

Original Research Article

Structure-Based Drug Repurposing of Clinically Approved Antivirals for Chikungunya Virus: A Target-centric Docking Approach Utilizing Alphavirus Therapeutic Insights

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ABSTRACT

Chikungunya virus (CHIKV) remains a major global health threat due to its rapid transmission, debilitating arthralgia, and absence of approved antiviral therapy. This study employed a structure-based drug repurposing strategy targeting key viral proteins (E1, E2, NSP2, and P62) and the host entry receptor MXRA8, which play essential roles in viral attachment, membrane fusion, replication, and virion maturation. Clinically approved antiviral and immunomodulatory compounds were systematically screened using molecular docking performed through AutoDock Vina, PyRx 0.8, Discovery Studio, and UCSF Chimera to identify potential inhibitors with favorable binding affinities and interaction profiles. Sixteen drugs were evaluated for their binding interactions, ADMET behavior, and toxicity characteristics. Paritaprevir exhibited the highest affinity toward the E1/E2 protease complex (−10.8 kcal/mol), while Ledipasvir demonstrated strong multi-target potential with notable affinity for E1/E2 (−10.7 kcal/mol) and NSP2 (−9.3 kcal/mol). Eptifibatide acetate showed the strongest binding to MXRA8 (−10.2 kcal/mol), forming extensive hydrogen bond networks, and Sorafenib displayed the best interaction with SQSTM1 (−8.8 kcal/mol) through unique halogen-mediated stabilization. ADMET profiling revealed that Rimantadine, Ruxolitinib, Naringenin, and silymarin derivatives possessed favorable bioavailability and safety margins, whereas Obatoclox exhibited the highest predicted toxicity. Collectively, these findings highlight Paritaprevir, Ledipasvir, and Eptifibatide acetate as potent multi-target antiviral candidates, while Rimantadine emerges as a low-risk repurposing option. This comprehensive target-centric screening provides mechanistic insights and establishes a strong foundation for further optimization, in vitro validation, and therapeutic development against CHIKV infections.

Introduction

The CHIKV is a mosquito-borne alphavirus that poses a significant threat to global public health. It belongs to the Togaviridae family, and was first

identified in Tanzania in 1952. The term “Chikungunya” originates from the Makonde language, meaning “to become contorted,” which reflects the severe joint pain and arthritis commonly observed in infected individuals [1,2]. Since its emergence, CHIKV has caused

multiple large-scale outbreaks worldwide, including those in the Indian Ocean islands during 2005–2006, India in 2006, and the Americas between 2013 and 2015, collectively affecting millions of people [3,4]. Transmission of CHIKV occurs primarily through *Aedes aegypti* mosquitoes, although *Aedes albopictus* also plays an important role in viral spread [5]. Clinically, CHIKV infection is characterized by sudden onset of high fever, severe arthralgia, myalgia, headache, and skin rash. While the acute phase of infection typically resolves within 7–10 days, a substantial proportion of patients develop chronic inflammatory arthritis that can persist for months or even years, leading to long-term morbidity [6–8]. Despite the significant disease burden, there are currently no FDA-approved drugs specifically targeting CHIKV infection, and treatment remains largely supportive [9]. This highlights the urgent need to identify effective antiviral targets and develop therapeutic strategies.

CHIKV is an enveloped virus with a positive-sense single-stranded RNA genome of approximately 11.8 kb. The viral genome encodes four non-structural proteins (nsP1–nsP4) responsible for viral replication and transcription, along with five structural proteins (capsid, E3, E2, 6 K, and E1) that form the viral particle and mediate host cell entry [10,11]. Among non-structural proteins, NSP2 is a multifunctional enzyme comprising 798 amino acids and plays a critical role in viral replication and pathogenesis. NSP2 contains several distinct functional domains, including an N-terminal region with methyltransferase-like features, an ATP-dependent RNA helicase domain, a cysteine protease domain responsible for polyprotein processing, and a C-terminal domain involved in protein–protein interactions [12–14].

The helicase domain of NSP2 facilitates ATP-dependent RNA unwinding, which is essential for viral RNA replication and transcription [15,16]. In addition, the protease domain mediates cleavage of the viral non-structural polyprotein through a cysteine protease mechanism, enabling the maturation of functional replication complexes [17–19]. Beyond its enzymatic roles, NSP2 exerts significant cytotoxic effects on host cells by inducing transcriptional shutoff, interfering

with host translation, causing cell cycle arrest, and promoting apoptosis [20–23]. Owing to its multifunctionality and essential role in the viral life cycle, NSP2 has emerged as a promising antiviral target, and recent studies have employed fragment-based screening, covalent inhibitors, and fluorogenic substrates for high-throughput screening [24,25].

In addition to viral proteins, host factors involved in antiviral defense have gained increasing attention as potential therapeutic targets. Sequestosome-1 (SQSTM1), also known as p62, is a multifunctional adaptor protein that plays a crucial role in linking the ubiquitin–proteasome system with selective autophagy [26–28]. SQSTM1/p62 contains several functional domains, including a PB1 domain for oligomerization, ZZ domain for binding receptor-interacting proteins, TRAF6-binding domain involved in NF- κ B activation, LC3-interacting region (LIR) for autophagosome targeting, and ubiquitin-associated (UBA) domain that recognizes polyubiquitinated proteins [29,30]. Through these domains, SQSTM1/p62 functions as a selective autophagy receptor, recognizing ubiquitinated protein aggregates and directing them to autophagosomes for degradation [31,32].

Recent studies have revealed a complex interplay between CHIKV infection and host autophagy machinery. SQSTM1/p62 has been shown to play a pivotal role in the host antiviral response by targeting ubiquitinated viral capsid proteins for autophagic degradation, thereby restricting viral replication [33–35]. This critical involvement of SQSTM1/p62 in CHIKV infection highlights its potential as a host-directed therapeutic target [36–38].

Viral entry is another key stage of the CHIKV life cycle, and offers opportunities for therapeutic intervention. MXRA8 has been identified as a major entry receptor for arthritogenic alphaviruses, including CHIKV, using comprehensive genetic screening approaches [39–41]. MXRA8 is a type I transmembrane protein consisting of an extracellular domain containing immunoglobulin-like and fibronectin type III domains, a single-pass transmembrane region, and a short cytoplasmic tail [42,43]. High-resolution crystal structures of MXRA8 have been determined, providing detailed

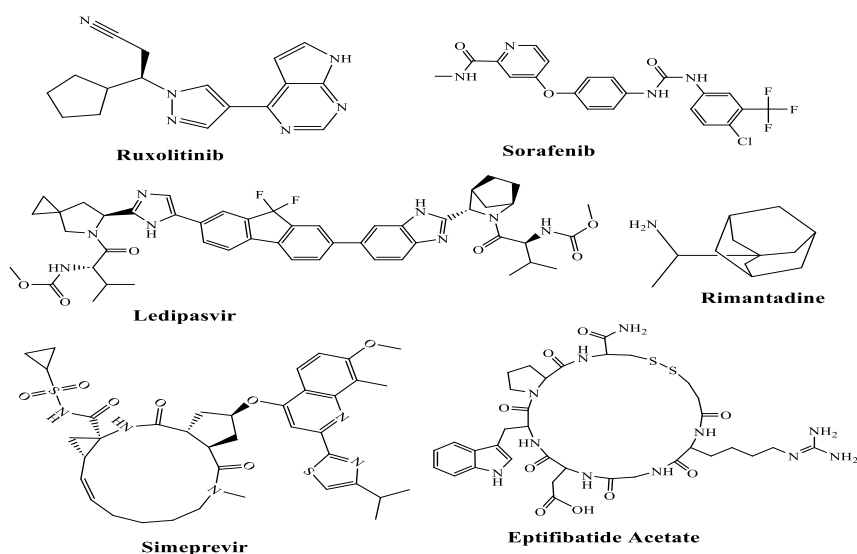
insights into its interaction with viral proteins [44,45].

MXRA8 facilitates CHIKV entry by mediating initial viral attachment by binding to envelope glycoproteins E1 and E2, followed by receptor-mediated endocytosis, pH-dependent membrane fusion, and subsequent release of the viral genome into the host cytoplasm [46,47]. The expression pattern of MXRA8 correlates strongly with CHIKV tissue tropism, particularly in joints, muscles, skin, and immune cells, further supporting its relevance as a therapeutic target [48,49]. Consequently, MXRA8 represents an attractive target for antiviral intervention aimed at blocking viral entry and tissue-specific infections [50,51].

CHIKV envelope glycoproteins E1 and E2 play central roles in viral attachment, membrane fusion, and host cell entry. E2 serves as the primary receptor-binding protein and is comprised of multiple functional domains, including Domain A containing the MXRA8-binding site, Domain B with a central β -barrel structure, and Domain C, which forms a C-terminal immunoglobulin-like domain [52,53]. In contrast, E1 functions as a viral fusion protein and consists of Domain I with a central β -sheet, Domain II containing a fusion loop, Domain III with an immunoglobulin-like fold, and a transmembrane domain anchoring the protein within the viral membrane [54,55].

