Computational Investigation of nsP2 Inhibitors for Chikungunya Virus Treatment: Advancing Therapeutic Strategies Against Viral Infections

.

By
Aisha Siddika
20346009

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm)

School of Pharmacy
BRAC University
May, 2025

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Aisha Siddika
20346009

Approval: The thesis titled "Computational Investigation of nsP2 Inhibitors for Chikungunya Virus Treatment: Advancing Therapeutic Strategies Against Viral Infections," submitted by Aisha Siddika (20346009) in May 2025, has been deemed satisfactory and accepted as part of the requirements for the Bachelor of Pharmacy degree.

Supervised By:

Asef Raj
Lecturer
School of Pharmacy
BRAC University

Approved By:

Acting Chairperson

Dr. Raushanara Akter, PhD
Acting Chairperson and Professor
Department of Pharmacy
School of Pharmacy
BRAC University

Acting Dean

A.F.M. Yusuf Haider, PhD
Acting Dean, School of Pharmacy
BRAC University

Ethics Statement

This study does not include any experiments or trials involving humans or animals.

Abstract

Chikungunya virus (CHIKV) is an insect-borne virus characterized by a positive-sense single-stranded RNA genome, primarily transmitted to humans through the bites of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Initially reported in Tanzania in 1952, the virus has since disseminated across various tropical and subtropical regions worldwide, leading to clinical manifestations such as high-grade fever, arthralgia, and myalgia. The virus encodes four non-structural proteins (nsP1–nsP4), which are integral to its replication cycle, immune evasion mechanisms, and RNA synthesis. Among these, nsP2 is particularly significant due to its dual functionality as a protease and helicase, making it a viable target for antiviral drug development in the absence of currently approved therapeutic agents. To explore potential inhibitors, a molecular docking study was undertaken utilizing the crystal structure of the nsP2 protease (PDB ID: 3TRK), resolved at a 2.4 Å resolution. A curated library of 202 FDA-approved compounds was subjected to virtual screening, resulting in the identification of five lead compounds with the highest binding affinities. These candidates exhibited robust interactions with critical residues in the nsP2 active site, highlighting their potential as promising antiviral agents against CHIKV.

Keywords: Chikungunya virus, molecular docking, antiviral, antiviral drug development, virtual screening

Dedication

This project is dedicated to all patients who suffer from viral diseases, in the hope that this work contributes, even in a small way, to advancing their care and well-being. I also dedicate this to my parents, whose unwavering love, support, and encouragement have been my greatest sources of strength**.**

Acknowledgement

I am profoundly grateful to the Almighty for providing me with the strength and determination to complete this project. My sincere thanks go to my supervisor, Mr. Asef Raj, Lecturer at the School of Pharmacy, BRAC University, for his invaluable guidance and constant support throughout this research.

I would also like to express my appreciation to Professor Dr. A.F.M Yusuf Haider, Acting Dean of the School of Pharmacy, for his exemplary leadership and commitment to academic excellence. Finally, I am truly thankful to my parents and loved ones for their unwavering encouragement and support, as well as to everyone who has contributed to the completion of this work.

Table of Contents

Table of Contents

LIST OF TABLES

LIST OF FIGURES

List of Acronyms

- CHIKV - Chikungunya Virus
- FDA - Food and Drug Administration
- ORF - Open Reading Frame
- Nsp1 - Non-Structural Protein 1
- Nsp2 - Non-Structural Protein 2
- Nsp3 - Non-Structural Protein 3
- Nsp4 - Non-Structural Protein 4
- SAR - Structure-Affinity Relationship

- QSAR - Quantitative Structure-Activity Relationships
- RNA - Ribonucleic Acid
- DNA - Deoxyribonucleic Acid
- NSAID - Non-Steroidal Anti-Inflammatory Drug
- RC - Replication Complex
- HADDOCK - High Ambiguity Driven Protein–Protein Docking
- UTA - Untranslated Region
- mRNA - Messenger Ribonucleic Acid
- NLS - Nuclear Localization Signal
- ADP - Adenosine Diphosphate
- BHK-21 - Baby Hamster Kidney-21
- RdRp - RNA-Dependent RNA Polymerase
- TATase - Adenylyltransferase
- KDA - Kilodalton
- PDB - Protein Data Bank
- VS - Virtual Screening
- DAA - Direct Acting Antiviral
- UCSF - University of California, San Francisco
- CPE - Cytopathic Effects
- CADD - Computer-Aided Drug Design
- DSV - Docking Scoring Vector
- SDF - Structure Data File
- HIV - Human Immunodeficiency Virus
- HCV - Hepatitis C Virus
- COVID-19 - Coronavirus Disease 2019
- SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2
- SFV - Score Function Value
- SBDD - Structure-Based Drug Design
- LBDD - Ligand-Based Drug Design
- RBVS - Receptor-Based Virtual Screening
- CoMFA - Comparative Molecular Field Analysis

- CoMSIA - Comparative Molecular Similarity Indices Analysis
- D-score - Druggability Score
- INSTI - Integrase Strand Transfer Inhibitor
- NS5A - Non-Structural Protein 5A

Chapter 1: Introduction

1.1 Overview of Chikungunya Virus (CHIKV)

Chikungunya virus (CHIKV) is a positive-sense, single-stranded RNA virus primarily transmitted by insect vectors, notably *Aedes aegypti* and *Aedes albopictus* mosquitoes, which facilitate the transmission of the virus to both humans and animals through their bites (Morrison,2014a).These illnesses are triggered by viruses, bacteria, or parasites that mosquitoes transmit when they bite an infected human or animal. The mosquito then transfers the pathogen to a new host, leading to the onset of disease. The Chikungunya virus is categorized within the Orthornavirae kingdom and is part of the alphavirus genus in the Togaviridae family(Schwartz & Albert, 2010).

CHIKV is a small, spherical virus, approximately 70 nanometers in diameter, surrounded by a lipid envelope(Silva & Dermody, 2017)Inside the nucleocapsid lies the single-stranded, positive-sense RNA genome, about 11.8 kilobases (kb) in length, which is complexed with multiple copies of a 30 kDa capsid protein (C), the only protein present in the nucleocapsid (Strauss & Strauss, 1994). The Chikungunya virus genome consists of two open reading frames (ORFs), designated as the 5′-ORF and 3′-ORF, which are connected by an intergenic junction region and flanked by untranslated regions at both ends, known as the 5′-UTR and 3′-UTR. The 3′-ORF, spanning roughly 3,700 nucleotides, encodes a short 6K peptide in addition to four structural proteins: the capsid (C) and the envelope proteins E1, E2, and E3. In contrast, the 5′-ORF, which is approximately 7,400 nucleotides in length, is responsible for synthesizing four non-structural proteins: nsP1, nsP2, nsP3, and nsP4 (Rabelo et al., 202).

1.2 Public Health Significance of Chikv

Chikungunya virus (CHIKV) leads a major global health concern owing to its capacity to trigger widespread epidemics and contribute to elevated mortality levels. The virus is thought to have emerged in the Central or Eastern regions of Africa (Sanyaolu et al., n.d.).Large-scale outbreaks of the Chikungunya virus (CHIKV) have been reported across several continents, especially in areas inhabited by Aedes mosquitoes, including Aedes aegypti and Aedes albopictus, which are the primary vectors for virus transmission. Regions such as Africa, Asia, and Europe have faced major CHIKV outbreaks(Mourad et al., 2022).Historical evidence suggests that the Chikungunya virus first emerged in the 18th century, with initial cases documented in Indonesia and possibly in the Americas(Weaver, 2014).

Although it was reported that In 1952 Tanzania a southern part of the country, first epidemic of Chikungunya virus (CHIKV) was discovered then chikungunya virus spreads all over the globe specifically in the regions of various tropical and subtropical countries specifically Africa, Asia, Indian ocean even America except antarctica where sub-tropical mosquito rarely found(Mathew et al., 2017). In the Southeast Asia region the first country where chikungunya virus first appeared was Thailand in 1958 but more than 20 years later the virus reemergence around 2001 in the tropical region of Indonesia called java Island. India experienced its first documented chikungunya outbreak during the 1960s (Mavalankar et al., 2007).

The Chikungunya virus (CHIKV) has been documented across West Africa, from Senegal to Cameroon, and in numerous other African nations. Similarly, Asia has witnessed several CHIKV outbreaks, especially in countries such as India, Thailand, and Indonesia, with notable epidemics occurring in the 1960s and 1990s. This underscores the broad geographic spread of CHIKV throughout Africa and Asia and its track record of triggering major epidemics(Caglioti et al., n.d.).The health impact of Chikungunya virus (CHIKV) affects not only infected individuals but also the communities they belong to. CHIKV infection results in a range of acute clinical manifestations, such as joint pain (arthralgia), skin rash, extreme tiredness (fatigue), high fever, and muscle aches (myalgia)(Sanyaolu et al., n.d.). A study conducted by (Fusco et al., 2006; Taubiz et al., 2007) since the outbreak began in the Indian Ocean region, over 1,000 imported cases of CHIKV have been identified in European and American travelers returning from affected areas (Caglioti et al., n.d.).The Chikungunya virus (CHIKV) results in long-lasting or delayed symptoms for 43%-75% of those infected, persisting for up to two years and causing chronic health issues. The risk of CHIKV

spreading globally is alarming, particularly due to the spread of Aedes aegypti mosquitoes into the Americas. This emphasizes CHIKV as a major global health concern, demanding immediate action from healthcare workers, researchers, and public health officials to combat its increasing impact (Sanyaolu et al., n.d.).

1.2 Public Health Significance of Chikv

Chikungunya virus (CHIKV) leads a major global health concern owing to its capacity to trigger widespread epidemics and contribute to elevated mortality levels. The virus is thought to have emerged in the Central or Eastern regions of Africa (Sanyaolu et al., n.d.).Large-scale outbreaks of the Chikungunya virus (CHIKV) have been reported across several continents, especially in areas inhabited by Aedes mosquitoes, including Aedes aegypti and Aedes albopictus, which are the primary vectors for virus transmission. Regions such as Africa, Asia, and Europe have faced major CHIKV outbreaks(Mourad et al., 2022).Historical evidence suggests that the Chikungunya virus first emerged in the 18th century, with initial cases documented in Indonesia and possibly in the Americas(Weaver, 2014).

Although it was reported that In 1952 Tanzania a southern part of the country, first epidemic of Chikungunya virus (CHIKV) was discovered then chikungunya virus spreads all over the globe specifically in the regions of various tropical and subtropical countries specifically Africa, Asia, Indian ocean even America except antarctica where sub-tropical mosquito rarely found(Mathew et al., 2017). In the Southeast Asia region the first country where chikungunya virus first appeared was Thailand in 1958 but more than 20 years later the virus reemergence around 2001 in the tropical region of Indonesia called java Island. India experienced its first documented chikungunya outbreak during the 1960s (Mavalankar et al., 2007).

The Chikungunya virus (CHIKV) has been documented across West Africa, from Senegal to Cameroon, and in numerous other African nations. Similarly, Asia has witnessed several CHIKV outbreaks, especially in countries such as India, Thailand, and Indonesia, with notable epidemics occurring in the 1960s and 1990s. This underscores the broad geographic spread of CHIKV throughout Africa and Asia and its track record of triggering major epidemics(Caglioti et al., n.d.).The health impact of Chikungunya virus (CHIKV) affects not only infected individuals but also the communities they belong to. CHIKV infection results in a range of acute clinical manifestations, such as joint pain (arthralgia), skin rash, extreme tiredness (fatigue), high fever, and muscle aches (myalgia)(Sanyaolu et al., n.d.). A study conducted by (Fusco et al., 2006; Taubiz et al., 2007) since the outbreak began in the Indian Ocean region, over 1,000 imported cases of CHIKV have been identified in European and American travelers returning from affected areas (Caglioti et al., n.d.).The Chikungunya virus (CHIKV) results in long-lasting or delayed symptoms for 43%-75% of those infected, persisting for up to two years and causing chronic health issues. The risk of CHIKV spreading globally is alarming, particularly due to the spread of Aedes aegypti mosquitoes into the Americas. This emphasizes CHIKV as a major global health concern, demanding immediate action from healthcare workers, researchers, and public health officials to combat its increasing impact (Sanyaolu et al., n.d.).

1.3 Clinical Symptoms

Chikungunya, which translates to "that which bends up the joints," is characterized by an abrupt onset of fever, followed by severe joint pain. This pain may last for weeks, months, or even years, resulting in extended discomfort and potential disability(Morrison, 2014b). After being bitten by an infected mosquito, the incubation period for Chikungunya ranges from 2 to 12 days, with symptoms typically emerging within 3 to 7 days. The onset of symptoms is usually sudden, and includes high fever, headache, back pain, muscle pain, and joint pain. The acute phase of the infection generally lasts from several days to a few weeks, with most patients making a full recovery. Prominent symptoms during the acute stage include a biphasic high fever lasting from days to weeks, along with back pain, fatigue, muscle pain (myalgia), and joint pain (arthralgia), which may be accompanied by joint swelling in some cases(Sanyaolu et al., n.d.)

