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REVIEW

Recent advances in antiviral drugs for Chikungunya virus (CHIKV): Targets, mechanisms, and development strategies

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Abstract Chikungunya virus (CHIKV), an alphavirus transmitted by *Aedes* mosquitoes, has frequently caused outbreaks in tropical and subtropical regions worldwide, posing a significant public health threat. CHIKV infection leads to chikungunya fever, characterized by fever, rash, and persistent joint pain, with approximately 30%−40% of patients developing chronic arthritis that severely impacts quality of life. Currently, no specific antiviral drugs or vaccines against CHIKV have been approved for clinical use, highlighting the urgency of drug development. This review systematically summarizes recent progress in antiviral research on CHIKV, focusing on key target proteins in the viral life cycle, such as non-structural proteins nsP1, nsP2, nsP3, nsP4, and structural protein E1−E2 complexes, as well as the mechanisms of action of their inhibitors. We analyze the current research status of various anti-CHIKV compounds, including suramin, baicalin, halofuginone, betulinic acid, andrographolide, and itraconazole. Additionally, we summarize host-directed antiviral strategies targeting pathways such as host cell oxidative folding, Na^+/K^+-ATPase, and MAPK signaling. This review aims to establish a theoretical foundation and outline potential research directions for the development of CHIKV-related therapeutics, thereby facilitating the discovery of effective treatment strategies against this pathogen.

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1. Introduction

Chikungunya virus (CHIKV), classified within the genus *Alphavirus* of the family Togaviridae, is a single-stranded, positive-sense RNA virus[1]. CHIKV is primarily transmitted by *Aedes* aegypti and *Aedes* albopictus mosquitoes. Since its initial identification in Tanzania in 1952, the virus has caused widespread epidemics across Africa, Asia, Europe, and the Americas[2]. According to the WHO, millions of CHIKV infections occur globally each year, with the 2013−2014 outbreak in the Caribbean region resulting in over one million infections[3,4]. China reported its first imported CHIKV case in 2008. According to the data of the Chinese Center for Disease Control and Prevention, since the CHIKV outbreak in July 2025, a total of 10,000 cases have been reported in China[5]. The cases are mainly in Guangdong Province, and there are no reports of severe cases or deaths.

CHIKV infection primarily presents as an acute febrile illness characterized by rash and severe joint pain. It is estimated that approximately 30%−40% of infected individuals develop chronic arthritis, with symptoms lasting for several months or even extending to years[6,7]. Although the disease is associated with low mortality, prolonged joint dysfunction results in considerable socioeconomic burdens. Importantly, the transmission range of CHIKV is expanding due to global climate change and increased international travel, thereby emerging as a significant global public health threat[8,9].

Despite its significant socioeconomic impact, there are currently no clinically approved specific antiviral therapies for CHIKV. Management primarily relies on supportive care, such as the administration of non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, which are unable to eradicate the virus and demonstrate limited efficacy in treating chronic arthritis[10,11]. Vaccination initiatives have led to the approval of two vaccine candidates: IXCHIQ, a live-attenuated vaccine, and VIMKUNYA, a virus-like particle vaccine. IXCHIQ, the first CHIKV vaccine to receive global approval, was granted FDA authorization in November 2023 but faced partial suspension for individuals over 65 years of age in May 2025 due to reported adverse events[12]. VIMKUNYA, which was approved in February 2025, is currently awaiting further safety evaluation[13,14]. Therefore, the development of safe and effective antiviral drugs against CHIKV remains a critical priority.

Recent advances in the understanding of CHIKV molecular biology and pathogenesis have facilitated significant progress in drug development. This review provides a systematic overview of current research, analyzes the mechanisms of both viral and host targets, and outlines potential strategies for drug discovery. The objective is to offer comprehensive insights that may contribute to the accelerated development of anti-CHIKV therapeutics.

2. CHIKV molecular biology and life cycle

2.1. Viral genome structure and encoded proteins

The CHIKV genome is approximately 11.8 kb in length and encodes two polyprotein precursors: the 5′ non-structural polyprotein (P1234) and the 3′ structural polyprotein (C−E3−E2−6K−E1)[15]. As a single-stranded positive-sense RNA virus, CHIKV contains two open reading frames (ORFs). The 5′ORF encodes the non-structural proteins (nsP1, nsP2, nsP3, and nsP4), which constitute the viral replication complex responsible for genome replication and the synthesis of subgenomic RNA. The 3′ORF encodes the structural proteins, including the capsid (C) and envelope glycoproteins (E3, E2, 6K, and E1), which are involved in virion assembly and release[16].

Non-structural proteins play crucial roles in viral replication and host interaction. nsP1 possesses guanylyltransferase (GTase) and methyltransferase (MTase) activities, which are essential for 5′-cap formation. nsP2 is a multifunctional protein that contains an N-terminal helicase/NTPase domain and a C-terminal cysteine protease domain; it is responsible for polyprotein processing and suppression of host transcription. nsP3 consists of an N-terminal macrodomain, a zinc-binding domain, and a C-terminal hypervariable region, all of which contribute to the assembly of the replication complex and the recruitment of host factors. nsP4 functions as the RNA-dependent RNA polymerase (RdRp) involved in viral RNA synthesis[17,18].

Structural proteins include the E1−E2 heterodimer, which forms trimeric spikes responsible for cell attachment and membrane fusion. Domain A of E2 (E2DA) serves as the primary neutralizing epitope and binds to the host receptor matrix remodeling-associated protein 8 (Mxra8)[19]. The capsid protein C is associated with the viral genomic RNA to form nucleocapsids, whereas the 6K protein plays a facilitating role in viral budding[20].

2.2. Viral life cycle

The CHIKV replication cycle encompasses five primary stages: (1) attachment and entry; (2) genome release and translation; (3) replication complex formation and RNA synthesis; (4) structural protein synthesis; (5) virion assembly, and release (Fig. 1). The detailed process is outlined below.

2.2.1. Viral attachment and entry

Following introduction into the human host *via* an infected mosquito bite, CHIKV initiates infection by attaching to host cells. This attachment is mediated by the viral envelope glycoproteins E1 and E2, which recognize and bind to cell surface molecules including glycosaminoglycans (*e.g.*, heparan sulfate) and specific receptors such as MXRA8, DC-SIGN, and TIM-1[21]. Receptor binding triggers clathrin-mediated endocytosis, internalizing the virion into an endosome[21]. Subsequent acidification of the endosome induces a conformational change in the E1 glycoprotein, facilitating fusion between the viral envelope and the endosomal membrane. This fusion event releases the viral nucleocapsid into the host cell cytoplasm.

2.2.2. Genome release and translation

Upon release into the cytoplasm, the nucleocapsid dissociates and the positive-sense, single-stranded RNA (+ssRNA) viral genome functions directly as mRNA. It is translated by host ribosomes to

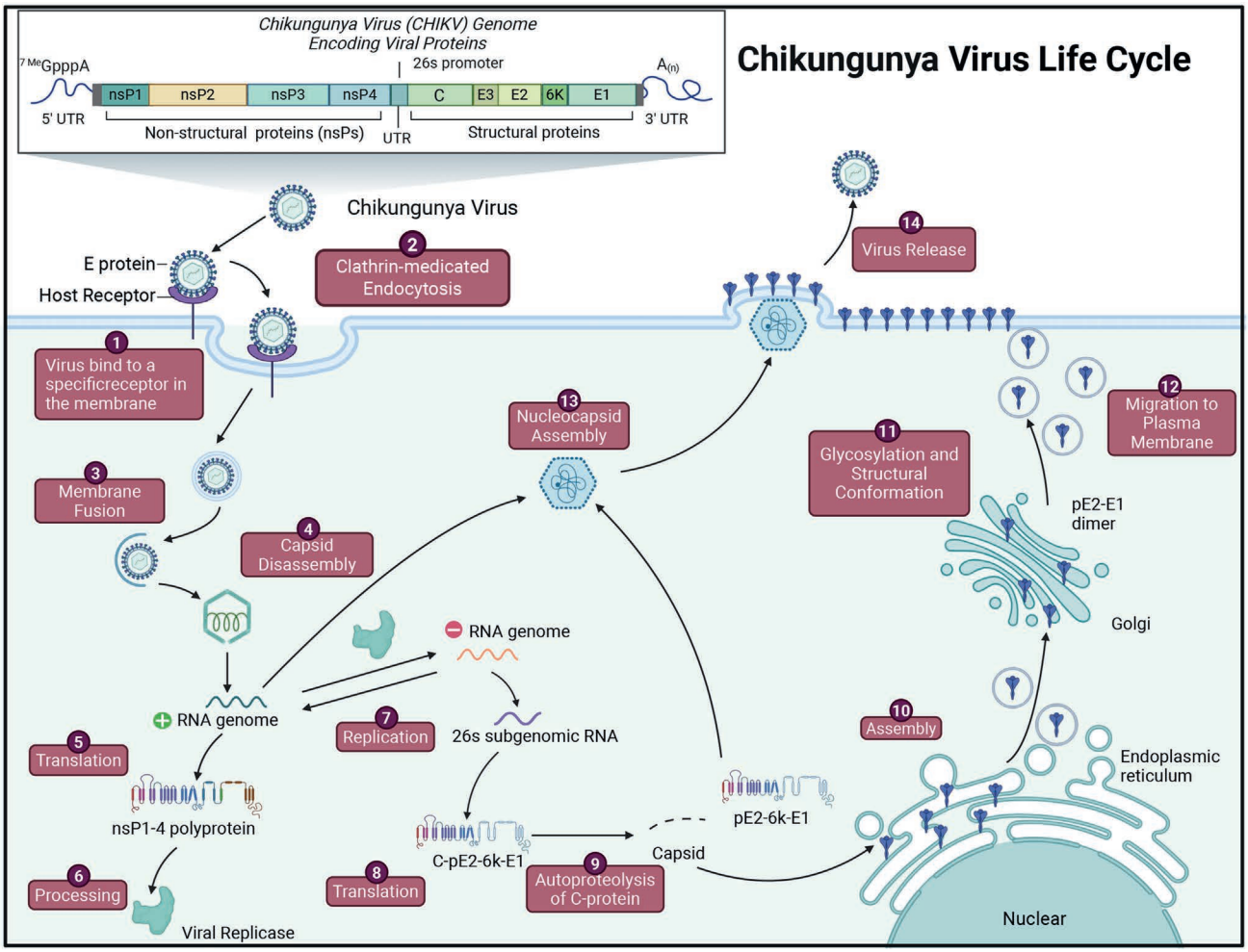

Figure 1 Chikungunya virus life cycle. (Step 1) Viral envelope glycoprotein E (E1/E2): CHIKV surface spikes composed of E1 and E2 glycoproteins. E2 Domain A (E2−DA) mediates attachment to host cell receptors (*e.g.*, MXRA8). (Step 2) CHIKV enters cells through clathrin-mediated endocytosis. (Step 3) Acidification of the vacuolar lumen leads to the exposure of the E1 fusion peptide, facilitating the fusion of viral and endosomal membranes. (Step 4) The viral capsid is released into the cytoplasm, where it undergoes rapid uncoating. (Step 5) The genomic RNA serves as mRNA for translation of the nsP1−nsP4 polyprotein. (Step 6) Viral protease nsP2 cleaves this polyprotein into individual non-structural proteins (nsP1, nsP2, nsP3, nsP4). nsP4 undergoes maturation and subsequently forms a complex with P123 and an RNA template, which is then transported to the plasma membrane. This complex induces structural remodeling of the cell membrane to facilitate the formation of replication organelles, also known as spherules, where a negative-stranded full-length RNA [(−)RNA] is synthesized (Step 6). (Step 7) The coordinated activity of these proteins leads to the formation of the viral replication complex, which facilitates the synthesis of the (−)RNA intermediate—a critical precursor for the generation of both subgenomic (26S) and genomic (49S) RNAs. Once synthesized, the 26S mRNA is translated into C−pE2−6K−E1 polyprotein precursors (Step 8), which are subsequently cleaved by a serine protease to generate the capsid (C) protein (Step9), as well as the pE2 and E1 glycoproteins. (Step 10) Structural proteins are translated from subgenomic RNA as a polyprotein, which is subsequently translocated to the endoplasmic reticulum (ER). (Step 11) C protein is released through autocatalytic processing. The E glycoproteins and the 6K protein are matured by host proteases (Step 11) and subsequently transported to the plasma membrane *via* the secretory pathway (Step 12). (Step 13) The C protein encapsulates the RNA genome to form an icosahedral nucleocapsid structure. (Step 14) The C/E2 interaction facilitates CHIKV particle budding and release.

produce a large non-structural polyprotein precursor (P1234)[22]. This precursor undergoes sequential proteolytic cleavage by the viral protease activity residing within nsP2, resulting in the formation of the mature individual non-structural proteins (nsP1, nsP2, nsP3, nsP4)[22]. nsP1 localizes to specialized plasma membrane microdomains, often lipid rafts, where it possesses guanylyltransferase and methyltransferase activities essential for capping viral RNAs[23].

2.2.3. Replication complex formation and RNA synthesis

The mature nsPs assemble into the viral replication complex (RC) on the cytoplasmic surfaces of modified host membranes, primarily derived from endosomes and lysosomes. These RCs are often organized within membrane invaginations termed "spherules", which provide a protected environment for RNA synthesis and shield double-stranded RNA (dsRNA) replication intermediates from host innate immune sensors[24]. Within the RC,

the full-length genomic RNA serves as a template for the synthesis of a complementary negative-sense RNA strand. This negative-sense strand then acts as the template to produce new positive-sense genomic RNA molecules and a subgenomic positive-sense 26S RNA molecule. The subgenomic RNA is transcribed from an internal promoter on the negative-sense template[24].

2.2.4. Structural protein synthesis and virion assembly

The subgenomic 26S RNA is translated into a structural polyprotein precursor (C−pE2−6K−TF−E1) in the cytoplasm. This polyprotein is co-translationally inserted into the endoplasmic reticulum (ER) lumen[25]. Within the ER, host signal peptidases cleave the capsid protein (C) from the N-terminus. The remaining polyprotein (pE2−6K−TF−E1) undergoes further processing: host proteases cleave pE2 into the mature E3 and E2 glycoproteins. E2 and E1 subsequently form heterodimers (pE2−E1, where pE2 is the E3−E2 precursor)[25,26]. This complex traffics through the Golgi apparatus. In the trans-Golgi network (TGN) or post-Golgi compartments, the E3 glycoprotein (which shields the fusion domain of E1 during transit) is cleaved from pE2 by host furin or furin-like proteases[26]. This cleavage event releases E3 and results in the formation of mature, metastable E2−E1 heterodimers, which assemble into trimeric spikes on the membrane. Concurrently, the cytoplasmic capsid (C) protein packages newly synthesized genomic + ssRNA to form nucleocapsids in the cytoplasm.

2.2.5. Virion assembly, budding, and release

Mature nucleocapsids are transported to sites on the plasma membrane enriched with viral E1/E2 glycoprotein spikes. Assembly involves interactions between the cytoplasmic domain of E2 and the nucleocapsid[27]. Progeny virions bud through these modified plasma membrane regions, acquiring their lipid envelope studded with E1/E2 spikes, and are released from the host cell[27]. Additionally, CHIKV can spread directly to neighboring cells *via* virus-induced intercellular long extensions (ILEs), a mechanism that may facilitate cell-to-cell transmission and potentially evade humoral immunity[28]. Released virions can either infect new cells within the host or be acquired by a feeding mosquito vector, thereby completing the transmission cycle[28].

3. Anti-CHIKV strategies targeting viral proteins

3.1. Inhibitors targeting viral non-structural proteins (nsPs)

Non-structural proteins (nsPs) play essential roles in the formation and function of the viral replication complex, thereby serving as key targets for antiviral drug development[29]. These proteins, which include enzymes and interaction interfaces, are critical for viral RNA synthesis, processing, and protection. Therefore, inhibiting their functions can effectively suppress viral replication within host cells[30] (Fig. 2).

3.1.1. nsP1 methyltransferase/guanylyltransferase: key to viral RNA stability

3.1.1.1. nsP1 protein inhibitors. nsP1 is the only CHIKV non-structural protein associated with the cell membrane and exhibits *N*7-methyltransferase (MTase) and guanylyltransferase (GTase) activities, which are responsible for the formation of the 5′ cap structure (Cap-0, m7GpppN) on viral RNA[31]. This cap structure plays a critical role in protecting viral RNA from degradation by host exonucleases, facilitating efficient translation initiation, and enabling the virus to evade detection by the host's innate immune system. Consequently, inhibition of the capping function of nsP1 represents a promising strategy for blocking viral replication.

3.1.1.1.1. Enzymatic characteristics and inhibition strategies of nsP1. The capping process mediated by nsP1 involves a two-step reaction: First, the GTase domain catalyzes the transfer of the GMP moiety from GTP to His37 on nsP1, forming a covalent intermediate, nsP1-GMP. Subsequently, the MTase domain facilitates the transfer of a methyl group from *S*-adenosylmethionine (SAM) to the N7 position of the GMP, ultimately resulting in the transfer of m7GMP to the 5′ end of the viral RNA[32]. Inhibitors targeting this capping mechanism can be classified into three major categories: (1) compounds that compete for binding at the GTP binding site; (2) analogs of SAM; and (3) molecules that interfere with nsP1−RNA interactions.

3.1.1.1.2. Strategies for the discovery of nsP1 inhibitors. Based on secondary structure prediction and cross-linking studies of recombinant SFV nsP1, the catalytic sites of CHIKV nsP1 are most likely H37 and D63 (Fig. 3). In the existing cryo-EM structure of apo-CHIKV nsP1, the D63 residue-considered the anchor for SAM-is buried too deeply within the protein structure to be accessible to endogenous ligands in docking simulations[33]. Structural analysis indicates that multiple conserved amino acid residues play a crucial role in this state. Among them, the side chain of D138 moves into the hydrogen-bonding interaction distance after binding to SAH, forming a stable contact with the amine group of the SAH base and thereby optimizing the SAH binding mode. Additionally, R92 acts as a bridge between the two substrates: one end interacts with the methionine sulfur atom of SAH, while the other end interacts with the methyl group of m^7GTP, thus stabilizing the methylated product in the active center. In the structural basis of nsP1 oligomerization-dependent activation, residues such as R41 and R70 collectively form an arginine network. This network directly coordinates the phosphate groups of m^7GTP, enabling it to function as a potential allosteric regulatory checkpoint[34].

3.1.1.1.3. Representative nsP1 inhibitors. Lobaric acid: Identified *via* high-throughput screening, lobaric acid competitively binds to the GTP-binding site of nsP1 with an IC_{50} value of 1.3 μmol/L, thereby inhibiting the guanylylation activity of nsP1[31]. In cellular assays, lobaric acid exhibited antiviral activity against both CHIKV and SINV, with EC_{50} values ranging from 5 to 10 μmol/L, and showed low cytotoxicity ($CC_{50} > 100$ μmol/L)[31]. Molecular docking studies revealed that lobaric acid acts as a ligand to recognize and bind to the active pocket of the GTase domain, forming a stable binding mode. Amino acids such as MET-73, LYS-99, and ALA-69 form hydrogen bond interactions with the carboxyl and hydroxyl groups of lobaric acid (Fig. 4). The aromatic structure of lobaric acid forms π−sulfur or π−alkyl interactions with residues such as MET-72. Lobaric acid was enclosed by a hydrophobic pocket formed by amino acids such as MET-73, ALA-69, and ARG-70, occupying the cavity of the GTase active center, indicating that its polyphenol structure and carboxylic acid group play important roles in the binding[35].

5-Iodotubercidin (5-IT): 5-IT is an adenosine analog originally identified as a protein kinase inhibitor. Research has demonstrated that 5-IT inhibits the methyltransferase (MTase) activity of CHIKV nsP1 with an IC_{50} of 0.8 μmol/L and suppresses CHIKV replication at the cellular level with an EC_{50} of 0.5 μmol/L[32]. Mechanistic studies suggest that 5-IT functions as a SAM-competitive inhibitor, interfering with the methyl transfer reaction. Notably, 5-IT also exhibits inhibitory effects against Venezuelan equine encephalitis virus (VEEV), indicating its

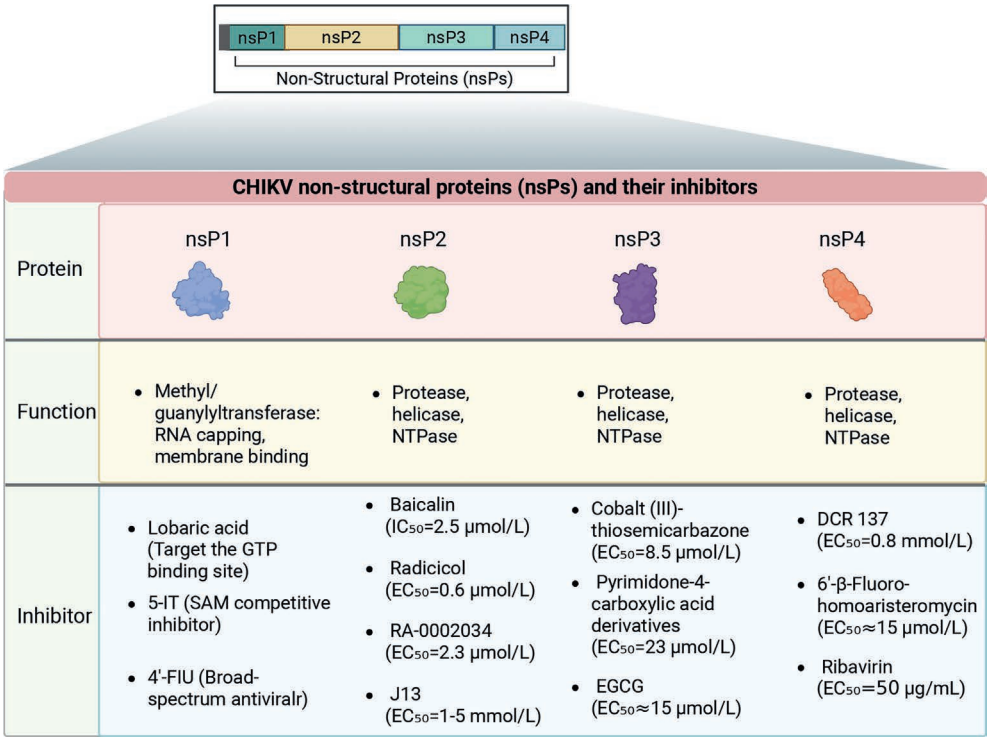

Figure 2 Representative inhibitors targeting CHIKV nondomain nsPs.

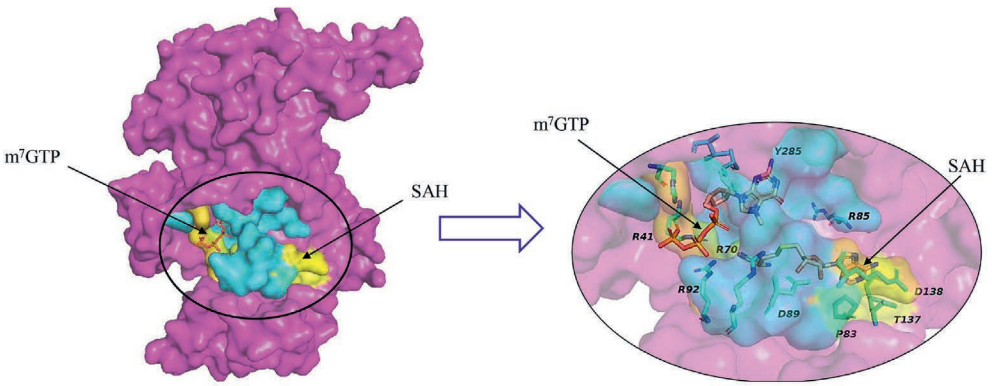

Figure 3 Active site structure of CHIKV nsP1 binding to SAH and m^7GTP in the post-methylated state. Detailed view of the nsP1 active site, showing details of SAH and m^7GTP in different colors for different regions, CHIKV nsP1 in pink, the active cavity in yellow, and the active region on the cavity surface in cyan (PDB#8AOW).

potential as a broad-spectrum antiviral agent against alphaviruses[32].

6′-β-Fluoro-isosorubicin: A carbon-ring nucleoside analogue that can directly inhibit the replication of CHIKV by targeting the MTase activity of nsP1[36]. This compound inhibits the replication of the chikungunya virus at the cellular level (EC_{50} = 0.12 μmol/L) and has resistance against SFV virus (EC_{50} = 4.0 μmol/L)[36]. Unlike traditional nucleoside analogues,6′-β-fluoro-isosorubicin undergoes structural changes, thereby preventing 5′-phosphorylation, and minimizes cytotoxicity (CC_{50} > 100 μmol/L)[37].

FHNA and FHA: 6′-β-Fluoro-homoaristeromycin (FHA) and 6′-fluoro-homoneplanocin A (FHNA) are carbocyclic nucleoside analogs that inhibit the replication of CHIKV and SFV by directly targeting the MTase activity of nsP1[36]. At the cellular level, FHA and FHNA inhibit CHIKV replication with EC_{50} = 0.12 ± 0.04 μmol/L and 0.18 ± 0.11 μmol/L, respectively. Unlike traditional nucleoside analogs, FHA has undergone structural modifications to avoid 5′-phosphorylation, thereby reducing cytotoxicity (CC_{50} > 250 μmol/L) and resulting in a SI > 1000. Additionally, both compounds exhibit potent inhibitory effects on SFV replication, with EC_{50} values of 3.9 ± 3.5 μmol/L (for FHA) and 5.2 ± 3.2 μmol/L (for FHNA), respectively.

3.1.1.1.4. Challenges and outlook. Despite progress in nsP1 inhibitor research, challenges remain: (1) The substrate binding pocket of nsP1 is shallow, making it difficult to design high-affinity small molecules; (2) Membrane-associated nsP1 is not easily accessible to drug molecules; (3) Balancing inhibitory activity with

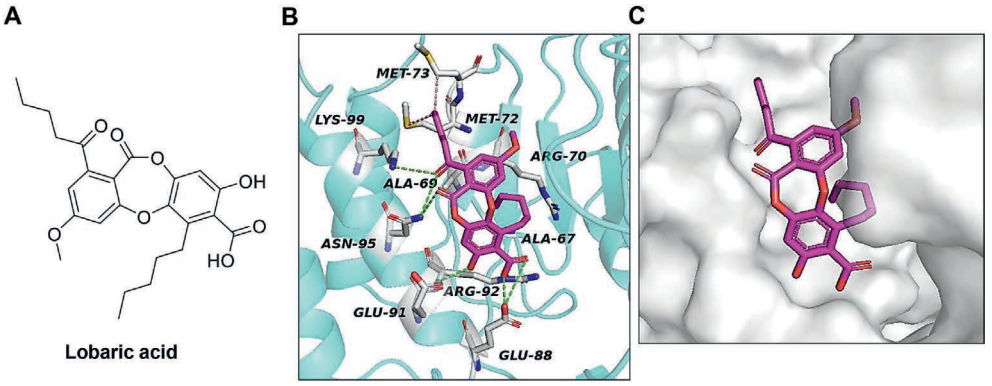

Figure 4 Predicted mode of action of lobaric acid with nsP1. (A) Chemical structure of lobaric acid. (B) The binding mode of lobaric acid to the active center of the GTase domain of nsP1 protein, nsP1 is shown in cyan, key amino acids are shown in a gray ball-and-stick model, lobaric acid is shown in a pink ball-and-stick model, lobaric acid is bound to a hydrophobic pocket formed by residues such as MET-73, ALA-69 and ARG-70, occupying the active cavity of GTase, mimicking the molecular interaction between lobaric acid and substrate. (C) Position of lobaric acid with active cavity, nsP1 is shown in gray (PDB#7DOP).

cellular permeability is required. Future research directions may include: (1) Structure-based rational drug design to optimize existing lead compounds; (2) Developing allosteric inhibitors; (3) Exploring interfaces of nsP1 interaction with other nsPs as targets.

