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Synthesis and Antibacterial Activities of Novel Antibiotics.

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Dissertation Approval

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Declaration

I declare that, except where explicit reference is made to the contribution of others, this dissertation is substantially my own work and has not been submitted for any other degree at the Arab American University or any other institution.

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Dedication

This dissertation is dedicated first and foremost to God Almighty for His endless blessings.

To the soul of my father, who inspired this journey, and to my mother, my constant source of love. To my husband, my unwavering partner, and to my daughters, Loreen, Dania, Lana, and Selina, my greatest motivation.

Finally, to my homeland, Palestine.

Nadine Mohammed Kamel Qalalweh

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Finally, I wish to thank every person who supported me, even with a single word of encouragement.

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Abstract

One of the biggest health threats in the world is antibiotic resistance, and patient non-compliance contributes to this issue because of the bad taste of oral antibiotics such as azithromycin and clarithromycin. The purpose of this work was to overcome these difficulties, by synthesizing and assessing new prodrugs and derivatives of macrolide (azithromycin, clarithromycin) and penicillins (amoxicillin, 6-aminopenicillanic acid) antibiotics. The major purposes were to cover the unpleasant macrolides taste and to improve the antimicrobial effect of beta-lactams. The parent antibiotics were reacted with five linkers, including, n-acetylbenzenesulfonyl chloride, p- nitrobenzoyl chloride, p-toluenesulfonyl chloride, 3,5-dinitrobenzoyl chloride and p- nitrobenzenesulfonyl chloride to produce 20 new derivatives. The synthesized products were identified by FTIR, NMR and Mass Spectrometry. Hydrolytic stability of Macrolide prodrugs were evaluated at pH 2.2, pH 5.5 and pH 7.4. Molecular docking in silico was conducted on penicillin derivatives to determine the interaction of the penicillin derivatives with penicillin-binding proteins (PBPs), and the in vitro antimicrobial activity of each derivative of penicillin against *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae,* and *Staphylococcus aureus* was assessed by determining the MIC, and IC50. The outcomes have shown that it was possible to produce the twenty derivatives successfully. The hydrolysis research indicated that the azithromycin derivative compound 5 exhibited optimal prodrug properties as it was easily transformed to the active parent drug with full pH-dependent hydrolysis. A number of derivatives had a marked improved antimicrobial activity as compared to the parent

drugs. Markedly, compounds 4 and 10 were found to be much stronger against *P. aeruginosa*, compound 8 was found to be highly active against *S. pneumoniae and S. aureus* compound 8 and the obvious wide-spectrum winner, whilst compounds 12 and 17 were found to be extremely active against *P. aeruginosa* and *S. aureus* more than the parent amoxicillin and consider abroad spectrum derivatives. This study was able to come up with promising antibiotics derivatives. One possible remedy to enhance patient compliance is the use of taste-masked pro drug forms of macrolide, especially compound 5. Moreover, the increased efficacy of certain derivatives of beta-lactam against pathogen resistance underscores their being the next generation therapeutic agents.

Keywords: Antibiotic Resistance, Prodrugs, Taste Masking, Macrolides, penicillins.

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List of Definitions of Abbreviations

Abbreviations	Title
6-APA	6-Aminopenicillanic Acid
ACD	Advanced Chemistry Development
CFU/mL	Colony Forming Units per Milliliter
CLSI	Clinical and Laboratory Standards Institute
DMF	Dimethylformamide
E. coli	*Escherichia coli*
FTIR	Fourier-Transform Infrared Spectroscopy
HCl	Hydrochloric Acid
HPLC	High-Performance Liquid Chromatography
IC50	Half-Maximal Inhibitory Concentration
IR	Infrared Spectroscopy
KOH	Potassium Hydroxide
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
MIC	Minimum Inhibitory Concentration
MOL	Molecular File Format
MS	Mass Spectrometry
NaH	Sodium Hydride
NMR	Nuclear Magnetic Resonance Spectroscopy
NO_2	Nitro Group
P. aeruginosa	*Pseudomonas aeruginosa*
PBP	Penicillin-Binding Protein
ΔG	Binding Free Energy
ΔG exp	Experimental Binding Free Energy
ΔG vina	Predicted Binding Affinity from AutoDock Vina

Chapter 1: Introduction

1.1. Background of the Problem

Antibiotic resistance by pathogenic microorganisms poses a formidable challenge to the global health arena, and the phenomenon is threatening to roll back much of the medical progress of the past century. The unstoppable resistance of pathogenic microorganisms against most of our trusted antibiotics has necessitated the need to come up with new and better antimicrobial agents (Organization et al., 2023). This lack of innovation is especially worrying because most of the big pharmaceutical firms have reduced their research and development activities in the anti-infective field, and as a result, there are now very few new antibiotics entering the market (Wright et al., 2014). Thus, the strategic use of the modern chemical synthesis to the discovery of antibacterial drugs should be given central role in case a crisis of global scale is to be prevented. The macrolides and the beta-lactams belong to the most clinically important categories of antibiotics. The broad use of macrolide antibiotics like azithromycin and clarithromycin in the treatment of different respiratory and skin infections is common. They have broad spectrums of activity and preferable pharmacokinetic profiles, thus becoming invaluable in the clinical setting (LeBel, 1993). Likewise, β-lactam antibiotics, such as amoxicillin and its precursor 6-aminopenicillanic acid (6-APA), are one of the most successful classes of antibiotics ever invented and have a long history of safe and effective use against a broad spectrum of bacterial pathogens (Geddes et al., 2007). Although they are quite effective, there is a serious issue with most oral antibiotic formulations: a strong, bitter taste. This is not a minor question of patient comfort but a significant factor in non-compliance, especially among pediatrics and geriatrics. According to the estimates of the American Academy of Pediatrics, the adherence level of medication in children may be as low as 50%, which leaves much to consider in terms of the level of treatment results (Matsui, 2007). The unpleasant mouth feel of a drug can cause the rejection of the drug, spitting, and also vomiting, and these lead to the suboptimal intake and the possibility of a greater risk of failure in the treatment. In its turn, it is likely to cause the development of chronic symptoms, the necessity for additional medical attention, and, most frighteningly, the development of drug-resistant bacteria (Karaman, 2014). The issue of taste-masking is particularly problematic with very bitter substances such as

azithromycin and clarithromycin, with traditional methods such as adding sweeteners and flavors being largely ineffective (Amin et al., 2018). To circumvent this problem, there has been a growing trend towards the use of the field of medicinal chemistry. The prodrug approach is a complex and efficient method of taste masking. A prodrug is a non-active or less active analog of a parent drug, which is destined to undergo a chemical change in the body to liberate the active pharmaceutical ingredient. One can chemically modify the parent drug to alter its physical and chemical characteristics, such as its flavor. Formation of a temporary covalent bond by the use of a linker. The major aspect of this method is the presence of a bond between the drug and a linker. The linker is designed so that it is stable to the oral cavity and the drug does not react with taste receptors, but to be digested in certain physiological conditions, in particular, in the acidic environment of the stomach or the enzymatic environment of the bloodstream and other sites, to liberate the active drug at its intended site of action (Blow et al., 1969). This study, thus, resolves a very serious and complex issue in the cross-section of medicinal chemistry, pharmacology, and community health. The research addresses two related yet independent issues. First, bitter taste of macrolide antibiotics, especially azithromycin and clarithromycin, since it is a critical obstacle to patient compliance and effective therapy. Second, the necessity to improve the therapeutic efficacy of the 6-APA and amoxicillin, focusing on the development of more powerful antimicrobial agents to overcome emerging resistance to antimicrobial therapy. Although the idea of a prodrug is known, a knowledge gap exists in the methodical analysis and evaluation of various linkers in overcoming both taste masking in macrolides as well as in enhancing efficacy in beta-lactams. This research seeks to bridge that gap by generating a collection of derivatives of azithromycin, clarithromycin, amoxicillin, and 6-APA by using five linkers, namely, n-acetyl benzene sulfonyl chloride, *p*-nitro benzoyl chloride, p-toluene sulfonyl chloride, 3,5-dinitrobenzoyl chloride, and *p*-nitro benzene sulfonyl chloride. A detailed test will be carried out on the synthesized derivatives, hydrolysis tests will be conducted in varying pH conditions to determine the *in vivo* behavior of macrolide derivatives, molecular docking will be done to learn about the way that penicillin derivatives interact with their biological targets, and *in vitro* biological activity will be tested to determine the efficacy. This broad strategy will offer a comprehensive evaluation of the potential of these new derivatives as better therapeutic options, as they solve taste issues with macrolides and improve antimicrobial efficacy with β-lactams.

1.2. Significance of the Study

The study that was conducted in this dissertation has the potential to be both theoretically and practically useful for the fields of medicinal chemistry and pharmaceutical sciences. The synthesis of the new derivative forms of antibiotics that will have a better taste is not just a matter of pure chemical synthesis but a direct response to an urgent clinical requirement that has far-reaching consequences to the health of the public (Liu et al., 2014). With regard to the practical and applied standpoint, this study has several implications. In the case of macrolide antibiotics, the benefit will be the possibility of creating more palatable azithromycin and clarithromycin formulations. Our ability to conduct successful synthesis of taste-masked derivatives may result in a new generation of patient-friendly drugs, especially important to pediatric and geriatric patients who are the most susceptible to medication non-compliance (Hanning et al., 2016). Studies have always indicated that adherence to medications among the pediatric population is more complicated compared to adults and that taste aversion is one of the leading factors that lead to non-compliance (El-Rachidi et al., 2017). Through increasing the palatability of these commonly used macrolides, we will be assured of high patient adherence, which translates to improved clinical outcomes and subsequent lack of treatment failure (Boateng, 2017). It is also vital that there is the possibility of creating more powerful beta-lactam analogs of amoxicillin and 6-APA. The development of beta-lactamase-producing bacteria and multidrug-resistant pathogens has greatly reduced the potency of the classical penicillin. An effective therapeutic alternative against resistant bacterial strains might be developed, as derivatives with increased antimicrobial action. This is especially important when considering that beta-lactam antibiotics are the most commonly prescribed group of antimicrobials in the world, and enhancements in their efficacy may have far-reaching consequences on treatment efficacy and alleviation of antibiotic resistance. The improved-biological-activity, improved-pharmacokinetic-activity, or new-mechanism-of-action derivatives might be of special interest to the pharmaceutical industry and, consequently, to the next-generation β-lactam antibiotics (Zeng & Lin, 2013). Theoretically speaking, the proposed study will have a considerable impact on our comprehension of the structure-activity relationships (SAR) of antibiotic derivatives (Subbaiah et al., 2024). With systematic synthesis and screening of a collection of compounds with well-defined structural changes, we can now start to uncover the inter-

relationship between chemical structure, taste, stability, and biological activity, which is complex. The obtained hydrolysis research data will be especially useful, as it will allow gaining essential information about the kinetics of drug release in various conditions of pH (Waterman et al., 2002). The information is not only necessary to predict the *in vivo* performance of the synthesized derivatives but also in the rational design of future prodrugs with custom release profiles. In addition, the molecular docking experiments will offer a theoretical background on the interaction of the binding with the new derivatives with their biological targets (Wong et al., 2022). This will not only assist in the explanation of the observed biological activity but also in designing even stronger and selective antibiotics in the future. This research will provide a valuable resource in terms of data and a complete analysis that will be highly beneficial to other scientists in the medicinal chemistry and drug discovery field.

1.3. Problem of the Study

The research touches upon two closely related yet separate issues in antibiotic treatment, which have a profound effect on clinical practice and population health. The initial issue is the low adherence rates of the oral treatment of macrolide antibiotics (azithromycin and clarithromycin) in patients, because of their bitter taste. which causes a chain of adverse outcomes that eventually affect the health of patients and expose them to the growing problem of antibiotic resistance (Salam et al., 2023). The issue is especially severe among the sensitive groups of patients that include children and the elderly, who tend to be more sensitive to taste and less tolerant to unpleasant medications (Mu et al., 2023). The second issue concerns the urgent need for increased therapeutic effect of β-lactam antibiotics, especially amoxicillin and 6-APA derivatives. Although these antibiotics have performed very well in the past, the current development of resistant bacterial strains and the development of β-lactamase enzymes have diminished their effectiveness (Drawz & Bonomo, 2010). It is clearly needed to create more potent derivatives of amoxicillin and new compounds on the 6-APA scaffold with the ability to overcome resistance mechanisms and offer better antimicrobial activity against new bacterial pathogens (Tooke et al., 2019).

1.4. Objectives of the Study

To direct this research and to make sure that the investigation is systematic and comprehensive, the following specific objectives were formulated:

1. To produce new analogs of macrolide and beta-lactam antibiotics: This will entail the chemical substitution of azithromycin, clarithromycin, amoxicillin, and 6-APA by reacting them with five different linkers, n-acetylbenzene sulfonyl chloride, 3,5-dinitrobenzoyl chloride, p-nitrobenzoyl chloride, p-toluenesulfonyl chloride, and p-nitrobenzene sulfonyl chloride. This will lead to the establishment of a 20 new chemical entities library.
2. To characterize the synthesized derivatives: The chemical structure and purity of each of the 20 newly prepared derivatives will be strictly confirmed with the help of various modern methods, such as Nuclear Magnetic Resonance (NMR) spectroscopy, Mass Spectrometry (MS), and Fourier-Transform Infrared (FTIR) spectroscopy.
3. To assess the hydrolytic stability of the synthesized derivatives: The hydrolysis of the azithromycin and clarithromycin derivatives will be at three pHs (7.4, 5.5, and 2.2). This will help give important data on the stability of the prodrugs in various physiological conditions, as well as how they may release the active parent drug in a regulated way.
4. To perform in silico molecular docking experiments: Molecular docking experiments will be carried out on penicillin's synthesized derivatives to determine the binding affinity and interaction with their biological targets. This will give useful insights into the molecular mechanisms of their biological activity and may help to justify the observed structure-activity relationships.
5. To establish the *in vitro* biological activity of the synthesized derivatives: The antimicrobial activity of all 20 derivatives will be evaluated based on their Minimum Inhibitory Concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) against a series of relevant bacterial strains. This will enable making a direct comparison of the biological activity of the derivatives with that of their parent antibiotics.

Through the attainment of these goals, this research will evaluate a novel class of new-generation of antibiotic derivatives. The findings will not merely help in finding

new promising candidates to build on, but will also going to aid in a better understanding of the principles of prodrug design to mask its taste.

1.5. Research Questions

This research is informed by a set of guiding research questions that aim to lead a systematic exploration of the possibility of a prodrug solution to the problem of bitter taste and a chemical modification for enhancing the biological activity of antibiotics. In answering these questions, the study will be able to present a thorough assessment of the newly synthesized derivatives and how they can be considered as better therapeutic agents.

1. Is it possible to successfully produce stable prodrugs and new structural forms of azithromycin, clarithromycin, amoxicillin, and 6-APA based on the chosen chemical linkers, and what are their structural features? This question deals with the basic viability of the chemical synthesis strategy and aims to find out whether the identified linkers are able to establish covalent bonds with the parent antibiotics. A successful synthesis and structural characterization of these derivatives will be the basis on which their potential as taste-masked formulations and improved therapeutic agents will be evaluated.
2. Which is the hydrolytic profile of the synthesized macrolide derivatives in the different pH conditions, and what can this tell us about whether they will be able to release the drugs *in vivo*? This question deals with the problematic question of prodrug activation. The optimum prodrug needs to be stable to be preserved in the mouth cavity and labile to induce the active drug in a precise location in the gastrointestinal system. Hydrolysis was observed by examining the hydrolysis of the derivatives at pH 7.4, 5.5, and 2.2, to assist in making some conclusions about how these compounds would behave *in vivo* and predict their oral administrability.
3. What happens at the molecular level of interaction between the synthesized penicillin derivatives and their different biological targets? The in silico molecular docking simulations will be used to answer this question. Through the modeling of the interaction between the derivatives and their target proteins, we can predict the binding affinity of the derivatives and the responsible key

molecular interactions that determine their biological activity, and whether their interactions are similar to the penicillin compounds.

4. What is the *in vitro* biological activity of the synthesized derivatives, and how does it compare with that of the parent antibiotics? This question aims to find out whether the antimicrobial activity of the parent drugs has been affected in any way by the chemical alterations conducted on them. The Minimum Inhibitory Concentration (MIC) and the IC_{50} of the derivatives against a panel of strains of the target bacteria can be determined, allowing us to directly compare the potency of the derivatives with that of the original antibiotics
5. Which of the produced derivatives have the most promising combination of chemical stability, their predicted *in vivo* drug delivery, and their biological activity? This last question can be taken as the conclusion of the research, and it aims to define the most promising leads to continue the development. The combination of the data obtained during the chemical synthesis, hydrolysis studies, molecular docking, and biological activity testing will allow us to conduct a comprehensive evaluation of the potential of each derivative and pick the most promising compounds to study further *in vivo*.

1.6. Hypotheses of the Study

The research is based on a number of central hypotheses, which will be systematically tested and measured. These hypotheses give a predictive basis for the probable findings of the study and give a direction to the analysis of the experimental results.

1. The derivation of prodrug analogs of azithromycin and clarithromycin to the covalent bond of the chosen linkers will lead to a considerable decrease in the bitter qualities of the raw materials. The principle behind this hypothesis is that the bitterness of the drug can be effectively masked by stopping the drug from binding to the taste receptors in the oral cavity, thus resulting in a more palatable formulation (Sohi et al., 2004).
2. The in silico molecular docking experiments will show that the synthesized derivatives can effectively bind to their biological targets. This is because this hypothesis relies on the assumption that the parent drug will not have its

interaction with the target protein blocked by the inclusion of the linker. It is assumed that the derivatives will assume a conformation that enables the central structure of the antibiotic to fit in the active site of the target, resembling that of the parent drug, and thus, maintain its biological activity (Ferreira et al., 2015; Kitchen et al., 2004).

3. The resulting derivatives will have significant *in vitro* biological action on a collection of specific bacterial strains, with certain derivatives possibly having superior or altered action patterns over the parent antibiotics. The rationale behind this hypothesis is that the chemical modifications to the parent drug can not only mask its taste in some prodrugs but also change its pharmacokinetic or pharmacodynamic action. Although the main purpose of these prodrugs is to maintain the activity of the parent drug, there is a possibility that the presence of the linker can cause increased cell permeability and/or altered metabolic stability and/or other effects that might lead to improved antimicrobial efficacy (Ettmayer et al., 2004; Huttunen et al., 2011).

1.7. Scope and Limitations of the Study

1.7.1 Scope of the Study

The theoretical framework of the study focuses on the design, synthesis, and analysis of innovative prodrug analogs of azithromycin, clarithromycin and penicillin derivatives of amoxicillin, and 6-aminopenicillanic acid (6-APA). The study is also characterized by the application of five particular chemical linkers, namely n-acetylbenzenesulfonyl chloride, p-nitrobenzoyl chloride, p-nitro benzenesulfonyl chloride, 3,5-dinitrobenzoyl chloride, and p-toluenesulfonyl chloride. The research problem is the application of a prodrug approach to the bitter taste issue of the macrolide antibiotics and the production of novel penicillin antibiotics with pronounced biological properties. The topical base of the research is limited to a set of specific experimental and computational research. They are chemical synthesis and characterization of the new derivatives, hydrolytic stability of the new derivatives in various pH conditions, in silico molecular docking to determine their binding behaviors, and *in vitro* research of their biological activity.

1.7.2 Limitations of the Study

1- The biggest limitation of the study is that it was not tested in animal models or human beings *in vivo*. Although the *in vitro* and in silico experiments are useful in understanding the potential of the synthesized derivatives, they are not capable of recreating the complicated biological setup of a living organism (Dafale et al., 2016). Thus, the results of the present research must be viewed as an indicator of a proof-of-concept, and additional *in vivo* experimentations will be necessary to establish the effectiveness and safety of the most promising candidates.

2- It is restricted to a maximum of five linkers and four antibiotics used in the study. they are not the entire possibility of prodrug design. The results of this study do not necessarily hold true for other linkers or other antibiotics. More development would require research on the possibility of other chemical modifications to mask taste and deliver a drug.

3- The synthesis of the synthesized derivatives is assessed as far as *in vitro* testing of the antimicrobial activity of the product is concerned. This gives an estimation of their potency against certain bacterial strains but does not state their pharmacokinetic characteristics, including absorption, distribution, metabolism, and excretion (ADME) (Schmidt et al., 2008). These are also important aspects that would have to be explored in further research to determine the general therapeutic potential of the new derivatives.

These limitations are recognized, which allows this study to be highly scientifically strict and give an honest view of its results. The findings of the study will form a strong background for further studies in the field and will play an important role in addressing the current question to come up with new and better antibiotic therapies.

1.8. Conceptual and Operational Definitions

To be clear and precise in this thesis, the main terms and concepts that will be employed should be defined. The conceptual and operational definitions given below give a precise and definite picture of the terminology applied in this study.

Molecular Docking is a computerized method of modeling that is utilized to forecast the preferred orientation position of a single molecule against a second larger molecule in the event that they are attached to one another to shape a stable complex. Molecular docking involves the computational modeling of ligand binding poses within a protein's specific binding region using scoring functions. These poses are then

improved to yield thermodynamic binding affinities, which are a form of activity prediction and structural information. The binding affinity and interaction of the synthesized derivatives to their biological targets will be predicted in the present thesis through molecular docking (Wong et al., 2022). This will give us an idea about the molecular mechanism of their biological activity.

MIC (Minimum Inhibitory Concentration) is the lowest concentration of an antimicrobial agent that is needed to prevent the visible growth of a microorganism following incubation overnight. In the present study, the determination of the MIC of the synthesized derivatives will be done against a panel of relevant bacterial strains to determine their in vitro biological activity (Forry et al., 2016).

Half-maximal inhibitory concentration (IC_{50}) is a parameter used to measure the strength of a compound in the inhibition of a certain biological or biochemical process. Drug concentration that is needed to reduce a biological process by 50% (W Caldwell et al., 2012).

Structure-Activity Relationship (SAR) is the association between the chemical structure of a molecule and its biological activity. SAR studies play a basic role in medicinal chemistry in finding out the effects of structural changes on the pharmacological characteristics of drugs (McKinney et al., 2000).

ADME (Absorption, Distribution, Metabolism, and Excretion) is the four most important processes of pharmacokinetics that influence the amount of the drug within the body. The properties of ADME play a key role in forecasting *in vivo* behavioral and pharmacological activity of pharmaceutical substances (Butina et al., 2002). Although the direct ADME studies are outside the scope of this study, the structural changes that have been performed on the parent antibiotics can affect these properties.

Penicillin-binding proteins (PBPs) are enzymes in bacteria that take part in the final stages of peptidoglycan synthesis in bacterial cell walls. The β-lactam antibiotics are the major agents that bind to the active site of these enzymes covalently and hence inhibit cell wall production, causing death of bacterial cells (Sauvage et al., 2008). In this research, molecular docking simulations will be used to predict the binding activities of the synthesized β-lactam derivatives with the target PBPs.

6-APA (6-aminopenicillanic acid) is the basic chemical structure of all penicillin antibiotics, which comprises a fused beta-lactam core connected to a thiazolidine ring bearing an amino group at the 6-position. It is the basic starting material of the semi-synthetic syntheses of various penicillin analogs with improved characteristics

(Carrington, 1971). Attention to 6-APA will be used in this research study because this compound will be chemically modified with different linkers to develop new derivatives that can have a better antimicrobial property.

A prodrug is a bio-reversible analog of a drug molecule that is enzymatically and /or chemically (like acidic pH) modified *in vivo* to release the active parent drug. The purpose of designing prodrugs is to enhance the pharmaceutical characteristics of solubility, stability, bioavailability, or to minimize side effects (Stella et al., 2007).

hydrolysis is a chemical reaction where one of more chemical bonds in a molecule is broken with the help of a molecule of water (common in esters, amides, carbamates, etc.). The release of the active antibiotic is referred to as hydrolysis in this research, and it is caused when the covalent bond between the linker and the parent drug is broken (Waterman et al., 2002). The hydrolysis will be examined at various pH levels to determine the *in vivo* action of the prodrugs.

Chapter 2: Literature Review

This chapter reviews previous studies and literature related to the topic of this research. It provides essential background information, clarifies key concepts, and highlights the main findings of earlier researchers. Through this review, the chapter establishes the context of the current study, identifies existing knowledge gaps, and demonstrates how the present research contributes to filling those gaps.

2.1. General introduction to Antibiotics and Penicillin Resistance.

Antibiotics were one of the most significant discoveries of the 20th century, as they revolutionized the treatment of bacterial infections and, most importantly, significantly reduced the death rates of diseases that were previously fatal (Dyary et al., 2023). Such antimicrobial agents, whether produced naturally or chemically synthesized, work by selectively targeting critical processes in a bacterium without harming the host organism (Dalhoff, 2021). The introduction of penicillin in 1928 marked the beginning of the antibiotic era. Through the development of additional classes of antimicrobial agents, such as beta-lactams, macrolides, aminoglycosides, fluoroquinolones, and tetracyclines, a diverse array of antimicrobial agents has accumulated, each with distinct mechanisms and specific therapeutic uses (Group, 1983; Mora-Ochomogo & Lohans, 2021). Macrolide antibiotics, including erythromycin, clarithromycin, and azithromycin, exert their antibacterial activity by binding to the 50S ribosomal subunit, thereby inhibiting protein synthesis. They demonstrate high activity against gram-positive bacteria and atypical pathogens (Dharmapalan, 2022). The predictable development of antimicrobial resistance has followed the clinical effectiveness of antimicrobials. This evolutionary mechanism has been exacerbated by the widespread misuse of antibiotics, leading to one of the most significant challenges in contemporary medicine (Nandy et al., 2024). The obstacles to antibacterial discovery have resulted in an exceptionally low output of novel antibacterial medication classes over the past 25 years, despite the existence of discovery programs at both major and small pharmaceutical companies, as well as academic laboratories, during this time. A logical approach to creating molecules with more potent activity against pathogens, improved absorption, distribution, metabolism, and excretion (ADME) properties, and

the ability to successfully evade or overcome the defensive adaptations made by resistant strains is to precisely modify the backbones of existing antibiotics (Silver, 2011; Wright, 2007). The efficacy of antibiotics is diminishing due to the increased emergence of resistance, which is evident across all antimicrobial agents. There is a growing number of studies on bacterial species that exhibit resistance to all known antibiotics, rendering us very susceptible to common diseases (Lobanovska & Pilla, 2017). For almost a century, the use of antibiotics has facilitated the rapid and widespread introduction of antibiotic resistance genes (ARGs) among bacteria globally. Bacterial antibiotic resistance emerges almost concurrently with the development and extensive application of novel antibiotics in society. Most recognized bacterial infections have developed resistance to antibiotics. The occurrence of resistance is increasingly noted not only in pathogenic bacteria originating from humans or animals but also in bacteria found in the environment (Li et al., 2023). The World Health Organization (WHO) estimates that 700,000 deaths occur globally each year due to Antimicrobial Resistance (AMR). By 2050, if the ongoing trend of growing antimicrobial resistance (AMR) is not restricted, it is anticipated that more than 10 million individuals may die annually worldwide from resistant bacterial infections (Hou et al., 2023). Resistance mechanisms include many processes such as modification of penicillin-binding proteins (PBPs), production of β-lactamases, changes in membrane permeability, and the existence of efflux pumps (Poole, 2004). β-lactamase inhibitors, such as clavulanic acid, sulbactam, and tazobactam, are employed with penicillins to prevent resistance induced by β-lactamase enzymes. Nonetheless, there is a necessity for innovative inhibitors to combat the rising resistance (Eiamphungporn et al., 2018).

2.2 Focus on β-Lactams

2.2.1. Penicillins

Penicillin is the oldest accessible pure antibiotic. In 1928, Alexander Fleming initially noted the suppression of staphylococci on an agar plate infected with Penicillium mold. Clinical research on his isolate began in the 1940s, when Florey and his colleagues at Oxford generated sufficient crude penicillin to initiate therapeutic trials in mice. However, the urgency of World War II postponed the drug's market launch until 1946. Subsequent research revealed that the product obtained from the industrial fermentation of Penicillium chrysogenum consisted of a group of closely comparable

chemicals that varied solely in the characteristics of the acyl side chain (Bush, 2010; Miller, 2002). The natural penicillins included penicillin F (pentenyl penicillin), G (benzyl penicillin), K (heptyl penicillin), and X (p-hydroxy benzyl penicillin). Benzylpenicillin (penicillin G) was chosen from this family as the most suitable penicillin for clinical development due to its biological properties and the simplicity of its commercial manufacture. The penicillin family of antibiotics consists of a substantial range of bicyclic penam compounds that vary based on the characteristics of the acyl side chain linked to the fused β-lactam–thiazolidine ring system. The majority are semisynthetic derivatives of the penicillin nucleus and 6-aminopenicillanic acid (6-APA), synthesized through the incorporation of acyl side chains at the 6-amino group (Bush, 2010). Penicillins are antibacterial agents, either natural or synthetic, derived from the fungus Penicillium. All penicillin types possess three fundamental chemical constituents: a thiazolidine ring, a covalently linked beta-lactam ring, and a side chain, as shown in **Figure 2.1.** Other antibiotics that include the beta-lactam structure similar to penicillin include cephalosporins, carbapenems, and monobactams. These types of antibiotics are together known as beta-lactams (Miller, 2002).

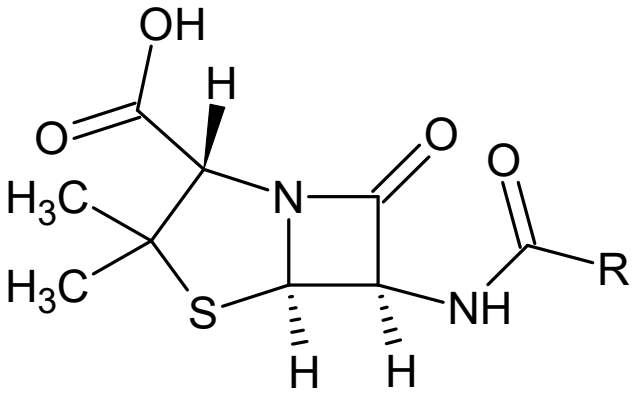


Figure 2.1: General structure of penicillins

2.2.2. Penicillin's Mechanism of Action & PBPs

The formation of the bacterial cell wall is a complex and precisely regulated process essential for cellular survival and morphogenesis. Peptidoglycan (PG), an essential constituent of the cell wall, is a heteropolymer that covers the bacterial membrane, protecting against osmotic lysis. The important function of the peptidoglycan biosynthetic machinery in bacterial proliferation made it a target for effective antibiotics, such as β-lactams, which have been utilized globally for decades

(Bertonha et al., 2023; Höltje, 1998; Matteï et al., 2010). The assembled peptidoglycan consists of polymerized disaccharide subunits, N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), interconnected by stem peptides, a disaccharide pentapeptide composed of (L-Ala-D-Glu-L-Lys-D-Ala-D-Ala). PBPs can catalyze transpeptidation processes (peptide cross-linking) (Egan et al., 2020; Leclercq et al., 2017; Miyachiro et al., 2019). The transpeptidation reaction is specifically blocked by β-lactam antibiotics, which obstructs peptide cross-linking, ultimately resulting in the weakening of peptidoglycan and cell lysis (Macheboeuf et al., 2006; Tipper & Strominger, 1965). Penicillins irreversibly bind to the active site of these enzymes, preventing the final cross-linking of the peptidoglycan layer and disrupting the formation of the cell wall (Ashraf et al., 2015). In the transpeptidation reaction, the active site of PBP identifies the terminal D-alanine residue of the peptide, resulting in the formation of an acyl-enzyme complex. The nucleophilic attacks on the carbonyl group of the penultimate D-alanine by the lateral amino group at position (3) of a neighboring chain produce a conventional "4–3" cross-link. β-lactam antibiotics structurally mimic D-alanine, D-alanine dipeptide, and thus form an acyl–enzyme complex with the active site serine of penicillin-binding proteins (PBPs); nonetheless, the relative stability of this complex against nucleophilic attack is crucial for its inhibitory effect **Figure 2.2** (Drawz & Bonomo, 2010; Macheboeuf et al., 2008).

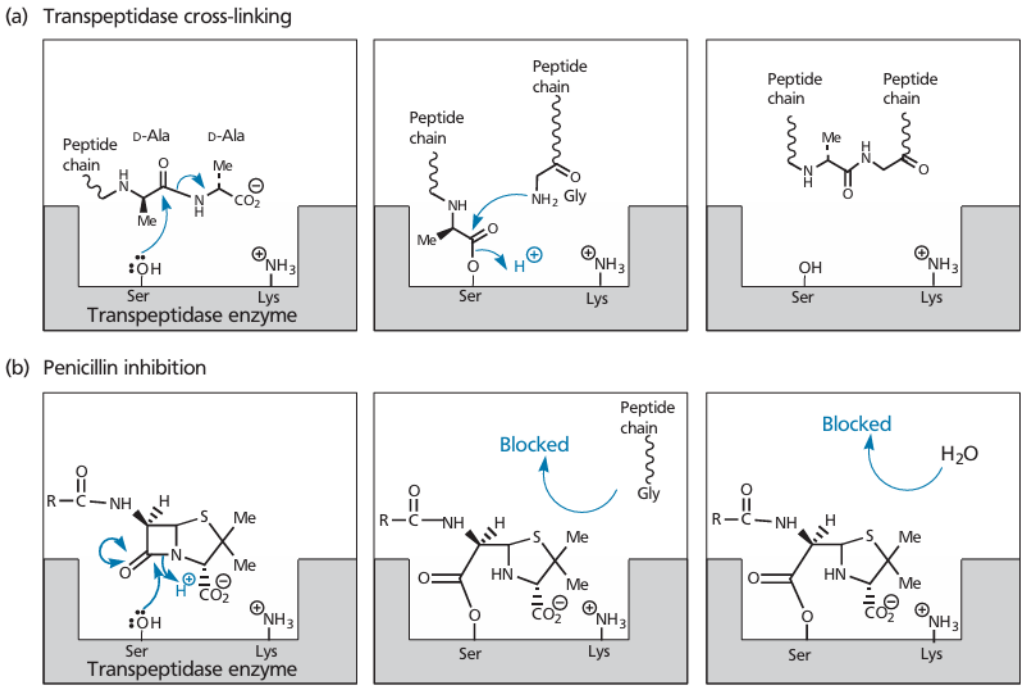


Figure 2.2: Mechanisms of transpeptidase cross-linking and penicillin inhibition (Patrick, 2023).

Penicillin-binding proteins (PBPs) are membrane-associated proteins in bacteria that enable the final step of peptidoglycan synthesis (Kim et al., 2023). Various organisms may exhibit different numbers of PBPs. High-molecular-mass penicillin-binding proteins (PBPs) can either facilitate both glycosyltransferase and transpeptidation (class A) or just the transpeptidation process (class B), as shown in **Table 2.1** (Sjodt et al., 2020). The other type is low-molecular-mass penicillin-binding proteins (PBPs). These are smaller, non-essential proteins that play a role in recycling, remodeling, and maintaining the cell wall. Although they are crucial for the general health of the cell, they are not the main targets for bacterial killing. Usually, they exhibit endopeptidase or carboxypeptidase activity. (Georgopapadakou & Liu, 1980; Goffin & Ghuysen, 1998; Laible & Hakenbeck, 1991; Typas et al., 2012; Veiga, 2012).

Table 2.1: Summary of types of PBPs in the four used bacteria

(Georgopapadakou & Liu, 1980; Goffin & Ghuysen, 1998; Laible & Hakenbeck, 1991; Typas et al., 2012; Veiga, 2012)

PBP Name	Bacterium	PBP Class	Primary Function(s)
PBP1	*Staphylococcus aureus*	Class B HMW	Transpeptidation
PBP3	*Pseudomonas aeruginosa*	Class B HMW	Transpeptidation
PBP3	*Escherichia coli*	Class B HMW	Transpeptidation
PBP2X	*Streptococcus pneumoniae*	Class B HMW	Transpeptidation

2.2.3. 6-Aminopencillic Acid & Amoxicillin

6-Aminopenicillanic acid (6-APA) is the basic framework underlying all penicillin antibiotics and is used in the production of semisynthetic penicillins (Batchelor et al., 1961; Rolinson & Stevens, 1961). This compound is analogous to penicillin, featuring the distinct beta-lactam ring joined to a thiazolidine ring to generate the penam nucleus and free amino group at position 6, to which acylation occurs to produce a variety of penicillin derivatives. This structural simplicity renders 6-APA an ideal model molecule to study the fundamental chemistry of 6-acyl beta-lactam modifications and the development of prodrugs. The free amino group at position 6 is very nucleophilic and reacts easily with a set of carboxyl acid derivatives that form the amide linkages that are

the hallmark of penicillin antibiotics. The role of 6-APA in antibiotic development cannot be overemphasized, and it has been used as the source of many important clinically active penicillins such as ampicillin, amoxicillin, and methicillin, among others. Commercially, the compound is synthesized by enzymatic degradation of penicillin G with penicillin G acylase, a reaction that has been very well optimized on an industrial scale. The commercial availability of 6-APA as a starting point material makes it eligible for prodrug development research studies (Sawant et al., 2020). The identification of 6-APA facilitated the development of semi-synthetic penicillins, which emerged as a crucial category of antibiotics in clinical use. The discovery of 6-APA stimulated the emergence of various other forms of β-lactam antibiotics (Rolinson & Geddes, 2007). The majority of currently available semisynthetic penicillins are derived from 6-aminopenicillanic acid (6-APA) as shown in **Figure 2.3**, which is predominantly generated through enzymatic or chemical deacylation of the natural benzyl penicillin (semisynthetic method) (Chisti & Moo-Young, 2020; Matsumoto, 1993; Parmar et al., 2000).

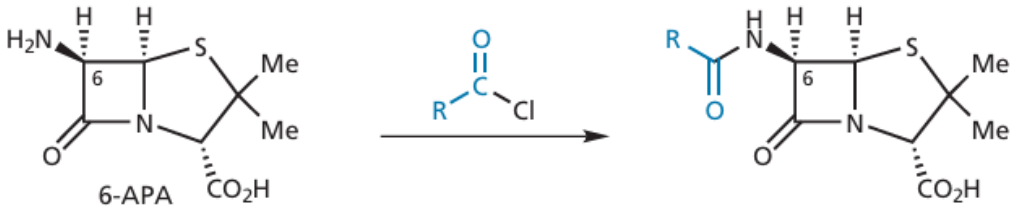


Figure 2.3: Penicillin analogues synthesized by acylating 6-APA (Patrick, 2023).

Amoxicillin is one of the most widely prescribed β-lactam antibiotics worldwide. It is a semisynthetic penicillin, which is produced by acylation of the 6-aminopenicillanic acid (6-APA). This is due to a structural alteration that makes amoxicillin different from ampicillin, in that the presence of a hydroxyl group on the phenyl ring enhances oral bioavailability and widens the spectrum of antimicrobial action (**Figure 2.4**). The amoxicillin molecule contains the typical beta-lactam ring attached to a thiazolidine moiety is required to exhibit antimicrobial activity (Huttner et al., 2020). Among the pharmacological capabilities of amoxicillin as a potential prodrug candidate are its broad-spectrum antimicrobial activity, high oral bioavailability, and good safety profile. Amoxicillin is highly active against Gram-positive microorganisms, such as Streptococcus species and non-beta-lactamase-producing *Staphylococcus*

aureus, in addition to various Gram-negative microorganisms. Amoxicillin exhibits its mechanism of action by inhibiting the synthesis of bacterial cell walls through covalent binding to penicillin-binding proteins (PBPs), which leads to lysis and killing of bacteria (Bereda, 2022). Nonetheless, the compound exhibits a significant bitter taste, a common issue for many β-lactam-based antibiotics (Gee & Hagemann, 2007; Uchida et al., 2025).

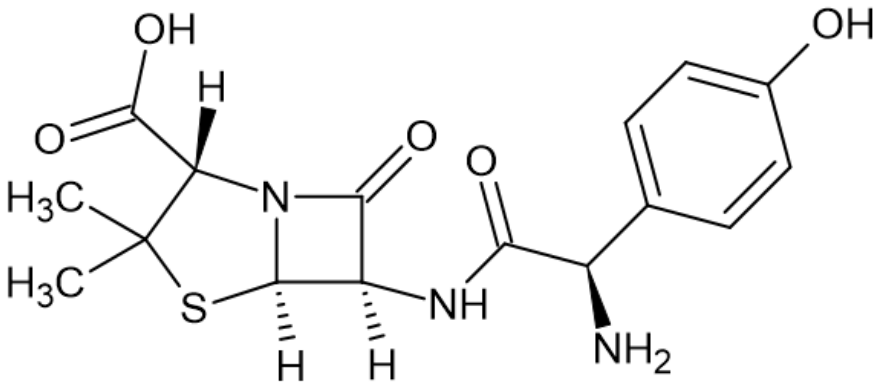


Figure 2.4: Chemical structure of amoxicillin

2.2.4. Structure-Activity Relationship of 6-APA & Amoxicillin

A crucial aspect is understanding the correlation between the chemical composition of penicillin derivatives and their ability to combat bacteria. Studies on SAR (Structure-Activity Relationship) inform and design the development of more potent compounds (Patrick, 2023). A significant quantity of penicillin analogues has been synthesized and examined.

The major structure-activity relationship in 6-APA derivatives is that the retention of the 6-beta-lactam ring structure is absolutely required. The critical pharmacophore associated with antimicrobial activity is the beta-lactam ring; any alteration to this structure will eliminate its activity. The 6-amino group of 6-APA is the structural point of chemical group modification and has been well studied regarding penicillin synthesis (Favre et al., 2012). As observed in the literature, based on the amino group, many acyl derivatives can be incorporated without compromising or enhancing the antimicrobial effect. Nevertheless, the characteristic of the acyl group also plays an important role in adding to the activity range on the one hand and the pharmaceutical quality of the product on the other hand. Unsubstituted alkyl acyl derivatives tend to have low antimicrobial potential and spectra. Comparatively, aromatic acyl groups, especially

those with other functional groups, e.g., amino or hydroxyl groups, can give broad-spectrum activity and superior potencies. This association is directly applicable to the proposed research study because it may indicate that modifications with benzoyl can contribute to better antimicrobial activity, rather than suppress it. The literature further shows that the stability and pharmacokinetics of 6-APA derivatives may be affected by the size and complexity of the acyl group. Extensive and bulky acyl derivatives offer superior anti-microbial activity, but poorer stability, and altered pharmacokinetics. This interaction indicates that the design of prodrugs requires a trade-off between advantages in terms of increased activity and those in favor of favorable stability and bioactivity traits (Perron et al., 1960). The structure-activity relationship of Penicillins is shown in **Figure 2.5.**

The findings of these investigations resulted in the subsequent SAR conclusions:

- The strained β-lactam ring is crucial
- The free carboxylic acid is necessary. This is often ionized, and penicillins are delivered as sodium or potassium salts. The carboxylate ion interacts with the charged nitrogen of a lysine residue within the binding site
- The bicyclic system is significant. This imposes additional stress on the β-lactam ring; increased strain correlates with enhanced activity.

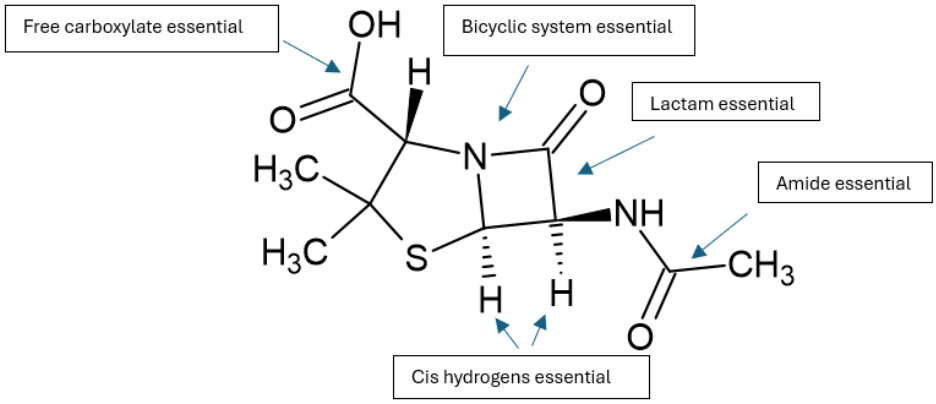


Figure 2.5: Structure Activity Relationships of Penicillins (Qalalweh, 2024).

The structure-activity relationship (SAR) that is most important to amoxicillin prodrug development is the imperative to maintain a β-lactam ring structure. The β-Lactam ring is the key pharmacophore conferring antibacterial action, and any alteration to this ring system will lead to total loss of activity (Abbas et al., 2022). This limitation severely limits the possibilities of chemical modification and requires special attention

when selecting the location of the modification and reaction conditions (Mora-Ochomogo & Lohans, 2021). The side chain of amoxicillin is the main target of chemical modification in the development of prodrugs. The literature shows that this side chain is able to accept a variety of chemical alterations and achieve antimicrobial properties, but the location and type of the alterations have profound effects on the properties. The amino group of the side chain is likely the most susceptible to chemical alteration since a wide range of acyl derivatives retain meaningful antimicrobial properties. That amino group's tolerance to modification is also directly applicable to the proposed research project, since it indicates that acylation with benzoyl chlorides would be compatible. Nevertheless, it should be altered in such a way that it can be bioactivated in physiological conditions. A potential site of chemical modification is also the phenolic hydroxyl group in the side chain of amoxicillin. There is evidence that esterification in this position can be done without losing any antimicrobial activity in general. The phenolic hydroxyl group lends itself especially well to prodrug applications since it is a clean site to form an ester without competing with the amino group (Li et al., 2021).

2.3. Focus on Macrolides

2.3.1. Introduction

Macrolides such as Spiramycin, tylosin and erythromycin are naturally occurring molecules consisting of a lactone ring with linked deoxy sugars. Some macrolides possess both antibiotic and antifungal characteristics and are used in pharmaceutical antimicrobial treatments. The first macrolide used in this capacity was the prototype erythromycin, introduced in 1952; it was frequently administered to patients with penicillin allergies or those with penicillin-resistant diseases (KLEIN, 1997). This discovery is attributed to Filipino scientist Dr. Abelardo Aguilar. He extracted the antibiotic from a strain of Streptomyces erythreus, which was discovered in soil samples from his home region of Iloilo, Philippines (Dy, 1997). Numerous derivatives have been synthesized, resulting in molecules with enhanced bioavailability, acid stability, and improved pharmacokinetics. These attempts led to the development of the second generation of macrolides, notable members of which include azithromycin and clarithromycin. Consequently, in response to the rising threat of antibiotic resistance, a

third generation of macrolides was developed, exhibiting enhanced efficacy against numerous macrolide-resistant bacteria (Dinos, 2017).

2.3.2. Mechanism & Resistance

Macrolides slow down bacterial protein synthesis. The mechanism of action of macrolides involves their capacity to attach to the bacterial 50S ribosomal subunit, resulting in the inhibition of bacterial protein synthesis. Upon binding, the medication inhibits mRNA translation, particularly the elongating peptide chain, by obstructing the enzyme peptidyl transferase from incorporating the next amino acid linked to the tRNA. The bacterial ribosome structure is extensively conserved among nearly all bacterial species, enabling it broad spectrum. Macrolides are bacteriostatic drugs that solely impede protein synthesis; yet, at elevated concentrations, they may have bactericidal properties. Macrolide antibiotics act as bacterial protein synthesis inhibitors by binding to the 50S ribosomal subunit and preventing the transfer of peptidyl-tRNA from the A site to the P site. This interaction is bacteriostatic against certain susceptible organisms and prevents protein chain from extending. The size and conformation of the macrolactone ring, a crucial pharmacophore, are crucial for ribosomal association. Alterations to the macrolactone ring or sugar components can influence antibacterial activities, pharmacokinetics, and resistance profiles, as evidenced by the structure-activity relationship in macrolides (Sohi et al., 2004). The synthesis of azithromycin and clarithromycin from erythromycin illustrates the potential of precise modifications for therapeutic improvement (Dinos, 2017; Kirst, 2010; Parnham et al., 2014; Tenson et al., 2003; Vázquez-Laslop & Mankin, 2018). Bacterial resistance to macrolide antibiotics is a significant clinical challenge, primarily driven by three main strategies. Bacteria most commonly develop resistance through target site modifications by producing enzymes encoded by erm genes, which methylate the 23S rRNA component of the 50S ribosomal subunit. The modification blocks macrolide binding to its target and typically results in resistance against multiple related antibiotics. A second common strategy is active drug efflux, mediated by membrane pumps. The bacterial pumps work to remove antibiotics from inside the cell, thus maintaining antibiotic concentrations below effective therapeutic levels. Certain bacteria employ enzymatic mechanisms to deactivate drugs through esterase enzymes or phosphotransferases, which either dismantle the macrolide ring structure or make modifications that eliminate its activity (Mandell, 1993).

2.3.3. Azithromycin & Clarithromycin

The semisynthetic, erythromycin-based azalide antibiotic azithromycin is one of the most clinically successful macrolide antibiotics to have been discovered in the previous several decades. It has the unusual 15-membered ring system of the azalide instead of the typical 14-membered ring of the macrolides by the introduction of an atom of nitrogen at 9a of the macrolactone ring (**Figure 2.6**). The presence of sugar substituents, especially the dimethylamino group, on the desosamine sugar makes the compound basic in nature and a bitter taste. The pharmacological capability and use of azithromycin as a potential candidate to find prodrugs and the specific reasons to make it an appealing possibility comprise a broad range of antimicrobial effects and efficacy, and a long half-life. Nonetheless, these useful capabilities are offset by substantial palatability issues, especially the overwhelmingly bitter flavor that restrains patient acceptance and compliance, especially with pediatric formulations (Amin et al., 1835; Amin et al., 2018; Mutak, 2007; Parnham et al., 2014).

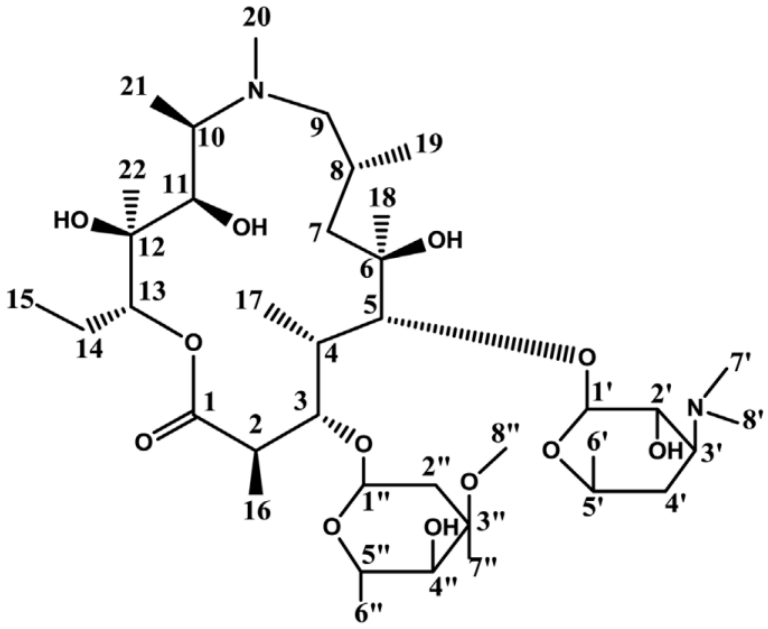


Figure 2.6: The chemical structure of azithromycin (Guo et al., 2021).

The 6-*O*-methyl derivative of erythromycin A, clarithromycin, is one of the most commercially successful semisynthetic macrolide antibiotics based on rational optimization of the natural product erythromycin (Neu, 1991). The most recent was in the 1980s when the compound was selectively methylated on the 6-hydroxyl group of erythromycin A, which vastly enhanced the acid stability and augmented the pharmacokinetic activity of the parent molecule (Omura et al., 1992). This mono methylation was to change erythromycin A with its acid liability and necessity to be

enterically coated into an acid-stable substance that could be administered as conventional oral medication (Veldkamp, 2022). Clarithromycin has a molecular structure comprising a 14-membered macrolactone ring to which are attached two sugar molecules: at the 3-position cladinose and at the 5-position desosamine (Peters & Clissold, 1992) (**Figure 2.7**). The important structural change compared to erythromycin A is the addition of the 6-O-methyl group, which blocks acid-catalyzed degradation by inhibiting the formation of the spiroketal of erythromycin A to confer acid stability to pharmaceutical formulations (Morimoto et al., 1984). Clarithromycin was selected as a favorable prodrug candidate based on the pharmacological properties of clarithromycin, specifically its broad spectrum of antimicrobial activity, good tissue penetration, and enhanced bioavailability relative to erythromycin A, as well as on palatability limitations; as with other macrolide antibiotics, clarithromycin has a bitter taste (Lu et al., 1991). This issue of palatability is especially of concern to pediatric formulations, wherein patient adherence is paramount to therapeutic success. The issue of bitter taste is made worse by the fact that the compound tends to exhibit an aftertaste effect, which may persist in the body even several hours following administration (Ntemi et al., 2019).

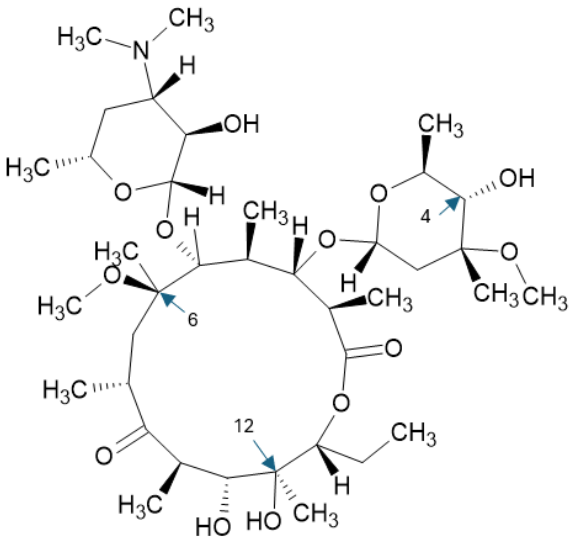


Figure 2.7:The chemical structure of clarithromycin

2.3.4. Structure Activity Relationship of Macrolides

All macrolides and their ketolide derivatives possess structures centered around a macrolactone ring, with the most therapeutically significant macrolides featuring a 14-, 15-, or 16-membered ring (**Figure 2.8**) (Douthwaite, 2001; Schlünzen et al., 2001).

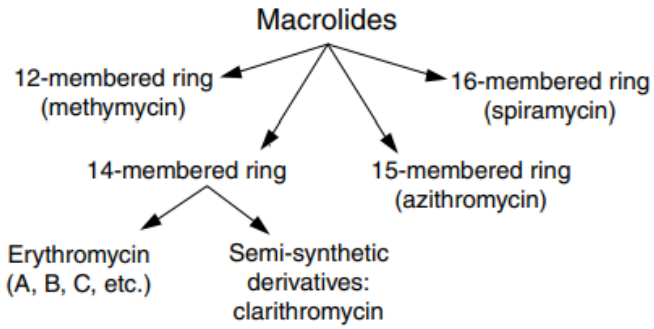


Figure 2.8: Ketolide and macrolide relationship (Douthwaite, 2001).

This diagram shows the Classification of macrolide antibiotics based on the size of their lactone ring, with examples for each category.

Table 2.2 shows Key Pharmacophore Features of Macrolide Antibiotics for Ribosomal Binding. The table summarizes the essential structural components required for macrolide activity, their respective localizations, and their critical interactions with the bacterial ribosome.

Table 2.2: Pharmacophore Features of Macrolide Antibiotics for Ribosomal Binding

Essential Part	Location	Reason It Cannot Be Modified	Key Interaction Type
Desosamine Sugar	C-5 of the lactone ring	Primary anchor to the ribosome; essential for binding affinity.	Ionic Bond
Macrolactone Ring	Core of the molecule	Provides the fundamental 3D scaffold to fit in the ribosomal tunnel.	Hydrophobic Interactions
C-2' Hydroxyl Group	On the desosamine sugar	Forms a critical secondary anchor (hydrogen bond) to the ribosome.	Hydrogen Bond

The 15-membered azalide ring is the primary structural motif of azithromycin, and its structural variations have been extensively explored. The literature is sufficient to demonstrate how the azalide ring can withstand certain chemical reactions without compromising its antimicrobial properties to some degree, with the position and nature of the modifications exerting a considerable influence. Investigations of novobiocin

analogs have demonstrated that a wide range of nitrogen-substituents is tolerated in the antibacterial agent, with alkyl, aryl, and acyl residues all being active. Such tolerance to N-substitution would also be of interest to prodrug development, since the nitrogen atom could potentially act as a point of prodrug attachment with little or no resultant antimicrobial impact. Replacements on the hydroxyl groups around the macrolactone ring have resulted in variable outcomes on the antibacterial activity, with some being more accepting of such substitutions than others. The 11-hydroxyl group is a center easily subject to modification without loss of much antimicrobial activity, so a variety of ester and ether derivatives have also been produced (Mutak, 2007). The sugar moieties on the azithromycin molecule, in particular the cladinose and desosamine sugars, are critical to antimicrobial activity and are key areas on the molecule that can serve as an attachment point to modify the drug at these positions (Patrick, 2023). It turns out that the alterations of these sugar moieties should require great caution to not damaging crucial specific connections with the bacterial ribosome (Kim et al., 2023). The existence of the cladinose sugar at position 3 also seems to be highly sensitive to modification, as studies have demonstrated that removal or significant alteration of this sugar can result in large reductions of antimicrobial activity (Sjodt et al., 2020). The azithromycin-sulfonamide conjugates investigation particularly observed that hydrolysis of the cladinose glycosidic bond led to inactive decladinosyl derivatives, and it was therefore crucial to keep this structural feature (Georgopapadakou & Liu, 1980). The cumulative literature of clarithromycin chemical modifications provides good insight into a few significant structure-activity relationships that are critical in the rational design of prodrugs. Such correlations help guide where to make modifications (sites) and reasonably estimate the probable effect of chemical modifications on antimicrobial activity and drug-like properties. The clarithromycin structure bearing the 14-membered macrolactone is the key structural aspect linked to the antimicrobial activity, with considerable investigations on the structural changes on the ring system (Seppälä et al., 1997). This observation is directly applicable to the proposed study, indicating that modifications to benzoyl and sulfonyl chlorides at these positions may be effective in the development of a prodrug. Of particular interest is the 6-*O*-methyl substituent that is unique to clarithromycin and may be vital to the acid stability of the molecule, and must be maintained in any prodrug design. Yet, other hydroxyl groups at the macrolactone, especially 11-hydroxyl, are not sensitive to modification. Research has indicated that significant antimicrobial activity can be retained when esterified at the 11-position, thus

making this position enticing as a place to make prodrugs.(Morimoto et al., 1984). It has been suggested that antimicrobial activity may particularly depend on the presence of cladinose sugar at position 3, as studies reveal that elongation or deletion of this sugar results in considerable loss of activity. But small functional group changes on the cladinose sugar, e.g., esterification of accessible hydroxyls, seem to be tolerated more favorably. The reason to believe that it might be possible to effect prodrug alterations at the cladinose hydroxyl functionality, thus is that this tolerance implies that alteration of the hydroxyl groups may indeed be possible, provided that it does not alter the structural features needed to maintain biological activity (Undheim, 2020). At position 5, the sugar is desosamine, which bears the dimethylamino group that largely achieves the bitter taste of clarithromycin. This category is a good target group for prodrug modification, as modification of the chemical property may work multifactorially on taste masking and lead to a prodrug linkage. It has been demonstrated that it is possible to alter the desosamine amine group whilst retaining certain antimicrobial activity, but this requires a careful design to provide adequate bioactivation (Lu et al., 1991).

2.4. The Prodrug Concept

2.4.1. General Prodrugs

Prodrugs are pharmacologically inactive (or less active) precursors that are engineered to be biotransformed *in vivo*, releasing the active drug at the desired location of action, thereby reducing side effects and maximizing therapeutic efficacy. The basic concept behind the prodrug strategy is to partially cover the functional groups involved in undesirable aspects, such as low bioavailability and rapid elimination, while retaining or increasing therapeutic activity (Hajnal et al., 2016; Rautio et al., 2008).

2.4.2. Acylation and Sulfonylation Reactions

Acylation methods include the introduction of acyl groups (R-CO-) into an organic molecule, typically via the reaction of acyl chlorides with nucleophilic sites such as amino or hydroxyl groups. The reaction is referred to as nucleophilic acyl substitution, wherein the nucleophile displaces the carbonyl carbon of the acyl chloride and then expels hydrogen chloride. In antibiotic derivatization, acylation has also been employed to derivatize amino and hydroxy groups in such compounds as 6-APA, amoxicillin, azithromycin, and clarithromycin. The reaction is often based on the Schotten-Baumann method, utilizing aqueous or biphasic conditions with inorganic bases to neutralize the

by-product hydrogen chloride (Carey & Sundberg, 2007; Clayden et al., 2012). The acylation has theoretical advantages that include potential for the introduction of a range of functional groups, modification of physicochemical properties, which modifies the interaction between antibiotics and enzymes (Bryskier, 2005).

Sulfonyl chlorides react with nucleophilic sites to form sulfonyl groups ($R\text{-}SO_2\text{-}$) during sulfonylation. It is similar to acylation but targets the sulfur center instead of the carbon. Sulfonamide bonds with amino groups and sulfonate ester bonds with hydroxyl groups are produced by sulfonylation (Carey & Sundberg, 2007). The formation of highly stable bonds, the incorporation of electron-withdrawing groups that can modify reactivity, and the potential for hydrogen bonding interactions via sulfonamide and sulfonate ester functionalities represent theoretical advantages of sulfonylation. The incorporation of sulfonamide groups into antibiotic scaffolds can yield synergistic antibacterial properties. Sulfonamides are a significant class of chemicals exhibiting a broad spectrum of biological activity. In recent decades, numerous pharmacological actions of sulfonamide conjugates have been documented. Furthermore, numerous lead compounds featuring sulfonamide activity are presently undergoing clinical trials for the treatment of various medical disorders. Consequently, the discovery of an effective procedure for synthesizing sulfonamides has consistently been a focal point in organic synthesis research. Sulfones ($R\text{-}SO_2\text{-}R$) serve as useful synthetic intermediates in organic chemistry, with compounds containing a sulfone unit utilized across numerous domains, including agrochemicals, medicines, and polymers (Das et al., 2018; Feng et al., 2016; Kołaczek et al., 2014; Liu et al., 2016; Posner et al., 1988; Shaaban et al., 2017; Wermuth, 2011).

2.4.3. Previous Studies on β-Lactam Prodrugs

β-Lactam antibiotics permanently affect the biosynthesis of bacterial cell walls. For PBP acylation to be effective, the β-lactam ring must possess appropriate reactivity and stereochemistry, which are the key features of β-lactam antibiotic structure-activity relationships. Side chains on the β-lactam nucleus have considerable influence on antimicrobial activity, potency, and resistance. Knowledge of these relationships is the fundamental aspect of rational design of modified derivatives with improved properties (Drawz & Bonomo, 2010; Zapun et al., 2008). This study directly builds upon the pioneering research conducted by Martinez and Martinez regarding the synthesis of novel β-lactam antibiotics using acyl chlorides. They utilized diphenyl acetyl chloride and 3,4,5-trimethoxybenzoyl chloride to acylate 6-APA and 7-aminocephalosporanic

acid. The study provided valuable advice for selecting reagents by identifying lower molar mass acyl chlorides with fewer functional groups that exhibit enhanced antibacterial activity. Martinez's research demonstrated that the steric and electronic characteristics of the acyl group significantly influence biological activity and confirmed the application of benzoyl chloride derivatives as acylating agents in β-lactam antibiotics. The researchers employed standard organic synthesis procedures and comprehensive antimicrobial testing, establishing a methodological framework applicable to the current investigation (Aldrich, 1999; Martinez & Martinez, 2021). According to the Bijev study, N-acylated derivatives of ampicillin and amoxicillin via chloro anhydrides have clinically effective antimicrobial activity with low toxicity in initial tests (Bijev & Hung, 2001). Key structure-activity relationship data from the study indicated that the nature of the acyl group determines potency as well as the spectrum of activity (Bijev & Hung, 2001; Yasuda et al., 1983). The single most innovative application of this research program was the study into the "Design of Novel Amoxicillin Prodrugs by Computational Methods," which used computational methods to design those prodrugs as specifically used in a taste masking application. A project described at this conference was a molecular modeling and computational chemistry prediction of the taste masking efficacy of several amoxicillin prodrug designs. This was done to show that it was possible to use computational techniques to predictively direct prodrug design and to save time and resources in performing similar experimental optimization. The computational design study applies specifically to the proposed research because it shows that it is possible to use computational techniques to suggest not only taste-masking efficacy but also prodrug characteristics. (Hourani, 2017). New semisynthetic penicillins and cephalosporins have been produced using the acylation of 6-aminopenicillanic and 7-aminocephalosporanic acids with ortho-substituted aromatic acids. Consequently, numerous syntheses have led to findings regarding the characteristics of the side chain at positions C-6 and C-7, respectively. Reports indicate that the inclusion of a substantial substituent at these sites yields penicillins and cephalosporins with enhanced antibacterial activity (Dürckheimer et al., 1985; Kaloyanov & Stoyanova, 2000; Milner et al., 1984; Nishida et al., 1999). Demirci and colleagues conducted a comprehensive investigation of hetero functionalized penicillanic acid derivatives starting with 6-APA. The general approach of introducing aromatic substituents at the 6-amino position provides valuable precedent. The anti-β-lactamase activity observed supports the hypothesis that appropriate modifications can

provide resistance to enzymatic degradation (Demirci et al., 2014). Pieper and others utilized tosyl chloride as a coupling reagent to define a general route of synthesis of antibiotics from cephalosporins. Their research gave answers to valuable problems in large-scale synthesis and confirmed the use of p-toluenesulfonyl chloride in the derivatization of antibiotics. The researchers proved that tosyl chloride is a good and cost-effective method for promoting amidation reactions during drug development (Pieper et al., 2019). Das *et al.* reported a comprehensive review on sulfonamide synthesis: they focused particularly on the use of various sulfonyl chlorides as sulfonylating agents. Their review highlighted the reagent versatility of sulfonyl chlorides and offered reaction conditions for antibiotic derivatization. It could be shown that most of the aromatic sulfonyl derivatives deliver favorable antibacterial activity profiles by means of structure-activity relationships (Das et al., 2018). Although there are ample studies on various amoxicillin functionalization, there are no systematic studies on sulfonyl chloride functionalization.

2.4.4. Previous Studies on Macrolides Prodrugs

An important investigation by Abualhasan and co-workers examined how adding benzoyl moieties to clarithromycin improves UV-Vis detection by anchoring an electron-accepting chromophore through an elegant derivatization. Notably, the benzoyl derivatization study revealed that the derivatization could be selective to hydroxyl functionalities on the clarithromycin molecule, indicating that derivative-selective prodrug development is viable (Bijev & Hung, 2001). This study also demonstrated that benzoyl-clarithromycin derivatives were, on average, more chemically stable than the parent agent, and this may be beneficial in the formulation and storage of the prodrug (Abualhasan et al., 2021). Lapa and his colleagues synthesized the ester of clarithromycin. The study found that 4″-*O*-modified clarithromycin demonstrated efficacy against gram-positive strains only, while compounds with C-9 substitution were more active than 4″-*O*-substituted antibiotics (Lapa et al., 2017).

Shutao Ma and his team developed, synthesized, and analyzed novel clarithromycin compounds featuring C-4 elongated arylalkyl groups to investigate the impact of varying lengths of their C-4 side chains on efficacy against resistant bacterial strains (Ma et al., 2011). The lack of sulfonyl modification studies of clarithromycin is especially striking, considering that it has been noted that the macrolide scaffold tolerates a wide range of chemical modifications. Sulfonyl chlorides exhibit distinct reactivity profiles and undergo hydrolysis, which could be utilized as an adjunct to

benzoyl chloride substitution as part of an integrated prodrug development platform (Fini, 2001; Undheim, 2020).

Mutak *et al*. created derivatives of azithromycin; the azalide scaffold has an amino group and several hydroxyl groups, which can be substituted or modified to yield novel molecules. Various derivatives were synthesized through nitrogen substitution, while a diverse array of derivatives, including ethers, esters, and carbamates, was produced via interactions with different hydroxyl groups. Substitutions using both nitrogen and hydroxyl groups, or two hydroxyl groups, produced novel bridging molecules. Research has focused on enhancing its antibacterial characteristics and addressing various pharmacological issues (Mutak, 2007). Krajačić and his colleagues developed novel hybrid compounds, conjugates of 15-membered azalides and sulfonamides, which exhibited enhanced effectiveness against inducible resistant Streptococcus pyogenes compared to the macrolide antibiotic azithromycin. This increased action was explained by the fact that the addition of a sulfonamide component that acts on the inhibition of folate synthesis, combined with the mechanism of the ribosomal protein inhibition action of the azithromycin. The most important finding of the study was that structural changes could be introduced to a significant extent into the 15-membered azalide ring without full loss of antimicrobial properties. It was, however, established that there are critical structural requirements in the research, highlighting especially the need to have the cladinose sugar glycosidic bond intact, as the hydrolysis of the bond gave rise to the inactive decladinosyl derivatives. This finding is significant to prodrug design in that it indicates that the changes that should be made should be such that they do not affect those parts of the drug that are critical to its activity. The precedent of the azithromycin-sulfonamide conjugates study is invaluable to the proposed research in a number of ways. It also supports, in direct form, the immersion idea that sulfonyl chlorides as modifying agents may be employed in combination with azithromycin. Second, it demonstrates that such alterations may increase and not disrupt antimicrobial activity, in keeping with the idea of dual-purpose prodrugs. Third, it presents high-quality structure-activity relationship information that may be used to direct the development of reversible sulfonyl prodrugs (Krajačić et al., 2007). Saris *et al.* produced azithromycin prodrugs by conjugating nitric oxide and acetate to its core structure. This novel chemical significantly improved the intracellular killing of MRSA compared to the uncontrolled variant (Saris et al., 2022). Regarding the perspective of azithromycin prodrugs, the synthesis and characterization of macrolide short-chain fatty acid

derivatives can be mentioned as an essential contribution, since they have been demonstrated by Ahmad *et al.* Also, Robbins and co-authors (2021) synthesized new macrolides esterified at 11, 2′, and 4″ positions on and in the form of azithromycin prodrugs. It also showed that ester bonds could be easily added to the azithromycin structure and retain the prospect of a slow release of the parent antibiotic. The fatty acid ester derivatives exhibited encouraging *in vitro* and *in vivo* distribution properties, as well as the ester bonds could be designed to be selectively bioreleased at desirable rates to meet specific therapeutic needs. This article is especially applicable to the proposed research since it has shown that ester chemistry will successfully be applied to azithromycin modification, offering precedence to benzoyl chlorides being used as acylating agents (Straß et al., 2021).

2.5. Methodological Background

2.5.1. Minimum Inhibitory Concentration (MIC)

Developing useful agents against microbes also entails the critical testing of their actions against the bacterial strains using uniform testing procedures. Minimum inhibitory concentration (MIC) has become the benchmark in the assessment of antimicrobial activity, as it is defined as the lowest concentration of the test antibiotic that prevents visible growth of the test organism (Barnes et al., 2023). A variety of methodological approaches to the determination of MIC have been developed, which contain certain advantages, as well as limitations related to the specifics of the application and needs of the laboratory. The broth microdilution process is the universal method and the gold standard of MIC testing that determines the serial dilution of an antimicrobial agent against a standardized inoculation of bacteria using a 96-well microtiter plate. The technique is highly quantitative and enables many compounds to be tested simultaneously, making it especially useful in research setups and large-scale screening projects (Andrews, 2001; Tan & Lim, 2015).