Acute phase of Chikungunya infection generally lasts a few days to several weeks, and the majority of patients recover completely after this period (Sanyaolu et al., n.d.)Skin involvement occurs in approximately 40-50% of cases, presenting as an itchy maculopapular rash, primarily on the chest (Caglioti et al., n.d.) In the late stage of chikungunya fever, persistent symptoms mainly include chronic joint and muscle pain, often linked to rheumatic conditions like rheumatoid arthritis and spondyloarthropathy. Other less common symptoms involve neurological, dermatological, and digestive issues. These prolonged effects can significantly impact quality of life and mental health, though standard lab tests typically remain normal, except in rheumatoid arthritis cases (Thiberville et al., 2013).

1.4 Importance of Viral Non-Structural Proteins (nsp2)

The enzymatic functions of alphavirus nsP1 are essential for capping viral RNA, a process critical for replication and immune evasion (Wang et al. 1996), highlight that capping protects viral RNA from degradation by host exonucleases, ensuring genome stability and functionality. The cap structure also enables efficient translation of viral proteins and helps the virus mimic host mRNA, evading immune detection. Furthermore, nsP1's capping activity anchors the viral replication complex to host membranes, facilitating RNA synthesis and replication (Ahola & Merits, 2016a). (Spuul et al. 2007) reported that nsP1 possesses a distinctive capability to attach to the inner surfaces of host cell membranes, enabled by specific domains that interact with lipid bilayers. By anchoring to the membrane, nsP1 plays a key role in assembling the viral replication complex (RC) by recruiting other viral non-structural proteins (nsP2, nsP3, and nsP4) as well as host factors. NsP1 possesses a distinctive capability to attach to the inner surfaces of host cell membranes, enabled by specific domains that interact with lipid bilayers. By anchoring to the membrane, nsP1 plays a key role in assembling the viral replication complex (RC) by recruiting other viral non-structural proteins (nsP2, nsP3, and nsP4) as well as host factors (Ahola & Merits, 2016a).

NsP2 is a versatile enzyme in CHIKV, vital for both viral replication and pathogenesis ,the N-terminal domain exhibits nucleoside triphosphatase, helicase, and RNA-dependent 5′-triphosphatase functions, while the C-terminal domain contains cysteine protease activity, also referred to as thiol protease (Nguyen et al., 2015). A research conducted by (Lulla et al. 2006, 2012; Vasiljeva et al. 2001) the protease activity of CHIKV nsP2 was recently studied

using a system originally designed for SFV protease. on the other side, a study conducted by (Das et al. 2014b) found that only full-length of nsP2 functions as a helicase as it could unwind RNA with an overhang of 12 bases or longer in a 5′–3′ direction. Additionally, nsP2 exhibited RNA strand annealing activity, with both functions relying on its C-terminal region. These findings suggest that functional cross-talk between different domains of nsP2 is crucial for its enzymatic activities (Ahola & Merits, 2016a).

nsP2 is referred to as a regulatory protein because it is essential for managing viral replication and host cell interactions. It possesses various enzymatic functions, including protease activity, RNA helicase function, transcriptional shutoff, and cytotoxic effects, as well as antiviral properties relevant to therapeutic drug development (Ahola & Merits, 2016a). The study by Bassetto et al. (2013) used computational drug screening with a homology-modeled nsP2 protease to identify potential CHIKV inhibitors. Molecular docking and screening led to a potent antiviral compound with an IC50 of ~3 μM and a high selectivity index, indicating strong efficacy and specificity (Ahola & Merits, 2016a).

The cytotoxicity of nsP2, driven by its capacity to disrupt host transcription and other functions, can be reduced through specific mutations. However, no single mutation entirely eliminates cytotoxicity while maintaining CHIKV replication. Instead, researchers have identified four combinations of mutations that allow sustained replication of CHIKV replicon RNA in BHK-21 cells. For instance, mutations that disrupt the nuclear localization signal (NLS) of nsP2 can lessen its ability to interfere with host transcription, impacting viral replication and cytotoxicity. These mutations also influence viral pathogenesis by modulating cell death, immune evasion, and the severity of the disease(Ahola & Merits, 2016a).

nsp2 also plays significant roles in viral pathogenesis according to (Fros et al. 2013) The NLS or nuclear localization signal region enables nsP2 to enter the nucleus and inhibit the JAK-STAT pathway, crucial for immune response. A mutation in this region prevents nsP2 from entering the nucleus, stopping its ability to block immune signaling (Ahola & Merits, 2016a).

nsP3 plays a pivotal role in RNA replication, viral assembly, host cell shutoff, protein synthesis, and virulence. It consists of three domains: an N-terminal macrodomain with phosphatase activity and the ability to bind ADP-ribose and nucleic acids; a central zinc-binding domain, known as the alphavirus unique domain; and a C-terminal hypervariable domain that is highly phosphorylated (Rabelo et al., 2020). (Rubach et al. 2009) reported that nsP4 functions as the catalytic subunit of the alphavirus replicase, serving as the RNA-

dependent RNA polymerase (RdRp).(Ahola & Merits, 2016b) nsP4 functions as the viral RNA-dependent RNA polymerase (RdRp) and consists of two separate regions: a C-terminal RdRp domain that carries out RdRp and adenylyltransferase (TATase) functions, and a partially unstructured N-terminal domain. This N-terminal region is thought to engage with other non-structural proteins (nsPs) in the replicase complex and plays a vital role in the virus's replication process (Rabelo et al., 2020).

1.5 the current status of FDA-approved antiviral and antimicrobial drugs

In the absence of an FDA-approved vaccine or antiviral drug for Chikungunya virus (CHIKV) infection, anti-inflammatory drugs are often prescribed to patients to alleviate symptoms. Researchers have explored various antiviral strategies, including antiviral compounds, inhibitors, virus-like particles, and antibodies, to effectively combat CHIKV infection (Ghildiyal & Gabrani, 2020). It is reported by (Taubitz et al., 2007) there are no targeted antiviral drugs available for CHIKV, so treatment focuses on managing symptoms. Patients are typically given nonsteroidal anti-inflammatory drugs (NSAIDs), fluids, and medications like ibuprofen, naproxen, acetaminophen, or paracetamol to alleviate fever and pain. Although steroids have been used in some cases, their effectiveness has not been proven to be significant (Caglioti et al., n.d.).

Repurposing existing medications is a promising strategy to address viral diseases, offering a faster route to treatment while also providing valuable insights for future drug discovery and preparedness against emerging viral threats. Our earlier research has demonstrated the potential of repurposing existing drugs to combat CHIKV infection effectively(Davuluri et al., 2024) The conventional drug discovery process is intricate, costly, and time-intensive. In contrast, drug repurposing offers a more efficient and cost-effective strategy for identifying treatments for infectious diseases. This approach involves exploring new therapeutic uses for already FDA-approved drugs, providing a promising avenue to expedite antiviral drug development (Mani et al., n.d.).

1.6 Molecular Docking in Drug Discovery

Molecular docking is a computational modeling technique used to predict the most favorable binding orientation between two molecules, such as a ligand and a receptor(Fan et al., 2019). The main concept of the text is that molecular docking, first introduced by Kuntz et al. (1982), is a computational technique used to simulate interactions between biological macromolecules (e.g., DNA, RNA, proteins, peptides) and small molecules (e.g., drugs, endogenous ligands). While modern docking methods like HADDOCK are designed for specific interactions (e.g., protein-protein docking), the primary focus remains on traditional small-molecule docking methods. Tools such as GOLD, Surflex-Dock, AutoDock, and Glide are widely used in structure-based drug design to predict how ligands interact with target proteins, making this technique a standard approach in drug discovery and molecular modeling (Pantsar & Poso, 2018).

There are several types of docking that was developed in 1982 by Irwin D. Kuntz is a trailblazing scientist in the area of molecular docking. In 1982, Kuntz and his research team at the University of California, San Francisco (UCSF) created DOCK, one of the earliest molecular docking programs. Docking evaluations are conducted to assess the interaction profile between a ligand and its target and to identify the optimal ligand conformation within the complex.Empirical scoring functions are employed to convert binding energy values into docking scores. A variety of free online tools, including Biovia DSV, PyMOL, Chimera, RasMol, and Swiss PDB Viewer, are available for generating 3D interaction profiles between ligands and targets. Furthermore, molecular docking can be classified into three main types: flexible docking, semi-flexible docking, and rigid docking (Azad, 2023). Docking is employed to identify the most favorable interaction profile of a ligand within a target protein, determining its optimal binding orientation and conformation. It also measures the energy involved or released during the ligand-protein interaction, offering valuable insights into the binding strength and stability. This information is essential for understanding molecular interactions and aiding in drug design.Various forces influence docking interactions, and the total energy released is calculated using an empirical formula, presented as the total binding energy. Based on these forces, docking interactions are classified into several categories for example Electro-dynamic forces,

Electrostatic forces, Steric forces, Solvent-related forces and Conformational changes that happen during ligand binding (Azad, 2023).

Molecular docking plays a crucial role in computational drug design by predicting binding affinities, screening large drug libraries, and identifying potential inhibitors. This method can predict both the binding affinity between a ligand and a protein and the structure of the protein-ligand complex, providing valuable insights for lead optimization. Over the past three decades, molecular docking has been extensively applied, leading to the discovery and development of numerous new drugs (Wang & Zhu, 2016).The primary objective of the docking score is to distinguish between active and inactive ligands in virtual screening, Estimate affinity constants (binding strengths) for ligand-protein interactions and Accurately rank compounds by their potency, which is the most crucial role of scoring functions(Guedes et al., 2014).Certain scoring functions are specifically designed to detect native and near-native binding modes. These functions do not use affinity data during their parameterization, a critical aspect to consider before performing a docking study (Guedes et al., 2014). Numerous methods, ranging in complexity, have been created to estimate the free energy to predict binding affinity(Guedes et al., 2014).

To estimate how well a ligand fits into a receptor's active site, predicting binding strength, stability, and interaction energy in docking method four type scoring functions are developed such as Force Field-Based Scoring Functions ,Empirical Scoring Functions, Knowledge-Based Scoring Functions, Machine Learning/Deep Learning-Based Scoring Functions (Kitchen et al.,2004). Docking based virtual screening (VS) is a computational tool used to analyze large libraries of compounds and identify the most likely active compounds for a specific molecular target based on predefined criteria. This approach serves as a complement to experimental high-throughput screening, enhancing the efficiency of lead compound discovery(Guedes et al., 2014). There is a constant demand for new drugs that offer improved efficacy and reduced toxicity. However, the drug discovery and development process is expensive, time-consuming, and fraught with challenges. Beyond issues like target validation and hit identification, a significant number of drug candidates fail during clinical trials due to poor pharmacokinetics, insufficient

efficacy (Chang et al., 2022),In-silico research has several advantages in drug development. By making it easier to screen, design, and predict the therapeutic potential of new drugs, they speed up the process of finding potential drug candidates (Roney & Mohd Aluwi, 2024),this techniques offer significant advantages in drug discovery by using computer-based methods to simulate and analyze biological and chemical processes also help reduce both time and cost in several ways for example (Roney & Mohd Aluwi, 2024). A Research conducted by Wong et al., which examined 406,038 trials, discovered that only 13.8% of all drugs (both those already on the market and those still in development) ultimately succeed. The research also highlighted that, on average, it takes about 2.6 years to move from synthesizing a drug to its first human testing, with the process costing close to USD 2.8 billion. This underscores the immense challenges, time, and financial investment required in drug development (Chang et al., 2022).According to (DiMasi et al. 2003; Song et al. 2009) On average, drug discovery and development require 10-15 years and cost approximately 800 million to 800 million to 1.8 billion (Macalino et al., 2015).

Computer-aided drug discovery and design helps lower drug development costs by ensuring that only the most promising lead compounds advance to animal studies. Additionally, it can shorten the time required for a drug to reach the consumer market. Computer-based drug programs offer multiple advantages, including cost efficiency, time savings, and optimized use of human resources (Leelananda & Lindert, 2016).

