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MINI REVIEW

An update on the development of antiviral against Mayaro virus: from molecules to potential viral targets

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Abstract

Mayaro virus (MAYV), first isolated in 1954 in Trinidad and Tobago islands, is the causative agent of Mayaro fever, a disease characterized by fever, rashes, headaches, myalgia, and arthralgia. The infection can progress to a chronic condition in over 50% of cases, with persistent arthralgia, which can lead to the disability of the infected individuals. MAYV is mainly transmitted through the bite of the female *Haemagogus* spp. mosquito genus. However, studies demonstrate that *Aedes aegypti* is also a vector, contributing to the spread of MAYV beyond endemic areas, given the vast geographical distribution of the mosquito. Besides, the similarity of antigenic sites with other *Alphavirus* complicates the diagnoses of MAYV, contributing to underreporting of the disease. Nowadays, there are no antiviral drugs available to treat infected patients, being the clinical management based on analgesics and non-steroidal anti-inflammatory drugs. In this context, this review aims to summarize compounds that have demonstrated antiviral activity against MAYV in vitro, as well as discuss the potentiality of viral proteins as targets for the development of antiviral drugs against MAYV. Finally, through rationalization of the data presented herein, we wish to encourage further research encompassing these compounds as potential anti-MAYV drug candidates.

Keywords Mayaro fever · Mayaro virus · Antiviral · Natural and synthetic compounds · Viral targets

Introduction

The Mayaro virus (MAYV) belongs to the *Togaviridae* family and *Alphavirus* genus, which is composed of a positive single-stranded RNA of 11,5 kb in length, covered by an icosahedral capsid and viral envelope (Fig. 1) (Diagne et al. 2020). It was first isolated in 1954 in Trinidad and Tobago islands from the blood of five febrile rural workers (Anderson et al. 1957). Since then, sporadic outbreaks have been identified in countries with tropical forests, including Brazil, Bolivia, Suriname, Peru, Equator, Venezuela, and recently Haiti (Aguilar-Luis et al. 2020). In addition, cases in North America (Taylor et al. 2005) and Europe (Theilacker et al. 2013) were reported in citizens who traveled to South America.

MAYV belongs to the Semliki Complex, a subgroup of the *Alphavirus* genus that includes Bebaru virus, Chikungunya virus (CHIKV), Getah virus, Una Forest virus (UNAV), O'nyong-nyong virus, among others. Viruses from this complex are classified into the same serological group, sharing common antigenic sites, resulting in cross-reactivity in conventional serological tests, complicating the accurate diagnosis of MAYV infections and the underreporting of the disease (Acosta-Ampudia et al. 2018).

MAYV infection is the causative agent of the Mayaro Fever, a disease characterized by acute fever, rashes, headache, retro-orbital pain, nausea, diarrhea, myalgia, and arthralgia (Acosta-Ampudia et al. 2018) that remain for months or years in over 50% of cases (Mackay and Arden

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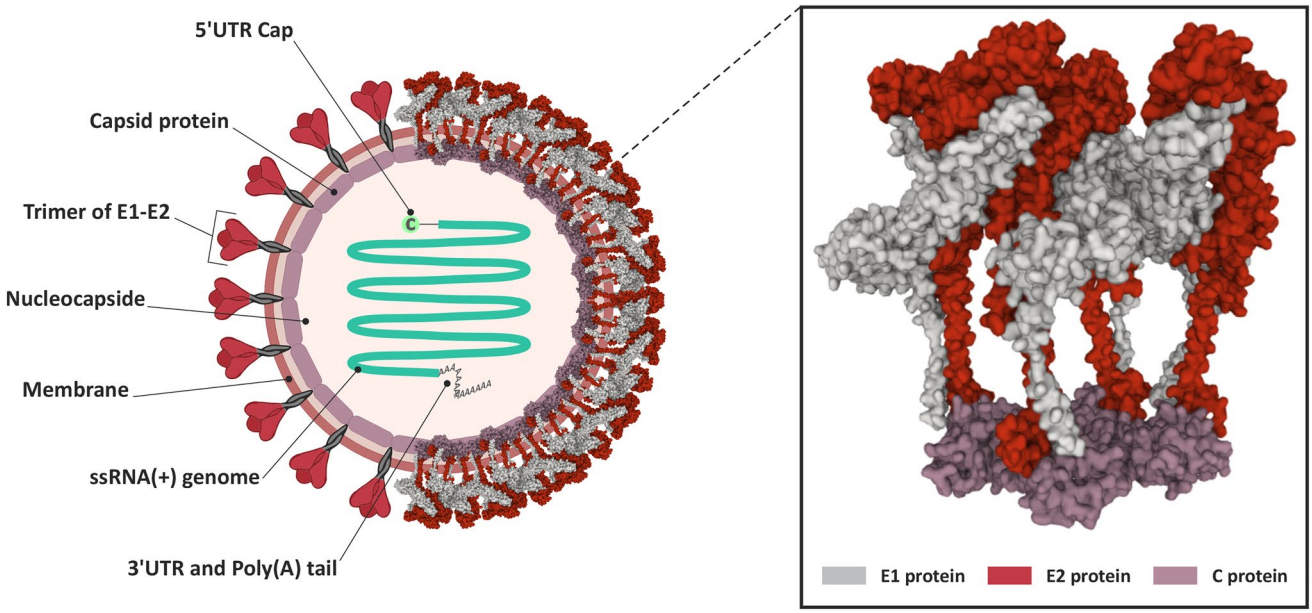


Fig. 1 Schematic structure of MAYV virion. Electron microscopy data show that the viral particle is about 70 nm in diameter. The virion is composed of a positive single-stranded RNA with an icosahedral capsid formed by the capsid protein (C), covered by an envelope membrane inserted of the E1, E2, and E3 glycoproteins. The E3 protein is not shown since it detaches from the E2 protein at the viral surface during maturation (Ribeiro-Filho et al. 2021). Viral structure was based on Cryo-EM structure of mature MAYV (PDB ID: 7KO8) (Ribeiro-Filho et al. 2021)

2016; Li et al. 2019), culminating in physical disability (Ferreira et al. 2018). In severe cases, Mayaro fever can cause intermittent fever, neurological complications, myocarditis, and ultimately death (Acosta-Ampudia et al. 2018).

MAYV is transmitted through the bite of the *Haemagogus* spp. mosquito, usually from infected non-human primates, birds, rodents, and small mammals to susceptible humans (Esposito and Fonseca 2017). The vector competence of Aedes aegypti mosquitoes in the transmission of MAYV is also known, and considering the wide geographic distribution of the Aedes genus, there is a concern about virus spread beyond endemic areas (Long et al. 2011; Choi et al. 2019).

The MAYV replication begins with virus entry into the host cells via specific receptors on the cell surface, such as MXRA8, triggering clathrin-mediated endocytosis or, alternatively, through a caveolin-coated pit (Carvalho et al. 2017; Zhang et al. 2018). Through pH changes in the endosome, the interaction between the endosome membrane and the viral envelope leads to the viral RNA release into the cytoplasm. MAYV RNA is composed of two open reading frames (ORFs). The first ORF is translated in the non-structural viral proteins (nsP) nsP1, nsP2, nsP3, and nsP4, which form the replicative complex (RC), responsible to produce a negative strand from the viral genome, that serves as templates for the synthesis of new positive-sense strands, besides the sub-genomic 26S RNA (Diagne et al. 2020). The other ORF is transcribed from the negative strand, and translated into a polyprotein which is cleaved to the structural proteins C, E1, E2, E3, and 6K (viroporin). Additionally, a frameshift event occurs during translation of the 6K gene, yielding the production of the transframe (TF) protein, comprised of a C-terminal extension of the 6K protein in the −1 ORF. TF protein has been associated with *alphavirus* budding efficiency and/or assembly (Firth et al. 2008; Snyder et al. 2013). The replication process is followed by the assembling of the viral components and virus release through budding in mammalian cells membrane (Jose et al. 2017; Brown et al. 2018) (Fig. 2), or through an alternative exocytosis pathway in insect cells (Acosta-Ampudia et al. 2018).

Despite the chronic condition of a high number of MAYV infections, there is no licensed antiviral against Mayaro fever. The treatment is palliative and consists in managing the symptoms using analgesics and/or non-steroidal anti-inflammatory drugs (Sun and Wu 2019). Therefore, research data have been produced on the potential of natural and synthetic molecules against MAYV. Here we summarize the natural and synthetic compounds previously described to possess antiviral activity against MAYV (Table 1). Thus, we critically compare molecules that could be further investigated in vivo and suggest protein targets to be further evaluated as anti-MAYV molecules. Finally, we aim to encourage further research encompassing these compounds as potential anti-MAYV drug candidates to treat infected patients.

