An *In Silico* Pharmacological And Molecular Docking Approach For The Development Of Potential Inhibitors Against CTX-M-9 Class A Beta-Lactamase From *Escherichia Coli*.

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Date of Submission: May 27, 2024

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A RESEARCH PROJECT

BY

JOHIRUL ISLAM

EXAM. ROLL NO.: ASH1913010M

SESSION: 2018-2019

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SUBMITTED TO

THE DEPARTMENT OF BIOTECHNOLOGY AND GENETIC ENGINEERING, NOAKHALI SCIENCE AND TECHNOLOGY UNIVERSITY, NOAKHALI-3814, BANGLADESH.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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CERTIFICATE OF APPROVAL

This is to certify that the project entitled **"*An In Silico Pharmacological And Molecular Docking Approach For The Development Of Potential Inhibitors Against CTX-M-9 Class A Beta- Lactamase From Escherichia Coli.*"** submitted in partial fulfillment of the requirements for the degree of Bachelor of Science(B.Sc.) in the Department of Biotechnology & Genetic Engineering, Noakhali Science & Technology University is a faithful record of the bona fide research work carried out by Johirul Islam, Examination Roll No.: ASH1913010M & Session: 2018-19 under my guidance & supervision. The style & the content of the project have been approved. No part of the work presented in the project paper has not been submitted for any other degree or qualifications.

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The Author

May, 2024

DECLARATION

I do hereby declare that the whole research presented as a project titled **"*An In Silico Pharmacological And Molecular Docking Approach For The Development Of Potential Inhibitors Against CTX-M-9 Class A Beta- Lactamase From Escherichia Coli.*"** submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (B.Sc.) in the Department of Biotechnology & Genetic Engineering, Noakhali Science and Technology University (NSTU). This research has been carried out by computer based software in the Bioinformatics & Genetic Engineering Laboratories, NSTU. No part of the work presented in the project paper has not been submitted for any other degree or qualification in any university or other institutes of learning.

……

Johirul Islam

B.Sc. (Hon's) in Biotechnology and Genetic Engineering

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ADMET	Absorption, Distribution, Metabolism, Excretion And Toxicity
AMR	Antimicrobial Resistance
BBB	Blood-Brain Barrier
CASTP	Computer Atlas Of Surface Topology Of Proteins
CADD	Computer-Aided Drug Design
CDC	Centers For Disease Control And Prevention
CNS	Central Nervous System
CTX	Tocefotaxime
DNA	Deoxy Ribonucleic Acid
ESBLS	Extended Spectrum Beta-Lactamases
ECDC	European Center For Disease Prevention And Control
E. coli	*Escherichia coli*
ETEC	Enterotoxigenic *E. coli*
EPEC	Enteropathogenic *E. coli*
Fu	Fraction Unbound
GLASS	Global Antimicrobial Resistance And Use Surveillance System
MDR	Multidrug Resistant
MSSA	*Methicillin Susceptible Staphylococcus aureus*
NDM	New Delhi MBL
NMR	Nuclear Magnetic Resonance Spectroscopy
PBP	Penicillin Binding Protein
pI	Isoelectric Point
PDB	Protein Data Bank
RNA	Ribonucleic Acid
TB	Tuberculosis
UTIS	Urinary Tract Infections
VIMS	Verona Integron Encoded Mbls
VRE	Vancomycin Resistant Enterococcus
WHO	World Health Organization
3D	Three-Dimensional

Abstract

Public health is greatly impacted by a variety of bacterial diseases. Diverse pathotypes of *Escherichia coli* strains are becoming more and more acknowledged as a serious threat to public health. Pathogenic *E. Coli* infections have been effectively treated with β-lactam antibiotics. However, a multitude of hydrolytic enzymes, known as β-lactamases secreted by bacteria, are currently seriously undermining the usefulness of β-lactams. *E. coli* produces ESBLs which are capable of breaking down extended spectrum beta-lactams such as penicillins, cephalosporins, monobactams, and carbapenems antibiotics and giving the bacteria tolerance to them. Although more than 150 different TEM- and SHV- type ESBLs are known, the CTX-M enzymes have been recognized as the most prevalent among Enterobacteriaceae. In fact, *E. coli* was the first species in which the CTX-M type ESBLs was found. One of the many beta-lactamases found in *E. coli*, CTX-M-9, is capable of breaking down monobactam and cephalosporin antibiotics and giving the bacteria tolerance to them. Hence, it is of crying need to develop alternative therapies, by exploiting novel targets, which should be devoid of the problems arising with currently available drugs. Computer-aided drug design could help to develop novel effective drugs against the infection. In this study we opted CTX-M-9 class A betalactamse enzyme of E. coli to devise novel inhibitors of the enzyme. Structure of CTX-M-9 of *E.coli* was collected from protein data bank server (PDB: 3G30). Homology modeling of CTX-M-9 was performed by Phyre2 web server and refined by Galaxy refiner. Structure assessment was done by using SWISS-MODEL through observing Ramachandran plot where 90.7% residues located in most favored regions. Active site was determined by CASTp server. For docking 500 ligands compound were selected from ZINC15 database and performed blind docking by PyRx software. Protein prepared by Discovery Studio and Swiss-PDB viewer. Ligands were prepared by Open Babel. The best ten ligands predicted to interact with protein have been selected according to their binding energy and ADEM/T analysis. The binding affinity of the ligands ranges between -9.8 Kcal/mol to (ligand-1:ZINC000811782179) to -8.9 Kcal/mol (ligand-9 ZINC000574208417), where ligand-1 showed the maximum and ligand-9 showed minimum affinity. With the exception of ligand-6, which did not exhibit p-glycoprotein II inhibitor, all of the ligands demonstrated hepatotoxicity and were inhibitors of p-glycoprotein I, p-glycoprotein II, and hERG II. The remaining ligands displayed p-glycoprotein substrates, but ligands 3, 6, and 10 did not. Eight ligands showed evidence of a hERG I inhibitor, whereas nine ligands did not. There were no ligands that exhibited skin sensitivity or AMES toxicity. The solubility of ligands 9 and 10 is low in water, with a moderate solubility remaining and high Caco2 permeability. But because of its lower overall clearing value, it is less suitable for speedy removal. With the exception of ligand-3, 6, and 10, all of the ligands are associated with lower toxicity and are safe based on the lethal

dose for 50% of the population (LD50). Oral Rat Chronic Toxicity is the source of this data (LOAEL). Therefore, these ligands have the potential to be employed as drugs to treat E. coli infection with some extent of modification.

Keywords: *E. coli*; CTX-M-9; ESBLs; Molecular Docking; ADME/T prediction

Chapter One
INTRODUCTI

1.1 General overview on bacteria and their effect on public health

Bacteria are single-celled, tiny microorganisms. They are some of the planet's oldest known life forms. Bacteria come in millions of varieties and inhabit every imaginable type of environment on the planet. They inhabit the earth's crust, the sea, and the soil [1]. Most bacteria don't cause harm. As a matter of fact, our skin and internal organs are teeming with microorganisms. Our intestines' (gut's) bacteria aid in food digestion. Yet some bacteria are capable of infecting people. By creating noxious compounds (toxins), infiltrating tissues, or doing both, bacteria can lead to disease. Certain bacteria have the ability to cause inflammation, which can impact the gastrointestinal tract, kidneys, neurological system, heart, or lungs. There are certain bacteria that raise the risk of cancer, like *Helicobacter pylori*. There is a chance that some germs could be employed as biological weapons. The germs responsible for anthrax, botulism, plague, and tularemia are among them [1, 2]. While most illnesses are not serious, some are [2].

A strange incident occurred in May 2017 at a Vancouver, British Columbia aquarium when a sea lion dragged a young girl into the water; the video quickly gained popularity on social media. Officials from the Aquarium speculate that the girl may have caught "Seal Finger," a rare bacterial illness. The oral cavity of marine mammals is home to a specific kind of bacteria known as Mycoplasma bacteria. The girl who was bitten by the sea lion in the Aquarium incident became infected with bacteria from the bite. The individual who has this condition may lose fingers or possibly entire limbs if treatment is not received. Thus, one of the most important scientific and medical concerns of our day is the widespread issue of infectious and fatal diseases brought on by bacteria [3].

The public's health is greatly impacted by these bacterial diseases. Since there is a larger variety of antimicrobial medicines with effectiveness against bacteria, treating bacterial infections is typically easier than treating viral infections. In contrast to infectious diseases resulting from viruses and parasites, antibiotic resistance in bacteria is an issue that is expanding quickly and has the potential to be extremely harmful. Additionally, microorganisms are becoming increasingly resistant to antibiotics these days [4].

1.2 Antibiotics, effect of antibiotics and it’s classification

1.2.1 Antibiotics

The word "antibiosis," which literally translates to "against life," is where the term "antibiotic" originated [5]. An antibiotic is a medication that either stops or kills bacterial growth. Antibiotics are one class of antimicrobials, a wider group which also includes anti-viral, anti-fungal, and anti-parasitic medications. Antibiotics are substances produced by or obtained from microorganisms (i.e. bugs or germs such as bacteria and fungus). In a major development for medical science, Alexander Fleming created the first antibiotic in 1928. In contemporary medicine, antibiotics are among the most commonly given drugs [6].

Certain antibiotics function by destroying bacteria; this is known as their "bactericidal" nature. Certain antibiotics function by preventing the growth of germs, a property known as "bacteriostatic action." Different bacteria are affected differently by each type of antibiotic. An antibiotic, for instance, may prevent a bacterium from using glucose as fuel or from building its cell wall. In this scenario, the bacterium perishes rather than proliferates. 'Broad-spectrum' antibiotics are those that have the ability to treat a variety of infections. 'Narrow-spectrum' antibiotics are those that work only against specific kinds of bacteria [6].

1.2.2 Effect of Antibiotic

Since the beginning of time, man and the microbial world have coexisted. While certain bacteria may coexist peacefully with humans and even work as resident microorganisms to stabilize and protect the body, harmful Bacteria enter and multiply in human tissues, resulting in illnesses and bodily damage that can occasionally be fatal. Antibiotics are a very efficacious treatment for bacterial infections and have the potential to literally save lives. Like any medications, they could have unfavorable side effects, though. Although many of these side effects are not harmful, they can make life unpleasant while the medication is being used. Antibiotics often don't have many major negative effects. The most typical antibiotic side effects include nausea, vomiting, and diarrhea. Antibiotics can also induce fungal infections in the mouth, digestive tract, and vagina because they kill the body's "good" flora, which helps prevent the proliferation of both the "bad" organisms (any one organism) and the one causing the infection need to be treated. Some people are allergic to antibiotics, particularly penicillins. Allergic reactions cause swelling of the face, itching and a skin rash and, in severe cases, breathing difficulties. Allergic reactions require prompt treatment [5, 6].

1.2.3 Classification of antibiotics

There are many different kinds of antibiotics. The type of antibiotics depends on the type of infection one have and what kind of antibiotics are known to be effective [6]. Some common classes of antibiotics based on chemical or molecular structures include:

- Aminoglycosides
- Macrolides
- Beta-lactams
- Fluoroquinolones
- Sulphonamides
- Tetracyclines
- Glycopeptides [5].

1.2.3.1 Aminoglycosides

Gram-negative bacterial infections are treated with aminoglycoside medicines. Streptomycin was the first medication in this class of antibiotics to be identified; it was originally isolated in 1943. Streptomycin has been extensively employed in the fight against Mycobacterium tuberculosis, the human tuberculosis causative agent. Compounds comprising typically three amino sugars joined by glycosidic linkages are known as aminoglycosides. They come from Actimomycetes found in soil. The scope of aminoglycoside's antibacterial activity is vast. They work against aerobic Gram-negative rods and some Gram-positive bacteria, and they can stop the synthesis of proteins in bacteria by attaching to one of the ribosomal subunits [6, 7].

1.2.3.2 Macrolides

Azithromycin and Clarithromycin are two new cousins of erythromycin that function in the same way, killing more bacteria and causing marginally fewer adverse effects. The term "macrolides" is another name for the erythromycin-like antibiotics. Macrolides are natural compounds that fall within the polyketide class. The purpose of macrolide antibiotics is to treat infections of the respiratory system, genitalia, gastrointestinal system, and soft tissues brought on by virulent strains of certain bacteria. Macrolides bind to vulnerable bacteria's ribosomes to stop them from producing proteins. At high concentrations, this activity can also be bactericidal, but its primary action is bacteriostatic. Compared to cephalosporins and penicillins, macrolides are much less likely to cause allergic issues; the main worry with these medications is that they can irritate the stomach [6].

1.2.3.3 Beta-lactams

This class of antibiotics has members with a highly reactive ring made up of one nitrogen and three carbons. They either kill the bacteria or stop their growth by interfering with proteins that are necessary for the formation of the bacterial cell wall. To put it more simply, during the manufacture of peptidoglycan, particular bacterial enzymes known as penicillin-binding protein (PBP) are in charge of cross-linking peptide units. Beta-lactam antibiotics have the ability to attach themselves to these PBP enzymes, interfering with peptidoglycan production and causing lysis and cell death in the process. The most well-known members of the beta-lactam class are carbapenems, monobactams, cephalosporins, and penicillins [8].

Penicillin

In 1929, Alexander Fleming made the first antibiotic discovery: penicillin. Penicillins are used to treat gonorrhea, respiratory tract infections, urinary tract infections, skin infections, dental infections, and ear infections. Penicillins are occasionally mixed with additional substances known as beta-lactamase inhibitors. These substances shield the penicillin from bacterial enzymes that could otherwise destroy it before it has a chance to accomplish its job. Penicillins are often quite secure. An allergic reaction carries the highest risk and can be quite serious. Penicillin allergies are common in those who have previously experienced cephalosporin allergies. Penicillins prevent bacteria from building their cell walls, which leads to the walls' disintegration and eventual bacterial death [6].

Cephalosporin

In terms of structure and method of action, members of this class of antibiotics resemble penicillin. They comprise one-third of all antibiotics given and provided by the National Health Scheme in the United Kingdom. They are part of the most frequently prescribed and administered antibiotics. Guiseppe Brotzu initially identified the first antibiotic in this class from the fungus Cephalosporium acremonium in 1945. Even though Guiseppe Brotzu was the one who isolated the medicine initially, Edward Abraham was the one who was able to remove the chemical and was given credit for patenting it. The side chain of cephalosporins contains 3,6-dihydro-2 H-1,3-thiazane rings, while the nucleus of the compound is 7-aminocephalosporanic acid. Cephalosporins are used in the treatment of bacterial infections and diseases arising from Penicillinase-producing, Methicillin-susceptible *Staphylococci* and *Streptococci, Proteus*

mirabilis, some *Escherichia coli, Klebsiella pneumonia, Haemophilus influenza, Enterobacter aerogenes* and some *Neisseria* [8, 9].

Carbapenems

In 1976, the need for antibiotics led to the discovery of this class. Before this point in the late 1960s, the rise of beta-lactamase in bacteria posed a serious threat to the efficiency of penicillin. Bacteria developed resistance to penicillin thanks to bacterial beta-lactamases. This seemingly unfavorable situation prompted researchers to launch a comprehensive hunt for beta-lactamase inhibitors. Their efforts paid off in 1976 when it was discovered that the Gram-positive bacterium Streptomyces clavuligerus produced olivanic acids, which inhibited beta-lactamase. Regretfully, these acids proved difficult to permeate the bacterial cell due to their chemical instability. These obstacles delayed the development of olivanic acids, but remarkably, two better beta-lactamase inhibitors were found not long after. These were clavulanic acid obtained also from *S. clavuligerus* , and thienamycin isolated from *Streptomyces cattleya* [5].

1.2.3.4 Fluoroquinolones

Since fluoroquinolones are broad-spectrum antibiotics, they work against a variety of microorganisms. The majority of common skin infections, respiratory infections (such as sinusitis, pneumonia, and bronchitis), and urinary tract infections are treated with fluoroquinolones. Fluoroquinolones' common negative effects mostly include the digestive tract: nausea, vomiting, diarrhea, and minor stomach discomfort. These are usually not so bad and eventually go away. It is not recommended to take fluoroquinolones when pregnant. By interfering with bacteria's capacity to produce DNA, fluoroquinolones restrict bacterial growth. Bacteria find it difficult to grow as a result of this action. It has a bacteriocidal action [6].

1.2.3.5 Sulfonamides

Sulfonamides, also called sulfa drugs, are a type of synthetic antimicrobial that doctors prescribe when first-line treatments are ineffective or contraindicated. The most common type is sulfamethoxazole with trimethoprim, called co-trimoxazole. It treats conditions such as pneumocystis pneumonia and nocardiosis in people with weakened immunity, as well as infections of the lower urinary tract in children [10].

1.2.3.6 Tetracyclines

Benjamin Duggar isolated tetracycline from a soil bacterium belonging to the genus Streptomyces in 1945. Tetracycline was a huge hit when it was originally found. A wide range of bacterial infections can be treated using tetracyclines. They frequently handle infections of the pelvis, urethra, and chest. Acne, rosacea, and perioral dermatitis are among the inflammatory skin disorders that tetracyclines also cure [5, 6, 10].

1.3 Antibiotic resistance and It's global concern

Numerous diseases and illnesses can be effectively treated with antibiotics. One of the most significant medical advancements of the 20th century was the invention of antibacterial therapy, which is now a cornerstone of contemporary medicine, helping to avert millions of preventable deaths from bacterial infections. Before the discovery of antibiotics, the mortality rate from *Streptococcus pneumoniae* pneumonia may reach 40% [11], the death rate from *Staphylococcus aureus* bacteraemia was 80% [12], and 97% of endocarditis patients did not survive [13]. Prior to the discovery of antibiotics, wound infections were frequently treated with amputation; in fact, 70% of amputations done during World War I were due to wound infections [14]. Antibiotics have significantly changed the prognosis for patients suffering from these infections, as well as how we treat and cure illnesses like syphilis and tuberculosis. Furthermore, the capacity to treat and cure infections has aided in the development of increasingly sophisticated surgical procedures, organ transplants, and chemotherapy in modern medicine [15]. Unfortunately, treating these conditions becomes more challenging due to antibiotic resistance. Globally, antibiotic resistance is a problem for public health. Antibiotic-resistant microorganisms are becoming more prevalent [16]. When bacteria adapt, they become resistant to the effects of antibiotics, making them more difficult to eradicate or suppress. Bacterial infections can become very challenging to treat [17].

Modern medicine is built on antibacterial medications. Drug-resistant bacteria are becoming more prevalent, endangering our capacity to treat common illnesses and carry out life-saving operations including organ transplants, hip replacements, cancer chemotherapy, and cesarean sections. Drug-resistant diseases also have an adverse effect on plant and animal health, lower farm productivity, and jeopardize food security. Both health systems and national economies as a whole bear heavy expenses as a result of antimicrobial resistance (AMR). For instance, it results in the need for more costly and intense care, impairs agricultural productivity, and reduces patient or caregiver productivity due to extended hospital admissions. Every nation, regardless of income level, faces the challenge of AMR [18].

Antibiotic-resistant bacteria are emerging in the field. Patients who are infected with multidrug-resistant organisms are not the only ones who suffer from their unfavorable effects. Antibiotic resistance has detrimental effects on empirical antibiotic regimens, employable antibacterial classes, and the use of medicines with lower efficacy, all of which affect patients. When resistance at the population level exceeds

a particular threshold, narrow-spectrum medications are no longer used for the treatment of common diseases, which has ramifications for antibiotic prescribing policies and guidelines [19].

The effectiveness of standard antibiotics against common bacterial infections is being threatened by the global growth of antibiotic resistance. Alarming rates of resistance among common bacterial infections are highlighted in the 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report. A significant worry is the median reported rates of 35% for *Methicillin-resistant Staphylococcus aureus* and 42% for third-generation cephalosporin-resistant *E. Coli* across 76 nations. In 2020, 1 in 5 cases of *E. Coli* urinary tract infections showed decreased susceptibility to common antibiotics such as ampicillin, co-trimoxazole, and fluoroquinolones. Effective treatment of common infections is becoming more difficult as a result [18].

1.4 β-Lactam Antibiotic Resistance

Because they can prevent the formation of bacterial cell walls, β-lactam antibiotics, such penicillins, have historically been the most often used antibiotics [20, 21]. The main component of the bacterial cell wall is a peptidoglycan polymer, which is cross-linked and mesh-like and whose basic unit is a disaccharide containing a pentapeptide [22, 23]. Specifically, penicillin binding proteins (PBPs) transpeptidases, which use pentapeptide as their substrate and are critical for peptidoglycan formation, are the target of β-lactam antibiotics [24–26]. Because the β-lactam ring resembles the D-Ala-D-Ala group at the pentapeptide's terminus, β-lactam antibiotics react with PBPs. Consequently, β-lactam molecules establish a steady acyl-enzyme link with the catalytic serine, permanently preventing the peptide substrate from accessing the active site [27–29]. The primary cause of the widespread development of bacterial resistance to β-lactam antibiotics, especially in Gram-negative bacteria, is the synthesis of β-lactamase, an enzyme that breaks down the β-lactam ring of several antibiotics, including cephalosporin and penicillin.

There are four families of beta-lactamases: classes A through D. In the clinical environment, class A and class C are the most often observed [30, 31]. Since the discovery of penicillin, new substances including cephalosporins and carbepenems have been developed to circumvent resistance against β-lactam antibiotics. Sadly, bacteria have developed resistance strategies by developing β-lactamase activity against these antibiotics with a wider spectrum, making most of these substances ineffective. Because they are metallo-enzymes, members of class B cannot function without a catalytic metal ion like zinc. As serine β-lactamases, classes A, C, and D hydrolyze the β-lactam ring using a catalytic serine, resulting in the formation of an acylenzyme complex resembling the PBPs. The serine βlactamases, on the other hand, catalyze a second hydrolytic deacylation event that releases the antibiotic, in contrast to the PBPs [32].

Not only is the Class A β-lactamase known as CTX-M active against first-generation penicillins and cephalosporins, but it is also highly effective against extended spectrum β-lactam antibiotics like cefotaxime. Since its discovery in the 1990s, CTX-M has emerged as the most commonly detected extended spectrum βlactamases (ESBLs) in numerous parts of the globe. Because of the great efficacy of ESBLs like CTX-M, therapy choices for bacterial infections will remain restricted [33-35].

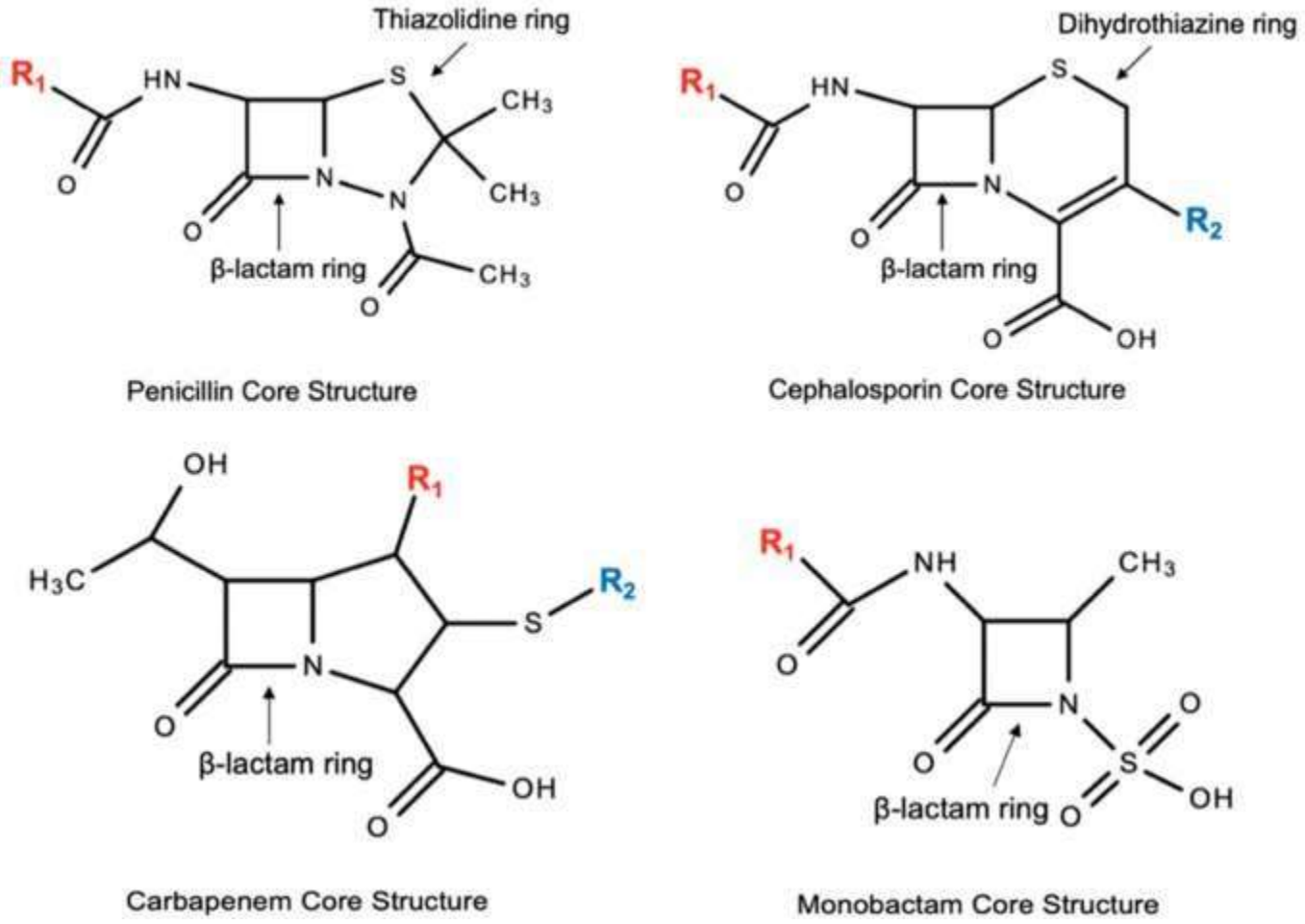


Fig 1. β-lactam Antibiotics

1.5 β-Lactamase Classification

A broad class of enzymes known as beta-lactamases is produced by bacteria and functions to break the beta-lactam ring, rendering the beta-lactam antibiotic inactive. Certain beta-lactamases are encoded on chromosomes, whereas others are encoded on mobile genetic elements (like plasmids). One of the most significant clinical resistance mechanisms for gram-negative bacterial infections is the generation of beta-lactamases. Comprehending the prevalent varieties of beta-lactamases generated by distinct pathogens can aid in the interpretation of susceptibility, aid in treatment decision-making, and support infection control protocols. There are thousands of different types of beta-lactamases; multiple classification schemes exist, but the Ambler classification scheme is the most widely used [36].

