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Review article

The medicinal chemistry of Chikungunya virus

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ABSTRACT

Arthropod-borne viruses (arboviruses) are an important threat to human and animal health globally. Among these, zoonotic diseases account for billions of cases of human illness and millions of deaths every year, representing an increasing public health problem. Chikungunya virus belongs to the genus *Alphavirus* of the family *Togariridae*, and is transmitted mainly by the bite of female mosquitoes of the *Aedes aegypti* and/or *A. albopictus* species. The focus of this review will be on the medicinal chemistry of Chikungunya virus, including synthetic and natural products, as well as rationally designed compounds.

Contents

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1. Introduction

During the evolution of the human species, infectious diseases have also evolved. The emergence of new diseases and the resurgence of old ones are a challenge to humanity.[1] Emerging infectious diseases are rapidly increasing in frequency and geographic range. Among these, zoonotic diseases account for billions of cases of human illness and millions of deaths every year, constituting a persistent health problem worldwide.[2]

Mosquitos (Diptera: *Culididae*) are vectors of pathogens and parasites of health diseases, such as dengue, zika, Chikungunya and lymphatic filariasis.[3] Additionally, mosquito control is of great importance throughout the world, especially in tropical and subtropical areas.[3]. However, the use of insecticides to kill mosquitoes has led to the development of resistance.[3]

In general, zoonotic viruses are transmitted to humans by hematophagous insects, such as mosquitoes, sandflies, ticks, and biting midges, and they are called arthropod-borne viruses (arboviruses).[2] In this sense, Chikungunya virus (CHIKV) is an arthropod-borne virus that belongs to the genus *Alphavirus* of the family *Togariridae*.[4–11] It is transmitted by the bite of female mosquitoes of the *Aedes aegypti*, *A. albopictus*, *A. furcifer* species[12] and *Culex* ssp.[2,4,5,12–17] Based on this, CHIKV can be transmitted through an urban cycle, man to mosquito to man, or through a sylvatic cycle, animal to mosquito to man.[18] Although in recent epidemic some cases were related to maternal-fetal transmission.[19] In 1952, CHIKV was firstly identified during an epidemic in Tanzania. In 2004, the global re-emergence of CHIKV started in Kenya, after which it spread to different islands in the Indian Ocean. Since the end of 1999, CHIKV infection has been reported in many countries in Central and South Americans, causing an estimated 11675,000 cases.[4,8,20,21] Actually, CHIKV is considered a real health problem where the Aedes mosquitoes thrive.[4]

In 2008, the United State National Institute of Allergy and Infectious Diseases (NIAID) included CHIKV into category C priority pathogen: this category is related to pathogens that could be utilized for mass dissemination in the future, due to their high morbidity and mortality rates and those with major health impacts.[12,22]

1.1. Clinical aspects of Chikungunya infection

During the acute phase, the viral load can reach 10^8 viral particles per mL of blood, and the plasma concentration of type I interferons (IFNs), ranging 0.5–2.0 ng mL^{-1}, associated with a robust induction of other pro-inflammatory cytokines and chemokines.[23–25]

Normally, the CHIKV symptoms begin between 4 and 7 days after a mosquito bite, it causes Chikungunya fever, which has symptoms, such as fever, arthralgia and, in some cases, a maculopapular rash.[4,5,7,9,26–30] It is rarely lethal, the disease proceeds in 60% of infected patients into a chronic stage that is characterized by persistent severe polyarthritis (predominantly Old World viruses).[4,12,31] Occasional cases of eye, heart, gastrointestinal, and neurological complications have been reported.[11,32–34] Basically, CHIKV illness severe joint pain in the ankles, fingers, toes, elbow, knees, and wrists. Additionally, the most of the patients fully recover, but in 10% of the cases, joint pain may persist for several for weeks, or months, or years.[35–37] In 40% of patients were found to still have anti-CHIKV immunoglobulin M (IgM), eighteen months after disease onset.[38,39]

In general, CHIKV attacks fibroblasts, suggesting its involvement with muscles, joints, and skin connective tissues. The high number of nerve endings within the joints and muscle tissues explains the pain associated with CHIKV.[40] Although, physical exercise are recommended to decrease the joint stiffness.[41]

In some cases, CHIKV has been associated with encephalitis in patients, leading to the increasingly strong suspicion of CHIKV being neurotropic (predominantly New World viruses).[7,8,12,25,31] Despite the propagation and the high morbidity rate of CHIKV infections, there is currently no approved vaccine or antiviral treatment available.[4,5,36] Although, researchers have recently reported on the development of a new candidate vaccine to protect against CHIKV infection.[42]

Basically, treatment has been limited to antipyretics, analgesics, corticosteroids, and non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate the symptoms.[4,37] In addition, disease-modifying antirheumatic drugs (DMARDs) such as methotrexate and sulphasalazine can be used in severe cases when NSAIDs are not

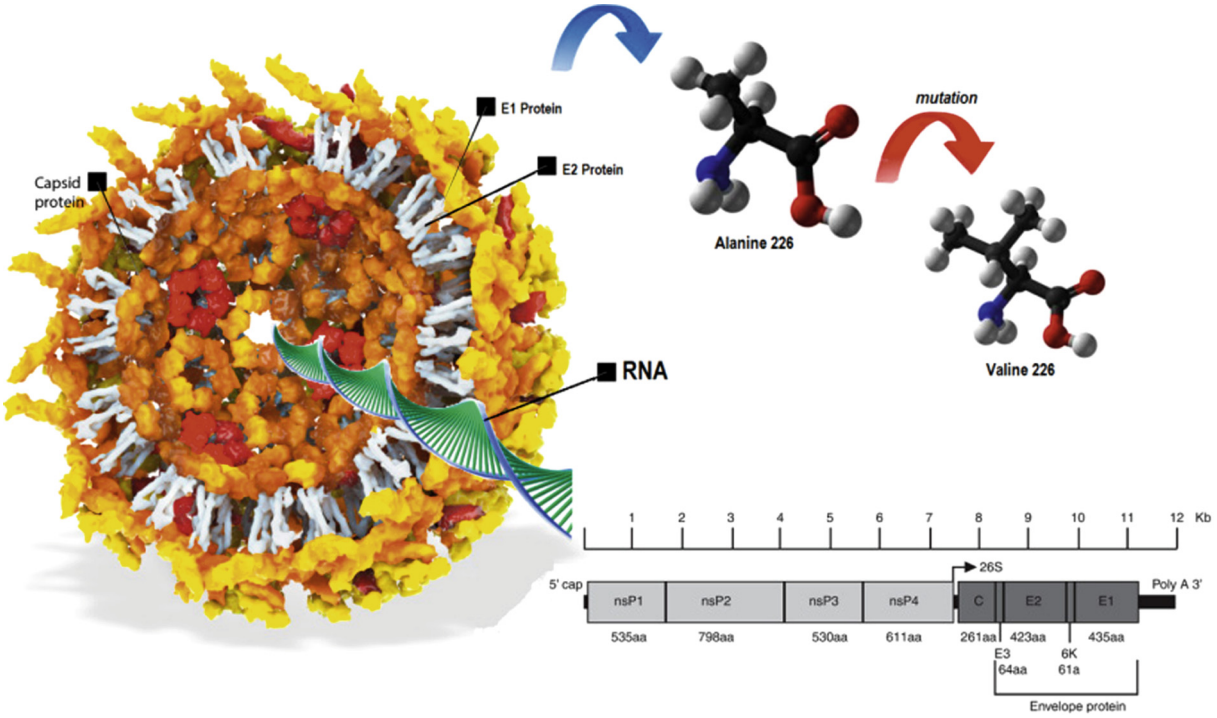


Fig. 1. Morphological visualization for Chikungunya virus, E_1 and E_2 proteins, genome length, and mutation in the Alanine residue.

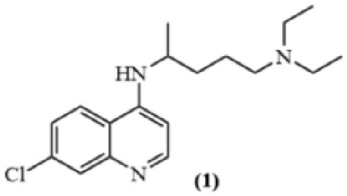


Fig. 2. Chloroquine (**1**) chemical structure.

effective.[30,43,44] Moreover, co-infection with Dengue (DENV), CHIKV, and Zika (ZIKV) has been reported in patients[9,45,46] Although, co-infection with ZIKV and CHIKV does not appear to increase the severity of the disease or the duration of arthritis.[47] Finally, chronic inflammatory rheumatisms following CHIKV infection are rare but potentially bone damaging. The most number of cases only require symptomatic treatments, mainly non-steroid anti-inflammatory drugs and physiotherapy.[36]

1.2. Virology of Chikungunya virus

Alphaviruses are membrane enveloped viruses, which consists of a single-stranded RNA as a genetic material and an icosahedral-like nucleocapsid of size (60–70 nm).[1] Genetically, the CHIKV possess an enveloped structure with a single-stranded, positive RNA genome.[25,45,48] Additionally, the CHIKV genome is approximately 11.8 kb long and comprises two open reading frames (ORFs) a 5′ end ORF capable to encode the four viral non-structural proteins (nsP_1-nsP_4) and a 3′ end ORF that encodes the viral structural proteins including capsid (C), two major enveloped (E) glycoproteins, E_1 and E_2, and two smaller accessories peptides, E_3 and 6K which and envelope proteins.[27,39,48–50] nsP_1 is involved in viral mRNA capping via its guanine-7-methyltransferase and guanylyltransferase enzymatic activities.[25,32] In addition, the viral nsP_2 protein has other enzymatic functions including RNA helicase, nucleoside triphosphatase (NTPase) and RNA-dependent 5′-triphosphatase activities which are located at the N-terminus of the protein, while the protease domain is located at the C-terminus nsP_3 acts as part of the replicase unit and an accessory protein involved in RNA synthesis.[15,27,51] Finally, nsP_4 acts as RNA-dependent-RNA polymerase.[27,32,52] The enveloped proteins E_1 and E_2 are responsible for formation of glycoproteins spikes on the viral particle surface and facilitate the binding of the viral particle to susceptible host cells (mutations of Alanine to Valine (Ala226Val) in the E_1 enveloped glycoprotein result in a new CHIKV strain that became more prevalent as the epidemic progressed)[48,53] (Fig. 1).

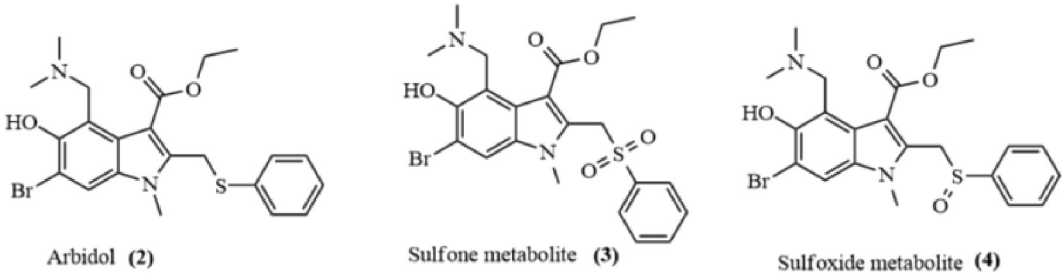
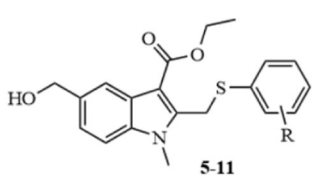


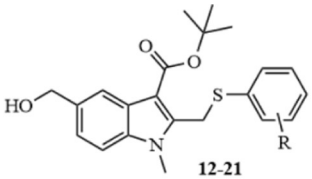
Fig. 3. Arbidol (**2**) and its sulfone (**3**) and sulfoxide (**4**) metabolites investigated by Delogu et al.

Table 1
Antiviral Activity of Arbidol Analogs Against CHIKV in Vero Cells.



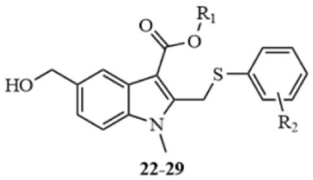
5-11

5=R= H
6=R= 4-OCH_3
7=R= 4-F
8=R= 4-CF_3
9=R= 4-OCF_3
10=R= 2,4-F
11=R= 2,6-Cl



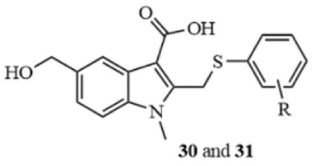
12-21

12=R= H
13=R= 4-OCH_3
14=R= 4-F
15=R= 4-CF_3
16=R= 4-OCH_3
17=R= 2,4-F
18=R= 2,6-Cl
19=R= 4-Cl
20=R= 2,4,6-CH_3
21=R= 2-CF_3



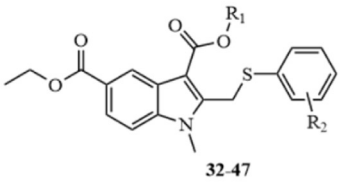
22-29

22=R_1= *t*-Bu; R_2=H
23=R_1= *t*-Bu; R_2= 4-F
24=R_1= *t*-Bu; R_2= 4-CF_3
25=R_1= *t*-Bu; R_2= 4-OCF_3
26=R_1= *t*-Bu; R_2= 2,6-CF_3
27=R_1= *t*-Bu; R_2= 2-CF_3
28=R_1= Et; R_2= H
29=R_1= Et; R_2= 2,6-Cl



30 and **31**

30=R= 4-CF_3
31=R= 4-Cl



32-47

32=R_1= Et; R_2= 4-OCH_3
33=R_1= Et; R_2= 4-F
34=R_1= Et; R_2= 4-CF_3
35=R_1= Et; R_2= 4-OCF_3
36=R_1= Et; R_2= 2,4-F
37=R_1= Et; R_2= 2,6-Cl
38=R_1= *t*-Bu; R_2= H
39=R_1= *t*-Bu; R_2= 4-OCH_3
40=R_1= *t*-Bu; R_2= 4-F
41=R_1= *t*-Bu; R_2= 4-CF_3
42=R_1= *t*-Bu; R_2= 4-OCF_3
43=R_1= *t*-Bu; R_2= 2,4-F
44=R_1= *t*-Bu; R_2= 2,6-Cl
45=R_1= *t*-Bu; R_2= 4-Cl
46=R_1= *t*-Bu; R_2= 2,4,6-CH_3
47=R_1= *t*-Bu; R_2= 2-CF_3

Compound	CHIKV inhibition (μM ± SD)[a]		SI[d]
	EC_{50}[b]	CC_{50}[c]	
(5)	>157	563 ± 12	3.6
(6)	>674	N.D	–
(7)	>691	N.D	–
(8)	>557	N.D	–
(9)	81.9 ± 12	464 ± 18	5.7
(10)	>654	N.D	–
(11)	35 ± 8	104 ± 12	2.9
(12)	N.A	N.C	–
(13)	N.A	N.C	–
(14)	14.4 ± 13	35 ± 8	2.4
(15)	11 ± 1.3	102 ± 39	9.0
(16)	28 ± 7	47 ± 18	1.7
(17)	N.A	N.C	–
(18)	18.5 ± 3	33 ± 4	1.8
(19)	11 ± 3	48 ± 7	4.4
(20)	N.A	N.C	–
(21)	>492	N.D	–
(22)	N.A	N.C	–
(23)	80 ± 11	527 ± 28	6.6
(24)	115 ± 1.2	>685	5.9
(25)	37 ± 4	180 ± 17	4.9
(26)	30 ± 4	397 ± 24	13.2
(27)	32 ± 1.1	>468	14.6
(28)	32 ± 3	172 ± 20	5.4
(29)	85 ± 4	203 ± 8	2.4
(30)	N.A	N.D	–
(31)	N.A	N.D	–
(32)	N.A	N.D	–
(33)	N.A	N.D	–
(34)	N.A	N.D	–
(35)	45.9 ± 3.1	>100	2.2
(36)	N.A	N.D	–
(37)	46.1 ± 2.7	68.8 ± 2.1	1.5
(38)	N.A	N.D	–
(39)	N.A	N.D	–
(40)	N.A	N.D	–
(41)	N.A	N.D	–
(42)	N.A	N.D	–
(43)	N.A	N.D	–

Table 1 (*continued*)

Compound	CHIKV inhibition (μM ± SD)[a]		
	EC_{50}[b]	CC_{50}[c]	SI[d]
(44)	N.A	N.D	–
(45)	N.A	N.D	–
(46)	N.A	N.D	–
(47)	N.A	N.D	–
(2)	35 ± 8	161 ± 18	4.6

N.A: not active. N.D: not determined.
[a] All data are mean values ± standard deviation for at least three independent experiments.
[b] 50% Effective concentration (concentration at which 50% inhibition of CPE is observed in Vero cells).
[c] 50% cytostatic/cytotoxic concentration (concentration at which 50% adverse effect is observed in the host cell).
[d] Selectivity index (CC_{50}/EC_{50}).