1.7 Rationale for Selecting FDA-Approved Drugs

Viruses are a diverse group of pathogens responsible for causing severe infectious diseases. Over the past 30 years, numerous antiviral agents targeting viral proteins or host factors have been successfully developed. The growing demand for new antiviral drugs is driven by the persistence of chronic viral infections, such as HIV, influenza, and hepatitis C; the re-emergence of new infections like picornaviruses and coronaviruses; and the increasing resistance to existing antiviral treatments (Mani et al., n.d.). Facing the absence of a definitive cure and high mortality rates among vulnerable groups, health authorities are prioritizing risk re-stratification and repurposing existing drugs to create timely, cost-effective treatments, particularly for hospitalized and critically ill patients (Lin et al., 2021). According to (Lin et al., 2021) Remdesivir a repurposed antiviral drug shows broad-spectrum antiviral activity against RNA viruses. In laboratory studies, remdesivir has demonstrated the ability to suppress viral replication in both Middle East Respiratory Syndrome Coronavirus and Severe Acute Respiratory Syndrome Coronavirus. In the latest and largest clinical trial, findings indicated that remdesivir treatment caused no significant harm, with 24.6% of patients in the remdesivir group experiencing adverse effects. Importantly, no deaths were linked to the use of remdesivir.

Favipiravir is Originally developed and approved for the treatment of influenza, it has been explored and repurposed for other viral infections such as Ebola, yellow fever, chikungunya, norovirus, and enterovirus. This drug has broad-spectrum antiviral and RNA-dependent RNA polymerase (RdRp) inhibitor that blocks viral RNA polymerase activity. It shows greater efficacy than lopinavir and ritonavir and has demonstrated 100% protection in mice against Ebola virus, as well as potential against COVID-19.Favipiravir is a versatile antiviral agent with wide-ranging activity(Munir et al., n.d.). In recent years, notable advancements have been achieved, with several existing drugs showing promise in treating SARS-CoV-2 infection and undergoing clinical trials. For example, remdesivir, initially developed for Ebola virus treatment, is now being tested for its efficacy against SARS-CoV-2 and has received emergency use authorization in several regions globally (Zhan et al., 2022). Nelfinavir, along with other viral

protease inhibitors such as Lopinavir and Ritonavir, has been repurposed for potential treatment against Dengue virus infection(Mani et al., 2019).

1.8 Research Gap and Objective

Despite the critical role of CHIKV nsP2 in the viral life cycle, there are no FDA-approved inhibitors specifically designed to target it. Current treatments for Chikungunya primarily address symptom relief rather than directly blocking viral replication, highlighting a significant gap in targeted antiviral therapies. Furthermore, the literature on CHIKV disease shows no evidence of FDA-approved antiviral or antimicrobial drugs that specifically target nsP2.Therefore, the main study of this objective is to identify potential inhibitors of chikungunya nsp2 protein using molecular docking FDA approved antiviral and antimicrobial drug.

1.9 Significance of the Study

The Role of nsP2 in CHIKV Replication is nsP2 protease of Chikungunya virus (CHIKV) is a vital component for viral replication, playing a key role in cleaving polyprotein precursors necessary for the replication process. NsP2 unwinds viral RNA and assists in RNA capping, both critical steps for viral RNA synthesis and translation**.** Showing its helicase activity. As a result, it is emerging as a promising target for drug development against CHIKV (Nguyen et al., 2015).
The nsP2 protein of Chikungunya virus (CHIKV) is crucial for evading the host immune system. Non-structural protein 2 (nsp2) suppressing the host antiviral response through inhibition of transcription and disruption of the JAK/STAT signaling pathway which is essential for interferon-mediated immunity, the nsP2 protein plays a key role in CHIKV infection. The discovery that a mutation (P718G) in nsP2 inhibits CHIKV replication underscores its potential as a promising target for antiviral drug development (Kaur & Chu, 2013).

Researchers led by Bassetto et al. created a homology model of CHIKV nsP2 using the VEEV nsP2 crystal structure and conducted a virtual screen of five million compounds. They identified Compound 1 as a potent antiviral candidate, which inhibited CHIKV-induced cytopathic effects (CPE) with an EC50 of 5 μM and a CC50 of 72 μM. Computational predictions suggested

Compound 1 binds to the nsP2 protease active site, and SAR studies highlighted the importance of its cyclopropylic moiety and hydrazone group for its anti-CHIKV activity (Kaur & Chu, 2013). In a separate study, researchers identified several nsP2 inhibitors through a computer-aided screening approach. Among these, one promising compound (referred to as compound 1) demonstrated notable antiviral activity against Chikungunya virus (CHIKV)(Abdelnabi et al., 2016)According to Veselovsky & Ivanov (2003), computational methods are essential at every stage of drug discovery and development, providing powerful and insightful tools. The advent of computer-aided drug design (CADD) has enabled researchers to explore the development of therapeutic agents by employing a variety of chemical, molecular, and quantum-based approaches to design potential drug candidates (Tiwari & Singh, 2022).This method used as computational tools and resources for handling, analyzing, storing, and modeling chemical compounds. It encompasses various aspects of drug discovery, including software for compound design, systematic evaluation of potential lead candidates, and the creation of digital databases to study chemical interactions (Ou-Yang et al., 2012). Computational methods are generally classified into structure-based (SBDD), ligand-based (LBDD), and sequence-based strategies. Structure-based techniques, including molecular docking and de novo drug design, rely on the three-dimensional configuration of target proteins to support the drug discovery process(Ou-Yang et al., 2012).

In Structure-Based Drug Design (SBDD), therapeutics are developed using information about the target's structure. Two widely utilized techniques in SBDD include molecular docking and de novo design of ligands (such as antagonists, agonists, and inhibitors) for the target(Leelananda & Lindert, 2016) in the late nineties structure-Based Drug Design played a crucial role in developing HIV protease inhibitors, greatly extending the life expectancy of individuals with HIV. Among the first HIV-1 protease inhibitors introduced to the market was Saquinavir, followed by Amprenavir, both of which were developed through SBDD (Leelananda & Lindert, 2016). Molecular docking is a widely adopted method in structure-based drug design (SBDD) and an essential part of molecular modeling. It is used to analyze the interactions between small molecules, referred to as ligands, and proteins(Bhagat et al., 2021).The success of this approach

depends on three key elements: docking accuracy (the ability to predict the correct binding orientation of a molecule), scoring accuracy (the ability to rank molecules based on their binding strength), and computational efficiency (the optimization of speed and resource utilization). These elements are largely determined by the approach used to explore the possible conformations of molecules during the docking process (Ou-Yang et al., 2012).

Docking algorithms are designed to predict the conformation and orientation of a ligand within the active site of a target receptor. Over time, these algorithms have advanced significantly, now accounting for flexibility in both the ligand and the protein's side chains, enhancing their accuracy and reliability(Ghosh et al., 2006). Since then, numerous docking algorithms have been developed. Among the most widely used molecular docking programs are Glide, Fred, AutoDock3, AutoDock Vina, GOLD, and FlexX. AutoDock3, in particular, employs an empirical scoring function composed of five distinct terms (Leelananda & Lindert, 2016). Virtual screening is transforming drug discovery by leveraging high-performance computing to efficiently analyze chemical libraries and prioritize compounds for further testing. It relies on two key strategies: ligand-based and receptor-based approaches. The ligand-based method identifies molecules similar to known ligands but is limited by its dependence on existing data, restricting hit diversity. Conversely, the receptor-based approach explores interactions between ligands and target proteins, using tools like docking and pharmacophore analysis to find compounds that bind effectively to active sites. This method is more flexible, enabling the discovery of new ligands that can replicate or create unique interactions with the target. The focus is on receptor-based virtual screening (RBVS) and its latest applications(Ghosh et al., 2006). In ligand-based design, a set of active molecules is analyzed to deduce the structural features responsible for their activity, which guides the design and optimization of new compound.It can be categorized into two main approaches: quantitative structure-affinity relationships (QSARs) and pharmacophore-based design (Hung & Chen, n.d.).This approach can be used in situations where the target or the exact mechanism of action of the molecules is unknown, as well as cases where the 3D structure of the target is known but the receptor's active

site remains unidentified. These methods are generally faster and easier to implement compared to structure-based drug design techniques (Prathipati et al., 2007).

Quantitative Structure-Activity Relationship (QSAR) is an essential computational technique in Ligand-Based Drug Design (LBDD) that utilizes mathematical modeling to forecast the biological effects of chemical compounds based on their molecular properties.Three-dimensional QSAR (3D-QSAR) is a significant component of QSAR, wherein the structural characteristics of bioactive ligands are organized to create a specific spatial model for target active sites. In 3D-QSAR, key chemical attributes such as hydrogen bonding, electrostatic interactions, and hydrophobic regions are taken into account.there two widely computational methods CoMFA (Comparative Molecular Field Analysis) and CoMSIA (Comparative Molecular Similarity Indices Analysis)(Hung & Chen, n.d.).

Chapter 2: Materials and method

For the preprocessing of the protein structure used in virtual screening, the selected target—nsP2 protease of the Chikungunya virus—was retrieved from the Protein Data Bank (PDB) with the accession ID 3TRK. This structure, resolved via X-ray crystallography at a resolution of 2.4 Å, served as the target for the molecular docking study. A curated collection of 202 FDA-approved compounds was screened against this viral protease.

To prepare the macromolecular structure, the three-dimensional coordinates of the nsP2 protease (PDB ID: 3TRK) were downloaded from the RCSB PDB. The structure was processed using PyMOL by removing all co-crystallized ligands and water molecules to refine the protein model. The resulting cleaned structure was then converted into PDBQT format using PyRx, enabling compatibility with molecular docking tools. For ligand preparation, the 3D structures of the selected 202 antiviral and antibiotic compounds were obtained from the PubChem database in SDF format. These were initially converted into PDB format using PyMOL, and then

subsequently transformed into PDBQT format to facilitate the docking simulations. The crystal structure of the nsP2 protease (PDB ID: 3TRK) displays an unoccupied binding cleft, indicating the absence of any co-crystallized ligand at the enzymatic active site. Based on the most druggable site, which exhibited a score of 1.016, a docking grid was defined and centered at the Cartesian coordinates **(6.1240, 30.4048, 22.8052)** to facilitate ligand binding analysis. All prepared compounds were subjected to molecular docking against the active site of the nsP2 protease using PyRx version 0.8, employing AutoDock Vina parameters. Through this computational approach, 202 ligands were virtually screened, and the compounds demonstrating the highest binding affinities were selected for subsequent in-depth analysis.

Chapter 3: Results and discussion

Identifying the active catalytic region of the nsP2 protease enzyme in the Chikungunya virus (CHIKV). Among the analyzed sites, the one that scored the highest Dscore (druggability score) was selected as it is Druggable and has reasonable chances of binding small molecules drugs (ligands) with selectivity and efficacy. According to Bassetto et al. demonstrated that Cys1013 and His1083 (based on CHIKV's nsP2 numbering) are critical catalytic residues within the protease's active site, identified through structural homology modeling and sequence alignment. These residues are structurally and functionally equivalent to Cys477 and His546 in VEEV's nsP2 protease, highlighting their evolutionary conservation and essential role across alphaviruses (Nguyen et al., 2015). The active site we choose contain conserved residues and surface groove characteristic of cysteine proteases suggest this particular active site is both functional and highly druggable.

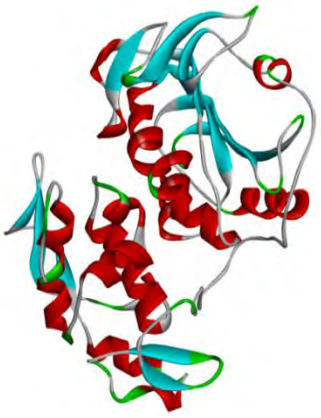


Figure 1: Representation of 3d diagram of selected active site of nsp2 protease (PDB ID :3TRK) of Chikungunya virus retrieved from RCSB

For performing molecular docking via PyRx and ligand interaction analysis, virtual screening was performed on an FDA-approved library containing 202 antiviral and antibiotic compounds. Five top-ranked compounds, based on their docking scores, were selected for further analysis. All corresponding ligand conformations were evaluated for potential interactions with the target protein under the defined grid conditions. In the below Table 1 presents the 202 antiviral and antibiotic compounds that were subjected to docking analysis using PyRx 0.8 version.

Table 1: Compiled list of 202 US FDA approved antiviral and antibiotic drugs. For each drug, the PubChem compound identifier, DrugBank accession number and therapeutic class information is provided.