Based on the functional characteristics of the enzymes and their amino acid sequence, beta-lactamases are categorized into classes A, B, C, and D. Based on their similarity in hydrolyzing substrates and the conservation of their amino acid sequence, the class A, C, and D β-lactamases are grouped together. These particular enzyme groups create an acyl enzyme complex by hydrolyzing the βlactam ring through a catalytic serine residue [30].

Conversely, class B β-lactamases are metalloenzymes that catalyze the hydrolysis of the beta-lactam ring by means of a metal ion, such as zinc ions in the active site [30, 33]. The most common classes of β-lactamases are A through C. There are four classes total. In the 1980s, class A, C, and D β-lactamase generating bacteria produced resistant Gram-negative infections, which were treated with third generation cephalosporins such cefotaxime and ceftazidime. The ESBLs, which are primarily made up of class A and D enzymes, emerged as a result of the repeated administration of these medications. It's interesting to note that among the class A β-lactamases, the class A CTX-M β-lactamases are the most common and frequently occurring ESBLs [32].

1.5.1 Beta-Lactamase Inhibitors

Sometimes used in conjunction with beta-lactam antibiotics, beta-lactamase inhibitors are medications that inhibit the activity of specific beta-lactamases. As an illustration, consider

- Medication such as clavulanate, sulbactam, and tazobactam inhibit penicillinases but not AmpC or carbapenemases. Although most medication combinations containing these compounds are not therapeutically effective against ESBL production, they do inhibit some ESBLs in vitro. Additionally, a small range of bacterial species, such as *Neisseria gonorrhoeae*, *Bacteroides fragilis*, and, most significantly, *A. baumanii* (a bacterium with the potential for significant antibiotic resistance), are susceptible to the antibacterial effects of sulbactam.

- Avibactam: This medication inhibits beta-lactamases of classes A (ESBLs, most KPCs), C (AmpC), and D (OXA), but not of class B (MBLs).
- Relebactam and vaborbactam are medications that inhibit classes A and C, but not D or B.

As of right now, there are no beta-lactamase inhibitors that are effective against MBLs, such as IMP (imipenem)-types, VIMs (Verona integron–encoded MBLs), and NDM-1 (New Delhi MBL-1), which are capable of inactivating all beta-lactam antibiotics save aztreonam. But a lot of strains that make MBLs also make other beta-lactamases that are capable of hydrolyzing aztreonam [36].

1.6 The origin of resistance to antibiotics

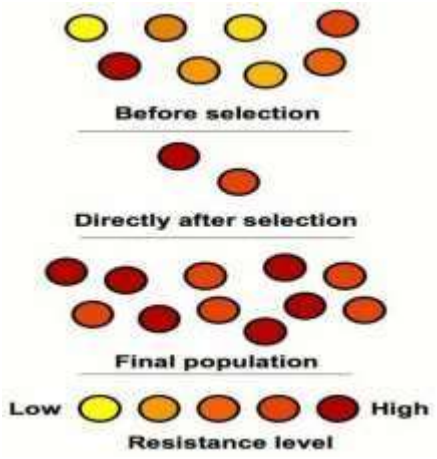
Antibiotic resistance has its roots in the evolutionary history of microorganisms. In essence, antibiotics are substances that either stop or eradicate bacterial development. Antibiotics influence the bacterial population selectively when they are introduced into a given environment. The majority of bacteria are destroyed by antibiotics, but some can have traits or genetic alterations that let them endure the onslaught.

Misuse of antibiotics is the main source of antibiotic resistance. The European Center for Disease Prevention and Control (ECDC) has identified three main categories of misuse:

1. The overprescription of antibiotics for viral infections even though these treatments are not effective against viruses.

2. The overuse of "broad-spectrum antibiotics" rather than more specifically tailored medications based on reliable diagnoses.

3. Inappropriate use by patients, such as not following the recommended treatment plan or dose instructions, which permits certain germs to persist and become resistant [37].



bacteria can survive antibiotic concentrations that would kill others.

1.7 Causes of antibiotic resistance

Over time, bacteria naturally become resistant to drugs. Nevertheless, a number of things can quicken this process:

1.7.1 Overuse of antibiotics:

Antibiotic resistance is a result of overuse of antibiotics. Antibiotics, for instance, do not work against the majority of viral sore throats. It's critical to take antibiotics only as directed by a medical professional.

1.7.2 Misuse of antibiotics:

Bacteria grab any opportunity to grow. Bacterial reproduction can occur as a result of missing doses, stopping therapy early, or taking someone else's prescription. Bacteria can undergo mutations during multiplication, which makes them more resistant to antibiotics. Antibiotics can eradicate bacteria that are not resistant, but they do not eradicate those that have become resistant.

1.7.3 Spontaneous resistance:

Bacteria occasionally undergo spontaneous mutations or changes in their genetic makeup (DNA). Antibiotics might not identify the recently altered bacteria in certain situations, making treatment useless. On the other hand, the mutation could make the germs more resistant to the effects of the medicine.

1.7.4 Transmitted resistance:

Drug-resistant bacterial illnesses that are contagious can spread to other people. After infection, the afflicted person develops a disease that is resistant to antibiotics. Alternative treatments might be accessible at first. On the other hand, treating these resistant germs can get harder with time [17].

1.8 Resistance mechanisms (Defense Strategies) of bacteria against antibiotics

The ability of a bacterium to withstand an antibiotic's effects to which it is normally vulnerable is known as antibiotic resistance [38]. Antibacterial resistance occurs when bacteria or other organisms learn to resist the medications meant to eradicate them. Treatment for resistant infections can be challenging and sometimes unattainable. Antibacterial resistance is a phenomenon that happens naturally [39].

On the other hand, a combination of bacteria exposed to antibiotics, their dissemination, and their resistance mechanisms are what cause rises in antibacterial resistance. The antibiotic is destroyed or its effects are neutralized by resistant microorganisms. Bacteria can encode antibiotic resistance at the chromosomal or plasmid level [15, 16]. Bacteria can evolve resistance mechanisms—defense mechanisms against antibiotics—in order to survive. Bacterial resistance mechanisms are determined by the specific proteins that are made possible by instructions found in DNA. Numerous resistance genes are carried by bacteria.

All antibiotics may become useless against bacteria that are already difficult to treat if they possess the appropriate combination of resistance mechanisms, rendering illnesses incurable. It is concerning to note that bacteria that are resistant to antibiotics can transfer their resistance mechanisms to other bacteria that have not been exposed to them [39].

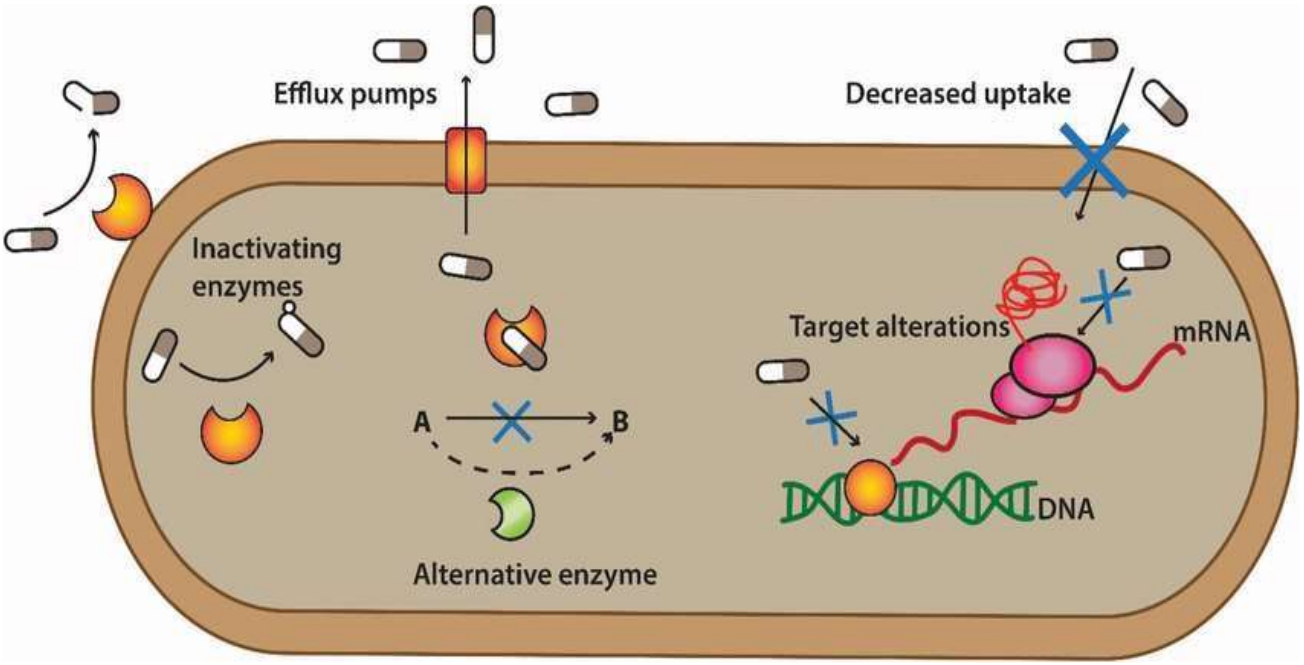


Fig 2. The mechanisms of antibiotic resistance in bacteria. Acquired enzymes inactivate the drugs, active efflux pumps transport specific or multiple antibiotics out of the cell, alternative metabolic pathways substitute those inhibited by the drug, modification of antibiotic target site leads to reduction of drug affinity to the binding sites and decreased drug accumulation due to decreased permeability. (Content courtesy of Springer Nature, terms of use apply) [40].

Table 1. Mechanisms of Antibiotic resistances

Resistance Mechanisms (Defense Strategies)	Description
Restrict access of the antibiotic	Germs restrict access by changing the entryways or limiting the number of entryways. **Example:** Gram-negative bacteria have an outer layer (membrane) that protects them from their environment. These bacteria can use this membrane to selectively keep antibiotic drugs from entering.
Get rid of the antibiotic	Germs get rid of antibiotics using pumps in their cell walls to remove antibiotic drugs that enter the cell. **Example:** Some *Pseudomonas aeruginosa* bacteria can produce pumps to get rid of several different important antibiotic drugs, including fluoroquinolones, beta-lactams, chloramphenicol, and trimethoprim.
Change or destroy the antibiotic	Bacteria change or destroy the antibiotics with enzymes, proteins that break down the drug. **Example:** *Klebsiella pneumoniae* bacteria produce enzymes called carbapenemases, which break down carbapenem drugs and most other beta-lactam drugs.
Change the targets for the antibiotic	Many antibiotic drugs are designed to single out and destroy specific parts (or targets) of a bacterium. Germs change the antibiotic's target so the drug can no longer fit and do its job. **Example:** *Escherichia coli* bacteria with the *mcr*-1 gene can add a compound to the outside of the cell wall so that the drug colistin cannot latch onto it.
Bypass the effects of the antibiotic	Germs develop new cell processes that avoid using the antibiotic's target. **Example:** Some *Staphylococcus aureus* bacteria can bypass the drug effects of trimethoprim [40].

This table-1 gives a few examples of defense strategies used to resist the effects of antibiotics which are accordance of CDC [39].

1.9 Consequences of antibiotic resistance

Globally, antibiotic resistance is a problem for public health. Antibiotic-resistant microorganisms are becoming more and more prevalent. Antibiotic resistance poses a risk of making manageable conditions like pneumonia, TB, or mild infections incurable. Families and our healthcare system would be burdened more financially and emotionally as a result [16].

The efficacy of many of the current treatments for common bacterial illnesses is declining. Consequently, there are instances in which none of the existing antibiotics can effectively treat sick people. Treatment may be hampered by this resistance, which could lead to problems or even death. In addition, a patient might require additional hospital stays, more intrusive procedures such intravenous injections, or the use of more costly and different antibiotics that might have more severe side effects [17]. According to a recent WHO research, common bacterium resistance to antibiotics has alarmingly increased in many regions of the world. For instance, common bacteria like *Escherichia coli*, which can cause urinary tract infections, *Staphylococcus aureus* (also known as *Methicillin-resistant Staphylococcus aureus*, or MRSA), *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are becoming more resistant to major antibiotics in Europe.

- According to WHO, this puts advancements in contemporary medicine at jeopardy since they depend on the availability of potent antimicrobial medications. For example:
- Cystitis, one of the most common bacterial infections in women, may become untreatable or require injection drugs, imposing additional costs on patients and the health system overall;
- Common infections like pneumonia that can occur in healthcare settings may not respond to available or recommended drugs like penicillin, putting the lives of patients at risk;
- Antibacterial medications may lose some of their potency or cease to work altogether when used to treat common infections in neonates and intensive care units or to prevent infections following procedures.

The WHO report highlights a problem: since 1985, there have been very few new antibiotics developed to replace the ones that are losing their effectiveness [37, 41, 42].

Here table-2 illustrates examples of the consequences of antibiotic resistance [43].

Table 2. Examples of the consequences of antibiotic resistance [43].

Problem	Example	Consequences	Responses to mitigate the impact of resistance	Problems associated with mitigating responses
Infections caused by MDR bacteria	ESBL *Escherichia coli* bacteraemia treated empirically with ceftriaxone	Inadequate therapy/delay in effective therapy	Guideline alteration, with carbapenems for empiric therapy Implementing rapid diagnosis and reporting	Overuse of broader spectrum agents for all patients Increased cost, only minimally reducing the delay
	Carbapenem-resistant *Acinetobacter baumannii* infection	Less efficacious or more toxic agents	Treatment with polymixins	Reduced efficacy, increased toxicity
	Infection with colistin-resistant *A. baumannii*	Infection with limited or no therapeutic options	Treatment with combination of agents each likely to be ineffective alone Surgical management	Likely ineffective therapy Toxicity Cost Resource utilization
Colonization with MDR bacteria	Failure of fluoroquinolone prophylaxis to prevent infection by resistant strains of *E. coli* after transrectal ultrasound-guided prostate biopsy	Additional infections	Guideline alteration, with fosfomycin, carbapenems or amikacin for prophylaxis Screening of all patients pre-biopsy and targeted prophylaxis	Overuse of broader spectrum agents and use of toxic agents for all patients Increased cost and burden on the healthcare system
Infections caused by non-MDR bacteria	Vancomycin for MSSA	Less efficacious treatment	Antimicrobial stewardship to limit use of vancomycin	Cost Under-treatment of MRSA
	Piperacillin/tazobactam empiric treatment for neutropenic sepsis where the causative organism is MSSA	Excessively broad-spectrum treatment	Antimicrobial stewardship to de-escalate from piperacillin/tazobactam	Under-treatment of MDR organisms
Hospitalization	Spread of epidemic/virulent VRE clones in a unit	Additional infections Lack of access to optimal or lifesaving procedures	VRE targeted infection control measures to prevent transmission	Cost, use of hospital resources such as isolation beds, negative effects on patients related to isolation

				Limitation of procedures such as transplantation
	Outbreak of carbapenem-resistant *Klebsiella spp*. in a unit	Lack of access to optimal or lifesaving procedures	Need for unit closure	Interruption of hospital activity Limitation of procedures

Abbreviations: ESBL, extended-spectrum β-lactamase; MDR, multidrug-resistant; MSSA, *Methicillin-susceptible Staphylococcus aureus*; VRE, *Vancomycin-resistant Enterococcus*.

1.10 Antibiotic-resistant bacteria that are in deadliest

Worldwide, the most common cause of nosocomial infections is the ESKAPE pathogens, which include Enterobacter faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species. One of the biggest problems in clinical practice is that the majority of them are isolates that are resistant to many drugs. One of the top three risks to global public health is multidrug resistance, which is typically brought on by prescription drug abuse, excessive medication use, improper antimicrobial use, and subpar pharmaceuticals [44]

Multidrug resistant bacteria are the most dangerous kind of all; they are especially dangerous in healthcare facilities, assisted living facilities, and among patients whose treatment necessitates the use of devices like blood catheters and ventilators. *Acinetobacter, Pseudomonas,* and several Enterobacteriaceae (*Klebsiella, E. Coli, Serratia,* and *Proteus*) are among them. They can result in serious infections including pneumonia and bloodstream infections, which are frequently fatal. Globally, these bacteria are linked to the greatest number of deaths from diseases resistant to antibiotics:

- *Escherichia coli* (*E. coli*).
- *Staphylococcus aureus* (*S. aureus*).
- *Klebsiella pneumoniae* (*K. pneumoniae*).
- *Streptococcus pneumonia* (*S. pneumoniae*).
- *Acinetobacter baumannii* (*A. baumannii*).
- *Pseudomonas aeruginosa* (*P. aeruginosa*) [17].

1.11 General overview on *E. coli* and it's CTX-M-9 class A beta-lactamase

German physician Theodor Escherich (1857–1911) found the bacteria *Escherichia coli* from infants' excrement in 1885 [45]. Gram-negative, non-sporulating, rod-shaped, facultative anaerobic and coliform bacteria belonging to the genus Escherichia, *E. coli* is frequently found in food, the environment, and the lower digestive tract of warm-blooded animals [46]. It is the most researched prokaryotic model organism in the fields of microbiology and biotechnology. It is often employed as an indicator organism for water contamination since it can survive for extended periods of time in feces, soil, and water. In new feces, the bacterium grows quickly under aerobic conditions for two to three days, but after that, its population steadily declines. *E.Coli* is a straight, non-sporing, non-acid fast bacillus that can be found alone or in pairs [47]. The status of *Escherichia coli* has changed significantly since it was discovered in 1885. Although the majority of commensal strains have been demonstrated to be advantageous to their host and lack specific virulence characteristics, pathogenic strains of *E. coli* have been linked to a variety of illnesses [48].

Enterotoxigenic *E. Coli* (ETEC) and enteropathogenic *E. Coli* (EPEC) are the intestine pathogenic *E. Coli* that are most frequently identified as the cause of bacterial gastroenteritis, particularly in low-income nations with inadequate sanitation [49]. However, traveler's diarrhea (induced by ETEC strains) or occasional occurrences are the most common ways that *E. coli*-related diarrhea is reported in affluent nations (Centers for Disease Control and Prevention, 2016). Treatment recommendations for infections brought on by various pathogenic *E. coli* have been made. For example, diarrhea caused by *Escherichia coli* is often treated with appropriate rehydration or is thought to be self-limiting. However, antibiotics are the cornerstone of treatment in the majority of cases, particularly those involving potentially fatal extraintestinal infections. β-lactam antibiotics have been successfully utilized for a long time in this regard. Antibiotics that are utilized worldwide include beta lactams. Antibiotics that are β-lactams possess a β-lactam ring inside their molecular structure. Penicillins, cephalosporins, monobactams, and carbapenems are a few of them.

However, β-lactamases that impart third generation cephalosporin resistance were reported in multiple investigations concurrent with their debut in the early 1980s [50]. The beta-lactamase enzyme produced by *E. coli* bacteria breaks down the betalactam ring of beta lactam antibiotics, rendering them useless and making *E. coli* resistant to them. The majority of them were the CTX-M enzymes [51] or the extended-spectrum β-lactamases (ESBLs), which were found as TEM-1, TEM-2, and SHV-1 variations [52]. The appearance of AmpC β-lactamases [53] and carbapenemases [54-56] was shortly followed by additional resistance to cephamycins and carbapenems. The CTX-M enzymes have been identified as the most common among Enterobacteriaceae, despite the fact that over 150 distinct TEM- and SHV-type ESBLs are known [50].

As early as 1990, reports of ESBLs of the CTX-M type were made, and E. coli was indeed the first species to have them [57]. Since then, nosocomial and community-acquired illnesses have been linked to the *E.*

Coli strains containing CTX-M. In recent times, reports of the CTX-M phenotype have also been made about *E. Coli* strains that were isolated from human health, cattle, companion animals, food products, and sewage. These reports suggest that these ESBLs are widely distributed and harbored in reservoirs.

One kind of extended-spectrum beta-lactamase (ESBL) that imparts resistance to some beta-lactam antibiotics, especially those in the cephalosporin class, is CTX-M-9 beta-lactamase, one of the many CTX-M betalactamses. Antibiotics such as cefotaxime, ceftriaxone, and ceftazidime are among them; certain beta-lactam antibiotics may exhibit restricted efficacy against it. As an example, carbapenems such as meropenem and imipenem [58]. The *E.Coli* betalactamse CTX-M-9 that results in drug resistance to antibiotics is the subject of our essay. Our goal is to identify a possible CTX-M-9 inhibitor.

1.12 Historical Perspective

Since ancient times, antibiotics have been used to cure diseases; yet, until recently, most people were unaware that bacteria are the actual cause of most infections. Some of the earliest civilizations used a variety of molds and plant extracts to cure diseases; the ancient Egyptians, for instance, placed moldy bread to wounds that were diseased [59]. Scientists didn't start seeing antibacterial compounds in action until the late 1800s. German scientist Paul Ehrlich discovered that some bacterial cells were colored by chemical dyes, but not by others. Based on this idea, he argued, it must be able to make compounds that selectively kill some bacteria without damaging other cells. He found that arsphenamine, a chemical, could effectively treat syphilis in 1909. Though Ehrlich called his discovery "chemotherapy"—the application of a chemical to cure a disease—it became the first modern antibiotic [59]. Salvadorsan, the first antibiotic, was introduced in 1910. The sulfonamide prodrug Prontosil, developed by Bayer bacteriologist Gerhard Domagk [60], who used the medication to prevent his daughter's arm from being amputated, supplanted salvarsan. Since dyes used to selectively label bacterial cells served as the model for the sulfa medicines, Domagk and colleagues were effectively carrying on Paul Ehrlich's work. Although penicillin was discovered in 1928 by Alexander Fleming on a contaminated Petri dish, sulfonamides were the first really effective, broad spectrum antimicrobials in clinical use and are still in use today [61]. In 1945, Dorothy Hodgkin was able to resolve the long-standing disagreement among prominent chemists including Chain, Abrahams, and Woodward—who thought the structure of penicillin was a beta-lactam—and Robert Robinson, who preferred a thiazolidine-oxazolone structure. Because it made it possible to create semi-synthetic derivatives that avoid penicillin resistance, this was a significant discovery [62].

The golden period of natural product antibiotic research began with the 1928 discovery of penicillin and ended in the mid-1950s. Since then, the current antimicrobial resistance dilemma has resulted from a slow downturn in the discovery and development of antibiotics as well as the emergence of drug resistance in numerous human infections. We have faced resistance for a long time. There is a sizable reservoir of antibiotic resistance genes in nature that have developed over millions of years, and bacteria have an astounding variety of genetic mechanisms for resistance to antibacterials [59, 62, 63]. The history of resistance among *S. aureus* provides an appropriate historical example. Before penicillin was used clinically [64] and while *S. aureus* penicillinase synthesis was still rare, Abrams and coworkers described penicillinase. But after penicillin was developed, it spread quickly, and by the late 1940s, about half of the isolates of *S. aureus* in the UK were resistant to the antibiotic [43]. Methicillin was first used in 1959 to treat *S. aureus* isolates that were resistant to penicillin. However, reports of *S. aureus* isolates that had developed methicillin resistance (MRSA) appeared in 1961. Multidrug-resistant (MDR) MRSA isolates were quickly discovered in hospitals across the globe and are currently spreading across the community. They were initially discovered in other European nations and subsequently in Japan, Australia, and the USA [65].

1.13 Objectives:

This study was performed with a view to develop novel inhibitors of CTX-M-9 class A beta-lactamase from *Escherichia coli*. The specific objectives were-

1. To perform molecular docking of potential ligands molecules from ZINC15 database with beta-lactamase (CTX-M-9 class A beta-lactamase from *Escherichia coli*)

2. To find out ligand molecules that can bind with enzyme with relatively higher affinity than beta-lactam drug. Thus these molecules could serve as the inhibitor of beta-lactamase enzyme which opts to bind with ligand molecule rather than beta-lactam antibiotics due to higher binding ability.

3. To perform ADME/T analysis of selected ligand molecules for analyzing the feasibility of the molecules to be used as drug candidates to combat infections caused by drug resistant *E. coli*

2. Literature Review

Single-celled, tiny organisms are called bacteria. They are some of the planet's oldest known life forms. Bacteria come in millions of varieties and inhabit every imaginable type of environment on the planet. They can be found deep within the earth's crust, in saltwater, and in soil. It has even been documented that certain bacteria may survive in radioactive waste. Numerous bacteria exist on human and animal bodies, both on the skin and in the mouth, airways, digestive, reproductive, and urinary tracts, all without harming them. These microorganisms are referred to as resident flora or the microbiome. Disease-causing microorganisms are quite rare. We refer to them as pathogens. Under some circumstances, the local bacterial flora can occasionally function as pathogens and induce illness. By creating noxious compounds (toxins), infiltrating tissues, or doing both, bacteria can lead to disease. Diverse pathotypes of *Escherichia coli* strains are becoming more and more acknowledged as a serious threat to public health. Pathogenic *E. coli* infections have been effectively treated with β-lactam antibiotics. However, a multitude of hydrolytic enzymes, known as β-lactamases secreted by bacteria, are currently seriously undermining the usefulness of β-lactams. One of the many beta-lactamases found in *E. coli*, CTX-M-9, is capable of breaking down monobactam and cephalosporin antibiotics and giving the bacteria tolerance to them. The goal of the current work was to evaluate and identify possible CTX-M-9 beta-lactamase inhibitors by molecular docking analysis.

The status of *Escherichia coli* has changed significantly since it was discovered in 1885. Although the majority of commensal strains have been demonstrated to be advantageous to their host and lack specific virulence characteristics, pathogenic strains of *E. coli* have been linked to a variety of illnesses [48]. Enterotoxigenic *E. Coli* (ETEC) and enteropathogenic *E. Coli* (EPEC) are the intestine pathogenic *E. Coli* that are most frequently identified as the cause of bacterial gastroenteritis, particularly in low-income nations with inadequate sanitation [49].

Treatment recommendations for infections brought on by various pathogenic *E. coli* have been made. For example, diarrhea caused by *Escherichia coli* is often treated with appropriate rehydration or is thought to be self-limiting. However, antibiotics are the cornerstone of treatment in the majority of cases, particularly those involving potentially fatal extraintestinal infections. β-lactam antibiotics have been successfully utilized for a long time in this regard [66].

However, β-lactamases that impart third generation cephalosporin resistance were reported in multiple investigations concurrent with their debut in the early 1980s. These mostly comprised the extended-spectrum β-lactamases (ESBLs), which were found as the CTX-M enzymes or as variations of TEM-1,

TEM-2, and SHV-1. They also featured extra resistance to cephamycins and carbapenems, which appeared shortly after the AmpC β-lactamases and carbapenemases [50].

