Synthesis, Characterization, and Bioevaluation of *N*-Protected Amino Acid Conjugates

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Master of Science in Chemistry

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Dedication

To my Parents

& my Siblings

Acknowledgments:

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Abstract

We have continually faced the challenge of diseases, which have evolved in complexity over time. The significance of medicine has increased proportionally, leading to progress in the development of novel drugs. However, the emergence of antibiotic-resistant diseases has a significant impact on our healthcare infrastructure. In response to this challenge, conjugates are formed by coupling *N*-protected amino acids with commercial drugs These conjugates contain amide moiety in their structure. The conjugates were synthesized by first protecting the amino group of amino acid with different protecting groups for example Boc, Cbz, and BzCl, and then coupled with commercial drugs such as 2-Aminobenzothiazole, Sulfadiazine, and Pyrimethamine in the presence of different coupling reagents like EDC and DCC. These synthesized compounds were characterized by FTIR and NMR. The biological activities of these compounds, including their anti-bacterial and cytotoxic assay were evaluated through testing. The anti-bacterial activity was assessed against two bacterial strains such as *Escherichia Coli* (Gram-negative) and *Staphylococcus Aureus* (Gram-positive) which were carried out by qualitative method using agar diffusion assay. The synthesized conjugates are 9-50% more potent against bacteria as compared to their starting reactant. NA-5 showed a maximum zone of inhibition of 23mm against *Escherichia Coli* and 19mm against *Staphylococcus Aureus*. The MTT assay was performed on the colon cell line to check the cytotoxicity of the synthesized compounds which had no adverse effects on healthy human cells.

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List of abbreviations:

Abbreviation	Explanation
Bn	Benzyl
Boc	Tert-butoxycarbonyl
Bz	Benzoyl
Cbz	Benzyl chloroformate
DCC	Dicyclohexylcarbodiimide
DCU	Dicyclohexylurea
DMSO	Dimethyl sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
FT-IR	Fourier transform infrared spectroscopy
Hz	Hertz
IBCF	Isobutyl chloroformate
IC_{50}	50 % Inhibitory capacity
PG	Protecting group
Ph	Phthaloyl
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin layer chromatography

Chapter 1

1.1 Introduction:

An amide linkage is sometimes referred to as an organic amide or a carboxamide linkage in organic chemistry. The amide bond can be thought of as a derivative of a carboxylic acid in which an amine group has taken the place of the hydroxyl group or vice versa. They are regarded as an important class of organic compounds because of their many applications[1].

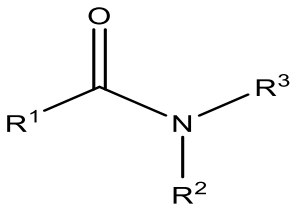


Figure-1: **General Structure of Amide Bond**

In proteins, amide bonds are also referred to as peptide bonds. They are essential for the structure and operation of biological macromolecules[2]. They connect the amino acids in proteins to form the polypeptide chain's backbone. Amide bonds are widely utilized in the production of medicinal compounds to enhance stability, bioavailability, and interactions with biological targets which can be achieved by incorporating amide functionalities into drug structures. Amide linkages are frequently found in peptide and peptidomimetic medicines[3].

Amide bonds are basic components of various polymers. Polyamides, for example, are synthetic polymers with repeating amide linkages in their backbone. Nylon is a well-known example of a polyamide. Amide bonds are found in many pigments and dyes. The color in certain dyes is a result of the conjugated systems that often involve amide groups[4].

Compounds containing amide moiety are employed in the formulation of emulsifiers and surfactants. These molecules help stabilize emulsions and also contribute to the detergent properties of certain compounds. Enzymes involved in forming or breaking amide bonds participate in crucial cellular functions[5].

Amide bonds also contribute to the properties of materials. The presence of amide linkages in certain polymers influences their mechanical and thermal properties. They can act as catalysts in

various reactions, contributing to the acceleration of chemical transformations. Amide bond formation is commonly employed in bioconjugation reactions, biosensors, and facilitating the attachment of molecules in biomaterials[6].

Understanding the properties and reactivity of amide bonds allows scientists and researchers to tailor molecules for specific purposes in a wide array of fields, contributing to advancements in chemistry, medicine, and materials science[7].

The present work is the coupling of N-amino acids with commercial drugs primarily focusing on enhancing their biological activities such as anti-bacterial. Drugs coupling with N-amino acids could modulate the metabolism of drugs, potentially reducing the formation of toxic metabolites or improving metabolic activation, leading to enhanced therapeutic effects while minimizing side effects[8]. Potential synergies between the N-amino acids and drugs lead to enhanced pharmacological activities compared to individual components alone[9].

Modifying the chemical structure of drugs through coupling with N-amino acids minimizes undesirable side effects commonly associated with these drugs, thereby improving their safety profile[10].

1.2 Protecting Groups:

A protecting group refers to a functional group temporarily added to a molecule to mask or shield a specific reactive site, preventing undesired reactions at that site during a chemical transformation, and they can be removed later under controlled conditions, revealing the original functional group.

Overall, protecting groups play a crucial role in synthetic chemistry, especially in the synthesis of complex molecules, and pharmaceuticals, with the flexibility to conduct intricate and multi-step syntheses by allowing them to control the reactivity of specific functional groups throughout the synthetic route[11].

1.2.1 Tert-butyloxycarbonyl:

In organic synthesis and peptide chemistry, the tert-butyloxycarbonyl (Boc) protecting group is a commonly utilized chemical moiety. During the synthesis of complex compounds, this protective group is used to temporarily mask the amino group ($-NH_2$) in amino acids and peptides, preventing undesirable reactions[12].

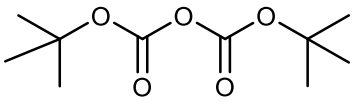
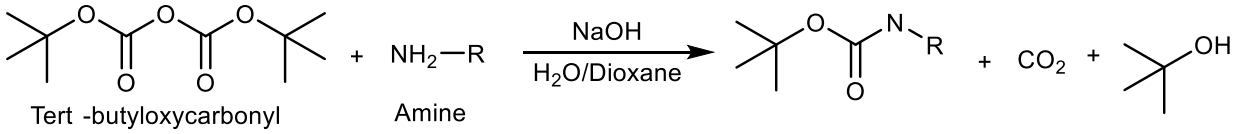


Figure-2: **Structure of Tert -butyloxycarbonyl**

A carbamate functional group's nitrogen is joined to a tert-butyl (t-Bu) group to form the Boc protecting group. The amino group is stabilized by this structural arrangement, which protects it against interactions with different reagents and environmental factors that are frequently encountered in organic synthesis. Effectively "protecting" the amino functionality, the tert-butyl group acts as a large, non-reactive barrier.



Scheme-1: **Amine protection by using Boc**

It is acid sensitive therefore mild acidic conditions such as Trifluoroacetic acid (TFA) is used to selectively remove it[13]. Because of its convenience and adaptability, the tert-butyloxycarbonyl protecting group has established itself as a standard instrument in the toolbox of organic chemists and biochemists. Although more advanced protective groups with distinct characteristics have been created, the Boc group is still a dependable option for a variety of uses in peptide chemistry and chemical synthesis. Chemical and pharmaceutical research has advanced greatly as a result of its successful application in the controlled manipulation of amino groups[14].

1.2.2 Benzyl Chloroformate:

The benzyl ester of chloroformic acid, or the chloride of the benzyloxycarbonyl (Cbz or Z) group, is another name for benzoyl chloroformate. In chemical synthesis, benzyl chloroformate, also known by its abbreviation Cbz-Cl, is a frequently used reagent for adding the benzyl-protecting group to amino groups[15].

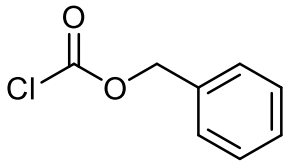
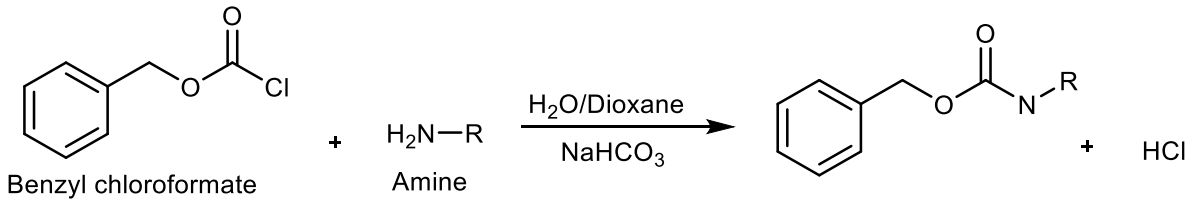


Figure-3: **Structure of Benzyl chloroformate**

A benzyloxycarbonyl molecule joined to an amino group's nitrogen under different reaction circumstances, offering protection.



Scheme-2: **Amine protection by using Cbz**

1.2.3 Phthaloyl:

The phthaloyl protecting group is common in peptide synthesis, where amino acids are joined to form peptides and proteins. It can be selectively eliminated when needed to return the amino group to its reactive state, just as other protective groups. Deprotection is usually achieved by cleaving the amino group from the phthaloyl group with hydrazine or another nucleophile[16].

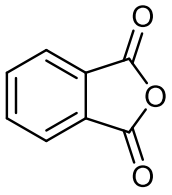
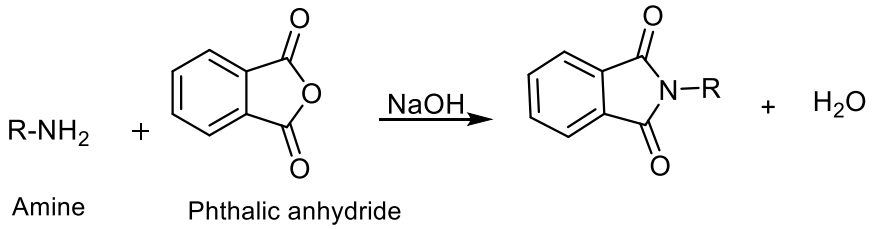


Figure-4: **Structure of Phthalic anhydride**

Phthalic anhydride reacts with amine in the presence of base to form amide[17].



Scheme-3: **Amine protection by using Phthalic anhydride**

1.2.4 Benzoyl (Bz):

Benzoyl is indeed used as a protecting group in organic synthesis, particularly in the protection of amine. The benzoyl group, often abbreviated as "Bz", can be attached to an amine functionality to temporarily block its reactivity while other chemical reactions are performed especially useful in multi-step organic synthesis where selective reactions are needed[18].

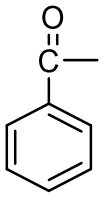
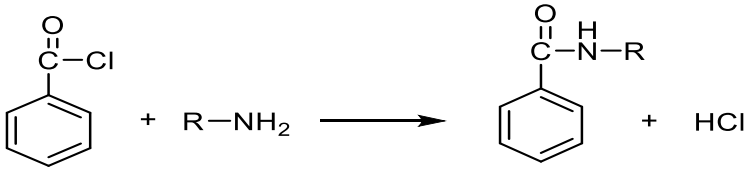


Figure-5: **Structure of Benzoyl**

The protection typically involves reacting the amine with benzoyl halides in the presence of a base, to form an amide linkage. This amide linkage can be removed later using appropriate conditions, such as acidic or basic hydrolysis, to regenerate the original amine functionality[19].



Scheme-4: **Amine protection by using Benzoyl chloride**

1.3 Coupling Reagents:

Chemical substances known as coupling reagents are employed in organic synthesis to help functional groups create covalent connections with one another. Their main goal is to increase reaction efficiency by encouraging the coupling of particular functional groups and reducing the number of undesirable byproducts that are formed[20].

1.3.1 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide(EDC):

EDC is a crucial coupling reagent that is widely used in peptide chemistry and other organic synthesis processes. A carbodiimide functional group including an ethyl and a 3-

(dimethylaminopropyl) group is present in its chemical structure. EDC is well known for its capacity to efficiently generate amide bonds in the presence of amines by activating carboxylic acids through the production of an O-acylisourea intermediate. By removing the kinetic and thermodynamic obstacles connected to the coupling reactions, this activation step increases the reactivity of carboxylic acids[21].

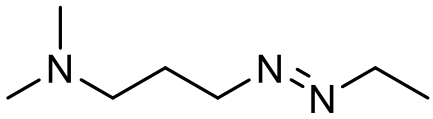


Figure-6: **Structure of EDC**

When it used in peptide synthesis, EDC is essential for connecting amino acids and facilitating the effective construction of peptide chains. Precise control over the synthesis of peptide bonds is made possible via a two-step method involving the production of the O-acylisourea intermediate and the amine's subsequent nucleophilic attack.

EDC has its share of difficulties even though it is quite successful. Side reactions are possible, including the production of byproducts called N-acylurea. EDC is frequently used with nucleophilic catalysts like N-hydroxysuccinimide (NHS) to overcome this problem and reduce unwanted byproducts. Because of its ability to activate carboxylic acids for the production of amide bonds, particularly in peptide synthesis, it is widely used and significant in many areas of organic chemistry[22].

1.3.2 Dicyclohexylcarbodiimide(DCC):

N,N'-Dicyclohexylcarbodiimide (DCC) is widely used in organic synthesis, especially when amide bonds are being formed. Two cyclohexyl groups surround a core carbodiimide functional group in its chemical structure. DCC is essential for the activation of carboxylic acids, which makes it easier for them to couple with amines to form amide bonds.

DCC is an important mediator in the peptide synthesis process, helping to assemble amino acids into polypeptide chains. When carboxylic acids are activated by DCC, an O-acylisourea intermediate is created. This intermediate then interacts with an amino group to generate the required amide bond. Peptide chains can be gradually extended using this method with a great degree of selectivity and precision[23].

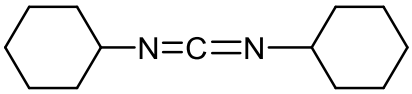


Figure-7: **Structure of DCC**

DCC's effectiveness in encouraging the formation of amide bonds in mild reaction circumstances is one of its main advantages. DCC does have several drawbacks, though, such as a propensity to produce N,N'-dicyclohexylurea byproducts. To address this problem, DCC is frequently employed in conjunction with co-catalysts like 1-hydroxybenzotriazole (HOBt) or nucleophilic scavengers like 4-dimethylaminopyridine (DMAP) to maximize coupling reaction efficiency and reduce side reactions[24]. It is regarded as a useful coupling reagent in organic chemistry, providing a dependable and popular technique for effective amide bond production. Its importance in the synthesis of complex compounds with a variety of applications is highlighted by its function in the synthesis of peptides and other chemical processes[25].

1.4 Drugs:

1.4.1 2-Aminobenzothiazole:

An amino group is joined to the benzene ring at the 2-position of 2-aminobenzothiazole, a heterocyclic molecule composed of a fused benzene ring and a thiazole ring. This organic molecule has a wide range of uses in industrial and medical chemistry[26].

2-amino-benzothiazole is an essential component in medicinal chemistry that is used to synthesize a wide range of medications and bioactive substances. Due to its structural characteristics, it can be used as a useful scaffold when designing possible therapeutic candidates, it shows pharmacological activities[27].

Multiple techniques are employed in the synthesis of 2-amino-benzothiazole and its derivatives, which enables the change of its chemical structure to customize its qualities for certain uses. The amino group also offers a reactive site for further functionalization, which makes it possible to create molecules with more potent biological activity[28].

Researchers are still looking into novel synthesis approaches and the biological effects of 2-aminobenzothiazole derivatives despite their wide range of uses. The goal of these initiatives is to

find new compounds with enhanced qualities for use in industrial applications and medication development.

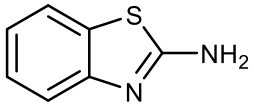


Figure-8: **Structure of 2-Aminobenzothiazole**

1.4.2 Sulfadiazine:

Sulfadiazine is a sulfonamide antibiotic that belongs to the class of antimicrobial agents known as sulfa drugs. Sulfadiazine exerts its antibacterial effects by inhibiting the synthesis of dihydrofolic acid, a key precursor in the biosynthesis of folic acid, which is essential for bacterial growth and replication[29]**.**

One notable application of sulfadiazine is in the treatment and prevention of various infections. It has been employed in combination with other antibiotics to enhance the spectrum of antimicrobial activity and reduce the likelihood of bacterial resistance. In combination with pyrimethamine, sulfadiazine is considered a standard therapy for toxoplasmosis, particularly in individuals with compromised immune systems, such as those with HIV/AIDS[30].

While sulfadiazine has proven effective in combating bacterial and parasitic infections, it is important to note that some individuals may experience adverse reactions or allergic responses to sulfa drugs. Common side effects include skin rashes and hypersensitivity reactions, and in rare cases, more severe adverse effects may occur[31]. Its clinical applications make it an important component in the arsenal of antimicrobial agents, contributing to the management of infections and the improvement of public health.

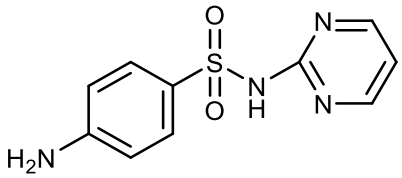


Figure-9: **Structure of Sulfadiazine**

1.4.3 Pyrimethamine:

Pyrimethamine is an antiparasitic medicine that is a member of the antifolate drug class. It is mostly used to treat and prevent malaria, an infectious disease spread by mosquitoes that is brought on by Plasmodium parasites[32].

Pyrimethamine works by preventing the parasite's dihydrofolate reductase (DHFR) enzyme from functioning. Pyrimethamine specifically targets the parasites, inhibiting their development and replication, by interfering with certain metabolic pathways[33].

To increase efficacy and lower the risk of drug resistance, pyrimethamine is frequently used in combination with other antimalarial medications, such as sulfadoxine, in the context of treating malaria. The capacity of malaria parasites to become resistant to individual medications makes combination therapy especially crucial[34].

Pyrimethamine is also used, frequently in conjunction with sulfadiazine, to treat toxoplasmosis[35]. Combination therapy works well against the parasite Toxoplasma gondii, which can lead to serious consequences, particularly in those with compromised immune systems[36]. Pyrimethamine can have side effects, such as rash, gastrointestinal distress, and hematologic abnormalities, even though it is usually well tolerated. Pyrimethamine use during pregnancy should be done very carefully since it could harm the growing fetus.

In recent years, the challenges of combating malaria, a significant global health concern, have been highlighted by the significance of combination therapy and the continuous research for novel antimalarial medications. With its unique method of action against the parasites, pyrimethamine is still an important weapon in the toolbox of antimalarial drugs.

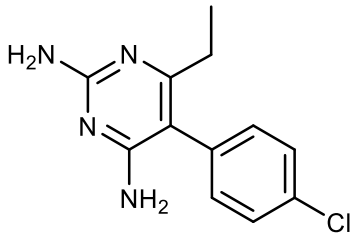


Figure-10: **Structure of Pyrimethamine**

1.5 History of Amide Linkage:

The story of organic chemistry is closely linked to the history of the amide bond, as notable advancements in the field occurred during the 1800s and 1900s. With a carbonyl group (C=O) bound to a nitrogen atom, the amide functional group is essential for several synthetic, biological, and medicinal uses. The earliest known studies on amides date from the middle of the 1800s. French scientist Auguste Laurent is recognized for his early research on the amide production process and his groundbreaking contributions to our understanding of organic molecules[37]. German scientist Emil Fischer made significant advances in this subject in the late 19th and early 20th centuries. Fischer won the 1902 Nobel Prize in Chemistry for his research on the synthesis and structure of amide bond-containing peptides and proteins[38].

With the development of methods, scientists were able to investigate the three-dimensional structures of molecules containing amides, providing insights into their characteristics and interactions[39]. The amide bond is still essential in chemical synthesis, medicinal chemistry, and biochemistry today[40]. Its importance in natural and manmade substances is highlighted by its presence in peptides, proteins, and medicines. The persistent significance of this functional group in the field of organic chemistry is demonstrated by the ongoing investigation of novel techniques for the effective production of amide bonds[41].

