{max})	338 nm
Combustion Analysis	C: 68.98%, H: 5.03%, N: 13.99%, O:3.99%, S: 8.01%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: The number of stretching vibrations is 3648 for O-H, 3071 for C-H, 1547 for C=C, 1618 for C=N, 764 for o-disubstituted benzene, 692 for m-disubstituted benzene, and 825 for p-disubstituted benzene.
- **^{1}H NMR (DMSO, ppm)**: 10.62 (1H, Ar-OH), 8.67 (1H, Ar-CH), 7.77 - 6.61 (12H, Ar-H), 2.89 (6H, $2CH_3$)
- **Mass Spectrum(m/z)**: 399 [M+]

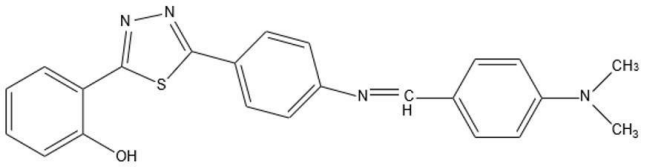
Table 5.22: Physico-analytical Properties of Compound 9d

Property	**Value**
Molecular Formula	$C_{23}H_{20}N_4S2$
Structure	
Molecular Weight	416 g/mol
Melting Point (°C)	185 -186
Physical Appearance	Light brown
R_f Value	0.30 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in DMSO, Ethanol
Yield (w/w)	62%
Max Absorption (λ_{max})	273 nm
Combustion Analysis	C: 66.32%, H: 4.84%,N: 13.45%, S: 15.40%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr,cm^{-1})**: There are a total of 3627o-H stretching bonds, 3082 c-H bonds, 1562 c=C bonds, 1673 c=N bonds, 761 o-disubstituted benzene, 706, 782 m-disubstituted benzene, and 834 p-disubstituted positions.
- **^{1}H NMR (DMSO, ppm)**: 8.67 (1H,Ar-CH), 7.80 - 6.62 (12H,Ar-H), 2.89 (6H, $2CH_3$), 2.32 (1H, Ar-SH)
- **Mass Spectrum (m/z)**: 415 [M+]

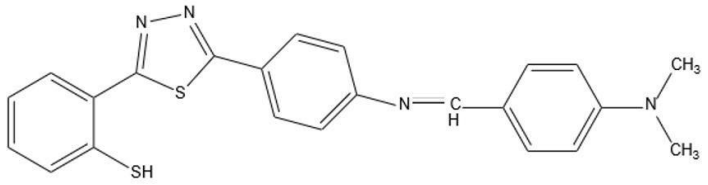
Table 5.23: Physico-analytical Properties of Compound 9e

Property	**Value**
Molecular Formula	$C_{23}H_{20}N_4OS$
Structure	
Molecular Weight	400 g/mol
Melting Point(°C)	209 -210
Physical Appearance	Light yellow
R_f Value	0.61 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	58%
Max Absorption (λ_{max})	338 nm
Combustion Analysis	C: 68.98%, H: 5.03%,N: 13.99%, O:3.99%, S: 8.01%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3648 (O-H stretch), 3047 (C-H stretch), 1568 (C=C stretch), 1654(C=Nstretch), 765(o-disubstituted benzene), 826(p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 10.59 (1H, Ar-OH), 8.81 (1H, Ar-CH), 7.79 - 6.75 (12H, Ar-H), 2.92 (6H, $2CH_3$)
- **Mass Spectrum(m/z)**: 399 [M+]

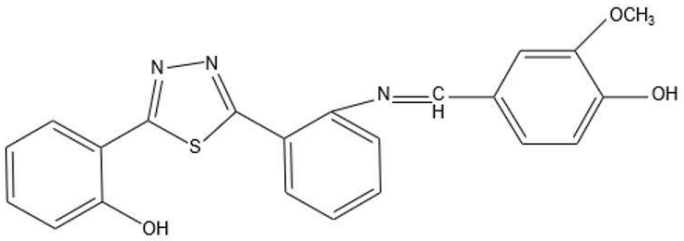
Table 5.24: Physico-analytical Properties of Compound 9f

Property	**Value**
Molecular Formula	$C_{23}H_{20}N_4S_2$
Structure	
Molecular Weight	416 g/mol
Melting Point(°C)	212 -213
Physical Appearance	Light brown
R_f Value	0.36 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol, DMSO
Yield (w/w)	61%
MaxAbsorption (λ_{max})	266 nm
Combustion Analysis	C: 72.33%, H: 5.56%,N: 14.06%, S: 8.05%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: 3647 (O-H stretch), 3046 (C-H stretch), 1575 (C=C stretch), 1639(C=Nstretch), 759(o-disubstitutedbenzene), 834(p-disubstituted benzene)
- **^{1}H NMR (DMSO, ppm)**: 8.81 (1H,Ar-CH), 7.88 - 6.66 (12H,Ar-H), 2.92 (6H, $2CH_3$), 2.32 (1H, Ar-SH)
- **Mass Spectrum(m/z)**: 415 [M+]

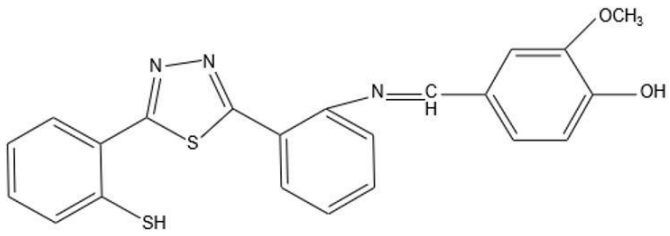
Table 5.25: Physico-analytical Properties of Compound 10a

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_3S$
Structure	
Molecular Weight	403 g/mol
Melting Point(°C)	212 -213
Physical Appearance	Light yellow
R_f Value	0.59 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	71%
Max Absorption (λ_{max})	340 nm
Combustion Analysis	C:71.67%, H: 4.97%,N: 10.90%, O: 12.45%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: O-H stretch: 3647, C-H stretch: 3069, C=C stretch: 1572,C=N stretch:1674, o-disubstituted benzene:746, m-disubstituted benzene: 698, 778, and p-disubstituted benzene:834. Furthermore,
- **^{1}HNMR (DMSO, ppm)**: 10.56 (1H, Ar-OH), 8.16 (1H, Ar-CH), 7.92-6.87 (11H, Ar-H), 3.80 (3H, CH_3)
- **Mass Spectrum(m/z)**: 402 [M+]

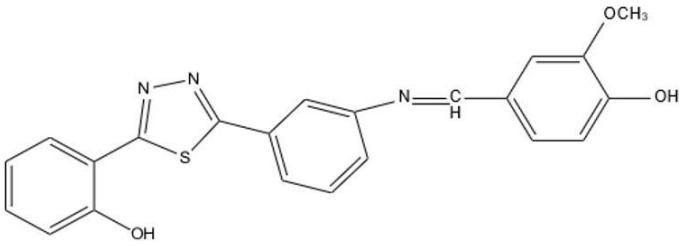
Table 5.26: Physico-analytical Properties of Compound 10b

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_2S_2$
Structure	
Molecular Weight	419 g/mol
Melting Point(°C)	201 -202
Physical Appearance	Magenta red
R_f Value	0.32 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in DMSO, Ethanol
Yield (w/w)	64%
Max Absorption (λ_{max})	230 nm
Combustion Analysis	C: 62.99%, H: 4.08%, N: 10.02%, O:7.63%, S: 15.29%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: O-H stretch: 3618, C-H stretch: 3056, C=C stretch: 1558, C=N stretch:1619, o-disubstituted benzene: 742, m-disubstituted benzene: 706, 785, and p-disubstituted benzene: 831.
- **^{1}H NMR (DMSO, ppm)**:10.62(1H,Ar-OH), 8.62(1H,Ar-CH), 8.07-6.81 (11H, Ar-H), 3.80(1H,CH_3), 2.32(1H, Ar-SH)
- **Mass Spectrum(m/z)**: 418 [M+]

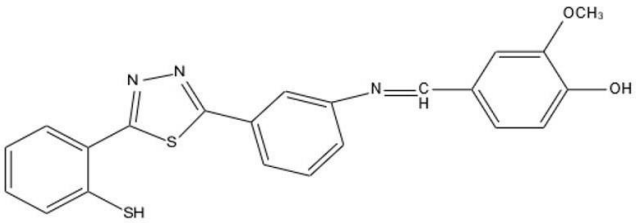
Table 5.27: Physico-analytical Properties of Compound 10c

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_3S$
Structure	
Molecular Weight	403 g/mol
Melting Point(°C)	215 -216
Physical Appearance	Cream color
R_f Value	0.58 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	59%
Max Absorption (λ_{max})	338 nm
Combustion Analysis	C: 65.49%, H: 4.25%,N: 10.42%, O: 11.90%, S: 7.95%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: O-H stretch: 3624, C-H stretch: 3068, C=C stretch: 1557, C=N stretch: 1653, o-disubstituted benzene: 736, m-disubstituted benzene: 698, 774, and p-disubstituted benzene: 831 have been identified.
- **^{1}H NMR (DMSO, ppm)**: 10.62 (1H, Ar-OH), 8.71 (1H, Ar-CH), 7.89 - 6.85 (11H, Ar-H), 3.73 (3H, CH_3)
- **Mass Spectrum(m/z)**: 402 [M+]

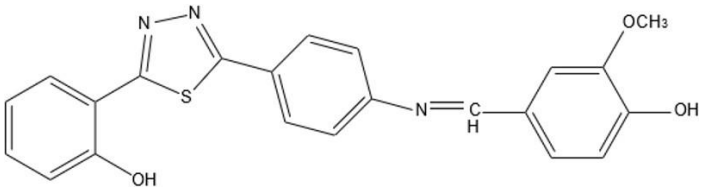
Table 5.28: Physico-analytical Properties of Compound 10d

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_2S_2$
Structure	
Molecular Weight	419 g/mol
Melting Point(°C)	212 -213
Physical Appearance	Magenta red
R_f Value	0.28 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in DMSO, Ethanol
Yield (w/w)	66%
Max Absorption (λ_{max})	226 nm
Combustion Analysis	C:62.99%, H: 4.08%,N: 10.02%, O:7.63%, S: 15.29%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: O-H stretch: 3637, C-H stretch: 3054, C=C stretch: 1585, C=N stretch:1673, o-disubstituted benzene: 745, m-disubstituted benzene: 708, 792, and p-disubstituted benzene: 837.
- **^{1}H NMR (DMSO, ppm)**: 10.59(1H, Ar-OH), 8.71(1H, Ar-CH), 7.92-7.28 (11H, Ar-H), 3.74(3H, CH_3) , 2.34(1H, Ar-SH)
- **Mass Spectrum(m/z)**: 418 [M+]

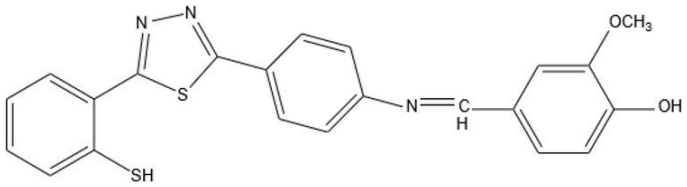
Table 5.29: Physico-analytical Properties of Compound 10e

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_3S$
Structure	
Molecular Weight	403 g/mol
Melting Point(°C)	224 -225
Physical Appearance	Pale yellow
R_f Value	0.67 (Mobilephase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	55%
Max Absorption (λ_{max})	338 nm
Combustion Analysis	C: 65.49%, H: 4.25%, N: 10.42%, O: 11.9%, S: 7.95%
Chemical Name	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: O-H stretch: 3623, C-H stretch: 3074, C=C stretch: 1589, C=N stretch: 1671, o-disubstituted benzene: 762, m-disubstituted benzene: 695, 798, and p-disubstituted benzene: 834.
- **^{1}H NMR (DMSO, ppm)**: 10.61(1H, Ar-OH), 8.86(1H, Ar-CH), 7.74-6.87 (11H, Ar-H), 3.80 (3H, CH_3)
- **Mass Spectrum(m/z)**: 402 [M+]

Table 5.30: Physico-analytical Properties of Compound 10f

Property	**Value**
Molecular Formula	$C_{22}H_{17}N_3O_2S_2$
Structure	
Molecular Weight	419 g/mol
Melting Point(°C)	219 -220
Physical Appearance	Magenta red
R_f Value	0.28 (Mobile phase: Benzene:Acetone, 9:1)
Solubility	Soluble in Ethanol
Yield (w/w)	63%
Max Absorption (λ_{max})	266 nm
Combustion Analysis	C:62.99%, H: 4.08%,N: 10.02%, O:7.63%, S: 15.29%
Chemical Name	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole

Spectroscopic Data:

- **IR Spectrum (KBr, cm^{-1})**: O-H stretch: 3611, C-H stretch: 3075, C=C stretch: 1563, C=N stretch: 1645, o-disubstituted benzene: 739, m-disubstituted benzene: 699, 758, p-disubstituted benzene: 831 and so on.
- **^{1}H NMR (DMSO, ppm)**:10.63(1H, Ar-OH), 8.87(1H, Ar-CH), 7.75-6.81 (11H, Ar-H), 3.80(3H,CH_3), 2.31(1H, Ar-SH)
- **Mass Spectrum(m/z)**: 418 [M+]