Additionally, it is known which alterations of Glycine407 to Arginine in the E_2 protein (Gly407Arg) are responsible for observed CHIKV resistance to arbidol drug, and they also regulate CHIKV adaptation.[54–56] Additionally, the CHIKV surface consists of 80 trimeric spikes composed of heterodimers of the envelope glycoproteins (E_1 and E_2) in the lipid bilayer.[57] CHIKV 6K is small, hydrophobic protein essential for the viral particle assembly, which acts as a signal sequence for the processing of the E_1 protein. The role of 6 K protein in viral replication is not fully resolved. Capsid protein is 261 amino acids (30 kDa) long, expressed as a part of the structural polyprotein, which has a conserved autoprotease domain at the C-terminal end that helps to release itself from the polypeptide string after synthesis of structural proteins.[1] In sequence, occurs the entry of the CHIKV virus via clathrin-dependent endocytosis and uncoating of the viral genome. Translation of viral RNA leads the viral non-structural protein complex, replicating the CHIKV RNA genome.[48] Then, this step is followed by translation of viral structural proteins and assembly of viral components within the cytoplasm.[48] Finally, viral particles bud out through the plasma membrane, forming mature, infectious progeny.[48] There are three genotypes, namely Asian (Asian), Eastern Central South African (ECSA), and West African (WA) strains.[45,58]

Furthermore, the amino acid sequence identity between CHIK and other alphavirus range from 58% to 85% and 42–85% in case of non-structural and structural proteins, respectively.[1]

2. Antiviral agents against Chikungunya virus

The proteins which mediate key steps in the virus life cycle can be targets for the design of new anti-CHIKV drugs. Inhibition of entry step presents an attractive therapeutic strategy because the damage caused by virulence factors during intracellular viral replication can be minimized. The identification of more CHIKV-specific receptors is crucial to driving future research into CHIKV entry inhibitors.[50]

Several previous studies have reported anti-CHIKV activities for some compounds and approved drugs, in vitro. These are chloroquine,[59,60] furin inhibitors,[61] arbidol,[31] mycophenolic acid,[62] picolinate,[63] interferon-α and ribavirin combination (considering that the IFN-α and IFN-β are mainly produced by leucocytes and fibroblasts, and those are associated with CHIKV physiopathology)[35,64–67] and others. Among these, only chloroquine has been tested *in vivo*, where proved to be poorly active.[27,61,68] Additionally, the CHIKV replication can be considered as a great starting point to identify potential targets during the development of novel antiviral compounds.[48] The antiviral mechanism of these agents is validated on the basis of their antioxidant and anti-inflammatory activities, scavenging capacities, immune-stimulatory properties inhibiting viral DNA and RNA synthesis, inhibition of viral entry, etc.[69] On the next pages, all these studies were organized and properly described.

2.1. CHIKV entry inhibitors

2.1.1. Chloroquine

Chloroquine (**1**) (Fig. 2), an antimalarial drug, has in vitro antiviral activity against a number of viruses, including HIV, severe acute respiratory syndrome, Alphaviruses and coronavirus. Chloroquine inhibits CHIKV replication in Vero A cells (IC_{50} = 7.0 μM, IC_{90} = 15.0 μM, SI = 37.14) in a dose-dependent manner.[70]

2.1.2. Arbidol and its analogs

The antiviral drug Arbidol (ARB) was originally developed for prophylaxis and treatment of acute respiratory infections including influenza.[31] Recently, it has been shown that ARB exhibits a wide range of activity against a number of RNA, DNA, enveloped and non-enveloped viruses. All these facts suggest that ARB targets a common critical step in virus-host cell interaction.[31] Additionally, ARB is capable of blocking virus entry target cells, in the case of influenza viruses or hepatitis C virus.[31]

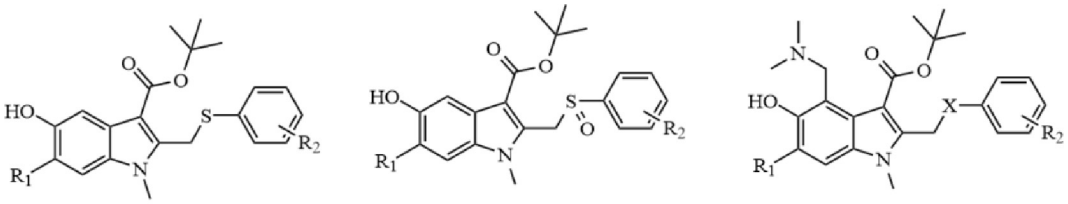
Delogu et al.[31] investigated the *in cellulo* antiviral ARB and its metabolites (Fig. 3) activity against CHIKV. Different cell lines were assayed (MCR-5 and Vero), in two conditions (pre- and post-infection treatment).

The effect of ARB (**2**) and its sulfone (**3**) and sulfoxide (**4**) metabolites on CHIKV replication were determined using specific indirect immunofluorescence. It was observed that (**2**) inhibits CHIKV infection with IC_{50} value at 12.2 μM. In addition, the CC_{50} values were obtained using Vero (679.9 μM) and MCR-5 (376 μM) cell lines. The (**2**) selectivity indices (CC_{50}/IC_{50}) were determined using Vero and MCR-5 cell lines, resulting in values about 28 and 36, respectively. In the case of ARB metabolites (**3** and **4**), some weak antiviral activity was observed, IC_{50} values at >56.4 and 54.5 μM concentration on Vero cell line. Finally, in order to investigate that direct inactivating effect of (**2**), the IC_{50} value was obtained 23.1 μM after 30 min incubation and 31.7 μM after 60 min incubation, indicating that the antiviral activity of (**2**) on CHIKV infection was not due to virucidal activity.[31]

Di Mola et al.[71] have used (**2**) to design novel analogs with anti-CHIKV activity, aiming to improve the (**2**) therapeutic index or to identify novel lead compounds. In Table 1 are summarized all results from inhibitory activity determination of these compounds.

With respect to the ethyl series (compounds **6**–**11**) it was noted that, when a substituent was introduced at the para position of the thiophenol ring, loss of anti-CHIKV activity was observed except for compound (**9**) (EC_{50} = 81.9 μM). The introduction of two bulky chlorine atoms at positions 2 and 6 (**11**) led to selective inhibition. The substitution of the ethyl ester at position 2 with a *tert*-butyl ester resulted in compounds more actives, with EC_{50} values ranging from 11 to 27.8 μM. The best compound was **15**, which is not only the most potent of this series (EC_{50} = 11 μM) but which also is less cytotoxic with selectivity index higher than (**2**). Finally, it obviously noted which the presence of an electron-withdrawing group at the para position of the thiophenol ring (**14**–**16**, and **19**)

Table 2
Novel Arbidol Derivatives and Its Anti-CHIKV Activities in Vero Cells.



Series 1 **48-54** | Series 2 **55-60** | Series 3 **61-64**

Compound	R_1	R_2	X	CC_{50} (μM)[a]	EC_{50} (μM)[b]	SI[c]
(48)	H	4-CF_3	–	11	3.6	3.1
(49)	H	2,6-Cl	–	7.8	2.9	2.7
(50)	H	2-CF_3	–	19	3.4	4.1
(51)	Br	2-CF_3	–	26	9.6	2.7
(52)	H	4-F	–	11	4.5	3.2
(53)	H	4-Cl	–	15	4.8	3.2
(54)	Br	2,6-Cl	–	13	5.7	3.2
(55)	H	4-CF_3	–	24	13	1.9
(56)	H	2,6-Cl	–	151	20	7.6
(57)	H	2-CF_3	–	156	6.5 ± 1	22
(58)	Br	2-CF_3	–	26	9.6	2.7
(59)	H	4-F	–	>100	17 ± 3	>5.7
(60)	H	4-Cl	–	93	8.2 ± 6	11
(61)	Br	2,6-Cl	S	17	≥17	–
(62)	H	2,6-Cl	S=O	9.2	4.1	2.2
(63)	H	2-CF_3	S=O	66	22	3
(64)	Br	2-CF_3	S=O	56	12	4.6
(2)	–	–	–	161 ± 18	35 ± 8	4.6

ND: Not determined. NC: Not possible to calculate. NA: not active.
[a] CC_{50}: 50% cytostatic/cytotoxic concentration (concentration at which 50% adverse effect is observed in the host cell).
[b] EC_{50}: 50% Effective concentration (concentration at which 50% inhibition of CPE is observed in Vero cells).
[c] SI: Selectivity index (CC_{50}/EC_{50}).

lead to molecules with an antiviral activity more than that (**2**). Derivatives (**17**) and (**18**) presented an opposite activity, suggesting that the position and the steric hindrance of the halogen can influence the antiviral activity. Therefore, derivatives with the lowest EC_{50} values were converted into their corresponding sulfoxide derivatives.[71]

The oxidation of compounds (**14**), (**15**) and (**16**) to sulfoxides (**23**), (**24**) and (**25**) led a decrease in anti-CHIKV activity and cytotoxicity. The replacement of the trifluoromethyl group from *para* (**24**) to *ortho* (**27**) position resulted in an increased potency and reduced cytotoxicity. Additionally, the similar effect was observed for the simultaneous presence of two chlorine atoms at ortho position (**26**).

The effect of oxidation of two compounds that belong to the ethyl series was also evaluated. For compound (**28**), an increase in activity was observed without a reduction of cytotoxicity. In addition, the introduction of two chlorine atoms at *ortho* position was responsible for a decrease in the activity, in fact, compound (**29**) presented a higher EC_{50} value and a smaller selectivity index than unsubstituted analog (**28**).

Subsequently, it was evidenced that carboxylic acid derivatives (**30** and **31**) of the most interesting *tert*-butyl esters (**15** and **19**) were completely inactive.

Finally, it was demonstrated that the compounds **32**–**37** and **38**–**47**, in which the hydroxymethyl group at position 5 was replaced by an ethyl ester, were completely inactives, except for (**35**) and (**37**) which presented moderate activity, however with a very low selectivity index.

Based on results obtained by Di Mola et al.,[71] new arbidol analogs were proposed by Scuotto et al.[55] In this study, three new series of compounds were synthesized and evaluated against CHIKV in Vero cells using CPE assay (Table 2). In addition, the E_2 unit of the crystal structure of the mature E_3-E_2-E_1 glycoprotein complex (PDB ID: 3N42) was used in the simulations (Gly407 corresponds to Gly82 in the crystal structure), two potential binding sites in the proximity of Gly82 were identified.

Compound (**57**) (*tert*-butyl-5-hydroxy-1-methyl-2-(2-trifluoromethysulfynyl) ethyl)-indole-3-carboxylate) presented the most high activity (IC_{50} of 156 μM) and selectivity index (SI 22). Additionally, BGM cell assays using CHIKV pseudoparticles (CHIKVpp) demonstrated which the molecular mechanism of action occurs in the entry and post-entry of the viral particle, probably also associated with other mechanisms.[55]

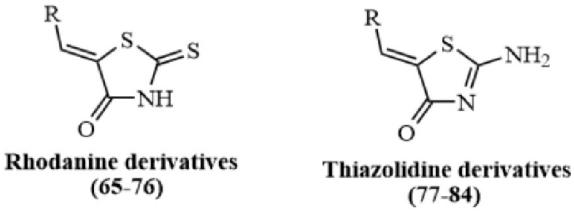
The closest site to Gly82 (site 1) indicates that this small hydrophilic and shallow pocket is probably not the binding site for (**2**). Site 2 consists of a more hydrophobic and well-defined pocket formed by Trp64, Arg80, Met97, Thr96, and Thr160. Gly82 is not directly involved in site 2 formation but it should be noted that this residue is located in a very flexible loop and it can be speculated that a substitution to Arginine, a significantly bigger residue, could change the loop conformation affecting the overall architecture of the proposed site 2. That way, (**2**) occupies the binding site inserting the ethyl ester group and the thiophenol deep in the pocket, whereas the dimethylamino moiety and the hydroxyl group are more solvents exposed.[55]

2.1.3. Rhodanine and thiazolidine derivatives

Jadav et al.[72] have performed an investigation about the anti-CHIKV activity of benzylidene rhodanine and thiazolidine derivatives (Table 3).

The compounds (**71**–**73**, **80**, and **83**) were assumed as actives against CHIKV. In addition, compound (**71**), containing the *ortho*-methyl group, was found most potent compound. Compound

Table 3
Antiviral Activity of Rhodanine and Thiazolidine Derivatives Against CHIKV.



Code	R	IC_{50} (μM)	CC_{50} (μM)
(65)	4-Hydroxyphenyl	N.D	>100
(66)	4-Acetophenone	N.D	>100
(67)	4-Chlorophenyl	N.D	>100
(68)	4-Dimethylaniline	N.D	>100
(69)	4-Benzonitrile	N.D	>100
(70)	2-Nitrophenyl	N.D	>100
(71)	2-Toluyl	0.42 (0.1 μg mL^{-1})	>100
(72)	4-Toluyl	4.2 (1 μg mL^{-1})	>100
(73)	$C_{10}H_7$(naphth-2-yl)	3.6 (1 μg mL^{-1})	>100
(74)	C_4H_3S(thiophen-2-yl)	N.D	>100
(75)	C_5H_4N(pyridine-2-yl)	N.D	>100
(76)	C_5H_4N(pyridine-3-yl)	N.D	>100
(77)	3-Hydroxyphenyl	N.D	>100
(78)	4-Hydroxyphenyl	N.D	>100
(79)	2,4-Dihydroxyphenyl	N.D	>100
(80)	2-Nitrophenyl	40.1 (10 μg mL^{-1})	>100
(81)	3-Nitrophenyl	N.D	>100
(82)	2-Methylphenyl	N.D	>100
(83)	3-Methylphenyl	6.8 (1.5 μg mL^{-1})	>100
(84)	4-Methylphenyl	N.D	>100

N.D: not showed activity at 100 μg mL^{-1}.

(**73**) with 2-naphthyl group inhibited CHIKV at a concentration similar to that of compound (**71**) and was followed by compound (**72**), containing a *para*-methyl group. It was observed that arylkylidene portion should be non-polar in nature to exert activity. This fact is supported by the inactivity of compounds (**65–70**) with polar groups at *para*-position and compounds (**74–76**) with heteroaryl rings, at 100 μg mL^{-1} concentration. Additionally, compounds (**80**) and (**83**) were found to be active. Compound (**80**) with *ortho*-nitro substitution and compound (**83**) with *meta*-methyl substitution presented active at 10 μg mL^{-1} concentration.[72]

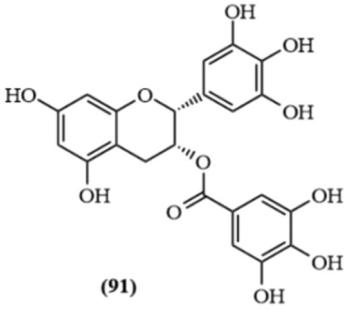


Fig. 4. Epigallocatechin gallate (**91**) chemical structure.

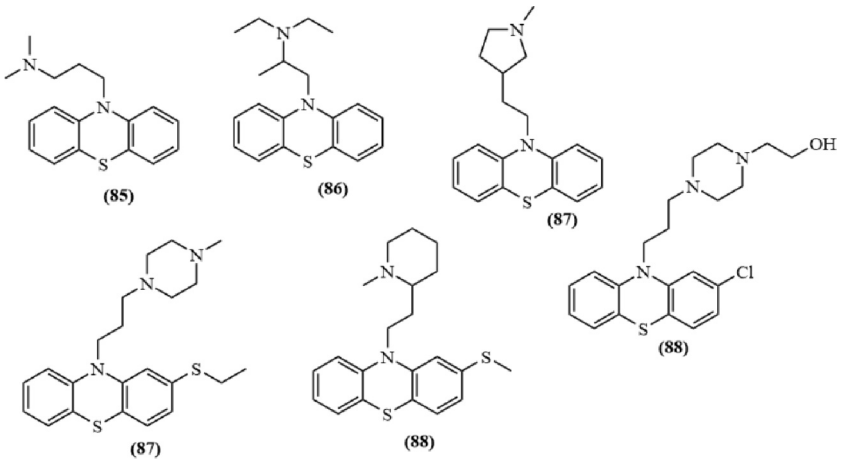
2.1.4. Phenothiazines

Applying a novel virus entry assay for the identification of Alphavirus entry inhibitors, Pohjala et al.[73] found six compounds with a 10*H*-phenothiazine moiety, including chlorpromazine (**85**), ethopropazine (**86**), methdilazine (**87**), perphenazine (**88**), thiethylperazine (**89**), and thioridazine (**90**) were found as possible entry inhibitors (Table 4). Although, the molecular mechanism by which these compounds inhibit viral entry yet remains obscured.[73]

2.1.5. Epigallocatechin gallate (EGCG)

Epigallocatechin gallate (EGCG) (**91**) (Fig. 4) represents the major constituent of green tea extract. It is known that EGCG has

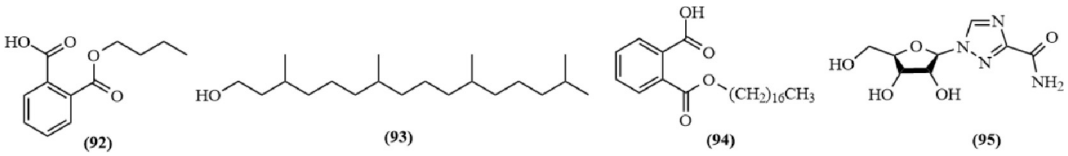
Table 4
Phenothiazine Derivatives and its Anti-CHIKV Activity.