3.1.2. nsP2 protein inhibitors: the important drug target of CKIKV

nsP2 is the largest CHIKV non-structural protein (~90 kDa), featuring an N-terminal helicase/nucleoside triphosphatase domain and a C-terminal cysteine protease domain, playing a central role in polyprotein processing and host defense evasion[38]. The nsP2 protease (nsP2pro) cleaves the P1234 polyprotein precursor, generating functional nsP1, nsP2, nsP3, and nsP4 proteins, which is essential for the correct assembly and function of the replication complex[8,39]. Additionally, nsP2 translocates to the nucleus to shut down host transcription and translation, thereby suppressing the host antiviral response[40]. Its core position in the viral lifecycle and unique enzymatic activity make it one of the most attractive antiviral drug targets[41].

3.1.2.1. Mechanism of action. CHIKV nsP2 is a multifunctional protein. It comprises an N-terminal helicase domain (nsP2h) and a C-terminal cysteine protease domain (nsP2p), which are connected by a 14-residue linker, thereby forming a multi-domain protein with structural flexibility[39]. This structural property allows it to play multiple roles in viral replication.

In terms of enzyme activity inhibition mechanisms, covalent inhibitors block protease activity by targeting catalytic cysteine residues to form covalent bonds. Studies have shown that such inhibitors can specifically modify the cysteine in the catalytic center, leading to conformational changes and functional inactivation of the protease[22]. In contrast, non-covalent inhibitors inhibit enzyme activity by competitively binding to the substrate pocket or regulatory sites. Covalent fragment inhibitors such as RA-0002034 exhibit nanomolar-level inhibitory activity; their inhibition kinetic studies indicate that the kinact/KI value reaches 6.4×10^3 $M^{-1} \cdot s^{-1}$, demonstrating high-efficiency covalent inhibitory capacity[42].

The inhibition of nsP2 helicase activity is primarily achieved by interfering with its nucleoside triphosphatase (NTPase) and RNA triphosphatase (RTPase) activities[43]. Nucleoside analogs can competitively bind to the NTPase site, blocking the ATP-dependent RNA unwinding process[44]; in contrast, decoy RNA oligonucleotides and peptide nucleic acids (PNAs) competitively block the interaction between nsP2 and viral RNA by mimicking viral RNA sequences[45]. These strategies can effectively inhibit viral RNA replication and may indirectly activate the host antiviral response.

In terms of host protein interactions, the interaction between nsP2 and heat shock protein 90β (Hsp90β) is crucial for viral replication[38]. Inhibitors of Hsp90β can block the interaction between nsP2 and Hsp90β, thereby inhibiting the assembly of the viral replication complex. This intervention strategy based on host protein interactions provides a new approach for antiviral therapy.

3.1.2.2. Structure and function of nsP2 protease. nsP2pro is a member of the cysteine protease family and contains a catalytic triad composed of Cys478, His548, and Asn562[46]. Distinct from other viral proteases, nsP2pro exhibits a unique folding pattern, with its active site situated in a cleft between two structural domains. This structural feature offers a specific opportunity for targeted drug design[22,47]. In addition to its proteolytic activity, nsP2 contributes to the host transcriptional shutdown through a nuclear localization signal (NLS), thereby suppressing cellular mRNA synthesis[48].

Inhibitors targeting nsP2pro can be broadly categorized into covalent and non-covalent types. Acrylamide derivatives, as covalent inhibitors, form irreversible covalent bonds with the catalytic cysteine residue in the active site, thereby achieving potent inhibition[22]. Other covalent inhibitors have also been developed, demonstrating strong *in vitro* activity and effective antiviral effects at the cellular level[23].

Among non-covalent inhibitors, compound J13 has been identified as a potent inhibitor of nsP2pro, significantly inhibiting viral replication[49]. Another compound, RA-0002034, also exhibits strong anti-CHIKV activity by targeting nsP2pro[42,50,51]. Moreover, ribostamycin, an aminoglycoside antibiotic, was found to competitively inhibit nsP2pro activity, suggesting potential for drug repurposing[52]. Natural products represent another valuable source of nsP2pro inhibitors. Researchers have isolated and identified several inhibitory compounds from plant extracts, including baicalin[6].

RNA helicases are a diverse class of ATP-dependent motor proteins that play essential roles in nearly every aspect of RNA metabolism, including transcription, splicing, translation, RNA transport, and decay. The N-terminal helicase domain of nsP2 is responsible for unwinding the double-stranded RNA (dsRNA) intermediates formed during viral RNA replication, thereby providing single-stranded templates for the movement of RNA polymerase. Alphaviruses encode an *SF1B* RNA helicase within the N-terminal domain of nonstructural protein 2 (nsP2hel) that is required for the synthesis of viral RNA. In addition to the helicase domain, nsP2 also contains a cysteine protease domain, which is connected to a C-terminal methyltransferase-like (MTL) domain. The alphavirus nsP2hel is a dynamic nonprocessive motor enzyme that detaches and rebinds as it unwinds the RNA. Recent studies suggest that the C-terminal MTL domain may stabilize the RNA−nsP2 interaction and is therefore required for efficient helicase unwinding activity. Although research on inhibitors targeting the helicase domain is relatively limited compared to that on the protease domain, its potential cannot be overlooked. Recent studies have begun to develop specific molecular probes for investigating and targeting the function of the nsP2 helicase, which lays a foundation for the screening and design of inhibitors against this domain[53]. The oxaspiropiperidine 1 was recently reported as a first-in-class nsP2hel inhibitor with broad-spectrum antialphaviral activity[54]. However, despite the increasing structural insights gained from X-ray crystallography and cryo-electron microscopy (cryoEM) studies, the development of selective small-molecule inhibitors for this dynamic and conformationally flexible enzyme has proven challenging. Furthermore, there are few chemical tools available to probe the function of nsP2hel or enable mechanistic studies of its activity.

3.1.2.3. Strategies for the discovery of nsP2 inhibitor. In the crystal structure of CHIKV, the active center of nsP2pro consists of a classical catalytic triad, comprising Cys478, Asn562, and His548 (Fig. 5). The key residue His548 is located on a flexible loop of the N-terminal protease subdomain, which lies between the β1 and β2 β-sheets. Notably, the loop connecting the catalytic residues, the β7 strand of the MTase-like domain, and the α9 helix functions to close the channel leading to the active site-thus acting like a "gate" for substrate entry. This loop also contains two conserved residues that are critical for substrate recognition: Trp549 and Asn547. Specifically, Trp549 has been identified as part of the glycine-specific motif, responsible for recognizing the glycine residue at the P2 position of the substrate. In contrast, Asn547 directly participates in substrate binding by forming a hydrogen bond network with the substrate peptide segment *via* its sidechain and main-chain atoms. Therefore, based on the conformational variability of the loop containing this residue in the CHIKV nsP2pro structure, Asn547 likely plays a central role in regulating the entry of substrates into the active site[47].

3.1.2.4. Representative nsP2 inhibitors. Radicicol: Radicicol is a macrolide antibiotic that exerts anti-CHIKV activity by targeting the nsP2 protein, with an EC_{50} of 0.6 μmol/L[38]. Mechanistic studies have shown that Radicicol binds to the helicase domain of nsP2, thereby inhibiting its ATPase activity and subsequently blocking viral RNA unwinding and replication. Importantly, Radicicol does not directly affect the protease activity of nsP2, suggesting that it specifically targets the helicase function[38]. Radicicol, functioning as an nsP2 inhibitor, recognizes and binds to the active pocket of nsP2-with CYS-478 and HIS-548 as the key residues-forming a stable binding conformation. ASN-547, CYS-478, and ASN-667 form hydrogen-bonding interactions with the ketone and hydroxyl groups of Radicicol (Fig. 6). The benzene ring of Radicicol establishes $\pi-\pi$ interactions with residues such as TRP-479. Additionally, the structure of radicicol is enclosed by a hydrophobic pocket formed by amino acids like CYS-478, ASN-547, and ASN-667, occupying the cavity of the active site. These observations indicate that the macrocyclic lactone structure and electrophilic ketone group of Radicicol play critical roles in the binding process[47].

J12 and J13: Identified through structure-based design, these compounds are potent nsP2pro inhibitors capable of suppressing CHIKV replication (EC_{50} = 1−5 μmol/L)[49]. Pharmacokinetic studies indicate that J13 possesses excellent oral bioavailability

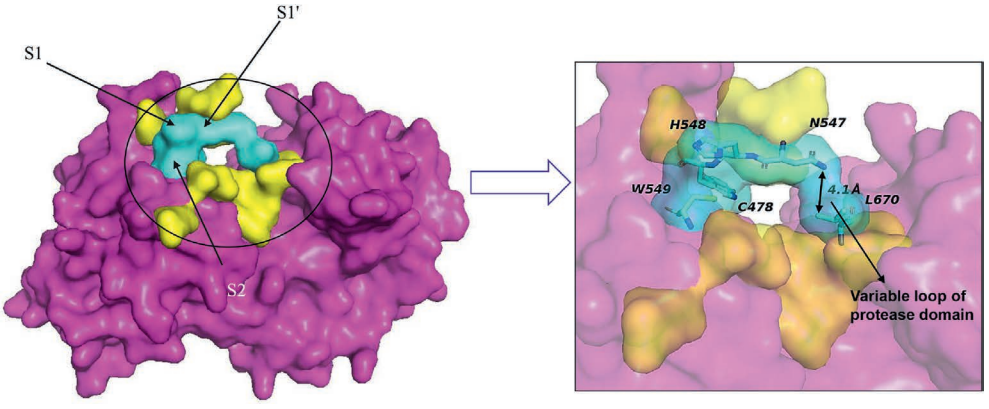

Figure 5 Schematic representation of the structure of the nsP2 protease active center and the substrate binding region. Detailed view of nsP2 subsites, with different regions indicated in different colors. The nsP2 protein is shown in pink, and the overall spatial structure of nsP2 protease is shown on the left. The S1, S1′, and S2 regions indicated by arrows in cyan are key subsites for protease interaction with substrate peptides. The Variable loop of protease domain located by N547 and L670 in the N-terminal protease domain was clearly observed. And the active cavity of the classical catalytic body consisting of Cys478, and His548, the cavity containing regions are shown in yellow[47] (PDB#4ZTB).

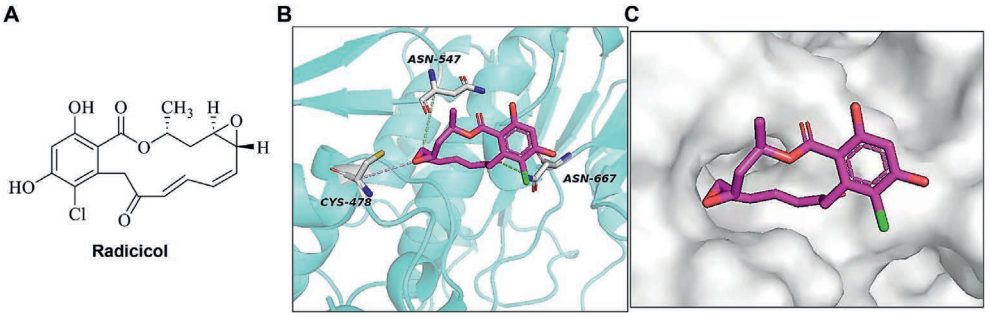

Figure 6 Predicted mode of action of radicicol with nsP2. (A) Chemical structure of Radicicol. (B) The binding mode of radicicol to the active pocket of nsP2 protein, nsP2 is shown in cyan, key amino acids are shown in a gray ball-and-stick model, radicicol is shown in a pink ball-and-stick model, radicicol binds to the active pocket formed by residues CYS-478 and HIS-548 to mimic the molecular interaction between radicicol and its substrate. (C) The location of radicicol with the active cavity, nsP2 is indicated in gray (PDB#4ZTB).

(>80%) and significantly alleviates symptoms of CHIKV infection in mouse models. Safety assessments demonstrated that J13 exhibited favorable results in *in vitro* safety pharmacology and off-target screening, suggesting its strong potential as a promising drug candidate[49].

Acc-CHIK15-dns: Based on the 15-peptide structure of nsP3/4 site, a novel class of fluorescent substrates, designated as acc-CHIK15-dns, was synthesized. Subsequent high-throughput screening using this substrate led to the identification of multiple active small-molecule inhibitors. Mechanistically, these inhibitors exert their antiviral effects by blocking the enzymatic activity of CHIK protease(nsP2), which in turn inhibits the cleavage of viral polyproteins. *In vitro* enzyme-substrate mimicry assays demonstrated that the IC_{50} of these inhibitors ranged from 1.2 to 10.6 μmol/L[55].

Oxasipiperidine derivative: Compound **2O** is an oxasipiperidine compound. Its mechanism of exerting antiviral effects is binding to the allosteric site of the nsP2hel, thereby interfering with the coupling process between ATP hydrolysis and RNA unwinding, and ultimately blocking the replication of viral RNA. Its cell experiments, the compound **2O** with EC_{50} = 130 nmol/L, while the EC_{50} for reducing the viral titer is 1−3 μmol/L; It is notable that it can reduce the viral titter by 10^3−10^8 times. Moreover, compound **2O** also shows inhibitory activity against MAYV and VEEV. In conclusion, these findings indicate that compound **2O** has broad-spectrum antiviral activity[41]. In another study, the (*R*)-enantiomer of oxaspiropiperidine1 (R-1) and (*S*)-enantiomer of oxaspiropiperidine1 (S-1) were isolated from Compound **2O** and performed pharmacological activity studies[54]. And FRET assay showed that (R)-1 exhibited an IC_{50} value of 130 nm for RNA helicase inhibition and was 200-fold more potent than (S)-1 (IC_{50} = 26 μmol/L). Against the Venezuelan equine encephalitis virus (VEEV, an alphavirus), (R)-1 demonstrated potent activity, whereas the (S)-1 enantiomer was inactive. Notably, (R)-1 showed no inhibitory effect on DENV or WNV.

RA-0002034: RA-0002034 is a covalent inhibitor that exerts its antiviral activity by specifically targeting and inactivating the catalytic cysteine residue of nsP2pro *via* its internal vinyl sulfone group. In CHIKV replication assays, RA-0002034 effectively inhibited viral replication, with EC_{50} = 160 nmol/L. Following oral administration, the compound significantly reduced CHIKV viral load in multiple tissue[56]. As a ligand, RA-0002034 recognizes and binds to the active pocket where the catalytic residues CYS-478 and HIS-548 are located, forming a specific binding mode (Fig. 7). MET-703, TYR-512, and TRP-479 form hydrogen-bonding interactions with the polar groups of RA-0002034. The aromatic structure of RA-0002034 establishes $\pi-\pi$ stacking interactions with TYR-544 and TRP-549. In addition, the structure of RA-0002034 is enclosed by a hydrophobic pocket formed by amino acids including MET-703, TYR-512, and TRP-479, occupying the core position of the binding cavity. These findings indicate that the polycyclic structure and polar side chains of RA-0002034 play important roles in the recognition and binding process[47].

3.1.2.5. Existing challenges. The development of nsP2 inhibitors faces multiple challenges. Target complexity is one of the most significant obstacles: as a multifunctional protein, nsP2 contains intrinsically disordered regions (IDRs) that lack a well-defined tertiary structure, rendering structure-based drug design extremely difficult[57]. Additionally, the synergistic interactions between different domains and dynamic conformational changes further increase the difficulty of achieving selective inhibition.

Selectivity represents another major challenge. nsP2 shares structural similarities with other host cysteine proteases; insufficient selectivity can lead to off-target effects and adverse reactions. The experimental methods themselves may also introduce biases-for instance, fluorescent labeling can significantly impair the accuracy of measurements for binding affinity and selectivity. A recent study identified a novel chemical probe, SGC-NSP2PRO-1, which exhibits over 30-fold higher selectivity for CHIKV nsP2pro compared to other cysteine proteases[57].

Drug resistance is a particularly prominent issue. Viruses develop resistance through gene mutations, which may occur in key regions of nsP2 and impair inhibitor binding. Studies have shown that drug-resistant mutations are often not isolated; instead, they involve the synergistic effects of multiple genes. For example, in CHIKV-resistant variants, combined mutations in nsP1, nsP2, and nsP3 collectively result in an approximately 18-fold increase in drug resistance[58]. Furthermore, the phenomenon of cross-resistance also limits the application prospects of single inhibitors.

3.1.2.6. Clinical translation potential. Preclinical studies have laid a crucial foundation for the development of nsP2 inhibitors. *In vitro* models, these inhibitors typically exhibit antiviral activity in the nanomolar to micromolar range, with a high selectivity index. *In vivo* studies have demonstrated that, in animal models

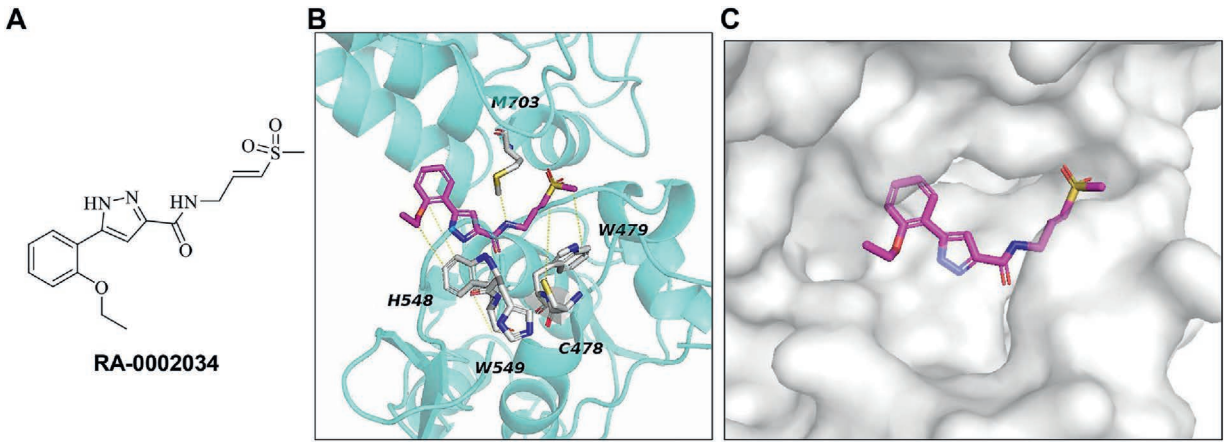

Figure 7 Predicted mode of action of RA-0002034 with nsP2. (A) Chemical structure of RA-0002034. (B) The binding mode of RA-0002034 to the active pocket of nsP2 protein, nsP2 is shown in cyan, key amino acids are shown in a gray ball-and-stick model, and RA-0002034 is shown in a pink ball-and-stick model. Radicicol binds to the active pocket formed by residues such as CYS-478 and HIS-548 and forms a specific binding mode to mimic the molecular interaction of RA-0002034 with its substrate by Pymol. (C) The position of RA-0002034 with the active cavity, nsP2 is shown in gray (PDB#4ZTB).

such as mice and hamsters, nsP2 inhibitors can significantly reduce viral load and alleviate joint inflammation as well as histopathological damage[59].

Innovations in drug delivery strategies have provided new perspectives for clinical translation. Nanoparticle delivery systems enable the precise release of drugs in specific tissues, extend circulation time, and improve bioavailability. Programmable multi-stage drug delivery systems are particularly suitable for antiviral drugs that require targeting of lymphoid tissues[60]. Prodrug design, on the other hand, can enhance drug solubility, stability, and membrane permeability, thereby significantly improving oral bioavailability.

The combination therapy strategy is critical for addressing drug resistance and improving therapeutic efficacy. Multi-target combination therapy can act synergistically through multiple mechanisms, significantly reducing the risk of drug resistance development. When combined with other inhibitors that target different viral proteins, it can simultaneously inhibit multiple stages of the viral replication cycle[61].

In summary, although CHIKV nsP2 inhibitors face multiple challenges, they still hold broad prospects for clinical translation through in-depth understanding of their mechanisms of action, optimization of drug design, innovation in delivery strategies, and adoption of combination therapy models. These advances are expected to provide an effective therapeutic option for CHIKV infections.

3.1.3. nsP3 protein inhibitors

nsP3 functions as a scaffold protein for the CHIKV replication complex and is composed of three primary domains: the N-terminal macrodomain (nsP3MD), a zinc-binding domain, and a C-terminal proline-rich hypervariable region[62]. nsP3MD exhibits ADP-ribosylhydrolase activity and plays a role in viral replication as well as in the evasion of the host's innate immune response, making it a promising new target for the development of anti-CHIKV therapeutics.

3.1.3.1. Function and inhibition strategies targeting nsP3. Strategies targeting nsP3 primarily aim to interfere with its essential protein-protein interactions or inhibit the enzymatic activity of its macrodomain. The nsP3 macrodomain (nsP3MD) can remove ADP-ribosylation modifications, thereby counteracting the antiviral effects mediated by host PARP proteins[62]. Research has demonstrated that CHIKV mutants lacking nsP3MD exhibit significantly diminished replication capacity, highlighting the critical role of this domain in viral pathogenesis[63]. Current approaches in inhibitor development for nsP3MD mainly focus on: (1) competing for the ADP-ribose binding pocket, and (2) disrupting interactions between nsP3 and other viral or host proteins.

Several small-molecule inhibitors targeting the nsP3 macrodomain have been reported[37]. Notably, strategies aimed at disrupting the nsP3−G3BP interaction have garnered increasing attention. Through high-throughput screening, a G3BP1-binding small molecule was identified, which effectively inhibits the interaction between nsP3 and G3BP. This disruption leads to suppressed replication of CHIKV and other viruses reliant on this interaction, demonstrating broad-spectrum antiviral potential[64].

3.1.3.2. Strategies for the discovery of nsP3 inhibitor. The ADP-ribose binding pocket of the CHIKV nsP3MD can be divided into three functional sub-sites: the adenine-binding site (Ad-site), the phosphate-binding site (Ph-site), and the distal ribose-binding site (Rib-site) (Fig. 8). This macrodomain is a highly conserved functional region with ADP-ribose binding and hydrolysis activities, which are crucial for viral replication and virulence. Asp-10, Gly-32, Val-33, and Arg-144 are directly involved in the specific recognition of the adenine base. The main-chain amino groups of Ser-110, Gly-112, and Tyr-114 form hydrogen bonds with the phosphate groups of ADP-ribose. The phenol ring of Tyr-114 undergoes $\pi-\pi$ stacking with the aromatic ring in the ligand, enhancing binding affinity. Additionally, Asn-24 and Asp-31 form hydrogen bonds with the hydroxyl groups of the distal ribose, further stabilizing the ligand orientation[64].

3.1.3.3. Representative nsP3 inhibitors. Pyrimidone-4-carboxylic acid derivatives: Identified through fragment-based screening, these compounds bind to the ADP-ribose binding site[64]. Among them, 2-oxo-5,6-benzoquinazoline-4-carboxylic acid displays the strongest anti-CHIKV activity

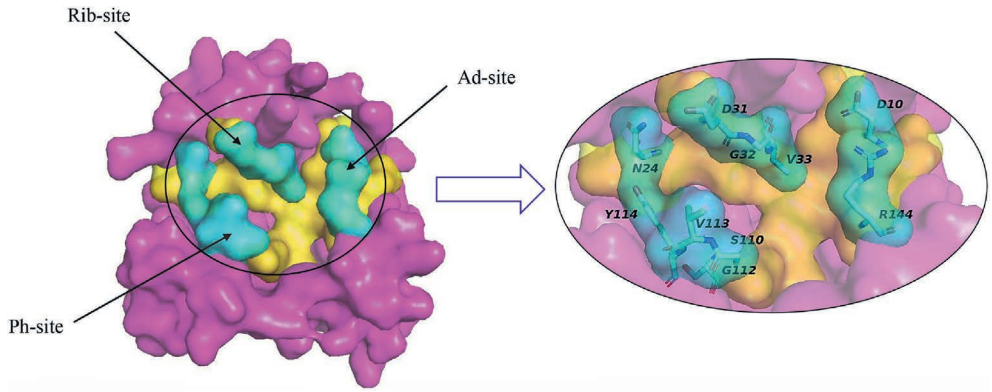

Figure 8 Subsites and key amino acids of CHIKV nsP3MD. A detailed view of the nsP3 subsite, with different regions in different colors, the adenine-binding site, phosphate-binding site, and distal ribose-binding site are shown in cyan. CHIKV nsP3 is shown in pink and the active cavity in yellow (PDB#4ZTB).

(IC_{50} = 23 μmol/L). Crystal structure analysis reveals that pyrimidone acts as an inhibitor to recognize and bind to the active pocket where the catalytic residues CYS-478 and HIS-548 are located and forms covalent or noncovalent binding modes (Fig. 9). Amino acids such as HIS-548, CYS-478, and LYS-481 form hydrogen-bonding interactions with the heterocyclic ring and polar groups of pyrimidone. The aromatic system of pyrimidone establishes $\pi-\pi$ stacking or $\pi-$alkyl interactions with residues like TRP-479. Additionally, the structure of pyrimidone is enclosed by a hydrophobic pocket formed by amino acids including CYS-478, HIS-548, and LYS-481, occupying the cavity of the catalytic center. These observations indicate that the heterocyclic structure and hydrogen bond donors/acceptors of pyrimidone play a central role in its inhibitory mechanism[64]. Interestingly, these compounds may also inhibit the macrodomains of other alphaviruses and coronaviruses, suggesting potential for broad-spectrum antiviral applications.