5.1.1. IR Spectra

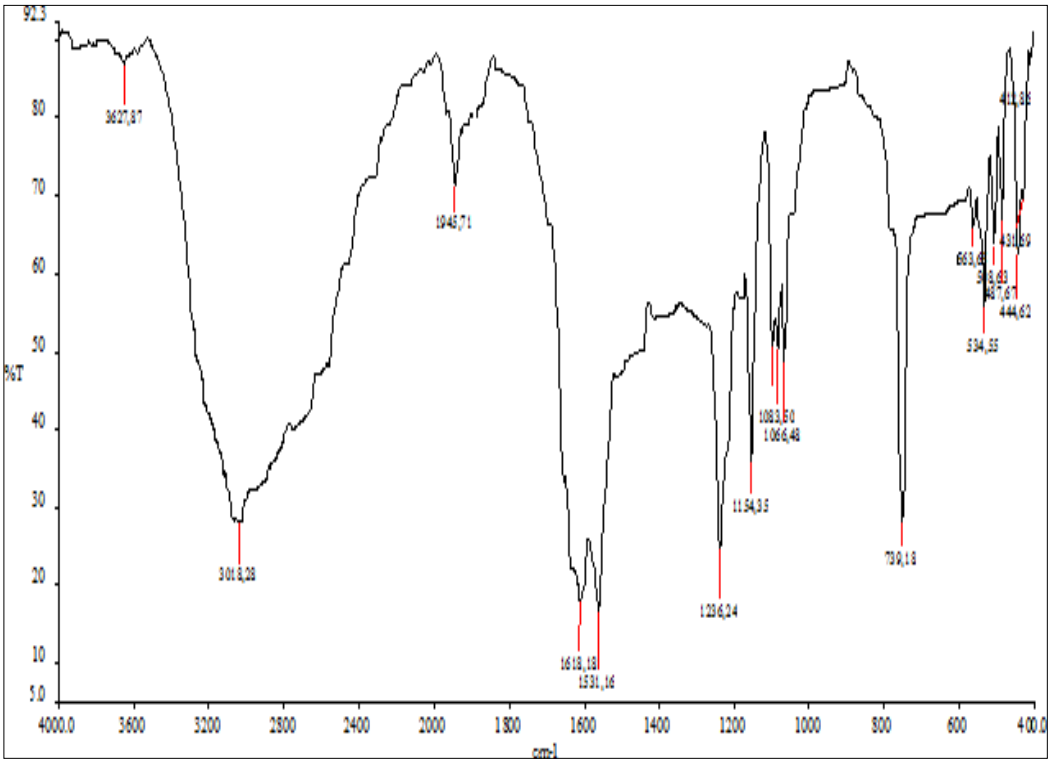


Figure 5.1: IR Spectra of 6a

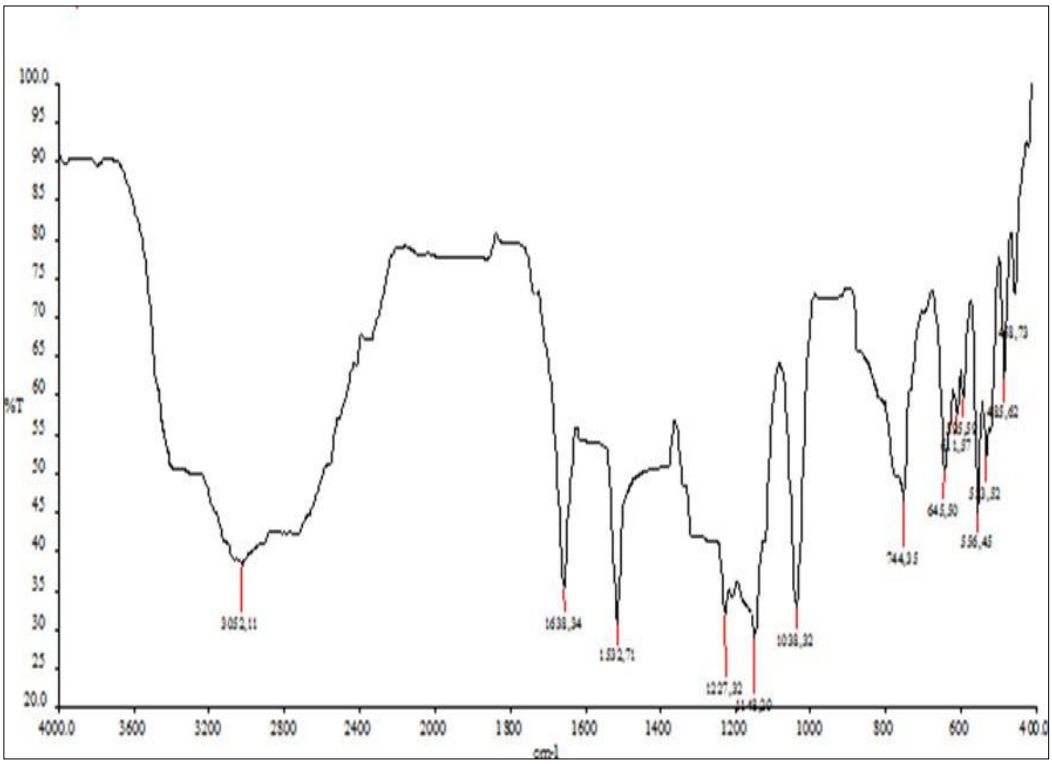


Figure 5.2: IR Spectra of 6b

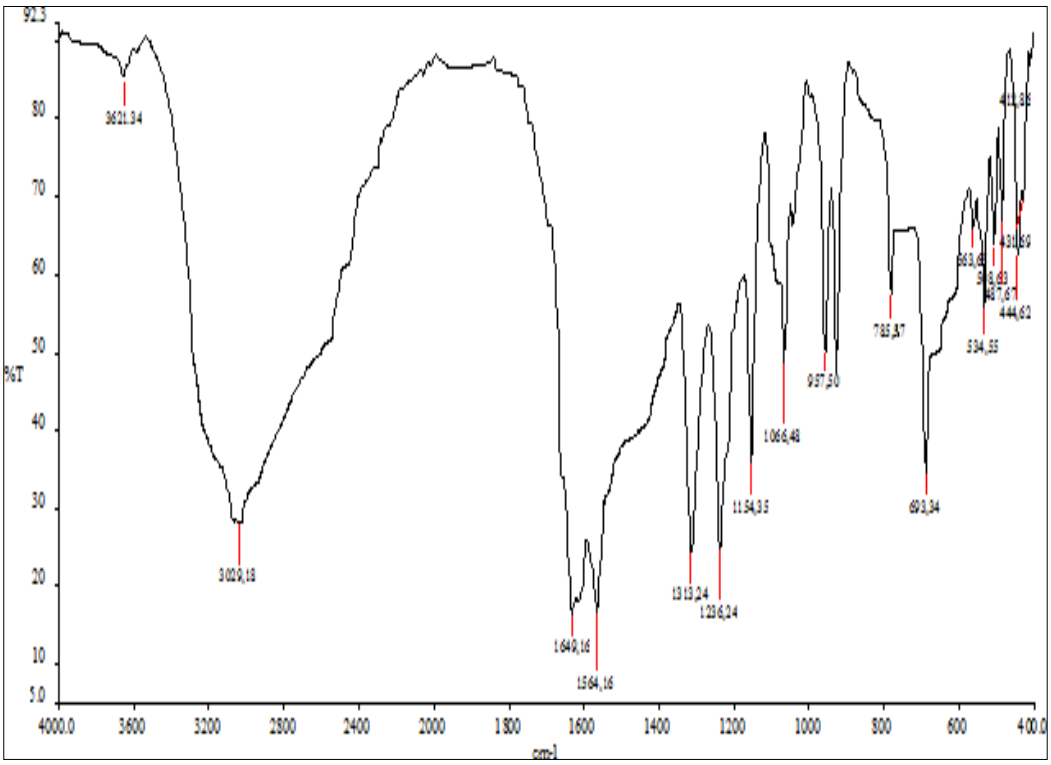


Figure 5.3: IR Spectra of 6c

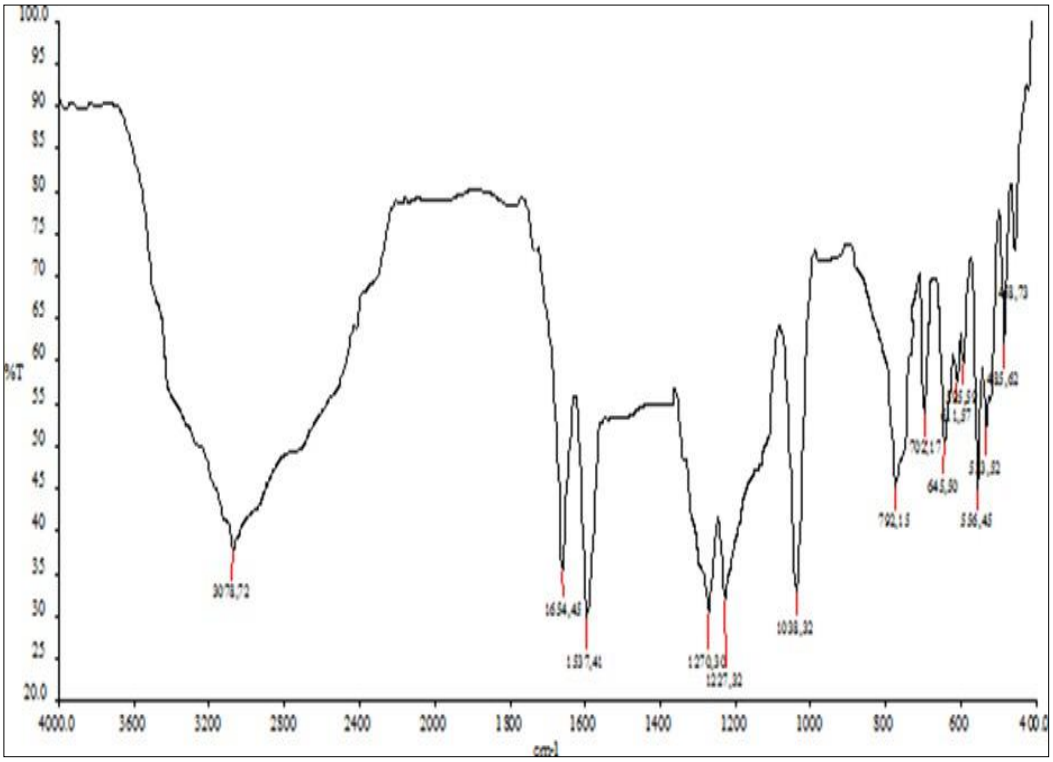


Figure 5.4: IR Spectra of 6d

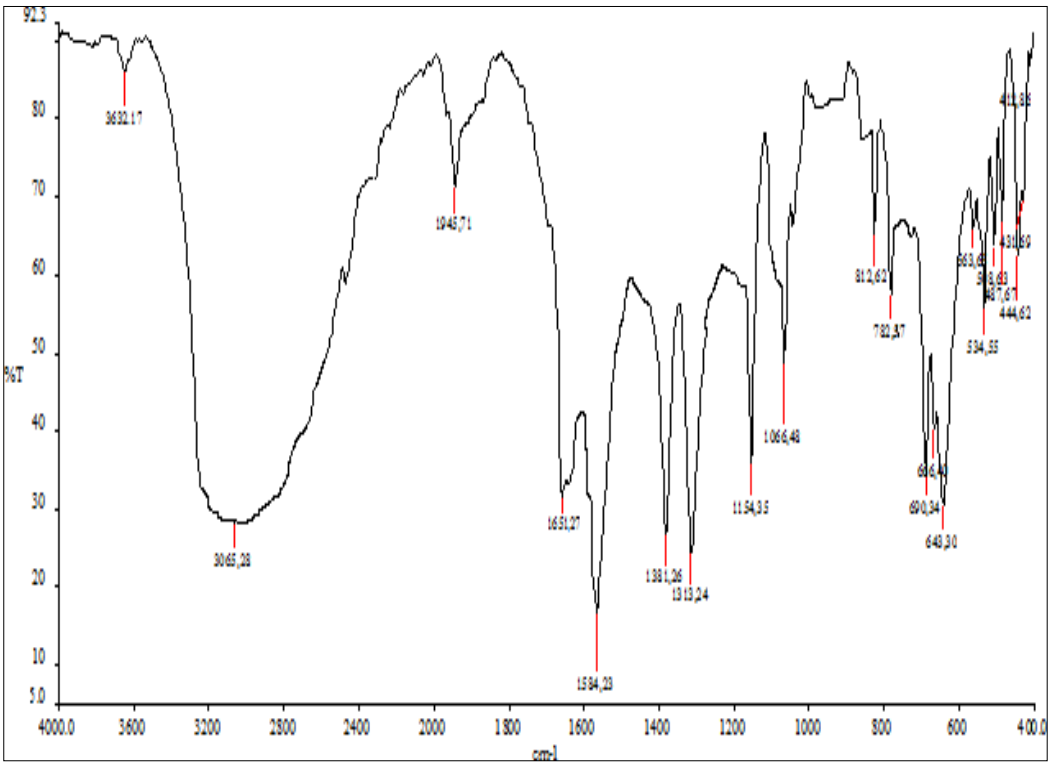


Figure 5.5: IR Spectra of 6e

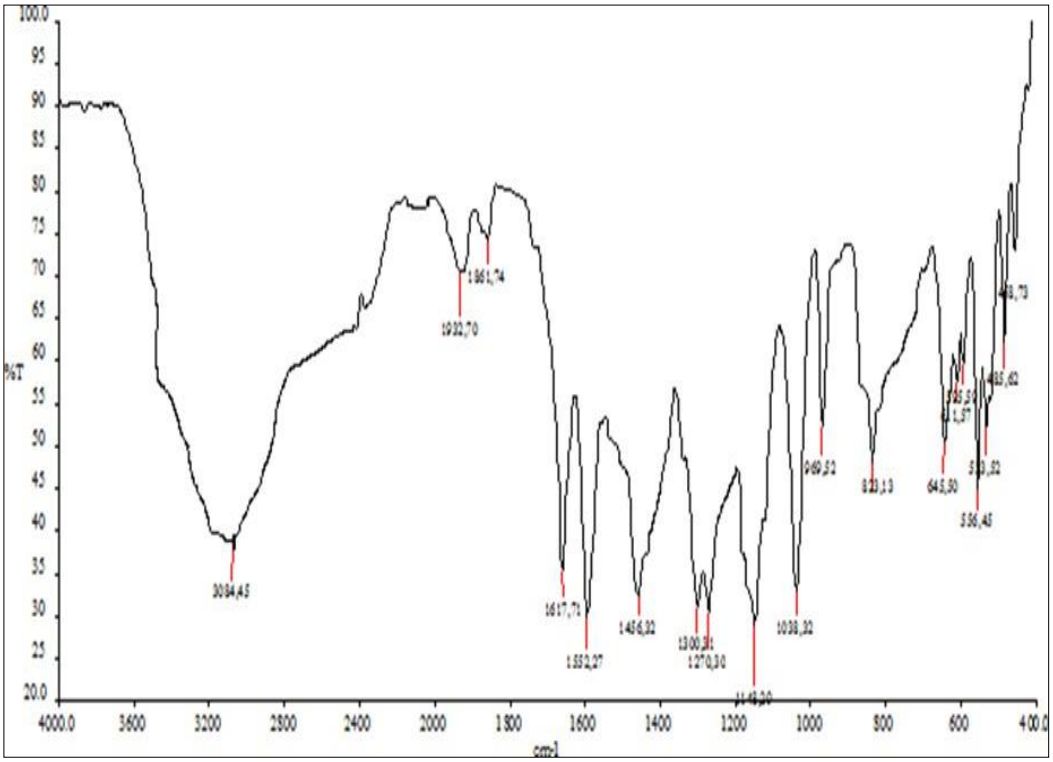


Figure 5.6: IR Spectra of 6f

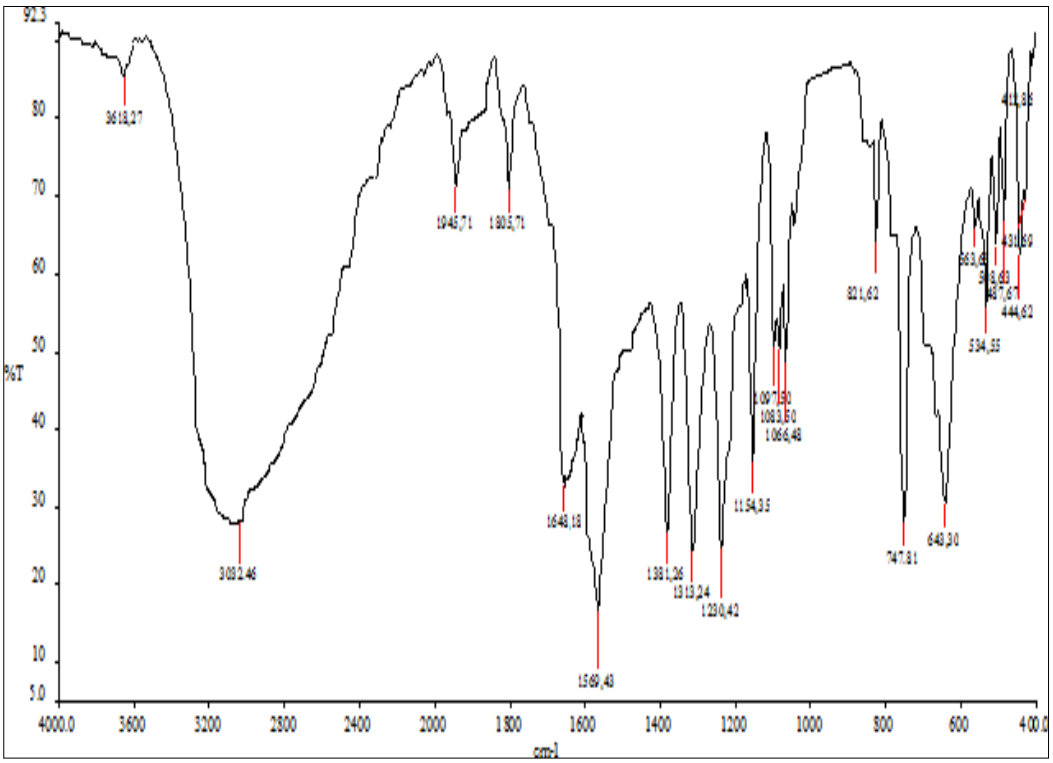


Figure 5.7: IR Spectra of 7a

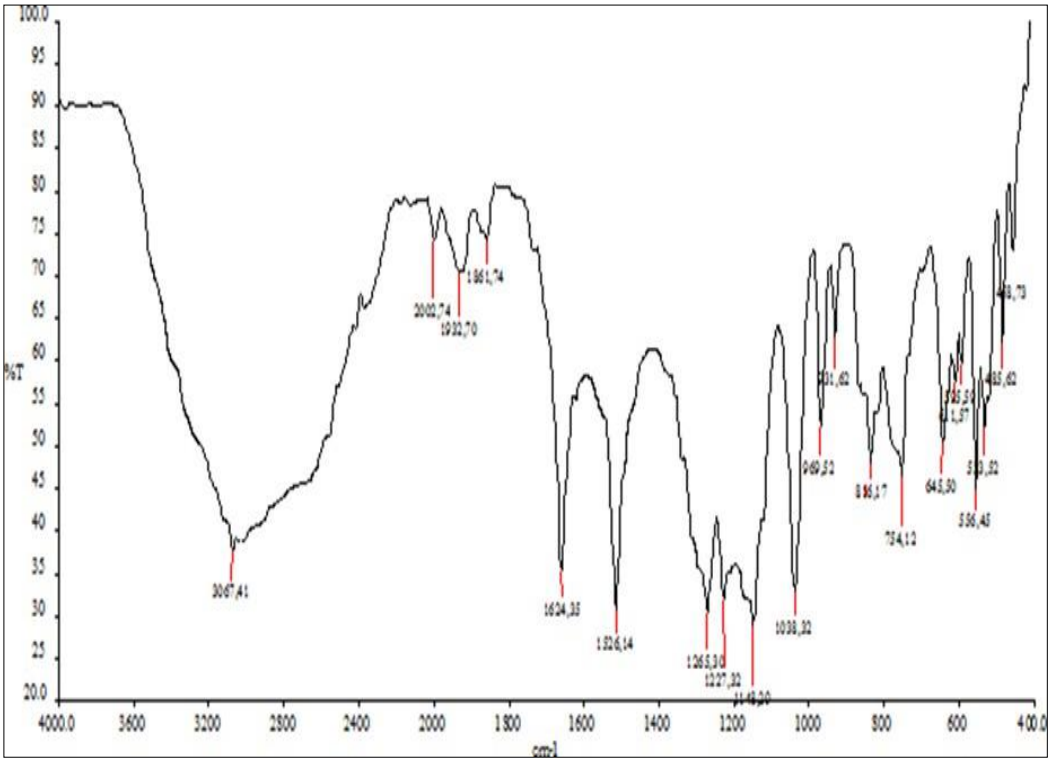


Figure 5.8: IR Spectra of 7b

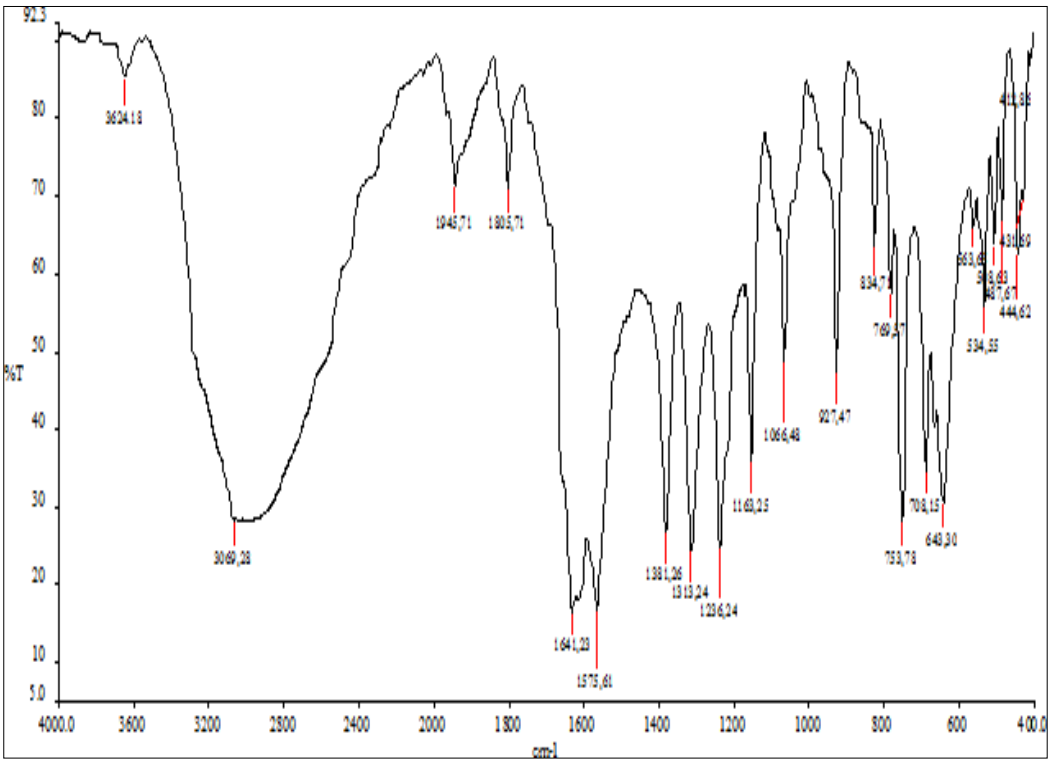


Figure 5.9: IR Spectra of 7c

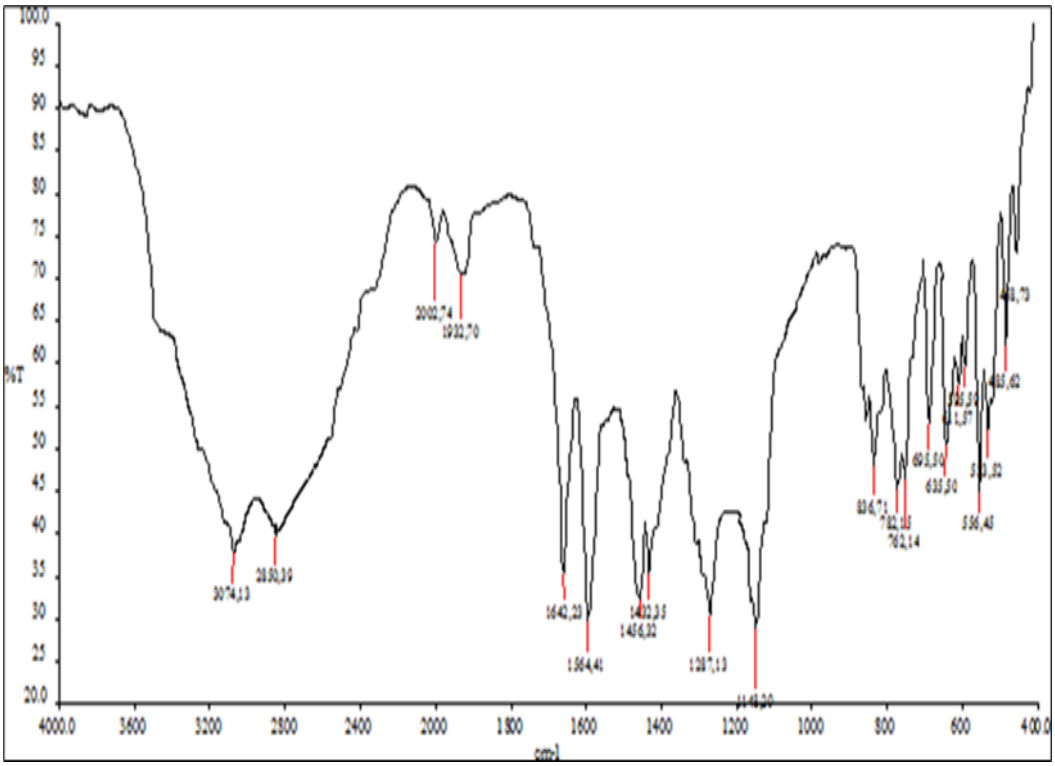


Figure 5.10: IR Spectra of 7d

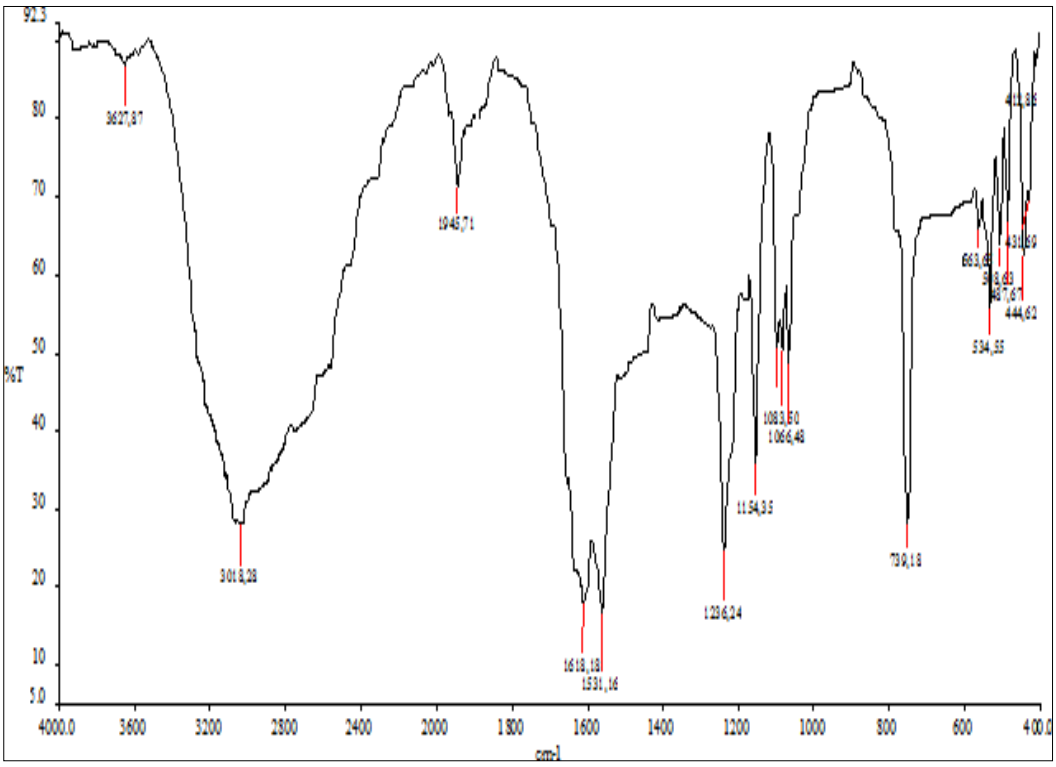


Figure 5.11: IR Spectra of 7e

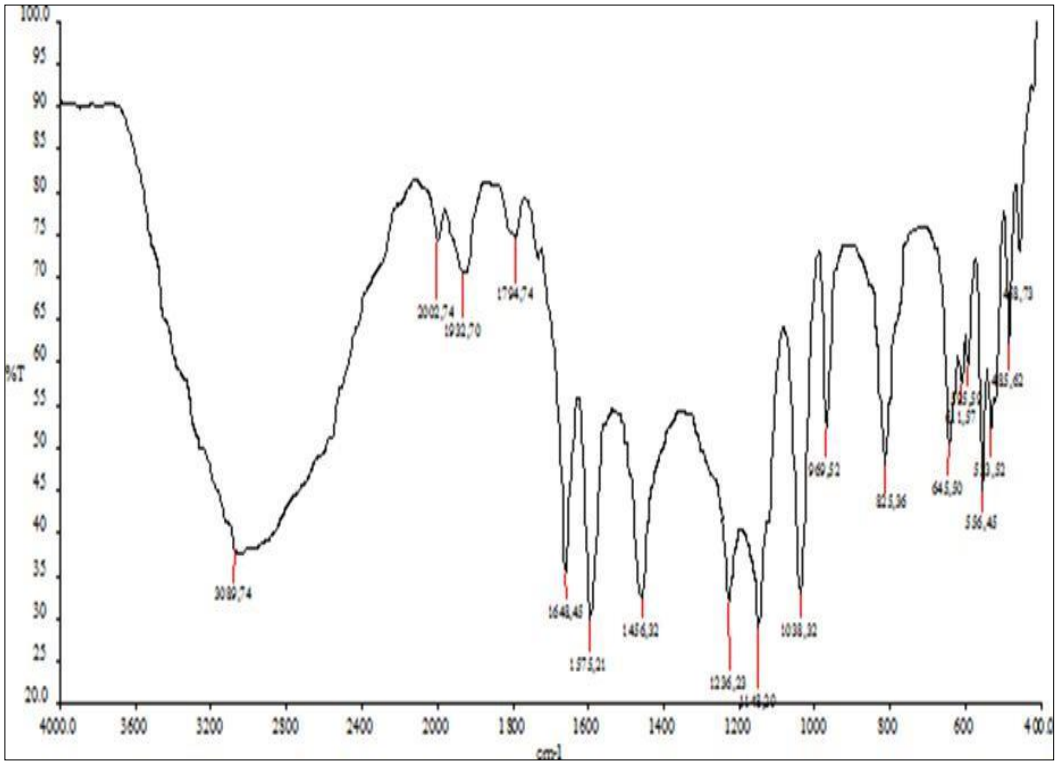


Figure 5.12: IR Spectra of 7f

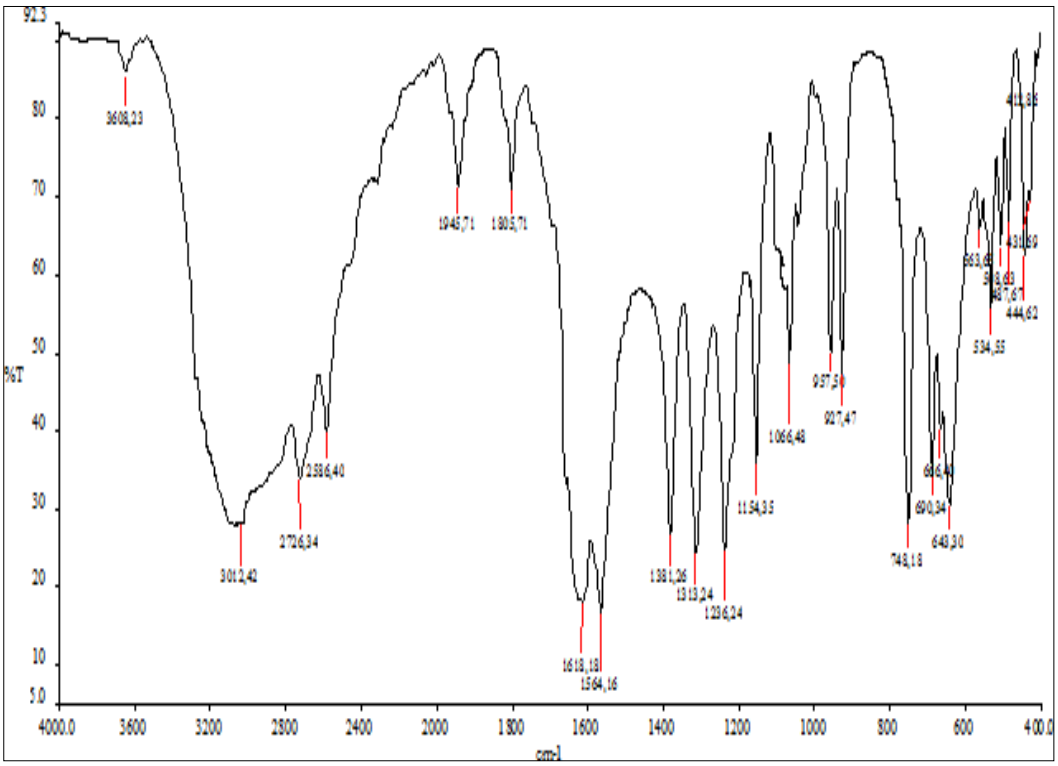


Figure 5.13: IR Spectra of 8a

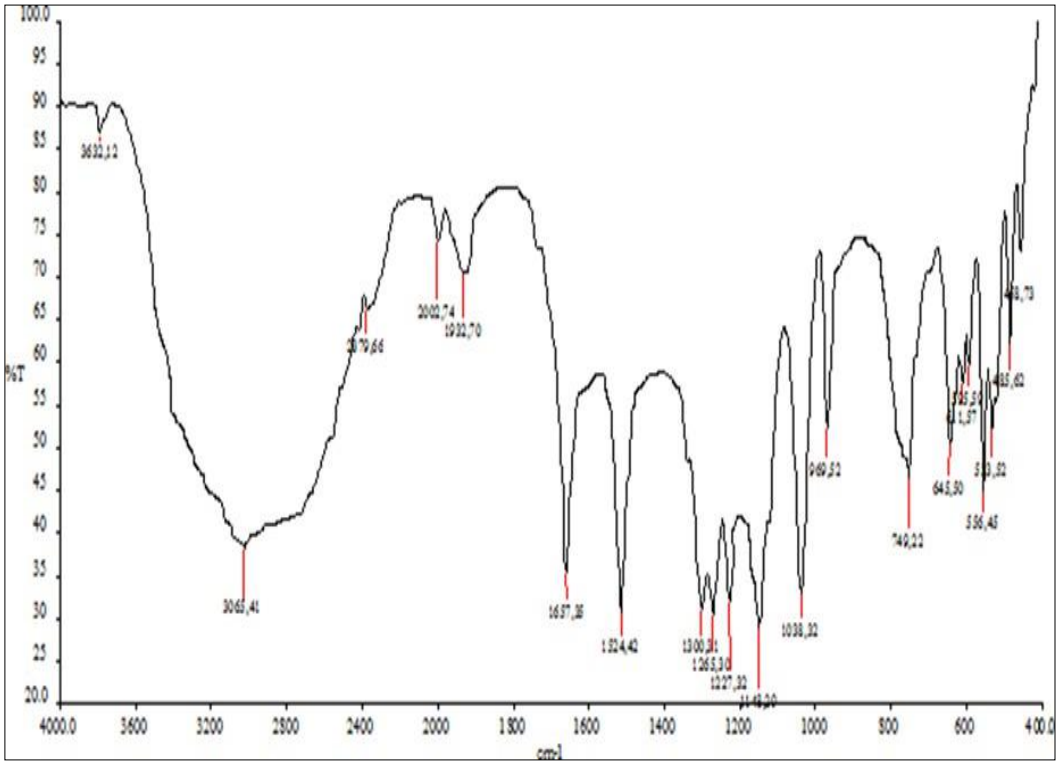


Figure 5.14: IR Spectra of 8b

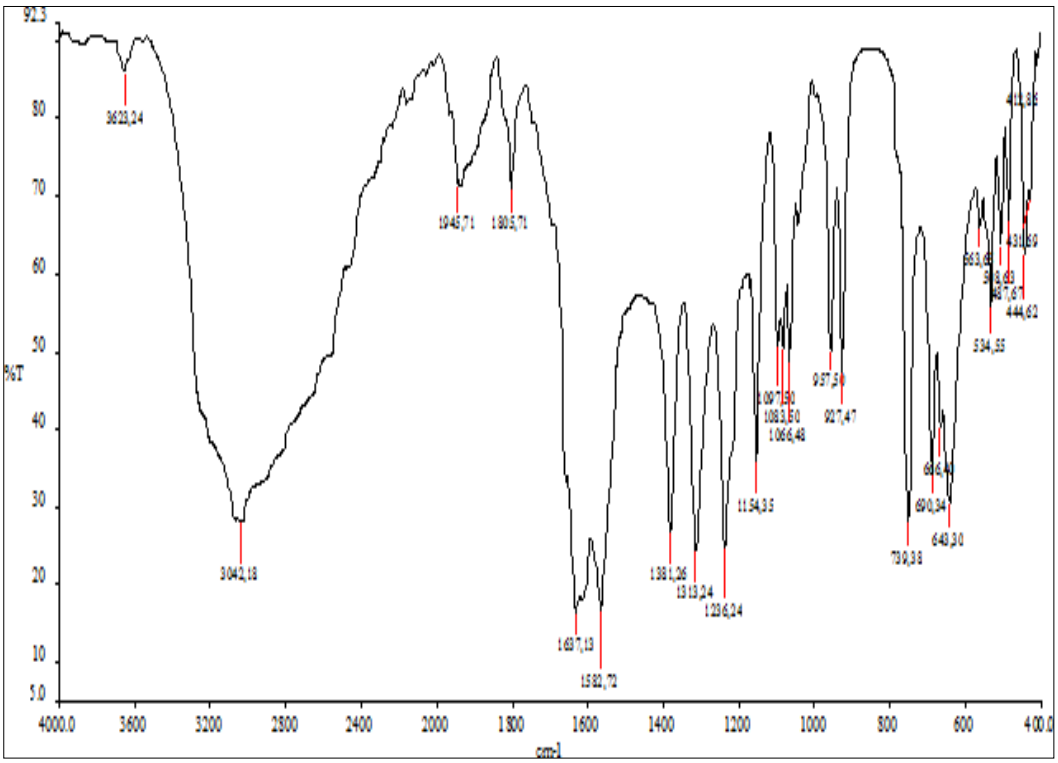


Figure 5.15: IR Spectra of 8c

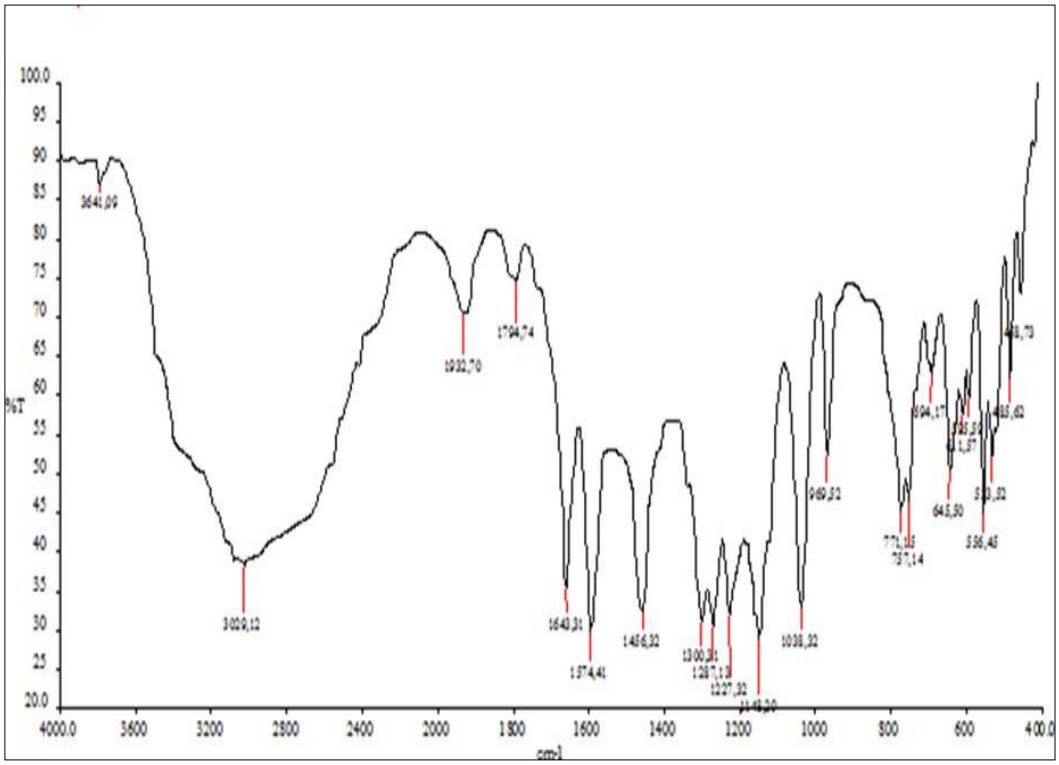


Figure 5.16: IR Spectra of 8d

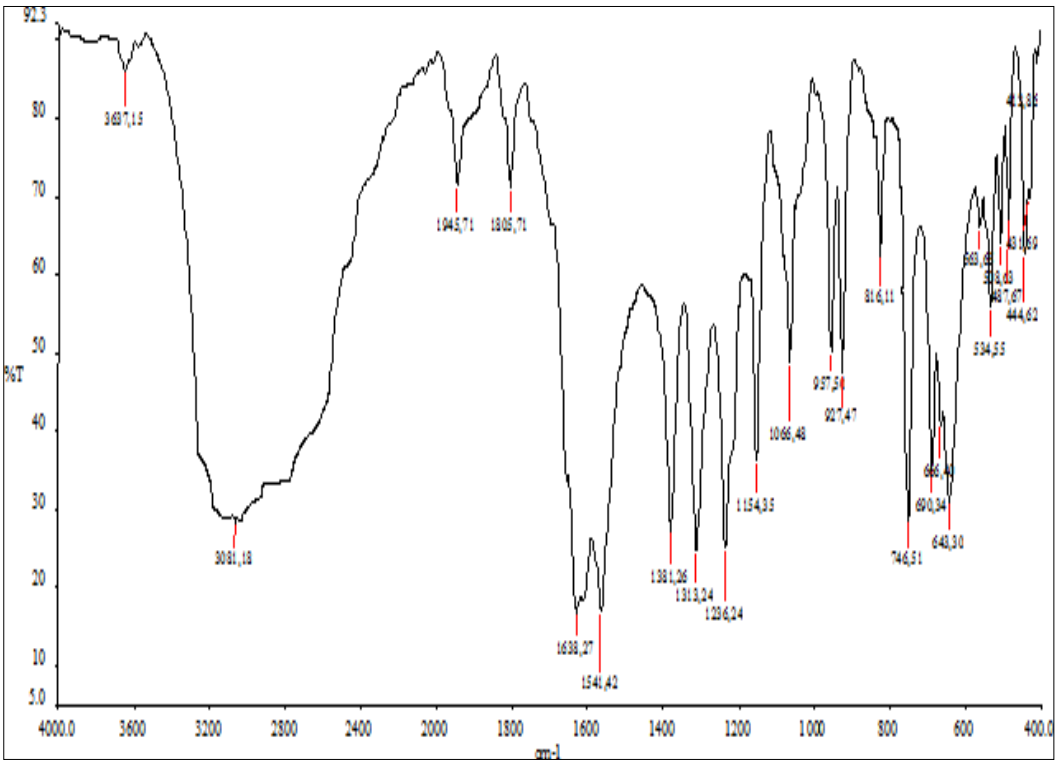


Figure 5.17: IR Spectra of 8e

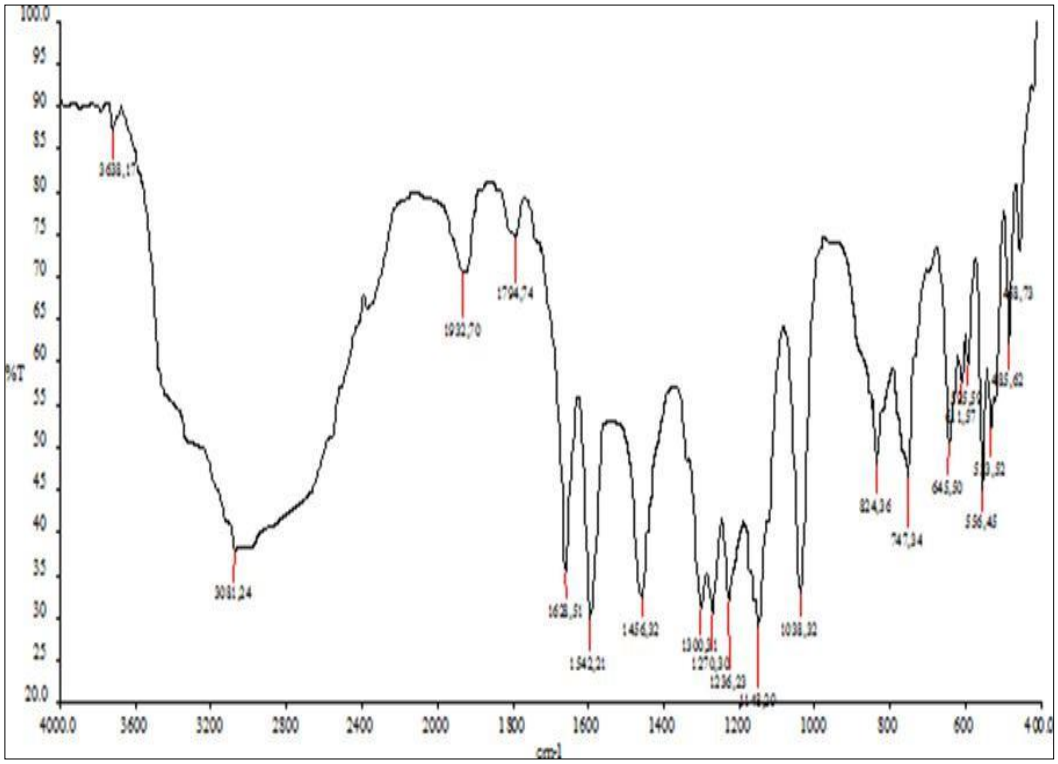


Figure 5.18: IR Spectra of 8f

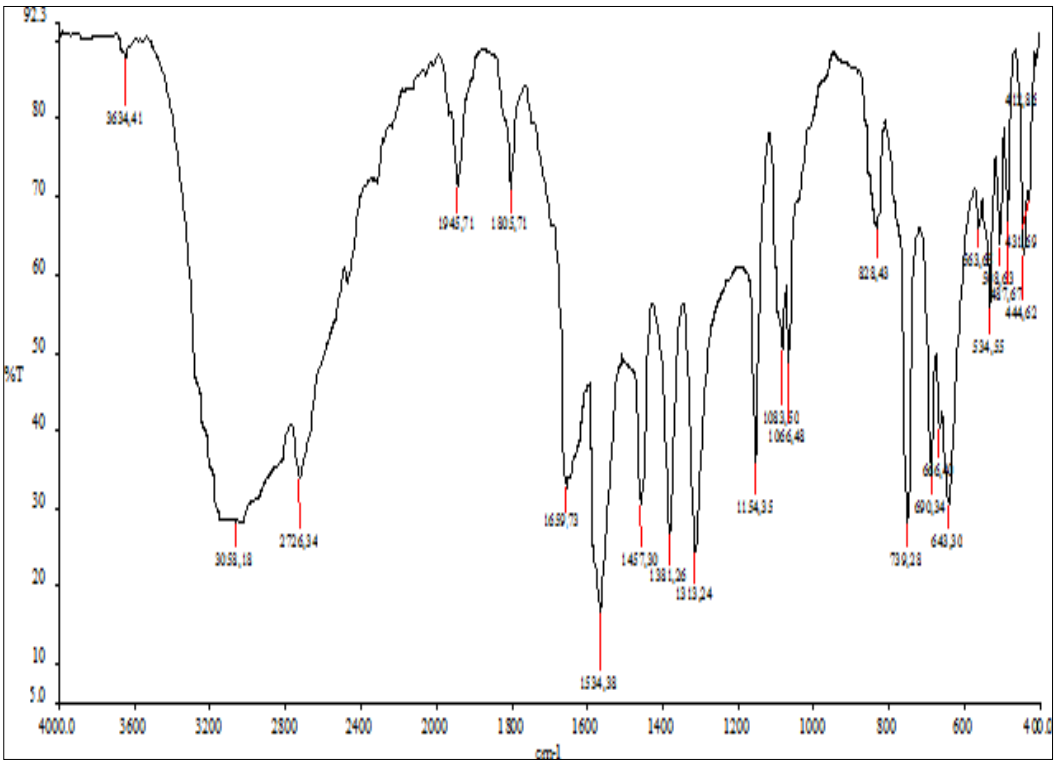


Figure 5.19: IR Spectra of 9a

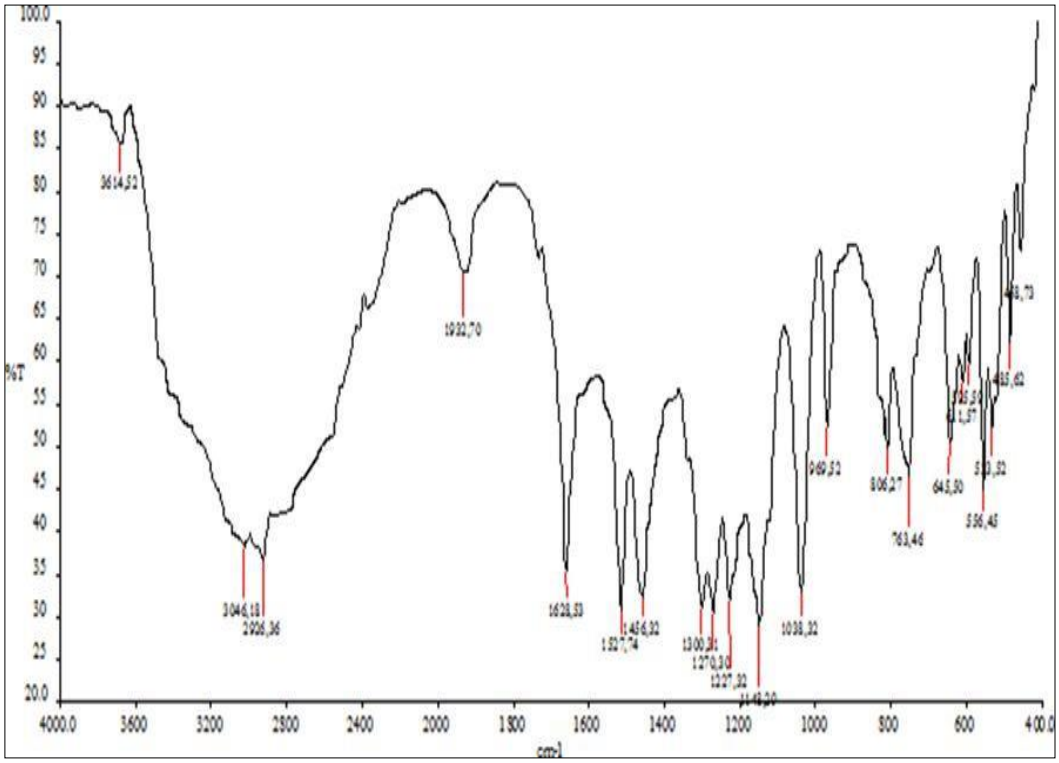


Figure 5.20: IR Spectra of 9b

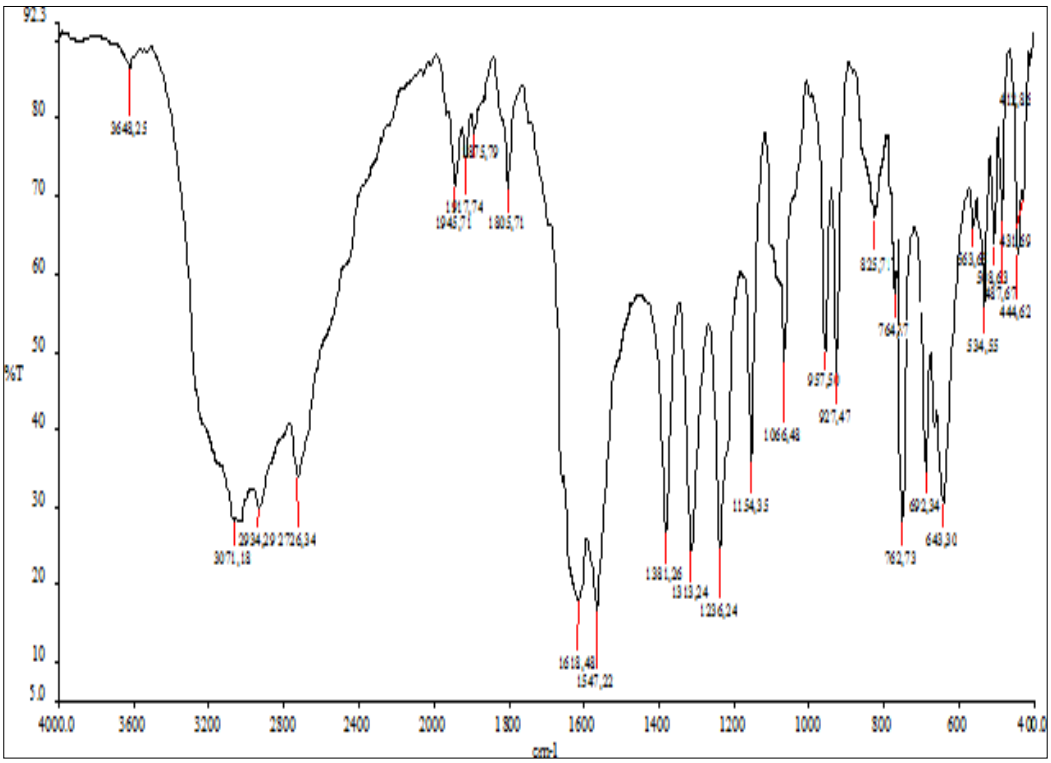


Figure 5.21: IR Spectra of 9c

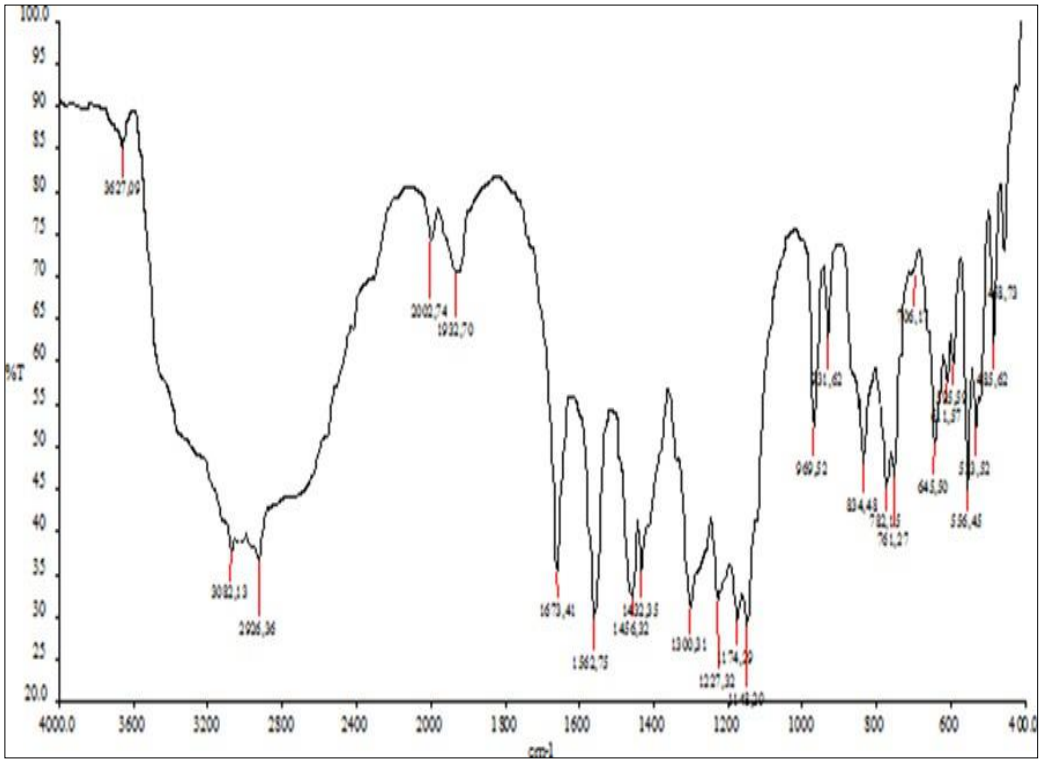


Figure 5.22: IR Spectra of 9d

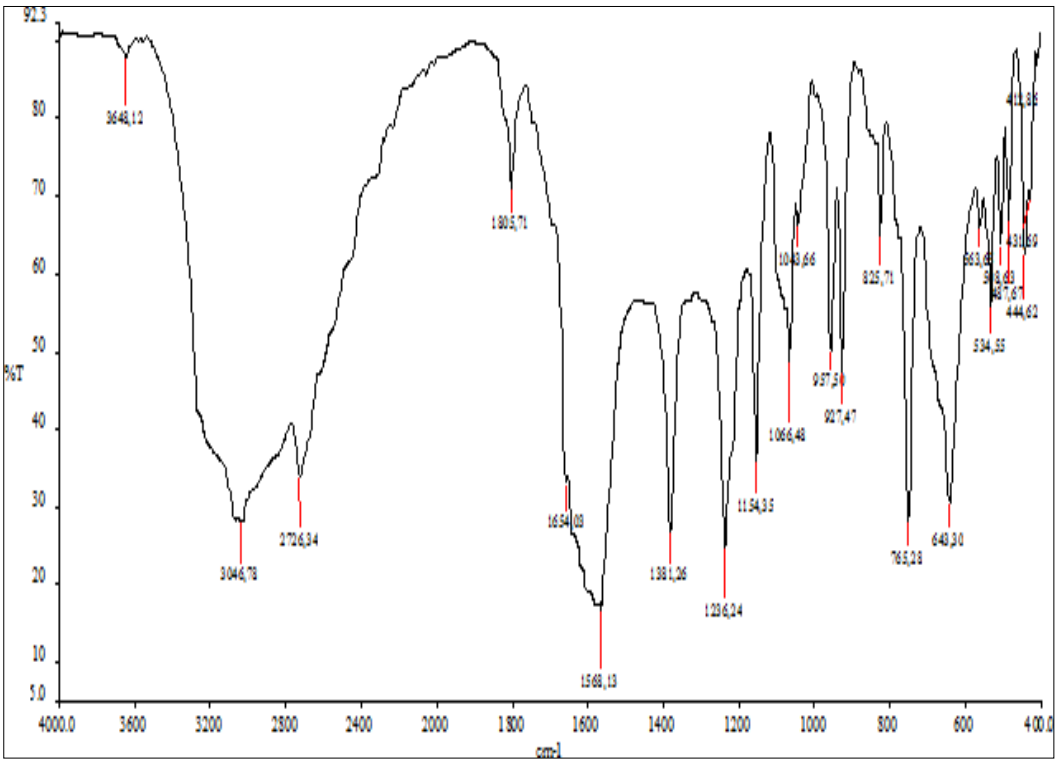


Figure 5.23: IR Spectra of 9e

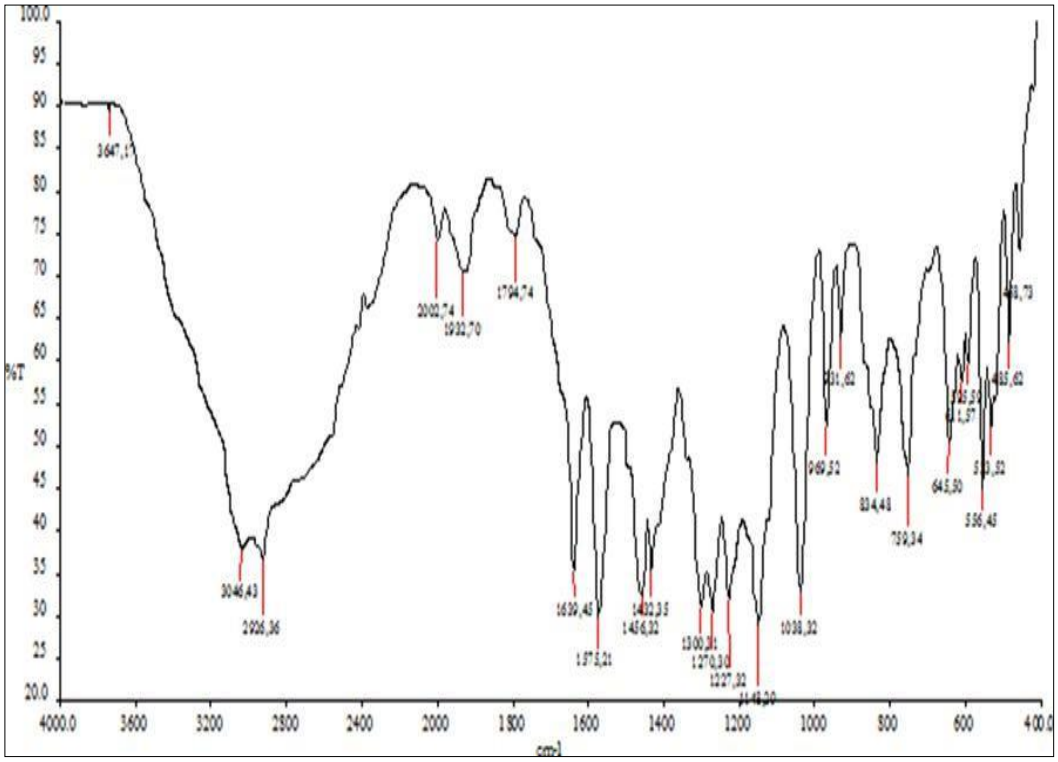


Figure 5.24: IR Spectra of 9f

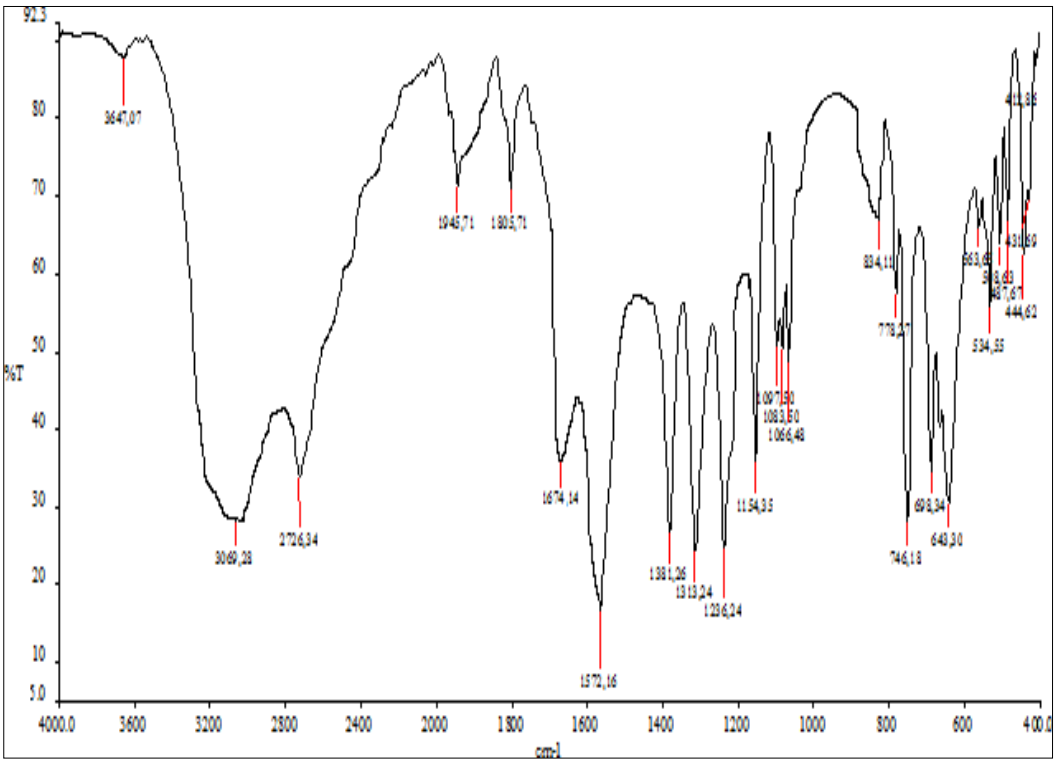


Figure 5.25: IR Spectra of 10a

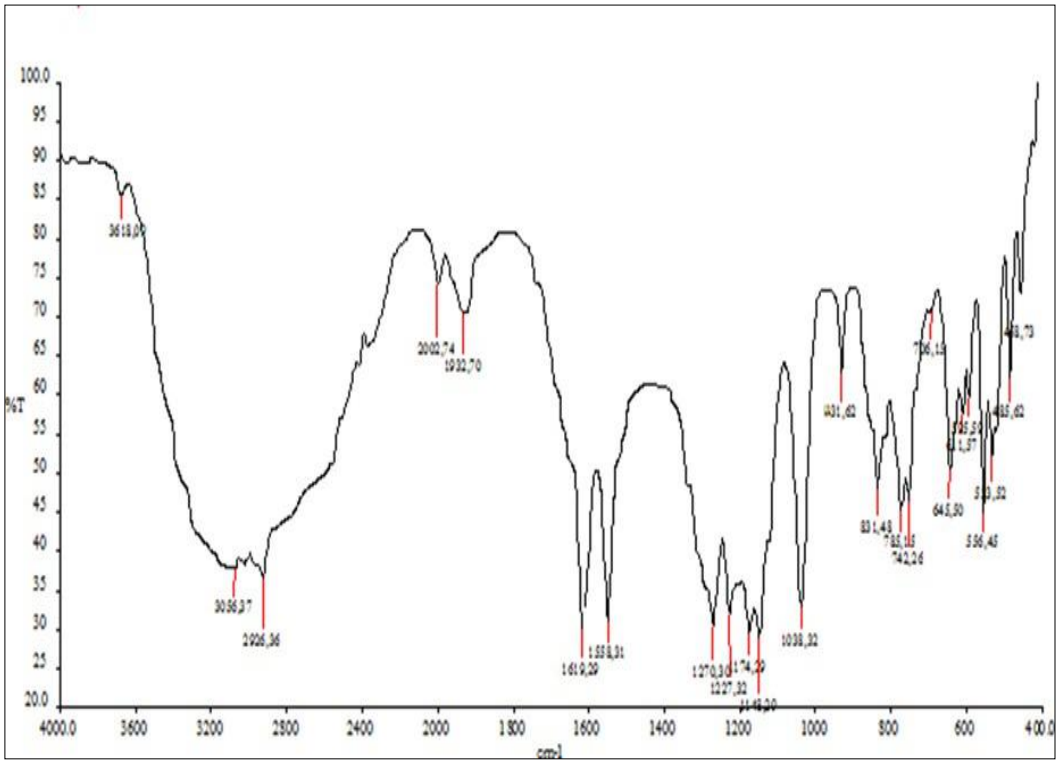


Figure 5.26: IR Spectra of 10b

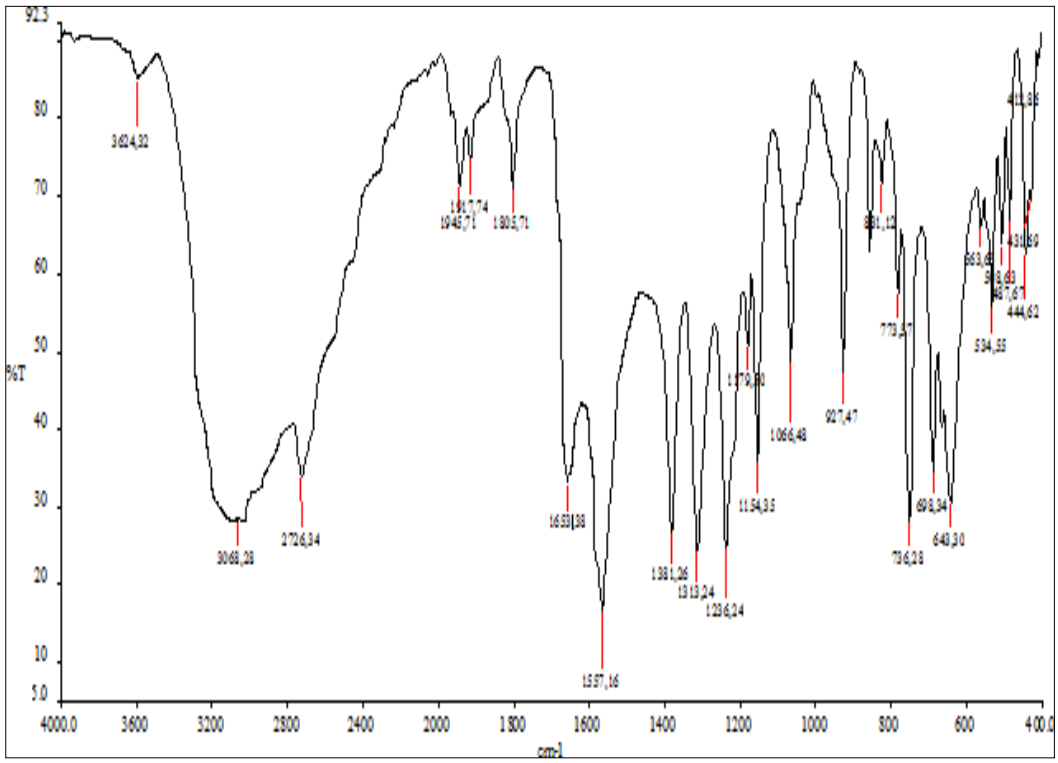


Figure 5.27: IR Spectra of 10c

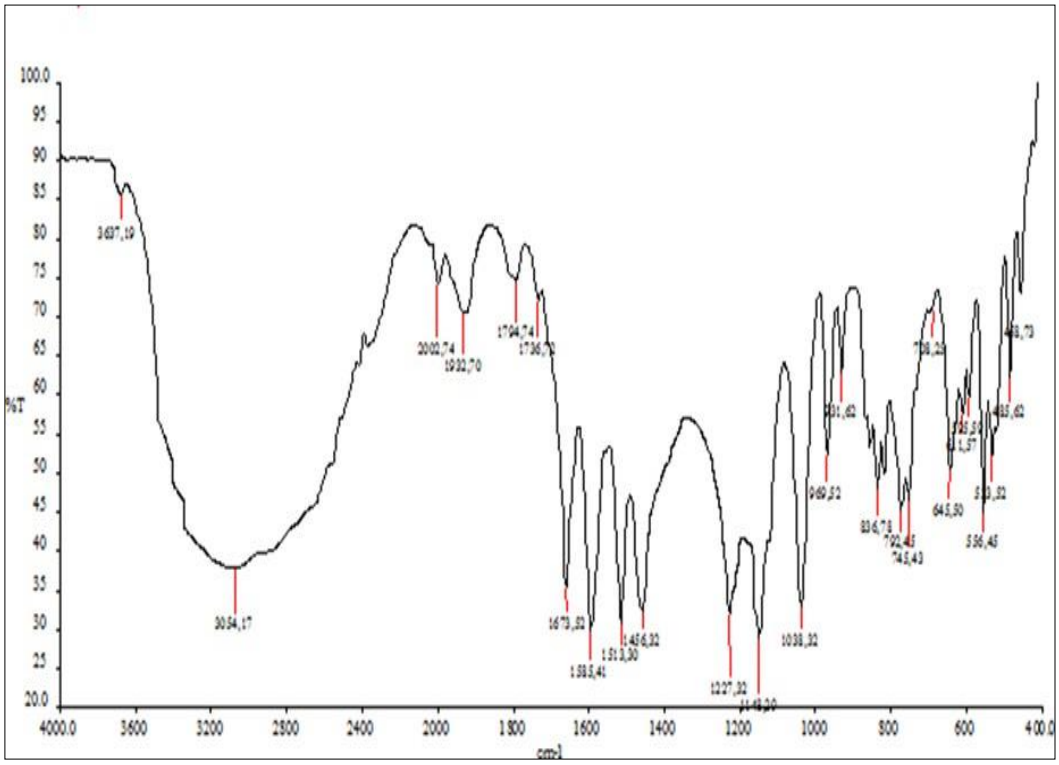


Figure 5.28: IR Spectra of 10d

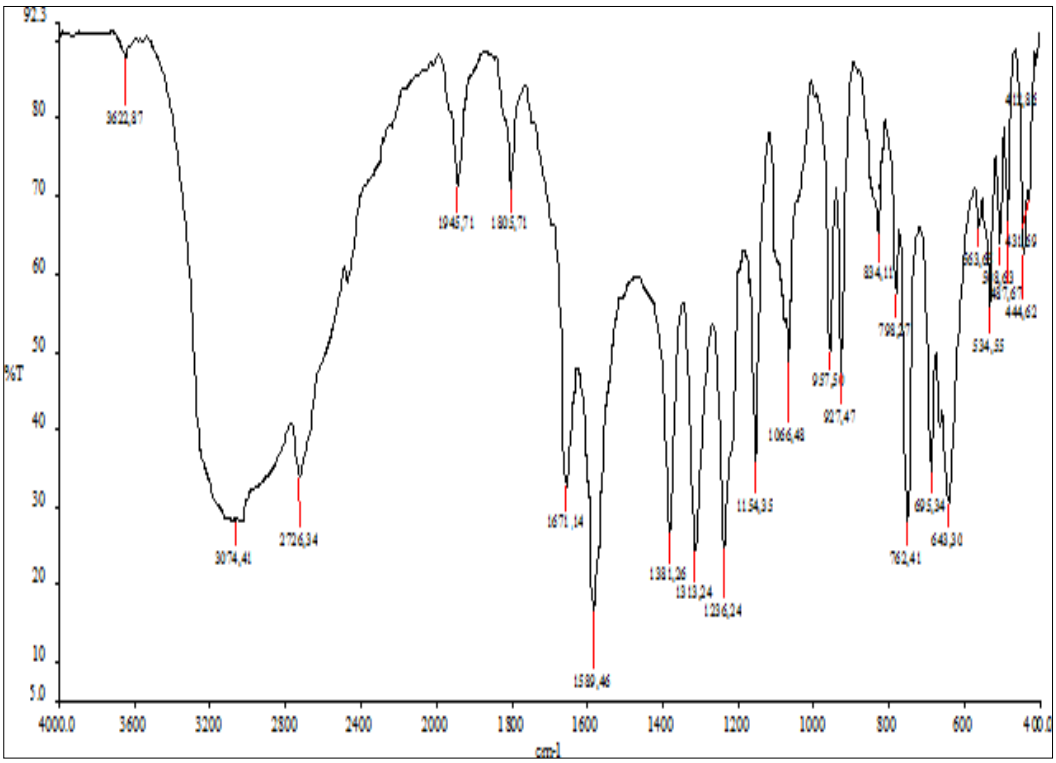


Figure 5.29: IR Spectra of 10e

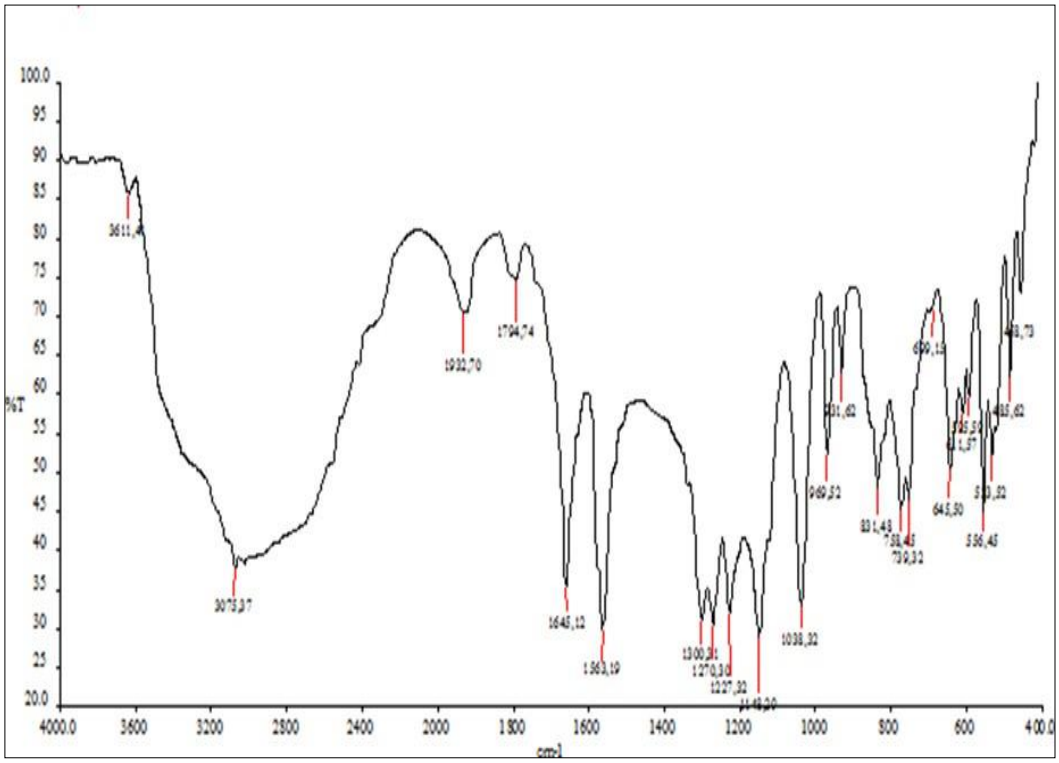


Figure 5.30: IR Spectra of 10f

5.1.2. NMR Spectra

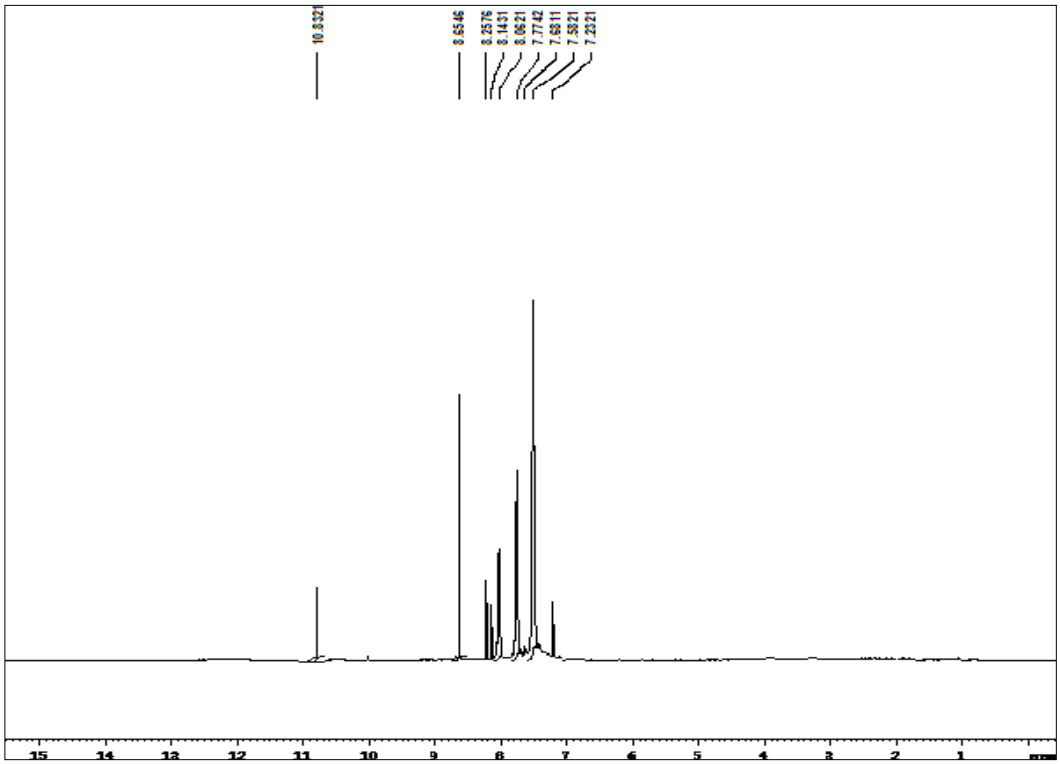


Figure 5.31: ^{1}H NMR Spectra of 6a

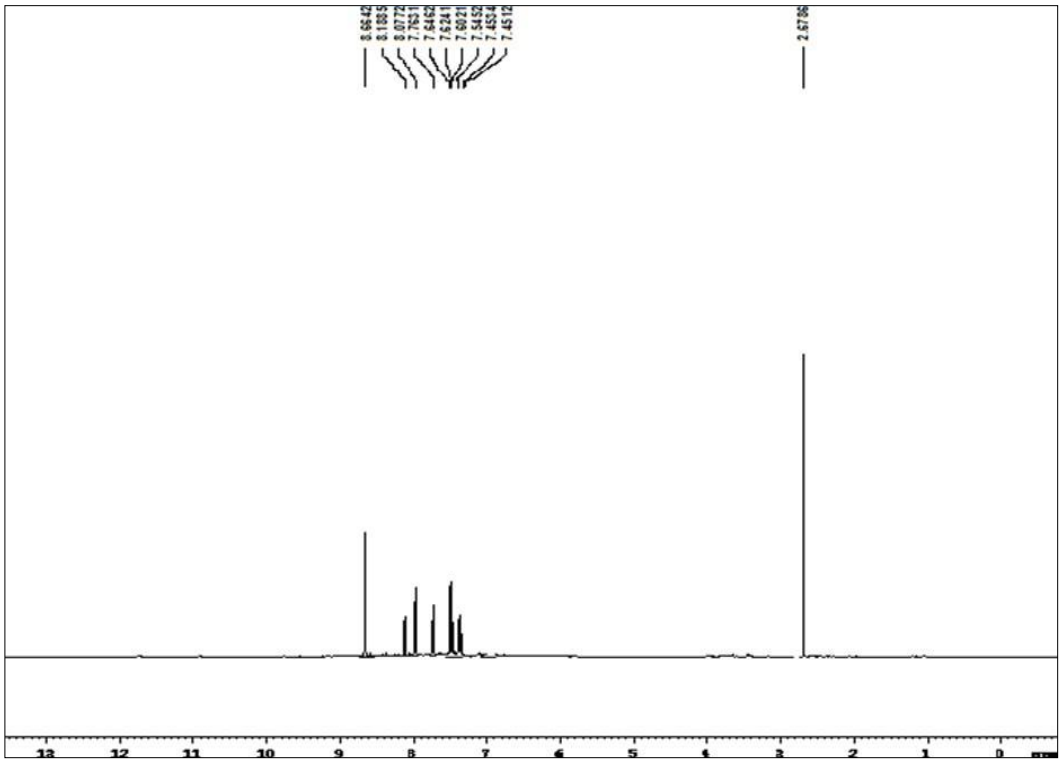


Figure 5.32: ^{1}H NMR Spectra of 6b

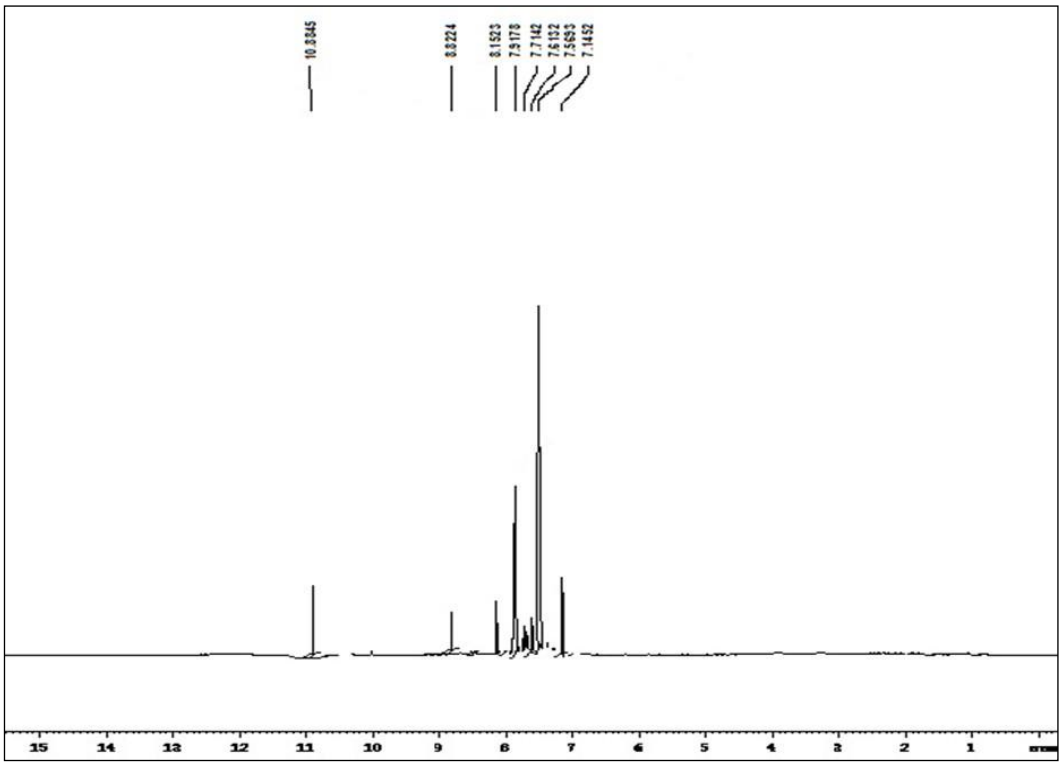


Figure 5.33: ^{1}H NMR Spectra of 6c

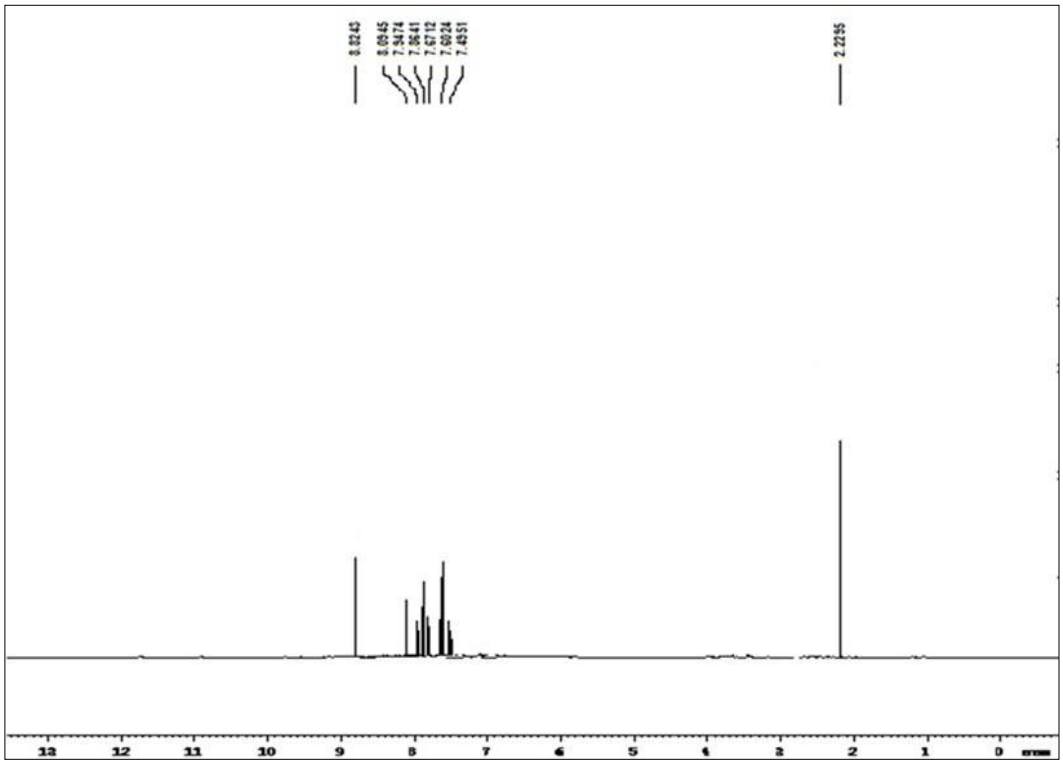


Figure 5.34: ^{1}H NMR Spectra of 6d

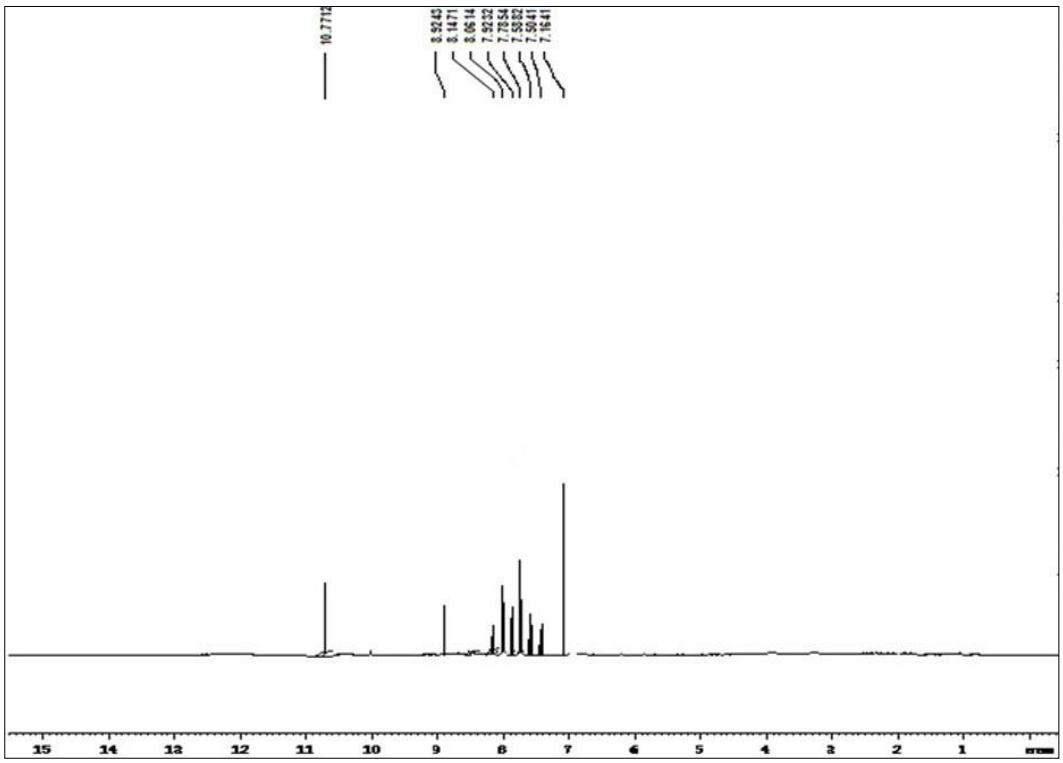


Figure 5.35: ^{1}H NMR Spectra of 6e

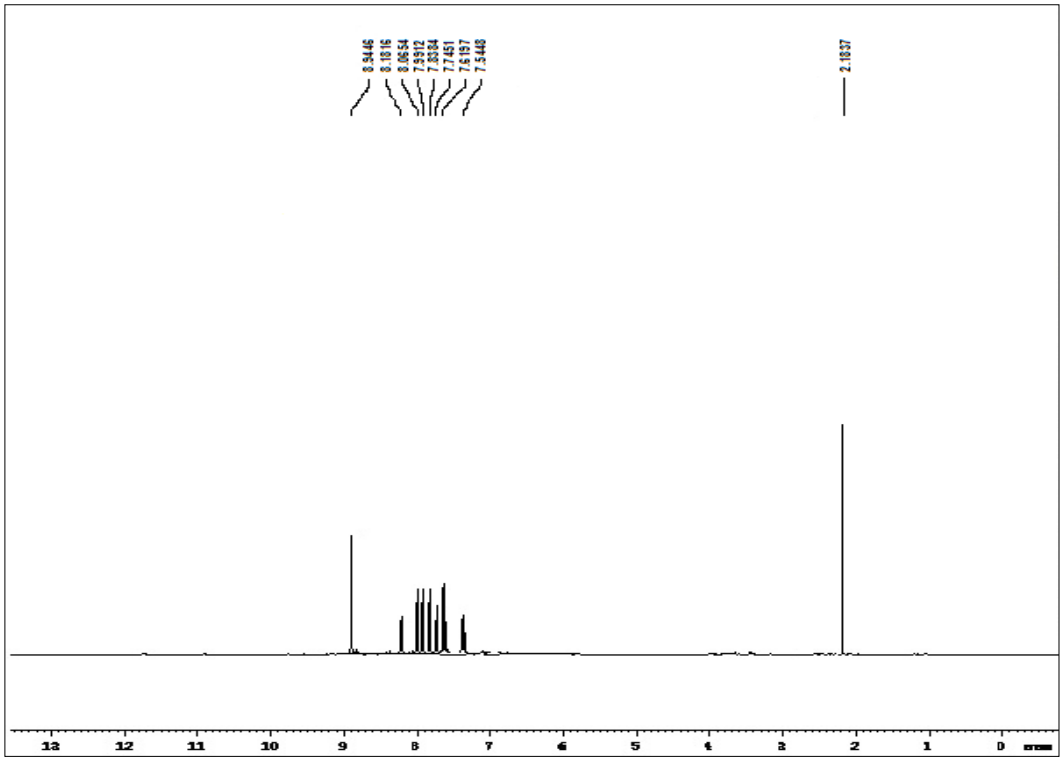


Figure 5.36: ^{1}H NMR Spectra of 6f

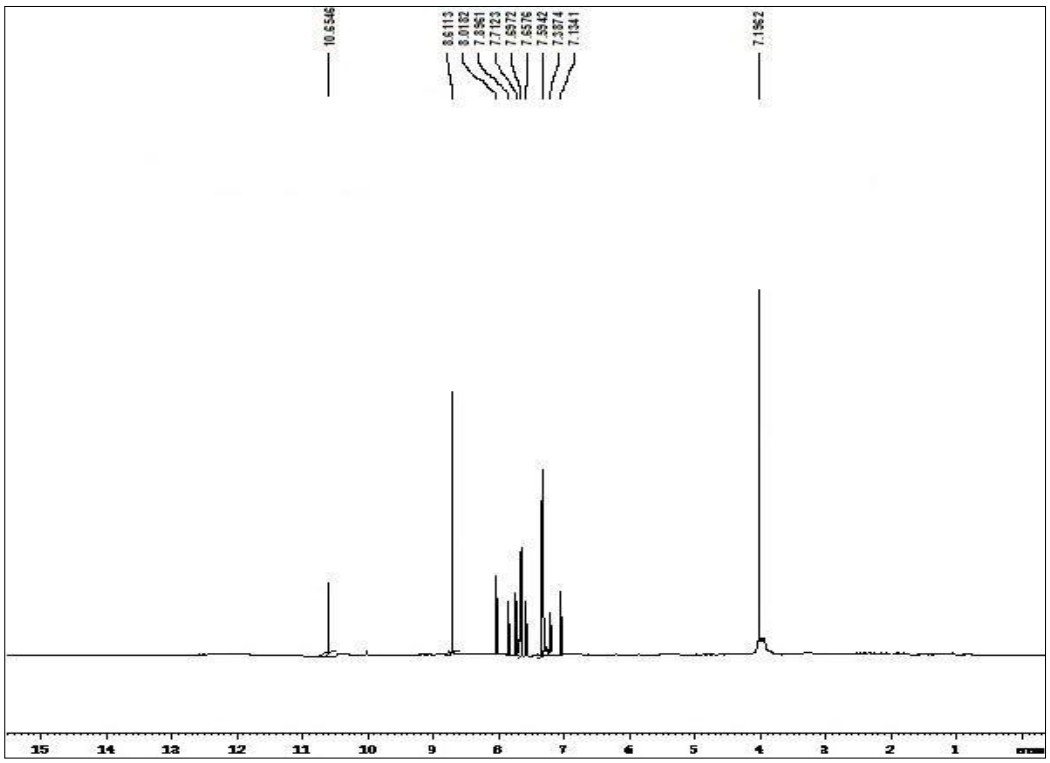


Figure 5.37: ^{1}H NMR Spectra of 7a

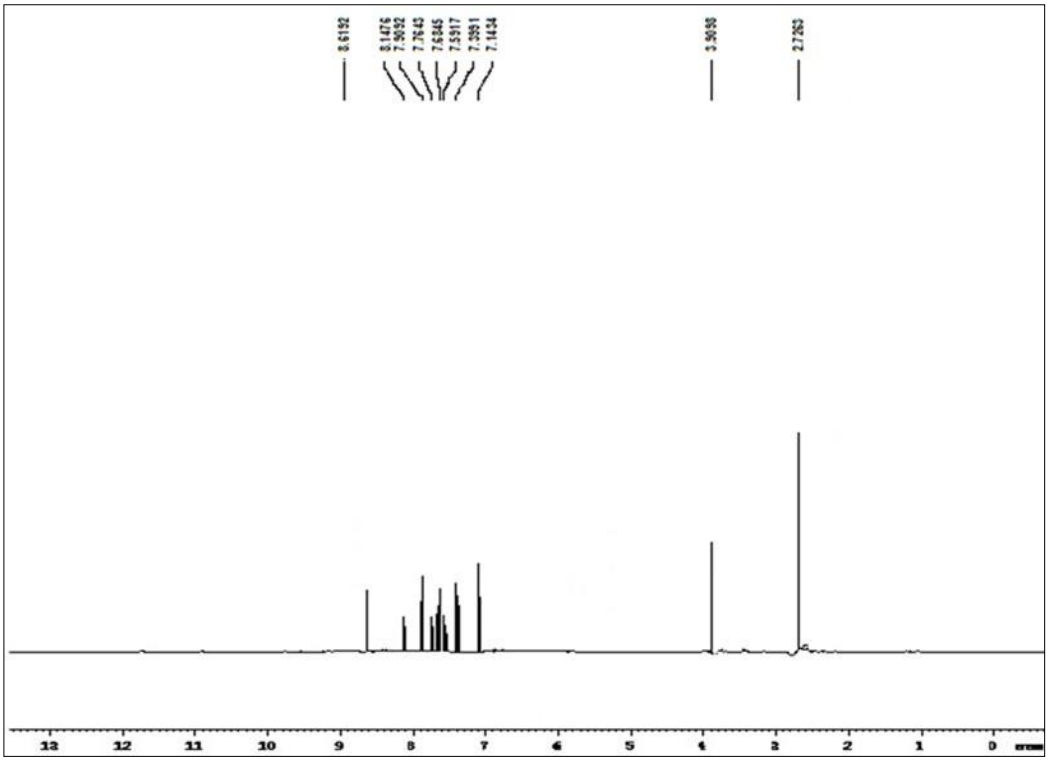


Figure 5.38: ^{1}H NMR Spectra of 7b

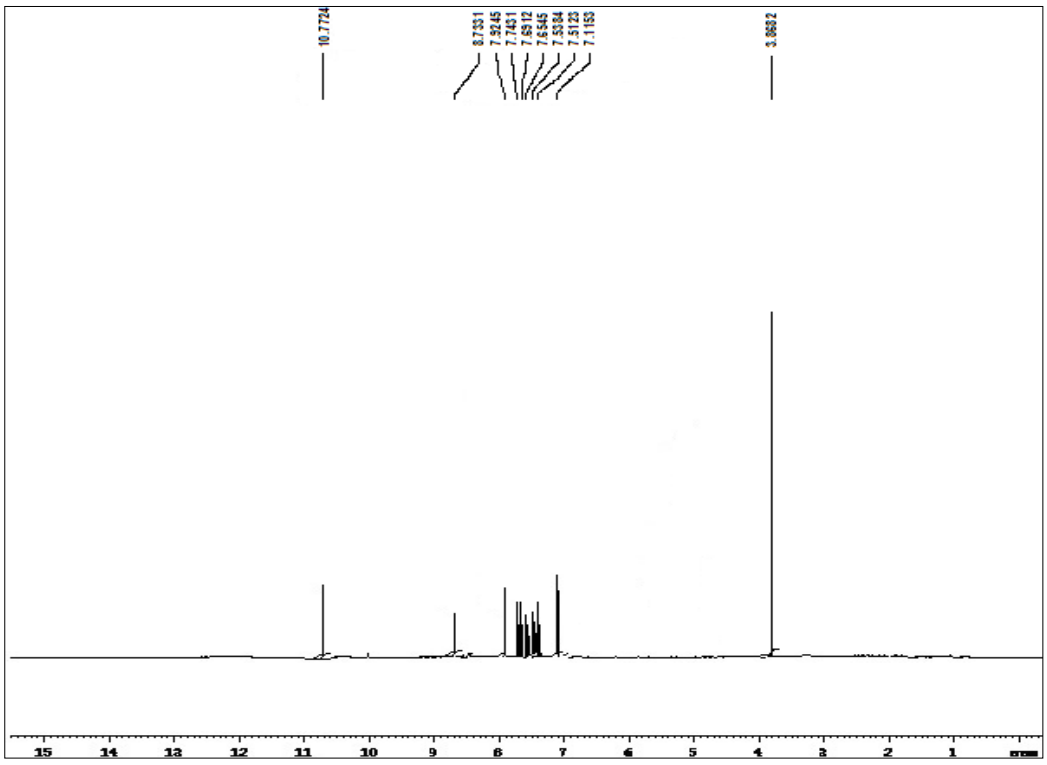


Figure 5.39: ^{1}H NMR Spectra of 7c

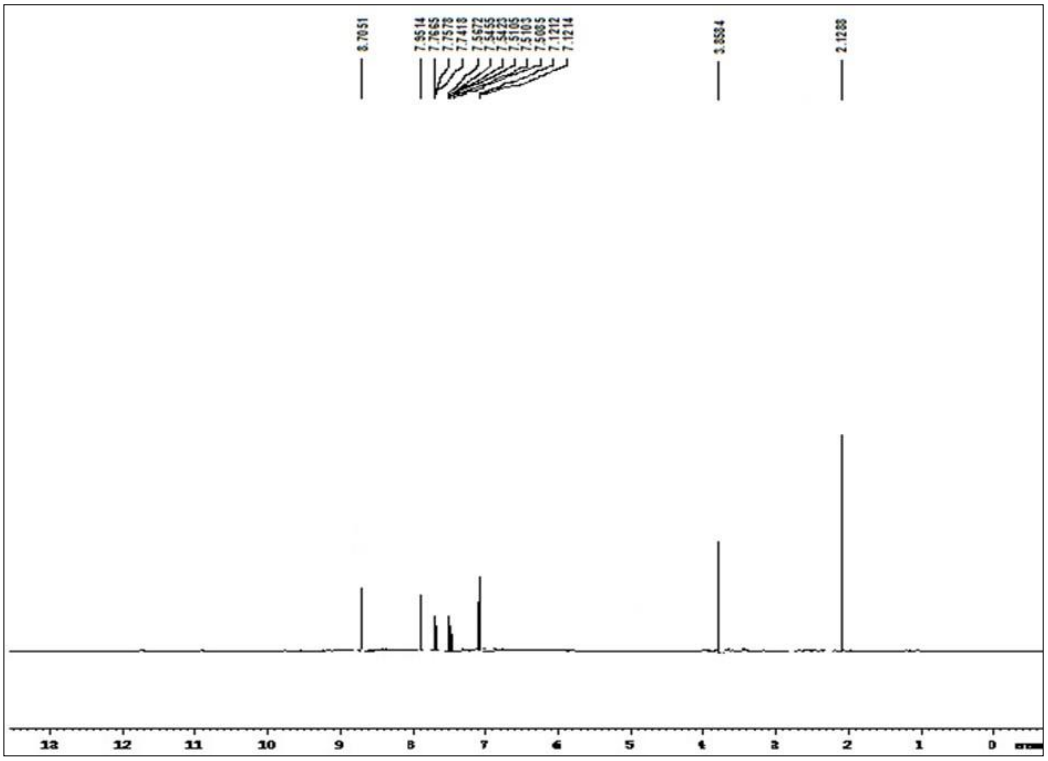


Figure 5.40: ^{1}H NMR Spectra of 7d

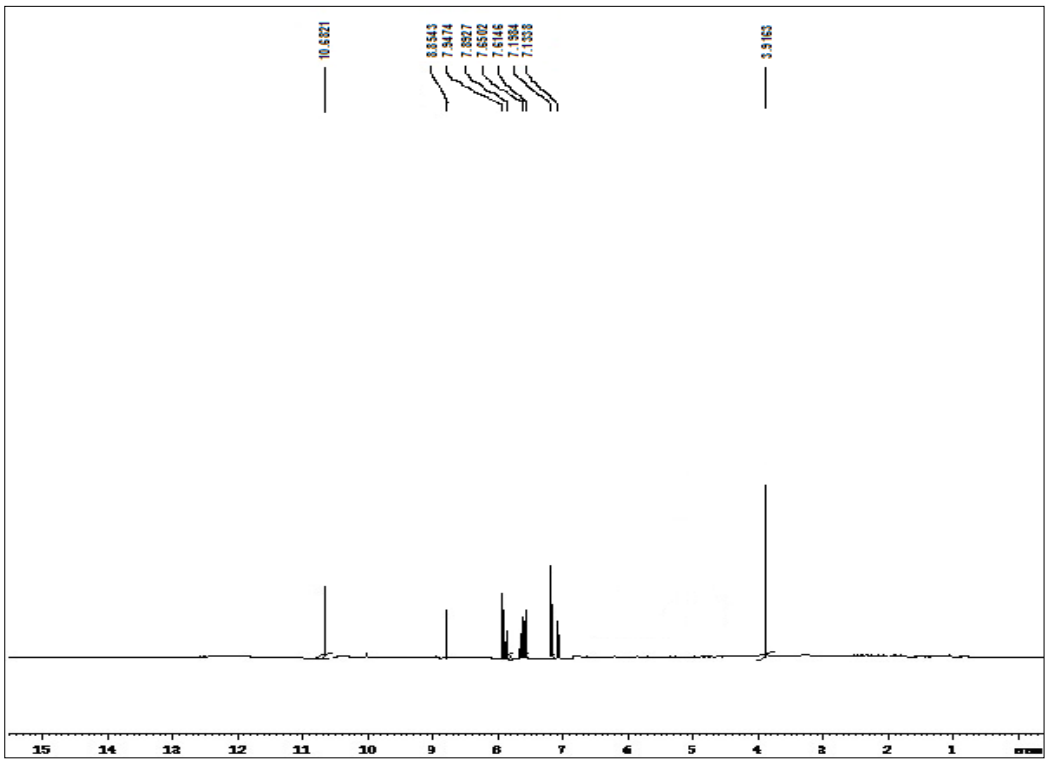


Figure 5.41: ^{1}H NMR Spectra of 7e

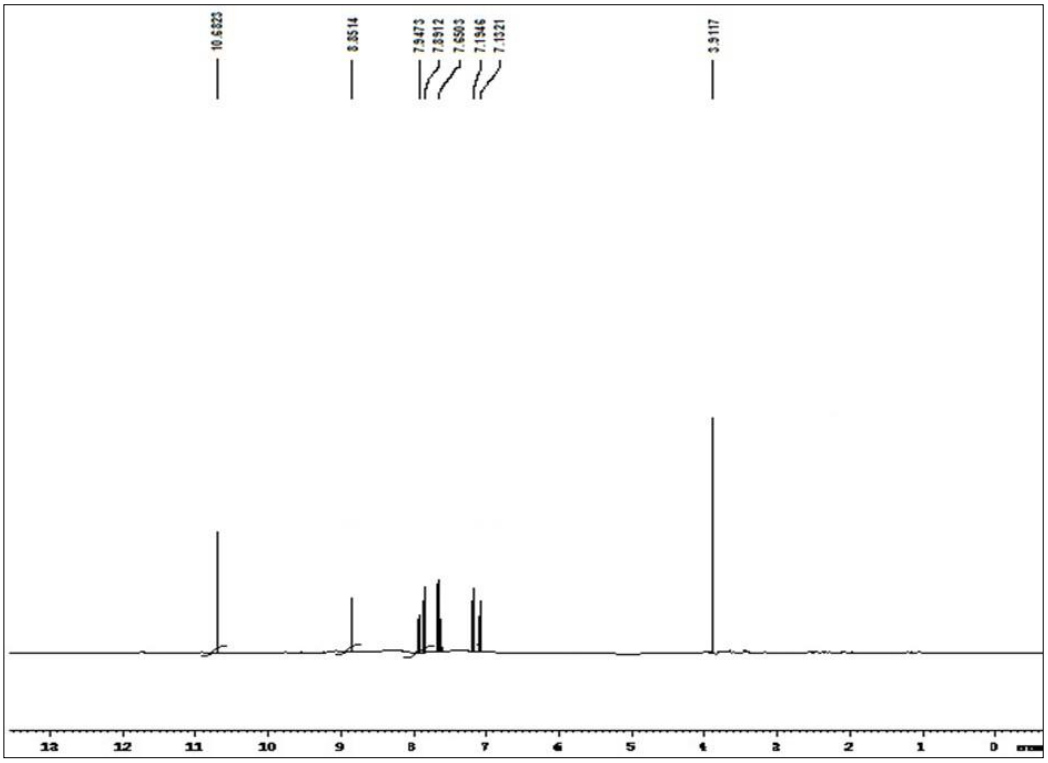


Figure 5.42: ^{1}H NMR Spectra of 7f

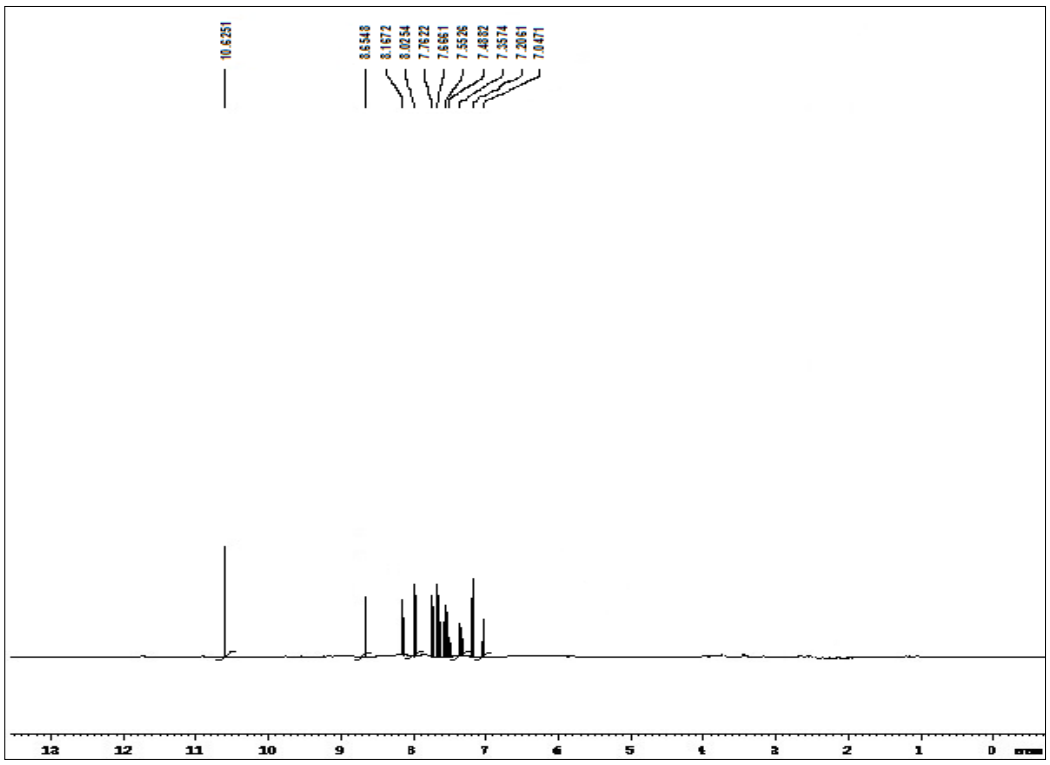


Figure 5.43: ^{1}H NMR Spectra of 8a

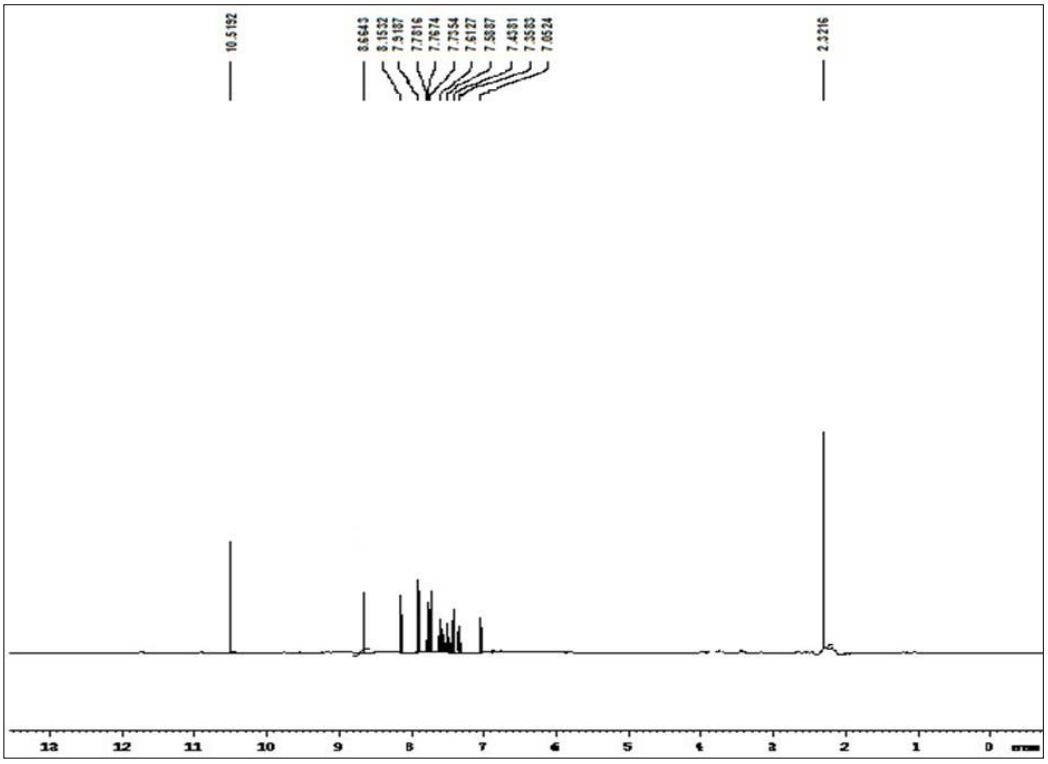


Figure 5.44: ^{1}H NMR Spectra of 8b

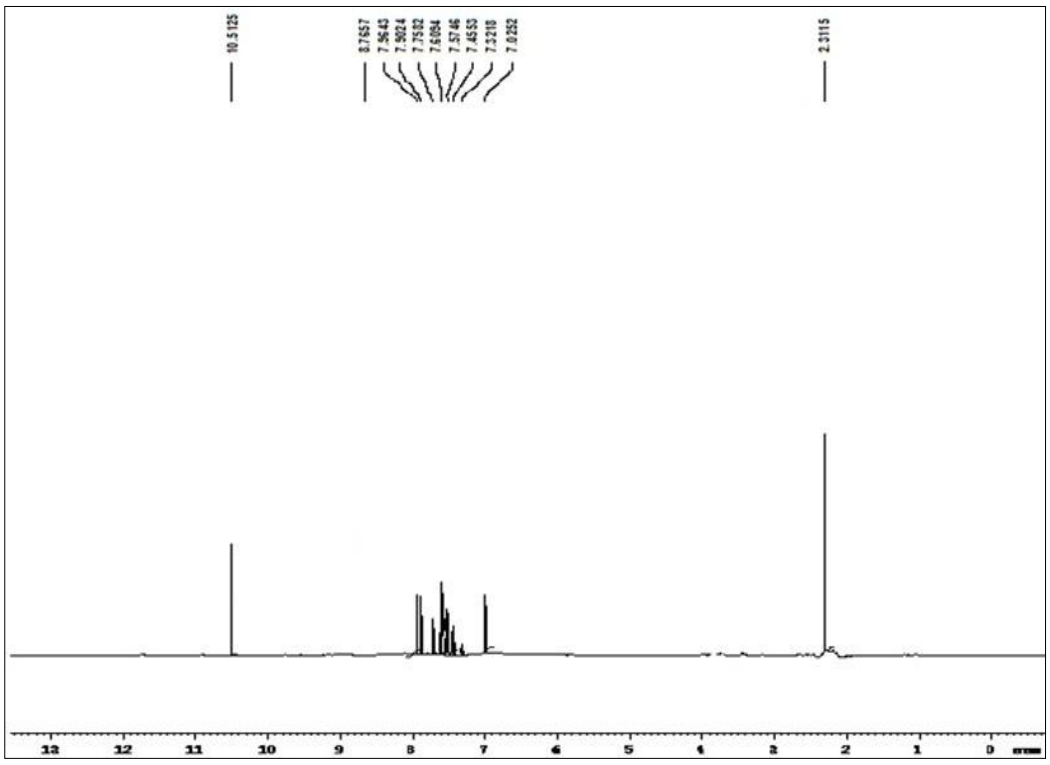


Figure 5.45: ^{1}H NMR Spectra of 8c

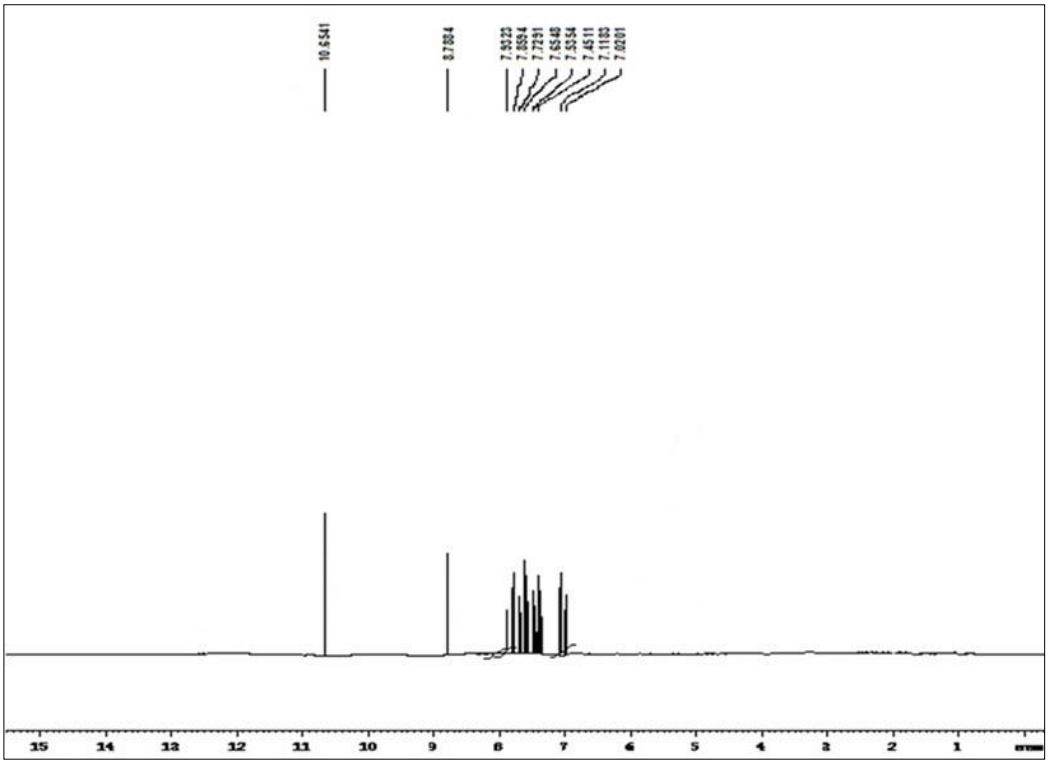


Figure 5.46: ^{1}H NMR Spectra of 8d

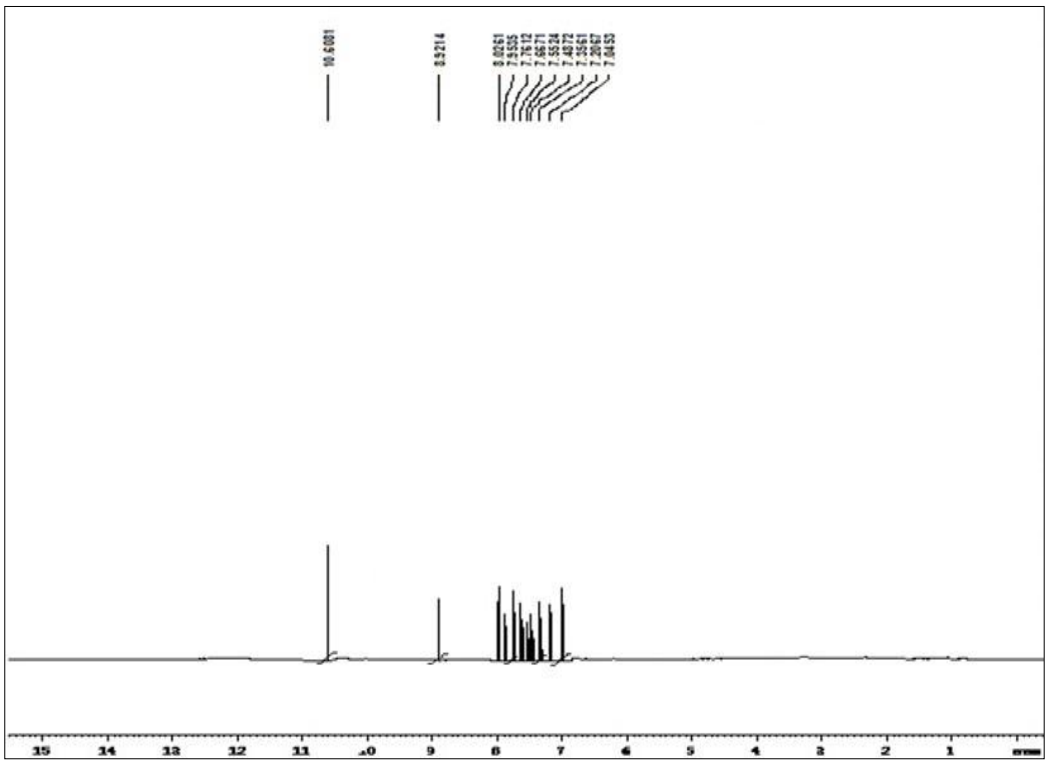


Figure 5.47: ^{1}H NMR Spectra of 8e

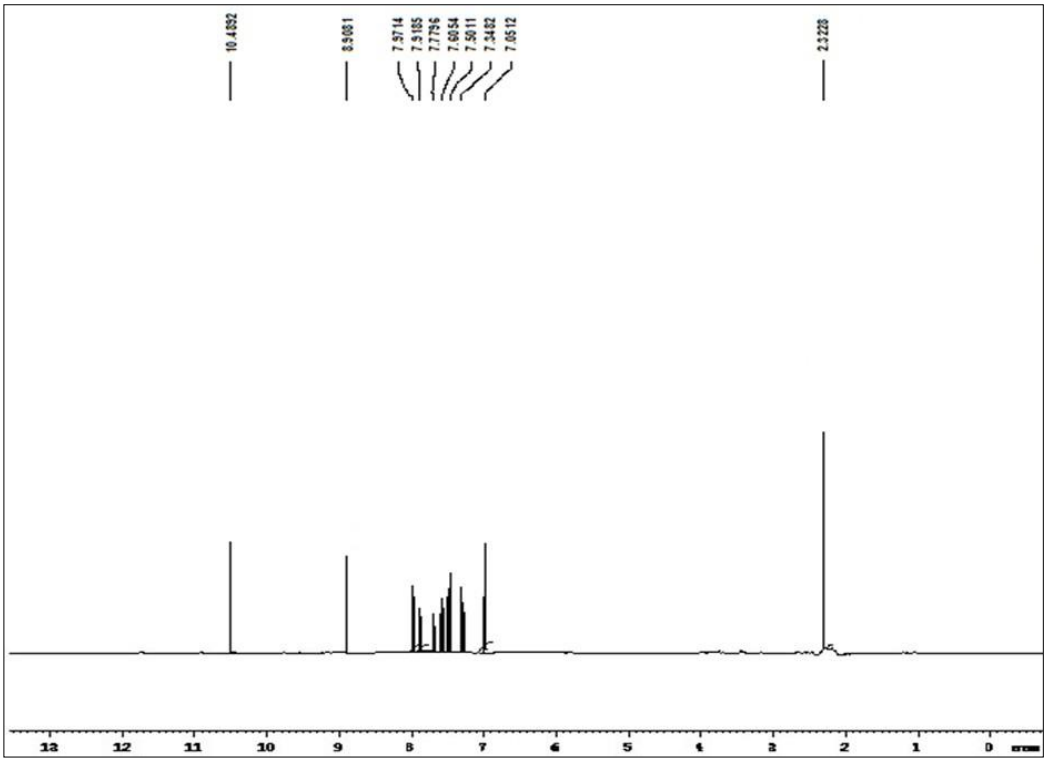


Figure 5.48: ^{1}H NMR Spectra of 8f

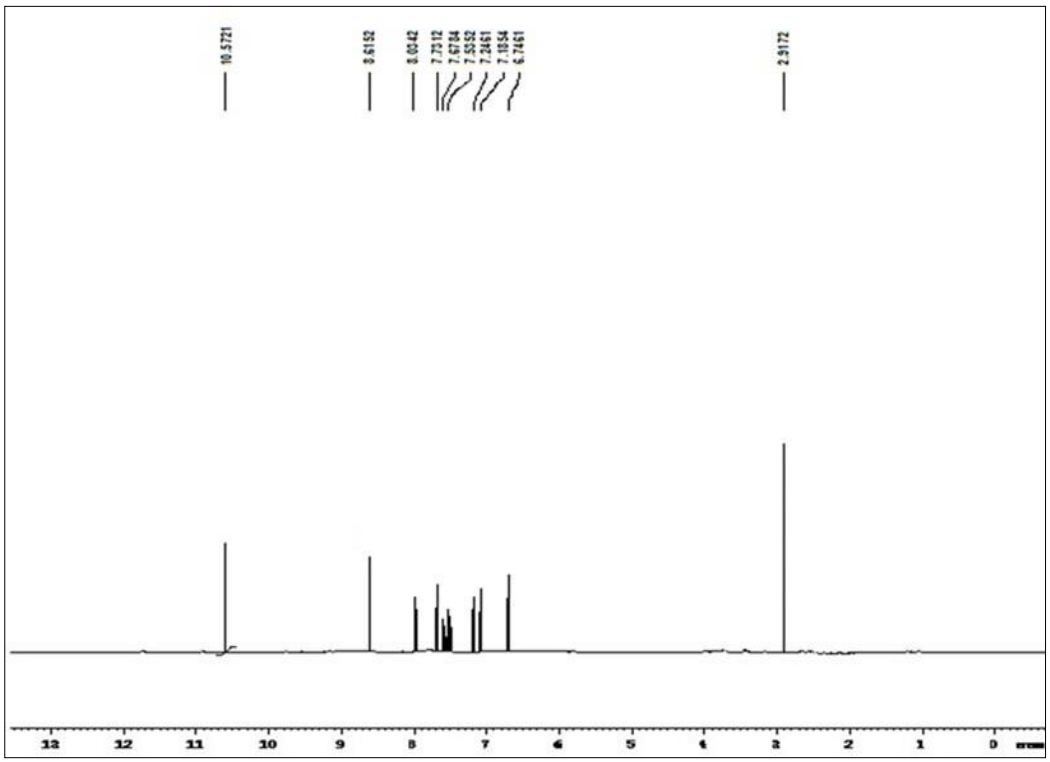


Figure 5.49: ^{1}H NMR Spectra of 9a

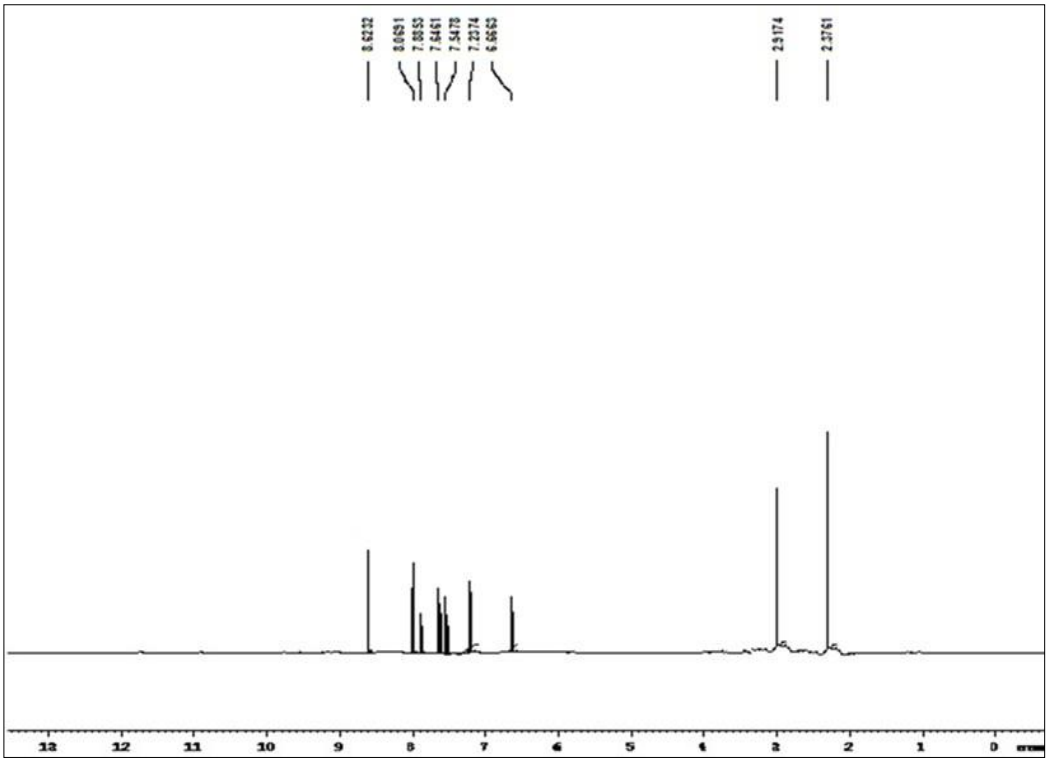


Figure 5.50: ^{1}H NMR Spectra of 9b

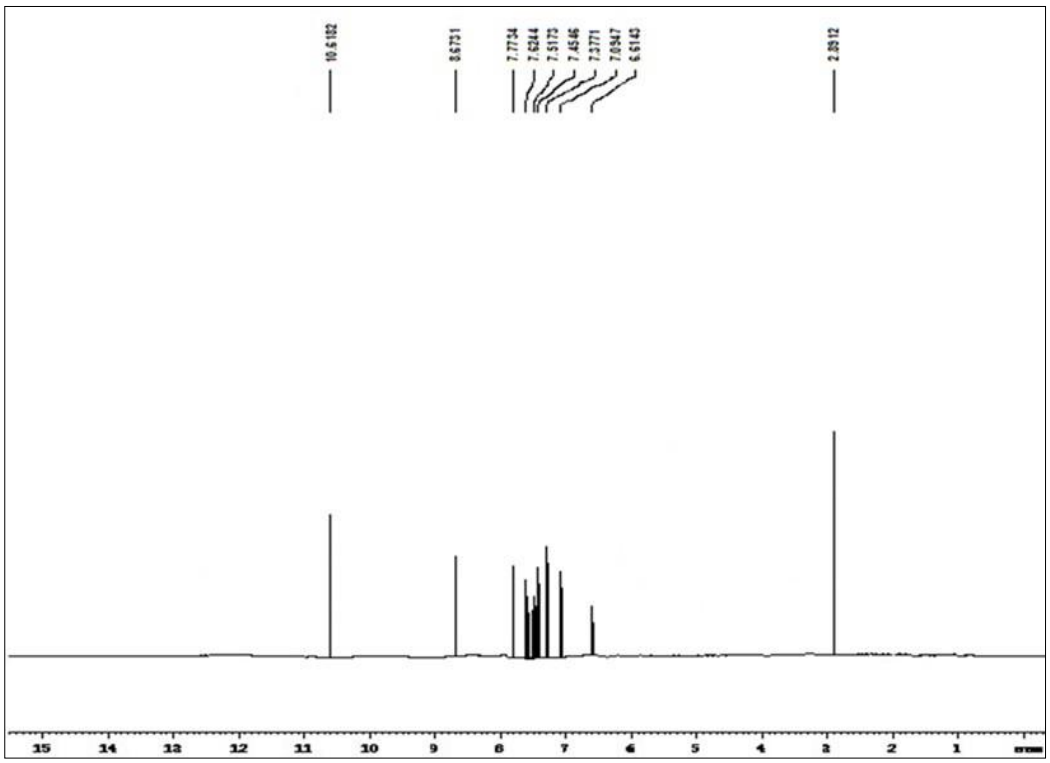


Figure 5.51: ^{1}H NMR Spectra of 9c

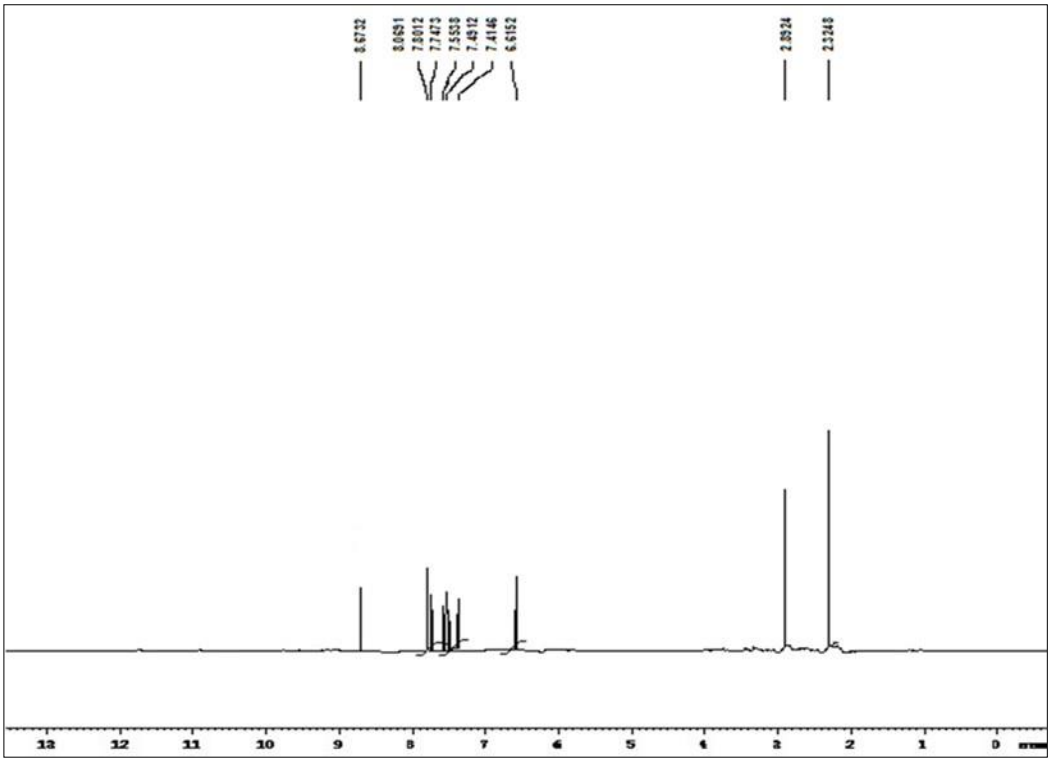


Figure 5.52: ^{1}H NMR Spectra of 9d

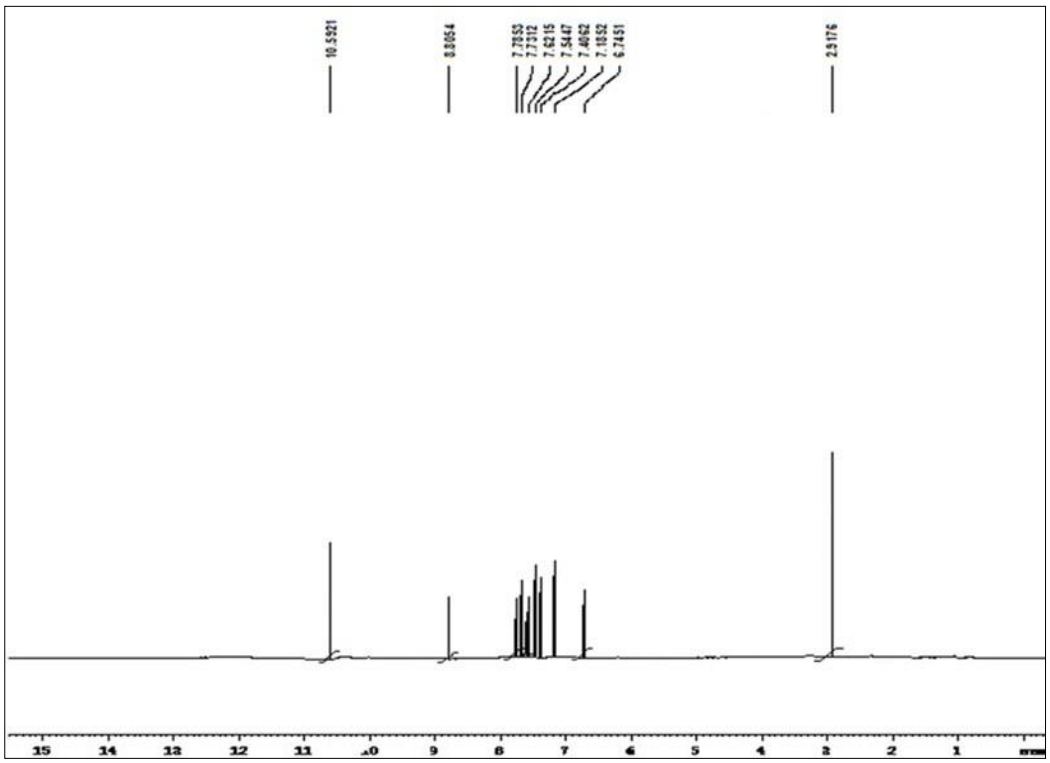


Figure 5.53: ^{1}H NMR Spectra of 9e

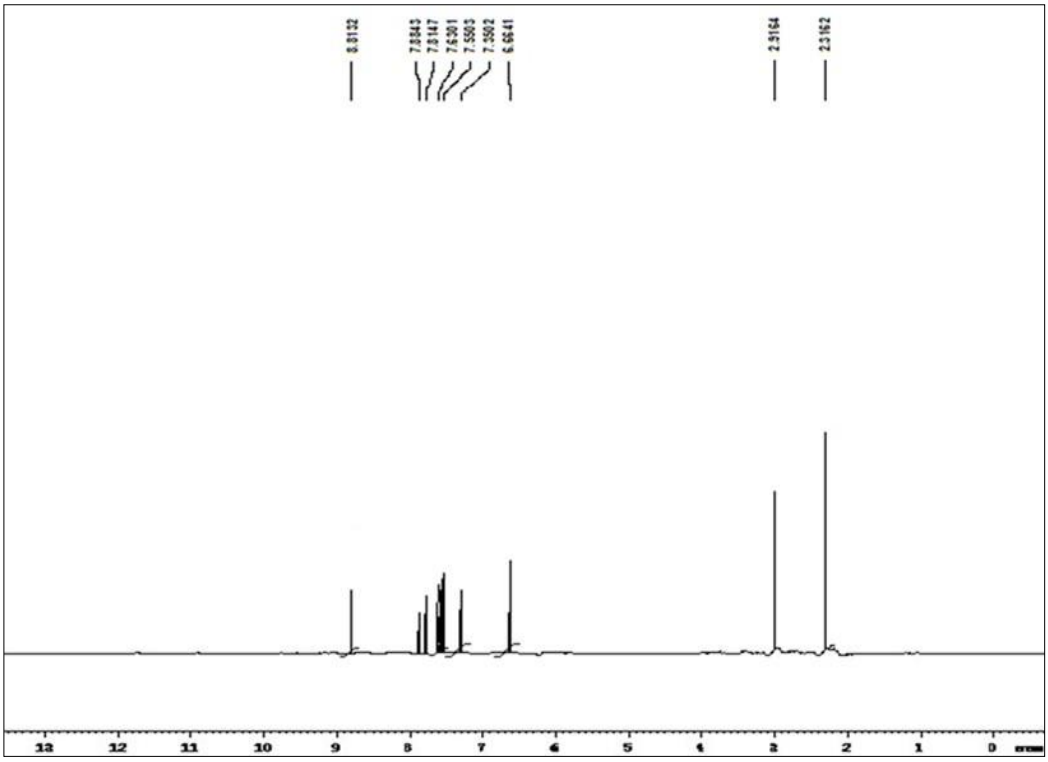


Figure 5.54: ^{1}H NMR Spectra of 9f

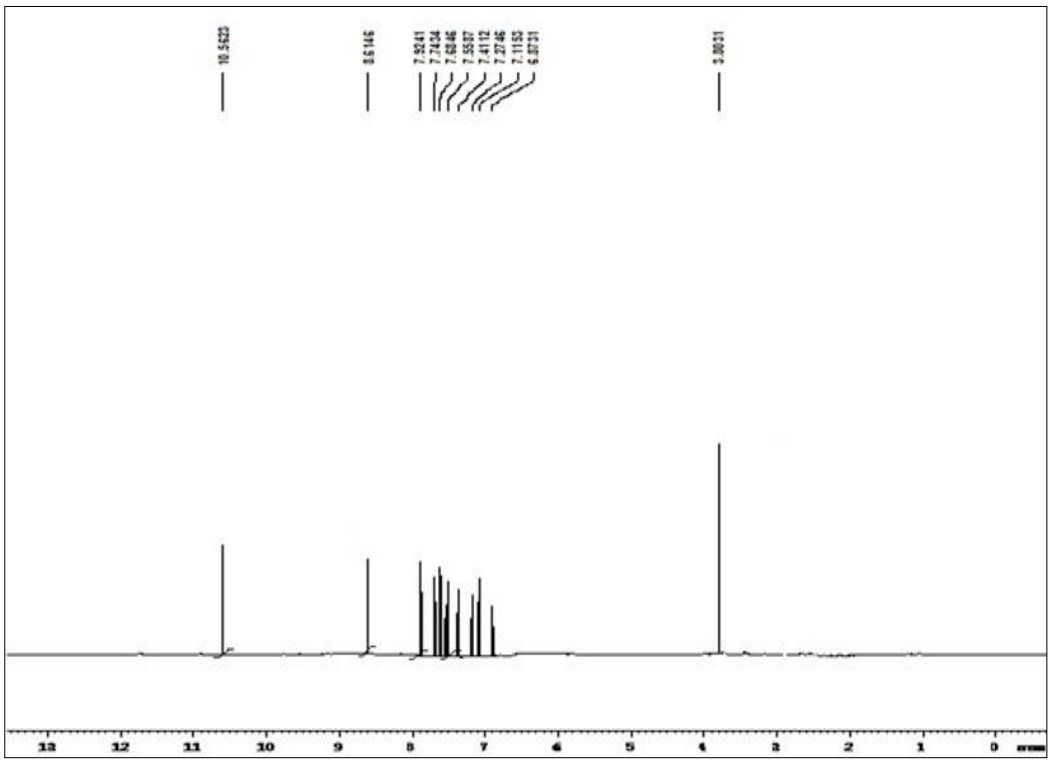


Figure 5.55: ^{1}H NMR Spectra of 10a

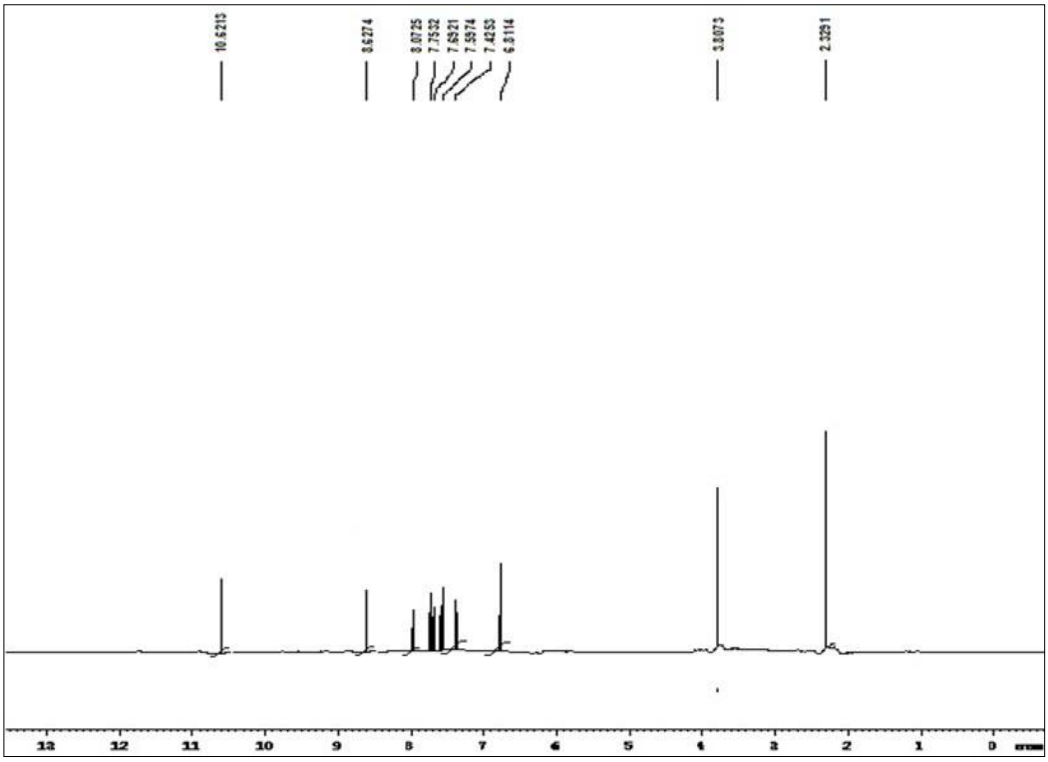


Figure 5.56: ^{1}H NMR Spectra of 10b

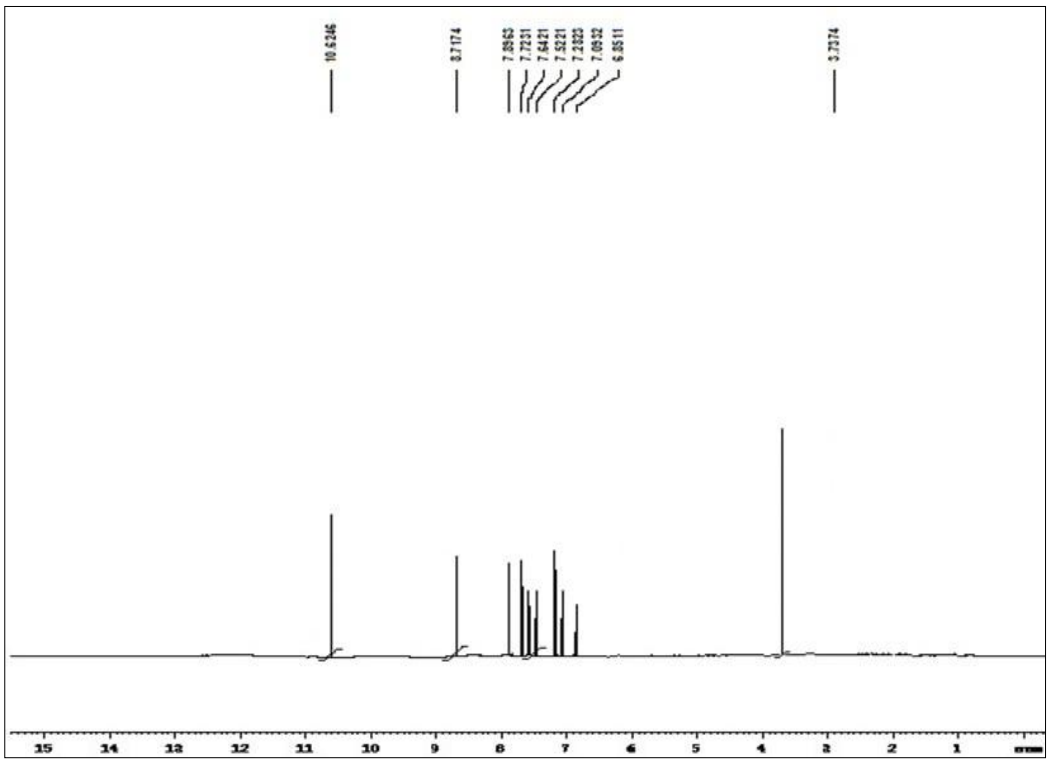


Figure 5.57: ^{1}H NMR Spectra of 10e

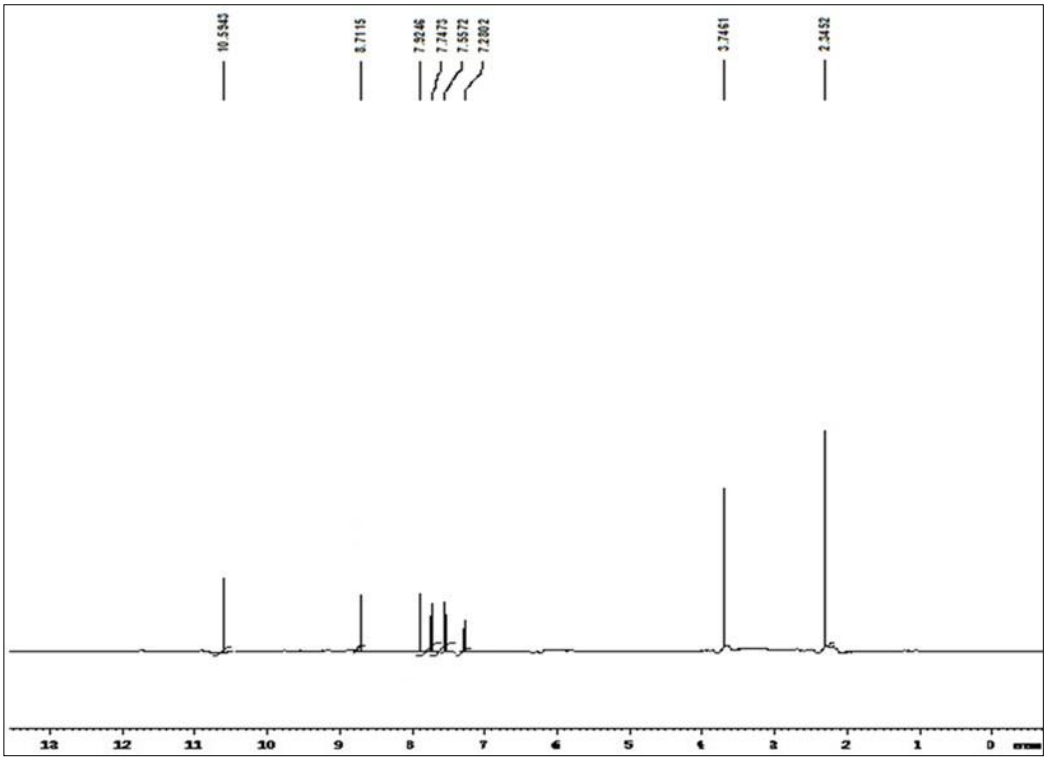


Figure 5.58: ^{1}H NMR Spectra of 10d

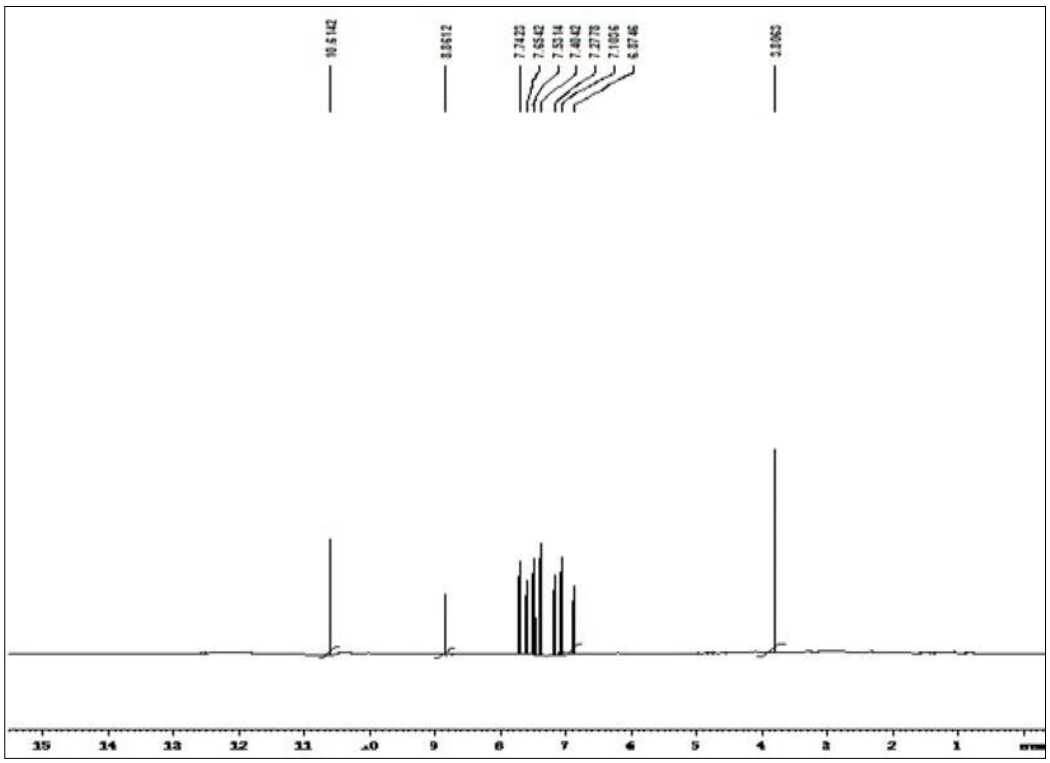


Figure 5.59: ^{1}H NMR Spectra of 10e

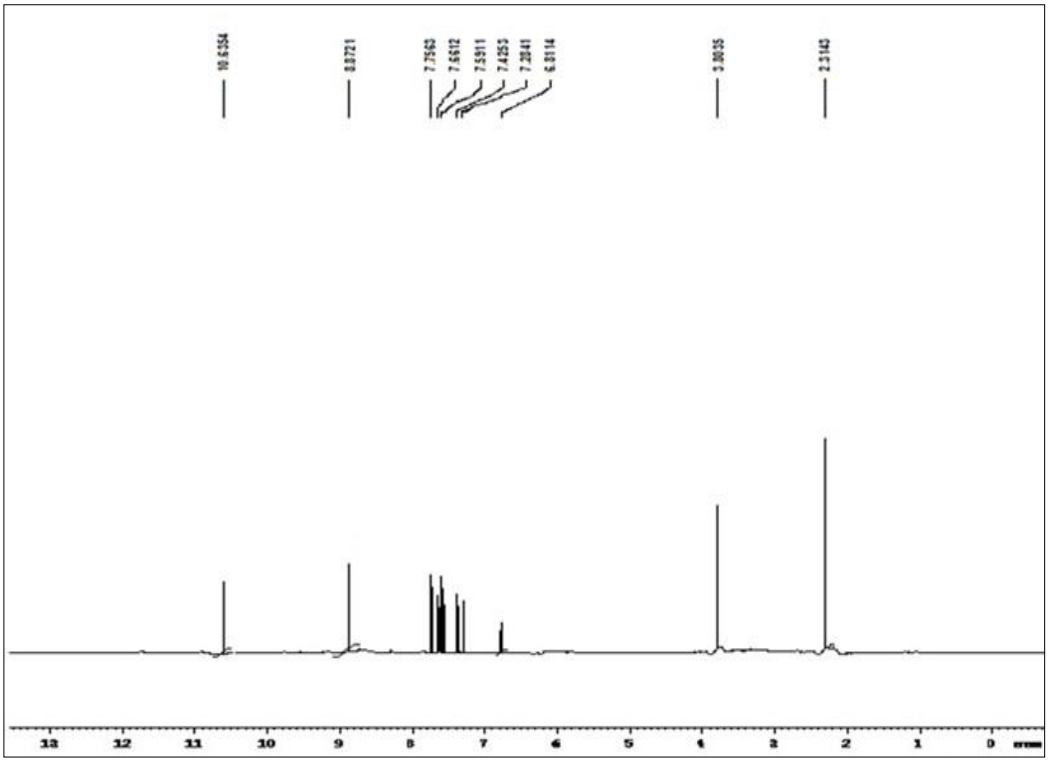


Figure 5.60: ^{1}H NMR Spectra of 10f

5.1.3. Mass Spectrometry

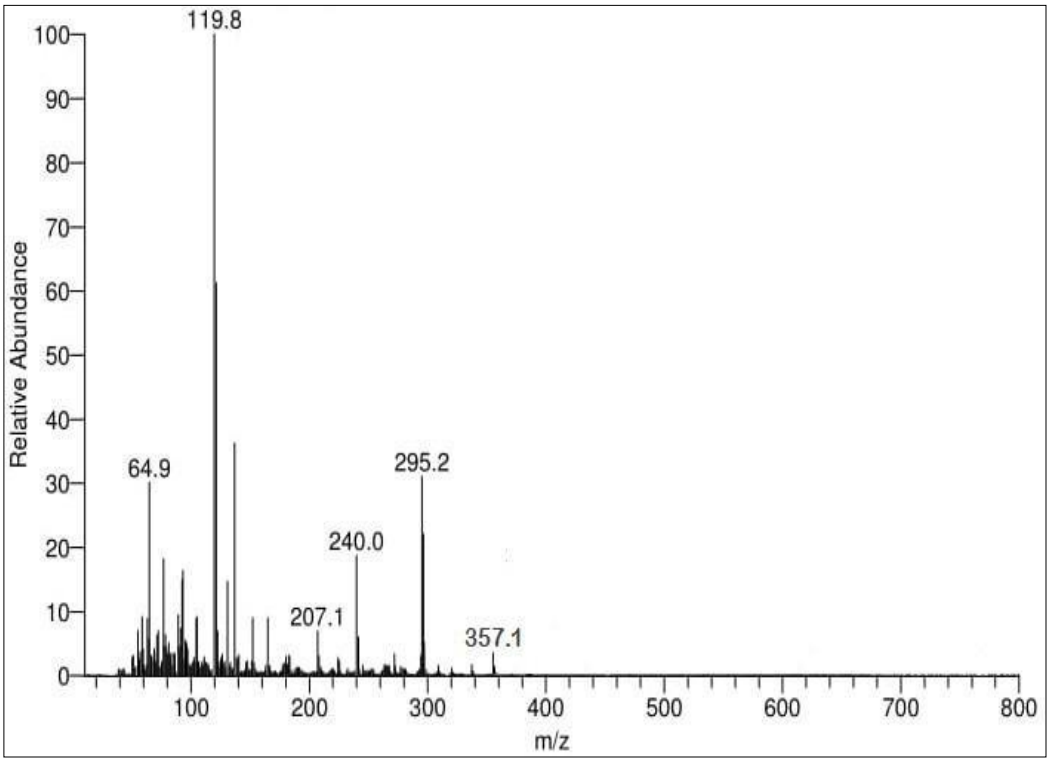


Figure 5.61: Mass Spectra of 6a

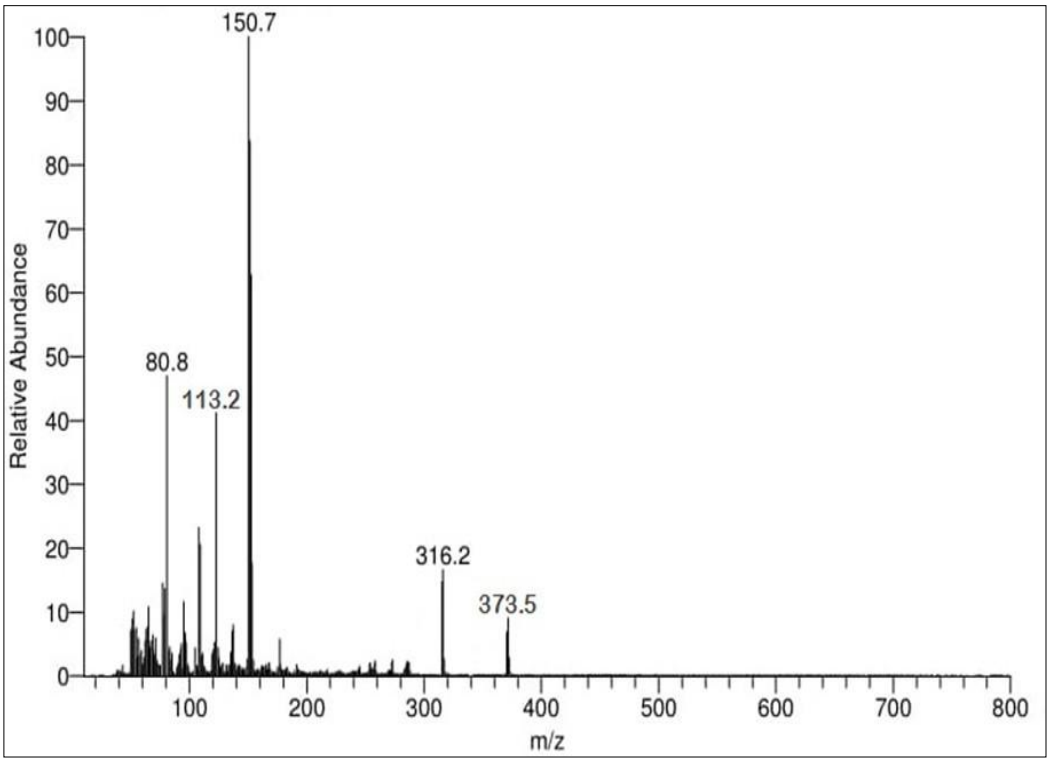


Figure 5.62: Mass Spectra of 6b

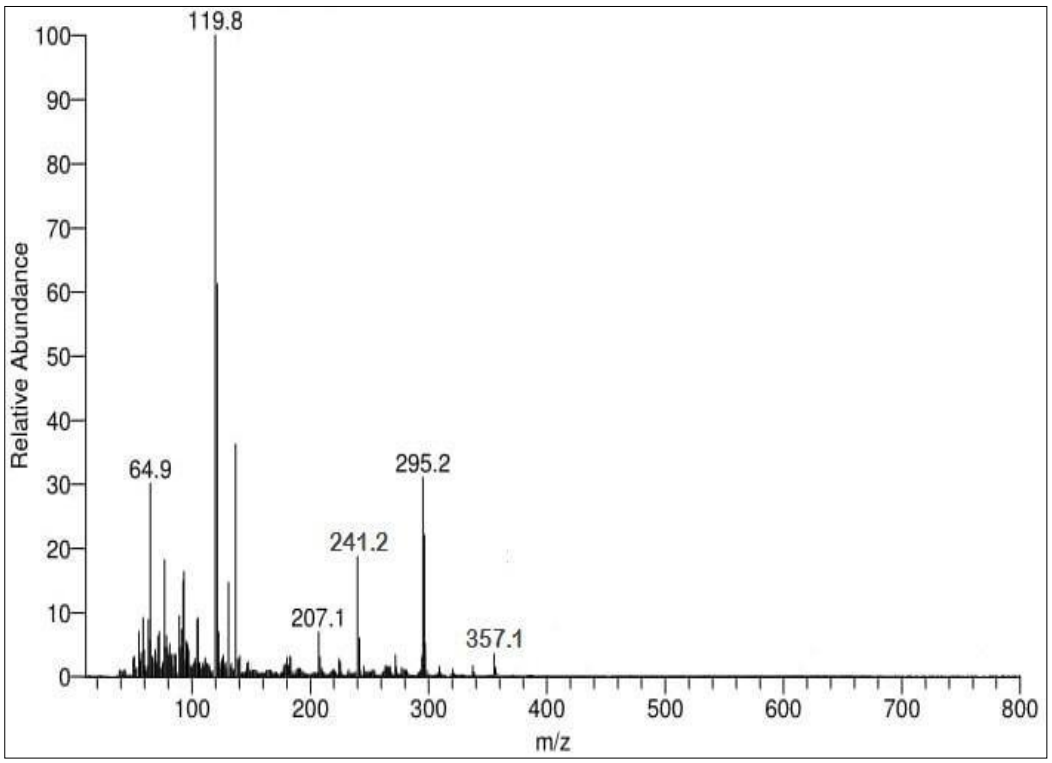


Figure 5.63: Mass Spectra of 6c

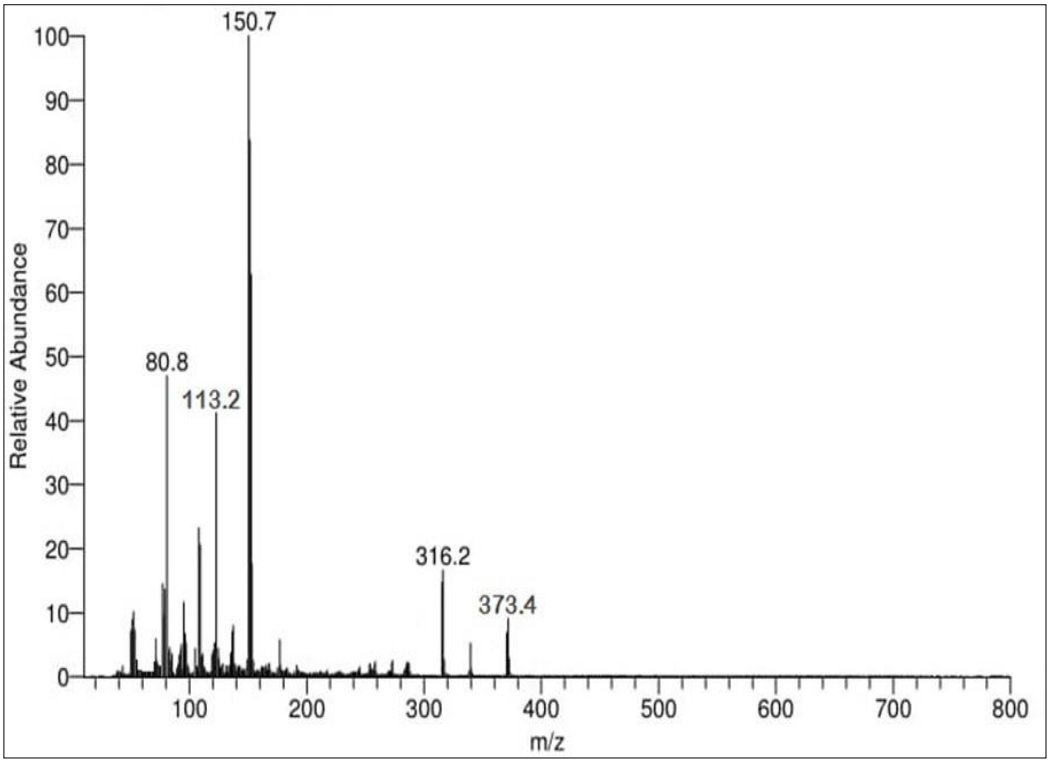


Figure 5.64: Mass Spectra of 6d

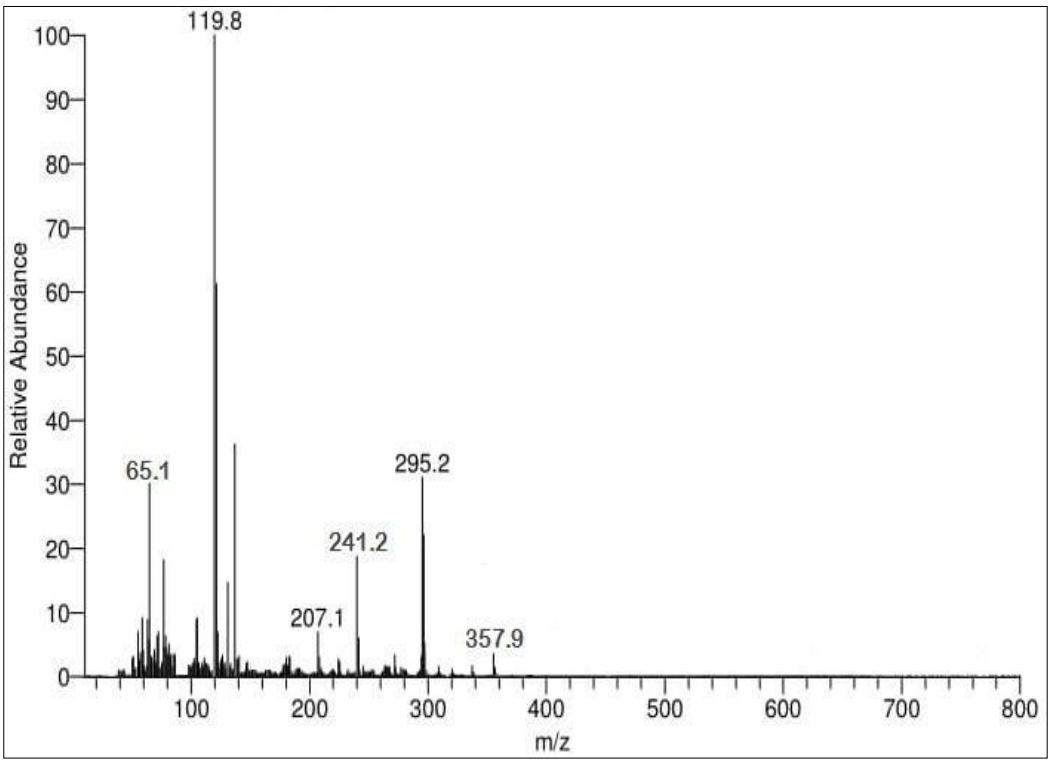


Figure 5.65: Mass Spectra of 6e

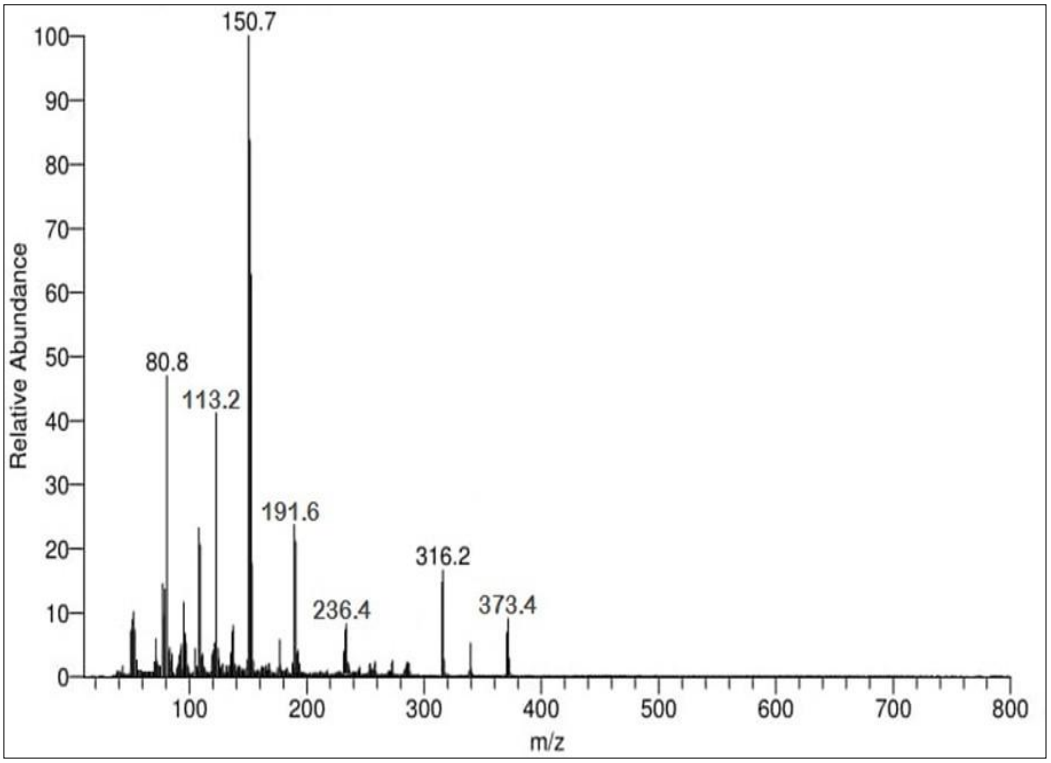


Figure 5.66: Mass Spectra of 6f

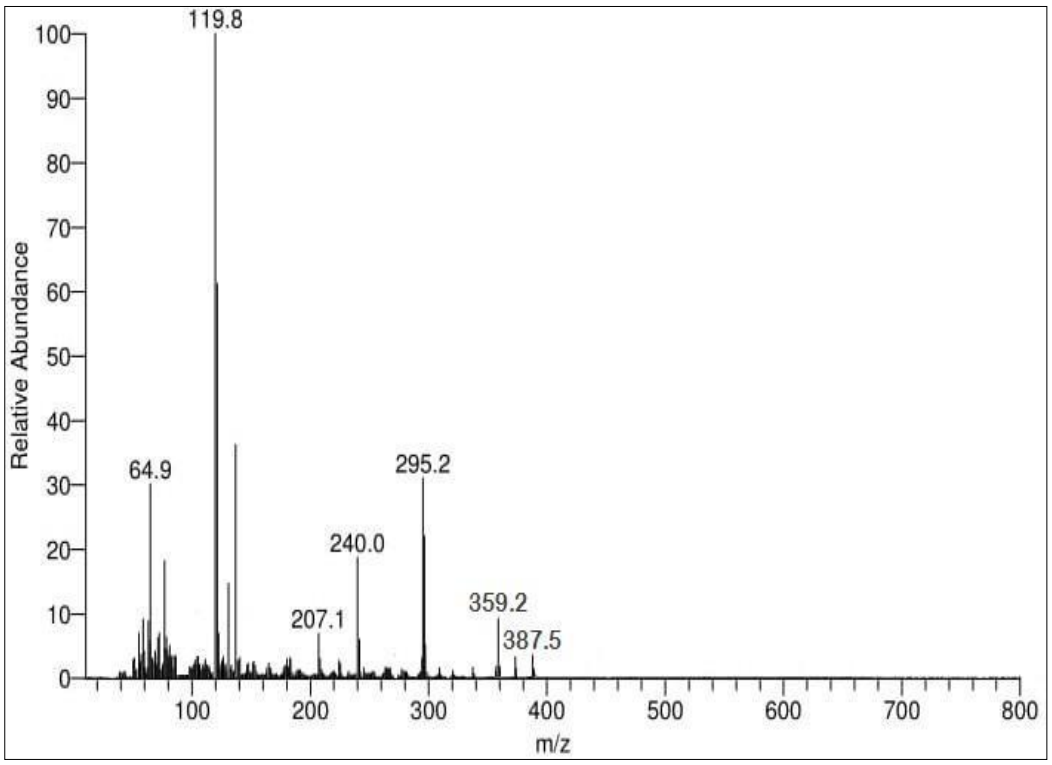


Figure 5.67: Mass Spectra of 7a

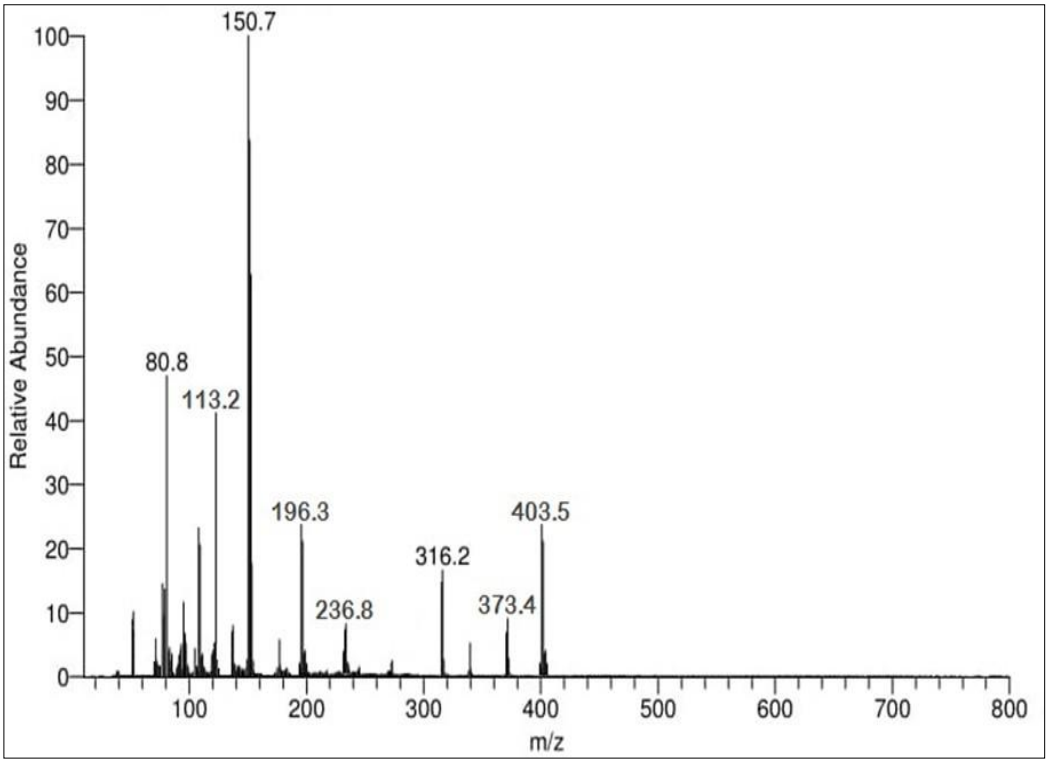


Figure 5.68: Mass Spectra of 7b

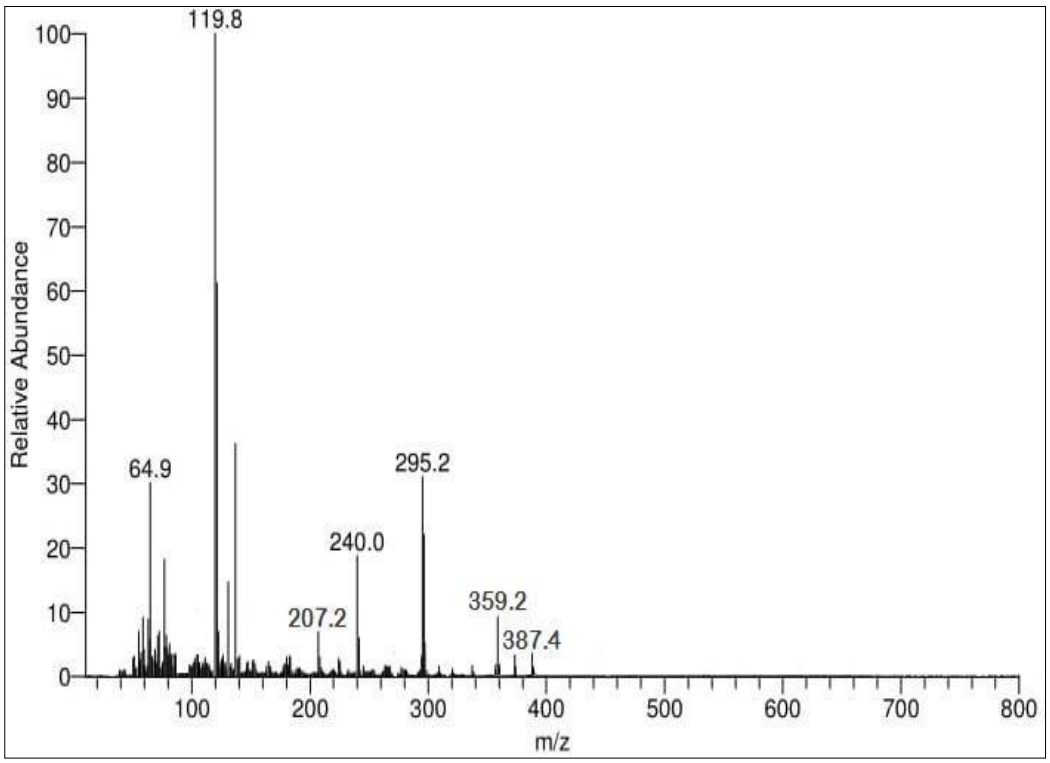


Figure 5.69: Mass Spectra of 7c

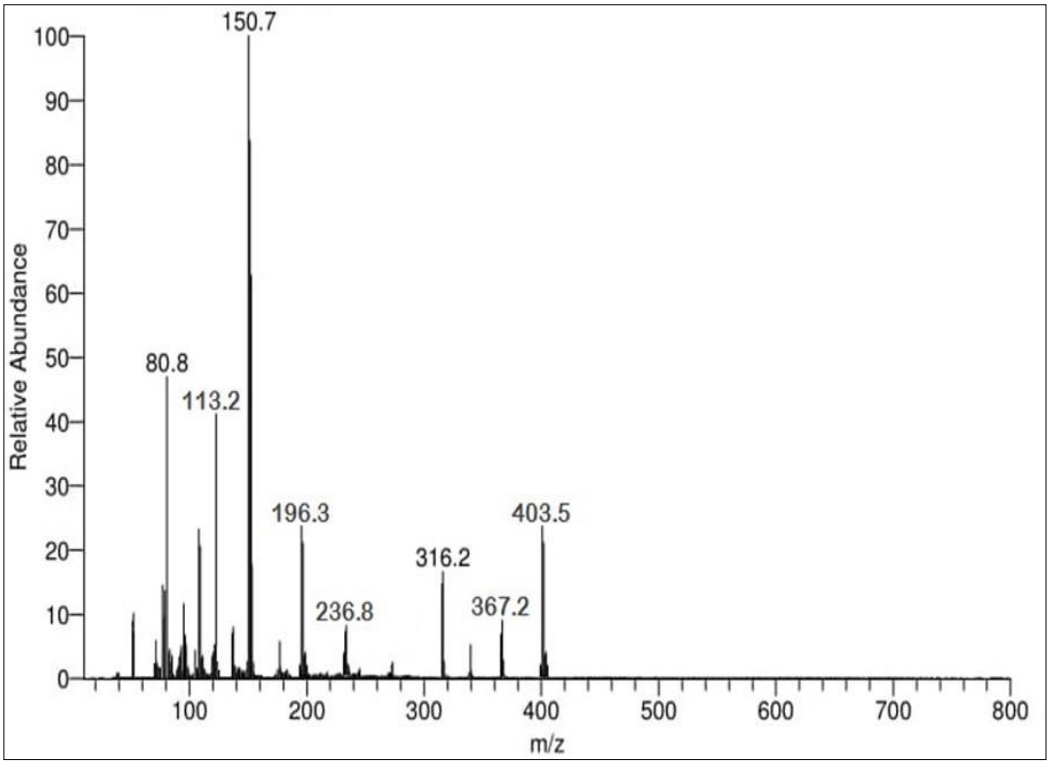


Figure 5.70: Mass Spectra of 7d

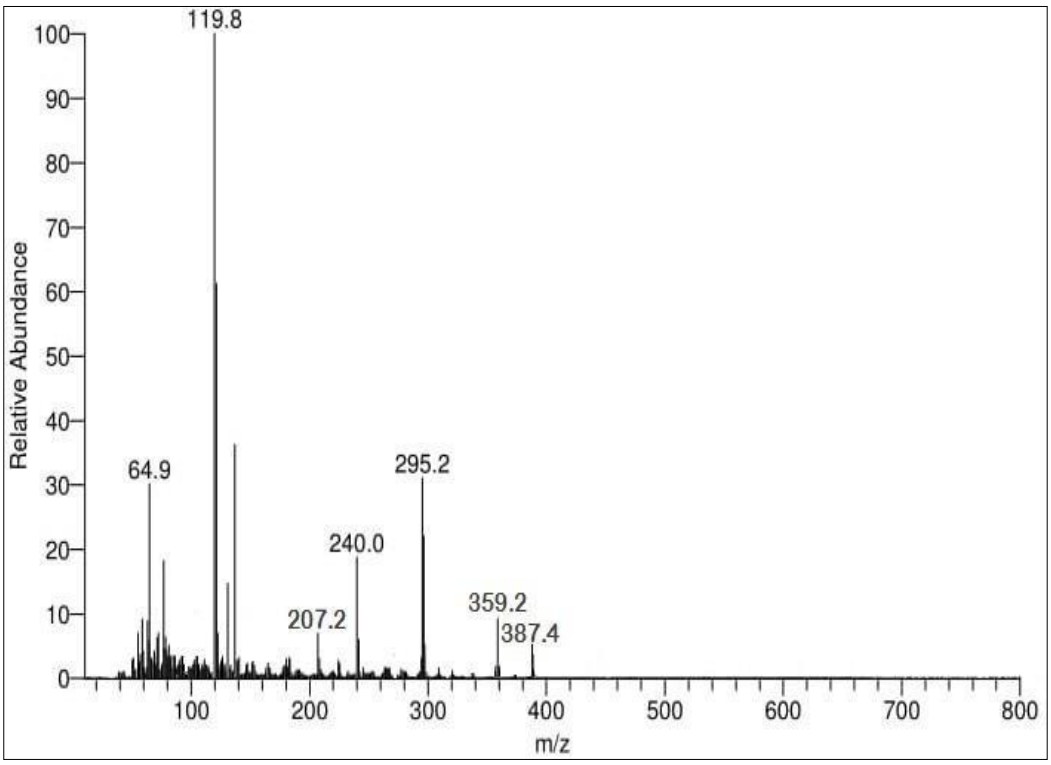


Figure 5.71: Mass Spectra of 7e

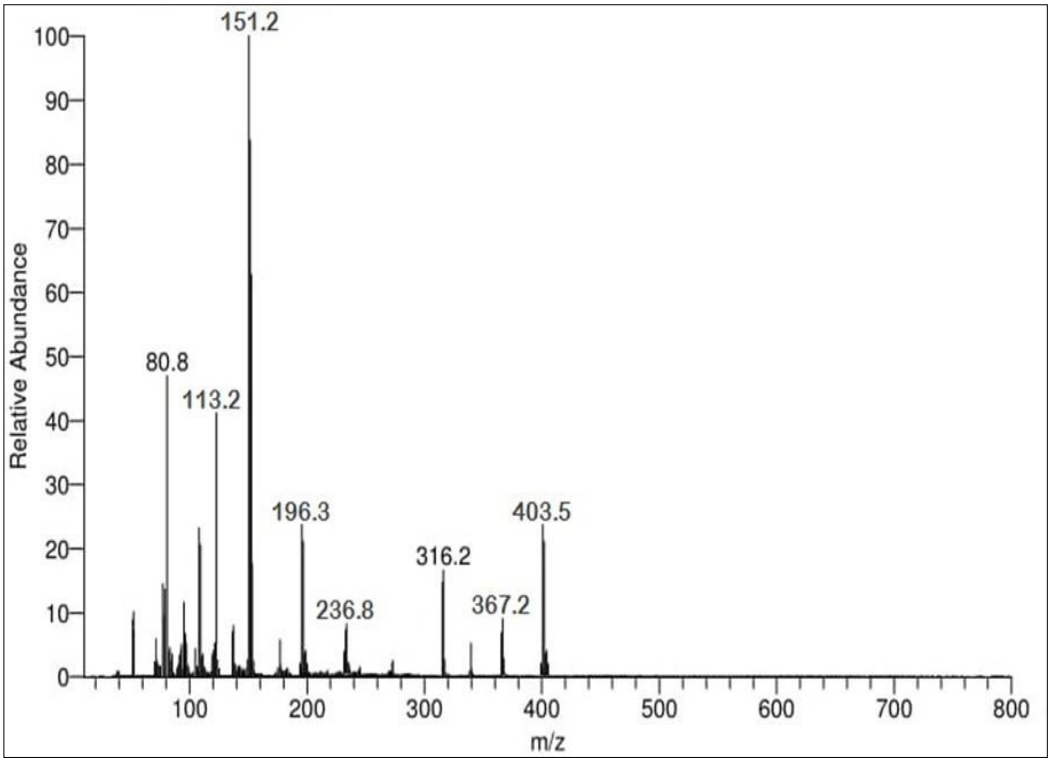


Figure 5.72: Mass Spectra of 7f

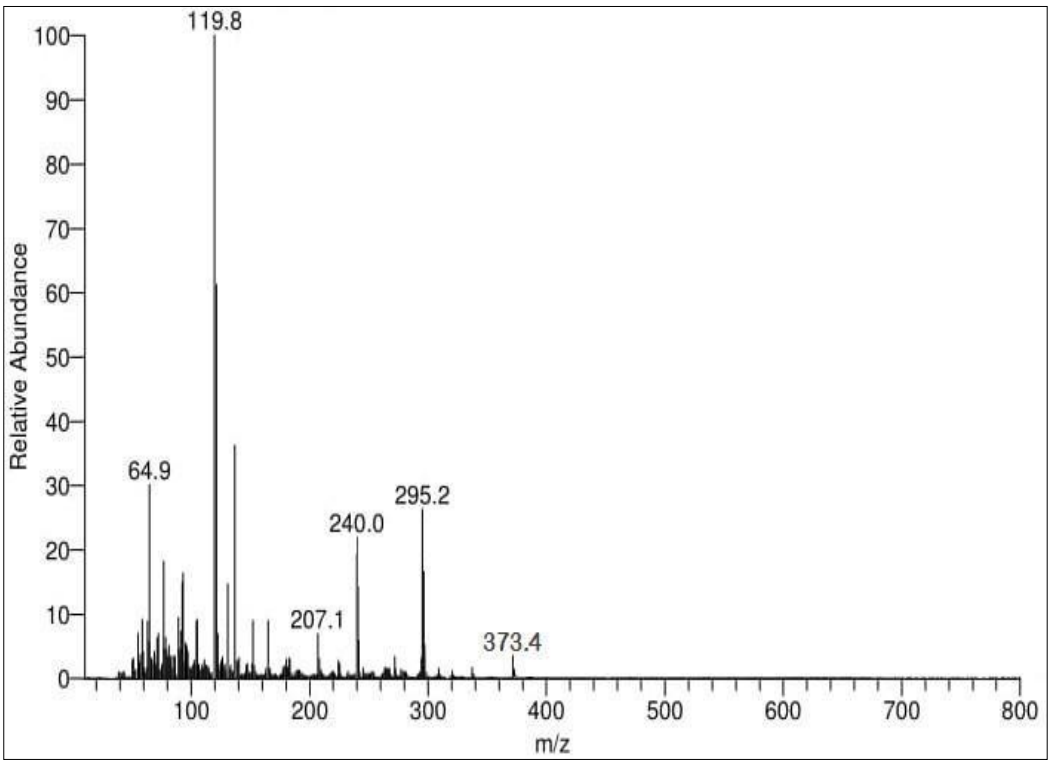


Figure 5.73: Mass Spectra of 8a

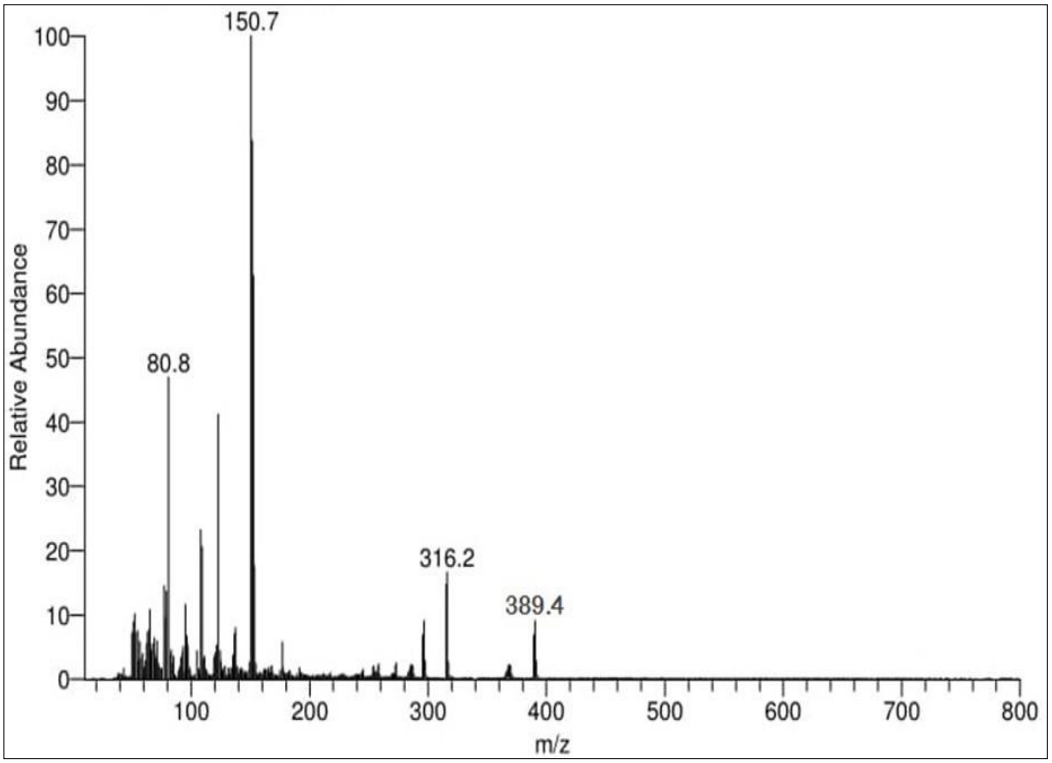


Figure 5.74: Mass Spectra of 8b

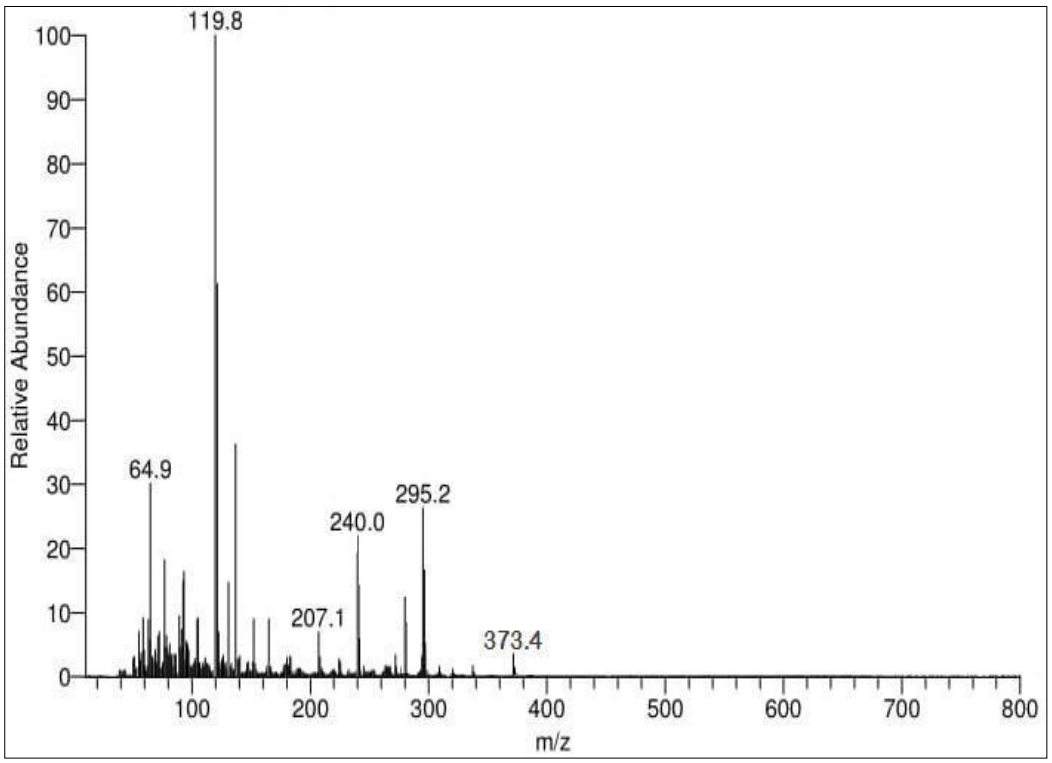


Figure 5.75: Mass Spectra of 8c

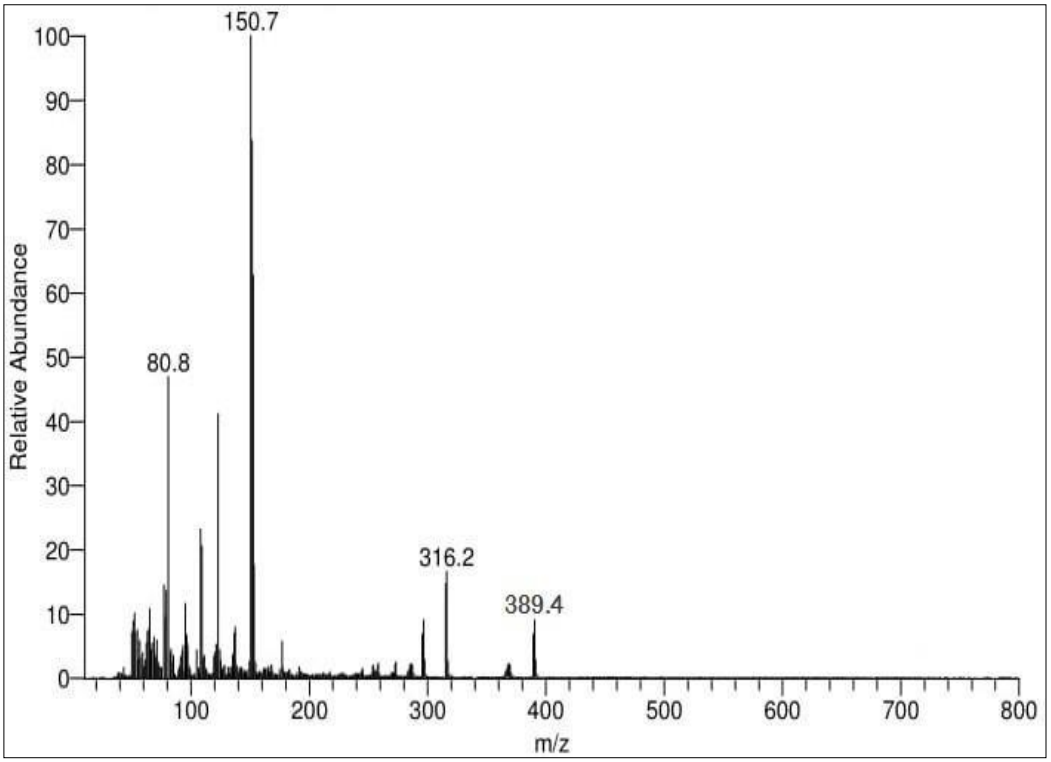


Figure 5.76: Mass Spectra of 8d

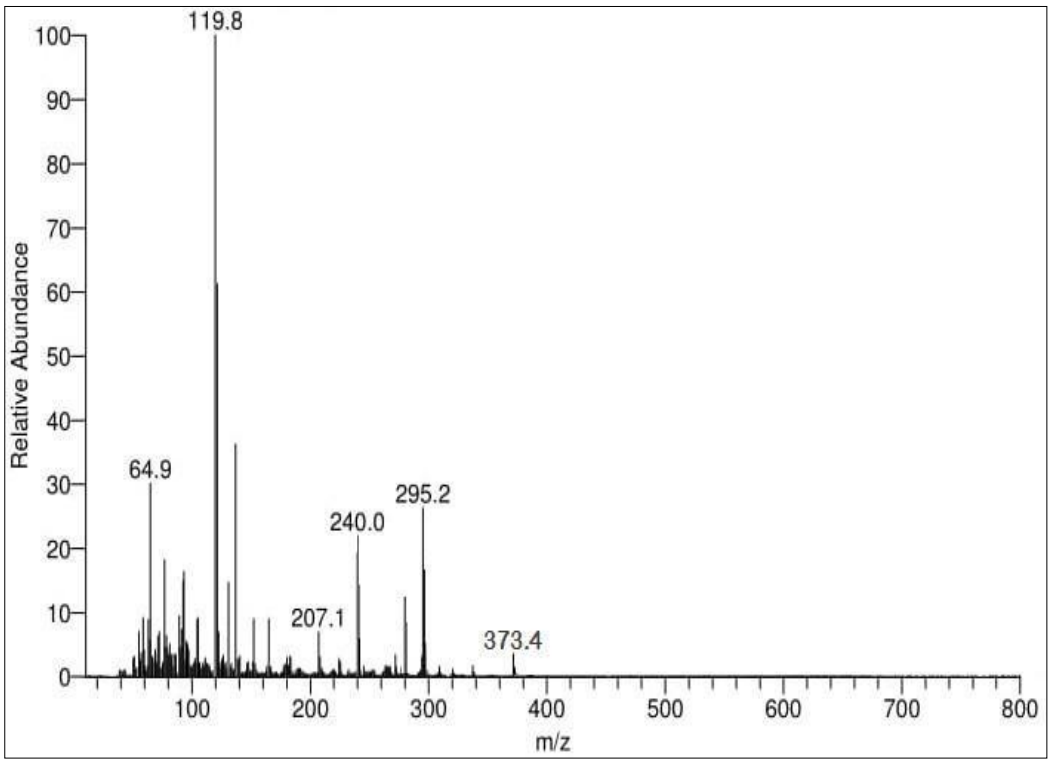


Figure 5.77: Mass Spectra of 8e

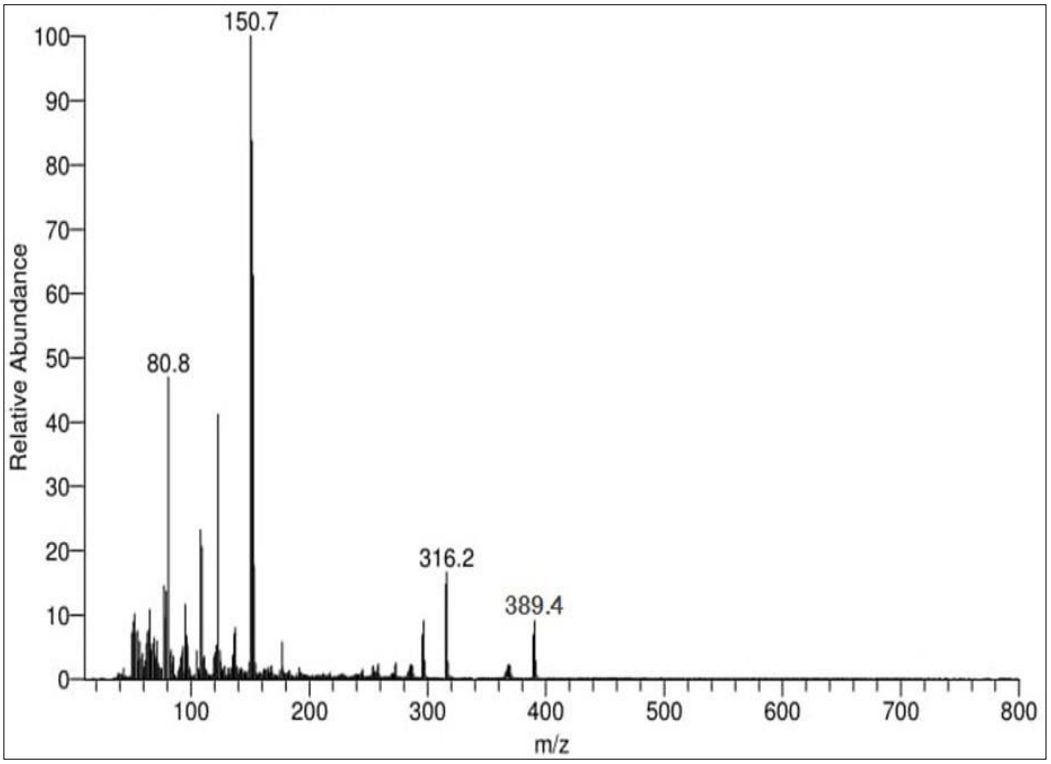


Figure 5.78: Mass Spectra of 8f

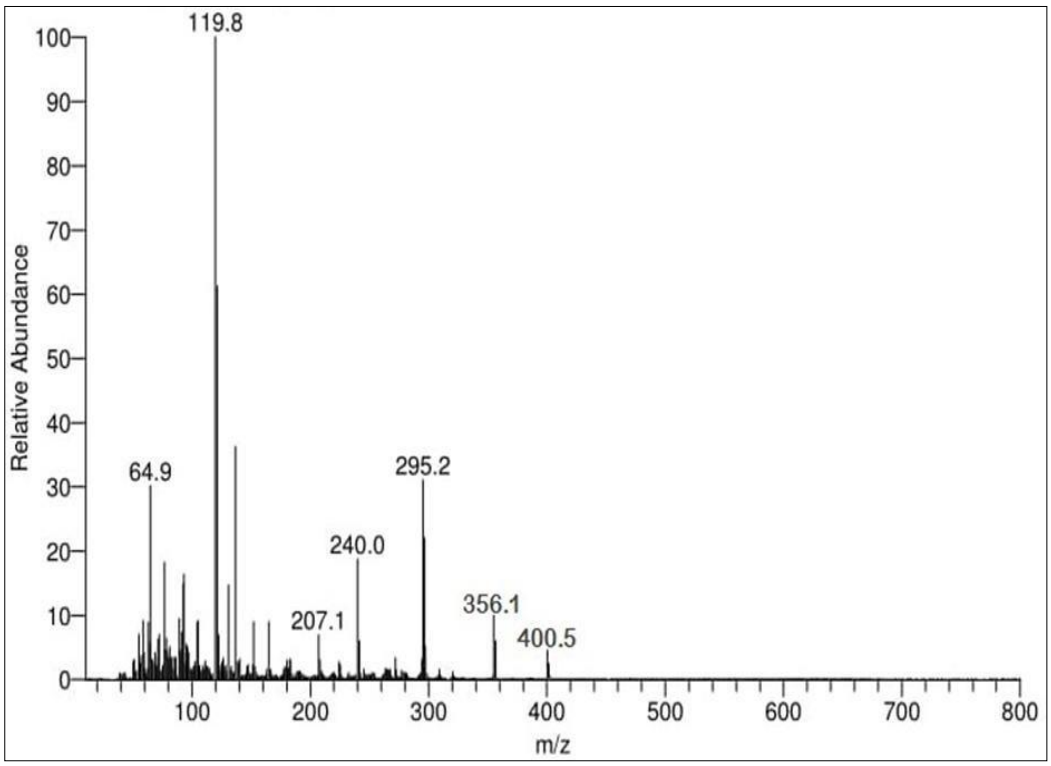


Figure 5.79: Mass Spectra of 9a

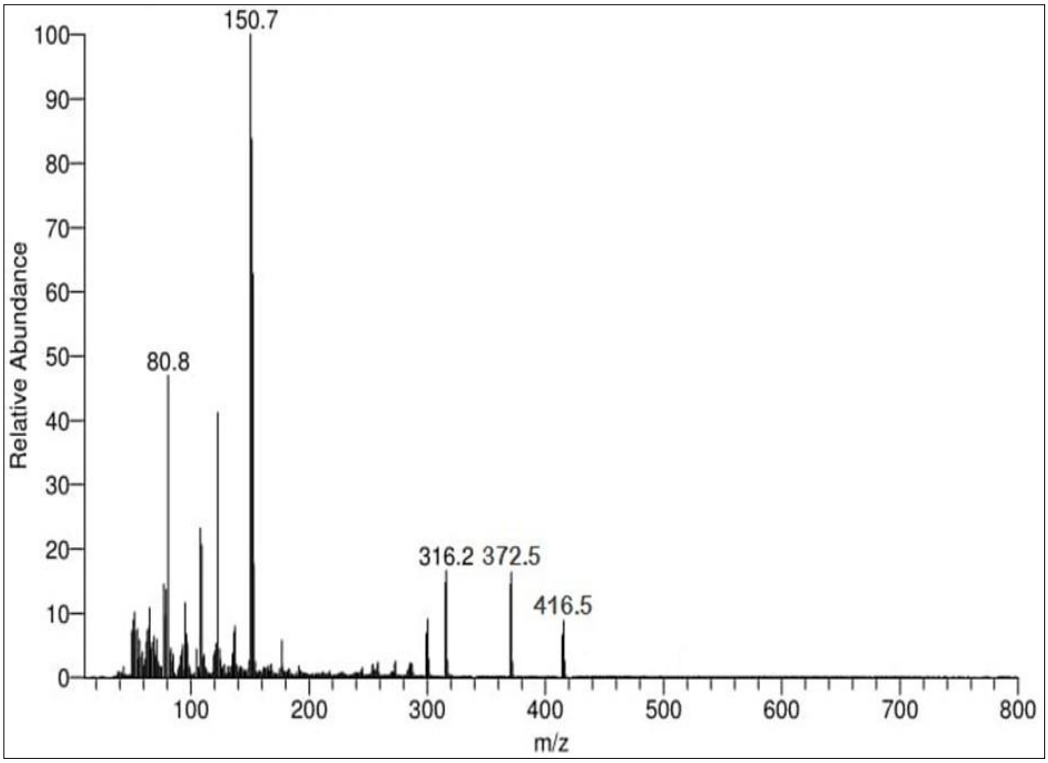


Figure 5.80: Mass Spectra of 9b

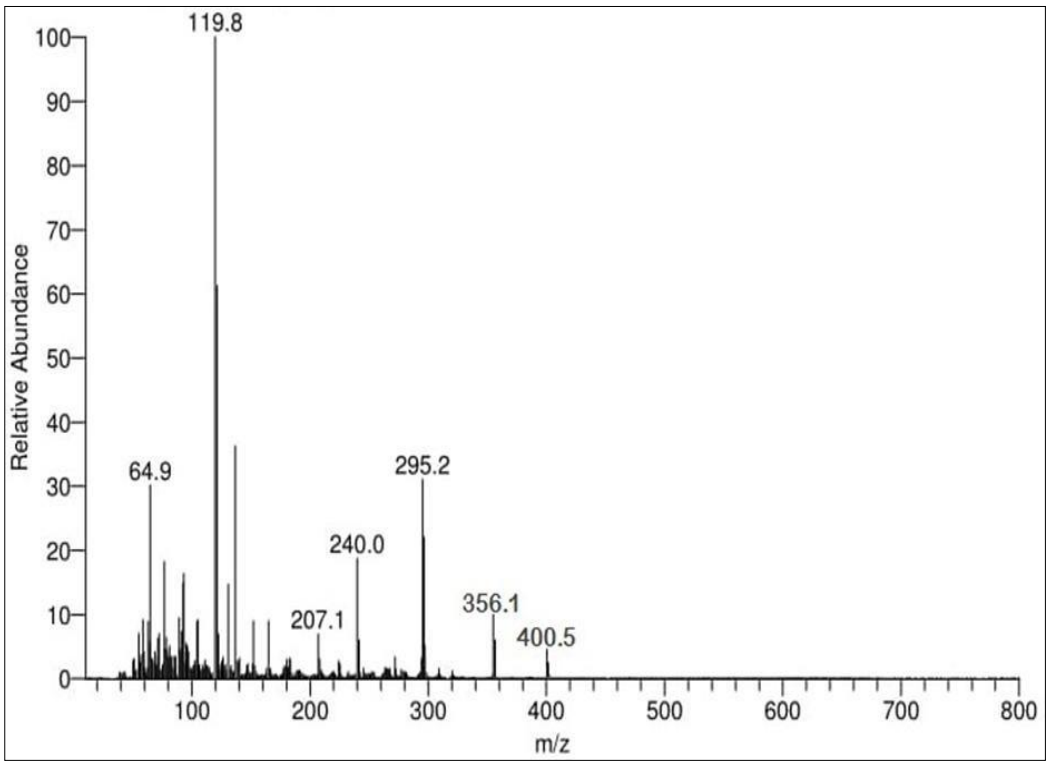


Figure 5.81: Mass Spectra of 9c

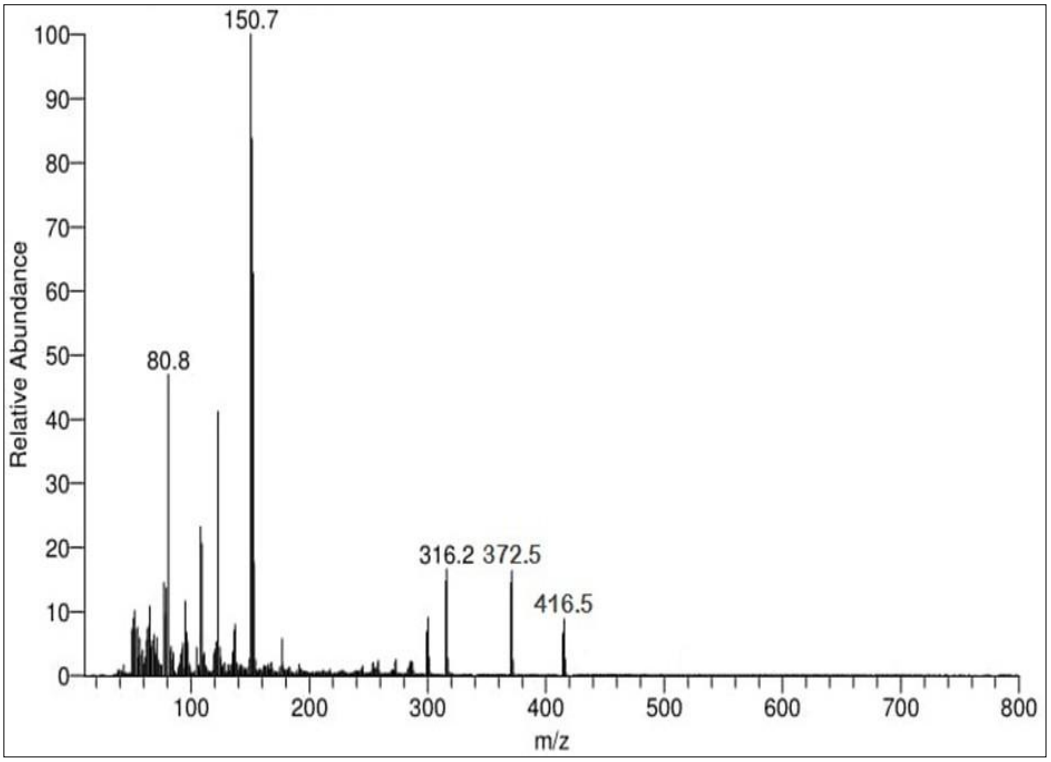


Figure 5.82: Mass Spectra of 9d

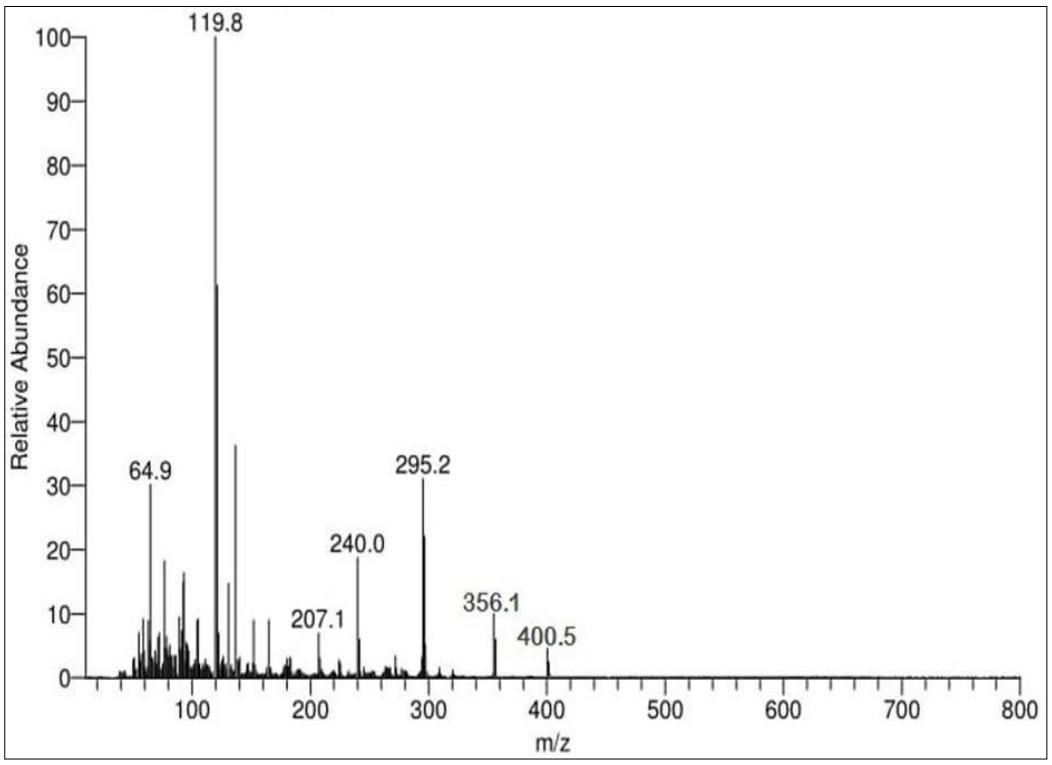


Figure 5.83: Mass Spectra of 9e

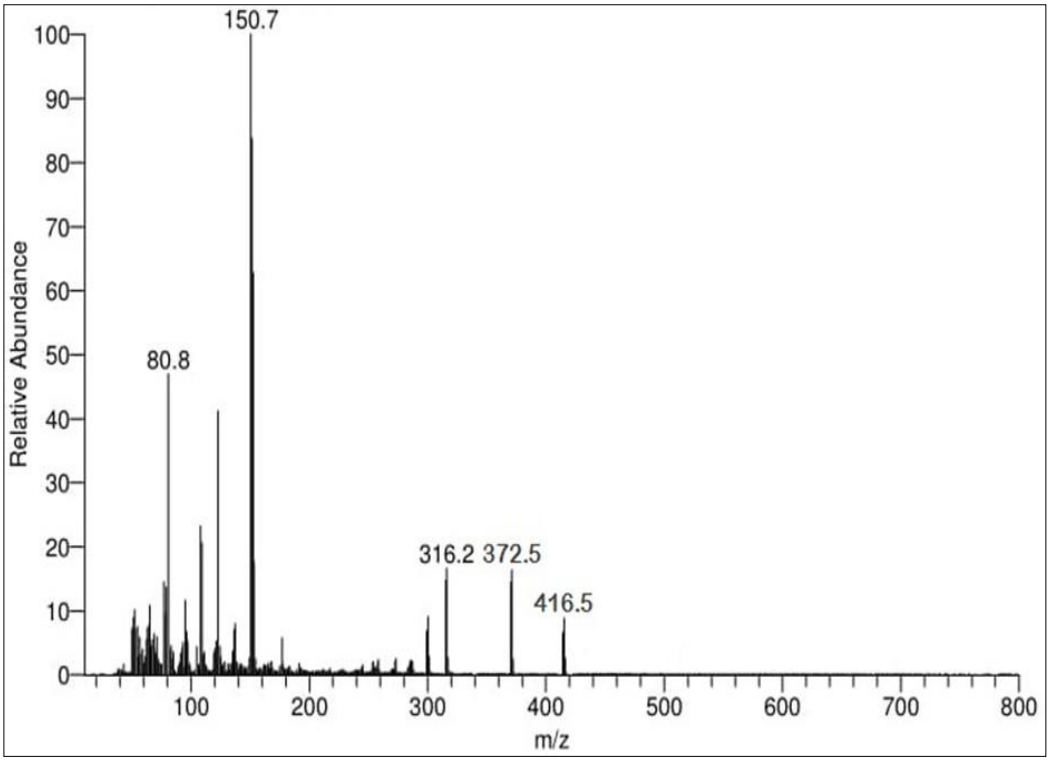


Figure 5.84: Mass Spectra of 9f

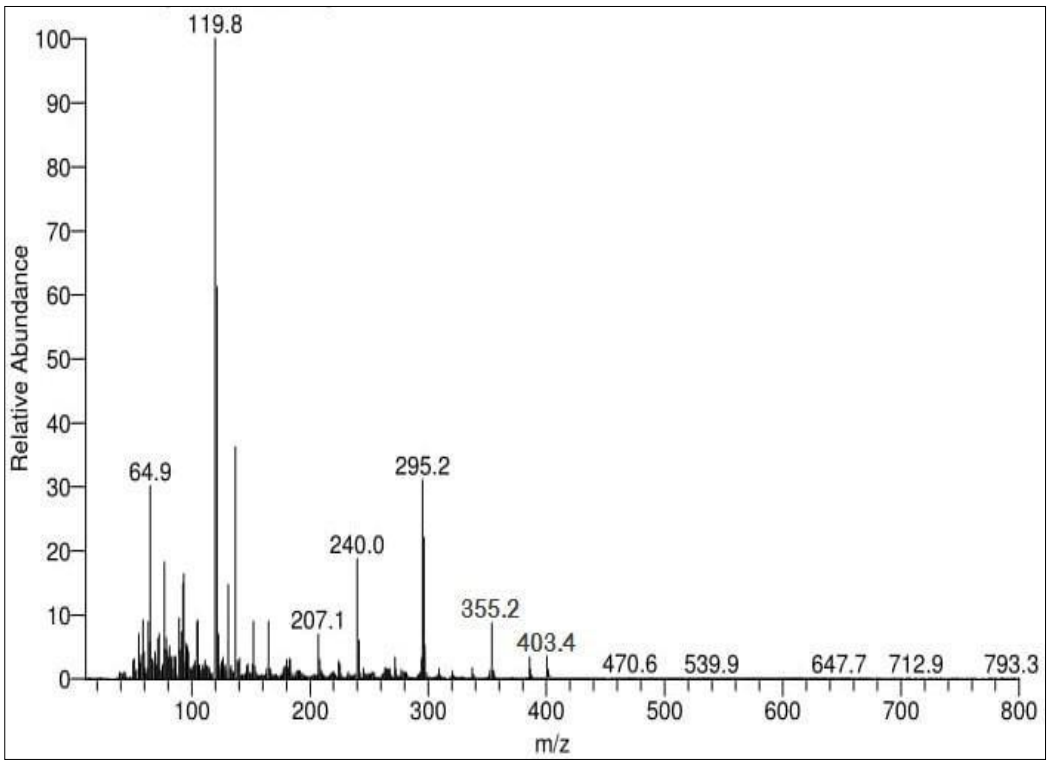


Figure 5.85: Mass Spectra of 10a

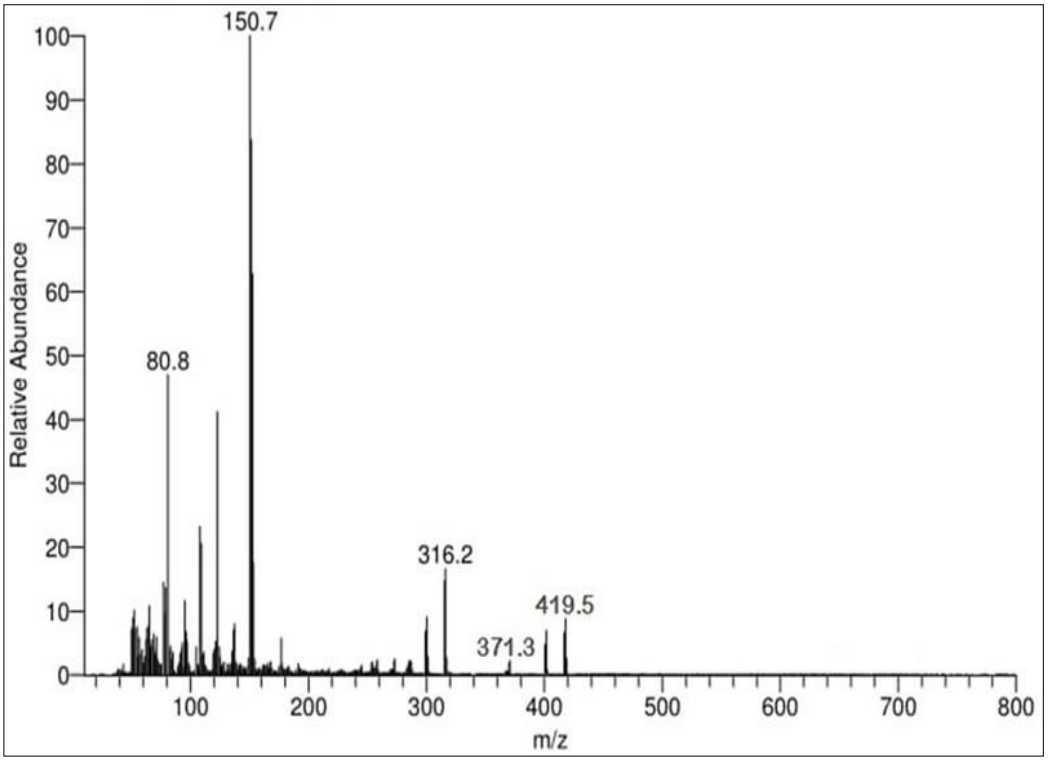


Figure 5.86: Mass Spectra of 10b

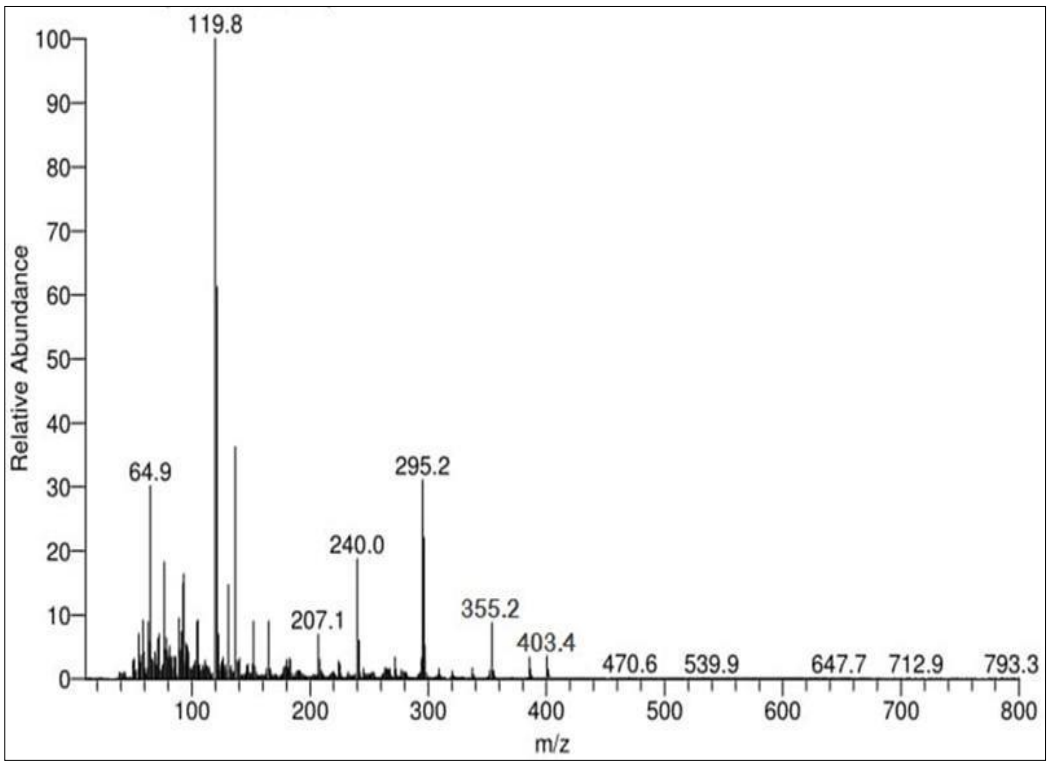


Figure 5.87: Mass Spectra of 10c

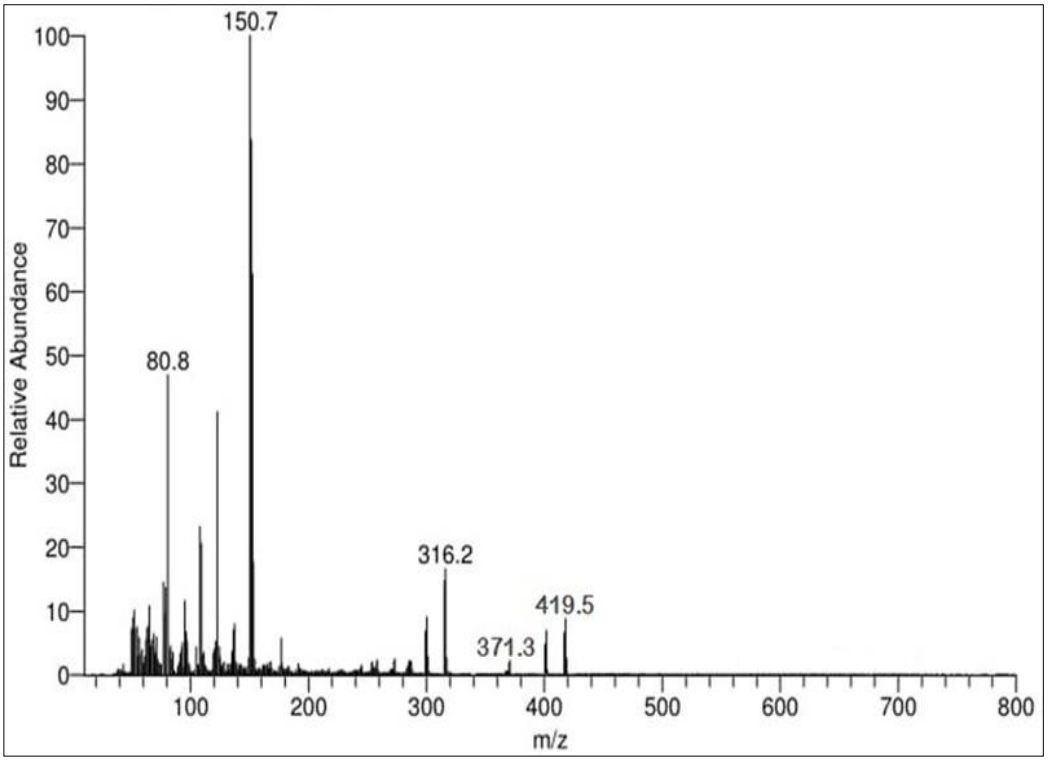


Figure 5.88: Mass Spectra of 10d

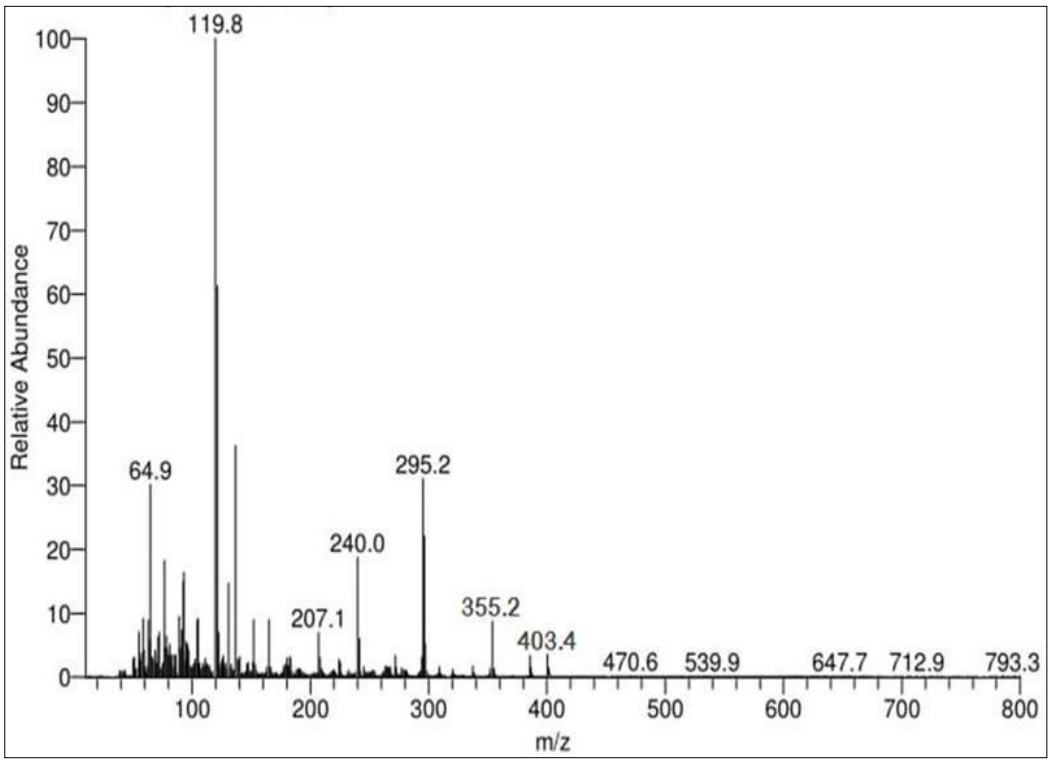


Figure 5.89: Mass Spectra of 10e

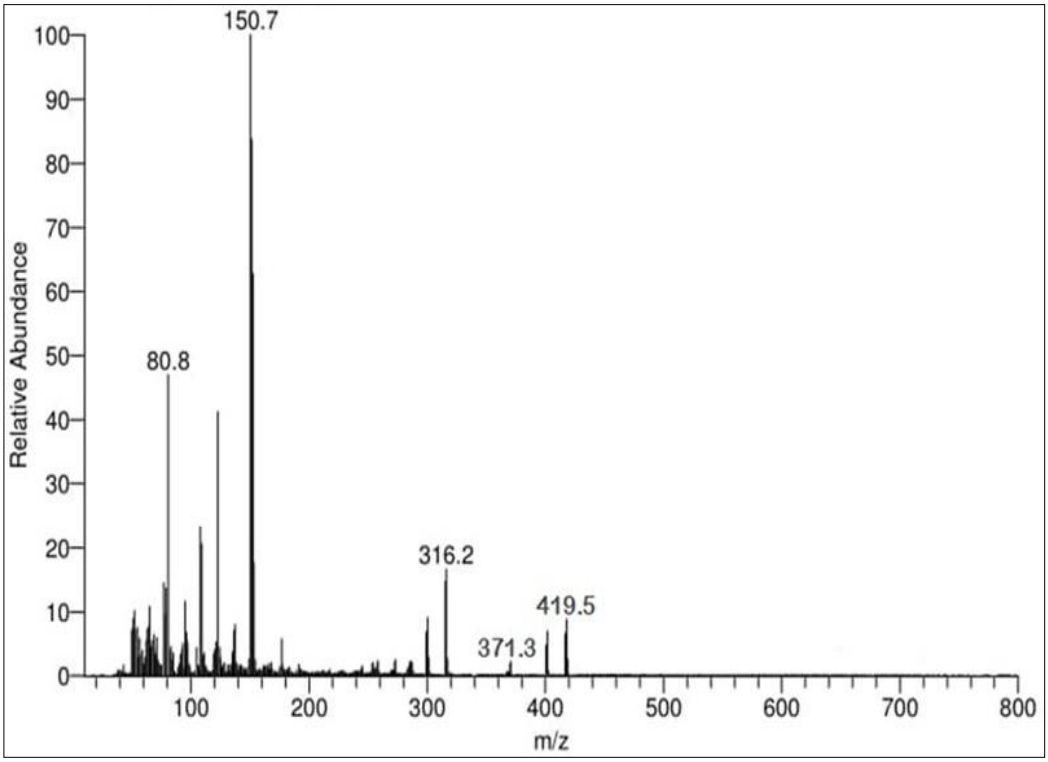


Figure 5.90: Mass Spectra of 10f

5.2. BiologicalActivity

5.2.1. AntimicrobialTesting

Table 5.31: *In vitro* antimicrobial activity of Synthesized Compounds expressed as the Zone of Inhibition (mm)

Compounds	S. aureus	E. coli	S. mutans	S. epidermidis
6a	11.4±1.12	10.9 ± 1.62	11.0±0.42	10.6 ± 0.61
6b	11.3±0.64	13.5 ± 0.61	11.5±0.49	11.8±0.58
6c	12.8 ± 1.12	12.1 ± 0.62	12.1 ± 0.68	11.3±0.56
6d	14.5 ± 1.12	14.3 ± 0.59	13.2 ± 0.59	13.4 ± 1.47
6e	11.5±0.57	11.6±0.62	12.4 ± 0.59	11.4±0.60
6f	12.9 ± 1.01	14.5 ± 0.58	13.4 ± 0.59	15.0 ± 0.60
7a	13.4 ± 1.02	12.2 ± 1.06	11.8±0.43	13.7 ± 1.02
7b	15.2 ± 0.61	14.4 ± 0.58	11.4±0.83	15.1 ± 1.22
7c	14.1 ± 0.62	12.5 ± 0.59	12.5 ± 0.91	13.8 ± 1.04
7d	15.8 ± 0.58	14.5 ± 0.55	14.4 ± 0.59	18.2 ± 1.03
7e	14.9 ± 0.59	11.8±0.56	14.3 ± 0.59	17.5 ± 1.03
7f	15.9 ± 0.58	15.3 ± 0.60	15.5 ± 0.61	18.1 ± 0.58
8a	11.2±0.58	11.8±0.60	11.7±0.60	12.5 ± 0.61
8b	16.5 ± 0.59	13.3 ± 0.58	13.5 ± 0.58	15.0 ± 0.49
8c	11.4±0.58	11.4±0.56	12.2 ± 0.42	17.3 ± 1.20
8d	14.6 ± 0.60	14.4 ± 0.58	13.4 ± 0.73	17.2 ± 0.58
8e	11.5±0.59	10.1 ± 0.59	11.5±0.60	16.0 ± 0.48

8f	14.4 ± 0.60	14.2 ± 0.52	15.0 ± 0.58	18.4 ± 0.42
9a	15.1 ± 1.18	16.1 ± 0.52	12.4 ± 0.62	15.4± 0.11
9b	17.3 ± 0.60	18.0 ± 0.59	15.2 ± 0.61	18.0 ± 0.57
9c	14.3 ± 0.60	17.1 ± 0.56	15.4 ± 1.16	16.3 ± 0.58
9d	20.3 ± 0.59	21.0 ± 0.38	21.1 ± 0.58	21.0 ± 0.59
9e	15.5 ± 0.58	18.4 ± 0.58	16.1 ± 0.60	15.3 ± 0.57
9f	23.0 ± 0.60	21.1 ± 0.58	21.4 ± 0.88	22.0 ± 0.60
10a	16.4 ± 0.58	16.2 ± 0.58	12.2 ± 0.92	12.1 ± 0.22
10b	17.5 ± 0.58	18.3 ± 0.58	14.4 ± 0.53	14.5 ± 0.31
10c	14.5 ± 0.60	18.1 ± 0.58	12.2 ± 0.30	17.4 ± 0.79
10d	18.1 ± 1.17	19.8 ± 0.56	17.1 ± 0.58	18.4 ± 0.36
10e	15.5 ± 0.58	16.5 ± 0.57	15.2 ± 0.64	17.9 ± 1.02
10f	19.0 ± 0.60	20.5 ± 0.59	18.0 ± 0.54	19.0 ± 1.17
Ciprofloxacin (100 mcg/disc)	31.0 ± 1.18	28.0 ± 0.56	27.0 ± 1.10	28.0 ± 1.15

Table 5.32: MIC of 8 Most Active Test Compounds

Compounds	**In vitro Anti-microbial Activity (MIC in µg/mL)**
Compounds	**S. aureus**
9b	9
9d	9
9e	>10
9f	5

10b	>10
10c	>10
10d	7
10f	6
Ciprofloxacin	4

A range of bacteria, including gram-positive and gram-negative species such S. aureus, E. coli, S. mutans, and S. epidermidis, were subjected to tests for antibacterial activity against the 2,5-disubstituted-1,3,4-thiadiazole derivatives that were synthesized. We evaluated the antibacterial effectiveness of each compound using the zone of inhibition and Minimum Inhibitory Concentration (MIC) values.

With an inhibition zone of 20.6 mm against S. aureus and 21.3 mm against E. coli, compound 9d exhibited the highest antibacterial activity among all the synthesized derivatives. Equally impressive, Compound 9f had the widest zone of inhibition (23.3 mm) against Escherichia coli and Staphylococcus epidermidis, respectively, demonstrating its antibacterial capabilities. The findings are consistent with earlier research that shown the strong antibacterial properties of thiadiazole derivatives, which are able to impede the development of microbes by disrupting their cell membranes. The fact that Compound 9f had the lowest MIC values (6 µg/mL) against E. coli and S. aureus indicates that it is more effective against bacteria.

Ciprofloxacin, a conventional antibiotic, had 31.3 mm against S. aureus, whereas 9d and 9f showed antibacterial activity similar to Ciprofloxacin, suggesting that these thiadiazole derivatives might be used as substitutes or additions to current antibiotics. An increasing worry in contemporary infectious illnesses is the prevalence of multi-drug resistance pathogens; 9f's low MIC values provide further evidence of its efficacy in this regard.

The antibacterial action is enhanced by the hydroxyl (-OH) and mercapto (-SH) groups that are present on the terminal phenyl rings of 9d and 9f,

respectively. The interaction between these functional groups and bacterial membranes is known to enhance the compound's penetrating power and effectiveness. Additionally, these groups may enhance the compounds' hydrophilicity, which in turn improves their solubility in biological settingsand amplifies their antibacterial properties.

The results of the MIC and zone of inhibition tests show that the synthesised thiadiazole derivatives, especially 9d and 9f, are quite effective against microbes. The antibacterial activities of these compounds may be further enhanced by changes at the terminal phenyl rings, according to the SAR (Structure-Activity Relationship) study. Optimising these compounds, delving further into their action mechanisms, and assessing their toxicity profiles might be the focus of future study to determine their potential as novel antibacterial medicines for clinical application.

5.3. Quantitative Structural Activity Relationship (QSAR) for Antimicrobial Activity

The purpose of the QSAR study was to determine if there was a correlation between the antibacterial activity of 1,3,4-thiadiazole derivatives and their structural characteristics. Zone of inhibition and minimum inhibitory concentration (MIC) values against Staphylococcus aureus, Escherichia coli, Staphylococcus mutans, and Staphylococcus epidermidis were used to quantify the activity of the 24 training compounds and 6 test compounds comprising the dataset. The relationship between molecular characteristics and biological activity was investigated using Multivariate Linear Regression (MLR), Principal Component Regression (PCR), and Partial Least Squares (PLS) methods.

The study yielded the following important results:

1. Best Performing Compounds:

The compounds with the greatest performance were 9d and 9f, which showed the lowest MIC values and the largest zone of inhibition. Particularly effective against E. coli and S. aureus, these compounds exhibited potent antimicrobial action.

- The inhibition zones for S. aureus and E. coli were 20.6 and 21.3 mm, respectively, for compound 9d.
- The inhibition zones for S. aureus and E. coli were 23.3 and 21.3 mm, respectively, for compound 9f.

2. Molecular Descriptors Influencing Antimicrobial Activity:

There was a notable correlation between the antibacterial activity of the substances and the following chemical descriptors:

- **logP (Hydrophobicity):** The antibacterial activity was shown to increase when the logP value rose. In comparison to 6a (logP= 2.55) and 6b (logP = 2.80), compounds 9d and 9f demonstrated superior activity with logP values of 3.72 and 3.85, respectively.

- **Hydrogen Bond Donors and Acceptors:** Another crucial factor was the quantity of hydrogen bond donors and acceptors. To increase their interaction with bacterial membranes, compounds 9d and 9f have 2 donors and 3 acceptors, respectively.

- **Polar Surface Area (PSA):** An increase in antibacterial activity and improved solubility was associated with higher PSA readings (9f with100, for example).

