



Chikungunya epidemiological survey and Current available inhibitors

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Abstract: Chikungunya is an insect borne disease virus spread through the mosquitoes to humans, causes the severe joint pains. The recent 2005-2007 outbreak was the most severe and one of the biggest eruptions caused by this virus. The cases of virus have been reported across the globe which was initially transmitted from African subcontinent, after its first outbreak in 1952 in Tanzania. Cause of disease remains to be explore and not well known. The complete epidemiology, mode of treatment and current research scenario through scientific literature on current CHIKV inhibitors was made to understand the in depth background of this disease. The current review posses the brief epidemiology, clinical manifestations, current mode of treatment and the number of antiviral agents (natural & synthetic) available up to date against the CHIKV infections along with vaccine development.

Keywords: Chikungunya; epidemiology; Antiviral therapy

1. Introduction

Chikungunya (CHIK) is one type pyrexia becoming important arbovirus disease, caused by bite of infected *Aedes (Ae)* mosquitoes^{1, 2} The latest upturn of symptoms associated with CHIK has strained worldwide population due to its drastic rapid onset, high morbidity, instantaneous spread and re-emergence across the globe by spreading its limits to newer areas.³⁻⁷ The most of imported cases to Europe, Americas was believed to be transmitted by the travelers as transmitters of the disease.^{3, 6} The fever, severe arthralgia, acute joint pains associated with CHIK has also been affected the millions of the population and made impact on socio-economy of India.^{1, 8} The ancient epidemics of CHIK fever and its symptoms was first described in 1952,⁹ in the border between Mozambique and Tanganyika. However, the causative pathogenic virus related to Chikungunya fever was first isolated in Tanzania.

1.1. Epidemiology and Worldwide Distribution of Chikungunya virus

The CHIKV first was arise in the countries of East/Central Africa,¹⁰ the pathogenic virus was originated in a sylvan cycle in the middle of forest-domicile mosquitoes in particular *Aedes* species and few

nonhuman primates. The urban areas of African countries and Asian continent was affected with CHIKV were transmitted from mosquitoes to humans and started its urban cycle which is quite similar to that of *Flaviviridae*. The two important disease carrying vectors which are responsible for transmission are identified i.e, *Ae. aegypti* and *Ae. albopictus* mosquitoes. The imported cases related to CHIKV transmission was documented in around 18 countries across the world, the virus is become localized in few countries of European Union.⁷

The urban outbreaks related CHIKV was reported in 1960s in the capital city of Thailand¹¹ and the urban areas of India was affected between 1963 to 1973.^{12, 13} Insignificant upsurges of disease happened over in next 30 years across the Asian and African countries and was neglected until 2004, after this a large epidemic outbreak was started on the coast of Kenya.^{7, 14} It led to transmit its limits to Asian countries along the Indian Ocean.¹⁴⁻¹⁶ The CHIKV strain responsible for large epidemic was throughout the period identified, and it was from Kenya.^{17, 18} Later, CHIKV was affected the 63% of the population and was documented in main island,¹⁶ almost 225,000 infections were reported with this virus.¹⁹

The re-emergence of virus was first detected early months of 2005 with least number of cases until 2006, during the period the virus has occupied its huge limits by affecting over 40,000 in La Reunion, almost 266,000 infection cases were reported, the neurological manifestations was also a part of them.^{20, 21} Infections associated, transmission of viral disease in La Reunion was believed to be through secondary vector i.e, *Ae. albopictus*.^{22, 23} The mutations in E1 glycoprotein associated with CHIKV virus is reported and was transmitted by *Ae. albopictus*.^{23, 24} The systemic immunological functions of individuals was responsible for the survival against viral transmission.²⁵ **Figure 1**

CHIKV virus was transmitted to Italy from India subcontinent, through the infected persons, become local by spreading through *Ae. albopictus* mosquitoes.²⁶ Therefore it has established the urban transmission

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cycle in Italy which can be maintained by humans as source. Moreover, from the major epidemic outbreak i.e period between 2004 to 2008, it was clearly indicated that virus can spread and infect humans across the Asia, Europe and now in Americas²⁷ and the worldwide spread of the disease through these vectors might be responsible for transmitting CHIKV.^{28,29}