2.5.2. Molecular Docking

Molecular docking has been used in antibiotic drug research studies, and it has given scientists significant support in decoding the interplay between molecules and predicting biological effects on drugs before their synthesis and experimentation. Docking is a computational technique that theorizes the position and orientation of small molecules (ligands) in their receptor on a macromolecule (receptor) (Kumar et al., 2014;

Pestana-Nobles et al., 2022). In the case of antibiotics, the technique can allow researchers to predict how antimicrobial agents may interact with their target and how to refine the interaction to enhance certain properties. To estimate the binding affinity between the ligand and the receptor, scoring functions are mathematical models that give us the estimated interaction between the predicted model and the binding affinity between them. Such functions normally include van der Waals interactions, electrostatic interactions, hydrogen bonding interactions, and the solvation effects. In the β-lactam antibiotics, special scoring functions might be needed to model the covalent bond formation that will take place during the acylation reaction. The precision with which the scoring functions model the protein-ligand interactions is key to ranking the various binding poses and is calculated to predict affinity relative to other related ligands (Modak et al., 2024). The molecular docking analysis of beta-lactam antibiotics with PBPs has helped to understand the molecular action of antimicrobials and their selectivity. The active sites of PBPs are characterized by a common serine residue upon which an acylation reaction occurs, yet the nearby amino acids are species- and type-specific. These differences determine the binding affinity/selectivity of various β-lactam antibiotics, which can be used in rational drug design (Reddy et al., 2023). Experimental studies are important to validate the prediction of molecular docking in order to determine the accuracy and role of the computational method in drug design. Experimental validation is usually of the form whereby predicted compounds are synthesized and their biological activity results are tested in the laboratory to confirm the predicted activity and affinity. The analysis of the consistency between docking output results and the measured outcomes gives hints about the accuracy of the computational schemes used and locates the areas requiring improvement in order of methodology. The most important application of molecular docking to date has been in SAR studies. The same effect can be used to test the quality of the computational modeling of various structural features by modifying the structures systematically until the observed biological activity clearly matches the computational prediction (Danishuddin & Khan, 2012; Pestana-Nobles et al., 2022).

Chapter 3: Materials and Methods

3.1. Chemicals

The chemicals used are of analytical grade. The N-acetylsulfanilyl chloride, Azithromycin, Clarithromycin, Amoxicillin, 6-aminopencilic acid, *p*-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride, p-nitrobenzenesulfonyl chloride, *p*-toluenebenzene sulfonyl chloride, sodium hydride (60%), ethyl acetate, hexane, dichloromethane, methanol, ethanol DMSO, DMF, THF, HPLC grade acetonitrile, phosphate buffer (pH 7.5, 5.5, 4.4 and 2.2), HPLC grade water, Potassium dihydrogen phosphate, di-potassium hydrogen phosphate, Mueller Hinton agar, Mueller Hinton broth. The tested microorganisms were obtained from the American Type Culture Collection (ATCC) biological laboratories. The bacteria included were *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus pneumoniae* (ATCC 49619).

3.2 Physical Measurements

The Stuart melting point apparatus (R00102618) was used for melting point determinations. Infrared spectroscopy was conducted via Fourier-transform infrared spectrophotometer (Shimadzu IR Spirit FTIR spectrophotometer), Nuclear Magnetic Resonance NMR spectra obtained using a Bruker Avance 500 MHz spectrometer (Bruker, Switzerland), (University of Jordan, Amman), mass spectra were obtained using LC-MS/MS facility (Sciex 4500 QTRAP equipped with UPLC-PDA-MS/MS), Al-Ahliyya Amman University, Jordan, The chromatographic separation was conducted using a Waters ACQUITY Arc high-performance liquid chromatography apparatus fitted with a C18 column, Autoclave (Tuttnauer), Incubator (Memmert), Microliter Pipettes, 96-well plates, ELISA reader (Thermo Fisher).

3.3. General Procedures for the Synthesis of Antibiotic Derivatives

3.3.1. *N*-Acetylsulfanilyl Azithromycin Derivative (Compound 1)

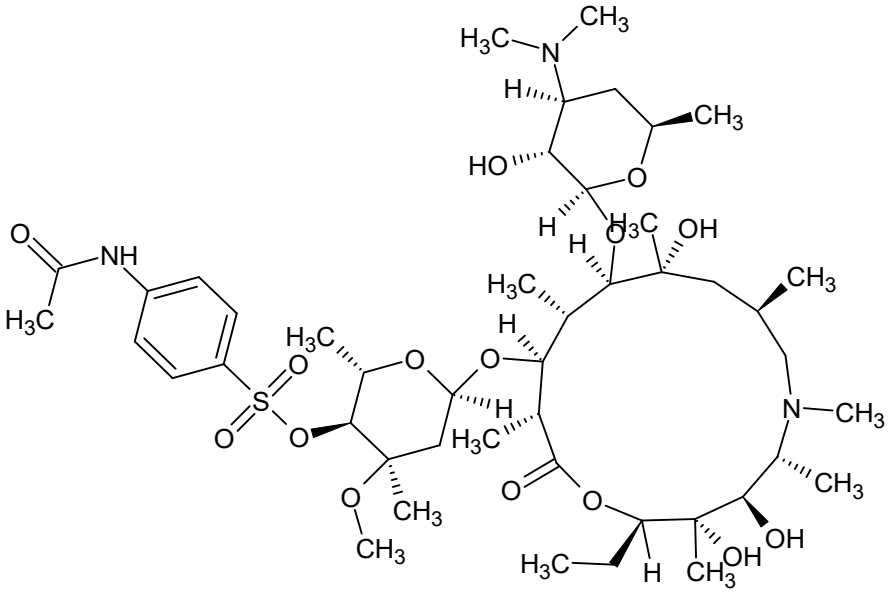


Figure 3.1 : Structure of N-Acetylsulfanilyl Azithromycin Derivative (Compound 1)

Nucleophilic acyl substitution of N-acetylsulfanilyl chloride (0.934 g, 3.99×10^{-3} mol, 3.5 eq) was conducted. Azithromycin (1g, 1.335×10^{-3}mol) was dissolved in 50 ml of THF, then sodium hydride (0.265 g, 0.011 mol, 5 eq) was added slowly with continuous stirring until all hydrogen bubbles were released. *N*-acetylsulfanilyl chloride was subsequently added with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 1 (Figure 3.1)**. The product was then filtered, collected, and recrystallized. The product's mass was 0.45 g (yield 36 %), m.p. = 203-205 °C, R_f= 0.43 (hexane: ethyl acetate: 1:4).

IR (v in cm^{-1}): 3388 NH, 3200-3600 OH, 3020 C-H aromatic, 1644 amide C=O, 1520 aromatic C=C, 1347 S=O asymmetric stretch, 1145 S=O symmetric stretch, 856 **(*para*-substituted aromatic ring)**. (**Appendix 1)**. ^{1}H NMR (500 MHz, MeOD) δ in ppm: δ 8.5 (s, 1H, NH amide group) 7.64 – 7.87 (m, 4H, aromatic), 5.06 (s, 1H, α-H of ester in lactone ring), 4.86 (s, 1H, OCHO desosamine sugar), 4.56 (proton on carbon bearing the sulfonyl group), 3.62 (s, 3H, $1OCH_3$), 3.38 (s, 6H, $2NCH_3$) (**Appendix 21)**. MS (ESI^+) m/z: 947.2 **(Appendix 41).**

3.3.2. 4-Nitrobenzoyl Azithromycin Derivative (Compound 2)

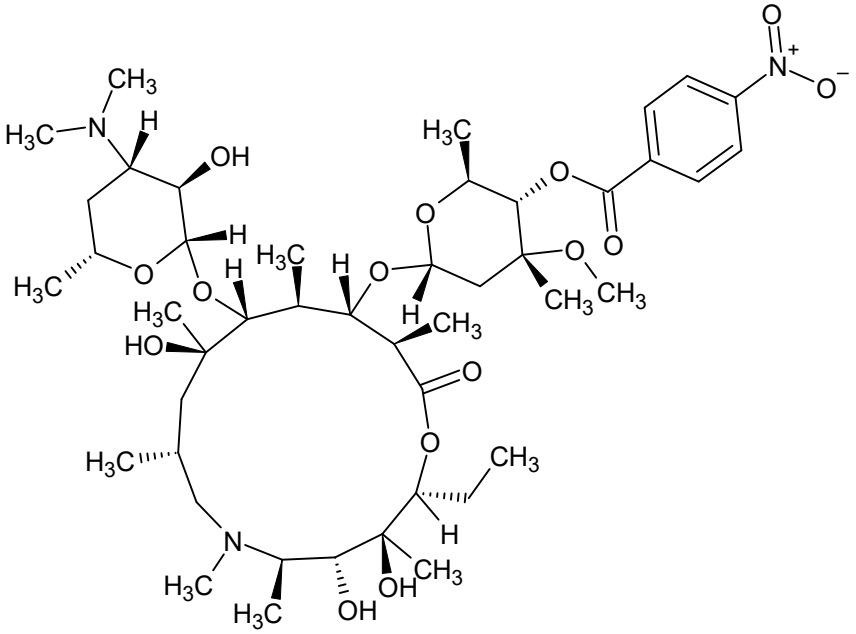


Figure 3.2 : Structure of 4-Nitrobenzoyl Azithromycin Derivative (Compound 2)

Nucleophilic acyl substitution of 4-nitrobenzoyl chloride (0.877 g, 4.72 $\times 10^{-3}$ mol, 3 eq) was conducted. Azithromycin (1g, 1.33 × 10^-3mol) was solubilized in 50 mL of THF, and sodium hydride (0.189 g, 0.0078 mol, 5 eq) was added incrementally with constant stirring until all hydrogen bubbles disappeared. Subsequently, 4-nitrobenzoyl chloride was introduced with continuous stirring. The reaction was performed once over two hours and once overnight, yielding the same **compound 2 (Figure 3.2)** in both cases. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 1 g (81% yield), m.p = 181-186 °C, R_f = 0.7 (hexane: ethyl acetate: 1:4).

IR (v in cm^{-1}): 3200-3600 OH, 3020 (C-H aromatic), 1730 (Ester C=O), 1620 C=C aromatic, 1340 (NO_2 symmetric stretch), 815 (*para*-substituted aromatic ring). (**Appendix 2**). ^{1}H NMR (500 MHz, acetone) δ in ppm: δ 8.24-8.25 (m, 4H, aromatic), 5.02 (s, 1H, α-H of ester in lactone ring), 4.81 (s, 1H, OCHO desosamine sugar), 4.62 (proton on carbon bearing the ester group), 3.40 (s, 3H, 1OCH_3), 2.57 (s, 6H, 2NCH_3) (**Appendix 42**). MS (ESI^+) m/z: 899.5 **(Appendix 22).**

3.3.3. 3,5-Dinitrobenzoyl Azithromycin Derivative (Compound 3)

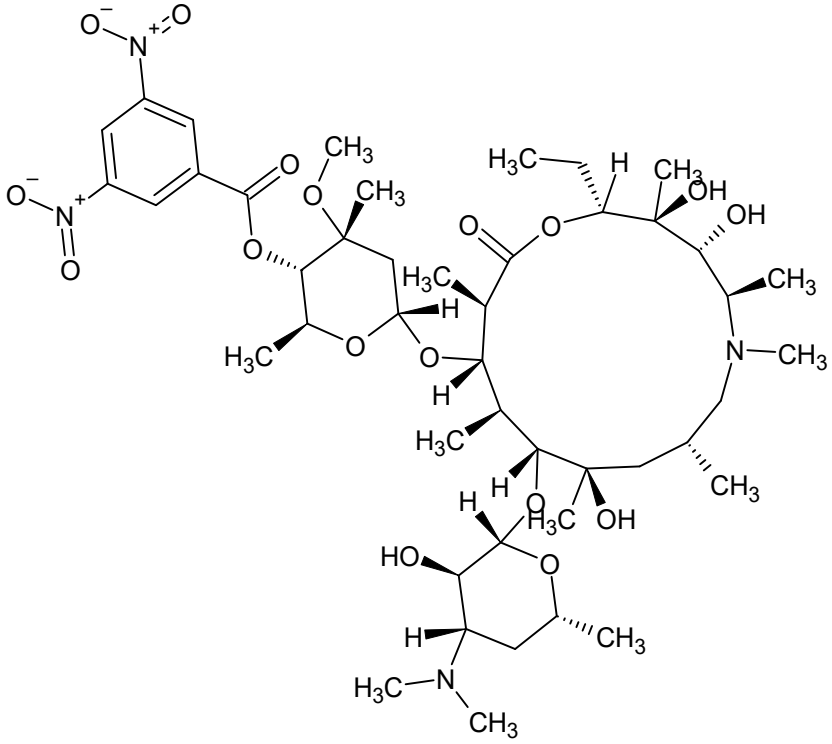


Figure 3.3. : Structure of 3,5-Dinitrobenzoyl Azithromycin Derivative (Compound 3)

Nucleophilic acyl substitution of 3,5-dinitrobenzoyl chloride (0.923 g, 4 $\times 10^{-3}$ mol, 3 eq) was conducted. azithromycin (1g, 1.33 $\times 10^{-3}$mol) was solubilized in 50 ml of THF, then sodium hydride (0.2935 g, 0.0122 mol, 5 eq) was incrementally introduced with constant stirring until all hydrogen bubbles disappeared. Subsequently, 3,5-dinitrobenzoyl chloride was introduced with continuous stirring. The reaction was performed once over two hours and once overnight, yielding the same **compound 3 (Figure 3.3)** in both cases. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.575 g (yield 46%), m.p = 244-247 °C, R_f = 0.7 (hexane: ethyl acetate: 1:4).

IR (v in cm^{-1}): 3200-3600 OH, 3060 (C-H aromatic), 1711 (ester C=O), 1640 C=C aromatic, 1380 (NO_2 symmetric stretch). **(Appendix 3).** ^{1}H NMR (500 MHz, DMF) δ in ppm: δ 9.26 (s, 2H, aromatic), 9.09 (s, 1H, aromatic), 5.17 (s, 1H, α-H of ester in lactone ring), 5.15 (s, 1H, OCHO desosamine sugar), 4.86 (proton on carbon bearing the ester group), 3.56 (s, 3H, 1OCH_3), 3.10 (s, 6H, 2NCH_3) **(Appendix 43).** MS (ESI^+) m/z: 944. **(Appendix 23).**

3.3.4. 4-Nitrobenzenesulfonyl Azithromycin Derivative (Compound 4)

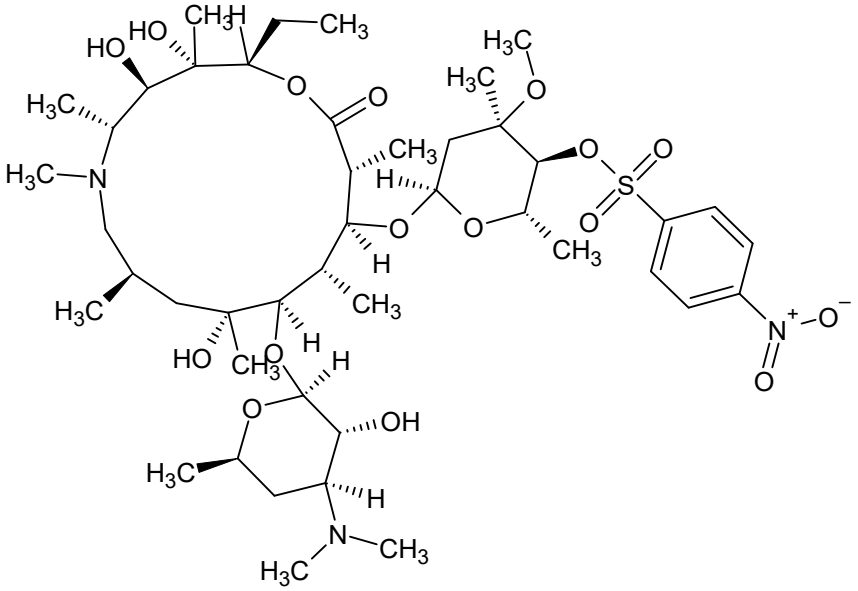


Figure 3.4. :Structure of 4-Nitrobenzenesulfonyl Azithromycin Derivative (Compound 4)

Nucleophilic acyl substitution of 4-nitrobenzenesulfonyl chloride (0.887 g, 4 $\times 10^{-3}$ mol, 3 eq) was conducted. Azithromycin (1g, 1.33 $\times$ 10^{-3}mol) was solubilized in 50 mL of THF, and sodium hydride (0.267 g, 0.011 mol, 5 eq) was added incrementally with constant stirring until all hydrogen bubbles disappeared. Subsequently, 4-nitrobenzenesulfonyl chloride was introduced with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 4 (Figure 3.4)**. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.99 g (yield 80%), m.p = 220-225 °C, R_f = 0.6 (hexane: ethyl acetate: 1:4).

IR (v in cm^{-1}): 3200-3600 OH, 3030 (C-H aromatic), 1621 (C=C aromatic), 1538 (N-O asymmetric stretch), 1380 (N–O symmetric stretch), 1166 (SO_2 symmetric stretch), 796 (*para*-substituted aromatic ring). **(Appendix 4).** ^{1}H NMR (500 MHz, acetone) δ in ppm: δ 8.24 - 8.05 (m, 4H, aromatic), 5.09 (s, 1H, α-H of ester in lactone ring), 4.97 (s, 1H, OCHO desosamine sugar), 4.95 (proton on carbon bearing the sulfonyl group), 3.82 (s, 3H, 1OCH_3), 3.06 (s, 6H, 2NCH_3) **(Appendix 24).** MS (ESI^+) m/z: 935. **(Appendix44).**

3.3.5 4-Toluenesulfonyl Azithromycin Derivative (Compound 5)

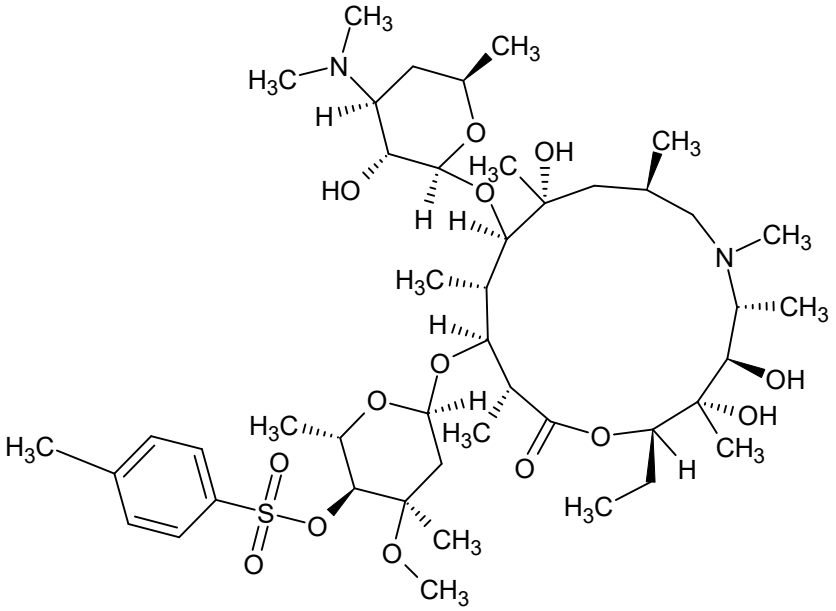


Figure 3.5. : Structure of 4-Toluenesulfonyl Azithromycin Derivative (Compound 5)

Nucleophilic acyl substitution of 4-toluenesulfonyl chloride (0.763 g, 4 $\times 10^{-3}$ mol, 3 eq) was conducted. Azithromycin (1g, 1.33 $\times$ 10^{-3} mol) was solubilized in 50 mL of THF, and sodium hydride (0.267 g, 0.011 mol, 5 eq) was added incrementally with constant stirring until all hydrogen bubbles disappeared. Subsequently, 4-toluenesulfonyl chloride was introduced with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 5 (Figure 3.5)**. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.5 g (yield 42%), m.p = 184-187 °C, R_f = 0.52 (hexane: ethyl acetate: 1:4).

IR (v in cm^{-1}): 3200-3600 OH, **3109 (**Aromatic C–H stretch), 1525 (C=C aromatic),1350 (asymmetric SO_2 stretch), 1150 Symmetric SO_2 stretch, 854 *para*-substituted aromatic rings. **(Appendix 5)**. ^{1}H NMR (500 MHz, acetone) δ in ppm: δ (7.36 - 7.57) (m, 4H, aromatic), 5.09 (s, 1H, α-H of ester in lactone ring), 4.97 (s, 1H, OCHO desosamine sugar), 4.95 (proton on carbon bearing the sulfonyl group), 3.3 (s, 3H, 1OCH_3), 3.04 (s, 6H, 2NCH_3), 2.41(s, 1H, CH_3 toluene) **(Appendix 25).** MS (ESI^+) m/z: 904. **(Appendix45).**

3.3.6. *N*-Acetylsulfanilyl Clarithromycin Derivative (Compound 6)

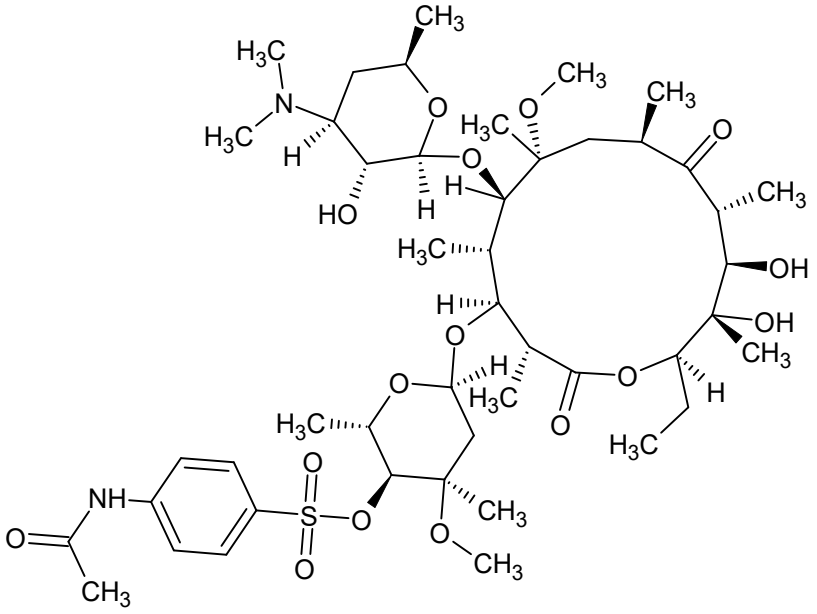


Figure 3.6. : Structure of N-acetylsulfanilyl Clarithromycin Derivative (Compound 6)

Nucleophilic acyl substitution of N-Acetylsulfanilyl chloride (0.9703 g, 4.15×10^{-3} mol, 3.1 eq) was conducted. Clarithromycin (1g, 1.34×10^{-3}mol) was solubilized in 50 ml of THF, then sodium hydride (0.265 g, 0.011 mol, 5 eq) was incrementally introduced with constant stirring until all hydrogen bubbles disappeared. Subsequently, *N*-acetylsulfanilyl chloride was introduced with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 6 (Figure 3.6)**. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.4 g (32% yield), m.p = 235-238, R_f = 0.24 (methanol: dichloromethane: 1:2).

IR (v in cm^{-1}): 3454 NH, 3200-3600 OH, 1686 (amide C=O), 1577 (aromatic C=C), 1313 (S=O asymmetric stretch), 1140 S=O symmetric stretch, 829 (*para*-substituted aromatic ring). **(Appendix 6).** ^{1}H NMR (500 MHz, MeOD) δ in ppm: δ 9 (s, 1H, NH amide group) 7.8 – 7.61 (m, 4H, aromatic), 5.26 (s, 1H, α-H of ester in lactone ring), 4.9 (s, 1H, OCHO desosamine sugar), 4.87 (proton on carbon bearing the sulfonyl group), 3.27 (s, 6H, $2OCH_3$), 2.97 (s, 6H, $2NCH_3$) **(Appendix 26).** MS (ESI^+) m/z: 946. **(Appendix 46).**

3.3.7. 4-Nitrobenzoyl Clarithromycin Derivative (Compound 7)

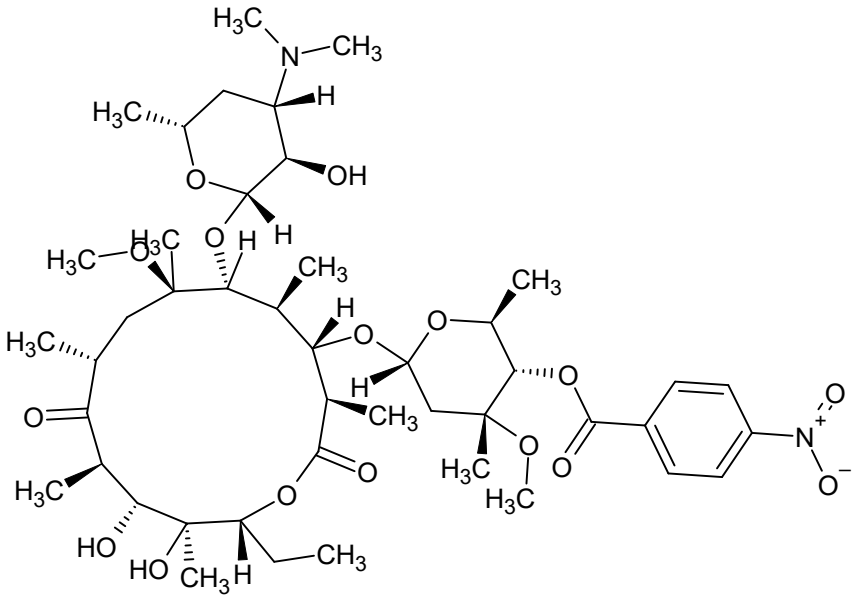


Figure 3.7. :Structure of 4-Nitrobenzoyl Clarithromycin Derivative (Compound 7)

Nucleophilic acyl substitution of 4-nitrobenzoyl chloride (0.372 g, 2×10^{-3} mol, 1.5 eq) was conducted. Clarithromycin (1g, 1.34×10^{-3}mol) was solubilized in 50 ml of THF, then sodium hydride (0.16 g, 0.0066 mol, 3 eq) was incrementally introduced with constant stirring until all hydrogen bubbles disappeared. Subsequently, 4-nitrobenzoyl chloride was introduced with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 7 (Figure 3.7)**. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.61 g (yield 51%), m.p = 195-198 °C, R_f= 0.76 (methanol: dichloromethane: 1:2).

IR (v in cm^{-1}): 3200-3600 OH, 3040 (Aromatic C–H,1711 (Ester C=O), 1644 C=C, 1380 (NO_2 symmetric stretch), 816 (*para*-substituted aromatic ring). **(Appendix 7).** ^{1}H NMR (500 MHz, acetone) δ in ppm: δ 8.25-8.3 (m, 4H, aromatic), 5.11 (s, 1H, α-H of ester in lactone ring), 4.93 (s, 1H, OCHO desosamine Sugar), 4.8 (proton on carbon bearing the ester group) 3.37 (s, 6H, $2OCH_3$), 2.50 (s, 6H, $2NCH_3$) **(Appendix 27).** MS (ESI^+) m/z: 898. **(Appendix 47).**

3.3.8. 3,5-Dinitrobenzoyl Clarithromycin Derivative (Compound 8)

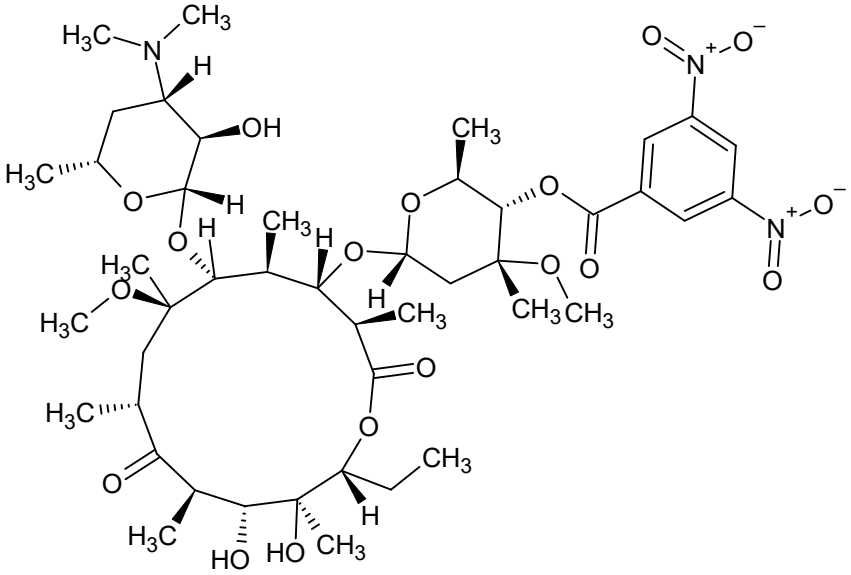


Figure 3.8. : Structure of 3,5-Dinitrobenzoyl Clarithromycin Derivative (Compound 8)

Nucleophilic acyl substitution of 3,5-dinitrobenzoyl chloride (0.923 g, 4 $\times 10^{-3}$ mol, 3 eq) was conducted. clarithromycin (1g, 1.33 $\times 10^{-3}$mol) was solubilized in 50 ml of THF, then sodium hydride (0.265 g, 0.011 mol, 5 eq) was incrementally introduced with constant stirring until all hydrogen bubbles disappeared. Subsequently, 3,5-dinitrobenzoyl chloride was introduced with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 8 (Figure 3.8)**. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.88 g (yield 70%), m.p = 244-247 °C, R_f=0.76 (methanol: dichloromethane: 1:2).

IR (v in cm^{-1}): 3115 (C-H aromatic), 1717 (Ester C=O), 1528 C=C aromatic, 1344 (NO_2 symmetric stretch). **(Appendix 8).** ^{1}H NMR (500 MHz, acetone) δ in ppm: δ 8.96-9.09 (d, 4H, aromatic), 5.12 (s, 1H, α-H of ester in lactone ring), 4.91 (s, 1H, OCHO desosamine sugar), 4.72 (proton on carbon bearing the ester group) 3.42 (s, 6H, $2OCH_3$), 3.18 (s, 6H, $2NCH_3$). **(Appendix 28).** MS (ESI^+) m/z: 943.1. **(Appendix 48).**

3.3.9. 4-Nitrobenzenesulfonyl Clarithromycin Derivative (Compound 9)

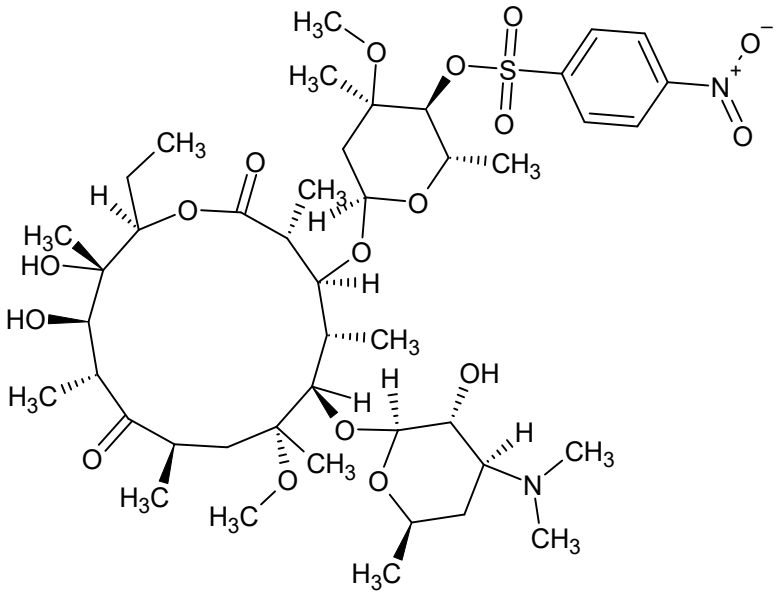


Figure 3.9. : Structure of 4-Nitrobenzenesulfonyl Clarithromycin Derivative (Compound 9)

Nucleophilic acyl substitution of 4-nitrobenzenesulfonyl chloride (0.888 g, 4 $\times 10^{-3}$ mol, 3 eq) was conducted. clarithromycin (1g, 1.33 $\times 10^{-3}$mol) was solubilized in 50 ml of THF, then sodium hydride (0.265 g, 0.011 mol, 5 eq) was incrementally introduced with constant stirring until all hydrogen bubbles disappeared. Subsequently, 4-nitrobenzenesulfonyl chloride was introduced with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 9 (Figure 3.9)**. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.65 g (yield 52%), m.p = 227-230 °C, R_f= 0.64 (methanol: dichloromethane: 1:2).

IR (v in cm^{-1}): 3200-3600 OH, 3111 (C-H aromatic), 1667 (C=C aromatic), 1529 (N-O asymmetric stretch), 1317 (N–O symmetric stretch), 1175 (SO_2 symmetric stretch), 835 (*para*-substituted aromatic ring). **(Appendix 9).** ^{1}H NMR (500 MHz, acetone) δ in ppm: δ 8.03- 8.22 (d, 4H, aromatic), 5.09 (s, 1H, α-H of ester in lactone ring), 4.86 (s, 1H, OCHO desosamine sugar), 4.77 (proton on carbon bearing the sulfonyl group), 3.25 (s, 6H, $2OCH_3$), 3.05 (s, 6H, $2NCH_3$). **(Appendix 29).** MS (ESI^+) m/z: 934. **(Appendix 49).**

3.3.10. 4-Toluenesulfonyl Clarithromycin Derivative (Compound 10)

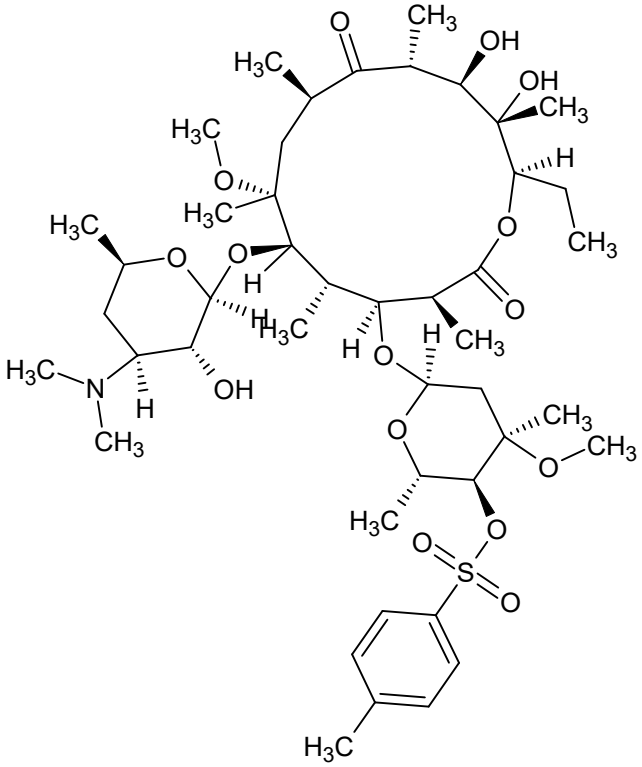


Figure 3.10. : Structure of 4-Toluenesulfonyl Clarithromycin Derivative (Compound 10)

Nucleophilic acyl substitution of 4-toluenesulfonyl chloride (0.764 g, 4 $\times 10^{-3}$ mol, 3 eq) was conducted. Clarithromycine (1g, 1.33 $\times 10^{-3}$mol) was solubilized in 50 ml of THF, then sodium hydride (0.265 g, 0.011 mol, 5 eq) was incrementally introduced with constant stirring until all hydrogen bubbles disappeared. Subsequently, 4-toluenesulfonyl chloride was introduced with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 10 (Figure 3.10)**. The product solution was extracted with ethyl acetate, the organic product filtered, collected, and recrystallized. The product's mass was 0.576 g (yield 48%), m.p = 239- 242 °C, R_f= 0.6 (methanol: dichloromethane: 1:2).

IR (v in cm^{-1}): 3200-3600 OH, 3047 **(**Aromatic C–H stretch), 1622 (C=C aromatic), 1340 **(**Asymmetric SO_2 stretch), 1165 (Symmetric SO_2 stretch), 796 (*para*-

substituted aromatic ring). **(Appendix 10).** ^{1}H NMR (500 MHz, acetone) δ in ppm: δ (7.71 - 7.18) (m, 4H, aromatic), 5.1 (s, 1H, α-H of ester in lactone ring), 4.86 (s, 1H, OCHO desosamine sugar), 4.61 (proton on carbon bearing the sulfonyl group), 3.15 (s, 6H, 2OCH$_3$), 2.99 (s, 6H, 2NCH$_3$), 2.34 (s, 1H, CH$_3$ toluene). **(Appendix 30).** MS (ESI$^+$) m/z: 903. **(Appendix 50).**

3.3.11. *N*-Acetylsulfanilyl 6-Aminopenicillanic acid Derivative (Compound 11)

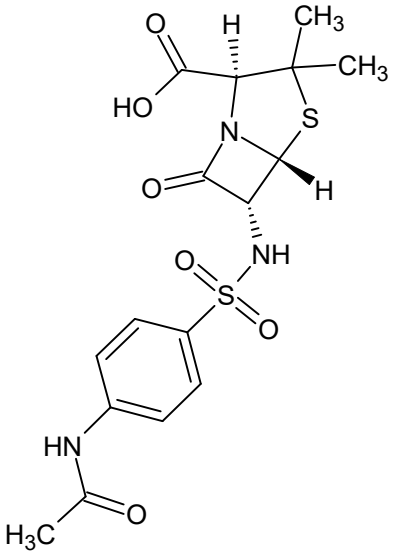


Figure 3.11. : Structure of N-Acetylsulfanilyl 6-Aminopenicillanic acid Derivative (Compound 11)

A mixture of 0.753 g (1.94 eq) sodium bicarbonate, 6 mL deionized water was left alone for 10-15 minutes, or until most sodium bicarbonate was dissolved. Proceeded by adding 2mL of acetone and 1g (0.00462 moles) of 6-APA until dissolved (took only minutes). 2.3 g (0.0098 moles) (2.13 eq) of N-acetylsulfanilyl chloride dissolved in 2 mL tetrahydrofuran with 0.64 mL triethylamine were added and left overnight to yield **compound 11 (Figure 3.11)**. The product was then filtered, collected, and recrystallized. The product's mass was 0.97 g (yield 51%), m.p = 211-214 °C, R_f = 0.75 (methanol: dichloromethane; 2:3).

IR (v in cm^{-1}): 3299 (NH), 1661 (C=O amide of acetyl group), 1596 (C=C, aromatic), 1318 (SO$_2$ asymmetric), 1184 (SO$_2$ symmetric stretch). **(Appendix 11).** ^{1}H NMR (500 MHz, MeOD) δ in ppm: δ 10.03 (s, 1H, COOH), (7.83-7.72) (m, 4H, aromatic), 7.16 (s, 1H, NH, sulfonamide group), 2.15 (s, 3H, acetyl group), 1.67 (s, 3H, CH$_3$), 1.5 (s, 3H, CH$_3$). **(Appendix 31).** MS (ESI$^+$) m/z: 414.5.**(Appendix 51).**

3.3.12. 4-Nitrobenzoyl 6-Aminopenicillanic acid Derivative (Compound 12)

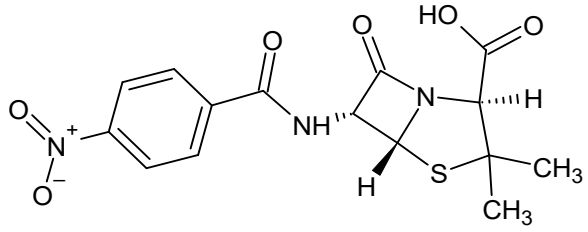


Figure 3.12.:Structure of 4-Nitrobenzoyl 6-Aminopenicillanic acid Derivative (Compound 12)

A mixture of 0.753 g (1.94 eq) sodium bicarbonate, 6 mL deionized water was left alone for 10-15 minutes, or until most sodium bicarbonate was dissolved. Proceeded by adding 2mL of acetone and 1g (0.00462 moles) of 6-APA until dissolved (took only minutes). 1.827 g (0.0098 moles) (2.13 eq) of 4-Nitrobenzoyl chloride and 2 mL of dichloromethane were added and left for two hours to yield **compound 12 (Figure 3.12)**. The product was then filtered, collected, and recrystallized. The product's mass was 1.29 g (yield 76%), m.p = 121-124 °C, R_f = 0.65 (methanol: dichloromethane; 2:3).

IR (v in cm^{-1}): 3513 (NH), 1762 (C=O carboxylic), 1650 (C=O amide), 1527 (C=C, aromatic), 1347 (N–O symmetric stretch), 852 (*para*-substituted aromatic ring). **(Appendix 12).** ^{1}H NMR (500 MHz, DMSO) δ in ppm: δ 9.68 (s, 1H, COOH), (8.07-8.31) (m, 2H, aromatic), 5.65 (s, 1H, NH), 1.62 (s, 3H, CH_3), 1.48 (s, 3H, CH_3). **(Appendix 32).** MS (ESI^+) m/z: 366.4. **(Appendix 52).**

3.3.13. 3,5-Dinitrobenzoyl 6-Aminopenicillanic acid Derivative (Compound 13)

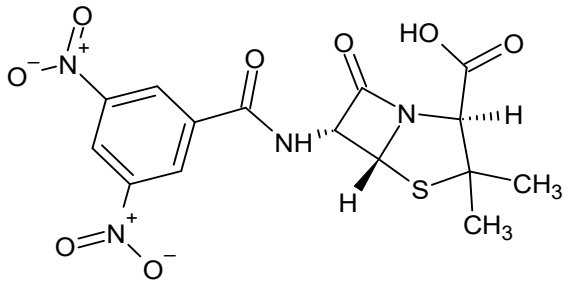


Figure 3.13.: Structure of 3,5-Dinitrobenzoyl 6-Aminopenicillanic acid Derivative (Compound 13)

A mixture of 0.753 g (1.94 eq) sodium bicarbonate, 6 mL deionized water was left alone for 10-15 minutes, or until most sodium bicarbonate was dissolved. Proceeded by adding 2mL of acetone and 1g (0.00462 moles) of 6-APA until dissolved (took only minutes). 2.27 g (0.0098 moles) (2.13 eq) of 3,5-dinitrobenzoyl chloride and 2 mL of THF were added and left for two hours to yield **compound 13 (Figure 3.13)**. The product was then extracted, collected, and recrystallized. The product's mass was 1.2 g (yield 63%), m.p =198-200 °C, R_f= 0.43 (methanol: dichloromethane: 2:3).

IR (v in cm^{-1}): 3275 (NH), 1730 (C=O carboxylic), 1628 (C=O amide), 1538 (C=C, aromatic), 1342 (N-O symmetric stretch). **(Appendix 13).** ^{1}H NMR (500 MHz, MeOD) δ in ppm: δ 9.15 (s, 1H, COOH), (8.17-9.15) (m, 3H, aromatic), 6.09 (s, 1H, NH), 1.55 (s, 3H, CH_3), 1.43 (s, 3H, CH_3). **(Appendix 33).** MS (ESI^+) m/z: 411.4. **(Appendix 53).**

3.3.14. 4-Nitrobenzenesulfonyl 6-Aminopenicillanic acid Derivative (Compound 14)

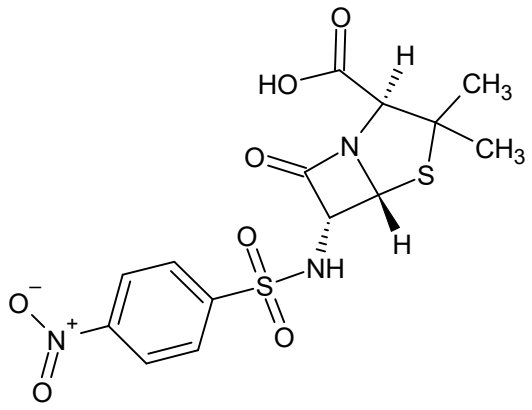


Figure 3.14.:Structure of 4-Nitrobenzenesulfonyl 6-Aminopenicillanic acid Derivative (Compound 14)

A mixture of 0.753 g (1.94 eq) sodium bicarbonate, 6 mL deionized water was left alone for 10-15 minutes, or until most sodium bicarbonate was dissolved. Proceeded by adding 2mL of acetone and 1g (0.00462 moles) of 6-APA until dissolved (took only minutes). 2.182 g (0.0098 moles) (2.13 eq) of 4-Nitrobenzenesulfonyl chloride dissolved in 2 mL of tetrahydrofuran with 0.64 mL triethylamine were added and left overnight to yield **compound 14 (Figure 3.14)**. The product was then filtered, collected,

and recrystallized. The product's mass was 1.62 g (yield 87.5%), m.p = 110-113 °C, R_f= 0.48 (methanol: dichloromethane: 2:3).

IR (v in cm^{-1}): 3331 (NH), 1768 (C=O carboxylic), 1526 (C=C, aromatic), 1348 (N–O symmetric stretch), 1161 (SO_2 symmetric stretch), 719 (*para*-substituted aromatic ring). **(Appendix 14).** ^{1}H NMR (500 MHz, MeOD) δ in ppm: δ (8.02-8.34) (m, 2H, aromatic), 5.65 (s, 1H, NH), 1.34 (s, 3H, CH_3), 1.31 (s, 3H, CH_3). **(Appendix 34).** MS (ESI^+) m/z: 402.4. **(Appendix 54).**

3.3.15. 4-Toluenesulfonyl 6-Aminopenicillanic acid Derivative (Compound 15)

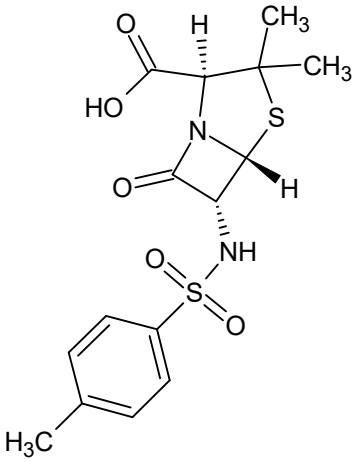


Figure 3.15. : Structure of 4-Toluenesulfonyl 6-Aminopenicillanic acid Derivative (Compound 15)

A mixture of 0.753 g (1.94 eq) sodium bicarbonate, 6 mL deionized water was left alone for 10-15 minutes, or until most sodium bicarbonate was dissolved. Proceeded by adding 2mL of acetone and 1g (0.00462 moles) of 6-APA until dissolved (took only minutes). 1.877 g (0.0098 moles) (2.13 eq) of 4-Toluenesulfonyl chloride in 2 mL of THF with 0.64 ml triethyl amine were added and left overnight to yield **compound 15 (Figure 3.15)**. The product was then extracted, collected, and recrystallized. The product's mass was 0.9 g (yield 52%), m.p = 180-183 °C, R_f= 0.66 (methanol: dichloromethane: 2:3).

IR (v in cm^{-1}): 3330 (NH), 1770 (C=O carboxylic), 1538 (C=C, aromatic), 1326 (SO_2 asymmetric stretch), 1157 (SO_2 symmetric stretch), 815 (*para*-substituted aromatic ring). **(Appendix 15).** ^{1}H NMR (500 MHz, D_2O) δ in ppm: δ (7.30-7.63) (m, 2H, aromatic), 5.6 (s,1H, NH), 2.31 (s, 3H, p-toluene), 1.47 (s, 3H, CH_3), 1.51 (s,3H, CH_3). **(Appendix 35).** MS (ESI^+) m/z: 371.5. **(Appendix 55).**

3.3.16. *N*-Acetylsulfanilyl Amoxicillin Derivative (Compound 16)

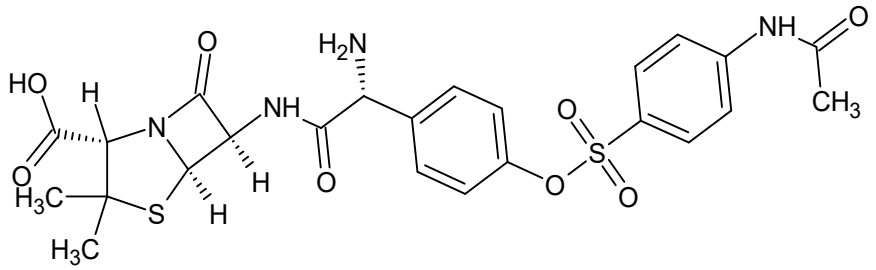


Figure 3.16.: Structure of N-Acetylsulfanilyl Amoxicillin Derivative (Compound 16)

Nucleophilic acyl substitution of N-acetylsulfanilyl chloride was conducted. Amoxicillin (1g, 2.74 $\times 10^{-3}$mol) was dissolved in 10 ml of tetrahydrofuran, and 2 ml of distilled water was added, stirred while being cooled at a temperature of 0-5 °C. Then, 2M potassium hydroxide solution was incrementally added, and the pH was adjusted to 6.8-7.2. Subsequently, N-acetylsulfanilyl chloride (1.28 g, 0.0054 mol, 2 eq) in 10 ml THF was added dropwise with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 16 (Figure 3.16)**. The product was then filtered, collected, and recrystallized. The product's mass was 0.89 g (yield 57.7%), m.p = 190-193 °C, R_f=0.8 (hexane: acetone 2:1).

IR (v in cm^{-1}): 3532-3600 (NH_2), 1624 (C=O amide group), 1364 (S=O asymmetric stretch), 1169 (S=O symmetric stretch). **(Appendix 16).** ^{1}H NMR (500 MHz, acetone) δ in ppm: δ 10.14 (s, 1H, COOH), 9.79 (s, 1H, NH acetyl group), (7.92-8.21) (m, 4H, aromatic), (7.16-7.68) (m, 4H, aromatic of amoxicillin), 4.2 (s, 2H, NH_2), 2.15 (s, 3H, p-toluene), 1.47 (s, 3H, CH_3), 1.51 (s, 3H, 1CH_3). **(Appendix 36).** MS (ESI^+) m/z: 563.6.***(Appendix 56).***

3.3.17. 4-Nitrobenzoyl Amoxicillin Derivative (Compound 17)

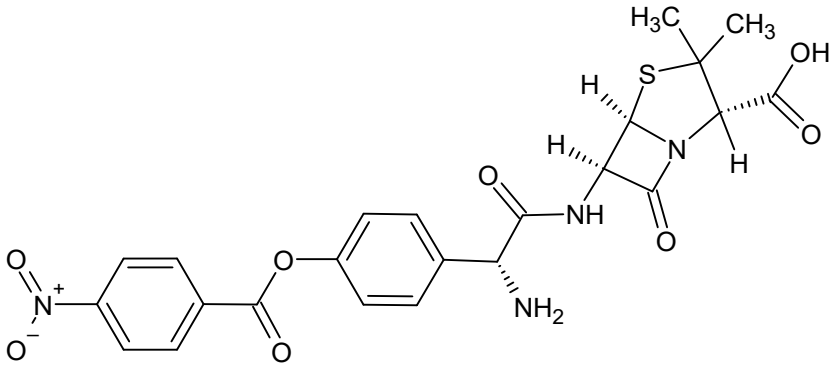


Figure 3.17.: Structure of 4-Nitrobenzoyl Amoxicillin Derivative (Compound 17)

Nucleophilic acyl substitution of 4-nitrobenzoyl chloride was conducted. Amoxicillin (1g, 2.73 $\times 10^{-3}$mol) was dissolved in 10 ml of tetrahydrofuran, and 2 ml of distilled water was added, stirred while being cooled at a temperature of 0-5 °C. Then, 2M potassium hydroxide solution was incrementally added, and the pH was adjusted to 6.8-7.2. Subsequently, 4-nitrobenzoyl chloride (1.067 g, 0.0058 moles, 2.13 eq) in 10 ml THF was added dropwise with continuous stirring, and the reaction mixture was maintained for two hours to yield **compound 17 (Figure 3.17)**. The product was then filtered, collected, and recrystallized. The product's mass was 0.93 g (yield 62%), m.p = 172-176 °C, R_f = 0.48 (hexane: acetone 2:1).

IR (v in cm^{-1}): 3454-3600 (NH_2), 1771 (C=O ester group), 1686 (C=O amide group), 1515 (N-O asymmetric stretch), 1347 (N-O symmetric stretch). **(Appendix 17).** ^{1}H NMR (500 MHz, MeOD) δ in ppm: δ 9.43 (s, 1H, COOH), (8.81-8.93) (m, 4H, aromatic), 6.87-6.96 (m, 4H, aromatic of amoxicillin) 3.68 (s, 2H, NH_2), 3.27 (s, 3H, CH_3), 3.09 (s, 3H, CH_3). **(Appendix 37).** MS (ESI^+) m/z: 515. **(Appendix 57).**

3.3.18. 3,5-Dinitrobenzoyl Amoxicillin Derivative (Compound 18)

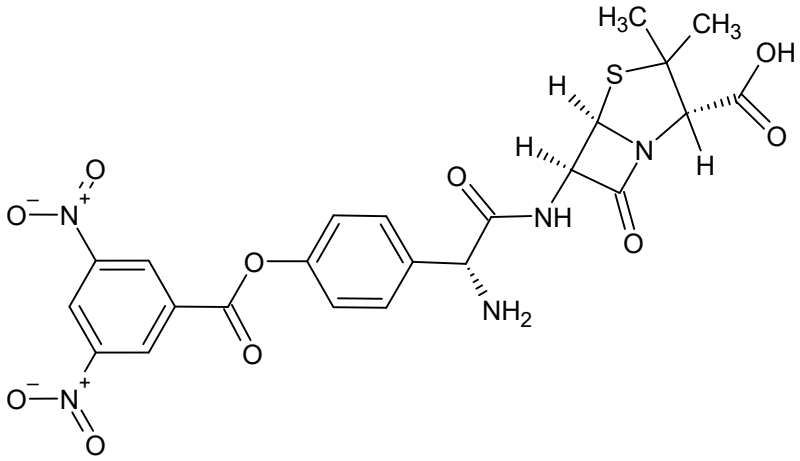


Figure 3.18.: Structure of 3,5-Dinitrobenzoyl Amoxicillin Derivative (Compound 18)

Nucleophilic acyl substitution of 3,5-dinitrobenzoyl chloride was conducted. Amoxicillin (1g, 2.73 $\times 10^{-3}$mol) was dissolved in 10 ml of tetrahydrofuran, and 2 ml of distilled water was added, stirred while being cooled at a temperature of 0-5 °C. Then, 2M potassium hydroxide solution was incrementally added, and the pH was adjusted to 6.8-7.2. Subsequently, 3,5-dinitrobenzoyl chloride. (1.34 g, 0.0058 moles, 2.13 eq) in 10 ml THF was added dropwise with continuous stirring, and the reaction mixture was maintained for two hours to yield **compound 18 (Figure 3.18)**. The product was then

extracted, collected, and recrystallized. The product's mass was 0.48 g (yield 29%), m.p =150-153 °C, R_f= 0.69 (hexane: acetone 2:1).

IR (v in cm^{-1}): 3447-3590 (NH_2), 1772 (C=O ester group), 1684 (C=O amide group), 1520 (N-O asymmetric stretch), 1376 (N-O symmetric stretch). **(Appendix 18).** ^1H NMR (500 MHz, D_2O) δ in ppm: δ 9.66 (s, 1H, COOH), (9.74-9.48) (m, 3H, aromatic), 8.6-8.61(amoxicillin aromatic), 2.22 (s, 2H, NH_2), 1.86 (s, 6H, $2CH_3$). **(Appendix 38).** MS (ESI^+) m/z: 560. **(Appendix 58).**

3.3.19. 4-Nitrobenzenesulfonyl Amoxicillin Derivative (Compound 19)

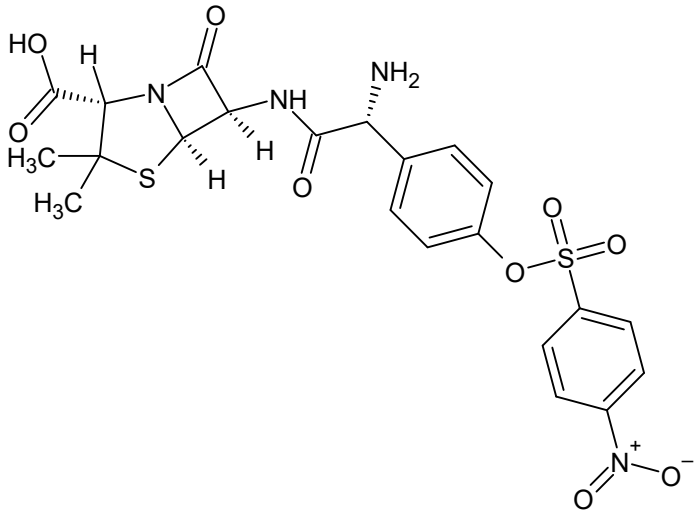


Figure 3.19.: Structure of 4-Nitrobenzenesulfonyl Amoxicillin Derivative (Compound 19)

Nucleophilic acyl substitution of 4-Nitrobenzenesulfonyl chloride was conducted. Amoxicillin (1g, 2.74 $\times 10^{-3}$mol) was dissolved in 10 ml of tetrahydrofuran, and 2 ml of distilled water was added, stirred while being cooled at a temperature of 0-5 °C, then 2M potassium hydroxide solution was incrementally added and the pH was adjusted to 6.8-7.2. Subsequently, 4-Nitrobenzenesulfonyl chloride (1.21 g, 0.0054 mol, 2 eq) in 10 mL THF was added dropwise with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 19 (Figure 3.19)**. The product was then filtered, collected, and recrystallized. The product's mass was 0.768 g (yield 67%), m.p = 222-225, R_f= 0.67 (hexane: acetone 2:1).

IR (v in cm^{-1}): 3449-3597(NH_2), 1639 (C=O amide group), 1519 (N-O asymmetric stretch), 1124 (S=O symmetric stretch). **(Appendix 19).** ^1H NMR (500 MHz, acetone) δ in ppm: δ 8.5 (s, 1H, COOH), (8.1-8.22) (m, 4H, aromatic), (7.0-7.52) (m, 4H, amoxicillin aromatic ring), 3.6 (s, 2H, NH_2), 1.29 (s, 6H, $2CH_3$). **(Appendix 39).** MS (ESI^+) m/z: 551.5. **(Appendix 59).**

3.3.20. 4-Toluenesulfonyl Amoxicillin Derivative (Compound 20)

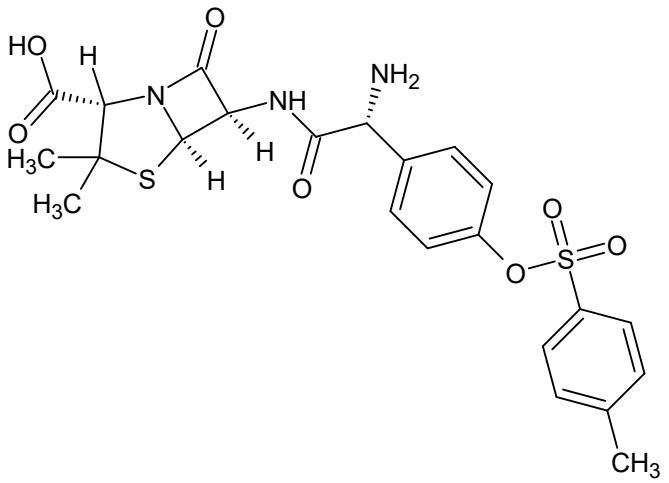


Figure 3.20.:Structure of 4-Toluenesulfonyl Amoxicillin Derivative (Compound 20)

Nucleophilic acyl substitution of 4-Nitrobenzenesulfonyl chloride was conducted. Amoxicillin (1g, 2.74×10^{-3}mol) was dissolved in 10 ml of tetrahydrofuran, and 2 ml of distilled water was added, stirred while being cooled at a temperature of 0-5 °C, then 2M potassium hydroxide solution was incrementally added, and the pH was adjusted to 6.8-7.2. Subsequently, 4-Toluenesulfonyl chloride (1.04 g, 0.0054 mol, 2 eq) in 10 mL THF was added dropwise with continuous stirring, and the reaction mixture was maintained overnight to yield **compound 20 (Figure 3.20)**. The product was then filtered, collected, and recrystallized. The product's mass was 0.33 g (yield 21.7%), m.p = 113-115 °C, R_f = 0.73 (hexane: acetone 2:1).

IR (v in cm^{-1}): 3368-3540 (NH_2), 1654 (C=O amide group), 1184 (S=O symmetric stretch). **(Appendix 20).** ^1H NMR (500 MHz, acetone) δ in ppm: δ 8.36 (s, 1H, COOH), (7.13-7.8) (m, 8H, aromatic), 5.6 (s, 1H, NH), 3.69 (s, 2H, NH_2), 2.55 (s, 3H, CH_3 in toluene), 1.3 (s, 6H, $2CH_3$). **(Appendix 40).** MS (ESI^+) m/z: 520.6. **(Appendix 60).**

3.4. Studying the hydrolysis of azithromycin and clarithromycin prodrugs on different pHs using the HPLC method.

3.4.1. Preparation of azithromycin and azithromycin stock solutions

A stock solution of azithromycin was made by dissolving 50 mg of azithromycin in 50 mL mobile phase (acetonitrile: phosphate buffer (pH 7.5) in 50:50 v/v ratio) using a 100 ml volumetric flask and the solution was sonicated for five minutes before adjusting the volume to the mark with mobile phase to achieve a concentration of 500 mg/L. The same procedure was followed to prepare the five azithromycin derivatives.

3.4.2. Preparation of prodrug solutions at different pHs

Five azithromycin prodrugs at a concentration of 500 ppm were obtained by dissolving 50 mg of each prodrug (1, 2, 3, 4, and 5) in 100 mL of solutions with pH values of 2.2, 5.5, and 7.4. The samples were incubated at 37 °C with continuous shaking for 24 hours. Subsequently, each sample was analyzed using HPLC to determine the retention time of the hydrolyzed azithromycin prodrugs.

3.4.3. Chromatographic conditions for azithromycin and azithromycin prodrugs solutions:

HPLC apparatus equipped with a C18 column (150 x 4.6 mm; 3.5 µm) with integrated UV detection at 215 nm. The mobile phase, comprising acetonitrile and phosphate buffer (pH 7.5) in a 50:50 v/v ratio, was freshly produced, filtered, and sonicated before use, and was given at a flow rate of 1.2 mL/min. The volume of each injection was 50 microliters. The column temperature was sustained at 40°C for the analysis and the runtime was around 9 minutes, corresponding to the retention time 7.33 minutes of Azithromycin. (Dewan et al., 2013).

3.4.4. Preparation of clarithromycin and clarithromycin prodrugs solutions:

A stock solution of clarithromycin was made by dissolving 40 mg of clarithromycin in 50 mL mobile phase using a 100 mL volumetric flask and the solution was sonicated for five minutes before adjusting the volume to the mark with mobile

phase to achieve a concentration of 400 mg/L. The same previous procedure was followed to prepare the five clarithromycin derivatives.

3.4.5. Preparation of clarithromycin prodrugs solutions at different pHs

Five clarithromycin prodrugs at a concentration of 400 ppm were obtained by dissolving 40 mg of each prodrug (1, 2, 3, 4, and 5) in 100 mL of solutions with pH values of 2.2, 5.5, and 7.4. The samples were incubated at 37 °C with continuous shaking for 24 hours. Subsequently, each sample was analyzed using HPLC to determine the retention time of the hydrolyzed clarithromycin prodrugs.

3.4.6. Chromatographic conditions for clarithromycin and clarithromycin prodrugs solutions

The chromatographic analysis was conducted using an isocratic separation mode with a C18 column (150 x 4.6 mm; 3.5 µm). The mobile phase included a homogeneous mixture of acetonitrile and potassium dihydrogen phosphate (0.035 M) at a 55:45 (v/v) ratio, adjusted to pH 4.4, and delivered at a flow rate of 0.5 ml/min. The column temperature was sustained at room temperature for the analysis. The effluent was measured at a wavelength of 210 nanometers. The injection volume was 20 µl, and the runtime was around 6 minutes, corresponding to the retention time 4.603 minutes of Clarithromycin. (Alam et al., 2017).

3.5. Minimum inhibitory concentration (MIC) assay and IC_{50} calculation

The minimum inhibitory concentration (MIC) of the compounds tested was determined using the broth microdilution method consistent with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Schuetz et al., 2025). Graphpad Prism version 8.0.2 (Graphpad Software, San Diego, CA, USA) was used to obtain the IC_{50} values. The data were input in an XY table where X was the log (concentration) and Y was the percentage of inhibition, with each concentration being tested in 5-6 replicates based on the compound. Dose-response curves (Nonlinear regression) were used to calculate IC_{50} values using the inhibitor vs. response (three parameters) model. Findings are given in the form of mean + S.D.

3.5.1 Preparation of prodrugs

A 5 mg/mL stock concentration of each sample was prepared using DMF. 0.05 grams (g) of each sample were weighed by using an electric balance, then 10 milliliters of DMF were added to make 5 milligrams/ milliliter (mg/mL) (Krajačić et al., 2007).

3.5.2. Bacterial Strains and Growth Conditions

With bacterial isolates, they were cultured on Mueller-Hinton agar (MHA) and incubated at 37 ºC 18-24 h. The fresh colony was then suspended in sterile saline and taken to a 0.5 McFarland standard (approximately 1.5 x 10^8 CFU/mL).

3.5.3. Preparation of Serial Dilutions

Serial dilutions were done 1:2 using Mueller-Hinton broth (MHB) to a final working concentration of 64 to 0.5 µg/mL with macrolides and 512 to 1 µg/mL with penicillin (**Figure 3.21**).

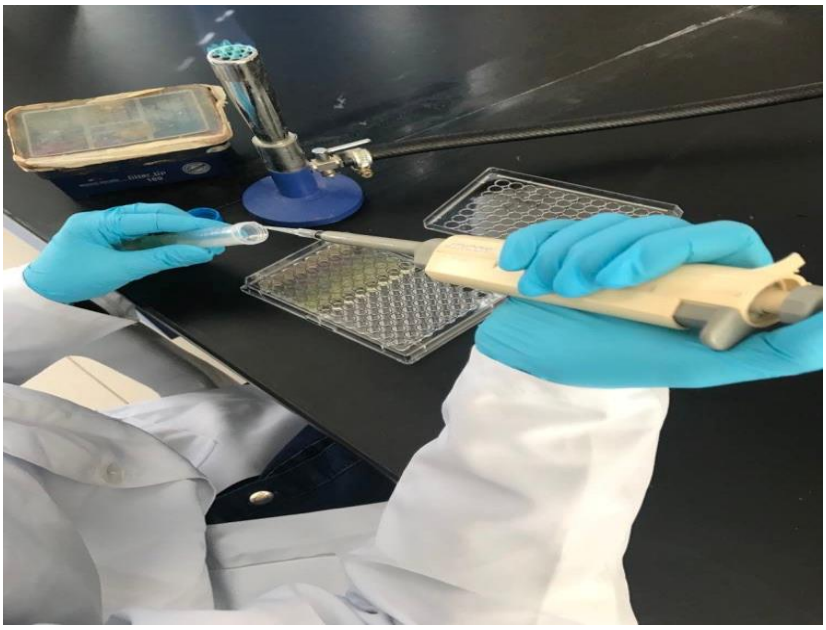


Figure 3.21.: Minimum inhibitory concentration (MIC) assay

3.5.4. Inoculation and Controls

An equal volume of the standardized bacterial suspension (usually 100 µL) was added to each well, resulting in a final bacterial concentration of approximately 5×10^5 CFU/mL.

The following controls were included:

Positive control: wells in which bacterial suspension was added but without the compound tested.

Negative control: wells with only MHB lacking bacteria.

3.5.5. Incubation

The microtiter plates were incubated at 37 °C for 18–24 h.

3.5.6. Reading of Results

At the end of the incubation period, it was observed whether the bacteria grew. Optical density was detected by measuring absorbance at 630 nm. MIC was determined

as the lowest concentration of the substance, in which no visible growth was evidenced relative to the positive control.

3.6. In silico study of penicillin prodrugs

All the chemical structures were sketched with ACD/ChemSketch (Advanced Chemistry Development, Inc.) (www.acdlabs.com). Molecular docking simulations were carried out by utilizing Autodock 4.2 (The Scripps Research Institute, San Diego, CA, USA) and AutoDock Vina 1.2.0 (The Scripps Research Institute, La Jolla, CA, USA). The results were then visualized and interpreted using Biovia Discovery Studio Visualizer (Dassault Systems) software (http://accelrys.com).

3.6.1 Validation and Reliability Experimental validation

Although computational models can be assessed using statistical and in silico validation methods, the biological relevance of their predictions must ultimately be confirmed experimentally. Molecular docking is an effective screening and hypothesis-generating methodology that should not substitute experimentation. The docking procedure may be improved by cross-validation of the protocol with known active and inactive compounds against the target protein, where such data is accessible in the literature (Shoichet, 2004). Before performing the molecular docking experiments on the ten compounds, a cross-validation procedure was undertaken to provide assurance of the reliability and accuracy of the computational methodology on the PBP2x protein target. A curated collection of known active and inactive compounds against PBP2x was compiled based on the literature to be used as a validation dataset. Docking studies of these reference compounds were cross-validated by docking these reference compounds to two independent crystal structures of PBP2x PDB IDs: 5OIZ (Bernardo-García et al., 2018) and 5OJ1 (Bernardo-García et al., 2018) using the same docking parameters. Experimental binding free energies (ΔG_{exp}) derived from IC_{50} values were correlated with the predicted binding affinities (ΔG_{vina}) from AutoDock Vina for each PBP2x structure, and Pearson correlation coefficients (r, R^2) were calculated to assess the predictive capability of each target structure.. It was found that the crystal structure 5OJ1 of PBP2x gave the best correlation between predictions and experimental binding affinities (R^2 = [0.5] and Pearson correlation coefficient (r): ≈ 0.696) (**Figure 3.22**), showing better predictive power than any other PBP2x structure examined.

In accordance with these validation results, the PBP2x structure 5OJ1 was chosen as the best target in all further molecular docking calculations with the new compounds. This stringent testing methodology helped to ensure that the selected docking protocol and PBP2x target construct could reliably be used to differentiate between active and inactive compounds and hence gives confidence in the predictions made on the compounds of interest.

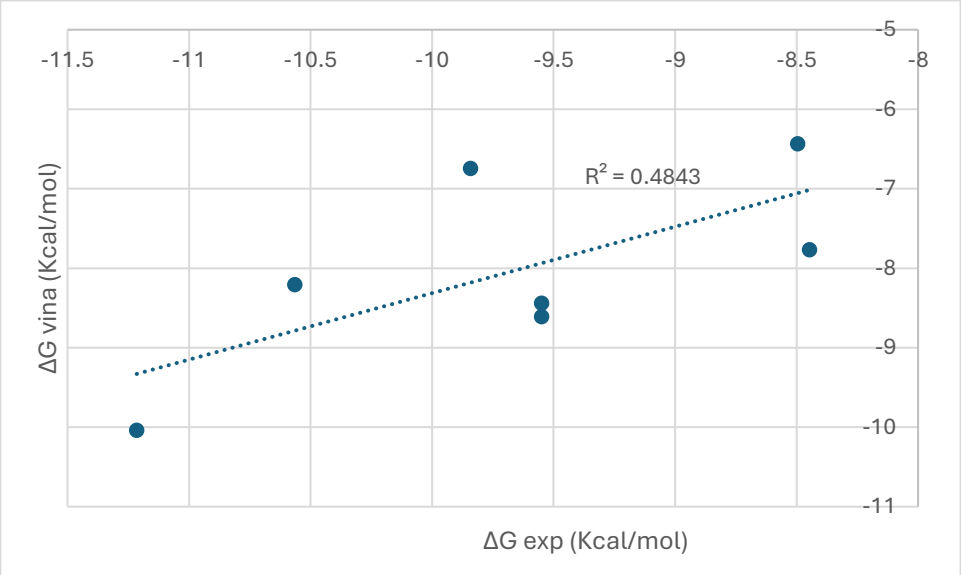


Figure 3.22.:Correlation Between Experimental ΔG and Predicted Docking Scores

3.6.2. Ligand Preparation and Molecular Docking

The simulations were conducted using the crystal structure of PBP1 complexed with penicillin G (PDB ID: 7O4B), *Staphylococcus aureus*, PBP3 complexed with temocillin (PDB ID: 6UN1), *Pseudomonas aeruginosa*, PBP3 complexed with Piperacillin (PDB ID: 6I1I), *Escherichia coli*, PBP2x complexed with oxacillin (PBP ID: 5OJ1), *Streptococcus pneumoniae* (**Table 3.1**). The X-ray crystallographic structures were obtained from the Protein Data Bank (**Figures 3.23-3.26**). The co-crystallized ligands indicated that the intermolecular interactions related to chains A, while the other chains were excluded from the crystal structure before docking. All synthesized compounds were first sketched with ACD/ChemSketch. The chemical structures produced were saved in a MOL file and subsequently converted to SDF format. These ligand files, along with the PDB file of the protein, were then prepared for docking simulations by using AutoDock Tools (version 4.2) and Autodock Vina (version 1.2). The protein structures and cocrystal structures were processed by adding polar hydrogen atoms and assigning Kollman charges for protein, Gasteiger charges for ligands to better representation (Gasteiger & Marsili, 1980). Finally, each prepared ligand molecule was

converted to the PDBQT file format utilizing AutoDock Tools, which was created specifically to prepare ligands to run AutoDock Vina calculations. The compounds were screened to identify the optimal docking candidate that binds comparably to cocrystal molecules. A grid box was employed to define the region of the protein structure for mapping purposes. The box dimensions were established at 20 Å in each axis (x, y, and z), with 100 simulations conducted per run. Upon completing the docking simulation, the resultant data regarding the docked coordinates and binding free energy in the docking out.pdbqt files were analyzed using Discovery Studio. The 10 compounds were sorted based on their optimal (highest negative) binding scores to determine the most promising candidates for further examination. The 2D intermolecular interactions between the four proteins and each chemical compound were studied based on binding energy and amino acid interactions.