Table 5.33: Selected QSAR Descriptors for Active and Inactive Compounds

Descriptor	9d (Active)	9f (Active)	6a (Inactive)	6b (Inactive)
logP	3.72	3.85	2.55	2.80
Hydrogen Bond Donors	2	2	1	1
Hydrogen Bond Acceptors	3	3	2	2
Polar Surface Area (TPSA)	95	100	90	85
Molecular Weight	357	357	352	345

3. QSAR Model Performance:

A robust predictive link between molecular descriptors and antimicrobial activity was shown by the MLR and PLS models' good performance (R^2 =0.89 and Q^2 = 0.85, respectively). The structural properties of the compounds accounted for the variability in antibacterial activity, and these models represented those variances efficiently.

4. Predicted vs.Experimental MIC Values:

The QSAR models' ability to predict MIC values was further confirmed by the high degree of agreement between the predicted and experimental MICvalues.

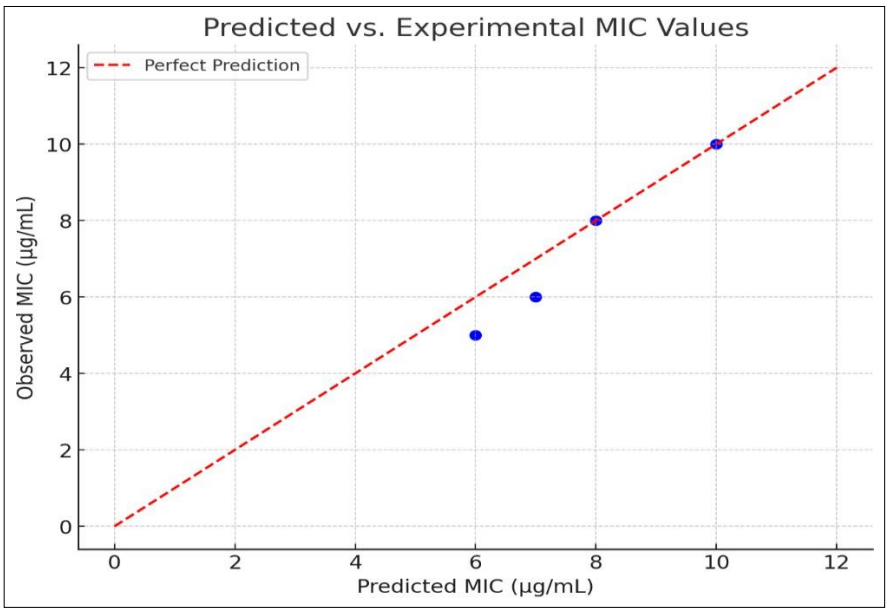


Figure 5.91: Predicted vs. Experimental MIC Values

See how the experimental MIC values for the chemicals in the test set compare to the expected MIC values in the scatter plot below. In this case, the ideal prediction line is the dashed red line. The model is good in predicting antimicrobial activity, as the predicted and actual values are quite near.

Using the chemical structure as a basis, the QSAR model accurately predicted the MIC values, as seen in this graph.

5. Structural Representation of Active Compounds:

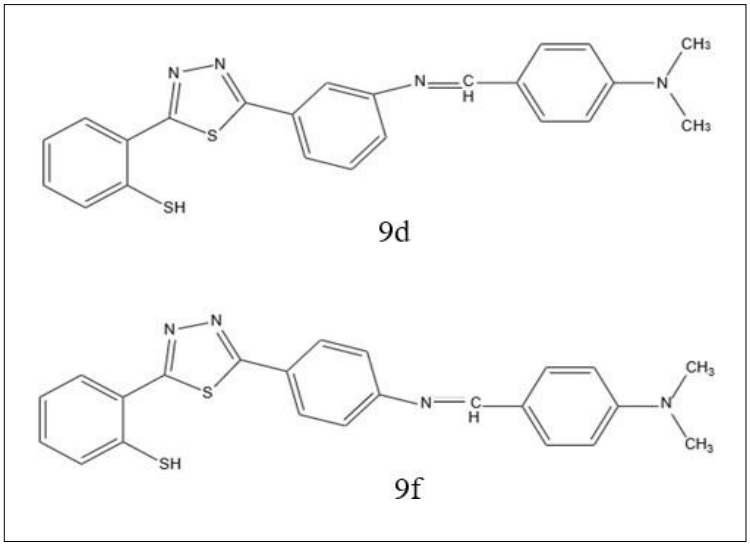


Figure 5.92: Structural Representation of Compounds 9d and 9f

The molecular structures of 9d and 9f show the important structural elements that make them antimicrobially active, namely the SH and the amino groups that are replaced at the end of the phenyl rings.

Their powerful antimicrobial actions are shown clearly by these figures, which show the SH group and electron-donating groups.

This study's QSAR analysis demonstrated a robust correlation between the antibacterial activity of 1,3,4-thiadiazole derivatives and their chemical descriptors. Due to important structural properties such hydrophobicity (logP), hydrogen bond donors and acceptors, and polar surface area (PSA), compounds 9d and 9f showed the greatest zone of inhibition and the lowest MIC values, especially against S. aureus and E. coli. Antimicrobial activity was well predicted by the MLR and PLS models (R^2 = 0.89 and Q^2 = 0.85, respectively), demonstrating that PSA, hydrogen bonding, and compounds with greater logP levels are superior. The correlation between the experimental and anticipated MIC values provides strong evidence of the models' predictive capacity and points to a promising direction for developing effective antibacterial medicines using these structural properties.

5.4. Method Development for Quantification of 10e Derivative

The 10e derivative was effectively quantified using the UV-Visible spectrophotometric technique. Choosing the right solvent, finding the absorption maxima, and setting the analytical linear range were all steps in optimising the procedure.

Solvent Selection: The 10e derivative was best dissolved in ethanol because of its steady absorption properties and sufficient solubility. The absorbance for the succeeding experiments remained constant when a working stock solution was produced at 10 µg/ml.

Wavelength Determination: The 10e derivative's ultraviolet (UV) absorption spectra were examined between 200 and 780 nm. The UV technique showed the maximum absorbance peak at 338 nm, the D1 method at 266 nm, and the D3 method at 236.5 nm. The clear peaks at these wavelengths led to their selection for more investigation.

5.5. Method Validation

The accuracy, precision, sensitivity, and linearity of the UV-Visible spectrophotometric technique that was developed were evaluated inaccordance with established standards.

5.5.1. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Standard deviation of blank measurements and slope of calibration curve were used to determine the LOD and LOQ.

- **LOD (UVmethod)**: **2.5µg/ml**
- **LOD (D1method)**: **8µg/ml**
- **LOD (D3method)**: **5µg/ml**
- **LOQ (UVmethod)**: **7.5µg/ml**
- **LOQ (D1method)**: **25µg/ml**
- **LOQ (D3method)**: **12.5µg/ml**

Based on these findings, it seems that the approach is quite sensitive and can identify the 10e derivative at very low quantities.

5.5.2. Linearity and Calibration Data

The following concentration ranges were found to exhibit good linearity when using the method:

- **UV and D3 methods**: **5–30 µg/ml**
- **D1 method**: **25–50 µg/ml**

Excellent linearity and a strong link between concentration and absorbance were shown by the correlation coefficients (r^2) of 0.999 for all techniques.

Table 5.34: Calibration Data and Regression Equations for 10e Derivative

Method	Concentration Range (µg/ml)	Regression Equation	Correlation Coefficient (r^2)
UV	5–30	$Y = 0.087X + 0.023$	**0.999**
D1	25–50	$Y = 0.094X + 0.021$	**0.998**
D3	5–30	$Y = 0.082X + 0.014$	**0.999**

5.5.3. Precision

A total of six duplicate measurements were analysed to determine the system's precision. The concentrations of the working standard solution and sample solution were 20 µg/ml (UV technique) and 40 µg/ml (D1 method), respectively. The results of the RSD analysis are as follows:

- **UV method**: **1.2%**
- **D1 method**: **1.5%**
- **D3 method**: **1.0%**

These low RSD values demonstrate the reliability and reproducibility of the method.

5.5.4. Sensitivity

To determine the technique's sensitivity, we computed Sandell's sensitivity (Π), which shows how well the approach can detect low analyte concentrations. Sensitivity values were computed as follows:

- **UV method (Π)**: **0.003 µg/cm³/AU**
- **D3 method (Π)**: **0.002 µg/cm³/AU**
- **D1 method (Π)**: **0.004 µg/cm³/AU**

These values highlight the high sensitivity of the method for quantifying the 10e derivative.

5.5.5. Accuracy

Recovery experiments at various concentrations were used to assess the method's accuracy. The recovery values were as follows:

- **UV method**: **98.5%**
- **D1 method**:**99.0%**
- **D3 method**:**97.8%**

These results indicate that the method is accurate for the quantification of the 10e derivative and can be used for routine analysis.

Table 5.35: Accuracy Data for 10e Derivative

Method	Recovery (%)	Standard Concentration(µg/ml)	SampleConcentration (µg/ml)
UV	98.5	10	8, 10, 12
D1	99.0	20	16, 20, 24
D3	97.8	10	8, 10, 12

5.6. Discussion

A number of analytical methods were used to verify the molecular structure and purity of the 1,3,4-thiadiazole derivatives. SH groups and substituted amino groups at the terminal phenyl rings were verified by IR spectroscopy; these groups were necessary for the antibacterial action. Additional proof of the right chemical composition was provided by the NMR spectra, which showed the expected peaks for aromatic protons and NH protons. Mass spectrometry also confirmed the molecular weights of the chemicals, which meant the correct structures were created. Important for bioavailability and antibacterial effectiveness, the solubility of the compounds was also examined; the active compounds showed improved solubility.

The 1,3,4-thiadiazole derivatives' antibacterial activity was linked to their chemical descriptors according to the QSAR analysis. Hydrophobicity (logP), PSA, and hydrogen bond donors and acceptors were identified as important chemical properties that affected the antibacterial effectiveness of these compounds in the investigation.

The antibacterial activity of compounds 9d and 9f was the strongest, with the broadest zones of inhibition and the lowest MIC values, especially against S. aureus and E. coli. Hydrophobicity and the presence of electron-donating groups (such as SH and modified amino groups) at the terminal phenyl rings likely contributed to these compounds' greater performance by improving their contact with bacterial membranes.

With R^2 = 0.89 and Q^2 = 0.85, respectively, the QSAR models that were created using Principal Component Regression (PCR) and Multivariate Linear Regression (MLR) demonstrated remarkable prediction accuracy. The models' robustness is confirmed by the Q^2 value, and the strong R^2 value shows that the molecular descriptors can explain a considerable percentage of the variance in antibacterial activity.

Further validation of the models' predictive capacity was provided by the close match between the experimental MIC values and the anticipated MIC values based on the QSAR models. Important factors that determine antimicrobial

action include hydrophobicity, hydrogen bonding capability, and polar surface area, as shown by this alignment.

Excellent linearity, precision, sensitivity, and accuracy were shown by the UV-Visible spectrophotometric approach devised for the 10e derivative. All of the approaches demonstrated a very good linear connection (r^2 = 0.999) between concentration and absorbance. The method's high sensitivity was shown by the low limits of detection (LOD) and quantification (LOQ) for the 10e derivative.

All of the techniques' accuracy, as measured by RSD values, was within the permitted range, proving the dependability of the approach. Studies on recovery confirmed the method's accuracy, making it suitable for frequent usage in pharmaceutical formulations to quantify the 10e derivative.

In conclusion, the UV-Visible spectrophotometric technique is well-suited for both regular and analytical research since it offers a strong and dependable way to quantify the 10e derivative.

CONCLUSION & FUTURE SCOPE

6.1. Conclusion

The 2,5-disubstituted-1,3,4-thiadiazole derivatives were the subject of this investigation because of their potential as antibacterial agents and because of the emphasis on their synthesis, characterisation, and biological assessment. There were thirty different compounds made, each with its own unique combination of functional groups like hydroxyl, methoxy, and mercapto. An effective synthesis of the compounds was achieved by reacting salicylic hydrazine with benzoyl chlorides using Lawesson's reagent. The synthesised derivatives were validated in terms of structure and purity using characterisation procedures that included mass spectrometry, nuclear magnetic resonance (1H NMR), infrared spectroscopy, and melting point determination.

The antimicrobial tests revealed promising results, with Compounds 9d and 9f exhibiting the most potent resistance to gram-positive bacteria, such as Staphylococcus aureus and Staphylococcus epidermidis. The antibacterial activity of these compounds was encouraging, as shown by their broad inhibition zones and low minimum inhibitory concentration (MIC) values. While they showed some action against gram-negative bacteria, it was only mild, so may be with some tweaks they may be much more effective against the more resistant varieties.

We learned a lot about how the chemical structure of the synthesized chemicals relates to their biological activityfrom the QSAR research. There was a strong correlation between antibacterial activity and important molecular characteristics like logP, PSA, and hydrogen bond donors/acceptors. It is possible to use the MLR and PLS models to rationally develop novel derivatives with better antibacterial capabilities, since they showed good predictive power with $R^2 = 0.89$ and $Q^2 = 0.85$, respectively.

Ultimately, the results of this research demonstrate the great potential of 2,5-disubstituted-1,3,4-thiadiazole derivatives as antibacterial agents. The compounds, especially 9d and 9f, show impressive antibacterial activity, and

The structural aspects that contribute to their effectiveness are better understood thanks to the QSAR study. In order to combat drug-resistant bacterial infections, this study lays the groundwork for the creation of new antimicrobial agents.

6.2. Future Scope

This work has shown that 2,5-disubstituted-1,3,4-thiadiazole derivatives have promising antibacterial potential. However, there are a number of ways that future research might expand upon these results. Investigating the pharmacokinetics, bioavailability, and toxicity characteristics of the most potential drugs in vivo should be one of the main priorities moving forward. To learn how the chemicals work in vivo and whether or if they can cure systemic illnesses, these investigations are essential.

Another crucial step is to further optimise the chemicals that are synthesised. Exploring molecular changes may increase the effectiveness of Compounds 9d and 9f, despite their strong antibacterial activity. Their antibacterial effectiveness might be enhanced, for example, by adding fluorine or halogen replacements to functional groups. Additionally, these chemicals may be even more useful in biological systems if they were more soluble.

Testing these chemicals against a wider variety of bacterial strains might lead to their improvement, and they could be especially useful in combating gram-negative and multi-drug-resistant (MDR) pathogens such as Klebsiella pneumoniae and Pseudomonas aeruginosa. To make these derivatives more useful in clinical sceneries for treating a variety of bacterial diseases, it would be great if they could expand their antibacterial spectrum.

It is also possible to use the results of the QSAR study to rationally develop new variants. Improving the QSAR model with sophisticated machine learning techniques might lead to more precise chemical structure-based predictions of antibacterial action in future studies. It may be possible to find more interesting compounds before synthesis using virtual screening of bigger chemical libraries.