1.6 Chemistry of Amide Linkage:

The bond between a nitrogen atom and a carbonyl group (C=O) is known as an amide bond, and it is a basic building block of organic chemistry. This functional group is present in many biological substances, including amides, proteins, and peptides, and it is essential to their structure and operation[42]. A condensation reaction between an amine and a carboxylic acid usually results in the synthesis of the amide linkage by eliminating water. The creation of peptides and proteins depends on this process, called amidation, in which amino acids are linked by amide bonds to form complex three-dimensional structures[43].

One important characteristic of the amide linkage that makes it so common in both natural and artificial compounds is its stability. The distribution of electron density by the amide group resonance structure creates a degree of double bond character that improves the overall stability. In

the case of proteins, this stability is essential because amide bonds support the tertiary structure and activity of proteins[44].

A flexible functional group with importance in medicinal chemistry is the amide bond, in addition. A comprehensive grasp of the hydrolysis of amide bonds is important for understanding drug metabolism and bioavailability, as amide linkages are present in many pharmaceutical products[45]. Scholars persistently investigate novel techniques for amide bond synthesis, encompassing eco-friendly and catalytic methodologies, to tackle obstacles in organic synthesis and pharmaceutical development.

In conclusion, the chemistry of amide linkage is complex and includes production, stability, and a variety of functions in both synthetic and biological environments. Its prevalence in biomolecules and its impact on medication development highlight how crucial it is to comprehend and work with the chemistry of amide bonds in modern research and applications[46].

1.7 Properties of Amide Linkage:

The amide bond has the following important properties:

Under many different circumstances, amide bonds are comparatively stable. The oxygen and nitrogen atoms resonance structure contribute to the distribution of electron density and gives the molecule some double-bond character. In biological structures like proteins, where amide bonds contribute to the structural integrity of the macromolecule, this stability is essential[47].

Hydrogen bonds can be formed by the nitrogen atom in the amide group. This characteristic is important for understanding how hydrogen bonds between amide groups in the peptide backbone produce alpha helices and beta sheets in the secondary structure of proteins. Amide bonds are found in many biological substances, including proteins and peptides. Proteins are composed of amide bonds that link amino acids sequentially. The arrangement of these bonds determines the three-dimensional structure and function of the protein[48].

The partial double-bond nature of the amide group restricts rotation around the C-N bond. This constraint is pertinent to the study of protein folding and peptide conformation and has consequences for the conformational flexibility of molecules containing amide bonds. Amides are typically polar substances, and the presence of hydrogen-bonding groups affects how soluble they

are in water. While bigger amides may show decreased solubility, smaller amides are frequently soluble in water[49].

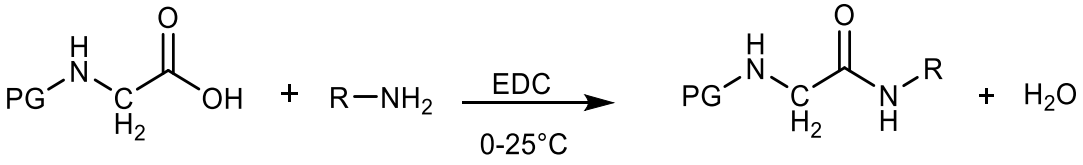
In the presence of acids or bases, amide bonds can go through hydrolysis processes that break the connection between the nitrogen atom and the carbonyl carbon. This is important for biological functions including metabolism and protein breakdown. An important tool in synthetic chemistry, amide bonds are versatile and stable[50].

Amide bond formation is an essential step in the synthesis of drugs, peptides, and other chemical molecules. Researchers as well as practitioners in disciplines including organic synthesis, medicinal chemistry, and biochemistry, where the manipulation and application of amide-containing compounds are crucial, must understand the characteristics of the amide bond[51].

1.8 Synthetic method of Amide bond:

1.8.1 General reaction:

The general reaction for amide synthesis involves the condensation of a carboxylic acid or its derivative (such as an acid chloride, anhydride, or ester) with an amine[52]. In the case of amino acids, the amino group is mostly protected with different protecting groups (such as Bn, Bz, Boc, and Cbz).



***Scheme-5*: General Reaction of Amide Bond Synthesis**

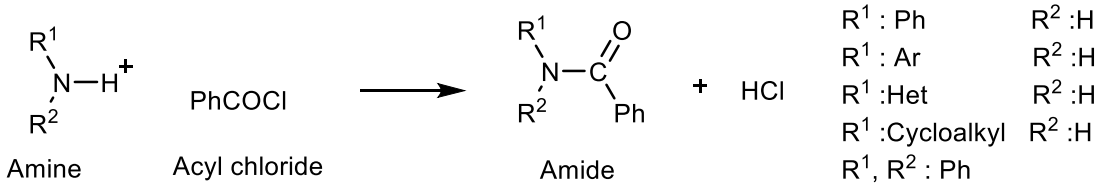
1.8.2 General Mechanism:

The carboxylic group is activated by coupling agents such as EDC or DCC to convert the carboxylic acid into a more reactive derivative. The activated carboxylic acid reacts with the amine, a step that involves a nucleophilic attack of the amine on the carbonyl carbon of the carboxylic acid derivative. This results in the amide bond formation where the nucleophilic amine attacks the acyl group, displacing the leaving group (which could be a proton or another group)[53].

Various methods are used to synthesize amide bonds which are as follows.

1.8.3 Synthesis from Acyl Chloride:

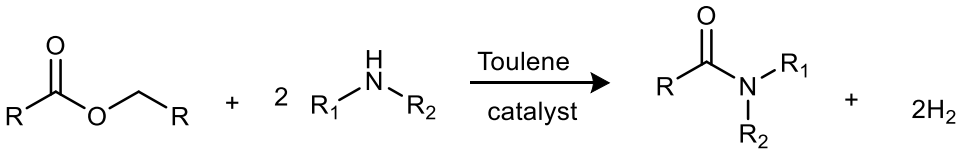
Using acyl chlorides for amide synthesis is a widely used and proven technique. An amine and an acid chloride react to generate an amide. Usually, the reaction is conducted at anhydrous conditions to avoid the development of undesirable by-products. The hydrochloric acid generated during the reaction is frequently neutralized by the application of a base[54]. To regulate the rate of reaction, the amine solution is gradually supplemented with acid chloride. Benzoyl chloride and amines are used by Jing Wang and his colleagues to create amide[55].



Scheme-6: **Synthesis of Amide from Acyl Chloride**

1.8.4 Synthesis from Ester:

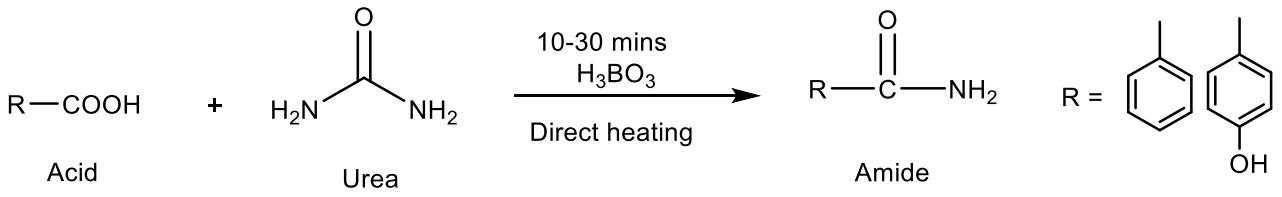
It is possible to synthesize amides efficiently from esters and amines directly while releasing molecular hydrogen in mild, neutral circumstances. In the presence of toluene, David Milstein and his colleagues use the ruthenium-pincer PNN complex as a catalyst to synthesize amide[56].



Scheme-7: **Synthesis of Amide from Ester**

1.8.5 Solvent Free Synthesis:

Synthesis of amide bond is also carried out in dry conditions without any solvent being used. It is an efficient method because utilization of a relatively cost-effective catalyst, yield efficiency, purity of synthesized product, and less consumption of time[57]. Chiragkumar J Gohil and his co-workers prepared amide bonds by the utilization of boric acid as a catalyst in solvent-free conditions[58].



Scheme-8: **Synthesis of Amide by Solvent-Free Method**

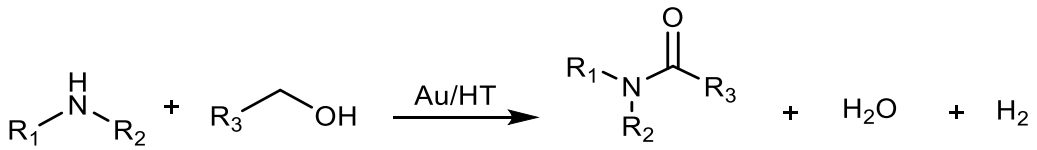
1.8.6 Synthesis of Amide by Coupling reagents:

The reaction of amines with amino acids in the presence of a coupling reagent is one widely used technique. A few common coupling reagents are IBCF, EDC, and DCC[59]. Ibrahim MA and his colleagues synthesized amide by combining 2-amino-4-phenylthiazole with Boc-protected amino acids and IBCF as coupling reagents[60].

Scheme-9: **Synthesis of Amide by using Coupling Reagent IBCF**

1.8.7 Dehydrogenative Amide Synthesis:

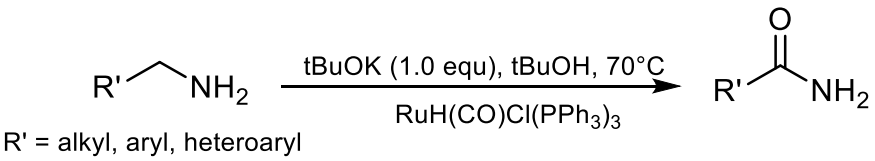
Dehydrogenative amide synthesis from alcohols involves the conversion of alcohols into amides. This reaction involves the use of a suitable catalyst[61]. The development in the methods for dehydrogenative amide synthesis from alcohols is an active area of research in organic chemistry, as it offers a direct and atom-economical approach to amide bond formation. Jiangling Zhu and his group synthesized amide from amine and alcohol by using Hydrotalcite-supported Nano-gold (Au/HT)[62].



Scheme-10: **Dehydrogenative Amide synthesis**

1.8.8 Oxidation of Amines:

Amines are readily available reagents that can be used to synthesize amide. They are relatively cheap. Methods are developed successfully for the catalytic oxidation of amines to their respective amide. This type of oxidation can be catalyzed by various transition metal catalysts under mild conditions. Ritwika Ray and co-workers oxidized amines to amide in the presence of ruthenium as a catalyst[63].



Scheme-11: **Amide synthesis by catalytic oxidation of amine**

1.9 Applications of Amide Linkage:

Amide linkage has versatile applications in various fields of life.

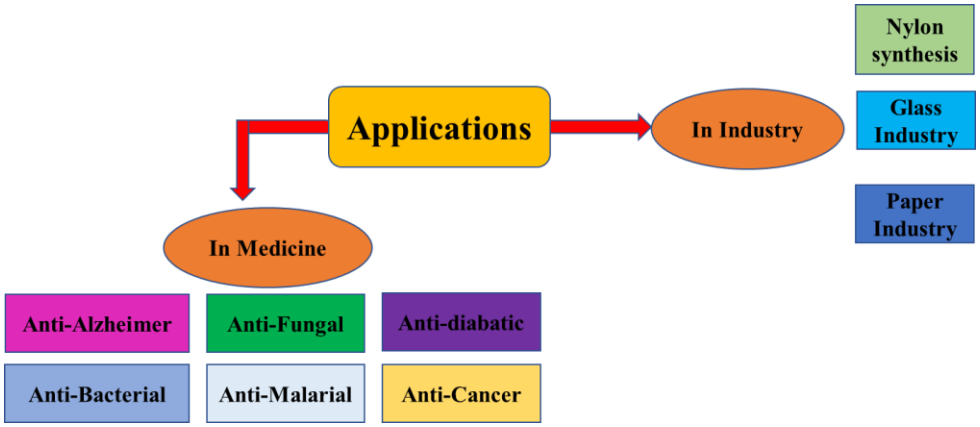


Figure 11: **Applications of Amide Bond**

1.9.1 Medicinal Applications:

Compounds containing amide moiety in structure play a pivotal role in medicinal applications due to their diverse and valuable properties. Their involvement extends to antibiotics, where amides contribute to the inhibition of bacterial cell wall synthesis, combating various bacterial infections[64].In the treatment of neurological disorders, amide linkage-containing compound serves as essential components in anticonvulsant medications, influencing neuronal activity.

Additionally, amide bonds are integral to the synthesis of antidepressants, contributing to the modulation of neurotransmitter levels[65].

These compounds with amide bonds in their structure find extensive use in analgesics and anti-inflammatory drugs, contributing to the alleviation of pain and reduction of inflammation. In the realm of anesthetics, amide linkage is utilized in local anesthetics, aiding in the temporary loss of sensation during medical procedures. These substances are widely used in analgesics and anti-inflammatory medications, where they help to lessen inflammation and relieve pain[66].

Furthermore, they play a crucial role in the production of antidepressants and the regulation of neurotransmitter levels. Their relevance to cardiovascular health is demonstrated by the fact that they are present in some antihypertensive medications. Amide linkage is also important in the development of anti-cancer drugs, that specifically target pathways that promote the growth of cancer cells[67].

Aside from this, amide linkage is present in hormones, vitamins, and other bioactive substances, helping to support their physiological processes. In conclusion, amide linkage has a broad spectrum of therapeutic applications in medicine, which makes it essential to the study of medicine and medication development[68].

1.9.1.1 Biological Activities:

A compound containing amide bonds in its structure offers a flexible framework in drug design that can be used to create compounds with particular biological activity. The inhibition of nerve impulses by amide-containing local anesthetics emphasizes the significance of amides in regulating neuronal activity[69]. The amide groups included in antibiotics help to prevent the production of bacterial cell walls as well as play a critical role in hormone structures, affecting signaling pathways and binding affinities. Moreover, their capacity to interfere with important biological processes is demonstrated by their interactions with enzymes, such as protease inhibitors included in antiviral medications[70]. A comprehensive understanding of amides biological action is crucial for maximizing medication efficacy and reducing adverse effects, which makes them invaluable in the complex field of medicinal chemistry.

1.9.1.2 Anti-Bacterial activity:

Compounds containing amide linkage in their structure are well recognized for their function in biological macromolecules like proteins and peptides, recent studies have focused a great deal of interest on their antibacterial properties. Several investigations have examined amide's potential as an antibacterial agent, yielding encouraging findings[71].

A few substances with amide groups may have antibacterial qualities. The antibacterial activity of these substances is frequently influenced by the amide functional groups and particular chemical structures. Utsab Manna and his team created L-phenylalanine bis-amide derivatives[72].

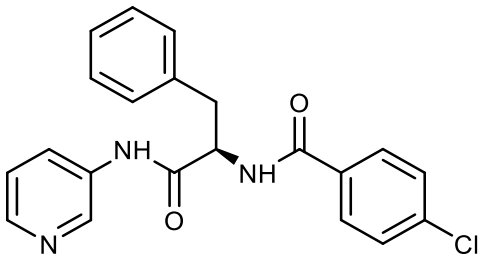


Figure-12: **4-Chlorophenyl substituted bis-amide as an Anti-Bacterial agent.**

1.9.1.3 Anti-Cancer Activity:

For instance, amide-containing drugs have been identified as inhibitors of specific enzymes and proteins involved in cancer progression that may target kinases, by inhibiting these kinases, amides can disrupt the signaling cascades that promote uncontrolled cell growth in cancer[73].

Research for new therapeutics and high-potency drugs with low toxicity is increasing and becoming important for biomedical sciences and pharmaceuticals. Nowadays, cancer is the major cause, leading to a large no. of deaths. Every year millions of cancer cases and deaths are reported. Therefore, many novel strategies for the development of new drugs have been developed. In recent times, amide-containing compounds have been reported as potential anticancer agents[74].

Afar Ali and his team synthesized pyridine amide-containing derivatives and their pharmacokinetics suggest that some derivatives have good anticancer activities[75].

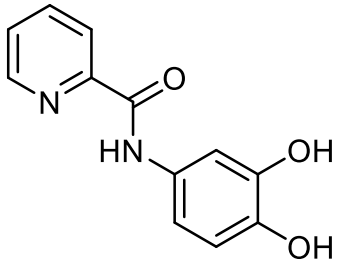


Figure-13: **N-(3, 4-dihydroxyphenyl)picolinamide as Anti-Cancer agent**

Greta Klejborowska and his team synthesized amides of 4-bromothiocolchicine. These derivatives were active against several different cancer cell lines with IC_{50} values of 5.3–14 nM[76].

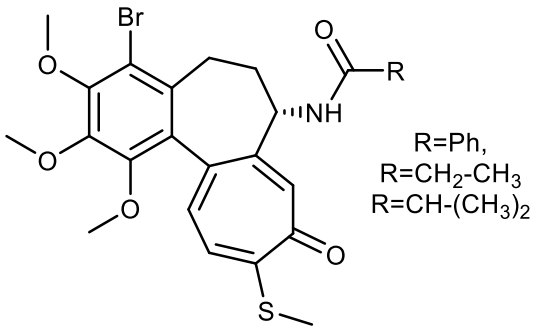


Figure-14: **Colchicine Derivatives as Anti-Cancer Agent**

1.9.1.4 Anti-Diabatic Activity:

The anti-diabetic activity of amide linkage in a drug is an area of interest in pharmaceutical research, as researchers explore new compounds for managing diabetes and related metabolic disorders. Some amides have shown promising effects in preclinical studies, suggesting their role in improving glucose homeostasis and insulin sensitivity. One mechanism through which amides may exert their anti-diabetic effects is by influencing key metabolic pathways. Amide moiety can modulate enzymes involved in glucose metabolism, such as those responsible for glycolysis and gluconeogenesis. By regulating these processes, amides may help maintain balanced blood glucose levels[77]. Furthermore, amide moiety in a compound may interact with receptors involved in insulin signaling, because of their ability to enhance insulin sensitivity, potentially mitigating insulin resistance and improving glucose uptake by cells[78].

Ning-bo Qin and co-workers synthesized N,N'-(butane-1,4-diyl)bis(4-hydroxy-benzamide) and this compound shows good α-Glucosidase inhibitory assay[79].

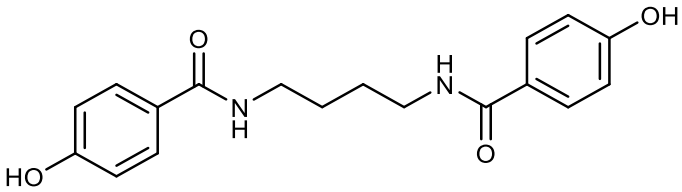


Figure-15: **N,N'-(butane-1,4-diyl)bis(4-hydroxybenzamide) as Anti-Diabatic agent**

1.9.1.5 Anti-Alzheimer Activity:

One avenue of exploration involves the anti-inflammatory and antioxidant properties of amide bonds. Amide base compound shows anti-inflammatory activity that may modulate immune responses and reduce inflammation in the brain, potentially slowing down the neurodegenerative processes associated with Alzheimer's[80].

They may interact with neurotransmitter systems involved in cognitive function. Modulation of neurotransmitter activity, such as acetylcholine, glutamate, or serotonin, could influence synaptic transmission and mitigate cognitive decline in Alzheimer's patients. Some have been investigated for their potential to enhance cholinergic function, which is known to be impaired in Alzheimer's disease[81].

Mehmet Koca and co-workers synthesized compounds from indene and tested their results of acetyl cholinesterase & butyl cholinesterase inhibition by various doses of indene-based scaffold. The compound shows good AChE & BuChE enzyme inhibition, giving IC_{50} values of 6.42±1.685 & 1.23±0.125μM[82].