Serial Number	Drug Name	PubChem Compound Identifier	DrugBank Accession Number	Therapeutic Class	Reference (kcal/mol)
1	Abacavir	441300	DB01048	Antiviral	-6.7
2	Acetosulfone	31400		Antibiotic	-6.7
3	Acyclovir	2022	DB00787	Antiviral	-5.9
4	Adefovir dipivoxil	60871	DB00718	Antiviral	-6.3
5	Amantadine	2130	DB00915	Antiviral	-4.7
6	Amikacin	37768	DB00479	Antibiotic	-6.3
7	Aminosalicylic acid	4649	DB00233	Antibiotic	-5.7

8	Amoxicillin	33613	DB01060	Antibiotic	-6.9
9	Ampicillin	6249	DB00415	Antibiotic	-6.1
10	Anisomycin	253602	DB07374	Antibiotic	-6.0
11	Lauryldimethyl-3,4-dichlorobenzylammonium	7606		Antibiotic	-5.3
12	Atazanavir	148192	DB01072	Antiviral	-7.2
13	Azithromycin	447043	DB00207	Antibiotic	-6.8
14	Aztreonam	5742832	DB00355	Antibiotic	-7
15	Baloxavir marboxil	124081896	DB13997	Antiviral	-8
16	Bedaquiline	5388906	DB08903	Antibiotic	-6.9
17	Benzoylpas	10718		Antibiotic	-7.2
18	Berberine	2353	DB04115	Antibiotic	-7.6
19	Besifloxacin	10178705	DB06771	Antibiotic	-6.9
20	Bictegravir	90311989	DB11799	Antiviral	-9.5
21	Biphenamine	21720	DB13815	Antibiotic	-6.6
22	Brincidofovir	483477	DB12151	Antiviral	-5.4
23	Carbomycin	6433412	DB11383	Antibiotic	-7.4
24	Cefaclor	51039	DB00833	Antibiotic	-6.9
25	Cefamandole nafate	23665731	DB14725	Antibiotic	-6.9
26	Cefazolin	33255	DB01327	Antibiotic	-6.7
27	Cefdinir	6915944	DB00535	Antibiotic	-6.4
28	Cefepime	5479537	DB01413	Antibiotic	-6.2
29	Cefiderocol	77843966	DB14879	Antibiotic	-7.5

30	Cefixime	5362065	DB00671	Antibiotic	-6.6
31	Cefoperazone	44187	DB01329	Antibiotic	-8
32	Cefotaxime	5742673	DB00493	Antibiotic	-6.8
33	Cefotetan	53025	DB01330	Antibiotic	-6.2
34	Cefoxitin	441199	DB01331	Antibiotic	-5.7
35	Cefpodoxime	6335986	DB01416	Antibiotic	-6.7
36	Cefprozil	5281006	DB01150	Antibiotic	-6.7
37	Ceftaroline fosamil	9852981	DB06590	Antibiotic	-7.9
38	Ceftazidime	5481173	DB00438	Antibiotic	-7
39	Ceftolozane	49863420	DB09050	Antibiotic	-7.9
40	Ceftriaxone	5479530	DB01212	Antibiotic	-6.7
41	Cefuroxime	5479529	DB01112	Antibiotic	-6.1
42	Cefacetrile	91562	DB01414	Antibiotic	-5.3
43	Chloramine-T	3641960	DB14708	Antibiotic	-6.2
44	Chloramphenicol palmitate	443382	DB14658	Antibiotic	-5.6
45	Chloramphenicol succinate	656580	DB07565	Antibiotic	-6.2
46	Chloroxylenol	2723	DB11121	Antibiotic	-4.8
47	Chlorquinaldol	6301	DB13306	Antibiotic	-6
48	Cidofovir	60613	DB00369	Antiviral	-5.9
49	Cilastatin	6435415	DB01597	Antibiotic	-5.8
50	Cimicifugin	45048779	DB13975	Antiviral	-7.3
51	Clarithromycin	84029	DB01211	Antibiotic	-7.1

52	Clavulanic acid	5280980	DB00766	Antibiotic	-5.5
53	Clindamycin	446598	DB01190	Antibiotic	-5.6
54	Cobicistat	25151504	DB09065	Antiviral	-7.1
55	m-Cresol	342	DB01776	Antibiotic	-4.7
56	Cycloserine	6234	DB00260	Antibiotic	-3.9
57	Dalfopristin	6323289	DB01764	Antibiotic	-7.2
58	Dapsone	2955	DB00250	Antibiotic	-6.2
59	Darunavir	213039	DB01264	Antiviral	-7
60	Delafloxacin	487101	DB11943	Antibiotic	-7.4
61	Demeclocycline	54680690	DB00618	Antibiotic	-7
62	Dequalinium	2993	DB04209	Antibiotic	-6.9
63	Dichlorophen	3037	DB11396	Antibiotic	-6.1
64	Dicloxacillin	18381	DB00485	Antibiotic	-6.7
65	Docosanol	12620	DB00632	Antiviral	-5.3
66	Dolutegravir	54726191	DB08930	Antiviral	-8
67	Domiphen	3149	DB11594	Antibiotic	-4.9
68	Doravirine	58460047	DB12301	Antiviral	-8.1
69	Doripenem	73303	DB06211	Antibiotic	-6.1
70	Doxorubicin	31703	DB00997	Antibiotic	-8
71	Doxycycline	54671203	DB00254	Antibiotic	-6.5
72	Efavirenz	64139	DB00625	Antiviral	-6.1
73	Elbasvir	71661251	DB11574	Antiviral	-9.8
74	Elvitegravir	5277135	DB09101	Antiviral	-7.7

75	Entecavir	135398508	DB00442	Antiviral	-6.2
76	Eravacycline	54726192	DB12329	Antibiotic	-8.5
77	Ertapenem	150610	DB00303	Antibiotic	-7.3
78	Erythromycin	2560	DB00199	Antibiotic	-7.5
79	Ethaverine	3280		Antiviral	-6.6
80	Ethionamide	2761171	DB00609	Antibiotic	-5.4
81	Etravirine	193962	DB06414	Antiviral	-7
82	Famciclovir	3324	DB00426	Antiviral	-6.3
83	Fidaxomicin	70678896	DB08874	Antibiotic	-7
84	Finafloxacin	11567473	DB09047	Antibiotic	-7
85	Floxuridine	5790	DB00322	Antiviral	-5.8
86	Formic acid	284	DB01942	Antibiotic	-3.1
87	Fosamprenavir	131536	DB01319	Antiviral	-6.7
88	Foscarnet	3415	DB00529	Antiviral	-4.3
89	Fosfomycin	446987	DB00828	Antibiotic	-4.2
90	Fostemsavir	11319217	DB11796	Antiviral	-7.6
91	Ganciclovir	135398740	DB01004	Antiviral	-5.4
92	Gatifloxacin	5379	DB01044	Antibiotic	-6.4
93	Gemifloxacin	9571107	DB01155	Antibiotic	-7
94	Gentamicin	3467	DB00798	Antibiotic	-6.9
95	Glecaprevir	66828839	DB13879	Antiviral	-8.9
96	Glucosulfone	487090		Antibiotic	-7.8
97	Grazoprevir	44603531	DB11575	Antiviral	-9.3

98	Haloprogin	3561	DB00793	Antibiotic	-4.7
99	Imipenem	104838	DB01598	Antibiotic	-5.8
100	Imiquimod	57469	DB00724	Antiviral	-6.5
101	Indinavir	5362440	DB00224	Antiviral	-8.5
102	Iodoform	6374	DB13813	Antibiotic	-2.5
103	Isocyclamine	7743		Antibiotic	-4.6
104	Isoniazid	3767	DB00951	Antibiotic	-5.3
105	Kanamycin	6032	DB01172	Antibiotic	-6.2
106	Lamivudine	60825	DB00709	Antiviral	-5.6
107	Ledipasvir	67505836	DB09027	Antiviral	-9.5
108	Lefamulin	25185057	DB12825	Antibiotic	-6.6
109	Letermovir	45138674	DB12070	Antiviral	-7.1
110	Levofloxacin	149096	DB01137	Antibiotic	-7.1
111	Lincomycin	3000540	DB01627	Antibiotic	-5.8
112	Linezolid	441401	DB00601	Antibiotic	-6.6
113	Lopinavir	92727	DB01601	Antiviral	-7.6
114	Mafenide	3998	DB06795	Antibiotic	-5.5
115	Maraviroc	3002977	DB04835	Antiviral	-8.3
116	Maribavir	471161	DB06234	Antiviral	-6.5
117	Meropenem	441130	DB00760	Antibiotic	-6.5
118	Methacycline	54675785	DB00931	Antibiotic	-7.2
119	Methenamine	4101	DB06799	Antibiotic	-3.3
120	Mezlocillin	656511	DB00948	Antibiotic	-8.3

121	Minocycline	54675783	DB01017	Antibiotic	-7.5
122	Moxifloxacin	152946	DB12329	Antibiotic	-6.9
123	Mupirocin	446596	DB00410	Antibiotic	-6.6
124	Lapyrium	22660	DB09376	Antibiotic	-6.7
125	Nafcillin	8982	DB00607	Antibiotic	-7.1
126	Nelfinavir	64143	DB00220	Antiviral	-7.4
127	Nevirapine	4463	DB00238	Antiviral	-6.5
128	Nifuroxime	6478035		Antibiotic	-5.6
129	Nifurtimox	6842999	DB11820	Antibiotic	-6
130	Nitrofurantoin	6604200	DB00698	Antibiotic	-6.1
131	p-Nitrosulfathiazole	10127		Antibiotic	-6
132	Norfloxacin	4539	DB01059	Antibiotic	-7.4
133	Novobiocin	54675769	DB01051	Antibiotic	-8.4
134	Ofloxacin	4583	DB01165	Antibiotic	-7.7
135	Omadacycline	54697325	DB12455	Antibiotic	-6.9
136	Oseltamivir	65028	DB00198	Antiviral	-5.4
137	Oxacillin	6196	DB00713	Antibiotic	-6.7
138	Oxolinic acid	4628	DB13627	Antibiotic	-7
139	Ozenoxacin	9863827	DB12924	Antibiotic	-7.2
140	Paritaprevir	45110509	DB09297	Antiviral	-8.6
141	Paromomycin	165580	DB01421	Antibiotic	-6.1
142	Penciclovir	4725	DB00299	Antiviral	-6.1
143	Penicillin	2349	DB01053	Antibiotic	-6.3

144	Penicillin V	6869	DB00417	Antibiotic	-6.8
145	Pentylphenol	61084		Antibiotic	-5.7
146	Peramivir	154234	DB06614	Antiviral	-5.6
147	Pheneticillin	272833	DB13337	Antibiotic	-6.8
148	Phthalylsulfathiazole	4806	DB13248	Antibiotic	-7.3
149	Piperacillin	43672	DB00319	Antibiotic	-7.8
150	Plazomicin	42613186	DB12615	Antibiotic	-6.6
151	Podofilox	10607	DB01179	Antiviral	-6.7
152	Pretomanid	456199	DB05154	Antibiotic	-7
153	Pyrazinamide	1046	DB00339	Antibiotic	-4.9
154	Quinupristin	5388937	DB01369	Antibiotic	-9.6
155	Raltegravir	54671008	DB06817	Antiviral	-8.5
156	Remdesivir	121304016	DB14761	Antiviral	-7.4
157	Retapamulin	6918462	DB01256	Antibiotic	-6.6
158	Rifabutin	6323490	DB00615	Antibiotic	-8.1
159	Rifampin	135398735	DB01045	Antibiotic	-8.3
160	Rifamycin	6324616	DB11753	Antibiotic	-7.4
161	Rifapentine	135403821	DB01201	Antibiotic	-9
162	Rifaximin	6436173	DB01220	Antibiotic	-8.1
163	Rilpivirine	6451164	DB08864	Antiviral	-7.5
164	Rimantadine	5071	DB00478	Antiviral	-5.1
165	Ritonavir	392622	DB00503	Antiviral	-7.5
166	Rolitetracycline	54682938	DB01301	Antibiotic	-7.3

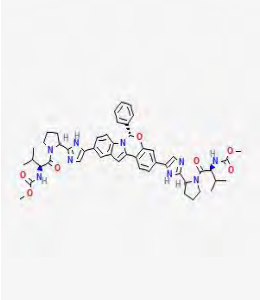
167	Rotenone	6758	DB11457	Antibiotic	-7.6
168	Saquinavir	441243	DB01232	Antiviral	-9.1
169	Sarecycline	54681908	DB12035	Antibiotic	-7
170	Simeprevir	24873435	DB06290	Antiviral	-9.1
171	Sisomicin	36119	DB12604	Antibiotic	-6.4
172	Sofosbuvir	45375808	DB08934	Antiviral	-7.2
173	Streptomycin	19649	DB01082	Antibiotic	-7
174	Succinyl sulfathiazole	5315	DB13580	Antibiotic	-6.9
175	Sulfacetamide	5320	DB00634	Antibiotic	-6.2
176	Sulfadiazine	5215	DB00359	Antibiotic	-6.2
177	Sulfaethidole	7181		Antibiotic	-6.1
178	Sulfaguanidine	5324	DB13726	Antibiotic	-6
179	Sulfalene	9047	DB00664	Antibiotic	-6.3
180	Sulfamerazine	5325	DB01581	Antibiotic	-6.5
181	Sulfanilamide	5333	DB00259	Antibiotic	-5.3
182	Sulfapyrazine	8309		Antibiotic	-6.2
183	Sulfisomidine	5343	DB13283	Antibiotic	-6.2
184	Tazobactam	123630	DB01606	Antibiotic	-5.3
185	Tecovirimat	16124688	DB12020	Antiviral	-8.4
186	Tedizolid phosphate	11476460	DB09042	Antibiotic	-7.7
187	Tenofovir alafenamide	9574768	DB09299	Antiviral	-6.3
188	Tenofovir disoproxil	5481350	DB00300	Antiviral	-6.3
189	Tetracycline	54675776	DB00759	Antibiotic	-6.6