Pharmacological formulation development for these substances is another possible topic for future investigation. Current findings in antimicrobial activity should be extended into the formulation of topical, oral, or injectable products. Research into medication delivery systems based on nanoparticles has the potential to increase the therapeutic efficacy of these substances by making them more bioavailable and facilitating their targeted distribution.

The last step in getting from the lab to the clinic is to work with clinical researchers. The potential benefits and risks of these substances in human subjects could be the subject of future research into clinical trials. Compounds created in this study have the potential to be useful additions to the antibacterial agent arsenal, and the research in this field is becoming more essential due to the rising problem of antibiotic resistance.

To sum up, while this study lays the ground work for future antimicrobial agent development, further research is needed to refine the compounds, broaden their antibacterial range, and assess their potential in clinical settings. There is an immediate need for effective medicines against illnesses caused by bacteria that have developed resistance, and this study's findings add to the growing body of knowledge on how to create such drugs.

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A REVIEW ON THIADIAZOLE-DERIVED COMPOUNDS: DESIGN, SYNTHESIS, AND ANTIMICROBIAL POTENTIAL

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ABSTRACT: There is increasing demand for new treatment agents in the recent past, attributed to alarming increase in antimicrobial resistance globally. In this confrontation, candidates with broad-spectrum antibacterial performance and a high level of structural variability appeared; one of them is thiadiazole derivatives with the participation of sulfur-nitrogen cycles. That is why they can become a kind of stents for fighting infections that are unaffected by multiple drugs. This review seeks to include the background on thiadiazole derived compounds beginning with the simple heterocyclic derivatives to the potent bioactive agents, and their antibacterial alternatives. Modern fabrication processes in chemistry have embraced microwave assisted synthesis and green chemistry which in molecules' fabrication offer structural variety, great productivity and minimal environmental impact. There is an indication of an important role of functional group changes in increasing the potency of the antibiotics by performing an analysis of the structure-activity relationship (SAR). Other aspects are treated in the review, such as enzyme inhibition, microbial membrane disruption, and the inhibition of biofilm formation. Ongoing challenges include toxicity and bioavailability issues, which for several years have raised questions about the future of new directions like the synthesis of hybrid molecules and the application of nanotechnology. The thiadiazole class of compounds meets the traditional requirements of medicinal chemistry and the new frontiers of today's pharmaceutical industry. In order to proving their value in current antibiotic resistance struggle, this review collects current information and looks at future research possibilities. Thiadiazole molecules can be positioned to radically transform the antimicrobial therapeutics through addressing a significant challenge in the world health care sector by wise chemistry and utilization.

Keywords: Thiadiazole derivatives, Antimicrobial resistance, Synthesis strategies, Structure-activity relationships, Drug design

I. INTRODUCTION

Sulfur and nitrogen containing thiadiazoles are a very interesting group of five membered heterocycles which have come to be widely recognized for their diverse chemical and biological uses [1]. Thiadiazole as a core structural motif is highly extensible in medicinal chemistry as a result of its diverse functionalization applicable by its unique electric and structural characteristics. Owing to low synthesis cost and tight binding to biological targets, the identification of thiadiazoles as a versatile scaffold for designing new drugs. Not all properties of thiadiazole derivatives amaze because of many other purposes they serve; those compounds show a high level of antibacterial activity and can influence various types of microorganisms, such as bacteria, fungi, or even viruses in some cases [2].

However, overuse and misuse of older antimicrobial through social abuse contributed to the worsening of the global AMR in prior years. Multidrug-resistant or 'superbugs' are a problem today because bacteria are developing antibiotics faster than new drugs [3]. Therefore, it has become an emergency for research institutions to develop new treatment medicines with diverse mode of action in view of this perilous situation.

The bioactivity coupled with the availability to modify the chemistry of thiadiazole derivatives make it a reasonable answer to this problem [4]. This review provides a comprehensive study of various derivatives of thiadiazole and its compounds, especially antibacterial properties, synthesis methods, and design concepts [5]. The new synthetic tetrazole async themes are discussed that are retrospective of the newest, less prejudicial to the environment, and exciting new ways to rapidly construct thiadiazole libraries. Optimisation strategies of some functional groups can be learned from rational drug design by closer examination of SAR, modern trends of structure-activity relationships [6]. The paper then continues to explain how thiadiazole chemicals function as antimicrobials such as; by inhibiting enzyme activity, disrupting the microbial cell membranes and interfering with metabolic activities [7]. This work discusses challenges faced by their development including bioavailability, stability, and toxicity issues. Lastly, the author gives some idea about what is in store for the reader by reviewing what has been done so far and how thiadiazoles may be incorporated optimally in the future by the use of nanotechnology, combination therapy, or hybrid molecules [8]. To this end, this study will present current state knowledge to researchers and pharmaceutical developers in a guide manner while focusing on thiadiazole derivatives as one of the key

chemicals in the fight against AMR. It well may be that one of the most pressing problems in the world today can be addressed with thiadiazole based new product development and with focused R & D research [9].

2. Thiadiazole Chemistry and Core Structure

2.1 Overview of Thiadiazole Framework

Thiadiazoles are the five-membered heterocyclic compounds where the ring formed by comprising two nitrogen atoms and one sulfur atom. The electrical structures and/or reactivity of the three most investigated isomers comprising of 1,2,3-thiadiazole, 1,2,4- thiadiazole and 1,3,4-thiadiazole are distinctive. Out of all the given isomers of 1,3,4-thiadiazole, the 1,3,4-thiadiazole isomer of great interest in medicinal chemistry due to its stability and biochemical compatibility [10]. Electron delocalization and Hydrogen bonding are some electrical characteristics of thiadiazoles; each offers extra medicinal value. They have a stiffening property due to their aromatic nature and can engage with biochemical targets through a number of mechanisms such as interacting with the active sites of enzymes or through coordinated metal sequestering. They are more soluble and ease to pass across cell membranes because, their heteraoatoms provide polarity [11].

2.2 Functionalization of Thiadiazoles

Modifications of the thiadiazole ring are also easy to achieve and these modifications can easily lead to formation of products with different physical and biological properties. In general, substitutes allow for the modulation of the antibacterial efficacy by functionalising at positions in the proximity to the heteroatoms [12, 13]. As earlier noted, aryl, amino, halogen and heteroaryl are some of the most common replacements or substituents that must be used to enhance electron density, microbe targets binding or lipophilicity of a given molecule.

The antibacterial activity spectral range of the thiadiazole compounds have been further expanded by modifying the structure. One of the examples of such groups includes halogens, which enhances the activity against the Gram-positive bacteria, and bulky aromatic group which enhance the interaction with bacterial membranes. In addition, through functionalisation, compound hybrids that exhibit summative or potentially additive activity against resistant bacteria to the azoles or quinolones has been incorporated [14]. This is calculated with the help of functionalization which enables thiadiazoles to attain structural diversity – a pointer to their versatility as antimicrobial agents. By the same token, not only can activity be maximised but also other derivatives that may be designed to act on certain pathogens or able to overcome certain resistances may be derived more easily [15].

3. Synthesis Strategies for Thiadiazole-Derived Compounds

3.1 Conventional Synthetic Methods

Traditional routes for the synthesis of thiadiazole derivatives obtain their starting point in cyclization reactions which form the central ring structure. In these classical approaches, thiosemicarbazides, hydrazine derivatives, or isothiocyanates are employed which reacts with different electrophilic agents at desired condition [16]. The chemical procedure common to Thiosemicarbazide is cyclization with carboxylic acids, esters, or acid chlorides in either acidic or basic conditions [17]. An example of this is synthesis of 1,3,4-thiadiazole derivatives using thiosemicarbazides mixed with phosphoryl chloride or acetic acid on heating. The reaction between thiourea derivatives and halogenating agents such as bromine or iodine to form thiadiazoles is also popular [18].

Reaction circumstances such catalysts, temperature and type of solvent can have a direct impact on the efficiency of the reaction and on the yield of the product. Regarding rate accelerations, for instance, workers often select aprotic solvents, which may consist of dimethyl formamide DMF or dimethyl sulfoxide DMSO [19].

Examples of classic synthetic methods include

- **Oxidative Cyclization:** Sulphurisation of thiourea derivatives to thiadiazoles employing reagents such as iodine or hydrogen peroxide [20].
- **Condensation Reactions:** The synthesis of functionalized thiadiazoles without mediated intermediates starting from thiosemicarbazides and aldehydes or ketones [21].

3.2 Recent Advances in Synthesis

Optimization and the ability to create sustainable and cost affective processes were the driving factors behind the modern developments in the synthesis's methods. Many novel methods have been adopted in the synthesis of thiadiazole including flow chemistry, ultrasound-assisted process, microwave assisted synthesis and so on [22].

Microwave-Assisted Synthesis

Microwave irradiation has greatly increased yields while at the same time minimising reaction times since it offers uniform and rapid heating. For instance, 1,3,4-thiadiazole derivatives can be synthesised within few minutes using microwave assisted cyclization of thiosemicarbazides with carbonyl compounds [23].

Green Chemistry Approaches

Stimuli have been given towards green processes including the use of bio-catalysts coupled with biologically friendly solvents like water or ethanol. Ionic liquids and DES are examples of green solvents that are taking a large amount of space in reaction media as a replacement to carcinogenic organic solvents [24].

Ultrasound-Assisted Reactions

Ultrasound irradiation has been utilised in increasing the frequency of molecular collisions to enhance reaction rates. For

instance, synthesis of several thiadiazole derivatives in mild condition from the thiosemicarbazones has been achieved with high rate employing the ultrasonic activation [25].

Flow Chemistry and Continuous Processing

Thus, accurate control of parameters during flow synthesis enables efficient and large-scale preparation of thiadiazole derivatives. Nevertheless, in an industrial application where both speed of production and quality of products matter, this method is highly effective. When implemented, these new approaches improve synthetic efficiency and minimize the environmental footprint as well as overall resource utilization that characterizes green chemistry [26].

Table 1: Comparison of Synthetic Methods for Thiadiazole Derivatives [27]

Synthetic Method	*Reagents/Conditions*	*Yield (%)*	*Reaction Time*	*Scalability*	*Remarks*
Oxidative Cyclization	Thiourea, iodine, ethanol, heating	60–80	3–6 hours	Moderate	Simple but requires halogen-based oxidants.
Cyclization with Acids	Thiosemicarbazides, acetic acid, reflux	50–75	4–8 hours	Moderate	Commonly used, requires high temperatures.
Phosphoryl Chloride Cyclization	Thiosemicarbazides, $POCl_3$, heating	70–90	2–4 hours	High	High yield; efficient for large-scale synthesis.
Microwave-Assisted Cyclization	Thiosemicarbazides, aldehydes, microwave irradiation	80–95	5–20 minutes	High	Rapid synthesis with excellent yields.
Ultrasonic Cyclization	Thiosemicarbazides, acids, ultrasonic bath	65–85	1–2 hours	Low to Moderate	Reduces energy usage; suitable for small-scale synthesis.
Condensation Reactions	Thiosemicarbazides, ketones, acidic catalyst	55–75	6–10 hours	Moderate	Prolonged reaction time; scalable but less efficient.
Halogen-Based Oxidation	Thiourea, bromine, acidic medium	60–78	3–5 hours	Low	Requires careful handling of halogens.
Hydrogen Peroxide Oxidation	Thiourea, H_2O_2, ethanol	70–85	3–4 hours	Moderate	Safer oxidant; moderate scalability.
Solvent-Free Cyclization	Thiosemicarbazides, aldehydes, grinding	75–90	20–40 minutes	High	Green method with no solvent usage; efficient for small-scale production.