Compound	Inhibition (μM) IC_{50}[a]	CC_{50}[b]	SI[c]
(85)	15.7	67.3	4.5
(86)	16.0	166.9	10.4
(87)	11.3	63.8	5.6
(88)	25.1	155.0	6.2
(89)	15.0	83.1	5.5
(90)	14.9	179.4	12.0

[a] Values determined using SFV-Rluc infection (MOI 0.001 in BHK cells and detection at 14 h post-infection.
[b] Viability values determined by ATP assay after 48 h exposure of BHK cells.
[c] Selectivity index.

Table 5
Cytotoxic and Activity of Extracted Compounds from Tectona grandis Lin.



	Asian CHIKV[a]			African CHIKV[a]	
Code	CC_{50} (Vero cells)	IC_{50}	SI[b]	IC_{50}	SI[b]
(92)	340.1 ± 0.022	2.49 ± 0.113	136	28.62 ± 0.115	11.88
(93)	259.2 ± 0.042	1.66 ± 0.093	156	112.4 ± 0.08	2.11
(94)	3252 ± 0.024	3.03 ± 0.109	116	76.46 ± 0.5	4.66
(95)	1622 ± 0.067	3.06 ± 0.115	529	–	–

[a] Mean ± Standard Deviation of at least three independent experiments (in μM).
[b] Selectivity index determined by CC_{50}/EC_{50}.

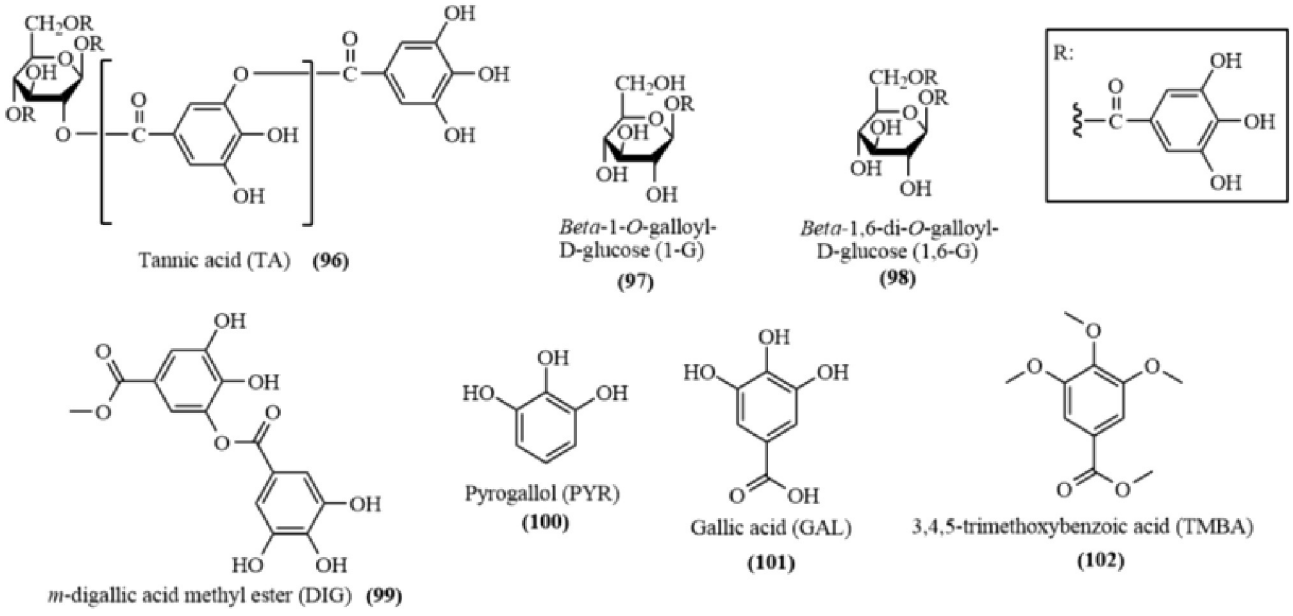


Fig. 5. The chemical structures of tannic acid and its derivatives.

in vitro antiviral activity against different viruses, including HIV, influenza and hepatitis C virus. Recently, Weber et al.[74] reported that EGCG is able to inhibit in vitro CHIKV replication. This study was capable of showing that this natural product inhibits (EC_{50} = 6.54 μg mL^{-1}) the entry of CHIKV pseudoparticles (carrying the CHIKV envelope proteins) into the target cell.[74]

2.1.6. Active metabolites from Tectona grandis Lin

Sangeetha et al.[35] performed a study about the anti-CHIKV capacity of extracted compounds from leaves of *Tectona grandis* Lin, commonly known as Teak in folklore medicines. In this study, three compounds were isolated and characterized, 2-(butoxycarbonyl)benzoic acid (**92**), 3,7,11,15-tetramethyl-1-hexadecanol (**93**), and benzene-1-carboxylic acid-2-hexadeconate (**94**). For these compounds, an evaluation of the activity against two strains of CHIKV (Asian and African Chikungunya viruses) was performed using ribavirin (**95**) as a positive control (Table 5).

The antiviral activities of the compounds were compared to the reference drug, ribavirin by MTT assay. Additionally, the selectivity indices of the compounds (**92**), (**93**), and (**94**) were 136, 156, and 116 to Asian strain and 11.88, 2.11, and 4.66 to the African strain.

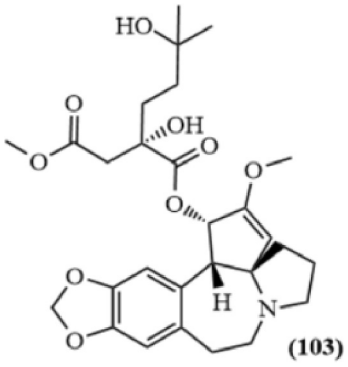
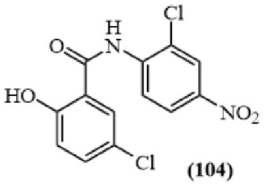


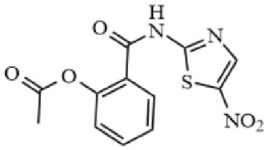
Fig. 6. Harringtonine (**103**) chemical structure.

Finally, compounds (**92**) and (**94**) were found to be highly significant as the therapeutic index >100 for Asian strain and >4 for the African strain.

Table 6
Antiviral Activity of Niclosamide and Nitazoxanide Against Different CHIKV Strains.



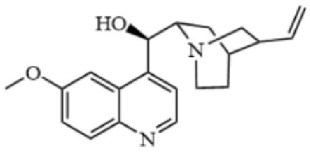
(104)



(105)

		Niclosamide (1 0 4) (μM)			Nitazoxanide (1 0 5) (μM)		
Cell Line	Virus Strain	EC_{50}[a]	CC_{50}[b]	SI[c]	EC_{50}	CC_{50}	SI
BHK-21	CHIKV	0.95 ± 0.22	>20	>21.0	2.96 ± 0.18	25	8.45
	CHIKV 0611aTw	0.85 ± 0.12	>20	>23.5	1.96 ± 0.48	25	12.76
	CHIKV 0810bTw	0.9 ± 0.12	>20	>22.2	4.95 ± 0.23	25	5.05
U2OS	CHIKV	0.36 ± 0.08	>20	>55.5	3.01 ± 0.61	25	8.31

[a] The EC_{50} values were determined using RT-qPCR and were presented as means ± SD ($n \geq 3$).
[b] The CC_{50} values were determined using a CCK-8 assay and were presented as means ± SD ($n \geq 3$).
[c] The SI (selectively index) represented the ratio of CC_{50} to EC_{50}.



Quinine (106)

Fig. 7. Chemical structure of quinine (**106**).

2.1.7. Tannic acid and its derivatives

Tannins are capable of inhibiting in vitro infectivity of animal viruses, such as herpes, influenza, and others. In addition, tannins are able to reduce the permeability of erythrocytes to anions or non-electrophiles, inhibition of enzyme actions, or agglutination of erythrocytes.[75] Based on this, Konishi and Hotta[75] performed a study to examine the mechanisms of virus-inactivating actions of tannins, by dealing with the interaction of CHIKV African strain with tannic acid and its related compounds (Fig. 5), in BHK-21 cells.

Compound (**96**) at 10 ppm concentration or less at pH 6.6 presented no cytotoxic effect on the cells and no morphological changes. Then, subsequent assays were carried out using (**96**) below 10 ppm concentration at pH 6.6. It was observed that (**96**) had no effect after the virus had invaded the cells under these conditions. The (**96**) (at 1 ppm) was capable of reducing the CHIKV infectivity, results confirmed by suppression using 0.1% of Bovine Serum Albumin (BSA), which is widely known to present the binding action of tannins to proteins. Additionally, (**99**) was almost as effective as (**96**). The effect of (**98**), as well as of (**101**), was significantly weaker than that of (**96**). The (**97**), (**102**), and (**100**) derivatives presented little effect. Finally, the results reported indicate that (**96**) inactivates CHIKV in vitro. Although (**96**) may also affect the culture cells in that it has an inactivation showed a close relation to phenolic hydroxyl groups since the displacement of the hydroxyl groups by methoxyl makes the chemicals ineffective (i.e. **101** and **102**) and reduction of these groups leads their effects weaker (i.e. **96**, **97**, and **98**). Ineffectiveness of (**100**) may possibly suggest that the existence of the carboxyl group is indirectly related to the action of the hydroxyl groups, in comparison with (**101**), but such a carboxyl groups do not seem to be directly active (i.e. **99**, **101**, and **102**).[75]

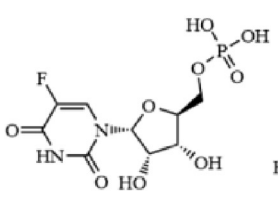
2.1.8. Harringtonine

In a study performed by Kaur et al.[48] it was verified that the harringtonine acts on the post-entry stage of the CHIKV replication cycle and strongly interferes in the process of viral proteins synthesis. The harringtonine (Fig. 6) is extracted from the Japanese plum yew, Cephalotaxus harringtonia, which belongs the cephalotaxine ester class.[48]

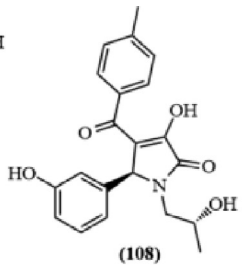
The inhibition of CHIKV replication observed when 5 μM harringtonine was added at a late time point, in time-of-addition studies may suggest an additional mechanism of inhibition at high concentrations of harringtonine, affecting later phases of the CHIKV replication cycle, in mock-infected BHK21 cells.[48]

2.1.9. Niclosamide and nitazoxanide

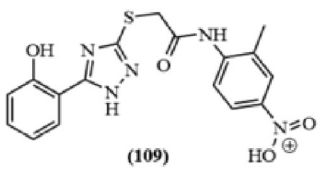
Wang et al.[76] shown CHIKV 26S mediated insect cell fusion assays could be used to search for anti-CHIKV drugs. From this platform, it was suggested the anti-alphaviruses properties of niclosamide (**104**) and nitazoxanide (**105**) also can affect CHIKV entry and transmission, which verified their potential use in human cells proved by U2OS cells (Table 6). Also found a significant inhibition of the cell-to-cell transmission of CHIKV infection,



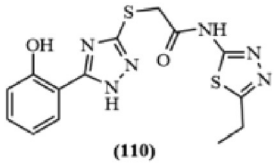
(107)



(108)



(109)



(110)

Fig. 8. *In silico* predicted CHIKV nsP_2 inhibitors.

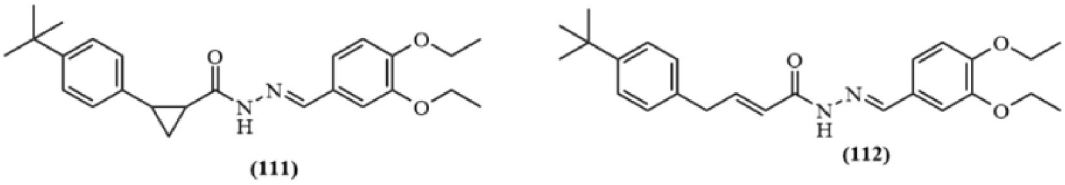


Fig. 9. Chemical structures of the CHIKV inhibitors identified from *in silico* screening.

which is considered an important transmission pathway and allows viruses to avoid attacks by the immune system. Niclosamide and nitazoxanide can be promising compounds for the further development of anti-CHIKV drugs.[76]

2.2. Inhibitors of Chikungunya non-structural protein 1 (nsP_1)

Quinine (**106**) (Fig. 7), an antimalarial drug is able to inhibit the CHIKV in vitro with an IC_{50} value of 0.1 μg mL^{-1}.[41] Finally, it was suggested that this compound (**106**) is capable of affecting the nsP_1, as mutations in this protein occur upon growing the virus in high concentrations of (**106**).[41,77]

2.3. Inhibitors of Chikungunya non-structural protein 2 (nsP_2)

Singh et al.[78] developed a homology model for the nsP_2 and screened a library of compounds *in silico*, leading to four compounds as promising inhibitors of the nsP_2 protease (Fig. 8).

Similarly, Bassetto et al.[79] performed an *in silico* study to identify CHIKV nsP_2 inhibitors using a virtual screening protocol with a large compounds library employing the developed homology model for the CHIKV nsP_2 protease. Among all compounds, the hit compound (**111**) was found bind to the central portion of the nsP_2 protease active site (Fig. 9). In vitro activity of this compound showed inhibition of virus at an EC_{50} of 5.0 μM and a selectivity index (SI) of 14, in the inhibition of virus-induced cytopathic effect (CPE).[79] Finally, a novel analog (**112**) (Fig. 9) was proposed based on the compound (**111**) structure. The antiviral activity was slightly improved with (**112**) displaying an EC_{50} of 3.2 μM and a SI of 32. Moreover, the binding poses of both compounds (**111** and **112**) were found to be similar.[77,79]

Analogously to the active site nsP_2, the hydrazone group is placed in the region defined by the catalytic dyad, Cys579, and His649, and also close to Trp650. The cyclopropane moiety is positioned in the space formerly occupied by the second Glycine residue of the $nsP_{3\text{-}4}$ linking peptide. The most relevant interactions observed for (**111**) are a hydrophobic contact between the 3,4-diethoxyphenyl ring and the Trp650 side chain, two hydrogen bonding bonds between the hydrazone function and the amide groups of the Tyr613 and Asn648 skeleton, and another interaction Hydrophobic group between the *t*-butyl group of (**111**) and His649. In addition, a substitution of the hydrazone group would be beneficial to overcome possible problems of chemical instability (susceptible to hydrolysis). That way, the compound (**112**) the cyclopropyl group was replaced by a *trans*-ethenyl function, which allows maintaining the length and geometry of the original ligand, avoiding the presence of any chiral center in the molecule. The simplified structure of compound (**112**) allows for a more rapid and efficient optimization of this class of treatment using a more accessible synthetic route, that way the of action of the novel inhibitors related is promising for representing an initial step towards a finding of a clinical candidate for the treatment of CHIKV infections.[79]

Sangeetha et al.[35] performed an *in silico* study using the crystal structures of the CHIKV proteins nsP_2 protease (PDB ID: 3TRK) and non-structural protein-3 (PDB ID: 3GPG) were obtained from Protein Data Bank. The envelope, capsid, and non-structural protein-1 were predicted by *in silico* modeling, using blast on-line platform. Finally, the results showed the compound (**113**) interacts with this same protein at Phe118, Val 179, Pro208, Lys181, Ser120, and Glu209 (Fig. 10), with low-affinity energy (−32.16 kcal mol^{-1}).

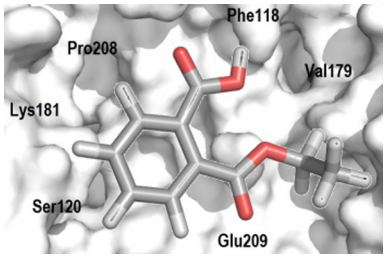


Fig. 10. Compound (**113**) in complex with nsP_2 protein from Chikungunya virus.

Table 7
Summary of Interactions Observed for Rhodanine and its Derivates.