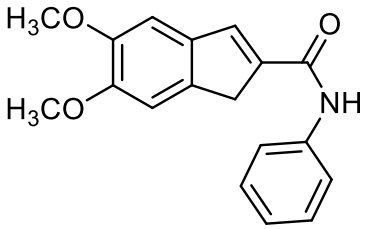


Figure-16: **5,6-dimethoxy-N-phenyl-1H-indene-2-carboxamide as Anti-Alzheimer agent**

1.9.1.6 Anti-Fungal Activity:

The cell wall is a crucial structure for fungal integrity and survival. The fungal cell wall can be weakened by inhibiting the enzymes that are involved in the manufacturing of certain components, such as chitin or beta-glucans, which makes the fungus more vulnerable to antifungal medications. Mepronil may also obstruct the creation of fungal proteins and other essential cellular functions[83]. Depending on their unique mechanism of action and dose, amides can have either fungicidal or fungistatic effects by targeting certain enzymes or proteins that are necessary for fungal growth and reproduction[84].

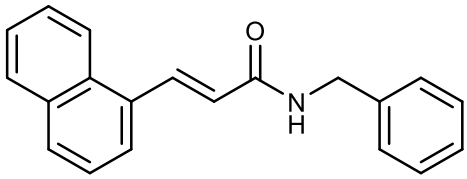


Figure-17: **(E)-N-benzyl-3-(naphthalen-1-yl) acrylamide as Anti-Fungal agent**

1.9.1.7 Anti-Malarial Activity:

Numerous derivatives, from benzamide, acetohydroxamic acid, and aryl sulfonamide, have been studied for their antimalarial properties[85]. These substances have demonstrated inhibitory effects on key enzymes required for the development and spread of plasmodium parasites.

Katja Kettler and his co-workers synthesized a compound by acylation of 2-amino-5-nitrobenzophenone by appropriate acid halides. The substituted derivative demonstrated an IC_{50} of 47 nM when tested against the multi-drug resistant strain Dd2 of Plasmodium falciparum[86].

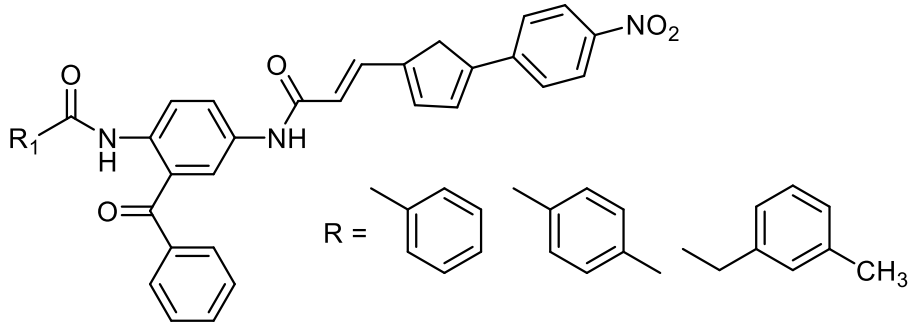


Figure-18: **[5-(4-nitrophenyl)-2-furyl]acrylic acid substituted benzophenone as an Anti-Malarial agent**

1.9.2 Applications in the Synthetic Industry:

A polymer that gets its special qualities from renewable resources is unsaturated biobased polyamides. Unsaturated biobased polyamides are produced using sustainable raw materials, such as plant-based feedstocks, in contrast to conventional polyamides, which are usually sourced from fossil fuels. This environmentally friendly strategy is in line with the expanding need across a range of industries for sustainable and ecologically conscious products[87]. These polyamides' unsaturation adds double bonds to the polymer structure, which increases the polymer's adaptability.

W. Malte and his colleagues have developed unsaturated biobased polyamides, which have versatility for various applications, such as automotive components, textiles, packaging materials, and others[88].

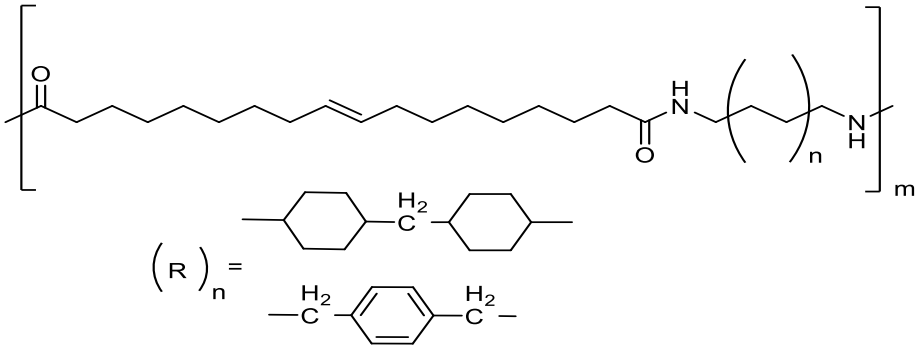


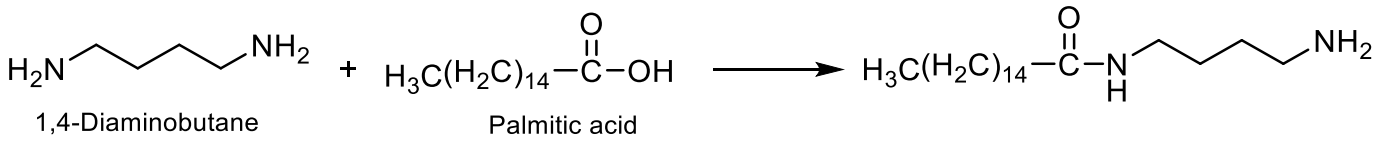
Figure-19: **(E)-N-(4-(methylamino)butyl)-18-oxononadec-9-enamide for Synthetic Industry**

1.9.3 Application in Corrosion Inhibition:

Metals deteriorate naturally as a result of chemical reactions with oxygen, contaminants, and moisture in the environment. This process is known as corrosion. Strong connections between amides and metal surfaces are well known, and their interaction helps the metal's surface form a solid, adherent layer. By keeping corrosive materials from getting to the metal and obstructing the electrochemical processes that cause corrosion, the layer serves as a barrier[89].

Hazim Saad Jabbar and his co-workers synthesized amide BAPA[N-(4-aminobutyl)palmitamide] by the reaction 1,4-Diaminobutane with Palmitic acid. When placed over the mild steel and exposed

to 1 M HCl, it delayed and reduced the corrosion by adhering to the steel surface and forming a protective layer. The efficiency in inhibition of corrosion is increased with increasing BAPA concentration, and maximum inhibition (of 90.5%) was observed at 0.5 mM BAPA[90].



Scheme-12: **Synthesis of N-(4-aminobutyl)palmitamide as Anti-Corrosion Agent**

Chapter 2

2.0 Experimental:

2.1 Chemicals:

Chemicals used are glycine, sodium chloride, pyrimethamine, hydrochloric acid, sulfadiazine, sodium bicarbonate, N,N′-dicyclohexylcarbodiimide, benzoyl chloride, magnesium sulfate, phthalic anhydride, potassium bisulfate, 2-aminobenzothiazole, magnesium sulfate, sodium hydroxide and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide. All the above chemicals were purchased from Merck, and Sigma Scientific (China).

2.2 Solvents:

The analytical grade solvents were used for experimentation, such as 1,4-dioxane ($C_4H_8O_2$), dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), n-hexane (C_6H_{14}), triethyl amine ($C_6H_{15}N$), tetrahydrofuran (C_4H_8O), diethyl ether/petroleum ether ($(C_2H_5)_2O$), dimethyl-formamide (C_3H_7NO), methanol (CH_3OH), acetone (C_3H_6O), acetonitrile (CH_3CN), chloroform ($CHCl_3$), ethyl acetate($C_4H_8O_2$), and ethanol (C_2H_5OH). By employing the following procedures, solvents mentioned previously, like triethylamine ($C_6H_{15}N$), and tetrahydrofuran (C_4H_8O), were subjected to drying.

2.2.1 Drying of Solvents:

2.2.1.1 Tetrahydrofuran (C_4H_8O):

Tetrahydrofuran was dehydrated by placing the necessary amount into a two-neck round-bottom flask equipped with a drying tube. After that add benzophenone and sodium metal in the solvent at intervals until the blue color appears which indicates the absence of oxygen and moisture from the solvent. Subsequently, it underwent 2-3 hours for reflux, then distillation was conducted in a nitrogen atmosphere. Afterward, the dry tetrahydrofuran (C_4H_8O), was stored in a dark brown, bottle.

2.2.1.2 Amine (Triethyl-amine ($C_6H_{15}N$):

The triethyl amine (TEA) was placed in a round bottom flask, along with an appropriate quantity of KOH pellets. The mixture was refluxed for three hours and subsequently subjected to distillation over a calcium hydride under nitrogen, any remaining water and other volatile impurities can be removed. After distillation, storing the purified triethylamine in a dark brown, sealed bottle helps to protect it from light and air, which can degrade the solvent.

2.3 Techniques:

2.3.1 Thin Layer Chromatography (TLC):

TLC is a versatile and widely used technique in various fields, including analytical chemistry, organic chemistry, biochemistry, and pharmaceuticals, due to its, speed, simplicity, and low cost. It is a separation technique that is used to monitor reaction progress and completion. The sample mixture is applied on the TLC plate, it is placed in a developing chamber with a solvent system, allowing the mobile phase to ascend the plate and separate the components based on their interactions with the stationary phase. The plate can be examined under a UV lamp to identify the product or other components[91].

2.3.2 $KMnO_4$ Stain:

To prepare the KMnO4 solution for TLC staining, mix 3 grams of potassium permanganate ($KMnO_4$) and 20 grams of K_2CO_3 in 250 mL of distilled water. Stir until all solids are dissolved to obtain a purple solution [92].

2.3.3 Ninhydrin Stain:

Ninhydrin solution is prepared by dissolving 0.1g of ninhydrin into 50 mL of absolute ethanol[93].

2.3.4 Column Chromatography:

Column chromatography is a separation method mostly employed to purify chemicals from a mixture. Silica gel 60 (0.063-0.200mm) E. Merck 70-230 mesh ASTM was used for performing column chromatography.

2.4 Instrumentation:

2.4.1 Melting Point:

The melting points of all solid compounds were measured using a capillary melting point apparatus SMP-10. This apparatus is commonly used in laboratories for accurately determining the melting points of solid products.

2.4.2 Infrared Spectroscopy:

FTIR of synthesized compounds was performed by ATR, ALPHA-20488, ranging from 500-4000 cm^{-1}.

2.4.3 ^{1}H-NMR:

H-NMR was performed by a BRUKER spectrometer using DMSO-d_6 as a solvent with a frequency of 400 MHz.

2.5 Solution Preparation of Cytotoxic Assay:

Using the MTT assay, the cytotoxic assay was carried out on the colon cell line. The cells were incubated at 37°C for 24 hours in 5% CO_2, under humid circumstances, to develop in 96-well plates of DMEM media supplemented with 10% fetal bovine serum and 1% antibiotics (streptomycin and penicillin-G). Trypsinization was performed after the actively dividing cells had formed a confluent monolayer. Cell suspension (105 cells per ml) was then sown in the wells containing culture medium and various concentrations (1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml 31.2µg/ml) of the 6 derivatives (NA-1, 2, 3, 7, 8, and 9). The plate was then incubated for 48 hours at 37°C with 5% CO_2. 20 µl of MTT (5 mg/ml PBS) was added to each well after the cellular viability for each concentration of the derivatives had been determined. The plates were then incubated once more at 37°C for 3 hours in a 5% CO_2 atmosphere. The medium was carefully removed after incubation. To dissolve the formazan crystals created by metabolically active cells, 100 µl of DMSO was applied. The optical density (O.D.) of the wells was then assessed using a microplate reader at 570 nm with a reference wavelength of 655 nm.

2.6 Solution Preparation of Anti-Bacterial Activity:

Qualitative Method using Agar Diffusion Assay

Bacterial strains are grown overnight in a nutrient broth medium until they reach a certain concentration, approximately 10^{-6} colony-forming units (CFU) per milliliter. The cultures are then swabbed onto nutrient agar plates using sterile cotton swabs. This step spreads the bacterial cultures evenly across the agar surface. The synthesized samples are spread on discs and these discs will be used in the experiment. The synthesized samples are treated as the experimental group, while untreated (control) samples are also included. Both treated and untreated are planted onto the nutrient agar plates inoculated with bacterial cultures. The agar plates are then incubated at 37°C for 24 hours[104]. This allows time for any antibacterial effects to occur. After the incubation period, the plates are examined for the presence of a zone of inhibition[105]. Clear zones indicate inhibition of bacterial growth due to the antibacterial properties of the synthesized compound. The diameter of the clear zones (inhibition zones) is measured and recorded as a relative measure of the antibacterial effectiveness of the compound. The means of three replicates are calculated and tabulated for statistical analysis.

2.7 Protection of Functional Groups of Amino acids:

In chemical synthesis, particularly, protecting groups are essential because they selectively mask specific functional groups to stop unwanted reactions while facilitating the desired transformations. When it comes to amino acid synthesis and amide bond formation protecting groups are especially important[94].

2.7.1 Amino (-NH_2) Group Protection of Amino acids:

The amino group (-NH_2) of glycine needs to be protected initially to prevent unwanted reactions during amide bond synthesis. Protecting groups helps to ensure that the desired bond formation occurs selectively and efficiently. Protecting groups for the protection of amino (-NH_2) group are Cbz or benzoyl chloroformate, Bz or benzoyl, Alloc or alloxycarbonyl, Boc or tert-butoxycarbonyl, Bn or benzyl, and Fmoc or fluorenylmethoxycarbonyl[95].

2.7.1.1 Synthesis of *N*-Boc-Glycine:

Glycine (10mmol, 0.75g) was dissolved in 1:1 water and dioxane (12mL). Subsequently, NaOH (10mmol, 0.4g) was added and then stirred for 15 minutes. The temperature was sustained at 0°C by cooling the reaction mixture in an ice bath. Then tertbutoxy-carbonyl (10mmol, 2.2g) was added to the reaction mixture at 0°C and continued the stirring for a further 18 hours. At the end of the reaction, dioxane was removed with the help of a rotary evaporator. After that reaction mixture was acidified by using $KHSO_4$ and then extracted with ethyl acetate (3 times). Then organic layer was combined and evaporated under reduced pressure. The purified product was obtained in good yield. Reaction time: 18-20 hours, yield: 88%, Physical appearance: white crystalline-shaped powder, M.P: 86-89°C, TLC: 10% methanol/chloroform solvent system. FTIR(ν_{max}): 2976(CH_2, str), 1737(C=O, acid), 1665(C=O, amide), 1408(N-H, amide), 1367[$C(CH_3)_3$], 1195(C-O) cm^{-1}.

2.7.1.2 Synthesis of *N*- Cbz-Glycine:

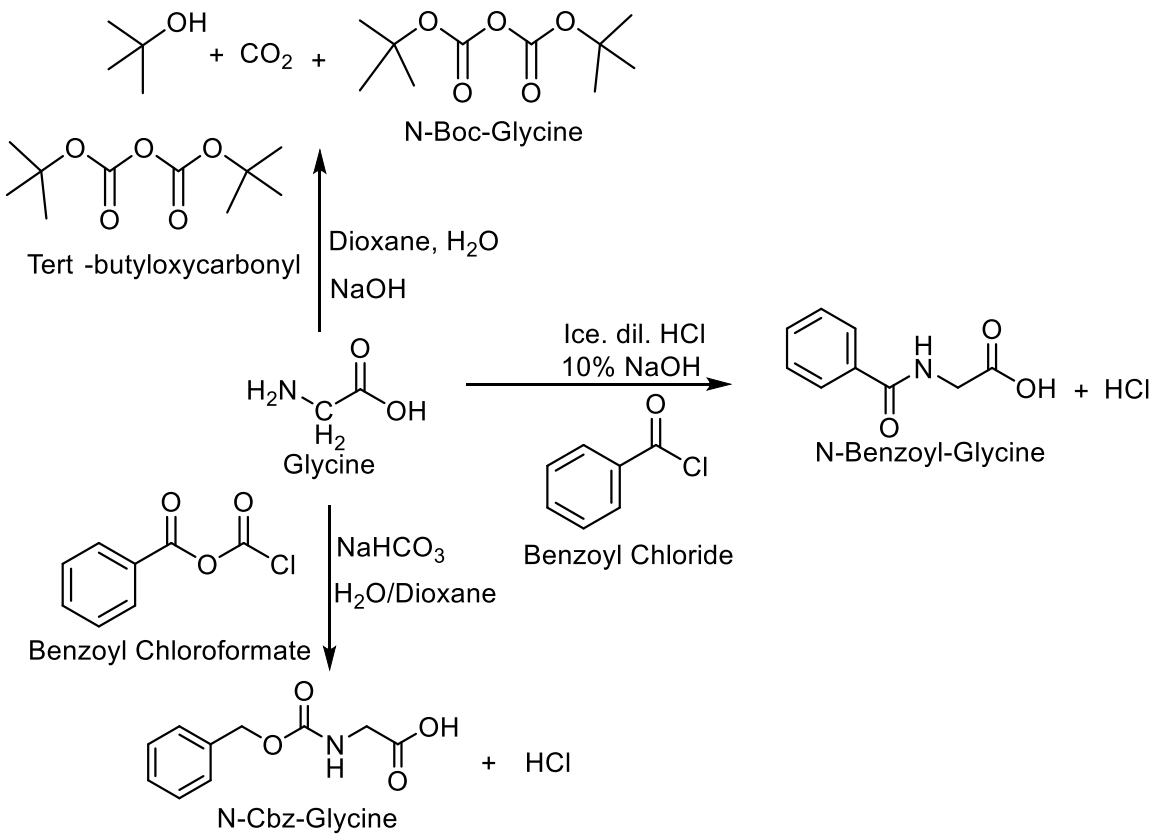
Glycine (2.28 g, 30.43mmol) was dissolved in a (50 mL) mixture of dioxane-water (1:1) in an ice bath and allowed to stir. $NaHCO_3$ (30.43mmol. 2.55g) was added to the reaction mixture and then benzoyl chloroformate (30.43mmol 4.4 mL) was added to stir overnight. First, the dioxane was evaporated with the rotary evaporator and the reaction mixture was acidified with 1N HCl. Extract the product with the help of solvent extraction. Then organic layer was collected and evaporated to obtain the final product.

Reaction time: 18-20 hours, yield: 81%, Physical appearance: off-white crystalline, M.P: 122-124°C, TLC: 10% methanol/chloroform solvent system, FTIR(ν_{max}): 3330(N-H), 2976(C-H), 1737(C=O, acid), 1665(C=O, amide), 1558(N-H) amide 1482(C=C), 1308(C-O-C) cm^{-1}.

2.7.1.3 Synthesis of *N*-Benzoyl-Glycine:

In a 10% NaOH solution add the 2.97g (39.75 mmol) of glycine and continue the stirring. Then add 3.8mL of benzoyl chloride (39.75mmol) in the reaction mixture. TLC is performed to monitor the reaction progress. On completion of the reaction, the solution is acidified by using HCl. The solution was filtered and wash the residue with cold water and dried to obtain the final product.

Reaction time: 16 hours, yield: 90%, Physical appearance: white crystalline shaped powder, M.P: 184-187°C, TLC: 10% methanol/chloroform solvent system, FTIR(ν_{max}): 3334 (N-H), 2976(C-H str), 1734(C=O, acid), 1600(C=O, amide), 1550(N-H, amide), 1412(C=C), 1195(C-O) cm^{-1}.



Scheme-13: **Synthesis of *N*-Protected-Glycine**

2.8 Synthesis of Target Molecule:

Target Molecules were synthesized by using different *N*-protected amino acids and amine-based drugs as starting materials according to the desired sequence. Synthesis was carried out in a stepwise schematic way where the amino group was protected first using Boc-anhydride, benzoyl chloride, phthaloyl, and benzyl chloroformate then further *N*-protected amino acid coupled with amine-based drugs such as 2-aminobenzothiazole, sulfadiazine, and pyrimethamine, resulting in the formation of respective Amides.

