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PHARMACOPHORE INSIGHTS AND MOLECULAR DOCKING OF CIPROFLOXACIN ANALOGUES AGAINST 2XE1: STRATEGIES FOR REDUCED ANTIBIOTIC RESISTANCE

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ABSTRACT
Background: Antibiotic resistance is a silent pandemic disease that is growing and causing a global threat. Existing antibiotics are less effective against infectious diseases, so we must discover more potent and effective drugs. The latest report from the World Health Organization (WHO) underscores the global nature of the situation, revealing that high levels of antibiotic resistance in bacteria worldwide lead to life-threatening bloodstream infections and resistance to treatment. **Methods:** This study focuses on the Molecular Docking and Pharmacophore Modeling of Ciprofloxacin and its analogs to explore ligand-protein interactions and identify potent drugs against AMR. Twenty ciprofloxacin analogs, designed using ChemDraw Pro12.0, were docked with the 2XE1 protein. Molecular docking assessed the binding affinity, with Arguslab 4.0 scoring the lowest docking scores to indicate strong interactions and biological activity. Pharmacophore modeling identified essential molecular features like HBA, HBD, and AI for optimal biological activity. **Results:** The computational screening identified several compounds with improved binding properties, showing greater affinity towards ALA129, TYR149, and PHE88 amino acids, essential for biological activity. **Conclusion:** The study identifies the best analog of ciprofloxacin, which can effectively combat antibiotic resistance. Compound 13 showed promising docking scores and relevant pharmacophoric features, outperforming the parent ciprofloxacin in binding affinity, suggesting it could be a potent drug candidate against AMR.

INTRODUCTION

Antimicrobial resistance (AMR) is a type of "silent pandemic" because of its danger to world health, even though its effects are not always immediately apparent or well-understood [1]. Microorganisms that are resistant to widely used antibiotics are called “Superbugs”. Antimicrobials prevent and cure infections

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in people, plants, and animals [2]. They include antibiotics, antivirals, antiparasitics, and antifungals. The development of antibiotic resistance in bacteria results in their resistance to the effects of antibiotics, which makes it challenging or impossible to treat illnesses brought on by these bacteria with conventional antibiotic therapy [3]. Due to drug resistance, infections become more complicated or impossible to cure, and antibiotics and other antimicrobial medications become ineffective, raising the risk of disease spread, severe sickness, disability, and death [4]. Three categories of AMR have been documented: total drug resistance (TDR), extensive drug resistance (XDR), and multidrug resistance (MDR) [5]. E. Coli is a group of bacteria that cause multidrug resistance and can cause infections in the gut (GI tract), urinary tract, and other body parts. This is the most common cause of Outbreaks and severe illness from E.coli, a critical challenge for Medical health science. 2XE1 is a Transport protein of E. coli and the main constituent of the body, essential for moving molecules across cell membranes and life growth. They act as gatekeepers, allowing specific substances to enter or exit the cell. This function is crucial for maintaining cellular homeostasis and carrying out various biological processes and plays a vital role in the life cycle, growth, and also in Multi-drug resistance (MDR), preventing an antibiotic attack [6]. E. Coli Gastrointestinal tract infections were treated by Fluoroquinolone medicine (Ciprofloxacin, Ofloxacin & Norfloxacin), but that was less susceptible after break repetition and also increased 5% risk of antibiotic resistance in 2020. This makes it more challenging to treat this common illness effectively [7]. Therefore, we want to discover more potent and best derivatives of these drugs that can combat more wholly and accurately. Being resistant to every agent in every class of antimicrobial drugs is known as TDR, nonsusceptibility to at least one agent in all but two or fewer antimicrobial groups is known as XDR, and an acquired lack of sensitivity to at least one agent in three or more antibiotic classes is known as MDR [8]. Ciprofloxacin, other than 2XE1, also has inhibitory properties against MRSA pyruvate enzyme, which has a synergetic effect with Baicalein [9].

The 2022 report by the Global AMR and Use Surveillance System (GLASS) reveals concerning rates of resistance in prevalent bacterial infections. Serious concerns arise from the median reported rates of 42% for third-generation cephalosporin-resistant E. Coli and 35% for methicillin-resistant Staphylococcus aureus across 76 economies. According to estimates, AMR may overtake all other causes of mortality worldwide by 2050 if preventative actions are not taken. Based on estimates from throughout the world, the number of fatalities directly related to AMR increased to over 1.2 million in 2019. If not enough effort is made to manage AMR, this figure is expected to climb to almost 10 million deaths annually by the year 2050 [10]. Furthermore, it is challenging to measure the economic impact of antibiotic resistance [11].

Increased resistance results in higher costs for more expensive antibiotics (treatment must shift to second or third-line medications, which are almost always more expensive) and associated items like specialized equipment, extended hospital stays, and patient isolation procedures. Death and lost production are examples of societal costs [12]. Based on economic forecasts, the global economy will lose USD 100 trillion due to AMR by 2050, with a 2-3.5 % decline in GDP and a 3–8% decline in livestock [13]. AMR poses a significant danger that directly impacts most of the 17 Sustainable Development Goals (SDGs) of the United Nations, including SDG3:As there are few effective anti-AMR drugs, reaching the third SDG of "good health and well-being" would be difficult [14].

Antibiotic Drug Resistance: A Growing Concern

Microbes have acquired antimicrobial resistance (AMR) to many drugs due to high selection pressure from the increasing use and misuse of antibiotics. A vast number of interdependent factors related to healthcare and agriculture govern the development of AMR through various drug-resistance mechanisms [15]**.** Antibiotic resistance (AR) can result from several circumstances, including inadequate storage, non-laboratory focused treatment, and subtherapeutic antibiotic usage [16]. AR is the genetic ability of microbes to flourish in the presence of high quantities of antibiotics. Antibiotic-resistant bacteria can proliferate and reproduce at antibiotic doses lethal to other strains of the same species. This phenomenon is often measured by determining the minimum inhibitory concentration (MIC) of a given antibiotic [17].

The abbreviation "ESKAPE" means "enterobacterales, Staphylococcus aureus, klebsiella pneumoniae, acinetobacter baumannii, pseudomonas aeruginosa, and enterobacter." Currently, the most commonly encountered superbugs globally are methicillin-resistant Staphylococcus aureus (MRSA),

carbapenem-resistant Enterobacter ales (CRE), vancomycin-resistant Enterococcus (VRE), multidrug-resistant Pseudomonas aeruginosa, and multidrug-resistant Acinetobacter [18].

The types of resistance that bacteria develop against antibiotics are:

a. Natural resistance

Natural resistance may be intrinsic or induced.

- **Intrinsic resistance:** The ability of bacteria to exhibit resistance to specific antibiotic classes owing to the existence of their chromosomal genes, without the need for mutation or gene acquisition, is known as intrinsic resistance. Intrinsic resistance involves both decreased permeability and efflux pumps in terms of drug-resistance mechanisms. Additionally, it commonly affects multidrug efflux pumps [19].
- **Induced resistance:** The bacteria, in this case, have naturally existing genes, but they don't express themselves to the point of resistance until they are exposed to an antibiotic [20].

b. Acquired resistance

It is an evolutionary mechanism in which a chromosomal gene mutation or the external acquisition of more genetic material through horizontal gene transfer (HGT) causes a previously susceptible bacterium to become resistant. The three main processes that underpin HGT are conjugation, transposition, and transformation. Temporary or permanent acquired resistance can be transmitted using conjugation-obtained plasmids [21].

The emergence of bacteria that are resistant to antibiotics restricts the available treatments for common diseases, which raises the risk of more extended sickness, more excellent death rates, and increased healthcare expenses. Treatment for infections brought on by resistant bacteria is more challenging and expensive; more substantial and more costly medications, extended hospital stays, and intensive care measures are frequently needed [22]. Furthermore, by raising the possibility of treatment failure and consequences, AR compromises the efficacy of life-saving medical treatments like organ transplants, chemotherapy, and surgery. AR calls for a multimodal strategy that includes worldwide cooperation, research and development of novel antibiotics, infection prevention and control measures, and antimicrobial stewardship [23]. Healthcare professionals, legislators, the pharmaceutical sector, agricultural stakeholders, and the general population must all be involved in the fight against AR to encourage responsible antibiotic use, minimize unneeded antibiotic exposure, and maintain the effectiveness of currently available antibiotics for the next generation [24].

Mechanism of Antibiotic Resistance

This portion highlights the few defense mechanisms bacteria use to resist the effects of antimicrobials or antibiotics. AR's main mechanisms include increasing active drug efflux, altering a drug target, inactivating a drug, and limiting drug uptake (Figure 1).