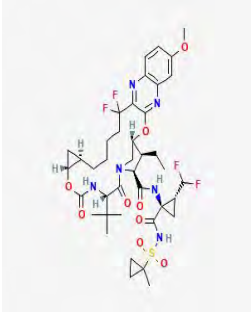
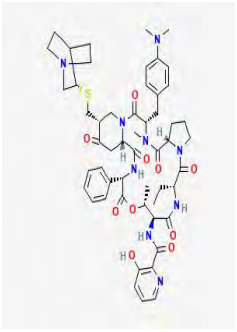
190	Thiazolsulfone	10124		Antibiotic	-5.9
191	Thymol	6989	DB02513	Antibiotic	-5.9
192	Tigecycline	54686904	DB00560	Antibiotic	-7.6
193	Tipranavir	54682461	DB00932	Antiviral	-6.3
194	Tobramycin	36294	DB00684	Antibiotic	-5.6
195	Trifluridine	6256	DB00432	Antiviral	-6.3
196	Trimethoprim	5578	DB00440	Antibiotic	-6
197	Trovafloxacin	62959	DB00685	Antibiotic	-8
198	Valganciclovir	135413535	DB01610	Antiviral	-6.3
199	Velpatasvir	67683363	DB11613	Antiviral	-9
200	Voxilaprevir	89921642	DB12026	Antiviral	-9.7
201	Zanamivir	60855	DB00558	Antiviral	-5.8
202	Zidovudine	35370	DB00495	Antiviral	-6.5

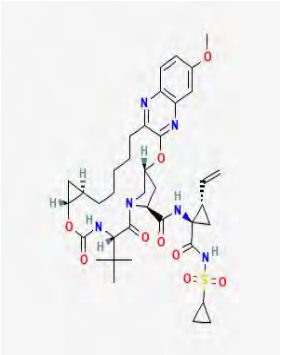
In table 2 it was reported that among 202 antiviral and antimicrobial compound we choose five compounds with highest docking score. Out of the 202 compounds analyzed, Elbasvir demonstrated the most significant predicted binding affinity, achieving a docking score of −9.8 kcal/mol. This FDA-approved antiviral drug is commonly prescribed for the treatment of chronic hepatitis C virus (HCV) infections, where it specifically targets the NS5A protein(Kiang, 2018). In the present study, Elbasvir exhibited the highest docking score against the nsP2 protease of the Chikungunya virus. Closely following, Voxilaprevir demonstrated a comparable binding affinity with a docking score of −9.7 kcal/mol. Voxilaprevir is a direct-acting antiviral (DAA) agent, clinically utilized in combination with Sofosbuvir and Velpatasvir for the treatment of chronic hepatitis C virus (HCV) infection. This orally administered medication undergoes hepatic metabolism primarily via cytochrome P450 enzymes and exhibits a high plasma protein

binding rate of approximately 99% following a single 100 mg dose (Heo & Deeks, 2018). Quinupristin, a streptogramin B antibiotic, has demonstrated a high docking score of -9.6 kcal/mol. Typically used in combination with dalfopristin, it is employed to treat severe infections caused by Gram-positive bacteria. When used together, quinupristin and dalfopristin exhibit strong bactericidal properties, which may vary based on concentration, against Gram-positive pathogens such as Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus epidermidis, and Streptococcus pneumoniae in laboratory settings. Although quinupristin has not been proven effective against Chikungunya virus (CHIKV) in clinical or notable preclinical trials, its potent bactericidal activity, as observed in vitro and animal infection studies, highlights its potential in treating serious bacterial infections(Finch, 1996). Bictegravir shows promising docking score with -9.5 kcal/mol with nsp2 protease this drug normally used to treat HIV-1 infection. This antiviral drug is an integrase strand transfer inhibitor (INSTI) that works by blocking the HIV integrase enzyme, which is crucial for the integration of viral DNA into the host's genome, thus preventing the replication of the virus (Tsiang et al., 2016).

Table 2. Illustration of binding of Food and Drug Administration (FDA) approved antiviral and antibiotic compounds with CHIKV nsp2 protease

SL. No.	Compound	Structure	Docking score(kcal/mol)	Hydrogen Bond and Other non-covalent interactions
1	Elbasvir		-9.8	Hydrogen bond LYS 1091, ALA 1201, ARG 1271 Electrostatic bond: ASP1246, GLU1050

2	Voxilaprevir		-9.7	Hydrogen bond TRP 1084, GLN1241,
3	Quinupristin		-9.6	Hydrogen bond ASN1082, GLN 1241, MSE 1242 Electrostatic bond: ASP 1246
4	Bictegravir		-9.5	Hydrogen bond: SER1048, TYR 1079 TRP 1084

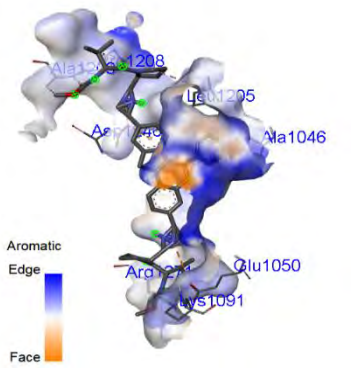
5	Grazoprevir		-9.3	Hydrogen bond: SER1048, GLN 1241 Electrostatic bond: ASP 1246

In the following step, the last compound Grazoprevir with the docking score -9.3 kcal/mol is an antiviral agent employed in managing chronic hepatitis C virus (HCV) infections. It functions as an NS3/4A protease inhibitor and is commonly administered alongside elbasvir as part of combination therapy. Elbasvir and grazoprevir exhibit extensive plasma protein binding, with more than 99.9% and 98.8% bound, respectively(Carrion & Martin, 2016). Grazoprevir and other four compound shows strong potential as an inhibitor of the Chikungunya virus (CHIKV) nsP2 protease based on in silico docking studies, but further experimental validation is needed to confirm its antiviral activity.

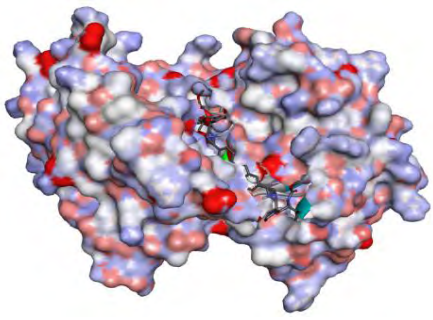
Elbasvir, exhibiting the highest docking score of -9.8 kcal/mol against the nsP2 protease, is an FDA-approved compound that demonstrates stable and favorable binding interactions. It forms several hydrogen bonds with key active site residues, including Lys1091, Ala1201, and Arg1271, with bond distances ranging from 1 to 2 Å. Additionally, it engages in electrostatic interactions with residues Asp1246 and Glu1050. The interaction profile of Elbasvir includes conventional hydrogen bonds, π-anion, π-alkyl interactions, and attractive charge interactions. In total, three hydrogen bonds and four hydrophobic interactions, with an approximate distance of 4 Å, contribute to the strong and stable binding between the ligand and the protease.

Multiple visual representations of Elbasvir's interaction with the nsP2 protease are provided, including the docked pose of Elbasvir within the protease's active site, a surface view depicting

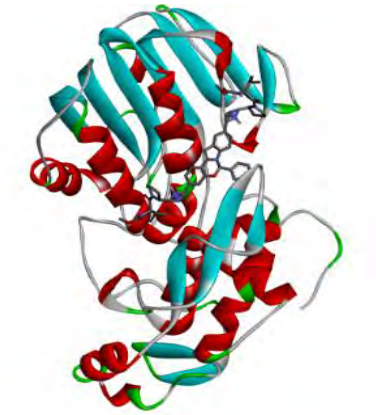
its binding orientation and molecular accessibility, a three-dimensional interaction diagram emphasizing key molecular contacts, and a two-dimensional schematic illustrating the specific ligand–protein interactions involved in its binding to the nsP2 protein.



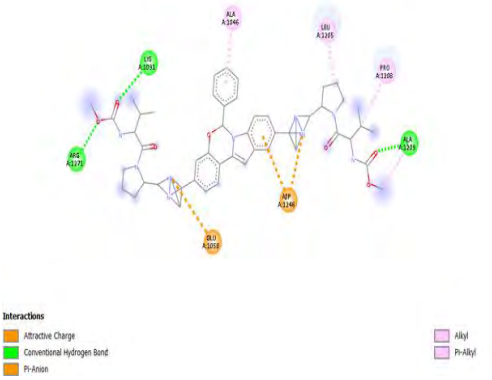
a) Docked pose of elbasvir



b) surface view of elbasvir



c) 3D ligand interaction of elbasvir

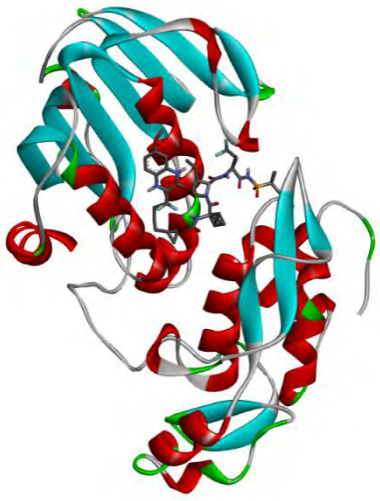


d) 2D ligand interaction of elbasvir

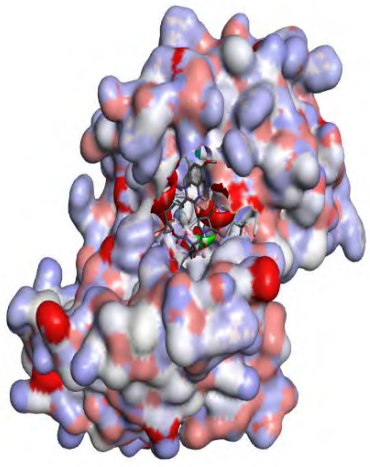
Figure 2: Different poses of elbasvir along with 3D and 2D interaction with nsp2

Voxilaprevir, exhibiting the second-highest docking score of -9.7 kcal/mol, demonstrates a strong binding affinity toward the nsP2 protease of the target protein. This antiviral agent engages in multiple hydrogen bonds with key active site residues Trp1084 and Gln1241, with interaction distances ranging from 1 to 3 Å. Additionally, it forms halogen interactions involving its fluorine atoms with Leu1205 and Asp1246, each at an approximate distance of 3 Å. Furthermore, hydrophobic interactions are observed with Trp1084 and MSE1242.

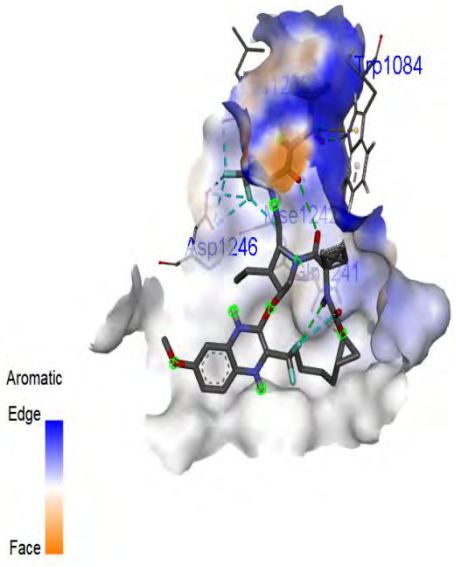
In total, Voxilaprevir establishes conventional hydrogen bonds, carbon–hydrogen bonds, seven halogen bonds, and two hydrophobic contacts, contributing to its stable binding at the active site. Below are multiple representations of Voxilaprevir illustrating its interaction with the nsP2 protease, including: a) the docked conformation of Voxilaprevir within the active site of nsP2 protease, b) the surface view depicting Voxilaprevir's binding orientation and accessibility within the protease, c) a three-dimensional interaction diagram highlighting the specific molecular interactions between Voxilaprevir and the nsP2 protease, and d) a two-dimensional schematic detailing the ligand–protein interactions involved in Voxilaprevir’s binding to the nsP2 protein.