Membrane fusion is initiated by endosomal acidification, which triggers conformational changes in E1, leading to the exposure and insertion of the fusion loop into the host membrane and subsequent fusion pore formation [56-59]. Although E1 and E2 do not possess intrinsic protease activity, their processing and activation by host proteases such as furin and cathepsin B are critical for efficient viral entry and infectivity [58-61].

In this study, a target-centric molecular docking strategy was employed based on well-established therapeutic insights from alphavirus biology. Key viral proteins (E1, E2, NSP2, and P62) and the host entry receptor MXRA8 were selected because of their critical and well-characterized roles in viral attachment, membrane fusion, replication, and virion maturation. These targets have been extensively implicated in the pathogenesis of alphaviruses, and represent validated intervention points for antiviral development. By leveraging prior mechanistic and structural knowledge from alphavirus research, the present approach strengthens the biological relevance of docking analysis and provides a robust rationale for repurposing clinically approved antivirals against CHIKV. The chemical structures of all drugs are shown in Figure 1.



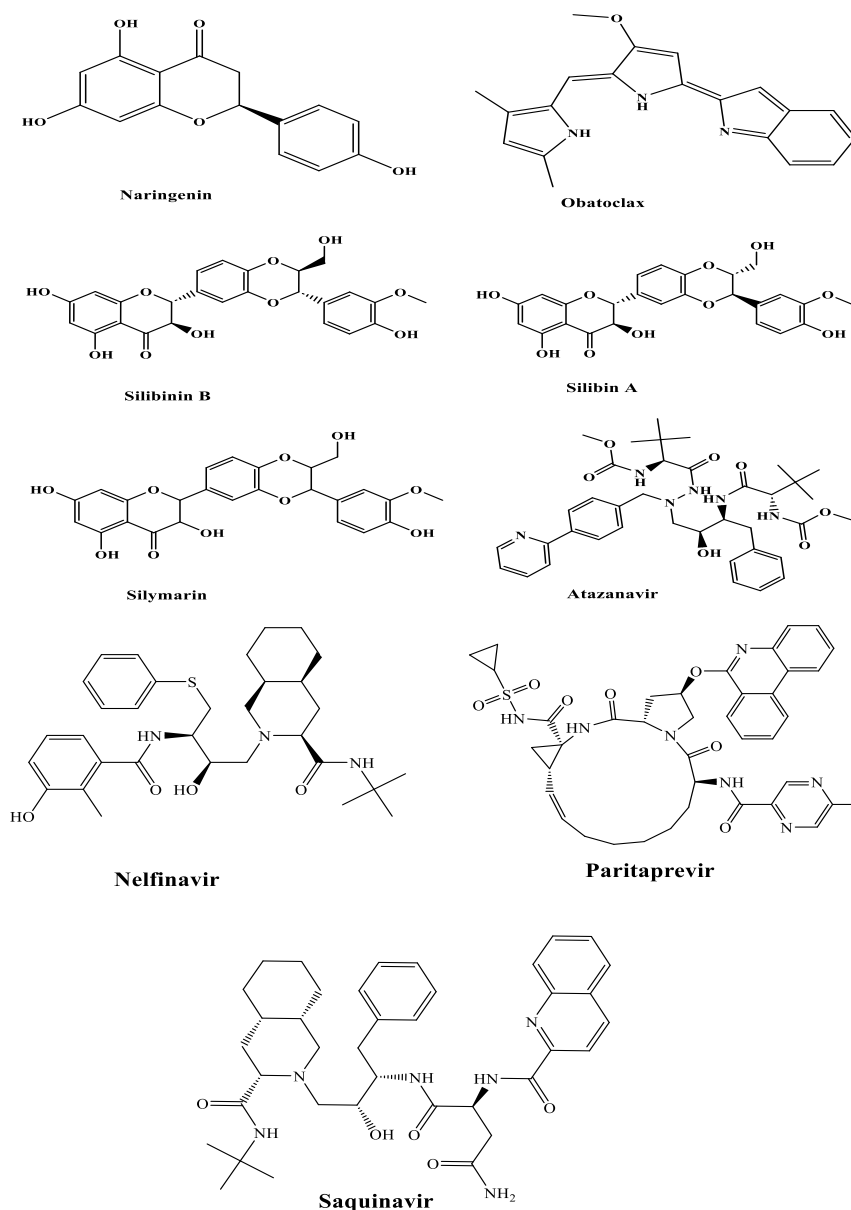


Figure 1. Chemical structure of Ruxolitinib, Sorafenib, Ledipasvir, Rimantadine, Simeprevir, Eptifibatide Acetate, Naringenin, Obatoclax, Silibinin B, Silybin A, Silymarin, Atazanavir, Nelfinavir, Paritaprevir, and Saquinavir

Materials and Methods

Chikungunya target selection and biological relevance

This study employed a systematic drug repurposing strategy targeting four critical CHIKV proteins: E1, E2, NSP2, and P62 (pE2), along with the host entry receptor MXRA8. [Figures 2](#) and [3](#) provide a comprehensive overview of the therapeutic targeting framework adopted in this study, highlighting how viral structural and non-structural proteins,

together with the host entry receptor MXRA8, were collectively exploited to disrupt the key stages of CHIKV pathogenesis. These targets were selected based on their well-established structural and functional roles in the life cycle of CHIKV.

The envelope glycoproteins E1 and E2 are essential for viral entry, where E2 mediates host cell receptor attachment, and E1 facilitates pH-dependent membrane fusion, enabling the release of the viral genome into the host cytoplasm. NSP2 was selected because of its

multifunctional enzymatic activities, including protease, helicase, and RNA triphosphatase functions, which are indispensable for viral replication and polyprotein processing. The precursor protein P62 (pE2) was targeted because it requires cleavage by host furin-like proteases for the maturation of the E1/E2 heterodimer; inhibition of this process can effectively disrupt viral assembly and infectivity. Additionally, MXRA8, a host receptor identified as a key mediator of CHIKV entry, represents a

crucial intervention point for blocking viral attachment and preventing infection.

By selecting both viral and host targets that operate at complementary stages of the viral life cycle, this study aimed to identify compounds with multitarget antiviral potential. Clinically approved drugs with known safety profiles were repurposed against validated alphavirus-related targets, thereby accelerating the identification of potential CHIKV inhibitors.

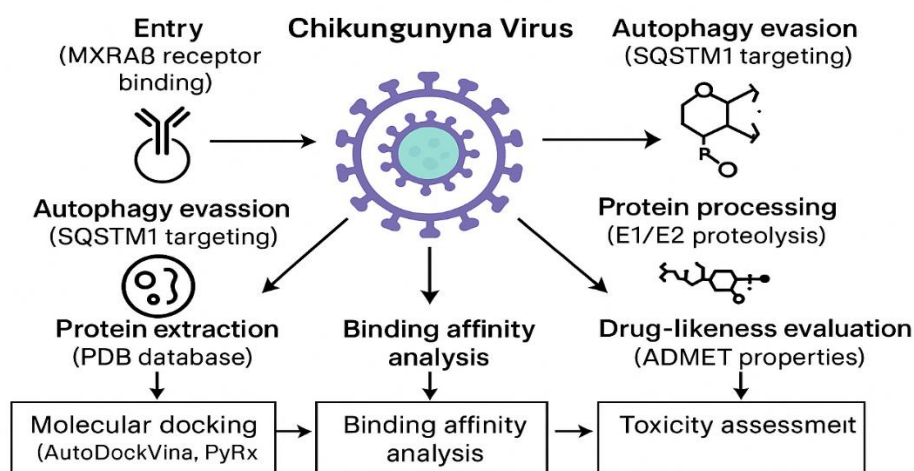


Figure 2. Computational pipeline for antiviral drug discovery targeting CHIKV infection mechanisms.

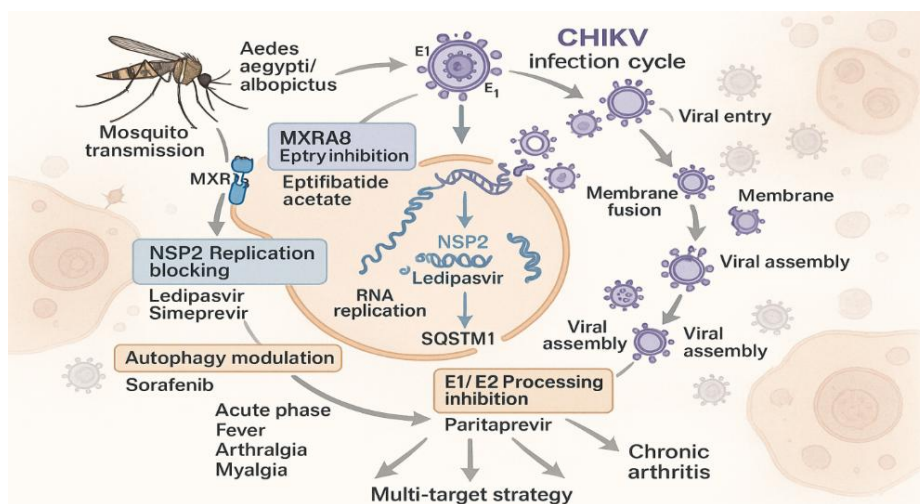


Figure 3. Comprehensive therapeutic targeting strategy for CHIKV infection integrating viral pathogenesis and multi-modal antiviral interventions.

