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Synthesis, Characterization, and Antibacterial Activity of Ciprofloxacin Prodrugs

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Synthesis, Characterization, and Antibacterial Activity of Ciprofloxacin Prodrugs

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Declaration

I certify that the thesis submitted for the degree of the master is the result of my research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

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Acknowledgment

First, thanks to Allah, who gave me the strength, endurance, and courage to complete this journey. "Praise to Allah, who has guided us to this; and we would never have been guided if Allah had not guided us." al A'raf 43

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Abstract

The increasing problem of antibiotic resistance highlights the critical need for the development of new antibiotics. Bacterial resistance has wide-ranging consequences, including the potential for longer and more severe infections, heightened rates of illness and death, and escalated healthcare expenses. In certain instances, infections caused by antibiotic-resistant bacteria can become untreatable, severely limiting effective treatment options. This poses a significant public health threat, as the management of common bacterial infections becomes more challenging and the risk of spreading resistant strains to others intensifies.

In the market and industry of antibiotics, the development of new antibiotics faces significant challenges. The process of bringing a new antibiotic to market has become increasingly slow and complex, with an average timeframe of a decade or more.

The rise of antibiotic resistance has necessitated a continuous search for novel solutions to combat infectious diseases effectively, and conventional methods for discovering antibiotics have largely run their course. Despite these obstacles, there are still chances to find new antibiotics by using medicinal chemistry and computational chemistry to create novel prodrugs as the prodrug strategy has been frequently used as a chemical approach for the enhancement of certain disadvantages of parent drugs.

In this thesis, two amide prodrugs have been synthesized of the anti-infective agent Ciprofloxacin, and their potential advantages in fighting bacteria over the respective parent compound have been demonstrated.

The two novel prodrugs of Ciprofloxacin (**ProD1** and **ProD2**) were synthesized to improve the antibiotic spectrum of the parent drug. The research details the synthesis of the prodrugs using the linkers cis-5-Norbornene-Endo-2,3-Diarboxylic Anhydride, and di-methyl-Maleic Anhydride respectively, utilizing nucleophilic acyl substitution. The final products of the produced prodrugs were obtained as powders and in high yields. Furthermore, they were characterized using melting point, and chromatographic methods in which UPLC, FT-IR, and ^{1}H-NMR analysis were carried out.

The *in vitro* susceptibility for both prodrugs was determined against *Escherichia coli*, *staphylococcus aureus*, and *pseudomonas*, and compared to that of the parent drug Ciprofloxacin, using disc diffusion method, and broth diffusion method. The results demonstrated that both novel

prodrugs exhibited superior efficacy compared to their parent drug in combating *pseudomonas* and *E. coli* infections. Furthermore, when administered at higher doses, they also displayed effectiveness against *staphylococcus aureus*. It is worth noting that those two novel prodrugs are stable compounds as they exert the antibiotic effect themselves without the need to undergo conversion to their corresponding parent drugs. The novel prodrugs exhibit their antibacterial activity against different types of bacterial strains due to the presence of the 4-pyridone ring with a 3- carboxylic acid group substitution on their structures.

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List of Abbreviations

5-FU	**5-fluorouracil**
0.1N HCl	**Normalized hydrochloric acid**
ADEPT	**Antibody Directed Enzyme Prodrug Therapy**
ADMET	**Absorption, distribution, metabolism, excretion and toxicity.**
AM	**Alveolar macrophage**
AZD	**Azithromycin-2',4''-diisovalerate**
BBB	**Blood-brain barrier**
Caco 2	**Colorectal adenocarcinoma cell lines**
CDC	**The center for disease control and prevention**
CIP	**Ciprofloxacin**
CLSI	**Clinical and Laboratory Standards Institute**
CNS	**Central nervous system**
CSF	**Cerebrospinal fluid**
CYP	**Cytochrome P450**
d	**Doublet**
DFT	**Density functional theory**
DNA	**Deoxyribonucleic acid**
D_2O	**Deuterium oxide**
dt	**Doublet of triplet of doublet**
DXR	**1-deoxy-D-xylulose-5-phosphate reductoisomerase**
E.coli	***Escherichia coli***
EPT	**Enzyme prodrug therapy**
FT-IR	**Fourier transform infrared spectrophotometer**
GABA	**Gamma-aminobutyric acid**

GI	**Gastrointestinal**
HEC	**Hydroxyethylcellulose**
HCl	**Hydrochloric acid**
HLB	**Hydrophilic lipophilic balance**
1H-NMR	**Proton nuclear magnetic resonance spectroscopy**
HPC	**Hydroxypropylcellulose**
HPLC	**High performance liquid chromatography**
IUPAC	**International Union of Pure and Applied Chemistry**
Log D	**Distribution coefficient**
Log P	**Partition coefficient**
Log S	**Aqueous solubility**
M	**Multiplet.**
mm	**Millimeter**
MM	**Molecular mechanics**
MBC	**Minimum bactericidal concentration**
MDRGNB	**Multiple-Drug-Resistant Gram-Negative *Bacilli***
MIC	**Minimum inhibitory concentration**
MPDs	**Macromolecular prodrugs**
MRSA	**Methicillin-resistant *Staphylococcus aurous***
NAC	**Near attack conformation**
NSAIDs	**Non-steroidal anti-inflammatory drugs**
PDA	**Photodiode array**
PEG	**Polyethylene glycol**
pH	**potential of hydrogen**

PK_a	**The negative base-10 logarithm of The acid dissociation constant (K_a)**
ppm	**Part per million**
ProD 1	**Prodrug 1**
ProD 1	**Prodrug 2**
ProD 3	**Prodrug 3**
PRSP	**Penicillin resistant *streptococcus pneumonia***
Q	**Quartet**
QM	**Quantum mechanics**
QSAR	**Quantitative structure-activity relationship**
RNA	**Ribonucleic acid**
RP	**Reverse phase**
S	**Singlet**
T	**Triplet**
THF	**Tetrahydrofuran**
TLC	**Thin Layer Chromatography**
UPLC	**Ultra- performance liquid chromatography**
VRE	**Vancomycin-resistant *Enterococcus***

Chapter one

Introduction

1. Introduction

Throughout history, infectious diseases have plagued humanity causing devastating epidemics and claiming countless lives. Diseases such as smallpox, cholera, pneumonia, tuberculosis, and syphilis were widespread contributing to an average life expectancy of only 47 years. The understanding of these diseases and the way to combat them was limited leading to unsuccessful attempts in prevention, treatment, and containment. Until the discovery of antibiotics, infectious diseases posed significant threats to global health.

The development of antibiotics is widely regarded as one of the most significant medical advancement of the 20th century. Antibiotics not only revolutionized the treatment of infectious diseases, but also enabled critical medical procedures like cancer treatment, organ transplants, and open heart surgery.

The history of antibiotics dates back thousands of years when antibiotic producing microbes were utilized to treat diseases. The use of ancient remedies such as moldy bread poultices and medicinal soil as a treatment for wounds was documented 1550BC. However, the development of anti-infective drugs and the concept of chemotherapy are credited to Paul Ehrlich, who developed synthetic arsenic based prodrugs to combat syphilis. The subsequent discoveries of sulfonamides and penicillin marked significant milestones in the field of antibiotics.

The discovery of penicillin by Alexander Fleming in 1928 marked a major breakthrough, followed by the purification and the beta-lactam structural elucidation of penicillin by Dorothy Hodgkin, paved the way of the development of semisynthetic derivatives. This period between the 1940s-1960s, were regarded as the "golden age" of antibiotic discovery, during which many new antibiotic classes were identified, many of which are still in clinical use today.

Despite the achievements of the antibiotic era, now a day, the development of new antibiotics has become increasingly challenging due to the rise of antibiotic resistant. Antibiotic resistance refers to the ability of bacteria to withstand the effects of antibiotics, to which they were once susceptible. It was realized that the bacteria have the ability to develop, acquire, and spread resistance mechanisms. This resistance results from natural factors in certain bacteria, genetic mutations, horizontal gene transfer, and selection pressure. The overuse and the misuse of antibiotics by healthcare professionals, inappropriate use by unskilled practitioners and the general public, poor quality medicines, overcrowding, inadequate hospital infection control practices, insufficient

surveillance, poverty, limited resources for combating antibiotic resistance, and a lack of political will contribute to the spread of resistance.

The rise of antibiotic resistance bacteria poses a significant threat to human health and life. Resistant organisms are difficult to treat, often requiring higher doses or alternative drugs that maybe more toxic and expensive. The problem is further compounded by emerging of superbugs; bacteria with resistance to nearly all available antibiotics. Penicillin resistant *streptococcus pneumonia* (PRSP), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Vancomycin-resistant Enterococcus* (VRE), and Multiple-Drug-Resistant Gram-Negative *Bacilli* (MDRGNB) are such examples.

The center for disease control and prevention (CDC) estimates that at least, 2 million people in the United States alone are infected with antibiotic resistant bacteria annually, resulting in over 23,000 deaths. The impact is expected to be even more sever in developing countries, where communicable diseases remain the main leading cause of death.

The alarming threat of post antibiotic era has urged policy makers, and promoted global attention to acknowledge the gravity of the situation and allocate additional funding for antibiotic research.

However, large pharmaceutical companies have shifted their focus to more profitable areas, leaving antibiotic discovery and development to small companies and biotechnology startups.

Large pharmaceutical companies are deterred from investing in antibiotic research and development due to numerous challenges: (i) Simple, small molecules that target specific enzymes in bacteria are prone to developing resistance quickly. In contrast, previous antibiotics were complex natural products with multiple binding sites, making it less likely for resistance to occur. Additionally, (ii) many novel targets for antibiotics are specific to certain bacterial species or strains, while clinical trials require coverage for multiple pathogens involved in a given infection. This specificity limits the commercial viability of pathogen-specific antibiotics compared to older, broader-use generics. Furthermore, (iii) antibiotics need to be highly selective for bacteria to avoid toxic effects on human cells, but many active molecules lack selectivity and can cause collateral damage. The development of rapid diagnostic tools to guide targeted therapy is hindered by high costs. As a result, new antibiotics are often reserved as a last resort for multi-drug resistant pathogens, which further reduces their market potential.

Small start-up companies believe they can succeed in finding new antibiotics despite the difficulties. They employ novel approaches to drug development, such as innovative natural product screening

methods, total synthesis of existing antibiotics to enable structural modifications, novel chemistries, and the synthesis of complex products through genetic manipulation. Start-ups have a focused and persistent approach to overcome development barriers in chemistry and bioavailability. Their smaller scale and lower infrastructure costs allow for more streamlined and cost-effective programs (Adedeji, 2016; Fernandes & Martens, 2017; Hutchings, Truman, & Wilkinson, 2019; Mohr, 2016).

In this research, the second-generation quinolone antibiotic Ciprofloxacin will be investigated, and the development of two new prodrugs derived from this antibiotic will be explored, aiming to address the growing problem of bacterial resistance.

Quinolones, one of the most commonly used groups of antibiotics today, were first discovered in the early 1960s. Initially, they were primarily used to treat urinary tract infections, with the first-generation quinolones like nalidixic acid being particularly effective in this regard. As research progressed, second-generation quinolones such as Ciprofloxacin were developed to have a broader spectrum of activity, targeting both Gram-negative and Gram-positive bacteria.

The development of quinolones continued with the introduction of third and fourth-generation variants that showed improved efficacy against bacterial strains that had developed resistance. Nalidixic acid, the first quinolone antibacterial agent, was derived indirectly from a natural product. It was originally named Negram as it was effective against Gram-negative bacteria.

Over time, modifications were made to quinolone structures, including the addition of a fluorine atom and the incorporation of aryl rings. These advancements led to the development of potent broad-spectrum fluoroquinolones like ciprofloxacin, levofloxacin, moxifloxacin, and others. The newer fluoroquinolones demonstrated enhanced pharmacokinetics and a wider range of activity against various bacterial strains.

However, concerns have emerged regarding the resistance of bacteria to ciprofloxacin and levofloxacin. In response, newer fluoroquinolones have been developed that target specific bacterial enzymes, such as DNA gyrase and topoisomerase IV (Rusu, Lungu, Moldovan, Tanase, & Hancu, 2021)

1.1. Ciprofloxacin

Nalidixic acid shares structural similarities with the synthetic antibacterial drug Ciprofloxacin ($C_{17}H_{18}FN_3O_3$), which belongs to the quinolone class. It is a second-generation fluroquinolone, quinolin-4(1H)-one, with substituents at positions 1, 3, 6, and 7, respectively, for cyclopropyl, carboxylic acid, fluoro, and piperazin-1-yl (National Center for Biotechnology Information, 2022, May 10). It was granted a patent by Bayer AG in 1983, and the US FDA approved its usage in 1987 (Sharma, Jain, Jain, Pahwa, & Yar, 2010). Since its invention, the majority of gram-negative bacteria continue to be extremely vulnerable to this drug *in vitro*. In general, or to a certain extent, gram-positive bacteria are vulnerable (Rick Davis, Anthony Markham, & Julia A. Balfour, 1996a).

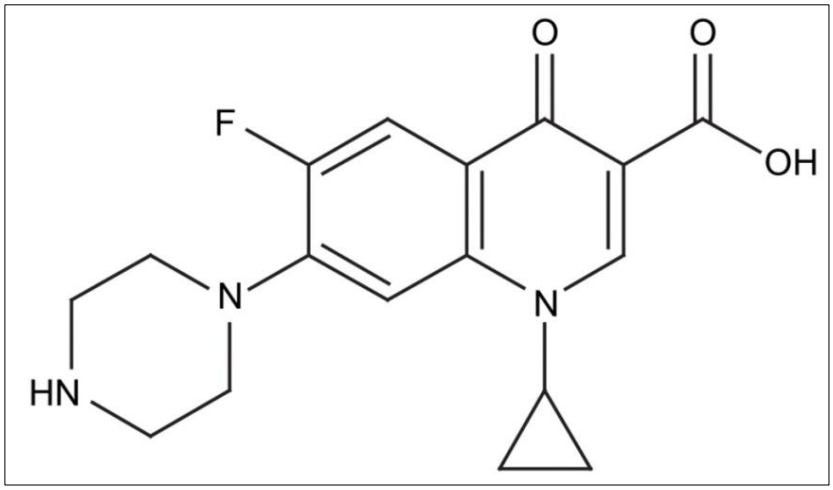


Figure 1-1: Ciprofloxacin; IUPAC name: 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid (National Center for Biotechnology Information, 2022,May 10)

1.1.1. Structure activity relationship

The 4-pyridone ring with a 3-carboxylic acid group is the bare minimum pharmacophore needed for strong antibacterial action; reducing the (2, 3) double bond renders activity ineffective. The presence of a fluorine atom at position C6 improves lipophilicity, enabling cell and deep tissue penetration. Additionally, it improves the inhibition of DNA gyrase and offers *staphylococci* activity.

The piperazine group on C7 broadens the spectrum, particularly to include gram-negative bacteria, but this group also causes an increase in GABA receptor affinity, which adds to the CNS adverse effects. This can be addressed by adding a methyl or ethyl group to piperazine or by attaching a bulky substituent to N1. It should be noted that adding a substituent to the piperazine group would help transfer the compound's excretion from the kidney to the liver and lengthen its half-life, both of which are advantageous for patients with poor renal function.

Additionally, ring alkylation at N1 increases the potency and half-life of gram-positives. Cyclopropyl at position N1 in Ciprofloxacin improves its efficacy against mycoplasma and chlamydia species (Abraham, 2003).

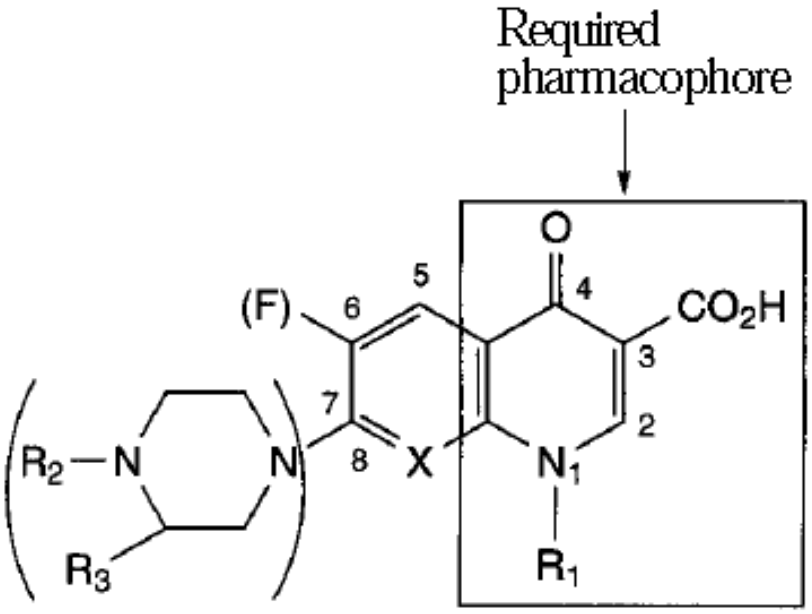


Figure 1-2: Quinolone pharmacophore and substituents.

1.1.2. Uses of Ciprofloxacin

Ciprofloxacin is a widely used broad spectrum antimicrobial agent for the treatment of sexually transmitted diseases (gonorrhea and chancroid), complicated urinary tract infections, prostatitis, enteric infections, biliary tract infections, skin and bone infections, gastrointestinal infections caused by multi-resistant organisms, lower respiratory tract infections, intra-abdominal infections (combined with the anti-anaerobic agent), and malignant external otitis.

There are ten approved uses for the adult population and two approved applications for the pediatric population in addition to several of veterinary uses. Off-label usage refers to other purposes. Furthermore, recent research on Ciprofloxacin has shown that it has anti-proliferative and apoptotic effects in a variety of cell lines (Davis *et al.*, 1996a; Sharma *et al.*, 2010).

1.1.3. Mechanism of Action

Quinolone antibiotics target DNA gyrase and topoisomerase IV, two crucial type II topoisomerases, to prevent DNA synthesis, which is necessary for bacterial reproduction.

Adenosine triphosphate hydrolyzing topoisomerase II enzyme DNA gyrase is crucial for eliminating positive super-helical twists and facilitating DNA replication. Along with its other crucial jobs that influence the start of DNA replication and the transcription of many genes. After DNA replication, the newly linked daughter chromosomes are separated by the decatenation enzyme topoisomerase IV. Supercoils are also relaxed by it. Both enzymes are bound by Ciprofloxacin, which inhibits their activity and kills the bacterial cell (Fàbrega, Madurga, Giralt, & Vila, 2009).

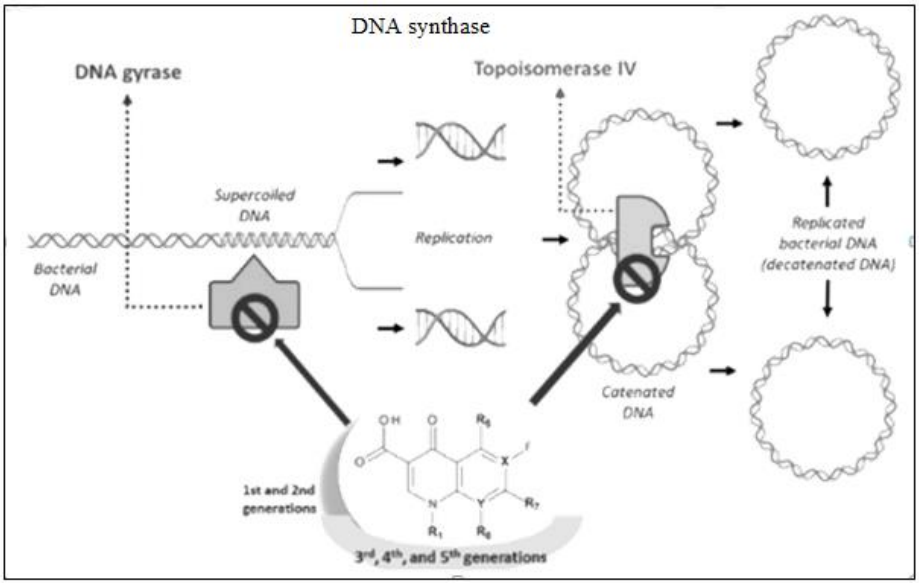


Figure 1-3: Mechanism of action of Ciprofloxacin and other quinolone drugs (Rusu, *et al.*, 2021).

1.1.4. Pharmacokinetic

- **Bioavailability**

Despite being easily absorbed after oral administration, it is not entirely absorbed. After a single 250-750 mg dose, the absolute bioavailability is between 70 and 80 percent after 1 to 2 hours of administration, and the maximum plasma concentration is between 0.8 and 3.9 mg/l. No notable loss was caused by first-pass metabolism.

The pharmacokinetics of Ciprofloxacin in cerebrospinal fluid (CSF) has been discussed in numerous researches. It was discovered that the concentration of Ciprofloxacin in brain tissues following an intravenous dose of 200 mg was 0.87–0.08 mg/kg, indicating that a greater dose is needed to ensure therapeutic concentration in brain tissue.

Interactions between a drug and a food may increase the time needed to achieve the highest plasma concentration and impact the area under the curve without significantly changing bioavailability.

- **Distribution**

Due to little binding to plasma components, the medication has a significant apparent volume of distribution following oral or intravenous administration. Tissue to serum ratios of above two is attained.

The kidney, liver, and lung all contain notable drug concentrations. Although it also reaches the CSF, the penetration is deemed to be subpar unless the meninges are inflamed.

A negligible portion of the medication crosses the placenta from the mother to the fetus, demonstrating that the human placenta acts as a barrier to fluoroquinolone transfer.

- **Metabolism and elimination**

The piperazine ring is easily metabolized in the liver and this reduces the antibacterial activity. This liver metabolism is predominantly by glucuronide conjugation at the 3-carboxylic group and is inactivating.

Both renal and non-renal pathways are used for elimination. The kidneys are the main method of elimination, and glomerulus filtration and tubular secretion generally result in un-metabolized excretion. So, dose adjustment is required for the elderly or those with impaired renal function. Insofar as it acts as a safety valve of excretion compensating for limited renal elimination, trans-

intestinal elimination represents an essential route and develops into a major route of Ciprofloxacin elimination in patients with impaired renal function. The medication is also eliminated through the bile duct.

Hemodialysis and peritoneal dialysis both perform subpar cleaning of Ciprofloxacin. Remember that diseases like hepatic cirrhosis have an impact on metabolism (Rohwedder, Bergan, Thorsteinsson, & Scholl, 1990); (Rick Davis, Anthony Markham, & Julia A Balfour, 1996b); (Nau, Sörgel, & Eiffert, 2010); (Sharma *et al.*, 2010); (Koltai, Reshkin, & Harguindey, 2020; Sullins & Abdel-Rahman, 2013).

1.1.5. Resistant mechanism of Quinolones

Antibiotic resistance has emerged as a global threat to public health, and its rise has been attributed to several factors. According to a literature review by Delcour *et al.* (2019), overuse and misuse of antibiotics are the leading causes of antibiotic resistance. Inappropriate use of antibiotics, such as prescribing them for viral infections or stopping treatment before completing the full course, can contribute to the emergence and spread of resistant bacteria. The lack of development of new antibiotics is another significant factor. While the demand for new antibiotics is increasing, the number of new drugs being developed has significantly declined due to a combination of factors, including the high cost of drug development, the regulatory challenges associated with getting new drugs approved, and the lack of financial incentives for drug companies to develop new antibiotics. Additionally, genetic mutations that lead to antibiotic resistance can occur naturally or be induced by exposure to antibiotics, allowing resistant bacteria to emerge and spread rapidly in which Bacteria can acquire resistance genes through various mechanisms that allow them to rapidly adapt to changing environments and develop resistance to antibiotics. Lastly, Inadequate infection control measures and their role in preventing the spread of antibiotic-resistant bacteria, such as poor hand hygiene practices in healthcare settings, can lead to the transmission of resistant bacteria from one patient to another.

The misuse and overuse of antibiotics have led to the development of multidrug-resistant strains of bacteria that pose a significant challenge to the medical community. Therefore, addressing the issue of antibiotic resistance requires a multifaceted approach that involves the appropriate use of antibiotics, the development of new antibiotics, and the implementation of infection prevention and control measures.