Baicalin: Baicalin is a flavonoid compound extracted from *Scutellaria baicalensis*, AutoDock Vina predicted a binding affinity of −9.8 kcal/mol for nsP3, suggesting that it is expected to be a potentially efficient candidate against chikungunya virus[65]. In cellular assays, Baicalin demonstrated significant anti-CHIKV activity ($EC_{50} \approx 7$ μmol/L) and effectively inhibited various CHIKV isolates[6]. Time-of-addition experiments suggest that Baicalin primarily interferes with the viral replication phase rather than the entry phase. Notably, baicalin also exhibits inhibitory effects against Dengue virus (DENV) and Zika virus (ZIKV), indicating its potential as a broad-spectrum antiviral agent, and nsP3 docking binding affinity = −9.8 kcal/mol[65]. As a substrate, baicalin recognizes and binds to the intrinsic substrate-binding

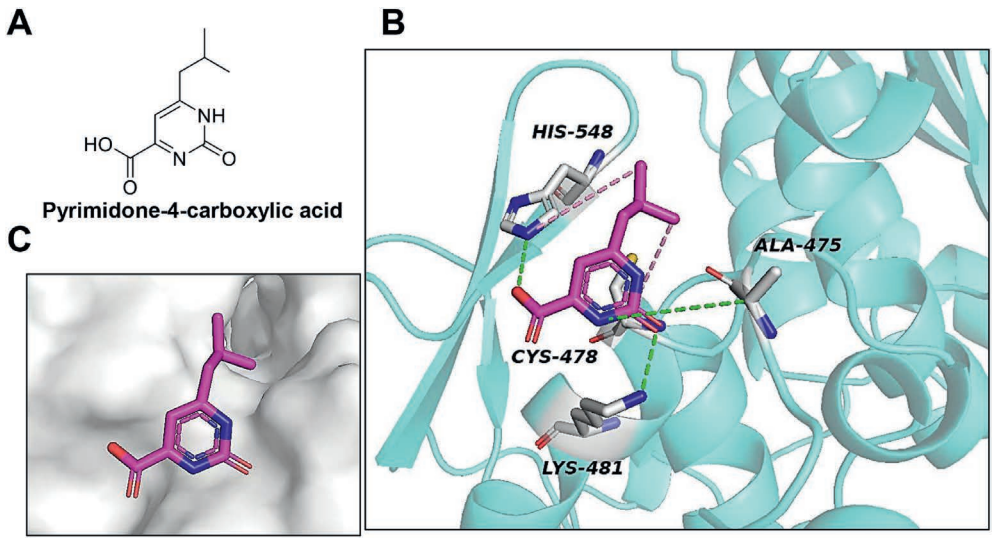

Figure 9 Predicted mode of action of pyrimidone-4-carboxylic acid with nsP3. Chemical structure of (A) pyrimidone-4-carboxylic acid. (B) The binding mode of pyrimidone-4-carboxylic acid to the active pocket in nsP2 protein, nsP2 is shown in cyan, the key amino acid is shown in a gray ball-and-stick model, and pyrimidone-4-carboxylic acid is shown in a pink ball-and-stick model, pyrimidone-4-carboxylic acid is bound to the active cavity formed by residues such as CYS-478 and HIS-548, and the molecular interaction of pyrimidone-4-carboxylic acid with the substrate is simulated by Pymol. Position of (C) pyrimidone-4-carboxylic acid with the active cavity, nsP2 is shown in off-white (PDB#4ZTB).

pocket of the nsP3 macrodomain, forming a stable binding conformation (Fig. 10). Amino acids such as LYS-13, LYS-35, LYS-39, and GLY-37 form relatively stable hydrogen-bonding interactions with the hydroxyl and carboxyl groups of baicalin. The benzene rings on baicalin establish π−alkyl interactions with the hydrophobic side chains of residues like LYS-35. Additionally, the structure of baicalin is enclosed by a hydrophobic pocket formed by amino acids including LYS-13, LYS-35, and GLY-37, occupying the central cavity of the binding pocket. These findings indicate that the phenolic hydroxyl groups and glucuronic acid moiety of baicalin make important contributions to substrate recognition and binding[64].

EGCG (Epigallocatechin gallate): The primary polyphenolic constituent of green tea, recently reported to inhibit CHIKV nsP2. Molecular docking suggests that as a polyphenolic ligand, EGCG recognizes and binds to the active pocket where the catalytic residues CYS-478 and HIS-548 are located, forming an extensive hydrogen bond network (Fig. 11). Amino acids including ALA-475, HIS-548, CYS-479, and LYS-481 form multiple hydrogen-bonding interactions with the phenolic hydroxyl groups and ester bonds of EGCG. The benzene rings of EGCG establish π−alkyl or $\pi-\pi$ interactions with residues such as ASN-521. Additionally, the structure of EGCG is enclosed by a hydrophobic pocket formed by amino acids like HIS-548, CYS-479, and LYS-481, occupying the cavity of the active center. These observations indicate that the galloyl group and catechol structure of EGCG play a dominant role in the binding process [66].

3.1.3.4. Challenges and outlook. The challenges in nsP3 inhibitor development lie in: (1) The substrate binding pocket of nsP3MD is shallow, making affinity optimization difficult; (2) nsP3 interacts with multiple host proteins, making its function complex; (3) Lack of highly sensitive enzymatic activity detection methods. Future research directions may include: (1) Developing allosteric inhibitors; (2) Designing compounds targeting other functional domains of nsP3 (*e.g.*, zinc-binding domain); (3) Exploring the role of nsP3 in virion assembly and developing corresponding inhibitors.

3.1.4. nsP4 RNA-dependent RNA polymerase (RdRp): the core engine of RNA synthesis

nsP4 of CHIKV is a critical member of the viral non-structural protein family. As an RNA-dependent RNA polymerase (RdRp), it plays a central role in the viral replication process. First, it is responsible for the replication of the 49S genomic positive-strand

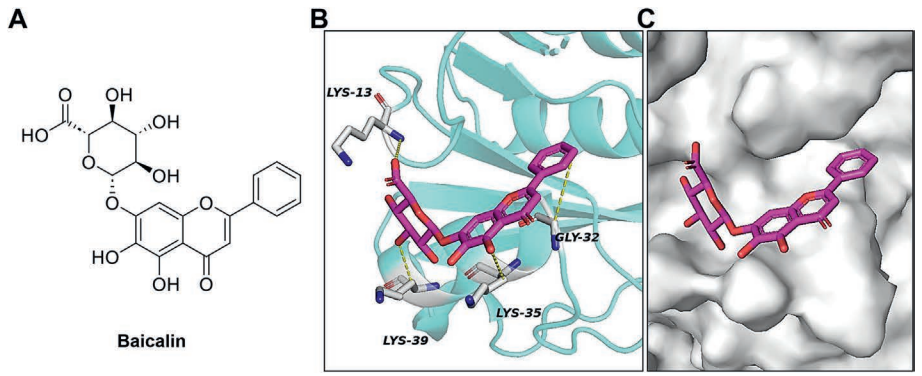

Figure 10 Predicted mode of action of Baicalin with nsP3. (A) Structure of baicalin (B) Binding mode of baicalin to the active pocket in nsP3 protein, nsP3 is shown in cyan, key amino acids are shown in a gray ball-and-stick model, baicalin is shown in a pink ball-and-stick model, baicalin is bound to a hydrophobic pocket formed by amino acids such as LYS-13, LYS-35 and GLY-37, and the molecular interaction of baicalin with the substrate was simulated by Pymol. (C) The position of Baicalin with the active cavity, nsP3 is indicated in gray (PDB#6W0T).

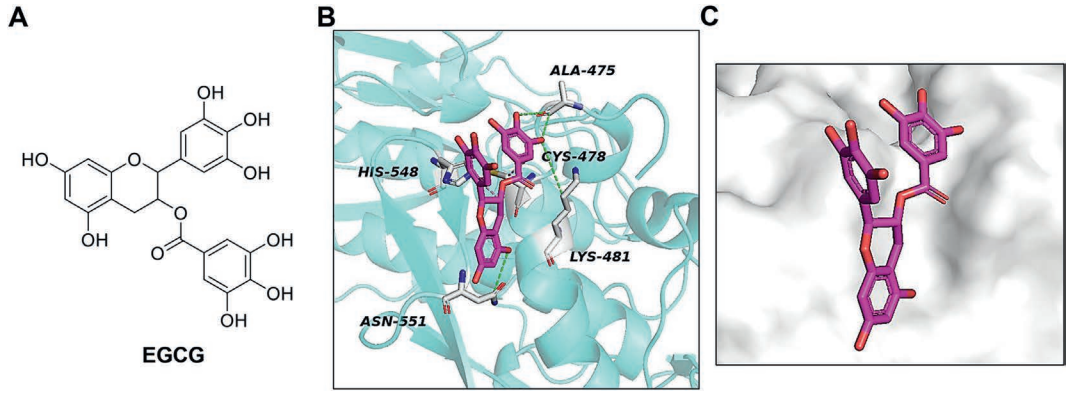

Figure 11 Predicted mode of action of EGCG with nsP3. (A) Chemical structure of EGCG. (B) The binding mode of EGCG to the active pocket of nsP2 protein, nsP2 is shown in cyan, the key amino acids are shown in a gray ball-and-stick model, and EGCG is shown in a pink ball-and-stick model. EGCG binds to the hydrophobic pocket formed by amino acids CYS-478 and HIS-548. Pymol was used to simulate the molecular interaction of EGCG with the substrate. (C) Location of EGCG with active cavity, nsP2 is indicated in off-white (PDB#4ZTB).

RNA (+ssRNA) and the transcription of the 26S subgenomic RNA (sgRNA)[67]. During the early stage of viral replication, nsP4 assembles into the early replication complex (RC) together with the incompletely processed precursor protein P123, which synthesizes the full-length negative-strand RNA intermediate. This negative-strand RNA then serves as a template for the synthesis of new positive-strand genomic RNA and subgenomic RNA[68]. Following the completion of polyprotein processing, the late RC—composed of fully cleaved nsP1 to nsP4—takes charge of synthesizing genomic RNA and 26S sgRNA[67]. Additionally, nsP4 possesses terminal adenylyl transferase (TATase) activity, which catalyzes the addition of a polyadenylate (polyA) tail to the 3′ end of the viral genomic RNA; this process is vital for maintaining the stability of viral RNA and ensuring efficient translation.

3.1.4.1. Structural characteristics of CHIKV nsP4. As a key enzyme in the CHIKV replication cycle, non-structural protein 4 (nsP4) exerts a core function in viral genome replication and transcription by acting as an RNA-dependent RNA polymerase (RdRp)[68,69]. As the most conserved protein in the Alphavirus genus, nsP4 consists of approximately 611 amino acids, which includes a C-terminal RdRp domain-this domain is critical for the replication and transcription of genomic RNA. Notably, nsP4 contains a unique GDD motif, a signature sequence of the RdRp catalytic core, and the functional integrity of this motif is indispensable for viral RNA synthesis[70].

nsP4 possesses a typical "right-hand" RNA-dependent RNA polymerase (RdRp) structure, consisting of finger, palm, and thumb domains, and contains the conserved GDD motif that plays a key role in the catalytic process[17]. Unlike RdRps of many other positive-sense RNA viruses, alphavirus nsP4 is inherently unstable and requires the assembly of a replication complex with other non-structural proteins (nsP1−nsP3) to maintain its RNA synthesis activity[23].

3.1.4.2. Mechanism of action of nsP4 inhibitors. Unlike other positive-strand RNA viruses, the alphavirus nsP4 is inherently unstable on its own. It requires association with other non-structural proteins (nsP1−3) to form a replication complex (RC) and thereby gain sustained RNA synthesis capability[71]. This unique structural characteristic provides multiple strategies for developing different types of inhibitors.

Competitive inhibitors primarily block the RNA synthesis process by mimicking natural substrates and binding to the active site of nsP4. Nucleoside analogs are typical representatives of such inhibitors; for instance, favipiravir (T-705) has been shown to exhibit inhibitory activity against CHIKV[72]. Structurally like natural nucleotides, these inhibitors can be recognized by nsP4 and incorporated into the nascent RNA chain, but they lack the ability for further extension, thereby terminating RNA synthesis. 4′-FlU is another potent nucleoside analog that can inhibit the RNA replication stage of multiple alphaviruses, including CHIKV[73,74]. Notably, drug resistance studies have revealed that the Q192L and C483Y mutations in nsP4 can reduce sensitivity to 4′-FlU. However, these variants can still be inhibited by higher concentrations of the drug, and the Q192L variant shows significant attenuation in mouse models-findings that support the clinical development value of this class of inhibitors[73].

Allosteric inhibitors bind to the allosteric sites of nsP4 and induce conformational changes in the enzyme, thereby inhibiting its activity. A key advantage of these inhibitors lies in their potential for higher specificity, as allosteric sites are generally more structurally diverse than active sites. Currently, research on allosteric inhibitors targeting CHIKV nsP4 is still ongoing; however, structural biology studies have revealed that the RdRp of CHIKV shares a certain degree of structural similarity with the RdRps of other arthropod-borne RNA viruses (*e.g.*, dengue virus and Zika virus). This finding provides a theoretical basis for the development of broad-spectrum RdRp inhibitors capable of combating multiple viruses simultaneously[75].

3.1.4.3. Representative nsP4 inhibitors. Cobalt(III)-thiosemicarbazone complex ([Co(III) (L1)2]Cl): A metal-organic compound that specifically targets nsP4, MST assays demonstrated a strong interaction between [CoIII(L1)2]Cl and CHIKV nsP4, and this binding affinity was further validated using molecular docking simulations[62]. Importantly, [CoIII(L1)2]Cl exhibited potent antiviral activity in cellular models, achieving an 80% inhibition rate against CHIKV replication[62]. Notably, the compound exhibits low cytotoxicity to host cells ($CC_{50} > 200$ μmol/L) and has good selectivity[62].

LabMol-309: The compound LabMol-309 has been identified as a potent ligand for CHIKV nsP4 and was found to bind to the active site of this protein. In BHK-21 cells infected with CHIKV-nanoluc, LabMol-309 exhibited $EC_{50} = 5.2$ μmol/L. In naive BHK-21 cells, it showed $CC_{50} = 52$ μmol/L. Over a 48-h incubation period, these values resulted in a selectivity index (SI) of 10[69].

4′-Fluorouridine (4′-FlU): 4′-FlU is a nucleoside analog recently reported to possess broad-spectrum antiviral activity against multiple RNA viruses, including SARS-CoV-2, respiratory syncytial virus, and influenza virus[76]. In CHIKV infection models, 4′-FlU primarily targets the nsP4 polymerase to exert its antiviral effect. However, recent studies have also shown that it inhibits the cap formation activity of nsP1[77]. 4′-FlU demonstrates high oral bioavailability and significantly reduces viral load and arthritis symptoms in mouse models, highlighting its promising therapeutic potential.

3.1.4.4. Challenges and outlook. The development of nsP4 inhibitors is confronted with three major challenges: (1) The intrinsic instability of nsP4 itself makes it difficult to obtain enough of the protein for biochemical analyses; (2) The lack of structural information on the replication complex (RC) hinders the design of specific inhibitors; (3) Nucleoside analogs may interfere with host RNA metabolism. Potential future research directions could include: (1) Developing methods to stabilize nsP4 for application in drug screening; (2) Resolving the high-resolution structure of the replication complex; (3) Exploring the interaction interfaces between nsP4 and other non-structural proteins as potential drug targets. As these challenges are gradually addressed, nsP4 inhibitors are expected to become an effective strategy for treating CHIKV infections.

3.2. Structural protein inhibitors

CHIKV structural proteins comprise the capsid protein (C) and envelope proteins (E3, E2, 6K, and E1), with the E1−E2 heterodimer playing a critical role in viral attachment and membrane fusion[78]. Targeting these structural proteins, particularly E2, with specific inhibitors can effectively block viral entry into host cells, thereby representing a significant strategy in the development of anti-CHIKV therapeutics.

3.2.1. Envelope glycoproteins (E1/E2): key to viral invasion and fusion

CHIKV's envelope is covered with spikes composed of heterodimeric glycoproteins E1 and E2. The E2 protein is responsible for recognizing and binding to receptors on the surface of host cells, thereby initiating the process of viral endocytosis. Once the viral particles enter the endosomes, the acidic environment induces an irreversible conformational change in the E2/E1 dimer, leading to the exposure of the E1 fusion peptide. This peptide then mediates the fusion of the viral envelope with the endosomal membrane, resulting in the release of the nucleocapsid into the cytoplasm[67].

The E1−E2 complex plays a central role in both receptor binding and membrane fusion during CHIKV infection: the A domain of E2 (E2DA) interacts with the host receptor Mxra8, which triggers viral internalization; under acidic conditions, E1 undergoes a structural rearrangement that exposes the fusion peptide, facilitating the fusion of the viral membrane with the endosomal membrane[79]. This entry mechanism offers multiple potential targets for therapeutic intervention: (1) molecules that inhibit the interaction between E2 and its receptor; (2) compounds that block the fusion activity of E1; and (3) agents that interfere with the assembly of the E1−E2 heterodimer. Importantly, many CHIKV vaccines currently in development, including mRNA-based vaccines, primarily utilize E1/E2 glycoproteins as antigenic components to elicit neutralizing antibodies capable of preventing viral entry[80].

3.2.1.1. Strategies for the discovery of E1−E2 inhibitor and vaccine. The E2 protein serves as the core component, within which Glu-166 and Asn-263-located in its domain B-are critical residues (Fig. 12A). As charged polar residues, they contribute to forming part of the receptor-binding interface: their side chains directly influence the affinity of the interaction between the virus and host cell receptors. Notably, Asn-263 is an experimentally validated N-linked glycosylation site. The "glycan shield" formed by the glycans attached to this site is one of the key mechanisms enabling the virus to achieve immune evasion. Residues Lys-47, Gln-49, and Val-50 collectively form a critical antigenic epitope region in this domain. They directly participate in the formation of salt bridges or hydrogen bonds with the CDRs of antibodies; meanwhile, they define the unique shape of this epitope by forming a specific spatial conformation, thereby allowing specific recognition by high-affinity antibodies. The synergistic effects of these residues make domain B a focal point for vaccine design and antibody drug development[81].

The E protein of CHIKV is composed of three E1−E2−E3 protomers. Three hMXRA8 receptor molecules bind to the E protein trimer respectively, forming a 3:3 complex. Each hMXRA8 molecule spans two adjacent E protein protomers and binds to the "canyon" region at the tip of the E protein spike (Fig. 12B). Structural analysis reveals that both the two immunoglobulin-like domains (D1 and D2) and the hinge region of hMXRA8 are involved in the binding process. Specifically, D2 and the hinge region mainly interact with domains A and B of E2, while D1 and the first hinge loop bind to the fusion loop and domain H of E1. These interactions highlight the critical role of MXRA8 in viral attachment and entry into host cells[79].

3.2.1.2. Representative structural protein inhibitors. Suramin: Suramin, a polysulfonated naphthylurea compound, was originally developed for the treatment of trypanosomiasis. It exerts its antiviral effect by competitively binding to domain A of the CHIKV E2DA, thereby inhibiting the interaction between the virus and cellular receptors[82]. At a concentration of 30 μmol/L, suramin can reduce viral binding by more than 50%, and it completely inhibits low-pH-induced virus-liposome fusion at a concentration as low as 0.3 μmol/L[82]. Its broad-spectrum antiviral activity has been confirmed in clinical isolates, including those harboring the E1-A226V mutation, with an EC_{50} ranging from 8.8 to 62.1 μmol/L[83]. However, suramin exhibits notable cytotoxicity at high concentrations (>100 μmol/L) in BHK-21 cells, and studies using a zebrafish model have further demonstrated that doses of ≥70 μmol/L may lead to developmental abnormalities[83]. Importantly, suramin also shows inhibitory effects against the Ebola virus, highlighting its broad antiviral potential. Despite these promising properties, its clinical use is limited by significant side effects, such as adrenal insufficiency and neurotoxicity, which necessitate further structural modifications to improve its safety profile[20].

As a polyvalent anionic ligand, suramin recognizes and binds to Domain A of the E2 protein, forming extensive electrostatic and hydrophobic interactions (Fig. 13). Its multiple sulfonate groups form strong hydrogen-bonding and salt-bridge interactions with basic or polar amino acids such as ARG-80 and THR-94. The naphthalene rings of suramin establish π−alkyl or hydrophobic stacking interactions with residues including THR-160 and GLN-138. Additionally, the structure of suramin is enclosed by multiple hydrophobic residues of Domain A and occupies the extended cavity of the binding pocket. These observations indicate that the polysulfonate groups and aromatic scaffold of suramin make prominent contributions to molecular recognition and binding[81].

2,4-Diaminoquinazoline derivatives (DCR 137): Discovered through cellular phenotypic screening, this inhibitor effectively suppresses CHIKV replication, with an EC_{50} = 37 μmol/L[84]. The compound also exhibits inhibitory activity against the Ross River virus, indicating broad-spectrum anti-alphavirus potential[84]. Recent studies have demonstrated that treatment of Vero cells with DCR137 leads to a significant reduction in the expression level of CHIKV envelope glycoprotein E2 compared to that in the virus control group[84]. As a ligand, DCR137 recognizes and binds to Domain A of the E2 protein, forming a specific binding pocket (Fig. 14). Amino acids such as THR-160, GLN-138, and THR-94 form stable hydrogen-bonding interactions with the polar groups of DCR137. The aromatic rings of DCR137 establish π−cation or π−alkyl interactions with residues like ARG-80. Additionally, the structure of DCR137 is enclosed by a hydrophobic environment formed by amino acids including THR-94, ARG-80, and GLN-138, occupying the key binding cavity of Domain A. These findings indicate that the aromatic rings and polar side chains of DCR137 play critical roles in the binding process[81].

CID 13680145: CID 13680145 was identified through computer-aided drug design as an inhibitor of the E1−E2 complex, exhibiting a docking score of −10.227 kcal/mol and a binding free energy of −51.53 kcal/mol[85]. Molecular dynamics simulations indicate that this compound stably interacts with the E1−E2 interface, particularly targeting the fusion loop region of E2.

Histamine: Histamine was identified *via* virtual screening as an inhibitor of Mxra8, capable of blocking the binding of CHIKV to its receptor[19]. Molecular docking studies demonstrate that histamine forms critical hydrogen bonds with Arg58 and Glu200 on Mxra8, thereby interfering with viral attachment.

Gambogic acid: Gambogic acid, a naturally occurring prenylated xanthone, has been shown through molecular docking to bind to the E2 protein with high affinity (binding energy = −10.9 kcal/mol)[86]. It may inhibit CHIKV binding to Mxra8 by interacting with the

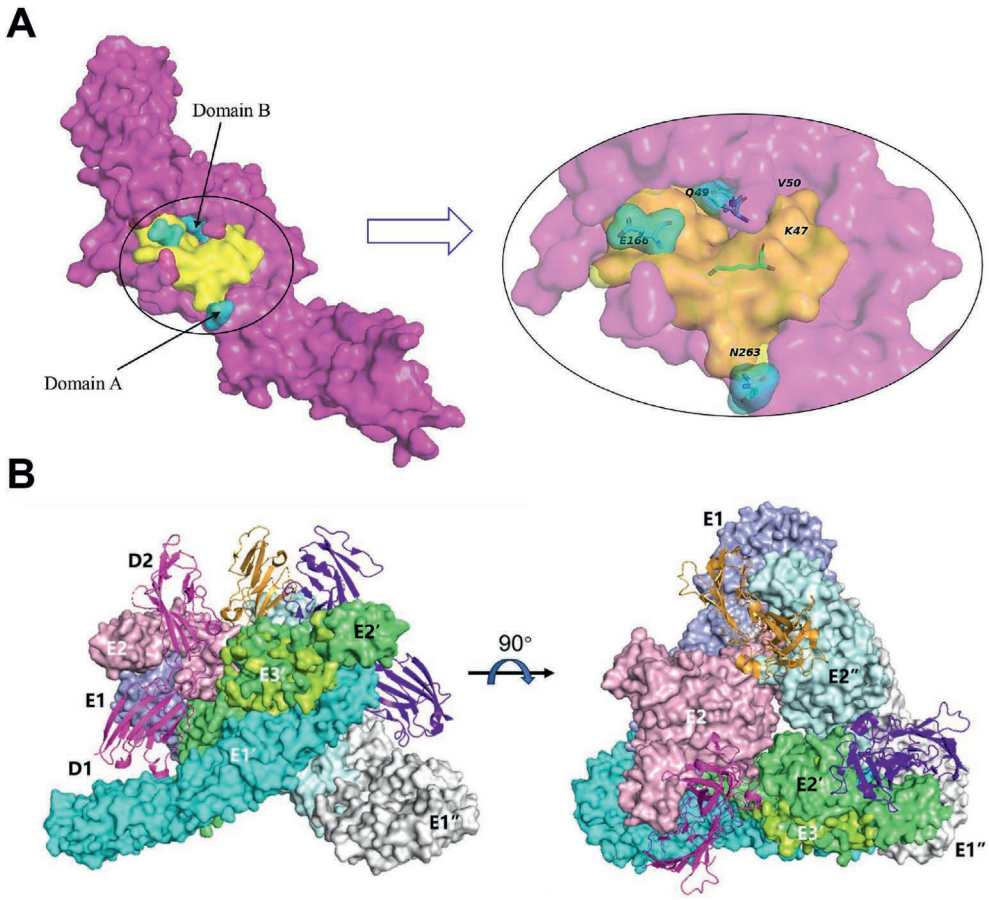

Figure 12 (A) Schematic representation of the spatial distribution of key domains and functional residues of E2 protein. The overall spatial structure of the E2 protein is shown on the left, where Domain A and Domain B, marked in cyan, are the core functional regions that mediate virus-host cell interaction and immune recognition. E166, K47, Q49 and V50 as key positioning amino acids are shown in cyan, which together form the key epitope region in Domain A is shown in yellow. In Domain B, N263 is also involved in the composition of the receptor binding interface is shown in cyan. The localized active cavity region connected to DomainA is indicated by yellow. (B) Global representation of the crystal structure of hMXRA8-E protein. The E1, E1′ and E1″ domains are shown in lavender, cyan, and white, respectively. The E2, E2′ and E2″ domains are shown in light pink, green, and light blue. Only one E3 domain was observed in the structure, indicated in lemon green. hMXRA8 is represented by ribbon and shown in magensia, yellow-orange, and dark purple, respectively. A 90-degree rotation on the right panel shows a top view of the complex (PDB ID #3N41).

receptor-binding domain of E2. Although experimental validation remains limited, gambogic acid, as a natural product, holds promise for further development as a potential anti-CHIKV therapeutic agent.

3.2.1.3. Challenges and outlook. The challenges in developing structural protein inhibitors include: (1) The E1−E2 complex undergoes significant conformational dynamics, making it difficult to design stably binding molecules; (2) Viruses can escape neutralization by monoclonal antibodies through mutation; (3) Balancing inhibitory activity with effects on host cells is necessary. Future research directions may include: (1) Developing small molecules targeting the E1−E2 interface or fusion peptide; (2) Designing multi-target inhibitors to simultaneously block attachment and fusion; (3) Exploring the capsid protein C as a potential target.

3.2.2. Capsid protein (C protein, CP): regulator of genome packaging and early replication

The capsid protein (C protein) primarily functions by binding to the viral genomic RNA, subsequently self-assembling into an icosahedral nucleocapsid structure to protect the RNA from enzymatic degradation. Following the translation of the structural polyprotein, the C protein undergoes auto-proteolytic cleavage from the polyprotein through its intrinsic serine protease activity[87]. Interfering with either the assembly of the C protein or its interaction with viral RNA has been shown to effectively inhibit the formation of infectious viral particles.

Research on inhibitors targeting the capsid protein has yielded promising results. Through high-throughput screening, benzimidazole derivatives, such as MBZM-N-IBT, have been identified to inhibit CHIKV replication, potentially by interfering with nucleocapsid formation[59,88]. Recent studies have further demonstrated that small molecules targeting the capsid protein can effectively suppress viral replication, suggesting that these inhibitors may exert their effects by disrupting capsid protein oligomerization or RNA-binding capabilities[89].