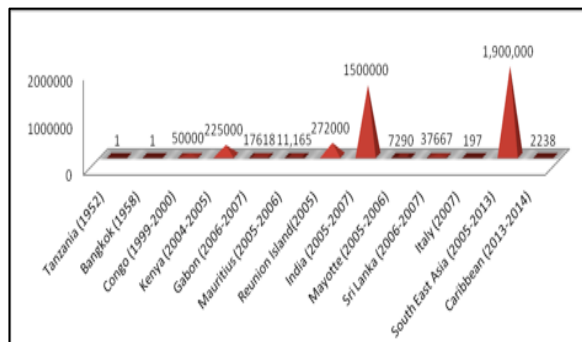


Figure 1 Reported cases of Chikungunya

1.2. CHIKV genome

CHIKV is a small, enveloped, positive-strand spherical RNA virus. The virion comprises with an envelope (E) and a nucleocapsid.³⁰ The CHIKV genome is made of 11,805 nucleotides in length and contains two essential polyproteins, the non structural polyprotein which intern consists of four nsP proteins (nsP1-nsP4) is responsible for viral replication and another named structural polyprotein consisting of five proteins (Capsid, E3, E2, 6K and E1). The 3' end of the RNA molecule is poly-adenylated and 5' end is capped with a 7-methylguanosine.³¹ The non structural polyproteins, nsP1 functions in viral mRNA capping, nsP2 codes for its protease activity and also helicase function, nsP3 has two roles i.e, replicase function and as additional protein for RNA synthesis, nsP4 involves in RNA-dependent-RNA polymerase activity. The structural polyproteins, which includes envelope glycoproteins of E2 and E3 responsible for the receptor binding, whereas E1 functions as fusion with cell membrane.

1.3. Clinical manifestations

CHIKV infections can cause acute, sub-acute, and chronic illness. Acute illness is most often characterized by sudden onset of high fever (typically greater than 102°F [39°C])⁷ and severe joint pain may be exhaustive and lasts for days to several months^{20,32,33}. The typical primary symptoms include the fever associated with arthralgia, polyarthralgias and rash. More than 90% cases were noticed with primary symptoms among the all investigated cases.^{20,33-35} Other common signs and symptoms may include muscle pain, acute arthralgia, nausea, headache, vomiting, rash, fatigue and myalgia.³⁶⁻³⁸ Diffuse back pain, polyarthritides and conjunctivitis.^{7,39-41} Onset of sickness due to CHIKV infection arises commonly between two to twelve days.³⁶ CHIKV and Dengue viral (DENV) infections are transmitted by the *Aedes* mosquitoes and causes the prolonged fever, may co-circulate, leading to both infections and simultaneous epidemics. CHIKV causes arthralgia which affects multiple joints are more consistent, but haemorrhage is quite similar in DENV. The specific symptoms or signs of both diseases help in

distinguish each one from other; it can be useful at the rural areas which lacks the diagnostic facility.⁴²

1.4. Treatment

Currently, the specific and precise antiviral therapy for CHIKV inhibition is not available but the limited supportive treatment is available to reduce the symptoms. The non-steroidal anti-inflammatory drugs (NSAIDs), anti-pyritics, analgesic agents are directed to treat the symptoms that includes fever associated with severe joint pains. Natural remedies and commercial vaccines to inhibit the CHIKV infections are also not available. CHIKV fever lasts no more than a few days but joint pains can be unbearable. The patient needs bed rest and supportive treatment.⁴³ Tremendous effort has been going on for the invention of antivirals, vaccine for the treatment of the present disease.

2. Current Available Inhibitors

The complete scientific literature was made in order to find the earlier reported inhibitors. Here we are listing the up to date antiviral agents including natural products, synthetic derivatives and vaccine candidates.