Although, more than 150 different TEM- and SHV- type ESBLs are known, the CTX-M enzymes have been recognized as the most prevalent among Enterobacteriaceae. In fact, *E. coli* was the first species in which the CTX-M type ESBLs were reported as early as 1990 [67].

The CTX-M-carrying *E. coli* strains have now been identified from nosocomial and community-acquired illnesses. The CTX-M phenotype has also been observed more recently in *E. coli* strains isolated from food goods, sewage, livestock, companion animals, and healthy humans, demonstrating the wide range of reservoirs that contain and spread these ESBLs [50].

Specifically, *E. Coli* strains harboring the ESBLs CTX-M-14 and CTX-M-15 have been identified in their respective provinces in China; these enzymes have not, however, been frequently reported in minority areas. The majority of CTX-M producers shown multiple resistance to extensive distribution [68].
It has also been noted that the *E. Coli* bacteria bearing the CTX-M-14 or CTX-M-15 ESBL exhibit differing susceptibilities to amoxicillin-clavulanic acid and fluoroquinolones [69].
The CTX-M-type extended-spectrum beta-lactamases (ESBLs) have been found to proliferate quickly and widely in *Escherichia coli*, and they are present in most β-lactam and non-β-lactam antibiotics [70].

The primary cause undermining antimicrobial treatment is the production of ESBL resistance to third generation cephalosporins in gram-negative bacteria, particularly Enterobacteriaceae. Globally, ESBLs are currently a significant issue in both hospital and community settings. Bacterial enzymes known as ESBLs have the ability to hydrolyze the β-lactam ring, making them resistant to a variety of the more recent β-lactam antibiotics, such as monobactams and third-generation extended-spectrum cephalosporins [71].

Cefotaximase (CTX-M), a novel class of plasmid-mediated ESBLs, was identified in the 1980s. Based on similarity in amino acid sequences, the CTX-M type is divided into five groups (CTX-M1, M2, M8, M9, and M25) [51].

The oxyimino cephalosporins, such as CTX, CAZ, ceftriaxone, cefuroxime, and cefepime, can all be hydrolyzed by CTX-M. Furthermore, aztreonam and other monobactams can be hydrolyzed by CTX-M [71]. The second largest category of β-lactamases is now the CTX-M type, a fast expanding family of β-lactamases [72], The CTX-M-type enzymes have so far been found and registered in over 100 different forms [73, 74].

Currently, the most common ESBL type in East Asia is CTX-M-type [75]. The CTX-M-1 and CTX-M-9 groups are the most prevalent CTX-M-types globally and in Asia among the several CTX-M-subtypes [76].

Reports of infections and epidemics brought on by ESBL-producing organisms, including TEM-, SHV-, and CTX-M ESBLs, have been made in Thailand [74, 77]. Furthermore, one of the most significant forms of resistance to a variety of antibiotic therapies is ESBL resistance. In Thailand, the CTX-M-9 group is the most common subtype of CTX-M [78, 79].

The class A plasmid-mediated enzymes known as Extended Spectrum Beta Lactamases (ESBLs) hydrolyze monobactams and oxyimino-cephalosporins but not cephamycins; clavulanic acid inhibits ESBL activity. The most prevalent ESBLs to be discovered in recent years are of the CTX-M type [80].
TEM and SHV enzyme mutations are the original source of ESBLs, which were initially identified in European *Klebsiella pneumoniae*. The CTX-M type ESBLs have recently been observed often on a global scale. The ESBLs of the CTX-M type mostly hydrolyze cefotaxime, while they have reduced activity against ceftazidime [81- 83].

Based on their amino acid sequences, the >70 different types of CTX-M ESBLs now make up this varied category, which is divided into five clusters [84].

These three types—CTX-M14, CTX-M3, and CTX-M2—are the most commonly observed. Additionally linked to resistance to aminoglycosides, cotrimoxazole, and fluoroquinolones is the presence of the CTX-M gene. *Escherichia coli* is the most prevalent bacterium that carries ESBLs, and in recent years, the CTX-M type of ESBLs has been isolated the most [84, 85].

Public health is seriously threatened by the advent of CTX-M class-A extended-spectrum β-lactamases, which confer resistance to second and third-generation cephalosporins. The β-lactam ring is hydrolyzed by CTX-M β-lactamases using a catalytic serine [86].
Globally, clinical isolates of the Enterobacteriaceae family are becoming more and more common with plasmid-mediated extended-spectrum β-lactamases (ESBLs). It has recently been reported that new enzyme groups that are non-TEM and non-SHV derivatives have developed with a characteristic ESBL resistance phenotype. A new class of enzymes known as CTX-M-type lactamases is encoded by Transferable plasmids. The CTX-M-type enzymes are a class of molecular class A extended-spectrum β-lactamases (ESBLs) that can hydrolyze broad-spectrum cephalosporins and are inhibited by clavulanic acid,

sulbactam, and tazobactam. They generally prefer tocefotaxime (CTX; hence the CTX-M name) and ceftriaxone. These enzymes, which can lead to resistance to CTX and other expanded-spectrum lactamases, are starting to appear in members of the Enterobacteriaceae family [87, 88].

A few changes in amino acids led to the formation of the early extended-spectrum β-lactamases (ESBLs) from the common plasmid-mediated TEM and SHV-1 β-lactamases. The earliest reports of the CTX-M-1 (MEN-1) enzyme at the start of the 1990s described a new class A kind of ESBL. The enzymes of the CTX-M type are often known as ESBLs, and they exhibit significantly higher hydrolytic activity against cefotaxime in comparison to ceftazidime. As a result, they significantly increase cefotaxime resistance while having negligible impact on ceftazidime's minimum inhibitory concentration [89].

Based on the similarities in their amino acid sequences, the family of CTX-M-type ESBLs consists of at least 12 members that fall into four major phylogenetic branches: the CTX-M-1 (MEN-1) branch, which includes CTX-M-1, CTX-M-3, and CTX-M-10; the CTX-M-2 branch, which includes CTX-M-2, Toho-1, and CTX-M-4 to CTX-M-6; the CTX-M-9 branch, which includes CTX-M-9 and Toho-2; and the CTX-M-8 branch. These enzymes have been found in *Vibrio cholerae* serovar El Tor as well as in a number of Enterobacteriaceae species [89, 90].
One significant and frequent cause of urinary tract infections (UTIs) is *Escherichia coli* (*E. coli*) [91, 92]. Antibiotic resistance is making it more difficult to treat UTIs brought on by *E. coli* [93]. Gram negative bacteria are known to be resistant by a variety of pathways, including modified target sites, enzymatic antibiotic inactivation, active efflux pump, and reduced permeability by the porins. The synthesis of Extended-Spectrum Beta-Lactamase (ESBL) enzymes, which hydrolyze all penicillins, early cephalosporins, oximino-cephalosporins, and monobactames but not carbapenemes or cephamycins, is one of the most prevalent resistance mechanisms. Inhibitors including clavolanic acid, sulbactam, and tazobactam can affect these enzymes [94].

Currently, ceftazidime and cefotaxime can be hydrolyzed by CTX-M-Beta Lactamases, which are encoded in a plasmid. However, they exhibit low action against ceftazidime and a high degree of resistance to cefotaxime [95, 96].

Bush classified group 2b of the traditional plasmid β-lactamases as the source of point mutations that give rise to TEM- and SHV-type ESBLs. These mutations cause alterations in their isoelectric point (pI) in addition to producing ESBL symptoms. Better hydrolyzation of cefuroxime, cefotaxime, and cefepime than

of ceftazidime is a characteristic of CTX-M-type ESBLs. When compared to other inhibitors, the ESBLs listed above are all more susceptible to tazobactam [97].

In recent decades, the number of bacteria that produce extended spectrum beta-lactamase (ESBL) has expanded substantially. The main ESBL-producing bacterium, *Escherichia coli*, is regarded as one of the major global causative agents of nosocomial infections. Global concerns have been highlighted by trends in the treatment of diseases caused by these kinds of bacteria.
[98].

By using PCR and sequencing assays, the most prevalent type of ESBL gene found was bla CTX-M-14a (n = 12). Additionally identified were the genes bla CTX-M-90 (n = 4), bla CTX-M-15 (n = 3), bla CTX-M-12 (n = 3), bla CTX-M-2 (n = 2), bla CTX-M-14b (n = 1), bla TEM-52 (n = 5), and bla SHV-12 (n = 1). AmpC β-lactamase genes, such as bla CMY-2 (n = 6) or bla DHA-1 (n = 2), were present in eight isolates. Every bla gene that codes for the enzymes CTX-M-1 and CTX-M-9 [99].

The primary method of acquiring resistance to extended-spectrum cephalosporins in Enterobacteriaceae involves the synthesis of plasmid-mediated AmpC β-lactamases (pAmpCs) and/or extended-spectrum β-lactamases (ESBLs). A decade ago, the blaSHV-type and blaTEM-type ESBLs were the most common enzymes worldwide. However, the blaCTX-M -type β-lactamases are now the dominating ESBL kinds in terms of epidemiology [100, 101].
Escherichia coli is a highly problematic member of the Enterobacteriaceae family of bacteria that are resistant to antibiotics. *E. Coli* is the second most prevalent Gram-negative bacteria that cause community-acquired bloodstream infections in people, accounting for 7.3% of all bloodstream infection isolates. It is also a prominent cause of urinary tract infections and intra-abdominal infections. The significance of ESBL-producing *E. Coli* isolates has grown in both nosocomial and community-onset infections. Over the past ten years, there has been a significant increase in the prevalence of fluoroquinolone and extended-spectrum cephalosporin resistance in *E. coli*, which has severely reduced the therapeutic choices available for treating these infections [102, 103].

Extremely broad spectrum β-lactamase enzymes, known as extended-spectrum β-lactamases (ESBLs), are produced by Gram-negative bacteria. The Enterobacteriaceae family is where they are mostly found. The TEM-1, TEM-2, and SHV-1 β-lactamases, which were initially identified in Western Europe and have been known since 1980–1990, are mutated to create ESBLs. TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA are among the nine distinct structural and evolutionary families based on amino acid sequence comparisons that have been used to classify the more than 350 known natural ESBL variants. The

four primary subtypes of ESBL variations are OXA, TEM, SHV, and CTX-M. It's interesting to note that blaCTX-M has grown quickly and is now frequently detected in clinically isolated *E. coli* worldwide. Particularly of the CTX-M type, ESBLs are strongly associated with specific clonal *E. coli* strains [104].

E. coli is one of the bacteria capable of producing ESBL enzymes. This bacterium is the key member of Enterobacteriaceae family and it is the main reason for many hospital-acquired infections such as gastroenteritis, enteritis, neonatal meningitis, sepsis and urinary tract infections. After being originally documented from Japan in 1986, ESBLs have now spread to other regions of the world. Although it has been identified in *Kluyvera ascorbata*, *K. pneumoniae*, and *E. coli*, this enzyme is also present in other Enterobacteriaceae. The five clusters of these β-lactamase enzymes are called CTX-M-1, and the principal ones are CTX-M 1, 3, 10, 12, 15, and UOE-1. They are encoded by Plasmid and arise from *Kluyvera ascorbata*. CTX-M-2 Consists of This cluster, formerly known as TOHO-1, also includes CTX-M 2, 4, 5, 6, 7, 20, and CTX-M44.FEC-1 is a member of this family as well. Additionally, they emerge from *Kluyvera ascorbata* and are Plasmid-encoded. CTX-M-8 is the most important member of the cluster CTX-M-40, and they emerge from *Kluyvera georgiana*. CTX-M-9 include 9, 13, 14, 16, 17, 18, 19 - 21, 24, 27, 46, 47, 48, 49 and 50 CTX-M. CTX-M-45 belongs to these families which are previously referred as TOHO-2 [105, 106].

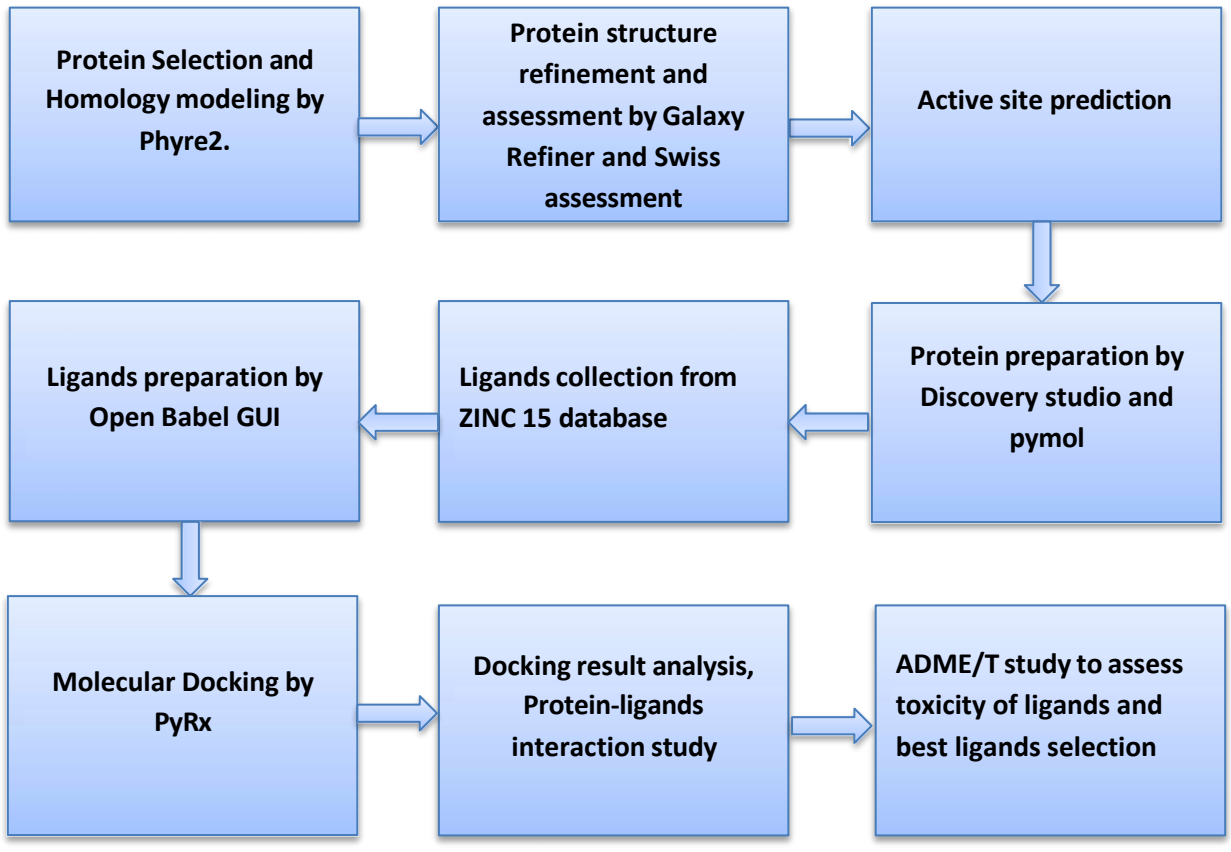
A particular AMR issue that has spread globally and affects both humans and animals is *E. coli* that produces extended-spectrum beta-lactamases (ESBLs). The blaCTX-M, blaSHV, and blaTEM genes, respectively, encode the CTX-M, TEM, and SHV β-lactamases that cause these bacteria to be resistant to penicillins, cephalosporins, and aztreonam. These genes may express themselves chromosomally or through plasmids. Of these three, CTX-M-enzymes are now the most common form of ESBL in both people and animals. These β-lactamases have strong hydrolytic activity against cefotaxime, which is reflected in their name CTX. They are not closely linked to TEM or SHV β-lactamases [107-109].

3. Materials and methods

This work was conducted using an in-silico (computer-aided drug design) technique. We have used methodical automated techniques to try and find suitable ligand candidates against the e. coli betalactamase, ctx-m-9. In order to achieve the goal, we generated the ctx-m-9 betalactamase and, with the aid of various software programs, identified suitable ligand candidates.

The total approach can be divided into following parts:

1. Protein Selection (*E.coli* CTX-M-9 betalactamse)
2. Protein Preparation
3. Ligands Collection
4. Ligands Preparation
5. Molecular Docking
6. Toxicity Assessment



Flow chart 1: Overview of the study

3.1 Protein Selection

E. coli is frequently found in food, the environment, and the lower digestive tract of warm-blooded animals [46]. It is the most researched prokaryotic model organism in the fields of microbiology and biotechnology, pathogenic strains of *E. coli* have been linked to a variety of illnesses [47, 48]. Enterotoxigenic *E. Coli* (ETEC) and enteropathogenic *E. Coli* (EPEC) are the intestine pathogenic *E. Coli* that are most frequently identified as the cause of bacterial gastroenteritis, particularly in low-income nations with inadequate sanitation [49]. β-lactam antibiotics have been successfully utilized for a long time in this regard.
However, β-lactamases that impart third generation cephalosporin resistance were reported in multiple investigations concurrent with their debut in the early 1980s [50]. The beta-lactamase enzyme produced by *E. coli* bacteria breaks down the betalactam ring of beta lactam antibiotics, rendering them useless and making *E. coli* resistant to them. The majority of them were the CTX-M enzymes [33]. The CTX-M enzymes have been identified as the most common among Enterobacteriaceae, despite the fact that over 150 distinct TEM- and SHV-type ESBLs are known [50].

One kind of extended-spectrum beta-lactamase (ESBL) that imparts resistance to some beta-lactam antibiotics, especially those in the cephalosporin class, is CTX-M-9 beta-lactamase, one of the many CTX-M betalactamses. Antibiotics such as cefotaxime, ceftriaxone, and ceftazidime are among them; certain beta-lactam antibiotics may exhibit restricted efficacy against it. As an example, carbapenems such as meropenem and imipenem [58]. The *E.Coli* betalactamse CTX-M-9 that results in drug resistance to antibiotics is the subject of our essay. In this study we opted CTX-M-9 betalactmase as protein. Protein PDB id was downloaded from RCSB PDB with code 3G30 and amino acid sequence downloaded from UniProtKB for further study.

- **The RCSB protein data bank**

The RCSB Protein Data Bank (RCSB PDB, http://rcsb.org) expands on PDB information to support structural biology, computational biology, and related fields of study through research and instruction. The only worldwide repository for experimentally determined, atomic-level, three-dimensional biological macromolecule structures (proteins, DNA, and RNA) is the Protein Data Bank (PDB). The PDB archive is overseen by the Worldwide Protein Data Bank organization (wwPDB; http://wwpdb.org), which consists of three founding regional data centers: RCSB Protein Data Bank (http://rcsb.org), Protein Data Bank Japan (http://pdbj.org), Protein Data Bank in Europe (http://pdbe.org), and a global nuclear magnetic resonance (NMR) specialist data repository called BioMagResBank, which is made up of deposition sites in the US (BMRB; http://www.bmrb.wisc.edu) and Japan (PDBj-BMRB; http://bmrbdep.pdbj.org). This information is managed by the Worldwide Protein Data Bank organization (wwPDB; http://wwPDB.org). These

wwPDB partners work together to gather, annotate, validate, and distribute standardized PDB data to the general public with no usage restrictions [110].

- **UniProt**

Over 120 million protein sequences and annotations from all areas of life are compiled in the UniProt Knowledgebase. The UniProt Knowledgebase (UniProtKB) integrates the unreviewed UniProtKB/TrEMBL entries, annotated by automatic methods, such as our rule-based systems, with the reviewed UniProtKB/Switzerland-Prot entries, to which data have been contributed by our skilled biocuration staff. Of the over 120 million entries in UniProtKB/TrEMBL, the majority are the results of large-scale sequencing operations. An increasing amount of high-quality metagenomic assembled genomes (MAGs) are represented in the database as a result of improved metagenomic assembly and binning technologies. Resources from UniProt can be accessed online at https://www.uniprot.org/ under a CC-BY (4.0) license [111].

3.1.1 Homology modeling

One technique for computational structure prediction that is used to infer the three-dimensional structure of proteins from their amino acid sequence is homology modeling. Among the techniques for predicting computational structure, it is thought to be the most precise. It is broken down into several simple phases that are simple to follow. The homology modeling process makes use of numerous servers and technologies. No single modeling program or server outperforms the others in every way. Optimizing the quality of homology modeling is essential because the model's functioning is reliant on the generated protein 3D structure's quality. There are numerous uses for homology modeling in the drug-discovery process. Drug development depends on the determination of the 3D structure of proteins and, consequently, on homology modeling, as medications interact with receptors that are primarily made of proteins. As a result, using 3D structures of proteins constructed using homology modeling, protein interactions have been clarified. This aids in the discovery of new therapeutic candidates. A significant factor in making drug development more realistic, affordable, quicker, and easier is homology modeling [112]. For homology model of our protein CTX-M-9, we used Phyre2 protein folding recognition server with amino acid sequence.

- **The Phyre2 web portal for protein modeling, prediction and analysis**

A set of online tools called Phyre2 is used to forecast and examine changes in protein structure, function, and mutation. Phyre2's main goal is to give biologists access to cutting-edge protein bioinformatics tools through an easy-to-use interface. Phyre2 builds 3D models, predicts ligand binding sites, and examines the

impact of amino acid changes (such as nonsynonymous SNPs, or nsSNPs) for a user's protein sequence using sophisticated distant homology detection techniques. A straightforward interface leads users through the results at a level of detail that they choose. This technique will let users understand their models' secondary and tertiary structures, domain composition, and quality when they submit a protein sequence. There is a description of a variety of other available programs that can be used to locate a protein structure inside a genome, submit several sequences at once, and do weekly searches automatically for proteins that are hard to model. Visit http://www.sbg.bio.ic.ac.uk/phyre2 to access the server. After submission, a typical structural prediction will be sent back between 30 minutes to two hours [113].

3.2 Protein structure prediction and refinement by Galaxy Refiner

After homology modeling, the CTX-M-9 structure was refined by Galaxy Refiner server.

- **GalaxyWEB server for protein structure prediction and refinement**

The knowledge of three-dimensional protein structures is crucial for comprehending and controlling the biological activities of proteins. The GalaxyWEB server refines loop or terminal areas by ab initio modeling and predicts protein structure from sequence using template-based modeling. This web server is based on the "Seok-server" approach, which was evaluated as one of the best template-based modeling servers in the CASP9 (9th Critical Assessment of Methods for protein Structure Prediction) tests. Using an optimization-based refinement strategy, the method re-builds unstable loops or termini and constructs reliable core structures from many templates. By giving a beginning model structure and the locations of loops or termini to refine, a user can submit a refinement-only task in addition to a structure prediction job. Open access to the web server is available at http://galaxy.seoklab.org/. When applied to refine the models produced by cutting-edge protein structure prediction servers, the approach can, on average, enhance both global and local structure quality [114, 115].

3.2.1 Structure assessment of refined protein

For the assessment of refined structure SWISS-MODEL server was used.

- **SWISS-MODEL server**

The molecular underpinnings of protein function can be better understood by using three-dimensional (3D) protein structures. This information can be used to effectively design investigations, such as site-directed mutagenesis, research of mutations linked to disease, or the structure-based design of particular inhibitors. Despite significant advancements in the field of experimental structure solution through the use of nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallography, the technique remains laborious and uncertain. Presently, the Protein Data Bank (PDB) has roughly 20,000 experimental protein structures

stored in it. A server for automated comparative modeling of three-dimensional (3D) protein structures is called SWISS-MODEL (http://swissmodel.expasy.org). Since its inception in 1993, it has been at the forefront of automated modeling and is currently the most popular free web-based tool for automated modeling. The server processed 120,000 requests for 3D protein models from users in 2002. In the EVA-CM project, the dependability of SWISS-MODEL is continually assessed. The goal of the SWISS-MODEL server's ongoing development is to enhance the effective integration of expert information into a user-friendly platform [116].

- **Ramachandran Plot**

The graphical representation of the dihedral angles (υ and ψ) of amino acid residues in protein structures is known as the Ramachandran Plot. Early on, the idea behind that layout was acknowledged as revolutionary, and it has remained crucial to our understanding of protein structure, energetics, and folding. The phi (ϕ) and psi (ψ) dihedral angles of amino acid residues are plotted in order to evaluate the stereochemical quality of protein structures. The ω angle at that specific peptide bond is always 180 degrees (180°) because the partial double bond maintains the peptide bond planar. Ramachandran Protein structures are verified using plots. Plot regions that show whether a protein structure is energetically advantageous or not aid in the verification and enhancement of three-dimensional protein representations' precision and caliber. It is a useful method for figuring out how accurate protein conformations are structurally. The Ramachandran plot's two dimensions are both necessary and sufficient to capture the main relationships: necessary because phi and psi only function as a joint distribution and do not exhibit meaningful preferences on their own, and sufficient because the approximation of ideal geometry and trans planar peptides is close enough to further effects that they are second order. Because the primary steric interactions happen between nearby peptides, the residue-centric phi, psi plot provides far-more information than a peptide-centric psi, phi plot. Additionally, if the two dimensions are appropriate, this type of plot can be effortlessly depicted, instantly comprehensible, and potentially even iconic [117].

3.3 Active site prediction

Active site predicted by Computed Atlas of Surface Topography of proteins (CASTp)

- **CASTp: Computed Atlas of Surface Topography of proteins**

Proteins interact with other molecules, including DNA, ligands, substrates, and other protein domains, to carry out their biological functions. The physicochemical texture and shape required for these interactions are provided by the three-dimensional structure of proteins. Comprehensive analyses of the correlation between protein structure and function are made possible by the structural data of protein surface areas.

The physicochemical characteristics that proteins require to carry out their functions are created by both the cavities on their surface and the precise placement of amino acids within them. Protein Surface Topography Computed Atlas CASTp (http://cast.engr.uic.edu), an online tool for identifying, defining, and quantifying concave surface regions on three-dimensional protein structures. These consist of voids hidden deep within proteins and pockets seen on the surfaces of proteins. The measurement comprises the volume and area of the pocket or void by the molecular surface model (Connolly's surface) and solvent accessible surface model (Richards' surface), which are all analytically determined [118, 119].

♦ 3.4 Protein preparation by Discovery studio visualizer and Swiss-PdbViewer

E. coli CTX-M-9 betalactamase protein was prepared by discovery studio and Swiss-PdbViewer. Discovery studio was exploited to clean the protein, for instance to remove the water, hetatm, and ligands groups, where Swiss-Pdb Viewer was used in energy minimization of clean protein.