Table 3.1: Selected PBPs crystal structures from the Protein Data Bank

	Penicillin Binding Protein	PDB Code	Resolution	Co-crystal ligand	IC50 (µg/mL)
1	Penicillin-Binding Protein 1	7O4B	2.59 Å	penicillin G	1
2	Penicillin-Binding Protein 3	6UN1	2.26 Å	Temocillin	128-256
3	Penicillin-Binding Protein 3	6I1I	1.75 Å	Piperacillin	0.01
4	Penicillin Binding Protein 2x	5OJ1	2.85 Å	Oxacillin	0.08

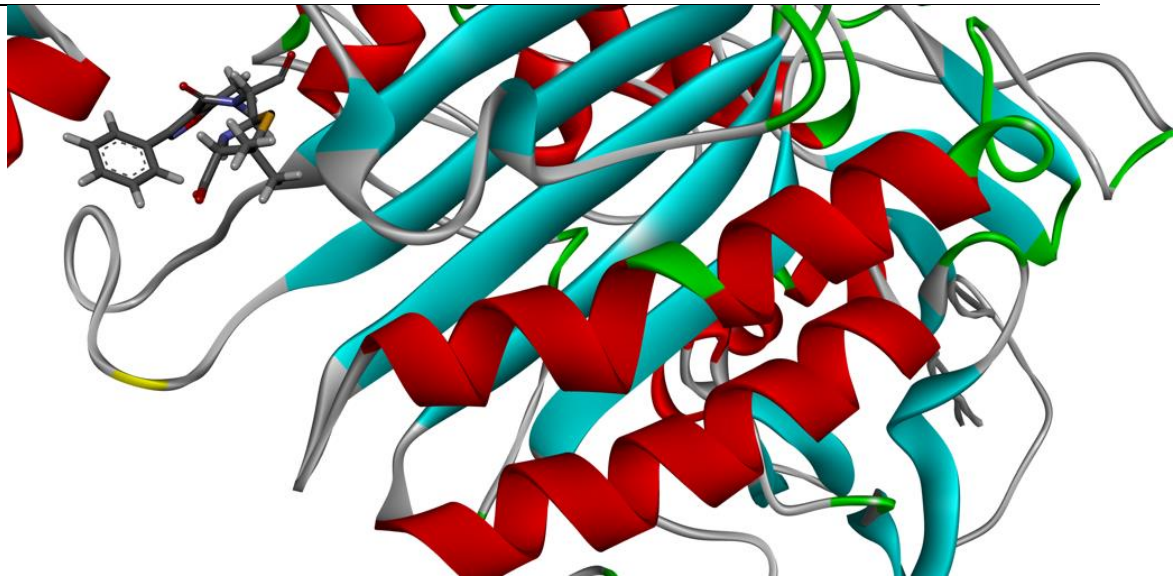


Figure 3.23.: solid ribbon representation of PBP2x crystal structure co-crystalised with Oxacillin (PDB ID: 5OJ1)

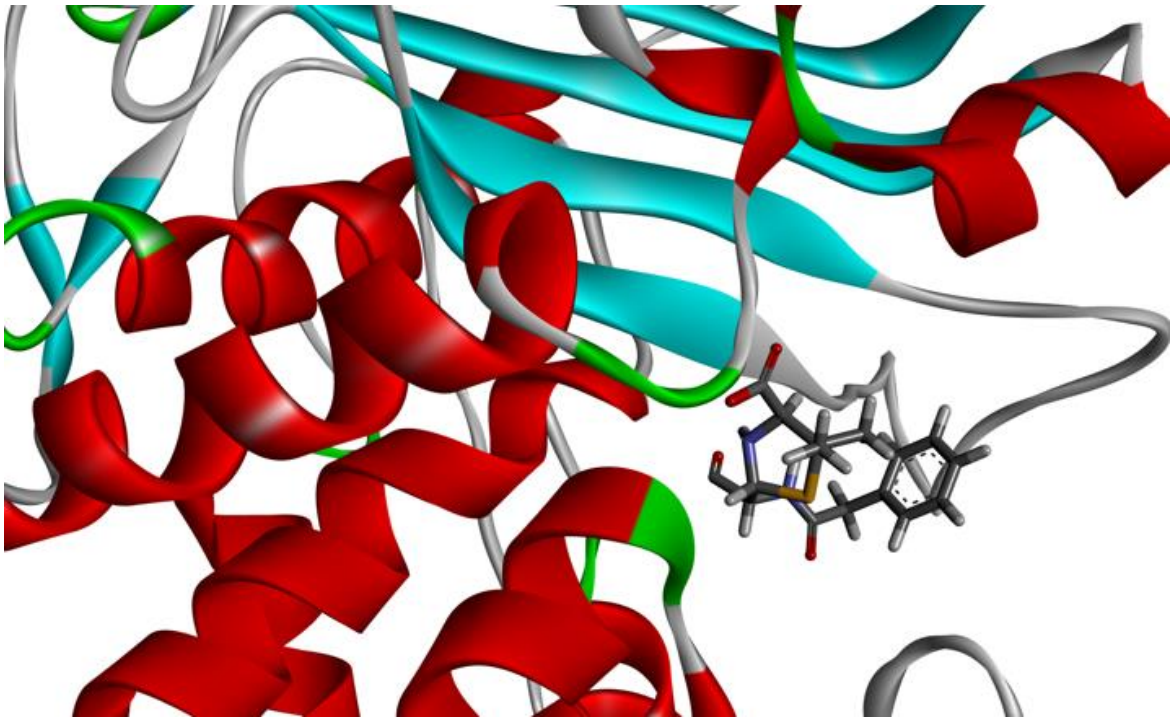


Figure 3.24.: solid ribbon representation of PBP1 crystal structure co-crystalised with penicillin G (PDB ID: 7O4B)

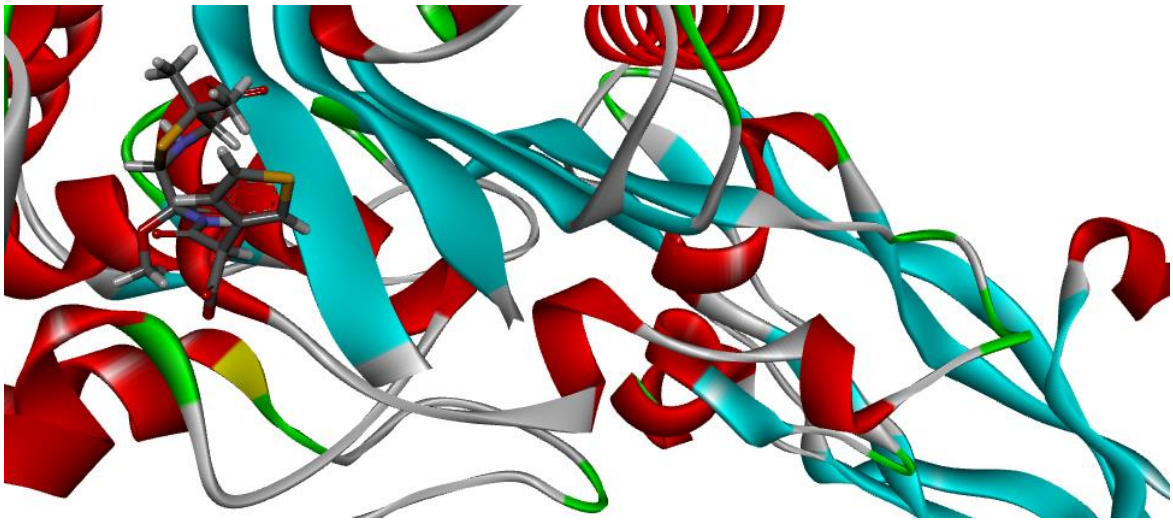


Figure 3.25.: solid ribbon representation of PBP3 crystal structure co-crystalised with Temocillin (PDB ID: 6UN1)

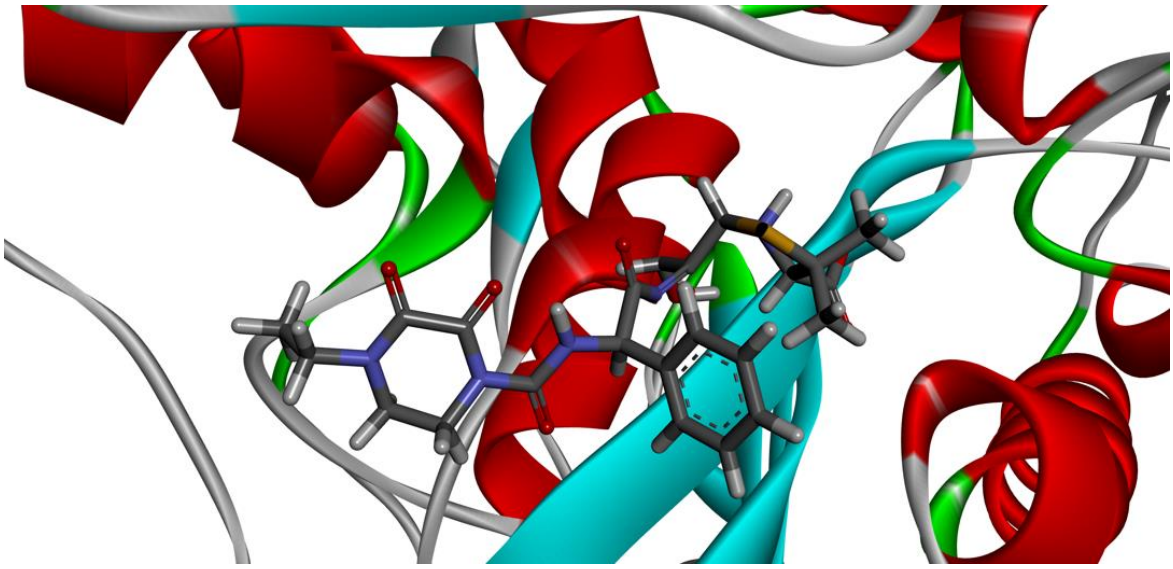


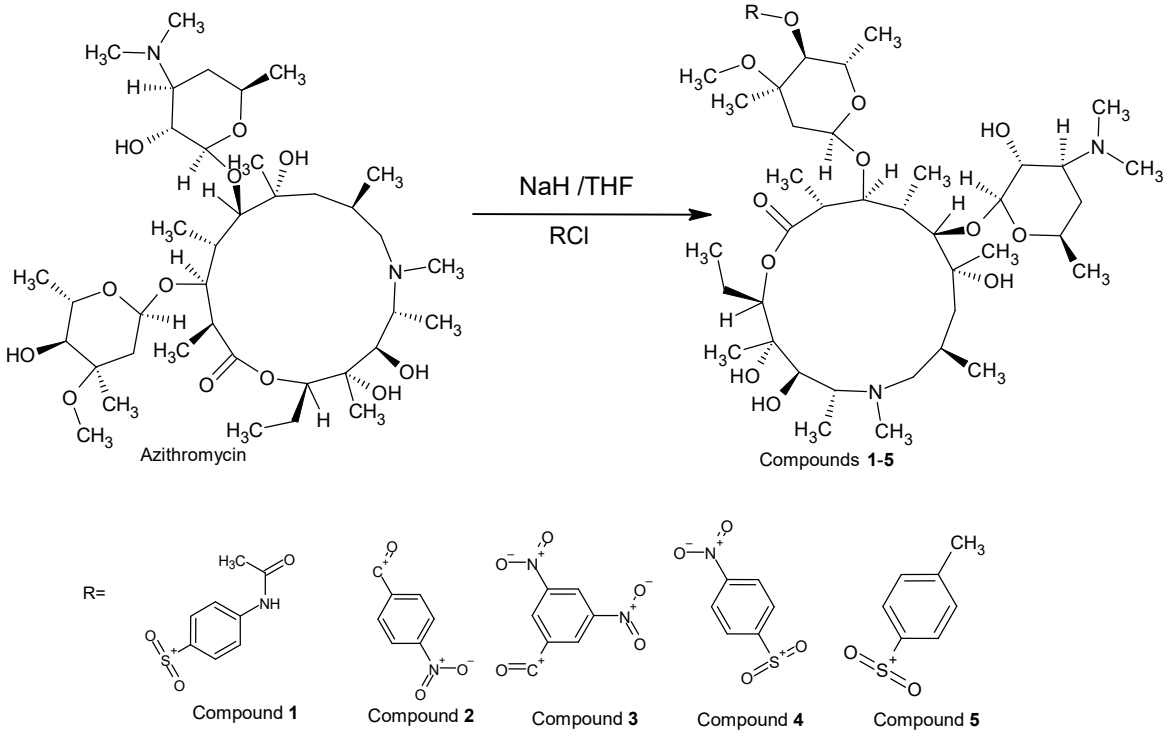
Figure 3.26.: solid ribbon representation of PBP3 crystal structure co-crystalised with Piperacillin (PDB ID: 6I1I)

Chapter 4: Results

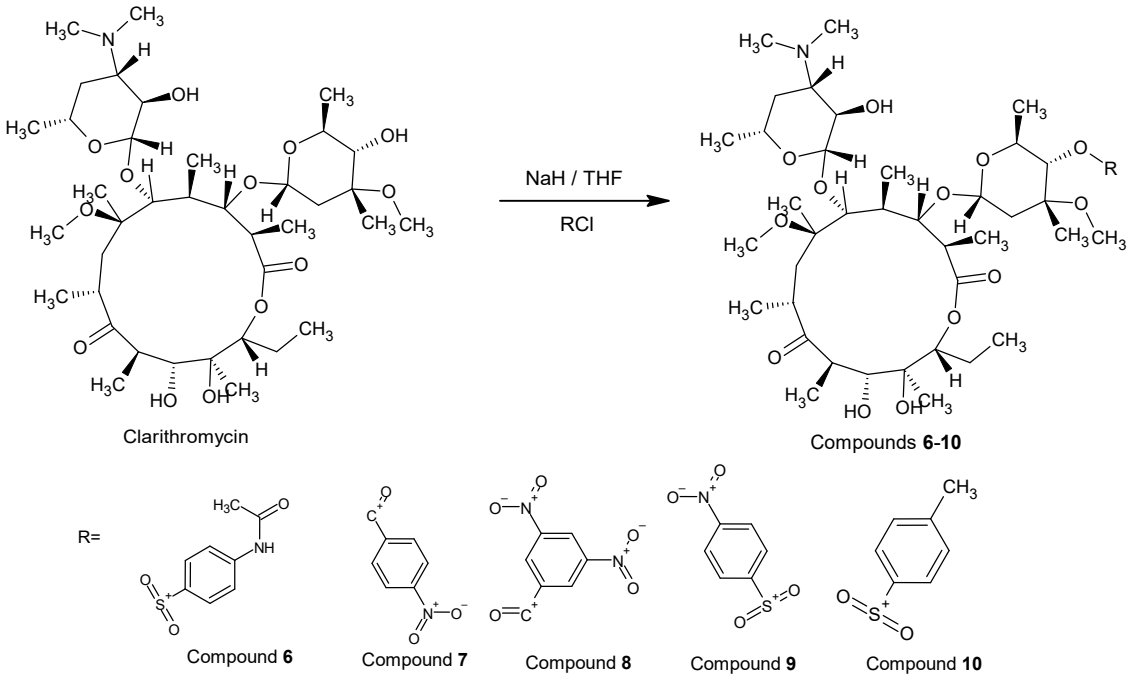
4.1. Chemical Synthesis

4.1.1. Synthesis of Azithromycin and Clarithromycin Prodrugs

Production of the prodrugs occurred in two steps in one pot. The macrolide antibiotic (azithromycin or clarithromycin) was dissolved in an appropriate anhydrous solvent, e.g., tetrahydrofuran (THF), and placed in an ice bath. Strong base (NaH) was added to the solution in portions. The mixture was stirred until no further evolution of hydrogen gas took place, which signals the full deprotonation of the hydroxyl groups on the macrolide and the generation of the corresponding alkoxide anions. The corresponding benzoyl chloride or sulfonyl chloride was added dropwise to the reaction mixture. The reaction was left to run for a few hours at room temperature, and its progress was followed by thin-layer chromatography (TLC). The reaction was quenched when complete, and the crude product was recrystallized (**Scheme 4.1,4.2**).



Scheme 4.1.: Synthesis of azithromycin derivatives



Scheme 4.2.: Synthesis of clarithromycin derivatives

4.1.2. Reaction Mechanism

The reaction goes through a nucleophilic acyl substitution reaction. The initial stage is the deprotonation of the hydroxyl groups of the macrolide by sodium hydride to produce highly nucleophilic alkoxide anions and release hydrogen gas. These anions of alkoxide then become the nucleophiles and strike the electrophilic carbon atom in the carbonyl group of the benzoyl chloride or the sulfur of the sulfonyl group of the sulfonyl chloride. This is replaced by the removal of the chloride ion as a leaving group to produce an ester or sulfonate ester bond, respectively.

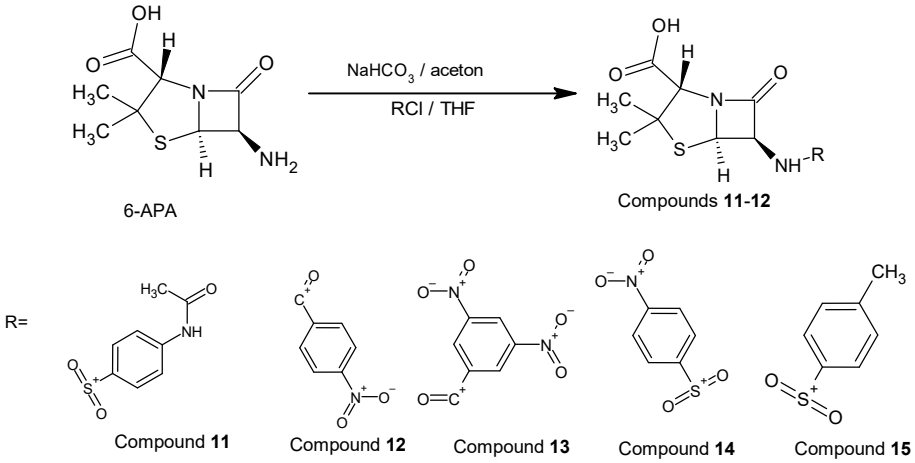
4.1.3. Characterization of Azithromycine and Clarithromycin Prodrugs

The structures of the synthesized prodrugs were determined by different spectroscopic methods, such as Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS). The main characteristic of IR Spectroscopy is: C=O bonds around 1735-1750 $cm^{-1,}$ ester bonds (benzoyl esters), S=O stretch, 1150-1360 cm^{-1} (asymmetric and symmetric S=O stretch), sulfonate esters. The aromatic C=C at 1500-1600 cm^{-1} of benzoyl and sulfonyl groups. Nitro group vibrations at 1520-1550 a (asymmetric NO_2 stretch) and 1340-1380 a (symmetric NO_2 stretch) of nitro-substituted derivatives. ^{1}H NMR Characteristic Signals: Aromatic protons: δ 7.2-8.2 ppm, Ester (sulfonyl)-linked protons: δ 4.5-6.0 ppm (proton on carbon near the ester group in lacton ring), Macrolide backbone: δ 0.8-4.5 ppm (complex

multiplets for azithromycin/clarithromycin structure). Mass Spectrometry: Molecular ion peaks: $[M+H]^+$ corresponding to derivative molecular weight.

4.1.4. Synthesis of 6-Amino Pencillinic Acid Derivatives

Sodium bicarbonate was placed in distilled water and stirred until dissolved, followed by the addition of the 6-APA, which dissolved in this solution, which then created a buffered aqueous solution pH (~8-9)which increased the solubility of 6-APA and prevented extreme pH changes that could degrade the β-lactam ring. Acetone was then added to give optimum conditions to the acylation reaction. Appropriate benzoyl chloride or sulfonyl chloride was dissolved in anhydrous tetrahydrofuran (THF) and was deposited drop-by-drop into the stirring solution of 6-APA. The benzoyl chloride reaction was carried out at room temperature for two hours, and the sulfonyl chloride reaction needed overnight stirring (12-16 hours) because they were less reactive. Triethylamine was also included in the case of sulfonyl chloride reaction as a base catalyst to activate the reaction and neutralize the hydrochloric acid product formed (**Scheme 4.3**). The reaction mixture was worked up on completion, and the crude product was purified using the recrystallization techniques to obtain the desired 6-APA derivatives.



Scheme 4.3.: Synthesis of 6-APA derivatives

4.1.5. Reaction Mechanism

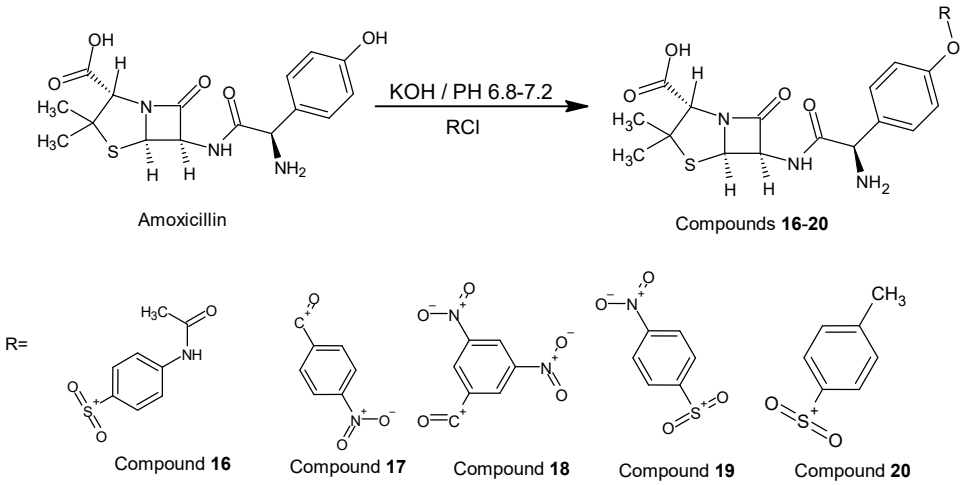
The reaction takes place through a nucleophilic acyl substitution mechanism. The amino group of 6-APA is a nucleophile, which reacts with the electrophilic carbon on a benzoyl chloride or sulfur center on a sulfonyl chloride. The chloride is used as a leaving group and removed to give the amide bond (benzoyl derivatives) or sulfonamide bond (sulfonyl derivatives).

4.1.6. Characterization of 6-Aminopenicillinic Acid Derivatives

The 6-APA prodrugs produced were profiled by several methods of analysis: Infrared (IR) Spectroscopy: Amide C=O: 1650-1680 cm-1 (in benzoyl derivatives), The aromatic C=C at 1500-1600 cm^{-1} of benzoyl and sulfonyl groups. Sulfonamide S=O: 1150-1360 cm^{-1} (asymmetric and symmetrical), N-H: 3200-3400 cm^{-1}, Nitro groups (where present): 1520-1550 cm^{-1} (asymmetric NO_2 stretch) and 1340-1380 cm^{-1} (symmetric NO_2 stretch). ^1H NMR Spectroscopy: Aromatic protons: 7.2-8.2 ppm (benzol derivatives), Amide NH: 6.0-7.0 ppm. Mass Spectrometry: Molecular ion peaks: $[M+H]^+$ corresponding to prodrug molecular weight.

4.1.7. Synthesis of Amoxicillin Derivatives

The reaction begins by dissolving amoxicillin in a mixed solution of tetrahydrofuran (THF) and deionized water, which renders amoxicillin soluble. This mixture is then cooled to 0-5 ^oC to prevent thermal breakdown of the heat-sensitive beta-lactam ring. The base 2M potassium hydroxide (KOH) is added in small portions. The PH is balanced at the best point of 6.8-7.2 so that the nucleophile could be basic enough and the beta-lactam core structure is not lost (**Scheme 4.4**). Acyl chloride reagent (either benzoyl chloride or sulfonyl chloride) is added in drop amounts into the reaction mixture in THF. The acyl chloride in solution reacts, benzoyl chloride reactions require 2 hours and sulfonyl chloride reactions are left overnight to ensure that the reaction is fully converted at room temperature.



Scheme 4.4: Synthesis of amoxicillin derivatives

4.1.8. Reaction Mechanism

These reactions of amoxicillin with sulfonyl and benzoyl chlorides proceed by the same general mechanism: nucleophilic substitution of an electrophilic center by the phenolic hydroxyl group of amoxicillin. The phenolic hydroxyl group is deprotonated to

a strong nucleophile (Ar-O-) rather than a weak nucleophile (Ar-OH), The acyl and sulfonyl chloride (E -Cl) is added, E is either R-SO - (sulfonyl) or R-CO - (benzoyl), The activated phenoxide anion approaches the electrophilic center - sulfur of sulfonyl chlorides, and the electrophilic center - carbon of benzoyl chlorides. The removal of chloride leads to the formation of the stable ester product (Ar-O-E), and the HCl is free and neutralized by the base to produce salt and water.

4.1.9. Characterization of Amoxicillin Derivatives

To ensure the effective synthesis of the amoxicillin derivatives and to determine their structures, various techniques of analysis were employed. The disappearance of the phenolic O-H stretching vibration is an obvious indication that the reaction took place at the phenolic hydroxyl group and not the amino group. Strong absorption bands associated with the symmetric and asymmetric stretching vibrations of the S=O bonds in the sulfonate ester group (usually in the ranges of 1150-1360 cm^{-1}) are likely to be observed in the FT-IR spectrum. Nitro groups (present where found): 1520-1550 cm-1 a (asymmetric NO_2 stretch) and 1340-1380 cm^{-1} a (symmetric NO_2 stretch). A new, effective absorption band of the ester carbonyl (C=O) stretching vibration (in benzoyl derivatives) should appear in the spectrum, usually at 1735-1750 cm^{-1}. The disappearance of the phenolic O-H proton signal (usually between 4.5-7 ppm with phenols) in the 1H NMR spectrum of the products will confirm that the reaction did not occur at the nitrogen center. The amino group protons (1-5) ppm will not disappear, which is an indication that the reaction took place at the nitrogen center. Mass Spectrometry: Molecular ion peaks: $[M+H]^+$ corresponding to derivative molecular weight.

4.2. Pro drugs Hydrolysis study

This chapter shows the experimental results of the study of pH-dependent hydrolysis of ten azithromycin and clarithromycin prodrugs. The findings are systematized to give a close examination of prodrug stability and degradation pattern at various pH conditions. The hydrolysis process was followed using a high-performance liquid chromatography (HPLC) to determine degraded products in 24 hours.

Table 4.1: Retention times of prepared prodrugs: Present the results of each prodrug tested alone (without pH adjustment). (Appendix 61-72).

Compounds	Retention times (minutes)
Azithromycin	7.33
Clarithromycin	4.603
Compound 1	3.662
Compound 2	3.797
Compound 3	3.801
Compound 4	1.265
Compound 5	2.934
Compound 6	2.7
Compound 7	3.341
Compound 8	3.056
Compound 9	2.957
Compound 10	2.851

4.2.1. Hydrolysis at pH 2.2 (Gastric Conditions)

Group I: Prodrugs with a Conversion to Parent Azithromycin.

Compounds: 2, 4, and **5.** The most promising hydrolysis characteristics were observed with **compounds 2, 4,** and **5,** which were partially converted to the starting azithromycin structure. These prodrugs were subjected to regulated hydrolysis, which led to the release of the parent antibiotic in confirmation of successful prodrug design in taste masking without the loss of therapeutic value (**Figures 4.1, 4.2, and 4.3**).

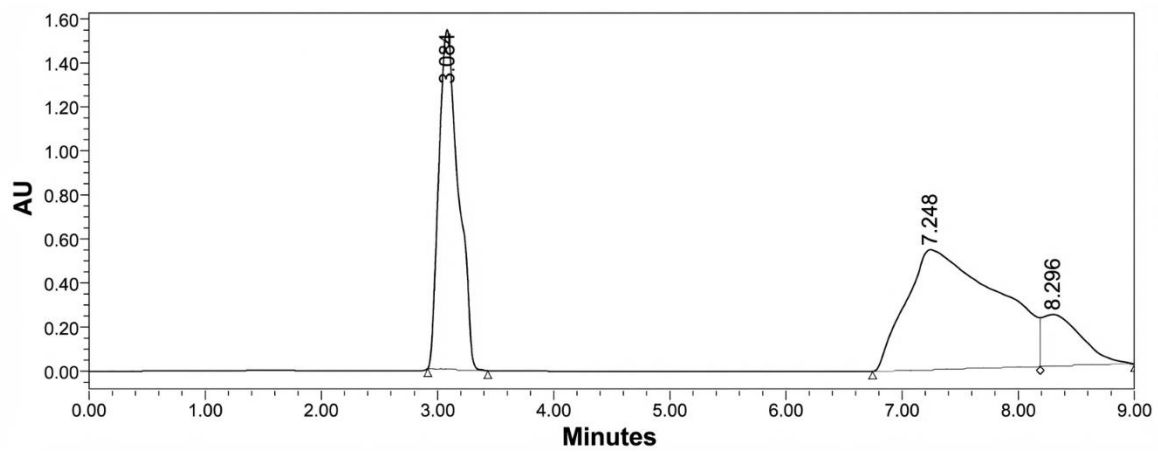


Figure 4.1.: Representative HPLC Chromatograms for compound 2

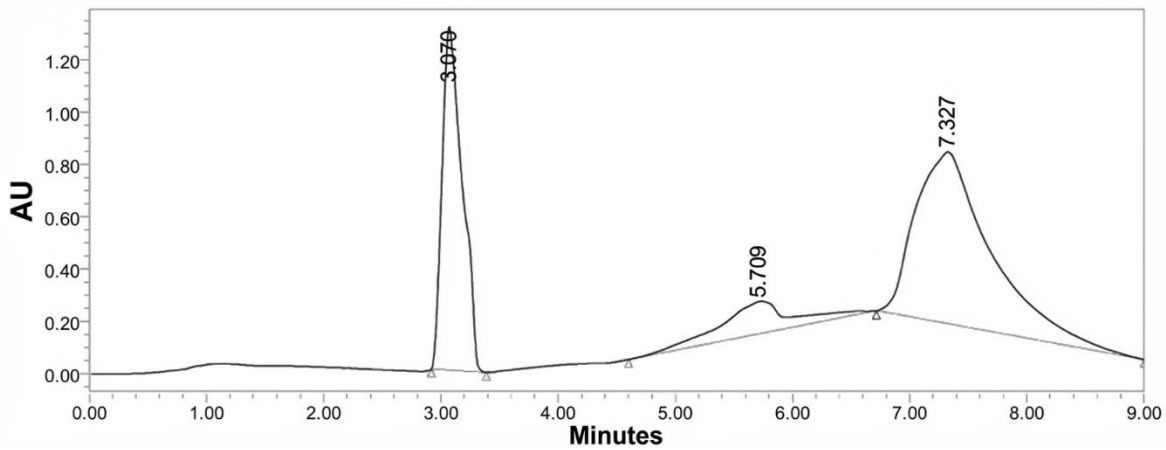


Figure 4.2. : Representative HPLC Chromatograms for compound 4

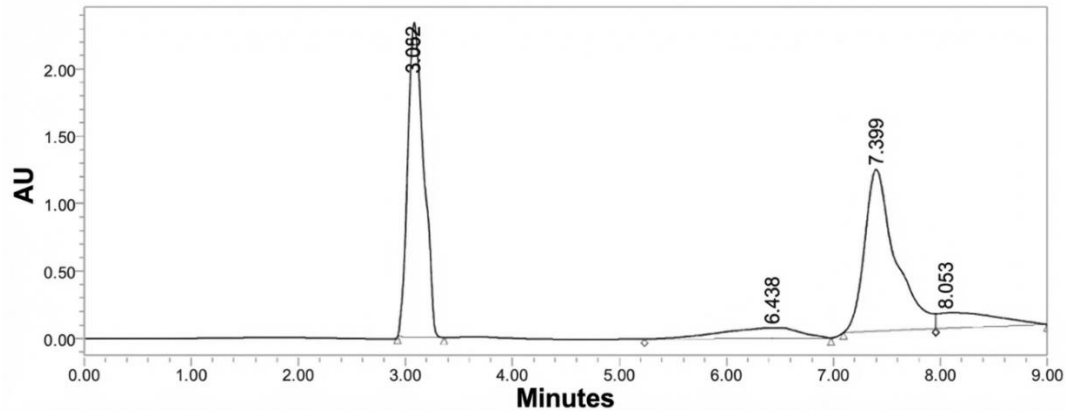


Figure 4.3.: Representative HPLC Chromatograms for compound 5

Group II: Prodrugs Exhibiting a partial or full dissociation to Non-Parent Products.

Compounds: 1, 3, 7, 8, 9, and **10.** Most of the prodrugs (**compounds 1, 3, 7, 8, 9,** and **10**) tested displayed an alternative hydrolysis profile due to partial or full

dissociation, leading to the non-formation of the original azithromycin molecule. Rather, there were different retention times in degradation products formed by these compounds, which pointed to other degradation pathways **(appendix 73, 74, 76 and 79).**

Group III: Prodrug Resistant to Hydrolysis.

Compound 6 was demonstrated to be extremely stable at pH 2.2, with no hydrolysis after 24 hours of incubation. This degradation resistance shows that the chemical bond in this prodrug is excessively stable to be useful as a drug **(appendix 75).**

4.2.2 Hydrolysis at pH 5.5 (Duodenal Transition Zone)

Group I: Prodrug with Successful Parent Azithromycin Conversion.

Compound 5 exhibited the most preferable hydrolysis, and the subsequent conversion to the original azithromycin molecule was successful (**Figure 4.4**). This prodrug was subjected to controlled hydrolysis, which led to the recovery of the parent antibiotic, indicating the successful prodrug design to mask the taste and also remain therapeutically active.

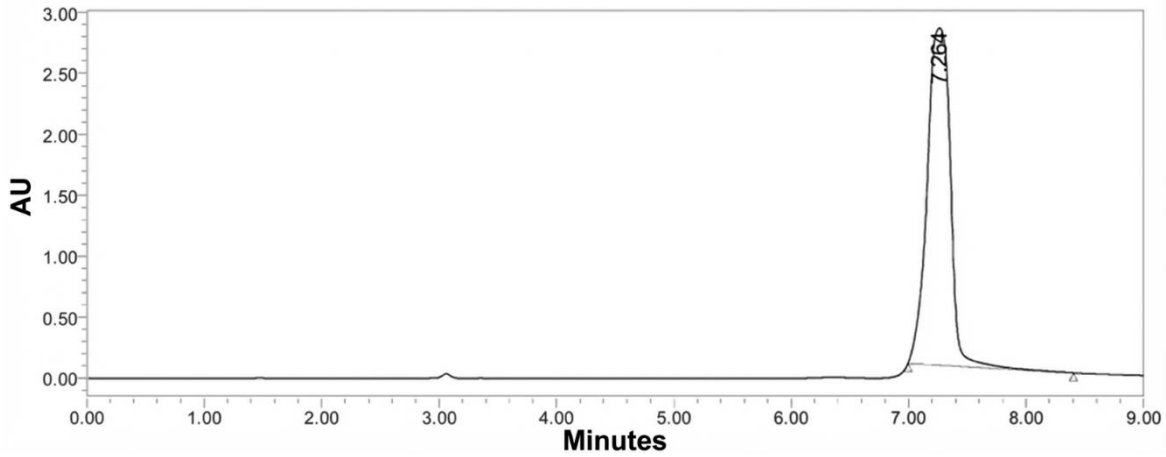


Figure 4.4.: HPLC Chromatograms for Compound 5 at pH 5.5

Group II: Prodrugs with full and Partially Hydrolyzed Group II features without giving the parent compound

Prodrugs that do not form parent drugs. Compounds: 1, 2, 3, 4, 8, 9 and 10. Most prodrugs which were tested (**compounds 1, 2, 3, 4, 8** and **9**) displayed partial or full hydrolysis, especially at and below pH 5.5 **(appendix 80 - 83 and appendix 86 – 87)**, and did not restore the original azithromycin molecule. Rather, the compounds produced

other degradation products with different retention times, which are unwanted degradation pathways.

Group III: Hydrolysis-resistant Prodrugs.

Compounds 6 and **7.** The clarithromycine derivatives **6** and **7** were found to be highly stable at pH 5.5, with insignificant hydrolysis observed after 24 hours of incubation. This is because such resistance to degradation shows that the chemical linkages of these prodrugs are too resistant to be used practically in pharmacy **(appendix 84 and 85).**

4.2.3 Hydrolysis Behavior at pH 7.4 (Physiological/Blood pH)

pH 7.4 corresponds to the physiological conditions of pH in the blood. Dramatic shifts in the behavior of hydrolysis were experienced at this pH, and previously resistant compounds started exhibiting considerable degradation.

Group I: Prodrug with Successful Parent Azithromycin Conversion.

The **compound 5** reached maximum conversion to azithromycin at pH 7.4, which proves it to be an effective prodrug (**Figure 4.5**). the complete hydrolysis percentage of group I compounds is presented in **Table 4.2**.

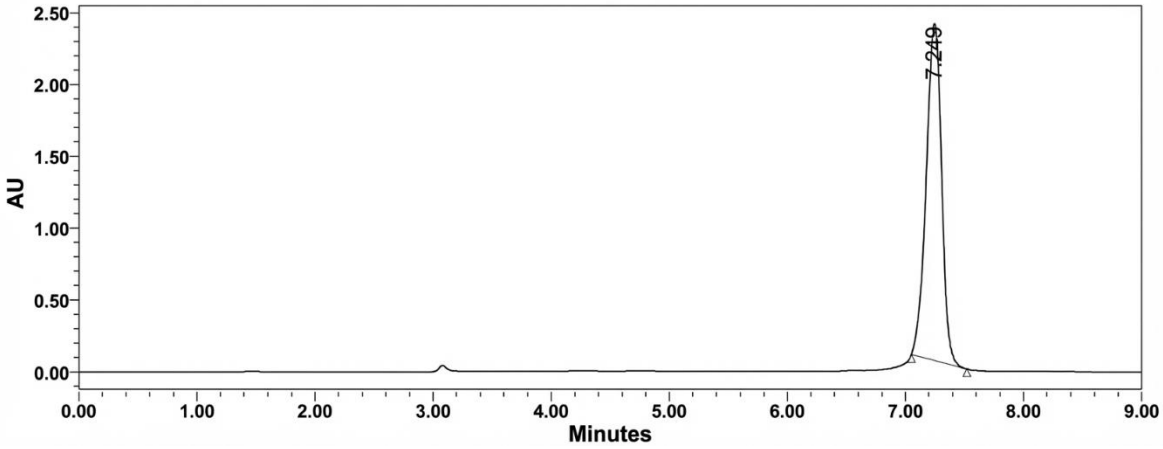


Figure 4.5.: HPLC Chromatograms for Compound 5 at pH 7.4

Group II: Prodrugs with Extensive Hydrolysis Without Parent Drug Formation

Compounds: 1, 2, 3, 4, 6, 7, 8, 9, 10. Nine products exhibit high degradation but no azithromycin or clarithromycin formation **(appendix 89- 92 and appendix 93-97).**

Table 4.2.: Complete Hydrolysis Results at pH 2.2, 5.5, and 7.4 for group I compounds

Compounds	pH	Remaining Prodrug (%)	Azithromycin Formed (%)	Other Products (%)
2	2.2	0	55.49	44.51
4	2.2	0	58.29	41.71
5	2.2	42.92	42.31	14.77
5	5.5	0	100	0
5	7.4	0	100	0

4.3. Biological Activity of Synthesized Prodrugs

The antimicrobial effectiveness of the compounds synthesized was measured by the Minimum Inhibitory Concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) values. The MIC is described as the minimal concentration of an antimicrobial agent that inhibits the visual growth of a microorganism when it is incubated overnight (Terwee et al., 2021). IC_{50} is a measure of the concentration of a drug needed to inhibit a biological process in vitro by 50 percent (Sebaugh, 2011). The two parameters are used to quantitatively determine the strength of the compounds being tested and can be used to compare the antimicrobial activity of the compounds. The antimicrobial activity of the 20 synthesized compounds was determined *in vitro* against a panel of four bacterial strains, two Gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria (**Tables 4.3-4.5**). The minimum inhibitory concentrations and IC_{50} values of these compounds are represented in **Tables 4.3-4.5**.

Table 4.3.: Minimum inhibitory concentrations of azithromycin and clarithromycin prodrugs against four test bacteria

Compound	*Escherichia coli* (MIC, μg/mL)	*Pseudomonas aeruginosa* (MIC, μg/mL)	*Streptococcus pneumoniae* (MIC, μg/mL)	*Staphylococcus aureus* (MIC, μg/mL)
Compound 1	4	8	4	4
Compound 2	4	4	4	4
Compound 3	4	8	4	4
Compound 4	4	8	8	8
Compound 5	4	8	8	8
Compound 6	4	4	4	4
Compound 7	8	4	4	4
Compound 8	4	8	0.5	4
Compound 9	4	8	8	8
Compound 10	4	8	8	8
Azithromycin	1	>16	2	1
Clarithromycin	4	>16	8	1

Table 4.4: Minimum inhibitory concentrations of amoxicillin and 6-APA derivatives against the four test bacteria.

Compound	*Escherichia coli* (MIC, μg/mL)	*Pseudomonas aeruginosa* (MIC, μg/mL)	*Streptococcus pneumoniae* (MIC, μg/mL)	*Staphylococcus aureus* (MIC, μg/mL)
Compound 11	32	32	64	64
Compound 12	32	2	32	1
Compound 13	32	32	64	64
Compound 14	128	32	64	128
Compound 15	32	32	64	128
Compound 16	32	16	64	64
Compound 17	32	2	32	32
Compound 18	32	32	32	128
Compound 19	32	32	64	128
Compound 20	32	32	64	128
Amoxicillin	16	>128	32	4

Table 4.5.: IC_{50} values of macrolide prodrugs against test bacteria. The values are reported as mean ± standard deviation. All dose-response curves had R^2 values greater than 0.95.

Compound	*Escherichia coli* (IC_{50}, µg/mL)	*Pseudomonas aeruginosa* (IC_{50}, µg/mL)	*Streptococcus pneumoniae* (IC_{50}, µg/mL)	*Staphylococcus aureus* (IC_{50}, µg/mL)
Compound 1	3.42 ± 0.18	1.65 ± 0.31	1.80 ± 0.25	15.66 ± 0.44
Compound 2	1.71 ± 0.10	0.81 ± 0.11	1.20 ± 0.21	6.11 ± 0.57
Compound 3	18.97 ± 0.36	2.79 ± 0.16	0.60 ± 0.30	1.25 ± 0.24
Compound 4	0.56 ± 0.29	0.10 ± 0.19	2.85 ± 0.21	61.56 ± 0.45
Compound 5	1.63 ± 0.13	11.71 ± 0.25	1.80 ± 0.37	9.83 ± 0.36
Compound 6	3.79 ± 0.22	0.38 ± 0.31	1.30 ± 0.08	15.16 ± 0.39
Compound 7	5.99 ± 0.22	0.93 ± 0.16	1.28 ± 0.07	21.22 ± 0.47
Compound 8	2.34 ± 0.29	0.32 ± 0.22	0.27 ± 0.28	0.15 ± 0.37
Compound 9	2.45 ± 0.16	0.23 ± 0.09	2.11 ± 0.15	11.46 ± 0.26
Compound 10	2.24 ± 0.13	0.11 ± 0.10	1.54 ± 0.08	40.31 ± 0.53
Azithromycin	0.34 ± 0.08	4.19 ± 0.54	0.41 ± 0.14	0.29 ± 0.12
Clarithromycin	1.01 ± 0.07	12.93 ± 0.62	0.60 ± 0.07	0.049 ± 0.04

Table 4.6.: IC_{50} values of amoxicillin and 6-APA derivatives against test bacteria. The values are reported as mean ± standard deviation. All dose-response curves had R^2 values greater than 0.95.

Compound	*Escherichia coli* (IC_{50}, µg/mL)	*Pseudomonas aeruginosa* (IC_{50}, µg/mL)	*Streptococcus pneumoniae* (IC_{50}, µg/mL)	*Staphylococcus aureus* (IC_{50}, µg/mL)
Compound 11	23.60 ± 0.47	279.30 ± 0.54	80.73 ± 0.59	83.16 ± 0.20
Compound 12	28.90 ± 0.34	0.66 ± 0.17	14.97 ± 0.41	0.11 ± 0.03
Compound 13	16.19 ±0.27	125.60 ± 0.47	25.21 ± 0.37	45.86 ± 0.13
Compound 14	25.14 ± 0.71	27.03 ± 0.45	44.67 ± 0.45	15.00 ± 0.15
Compound 15	19.09 ± 0.40	47.40 ± 0.49	13.71 ± 0.54	36.48 ± 0.20
Compound 16	22.47 ± 0.47	23.79 ± 0.36	18.95 ±0.34	9.70 ± 0.15

Compound 17	23.77 ± 0.39	2.336 ± 0.69	57.72 ± 0.76	2.46 ± 0.13
Compound 18	24.76 ± 0.24	75.67 ± 0.46	22.65 ± 0.42	31.59 ± 0.15
Compound 19	27.58 ± 0.82	121.30 ± 0.47	29.92 ± 0.35	114.70 ± 0.14
Compound 20	44.39 ±0.35	108.30 ± 0.62	7.22 ± 0.28	74.99 ± 0.08
Amoxicillin	17.19 ± 0.54	122.50 ± 0.89	17.23 ± 0.39	1.011 ± 0.01

Dose-response analysis was used to determine the antimicrobial activity of the derivatives, and the results are in (**appendix 98-105)** as IC_{50} curves that indicate percent inhibition in relation to log (concentration). Derivatives of all the mixtures showed concentration-dependent effects of inhibiting the tested bacterial strains, with clear IC_{50} values being represented on the sigmoidal dose-response curves. The R^2 was good, and the dose-response curves were good fits.

4.3.1. Comparison with Azithromycin and Clarithromycin Standard

To test the antibacterial activity of the newly synthesized compounds, their inhibitory potentials against four pathogenic bacterial strains, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Streptococcus pneumoniae,* were evaluated and compared with two commonly used macrolide antibiotics, azithromycin and clarithromycin.

Table 4.7 gives the IC_{50} values against *E. coli*. The tested compounds showed the highest potency, with the IC_{50} being 0.3379 µg/mL for azithromycin. In **compound 4**, there was a promising activity with an IC_{50} of 0.5639 µg/mL (**Figure 4.6**), which is comparable to azithromycin and stronger than clarithromycin IC_{50} = 1 µg/mL.

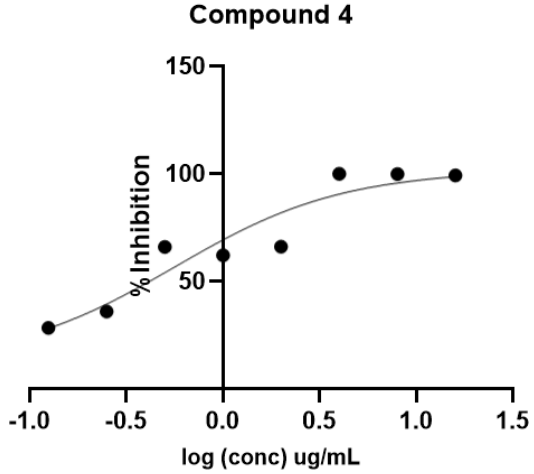


Figure 4.6.: IC50 Determination of Compound 4 on Escherichia coli

Table 4.7 : IC_{50} values of macrolide derivatives against *Escherichia coli* ranked from highest to lowest activity.

Compound	*Escherichia coli* (IC50, µg/mL)
Azithromycin	0.34 ± 0.08
Compound 4	0.56 ± 0.29
Clarithromycin	1.01 ± 0.07
Compound 5	1.63 ± 0.13
Compound 2	1.71 ± 0.10
Compound 10	2.24 ± 0.13
Compound 8	2.34 ± 0.29
Compound 9	2.45 ± 0.16
Compound 1	3.42 ± 0.18
Compound 6	3.79 ± 0.22
Compound 7	5.99 ± 0.22
Compound 3	18.97 ± 0.36

Table 4.8 shows the activities of the compounds against ***P. aeruginosa***. Interestingly, **Compounds 10** and **4** were remarkably potent, with IC_{50} values of 0.11 and 0.1 µg/mL, respectively (**Figures 4.7, 4.8**). These were much better than azithromycin (IC_{50} = 4.188 ug/mL) and clarithromycin (IC_{50} = 12.93 ug/mL). There were also other compounds with higher potency than the references, except **compound 5,** which is more potent than clarithromycin but less potent than azithromycin.

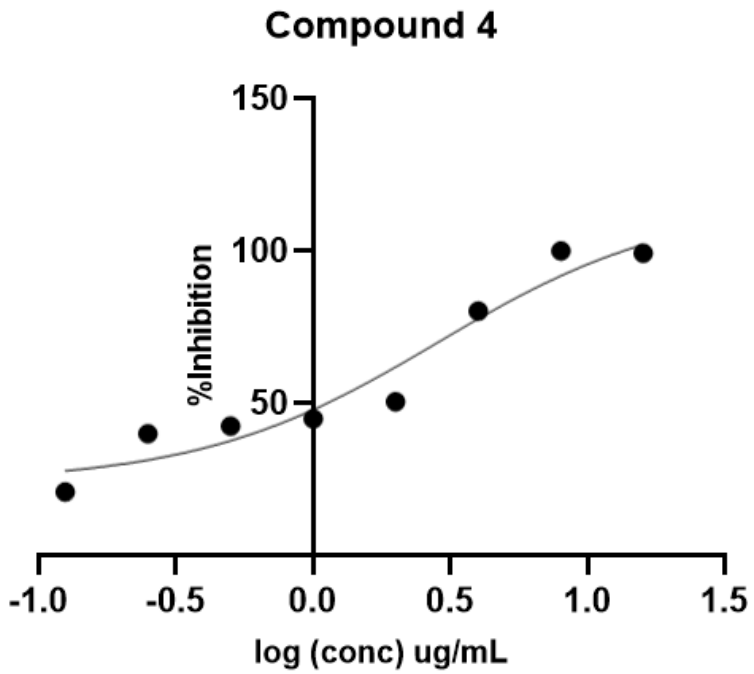


Figure 4.7.: IC50 Determination of Compound 4 on *Pseudomonas aeruginosa*

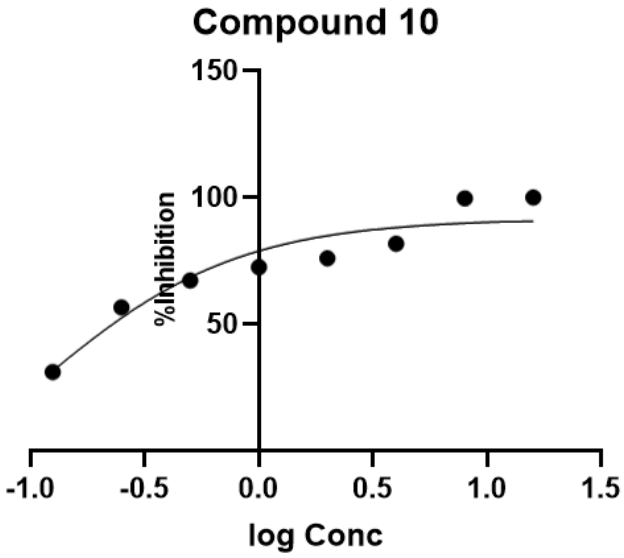


Figure 4.8.: IC50 Determination of Compound 10 on *Pseudomonas aeruginosa*

Table 4.8.: IC_{50} values of macrolide derivatives against *Pseudomonas aeruginosa* ranked from highest to lowest activity.

Compound	*Pseudomonas aeruginosa* (IC_{50}, µg/mL)
Compound 4	0.10 ± 0.02
Compound 10	0.11 ± 0.01
Compound 9	0.23 ± 0.09
Compound 8	0.32 ± 0.22
Compound 6	0.38 ± 0.03
Compound 2	0.81 ± 0.11
Compound 7	0.93 ± 0.16
Compound 1	1.65 ± 0.31
Compound 3	2.79 ± 0.16
Azithromycin	4.19 ± 0.54
Compound 5	11.71 ± 0.25
Clarithromycin	12.93 ± 0.62

Compound 8 had the most potent activity against ***S. pneumonia***e and an IC_{50} of 0.27 µg/mL as shown in **Table 4.9** (**Figure 4.9**). It was succeeded by azithromycin and clarithromycin, which had IC_{50} values of 0.41 and 0.6 µg/mL, respectively. Also, **Compound 3** has very close activity to clarithromycin.

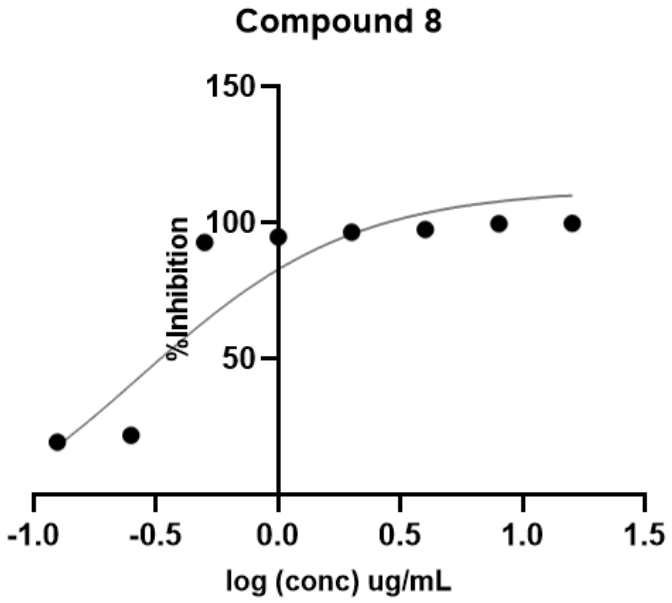


Figure 4.9.: IC_{50} Determination of Compound 8 on *Streptococcus pneumoniae*

Table 4.9. : IC_{50} values of macrolide derivatives against *Streptococcus pneumoniae* ranked from highest to lowest activity.

Compound	*Streptococcus pneumoniae* (IC_{50}, µg/mL)
Compound 8	0.27 ± 0.03
Azithromycin	0.41 ± 0.14
Clarithromycin	0.60 ± 0.07
Compound 3	0.60 ± 0.30
Compound 2	1.20 ± 0.21
Compound 7	1.28 ± 0.07
Compound 6	1.30 ± 0.08
Compound 10	1.54 ± 0.08
Compound 5	1.80 ± 0.37
Compound 1	1.80 ± 0.25
Compound 9	2.12 ± 0.15
Compound 4	2.85 ± 0.21

Clarithromycin was the best agent against ***S. aureus*** with the IC_{50} of 0.049 µg/mL (**Table 4.10**). **Compound 8** was also active with an IC_{50} of 0.15 µg/mL (**Figure 4.10**), which is better than azithromycin (IC_{50} = 0.29 µg/mL). The other compounds possessed diverse activity with IC_{50} of 1-61 µg/mL.

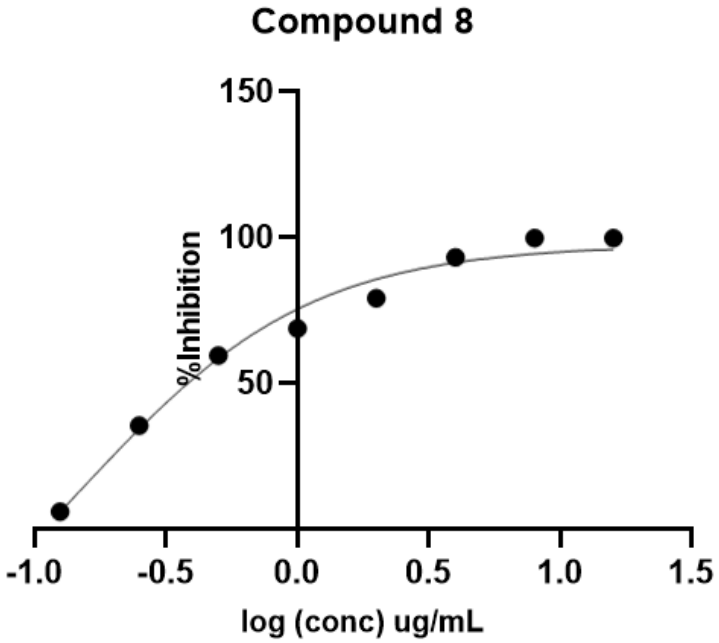


Figure 4.10.: IC50 Determination of Compound 8 on *Staphylococcus aureus*

Table 4.10. : IC_{50} values of macrolide derivatives against *Staphylococcus aureus* ranked from highest to lowest activity.

Compound	*Staphylococcus aureus* (IC_{50}, µg/mL)
Clarithromycin	0.049 ± 0.001
Compound 8	0.15 ± 0.04
Azithromycin	0.29 ± 0.01
Compound 3	1.25 ± 0.24
Compound 2	6.11 ± 0.57
Compound 5	9.83 ± 0.36
Compound 9	11.46 ± 0.26
Compound 6	15.16 ± 0.39
Compound 1	15.66 ± 0.44
Compound 7	21.22 ± 0.47
Compound 10	40.31 ± 0.53
Compound 4	61.56 ± 0.45

4.3.2. Comparison with Amoxicillin Standard

The products of the synthetically modified penicillins were tested in comparison to amoxicillin as a control standard in order to recognize compounds with better or equal antibacterial activity. In the analysis below, derivatives that were more potent or had promising activity profiles compared to amoxicillin are identified

Compound 13, 16.19 µg/m (**Figure 4.11**), showed greater activity than amoxicillin (17.19 µg/mL). As well, **Compound 15** was also reminiscent of the standard drug in terms of activity, IC_{50} = 19.09 µg/mL (**Figure 4.12**) with only slight discrepancies (**Table 4.11**).

Table 4.11.: values of penicillin derivatives against *Escherichia coli* ranked from highest to lowest activity.

Compound	*Escherichia coli* (IC_{50}, µg/mL)
Compound 13	16.19 ±0.27

Amoxicillin	17.19 ± 0.54
Compound 15	19.09 ± 0.40
Compound 16	22.47 ± 0.47
Compound 11	23.60 ± 0.47
Compound 17	23.77 ± 0.39
Compound 18	24.76 ± 0.24
Compound 14	25.14 ± 0.71
Compound 19	27.58 ± 0.82
Compound 12	28.90 ± 0.34
Compound 20	44.39 ±0.35

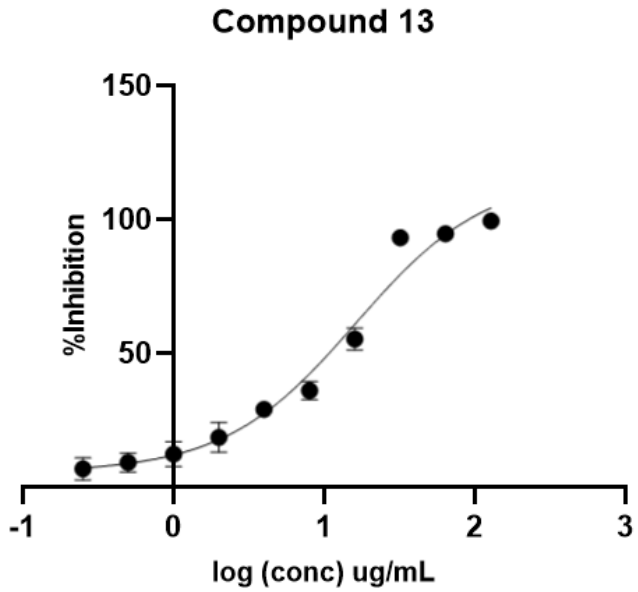


Figure 4.11.: IC50 Determination of Compound 13 on *Escherichia coli*

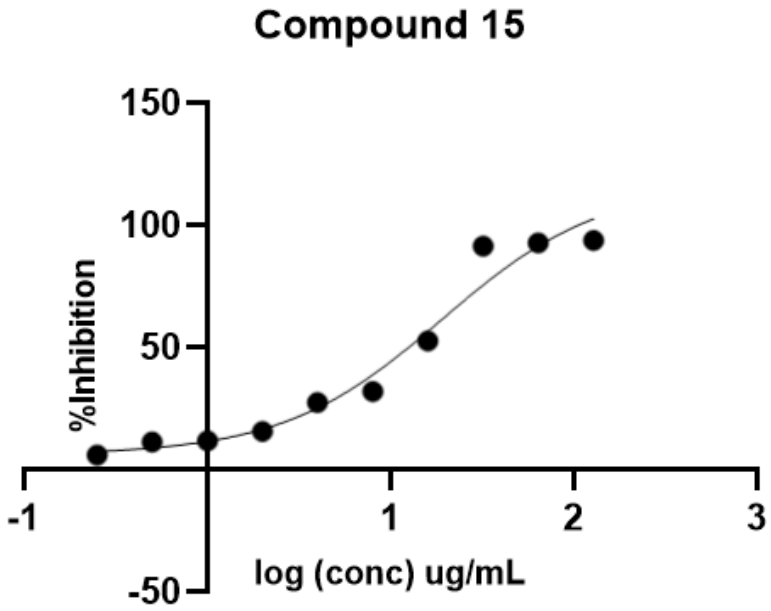


Figure 4.12.: IC50 Determination of Compound 15 on *Escherichia coli*

Compound 12 was the most promising result, with a higher outcome than that of amoxicillin by 185-fold, $\boldsymbol{IC_{50}}$ = 0.66 µg/mL compared to 122.5 µg/mL (**Figure 4.13**). **Compounds 14, 15, 16, 17, 18, 20,** and **19** also give higher activity than the reference. **Compound 13** was also found to have similar efficacy with an IC_{50} of 125.6 µg/mL, and within the same range as amoxicillin 122.5 µg/mL (**Table 4.12**).

Table 4.12.: IC_{50} values of penicillin derivatives against *Pseudomonas aeruginosa* ranked from lowest to highest activity.

Compound	*Pseudomonas aeruginosa* (IC_{50}, µg/mL)
Compound 12	0.66 ± 0.17
Compound 17	2.34 ± 0.69
Compound 16	23.79 ± 0.36
Compound 14	27.03 ± 0.45
Compound 15	47.40 ± 0.49
Compound 18	75.67 ± 0.46
Compound 20	108.30 ± 0.62
Compound 19	121.30 ± 0.47
Amoxicillin	122.50 ± 0.89

Compound 13	125.60 ± 0.47
Compound 11	279.30 ± 0.54

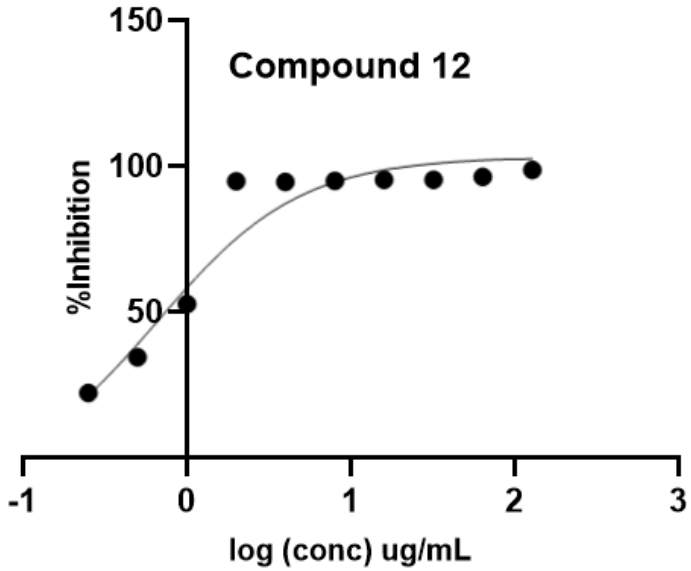


Figure 4.13.: IC50 Determination of Compound 12 on *Pseudomonas aeruginosa*

Compound 20, IC_{50} = 7.22 µg/mL (**Figure 4.14**), **Compound 15,** IC_{50} = 13.71 µg/mL (**Figure 4.15**), and **Compound 12,** IC_{50} = 14.97 µg/mL (**Figure 4.16**) were found to show significant improvement over amoxicillin, and were observed to have a greater potency than the reference standard (IC_{50} = 17.23 µg/mL). **Compound 16** (IC_{50} = 18.95 µg/mL) showed similar activity levels as those of amoxicillin and, therefore, it may be therapeutic equivalent (**Table 4.13**).

Table 4.13: IC_{50} values of penicillin derivatives against [*Streptococcus pneumoniae*] ranked from lowest to highest activity.

Compound	*Streptococcus pneumoniae* (IC_{50}, µg/mL)
Compound 20	7.22 ± 0.28
Compound 15	13.71 ± 0.54
Compound 12	14.97 ± 0.41
Amoxicillin	17.23 ± 0.39
Compound 16	18.95 ±0.34
Compound 18	22.65 ± 0.42

Compound 13	25.21 ± 0.37
Compound 19	29.92 ± 0.35
Compound 14	44.67 ± 0.45
Compound 17	57.72 ± 0.76
Compound 11	80.73 ± 0.59

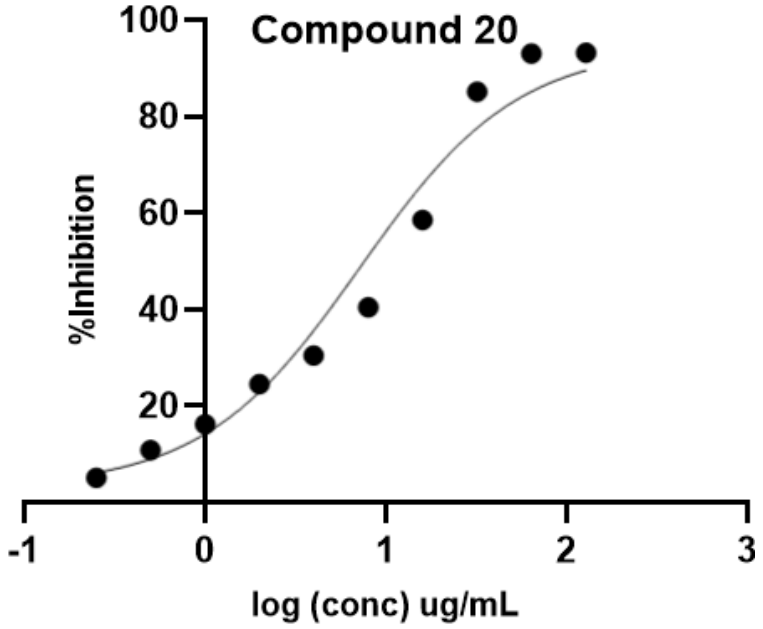


Figure 4.14. :IC50 Determination of Compound 20 on *Streptococcus pneumoniae*

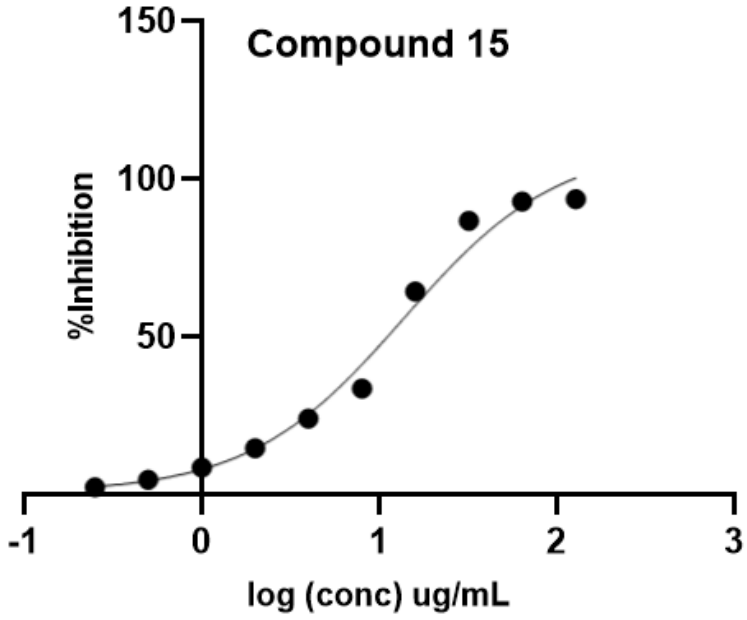


Figure 4.15: IC50 Determination of Compound 15 on *Streptococcus pneumoniae*

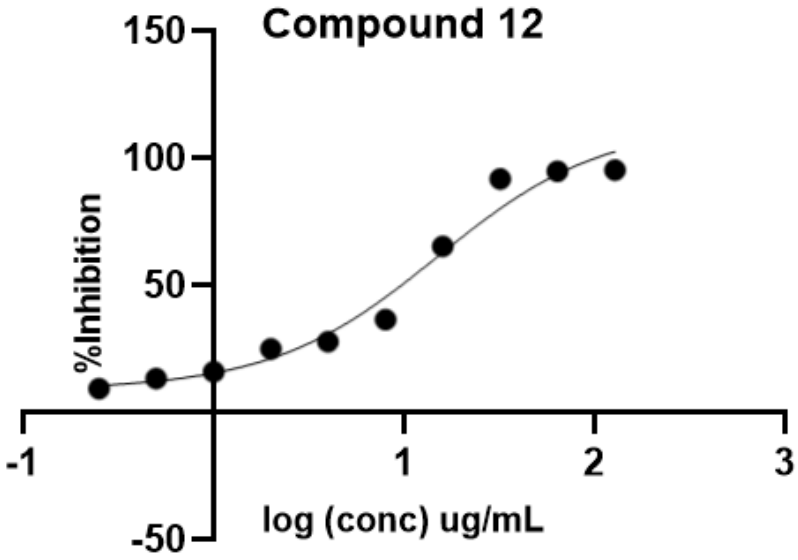


Figure 4.16:IC50 Determination of Compound 12 on *Streptococcus pneumoniae*

Compound 12 showed the greatest change with an IC_{50} of 0.11 µg/mL in comparison to amoxicillin, with amoxicillin being 1.01 µg/mL (**Figure 4.17**). **Compound 17** was similarly a potent antibiotic with IC_{50} = 2.46 µg/mL, but less than the known antibiotic (**Table 4.14**).

Table 4.14. : IC_{50} values of penicillin derivatives against [*Staphylococcus aureus*] ranked from lowest to highest activity.

Compound	*Staphylococcus aureus* (IC_{50}, µg/mL)
Compound 12	0.11 ± 0.03
Amoxicillin	1.01 ± 0.01
Compound 17	2.46 ± 0.13
Compound 16	9.70 ± 0.15
Compound 14	15.00 ± 0.15
Compound 18	31.59 ± 0.15
Compound 15	36.48 ± 0.20

Compound 13	45.86 ± 0.13
Compound 20	74.99 ± 0.08
Compound 11	83.16 ± 0.20
Compound 19	114.70 ± 0.14

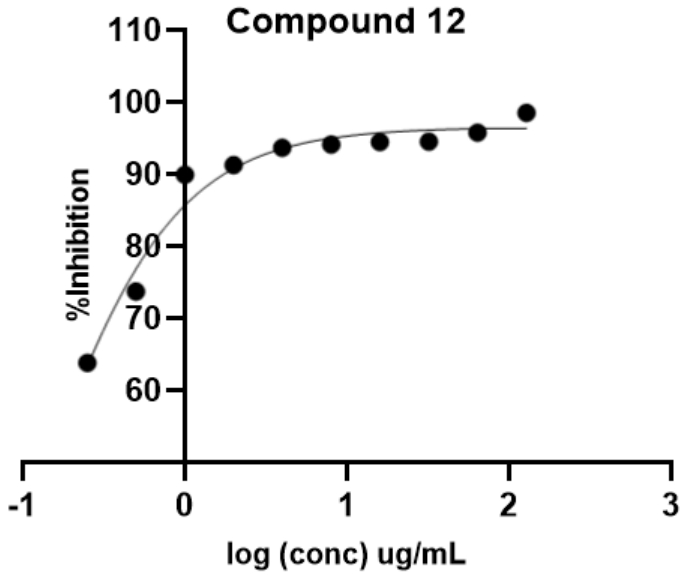


Figure 4.17 :IC_{50} Determination of Compound 12 on *Staphylococcus aureus*

4.4. Molecular Docking Studies

This study is aimed at developing and analyzing five original amoxicillin compounds and five original 6-aminopenicillin compounds (6-APA) as prospective bacterial protein inhibitors. Amoxicillin is a broad-spectrum beta-lactam antibiotic; 6-APA is the core nucleus of all penicillins, and a valuable template to address chemical modification. It is hoped that through the synthesis and testing of a collection of derivatives, we will find compounds with a higher antimicrobial activity and a better binding affinity to their target proteins.