Various mechanisms contribute to bacterial resistance to antibiotics. One of the most common mechanisms is the alteration or mutation of the target site of the antibiotic, which may prevent the antibiotic from binding to its intended target. Another mechanism is the modification or degradation of the antibiotic molecule by enzymes produced by the bacteria, which can render the antibiotic inactive. Bacteria may also develop efflux pumps that can expel the antibiotic from the bacterial cell before it can exert its effect. Furthermore, bacteria can acquire antibiotic resistance genes through horizontal gene transfer, which allows for the spread of resistance within bacterial populations. Additionally, bacteria can enter a dormant state, such as biofilms, which can limit the efficacy of antibiotics by reducing the exposure of bacteria to the drug. Finally, bacteria can develop metabolic pathways that bypass the target of the antibiotic, allowing them to continue to grow and divide despite the presence of the drug.

The essential points of gram-negative bacteria's method of resistance can be summed up as follows:

1. Due to the existence of a thin layer of peptidoglycan shielded by an outer membrane made of proteins, phospholipids, and lipopolysaccharides, gram-negative bacteria have a special mechanism of resistance. They are more resistant to antibiotics than Gram-positive bacteria because the outer membrane serves as a barrier and protection.
2. A significant factor that makes treating Gram-negative bacteria challenging is efflux pumps. These pumps actively remove antibiotics from the bacterial cell, which hinders the antibiotic's ability to work. Energy is used by efflux pumps to remove a variety of substrates from the cell, including antibiotics.
3. Antibiotics can lose their efficacy when modified or degraded by gram-negative bacteria. These enzymes alter the structure of the antibiotic molecule or completely degrade it, preventing it from binding to its target site and acting as intended. The creation of beta-lactamases, which break down beta-lactam antibiotics, is an illustration of this.
4. Other types of resistance mechanisms in Gram-negative bacteria include the ability to take up resistance genes from other bacteria, such as by plasmid transfer, lower drug permeability caused by alterations in the bacterial outer membrane, and modification of the antibiotic target site.
5. Gram-negative bacteria that produce biofilms are another type of organism that increases resistance to antibiotics. Complex bacterial communities known as biofilms can develop on a variety of surfaces, including the human body. Because they create a physical barrier that may prevent antibiotics from reaching the bacteria and thus limit their effectiveness,

biofilms play a significant role in the development of antibiotic resistance in gram-negative bacteria. Additionally, the bacteria within the biofilm may experience modifications to their metabolism and gene expression, which could increase their susceptibility to antibiotics. A favorable environment for horizontal gene transfer is provided by biofilms, and this can result in the acquisition of genes for antibiotic resistance. It might be challenging to entirely remove the bacteria with antibiotics when they are present in a biofilm because they can act as a reservoir for recurring infections. The unique characteristics of Gram-negative bacteria make them particularly difficult to treat and contribute to the growing problem of antibiotic resistance. Researchers are working to develop new antibiotics and other strategies to overcome these challenges and improve the treatment of Gram-negative bacterial infections (Breijyeh, *et al.*, 2020).

On the other hand, Gram-positive bacteria also have a unique defense system, which consists of:

Modification or inactivation of the target site of the antibiotic: Gram-positive bacteria can modify the target site of the antibiotic by altering it or inactivating it, which prevents the antibiotic from binding to the site and exerting its effect.

1. The antibiotic is modified or degraded by bacteria-produced enzymes, rendering the antibiotic inactive and unable to exert its intended action.
2. Lessened permeability: The thick peptidoglycan layer on the cell walls of some gram-positive bacteria lessens the permeability of antibiotics, preventing them from penetrating the bacterial cell and having an impact.
3. Active efflux of the antibiotic: Bacteria can also create pumps called efflux systems that actively remove the antibiotic from the bacterial cell, blocking its action.
4. Gram-positive bacteria have the ability to create biofilms, which can reduce antibiotics' exposure to bacteria and reduce their effectiveness.
5. Acquisition of resistance genes: By way of horizontal gene transfer, bacteria can pick up resistance genes, enabling the spread of resistance within bacterial populations (Karaman, *et al.*, 2020b).

One of the most concerning examples of Gram-positive resistance is the resistance to glycopeptide antibiotics, such as vancomycin. Vancomycin is a powerful antibiotic that is used to treat infections caused by Gram-positive bacteria, such as *Staphylococcus aurous* and *Enterococcus faecalis*. It works by binding to the bacterial cell wall and preventing the bacteria from building new cell walls, which ultimately leads to bacterial death.

However, in recent years, there has been an increase in the number of Gram-positive bacteria that are resistant to vancomycin. This is particularly concerning as vancomycin is often used as a last resort for treating serious infections that are resistant to other antibiotics.

The emergence of vancomycin-resistant strains, such as vancomycin-resistant *Enterococcus* (VRE) and methicillin-resistant *Staphylococcus aurous* (MRSA), has become a significant issue in clinical settings. The development of resistance to glycopeptide antibiotics is primarily due to the bacteria modifying the cell wall structure, which prevents the drug from binding to its target site. This modification is achieved through the expression of specific genes, such as the vanA and vanB genes, which encode for enzymes that alter the target site. Additionally, some strains of bacteria can acquire resistance through horizontal gene transfer, which allows for the spread of resistance within bacterial populations.

Moreover, some bacteria can produce biofilms, which are communities of bacteria that are surrounded by a protective matrix. These biofilms can prevent the antibiotic from reaching the bacteria and contributing to the development of resistance.(Jubeh *et al.*, 2020b)

This all highlights the urgent need for the development of new antibiotics and alternative therapies to combat the growing issue of antibiotic resistance.

As for quinolones, three resistance mechanisms predominate (i) chromosomal changes in the target protein's encoding genes. (ii) Mutations that result in decreased drug accumulation through either increased efflux or decreased absorption. Plasmid-based genes linked to quinolone resistance (iii) (Fàbrega *et al.*, 2009). There have been reports of rising Ciprofloxacin resistance from various nations for a variety of bacterial species (Davis *et al.*, 1996b). Moellering concluded that "The future viability of the quinolones will in large part depend on the ability of the medical community to use them wisely" after taking the issue of quinolone resistance into account (Russell, 1998).

Understanding these mechanisms of resistance is crucial in developing effective strategies to combat the growing threat of antibiotic resistance in both Gram-positive and Gram-negative bacteria.

Various approaches are being taken to address the issue of antibiotic resistance. One approach is the development of new antibiotics or modification of existing antibiotics to increase their efficacy against resistant bacteria. This can involve targeting new bacterial pathways or targets or modifying the chemical structure of existing antibiotics to increase their potency or ability to bypass bacterial resistance mechanisms. However, the development of new antibiotics is a time-consuming and costly process, and there is a risk that bacteria will eventually develop resistance to new antibiotics as well.

Another approach is the use of combination therapies, which involves using two or more antibiotics together to increase their efficacy and reduce the likelihood of resistance developing. Combination therapies can also target multiple pathways or mechanisms of bacterial resistance simultaneously, making it more difficult for bacteria to develop resistance.

In addition to developing new antibiotics and combination therapies, efforts are also being made to improve antibiotic stewardship, which involves ensuring that antibiotics are used appropriately and only when necessary. This includes efforts to educate healthcare providers and the public about the proper use of antibiotics, as well as implementing policies and guidelines to reduce the overuse and misuse of antibiotics. Moreover the use of rapid diagnostic tests to identify the specific bacteria causing an infection and its susceptibility to different antibiotics allows for targeted and personalized treatment.

Lastly is the use of alternative therapies, such as bacteriophages, Phage therapy involves using bacteriophages, which are viruses that infect and kill bacteria, as a treatment for bacterial infections. Phages are specific to particular bacterial strains, so they can be targeted to the specific bacteria causing an infection. This specificity can help avoid the disruption of the beneficial bacteria in the body that can occur with broad-spectrum antibiotics. However, phage therapy has some limitations, such as the potential for the development of phage resistance by bacteria.

Immunotherapy involves using the body's immune system to fight bacterial infections. This can be achieved through the use of monoclonal antibodies, which are laboratory-produced antibodies that

can target specific components of the bacteria, such as their outer membrane or efflux pumps. These antibodies can help the immune system to better recognize and target the bacteria, leading to improved clearance of the infection.

Probiotics have shown that they can confer health benefits when consumed in adequate amounts. Probiotics can be used to prevent or treat infections caused by antibiotic-resistant bacteria by competing with pathogenic bacteria for nutrients and space in the gut. However, the use of probiotics as a standalone therapy for bacterial infections is still controversial, and more studies are needed to establish their efficacy.

Researchers are also exploring the use of alternative therapies, such as antimicrobial peptides and natural products (Breijyeh *et al.*, 2020; Jubeh *et al.*, 2020b).

To combat antibiotic resistance in gram-negative bacteria here are the main approaches used:

- **Combination therapy:** Using two or more antibiotics that target different mechanisms can increase their effectiveness against resistant gram-negative bacteria. For example, combination therapy with carbapenems and aminoglycosides can be used to treat infections caused by carbapenem-resistant *Enterobacteriaceae.*
- **Targeting efflux pumps:** Efflux pumps are a significant contributor to antibiotic resistance in gram-negative bacteria. Inhibiting these pumps with drugs can increase the efficacy of antibiotics against these bacteria. For instance, efflux pump inhibitors such as phenylalanine arginine beta-naphthylamide (PAβN) can enhance the activity of various antibiotics against gram-negative bacteria.
- **Developing new antibiotics:** Developing new antibiotics with novel mechanisms of action is crucial for combating antibiotic resistance. For example, new antibiotics such as ceftazidime-avibactam and meropenem-vaborbactam have been developed to target carbapenem-resistant bacteria.
- **Using bacteriophages:** Bacteriophages are viruses that can infect and kill bacteria. Phage therapy has shown promise as an alternative approach for treating infections caused by antibiotic-resistant gram-negative bacteria.
- **Immunotherapy:** Immunotherapy involves using antibodies or other immune-based therapies to enhance the host immune response against bacterial infections. Monoclonal antibodies against bacterial targets such as lipopolysaccharides have been developed as a potential strategy to treat gram-negative bacterial infections (Breijyeh *et al.*, 2020).

In the case of Gram-positive bacteria overcoming antibiotic resistance can be achieved through this:

- **Development of new antibiotics:** One approach to combat antibiotic resistance in gram-positive bacteria is to develop new antibiotics. This can be done by identifying novel drug targets or by modifying existing antibiotics to make them more effective. For example, the antibiotic daptomycin was developed to combat gram-positive bacteria by targeting their cell membranes, while linezolid was designed to target ribosomes, the cell structures responsible for protein synthesis. Another example is teixobactin, a newly discovered antibiotic that targets the cell wall of gram-positive bacteria.
- **Modification of existing antibiotics:** Modifying existing antibiotics can also help overcome antibiotic resistance in gram-positive bacteria. One way to do this is to use combination therapy, where two or more antibiotics are used together to target different aspects of bacterial growth and survival. For example, the combination of ampicillin and sulbactam is used to treat infections caused by bacteria that produce beta-lactamases, enzymes that can break down beta-lactam antibiotics. Another approach is to modify existing antibiotics to make them more effective or resistant to bacterial enzymes. For example, the antibiotic fosfomycin was modified to increase its stability in the presence of bacterial enzymes that can degrade it.
- **Alternative therapies:** Alternative therapies such as phage therapy, immunotherapy, and bacteriocins can also be used to overcome antibiotic resistance in gram-positive bacteria. Phage therapy involves the use of bacteriophages, viruses that can infect and kill bacteria, to target specific strains of bacteria. Immunotherapy involves the use of antibodies or vaccines to boost the immune system's ability to fight bacterial infections. Bacteriocins are small proteins produced by some bacteria that can kill other bacteria. For example, nisin, a bacteriocin produced by Lactococcus lactis, has been used to treat infections caused by gram-positive bacteria.
- **Prevention:** Preventing infections in the first place can also help combat antibiotic resistance in gram-positive bacteria. This can be done through good hygiene practices, such as hand washing, and vaccination against bacterial infections. Vaccines can help prevent bacterial infections, reducing the need for antibiotics and limiting the development of antibiotic resistance. For example, the pneumococcal vaccine can prevent infections caused

by *Streptococcus pneumonia*, Gram-positive bacteria responsible for *pneumonia*, meningitis, and sepsis (Jubeh *et al.*, 2020b).

1.1.6. Dosage and administration

Both oral (in the form of oral solution, pills, and extended-release tablets) and intravenous (premixed injection) forms of Ciprofloxacin are offered. Two doses of each type are given each day. Ophthalmic solutions, ophthalmic ointments, and otic suspensions are some other dosing forms (Hospital, Kleinman, McDaniel, & Molloy, 2020).

Depending on the location and the severity of the illness, a daily dose of 500 mg to 1500 mg of oral Ciprofloxacin is advised. To lessen irritability, intravenous dosages typically range from 200 mg to 400 mg/twice daily and are injected over at least 60 minutes; larger doses have been used with life-threatening illnesses. Ciprofloxacin is a well-tolerated medication with most gastrointestinal side effects when used within the therapeutic range.

As a broad-spectrum antibiotic, oral Ciprofloxacin can be used as an alternative to conventional parenteral regimens, which lowers the overall cost of treatment (Thai, Salisbury, & Zito, 2021); (Davis *et al.*, 1996a).

1.2. The need of novel antibiotic discovery research

Due to the dearth of new and potent molecules, antibiotic discovery research is currently in a problematic situation. It is vital to create new antibiotics to combat the problem of antibiotic resistance, which is getting worse. However, the conventional methods of antibiotic discovery have largely run their course and new approaches must be investigated.

The complexity of the microbial world, which contains numerous uncultured and understudied microorganisms, is one of the major obstacles to antibiotic discovery. Additionally, the effectiveness of screening natural product libraries is declining, necessitating the development of new techniques for locating potential antibiotic compounds.

In the past, discovering novel antibiotics relied on testing natural items for their antibacterial action, such as soil or marine organisms. This method, meanwhile, has drawbacks because it frequently produces low-potency or already-known chemicals. As a result, alternate techniques have been

created, including target-based treatments that include locating and concentrating on crucial bacterial proteins or enzymes.

To find novel compounds having antibiotic characteristics, cutting-edge techniques like machine learning and artificial intelligence have been used recently. Using algorithms and prediction models, these techniques examine enormous databases of chemicals and forecast their biological activity. Such methods have demonstrated potential in locating new compounds with potent antibiotic activity that might have gone undiscovered by means of conventional screening techniques.

There are still opportunities in this profession despite the difficulties. Novel antibiotics can now be found thanks to developments in synthetic biology, metagenomics, and genomics. Additionally, there is growing interest in developing new antimicrobial therapies that can complement antibiotics and target the microbiome.

New antibiotic development is a continuous problem, though, and it can be prohibitively expensive for many small biotech firms. Additionally, there aren't enough financial incentives to create new antibiotics because the long-term costs of resistance frequently outweigh the immediate financial gains (Livermore *et al.*, 2011).

1.3. Prodrugs

Drugs are chemical substances used to treat, diagnose, relieve, and prevent diseases. In order to provide the required systemic concentration by optimizing the drug's absorption, distribution, metabolism, and excretion (ADME), a medicine must have balanced physiochemical properties. Poor absorption, quick metabolism, or high excretion of a medicine will not result in a favorable therapeutic profile.

Drug development and design are influenced by the pharmacokinetic and toxicity profiles, which are affected by the physiochemical characteristics of the treatments. The process of creating new drugs is sequential and starts with the discovery of targets and leads. Next, lead optimization and preclinical studies *in vitro* and *in vivo* are conducted to see if a compound satisfies certain requirements before moving forward with clinical development.

Poor pharmacokinetics and toxicity have been linked to high attrition rates in drug development. Therefore, it is generally agreed that these issues should be dealt with at an early stage of the drug discovery process to increase the sector's efficiency and cost-effectiveness.

However, resolving the pharmacokinetic and toxicological properties of drug candidates continues to be a significant challenge for drug developers, as it takes over 10 years and costs more than $1 billion to introduce a drug to the pharmaceutical market (Ala'Abu-Jaish, *et al.*, 2014; Karaman, 2014).

So, in order for a drug to effectively exert its effect, it must possess the physical and chemical qualities listed below:

(i) Chemical stability in aqueous solutions, such as those found in the stomach, intestine, and blood circulation environments; (ii) Metabolic stability; the drug must withstand liver digestive and metabolic enzymes; and (iii) Successful absorption; diffusion across membranes (solubility and permeability, size, hydrogen bonding).

The prodrug design strategy, which is used to optimize (ADME) and increase the medications' selectivity for their intended targets, has been an important method for enhancing the pharmacological qualities of drugs.

The term "prodrug strategy" was first used to describe a chemical moiety that is pharmacologically inactive but can be used to temporarily change a drug's physicochemical characteristics to improve its usability and lessen its associated toxicity. This is done by creating a covalent bond between the drug and the chemical entity. In addition to other prodrugs that release their active drug following molecular modification such as oxidation or reduction reaction, they would either be destroyed chemically or enzymatically to give the parent active drug and remove the additional linker once they were in vivo. Prodrugs may also be manufactured as photodynamic treatment agents, double prodrugs, or codrugs.

Because any of the ADME properties for a potential drug candidate can be optimized, the prodrug approach is regarded as a flexible method to increase the utility of biologically active compounds. The following methods can be used to design a prodrug:

1. Increasing the solubility of active drugs, which will increase their bioavailability; the dissolution of a drug's molecule is thought to be the rate-limiting step in the absorption process. So improving the parent drug's aqueous solubility through the use of ionizable or polar neutral functions like phosphates, amino acids, or sugar moieties would not only improve oral bioavailability but also aid in the preparation of parenteral/injectable drugs' delivery. Take note that only 30% of chemicals used in drug research are poorly soluble in water.

2. Increasing permeability and absorption; membrane permeability has a significant impact on drug efficacy in this scenario. Since the passive transport mechanism accounts for the majority of the drug's absorption during oral drug delivery, its HLB value will have an impact on absorption. Drugs that are extremely lipophilic and have a low HLB value are poorly soluble in aqueous solutions and difficult to absorb through membranes. They are more likely to remain in fat tissues after injection. On the other hand, medications that are highly polar or strongly ionized and have a high HLB value are unable to effectively cross the GI (gastrointestinal) barrier and must instead be given intravenously (IV). They are unfavorable, though, because they disappear quickly. Masking a drug's polar ionized or non-ionized functional groups also enhances both oral and topical absorption.

Modifying a drug's substituents is one method for changing a drug's polarity and/or ionization, and this strategy is known as a quantitative structure-activity relationship (QSAR). There are numerous ways to do this, including changing the polar functional groups and alkyl or acyl substituents to adjust polarity, changing the N-alkyl substituents to change the pK_a, and changing the aromatic substituents to add or remove electron-donating or -withdrawing groups. If the substituent interacts with the ring *via* resonance, the substituent's location may also be important. Additionally, bioisosteres can be used for polar groups, for example, replacing carboxylic acid with a bioisostere group like 5-substituted tetrazoles, which have comparable physiochemical properties but a more palatable pK_a, or using ester prodrugs. Alkyl and aryl carboxylic groups typically have a pK_a between 2-5.
The most extensively researched and productive area of prodrugs has been lipophilicity improvement.

3. Altering the distribution profile; in order for a medicine to be effective, it must get past a number of pharmacological and pharmacokinetic obstacles. One of the most promising sit-selective approaches is the prodrug method, which employs endogenous enzymes or transporters that are unique to the target cell or tissue.

4. Keeping a quick metabolism and excretion in check for oral medications, first-pass metabolism in the GI and liver reduces the amount of medication that reaches circulation and the drug target, rendering the medication ineffective or necessitating frequent dosing. This issue was resolved by making the medication available in controlled release formulations as well as sublingual and buccal dose forms. However, it can also be beaten by utilizing the prodrug tactic.

On the other hand, medications that are very slow to leave the body and are extremely stable to metabolism can also be problematic since they increase toxicity and side effects.

Several strategies, including those for enhancing drug metabolism and those for making medications less resistant to drug metabolism, can be used to address these problems.

To boost the stability of the drug molecule's weak functional groups, including esters and amides, one method is to add steric shields to them. In order to prevent nucleophiles or nucleophilic centers on enzymes from approaching the susceptible group, a bulky alkyl group, such as t-butyl, must be added.

Utilizing the electronic properties of bioisosteres to protect a labile functional group through electronic stabilization is another strategy. For instance, a urethane functional group, which is more stable than the parent ester, is produced when an ester's methyl group is swapped out for an amine group. By using its inductive action to donate electrons to the carbonyl group, the amine group lowers the electrophilicity of the carbonyl group, stabilizing it against hydrolysis.

Labile groups are stabilized through stereoelectronic modification, which combines electronic stability with steric hindrance. For instance, the ester medication procaine is quickly hydrolyzed, but converting the ester to the less reactive amide group, as in the examples of procainamide and lidocaine, slows down hydrolysis.

To obstruct the metabolism of a medicine, polar functional groups can be added at specific locations in the drug's structure using metabolic blockers. For instance, megestrol acetate undergoes position 6 oxidation to yield a hydroxyl group, but when the hydrogen at position 6 is replaced with a methyl group, the metabolism of the compound is blocked, extending the duration of action.

By removing or replacing a metabolically vulnerable group, which is conceivable if the group in question isn't involved in crucial binding interactions within the active site of the receptor or enzyme, susceptible metabolic groups can also be removed. If the group is significant, a different approach is adopted, such as masking the weak group with a prodrug or moving the weak group within the molecular skeleton.

Since some ring systems are frequently found to be vulnerable to metabolism, ring variation can also increase metabolic stability. As with fluconazole, which substitutes a 1,2,4-triazole ring for the imidazole ring that is susceptible to metabolism in tioconazole, changing the ring can frequently improve metabolic stability.

In order to create medications with lessened chemical and metabolic stability, A good strategy to reduce a drug's half-life is to include groups that are susceptible to metabolism, such as a methyl group that can metabolically undergo oxidation to a polar alcohol or a carboxylic acid. Another choice is a self-destructive drug, which is chemically stable under some circumstances but spontaneously cleaves under other circumstances. An example of a self-destruct drug is atracurium, a neuromuscular blocking agent. Atracurium is stable at acidic pH but self-destructs when exposed to the slightly alkaline blood conditions, resulting in a short duration of action that can be controlled during surgery by administering it as a continuous intravenous drip.
5. Reducing toxicity; this can be done by changing one or more DAME barriers, but it's frequently done by using site-specific drug delivery to target drugs to desired sites and tissues. In this method, the designed prodrug must be transported to the site of action in order to be selectively and quantitatively transformed into the active drug and retained in the target tissue to produce the desired therapeutic effect.

6. Make a prodrug that slowly transforms into the active drug, attaining sustained action, to prolong drug activity (Fattash , *et al.*, 2014).

The targeted drug design strategy, which focuses on certain enzymes or carriers, and the chemical approach, which involves the enzymatic or chemical breakdown of the prodrug into its active form, are the two main prodrug design strategies.

The chemical approach can be further broken down into two main categories: (i) carrier-linked prodrugs, which contain a group that can be easily removed enzymatically, and (ii) intramolecular chemical approach, which uses no enzyme and relies solely on the rate-limiting step of the intramolecular reaction to control the inter-conversion of the prodrug.

- **The chemical approach that uses carrier-linked prodrugs**

This approach involves the use of carrier-linked prodrugs, which can be subdivided into bipartite, tripartite, and mutual prodrugs.

While tripartite prodrugs have a carrier group linked to the drug, bipartite prodrugs only have one carrier group attached to the drug. Contrarily, mutual prodrugs are made up of two drugs that are connected. The interconversion rate in this method depends on enzyme catalysis.

The aim of the chemical method is to enhance the drug molecule's physicochemical and pharmacokinetic qualities. The carrier group acts as a pro-moiety that the physiological environment may easily remove enzymatically, giving the parent drug. For effective activation *in vivo*, the promoiety must be pharmacologically inert and non-toxic, and the coupling between the medication and promoiety must be unstable.