♦ Discovery Studio visualizer

Because it can predict and validate the binding of small molecule ligands to the right target binding site, molecular docking is one of the most widely utilized techniques in structure-based drug discovery. Drug research and discovery is a very difficult, costly, and time-consuming procedure. The advancement of computational techniques and technologies has expedited it. There are several molecular docking programs on the market right now, however after reviewing the literature, it was discovered that Discovery Studio is a suite of programs for stimulating macromolecule and small molecule systems. Accelarys develops and distributes it. The time and cost associated with bringing products to market can be decreased by investigating and testing hypotheses in silico before implementing expensive experiments. It is produced and marketed by Accelerys, a division of Dassault System BIOVIA [120].

♦ Swiss-PdbViewer

The Swiss PDB viewer, or SPBDV, was utilized to minimize energy in order to obtain the lowest possible energy conformation for structures that were predicted and assessed. Swiss-PdbViewer is intended for experimental scientists who lack the time to learn intricate computer instructions and was created with the goal of maximizing interactivity. The robust interface of Swiss-PdbViewer is one of its strongest points. It enables easy modification of the display of specific protein segments, rapid "navigation" inside protein structures, and measurement of atom-to-atom distances, angles, and dihedral angles. You can load many files at once. Every loaded file shows up in a different layer, which is made up of distinct chains that are made up of groups. Amino acids, nucleotides, or heterogeneous groups (like NAD and HEME) are examples of these groups [121].

3.5 Ligands collection from ZINC 15 database

Random ligands were collected from ZINC 15 database.

- **ZINC 15 database**

ZINC, which stands for "ZINC Is Not Commercial," is a publicly accessible database and toolkit that was first created to facilitate easy access to compounds for virtual screening. It has now gained widespread usage in ligand discovery pharamcophore screens, force field construction, benchmarking, and virtual screening. You can get ZINC for free at http://zinc15.docking.org. Almost 120 million buyable "drug-like" compounds—basically, all chemical molecules that are offered for sale—are present in the updated edition, with 25% of them being delivered right away. ZINC links marketable chemicals to valuable ones, including medicines, natural products, metabolites, and compounds with annotations from published works.The major and minor target classes that the annotated genes belong to, as well as the genes themselves, can access the compounds. It provides new analytical tools with minimal limits for professionals and ease of use for nonspecialists. ZINC has not forgotten its 3D origins; all molecules are offered in ready-to-dock formats that are important to biology. In order to provide a tool that is user-friendly for non-experts and completely programmable for chemoinformaticians and computational biologists, ZINC15 combines the fields of biology and chemoinformatics [122].

3.6 Ligands preparation by Open Babel GUI

We took ligands molecules from ZINC 15 database, for energy minimization and formation of pdbqt format Open Babel GUI was exploited.

- **Open Babel GUI**

The Babel chemical file translation tool is available for free as Open Babel, an open-source version. As a cross-platform application and library created to interconvert between numerous file formats used in molecular modeling, computational chemistry, and many other related fields, Open Babel is a project meant to continue where Babel left off. There are two parts to Open Babel: a C++ library and a command-line tool. The command-line tool is meant to be used to translate between different chemical file formats in place of the original Babel application. All of the file-translation code and a host of tools to support the creation of additional open-source scientific software are included in the C++ library.Open Babel now supports 111 different chemical file types as of Open Babel 2.3. It has 85 writing and 82 reading styles. These include common cheminformatics file formats (SMILES, InChI, MOL, MOL2), as well as files from a range of computational chemistry packages (GAMESS, Gaussian, MOPAC), crystallographic file formats (CIF, ShelX), reaction formats (MDL RXN), molecular dynamics and docking package file formats (AutoDock, Amber), 2D drawing package file formats (ChemDraw), 3D viewers, and chemical kinetics

and thermodynamics, among others. New file formats can be easily contributed by users because Open Babel implements formats as "plugins". Open Babel extracts not just the molecular structure but also other data, depending on the format; for instance, it reads property fields from SDF files, extracts vibrational frequencies from computational chemistry log files, and extracts unit cell information from CIF files [123, 124].

3.7 Lipinski Rule of Five

Lipinski rule of 5 helps in distinguishing between drug like and non-drug like molecules. It predicts high possibility of success or failure due to drug likeness for molecules complying with two or more of the following rules.

- Molecular mass less than 500 Dalton
- High lipophilicity (expressed as MLogP less than 5)
- Less than 5 hydrogen bond donor
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130

These filters can help in the early preclinical development and could help avoid costly late-stage preclinical and clinical failures [125].

3.8 Molecular Docking by PyRx

Our aim is molecular docking between *E. coli* CTX-M-9 betalactamase and ligands. Docking was run by PyRx.

- **PyRx: virtual screening tool**

A crucial tool for researching and comprehending protein-ligand interaction is protein-ligand docking. The manner and affinity of protein-ligand interaction are predicted via docking. Numerous free docking applications, including PyRx, ArgusLab, AutoDock, and AutoDock Vina, have been utilized in molecular modeling. The docking investigation usually starts with a re-docking of the protein's ligand from the PDB file to validate the docking methodology. It is a quick method to assess a docking process before interacting with the intended ligands. PyRx is a computational drug discovery tool that simulates screening libraries of compounds against putative therapeutic targets. Because PyRx has an intuitive user interface and docking wizard, it is a useful tool for computer-aided drug design (CADD). In addition, PyRx has a strong visualization engine and features akin to chemical spreadsheets, which are critical for logical drug design [126, 127].

3.9 Docking result analysis

Protein-ligands interaction was analyzed by Discovery studio and Pymol. Ligand which binds with higher binding energy is regards as best ligands. Interaction of CTX-M-9 betalactamse and ligands molecules was checked by Pymol and Discovery Studio visualizer.

3.10 ADME/T study to assess toxicity of ligands and best ligands selection

The potential CTX-M-9 betalactamase inhibitors were analysed their effectiveness in human body through pharmacokinetics (ADME) and toxicity study by using pkCSM and SwissADME

- **pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures**

The process of creating new medications is getting harder, more expensive, riskier, and less successful every time. Because of either unacceptably high side effects or insufficient efficacy, the great majority of medications tested in clinical trials never make it to market. A delicate balance must be struck while developing new drugs to optimize their pharmacokinetics, safety, and efficacy. The goal of many early-stage drug discovery initiatives is to find compounds that bind to desired targets. Although potency is important in these early phases, the pharmacokinetic and toxicological characteristics ultimately determine if a substance will ever become more effective and successful in therapy. Effective medications depend on the interplay of pharmacokinetics, toxicity, and potency. A compound's absorption, distribution, metabolism, and excretion (ADME) characteristics are determined by its pharmacokinetic profile. Lackluster pharmacokinetic and safety characteristics are a major barrier to drug development, which has a high attrition rate. The use of computational methods could reduce these dangers. We have created a unique method (pkCSM) to create predictive models of core ADMET features for drug development using graph-based signatures. pkCSM outperforms existing techniques by a significant margin. A freely available web server (http://structure.bioc.cam.ac.uk/pkcsm) offers an integrated platform for quickly assessing pharmacokinetic and toxicological properties [128].

- **Absorption**

  - **Caco-2 Permeability**

Cells from human epithelial colorectal adenocarcinomas make up the Caco-2 cell line. In vitro models of the human intestinal mucosa, such as the Caco-2 monolayer of cells, are frequently used to forecast the absorption of oral medications. This model predicts the logarithm of the apparent permeability coefficient (log Papp; log cm/s) and is based on 674 drug-like compounds with Caco-2 permeability values. If a compound's Papp is greater than 8x10-6 cm/s, it is regarded as having a high Caco-2 permeability. High

Caco-2 permeability would result in projected values > 0.90 for the pkCSM prediction model. High Caco-2 permeability is generally considered good for a drug

- **Intestinal Absorption (Human)**

When a medication is taken orally, its principal site of absorption is typically the stomach. This technique is designed to estimate the percentage of substances that passed through the small intestine of a human. It forecasts the percentage of a given substance that will pass through the human intestines for absorption. A chemical is deemed poorly absorbed if its absorbance is less than thirty percent.

- **Water Solubility**

A compound's water solubility (logS) indicates how soluble the molecule is in water at 25 °C. Particularly when administered enterally, lipid-soluble medications are less well absorbed than water-soluble ones. Experimental observations of 1708 molecules' water solubility were used to build this model. Logarithm of the molar concentration (log mol/L) is the anticipated water solubility of a chemical.

- **P-glycoprotein substrate**

An ABC (ATP-binding cassette) transporter is the P-glycoprotein. It keeps poisons and other substances out of cells, acting as a biological barrier in the process. In vitro cell cultures and transgenic mdr knockout mice are used for P-glycoprotein transport screening. 332 chemicals whose capacity to be transported by Pgp has been characterized were used to construct this model. The model makes predictions about a compound's likelihood of serving as a Pgp substrate.

- **P-glycoprotein I and II inhibitors**

Significant pharmacokinetic effects of altering P-glycoprotein-mediated transport exist for Pgp substrates, which may confer specific therapeutic benefits or lead to contraindications. 1273 and 1275 substances that have been shown to block P-glycoprotein I and P-glycoprotein I transport, respectively, were used to construct these predictive models. A particular compound's likelihood of being a P-glycoprotein I/Il inhibitor will be ascertained by the predictor.

- **Skin Permeability**

Skin permeability is an important factor in determining the effectiveness of many consumer goods and is being studied in relation to transdermal medicine administration. 211 substances whose in vitro human skin permeability has been assessed were used to create this prediction. The skin permeability constant, logKp

(cm/h), is used to forecast the likelihood that a given substance would be skin permeable. If a compound has a logKp > -2.5, it is regarded as having a relatively low skin permeability [128].

- **Distribution**

➢ **VDss (Human)**

The volume of distribution (VD) is the theoretical volume required for a drug's complete dose to be evenly dispersed in order to produce a concentration equivalent to that of blood plasma. A medication is more widely disseminated in tissue than in plasma the higher the VD. Both dehydration and renal failure may have an impact. The estimated steady state volume of disdribulion (VDss) in humans from 670 medications was used to construct this predictive model. The log Ukg is the expected logarithm of VDss for a certain substance. VDss is classified as high above 2.81 Ukg (log VDss > 0.45) and low below 0.71 Ukg(logVDss<-0.15).

➢ **BBB permeability**

The blood-brain barrier shields the brain from external substances (BBB). When determining a medication's capacity to reduce side effects and opioids or enhance the effectiveness of medications whose pharmacological activity occurs in the brain, the drug's ability to penetrate the brain is a crucial factor to take into account. The logarithmic ratio of brain to plasma drug concentrations, or logBB, is used to quantify blood-brain permeability in vivo in animal models. Using 320 chemicals whose logBB has been empirically determined, this predictive model was constructed.Molecules with a logBB < -1 are poorly distributed to the brain, whereas those with a logBB > 0.3 are thought to penetrate the blood-brain barrier more easily.

➢ **Fraction Unbound (Human)**

A drug's efficacy may be influenced by how much it binds to blood proteins; the more bound a drug is, the less effectively it can diffuse or traverse cellular membranes. This predictive model was constructed using the measured free proportion of 552 compounds in human blood (Fu). For each compound, the predicted fraction that would be unbound in plasma will be calculated. The majority of drugs in plasma will exist in equilibrium between either an unbound state or bound to serum proteins.

➢ **CNS permeability**

When there are confounding variables, measuring blood brain permeability might be challenging. A more straightforward measurement is the blood-brain permeability-surface area product (logPS). It is achieved

by directly injecting the chemical into the carotid artery during in situ brain perfusions. The systemic distribution effects that could skew brain penetration are absent from this. For the construction of this predictive model, 153 substances with experimentally observed logPS were used.Compounds with a logPS > -2 are thought to be able to enter the central nervous system (CNS), whereas those with a logPS < -3 are thought to not be able to do so [128].

- **Metabolism**

➢ **Cytochrome P450 inhibitors**

The body's major detoxifying enzyme, cytochrome P450, is mostly located in the liver. To aid in xenobiotics' excretion, it oxidizes them. The cytochrome P450 enzymes have the ability to both activate and deactivate a variety of medicines. It is not advisable to use drugs that inhibit this enzyme, including grapefruit juice, as they may alter their metabolism. Therefore, evaluating a compound's capacity to inhibit cytochrome P450 is crucial. Using between 14,000 and 18,000 different chemicals whose ability to inhibit cytochrome P450 has been shown, models were constructed for distinct isoforms (CYP1A2/CYP2C19/CYP2C9/CYP2D6 ICYP3A4). If a compound's concentration is less than 10 uM and results in 50% inhibition, it is deemed to be a cytochrome P450 inhibitor. For a specific isoform, the predictors v.11 evaluate a given chemical to determine the likelihood that it will be a cytochrome P450 inhibitor.

➢ **CYP2D6/CYP3A4 substrate**

Numerous medications are metabolized by cytochrome P450s. P450 inhibitors, however, have the ability to significantly change the pharmacokinetics of these medications. In order to determine if a particular molecule is likely to be a cytochrome P450 substrate, II is crucial. 2D6 and 3M are the two primary isoforms in charge of drug metabolism. 671 substances whose metabolism by each cytochrome P450 isoform has been examined were used to construct these models. The predictor will determine the likelihood that a particular molecule will be metabolized by either P450 [128].

- **Excretion**

➢ **Renal OCT2 substrate**

The renal uptake transporter known as Organic Cation Transporter 2 is crucial for the renal clearance and disposal of medications and exogenous substances. Adverse interactions between OCT2 substrates and concurrently given OCT2 inhibitors are also a possibility. Evaluating a candidate's suitability for OCT2 transport yields important information about both the procedure's clearance and any possible contraindications. 906 chemicals whose transit by OCT2 has been experimentally tested were used to construct this model. The predictor will determine the likelihood that a particular molecule is an OCT2 substrate.

- **Total Clearance**

The proportionality coastant Cltot is used to evaluate drug clearance, which mostly comes from a mixture of hepatic clearance (liver metabolism and biliary clearance) and renal clearance (kidney excretion). It has to deal with bioavailability and is crucial for reducing dosage rates in order to reach steady-state concentrations. Utilizing the complete clearance data for 398 chemicals, this predictor was constructed. For a given drug, the estimated total clearance log (Cltot) is expressed in log(ml/min/kg) [128].

- **Toxicity**

- **Rat LD50**

It is importaat to consider the toxic potency of a poteatial compound. The lethal dosage values (LD50) are a standard measurement of acute toxicity used to assess the relative toxicity of different molecules. The LD50 is the amount of a compound given all at once that causes the death of 50% of a group of test animals. The model was built on over 10000 compounds tested in rats and predicts the LD50 (in mollkg).

- **AMES toxicity**

The Ames test is a widely employed method to assess a compounds mutageneic potential using bacteria. A positive test indicates that the compound is mutagenic and therefore may act as a carcinogen. This predictive model was built on the results of over 8000 compounds Ames tests. It predicts whether a given compound is likely to be Ames posrtive and hence mutagenic.

- **T. Pyriformis toxicity**

T. Pyriformis is a protozoa bacteria, with rts toxicity often used as a toxic endpoint. This method was build using the concentration of 1571 compounds required to inhibit 50% of growth (IGC50). For a given compound, the plGC50 (negative logarithm of the concentration required to inhibit 50% growth in log ug/L) is predicted, wrth a value > -0.5 log ug/L is considered toxic.

- **Minnow toxicity**

The lethal concentration values (LC50) represent the concentration of a molecule necessary to cause the death of 50% of the Flathead Minnows. This predictive model was built on LC50 measurements for 554 compounds. For a given compound, a log LC50 will be predicted. LC50 values below 0.5 mM (log LCS0 < -0.3) are regarded as high acute toxicity.

- **Maximum Tolerated Dose**

The hazardous dosage threshold of substances in humans can be estimated using the maximum recommended tolerated dose (MRTD). 1222 experimental data points from human clinical trials are used to train the model, which then forecasts the logarithm of the MRTD (log mg/kg/day). This will assist in directing phase I clinical trial maximum recommended starting doses for medications, which are currently determined by extrapolating data from animal studies. A chemical is classified as high if its MRTD is greater than 0.477 log(mg/kg/day) and low if its MRTD is less than or equal to 0.477 log(mg/kg/day).

- **Hepatotoxicity**

One of the main causes of drug attrition and a critical safety concern for drug development is drug-induced liver harm. This predictor was developed based on 531 drugs' liver-related side effects that were recorded in human subjects. A substance was deemed hepatotoxic if it caused at least one physiological or pathological liver event, which is closely linked to impaired liver function.
It makes predictions about a compound's propensity to be linked to abnormal liver function disruption.

- **Oral Rat Chronic Toxicity**

Long-term exposure to low-to-moderate chemical dosages is a major problem in several therapeutic approaches. The goal of chronic studies is to determine the compound's lowest dose at which an adverse impact is detected (LOAEL) and its highest dose at which no adverse effects are observed (NOAEL). Using the LOAEL values from 445 chemicals, this predictor was constructed. The estimated log Lowest Observed Adverse Effect (LOAEL) for a specific chemical will be produced in log(mg/kg_bw/day). It is necessary to interpret the LOAEL results in light of the necessary bioactive concentration and treatment durations.

- **Skin Sensitization**

Skin sensitisation is a potential adverse effect for dermally applied products. The evaluation of whether a compound, that may encountered the skin, can induce allergic contact dermatitis is an important safety concern. This predictor was built using 254 compounds which have been evaluated for their ability to induce skin sensitisation. It predicts whether a given compound is likely to be associated with skin sensitization.

- **hERG I and II Inhibitors**

The hERG (human ether-a-go-go gene) expressed potassium channels are primarily inhibited, which can result in progressive long QT syndrome and ultimately deadly ventricular arrhythmia. Numerous drugs have been taken off the pharmaceutical market due to their inhibition of hERG channels. Utilizing data on hERG I and II inhibition for 368 and 806 chemicals, respectively, these models were constructed. The predictor will ascertain the likelihood that a particular substance is a hERG 1/11 inhibitor [128].

- **SwissADME**

A potent chemical needs to reach its target in the body at a high enough concentration and remain there in a bioactive form for the anticipated biologic activities to take place in order for it to be successful as a medication. In the process of developing new drugs, absorption, distribution, metabolism, and excretion (ADME) are evaluated at progressively earlier stages of the discovery phase, when the number of potential compounds is high but physical sample access is constrained. Within that framework, computer simulations are legitimate substitutes for experiments. The goal of many different in-silico techniques is to predict ADME parameters based on molecular structure. Notably, Lipinski et al.'s groundbreaking research considered molecules that are active when taken orally in order to identify physicochemical ranges that have a high likelihood of becoming an oral medication (i.e., drug-likeness). The link between pharmacokinetic and physicochemical factors was outlined by the so-called Rule-of-five.The SwissADME web application provides free access to a variety of quick and reliable predictive models for physicochemical characteristics, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness. These models include in-house, highly skilled approaches like the iLOGP, Bioavailability Radar, and BOILED-Egg. Through the login-free website http://www.swissadme.ch, a user-friendly interface ensures easy, efficient input and interpretation. In order to aid in their drug development efforts, experts in cheminformatics and computational chemistry as well as non-experts can quickly estimate critical parameters for a group of molecules [129].

4.1 Protein Selection and Homology modeling by Phyre2

We select *E.coli* betalactamase CTX-M-9 (figure-3) for our study. The pdb format of this betalactamase is available in RCSB PDB (https://www.rcsb.org/). The PDB id of CTX-M-9 betalactamase is 3G30. The amino acid sequences of this protein downloaded from UniprotKB (https://www.uniprot.org/). *E.coli* CTX-M-9 betalactamse is single chain enzyme denoted by A chain with length 263. The amino acid sequence is given below;

>tr|Q9L5C8|Q9L5C8_ECOLX Beta-lactamase OS=Escherichia coli OX=562 GN=blaCTX-M-9a PE=1 SV=1

MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDTADNTQVLYR
GDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTMTLAELSAA
ALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDPRDTTTPRAMAQTL
RQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTAGDKTGSGDYGTTNDIAVIWPQGR
APLVLVTYFTQPQQNAESRRDVLASAARIIAEGL

And structure of CTX-M-9 betalactamase is given below;

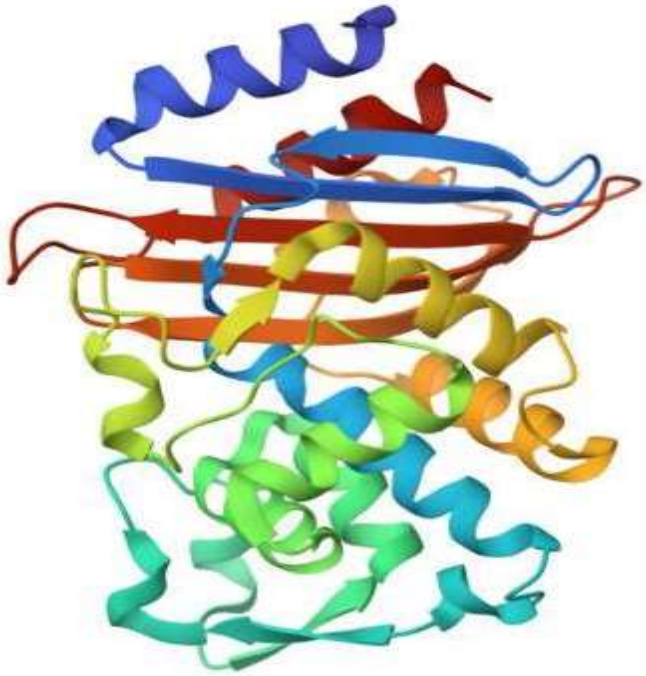


Figure 3: *E. coli* CTX-M-9 class A beta-lactamase

4.1.1 Homology modeling

The structure of CTX-M-9 class-A betalactamase generated by X-RAY DIFFRACTION with Resolution: 1.80 Å, we have done docking with in-silico approach which needs facilitation of homology modeling for determination of structure. The homology modeling done by Phyre2 server with Model dimensions (Å): X: 46.102 Y: 59.892 Z: 38.271. According to homology model by Phyre2 CTX-M-9 betalactamase is belong in beta-lactamase/transpeptidase-like superfamily with 100% confidence and 89% coverage.

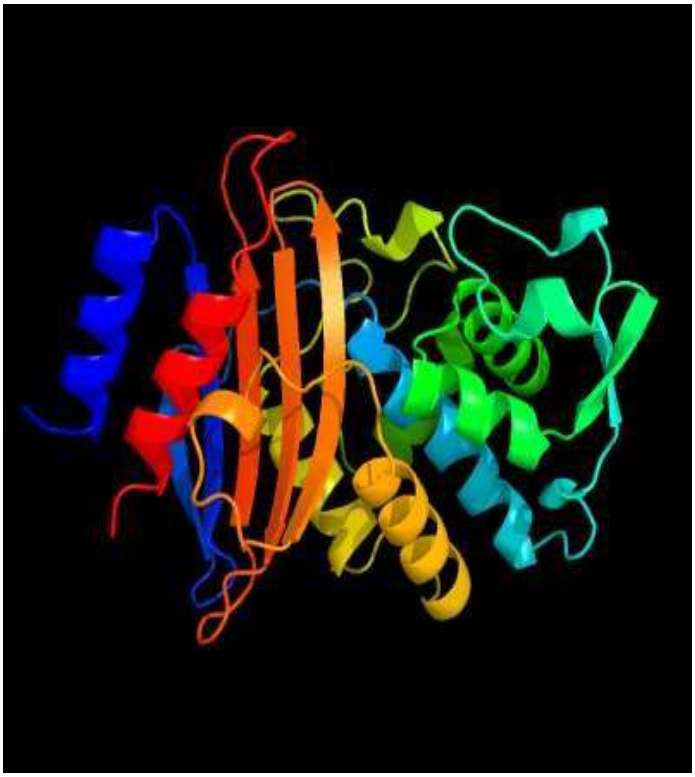


Figure 4: Homology modeled of CTX-M-9 Class A betalactamse of *E.coli* by Phyre2 server.

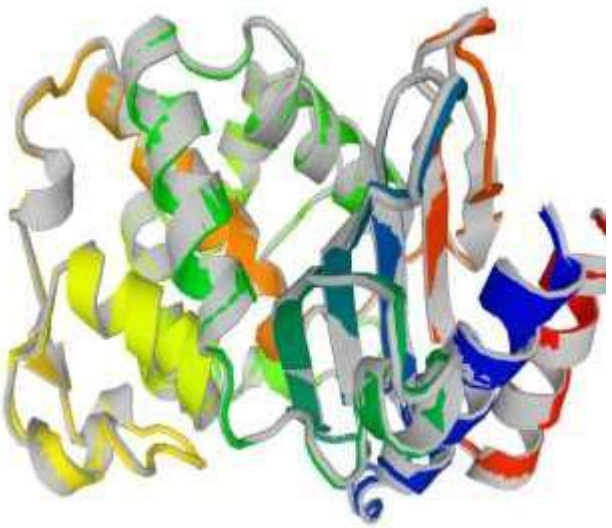


Figure 5: Refined structure by Galaxy refiner

4.2 Protein structure refinement and assessment by Galaxy Refiner and Swiss assessment

The modeled protein is then refined (figure-5) by using galaxy refiner. Protein structure refinement aims to bring moderately accurate template-based protein models closer to the native state through conformational sampling. The refined homology modeled CTX-M-9 class A betalactamase protein assessment was carried

out by SWISS-MODEL structure assessment tool. A Ramachandran plot is a method visualizing energetically favored regions for backbone dihedral angles of amino acid residues in protein structure. It analysis for the modeled CTX-M-9 protein revealed that 90.7% of the residues in the allowed regions confirming the accuracy of the modeled structure (figure-6).