Molecular docking

Prior to being saved in the PDB format, the target proteins were extracted from the [Protein Data Bank](#) and docked using Discovery Studio Visualizer 2020, which eliminated unnecessary

ligands and water molecules. Using AutoDock Vina 1.1.2 in PyRx 0.8, molecular docking of the natural ligand with a few chosen compounds was performed. UCSF Chimera and Discovery Studio Visualizer 2020 were used to examine and visualize interactions with amino acids [62-

66]. To ensure reliable docking outcomes, all the selected drug molecules were first standardized and energetically optimized through a systematic ligand preparation process, as described below.

Ligand preparation

In this study, the chikungunya and present drugs (SDF file) were obtained from the official website of the US National Library of Medicine PubChem. Afterward, using the Open Babel tool, structures were imported into PyRx Virtual Screening Tool 0.8 software, and the energy minimization process was performed by considering fundamental parameters based on the elements and their hybridization by a universal force field (UFF) [67]. Discovery Studio software was used to predict the active sites of the selected chikungunya receptor. Following ligand optimization, the next step involved careful preparation of the target protein structures to ensure an accurate representation of their active conformations for docking simulations.

Target preparation

The complete molecular crystal structure of chikungunya Receptor Protein Kinase with PDB IDs— (3N40, 4ZTB, 6JO8, 8FCG) has been utilized study, which was developed by Chitnumsub et al. For this study, a three-dimensional grid box (size_x = 26.3786 Å ;size_y = 29.0004 Å ; size_z = 20.8096 Å) was designed to define the area for interactions in the occupied cavity of the chikungunya receptor using the AutoDock tool 1.5.6, with an exhaustiveness value of 8. The cavity was defined using the Toggle Selection Spheres option provided in the Vina Wizard Tool of PyRx 0.8. This option was used to select drugs. The grid box was precisely positioned to occupy all active binding sites as well as critical residues. All the present drugs and chikungunya receptors were then subjected to docking to obtain the possible affinities/interactions with each other. The complete molecular docking procedure was performed as described by S. L. Khan *et al.* [68,69]. Once the receptor structures were prepared and the active-site grid parameters were defined, molecular docking simulations

were performed to evaluate the ligand-target binding affinities and interaction profiles.

Docking procedure

The "load molecule" option was used to import the target protein structures into PyRx 0.8. The right-click menu was then used to convert the receptor structure into the PDBQT format. Both ligand and target PDBQT files were chosen for docking, and the binding affinity was examined using the Vina Wizard Tool. AutoDock 1.5.6, 118 Journal of Pharmaceutical Sciences and Computational Chemistry 2025, Volume 1, Issue 2 3D grid box centered on the active sites of the target proteins, and an exhaustiveness value of 8 were used to conduct molecular docking simulations. Based on earlier research techniques, this study employed an integrated experimental approach that included molecular docking. Based on the docking results, the most promising compounds were subjected to ADMET analysis to assess their pharmacokinetic behavior, drug-likeness, and safety profiles.

ADMET analysis

ADMET profiling of the selected compounds was performed to evaluate their pharmacokinetic behavior, drug-likeness, and toxicity parameters. The two-dimensional structures of all compounds were first sketched and optimized using ChemDraw, after which canonical SMILES and physicochemical data were retrieved from the PubChem database. These optimized structures were imported into the ADMETlab 3.0 web server to predict their absorption, distribution, metabolism, excretion, and toxicity properties following standard computational filters. The analyses included gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein interaction tendency, cytochrome P450 enzyme inhibition capacity, bioavailability score, skin permeation (LogKp), hydrogen bond donors/acceptors, molecular weight, topological polar surface area, and lipophilicity parameters, consistent with the drug-likeness evaluation workflow used in the selected reference. Toxicity predictions—including LD₅₀ values, hepatotoxicity, carcinogenicity, immunotoxicity, and cytotoxicity—were similarly obtained through

the ADMETlab toxicity module. Nuclear receptor signaling interactions (androgen receptor, aromatase, and estrogen receptor alpha) were also screened to assess the potential endocrine-related risks. All computational predictions were generated using default model settings, and the resulting ADMET descriptors were tabulated for the comparative evaluation of compound suitability for further *in silico* and *in vivo* investigations.

Results and Discussion

Molecular docking study of drug compounds with SQSTM1

Molecular docking studies were performed to assess the binding interactions of Ruxolitinib and Sorafenib with the sequestosome-1 protein (SQSTM1; PDB ID: 3N40), a key adaptor protein involved in autophagy regulation and protein aggregation. Comparative evaluation of docking scores and interaction profiles revealed distinct differences in the binding affinity and molecular stabilization between the two ligands.

Sorafenib demonstrated a higher binding affinity toward SQSTM1 with a docking score of -8.8 kcal/mol, compared with -7.1 kcal/mol for Ruxolitinib, indicating a more stable and energetically favorable ligand-protein complex. The enhanced binding performance of Sorafenib can be attributed to its ability to form a diverse and extensive interaction network within the SQSTM1 binding pocket. Sorafenib established multiple conventional hydrogen bonds with key residues HIS206, ILE200, and ARG208, with short bond distances (approximately 2.0–2.45 Å), suggesting strong and directional interactions. Additional stabilization was provided by carbon hydrogen bonds with CYS330 and ARG331. Notably, Sorafenib exhibits distinct halogen (fluorine) interactions with PRO198, VAL199, and GLU203, which are known to enhance binding specificity and stability by improving molecular

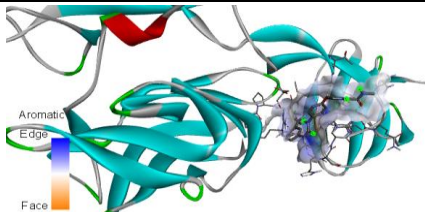
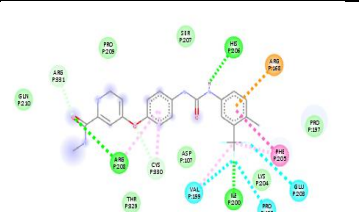
complementarity. Furthermore, a π -cation electrostatic interaction with ARG168 reinforced electrostatic anchoring within the binding site. Hydrophobic contributions, including π - π T-shaped interactions with PHE205 and alkyl and π -alkyl contacts involving VAL199, ARG208, CYS330, and PHE205, further strengthened the Sorafenib-SQSTM1 complex.

In contrast, Ruxolitinib showed moderate binding affinity, forming a more limited interaction profile. Two conventional hydrogen bonds with PHE205 and PRO197 contributed to polar stabilization, whereas a π -cation interaction with LYS204 provided additional electrostatic support. Hydrophobic interactions, including an alkyl contact with ILE200 and π -alkyl interactions with LYS204 and PHE205, contributed to overall binding; however, the reduced number and diversity of interactions likely accounted for its lower docking score relative to Sorafenib. Comparative analysis revealed that residues such as ILE200, PHE205, ARG208, and CYS330 play critical roles in ligand recognition within the SQSTM1 binding cavity. The ability of Sorafenib to simultaneously engage these residues through hydrogen bonding, hydrophobic interactions, electrostatic forces, and unique halogen bonding underscores its superior binding efficiency. In contrast, the absence of halogen interactions and fewer stabilizing contacts in ruxolitinib resulted in a comparatively weaker binding.

Overall, the docking results clearly indicate that Sorafenib exhibits exceptional binding affinity and interaction diversity with SQSTM1, outperforming Ruxolitinib. The strong and multifaceted binding profile of Sorafenib supports its potential as an SQSTM1-targeting therapeutic agent, particularly for conditions involving dysregulated autophagy and protein aggregation. Detailed interaction parameters and binding affinities are summarized in [Table 1](#), and the corresponding binding conformations are shown in [Figure 4](#).

Table 1. MD study of present drug with (sequestosome-1) (SQSTM1) representing the binding affinities (kcal/mol), hydrogen bonds, and active amino acid residues with their bond length (Å)

Compound name	Molecular formula/Molecular weight (mg/mol)	Binding affinity (kcal/mol)	Active amino acid residue	Bond length (Å)	Bond category	Bond types
Ruxolitinib	C ₁₇ H ₁₈ N ₆ /306.36	-7.1	PHE205	2.79064	Hydrogen bond	Conventional hydrogen bond
			PRO197	2.65795		
			LYS204	4.66596	Electrostatic	Pi-Cation
			ILE200	5.29234	Hydrophobic	Alkyl
			LYS204	3.81448		Pi-Alkyl
			PHE205	4.83629		
			HIS206	2.10015	Hydrogen bond	Conventional hydrogen bond
			ILE200	2.04065	Hydrogen bond;Halogen	Conventional hydrogen bond;Halogen (Fluorine)
			ARG208	2.45	Hydrogen bond	Conventional hydrogen bond
			CYS330	3.55062		Carbon hydrogen Bond
Sorafenib	C ₂₁ H ₁₆ ClF ₃ N ₄ O ₃ /464.82	-8.8	ARG331	3.43858	Halogen	Halogen (Fluorine)
			PRO198	3.24216		
			VAL199	3.515		
			GLU203	3.355	Electrostatic	Pi-Cation
			ARG168	4.66686		Pi-Pi T-shaped
			PHE205	5.16828		Alkyl
			VAL199	4.98524	Hydrophobic	
			ARG208	4.1321		
			CYS330	4.35153		Pi-Alkyl
			PHE205	4.52416		