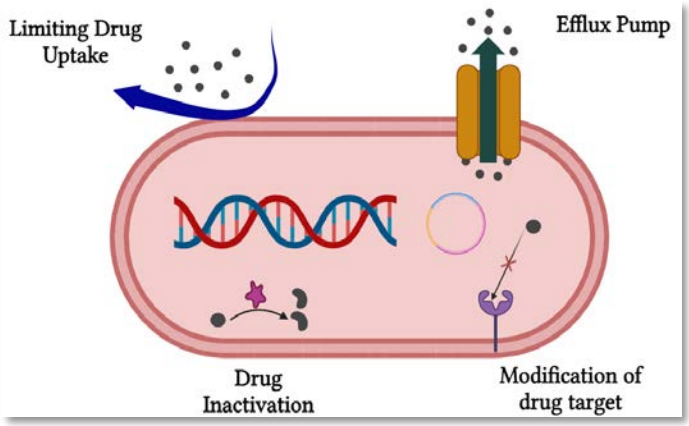


Figure 1: Mechanism of Antibiotic Resistance

a) Limiting drug uptake

The capacity of bacteria to restrict the absorption of antimicrobial drugs varies naturally [25]. Gram-negative bacteria have an exterior membrane that acts as a permeability barrier because they contain a lipopolysaccharide (LPS) layer, which makes them inherently less susceptible to certain antibiotics than Gram-positive bacteria [26]. When bacteria have thick outer membranes, substances frequently enter the cell through porin channels. The porin channels in gram-negative bacteria may often be used to access hydrophilic substances. The porins (OmpA, OmpC, OmpF, OmpW, and OmpX) present in gram-negative bacteria differ in their weights and structures [27]. Porin alterations may limit the uptake of drugs in two primary ways: by lowering the number of porins present there or altering the selectivity of the porin channel through mutations [25].

For example, it is known that Enterobacteriaceae members develop resistance due to a decrease in porin number. Collectively, these bacteria lower the number of porins as a form of resistance against carbapenems [28]. *E. aerogenes* has been observed to have mutations that alter the porin channel, leading to resistance to imipenem and certain cephalosporins [29].

An additional common step of bacterial colonization is the production of a biofilm by a colony. For pathogenic organisms, biofilm development shields the bacterium from antimicrobial agents and the human immune system. The dense, sticky biofilm matrix, which is composed of proteins, polysaccharides, and DNA from the resident bacteria, makes it difficult for antimicrobial medications to reach the bacteria [30].

b. Drug Efflux

Efflux pumps are membrane proteins that export antibiotics from the cell while preserving their low intracellular concentrations. Efflux mechanisms release these antimicrobials at the same rate as they are entering the cell, preventing them from reaching their intended target [31]. The ATP-Binding Cassette (ABC) superfamily, Multidrug and Toxic Compound Extrusion (MATE) superfamily, Major Facilitator Superfamily (MFS), Resistance Nodulation and Cell Division (RND) superfamily, and Small Multidrug Resistance (SMR) superfamily are the five prominent families of efflux pumps. These families are classified based on their structure and energy source [32]. ABC efflux pumps, also known as "primary active transporters," remove substrates by using the energy released during ATP hydrolysis, whereas "secondary active transporters" (MATE, MFS, RND, and SMR) pump hydrogen and sodium out of the membrane to use the proton motive force (PMF) as an energy source [33]. AMR resulting from this mechanism frequently develops resistance to many classes of antibiotics, particularly the fluoroquinolone, tetracycline, and macrolide classes.

One of the best-known instances of efflux-mediated resistance is tetracycline resistance, which is caused by the Tet efflux pumps (which are members of the MFS family) extruding tetracyclines by using proton exchange as their energy source [34].

c. Modification of drug target

Target modification typically causes the original drug target's structure to change, resulting in either poor or no drug binding. Target site alterations frequently arise from a bacterial gene on a chromosome spontaneously changing [35]. Minor changes to the target molecule can significantly impact antibiotic binding since the interaction between antibiotics and their targets is often very selective. Gram-positive bacteria nearly exclusively use β-lactam antibiotics. However, they become resistant to them by altering the structure and amount of PBPs (penicillin-binding proteins) [31]. PBPs are transpeptidases that help the cell wall's peptidoglycan to form. Variations in the PBP count affect the quantity of medication that may bind to that target. A structural alteration (Such as PBP2a in S. aureus due to the mecA gene acquisition) may lessen or stop the medication's binding ability [36]. The 50S ribosomal subunit is the binding site for MLS (macrolides, lincosamides, and streptogramins) antibiotics, exhibiting target modification. 23S rRNA methyltransferases methylate 23S rRNA at position A-2058 as part of this process [37]. Dimethylation (MLS type II) offers significant resistance, but monomethylation (MLS type I) usually provides a moderate amount of resistance q1A [38]. Topoisomerase IV or DNA gyrase mutations mediate resistance to medications that block nucleic acid synthesis, such as fluoroquinolones. These alterations change the structure of topoisomerase and gyrase, which decreases or eliminates the drug's ability to bind to these components [39].

d. Drug inactivation

Bacteria have two main ways of rendering drugs inactive: physically breaking down the medication or adding a chemical group. An enormous class of enzymes that hydrolyze drugs is known as the β-lactamases. Another drug that can be hydrolyzed through the tetX gene to become inactive is tetracycline [40].

The mechanism by which the β-lactamases (formerly known as penicillinases and Cephalosporinases) hydrolyze a particular location in the β-lactam ring structure and open the ring renders β-lactam medications inactive. The open-ring medications cannot bind the target PBP proteins [41]. Enzyme families comprise bacterial β-lactamases, subdivided according to two main categorization methods (Ambler and Bush classifications). Whereas the Bush classification is based on functionality (substrate and inhibition profile), the Ambler classification is based on similarities in amino acid sequences (protein homology) [42,43]. β-lactamases may be further subdivided into four molecular classes (A–D) based on the Ambler classification. Class B comprises a metalloenzymatic zinc ion at its active site, whereas classes A, C, and D use a serine moiety. Three main categories are developed based on the Bush classification: Group 1 is made up of Cephalosporinases; Group 2 is made up of serine β-lactamases; and Group 3 is made up of metallo-β-lactamases (MBL) [44].

Class A beta-lactamases: These enzymes show some vulnerability to a variety of β-lactamase inhibitors that are sold commercially, such as Tazobactam, Sulbactam, and Clavulanate—additional reports of other students in this class,

including VH5, PER, and SHV. Bacterial enzymes known as Class B β-lactamases work in conjunction with a metal cofactor, such as natural divalent zinc, to break down β-lactam antibiotics [45]. AmpC β-lactamases are among the class C enzymes; these enzymes are typically expressed by bla genes found on bacterial chromosomes, while plasmid-borne AmpC enzymes are becoming increasingly common. The majority of cephalosporins, such as cefoxitin, cefotetan, ceftriaxone, and cefotaxime, as well as penicillins and β-lactamase inhibitors (clavulanate and tazobactam), are usually resistant to organisms expressing the AmpC β-lactamase [46]. Class D β-lactamases initially characterized as "oxacillinases" due to their capacity to hydrolyze oxacillin at a rate at least 50% faster than benzylpenicillin, in contrast to classes A and C's comparatively slower oxacillin hydrolysis [45].

Current Studies and Strategies to Combat AMR

Several promising alternatives to traditional antibiotics have been studied extensively to combat AMR. While some of these approaches have yet to yield practical impacts, others show significant potential in addressing the challenges posed by AMR. Here, we review some of the most promising alternatives that have been explored:

a. Combination therapy

This strategy has been used to treat infections caused by bacteria resistant to multiple drugs. It involves either combining antibiotics with other antibiotics or using adjuvants that either directly target resistance mechanisms or indirectly increase the effect of antibiotics by blocking their efflux or targeting bacterial signaling mechanisms [47].

R. Chiara et al. investigated combining antivirulence drugs with antibiotics against Pseudomonas aeruginosa. They tested gallium and furanone C-30 with ciprofloxacin, colistin, meropenem, and tobramycin. Synergies were observed at intermediate drug concentrations, and antivirulence compounds acted as potent adjuvants, restoring growth inhibition for resistant clones and reversing resistance selection in some cases. The study highlights the potential of antivirulence-antibiotic combinations against Pseudomonas aeruginosa infections and in limiting AR spread [48]. *L. Quin et al.'s* research demonstrates the potent antimicrobial activity of peptide D-11 and vancomycin against Gram-negative pathogens, even at low concentrations. The combination maintains effectiveness in biological fluids while being non-hemolytic and non-toxic to cells. The combination significantly reduced pathogen levels in a mouse model of Pseudomonas aeruginosa infection. This study suggests that combining peptide D-11 and vancomycin could offer a promising alternative for addressing drug-resistant Gram-negative pathogens in both humans and mammals [49]. *M. Prasanth et al.* used phage MRM57 and Citrobacter amalonaticus to study the synergy of phage-antibiotic combinations. They found that even with low phage counts, antibiotic synergism is concentration-dependent. This implies that phages may function as adjuvants in conjunction with sublethal antibiotic dosages, presenting a potentially effective treatment approach [50].

b. Phage Therapy

Using bacteriophages—viruses that infect and destroy bacteria—to treat bacterial illnesses is known as phage treatment, an inventive strategy to tackle AR [51]. Phages can cause lytic or lysogenic infections in bacteria by attaching themselves to a receptor or receptors on the bacterial surface and inserting their genome into the bacterium. When a phage reproduces, it creates additional phage particles that lyse the bacterium and spread to other bacteria, resulting in a lytic infection [52]. During a lysogenic infection, a DNA phage inserts its genetic material into the bacterial chromosome; when the bacterium multiplies, the genome is passed on to the daughter cells. Liquid phage particles can be produced when integrated DNA separates from the chromosome due to environmental changes [53]. The main approaches for phage therapy currently in use are the development of phage-derived enzymes, cocktails, phage and antibiotic combinations, phage engineering, and the recently discovered phage-associated clustered regularly interspaced short palindromic repeats system (CRISPR-Cas).