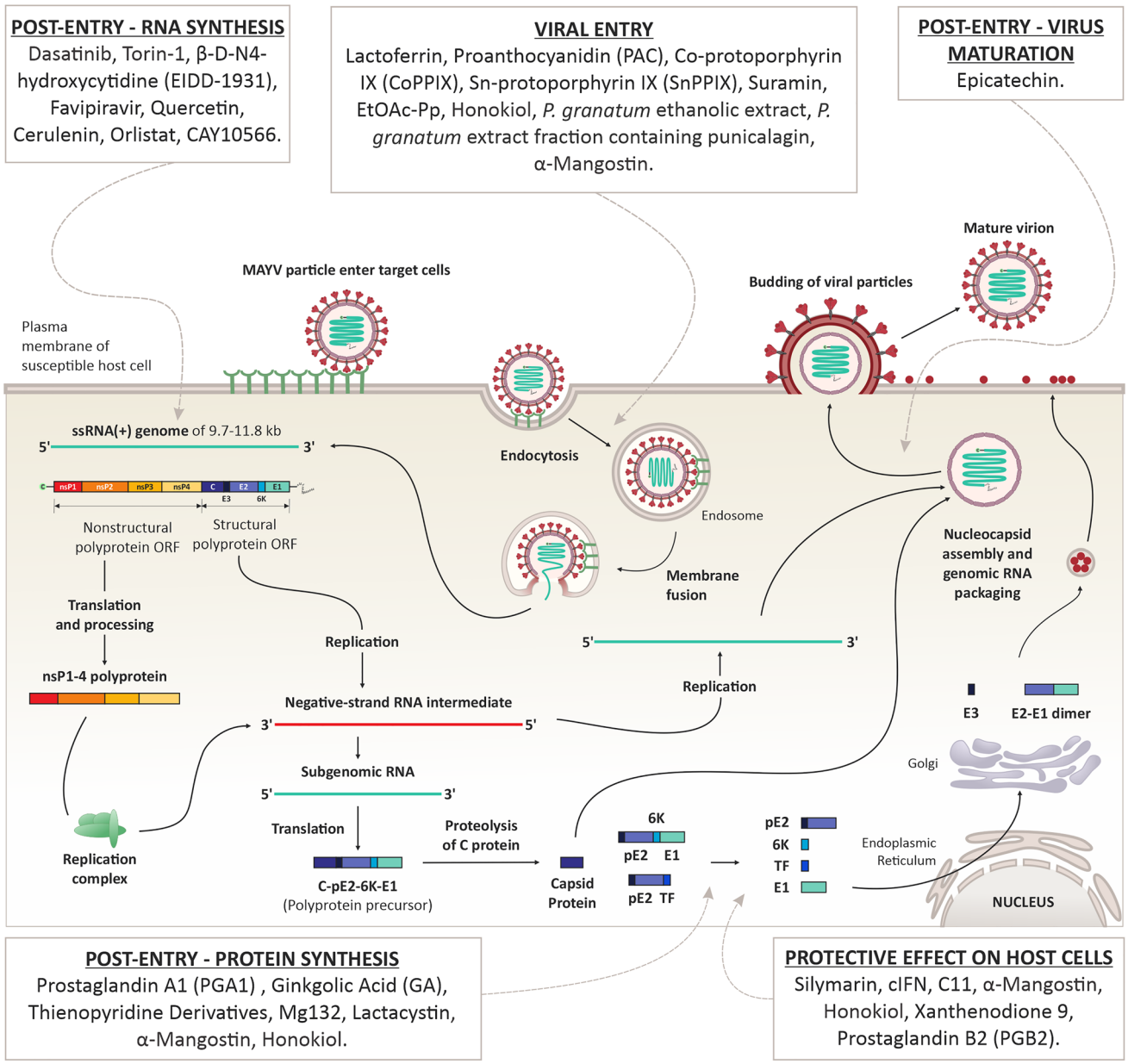
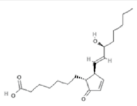
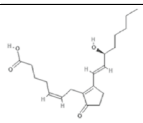
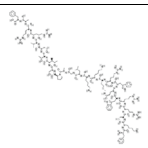
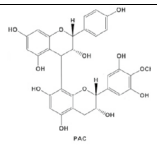
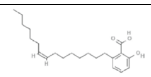
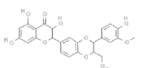
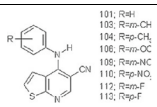
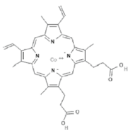
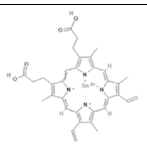
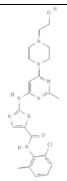


Fig. 2 Schematic representation of MAYV replicative cycle. The replicative cycle and genomic structure of MAYV. *E* envelope, *nsP* nonstructural protein, *UTR* untranslated region, *nt* nucleotides. MAYV attaches to the host cells through the interaction between the host receptors and glycoproteins. Endocytosis occurs through a clathrin-coated pit or, alternatively, a caveolin-coated pit; virus internalization starts after interaction with host cell receptors; the low pH in the endosome leads to structural modifications in the viral envelope that reveal the E1. The latter mediates cell membrane-virus fusion, and later, endosomal cell membrane-virus fusion. Viral uncoating results in the release of the viral genome, and the replication stage occurs (translation and transcription). From the RNA of the virus, nsP precursors are generated; the replication complex is obtained from nsP proteins. This complex thus allows the synthesis of a minus-strand RNA, and it is used as the template to generate both genomic and subgenomic RNAs. Polyprotein precursor is cleaved by an autoproteolytic serine protease; and genomic RNA involved in nucleocapsid core assembly and genomic RNA packaging. Maturation of glycoproteins pE2 and E1 occurs. In the Golgi, processed glycoproteins are associated. Then they are transported into the cell membrane. At the cell membrane, the pE2 is split into E2 and E3; there is also the association of the viral RNA with the capsid C and the recruitment of E1 allows viral assembly. Last, particles of MAYV associated with the core are released outside of the host cell through the membrane. Compounds with antiviral activity against MAYV are indicated in each step of the virus replication cycle: (i) Viral Entry; (ii) Post-Entry (Maturation, RNA and Protein Synthesis); and (iii) Protective Effect on Host Cells. Viral cycle was based on the work of Diagne and colleagues (Diagne et al. 2020)

Table 1 Compounds with antiviral activity against MAYV

Compound*	Chemical Structure	Structure Reference	Antiviral activity Reference	Inhibition	Mechanism	EC_{50}	CC_{50}	Cell lineage
Prostaglandin A1 (PGA1)		PubChem CID: 5281912 (Kim et al. 2021)	(Ishimaru et al. 1998; Burlandy and Rebello 2001; Caldas et al. 2018)	Post-entry	Reduction in the synthesis of E1, E2, C, and in the precursors of virus proteins	3 µg/mL	ND	Vero
						1 µg/mL	ND	Hep-2
						3 µg/mL	ND	Vero
Prostaglandin B2 (PGB2)		PubChem CID: 5280881 (Kim et al. 2021)	(Ishimaru et al. 1998)	Host cells	ND	8 µg/mL	ND	Vero
Lactoferrin		PubChem CID: 126456119 (Kim et al. 2021)	(Carvalho et al. 2014)	Viral Entry	Prevents the viral entrance	ND	ND	Vero
Proanthocyanidin (PAC)		(Ferraz et al. 2019)	(Ferraz et al. 2019)	Viral Entry	Interacts with proanthocyanins on the envelope glycoproteins, preventing viral entry	37.9 µM	> 1640 µM	Vero
Ginkgolic Acid (GA)		PubChem CID: 5281858 (Kim et al. 2021)	(Campos et al. 2020)	Post-entry	Inhibits the initial stages of the viral cycle, and suppresses the expression of E1 and E2 viral proteins	ND	ND	Vero, HeLa
Silymarin		PubChem CID: 5213 (Kim et al. 2021)	(Camini et al. 2018)	Host cells	Decreases virus-induced oxidative stress on host cells associated with the antiviral activity	3.58 µg/ml	103.5 µg/mL	HepG2
Thienopyridine Derivative 104		(Amorim et al. 2017)	(Amorim et al. 2017)	Post-entry	Decreases viral protein synthesis	20.0 µM	2500 µM	Vero
Co-protoporphyrin IX (CoPPIX)		PubChem CID: 3000479 (Kim et al. 2021)	(Neris et al. 2018)	Viral Entry	Impairs viral entry and adsorption	5.94 µM - without light	762.3 µM - without light	BHK-21, Vero
						7.48 µM - with light	1018 µM - with light	
Sn-protoporphyrin IX (SnPPIX)		PubChem CID: 3000478 (Kim et al. 2021)	(Neris et al. 2018)	Viral Entry	Impairs viral entry and adsorption	5.99 µM - without light	816 µM - without light	BHK-21, Vero
					Generates ROS	0.09 µM - with light	20.7 µM - with light	
Dasatinib		PubChem CID: 3062316 (Kim et al. 2021)	(Broeckel et al. 2019)	Post-entry	Blocks the subgenomic RNA translation, impairing viral replication	ND	ND	ND