Compounds	3D Diagram	2D Diagram
Sorafenib		

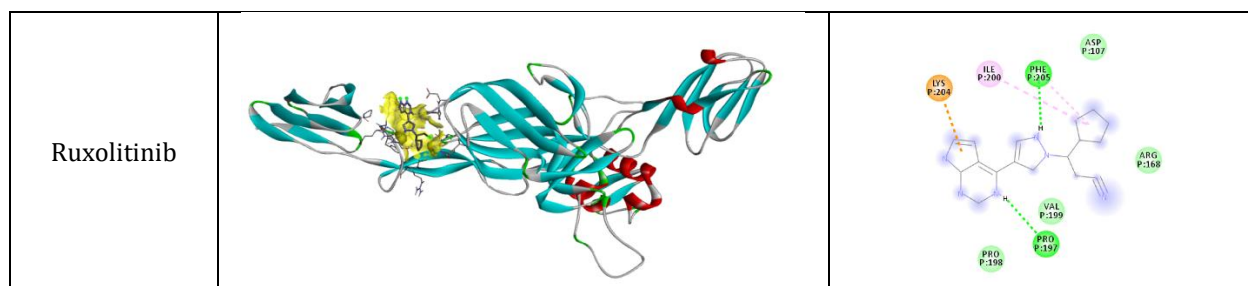


Figure 4. 2D and 3D binding interactions of Sorafenib, Ruxolitinib with SQSTM1.

Molecular docking study of drug compounds with NSP2 (Helicase Inhibitor)

Molecular docking studies were performed to evaluate the binding potential of Ledipasvir, Rimantadine, and Simeprevir against the NSP2 helicase protein (PDB ID: 4ZTB), a key viral component involved in RNA processing and replication. Comparative assessment of docking scores and interaction profiles revealed clear differences in the binding affinity and molecular interaction patterns among the tested compounds.

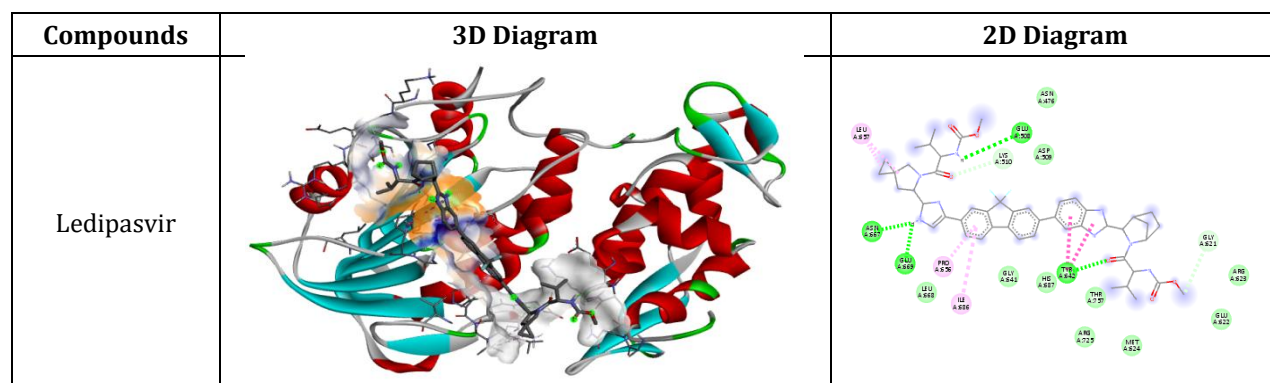
Among the evaluated ligands, Simeprevir exhibited the highest binding affinity, with a docking score of -9.6 kcal/mol, followed closely by Ledipasvir (-9.3 kcal/mol), while Rimantadine showed substantially weaker binding (-5.2 kcal/mol). These variations in affinity reflect differences in the extent and nature of ligand-protein interactions within the NSP2 binding pocket. Ledipasvir formed an extensive and well-distributed interaction network with NSP2, dominated by multiple conventional hydrogen bonds involving residues ASN667, GLU669, GLU508, and TYR642, with bond lengths of 2.0–2.7 Å, indicative of strong polar interactions. Additional stabilization was provided by carbon hydrogen bonds with GLY621 and LYS510. Hydrophobic contributions, including π - π T-shaped interactions with TYR642, alkyl interactions with LEU657, and π -alkyl contacts with PRO656 and ILE686 further anchored Ledipasvir within the helicase binding site. The combination of multiple hydrogen bonds and hydrophobic interactions explains the high binding affinity and stable complex formation. In contrast, Rimantadine demonstrated a limited binding profile, characterized by a single

conventional hydrogen bond with ARG746. Its stabilization relied primarily on hydrophobic alkyl interactions with VAL780 and π -alkyl contact with PHE776. The absence of multiple hydrogen bonds and electrostatic interactions resulted in reduced stabilization, accounting for the markedly lower docking score. Simeprevir displays a distinct and highly favorable binding pattern, primarily driven by electrostatic and sulfur-based interactions. A π -anion interaction with ASP711 and a π -sulfur bond with MET707 contributed significantly to electrostatic stabilization. Additional hydrophobic interactions, including alkyl contacts with LEU670 and LYS556, and π -alkyl interactions involving MET707 and TYR544 further reinforced binding. Although Simeprevir formed fewer conventional hydrogen bonds than Ledipasvir, the presence of strong electrostatic and sulfur-mediated interactions likely accounts for its superior binding affinity. Comparative analysis highlighted the importance of residues such as TYR642, GLU669, ASN667, ASP711, and MET707 in NSP2 ligand recognition and stabilization. Compounds capable of engaging these residues through diverse interactions, including hydrogen bonding, electrostatic forces, and hydrophobic contacts, exhibited enhanced binding affinity.

Overall, the docking results clearly demonstrated that Simeprevir and Ledipasvir possess superior binding characteristics toward NSP2 compared to Rimantadine. Their strong and diverse interaction profiles suggest the effective targeting of critical helicase residues, supporting their potential as NSP2-targeting therapeutic agents. The detailed binding parameters are summarized in Table 2 and the corresponding binding conformations are shown in Figure 5.

Table 2. MD study of present drug with NSP2(Helicase Inhibitor) representing the binding affinities (kcal/mol), hydrogen bonds, and active amino acid residues with their bond length (Å)

Compound name	Molecular formula/Molecular weight (mg/mol)	Binding affinity (kcal/mol)	Active amino acid residue	Bond length (Å)	Bond category	Bond types
Ledipasvir	C ₄₉ H ₅₄ F ₂ N ₈ O ₆ /889	-9.3	ASN667	2.10567	Hydrogen bond	Conventional hydrogen bond
			GLU669	2.46528		
			GLU508	2.68721		
			TYR642	2.06928		
			GLY621	3.43163	Carbon hydrogen bond	
			LYS510	3.09594		
			TYR642	4.59644	Pi-Pi T-shaped	
			TYR642	4.72447		
			LEU657	5.1048	Hydrophobic	Alkyl
			LEU657	4.44939		
PRO656	3.85769					
Rimantadine	C ₁₂ H ₂₁ N/179.30	-5.2	ILE686	5.37313	Pi-Alkyl	
			ARG746	2.03187		Hydrogen bond
			VAL780	4.18422		
			VAL780	4.61867	Hydrophobic	Alkyl
			VAL780	4.43679		
			PHE776	4.58888	Electrostatic Other	Pi-Alkyl
ASP711	3.93256	Pi-Anion				
MET707	3.62021	Pi-Sulfur				
Simeprevir	C ₃₈ H ₄₇ N ₅ O ₇ S ₂ /749.94	-9.6	LEU670	4.86132	Hydrophobic	Alkyl
			LYS556	5.41301		
			MET707	4.95961		
			TYR544	5.43656		
			TYR544	5.37902	Pi-Alkyl	