4.4.1. Docking Score Analysis

Simulations of molecular docking were done to determine the binding modes and affinities of the ten derivatives to four target proteins chosen out of the four bacteria mentioned above. The molecular docking results were compared with the in vitro determination of the minimum inhibitory concentration (MIC) and the half-maximal inhibitory concentration (IC50) to identify consistent trends. Besides, the 2D

interactions of the most active derivatives to know the molecular basis of their activity, and especially the possibility of covalent bonding with a key serine residue in the active site of the target proteins, provide useful information in the rational design of the next-generation beta-lactam antibiotics. **Table 4.15** summarizes the docking scores (binding energies) of the five amoxicillin and five 6-APA analogs of the four target proteins. The scores are used to indicate the predicted binding affinity, the binding energy of the compound, the similarity between its interactions and the co-crystal molecules and the distance at which the serine oxygen and the carbonyl carbon of the beta lactam moiety were the criteria by which the compounds were classified and compared to amoxicillin in the docking study and would indicate a favorable conformation for each derivative. The derivatives, as demonstrated in **Table 4.14**, had a wide range of binding affinities, with some compounds displaying much higher docking scores than amoxicillin and some giving higher docking scores than co-crystal molecules.

Table 4.15. : Docking scores (kcal/mol) of amoxicillin and 6-APA derivatives against the four target proteins.

Compound	*Escherichia coli* (PDB ID: 6I1I)	*Pseudomonas aeruginosa* (PDB ID: 6UN1)	*Streptococcus pneumoniae* (PDB ID: 5OJ1)	*Staphylococcus aureus* (PDB ID: 7O4B)
Compound 11	-8.068	-6.040	-6.813	-6.310
Compound 12	-7.762	-10.182	-7.778	-8.365
Compound 13	-8.461	-6.500	-6.590	-6.994
Compound 14	-7.971	-8.132	-6.455	-6.410
Compound 15	-8.537	-7.790	-7.612	-6.266
Compound 16	-8.099	-9.323	-9.233	-7.991
Compound 17	-7.345	-8.008	-6.811	-8.285
Compound 18	-7.951	-8.462	-9.170	-7.871
Compound 19	-8.114	-7.810	-7.741	-7.810
Compound 20	-6.817	-7.674	-8.491	-8.003
Co-crystal molecule	-9.273	-7.260	-9.196	-7.653
Amoxicillin	-8.13	-7.201	-7.030	-8.057

In the case of the four proteins, the compounds are ranked in the tables below in terms of their binding energies, with those considered better than amoxicillin and the co-crystal. Each protein has compounds in a decreasing order, from the highest to the lowest. In *Escherichia coli* **compound 15** and **compound 13** give higher affinity than amoxicillin (**Table 4.16**), in *Pseudomonas aeruginosa* **compounds 12, 16, 18, 14, 17, 19, 15** and **20** (**Table 4.17**), in *Streptococcus pneumoniae* **compounds 16, 18, 20,12,19** and **15** (**Table 4.18**), While in *Staphylococcus aureus* **compounds 12** and **17** (**Table 4.19**).

Table 4.16.: Molecular docking energies of penicillin derivatives against PBP 3 from *Escherichia coli:* binding affinities ranked from highest to lowest.

Compound	*Escherichia coli* (PDB ID: 6I1I)
Co-crystal molecule	-9.273
Compound 15	-8.537
Compound 13	-8.461
Amoxicillin	-8.130
Compound 19	-8.114
Compound 16	-8.099
Compound 11	-8.068
Compound 14	-7.971
Compound 18	-7.951
Compound 12	-7.762
Compound 17	-7.345
Compound 20	-6.817

Table 4.17. : Molecular docking energies of penicillin derivatives against PBP 3 from *Pseudomonas aeruginosa:* binding affinities ranked from highest to lowest.

Compound	*Pseudomonas aeruginosa* (PDB ID: 6UN1)
Compound 12	-10.182
Compound 16	-9.323
Compound 18	-8.462

Compound 14	-8.132
Compound 17	-8.008
Compound 19	-7.810
Compound 15	-7.790
Compound 20	-7.674
Co-crystal molecule	-7.260
Amoxicillin	-7.201
Compound 13	-6.500
Compound 11	-6.040

Table 4.18: Table 4.8 Molecular docking energies of penicillin derivatives against PBP 2x from *Streptococcus pneumoniae:* binding affinities ranked from highest to lowest.

Compound	*Streptococcus pneumoniae* (PDB ID: 5OJ1)
Compound 16	-9.233
Co-crystal molecule	-9.196
Compound 18	-9.170
Compound 20	-8.491
Compound 12	-7.778
Compound 19	-7.741
Compound 15	-7.612
Amoxicillin	-7.030
Compound 11	-6.813
Compound 17	-6.811
Compound 13	-6.590
Compound 14	-6.455

Table 4.19.: Molecular docking energies of penicillin derivatives against PBP 1 from *Staphylococcus aureus:* binding affinities ranked from highest to lowest.

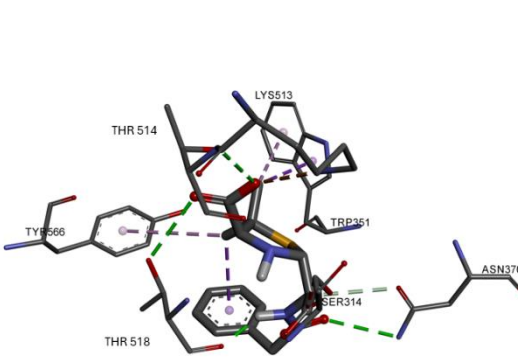
Compound	*Staphylococcus aureus*

	(PDB ID: 7O4B)
Compound 12	-8.365
Compound 17	-8.285
Amoxicillin	-8.057
Compound 20	-8.003
Compound 16	-7.991
Compound 18	-7.871
Compound 19	-7.810
Co-crystal molecule	-7.653
Compound 13	-6.994
Compound 14	-6.410
Compound 11	-6.310
Compound 15	-6.266

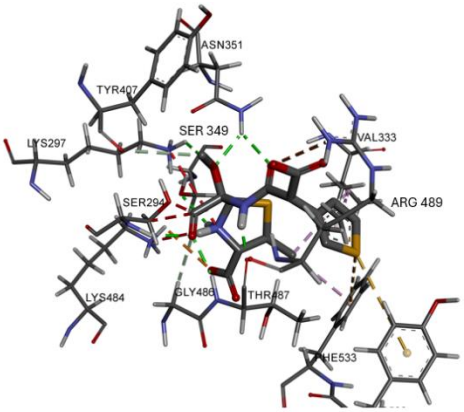
4.4.2. Binding Mode Analysis

In order to interpret the observed binding affinities using the structural basis, we examined the binding modes of the most effective derivatives in the active sites of the target proteins. **Figure 4.18** indicates the binding interactions of the co-crystal molecules, penicillin G in the active site of PBP1, temocillin in the active site of PBP3, Piperacillin in the active site of PBP3, and oxacillin in the active site of PBP2x. **Figure 4.19** shows the binding interactions of amoxicillin with four target proteins. The ligands are held together through a hydrogen bond, van der Waals, and dipole-dipole interactions.

(a) (b)



(c)



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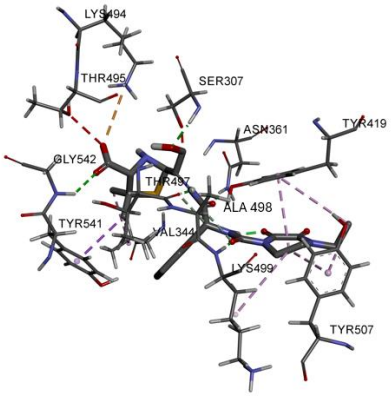


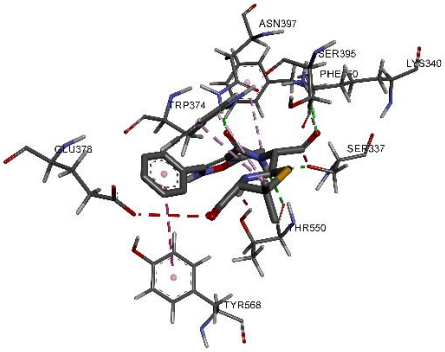
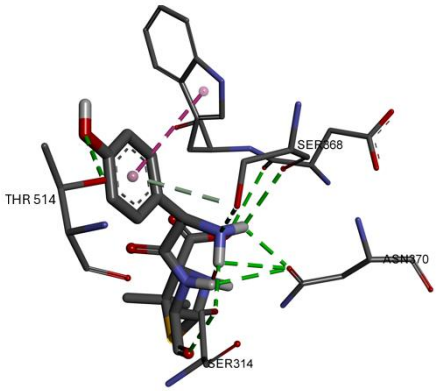


Figure 4.18 (a-d): 2D interaction of co-crystal molecules and their proteins (a) 2D interactions of penicillin G and PBP1 (PDB ID: 7O4B) (b) 2D interactions of temocillin and PBP3 (PDB ID: 6UN1) (c) 2D interactions of Piperacillin and PBP3 (PDB ID: 6I1I) (d) 2D interactions of oxacillin and PBP2x (PDB ID: 5OJ1).

(a)



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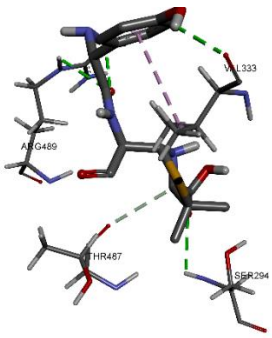


(d)

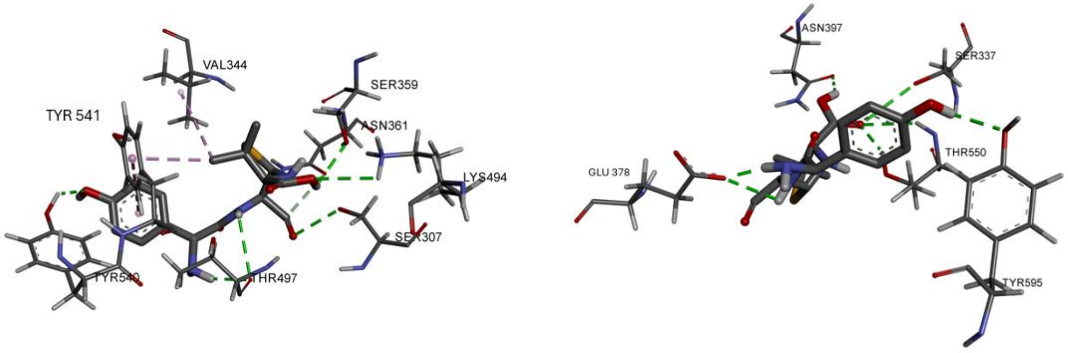
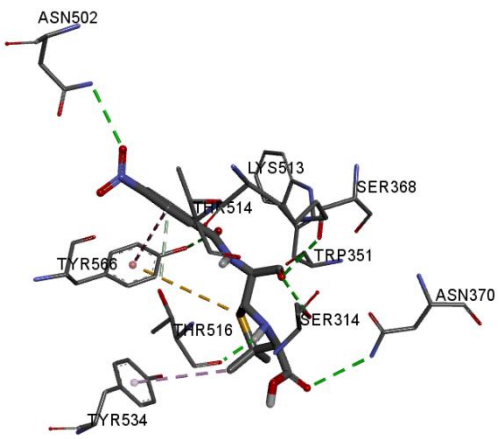


Figure 4.19 (a-d): 2D interaction of amoxicillin molecules and their proteins (a) 2D interactions of Amoxicillin and PBP1 (PDB ID: 7O4B) (b) 2D interactions of amoxicillin and PBP3 (PDB ID: 6UN1) (c) 2D interactions of amoxicillin and PBP3 (PDB ID: 6I1I) (d) 2D interactions of amoxicillin and PBP2x (PDB ID: 5OJ1).

In order to explore the interactions between the active site residues and the derivatives, 2D interaction diagrams were created. The hydrogen bonds, hydrophobic interactions, and other noncovalent interactions that aid in the binding are clearly seen. Of special interest is the interaction with the catalytic serine residues, Ser 314, Ser 294, Ser 307, and Ser 337 in **Figures 4.20- 4.23**, respectively, which are in the close vicinity of the beta-lactam ring of the derivative. The distance between the hydroxyl group of the serine and the carbonyl carbon of the beta-lactam is [≤ 3.0–3.5] Å, which indicates the high possibility of an attack by nucleophilic and a resulting formation of a covalent bond. **The tables (4.20 - 4.23)** below demonstrate the amino acids making interactions between the most potent compounds and the target protein, and some 2D interactions of them are shown in **Figures 4.20- 4.23.**

(a): Compound 12



(b): Compound 17

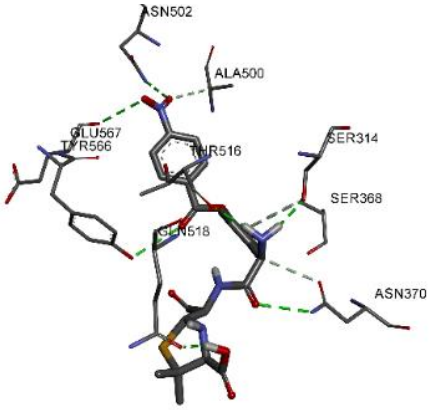


Figure 4.20. (a-b): Stick representation of docked modes interactions of the most potent penicillin derivative in PBP1 (PDB ID: 7O4B)

(a): Compound 17 (b): Compound 12

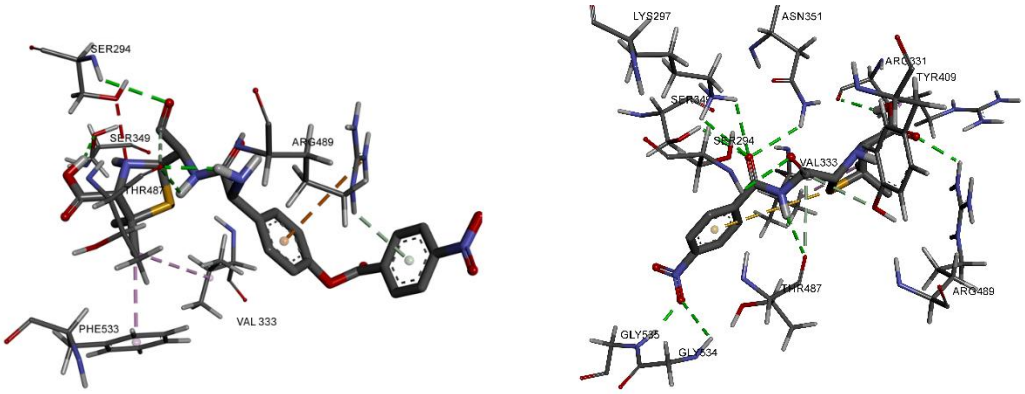


Figure 4.21.(a-b): 2D interactions of the most potent penicillin derivative in PBP3 (PDB ID: 6UN1)

(a): Compound 15 (b): Compound 13

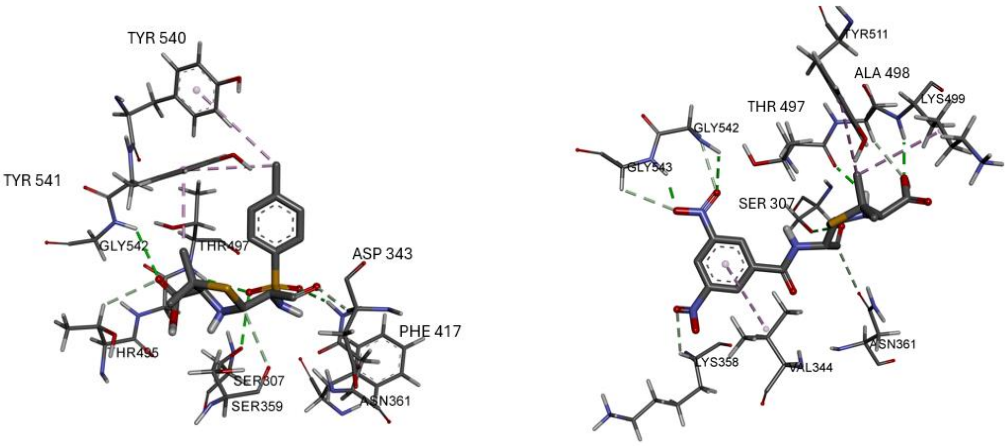


Figure 4.22 (a-b). : Stick representation of docked modes interactions of the most potent penicillin derivative in PBP3 (PDB ID: 6I1I)

(a): Compound 12 (b): Compound 20

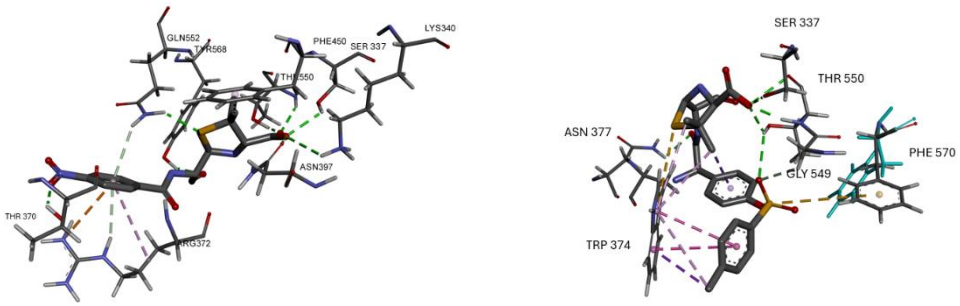


Figure 4.23 (a-b) : Stick representation of docked modes interactions of the most potent penicillin derivative in PBP2x (PDB ID: 5OJ1).

Table 4.20.: AutoDock yields the lowest binding energies determined from AutoDock 4.2 and interacting amino acids in *Staphylococcus aureus* (PDB ID: 7O4B).

Compounds	Docking Energy (kcal/mol)	Interacting Amino Acids in hydrophobic interaction	Interacting Amino Acids in Hydrogen bonds
Compound 12	-8.365	TYR 566, TYR 534, THR 514, TRP 351	ASN 502, ASN 370, LYS 513, **SER 314,** THR 516, SER 368, TYR 566
Compound 17	-8.285	ALA 500, ASN 370 SER 368	TYR 566, THR 516, ASN 502, GLN 518, **SER 314**

Table 4.21.: AutoDock yields the lowest binding energies determined from AutoDock 4.2 and interacting amino acids in *Pseudomonas aeruginosa* (PDB ID: 6UN1).

Compounds	Docking Energy (kcal/mol)	Interacting Amino Acids in hydrophobic interaction	Interacting Amino Acids in Pi-sulfer interaction	Interacting Amino Acids in Hydrogen bonds	Interacting Amino Acids in Ionic interaction

Compound 12	-10.182	VAL 333, THR 487, ARG 331	_____	THR 487, LYS 297, SER 345, **SER 294**, GLY 535, GLY 334, ASN 351, TYR 409, ARG 489	____
Compound 16	-9.323	LYS 348, VAL 333	PHE 533	SER 334, SER 349, THR 487, **SER 294**, ARG 489, TYR 503,	____
Compound 18	-8.462	SER 485, GLY 534, GLY 486, VAL 333, ASN 351	____	VAL 333, GLY 535, GLY 534, GLY 486, THR 487, SER 349, **SER 294**	____
Compound 14	-8.132	PHE 533, THR 487, VAL 333, TYR 503	____	SER 485, **SER 294**, ARG 335, ARG 489, TYR 409,	____
Compound 17	-8.008	PHE 533, VAL 333, ARG 489	____	**SER 294**, SER 349, THR 487	Arg 372
Compound 19	-7.810	VAL 333, ARG 335	____	SER 485, PHE 533, THR 487, **SER 294**, ARG 331	ARG 489
Compound 15	-7.790	PHE 533, VAL 333	____	LYS 484, SER 485, SER 294 THR 487, TYR 407, **SER 294**	____

Compound 20	-7.674	VAL 333	PHE 533	VAL 471, TYR 409, ARG 489, TYR 503, **SER 294** THR 487	LYS 297

Table 4.22.: AutoDock yields the lowest binding energies determined from AutoDock 4.2 and interacting amino acids in *Escherichia coli* (PDB ID: 6I1I).

Compounds	Docking Energy (kcal/mol)	Interacting Amino Acids in hydrophobic interaction	Interacting Amino Acids in Hydrogen bonds
Compound 15	-8.537	TYR 540, TYR 541, ASN 361, THR 497	**SER 307**, SER 359, ASP 343, PHE 417 GLY 542, THR 495
Compound 13	-8.461	LYS 358, VAL 344, TYR 511, ASN 361, LYS 499	LYS 499, GLY 543, GLY 542, THR 587, ALA498, **SER 307**.

Table 4.23.: AutoDock yields the lowest binding energies determined from AutoDock 4.2 and interacting amino acids in *Streptococcus pneumoniae* (PDB ID: 5OJ1).

Compounds	Docking Energy (kcal/mol)	Interacting Amino Acids in hydrophobic interaction	Interacting Amino Acids in Pi-sulfer interaction	Interacting Amino Acids in Hydrogen bonds	Interacting Amino Acids in ionic interaction
Compound 16	-9.233	PHE 450	TYR 595, TYR 568	GLN 552, THR550, **SER 337**, GLU 378, ASN 397	——
Compound 18	-9.170	ALA 551, ASN 397, TRP 374	TYR 595	THR 550, LYS 340, GLN 552, GLU 378 **SER 337**	——

Compound 20	-8.491	GLY 549, PHE 570	____	TRP 374, **SER 337**, THR 550, ASN 397	____
Compound 12	-7.778	ARG 372, PHE 450 GLN 552	____	THR 370, GLN 552, TYR 568, ASN 397, THR 550, **SER 337**, LYS 340	ARG 372
Compound 19	-7.741	TRP 374, PHE 450	TYR 595, TYR 568	THR 550, THR 526, **SER 337** GLU 378, ASN 377, ASN 397	____
Compound 15	-7.612	TYR 568, TRP 374, PHE 450,	___	ASN 377, THR 550, GLY 549, **SER 337**, SER 395, ASN 397	GLU 378

Chapter 5: Discussion

The design of new drugs and prodrugs is an important frontier in medicinal chemistry, as it is supposed to eliminate the constraint of traditional therapeutics. The underlying assumption of this research was that new drugs and prodrug strategies, which involve the chemical modification of the original structure with the use of well-designed chemical linkers, could successfully hide many of the limitations of drugs.

5.1. Background

Antibiotics are the backbone of contemporary medicine, without which it is impossible to treat bacterial infections. Their modes of action are varied, with key classes of the macrolides and β-lactams that are used to disrupt key bacterial activities, including protein synthesis and cell wall generation, respectively (Patel & Hashmi, 2023). Nonetheless, the two significant factors that continuously question the clinical effectiveness of these essential medications are the development of bacterial resistance and the problem of patient compliance. Poor palatability is a compliance barrier to some antibiotics, especially in the pediatric and geriatric populations, which may result in incomplete therapy and resistance development (Elgammal et al., 2025; Soares et al., 2022).

Clarithromycin and azithromycin are the leading representatives of the macrolide group of antibiotics. Their therapeutic effect is because they are able to bind to the 50S subunit of the bacterial ribosome and prevent the synthesis of proteins (Patel & Hashmi, 2023). It is an extensive mechanism of activity against a wide range of pathogens that cause respiratory tract, skin, and sexually transmitted infections (Vázquez-Laslop & Mankin, 2018). The major weakness of both azithromycin and clarithromycin, however, is that they have an extremely bitter flavor. This property often contributes to non-adherence, particularly to those liquid suspensions that are developed in children and highlights the clinical importance of having formulations with better taste characteristics (Mennella et al., 2013; Soares et al., 2022). Another highly sensitive group of antibacterial agents is the β-lactam group of antibiotics, such as amoxicillin. Amoxicillin is a semi-synthetic penicillin analog of 6-aminopenicillinic acid (6-APA), the backbone structure of an enormous range of penicillin analogs designed and produced during the

past 50 years (Akhavan et al., 2023; Rolinson & Geddes, 2007). Although amoxicillin tends to be more palatable than the macrolides, the major risk to the effectiveness of the former is the phenomenon of widespread bacterial resistance, which appears mostly as a result of the formation of β-lactamase enzymes. The enzymes break the amide bond of the β-lactam ring, which inactivates the antibiotic and makes it useless. This has led to significant interest in medicinal chemistry in this field to develop new 6-APA analogs and amoxicillin analogs such that they are more potent and exhibit increased stability to degradation to counteract resistance and increase their activity (Akhavan et al., 2023). The taste of bitterness is a complicated sense mechanism that is acted upon by a family of G protein-coupled receptors, the taste 2 receptors (T2Rs), among which 25 known functional types exist in humans (Tuzim & Korolczuk, 2021). These receptors are on the tongue, and they act as a protective system, where the receptors indicate the existence of possibly harmful substances (Clark et al., 2012). These receptors are sensitive and have a wide-tuning range, which implies numerous structurally diverse pharmacological agents, such as azithromycin and clarithromycin, are able to stimulate them, and leave a bad taste. Different chemical approaches are needed to solve the specific problems presented by such groups of antibiotics. In the case of macrolides such azithromycin and clarithromycin, the predominant problem is bitterness, in which case prodrug development is a complex and efficient method of masking the taste. A prodrug may be chemically modified to exhibit different physicochemical characteristics of the parent drug, including a different molecular structure that does not permit the prodrug to interact with and activate T2Rs (Gala & Chauhan, 2014; Karaman, 2014). The given strategy represents a good way to conceal the bitter taste at the molecular level, which will be a promising direction to enhance patient compliance and treatment outcomes in case of macrolide therapies. The idea behind this prodrug solution is that the functional groups causing the bitter taste are blocked only temporarily. It has been suggested that hydrogen bonding between the hydroxyl groups of the macrolide structure and the bitter taste receptors on the tongue is the cause of the bitter taste. This interaction is avoided by changing these hydroxyl groups to either esters or sulfonate esters, thereby disguising the bitter taste. These prodrugs are intended to be metabolized by enzymes in the body after being ingested to release the active parent drug, which can then take on its therapeutic action (**Figure 5.1**).

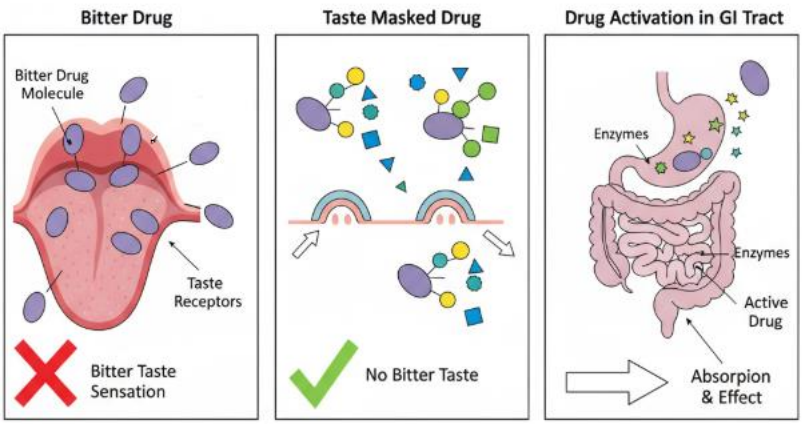


Figure 5.1: Conceptual illustration of the taste-masking mechanism of a prodrug.

5.2. Rationale of Choice of Chemical Linkers.

The choice of the chemical linkers used in the synthesis of the azithromycin and clarithromycin derivatives in this study was based on the principles of prodrug design, and the goal of obtaining the pH-dependent hydrolysis in particular. This aimed to produce derivatives that would readily hydrolyze either at acidic or basic conditions or at the physiologic pH to liberate the active macrolide. In this regard, a chain of chloride sulfonyl and benzoyl promoieties was selected. The derivatives of the sulfonyl chloride, such as *N*-acetylbenzenesulfonyl chloride, **Compound 1** and **6**, 4-nitrobenzenesulfonyl chloride, **Compound 4** and **9**, *p*-toluenesulfonyl chloride, **Compound 5** and **10**, were chosen due to their possible ability to form acid or base-labile sulfonate ester linkages. These bonds are also reported to be subject to hydrolysis in an acidic and basic environment, and the hydrolysis can be adjusted to the electronic characteristics of the substituents on the aromatic ring. As the electron-withdrawing nitro groups were incorporated into the reaction, as 4-nitrobenzenesulfonyl chloride was used, the sulfonamide and sulfonate ester bonds were expected to become more labile, and the hydrolysis was expected to speed up. On the other hand, the methyl group used in the *p*-toluene sulfonyl chloride that donates electrons was expected to stabilize. The compounds 4-nitrobenzoyl chloride (**Compound 2** and **7)** and 3,5-dinitrobenzoyl chloride (**Compound 3** and **8**), which are benzoyl chloride derivatives, were selected to examine the nature of ester or amide linkages. These bonds are also susceptible to hydrolysis, and their stability can equally be determined by the electronic nature of the substituents in the benzoyl ring. It was predicted that the strong electron-withdrawing

properties of nitro groups would be of great help in affecting the rate of hydrolysis because of the one or two nitro groups present.

5.3. pH-Sensitive Prodrugs: A Theoretical Framework

The efficacy of the design of a prodrug to be delivered orally is dependent on whether or not it can survive the various chemical conditions of the gastrointestinal (GI) tract intact, where it is required, and it releases its active moiety at the most favorable location where it can be absorbed or cause an effect. The natural pH gradient along the GI tract, which moves through a highly acidic environment of the stomach (pH 1.5-3.5) to the increasingly more neutral, less acidic and slightly alkaline environment of the small and large intestines (pH 5.5 -7.5), provides a naturally occurring and accessible source of a unique drug delivery mechanism. This is the basis of the development of pH-sensitive prodrugs, which are designed to have different stability within this pH range (Devnarain et al., 2021; Hassan et al., 2020; Osman et al., 2024).

5.4. Experimental Results Analysis of Hydrolysis

The hydrolysis experiments were performed at pH 2.2, 5.5, and 7.4 in order to mimic the conditions in the stomach, proximal small intestine, and systemic circulation /distal small intestine, respectively. The findings showed a very fascinating and varied variation of the hydrolysis profiles of the ten derivatives, indicating the significant influence of the various chemical linkers.

In pH 2.2, when simulating the acidic conditions of the stomach, the partial hydrolysis of **Compounds 2, 4,** and **5**, which are all derivatives of azithromycin, to the parent drug was deemed important. The therapeutic implications of this discovery are of great importance since it indicates that these compounds may start to liberate the active antibiotic in the stomach, which might result in a subsequent faster systemic uptake and action. The controlled release mechanism is suggested by the fact that the hydrolysis was not complete, and the same could be used as a benefit of keeping the therapeutic drug concentrations on a long-term basis. The ability of the other compounds to be resistant to hydrolysis at this pH indicates that their linkers are not readily degraded at extreme acidic pH, which might be an advantageous feature in the event that the objective is to be able to target drug release only to the small intestine.

In a pH of 5.5, which was close to the pH of the upper small intestine, the most significant finding was that, at this pH, **Compound 5** (azithromycin with *p*-toluene sulfonyl chloride) was fully hydrolyzed to the parent azithromycin. This implies that this type of derivative will be best suited to be released specifically in the main area of drug absorption. Hydrolysis of the other compounds at this pH is also a variable behavior, which further indicates the extent to which drug release can be adjusted by making slight changes to the promoiety.

When pH was at 7.4, which is the physiological pH of physiological blood and the lower small intestine, **Compound 5** again showed full hydrolysis. This is an important discovery since it will guarantee that any of the prodrug absorbed will be effective in converting to the active form in the systemic circulation. It is also informative that the other compounds were either resistant to hydrolysis or were, to some extent, transformed to other compounds other than the parent drug. This implies that they could exist on different metabolic routes or that their linkers are created to be released in response to different physiological stimuli, and, potentially, these could be investigated in future research. The appearance of non-parent metabolites suggests such significant questions as what possibility there is of such compounds having their own distinct pharmacological profiles.

5.5. Structure-Hydrolysis Relationships.

The different hydrolysis ways of the ten derivatives offer abundant data to explain the structure-hydrolysis correlation. The findings clearly lead to the effect of the nature of the linking group (sulfonyl vs. benzoyl), as well as the nature of the substituents on the aromatic ring. Notably, the full and fast hydrolysis of **Compound 5** (azithromycin with *p*-toluene sulfonyl chloride) was observed at pH 5.5 and 7.4. Contrary to expectations it is found that the *p*-methyl substitution gives an ideal electronic environment, balancing the stability of bonds and hydrolytic instability forms positive kinetics of hydrolysis. Also, the aspects of the macrolide-sulfonate conjugate could enable nucleophilic attack on the sulfonyl center to produce the desired full conversion of the parent azithromycin. The fact that **Compounds 2** (azithromycin with 4-nitrobenzoyl chloride) and **4** (azithromycin with 4-nitrobenzene sulfonyl chloride) are partially hydrolyzed at pH 2.2 is in line with the fact that the nitro group is an electron-withdrawing group that should be expected to make the ester and sulfonate ester bonds

more labile. The observation that the related clarithromycin analogues (compounds **7** and **9**) were not found to undergo the same extent of hydrolysis under these conditions implies that even the nature of the macrolide core of a prodrug also affects the stability of the prodrug linkage. This may be as a result of variation in the steric environment surrounding the site of attachment or the slight variation in the electronic properties of the two macrolides. It is also interesting to note that **Compounds 3** and **8** (the 3,5-dinitrobenzoyl derivatives) are resistant to hydrolysis. Although one would have anticipated a strong electron-withdrawing effect when the two nitro groups are in the meta position on the benzoyl ring, they might exert a reduced direct electronic effect on the ester bond when compared to a para-substituted nitro group. Also, the two large nitro groups may offer steric hindrance to block the nucleophilic attack of water on the ester and sulfonate ester bonds. This shows that it is not only the electronic properties of the substituents that are important, but also the steric and positional effects.

The Structure-Activity Relationship (SAR) study indicates that the location of electron-withdrawing groups (EWGs) on the aromatic promoiety is the key factor in hydrolytic stability and, consequently, is the ability to activate the prodrug. The comparison of the various nitro-substituted benzoyl esters has provided a clear azithromycin pro-drugs's SAR:

Major Characteristic: Introduction of a strong EWG, e.g., a nitro group, in the para-position of the benzoyl ring (noted in Compound 2 and Compound 4).

Mechanism: The *para*-nitro group has a strong electron-withdrawing resonance and induction effect. This is directly extended to the carbonyl carbon in the ester, which is greatly made electrophilic.

Outcome: The increased electrophilicity of the carbonyl center renders it very susceptible to nucleophilic attack by water, thereby enabling the targeted acid-catalyzed hydrolysis at pH 2.2. while the meta position of nitro inhibits the hydrolysis of ester and sulfonate ester. This shows an effective approach to developing a promoiety which can undergo cleavage under certain conditions to set free the parent drug.

5.6. Antibacterial activity of macrolide derivatives

5.6.1. Sustaining Effectiveness Against *E. Coli*

All the derivatives showed a steady activity against *E. coli,* with the majority of compounds attaining an MIC of 4 μg/mL. **Compound 5 (**1.63 μg/mL) is a close

analogue to clarithromycin (1.01 μg/mL), whereas **Compound 4** (0.56 μg/mL) is superior to it.

5.6.2. Antibacterial activity against *Pseudomonas aeruginosa*

The parent compounds, azithromycin and clarithromycin, were both found to have poor activity against *P. aeruginosa* with an MIC >16 μg/mL, which classifies them in the resistant category compared to other new derivatives. Comparatively, the ten derivatives all had the lowest MIC of 8 μg/mL or less, with the highest activity than the parents. The most amazing observation in this category is **Compound 4** and **10**, which exhibited very good IC_{50} values of 0.1 and 0.11 μg/ml, respectively, against *P. aeruginosa* with an MIC of 8 μg/mL.

5.6.3. Exceptional Performance of *Streptococcus pneumoniae*.

Compound 8 proved to be the winner in both *S. pneumoniae* assays with an excellent IC_{50} value of 0.27 μg/mL and an excellent MIC of 0.5 μg/mL compared to the parent macrolides (azithromycin 0.41 μg/mL, clarithromycin 0.60 μg/mL). **Compound 3** (0.6 μg/mL) also demonstrated a result equivalent to that of clarithromycin.

5.6.4. Well performance against *Staphylococcus aureus*

Compound 8 (0.15 μg/mL) showed superior activity against (azithromycin 0.29 μg/mL) while **compound 3** (1.25 μg/mL) gave a low IC_{50} and good activity in comparison.

5.7. Dual Functionality derivatives: Taste Masking pro-drugs and Antimicrobial Efficacy derivatives.

5.7.1. Effective Prodrug Design.

The end objective of the study was to develop and prepare macrolide analogs that would effectively overcome the two-fold problem of flavoring and antimicrobial activity. These outcomes have proven that two different, but equally useful mechanisms have been used to achieve this objective. On the one hand, we have discovered a compound that can be used as a real prodrug, as the unpleasant taste of the original antibiotic is covered and then released in a controlled form. Conversely, we have learned a few compounds, which, though also presumably less bitter than the parent drugs, have proven themselves worthy as potent antimicrobial agents, with their own unique and impressive activity profiles.

The *p*-toluyl sulfonyl chloride analogue of azithromycin that is compounded as **Compound 5** is the ultimate appearance of an effective prodrug in this work. The fact that it was fully hydrolyzed to the parent azithromycin at 5.5 and 7.4 pH shows that it would be effective at releasing the active drug in the small intestine and in the systemic circulation. This, coupled with its probable poorer bitterness owing to the covalent alteration, makes it a good choice for a taste-masked formulation of azithromycin. Its ability to partially hydrolyze at pH 2.2 also indicates that it might be able to offer a rapid onset of action, which is a desirable characteristic of the treatment of acute infections. **Compound 5** will be as active against microorganisms as parent azithromycin after dissociation.

Compounds 8, in contrast, are a different and, possibly, even more significant result of this study. 3,5-dinitrobenzoyl derivative of clarithromycin has shown outstanding antimicrobial activity that is far better than that of the parent clarithromycin. As was mentioned previously, **Compound 8** had excellent potency against *Pseudomonas aeruginosa, Streptococcus pneumoniae,* and *Staphylococcus aureus,* giving a broad spectrum of activity. This compound is so strong on its own that it is unlikely that it is a prodrug but is actually a new chemical entity and has its own mechanisms of action. Although the likelihood that its taste properties are much less bitter than the parent clarithromycin is high due to the change in their chemical structure. Therefore, the compounds are a two-fold win: they should be more taste acceptable as compared to the parent medication, and they are more potent as well. This makes them very promising developmental new, independent antibiotics.

5.7.2. Summary: Broad-Spectrum Champion - Compound 8.

Compound 8 (Clarithromycin + 3, 5-Dinitrobenzoyl Chloride) comes out as the obvious wide-spectrum winner in this thesis and exhibits:

1- Better activity on 3/4 pathogens than parent.
2- High anti-*Streptococcus pneumoniae* potency against azithromycin and clarithromycin.
3- Good anti-staphylococcal effect, more than azithromycin.
4- The impressive anti-pseudomonal improvement in comparison with azithromycin and clarithromycin.
5- Full hydrolysis to unknown compound (new mechanism)

Probably masking of taste as a result of structural modification. The given compound is the best ever result of this study: the solution, which is based on the need to mask the taste of the drug, and represents better antimicrobial properties and a broad spectrum, thus, a good choice for becoming a new and improved version of macrolide-based antibiotics.

Compound 5: The Ideal Prodrug.

Compound 5 (Azithromycin + *p*-Toluene Sulfonyl Chloride) prodrug

1- Design of an ideal prodrug with full pH-dependent hydrolysis.
2- Good taste masking by chemical modification.
3- Known safety and efficacy profile (azithromycin)

5.8. Penicillin derivatives as a step toward enhanced potency

Biological analysis of these compounds was performed in terms of minimum inhibitory concentrations (MICs) and half-maximal inhibitory concentrations (IC_{50}) using four pathogenic bacteria that represent both Gram-positive and Gram-negative bacteria. To clarify the molecular pathway of the observed biological activities and inform rational drug design, we conducted extensive molecular docking experiments with the respective PBPs of each bacterial strain. The docking protocol was also carefully computed to determine binding conformations that most closely resemble those of co-crystallized ligands, but with special consideration to the distance between the beta-lactam carbonyl carbon and the catalytic serine hydroxyl group; this distance is crucial to forming covalent bonds.

5.8.1. Validation results:

A moderate correlation was obtained ($r = 0.70$, $R^2 = 0.48$, $n = 7$). This level of agreement is consistent with the empirical nature of the Vina scoring function, which is primarily parameterized for pose ranking and qualitative affinity ordering rather than for reproducing absolute experimental binding free energies. In addition, the limited number of compounds and the uncertainty associated with converting IC_{50} values to ΔG under the assumption $Ki \approx IC_{50}$ introduce additional variability. Nevertheless, compounds with stronger experimental affinity generally exhibited more favorable docking scores, supporting the use of this docking protocol as a qualitative, hypothesis-generating tool rather than a fully quantitative predictor of binding free energy.

5.9. Biological activity and molecular docking results

5.9.1 Activity Against Escherichia coli

The MIC values against *E. coli* showed that the majority of the synthesized compounds demonstrated moderate activity, with **Compounds 11, 12, 13, 15, 16, 17, 18, 19,** and **20** showing MIC of 32 µg /mL. It is worth noting that amoxicillin had a higher activity with an MIC of 16 µg/mL. The values of the IC_{50} have given more quantitative data regarding the inhibiting activity of each compound, so IC_{50} values will be used to interpret the results and to make the necessary comparisons in a more precise way in the process of my discussion.

The IC_{50} data against *E. coli* revealed that **Compound 13** exhibited the most potent activity with an IC_{50} of 16.19 µg/mL, closely followed by amoxicillin (17.19 µg/mL) and **Compound 15** (19.09 µg/mL). The molecular docking studies against *E. coli* PBP (PDB ID: 6I1I) showed generally good consistency between docking scores and biological activity. **Compound 15** achieved the most favorable docking score of -8.537 kcal/mol, while **Compound 13** showed a docking score of -8.461 kcal/mol compared to amoxicillin (-8.13 kcal/mol), consistent with their low IC_{50} values.

Compound 13 has two nitro groups, one of which interacts with the hydrogen atom of CH in LYS 358 and the oxygen atom in the nitro group, forming a hydrophobic interaction, and the other between the oxygen of the nitro group and the hydrogen in NH of GLY 542 and GLY 543, forming an H-bond interaction, and a π-π interaction between the aromatic ring and the aromatic in VAL 344, thus stabilizing the enzyme-inhibitor complex, and this seems to give **Compound 13** increased activity against *E. coli.* also, nitro groups are electron-withdrawing groups, which can make and enhance the electrophilicity of the beta-lactam carbonyl carbon, allowing the active site serine residue to do nucleophilic attack. **Compound 15**, theoretically, showed better performances than amoxicillin, but in the experiment, it showed an activity that was almost less as but near amoxicillin. Theoretical research showed that the methyl group created more hydrophobic (pi-alkyl) interactions with TYR 540 and TYR 541. The additional interaction with the oxygen atom of the sulfonyl group that interacted by hydrophobic interaction with the hydrogen of CH in THR 497. SER 307 forms an H-bond with the carbonyl of the open β-lactam ring. Surprisingly, **Compound 12,** which was found to be really active against other bacterial strains, had a comparatively low activity against *E. coli* (IC_{50} = 28.90 µg/ml) and low binding affinity (-7.762 kcal/mol).

This implies that different bacterial species may have different structural conditions to be optimal, perhaps because of differences in the structure of the PBPs, outer membrane permeability, or the activity of efflux pumps. The activity of **Compound 20** (IC_{50} = 44.39 μg/ml) is relatively low with low binding activity (-6.817 kcal/mol), showing that the p-toluene sulfonyl chloride modification is not an ideal one regarding activity against *E. coli*, maybe because of a steric hindrance or a poor hydrophobic reaction.

5.9.2 Activity Against *Pseudomonas aeruginosa*

The findings against *P. aeruginosa* were quite impressive and symbolize the most important findings of this research. **Compound 12 and Compound 17** exhibited remarkable activity with a MIC of 2 μg /mL, which is an incredible improvement compared to amoxicillin, which had a MIC of >128 μg /mL. This indicates over 64 times better potency, which reflects the important effect of the 4-nitrobenzoyl modification. These results were supported by the IC_{50} determinations, with **Compound 12** having an exceptionally low IC_{50} of 0.66 μg/mL, the strongest activity recorded in this part of investigation. **Compound 17** was also found to be very active with an IC_{50} of 2.34 μg /mL compared to amoxicillin (122.50 μg /mL). The molecular docking experiments allowed mechanistic explanations of this impressive potency. **Compound 12** had the best docking score of -10.182 kcal/mol with *P. aeruginosa* PDP (PDB ID: 6UN1) compared to amoxicillin (-7.201 kcal/mol), which was definitely the most favorable score of all the compounds tested. The remarkable *P. aeruginosa* activity of **Compounds 12** and **17 (**-8.008 kcal/mol) can be explained by a number of factors. To begin with, the 4-nitrobenzoyl moiety has a nitro group in the para position that has the power to withdraw electrons inductively and resonantly. This increases the electrophilicity of the beta-lactam carbonyl carbon, thus becoming more reactive to the nucleophilic serine residue of the PBP active site. Second, the nitro group in **compound 12** also made additional stabilizing H-bond contacts between the oxygen in nitro group and hydrogen atoms in NH of both GLY 534 and GLY 535 in the enzyme, pi-sulfur interaction between aromatic ring of benzoyl moiety and sulfur in thiazolidine ring, hydrogen bonds between the carbonyl oxygen atom of amide bond and hydrogen atoms in NH in both (ASN 351 and LYS 297) and hydrogen bond interaction between the hydrogen atom of NH of amide bond and oxygen carbonyl of THR 487 which also make hydrophobic interaction with hydrogen of CH near amide group while in **Compound 17** π-doner hydrogen interaction (hydrophobic interaction) between aromatic ring of benzoyl moiety and hydrogen atom of NH in ARG 489. It is noticeable that the 4-

nitrobenzoyl modification **(Compounds 12** and **17**) was the most effective against *P. aeruginosa* in comparison to the 3,5-dinitrobenzoyl modification (**Compound 13**). **Compound 13** was found to be active against *E. coli*, but was inactive against *P. aeruginosa* (IC_{50} = 125.60 μg/mL) with a binding energy (-6.500 kcal/mol). This implies that the location of the nitro group is important in activity against *P.* aeruginosa, with the para position being the best. The addition of two nitro groups on the 3,5-positions can cause steric or undesired electronic effects leading to a decrease in binding affinity to the *P. aeruginosa* PBP, while in **compound 18 (**75.67 μg /mL) it is good maybe due to the amoxicillin scaffold. **Compound 16** also showed considerable activity against *P. aeruginosa* with an MIC of 16 μg /mL and an IC_{50} of 23.79, which is a significant improvement compared to parent amoxicillin. **Compound 16** had the second-best docking score of -9.323 kcal/mol, which indicates that the N-acetyl benzene sulfonyl moiety has good binding interactions with the target enzyme in amoxicillin derivatives. The low potency of **Compound 11** (IC_{50}= 279.30 μg/mL) and low docking score (-6.040 kcal/mol) suggests that the 6-APA scaffold, when substituted with N-acetyl benzene sulfonyl moiety, is not sufficient to inhibit the growth of *P. aeruginosa*.

5.9.3 Activity Against *Streptococcus pneumoniae*

Compared to *S. pneumoniae*, the MIC results indicated that the majority of compounds had moderate activity, with the range of MIC values between 32 and 64 μg/mL. The determinations of the IC_{50}, however, did show more significant differences in potency amongst the compounds. Interestingly, **Compound 20** had the highest potency against *S. pneumoniae* and an IC_{50} of 7.22 μg/mL, showing better activity than amoxicillin (IC_{50} = 17.23 μg/mL). This will be a 2.4-fold potency improvement. **Compound 15** was also an active compound with an IC_{50} of 13.71 μg/mL. The results indicate that the *p*-toluene sulfonyl functional group (both in **compound 15** and **compound 20**) is especially compatible with activity against *S. pneumoniae*, which may be a consequence of an ideal fit in the PBP2x binding pocket. Interestingly, **Compound 12** was also active against *S. pneumoniae* (*IC50* 14.97 μg/mL). Molecular docking against *S. pneumoniae* PDB (PDB ID: 5OJ1) showed that there was a significant consistency between calculations and experimental findings. Analysis of the docking scores reveals that **Compounds 12, 15, 16, 18, 19,** and **20** obtained good binding energies, and are some of the best compounds in theory. Notably, it is well consistent with the experimental results because **Compounds 12, 15,** and **20** exhibited the lowest IC_{50} values (14.97, 13.71, and 7.22 μg/mL, respectively), validating that they are the

most active choices based on docking. **Compound 16** had the best docking score (-9.233 kcal/mol), and **Compound 18** (-9.170 kcal/mol) had better docking score than amoxicillin (-7.030 kcal/mol). **Compound 16** exhibited an IC_{50} of 18.95 µg/mL, not better than amoxicillin (17.23 µg /mL), but very close. These docking outcomes were correct in their prediction that **Compound 16** would have an activity similar to that of amoxicillin, with a similar IC_{50} value as seen between the two compounds, with just a 1.1-fold difference. This activity similarity with the high docking score confirms the computational method and gives reason to believe that **Compound 16** is an effective active site binder of the *S. pneumoniae* PDB active site. Likewise, **Compound 18** had an IC_{50} of 22.65 µg/mL, not very high and not better than amoxicillin, but it has a good docking score and has good activity against this pathogen. **Compound 19** (IC_{50} = 29.92 µg/mL) and **Compound 20** were also found to dock with good scores (-7.741 and -8.491 kcal/mol, respectively), **Compound 20** having the best experimental activity, also indicating the consistency between computational and experimental data, its higher affinity due to π–π interaction between the extra aromatic ring and TRP 374, π-alkyl interaction between the extra methyl group and pyrrole ring in TRP 374, pi-sulfur interaction between the sulfur atom in sulfonyl group and the aromatic ring in PHE 570, and hydrogen bond and hydrophobic interaction between the oxygen of the sulfonyl group and the hydrogens in OH of THR 540 and CH of GLY 549 respectively. **Compound 12** has high activity due to an extra hydrogen bond between the nitro group and hydrogen atom in OH of THR 370, a π-doner interactions between the aromatic ring in benzoyl moiety and hydrogen atom of NH in GLN 552, a π-doner interactions between the aromatic ring in benzoyl moiety and hydrogen atom of NH in ARG 372, pi-alkyl interaction between aromatic ring of benzoyl with alkyl group of ARG 372 and pi-cation interaction between aromatic ring of benzoyl group and nitrogen atom in ARG 372. Conversely, **Compound 17** had a comparatively low degree of activity (IC_{50} = 57.72 µg/mL), binding energy despite its outstanding performance versus other bacterial strains, and its docking score (-6.811 kcal/mol) was one of the lowest energy, which accurately predicted its poor activity. Such a species-specific difference in activity demonstrates the structural diversity of PBPs of different bacterial species and the relevance of testing antimicrobial agents against various pathogens. The success of the docking studies to implement the compounds that exhibited the most favorable experimental activity (**Compounds 12**, **15**, and **20**) and to be able to predict the relative

performance of **Compound 16** in comparison to that of amoxicillin indicates the effectiveness of molecular docking as a predictive measure of this bacterial target.

5.9.4. Activity Against *Staphylococcus aureus*

Values of the results against *S. aureus* showed the most significant variations in activity between the synthesized compounds. **Compound 12** also displayed unusual potency of MIC of 1 μg /mL, and IC_{50} of 0.11 μg /mL, which is the lowest IC_{50} of this whole study. This is a 9-fold increase over amoxicillin (MIC = 4 μg/mL, IC_{50} 1.01 μg/mL), which is, in turn, considered very active against susceptible strains of *S. aureus*. **Compound 17** had an IC_{50} value (2.46 μg /mL) that is nearly near to that of amoxicillin (1.01 μg /mL). The docking results support this near value in biological activity because **Compound 17** had a better docking score (-8.285 kcal/mol) than amoxicillin (-8.057 kcal/mol). Such good agreement between the computational predictions and experimental findings further justifies the validity of the molecular docking method in predicting antibacterial activity against *S. aureus*. **Compound 12** recorded the best docking score of -8.365 kcal/mol, with **Compound 17** recording -8.285 kcal/mol. It is possible to explain the outstanding activity of **Compound 12** against *S. aureus* by the presence of the most favorable interactions between the nitrobenzoyl group and the active site of PBP. This effect is caused by the electron-withdrawing nitro group that increases the reactivity of the beta-lactam ring to a nucleophilic attack by the catalytic serine residue, leading to the rapid and efficient acylation of the enzyme. Also, in **compound 12**, the 4-nitrobenzoyl group obviously forms hydrogen bond interactions between the oxygen atom in a nitro group and hydrogen atom of NH in ASN 502, π-π stacking interactions between the aromatic ring of benzoyl moiety and the benzene ring TYR 566, and hydrogen bond interactions between the oxygen atom of carbonyl of the benzoyl group and hydrogen atom in OH of TYR 566 residues in the binding pocket, while **compound 17** has hydrogen bond interactions between the oxygen atom in nitro group and hydrogen atom in NH in ASN 502 and hydrogen bond interaction between the oxygen atom in carbonyl group of the benzoyl group and hydrogen atom in OH of TYR 566. When **Compound 12** (6-APA-based) and **Compound 17** (amoxicillin-based), both with the same 4-nitrobenzoyl modification, are compared, it is evident that the 6-APA scaffold would be much more active against *S. aureus* (IC_{50} = 0.11 μg /mL vs. 2.46 μg /mL). This 22-fold difference in potency indicates the possibility of a steric constraint or bad interaction with the presence of the amoxicillin structure, which lowers the binding affinity to the *S. aureus* PBP. The simpler design of the 6-APA-based

Compound 12 could also enable a more favorable orientation of the 4-nitrobenzoyl group of the active site in the 6-APA-based structure. This may be due to ineffective membrane penetration or fast efflux.

5.10. Structure-Activity Relationships and Mechanistic Insights.

A high-quality comparison of 10 structurally related compounds with four bacterial species has shown some interesting structure-activity correlations:

1. The 4-nitrobenzoyl variant is best suited to wide-spectrum action. Both **compounds 12** and **17,** containing the 4-nitrobenzoyl group, were highly active against *P. aeruginosa*, *S. aureus* and *S. pneumoniae* for **compound 12**. The para-positioned nitro group exhibits the best electronic and steric characteristics to inhibit PBP.

2. Sulfonyl modifications are variable. The p-toluene sulfonyl reaction (**Compounds 15** and **20**) proved most advantageous in activity against *S. pneumoniae, P. aeruginosa and E. coli* for **compound 15** but less so against other species. The 4-nitrobenzene sulfonyl functional group (**Compounds 14** and **19**) tended to give low activity in all species except *P. aeruginosa*, implying a lack of an optimal combination of a sulfonyl functional group and a nitro group.

3. β-Lactam reactivity is increased by electron-withdrawing groups. The enhanced reactivity of the nitro-containing compounds consistent with the hypothesis that electron-withdrawing groups enhance the electrophilicity of the β-lactam carbonyl carbon, which can be attacked by the active site serine residue in a nucleophilic attack of PBP. This increased reactivity corresponds to increased efficiency of enzyme acylation and increased antibacterial activity.

5.11. Broad-Spectrum Activity and Clinical Implications (the champions of the penicillin derivatives)

An antimicrobial agent with activity toward Gram-positive and Gram-negative bacteria is referred to as a broad-spectrum antibiotic (Acar, 1997). The findings of this research suggest that **Compound 12** and **Compound 17** have true broad-spectrum activity, and that they are active against most of the four bacterial species used, but **Compound 12** demonstrated superior performance. This is especially important considering the fact that amoxicillin, the parent compound to **Compound 17,** displays low activity against P. aeruginosa (MIC >128 μg/mL).

The fact that these compounds could bypass the intrinsic resistance *of P. aeruginosa*, but remain highly active against Gram-positive pathogens, is a major achievement. *P. aeruginosa* is well-known hard to cure because its outer membrane is impermeable, it expresses efflux pumps constitutively and has chromosomal β-lactamases. **Compounds 12** and **17** reached MIC values of 2 μg /mL against this difficult pathogen is an indication that the 4-nitrobenzoyl modification imparts properties that allow the compounds to circumvent these resistance mechanisms.

Global health has given high priority to the development of new β-lactam antibiotics that are more active against these pathogens. These compounds are lead compounds, which have a good chance of developing into therapeutic agents. And consider the basis for the future to face the increase in antibiotic resistance.

Taken together, this work demonstrates that rational prodrug and analogue design, guided by pH-sensitive linkers and electronic design, can simultaneously address poor palatability and limited antimicrobial performance of key macrolide and β-lactam antibiotics. By introducing sulfonyl and benzoyl promoieties onto azithromycin and clarithromycin, we showed that hydrolysis behavior can be finely tuned along the gastrointestinal pH gradient, with **Compound 5** (azithromycin–*p*-toluene sulfonyl chloride) emerging as an exemplary taste-masked prodrug that is partially hydrolyzed under gastric conditions and fully converted to the active drug at intestinal and physiological pH, thereby supporting both rapid onset and complete systemic availability. In parallel, the 3,5-dinitrobenzoyl clarithromycin derivative (**Compound 8**) displayed markedly enhanced activity against *Pseudomonas aeruginosa*, Streptococcus *pneumoniae*, and *Staphylococcus aureus*, suggesting a promising new macrolide-like chemical entity that likely combines reduced bitterness with a broadened, high-potency antimicrobial profile. Extending this strategy to β-lactam scaffolds, benzoyl and sulfonyl modifications of 6-APA and amoxicillin generated penicillin derivatives with substantial gains in potency and spectrum, most notably **Compounds 12** and **17**, which achieved true broad-spectrum activity and overcame the intrinsic resistance of *P. aeruginosa*, and **Compound 20**, which significantly improved activity against *S. pneumoniae*. Structure–activity analysis, supported by molecular docking with relevant PBPs, confirmed that *para*-nitrobenzoyl and selected sulfonyl groups enhance β-lactam electrophilicity and optimize active-site interactions, accounting for the superior MIC and IC_{50} profiles of these leads. Overall, the integrated experimental and computational findings highlight **Compounds 5** and **8** among the macrolides and **Compounds 12, 17,** and **20** among the

penicillin derivatives as promising candidates for further preclinical development, and more broadly validate linker-based, electronically tuned modification of existing antibiotics as a viable strategy to improve taste, potency, and spectrum in the face of rising antimicrobial resistance.

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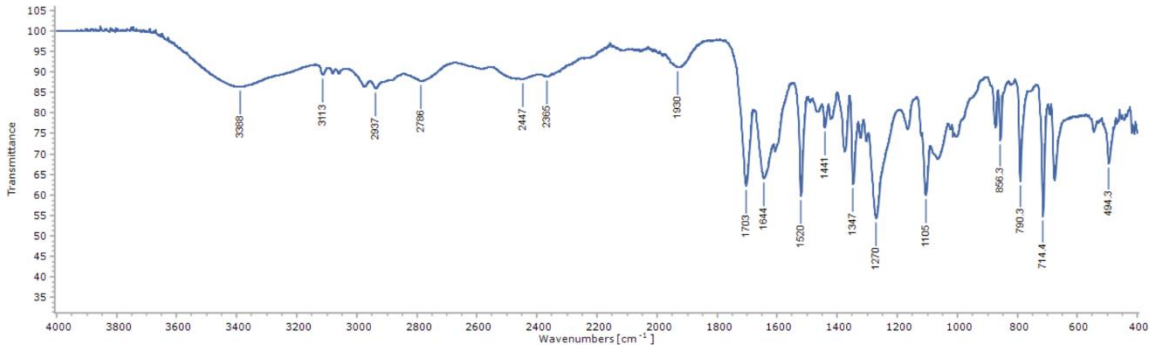
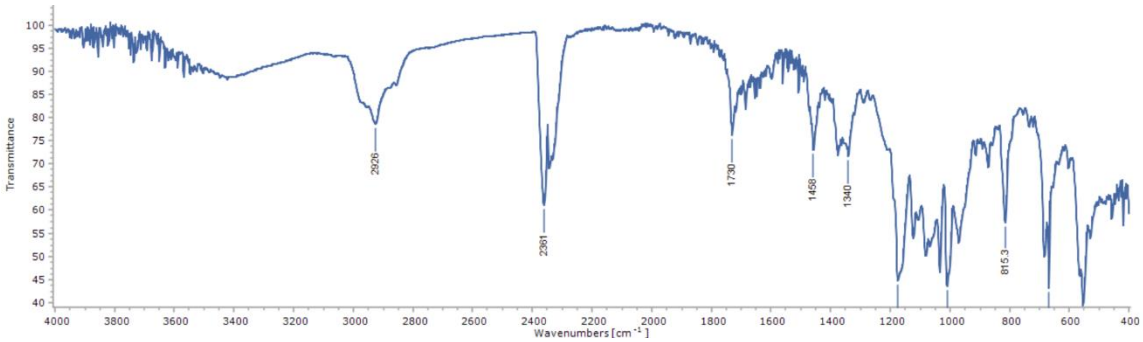
Appendices

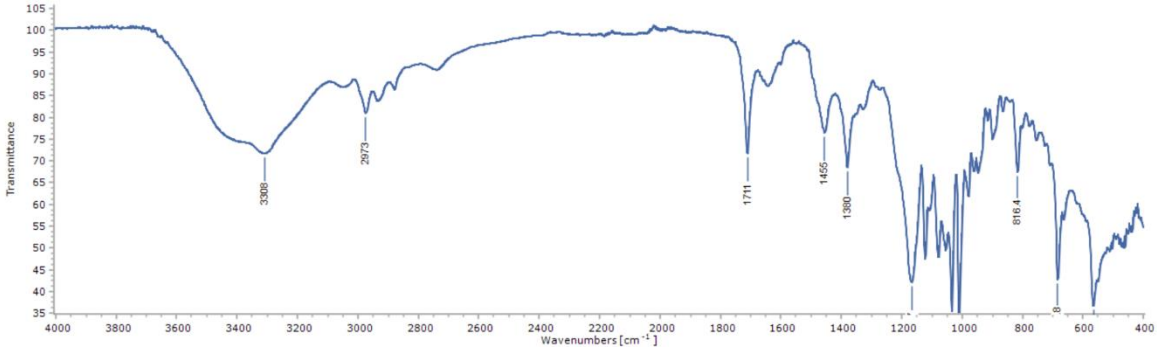
Appendix 1: *FT-IR* Spectra of compound (1)



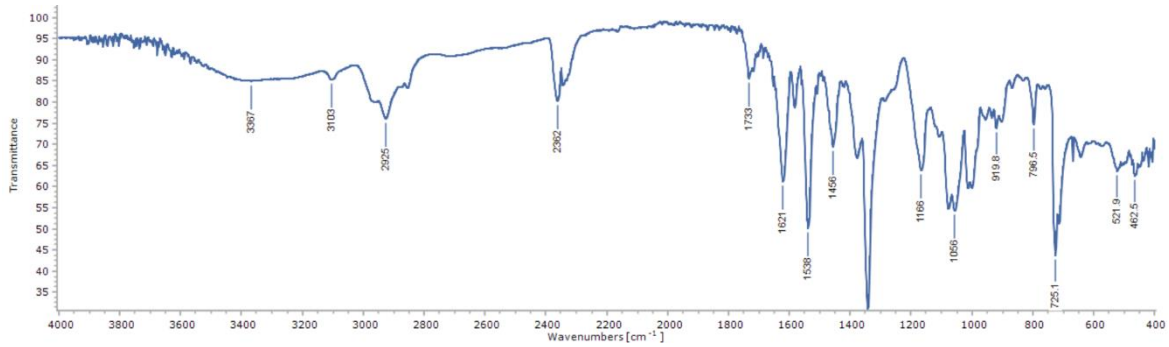
Appendix 2: *FT-IR* Spectra of compound (2)



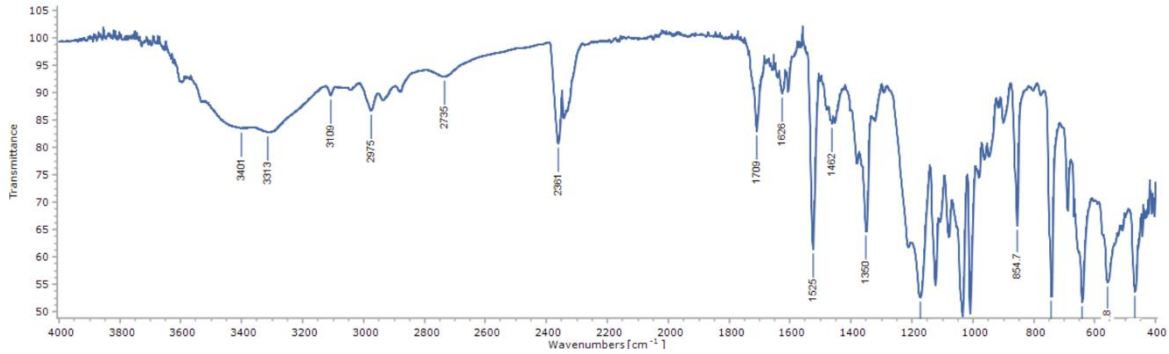
Appendix 3:*FT-IR* Spectra of compound (3)



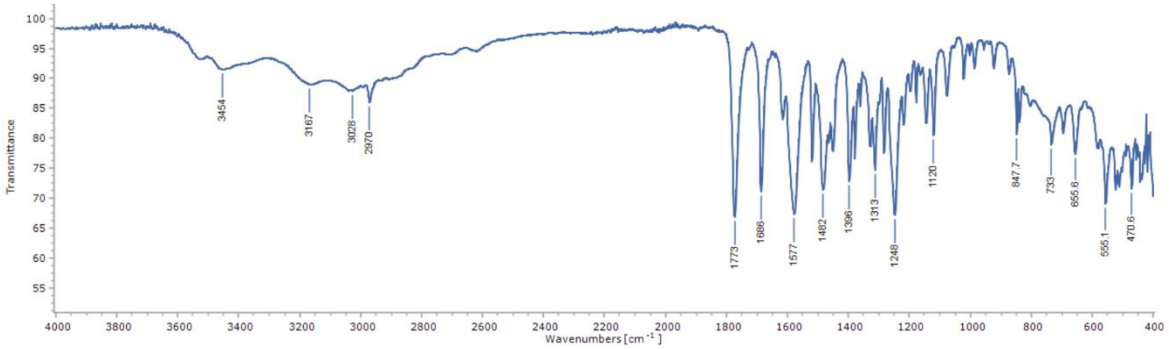
Appendix 4: *FT-IR* Spectra of compound (4)



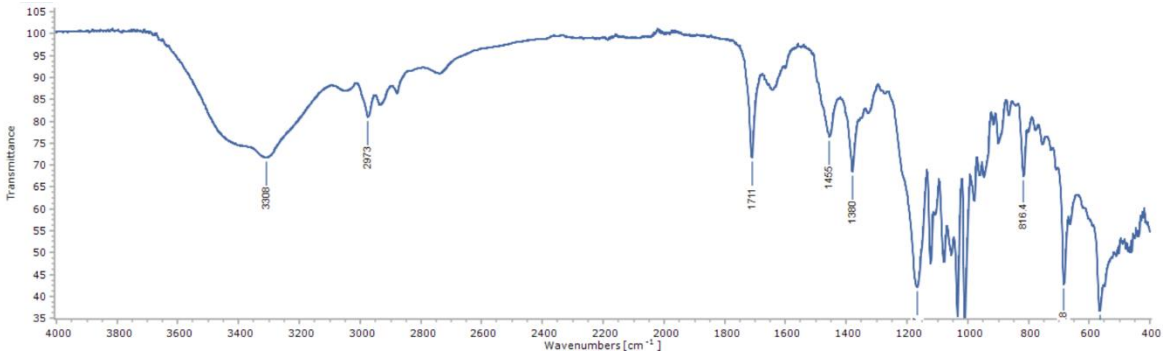
Appendix 5:*FT-IR* Spectra of compound (5)



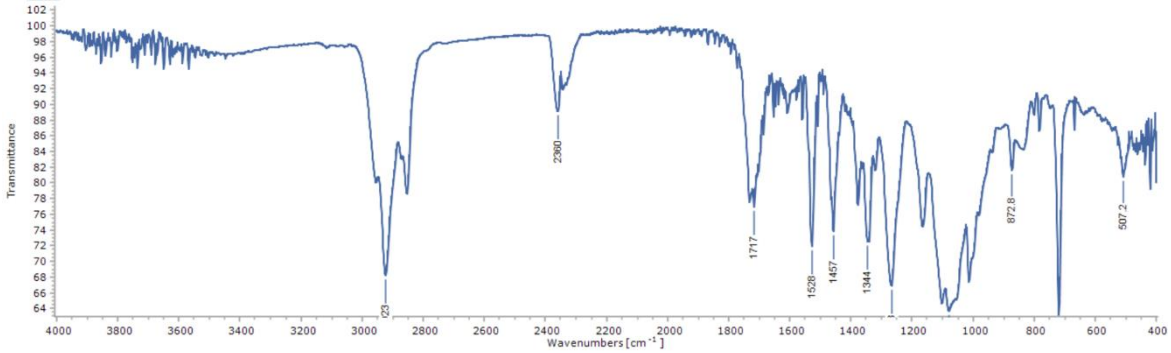
Appendix 6:*FT-IR* Spectra of compound (6)



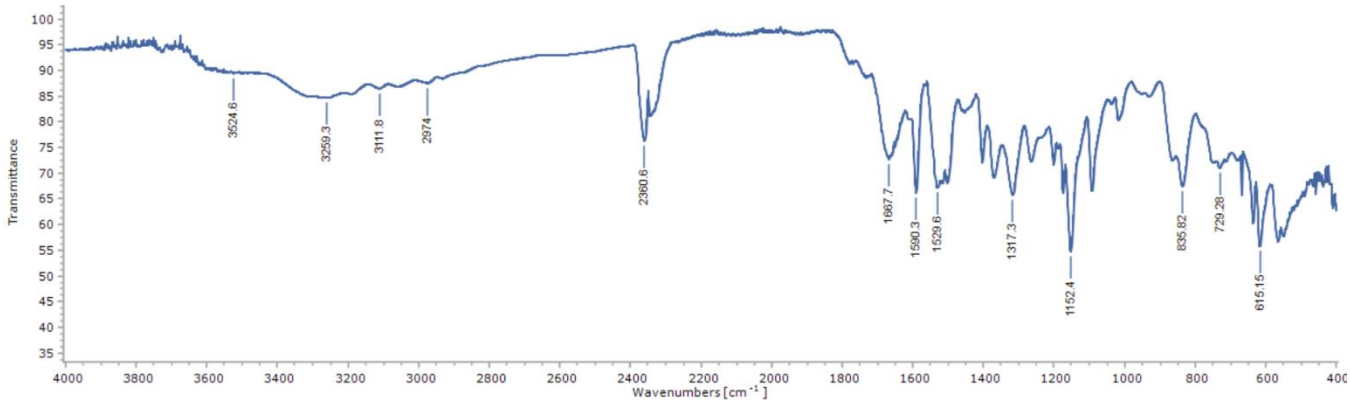
Appendix 7:*FT-IR* Spectra of compound (7)