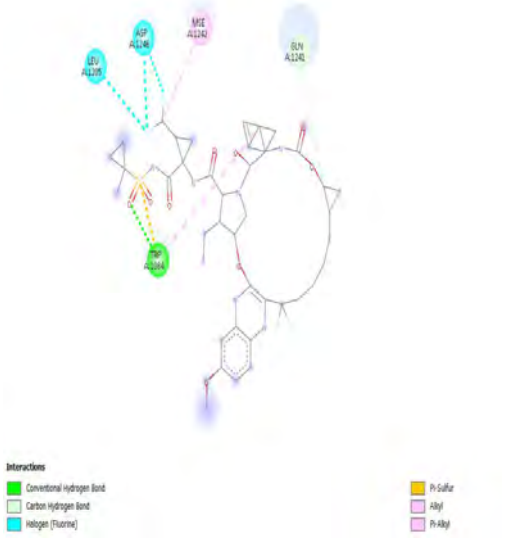
a) docked pose of voxilaprevir



b) surface view of voxilaprevir



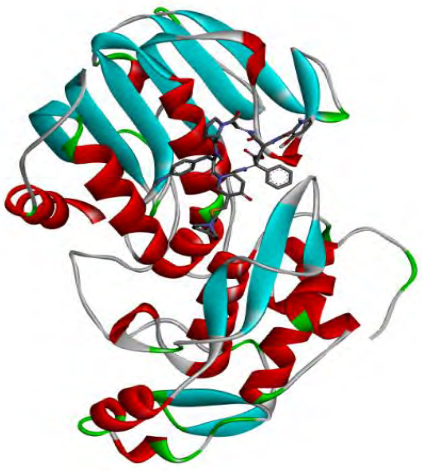
c) 3D ligand interaction of Voxilaprevir



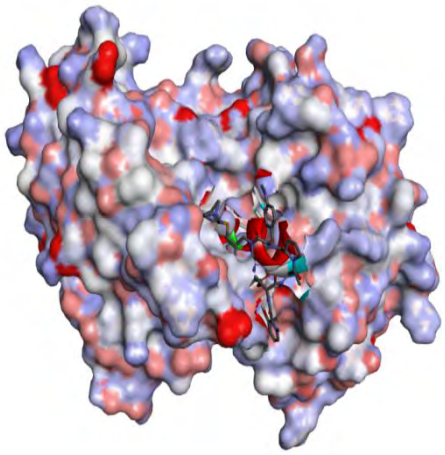
d) 2D ligand interaction of Voxilaprevir

Figure 3: Different poses of Voxilaprevir along with 3D and 2D interaction with nsp2

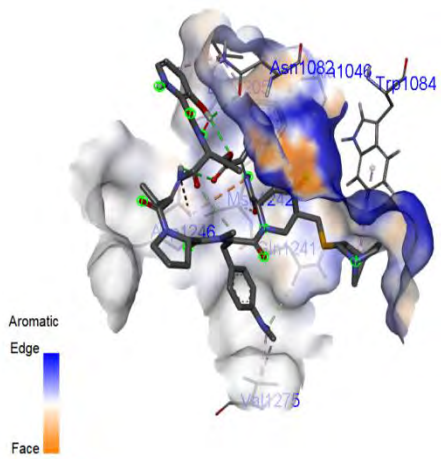
Quinupristin, an antibiotic from the streptogramin B class, exhibits the third-highest docking score of -9.6 kcal/mol against the nsP2 protease. This compound forms multiple hydrogen bonds with key active site residues Asn1082, Gln1241, and MSE1242, with interaction distances around 3 Å. Additionally, it establishes electrostatic interactions with Asp1246, similar to the interaction profile observed with Elbasvir. Quinupristin also engages in hydrophobic interactions with residues Leu1205, Val1275, Ala1046, and MSE1242, at distances ranging from 4 to 5 Å. Overall, the compound forms three hydrogen bonds and seven hydrophobic interactions, indicating a stable and significant binding affinity at the active site of the target protease. below are multiple representations of Quinupristin illustrating its interaction with the nsP2 protease, including: a) the docked conformation of Quinupristin within the active site of nsP2 protease, b) the surface view depicting Quinupristin's binding orientation and accessibility within the protease, c) a three-dimensional interaction diagram highlighting the specific molecular interactions between Quinupristin and the nsP2 protease, and d) a two-dimensional schematic detailing the ligand–protein interactions involved in Quinupristin's binding to the nsP2 protein

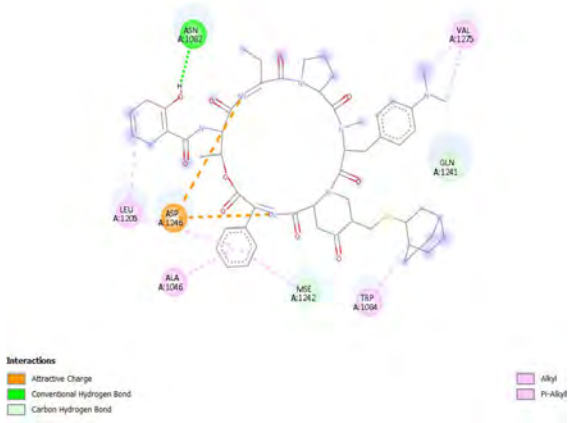


a)docked pose of Quinupristin



b)surface view of Quinupristin





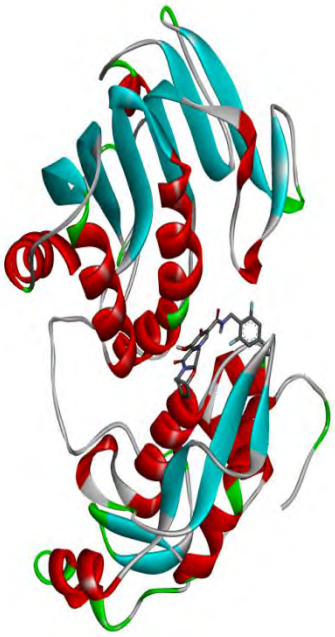
c) 3D ligand interaction of Quinupristin

d) 2D ligand interaction of Quinupristin

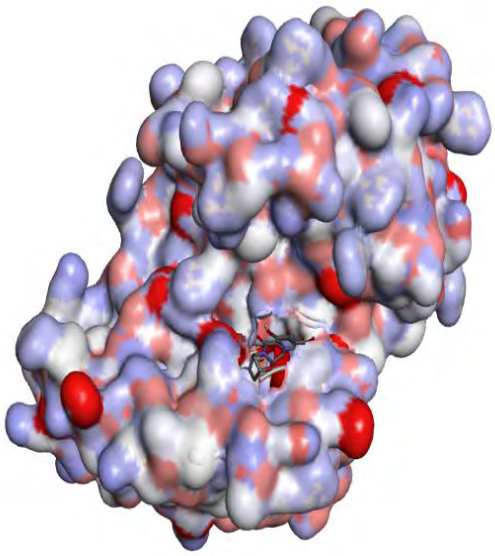
Figure 4: different poses of Quinupristin along with 3D and 2D interaction with nsp2

Bictegravir, an antiviral agent primarily prescribed for the treatment of HIV-1 infection, exhibits the fourth-highest docking score of -9.5 kcal/mol against the nsP2 protease. The compound forms multiple hydrogen bonds with key active site residues, including Ser1048, Tyr1079, and Trp1084, with bond distances of approximately 2 Å. Additionally, it engages in halogen interactions with Tyr1047 and Asn1082, at distances close to 3 Å. Bictegravir also establishes hydrophobic interactions with several residues—Ala1046, Tyr1079, Trp1084, and Lys1091—ranging from 3 to 5 Å. In total, the compound forms five conventional hydrogen bonds, four halogen bonds, and five hydrophobic contacts with active site residues, indicating a stable and well-defined interaction between Bictegravir and the nsP2 protease. Below are several visual representations of Bictegravir that illustrate its interaction with the nsP2 protease. a) These include the docked conformation of Bictegravir within the active site of the protease, b) a surface view showing its binding orientation and accessibility,c) a three-dimensional interaction diagram

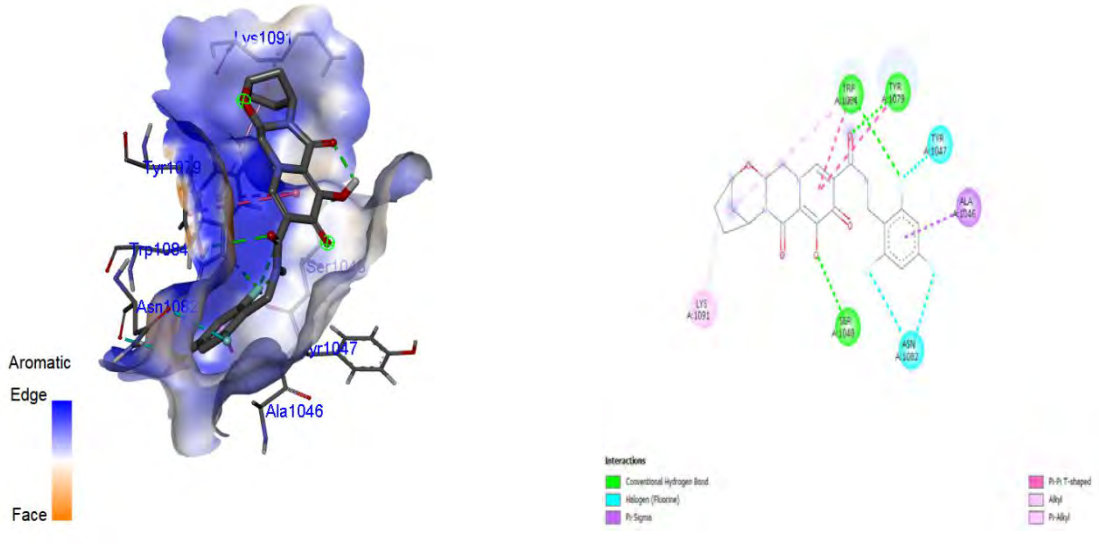
highlighting the specific molecular contacts between the ligand and the protease, and d) a two-dimensional schematic that outlines the key ligand–protein interactions involved in Bictegravir's binding to the nsP2 protein.



a)docked pose of Bictegravir



b)surface view of Bictegravir



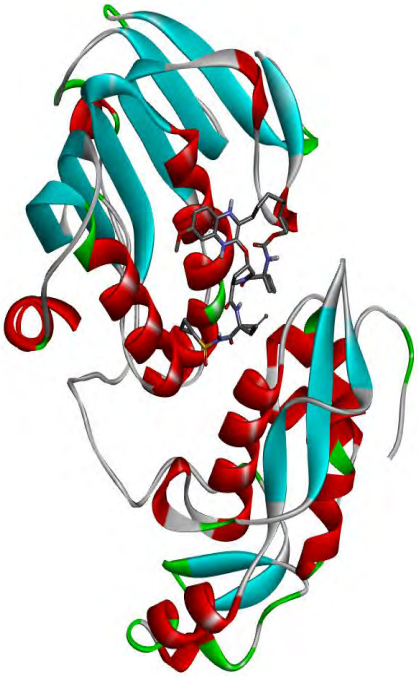
c)3D ligand interaction of Bictegravir

d)2d ligand interaction of Bictegravir

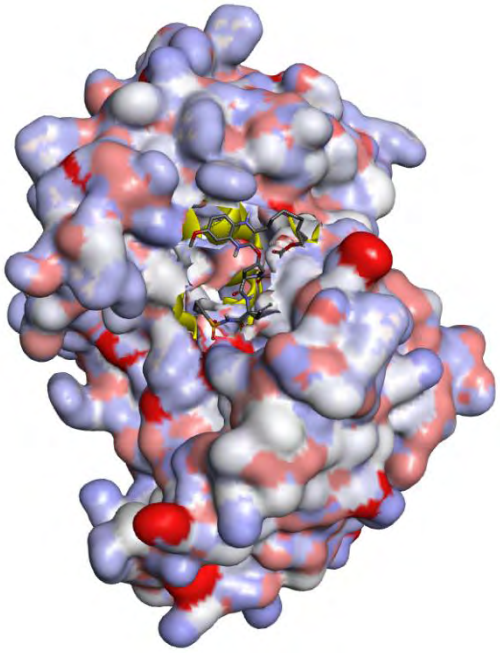
Figure 5: different poses of Bictegravir along with 3D and 2D interaction with nsp2

and Grazoprevir, an antiviral compound indicated for the treatment of chronic Hepatitis C virus (HCV) infection, demonstrates the fifth-highest docking score of -9.3 kcal/mol against the nsP2 protease. This compound establishes several hydrogen bonds with critical active site residues, namely Ser1048 and Gln1241, at a distance of approximately 2 Å. It also forms electrostatic interactions with Asp1246, measured at 3 Å. Furthermore, Grazoprevir engages in hydrophobic interactions with residues such as Leu1248, Val1275, Tyr1079, and Trp1084, with interaction distances ranging from 4 to 5 Å. All things considered, the compound forms five conventional hydrogen bonds, a π-anion interaction, and four hydrophobic interactions, including two alkyl and two π-alkyl bonds, indicating a strong and stable association with the target protease. Included below are several visual representations of Grazoprevir's interaction with the nsP2 protease. a) These include the docked conformation of Grazoprevir within the active site of the protease, b) a surface view of Grazoprevir showing its binding orientation and accessibility, c) a three-dimensional of Grazoprevir interaction diagram highlighting the specific molecular

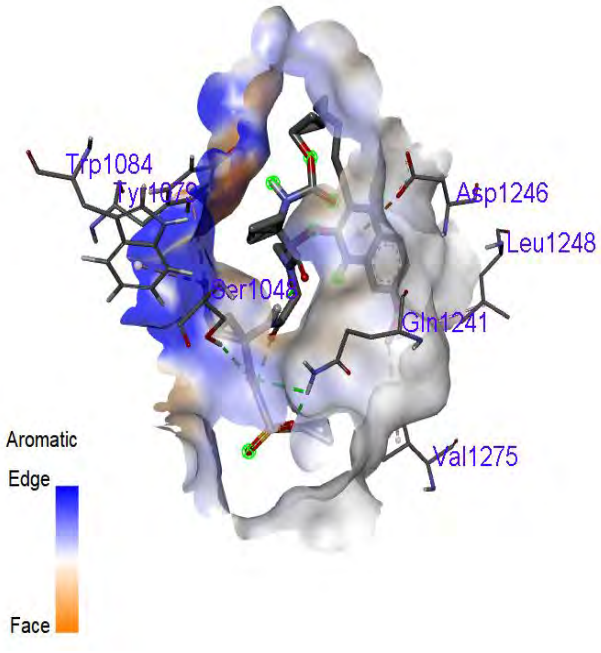
contacts between the ligand and the protease, and d) a two-dimensional of Grazoprevir schematic that outlines the key ligand–protein interactions involved in Grazoprevir's binding to the nsP2 protein.