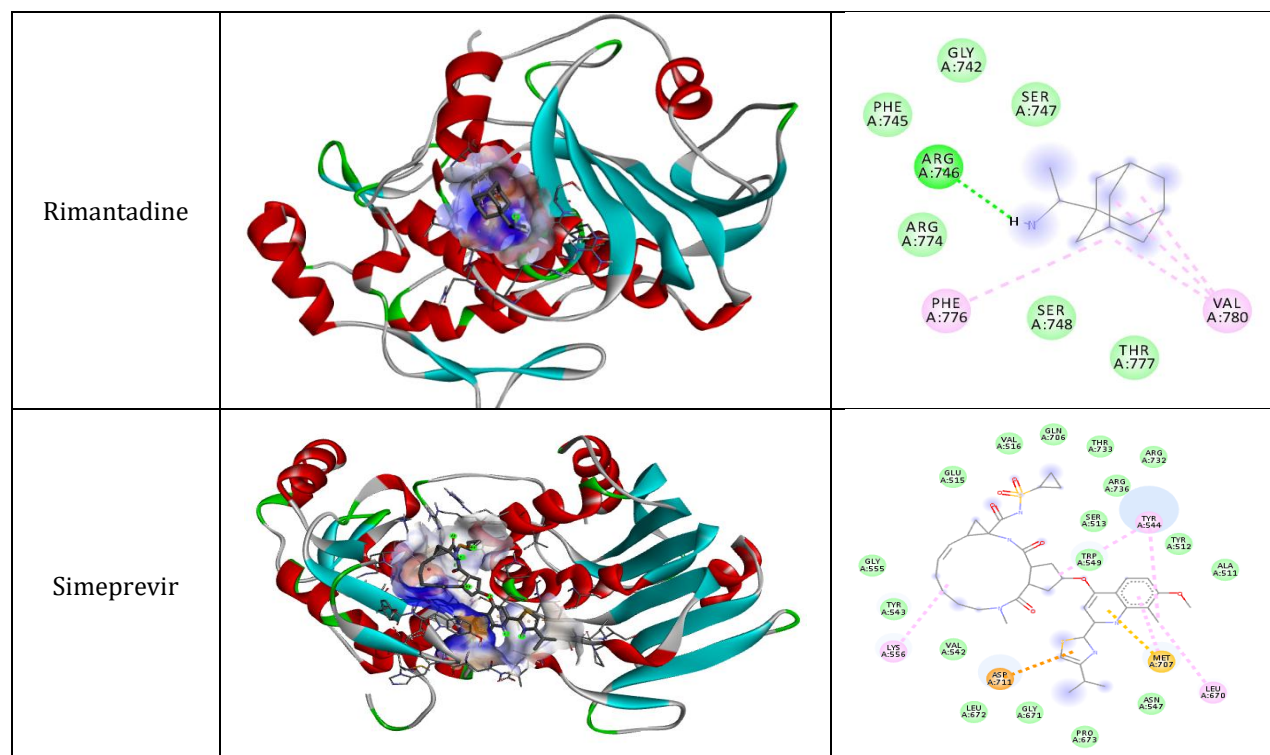


Figure 5. 2D and 3D binding interactions of Ledipasvir, Rimantadine, and Simeprevir with NSP2 helicase protein.

Molecular docking study of drug compounds with MXRA8 (Protease Alpha, Membrane fusion, and Capsid Protein)

Molecular docking studies were conducted to evaluate the binding potential of the selected drug compounds, eptifibatide acetate, naringenin, obatoclax, silibinin B, silybin A, and silymarin, against the MXRA8 protein (PDB ID: 6J08), a key mediator involved in viral membrane fusion and capsid-related interactions. The comparative docking scores and interaction profiles highlighted marked differences in binding affinity and molecular recognition among the tested ligands.

Among all compounds, eptifibatide acetate exhibited the strongest binding affinity with a docking score of -10.2 kcal/mol, indicating a highly favorable and stable interaction with MXRA8. This superior affinity is supported by the formation of multiple conventional hydrogen bonds involving critical residues, such as ARG168, PRO154, LYS171, ARG208, ARG331, and HIS206. The presence of several hydrogen bonds with bond lengths predominantly below 2.5 Å suggests strong electrostatic stabilization within the active site. Additionally, extensive

hydrophobic interactions, including π - π T-shaped, alkyl, and π -alkyl interactions with residues such as PHE205, ILE200, CYS330, PRO197, VAL199, and LYS204, further reinforced the ligand-protein complex. The combined hydrogen bonding and hydrophobic network indicated that eptifibatide acetate engages deeply within the MXRA8 binding pocket, making it the most promising candidate among the evaluated compounds. Silibinin B and silybin A demonstrated the second-highest binding affinities, each with a docking score of -8.7 kcal/mol, reflecting strong and comparable interactions with MXRA8. Silibinin B formed conventional hydrogen bonds with GLN222 and ARG331, along with π -sulfur interactions with CYS330 and multiple hydrophobic contacts involving PHE205, VAL328, ILE200, and CYS330. These interactions suggest stable anchoring of the ligand within the protein cavity. Similarly, silybin A established multiple hydrogen bonds with LYS171, ARG208, and ASP107, including short bond distances, that are indicative of strong interactions. Additional stabilization was achieved through π -S and hydrophobic π -alkyl contacts. The overlap of

interacting residues, such as LYS171, ARG208, ASP107, and CYS330, with those involved in eptifibatide acetate binding highlights the functional importance of these amino acids in MXRA8 ligand recognition.

Obatoclox showed moderate binding affinity (-8.0 kcal/mol) and a diverse interaction pattern dominated by hydrophobic forces. Although only one conventional hydrogen bond with PHE70 and a carbon hydrogen bond with THR224 were observed, obatoclox exhibited extensive π - π stacking, π - π T-shaped, π -sigma, and π -alkyl interactions involving residues such as HIS320, PHE70, VAL72, and ALA226. This suggests that hydrophobic interactions play a dominant role in stabilizing the obatoclox-MXRA8 complex, compensating for relatively fewer hydrogen bonds. Naringenin displayed comparatively weaker binding (-7.5 kcal/mol), characterized by a limited interaction profile. Only a single conventional hydrogen bond with TYR73 was observed, along with one π -sulfur interaction and several hydrophobic contacts. The reduced number of stabilizing interactions indicated a less optimal fit within the MXRA8 binding site, which may account for its lower docking score. In contrast, silymarin exhibited the weakest binding affinity (-0.5 kcal/mol)

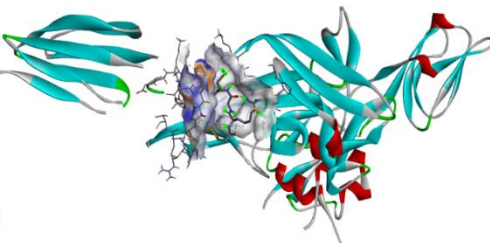
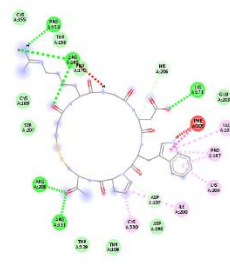
among all tested compounds. Its interaction was restricted to two carbon hydrogen bonds with PRO59, and no significant hydrophobic or π -based interactions were detected. This minimal interaction profile suggests poor accommodation of silymarin within the MXRA8 active site and a limited potential for effective inhibition.

Overall, the docking results clearly demonstrated that eptifibatide acetate possesses the most favorable binding characteristics toward MXRA8, followed by silibinin B and silybin A. The strong binding affinity, extensive hydrogen bonding, and involvement of key residues associated with membrane fusion and protease-related regions support their potential as MXRA8-targeting agents. These findings suggest that compounds capable of engaging critical amino acids, such as ARG168, LYS171, ARG208, ASP107, and CYS330, may effectively interfere with MXRA8-mediated viral entry mechanisms. The detailed binding profiles presented in Table 3 and visualized in Figure 6 provide a strong molecular basis for further experimental validation of these compounds as potential inhibitors of viral membrane fusion.

Table 3. MD study of present drug with MXRA8 (Protease Alpha, Membran fusion, and Capside Protein) representing the binding affinities (kcal/mol), hydrogen bonds, and active amino acid residues with their bond length (Å)

Compound name	Molecular formula/Molecular weight (mg/mol)	Binding affinity (kcal/mol)	Active amino acid residue	Bond length (Å)	Bond category	Bond types
Eptifibatide Acetate	$C_{35}H_{49}N_{11}O_9S_2$ /831.96	-10.2	ARG168	2.14975	Hydrogen Bond	Conventional Hydrogen Bond
			PRO154	2.18747		
			ARG168	2.72675		
			LYS171	2.36552		
			ARG208	2.12433		
			ARG208	2.20618		
			ARG331	2.02373	Hydrophobic	Carbon Hydrogen Bond
			HIS206	3.6056		
			ASP107	3.41568		
			PHE205	5.23401		
			ILE200	4.41785		Alkyl
			CYS330	4.4929		
			PRO197	5.27358		
			VAL199	5.42085		
						Pi-Alkyl

			PRO197	5.09068		
			LYS204	5.32011		
			TYR73	2.24407	Hydrogen Bond	Conventional Hydrogen Bond
			MET161	5.74181	Other	Pi-Sulfur
			HIS320	4.37857		Pi-Pi Stacked
			PHE70	4.85276	Hydrophobic	Pi-Pi T-shaped
			ALA226	4.73937		Pi-Alkyl
			ALA226	4.67455		
			PHE70	2.34775	Hydrogen Bond	Conventional Hydrogen Bond
			THR224	3.76561		Carbon Hydrogen Bond
			VAL72	3.56385		Pi-Sigma
			ALA226	3.98109		
			HIS320	4.89669		Pi-Pi Stacked
			HIS320	3.91538		
			PHE70	4.76051	Hydrophobic	Pi-Pi T-shaped
			PHE70	5.28711		
			HIS126	4.79639		
			ALA226	3.9203		
			VAL72	5.41586		Pi-Alkyl
			ALA226	5.32096		
			GLN222	2.77147	Hydrogen Bond	Conventional Hydrogen Bond
			ARG331	2.70462	Other	Pi-Sulfur
			CYS330	3.92531		Pi-Pi T-shaped
			PHE205	5.04036		
			VAL328	5.1889	Hydrophobic	Pi-Alkyl
			ILE200	5.42341		
			CYS330	5.38546		
			LYS171	2.27526		
			LYS171	2.06329	Hydrogen Bond	Conventional Hydrogen Bond
			ARG208	1.82836		Carbon Hydrogen Bond
			ASP107	3.64003		Pi-Sulfur
			ASP107	3.243	Other	Pi-Pi T-shaped
			CYS330	4.02327		
			HIS206	5.08849		
			PRO197	5.03166	Hydrophobic	Pi-Alkyl
			ARG208	4.89063		
			PRO59	3.58716	Hydrogen Bond	Carbon Hydrogen Bond
			PRO59	3.55511		