In comparison to other prodrug design strategies, the chemical approach has a number of benefits. It can promote medication selectivity, improve drug solubility, and boost the bioavailability of pharmaceuticals that are poorly absorbed. The strategy can also extend the duration of the drug's activity, lower its toxicity, and lessen its negative effects.

When creating carrier-linked prodrugs, several things need to be taken into account. To ensure the proper delivery of the parent drug, it is imperative to improve the chemical stability, solubility, and permeability of the prodrugs. It is also important to consider the prodrug's enzymatic stability because particular physiological conditions may make some enzymes inactive or unavailable.

The creation of a successful carrier system is one of the main difficulties with the chemical method. The medication must be able to reach the target site through the carrier group and must be effectively cleaved to release the parent drug. In order to achieve effective activation in vivo, the linkage between the drug and promoiety must also be stable during drug storage and administration while being labile.

- **The intramolecular chemical approach**

Adding a labile group to drug molecules, which can then undergo an intramolecular reaction to produce the parent drug in vivo, is one method for prodrug design.

The molecular orbital (MO) and molecular mechanics (MM) methods for computation, as well as correlations between experimental and computed data, are used to construct the intramolecular chemical approach. In this method, a prodrug's intraconversion chemical reaction to its parent drug does not involve an enzyme. The intramolecular reaction's rate-limiting step is the only factor affecting how the prodrug interconverts.

The design of numerous prodrugs, including anti-inflammatory, anti-cancer, anti-viral, and anti-hypertensive drugs, has utilized the intramolecular chemical method. For instance, prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs) like indomethacin and ketoprofen, which are frequently used to treat pain and inflammation, have been created using the intramolecular chemical

method. By adding a labile ester or amide group, which then undergoes an intramolecular reaction to produce the parent drug in vivo, the prodrugs of indomethacin and ketoprofen were created.

Similar to this, prodrugs of anti-cancer medications like 5-fluorouracil (5-FU) and doxorubicin have been created using the intramolecular chemical method. By including a labile carbonate or carbamate group, which undergoes an intramolecular reaction to generate the parent drug in vivo, the prodrug of 5-FU was created. By including a labile hydrazone group, which undergoes an intramolecular reaction to produce the parent drug in vivo, the prodrug of doxorubicin was created.

In conclusion, the intramolecular chemical approach is a viable method for designing prodrugs and has a number of benefits over traditional prodrug methods. The design of prodrugs that are activated by an intramolecular reaction can be regulated by molecular calculations and correlations between experimental and estimated values thanks to the intramolecular chemical method.

1.3.1. Applications of prodrug design in drug development

For medications with poor water solubility, prodrug design can improve drug solubility and bioavailability. More than 30% of chemicals used in drug research have low water solubility. By binding to ionizable or polar groups, such as phosphates, sugars, or amino acid moieties, prodrugs can improve their solubility in water. As a result, the drug's molecule may dissolve more quickly and have a higher bioavailability when administered orally, parenteral, or intravenously.

Additionally, it may improve drug absorption and permeability. Poorly permeable medications may not be able to reach their target areas since membrane permeability has a considerable impact on drug effectiveness. Poorly permeable medicines can become more lipophilic by being linked to lipophilic groups through prodrug design. This can enhance their bioavailability and absorption when taking oral or topically applied medications. Increased oral or topically applied absorption can also be achieved by masking polar ionized or non-ionized functional groups.

Prodrug design can also alter the medicines' distribution profiles. The medicine must go past a number of pharmacological and pharmacokinetic obstacles before it can reach its physiological target. The prodrug strategy makes use of endogenous enzymes and transporters that are unique to the target cell or tissue and can deliver the drug to the desired location. One of the most effective site-specific drug delivery methods, it can increase the effectiveness and safety of medications (Ala'Abu-Jaish ,*et al.*, 2014; Karaman, 2013). The following prodrugs have been created, manufactured, and tested utilizing a chemical-based methodology:

- **Ester prodrugs**

In order to promote membrane penetration and enhance medication delivery, ester prodrugs have been designed to boost lipophilicity (Li, Maag, & Alfredson, 2008). This is accomplished by enzymatic catalysis, specifically by enzymes like carboxyl esterases, acetylcholinesterases, butyrylcholinesterases, paraoxonases, arylesterases, and biphenyl hydrolase-like protein, which hydrolyze functional groups like carboxylic acid, hydroxyl, phosphate, and thiol (Del Amo, Urtti, & Yliperttula, 2008; Tammara, Narurkar, Crider, & Khan, 1994). By lengthening the ester alkyl chain, the usage of ester prodrugs can also enhance binding to the hydrophobic region of carboxylesterase. These prodrugs have been used for a variety of purposes, such as enhancing the oral bioavailability of medications like acyclovir and tenofovir (Bando, Takagi, Yamashita, Takakura, & Hashida, 1996; Kim *et al*., 2003), lowering GI side-effects related to NSAIDs, and addressing issues with therapeutic medications used in clinical practice. Benorylate and naproxen-propyphenazone are examples of mutual prodrugs based on ester linkage that have been developed to provide synergistic benefits while lowering gastric irritancy (Bhosle, Bharambe, Gairola, & Dhaneshwar, 2006). Furthermore, organic NSAID nitrate esters that release NO have been shown to have gastroprotective effects and can be used to prevent GI side effects. (Muscará, McKnight, Del Soldato, & Wallace, 1998)

- **Amide prodrugs**

A technique for improving drug stability, providing targeted drug administration, and changing the lipophilicity of medications that contain carboxylic acid or amine groups is the use of amide prodrugs (Kalgutkar, Marnett, Crews, Remmel, & Marnett, 2000). Although the high *in vivo* stability of amide prodrugs prevents them from being widely used, some techniques for producing them have been developed to get around this problem. For instance, amide prodrugs that are more easily transformed back into the parent drug in vivo can be created through intramolecular cyclization processes (Shan, Nicolaou, Borchardt, & Wang, 1997).

Using amide connections, such as atorvastatin and amlodipine, mutual prodrugs can also be produced that, upon in vivo amide hydrolysis, release the corresponding active parent drug. Both nonspecific amidases and specialized enzymatic activation, such as renal-glutamyl transpeptidase, can convert amide prodrugs back to the parent drug. For instance, renal -glutamyl transpeptidase activates the dopamine double prodrug -glutamyl-l-dopa (gludopa) to generate a roughly fivefold rise in dopamine levels compared to L-dopa prodrug. Dopamine [N-(N-acetyl-L-methionyl)-O, O-

bis(ethoxycarbonyl)dopamine] was developed and has demonstrated better oral absorption because gludopa has limited oral bioavailability. Since oral administration of dopamine can result in dopamine inactivation due to COMT and MAO activity, this prodrug is used to treat renal and cardiovascular diseases (Casagrande, Merlo, Ferrini, Miragoli, & Semeraro, 1989; Lee, 1990).

Dapsone is a good example of how amide-linked amino acid conjugates have been employed to increase medication solubility (Pochopin, Charman, & Stella, 1994). Additionally, amide-based prodrugs based on allopurinol N-acyl derivatives have been created; these prodrugs are more lipophilic than allopurinol itself (Simplício, Clancy, & Gilmer, 2008). A useful method for drug creation overall, amide prodrugs have the potential for improved solubility, altered lipophilicity, and targeted administration.

- **Carbonates and carbamates prodrugs**

Carbonates and carbamates are two types of prodrugs that are more stable than esters but less stable than amides. They do not have specific enzymes for their hydrolysis reactions; however, they are degraded by esterases to give the corresponding active parent drugs (Safadi *et al.*, 1993). Carbamates prodrugs are considered double prodrugs (pro-prodrug) because they are enzymatically activated at first, followed by spontaneous cleavage of the resulting carbamic acid (Li *et al.*, 2008).

One example of carbamates prodrug is the co-carboxymethylphenyl ester of amphetamine, which can be hydrolyzed by esterase to yield amphetamine(Safadi *et al.*, 1993). Another example is fluorenylmethoxycarbonyl]-3 derivatives of insulin and exenatide that undergo slow interconversion *via* carbamate bond breakdown, providing glucose-controlling agents in an adequate rate, which results in lowering the risk of hypoglycemia (Safadi *et al.*, 1993).

Carbamates prodrugs have been used to increase the solubility of active drugs such as cephalosporins (Hecker *et al.*, 2003). They have also been exploited in targeted therapy such as ADEPT, where the carbamate group is susceptible to the action of tyrosinase enzyme present in melanomas. This approach is usually utilized in cancer targeted therapy (Karaman, 2013) .

One example of a carbonate prodrug is estramustine, which is obtained by linking phosphorylated steroid, an estradiol, to normustard, an alkylating agent, through a carbamate linkage. The steroid portion has an antiandrogenic action and acts to concentrate the prodrug in the prostate gland where prodrug hydrolysis takes place and normustard action can then be exerted(Bhosle *et al.*, 2006).

Loratadine is another example of a carbamate prodrug. It is an ethylcarbamate that undergoes *in vivo* interconversion to its active form, desloratadine, through the action of CYP450 enzymes (Yumibe et al., 1996). Capecitabine, an anticancer agent, is also a carbamate prodrug that undergoes a multistep activation to finally yield 5-fluorouracil in the liver. Capecitabine is less toxic than 5-fluorouracil, more selective, and widely used in clinical practice (Alexander *et al.* , 1988).

- **Oximes prodrugs**

Oxime prodrugs are a type of prodrug that can increase the permeability of the corresponding active drugs by introducing an oxime group to the drug molecule. The oxime group can act as a hydrogen bond donor and acceptor, which can increase the water solubility and lipophilicity of the drug. In addition, oxime prodrugs can also increase the stability of drugs by protecting them from degradation.

The conversion of oxime prodrugs to the corresponding active drugs is typically catalyzed by microsomal cytochrome P450 enzymes (CYP450) present in the liver. These enzymes can cleave the oxime group, thereby releasing the active drug.

An example of an oxime prodrug is 6-(N,N-Di-n-propylamino)-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one, which is a dopaminergic prodrug. This prodrug is converted to its active form, 6-(N,N-Di-n-propylamino)-2,3,4,5-tetrahydro-1H-benzo[d]azepin-8-ol (apomorphine), by microsomal cytochrome P450 enzymes in the liver. Apomorphine is used in the treatment of Parkinson's disease and other dopaminergic disorders. The use of an oxime prodrug of apomorphine can improve its oral bioavailability and reduce its side effects, making it a more effective and safe drug (Li *et al.*, 2008; Venhuis *et al.*, 2003).

- **N-Mannich Bases, Enaminones, and Schiff Bases (Imines)**

N-Mannich bases, Enaminones, and Schiff bases (imines) are classes of prodrugs that are used to enhance the solubility and bioavailability of active drugs. N-Mannich bases are prepared by a reaction known as the Mannich reaction, which involves the reaction of an NH-acidic compound, an aldehyde, and an amine in ethanol. These prodrugs are water-soluble and suitable for parenteral administration, and they are converted to their parent drugs in the stomach when administered orally. However, some N-Mannich base prodrugs have poor in vitro stability, and their enzymatic

breakdown can lead to the formation of formaldehyde, which is a limitation of this prodrug approach (Simplício *et al.*, 2008).

Enamines, which are α, β-unsaturated amines, are generally unstable at low pH, making them unsuitable for oral administration. Nonetheless, an ampicillin prodrug based on enamines was prepared for rectal use and showed increased absorption compared to its active parent drug. Enaminones are enamines of β-dicarbonyl compounds that undergo a ketoenolimine-enamine tautomeric equilibrium. These prodrugs are more lipophilic than their parent drugs and have an improved oral absorption. Enaminones are generally chemically stable, but some enaminones derived from ketoesters and lactone may undergo enzymatic degradation, which could improve their conversion rate to the active drug.

Schiff bases, also known as imines, are another class of prodrugs used to enhance the solubility and bioavailability of active drugs. Schiff bases are formed by the reaction between an amine and an aldehyde or ketone. These prodrugs are generally stable but can undergo hydrolysis in the body to release the active drug (Karaman, 2013).

- **Phosphate and Phosphonate Prodrugs**

Phosphate and phosphonate prodrugs are types of prodrugs that utilize phosphorylation to increase the aqueous solubility of the parent drug. This can improve the bioavailability and patient compliance of the drug (Huttunen *et al.*, 2011).

Phosphate prodrugs, such as prednisolone sodium phosphate and fosamprenavir, are created by linking a phosphate group to a free hydroxyl group on the parent drug. These prodrugs are often more water soluble than their active forms, making them easier to administer and more effective in the body (Chapman *et al.*, 2004; Huttunen *et al.*, 2011). For example, fosamprenavir is 10 times more water soluble than its active form, amprenavir, and can be administered twice a day instead of eight times a day (Chapman *et al.*, 2004).

Phosphate prodrugs are cleaved back into their corresponding active drugs in the gut through the action of alkaline phosphatases. The active drugs are then absorbed into the systemic circulation.

Phosphonate prodrugs, such as fosphenytoin, are similar to phosphate prodrugs, but utilize a phosphonate group instead of a phosphate group to enhance solubility. Fosphenytoin is a prodrug of the anticonvulsant agent phenytoin and has enhanced solubility compared to its corresponding drug (Boucher, 1996).

- **Azo Compounds**

Azo compounds are a type of prodrug approach that utilizes colonic bacteria as a means of prodrug activation. This approach is used specifically in targeted drug strategy, where the drug is designed to be released in a specific part of the body (Liljefors, Krogsgaard-Larsen, & Madsen, 2002).

Sulfasalazine is an example of an azo compound prodrug used in the treatment of ulcerative colitis. It is a prodrug of 5-aminosalicylic acid and sulfapyridine. Once it reaches the colon, sulfasalazine undergoes azo bond cleavage to release the active parent drug (Bhosle *et al.*, 2006).

Other examples of prodrugs that are activated by azo-reductases include Osalazine, Balsalazide, and Ipsalazide. These prodrugs have 5-aminosalicylic acid moiety conjugated to 4-aminobenzoyl-β-alanine and 4-aminobenzoylglycine, respectively (Sandborn, 2002).

Another example of this class of prodrug is one in which 5-aminosalicylic acid is linked to L-aspartic acid. This prodrug has shown a desirable colon-specific delivery and a 50% release of 5-aminosalicylic acid from an administered dose (Jung, *et al.*, 2001).

It is important to note that this approach is limited to aromatic amines, as azo compounds of aliphatic amines exhibit significant instability.

- **Poly Ethylene Glycol (PEG) Conjugates**

Polyethylene glycol (PEG) is a water-soluble, non-toxic polymer that has been widely used in drug delivery systems. PEG conjugation involves linking a drug molecule to the PEG polymer, either to increase drug solubility or to prolong drug plasma half-life (Simplício *et al.*, 2008).

There are several spacers that can be used to link the drug to PEG, including esters, carbamates, carbonates, or amides. These spacers can be enzymatically broken down, resulting in the release of the active drug.

For example, daunorubicin can be conjugated to PEG via a spacer attached to the phenol group of the open lactone. The rate of drug release can be controlled by manipulating the substituents on the aromatic ring.

Upon enzymatic breakdown of the spacer, the resultant ester or carbamate drug can be liberated by 1,4- or 1,6-benzyl elimination. This strategy allows for controlled release of the drug over a longer period of time and can lead to improved therapeutic efficacy.

Overall, PEG conjugation is a promising approach for drug delivery that offers improved solubility, prolonged plasma half-life, and controlled drug release (Karaman, 2013).

1.3.2. Antibacterial prodrugs to overcome bacterial resistance

Bacterial resistance to traditional antibiotics is a growing concern, and prodrug-based approaches have been identified as a promising strategy to combat this issue. Prodrug-based approaches involve modifying an antibiotic to create a prodrug that is inactive until it reaches the target bacterial cell, where it is activated by bacterial enzymes.

Several types of prodrugs can be used to target different bacterial components, including:

1. **Prodrugs that target the bacterial cell wall:** These prodrugs are designed to be inactive until they reach the bacterial cell wall, where they are activated by bacterial enzymes. Such examples are beta-lactams and glycopeptides, which are commonly used in clinical practice, but resistance has emerged against these antibiotics because bacteria have developed mechanisms to resist the action of antibiotics that target the cell wall, such as by producing beta-lactamases or by altering the structure of the cell wall.

However there have been prodrugs developed to overcome resistance, one example is fosfomycin trometamol, which is a prodrug that is converted to the active form, fosfomycin, by alkaline phosphatases present in bacterial cells. Fosfomycin is then able to inhibit bacterial cell wall synthesis, making it an effective antibacterial agent. Another example includes ceftolozane/tazobactam, which is a cephalosporin antibiotic that inhibits cell wall synthesis in Gram-negative bacteria. Ceftaroline fosamil, a prodrug of the antibiotic ceftaroline that is used to treat methicillin-resistant *Staphylococcus aurous* (MRSA) infections.

2. **Prodrugs that target bacterial enzymes:** These prodrugs are designed to be metabolized by bacterial enzymes to produce active drugs by targeting bacterial enzymes involved in the biosynthesis of essential molecules, such as nucleotides and amino acids One example is sulbactam, which is a prodrug that is cleaved by beta-lactamases, which are enzymes produced by some bacteria that can degrade beta-lactam antibiotics. Sulbactam is able to inhibit the beta-lactamases, thus restoring the activity of beta-lactam antibiotics and overcoming bacterial resistance. Other Examples include the prodrug nitrocefin, which is converted to a chromophoric β-lactam upon cleavage by β-lactamase enzymes produced by many Gram-negative bacteria, and avibactam, which inhibits β-lactamases and restores the activity of

existing antibiotics. Moreover, fosfomycin tromethamine a prodrug of the antibiotic fosfomycin inhibits the enzyme MurA, which is involved in the biosynthesis of peptidoglycan.

3. **Prodrugs that target bacterial nucleic acid synthesis:** These prodrugs are designed to be metabolized by bacterial enzymes involved in nucleic acid synthesis to produce active drugs. Upon activation they target bacterial DNA or RNA synthesis, one example is rifampin, which is a prodrug that is converted to the active form by bacterial RNA polymerase. Rifampin is able to inhibit bacterial RNA polymerase, which is essential for bacterial nucleic acid synthesis. Other example includes azithromycin-based prodrugs that release azithromycin, an antibiotic that targets bacterial protein synthesis, and a quinolone that targets bacterial DNA gyrase, upon activation. Other Examples include prodrugs of fluoroquinolones, such as Ciprofloxacin and levofloxacin, which are converted into their active form by bacterial enzymes, and metronidazole, which is a prodrug that is activated by reduction of its nitro group in anaerobic bacteria.
4. **Efflux pump-targeting prodrugs:** These prodrugs are designed to release antibiotics that are not substrates of bacterial efflux pumps, upon activation. One example is fluoroquinolone-based prodrugs that are not effluxed by the AcrAB-TolC efflux pump in Escherichia coli, thereby increasing their antibacterial activity.

Despite the success of prodrug-based approaches, resistance has emerged against prodrugs. To combat this issue, the design and development of new prodrugs, such as ester and amide prodrugs, are discussed as alternative strategies.

Ester prodrugs are a type of prodrug that can improve the oral bioavailability and stability of antibiotics. They are designed to be converted into the active drug through hydrolysis of the ester bond in which the prodrug molecule contains an ester group that is cleaved by esterases in the intestinal mucosa, blood, or tissues. This cleavage results in the release of the active drug, which can then be distributed to the target site.

One example of an ester prodrug is pivmecillinam. It is a prodrug of mecillinam and is used to treat urinary tract infections caused by Escherichia coli. Pivmecillinam is administered orally and is absorbed from the intestine. It is then transported to the liver, where it is hydrolyzed by esterases to release the active drug, mecillinam. Another example of an ester prodrug is azithromycin-2',4''-diisovalerate (AZD-I). It is a prodrug of azithromycin and has been shown to have improved pharmacokinetic properties compared to azithromycin. *In vitro* studies have shown that AZD-I is

rapidly hydrolyzed by esterases to release azithromycin. Ester prodrugs have also been developed to improve the pharmacokinetic properties of fluoroquinolones. Levofloxacin-5-O-(1-carboxymethyl) oxime (LFX-CMO) is a prodrug of levofloxacin that has been shown to have improved oral bioavailability and to exhibit prolonged elimination half-life compared to levofloxacin. LFX-CMO is hydrolyzed by esterases to release levofloxacin.

Amide prodrugs on the other hand are designed to improve the water solubility and membrane permeability of antibiotics, which can increase their effectiveness against resistant bacteria.

One example of an amide prodrug is fosmidomycin trometamol. Fosmidomycin is an antibiotic that inhibits the enzyme 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) in the bacterial isoprenoid biosynthesis pathway. Fosmidomycin trometamol is a prodrug form of fosmidomycin that is metabolized by esterases to release the active drug. The trometamol moiety of the prodrug enhances its solubility and stability, which improves its pharmacokinetic properties.

Another example of an amide prodrug is AZD5847, which is a prodrug of the antibiotic azithromycin. AZD5847 has been shown to be effective against multidrug-resistant strains of bacteria, including Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA). The amide linkage in AZD5847 improves the lipophilicity of the prodrug, which enhances its ability to penetrate bacterial membranes and reach its target.

Prodrug-based approaches offer a promising strategy to overcome bacterial resistance. However, there are both advantages and limitations associated with this approach.

Advantages of prodrug-based approaches

- **Improved bioavailability:** Prodrugs can improve the oral bioavailability of antibiotics, allowing for increased absorption and improved distribution to the target site.
- **Enhanced stability:** Prodrugs can increase the stability of antibiotics in the bloodstream, improving their half-life and overall efficacy.
- Reduced toxicity: Prodrugs can reduce the toxicity of antibiotics by decreasing their side effects and adverse reactions.
- **Overcoming resistance mechanisms:** Prodrugs can be designed to overcome bacterial resistance mechanisms by targeting specific components of bacteria and utilizing different prodrug designs.

- **Potential for combination therapy:** Prodrugs can be used in combination with other antibiotics or therapies, providing synergistic effects and potentially reducing the development of resistance.

Limitations of prodrug-based approaches

- **Complexity of design:** Prodrug design can be complex and requires a thorough understanding of bacterial enzymes and resistance mechanisms.
- **Limited spectrum of activity:** Prodrugs may only be effective against specific bacterial species or strains, limiting their overall utility.
- **Variable pharmacokinetic properties:** Prodrugs can have variable pharmacokinetic properties, leading to variability in efficacy and potential adverse effects.
- **Risk of unintended consequences:** Prodrug modifications may lead to unintended consequences, such as the activation of resistance mechanisms or the creation of toxic metabolites.
- **Development and regulatory challenges:** Developing and gaining regulatory approval for prodrug-based approaches can be challenging and time-consuming, requiring extensive preclinical and clinical testing.

The future of prodrug-based approaches appears promising, but further research is needed to optimize their design and improve their clinical efficacy. While prodrugs have shown to be effective in overcoming bacterial resistance, challenges such as bioavailability, toxicity, and pharmacokinetic properties must be addressed for successful clinical application.

One potential future direction is the development of prodrugs that are activated specifically by bacterial enzymes or cellular processes. This could lead to increased selectivity and reduced toxicity for the prodrug, as it would only be activated by the target bacteria. Additionally, the use of nanotechnology and drug delivery systems to improve the targeting and release of prodrugs to bacterial cells could further enhance their efficacy and reduce potential side effects.

Another future direction is the combination of prodrug-based approaches with other therapeutic strategies, such as immunotherapy or bacteriophage therapy. The use of combination therapies could potentially enhance the efficacy of prodrugs and overcome resistance mechanisms that may arise during treatment (Jubeh, *et al.*, 2020a).

1.4.Problem statement

To begin with, there are two main problems of Ciprofloxacin drug:

(i) It is an Antibiotic drug that is exposed to bacterial resistance as described earlier.

(ii) Its physiochemical properties come with drawbacks.