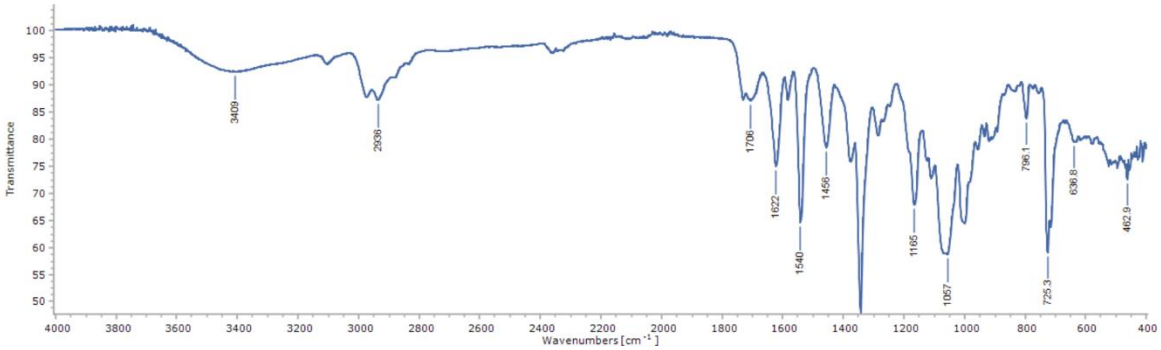
Appendix 8:*FT-IR* Spectra of compound (8)



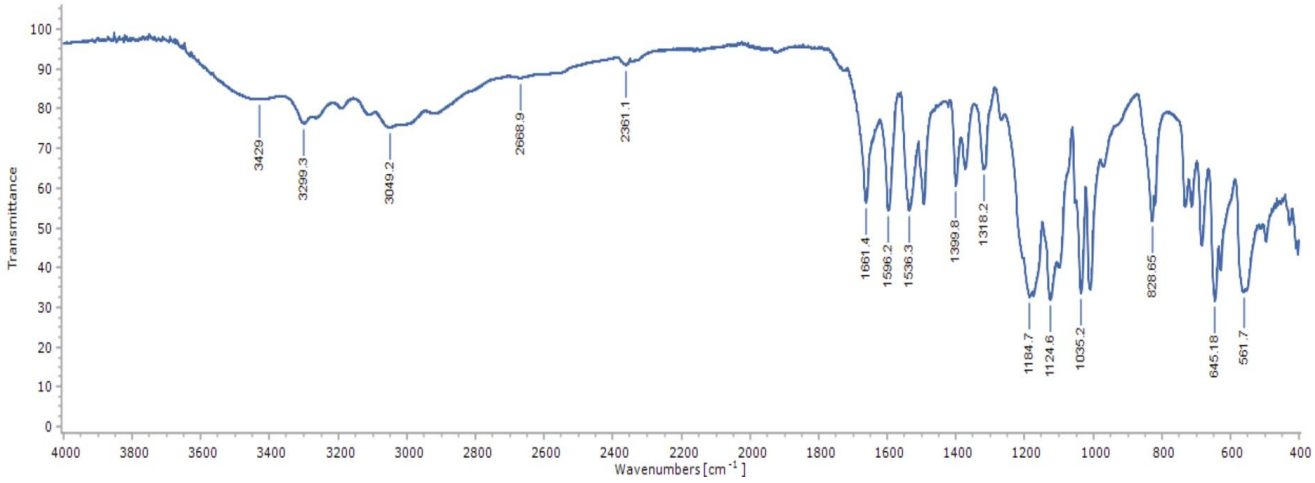
Appendix 9: *FT-IR* Spectra of compound (9)



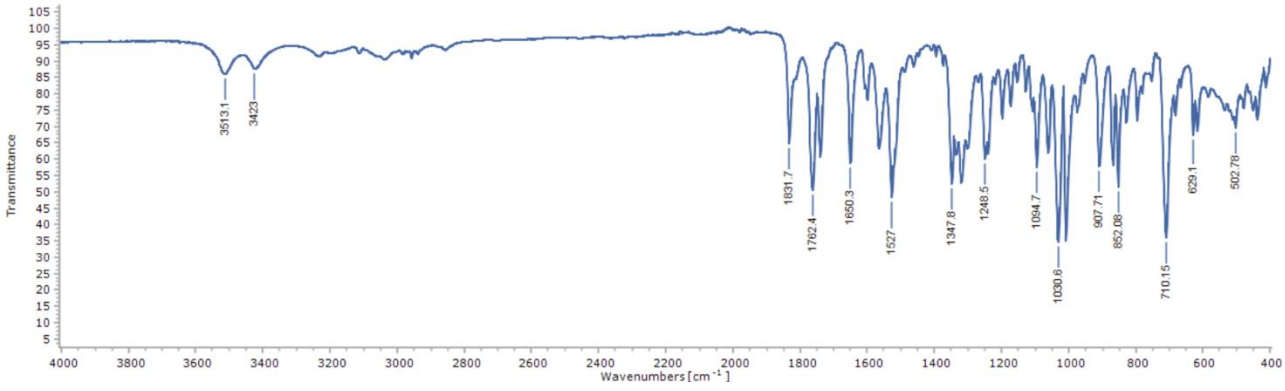
Appendix 10: *FT-IR* Spectra of compound (10)



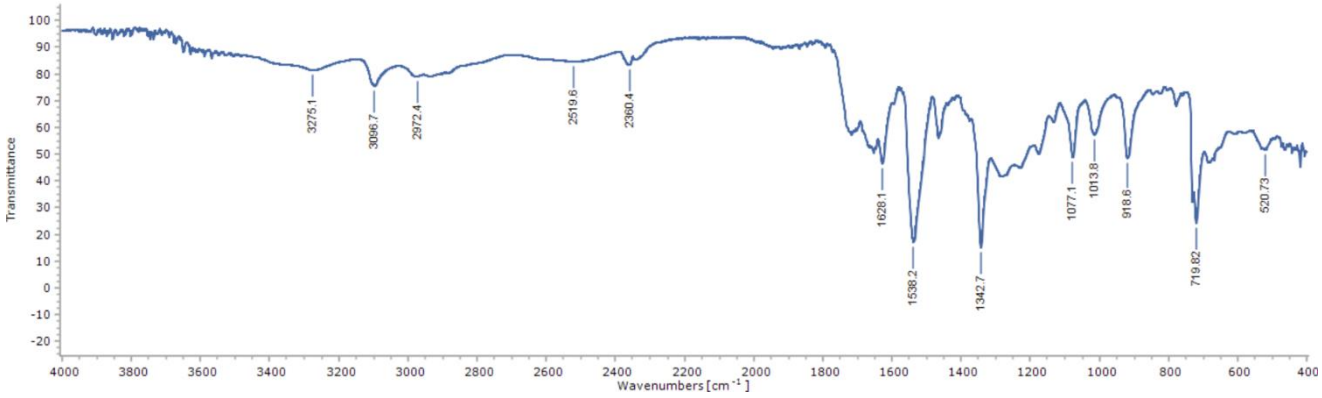
Appendix 11: *FT-IR* Spectra of compound (11)



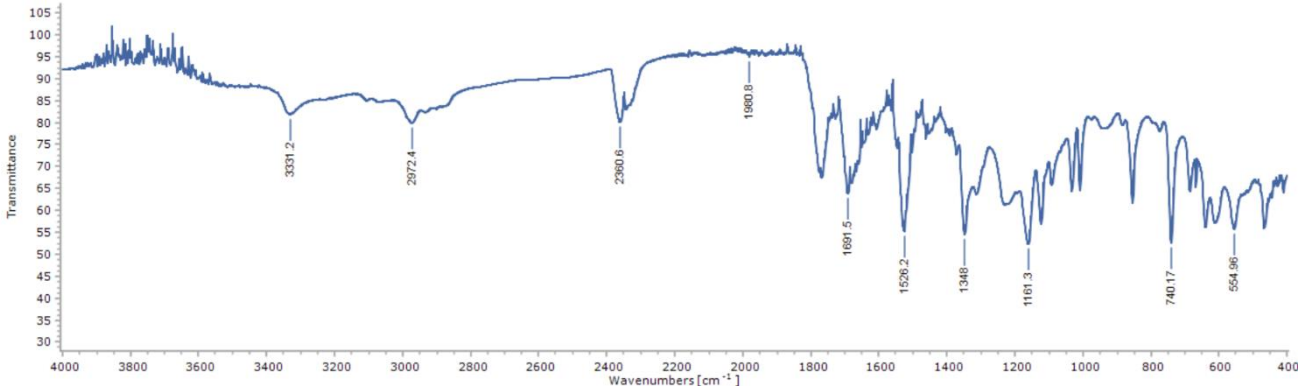
Appendix 12: *FT-IR* Spectra of compound (12)



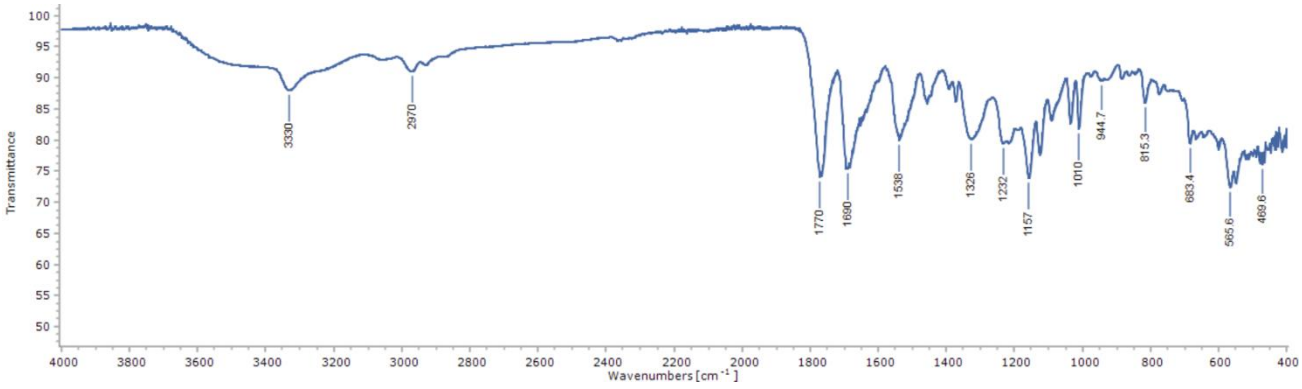
Appendix 13: *FT-IR* Spectra of compound (13)



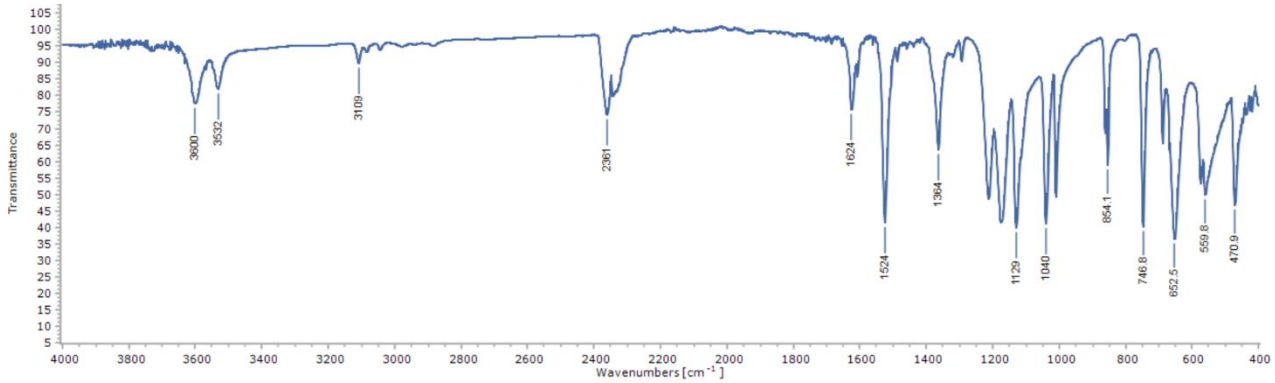
Appendix 14: *FT-IR* Spectra of compound (14)



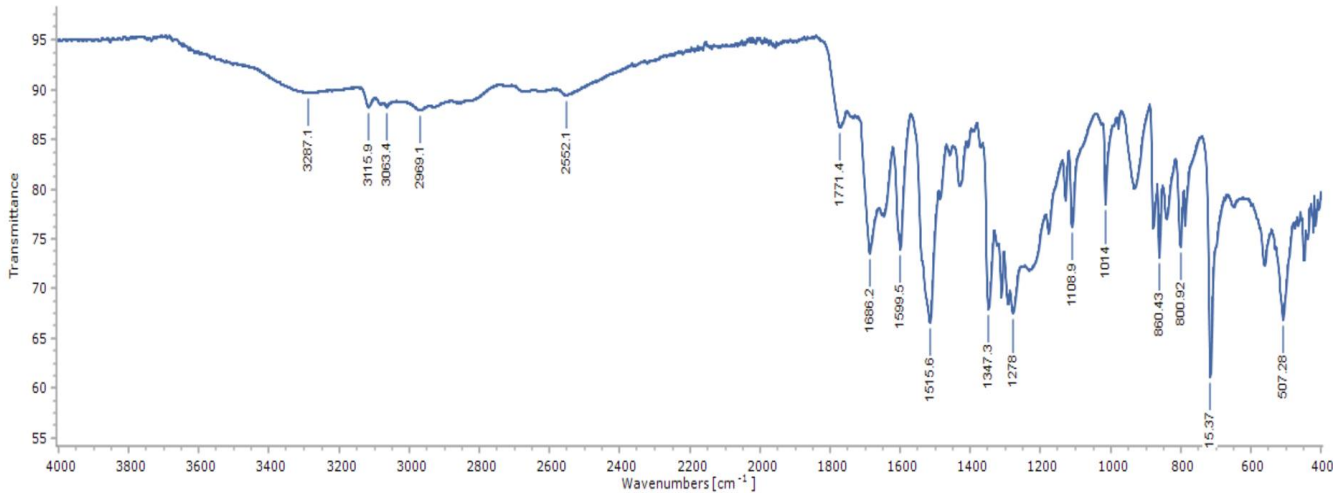
Appendix 15: *FT-IR* Spectra of compound (15)



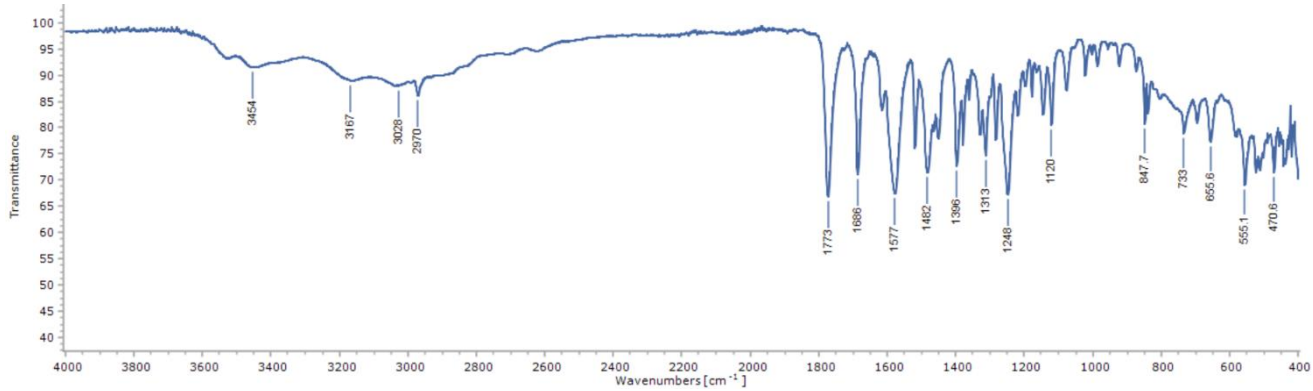
Appendix 16: *FT-IR* Spectra of compound (16)



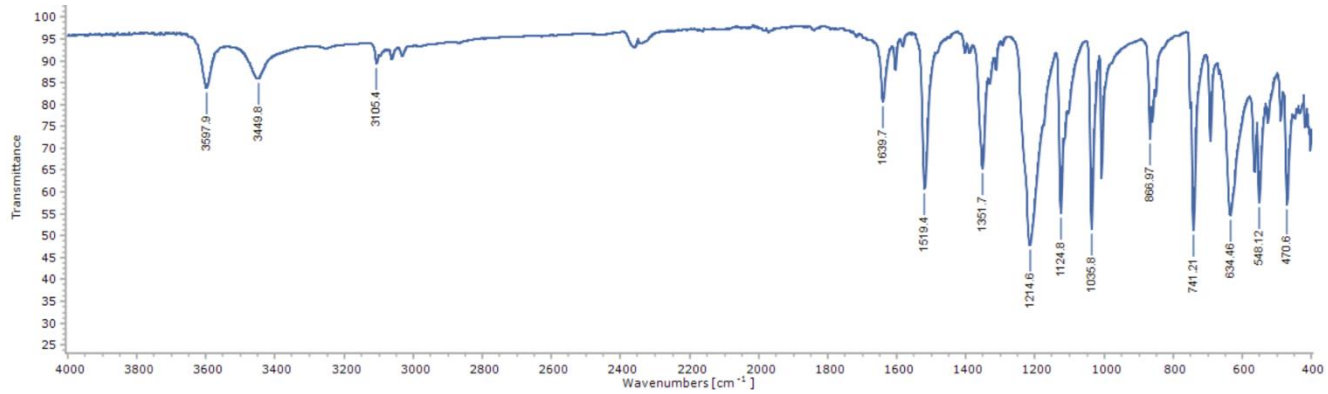
Appendix 17: *FT-IR* Spectra of compound (17)



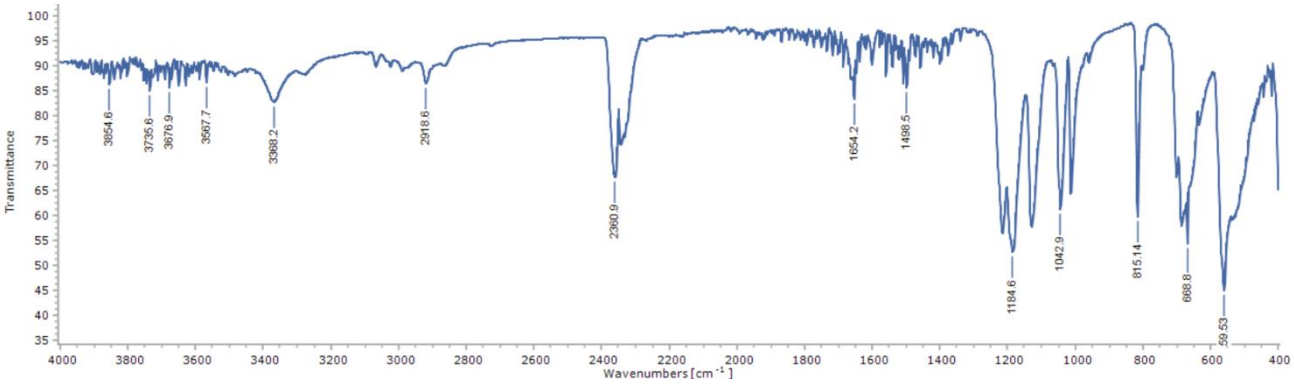
Appendix 18: *FT-IR* Spectra of compound (18)



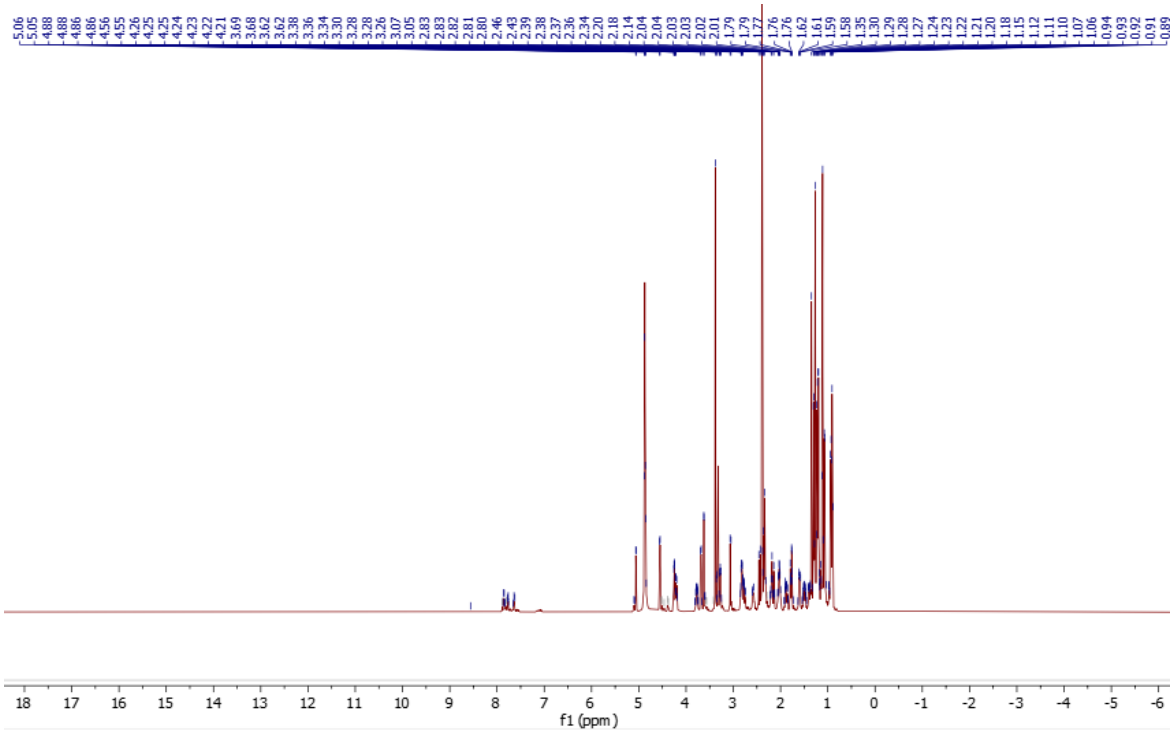
Appendix 19: *FT-IR* Spectra of compound (19)



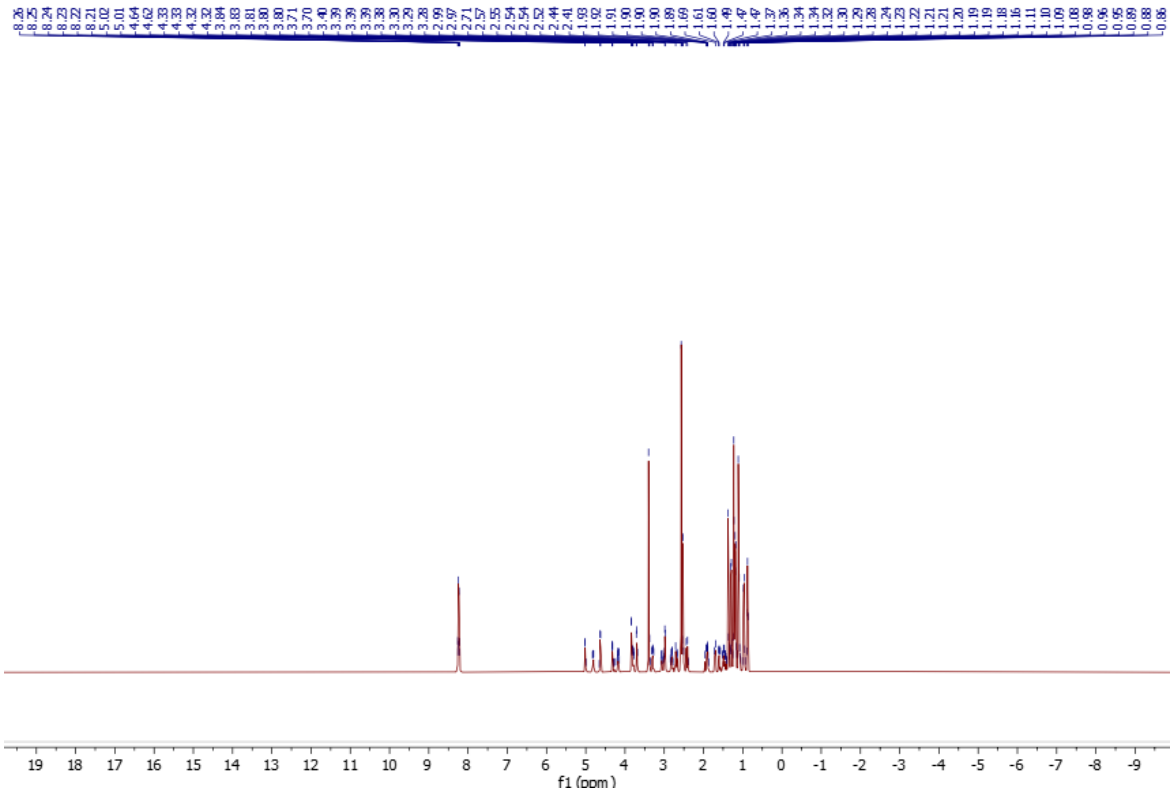
Appendix 20: *FT-IR* Spectra of compound (20)



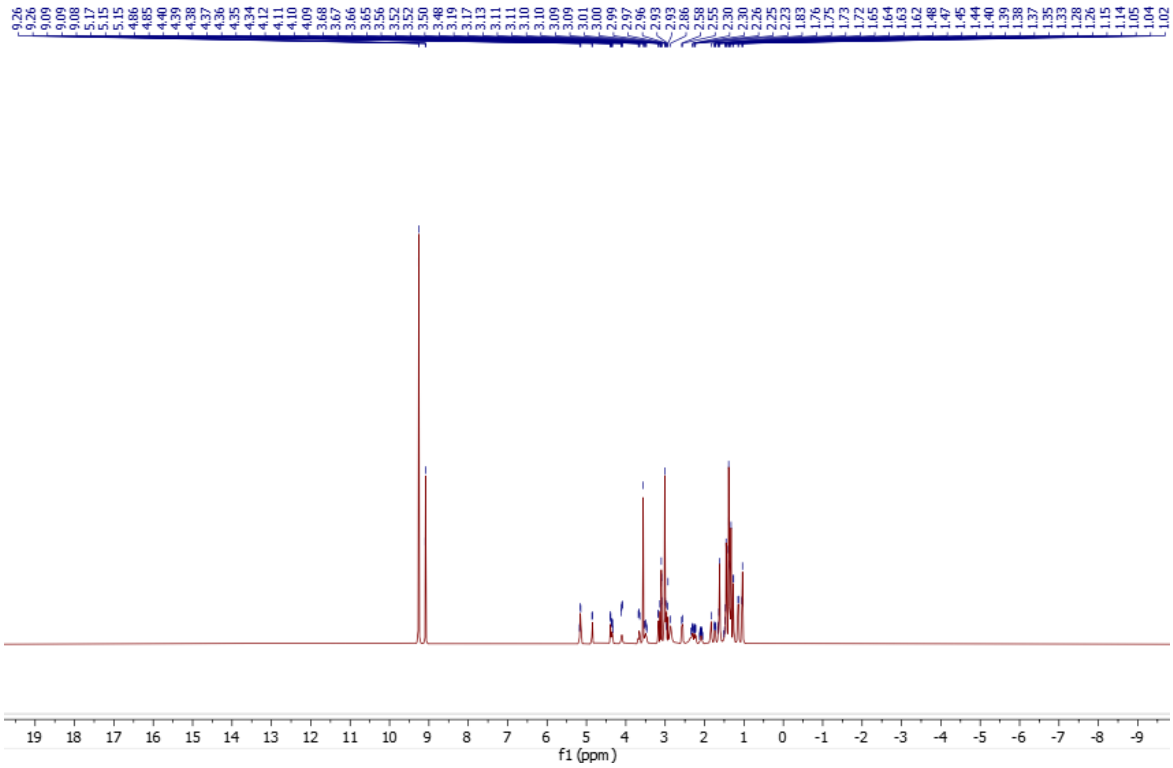
Appendix 21:*[1]H-NMR* Spectra of compound (1)



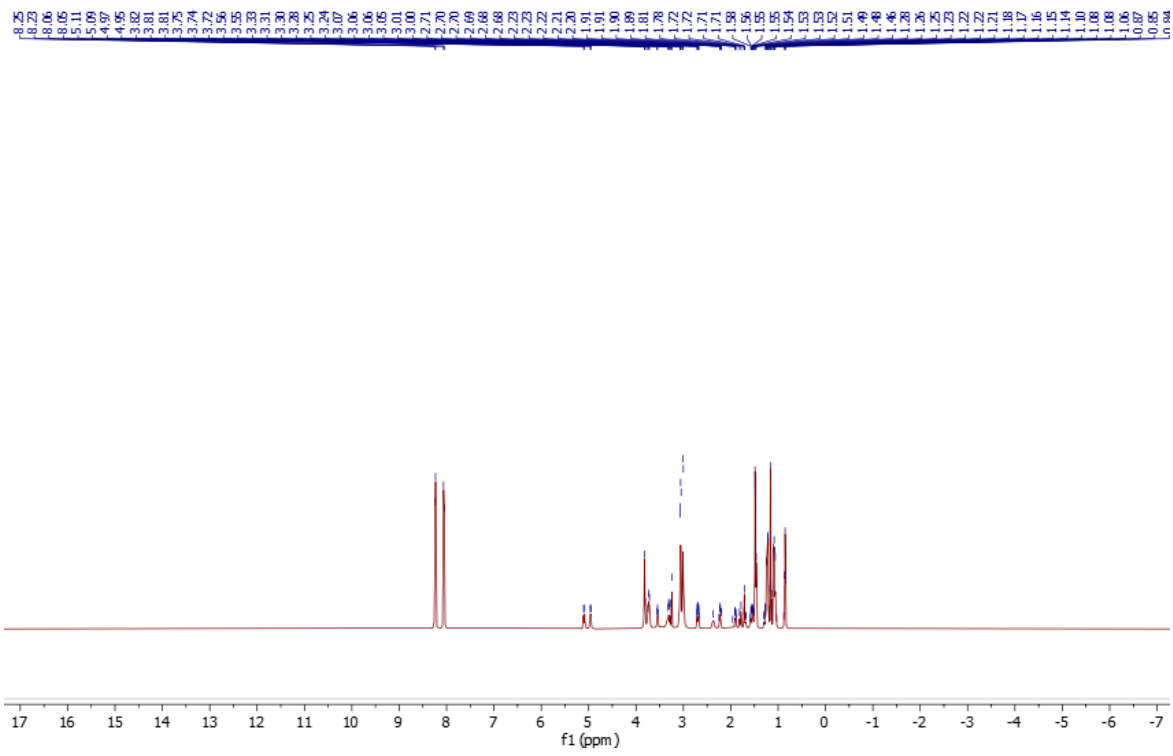
Appendix 22:*[1]H-NMR* spectra of compound (2)



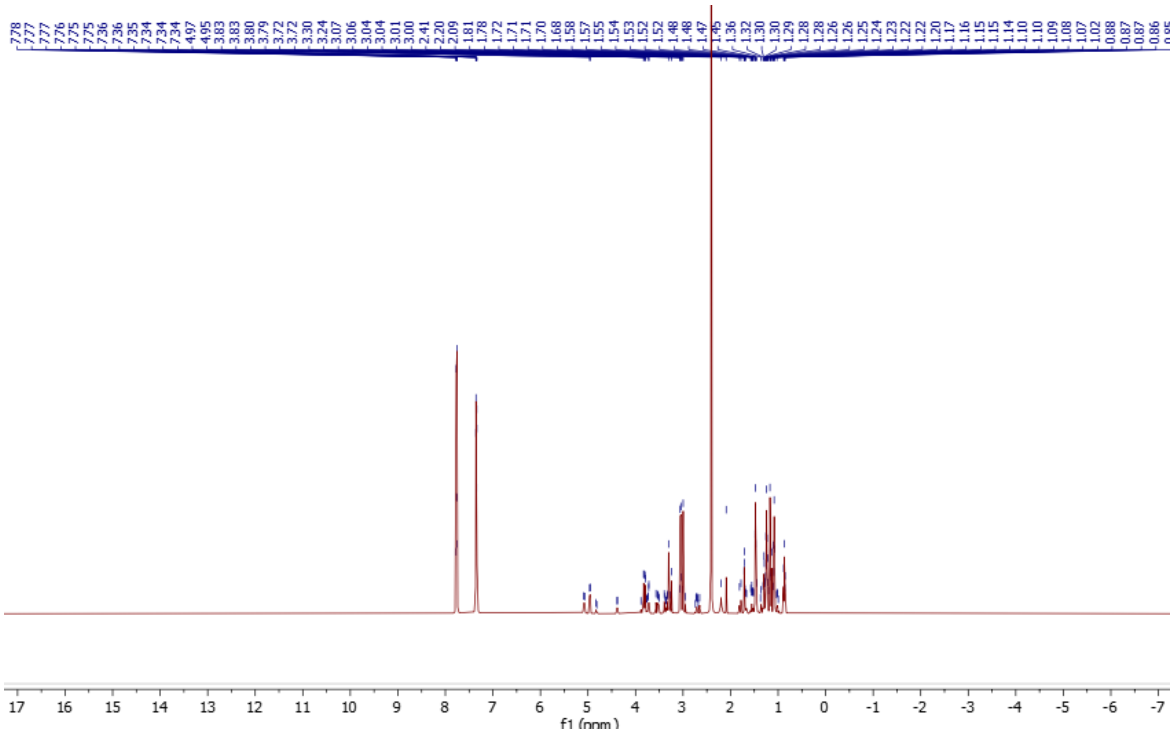
Appendix 23: *[1]H-NMR* spectra of compound (3)



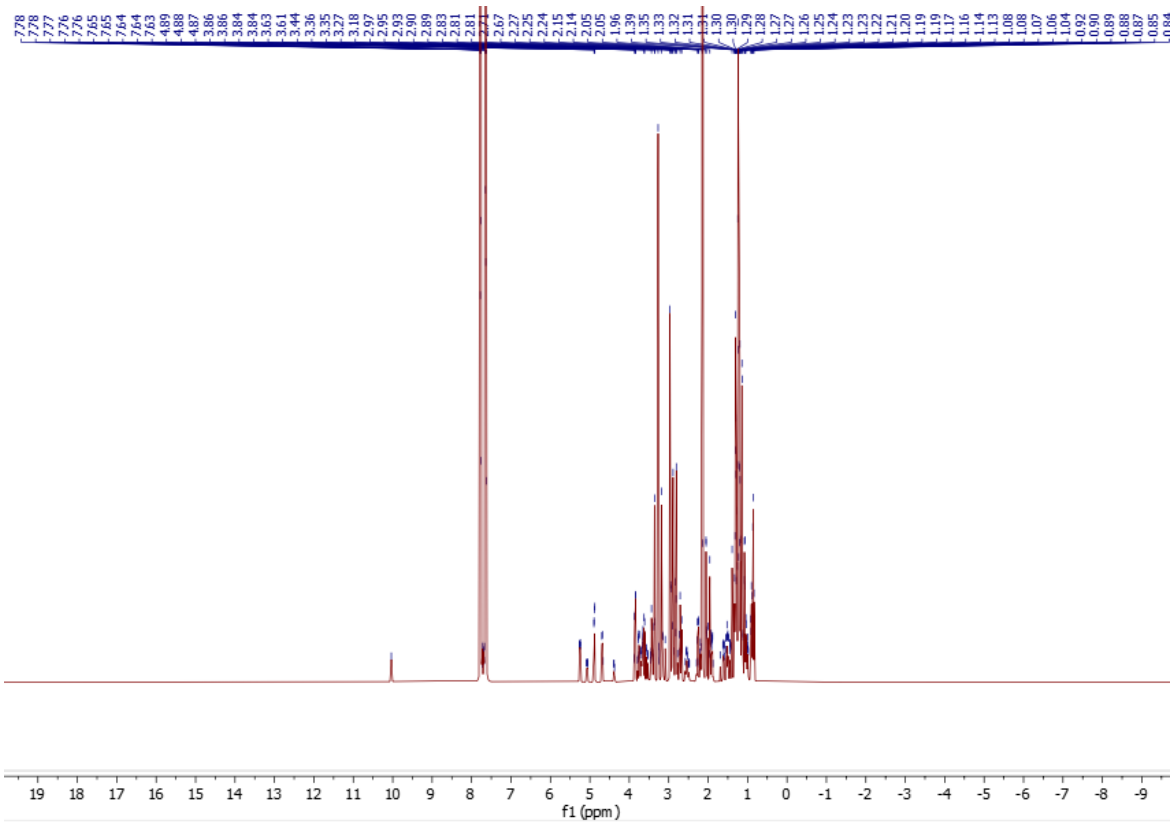
Appendix 24:*[1]H-NMR* spectra of compound (4)



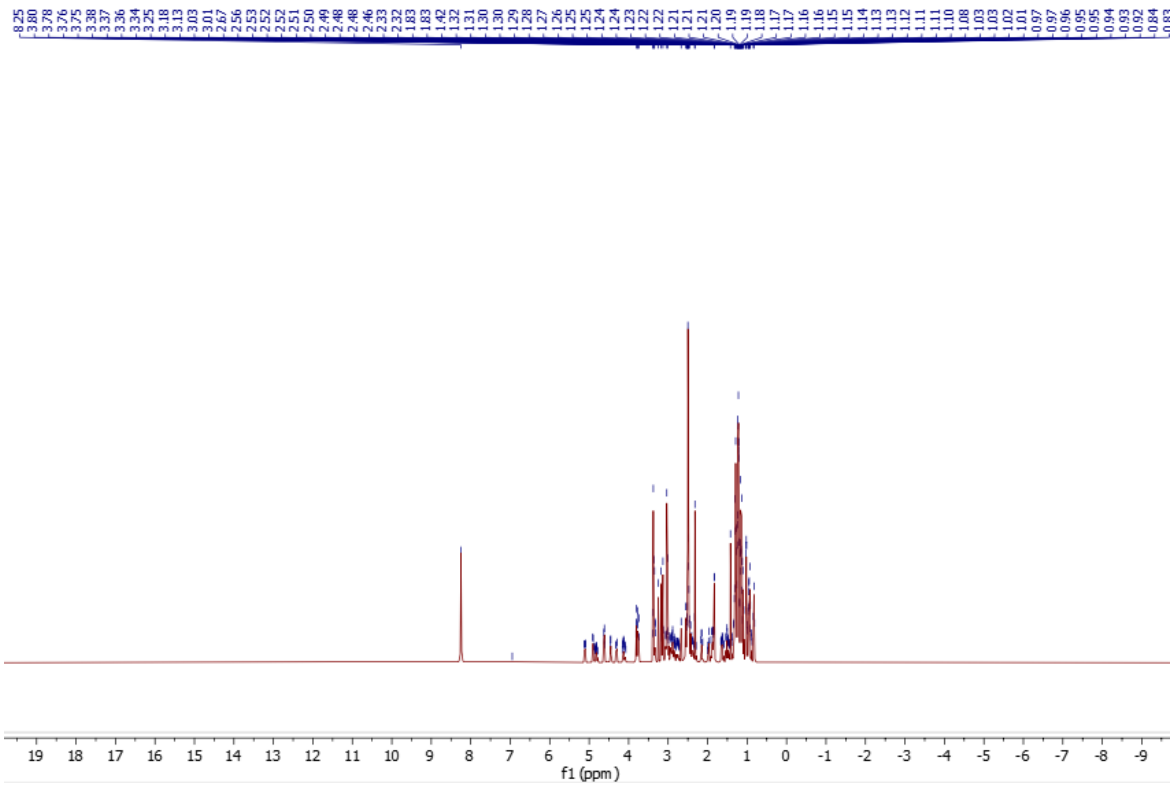
Appendix 25:*^{1}H-NMR* spectra of compound (5)



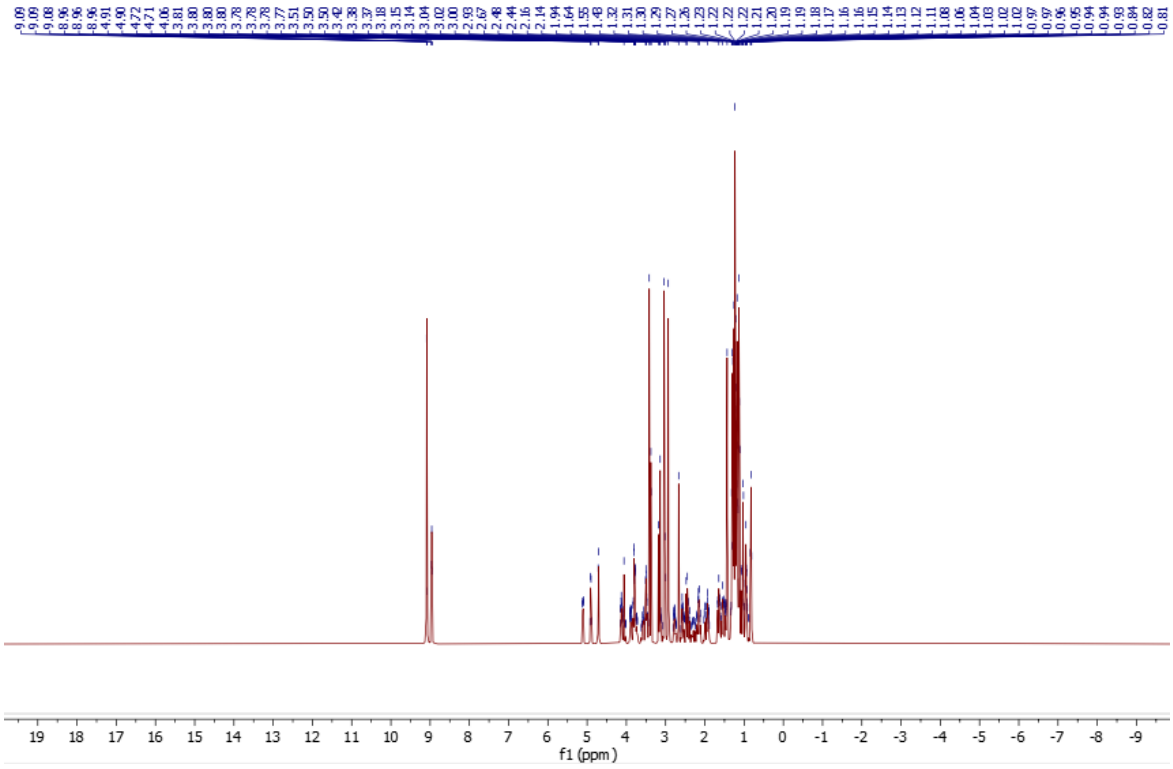
Appendix 26:*[1]H-NMR* spectra of compound (6)



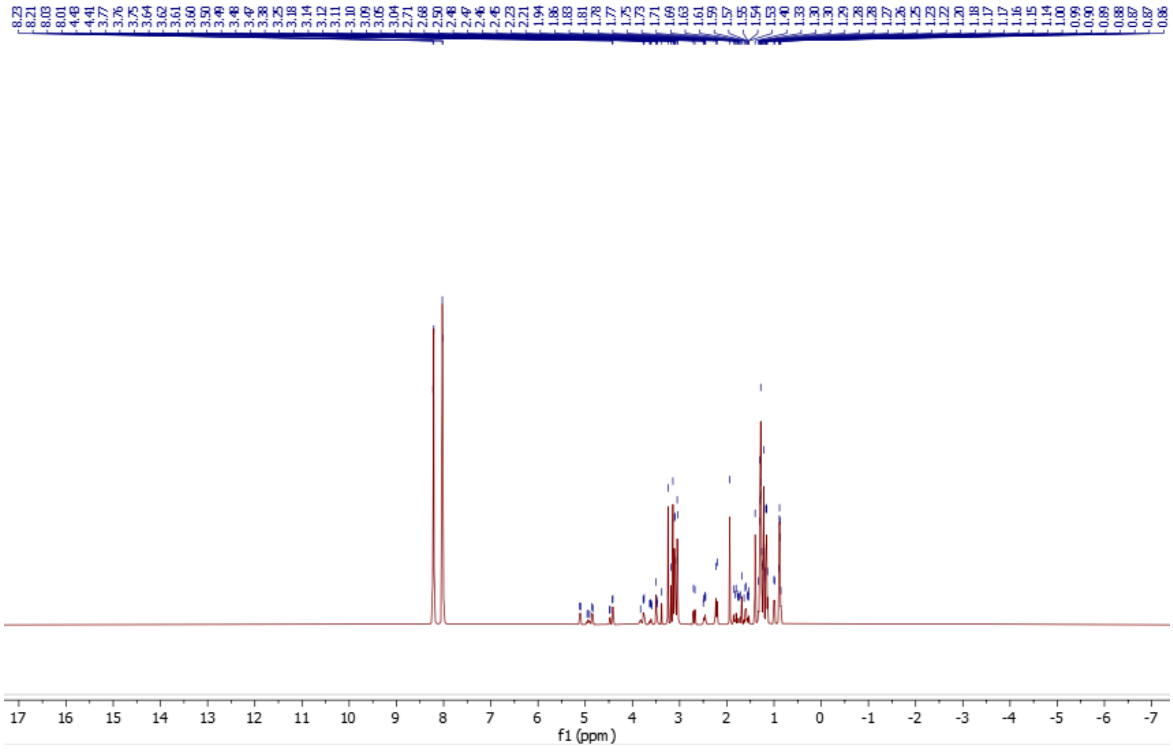
Appendix 27:*[1]H-NMR* spectra of compound (7)



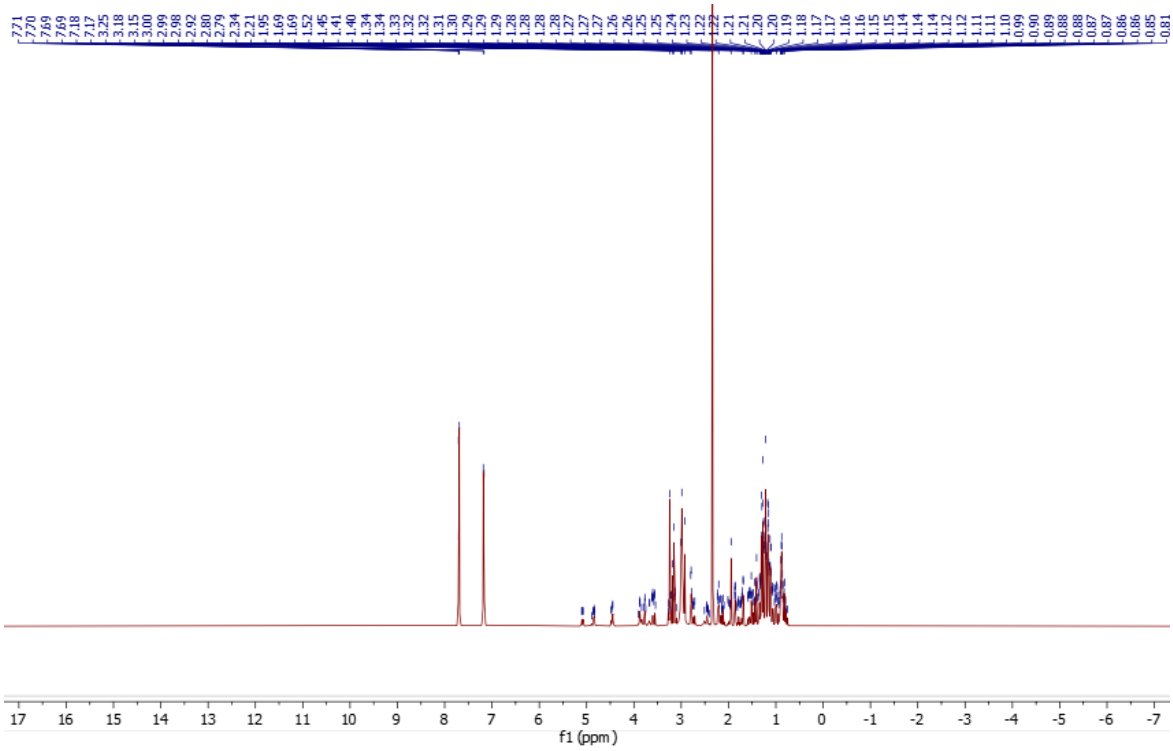
Appendix 28:*[1]H-NMR* spectra of compound (8)



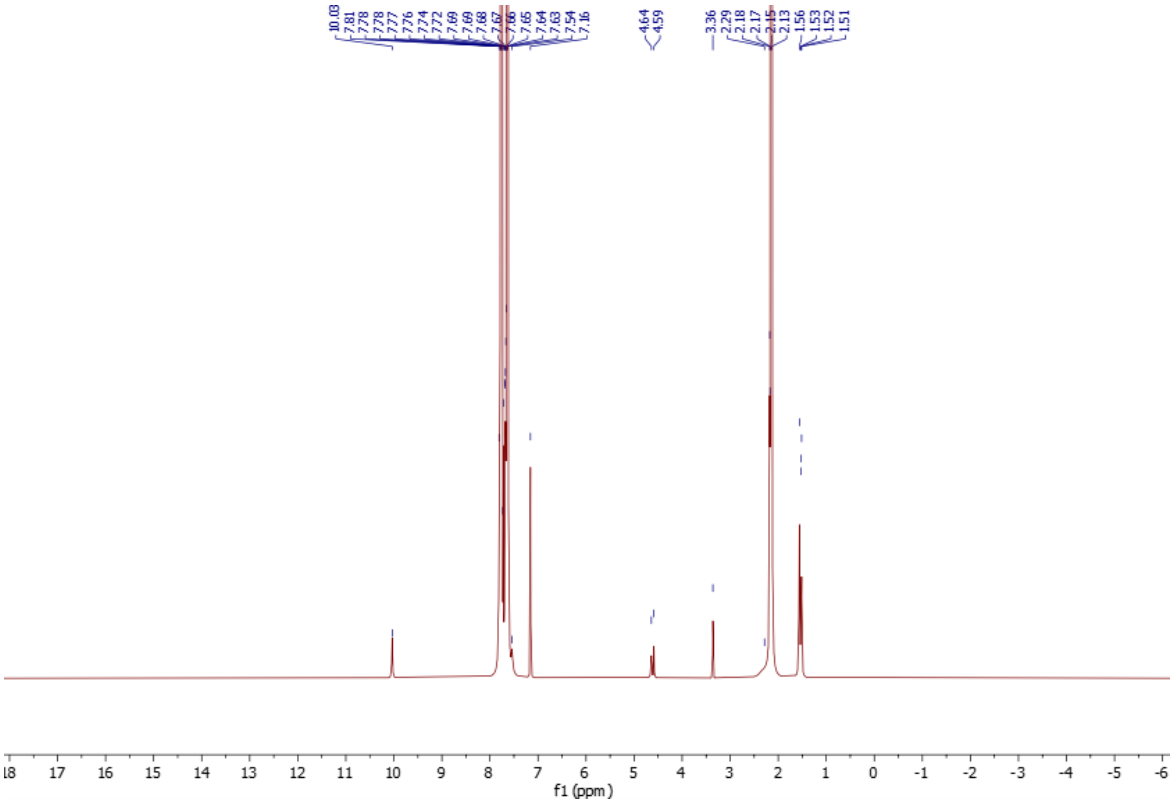
Appendix 29:*[1]H-NMR* spectra of compound (9)



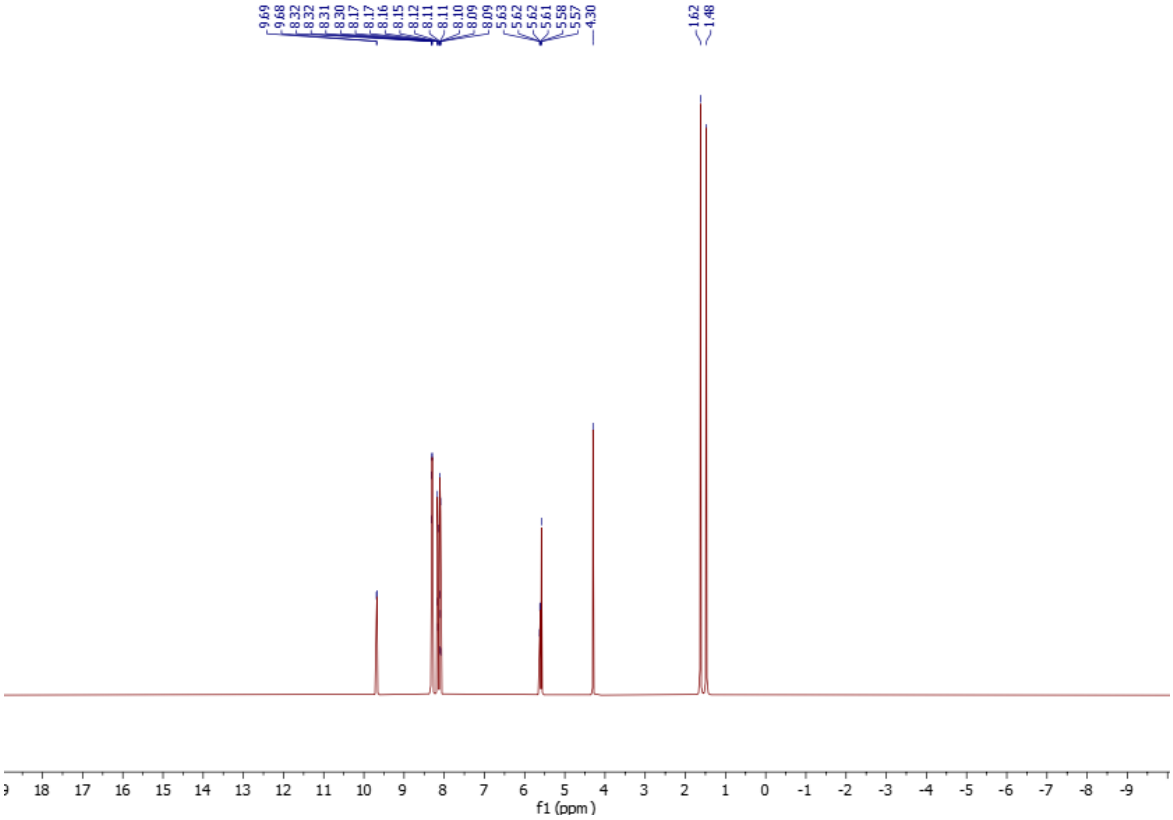
Appendix 30:*[1]H-NMR* spectra of compound (10)



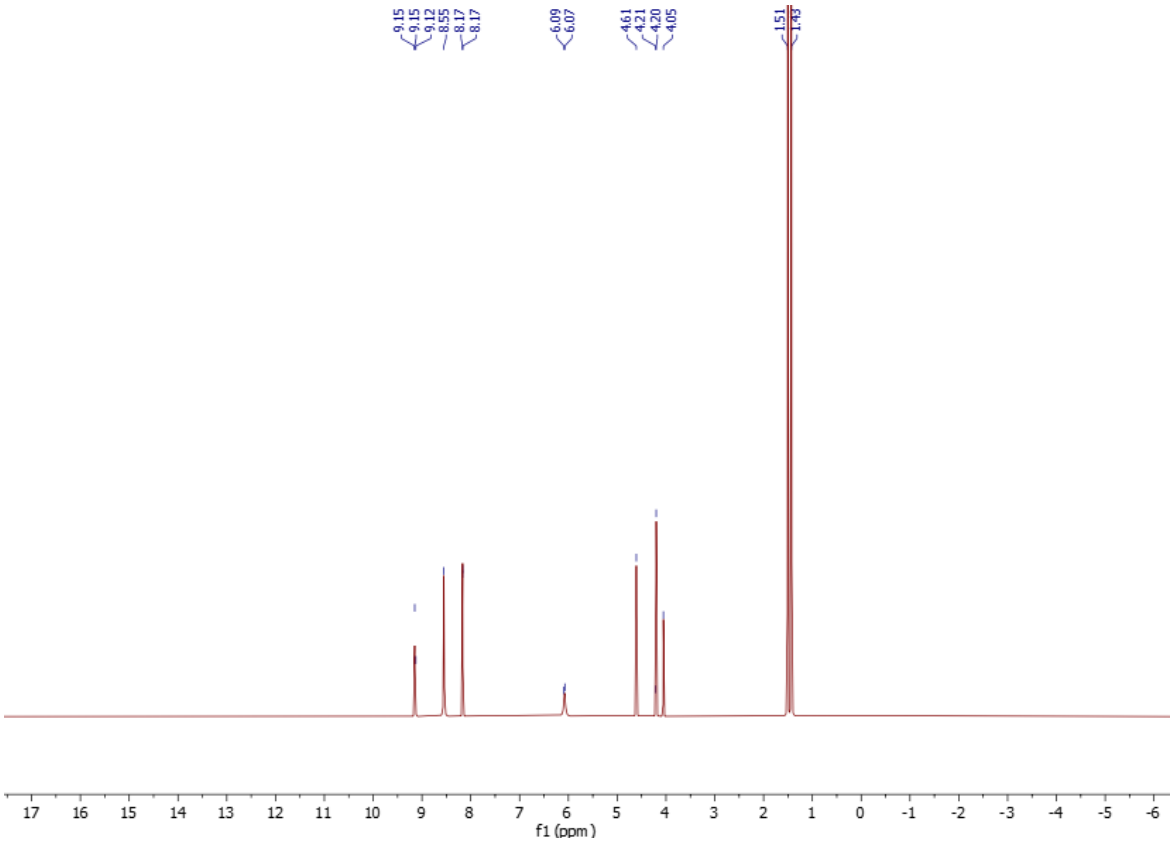
Appendix 31:*[1]H-NMR* spectra of compound (11)



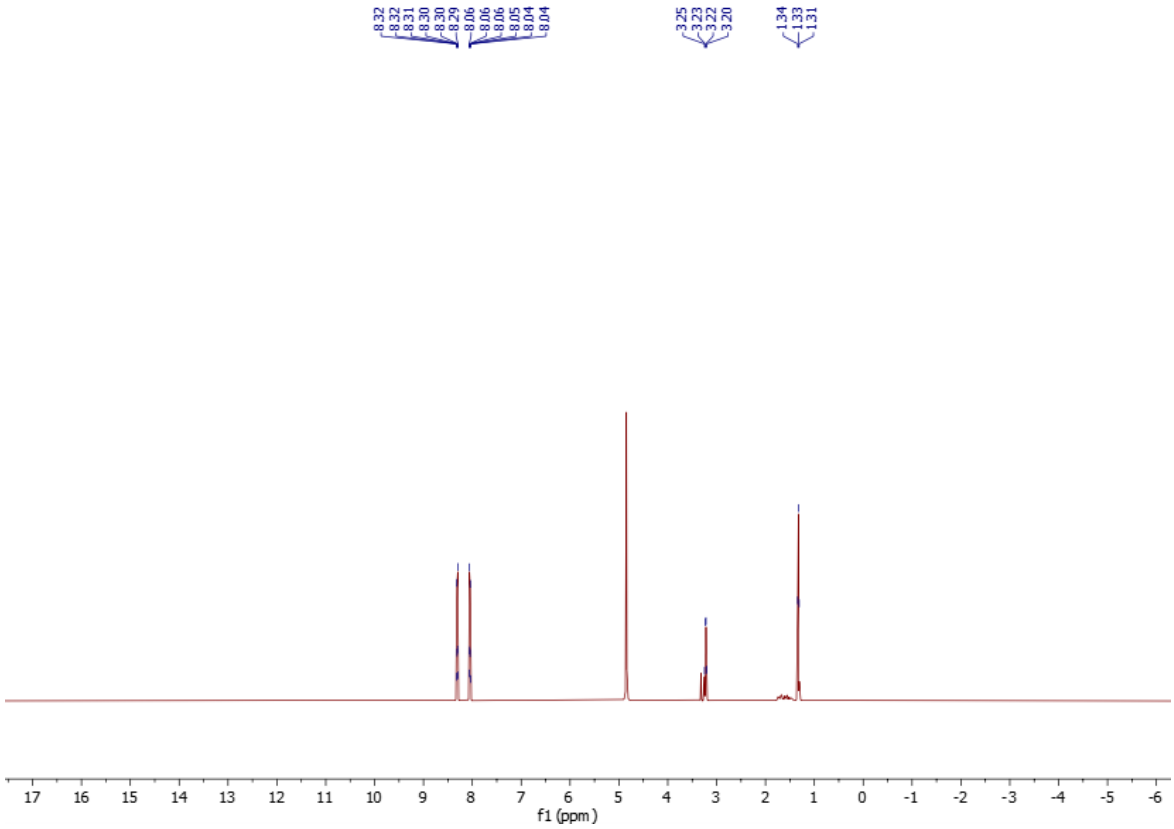
Appendix 32:*[1]H-NMR* spectra of compound (12)



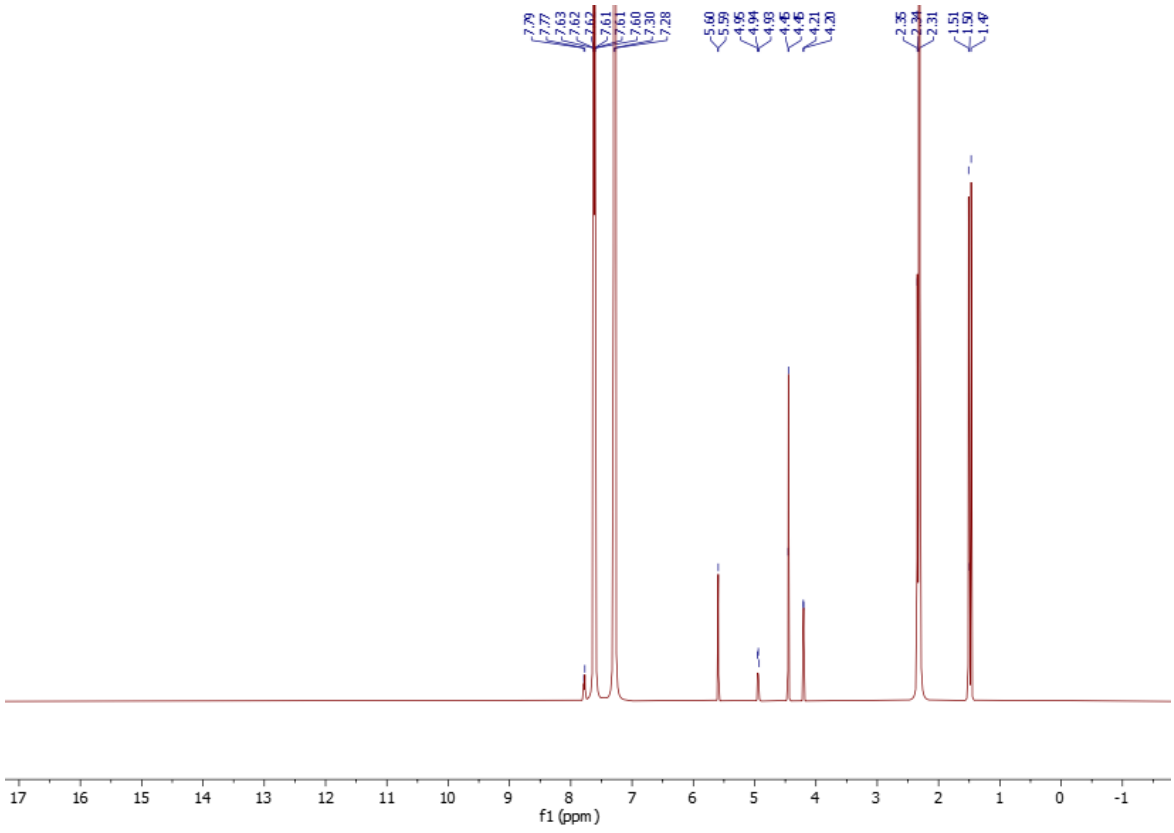
Appendix 33:*[1]H-NMR* spectra of compound (13)



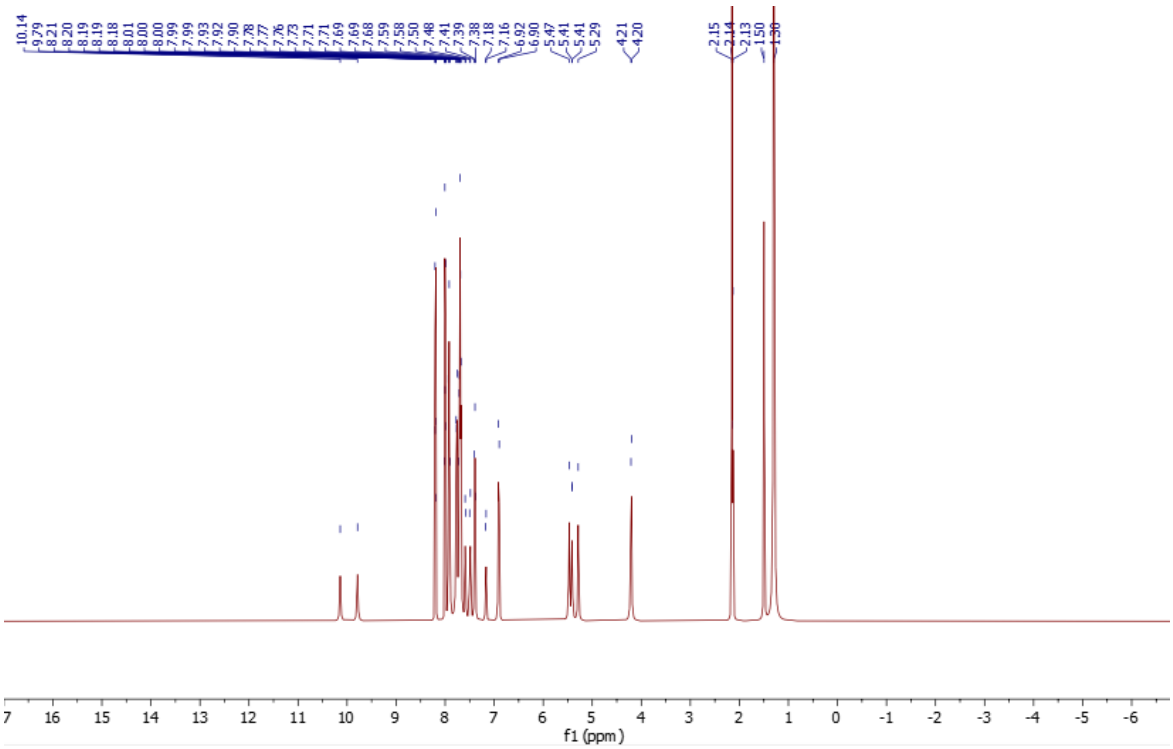
Appendix 34:*[1]H-NMR* spectra of compound (14)



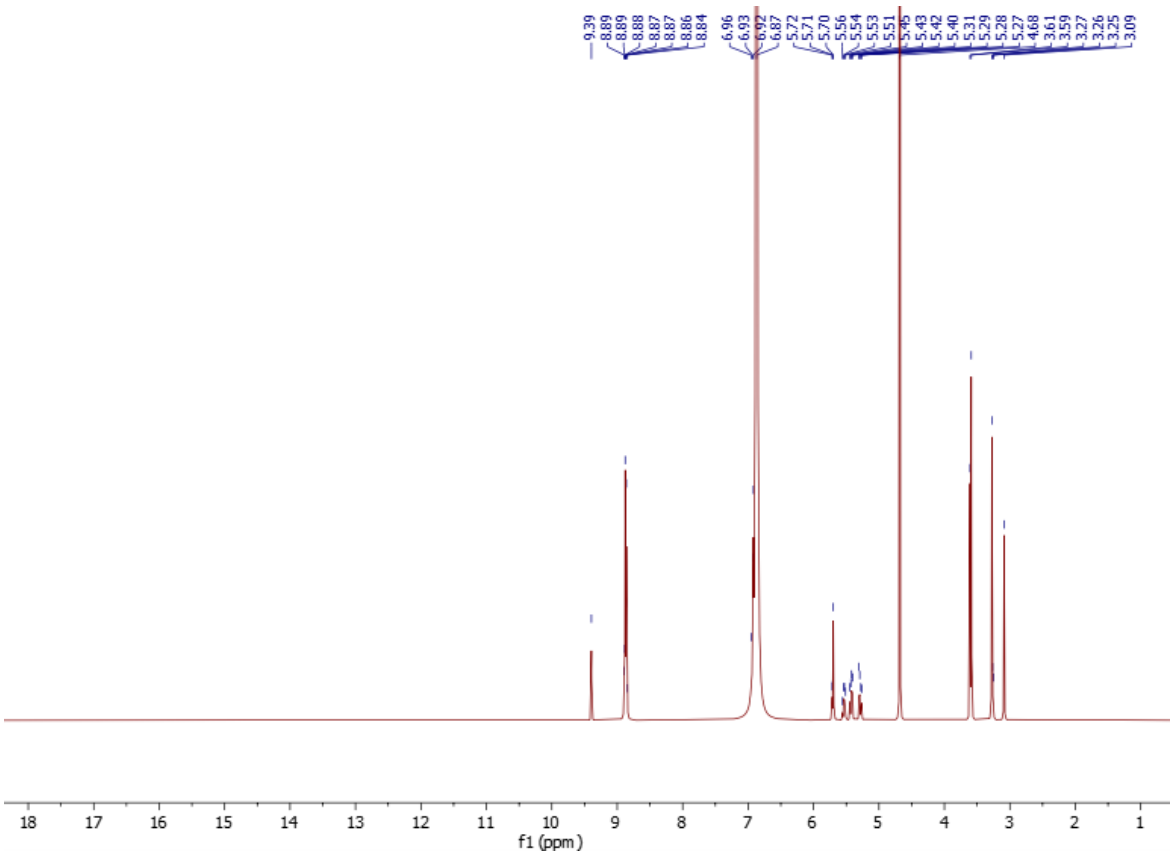
Appendix 35:*[1]H-NMR* spectra of compound (15)



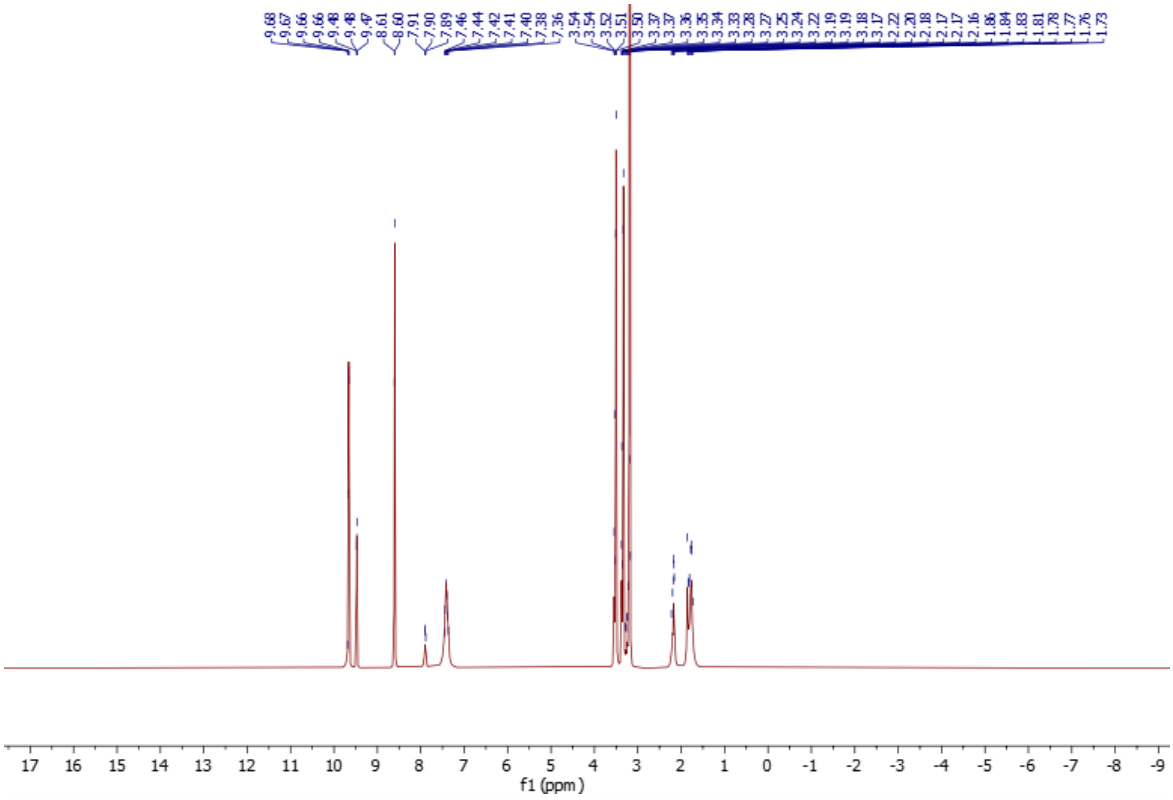
Appendix 36:*[1]H-NMR* spectra of compound (16)