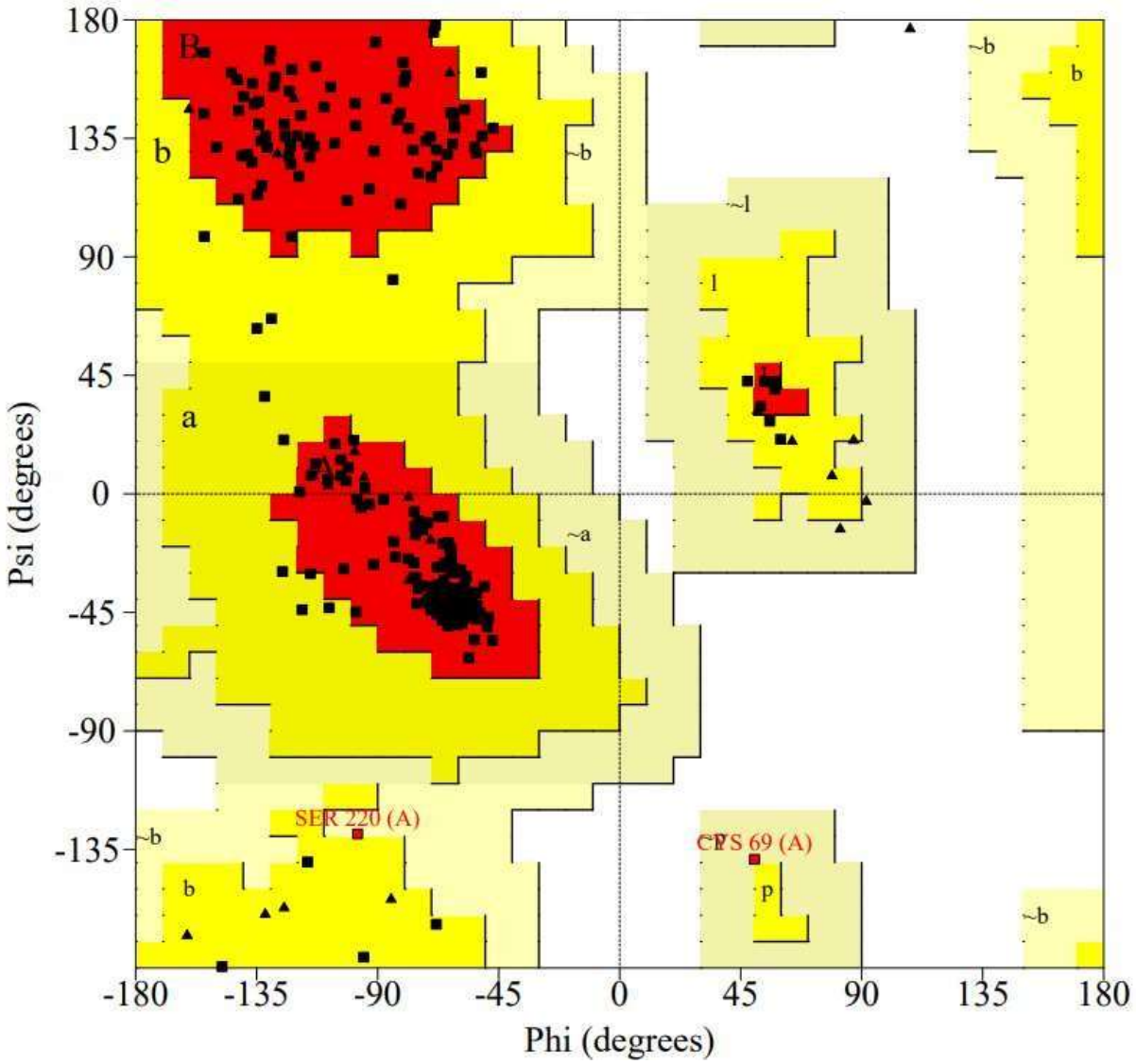


Figure 6: Ramachandran Plot (RAMPAGE: Residues in Most Favored Regions [A, B, L] = 90.7%, Residues in Additional and Generously Allowed Regions [a, b, l, p] = 8.4%, and Residues in Disallowed Regions = 0%. All Nonglycine and Proline Residues Are Shown as Filled Black Squares, Whereas Glycines (Non-end) Are Shown as Filled Black Triangles.

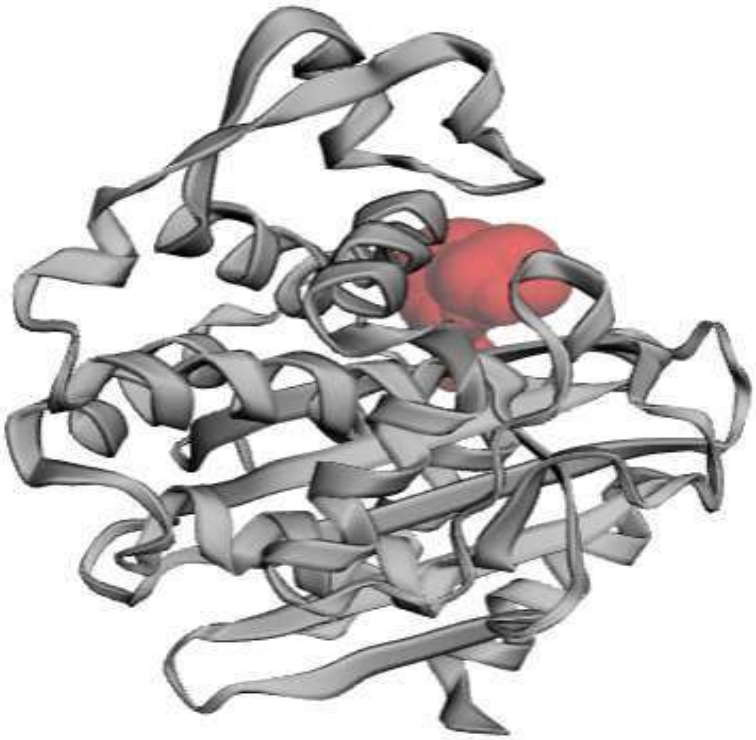
The statistical distribution of the combinations of the backbone dihedral angles υ and ψ that are permitted, unpermitted, and favorable was shown by the Ramachandran plot. The potential values of ϕ/ψ angles for an amino acid are represented by the plot's allowed regions. The distribution of υ/ψ values in a protein structure serves as the basis for structure validation. Dihedral angles seen in the prohibited areas of the Ramachandran plot indicate homology models of poor quality that depict the structural issue like clashes or steric hindrance are present. All of the permitted and preferred regions in the current model match the amino acid conformations and show no signs of steric hindrance. In the most preferred locations, a high-quality model should have a threshold value of more than 90%, where our protein occupied more than 90%.

4.3 Active site prediction

The protein CTX-M-9 class A betalactamase has active sites which was determined by CASTp(Computed Atlas of Surface Topography of proteins). Amino acids position in active site is in table -4. Active site and amino acid of active site is given in figure-7 and figure-8. Pocket ID, Area and Volume is given in Table-3.

Table 3: Volume and Area of best active site

Pocket ID	Area(SA)	Volume(SA)
1	129.080	78.212



A betalactamase in complex with G30

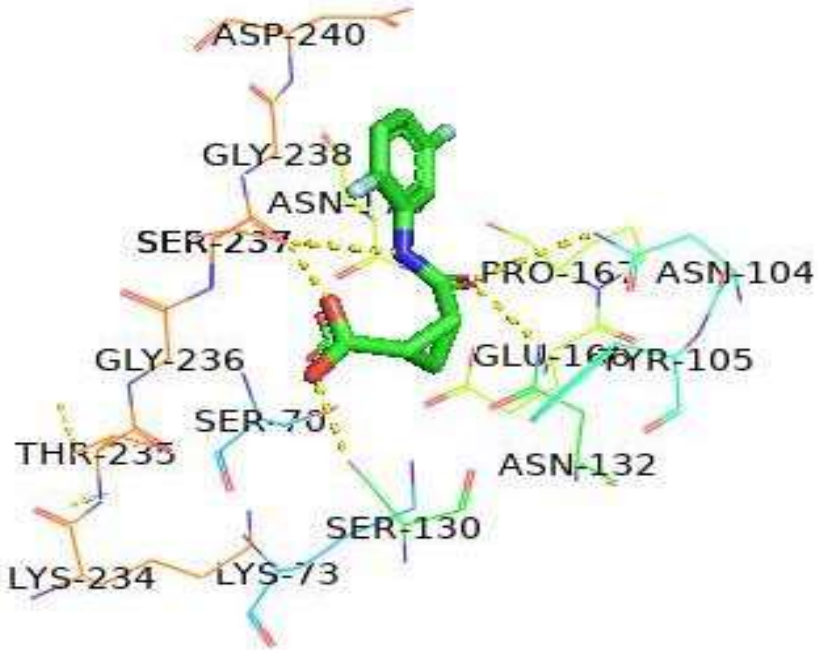


Figure 8: Amino acids in active site in complex with G30

Table 4: Active site amino acid and their position

Pocket ID	Chain	Sequence ID	Amino Acid
1	A	70	SER
1	A	104	ASN
1	A	105	TYR
1	A	129	TYR
1	A	130	SER
1	A	132	ASN
1	A	170	ASN
1	A	216	THR
1	A	220	SER
1	A	235	THR
1	A	236	GLY
1	A	237	SER
1	A	244	THR
1	A	245	ASN
1	A	276	ARG
1	A	279	LEU

4.4 List of Ligands with binding energy

To perform our study we have used ligands from ready to dock ligand database ZINC15. Approximately five hundreds ligands were collected from ZINC15 with criteria such as, 3D ready to dock tranches, ligands are standard and available in stock. pH of these ligands were neutral all value were standard. And most importantly ligands are drug-like molecules and have passed “Lipinski Rule of Five”. Among 500 ligands, best 60 ligands is given below (Table-6) with their binding energy or affinity. For control we also kept

Penicillins, Cephalosporins, Monobactam, Carbaphenem, Traditional Inhibitors and a Newer Inhibitor with their binding energy (table-5) . After considering the binding energy of protein-ligands interaction, pharmacokinetics and toxicity in human body, the ligands with higher binding energy and less toxicity were selected as potential inhibitor against CTX-M-9 class A betalactamase.

Table 5: Control drug and their binding energy with CTX-M-9 class A betalactamase of *E.coli*

Control compounds		**Binding Energy**
Penicillins	Benzylpenicillin(ZINC3871701)	-6.9
	Oxacillin(ZINC3875439)	-7.0
	Carbenicillin(ZINC3871880)	-7.6
Cephalosporins	Ceftriaxone(ZINC28467879)	-8.8
	Cefalotin(ZINC3830507)	-7.3
	Cefepime (ZINC3871923)	-8.1
	Cefoperazone (ZINC3830432)	-8.8
Monobactam	Aztreonam (ZINC3830264)	-8.8
Carbaphenem	Imipenem (ZINC4097225)	-5.6
Traditional Inhibitors	Clavulanate (ZINC3830569)	-6.4
	Sulbactam (ZINC897244)	-5.1
	Tazobactam (ZINC3787060)	-6.2
Newer Inhibitor	Relebactam (ZINC43206319)	-7.3

Table 6: List of best 60 ligands with their binding energ

Sr. No.	Ligand ID (ZINC 15)	Binding Energy
1	ZINC000853516030	-10.2
2	ZINC000575171306	-9.9
3	ZINC000811782179	-9.8
4	ZINC000685890317	-9.6
5	ZINC000514764724	-9.5
6	ZINC000114619616	-9.5
7	ZINC000003806288	-9.5
8	ZINC000044135896	-9.5
9	ZINC000034284849	-9.4
10	ZINC000555114280	-9.4
11	ZINC000811783254	-9.4
12	ZINC000022063671	-9.4
13	ZINC000811780992	-9.3
14	ZINC000811779605	-9.3
15	ZINC000000538119	-9.3
16	ZINC000811782810	-9.3
17	ZINC000571933834	-9.3
18	ZINC000044136143	-9.2
19	ZINC000303543061	-9.2
20	ZINC000579766740	-9.2
21	ZINC000289891555	-9.1
22	ZINC000811782792	-9.1
23	ZINC000816615556	-9.1
24	ZINC000117341031	-9.0
25	ZINC000811780886	-9.0
26	ZINC000114619614	-9.0
27	ZINC000329855499	-9.0
28	ZINC000811782228	-9.0
29	ZINC000848473943	-8.9
30	ZINC000840074718	-8.9

Sr. No.	Ligand ID (ZINC 15)	Binding Energy
31	ZINC000811783663	-8.9
32	ZINC000811782242	-8.9
33	ZINC000583665838	-8.9
34	ZINC000811603582	-8.9
35	ZINC000660565144	-8.9
36	ZINC000574208417	-8.9
37	ZINC000032093045	-8.8
38	ZINC000292761660	-8.8
39	ZINC000290907431	-8.8
40	ZINC000853510372	-8.8
41	ZINC000581556260	-8.8
42	ZINC000685890693	-8.8
43	ZINC000289640381	-8.7
44	ZINC000582638235	-8.7
45	ZINC000811782792	-8.7

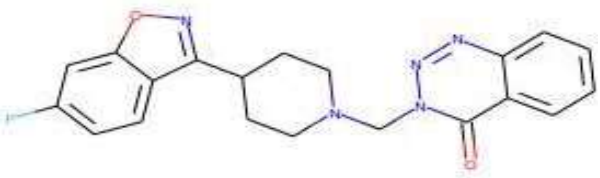
Sr. No.	Ligand ID (ZINC 15)	Binding Energy
46	ZINC000581102888	-8.7
47	ZINC000172242594	-8.7
48	ZINC000558729767	-8.7
49	ZINC000571217794	-8.6
50	ZINC000034284854	-8.6
51	ZINC000103233603	-8.6
52	ZINC000572056321	-8.6
53	ZINC000000589280	-8.6
54	ZINC000562028971	-8.5
55	ZINC000000786053	-8.5
56	ZINC000000002203	-8.5
57	ZINC000303543061	-8.5
58	ZINC000426775250	-8.5
59	ZINC000579766740	-8.5
60	ZINC000811783224	-8.5

10 among 60 ligands is given below (table7) which have relatively high binding energy.

Table 7: Best 10 ligands with their characteristics.

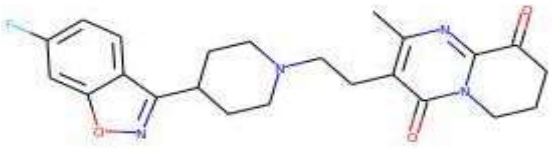
Sr. No.	ZINC15 ID	Molecular formula	Molecular weight(g/mol)	H bond donor	H bond acceptor	LogP
1	ZINC000811782179	C20H18FN5O2	379.395	0	7	2.909
2	ZINC000003806288	C22H20FN3O3	393.418	1	4	3.443
3	ZINC000044135896	C23H25FN4O3	424.476	1	6	3.231
4	ZINC000022063671	C24H25FN4O2	420.488	1	5	4.014
5	ZINC000811780992	C22H21FN4O2	392.434	0	6	3.822
6	ZINC000811779605	C19H18FN5O2	367.384	0	7	2.613
7	ZINC000000538119	C24H25FN4O2	420.488	1	5	4.014
8	ZINC000811782810	C20H18FN3O2S	383.448	0	6	4.18
9	ZINC000574208417	C23H20FN3O3	405.429	0	5	3.688
10	ZINC000811782792	C20H18FN5OS	395.463	0	7	4.278

Structure of these ten ligands is given (figure-9) below. Structure was retrieved from ZINC15 database. The quality of protein–ligand structures is of high importance for structure-based design since the information is used to guide synthetic strategies aimed at improving protein–ligand interactions.

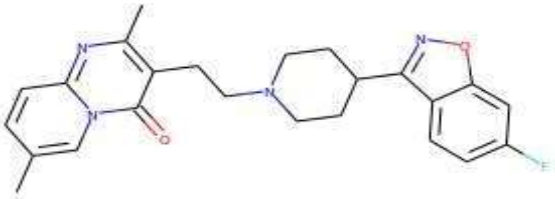


1. ZINC000811782179

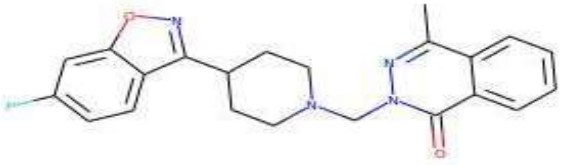
2. ZINC000003806288



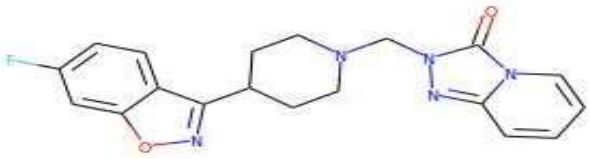
3. ZINC000044135896



4. ZINC000022063671

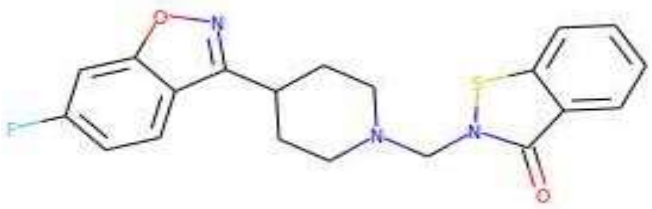


5. ZINC000811780992

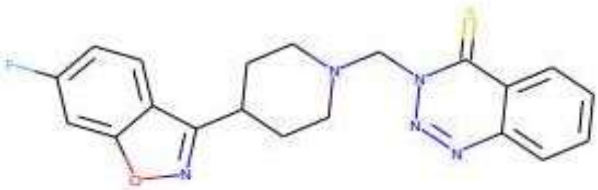


6. ZINC000811779605

7. ZINC000000538119



8. ZINC000811782810



9. ZINC000574208417

10. ZINC000811782792

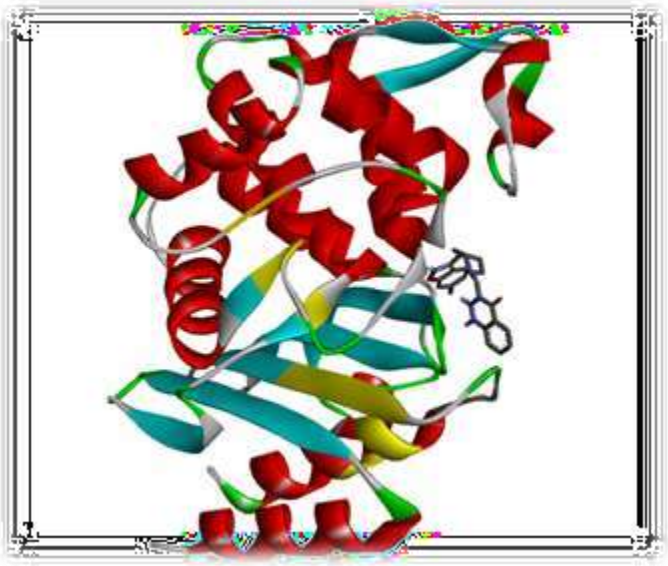
Figure 9: Structure of selected ten ligands

4.5 Molecular Docking and Validation of Docking

To find the potential ligands molecules molecular docking study has been run by PyRx software. For the formation of pdfqt format ligands, Open Babel has been used. After docking, PyRx software gives value of binding energy. The higher negative value of binding energy denotes the higher affinity. Among 60 of best binding energy of ligands suitable 10 were taken for further study. The pharmacokinetics (ADME) and toxicity analysis has been run after the docking. For the validation of docking Discovery Studio was exploited to reveal the binding site and suitable pose.

4.5.1 The docking of the Ligand 1(ZINC000811782179)

Ligand molecule-1(ZINC000811782179) has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.8. Ligand-1 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-1 bind with SER amino acid (position273) of CTX-M-9 betalactamase by conventional hydrogen bond(figure-10). A ligand can be bind with a single amino acid like Ligand-1 which bind to SER which located at position 273. Ligand bind with SER amino acid by strong hydrogen bond. Here figure-10 shows the 3D structure of protein-ligands interaction as well as 2D structure to get the perfect intuition of binding properties.



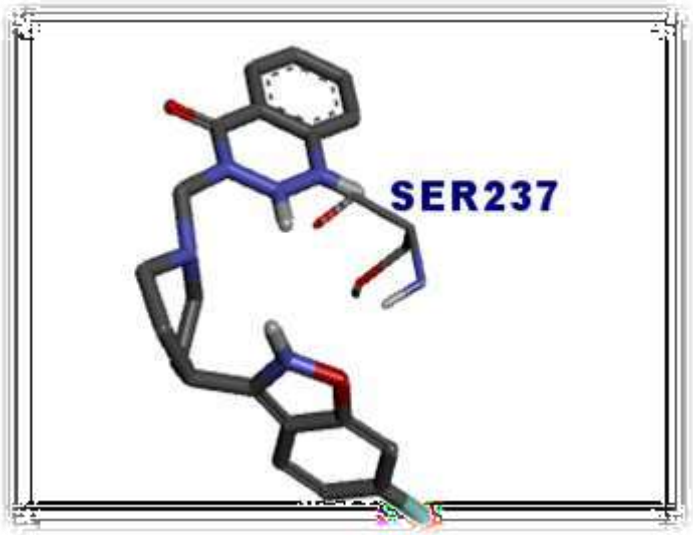


Figure 10: Observation of Ligand 1(ZINC000811782179) interaction with CTX-M-9 in 2D and 3D using Discovery studio

4.5.2 The docking of the Ligand 2(ZINC000003806288)

Ligand molecule-2(ZINC000003806288) has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.5. Ligand-2 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-2 bind with 4 amino acid (table-8) given below as well structure is given in figure 11.

Table 8: Interaction properties of Ligand 2(ZINC000003806288)

	Drug binding Amino acid residues	position	Bond types
Ligand 2(ZINC000003806288)	SER	237	Conventional Hydrogen Bond
	ASN	104	Conventional Hydrogen Bond
	ASP	240	Conventional Hydrogen Bond
	PRO	167	Pi-Alkyl

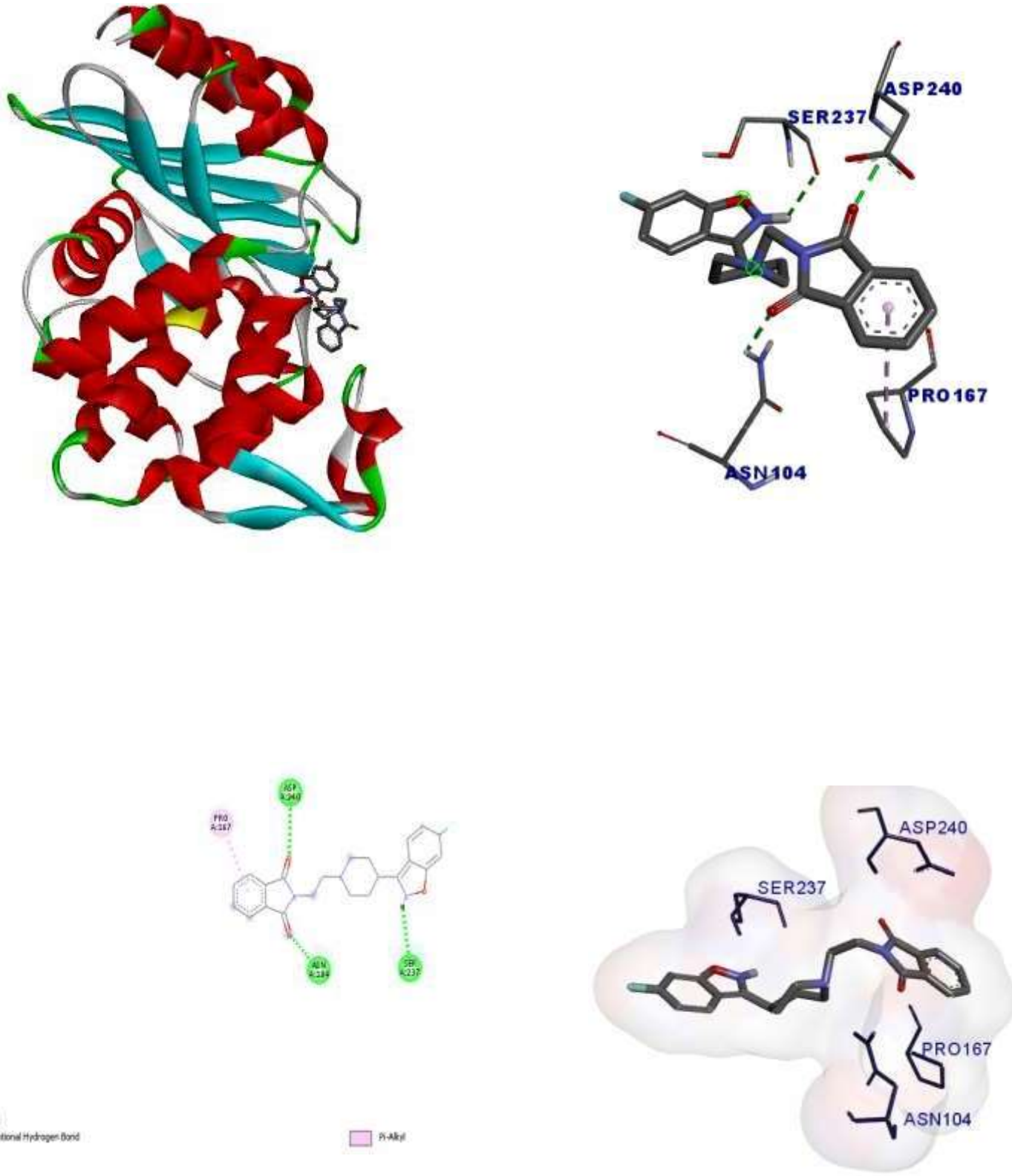


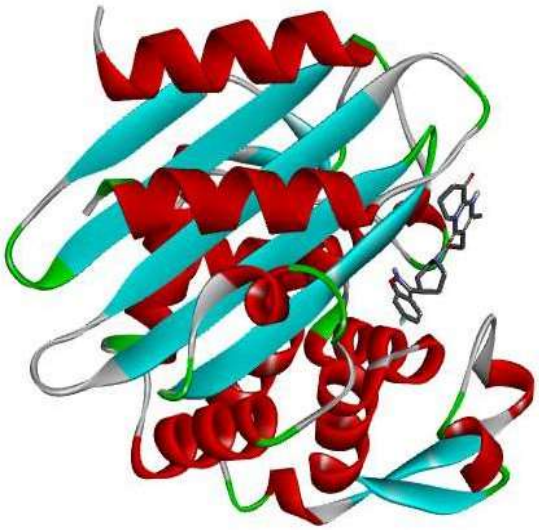
Figure 11: Observation of Ligand 2(ZINC000003806288) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

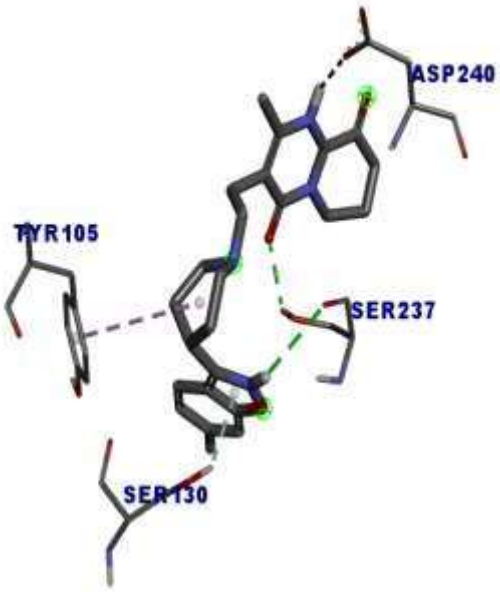
4.5.3 The docking of the Ligand 3(ZINC000044135896)

Ligand molecule-3(ZINC000044135896**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.5. Ligand-3 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-3 bind with 4 amino acid (table-9) given below as well structure is given in figure 12

Table 9: Interaction properties of Ligand 3(ZINC000044135896)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 3(ZINC000044135896)	SER	237	Conventional Hydrogen Bond
	ASP	240	Conventional Hydrogen Bond
	SER	237	Conventional Hydrogen Bond
	SER	130	Pi-Donor Hydrogen Bond
	TYR	105	Pi-Alkyl





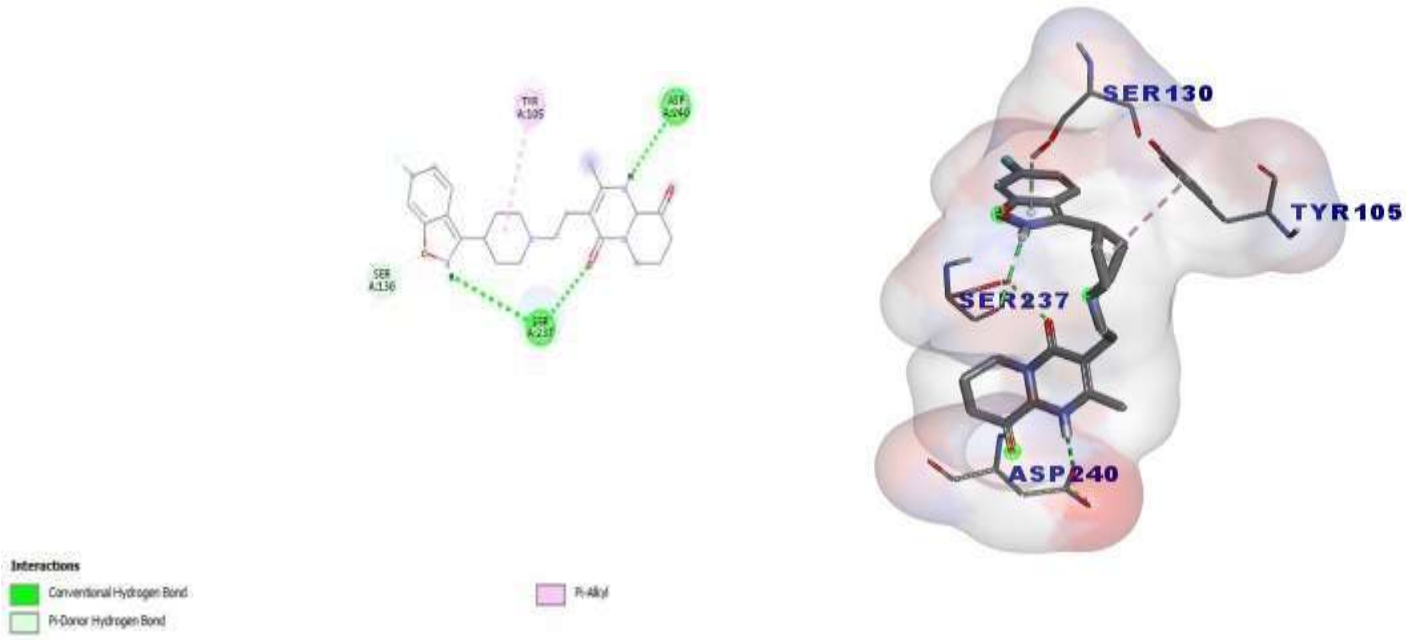


Figure 12: Observation of Ligand 3(ZINC000044135896) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

4.5.4 The docking of the Ligand 4(ZINC000022063671)

Ligand molecule-4(ZINC000022063671**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.4. Ligand-4 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-4 bind with 8 amino acid (table-10) given below as well structure is given in figure 13.