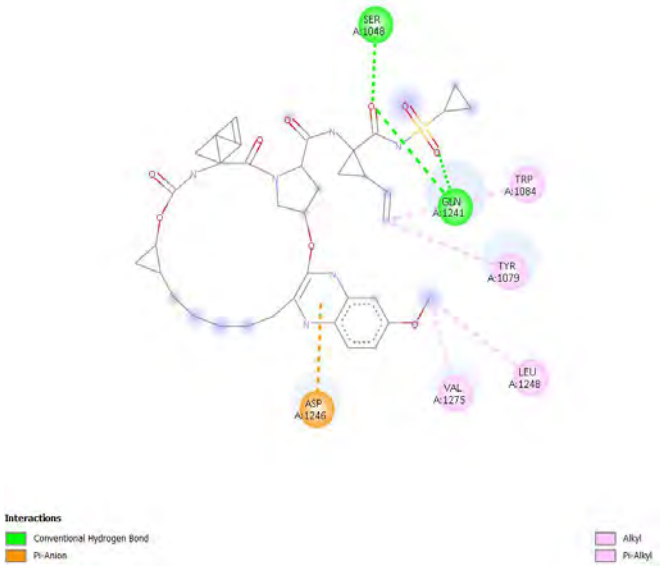


a) docked pose of Grazoprevir



b) surface view of Grazoprevir





c) 3D ligand interaction of Grazoprevir

d) 2D ligand interaction of Grazoprevir

Figure 6: different poses of Grazoprevir along with 3D and 2D interaction with nsp2

Chapter 4: Conclusions

In the present study, computational approaches were employed to investigate the binding affinity of 202 FDA-approved compounds, along with a selection of cysteine protease inhibitors, against the nsP2 protease of the Chikungunya virus. The rationale for choosing FDA-approved drugs lies in their well-established safety profiles, with documented data on toxicity, pharmacokinetics, and side effects, thereby minimizing the risk associated with drug repurposing.

Moreover, these compounds offer the advantage of a shortened regulatory timeline, which could potentially accelerate their clinical deployment. From a developmental standpoint, this approach is also cost-effective, as de novo drug discovery is often resource-intensive and time-consuming. Thus, virtual screening and docking of approved drugs provide a strategic alternative, especially in urgent public health scenarios such as Chikungunya virus outbreaks, where rapid identification of effective therapeutic candidates is critical.

Based on the docking analysis, the top five compounds demonstrating the highest binding scores were identified. These compounds exhibited strong interactions with key active site residues of the nsP2 protease, particularly GLN1241 and TRP1084. Given these promising results, it is recommended that these top candidates undergo in vitro validation to assess their inhibitory potential, supporting their consideration for further experimental and clinical evaluation.

Chapter 5: References

Carrion, A. F., & Martin, P. (2016). Safety and efficacy of elbasvir and grazoprevir for treatment of hepatitis C. *Expert Opinion on Drug Safety*, *15*(6), 883–890. https://doi.org/10.1080/14740338.2016.1179278

Finch, R. G. (1996). Antibacterial Activity of Quinupristin/Dalfopristin: Rationale for Clinical Use. *Drugs*, *51*(Supplement 1), 31–37. https://doi.org/10.2165/00003495-199600511-00007

Heo, Y.-A., & Deeks, E. D. (2018). Sofosbuvir/Velpatasvir/Voxilaprevir: A Review in Chronic Hepatitis C. *Drugs*, *78*(5), 577–587. https://doi.org/10.1007/s40265-018-0895-5

Kiang, T. K. L. (2018). Clinical Pharmacokinetics and Drug–Drug Interactions of Elbasvir/Grazoprevir. *European Journal of Drug Metabolism and Pharmacokinetics*, *43*(5), 509–531. https://doi.org/10.1007/s13318-018-0471-0

Nguyen, P. T. V., Yu, H., & Keller, P. A. (2015). Identification of chikungunya virus nsP2 protease inhibitors using structure-base approaches. *Journal of Molecular Graphics and Modelling*, *57*, 1–8. https://doi.org/10.1016/j.jmgm.2015.01.001

Tsiang, M., Jones, G. S., Goldsmith, J., Mulato, A., Hansen, D., Kan, E., Tsai, L., Bam, R. A., Stepan, G., Stray, K. M., Niedziela-Majka, A., Yant, S. R., Yu, H., Kukolj, G., Cihlar, T., Lazerwith, S. E., White, K. L., & Jin, H. (2016). Antiviral Activity of Bictegravir (GS-9883), a Novel Potent HIV-1 Integrase Strand Transfer Inhibitor with an Improved Resistance Profile. *Antimicrobial Agents and Chemotherapy*, *60*(12), 7086–7097. https://doi.org/10.1128/AAC.01474-16

Zhan, P., Yu, B., & Ouyang, L. (2022). Drug repurposing: An effective strategy to accelerate contemporary drug discovery. *Drug Discovery Today*, *27*(7), 1785–1788. https://doi.org/10.1016/j.drudis.2022.05.026

Morrison, T. E. (2014). Reemergence of Chikungunya Virus. *Journal of Virology*, *88*(20), 11644–11647. https://doi.org/10.1128/JVI.01432-14

Strauss, J. H., & Strauss, E. G. (1994). The alphaviruses: Gene expression, replication, and evolution. *Microbiological Reviews*, *58*(3), 491–562. https://doi.org/10.1128/mr.58.3.491-562.1994

Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., Ugwuja, E. I., & Aja, P. M. (2023). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. *Scientific Reports*, *13*(1), 13398. https://doi.org/10.1038/s41598-023-40160-2

Abdelnabi, R., Neyts, J., & Delang, L. (2016). Antiviral Strategies Against Chikungunya Virus. In J. J. H. Chu & S. K. Ang (Eds.), *Chikungunya Virus* (Vol. 1426, pp. 243–253). Springer New York. https://doi.org/10.1007/978-1-4939-3618-2_22

Bhagat, R. T., Butle, S. R., Khobragade, D. S., Wankhede, S. B., Prasad, C. C., Mahure, D. S., & Armarkar, A. V. (2021). Molecular Docking in Drug Discovery. *Journal of Pharmaceutical Research International*, 46–58. https://doi.org/10.9734/jpri/2021/v33i30B31639

Davuluri, K. S., Ghanghav, R., Ahire, G., Kakade, M., Cherian, S., Alagarasu, K., & Parashar, D. (2024). Repurposed drugs in combinations exert additive anti-chikungunya virus activity: An in-vitro study. *Virology Journal*, *21*(1), 5. https://doi.org/10.1186/s12985-023-02271-0

Sanyaolu, A., Okorie, C., Badaru, O., Wynveen, E., White, S., Wallace, W., Akl, J., Freeze, A., Kamel, A., Madonna, M., Mathur, A., Moran, R., & Perry, C. (n.d.). *Chikungunya Epidemiology: A Global Perspective*.

Mavalankar, D., Shastri, P., & Raman, P. (2007). Chikungunya epidemic in India: A major public-health disaster. *The Lancet Infectious Diseases*, *7*(5), 306–307. https://doi.org/10.1016/S1473-3099(07)70091-9

Ghosh, S., Nie, A., An, J., & Huang, Z. (2006). Structure-based virtual screening of chemical libraries for drug discovery. *Current Opinion in Chemical Biology*, *10*(3), 194–202. https://doi.org/10.1016/j.cbpa.2006.04.002

Ghildiyal, R., & Gabrani, R. (2020). Antiviral therapeutics for chikungunya virus. *Expert Opinion on Therapeutic Patents*, *30*(6), 467–480. https://doi.org/10.1080/13543776.2020.1751817

Galán-Huerta, K. A., Rivas-Estilla, A. M., Fernández-Salas, I., Farfan-Ale, J. A., & Ramos-Jiménez, J. (2015). Chikungunya virus: A general overview. *Medicina Universitaria*, *17*(68), 175–183. https://doi.org/10.1016/j.rmu.2015.06.001

Freppel, W., Silva, L. A., Stapleford, K. A., & Herrero, L. J. (2024). Pathogenicity and virulence of chikungunya virus. *Virulence*, *15*(1), 2396484. https://doi.org/10.1080/21505594.2024.2396484

Finch, R. G. (1996). Antibacterial Activity of Quinupristin/Dalfopristin: Rationale for Clinical Use. *Drugs*, *51*(Supplement 1), 31–37. https://doi.org/10.2165/00003495-199600511-00007

Faver, J. C., Ucisik, M. N., Yang, W., & Merz, K. M. (2013). Computer-Aided Drug Design: Using Numbers to Your Advantage. *ACS Medicinal Chemistry Letters*, *4*(9), 812–814. https://doi.org/10.1021/ml4002634

Farha, M. A., & Brown, E. D. (2019). Drug repurposing for antimicrobial discovery. *Nature Microbiology*, *4*(4), 565–577. https://doi.org/10.1038/s41564-019-0357-1

Fan, Q., Zhang, B., Ma, J., & Zhang, S. (2020). Safety profile of the antiviral drug remdesivir: An update. *Biomedicine & Pharmacotherapy*, *130*, 110532. https://doi.org/10.1016/j.biopha.2020.110532

Fan, J., Fu, A., & Zhang, L. (2019). Progress in molecular docking. *Quantitative Biology*, *7*(2), 83–89. https://doi.org/10.1007/s40484-019-0172-y

Deng, Q., Guo, Z., Hu, H., Li, Q., Zhang, Y., Wang, J., Liao, C., Guo, C., Li, X., Chen, Z., & Lu, J. (2022). Inhibition of Chikungunya virus early replication by intracellular nanoantibodies targeting nsP2 Epitope Rich Region. *Antiviral Research*, *208*, 105446. https://doi.org/10.1016/j.antiviral.2022.105446

De Ruyck, J., Brysbaert, G., Blossey, R., & Lensink, M. (2016). Molecular docking as a popular tool in drug design, an in silico travel. *Advances and Applications in Bioinformatics and Chemistry*, *Volume 9*, 1–11. https://doi.org/10.2147/AABC.S105289

De Lamballerie, X., Ninove, L., & Charrel, R. (2009). Antiviral Treatment of Chikungunya Virus Infection. *Infectious Disorders - Drug Targets*, *9*(2), 101–104. https://doi.org/10.2174/187152609787847712

De Clercq, E., & Li, G. (2016). Approved Antiviral Drugs over the Past 50 Years. *Clinical Microbiology Reviews*, *29*(3), 695–747. https://doi.org/10.1128/CMR.00102-15

Cortegiani, A., Ingoglia, G., Ippolito, M., Giarratano, A., & Einav, S. (2020). A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. *Journal of Critical Care*, *57*, 279–283. https://doi.org/10.1016/j.jcrc.2020.03.005

Cherkashchenko, L., Rausalu, K., Basu, S., Alphey, L., & Merits, A. (2022). Expression of Alphavirus Nonstructural Protein 2 (nsP2) in Mosquito Cells Inhibits Viral RNA Replication in Both a Protease Activity-Dependent and -Independent Manner. *Viruses*, *14*(6), 1327. https://doi.org/10.3390/v14061327

Chang, Y., Hawkins, B. A., Du, J. J., Groundwater, P. W., Hibbs, D. E., & Lai, F. (2022). A Guide to In Silico Drug Design. *Pharmaceutics*, *15*(1), 49. https://doi.org/10.3390/pharmaceutics15010049

Carrion, A. F., & Martin, P. (2016). Safety and efficacy of elbasvir and grazoprevir for treatment of hepatitis C. *Expert Opinion on Drug Safety*, *15*(6), 883–890. https://doi.org/10.1080/14740338.2016.1179278

Caglioti, C., Lalle, E., Castilletti, C., Carletti, F., Capobianchi, M. R., & Bordi, L. (n.d.). *Chikungunya virus infection: An overview*.