*3.2.2.1. Representative capsid inhibitors. Thienopyrrole derivatives (Compound **15c**):* Compound **15c** inhibit viral RNA synthesis or the expression of viral proteins (nsP1, capsid, nsP3,

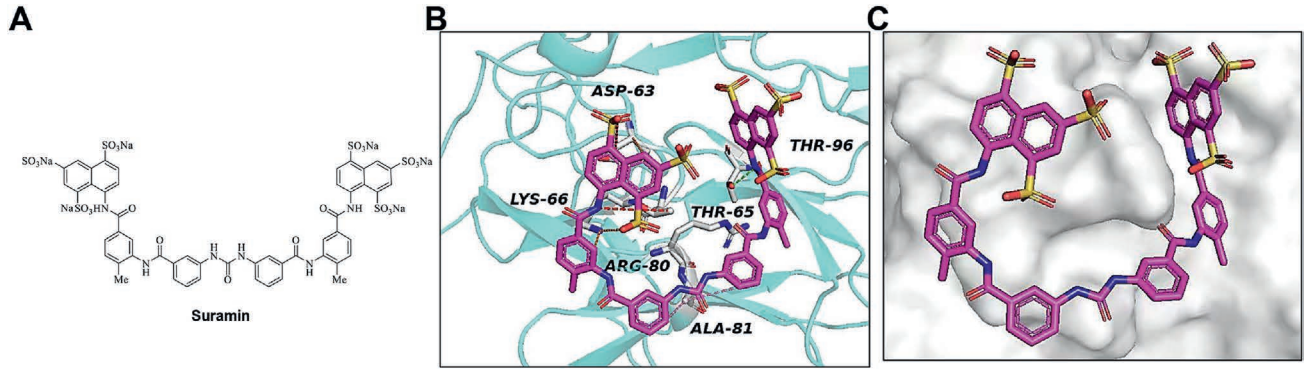

Figure 13 The predicted mode of action of surami and E2. (A) Chemical structure of suramin. (B) The binding mode of Suramin to the active pocket of E2 protein, E2 is shown in cyan, the key amino acids are shown in a gray ball-and-stick model, suramin is shown in a pink ball-and-stick model, E2 is bound to the active pocket formed by amino acids such as Arg80 and THR-94, etc. Pymol was used to model the molecular interaction of E2 with the substrate. (C) Suramin with the active cavity, E2 is shown in gray (PDB#3N41).

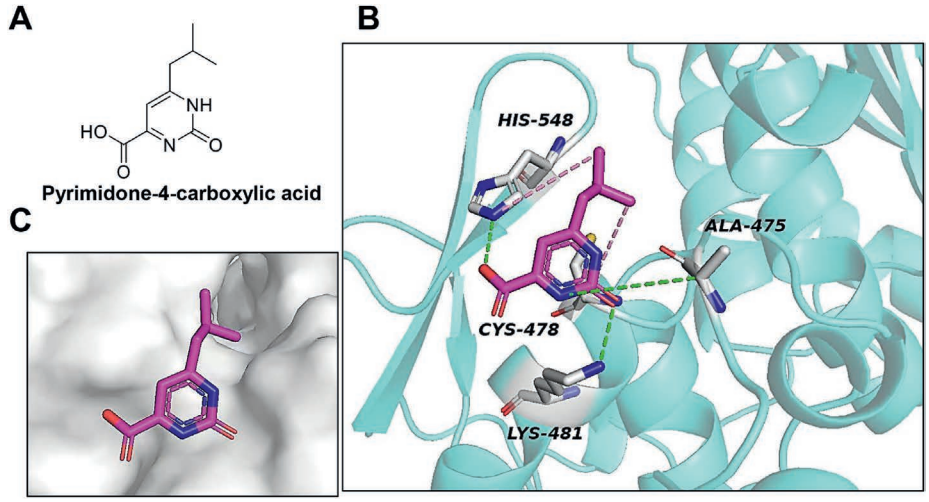

Figure 14 Predicted mode of action of DCR137 with E2. (A) Chemical structure of DCR137. (B) The binding mode of DCR137 to the active pocket of E2 protein, E2 is shown in cyan, key amino acids are shown in a gray ball-and-stick model, and DCR137 is shown in a pink ball-and-stick model. E2 binds to the hydrophobic pocket formed by amino acids such as Arg80 and THR-94. Pymol was used to model the molecular interaction of E2 with the substrate. (C) The position of DCR137 with the active cavity, E2 is shown in gray (PDB#3N41).

E3), thereby significantly suppressing viral replication at a concentration of 2.5 μmol/L[90].

Picolinic acid (PCA): PCA binds to the hydrophobic pocket (HP pocket) of the capsid protein, which leads to the inhibition of viral RNA and protein synthesis. Quantitative real-time PCR (qRT-PCR) and plaque reduction assays demonstrated that PCA significantly reduces viral load[91].

(S)-(+)-Mandelic acid (MDA) and ethyl 3-aminobenzoate (EAB): MDA and EAB were analyzed using *in vitro* assays, including fluorescence spectroscopy and surface plasmon resonance (SPR). These experiments confirmed the interaction of both compounds with CP. The binding constants (K_d) determined by SPR were 1.2×10^{-3} mol/L for MDA and 0.2×10^{-9} mol/L for EAB[92].

3.2.3. 6K protein and ion channel activity

The 6K protein is a small, hydrophobic transmembrane protein that is believed to function as a viroporin by forming ion channels in the cell membrane. This ion channel activity is thought to modify membrane permeability, thereby facilitating the efficient budding and release of viral particles from the host cell membrane[93,94]. Consequently, inhibiting the ion channel function of the 6K protein represents a viable strategy for blocking the final stage of the viral life cycle.

Research on inhibitors targeting the CHIKV 6K protein remains limited. To date, the only well-documented inhibitor is amantadine. amantadine suppresses the ion channel activity of the CHIKV 6K protein, thereby preventing viral envelope membrane fusion. This inhibition results in the production of morphologically abnormal and heterogeneous viral particles, ultimately reducing viral release and demonstrating a moderate antiviral effect (EC_{50} = 29.51 μmol/L)[94] (see Table 1).

4. Host-targeting anti-CHIKV strategies

In addition to directly targeting viral proteins, modulating host cell pathways to create an environment that is unfavorable for viral

replication represents another effective strategy for combating CHIKV[71] (Table 2). The advantages of this approach include: (1) Conservation of host targets reduces the likelihood of drug resistance development; (2) Potential for broad-spectrum antiviral efficacy; (3) Opportunity to develop drugs targeting host proteins that are exploited by the virus. This section provides an overview of the major research advancements in host-targeted anti-CHIKV strategies.

4.1. Host oxidative folding pathway inhibitors

The proper folding and formation of disulfide bonds in viral proteins, particularly envelope proteins, are essential for CHIKV replication and depend on the host cell's endoplasmic reticulum (ER) redox system, which includes members of the protein disulfide isomerase (PDI) family[71]. Targeting these enzymes may interfere with the maturation and functional integrity of viral proteins (Fig. 15).

4.1.1. Key targets and mechanisms

PDI family proteins catalyze both the formation and isomerization of disulfide bonds. The CHIKV E1 and E2 proteins contain multiple conserved cysteine residues, the correct pairing of which depends on PDI activity[95]. ER oxidoreductin 1 (ERO1) supplies oxidizing equivalents to PDI, thereby jointly maintaining the oxidative environment of the endoplasmic reticulum (ER)[96]. Furthermore, thioredoxin reductase (TrxR) also plays a role in regulating cellular redox homeostasis, thereby influencing the proper folding of viral proteins[71].

4.1.2. Representative inhibitors

3-Methyltoxoflavin: A PDI inhibitor that significantly inhibits CHIKV replication (EC_{50} = 1.2 μmol/L). Mechanistic studies have demonstrated that this compound interferes with the formation of the E1−E2 dimer, thereby reducing the production of infectious viral particles. Notably, treatment with 3-methyltoxoflavin results in an increased ratio of viral genome to infectious particles, suggesting that it may affect the correct folding of viral proteins[95].

Auranofin: A clinically approved TrxR inhibitor that was initially developed for the treatment of rheumatoid arthritis. Auranofin has been shown to inhibit CHIKV replication, with a therapeutic index (TI) of up to 104.5 (at 12 h) and can alleviate arthritis symptoms in mouse models. In contrast to PDI inhibitors, Auranofin primarily exerts its antiviral effects indirectly by depleting cellular reducing equivalents and altering the cellular redox environment[71].

16F16: 16F16 is a selective PDI inhibitor that effectively inhibits CHIKV infection at the cellular level and suppresses E1-mediated cell fusion without affecting intracellular viral RNA levels, although it reduces viral infectivity. However, this compound typically exhibits a TI < 2, which is significantly lower than that of auranofin, potentially limiting its utility in therapeutic settings[71].

4.1.3. Challenges and outlook

The challenges of targeting the oxidative folding pathway include: (1) Functional redundancy among PDI family members requires multi-target inhibition; (2) Altering redox status may affect host cell functions; (3) Balancing antiviral activity with cytotoxicity is necessary. Future directions may include: (1) Developing inhibitors with higher specificity for PDI subtypes; (2) Exploring virus-specific disulfide bonds as targets; (3) Combining oxidative folding modulators with different mechanisms.

4.2. Sodium-potassium ATPase inhibitors

Sodium-potassium ATPase (Na^+/K^+-ATPase) is a membrane-bound ion transporter responsible for maintaining the electrochemical gradients of sodium and potassium ions across the cell membrane. CHIKV infection is dependent on the functional activity of Na^+/K^+-ATPase, and pharmacological inhibition of this enzyme has been shown to effectively suppress viral replication[97].

4.2.1. Mechanism of action

Na^+/K^+-ATPase may influence the CHIKV lifecycle through multiple mechanisms: (1) regulating endosomal acidification and thereby affecting viral fusion; (2) maintaining an optimal ionic environment that supports the assembly of viral replication complexes; and (3) functioning as a signaling scaffold involved in host antiviral responses. Importantly, different cell types exhibit varying degrees of sensitivity to Na^+/K^+-ATPase inhibition, suggesting the presence of cell-specific regulatory mechanisms[97].

4.2.2. Representative inhibitors

Digoxin: Digoxin is a cardiac glycoside and a Na^+/K^+-ATPase inhibitor. It demonstrates significant antiviral activity against CHIKV infection with an EC_{50} = 48.8 nmol/L in human cell lines such as U-2 OS cells and similarly reduced CHIKV infection in primary human synovial fibroblasts (HSFs) and Vero African green monkey kidney cells (Vero cells), with EC_{50} = 43.9 and 67.3 nmol/L, respectively[97]. Resistance studies have shown that mutations in nsP2 and nsP4 reduce viral susceptibility to digoxin, thereby confirming its antiviral mechanism through targeting the viral replication complex. Notably, digoxin also exhibits inhibitory effects against other alphaviruses, such as Ross River virus, as well as rhabdoviruses, including Vesicular Stomatitis Virus, indicating broad-spectrum antiviral potential.

Ouabain: Ouabain is another cardiac glycoside that acts as a Na^+/K^+-ATPase inhibitor, and its anti-CHIKV activity is comparable to that of digoxin. In human cells (*e.g.*, U-2 OS cells, primary human synovial fibroblasts), ouabain significantly reduces CHIKV infection, with an EC_{50} = 43.9−67.3 nmol/L[97]. Time-course experiments have demonstrated that ouabain primarily affects the post-entry replication steps of the virus, rather than the initial attachment or internalization processes. However, cardiac glycosides have a narrow therapeutic window, and their clinical application requires careful evaluation of cardiotoxicity risks[97].

4.2.3. Challenges and outlook

The primary challenge in targeting Na^+/K^+-ATPase lies in its essential physiological role as an ion pump, whereby its inhibition may result in significant adverse effects. Potential future research directions could involve: (1) developing molecules that selectively target virus-dependent subtypes or localized forms of Na^+/K^+-ATPase; (2) investigating low-dose combination therapy strategies; and (3) elucidating the molecular mechanisms underlying virus-Na^+/K^+-ATPase interactions to facilitate the design of specific interfering compounds.

4.3. Signaling pathway modulators

The mitogen-activated protein kinase (MAPK) signaling pathway regulates a variety of cellular processes, such as inflammation and

Table 1 Inhibitors targeting CHIKV.

Non-structural protein (nsPs)				
Compd.	Target	Effect	Experimental model	Ref.
Lobaric acid	nsP1 (GTP binding site)	Competitively binds to the GTP site (IC_{50} = 1.3 μmol/L) and inhibits the guanylation activity of nsP1; *in vitro* antiviral activity, EC_{50} = 5 −10 μmol/L).	*In vitro*	31
5-IT	nsP1 (SAM competitive inhibitor)	Inhibit the replication of CHIKV EC_{50} = 0.5 μmol/L; broad-spectrum anti-alphavirus activity.	*In vitro*	32
6′-β-Fluoro-homoaristeromycin	nsP1	Inhibit the replication of CHIKV $EC_{50} \approx$ 0.12 μmol/L.	*In vitro*	36
FHA and FHNA	nsP1	*In vitro* antiviral activity, EC_{50} = 0.12 ± 0.04 μmol/L and 0.18 ± 0.11 μmol/L.	*In vitro*	36
Radicicol	nsP2 protease	Targeting nsP2 exerts an anti-CHIKV effect; EC_{50} = 0.6 μmol/L.	*In vitro*	38
Compound **2O**	nsP2hel	*In vitro* antiviral activity, EC_{50} = 130 nmol/L; In the virus titer experiment, EC_{50} = 1−3 μmol/L.	*In vitro*	41
J13	nsP2 protease	Effectively inhibits CHIKV replication, EC_{50} = 1 −5 mmol/L; with an oral bioavailability of >80%, and alleviates arthritis symptoms in mice.	*In vitro* Mouse model	49
R-1	nsP2hel	*In vitro* antiviral activity, EC_{50} = 0.13 μmol/L.	*In vitro*	54
Acc-CHIK15-dns	CHIKVP (nsP2)	IC_{50} = 1.2−10.6 μmol/L.	*In vitro*	55
RA-0002034	nsP2pro	*In vitro* antiviral activity, EC_{90} = 160 nmol/L.	*In vitro*	56
Pyrimidone-4-carboxylic acid derivatives	nsP3 (ADP-ribose binding site)	*In vitro* antiviral activity, IC_{50} = 23 μmol/L.	*In vitro*	64
EGCG	nsP2 protease	EGCG showed strong binding affinity (−41.50 and −33.29 in AutoDock Vina and AutoDock4 MM/GBSA, respectively).	Docking	66
Baicalin	nsP3	Baicalin and nsP3 showed a strong binding affinity (−9.8 kcal/mol); *in vitro*: EC_{50} = 7 μmol/L; has inhibitory activity against DENV and Zika.	Docking	214
Cobalt(III)-thiosemicarbazone	nsP4	Replication with 80% inhibition of CHIKV, CC_{50} > 200 μmol/L.	*In vitro*	62
LabMol-309	nsP4	*In vitro* antiviral activity, EC_{50} = 5.2 μmol/L; CC_{50} = 52 μmol/L.	*In vitro*	69
4′-FlU	nsP4	Effective against a variety of RNA viruses; reduce the viral load and arthritis symptoms in the mouse model.	*In vitro* Mouse model	76,77
Structural protein inhibitorrowhead				
DCR 137	E2 protein	Effectively inhibits CHIKV replication, EC_{50} = 37 μmol/L; broad-spectrum activity against a variety of alphaviruses.	*In vitro*	84
CID 136801451	E1−E2 complex interface	Binding energy = −51.53 kcal/mol	*In silicon*	85
Gambogic acid	E2 prorein	Binding energy = −10.9 kcal/mol	*In silicon*	86
Suramin	E2DA receptor binding domain	*In vitro* antiviral activity, EC_{50} = 8.8−62.1 μmol/L; broad-spectrum activity against a variety of alphaviruses; zebrafish models show that it can cause malformations.	*In vitro* Zebrafish model	15,82,83
PCA	C Protein (HP pocket)	Significantly reduce the viral load.	*In vitro*	91
MDA & EAB	C Protein	K_d: 1.2 × 10^{-3} mol/L for MDA; 0.2 × 10^{-9} mol/L for EAB.	SPR	92
Compound **15c**	C Protein	Effectively inhibits CHIKV replication, EC_{50} = 2.5 μmol/L.	*In vitro*	215
Amantadine	6K	*In vitro* antiviral activity, EC_{50} = 29.51 μmol/L.	*In vitro*	94
Histamine	Mxra8	The antiviral activity is relatively weak ($EC_{50} \approx$ 100 μmol/L).	*In silicon*	19

4′-FlU, 4′-Fluorouridine; 5-IT, 5-iodotubercidin; EAB, ethyl 3-aminobenzoate; EGCG, epigallocatechin gallate; DCR 137, 2,4-diaminoquinazoline derivatives; MDA, (*S*)-(+)-mandelic acid; PCA, picolinic acid.

Table 2 Inhibitors targeting host factors.

Compd.	Target	Effect	Experimental model	Ref.
16F16	PDI	Inhibits CHIKV replication, TI < 2, limit its therapeutic application.	*In vitro*	71
3-Methyltoxoflavin	PDI	Effectively inhibits CHIKV replication, EC_{50} = 1.2 μmol/L.	*In vitro*	95
Auranofin	TrxR	Effectively inhibits CHIKV replication, TI = 104.5; alleviate the symptoms of arthritis in mouse models.	*In vitro* Mouse model	71
Digoxin	Na^+/K^+-ATPase	Effectively inhibits CHIKV replication in different cells, EC_{50} = 43.9−67.3 nmol/L; broad-spectrum activity against a variety of alphaviruses.	*In vitro*	97
CMPD1	MK2	The viral titer decreased by approximately 80% at 10 μmol/L.	*In vitro*	98
SB203580	p38 MAPK	The number of new virus offspring released decreased by approximately 2.5 times (1.5 μmol/L).	*In vitro*	99
MG132	Proteasome	Promote the degradation of viral proteins.	*In vitro*	100
PR619, WP1130	Deubiquitinating-proteasomal enzymes	In Vero cells, treated with, viral yield decreased by 1.4 log units (WP1130, 250 nmol/L); viral yield decreased by 2.5 log units (PR619, 5 μmol/L).	*In vitro*	102
NCAO	Polyamine synthesis	In Vero and C6/36 cells, the virus titer decreased by at least 4 log units.	*In vitro*	103
Ivermectin	Nuclear transport proteins	Inhibits CHIKV replication: In BHK-21 cell, EC_{50} = 0.6 μmol/L; In Huh-7.5 cell, EC_{50} = 0.6 μmol/L;	*In vitro*	73
MCC950	NLRP3 inflammasome	No significant effect on the viral titer and viral RNA load. Reduce CHIKV-induced inflammation and bone loss.	Mouse model	75

PDI, protein disulfide isomerase; TrxR, thioredoxin reductase; TI, therapeutic index ($CC_{50}/EClog_{50}$).

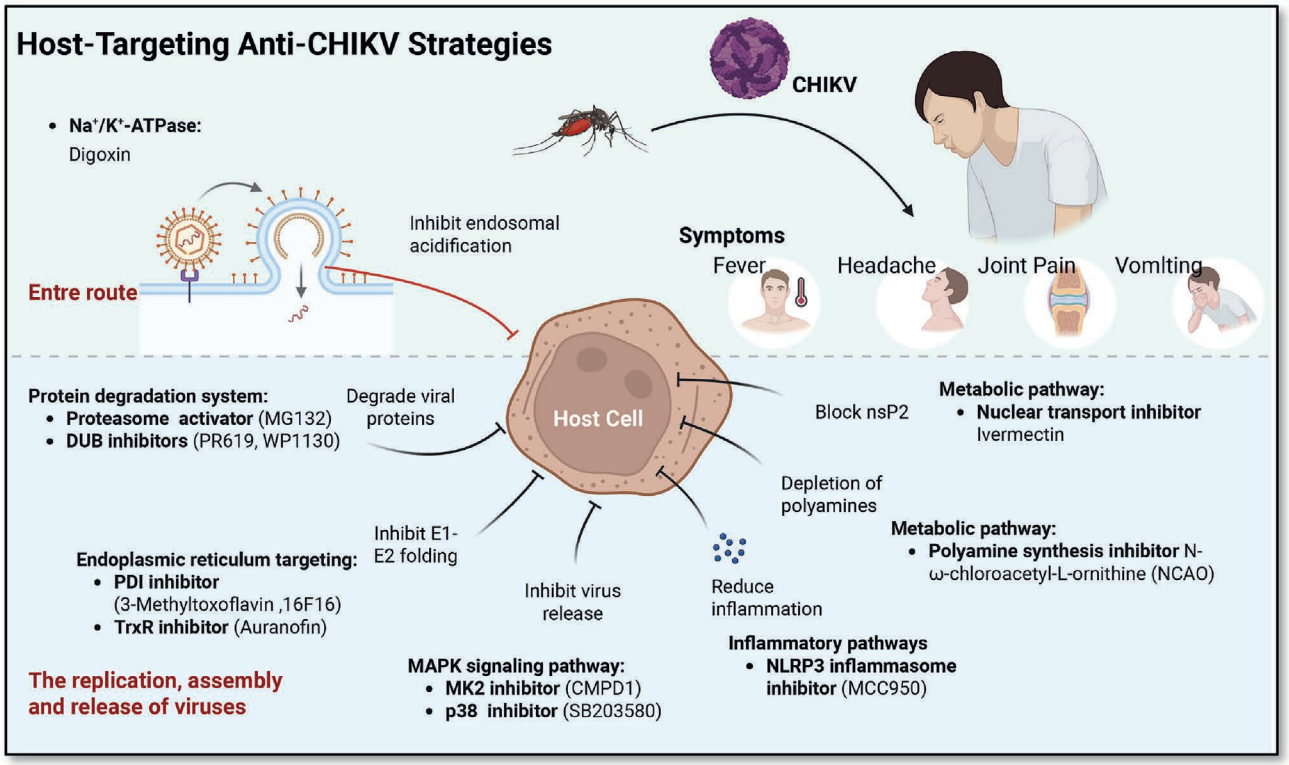

Figure 15 Host-targeting anti-CHIKV strategies.

viral replication. Research has demonstrated that CHIKV infection activates the p38 and JNK MAPK pathways, thereby enhancing the production of inflammatory cytokines and facilitating viral replication[98].

4.3.1. Key targets and mechanisms

MK2/3 (MAPK-activated protein kinase 2/3) are downstream effectors of p38 MAPK and play a role in regulating cytoskeletal reorganization and inflammatory responses. CHIKV infection has been shown to upregulate MK2/3 expression, thereby enhancing viral release; conversely, inhibition of MK2/3 can decrease viral production and mitigate inflammatory damage[98]. Furthermore, CHIKV infection in macrophages activates p38 and JNK phosphorylation, which contributes to TNF production. Both p38 and JNK are also directly involved in the inflammatory responses mediated by CHIKV nsP2, making them potential therapeutic targets[99].

4.3.2. Representative modulators

CMPD1: CMPD1 is an inhibitor of MK2 activation that suppresses CHIKV replication *in vitro* (68% inhibition) and alleviates arthritis symptoms in mouse models[98]. Mechanistic studies have demonstrated that CMPD1 interferes with viral release by inhibiting MK2-mediated lamellipodia formation. Moreover, CMPD1 treatment reduces the expression of key inflammatory cytokines, including CXCL13, RAGE, FGF, and MMP9, while increasing the levels of the recovery marker HGF.

SB203580 and SP600125: SB203580 and SP600125 are specific inhibitors of p38 and JNK, respectively, which can attenuate TNF production induced by CHIKV. Research has shown that CHIKV nsP2 directly interacts with phosphorylated p38 and phosphorylated JNK, thereby activating the c-jun transcription factor and promoting inflammatory responses. Inhibition of p38 or JNK can block this pathway, leading to a reduction in viral pathogenicity[99].

4.3.3. Challenges and outlook

The challenge of targeting the MAPK pathway is its involvement in multiple physiological processes; inhibition may cause widespread side effects. Future research directions may include: (1) Developing virus-specific MAPK modulators (2) Targeting downstream effectors (*e.g.*, MK2/3); rather than upstream kinases; (3) Exploring crosstalk between MAPK and other signaling pathways to design combination intervention strategies.

4.4. Protein degradation system

In addition to the main pathways mentioned above, researchers have explored various other host targets for anti-CHIKV therapy.

4.4.1. Proteasome inhibitors

Proteasome inhibitors such as MG132 enhance the ubiquitination and subsequent degradation of viral proteins, including nsP4, thereby inhibiting CHIKV replication[100]. Additionally, Bortezomib, an anti-tumor drug approved by the FDA, can interfere with CHIKV replication through multiple pathways[101].

4.4.2. Deubiquitinating enzyme (DUB) inhibitors

Deubiquitinating enzyme (DUB) inhibitors, such as PR619 and WP1130, impair the stability of viral proteins and suppress CHIKV replication[102]. PR619 and WP1130 inhibit CHIKV replication by compromising the stability of viral proteins. In Vero cells treated with 250 nmol/L WP1130, viral yield decreased by 1.4 log units, corresponding to an approximate 25-fold reduction. Similarly, treatment with 5 μmol/L PR619 resulted in a significant reduction in viral titter by 2.5 log units, representing a decrease of over 300-fold. These findings indicate that both compounds effectively suppress viral replication. Furthermore, administration of PR619 and WP1130 was associated with marked dysregulation of viral RNA and protein synthesis, suggesting that DUB enzyme activity plays a critical role in the CHIKV life cycle.

4.4.3. Synthesis inhibitor

N-ω-Chloroacetyl-L-ornithine (NCAO) can inhibit the replication of CHIKV in both Vero and C6/36 cells in a dose-dependent manner, reducing the viral titer per milliliter by a minimum of 4 log units in these cell lines. This inhibitory effect is mediated through the suppression of ornithine decarboxylase activity and the subsequent depletion of intracellular polyamines, which are essential for CHIKV replication. Furthermore, the antiviral effect of NCAO can be counteracted by the addition of exogenous polyamines, thereby confirming its mechanism of action[103]. In another study, a derivative of serpentine alkaloid called harringtonine exhibited potent inhibitory activity against CHIKV infection (EC_{50} = 0.24 μmol/L), while showing extremely low cytotoxicity. Mechanistically, this compound exerts its antiviral effect by interfering with the RNA replication and the expression of viral proteins[104].

4.4.4. Nuclear transport inhibitor

Ivermectin inhibits CHIKV replication by blocking the IMPα/β1-mediated nuclear import of viral proteins[103]. In BHK-21 cells, the EC_{50} of ivermectin for inhibiting CHIKV replication is 0.6 μmol/L. In human hepatocellular carcinoma cells (Huh-7.5), the EC_{50} = 1.2 μmol/L[73]. It is also effective against other alphaviruses (such as Sindbis virus SINV, Semliki Forest virus SFV, and Yellow Fever virus YFV), reducing the virus titer by 2−4 log levels.

4.4.5. LRP3 inflammasome inhibitor

CHIKV infection activates the NLRP3 inflammasome, thereby promoting the production of IL-1β and IL-18[105]. MCC950 treatment did not significantly affect the viral titer or viral RNA load in CHIKV-infected samples, suggesting that its mechanism of action is primarily through immunomodulation rather than direct antiviral activity[75]. Nevertheless, a marked reduction in foot swelling was observed in mice treated with MCC950, particularly during the early phase of infection (Days 2−3), where the therapeutic effect was most pronounced. MCC950 effectively mitigates CHIKV-induced inflammation and bone degradation without interfering with viral replication. This anti-inflammatory approach also demonstrates efficacy against Ross River virus (RRV), but not against West Nile virus (WNV), indicating a certain level of specificity in its mode of action.

These diverse host-targeting strategies offer a wide range of possibilities for the development of anti-CHIKV therapeutics. When used in combination with direct-acting antiviral agents, these approaches may exert synergistic effects and potentially lower the likelihood of drug resistance emergence.