Table 10: Interaction properties of Ligand 4(ZINC000022063671)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 4(ZINC000022063671)	ASN	104	Conventional Hydrogen Bond
	ASN	170	Conventional Hydrogen Bond
	ASP	240	Conventional Hydrogen Bond
	SER	237	Conventional Hydrogen Bond
	SER	237	Carbon Hydrogen Bond
	PRO	167	Carbon Hydrogen Bond
	THR	216	Pi-Sigma
	ASP	240	Amide-Pi Stacked
	PRO	167	Alkyl
	TYR	105	Pi-Alkyl

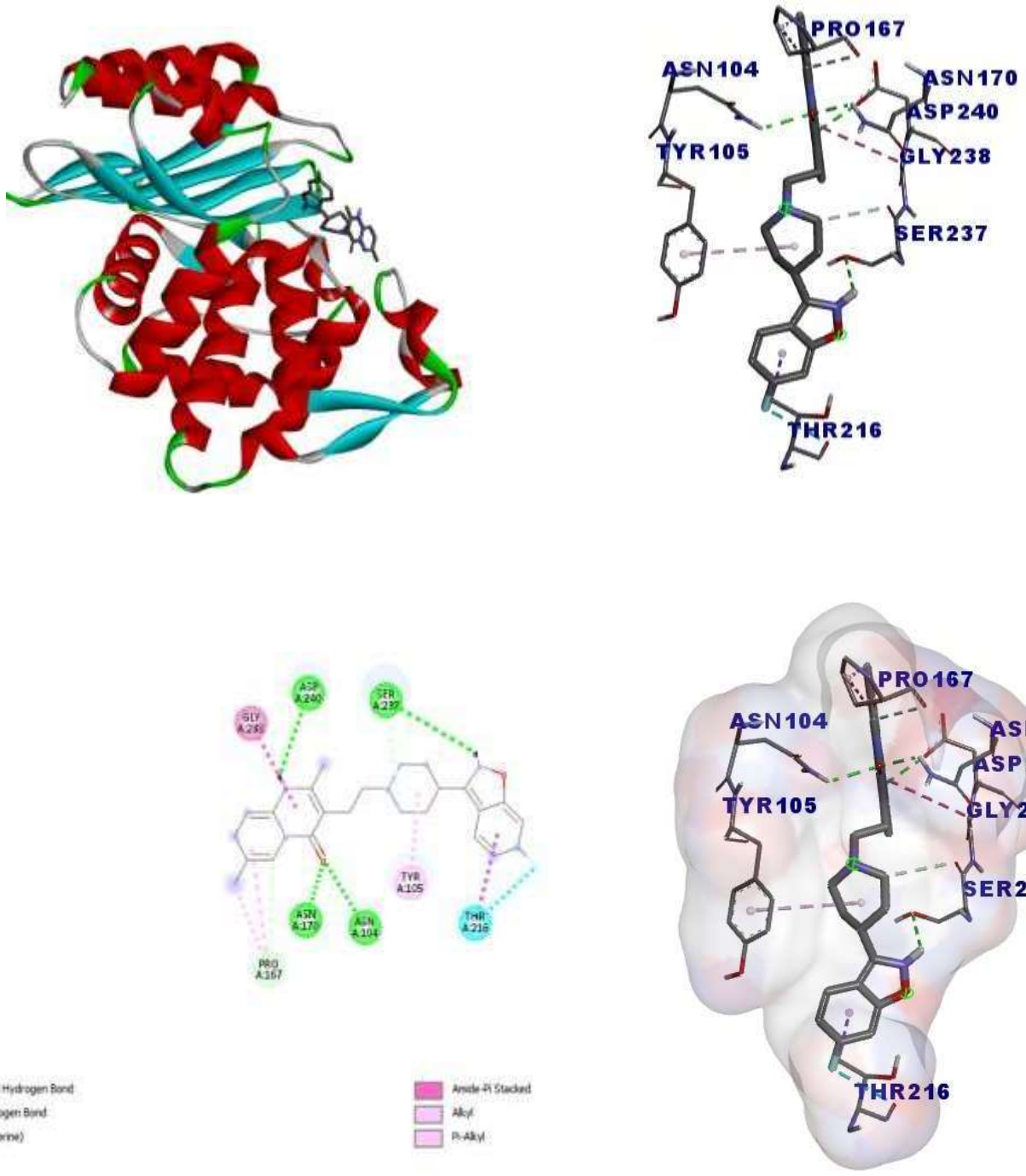


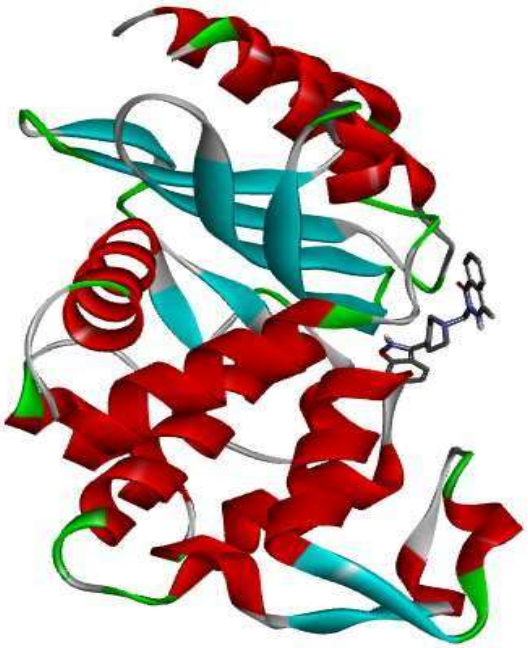
Figure 13: Observation of Ligand 4(ZINC000022063671) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

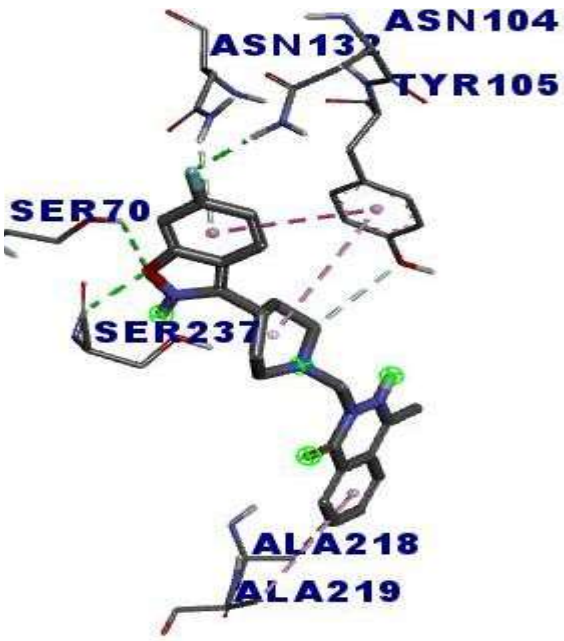
4.5.5 The docking of the Ligand 5(ZINC000811780992)

Ligand molecule-5(ZINC000811780992**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.3. Ligand-5 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-5 bind with 7 amino acid (table-11) given below as well structure is given in figure 14.

Table 11: Interaction properties of Ligand 5(ZINC000811780992)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 5(ZINC000811780992)	SER	70	Conventional Hydrogen Bond
	ASN	104	Conventional Hydrogen Bond; Halogen (Fluorine)
	SER	237	Conventional Hydrogen Bond
	TYR	105	Carbon Hydrogen Bond
	ASN	132	Pi-Donor Hydrogen Bond
	TYR	105	Pi-Pi Stacked
	ALA	218	Alkyl
	ALA	219	Alkyl
	TYR	105	Pi-Alkyl





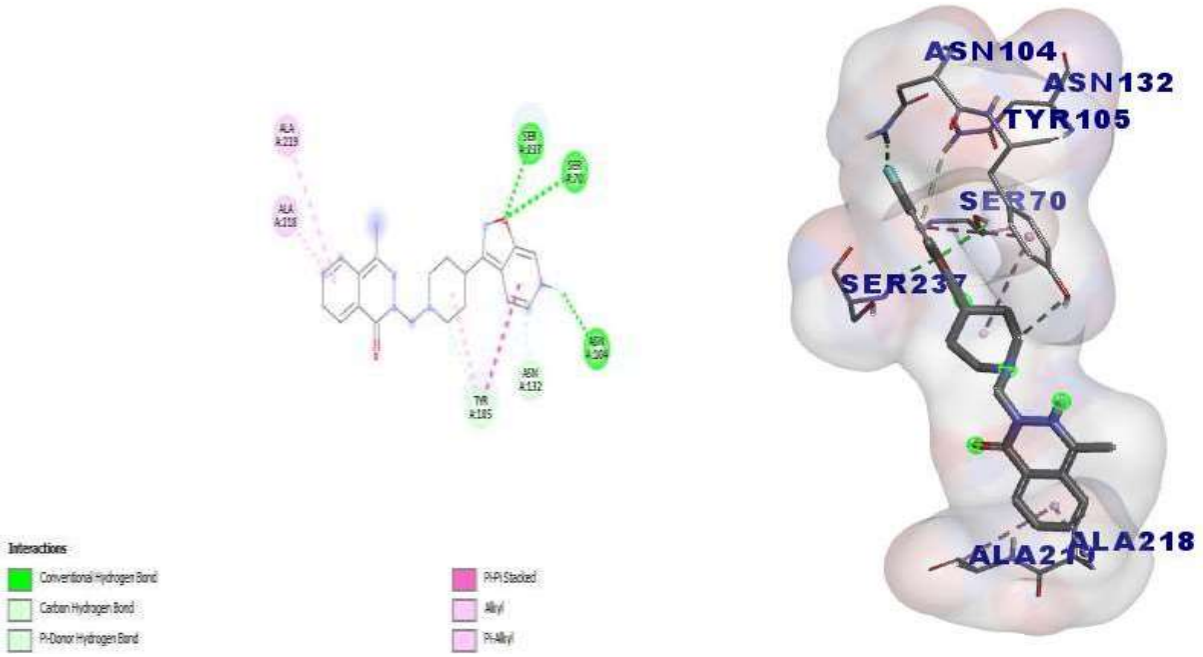


Figure 14: Observation of Ligand 5(ZINC000811780992) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

4.5.6 The docking of the Ligand 6(ZINC000811779605)

Ligand molecule-6(ZINC000811779605**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.3. Ligand-6 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-6 bind with 6 amino acid (table-12) given below as well structure is given in figure 15.

Table 12: Interaction properties of Ligand 6(ZINC000811779605)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 6(ZINC000811779605)	ASN	104	Conventional Hydrogen Bond; Halogen (Fluorine)
	SER	237	Conventional Hydrogen Bond
	ASN	132	Pi-Donor Hydrogen Bond
	ALA	218	Alkyl
	ALA	219	Alkyl
	TYR	105	Pi-Alkyl

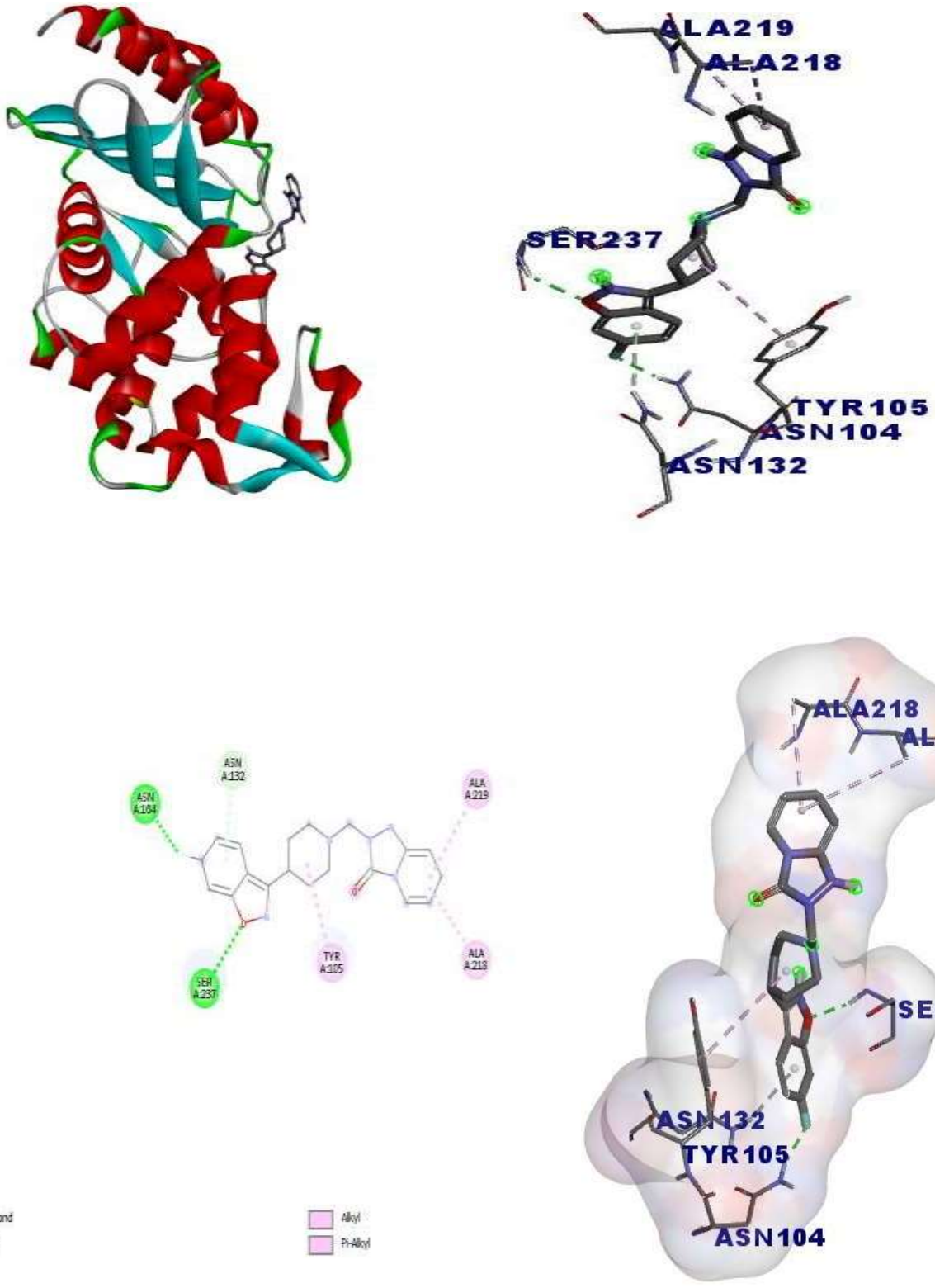


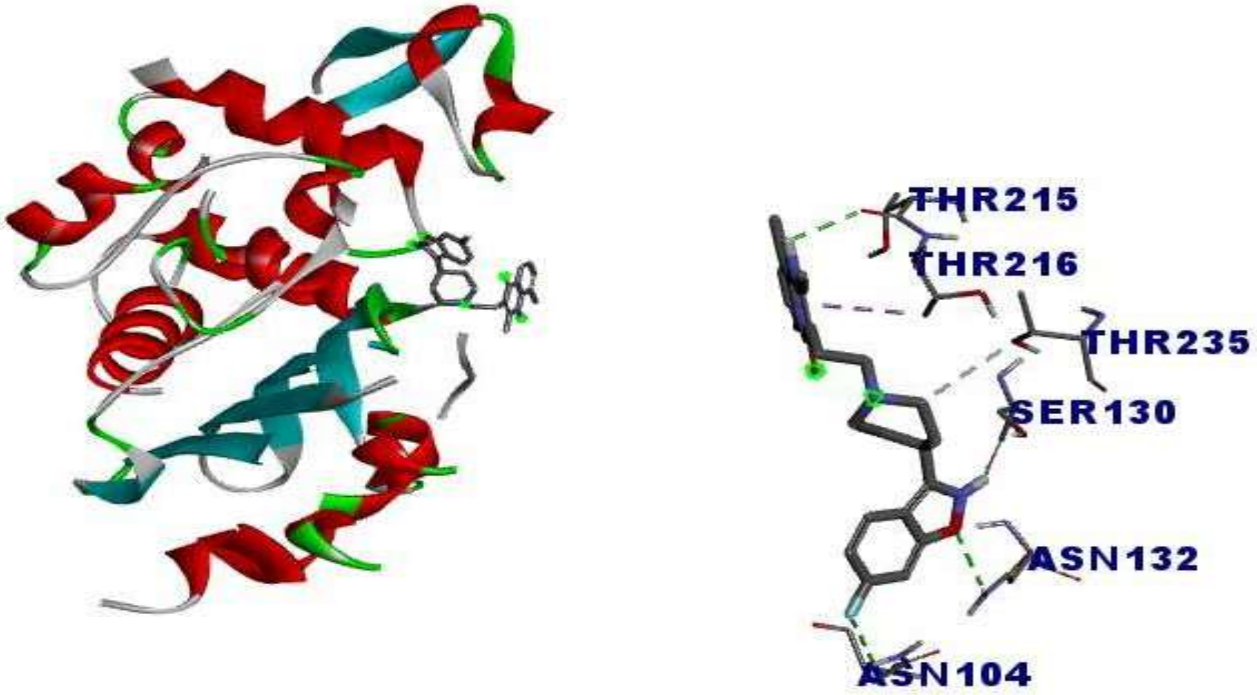
Figure 15: Observation of Ligand 6(ZINC000811779605) interaction with CTX-M-9 in 2D and 3D using Discovery studio

4.5.7 The docking of the Ligand 7(ZINC000000538119)

Ligand molecule-7(ZINC000811779605**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.3. Ligand-7 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-7 bind with 6 amino acid (table-13) given below as well structure is given in figure 16.

Table 13: Interaction properties of Ligand 7(ZINC000000538119)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 7(ZINC000000538119)	THR	215	Conventional Hydrogen Bond
	SER	130	Conventional Hydrogen Bond
	ASN	104	Conventional Hydrogen Bond; Halogen (Fluorine)
	ASN	132	Conventional Hydrogen Bond
	THR	235	Carbon Hydrogen Bond
	THR	216	Pi-Sigma



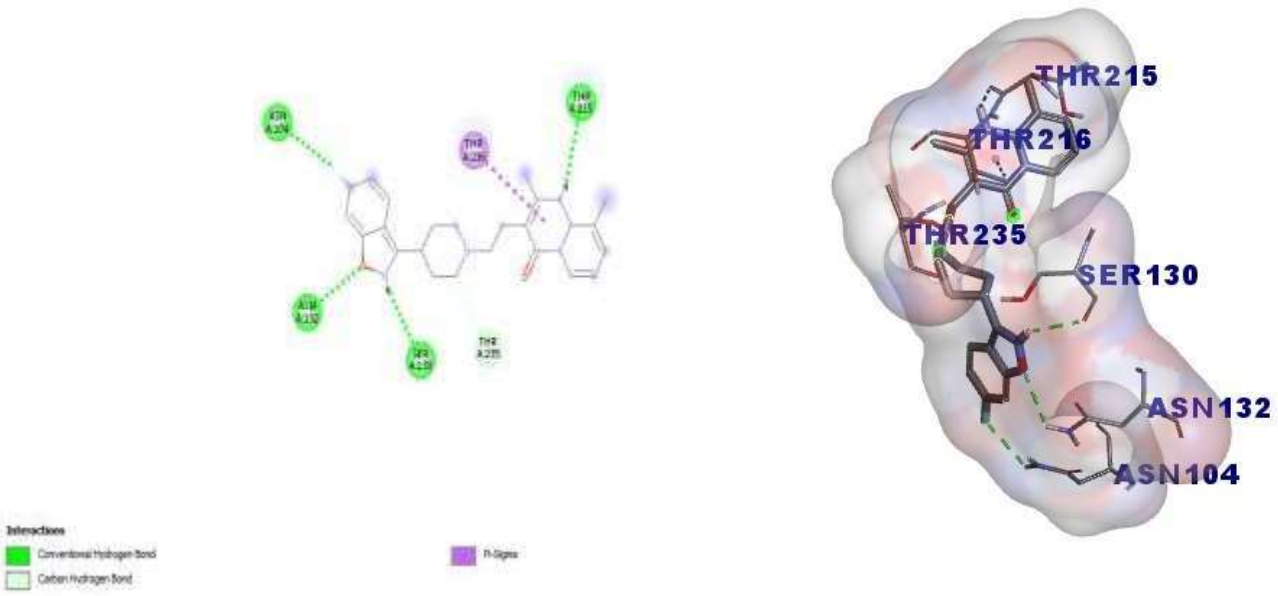


Figure 16: Observation of Ligand 7(ZINC000000538119) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

4.5.8 The docking of the Ligand 8(ZINC000811782810)

Ligand molecule-8(ZINC000811782810**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.3. Ligand-8 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli*. Ligand-8 bind with 10 amino acid (table-14) given below as well structure is given in figure 17.