Bhagat, R. T., Butle, S. R., Khobragade, D. S., Wankhede, S. B., Prasad, C. C., Mahure, D. S., & Armarkar, A. V. (2021). Molecular Docking in Drug Discovery. *Journal of Pharmaceutical Research International*, 46–58. https://doi.org/10.9734/jpri/2021/v33i30B31639

Battisti, V., Urban, E., & Langer, T. (2021). Antivirals against the Chikungunya Virus. *Viruses*, *13*(7), 1307. https://doi.org/10.3390/v13071307

Ballester, P. J., & Mitchell, J. B. O. (2010). A machine learning approach to predicting protein–ligand binding affinity with applications to molecular docking. *Bioinformatics*, *26*(9), 1169–1175. https://doi.org/10.1093/bioinformatics/btq112

Azad, I. (2023). Molecular Docking in the Study of Ligand-Protein Recognition: An Overview. In E. Salih Istifli (Ed.), *Biomedical Engineering* (Vol. 15). IntechOpen. https://doi.org/10.5772/intechopen.106583

Antonelli, G., & Turriziani, O. (2012). Antiviral therapy: Old and current issues. *International Journal of Antimicrobial Agents*, *40*(2), 95–102. https://doi.org/10.1016/j.ijantimicag.2012.04.005

Ahola, T., & Merits, A. (2016). Functions of Chikungunya Virus Nonstructural Proteins. In C. M. Okeoma (Ed.), *Chikungunya Virus* (pp. 75–98). Springer International Publishing. https://doi.org/10.1007/978-3-319-42958-8_6

Aguiar, C., & Camps, I. (2024). *Molecular Docking in Drug Discovery: Techniques, Applications, and Advancements*. Chemistry. https://doi.org/10.26434/chemrxiv-2024-gmhtx

Wermuth, C. G., Villoutreix, B., Grisoni, S., Olivier, A., & Rocher, J.-P. (2015). Strategies in the Search for New Lead Compounds or Original Working Hypotheses. In *The Practice of Medicinal Chemistry* (pp. 73–99). Elsevier. https://doi.org/10.1016/B978-0-12-417205-0.00004-3

Weaver, S. C. (2014). Arrival of Chikungunya Virus in the New World: Prospects for Spread and Impact on Public Health. *PLoS Neglected Tropical Diseases*, *8*(6), e2921. https://doi.org/10.1371/journal.pntd.0002921

Wang, G., & Zhu, W. (2016). Molecular Docking for Drug Discovery and Development: A Widely Used Approach but Far from Perfect. *Future Medicinal Chemistry*, *8*(14), 1707–1710. https://doi.org/10.4155/fmc-2016-0143

Walters, W. P., Stahl, M. T., & Murcko, M. A. (1998). Virtual screening—An overview. *Drug Discovery Today*, *3*(4), 160–178. https://doi.org/10.1016/S1359-6446(97)01163-X

Tiwari, A., & Singh, S. (2022). Computational approaches in drug designing. In *Bioinformatics* (pp. 207–217). Elsevier. https://doi.org/10.1016/B978-0-323-89775-4.00010-9

Thiberville, S.-D., Moyen, N., Dupuis-Maguiraga, L., Nougairede, A., Gould, E. A., Roques, P., & De Lamballerie, X. (2013). Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Research*, *99*(3), 345–370. https://doi.org/10.1016/j.antiviral.2013.06.009

Stanzione, F., Giangreco, I., & Cole, J. C. (2021). Use of molecular docking computational tools in drug discovery. In *Progress in Medicinal Chemistry* (Vol. 60, pp. 273–343). Elsevier. https://doi.org/10.1016/bs.pmch.2021.01.004

Sreejith, R., Rana, J., Dudha, N., Kumar, K., Gabrani, R., Sharma, S. K., Gupta, A., Vrati, S., Chaudhary, V. K., & Gupta, S. (2012). Mapping interactions of Chikungunya virus nonstructural proteins. *Virus Research*, *169*(1), 231–236. https://doi.org/10.1016/j.virusres.2012.08.006

Silva, L. A., & Dermody, T. S. (2017). Chikungunya virus: Epidemiology, replication, disease mechanisms, and prospective intervention strategies. *Journal of Clinical Investigation*, *127*(3), 737–749. https://doi.org/10.1172/JCI84417

Schwartz, O., & Albert, M. L. (2010). Biology and pathogenesis of chikungunya virus. *Nature Reviews Microbiology*, *8*(7), 491–500. https://doi.org/10.1038/nrmicro2368

Roney, M., & Mohd Aluwi, M. F. F. (2024). The importance of in-silico studies in drug discovery. *Intelligent Pharmacy*, *2*(4), 578–579. https://doi.org/10.1016/j.ipha.2024.01.010

Rabelo, V. W.-H., Paixão, I. C. N. D. P., & Abreu, P. A. (2020). Targeting Chikungunya virus by computational approaches: From viral biology to the development of therapeutic strategies. *Expert Opinion on Therapeutic Targets*, *24*(1), 63–78. https://doi.org/10.1080/14728222.2020.1712362

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C., Norris, A., Sanseau, P., Cavalla, D., & Pirmohamed, M. (2019). Drug repurposing: Progress, challenges and recommendations. *Nature Reviews Drug Discovery*, *18*(1), 41–58. https://doi.org/10.1038/nrd.2018.168

Pantsar, T., & Poso, A. (2018). Binding Affinity via Docking: Fact and Fiction. *Molecules*, *23*(8), 1899. https://doi.org/10.3390/molecules23081899

Ou-Yang, S., Lu, J., Kong, X., Liang, Z., Luo, C., & Jiang, H. (2012). Computational drug discovery. *Acta Pharmacologica Sinica*, *33*(9), 1131–1140. https://doi.org/10.1038/aps.2012.109

Munir, M. A., Kuganda, H., & Basry, A. (n.d.). *The Efficacy and Safety of Antivirus Drugs for COVID-19: A Systematic Review*.

Mourad, O., Makhani, L., & Chen, L. H. (2022). Chikungunya: An Emerging Public Health Concern. *Current Infectious Disease Reports*, *24*(12), 217–228. https://doi.org/10.1007/s11908-022-00789-y

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Medina-Franco, J. L. (n.d.). *ADVANCES IN COMPUTATIONAL APPROACHES FOR DRUG DISCOVERY BASED ON NATURAL PRODUCTS*.

Mathew, A. J., Ganapati, A., Kabeerdoss, J., Nair, A., Gupta, N., Chebbi, P., Mandal, S. K., & Danda, D. (2017). Chikungunya Infection: A Global Public Health Menace. *Current Allergy and Asthma Reports*, *17*(2), 13. https://doi.org/10.1007/s11882-017-0680-7

Mani, D., Wadhwani, A., & Krishnamurthy, P. T. (2019). Drug Repurposing in Antiviral Research: A Current Scenario. *Journal of Young Pharmacists*, *11*(2), 117–121. https://doi.org/10.5530/jyp.2019.11.26

Macalino, S. J. Y., Gosu, V., Hong, S., & Choi, S. (2015). Role of computer-aided drug design in modern drug discovery. *Archives of Pharmacal Research*, *38*(9), 1686–1701. https://doi.org/10.1007/s12272-015-0640-5

Lin, H. X. J., Cho, S., Meyyur Aravamudan, V., Sanda, H. Y., Palraj, R., Molton, J. S., & Venkatachalam, I. (2021). Remdesivir in Coronavirus Disease 2019 (COVID-19) treatment: A review of evidence. *Infection*, *49*(3), 401–410. https://doi.org/10.1007/s15010-020-01557-7

Leelananda, S. P., & Lindert, S. (2016). Computational methods in drug discovery. *Beilstein Journal of Organic Chemistry*, *12*, 2694–2718. https://doi.org/10.3762/bjoc.12.267

Leach, A. R., Shoichet, B. K., & Peishoff, C. E. (2006). Prediction of Protein−Ligand Interactions. Docking and Scoring: Successes and Gaps. *Journal of Medicinal Chemistry*, *49*(20), 5851–5855. https://doi.org/10.1021/jm060999m

Kumar, P., Kumar, D., & Giri, R. (2019). Targeting the nsp2 Cysteine Protease of Chikungunya Virus Using FDA Approved Library and Selected Cysteine Protease Inhibitors. *Pathogens*, *8*(3), 128. https://doi.org/10.3390/pathogens8030128

Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nature Reviews Drug Discovery*, *3*(11), 935–949. https://doi.org/10.1038/nrd1549

Kort, E., & Jovinge, S. (2021). Drug Repurposing: Claiming the Full Benefit from Drug Development. *Current Cardiology Reports*, *23*(6), 62. https://doi.org/10.1007/s11886-021-01484-5

Kontoyianni, M. (2017). Docking and Virtual Screening in Drug Discovery. In I. M. Lazar, M. Kontoyianni, & A. C. Lazar (Eds.), *Proteomics for Drug Discovery* (Vol. 1647, pp. 255–266). Springer New York. https://doi.org/10.1007/978-1-4939-7201-2_18

Kiang, T. K. L. (2018). Clinical Pharmacokinetics and Drug–Drug Interactions of Elbasvir/Grazoprevir. *European Journal of Drug Metabolism and Pharmacokinetics*, *43*(5), 509–531. https://doi.org/10.1007/s13318-018-0471-0

Kaur, P., & Chu, J. J. H. (2013). Chikungunya virus: An update on antiviral development and challenges. *Drug Discovery Today*, *18*(19–20), 969–983. https://doi.org/10.1016/j.drudis.2013.05.002

Ivanova, L., Rausalu, K., Ošeka, M., Kananovich, D. G., Žusinaite, E., Tammiku-Taul, J., Lopp, M., Merits, A., & Karelson, M. (2021). Novel Analogues of the Chikungunya Virus Protease Inhibitor: Molecular Design, Synthesis, and Biological Evaluation. *ACS Omega*, *6*(16), 10884–10896. https://doi.org/10.1021/acsomega.1c00625

Guedes, I. A., De Magalhães, C. S., & Dardenne, L. E. (2014). Receptor–ligand molecular docking. *Biophysical Reviews*, *6*(1), 75–87. https://doi.org/10.1007/s12551-013-0130-2

Faver, J. C., Ucisik, M. N., Yang, W., & Merz, K. M. (2013). Computer-Aided Drug Design: Using Numbers to Your Advantage. *ACS Medicinal Chemistry Letters*, *4*(9), 812–814. https://doi.org/10.1021/ml4002634

Deng, Q., Guo, Z., Hu, H., Li, Q., Zhang, Y., Wang, J., Liao, C., Guo, C., Li, X., Chen, Z., & Lu, J. (2022). Inhibition of Chikungunya virus early replication by intracellular nanoantibodies targeting nsP2 Epitope Rich Region. *Antiviral Research*, *208*, 105446. https://doi.org/10.1016/j.antiviral.2022.105446

De Ruyck, J., Brysbaert, G., Blossey, R., & Lensink, M. (2016). Molecular docking as a popular tool in drug design, an in silico travel. *Advances and Applications in Bioinformatics and Chemistry*, *Volume 9*, 1–11. https://doi.org/10.2147/AABC.S105289

De Lamballerie, X., Ninove, L., & Charrel, R. (2009). Antiviral Treatment of Chikungunya Virus Infection. *Infectious Disorders - Drug Targets*, *9*(2), 101–104. https://doi.org/10.2174/187152609787847712

De Clercq, E. (2004). Antiviral drugs in current clinical use. *Journal of Clinical Virology*, *30*(2), 115–133. https://doi.org/10.1016/j.jcv.2004.02.009

Chang, Y., Hawkins, B. A., Du, J. J., Groundwater, P. W., Hibbs, D. E., & Lai, F. (2022). A Guide to In Silico Drug Design. *Pharmaceutics*, *15*(1), 49. https://doi.org/10.3390/pharmaceutics15010049

Prathipati, P., Dixit, A., & Saxena, A. (2007). Computer-Aided Drug Design: Integration of Structure-Based and Ligand-Based Approaches in Drug Design. *Current Computer Aided-Drug Design*, *3*(2), 133–148. https://doi.org/10.2174/157340907780809516