Compounds	3D Diagram	2D Diagram
Eptifibatide Acetate		

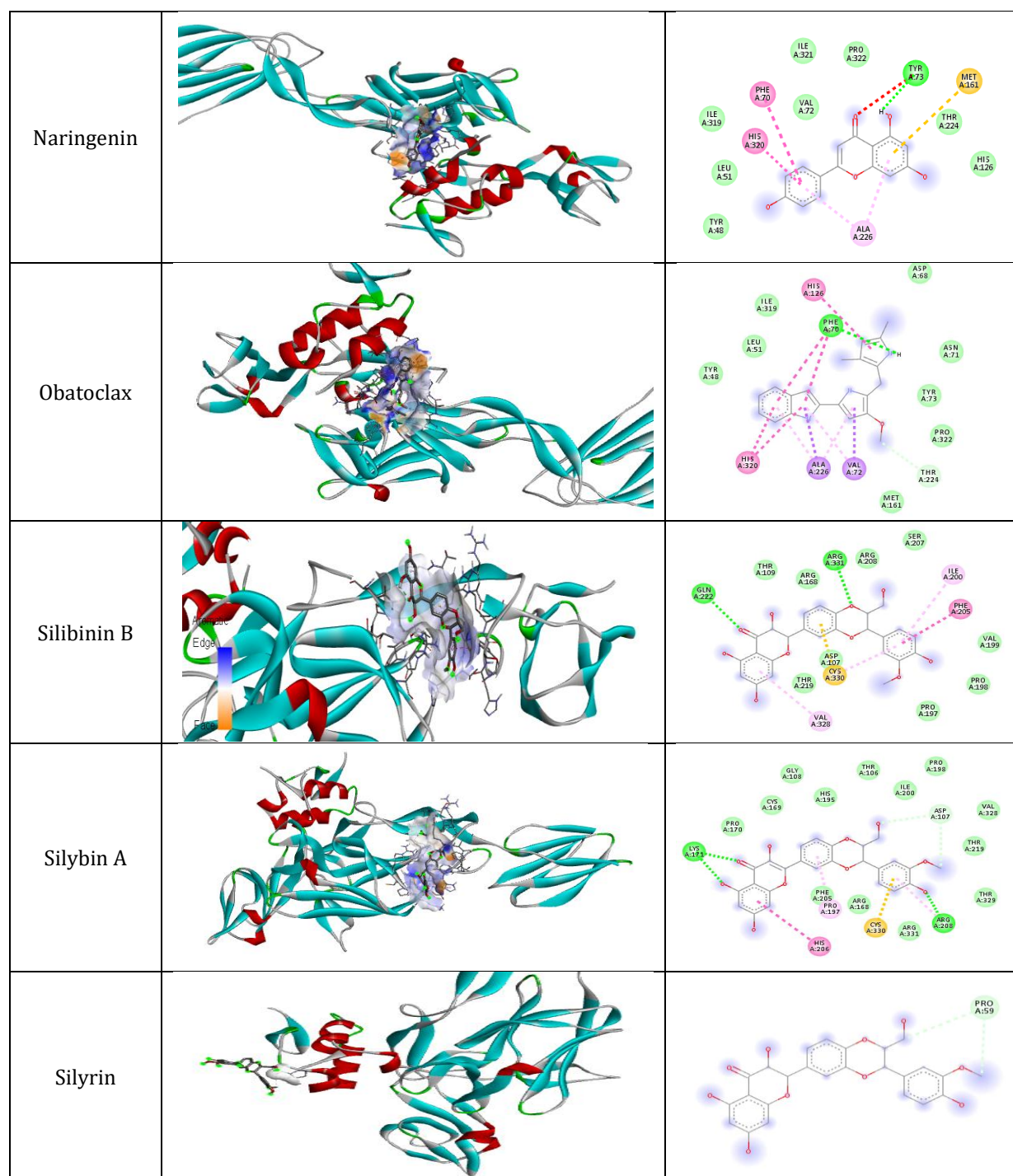


Figure 6. 2D and 3D binding interactions of Eptifibatide Acetate, Naringenin, Obatoclax, Silibinin B, Silybin A, Silymarin with MXRA8 protein

Molecular docking study of drug compounds with E1 and E2 (protease inhibitor)

Molecular docking analysis was performed to investigate the binding interactions of selected antiviral compounds (Atazanavir, Ledipasvir, Nelfinavir, Paritaprevir, Rimantadine,

Saquinavir, and Simeprevir) with the E1/E2 protease inhibitor protein (PDB ID: 8FCG). Comparative docking scores and interaction profiles revealed substantial differences in binding affinity, interaction diversity, and stabilization mechanisms among the evaluated

ligands, highlighting their relative inhibitory potential against protease targets.

Among all tested compounds, Paritaprevir demonstrated the highest binding affinity, with a docking score of -10.8 kcal/mol, indicating a highly stable ligand-protein complex. This strong affinity was supported by multiple conventional hydrogen bonds with key residues, LYS16 and THR395, including short bond lengths (as low as 1.89 Å), suggesting robust electrostatic interactions. Additional stabilization was achieved through π -donor hydrogen bonding with THR395, π -sigma interactions with VAL6, and extensive hydrophobic interactions involving TYR15 and PRO8. The convergence of hydrogen bonding and hydrophobic contacts suggests the optimal accommodation of Paritaprevir within the E1/E2 protease active region. Ledipasvir exhibited comparable binding strength, ranking second with a docking score of -10.7 kcal/mol. The binding profile was distinguished by a complex interaction network comprising multiple hydrogen bonds with ASN335, GLN260, and LYS16, along with unique halogen (fluorine) interactions with ALA392 and HIS394. These halogen bonds likely contribute significantly to the binding stability. Moreover, extensive hydrophobic interactions with residues such as VAL11, ILE7, PRO8, PRO391, HIS3, and TYR15 further reinforce ligand anchoring. The combination of hydrogen bonding, halogen bonding, and hydrophobic interactions explains the high binding affinity of Ledipasvir toward the E1/E2 protease.

Atazanavir showed strong binding affinity (-8.5 kcal/mol) with a well-distributed interaction profile. Multiple conventional hydrogen bonds with ASN335, HIS394, THR334, and THR338 were observed with bond lengths indicative of stable interactions. Hydrophobic contributions, including π - π T-shaped interactions with TYR15 and π -alkyl interactions with PRO8 and VAL13, further stabilized the complex. These interactions suggest that Atazanavir effectively occupies functionally important regions of the protease active site. Simeprevir and Nelfinavir demonstrated moderate binding affinities of $-$

7.7 and -7.2 kcal/mol, respectively. Simeprevir forms multiple conventional hydrogen bonds with TYR233 and displays a distinctive π -sulfur interaction, along with π -sigma, π - π T-shaped, and alkyl contacts primarily involving ILE55 and TYR233. These interactions indicated a mixed stabilization pattern driven by both polar and hydrophobic forces. Nelfinavir, on the other hand, established fewer hydrogen bonds, mainly with THR53 and SER238, supported by carbon hydrogen bonds and hydrophobic alkyl and π -alkyl interactions. The comparatively reduced interaction density accounted for the moderate docking score.

Saquinavir exhibited a binding affinity of -7.0 kcal/mol, characterized by multiple hydrogen bonds predominantly involving ASN335, THR5, and VAL6. Despite the presence of several hydrogen bonds, limited hydrophobic interactions were observed, suggesting less effective stabilization within the binding pocket compared to higher-ranking ligands. Rimantadine showed the weakest binding affinity (-6.2 kcal/mol) among the studied compounds. Its interaction profile was restricted primarily to conventional hydrogen bonds with THR334, VAL337, and SER332 without significant hydrophobic or aromatic interactions. This limited interaction network may explain the reduced binding stability and inferior docking performance.

Overall, the docking results clearly indicated that Paritaprevir and Ledipasvir possessed superior binding affinity and interaction complexity toward the E1/E2 protease inhibitor, followed by Atazanavir. The consistent involvement of residues such as LYS16, ASN335, THR395, TYR15, and PRO8 across high-affinity ligands highlights their critical roles in protease recognition and inhibition. The strong binding profiles of Paritaprevir and Ledipasvir support their potential as effective E1/E2 protease targeting agents for viral inhibition. The detailed interaction parameters and binding affinities are summarized in [Table 4](#), and the three-dimensional binding conformations are illustrated in [Figure 7](#).