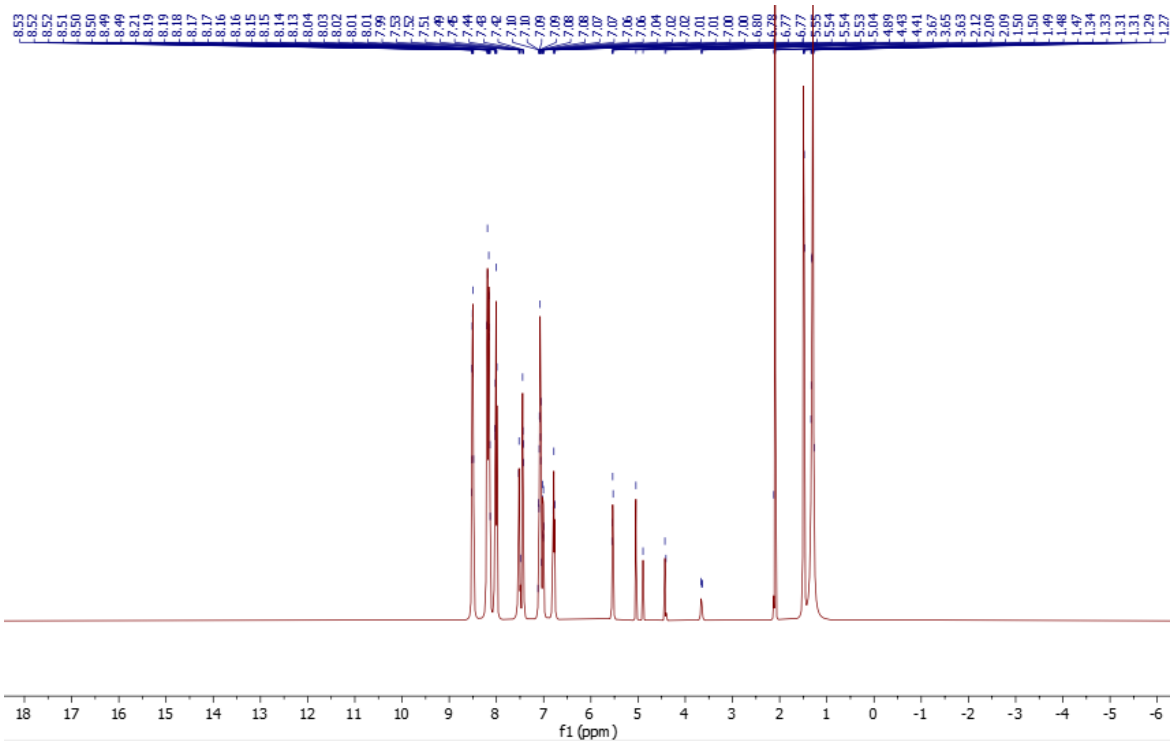
Appendix 37:*[1]H-NMR* spectra of compound (17)



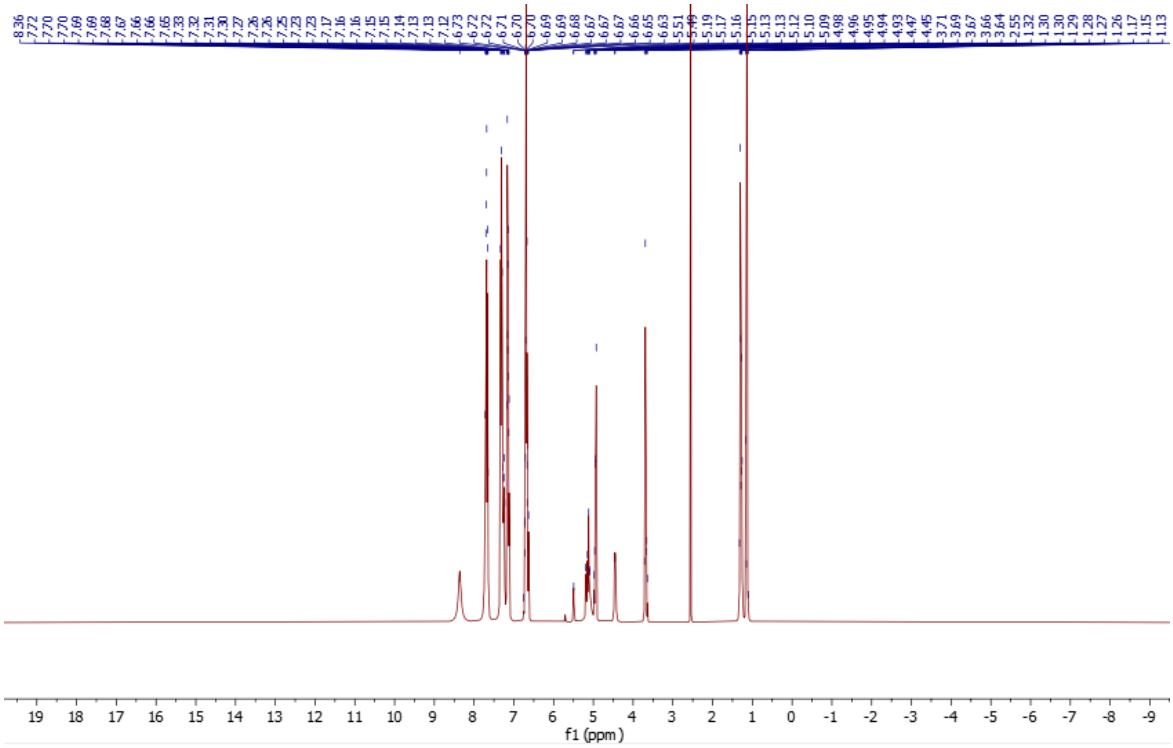
Appendix 38:*[1]H-NMR* spectra of compound (18)



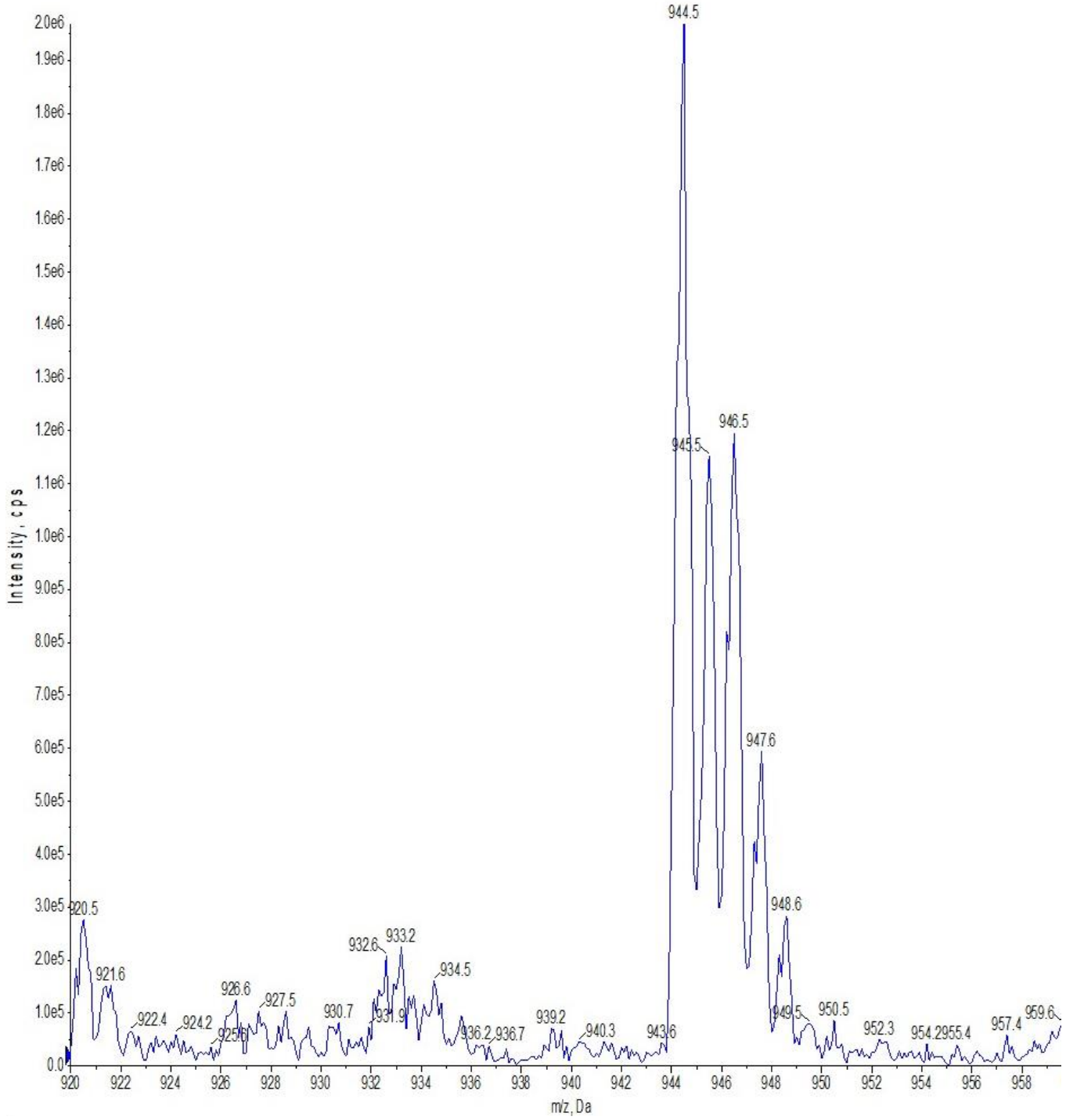
Appendix 39:*[1]H-NMR* spectra of compound (19)



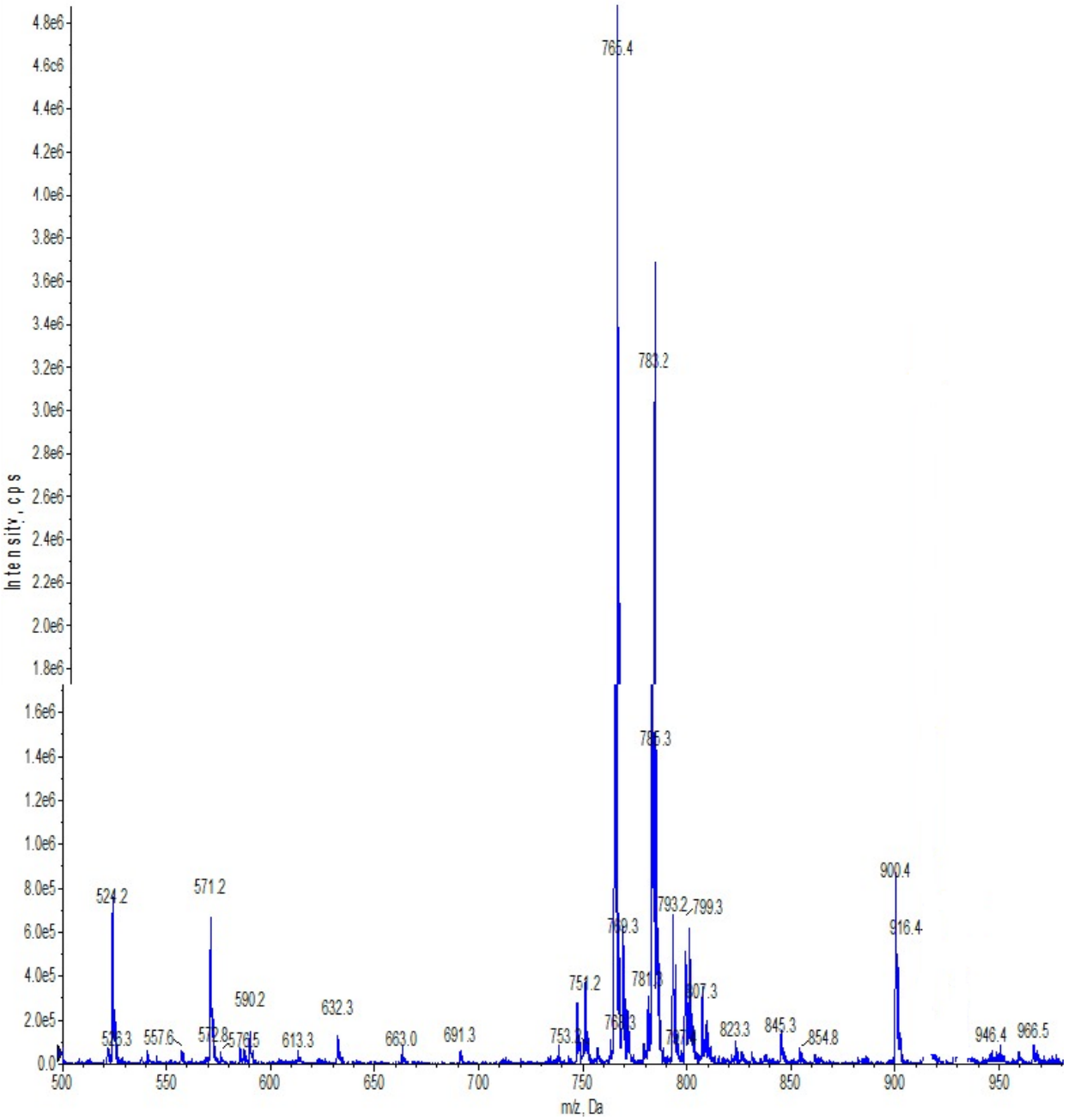
Appendix 40:*[1]H-NMR* spectra of compound (20)



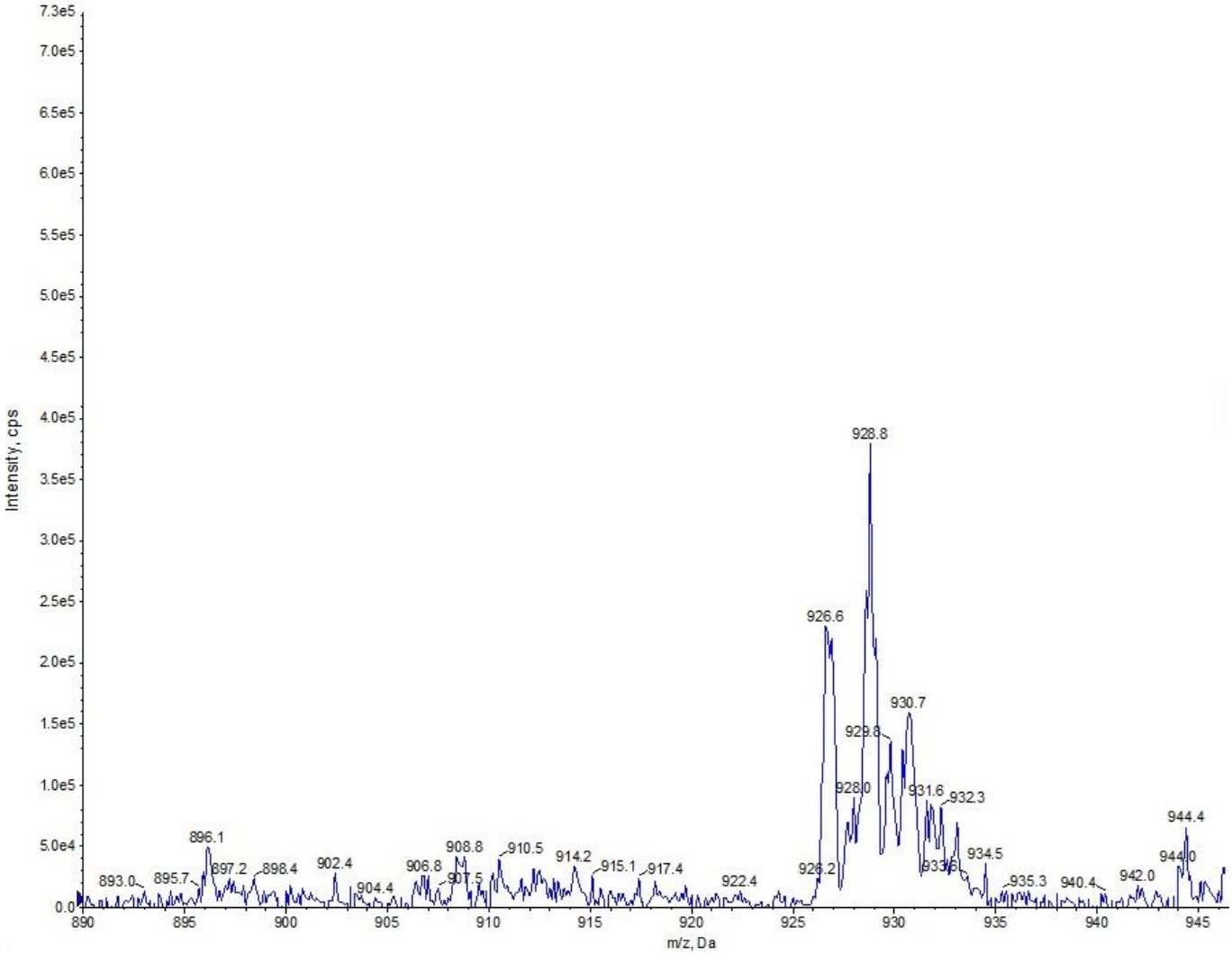
Appendix 41:*MS* Spectra of compound (1)



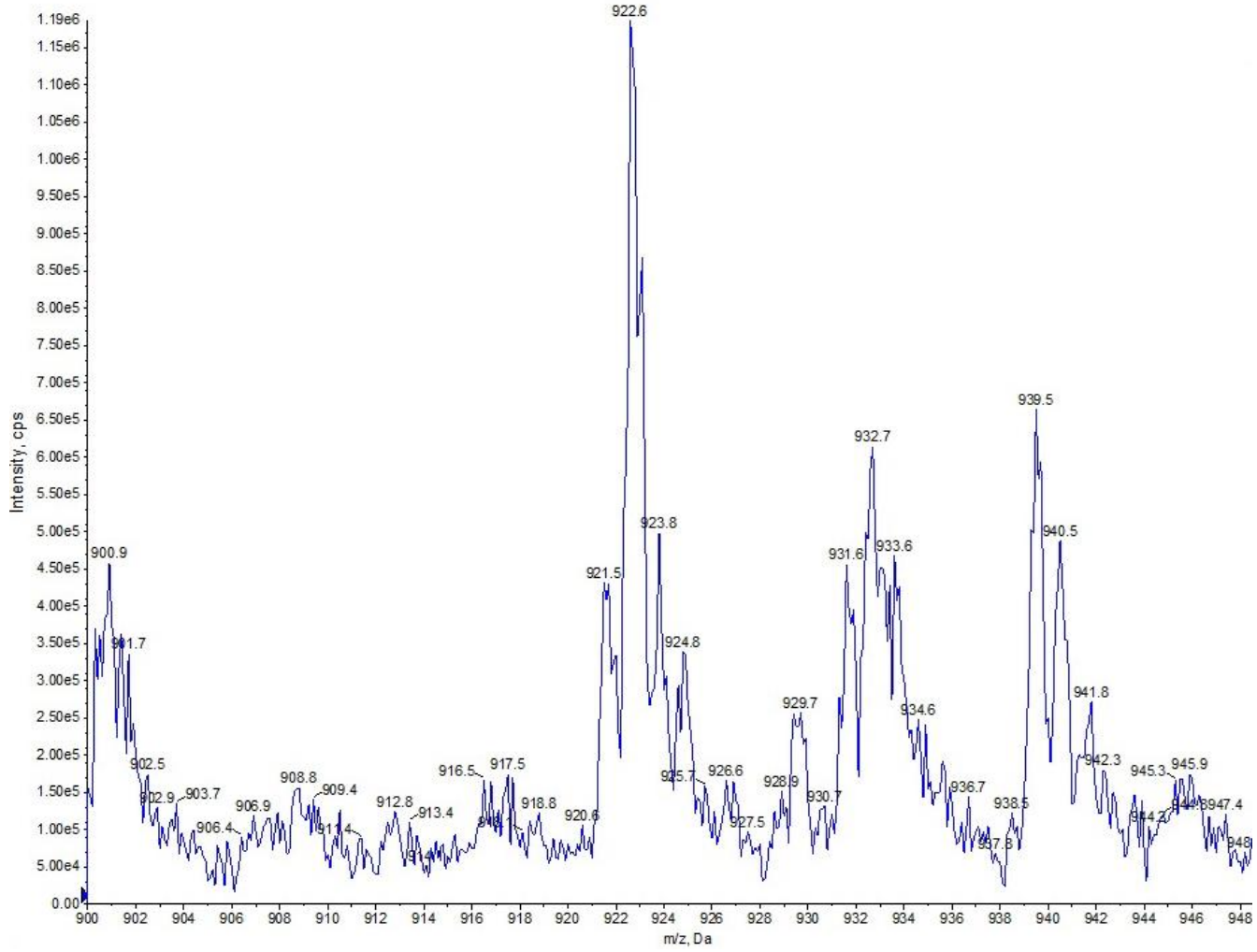
Appendix 42:*MS* Spectra of compound (2)



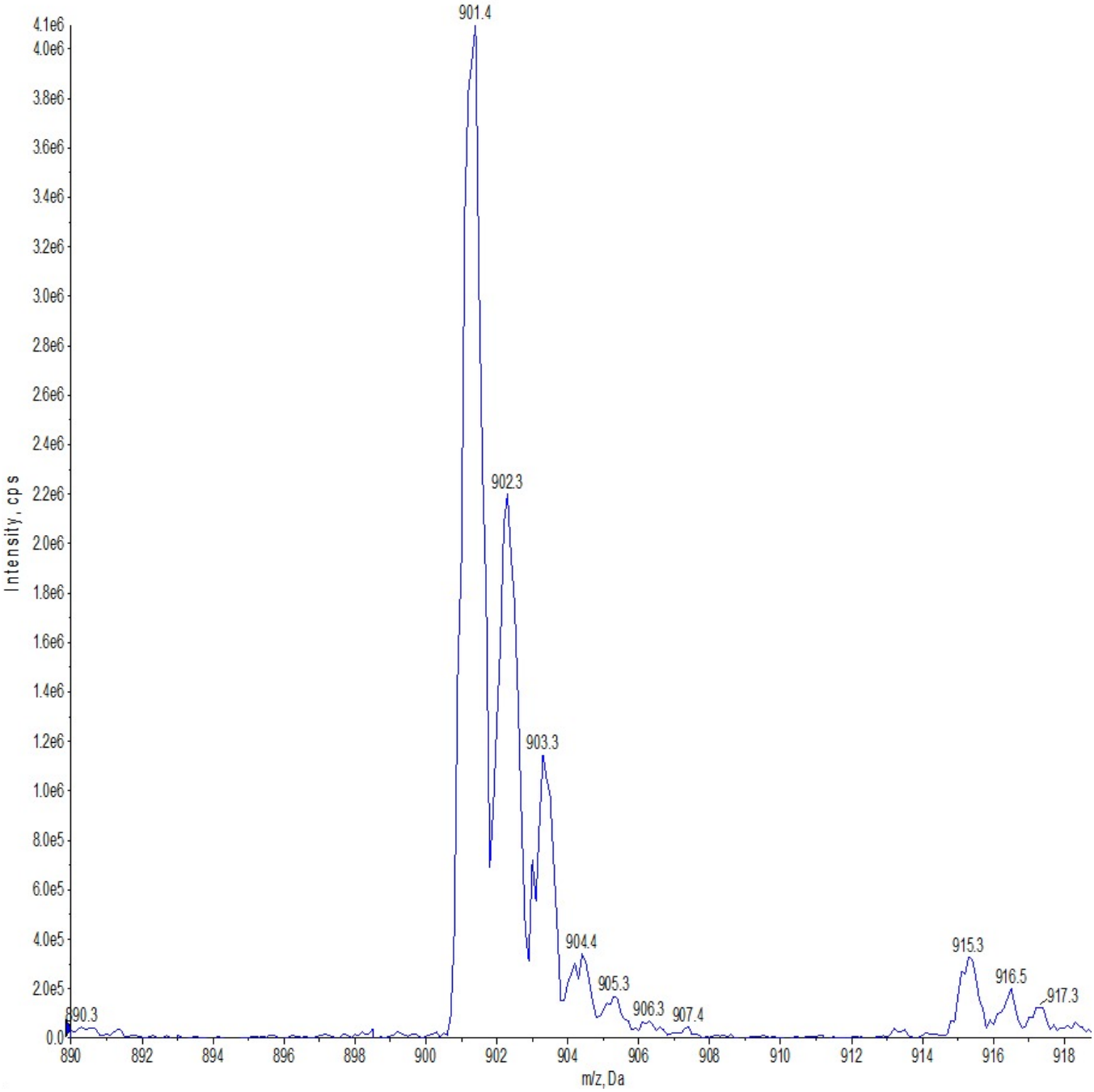
Appendix 43:*MS* Spectra of compound (3)



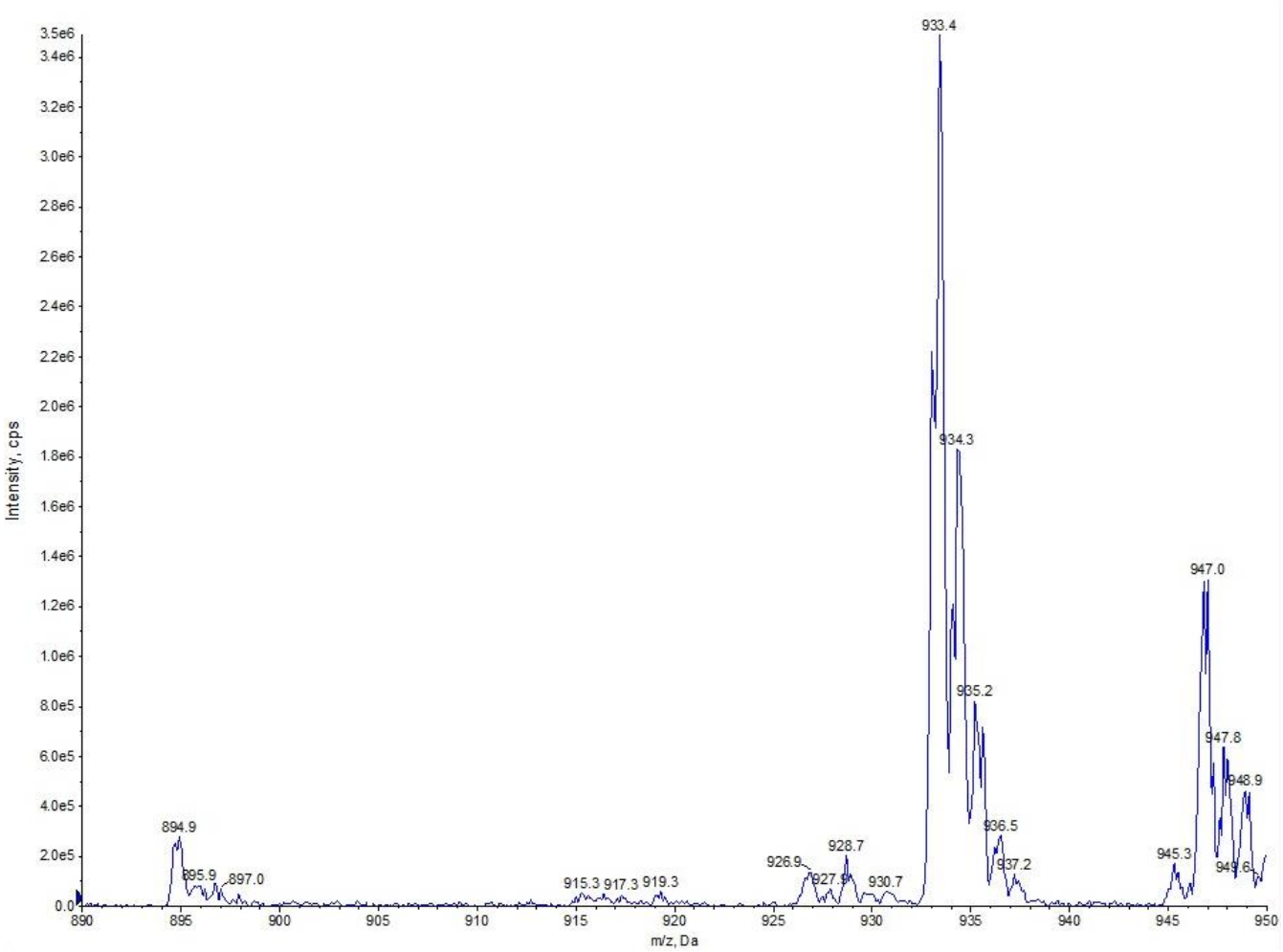
Appendix 44:*MS* Spectra of compound (4)



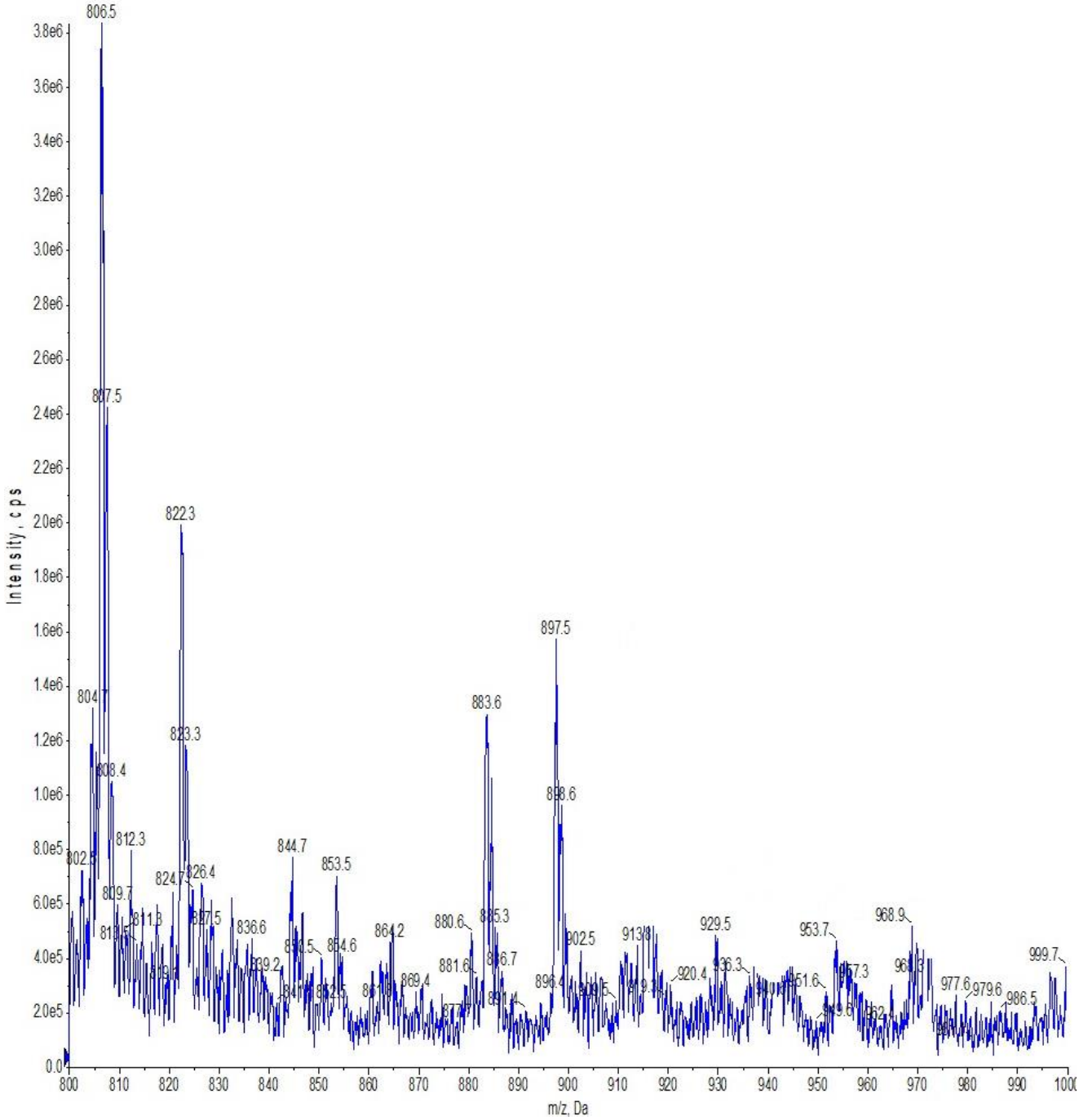
Appendix 45:*MS* Spectra of compound (5)



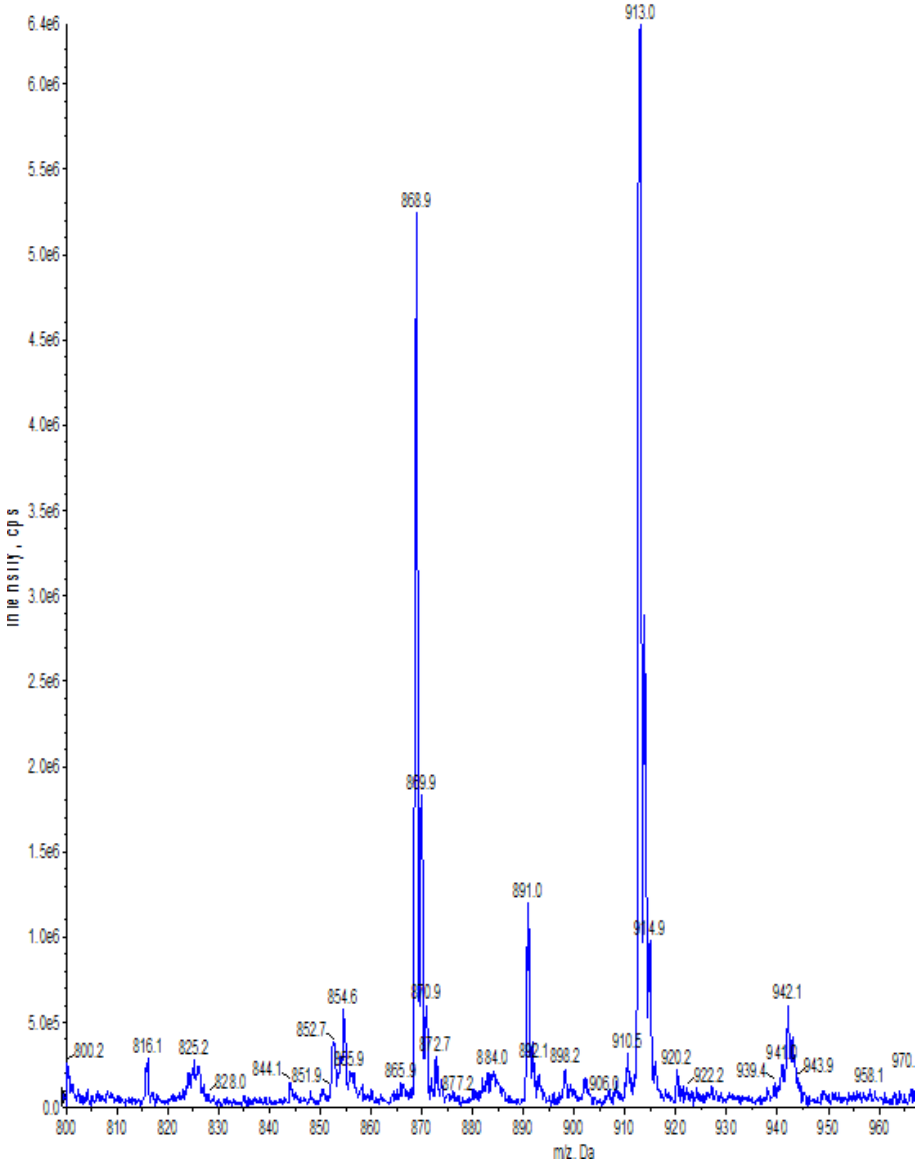
Appendix 46:*MS* Spectra of compound (6)



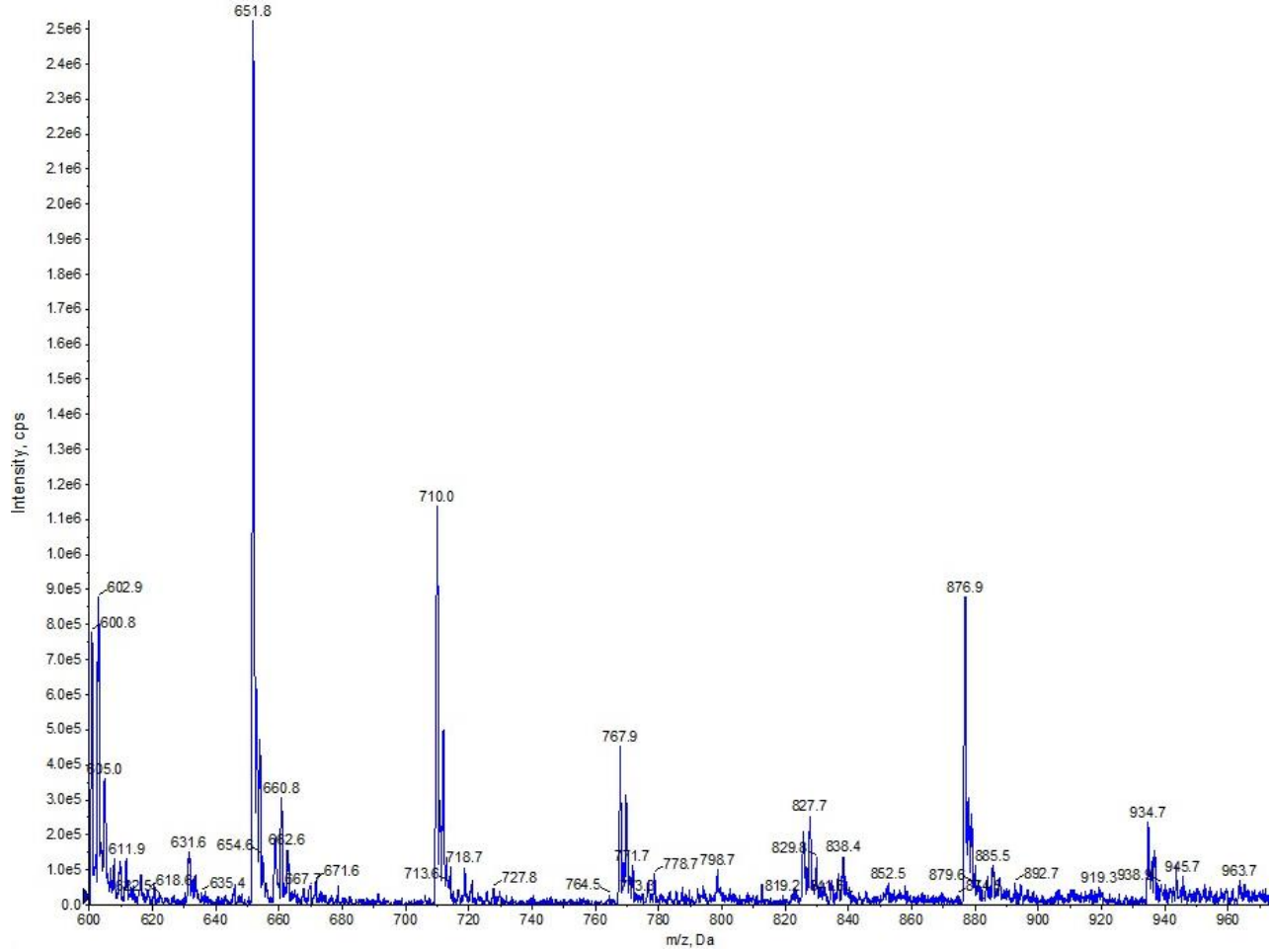
Appendix 47:*MS* Spectra of compound (7)



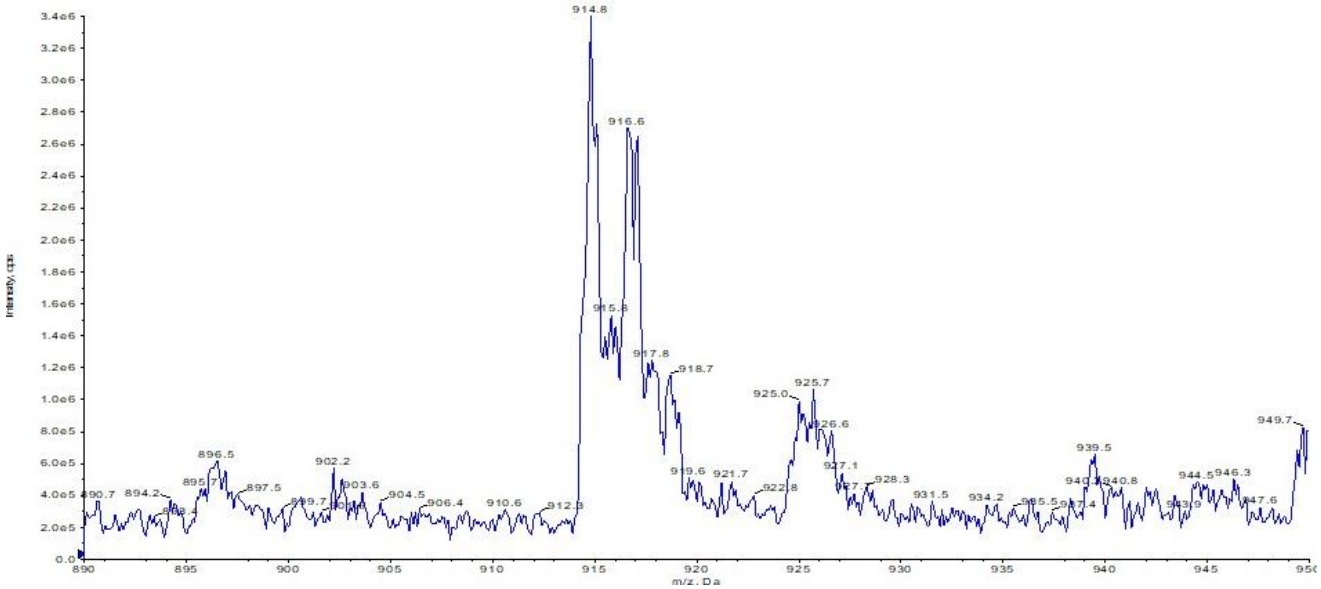
Appendix 48:*MS* Spectra of compound (8)



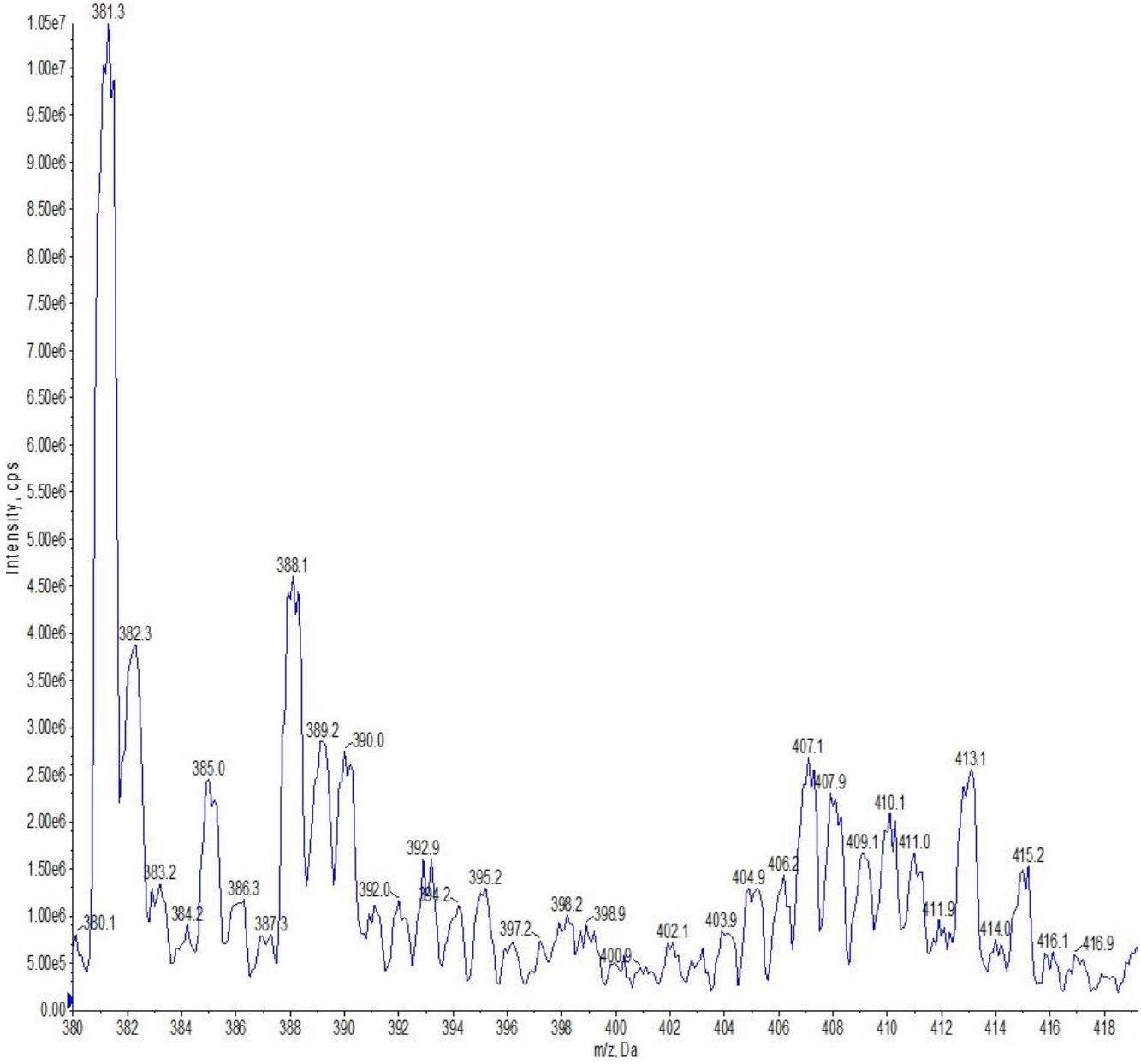
Appendix 49:*MS* Spectra of compound (9)



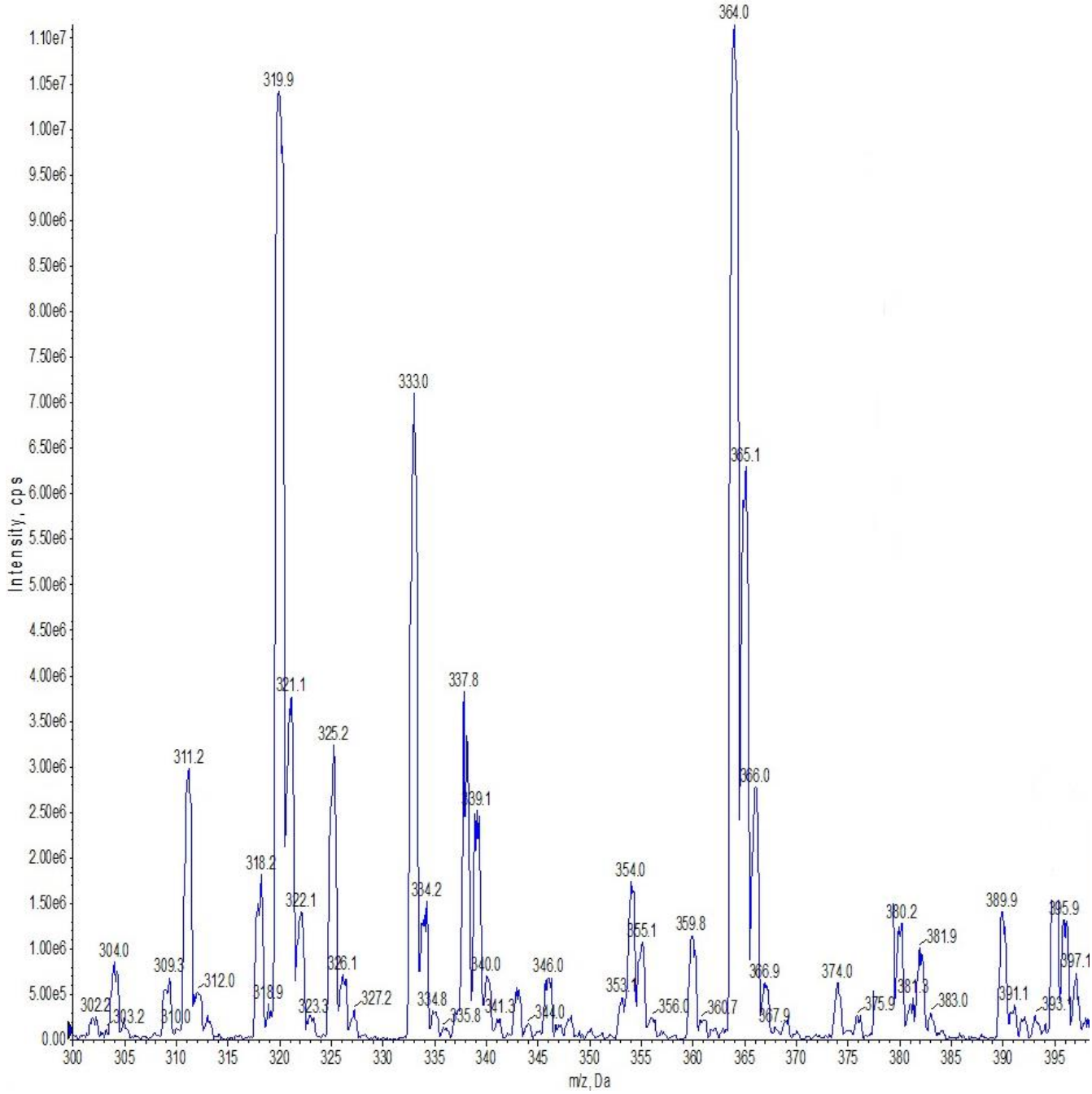
Appendix 50:*MS* Spectra of compound (10)



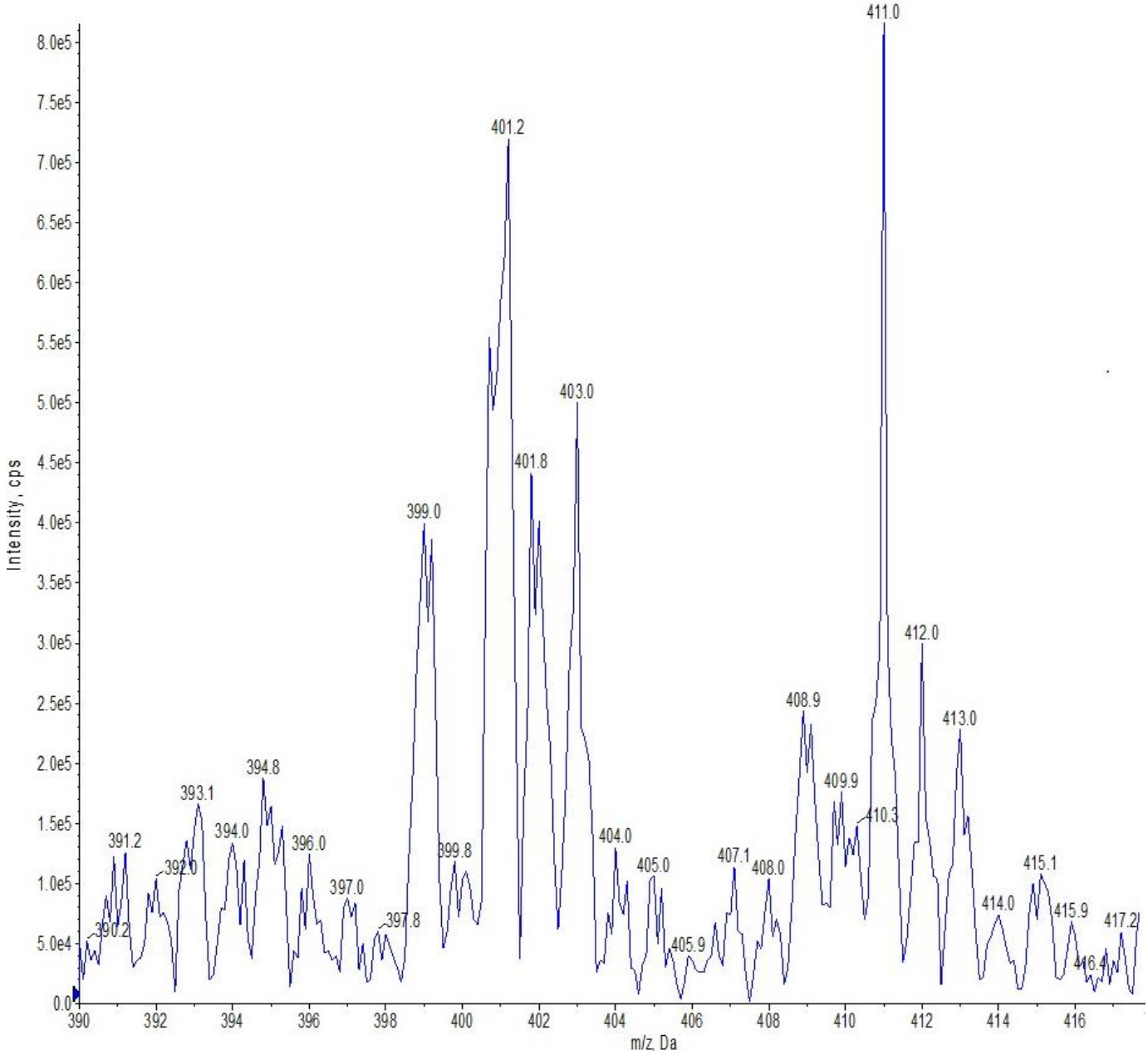
Appendix 51:*MS* Spectra of compound (11)



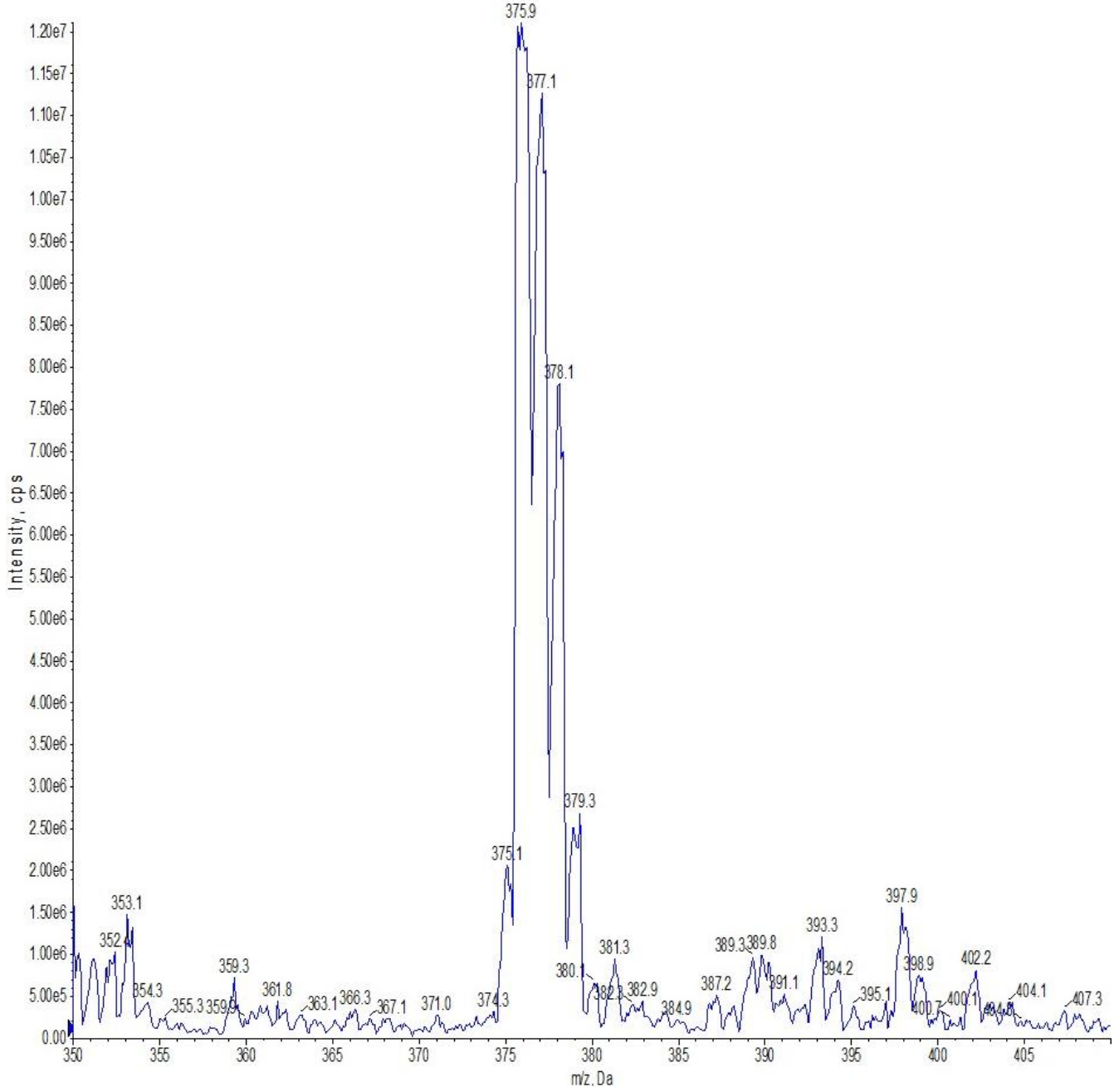
Appendix 52:*MS* Spectra of compound (12)



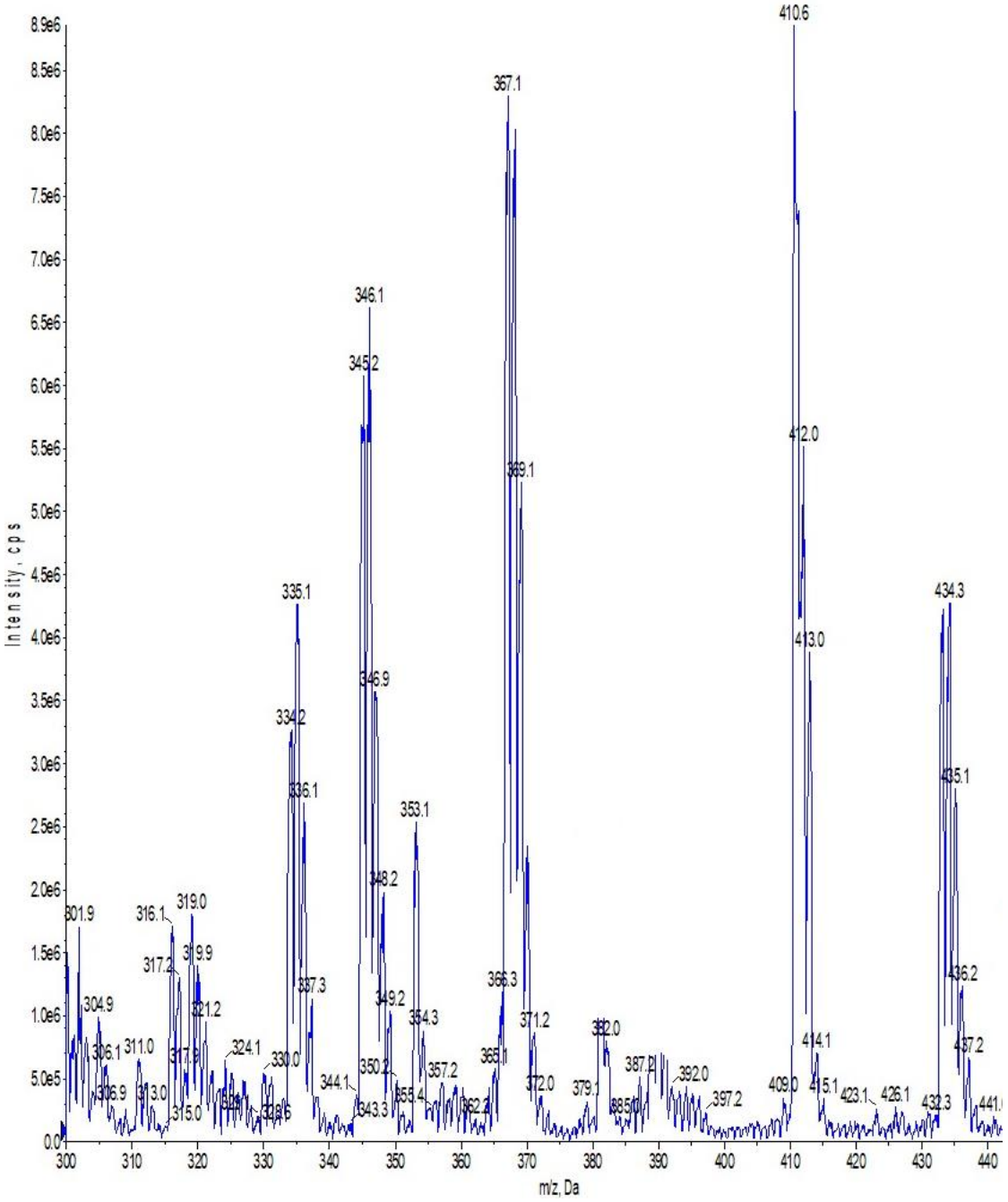
Appendix 53:*MS* Spectra of compound (13)



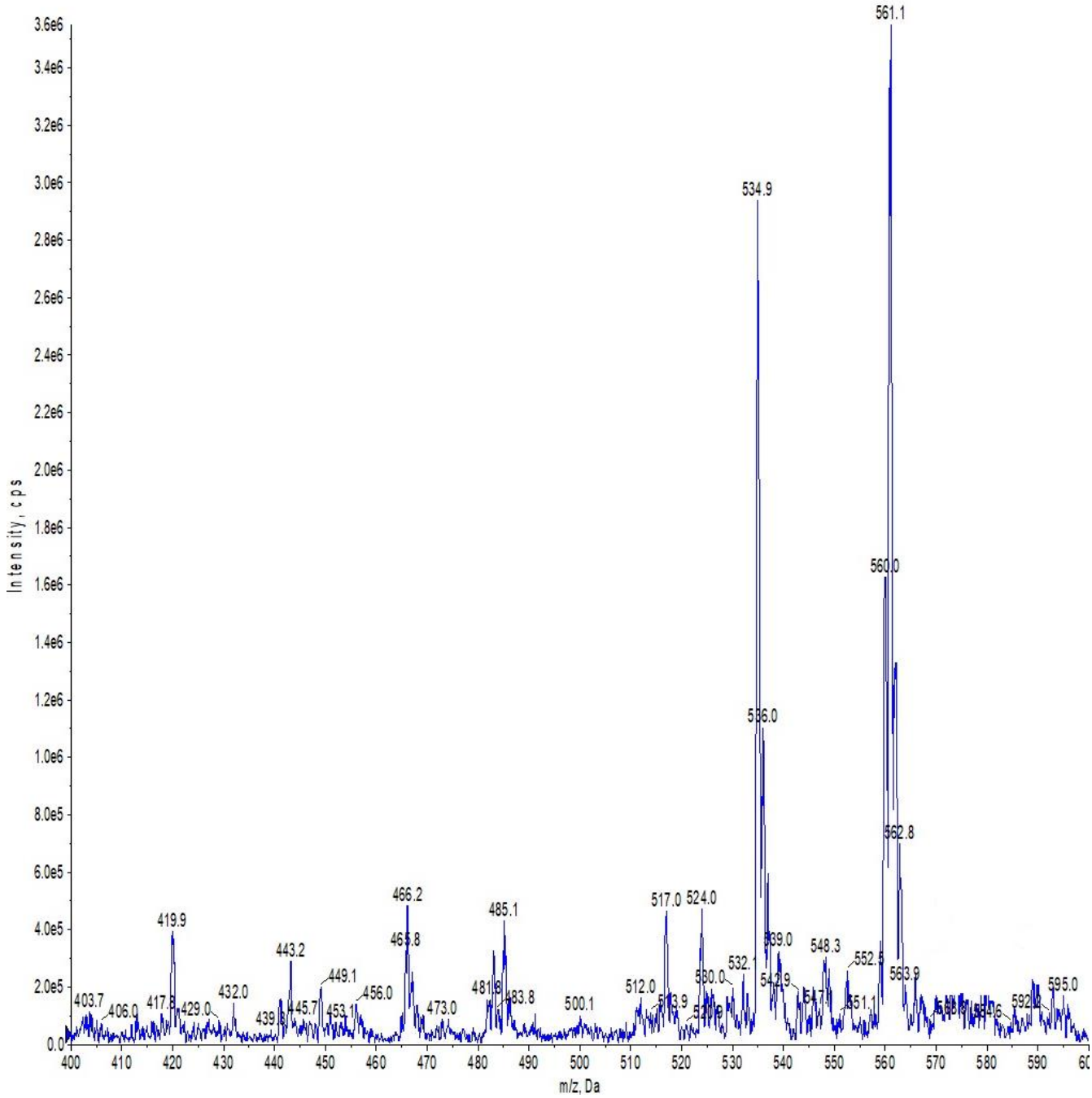
Appendix 54: *MS* Spectra of compound (14)



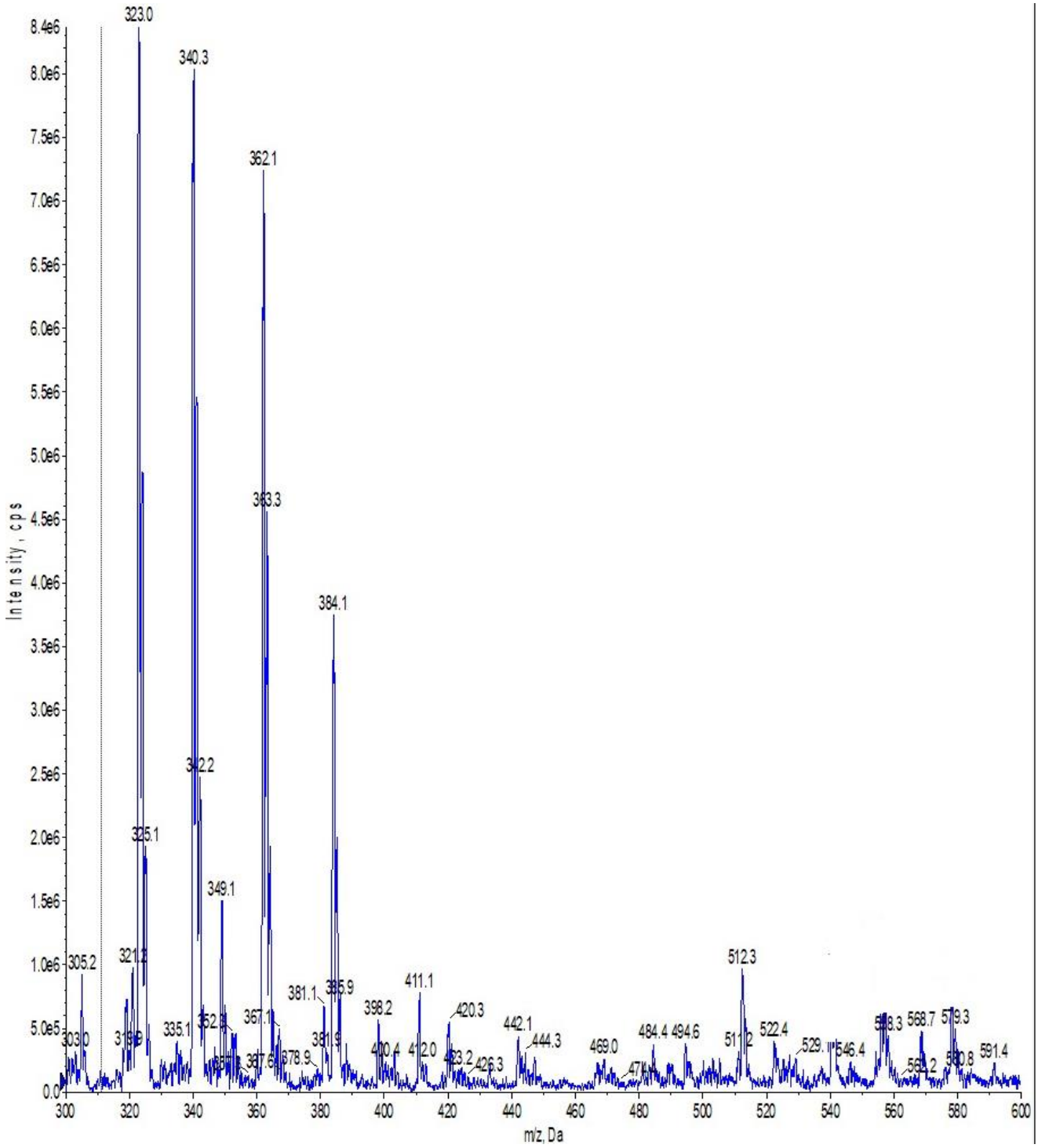
Appendix 55:*MS* Spectra of compound (15)



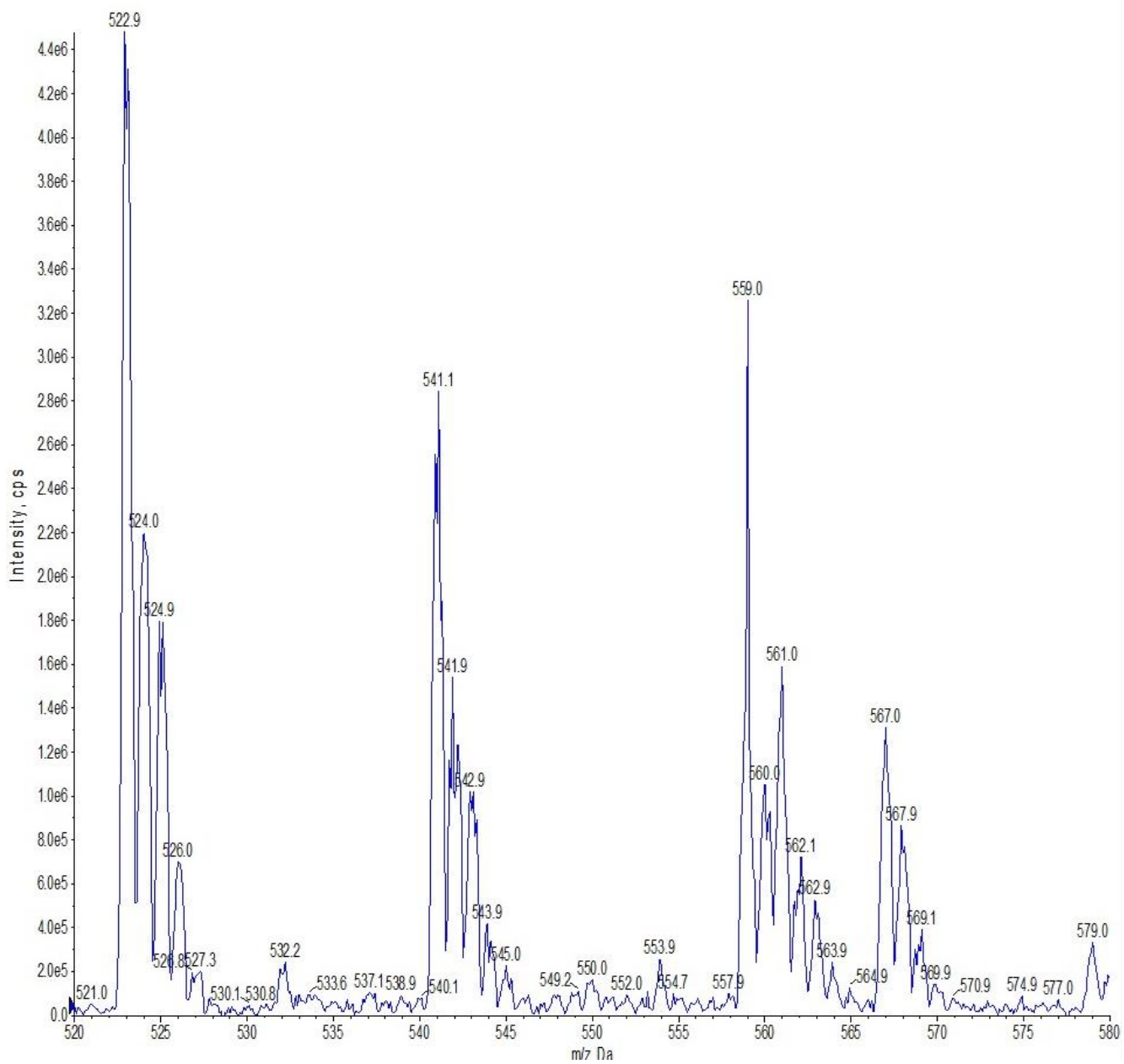
Appendix 56:*MS* Spectra of compound (16)