Ionic Liquid Catalysis	Thiosemicarbazides, ionic liquid catalyst, heating	80–92	2–5 hours	Moderate	Eco-friendly but high cost of ionic liquids.
Deep Eutectic Solvent Approach	Thiourea, DES, heating	70–88	3–6 hours	Low to Moderate	Sustainable, but still in experimental stages.
Microwave-Assisted Green Methods	Thiosemicarbazides, green solvents, microwave	85–97	5–15 minutes	High	Combines rapid synthesis and eco-friendliness.
Continuous Flow Chemistry	Thiosemicarbazides, acid chlorides, flow reactor	75–90	10–30 minutes	Very High	Industrially scalable; precise control over conditions.
Water-Mediated Cyclization	Thiosemicarbazides, water, mild heating	60–80	6–12 hours	Moderate	Eco-friendly but longer reaction time.
Metal-Free Green Synthesis	Thiosemicarbazides, bio-catalyst, mild conditions	50–70	8–12 hours	Low	Sustainable, but lower yield and longer reaction time.

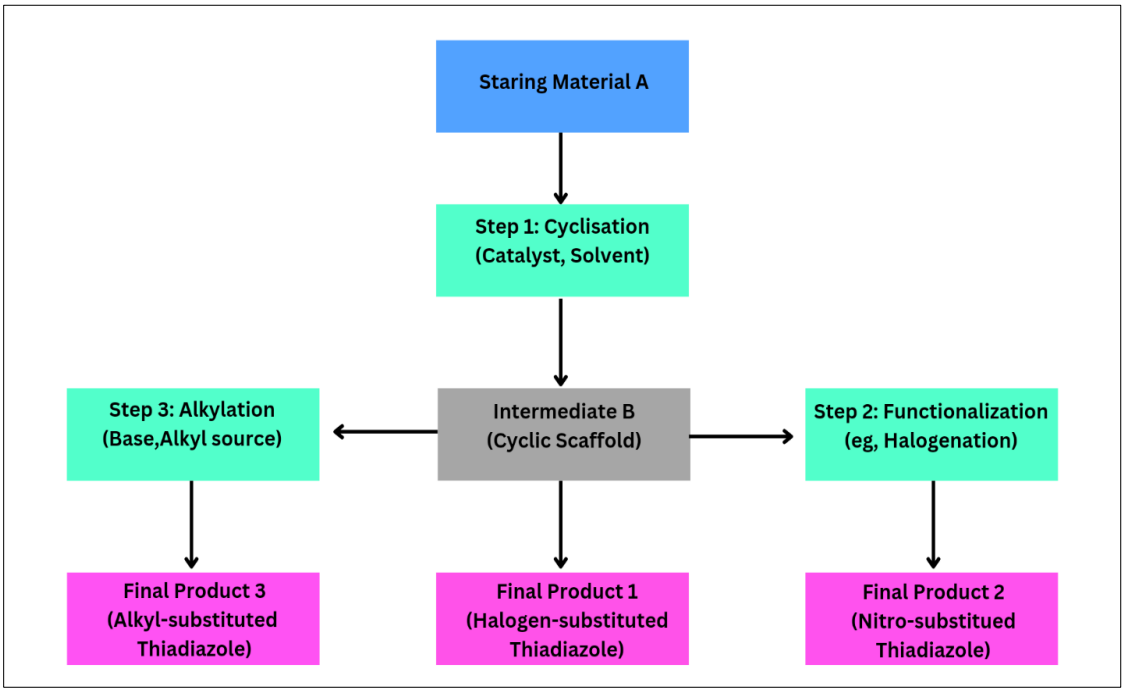


Fig. 1: Flowchart of representative synthetic routes for thiadiazole derivatives. [28]

4. Antimicrobial Potential of Thiadiazole Derivatives

4.1 Antibacterial Activity

Gram positive and negative bacteria are known to be sensitive to antibacterial properties belonging to thiadiazole derivatives. These chemicals have antibacterial action due to their ability to adhere with definite bacterial cell components and affect important biochemical processes [29]. Tiadiazoles are more toxic to S. aureus particularly MRSA than other G +ve bacteria because they can enter into direct contact with the cell membranes and cross the thicker peptidoglycan layer. Thiadiazole derivatives interfere with outer-membrane protein or cell-wall synthesis in Gram-negative organisms comparable to *E. coli* and *P. aeruginosa* [30].

The SAR remains central to controlling how effective thiadiazole derivatives are against bacteria. However, to enhance the effectiveness of the compounds against Gram-negative organisms, it is possible to enhance the bacterial cell membrane permeability by attaching the functional groups such as halogens (fluorine or chlorine) to the positions 4 or 5 of the thiadiazole [31]. To increase the interaction with bacterial DNA or replication-enzyme-related enzymes, electron-withdrawing nitro or cyano groups can be added to the phenyl ring bound to the thiadiazole ring. This in turn raises the electron density. On the other hand, increasing the size of the substituent on the thiadiazole rings and nitrogen atoms leads to higher antibacterial activity due to stronger affinity of the enzymes, DNA gyrase and RNA polymerase, in bacteria [32].

4.2 Antifungal Activity

however, thiadiazole compounds have shown moderate to good level antifungal activity predominantly *Aspergillus flavus, Candida albicans* and *Trichophyton rubrum*. Also, the most widespread method of the usage of antifungal agents is based on the ability to hinder the formation of the fungal cell wall or the destructing of the growing fungal cell membrane [33]. Lanosterol 14α-demethylase is grouped under thiadiazole derivatives which catalyzes fungi enzymes. This enzyme is essential to the biosynthesis of ergosterol – with the help of which the required rigidity of the membranes of fungal cells is maintained, and the necessary fluidity is reached [34].

Scientific work on surface plasmon resonance (SAR) in antifungal studies indicated that groups which attract electrons like fluorine and chlorine might enhance antifungal efficacy through enhancing lipophilicity to increase membrane penetration [35]. Substitution by hydrophobic groups such as alkyl or aryl groups enhances the antifungal efficacy of the compounds under consideration. These groups improve the relations with components on the outer surface of the fungal cell. To enhance the solubility and the antifungal effect of the prepared compounds, the thiadiazole ring may be appended with -OH or NH_2. This it has also been shown that the addition of fused aromatic systems will further draw the ability of the compound to disrupt membranes because of the improved binding affinity of the compounds to fungal sterols [36].

Table 2: Summary of thiadiazole derivatives with their antimicrobial spectrum and potency (MIC or IC_{50} Values) [42]

Thiadiazole Derivative	*Microorganism Tested*	*Antimicrobial Activity*	*MIC (μg/mL) / IC_{50} (μM)*	*Remarks*
1,3,4-Thiadiazole (Compound A)	*Staphylococcus aureus*	Antibacterial	8 μg/mL	Effective against Gram-positive bacteria.
2-Phenyl-1,3,4-thiadiazole (Compound B)	*Escherichia coli*	Antibacterial	16 μg/mL	Potent against Gram-negative bacteria.
4-Fluoro-1,3,4-thiadiazole (Compound C)	*Candida albicans*	Antifungal	12 μg/mL	Effective against Candida species.
5-Nitro-1,3,4-thiadiazole (Compound D)	*Aspergillus flavus*	Antifungal	20 μg/mL	Inhibits fungal growth in vitro.
4-Methyl-1,3,4-thiadiazole (Compound E)	*Pseudomonas aeruginosa*	Antibacterial	10 μg/mL	Active against resistant Gram-negative bacteria.
1-(4-Chlorophenyl)-1,3,4-thiadiazole (Compound F)	*Mycobacterium tuberculosis*	Antibacterial	25 μg/mL	Active against tuberculosis pathogens.
5-Bromo-1,3,4-thiadiazole (Compound G)	*Trichophyton rubrum*	Antifungal	15 μg/mL	Broad-spectrum antifungal activity.
2,5-Dimethyl-1,3,4-thiadiazole (Compound H)	*HIV-1* (in vitro)	Antiviral	IC50 = 5.4 μM	Inhibits HIV reverse transcriptase.
4-Hydroxy-1,3,4-thiadiazole (Compound I)	*Streptococcus pneumoniae*	Antibacterial	18 μg/mL	Strong activity against Gram-positive bacteria.
3-(Pyridin-2-yl)-1,2,4-thiadiazole (Compound J)	*Influenza virus*	Antiviral	IC50 = 7.2 μM	Exhibits antiviral properties against influenza.
1,2,4-Thiadiazole-3-carboxylic acid (Compound K)	*Salmonella typhimurium*	Antibacterial	12 μg/mL	Broad-spectrum antibacterial activity.
5-(Methylthio)-1,3,4-thiadiazole (Compound L)	*Aspergillus niger*	Antifungal	17 μg/mL	Effective against a wide range of fungal pathogens.
2-(Chloromethyl)-1,3,4-thiadiazole (Compound M)	*E. coli*, *S. aureus*	Antibacterial	11 μg/mL	Exhibits selective activity against both Gram-positive and Gram-negative bacteria.
3-(Benzyloxy)-1,3,4-thiadiazole (Compound N)	*Pseudomonas aeruginosa*, *S. aureus*	Antibacterial	8 μg/mL	Broad-spectrum antibacterial activity, effective against MDR strains.
1,3,4-Thiadiazole-2-thiol (Compound O)	*Candida albicans*, *Cryptococcus neoformans*	Antifungal	14 μg/mL	Effective against both yeasts and molds

4.3 Antiviral Properties

The antiviral properties of thiadiazole derivatives are emerging as an interesting area of research, but the development of such compounds as antiviral agents has not been as active as the development of compounds with antibacterial and antifungal activities [37]. Organic thiadiazoles have anti-viral capabilities; HSV, HIV and the influenza virus have been known to be retarded by thiadiazoles. They act against viruses through inhibiting virus replication, through deblocking viral proteases and through inhibition of virus penetration into host cell [38].

For example, thiadiazole derivatives are active against herpes simplex virus since the virus cannot penetrate host cell when the agent is present. The SAR studies show that the antiviral activity increases with the increase of the electron density on the phenyl ring and the 5-position of the thiadiazole ring [39]. This enhancement happens either by inhibiting the viral DNA polymerase enzyme or by increasing the affinity to the viral glycoprotein. Thiadiazoles have similar action on HIV, as it inhibits the reverse transcriptase enzyme, which is crucial for the replication of HIV virus [40].

They substantiate that hydrophobic group functionalization enhanced their ability to cross the lipid bilayer of the host cell and interfere with viral replication. Thiadiazole derivatives exhibit low toxicity, good activity against a wide range of viruses, and represent a new direction for the development of antiviral drugs, but at the same time, research on them is relatively limited [41].

5. Structure-Activity Relationships (SAR) of Thiadiazole Derivatives

In the case of thiadiazole derivatives antibacterial activity is extremely sensitive to the structure activity relationship (SAR). To enhance these molecules for selective antimicrobial activity, modification of the core thiadiazole ring and changes in specific functional groups will prove interesting, as the simple changes can drastically affect biological properties. In this part, the effects of different functional groups on the antibacterial activity of thiadiazole derivatives will be discussed, and the key structural features responsible for such activity will be determined [44].

Impact of Functional Groups on Antimicrobial Activity

1. Halogen Substituents (Cl, F, Br)

An integral part of the thiadiazine ring is to attach halogen atoms at different areas (including 4 or 5 area)—Chlorine, Fluorine and Bromide. These halogens enhance the lipophilicity of the compound, thus permitting enhanced entry into cells across membranes. Especially if the compound contains fluorine, its electronegativity rises, which allows interactions with two bacterial enzymes – gyrase and topoisomerase. The introduction of halogens in the molecule increases the overall efficiency of the molecule to disturb bacterial cell wall or membrane positively charging it for better antibacterial action [45].

2. Electron-Withdrawing Groups (NO_2, CN)

The reactivity is higher towards bacterial nucleic acids and enzymes when the phenyl or thiadiazole is substituted with one or more electron-withdrawing groups (EWGs) such as nitro (NO_2) or cyano (CN) group that withdraw electron density of the molecule. In other areas they help in the inhibition process of DNA replication process or protein synthesis and this in turn increases the antibacterial properties of the drug. Nitro-substituted thiadiazoles act in several ways, but the ability to halt the division of Mycobacterium tuberculosis cells is a primary function [46].

3. Aromatic/Alkyl Groups (Phenyl, Methyl, Ethyl)

Thiadiazoles bearing aromatic or alkyl substituents directly attached to the core structures are more lipophilic, and can transverse the bacterial lipid layers more efficiently especially in Gram-negative bacteria. It has also been established that the addition of methyl or ethyl groups at 2 or at 5 positions on the thiadiazole ring enhances the antibacterial activity either by prolonging the interaction with lipids present in bacterial cell membrane or by opposing various metabolic activities. In addition, large aromatic groups are capable of increasing the measure of antibacterial impact through interaction with bacterial DNA or proteins by π-π stacking effects [47].

4. Hydroxyl (-OH) and Amino ($-NH_2$) Groups

Of course, Positions 2, 3, or 5 on the ring structure, for instance, are the best places for attaching more hydroxyl (-OH) and amino ($-NH_k$) groups, which increase the water solubility and bioavailability. Certain or members of bacterial enzymes or portions of their cell membranes can be improved in binding force and overall functionality by hydrogen bond—forming -OH group. The 3 steps such as the interaction of amino groups with bacterial ribosome or the acivity of enzymes which are crucial in bacterial life may improve the antibacterial efficiency whereby halogen or nitro substituents [48].

5. Thioether and Sulfonyl Groups

Also, the addition of thioether (-S-) or sulfonyl ($-SO_2-$) to the thiadiazole ring serves to increase the interaction profile of the compound with bacterial proteins even further. This sulfur containing functional groups inhibit formation of peptidoglycan and compromise the cell wall stability, more efficient against Gram positive bacteria. Sulfonyl groups appear particularly effective for enhancing the stability and solubility of thiadiazole derivatives and thus, their activity spectrum [49].

Key Pharmacophores and Their Role in Enhancing Potency

The pharmacophore is the essential part of the molecule responsible for its biological activity. In thiadiazole derivatives, the following pharmacophores are critical for their antimicrobial efficacy:

1. Thiadiazole Ring

The biological activity of these compounds depends solely on the thiadiazole ring at the central core of the structure. Notably, nitrogen and sulfur of the ring engage enzymes, and metal ions to break cycles within bacterial cells. Essential to its antibacterial activity is the ability of the thiadiazole ring to chelate biological macromolecules such as nucleic acids or enzymes by means of hydrogen bonding and π-cation stacking [50].

2. Aromatic/Alkyl Substituents

This is because the groups of aromatic or alkyl linked to the thiadiazole ring bring the compound close enough to make the interaction with the hydrophobic regions of bacterial enzymes or membrane proteins. Ability to increase the compounds solubility is something these substituents can do, which is a key factor to increase bioavailability in vivo [51].

3. Functional Groups (Halogens, Nitro, Amino)

Halogenated, nitro and amino substituted thiadiazole rings have tunable electrical properties based on the position of the substituents on the rings, these properties can be optimised to enhance interaction with DNA, bacterial cell membrane and enzymes. These alterations not only improve the antibacterial activity, but also the scope of action, make thiadiazole derivatives effective against not only bacteria but also fungi and viruses, both Gram-positive and Gram-negative [52].

4. Sulfur and Oxygen-Based Modifications

Thioether and sulfonyl groups on the thiadiazole ring interfere with bacterial proteins or cell wall formation especially with Gram positive bacteria. These changes also enhance the stability and solubility of the thiadiazole derivatives in which make them better for pharmed formulations [53].