Table 4. MD study of present Drug with E1, E2 (protease inhibitor) representing the binding affinities (kcal/mol), hydrogen bonds, and active amino acid residues with their bond length (Å)

Compound name	Molecular formula/Molecular weight (mg/mol)	Binding affinity (kcal/mol)	Active amino acid residue	Bond length (Å)	Bond category	Bond types
Atazanavir	C ₃₈ H ₅₂ N ₆ O ₇ /704.85	-8.5	ASN335	2.70311	Hydrogen Bond	Conventional Hydrogen Bond
			HIS394	1.9634		
			THR334	2.6891		
			ASN335	2.37958		
			THR338	2.03938	Hydrophobic	Pi-Pi T-shaped Pi-Alkyl
			TYR15	4.85284		
			PRO8	4.12213		
			VAL13	5.37163		
			ASN335	2.6791	Hydrogen Bond	Conventional Hydrogen Bond
			GLN260	2.63098		
Ledipasvir	C ₄₉ H ₅₄ F ₂ N ₈ O ₆ /889	-10.7	ASN335	3.41086	Hydrogen Bond	Carbon Hydrogen Bond
			LYS16	3.23665		
			LYS16	3.55979		
			ALA392	2.78577	Halogen	Halogen (Fluorine)
			ALA392	3.69449		
			HIS394	2.87479		
			VAL11	3.7008		
			VAL11	3.90026	Hydrophobic	Alkyl
			LYS16	4.18038		
			ILE7	4.74836		
			ILE7	4.8918		
			PRO8	4.82102		Pi-Alkyl
			PRO8	4.31561		
			PRO391	4.6869		
			PRO391	4.85418		
Nelfinavir	C ₃₂ H ₄₅ N ₃ O ₄ S/567.78	-7.2	PRO8	5.45224	Hydrophobic	Pi-Alkyl
			HIS3	5.22818		
			TYR15	4.62589		
			THR53	2.05258		
			SER238	2.15986	Hydrogen Bond	Conventional Hydrogen Bond
			TYR233	3.70342		
			GLN235	3.75018		
			LYS52	3.93752		
			PRO237	4.94935	Hydrophobic	Alkyl
			LYS52	3.93508		
Paritaprevir	C ₄₀ H ₄₃ N ₇ O ₇ S/765.88	-10.8	ILE55	5.49353		
			LYS16	2.9973		
			LYS16	1.89874	Hydrogen Bond	Conventional Hydrogen Bond
			THR395	2.8317		
			THR395	2.39		

[illegible]

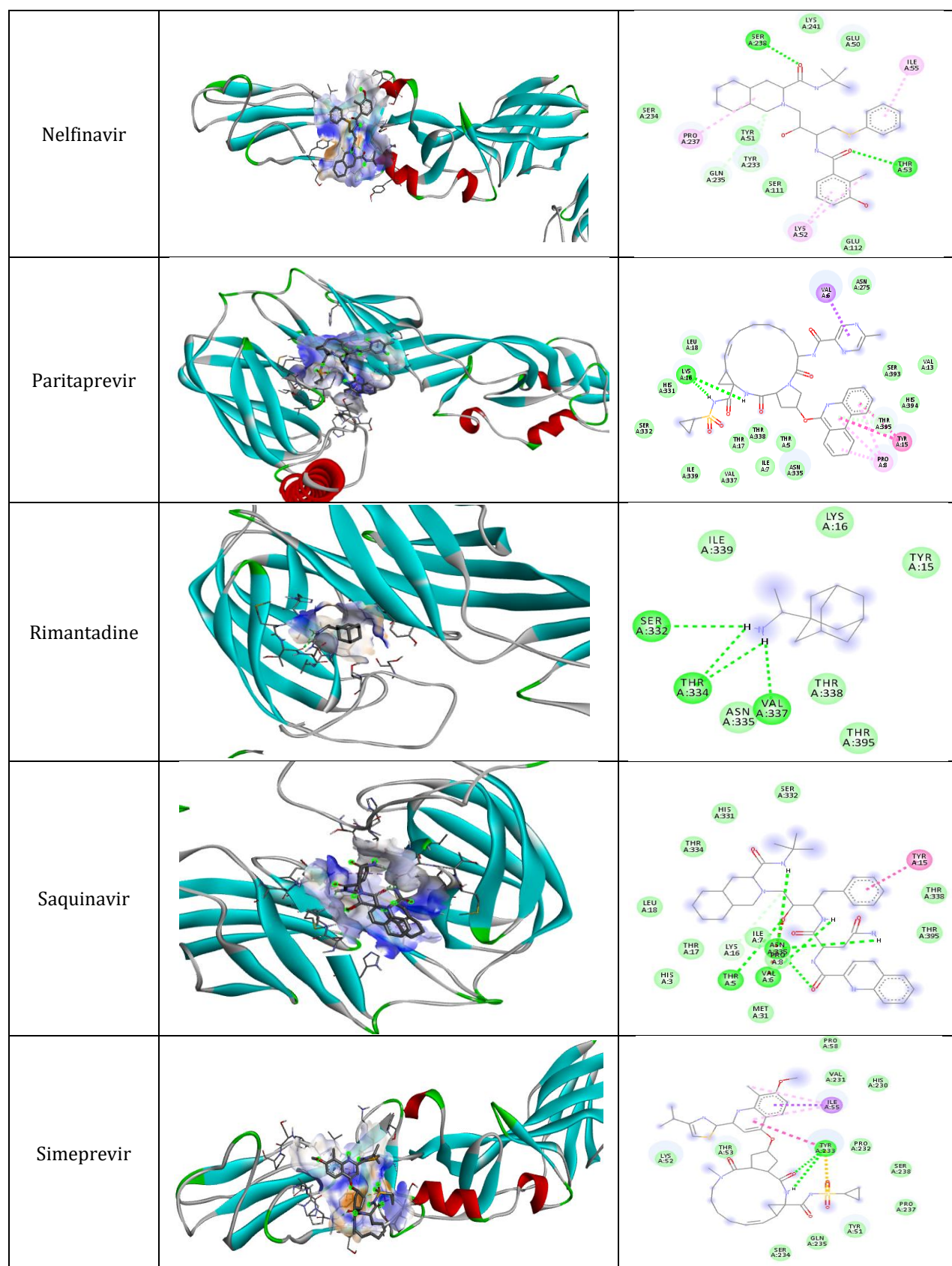


Figure 7. 2D and 3D binding interactions of Atazanavir, Ledipasvir, Nelfinavir, Paritaprevir, Rimantadine, Saquinavir, and Simeprevir with E1 and E2 protease inhibitor proteins

ADMET analysis

ADMET profiling was conducted for selected compounds (Tables 5–7) to evaluate their pharmacokinetic behavior, drug likeness, and toxicity, with the specific aim of integrating these parameters with molecular docking performance for rational lead prioritization. Rather than relying solely on binding affinity, the compounds were assessed using a balanced decision framework that jointly considered (i) docking strength and multi-target engagement, (ii) pharmacokinetic feasibility, and (iii) predicted toxicity risk.

Ruxolitinib exhibited favorable pharmacokinetic characteristics, including high gastrointestinal absorption, blood–brain barrier permeability, absence of P-glycoprotein substrate behavior, and a relatively high bioavailability score (0.71), supported by its moderate molecular weight and lipophilicity. Although its docking performance was moderate, the ADMET profile was found to be a pharmacokinetically reliable reference compound.

In contrast, Sorafenib and Ledipasvir demonstrated strong docking affinities against key viral and host targets but were associated with notable pharmacokinetic limitations. Sorafenib showed low gastrointestinal absorption, P-glycoprotein substrate liability, CYP inhibition, and reduced bioavailability (0.35), while Ledipasvir displayed the most unfavorable ADMET profile, characterized by extremely low bioavailability (0.17), very high molecular weight, and excessive lipophilicity. These findings indicate that despite excellent docking performance, both compounds would require formulation strategies or structural optimization to improve systemic exposure.

Rimantadine emerged as the most pharmacokinetically favorable compound, exhibiting high gastrointestinal absorption, blood–brain barrier permeability, absence of CYP inhibition, and the highest bioavailability score (0.85), supported by its low molecular weight and minimal hydrogen bonding. Although its docking scores were comparatively weaker, its excellent ADMET and safety profile

justify its prioritization as a low-risk repurposing candidate, particularly for rapid translational evaluation. Similarly, Naringenin demonstrated a favorable balance among acceptable docking performance, high absorption, good bioavailability (0.72), and minimal toxicity, supporting its consideration as a safe adjunct or optimization scaffold.

Protease inhibitors, including simeprevir, atazanavir, nelfinavir, paritaprevir, and saquinavir, generally show strong to moderate docking affinities but are limited by poor gastrointestinal absorption, P-glycoprotein substrate behavior, extensive CYP inhibition, and low bioavailability scores. Among these, paritaprevir was retained as a lead candidate because of its consistently superior docking performance across critical viral targets, with the acknowledgment that pharmacokinetic enhancement is required. Eptifibatide acetate, despite its excellent docking to MXRA8, exhibited extremely poor bioavailability and membrane permeability, restricting its potential to non-oral or targeted delivery strategies.