2.1. Natural products

The detailed natural product isolates searched for their anti-CHIKV activity, some research groups reported natural compounds which were isolated from few plant sources. The *in vitro* inhibition of CHIKV by Tannic acid and its related compounds was reported in early 1980s. The CHIKV inhibitory activity of tannic acid was pH dependant, suggested that virus-inactivating might be due to presence of phenolic-OH groups. The consecutive behavior of concavalin A on the growth of the CHIKV, it was stopped the release of virus from the infected cells followed by treatment, the plant *Canavalia ensiformis* is belongs to the family *legume* lectin.⁴⁴ The betulin and their derived compounds were screened for their inhibitory activity against CHIKV and few other alphaviruses.⁴⁵ A virus cell based assay was performed on the leaves of *Anacolasa pervilleana*, the triterpenoid compounds were isolated, lupenone and β -amyrone were showed anti CHIKV activity of $EC_{50}=77 \mu\text{m}$ and $86 \mu\text{m}$ respectively.^{46,47} The investigation of anti-CHIKV activity of a few 5,7-dihydroxy flavones and their derivatives were carried out and compounds named apigenin, chrysin, naringenin and silybin were showed the remarkable inhibition of the CHIKV infections.⁴⁵ The first series of halogenated active constituents trigocherrin A, B & F and trigocherriolide A, B & C from the bark of *Trigonostemon cherrieri* were recently isolated, later on these derivatives were described as the selective inhibitors against the CHIKV.^{48,49} The novel lead molecules belonging to Vietnamese plant *Trigonostemon howii*, the isolated active constituents, which were identified to inhibit the CHIKV infections with remarkable inhibitory activity. The trigowiin A is structurally similar to prostaticin, 12-O-Tetradecanoylphorbol-13-acetate (TPA) and various miscellaneous which were also reported here with their antiviral activity.⁴⁷

Harringtonine is a cephalotoxin alkaloid obtained from *Cephalotaxus harrintonica* trees, was reported to be inhibiting the synthesis of nonstructural nsP3 and structural E2 envelop proteins, belongs to both positive and negative sense CHIKV RNA, and alkaloid showed the minimal cytotoxicity. An analogue of harringtonine,

homoharringtonine, with an extra methyl functional group, also posses to have anti-CHIKV activity.^{50, 51} The anti-CHIK activity of harringtonine was described, which has been prescribing for the curing of chronic myeloid leukemia and approved by FDA. It became a promising lead molecule for the discovery of anti-CHIKV drugs. Recently the compounds 12-O-decanoyl-7-hydroperoxy-phorbol-5-ene-13-acetate and 12-O-decanoylphorbol-13-acetate were isolated from fresh leaves of *Croton mauritanus*. These compounds were structurally resembled with earlier reported natural isolates. The both isolates were inhibited the CHIKV strain by induced cell death mechanism having the EC₅₀s of 4.0 ± 0.8 μM and 2.4 ± 0.3, respectively.⁵² The selective antiviral activity of jatropene esters of plant *Euphorbia amygdaloides* was proved that the CHIKV virus is sensitive to this type of compounds which was having the same basic moiety with compare to earlier, among all, only single compound with EC₅₀ = 0.76 μM inhibited the CHIKV infection selectively and it was also having anti-HIV potency.⁵³ The detailed chemical structures and their inhibitory concentration values were given in **Table 1 & 2** respectively.

2.2. Synthetic Molecules

Chloroquine and Quinine

The anti malarial drug successfully used and reported 35 years ago. The alpha viruses shows the susceptibility to chloroquine and found to be effective *in vitro*, it has been reported that the EC₅₀ values of chloroquine in various clinically tested candidates were similar to the plasma concentrations during the treatment of malaria.⁵⁴ Based on the research, Chloroquine has classified under entry inhibitor, both chloroquine and chloroquine phosphate have been indicated for curing chikungunya Fever (CHIKVF),⁵⁵ overall report studies suggested that chloroquine is an effective drug lead molecule.

6-Azauridine

The antimetabolite shows the antiviral activity by inhibition of orotidine monophosphate decarboxylase,

which is required for biosynthesis of nucleosides through the *De novo* biosynthesis process. Its analogue generally used in the treatment of psoriasis without any side effects. At low concentration, it shown a significant activity of CHIKV.⁵⁶ Further careful evaluation leads *in vivo* as a CHIKV inhibitor.