2.9 Synthesis of Amide by using *N*-Protected Amino Acids with Drugs:

2.9.1 Coupling of *N*-Benzoyl-Glycine with Drugs:

An experiment was conducted by utilizing a two-neck round bottom flask fitted with a 3-way stopper. The vacuum was created in the flask by using a vacuum pump, and then nitrogen gas was introduced. Next dissolve the *N*-benzoyl-glycine (0.51g, 2.85mmol) in the dry THF (20-25mL) stirring until the *N*-benzoyl-glycine is dissolved. Using a syringe, add triethylamine (3.97 mL, 2.85 mmol) as the base and continue stirring. Afterward, regulate the reaction temperature to a range of 0-25°C using an ice bath. Subsequently, add coupling reagent EDC (0.55g, 2.85 mmol) to the reaction mixture. Finally, add the subsequent amount of the drug to a flask and continue stirring for a further 5 to 6 hours. Three different drugs such as 2-aminobenzothiazole (0.42g, 2.85mmol), sulfadiazine (0.72g, 2.85mmol), and pyrimethamine (0.35g, 1.42mmol) are coupled with *N*-benzoyl-glycine in three different reactions. The reaction progress is monitored with the help of TLC. Evaporate the solvent (THF) using a rotary evaporator. Separate the product by using solvent extraction. Then wash the organic layer with concentrated solutions of NaCl and $NaHCO_3$. Collect the organic layer and concentrate it with a rotary evaporator. Further, purification of crude product was done by Column chromatography in 3-5% methanol and chloroform to obtain a desired pure product.

Physical Data of NA-1:

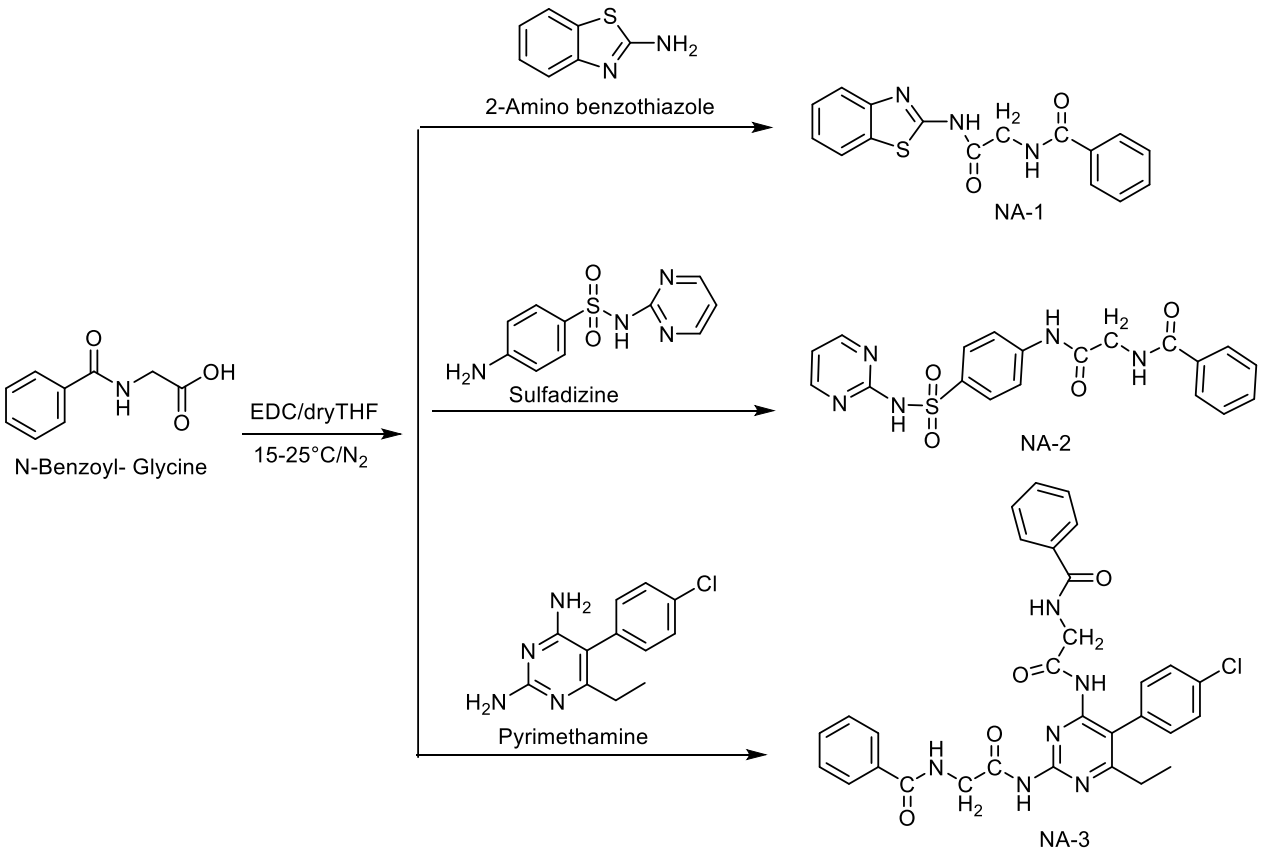
Physical appearance: Dark yellow powder, Melting point: 200-205°C, Reaction time: 8 hours, TLC: 12% (methanol/chloroform), FTIR(ν_{max}): 3311(secondary N-H), 2936(C-H), 1690(C=O), 1605(N-H), 1517(C=N), 1452(C=C), 1287(C-N) cm^{-1}.

Physical Data of NA-2:

Physical appearance: Dark yellow powder, Melting point: 197-201°C, Reaction time: 8 hours, TLC: 12% (methanol/chloroform), FTIR(ν_{max}): 3337(secondary N-H), 2921(C-H), 1686(C=O), 1595(N-H), 1433(C=C), 1287(C-N), 1086(S=O) cm^{-1}.

Physical Data of NA-3:

Physical appearance: Dark yellow powder, Melting point: 208-212°C, Reaction time: 8 hours, TLC: 12% (methanol/chloroform), FTIR(ν_{max}): 3320(N-H), 2906(C-H), 1686(C=O), 1595(N-H), 1517(C=N), 1452(C=C), 1287(C-N) cm^{-1}.



Scheme-14: **Coupling of *N*-Benzoyl-Glycine with Drugs**

2.9.2 Coupling of *N*-Boc-Glycine with Drugs:

First, create a vacuum in a flask equipped with a 3-way stopper with the help of a vacuum pump. After that nitrogen gas was introduced in a flask by the balloon. Dissolve the *N*-Boc-glycine (0.49g, 2.85mmol) in the dry THF (20-25mL) on continuous stirring until the *N*-Boc-glycine is dissolved. Then subsequently add triethylamine (3.97 mL, 2.85 mmol) as the base and continue the stirring. The reaction temperature was then carefully controlled within a specified range (0-25°C) using an ice bath. Following temperature regulation, coupling reagent EDC (0.55g, 2.85 mmol) was introduced into the reaction mixture. Finally, add a drug to the reaction mixture and continue the stirring for 7-8 hours. Three different drugs such as 2-aminobenzothiazole (0.42g, 2.85mmol), sulfadiazine (0.72g, 2.85mmol), and pyrimethamine (0.35g, 1.42mmol) are coupled with *N*-Boc-glycine in three different reactions. Upon completion, solvent is evaporated and ethyl acetate is added to dilute the reaction mixture and wash it with concentrated solutions of NaCl and $NaHCO_3$. Extract the organic layer and concentrate it with a rotary evaporator under a vacuum. The crude is purified with the help of column chromatography in 3-6% methanol and chloroform to obtain a pure product.

Physical Data of NA-4:

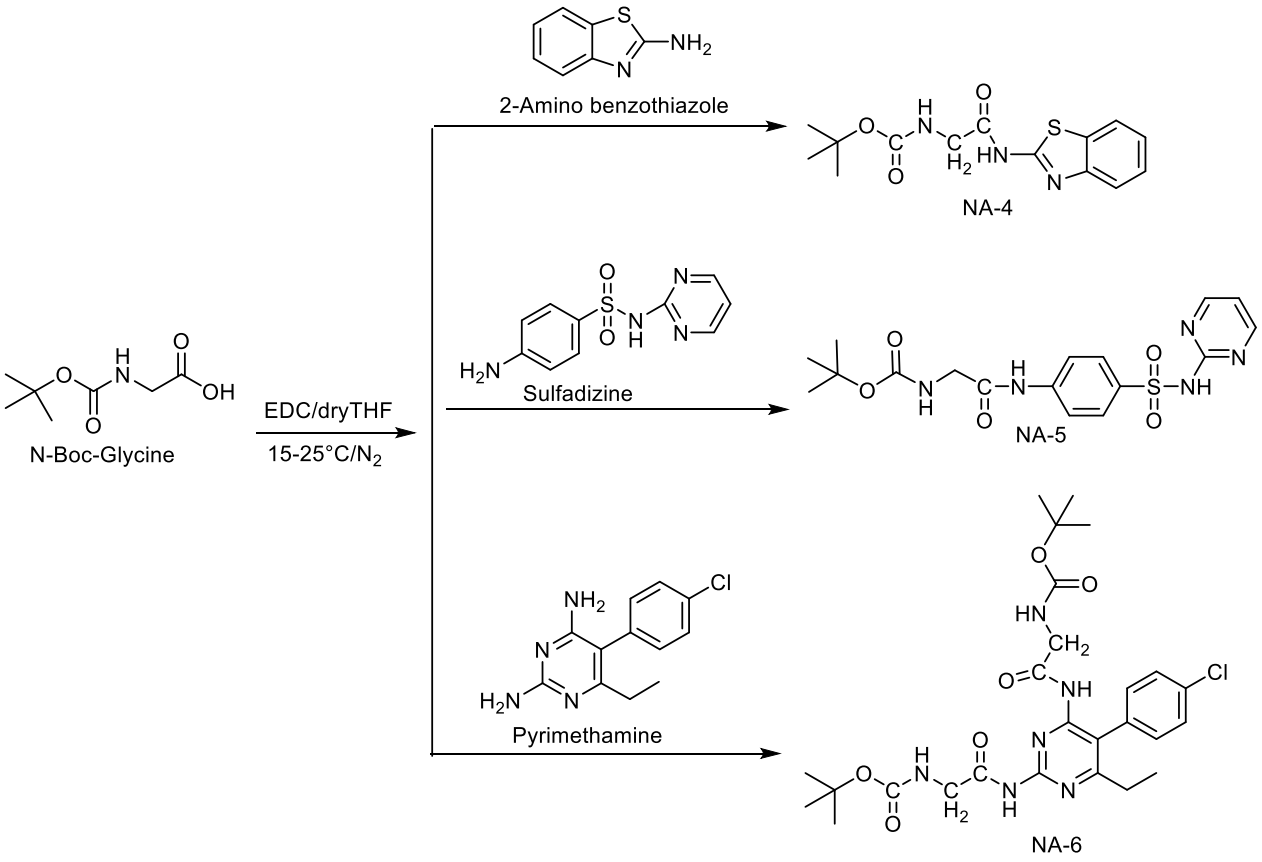
Physical appearance: Yellow powder, Melting point: 187-190°C, Reaction time: 10 hours, TLC: 12% (methanol/chloroform), FTIR(ν_{max}): 3303(N-H), 2936(C-H), 1686(C=O), 1565(N-H), 1467(C=C), 1329[$C(CH_3)_3$], 1287(C-N) cm^{-1}.

Physical Data of NA-5:

Physical appearance: Yellow powder, Melting point: 201-204°C, Reaction time: 10 hours, TLC: 12% (methanol/chloroform), FTIR(ν_{max}): 3343(secondary N-H), 2978(C-H), 1662(C=O), 1595(N-H), 1516(C=N), 1452(C=C), 1369[$C(CH_3)_3$], 1287(C-N), 1068(S=O) cm^{-1}.

Physical Data of NA-6:

Physical appearance: Yellow powder, Melting point: 207-211°C, Reaction time: 10 hours, TLC: 12% (methanol/chloroform), FTIR(ν_{max}): 3343(N-H), 2986(C-H), 1689(C=O), 1595(N-H), 1452(C=C), 1369[$C(CH_3)_3$], 1287(C-N) cm^{-1}.



Scheme-15: **Coupling of *N*-Boc-Glycine with Drugs**

2.9.3 Coupling of *N*-Cbz-Glycine with Drugs:

N-Cbz-glycine (0.59g, 2.85mmol) and coupling agent DCC (0.63g, 3.00mmol) were added to a flask containing absolute THF (25mL) and stirred until it was completely dissolved at room temperature. Then add the subsequent amount of drug into the round-bottom flask and stir it continuously for 7-8 hours. Three different drugs such as 2-aminobenzothiazole (0.42g, 2.85mmol), sulfadiazine (0.72g, 2.85mmol), and pyrimethamine (0.35g, 1.42mmol) are coupled with *N*-Cbz-glycine in three different reactions. The TLC was performed to monitor the progress of the reaction. Ethyl acetate was added to the reaction mixture to further dilute it. Afterward, the urea is removed from the solution by filtrating the reaction mixture. Concentrate the filtrate by using a rotary evaporator, and white powder is obtained as a final product.

Physical Data of NA-7:

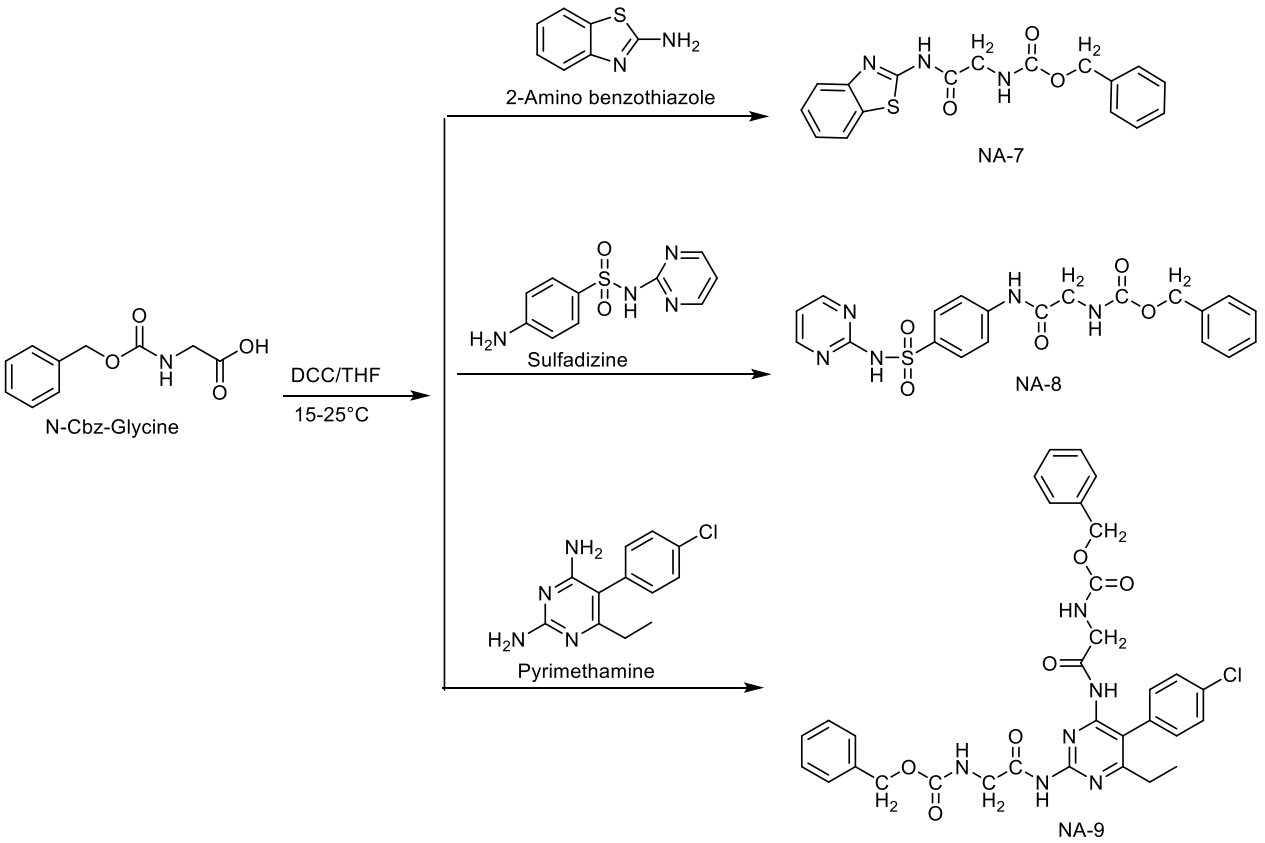
Yield: 78%, Reaction time: 9 hours, Physical appearance: White powder, M.P: 198-202 ℃, TLC: 12%(methanol/chloroform) FTIR(ν_{max}): 3316(sec N-H, str), 2912(C-H), 1680(C=O, Amide), 1653(N-H, Amide), 1531(C=N), 1418(C=C, Aromatic), 1293(C-N), 1258(C-O) cm^{-1}.

Physical Data of NA-8:

Yield: 78%, Reaction time: 9 hours, Physical appearance: White powder, M.P: 202-207 ℃, TLC: 12%(methanol/chloroform) FTIR:(ν_{max}): 3324(N-H, str), 2927(C-H), 1680(C=O, Amide), 1576(N-H, Amide), 1448(C=C, Aromatic), 1284(C-N), 1224(C-O) cm^{-1}.

Physical Data of NA-9:

Yield: 78%, Reaction time: 9 hours, Physical appearance: White powder, M.P: 231-235 ℃, TLC: 12%(methanol/chloroform) FTIR:(ν_{max}): 3323(N-H, str), 2922(C-H), 1689(C=O, Amide), 1615(N-H, Amide), 1442(C=C, Aromatic), 1293(C-N), 1258(C-O) cm^{-1}.



Scheme-16: **Coupling of *N*-Cbz-Glycine with Drug**

Chapter 3

3.0 RESULTS AND DISCUSSION:

3.1 Protection of Functional Groups of α-Amino acid:

Using the amino acid protection method, target amides were produced step-by-step from the C-terminus to the N-terminus during the solution phase. Additionally, side-chain functional group protection was done to prevent contaminants and get chemo-selective results[96].

3.1.1 Amino (-NH_2) Group Protection of Amino acids:

It is necessary to protect the amino group (-NH_2) of amino acid during the synthesis of peptides to stop side reactions and polymerization. Since the targeted medication is manufactured from the C-terminus to the N-terminus, it is possible to remove temporary amino-protecting groups, during the synthesis process as needed[97]. To avoid affecting additional protective groups (permanent protecting groups, which are typically removed after the synthesis process), removal is thus carried out under mild conditions[98]. To generate peptides in a peptide chain, amino acids must normally be linked, which requires the removal of some semi-permanent amino-protecting groups at the C-terminus[99]. Characteristics of amino-protecting groups include being sufficiently stable, conveniently soluble in a variety of common solvents, and free of side products.

3.1.2 Characterization of *N*-Protected-Glycine:

Different methods were used to analyze the *N*-protected glycine such as melting point and FTIR. The melting point of *N*-protected glycine ranges from 87-197^{o}C. The purity of the compounds was demonstrated by their sharp melting point. Further calculations were made to find their molecular weight which ranges from 175 to 210 g/mol and percentage yield ranges from 83 to 90%. The physical appearance of the compounds was a white powder. The table provides physical data on *N*-protected glycine.

Table-1: **Physical Data of *N*-Protected-Amino acid**

***N*-Protected-Glycine**	**Molecular weight (g/mol)**	**Color**	**Melting point (^{0}C)**	**Yield (%)**
N-Boc-Glycine	175.18	White	87	88
N-Cbz-Glycine	209.20	White	119	83
N-Benzoyl-Glycine	179.17	White	187	90

3.1.2.1 Characterization of *N*-Boc-Glycine:

N-Boc-Glycine is created when the amino group of glycine combines with Boc anhydride in the presence of a base. *N*-Boc-glycine typically appears as a white to off-white crystalline solid. FTIR analysis of *N*-Boc-glycine shows band at 3376 cm^{-1} is due to the N-H bond of secondary amine while 2997 cm^{-1} is due to the C-H bond. The band at 1737 cm^{-1} is due to C=O of acid while signal 1666 cm^{-1} is due to C=O of amide and 1529 cm^{-1} N-H of amide. The signal at 1408cm^{-1} indicates the presence of the C=C bond while the band at 1196 cm^{-1} is because of the C-O bond.