Natural compounds as inhibitors of the replicative cycle of MAYV

Some of the first natural molecules tested for their potential to inhibit MAYV replication in vitro were Prostaglandin A1 (PGA1) and Prostaglandin B2 (PGB2) (Ishimaru et al. 1998). PGA1, a natural molecule that possesses a hormone-like lipid structure, is produced by cells in the presence of injury and/or inflammation (Caldas et al. 2018). Alternatively, PGB2 is a lipid mediator

Table 1 (continued)

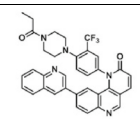
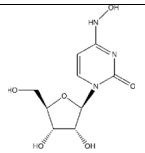
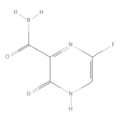
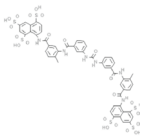
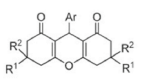
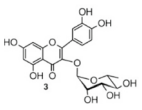
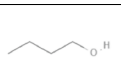
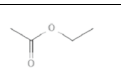
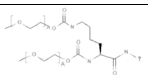
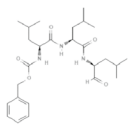
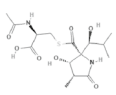
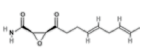
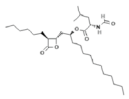
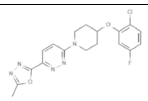
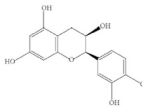
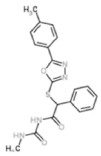
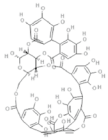
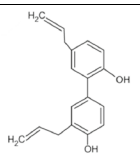
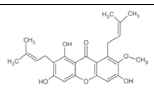
Torin-1		(Sun et al. 2015)	(Broeckel et al. 2019)	Post-entry	Blocks the subgenomic RNA translation, impairing viral replication	ND	ND	ND
β-D-N4-hydroxycytidine (EIDD-1931)		(Agostini et al. 2019)	(Langendries et al. 2021)	Post-entry	Impairs viral RNA synthesis, resulting in non-functional copied viruses	1.6 µM	> 100 µM	Vero
Favipiravir		PubChem CID: 492405 (Kim et al. 2021)	(Langendries et al. 2021)	Post-entry	Impairs viral RNA synthesis, resulting in non-functional copied viruses	79 µM	2837 µM	Vero
Suramin		PubChem CID: 5361 (Kim et al. 2021)	(Langendries et al. 2021)	Viral Entry	Inhibits viral entry	124 µM	> 2000 µM	Vero
9-(5-(4-chlorophenyl]furan-2-yl)-3,6-dimethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2))-dione (Xantenedione)		(Fernandes et al. 2021)	(Fernandes et al. 2021)	Host Cells and post-entry	Inhibits viral in pretreatment and post-entry assay.	21,5 µM	338.8 µM	Vero
Quercetin		(dos Santos et al. 2014)	(dos Santos et al. 2014)	Post-entry	Binds to viral RNA, inhibition RNA metabolism	10 µM ND	941 µM ND	Vero HEK293T
***n*-BuOH**		PubChem CID: 263 (Kim et al. 2021)	(dos Santos et al. 2014)	ND	ND	7.1 µM 3.0 µM	2614 µM 418 µM	Vero Vero
EtOAc		PubChem CID: 8857 (Kim et al. 2021)	(dos Santos et al. 2014)	ND	ND	8.2 µM 5.0 µM	457.7 µM 3116 µM	Vero Vero
EtOAc-Pp	NA	NA	(dos Santos et al. 2014)	Viral Entry	Impairs viral entry and adsorption	2.5 µM	324.1 µM	Vero
cIFN		PubChem SID: 135347948 (Kim et al. 2021)	(Grabarz et al 2021)	Host cells	ND	3,57 X 10^{-7} µg/mL	ND	Vero
MG132		PubChem CID: 462382 (Kim et al. 2021)	(Llamas-González et al. 2019)	Post entry	Reduces the expression of viral proteins nsP1 and E1	ND	ND	Vero-E6, HeLa
Lactacystin		PubChem CID: 6610292 (Kim et al. 2021)	(Llamas-González et al. 2019)	Post entry	Reduces the expression of viral proteins nsP1 and E1	ND	ND	Vero-E6, HeLa
Cerulenin		PubChem CID: 5282054 (Kim et al. 2021)	(Bakhache et al. 2019)	Post entry	Decreases genome replication due to manipulation of FASN enzymatic activity.	ND	ND	HEK293T

Table 1 (continued)

Orlistat		PubChem CID: 3034010 (Kim et al. 2021)	(Bakhache et al. 2019)	Post entry	Decreases genome replication due to manipulation of FASN enzymatic activity.	ND	ND	HEK29 3T
CAY10566		PubChem CID: 16732433 (Kim et al. 2021)	(Bakhache et al. 2019)	Post entry	Decreases genome replication due to manipulation of SCD1 enzymatic activity.	ND	ND	HEK29 3T
Epicatechin		(Hosseinimehr et al. 2013)	Ferreira et al. 2018)	Post entry	Binds to mature virions released into the extracellular environment	0.247 µM	> 1.723 µM	Vero
C11		(Gall et al. 2018)	(Gall et al. 2018)	Host cells	Acts as an agonist of the STING Pathway	ND	ND	THF, MM6
α-punicalagin (*P. granatum* extract fraction containing punicalagin)		PubChem CID:16149716 (Kim et al. 2021)	(Salles et al. 2021)	Viral Entry	ND	523.1 µg/ml	62.5 µg/ml	Vero
***P. granatum* ethanolic extract**	NA	NA	(Salles et al. 2021)	Viral Entry	Virucidal	590.8 µg/ml	12.3 µg/ml	Vero
Honokiol		(Valdés-Torres et al. 2022)	(Valdés-Torres et al. 2022)	Host cells and post-entry	Downmodulates the expression of E1 and nsP1 proteins decreases viral RNA and increases type I interferon cellular response	ND	ND	Vero-E6, HeLa
α--Mangostin		(Valdés-Torres et al. 2022)	(Valdés-Torres et al. 2022)	Host cells, Viral Entry and Post-entry	Downmodulates the expression of E1 and nsP1 proteins decreases viral RNA and increases type I interferon cellular response	ND	ND	Vero-E6, HeLa

ND not described, EC_{50} effective concentration of 50%, CC_{50} cytotoxic concentration of 50%, *NA* not applicable

*A list of abbreviations was provided as supplementary material

that acts as an auxiliary signal to T lymphocyte activation (Ishimaru et al. 1998; Raulin 2002). The antiviral effects of PGA1 and PGB2 against MAYV were investigated by using the plaque reduction assay in infected green monkey kidney cells (Vero cells) (Ishimaru et al. 1998). Cells were infected with MAYV at a concentration of 1 plaque-forming unit (pfu) per cell for 1 h, followed by the administration of PGs at concentrations ranging from 0 to 10 µg/ml for 1 h. The EC_{50} was observed with doses of 3 µg/ml for PGA1 and 8 µg/ml for PGB2. A higher antiviral effect was observed when compounds were added up to 2 h post-infection (h.p.i.) (Ishimaru et al. 1998). PGA1 was more effective than PGB2, being able to inhibit the production of glycoproteins E1 and E2 by over 50% at 10 µg/ml, resulting in a decrease of infectious particles (Ishimaru et al. 1998).

Later, Burlandy and Rebello corroborated the anti-MAYV activity of PGA1 that reduced 99% of MAYV replication in vitro at 10 µg/mL through a reduction plaque assay (Burlandy and Rebello 2001). Moreover, to suggest which stage of the replicative cycle was affected, a time-of-drug addition assay was performed. The results showed that PGA1 possesses the strongest effect when added to the cells simultaneously to the virus. The authors also identified a reduction in the synthesis of E1, E2, and C, and in the precursors of virus proteins, which suggests the effect in the early stages of viral replication. Additionally, an increase in the heat shock protein 70 (HSP70) was detected, and since this molecule is

responsible for protein folding, disaggregation, and degradation to ensure proper cell function (Holbrook et al. 1992), it is possible to suggest a mode of action for PGA1(Burlandy and Rebello 2001). Caldas and coworkers also assessed the effect of PGA1 on MAYV replication and the relevance of HSP70 in this process (Caldas et al. 2018). Human cervix carcinoma cells (Hep-2 cells) were infected with MAYV with a multiplicity of infection (MOI) of 1 for 1 h and treated with different concentrations of the compound. According to the authors, the PGA1 was not cytotoxic, presented an EC_{50} of 1 µg/mL, and inhibited up to 95% of viral production at 6 µg/mL. In agreement with the previous studies, PGA1 impaired p110, p62, E1/E2, and C synthesis, as well as a significantly increased HSP70 expression (Ishimaru et al. 1998; Caldas et al. 2018). The HSP70 increase seems to be related to the PGA1 antiviral effect since the inhibition of viral replication was only observed in PGA1 concentrations that induced the production of this HSP. In this context, the authors proposed that PGA1 may act in different stages of viral replication due to the different strategies presented by the molecule.