Ribavirin and Interferon-α

The clinically approved ribavirin is a nucleoside drug that shows inhibitory activity against the classified RNA and DNA viruses.^{57, 58} Interferon-α and ribavirin on combination helps in the curing of chronic Hepatitis C in association with interferon, respiratory syncytial virus, Lassa fever virus, and Hanta virus.⁵⁷ The combination of these two compounds exhibited the synergistic effect via *in vitro* in the inhibition of pathogenic CHIKV. Ribavirin alone showed inhibition of CHIKV, but in combination it inhibits the CHIKV replication by 50%.⁵⁹ Further scope showed a synergistic effect of Ribavirin and interferon-α is suitable *in vivo* models.

Mycophenolic acid (MPA)

Mycophenolate mofetil (MMF) is the active drug constituent reported with anti-proliferative activity which inhibits the Type II inosine monophosphate dehydrogenase (IMPDH-II), which is a vital enzyme for purine biosynthesis. MPA (Mycophenolic acid) is a non-competitive inhibitor and an immunosuppressive agent has an effective use for the treatment organ transplant recipients which prevent the organ rejection.⁶⁰ Recent studies stated that, MPA can inhibit the CHIKV replication by virus induced cell death.⁵⁰ The authors suggesting it is a good lead compound, the treatment with MPA lowers the CHIKV infection.

Arbidol

Arbidol is a potent wide range of antiviral drug compound has been indicated for the curing of both form of HCV infection from past 24 years. The two cell lines under various conditions via *in vitro* test on the CHIKV were indicated that arbidol can effectively inhibit CHIKV infection. In HCV, influenza, common cold