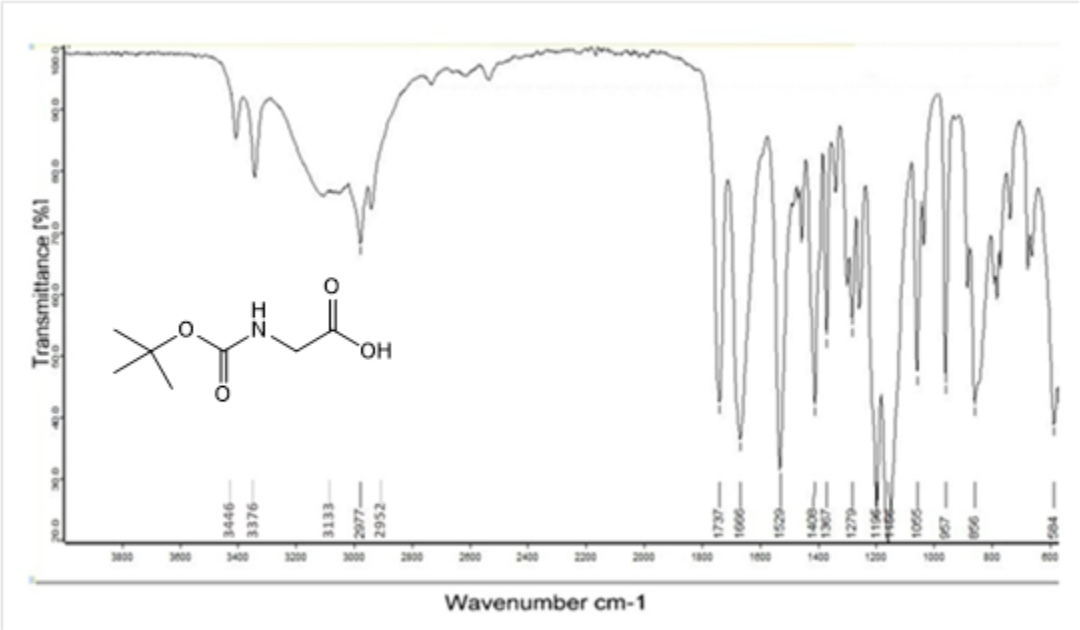


Figure-20: **FTIR Spectrum of *N*-Boc-Glycine**

3.1.2.2 Characterization of *N*-Cbz-Glycine:

N- Cbz-glycine is created when the amino group of glycine combines with benzyl chloroformate in the presence of a base. *N*-Cbz-glycine typically appears as an off-white crystalline solid. FTIR analysis of *N*-Cbz-glycine shows a band at 3600 cm^{-1} is due to the O-H bond while the band at 3331 cm^{-1} is due to N-H bond stretching. The signal at 1736 cm^{-1} is due to C=O of acid while 1681 cm^{-1} is due to C=O of amide and 1581 cm^{-1} is due to N-H stretching of amide. The signal that appeared at 1444 cm^{-1} is due to the C=C bond. The C-N bond gives a signal at 1221 cm^{-1}.

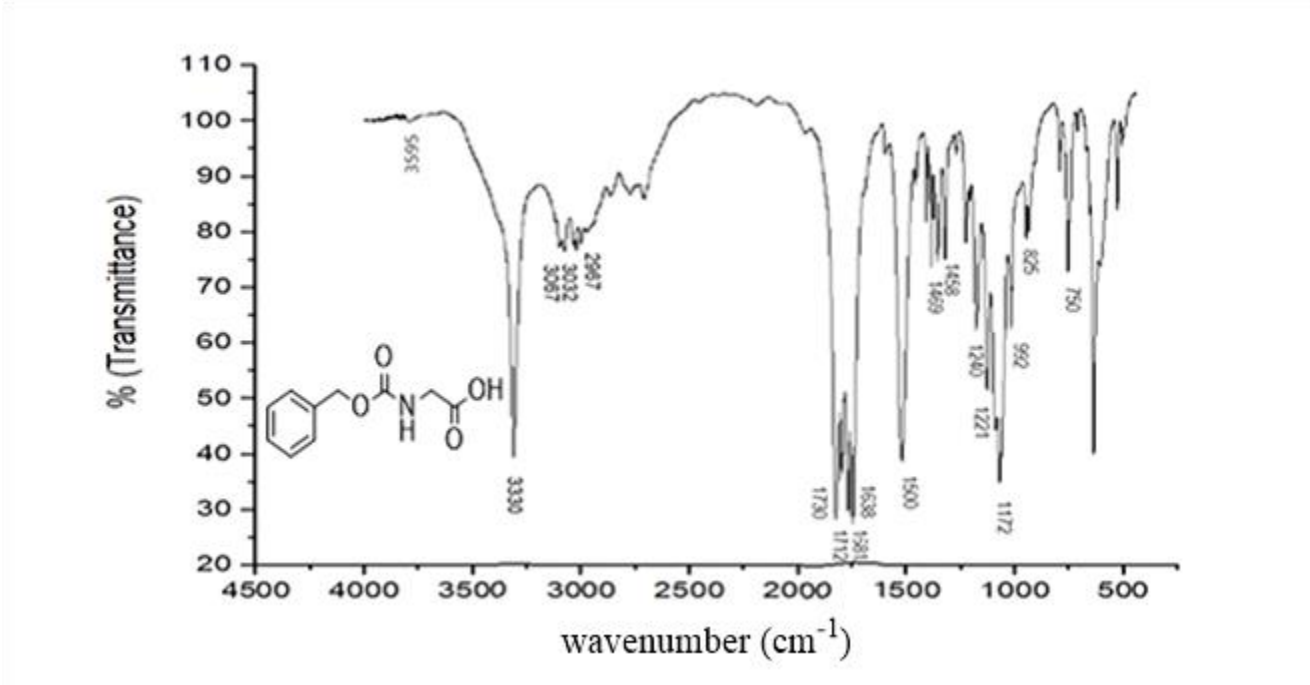


Figure-21: **FTIR Spectrum of *N*-Cbz-Glycine**

3.1.2.3 Characterization of *N*-Benzoyl-Glycine

N-benzoyl-glycine is created when the amino group of glycine combines with benzoyl chloride in the presence of a base. *N*-benzoyl-glycine typically appears as a white to off-white crystalline solid. FTIR analysis of *N*-benzoyl-glycine shows band at 3334 cm^{-1} is due to N-H stretching while 2976 cm^{-1} is due to the C-H bond. The band at 1734 cm^{-1} is due to C=O of acid while signal 1600 cm^{-1} is due to C=O of amide and 1550 cm^{-1} N-H of amide. The band at 1412 cm^{-1} was attributed to the presence of the C=C bond while the band that appeared at 1195 cm^{-1} is due to the C-O bond.

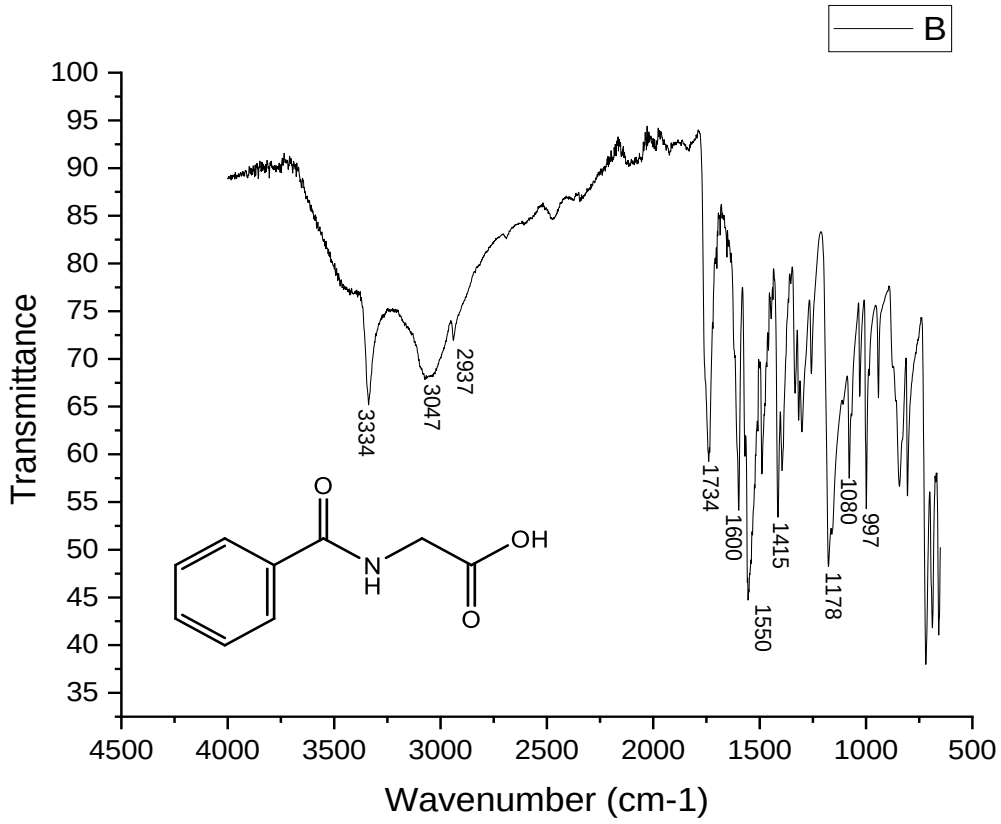


Figure-22: **FTIR of *N*-Benzoyl-Glycine**

3.2 Coupling of Amines with *N*-Protected-Glycine to form Amide:

Different N-protected amino acids and amine-based medications were used as starting materials to create target molecules in the correct order. The process of synthesis was carried out in a step-by-step schematic manner. First, amino groups were protected using benzoyl chloride, Boc anhydride, and benzoyl chloroformate. Next, *N*-protected amino acids were combined with amine-based medications, such as pyrimethamine, sulfadiazine, and 2-aminobenzothizole, to form the corresponding amides. Following each stage of synthesis, the produced products were purified.

3.2.1 Characterization of Amide:

Different methods were used to analyze the synthesis and purity of amide such as Melting point, FTIR, and H-NMR. The melting point of synthesized amide ranges from 185 to 245^{o}C. The purity of the compounds was demonstrated by their sharp melting point. Further calculations were made to find their molecular weight which ranges from 307 to 630 g/mol and percentage yield ranges

from 69 to 79%. The physical appearance of the compounds was white and yellow powder. The table provides physical data of synthesized compounds.

Table-2: **Physical Data of synthesized compounds (NA-1 to NA-9)**

Compounds	**Molecular weight (g/mol)**	**Color**	**Melting point (^{0}C)**	**Yield (%)**
NA-1	311	Dark yellow	205	79
NA-2	411	Dark yellow	197	73
NA3	572	Dark yellow	211	78
NA-4	307	Yellow	189	72
NA-5	407	Yellow	199	68
NA-6	563	Yellow	209	70
NA-7	341	White	202	76
NA-8	441	White	205	71
NA-9	630	White	233	74

3.2.1.1 Characterization of NA-1:

FTIR-Analysis

The FTIR of NA-1 shows the band at 1650 cm^{-1} which indicates the C=O of amide-I while the signal at 1597 cm^{-1} shows N-H of amide-II. These signals indicate the synthesis of an amide bond. The single band at 3312 cm^{-1} indicates the presence of secondary amine. The band at 2935 cm^{-1} is due to the C-H bond. The signal at 1517 cm^{-1} indicates the C=N. The IR band at 1480 cm^{-1} indicates C=C stretching. The band at 1240 cm^{-1} is due to C-N.

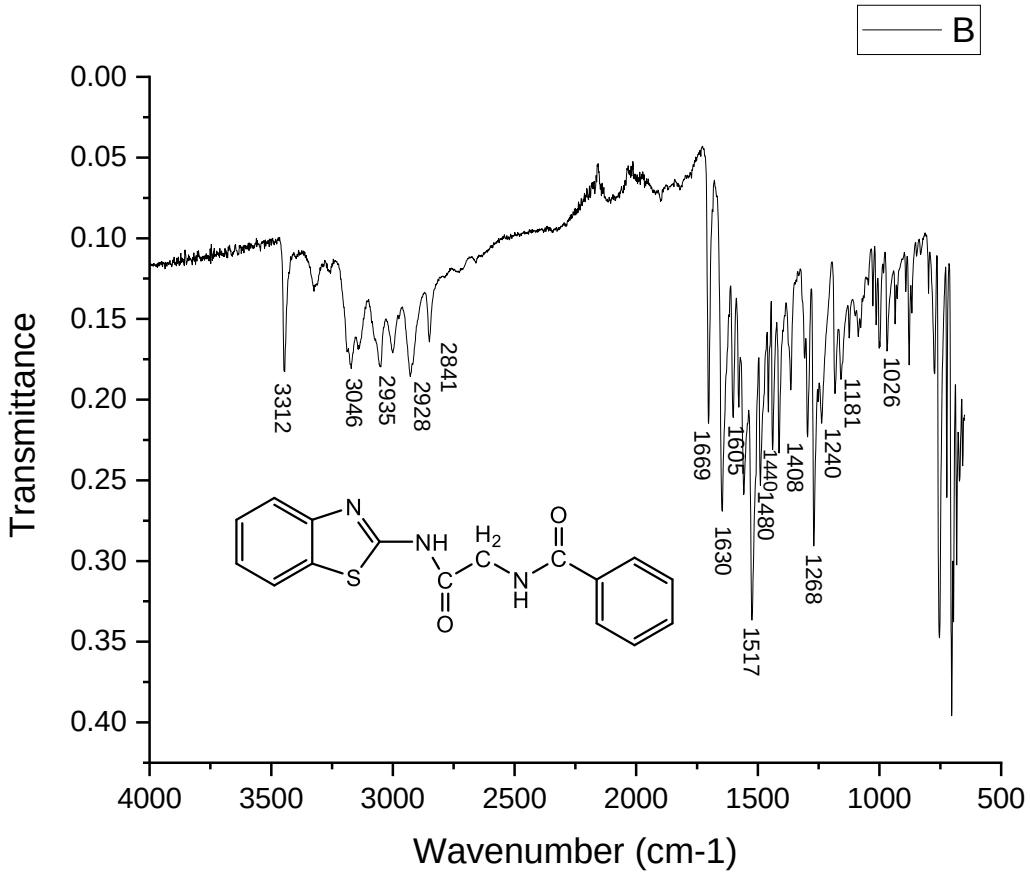


Figure-23: **FTIR Spectrum of NA-1**

[1]H-NMR Analysis:

Proton NMR of NA-1 was performed in DMSO-d6 as a solvent at the 400-MHz instrument. The singlet with a chemical shift of 4.08 ppm is due to the Ha proton. The Hb and Hc protons show triplet of doublet with a chemical shift of 7.08 (J=9.6, J=1.8 Hz) and 7.21 ppm (J=9.2, J= 2 Hz) due to adjacent protons of the aromatic ring. The Hd protons of the aromatic ring show multiplet at 7.48-7.55 ppm. The He and Hf protons show doublet with a chemical shift of 7.74 (J=9.2 Hz) and 7.86 ppm (J=9.8 Hz) due to adjacent protons of an aromatic ring. The Hg proton shows a singlet at 8.39 ppm and the Hh proton shows a singlet at 11.342 ppm. This singlet confirms the formation of amide linkage.

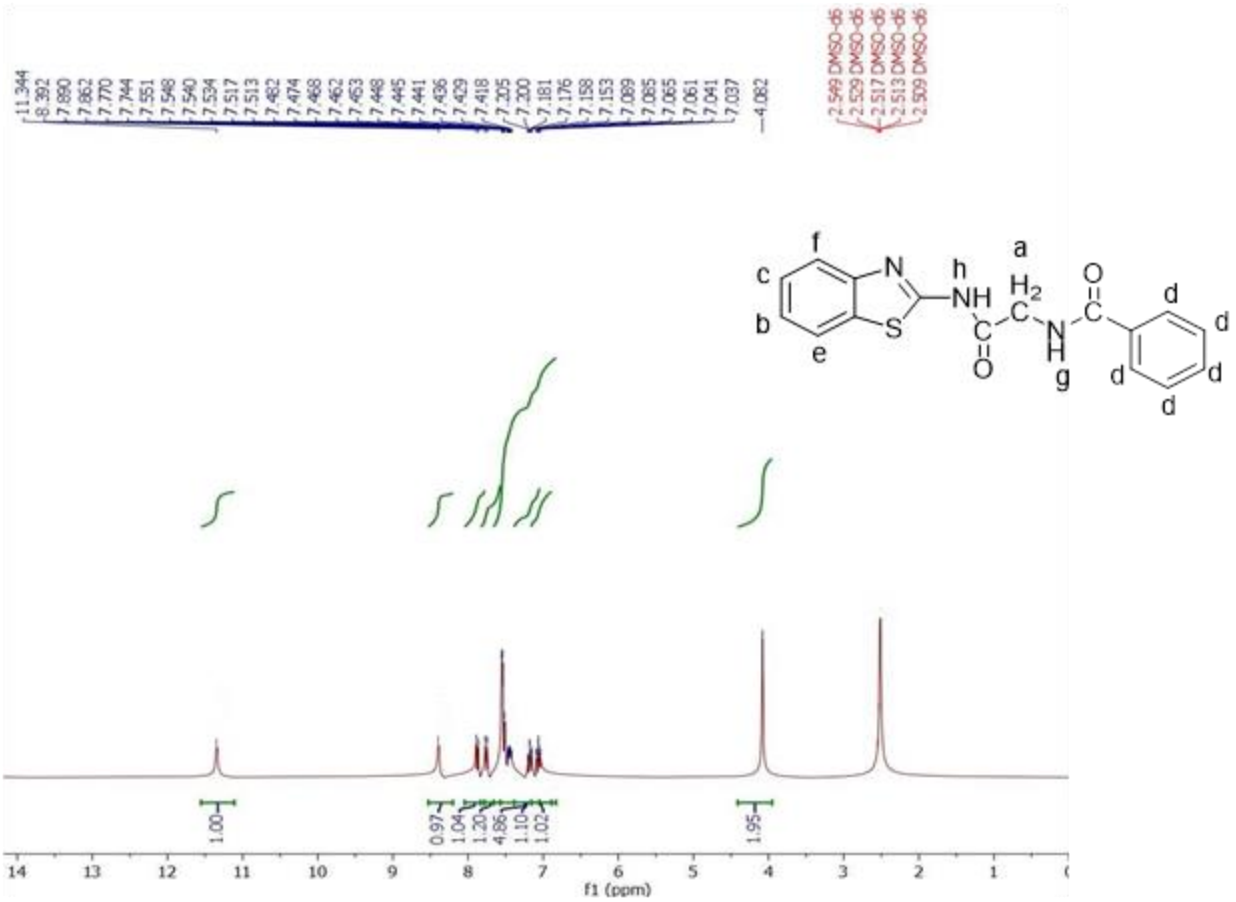


Figure-24: **^{1}H-NMR Spectrum of NA-1**

3.2.1.2 Characterization of NA-2:

FTIR-Analysis

The FTIR of NA-2 shows the band at 1682 cm^{-1} indicating the C=O of amide-I while at 1592cm^{-1} shows N-H of amide-II. These signals indicate the synthesis of an amide bond. The single band at 3337 cm^{-1} indicates the presence of secondary amine. The band at 2921 cm^{-1} is due to the C-H bond. The band at 1442 cm^{-1} indicates C=C stretching. The 1305 cm^{-1} band of IR indicates the presence of C-O-C. The band at 1249 cm^{-1} is due to C-N. The band at 1130 cm^{-1} indicates the SO_2 bond.