5. Natural products derived anti-CHIKV compounds

Natural products serve as a crucial reservoir for antiviral drug discovery, owing to their structural diversity and biocompatibility[106]. In recent years, a number of naturally derived compounds exhibiting anti-CHIKV activity have been isolated from plant, microbial, and marine sources, some of which have progressed to preclinical evaluation[68].

5.1. Flavonoids

5.1.1. Baicalin

Extracted from *Scutellaria baicalensis*, baicalin directly virucidal activity (EC_{50} = 7 μmol/L)[6]. It demonstrates significant anti-CHIKV activity (EC_{50} = 77−350 μmol/L) in Vero, BHK-21, and HEK293T cells with low cytotoxicity (CC_{50} > 600 μmol/L)[107]. Time-of-addition experiments indicate it primarily affects the replication stage, with activity being cell-type-independent.

5.1.2. Silymarin

Silymarin, an active small-molecule compound extracted from *Silybum marianum*, significantly inhibited CHIKV replication in Vero cell models. Further experiments demonstrated that silymarin exerts its antiviral effect by reducing the replication efficiency of CHIKV and downregulating the production of relevant viral proteins[108].

5.1.3. Naringin

Naringin, identified as an antiviral active constituent of *Chiococca alba* (L.) Hitchc., demonstrates a plaque formation inhibition rate exceeding 70% against CHIKV at a concentration of 60 μg/mL. Molecular docking analysis reveals that naringin exhibits strong binding affinity to the nsP2 of both CHIKV and Mayaro virus (MAYV), with a binding affinity energy of −7.9 kcal/mol. This interaction involves van der Waals forces with amino acid residues such as SER44, PRO204, and LEU203, π−cation interactions with TRP80, and carbon-hydrogen bonds with LEU201 and ASP229 in CHIKV-nsP2 [109]. In addition, naringin exhibits an IC_{50} = 168 μg/mL against Dengue virus type 2 (DENV-2), exerting its antiviral effect through the inhibition of viral adsorption and RNA polymerase activity[110].

5.2. Triterpenoids

5.2.1. Betulinic acid

This pentacyclic triterpenoid inhibits the post-CHIKV infection stage[3]. Time-course studies indicate that it effectively blocks viral RNA synthesis and protein production and demonstrates efficacy against other mosquito-borne RNA viruses, such as DENV and ZIKV, suggesting its potential as a broad-spectrum antiviral agent.

5.2.2. Andrographolide derivatives

Andrographolide exhibits strong inhibitory effects against CHIKV infection, reducing virus production by approximately 3 $\log_{10}$ with an EC_{50} of 77 μmol/L, and shows no cytotoxicity[2,111]. Structural modifications of andrographolide have led to the development of the oxindole-rhodanine hybrid 7-chloro-oxindole (E)-42, which demonstrates potent antiviral activity against clinical CHIKV isolates (EC_{50} = 0.6−1.2 μmol/L)[112]. With a selectivity index (SI) greater than 100, and the capacity to inhibit viral RNA synthesis and protein expression, this compound represents one of the most promising plant-derived candidates for anti-CHIKV therapy.

5.3. Alkaloids

5.3.1. Lycorine

Identified through screening of 109 natural compounds, lycorine effectively inhibits CHIKV and DENV at low micromolar concentrations[23]. Time-of-addition experiments indicate that it does not interfere with viral entry but rather suppresses the synthesis of negative-strand viral RNA. Molecular docking studies suggest that lycorine binds to the palm and finger domains of the CHIKV and DENV RdRp, located in proximity to the catalytic site.

5.3.2. 8-Bromobaicalein

Originally identified as a flavivirus polymerase inhibitor, 8-bromobaicalein also demonstrates anti-CHIKV activity (EC_{50} = 3.5 μmol/L)[113]. In adult mouse models, it mitigates musculoskeletal inflammation and decreases viral load. By targeting nsP4, it reduces the likelihood of resistance development.

5.4. Other active plant components

5.4.1. Magnolol triazole-chloroquine derivatives

Derivatives of magnolol, obtained through click chemistry modification (*e.g.*, compound **7**), demonstrate significant anti-CHIKV activity (EC_{50} = 9.05 μmol/L, SI > 16.8)[114]. These derivatives exhibit more than 46-fold higher potency compared to the positive control and effectively inhibit CHIKV replication, without exerting a direct effect on viral particles.

6. Drug repurposing strategies and the PAINS challenge

Drug repurposing presents notable advantages compared to conventional drug development approaches: 1) established safety profiles, based on comprehensive pharmacokinetic and toxicity data from previously approved drugs, which significantly reduce development risks; 2) abbreviated development timelines, with an estimated reduction of 5−7 years; 3) reduced costs, projected to be only 30%−50% of those associated with de novo drug development; and 4) the potential for direct entry into Phase II clinical trials, thereby accelerating clinical translation[115,116]. By leveraging existing safety data, drug repurposing facilitates the expedited development of anti-CHIKV therapies. Several approved drugs have demonstrated anti-CHIKV activity.

Although high-throughput screening (HTS) of approved drugs and fragment-based drug design for multi-target-directed ligands (MTDLs) have become routine strategies to achieve optimal multi-target combinations, the unexpected occurrence of pan-assay interference compounds (PAINS) concerns during MTDL development often leads to non-specific interactions or other adverse effects, thereby resulting in artifacts or false-positive data in biological assays[117]. The chemical structure of PAINS typically contains specific reactive groups that are capable of interacting non-specifically with a variety of target proteins[118].

6.1. Antifungal drugs

6.1.1. Itraconazole (ITZ)

Identified through replicon-based screening, the antifungal drug ITZ is a highly selective inhibitor of CHIKV, exhibiting a SI greater than 312 in replicon assays and greater than 294 in virus infection assays[40]. ITZ may interfere with the formation of viral replication organelles by modulating cholesterol metabolism;

however, its exact mechanism of action remains to be fully elucidated. Due to its favorable oral bioavailability, established safety profile, and low cost, ITZ represents a promising candidate for rapid clinical translation. It should be noted that the high hydrophobicity of ITZ (Log$P > 5$) makes it prone to form colloidal aggregates. Since detergents may affect their solubility and activity, verification through detergent sensitivity experiments (*e.g.*, treatment with 0.01% Triton X-100) is required, or dynamic light scattering (DLS) can be used to detect the particle size distribution. In addition, ITZ has OSBP inhibitory activity, which may interfere with cholesterol transport, so attention should be paid *in vivo* experiments. Oxysterol-binding protein (OSBP) is a human lipid transport protein that is essential for the cellular replication of many types of viruses[119]. It is particularly noteworthy that ITZ can inhibit viral replication through interaction with the OSBP protein. Therefore, the confirmation of ITZ's anti-CHIKV target requires caution and can be verified by X-ray crystallography or chemical proteomics (such as CITe-Id) to confirm covalent binding[120].

6.1.2. Micafungin

This echinocandin-class antifungal agent inhibits CHIKV replication, viral release, and cell-to-cell transmission[121]. It also demonstrates efficacy against SINV and SFV, with high doses exerting only minimal effects on viral stability. Given its FDA-approved status, micafungin offers a novel therapeutic option for the treatment of CHIKV.

6.2. Antiretroviral drugs

6.2.1. Etravirine

Screening of an FDA-approved reverse transcriptase inhibitor library identified etravirine as an inhibitor of WNV and CHIKV infection[122]. The drug primarily exerts its effect during the viral replication phase. Molecular docking studies suggest that etravirine has strong binding potential with the RdRp of WNV and alphaviruses. In mouse models, etravirine significantly alleviated weight loss and neurological symptoms induced by WNV infection and showed efficacy in CHIKV-induced arthritis and lethal infection models.

6.2.2. Efavirenz

The HIV antiretroviral drug efavirenz exhibits anti-CHIKV activity (EC_{50} = 8.33 μmol/L, SI = 9.1)[123]. Mechanistic studies indicate that it acts through dual inhibition of both viral entry and replication, while also reducing the production of pro-inflammatory cytokines, thereby offering a multi-target therapeutic advantage. Efavirenz exhibits fluorescent properties (λ_{ex} 290 nm), so false positives need to be excluded in the HTS fluorescence polarization assay.

6.3. Antiparasitic drugs

The anthelmintic compound oxibendazole, identified through virtual screening, demonstrates inhibitory activity against the CHIKV nsP2 protease and exhibits efficacy against both the Asian and ECSA genotypes, with EC_{50} values ranging from 1.2 to 2.3 μmol/L[124]. Similarly, the antimalarial agent chloroquine inhibits membrane fusion by increasing the pH of endosomes; however, its clinical effectiveness remains limited[125]. Ivermectin exerts its antiviral effect by targeting nuclear transport proteins, thereby inhibiting the nuclear import of nsP2 and reducing viral replication[61,115].

6.4. Anti-inflammatory and immunomodulatory agents

Corticosteroids such as dexamethasone may help alleviate clinical symptoms but carry the potential to prolong viremia[126,127]. The Janus kinase (JAK) inhibitor baricitinib attenuates inflammatory responses by suppressing IFN-γ signaling pathways and is currently undergoing clinical evaluation[127,128]. Furthermore, the IL-2/JES6-1 antibody complex has been shown to mitigate joint inflammation through the expansion of regulatory T cells; however, the potential risk of immune activation necessitates cautious application[129-131].

6.5. Antihypertensive drugs

Angiotensin-converting enzyme (ACE) inhibitors, such as ramipril and enalaprilat, exhibit anti-CHIKV activity, potentially through interference with viral attachment or internalization[115]. Notably, enalaprilat in combination with 2-fluoroadenine demonstrates an additive effect, resulting in a 3-log reduction in viral titers [132]. Molecular docking studies suggest that these compounds may bind to the E2 protein, thereby inhibiting its interaction with host cell receptors[133]. Computational screening has also identified the antihypertensive agent telmisartan and the antibiotic neomycin as inhibitors of nsP2 protease activity, with an IC_{50} of approximately 100 μmol/L[134]. Small-angle X-ray scattering (SAXS) analyses indicate that these compounds induce structural compression of nsP2 while preserving its monomeric state, serving as an example of drug repurposing. The corticotropin-releasing factor receptor type 1 (CRF-R1) antagonist CP-154526 was identified as a CHIKV inhibitor through the use of a replicon system[135]. Time-of-addition and virus entry assays suggest that this compound acts on intracellular targets during the viral replication phase. Additional CRF-R1 antagonists, including R121919 and NGD 98-2, have also shown antiviral potential.

It is noteworthy that EGCG, baicalin, and suramin are high-risk PAINS. Both EGCG and baicalin have polyphenolic structures and belong to redox-cycling PAINS; their activity may stem from ROS generation rather than genuine inhibition. Therefore, after obtaining positive results in the primary screening, catalase (CAT) should be added to verify ROS dependence. In addition, these two compounds exhibit intrinsic fluorescent properties, which may interfere with fluorescence-based detection, so the detection conditions need to be controlled. Suramin contains naphthylamine and polysulfonic acid groups; its multiple benzene rings and conjugated structure may lead to aggregation, and its strong negative charge may interfere with biochemical assays. Thus, detergent sensitivity experiments are required for further verification.

Therefore, after online filtering, many offline experiments should be conducted to distinguish undesirable PAINS, to avoid misassessment of promising scaffolds. The adoption of a fair trial strategy to identify interesting molecules among PAINS can provide certain structure-function insights for MTDL development[136].

7. Structure-based rational drug design

In the medicinal chemistry research targeting CHIKV, various innovative strategies have been employed to address its specific challenges[137,138]. Structure-based design strategies have focused on key targets involved in the viral replication process[139]. Through virtual screening of FDA-approved drug libraries, researchers have identified several potential CHIKV inhibitors. Among them,

cephalosporin drugs such as cefmenoxime and ceforanide can bind to the E1−E2 interface, disrupting the function of envelope glycoproteins[133]. Molecular docking results showed that cefmenoxime forms multiple conventional hydrogen bonds with the conserved residue Ser238 on the E1 protein and also exhibits interactions such as C−H bonds with residues including Glu50 (Fig. 16). Meanwhile, it forms strong electrostatic interactions-including hydrogen bonds and even salt bridges-with the key residue Arg36 on the E2 protein[140]. Similarly, antihistamines like chlorpromazine inhibit protease activity by binding to the allosteric site of nsP2[141]. A pharmacophore model built based on the active site of nsP2 protease has further guided the design of a series of peptidomimetic inhibitors[142]. These compounds contain α/β-unsaturated ketones, which can form covalent bonds with catalytic cysteines, with IC_{50} reaching nanomolar levels[22].

Molecular design has been performed targeting the catalytic triad Cys478/His548/Asn562 of the nsP2 protease[42,143]. Through molecular docking and molecular dynamics simulation studies, researchers have been able to precisely identify compounds that interact with the catalytic active site and design peptidomimetic inhibitors with high affinity[42]. The advantage of this approach lies in its ability to directly target the active site of a key enzyme involved in the viral replication mechanism, thereby effectively inhibiting the function of viral RNA polymerase while reducing the risk of drug resistance caused by viral mutations. Using long-time scale molecular dynamics simulations, researchers optimized the binding mode of nsP2 inhibitors with their targets[66,144]. Key findings include significant conformational dynamics of nsP2; inhibitor binding affects the collective motion patterns of the protein; and non-polar interactions are the main driving force for binding. These insights provide a theoretical basis for optimizing inhibitors, enabling fragment-based screening strategies to demonstrate unique advantages and value in CHIKV drug development.

Fragment-based screening strategies have provided a new avenue for CHIKV drug discovery, particularly demonstrating significant efficacy in studies targeting the nsP3 macrodomain[64]. Through virtual screening of fragment libraries combined with X-ray crystallographic analysis, researchers identified compounds sharing a 2-pyrimidinone-4-carboxylic acid scaffold, which can specifically bind to the ADP-ribose binding site of the nsP3 macrodomain[64]. Among them, 2-oxo-5,6-benzopyrimidine-4-carboxylic acid exhibited anti-CHIKV activity with an IC_{50} value of 23 μmol/L, laying the foundation for the development of specific and potent nsP3 inhibitors (Fig. 17). Additionally, fragment design based on the MXRA8 structure has also identified several promising hit compounds[145]. The advantage of this strategy lies in its ability to efficiently explore chemical space and discover lead compounds with novel mechanisms of action[64].

Natural product optimization strategies have provided abundant research resources for CHIKV drug development, particularly the studies on andrographolide and its derivatives[146]. As a diterpenoid compound extracted from *Andrographis paniculata*, andrographolide and its derivatives have shown inhibitory activity against a variety of viruses, including CHIKV[146]. A pivotal scaffold hopping was performed on the andrographolide scaffold. Specifically, the ent-labdane core of compound (*E*)-**2**-derived from natural andrographolide-was converted to its stereoisomer, the normal-labdane core. This modification aimed to enhance interactions with the target through a more favorable stereoconformation. Subsequently, based on the optimized core, further substituent modification was conducted on the key oxindole pharmacophore. A chlorine atom was introduced at the 7-position of the benzene ring in the oxindole moiety, forming a 7-chlorooxindole structure. Through the synergistic effect of these two-step structural optimizations, the finally obtained compound (*E*)-**42** achieved a comprehensive leap in key pharmaceutical indicators compared with the initial compound (*E*)-**2**: antiviral activity was increased by approximately 3.5-fold; cytotoxicity was significantly reduced; selectivity index was improved by nearly 6-fold (Fig. 18).

The advantage of natural product strategies lies in their ability to provide diverse chemical scaffolds and relatively low cytotoxicity, offering important resources for the development of safe and effective antiviral drugs.

The integrated application of medicinal chemistry strategies can effectively address the specific challenges posed by CHIKV. A research approach involving enhance allel implementation of multiple strategies not only enhances the efficiency of drug

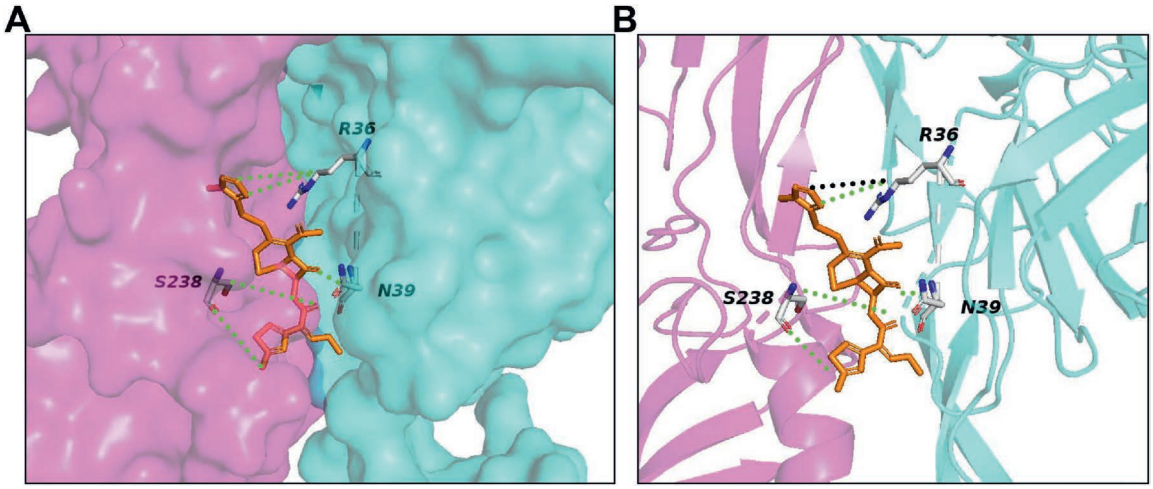

Figure 16 Predicted mode of action of cefmenoxime with E1−E2. The E1 protein chains are shown as pink surfaces and the E2 protein chains as cyan surfaces, clearly outlining the topology of the binding pocket located at the E1−E2 interface. The cefoxime molecule is shown as an orange sticks ball-and-stick model embedded in a binding cavity formed by both E1 and E2 proteins. Key interactions are indicated by yellow dashed lines (PDB: 3N41).

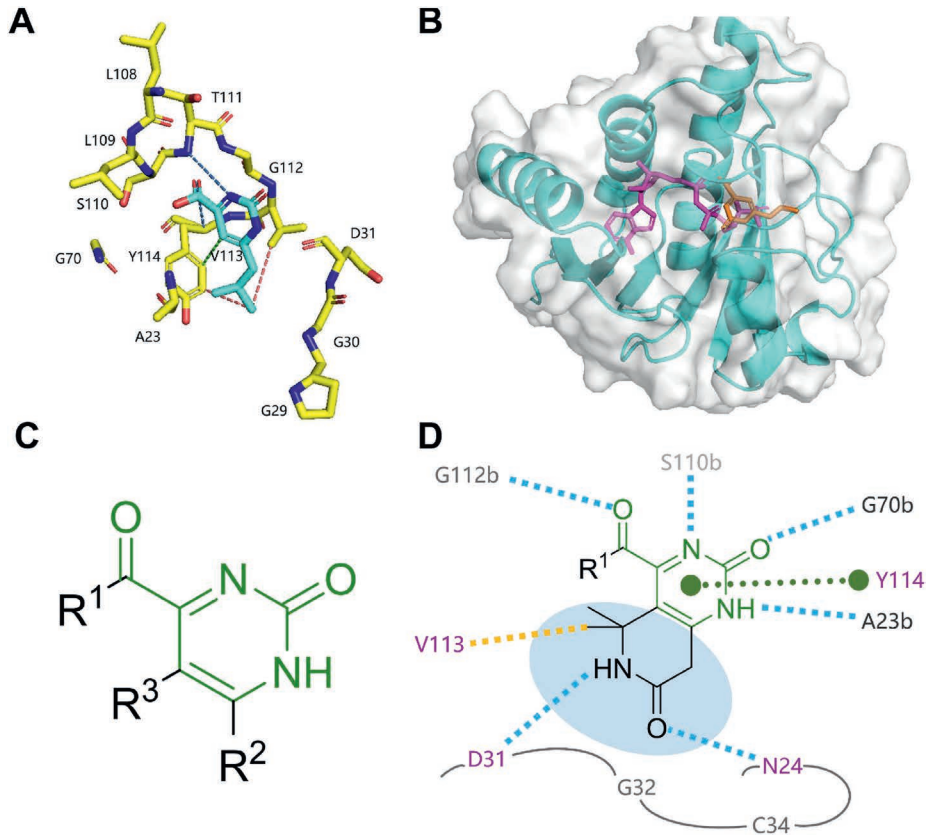

Figure 17 (A) The binding patterns of pyrimidinones and key residues (*e.g.*, A23, G29, D31, G70, S110, L108, L199, T111, G112, Y114, etc.) of nsP3 target proteins, showing intermolecular hydrogen bonds (blue dashed line) and other interactions. (B) 3D structure of the target protein (yellow helix and fold structure), reflecting the binding conformation of the pyrimidinone compound in the active pocket of the protein. (C) The general chemical formula of the core structure of pyrimidinone, marked R1, R2, and R3 as the structural modification sites, in which 2-pyrimidinone-4-carboxylic acid is the "core pharmacophore". (D) Schematic representation of hydrogen bonds (blue dashed lines) and other interactions between pyrimidinones and target protein residues such as G112b, G70b, A23b, D31, G32, C34, etc. (PDB #3GPO).

discovery but also opens more possibilities for the development of broad-spectrum antiviral drugs. This provides a crucial scientific foundation for combating the threats posed by CHIKV and its related alphaviruses.

8. Monoclonal antibodies

In the development of CHIKV mAbs, research has focused most extensively on antibodies targeting the viral envelope glycoproteins E1 and E2. DC2.112 is an mAb that targets the E1 protein; it binds to the fusion loop of E1 domain II and inhibits viral release[147]. The E2 glycoprotein serves as the primary antigenic determinant of CHIKV and is also the main target for antibody recognition. Kim et al.[148] developed two monoclonal antibodies, **19-1** and **21-1**, against the CHIKV-E2 protein. Among these, the **19-1** mAb exhibits the strongest binding affinity to inactivated CHIKV and extremely low cross-reactivity with other mosquito-borne viruses such as ZIKV, JEV, and DENV. Other mAbs targeting the E2 protein include C9, CHK-124, CHK-152, and CHK-265[149-151]. Additionally, there are several highly potent mAbs targeting the E1−E2 complex: CHK-263 locks the conformation of the E1/E2 complex and inhibits virus−cell fusion ($PRNT_{50}$ = 3.8 ng/mL); 8B10/5F10 inhibits extracellular viral spread, with IC_{50} = 46−72 ng/mL[152,153].

The evaluation of antibody binding affinity and specificity is a critical step in the characterization of mAbs. Binding affinity is typically quantified using techniques such as SPR or BLI, which can provide kinetic parameters for antibody-antigen interactions[148]. Specificity assessment is conducted *via* cross-reactivity testing to ensure that the antibody binds exclusively to the target antigen without cross-reacting with other related viruses. For example, the **19-1** mAb exhibits extremely low cross-reactivity with mosquito-borne viruses like Zika virus (ZIKV), Japanese encephalitis virus (JEV), and dengue virus (DENV), making it an ideal candidate for CHIKV diagnosis[148].

Antibody epitope mapping is crucial for understanding the mechanism of antibody action and guiding antibody optimization. Epitope mapping can be achieved through a variety of methods, including X-ray crystallography, HDX-MS, peptide scanning, and viral escape mutation analysis[154]. In a study by Quiroz et al.[154], viral escape mutation analysis was used to identify that the binding epitopes of two E1-specific mAbs are in domain I or the linker region between domains I and III; in contrast, the binding epitopes of two E2-specific mAbs are situated between the β-linker region and domain B. Such precise epitope mapping facilitates understanding of how antibodies interact with the virus and provides guidance for the design of more effective therapeutic antibodies.

Viral escape mutation analysis is a key method for evaluating the therapeutic potential of mAbs. By culturing viruses in the presence of antibodies and screening for mutant strains that can escape antibody neutralization, the mechanism of antibody action

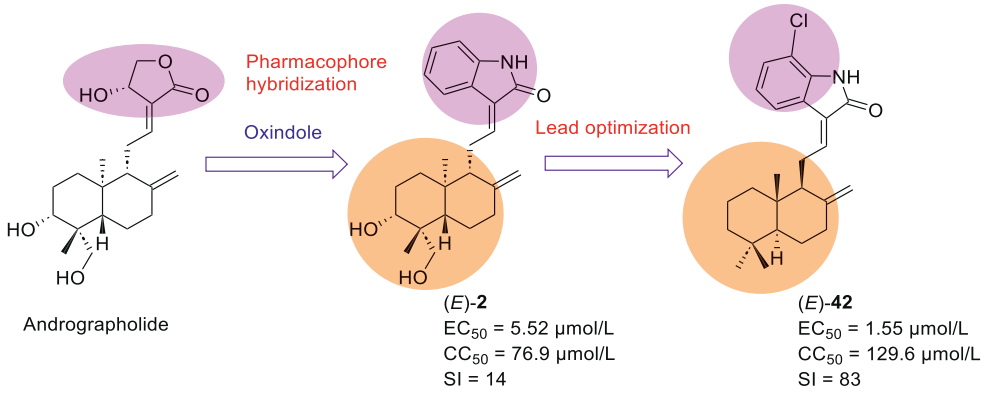

Figure 18 Derivative optimization strategy based on andrographolide stent.

and potential emergence of drug resistance can be determined[154]. In CHIKV research, Quiroz et al.[154] used this method to identify the precise epitope locations bound by antibodies. They further found that two E2-specific mAbs provided *in vivo* protection in a stringent lethal CHIKV infection mouse model, whereas E1 mAbs did not. These findings not only shed light on the characteristics of the human antibody response to CHIKV but also identified candidate mAbs with therapeutic potential.

Mechanistically, the Fc effector function of CHIKV mAbs plays a pivotal role in viral clearance, primarily by activating FcγRs to mediate interactions with immune cells. Studies have demonstrated that the therapeutic efficacy of CHIKV mAbs is significantly dependent on their Fc effector function. Using antibody engineering techniques and mouse strains deficient in C1q or FcγRs, Fox et al.[155] showed that the therapeutic effect of CHIKV-targeting monoclonal IgG antibodies relies on the expression of FcγRs. Further research revealed that when these antibodies were used as post-exposure therapy in mice, those with intact Fc effector function significantly enhanced viral clearance from infected cells and effectively reduced joint swelling. The Fc effector function of CHIKV mAbs, by activating FcγRs-particularly through interactions with monocytes-markedly promotes viral clearance and alleviates clinical symptoms. This finding not only deepens our understanding of the immune defense mechanisms against CHIKV but also provides critical guidance for developing more effective CHIKV therapeutic strategies. Future studies could further explore the optimization of the antibody Fc region to enhance specific effector functions, thereby developing therapeutic antibodies against CHIKV and other related viruses[155,156].

9. mRNA vaccine nanodelivery technology

Drawing on the groundbreaking advancements in mRNA vaccines and nanodelivery technologies during the COVID-19 pandemic, we can gain valuable experience and define technical pathways for CHIKV vaccine development. The success of COVID-19 mRNA vaccines is largely attributed to the mature application of LNP technology. These nanocarriers can effectively protect mRNA from ribonuclease degradation while enhancing cellular penetration and stability[157,158]. Compared with traditional liposomes, LNPs possess unique rigid morphology and improved cargo stability, which provide unprecedented delivery efficiency for mRNA vaccines[158,159]. The rapid development and success of COVID-19 mRNA vaccines (with an efficacy of approximately 95%) have demonstrated the pivotal role of nanotechnology in vaccine development, offering a critical template for addressing future pandemics[158,160].

