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A compendium of small molecule direct-acting and host-targeting inhibitors as therapies against alphaviruses

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Alphaviruses were amongst the first arboviruses to be isolated, characterized and assigned a taxonomic status. They are globally widespread, infecting a large variety of terrestrial animals, birds, insects and even fish. Moreover, they are capable of surviving and circulating in both sylvatic and urban environments, causing considerable human morbidity and mortality. The re-emergence of Chikungunya virus (CHIKV) in almost every part of the world has caused alarm to many health agencies throughout the world. The mosquito vector for this virus, *Aedes*, is globally distributed in tropical and temperate regions and capable of thriving in both rural and urban landscapes, giving the opportunity for CHIKV to continue expanding into new geographical regions. Despite the importance of alphaviruses as human pathogens, there is currently no targeted antiviral treatment available for alphavirus infection. This mini-review discusses some of the major features in the replication cycle of alphaviruses, highlighting the key viral targets and host components that participate in alphavirus replication and the molecular functions that were used in drug design. Together with describing the importance of these targets, we review the various direct-acting and host-targeting inhibitors, specifically small molecules that have been discovered and developed as potential therapeutics as well as their reported *in vitro* and *in vivo* efficacies.

1. Introduction

1.1 The alphaviruses as human pathogens

Alphaviruses belong to the *Togaviridae* family, are mainly arthropod-borne viruses that are transmitted by vectors such as mosquitoes and can be found widely throughout the world except Antarctica.[1–3] Alphaviruses cause various clinical manifestations ranging from febrile illnesses to neurological diseases.[4] Infections with Old World alphaviruses such as Chikungunya virus (CHIKV), Semliki Forest virus (SFV), O'nyong nyong virus (ONNV), Sindbis virus (SINV), Mayaro virus (MAYV) and Ross River virus (RRV) commonly cause febrile illness and painful arthralgia or polyarthralgia.[5] In contrast, encephalitis is mainly caused by New World alphaviruses such as Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV) and Western equine encephalitis virus (WEEV).[6]

Functionally, an alphavirus particle comprises a single-stranded positive-sense RNA and delivery system.[7] The delivery system involves a protein shell that consists of capsid protein (CP), glycoproteins and a host-derived envelope that is acquired when the virus buds through a cellular membrane.[7,8] This protein shell surrounds, stabilizes and protects the positive-sense RNA genome, which encodes non-structural proteins (nsPs) and structural proteins.[2] The nsPs (i.e. nsP1, nsP2, nsP3 and nsP4) aid in the production of new viral RNA strands, while the structural proteins consist of the CP, envelope glycoproteins E1, E2, E3 and the residual polypeptide 6K, which ultimately make up part of the mature virion.[2] Table 1 illustrates the essential known functions of each alphavirus protein.[9]

For any given virus, a detailed understanding of the processes involved in its replication cycle is vital for the design of drugs that selectively inhibit viral replication without interfering with host cell function. As illustrated in Figure 1, the replication cycle of alphavirus can be summarized in three main stages, which are virus entry, intracellular replication, and maturation. At the beginning of the replication cycle, alphavirus infects host cells by engaging its E2 glycoproteins with the cell surface receptors and then enters the cells via clathrin-mediated endocytosis.[10] As the virus-containing endosome matures, the acidic environment that develops within the endocytotic vesicle destabilizes the envelope glycoprotein structure.[2,11] The resulting conformational change initiates fusion between the virus and late endosomal membranes, leading to the emptying of the nucleocapsid into the cytosol (Event 1).

Table 1. Proteins encoded by alphavirus genome

Protein	Structural and/or enzymatic functions	Role in virus replication cycle
nsP1	membrane association, guanosine-7-methyltransferase (MT), guanylyltransferase (GT)	RNA capping activity
nsP2	nucleotide triphosphatase, helicase, protease	P1234 polyprotein processing
nsP3	macro domain	di-phosphoribose 1′-phosphate phosphatase activity
nsP4	RNA-dependent RNA polymerase	production of viral RNAs
CP	forms nucleocapsid core with the genomic RNA, trypsin-like protease	formation of nucleocapsid
E1	forms part of a continuous isocahedral protein shell on the virion, glycoprotein	mediation of membrane fusion
E2	forms part of a continuous isocahedral protein shell on the virion, glycoprotein	interactions with host cell surface receptors
E3	peripheral glycoprotein	regulates spike assembly[147]
6K	residual polypeptide chain	assists in E1 folding