Table 1. List of previously reported Natural products

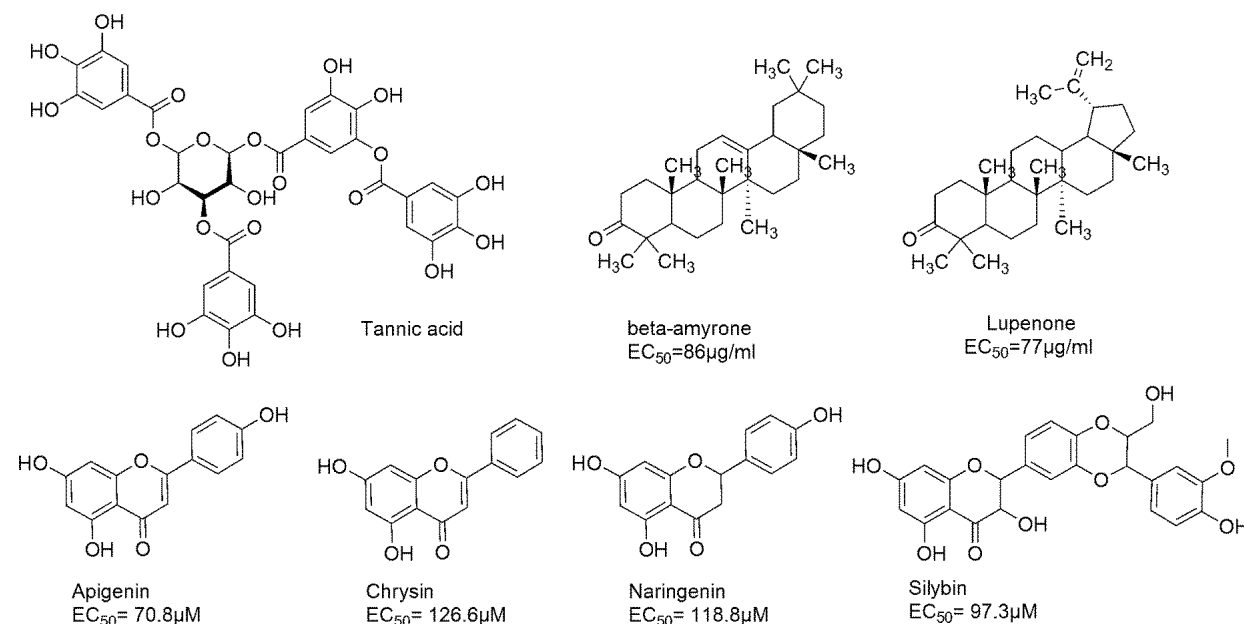
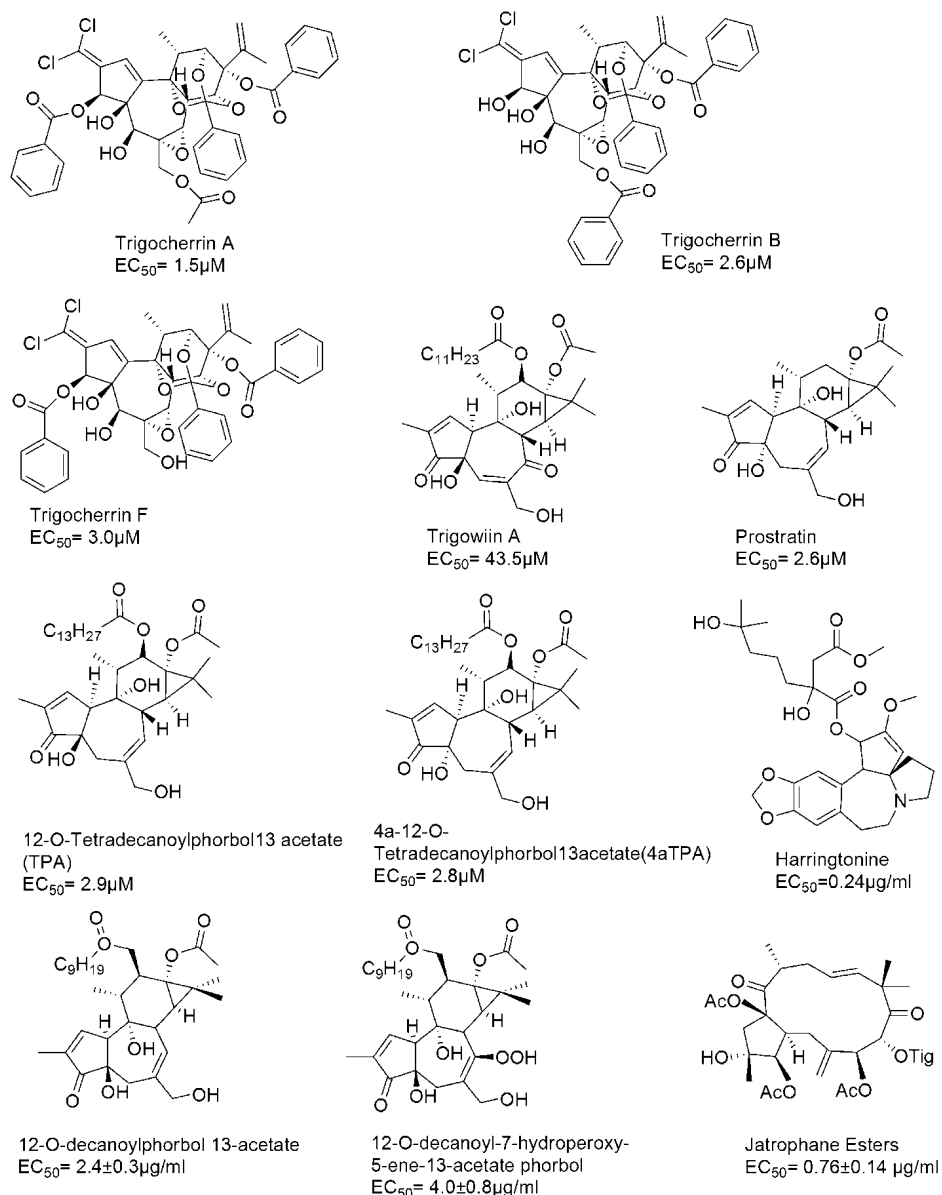


Table 2. List of recent reported Natural Product Isolates against Chikungunya

it shows the mode of action of arbidol is creating hindrance by blocking the virus at entry levels into the target cells *via* the glycoprotein conformational change inhibition.⁶¹ Overall, it blocks the early stages of virus life cycle which includes replication, attachment and virus entry.^{61,62} These studies imply that it is a potent antiviral compound and also it works for the CHIKV by targeting the cellular membranes.