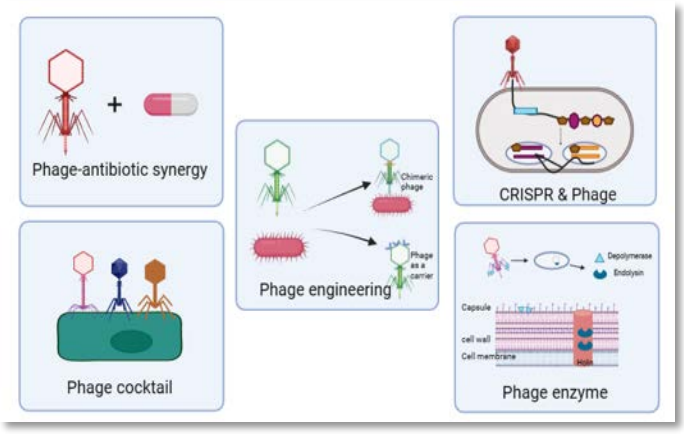


Figure 2: Phage Therapy approach to combat AMR

- **Phage Cocktails:** Combining multiple phages targeting different strains or species of bacteria to broaden the spectrum of activity and reduce the chance of bacterial resistance emergence (Figure 2) [54].
- **Phage-derivedEnzymes:** Utilizing enzymes produced by phages, such as endolysins and depolymerizes, to degrade bacterial cell walls or disrupt biofilms, thereby enhancing the efficacy of phage treatment, especially against antibiotic-resistant bacteria (Figure 2) [51].
- **Combination Therapy with antibiotics:** Pairing phages with antibiotics to achieve synergistic effects, potentially enhancing the effectiveness of both treatments while reducing the likelihood of bacterial resistance development, particularly valuable in complex infections or against highly resistant bacterial strains (Figure 2) [51,52].
- **Phage Engineering:** Modifying or engineering phages to enhance their therapeutic properties, such as improving specificity, increasing replication rates, or overcoming bacterial resistance mechanisms, promising for tailoring phage therapy to specific clinical needs and challenges (Figure 2) [54].
- **CRISPR-Cas and Phage Therapy:** Exploring the integration of the CRISPR-Cas system with phage therapy to potentially enhance treatment effectiveness by making bacteria more susceptible to phage infection or preventing the emergence of resistance through targeted bacterial gene editing (Figure 2) [54].

In the research study, *R.M. Dedrick et al.* treated a cystic fibrosis patient with disseminated Mycobacterium abscessus infection post-lung transplantation using a three-phage cocktail. They developed effective lytic phage derivatives through genome engineering and forward genetics. Intravenous phage treatment was well tolerated and led to clinical improvements, including sternal wound closure, improved liver function, and resolution of infected skin nodules [55]. In another study, *K. Kotaro et al.* developed CapsidCas13a(s), CRISPR-Cas13a based nucleocapsids targeting antibiotic-resistant E. coli and S. aureus by recognizing AMR genes. Packaged into bacteriophage capsids, these constructs effectively kill the bacteria and detect bacterial genes without additional manipulation or visual aids. The study highlights the potential of CapsidCas13a(s) as both therapeutic agents against antibiotic-resistant bacteria and as simple tools for bacterial gene detection [56]. A 63-year-old female patient with extensively drug-resistant Klebsiella pneumonia (ERKp) developed a recurrent urinary tract infection (UTI), as reported by *B. Juan et.al.* Within days after the first two rounds of phage treatment, phage-resistant mutants emerged. While ERKp strains were resistant to sulfamethoxazole-trimethoprim, the patient's UTI was successfully treated with sulfamethoxazole-trimethoprim and phage cocktail. This combination suppressed the emergence of phage-resistant mutants in vitro [57]. Nir-Paz et al. successfully cured a patient infected with extensively drug-resistant *A. baumannii* and multidrug-resistant *K. pneumoniae* by a combination of a phage cocktail and intravenous meropenem and colistin [58].

Anti-microbial Peptides

Small proteins called AMPs are made by living things and function as the first line of defense against bacteria, viruses, and fungi. The rising problem of AR has brought them to light as possible substitutes for traditional antibiotics. They exhibit a wide range of antimicrobial action, efficiently restricting or eliminating a variety of pathogens, such as viruses, fungi, and bacteria, both Gram-positive and Gram-negative [59]. They are effective against drug-resistant strains because of their distinct action method, which targets several locations on the plasma membrane and intracellular targets of pathogenic bacteria. Furthermore, AMPs have a variety of biological properties, such as the ability to regulate the immune system, angiogenesis, healing of wounds, and tumor suppression [60].

Xiaorui Wang et al. discovered that peptide W3R6 and its analogs have strong antimycobacterial action against *M. smegmatis* while not affecting the erythrocytes of humans. These peptides target both the mycobacterial membrane and genomic DNA, lowering the possibility of resistance development. Furthermore, they successfully remove *M. smegmatis* from infected macrophages. This implies that W3R6 may be an excellent lead chemical for treating drug-resistant strains of M. tuberculosis, suggesting potential therapeutic uses in TB therapy [61]. *Chao Zhong et al.* synthesized new AMPs by adding various lengths of fatty acid chains to D-amino acids at sites 4 or 7 of Ano-D4,7, an analog of anoplin. These peptides displayed significant antibacterial action against a diverse spectrum of bacteria, including multidrug-resistant species, outperforming conventional antibiotics effectively. Combining fatty acids with D-amino acid side chains is a viable technique for developing effective antibacterial options to overcome

bacterial resistance [62]. *Jingru Shi et al.* revealed that peptide WW307 had high antibacterial action towards MRSA and Gram-negative bacteria with resistance genes such as blaNDM-5, mcr-1, and tet (X4). WW307 successfully suppressed and eliminated bacterial biofilms while maintaining low toxicity and resistance to physiological circumstances. Mechanistic investigations confirmed WW307's potential to disrupt bacterial membranes by targeting particular components and producing ROS. Overall, WW307 is a good candidate for treating organisms with multidrug resistance[63].

METHODOLOGY

To know the ligand-protein interaction and Pharmacophoric features of the Ciprofloxacin drug, in this work, an attempt has been made to focus on the Molecular docking and Pharmacophore modeling of the drug that reveals ligand-protein interaction properties and Pharmacophore features, which is necessary for the optimum biological activity of the compound. After knowing the drug's ligand-protein interaction properties and pharmacophoric features, we can design more potential compounds based on interaction properties and pharmacophoric features than the parent ciprofloxacin drug against AR. And it will be much more helpful for novel drug discovery. Based on molecular docking, this work is done hypothetically to explain the interaction of Ciprofloxacin antibiotic with highly causing AR protein 2XE1, a transport protein of E. coli, using Computer-aided drug design technique (CADD) [64].

Several software tools are available for docking studies, catering to diverse research needs. AutoDock, developed by the Olson Lab at The Scripps Research Institute, stands out for its versatile ligand docking capabilities, recognized for precision and reliability [65]. DOCK, from the Shoichet Laboratory at the University of California, San Francisco, offers molecular docking solutions underpinned by cutting-edge research [66]. Genetic Optimization for Ligand Docking (GOLD), a commercial offering from the Cambridge Crystallographic Data Centre (CCDC), employs ligand docking and scoring genetic algorithms. Glide, crafted by Schrödinger, Inc., is esteemed for its speed and accuracy, serving academia and industry alike [67]. FlexX, by BioSolveIT GmbH, excels in handling protein flexibility during docking computations. Surflex-Dock, from Tripos International, integrates patented search methods and proprietary scoring mechanisms for efficient ligand docking [68]. These tools collectively provide a robust suite for molecular docking studies, empowering drug discovery and molecular biology researchers. As we know, molecular docking is a computational approach used to identify the interaction of ligands with proteins or a computer-based procedure that predicts a ligand's ability to bind with the receptor protein [69]. It is employed in the development and discovery of pharmaceuticals. In this review, we use Argus Lab 4.0 Molecular docking software, which is appropriate, non-paid, easy to operate, and gives more precise results [70]. Arguslab 4.0 was chosen based on availability, and it is faster, cheaper, and more effective. It provides automatically generated Dock scores representing drug interaction and Amino acid affinity towards drug molecules. This Docking score can be used to assess enormous chemical libraries and identify potential novel treatments. Arguslab 4.0 is a valuable tool for Molecular docking and visualization but has some limitations in accuracy and flexibility [71].

Arguslab 4.0 is a valuable tool for molecular docking, but it has some limitations, particularly when handling the flexibility of ligands and receptors. Ligand flexibility and receptor flexibility are the major issues for docking as less accurate predictions, and they also have a limit of exploration of sufficiently sizeable conformational space. Molecular docking has many applications at various stages in drug discovery. Although it has multiple application areas, it is commonly applied in virtual screening and drug repurposing. As a result, it is playing a substantial role in the endeavor to discover a potent drug against COVID-19 [73]. There are also approved drugs in the pharmaceutical market developed through molecular docking. As accessible data and methods advance with the contribution of the latest computational developments, their use in drug discovery also increases [72].