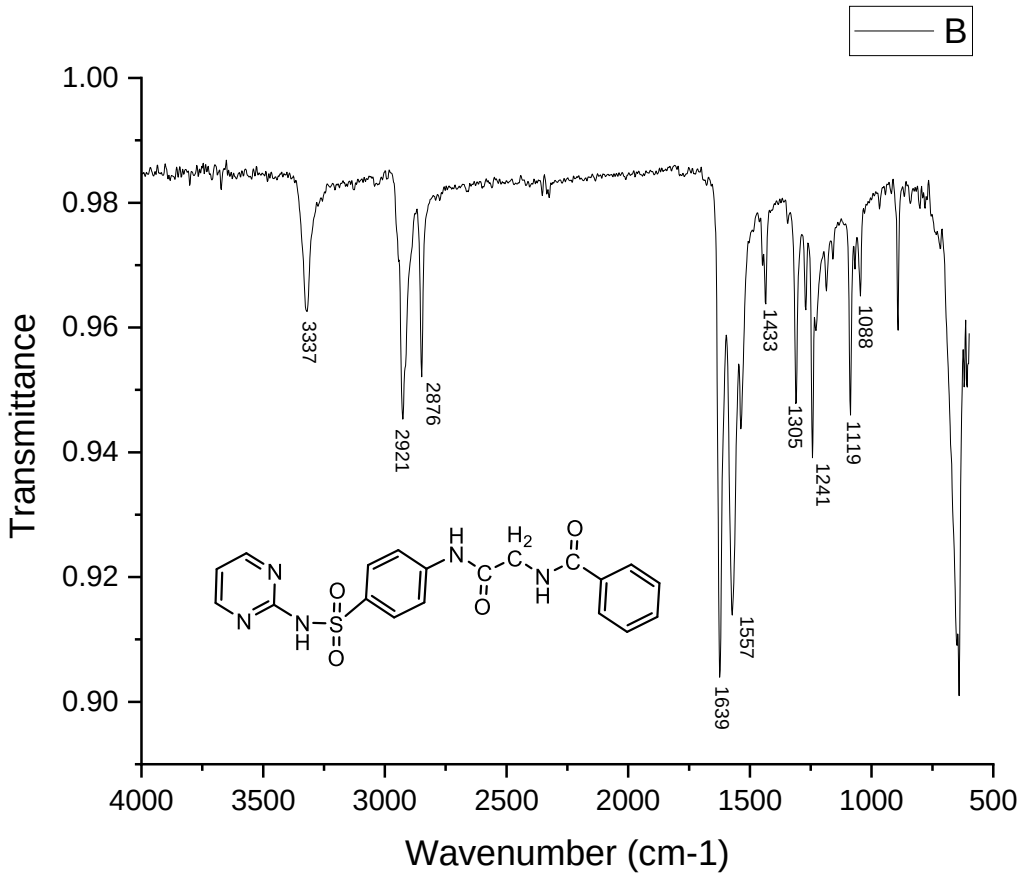


Figure-25: **FTIR Spectrum of NA-2**

^{1}H-NMR Analysis:

Proton NMR of NA-2 was done at the 400-MHz instrument in the presence of DMSO-d6 as a solvent. The Ha protons appear as a singlet with a chemical shift of 4.098 ppm. The Hb proton of the heterocyclic aromatic ring appears as a triplet with a chemical shift of 6.92 ppm (J=4.4 Hz) due to two adjacent protons. The Hc protons of the mono-substituted aromatic ring appear as a multiplet with chemical shift 7.23-7.35 ppm. The Hd and He protons of the para-di-substituted aromatic ring appear as a doublet with a chemical shift of 7.67 (J=8.6 Hz) and 7.79 ppm (8.8 Hz). The Hf proton appears as a singlet with a chemical shift of 8.01 ppm. The Hg protons of the heterocyclic aromatic ring appear as a doublet with a chemical shift of 8.38 ppm (J=4Hz). The Hh proton appears as a

singlet with a chemical shift of 10.43 ppm. This singlet confirms the formation of amide linkage. The Hi proton shows a singlet with a chemical shift of 11.26 ppm.

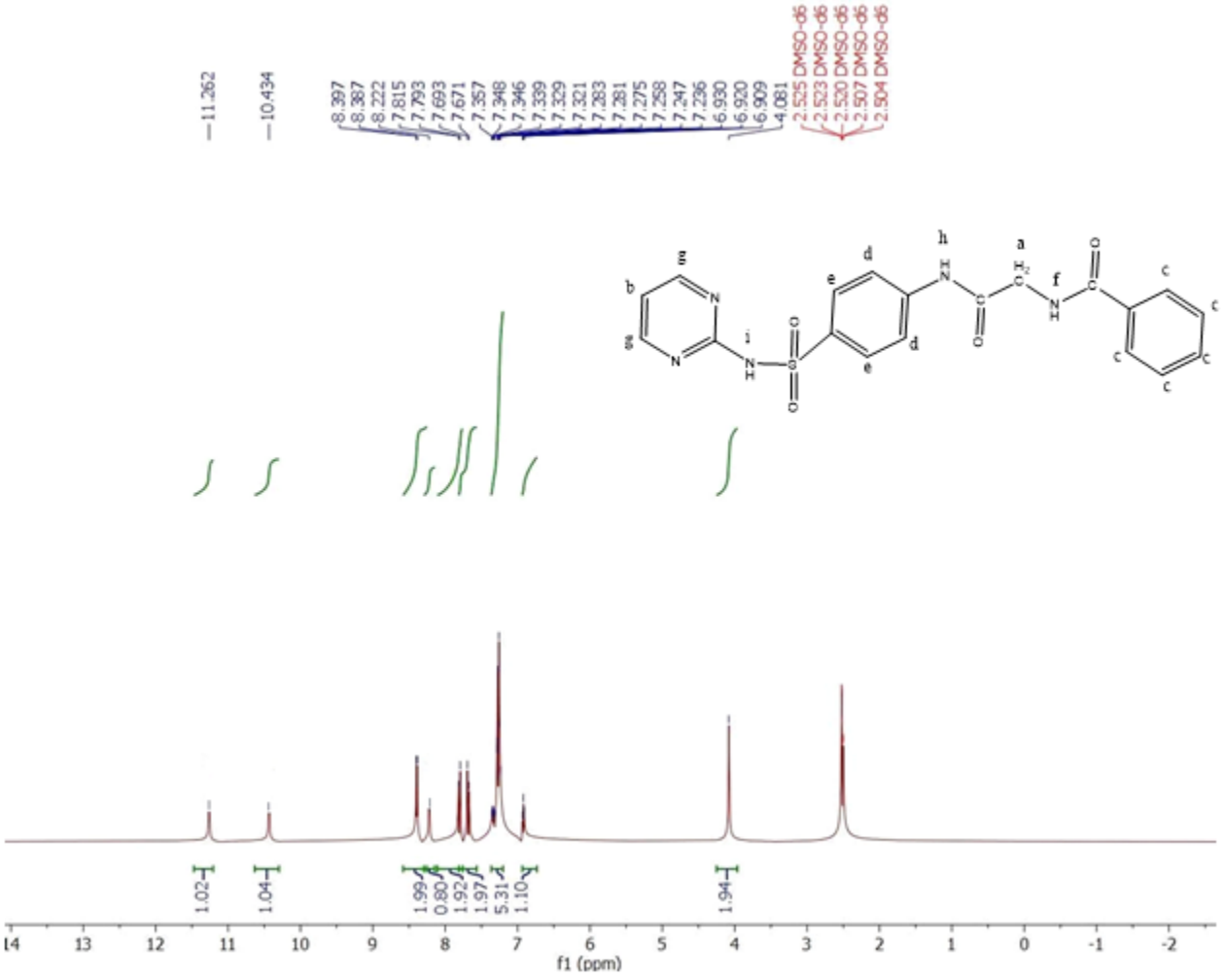


Figure-26: **^{1}H-NMR Spectrum of NA-2**

3.2.1.3 Characterization of NA-3:

FTIR-Analysis

The FTIR of NA-3 shows the band at 1672 cm^{-1} indicating the C=O of amide-I and 1580 cm^{-1} shows N-H of amide-II. These signals indicate the synthesis of an amide bond. The single band at 3320 cm^{-1} indicates the presence of secondary amine. The band at 2907 cm^{-1} is due to the presence of a C-H bond. The band at 1452 cm^{-1} indicates the presence of C=C stretching. The band at 1249

cm^{-1} is due to the C-N bond. The band at 1130 cm^{-1} indicates the presence of a C-O-C bond. The band is due to the C-Cl bond present at 908 cm^{-1}.

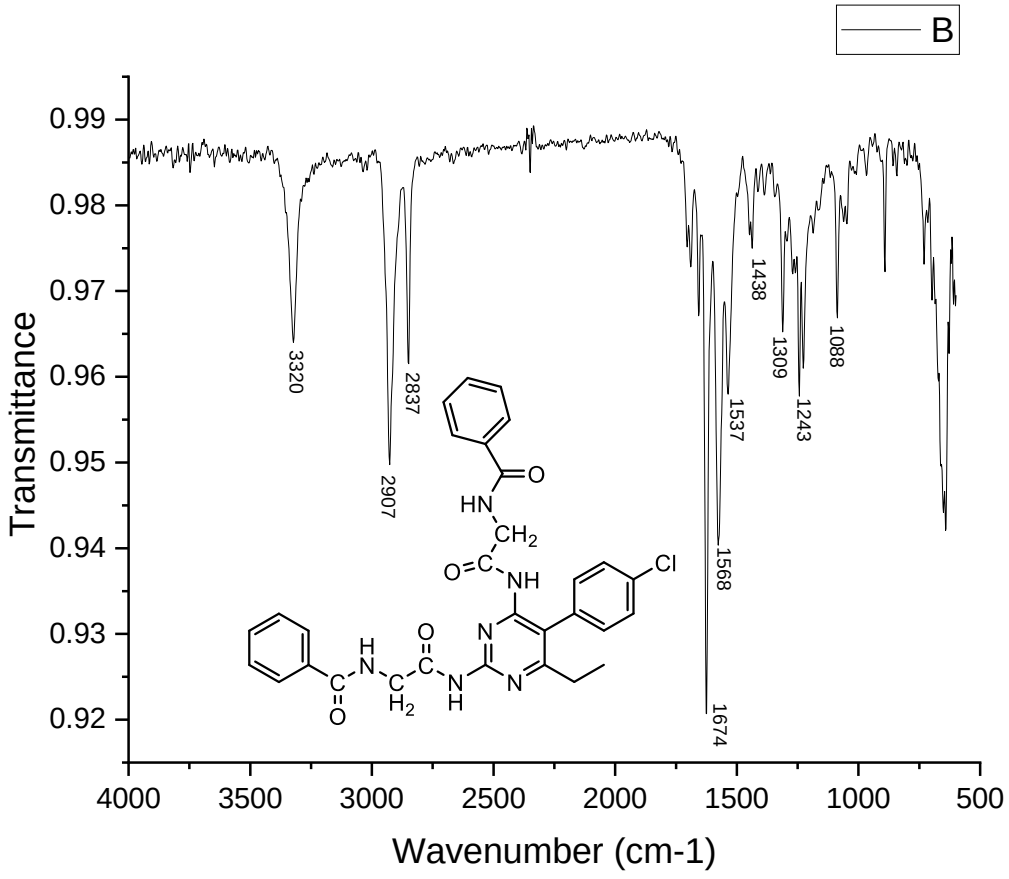


Figure-27: **FTIR Spectrum of NA-3**

3.2.1.4 Characterization of NA-4:

The FTIR of NA-4 shows the signal at 1682 cm^{-1} indicating the presence of C=O of amide-I while 1592 cm^{-1} shows N-H of amide-II. These signals indicate the synthesis of an amide bond. The single band at 3342 cm^{-1} indicates the presence of secondary amine. The band at 2938 cm^{-1} shows the presence of C-H. The C=N of the aromatic ring shows the band at 1518 cm^{-1}. The IR band at 1452 cm^{-1} indicates C=C. The 1367 cm^{-1} band of IR shows the $[C(CH_3)_3]$ presence. The band at 1249 cm^{-1} is due to C=S. The presence of C-O-C is indicated by the signal at 1151cm^{-1}.

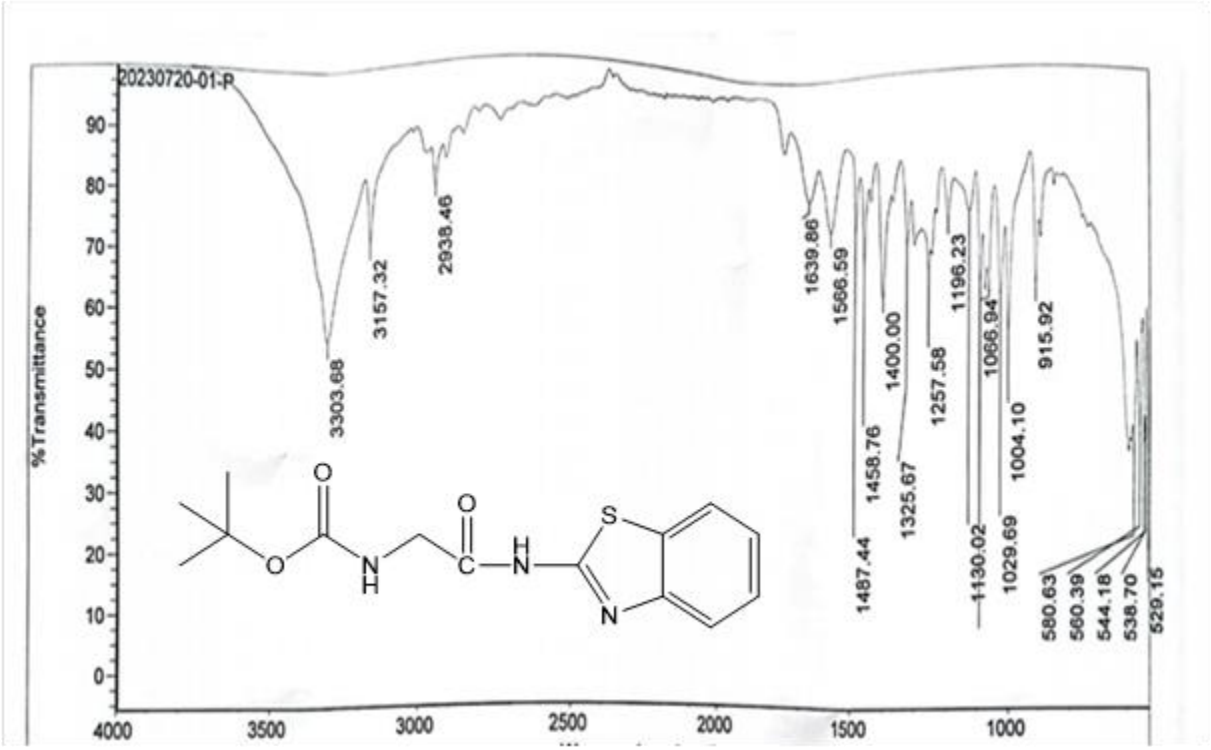


Figure-28: **FTIR Spectrum of NA-4**

3.2.1.5 Characterization of NA-5:

FTIR-Analysis

The FTIR of NA-2 shows the band at 1632 cm^{-1} indicates the C=O of amide-I while 1562 cm^{-1} shows N-H of amide-II. These signals indicate the synthesis of amide linkage. The single band at 3302 cm^{-1} indicates the presence of a secondary amine. The band at 2978 cm^{-1} shows the presence of a C-H bond. The IR band at 1458 cm^{-1} indicates C=C stretching while the band at 1366 cm^{-1} shows the $[C(CH_3)_3]$ presence. The band at 1257 cm^{-1} is due to the C-N bond. The band at 1090 cm^{-1} indicates the presence of the S-O bond.

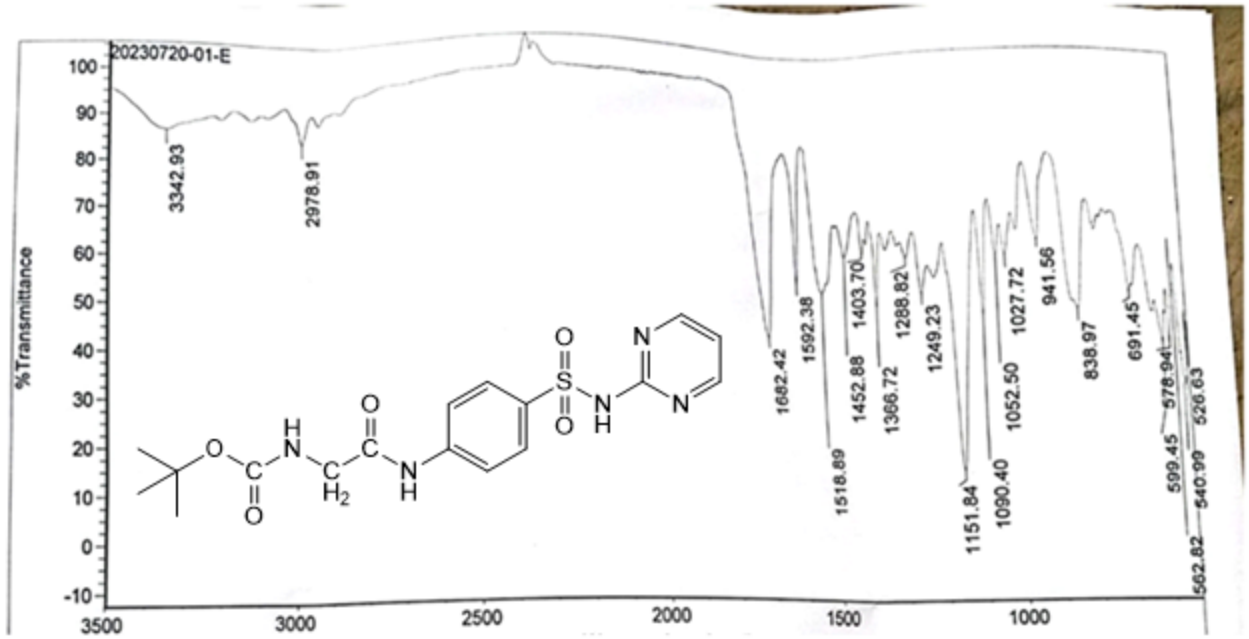


Figure-29: **FTIR Spectrum of NA-5**

3.2.1.6 Characterization of NA-7:

FTIR-Analysis

The FTIR of NA-7 shows the band at 1682 cm^{-1} indicating the C=O of amide-I and 1623 cm^{-1} shows N-H of amide-II. These signals indicate the synthesis of an amide bond. The single band at 3316 cm^{-1} indicates the presence of secondary amine. The band at 2922 cm^{-1} is due to the C-H bond. The presence of the C=N bond shows the signal at 1531 cm^{-1}. The IR band at 1452cm^{-1} indicates C=C. The band at 1259 cm^{-1} is due to C-N. The band at 1080 cm^{-1} shows the presence of S-O.