To address the second issue, the solubility and the permeability of Ciprofloxacin must be understood. Ciprofloxacin is regarded as a substance with limited solubility. Despite having a relatively high solubility at both low pH (4) and high pH (> 10), it has a very low solubility at the pH that is close to its isoelectric point, which also corresponds to the physiological pH range (6–8). It is 0.070 to 0.088 mg/mL soluble at pH 6.8 to 7.5 and 25 °C. The drug's ionization profile may be a contributing factor to its limited solubility. The molecules in the solid state are tiny, stiff, and frequently flat, enabling them to pack tightly and form strong intermolecular bonds (Van Waals interaction, II-II staking, and hydrogen bonds) within the crystal lattice (Tehler *et al.*, 2013). This could explain the reason for low solubility of Ciprofloxacin.

The issue of the poor solubility gets worse because commercially available formulae frequently use film coating to hide the drug's awful, extremely bitter taste, which is the dug's second disadvantage. Most of the Ciprofloxacin is left undissolved until it reaches the small intestine, where the luminal pH (7.5) becomes unfavorable for its solubilization. The coating inhibits the drug product from successfully dissolving in the comparatively acidic gastric fluid (pH 1.5–3.5).

The drug's limited permeability is the cause of another issue. Passive diffusion plays a major role in the absorption of Ciprofloxacin in the digestive system. At a pH of around 7.5, its pH-distribution coefficient (logD) peaks. Since it is generally accepted that only the neutral, un-ionized form of an ionizable compound can enter the lipophilic environment of biological membranes, this could be explained by the relationship between the pH and the fraction of the neutral form of Ciprofloxacin. The peak of Ciprofloxacin's logD and membrane permeability occur near the isoelectric point, where the percentage of the neutral form is at its maximum concentration. The fraction of the neutral form at pH 7.5 is nevertheless theoretically just 0.23%, which leads to low absolute values of logD and passive diffusivity. Last but not least, Ciprofloxacin's efflux mechanism, which some research utilizing caco2 cells and some animal models revealed may be a potential substrate for efflux transporters, is another component in the drug's poor intestinal permeability (Sun, 2013).

All of the aforementioned factors, which led to the medicine's classification as class IV in the biopharmaceutical classification system and decreased the drug's bioavailability, caused the drug to be poorly absorbed.

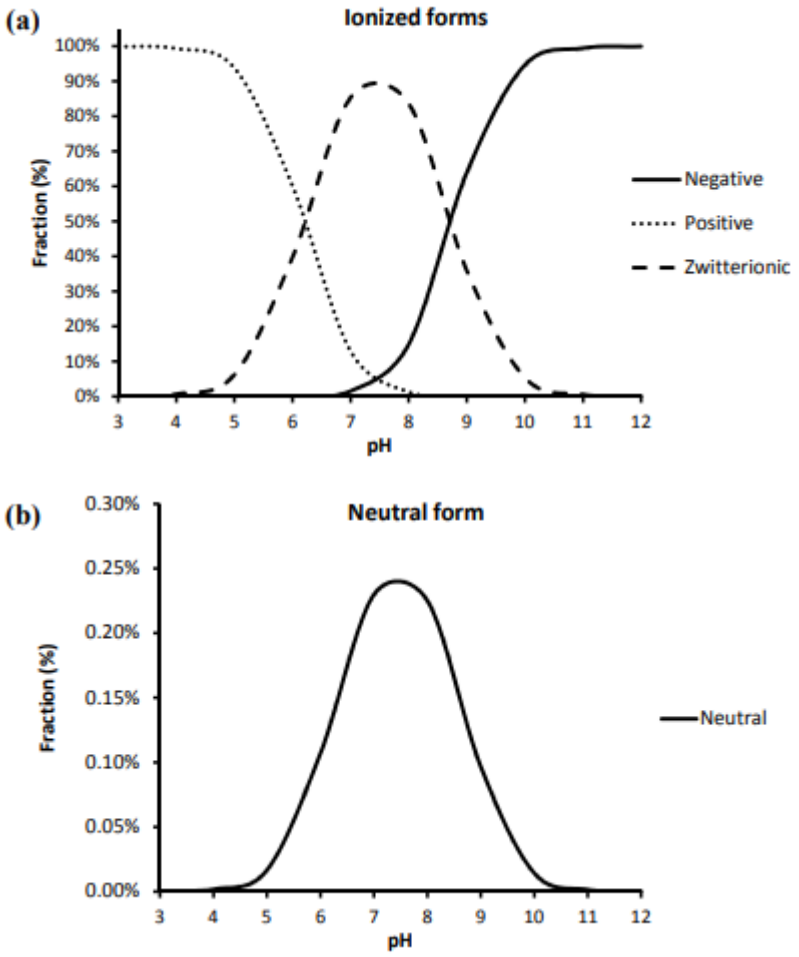


Figure1-4: Theoretical relationship between the solution pH and the distribution of (a) the three ionized species and (b) the neutral form of Ciprofloxacin

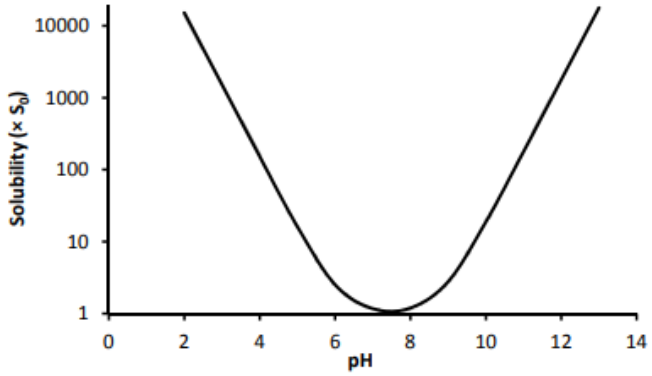


Figure 1-5: Theoretical relationship between the solution pH and the aqueous solubility of Ciprofloxacin, expressed in multiples of intrinsic solubility (× S0). The y-axis is shown in the log10 scale

1.5.Thesis objectives

The major objective of the research is to create novel Ciprofloxacin prodrugs with improved antibacterial activity to overcome the prevalent bacterial resistance issue. These prodrugs would have greater antibacterial activity than Ciprofloxacin and they will poses better physiochemical properties.

• Specific objectives of the work are

1. To assess the antibacterial effects of prodrugs.
2. To characterize the suggested prodrugs using a variety of methods, including melting point, chromatographic methods including UPLC, and NMR.

1.6.Research question

- Could Ciprofloxacin be chemically synthesized to attach to the linkers?
- Are the manufactured Ciprofloxacin prodrugs more effective against bacteria than the parent medication?
- Would the synthetic prodrugs cleave into their parent compounds?
- Are there any physiochemical characteristics in the suggested Ciprofloxacin prodrugs that would make them more soluble and palatable than the parent medication?

Chapter two

Literature review

Chapter two

2. Literature review

There are two primary sections in this chapter: Part 1 highlights the clinical studies that demonstrate how Ciprofloxacin is a medicine that has great potential in a variety of therapeutic areas. As a result, much research has been done to find ways to extend the drug's shelf life through synthetic development utilizing the prodrug technique. There are headlines that categorize the Ciprofloxacin prodrug studies according to the rationale for their synthesis.

The second section discusses prodrugs based on a technique called intramolecular processes (enzyme models).

Part one

- **Prodrug approaches to increase solubility and improve the physiochemical properties of the parent drug:**

According to the biopharmaceutical classification system, Ciprofloxacin is categorized as class IV, which denotes that it has low solubility and permeability. If you look closely at the molecules in the solid state, you will notice that they are small, rigid, and frequently flat, allowing them to pack tightly and form strong intermolecular bonds (Van Waals interaction, II-II staking, and hydrogen bonds) within the crystal lattice (Tehler *et al.*, 2013). This may help to explain why the solubility is so low. Due to the physiochemical characteristics that affect the drug's kinetics, researchers have made several efforts to enhance the drug utilizing the prodrug technique.

By adding thick, flexible side chains and lowering the ability of molecules to form hydrogen bonds that could fracture the crystal lattice, seven Ciprofloxacin analogs were created by the esterification reaction. The goal was to increase the drug's solubility in water. Studies on melting point, solubility, and permeability were done(Tehler *et al.*, 2013).

A different study proposed creating a prodrug by joining two different pharmacophores so that each one serves as a promoiety or carrier for the other. The linkage should be cleavable under physiological circumstances. This method has the advantage of increasing the physiochemical proprieties of the drugs when compared to each drug agent alone, rather than co-administering medications in separate doses when combination therapy is required. In this work, Ciprofloxacin and Secnidazole were conjugated to produce two prodrugs (one with ester coupling and the other

with amide coupling), which were then evaluated for solubility, lipophilicity, taste, and antibacterial activity and contrasted with the parent drug(Khalid *et al.*, 2021).

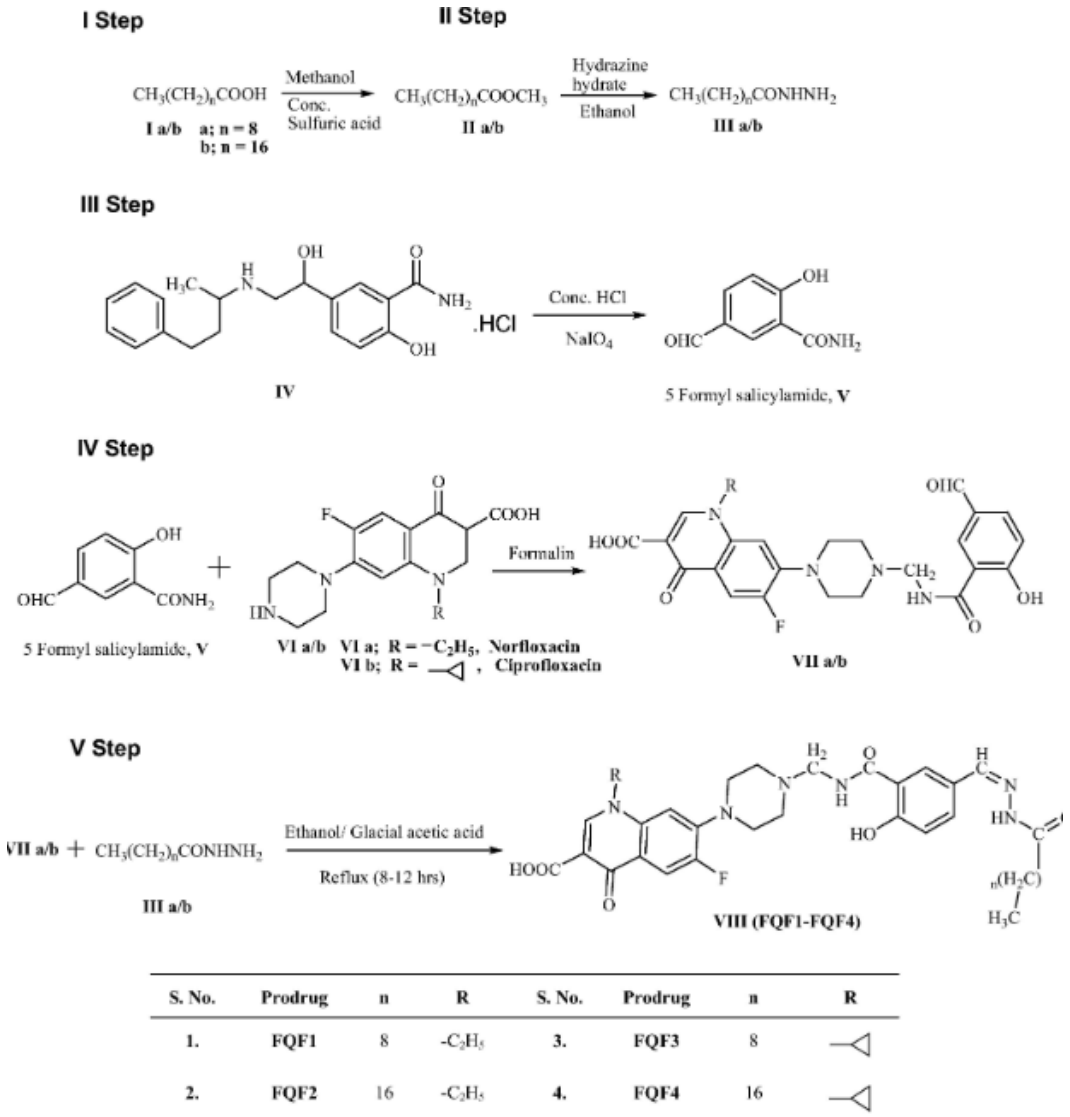
Ciprofloxacyl-glycine methyl ester, an amide prodrug of Ciprofloxacin, was created and showed the potential to have a considerably higher solubility than Ciprofloxacin does in the physiological pH range. Additionally, compared to the parent compound, Cipro-Gly-OMe may have a higher intestinal permeability at neutral pH. Chemically speaking, Ciprofloxacin's poor biopharmaceutical qualities are partially a result of the carboxyl group and secondary amine in the compound's inherent dissociation constants, which both control the equilibrium between the drug's four solute species. The main reason for Ciprofloxacin's low solubility and intestinal permeability at neutral pH is the prevalence of the zwitterionic form of the medication at pH levels close to its isoelectric point. In order to change the solute species' equilibria and maybe improve the compound's solubility and intestinal permeability, this study uses the prodrug technique. The idea was that the equilibria would be significantly changed, potentially altering the compound's biopharmaceutical properties, if one or both of the ionizable groups could be concealed by a promoiety (or promoieties).

The study's final findings showed that the prodrug Cipro-Gly-OMe had potentially considerably higher solubility than the parent molecule Ciprofloxacin does at physiological pH ranges, as well as better Caco-2 permeability compared to the parent drug at neutral pH. The lack of effective biotransformation of the prodrug to Ciprofloxacin, however, could cause the antibacterial pharmacological properties of the drug to start acting later or never at all. Therefore, other promoiety structures for masking the carboxyl group of Ciprofloxacin should be taken into consideration in future prodrug designs (Sun, 2013).

Al Najah National University published a study in 2016 intending to creat Ciprofloxacin prodrugs with a higher solubility profile. Three ethylene glycol derivatives are successfully used in an esterification reaction to produce three prodrugs with improved solubility and antibacterial activity by up to 40%.(Assali *et al.*, 2016)

This 2017 study was based on the hypothesis that the synthesis of N-Mannich bases lowers the amines' pKa by around 3 points, increasing the parent drug's lipophilicity and, subsequently, its diffusion potential. Additionally, N-Mannich bases hydrolyze in buffers at varying rates, depending on their pH, to release the bioactive compound. As a result, it is possible to assume that the produced prodrugs may go through a hydrolysis step to release the active drug molecule.

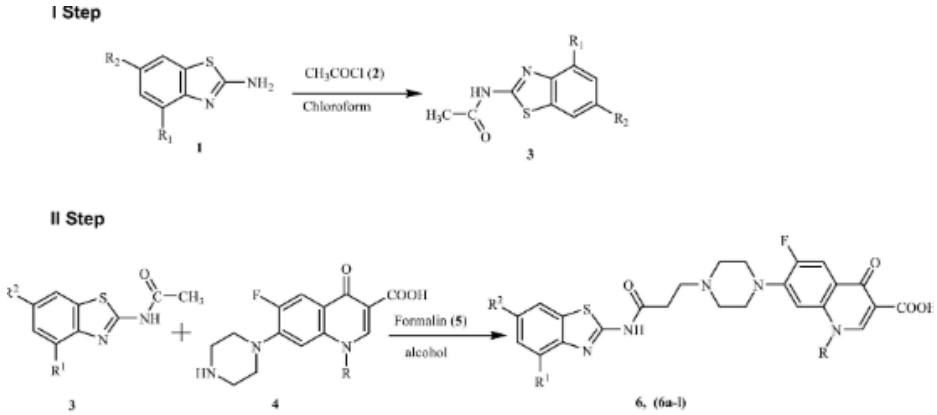
Using two approaches, several prodrugs of Ciprofloxacin were created. In **Scheme 2-1**, the synthesis of lipid-based prodrugs was carried out in five steps, resulting in the synthesis of four prodrugs (FQF1-FQF4).



S. No.	Prodrug	n	R	S. No.	Prodrug	n	R
1.	FQF1	8	$-C_2H_5$	3.	FQF3	8	
2.	FQF2	16	$-C_2H_5$	4.	FQF4	16	

Scheme 2-1: Synthetic scheme 1 for preparation of prodrugs FQF1-FQF4.

In **Scheme 2-2**, the synthesis of benzothiazoles clubbed prodrugs of Ciprofloxacin was carried out in two steps, resulting in the synthesis of twelve prodrugs. These prodrugs were assessed for their biological activity (cytotoxic action against cancer and anthelmintic activity), spectral characterisation, and physiochemical traits (Piplani *et al.*, 2017).



Prodrug	R	R^1	R^2	Prodrug	R	R^1	R^2
6a	$-C_2H_5$	-H	$-OCH_3$	**6g**		-H	$-OCH_3$
6b	$-C_2H_5$	-H	$-OC_2H_5$	**6h**		-H	$-OC_2H_5$
6c	$-C_2H_5$	-H	$-CH_3$	**6i**		-H	$-CH_3$
6d	$-C_2H_5$	-H	-Cl	**6j**		-H	-Cl
6e	$-C_2H_5$	-H	$-NO_2$	**6k**		-H	$-NO_2$
6f	$-C_2H_5$	-Cl	-H	**6l**		-Cl	-H

Scheme 2-2: Synthetic scheme 2 for preparation of prodrugs 6a-l

The last example of research in this area is a study on self-assembling prodrugs, which is created by conjugating a drug to a carrier molecule like a polymer to produce an amphiphile that self-assembles into nanoparticles. These nanoparticles exhibit passive targeting and extended blood circulation. A variety of linkers between the parent drug and the carrier molecule can also be used to adjust the drug release. The work describes the synthesis of macromolecular prodrugs (MPDs) of Ciprofloxacin (CIP) based on hydroxypropylcellulose (HPC) and hydroxyethylcellulose (HEC) (Amin, Abbas, Hussain, Sher, & Edgar, 2018).

- **Using the prodrug approach for targeted therapy**

In order to eliminate or reduce drug side effects resulting from the pharmacological and biological performance of commercially available medications, the pharmaceutical industry has undergone significant changes. The best way to reduce side effects was to design medication delivery devices,

however, this strategy is difficult to implement and has downsides. Prodrugs were now being used as a novel strategy for focused therapy.

Ciprofloxacin is used to treat periodontitis; however, it takes several grams during the course of treatment to completely cure the condition. Therefore, a prodrug that can achieve the desired therapeutic outcome with the least amount of drug was required; as a result, a commercially available biotinylation reagent was coupled to maleimides of either the prodrug or fluorescein analogue *via* glutathione to create a biotinylated prodrug and biotinylated fluorescent analogue of Ciprofloxacin. Targeting aggregatibacter actinomycetemcomitans (A.a) biofilms required biotinylation of the Ciprofloxacin prodrug using a glutathione linker in between. To help in measuring the concentration of Ciprofloxacin prodrug that enters the biofilms via the biotin-streptavidin pair, a fluorescent analogue was created. An isotype mIgG2b monoclonal antibody (A.a-mAb) 325AA2 against A.a was utilized as the A.a-specific targeting reagent(Amly & Karaman, 2016).

In order to combat infections that can occur after implanting biomedical devices, this study focused on another type of biofilm. Their strategy was to design a localized drug delivery using "enzyme prodrug therapy" (EPT), in which drug synthesis takes place locally at the treatment site. They created Ciprofloxacin glucuronide prodrugs for EPT by synthesizing carbamoyl-linked fluoroquinolone glucuronides, and they then designed surface coatings based on multilayered polyelectrolyte coatings, an easy method of surface modification. Then they used planktonic bacteria and biofilms to examine enzyme-containing coatings in the context of prodrug conversion and subsequent antibacterial effects. They looked into both the prevention and therapy of biofilms for the latter (Walther, *et al.*, 2018).

Another targeted therapy study attempted to create a Ciprofloxacin prodrug to treat osteomyelitis, a bone inflammation or swelling caused by bacterial infections that is difficult to treat and necessitates high doses of systemically administered antibiotics. They created bisphosphonate antibacterial prodrugs based on eight distinct linkers attached to the free amino functionality on fluoroquinolone-antibiotics in an effort to capitalize on the bisphosphonate group's affinity for bone minerals. The synthesized prodrugs were examined for their capacity to release the parent medication, as well as for their capacity to fend off infection in rat models (Houghton *et al.*, 2008).

Research on targeted therapy had some application to the respiratory system. One of the most difficult infectious disorders, alveolar intracellular bacterial infections localized to the lung's

alveolar macrophage (AM), still have a significant unmet clinical need for effective therapy. The three papers that follow go into great length about this subject.

The creation of a novel intracellular enzyme-cleavable polymeric prodrug with specific Ciprofloxacin release profiles in the lungs and alveolar macrophages (AM) was shown in the first investigation. The hydrophilic mannose residues in the targeted polymeric prodrug (also known as drugamer) help to solubilize the antibiotic payload, target and increase AM uptake and intracellular delivery, and offer high and sustained intracellular AM drug dosage. From the antibiotic Ciprofloxacin, prodrug monomers were created using either a hydrolytic phenyl ester linker or an intracellular protease cleavable dipeptide linker. The effectiveness of the newly created prodrug in comparison to the parent medication was tested by intra-tracheal administration, and the results were highly encouraging (Su *et al.*, 2018).

In order to deploy "drugamer" as an unexpectedly successful pre-exposure preventive in very lethal murine models of aerosolized human pulmonary melioidosis, the second investigation adopts the same methodology as the first.

An intracellular depot of Ciprofloxacin was targeted to the alveolar macrophage compartment by a single dosage of the macrophage-targeted poly-Ciprofloxacin prodrug, and this intracellular depot was sustained over a period of seven days above minimal inhibitory values. They presented evidence for the first time that pre-exposure prophylaxis in a model of pulmonary melioidosis can be achieved by targeting the intracellular macrophage compartment with sustained antibiotic treatment (Chavas *et al.*, 2021).

In the third investigation, they identified inhalable macromolecular prodrugs made from polymerizable prodrug monomers as a viable modular platform technology that may be used to treat various invasive alveolar intracellular bacteria in addition to aerosolized F. tularensis. From polymerizable drug monomers, they created polymeric prodrugs with high drug densities of Ciprofloxacin, a model antibiotic that can treat respiratory tularemia quickly and with less medication. The designed chemically labile drug-linkers used in the macromolecular Ciprofloxacin prodrugs provide sustained release of antibiotics via ester hydrolysis. By selecting different linkers and polymer architectures, it is possible to regulate the kinetics of the drug release (Das, 2017).

The study that follows outlines a strategy for using targeted to combat bacterial resistance. The development of a brand-new cephalosporin-fluoroquinolone prodrug. The prodrug was created to have limited effects on bacteria that do not produce the -lactamase enzymes, a characteristic of

resistance, while selectively delivering the broad-spectrum antibiotic Ciprofloxacin to only lactamase expressing bacteria. The prodrug demonstrated selectivity toward *E. coli* that expresses lactamases, no activity on non-lactamase expressing bacteria, -lactamase-mediated intracellular release of Ciprofloxacin upon cleavage of the cephalosporin, and very low activity of the intact prodrug.(Evans *et al.*, 2019)

- **The use of prodrug approach to fight against cancer, bacterial resistance and malaria**

Due to the significance of combating the emergence of bacterial resistance and the excellent chance to employ a medicine with such few side effects as a cytotoxic agent to fight malignancy, there are an incredible number of publications that can be found on this subject. It can also be used to treat ailments like malaria and ADIS because it is a cost-effective medication.

Only recently published publications will be attempted to be summarized in this area; however, there are many earlier research trials go unmentioned that may be helpful to those working on the same topic.