	Pocket 3	
Code	Hydrophobic	H-Bond
(71)	Leu1205 Asn1082 Cys1013 Ala1046 Glu1204 Ser1048 Tyr1079 Trp1084	CO at thiazolidine ring with NH_2 at Tyr1047
(72)	–	Oxygen at thiazolidinone ring with backbone NH of Tyr1047; H at thiazolidine ring with Asn1011
(73)	–	Carbonyl oxygen at thiazolidine ring with backbone NH of Ala1046 and H at thiazolidine with Glu1204
(80)	CO at thiazolidine ring with Cys1013 and H at thiazolidine with Asn1082	–
(83)	CO at thiazolidine ring with Ala1046 and H thiazolidine with Asn1011	–
	Pocket 2	
	Hydrophobic	H-Bond
(71)	Cys1013 Tyr1047 Tyr1049 Trp1084	Backbone-amide NH of Tyr1047
	Pocket 1	
	Hydrophobic	H-Bond
(72)	–	H at thiazolidine ring with Asn1011
(80)	–	Interactions is largely confined to S1 pocket
(83)	–	Interactions is largely confined to S1 pocket

(–): None observed contacts.

Jadav et al.[72] have employed molecular docking simulation in order to understand the possible mechanism of action of the best rhodanine derivatives (see Table 3) in the CHIKV nsP_2 protease (PDB ID: 3TRK). After docking analyses, all interactions observed were summarized in Table 7.

Based on all results obtained from docking analysis, it was concluded that the hydrophobic interactions with S2 and S3 pockets and H-bond (at distance of 3.9 Å) in interaction with Tyr1047 are critical for activity and potency of compounds[72] (Fig. 11).

2.4. Inhibitors of Chikungunya non-structural protein 3 (nsP_3)

2.4.1. Flavonoids

In comparison with other CHIKV proteins, the function of Alphavirus replicase protein (nsP_3) is still uncertain, and there is presently no discovered inhibitor against this protein.[27]

Flavonoids from plants are polyphenolic compounds that possess a wide range of biological properties to human health, such as antioxidant, anti-inflammatory, antibacterial, and antifungal activities.[27] Actually, it is known that various types of flavonoids such as rutin, naringin, baicalein, quercetin, and kaempferol are potential antiviral agents against Dengue virus, H5N1 influenza A viruses, HIV, Coxsackie virus and Japanese encephalitis virus.[27] In this sense, and considering that molecular docking accelerates the drug design process, and is broadly used in the biopharmaceutical industry to discover and develop new lead compounds, Seyedi et al.,[27] performed a study to predict a valid pose from a receptor conformation (nsP_3, PDB ID: 3GPO), and a set containing four ligands (ADP-ribose (**114**), Baicalin (**115**), Naringenin (**116**), and Quercetagetin (**117**)) using scoring based on their binding affinity.

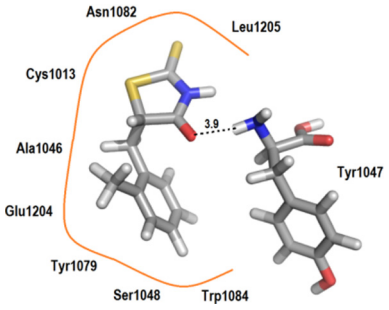


Fig. 11. 3D-view showing the interaction of compound (**71**) with CHIKV nsP_2 protease active site. In orange: hydrophobic contacts; in green: hydrogen-bond (value in Ångström).

All ligand conformations were ranked according to their predicted binding affinities using the default scoring function in AutoDock Vina. The best docking conformation of (**114**) showed a binding affinity of −8.7 kcal mol^{-1}, whereas, among the three other ligands analyzed, (**115**) presented the most potent antiviral activity with a binding affinity of -9.8 kcal mol^{-1} (Table 8).

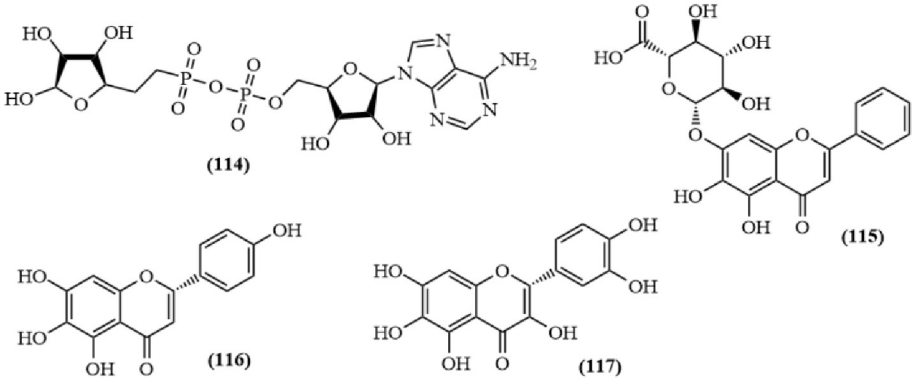
Additionally, it was evidenced that the flavonoids were capable of interacting with 10 residues in the active site of nsP3 (115: Leu108, Tyr142, Ser110, Thr111; 116: Ser110, Thr111; 117: Cys34, Leu108, Arg144, and Asp145). Finally, it was also observed one π-π stacking interaction between (115) with nsP3 residue Tyr114. With respect to this flavonoid, it showed H-bonds with distance ranges from 1.81 to 2.42 Å. A review by Szatylowicz[80] classified the energy borders setting for strong (1.2–1.5 Å), moderate (>1.5–2.2 Å), and weak (>2.2 Å) H-bonds. Considering the involvement of CHIKV nsP3 in the intracellular replication cycle, these results suggest that (115) could potentially interfere with the post-entry stage(s) of CHIKV infection.[27]

2.5. Inhibitors of E_1-E_2 complex proteins

Advances in high-throughput approaches have shown an alternative pathway to efficiently identify novel molecules that can be utilized for designing of new strategies to combat the spread of this virus.[45]

The possible binding target sites of the CHIKV envelope proteins had not previously been investigated. In sense, Rashad and Keller[81] have investigated for the first time the identification of possible antagonist for the E_1 and E_2 sites through virtual screening using two success docking scores; FRED docking for fast precise screening using top hits then subjected to a ranking scoring using the AutoDock algorithm.[81] Additionally, both the immature (PDB ID:

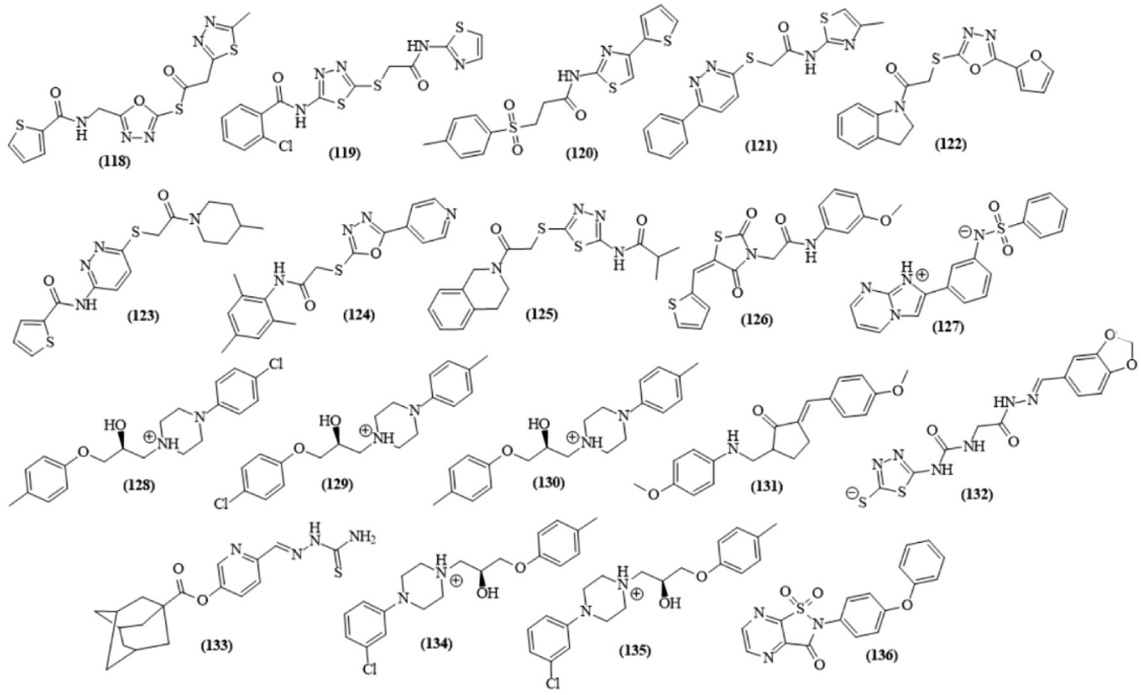
Table 8
Binding Affinity and Interaction Energy of Best Docking Pose Against CHIKV nsP_3 Target.



Code	K_i value[a]	Affinity[b]	Interaction[c]	Van der Waals[d]	Electrostatic[e]
(114)	–	−8.7	−967.55	−183.61	−783.94
(115)	0.064	−9.8	−552.4	105.18	−657.59
(116)	0.685	−8.4	−647.04	−24.71	−622.32
(117)	0.489	−8.6	−459.84	12.04	−471.88

[a] Inhibitory constant, in μM.
[b,c,d,e] Energy values in kcal mol^{-1}.

Table 9
Top Hit Compounds Identified by Virtual Screening Research.



Code	Target	$E_{binding}$[a]	K_i[b]	Interactions (protein)
(118)	Site 2 immature	−10.06	42.15	Lys52 (E_1), Ile55 (E_1), Thr53 (E_1), Tyr301 (E_2), Arg100 (E_2)
(119)	Site 2 immature	−9.43	121.87	Lys52 (E_1), Ile55 (E_1), Thr53 (E_1), Tyr301 (E_2), Arg100 (E_2)
(120)	Site 2 immature	−9.36	138.19	Lys52 (E_1), Ile55 (E_1), Tyr301 (E_2), Glu232 (E_2)
(121)	Site 2 immature	−9.18	187.59	Lys52 (E_1), Ile55 (E_1), Tyr301 (E_2), Glu232 (E_2), Arg100 (E_2)
(122)	Site 2 immature	−8.99	225.48	Lys52 (E_1), Ile55 (E_1), Tyr301 (E_2), Arg100 (E_2)
(123)	Site 2 mature	−9.98	48.38	Lys52 (E_1), Thr53 (E_1), Ile55 (E_1), Arg36 (E_2), Glu168 (E_2)
(124)	Site 2 mature	−9.71	75.78	Lys52 (E_1), Ile55 (E_1), Arg36 (E_2), Glu168 (E_2), Tyr237 (E_2)
(125)	Site 2 mature	−9.36	138.07	Lys52 (E_1), Ile55 (E_1), Tyr237 (E_2)
(126)	Site 2 mature	−9.26	163.4	Lys52 (E_1), Thr53 (E_1), Ile55 (E_1), Arg36 (E_2), Tyr237 (E_2)
(127)	Site 2 mature	−9.17	190.7	Lys52 (E_1), Thr53 (E_1), Ile55 (E_1), Arg36 (E_2), Tyr237 (E_2)
(128)	Site 4 immature	−11.3	5.18	Val229 (E_1), His82 (E_2), His93 (E_2), Leu80 (E_2), Leu305 (E_2)
(129)	Site 4 immature	−11.23	5.91	Val229 (E_1), His82 (E_2), His93 (E_2), Leu80 (E_2), Leu305 (E_2)
(130)	Site 4 immature	−11.2	6.19	Val229 (E_1), His82 (E_2), His93 (E_2), Leu80 (E_2), Leu305 (E_2)
(131)	Site 4 immature	−10.69	14.49	His82 (E_2), His93 (E_2), Leu80 (E_2)
(132)	Site 4 immature	−10.45	21.98	Phe87 (E_1), His82 (E_2), His93 (E_2), Ser91 (E_2), Leu80 (E_2), Leu305 (E_2)
	Site 4 mature	−10.25	30.49	Phe87 (E_1), His18 (E_2), His29 (E_2), Ser27 (E_2), Leu16 (E_2), Leu241 (E_2)
(133)	Site 4 mature	−10.03	10.03	Thr228 (E_1), Gly229 (E_1), His18 (E_2), His29 (E_2)
(134)	Site 4 mature	−10.0	46.61	Val229 (E_1), His18 (E_2), His29 (E_2)
(135)	Site 4 mature	−9.98	48.35	Val229 (E_1), His18 (E_2), His29 (E_2)
(136)	Site 4 mature	−9.88	57.61	Trp89 (E_1), His18 (E_2), His29 (E_2), Leu16 (E_2)

[a] Energy binding in kcal mol^{-1}.
[b] Predicted value in μM.

3N40) and the mature (PDB ID: 3N42) E_1-E_2 protein complex structures from CHIKV were included in the study to increase the probability of finding positive and reliable hits. Finally, several molecules have been identified as good *in silico* Chikungunya virus enveloped proteins inhibitors (Table 9).

Initially, two chemical compounds libraries were used - NCI (265,242 compounds) and Life chemicals protein-protein interactions inhibitors library of 31,143 compounds. In addition, some filters were applied, such as Molecular Weight $\leq$ 500, cLogP $\leq$ 5, hydrogen bond donors (HBD) and acceptor (HBA) (OH and NH $\leq$ 5; N and O $\leq$ 10).[81] Subsequently, molecular docking calculations were performed using previously selected compounds by FRED program. After docking analyses, the poses retrieved were scored and ranked with Gaussian shape function independently by the five available scoring functions (PLP, ChemGauss3, ChemScore, OEChemScore, and ScreenScore).[81]

In total, seven sites were detected at E_1-E_2 protein complex, where the sites 2 and 4 showed interesting in this study. Site 2 represents a surface activity that lies between the E_1 domain II and E_2 β-ribbon that connects E_2 domain A to E_2 domain C. Additionally, it makes close contact with residues from E_1 and E_2 proteins. E_1 residues are Glu50-Val60, Val229-Pro237; the E_2 residues are Ala97-Arg102 (which corresponds to Ala33-Arg38 in the mature form) and Gln300-Arg308 (Gln236-Arg244 in the mature form). In general, valine, alanine, and proline amino acids within this pocket are also able to participate in the hydrophobic interactions.[81]

At site 2, the presence of an electron rich system leads in strong non-covalent molecular interactions. Additionally, the heterocyclic ring adjacent to the sulfur in most of the top-ranked poses can accept hydrogen bond and with E_1 Lys52, Ile55, and Thr53.

Site 4 can be described as a narrow channel extending just behind the fusion loop and surrounded by both E_2 domains A

and B. It makes close contacts with the E_1 fusion loop residues Pro86-Gly91, E1 Gly227-His230. The fusion loop Gly91 and His23 were found to be critical for fusion. For E_2, residues are Arg77-His82 (Arg13-His18 in the mature form), Ser91-Val96 (Ser27-Val32 in the mature form) form close contacts with the active site.[81]

Moreover, the predicted binding affinity and inhibitory constant (Ki, in the nM range), along with the cLogP value of 1.8 make it (compound **132** at site 4 mature and immature proteins) an attractive candidate for developing an anti-CHIKV drug targeting the envelope proteins.[81]

2.6. Inhibitors of viral genome replication

2.6.1. Ribavirin

Ribavirin (**95**) is a synthetic guanosine analog with broad-spectrum antiviral activity. The compound (**95**) showed to exert in vitro anti-CHIKV (EC_{50} = 341 μM) and resulted in a synergistic effect when in combination with IFN-α.[64] Additionally, a combination of doxycycline (**137**) (Fig. 12) and (**95**) led to good antiviral effect against CHIKV replication in Vero cells and also reduced the viral load and inflammatory process in infected 1CR mice.[82]

2.6.2. Mycophenolic acid (MAP)

Mycophenolic acid (MAP) (**138**) (Fig. 13) is broadly used as an immunosuppressant to prevent the rejection of transplant of transplant organs. In sense, Khan et al.[62] reported the effectiveness of this compound in inhibit CHIKV (IC_{50} = 0.1 μM, CC_{50} = 30 μM, SI = 300) replication (in Vero cells).[70]

2.6.3. 6-Azauridine

6-Azauridine (**139**) (Fig. 14) is a uridine derivative with broad-spectrum antiviral activity against both DNA and RNA viruses, capable of inhibiting in vitro CHIKV replication (EC_{50} = 0.82 μM) in Vero cells.[64] Basically, it depletes intracellular UTP-pools, justifying its activity on quickly replicating virus such as CHIKV.[83]

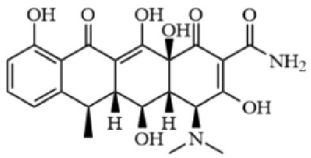
2.6.4. Favipiravir T-705 and T-1105

Favipiravir (T-705) (**140**) is a broad-spectrum antiviral agent that was recently approved in Japan for the treatment of influenza virus infections. Based on this, Delang et al.[84] reported that (**140**) and its analogs T-1105 (**141**) inhibited the replication of different laboratory strains and clinically isolated of CHIKV in Vero cells (Table 10).