Table 14: Interaction properties of Ligand 8(ZINC000811782810)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 8(ZINC000811782810)	SER	70	Conventional Hydrogen Bond
	ASN	104	Conventional Hydrogen Bond; Halogen (Fluorine)
	SER	237	Conventional Hydrogen Bond
	TYR	105	Carbon Hydrogen Bond
	ASN	170	Halogen (Fluorine)
	ASN	132	Pi-Donor Hydrogen Bond
	TYR	105	Pi-Pi Stacked
	THR	216	Amide-Pi Stacked
	ALA	218	Pi-Alkyl
	TYR	105	Pi-Alkyl

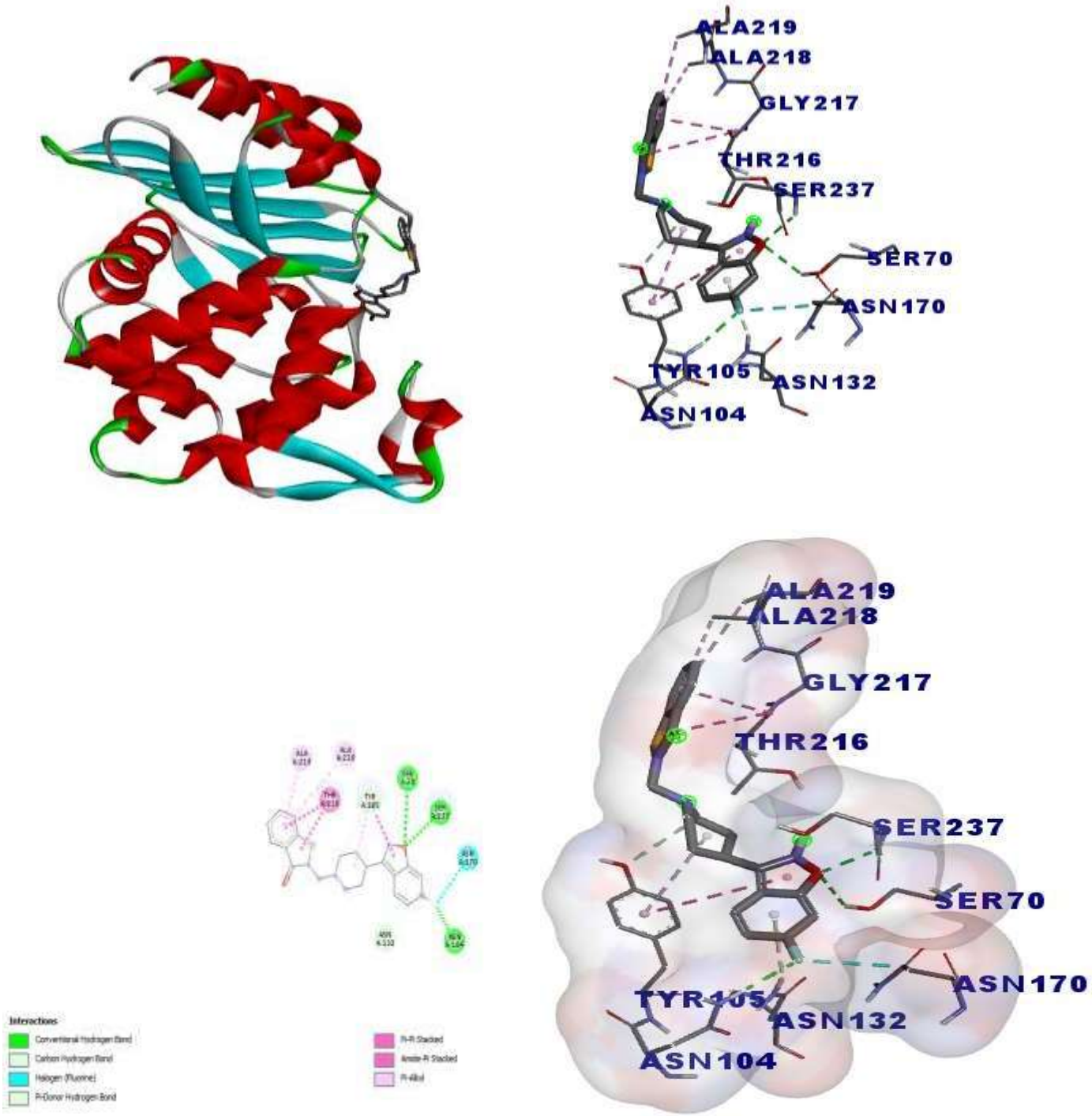


Figure 17: Observation of Ligand 8(ZINC000811782810) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

4.5.9 The docking of the Ligand 9(ZINC000574208417)

Ligand molecule-9(ZINC000574208417**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -8.9. Ligand-9 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli.* Ligand-9 bind with 2 amino acid (table-15) given below as well structure is given in figure 18.

Table 15: Interaction properties of Ligand 9(ZINC000574208417)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 9(ZINC000574208417)	ASP	240	**Pi-Anion**
	TYR	105	**Pi-Alkyl**

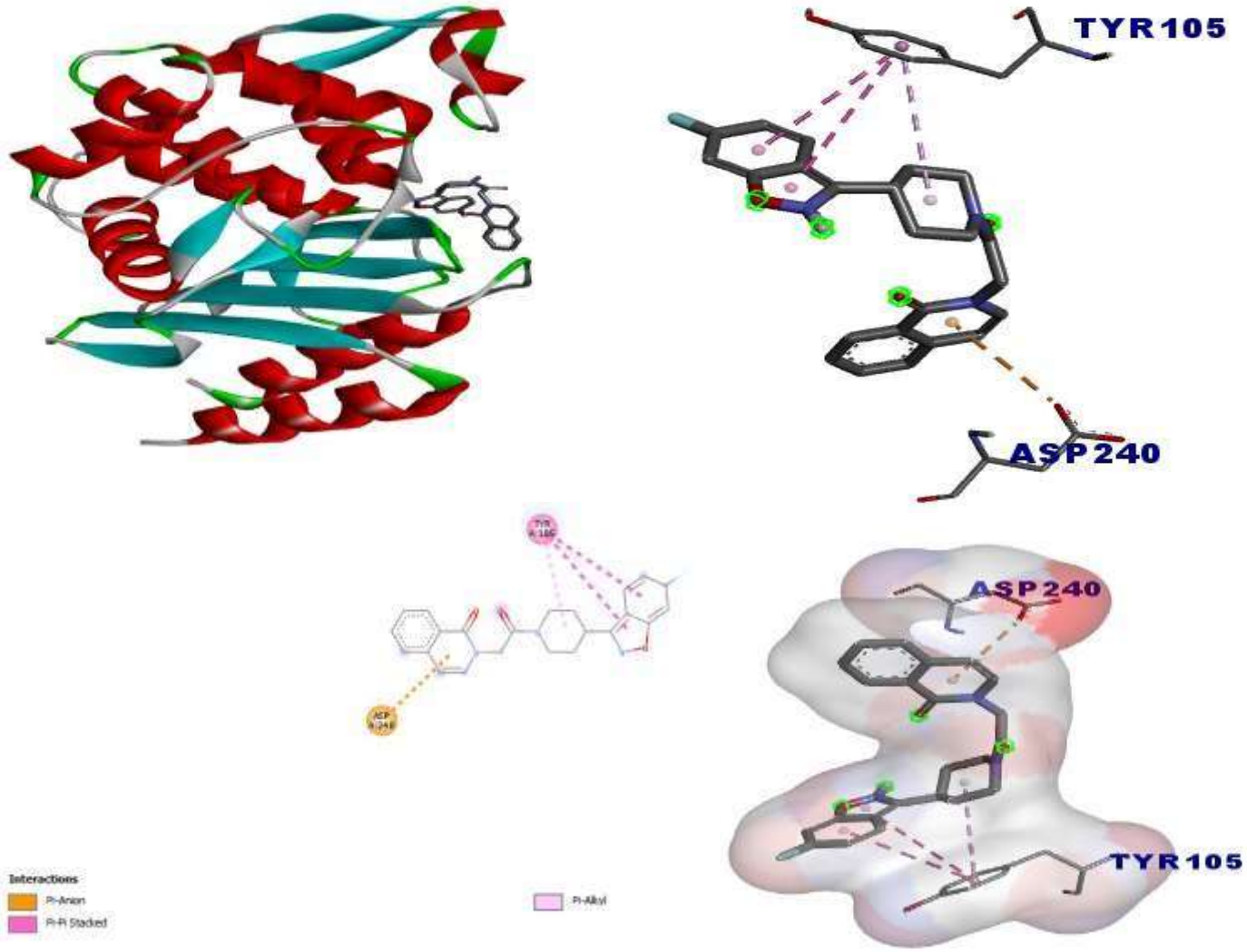


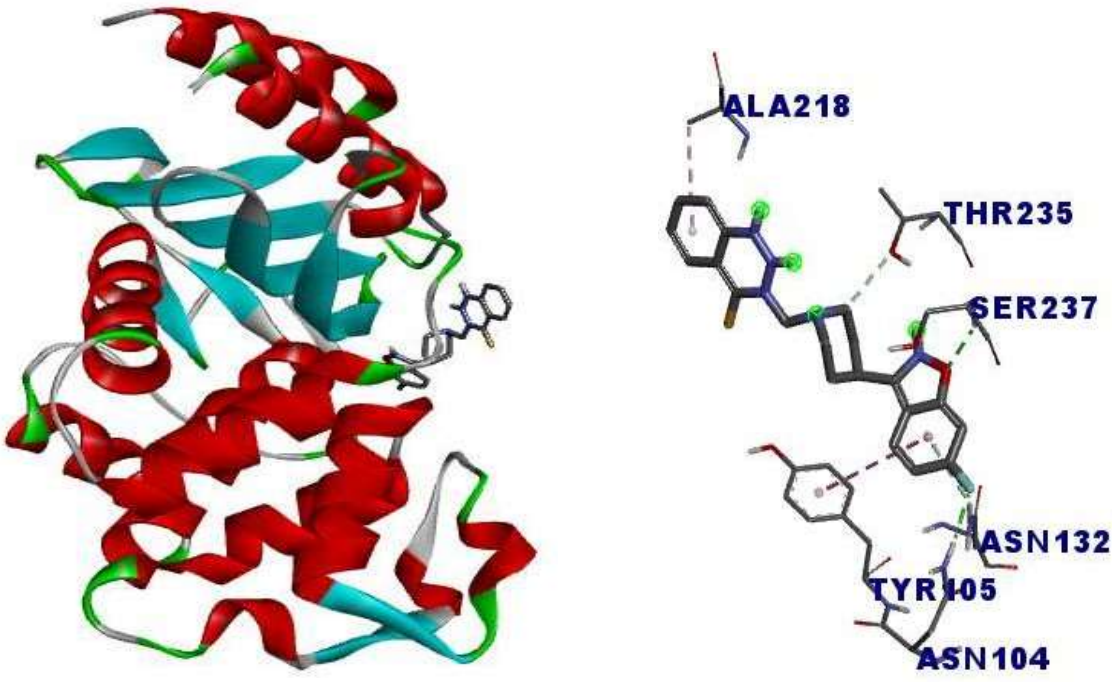
Figure 18: Observation of Ligand 9(ZINC000574208417) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

4.5.10 The docking of the Ligand 10(ZINC000811782792)

Ligand molecule-10(ZINC000811782792**)** has high affinity to target CTX-M-9 class A betalactamase and bind with binding energy -9.1. Ligand-10 could be a potential drug candidate against CTX-M-9 class betalactamase of *E.coli*. Ligand-10 bind with 6 amino acid (table-16) given below as well structure is given in figure 19.

Table 16: Interaction properties of Ligand 10(ZINC000811782792)

	Drug binding Amino acid residues	**position**	**Bond types**
Ligand 10(ZINC000811782792)	ASN	104	Conventional Hydrogen Bond; Halogen (Fluorine)
	SER	237	Conventional Hydrogen Bond
	THR	235	Carbon Hydrogen Bond
	ASN	132	Pi-Donor Hydrogen Bond
	TYR	105	Pi-Pi Stacked
	ALA	218	Alkyl



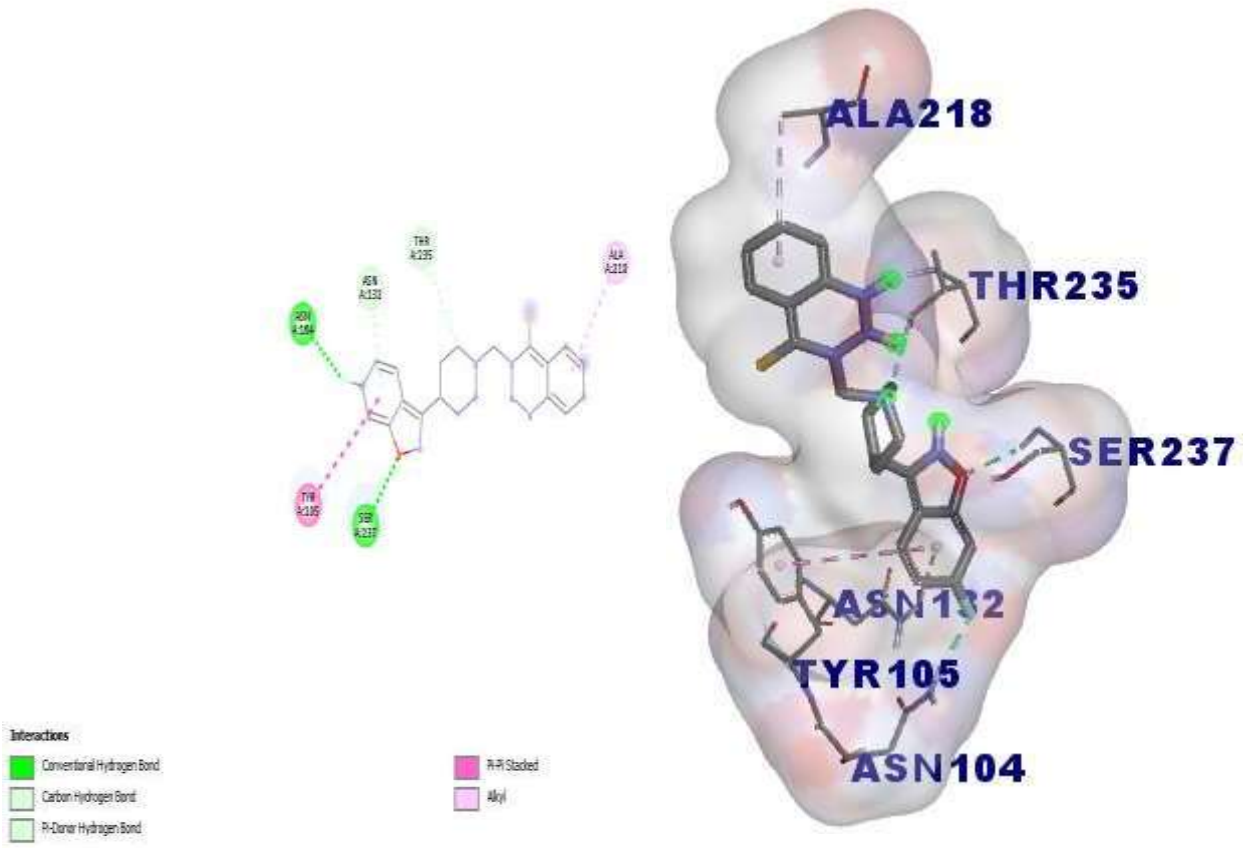


Figure 19: Observation of Ligand 10(ZINC000811782792) interaction with CTX-M-9 in 2D and 3D using Discovery studio.

4.6 Pharmacokinetics (ADME) and Toxicity analysis.

PkCSM server was exploited to analysis ADME/T properties of ligands. ADME/T test of ligands is very crucial as it show the pharmacokinetics and toxicity information of a ligands which are require for ligands to be a drug. ADME/T test are given in following table (table 17-27) of our selected ligands.

Table 17: ADME/T report of Ligand 1 (ZINC811782179)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.013**	Numeric (log mol/L)
	Caco2 permeability	**1.386**	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	**93.879**	Numeric (% Absorbed)
	Skin Permeability	**-2.751**	Numeric (log Kp)
	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**0.644**	Numeric (log L/kg)
	Fraction unbound (human)	**0.196**	Numeric (Fu)
	BBB permeability	**-0.785**	Numeric (log BB)
	CNS permeability	**-2.259**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**Yes**	Categorical (Yes/No)
Excretion	Total Clearance	**0.538**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**Yes**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**0.297**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**3.059**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**1.025**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.286**	Numeric (log ug/L)
	Minnow toxicity	**-1.406**	Numeric (log mM)

Table 18: ADME/T report of Ligand 2(ZINC000003806288)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.59**	Numeric (log mol/L)
	Caco2 permeability	**1.346**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**92.022**	Numeric (% Absorbed)
	Skin Permeability	**-2.985**	Numeric (log Kp)
	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**1.075**	Numeric (log L/kg)
	Fraction unbound (human)	**0.118**	Numeric (Fu)
	BBB permeability	**-0.1**	Numeric (log BB)
	CNS permeability	**-2.21**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**Yes**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**No**	Categorical (Yes/No)
	CYP2C19 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**Yes**	Categorical (Yes/No)
Excretion	Total Clearance	**0.813**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**Yes**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**0.006**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**2.565**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**1.099**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.333**	Numeric (log ug/L)
	Minnow toxicity	**1.849**	Numeric (log mM)

Table 19: ADME/T report of Ligand 3(ZINC000044135896)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.404**	Numeric (log mol/L)
	Caco2 permeability	**1.301**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**94.599**	Numeric (% Absorbed)
	Skin Permeability	**-2.779**	Numeric (log Kp)
	P-glycoprotein substrate	**No**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**0.697**	Numeric (log L/kg)
	Fraction unbound (human)	**0.301**	Numeric (Fu)
	BBB permeability	**-0.771**	Numeric (log BB)
	CNS permeability	**-2.885**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**Yes**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**No**	Categorical (Yes/No)
	CYP2C19 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C9 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**Yes**	Categorical (Yes/No)
Excretion	Total Clearance	**0.858**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**Yes**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**-0.131**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**2.912**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**0.981**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.289**	Numeric (log ug/L)
	Minnow toxicity	**0.004**	Numeric (log mM)

Table 20: ADME/T report of Ligand 4(ZINC000022063671)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.533**	Numeric (log mol/L)
	Caco2 permeability	**1.406**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**95.705**	Numeric (% Absorbed)
	Skin Permeability	**-2.759**	Numeric (log Kp)
	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**1.088**	Numeric (log L/kg)
	Fraction unbound (human)	**0.238**	Numeric (Fu)
	BBB permeability	**0.126**	Numeric (log BB)
	CNS permeability	**-2.768**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**Yes**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**Yes**	Categorical (Yes/No)
	CYP3A4 inhibitior	**Yes**	Categorical (Yes/No)
Excretion	Total Clearance	**0.672**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**Yes**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**-0.155**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**3.026**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**1.441**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.305**	Numeric (log ug/L)
	Minnow toxicity	**0.667**	Numeric (log mM)

Table 21: ADME/T report of Ligand 5(ZINC000811780992)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.78**	Numeric (log mol/L)
	Caco2 permeability	**1.384**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**94.332**	Numeric (% Absorbed)
	Skin Permeability	**-2.739**	Numeric (log Kp)
	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**0.527**	Numeric (log L/kg)
	Fraction unbound (human)	**0.207**	Numeric (Fu)
	BBB permeability	**0.01**	Numeric (log BB)
	CNS permeability	**-2.013**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**Yes**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.753**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**Yes**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**0.337**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**2.815**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**1.029**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.298**	Numeric (log ug/L)
	Minnow toxicity	**-1.011**	Numeric (log mM)

Table 22: ADME/T report of Ligand 6(ZINC000811779605)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.28**	Numeric (log mol/L)
	Caco2 permeability	**1.293**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**97.308**	Numeric (% Absorbed)
	Skin Permeability	**-2.872**	Numeric (log Kp)
	P-glycoprotein substrate	**No**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**No**	Categorical (Yes/No)
Distribution	VDss (human)	**0.512**	Numeric (log L/kg)
	Fraction unbound (human)	**0.304**	Numeric (Fu)
	BBB permeability	**-0.912**	Numeric (log BB)
	CNS permeability	**-2.923**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**Yes**	Categorical (Yes/No)
Excretion	Total Clearance	**0.639**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**-0.644**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**2.957**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**0.761**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.344**	Numeric (log ug/L)
	Minnow toxicity	**1.978**	Numeric (log mM)

Table 23: ADME/T report of Ligand 7(ZINC000000538119)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.456**	Numeric (log mol/L)
	Caco2 permeability	**1.159**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**95.587**	Numeric (% Absorbed)
	Skin Permeability	**-2.753**	Numeric (log Kp)
	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**0.987**	Numeric (log L/kg)
	Fraction unbound (human)	**0.308**	Numeric (Fu)
	BBB permeability	**0.178**	Numeric (log BB)
	CNS permeability	**-2.788**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**Yes**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**Yes**	Categorical (Yes/No)
Excretion	Total Clearance	**0.668**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**Yes**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**0.091**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**3.183**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**1.378**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.287**	Numeric (log ug/L)
	Minnow toxicity	**-0.985**	Numeric (log mM)

Table 24: ADME/T report of Ligand 8(ZINC000811782810)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.529**	Numeric (log mol/L)
	Caco2 permeability	**0.735**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**90.917**	Numeric (% Absorbed)
	Skin Permeability	**-2.781**	Numeric (log Kp)
	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**0.803**	Numeric (log L/kg)
	Fraction unbound (human)	**0.198**	Numeric (Fu)
	BBB permeability	**0.14**	Numeric (log BB)
	CNS permeability	**-1.823**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**Yes**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**Yes**	Categorical (Yes/No)
	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.699**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**0.236**	Numeric (log mg/kg/day)
	hERG I inhibitor	**Yes**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**3.143**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**1.816**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.31**	Numeric (log ug/L)
	Minnow toxicity	**-0.257**	Numeric (log mM)

Table 25: ADME/T report of Ligand 9(ZINC000574208417)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-4.052**	Numeric (log mol/L)
	Caco2 permeability	**1.386**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**94.774**	Numeric (% Absorbed)
	Skin Permeability	**-2.769**	Numeric (log Kp)
	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**0.266**	Numeric (log L/kg)
	Fraction unbound (human)	**0.09**	Numeric (Fu)
	BBB permeability	**-0.607**	Numeric (log BB)
	CNS permeability	**-1.975**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C9 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.459**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**0.151**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**2.245**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**2.113**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.348**	Numeric (log ug/L)
	Minnow toxicity	**-0.396**	Numeric (log mM)

Table 26: ADME/T report of Ligand 10(ZINC000811782792)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-4.351**	Numeric (log mol/L)
	Caco2 permeability	**1.01**	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	**91.16**	Numeric (% Absorbed)
	Skin Permeability	**-2.733**	Numeric (log Kp)
	P-glycoprotein substrate	**No**	Categorical (Yes/No)
	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**0.532**	Numeric (log L/kg)
	Fraction unbound (human)	**0.08**	Numeric (Fu)
	BBB permeability	**0.213**	Numeric (log BB)
	CNS permeability	**-1.955**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.567**	Numeric (log ml/min/kg)
	Renal OCT2 substrate	**Yes**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
	Max. tolerated dose (human)	**0.377**	Numeric (log mg/kg/day)
	hERG I inhibitor	**No**	Categorical (Yes/No)
	hERG II inhibitor	**Yes**	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	**2.671**	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	**0.758**	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	**Yes**	Categorical (Yes/No)
	Skin Sensitisation	**No**	Categorical (Yes/No)
	T.Pyriformis toxicity	**0.332**	Numeric (log ug/L)
	Minnow toxicity	**-0.876**	Numeric (log mM)

4.7 Discussion

For a medicine to be approved and be considered a feasible treatment for disease mitigation, ADME/T study is essential. Our ten ligands use the pkCSM server to display a range of ADME/T statistics. A comparison of the ligands' ADME/T studies is displayed in Table-27.

Table 27: Comparison of ADME/T among ligands

Ligands	Solubility (log mol/L)	Caco2 permeability(log Papp in 10-6 cm/s)	Total Clearance (log ml/min/kg)	Skin Permeability(logKp)	VDss (human) logL/Kg	LD50 (mol/kg)	LOAEL (log mg/kg)
1	-3.013	1.386	0.538	-2.751	0.644	3.059	1.025
2	-3.59	1.346	0.813	-2.985	1.075	2.567	1.099
3	-3.404	1.301	0.858	-2.779	0.697	2.912	0.981
4	-.533	1.406	0.672	-2.759	1.088	3.026	1.441
5	-3.78	1.334	0.753	-2.739	0.527	2.815	1.029
6	-3.28	1.293	0.639	-2.872	0.512	2.967	0.761
7	-3.456	1.159	0.668	-2.753	0.982	3.138	1.378
8	-3.529	0.735	0.699	-2.781	0.803	3.143	1.816
9	-4.052	1.386	0.459	-2.769	0.206	2.245	2.113
10	-4.351	1.01	0.567	-2.733	0.532	2.671	0.758

All ten ligands displayed hepatotoxicity, as well as inhibitors of p-glycoprotein I, p-glycoprotein II, and hERG II with the exception of ligand-6 which show doesn’t showed p- glycoprotein II inhibitor. Ligands 3, 6 and 10 did not exhibit any p-glycoprotein substrate; the remaining ligands did. Nine ligands did not exhibit a hERG I inhibitor, while ligands-8 did. Skin sensitivity and AMES toxicity were absent from all ligands. So some sort of ligand modification is necessary to reduce the toxicity.

Table 27 indicates that ligands 9 and 10 have low solubility in water, while the remaining solubility is moderate and they have good Caco2 permeability. However, the overall clearance value is lower, making it less ideal for quick removal. Every ligand increases the permeability of the skin. VDss shows that while all of the ligands disperse somewhat into tissues and plasma, ligands 2 and 4 diffuse extensively. All of the ligands are safe based on the lethal dose for 50% of the population, and all of them are associated with decreased toxicity except for 3, 6, and 10. This information is derived from Oral Rat Chronic Toxicity (LOAEL). Thus with some extent of modification, these ligands can be used as potential drug for treatment of *E.coli* bacteria infection.

Public health is seriously threatened by the advent of CTX-M class-A extended-spectrum β-lactamases, which confer resistance to second and third-generation cephalosporins. The β-lactam ring is hydrolyzed by CTX-M β-lactamases using a catalytic serine. One of the many beta-lactamases found in *E. coli*, CTX-M-9, is capable of breaking down monobactam and cephalosporin antibiotics and giving the bacteria tolerance to them. To evade such circumstances, there is need to design novel inhibitor against CTX-M-9 class A betalactamse of *E.coli.* The goal of this study was to find novel and potent ligand (inhibitor) molecules against CTX-M-9 betalactamse. In this study, ligands' properties analysis, ligand-protein interaction, molecular docking and ADME/T property analysis were applied successfully to propose ten novel ligands molecule against the CTX-M-9 class A betalactamase. From total 500 ligands molecules, we have taken best ten ligands that showed highest binding affinity with good ADME/T. But these ten ligands have some extent of toxicity. As the proposed ligands are following good pharmacokinetics and toxicity properties with greater binding affinity, they would inhibit the CTX-M-9 betalactamase. Consequently, the proposed ligands could be potential inhibitor against CTX-M-9 class A betalactamase of *E.coli* which are responsible for gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease.

In addition to CTX-M-9 class A betalactamas, *E. coli* bacteria also contain a variety of betalactamse to help them fight antibiotics. Every day, bacteria develop novel resistance mechanisms that render medications ineffective. A potential cure for bacterial infections in the future would be the creation of a new inhibitor against bacterial betalactamase. Both in vitro and in vivo research are needed for the creation and enhancement of a novel possible inhibitor against *E. Coli* CTX-M-9 class A betalactamase. Before being used in practice, potential candidates for in vitro analysis with good ADME/T should be tested in animal models.