This study first introduces a novel class of Ciprofloxacin-based prodrugs containing benzothiazoles that were developed, produced, and tested for antibacterial activity. The research found that these compounds have promising antibacterial activity while outperforming their parent medication in terms of solubility. In order to create six different analogs of the parent drug, Prodrug[7-(4-(2-(6-Methoxybenzo[d]thiazol-2-yl) ethyl)piperazin-1-yl)]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid], Ciprofloxacin was dissolved in methanol and added to formalin has been demonstrated to be the most effective antibacterial agent, with MIC values of 12.5 and 25 g/ml against S. aureus MTCC 96 and S. pyogenus MTCC 442, respectively. Prodrugs [7-(4-(2-(6-Nitrobenzo[d]thiazol-2-yl) ethyl)piperazin-1-yl)-6-fluoro-1,4-dihydro-4- oxoquinoline-3-carboxylic acid] and [7-(4-(2-(4-Chlorobenzo[d]thiazol-2-yl)carbamoyl) ethyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4- oxoquinoline-3-carboxylic acid] showed antibacterial efficacy against several bacterial strains that was comparable to those of reference medications. When compared to conventional medications, some of the synthesized prodrugs demonstrated good antifungal activity against particular strains (Piplani *et al.* , 2016).

This study used the same methodology as the first by combining Ciprofloxacin with Mannich bases to enhance its physicochemical characteristics, antibacterial, and anticancer effects, as well as caspase-3 induced apoptosis. By refluxing Ciprofloxacin with the proper phenolic compound in the presence of too much formaldehyde in ethanol, ten prodrugs were created. While the remaining

derivatives displayed moderate to weak activity against the tested 60 cancer cell lines, the naphtol Mannich bases 2a and 2b demonstrated remarkable cytotoxic activities. When it comes to antimicrobial activity, it is clear that the addition of Mannich's Ciprofloxacin base boosted antibacterial activity against both Gram-positive and Gram-negative species. The naphthol Mannich bases[1-Cyclopropyl-6-fluoro-7-(4-((1-hydroxynaphthalen-2-yl)methyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid] and[1-Cyclopropyl-6-fluoro-7-(4-((2-hydroxynaphthalen-1-yl) methyl) piperazin-1-yl)-4-oxo- 1,4-dihydroquinoline-3-carboxylic acid] exhibited excellent activities against MRSA (clinical strain), *Staphylococcus aurous* and *Pseudomonas aeruginosa* compared with Ciprofloxacin. Surprisingly, against *Staphylococcus aurous* and MRSA (reference strain), [7-(4-(5-Chloro-2-hydroxybenzyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid] showed improved antibacterial activity by 27 and 22 times compared to Ciprofloxacin. The antimicrobial efficacy of [1-Cyclopropyl-6-fluoro-7-(4-(2-hydroxy-5(methoxycarbonyl) benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid] against pseudomonas aeruginosa and *E. coli* was also quite impressive(Abdel-Rahman *et al.*, 2021).

This study clarifies the two potential mechanisms by which Ciprofloxacin exerts its cytotoxic effect. They then attempted to synthesize 31 7-((4-substituted) piperazin-1-yl) derivatives of CP using a quick and inexpensive process. Two of these derivatives, specifically compounds [7-(4-(2-Chloroacetyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylic acid] and [7-(4-Decanoylpiperazin-1-yl)] showed strong in vitro antitumor activity. This study thus demonstrates that adding straightforward substituents to the piperazinyl group can positively modulate the cytotoxicity of CP (Azéma *et al.*, 2009).

To boost Ciprofloxacin's anticancer effectiveness even more, dimeric prodrugs of the antibiotic were created. It is clear from this study that one of the Ciprofloxacin dimeric prodrugs has demonstrated greater anticancer activity in comparison to parent medications. Dimeric Ciprofloxacin prodrugs had IC_{50} values that were noticeably lower than those of Ciprofloxacin. They also evaluated the monomers and dimer prodrugs for their antibacterial activity and discovered that the majority of them had minimum inhibitory concentrations (MIC) against *S. aurous* that were comparable to Ciprofloxacin (0.97 M). However, most prodrugs lost their effectiveness against *E. coli* and *P. aeruginosa*. Strong antibacterial efficacy against *S. aurous* was demonstrated by the monomeric oxoethyloctanoate-based Ciprofloxacin prodrug (MIC 1 nM) (Azéma *et al.*, 2011).

The antimalarial and anti-toxoplasma activity of new ester prodrugs of Ciprofloxacin was examined in this work. Therefore, molecular modeling and computational calculations were used to understand the mechanisms of action of these drugs. These new compounds proved to be incredibly effective against these parasites. Their earlier research improved the antimalarial activity of Ciprofloxacin by adopting a dual method that combined a prodrug/bio-organometallic approach. They continue their previous work in this piece and attempt to create more effective ester prodrugs of Ciprofloxacin (Dubar *et al.*, 2011).

Part two

- **Prodrugs Based on Intramolecular Processes (Enzyme Models)**

One method for creating prodrugs is called the "enzyme model approach," which relies on the intraconversion chemical interaction between the prodrug and its parent drug. No enzymes are employed in this method; instead, the rate-limiting step is an intramolecular reaction.

Because enzyme catalysis is frequently unpredictable and because there are numerous intrinsic and extrinsic factors that influence the bioconversion mechanisms, it has become necessary to develop prodrugs that do not rely on enzymes to give their parent drugs. For instance, genetic polymorphisms, aging-related physiological changes, or drug interactions may alter the activity of various prodrug-activating enzymes, resulting in negative pharmacokinetic, pharmacodynamic, and clinical outcomes. In addition, the presence of significant interspecies variability in the expression and operation of the majority of the enzyme systems activating prodrugs would provide significant difficulties for preclinical optimization (Karaman, 2012).

The goal of the enzyme model technique is to replicate the catalytic mechanism of an enzyme. Over the past 50 years, Bruice and Bnkouic, Jenks, Menger, Kirby, Walsh, and Bender have all extensively studied the mechanisms of enzymes. Their work has helped us grasp how and to what extent enzymes accelerate biological transformation.

Today, scientists generally agree that the basis of enzyme catalysis lies in the interaction between the catalytic effects of functional groups and the capacity to reroute intermolecular reactions through different routes where the substrate can bind to a pre-organized active site.

Due to the way enzymes catalyze reactions and increase the rate of chemical processes, scientists spent more than 50 years developing chemical models that could provide reaction rates that were

comparable to those of enzymes. For instance, it has been noted that the rate of the majority of enzymatic processes exceeds 10^{10} -10^{18} folds that of their bimolecular catalyzed non-enzymatic counterparts. Utilizing cyclophilin-catalyzed reactions is increased by 10^5 and orotidine monophosphate decarboxylase-catalyzed reactions is increased by 10^{17}.

Covalently enforced proximity is one method for speeding up chemical reactions. In this method, functional groups are brought near together to encourage interactions (Karaman, 2013).

The work by Bruice *et al.*, which showed the effectiveness of intramolecular ring closing processes in boosting chemical reactions, is the most frequently cited example of this. They developed a model that uses dicarboxylic semi-esters to create aldehydes, and they were able to create anhydrides at a pace that was 5 x 10^7 times faster than that of comparable intermolecular interactions. The ground state molecules' close closeness of the nucleophile and electrophile caused enthalpic effects, which caused acceleration.

The phrase "near attack conformation" (NAC) was coined by Bruice and Lightstone to refer to the necessary conformation for reactants to enter a transition state. The mole fraction of reactant conformations that are present as the NAC affects how quickly the intramolecular reaction proceeds. This study demonstrated that chemical simulations can imitate the effectiveness of enzymes in catalyzing chemical processes (Lightstone & Bruice, 1996) (Bruice & Pandit, 1960).

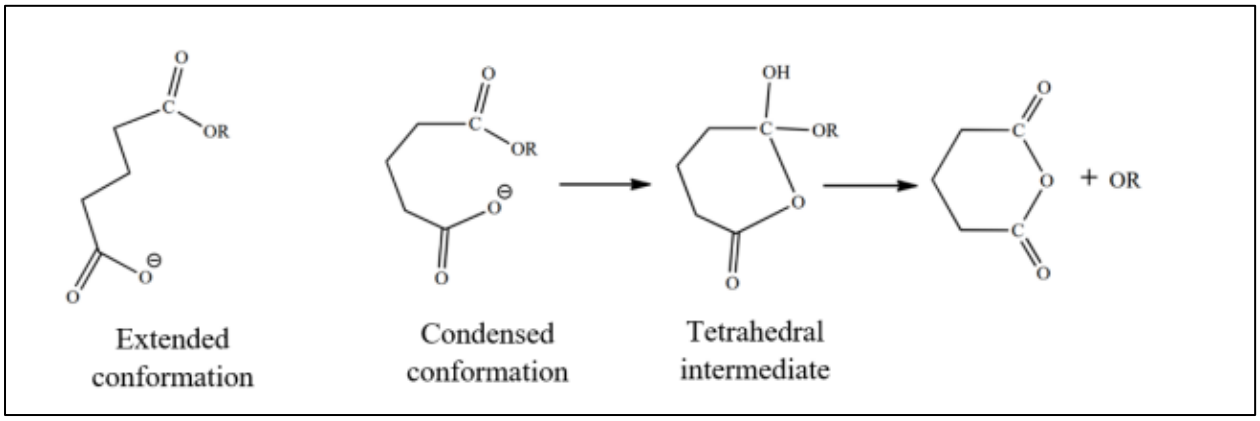


Figure 2-1: The figure illustrates how intramolecular nucleophilic catalysis promotes ester hydrolysis in Bruice's enzyme model. The nucleophile (O- of COO-) and the electrophile (C of COOR) are closer in the condensed conformation compared to the extended conformation. This closer proximity leads to a more efficient cyclization reaction

Another method for achieving rate acceleration is proximity orientation, which involves bringing two reactive centers closer to one another to speed up the reaction time. This strategy is

demonstrated by Koshland's orbital steering theory. It implies that the intramolecular localization reaction rate can be greatly influenced by the ground state angle of attack value of the hydroxyl group in hydrocarboxylic acids. This implies that the reaction rate can be influenced by the angle at which the hydroxyl group approaches the other reactive center (Dafforn & Koshland Jr, 1973).

According to Mengers' spatiotemporal hypothesis, whether an intermolecular or intramolecular reaction took place, the pace at which protons were transferred was greatly influenced by the distance between the two reactive centers involved (Menger, 2005).

Acid-catalyzed lactonization can also hasten intramolecular processes. This was the enzyme model that Cohen and Milstien proposed after researching the trimethyl-lock system that contains hydroxycarboxylic acid. The comparatively high amplification rate in hydroxycinnamic acid lactonization reactions containing two methyl groups on the beta position of their carboxylic groups is explained by Cohen's stereopopulation control theory. This hypothesis states that methyl groups can boost the pace of the reaction by regulating the stereopopulation of the reaction intermediate (Milstien & Cohen, 1972).

The significance of hydrogen bonding creation in the products and transition states leading to the acid-catalyzed hydrolysis of acetals and maleamic acid amides is explained by Kirbys' proton transfer models. Accordingly, it is possible for hydrogen bonds to form, bringing the reactive centers closer together and speeding up the reaction process (Barber, Dean, & Kirby, 1999; Kirby, 1997).

Making prodrugs based on enzyme models has had several successful pharmaceutical uses during the past fifteen years. Since it can release the biologically active amine (drug) upon acetate hydrolysis by enzyme-triggering, 3-(2′-acetoxy-4′, 6′-dimethyl dimethyl)-phenyl-3, 3-dimethylpropionamide derivative (pro-prodrug) was used by Brochardt et al. in their prodrug based on hydroxyhydrocinnamic acid.

Prodrug taxol is another application based on the stereopopulation control paradigm, which improved the drug's water solubility to enable intravenous administration of the medication.

Such prodrugs are created using a computational approach. Chemistry employs computer science techniques to assist in problem-solving. This is accomplished by computing the structure of molecules as well as their physical and chemical properties utilizing results from theoretical chemistry assimilation onto computer systems.

The use of computational chemistry for computing the properties of molecules in their ground and transitional states has been growing over the past 60 years and has become crucial in many areas of chemistry, including organic, bioorganic, and pharmaceutical chemistry. Reaction rates and equilibrium energy-based calculations are needed for biological systems with pharmaceutical and bio-medical interests as they present a significant challenge to the medical community.

To give structural energy calculations for an accurate prediction of possible medications and prodrugs, it is now reliable and acceptable to apply tools from quantum mechanics (QM), molecular mechanics (MM), and approaches like ab-nitio, semi-empirical, and density functional theory (DFT).

Both static and dynamic scenarios can be solved using ab-initio and semi-empirical methods, but the computational cost skyrockets as the size of the system under study.

When approximations are sufficiently small and the finite set of basic functions approaches the limit of a complete set, ab-initio methods, which are entirely based on theory from first principles, can converge to exact solutions. However, the computational cost of these methods is a drawback as they frequently require enormous amounts of computer time, memory, and disk space. In contrast, semi-empirical approaches minimize processing costs by approximating a system's electrical structure using a combination of theory and experimental evidence (Karaman, 2015).

Based on computational investigations, Karaman and his research group have examined several enzyme models and created new prodrug designs. They established relationships between observed and computed rate values for some intramolecular events using various molecular orbital and MM approaches. Their research demonstrated that entropy and enthalpy both affect how quickly intramolecular reactions accelerate (Karaman, 2012, and 2015) (Karaman, 2010, 2011; R Karaman, Fattash, & Qtait, 2013).

This research will examine the synthesis, characterization and the assessment of the antibacterial activity of two novel Ciprofloxacin prodrugs that Karaman proposed based on his calculations.

Chapter three

Experimental part

Chapter Three

3. Experimental Part

There are three primary sections in this chapter: All of the equipment, chemicals, and materials utilized in this study are described in part one. The second part is the synthetic section, which is concerned with the identification of the synthesized prodrugs and the synthesis processes. The third one addresses the necessary microbiological testing for this study.

3.1. Part one

3.1.1. Chemicals and Instrumentation

3.1.1.1. Reagents

A pure standard of Ciprofloxacin HCl was obtained from Pharmacare PLC Pharmaceutical Company. Di-methyl-maleic anhydride, Cyclohexene-1, 2-dicarboxylic anhydride and cis-5-Norbornene-endo-2, 3-dicarboxylic anhydride, potassium carbonate (K_2CO_3), and thin layer chromatography were all purchased from Sigma Aldrich.

3.1.1.2. Solvents

Tetrahydrofuran, Hexane, and Ethylacetate were all obtained from Sigma Aldrich. Distilled water was obtained from a reverse osmosis purifier available at Al-Quds University-Faculty of Pharmacy labs. HPLC grade solvents of methanol, acetonitrile, and water were supplied from Al-Quds University-Faculty of Pharmacy labs.

3.1.1.3. Instrumentation and substance identification

Chemical hazards fuming hood, hotplates, pH meter, and rotary evaporator are available at Karaman's Lab in the Faculty of Pharmacy, Al-Quds University as well as melting point and FT-IR instrumentation. UPLC was done at Pharmacare Pharmaceutical Company in Ramallah and at Karaman's Lab in the Faculty of Pharmacy. Proton nuclear magnetic resonance spectroscopy(^{1}H-NMR) was done at the Hebrew University.

3.1.1.4. pH meter

pH meter model HM-30G: TOA electronics™ was used to measure the pH values for all buffers involved in this research.

3.1.1.5. FT-IR

All infrared spectra (FTIR) were obtained from potassium bromide matrix (4000–400 cm^{-1}) using BRUKER TENSOR FT-IR spectrometer equipped with a platinum attenuated total reflectance (ATR) unit.

3.1.1.6. UPLC

An analytical method for drug identification was developed on UPLC-UV/Vis for Ciprofloxacin and each synthesized prodrug using Shimadzu's Nexera X2 UPLC system with Shimadzu Prominence SPD-M20A Diode Array Detector. Data acquisition and control were carried out using Shimadzu's LabSolutions software.

Analytes were separated using RP-8 endcapped column (150mm x 4.6 mm, 5 μm). The mobile phase used was a methanol: phosphate buffer (pH 3) mixture (40:60, v/v) with a flow rate of 1.2 mL/min. the detection wavelength (λ Detection) was 257 nm. All the UPLC analysis in the following sections was performed using this method.

3.1.1.7. 1H-NMR

All ^{1}H-NMR spectra were conducted using the 500 MHz Varian NMR spectrometer. Samples were run in deuterium oxide (D_2O). For ^{1}H-NMR, chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Spin multiplicities are described as doublet (d), doublet of triplet of doublet (dt), singlet (s), triplet (t), quartet (q), and multiplet (m).

3.2. Part two

3.2.1. Synthesis of Ciprofloxacin Prodrugs

The reaction would start by dissolving 1 equivalent of Ciprofloxacin in THF, then an excess of the aqueous solution of potassium carbonate was added. The mixture then was sonicated.

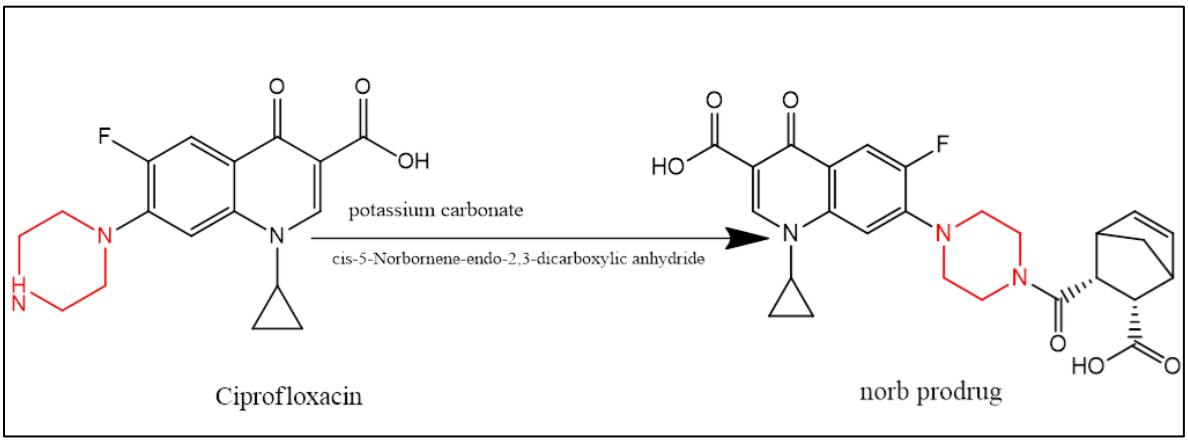
1.1 equivalent of the linker was added while sonicating for 15 minutes. The solid formed was filtered and dried by a rotary evaporator. The reaction progress was monitored by TLC.

UPLC was used lastly to make sure that the synthesis process was completed.

(The linkers to be used are cis-5-Norbornene-endo-2,3-dicarboxylic, and anhydride di-methyl-maleic anhydride).

- **Ciprofloxacin ProD 1 preparation**

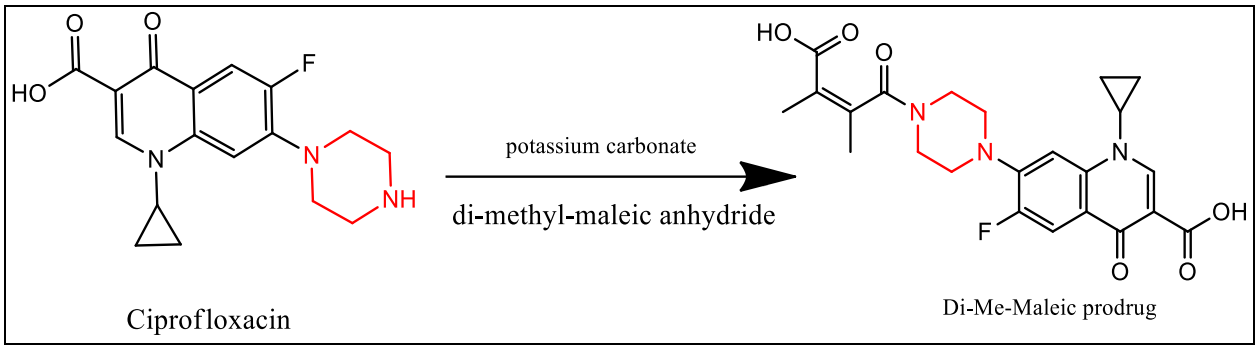
Following the previous procedure, an off white powder product was obtained. Thereafter, the product was characterized by melting point, FT-IR spectroscopy, and ^{1}H NMR. Melting point 172C, ^{1}H-NMR (500 MHz, D_2O) δ ppm: 1.16(td, 2 H, C$\underline{H_2}$CHCH_2), 1.328(td, 1H,CHC$\underline{H_2}$CH), 1.347(td, 1H, CHC$\underline{H_2}$CH), 3.003(dt,2H,C$\underline{H}$COOH), 3.187(dd,2H,C$\underline{H}$CHCOOH), 1.42 (td, 2H, CH_2CHC$\underline{H_2}$), 3.36 (d, 1H, CH_2C$\underline{H}$$CH_2$) 3.56 (m, 2H, NC$\underline{H_2}$), 3.61 (td, 2H, HNC$\underline{H_2}$), 6.103(td, 2H,C$\underline{H}$=C$\underline{H}$), 7.19 (d, 1H, C=CC$\underline{H}$) 7.37 (d, 1H, COC=$\underline{H}$), 8.44(s, 1H, NC$\underline{H_2}$). IR (KBr/$\nu_{max}$cm^{-1}) 675-730 cm^{-1} (C=C), 1700 cm-1 (C=O), 3500- 3450cm-1(OH).



Scheme 3-1: Ciprofloxacin ProD 1; synthesis scheme for the formation of ProD 1 using cis-5-Norbornene-endo-2, 3-dicarboxylic.

- **Ciprofloxacin ProD 2 preparation**

We followed the same procedure for Ciprofloxacin **ProD 1** but instead of using cis-5-Norbornene-endo-2, 3-dicarboxylic anhydride linker, di-methyl-maleic anhydride was used (**Scheme 3-2**).



Scheme 3-2: Ciprofloxacin ProD 2; synthesis scheme for the formation of Ciprofloxacin prodrug 2 using dimethyl maleic anhydride.

A white precipitate resulted as a product of this reaction that had a melting point of 250C. ^{1}H-NMR (500 MHz, D2O) δ ppm: 1.153 (td, 2 H, C$\underline{H_2}$CHCH$_2$), 1.341 (td, 2H, CH$_2$CHC$\underline{H_2}$), 1.890(m,3H, C$\underline{H_3}$) 3.082(m, 2H, NC$\underline{H_2}$), 3.299(td, 2H, HNC$\underline{H_2}$), 3.6 (d, 1H, C=CC$\underline{H}$) (d, 1H, CH$_2$C$\underline{H}$CH$_2$) 7.66(d, 1H, COC=C$\underline{H}$),7.928(q,3H,C$\underline{H_3}$) 8.5(s, 1H, NC$\underline{H_2}$). (KBr/ν_{max}cm^{-1}) 1557 cm^{-1} (C=O), 3351 cm-1 (OH).

3.3. Part three

3.3.1. Microbiology

3.3.1.1. Buffer Preparation:

0.1 Normalized hydrochloric acid (0.1 N HCl) was prepared by diluting 8.5 ml of hydrochloric acid with water to 1000 ml. Moreover, pH 3 was prepared by dissolving 6.8 g of potassium dihydrogen phosphate in 900 ml water for HPLC then it was adjusted by diluted ortho-phosphoric acid and water was added to a final volume of 1000 ml.

3.3.1.2. **Media preparation**

Muller Hinton agar and nutrient broth were prepared in standard concentrations.

3.3.1.3. **Inocula preparation**

The following bacterial strains were used to test the effectiveness of the prepared prodrugs of Ciprofloxacin:

- *Staphylococcus aureus*
- *Escherichia coli*
- *pseudomonas aeruginosa*

All were obtained from microbiological labs (Al-Quds University)

By using an isolated bacterial colony, inocula was prepared in which it was inoculated in 5 ml nutrient broth & incubated for 24 hours at 37^{o}C, the growth turbidity in nutrient broth was adjusted by further incubation or dilution with sterile physiological saline; after comparison with that of a McFarland nephlometer tube no. 0.5 (10^{8} cfu/ml) using a spectrophotometer at 625 nm (optical density of 0.08-0.1). By combining 9.9 ml of sterile saline with 0.1 ml of the generated bacterial broth culture, an inoculum with 106 cfu/ml of bacterial suspension was created.