Alternatively, Carvalho and colleagues proposed the natural macromolecule Lactoferrin, a globular glycoprotein, as a candidate for treatment against MAYV (Carvalho et al. 2014). This molecule is produced by exocrine glands, such as mammary and lacrimal glands (Kell et al. 2020) and has been used as apolactoferrin, its apo form found in bovine whey (bLf). To assess the effect of Lactoferrin against MAYV, Vero cells were infected with viral particles labeled with DiD (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbo cyanine,4-chlorobenzenesulfonate salt), a lipophilic carbocyanine marker that expresses red fluorescence, and then incubated with bLf at concentrations ranging from 0.2 to 1.0 mg/mL. BLf inhibited viral replication in a dose-dependent manner, with 1 mg/mL being able to inhibit 85% of viral infection. Furthermore, a time-of-drug addition assay was also carried out to investigate which stage of the replication cycle was affected (Carvalho et al. 2014). The bLf mainly acted on virus entry, suggesting that this molecule may interact with the sulfate groups of the glycosaminoglycans layer (GAGs) of the host cells, preventing the attachment of the virus. However, since the specific MAYV receptor at the time of the study was unknown (Carvalho et al. 2014), further studies involving the Mrxa8 are needed to corroborate this mechanism of action.

Other potential antiviral candidates tested against MAYV were the flavonoids 4′-*O*-methylepigallocatechin (MEP) and the proanthocyanidin [(−)-epicatechin-(4β → 8)-(−)-4′-methylepigallocatechin] (PAC), an MEP dimerized with the epicatechin, isolated from *Maytenus imbricata* roots (Ferraz et al. 2019). The viability of MEP and PAC was evaluated through an MTT ([(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide)] assay using Vero cells, and both demonstrated no cytotoxic effect, with CC_{50} over 1640 µM. To assess the antiviral effect, cells were infected with MAYV and treated with concentrations of MEP and PAC. No activity was observed against MAYV with MEP, even at the highest tested concentration (1000 µM). However, the treatment of MAYV-infected cells with PAC resulted in EC_{50} of 37.9 µM and a SI (selectivity index) higher than 43. The treatment at different stages of the virus cycle demonstrated the virucidal activity of PAC, which was confirmed by a dialysis membrane assay. The authors proposed that the mechanism of action may be related to the interaction of proanthocyanins with the envelope glycoproteins, decreasing the infectivity of viral particles (Ferraz et al. 2019).

The antiviral effect of the flavonoid Quercetin, as well as the EtOAc, and n-BuOH fractions, isolated from *Bauhinia longifólia,* was investigated by employing the viral yield inhibition assay. Vero cells with MAYV (MOI of 0.1) for 1 h, and then treated for 24 h with the substances (0–100 µg/ml). Subsequently, the culture supernatants were recovered and tittered to calculate the extracellular infectious viral particles using the plaque reduction assay. Among the tested compounds, quercetin and the fractions of EtOAc, and n-BuOH had an EC_{50} of 10 µM, 5 µM, and 3 µM, respectively. EtOAc had the highest SI (623), followed by n-BuOH (208) and quercetin (94). The mechanisms of action of these substances still need to be elucidated. It has been suggested that quercetin may act inhibiting viral RNA polymerase (dos Santos et al. 2014).

The antiviral activity of Quercetin against MAYV was also studied by Bakhache and coworkers, in addition to the compounds Cerulenin, Orlistat, and CAY10566 (Bakhache et al. 2019). These molecules are known to interfere with fatty acid synthase (FASN) or stearoyl-CoA desaturase (SCD1), cofactors of MAYV infection. To assess the their antiviral activity, human embryonic epithelial kidney cells (HEK293T) were infected with the MAYV-Luc reporter virus in an MOI of 1, and Quercetin, Cerulenin, Orlistat, and CAY10566 were added 1.5 h before or 1.5 h after the cell infection at concentrations of 0–150 µM, 0–25 µM, 0–100 µM, and 0–7.5 µM, respectively. Luciferase activity was then monitored for 6, 16, and 24 h.p.i.. As an outcome, the inhibitors induced a dose-dependent inhibitory effect on MAYV, when added before or after the infection. Among the tested drugs, Cerulenin presented the highest antiviral activity, demonstrating an increased ability to inhibit MAYV infectivity when added before the viral infection. The authors also screened these molecules against CHIKV, which demonstrated to decrease CHIKV genome replication due to the interference with FASN or SCD1 enzymatic activity, proposing that these compounds act in a similar mechanism against MAYV.

Similarly, the antiviral activity of EtOAc, n-BuOH, and EtOAc-Pp, extracted from *Cassia australis*, against MAYV were evaluated (Spindola et al. 2014). The production of MAYV in Vero cells decreased by about 70% and 85% in the presence of EtOAc and n-BuOH at 25 µg/mL, respectively, while EtOAc-Pp at 10 µg/mL decreased 90% the viral progeny production. The authors proposed that the highest antiviral effect of EtOAc-Pp may be due to the condensed tannins, which are present only in this substance. Tannins are a class of polyphenols, characterized by having different molecular sizes and degrees of polymerization, which affect their pharmacokinetics and antiviral activity (Maugeri et al. 2022). Condensed tannins have previously demonstrated antiviral activity against other viruses, such as the respiratory syncytial virus (RSV), influenza A virus (FLU-A), and parainfluenza virus (PIV) (Bruyne et al. 1999). The authors suggested that the mechanism of action of EtOAc-Pp is related to the binding of tannins with viral envelope proteins, resulting in the inhibition of viral binding and penetration into host cells.

What is more, the EtOH fruit extract from *Punica granatum* (Pomegranate), and its two main fractions, one containing the α and β anomers of Punicalagin and another the Pomegranate ethanol extract, were assessed by their anti-MAYV activity (Salles et al. 2021). To this, Vero cells were infected with MAYV at an MOI of 0.1 for 1 h, and then added of medium-containing substances at concentrations of 12.5, 25, 50, and 100 µg/ml. Virus was titrated 24 h.p.i. and titrated by the $TCID_{50}$. As a result, *P. granatum* ethanolic extract demonstrated strong antiviral activity against MAYV infection, with a CC_{50} of 590.8 µg/mL and an EC_{50} of 12.3 µg/mL. The fraction containing punicalagin as the main component also showed strong antiviral activity, with a CC_{50} of 523.1 µg/mL and an EC_{50} of 62.5 µg/mL. The authors performed a virucidal assay, incubating the virus with each substance at 200 µg/mL for 1 h at 37 °C. Pomegranate ethanol extract showed 98% virucidal activity against MAYV particles, and punicalagin showed no visible virucidal activity, being the results confirmed by immunofluorescence microscopy.

Another natural compound identified to possess anti-MAYV activity is Epicatechin, isolated from extracts of *Salacia crassifolia*, which belongs to a genus of plants known by its use against diabetes, and as an anti-inflammatory, in folk medicine (Ferreira et al. 2018). Ferreira and coworkers selected Epicatechin for in vitro analysis after performing in silico screening tests that suggested a potential binding interaction with MAYV C protein. To assess the in vitro antiviral activity, cells were pretreated for 30 min with dilutions of the compound (0.013 to 0.574 µmol/mL), and then infected with MAYV at an MOI of 0.1 or 5 for 48 h. Epicatechin presented EC_{50} of 0.247 µmol/mL, and prevented approximately 100% of the cytopathic effect in MAYV-infected cells at 0.430 µmol/mL. A time-of-drug addition assay was also performed, and when added after infection, epicatechin reduced virus production by two logs in the first 12 h. The authors suggested that the compound acts in the second cycle of infection, when mature virions are released into the extracellular environment, but did not exclude an intracellular action in the late stages of viral replication, considering the MOI used in the assay, concluding that epicatechin acts by binding to components of the viral particle, but not to cellular components.

Ginkgolic Acid (GA), a compound isolated from *Ginkgo biloba*, is widely used in traditional Chinese medicine (Campos et al. 2020), reported to possess biological activities, such as anti-tumor (Ma et al. 2015; Qiao et al. 2017), antibacterial (Hua et al. 2017), anti-parasitic (Chen et al. 2008), and antiviral (Lü et al. 2012). Campos and colleagues evaluated the effects of GA on CHIKV, MAYV, and UNAV replication. Through a plaque reduction assay using epithelial cervical cancer cells (HeLa cells), it was found that GA at 10 µM reduced the MAYV viral progeny in almost 2 log_{10} at 16 h.p.i.. To confirm these results, an immunofluorescence assay was performed, demonstrating that treated cells presented a decrease in viral antigens. Additionally, a time-of-drug-addition assay was performed by adding GA to the cells hourly after adsorption, and supernatants were collected 24 h.p.i. to measure viral progeny yields. As a result, the compound was able to mainly inhibit the initial stages of the viral cycle, which was confirmed by the suppression of E1 and E2 viral proteins in western blot (Campos et al. 2020). Despite the promising effects of GA against MAYV in vitro, the molecule demonstrated to be toxic in other cell lines and animal studies (Berg et al. 2015; Qian et al. 2017). Therefore, the authors suggest that further investigations are needed to confirm the safety of the treatment, and the antiviral activity against MAYV.