However, the development of CHIKV vaccines still faces numerous unmet needs. Although CHIKV has caused millions of infections worldwide, only two vaccines, IXCHIQ and VIMKUNYA, have been approved for marketing by the FDA so far-and IXCHIQ is associated with severe adverse reactions in the elderly population[78,161,162]. This "unmet medical need" is also confirmed by patent analysis, which points out that the development of CHIKV vaccines is relatively lagging[161]. While the mortality rate of CHIKV infection is relatively low (approximately 0.1%), it causes severe joint pain that can last for months or even years, imposing long-term health burdens and economic losses on patients. Currently available treatment options are limited to supportive care, with a lack of specific antiviral drugs[161-163].

The successful experience of COVID-19 vaccines offers crucial insights for the development of CHIKV vaccines. First, the nanodelivery technologies can significantly enhance vaccine stability and delivery efficiency[159,164]. Second, the mRNA platform boasts advantages in rapid development and production, enabling rapid responses to emerging outbreaks[164,165]. Furthermore, the manufacturing processes and quality control experience accumulated during the COVID-19 pandemic can be directly applied to the large-scale production of CHIKV vaccines[159,165]. Most importantly, the development of COVID-19 vaccines has established a comprehensive regulatory pathway and clinical trial framework, providing a reference for the accelerated approval of CHIKV vaccines[158,165].

9.1. RNA interference technology

RNAi mediates the degradation of viral mRNA through siRNA, thereby achieving specific gene silencing[166,167]. siRNAs targeting the nsP1, nsP2, and E1 genes of CHIKV can effectively inhibit viral replication (>90%). However, this approach is hindered by challenges such as inefficient delivery and off-target effects[168].

Recent advances include: 1) chemical modification to improve siRNA stability; 2) Nanocarrier improves delivery efficiency; 3) Multiplex siRNA cocktails reduce the risk of resistance[166]. In addition, viral replicon-based reporter gene systems provide a convenient tool for screening efficient sirnas[135,169].

9.2. *Aptamer-based therapeutics*

RNA aptamer Apt#1, selected *via* SELEX technology, can specifically bind to the domain A of the E2 protein and neutralize CHIKV infection (with an $IC_{50} = 2.59$ nmol/L)[83]. Bivalent aptamers (BiNb-2E8 and BiNb-3C5) have further enhanced binding affinity and neutralizing activity, providing new options for diagnosis and treatment[170].

9.3. *Antisense oligonucleotides*

Locked nucleic acid (LNA)-modified antisense oligonucleotides target the conserved regions of the CHIKV genome and can inhibit viral replication, with $EC_{50} = 50$ nmol/L[168,171]. Compared with siRNAs, antisense oligonucleotides exhibit superior tissue distribution properties; however, they also face challenges related to delivery.

9.4. *Nanoparticle delivery systems*

AuNPs and AgNPs possess inherent antiviral activity and can also serve as drug carriers[172,173]. Studies have shown that AuNPs inhibit CHIKV infection by interfering with viral attachment and membrane fusion, with $EC_{50} = 5$ μg/mL[174,175]. In contrast, poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles enhance the intracellular delivery efficiency of siRNAs[176].

9.5. *Nanodiagnostic technologies*

Nanomaterial-based biosensors enable the ultra-sensitive detection of CHIKV. For instance, GFET biosensors achieve a LOD of 1 pfu/mL, which is 1000 times more sensitive than ELISA[174,177]. Additionally, SERS nanotags are also applied for the rapid genotyping detection of CHIKV[178].

9.6. *Nano-vaccines*

VLP-based nanovaccines can effectively induce neutralizing antibodies. A novel CHIKV VLP platform can display 480 antigen copies on its surface, resulting in significantly enhanced immunogenicity. Additionally, nanoadjuvants such as monophosphoryl lipid A (MPLA)-modified liposomes can also potentiate vaccine-induced immune responses[78,161-163].

Several aspects require prioritization in the development of CHIKV vaccines: optimizing nanodelivery systems to enhance immunogenicity, establishing appropriate surrogate endpoints for immune protection, and developing broad-spectrum vaccines targeting different CHIKV genotypes[78,162,179]. Drawing on the experience from COVID-19 vaccine development, the mRNA-LNP platform can be adopted for CHIKV vaccines, combined with VLP technology, to achieve improved immune protection. Meanwhile, it is essential to strengthen international collaboration, establish unified clinical trial standards and regulatory frameworks, accelerate the marketing process of CHIKV vaccines, and ultimately achieve effective prevention and control of this significant infectious disease[165].

10. Viral protein mutations and drug resistance

The association between CHIKV protein mutations and drug resistance represents a critical focus in antiviral therapy research. CHIKV evades the inhibitory effects of antiviral drugs primarily through mutations in key viral proteins, a mechanism that is particularly prominent in the non-structural proteins nsP1 and nsP2.

nsP1 is a critical protein in the replication process of CHIKV, possessing both MTase and GTase activities. It is responsible for the capping of viral RNA and serves as a vital target for antiviral drugs[180]. Studies have shown that inhibitors targeting nsP1 can be categorized into several classes with distinct functions. These inhibitors bind to different structural domains of nsP1, leading to varied drug resistance mechanisms (Fig. 19). Specifically, inhibitors of the MADTP and CHVB series bind to the SAM binding site within the capping domain of nsP1, acting as competitive or non-competitive inhibitors. In contrast, inhibitors of the FHNA series bind to a secondary binding pocket in the cyclic structure of the nsP1 dodecamer. This pocket is in the Membrane-Associated Oligomerization (RAMBO) domain and may interfere with the membrane binding and oligomerization of nsP1[180]. These distinct binding sites enable the virus to evade different types of inhibitors through corresponding point mutations, resulting in the development of drug resistance[180].

nsP2 is a multifunctional core non-structural protein in CHIKV, consisting of an nsP2h and a nsP2p, which are connected by a 14-residue linker[181]. The interaction between the helicase domain of nsP2 and viral RNA is essential for viral replication. Studies have identified that three hydrophobic residues (Y161, F164, and F287) in nsP2h form stacking interactions with RNA bases, thereby bending the RNA backbone[182]. The F287A mutation disrupts these stacking interactions: it increases basal ATPase activity but reduces RNA-binding affinity, which in turn impairs subgenomic RNA synthesis and decreases viral infectivity[182]. Notably, this replication defect can be rescued by either a pseudorevertant mutation (A287V) or adaptive mutations in the helicase domain (T358S or V410I)[182]. Furthermore, the flexible link between the nsP2 domains plays a critical role in the assembly efficiency of the viral replication complex. Shortening the linker (by deleting 3 or 5 amino acids) results in moderate defects in viral RNA replication and transcription, while completely abolishing viral infectivity. In contrast, increasing the flexibility of the linker enhances both genomic RNA replication and viral infectivity[181]. These findings indicate that the domain conformation and flexibility of nsP2 are key determinants of viral replication and infectivity, making it a vital target for antiviral drug design and drug resistance research.

Currently, research on the direct association between mutations in nsP3 and nsP4 of CHIKV and drug resistance remains relatively limited. However, mutations in the structural domains of these proteins may alter viral susceptibility to nucleotide analogs or other drugs targeting viral RNA polymerase. Specifically, studies have shown that the Q192L and C483Y mutations in nsP4 reduce CHIKV susceptibility to 4′-FlU. CHIKV structural proteins, including capsid protein (C), E3, E2, 6K, and E1, play pivotal roles in viral assembly and entry[164]. Mutations in these proteins may affect viral sensitivity to drugs that target the viral assembly or entry processes. For instance, mutations in the E1 protein could impact viral susceptibility to membrane fusion inhibitors, while mutations in the capsid protein may influence sensitivity to assembly inhibitors. Nevertheless, current research on the relationship between CHIKV structural protein mutations and drug resistance is still relatively scarce, warranting further investigation.

To tackle the issue of CHIKV drug resistance, researchers have adopted multiple strategies: 1) Developing multi-target inhibitors

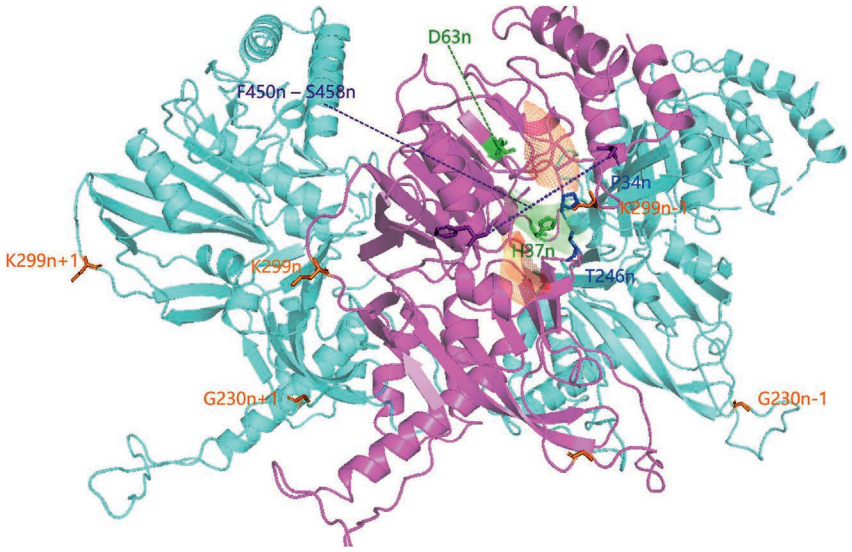

Figure 19 Resistance sites based on the CHIKV nsP1 structure. Residues in different functional regions are color-coded in the figure. Catalytic residues H37 and D63 are shown in green. Residues P34 and T246 associated with resistance to MADTP series inhibitors are marked in blue. Residues G230 and K299 associated with resistance in the FHNA series are shown in orange, residues F450 to S458 (purple) are in the region where residues S454 and W456 are located, and the orange and green dashed volumes represent the proposed binding pockets of GTP and SAM, respectively (PDB# 6Z0V).

to reduce resistance caused by mutations in a single target; 2) Designing next-generation inhibitors capable of overcoming known resistance mutations. 3) Implementing combination therapy strategies that simultaneously target multiple stages of the viral life cycle. Additionally, studies on resistance to kinase inhibitors have shown that resistance mutations typically occur at drug-binding sites. By optimizing the structure of inhibitors to enhance their binding ability to mutated sites, drug resistance can be effectively overcome.

In summary, small-molecule inhibitors represent a critical direction in anti-CHIKV drug development. By targeting different stages of viral replication, they can effectively inhibit CHIKV replication. Future research should continue to focus on the discovery of novel inhibitors, the elucidation of resistance mechanisms, and the optimization of combination therapy strategies, thereby providing more options for the treatment of CHIKV infection.

11. Drug combination strategies and synergistic effects

11.1. Principles and advantages of combination therapy

Combination therapy acts on multiple stages of the viral life cycle simultaneously, thereby enhancing therapeutic efficacy, minimizing the risk of drug resistance, and allowing for reduced dosages of individual agents[72,183]. For CHIKV, effective combination strategies should be based on the following key considerations: (1) complementary mechanisms of action; (2) compatible pharmacokinetic profiles; and (3) non-overlapping toxicity profiles[184].

11.2. Synergistic drug combinations

11.2.1. 2-Fluoroadenine with metabolic modulators

The combination of 2-fluoroadenine with either metyrapone or enalaprilat results in significantly enhanced anti-CHIKV activity, exhibiting additive effects (combination index = 0.8−1.2)[132]. Mechanistically, 2-fluoroadenine interferes with viral RNA synthesis, whereas metyrapone inhibits viral replication by modulating host metabolic pathways[132,185].

11.2.2. EGCG with suramin

EGCG with Suramin: Epigallocatechin gallate (EGCG) and suramin target different stages of the CHIKV life cycle-viral entry and replication, respectively. Their combined application produces a synergistic antiviral effect (combination index = 0.62)[184]. Specifically, EGCG prevents viral attachment by binding to the E2 envelope protein, while suramin competitively inhibits the activity of the nsP2 protease.

11.2.3. Zinc and magnesium salts

Zinc salts (*e.g.*, zinc sulfate and zinc acetate) and magnesium salts (*e.g.*, magnesium sulfate) exhibit antiviral activity through distinct mechanisms. Zinc salts inhibit viral entry and capsid uncoating, whereas magnesium salts suppress RNA polymerase activity[186]. Preclinical studies in animal models indicate that oral administration of these salts significantly reduces viral load and mitigates arthritis-related symptoms.

11.2.4. Favipiravir + MCC95

Favipiravir (FAV), an RNA polymerase inhibitor, directly inhibits CHIKV replication by inducing lethal mutagenesis. It exhibits inhibitory effects on CHIKV replication *in vitro* (EC_{50} = 143 μmol/L) and alleviates joint swelling in mice[187,188]. In contrast, MCC950 specifically blocks the NLRP3 inflammasome, which significantly reduces joint inflammation and bone destruction, while also markedly improving bone mineral density and myositis[75]. The combination therapy of FAV and MCC950 is expected to achieve a dual effect of "antiviral + anti-inflammatory", making it particularly suitable for patients with high viral loads. Efficacy may be optimized through sequential administration (FAV first, followed by MCC950), which avoids the

limitations of single-drug treatment and provides a new strategy for preventing CHIKV-induced chronic arthritis.

11.2.5. Challenges and optimization of combination therapy

Despite its therapeutic potential, combination therapy encounters several critical challenges: 1) the risk of drug−drug interactions; 2) complex pharmacokinetic and pharmacodynamic relationships; and 3) difficulties in achieving optimal dosing regimens[132]. Systems pharmacology and quantitative systems pharmacology (QSP) models offer promising approaches to address these challenges and enhance the development of effective combination therapies[189].

12. Preclinical and clinical research progress

12.1. Mouse models

Wild-type C57BL/6 mice infected with CHIKV develop acute arthritis and chronic joint lesions, recapitulating key features of the human disease[190]. Transcriptomic analysis demonstrates a high degree of similarity between gene expression profiles in infected mice and humans (50% gene overlap), thereby validating the translational relevance of this model[191]. Prophylactic administration of an IFN-α adenovirus vector (mDEF201) completely protected mice from a lethal CHIKV challenge, reducing viral load by more than 3 log[192]. Similarly, andrographolide (50 mg/kg/day) significantly alleviated joint inflammation and inhibited viral replication.

12.2. Non-human primate models

Rhesus macaques infected with CHIKV exhibit symptoms including fever, rash, and viremia that typically persist for approximately 5−7 days[193]. This model is primarily utilized for evaluating vaccine immunogenicity, with limited application in antiviral drug testing. Notably, chronic arthritis is less pronounced in non-human primates compared to humans.

12.3. Clinical research status

During the development of drugs for CHIKV infection, the combination therapy of methotrexate and dexamethasone was once regarded as a potential treatment regimen. This regimen was designed based on the anti-inflammatory and immunomodulatory effects of the two drugs, which could theoretically alleviate joint inflammation and pain caused by CHIKV infection. However, clinical trial results have shown that methotrexate (10−15 mg/week) in combination with dexamethasone (4 mg/day) was evaluated in randomized, double-blind clinical trials for the treatment of chronic CHIKV arthritis but failed to demonstrate significant improvement (NCT03058471)[126]. This lack of efficacy may be attributed to the potent anti-inflammatory effects of dexamethasone potentially masking the therapeutic impact of methotrexate. Chloroquine, another therapeutic agent previously explored for CHIKV treatment, has shown antiviral activity *in vitro*. However, chloroquine (250 mg/day) did not significantly reduce viremia or symptom duration in early clinical trials[125]. Immunomodulatory agents such as tocilizumab and baricitinib are currently under investigation for the treatment of chronic arthritis[194]. In addition, some other drugs, such as ribavirin and interferon, have also been evaluated in small clinical trials for their efficacy against CHIKV infection. Most of these trials were limited by small sample sizes and short follow-up periods, which failed to provide sufficient evidence of efficacy.

12.4. Challenges in clinical trial design for CHIKV drugs

Clinical trials for CHIKV drugs face numerous design challenges, which directly impact the efficiency and success rate of drug development. First, disease heterogeneity is one of the major obstacles in CHIKV drug development. CHIKV infection exhibits significant interindividual variability-different patients may present with distinct clinical symptoms and disease progression trajectories, making it difficult to establish unified efficacy assessment criteria[195]. Like other rare diseases, the CHIKV patient population is small and highly heterogeneous, which further increases the complexity and difficulty of clinical trials[196].

Second, patient recruitment difficulties constitute another major challenge in CHIKV drug clinical trials. Due to the regional and seasonal nature of CHIKV epidemics, patients are unevenly distributed, making it difficult to recruit enough eligible participants within a short timeframe[197]. Additionally, patients' awareness of clinical trials and their willingness to participate also affect the recruitment progress. As noted in the literature, Despite legislative requirements or incentive programs, advancing trials for pediatric treatments has proven challenging for drug developers, and this dilemma is equally applicable to CHIKV drug development[198].

The lack of reliable biomarkers represents a critical bottleneck in clinical trials for CHIKV drugs. Biomarkers are essential for disease diagnosis, staging, and efficacy assessment; however, CHIKV infection currently lacks specific biomarkers to predict disease progression or treatment response[199]. The absence of standardized metrics for measuring treatment response directly undermines the scientific rigor of clinical trial design and the credibility of trial results.

Resolving these challenges necessitates multidimensional efforts, including refining clinical trial designs, optimizing patient recruitment strategies, enhancing research on disease biology, developing specific biomarkers, and establishing closer collaborative relationships with regulatory authorities. Only by overcoming these challenges can the development process of effective CHIKV drugs be accelerated.

13. Challenges and future perspectives

The prevention and control of CHIKV and the development of new therapies face multiple scientific challenges, but at the same time, it also benefits from the vigorous development of emerging technologies, which brings hope for the future.

13.1. Key scientific challenges

13.1.1. Viral genetic diversity and resistance potential

CHIKV circulates as three major genotypes: Asian, West African, and East/Central/South African (ECSA)[200]. The genetic variation of CHIKV enables it to adapt to new mosquito vector species-most notably, expanding from its traditional vector, *Aedes aegypti*, to *Aedes albopictus*. This adaptation has significantly expanded its transmission range to non-tropical and non-endemic regions. Such adaptive variation not only increases the risk of viral transmission but also limits the effectiveness of vector-specific prevention and

control strategies. Meanwhile, viral variation may reduce or even completely abolish the efficacy of existing drugs. This is because drug targets (*e.g*., key viral proteins) may undergo mutations that alter their structure, thereby decreasing the binding affinity between the drug and its target[183]. Notably, as an RNA virus, CHIKV possesses an inherently high mutation rate, which constitutes a fundamental challenge for antiviral development by facilitating the rapid emergence of drug resistance[200]. This concern is supported by recent studies showing that a single M2295T mutation in the viral nsP4 protein confers resistance to benzimidazole-based compounds, a promising class of antiviral candidates[201]. The high mutation rate and mutation characteristics of CHIKV pose a serious challenge for drug development[201]. To address this challenge, it is essential to develop therapeutics or vaccines that exhibit broad-spectrum activity or target highly conserved regions of the viral genome.

13.1.2. Complexity of host-pathogen interactions

CHIKV pathogenesis involves a dual mechanism: direct cytopathic effects caused by viral infection and the induction of a strong, often dysregulated, host inflammatory response. This dual nature of pathogenesis makes purely antiviral or immunomodulatory strategies frequently insufficient[202]. Moreover, a significant proportion of patients develop persistent chronic arthritis. The underlying mechanisms of this condition remain poorly understood but may involve persistent viral infection, retention of viral antigens, or the induction of autoimmune responses-actors that significantly complicate long-term therapeutic strategies[199,203].

13.1.3. Therapeutic needs in special populations

CHIKV infection exerts a particularly severe impact on special populations, including neonates, the elderly, immunocompromised patients, as well as pregnant and lactating women. Due to the uniqueness of their physiological status and immune systems, these groups often face a higher risk of severe disease and more adverse clinical outcomes[204]. Neonates and the elderly are two populations with the highest susceptibility to infectious diseases, necessitating age-specific therapeutic regimens tailored to their distinct immune characteristics[204]. In the context of CHIKV infection, these special populations may experience prolonged periods of joint pain, higher rates of complications, and extended recovery times-posing unique challenges for drug development. Notably, pregnant women are frequently excluded from clinical trials due to concerns about potential adverse maternal-fetal outcomes and the risk of drug-induced harm to the fetus[205]. However, CHIKV infection in pregnant women can lead to more severe symptoms, such as high fever, severe arthralgia, and may even increase the risk of adverse pregnancy outcomes. Similarly, lactating women face limitations in medication options, as the potential transfer of drugs to infants *via* breast milk must be considered. The lack of safety data for these populations leaves clinicians in a dilemma when formulating therapeutic strategies for CHIKV-infected individuals in these groups, as they must balance the need to treat infection against the risk of unintended harm to vulnerable individuals.

13.2. Emerging technologies and integrated strategies

13.2.1. AI-based target discovery and drug design

Traditional drug development typically requires substantial time and financial investment, but the introduction of AI technology is significantly transforming this landscape[75]. The combination of computational biology, CADD, and AI can accelerate the identification and validation of CHIKV drug targets by analyzing large volumes of biological data. For instance, Chemistry42-an AI-driven molecular design platform-integrates AI technology with computational and medicinal chemistry methodologies to efficiently generate novel molecular structures with optimized properties[206]. Such platforms can conduct virtual screening against key proteins in the CHIKV viral life cycle, predict the activity and selectivity of potential inhibitors, and thereby drastically reduce the workload of experimental screening.

The application of AI technology in CHIKV drug development extends beyond target identification, encompassing lead compound discovery and optimization as well[75]. By leveraging deep learning algorithms to analyze the SARs of known antiviral compounds, AI systems can predict the chemical structures of novel CHIKV inhibitors and guide their structural optimization. This approach not only shortens the research and development cycle but also reduces costs, enabling more promising candidate compounds to advance to the preclinical evaluation stage.

13.2.2. Organ-chip-based high-throughput screening

Progress in microfluidic technology has provided a revolutionary tool for CHIKV drug screening[207]. Microfluidic chips can integrate complex drug testing and pharmacology experiments into a single chip system, realizing the concept of a "lab-on-a-chip". This technology is particularly well-suited for high-throughput screening of CHIKV drugs, as it enables the simultaneous testing of many compounds within a tiny spatial volume, significantly improving screening efficiency. Advanced synthesis methods for microfluidic biochips are also continuously being optimized. Synthesis approaches based on relative scheduling can optimize bioassay scheduling and the use of on-chip devices, substantially reducing the total execution time of biological measurements without violating biological constraints[208]. This enhancement of efficiency is particularly critical for CHIKV drug development, which requires the rapid screening of a large pool of candidate compounds to accelerate the identification of potential antiviral agents.

The use of joint organoids derived from induced pluripotent stem cells (iPSCs) has made it possible to investigate the long-term mechanisms of chronic CHIKV arthritis *in vitro*, paving the way for identifying targets to intervene in disease chronicity. Sonal et al. used laboratory-cultured organs (recellularized scaffolds of sheep kidneys) for CHIKV cultivation and to study the virus's interaction with the extracellular matrix (ECM) of host tissues. Prior to the use of animal models, this model serves as a pharmacodynamic evaluation platform for assessing the effects of vaccines or antiviral drugs on the recombinant organ matrix[209].

13.2.3. Formulation optimization based on novel delivery systems

Smart responsive drug delivery systems-novel technologies capable of achieving site-specific drug release in response to microenvironmental changes at the infection site-exhibit great potential in CHIKV treatment. By sensing changes in biological signals such as pH, temperature, and enzymes, these systems precisely control the release of drugs at the infection site, thereby increasing local drug concentration and reducing toxicity to normal tissues[210-212]. Exosomes or small extracellular vesicles have attracted attention as natural nanoparticles for the treatment of CNS diseases due to their natural blood−brain barrier crossing potential and extensive surface engineering capabilities. Rmt-

mediated exosome transport of expressed ligands, such as targeting LDLR apolipoprotein B, has shown promising results. Thus, CHIKV-induced brain diseases can be treated with the help of neurotropic exosome delivery system[213]. Through precise design and optimization *via* microfluidic technology, these delivery systems can protect drugs from degradation, enhance their accumulation at the infection site, and potentially enable targeted delivery to specific cell types. This not only improves therapeutic efficacy but also minimizes side effects, addressing key limitations of conventional antiviral formulations in CHIKV treatment.

CHIKV drug development pipeline requires the integration of a variety of innovative technologies to address challenges in traditional drug development, such as high costs, long cycles, and low efficiency. Emerging technologies-including AI-based target discovery, organ-on-a-chip-enabled high-throughput screening, and formulation optimization based on novel delivery systems-are reshaping the paradigm of interdisciplinary CHIKV drug development strategies (Fig. 20).

14. Conclusions

Significant progress has been achieved over the past decade in the development of antiviral drugs targeting CHIKV. The integration of structural biology, computational drug design, and translational research has facilitated the identification of multiple promising therapeutic candidates. These include viral entry inhibitors (*e.g.*, molecules targeting MXRA8), protease inhibitors (*e.g.*, covalent inhibitors directed against nsP2), and replicase inhibitors (*e.g.*, compounds targeting nsP4). Drug repurposing strategies have successfully identified several approved pharmaceutical agents, such as oxfendazole and posaconazole, which exhibit anti-CHIKV activity, thereby expediting their potential transition to clinical application. Natural products and their derivatives (*e.g.*, α-mangostin, andrographolide) offer a source of structurally diverse lead compounds. Moreover, combination therapies that target multiple stages of the viral life cycle demonstrate synergistic effects and represent a promising strategy to address the emergence of drug resistance.