3.3.2. Antimicrobial Activity screening method

An antibacterial medicine is typically evaluated *in vitro* environments to see if it will be a successful drug or not. It is understood that the *in vitro* environment is distinct from the *in vivo* environment since it is devoid of all elements that can affect drug activity. Despite this, studies attempting to show how antibacterial medicines affect bacterial growth in a lab setting have been found to closely

match the clinical results of infected individuals treated with those substances. The three most common ways to conduct an antimicrobial susceptibility test are as follows:

- The broth dilution method which entails preparing various antimicrobial agent concentrations in broth tubes before adding the test organisms. The concentration of the antimicrobial drug can be directly linked to the suppression of bacterial growth that results. The minimal inhibitory concentration (MIC) is the lowest concentration at which growth is inhibited.
- Agar dilution method is conceptually similar to the previously mentioned method, but it differs in that the microbial organism would be grown on the surface of the agar and different concentrations of the antimicrobial agent would be diluted in agar instead of broth. The result of the bacterial growth inhibition or inhibition would then be assessed based on the agent's concentration in addition to the ability to determine MIC.
- Methods for disc diffusion include laying paper discs with antimicrobial impregnation on top of agar that has already been cultured with the bacteria that will be tested; this method is said to be the simplest and most common. The antimicrobial substance would permeate the agar and maybe prevent bacterial development in the area around the disc. However, there are numerous variables that could affect the outcomes, so this method was altered with standardized conditions to enable generalization. The Kirby-Bauer method, which was created as a result of the change, has been widely used by clinical laboratories ever since. According to the Clinical and Laboratory Standards Institute's (CLSI) guidelines, the antimicrobial agent would be categorized as either Resistant (R), Intermediate (I), or Susceptible (S) based on the measurement of the inhibition zone (In Biemer, 1973).

In this work, the Kirby-Bauer method and the broth dilution methods were used.

3.3.2.1. Disc diffusion method (Kirby-Bauer method)

With a sterile cotton applicator, 10^8 cfu/ml of each bacterial strain (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) were swabbed on Muller Hinton agar using the following procedure:

1. The cotton applicator was dipped into the bacterial suspension, rotated several times, and pressed against the inside wall of the tube to remove excess inoculum.

2. The agar plate was then streaked in three different directions and around the agar margin to ensure an even distribution of the inoculum.

3. The plates were left to dry for 3-5 minutes.

4. Using sterile forceps the disks, which contained prodrugs, Ciprofloxacin drug, and buffer (which was used as negative control), were then distributed evenly on the surface of the agar plates.

5. The plates were incubated upside-down at 37^{o}C for 24hrs.

6. The inhibition zone around each disk was measured using a transparent ruler (Patel, 2012).

3.3.2.2. Broth diffusion method

- **Preparation of media**

A total of 75 broth-containing tubes were created, each having an inoculum of 10^6 cfu/ml of bacterial suspension and a total volume of 9.9 ml nutrient broth that was produced and autoclaved before adding bacteria. This was done to examine the efficacy of the prodrugs and to compare them to Ciprofloxacin.

Each medication was tested using five different concentrations on three distinct bacterial strains, and the outcomes were compared using a positive control tube (carrying a suspension of the tested bacterial strains) and a negative control tube (carrying an autoclaved nutritional broth).

- **Preparation of the active ingredient dilutions**

Three stock solutions of (Ciprofloxacin, **ProD1**, **and ProD 2**) were prepared to give a final concentration of 3000mcg/ml by dissolving them using buffer (pH 3) and methanol as co-solvent to increase solubility. Then a series of serial solutions were done as shown in **Table 3-1**.

Table 3-1: series of serial solutions of active ingredient stock solutions

Active ingredient	Stock solution(mcg/ml)	Dilution 1 (mcg/ml)	Dilution 2 (mcg/ml)	Dilution 3 (mcg/ml)	Dilution 4 (mcg/ml)
Ciprofloxacin	3000	1500	750	375	187.5
Prodrug 1	3000	1500	750	375	187.5
Prodrug 2	3000	1500	750	375	187.5

- **Incorporation of active ingredient into media**

For the incorporation of the active ingredient into the media, (0.1ml) of each prepared solution was added to nutrient broth tubes to yield a total of (10 ml) final volume. **Table 3-2** shows the final concentration of active ingredients in the medium.

Table 3-2: the final concentration of active ingredients in the medium.

Tube number	1	2	3	4	5
Concentration mcg/ml	30	15	7.5	3.75	1.875

- **Determination of the minimum inhibitory concentration (MIC)**

All 75 test tubes containing broth (which were prepared for the broth dilution procedure) are incubated for 24 hours at 37 degrees Celsius in order to determine the minimum concentration of the active substances stated above that is required to suppress the bacterial growth. Visual turbidity will not be present, which is a sign of MIC.

The minimal bactericidal concentration (MBC) of tubes that would look clear to the naked eye would be determined after additional investigation.

Samples from the tubes that looked to be clear would be grown on Molar Hinton Agar-coated petri dishes to create subcultures, which would then be checked for healthy, visible colonies.

After overnight incubation at 37° C results would be recorded as the following:

- Bacteriostatic if similar number of colonies are present.
- Partial bactericidal if the number of colonies found was reduced.
- No growth indicates that the whole inoculums have been killed.

Chapter four

Results and discussion

Chapter four

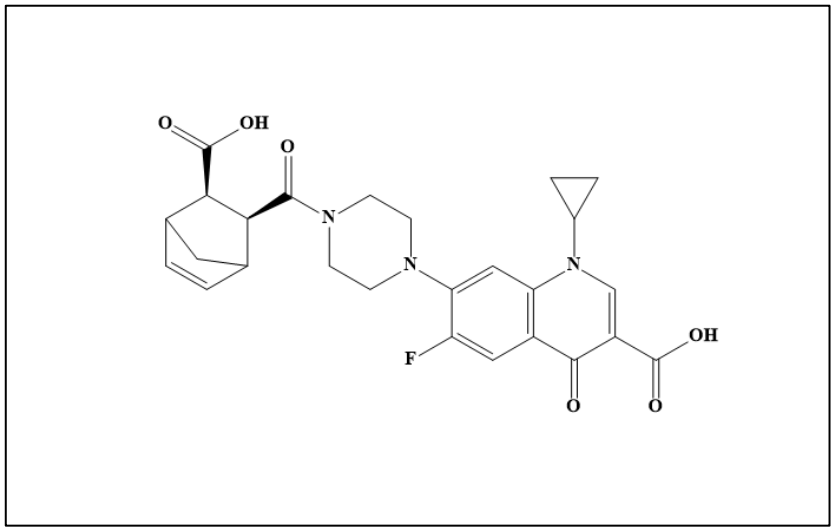
4. Results and discussion

4.1. Synthesis of Ciprofloxacin prodrugs

The innovative Ciprofloxacin prodrugs were effectively synthesized by reacting the amine with an acid hydride to produce an amide link through nucleophilic acyl substitution **(Figure 4-1)**. The Ciprofloxacin amine's nucleophilic attack on the anhydride's carbonyl caused the reaction, which led to conjugation and the opening of the anhydride cycle **(Scheme4-1)**. The prodrug linkers cis-5-Norbornene-endo-2, 3-dicarboxylic anhydride, and di-methyl-maleic anhydride were used to create the prodrugs Ciprofloxacin 1 (**ProD1**), and Ciprofloxacin 2 (**ProD2**) respectively **(scheme 4-2)**. Prior to adding extra potassium carbonate aqueous solution to the reaction pot as a catalyst, Ciprofloxacin was first dissolved in THF.

The finished products were powder: **ProD1** is an off-white powder with a melting point of 172 °C, and **ProD2** is a white powder with a melting temperature of 250 °C. The two powders nevertheless possess the acrid taste of Ciprofloxacin while having a seemingly better solubility than the parent medication. Moreover, **ProD1** and **ProD2** proved to be very stable prodrugs.

a)



b)

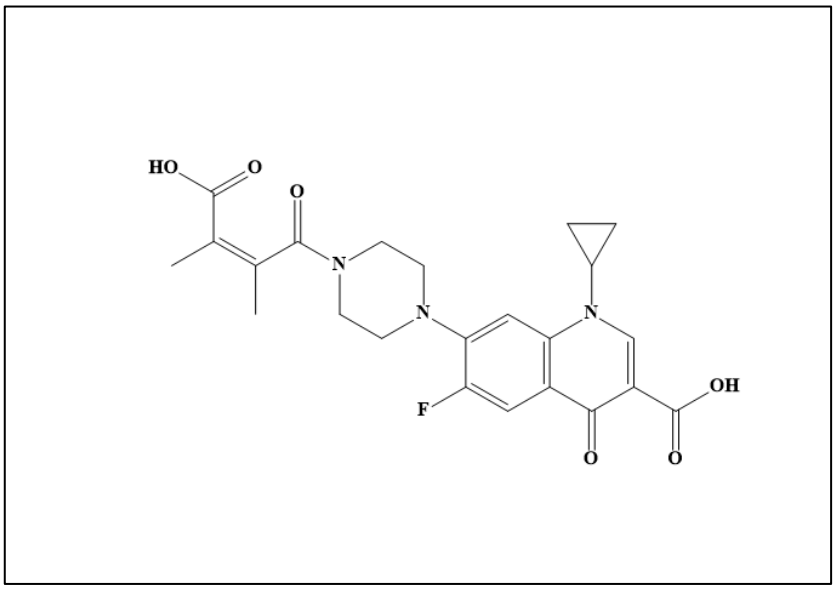
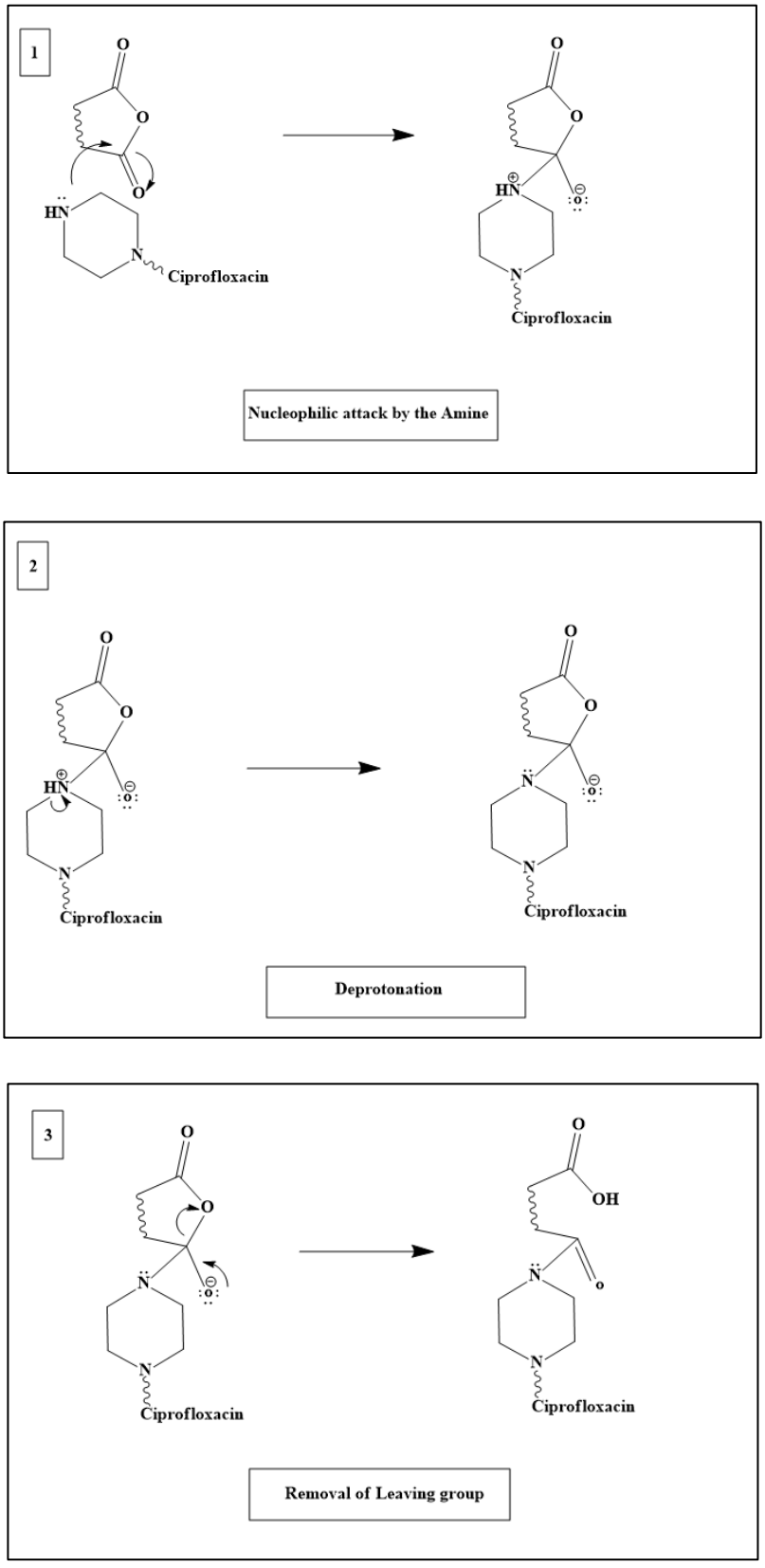
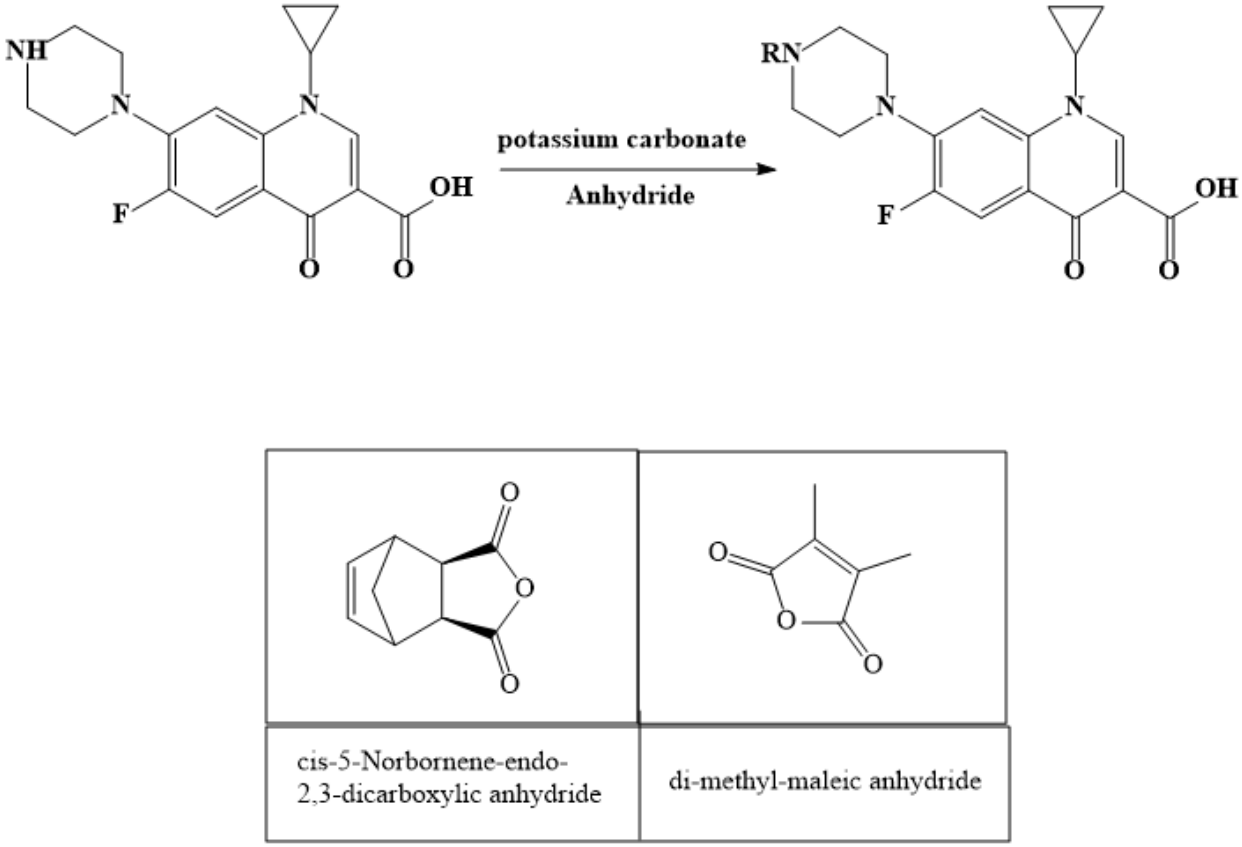


Figure 4-1: Chemical structures of ProD1 (a), and ProD2 (b)



Scheme 4-1: Mechanism of reaction of Ciprofloxacin amine's nucleophilic with the anhydride's carbonyl carbon



Scheme 4-2: Reaction of Ciprofloxacin with different anhydrides for the synthesis of Ciprofloxacin ProD1, and ProD2

4.1.1. Characterization of Ciprofloxacin standard

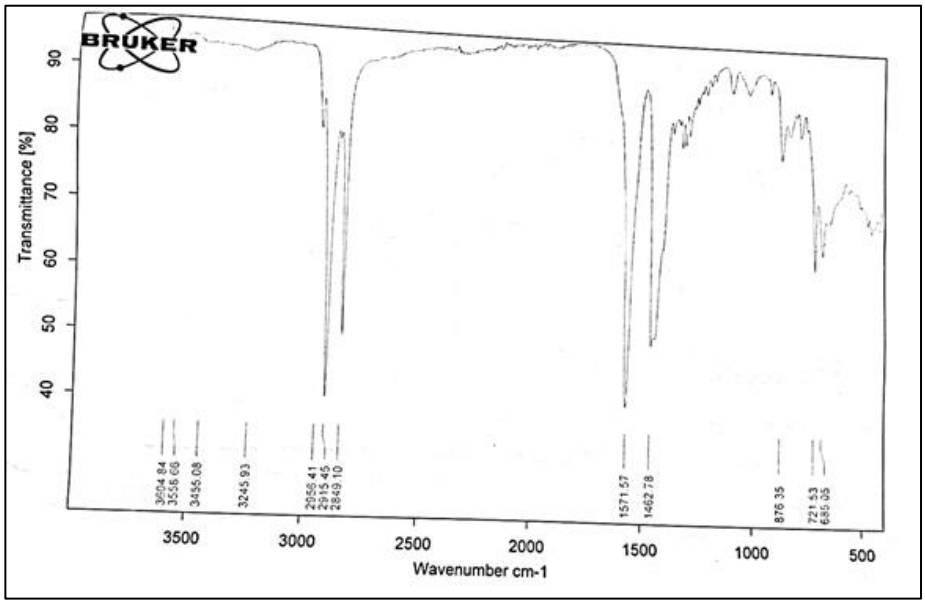
To enable a comparison between the reactant and product of the synthesis, the Ciprofloxacin standard, which was utilized in the prodrugs' synthesis, was characterized in the same way as the prodrugs. The melting point of Ciprofloxacin is 360℃. **Figure 4-2** show the chemical characterization of Ciprofloxacin. The FTIR spectra of Ciprofloxacin standard shows that the prominent characteristic peaks were found between 3500- 3450cm-1, which was assigned to the stretching vibration of the hydroxyl (OH) group and intermolecular hydrogen bonding. The possible peak of imino moiety of the piperazinyl group was less prominent due to intense O-H stretching vibration. Another band at 3000-2950cm-1 represented the alkene and aromatic C-H stretching, especially υ=C-H.

The range 1950 to 1450 cm^1 region exhibited FTIR absorption from a wide variety of double-bonded functional groups. The peak at 2900cm-1 was assigned to C-H stretching vibration of the cyclopropyl group. The region from 1750 to 1700cm-1 represented the carbonyl C=O stretching. The band at 1650 to 1600cm-1 was assigned to quinolones. The band at 1450 to 1400 cm-1 represented υC-O and at 1300 to 1250 cm-1 suggested bending vibration of the O-H group which proved the presence of carboxylic acid. A strong absorption peak between 1050 an d 1000 cm-1 was assigned to the C-F group **(Figure 4-2 a).** Ciprofloxacin was detected by UPLC-UV/Vis at a retention time of 2.39 minutes as the sample contained a mixture of the pure standard Ciprofloxacin with the synthesized prodrugs **(Figure 4-2 b).**

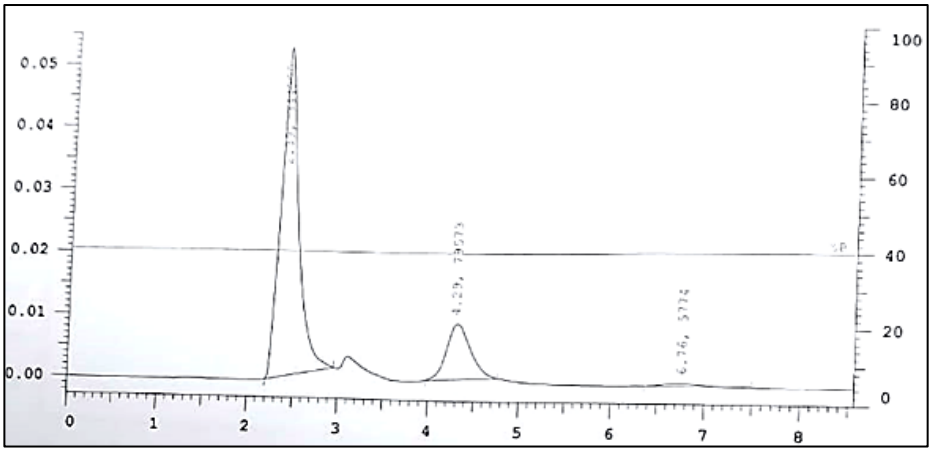
Ciprofloxacin: 1H-NMR (500 MHz, D2O) δ ppm: 1.16 (td, 2 H, $C\underline{H_2}CHCH_2$), 1.42 (td, 2H, $CH_2CHC\underline{H_2}$), 3.36 (d, 1H, $CH_2C\underline{H}CH_2$) 3.56 (m, 2H, $NC\underline{H_2}$), 3.61 (td, 2H, $HNC\underline{H_2}$), 7.19 (d, 1H, C=CC<u>H</u>) 7.37 (d, 1H, COC=<u>H</u>), 8.44(s, 1H, $NC\underline{H_2}$) **(Figure 4-2 c)**.

By comparing Ciprofloxacin characteristics with those of the newly synthesized prodrugs, it would appear to the reader that the synthesis reaction was successful in creating novel drugs.

a)



b)



c)

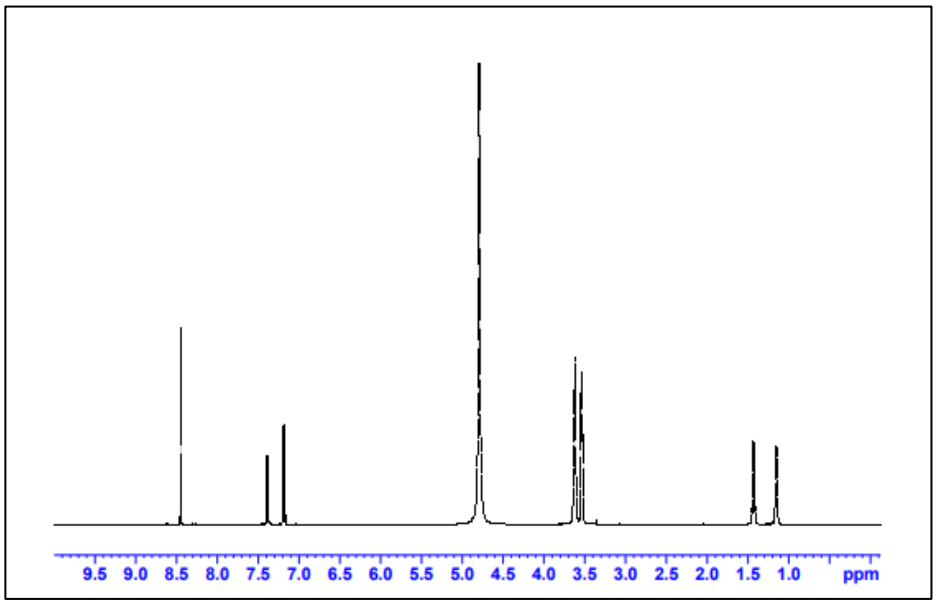


Figure 4-2: Ciprofloxacin chemical characterization. FT-IR spectrum of Ciprofloxacin (a). UPLC detection of Ciprofloxacin showing the peak of Ciprofloxacin at 2.39 minutes adjacent to prodrug peaks (b). 1H-NMR spectrum of Ciprofloxacin in D_2O).