Several crucial processes are involved in molecular docking, such as preparation, scoring, analysis, and search strategies. Charge assignment, hydrogen addition, and structural optimization are the steps in getting ligands and receptors ready. Throughout the receptor binding site, the search algorithm looks at potential ligand conformations and orientations[74]. The ligand-receptor interactions are ranked and evaluated by scoring functions according to several parameters, such as electrostatic interactions, hydrogen bonding, and shape complementarity. The most advantageous binding positions and interactions between the ligand and receptor are then determined by analyzing the docking findings [69].

Arguslab is a drug design, graphics, and molecular modeling software. ArgusLab4.0's ArgusDock docking engine, comparable to Glide and DOCK, simulates an exhaustive search technique. ArgusLab allows for flexible ligand docking, where grids are built over the binding site, and the ligand is defined as a torsion tree [75].

For Docking, we downloaded E.coli 2XE1 protein from Protein Data Bank (PDB), an international database containing 3-D structural information on biological entities, including nucleic acids and proteins. This is an experimentally determined structure from techniques such as X-ray crystallography and NMR spectroscopy contributed by scientists worldwide[76]. The PDB has tools for viewing and evaluating structural data in addition to providing insightful information on a variety of biological processes [77].

B. I. Esra et al. studied NorA, a protein in Staphylococcus aureus that contributes to antibiotic AR. The study identified critical residues required for inhibitor interaction by combining known NorA inhibitors into chemical clusters and docking them into NorA binding pockets using molecular dynamics simulations. Notably, residues I23, E222, and F303 are implicated in inhibitor binding, but others, such as I244, T223, F303, and F140, interact strongly with specific inhibitor clusters. This insight into NorA's structural promiscuity in detecting various ligands improves knowledge of AR processes. It informs the creation of more potent efflux pump blockers vital for tackling multidrug resistance in S. aureus [78]. *Fangfang Jiao et al.* investigated how ceftaroline (CFT), a fifth-generation cephalosporin, stimulates allosteric modulation of the active site of PBP2a, a protein responsible for resistance to antibiotics in MRSA. Computational simulations show that CFT stabilizes the allosteric domain while improving the catalytic domain. The work documents the opening of the active pocket in CFT-bound systems and reveals altered signal-propagating routes from the allosteric to active sites. These results illuminate the CFT-mediated allostery mechanism in PBP2a and provide insights for dual-site drug design or combination treatment toward MRSA by targeting PBP2a[79]. *K. Hithesh et al.* discovered prevalent and emergent mutations in Salmonella drug targets, with MDR variants exhibiting conservation and local INDEL alterations. The study identified Nimbolide, a phytochemical, as a potent inhibitor of primary Salmonella targets (PBP2, DNA gyrase subunit A, and parC) by virtual screening, Chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET)tests, and structural dynamics. Nimbolide displayed a stronger affinity for pharmacological targets than traditional antibiotics, indicating an opportunity for future clinical studies despite mutations [80].

The fluoroquinolone (FQ) antibiotic ciprofloxacin is a broad-spectrum antibiotic that is used to treat a variety of illnesses, such as gastrointestinal tract infections, urinary tract infections, endocarditis, lower respiratory tract infections, caused by E.coli but less susceptible and causing Antibiotic resistance when a patient repeatedly taking by dose gap or when the drug does not interact ultimately receptor due less binding affinity towards receptor protein [81]. Therefore, we want to design a more potent and active drug than its parent drug, Ciprofloxacin, by improving the drug receptor affinity feature that is clear after the docking and Pharmacophore modeling and can effectively interact with its transport protein 2XE1.

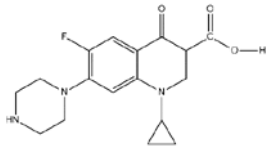
Pharmacophore modeling is a computational method used to determine the spatial features of drugs necessary for optimum biological activity. In this work, we use Ligand Scout software. This non-paid or more precise computational tool will define similar spatial features in favor of protein binding, which is responsible for biological action.

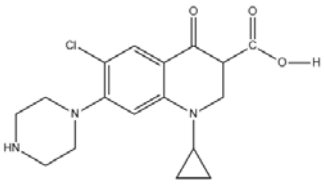
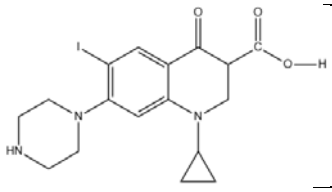
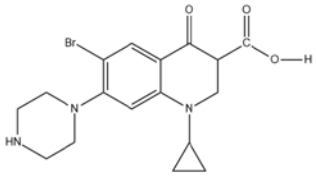
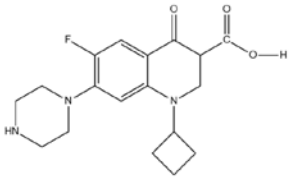
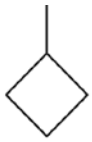
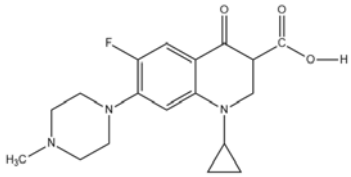
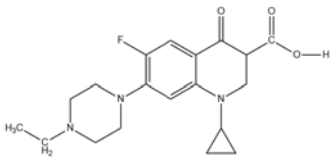
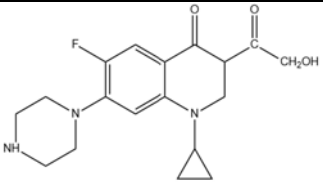
The main cause of bacterial resistance to ciprofloxacin is an alteration in the bacterial DNA, leading to AR to its mode of action [82]. Ciprofloxacin inhibits the bacterial enzyme DNA gyrase, which is necessary for DNA replication and repair in bacteria. Usually, resistance results from changes in the genes that control drug absorption or efflux or in the genes that encode DNA gyrase [81]. Furthermore, bacteria can develop resistance to ciprofloxacin through a process known as horizontal gene transfer, in which resistance genes are transferred from one bacterial population to another. First, in this work, we designed 20 different analogs of Ciprofloxacin designed computationally by Chem draw 2D software and named Compound-1, Compound-2, Compound-3, to Compound-20 in a serial way, and then converted them in 3D through Chem draw 3D ultra software, which is an acceptable form for docking and Pharmacophore Modeling software. After this, Ciprofloxacin and its 20 different analogs docked with 2XE1 protein, which is a receptor protein or binding site, through Arguslab 4.0 software, which yields ligand-protein interaction resulting in the form of

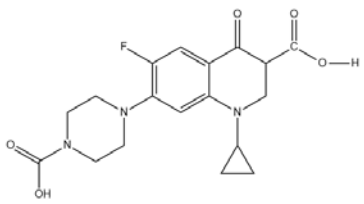
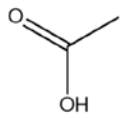
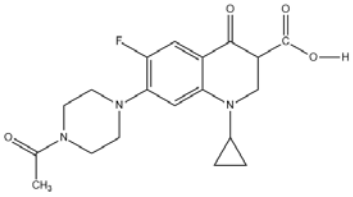
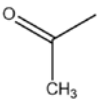
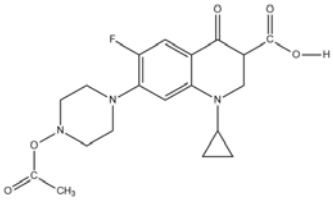
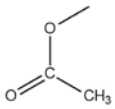
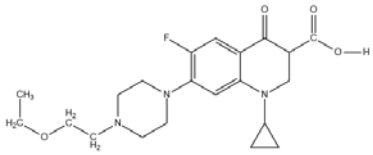
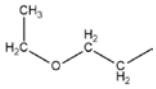
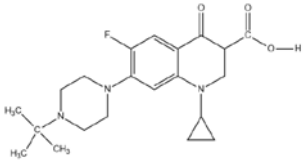
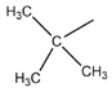
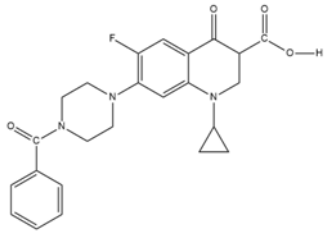
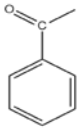
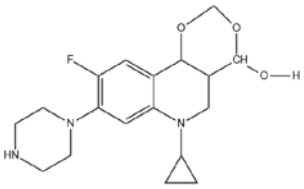
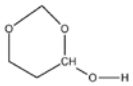
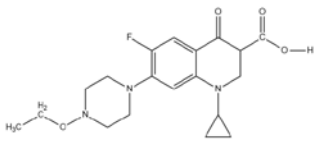
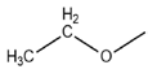
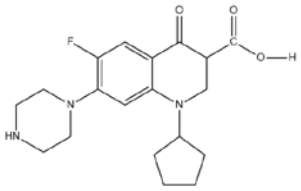
docking score and affinity of amino acid towards the active compound, which is shown in the table 1. Pharmacophore Modeling was also done after the docking of Ciprofloxacin and a suitable scoring ligand for the comparative study between Ciprofloxacin and its derivative [83].