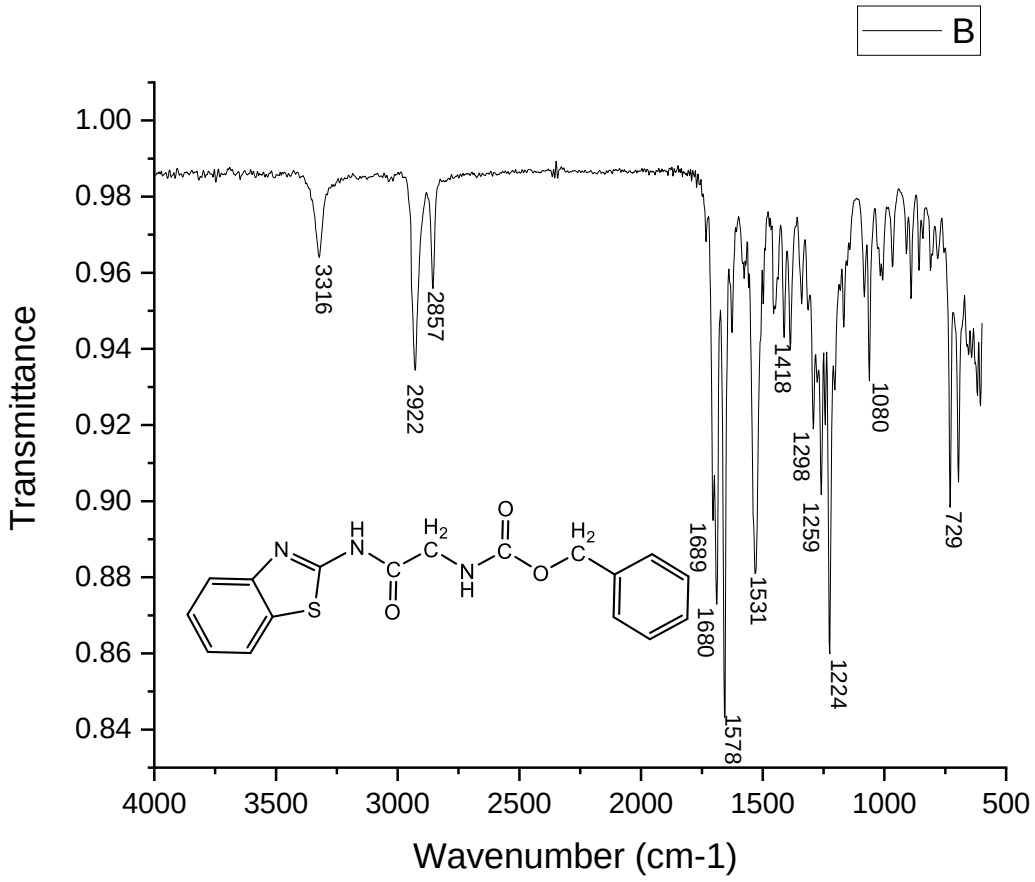


Figure-30: **FTIR Spectrum of NA-7**

3.2.1.7 Characterization of NA-8:

FTIR-Analysis

The FTIR of NA-8 shows the band at 1682 cm^{-1} indicating the C=O of amide-I and at 1561 cm^{-1} shows the N-H stretching of amide-II. These signals indicate the synthesis of an amide bond. The single band at 3324 cm^{-1} demonstrates the secondary amine. The band at 2927 cm^{-1} shows the C-H stretching. The IR band at 1447 cm^{-1} demonstrated the presence of C=C stretching. The IR band at 1284 cm^{-1} is due to C-N. The band at 1161 cm^{-1} indicates the presence of the S-O bond.

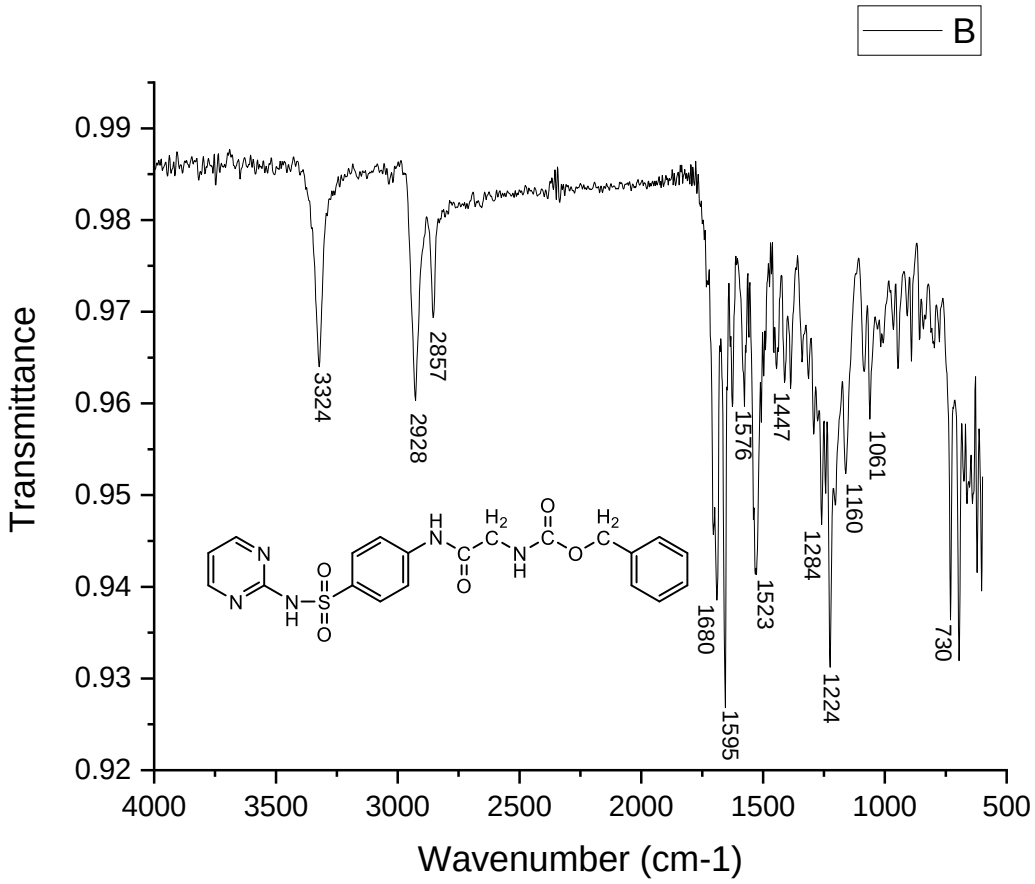


Figure-31: **FTIR Spectrum of NA-8**

[1]H-NMR Analysis:

Proton NMR of NA-2 was performed at the 400-MHz instrument in DMSO-d6 as a solvent. The Ha protons appear as a singlet with a chemical shift of 4.04 ppm. The protons Hb show a singlet with a chemical shift of 5.13 ppm. The proton Hc of the heterocyclic aromatic ring appears as a triplet with a chemical shift of 6.91 ppm (J=4.4Hz) due to two adjacent protons. The Hd protons of the mono-substituted aromatic ring appear as a multiplet with chemical shift 7.23-7.35 ppm. The He and Hf protons of the para-di-substituted aromatic ring appear as a doublet with a chemical shift of 7.67 (J=8.6Hz) and 7.79 ppm (J=8.8Hz). The Hg proton appears as a singlet with a chemical shift of 8.25 ppm. The Hh protons of the heterocyclic aromatic ring appear as a doublet with a chemical shift of 8.47 ppm (J=4.4Hz). The Hi proton appears as a singlet with a chemical shift of

10.41 ppm. This singlet confirmed the formation of an amide linkage. The Hj proton shows a singlet with a chemical shift of 11.21 ppm.

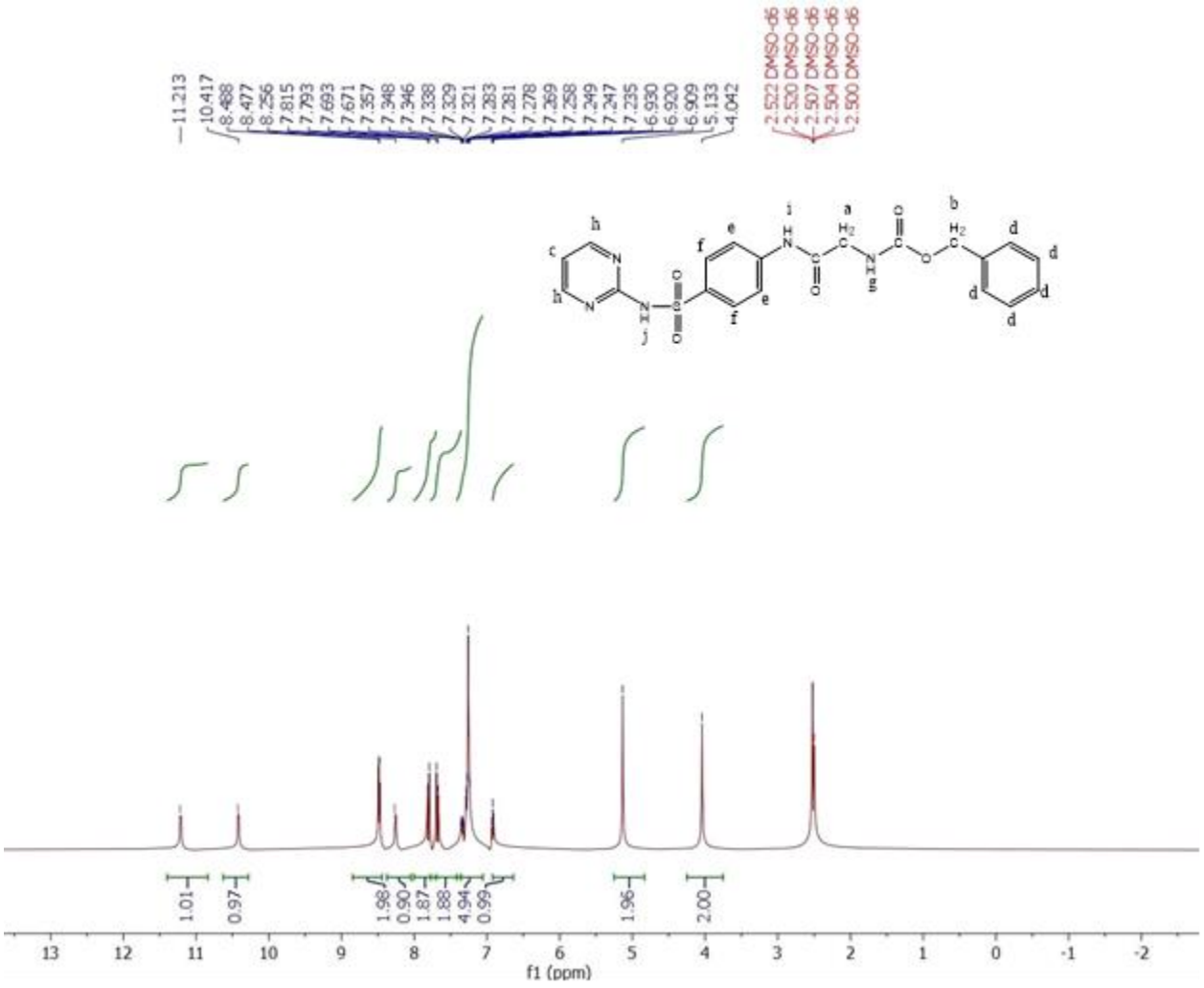


Figure-32: **^{1}H-NMR Spectrum of NA-8**

3.2.1.8 Characterization of NA-9:

FTIR-Analysis

The FTIR of NA-9 shows the band at 1693 cm^{-1} which indicates the presence of C=O of amide-I while 1642 cm^{-1} shows N-H of amide-II. These signals indicate the formation of an amide bond. The single band at 3323 cm^{-1} specifies the presence of a secondary amine. The band at 2922 cm^{-1} is due to the C-H bond stretching. The signal at 1537 cm^{-1} is due to the C=N bond. The weak signal

at 1415 cm^{-1} indicates C=C stretching. The band at 1252 cm^{-1} is due to C-N. The C-O-C shows the band at 1218 cm^{-1}. The IR band at 879 cm^{-1} is due to C-Cl.

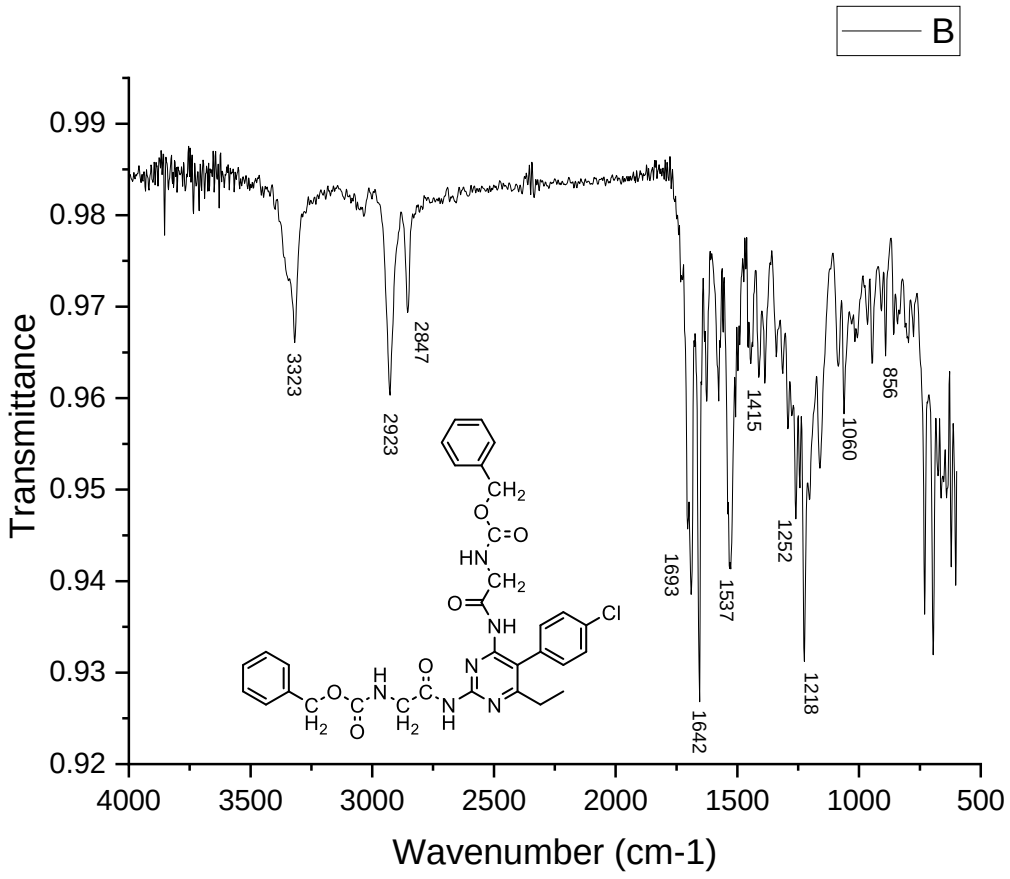


Figure-33: **FTIR Spectrum of NA-9**

3.3 Applications

3.3.1 Biological Activity

Amid linkage plays a prevalent role in the pharmaceutical industry; amide moiety is present in many drugs[100]. They play diverse and indispensable roles in numerous biological activities, spanning from the structural components of biomolecules to signaling and regulation within living systems[101].

3.3.1.1 Cytotoxic Assay

The following observations were made in light of the MTT results that were provided:

1. The "percentage survival" represents the rate of healthy cell survival after treatment with a synthesized compound.
2. These compounds exhibited relatively low cytotoxicity.
3. The % survival increases with increasing the concentration of synthesized compounds.

Table-3: **Numerical data of Cytotoxic Assay of Compounds NA-1,2,3 and NA-7,8,9**

Conc NA1	% Survival	Conc NA2	% Survival	Conc NA3	% Survival	Conc NA7	% Survival	Conc NA8	% Survival	Conc NA9	% Survival
0.181	59.803	0.268	66.665	0.383	75.291	0.372	78.437	0.154	55.354	0.242	63.465
0.232	63.196	0.329	72.431	0.223	60.713	0.227	61.839	0.238	64.867	0.201	59.155
0.366	76.431	0.484	86.274	0.156	54.881	0.293	68.809	0.314	72.582	0.458	84.186
0.425	83.254	0.289	69.117	0.312	70.823	0.476	88.843	0.392	79.725	0.381	79.465
0.345	72.333	0.191	58.091	0.481	89.764	0.339	74.827	0.485	88.824	0.492	90.155
0.393	79.215	0.361	74.093	0.428	83.921	0.242	63.747	0.379	75.663	0.312	70.165
0.329	70.451	0.431	82.354	0.369	74.317	0.395	79.809	0.439	82.402	0.365	74.604
0.275	65.682	0.389	77.458	0.272	64.392	0.368	77.764	0.278	68.325	0.145	52.121

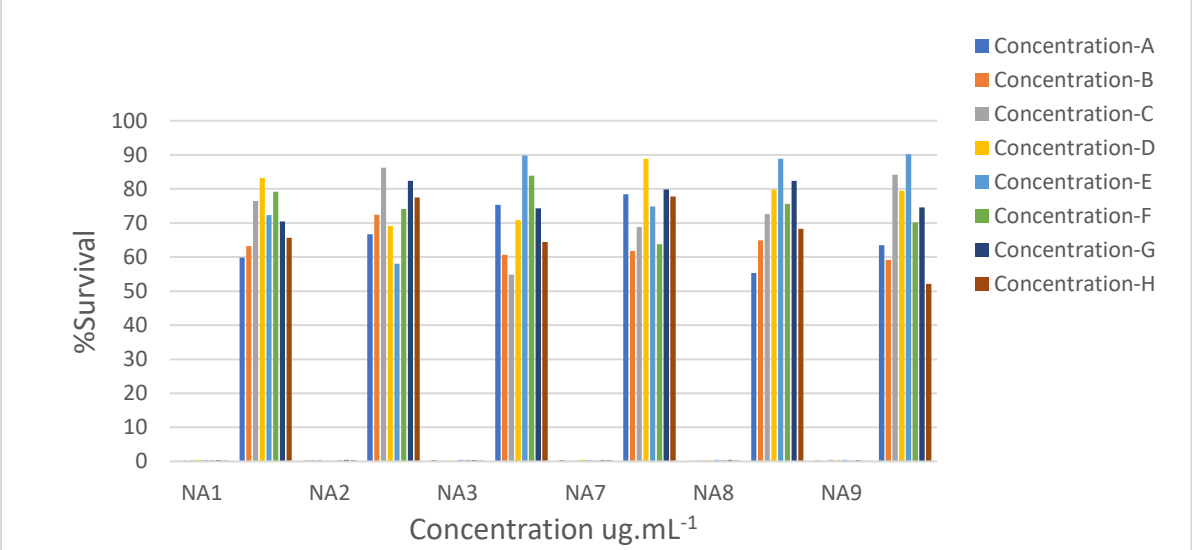


Figure-34: **Healthy Cell Percentage Survival of Synthesized Compounds.**

3.3.1.2 Anti-Bacterial Activity:

All the synthesized compound was subjected to screening against two bacterial strains such as *Escherichia Coli*, and *Staphylococcus Aureus*[102, 103]. Their results show that the synthesized compound containing amide linkage in their structure shows very good results as compared to the starting reactants and standard drugs norfloxacin in the case of *Escherichia Coli* (Gram-negative) and amikacin in the case of *Staphylococcus Aureus* (Gram-positive).

3.3.1.2.1 Anti-Bacterial activity of NA-1, 4 & 7:

The synthesized sample was screened against two strains of bacteria. Their results are summarized in Tables 4 and 5. These conjugates were formed by the coupling of 2-aminobenzothiazole with *N*-protected glycine. The results show that these conjugates are more active against bacteria than their starting reactant 2-aminobenzothiazole. In the case of *Escherichia Coli*, the NA-1, 4 & 7 with a zone of inhibition 19, 21, and 16 mm respectively are approximately 12-38% more potent to *Escherichia Coli* than the 2-aminobenzothiazole. In the case of *Staphylococcus Aureus*, the NA-1, 4 & 7 with zones of inhibition 14, 17, and 15 mm respectively are approximately 15-30% more potent to *Staphylococcus Aureus* than the 2-aminobenzothiazole.

Sahib HA and his team synthesized derivatives of benzothiazole containing amide moiety in their structure and evaluated them against bacteria. Their results show that only a few derivatives show good activity against *Escherichia Coli* (with the zone of inhibition less than 15mm) as compared to the standard drug[106]. However, in this study, all the compounds show good activity against both strains of bacteria.