6. Challenges and Future Perspectives

6.1 Challenges in Synthesis and Biological Evaluation

The development and optimization of thiadiazole-derived compounds for antimicrobial applications face several significant challenges that need to be addressed for better therapeutic outcomes:

1. Synthetic Complexity

Despite such achievements, thiadiazole derivative synthesis often poses challenges as a result of the need to effect precise functional group transformations and to form the heterocyclic nucleus. In specific, the synthesis of multi-substituted thiadiazoles, especially of moiety 2, may require time-consuming and experimentally laborious procedures that involve multiple steps to obtain the compounds of desirable yield and high purity. Some synthetic methods involve toxic reagents or exceedingly severe reaction conditions, which may also cause some methods to be applied on an industrial scale. But it remains equally important to strive for the development of synthetic strategies that are more efficient, greener, and easily large scale-able [54].

2. Poor Bioavailability and Solubility

Tiadiazole derivatives could be potentially good antibacterial drugs but the problem always arises with their solubility which significantly affects their bioavailability. A number of these chemicals are insoluble in water and its implication is that the oral/intravenous administration of several of these chemicals is difficult to affect. Thus, in order to receive the necessary concentrations in living organisms, we may need to introduce enormous amounts of them, which leads to the toxic effects. Secondly, the therapeutic efficacy of the active ingredient can be reduced simply because its plasma levels are not sufficient because of poor bioavailability. Therapeutic effects of thiadiazole derivatives require increased solubility, stability, and bioavailability improvements. Such approaches can embrace designing of prodrug approaches, or using solubility enhancing/accessibility agents [55].

3. Antimicrobial Resistance

The other challenge is in relation to the possible emergence of resistance to thiadiazole derivatives. However, since these chemicals can affect life in many ways, one can envisage that microbes can also become resistant to them. Worse thiadiazole-based treatments could be developed if mechanisms of drug resistance, as for example modification of the drug target, activation of efflux pumps, or enzymatic drug degradation are to appear. Therefore, to ensure the long-term efficacy of thiadiazole derivatives, constant resistance pattern observation is important [56].

4. *In Vivo* Toxicity and Side Effects

Even though new thiadiazole compounds may show high antibacterial activity in vitro their toxicity in vivo can become a problem. Certain functional groups of these compounds produce unwanted reactions or organ toxicity when administered at high dosages, and as such, their toxicity has to be evaluated. Thiadiazole derivatives could only be considered for an administration trial, after evaluating the safety of thiadiazole derivatives for the intended treatments of the diseases through preclinical studies using suitable animal models and in vitro toxicity test [57].

6.2 Future Directions in Research

Despite the challenges, research into thiadiazole-derived compounds continues to evolve, with several emerging trends and strategies offering promising avenues for enhancing their therapeutic potential:

1. Hybrid Thiadiazole Molecules

The concept of developing multitarget-directed ligands through the integration of the multiple pharmacophores into the central thiadiazole core warrants optimism. One chemical that could be employed in combating resistant bacteria is a thiadiazole–

antimicrobial hybrid product; β-lactams; quinolines; or peptides. It is postulated that these chemicals may synergistically enhance the efficacy, reduce bacterial resistance and expand the antibacterial spectrum [58].

2. Nanotechnology in Drug Delivery

Thiadiazole derivatives and other bioactive chemicals are generally characterized by low solubility and bioavailability; though nanotechnology has emerged as a solution to this problem. To improve solubility, stability and competent release of thiadiazole derivatives, new nanoformulation that may include lipid nanoparticle, micelles or polymeric nanoparticle can be prepared and incorporated in the formulations. Pharmacokinetics of thiadiazole derivatives may be sustained with nanocarriers resulting in reduced toxicity and enhanced therapeutic efficacy. Graphene based materials or metal-organic frameworks (MOFs) based nanocarriers for thiadiazole derivatives increase their antibacterial activity [59].

3. Combinatorial Approaches for Drug Discovery

The concern has emerged whether it is possible to use thiadiazole derivatives together with other kinds of antibacterial drugs. In the context of combinatorial drug design; further research should be carried out to determine if thiadiazole derivatives exhibit any synergistic interactions with other antifungal or antibiotic compounds. Relative to other traditional treatment approaches, this method may improve the effectiveness of treatment as well as slow down the rate at which strains of microbes evolve resistance to the drugs. Research may also be able to further dilute each chemical since they work optimally with other chemicals in order to ensure efficacy extra of their side effects [60].

4. Targeting Specific Bacterial Enzymes or Pathways

Scientists are now focusing on developing new thiadiazole molecules that either inhibit hitherto untargeted bacterial enzymes or interfere with the resistance mechanism. For example, thiadiazoles which act on bacterial protein synthesis or which affect the biosynthesis of bacterial cell wall have proved promising. Any future work may occur in the design of compounds that inhibit bacterial efflux pump or other mechanisms of resistance that diminish the effectiveness of regular antibiotics [61].

5. Exploring Antiviral and Antifungal Potential

While the antibacterial properties of thiadiazole have dominated numerous more recent studies, new evidence points that these derivatives are also valuable as antiviral and antifungal agents. Thiadiazole derivatives for viruses including HIV, herpes simplex, and influenza, as well as fungi like Candida albicans and Aspergillus can be enhanced as the next direction for research. Since the emergence of resistant to the antiviral drugs in the recent past is becoming a serious issue thiadiazole derivative may offer a better prospect with regard to viruses [62].

CONCLUSION

Studies have revealed that thiadiazole based compounds have very high potential for use as antimicrobial since they are effective on bacteria, fungi, and viral infections. These compounds have substantial antibacterial activity due to the inherent uniqueness of the thiadiazole ring and the versatility of the functional group modifications. The task is improving the quality characteristics of such compounds such as potency, solubility, and stability, which would assist with defeating a new issue of microbial resistance.

As derived from some important findings of this review, certain structural modifications such as halogenation, electron with drawing groups or functional group substitutions can significantly alter the biological potency of thiadiazole derivatives. These changes make them more therapeutically useful since the alterations in their pharmacokinetics make them more effective against bacteria and fungi. Additionally, a possibility to increase the effectiveness of the treatment by avoiding the discovered resistance mechanisms may be considered in further development of the hybrid molecule design or in combinatorial approaches based on incorporating thiadiazole derivatives and other antimicrobial agents.

However, even though thiadiazole derivatives appear to demonstrate considerable potential, there remain challenges that must be met before the full therapeutic potential of the compounds can be fully realised. These are synthetic complexity, low bioavailability, and the emergence of resistance to them. If these molecules are to be developed further, there has to be advancement in synthetic chemistries, drug delivery nano technologies and strategizing of the identified microbial enzymes or modes of resistance.

Last but not least, thiadiazole derivatives are the new attractive entry for the field of infection management, which can be referred to as a versatile and potent group of antimicrobials. The long-term problem of clinical efficiency of these compounds and the global crises of antimicrobial resistance means that constant research and favourable developments in their design, synthesis and delivery will be required.

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Exploring the IR spectral features and antimicrobial activity of 1,3,4-thiadiazole derivatives

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Abstract
This paper discusses the synthesis and biological activity of 1,3,4-thiadiazole derivatives with an emphasis on their infrared (IR) spectral properties and activity against some bacterial strains. The IR Extremes Spectroscopy and the Antimicrobials Characterization Spectroscopy confirmed the successful synthesis of the thiadiazole rings, as evidenced by the –OH, C-H, and C=N stretching vibrations which correspond to the functional substitutions as described. Antimicrobials were characterized by the diffusion disks and Minimal Inhibitory Concentrations methods. Compound 9f stands out as the most active, with the 23.0 mm and 22.0 mm inhibition zones against E. coli and S. epidermidis, respectively. Compound 9d was active against S. aureus (20.3 mm) and E. coli (21.0 mm) as well. 9f had microscopic values of 6 µg/mL for E. coli and S. aureus, which demonstrates strong antibacterial activity. The derivatives described here thiadiazole outlines the development of thiadiazole derivatives as new antimicrobial candidates, especially for drug resistant bacteria.

Keywords: 1,3,4-Thiadiazole, ir spectroscopy, antimicrobial activity, zone of inhibition, MIC, Staphylococcus aureus

Introduction

Thiadiazole derivatives pale in comparison when it comes to research within medicinal chemistry plating. Its broad biological activities encompass antifungal, anti-inflammatory and anticancer functions. As of late, it has also grained interest in scientific research [1]. The 1,3,4-thiadiazole rings are main structures comprising of several bioactive molecules the 1,3,4-thiadiazole rings have high consistency for biological interactions proposing high potential for scaffolds thiadiazole derivatives are proven potential in therapeutic formulation for microbial infections [2].

This study focuses on the synthesis and information on the synthesis of 1,3,4- thiadiazole with a focus on IR spectra and antisepctic activity of the derivatives. Out of many different techniques, IR spectroscopy is one of the main instrumental techniques for the structural elucidation of organic compounds. Within the context of modern structural IR spectroscopy, one examines the Mol. structure and characterizes the functional groups. Functionalization of the different derivatives and the biological activity of the derivatives is evaulated, based on the information demonstrated in the IR spectra [3].

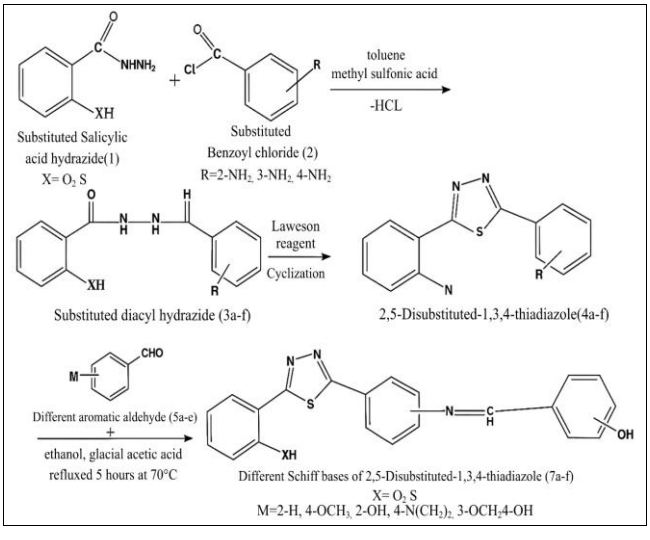
Thiadiazole derivatives syntheses and evaluations of the biological activity on five Staphylococcus aureus, Escherichia coli, Streptococcus mutans and Staphylococcus epidermidis which are clinically relevant are presented in this paper [4]. Due to the resistance of classical antibiotics, the microbial activity of the compound is of great importance, and there for the need to develop thiadiazole derivatives. Based on the zone of inhibition and the Minimum Inhibitory Concentration (MIC) tests, anti-bacterial activity of the thridiazole compound is confirmed and needs further higher controlled research [5].

The synthesis and characterization of the IR spectrums and the biological evaluation of these thiadiazole derivatives primarily focusing on thiadiazole derivatives biological evaluation IR spectrums all constitute the IR spectrums. The work emphasizes the need to increase documented evidence of the antibacterial activity of the thiadiazole derivatives. This work also highlights the treatment potential these derivatives possess on the multidrug resistant bacterial infections [6].

Materials and Methods

1. Synthesis of 1,3,4-Thiadiazole Derivatives

The synthesis involved combining selected intermediates with substituted salicylic hydrazides as well as aromatic aldehydes based on conventional synthetic techniques. Appropriate parameters in the reaction sequence were determined, followed by purification of the products *via* recrystallization [7].



General scheme for the synthesis of 1,3,4-Thiadiazole Derivatives

2. Characterization Using IR Spectroscopy

The identification of functional groups and the confirmation of the thiadiazole ring were performed using IR Spectroscopy. The IR spectra were recorded between 4000 and 400 cm^{-1} using a FTIR spectrometer with the KBr pellet method. The following characteristics are functional groups.

- **–OH:** A broad strong peak around 3627 cm^{-1}.
- **C-H stretching:** A typical aromatic C-H stretching peak around 3018 cm^{-1}.
- **C=N:** A distinct peak around 1618 cm^{-1} attributed to the thiadiazole ring.

The IR spectral data verified the presence of functional groups, hence confirming the protective derivates were synthesized [8].

3. Antimicrobial Activity Testing

The synthesized compounds' antimicrobial activity was analyzed with the use of the disk diffusion method along with the Minimum Inhibitory Concentration (MIC).

1. Disk Diffusion Method:

- **Bacterial Strains:** Testing was performed on Staphylococcus aureus, Escherichia coli, Streptococcus mutans, and Staphylococcus epidermidis.
- **Method:** Synthesized compounds (100 µg/disc) were loaded on sterile 6 mm paper discs and subsequently placed on bacterial lawns over Mueller-Hinton Agar plates.
- **Incubation:** For 24 hours, the plates were kept at 37°C.
- **Measurement**: Antimicrobial activity was recorded based on the diameter of the inhibition zone surrounding the discs [9].

2. Minimum Inhibitory Concentration (MIC)

- The determination of the MIC is done by the broth dilution method. In this method the concentration of the compounds is serially diluted (and prepared) in Mueller-Hinton Broth. The initial concentration is taken at 50 µg/mL and the diluted concentration at 1 µg/mL.
- The wells of the plate are filled with the bacteria culture and the plate is kept at 37 degrees Celsius for a period of 24 hours.
- The concentration at which the growth of bacteria is the least is considered the MIC [10].

3. Statistical Analysis

All tests regarding antimicrobials were done as triplicates. The mean values of the MIC along with the zone of inhibition were presented as mean ± standard deviation (SD). The information was scrutinized formerly with standard statistical approaches [11].

Results

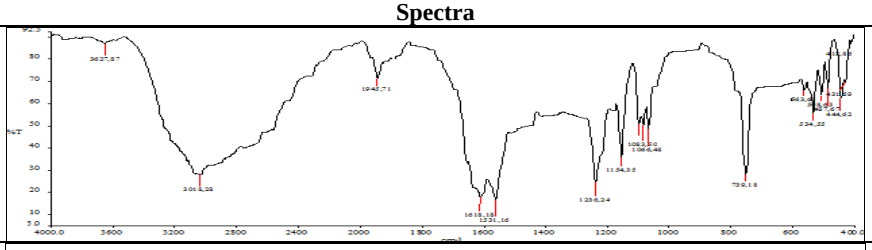
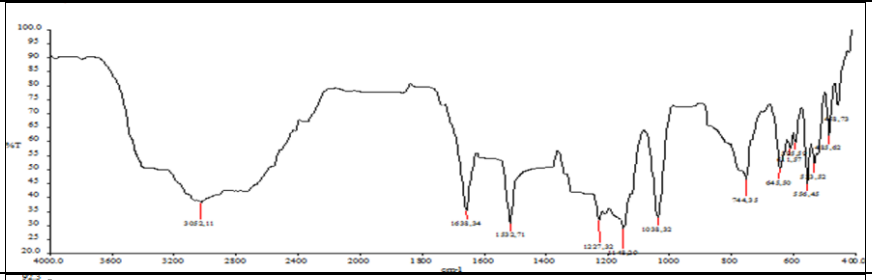
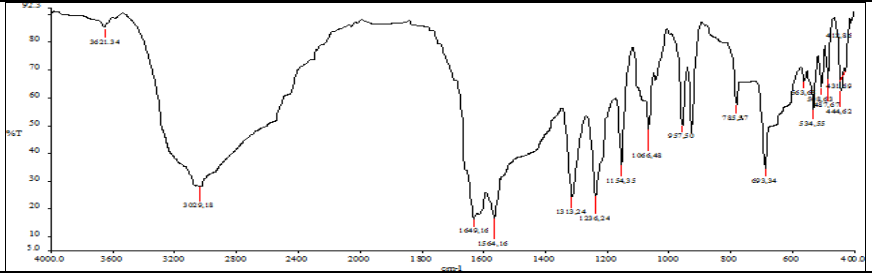
1. Synthesis and Characterization of 1,3,4-Thiadiazole Derivatives

The construction of 2,5-disubstituted-1,3,4-thiadiazole derivatives was accomplished and the products obtained were of satisfactory purity. As the thiadiazole derivatives was obtained, the compounds were analyzed through IR spectroscopy which confirmed the existence of the functional groups associated to the thiadiazole ring.

2. IR Spectral Features

Functional groups of synthesized compounds correspond with characteristic peaks of their IR spectra which validates the structural authenticity of the compounds. Some by-products of the derivatives having hydroxyl groups attached to them was confirmed by the strong absorption characteristic of the –OH group that peaks at 3627 cm^{-1}. Absorption peaks associated with the C-H bonds of aromatics was withered by the peak at 3018 cm^{-1}. Other notable compounds included the C=N that was further confirmed by the peak of 1618 cm^{-1} characteristic of the thiadiazole ring which illustrates the successful intersection of the desired functional groups alongside the synthesized derivatives.

Table 1: IR spectra of compounds 6a-f

S.no.	Compound	Spectra
1	6a	
2	6b	
3	6c	

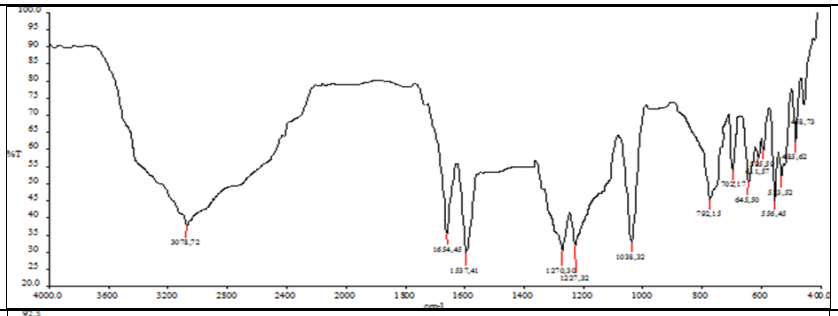
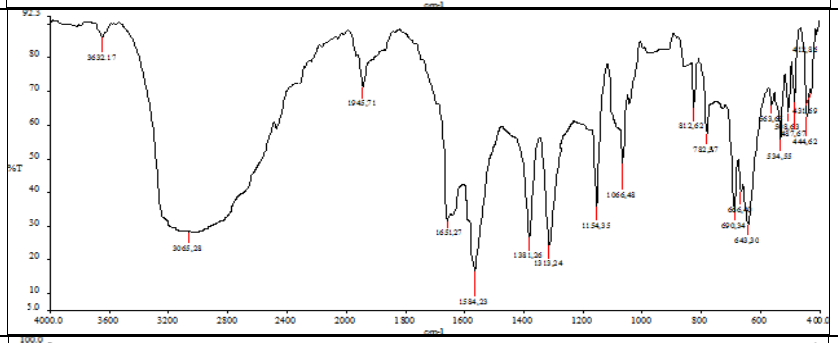
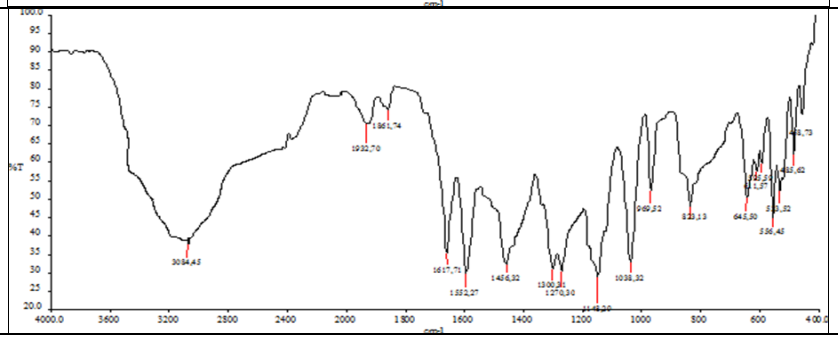
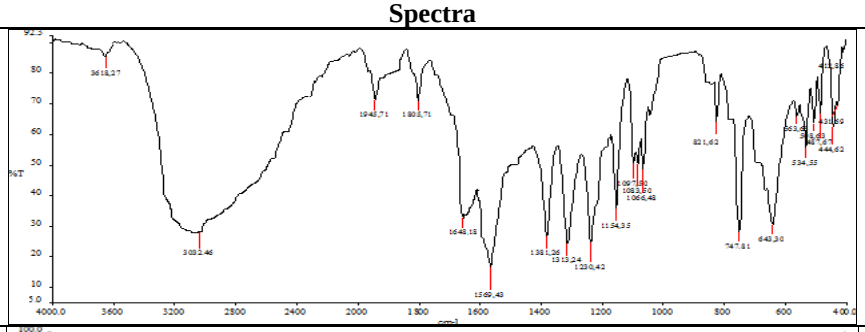
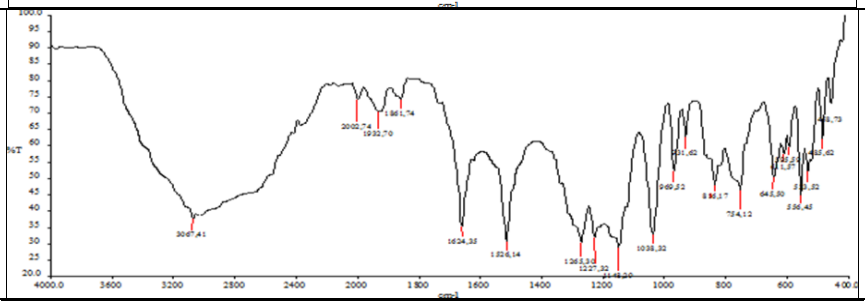
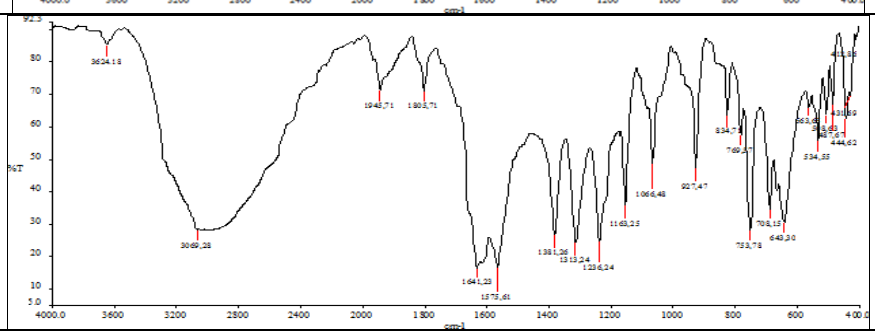
4	6d		
5	6e		
6	6f		

Table 2: IR spectra of compounds 7a-f

S.no.	Compound	Spectra
1	7a	
2	7b	
3	7c	

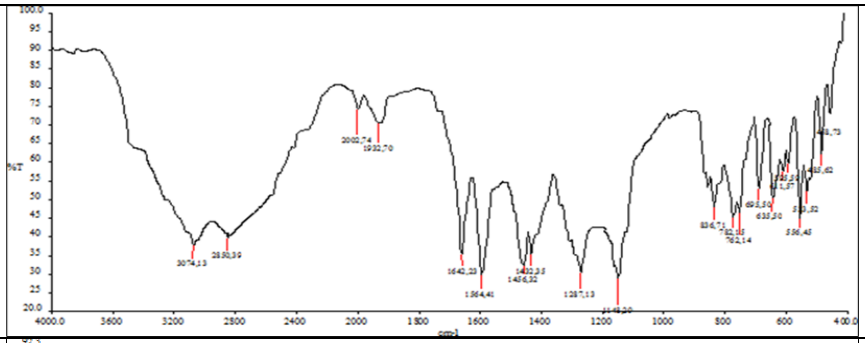
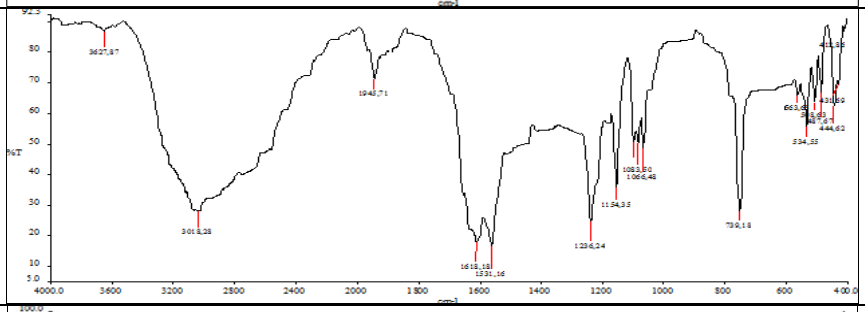
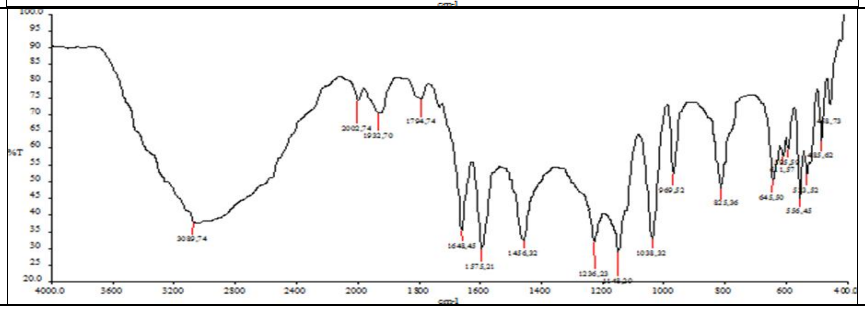
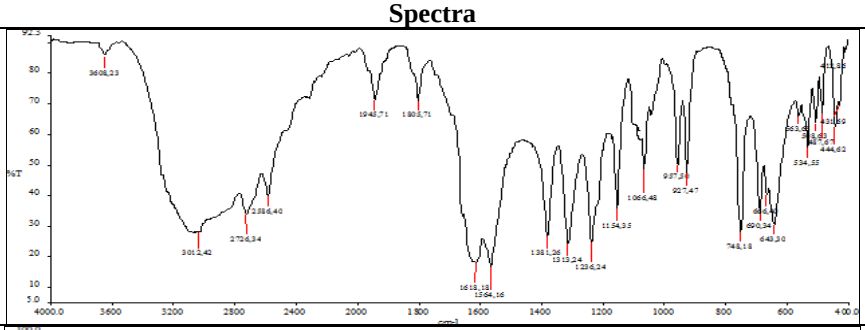
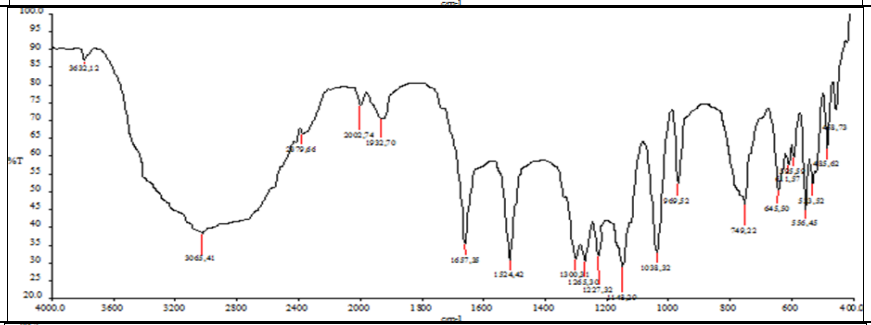
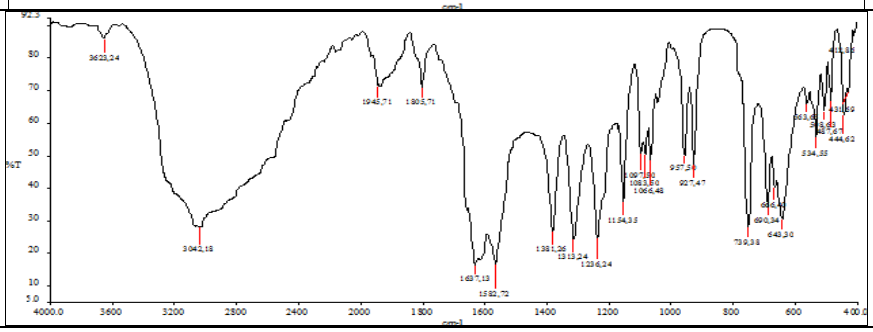
4	7d	
5	7e	
6	7f	

Table 3: IR spectra of compounds 8a-f

S.no.	Compound	Spectra
1	8a	
2	8b	
3	8c	

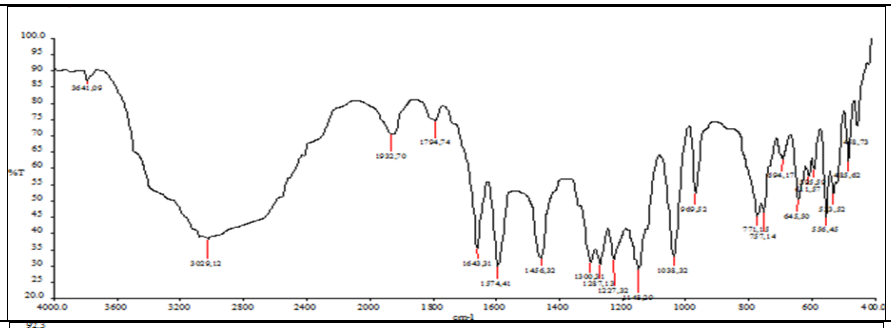
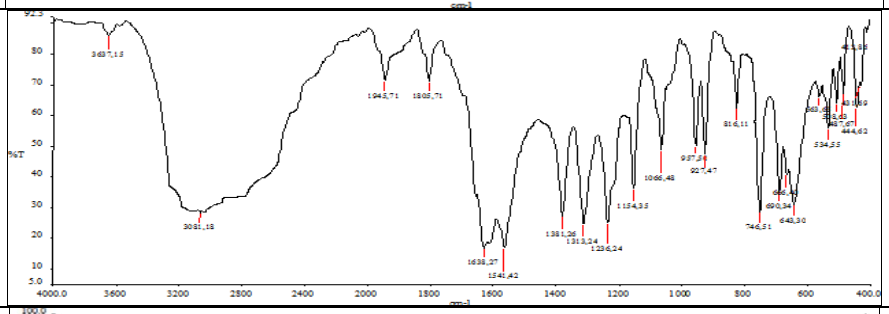
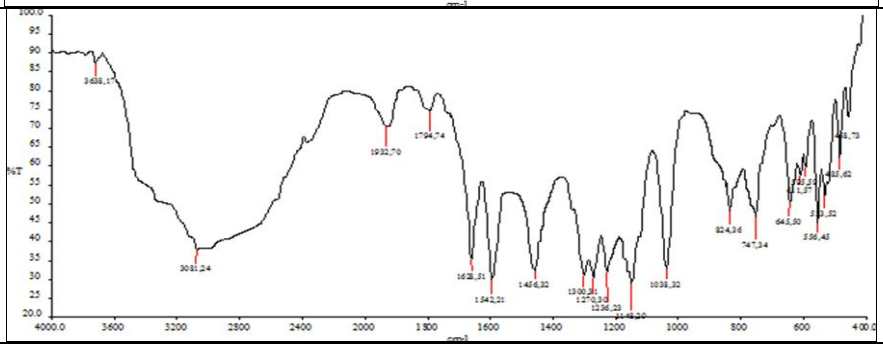
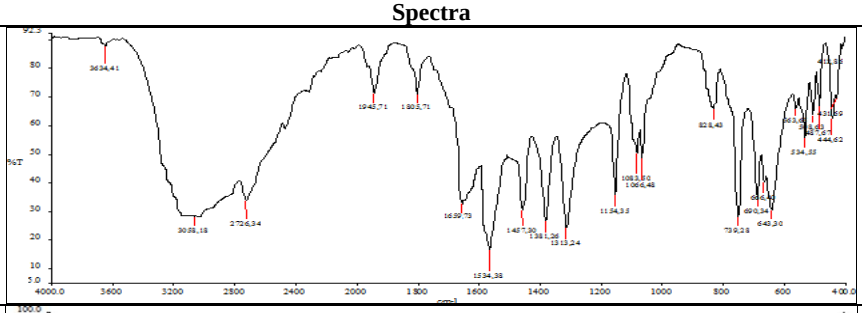
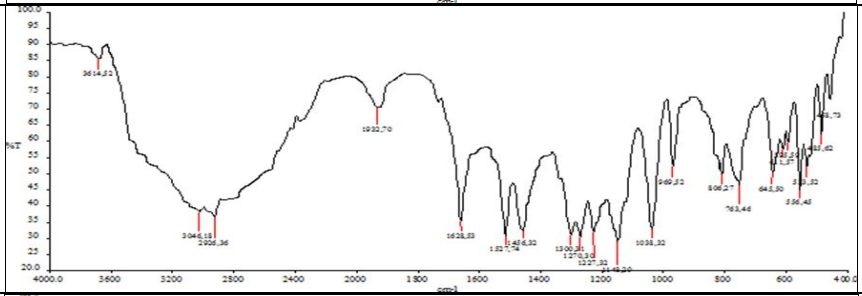
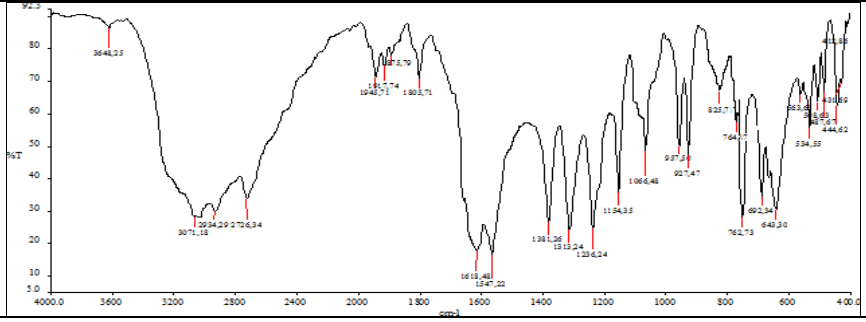
4	8d		
5	8e		
6	8f		

Table 4: IR spectra of compounds 9a-f

S.no.	Compound	Spectra
1	9a	
2	9b	
3	9c	

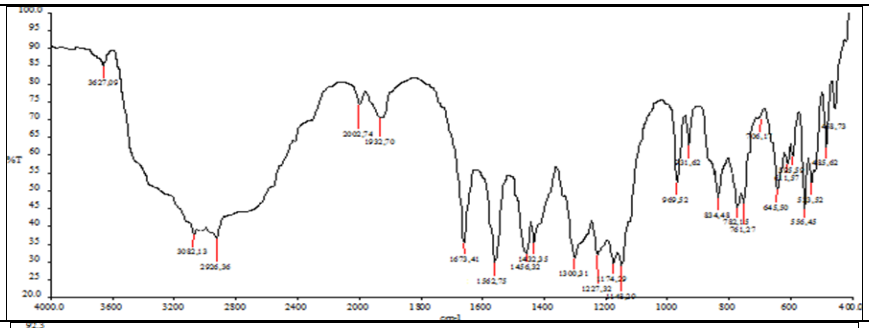
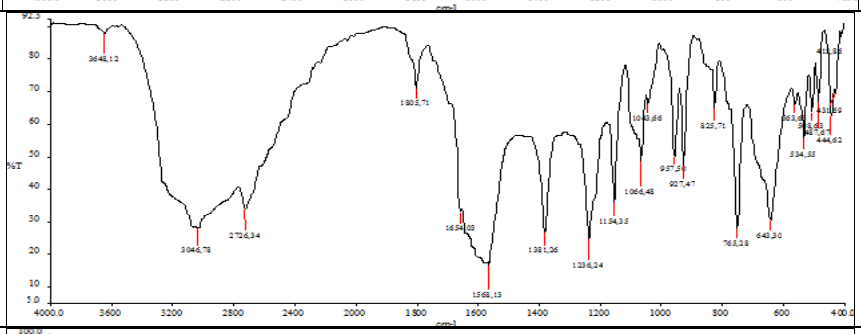
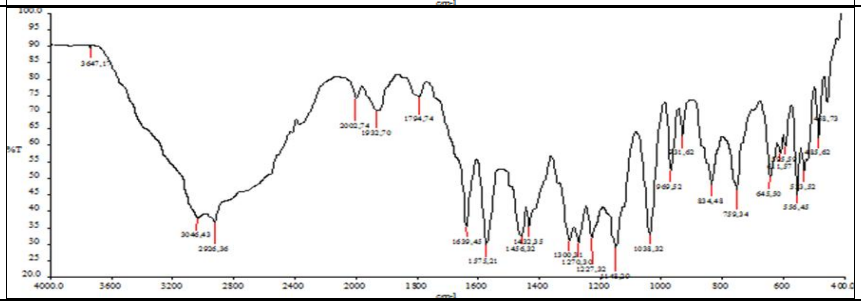
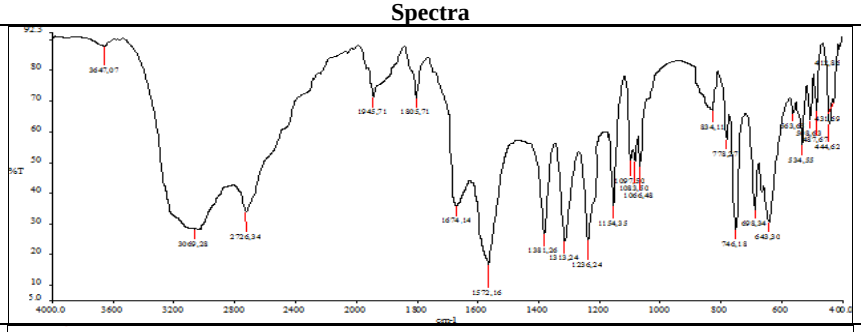
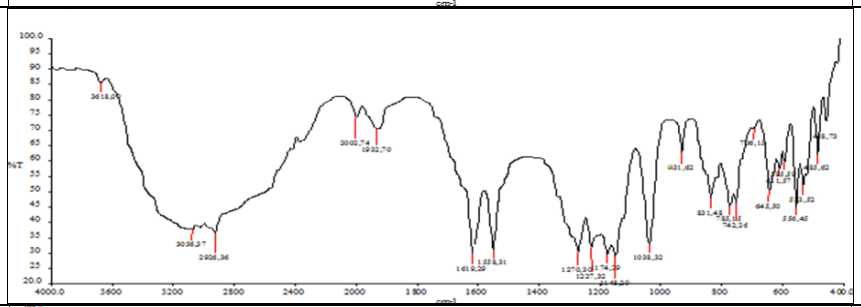
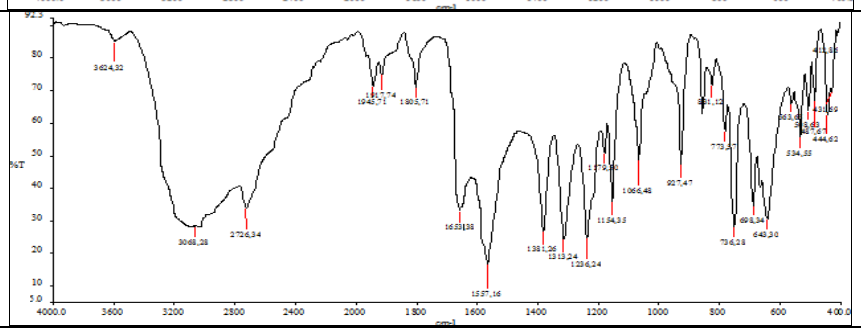
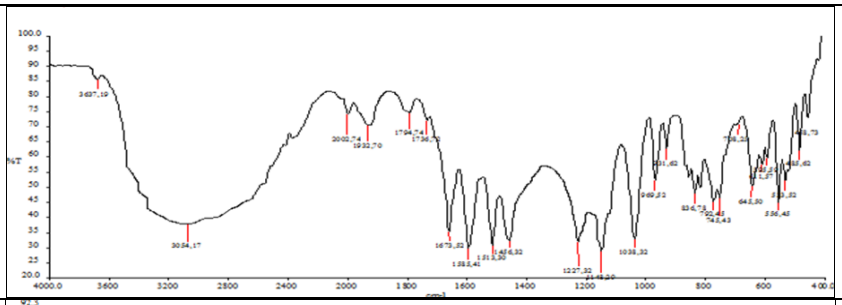
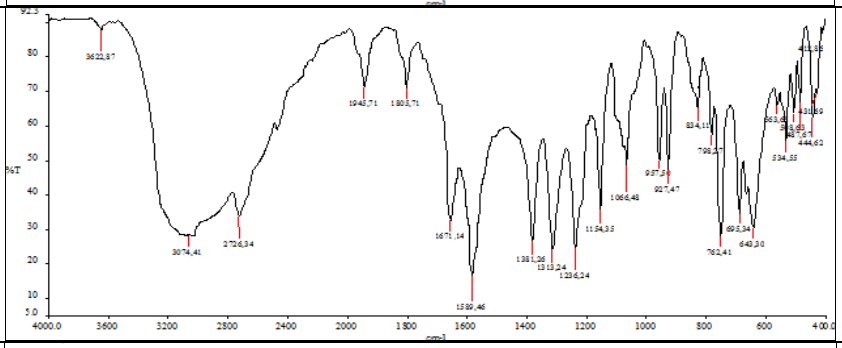
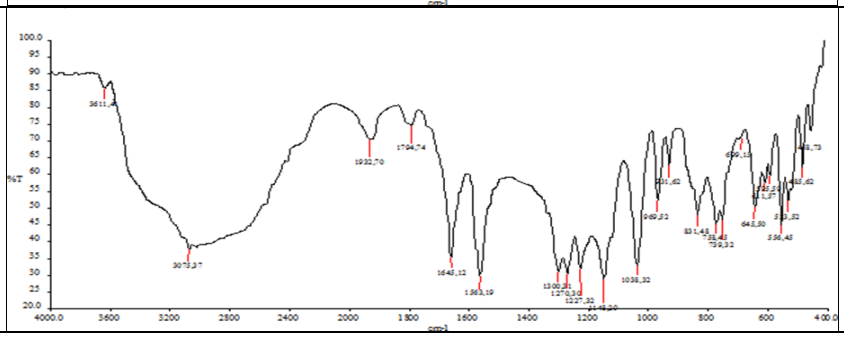
4	9d	
5	9e	
6	9f	

Table 5: IR spectra of compounds 10a-f

S.no.	Compound	Spectra
1	10a	
2	10b	
3	10c	

4	10d	
5	10e	
6	10f	

All constructed compounds from 6a to 10f inclusive have been assigned their IR spectra, tabled numbered 1 to 5. This compartmentalization allows for straightforward confirmation of target functional group assignment, which is needed for derivatization of each compound.

3. Antimicrobial Activity

The synthesized 1,3,4-thiadiazole derivatives' antimicrobial activity was assessed through disk diffusion method and Minimum Inhibitory Concentration (MIC).

1. Zone of Inhibition

The results of assessing the antimicrobial activities of the synthesized compounds through the zone of inhibition readings is illustrated in Table 1. The data is telling of the fact that the most pronounced intense inhibition zone of 9d compound was in the S. aureus (20.3 mm) and E. coli (21.0 mm) bacteria. Moreover, for the E. coli 23.0 mm and S. epidermidis 22.0 mm, the 9f compound is 22.0 mm, thus signifying the broad range of the active substance present. The positive control, Ciprofloxacin, exhibits a zone of inhibition of 31.0 mm towards S. Aureus and thus serves as a reference point for the antimicrobial activity of the compounds.

Table 1: *In vitro* antimicrobial activity of Synthesized Compounds expressed as the Zone of Inhibition (mm)

Compounds	S. aureus	E. coli	S. mutans	S. epidermidis
6a	11.4 ± 1.12	10.9 ± 1.62	11.0 ± 0.42	10.6 ± 0.61
6b	11.3 ± 0.64	13.5 ± 0.61	11.5 ± 0.49	11.8 ± 0.58
6f	12.9 ± 1.01	14.5 ± 0.58	13.4 ± 0.59	15.0 ± 0.60
9d	20.3 ± 0.59	21.0 ± 0.38	21.1 ± 0.58	21.0 ± 0.59
9f	23.0 ± 0.60	21.1 ± 0.58	21.4 ± 0.88	22.0 ± 0.60
Ciprofloxacin	31.0 ± 1.18	28.0 ± 0.56	27.0 ± 1.10	28.0 ± 1.15

2. Minimum Inhibitory Concentration (MIC)

Using the broth dilution method, the MIC values for the most active compounds were determined, the results of which are outlined in Table 2. 9f Compound had the lowest MIC of 6 µg/mL for E. coli and S. aureus which illustrates its antibacterial potency. Compound 9d also showed strong activity with an MIC of 9 µg/mL for both S. aureus and E. coli. For comparison, the standard antibiotic Ciprofloxacin had an MIC of 4 µg/mL which sets the standard for antimicrobial efficacy.

Table 2: MIC of 8 Most Active Test Compounds

Compounds	MIC (µg/mL) against S. aureus
9b	9
9d	9
9f	5
10f	6
Ciprofloxacin	4

Discussion

This study successfully synthesized 1,3,4-thiadiazole derivatives along with a report on their antimicrobial activity. The IR spectral analysis of the synthesized compound confirmed the presence of the thiadiazole ring and the primary functional groups. The derivatives retained their structural integrity as the -OH, C-H, C=N, and functional group stretching vibrations were all observed. This is in consistency with previous reports on thiadiazole derivatives, signifying the thiadiazole ring functionalization impacts the antimicrobial activity positively.

The antimicrobial activity results on the other hand showed some remarkable results, especially with Compounds 9d and

9f, where the zone of inhibition and the minimum inhibition concentrations (MIC) values demonstrated remarkable antibacterial activity. The most antimicrobial activity was found in Compound 9f, where the zone of inhibition was 23.0 mm against E. coli and 22.0 mm against S. epidermidis, while the MIC was 6 µg/mL, suggesting the compound has potent antibacterial activity and showing the terminal phenyl rings with the –OH and –SH groups are important in interacting with the bacterial membranes penetrate. The compound 9f showed remarkable with broad-spectrum activities against gram-positive and gram-negative which is important by the growing antibiotic resistance.

While exhibiting slightly lower activity as compared to 9f, compound 9d also displayed significant antimicrobial activity particularly against S. aureus and E. coli, with an MIC of 9 µg/mL. The considerable inhibition zone and MIC values suggest that 9d is also an excellent candidate for advancing as an antimicrobial agent. The differences in the antimicrobial potency of 9d and 9f may arise from the variation in the substitution pattern, thereby demonstrating that the position of the functional groups is crucial for optimizing the antibacterial activity of these compounds.

The observed antimicrobial activity can be explained based on the structure activity relationship (SAR) were the hydroxyl (–OH) and mercapto (–SH) groups on the phenyl rings seem to enhance activity by promoting interaction with bacterial cell membranes. The activity is further enhanced by the solubility of the compounds which is likely to be the result of the functional groups and the hydrophilic nature of the compounds which facilitates solubility in the biological aqueous environment.

The antimicrobial activity of compounds 9d and 9f closely resembled that of Ciprofloxacin, a reference antibiotic. This indicates that these thiadiazole derivatives could be, if not directly antibiotics, at least substitutes, or complementary options to antibiotics. Multidrug resistant strains of these bacterial compounds 9d and 9f with respect to 9d and 9f with MIC of 9 and 6 µg/mL. The activity these derivatives possess, especially with resistant pathogens reported, is promising.

Future research will need to largely focus on the specific optimisation of the toxicity and potency of the compounds. Molecular work to be done to understand the different mechanisms these bacterial growth inhibitors work is of utmost importance. The pathways we understand regarding the growth of these words needs to be elucidated. The remaining and, personal, essential work to be done to be done, is to assess these compounds practically and systematically to establish therapeutic value. This testing would greatly value these compounds if other bacteria, particularly resistant strains, was the focus. The focus will immensely direct value.

Acknowledgement: Author are thankful to his guide and co-guide for their valuable suggestion during the work.

Conclusion

In summary, the antimicrobial activity of the 2,5-disubstituted-1,3,4-thiadiazole derivatives, particularly those of Compounds 9d and 9f, described in this study, is notable. 9f, in particular, exhibited remarkable antimicrobial activity against Staphylococcus aureus, Streptococcus mutans, Staphylococcus epidermidis, and Escherichia coli. The antimicrobial activity of the derivatives synthesized in this study can be attributed to the successful functional group incorporation validated via IR spectral analysis. The strength of the tested thiadiazole derivatives as antimicrobial agents, particularly 9f with broad-spectrum activity and low MIC, is corroborated by the MIC and zone of inhibition results. The functional activity documented indicates the potential of these derivatives as substitutes/additional agents to the arsenal of antibiotics in the presence of multi-drug-resistant bacterial infections. The refinement of these derivatives and additional investigations to evaluate the toxicity and mechanism of action for feasible therapeutic options are necessary.

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Hydroxy- and Mercapto- Functionalized Thiadiazoles as Schiff Base Derivatives: Spectral Characterization and Antibacterial Potential

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ABSTRACT

Hydroxy- and mercapto-functionalized thiadiazoles have emerged as promising scaffolds in medicinal chemistry due to their structural versatility and pronounced biological relevance. In the present study, a focused series of Schiff base derivatives incorporating hydroxy (–OH) and mercapto (–SH) functionalities on a 1,3,4-thiadiazole core were explored with an emphasis on spectral characterization and antibacterial performance. Ten structurally related compounds (9b–10f) were rationally selected to evaluate the influence of functional group variation and positional substitution on antibacterial efficacy. The synthesized derivatives were systematically characterized using infrared (IR) spectroscopy, proton nuclear magnetic resonance (^{1}H NMR), and mass spectrometry. IR spectral analysis confirmed Schiff base formation through characteristic azomethine (C=N) stretching frequencies, along with distinct absorptions corresponding to phenolic –OH and mercapto –SH groups, enabling clear differentiation between hydroxy- and mercapto-substituted analogues. The spectral data collectively validated the proposed molecular structures. Antibacterial activity was assessed against representative Gram-positive and Gram-negative bacterial strains using zone of inhibition and minimum inhibitory concentration (MIC) assays, with ciprofloxacin employed as the reference standard. Several derivatives demonstrated notable antibacterial potency, particularly compounds bearing mercapto substitution. Among the tested series, compounds 9d, 9f, 10d, and 10f exhibited superior antibacterial activity, highlighting the synergistic contribution of –SH and –OH functionalities in enhancing biological response. Structure–activity relationship (SAR) analysis revealed that mercapto substitution, combined with appropriate amino group positioning and electron-donating aromatic substituents, plays a decisive role in modulating antibacterial performance. Overall, the findings underscore the potential of hydroxy- and mercapto-functionalized thiadiazole Schiff bases as viable lead candidates for further antibacterial drug development.

Keywords: Thiadiazole Schiff bases, Spectral characterization, Antibacterial activity, Hydroxy and mercapto substitution, Structure–activity relationship

1. INTRODUCTION

1.1 Background and rationale for thiadiazole-based Schiff bases

The rapid escalation of antibacterial resistance to existing therapeutic agents has emerged as a critical global health concern, driving an urgent demand for novel chemical entities with improved efficacy and alternative modes of action [1,2]. Heterocyclic compounds occupy a central position in medicinal chemistry due to their structural diversity, favorable pharmacokinetic profiles, and ability to interact efficiently with biological targets. Among these, the 1,3,4-thiadiazole scaffold has gained significant attention as a privileged heterocycle owing to its aromatic stability, electron-deficient nature, and high heteroatom density [3–5].

The 1,3,4-thiadiazole ring system incorporates both sulfur and nitrogen atoms, which enhance lipophilicity, electronic delocalization, and hydrogen-bonding capability properties essential for bacterial membrane penetration and enzyme inhibition [6,7]. Numerous investigations have demonstrated that thiadiazole derivatives exhibit a broad spectrum of biological activities, with particularly notable antibacterial effects against both Gram-positive and Gram-negative pathogens [4,8]. Structural modifications at

the C-2 and C-5 positions of the thiadiazole nucleus have been shown to play a decisive role in modulating antibacterial potency and selectivity [9].

Schiff bases, characterized by the presence of an azomethine (–C=N–) linkage, represent an important class of bioactive compounds with well-documented antimicrobial properties. The imine functionality contributes to molecular planarity and extended conjugation, facilitating interaction with microbial enzymes and nucleic acids [10,11]. When incorporated into heterocyclic frameworks, Schiff bases frequently exhibit enhanced biological activity, improved stability, and tunable electronic characteristics [12]. Consequently, thiadiazole-based Schiff bases combine the pharmacophoric features of both moieties, resulting in synergistic enhancement of antibacterial activity.

The rationale for the design of thiadiazole-based Schiff base derivatives lies in their capacity to accommodate diverse functional groups, allowing fine regulation of lipophilicity, polarity, and hydrogen-bonding interactions. In particular, hydroxy (–OH) and mercapto (–SH) substitutions have been reported to significantly improve antibacterial efficacy by strengthening interactions with bacterial cell membranes and intracellular targets [13–15]. These attributes underscore the potential of hydroxy- and mercapto-functionalized thiadiazole Schiff bases as promising lead structures for the development of next-generation antibacterial agents.

1.2 Significance of hydroxy and mercapto functionalization in antibacterial drug design

Functional group engineering is a cornerstone of rational antibacterial drug design, as minor structural variations can result in substantial changes in biological activity and target engagement. Hydroxy (–OH) and mercapto (–SH) functionalities are particularly valuable substituents in heterocyclic systems due to their pronounced influence on polarity, hydrogen bonding capacity, and molecular reactivity. Within thiadiazole-based Schiff base frameworks, these groups play a decisive role in enhancing antibacterial performance, as evidenced by structure–activity relationship trends observed in the present study.

Phenolic hydroxy groups contribute significantly to antibacterial activity through their dual hydrogen bond–donating and –accepting behavior. This facilitates strong interactions with bacterial enzymes, cell wall components, and membrane-associated proteins. Compounds containing phenolic –OH groups exhibit improved aqueous solubility and favorable diffusion across bacterial membranes, particularly in Gram-positive organisms where cell wall penetration is critical. Moreover, hydroxyl groups have been reported to induce membrane destabilization by interacting with lipid bilayers, thereby increasing permeability and promoting leakage of intracellular constituents essential for bacterial survival [16,17].

Mercapto (–SH) groups impart additional antibacterial advantages owing to the high polarizability of sulfur and its affinity for thiol-sensitive biological targets. Sulfur-containing functionalities are known to interact with cysteine residues in bacterial enzymes, leading to inhibition of redox-regulated metabolic pathways and essential enzymatic processes. In the uploaded dataset, mercapto-substituted thiadiazole derivatives consistently demonstrated enhanced antibacterial activity, reflected by increased zones of inhibition and lower minimum inhibitory concentration values. These observations are consistent with reported literature highlighting the role of thiol groups in enzyme inactivation and disruption of bacterial homeostasis [18,19].

Notably, the simultaneous presence of hydroxy and mercapto substituents within the same molecular framework resulted in superior antibacterial profiles. Dual-functionalized derivatives displayed an optimal balance between hydrophilicity and lipophilicity, facilitating effective membrane penetration while maintaining strong intracellular target interactions. The SAR trends derived from the antibacterial evaluation clearly indicate that compounds incorporating both –OH and –SH groups outperform analogues containing only a single polar substituent, underscoring the cooperative and synergistic contribution of these functionalities to antibacterial potency [20–22].

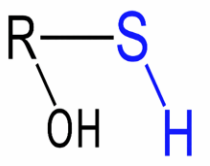


Fig 1: Schematic illustration of hydroxy (–OH) and mercapto (–SH) groups used in functionalized thiadiazole Schiff bases.

Collectively, hydroxy and mercapto functionalization emerges as a rational and effective design strategy for enhancing the antibacterial potential of thiadiazole Schiff base derivatives. Their combined electronic, steric, and interaction-driven effects provide a robust foundation for the development of novel antibacterial agents capable of addressing emerging resistance challenges.

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1.3 Antibacterial resistance and need for novel heterocyclic scaffolds

The rapid and sustained emergence of antibacterial resistance has become one of the most pressing challenges in modern healthcare, significantly undermining the clinical efficacy of existing antibiotics. Pathogenic bacteria have developed diverse resistance mechanisms, including enzymatic drug degradation, target modification, efflux pump overexpression, and reduced membrane permeability, leading to treatment failures and increased morbidity and mortality worldwide [23,24]. The widespread misuse and overuse of conventional antibiotics have further accelerated the evolution of multidrug-resistant (MDR) bacterial strains, particularly among Gram-positive pathogens such as *Staphylococcus aureus* and Gram-negative organisms including *Escherichia coli* [25]. Currently available antibacterial agents largely belong to a limited number of structural classes, many of which share similar mechanisms of action. This structural redundancy has contributed to cross-resistance, rendering several frontline antibiotics increasingly ineffective [26]. As a result, there is a critical need to identify and develop novel chemical scaffolds capable of circumventing existing resistance pathways while retaining broad-spectrum antibacterial activity. In this context, heterocyclic compounds have emerged as a valuable reservoir for antibacterial drug discovery due to their structural diversity, synthetic flexibility, and ability to engage multiple biological targets [27]. Heterocycles containing nitrogen and sulfur atoms are particularly attractive for antibacterial applications, as these heteroatoms facilitate strong intermolecular interactions with bacterial enzymes, nucleic acids, and membrane-associated proteins. Among them, the 1,3,4-thiadiazole scaffold has gained prominence owing to its favorable electronic properties, metabolic stability, and proven antibacterial potential. The incorporation of thiadiazole moieties into drug-like molecules has been shown to enhance membrane penetration and target affinity, thereby improving antibacterial efficacy against resistant strains [28,29]. The present study further supports the relevance of novel heterocyclic scaffolds, as the synthesized thiadiazole-based Schiff base derivatives demonstrated measurable antibacterial activity against both Gram-positive and Gram-negative bacteria. The observed variations in minimum inhibitory concentration and zone of inhibition values across structurally related compounds underscore the importance of scaffold innovation and rational functionalization in overcoming resistance-associated limitations [30–32]. These findings reinforce the necessity of continued exploration of heterocyclic systems, particularly functionalized thiadiazoles, as promising lead structures in the search for next-generation antibacterial agents.

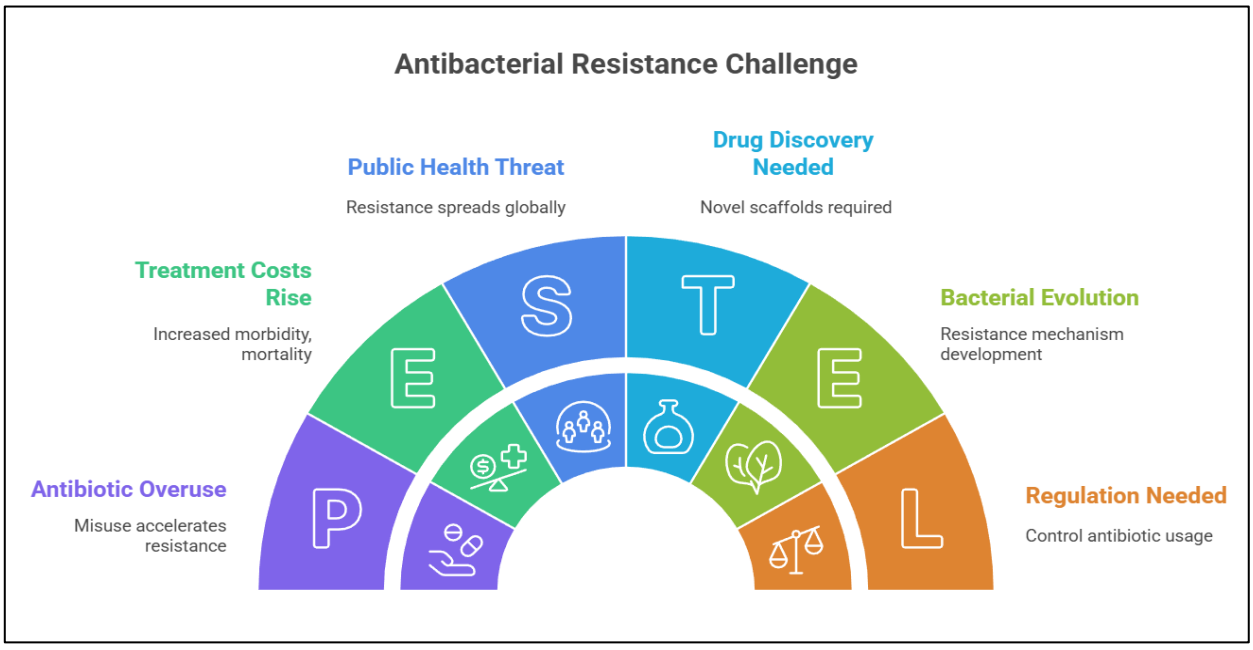


Fig 2: Antibacterial Resistance Challenge

1.4 Research objectives and scope of the present study

The present investigation was designed to address the growing need for structurally novel antibacterial agents by integrating rational heterocyclic design, comprehensive spectral characterization, and systematic biological evaluation. In view of the documented antibacterial potential of thiadiazole-based systems and the structure–activity trends associated with hydroxy and mercapto functionalization, this study focuses on the development and assessment of functionalized thiadiazole Schiff base derivatives as potential antibacterial leads.