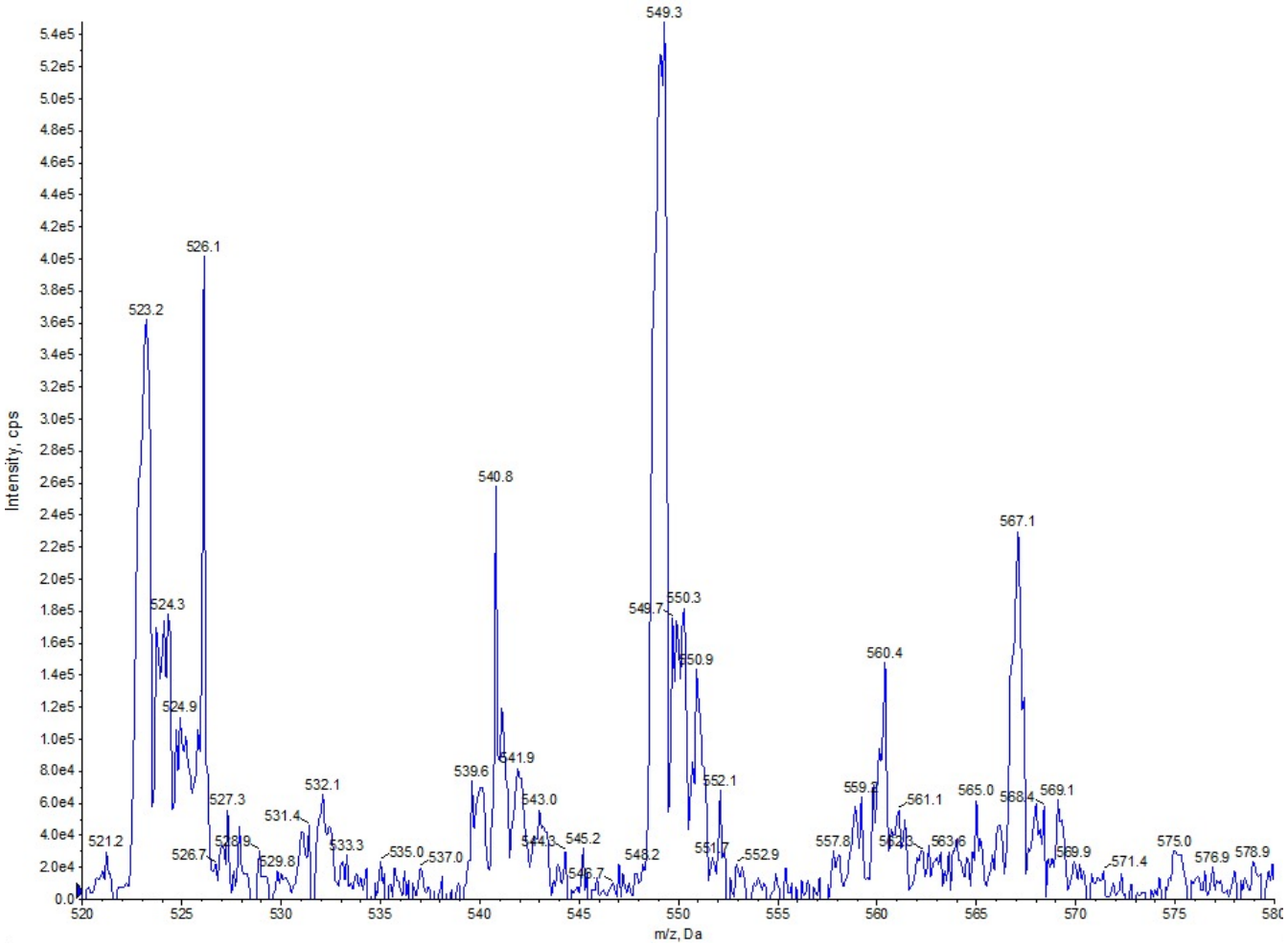
Appendix 57:*MS* Spectra of compound (17)



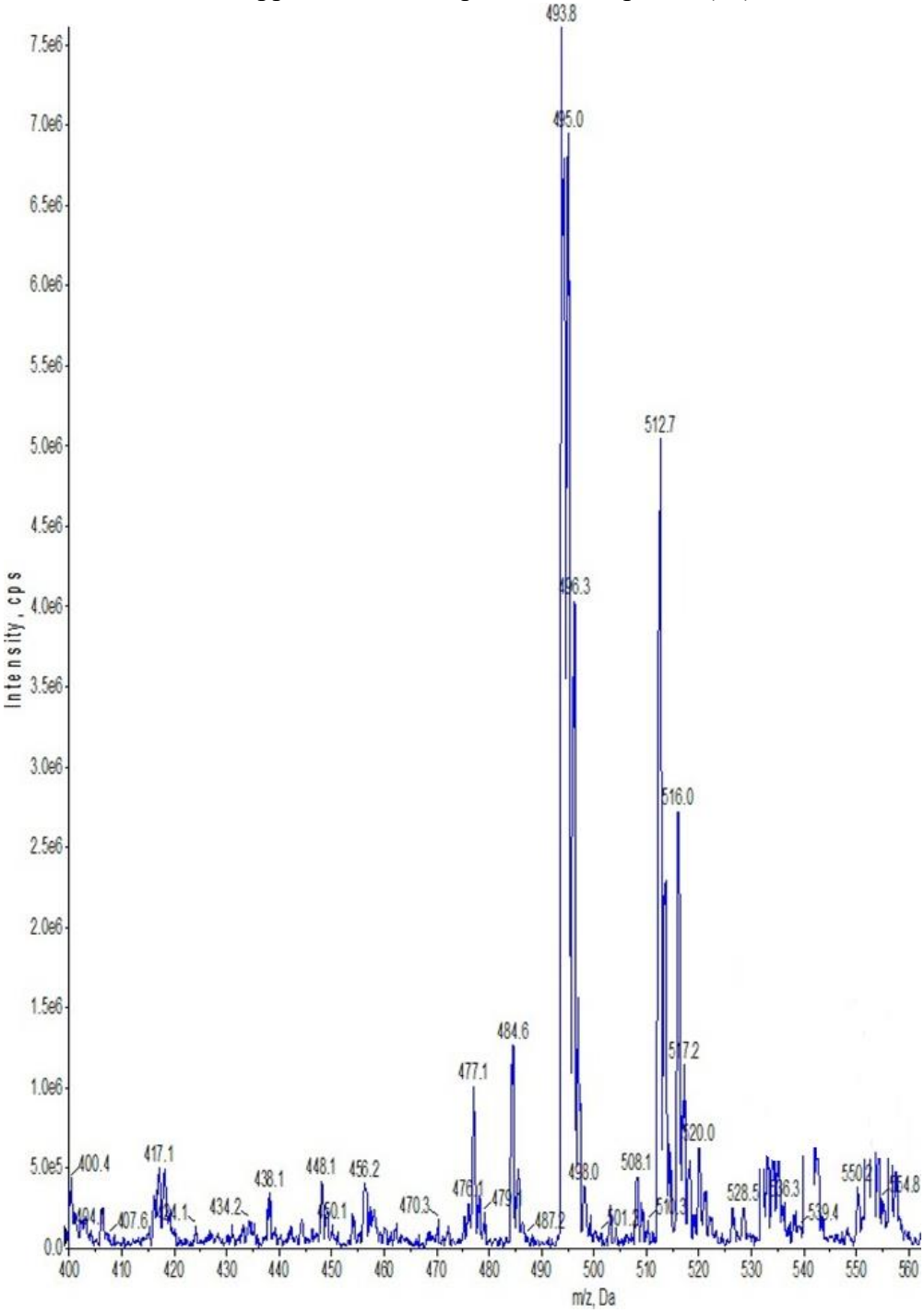
Appendix 58:*MS* Spectra of compound (18)



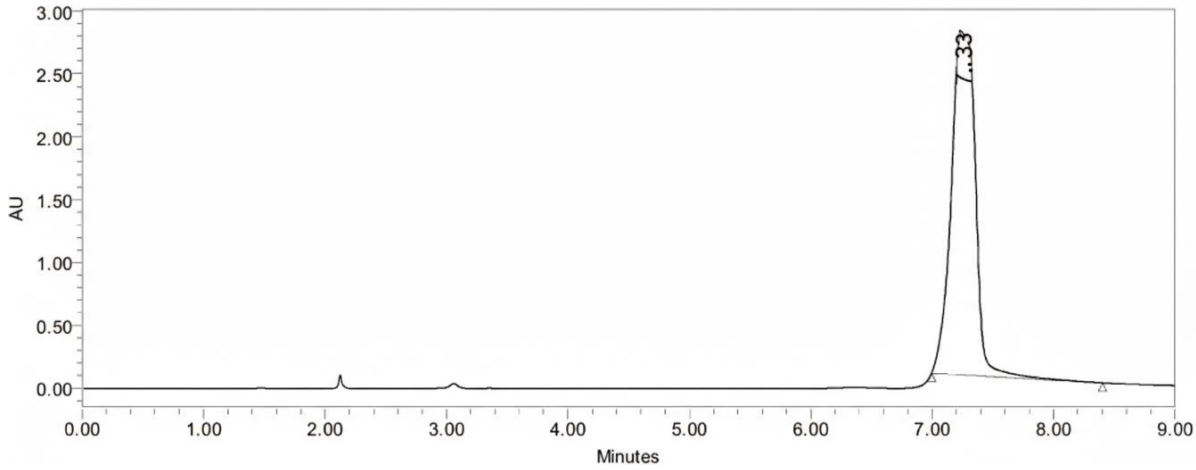
Appendix 59: *MS* Spectra of compound (19)



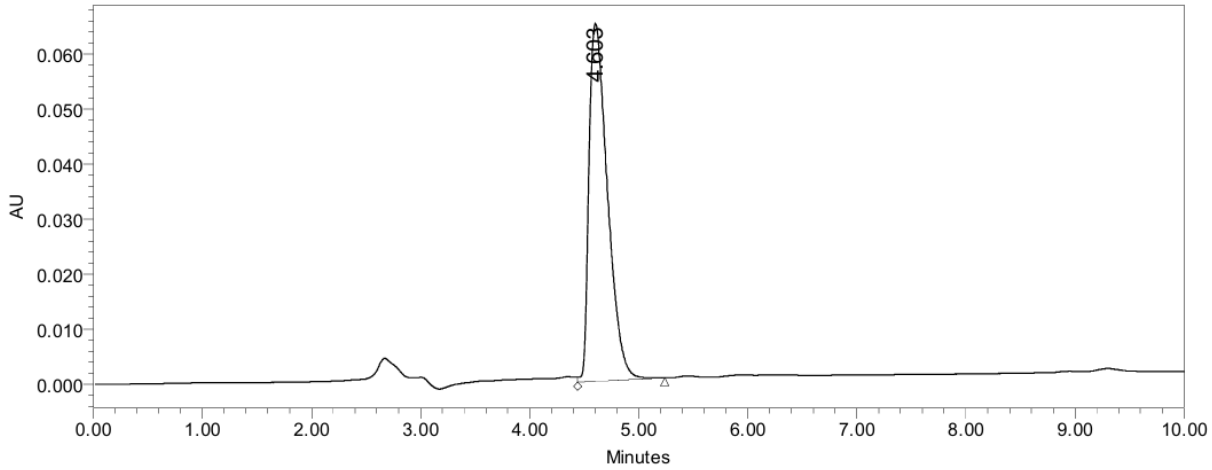
Appendix 60:*MS* Spectra of compound (20)



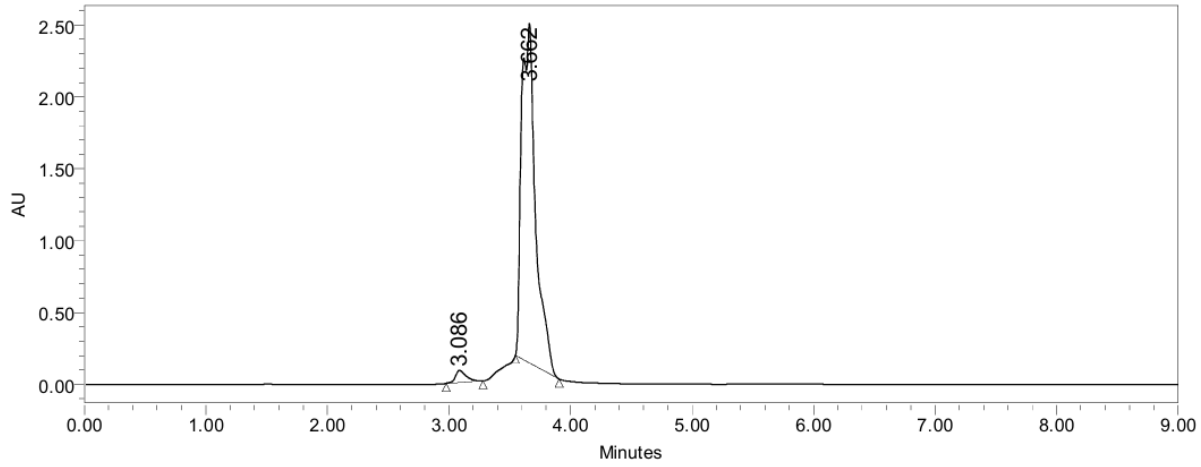
Appendix 61HPLC Chromatogram of azithromycin



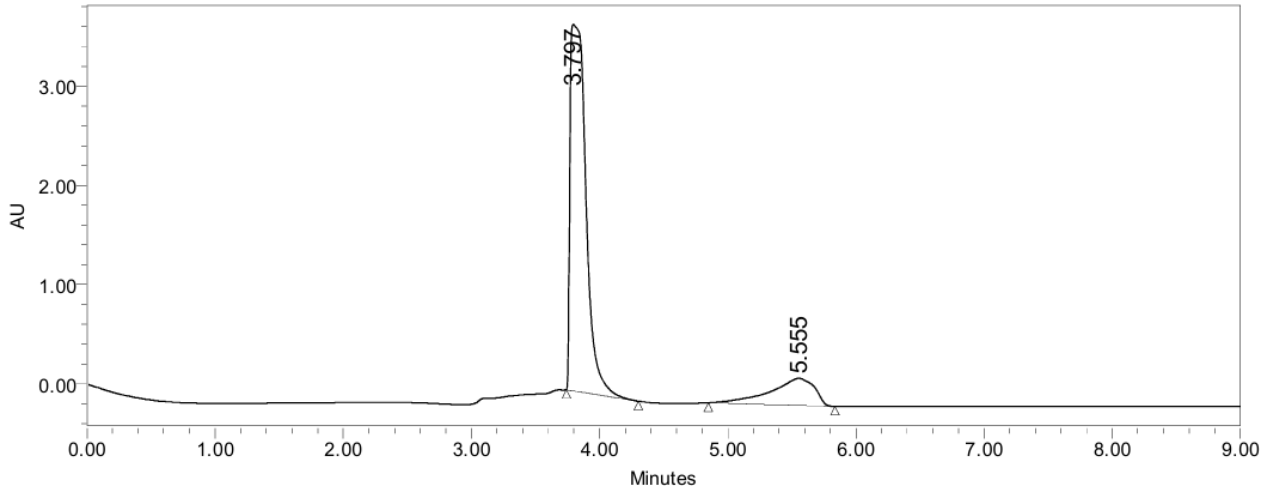
Appendix 62: HPLC Chromatogram of clarithromycin



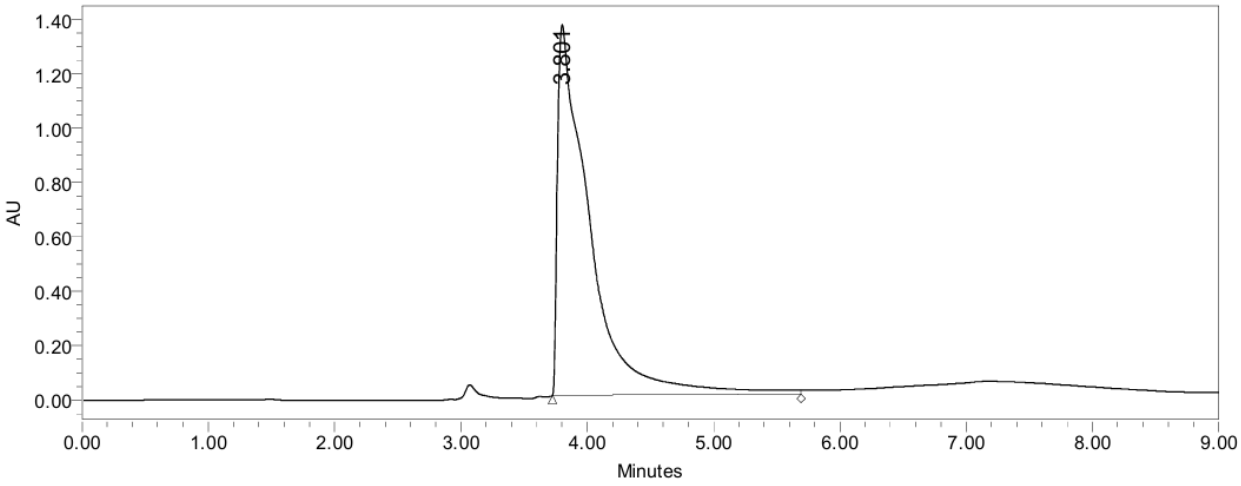
Appendix 63:HPLC Chromatogram of compound 1



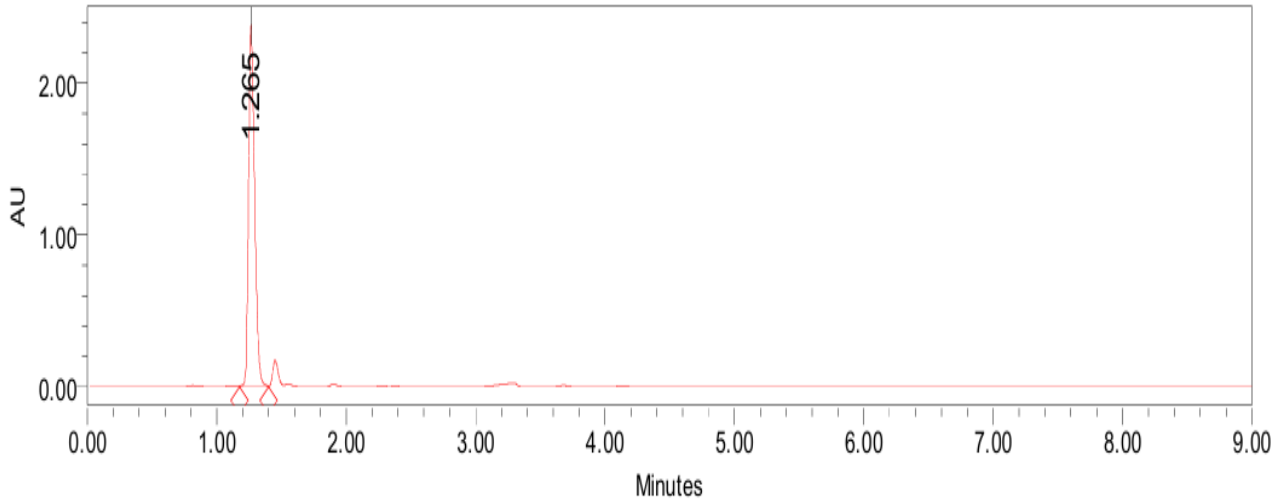
Appendix 64: HPLC Chromatogram of compound 2



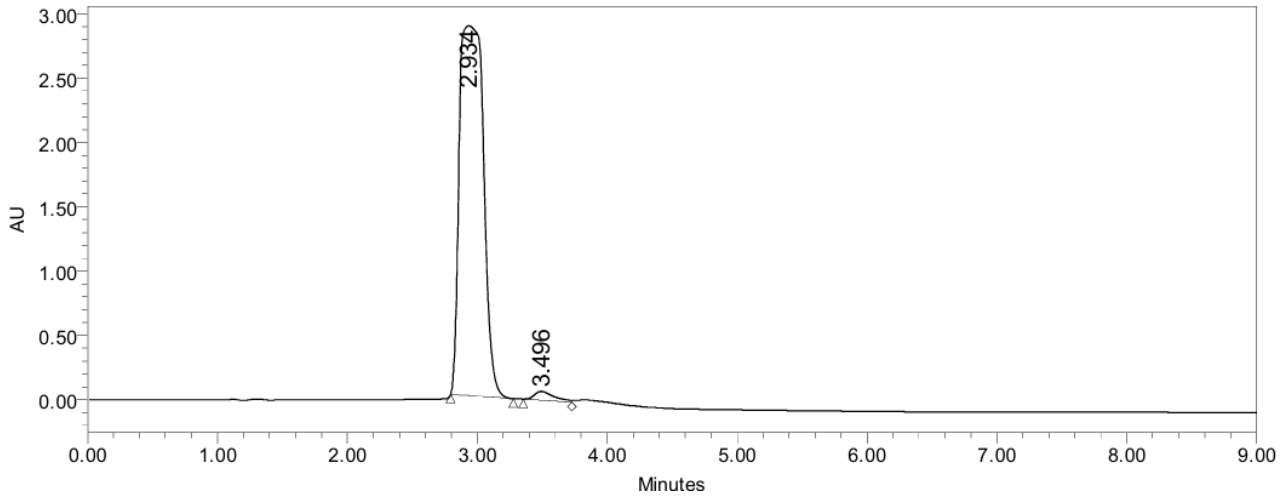
Appendix 65:HPLC Chromatogram of compound 3



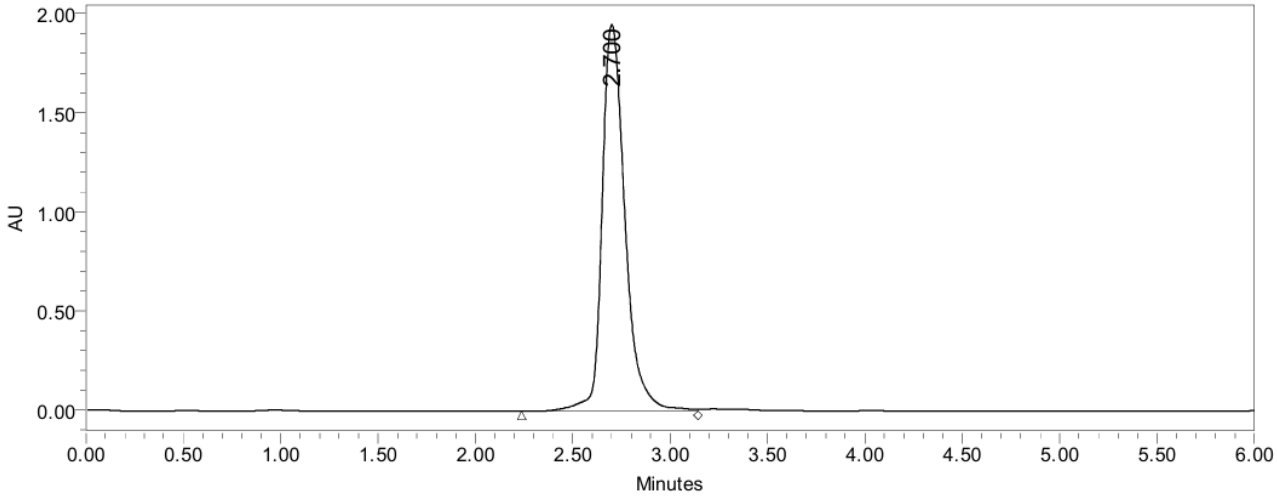
Appendix 66: HPLC Chromatogram of compound 4



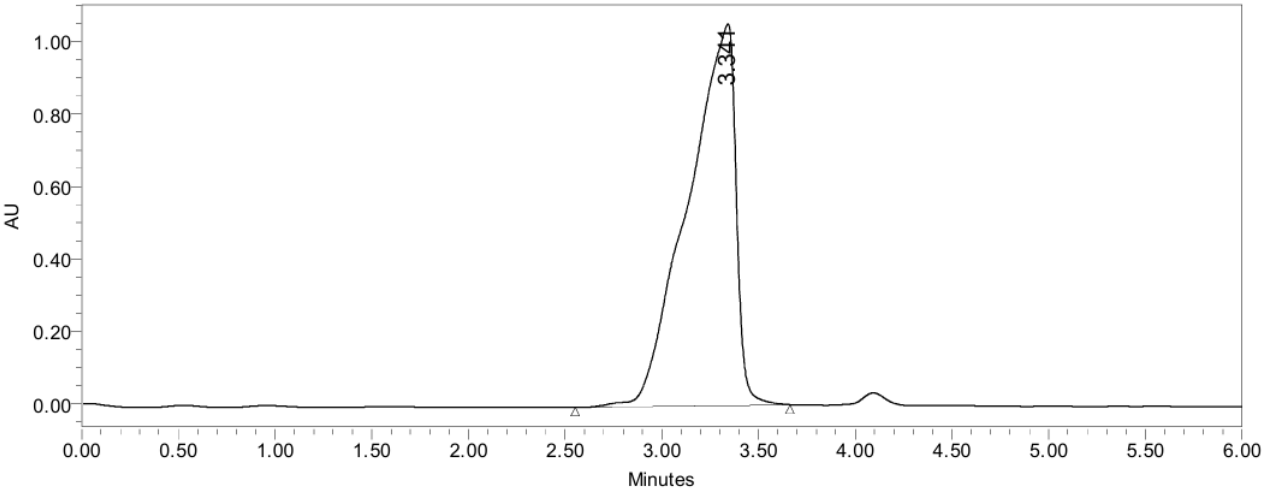
Appendix 67: HPLC Chromatogram of compound 5



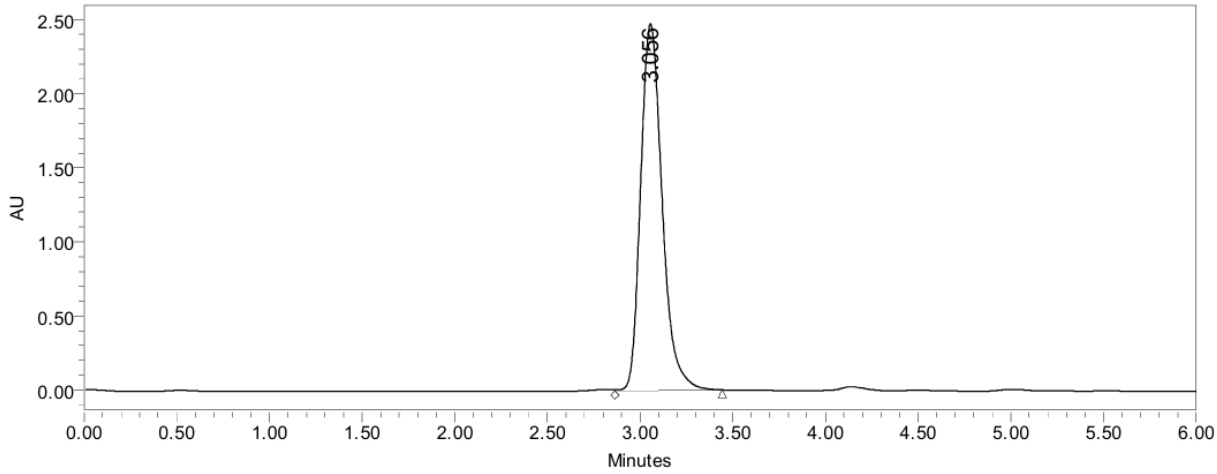
Appendix 68:HPLC Chromatogram of compound 6



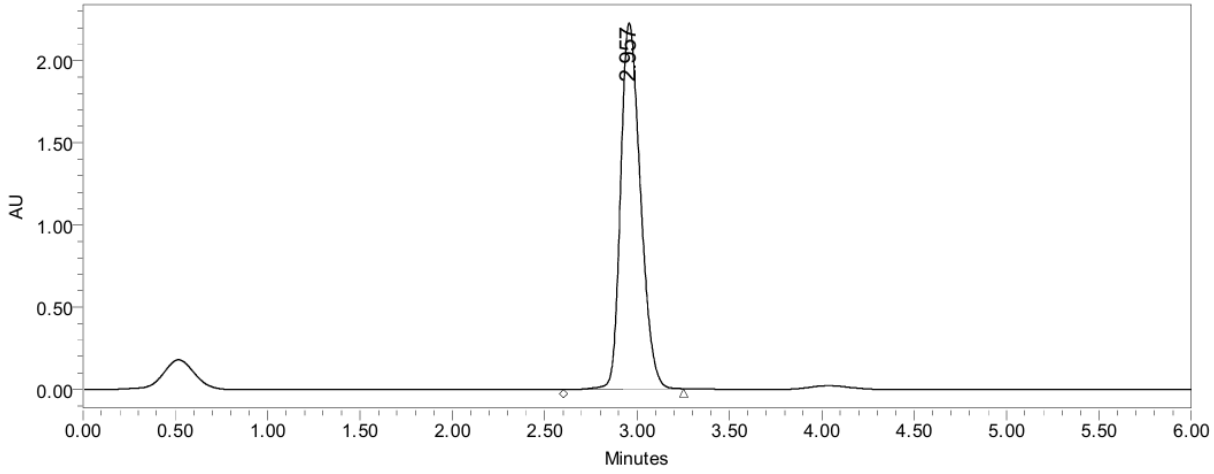
Appendix 69: HPLC Chromatogram of compound 7



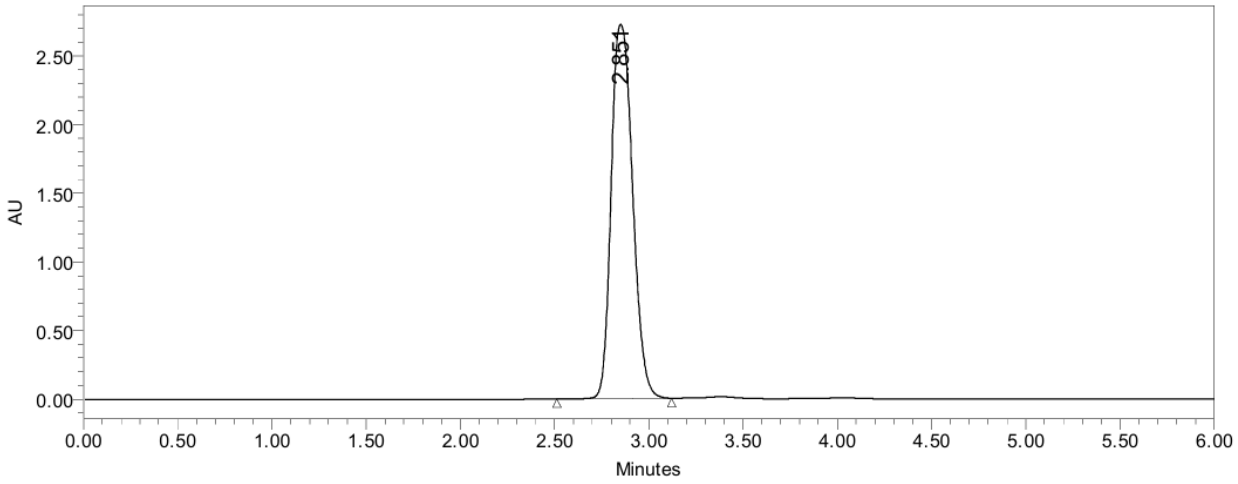
Appendix 70:HPLC Chromatogram of compound 8



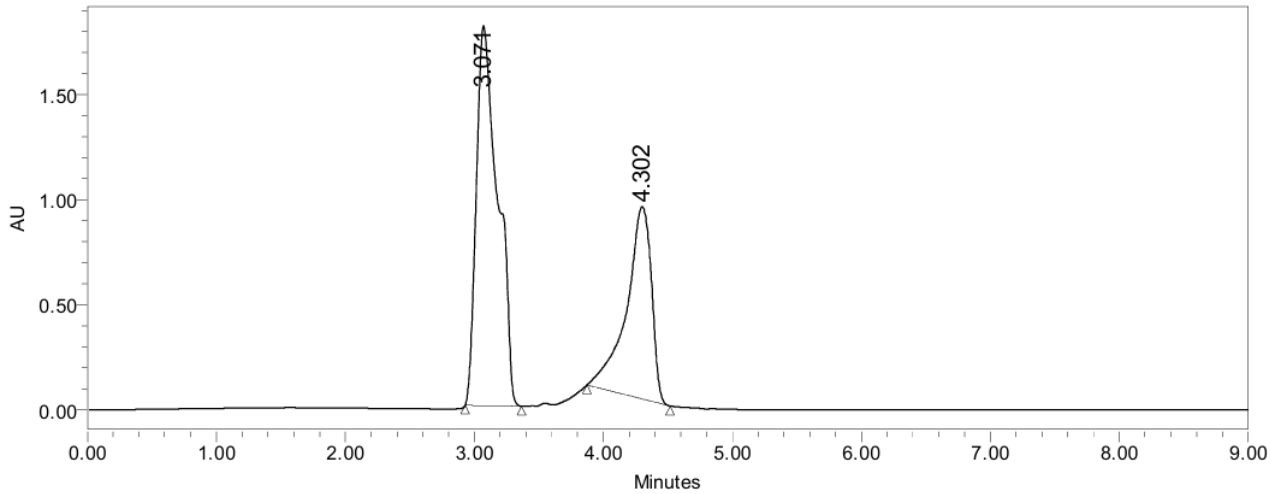
Appendix 71:HPLC Chromatogram of compound 9



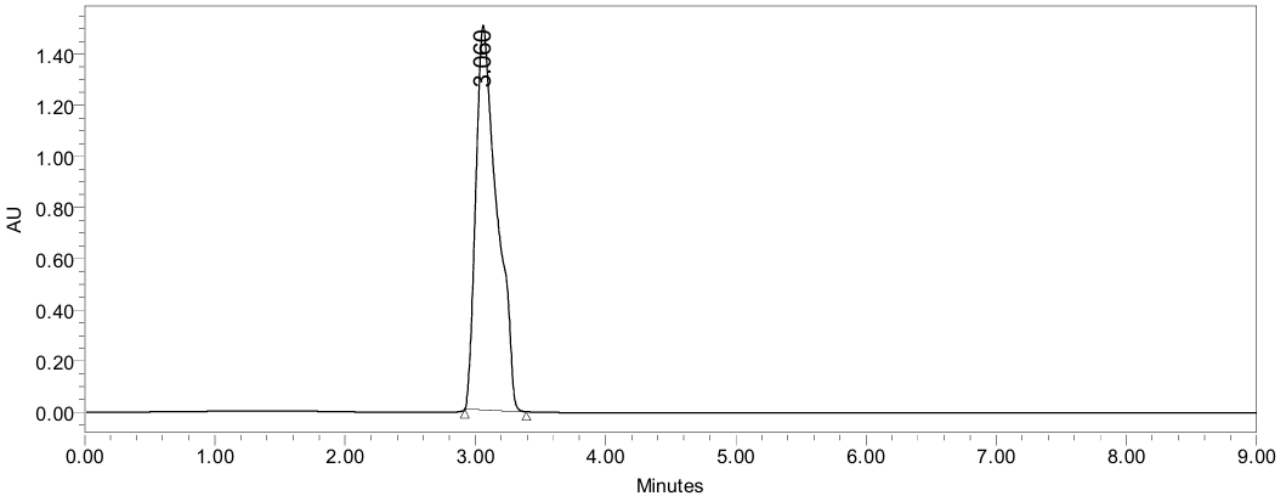
Appendix 72:HPLC Chromatogram of compound 10



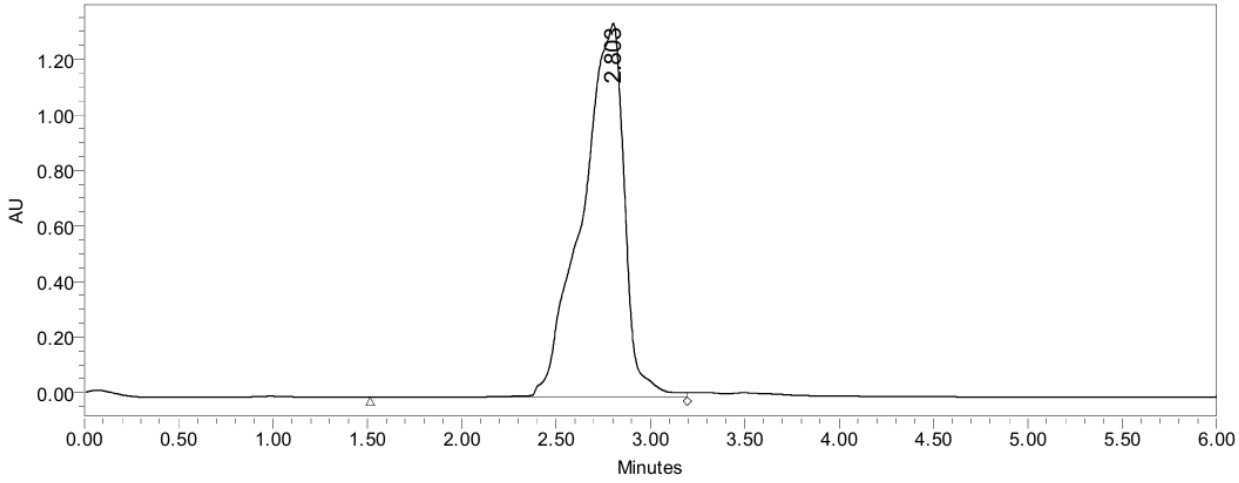
Appendix 73: HPLC Chromatogram of compound 1 PH 2.2



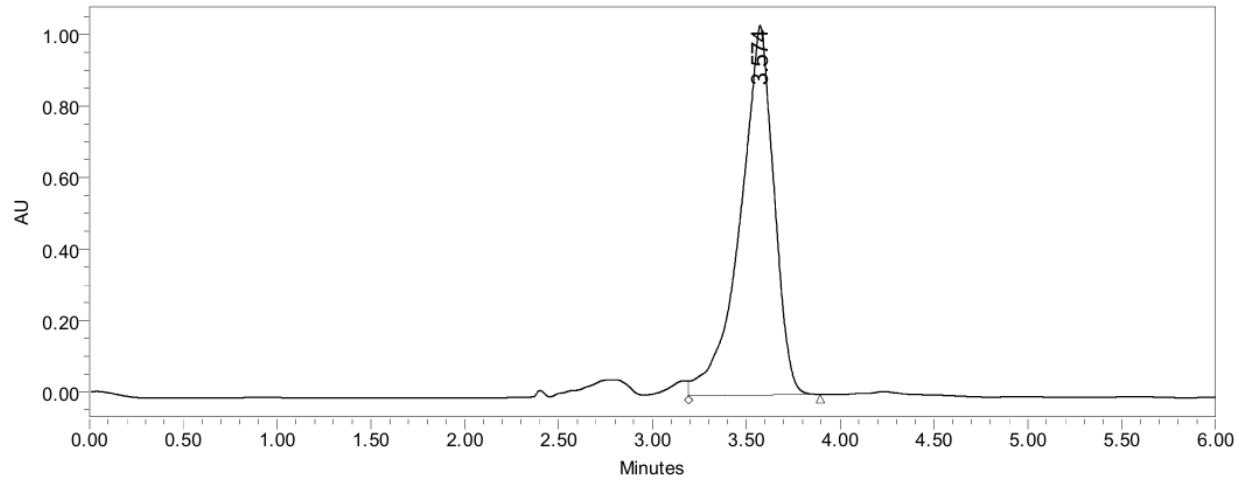
Appendix 74: HPLC Chromatogram of compound 3 PH 2.2



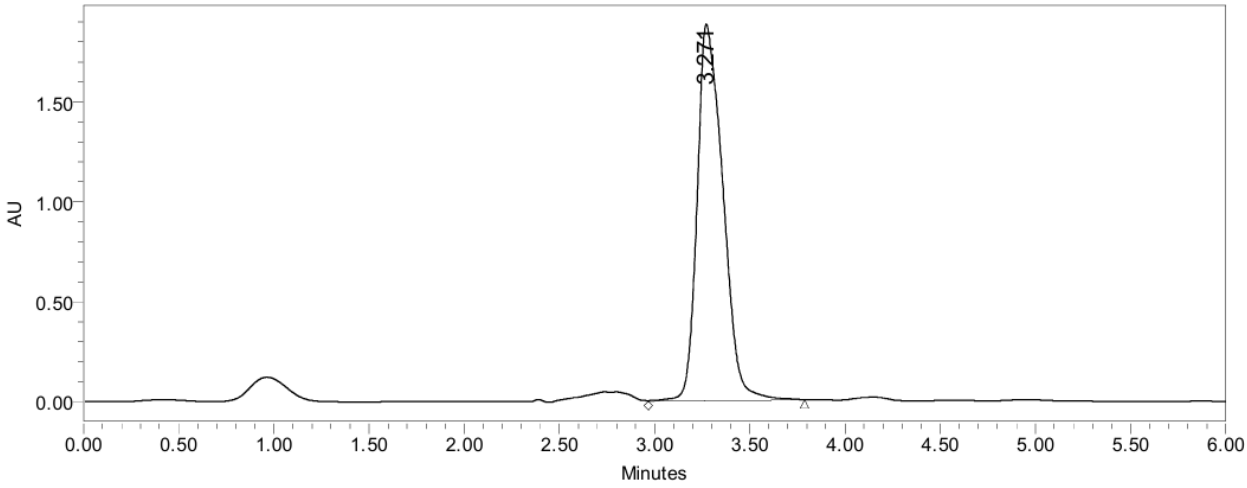
Appendix 75:HPLC Chromatogram of compound 6 PH 2.2



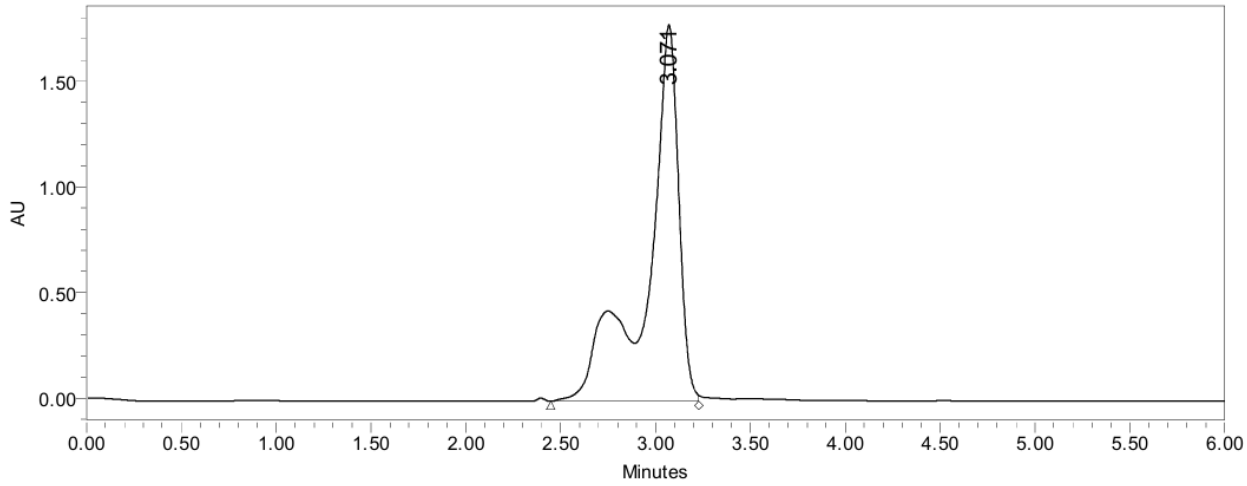
Appendix 76:HPLC Chromatogram of compound 7 PH 2.2



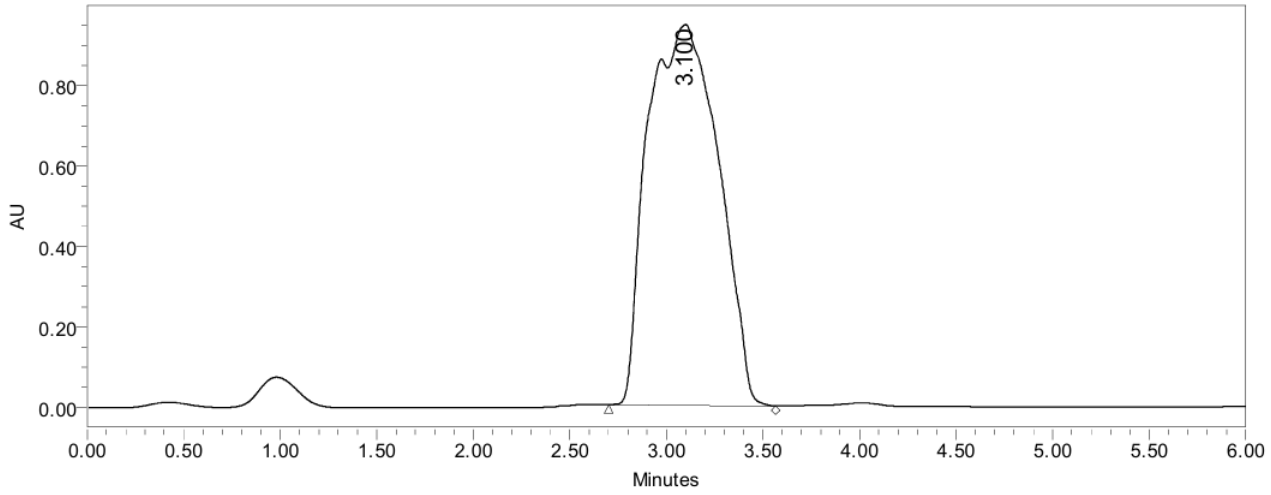
Appendix 77: HPLC Chromatogram of compound 8 PH 2.2



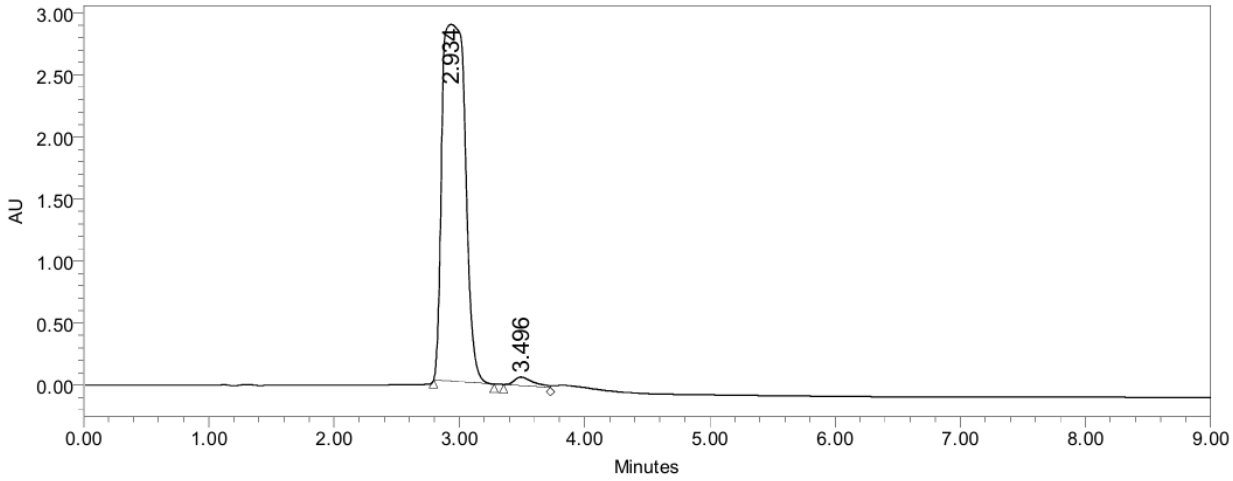
Appendix 78:HPLC Chromatogram of compound 9 PH 2.2



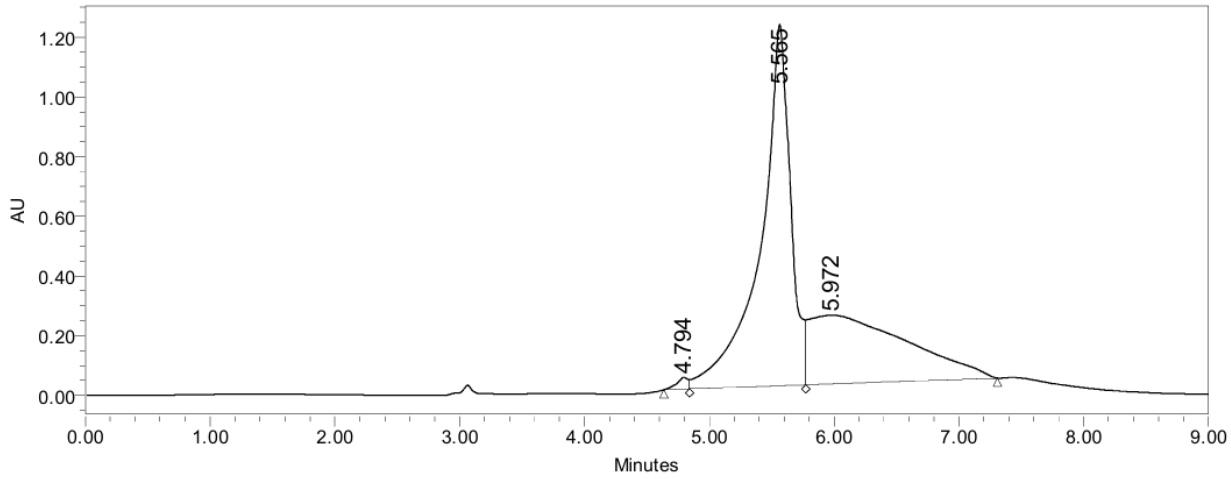
Appendix 79: HPLC Chromatogram of compound 10 PH 2.2



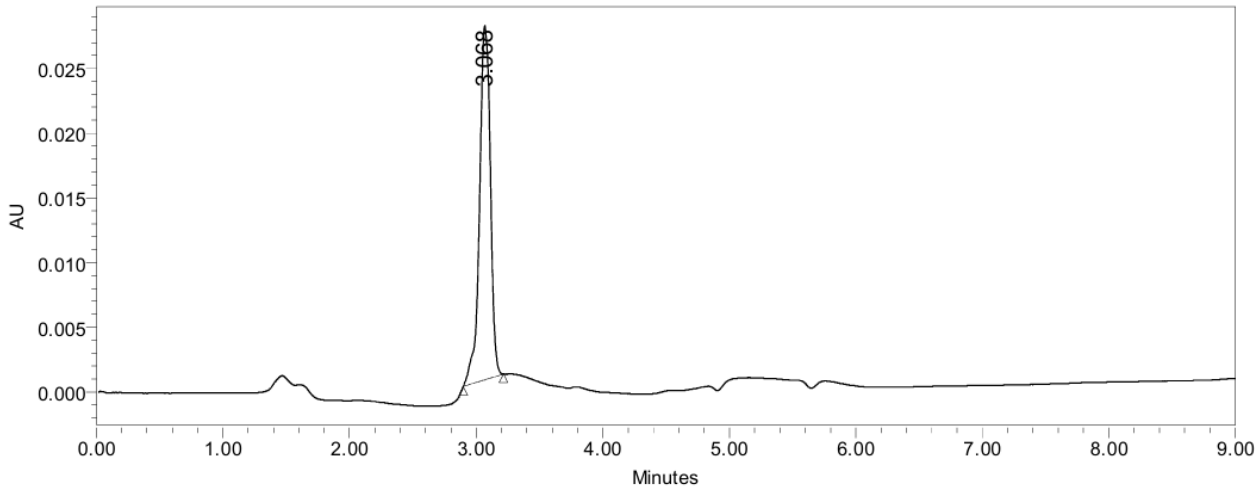
Appendix 80:HPLC Chromatogram of compound 1 PH 5.5



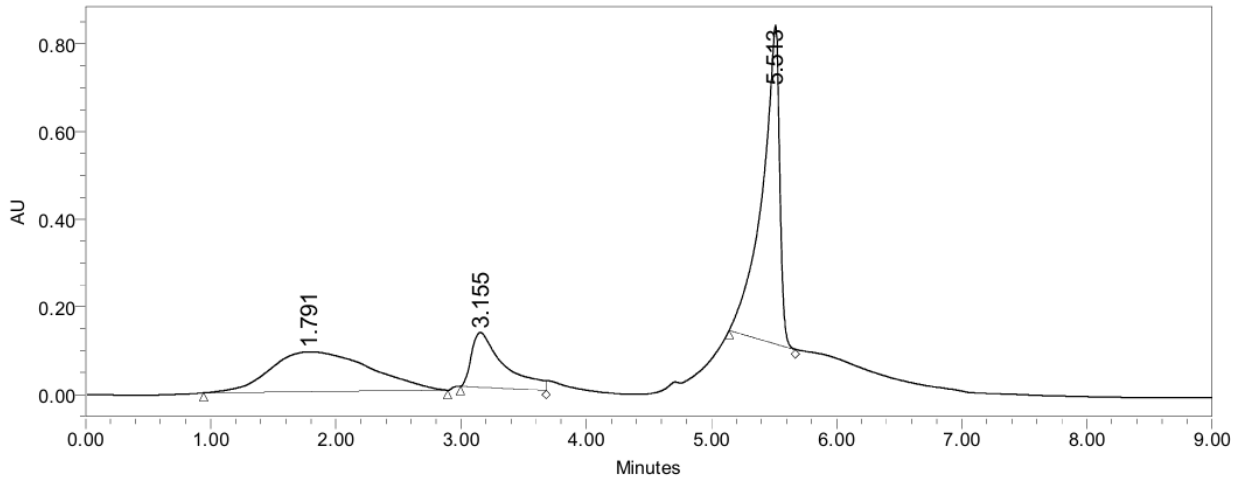
Appendix 81:HPLC Chromatogram of compound 2 PH 5.5



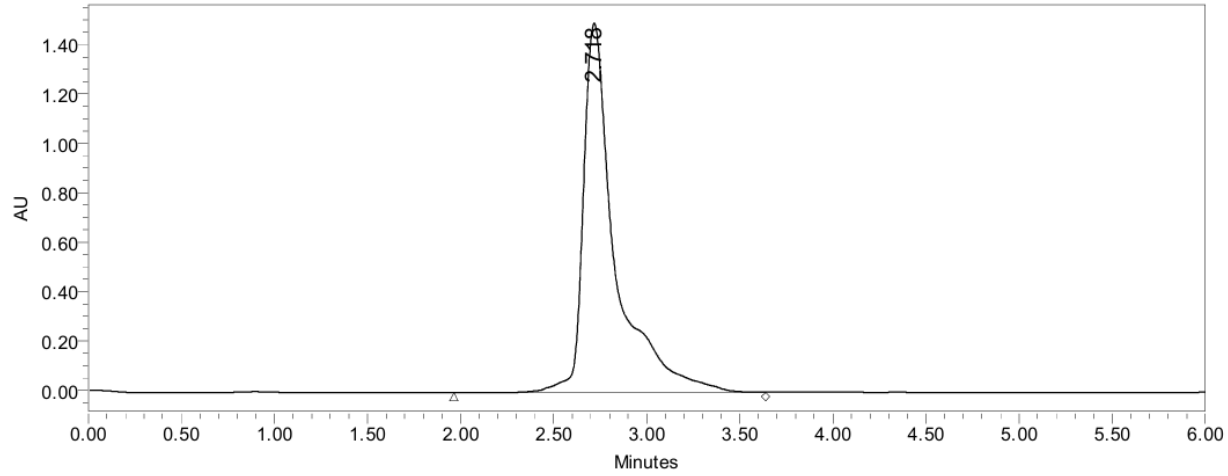
Appendix 82:HPLC Chromatogram of compound 3 PH 5.5



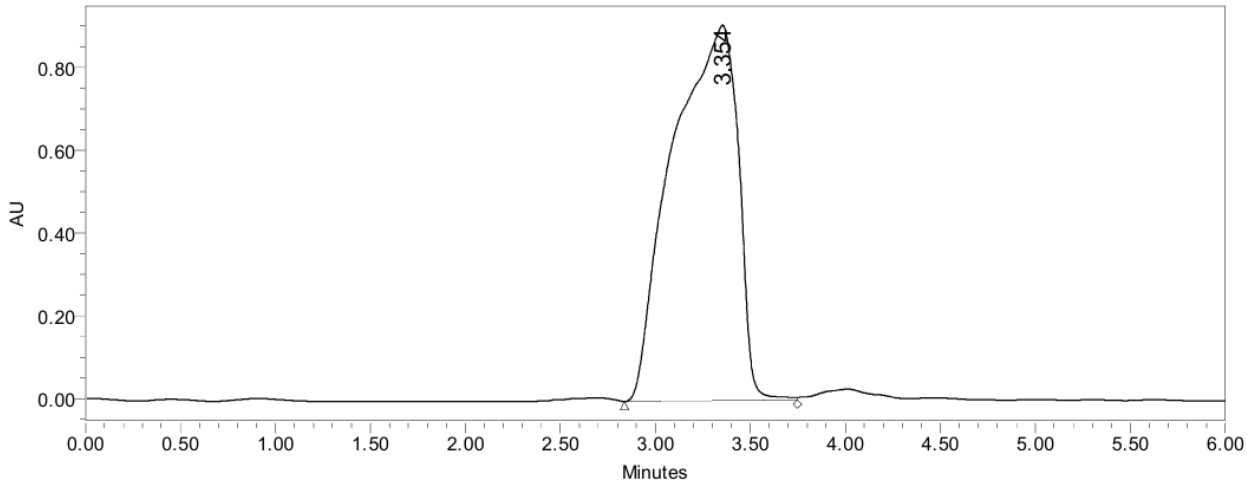
Appendix 83:HPLC Chromatogram of compound 4 PH 5.5



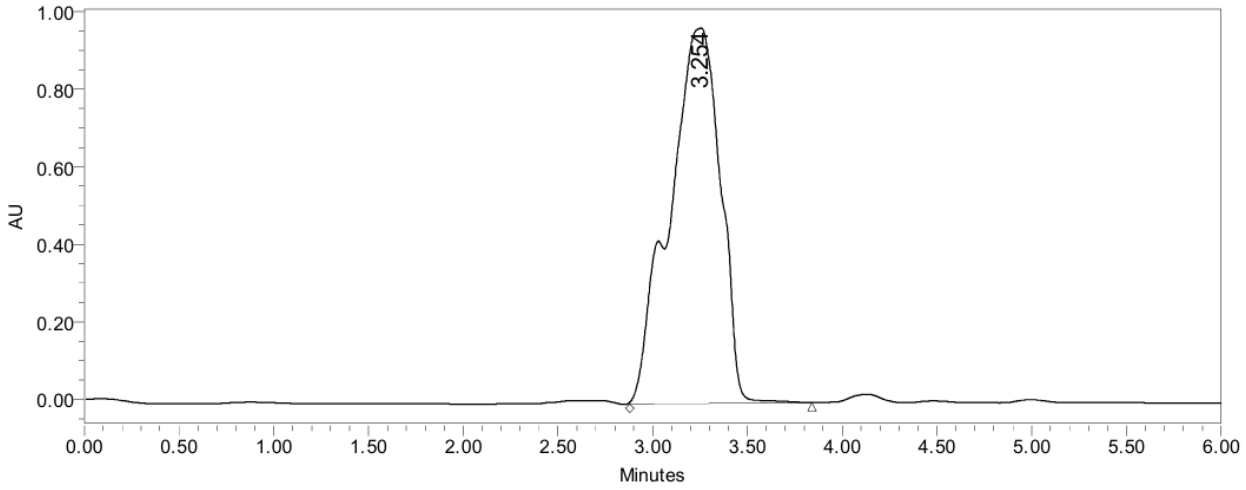
Appendix 84:HPLC Chromatogram of compound 6 PH 5.5



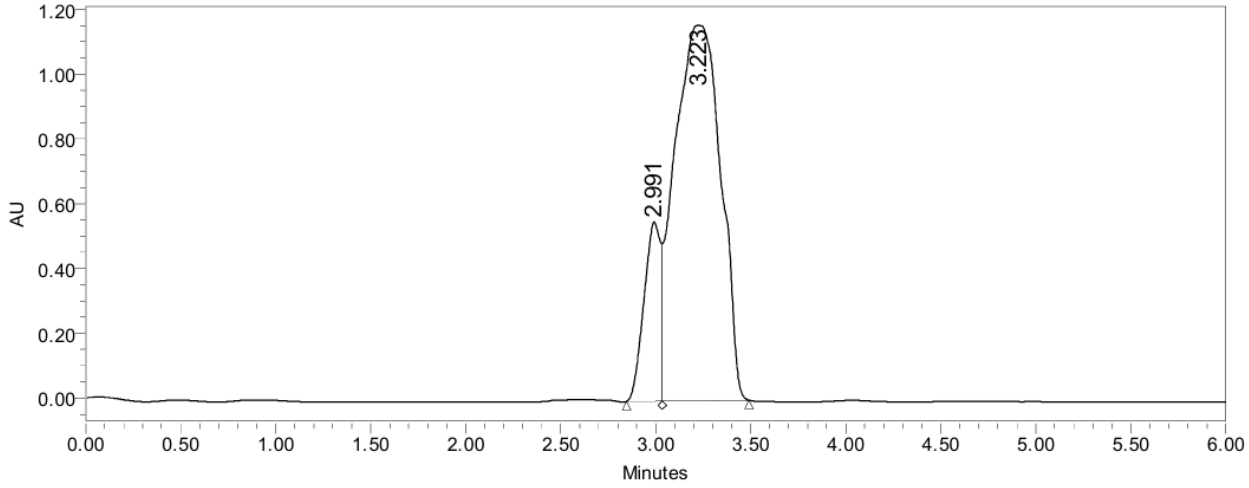
Appendix 85: HPLC Chromatogram of compound 7 PH 5.5



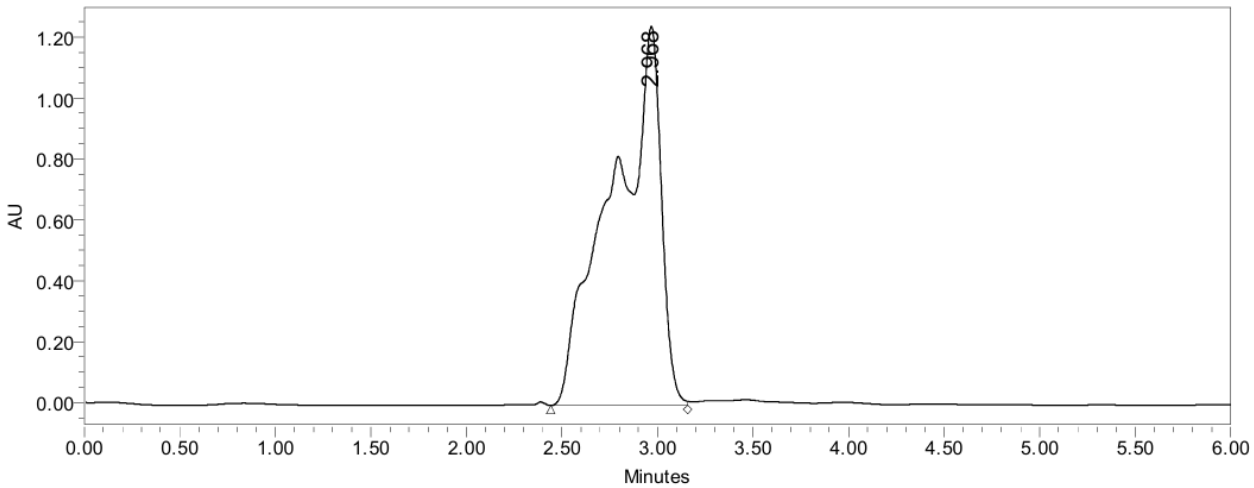
Appendix 86: HPLC Chromatogram of compound 8 PH 5.5



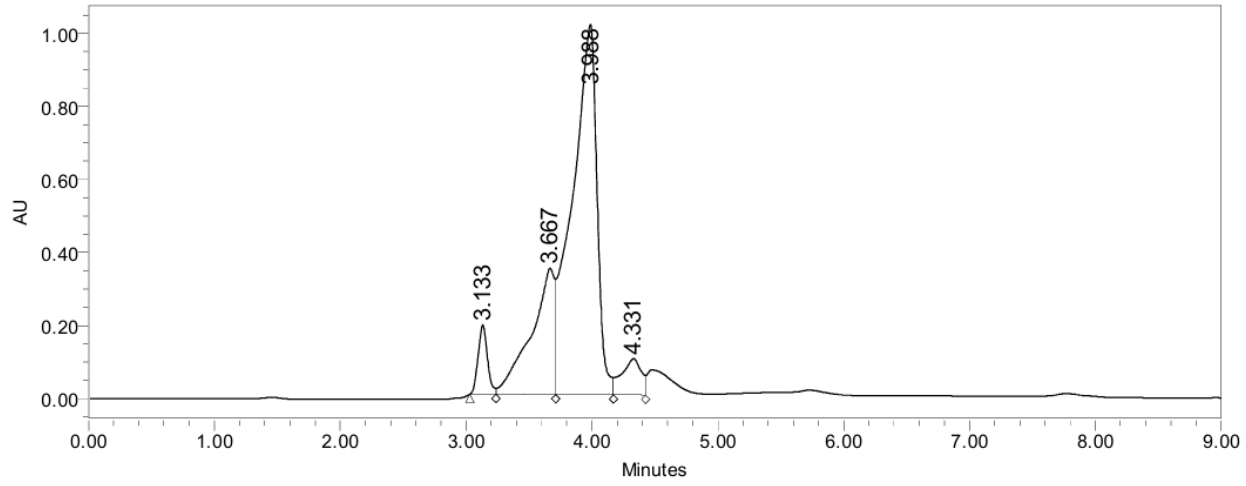
Appendix 87:HPLC Chromatogram of compound 9 PH 5.5



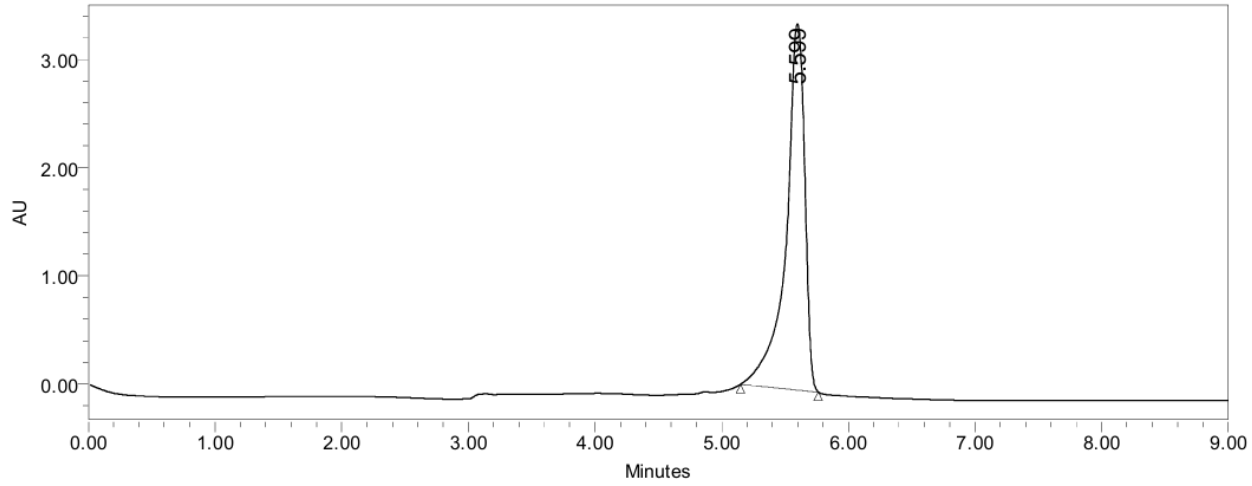
Appendix 88: HPLC Chromatogram of compound 10 PH 5.5



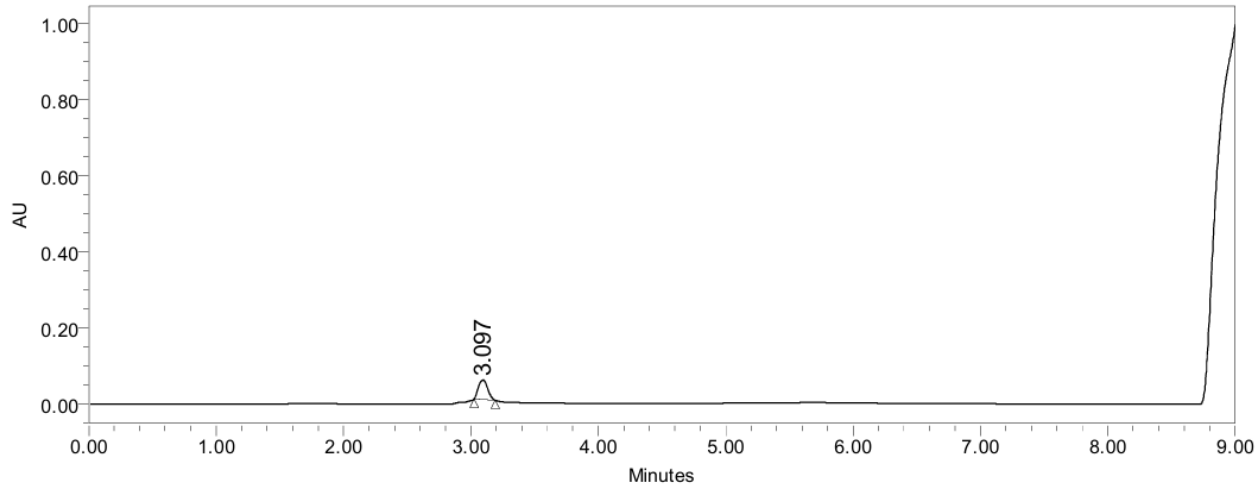
Appendix 89: HPLC Chromatogram of compound 1 PH 7.4



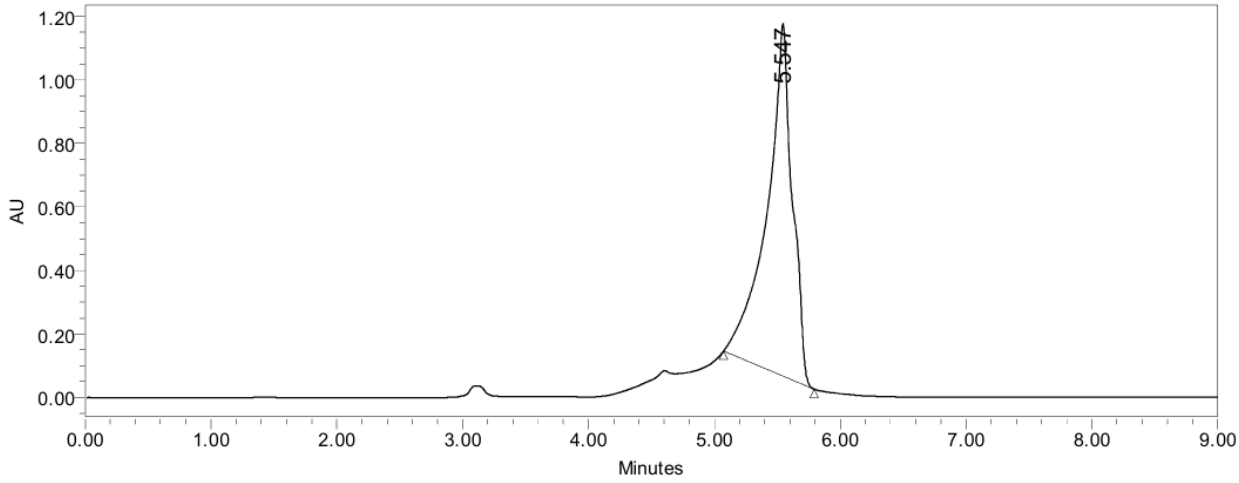
Appendix 90:HPLC Chromatogram of compound 2 PH 7.4