Toxicity profiling further refined the lead selection. Rimantadine, Ledipasvir, Naringenin, and silymarin derivatives displayed high LD₅₀ values and minimal toxicity concerns, whereas obatoclox showed severe predicted toxicity, including hepatotoxicity and carcinogenicity, leading to its exclusion despite moderate docking performance. Recurrent hepatotoxicity among several protease inhibitors has been recognized as a class-specific limitation and factored into the lead ranking.

Overall, this integrated ADMET–docking prioritization framework highlights Rimantadine and Naringenin as the most balanced candidates combining safety and bioavailability, while Paritaprevir and Ledipasvir were prioritized for their exceptional multi-target docking performance despite their pharmacokinetic drawbacks. This transparent balance between potency and developability strengthens the translational relevance of the proposed lead candidates and provides a clear rationale for future optimization and experimental validation.

Table 5. Pharmacokinetic and drug-likeness properties

Parameter and compound name	GI absorption	BBB permeability	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log-Kp	Ghose	Egan	Bioavailability
Ruxolitinib	High	Yes	No	Yes	Yes	Yes	No	No	-5.53	Passed	Good	0.71
Sorafenib	Low	No	Yes	No	No	Yes	No	Yes	-5.38	Failed	Good	0.35
Ledipasvir	Low	No	Yes	No	No	Yes	No	Yes	-5.12	Failed	Poor	0.17
Rimantadine	High	Yes	No	No	No	No	No	No	-5.92	Passed	Good	0.85
Simeprevir	Low	No	Yes	No	No	Yes	No	Yes	-4.86	Failed	Poor	0.24
Eptifibatide Acetate	Low	No	No	No	No	No	No	No	-10.25	Failed	Poor	0.11
Naringenin	High	No	No	Yes	No	Yes	No	No	-6.14	Passed	Good	0.72
Obatoclax	High	Yes	Yes	No	No	No	No	Yes	-5.04	Failed	Good	0.63
Silibinin B	Low	No	No	No	No	Yes	No	No	-7.93	Failed	Poor	0.30
Silybin A	Low	No	No	No	No	Yes	No	No	-7.89	Failed	Poor	0.31
Silymarin	Low	No	No	No	No	Yes	No	No	-7.91	Failed	Poor	0.30
Atazanavir	Low	No	Yes	No	No	No	Yes	Yes	-6.42	Failed	Poor	0.29
Nelfinavir	High	No	Yes	No	No	Yes	Yes	Yes	-5.63	Failed	Good	0.45
Paritaprevir	Low	No	Yes	No	No	Yes	No	Yes	-5.32	Failed	Poor	0.22

Table 6. Molecular formula and drug-likeness properties

Compounds	Molecular Formula	Molecular weight	HBA	HBD	Log P	TPSA (Å ²)	Number of Rotatable Bonds
Ruxolitinib	C ₁₇ H ₁₈ N ₆	306.36	6	1	1.87	97.98	4
Sorafenib	C ₂₁ H ₁₆ ClF ₃ N ₄ O ₃	464.82	7	3	3.76	92.35	6
Ledipasvir	C ₄₉ H ₅₄ F ₂ N ₈ O ₆	889	14	4	8.41	174.57	13
Rimantadine	C ₁₂ H ₂₁ N	179.3	1	1	3.4	26.02	1
Simeprevir	C ₃₈ H ₄₇ N ₅ O ₇ S ₂	749.94	12	4	4.84	167.81	12
Eptifibatide Acetate	C ₃₅ H ₄₉ N ₁₁ O ₉ S ₂	831.96	20	12	-2.67	286.75	19
Naringenin	C ₁₅ H ₁₂ O ₅	272.25	5	3	2.12	86.99	1
Obatoclax	C ₂₀ H ₁₉ N ₃ O	317.38	4	2	4.92	43.35	4
Silibinin B	C ₂₅ H ₂₂ O ₁₀	482.44	10	5	1.47	155.14	4
Silybin A	C ₂₅ H ₂₂ O ₁₀	482.44	10	5	1.42	155.14	4
Silymarin	C ₂₅ H ₂₂ O ₁₀	482.44	10	5	1.45	155.14	4
Atazanavir	C ₃₈ H ₅₂ N ₆ O ₇	704.85	13	5	4.54	171.22	18
Nelfinavir	C ₃₂ H ₄₅ N ₃ O ₄ S	567.78	7	4	4.72	101.9	11
Paritaprevir	C ₄₀ H ₄₃ N ₇ O ₇ S	765.88	14	3	4.17	189.15	14
Saquinavir	C ₃₈ H ₅₀ N ₆ O ₅	670.84	11	5	3.16	166.75	13

Table 7. Toxicity assessment and nuclear receptor signaling activation

Compound	LD50 value (mg/kg)	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Cytotoxicity	Androgen Receptor (AR)	Aromatase	Estrogen Receptor Alpha (ER)
Ruxolitinib	847.2	No	No	Yes	No	No	No	No
Sorafenib	263.4	Yes	No	No	Yes	No	No	No
Ledipasvir	1000.0	No	No	No	No	No	No	No
Rimantadine	2300.0	No	No	No	No	No	No	No
Simeprevir	750.0	Yes	No	No	No	No	No	No
Eptifibatide Acetate	5000.0	No	No	Yes	No	No	No	No
Naringenin	2000.0	No	No	No	No	No	Yes	Yes
Obatoclox	89.5	Yes	Yes	No	Yes	No	No	No
Silibinin B	5000.0	No	No	No	No	No	Yes	Yes
Silybin A	5000.0	No	No	No	No	No	Yes	Yes
Silymarin	5000.0	No	No	No	No	No	Yes	Yes
Atazanavir	1045.0	Yes	No	No	No	No	No	No
Nelfinavir	1250.0	Yes	No	No	No	No	No	No
Paritaprevir	873.5	Yes	No	No	No	No	No	No
Saquinavir	978.6	Yes	No	No	No	No	No	No

Conclusion

This study systematically evaluated clinically relevant compounds against four key CHIKV-associated targets (SQSTM1, NSP2, MXRA8, and E1/E2), identifying several candidates with strong target-specific and multi-target binding potential. Paritaprevir showed the highest affinity toward the E1/E2 complex; Ledipasvir demonstrated consistently high-affinity binding across E1/E2 and NSP2; Eptifibatide acetate exhibited the strongest interaction with MXRA8; and Sorafenib displayed superior binding to SQSTM1. Docking analyses consistently highlighted critical binding residues across the targets, supporting the structural relevance of the selected sites. The integration of ADMET and toxicity profiling refined lead prioritization by

balancing potency with developability. Although Paritaprevir and Ledipasvir emerged as the most potent multi-target candidates, their pharmacokinetic limitations were clearly identified, indicating the need for formulation or optimization strategies. In contrast, Rimantadine and Naringenin showed favorable safety and bioavailability profiles, supporting their consideration as low-risk candidates for repurposing. Obatoclox was deprioritized because of its predicted toxicity. Overall, the findings provide a focused set of prioritized compounds supported directly by docking and ADMET results, offering a rational and evidence-based foundation for further experimental validation and optimization of Chikungunya antiviral development.

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None.

Ethical Issues

None.

Competing Interests

The authors declared no conflict of interest in this work.

Authors' Contributions

Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; and writing – review and editing by CNU, IMS, KAAR, KAI, and MHS.

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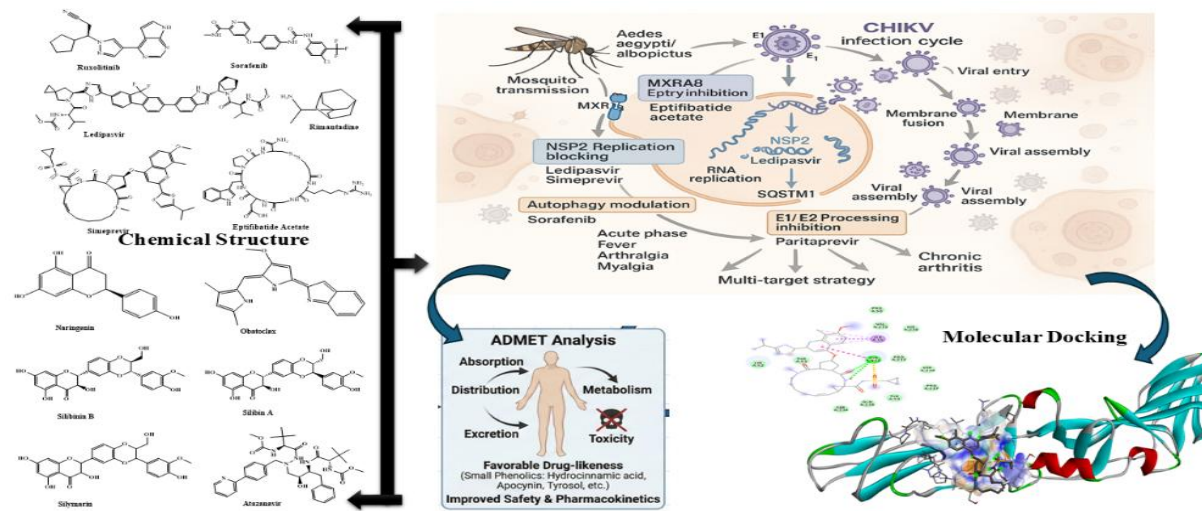
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