2.6.5. Thieno[3,2-b]pyrrole derivatives

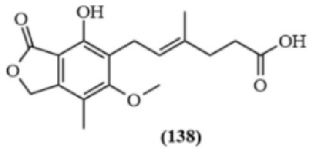
In a study performed by Ching et al.,[85] the literature review for known inhibitors against related arboviruses was examined. Starting from this, they have synthesized a small library of 3 different classes of compounds (**142**–**187**), namely, the pyrazolines, thieno [3,2-*b*]pyrroles and pyrimidinones-pyrimidinethiones, which were reported as inhibitors of flavivirus and alphaviruses. Based on screening studies, the thieno[3,2-*b*]pyrrole family presented promising anti-CHIKV activity, and this was used to prepare different active analogs (Table 11). Initially, the primary screening of the anti-CHIKV activities was measured using a Gaussia luciferase-based gene assay (CHIKV-Gluc).[85]

The compounds (**142**) (ester), (**143**) (acid), and (**144**) (amide) showed that amide (**144**) had the lowest EC_{50} value of 36 μM. The secondary amides (**145**–**147**) did not show any activity (EC_{50} values > 100 μM), while tertiary amides were better (EC_{50} values ranging from 32 to 80 μM). Additionally, the best activity was observed for compound (**152**) (piperazine amide), with an IC_{50} value of 32.5 μM). The benzyl substituent on the nitrogen atom at piperazine ring of (**150**) resulted in compound (**153**), which



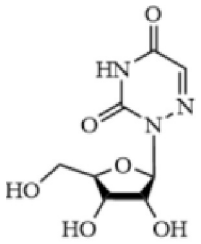
Doxycycline (137)

Fig. 12. Chemical structure of Doxycycline (**137**).



(138)

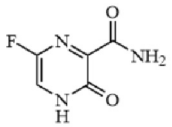
Fig. 13. Chemical structure of Mycophenolic acid (**138**).



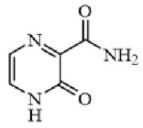
(139)

Fig. 14. Chemical structure of 6-Azauridine (**139**).

Table 10
Antiviral Activity of T-705 (**140**) and T-1105 (**141**) Against Different CHIKV Strains.



(140)



(141)

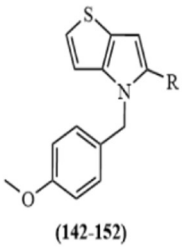
Strain	Inhibition ± SD[a]	
	(140)	**(141)**
Indian Ocean 899 (lab)	25 ± 3	7 ± 1
LR2006-OPY1 (lab)	25 ± 1	N.D
Italy 2008 (clinical)	16 ± 6	N.D

N.D: not determined.
[a] All data are mean values ± standard deviation for at least three independent experiments.

showed a slight decrease in activity (EC_{50} value of 41.7 μM) in comparison with (**152**). Notably, compound (**154**) which possess an ester moiety at the 4-position of the piperidine resulted in significant improvement in activity (EC_{50} value of 13.1 μM) when compared to the corresponding compound (**150**). In contrast, the compound (**155**) (acid) is inactive.

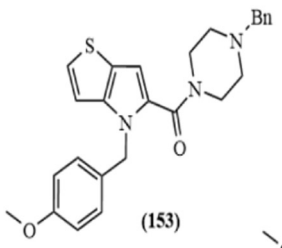
The secondary amides, those amides possessing an aryl group (**157**–**159** and **165**) were not cytotoxic (CC_{50} values > 100 μM) and provided a selectivity index > 8, while those amides with an alkyl group (**161**–**164**) show some cytotoxicity (CC_{50} values ranging from 18 to 30 μM). Among the amides (**161**–**164**), compound (**1 6 1**) exhibited the best activity (EC_{50} value of 7 μM).

Table 11
Biological Evaluation for Thieno[3,2-*b*]pyrroles Against CHIK-Gluc and CHIKV-IMT Infections.

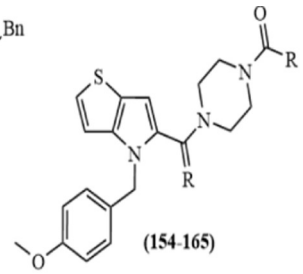


(142-152)

142=R= COOEt
143=R= COOH
144=R= $CONH_2$
145=R= CONH(*n*-Pr)
146=R= CONH(Bn)
147=R= $CONHCHCH_3(C_6H_5)$
148=R= $CON(Et)_2$
149=R= CO(pyrrolidine)
150=R= CO(piperidine)
151=R= CO(morpholine)
152=R= CO(piperazine)

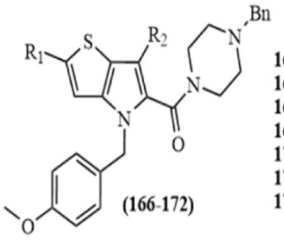


(153)



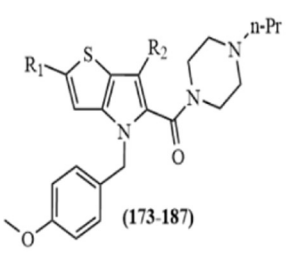
(154-165)

154=R= OEt
155=R= OH
156=R= NH_2
157=R= $NHCH_2(C_6H_5)$
158=R= $NHCHCH_3(C_6H_5)$
159=R= $NHCH_2(4\text{-}Br(C_6H_5))$
160=R= *n*-Bu
162=R= NH(*n*-Pr)
163=R= NH(cyclopropane)
164=R= $NHCH_2CH_2CH(CH_3)_2$
165=R= $NHCH_2CH_2(C_6H_5)$



(166-172)

166=R_1= $CO(C_6H_5)$; R_2= H
167=R_1= Acetyl; R_2= H
168=R_1= Phenyl; R_2= H
169=R_1= $CH_2NHCHCH_3(C_6H_5)$; R_2= H
170=R_1= $CH_2OCH_2(C_6H_5)$; R_2= H
171=R_1= H; R_2=$CH_2NHCHCH_3(C_6H_5)$
172=R_1= H; R_2= $CH_2OCH_2(C_6H_5)$



(173-187)

173=R_1= $CO(C_6H_5)$; R_2= H
174=R_1= Acetyl; R_2= H
175=R_1= Br; R_2= H
176=R_1= CH_2NH(*n*-Pr); R_2= H
177=R_1= $CH_2NHCHCH_3(C_6H_5)$; R_2= H
178=R_1= $CH_2NHCH_2(C_6H_5)$; R_2= H
179=R_1= CH_2OH; R_2= H
180=R_1= CH_3; R_2= H
181=R_1= Phenyl; R_2= H
182=R_1= CN; R_2= H
183=R_1= H; R_2= Br
184=R_1= H; R_2= CH_2OH
185=R_1= H; R_2= CH_2NH(*n*-Pr)
186=R_1= H; R_2= CH_2NH(*n*-Pr)
187=R_1= H; R_2= $CH_2NHCH_2(C_6H_5)$

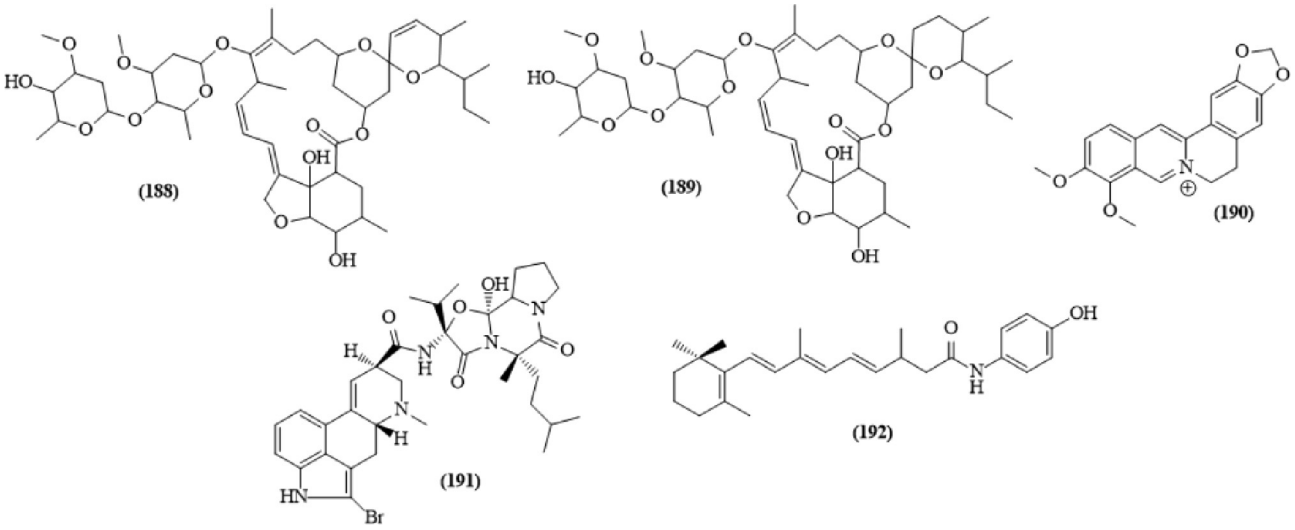
	Inhibition (μM) ± SD[a]			
Compound	EC_{50} CHIKV-*Gluc*[b]	EC_{50} CHIKV-IMT[c]	CC_{50}[d]	SI[e]
(142)	>100	–	>100	–
(143)	>100	–	>100	–
(144)	36 ± 5.4	–	>100	>2.8
(145)	>100	–	>100	–
(146)	>100	–	>100	–
(147)	>100	–	>100	–
(148)	49.6 ± 1.3	–	>100	>2.0
(149)	35.4 ± 3.3	–	>100	>2.8
(150)	49.7 ± 2.5	–	>100	>2.0
(151)	79.7 ± 13.3	–	>100	>1.3
(152)	32.5 ± 2.2	–	>100	>3.1
(153)	41.7 ± 1.4	–	>100	>2.4
(154)	13.1 ± 1.1	–	39	3.0
(155)	>100	–	>100	–
(156)	70.9 ± 4.6	–	>100	>1.4
(157)	12.3 ± 0.6	–	>100	>8.1
(158)	13.0 ± 2.8	–	>100	>7.7
(159)	10.9 ± 1.3	–	>100	>9.2
(160)	13.3 ± 0.1	–	38.9	2.9
(161)	7.0 ± 1.51	6.55 ± 0.49	18.8	2.7
(162)	7.59 ± 2.17	5.59 ± 1.65	21.4	2.8
(163)	10.4 ± 0.8	–	24.6	2.4
(164)	11.0 ± 0.4	–	30.9	2.8
(165)	11.1 ± 0.1	–	>100	>9.0
(166)	>100	–	>100	–
(167)	17 ± 0.6	–	>100	>5.9
(168)	42.4 ± 3.7	–	>100	>2.4
(169)	9.44 ± 0.06	25.5 ± 2.9	>100	>11
(170)	>100	–	>100	–
(171)	23.0 ± 0.9	–	>100	>4.3
(172)	>100	–	>100	–
(173)	>100	–	>100	–
(174)	20.9 ± 2.8	–	>100	>4.8
(175)	3.1 ± 0.71	1.96 ± 0.63	>100	>32
(176)	40 ± 4.0	–	>100	>2.5
(177)	8.44 ± 2.21	13.5 ± 3.1	33.9	4.0
(178)	11.2 ± 3.4	–	46.8	4.2
(179)	4.33 ± 0.86	2.39 ± 0.11	15.5	3.6
(180)	3.6 ± 0.41	3.27 ± 0.62	36.3	10
(181)	3.85 ± 0.06	3.08 ± 0.47	>100	>2.6
(182)	4.9 ± 0.16	8.23 ± 0.65	>100	>2.0

Table 11 (*continued*)

Compound	Inhibition (μM) ± SD[a]			
	EC_{50} CHIKV-*Gluc*[b]	EC_{50} CHIKV-IMT[c]	CC_{50}[d]	SI[e]
(183)	7.75 ± 0.23	14.0 ± 1.5	31.6	4.1
(184)	47.6 ± 2.3	–	>100	>2.1
(185)	27.7 ± 4.5	–	>100	>3.6
(186)	11.9 ± 1.9	–	57.5	4.8
(187)	15.4 ± 2.3	–	77.6	5.0

[a] The values are the mean ± SD from 3 independent experiments.
[b,c] EC_{50} values against CHIKV-Gluc were determined by the inhibition of Gaussia luciferase expression in the antiviral assay. EC_{50} values against wild-type (WT) CHIKV-IMT were calculated effective concentrations of compounds required to inhibit 50% CHIKV-IMT infectivity. Cell viability CC_{50} values were determined by CellTiter-Glo luminescent assay after 24 h exposure of HEK 293T cells.
[d] CC50 is defined as the compound's concentration required for the reduction of cell viability by 50% as compared to the untreated control.
[e] Selectivity index.

Table 12
Anti-CHIKV Evaluation in Huh-7.5 and BHK-21 cells.



Code	HUH-7.5			BHK-21		
	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
(188)	1.4 ± 0.9	15.2 ± 1.0	10.9	1.5 ± 0.6	28.2 ± 1.1	19.2
(189)	1.9 ± 0.8	8 ± 0.2	4.1	0.6 ± 0.1	37.9 ± 7.6	62.4
(190)	1.9 ± 0.9	>100	>52.6	1.8 ± 0.5	>100	>55.6
(191)	1.4 ± 0.3	>100	>71.4	3.7 ± 0.4	>100	>27
(192)	0.5 ± 0.01	15.6 ± 0.2	29.9	3.1 ± 0.5	31.5 ± 0.6	10.3

[a] Concentration causing 50% inhibition of CHIKV replication.
[b] Cytotoxic concentration causing 50% inhibition of cell survival.
[c] SI, selectivity index is the ratio of Toxicity CC_{50}: Antiviral EC_{50}.

The amides (**166**–**170**) and (**171** and **172**) required high concentrations to effect 50% cell death, suggesting that these compounds have low toxicities. Amides (**166**) and (**170**) exhibited EC_{50} values > 100 μM, suggesting that the poor activity is related to the nature of the substituents.

The compound (**171**) showed an improvement in activity as compared to the corresponding amide (**153**), while ether (**172**) was inactive. Interestingly, compounds (**175**) (bromo), (**179**) (methyl ether), (**180**) (methyl), (**181**) (phenyl), and (**182**) (ciano) substituted thieno[3,2-*b*]pyrroles show enhanced activities as compared to the amide (**161**). Among these, compared (**175**) showed the best selectivity index (SI > 32). Additionally, no direct relationship was observed between the electronegativity of the substituent and the antiviral activity or cytotoxicity. Finally, it was also observed that thieno[3,2-*b*]pyrroles with similar substituents (**183**–**184** and **186**–**187**) showed poorer activity as compared to their corresponding amides (**175** and **177**–**179**).

Subsequently, the most potent compounds (**161**, **162**, **169**, **175**, **177**, and **179**–**183**) from the CHIKV-Gluc screen were selected for a study involving the antiviral activity against wild-type CHIKV (CHIKV-IMT). The compounds (**169**), (**177**), and (**183**) (amides) showed poorer activity (EC_{50} values > 10 μM) against CHIKV-IMT infection compared to their activity against CHIKV-Gluc, others (**175** and **179**–**181**) retained their potency, possessing EC_{50} values as low as 2 μM. Finally, the compound (**175**) was found to be as a potent lead compound, it was equally potent against CHIKV-Gluc and CHIKV-IMT infections. Furthermore, studies using Western Blot analyses and qRT-PCR demonstrated that compound (**175**) is capable of inhibiting both protein synthesis and viral RNA synthesis at 2.5 μM concentration.

2.6.6. Abamectin, berberine, bromocriptine, fenretinide, and ivermectin

In order to identify active compound against CHIKV replication, Varghese et al.[86] investigated approximately 3000 compounds, including approved drugs and substances in clinical trials. The viral replication evaluation using *Renilla reniformis* luciferase (Rluc) revealed five hit compounds. While abamectin (**188**), ivermectin (**189**) and berberine (**190**) showed high inhibition activity against CHIKV (>85%), bromocriptine (**191**) and fenretinide (**192**) inhibited 40 and 65%, respectively. In addition, toxicity and inhibitory concentrations were evaluated upon two different cell lines, Baby Hamster Kidney (BHK-21) and Human Hepatocellular (Huh-7.5) (Table 12).

As observed in Table 12, IC_{50} values ranging from 0.5 to 1.9 μM in a Huh-7.5 cell line, and from 0.6 to 3.7 μM in BHK-21. The compounds (**188**) and (**189**) have presented low toxicity only for BHK-21 cell line, while (**190**) and (**191**) for both cell lines.