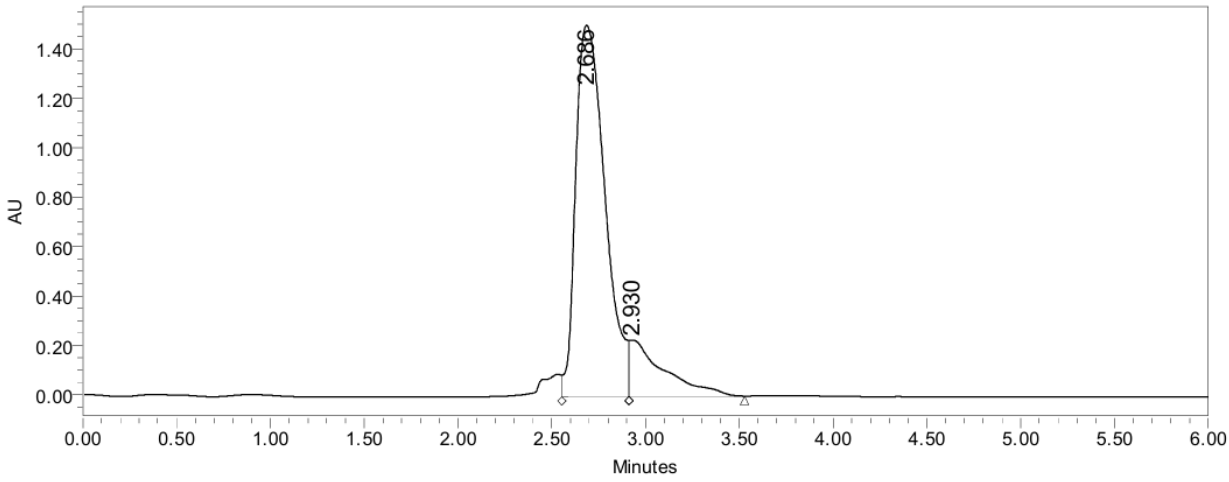
Appendix 91: HPLC Chromatogram of compound 3 PH 7.4



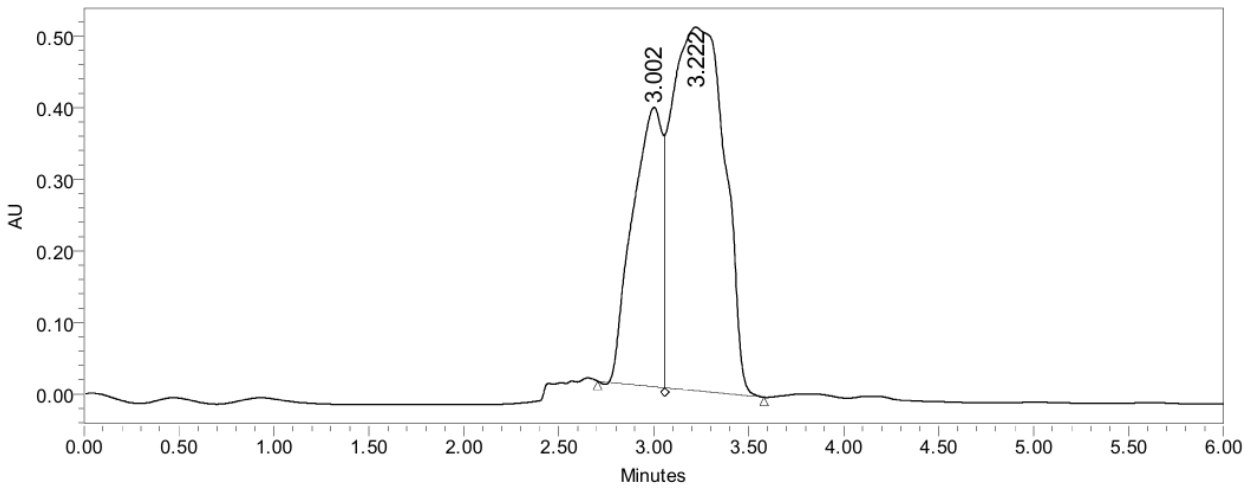
Appendix 92: HPLC Chromatogram of compound 4 PH 7.4



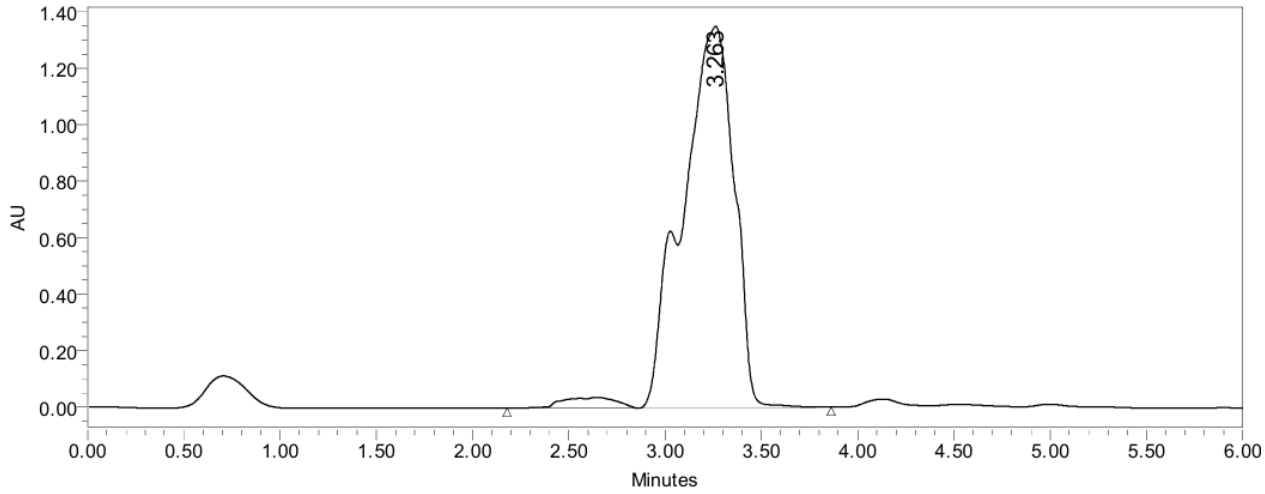
Appendix 93:HPLC Chromatogram of compound 6 PH 7.4



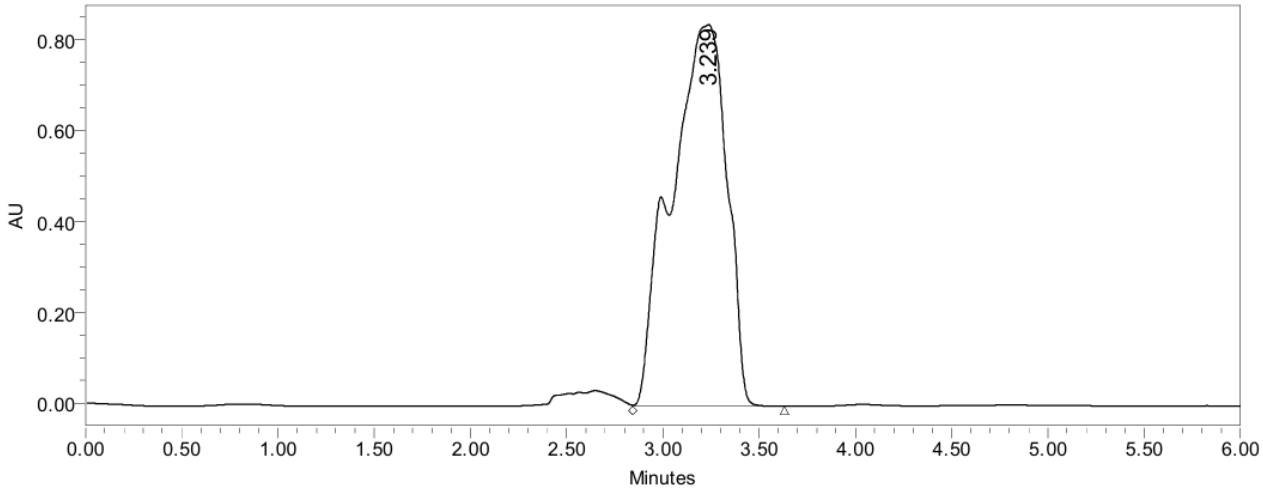
Appendix 94: HPLC Chromatogram of compound 7 PH 7.4



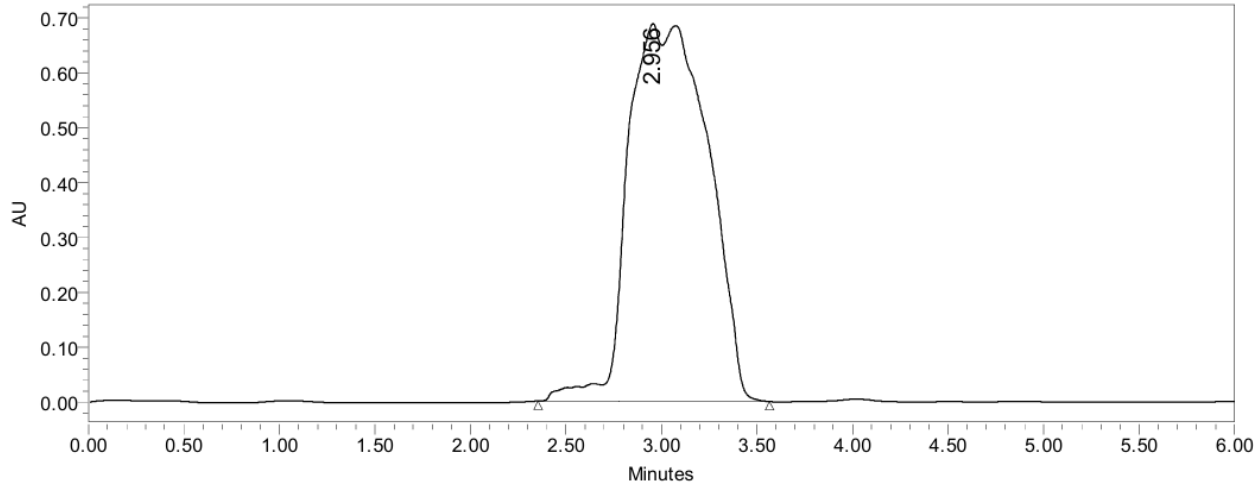
Appendix 95: HPLC Chromatogram of compound 8 PH 7.4



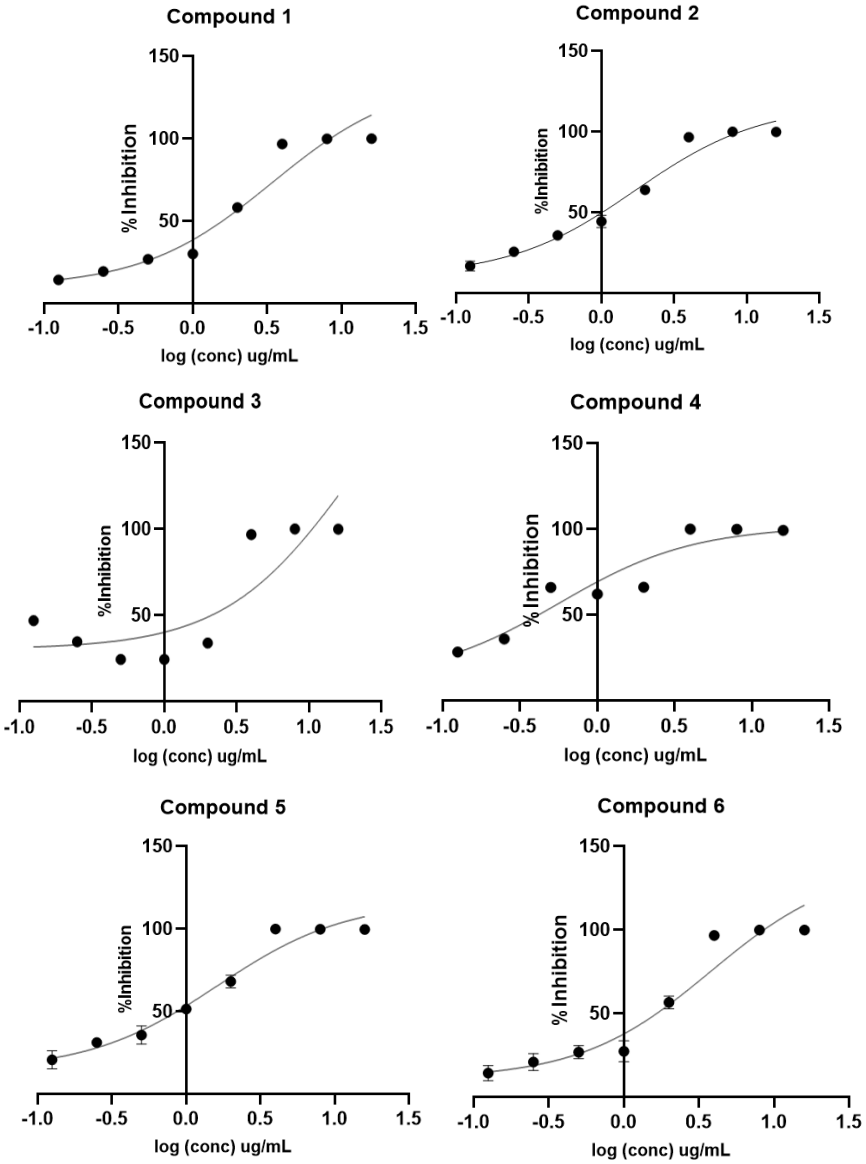
Appendix 96: HPLC Chromatogram of compound 9 PH 7.4

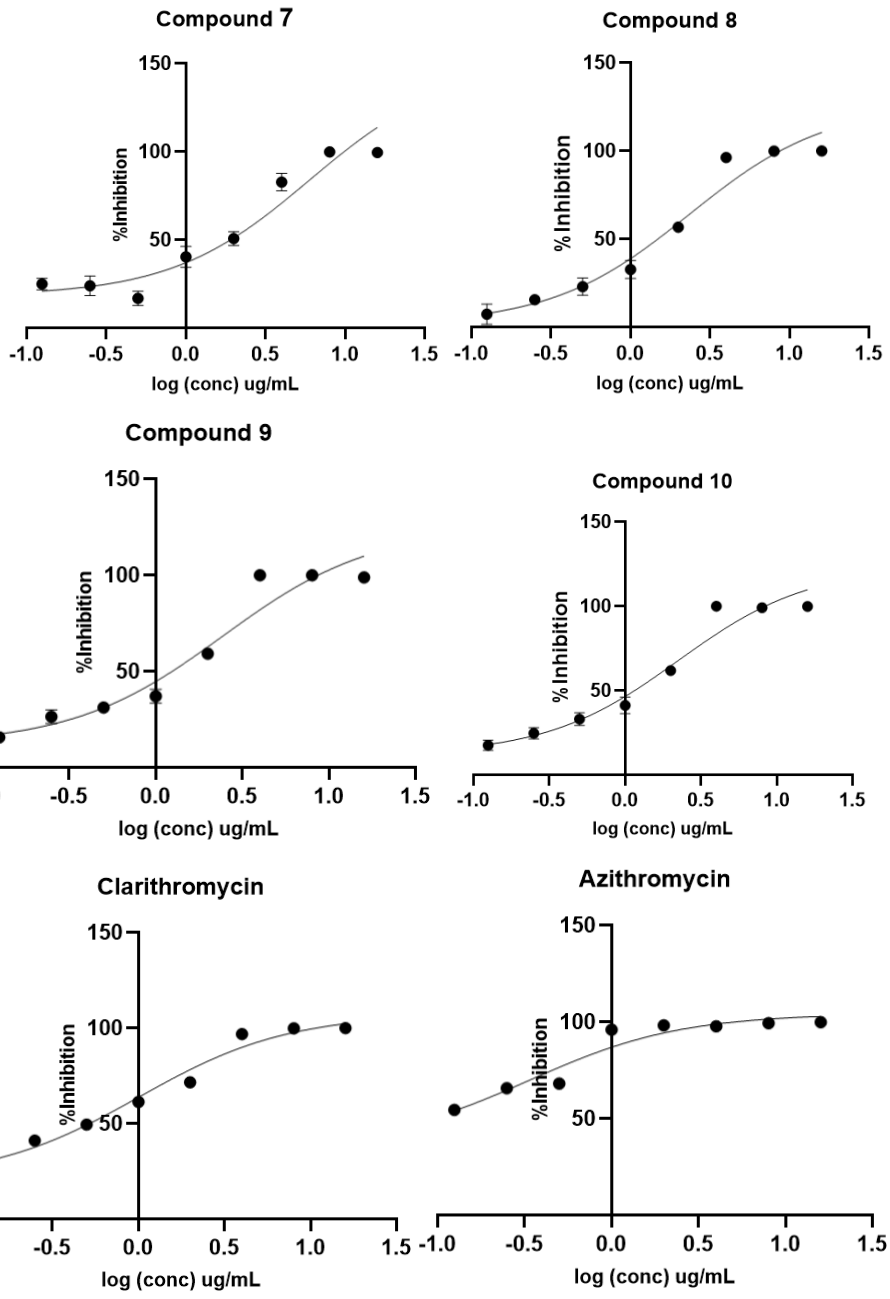


Appendix 97:HPLC Chromatogram of compound 10 PH 7.4

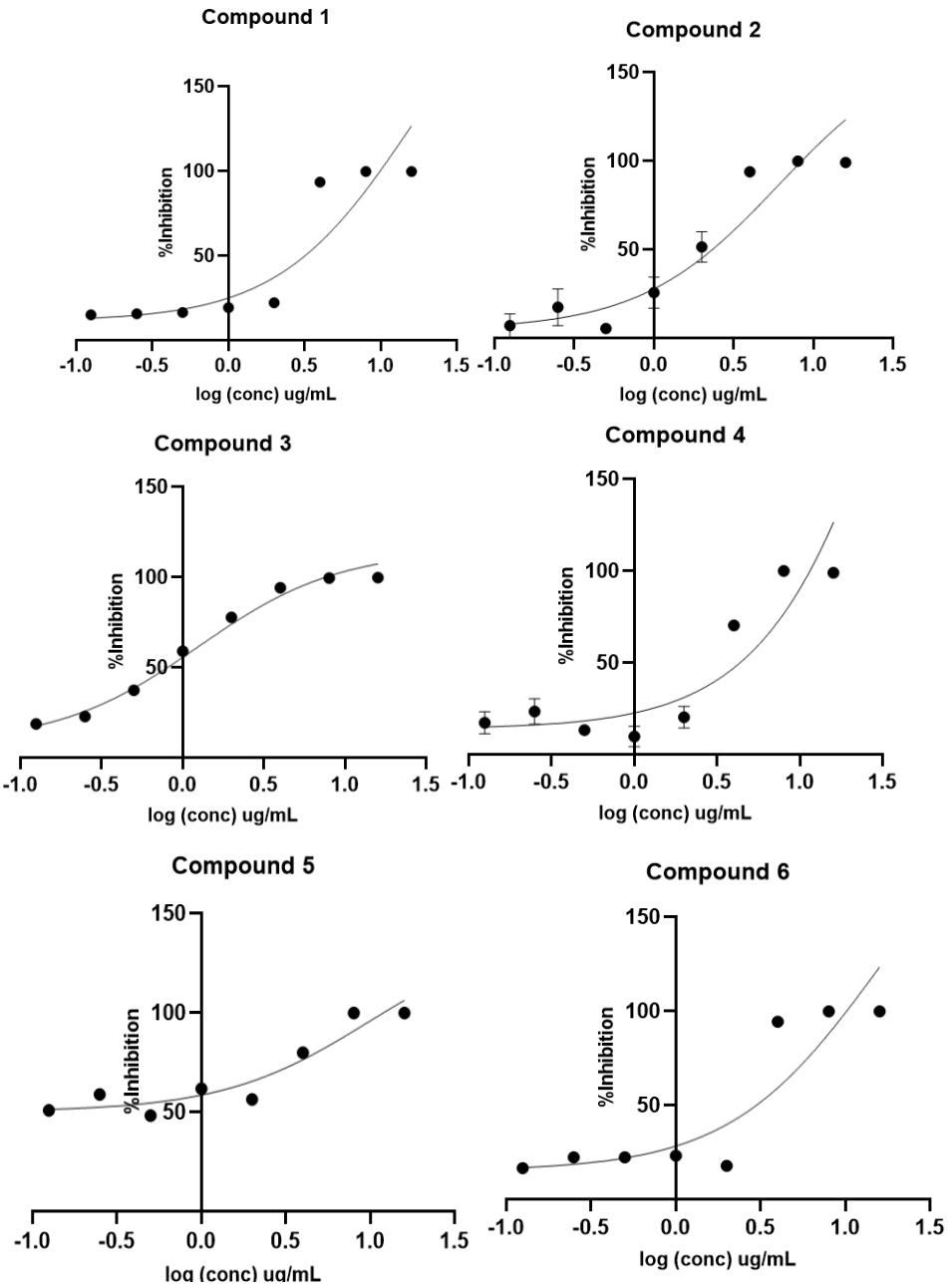


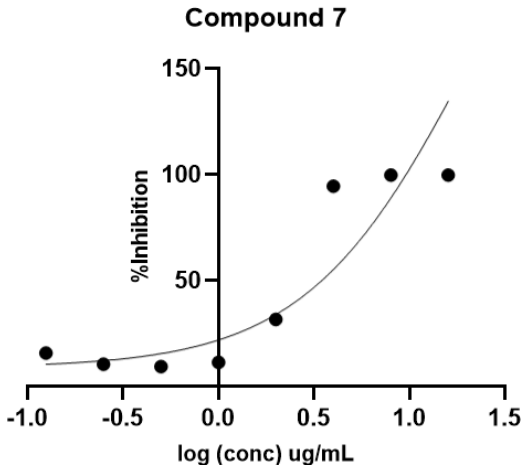
Appendix 98:Dose-response curve of macrolide derivatives in *Escherichia coli*

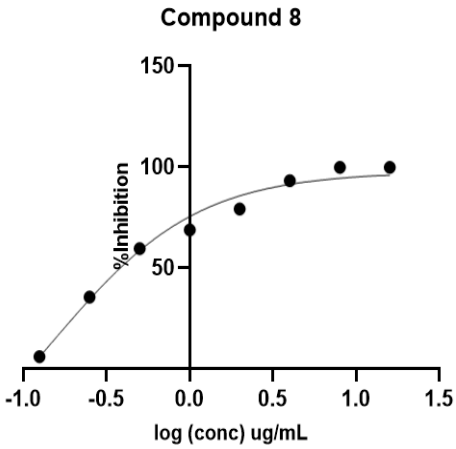


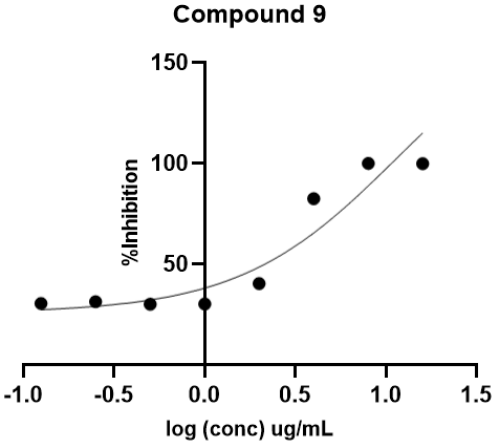

Compound 7
%Inhibition
log (conc) ug/mL
Compound 8
% Inhibition
log (conc) ug/mL
Compound 9
%Inhibition
log (conc) ug/mL
Compound 10
% Inhibition
log (conc) ug/mL
Clarithromycin
%Inhibition
log (conc) ug/mL
Azithromycin
%Inhibition
log (conc) ug/mL

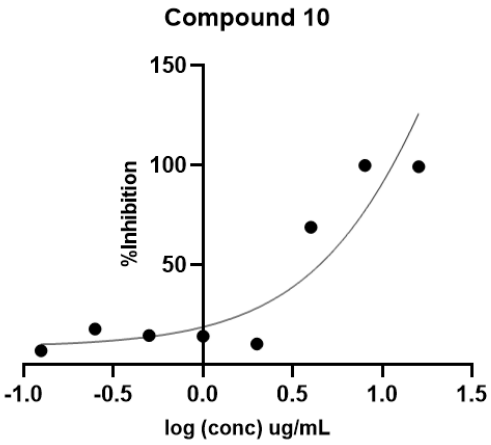
Appendix 99:Dose-response curve in macrolide derivatives in *Staphylococcus aureus*

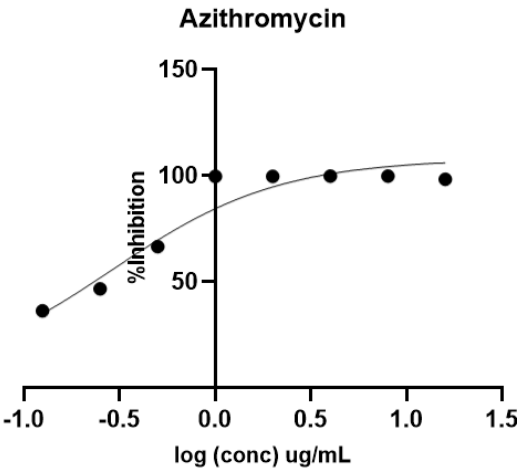


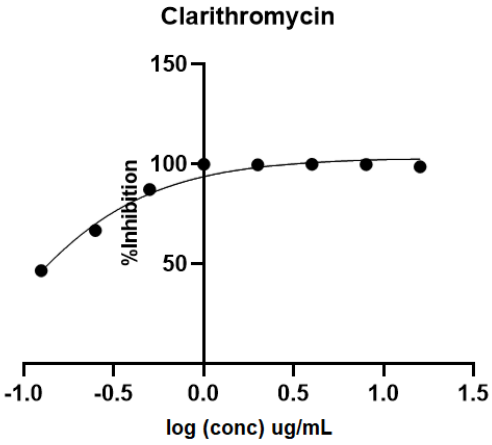

Compound 7
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL


Compound 8
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

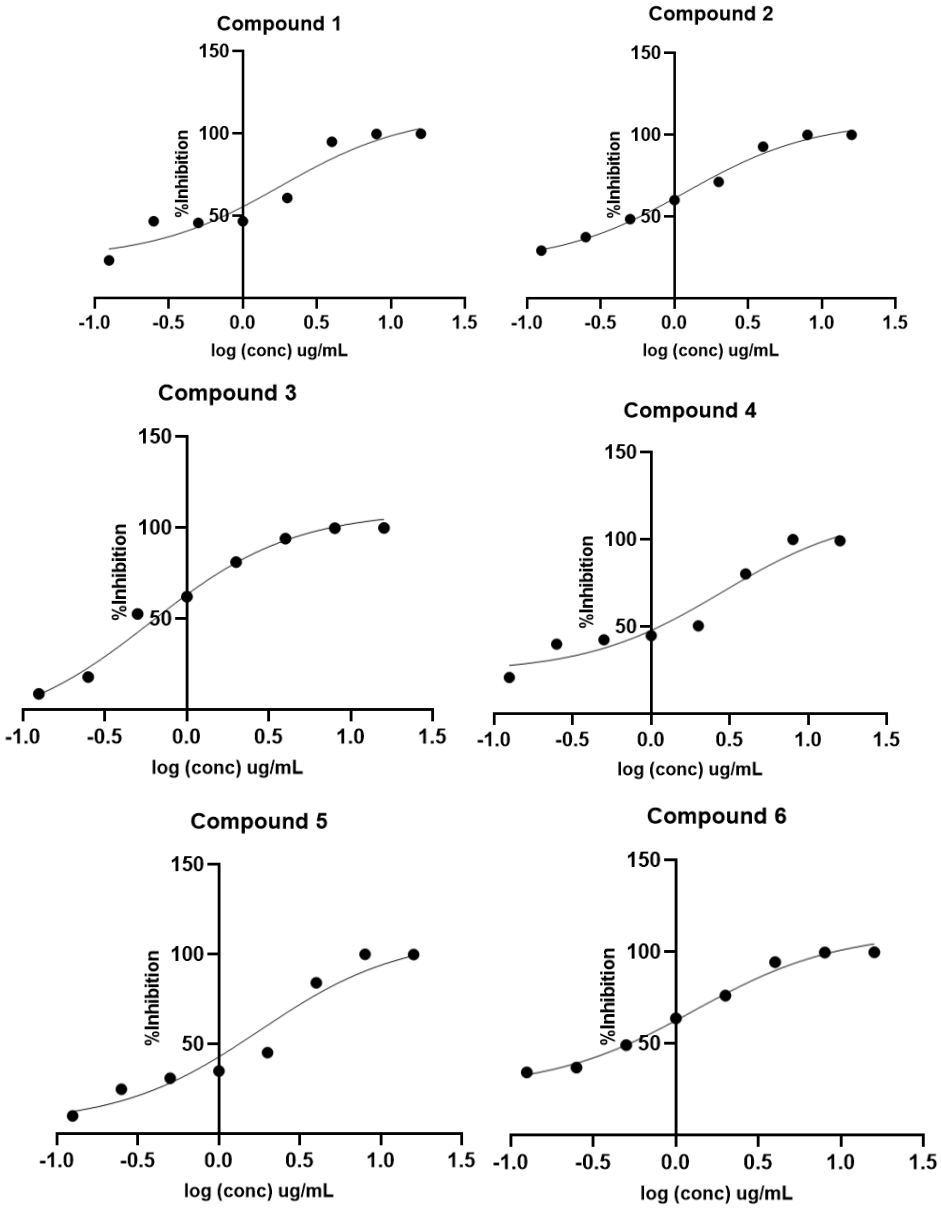

Compound 9
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

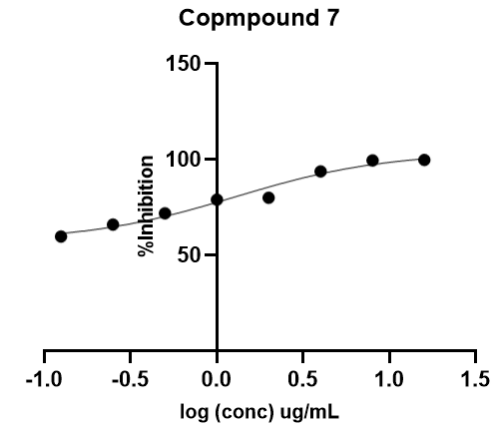

Compound 10
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

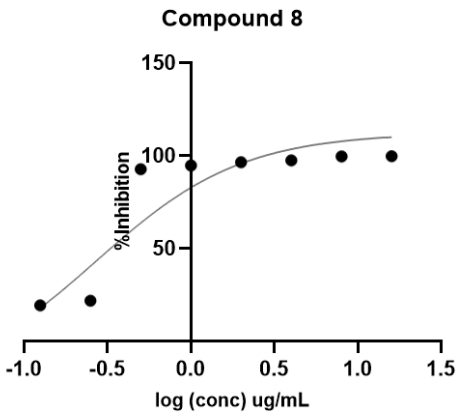

Azithromycin
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

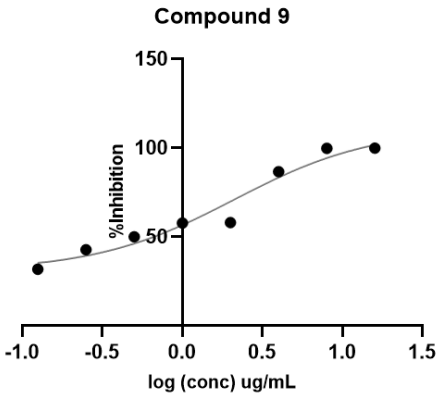

Clarithromycin
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

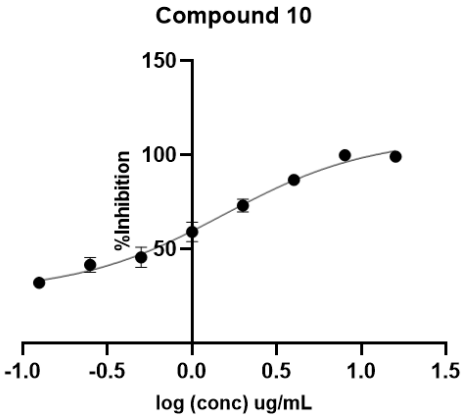
Appendix 100: Dose-response curve in macrolide derivatives in *Streptococcus pneumoniae*

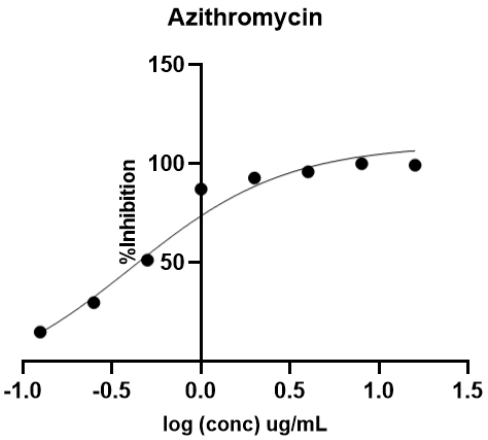


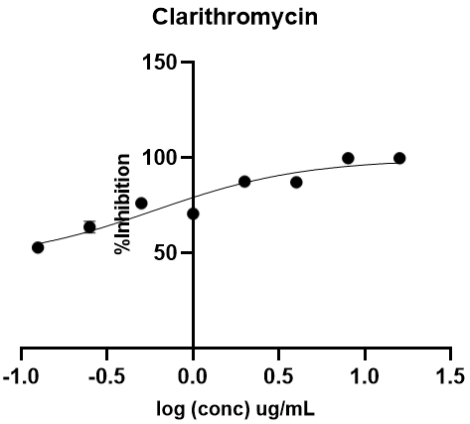

Copmpound 7
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL


Compound 8
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

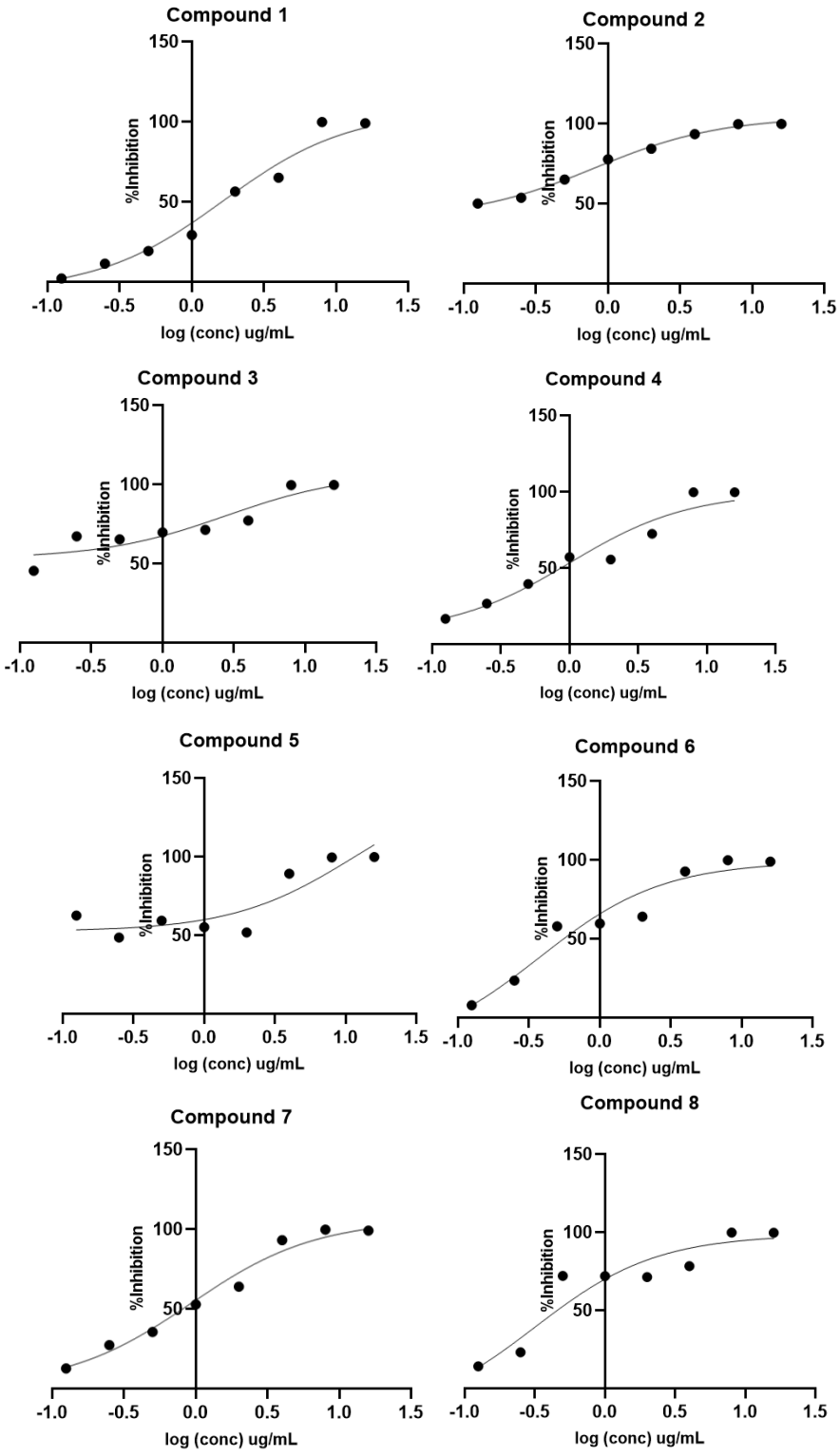

Compound 9
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

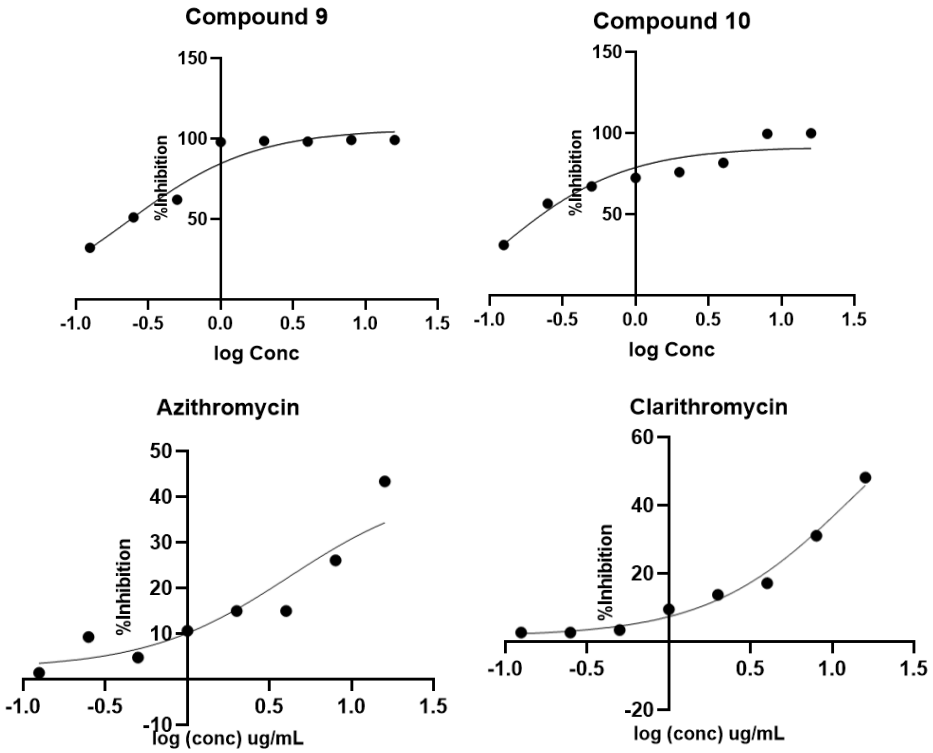

Compound 10
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL


Azithromycin
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

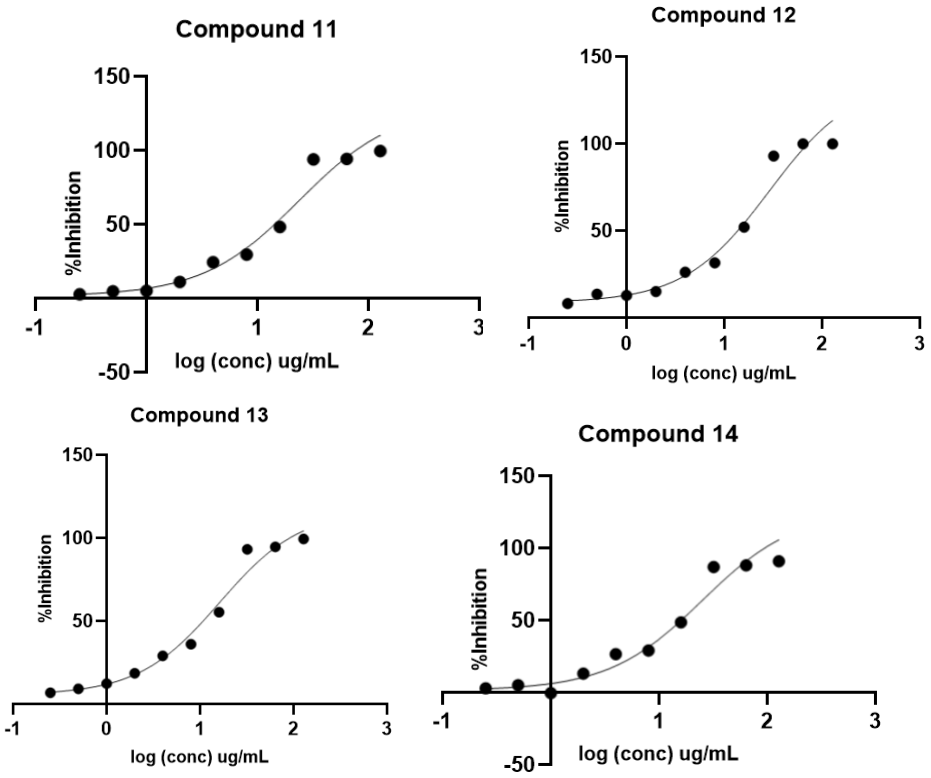

Clarithromycin
150
100
50
%Inhibition
-1.0
-0.5
0.0
0.5
1.0
1.5
log (conc) ug/mL

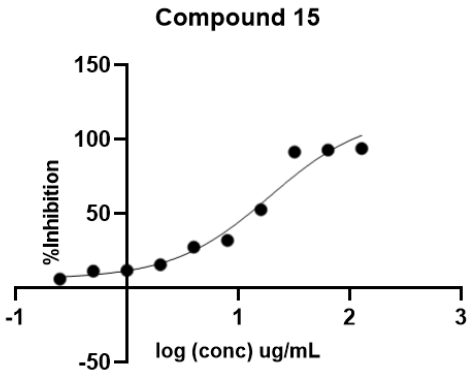
Appendix 101:Dose-response curve in macrolide derivatives in *Pseudomonas aeruginosa*

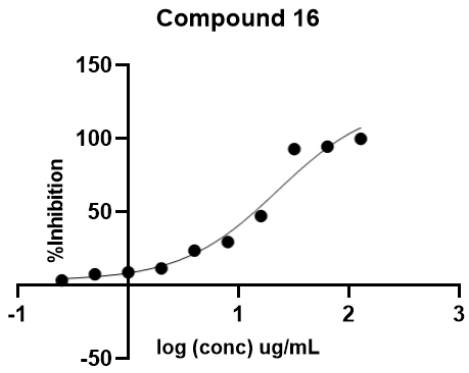


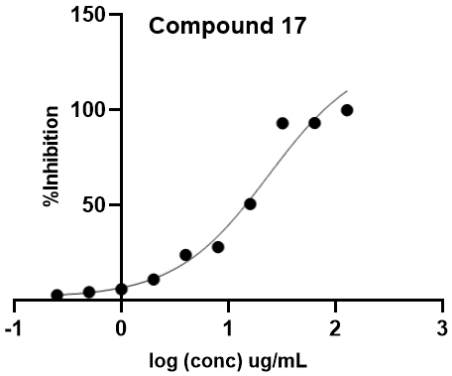


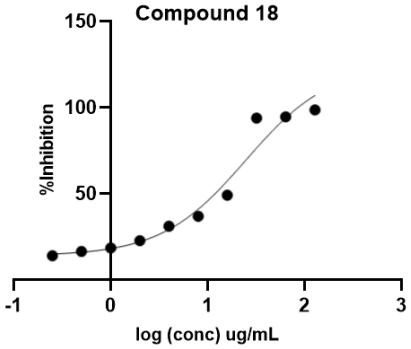
Appendix 102:Dose-response curve in penicillin derivatives in *Escherichia coli*

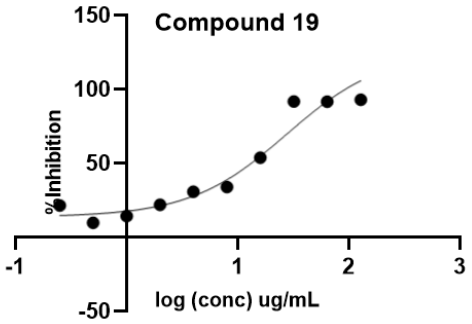


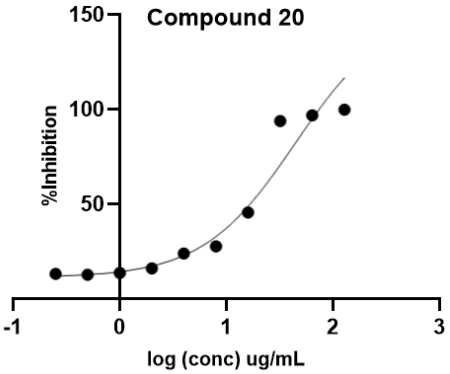

Compound 15
150
100
50
-50
%Inhibition
-1
1
2
3
log (conc) ug/mL

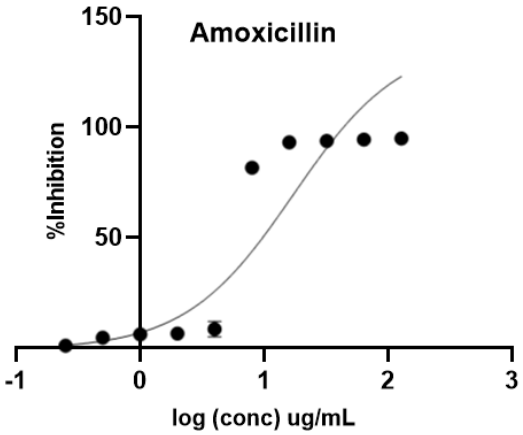

Compound 16
150
100
50
-50
%Inhibition
-1
1
2
3
log (conc) ug/mL


Compound 17
150
100
50
%Inhibition
-1
0
1
2
3
log (conc) ug/mL

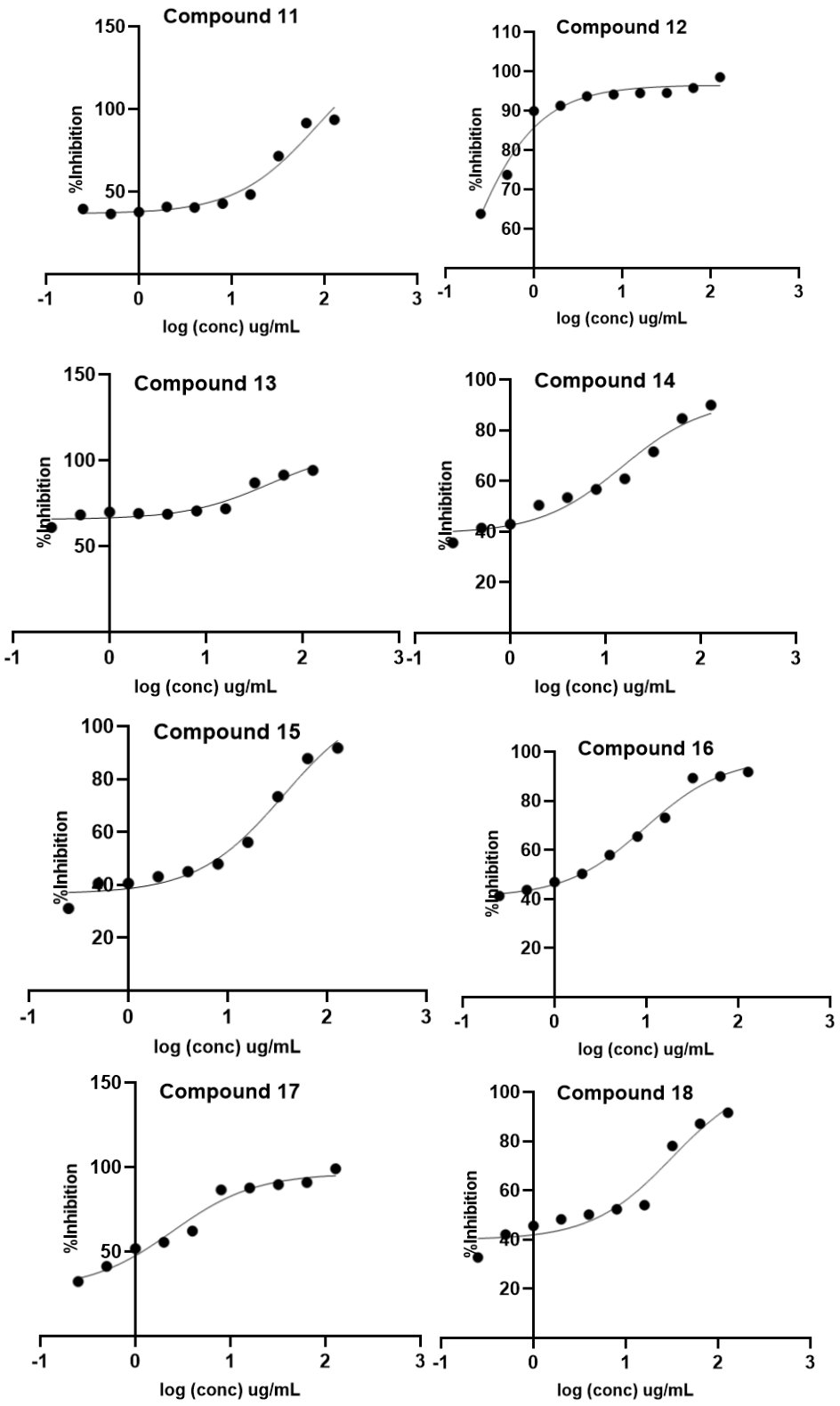

Compound 18
150
100
50
%Inhibition
-1
0
1
2
3
log (conc) ug/mL

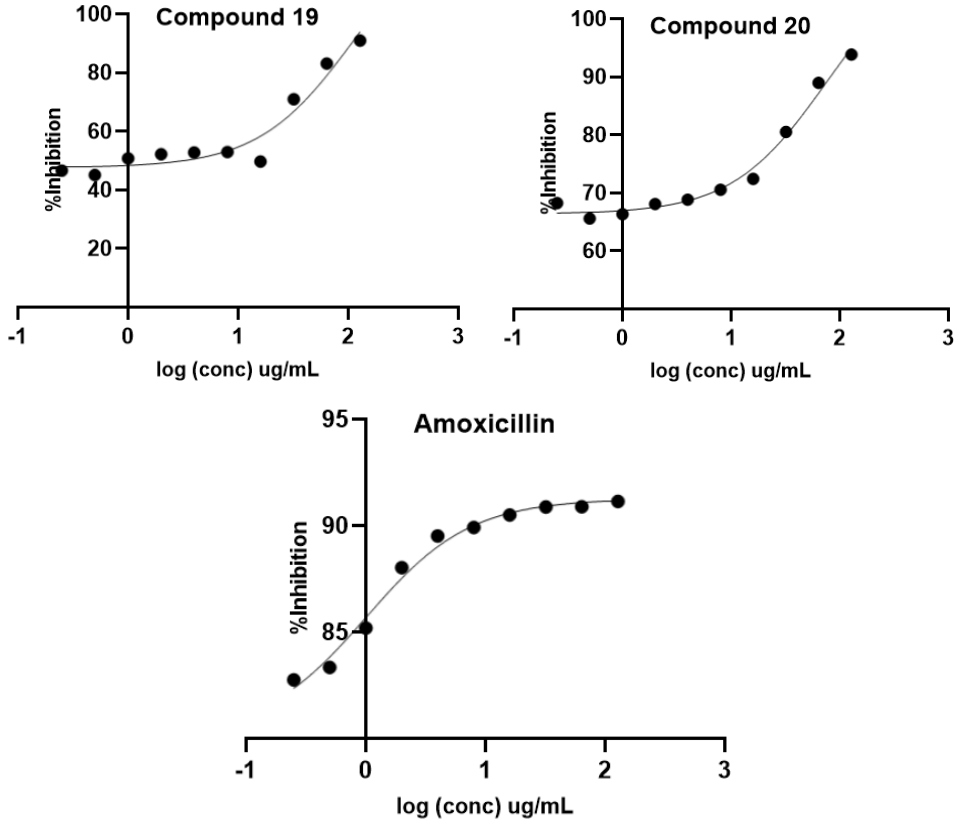

Compound 19
150
100
50
-50
%Inhibition
-1
1
2
3
log (conc) ug/mL


Compound 20
150
100
50
%Inhibition
-1
0
1
2
3
log (conc) ug/mL

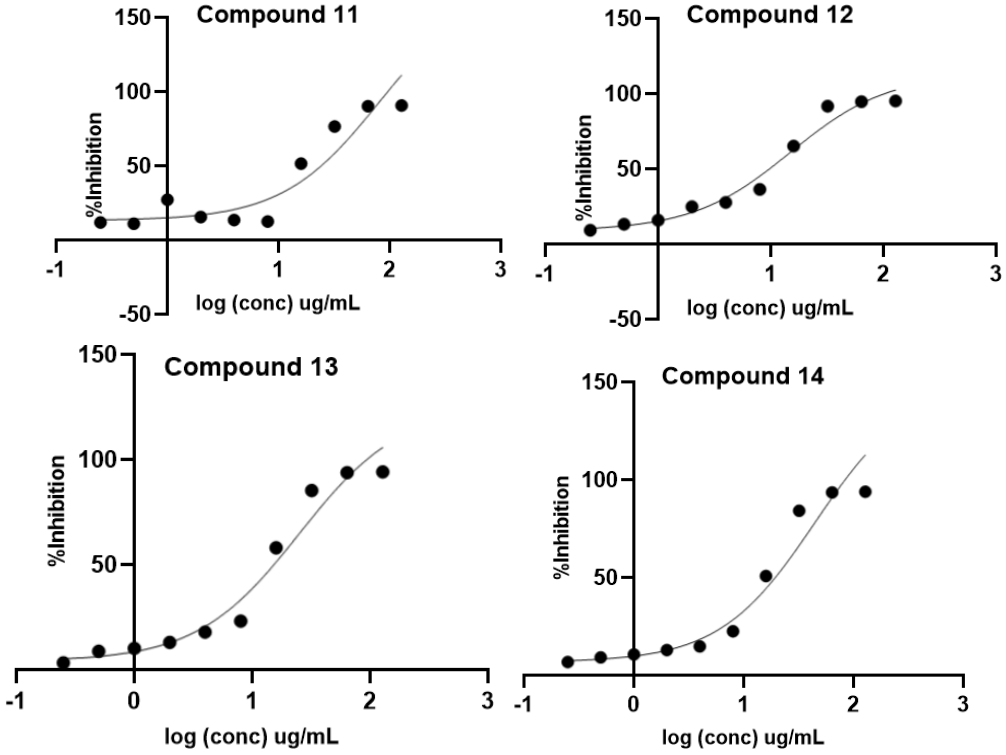

Amoxicillin
150
100
50
%Inhibition
-1
0
1
2
3
log (conc) ug/mL

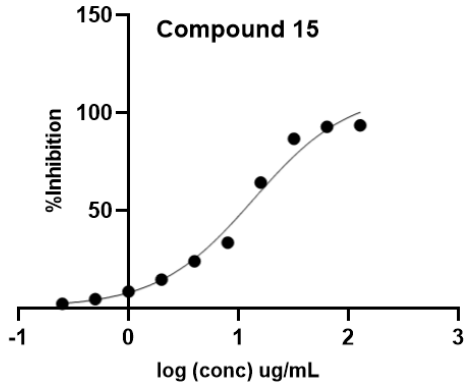
Appendix 103: Dose-response curve in penicillins derivatives in *Staphylococcus aureus*

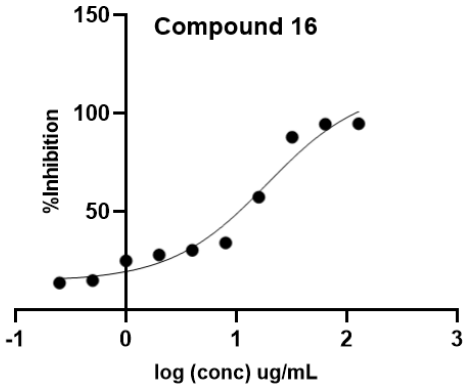


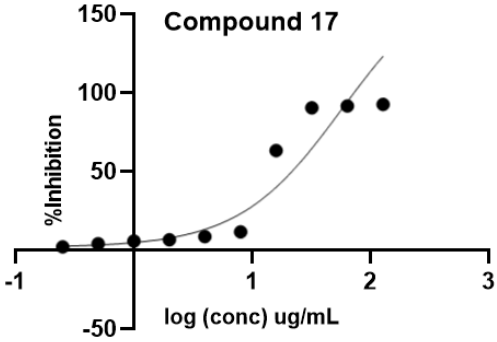


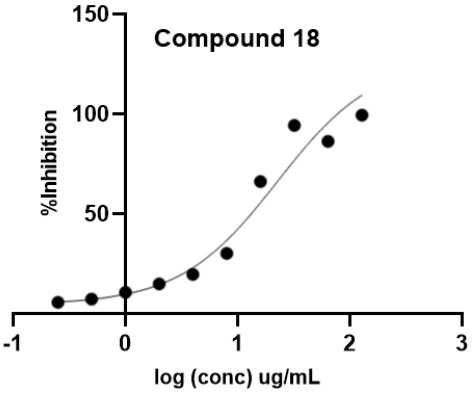
Appendix 104: Dose-response curve in penicillin derivatives in *Streptococcus pneumoniae*

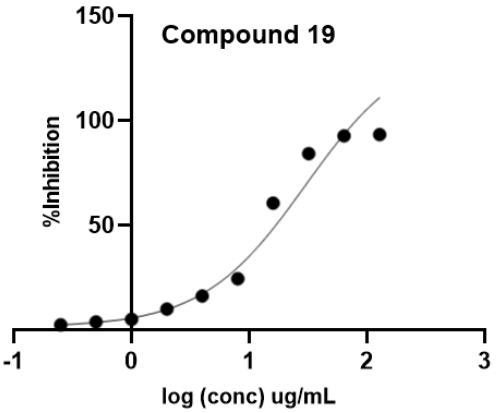


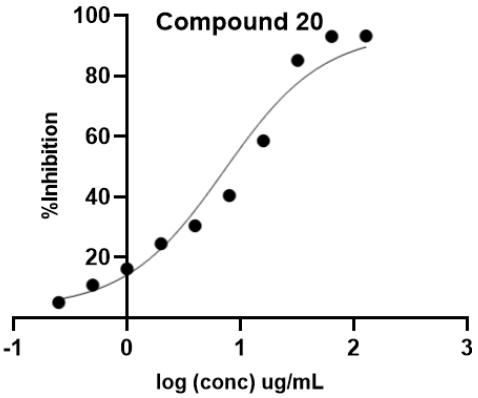

Compound 15
%Inhibition
150
100
50
-1
0
1
2
3
log (conc) ug/mL

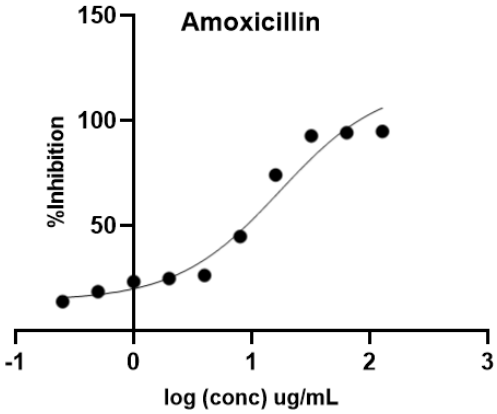

Compound 16
%Inhibition
150
100
50
-1
0
1
2
3
log (conc) ug/mL


Compound 17
%Inhibition
150
100
50
-50
-1
1
2
3
log (conc) ug/mL

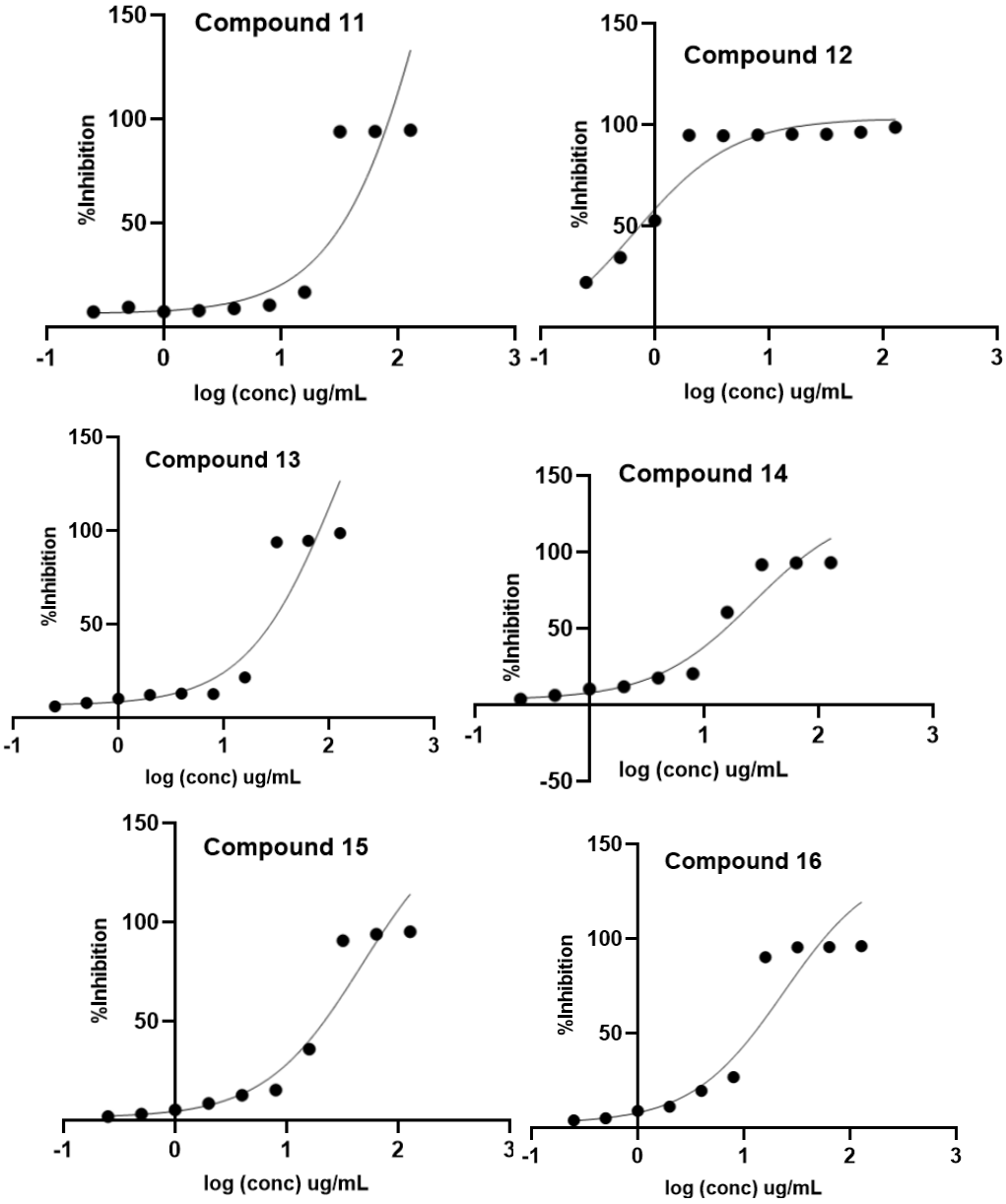

Compound 18
%Inhibition
150
100
50
-1
0
1
2
3
log (conc) ug/mL

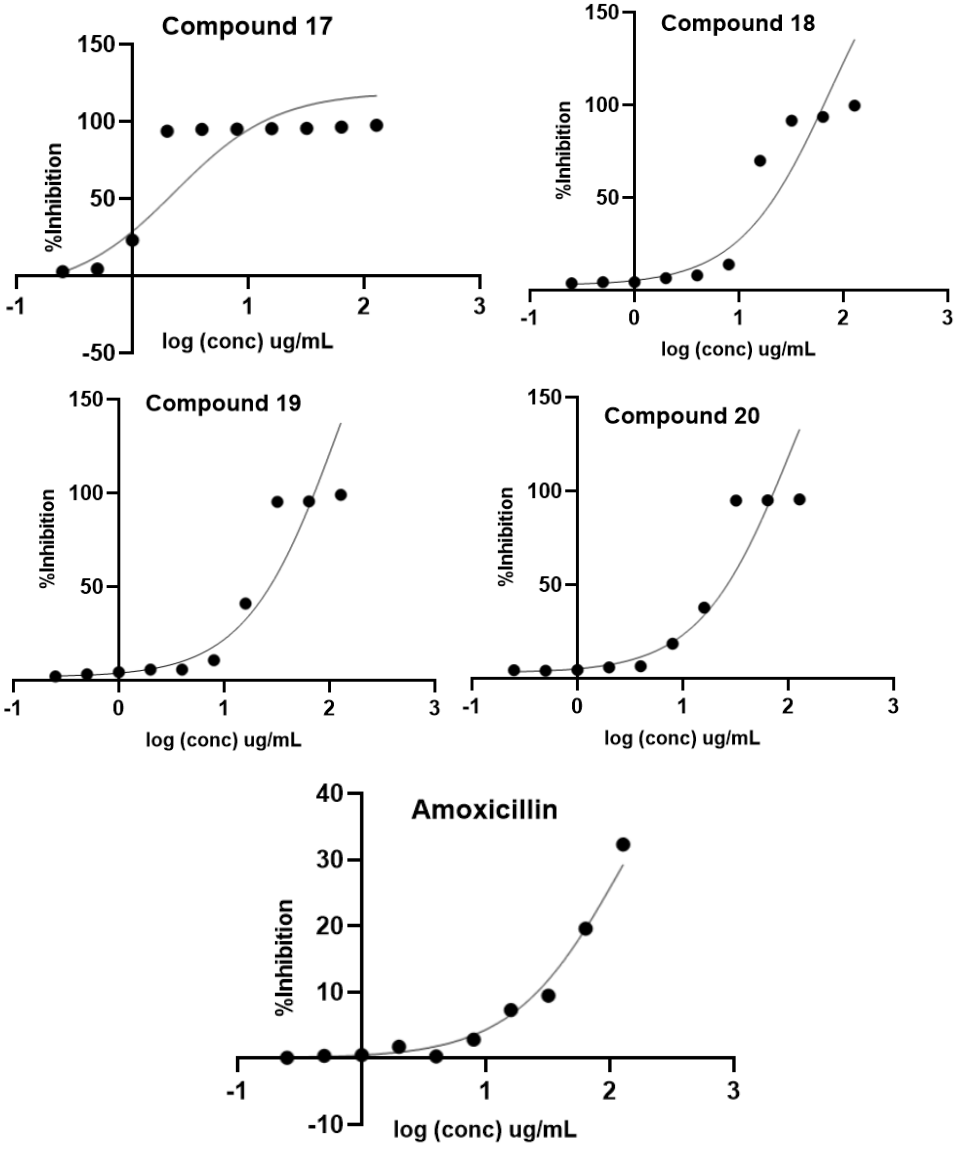

Compound 19
%Inhibition
150
100
50
-1
0
1
2
3
log (conc) ug/mL


Compound 20
%Inhibition
100
80
60
40
20
-1
0
1
2
3
log (conc) ug/mL


Amoxicillin
%Inhibition
150
100
50
-1
0
1
2
3
log (conc) ug/mL

Appendix 105:Dose-response curve in penicillin derivatives in *Pseudomonas aeruginosa*




Compound 17
%Inhibition
log (conc) ug/mL
Compound 18
%Inhibition
log (conc) ug/mL
Compound 19
%Inhibition
log (conc) ug/mL
Compound 20
%Inhibition
log (conc) ug/mL
Amoxicillin
%Inhibition
log (conc) ug/mL

التحضير والنشاط المضاد للبكتيريا لمضادات حيوية جديدة.

ندين محمد كامل قلالوة

لجنة الإشراف:

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الملخص باللغة العربية:

تمثل مقاومة المضادات الحيوية أحد أكبر التهديدات الصحية العالمية في العصر الحالي، حيث يسهم عدم التزام المرضى بالعلاج في تفاقم هذه المشكلة بسبب الطعم غير المستساغ للمضادات الحيوية الفموية مثل الأزيثرومايسين والكلاريثرومايسين. استهدفت هذه الدراسة التغلب على هذه الصعوبات من خلال تصنيع وتقييم أدوية أولية ومشتقات جديدة من المضادات الحيوية الماكروليدية (الأزيثرومايسين والكلاريثرومايسين) والبنسلينية (الأموكسيسيلين وحمض 6-أمينوبنسيلانيك). تمثلت الأهداف الرئيسية في إخفاء الطعم غير المستساغ للماكروليدات وتحسين الفعالية المضادة للميكروبات لمشتقات البيتا-لاكتام.

تم مفاعلة المضادات الحيوية الأم مع خمسة روابط كيميائية شملت 4-أسيتيل بنزين سلفونيل كلورايد، و4-نيترو بنزويل كلورايد، و 4-تولوين سلفونيل كلورايد، و3,5-ثنائي نيترو بنزويل كلورايد، و 4-نيترو بنزين سلفونيل كلورايد، مما أسفر عن إنتاج عشرين مشتقاً جديداً. تم

تحديد هوية المنتجات المصنعة باستخدام تقنيات الأشعة تحت الحمراء (FTIR)، والرنين النووي المغناطيسي (NMR)، ومطيافية الكتلة (MS). تم تقييم استقرار التحلل المائي لأدوية الماكروليد الأولية عند درجات حموضة مختلفة (pH 2.2 و pH 5.5 و pH 7.4). أجريت دراسات الالتحام الجزيئي الحاسوبية على مشتقات البنسلين لتحديد آلية تفاعل مشتقات البنسلين مع بروتينات ربط البنسلين (PBPs)، وتم تقييم النشاط المضاد للميكروبات في المختبر لكل مشتق بنسلين ضد بكتيريا الإشريكية القولونية والزائفة الزنجارية والعقدية الرئوية والعنقودية الذهبية من خلال تحديد التركيز المثبط الأدنى (MIC) والتركيز المثبط النصفي (IC50).

أظهرت النتائج إمكانية تصنيع المشتقات العشرين بنجاح. أشارت دراسات التحلل المائي إلى أن مشتق الأزيثرومايسين رقم 5 أبدى خصائص مثالية كدواء أولي حيث تحول بسهولة إلى الدواء الأم الفعال مع تحلل مائي كامل يعتمد على درجة الحموضة. أظهرت عدة مشتقات تحسناً ملحوظاً في النشاط المضاد للميكروبات مقارنة بالأدوية الأم. بشكل خاص، ثبت أن المركبين 4 و 10 أقوى بكثير ضد الزائفة الزنجارية، وكان المركب 8 فعالاً للغاية ضد العقدية الرئوية والعنقودية الذهبية ويعتبر الفائز الواضح ذو الطيف الواسع، بينما ثبت أن المركبين 12 و 17 فعالين للغاية ضد الزائفة الزنجارية والعنقودية الذهبية أكثر من الأموكسيسيلين الأم ويعتبران مشتقات ذات طيف واسع. تمكنت هذه الدراسة من التوصل إلى مشتقات واعدة للمضادات الحيوية. يمثل استخدام أشكال أدوية الماكروليد الأولية ذات الطعم المخفي، خاصة المركب 5، حلاً محتملاً لتحسين التزام المرضى بالعلاج. علاوة على ذلك، يؤكد تحسن الفعالية لمشتقات معينة من البيتا–لاكتام ضد مقاومة مسببات الأمراض على أنها تمثل جيلاً جديداً من العوامل العلاجية.

الكلمات المفتاحية: مقاومة المضادات الحيوية، الأدوية الأولية، إخفاء الطعم، الماكروليدات، البنسلينات.