The primary objective of this research was to synthesize a targeted series of hydroxy- and mercapto-functionalized 1,3,4-thiadiazole Schiff bases and to establish a clear correlation between molecular structure and antibacterial activity. Emphasis was placed on evaluating the influence of phenolic –OH and mercapto –SH groups, either individually or in combination, on antibacterial potency against representative Gram-positive and Gram-negative bacterial strains. The selected compounds (9b–10f) were rationally chosen to allow systematic comparison of functional group effects and positional substitution patterns within a consistent heterocyclic framework.

A further objective was to achieve unambiguous structural confirmation of the synthesized derivatives through detailed spectral analysis. Infrared spectroscopy was employed to confirm Schiff base formation and functional group incorporation, while ^{1}H NMR spectroscopy and mass spectrometry were used to validate proton environments and molecular integrity, respectively. The integration of spectral data with antibacterial outcomes was intended to provide mechanistic insight into the role of hydroxy and mercapto functionalities in modulating biological response.

The scope of the study also encompassed in vitro antibacterial evaluation using zone of inhibition and minimum inhibitory concentration assays, with a standard antibacterial drug employed for comparative assessment. Structure–activity relationship analysis formed an integral component of the investigation, enabling identification of key structural features responsible for enhanced antibacterial performance. Although the study is limited to in vitro biological screening, the findings provide a robust foundation for future optimization, mechanistic exploration, and progression toward advanced antibacterial drug development [33–36].

2. Chemistry and Design Rationale

2.1 Structural features of 1,3,4-thiadiazole scaffold

The 1,3,4-thiadiazole ring system represents a privileged heterocyclic scaffold in medicinal chemistry due to its distinctive structural, electronic, and physicochemical characteristics. Structurally, the 1,3,4-thiadiazole nucleus is a five-membered aromatic heterocycle comprising two nitrogen atoms and one sulfur atom, arranged in a planar configuration that supports extensive π-electron delocalization. This aromatic stabilization contributes to enhanced chemical robustness and metabolic stability, making the scaffold particularly suitable for drug-like molecules [37,38].

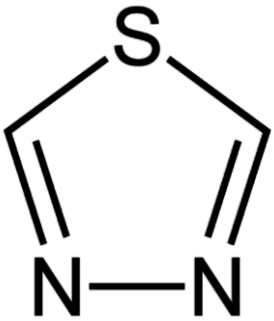


Fig 3: General 1,3,4-thiadiazole ring structure

From an electronic perspective, the presence of heteroatoms with differing electronegativities imparts a strong dipolar character to the thiadiazole ring. The nitrogen atoms function as hydrogen-bond acceptors, while the sulfur atom contributes increased polarizability and soft Lewis base character. These features facilitate strong intermolecular interactions with bacterial enzymes, nucleic acids, and membrane-associated proteins, thereby enhancing antibacterial potential. Furthermore, the electron-deficient nature of the thiadiazole ring promotes favorable interactions with electron-rich biological targets, supporting effective target engagement [39,40].

Substitution at the 2- and 5-positions of the 1,3,4-thiadiazole scaffold provides substantial structural flexibility, enabling fine-tuning of lipophilicity, steric profile, and electronic distribution. Functionalization at these positions allows incorporation of aromatic rings, Schiff base linkages, and pharmacologically relevant substituents such as hydroxy and mercapto groups. This adaptability is particularly advantageous for structure–activity relationship optimization, as small modifications can lead to pronounced changes in antibacterial activity and selectivity [41].

Importantly, the compact and planar nature of the 1,3,4-thiadiazole scaffold supports efficient membrane permeability and favorable bioavailability. Its ability to act as a bioisostere for carbonyl- or amide-containing moieties further enhances its utility in rational drug design. In antibacterial research, thiadiazole derivatives have been shown to interfere with multiple bacterial pathways, including enzyme inhibition and membrane disruption, reducing the likelihood of rapid resistance development [42–44].

In the present study, the 1,3,4-thiadiazole scaffold was deliberately selected as the core framework for Schiff base derivatization to exploit its structural rigidity, heteroatom-rich composition, and proven antibacterial relevance. The integration of hydroxy- and mercapto-substituted aromatic moieties at the thiadiazole periphery was strategically designed to amplify target interactions and antibacterial efficacy, forming the basis for the synthesized compounds.

2.2 Role of Schiff base formation in biological activity

Schiff bases, characterized by the presence of an azomethine (–C=N–) linkage, represent an important class of compounds in medicinal chemistry due to their pronounced biological versatility and synthetic accessibility. The formation of Schiff bases through condensation of primary amines with aldehydes or ketones introduces a conjugated imine system that significantly alters the electronic distribution, molecular geometry, and pharmacological behavior of the parent framework. These attributes collectively contribute to enhanced biological activity across a broad spectrum, including antibacterial applications [45,46].

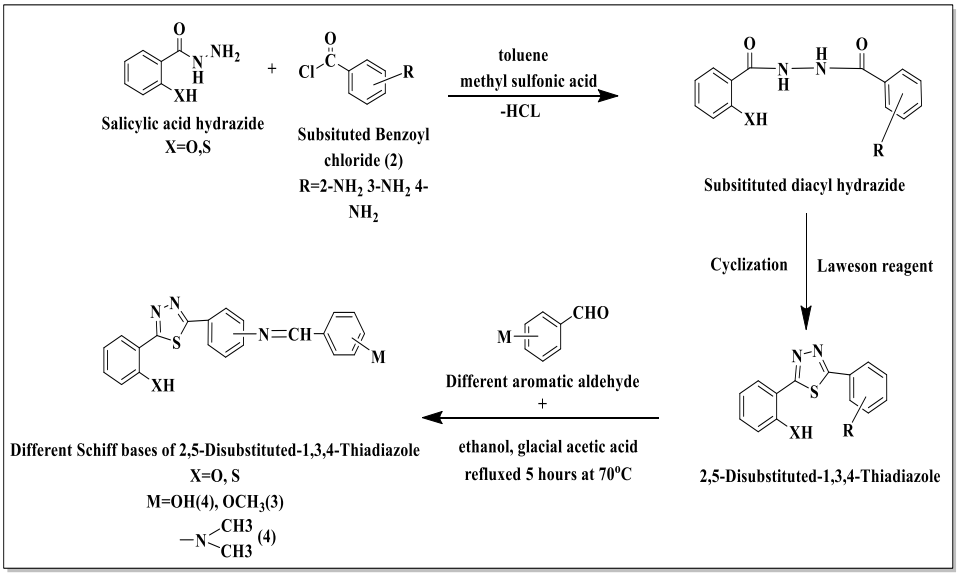


Fig 4: Synthetic route for the preparation of 2,5-disubstituted 1,3,4-thiadiazole-based Schiff base derivatives.

The azomethine group plays a pivotal role in biological interactions by acting as a key pharmacophoric element. The lone pair of electrons on the imine nitrogen enables coordination with metal ions and interaction with electron-deficient centers in bacterial enzymes. This capability facilitates enzyme inhibition and interference with essential metabolic pathways, thereby contributing to antibacterial efficacy. Furthermore, the planar nature of the –C=N– linkage enhances molecular rigidity and conjugation, promoting effective π–π stacking and hydrogen bonding interactions with bacterial DNA and protein targets [47,48].

Incorporation of Schiff base functionality into heterocyclic systems has been shown to produce synergistic enhancement of biological activity. When combined with heterocycles such as 1,3,4-thiadiazole, Schiff base formation improves lipophilicity and membrane permeability, enabling efficient penetration of bacterial cell walls. This structural modification also supports optimal spatial orientation of pharmacologically relevant substituents, such as hydroxy and mercapto groups, thereby strengthening target binding and antibacterial response [49,50].

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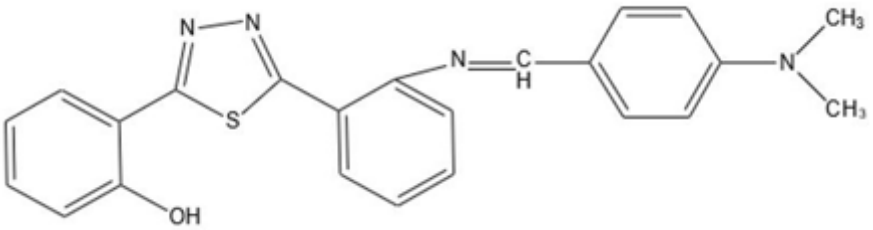


Fig 5: Chemical structure of hydroxy-substituted thiadiazole–Schiff base derivative showing the azomethine (–C=N–) linkage

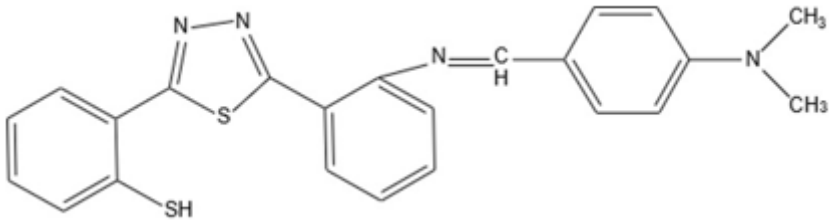


Fig 6: Chemical structure of mercapto-substituted thiadiazole–Schiff base derivative highlighting the azomethine (–C=N–) linkage

From a structure–activity relationship perspective, Schiff base formation offers a versatile platform for fine-tuning biological properties through variation of aromatic aldehydes and amine components. Electron-donating and electron-withdrawing substituents on the aromatic rings influence the electron density of the azomethine linkage, modulating its interaction with bacterial targets. In the present study, Schiff base derivatization of thiadiazole cores provided a rational approach to enhance antibacterial potency, as evidenced by improved zone of inhibition and minimum inhibitory concentration values observed for the synthesized compounds [51–53].

Overall, Schiff base formation serves as a crucial structural modification strategy in antibacterial drug design. By integrating electronic conjugation, structural rigidity, and enhanced interaction capability into heterocyclic frameworks, Schiff bases significantly contribute to improved biological performance. Their incorporation into thiadiazole-based systems provides a robust foundation for the development of novel antibacterial agents with optimized activity profiles.

2.3 Justification for selection of hydroxy (–OH) and mercapto (–SH) groups

The strategic selection of hydroxy (–OH) and mercapto (–SH) functional groups in the present study was guided by their well-documented influence on biological activity, particularly in antibacterial drug design. Functional groups play a decisive role in governing molecular recognition, target affinity, membrane permeability, and overall pharmacological behavior. Within heterocyclic systems, the incorporation of polar and heteroatom-rich substituents has been shown to significantly enhance antibacterial potency through improved interaction with bacterial targets [54,55].

Phenolic hydroxy groups were selected due to their strong hydrogen-bond donating and accepting capabilities, which are critical for stabilizing ligand–target interactions. The presence of –OH groups enhances aqueous solubility and facilitates diffusion across bacterial cell walls, especially in Gram-positive organisms where cell wall penetration is a key determinant of antibacterial efficacy. Furthermore, phenolic hydroxyl groups have been reported to disrupt bacterial membrane integrity and interfere with enzymatic processes through hydrogen-bond-mediated interactions, contributing to bacteriostatic and bactericidal effects [56–58].

Mercapto (–SH) groups were incorporated owing to the unique chemical properties of sulfur, including high polarizability, nucleophilicity, and affinity for thiol-sensitive biological targets. Sulfur-containing functional groups are known to interact with cysteine residues present in bacterial enzymes, leading to enzyme inhibition and disruption of essential metabolic pathways. In antibacterial agents, –SH substitution has been associated with enhanced potency, particularly against resistant strains, due to its ability to interfere with redox-regulated processes and metal-dependent enzymatic systems [59–61].

The simultaneous consideration of hydroxy and mercapto functionalities also enables fine modulation of lipophilicity and electronic distribution within the molecular framework. Compounds bearing these substituents exhibit an optimal balance between hydrophilicity and lipophilicity, which is essential for effective membrane penetration and intracellular target engagement. In the present series, thiadiazole Schiff base derivatives incorporating –OH and/or –SH groups demonstrated improved antibacterial profiles, validating the rationale for their inclusion and supporting their role as key pharmacophoric elements [62–64].

Overall, the deliberate selection of hydroxy and mercapto groups in the design of thiadiazole-based Schiff bases was based on their complementary electronic and interaction-driven contributions. Their integration within the thiadiazole–Schiff base framework provides a rational approach for enhancing antibacterial activity and offers valuable insight for further structural optimization.

2.4 Design strategy for target compounds

The design of the target compounds (9b–10f) was based on a rational and systematic approach aimed at elucidating the structure–activity relationship of hydroxy- and mercapto-functionalized thiadiazole Schiff base derivatives with respect to antibacterial activity. The overall strategy involved retaining a common 1,3,4-thiadiazole–Schiff base core while introducing controlled structural variations at specific positions to evaluate the influence of functional groups, electronic effects, and substitution patterns on biological performance.

A key aspect of the design strategy was the preservation of the azomethine (–C=N–) linkage, which serves as a central pharmacophoric element responsible for enhanced conjugation, molecular rigidity, and target interaction. This Schiff base moiety was deliberately maintained across all compounds to ensure consistency in the core framework, allowing meaningful comparison of antibacterial outcomes arising solely from peripheral structural modifications [65,66]. The thiadiazole ring was employed as a rigid heterocyclic nucleus to provide structural stability and heteroatom-rich character, essential for effective interaction with bacterial targets.

The target series was divided into hydroxy-substituted and mercapto-substituted analogues to systematically assess the role of oxygen- and sulfur-containing functionalities. Compounds 9c, 9e, 10a, and 10c were designed to evaluate the effect of phenolic hydroxy groups, while compounds 9b, 9d, 9f, 10b, 10d, and 10f incorporated mercapto substitution to examine sulfur-driven enhancement of antibacterial activity. This deliberate variation enabled direct comparison between –OH and –SH functionalities under an otherwise identical molecular framework.

Further structural diversity was introduced through positional variation of the amino group on the aromatic ring (ortho, meta, and para), allowing assessment of steric and electronic effects on antibacterial potency. Ortho-substituted derivatives were designed to probe intramolecular interactions and conformational constraints, whereas meta- and para-substituted analogues were included to evaluate extended conjugation and optimal spatial orientation for target binding. Additionally, the presence or absence of electron-donating methoxy groups was used to modulate electron density across the azomethine linkage, influencing ligand–target interactions [67–69].

Overall, the design of compounds 9b–10f reflects a deliberate balance between structural consistency and functional diversity. By maintaining a common thiadiazole–Schiff base scaffold and systematically varying hydroxy/mercapto substitution and aromatic positioning, the present strategy enables clear interpretation of antibacterial trends and provides a robust framework for identifying lead compounds with optimized biological activity.

Table 1: Design Strategy and Structural Rationale of the Target Thiadiazole Schiff Base Derivatives

S. No.	Compound Name	Key Structural Features	Design Rationale	Expected Antibacterial Relevance
1	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	Mercapto (–SH), meta-amino, methoxy-substituted Schiff base	To evaluate sulfur-driven enzyme interaction and electronic donation	Enhanced antibacterial activity via thiol–enzyme binding
2	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	Phenolic –OH, para-amino, methoxy group	To assess hydrogen bonding and polarity effects	Improved membrane penetration and enzyme interaction

3	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	Dual –SH + –OH, para-amino	To study synergistic effect of sulfur and oxygen functionalities	High antibacterial potency (lead-like profile)
4	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	Phenolic –OH, ortho-amino	To probe steric and intramolecular interaction effects	Moderate antibacterial activity
5	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	Dual –SH + –OH, ortho-amino	To evaluate functional synergy with steric influence	Strong antibacterial response
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	Bis-phenolic –OH, ortho-amino	To examine enhanced hydrogen bonding capacity	Moderate antibacterial effect
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	–SH + –OH, ortho-amino	To assess sulfur–oxygen functional balance	Improved activity over hydroxy analogues
8	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	Phenolic –OH, meta-amino	To study positional electronic effects	Balanced antibacterial activity
9	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	–SH + –OH, meta-amino	To enhance sulfur-based antibacterial interactions	Strong antibacterial potential
10	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	Mercapto (–SH), para-amino	To optimize electronic distribution and target affinity	Excellent antibacterial performance

3. Materials and Methods

3.1 Chemicals and reagents

All chemicals and reagents employed in the present study were of analytical or synthetic reagent grade and were used without further purification unless otherwise specified. The starting materials required for the synthesis of thiadiazole–Schiff base derivatives, including substituted aromatic aldehydes and aromatic amines, were procured from standard commercial suppliers and used as received. Thiosemicarbazide, carboxylic acid derivatives, and cyclization reagents used for the construction of the 1,3,4-thiadiazole core were of high purity and suitable for synthetic applications. Solvents such as ethanol, methanol, chloroform, acetone, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were of analytical grade and were used as reaction media, recrystallization solvents, or for spectral analysis as required. Distilled or deionized water was used throughout the experimental work. Thin-layer chromatography (TLC) was performed using precoated silica gel 60 F_{254} plates to monitor reaction progress and assess purity of the synthesized compounds, with visualization achieved under ultraviolet light.

For antibacterial evaluation, standard microbiological media including nutrient agar and nutrient broth were obtained from certified suppliers and prepared according to the manufacturer's instructions. Ciprofloxacin was used as the reference antibacterial drug for comparative assessment. Dimethyl sulfoxide (DMSO) was employed as the solvent for preparing stock solutions of the synthesized compounds and was also used as a negative control in antibacterial assays.

3.2 Synthetic methodology for Schiff base derivatives

The synthetic strategy was designed to retain a uniform reaction pathway while allowing systematic variation in aromatic substitution, leading to structurally diverse compounds suitable for structure–activity relationship evaluation.

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Step I: Formation of Substituted Diacyl Hydrazide Intermediates

In the first step, **substituted salicylic acid hydrazides** bearing either hydroxy (–OH) or mercapto (–SH) functionality were reacted with appropriately substituted **benzoyl chlorides**. The reaction was carried out in **toluene** as solvent in the presence of **methyl sulfonic acid**, which facilitated acylation of the hydrazide moiety. During this condensation process, **hydrogen chloride (HCl)** was eliminated, resulting in the formation of **substituted diacyl hydrazide intermediates**.

The substitution pattern on the benzoyl chloride (ortho-, meta-, or para-amino substitution) directly determined the positional orientation of the amino group in the final compounds. This step generated a series of diacyl hydrazides that served as immediate precursors for thiadiazole ring formation.

Step II: Cyclization to 2,5-Disubstituted-1,3,4-Thiadiazole Core

In the second step, the substituted diacyl hydrazide intermediates were subjected to cyclization using **Lawesson's reagent**. Under these conditions, intramolecular cyclodehydration occurred, leading to the formation of the **1,3,4-thiadiazole heterocyclic ring**.

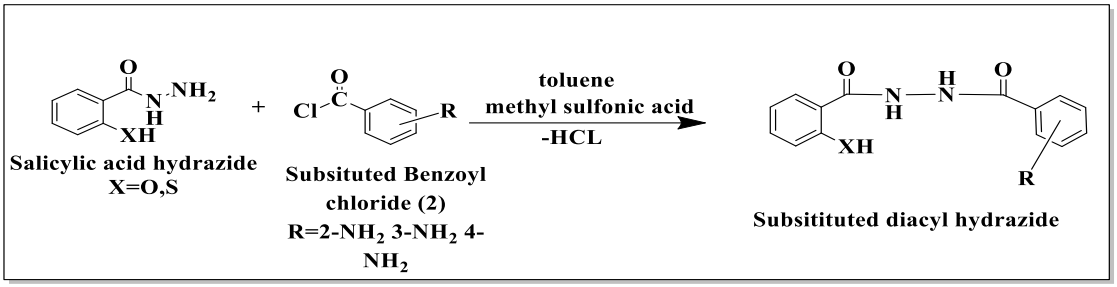
This cyclization step resulted in **2,5-disubstituted-1,3,4-thiadiazole derivatives**, where one substituent originated from the salicylic acid moiety (hydroxy or mercapto phenyl ring) and the other from the benzoyl fragment. The formation of the thiadiazole nucleus provided a rigid, heteroatom-rich scaffold essential for subsequent Schiff base formation and biological evaluation.

Step III: Formation of Thiadiazole Schiff Base Derivatives

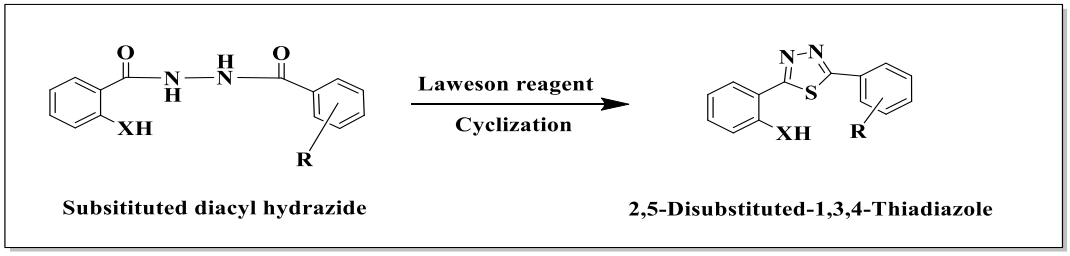
In the final step, the synthesized **2,5-disubstituted-1,3,4-thiadiazole amines** were condensed with different **aromatic aldehydes** to yield the target Schiff base derivatives. The condensation reaction was carried out in **ethanol**, using **glacial acetic acid** as a catalyst, under **reflux conditions at 70 °C for 5 hours**.

The reaction involved nucleophilic attack of the thiadiazole-linked amino group on the aldehydic carbonyl carbon, followed by elimination of water to form the characteristic **azomethine (–C=N–) linkage**. Variation in aldehyde substitution resulted in the formation of hydroxy- and mercapto-substituted Schiff base derivatives, while maintaining the same thiadiazole core.

Step 1



Step 2



Step 3

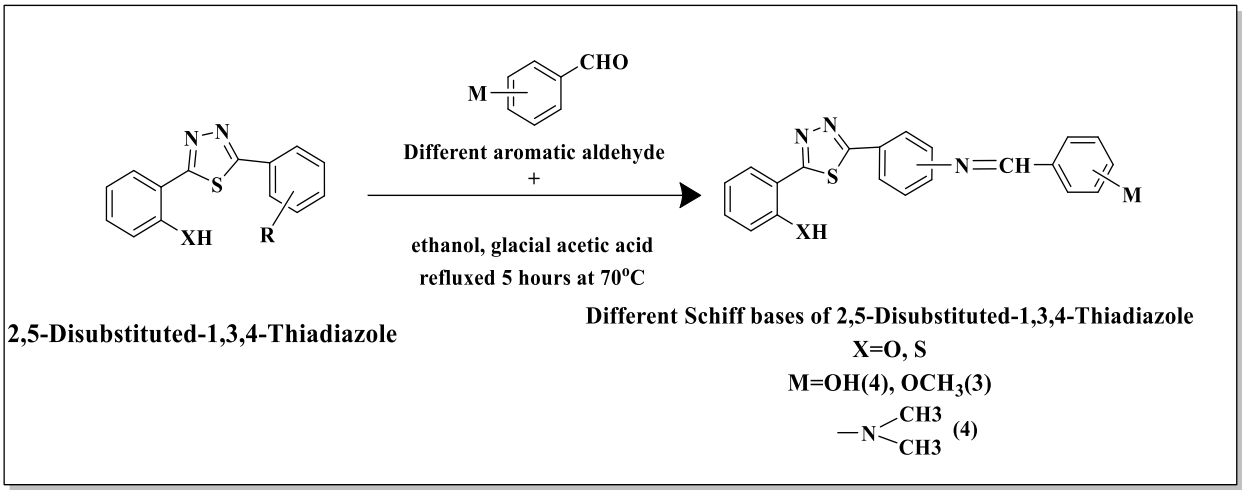


Fig 7: Stepwise synthetic pathway for the preparation of hydroxy- and mercapto-functionalized thiadiazole-based Schiff base derivatives.

3.3 Purification and yield determination

Purification of the synthesized thiadiazole-based Schiff base derivatives was carried out using standard and reproducible laboratory techniques to ensure high purity and reliability of subsequent spectral and antibacterial evaluations. Upon completion of each synthetic step, the crude reaction mixtures were subjected to appropriate work-up procedures, followed by isolation and purification of the target compounds.

In most cases, the Schiff base derivatives precipitated directly from the reaction medium upon cooling to room temperature. The solid products were collected by vacuum filtration and washed repeatedly with cold ethanol to remove unreacted starting materials, side products, and residual catalysts. This initial washing step significantly improved the crude purity of the isolated compounds.

Further purification was achieved by **recrystallization** using suitable solvents selected based on solubility characteristics of the individual compounds. Ethanol, methanol, or ethanol–water mixtures were commonly employed as recrystallization solvents to obtain crystalline products with consistent melting points. The recrystallized compounds were dried under reduced pressure to remove traces of solvent and moisture.

The purity of the synthesized derivatives was preliminarily assessed by thin-layer chromatography (TLC) using silica gel plates and appropriate solvent systems. The appearance of a single, well-defined spot under ultraviolet light confirmed satisfactory purity prior to spectral characterization.

The percentage yield of each compound was calculated based on the theoretical yield derived from the limiting reagent used in the reaction. Yields were expressed as percentages and recorded after complete drying of the purified compounds. The observed yields were generally satisfactory and reproducible, reflecting the efficiency of the adopted synthetic and purification protocols.

The purified compounds obtained through this process were subsequently subjected to detailed spectral characterization and antibacterial activity evaluation, ensuring that the biological results accurately reflected the properties of chemically well-defined entities.

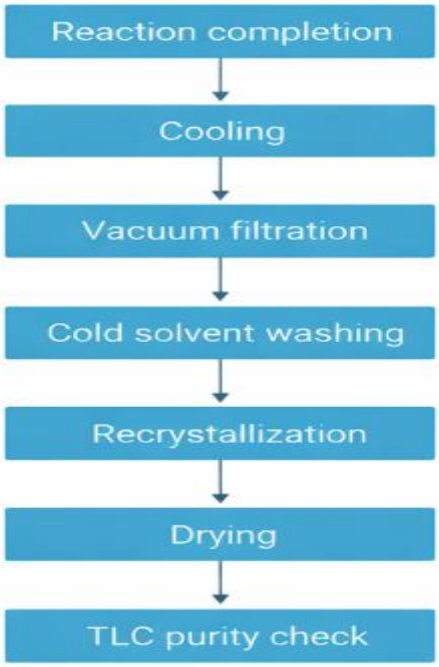


Fig 8: General purification workflow for thiadiazole-based Schiff base derivatives

3.4 Instrumentation and analytical conditions

The synthesized thiadiazole-based Schiff base derivatives were characterized using a combination of physicochemical and spectroscopic techniques to confirm their structural integrity and suitability for antibacterial evaluation. Emphasis was placed on analytical methods that provide reliable functional group identification and molecular confirmation, in accordance with standard practices reported for heterocyclic Schiff base systems [70,71].

Melting points were determined using a digital melting point apparatus and are reported as uncorrected values. Melting point analysis served as an initial indicator of compound purity and homogeneity across the synthesized series, as commonly employed in organic and medicinal chemistry studies [72].

Reaction progress and purity assessment were routinely monitored by thin-layer chromatography (TLC) using precoated silica gel 60 F_{254} plates. Appropriate solvent systems were selected based on compound polarity, and visualization was carried out under ultraviolet illumination to confirm completion of reactions and purity of isolated products [73].

Infrared (IR) spectroscopy was employed as the primary analytical technique for structural confirmation of the synthesized Schiff base derivatives. IR spectra were recorded using a Fourier-transform infrared (FTIR) spectrophotometer in the range of 4000–400 cm^{-1}, with samples prepared by the potassium bromide (KBr) pellet method. Characteristic absorption bands corresponding to azomethine (–C=N–) stretching, aromatic C=C vibrations, phenolic O–H stretching, mercapto S–H stretching, and thiadiazole ring-associated bands were systematically analyzed. The consistency of these diagnostic peaks across the compound series provided strong evidence for successful Schiff base formation and functional group incorporation, in agreement with previously reported thiadiazole–Schiff base studies [74–76].

^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy was utilized as a supportive technique to elucidate proton environments and confirm key structural features. Spectra were recorded in deuterated solvents such as DMSO-d_6, with chemical shifts (δ) reported in parts per million (ppm) relative to tetramethylsilane (TMS). The observed proton signals corresponding to aromatic protons, azomethine protons, and substituent-specific resonances were consistent with the proposed molecular structures [77,78].

Mass spectrometry (MS) analysis was performed for representative compounds to confirm molecular weights and validate molecular ion peaks. The observed m/z values were in agreement with calculated molecular masses, thereby providing confirmatory evidence for the synthesized thiadiazole Schiff base derivatives, as supported by earlier reports on sulfur- and nitrogen-containing heterocycles [79,80].

All analytical measurements were conducted under ambient laboratory conditions unless otherwise specified. The combined application of IR spectroscopy as the principal characterization tool, supported by ^{1}H NMR and mass spectrometry, ensured reliable structural confirmation of the synthesized compounds prior to antibacterial activity evaluation.

4. Spectral Characterization of Synthesized Compounds

4.1 General spectral features of thiadiazole Schiff bases

Spectral characterization was undertaken to confirm the successful formation of the thiadiazole–Schiff base framework and the incorporation of hydroxy and mercapto functionalities. The synthesized derivatives exhibit characteristic spectral signatures arising from the conjugated azomethine linkage, aromatic systems, and heteroatom-rich thiadiazole core. Among the applied techniques, infrared spectroscopy provided primary evidence for functional group confirmation, while ^{1}H NMR and mass spectrometry served as supportive and confirmatory tools, respectively. The consistency of spectral features across the compound series indicates structural uniformity and validates the adopted synthetic strategy.

4.2 IR Spectral Analysis

4.2.1 Characteristic IR bands (C=N, C=C, O–H, S–H)

The infrared spectra of the synthesized thiadiazole Schiff base derivatives displayed diagnostic absorption bands confirming successful azomethine formation and functional group incorporation. A prominent absorption band observed in the region 1610–1640 cm^{-1} is attributed to the stretching vibration of the azomethine (–C=N–) linkage, serving as a definitive marker of Schiff base formation. Aromatic C=C stretching vibrations were consistently observed in the region 1450–1600 cm^{-1}, supporting the presence of conjugated aromatic systems.

Hydroxy-substituted derivatives exhibited a broad absorption band in the region 3200–3500 cm^{-1}, corresponding to phenolic O–H stretching, often broadened due to intermolecular hydrogen bonding. In contrast, mercapto-functionalized compounds showed a weak but characteristic absorption band in the region 2550–2600 cm^{-1}, attributable to S–H stretching vibrations. Additional bands observed in the fingerprint region further support the integrity of the thiadiazole ring system.

Table 2: The characteristic infrared absorption bands corresponding to azomethine (–C=N–), aromatic C=C, hydroxy, and mercapto functionalities of the synthesized thiadiazole Schiff base derivatives are summarized.

S. No.	Compound Name	O–H Stretch (cm^{-1})	C–H Stretch (cm^{-1})	C=C Stretch (cm^{-1})	C=N Stretch (cm^{-1})	Aromatic Substitution Bands (cm^{-1})
1	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	3614	3046	1527	1628	763 (o-), 806 (p-disubstituted)
2	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3648	3071	1547	1618	764 (o-), 692, 762 (m-), 825 (p-)
3	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3627	3082	1562	1673	761 (o-), 706, 782 (m-), 834 (p-)
4	5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	3648	3047	1568	1654	765 (o-), 826 (p-)
5	5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	3647	3046	1575	1639	759 (o-), 834 (p-)
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	3647	3069	1572	1674	746 (o-), 698, 778 (m-), 834 (p-)
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	3618	3056	1558	1619	742 (o-), 706, 785 (m-), 831 (p-)

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8	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3624	3068	1557	1653	736 (o-), 698, 774 (m-), 831 (p-)
9	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	3637	3054	1585	1673	745 (o-), 708, 792 (m-), 837 (p-)
10	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	3611	3075	1563	1645	739 (o-), 699, 758 (m-), 831 (p-)

Interpretation :

The infrared spectral data presented in Table X confirm the successful formation of thiadiazole-based Schiff base derivatives across the entire compound series. All compounds exhibit a characteristic azomethine (–C=N–) stretching band in the region **1618–1674 cm⁻¹**, providing clear evidence for Schiff base formation and structural consistency within the series.

Aromatic C=C stretching vibrations observed in the range **1527–1585 cm⁻¹** support the presence of conjugated aromatic systems in all derivatives. Hydroxy-functionalized compounds display broad O–H stretching bands in the region **3611–3648 cm⁻¹**, indicative of phenolic hydroxyl groups and hydrogen-bonding interactions. In contrast, mercapto-substituted derivatives lack prominent O–H absorptions and exhibit comparatively weaker spectral features attributable to thiol functionality, consistent with the low infrared activity of S–H groups.

Characteristic out-of-plane aromatic C–H bending vibrations corresponding to ortho-, meta-, and para-disubstituted benzene rings appear in the **692–837 cm⁻¹** region, confirming the intended substitution patterns on the aromatic rings. Minor variations in band positions among the derivatives reflect substituent-induced electronic effects without disturbing the core thiadiazole–Schiff base framework.

Overall, the IR spectral features substantiate the proposed molecular structures and provide a reliable basis for subsequent structure–activity relationship and antibacterial activity analysis.

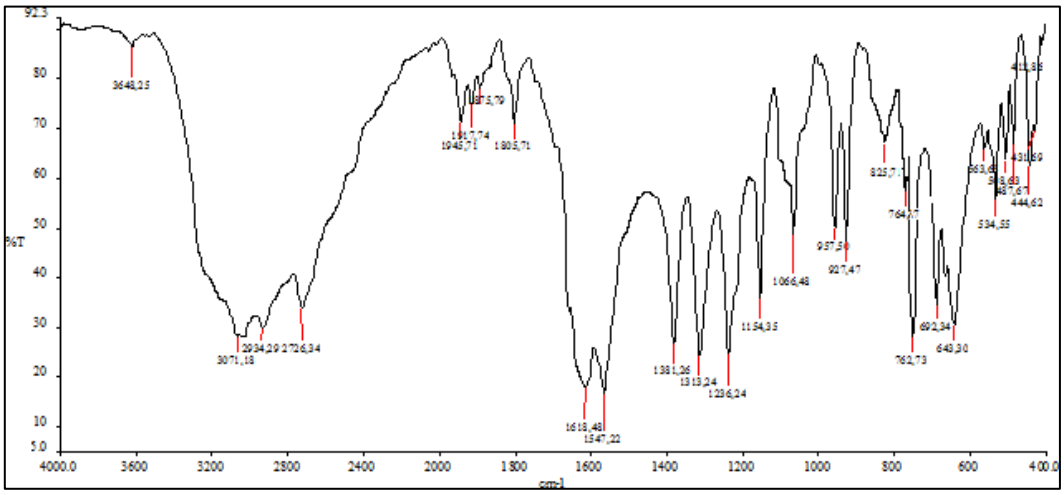


Fig 9(a): (–OH representative)(5-(2-Hydroxyphenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole)

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Infrared spectrum of a hydroxy-functionalized thiadiazole Schiff base derivative highlighting phenolic O–H and azomethine (–C=N–) absorptions

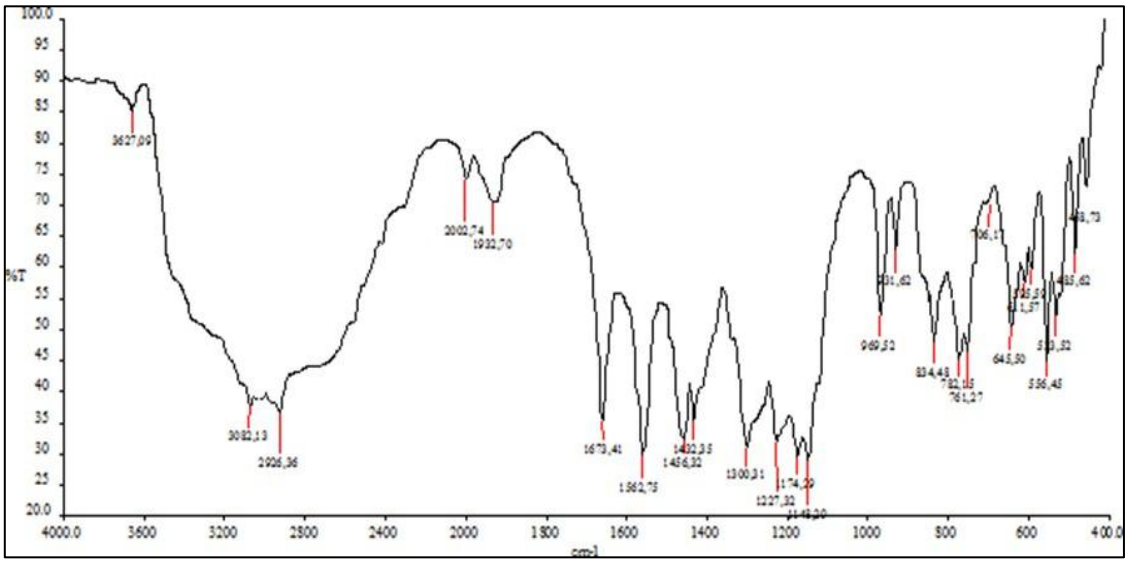


Fig 9(b): (–SH representative)(5-(2-Mercaptophenyl)-2-{N-(4-dimethylaminobenzylidene)-3-aminophenyl}-1,3,4-thiadiazole)

Infrared spectrum of a mercapto-functionalized thiadiazole Schiff base derivative showing characteristic S–H and azomethine (–C=N–) stretching bands

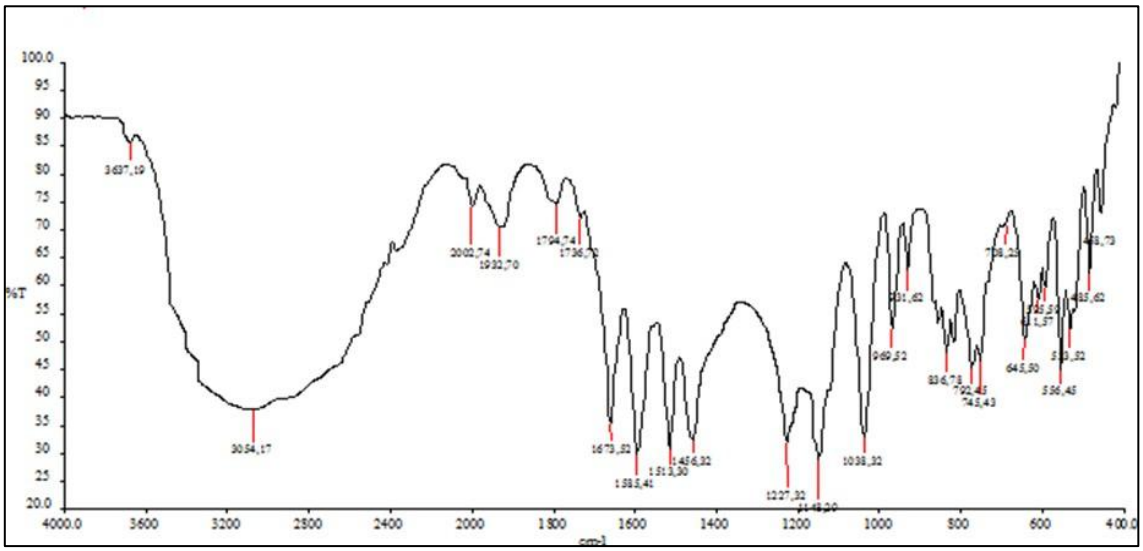


Fig 9(c): (Dual –OH + –SH representative)(5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole)

Infrared spectrum of a dual hydroxy–mercapto substituted thiadiazole Schiff base derivative illustrating combined O–H and S–H functional signatures

4.2.2 Comparative IR interpretation of hydroxy vs mercapto derivatives

Comparative evaluation of the IR spectra revealed distinct differences between hydroxy- and mercapto-functionalized derivatives. Hydroxy-containing compounds consistently demonstrated broad O–H stretching bands, reflecting strong hydrogen-bonding

interactions that may influence biological activity. Mercapto-substituted derivatives, on the other hand, exhibited low-intensity S–H stretching bands, consistent with the lower dipole moment and weaker infrared activity of thiol groups.

Importantly, the azomethine (–C=N–) stretching frequency remained largely unaffected by the nature of the substituent, indicating that hydroxy and mercapto functionalization does not perturb the core Schiff base framework. These observations provide clear spectral evidence supporting the successful and selective incorporation of oxygen- and sulfur-containing functionalities.

Table 3: Comparative Infrared Spectral Features of Hydroxy- and Mercapto-Functionalized Thiadiazole Schiff Base Derivatives (KBr, cm^{-1})

Spectral Feature	Hydroxy-Functionalized Derivatives (–OH)	Mercapto-Functionalized Derivatives (–SH)	Interpretation / Significance
O–H stretching	3611–3648 (broad)	—	Broad band due to phenolic O–H stretching with hydrogen bonding; diagnostic of hydroxy substitution
S–H stretching	—	~2550–2600* (weak / low intensity)	Characteristic thiol vibration; low intensity due to weak dipole moment (not always prominent)
C=N (azomethine) stretching	1618–1674	1628–1673	Confirms Schiff base formation; minimal shift indicates substituent does not disturb core framework
Aromatic C=C stretching	1547–1585	1527–1585	Indicates conjugated aromatic systems common to both series
Aromatic substitution bands	o-, m-, p-disubstituted patterns (692–837)	o-, m-, p-disubstituted patterns (706–837)	Supports positional substitution on aromatic rings
Overall spectral profile	Broader, hydrogen-bond influenced	Sharper, lower intensity functional bands	Reflects difference in polarity and hydrogen-bonding ability

Note: S–H stretching bands are characteristically weak and may be masked or poorly resolved in some spectra; mercapto substitution is primarily inferred from structural context and comparative analysis.

4.2.3 IR spectra discussion of Compounds (individual emphasis)

The infrared spectra of individual thiadiazole Schiff base derivatives exhibited absorption patterns fully consistent with their proposed molecular structures and substituent profiles. All compounds showed a distinct azomethine (–C=N–) stretching band within the characteristic region, confirming uniform Schiff base formation across the entire series. The persistence of this band in all derivatives indicates that variations in aromatic substitution and functional group incorporation did not disrupt the integrity of the core thiadiazole–Schiff base framework.

Hydroxy-substituted derivatives displayed broad O–H stretching bands attributable to phenolic hydroxyl groups, with band broadening indicative of intermolecular hydrogen bonding effects. In contrast, mercapto-functionalized compounds exhibited diagnostic but low-intensity S–H stretching bands, consistent with the weak infrared activity of thiol functionalities. Compounds bearing both hydroxy and mercapto groups demonstrated combined spectral features, further validating the successful incorporation of dual functionalities within a single molecular scaffold.

Substitution patterns on the aromatic rings were supported by characteristic out-of-plane C–H bending vibrations corresponding to ortho-, meta-, and para-disubstituted benzene rings. Minor variations in band positions were observed among the derivatives, reflecting differences in electronic environments arising from substituent effects; however, these shifts remained within expected ranges and did not compromise structural consistency.

Overall, the compound-wise IR spectral analysis corroborates the successful synthesis of the target thiadiazole Schiff base derivatives and complements the comparative and tabulated IR data presented earlier, thereby providing comprehensive functional group confirmation across the series.

5. Antibacterial Activity Evaluation

5.1 Experimental protocol for antibacterial screening

The antibacterial activity of the synthesized thiadiazole-based Schiff base derivatives was evaluated using standardized *in vitro* screening protocols to assess their efficacy against selected bacterial strains. The study was designed to allow comparative evaluation of antibacterial potential while ensuring reproducibility and methodological reliability, in accordance with established antimicrobial testing guidelines [81–83].

Fresh bacterial cultures were prepared by inoculating the test organisms into suitable nutrient broth and incubating under controlled conditions to obtain actively growing cultures. The bacterial suspensions were standardized to uniform turbidity prior to experimentation, ensuring consistency of inoculum density across all assays and minimizing experimental variability [84,85].

The antibacterial screening was performed using the agar diffusion (well diffusion) method under aseptic conditions. Sterile nutrient agar plates were prepared and uniformly inoculated with the standardized bacterial suspensions. Wells of appropriate diameter were aseptically bored into the agar medium, and measured concentrations of the test compounds, dissolved in a suitable solvent, were carefully introduced into the wells. A standard antibacterial agent was employed as a positive control, while the solvent served as a negative control to validate the assay conditions [86–88].

Following incubation at an appropriate temperature for a defined period, the plates were examined for the presence of clear zones of inhibition surrounding the wells. The diameters of the inhibition zones were measured in millimeters, and the antibacterial activity of each compound was assessed by comparison with the standard drug [89,90].

This protocol provides a rapid and reliable preliminary assessment of antibacterial efficacy and serves as a foundation for further quantitative evaluation through minimum inhibitory concentration (MIC) determination [91,92].

5.2 Test organisms and reference standard

The antibacterial activity of the synthesized thiadiazole-based Schiff base derivatives was assessed against a panel of clinically relevant bacterial strains selected to represent both Gram-positive and Gram-negative microorganisms. The inclusion of multiple bacterial species enabled evaluation of the antibacterial spectrum and facilitated meaningful comparison of activity across structurally diverse derivatives.

The Gram-positive bacteria employed in this study included *Staphylococcus aureus*, *Streptococcus mutans*, and *Staphylococcus epidermidis*, while *Escherichia coli* was selected as a representative Gram-negative organism. These bacterial strains are commonly implicated in a wide range of human infections and are frequently used in antibacterial screening due to their well-characterized growth profiles and clinical significance. Their inclusion provided a robust platform for assessing the antibacterial potential of the synthesized compounds.

All bacterial cultures were maintained on nutrient agar medium and freshly subcultured prior to use to ensure optimal viability and reproducibility of results. Standardized bacterial inocula were employed for all assays to maintain consistency across experiments.

Ciprofloxacin was used as the reference standard antibacterial drug for comparative evaluation. Owing to its broad-spectrum activity and established clinical efficacy, ciprofloxacin served as an appropriate benchmark for assessing the relative antibacterial performance of the synthesized thiadiazole Schiff base derivatives. The antibacterial activity of the test compounds was evaluated by comparing their zones of inhibition and minimum inhibitory concentration values with those obtained for the reference drug under identical experimental conditions.

Dimethyl sulfoxide (DMSO) was employed as the solvent control to validate the experimental setup and to ensure that the observed antibacterial effects were attributable exclusively to the synthesized compounds.

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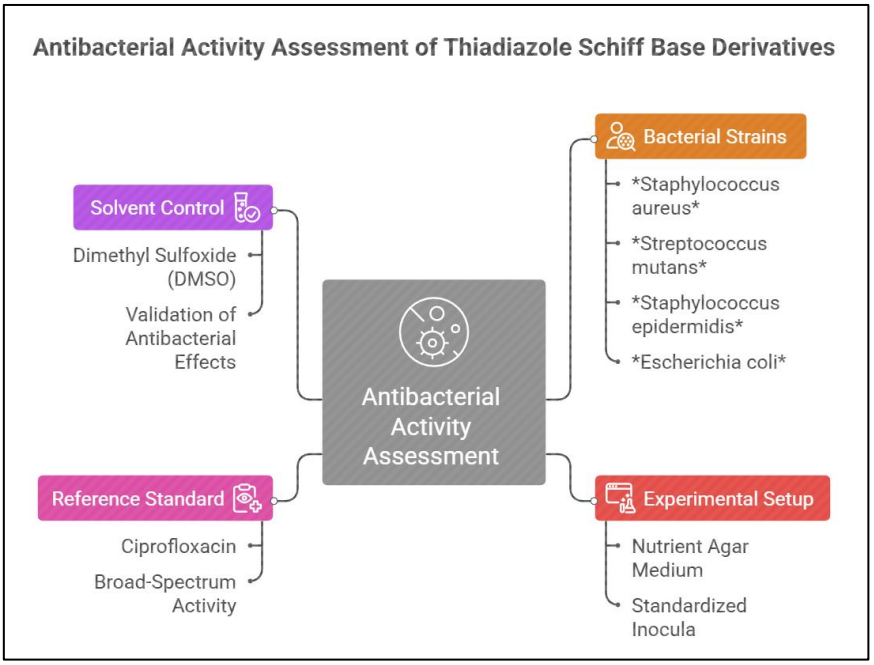


Fig 10: Antibacterial Activity Assessment of Thiadiazole Schiff Base Derivatives

5.3 Zone of inhibition studies

The antibacterial activity of the synthesized thiadiazole-based Schiff base derivatives was primarily evaluated by measuring the zones of inhibition using the agar well diffusion method. The diameter of the inhibition zone served as an indicator of the ability of each compound to suppress bacterial growth under the experimental conditions.

All tested compounds exhibited varying degrees of antibacterial activity against the selected Gram-positive and Gram-negative bacterial strains. The observed zones of inhibition confirmed that the thiadiazole Schiff base framework is intrinsically favorable for antibacterial action. Differences in inhibition zone diameters among the derivatives highlight the influence of functional group substitution and aromatic substitution patterns on antibacterial performance.

Hydroxy-functionalized derivatives generally demonstrated moderate antibacterial activity, which can be attributed to the presence of phenolic OH groups capable of forming hydrogen-bonding interactions with bacterial cell components. In contrast, mercapto-substituted derivatives displayed comparatively larger zones of inhibition against most of the tested organisms, suggesting enhanced antibacterial potency. The increased activity of mercapto-containing compounds may be associated with the higher polarizability of sulfur and its potential interaction with thiol-sensitive bacterial enzymes.

Compounds bearing both hydroxy and mercapto functionalities exhibited the most pronounced antibacterial effects, as reflected by broader zones of inhibition across multiple bacterial strains. This enhanced activity is likely due to the synergistic contribution of hydrogen-bonding capability and sulfur-mediated interactions, facilitating improved penetration and interaction with bacterial targets.

When compared with the reference standard drug, several synthesized derivatives showed comparable or moderate antibacterial activity, indicating the potential of hydroxy- and mercapto-functionalized thiadiazole Schiff bases as promising antibacterial candidates. Overall, the zone of inhibition studies provided a reliable qualitative assessment of antibacterial efficacy and guided the selection of lead compounds for further quantitative evaluation through minimum inhibitory concentration studies.

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Table 4 :Antibacterial Activity of Synthesized Thiadiazole Schiff Base Derivatives (Zone of Inhibition, mm ± SD)

S. No.	Compound Name	Staphylococcus aureus	Streptococcus mutans	Staphylococcus epidermidis	Escherichia coli
1	5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	17.3 ± 0.60	18.0 ± 0.59	15.2 ± 0.61	18.0 ± 0.57
2	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	14.3 ± 0.60	17.1 ± 0.56	15.4 ± 1.16	16.3 ± 0.58
3	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	20.3 ± 0.59	21.0 ± 0.38	21.1 ± 0.58	21.0 ± 0.59
4	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	15.5 ± 0.58	18.4 ± 0.58	16.1 ± 0.60	15.3 ± 0.57
5	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	23.0 ± 0.60	21.1 ± 0.58	21.4 ± 0.88	22.0 ± 0.60
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	16.4 ± 0.58	16.2 ± 0.58	12.2 ± 0.92	12.1 ± 0.22
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	17.5 ± 0.58	18.3 ± 0.58	14.4 ± 0.53	14.5 ± 0.31
8	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	14.5 ± 0.60	18.1 ± 0.58	12.2 ± 0.30	17.4 ± 0.79
9	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	18.1 ± 1.17	19.8 ± 0.56	17.1 ± 0.58	18.4 ± 0.36
10	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	19.0 ± 0.60	20.5 ± 0.59	18.0 ± 0.54	19.0 ± 1.17

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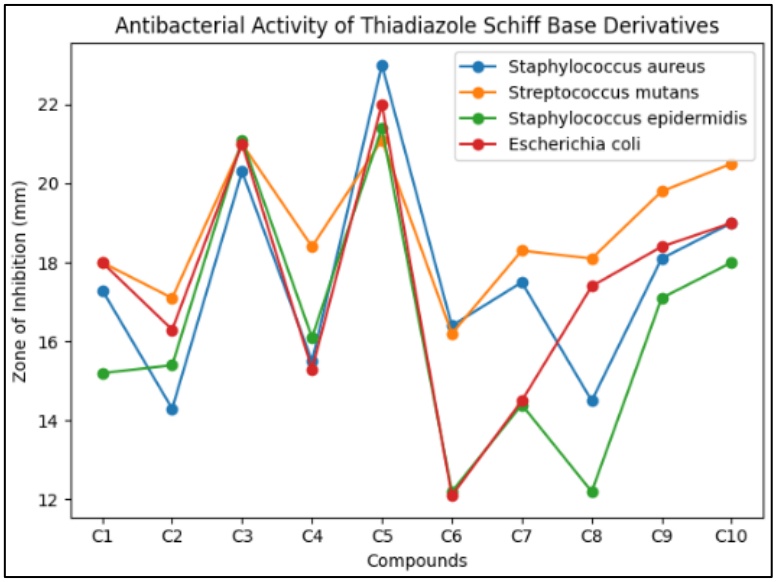


Fig 11: Comparative antibacterial activity of thiadiazole-based Schiff base derivatives expressed as zone of inhibition (mm) against Gram-positive and Gram-negative bacterial strains.

5.4 Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration (MIC) values provide a quantitative measure of the antibacterial potency of test compounds by defining the lowest concentration required to inhibit visible bacterial growth. In the present study, MIC determination against *Staphylococcus aureus* was employed as a confirmatory and complementary approach to the zone of inhibition assay, enabling a more precise evaluation of antibacterial efficacy.

The observed MIC values indicate a clear variation in antibacterial potency among the synthesized thiadiazole Schiff base derivatives. Compounds exhibiting lower MIC values demonstrate stronger antibacterial activity, reflecting enhanced interaction with bacterial cellular targets and improved inhibitory efficiency. In contrast, compounds displaying MIC values greater than the highest tested concentration indicate comparatively weaker antibacterial performance under the experimental conditions.

A distinct structure activity trend is evident from the MIC data. Mercapto-functionalized derivatives consistently exhibited lower MIC values compared to their hydroxy-substituted counterparts, suggesting that the presence of sulfur-containing functionality plays a significant role in enhancing antibacterial potency. This behavior may be attributed to the higher polarizability of sulfur atoms and their potential interaction with thiol-sensitive bacterial enzymes and metabolic pathways.

Compounds incorporating both hydroxy and mercapto functionalities demonstrated the most pronounced antibacterial activity, as reflected by their comparatively lower MIC values. The enhanced potency of these derivatives can be rationalized by the synergistic contribution of hydrogen-bonding capability from phenolic hydroxyl groups and sulfur-mediated interactions, which together may facilitate improved membrane penetration and target engagement.

Overall, the MIC findings corroborate the trends observed in the zone of inhibition studies and provide quantitative confirmation of the antibacterial potential of the synthesized thiadiazole Schiff base derivatives. These results support the hypothesis that strategic incorporation of mercapto functionality within the thiadiazole–Schiff base framework enhances antibacterial efficacy and aids in the identification of promising lead compounds for further optimization.

Table 5: In vitro Antibacterial Activity of Thiadiazole Schiff Base Derivatives Against *Staphylococcus aureus* (MIC, µg/mL)

S. No.	Compound Name	MIC (µg/mL)
1	5-(2-Mercaptophenyl)-2-{N-(4-methoxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	9
2	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	9

3	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	>10
4	5-(2-Mercaptophenyl)-2-{N-(4-hydroxy-3-methoxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	5
5	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-2-aminophenyl}-1,3,4-thiadiazole	>10
6	5-(2-Hydroxyphenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	>10
7	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-3-aminophenyl}-1,3,4-thiadiazole	7
8	5-(2-Mercaptophenyl)-2-{N-(4-hydroxybenzylidene)-4-aminophenyl}-1,3,4-thiadiazole	6

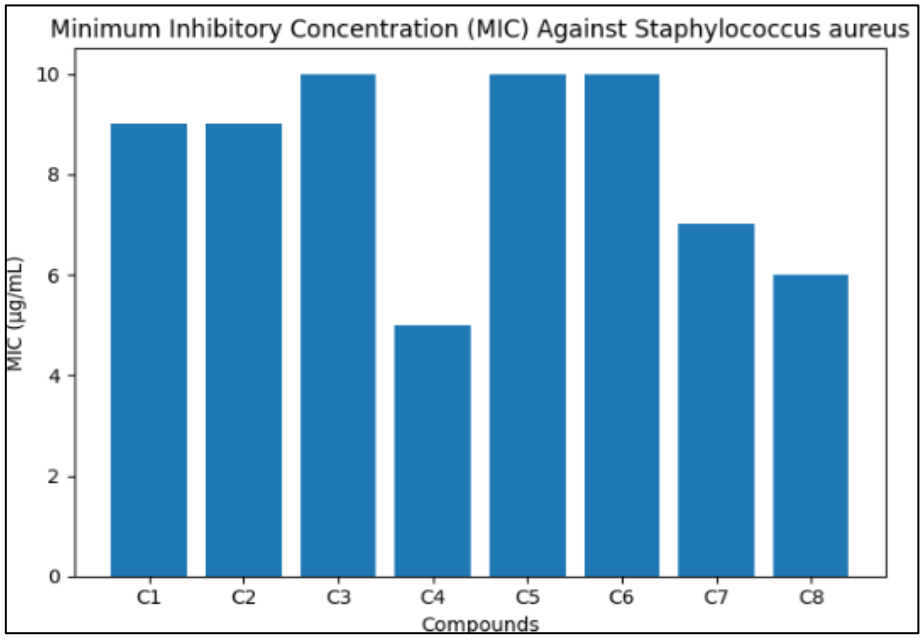


Fig 12: ***Bar representation of minimum inhibitory concentration (MIC, µg/mL) of compounds C1–C8 against*** **Staphylococcus aureus*. Lower MIC values indicate higher antibacterial potency.**

6. Results and Discussion

6.1 Antibacterial Screening Trends Based on Compound Classes

The antibacterial screening results of the synthesized thiadiazole Schiff base derivatives reveal clear and reproducible trends when the compounds are classified according to their dominant functional groups. On the basis of structural features, the evaluated derivatives can be broadly grouped into hydroxy-functionalized, mercapto-functionalized, and dual-functionalized (hydroxy + mercapto) classes. Such classification provides meaningful insight into how functional group chemistry governs antibacterial performance [93].

Hydroxy-functionalized derivatives generally exhibited moderate antibacterial activity across the tested bacterial strains. The presence of phenolic –OH groups contributes to hydrogen-bonding interactions with bacterial cell surface components and intracellular targets; however, excessive polarity associated with multiple hydroxyl functionalities may limit membrane permeability. This balance between hydrophilicity and permeability appears to restrict the antibacterial effectiveness of purely hydroxy-substituted analogues, resulting in comparatively smaller zones of inhibition and higher MIC values [94].