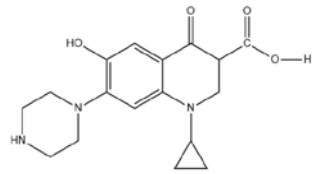
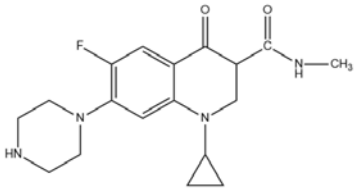
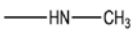
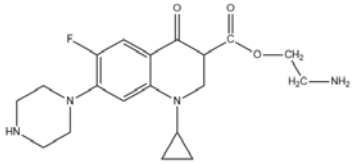
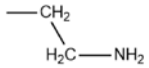
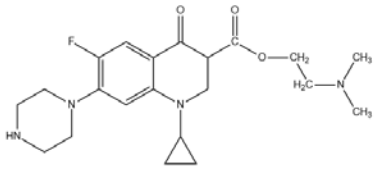
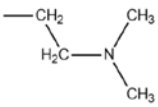
Table 1: Ciprofloxacin derivative along with their mode of interactions in the cavity of 2XE1

Structure of Ciprofloxacin
Ciprofloxacin interacted Amino acid- PHE88, TYR149 &TYR90
Ciprofloxacin docking score: (-7.06336 kcal/mol)



Compound No.	2D Ciprofloxacin Derivative	Substitution	Amino acid	Amino acids involved in Hydrogen interaction	Amino acids involved in Hydrophobic interaction	Docking Scores (kcal/mol)
1		-Cl	**ALA129, TYR149,** GLY127, GLY151, TYR90, GLN150	GLY150, GLN151	**ALA129, TYR149**	-7.31
2		-I	**TYR149, PHE88**, GLY 151, TYR 90	GLY151	**TYR149, PHE88**	-7.21
3		-Br	**ALA129, TYR149**	ALA129	**TYR149**	-7.35
4			**ALA129, TYR149, PHE88,** TYR131, THR130	**PHE88,** TYR131, THR130	**ALA129**	-7.23
5		-CH_3	**ALA129, TYR149, PHE88,** TYR131, TYR90, GLN148	TYR90, **TYR149,** GLN148	**PHE88,** TYR90	-7.03
6		-CH_2CH_3	**ALA129, TYR149, PHE88,** THR130, TYR131	THR130, TYR131	**ALA129, PHE88**	-6.79
7		-CH_2OH	**ALA129, PHE88,** GLY127, TYR90, GLY151, GLN 150	GLY151, GLN 150	**PHE88**	-7.09

8			**ALA 129, PHE 88,** TYR131, GLN148, **TYR149**	GLN148, **TYR149**	**PHE 88**	-7.57
9			**ALA129, TYR149, PHE88,** TYR131, VAL147, TYR 90	**TYR149,** TYR131	**PHE88,** TYR 90	-7.29
10			**ALA129, TYR149, PHE88,** TYR90 TYR131 GLY151	**ALA129,** TYR90**, PHE88**	**TYR149,** TYR90 TYR131 GLY151	-7.32
11			**ALA129, TYR149,** GLN148, VAL147, VAL184, TYR90	**ALA129, TYR149**	VAL147, VAL184, **TYR149**	-6.84
12			**ALA129,** VAL147, TYR90	**ALA129**	VAL147	-7.54
13			**ALA 129, PHE 88**, GLY 151, GLN 152	GLY 151, GLN 152	**ALA 129, PHE 88**	-7.94
14			**PHE88, ALA129, TYR149,** VAL147	**ALA129,** VAL147	**PHE88, ALA129**	-7.23
15			**PHE88, ALA129, TYR149,** VAL147, TYR90	**ALA129, TYR149,** VAL147, TYR90	**ALA129, PHE88,** TYR90, **TYR149,**	-7.25
16			**ALA129, TYR149, PHE88,** TYR131, TYR90	**ALA129, TYR149,** TYR131	TYR90	-7.59

17			**ALA129, TYR149, PHE88,** TYR131	TYR131	**ALA129, PHE88**	-6.31
18			**ALA129, TYR149, PHE88,** GLY127, GLY151, GLN150	GLY151, GLN150	**ALA129, PHE88**	-7.22
19			**ALA129, TYR149, PHE88,** GLY127, TYR90, GLY151	GLY127, **TYR149,**	**ALA129, PHE88**	-7.68
20			**ALA129, PHE88,** TYR90	TYR90	**ALA129, PHE88**	-6.69

RESULT & DISCUSSION

Generally, the biological activity of any Analogue is computationally represented by ligand-protein interaction properties and binding affinity, which is related to the docking score. A compound with a minimum docking score indicates that it interacts well with the target protein and gives biological activity. The above result shows that the Ciprofloxacin drug interacts with **PHE 88, TYR149 & TYR 90** amino acids of **2XE1** protein and scoring (-7.006 kcal/mol) docking score and also its derivative showing interaction with the above-mentioned amino acid, which means they involve in ligand-protein interaction and have an affinity towards common drug nucleus. A comparative study of the docking score of standard and analog drugs helps find the most interactive compound. It means analogs that have minimum docking score perform greater binding affinity or have greater affinity because they are involved in interaction with PHE 88, TYR149 & TYR 90 amino acids, resulting in more biological activity because the absence of these results in a lousy docking score. Second, most derivatives show binding affinity towards these **ALA129**, **TYR 149,** and **PHE 88** amino acids. Two of these, **PHE88** and **TYR149,** are also involved in the interaction of the Parent Ciprofloxacin drug, which means that these two amino acids have binding affinity to the common Ciprofloxacin nucleus, which is necessary for optimum biological activity. If amino acids are absent in binding, this derivative's biological activity will decrease. Secondly, the maximum derivative has a more excellent docking score than the ciprofloxacin parent nucleus, meaning they have more biological activity. Compound no-13, with a more excellent docking score, can produce more biological activity than the ciprofloxacin parent nucleus. This work's main point of view is that **ALA 129** docks with 19 different compounds, which means the **ALA129** amino acid has the highest binding affinity. In contrast, **VAL 184** has the lowest binding affinity because that shows interaction with only one compound). This means that **ALA 129** is essential for biological activity because biological activity directly depends on ligand-receptor interaction. Another thing is that **ALA129 & PHE88** have an excellent affinity toward hydrophobic interaction. Compound no-11, which has protein interaction with **VAL 184,** has the lowest affinity towards Ciprofloxacin derivative & also has a lower docking score.

Standard Compound: Ligand protein interaction shows the Cyclopropyl group interacts with **PHE88 & TYR90,** two amino acids through the hydrophobic bond. From docking interaction, tabulation data also represent that these two amino acids are essential for biological activity(Figure3& 4). A standard compound does not make hydrogen bonds with any amino acid, but all the derivatives make hydrogen bonds with different

amino acids. The docking simulation of interaction is shown below in Figure 3, 4, which is the 2D and 3D representation of interaction & the docking score of Parent ciprofloxacin with 2XE1 is **-7.06 kcal/mol**(Table 1)**.** Figure 3 is a 3D representation of drug-protein interaction that explains the Cyclopropyl moiety of Ciprofloxacin interacts hydrophobically with PHE 88, TYR90 amino acids of 2XE1 protein. It produces biological activity, which means the interaction of drug molecules with these amino leads to biological activity. This means that analogs that have an affinity for this amino acid can also produce biological activity because they will be involved in the interaction. In other words, they have an affinity towards the ciprofloxacin nucleus.

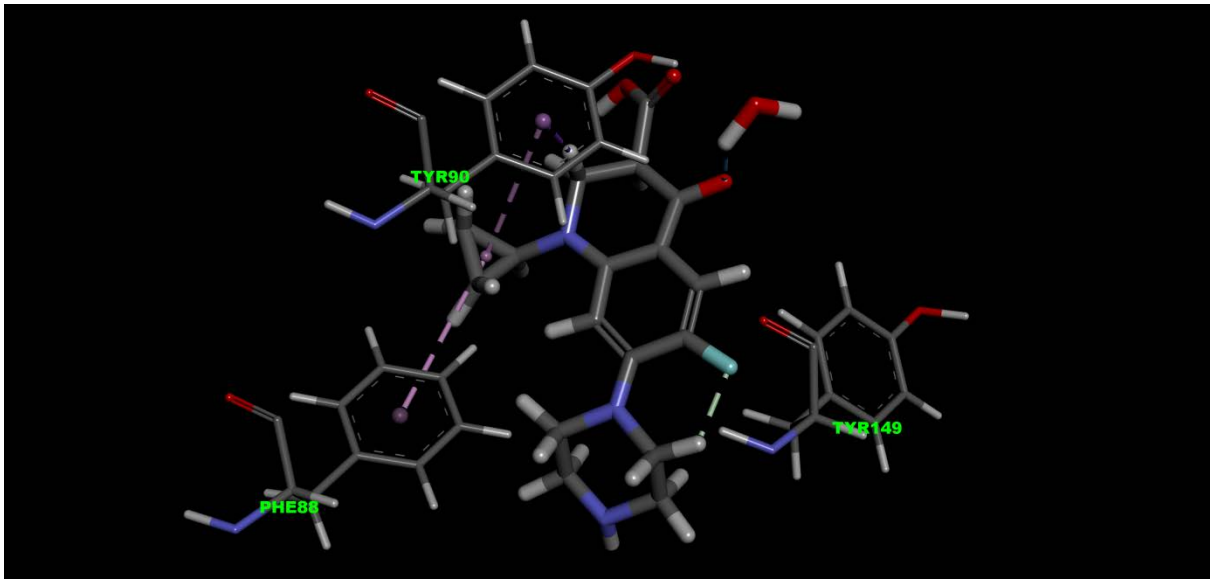


Figure 3: 3D visual representation of Docking (ligand-protein interaction) of Standard Ciprofloxacin with 2XE1