Nonetheless, the development of CHIKV-specific therapeutics continues to face considerable challenges, including viral genetic variability, limitations associated with current animal models, and complexities in clinical trial design. Future research should prioritize the following areas: 1) AI-assisted design of multi-target agents; 2) elucidation of the molecular mechanisms underlying chronic arthritis and the development of targeted therapeutic interventions; 3) implementation of innovative clinical trial

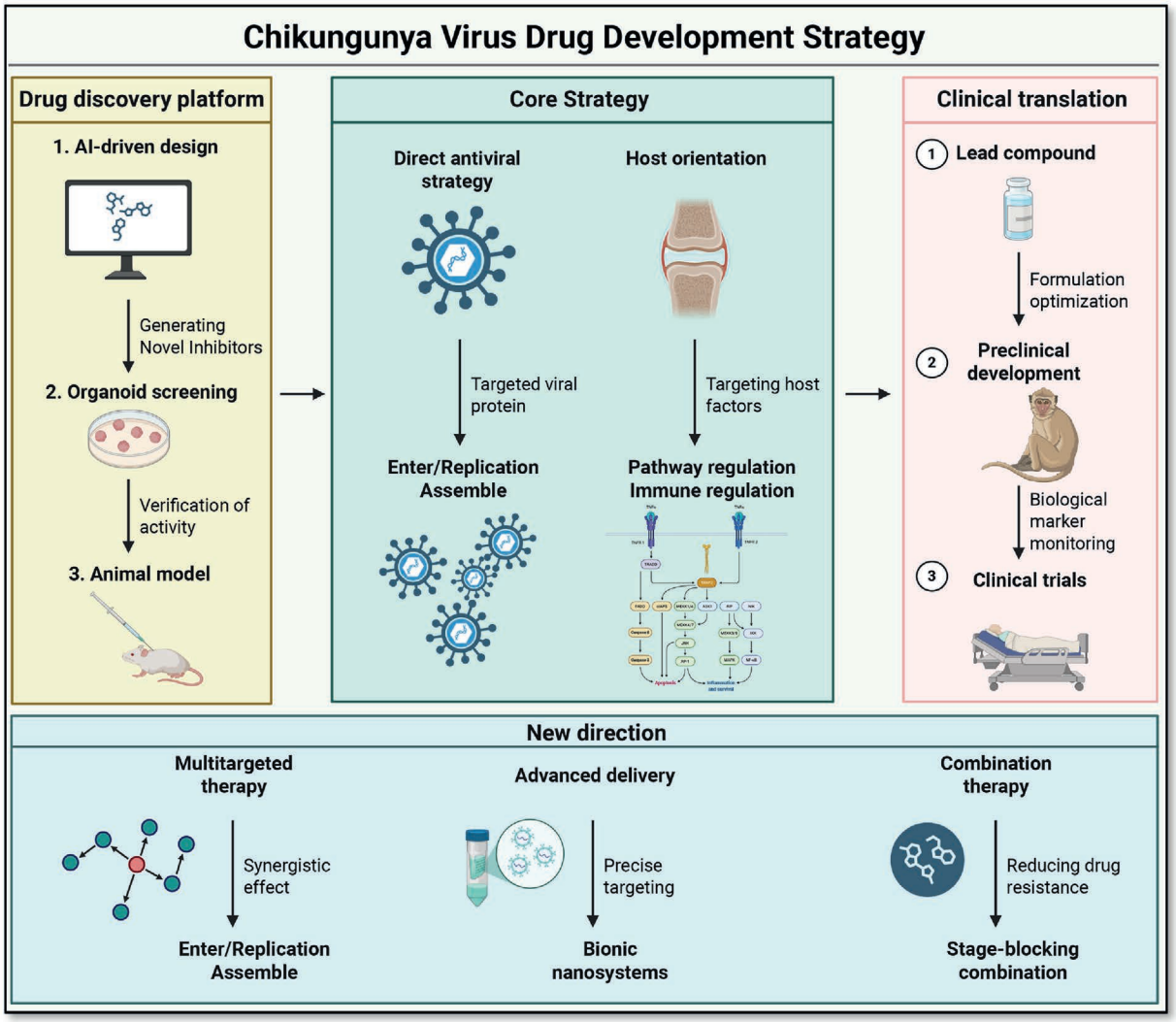

Figure 20 CHIKV drug development strategy.

frameworks; and 4) strengthening international collaboration and resource-sharing initiatives. Through sustained interdisciplinary efforts and strategic investment, the clinical translation of specific antiviral therapies for CHIKV is anticipated soon, offering the potential to reduce the global burden of this important emerging infectious disease.

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Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

References

1. Lin D, Tang C, Wang J, Yang Y, Yang H, Zhou Y, et al. Multiorgan proteomic analysis of infected animal models predict potential host factors for chikungunya virus. *MedComm* 2025;**6**:e70013.
2. Wintachai P, Kaur P, Lee RC, Ramphan S, Kuadkitkan A, Wikan N, et al. Activity of andrographolide against chikungunya virus infection. *Sci Rep* 2015;**5**:14179.
3. Loe MWC, Hao E, Chen M, Li C, Lee RCH, Zhu IXY, et al. Betulinic acid exhibits antiviral effects against dengue virus infection. *Antiviral Res* 2020;**184**:104954.
4. Henderson Sousa F, Ghaisani Komarudin A, Findlay-Greene F, Bowolaksono A, Sasmono RT, Stevens C, et al. Evolution and immunopathology of chikungunya virus informs therapeutic development. *Dis Model Mech* 2023;**16**:dmm049804.
5. Sun C, Zhang L, Yi H, Wan S, Zhu A, Yuan B, et al. Isolation and genomic characterization of Chikungunya virus during the 2025 outbreak in Foshan, China. *Emerg Microbes Infect* 2025;**14**:2595790.
6. Oo A, Rausalu K, Merits A, Higgs S, Vanlandingham D, Bakar SA, et al. Deciphering the potential of baicalin as an antiviral agent for Chikungunya virus infection. *Antiviral Res* 2018;**150**:101−11.
7. Soares AP, de Lima Neto DF, Pour SZ, Passos SD, Cunha MDP, Zanotto PMA. Evaluation of renal markers and liver enzymes in patients infected with the Chikungunya virus. *J Med Virol* 2023;**95**:e29276.
8. Battisti V, Urban E, Langer T. Antivirals against the Chikungunya virus. *Viruses* 2021;**13**:1307.
9. Moscona A. Chikungunya infection: de-linking replication from symptomatology reveals the central role of muscle. *J Clin Investig* 2020;**130**:1099−101.
10. Nicacio JM, Souza CDF, Gomes OV, Souza BV, Lima JAC, Carmo RFD, et al. Cardiac biomarkers in a Brazilian Indigenous population exposed to arboviruses: a cross-sectional study. *Viruses* 2024;**16**:1902.
11. Gupta S, Mishra KP, Kumar B, Singh SB, Ganju L. Andrographolide mitigates unfolded protein response pathway and apoptosis involved in Chikungunya virus infection. *Comb Chem High Throughput Screen* 2021;**24**:849−59.
12. Iacobucci G. UK pauses chikungunya vaccine (IXCHIQ) in over 65s to conduct safety review. *Bmj* 2025;**389**:r1277.
13. Tindale LC, Richardson JS, Anderson DM, Mendy J, Muhammad S, Loreth T, et al. Chikungunya virus virus-like particle vaccine safety and immunogenicity in adults older than 65 years: a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet (London, England)* 2025;**405**:1353−61.
14. Richardson JS, Anderson DM, Mendy J, Tindale LC, Muhammad S, Loreth T, et al. Chikungunya virus virus-like particle vaccine safety and immunogenicity in adolescents and adults in the USA: a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet (London, England)* 2025;**405**:1343−52.
15. Albulescu IC, van Hoolwerff M, Wolters LA, Bottaro E, Nastruzzi C, Yang SC, et al. Suramin inhibits chikungunya virus replication through multiple mechanisms. *Antiviral Res* 2015;**121**:39−46.
16. Yin H, Yin P, Zhao H, Zhang N, Jian X, Song S, et al. Intraviral interactome of Chikungunya virus reveals the homo-oligomerization and palmitoylation of structural protein TF. *Biochem Biophys Res Commun* 2019;**513**:919−24.
17. Ghildiyal R, Gupta S, Gabrani R, Joshi G, Gupta A, Chaudhary VK, et al. *In silico* study of chikungunya polymerase, a potential target for inhibitors. *Virusdisease* 2019;**30**:394−402.
18. Monteiro S, Nunes F, Dosse M, Cangi Vaz N, Nhantumbo C, Juízo DL, et al. Environmental surveillance of vector-borne diseases in a non-sewered system: a case study in Mozambique. *Environ Sci Technol* 2025;**59**:3411−21.
19. Kumar A, Rathi E, Kini SG. Exploration of small-molecule entry disruptors for chikungunya virus by targeting matrix remodelling associated protein. *Res Pharm Sci* 2020;**15**:300−11.
20. Henß L, Beck S, Weidner T, Biedenkopf N, Sliva K, Weber C, et al. Suramin is a potent inhibitor of Chikungunya and Ebola virus cell entry. *Virol J* 2016;**13**:149.
21. Izumida M, Hayashi H, Tanaka A, Kubo Y. Cathepsin B protease facilitates Chikungunya virus envelope protein-mediated infection *via* endocytosis or macropinocytosis. *Viruses* 2020;**12**:722.
22. Das PK, Puusepp L, Varghese FS, Utt A, Ahola T, Kananovich DG, et al. Design and validation of novel Chikungunya virus protease inhibitors. *Antimicrob Agents Chemother* 2016;**60**:7382−95.
23. Agrawal T, Siddqui G, Dahiya R, Patidar A, Madan U, Das S, et al. Inhibition of early RNA replication in Chikungunya and Dengue virus by lycorine: *in vitro* and *in silico* studies. *Biochem Biophys Res Commun* 2024;**730**:150393.
24. Laurent T, Kumar P, Liese S, Zare F, Jonasson M, Carlson A, et al. Architecture of the chikungunya virus replication organelle. *eLife* 2022;**11**:e83042.
25. Zhang N, Zhang L. Key components of COPI and COPII machineries are required for chikungunya virus replication. *Biochem Biophys Res Commun* 2017;**493**:1190−6.
26. Ozden S, Lucas-Hourani M, Ceccaldi PE, Basak A, Valentine M, Benjannet S, et al. Inhibition of Chikungunya virus infection in cultured human muscle cells by furin inhibitors: impairment of the maturation of the E2 surface glycoprotein. *Biol Chem* 2008;**283**:21899−908.
27. Uchime O, Fields W, Kielian M. The role of E3 in pH protection during alphavirus assembly and exit. *J Virol* 2013;**87**:10255−62.
28. Martin CK, Wan JJ, Yin P, Morrison TE, Messer WB, Rivera-Amill V, et al. The alphavirus determinants of intercellular long extension formation. *mBio* 2025;**16**:e0198624.
29. Ivanova L, Rausalu K, Žusinaite E, Tammiku-Taul J, Merits A, Karelson M. 1,3-Thiazolbenzamide derivatives as Chikungunya virus nsP2 protease inhibitors. *ACS Omega* 2021;**6**:5786−94.
30. Miao X, Law MCY, Kumar J, Chng CP, Zeng Y, Tan YB, et al. Saddle curvature association of nsP1 facilitates the replication

complex assembly of Chikungunya virus in cells. *Nat Commun* 2025; **16**:4282.
31. Feibelman KM, Fuller BP, Li L, LaBarbera DV, Geiss BJ. Identification of small molecule inhibitors of the Chikungunya virus nsP1 RNA capping enzyme. *Antiviral Res* 2018;**154**:124−31.
32. Mudgal R, Mahajan S, Tomar S. Inhibition of Chikungunya virus by an adenosine analog targeting the SAM-dependent nsP1 methyltransferase. *FEBS (Fed Eur Biochem Soc) Lett* 2020;**594**:678−94.
33. Ahola T, Laakkonen P, Vihinen H, Kääriäinen L. Critical residues of Semliki Forest virus RNA capping enzyme involved in methyltransferase and guanylyltransferase-like activities. *J Virol* 1997;**71**: 392−7.
34. Jones R, Hons M, Rabah N, Zamarreño N, Arranz R, Reguera J. Structural basis and dynamics of Chikungunya alphavirus RNA capping by nsP1 capping pores. *Proc Natl Acad Sci U S A* 2023;**120**: e2213934120.
35. Zhang K, Law YS, Law MCY, Tan YB, Wirawan M, Luo D. Structural insights into viral RNA capping and plasma membrane targeting by Chikungunya virus nonstructural protein 1. *Cell Host Microbe* 2021;**29**:757−64.e3.
36. Kovacikova K, Morren BM, Tas A, Albulescu IC, van Rijswijk R, Jarhad DB, et al. 6′-β-Fluoro-homoaristeromycin and 6′-fluoro-homoneplanocin A are potent inhibitors of Chikungunya virus replication through their direct effect on viral nonstructural protein 1. *Antimicrob Agents Chemother* 2020;**64**:e02532-19.
37. Shin YS, Jarhad DB, Jang MH, Kovacikova K, Kim G, Yoon JS, et al. Identification of 6′-β-fluoro-homoaristeromycin as a potent inhibitor of chikungunya virus replication. *Eur J Med Chem* 2020;**187**:111956.
38. Nam S, Ga YJ, Lee JY, Hwang WY, Jung E, Shin JS, et al. Radicicol inhibits Chikungunya virus replication by targeting nonstructural protein 2. *Antimicrob Agents Chemother* 2021;**65**:e0013521.
39. Wang M, Wang L, Leng P, Guo J, Zhou H. Drugs targeting structural and nonstructural proteins of the chikungunya virus: a review. *Int J Biol Macromol* 2024;**262**:129949.
40. Policastro LR, Dolci I, Godoy AS, Silva Júnior JVJ, Ruiz UEA, Santos IA, et al. The antifungal itraconazole is a potent inhibitor of Chikungunya virus replication. *Viruses* 2022;**14**:1351.
41. Muthu Ramalingam B, Sears JD, Talbot KM, Su YN, Houliston S, Hossain MA, et al. Identification of direct-acting nsP2 helicase inhibitors with antialphaviral activity. *J Med Chem* 2025;**68**:14514−36.
42. Merten EM, Sears JD, Leisner TM, Hardy PB, Ghoshal A, Hossain MA, et al. Discovery of a cell-active chikungunya virus nsP2 protease inhibitor using a covalent fragment-based screening approach. *bioRxiv* 2024. Available from: https://doi.org/10.1101/2024.03.22.586341.
43. Das PK, Merits A, Lulla A. Functional cross-talk between distant domains of chikungunya virus non-structural protein 2 is decisive for its RNA-modulating activity. *J Biol Chem* 2014;**289**:5635−53.
44. Wang Q, Wu J, Wang H, Gao Y, Liu Q, Mu A, et al. Structural basis for RNA replication by the SARS-CoV-2 polymerase. *Cell* 2020;**182**: 417−28.e13.
45. Denichenko P, Mogilevsky M, Cléry A, Welte T, Biran J, Shimshon O, et al. Specific inhibition of splicing factor activity by decoy RNA oligonucleotides. *Nat Commun* 2019;**10**:1590.
46. Singh H, Mudgal R, Narwal M, Kaur R, Singh VA, Malik A, et al. Chikungunya virus inhibition by peptidomimetic inhibitors targeting virus-specific cysteine protease. *Biochimie* 2018;**149**:51−61.
47. Narwal M, Singh H, Pratap S, Malik A, Kuhn RJ, Kumar P, et al. Crystal structure of chikungunya virus nsP2 cysteine protease reveals a putative flexible loop blocking its active site. *Int J Biol Macromol* 2018;**116**:451−62.
48. Akhrymuk I, Lukash T, Frolov I, Frolova EI. Novel mutations in nsP2 abolish Chikungunya virus-induced transcriptional shutoff and make the virus less cytopathic without affecting its replication rates. *J Virol* 2019;**93**:e02062-18.
49. Metibemu DS, Adeyinka OS, Falode J, Hampton T, Crown O, Ojobor JC, et al. Inhibitor of the non-structural protein 2 protease shows promising efficacy in mouse models of chikungunya. *Eur J Med Chem* 2024;**278**:116808.
50. Merten EM, Sears JD, Leisner TM, Hardy PB, Ghoshal A, Hossain MA, et al. Identification of a cell-active chikungunya virus nsP2 protease inhibitor using a covalent fragment-based screening approach. *Proc Natl Acad Sci U S A* 2024;**121**:e2409166121.
51. Ghoshal A, Asressu KH, Hossain MA, Brown PJ, Merten EM, Sears JD, et al. Structure activity of β-amidomethyl vinyl sulfones as covalent inhibitors of Chikungunya nsP2 cysteine protease with antialphavirus activity. bioRxiv 2024. Available from: https://pubs.acs.org/doi/10.1021/acs.jmedchem.4c01346.
52. Kumar P, Kumar D, Giri R. Targeting the nsp2 cysteine protease of Chikungunya virus using FDA approved library and selected cysteine protease inhibitors. *Pathogens* 2019;**8**:128.
53. Merten EM, Sears JD, Leisner TM, Hardy PB, Ghoshal A, Hossain MA, et al. Identification of a cell-active chikungunya virus nsP2 protease inhibitor using a covalent fragment-based screening approach. *Proc Natl Acad Sci U S A* 2024;**121**:e2409166121.
54. Muthu Ramalingam B, Oh HJ, Sears JD, Chen CH, Vala A, Liu S, et al. Enantioselective chemical probe for Chikungunya nsP2 helicase with antialphaviral activity. *ACS Infect Dis* 2025;**11**:2331−41.
55. Makhaik S, Rut W, Choudhary S, Upadhyay T, Hao C, Westberg M, et al. A robust fluorogenic substrate for Chikungunya virus protease (nsP2) activity. *Protein Sci* 2025;**34**:e70069.
56. Hossain MA, Mayo AK, Ghoshal A, Taft-Benz S, Anderson E, Morales NL, et al. Species-dependent metabolism of a covalent nsP2 protease inhibitor with *in vivo* antialphaviral activity. *J Med Chem* 2025;**68**:10473−85.
57. Ghoshal A, Tse EG, Hossain MA, Asressu KH, Merten EM, Sears JD, et al. A covalent chemical probe for Chikungunya nsP2 cysteine protease with antialphaviral activity and proteome-wide selectivity. *Sci Rep* 2025;**15**:7264.
58. Abdelnabi R, Kovacikova K, Moesslacher J, Donckers K, Battisti V, Leyssen P, et al. Novel class of Chikungunya virus small molecule inhibitors that targets the viral capping machinery. *Antimicrob Agents Chemother* 2020;**64**:e00649-20.
59. De S, Ghosh S, Keshry SS, Mahish C, Mohapatra C, Guru A, et al. MBZM-N-IBT, a novel small molecule, restricts Chikungunya virus infection by targeting nsP2 protease activity *in vitro, in vivo*, and *ex vivo*. *Antimicrob Agents Chemother* 2022;**66**:e0046322.
60. Schudel A, Chapman AP, Yau MK, Higginson CJ, Francis DM, Manspeaker MP, et al. Programmable multistage drug delivery to lymph nodes. *Nat Nanotechnol* 2020;**15**:491−9.
61. Betancur-Galvis L, Jimenez-Jarava OJ, Rivas F, Mendoza-Hernández WE, González-Cardenete MA. Synergistic *in vitro* antiviral effect of combinations of ivermectin, essential oils, and 18-(phthalimid-2-yl) ferruginol against arboviruses and herpesvirus. *Pharmaceuticals* 2023;**16**:1602.
62. Martins DOS, Souza RAC, Freire M, de Moraes Roso Mesquita NC, Santos IA, de Oliveira DM, et al. Insights into the role of the cobalt(III)-thiosemicarbazone complex as a potential inhibitor of the Chikungunya virus nsP4. *J Biol Inorg Chem* 2023;**28**:101−15.
63. Chaudhary M, Kumar A, Bala Sharma K, Vrati S, Sehgal D. *In silico* identification of Chikungunya virus replication inhibitor validated using biochemical and cell-based approaches. *FEBS J* 2024;**291**:2656−73.
64. Zhang S, Garzan A, Haese N, Bostwick R, Martinez-Gzegozewska Y, Rasmussen L, et al. Pyrimidone inhibitors targeting Chikungunya virus nsP3 macrodomain by fragment-based drug design. *PLoS One* 2021;**16**:e0245013.
65. Seyedi SS, Shukri M, Hassandarvish P, Oo A, Shankar EM, Abubakar S, et al. Computational approach towards exploring potential anti-chikungunya activity of selected flavonoids. *Sci Rep* 2016;**6**:24027.
66. Setyawati I, Setiawan AG, Nemchinova M, Vidilaseris K. The potential inhibitory mechanism of EGCG against the Chikungunya virus targeting non-structural protein 2 through molecular dynamics simulation. *Sci Rep* 2024;**14**:29797.
67. Kovacikova K, van Hemert MJ. Small-molecule inhibitors of Chikungunya virus: mechanisms of action and antiviral drug resistance. *Antimicrob Agents Chemother* 2020;**64**:e01788-20.

68. Dansana J, Purohit P, Panda M, Meher BR. Recent advances in phytocompounds as potential Chikungunya virus non-structural protein 2 protease antagonists: a systematic review. *Phytomedicine* 2025;**136**:156359.
69. Freire M, Basso LGM, Mendes LFS, Mesquita N, Mottin M, Fernandes RS, et al. Characterization of the RNA-dependent RNA polymerase from Chikungunya virus and discovery of a novel ligand as a potential drug candidate. *Sci Rep* 2022;**12**:10601.
70. Alguridi HI, Alzahrani F, Almalki S, Zamzami MA, Altayb HN. Identification and molecular docking of novel Chikungunya virus NSP4 inhibitory peptides from camel milk proteins. *J Biomol Struct Dyn* 2024;**42**:9961−76.
71. Langsjoen RM, Auguste AJ, Rossi SL, Roundy CM, Penate HN, Kastis M, et al. Host oxidative folding pathways offer novel anti-chikungunya virus drug targets with broad spectrum potential. *Antiviral Res* 2017;**143**:246−51.
72. Verwimp S, Wagoner J, Arenas EG, De Coninck L, Abdelnabi R, Hyde JL, et al. Combinations of approved oral nucleoside analogues confer potent suppression of alphaviruses *in vitro* and *in vivo*. *Antivir Res* 2025;**239**:106186.
73. Varghese FS, Kaukinen P, Gläsker S, Bespalov M, Hanski L, Wennerberg K, et al. Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses. *Antiviral Res* 2016;**126**:117−24.
74. Julander JG, Dagley A, Gebre M, Komeno T, Nakajima N, Smee DF, et al. Strain-dependent disease and response to favipiravir treatment in mice infected with Chikungunya virus. *Antiviral Res* 2020;**182**:104904.
75. Chen W, Foo SS, Zaid A, Teng TS, Herrero LJ, Wolf S, et al. Specific inhibition of NLRP3 in chikungunya disease reveals a role for inflammasomes in alphavirus-induced inflammation. *Nat Microbiol* 2017;**2**:1435−45.
76. Yin P, May NA, Lello LS, Fayed A, Parks MG, Drobish AM, et al. 4′-Fluorouridine inhibits alphavirus replication and infection *in vitro* and *in vivo*. *mBio* 2024;**15**:e0042024.
77. Yin P, Sobolik EB, May NA, Wang S, Fayed A, Vyshenska D, et al. Mutations in chikungunya virus nsP4 decrease viral fitness and sensitivity to the broad-spectrum antiviral 4′-fluorouridine. *PLoS Pathog* 2025;**21**:e1012859.
78. Battini L, Fidalgo DM, Álvarez DE, Bollini M. Discovery of a potent and selective chikungunya virus envelope protein inhibitor through computer-aided drug design. *ACS Infect Dis* 2021;**7**:1503−18.
79. Song H, Zhao Z, Chai Y, Jin X, Li C, Yuan F, et al. Molecular basis of arthritogenic alphavirus receptor MXRA8 binding to Chikungunya virus envelope protein. *Cell* 2019;**177**:1714−24.e12.
80. Ge N, Sun J, Liu Z, Shu J, Yan H, Kou Z, et al. An mRNA vaccine encoding Chikungunya virus E2-E1 protein elicits robust neutralizing antibody responses and CTL immune responses. *Virol Sin* 2022;**37**:266−76.
81. Voss JE, Vaney MC, Duquerroy S, Vonrhein C, Girard-Blanc C, Crublet E, et al. Glycoprotein organization of Chikungunya virus particles revealed by X-ray crystallography. *Nature* 2010;**468**:709−12.
82. Albulescu IC, White-Scholten L, Tas A, Hoornweg TE, Ferla S, Kovacikova K, et al. Suramin inhibits Chikungunya virus replication by interacting with virions and blocking the early steps of infection. *Viruses* 2020;**12**:314.
83. Goto K, Amano R, Ichinose A, Michishita A, Hamada M, Nakamura Y, et al. Generation of RNA aptamers against chikungunya virus E2 envelope protein. *J Virol* 2025;**99**:e0209524.
84. Saha A, Acharya BN, Parida M, Saxena N, Rajaiya J, Dash PK. Identification of 2,4-diaminoquinazoline derivative as a potential small-molecule inhibitor against Chikungunya and ross river viruses. *Viruses* 2023;**15**:2194.
85. Rahman MM, Limon MBH, Saikat TA, Saha P, Nahid AH, Alam MM, et al. Potent inhibition of Chikungunya virus entry by a pyrazole-benzene derivative: a computational study targeting the E1-E2 glycoprotein complex. *Int J Mol Sci* 2025;**26**:6480.
86. Qamar S, Syeda A, Amjad Beg M, Irfan Qureshi M. Insights from the molecular docking analysis of gambogic acid with the Chikungunya spike glycoprotein E2. *Bioinformation* 2023;**19**:525−30.
87. Fatma B, Kumar R, Singh VA, Nehul S, Sharma R, Kesari P, et al. Alphavirus capsid protease inhibitors as potential antiviral agents for Chikungunya infection. *Antiviral Res* 2020;**179**:104808.
88. Moharana AK, Gaur M, Mohapatra TK, Dash RN, Subudhi BB. Network pharmacology, molecular docking and *in vivo*-based analysis on the effects of the MBZM-N-IBT for arthritis. *Curr Comput Aided Drug Des* 2025;**21**:194−210.
89. Wan JJ, Brown RS, Kielian M. Berberine chloride is an alphavirus inhibitor that targets nucleocapsid assembly. *mBio* 2020;**11**:e01382-20.
90. Ching KC, Kam YW, Merits A, Ng LF, Chai CL. Trisubstituted thieno[3,2-*b*]pyrrole 5-carboxamides as potent inhibitors of alphaviruses. *J Med Chem* 2015;**58**:9196−213.
91. Sharma R, Fatma B, Saha A, Bajpai S, Sistla S, Dash PK, et al. Inhibition of chikungunya virus by picolinate that targets viral capsid protein. *Virology* 2016;**498**:265−76.
92. Sharma R, Kesari P, Kumar P, Tomar S. Structure−function insights into chikungunya virus capsid protein: small molecules targeting capsid hydrophobic pocket. *Virology* 2018;**515**:223−34.
93. Jing X, Ma C, Ohigashi Y, Oliveira FA, Jardetzky TS, Pinto LH, et al. Functional studies indicate amantadine binds to the pore of the influenza A virus M2 proton-selective ion channel. *Proc Natl Acad Sci U S A* 2008;**105**:10967−72.
94. Dey D, Siddiqui SI, Mamidi P, Ghosh S, Kumar CS, Chattopadhyay S, et al. The effect of amantadine on an ion channel protein from Chikungunya virus. *PLoS Negl Trop Dis* 2019;**13**:e0007548.
95. Puhl AC, Fernandes RS, Godoy AS, Gil L, Oliva G, Ekins S. The protein disulfide isomerase inhibitor 3-methyltoxoflavin inhibits Chikungunya virus. *Bioorg Med Chem* 2023;**83**:117239.
96. Matsusaki M, Okuda A, Matsuo K, Gekko K, Masuda T, Naruo Y, et al. Regulation of plant ER oxidoreductin 1 (ERO1) activity for efficient oxidative protein folding. *J Biol Chem* 2019;**294**:18820−35.
97. Ashbrook AW, Lentscher AJ, Zamora PF, Silva LA, May NA, Bauer JA, et al. Antagonism of the sodium-potassium ATPase impairs Chikungunya virus infection. *mBio* 2016;**7**:e00693-16.
98. Mamidi P, Nayak TK, Kumar A, Kumar S, Chatterjee S, De S, et al. MK2a inhibitor CMPD1 abrogates Chikungunya virus infection by modulating actin remodeling pathway. *PLoS Pathog* 2021;**17**:e1009667.
99. Nayak TK, Mamidi P, Sahoo SS, Kumar PS, Mahish C, Chatterjee S, et al. P38 and JNK mitogen-activated protein kinases interact with chikungunya virus non-structural protein-2 and regulate TNF induction during viral infection in macrophages. *Front Immunol* 2019;**10**:786.
100. Yan Y, Zhang F, Zou M, Chen H, Xu J, Lu S, et al. Identification of RACK1 as a novel regulator of non-structural protein 4 of chikungunya virus. *Acta Biochim Biophys Sin* 2024;**56**:1425−36.
101. Kaur P, Lello LS, Utt A, Dutta SK, Merits A, Chu JJH. Bortezomib inhibits chikungunya virus replication by interfering with viral protein synthesis. *PLoS Negl Trop Dis* 2020;**14**:e0008336.
102. López LS, Calvo EP, Castellanos JE. Deubiquitinating enzyme inhibitors block chikungunya virus replication. *Viruses* 2023;**15**:481.
103. Rojas-Luna L, Posadas-Modragón A, Avila-Trejo AM, Alcántara-Farfán V, Rodríguez-Páez LI, Santiago-Cruz JA, et al. Inhibition of chikungunya virus replication by *N*-ω-chloroacetyl-L-ornithine in C6/36, Vero cells and human fibroblast BJ. *Antivir Ther* 2023;**28**:13596535231155263.
104. Kaur P, Thiruchelvan M, Lee RC, Chen H, Chen KC, Ng ML, et al. Inhibition of chikungunya virus replication by harringtonine, a novel antiviral that suppresses viral protein expression. *Antimicrob Agents Chemother* 2013;**57**:155−67.
105. Oyewole OO, Dunnavant K, Bhattarai S, Kharel Y, Lynch KR, Santos WL, et al. A novel sphingosine kinase inhibitor suppresses Chikungunya virus infection. *Viruses* 2022;**14**:1123.
106. Mishra S, Pandey A, Manvati S. Coumarin: an emerging antiviral agent. *Heliyon* 2020;**6**:e03217.