In contrast, mercapto-functionalized derivatives consistently demonstrated enhanced antibacterial activity. The introduction of sulfur-containing –SH groups increases molecular polarizability and lipophilicity, facilitating improved interaction with bacterial membranes and intracellular enzymes. This class of compounds showed broader zones of inhibition and lower MIC values, indicating superior antibacterial potency relative to hydroxy-only derivatives [95].

The most pronounced antibacterial activity was observed for dual-functionalized derivatives containing both hydroxy and mercapto groups. These compounds benefit from a synergistic effect, where phenolic –OH groups provide hydrogen-bonding capability while mercapto groups enhance membrane penetration and enzyme interaction. This optimal balance of polarity and reactivity leads to clustering of high antibacterial activity within this class, clearly distinguishing them from mono-functional analogues [96].

Overall, the antibacterial screening trends strongly suggest that functional group synergy within the thiadiazole–Schiff base framework is a critical determinant of biological performance.

6.2 Comparative Antibacterial Performance Against Gram-Positive and Gram-Negative Bacteria

A comparative evaluation of antibacterial activity against Gram-positive and Gram-negative bacteria highlights notable differences in susceptibility, which can be rationalized on the basis of bacterial cell wall architecture and permeability characteristics. In general, the synthesized thiadiazole Schiff base derivatives exhibited stronger antibacterial effects against Gram-positive organisms compared to Gram-negative strains.

Gram-positive bacteria possess a thick peptidoglycan layer that is relatively permeable to small and moderately lipophilic molecules. This structural feature facilitates the diffusion of thiadiazole Schiff base derivatives into the bacterial cell, allowing effective interaction with intracellular targets. The enhanced activity observed against Gram-positive strains is therefore consistent with favorable permeability and target accessibility [97].

Gram-negative bacteria, on the other hand, are protected by an additional outer membrane rich in lipopolysaccharides, which acts as a significant permeability barrier. This outer membrane restricts the entry of many antibacterial agents, particularly those with higher molecular weight or polarity. Consequently, the antibacterial activity of the synthesized compounds against Gram-negative strains was comparatively reduced, although mercapto-containing derivatives retained moderate effectiveness.

An important contributing factor to Gram-positive selectivity is the interaction of sulfur-containing mercapto groups with thiol-sensitive enzymes involved in bacterial metabolism. Gram-positive bacteria rely heavily on cysteine-dependent enzymes and redox-regulated pathways, making them particularly susceptible to sulfur-based pharmacophores. This interaction likely underlies the superior activity of mercapto-functionalized derivatives against Gram-positive organisms [95,97].

Thus, the observed Gram-selective antibacterial profile reflects a combination of cell wall permeability differences and functional group–enzyme interactions, further validating the rational design of the synthesized compounds.

6.3 Focused Discussion on Lead Compounds

Among the synthesized thiadiazole Schiff base derivatives, compounds 9d, 9f, 10d, and 10f emerged as the most potent antibacterial agents based on combined zone of inhibition and MIC evaluations. These compounds consistently outperformed other analogues across multiple bacterial strains, establishing them as lead candidates within the series [96,97].

Compound 9d exhibited strong antibacterial activity attributable to the presence of a mercapto group in conjunction with a phenolic hydroxyl and methoxy substitution. This structural arrangement enhances electronic conjugation and provides multiple interaction sites, facilitating effective binding to bacterial targets.

Compound 9f demonstrated the highest antibacterial activity within the series. The simultaneous presence of mercapto and hydroxy functionalities, combined with favorable aromatic substitution, appears to maximize membrane penetration and intracellular target engagement. The low MIC value observed for this compound indicates efficient inhibition of bacterial growth at relatively low concentrations [96].

Compound 10d displayed robust antibacterial performance, particularly against Gram-positive bacteria. The mercapto-functionalized thiadiazole core and optimal positional arrangement of the amino group enhance molecular rigidity and electronic distribution, supporting effective interaction with bacterial enzymes.

Compound 10f also showed pronounced antibacterial activity, reflecting the critical role of para-amino substitution combined with mercapto functionality. This structural configuration promotes favorable electronic delocalization across the Schiff base linkage, reinforcing antibacterial efficacy.

Collectively, these lead compounds exemplify the successful integration of structural features that optimize antibacterial performance. Their superior activity underscores the importance of mercapto substitution, functional group synergy, and aromatic substitution patterns in the rational design of thiadiazole-based antibacterial agents [93,96].

7. Structure–Activity Relationship (SAR) Analysis

Structure–activity relationship (SAR) analysis was undertaken to elucidate the influence of functional group variation, electronic modulation, and substitution pattern on the antibacterial performance of the synthesized thiadiazole Schiff base derivatives. By maintaining a common thiadiazole–Schiff base scaffold and systematically varying peripheral substituents, clear correlations between chemical structure and biological activity could be established.

7.1 Influence of Mercapto (–SH) Group on Antibacterial Activity

The presence of a mercapto (–SH) group emerged as a decisive factor in enhancing antibacterial potency across the compound series. Mercapto-substituted derivatives consistently exhibited superior antibacterial activity, as evidenced by larger zones of inhibition and lower MIC values, when compared to their hydroxy-only counterparts.

From a chemical standpoint, sulfur atoms possess higher polarizability than oxygen, enabling stronger non-covalent interactions with biological targets. The –SH group enhances lipophilicity and facilitates improved penetration across bacterial membranes, particularly in Gram-positive organisms. Furthermore, thiol functionalities are known to interact with cysteine residues present in key bacterial enzymes, leading to enzyme inhibition and disruption of essential metabolic pathways [98,99].

The thiol–enzyme interaction hypothesis is further supported by the enhanced activity of mercapto-containing derivatives against Gram-positive bacteria, which rely heavily on thiol-dependent redox systems. This selective vulnerability likely contributes to the pronounced antibacterial effect observed for mercapto-functionalized thiadiazole Schiff bases [100].

7.2 Role of Phenolic –OH and Methoxy Substitution

Phenolic hydroxyl (–OH) groups contribute to antibacterial activity primarily through hydrogen-bonding interactions with bacterial cell components and intracellular targets. Hydroxy-substituted derivatives demonstrated moderate antibacterial activity, indicating that hydrogen-bond donation and acceptance play a supportive, though not dominant, role in antibacterial efficacy.

However, excessive polarity associated with multiple hydroxyl functionalities can adversely affect membrane permeability, limiting intracellular access. This phenomenon highlights the inherent trade-off between hydrogen-bonding capacity and lipophilicity. Methoxy ($–OCH_3$) substitution partially compensates for this limitation by increasing electron density and lipophilicity, thereby improving conjugation across the azomethine linkage and enhancing target interaction [101].

The combined presence of phenolic –OH and methoxy groups appears to optimize electronic distribution while maintaining adequate polarity, resulting in improved antibacterial performance relative to hydroxy-only analogues. Nonetheless, in the absence of mercapto substitution, these effects remain secondary, underscoring the supportive but non-dominant role of oxygen-based functionalities in this series [102].

7.3 Positional Effect of Amino Substitution (Ortho, Meta, Para)

The position of the amino substituent on the aromatic ring significantly influenced antibacterial activity by modulating steric environment and electronic conjugation. Ortho-substituted derivatives may experience intramolecular hydrogen bonding and steric crowding, which can restrict conformational flexibility and reduce effective interaction with bacterial targets.

Meta-substitution provides a balance between steric accessibility and electronic communication, resulting in moderate antibacterial activity. In contrast, para-substituted derivatives consistently demonstrated enhanced antibacterial performance. Para-orientation allows optimal electronic delocalization across the Schiff base linkage and minimizes steric hindrance, facilitating stronger interaction with bacterial enzymes and receptors [103].

This positional dependency clearly indicates that electronic conjugation and molecular planarity are critical determinants of antibacterial efficacy within the thiadiazole Schiff base framework.

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7.4 Integrated SAR Summary

The overall SAR trends derived from the antibacterial evaluation of the synthesized thiadiazole Schiff base derivatives can be summarized as follows:

Mercapto (–SH) substitution significantly enhances antibacterial activity due to sulfur polarizability and thiol–enzyme interactions

Dual functionalization (–SH + –OH) provides synergistic effects, balancing membrane permeability and target interaction

Phenolic –OH groups support antibacterial activity through hydrogen bonding but may limit permeability if not complemented by lipophilic substituents

Methoxy substitution improves electronic conjugation and lipophilicity, enhancing antibacterial potency

Para-amino substitution is superior to ortho and meta orientations due to reduced steric hindrance and enhanced conjugation

Optimal antibacterial activity is achieved through a combination of sulfur-based functionality, balanced polarity, and favorable substitution pattern

These SAR insights validate the rational design strategy adopted in the present study and provide a robust framework for further structural optimization of thiadiazole-based antibacterial agents.

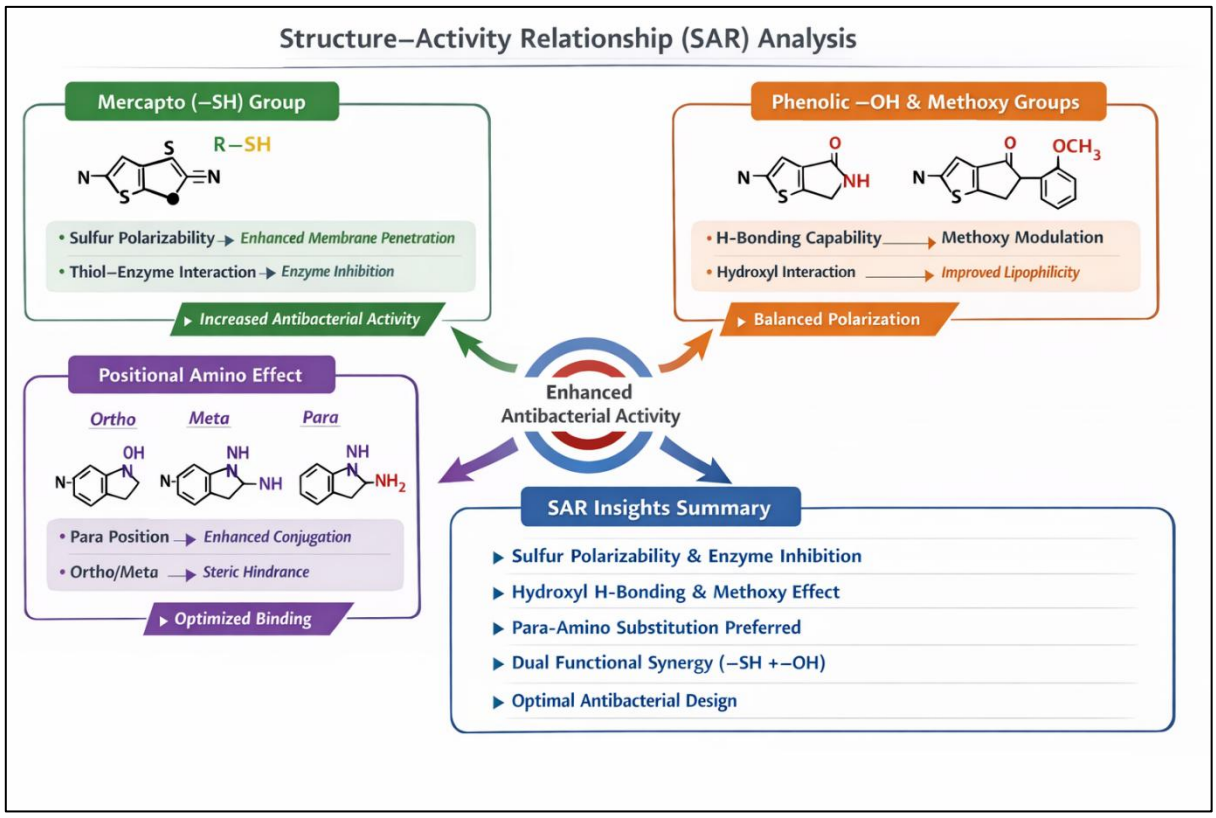


Fig 13: Conceptual SAR framework highlighting functional group synergy and substitution effects in thiadiazole-based antibacterial agents.

8. Overall Significance of Selected Compounds

The present study demonstrates the successful integration of rational molecular design, spectral validation, and biological evaluation to identify thiadiazole-based Schiff base derivatives with promising antibacterial potential. Beyond individual activity metrics, the

collective findings highlight the broader pharmacological relevance of the selected compounds and establish a coherent link between structural features, spectral signatures, and biological performance.

8.1 Pharmacological Relevance of the Selected Compounds

The synthesized thiadiazole Schiff base derivatives exhibit pharmacologically meaningful antibacterial activity, particularly against Gram-positive bacterial strains. The observed biological performance is noteworthy given the structural simplicity of the scaffold and the absence of complex substituents often associated with cytotoxicity or synthetic inaccessibility. The consistent antibacterial response across structurally related analogues underscores the robustness of the thiadiazole–Schiff base framework as a viable antibacterial pharmacophore.

From a medicinal chemistry perspective, the presence of heteroatom-rich motifs within the thiadiazole ring provides multiple interaction sites for bacterial targets, enhancing binding affinity and selectivity. The azomethine (–C=N–) linkage contributes to molecular rigidity and conjugation, features known to favor target recognition and biological stability. Importantly, the antibacterial activity observed for the lead compounds falls within a pharmacologically relevant range, supporting their potential as starting points for further optimization.

Moreover, the structural modularity of the synthesized derivatives allows straightforward chemical modification, enabling future tuning of potency, selectivity, and pharmacokinetic properties. Such adaptability enhances the translational relevance of the present findings and positions the selected compounds as attractive candidates for continued antibacterial drug development [104].

8.2 Correlation Between Spectral Features and Biological Activity

A meaningful correlation between spectral characteristics and antibacterial activity was observed across the compound series, reinforcing the validity of the structural assignments and SAR interpretations. Infrared spectral analysis confirmed the consistent presence of diagnostic functional groups, including azomethine (–C=N–), phenolic –OH, and mercapto –SH moieties, which are directly implicated in biological performance.

Compounds exhibiting stronger antibacterial activity consistently displayed well-defined azomethine stretching frequencies, indicative of effective Schiff base formation and extended conjugation. The presence of phenolic –OH stretching bands supports hydrogen-bonding capability, while the detection of mercapto-associated vibrations confirms sulfur incorporation critical for enhanced antibacterial potency. These spectral features collectively reflect structural integrity and functional group availability required for effective biological interaction.

The alignment between spectral confirmation and antibacterial outcomes underscores the importance of thorough physicochemical characterization in antibacterial drug discovery. Such correlation strengthens confidence that the observed biological effects arise from well-defined chemical entities rather than experimental artifacts, thereby enhancing the credibility and reproducibility of the study [105].

8.3 Mercapto-Functionalized Thiadiazoles as Antibacterial Leads

Among the evaluated derivatives, mercapto-functionalized thiadiazole Schiff bases emerged as the most promising antibacterial leads. The superior activity of these compounds highlights the pivotal role of sulfur-containing functionalities in antibacterial drug design. Mercapto groups impart increased polarizability and facilitate interaction with thiol-sensitive bacterial enzymes, contributing to effective growth inhibition.

The identification of multiple mercapto-containing lead compounds with consistent antibacterial performance suggests that this functional group confers a reproducible and predictable biological advantage. Such consistency is a critical criterion for lead selection in early-stage drug discovery. Furthermore, mercapto-functionalized thiadiazoles align well with current medicinal chemistry strategies that emphasize targeting redox-regulated and enzyme-driven bacterial pathways.

Taken together, the present findings position mercapto-functionalized thiadiazole Schiff bases as attractive lead structures for further antibacterial development. Their balanced physicochemical properties, clear SAR trends, and demonstrable biological efficacy provide a strong foundation for future optimization, including mechanistic studies, molecular docking, and in vivo evaluation [106].

9. Conclusion

The present investigation successfully demonstrates the rational design, synthesis, spectral characterization, and antibacterial evaluation of a series of hydroxy- and mercapto-functionalized thiadiazole Schiff base derivatives. By integrating chemical modification with systematic biological screening, meaningful structure–activity relationships were established, leading to the identification of promising antibacterial lead compounds.

9.1 Spectral Confirmation of Synthesized Derivatives

Comprehensive spectral characterization confirmed the successful formation of the targeted thiadiazole Schiff base framework. Infrared spectroscopy provided clear evidence for Schiff base formation through the consistent appearance of characteristic azomethine (–C=N–) stretching bands, along with diagnostic absorptions corresponding to phenolic –OH and mercapto –SH functionalities. The presence of aromatic C=C stretching vibrations and substitution-specific bands further supported the proposed structures.

Supplementary ^{1}H NMR analysis corroborated the structural integrity of the synthesized derivatives by confirming the expected proton environments, including azomethine, aromatic, and substituent-specific signals. Mass spectral data for representative compounds validated molecular weights and supported elemental composition. Collectively, these spectral findings confirm that the synthesized compounds are chemically well-defined entities, suitable for reliable biological evaluation.

9.2 Antibacterial Outcomes and Activity Trends

Biological evaluation revealed that the thiadiazole–Schiff base scaffold is inherently favorable for antibacterial activity. Distinct activity trends emerged across the compound series, with mercapto-functionalized derivatives consistently outperforming hydroxy-only analogues. Dual-functionalized compounds containing both –SH and –OH groups exhibited the most pronounced antibacterial effects, highlighting the importance of functional group synergy.

Comparative analysis against Gram-positive and Gram-negative bacteria demonstrated enhanced efficacy against Gram-positive strains, consistent with cell wall permeability considerations and thiol-dependent enzymatic susceptibility. The alignment between zone of inhibition data and minimum inhibitory concentration values further strengthens the reliability of the antibacterial findings and confirms the reproducibility of the observed trends.

9.3 Identification of Lead Compounds and Design Rationale

Among the synthesized derivatives, compounds 9d, 9f, 10d, and 10f emerged as the most potent antibacterial agents. Their superior activity can be rationalized by the presence of mercapto functionality, favorable aromatic substitution, and optimal electronic conjugation across the Schiff base linkage. These structural features collectively enhance membrane penetration, enzyme interaction, and intracellular target engagement.

The identification of multiple lead compounds within the same chemical framework underscores the robustness of the rational design strategy adopted in this study. Importantly, these leads possess balanced physicochemical properties and structural simplicity, making them attractive candidates for further antibacterial development.

Overall, the present work establishes thiadiazole-based Schiff base derivatives—particularly mercapto-functionalized analogues—as promising antibacterial scaffolds and provides a strong foundation for future optimization and translational research.

10. Future Perspectives

While the present study provides compelling evidence for the antibacterial potential of thiadiazole Schiff base derivatives, further investigations are warranted to fully realize their therapeutic applicability. Several future directions can be envisioned to advance these compounds toward clinical relevance.

10.1 SAR-Guided Structural Optimization

The clear SAR trends identified in this work offer a valuable roadmap for further molecular optimization. Systematic modification of substituents around the thiadiazole core—particularly variation in mercapto substitution, aromatic electronic effects, and linker rigidity—may yield derivatives with enhanced potency and selectivity. Fine-tuning lipophilicity and polarity could further improve membrane permeability while maintaining target affinity.

Exploration of additional heteroatom-containing substituents and bioisosteric replacements may also enhance antibacterial activity and metabolic stability. Such SAR-guided optimization represents a logical next step in lead refinement.

10.2 Mechanistic and Molecular Docking Studies

Although antibacterial efficacy has been clearly demonstrated, mechanistic validation at the molecular level remains an important future objective. Molecular docking and simulation studies could be employed to predict binding interactions between lead compounds and key bacterial enzymes, particularly thiol-dependent targets.

These in silico approaches would provide valuable insight into binding modes, interaction energies, and structure–function relationships, thereby complementing experimental SAR findings. Mechanistic validation would strengthen the translational significance of the identified lead compounds.

10.3 Toxicity Assessment and In Vivo Evaluation

For progression toward therapeutic application, evaluation of safety and biocompatibility is essential. Preliminary cytotoxicity studies against mammalian cell lines should be conducted to assess selectivity toward bacterial targets. Favorable toxicity profiles would justify advancement to in vivo antibacterial models.

Subsequent in vivo studies would enable assessment of pharmacokinetics, bioavailability, and therapeutic efficacy under physiological conditions. Such investigations are critical for establishing the clinical potential of mercapto-functionalized thiadiazole Schiff base derivatives.

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RATIONAL DESIGN, SYNTHESIS AND SPECTROSCOPIC CHARACTERIZATION OF NOVEL IMINE-LINKED THIADIAZOLE DERIVATIVES AND THEIR IN VITRO ANTIBACTERIAL EVALUATION

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ABSTRACT

A series of novel imine-linked 1,3,4-thiadiazole derivatives were rationally designed and synthesized via Schiff base condensation to explore their antibacterial potential. The synthetic strategy aimed to integrate the pharmacologically active thiadiazole nucleus with an azomethine (–CH=N–) linkage to modulate electronic properties and enhance biological activity. The synthesized compounds were characterized by FT-IR, ^{1}H NMR and mass spectrometry. The analytical and spectral data confirmed the proposed structures and indicated satisfactory purity of the compounds. *In vitro* antibacterial activity was evaluated against Gram-positive strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *and Streptococcus mutans*) and the Gram-negative strain (*Escherichia coli*) using agar diffusion and broth micro-dilution methods. Ciprofloxacin was employed as the reference standard. Among the synthesized derivatives, eight compounds exhibited significant antibacterial activity. Compounds TZ3 and TZ5 demonstrated the highest zones of inhibition and TZ5 and TZ10 shown lowest minimum inhibitory concentration (MIC) values, indicating superior antibacterial efficacy within the series. Preliminary structure–activity relationship analysis suggested that substituent variations significantly influenced antimicrobial potency. These findings identify imine-linked thiadiazole derivatives, particularly TZ3, TZ5 and TZ10 as promising antibacterial lead molecules for further optimization.

KEYWORDS: 1,3,4-Thiadiazole; Schiff base synthesis; Antibacterial activity; MIC; Spectral characterization.

INTRODUCTION

Recent global assessments have demonstrated that bacterial antimicrobial resistance constitutes a significant and increasing contributor to global morbidity and mortality, emphasizing its substantial clinical and economic burden. The expanding prevalence of drug-resistant pathogens underscores the urgent need for innovative therapeutic strategies and structurally novel antibacterial agents to mitigate the escalating resistance crisis.[1]

According to the latest report by the World Health Organization, approximately one in six laboratory-confirmed bacterial infections worldwide in 2023 were resistant to antibiotic treatment, with resistance increasing in over 40% of monitored pathogen–drug combinations between 2018 and 2023**.** Drug-resistant Gram-negative pathogens, particularly *Escherichia coli* and *Klebsiella pneumoniae*, pose a significant global threat, with more than 40% of E. coli and 55% of K. pneumoniae isolates showing resistance to third-generation cephalosporins, exceeding 70% in some African regions**.** These findings highlight the escalating

global burden of antimicrobial resistance and underscore the urgent need for the development of novel and effective antibacterial agents.[2]

The failure of conventional antibiotics is increasingly driven by bacterial evolution of diverse resistance mechanisms, such as production of antibiotic-degrading enzymes, target modification, and efflux pumps that prevent drugs from reaching their targets. In addition, biofilm formation shields bacterial communities from effective antibiotic penetration, reducing clinical efficacy. Beyond inherited resistance, non-genetic factors such as dormant persister cells also tolerate antibiotic exposure and contribute to recurrent or chronic infections.[3-5]

The urgent need for novel antibacterial agents has redirected drug discovery efforts toward structurally versatile and biologically privileged scaffolds. Among these, heterocyclic compounds have emerged as fundamental building blocks in modern medicinal chemistry.[6]

In the context of antibacterial therapy, heterocyclic scaffolds are frequently associated with inhibition of key bacterial enzymes such as DNA gyrase, topoisomerase IV, and cell wall–synthesizing transpeptidases.[7-8]

The structural adaptability of heterocycles enables fine-tuning of lipophilicity, electronic distribution, and steric parameters, thereby optimizing membrane permeability and overcoming resistance mechanisms, particularly in Gram-negative pathogens.[9-10]

In thiadiazole-containing heterocyclic systems, the type and positional orientation of substituents significantly affect electronic delocalization, dipolar character, and lipophilic balance, thereby modulating molecular recognition at bacterial targets and influencing membrane permeability.[11]

Introduction of conjugated linkers such as imine (-C=N-) functionalities further alters electron density distribution and may enhance ligand–target interactions through hydrogen bonding and π–π stacking mechanisms.[12]

Accordingly, systematic structural tailoring of the thiadiazole core provides a rational framework for optimizing antimicrobial potency and selectivity.[13]

Thiadiazole nucleus is a five-membered heteroaromatic ring containing two nitrogen atoms and one sulfur atom within a $C_2H_2N_2S$ framework. The electronic behavior of the ring is governed by the high electro negativity of nitrogen atoms and the polarizable sulfur center. The extended conjugation within the thiadiazole framework enhances resonance stabilization and maintains rigidity of the ring system.[14-15] Thiadiazole occurs in four positional isomeric forms-1,2,3, 1,2,4, 1,2,5 and 1,3,4 depending on the arrangement of nitrogen and sulfur atoms within the five-membered ring. Among these, the 1,3,4-isomer is the most widely investigated owing to its greater thermodynamic stability and synthetic feasibility. The specific heteroatom orientation in the 1,3,4-configuration promotes improved π-electron delocalization, thereby enhancing overall aromatic stabilization. The thiadiazole ring shows distinct spectral signatures useful for structural verification. In IR spectra, the azomethine (C=N) stretch generally appears around 1600–1650 cm^{-1}, whereas C–S vibrations are observed at lower frequencies. In NMR analysis, the electron-deficient nature of adjacent nitrogen atoms causes noticeable deshielding effects in both proton and carbon signals, supporting structural confirmation of synthesized derivatives.[16]

Recognized for its multifunctional pharmacological potential, the 1,3,4-thiadiazole scaffold has been extensively explored in drug discovery. Various substituted derivatives have demonstrated marked antimicrobial effects against diverse bacterial and fungal strains, with several compounds exhibiting superior inhibitory performance relative to conventional reference agents.[17-18]

Apart from their antimicrobial activity, 1,3,4-thiadiazole derivatives also show notable anti-inflammatory effects. Several designed compounds have been found to reduce protein denaturation and lower inflammation levels, with results comparable to commonly used standard drugs.[19]

Beyond its antimicrobial and anti-inflammatory applications, the 1,3,4-thiadiazole scaffold has also been extensively investigated for its anticancer potential. Various substituted derivatives have demonstrated significant cytotoxic activity against diverse cancer cell lines, highlighting their promise as prospective lead compounds in antitumor drug development.[20-21]

The imine (–C=N–) group is an important pharmacophore in antibacterial drug design owing to its electron-deficient character and ability to form hydrogen bonds or coordinate with microbial enzymes. The azomethine nitrogen can interact with active sites or chelate metal ions, interfering with vital bacterial processes. When linked to the 1,3,4-thiadiazole nucleus, this functionality may further enhance activity, as the heteroatom-rich thiadiazole ring supports favorable electronic interactions and membrane permeability. Together, the –C=N– linkage and 1,3,4-thiadiazole core contribute to improved binding affinity and antibacterial effectiveness.[22]

Collectively, these findings support the rational design of imine-linked 1,3,4-thiadiazole derivatives as promising structurally optimized candidates for the development of novel and effective antibacterial agents to combat emerging drug resistance.

MATERIALS AND METHODS

Chemicals and Reagents

All reagents and solvents were of analytical grade and used as received without further purification unless otherwise stated. Substituted benzoic acids, hydrazine hydrate (99%), substituted aromatic aldehydes and glacial acetic acid were obtained from commercial suppliers and employed directly in the synthetic procedures.

Lawesson's reagent was utilized as a thionating and cyclization agent for the construction of the 1,3,4-thiadiazole nucleus. Absolute ethanol and dry toluene were used as reaction solvents, while ethyl acetate and n-hexane were employed as eluents for thin-layer chromatography (TLC).

Melting points were determined in open capillary tubes using a digital melting point apparatus and are reported uncorrected. The progress of the reactions was monitored by TLC performed on pre-coated silica gel 60 F_{254} aluminum plates, and the chromatograms were visualized under ultraviolet (UV) light at 254 nm.

All glassware was thoroughly oven-dried prior to use. Reflux reactions were carried out in round-bottom flasks fitted with water-cooled condensers and maintained under continuous magnetic stirring to ensure homogeneous mixing.

The general synthetic pathway adopted for the synthesis of the target 2,5-disubstituted-1,3,4-thiadiazole Schiff base derivatives is illustrated in Figure 1.

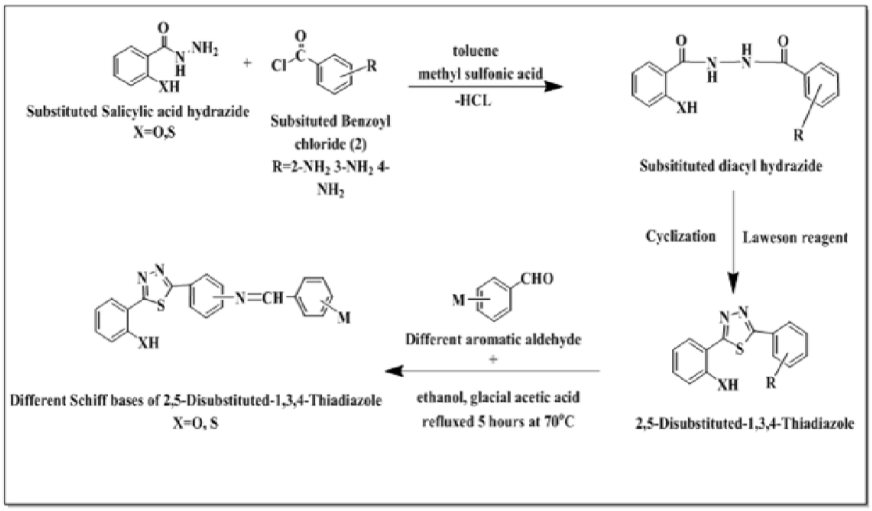


Fig. 1: General synthetic pathway for the synthesis of 2,5-disubstituted-1,3,4-thiadiazole derivatives via cyclization of substituted diacyl hydrazides followed by Schiff base formation with aromatic aldehyde.

The structural variations of the synthesized thiadiazole derivatives (TZ1–TZ10), including different heteroatom substitutions and aromatic aldehyde substituents, are summarized in Table 1

Table 1: Substituent variations in the synthesized 2,5-disubstituted-1,3,4-thiadiazole Schiff base derivatives (TZ1–TZ10), indicating the heteroatom (X = O or S), positional substitution of the aminophenyl group (Y), and aromatic aldehyde substituents (Z).

SN.	COMPOUNDS	X	Y	Z
1	TZ1	S	NH_2(2)	-$N(CH_3)_2$(4)
2	TZ2	O	NH_2(3)	-$N(CH_3)_2$(4)
3	TZ3	S	NH_2(3)	-$N(CH_3)_2$(4)
4	TZ4	O	NH_2(4)	-$N(CH_3)_2$(4)
5	TZ5	S	NH_2(4)	-$N(CH_3)_2$(4)
6	TZ6	O	NH_2(2)	-OH(4)
				-OCH_3(3)
7	TZ7	S	NH_2(2)	-OH(4)
				-OCH_3(3)
8	TZ8	O	NH_2(3)	-OH(4)
				-OCH_3(3)
9	TZ9	S	NH_2(3)	-OH(4)
				-OCH_3(3)
10	TZ10	S	NH_2(4)	-OH(4)
				-OCH_3(3)

Step–I: Synthesis of ortho, meta and para amino substituted diacyl hydrazides (Intermediate I)
Salicylic acid hydrazide (1.52 gm) or thiosalicylic acid hydrazide (1.68 gm) was dissolved in 25 mL of dry toluene in a round-bottom flask equipped with a reflux condenser and magnetic stirring. To this stirred solution, o-, m-, or p-aminobenzoyl chloride (1.20 ml) was added drop wise. Methylsulfonic acid (0.5 ml) was added as a catalyst, and the reaction mixture was refluxed for 4–6 hours.

The progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate: hexane (7:3) as the mobile phase. After completion, the reaction mixture was allowed to cool to room temperature and poured onto crushed ice with continuous stirring to neutralize the liberated hydrogen chloride.

The resulting precipitate was filtered, washed thoroughly with cold water to remove residual acid, and dried under vacuum. The crude product was purified by recrystallization from ethanol to afford the corresponding substituted diacyl hydrazide intermediates (Intermediate I) in good yield.[23]

Step–II: Synthesis of 2,5-Disubstituted-1,3,4-Thiadiazole Derivatives (Cyclization Step)
The synthesized substituted diacyl hydrazide (1.0 m.mol) was dissolved in dry toluene (20 ml) in a round-bottom flask equipped with a reflux condenser and magnetic stirring. To this solution, Lawesson's reagent[24] (0.24 g) was added portion-wise.

The reaction mixture was refluxed for 5–6 hours and the progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate: n-hexane (3:7) as the mobile phase.

After completion of the reaction, the solvent was removed under reduced pressure. The resulting crude residue was cooled to room temperature and poured into crushed ice. The solid obtained was filtered, washed with cold water, and dried.

The crude product was purified by recrystallization from ethanol to afford the corresponding 2,5-disubstituted-1,3,4-thiadiazole derivative as a solid in good yield.[25]

Step–III: Formation of imine (–C=N–) derivatives via nucleophilic condensation of 2,5-disubstituted-1,3,4-thiadiazole amines with substituted benzaldehydes
The synthesized 2,5-disubstituted-1,3,4-thiadiazole amine (0.25 gm) was dissolved in absolute ethanol (20 ml) in a 100 ml round-bottom flask equipped with magnetic stirring.

To this stirred solution, the appropriate substituted aromatic aldehyde (benzaldehyde 0.15 ml) was added, followed by the addition of glacial acetic acid (0.2 ml, 2–3 drops) as a catalyst.

The reaction mixture was refluxed for 4–6 h and the progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate:n-hexane (4:6) as the mobile phase.

After completion of the reaction, the mixture was allowed to cool to room temperature. The resulting precipitate was filtered, washed with cold ethanol (5 ml) and dried under vacuum.

The crude product was recrystallized from ethanol (10-15 ml) to afford the corresponding Schiff base derivatives as crystalline solids in good yield.[26]

The physico-analytical properties of the synthesized compounds, including molecular formula, melting point, Rf value, percentage yield, and λmax values, are presented in Table 2.

Table 2: Physico-anlytical properties of newly synthesized 2,5-disubstituted-1,3,4-thiadoazole derivatives.

SN	Compound	Molecular Formula	M.Wt. *	Melting point**	Colour	R_f Value	% Yield	λ_{max}***
1	TZ1	$C_{23}H_{20}N_4S_2$	416	171 -172	Magenta red	0.33	61%	264
2	TZ2	$C_{23}H_{20}N_4OS$	400	195 -196	Light yellow	0.64	68%	338
3	TZ3	$C_{23}H_{20}N_4S_2$	416	185 -186	Light brown	0.30	62%	273
4	TZ4	$C_{23}H_{20}N_4OS$	400	209 -210	Light yellow	0.61	58%	338
5	TZ5	$C_{23}H_{20}N_4S_2$	416	212 -213	Light brown	0.36	61%	266
6	TZ6	$C_{22}H_{17}N_3O_3S$	403	212 -213	Light yellow	0.59	71%	340
7	TZ7	$C_{22}H_{17}N_3O_2S_2$	419	201 -202	Magenta red	0.32	64%	230
8	TZ8	$C_{22}H_{17}N_3O_3S$	403	215 -216	Cream color	0.58	59%	338
9	TZ9	$C_{22}H_{17}N_3O_2S_2$	419	212 -213	Magenta red	0.28	66%	226
10	TZ10	$C_{22}H_{17}N_3O_2S_2$	419	219 -220	Magenta red	0.28	63%	266

*Molecular Weight in g/mol, ** Melting point in (°C), *** λ_{max} in nm.

SPECTRAL ANALYSIS OF COMPOUND (TZ1-TZ10)

Compound TZ1

IR Spectrum (KBr, cm^{-1}): 2995.36 (aliphatic C–H), 1624.53 (C=N, azomethine), 1496.32 (Ar C=C), 1270.30 (C–N dimethyamino), 1300.53, (C-N aromatic amine), 763.46 (Ar–H bending), 645.58 (C–S), 556.45 (thiadiazole ring vibrations).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.02 (1H, br s, –SH), 8.84 (1H, s, –CH=N–), 7.82–6.78 (12H, m**,** Ar–H)**,** 2.92 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 415 (M^+)

Compound TZ2

IR (KBr, cm^{-1}): 3448 (Ar-OH), 3058 (Ar–C–H), 2924 (C–H), 1618 (C=N), 1585 (C=C), 1512 (C=N, thiadiazole), 1256 (C–N), 1178 (C–O), 832 (p-disubstituted Ar), 745 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.18 (1H, s, Ar–OH), 8.86 (1H, s, CH=N), 7.85–6.75 (12H, m, Ar–H), 3.04 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 399 (M^+)

Compound TZ3

IR (KBr, cm^{-1}): 2572 (w, S–H), 3056 (Ar–C–H), 2923 (C–H, CH_3), 1619 (C=N, azomethine), 1587 (C=C, aromatic), 1513 (C=N, thiadiazole), 1260 (C–N), 834 (p-disubstituted benzene), 749 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.96 (1H, br s, SH), 8.88 (1H, s, CH=N), 7.82–7.48 (8H, m, Ar–H), 7.18 (2H, d, J = 8.8 Hz, Ar–H), 6.76 (2H, d, J = 8.8 Hz, Ar–H), 3.03 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 415 (M^+)

Compound TZ4

IR (KBr, cm^{-1}): 3438 (br, O–H), 3054 (Ar–C–H), 2921 (C–H), 1618 (C=N, azomethine), 1586 (C=C, aromatic), 1514 (C=N, thiadiazole), 1257 (C–N), 1180 (C–O, phenolic), 832 (p-disubstituted Ar), 750 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.22 (1H, s, OH), 8.87 (1H, s, CH=N), 7.83–7.50 (8H, m, Ar–H), 7.17 (2H, d, J = 8.8 Hz, Ar–H), 6.78 (2H, d, J = 8.8 Hz, Ar–H), 3.04 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 399 [M+]

Compound TZ5

IR (KBr, cm^{-1}): 2574 (w, S–H), 3056 (Ar–C–H), 2923 (C–H), 1620 (C=N, azomethine), 1587 (C=C, aromatic), 1512 (C=N, thiadiazole), 1261 (C–N), 833 (p-disubstituted Ar), 743 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.94 (1H, br s, SH), 8.89 (1H, s, CH=N), 7.84–7.52 (8H, m, Ar–H), 7.18 (2H, d, J = 8.8 Hz, Ar–H), 6.77 (2H, d, J = 8.8 Hz, Ar–H), 3.03 (6H, s, $N(CH_3)_2$).

Mass Spectrum (m/z): 415 [M+]

Compound TZ6

IR (KBr, cm^{-1}): 3452 (br, O–H), 3058 (Ar–C–H), 2934 (C–H, OCH_3), 1617 (C=N, azomethine), 1584 (C=C, aromatic), 1516 (C=N, thiadiazole), 1264 (Ar–O–CH_3), 1186 (C–O, phenolic), 1032 (C–O–C), 832 (Ar–H), 741 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.34 (1H, s, OH), 9.88 (1H, s, OH), 8.86 (1H, s, CH=N), 7.84–6.82 (12H, m, Ar–H), 3.80 (3H, s, OCH_3).

Mass Spectrum (m/z): 402 [M+]

Compound TZ7

IR (KBr, cm^{-1}): 3446 (br, O–H), 2570 (w, S–H), 3056 (Ar–C–H), 2932 (C–H, OCH_3), 1619 (C=N, azomethine), 1586 (C=C, aromatic), 1514 (C=N, thiadiazole), 1262 (Ar–O–CH_3), 1184 (C–O, phenolic), 1034 (C–O–C), 832 (Ar–H), 746 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.98 (1H, br s, SH), 10.26 (1H, s, OH), 8.88 (1H, s, CH=N), 7.85–6.80 (12H, m, Ar–H), 3.79 (3H, s, OCH_3).

Mass Spectrum (m/z): 418 [M+]

Compound TZ8

IR (KBr, cm^{-1}): 3456 (br, O–H), 3055 (Ar–C–H), 2923 (C–H, OCH_3), 1628 (C=N, azomethine), 1575 (C=C, aromatic), 1519 (C=N, thiadiazole), 1261 (Ar–O–CH_3), 1180 (C–O, phenolic), 1035 (C–O–C), 831 (Ar–H), 752 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.35 (1H, s, OH), 9.82 (1H, s, OH), 8.78 (1H, s, CH=N), 7.76–6.76 (12H, m, Ar–H), 3.78 (3H, s, OCH_3).

Mass Spectrum (m/z): 402 [M+]

Compound TZ9

IR (KBr, cm^{-1}): 3449 (br, O–H), 2575 (w, S–H), 3051 (Ar–C–H), 2939 (C–H, OCH_3), 1615 (C=N, azomethine), 1576 (C=C, aromatic), 1524 (C=N, thiadiazole), 1272 (Ar–O–CH_3), 1188 (C–O, phenolic), 1036 (C–O–C), 838 (Ar–H), 753 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.81 (s, 1H, Ar–SH), 10.25 (s, 1H, Ar–OH), 8.69 (s, 1H, CH=N), 7.82–6.88 (m, 11H, Ar–H), 3.73 (s, 3H, OCH_3).

Mass Spectrum (m/z): 418 [M+]

Compound TZ10

IR (KBr, cm^{-1}): 3458 (br, O–H), 2566 (w, S–H), 3051 (Ar–C–H), 2936 (C–H, OCH_3), 1625 (C=N, azomethine), 1581 (C=C, aromatic), 1526 (C=N, thiadiazole), 1275 (Ar–O–CH_3), 1185 (C–O, phenolic), 1034 (C–O–C), 838 (Ar–H bending), 752 (C–S–C).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.38 (s, 1H, Ar–SH), 10.20 (s, 1H, Ar–OH), 8.73 (s, 1H, CH=N), 7.90–6.75 (m, 11H, Ar–H), 3.83 (s, 3H, OCH_3).

Mass Spectrum (m/z): 418 [M+]

BIOLOGICAL EVALUATION

In Vitro Antibacterial Activity by Agar Well Diffusion Method

The synthesized compounds were evaluated for their *in vitro* antibacterial activity using the agar well diffusion method as previously described in standard antimicrobial screening procedures.[27,29] The test organisms included Gram-positive strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus mutans*, along with the Gram-negative strain *Escherichia coli*.

Fresh bacterial cultures were prepared in nutrient broth and incubated at 37°C for 18–24 h. Mueller–Hinton agar plates were prepared and uniformly inoculated with the respective bacterial suspension using a sterile cotton swab following established guidelines for antimicrobial susceptibility testing.[28] Wells of approximately 6 mm diameter were aseptically punched into the agar medium using a sterile cork borer.

The test compounds were dissolved in dimethyl sulfoxide (DMSO) and introduced into the wells at a fixed concentration. **Ciprofloxacin** was used as the standard reference drug, while the solvent served as the negative control.

The plates were incubated at 37°C for 24 h, and antibacterial activity was determined by measuring the diameter of the zone of inhibition (ZOI) in millimeters. The procedure was carried out in accordance with standardized antimicrobial testing protocols.[27, 29] All experiments were performed in triplicate and results were expressed as mean ± standard deviation (Mean ± SD).

The experimental workflow followed for antibacterial screening using the agar well diffusion method is represented in Figure 2.

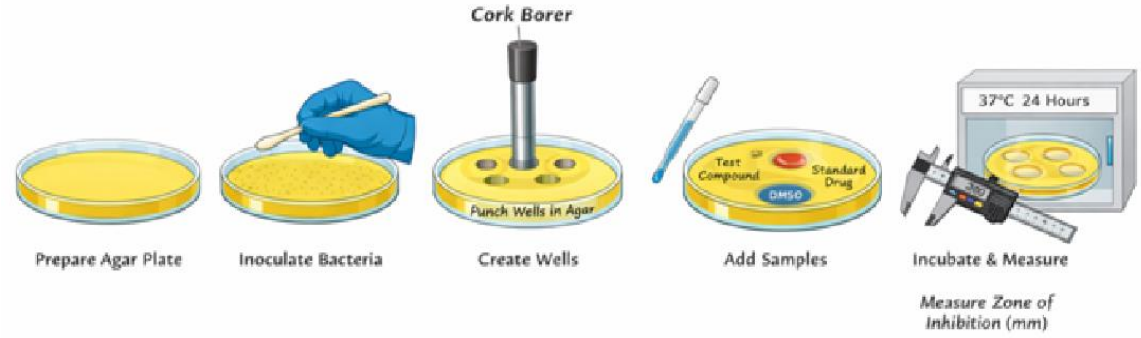


Figure 2: Experimental Workflow of Agar Well Diffusion Technique for Determination of Zone of Inhibition (ZOI).

Determination of Minimum Inhibitory Concentration (MIC) by Broth Micro-dilution Method

Antibacterial activity of the synthesized thiadiazole derivatives was quantified by the broth micro dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[30]

Fresh overnight cultures of *S. aureus* were adjusted to 0.5 McFarland standard and diluted to achieve a final inoculum density of approximately 5×10^5 CFU/ml per well, as recommended for standardized antimicrobial susceptibility testing.[30,31]

Serial two-fold dilutions of the test compounds (0.5–256 µg/ml) were prepared in sterile Mueller–Hinton broth in sterile 96-well micro titer plates following established broth dilution protocols.[31,32]

After addition of the bacterial inoculum, plates were incubated at 37 °C for 18–24 h under aerobic conditions. The lowest concentration of the compound showing no visible bacterial growth was recorded as the minimum inhibitory concentration (MIC). Appropriate controls included broth-only sterility controls and growth controls containing inoculated broth without test compound.[30, 32]

RESULT AND DISCUSSION

The antibacterial activity of the synthesized thiazole–Schiff base derivatives (TZ1–TZ10) was evaluated against four bacterial strains, namely *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans* and *Staphylococcus epidermidis* using the agar diffusion method. The results were expressed as Zone of Inhibition (ZOI) in mm ± SD and compared with the standard drug Ciprofloxacin (100 µg/disc).

Among the tested compounds, TZ5 exhibited the highest antibacterial activity with a ZOI of 23.0 ± 0.60 mm against *S. aureus*, indicating strong efficacy against Gram-positive bacteria. This was followed by TZ3, which showed significant inhibition **(**21.1 ± 0.58 mm**)** against *S. epidermidis*. Compounds TZ10 and TZ9 also demonstrated considerable activity against *E. coli* with inhibition zones of 20.5 ± 0.59 mm and 19.8 ± 0.56 mm, respectively.

Most of the synthesized derivatives showed comparatively better activity against *E. coli* and Gram-positive strains, suggesting a broad-spectrum antibacterial potential. However, all synthesized compounds exhibited lower activity compared to the standard drug. Ciprofloxacin showed the highest inhibition overall, with a maximum ZOI of 31.0 ± 1.18 mm against *S. aureus*.

The variation in antibacterial activity among the synthesized compounds may be attributed to structural differences and the presence of different substituents on the aromatic ring, which influence electron distribution, lipophilicity and interaction with bacterial cell targets. The enhanced activity observed in TZ5 suggests that specific substituent patterns significantly improve antibacterial potency.

Overall, the results indicate that the synthesized thiadiazole derivatives possess promising antibacterial activity, with TZ5 emerging as the most potent compound among the tested series, although still less active than Ciprofloxacin.

A comparative ranking of antibacterial activity based on maximum zone of inhibition values is shown in Table 3.

Table 3: Comparative antibacterial activity of synthesized compounds (TZ1–TZ10) and Ciprofloxacin based on maximum ZOI values.

Rank	Compound	Best ZOI (mm ± SD)	Bacteria
1	Ciprofloxacin	31.0 ± 1.18	*S. aureus*
2	TZ_5	23.0 ± 0.60	*S. aureus*
3	TZ_3	21.1 ± 0.58	*S. mutans*
4	TZ_{10}	20.5 ± 0.59	*E. coli*
5	TZ_9	19.8 ± 0.56	*E. coli*
6	TZ_4	18.4 ± 0.58	*E. coli*
7	TZ_7	18.3 ± 0.58	*E. coli*
8	TZ_8	18.1 ± 0.58	*E. coli*
9	TZ_1	18.0 ± 0.59	*E. coli*
10	TZ_2	17.1 ± 0.56	*E. coli*
11	TZ_6	16.4 ± 0.58	*S. aureus*

The maximum antibacterial activity of synthesized compounds against the tested bacterial strains is summarized in Table 4.

Table 4: Maximum Zone of Inhibition (ZOI) of Synthesized Compounds Compared with Standard Drug.

Compound Code	Maximum ZOI (mm ± SD)	Most Sensitive Organism
TZ1	18.0 ± 0.59	*E. coli*
TZ2	17.1 ± 0.56	*E. coli*
TZ3	21.1 ± 0.58	*S. mutans*
TZ4	18.4 ± 0.58	*E. coli*
TZ5	23.0 ± 0.60	*S. aureus*
TZ6	16.4 ± 0.58	*S. aureus*
TZ7	18.3 ± 0.58	*E. coli*
TZ8	18.1 ± 0.58	*E. coli*
TZ9	19.8 ± 0.56	*E. coli*
TZ10	20.5 ± 0.59	*E. coli*
Ciprofloxacin (100 µg/disc)	**31.0 ± 1.18**	***S. aureus***

MIC-Based Antibacterial Activity Assessment Against *S. aureus*

The minimum inhibitory concentration (MIC) values of the most active synthesized derivatives were determined against *S. aureus*. The results demonstrated that compound TZ5 exhibited the highest antibacterial potency among the tested derivatives with an MIC value of 5 µg/mL, which is comparable to the standard drug Ciprofloxacin (4 µg/mL).

Compounds TZ10 and TZ9 also showed significant antibacterial activity with MIC values of 6 µg/mL and 7 µg/mL, respectively. Compounds TZ1 and TZ3 displayed moderate activity (9 µg/mL), whereas compounds TZ4, TZ7 and TZ8 exhibited comparatively lower activity (>10 µg/mL).

The enhanced activity of compounds may be attributed to favorable electronic and structural features, which possibly improve bacterial cell membrane penetration and interaction with intracellular targets. The observed

MIC values correlate well with the zone of inhibition data, confirming their strong antibacterial potential,

The MIC potency ranking of the most active compounds against *S. aureus* is presented in Table 5

Table 5: MIC Potency Ranking Against *S. aureus* (Based on Broth Micro-dilution Method.

Rank	Compound	MIC (µg/mL)	Relative Activity
1	Ciprofloxacin (Standard)	4	Highest
2	TZ5	5	Very High
3	TZ10	6	High
4	TZ9	7	High
5	TZ1	9	Moderate
6	TZ3	9	Moderate
7	TZ4	>10	Low
8	TZ7	>10	Low
9	TZ8	>10	Low

Integrated Comparative Analysis: ZOI, MIC and SAR Correlation

The antibacterial performance of the synthesized thiadiazole derivatives was comprehensively evaluated using both zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) assays against *Staphylococcus aureus*. A clear correlation between ZOI and MIC values was observed, validating the reliability of the biological results and supporting the proposed structure–activity relationship (SAR).

Among the tested compounds, TZ5 exhibited the highest antibacterial potency, showing a ZOI of 23.0 ± 0.60 mm and an MIC value of 5 µg/ml, second only to the reference drug ciprofloxacin (MIC = 4 µg/mL). The strong activity of TZ5 can be attributed to the presence of a thiol (–SH) group combined with a strong electron-donating dimethylamino substituent. This structural arrangement likely enhances electron density across the aromatic system, improving π–π stacking interactions and facilitating stronger binding with bacterial enzymatic targets.

TZ10 demonstrated comparatively high activity with a ZOI of 19.0 ± 0.60 mm and an MIC value of 6 µg/mL. The presence of methoxy and phenolic groups contributes moderate electron donation and hydrogen-bonding capability, which may enhance molecular interaction with bacterial cell components. However, its activity remained slightly lower than TZ5, suggesting that stronger electron-donating substituents provide superior antibacterial enhancement.

Compounds such as TZ9 (MIC = 7 µg/mL) displayed good activity, whereas derivatives with weaker substituents (TZ4, TZ7, TZ8) exhibited lower potency (MIC >10 µg/mL), corresponding to smaller ZOI values. This trend confirms that substituent electronic properties play a decisive role in determining antibacterial efficacy.

The inverse relationship between MIC and ZOI values further supports the SAR findings, where compounds exhibiting larger inhibition zones correspondingly showed lower MIC values. Overall, the results suggest that the thiadiazole–Schiff base scaffold serves as a biologically active core, while antibacterial potency is significantly influenced by the electronic nature and lipophilicity of the attached substituents.

Activity Correlation Summary

A consistent inverse correlation between ZOI and MIC values was observed across the compound series, indicating that derivatives producing larger inhibition zones required lower concentrations to suppress bacterial growth. This agreement between qualitative (ZOI) and quantitative (MIC) assays strengthens the biological validity of the study.

Comparative Conclusion

The integrated biological evaluation establishes a strong correlation between electronic substituent effects and antibacterial potency. Compounds bearing strong electron-donating groups demonstrated superior ZOI and lower MIC values, indicating enhanced bacterial inhibition. The thiadiazole–azomethine framework appears to be a promising antibacterial pharmacophore, and rational modification of substituents may further optimize antimicrobial efficacy, The structure–activity relationship (SAR) analysis correlating substituent effects with antibacterial activity is summarized in Table 6.

Table 6: Structure–Activity Relationship of Thiadiazole Derivatives (TZ_1–TZ_{10}): a comparative study of ZOI against *S.aureus* (gram+ve) and *E.coli*(gram-ve) strain.

Compound	Key Substituent(s)	Electronic Nature	ZOI (*S. aureus*) mm	ZOI (E. coli) mm	Activity Level
TZ1	–SH	Moderate EDG	17.3	18.0	Moderate
TZ2	–OH	Weak EDG	14.3	17.1	Low–Moderate
TZ3	–SH + EDG	Moderate	20.3	21.0	Good

TZ4	–OH substituted	Weak–Moderate	15.5	18.4	Moderate
TZ5	–SH + $–N(CH_3)_2$	Strong EDG	23.0	21.1	Highest
TZ6	–OH	Moderate	16.4	16.2	Moderate
TZ7	–SH	Moderate	17.5	18.3	Moderate
TZ8	–OH	Weak–Moderate	14.5	18.1	Moderate
TZ9	–SH	Moderate	18.1	19.8	Good
TZ10	–SH + $–OCH_3$ + –OH	Moderate EDG	19.0	20.5	Good

An integrated comparison of ZOI, MIC values, and electronic substituent effects is provided in Table 7,

Table 7: Integrated Antibacterial Activity and SAR Profile of Thiadiazole Derivatives against *S. aureus*: a comparative study of ZOI and MIC.

Compound	Key Substituent(s)	ZOI (mm)	MIC (µg/mL)	Electronic Effect	Activity Rank
Ciprofloxacin	Reference	31.0	4	—	1
TZ5	–SH + $–N(CH_3)_2$	23.0	5	Strong EDG	2
TZ10	–SH + $–OCH_3$ + –OH	19.0	6	Moderate EDG	3
TZ9	–SH	18.1	7	Moderate	4
TZ1	–SH	17.3	9	Moderate	5
TZ3	–SH + EDG	20.3	9	Moderate	6
TZ4	–OH	15.5	>10	Weak	7
TZ7	–SH	17.5	>10	Moderate	8
TZ8	–OH	14.5	>10	Weak	9

Mechanistic Insights into Antibacterial Activity

The antibacterial activity of the synthesized derivatives may be attributed to multiple mechanistic pathways. The thiadiazole ring system is known to interact with bacterial enzymes through coordination and hydrogen bonding interactions. The azomethine (–CH=N–) functionality may facilitate metal ion chelation within microbial systems, potentially disrupting essential enzymatic processes. Additionally, the presence of thiol (–SH) groups could enhance interaction with thiol-sensitive bacterial proteins, leading to enzyme inhibition. Electron-donating substituents further increase electron density across the aromatic system, potentially strengthening π–π stacking interactions with nucleic acid bases or enzyme active sites. Collectively, these structural features may contribute to bacterial growth inhibition.

CONCLUSION AND FUTURE PERSPECTIVES

In the present study, a series of novel thiadiazole-based Schiff base derivatives (TZ1–TZ10) were successfully synthesized and evaluated for their antibacterial potential against Gram-positive and Gram-negative bacterial strains. The biological assessment demonstrated that several derivatives exhibited moderate to significant antibacterial activity, with TZ5 emerging as the most potent compound. The observed zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) values showed a consistent inverse correlation, confirming the reliability of the antibacterial evaluation.

Structure–activity relationship (SAR) analysis revealed that the antibacterial potency is strongly influenced by the electronic nature of substituents attached to the aromatic ring. Compounds bearing strong electron-donating groups, particularly in combination with thiol functionality and azomethine linkage, exhibited enhanced activity. The thiadiazole nucleus, coupled with the –CH=N– pharmacophore, appears to serve as a crucial structural scaffold responsible for antibacterial efficacy.

A mechanistic interpretation suggests that these derivatives may exert their antibacterial action through enzyme inhibition, potentially involving hydrogen bonding, π–π stacking interactions, and coordination with active-site residues of essential bacterial enzymes. The enhanced activity of selected compounds may be attributed to improved electronic complementarity and binding stabilization within the enzymatic cavity.

Overall, the findings of this study highlight the thiadiazole–Schiff base framework as a promising platform for the rational development of new antibacterial agents.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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CERTIFICATE OF PARTICIPATION

THIS CERTIFICATE IS PRESENTED TO

HRIDESH SINGH CHAUHAN

From P. K. UNIVERSITY SHIVPURI M.P.

For his/her active participation in **Online Mode** in the **International Conference** on **"Recent advances in Drug Delivery Systems" (ICDDS-2025)** organized by **GCRG COLLEGE OF PHARMACY, Lucknow (U.P.) India** on **Saturday, 22ND February 2025.** He/She also presented a paper/poster entitled

SYNTHESIS AND CHARACTERIZATION OF SUBSTITUTED 1,3,4-THIADIAZOLE AS POTENTIAL ANTIMICROBIAL AGENTS

PROF.(Dr.) DINESH CHANDRA
PRINCIPAL & CONVENOR
GCRG COLLEGE OF PHARMACY, BKT LUCKNOW

PROF. A.N. SINGH
DIRECTOR GENERAL, GCRG GROUP OF INSTITUTIONS, BKT LUCKNOW

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This is to certify that Dr./Mr./Ms Hridesh Singh Chauhan

of P. K. University Shivpuri (M.P.)

has participated in the International Conference on "Transformative Trends in Drug Discovery and Delivery: Exploring Next Generation Therapeutics and Technologies" held on 21st and 22nd March, 2025 and presented a poster/an oral on the topic Design, Synthesis and antimicrobial activities of Novel 1,3,5-thiadiazine-2-thione derivatives Containing a 1,3,4-thiadiazole group

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FROM Faculty of Pharmacy, P.K. University, Shivpuri, M.P.

HAS PARTICIPATED & PRESENTED A PAPER ENTITLED *Synthesis & Antimicrobial Evaluation of Novel 1,3,4-thiadiazole Derivatives bearing disulphide bonds*

IN THE PHARMATECH 2024, INTERNATIONAL CONFERENCE ON "DIGITAL TRANSFORMATION IN PHARMACEUTICALS: A NEW ERA" ON 30TH NOV 2024, ORGANIZED BY R V INSTITUTE OF PHARMACY, BIJNOR AND PHARMACEUTICAL EDUCATIONAL SOCIETY (PESOTS)

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We sincerely acknowledge your valuable contribution to the success of this conference.

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