The compounds (**188**) and (**189**) are macrocycle lactones produced by fermentation of *Streptomyces avemitilis* fungus. Both these compounds are able to inhibit RNA synthesis and viral protein expressions (down-regulation), such as nsP_1 and nsP_3, in BHK-21 cells. In addition, (**190**) and (**191**) were also able to inhibit RNA synthesis at same conditions, although, (**191**) and (**192**) were not capable of interfering with viral protein expression, as observed for (**190**). Finally, (**188**), (**189**), and (**190**) were able to inhibit CHIKV in dose-dependent response. Furthermore, these compounds demonstrated to be found as promising antiviral agents against others Alphaviruses, such as Semliki Forest and Sindbis viruses.[86]

2.6.7. Flavonoids

Flavonoids of natural compounds with a 5,7-dihydroxyflavone structure such as (**1 1 6**), apigenin (**193**), chrysin (**194**), and silybin (**195**) (Fig. 15) are reported to inhibit replication of rhinoviruses, picornaviruses, CHIKV, HIV, and Enterovirus-71. It was shown that these flavonoids inhibited CHIKV replication with IC_{50} value in the range from 22.5 to 126.6 μM, once they gain entry into the host cells but were ineffective in preventing the entry of the virus into the host cell. Additionally, studies from the ethanol extract of *Cynodon dactylon* provided a preparation of fraction rich in (**193**) and luteolin (**196**) (Fig. 15) flavonoids exhibiting potential anti-CHIKV activity, can be effectively used in reducing the inflammation in joints and thereby reducing the severity of the disease.[69]

2.6.8. Flavonols

Studies with (**115**) extracted from Huangchin plants (*Scutellaria baicalensis* and *Scutellaria lateriflora*) demonstrated inhibition of different stages of DENV type-2 replication in vitro. Flavonols, fisetin (**197**) and quercetagetin (**198**) (Fig. 16) shown are variants based on the position of the substituents that are attached to the basic structure of the flavane nucleus, 2-phenyl-benzo[*a*]pyrane. The compound (**197**) has shown significant in vitro antiviral activity against the replication of dengue virus type-2 and enterovirus A71. The compound (**198**) has potential antiviral activity against HCMV and the hepatitis C virus. Based on these results, an investigation toward anti-CHIKV was performed. The compounds (**115**) and (**197**), exhibited stronger antiviral activity against CHIKV intracellular replication than (**198**), with IC_{50} and SI values of 6.997 μM/188.4 and 29.5 μM/23.02, respectively. The compound (**198**) showed significant effects on CHIKV binding to Vero cells, with an IC_{50} of 25.3 mM and an SI of 16.3. It was identified roughly the mechanism of action of the compounds in intracellular replication, the compounds interfere at the stage of converting negative-strand RNA to positive-strand RNA. The data from Western Blot analyses suggest that the antiviral function of (**115**) and (**197**) began with the inhibition of translation of nonstructural proteins, leading to a decrease in the production levels of the replicase units.[87]

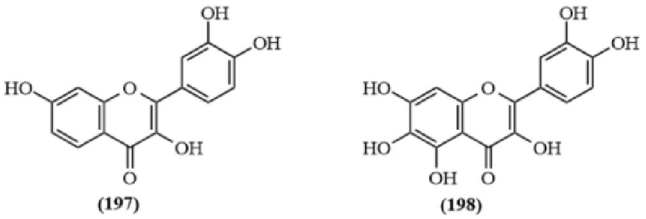


Fig 16. Chemical structures of fisetin (**197**) and quercetagetin (**198**) flavonols.

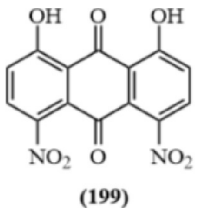


Fig. 17. Chemical structure of anthraquinone ARDP0006 (**199**).

2.6.9. Anthraquinone derivative (ARDP0006)

CHIKV tests based on the NS2B-NS3 protease inhibitors found in DENV, identified as a plausible drug target because of their involvement in viral replication in mammalian host cells, showed an inability to penetrate the cell membrane or lack cellular antiviral activity with the exception of the anthraquinone compound ARDP0006 (**199**) (Fig. 17) was the most potent inhibitor that reduced the dengue viral titer by more than 1 log PFU/mL to 1 μM in the assays involving HuH-7 and K562 cell lines, being highly permeable to the membrane and without cytotoxicity. Human hepatocarcinoma cells (HuH-7) infected with CHIKV shown minimal inhibition of infection at 10 μM, with an approximate reduction of 1 log-unit PFU/ml. That way, anthraquinone could serve as the basis for further studies in the medicinal chemistry of CHIKV.[88]

2.6.10. Lanatoside C

It was recently reported that lanatoside C (**200**) (Fig. 18) to inhibit various negative-strand RNA viruses. Being a Food and Drug

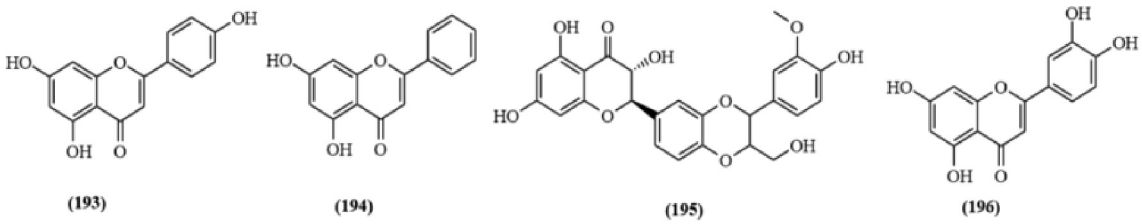


Fig. 15. Chemical structures of apigenin (**193**), chrysin (**194**), silybin (**195**), and luteolin (**196**) flavonoids.

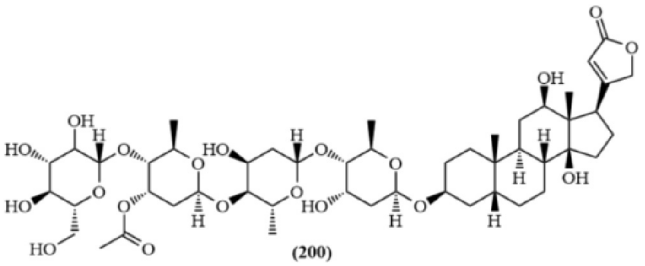


Fig. 18. Chemical structure of lanatoside C (**200**).

Administration (FDA) approved drug also makes (**200**) an ideal antiviral candidate since it has been approved for human usage. BHK21 cells were infected with CHIKV and then treated with 0.5 μM and 1.0 μM of (200) shown inhibitory effects, was reduced by 38.66% at 1.0 μM. The compound (**200**) as a promising antiviral drug that should be further examined.[89]

2.6.11. Bryostatin 1 and its analogs

Bryostatin 1 (**201**) is a potent modulator of both conventional (α, βI/βII, and γ) and novel (δ, ε, η, and θ) protein kinase C (PKCs). Was recently discovered bryostatin analogs, incorporating different A- or B-ring functionalities or a salicylate group in place of the AB ring system, protect cells from CHIKV-induced cell death with EC_{50} values in the low μM range (Table 13). It was related salicylate-based (**201**) analogs (compounds **202**–**204**) have been developed that inhibit CHIKV replication through a novel, yet still elusive, non-PKC dependent pathway. Further studies are ongoing to unravel the precise molecular mechanism by which the bryostatin analogs inhibit CHIKV replication.[90]

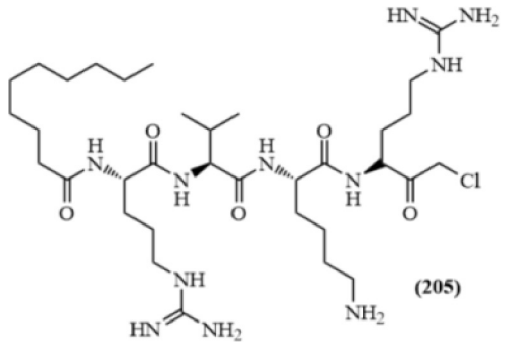


Fig. 19. Chemical structure of dec-RVKR-cmk (**205**).

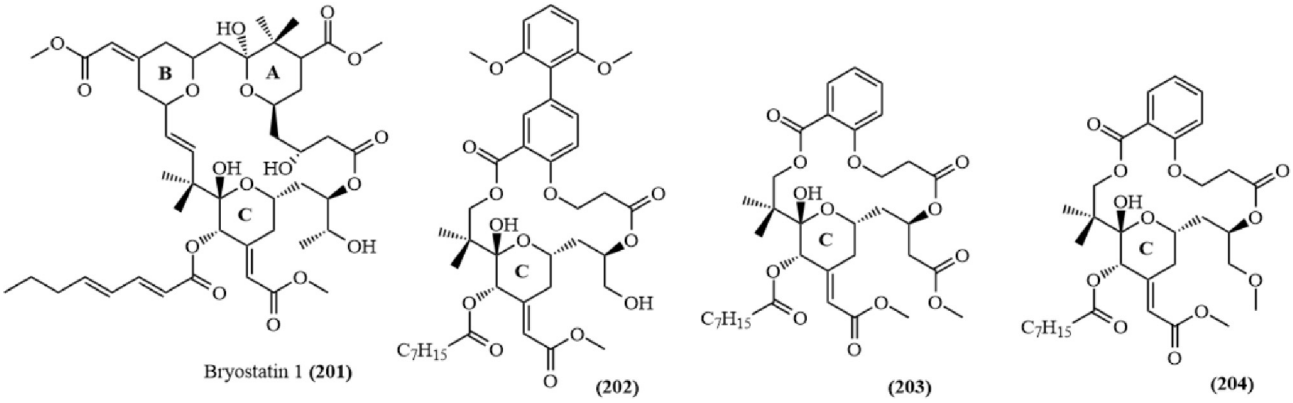
2.7. Host-targeting antivirals

2.7.1. Furin inhibitors

Besides chloroquine, infection by Alphaviruses can be inhibited in vitro by blocking the intracellular cleavage of viral envelope glycoproteins. Alphavirus envelope glycoproteins are initially produced as precursors (E_3E_2 or p62) and during viral maturation further cleaved at short multibasic motifs.[61] Among cellular proteases, the basic amino acid-specific furin or furin-like pro-proteins convertases (PCs) have been considered to be involved in Ca^{2+}-dependent processes, resulting in the cleavage of surface glycoproteins at the C-terminal of the consensus sequence (K/R)X(K/R)R↓.[61]

4110990-7529830In a study performed by Ozden et al.[61] have shown that among a large panel of PCs, furin, but also other furin-like proteases, could process CHIKV E_3E_2 in vitro and *in vivo*. Additionally, it was investigated the inhibitory effects of

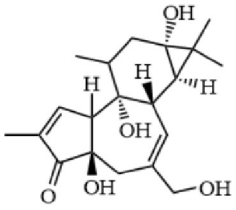
Table 13
Effect of Bryostatin 1 Analogs on the Replication of CHIKV in Vero Cells.



Compound	CHIKV-899 Inhibition ± SD[a]	
	CC_{50} (μM)	EC_{50} (μM)
(202)	>50[b]	4.0 ± 0.4[b]
		2.1 ± 0.1[c]
		2.0 ± 0.4[d]
(203)	>50[b]	8.0 ± 0.4[b]
		4.6 ± 0.8[c]
		4.5 ± 1.0[d]
(204)	>50[b]	7.5 ± 2[b]
		6.0 ± 0.4[c]
		4.1 ± 0.2[d]

[a] Data are obtained from at least three independent experiments. Values were determined by.
[b] CPE reduction assay (MTS readout).
[c] qRT-PCR or.
[d] End-point titration assay.

Table 14
Antiviral Activity of Prostratin Against Different CHIKV Strains.



(206)

Virus strain	EC_{50} (μM) ± SD[a]
CHIKV-899	8 ± 1.2[b]
	7.6 ± 1.3[c]
	7.1 ± 0.6[d]
CHIKV-SGP011	0.2 ± 0.05[e]
	0.3 ± 0.06[c]
	0.4 ± 0.08[d]
CHIKV-LR2006 OPY1	0.2 ± 0.03[e]
CHIKV-CNR20235	0.5 ± 0.2[e]

[a] Mean values ± Standard Deviation of three independent experiments.
[b] Cytopathic effects (CPE) assay.
[c] Quantitative reverse transcription PCR (qRT-PCR) assay.
[d] Titration assay;
[e] Luciferase assay.

an irreversible furin peptide, decanoyl-Arg-Val-Lys-Arg-chloromethyl ketone (dec-RVKR-cmk (**205**), Fig. 19) on CHIKV infection of a human muscle satellite cells. Interestingly, it was observed a strong inhibition of CHIKV entry when pretreating the cells with convertase inhibitor dec-RVKR-cmk. Consequently, pretreatment of muscle cells with dec-RVKR-cmk should not have affected virus entry and the number of infected foci.[61]

2.7.2. Protein kinase C (PKC) inhibitors - prostratin

Protein kinase C (PKC) is a family of related serine/threonine kinases that regulate several cellular processes, such as proliferation, differentiation, and apoptosis through phosphorylation of pathway signaling.[4,5,91] In according to Abdelnabi et al.,[4] prostratin (**206**), a phorbol ester originally isolated from croton oil (*Croton tiglium*), have been reported to inhibit the entry of HIV and to compromise latent HIV viral reservoirs. Additionally, (**206**) was shown to inhibit the replication of CHIKV in vitro, however, the mechanism remains poorly elucidated. Table 14 summarizes the results for the anti-CHIKV activity upon the CHIKV Indian Ocean strain 899 (CHIKV-899), CHIKV isolates from Singapore (SGP011), Reunion Island (LR2006 OPY1), and Caribbean (CNR20235).

The compound (**206**) inhibited CHIKV RNA synthesis and the production of infectious virus progeny. In addition, it reduced the accumulation of viral proteins (nsP1 and Capsid) in a dose-dependent manner. These data strongly indicated that prostratin not only has a cell-protective effect but that it has a direct inhibitory effect on CHIKV replication too, through PKC inhibition.

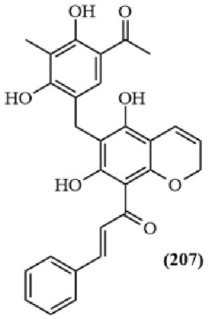
Based on these results, the antiviral effect of different PKC inhibitors, such as Rottlerin (**207**) (known as mallotoxin), Gö6976 (**208**), Ro-32-0432 (**209**), and Sotrastaurin (**210**), was determined in buffalo green monkey kidney cells (BGM) by a CPE reduction assay. None of the tested PKC inhibitors inhibited CHIKV replication at the highest non-toxic concentration[4] (Table 15).

2.7.3. Tigliane diterpenes

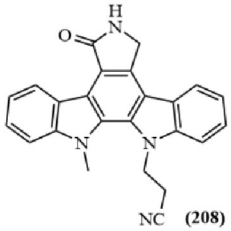
Bourjot et al.[5] performed a chemical study of the Vietnamese plant species *Trigonostemon howii* led to the isolation of a new tigliane-type diterpenoid, trigowiin A (**211**), along with several know coumarins and phenylpropanoids. Additionally, this study included four structurally closely related tigliane diterpenes, such as (**206**), phorbol (**212**), 12-O-tetradecanoylphorbol 13-acetate (TPA) (**213**), and 4-α-12-O-tetracarbonylphorbol 13-acetate (4α-TPA) (**214**) in anti-CHIKV assays, employing (**1**) as a positive control. The results are shown in Table 16.

In the CHIKV assay, it was observed that (**206**) and (**213**) proved to be the most potent inhibitors, as apparent from their lower potent EC_{50} values and higher selectivity indices, in comparison to the reference compound (**1**), as shown above. Additionally, the (**213**) proved to be on average 65 times more potent than (**206**) and 1000 times more potent than its (**214**), while (**212**) was found to be completely inactive. Finally, taking into account that the antiviral assays, the probable mechanism was suggested by the authors. This mechanism could be associated with the activation of the signal transduction enzyme PKC, similar to the mechanism of inhibition of HIV replication performed by (**213**).[5]

Table 15
Cytotoxic and Anti-CHIKV Activity of PKC Inhibitors.



(207)



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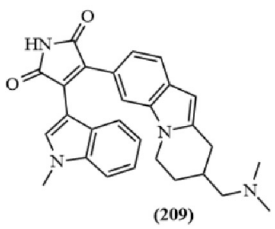
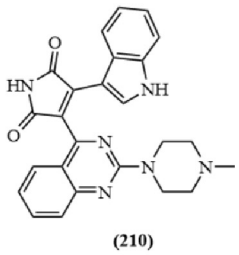


(209)

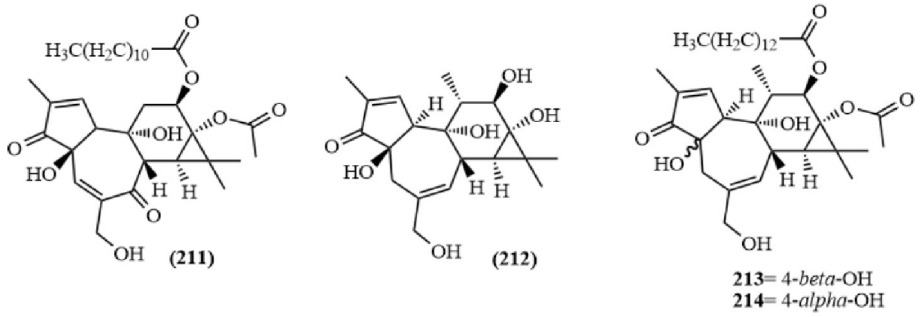


(210)

PKC inhibitor	Inhibition (μM) ± SD[a]	
	CC_{50} (Vero cells)	EC_{50} (CHIKV)
(207)	0.2 ± 0.001	>0.2
(208)	6.0 ± 0.07	>6.0
(209)	35.0 ± 1.0	>6.0
(210)	66.0 ± 3.0	>66.0

[a] Mean ± Standard Deviation of at least three independent experiments.