10H-phenothiazines

Phenothiazines are viral entry inhibitors and reported with effective inhibition profile against the recombinant strain of CHIKV carrying a Luciferase gene. The compounds like ethopropazine, perphenazine, chlorpromazine, thioridazine, thiethylperazine and methdilazine are useful in CHIKV cases along with few neurological complications. The same class of compounds were inhibited Semliki Forest virus they are expecting to inhibit the CHIKV entry.⁶³

Polyinosinic acid-Polycytidylic acid

It is a most potent immunostimulant and its sodium salt used to simulate viral infections and it is synthetic

double stranded RNA analogue. Upon treatment of poly inosinic acid induced secretion of IFN-β and upregulated Toll like receptor (TLR3) used in CHIKV infection. It can be used as an auxiliary agent in vaccine development, and its sensitivity helps in reduction of cytopathic effect leads to inhibition of the virus replication in the medium cell lines.^{56,64}

Small Synthetic Molecules

The hydrazine derivatives with good inhibitory activity against CHIKV were published earlier. The hydrazine derivatives were identified based on the structure based drug design techniques by employing the nsP2 protease as target. The lead molecules obtained were further modified and screened for their efficiency. However, they were not showed the exact mechanism of action of these molecules.⁶⁵

Small molecule inhibitors for CHIKV infection: Kinase Inhibitors Library

Along with the kinase inhibitory profile a few promising lead molecules comprising of benzofuran, pyrrolopyridine and thiazole carboxamide derivatives

were identified through the combination of cell-based HTS assay using resazurin and image-based high content assay approach against CHIKV infections with novel anti-CHIK activity.⁶⁶ The elaborated details with

structures of all synthetic inhibitors were given in **Table 3 & 4** respectively.