Table-4: **Anti-Bacterial activity of NA-1, 4 & 7 against *Escherichia Coli***

S. No	Sample	*Escherichia Coli* (Zone of Inhibition)
1	Norfloxacin	25mm
2	2-Aminobenzothiazole	13mm
3	NA-1	19mm
4	NA-4	21mm
5	NA-7	16mm

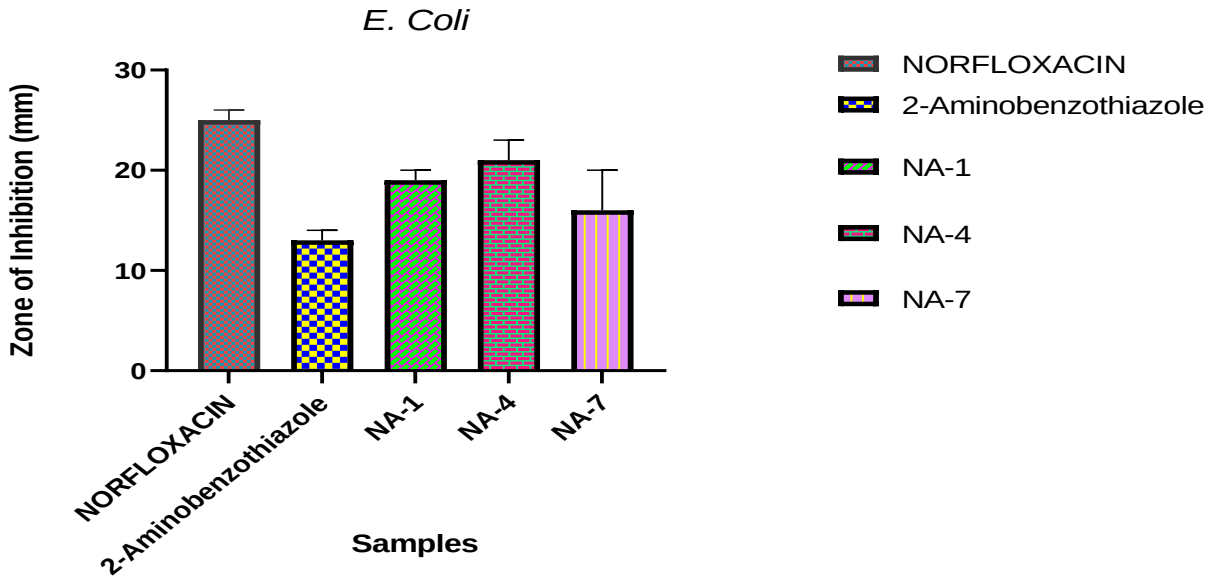


Figure-35: **Anti-Bacterial activity of NA-1, 4 & 7 against *Escherichia Coli***

Table-5: **Anti-Bacterial activity of NA-1, 4 & 7 against *Staphylococcus Aureus***

S. No	Sample	*Staphylococcus Aureus* (Zone of Inhibition)
1	Amikacin	20mm
2	2-Aminobenzothiazole	11mm
3	NA-1	14mm
4	NA-4	17mm
5	NA-7	15mm

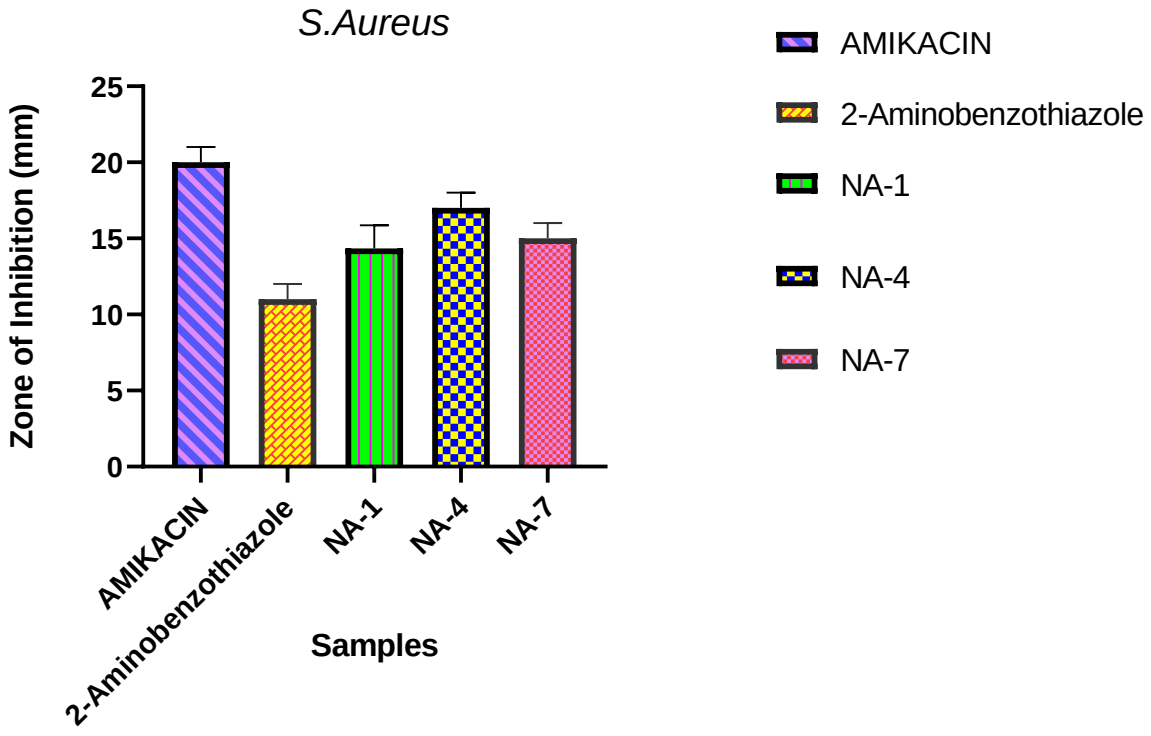


Figure-36: **Anti-Bacterial activity of NA-1, 4 & 7 against *Staphylococcus Aureus***

3.3.1.2.2 Anti-Bacterial activity of NA-2, 5 & 8:

The synthesized conjugates NA-2, 5, & 8 demonstrate notable antibacterial efficacy. Their results are summarized in Tables 6 and 7. These conjugates were formed by coupling sulfadiazine with *N*-protected glycine. The results show that these conjugates are more active against bacteria than their starting reactant sulfadiazine. Specifically, against *Escherichia coli*, NA-2, 5 & 8 displayed zones of inhibition with values of 20, 23, and 19 mm, respectively, representing an enhancement of approximately 8-24% over sulfadiazine. Similarly, concerning *Staphylococcus aureus*, NA-2, 5 & 8 demonstrated zone of inhibition with values of 18, 19, and 17 mm, respectively, indicating an improvement of around 9-19% as compared to sulfadiazine.

Radhiyah A. Khdu and his team synthesized derivatives of sulfadiazine and evaluated them against their *Staphylococcus aureus*. Their results show that these derivatives have a very low zone of inhibition approximately less than 10mm[107]. Manu Lahtinen and his team synthesized *N*-substituted sulfanilamide derivatives and studied their antimicrobial activities. Their derivatives show moderate results against bacteria but show very good anti-fungal activities[108]. On the other hand, in this research, all the compounds exhibit significant efficacy against both strains of bacteria.

Table-6: **Anti-Bacterial activity of NA-2, 5 & 8 against *Escherichia Coli***

S. No	**Sample**	***Escherichia Coli*** **(Zone of Inhibition)**
1	Norfloxacin	25mm
2	Sulfadiazine	17mm
3	NA-2	20mm
4	NA-5	23mm
5	NA-8	19mm

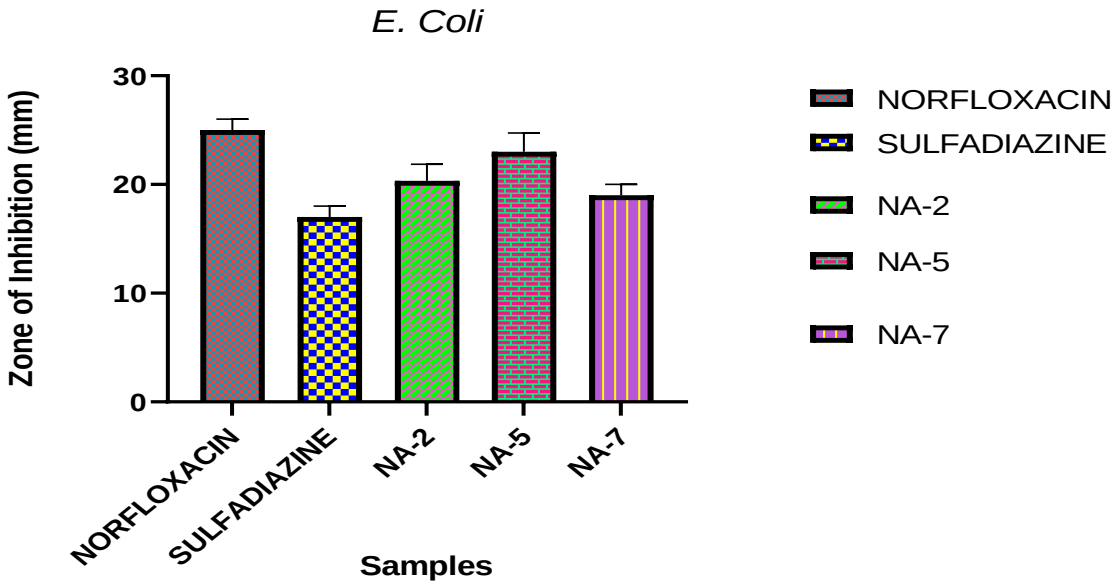


Figure-37: **Anti-Bacterial activity of NA-2, 5 & 8 against *Escherichia Coli***

Table-7: **Anti-Bacterial activity of NA-2, 5 & 8 against *Staphylococcus Aureus***

S. No	**Sample**	***Staphylococcus Aureus* (Zone of Inhibition)**
1	Amikacin	20mm
2	Sulfadiazine	14mm
3	NA-2	18mm
4	NA-5	19mm
5	NA-8	17mm

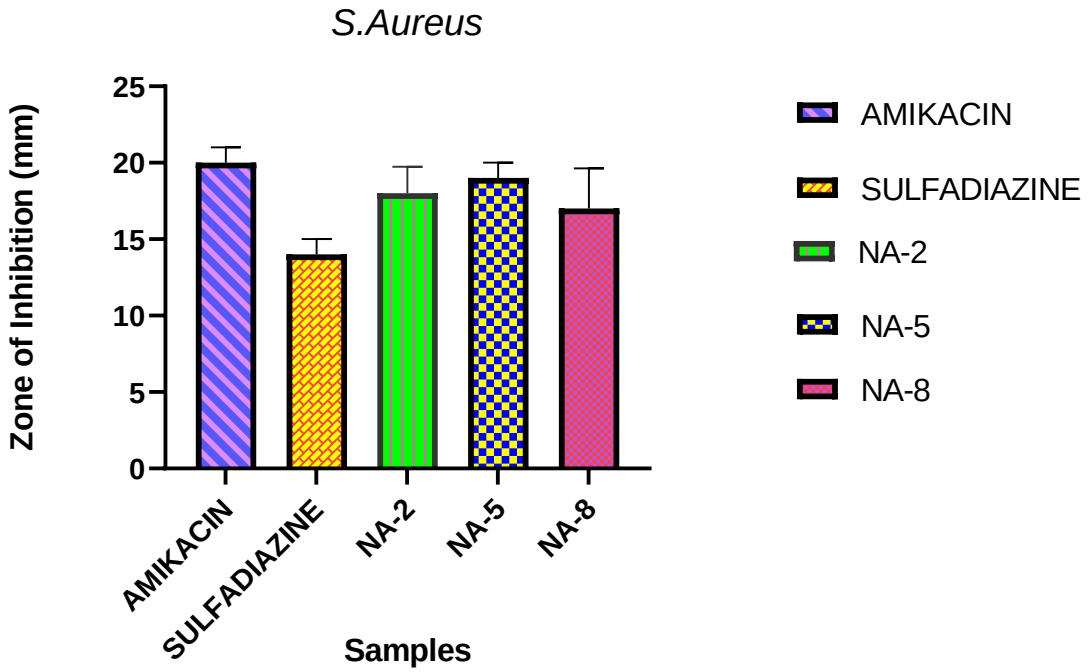


Figure-38: **Anti-Bacterial activity of NA-2, 5 & 8 against *Staphylococcus Aureus***

3.3.1.2.3 Anti-Bacterial activity of NA-3, 6 & 9:

The synthesized conjugates NA-3, 6 & 9 resulting from the coupling of pyrimethamine with *N*-protected glycine, demonstrate notable antibacterial efficacy. Their results are summarized in Tables 8 and 9. The results show that these conjugates are more active against bacteria than their starting reactant pyrimethamine. In the case of *Escherichia Coli*, the NA-3, 6 & 9 with a zone of inhibition 11, 12, and 9 mm respectively are approximately 36-48% more effective in *Escherichia Coli* than the pyrimethamine. In the case of *Staphylococcus Aureus*, the NA-3, 6 & 9 with zones of inhibition 8, 10, and 9 mm respectively are approximately 40-50% more effective to *Staphylococcus Aureus* than the pyrimethamine.

Pyrimethamine is an anti-malarial drug that is not effective against bacterial strains[109]. It shows very good results against malaria when it forms conjugates with other drugs such as azithromycin and sulfadiazine[110].

Table-8: **Anti-Bacterial activity of NA-3, 6 & 9 against *Escherichia Coli***

S. No	Sample	*Escherichia Coli* (Zone of Inhibition)
1	Norfloxacin	25mm
2	Pyrimethamine	0mm
3	NA-3	11mm
4	NA-6	12mm
5	NA-9	9mm

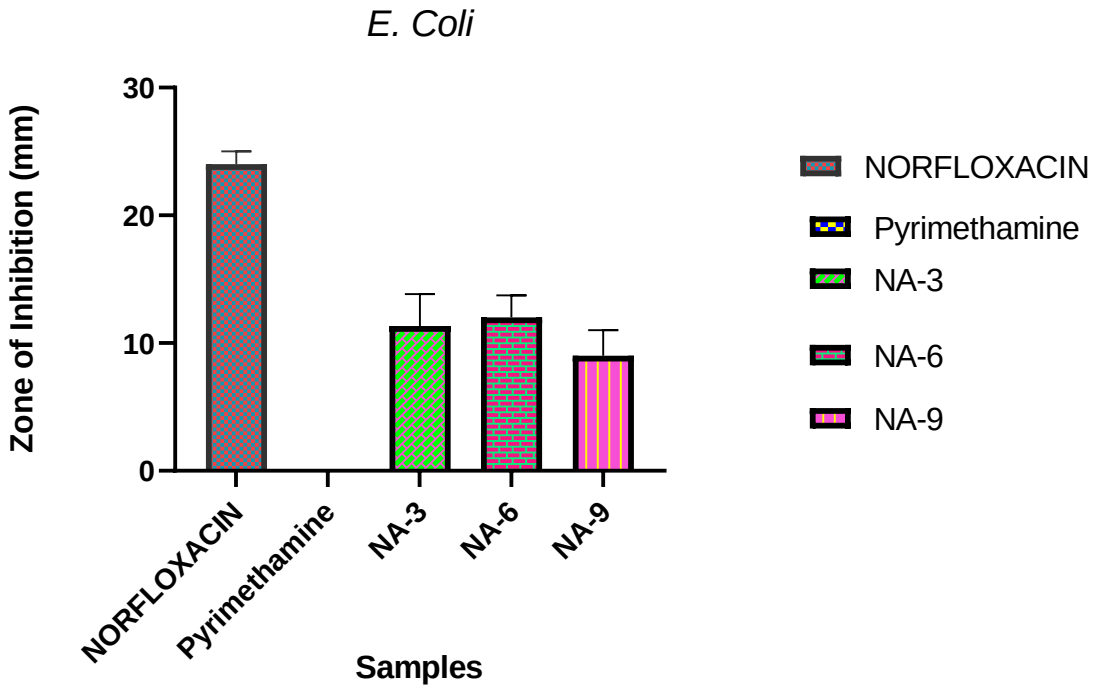


Figure-39: **Anti-Bacterial activity of NA-3, 6 & 9 against *Escherichia Coli***

Table-9: **Anti-Bacterial activity of NA-3, 6 & 9 against *Staphylococcus Aureus***

S. No	**Sample**	***Staphylococcus Aureus* (Zone of Inhibition)**
1	Amikacin	20mm
2	Pyrimethamine	0mm
3	NA-3	8mm
4	NA-6	10mm
5	NA-9	9mm

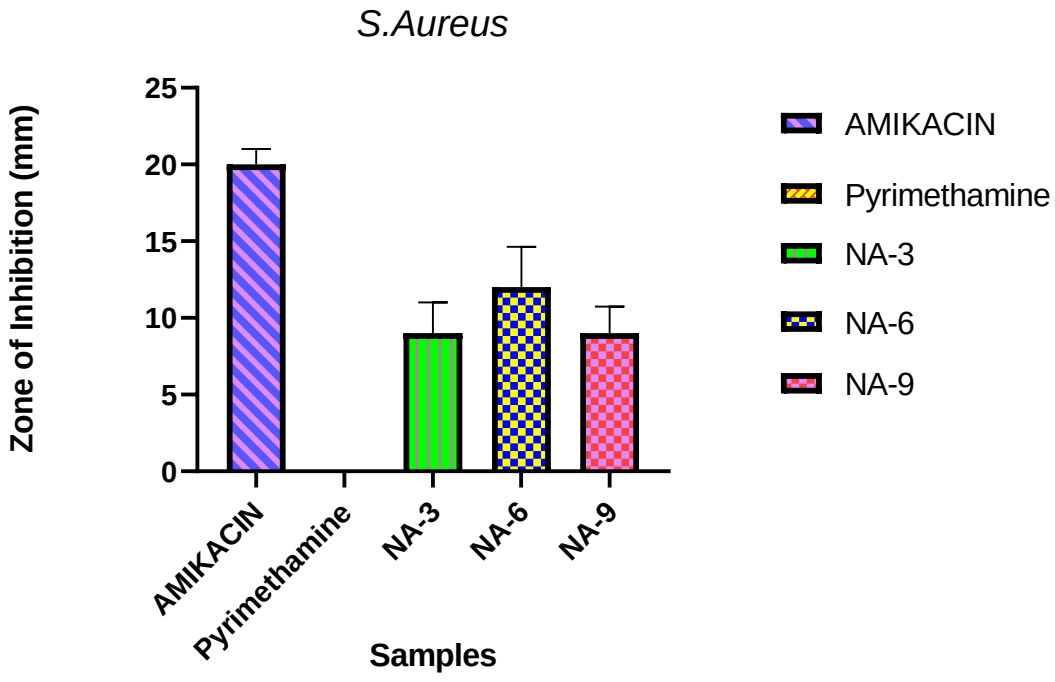


Figure-40: **Anti-Bacterial activity of NA-3, 6 & 9 against *Staphylococcus Aureus***

Different functional groups in a compound contribute to their biological activities. Gaurav Alang and his team synthesized new derivatives of benzothiazole and evaluated them against *Escherichia Coli* and *Staphylococcus Aureus*. Their results conclude that the sulfur-containing compounds and side chains possess moderate-to-potent antimicrobial activity[111]. B. Andrews and his team synthesized different derivatives of pyrimidine and their results show that heterocyclic compound with nitrogen atom shows good activity against different strains of bacteria[112].

The present work also shows that the synthetic compound has very good antibacterial activity. The presence of aliphatic region, sulfur, nitrogen, and amide moieties in the synthesized compound might contribute to their antibacterial activity[113]. Moreover, comparison with existing antibacterial agents highlights the potential of our compound as a promising candidate for further development in antibacterial drug discovery

Conclusion

A set of nine compounds containing amide moiety has been synthesized successfully with a high yield. Characterization of the synthesis compound was conducted through FTIR and ^{1}H-NMR analyses. Importantly, our findings also reveal that the compound demonstrates low cytotoxicity, suggesting a beneficial and advantageous safety profile for potential therapeutic applications. The compound's ability to effectively combat bacterial infections while minimizing harm to host cells highlights its potential as a valuable addition to the antibacterial agents. Among the conjugates of 2-aminobenzothiazole NA-4 shows the maximum zone of inhibition of 21mm against *Escherichia Coli* and 17mm against *Staphylococcus Aureus.* NA-5 exhibits the maximum zone of inhibition among the conjugates of sulfadiazine with a measurement of 23mm against *Escherichia Coli* and 19mm against *Staphylococcus Aureus.* Lastly, within the pyrimethamine conjugates, NA-6 shows the maximum zone of inhibition of 12mm against *Escherichia Coli* and 10mm against *Staphylococcus Aureus*. However, all the synthesized compounds exhibited excellent anti-bacterial activity compared to their starting reactant. Overall, our findings hold promise for the development of antibacterial treatment that addresses the critical need for alternatives to combat antibiotic-resistant bacteria, while ensuring minimal harm to patients.

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