Another promising inhibitor of MAYV is Silymarin, a compound extracted from *Silybum marianum*. This molecule is widely used for the treatment of liver disorders (Camini et al. 2018), and was described to have antiviral activity against the Hepatitis C virus (Wagoner et al. 2010) and CHIKV (Lani et al. 2015). Camini and coworkers assessed the effects of Silymarin using hepatocarcinoma cells (HepG2 cells), which resulted in CC_{50} of 103.5 µg/mL and EC_{50} of 3.58 µg/mL. Then, HepG2 cells were treated with 25 µg/mL of Silymarin and simultaneously infected with MAYV at an MOI of 5. Supernatants were collected 48 h.p.i. and used to infect naïve Vero cells. The results demonstrated a reduction of up to two logs in MAYV replication, inhibiting almost 100% of the cytopathic effect. The production of reactive species of oxygen (ROS) was measured by employing infected HepG2 cells with a fluorogenic marker, confirming that MAYV infection increased ROS generation in a time-dependent curve. Other oxidative stress biomarkers were quantified, such as malondialdehyde (MDA) and

carbonyl protein, in the presence of MAYV and Silymarin, which culminated in a drop of these biomarkers, suggesting a protective effect on host cells, associated with the antiviral activity (Camini et al. 2018).

Finally, Valdés-Torres and collaborators investigated the potential of Honokiol and α-Mangostin against MAYV (Valdés-Torres et al. 2022). Primary human dermal fibroblasts (HDFs), Vero-E6, and HeLa cells were infected with MAYV at an MOI of 1 for 1 h, and then treated with Honokiol (5 to 10 µM) or α-Mangostin (1 to 5 µM) for 24 h. Honokiol and α-Mangostin reduced MAYV-induced cytopathic effects in a dose-dependent manner and inhibited the production of viral progeny in pretreatment assay. MAYV-infected HDFs revealed that α-Mangostin at 1 µM affected MAYV binding to the host cells, while Honokiol showed a modest effect at 10 µM. The viral entry was partially disturbed by α-Mangostin, but not by Honokiol. Both compounds affected the post-entry stage of MAYV infection. The authors also evaluated the expression of Interferon (IFN) type I and specific genes stimulated by IFN in treated or untreated HeLa cells through quantitative RT-PCR. As an outcome, treatment with Honokiol and α-Mangostin promoted a significant increase in IFN type I and interferon-stimulated genes. In addition, the compounds decreased the expression of MAYV E1 and nsP1, and affected viral RNA replication. The authors pointed out that Honokiol and α-Mangostin are post-entry inhibitors impairing MAYV replication through different mechanisms of action, and may act as potential broad-spectrum antivirals, also inhibiting UNAV, CHIKV, and Zika virus (ZIKV).

Synthetic and semi-synthetic inhibitors of MAYV replicative cycle

Natural compounds may present limitations in terms of large-scale production and patentability, and therefore, the production of synthetic and semi-synthetic compounds is an alternative and attractive approach to the development of antiviral candidates (Ortholand and Ganesan 2004).

Among the synthetic molecules, the Thienopyridine derivatives were described as antimicrobial agents against bacteria (Aly et al. 2011), protozoa (Pinheiro 2012), viruses (Bernardino et al. 2004), and parasites (Rolim Bernardino et al. 2006). In this context, Amorim and coworkers conducted a study based on the antiviral activity of thieno[2,3-b]pyridine derivatives 101 to 113 on the MAYV replicative cycle in vitro, using Vero cells (Amorim et al. 2017). Among the non-cytotoxic compounds, molecule 104 was the most viable compound, presenting CC_{50} of 2500 µM, EC_{50} of 20.0 µM, and an SI of 125. To further assess the effect of molecule 104 on viral protein synthesis, Vero cells were infected with MAYV at an MOI of 0.05 and treated with 100 µM of the compound in a time-of-drug addition assay. Through the quantification of the plaque-forming assay, it was observed that 104 presented a higher effect when added prior or during the virus infection. Therefore, a virucidal assay was performed by incubating the virus and the compound for 1 h prior to the infection, confirming the virucidal effect of 104 at concentrations higher than 25 µM. The authors also showed a decrease in protein synthesis in the presence of 104, by labeling viral proteins with 35S-methionine, confirmed by transmission electron microscopy, which demonstrated a reduction in the number of intracytoplasmic nucleocapsids and mature virus particles in treated cells.

Porphyrins are organic molecules that can bear a metal ion in the center of their tetrapyrrolic structure (Assunção-Miranda et al. 2016). These molecules are capable of absorbing light and are widely used in photodynamic therapies. The porphyrins have also been shown to inactivate viral particles by targeting the viral envelope of Dengue virus (DENV) and Yellow Fever virus (YFV) (Neris et al. 2018). Neris and coworkers evaluated the antiviral potential of the heme porphyrins Co-protoporphyrin IX (CoPPIX) and Sn-protoporphyrin IX (SnPPIX) in the presence or absence of light against MAYV using Baby Hamster Kidney Fibroblast (BHK-21) or Vero cells (Neris et al. 2018). With no light stimulation, CoPPIX and SnPPIX presented a CC_{50} of 762.3 µM and 816 µM, respectively, while in the presence of light, the compounds demonstrated a CC_{50} of 1018 µM and 20.7 µM, respectively. Later, 10^7 PFU of viruses were pre-incubated with different concentrations of each compound in the absence of light for 1 h at 37 °C, and then used to infect the cells with an MOI of 0.1. CoPPIX and SnPPIX at 300 µM were able to completely inactivate MAYV, with an EC_{50} of 5.94 µM and 5.99 µM, respectively. Additionally, light-stimulated for 10 min before incubation for 1 h in the dark resulted in EC_{50} of 0.09 µM for SnPPIX and 7.48 µM for CoPPIX. To further investigate the CoPPIX and SnPPIX inhibition, MAYV was labeled with DiD to follow fusion between virus and endosomal membranes during endocytosis. After treatment with CoPPIX or SnPPIX at 300 µM with no light stimulation, and SnPPIX at 10 µM with light stimulation, MAYV was unable to efficiently fuse with the endosomal membrane. The authors highlighted that the porphyrins are hydrophobic molecules, that might interact with viral envelope lipids, impairing the attachment and entry of the virus into the cells. Furthermore, the photoactivation of SnPPIX generates ROS, which can modify viral proteins in the envelope environment, producing an antiviral activity. The authors emphasized that a single intensity of light stimulation was performed and suggested that an increase in luminous intensity might increase SnPPIX virucidal efficiency (Neris et al. 2018).

Another promising compound is β-D-N4-hydroxycytidine (EIDD-1931), a ribonucleoside analogue which previously

demonstrated antiviral activity against RNA viruses, such as SARS-COV-2, Equine Encephalitis Virus, and CHIKV (Yousefi et al. 2021; Langendries et al. 2021). EIDD-1931 can rapidly reach plasma, is efficiently distributed in mice organs, and also presents a high genetic barrier for the development of viral resistance (Painter et al. 2019; Yousefi et al. 2021). A study conducted by Langendries and coworkers screened compounds with previously reported antiviral activity against other arboviruses, such as EIDD-1931, Favipiravir, and Suramin, against MAYV (strain TC625, MOI of 0.01), using Vero cells (Langendries et al. 2021). The experiments demonstrated CC_{50} of > 100 µM, > 2000 µM, and 2837 µM, and EC_{50} of 1.6 µM, 124 µM, and 79 µM for EIDD-1931, Suramin, and Favipiravir, respectively. To evaluate which stage of the replicative cycle was affected by these compounds, a time-of-drug-addition assay was carried out. Vero cells were incubated with 300 µM Favipiravir, 500 µM Suramin, and 25 µM EIDD-1931 2 h before infection, and 0, 2, 4, 6, and 8 h.p.i. at an MOI of 1. Through end-point titration at 10 h.p.i and qRT-PCR quantification, the authors demonstrated that EIDD-1931 impaired viral replication when added at 0, 2, and 4 h.p.i., with a moderated effect if added at 6 h.p.i. Differently, Suramin and Favipiravir reduced viral titers at 0 and 2 h.p.i., respectively. From the results and previous data in the literature, the authors suggested that EIDD-1931 and Favipiravir impair viral RNA synthesis, resulting in non-functional copied viruses due to a large number of mutations in the viral genome (Joshi et al. 2021; Langendries et al. 2021). It was also proposed that Suramin acts during the early steps of the MAYV replicative cycle, more specifically in the viral entry, in a similar mechanism demonstrated by studies with CHIKV (Albulescu et al. 2020) and SARS-CoV-2 (Salgado-Benvindo et al. 2020).