107. Oo A, Rausalu K, Merits A, Higgs S, Vanlandingham D, Bakar SA, et al. Deciphering the potential of baicalin as an antiviral agent for Chikungunya virus infection. *Antiviral Res* 2018;**150**:101−11.
108. Lani R, Hassandarvish P, Chiam CW, Moghaddam E, Chu JJ, Rausalu K, et al. Antiviral activity of silymarin against chikungunya virus. *Sci Rep* 2015;**5**:11421.
109. Tripathi PK, Singh J, Gaurav N, Garg DK, Patel AK. *In silico* and biophysical investigation of biomolecular interaction between naringin and nsP2 of the chikungunya virus. *Int J Biol Macromol* 2020; **160**:1061−6.
110. Zandi K, Teoh BT, Sam SS, Wong PF, Mustafa MR, Abubakar S. Antiviral activity of four types of bioflavonoid against dengue virus type-2. *Virol J* 2011;**8**:560.
111. Gupta S, Mishra KP, Gupta R, Singh SB. Andrographolide—a prospective remedy for chikungunya fever and viral arthritis. *Int Immunopharmacol* 2021;**99**:108045.
112. Tran QTN, Lee RCH, Liu HJ, Ran D, Low VZL, To DQ, et al. Discovery and development of labdane-oxindole hybrids as small-molecule inhibitors against chikungunya virus infection. *Eur J Med Chem* 2022;**230**:114110.
113. Cao V, Loeanurit N, Hengphasatporn K, Hairani R, Wacharachaisurapol N, Prompila N, et al. The 8-bromobaicalein alleviated Chikungunya-induced musculoskeletal inflammation and reduced the viral load in healthy adult mice. *Emerg Microbes Infect* 2023;**12**:2270074.
114. Reis ACC, de Oliveira CM, Rangel BC, de Carvalho LVB, Portruneli C, Agostini LDC, et al. Paulownin triazole-chloroquinoline derivative: a promising antiviral candidate against chikungunya virus. *Lett Appl Microbiol* 2025;**78**:ovaf092.
115. Rajput A, Thakur A, Rastogi A, Choudhury S, Kumar M. Computational identification of repurposed drugs against viruses causing epidemics and pandemics *via* drug−target network analysis. *Comput Biol Med* 2021;**136**:104677.
116. Kalam N, Ali R, Balasubramaniam VRMT. Exploring the potential of direct-acting antivirals against Chikungunya virus through structure-based drug repositioning and molecular dynamic simulations. *Comput Biol Med* 2025;**189**:109989.
117. Alcaro S, Bolognesi ML, García-Sosa AT, Rapposelli S. Editorial: Multi-Target-Directed Ligands (MTDL) as challenging research tools in drug discovery: from design to pharmacological evaluation. *Front Chem* 2019;**7**:71.
118. chneider P, Schneider G. Privileged structures revisited. *Angew Chem Int Ed Engl* 2017;**56**:7971−4.
119. Kobayashi J, Arita M, Sakai S, Kojima H, Senda M, Senda T, et al. Ligand recognition by the lipid transfer domain of human OSBP is important for enterovirus replication. *ACS Infect Dis* 2022;**8**:1161−70.
120. Roberts BL, Severance ZC, Bensen RC, Le-McClain AT, Malinky CA, Mettenbrink EM, et al. Differing activities of oxysterol-binding protein (OSBP) targeting anti-viral compounds. *Antiviral Res* 2019;**170**:104548.
121. Ho YJ, Liu FC, Yeh CT, Yang CM, Lin CC, Lin TY, et al. Micafungin is a novel anti-viral agent of chikungunya virus through multiple mechanisms. *Antiviral Res* 2018;**159**:134−42.
122. Zheng X, He Y, Xia B, Tang W, Zhang C, Wang D, et al. Etravirine prevents west Nile virus and Chikungunya virus infection both *in vitro* and *in vivo* by inhibiting viral replication. *Pharmaceutics* 2024;**16**:1111.
123. Nehul S, Rani R, Walia P, Panda PK, Singh S, Chattopadhyay S, et al. Repurposing efavirenz, the HIV antiretroviral drug for Chikungunya virus infection. *ACS Infect Dis* 2025;**11**:963−76.
124. Rabelo VW, Sanchez-Nuñez ML, Corrêa-Amorim LS, Kuhn RJ, Abreu PA, Paixão I. *In silico* drug repurposing uncovered the antiviral potential of the antiparasitic drug oxibendazole against the Chikungunya virus. *ACS Omega* 2024;**9**:27632−42.
125. Martins YA, Gonçalves TM, Lopez RFV. HPLC methods for choloroquine determination in biological samples and pharmaceutical products. *Daru* 2021;**29**:223−39.
126. Amaral JK, Lucena G, Schoen RT. Chikungunya arthritis treatment with Methotrexate and dexamethasone: a randomized, double-blind, placebo-controlled trial. *Curr Rheumatol Rev* 2024;**20**:337−46.
127. Kubo S, Nakayamada S, Sakata K, Kitanaga Y, Ma X, Lee S, et al. Janus kinase inhibitor baricitinib modulates human innate and adaptive immune system. *Front Immunol* 2018;**9**:1510.
128. Almulhim M, Ghasemian A, Memariani M, Karami F, Yassen ASA, Alexiou A, et al. Drug repositioning as a promising approach for the eradication of emerging and re-emerging viral agents. *Mol Divers* 2025;**29**:5465−85.
129. Lee WW, Teo TH, Her Z, Lum FM, Kam YW, Haase D, et al. Expanding regulatory T cells alleviates chikungunya virus-induced pathology in mice. *J Virol* 2015;**89**:7893−904.
130. Lee WW, Teo TH, Lum FM, Andiappan AK, Amrun SN, Rénia L, et al. Virus infection drives IL-2 antibody complexes into pro-inflammatory agonists in mice. *Sci Rep* 2016;**6**:37603.
131. Lee SY, Cho ML, Oh HJ, Ryu JG, Park MJ, Jhun JY, et al. Interleukin-2/anti-interleukin-2 monoclonal antibody immune complex suppresses collagen-induced arthritis in mice by fortifying interleukin-2/STAT5 signalling pathways. *Immunology* 2012;**137**: 305−16.
132. Davuluri KS, Ghanghav R, Ahire G, Kakade M, Cherian S, Alagarasu K, et al. Repurposed drugs in combinations exert additive anti-chikungunya virus activity: an *in-vitro* study. *Virol J* 2024;**21**:5.
133. Agarwal G, Gupta S, Gabrani R, Gupta A, Chaudhary VK, Gupta V. Virtual screening of inhibitors against Envelope glycoprotein of Chikungunya virus: a drug repositioning approach. *Bioinformation* 2019;**15**:439−47.
134. Tripathi PK, Soni A, Singh Yadav SP, Kumar A, Gaurav N, Raghavendhar S, et al. Evaluation of novobiocin and telmisartan for anti-CHIKV activity. *Virology* 2020;**548**:250−60.
135. Watanabe Y, Suzuki Y, Emi A, Murakawa T, Hishiki T, Kato F, et al. Identification of the corticotropin-releasing factor receptor 1 antagonists as inhibitors of Chikungunya virus replication using a Gaussia luciferase-expressing subgenomic replicon. *Biochem Biophys Res Commun* 2022;**637**:181−8.
136. Sun J, Zhong H, Wang K, Li N, Chen L. Gains from no real PAINS: where 'Fair Trial Strategy' stands in the development of multi-target ligands. *Acta Pharm Sin B* 2021;**11**:3417−32.
137. Gao S, Huang T, Song L, Xu S, Cheng Y, Cherukupalli S, et al. Medicinal chemistry strategies towards the development of effective SARS-CoV-2 inhibitors. *Acta Pharm Sin B* 2022;**12**:581−99.
138. Song L, Gao S, Ye B, Yang M, Cheng Y, Kang D, et al. Medicinal chemistry strategies towards the development of non-covalent SARS-CoV-2 Mpro inhibitors. *Acta Pharm Sin B* 2024;**14**:87−109.
139. Bassetto M, Brancale A. Chapter Four—The search for antivirals to treat alphavirus infections. In: Seley-Radtke Keditor. *Coronaviruses and other novel antiviral targets, annual reports in medicinal chemistry.* Academic Press. pp. 133−151.
140. Agarwal G, Gabrani R. Antiviral peptides: identification and validation. *Int J Pept Res Ther* 2021;**27**:149−68.
141. Pohjala L, Utt A, Varjak M, Lulla A, Merits A, Ahola T, et al. Inhibitors of alphavirus entry and replication identified with a stable Chikungunya replicon cell line and virus-based assays. *PLoS One* 2011;**6**:e28923.
142. El-labbad EM, Ismail MA, Abou Ei Ella DA, Ahmed M, Wang F, Barakat KH, et al. Discovery of novel peptidomimetics as irreversible CHIKV NsP2 protease inhibitors using quantum mechanical-based ligand descriptors. *Chem Biol Drug Des* 2015;**86**: 1518−27.
143. Dhindwal S, Kesari P, Singh H, Kumar P, Tomar S. Conformer and pharmacophore based identification of peptidomimetic inhibitors of chikungunya virus nsP2 protease. *J Biomol Struct Dyn* 2017;**35**: 3522−39.
144. Rabelo VW, de Palmer Paixão ICN, Abreu PA. Structural insights into the inhibition of the nsP2 protease from Chikungunya virus by molecular modeling approaches. *J Mol Model* 2022;**28**:311.

145. Qamar S, Beg M, Qureshi MI. Insights from the molecular docking analysis of gambogic acid with the Chikungunya spike glycoprotein E2. *Bioinformation* 2023;**19**:525−30.
146. Wardana AP, Aminah NS, Rosyda M, Abdjan MI, Kristanti AN, Tun KNW, et al. Potential of diterpene compounds as antivirals, a review. *Heliyon* 2021;**7**:e07777.
147. Kim AS, Kafai NM, Winkler ES, Gilliland Jr TC, Cottle EL, Earnest JT, et al. Pan-protective anti-alphavirus human antibodies target a conserved E1 protein epitope. *Cell* 2021;**184**:4414−29.e19.
148. Kim J, Yang J, Kim YB, Lee HJ, Kim S, Poo H. Development of a specific CHIKV-E2 monoclonal antibody for Chikungunya diagnosis. *Virol Sin* 2019;**34**:563−71.
149. Selvarajah S, Sexton NR, Kahle KM, Fong RH, Mattia KA, Gardner J, et al. A neutralizing monoclonal antibody targeting the acid-sensitive region in chikungunya virus E2 protects from disease. *PLoS Negl Trop Dis* 2013;**7**:e2423.
150. Pal P, Dowd KA, Brien JD, Edeling MA, Gorlatov S, Johnson S, et al. Development of a highly protective combination monoclonal antibody therapy against Chikungunya virus. *PLoS Pathog* 2013;**9**: e1003312.
151. Fox JM, Long F, Edeling MA, Lin H, van Duijl-Richter MKS, Fong RH, et al. Broadly neutralizing alphavirus antibodies bind an epitope on E2 and inhibit entry and egress. *Cell* 2015;**163**:1095−107.
152. Zhou QF, Fox JM, Earnest JT, Ng TS, Kim AS, Fibriansah G, et al. Structural basis of Chikungunya virus inhibition by monoclonal antibodies. *Proc Natl Acad Sci U S A* 2020;**117**:27637−45.
153. Warter L, Lee CY, Thiagarajan R, Grandadam M, Lebecque S, Lin RT, et al. Chikungunya virus envelope-specific human monoclonal antibodies with broad neutralization potency. *J Immunol* 2011; **186**:3258−64.
154. Quiroz JA, Malonis RJ, Thackray LB, Cohen CA, Pallesen J, Jangra RK, et al. Human monoclonal antibodies against chikungunya virus target multiple distinct epitopes in the E1 and E2 glycoproteins. *PLoS Pathog* 2019;**15**:e1008061.
155. Fox JM, Roy V, Gunn BM, Huang L, Edeling MA, Mack M, et al. Optimal therapeutic activity of monoclonal antibodies against chikungunya virus requires Fc−FcγR interaction on monocytes. *Sci Immunol* 2019;**4**:eaav5062.
156. Wang XY, Wang B, Wen YM. From therapeutic antibodies to immune complex vaccines. *npj Vaccines* 2019;**4**:2.
157. Chaudhary N, Weissman D, Whitehead KA. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Nat Rev Drug Discov* 2021;**20**:817−38.
158. Khurana A, Allawadhi P, Khurana I, Allwadhi S, Weiskirchen R, Banothu AK, et al. Role of nanotechnology behind the success of mRNA vaccines for COVID-19. *Nano Today* 2021;**38**:101142.
159. Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles−from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano* 2021;**15**:16982−7015.
160. Shin MD, Shukla S, Chung YH, Beiss V, Chan SK, Ortega-Rivera OA, et al. COVID-19 vaccine development and a potential nanomaterial path forward. *Nat Nanotechnol* 2020;**15**:646−55.
161. Dapurkar D, Telang M. A patent perspective on chikungunya. *Acta Trop* 2019;**199**:105131.
162. Saxena SK. Re-emergence of the Knotty Chikungunya virus: facts, fear or fiction. *Future Virol* 2007;**2**:121−6.
163. Gong EY, Bonfanti JF, Ivens T, Van der Auwera M, Van Kerckhove B, Kraus G. Development of a high-throughput antiviral assay for screening inhibitors of chikungunya virus and generation of drug-resistant mutations in cultured cells. *Methods Mol Biol* 2013; **1030**:429−38.
164. Chaudhary N, Weissman D, Whitehead KA. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Nat Rev Drug Discov* 2021;**20**:817−38.
165. Shin MD, Shukla S, Chung YH, Beiss V, Chan SK, Ortega-Rivera OA, et al. COVID-19 vaccine development and a potential nanomaterial path forward. *Nat Nanotechnol* 2020;**15**:646−55.
166. Panda K, Alagarasu K, Tagore R, Paingankar M, Kumar S, Jeengar MK, et al. RNAi-induced gene silencing against Chikungunya and COVID-19: what have we learned so far, and what is the way forward?. *Viruses* 2024;**16**:1489.
167. Karlas A, Berre S, Couderc T, Varjak M, Braun P, Meyer M, et al. A human genome-wide loss-of-function screen identifies effective chikungunya antiviral drugs. *Nat Commun* 2016;**7**:11320.
168. Agarwal A, Sarma DK, Chaurasia D, Maan HS. Novel molecular approaches to combat vectors and vector-borne viruses: special focus on RNA interference (RNAi) mechanisms. *Acta Trop* 2022;**233**: 106539.
169. Huesken D, Lange J, Mickanin C, Weiler J, Asselbergs F, Warner J, et al. Design of a genome-wide siRNA library using an artificial neural network. *Nat Biotechnol* 2005;**23**:995−1001.
170. Li Q, Zhang F, Lu Y, Hu H, Wang J, Guo C, et al. Highly potent multivalent VHH antibodies against Chikungunya isolated from an alpaca naïve phage display library. *J Nanobiotechnology* 2022;**20**:231.
171. Prosser O, Stonehouse NJ, Tuplin A. Inhibition of Chikungunya virus genome replication by targeting essential RNA structures within the virus genome. *Antiviral Res* 2023;**211**:105523.
172. Ayipo YO, Bakare AA, Badeggi UM, Jimoh AA, Lawal A, Mordi MN. Recent advances on therapeutic potentials of gold and silver nanobiomaterials for human viral diseases. *Curr Res Chem Biol* 2022;**2**:100021.
173. Villeret B, Dieu A, Straube M, Solhonne B, Miklavc P, Hamadi S, et al. Silver nanoparticles impair retinoic acid-inducible gene I-mediated mitochondrial antiviral immunity by blocking the autophagic flux in lung epithelial cells. *ACS Nano* 2018;**12**:1188−202.
174. Shahrtash SA, Ghnim ZS, Ghaheri M, Adabi J, Hassanzadeh MA, Yasamineh S, et al. Recent advances in the role of different nanoparticles in the various biosensors for the detection of the Chikungunya virus. *Mol Biotechnol* 2025;**67**:54−79.
175. Sharma P, Hasan MR, Naikoo UM, Khatoon S, Pilloton R, Narang J. Aptamer based on silver nanoparticle-modified flexible carbon ink printed electrode for the electrochemical detection of Chikungunya virus. *Biosensors* 2024;**14**:344.
176. Duarte JL, Filippo LDD, Araujo VHS, Oliveira A, de Araújo JTC, Silva F, et al. Nanotechnology as a tool for detection and treatment of arbovirus infections. *Acta Trop* 2021;**216**:105848.
177. Xu L, Ramadan S, Akingbade OE, Zhang Y, Alodan S, Graham N, et al. Detection of glial fibrillary acidic protein in patient plasma using on-chip graphene field-effect biosensors, in comparison with ELISA and single-molecule array. *ACS Sens* 2022;**7**:253−62.
178. Cardoso-Lima R, Filho J, de Araujo Dorneles ML, Gaspar RS, Souza PFN, Costa Dos Santos C, et al. Nanomechanical and vibrational signature of Chikungunya viral particles. *Viruses* 2022;**14**:2821.
179. Kandaswamy S, Srinet S, Praturi U, Pydigummala JS, Ella K. Vaccines for emerging infections: chikungunya vaccine. *Int J Infect Dis* 2016;**45**:420.
180. Kovacikova K, Gorostiola González M, Jones R, Reguera J, Gigante A, Pérez-Pérez MJ, et al. Structural insights into the mechanisms of action of functionally distinct classes of Chikungunya virus nonstructural protein 1 inhibitors. *Antimicrob Agents Chemother* 2021;**65**:e0256620.
181. Law YS, Wang S, Tan YB, Shih O, Utt A, Goh WY, et al. Interdomain flexibility of Chikungunya virus nsP2 helicase-protease differentially influences viral RNA replication and infectivity. *J Virol* 2021;**95**:e01470-20.
182. Law YS, Utt A, Tan YB, Zheng J, Wang S, Chen MW, et al. Structural insights into RNA recognition by the Chikungunya virus nsP2 helicase. *Proc Natl Acad Sci U S A* 2019;**116**:9558−67.
183. Ghildiyal R, Gabrani R. Antiviral therapeutics for chikungunya virus. *Expert Opin Ther Pat* 2020;**30**:467−80.
184. Lu JW, Hsieh PS, Lin CC, Hu MK, Huang SM, Wang YM, et al. Synergistic effects of combination treatment using EGCG and suramin against the chikungunya virus. *Biochem Biophys Res Commun* 2017;**491**:595−602.

185. Kasabe B, Ahire G, Patil P, Punekar M, Davuluri KS, Kakade M, et al. Drug repurposing approach against chikungunya virus: an *in vitro* and *in silico* study. *Front Cell Infect Microbiol* 2023;**13**:1132538.
186. Davuluri KS, Shukla S, Kakade M, Cherian S, Alagarasu K, Parashar D. Explorations on the antiviral potential of zinc and magnesium salts against chikungunya virus: implications for therapeutics. *Front Cell Infect Microbiol* 2024;**14**:1335189.
187. Verwimp S, Wagoner J, Arenas EG, De Coninck L, Abdelnabi R, Hyde JL, et al. Combinations of approved oral nucleoside analogues confer potent suppression of alphaviruses *in vitro* and *in vivo*. *Antiviral Res* 2025;**239**:106186.
188. Logiudice J, Tiecco G, Pavesi A, Bertoni F, Gerami R, Zanella I, et al. Novel and repurposed antiviral molecules for arbovirus infections with epidemic potential: a systematic review. *New Microbes New Infect* 2025;**66**:101614.
189. España G, Grefenstette J, Perkins A, Torres C, Campo Carey A, Diaz H, et al. Exploring scenarios of chikungunya mitigation with a data-driven agent-based model of the 2014-2016 outbreak in Colombia. *Sci Rep* 2018;**8**:12201.
190. Chang AY, Tritsch SR, Porzucek AJ, Schwartz AM, Seyler-Schmidt M, Glass A, et al. A mouse model for studying post-acute arthritis of Chikungunya. *Microorganisms* 2021;**9**:1998.
191. Bishop CR, Caten FT, Nakaya HI, Suhrbier A. Chikungunya patient transcriptional signatures faithfully recapitulated in a C57BL/6J mouse model. *Front Immunol* 2022;**13**:1092370.
192. Chen H, Min N, Ma L, Mok CK, Chu JJH. Adenovirus vectored IFN-α protects mice from lethal challenge of Chikungunya virus infection. *PLoS Negl Trop Dis* 2020;**14**:e0008910.
193. Dupuis-Maguiraga L, Noret M, Brun S, Le Grand R, Gras G, Roques P. Chikungunya disease: infection-associated markers from the acute to the chronic phase of arbovirus-induced arthralgia. *PLoS Negl Trop Dis* 2012;**6**:e1446.
194. Osiri M, Dilokthornsakul P, Chokboonpium S, Suthipinijtham P, Koolvisoot A. Budget impact of sequential treatment with biologics, biosimilars, and targeted synthetic disease-modifying antirheumatic drugs in thai patients with rheumatoid arthritis. *Adv Ther* 2021;**38**: 4885−99.
195. Webb E, Michelen M, Rigby I, Dagens A, Dahmash D, Cheng V, et al. An evaluation of global Chikungunya clinical management guidelines: a systematic review. *eClinicalMedicine* 2022;**54**:101672.
196. Fonseca DA, Amaral I, Pinto AC, Cotrim MD. Orphan drugs: major development challenges at the clinical stage. *Drug Discov Today* 2019;**24**:867−72.
197. Soares-Schanoski A, Baptista Cruz N, de Castro-Jorge LA, de Carvalho RVH, Santos CAD, Rós ND, et al. Systems analysis of subjects acutely infected with the Chikungunya virus. *PLoS Pathog* 2019;**15**:e1007880.
198. Khurana M, Egger GF, Yao L, Thompson A, Pallidis C, Goldstein SL, et al. Overcoming barriers to drug development in children with CKD. *Clin J Am Soc Nephrol CJASN* 2023;**18**:1101−7.
199. Chopra A, Venugopalan A. Chikungunya and other viral arthritis. *Best Pract Res Clin Rheumatol* 2025;**39**:102068.
200. Christofferson RC, Chisenhall DM, Wearing HJ, Mores CN. Chikungunya viral fitness measures within the vector and subsequent transmission potential. *PLoS One* 2014;**9**:e110538.
201. Wada Y, Orba Y, Sasaki M, Kobayashi S, Carr MJ, Nobori H, et al. Discovery of a novel antiviral agent targeting the nonstructural protein 4 (nsP4) of Chikungunya virus. *Virology* 2017;**505**: 102−12.
202. Joubert PE, Werneke SW, de la Calle C, Guivel-Benhassine F, Giodini A, Peduto L, et al. Chikungunya virus-induced autophagy delays caspase-dependent cell death. *J Exp Med* 2012;**209**:1029−47.
203. Amaral JK, Schoen RT, Bingham CO, Chang A, Cândido EL. Immunomodulatory therapy of chikungunya arthritis: systematic review and meta-analysis. *J Travel Med* 2025;**32**:taaf067.
204. Keener A. Tailoring vaccines for older people and the very young. *Nature* 2019;**575**:S48−50.
205. Kaushik SB, Lebwohl MG. Psoriasis: which therapy for which patient: focus on special populations and chronic infections. *J Am Acad Dermatol* 2019;**80**:43−53.
206. Eberle RJ, Olivier DS, Pacca CC, Avilla CMS, Nogueira ML, Amaral MS, et al. *In vitro* study of hesperetin and hesperidin as inhibitors of Zika and Chikungunya virus proteases. *PLoS One* 2021; **16**:e0246319.
207. Jia X, Yang X, Luo G, Liang Q. Recent progress of microfluidic technology for pharmaceutical analysis. *J Pharm Biomed Anal* 2022; **209**:114534.
208. Zuo F, Li M, Tseng TM, Ho TY, Schlichtmann U. Relative-scheduling-based high-level synthesis for flow-based microfluidic biochips. In: *2021 IEEE/ACM international conference on computer aided design (ICCAD)*; 2021. p. 1−9. Available from: https://doi.org/10.1109/ICCAD51958.2021.9643477. Munich, Germany.
209. Walawalkar S, Almelkar S. Re-cellularised kidney scaffold for Chikungunya virus propagation: a novel approach. *Tissue Eng Regen Med* 2022;**19**:769−79.
210. Ding H, Tan P, Fu S, Tian X, Zhang H, Ma X, et al. Preparation and application of pH-responsive drug delivery systems. *J Control Release* 2022;**348**:206−38.
211. Constantin M, Bucatariu S, Ascenzi P, Butnaru M, Fundueanu G. Smart drug delivery system activated by specific biomolecules. *Mater Sci Eng C Mater Biol Appl* 2020;**108**:110466.
212. Tian B, Liu Y, Liu J. Smart stimuli-responsive drug delivery systems based on cyclodextrin: a review. *Carbohydr Polym* 2021;**251**: 116871.
213. Choi H, Choi K, Kim DH, Oh BK, Yim H, Jo S, et al. Strategies for targeted delivery of exosomes to the brain: advantages and challenges. *Pharmaceutics* 2022;**14**:672.
214. Seyedi SS, Shukri M, Hassandarvish P, Oo A, Shankar EM, Abubakar S, et al. Computational approach towards exploring potential anti-chikungunya activity of selected flavonoids. *Sci Rep* 2016;**6**:24027.
215. Ching KC, Kam YW, Merits A, Ng LFP, Chai CLL. Trisubstituted thieno[3,2-*b*]pyrrole 5-carboxamides as potent inhibitors of alphaviruses. *J Med Chem* 2015;**58**:9196−213.