Table 16
Cytotoxic and Anti-CHIKV Profile of Tigliane Diterpenes.



Compound	Inhibition (μM) ± SD[a]		SI[b]
	CC_{50} (Vero cells)	EC_{50} (CHIKV)	
(206)	7.9 ± 17.4	2.6 ± 1.5	30.3
(211)	>100	43.5 ± 12.8	>2.3
(212)	>343	>343	>1.0
(213)	5.7 ± 1.7	0.0029 ± 0.0003	1965
(214)	5.3 ± 0.6	2.8 ± 0.5	1.9
(1)	89.0 ± 28.0	11.0 ± 7.0	8.1

[a] Mean ± Standard Deviation of at least three independent experiments.
[b] Selectivity index determined by CC_{50}/EC_{50}.

2.7.4. Polyinosinic acid

Polycytidylic acid [poly(I:C)] (**215**) (Fig. 20) is a synthetic double-stranded RNA (dsRNA) analog. In addition, it is an immunostimulant acting as an inducer for the most potent interferon (INF), via interaction with the Toll-like receptor 3 (TRL3).[77,92] Moreover, activation of the TRL3 leads to an innate immune response against many different types of viruses.[93] In according with Li et al.,[94] the CHIKV was found to be sensitive to the innate immune response induced by (**215**). Finally, this sensitivity was assumed to be related to a decreased cytopathic effect and inhibition of the virus replication in the infected cell lines. Furthermore, these steps are involved with the overstimulation of the TRL3 as well as the other antiviral genes by (**215**).[77,94]

2.8. Inhibitors with unidentified targets

2.8.1. Trigocherrins and Trigocherriolides

In studies performed by Allard et al.,[59,95] Trigocherrins A-D (216–219) and Trigocherriolides A-D (**220**–**223**) compounds, possessing a daphnane diterpenoids skeleton, were isolated from Trigonostemon cherrier (*Euphorbiaceae*), and some of them have presented to be selective inhibitors of CHIKV replication. The results are grouped into Table 17.

2.8.2. 6-Mercaptopurine

It is known that the 6-mercaptopurine (6-MP) (**224**) and azathioprine (**225**) (Fig. 21) are responsible for changing the resistance of the intact host to viral infection.[96] Based on this, Geme et al.[96] performed about the potential of (**224**) as an inhibitor against CHIKV strain S-27. It was observed that the titers of interferon in cultures treated with (**224**) were uniformly less than that of control cultures, ranging from 47 to 67 units mL^{-1}. Finally, it was considered that (**224**) failed to inhibit the synthesis and the action of interferon. Different aspects of host resistance to viral infection should be considered, and the delayed hypersensitivity may be considered too.[96]

2.8.3. Macrocycle C15-type metabolites

Techer et al.[26] developed a bio-assay guided fractionation of the ethyl acetate bark extract from *Stillingia lineata* ssp. *lineata* in a virus-cell-based assay for CHIKV on Vero cells, as well as the characterization of diterpenes using 500 MHz 2D-NMR (COSY, NOESY, HSQC, and HMBC) and mass spectrometry.

The *S. lineata* ssp. *lineata* ethyl acetate bark extract was selected due its potent anti-CHIKV activity ($EC_{50} < 0.8$ μg mL^{-1}) and weak cytotoxicity on Vero cells ($CC_{50} = 60.9$ μg mL^{-1}). In addition, the use of preparative and semipreparative C18 HPLC allowed purifying three rare macrocycle C15-type diterpenes: tonantzitlolone (**226**), tonantzilolone (**227**), and 4′-hydroxytonantzitolone (**228**), and one pimarane, ent-12α-hydroxy-3,7-dioxoisopimara-8,15-diene (**229**) (Fig. 22).

In according with Techer et al.,[26] only the (**227**) selectively inhibited virus-induced cell death, with EC_{50} and selectivity index (SI) values of 7.0 μM and 8.8, respectively. In addition, (**228**) was not selective ($EC_{50} = 34.0$ μM and SI = 3.2). Where a comparison between these two structures was performed, and it was observed that the presence of an acetyl group is important in improving the activity.

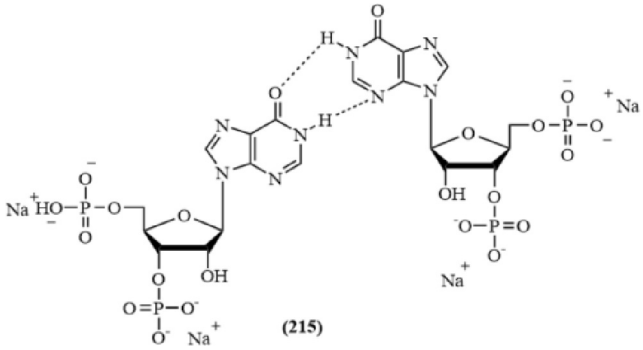
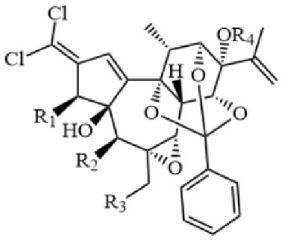


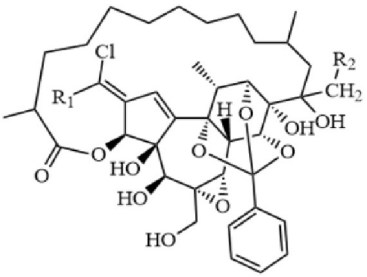
Fig. 20. The structure of the dsRNA analog (**215**).

Table 17
Cytotoxic and Activity of Trigocherrin-A, -B and -D and Trigocherriolide-A, -B and -C against CHIKV.



Trigocherrins (216-219)

	R_1	R_2	R_3	R_4
216=A=	$(C_6H_6)COO$	Ac	Ac	OH
217=B=	H	H	Ph	Ph
218=C=	H	Ph	H	Ph
219=D=	Ph	H	H	Ph

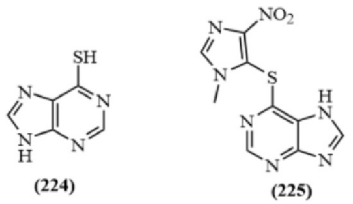


Trigocherriolides (220-223)

	R_1	R_2
220=A=	H	2-Hydroxibenzyl acetate
221=B=	H	H
222=C=	H	$(C_6H_6)COO$
223D=	Cl	H

		Inhibition (μM) ± SD[a]	
Class	Code	CC_{50} (Vero cells)	EC_{50} (CHIKV)
Trigocherrins	**(216)**	35.0 ± 8.0	1.5 ± 0.6
	(217)	93.0 ± 3.0	2.6 ± 0.7
	(219)	23.1 ± 0.6	3.0 ± 1.2
Trigocherriolides	**(220)**	4.6 ± 0.8	1.9 ± 0.6
	(221)	5.3 ± 0.2	2.5 ± 0.3
	(222)	10.5 ± 0.1	3.9 ± 1.0

[a] Values are the median ± median absolute deviation calculated from at least three independent assays.



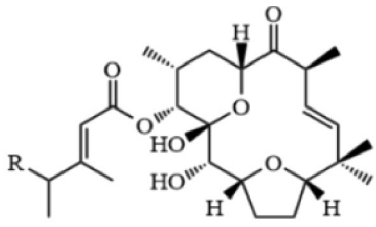
(224) **(225)**

Fig. 21. Chemical structures of 6-Mercaptopurine (**224**) and azathioprine (**225**).

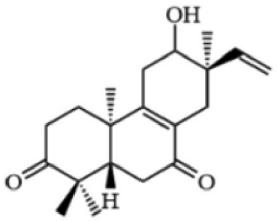
2.8.4. [1,2,3]Triazolo[4,5-d]pyrimidin-7-(6H)-ones

Gigante et al.[37] have employed a joint screening program to identify novel compounds against CHIKV. The selective antiviral activity of a structurally diverse collection of chemical compounds from CSIC (Madri) was found as selective in a virus-cell-based assay for CHIKV replication at KU Leuven. One of the chemical samples, coded by TR247, portrayed an interesting activity/cytotoxicity. Applying high-performance liquid chromatography-mass spectrometry (HPLS-MS) analysis was found that the hit sample was a 1:1 mixture of two compounds. Subsequently, both entities were separated using chromatography, structurally characterized, and individually evaluated for anti-CHIKV activity. This last, revealed that the chloro-compound (**230**) was inactive while the 7-oxo derivative (**231**) presented the antiviral activity (EC_{50} value of 19 μM).

Based on these results, compound (**231**) was selected to prepare structural analogs and to explore the structure-activity relation-



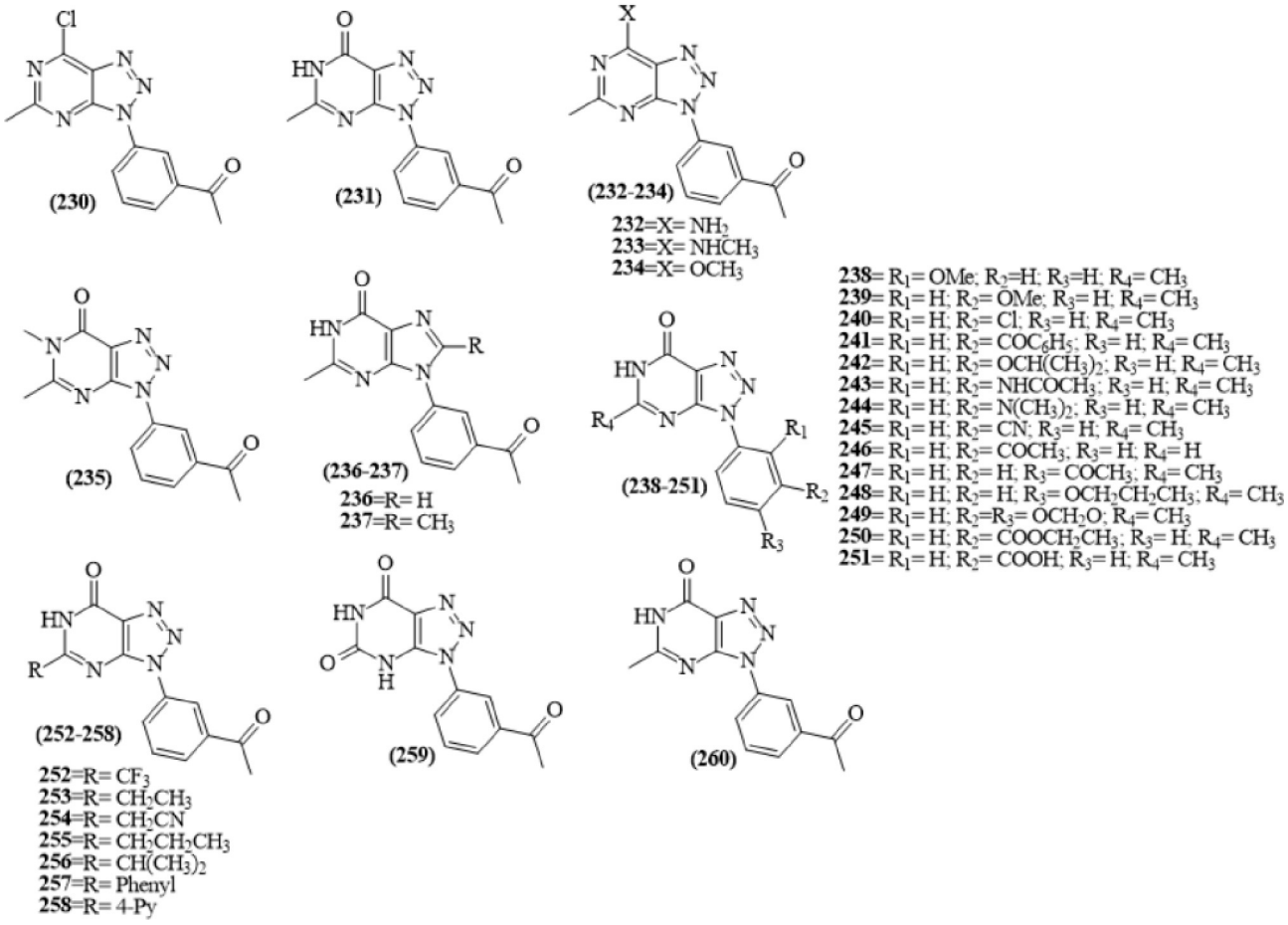
R:
H= tonantzitlolone **(226)**
Acetyl= 4'-acetoxytonantzitolone **(227)**
OH= 4'-hydroxytonantzitlolone **(228)**



ent-12a-hydroxy-3,7-dioxoisopimara-8,15-diene **(229)**

Fig. 22. Compounds isolated from *S. lineata* ssp. *lineata* stem bark.

Table 18
Antiviral Evaluation of the Triazolopyrimidines and Analogs Against CHIKV in Vero Cells.



Compound	Inhibition (μM ± SD)[a]		
	EC_{50}[b]	EC_{90}[c]	CC_{50}[d]
(230)	>174	>174	>174
(231)	19 ± 2	38 ± 16	>743
(232)	225 ± 33	309 ± 48	>746
(233)	>443	>443	514 ± 55
(234)	>441	>441	495 ± 34
(235)	>441	>441	>706
(236)	127 ± 10	161 ± 27	491
(237)	>440	>440	>703
(238)	348 ± 36	460 ± 13	>777
(239)	28 ± 6	179 ± 44	>777
(240)	32 ± 11	235 ± 7	>764
(241)	23 ± 6	47	>604
(242)	12 ± 4	156 ± 43	>704
(243)	318	425	206 ± 78
(244)	>370	>370	538
(245)	131 ± 11	187 ± 21	>793
(246)	>490	>490	>784
(247)	326 ± 53	>743	>743
(248)	169	>701	594 ± 100
(249)	>461	>461	>737
(250)	202 ± 53	331 ± 64	322
(251)	>399	>399	>638
(252)	68 ± 4	>147	104 ± 32
(253)	3 ± 1	18 ± 18	>668
(254)	115 ± 16	137	215 ± 63
(255)	>399	>399	>638
(256)	280	>638	>638
(257)	204 ± 72	>360	227 ± 128
(258)	75 ± 19	>144	82 ± 22

(*continued on next page*)

Table 18 (*continued*)

Compound	Inhibition (μM ± SD)[a]		
	EC_{50}[b]	EC_{90}[c]	CC_{50}[d]
(259)	>435	>435	>696
(260)	167 ± 10	232 ± 62	>872
(1)	11 ± 7	21 ± 18	89 ± 28

[a] All data are mean values ± standard deviation for at least three independent experiments.
[b] 50% effective concentration or calculated concentration of compound that is required to protect 50% of the cells against cytopathic effects caused by the viral infection.
[c] 90% effective concentration or calculated concentration of compound that is required to protect 90% of the cells against cytopathic effects caused by the viral infection.
[d] 50% cytotoxic concentration or calculated concentration of compound that reduces the overall cell metabolic activity (by a combined cytotoxic, cytostatic, and anti-metabolic effect) to 50%.

ship of this compound class in more detail. In total, 31 compounds (**230**–**260**) were synthesized and evaluated for anti-CHIKV activity upon Vero cells (Table 18).

With respect to the first series of compounds (**232**–**237**), it was revealed that a carbonyl group was required at position 7 of the heterocyclic base to exhibit antiviral activity, as compound (**231**). In addition, those compounds with NH_2, NHMe, or OMe groups at position 7 (compounds **232**–**234**, respectively) were significantly less active or completely inactive. Also, the NH group at position 6 showed be unsubstituted based on the lack of activity of the N-Me derivative (**235**). Additionally, when the triazolopyrimidine in compound (**231**) is replaced by the analogous imidazopyrimidine (**236**), an antiviral activity could be observed but at EC_{50} values 7-fold higher than that of compound (**231**). Also, when a methyl group is introduced at position 8 of the purine (**237**), the antiviral activity is lost.