Cyclic ketones are natural and synthetic compounds with antiviral (Sivropoulou et al. 1997; Chung 2017), antioxidant (Pombal et al. 2017), and antifungal (Pizzolitto et al. 2015) properties. The antiviral activity of 24 cyclic ketones was assessed by Fernandes and coworkers, by screening these compounds, using MAYV-infected Vero cells (MOI 1), treated with each compound at concentrations ranging from 1 to 100 µM for 24 h. Among them, 8 compounds showed viability ≥ 50% and were selected for viral inhibition assay. A plaque-forming assay was performed, and among the 8 compounds, the Xanthenodione 9-(5-(4-chlorophenyl]furan-2-yl)-3,6-dimethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2))-dione inhibited the MAYV with EC_{50} of 21.5 µM and SI of 15.8. The authors also performed a time-of-drug addition assay and found that the compound inhibits viral replication in the prior and post-treatment assays (Fernandes et al. 2021).

The Proteasome inhibitors MG132 and Lactacystin are also antiviral candidates against MAYV (Llamas-González et al. 2019). MG132 acts as a reversible inhibitor of the chymotryptic activity of the proteasome, and Lactacystin is an irreversible inhibitor capable of binding to the catalytic β subunits of the 20S proteasome (Llamas-González et al. 2019). The anti-MAYV activity of these compounds was evaluated by Llamas-González and colleagues. Vero-E6 or HeLa cells were treated with MG132 at 10 µM or Lactacystin at 25 µM for 1 h, and then infected with MAYV (MOI of 1 or 10) for 1 h, when the inoculum was replaced by fresh medium-containing compounds. Viruses were titrated by plaque-forming assay 24 h.p.i.. MG132 and Lactacystin low cytotoxicity and significant antiviral effect using either cell lines. The authors also performed immunofluorescence of MAYV-infected HeLa cells, which demonstrated a significant reduction in the percentage of MAYV-positive cells under treatment, resulting in < 5% for MG132 and < 10% for Lactacystin, versus > 20% in the untreated control group. Viral titers were lower when compounds were added in the early stages of infection, and the treatment with MG132 or Lactacystin affected the production of viral proteins nsP1 and E1, showed by the reduced expression of MAYV proteins under the treatment with these proteasome inhibitors.

Exploiting host factors, the Src family of protein tyrosine kinases (SFK) are involved in intracellular signaling pathways related to virus elimination (Broeckel et al. 2019). However, during infection, the virus can interact with kinases favoring viral replication. Therefore, cell signaling inhibitors, including SFK inhibitors, have been tested for their antiviral activity against *Alphavirus* in human fibroblasts (Broeckel et al. 2019). Since the knowledge of the signaling profile of SFK during *Alphavirus* is limited, the authors carried out a range of experiments using CHIKV infection, as a base to identify the kinase pathways involved in this genus infection. The SFK-phosphatidylinositol 3-kinase (PI3K)-Akt-mTOR signaling was demonstrated to be the main pathway during CHIKV infection. Then, the authors evaluate the effects of dasatinib and Torin, inhibitors of this pathway, on fibroblasts infected with MAYV. Through the viral titration of the supernatant collected from infected and treated cells, it was shown that MAYV was significantly reduced by these inhibitors. The authors demonstrated that the SFK inhibitors blocked the subgenomic RNA translation, resulting in the impairment of *Alphaviruses* replication (Broeckel et al. 2019).

A non-natural interferon (cIFN) that was designed according to the analysis of the amino acid sequences present in several subtypes of IFN-α was investigated by its anti-MAYV activity by Grabarz et al. (2021). Vero cells were treated with increasing concentrations of cIFN for two hours and then infected with MAYV (MOI = 0.1) for 48 h. MAYV infection was inhibited by cIFN inhibited, with EC50 of 35.7 fg/mL, being possible to obtain about 80% and 100% cell confluency with concentrations of 81.4 fg/mL, and 162.8 fg/mL of the compound, respectively. In comparison,

untreated cells showed almost complete destruction of the cell monolayer 24 h.p.i. with MAYV. In this study, cIFN was also able to inhibit the cytopathic effect of other viruses, such as ZIKV, CHIKV, and SARS-CoV-2.

Another promising compound is N-(methylcarbamoyl)-2-{[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-2-phenylacetamide (known as C11), a molecule that induces type I IFN-dependent activity in human cell lines (Gall et al. 2018). In this context, Gall and collaborators produced THF cells and MM6 cells with deletion of the stimulator of interferon genes (STING), such as the RIG-1 mitochondrial antiviral signaling (MAVS) and the TIR-domain-containing adapter-inducing interferon-β gene (TRIF), related to the activation of the immune response. To assess the antiviral activity of C11, these cells were treated for 2 h with C11, IFN-β, or DMSO at non-cytotoxic concentrations and then infected with MAYV at an MOI of 1. The results demonstrated that C11 had an effective concentration of 90% (EC_{90}) of 25.19 µM against MAYV. STING-deleted cells showed no inhibition of virus replication in the presence of the compound. C11 was also evaluated against CHIKV, VEEV, and RRV viruses in human cells, resulting in reductions in the virus titer, but when evaluated using non-human cells (RAW264.7 monocytic cells), a lack of antiviral activity in the murine RAW264.7 cell line was noticed. The authors proposed that C11 acts by activating specifically the human type I IFN response through a STING-dependent process, leading to the generation of an IFNAR-mediated antiviral cellular state.

Molecular target to drug development against MAYV

Drug development can be a time-consuming process, which can result in unexpected outcomes (Adamson et al. 2021). Additionally, the lack of treatment against viral infections, mainly for neglected diseases, may jeopardize the control of the disease, as well as its consequences, such as the loss of human lives (Young et al. 2019). In this context, several strategies can be applied to accelerate the progress toward the development of novel molecules. Therefore, this section focuses on the MAYV proteins which could be exploited as a target for antiviral development, considering the factors: (i) previously described compounds with antiviral activity against MAYV and/or other *Alphaviruses*, (ii) resolved structure of viral proteins deposited in databanks, and (iii) structural similarity and conservation among viruses.

The glycoprotein E1 possesses an ectodomain, subdivided into subdomains I, II, and III, and a transmembrane subdomain (TM). Similarly, the E2 ectodomain is composed of subdomains A, B, C, and D, and a TM portion (Filho et al. 2020). The ectodomains of E1 and E2 interact with each other to form a portion of the spike structure outside the envelope: the glycoproteins E1 and E2 interact forming a dimeric unit (Fig. 3A) that is further associated with additional two dimers to form the spike (Fig. 3B–D). The TM portions of E1/E2 bind the spike to the lipid membrane, while E2 C-terminal connects the spike to the capsid protein, through a well-described TPY consensus motif (West et al. 2006; Ribeiro-Filho et al. 2021), forming an E1–E2-capsid unit (Fig. 3C, E). As observed for other *Alphaviruses*, MAYV possesses an asymmetric unit formed by the assembling of four E1–E2-capsid units resulting in 80 spikes (Filho et al. 2020). MAYV belongs to the arthritogenic class of viruses, and, therefore, it is possible to suggest that Mxra8 is the main receptor related to the MAYV entry into host cells (Fig. 3E) (Powell et al. 2020). In this context, considering that the glycoprotein protein complex interacts with Mxra8, mainly through E2, this interaction could be impaired by active molecules through interference or destabilization of E2 binding sites (Zhang et al. 2018; Filho et al. 2020). Molecules were previously described to bind to viral glycoproteins of other arthritogenic viruses, such as CHIKV, consequently inhibiting viral entry to the host cells. For instance, Oliveira and coworkers described the in vitro virucidal activity of a coordinated organic compound against CHIKV, and demonstrated that in silico analysis suggested interactions between the compound and CHIKV E2 domain (Oliveira et al. 2020). Additionally, studies encompassing the compounds PAC (Ferraz et al. 2019), bLf (Carvalho et al. 2014), CoPPIX, and SnPPIX (Neris et al. 2018), which are discussed here, also demonstrated a virucidal activity against MAYV infection. Even though these molecules were not rationally designed for specific targets, further studies could investigate whether their mode of action is related to these hint targets.