Table 3. List of synthetic compounds reported against Chikungunya infections

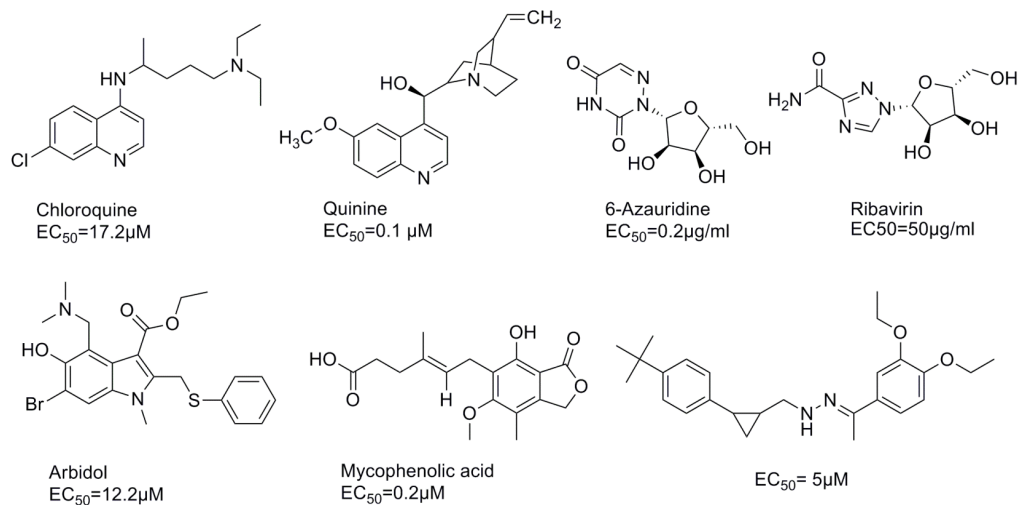


Table 4. List of Phenothiazenes & Kinase library inhibitors

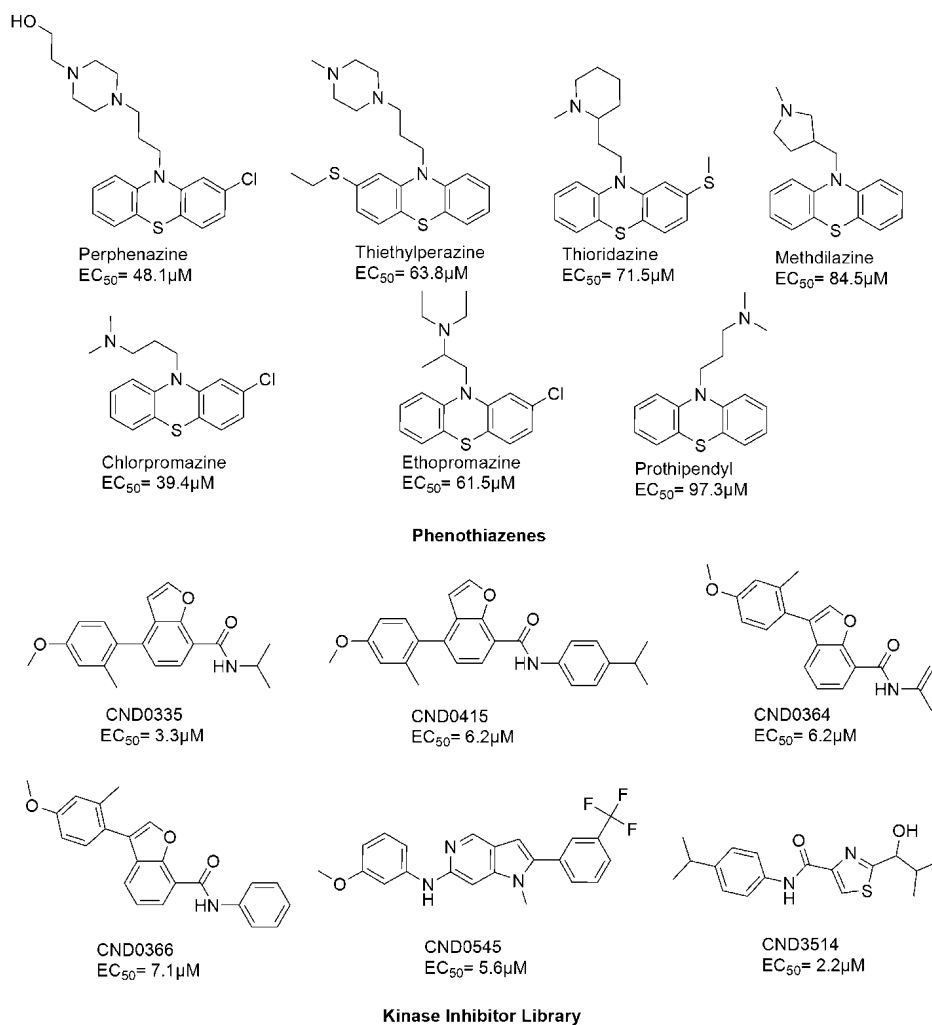


Table 5. The list of Vaccine under preclinical and clinical trials (Chronological)

S.No	Approach	Method	Status	Reference
1	Inactivated vaccine	Tween ether-inactivated CHIKV strains (African 168, Asian BAH-306, and Indian C-266) grown on GMK cells	Preclinical	67
	Inactivated vaccine	Formalin-inactivated CHIKV Thailand strain 15561 grown on GMK cells	Phase I	68
3	Live-attenuated vaccine	Attenuated CHIK 181/clone 25 developed by serial passage of CHIKV Thailand strain 15561 in MRC-5 cells	Preclinical	69
4	Live-attenuated vaccine	Live, attenuated TSI-GSD-218, CHIKV vaccine-infected with an attenuated strain, CHIK 181/clone 25	Completed Phase II	70
5	Genetically engineered vaccines	Chimeric vaccine: Using three alphavirus vaccine backbones; VEEV, EEEV, SINV and replacing the specific structural protein coding sequence with La Reunion strain	Preclinical	71
6	Genetically engineered vaccines	DNA vaccine: encoding C, E1, E2 genes of CHIKV by using three individual plasmids	Preclinical	72
7	Live-attenuated vaccine	Formalin-inactivated 2006 Indian strain grown on Vero cells adjuvanted by Alhydrogel	Preclinical	73
8	Genetically engineered vaccines	CHIKV genes inserted into nonreplicating adenovirus vectors produce recombinant expressing structural sequence from Asian and ECSA genotype isolates	Preclinical	74
9	Genetically engineered vaccines	CHIK-IRES: replacement of structural proteins for altering levels and host specific mechanism, CHIKV/IRES by EMCV/IRES	Preclinical	74
10	Genetically engineered vaccines	DNA vaccine encoding envelope glycoprotein by using single plasmid	Preclinical	75