Regarding the substituents at the aryl ring (compounds **238**–**251**), the molecular features for antiviral activity are also quite strict. Compounds with OMe (**239**), Chloro (**240**), benzoyl (**241**), or isopropoxy (**242**) groups at position 3, associated with the acetyl of the hit compound (**231**), produced the best antiviral activity. In addition, when the OMe group was introduced at position 2, the EC_{50} was more than 10-fold higher than the OMe group at position 3 (**238** in comparison with **239**). Furthermore, moving the acetyl group from position 3 to position 4 (compound **231** in comparison with **247**) led to a 17-fold decrease in antiviral profile. The best EC_{50} values were observed when this substituent was an isopropoxy (**242**). Compound (**246**), with an acetyl at position 3 at the aryl ring but a hydrogen at position 5 of the base, was inactive, suggesting the importance of this position of the heterocycle in this antiviral activity. This fact was better observed with the evaluation of compounds (**252**–**258**) and (**259**). For these, some compounds presented antiviral activity (**252**–**254**, **257** or **258**), although in many cases, this fact was accompanied by additional cytotoxicity according to the CC_{50} values. Interestingly, compound (**253**), containing an ethyl group at position 5, presented interesting EC_{50} and EC_{90} values, with no cytotoxicity up to 668 μM. In sense, compound (**253**) with a selectivity index (SI) of 222 was the most potent and selective compound among all these triazolopyrimidines and has a better profile than the control compound chloroquine. Finally, the benzyl derivative (**260**) was at least 9-fold less potent than its aryl analog (**231**).

The hit compound (**231**) and the most potent compound of this series (**258**) were evaluated for selective antiviral assay against different CHIKV strains (Table 19).

Compound (**253**) was 10 times more active than the hit compound (**231**), with EC_{50} values in the low micromolar range and the best value of 0.75 μM for CHIKV Cong 95 strain. Both compounds were significantly more active by a factor of 4 against the African Congo strain than against the other strains. Additionally, antiviral activity was found for compound (**231**) and (**253**) against the recently emerged St. Martin strain. Finally, no cytotoxicity up to 300 μM was observed in Vero E6 line cells employing the same experimental conditions as the antiviral assay.

2.8.5. Benzouracil-coumarin-arene conjugates

Hwu et al.[97] have synthesized new uracil-coumarin-arene conjugates and evaluated for their antiviral activity against CHIKV replication upon Vero cells (Table 20). Among these, five conjugates showed significant inhibition of the in vitro CHIKV replication with low toxicity. Additionally, structure-activity relationship studies revealed that the compounds with a benzouracil-SCH_2-coumarin-OSO_2-arene scaffold were the most potent inhibitors in this series.

2.8.6. Lupenone and β-Amyrone

Bourjot et al.[99] performed an investigation about the anti-CHIKV potential of triterpenoid metabolites isolated (Lupenone (**286**) and β-Amyrone (**287**), Fig. 23) from *Anacolosa pervilleana*, namely Madagascan plant. In a virus-cell based assay for CHIKV was observed that compounds showed moderate anti-CHIKV activity, IC_{50} values of 77 and 86 μM, respectively.[77,99]

2.8.7. Purine-based inhibitors

D' Hooghe et al.[100] reported the design and synthesis of a novel series of purine-β-lactam hybrids and purine-aminopropanol hybrids and their potential antiviral activities. In total, nine different viruses were included in this study. The results showed that two purine-β-lactam hybrids and one purine-aminopropanol hybrid (Fig. 24) presented promising activity and cytotoxicity profiles, the purine-β-lactam (**288**) with EC_{50} value of 17.11 μM and SI > 5.75 the purine-β-lactam (**289**) with EC_{50} value of 13.01 μM and SI > 4, and the purine-aminopropanol (**290**) with EC_{50} = 11.51 - μM and SI > 6. Finally, the mechanism of action has not been investigated.[77,100]

Table 19
Antiviral Evaluation of the Triazolopyrimidine (**231**) and (**253**) Against Different Laboratory and/or Isolated Strains of CHIKV in Vero Cells.

CHIKV strain	Inhibition (μM ± SD)[a]	
	Compound **(231)** EC_{50}	Compound **(253)** EC_{50}
899	19 ± 2	2.6 ± 1
LR2006-OPY1	25	2.6 ± 0.5
Venturini	26 ± 2	1.4 ± 0.01
Congo 95	6.4 ± 0.05	0.75 ± 0.4
St. Martin	24 ± 0.5	2.9 ± 0.05

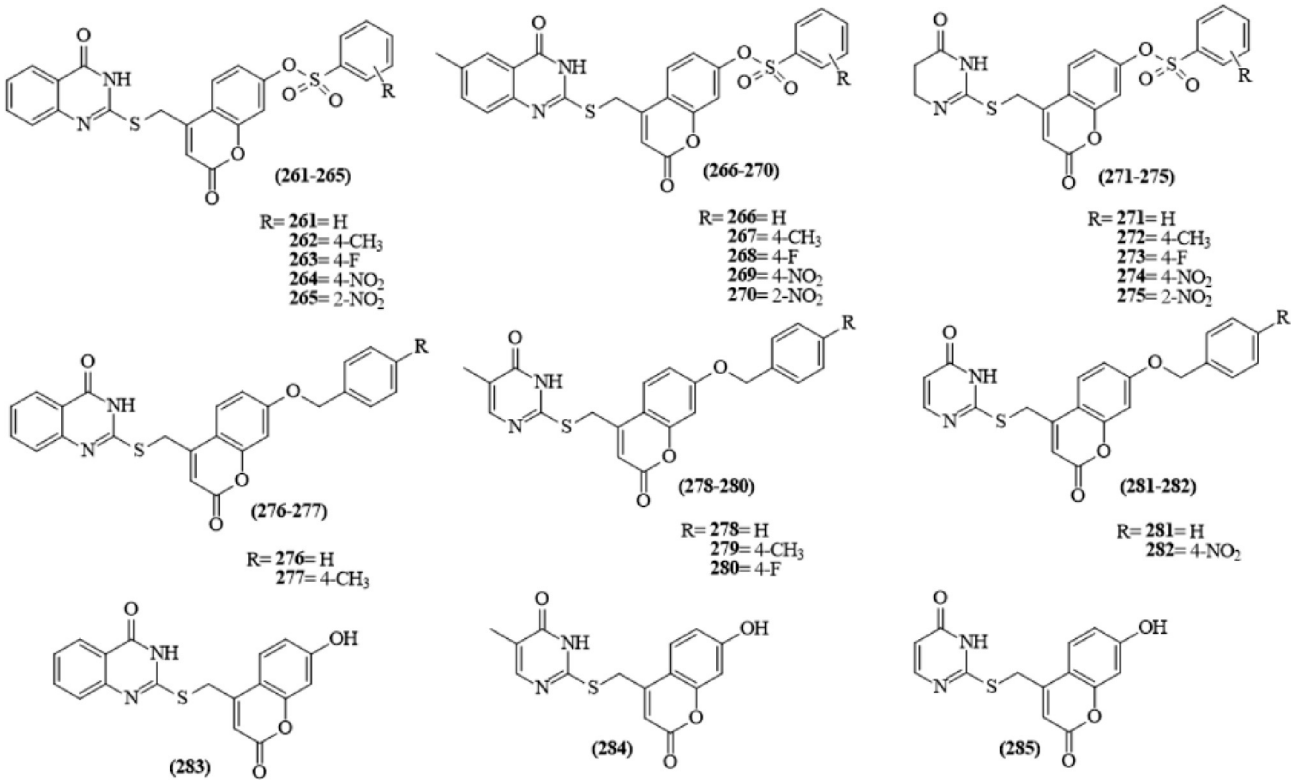
[a] All data are mean values ± standard deviation for at least three independent experiments.

2.9. Larvicidal, ovicidal and oviposition deterrent agents

2.9.1. Hedychium larsenii essential oil

AlShebly et al.[101] reported the larvicidal and oviposition deterrent activity of the *Hedychium larsenii* essential oil against the *A. aegypti* vector. In addition, the two major constituents of this essential oil were isolated, *ar*-curcumene (**291**) (28.6%) and *epi*-*β*-bisabolol (**292**) (10.3%). The essential oil extract from the leaves of *H. larsenii* presented moderate larvicidal activity (Table 21).

Table 20
Antiviral Activity of Conjugated Compounds on CHIKV (899 Strain) in Vero Cells.



Compound	Inhibition (μM) EC_{50}[a]	CC_{50}[b]	SI
(261)	19.1	178	9.3
(262)	10.2	117	11.5
(263)	18.4	30	1.6
(264)	54.5	117	2.2
(265)	17.2	144	8.8
(266)	58	126	2.2
(267)	26.4	114	4.3
(268)	116	86.4	–
(269)	>199	–	–
(270)	19.0	107	5.6
(271)	57.4	–	–
(272)	23.1	60.2	2.6
(273)	128	111	–
(274)	>205	–	–
(275)	13	75.2	5.8
(276)	>45.2	–	–
(277)	>2.19	102	–
(278)	>246	–	–
(279)	>48	–	–
(280)	45.1	104	2.3
(281)	>255	–	–
(282)	4.6	13.8	3.0
(283)	192	>284	>1.5
(284)	>316	–	–
(285)	>331	–	–

[a] The concentration of a compound with an adverse effect of 50% was observed on the host cell metabolism as determined by the MTS method.
[b] The concentration of a compound at with virus replication was inhibited by 50% was observed, as determined by real-time quantitative RTq-PCR.

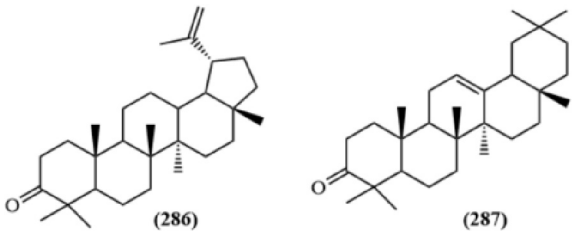


Fig. 23. Chemical structures of lupenone (**286**) and β-amyrone (**287**) extracted from A. pervilleana.

Additionally, the (**291**) showed LC_{50} of 11.24 μg mL^{-1}, while the (**292**) 15.83 μg mL^{-1} (Table 22). Finally, the oviposition deterrent activity of *H. larsenii* essential oil, (**291**), and (**292**) was evaluated, results shown in Table 23.

2.9.2. Arylhydrazone ester derivatives

Bandyopadhyay et al.[13] have performed the synthesis of arylhydrazone esters (Scheme 1). Initially, it was found better attractant results in the case of unsymmetrical arylhydrazone esters than that of the symmetrical structures. The oviposition response studies in *A. albopictus* were performed in accord with methods described by Linley.[102]

The oviposition activity was assumed as oviposition activity index (OAI), those compounds with an OAI of +0.3 and superior are considered as attractants, where those with −0.3 and inferior are considered as repellents (Table 24).

Among these 13 compounds, (**304**) showed great positive oviposition stimulation with OAI value of 0.299 in *A. albopictus*. Additionally, it was observed that when the phenyl ring of (**293**) was replaced by naphthalene ring in (**304**) the oviposition attractant activity increased of +0.157 to +0.299, in OAI terms. The (**302**) presented highest oviposition deterrent activity with OAI value of −0.247. Finally, the oviposition response was found to be neutral for (**294**), (**299**), (**300**), and (**305**) compounds.

3. Conclusion

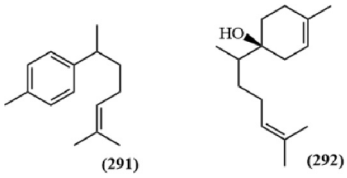
Actually, therapeutic alternatives involving antiviral drugs, natural products, and metabolites, and immunomodulatory strategies need to be better evaluated but in subgroups of chronic patients. However, all these alternatives are expected to be improved and evidence-based in scientific and clinical studies. Despite several studies involving biological targets and biomolecular approaches, the physiopathologic mechanism remains poorly understood. Additionally, the major problem is the lack of an animal model that can perfectly reproduce the human chronic stage of Chikungunya disease. Finally, Chikungunya virus represents a serious public health problem in the world that can no longer be ignored. In sense, there is an urgent need for the development of safe and effective antivirals against CHIKV to control symptoms and minimize complications in future epidemics, as well as to combat the vector in different stages of its life cycle.

Table 21
Larvicidal Activity of the Essential Oil from *Hedychium larsenii* Against Aedes aegypti.

Concentration (μg/mL)	Mortality (%) ± SD[a]	Inhibition (μg/mL) LC_{50}	LC_{90}	Slope
40	25.9 ± 1.2	88.6	171.85	2.95
80	42.5 ± 0.8			
120	66.2 ± 0.6			
160	84.6 ± 0.4			
180	98.1 ± 0.8			

[a] SD: standard deviation calculated from three independent experiments.

Table 22
Larvicidal Activity of *ar*-curcumene and *epi*-*β*-bisabolol Against *Aedes aegypti*.



Code	Concentration (μg/mL)	Mortality (%) ± SD[a]	Inhibition (μg/mL) IC_{50}	IC_{90}
(291)	5	24.5 ± 1.2	11.24	21.99
	10	45.3 ± 0.6		
	15	62.7 ± 0.4		
	20	84.2 ± 0.8		
	25	97.1 ± 0.6		
(292)	7	24.8 ± 1.2	15.83	30.31
	14	42.6 ± 0.8		
	21	63.2 ± 0.4		
	28	84.7 ± 0.6		
	35	98.1 ± 1.2		

[a] Standard deviation calculated from three independent experiments.

Table 23
Oviposition Deterrent Activity of *Hedychium larsenii* Essential Oil, *ar*-curcumene, and *epi*-*β*-bisabolol Against the Dengue/Chikungunya Vector *Aedes aegypti*.

Treatment	Concentration (μg/mL)	Effective repellency (%)	OAI[a]
H. larsenii oil	50	74.1	−0.58
	100	78.83	−0.65
	150	83.4	−0.71
	200	86.98	−0.76
	250	89.58	−0.81
(291)	10	70.28	−0.54
	20	75.62	−0.6
	30	80.87	−0.67
	40	86.0	−0.75
	50	89.99	−0.81
(292)	15	72.3	−0.56
	30	77.03	−0.62
	45	81.13	−0.68
	60	85.45	−0.74
	75	88.99	-0.8

[a] Oviposition activity index.

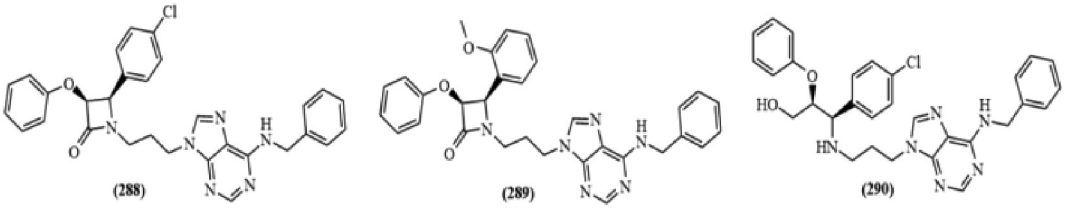
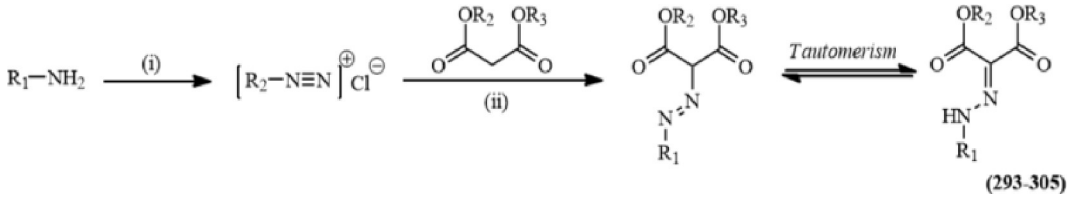


Fig. 24. Purine-Based Inhibitors with Anti-CHIKV activity.



Scheme 1. The synthetic route for obtaining of arylhydrazone esters performed by Bandyopadhyay et al. Reagents and conditions: (i): $NaNO_2$/HCl, 0 → 5 °C; (ii) $H_3CCOONa$/H_3CCH_2OH.

Table 24
Oviposition Response of *A. albopictus* to Arylhydrazone Esters.

Compound	R_1	R_2	R_3	OAI[a]
(293)	Phenyl	CH_3	H_3CCH_2	+0.157
(294)	4-Nitrophenyl	CH_3	H_3CCH_2	+0.003
(295)	4-Methylphenyl	CH_3	H_3CCH_2	-0.197
(296)	4-Hydroxyphenyl	CH_3	H_3CCH_2	+0.076
(297)	2-Tolylethanone	CH_3	H_3CCH_2	-0.124
(298)	2-Tolylmethanol	CH_3	H_3CCH_2	+0.114
(299)	4-Tolylethanone	CH_3	H_3CCH_2	−0.032
(300)	4-Fluorophenyl	CH_3	H_3CCH_2	−0.015
(301)	4-Fluorophenyl	CH_3	$CH_2CH(CH_3)_2$	−0.199
(302)	3-Chlorophenyl	CH_3	H_3CCH_2	−0.247
(303)	4-Methoxylphenyl	CH_3	H_3CCH_2	+0.147
(304)	Naphthalen-1-yl	CH_3	H_3CCH_2	+0.299
(305)	Ethyl 4-methylbenzoate	CH_3	H_3CCH_2	+0.073

[a] Oviposition activity index.

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