1. MSD manual Overview of Bacteria Available from; https://www.msdmanuals.com/home/infections/bacterial-infections-overview/overview-of-bacteria (last accessed 04.05.2024).
2. Health direct Bacterial infections Available from; https://www.healthdirect.gov.au/bacterial-infections (last accessed 04.05.2034)
3. Top 14 Most Infectious and Deadliest Diseases Caused By Bacteria," in Bio Explorer, June 12, 2021, Available from:https://www.bioexplorer.net/bacterial-diseases.html/(last accessed 04.05.2024)
4. Doron, S., & Gorbach, S. L. Bacterial Infections: Overview. International Encyclopedia of Public Health, (2008) 273–282. https://doi.org/10.1016/B978-012373960-5.00596-7
5. Etebu, E., & Arikekpar, I. (2016). Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. Int. J. Appl. Microbiol. Biotechnol. Res, 4(2016), 90-101.
6. Bayarski, Y. (2006). Antibiotics and Their Types, Uses and Side Effects. Dari: http://hamiltoncountypreppers. org/. Diakses, 12.
7. Peterson L. R. (2008). Currently available antimicrobial agents and their potential for use as monotherapy. Clin Microbial. Infect. 14(6):30-45
8. Heesemann J. (1993). Mechanisms of resistance to beta-lactam antibiotics. Infection. 21(1):S4-9.
9. Pegler S. & Healy B. (2007). In patients allergic to penicillin, consider second and third generation cephalosporins for life threatening infections. BMJ. 335(7627): 991.
10. What Are the Most Common Antibiotics? Available from; https://www.healthline.com/health/antibiotics/names-of-common-antibiotics (last accessed 17.05.2024).
11. J.G. Bartlett, L.M. Mundy. (1995), Community-acquired pneumonia N Engl J Med, 333, pp. 1618-1624
12. A.W. Karchmer. (1991), Staphylococcus aureus and Vancomycin: the Sequel Ann Intern Med, 115, pp. 739-741
13. W. Newman, J.M. Torres, J.K. (1954), Guck Bacterial endocarditis: an analysis of fifty-two cases Am J Med, 16 ,pp. 535-542
14. E.F. Hirsch. (2008), The treatment of infected wounds, Alexis Carrel’s contribution to the care of wounded soldiers during world war I J Trauma Acute Care Surg, 64, pp. S209-S210
15. P.M. Hawkey. (2008), The growing burden of antimicrobial resistance J Antimicrob Chemother, 62 (s1), pp. i1-i9

16. Government of Canada Antibiotic (Antimicrobial) resistance Available from; https://www.canada.ca/en/public-health/services/antibiotic-antimicrobial-resistance/impacts-antibiotic-resistance.html (last accessed 04.05.2024)
17. Cleveland Clinic Antibiotic Resistance Available from; https://my.clevelandclinic.org/health/articles/21655-antibiotic-resistance (last accessed 04.05.2024)
18. Antimicrobial resistance," in WHO, 21 November 2023, Available from; https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance#:~:text=As%20a%20result%20of%20drug,through%20genetic%20changes%20in%20pathogens (last accessed 04.03.2024)
19. G.M. Eliopoulos, S.E. Cosgrove, Y. Carmeli. (2003), The impact of antibacterial resistance on health and economic outcomes Clin Infect Dis, 36, pp. 1433-1437
20. Tipper DJ, Strominger JL.b (1965), Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proceedings of the National Academy of Sciences of the United States of America, 54(4):1133-41.
21. Chen Y, Zhang W, Shi Q, Hesek D, Lee M, Mobashery S, et al. (2009), Crystal structures of penicillin-binding protein 6 from Escherichia coli. J Am Chem Soc, 131(40):14345-54. Epub 2009/10/08. doi: 10.1021/ja903773f. PubMed PMID: 19807181.
22. Silvaggi NR, Anderson JW, Brinsmade SR, Pratt RF, Kelly JA. (2003), The crystal structure of phosphonate-inhibited D-Ala-D-Ala peptidase reveals an analogue of a tetrahedral transition state. Biochemistry, 42(5):1199-208.
23. Tipper DJ, Strominger JL. (1965), Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proc Natl Acad Sci U S A, 54(4):1133-41.
24. Bush K, Jacoby GA, Medeiros AA. (1995), A functional classification scheme for betalactamases and its correlation with molecular structure. Antimicrob Agents Chemother, 39(6):1211-33.
25. Livermore DM. (1995), beta-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev, 8(4):557-84. PubMed PMID: 0008665470.
26. Delmas J, Leyssene D, Dubois D, Birck C, Vazeille E, Robin F, et al. Structural insights into substrate recognition and product expulsion in CTX-M enzymes. J Mol Biol. 2010;400(1):108-20. Epub 2010/05/11. doi: S0022-2836(10)00463-8 [pii] 10.1016/j.jmb.2010.04.062. PubMed PMID: 20452359.

27. Bradford PA. (2001), Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev, 14(4):933-51. PubMed PMID: 11585791.
28. Tsao SG, Brunk CF, Pearlman RE. (1983), Hybridization of nucleic acids directly in agarose gels. Anal Biochem, 131(2):365-72. [PubMed ID: 6193737].
29. Safari M, Shojapour M, Akbari M, Pourbabaee A, Abtahi H. (2013), Dissemination of CTX-M-Type Beta-lactamase Among Clinical Isolates of Enterobacteriaceae in Markazi Province, Iran. Jundishapur J Microbiol, 6(8):e7182. https://doi.org/10.5812/jjm.7182.
30. Dutour C, Bonnet RMarchandin H, Boyer M, Chanal C, Sirot D, Sirot J.(2002), CTX-M-1, CTX-M-3, and CTX-M-14 β-Lactamases from Enterobacteriaceae Isolated in France. Antimicrob Agents Chemother46:.https://doi.org/10.1128/aac.46.2.534 -537.2002
31. Bonnet, R., J. L. M. Sampaio, R. Labia, C. De Champs, D. Sirot, C. Chanal, and J. Sirot. (2000), A novel CTX-M β-lactamase (CTX-M-8) in cefotaxime-resistant Enterobacteriaceae isolated in Brazil. Antimicrob. Agents Chemother.44:1936 –1942.
32. Silvaggi NR, Anderson JW, Brinsmade SR, Pratt RF, Kelly JA. (2003), The crystal structure of phosphonate-inhibited D-Ala-D-Ala peptidase reveals an analogue of a tetrahedral transition state, Biochemistry, 42(5):1199-208.
33. 28. I.I. Shabana, A.T. Al-Enazi. (2020), Investigation of plasmid-mediated resistance in E. coli isolated from healthy and diarrheic sheep and goats, Saudi Journal of Biological Sciences, 27, pp. 788-796, https://doi.org/10.1016/j.sjbs.2020.01.009.
34. Thomas M, Hooton MD. (2012) Uncomplicated urinary tract infection. N Engl J Med, 366:1028–37. doi: 10.1056/NEJMcp1104429.
35. Dormingy JA, Nabeth P, Juergens-Behr A, Perrier-Gros-Claude JD. (2005), Risk factors for antibiotic resistance Escherchia coli isolated from community–aquired urinary tract infections in Dakar, Senegal. J Antimicrob Chemother, 56(1):236–9. doi: 10.1093/jac/dki158.
36. Ko, J., Park, H., Heo, L., & Seok, C. (2012). GalaxyWEB server for protein structure prediction and refinement. Nucleic acids research, 40(W1), W294-W297.
37. 14. N.D. Friedman, E. Temkin, Y. Carmeli, (2016), The negative impact of antibiotic resistance, Clinical Microbiology and Infection, 22, Pages 416-422, https://doi.org/10.1016/j.cmi.2015.12.002.
38. Campbell NA, Reece JB. Biology. (2002), San Francisco: Pearson Education Inc.
39. Feng P, Weagant S, Grant M. (2007), Enumeration of Escherichia coli and the Coliform Bacteria. Bacteriological Analytical Manual. 8th ed. USA: FDA/Center for Food Safety & Applied Nutrition.

40. Basavaraju, M., & Gunashree, B. S. (2023). Escherichia coli: An Overview of Main Characteristics. IntechOpen. doi: 10.5772/intechopen.105508.
41. Kaper J. B., Nataro J. P., Mobley H. L. (2004). Pathogenic Escherichia coli. Nat. Rev. Microbiol. 2 123–140. 10.1038/nrmicro818.
42. Qadri F., Svennerholm A. M., Faruque A. S., Sack R. B. (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin. Microbiol. Rev. 18 465–483. 10.1128/CMR.18.3.465-483.2005.
43. Bajaj, P., Singh, N. S., & Virdi, J. S. (2016). Escherichia coli β-Lactamases: What Really Matters. Frontiers in microbiology, 7, 417. https://doi.org/10.3389/fmicb.2016.00417 .
44. Santajit S, Indrawattana N. (2016) Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. Biomed Res Int, 2016:2475067. doi: 10.1155/2016/2475067. Epub 2016 May 5. PMID: 27274985; PMCID: PMC4871955.
45. Feng P, Weagant S, Grant M. (2007), Enumeration of Escherichia coli and the Coliform Bacteria. Bacteriological Analytical Manual. 8th ed. USA: FDA/Center for Food Safety & Applied Nutrition.
46. Campbell NA, Reece JB. Biology. (2002), San Francisco: Pearson Education Inc.
47. Basavaraju, M., & Gunashree, B. S. (2023). Escherichia coli: An Overview of Main Characteristics. IntechOpen. doi: 10.5772/intechopen.105508
48. Kaper J. B., Nataro J. P., Mobley H. L. (2004). Pathogenic Escherichia coli. Nat. Rev. Microbiol. 2 123–140. 10.1038/nrmicro818
49. Qadri F., Svennerholm A. M., Faruque A. S., Sack R. B. (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin. Microbiol. Rev. 18 465–483. 10.1128/CMR.18.3.465-483.2005
50. Bajaj, P., Singh, N. S., & Virdi, J. S. (2016). Escherichia coli β-Lactamases: What Really Matters. Frontiers in microbiology, 7, 417. https://doi.org/10.3389/fmicb.2016.00417
51. Bonnet R. (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. 48 1–14. 10.1128/AAC.48.1.1-14.2004
52. Bradford P. A. (2001). Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. 14 933 – 951. 10.1128/CMR.14.4.933-951.2001
53. Philippon A., Arlet G., Jacoby G. A. (2002). Plasmid-determined AmpC-type β-lactamases. Antimicrob. Agents Chemother. 46 1–11. 10.1128/AAC.46.1.1-11.2002
54. Livermore D. M. (2009). Has the era of untreatable infections arrived? J. Antimicrob. Chemother. Suppl. 1 I29–I36. 10.1093/jac/dkp255

55. Kumarasamy K. K., Toleman M. A., Walsh T. R., Bagaria J., Butt F., Balakrishnan R., et al. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect. Dis. 10 597–602. 10.1016/S1473-3099(10)70143-2
56. Nordmann P., Naas T., Poirel L. (2011), Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg. Infect. Dis. 17 1791–1798. 10.3201/eid1710.110655
57. Bauernfeind A., Grimm H., Schweighart S. (1990). A new plasmidic cefotaximase in a clinical isolate of Escherichia coli. Infection 18 294–298. 10.1007/BF01647010
58. I.I. Shabana, A.T. Al-Enazi. (2020), Investigation of plasmid-mediated resistance in E. coli isolated from healthy and diarrheic sheep and goats, Saudi Journal of Biological Sciences, 27, pp. 788-796, https://doi.org/10.1016/j.sjbs.2020.01.009.
59. MICROBIOLOGY SOCIETY, THE HISTORY OF ANTIBIOTICS, from; https://microbiologysociety.org/membership/membership-resources/outreach-resources/antibiotics-unearthed/antibiotics-and-antibiotic-resistance/the-history-of-antibiotics.html (last accessed 06.05.2024)
60. H. Otten (1986), Domagk and the development of the sulphonamides J Antimicrobial Chemother, 17 pp. 689-690
61. A. Fleming. (1929), On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenza Br J Exp Pathol, 10. pp. 226-236
62. 35. M. I. Hutchings, A. W Truman, B. Wilkinson. (2019), Antibiotics: past, present and future, Current Opinion in Microbiology, 51, pp. 72-80, https://doi.org/10.1016/j.mib.2019.10.008.
63. D.M. Shlaes, D.N. Gerding, J.F. John, W.A. Craig, D.L. Bornstein, R.A. Duncan, et al. (1997), Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: guidelines for the prevention of antimicrobial resistance in hospitals Clin Infect Dis, 25, pp. 584-599
64. W.A. Bolhofer, J.C. Sheehan, E.L.A. (1960), Abrams Totally synthetic penicillins J Am Chem Soc, 82, pp. 3437-3441
65. M.C. Enright, D.A. Robinson, G. Randle, E.J. Feil, H. Grundmann, B.G. Spratt (2002), The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA) Proc Natl Acad Sci USA, 99, pp. 7687-7692
66. Paterson, D. L., and Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: a clinical update. Clin. Microbiol. Rev. 18, 657–686. doi: 10.1128/CMR.18.4.657- 686.2005

67. Bauernfeind, A., Grimm, H., and Schweighart, S. (1990). A new plasmidic cefotaximase in a clinical isolate of Escherichia coli. Infection 18, 294–298. doi:10.1007/BF01647010
68. Nicolas-Chanoine, M. H., Blanco, J., Leflon-Guibout, V., Demarty, R., Alonso, M. P., Caniça, M. M., et al. (2008). Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J. Antimicrob. Chemother. 61, 273–281. doi: 10.1093/jac/dkm46
69. Freeman, J. T., Williamson, D. A., Heffernan, H., Smith, M., Bower, J. E., and Roberts, S. A. (2012). Comparative epidemiology of CTX-M-14 and CTX-M-15 producing Escherichia coli: association with distinct demographic groups in the community in New Zealand. Eur. J. Clin. Microbiol. Infect. Dis. 31, 2057–2060. doi: 10.1007/s10096-011-1540-3
70. Zhao, W.-D.; Yan, P.; Guan, H.-N.; Zhang, Q.-Z. (2014) Characterization of CTX-M-type extended-spectrum beta-lactamase in clinical clones of Escherichia coli in Southwest China. J. Basic Microbiol. 54.
71. Pitout J.D., Laupland K.B. (2008), Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 8: 159–66.
72. Rawat D., Nair D. (2010) Extended-spectrum beta-lactamases in Gram negative bacteria. J Glob Infect Dis 2: 263–74.
73. Lahey Clinic. OXA-type β-lactamases. [Cited 20 March 2014] Available from URL: http://www.lahey.org/studies/other.asp#tabl
74. Shibata N., Kurokawa H., Doi Y., Yagi T., Yamane K., Wachino J., Suzuki S., Kimura K., Ishikawa S., Kato H., Ozawa Y., Shibayama K., Kai K., Konda T., Arakawa Y. (2006) PCR classification of CTX-M-type β-lactamase genes identified in clinically isolated Gram-negative bacilli in Japan. Antimicrob Agents Chemother 50: 791–95.
75. Kiratisin P., Apisarnthanarak A., Laesripa C., Saifon P. (2008) Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob Agents Chemother 52: 2818–24.
76. Ewers C., Bethe A., Semmler T., Guenther S., Wileler LH. (2012) Extended-spectrum beta-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18: 646–55.
77. Kiratisin P., Chattammanat S., Sa-Nguansai S., Dansubutra B., Nangpatharapornthawee P., Patthamalai P., Tirachaimongkol N, Nunthanasup T. (2008) A 2-year trend of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Thailand: an alert for infection control. Trans R Soc Trop Med Hyg 102: 460–64.

78. Chanawong A, Lulitanond A, Kaewkes W, Lulitanond V, Srigulbutr S, Homchampa P. (2007) CTX-M extended-spectrum beta-lactamases among clinical isolates of Enterobacteriaceae in a Thai university hospital. Southeast Asian J Trop Med Public Health 38: 493–500.
79. Luvsansharav UO, Hirai I, Nakata A, Imura K, Yamauchi K, Niki M, Komalamisra C, Kusolsuk T, Yamamoto Y. (2012) Prevalence of and risk factors associated with faecal carriage of CTX-M β-lactamase-producing Enterobacteriaceae in rural Thai communities. J Antimicrob Chemother 67: 1769–74.
80. BHAT, M. A., Sageerabanoo, S., Kowsalya, R., & SARKAR, G. (2012). The Occurrence of CTX-M3 Type Extended Spectrum Beta Lactamases among Escherichia Coli Causing Urinary Tract Infections in a Tertiary Care Hospital in Puducherry. Journal of Clinical & Diagnostic Research, 6(7).
81. Jemima SA, Verghese S. (2008) Multiplex PCR for bla CTX-M and bla SHV in the extended spectrum beta lactamase (ESBL) producing gramnegative isolates. Indian J Med Res. 128:313-17
82. Lal P, Kapil A, Das BK, Sood S. (2007) The occurrence of the TEM and the SHV genes in the extended spectrum β-lactamases (ESBLs) producing Klebsiella species which were isolated from a tertiary care hospital. Indian J Med Res. 125:173-78.
83. Ryoo NH, Kim EC, Hong SG, Park YJ, Lee K, Bae IK et al. (2005) The dissemination of the SHV-12 and the CTX-M type extendedspectrum β-lactamases among the clinical isolates of Escherichia coli and Klebsiella pneumonia and the emergence of GES-3 in Korea. J Antimicrobial Chemother. 56: 698-702
84. Ruppe E, Hem S, Lath S, Gautier V, Ariey F, Sarthou J, et al. (2009), The CTX-M β-lactamases in Escherichia coli which were isolated from community-acquired urinary tract infections in Cambodia. Emerg Inf Dis, 15(5):741-48
85. Jacoby GA, Munoz-Price LS. (2005), The new β-lactamases. New Eng J Med, 352(4):380-90
86. Nichols, D. A. (2014). Structure-Based Design of Novel Inhibitors and Ultra High Resolution Analysis of CTX-M Beta-Lactamase. University of South Florida.
87. Tsao SG, Brunk CF, Pearlman RE. (1983), Hybridization of nucleic acids directly in agarose gels. Anal Biochem, 131(2):365-72. [PubMed ID: 6193737].
88. Safari M, Shojapour M, Akbari M, Pourbabaee A, Abtahi H. (2013), Dissemination of CTX-M-Type Beta-lactamase Among Clinical Isolates of Enterobacteriaceae in Markazi Province, Iran. Jundishapur J Microbiol, 6(8):e7182. https://doi.org/10.5812/jjm.7182.

89. Dutour C, Bonnet RMarchandin H, Boyer M, Chanal C, Sirot D, Sirot J. (2002), CTX-M-1, CTX-M-3, and CTX-M-14 β-Lactamases from Enterobacteriaceae Isolated in France. Antimicrob Agents Chemother46:.https://doi.org/10.1128/aac.46.2.534 -537.2002
90. Bonnet, R., J. L. M. Sampaio, R. Labia, C. De Champs, D. Sirot, C. Chanal, and J. Sirot. (2000), A novel CTX-M β-lactamase (CTX-M-8) in cefotaxime-resistant Enterobacteriaceae isolated in Brazil. Antimicrob. Agents Chemother.44:1936 –1942.
91. Thomas M, Hooton MD. (2012), Uncomplicated urinary tract infection. N Engl J Med, 366:1028–37. doi: 10.1056/NEJMcp1104429.
92. Dormingy JA, Nabeth P, Juergens-Behr A, Perrier-Gros-Claude JD.(2005), Risk factors for antibiotic resistance Escherchia coli isolated from community–aquired urinary tract infections in Dakar, Senegal. J Antimicrob Chemother, 56(1):236–9. doi: 10.1093/jac/dki158.
93. Giske CG, Sundsfjord AS, Kahlmeter G, Woodford N, Nordmann P, Paterson DI, et al. (2009) Redefining extended-spectrum beta lactamase: Balancing science and clinical need. J Antimicrob Chemother, 63(1):1–4. doi: 10.1093/jac/dkn444.
94. Tabar, M. M., Mirkalantari, S., & Amoli, R. I. (2016). Detection of ctx-M gene in ESBL-producing E. coli strains isolated from urinary tract infection in Semnan, Iran. Electronic physician, 8(7), 2686–2690. https://doi.org/10.19082/2686
95. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchoun Sh. (2003), Prevalence and molecular epidemiology of CTXM extended–Spectrum B-lactamase-producing Escherchia coli and Klebsiellapneumoniae in Russian hospitals. Antimicrob Agents Chemother, 47(12):3724–32. doi: 10.1128/AAC.47.12.3724-3732.2003.
96. Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ. (2000), CTX-M type B-lactamases: an emerging group of extended–Spectrum enzymes. Int J Antimicrob Agents, 14(2):137–42. doi: 10.1016/S0924-8579(99)00165-X.
97. S. Antonio, G. José, L. D. Juan de , O. Jesús, L. José, María, S. José, P. Gonzalo, (2007), High presence of extended-spectrum β-lactamases and resistance to quinolones in clinical isolates of Escherichia coli, Microbiological Research, 162(4), P:347 -354,https://doi.org/10.1016/j.micres.2006.02.003.
98. K.R. Kolsom, K.R. Ashrafali, K.R. Leili, K. Maryam, K.R. Khadijeh, M.N. Maryam, (2020), Isolation of Beta-Lactamase Producing Genes (SHV, CTX-M1, CTX-M2, CTX-M3) in Escherichia coli Isolated from Pregnant Woman Patients, World Journal of Peri and Neonatology, 1(1). DOI: https://doi.org/10.18502/wjpn.v1i1.2782.
99. Song W, Kim J, Bae IK, Jeong SHSeo YH, Shin JH, Jang SJ, Uh Y, Shin JH, Lee M, Lee K. (2011), Chromosome-Encoded AmpC and CTX-M Extended-Spectrum β-Lactamases in

Clinical Isolates of Proteus mirabilis from Korea . Antimicrob Agents Chemother55:.https://doi.org/10.1128/aac.01835-09.

100. Batchelor M, Threlfall EJ, Liebana E. (2005), "Cephalosporin resistance among animal-associated enterobacteria: a current perspective" . Expert Rev Anti Infect Ther, 3(3):403-417

101. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. (2007), "CTX-M: changing the face of ESBLs in Europe" . J Antimicrob Chemother, 59(2):165-174.

102. Musicha P, Feasey NA, Cain AK, et al.(2017), Genomic landscape of extended-spectrum β-lactamase resistance in Escherichia coli from an urban African setting. J Antimicrob Chemother, 72(6):1602–1609.

103. Chong Y, Shimoda S, Shimono N. (2018), Current epidemiology, genetic evolution and clinical impact of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infect Genet Evol, 61:185–188.

104. Bubpamala, J., Khuntayaporn, P., Thirapanmethee, K., Montakantikul, P., Santanirand, P., & Chomnawang, M. T. (2018). Phenotypic and genotypic characterizations of extended-spectrum beta-lactamase-producing Escherichia coli in Thailand. Infection and Drug Resistance, 11, 2151–2157. https://doi.org/10.2147/IDR.S174506

105. Emery CL WL. (1997), Detection and clinical significance of extended-spectrum b-lactamase in a tertiary-care medical center. J Clin Microbiol, 35(8) p: 2030-2036.

106. TM. CRC. (20060, The CTX-M b-lactamase pandemic. Curr Opin Microbiol, 9(5), p: 466-75.

107. Chong Y, Shimoda S, Shimono N. (2018), Current epidemiology, genetic evolution and clinical impact of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infect Genet Evol, 61:185–188.

108. Michael GB, Freitag C, Wendlandt S, Eidam C, Feßler AT, Lopes GV, et al. (2015), Emerging issues in antimicrobial resistance of bacteria from food-producing animals. Future Microbiol.;10:427–43.

109. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. (2015). Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Biol Sci.22, 90–101.

110. Rose, P. W., Prlić, A., Altunkaya, A., Bi, C., Bradley, A. R., Christie, C. H., Costanzo, L. D., Duarte, J. M., Dutta, S., Feng, Z., Green, R. K., Goodsell, D. S., Hudson, B., Kalro, T., Lowe, R., Peisach, E., Randle, C., Rose, A. S., Shao, C., Tao, Y. P., … Burley, S. K. (2017).

The RCSB protein data bank: integrative view of protein, gene and 3D structural information. Nucleic acids research, 45(D1), D271–D281. https://doi.org/10.1093/nar/gkw1000

111. UniProt Consortium (2019). UniProt: a worldwide hub of protein knowledge. Nucleic acids research, 47(D1), D506–D515. https://doi.org/10.1093/nar/gky1049.
112. Muhammed, M. T., & Aki-Yalcin, E. (2019). Homology modeling in drug discovery: Overview, current applications, and future perspectives. Chemical biology & drug design, 93(1), 12-20.
113. Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. Nature protocols, 10(6), 845 - 858
114. Heo, L., Park, H., & Seok, C. (2013). GalaxyRefine: Protein structure refinement driven by side-chain repacking. Nucleic acids research, 41(W1), W384-W388.
115. Ko, J., Park, H., Heo, L., & Seok, C. (2012). GalaxyWEB server for protein structure prediction and refinement. Nucleic acids research, 40(W1), W294 -W297.
116. Schwede, T., Kopp, J., Guex, N., & Peitsch, M. C. (2003). SWISS-MODEL: An automated protein homology-modeling server. Nucleic acids research, 31(13), 3381–3385. https://doi.org/10.1093/nar/gkg520
117. Richardson, J. S., Keedy, D. A., & Richardson, D. C. (2013). “The plot” thickens: more data, more dimensions, more uses. In Biomolecular Forms and Functions: A Celebration of 50 Years of the Ramachandran Map (pp. 46-61).
118. Binkowski, T. A., Naghibzadeh, S., & Liang, J. (2003). CASTp: computed atlas of surface topography of proteins. Nucleic acids research, 31(13), 3352-3355.
119. Dundas, J., Ouyang, Z., Tseng, J., Binkowski, A., Turpaz, Y., & Liang, J. (2006). CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. Nucleic acids research, 34(suppl_2), W116-W118.
120. Wang, Q. (2017). Protein-ligand Docking Application and Comparison Using Discovery Studio and AutoDock (Doctoral dissertation, North Dakota State University).
121. Guex, N., & Peitsch, M. C. (1997). SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. electrophoresis, 18(15), 2714 -2723.
122. Sterling, T., & Irwin, J. J. (2015). ZINC 15–ligand discovery for everyone. Journal of chemical information and modeling, 55(11), 2324-2337.
123. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. Journal of cheminformatics, 3, 1 -14.
124. Hutchison, G. R., Morley, C., James, C., Swain, C., De Winter, H., Vandermeersch, T., & O’Boyle, N. M. (2011). Open Babel Documentation.

125. 126. Lipinski, C. A. (2004). Lead-and drug-like compounds: the rule-of-five revolution. Drug discovery today: Technologies, 1(4), 337-341

126. Ounthaisong, U., & Tangyuenyongwatana, P. (2017). Cross-docking study of flavonoids against tyrosinase enzymes using PyRx 0.8 virtual screening tool. TJPS, 41(2017).

127. Kondapuram, S. K., Sarvagalla, S., & Coumar, M. S. (2021). Docking-based virtual screening using PyRx Tool: autophagy target Vps34 as a case study. In Molecular Docking for Computer-Aided Drug Design (pp. 463-477). Academic Press.

128. Pires, D. E., Blundell, T. L., & Ascher, D. B. (2015). pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. Journal of medicinal chemistry, 58(9), 4066-4072.

129. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports, 7, 42717. https://doi.org/10.1038/srep42717