4.1.2. Characterization of ProD1

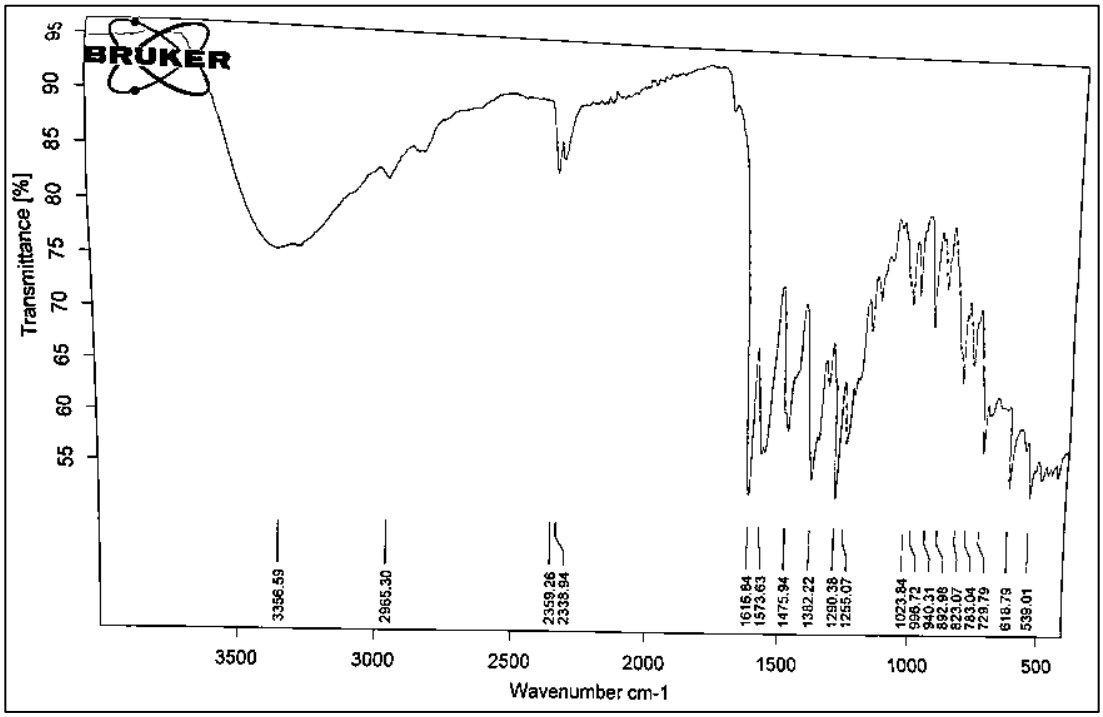
^{1}H-NMR (500 MHz, D2O) δ ppm: 1.16(td, 2 H, C$\underline{H_2}$CHCH_2), 1.328(td, 1H,CHC$\underline{H_2}$CH), 1.347(td, 1H, CHC$\underline{H_2}$CH), 3.003(dt,2H,C$\underline{H}$COOH), 3.187(dd,2H,C$\underline{H}$CHCOOH), 1.42 (td, 2H, CH_2CHC$\underline{H_2}$), 3.36 (d, 1H, CH_2C$\underline{H}$CH_2) 3.56 (m, 2H, NC$\underline{H_2}$), 3.61 (td, 2H, HNC$\underline{H_2}$), 6.103(td, 2H,C$\underline{H}$=C$\underline{H}$), 7.19 (d, 1H, C=CC$\underline{H}$) 7.37 (d, 1H, COC=$\underline{H}$), 8.44(s, 1H, NC$\underline{H_2}$) **(Figure 4-4)**.

Chemical characterization of **ProD1**, including FT-IR spectrum, and UPLC detection, are illustrated in **Figure 4-3**.

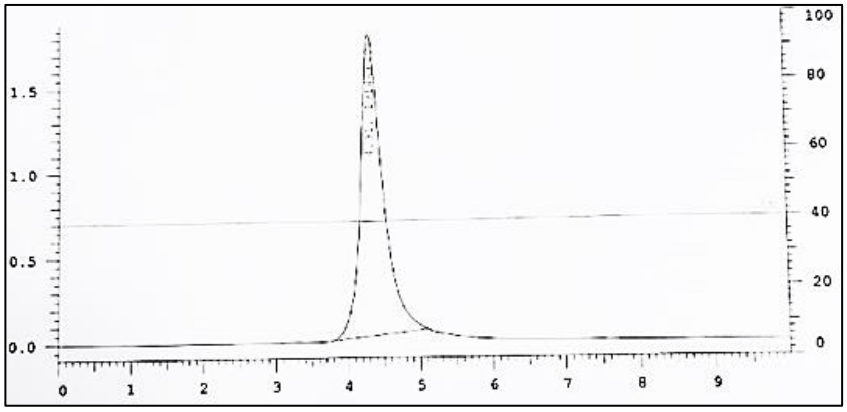
ProD1 was detected by UPLC at 4.52 minutes (**Figure 4-3 b**). The prodrug has longer retention times in RP chromatography compared to the parent drug (**Figure 4-3 c**), because of the formation of the amide bond.

(Figure 4-3 a) shows an absorbance band at 675-730 cm^{-1}) that represented the Cis alkene peak of the linker. Additionally, carbonyl absorption at the carboxylate stretch (1700 cm-1) is intensified because of the additional carbonyl group of the linker. The OH band appeared wider and stronger (3500- 3450cm-1) because of the moisture present in the analyzed sample. The new bond formed between the drug and the linker (tertiary amide) was difficult to detect via infrared spectroscopy. 1H-NMR confirmed the structure of **ProD1** as well, signals at 1.328, 1.347, 3.003, 3.187, and 6.103 *ppm* represent the five protons of the cis-5-Norbornene-endo-2,3-dicarboxylic linker.

a)



b)



c)

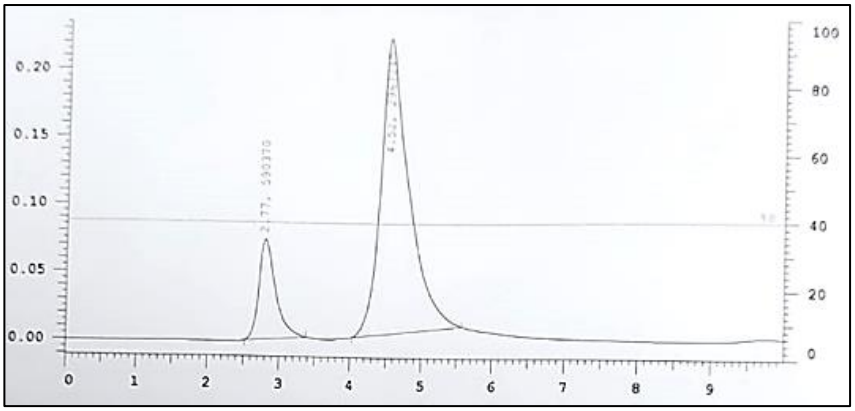
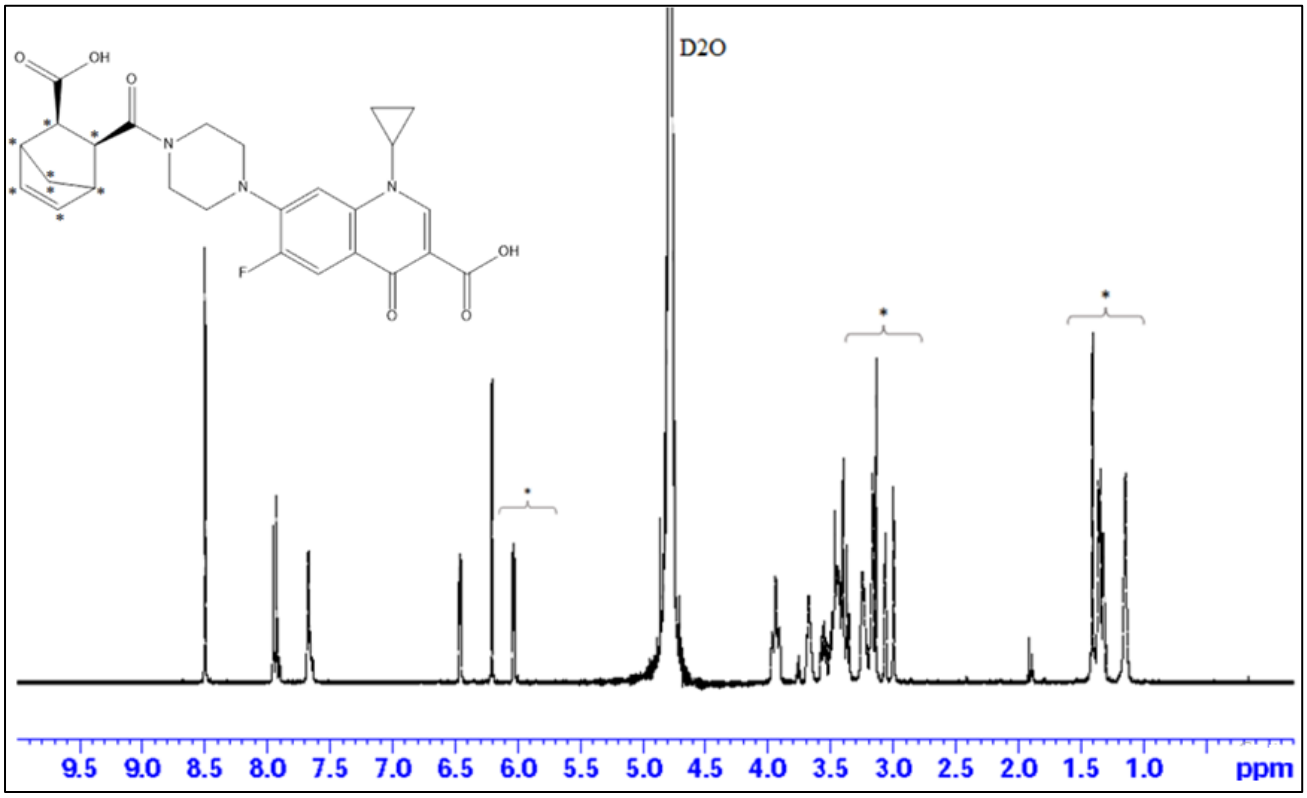


Figure 4-3: Chemical characterization of ProD1. FT-IR spectrum (a), and UPLC detection (b,c) of ProD1.



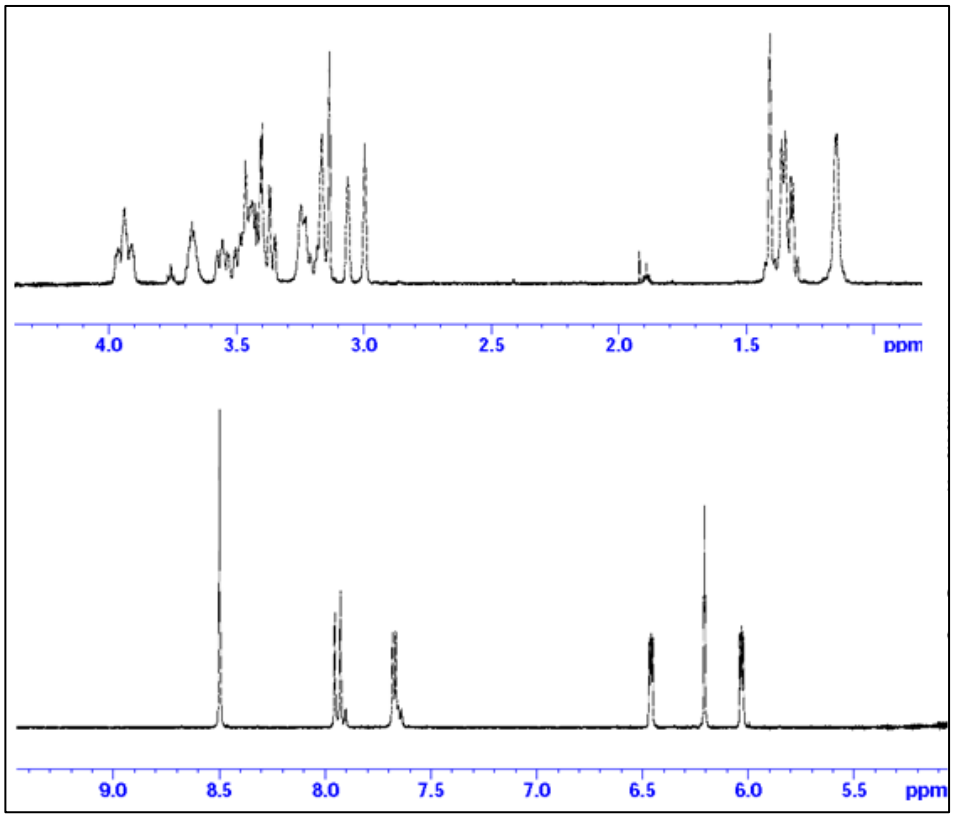


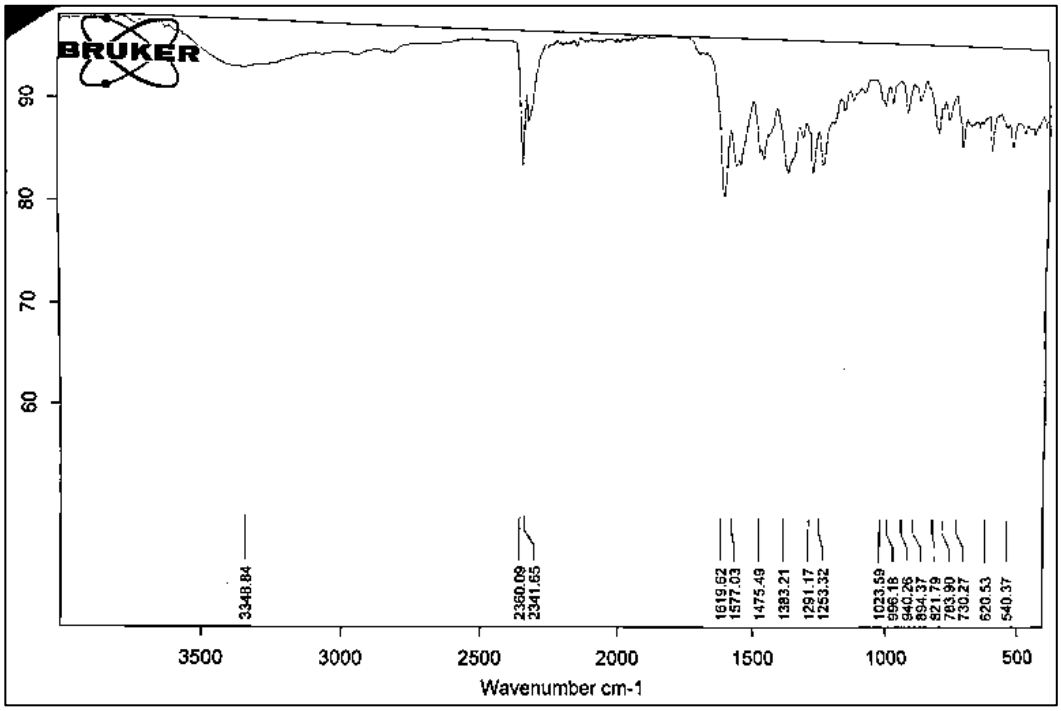
Figure 4-4: ^{1}H-NMR spectrum of ProD1 in D_2O. The protons of the cis-5-Norbornene-endo-2, 3-dicarboxylic linker were detected at 1.328, 1.347, 3.003, 3.187, and 6.103 *ppm*.

4.1.3. Characterization of ProD2

ProD2: ^{1}H-NMR (500 MHz, D2O) δ ppm: 1.153 (td, 2 H, C$\underline{H_2}$CHCH$_2$), 1.341 (td, 2H, CH$_2$CHC$\underline{H_2}$), 1.890(m,3H, C$\underline{H_3}$) 3.082(m, 2H, NC$\underline{H_2}$), 3.299(td, 2H, HNC$\underline{H_2}$), 3.6 (d, 1H, C=CC$\underline{H}$) (d, 1H, CH$_2$C$\underline{H}$CH$_2$) 7.66(d, 1H, COC=C$\underline{H}$),7.928(q,3H,C$\underline{H_3}$) 8.5(s, 1H, NC$\underline{H_2}$) (**Figure 4-6**). 1H-NMR confirmed the successful synthesis of proD2, in which new chemical shifts appeared in the spectrum representing two types of protons of the dimethyl maleic linker that can be identified at 1.890 and 7.928 ppm.

FT-IR characterization of **ProD2** (**Figure 4-5 a**) expressed the absorbance band of the carbonyl stretching at 1557 cm^{-1} , and the hydroxyl group band appeared wider and stronger around 3351 cm-1 due to the presence of other OH bonds (acid OH of the linker). **ProD2** was detected by UPLC at a retention time of 4.3 minutes when injected with and without Ciprofloxacin (**Figure 4-5 b**) (**Figure 4-5 c**).

a)



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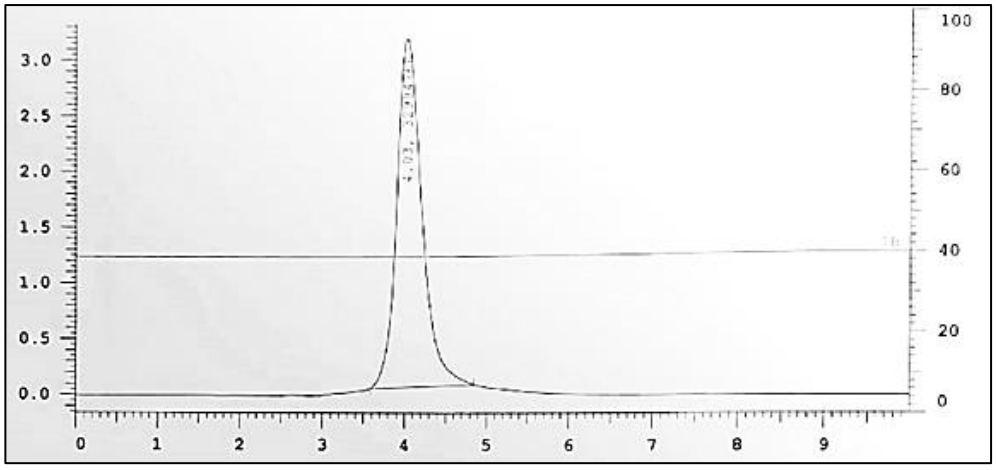


c)

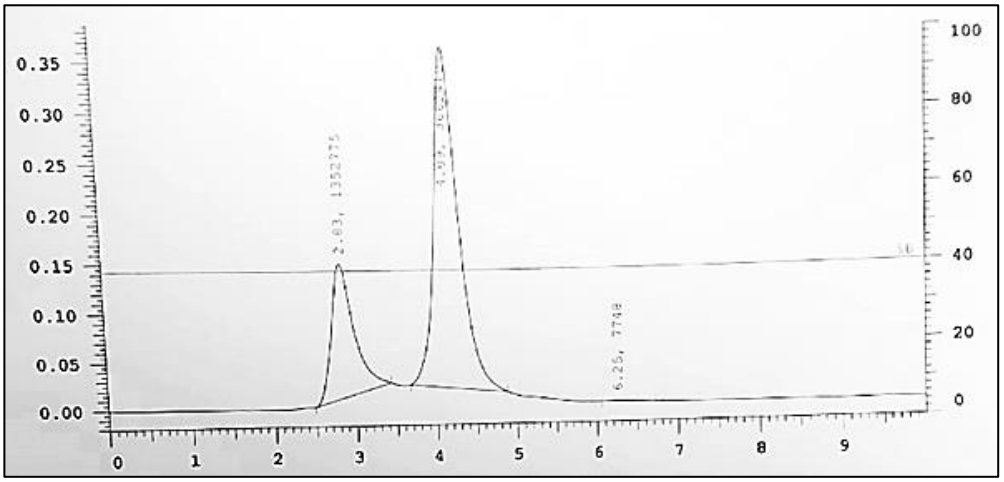


Figure 4-5: Chemical characterization of ProD2. FT-IR spectrum (a), and UPLC detection (b/c) of ProD2

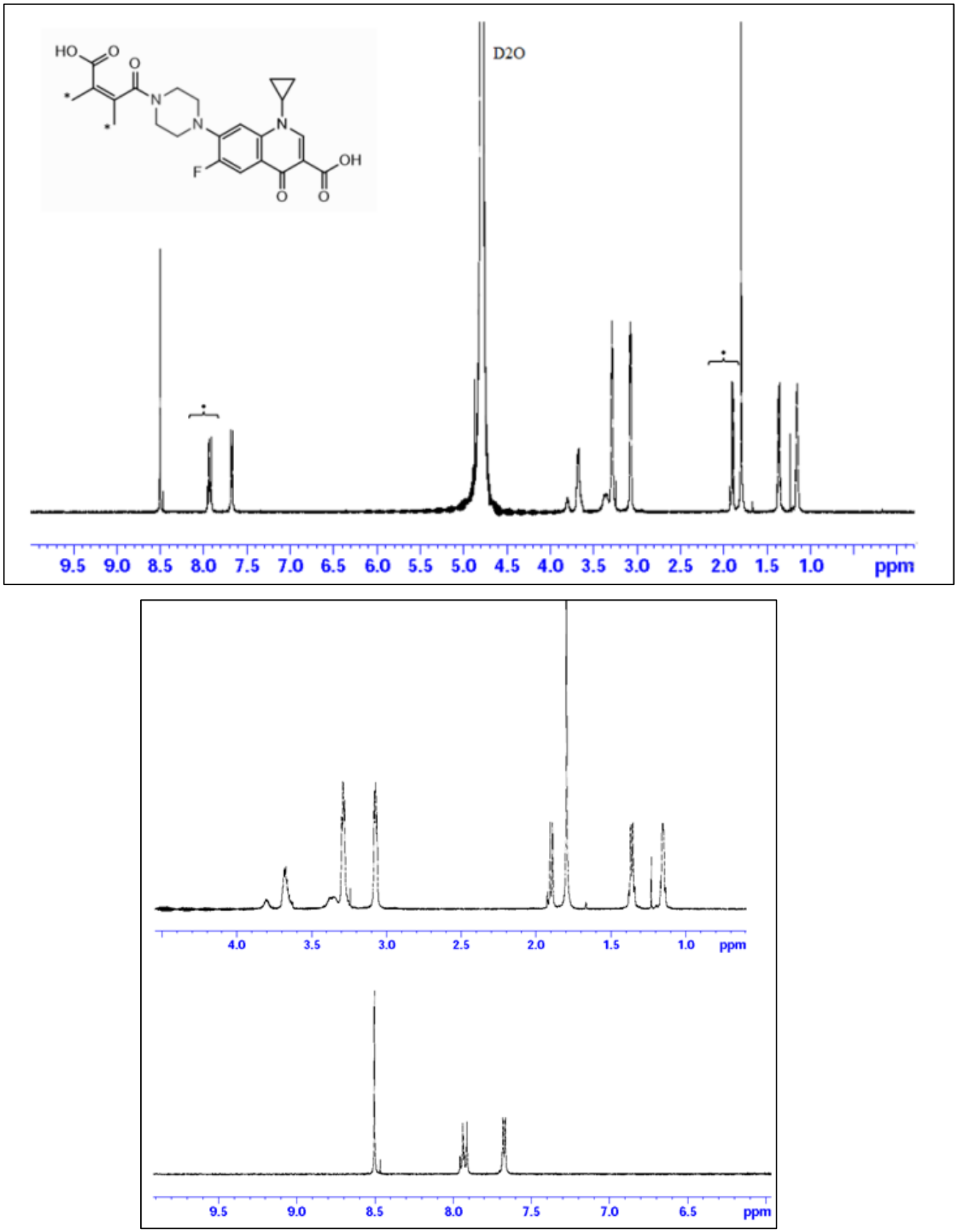


Figure 4-6: ^{1}H-NMR spectrum of ProD2 in D_2O

Part two

Results and discussion of the microbiology part

4.2.Screening of drug and prodrugs inhibition of bacteria showing the mean zone of inhibition diameter in mm

Table 4-1: Mean zone of inhibition diameter in mm

Drug(30 mcg)/Bacterial strains inhibition zone(mm)	*S. aureus*	*Pseudomonas*	*E.coli*
Ciprofloxacin	No inhibition zone	33.93	37.93
ProD1	No inhibition zone	36.38	40
ProD2	No inhibition zone	34.07	40.27

Novel Ciprofloxacin prodrugs were created because, as previously mentioned, chemical alteration to the piperazine group can alter the chemical characteristics and the activity spectrum of quinolone medicines.