Another spot protein in MAYV replication is the nsp4, an RNA-dependent RNA polymerase (RdRp) essential to the viral replication (Rubach et al. 2009). Therefore, it is an attractive target to the development of antiviral drugs. To the best of our knowledge, the RdRp crystal structure of MAYV has not been determined and characterized yet, which might postpone drug design applied to this target. Regardless, the RdRp amino acid sequence is commonly conserved among viruses from the A*lphaviruses* genus, and especially between the Semliki Forest serocomplex members (Rupp et al. 2015). Therefore, the RdRp structure from the Ross River virus (RRV) (Fig. 4), another member of the *Alphavirus* genus might be employed as a template for drug development. Its structure is composed of fingers, the palm containing the GDD active site, and thumb domains (Rubach et al. 2009). Therefore, it is possible to hypothesize that compounds with antiviral activity described against the RdRp of other viruses could act against MAYV infection (Chen et al. 2017). Indeed, most of the described and approved drugs are

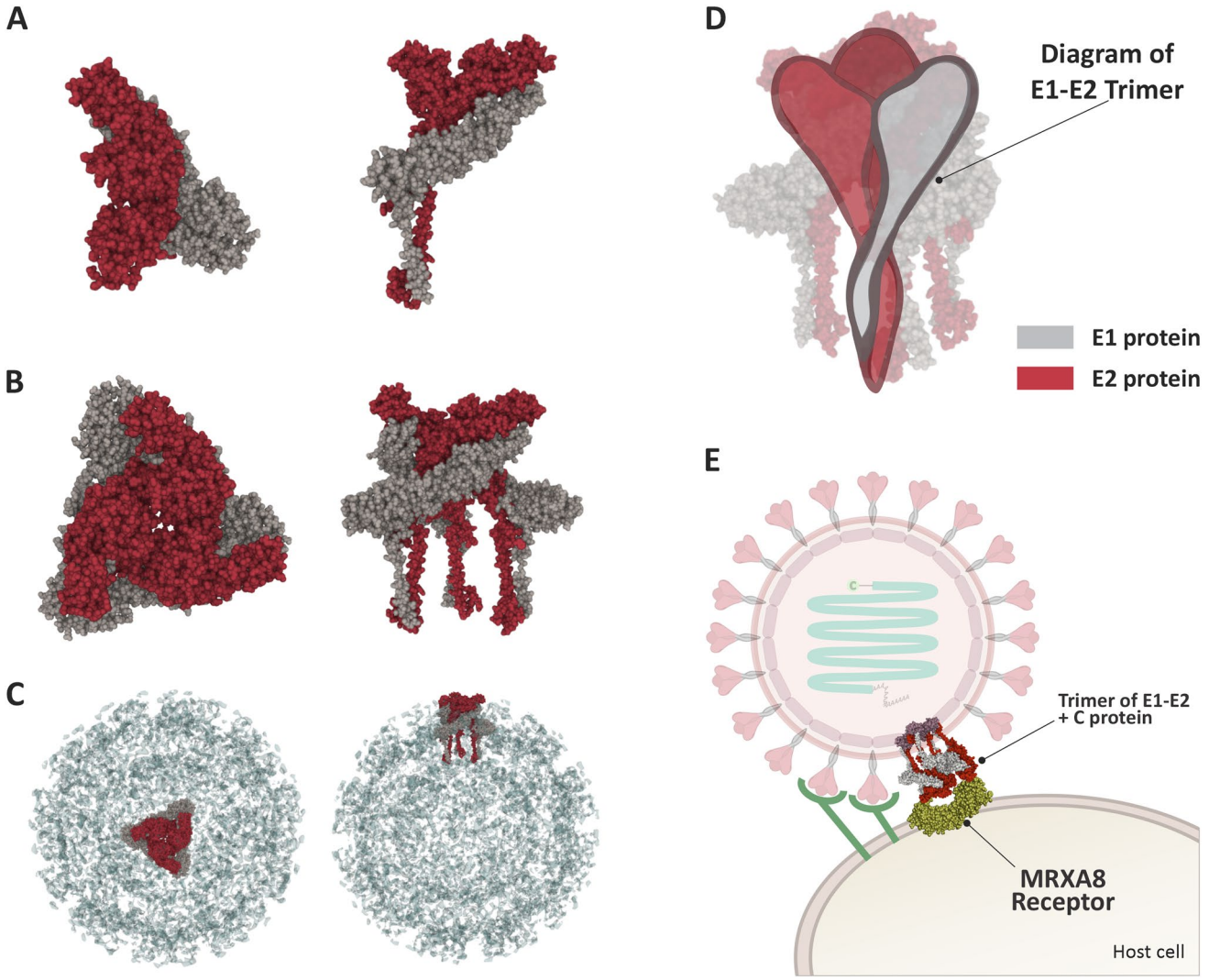


Fig. 3 Schematic structure of MAYV glycoprotein. The glycoproteins E1 and E2 interact forming a dimeric unit (**A**), that is further associated to two additional dimeric units to form a trimer (**B**). The localization of the trimers is demonstrated in (**C**), and a diagram of the E1–E2 trimer is shown in (**D**). The interaction between the glycoproteins and the MRXA8 receptor in host cells is demonstrated in **E**. MAYV E1 and E2 structure were based on Cryo-EM structure of mature MAYV (PDB ID: 7KO8) (Ribeiro-Filho et al. 2021), and the receptor was based on human MXRA8 (PDB ID: 6JO8) (Powell et al. 2020)

nucleoside and non-nucleoside inhibitors that impair viral infections through RNA synthesis inhibition. Among them, Remdesivir, Azidothymidine (AZT), Sofosbuvir, and Lopinavir/Ritonavir, are licensed antivirals used to treat Ebola virus, Hepatitis C virus, and Human immunodeficiency virus infections (Tian et al. 2021).

The MAYV nsP3 is another protein that represent a target for antiviral development (Tsika et al. 2019). It is a modular protein with three domains: the N-terminal macro domain, a central zinc-binding domain, and the C-terminal hypervariable domain (Fig. 4) (Götte et al. 2018). Each domain plays a different role in viral replication and interferes with the virulence among Old and New World Alphaviruses, being necessary for RNA synthesis (Rupp et al. 2015). The MAYV macro domain possesses four alpha-helices and six beta-strands (Malet et al. 2009; Melekis et al. 2015). On the other hand, the central zinc-binding domain plays an undefined, but essential role in minus-strand RNA synthesis, through an association with the macro domain, forming a ring-like structure to RNA binding (Shin et al. 2012). Alternatively, the hypervariable domain is a large protein that upholds several insertions and deletions, and is responsible for interactions with multiple host proteins during viral replication, probably favoring virulence (Davis et al. 1989; Dé et al. 2003; Uversky 2013). However, the macro domain is the most conserved site in the protein structure and could be exploited for drug development, particularly as a broad-spectrum antiviral. The macro domain site binds to ADP-ribose,