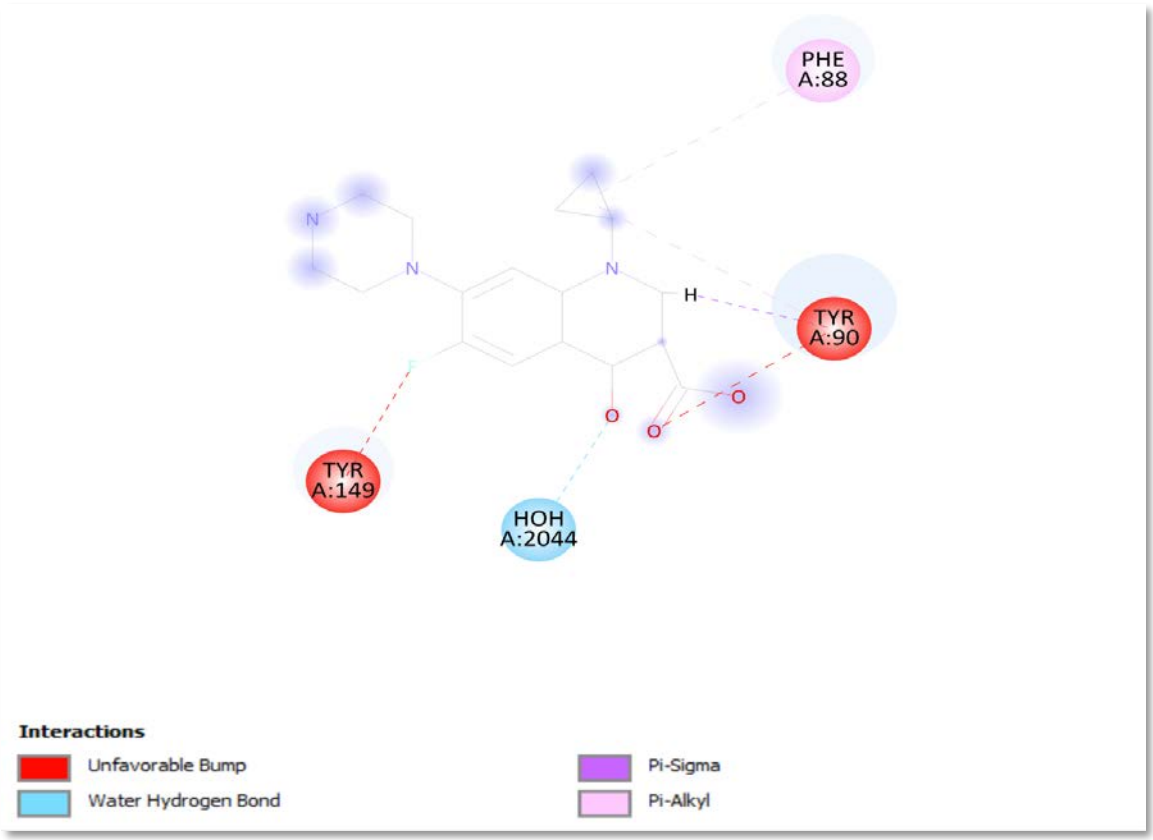


Figure 4: 2D representation ligand-protein interaction of Standard Ciprofloxacin with 2XE1

Compound No-1: Compoundno-1 is Fluorine substituted with a Chlorine halogen atom that shows interaction withALA129, TYR149, GLY127, GLY151, TYR90, and GLN150 with docking score (-7.31). **ALA129 & TYR149** amino acids hydrophobically interact with the Cyclopropyl group, and this compound's carboxylic group interacts with **GLY 151 & GLU 150** amino acids through hydrogen bonds (Table 1).

Compound Similar to compound no-1Flourine substituted with Iodine halogen atom shows interaction with TYR149, PHE88, GLY151, and TYR 90 with docking score (-7.21). In this compound, **PHE88 & TYR90** amino acids hydrophobically interact with Iodine. Adding another halogen, the Iodine atom, can replace Fluorine in hydrophobic interaction from cyclopropyl to the Iodine atom. This means this compound shows more interaction affinity towards the Iodine group than the cyclopropyl group (Table 1).

Compound No-3: Similar to compound no-1, fluorine is substituted with a chlorine halogen atom, which shows interaction with ALA129 and TYR149 with a docking score (-7.35).In this compound, some different interactions have been seen, such as the Piperazyl group interacting with **ALA129** and the Bromine group interacting with **TYR149** through hydrophobic bonding, and due to this, this compound shows more significant docking interaction in comparison to the above three compounds along with standard compounds. This means that the interaction of the piperazinyl group with **ALA129 and** Bromine with **TYR 149 increases** the interaction affinity. This means chlorine atoms, compared to fluorine, show good interaction (Table 1).

Compound No-4: In this compound, the cyclopentyl group is replaced by the cyclobutyl group, which interacts with PH88amino acid, while TYR131 and THR130 interact with the carboxylic group through hydrogen bonding and the **ALA129** quinoline group through hydrophobic interaction. Adding the Cyclobutyl group lowers the docking score, which means replacing the cyclopropyl group with another cyclic ring, such as cyclobutyl and cyclopropyl, can decrease the biological activity (Table 1).

Compound No-5: Adding a methyl group to the Piperzyl ring can decrease the biological activity. Because of this, the substituted methyl group interacts with TYR90 through hydrogen bonds, while TYR149 and GLY148 interact with the carboxylic group and lower the interaction. **PHE88** hydrophobically interacts with the cyclopropyl group **TYR90** with the Piperzyl ring (Table 1).

Compound No-6: This compound is an ethylated substituted compound.**THR130 and TYR131 interact with the carboxylic group through hydrogen bonds, and PHE88** and **ALA129** interact with cyclopropyl and quinoline rings through hydrophobic bonds (Table 1).

Compound No-7: GLY151 and GLN150 amino acids interact with the carboxylic group through a hydrogen bond, and **PHE88** similarly interacts with cyclopropyl through the hydrophobic bond. Adding methanol does not produce any specific activity (Table 1).

Compound No-8: TYR149 &GLN148 amino acids interact with the oxo group through a hydrogen bond, and **PHE88** amino acid interacts with the cyclopropyl group through the hydrophobic bond. The addition of the carboxylic group on the piperazine ring shows good interaction with protein and produces a good docking score (Table 1).

Compound No-9: TYR149 and TYR131 will interact with the oxo and carboxylic groups through hydrogen bonds. Similarly, PHE88 and TYR90 will interact with the Cyclopropyl and piperazyl groups, respectively (Table 1).

Compound No-10: PHE88, TYR90 & ALA129 will interact with the cyclopropyl group through the hydrophobic bond. ALA129 also makes hydrophobic bonds with the quinoline ring and piperazyl ring. **TYR149** also interacts hydrophobically with the quinoline ring—**TYR131 interacts** with the acetyl group through a hydrogen bond. **TYR149** also interacts through hydrogen bonds with the quinoline ring. **GLY151& TYR 90** interact with the carboxylic group through the hydrophobic bond (Table 1).

Compound No-11: ALA129 interacts with the piperazinyl ring and **TYR149** with the ether group through the hydrogen bond. That compound shows interaction with two amino acids, **ALA129** and **TYR149,** which are common in the interaction of all compounds. Adding an ether group can resist the compound's constant biological activity level, which is essential. **VAL147, VAL184,** and **TYR149** will interact with the ether group through the hydrophobic bond (Table 1).

Compound No-12: For this compound, **VAL 147,** only one amino acid interacts hydrophobically with the tertiary butyl group to show interaction, which means the addition of tertiary butyl to the para position of the piperazinyl ring can produce good biological activity. **ALA129** interacts with the piperazinyl ring through a hydrogen bond (Table 1).

Compound No-13: GLY151 & GLY152 will interact with the carboxylic group through hydrogen bonds. Before it,

Compound-1 interacted with **GLY151** through hydrogen bonds and showed good biological activity. This means that GLY151hasa has a good affinity for the carboxylic group. **ALA129** and **PHE88** interact with the piperazyl ring through hydrophobic bonds. Simultaneously, PHE88 also interacts with the Acetophenone group. This compound shows good interaction with **ALA129, PHE88, GLY151,** and **GLY152** amino acids(Figure 5& 6). Two of these amino acids, ALA129 & PHE88, are the most common amino acids that interact with most of the derivatives of Ciprofloxacin and standard drugs. So therefore, Compound-13 performs well in interaction with protein and results in a good docking score (-7.94) (Table 1), a comparatively better docking score than the standard. This means that adding the acetophenone group to the para position of the piperazyl group can give the most active compound and produce more biological activity.