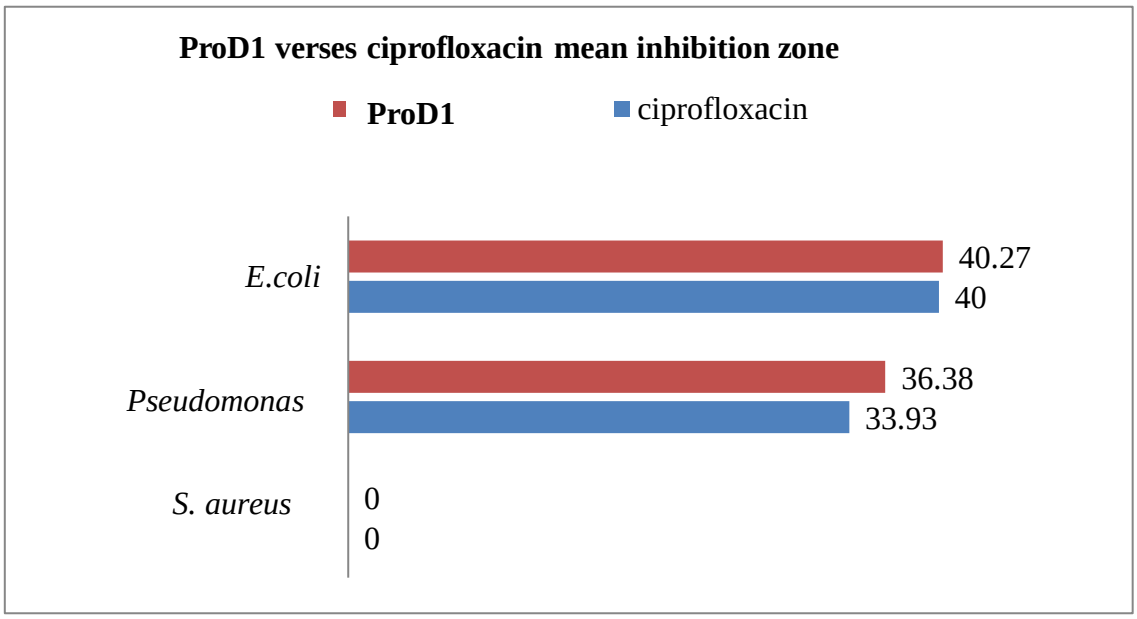
The antibacterial activity and bacterial spectrum of the synthesized prodrugs of Ciprofloxacin are examined in this section.

Using a two-sample t-test model to calculate the *p*-values for the antimicrobial agents, it was discovered that there is evidence of a statistically significant difference between the mean inhibition zones of Ciprofloxacin and **ProD1** against *Pseudomonas* bacteria (*p*-value 0.024) and that there is a significant difference between the mean inhibition zones of Ciprofloxacin and **ProD1** against *Pseudomonas* bacteria.

Additionally, there was a significant difference between the inhibition zones produced by Ciprofloxacin and **ProD2** against *E. coli* bacteria (*p* 0.05), but there is insufficient data to conclude that there was a significant difference between the inhibition zones produced by Ciprofloxacin and **ProD2** against *pseudomonas* bacteria (*p*-value > 0.05).

Followings are **Figure 4-7**, and **Figure 4-8** that illustrates the correlations of Ciprofloxacin and the newly synthesized prodrugs. *S. aureus* strain exhibited resistance to Ciprofloxacin and its prodrugs at the specified concentration, although it did exhibit inhibitory activity at higher concentrations of the antibiotic and its prodrugs **(figure 4-9).**

a)



b)

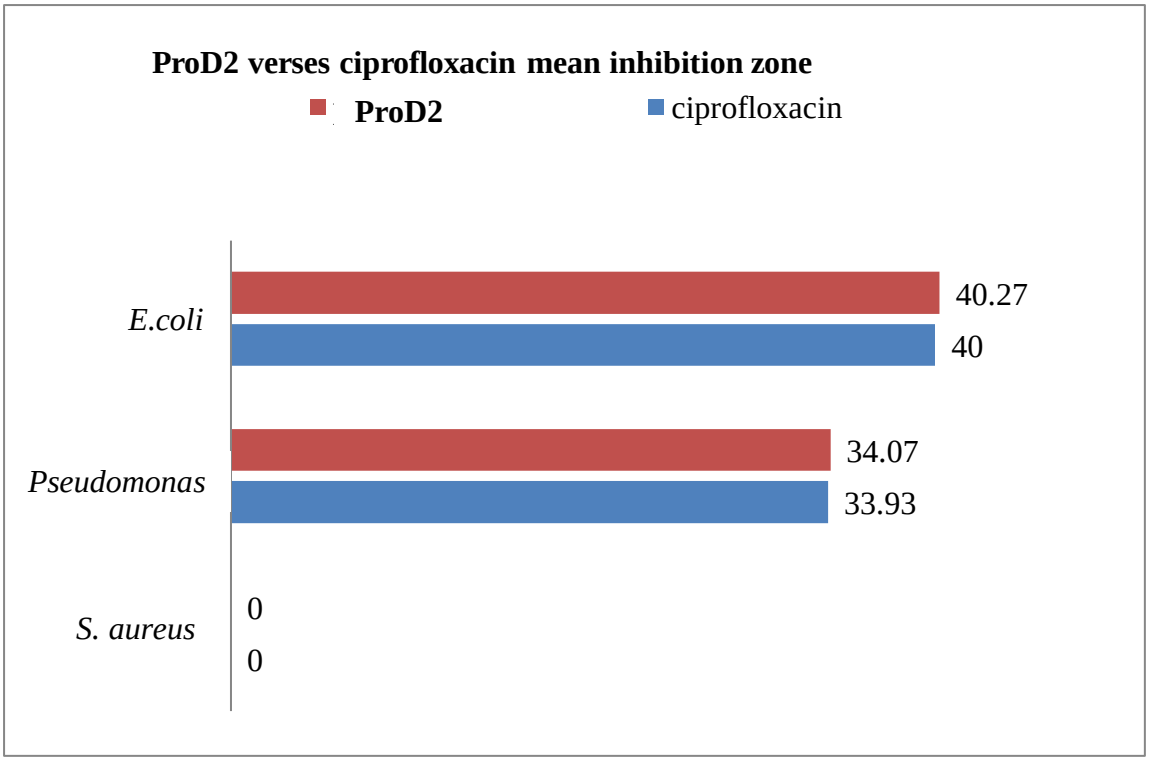
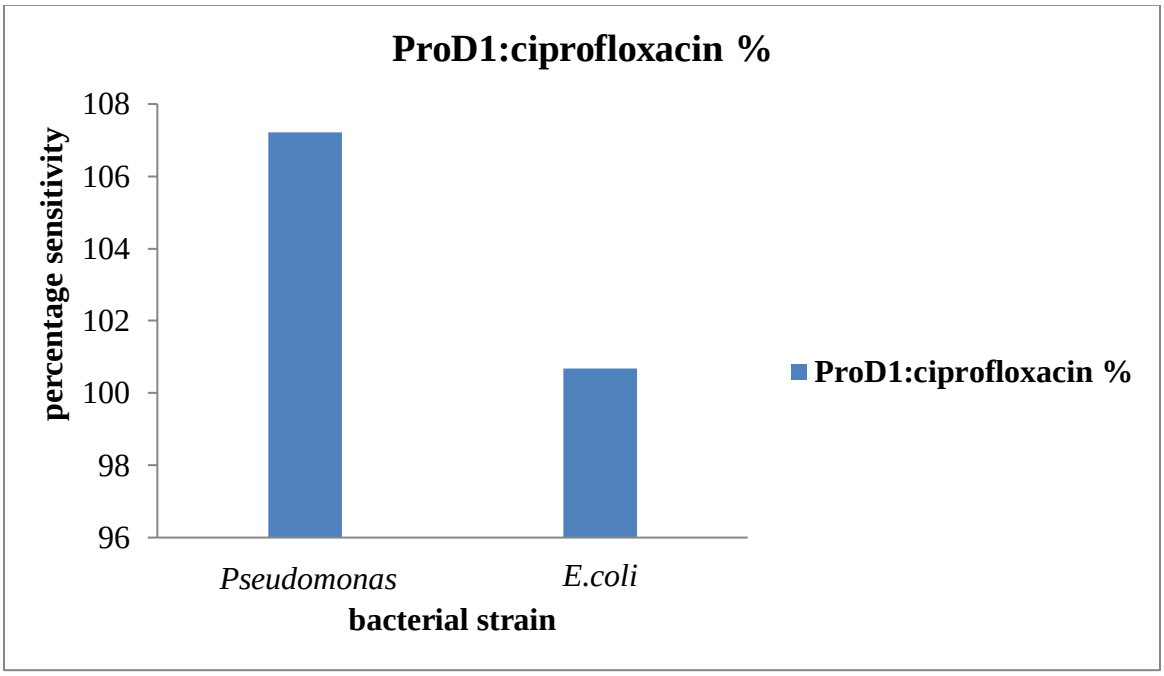


Figure 4-7: Antibacterial activity of drug and prodrugs of Ciprofloxacin against bacterial strains: a) Antibacterial activity of Ciprofloxacin and ProD1 against bacterial strains; b) Antibacterial activity of Ciprofloxacin and ProD2 against bacterial strains

a)



b)

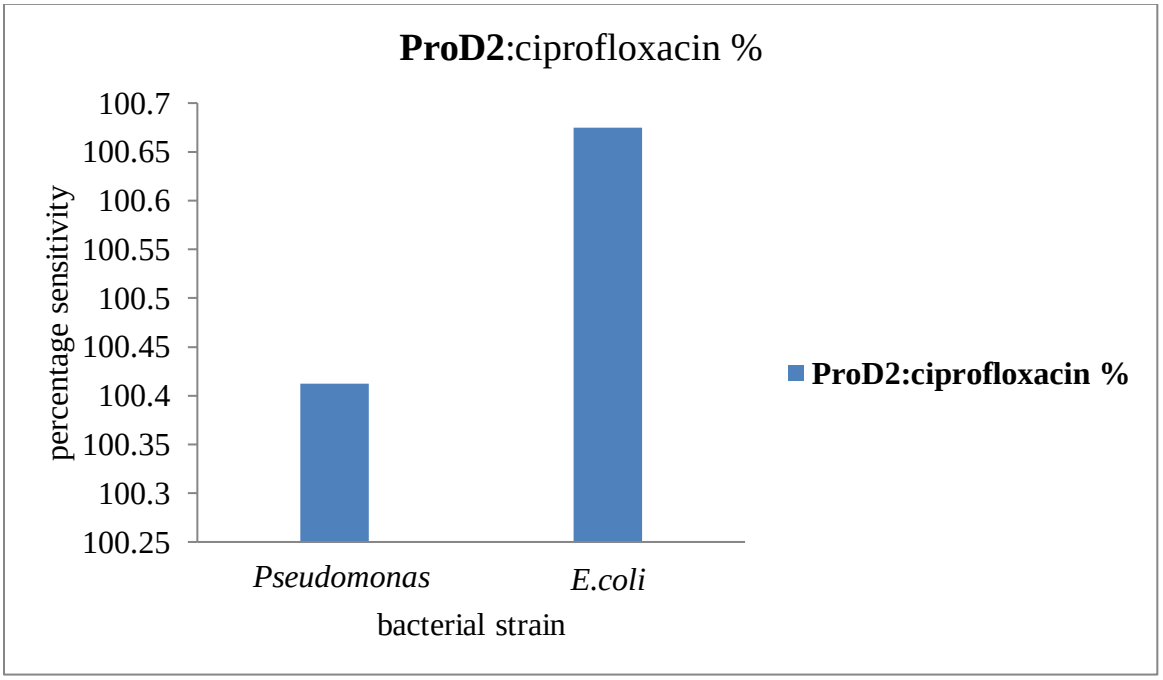


Figure 4-8: Percentage of the prodrug to drug of Ciprofloxacin against bacteria: a) Percentage of ProD1 to drug of Ciprofloxacin against bacteria; b) Percentage of ProD2 to drug of Ciprofloxacin against bacteria.

a)  b) 

Figure 4-9: a) Zone of inhibition in mm determined for each agents against *staphylococcus aureus*: (1) Ciprofloxacin drug, (2) ProD1, (3) ProD2, (5) negative control (buffer).

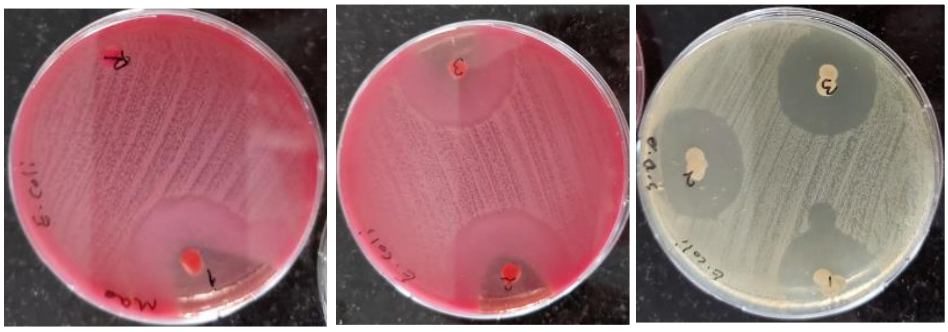


Figure 4-10: Zone of inhibition in mm determined for each agent against *E.coli*: (R) Ciprofloxacin drug, (1) ProD1, (2) ProD2.

Figure 4-11: Zone of inhibition in mm determined for each agent against *Pseudomonas*: (1) Ciprofloxacin drug, (2) ProD1, (3) ProD2, and (5) negative control (buffer).

4.3. Finding MIC values

Table 4-2: MIC and MBC values for different bacterial strains

Bacterial strains/Drug	**Ciprofloxacin**		**ProD1**		**ProD2**	
concentration(mcg/ml)	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
Pseudomonus	7.03	No inhibition	7.03	No inhibition	17.5	1.875
E.coli	8.75	No inhibition	8.75	15	27.66	No inhibition

The MIC and MBC values for Ciprofloxacin and its prodrugs were calculated and compared in this study.

The Kruskal-Walli's test, a non-parametric test that compares the medians of various independent groups, is frequently employed for this type of data in order to obtain the P-value for MIC of the antibiotics.

The lowest inhibitory concentration of Ciprofloxacin and two different prodrugs antibiotics against *pseudomonas* bacteria was compared using the Kruskal-Walli's test, and *p*-value of approximately 0.018, using a significance level of 0.05. This shows that the median minimum inhibitory concentrations of the four medicines against *pseudomonas* varied significantly from one another.

The MIC data for Ciprofloxacin and its prodrugs against *E. coli* would be examined using a one-way ANOVA test, and the resulting p-value of 0.005 is less than the significance level of 0.05, which indicates that there is a statistically significant difference between the MIC values of Ciprofloxacin, **ProD1**, and **ProD2** against *E.coli*.

S. aureus showed no inhibition at all indicating that the concentration used to conduct the research was too low. *Pseudomonus* and *E. coli* were susceptible to Ciprofloxacin, **ProD1**, and **ProD2**, however, they were only effective in inhibiting their growth, and higher concentrations are required for killing the bacteria.

In conclusion, the MIC study and the Kirby Bauer method study both offer important details on the antibacterial activity of Ciprofloxacin and its prodrugs against *Pseudomonas* and *E. coli*. The Kirby Bauer method investigation reveals statistically significant variations in the mean inhibition zones between Ciprofloxacin and its prodrugs against both *Pseudomonas* and *E. coli*, demonstrating that the prodrugs have a different antimicrobial activity spectrum from Ciprofloxacin. This conclusion is further supported by the MIC analysis, which demonstrates that there are no variations in the minimum inhibitory concentrations of the two new antibiotics against *Pseudomonas* and *E. coli*. According to these findings, the newly synthesized prodrugs may be useful as antibacterial agents against *Pseudomonas* and *E. coli*.

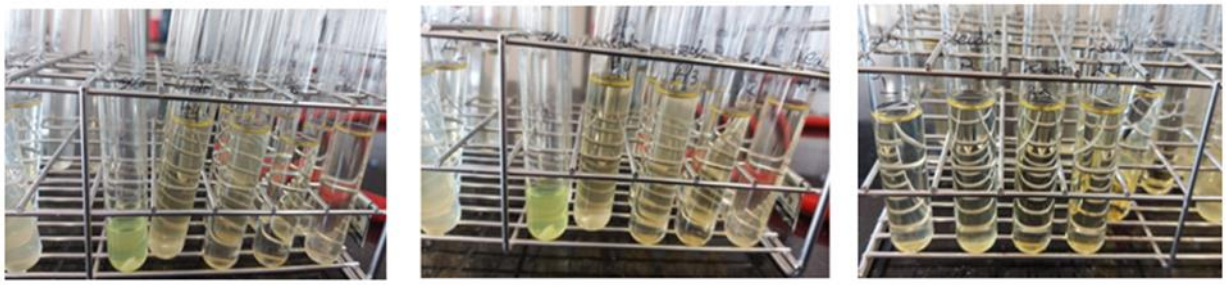


Figure 4-12: testing different prodrugs on *S. aureus, Pseudomonas, and E.coli* using four different concentrations to determine the MIC and MBC.

Chapter Five

Conclusion and Future Direction

Chapter five:

5. Conclusion and future direction

This thesis describes the synthesis of two novel Ciprofloxacin amide prodrugs, namely **ProD1** and **ProD2**, with the aim of addressing the growing bacterial resistance to Ciprofloxacin. The prodrugs were synthesized using the prodrug linkers Cis-5-Norbornene-endo-2,3-dicarboxylic anhydride and di-methyl-maleic anhydride respectively, resulting in the successful production of stable compounds. To establish their identity, the prodrugs characterization was carried out using melting point determination, UPLC, FT-IR, and ^{1}H-NMR analysis.

To assess their effectiveness, the *in vitro* susceptibility of both prodrugs was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas*, comparing them to the parent drug Ciprofloxacin. This assessment was conducted using the disc diffusion method and broth diffusion method. The results clearly demonstrated that both novel prodrugs exhibited superior efficacy compared to the parent drug in combating *pseudomonas* and *E. coli* infections. Furthermore, at higher doses, they also demonstrated effectiveness against *Staphylococcus aureus*. Notably, these novel prodrugs are stable compounds capable of exerting their antibiotic effect without the need for conversion into their corresponding parent drugs.

To further evaluate the prodrugs' effectiveness, future research should focus on assessing their performance *in vivo* and testing them against various bacterial strains.

It is advised to study intraconversion of the prodrugs *in vitro* in various pH media and blood plasma and Future research on the kinetics of prodrug absorption and conversion needs to be done *in vivo*.

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تصنيع ودراسة مواصفات طلائع الأدوية لمركب السيبروفلوكساسين بالإضافة لدراسة الفعاليّة المضّادة للبكتيريا

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الملخص

يعد اكتشاف مضادات حيوية جديدة أمرًا بالغ الأهمية و حاجة ملحة، في ضوء المشكلة المتزايدة لمقاومة المضادات الحيوية المتوفرة. حيث أن لمقاومة البكتيريا للمضادات الحيوية المتواجدة تبعات واسعة النطاق، بما في ذلك احتمالية حدوث عدوى طويلة الأمد وأكثر شدة، وارتفاع معدلات المرض والوفيات، وتزايد تكاليف الرعاية الصحية. وفي بعض الحالات، يمكن أن تصبح العدوى الناجمة عن البكتيريا المقاومة للمضادات الحيوية لا يمكن علاجها، مما لا يترك خيارات متاحة لعلاج فعال. وهذا يشكل تهديداً كبيراً للصحة العامة، حيث تصبح إدارة العدوى البكتيرية الشائعة أكثر تحديًا ويزيد خطر انتشار سلالات مقاومة للمضادات الحيوية إلى الآخرين.

في سوق وصناعة المضادات الحيوية، يواجه تطوير المضادات الحيوية الجديدة تحديات كبيرة. أصبحت عملية إطلاق مضاد حيوي جديد في السوق أكثر بطئًا وتعقيدًا، مع متوسط فترة زمنية تستغرق عقدًا من الزمان أو أكثر.

ارتفاع مقاومة المضادات الحيوية قد استدعى إلى البحث المستمر عن حلول جديدة لمكافحة الأمراض المعدية بفعالية، وأصبحت الطرق التقليدية لاكتشاف المضادات الحيوية قد استنفذت معظم إمكاناتها. على الرغم من هذه العقبات، لا تزال هناك فرص لاكتشاف مضادات حيوية جديدة باستخدام الكيمياء الدوائية والكيمياء الحاسوبية لإنشاء طلائع مضادات حيوية جديدة، حيث تم استخدام استراتيجية تصنع الطلائع الدوائية لتحسين بعض عيوب العقاقير الأصلية.

في هذه الأطروحة، تم تخليق اثنان من طلائع الأميد لدواء سيبروفلوكساسين المستخدم كمضاد بكتيري باستخدام cis-5-Norbornene-Endo-2,3-Diarboxylic Anhydride, and di-methyl-Maleic Anhydride عن طريق nucleophilic acyl substitution ، وتم إظهار مزاياهما المحتملة في مكافحة البكتيريا مقارنة بالمركب الأم. أثناء قراءتك للعمل، ستجد توصيفًا دقيقا يشرح طريقة تخليق هذه المركبات الدوائية، بالاضافة إلى توصيفٍ لهذه المركبات عن طريق تحليلها باستخدام بعض الفحوصات المعياريةفي الابحاث مثل درجة الانصهار و تقنيات الفصل اللوني (الفصل الكروماتوغرافي) مثل UPLCو FT-IR و ^{1}H-NMR وتم الحصول على المنتجات النهائية للطلائع بشكل مسحوقي بعائد عالٍ.

تم تحديد الحساسية في المختبر لكلا المشتقتين بالنسبة لثلاث أنواع من البكتيريا:

Escherichia coli, *Staphylococcus aureus*, and *Pseudomonas*

ومقارنتها بتلك للمركب الأم سيبروفلوكساسين، باستخدام طريقتي:

Disc diffusion method and broth diffusion method

. أظهرت النتائج أن كلا المشـتقتين الجديدين تفوقا بفاعلية على المركب الأم في مكافحة العدوى *Escherichia coli* و*Pseudomonas* . علاوة على ذلـك، عنـدمـا تم إعطـاؤهـا بجرعـات أعلى، أظهرت فعـاليـة أيضًـــا ضـــد *Staphylococcus aureus*. يجدر بالذكر أن هذه الطلائع الجديدة هي مركبات مسـتقرة، حيث يظهر تأثير المضـاد الحيوي بذاته دون الحاجة إلى التحول إلى المركبات الأم.

عمادة الدراسات العليا

جامعة القدس

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المشرف الرئيسي: بروفيسور رفيق قرمان

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الصيدلانية من كلية الدراسات العليا جامعة القدس- فلسطين.

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