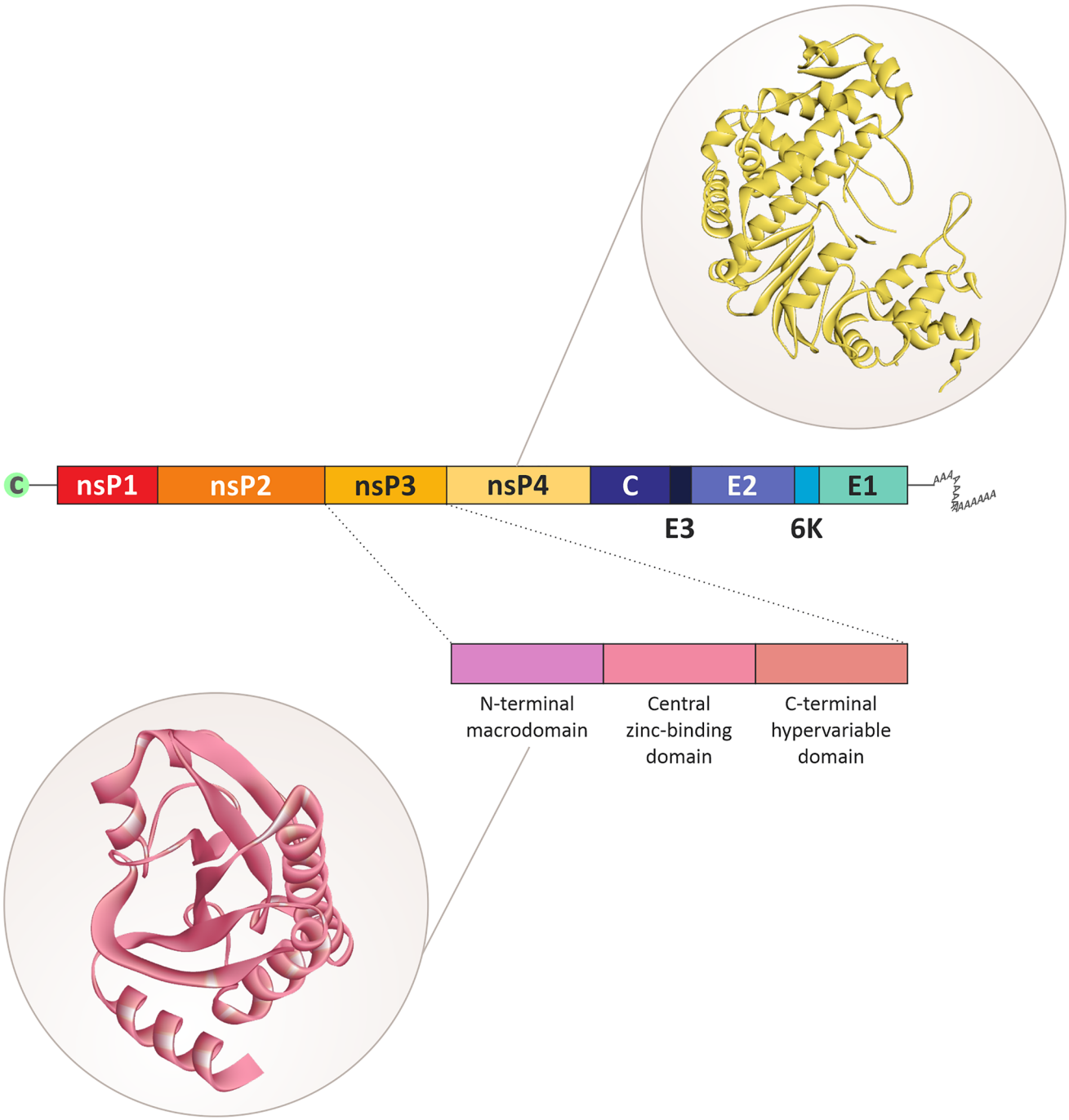


Fig. 4 Schematic structure of MAYV genome and the crystal structure of nsP3 macrodomain and nsP4. Nsp3 is a modular protein with three domains: the N-terminal macro domain, a central zinc-binding domain, and the C-terminal hypervariable domain, being the macro domain constituted by four alpha-helices and six beta-strands. The nsP4 protein acts as an RNA-dependent RNA polymerase (RdRp), and its structure is composed of fingers, the palm containing the GDD active site, and thumb domains. Since RdRp crystal structure of MAYV has not been determined and characterized yet, the template used in the figure was obtained from Ross River virus RdRp (PDB ID: 7F0S) (Tan et al. 2022). The nsP3 structure was based on MAYV macro domain structure (PDB ID: 5IQ5) (Tsika et al. 2019)

dephosphorylate ADP-ribose-1′-phosphate, and possesses a de-ADP-ribose hydrolase activity, which is essential to viral replication (McPherson et al. 2017). Several compounds were virtually screened employing the nsP3 macrodomain of CHIKV (Nguyen et al. 2014; Shimizu et al. 2020; Subudhi et al. 2018), SARS-CoV-2 (Jung et al. 2020), and the Venezuelan equine encephalitis virus (VEEV) (Atasheva et al. 2014) as targets. Shimizu and collaborators selected

compounds that targeted the nsP3 macro domain of CHIKV by in silico analysis, and validated their antiviral activity performing in vitro assays (Shimizu et al. 2020). Therefore, it is possible to suggest the nsP3 macrodomain of MAYV as a relevant target for future antiviral discovery.

Furthermore, components of virus lipidic membrane are also potential targets yet to be explored. Among membrane components, the cholesterol is an important molecule related to *alphavirus* stability, infectivity, and assembly (Sousa et al. 2020). This molecule was recently associated with the successful infection in several viruses (Sun and Whittaker 2003; Huang et al. 2006), being also modulated to increase the infection efficiency (Zhang et al. 2019). Cholesterol can be found in increased proportions in MAYV viral particles isolated from vertebrate cells, being associated with the lateral organization of the viral envelope and binding to the host membrane (Sousa et al. 2020). Additionally, a decrease in *alphavirus* infectivity is observed when the arrangement of the viral envelope is disturbed, suggesting that the lateral membrane organization is important for the physical stability of the viral particle and, consequently, for virus-cell interactions (Kielian et al. 2010). Cholesterol is also associated with MAYV entry, being used as an alternative pathway for its entry through cholesterol-enriched caveolae-derived vesicles and release (Carvalho et al. 2017). Therefore, targeting cholesterol from the viral lipidic membrane represent an interesting approach for antiviral drug development. However, designing and identifying active compounds focusing on these components can be challenging, due to their similarity to the host cell membrane (Sousa et al. 2020). Interestingly, the compound 25-hydroxycholesterol (25HC), a reactive oxysterol catalyzed by cholesterol-25-hydroxylase, is a promising molecule that can interact with lipids on viral membrane, and demonstrated broad-spectrum antiviral activity, low toxicity, and ability to reduce viremia and protect embryonic mice against ZIKV in vivo (Li et al. 2017; Mao et al. 2022). In this context, molecules focused on interacting with cholesterol could be employed to drug development against MAYV. ed to drug development against MAYV.

Perspectives

MAYV infection can impact the quality of life of infected patients, due to the chronic condition that can result in progressive arthralgia and disabling disease. Besides that, the lack of approved antiviral treatment and vaccines against MAYV, associated with the presence of susceptible vectors in tropical and subtropical countries, can lead to future outbreaks.

In this context, this review summarized and discussed the literature concerning the compounds with anti-MAYV activity, as well as presented possible viral proteins that could be further exploited as targets for drug development. To the best of our knowledge, only the studies described here reported antiviral activity against MAYV infection, demonstrating that further research on treatments against Mayaro fever is urgent. It is important to emphasize that the data presented here employed different methodologies, including cell lines, MOI for infection assays, and the design of the assays. However, these studies present relevant information on the antiviral activity of a range of molecules (natural and synthetic) against MAYV, and suggested possible mechanisms of action (MOA) related to each compound. The characterization of the MOA of these molecules represents an advance for further studies, providing information concerning the interactions among host cells, viruses, and compounds (Iorio et al. 2010; Salters-Pedneault 2014). Besides, the MOA of several compounds against MAYV are still unknown (Figueiredo et al. 2019). From the compounds discussed here, Lactoferrin, molecule 104 (thienopyridine derivative), and Silymarin presented significant antiviral activity against MAYV in vitro, with low or no cytotoxicity. The repurposing antiviral agent EIDD-1931 is also a promising treatment since it was able to completely inhibit MAYV replication in vitro. However, these compounds need to be further investigated in vivo against MAYV. Ginkgolic Acid (GA) was one of the most active compounds, and the only molecule tested in vivo, however demonstrating hepatotoxicity and nephrotoxicity effects, as well cytotoxicity in different cell lines. Qian and collaborators also observed that rats treated with either, high or low doses of GA, showed histopathological changes in the liver and kidneys. In addition, the serum levels of urea nitrogen and creatinine increased in the blood, and total protein and albumin decreased, indicating kidney dysfunction and liver damage (Qian et al. 2017). On the other hand, the genotoxicity evaluation of GA evidenced a low risk of genotoxicity in vivo (Berg et al. 2015). Therefore, more studies in human cells and animal models are necessary to analyze the safety of this compound.

Furthermore, we discussed and highlighted MAYV proteins that could represent relevant targets for drug development, acknowledging that focusing on host factors might produce cytotoxicity in vitro, and adverse effects in vivo. Here, we suggested the glycoproteins complex, the nsP4 (RdRp), and the nsP3 macro domain as targets. The lack of resolved structures of several MAYV proteins, such as nsP1, nsP2, C, and E, invalidate them as targets for drug development.

Finally, licensed drugs employed in the treatment of several diseases have the potential to be repurposed to target the viral molecules underlined here. In this sense, Langendries and colleagues identified EIDD-1931, favipiravir, and suramin, three licensed antivirals, with activity against MAYV (Langendries et al. 2021). Additionally, the

potential of Lactacystin (Llamas-González et al. 2019), Cerulenin, Orlistat (Bakhache et al. 2019), and Quercetin (dos Santos et al. 2014), as repurposing therapy against MAYV was also discussed. The compounds were active with low EC_{50} and low cytotoxicity, and showed potential to be applied as antiviral therapy in the treatment of infections caused by this pathogen, being a prospective target for further research. The use of approved drugs as repurposing therapy may favor patient access to drugs due to the potential to result in shorter clinical studies, since these molecules have their pharmacological profile and adverse effects already described (Santos et al. 2022). Bearing that in mind, it is an inconsistency that repurposing drugs against MAYV have been understudied. Therefore, we further emphasize and encourage researchers to screen licensed drugs against MAYV.

Conclusions

The spread of MAYV infections in tropical and subtropical regions represents a threat to developing countries. Additionally, the lack of epidemiological data on Mayaro fever might underestimate the importance of this disease and its consequences. There is an urgent need for studies to understand the biology of the virus, thus creating paths to combat future outbreaks. In this context, the compounds described to possess anti-MAYV activity, and the possible targets discussed herein, may contribute to the future development of antiviral drugs against MAYV.

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Authors contribution Literature research: MP, MdSM, IAS, VRG, and DOSM. Drafting of the manuscript: MP, MdSM, and DOSM. Figures: VRG. Design and Critical revision: IAS, RBR, and ACGJ. All authors reviewed the manuscript.

Data availability statement All data generated or analysed during this study are included in this published article (and its supplementary information files).

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential direct or indirect conflict of interest.

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