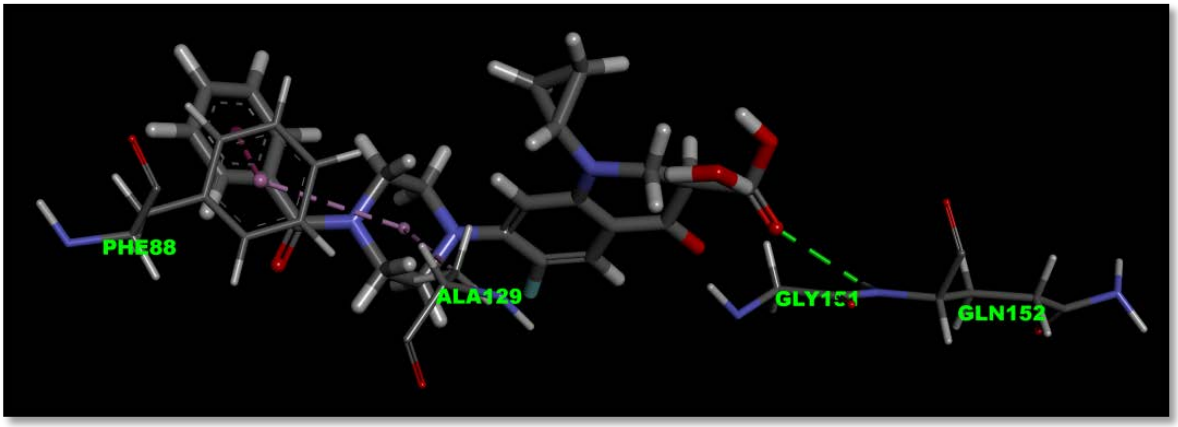


Figure 5: a 3D visual representation of docking of the Best pose of Compound no-13.

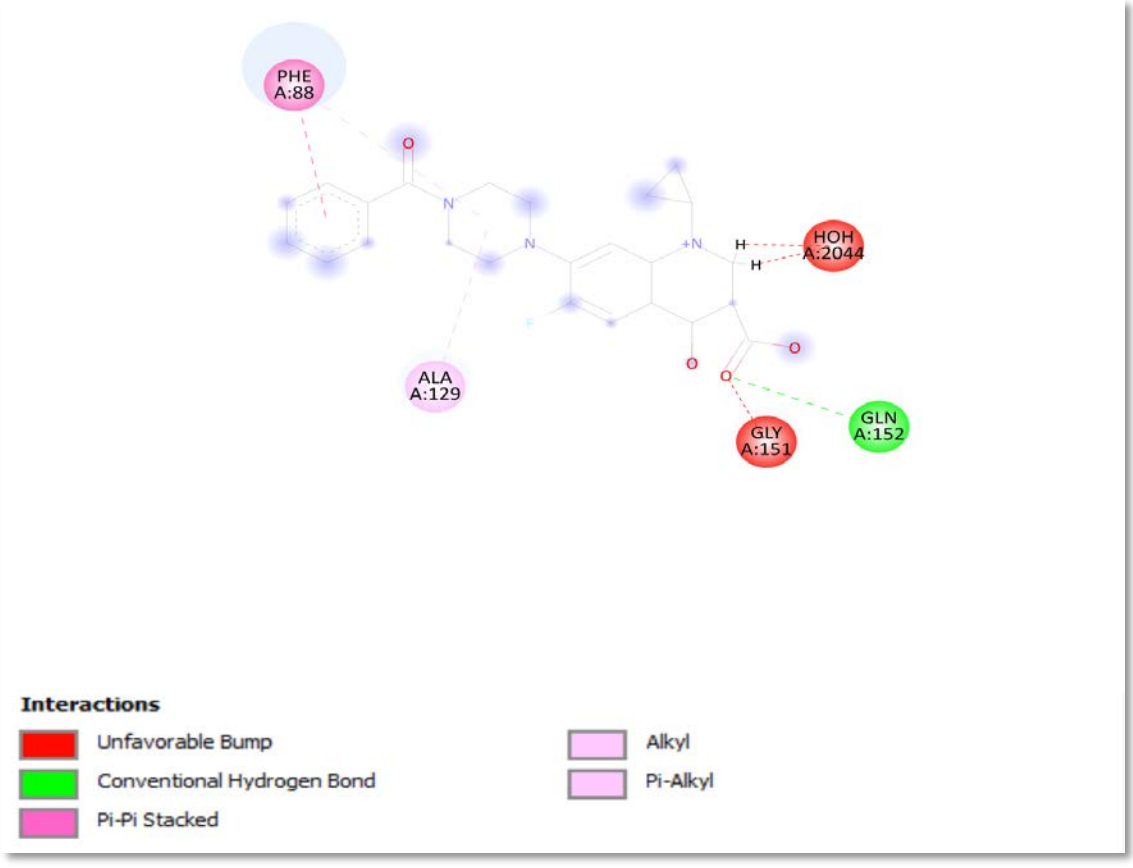


Figure 6: 3D Best pose of Ligand-protein interaction of Compound no 13.

Compound No-14: ALA129 & VAL147 will interact with the piperazyl ring through a hydrogen bond. In the modified group, this ligand hydrophobically does not show interaction with amino acids but hydrophobically interacts with **PHE88** amino

acid, which is a typical interaction amino acid. **ALA129** interacts with three groups, the piperazyl ring, benzoquinone, and cyclopropyl ring, through the hydrophobic bond. **TYR149** also interacts with the cyclopropyl group through the hydrophobic bond (Table 1).

Compound No-15: ALA129 interacts simultaneously with three groups, quinoline, piperazyl, and Cyclopropyl ring, through the hydrophobic bond. **TYR90 & PHE88** both interact with cyclopropyl rings through hydrophobic bonds. **TYR149** interacts hydrophobically with Quinoine and piperazyl ring. **VAL147** interacting with the ethanolic group. **ALA129** interacts with the piperazyl ring through the hydrogen bond. **TYR149** will interact with the quinoline ring through a hydrogen bond—similarly, **TYR90** interacts with the carboxylic group through hydrogen bonds. And **VAL147** amino acid interacts with the substituted ethanolic group (Table 1).

Compound No-16: TYR149 interacts with the oxo group through hydrogen bonds and TYR131 with the carboxylic group. **ALA129 is** going to interact with quinoline and piperazyl ring. **TYR90** single amino acid interacts with the piperazyl ring through the hydrophobic bond. The most important thing is that the most common amino acid, **PHE88,** interacts with the pyrazole ring, which is substituted for this compound. Replacing the cyclopropyl group with the Pyrazole ring can yield the most active compound (Table 1).

Compound No-17: ALA129 interacts with the Quinolne ring through the hydrophobic bond. Similarly, **PHE88,** an amino acid, binds with the cyclopropyl group through the hydrophobic bond—**TYR131**interacts with the carboxylic group through hydrogen (Table 1).

Compound No-18: GLY150 and GLY151 interact with the amide group through hydrogen bonds. **ALA129** and **PHE88** interact with the Cyclopropyl group through a Hydrophobic bond—similarly, **TYR149** interacts with the methyl group of amide substitution (Table 1).

Compound No-19: ALA129 and PHE88 amino acids interact with the cyclopropyl group through a hydrophobic bond, and GLY127 and TYR149 interact with the amine group through hydrogen bonding. This compound also interacts with protein with a second good dock score. This means it can also produce good biological activity and can reduce the probability of AR due to its high drug potency (Table 1).

Compound No-20: TYR90 interacts with the methyl group of the amine substituent through a hydrogen bond. ALA129 and PHE88 interact with the Cyclopropyl group through a hydrophobic bond. Adding the longest aliphatic chain with the amine group to replace the carboxylic group did not present a good docking score (Table 1).

Pharmacophore Modeling

Pharmacophore is a fundamental concept of 3-dimensional structure that reveals the essential structural and spatial features, including Hydrogen bonding acceptor, Hydrogen bonding donor, aromatic ring, and hydrophobic moieties within the molecule, which is necessary for interaction with receptor protein DNA gyrase & topoisomerase-IV enzyme in bacteria to produce our biological activity. Therefore, to determine the arrangement, features, and structural similarities of Ciprofloxacin and its analogy, the Pharmacophore modeling was done using the Portable software Ligandsout. This comparative analysis of the models highlighted similarities and subtle differences in their spatial arrangements, which provide brief insights into structural modification that could enhance efficacy & selectivity, as shown below (Figures 7 & 8).