2.3. The Anti-Chikv Vaccine Development Programme

The ideal vaccine therapy for the treatment of CHIKV infections is still not existing, and a few vaccine candidates are under clinical trials and preclinical trials.^{67, 76} The detail vaccine development program was listed in **Table 5**.

In year 1970, the vaccine development trials against CHIKV were initiated using tissue culture cells of green monkey kidneys. The vaccines have been developed by using Tween 80, and found that it protects from three CHIKV strains (African 168, Indian C-266 and Asian BAH-306).⁶⁷ Later, the four CHIKV isolates from human (Thailand strains CHIK6461, 6348, 23337 and 15561) were killed by the experimental formalin. The individual potency tests of all strains in mice and monkeys, CHIK 15561 entered in Clinical trials (Phase I).⁶⁸ In mid 1980s, the live attenuated vaccines were discovered for the first time by passaging the CHIK strain 15661 in to MRC-25 cells. This mimics the cellular responses followed by natural infections, gives the prolonged immunity by neutralizing the antibodies (Mice and rhesus monkeys).⁶⁹ In year 2000, the side effects of the live attenuated vaccine candidate include rheumatism was reported.⁷⁰

The chimeric vaccine candidate was manufactured by genetic recombination process using backbones of alphavirus (VEEV, EEEV and SINV), the test results in mice, signs of infections caused by the CHIKV were absent; whereas live vaccine caused the rheumatism.⁷¹ A few reports of immunogenic effects of vaccine which was developed based on consensus-DNA vaccine methods were reported.⁷² Inactivated vaccine using aluminum hydroxide as adjuvant, reported the potency in mice with remarkable immunogenicity to neutralize the virus infectivity in against ECSA genotype strain,

which was obtained during outbreak 2006 in India.⁷³ The chimeric vaccine candidates from the structural proteins of CHIKV, with lowest antiviral responses with the capability of interference were studied.⁷⁴ The new vaccine was constructed by using IRES (internal ribosome entry site) of encephalomyocarditis virus (EMCV), later found that it is effective with immunogenicity.⁷⁴ Synthetic DNA vaccine candidate which was provided the immunogenicity against infections caused by the CHIKV.⁷⁵ The new vaccine candidates, who were produced from the CHIK strain 37997 structural proteins by using the safe virus like particles (VLP) in human kidney cells (293T). The proteins are responsible for virus replication were absent in viruses like particles (VLP). These are the major composition of the CHIK vaccine, makes easy for passaging of the CHIKV through the cell wall. (<http://clinicaltrials.gov/ct2/show/NCT01489358>).

Conclusion

The CHIKV viral infections have drawn the universal recognition due to its inception, expeditious spread, and high morbidity. Up to the present time, there is no possible drug discovery for the chikungunya virus due to paucity of scientific knowledge. Recently a few promising lead molecules were discovered through high throughput virtual screening, including synthetic and natural, which can be a starting point towards effective treatment. The lack of information about enzymatic functions of non structural poly proteins makes inexpedient to design the small molecule inhibitors, further it can lead to have a detailed SAR studies. Ongoing studies will help to design safer and potent novel small molecule inhibitors for the chikungunya virus. The present study provides the list of various synthetic and natural lead molecules reported against the chikungunya virus infections till date.

List of Abbreviations:

CHIKV- Chikungunya virus; SAR- Structure activity relationship; RNA- Ribose nucleic acid; EC₅₀- Half maximal effective concentration; MPA- Mycophenolic acid; TLR- Toll like receptor; CHIKVF- Chikungunya fever; IFN-Interferon

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