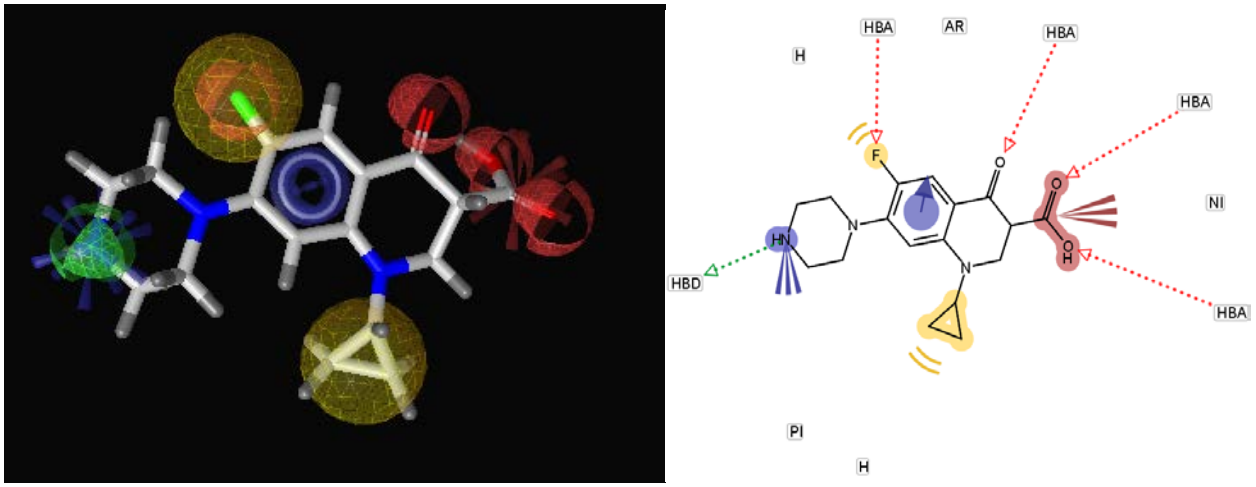


Figure 7: 2D, and 3D representation of Pharmacophoric Feature of Ciprofloxacin

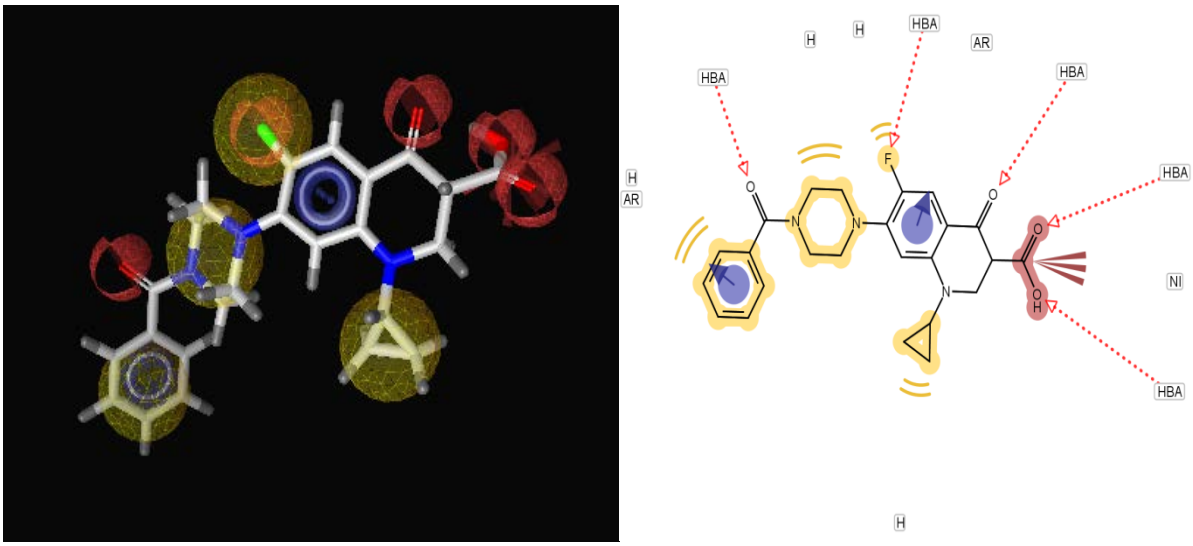


Figure 8: 2D, and 3D representation of Pharmacophore feature of Compound no-13

The Pharmacophore model generated using LigandScout software offers valuable insights into the molecular basis of ciprofloxacin's antibacterial activity. The above Pharmacophore model represents that Ciprofloxacin and its analog have similar spatial features in favor of protein binding, which is responsible for biological action. Both compounds have the same hydrogen bonding acceptor group and one negative ionization group, which is essential for biological activity by elucidating the essential structural key feature required for drug target interaction and offering the rational design of new ciprofloxacin analogs with the improved pharmacological profile. Future research could focus on synthesizing and testing analog no 13 to validate their predicted activities and explore their potential in clinical practice (Figures 7 & 8).

CONCLUSION

Ciprofloxacin is a broad-spectrum antibiotic drug used to treat many bacterial infections, such as Gastrointestinal infections, Urinary tract infections, and other bacterial infections. It will most commonly interact with 2XE1 transport protein to give its biological activity. Similarly, in Docking, all analogs of ciprofloxacin interact well with the 2XE1 protein. After docking, we found that amino acids ALA 129, TYR 149, and PHE 88 are common in every ligand-protein interaction**.** If any of these do not participate in the interaction, the docking score will not be good, which means these amino acids are essential for optimum biological activity. For example, when Compound no 13 is docked with 2XE1 protein, it shows interaction with ALA129, PHE88, GLY151, and GLY152. Here, two amino acids, ALA129 and PHE88, are familiar and involved with standard drugs, whereas when compound-11 is docked with 2XE1 protein, then it shows interaction with ALA129, TYR149, GLN148, TYR90, VAL149, and VAL184 amino acids. Still, only one common acid, ALA129, is involved here, so it has a docking score higher than standard compound and compound 13. Simultaneously, Pharmacophore modeling of ciprofloxacin and its analogs, a powerful approach, reveals essential for good drug discovery & design. So from this approach, we can increase the Ciprofloxacin affinity towards these common amino acids for good interaction & can develop a more potent compound. The above tabulation shows that **PHE88** and **ALA129,** two amino acids, show the maximum time involved in hydrophobic interaction that declares the drug must have an affinity toward these two amino acids so that the drug can easily hydrophobically interact with the protein and can produce biological activity. More importantly, this work revealed spatial docking and pharmacophoric features required for Quinoline derivative activity. Based on these features, we can design more potent & effective compounds that have a greater affinity towards this common amino acid and produce biological activity. For example, Compound-13, which has a good docking score, good interaction properties, and spatial pharmacophoric features similar to the standard, can make more biological activity and become a more potent novel drug in the future. This work also reveals good interaction between Ciprofloxacin and its other analogs. The affinity of ligands towards **ALA129, TYR149 & PHE88** amino acids is essential. Secondly, Compound No-19 also represents good interaction with a good docking score after compound no-13. For future drug development, this study will be beneficial in designing new

drugs. These two compounds, Compound 13 & Compound 19, have become more potentially active new drugs. Recently, the pharmacophore Anchor model complied 89 known NS3 Protease inhibitors, and this method was applied to the DENV NS3 protease to screen FDA drugs, discovering boceprevir, telaprevir, and asunaprevir as promising anti-DENV candidates. The insight gained from this study provides a foundation for novel antibiotics that effectively combat bacterial infection while minimizing resistance and adverse effects.

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FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Sanjana Katlaria and Ashish Singh Chauhan wrote the main manuscript text, while Krishna Kumar, Bhumika Chauhan, and Mohit Kumar wrote the Conceptualization and Methodology. Sanjana Katlaria prepared figures 1-2, and Ashish Singh Chauhan and Vikash Jakhmola reviewed and edited them. All authors reviewed the manuscript.

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LIST OF ABBREVIATION

Antimicrobial resistance (AMR)
Antibiotic resistance (AR)
Total drug resistance (TDR)
Extensive drug resistance (XDR)
Multidrug resistance (MDR)
Global Antimicrobial Resistance and Use Surveillance System (GLASS)
Sustainable Development Goals (SDGs)
Minimum inhibitory concentration (MIC)
Enterobacterales
Staphylococcus aureus
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
and Enterobacter (ESKAPE)
Methicillin-resistant Staphylococcus aureus (MRSA)
Carbapenem-resistant enterobacterales (CRE)
Vancomycin-resistant Enterococcus (VRE)
horizontal gene transfer (HGT)
Lipopolysaccharide (LPS)
ATP-Binding Cassette (ABC)
Multidrug and Toxic Compound Extrusion (MATE)
Major Facilitator Superfamily (MFS)
Resistance Nodulation and Cell Division (RND)
Small Multidrug Resistance (SMR)
Proton motive force (PMF)
Penicillin-binding proteins (PBPs)
Metallo-β-lactamases (MBL)
Clustered regularly interspaced short palindromic repeats system (CRISPR-Cas)
Extensively drug-resistant Klebsiella pneumonia (ERKp)
Recurrent urinary tract infection (UTI)
Scanning electron microscopy (SEM)
Confocal laser scanning microscopy(CLSM)
Chitosan oligosaccharide (COS-AuNPs)
copper oxide nanoparticles (CuONPs)
Norfloxacin (NOR)
Thymoquinone (TQ)
Antimicrobial peptides (AMPs)
Poly(ethylene glycol) methyl ether-block-poly (lactide-co-glycolide) (PEG-PLGA)
Genetic Optimization for Ligand Docking (GOLD)
Cambridge Crystallographic Data Centre (CCDC)
Protein Data Bank (PDB)
Ceftaroline (CFT)
Chemical absorption, distribution, metabolism, excretion and toxicity (ADMET)
Fluoroquinolone (FQ).