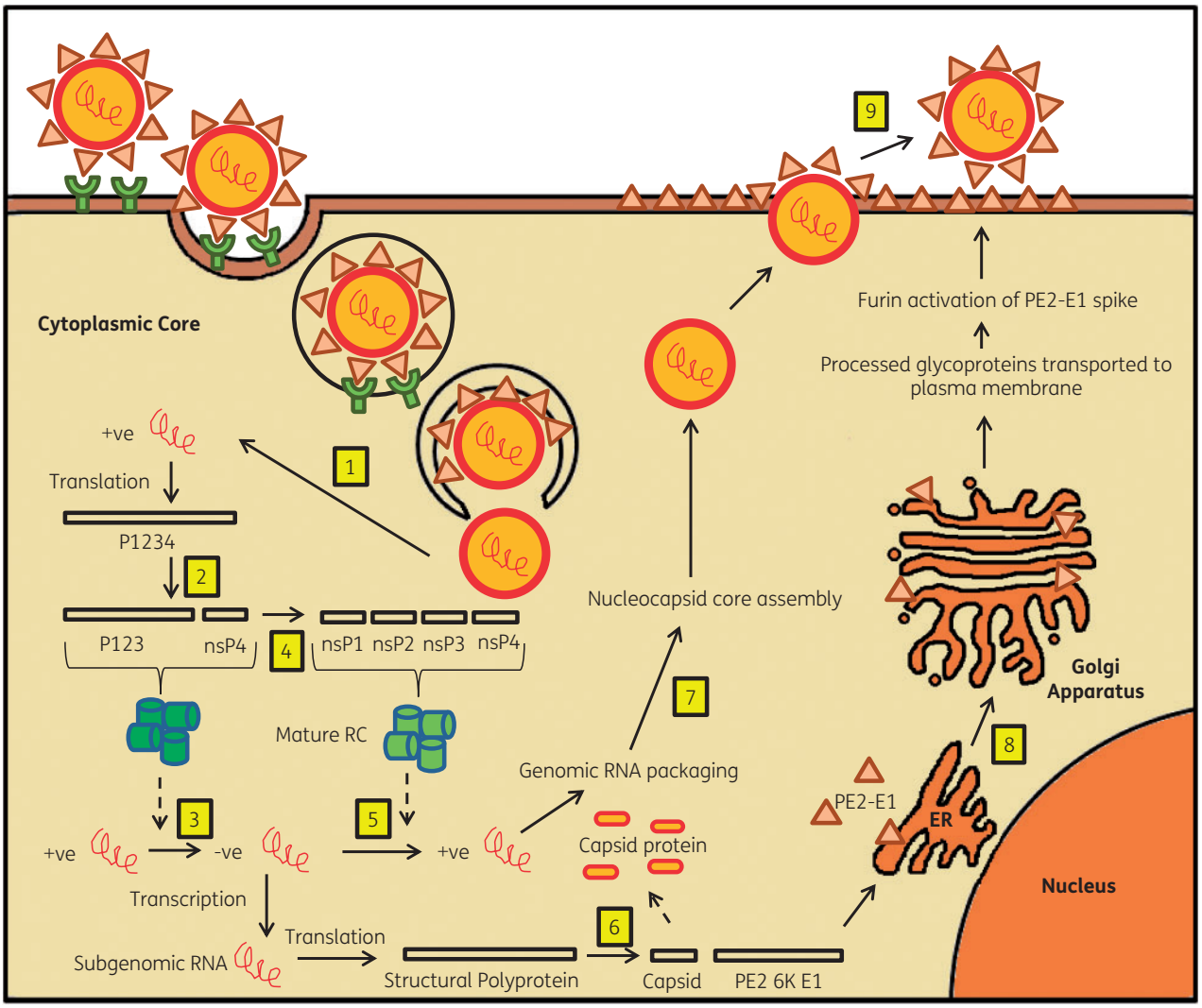

Figure 1. Schematic representation of the replication cycle of alphaviruses. Description of each numbered event indicated in the yellow squares is outlined in the main article text. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Once the nucleocapsid disassembles in the cytosol, the encapsidated genome gains access to the synthetic machinery of the cell and intracellular replication proceeds. Alphaviruses use the cellular machinery for the translation of viral polyprotein P1234 from its full-length genomic RNA.[2] This polyprotein is then processed stepwise into individual nsPs through proteolytic cleavage by nsP2 (Event 2).[12] Early processing of P1234 produces P123 and nsP4, which associate to form the primary replication complex (RC), which performs negative-sense RNA synthesis (Event 3).[13] P123 is further processed to produce the individual nsPs, which associate with nsP4 to form a mature RC (Event 4).[13,14] The mature RC regulates the synthesis of positive-sense RNA as well as the transcription of subgenomic 26S mRNA using the negative-sense RNA as template (Event 5).[15] Simultaneously, the CP-pE2–6K-E1 structural precursor is also translated from the subgenomic 26S mRNA.[16]

At the maturation stage, the CP is first cleaved from the structural polyprotein by polyprotein processing[17] and assembled with the newly synthesized positive-sense single-stranded RNA molecule to form the nucleocapsid (Events 6 and 7, respectively).[18] The pE2 and E1 molecules are then translocated into the endoplasmic reticulum for post-translational modifications (Event 8).[19] Prior to the arrival of the pE2–E1 heterodimer at the plasma membrane, the pE2 is cleaved to E2 and E3 by furin in the Golgi apparatus to activate the infectivity of the virus.[20] The post-modified envelope glycoproteins then translocate to the plasma membrane[21] and assemble with the mature nucleocapsid.[22] During the budding phase, the assembled virion exits the host cell and acquires a host-derived lipid envelope containing the integral membrane glycoproteins E1 and E2 (Event 9).[23]

1.2 Re-emergence of CHIKV

Prior to 2005, CHIKV was considered a relatively less important viral infection because it was mainly confined to localized outbreaks in Asia and Africa.[24] It was not until 2005–06, when CHIKV re-emerged and caused a large outbreak and infected up to 40% of the population on the French island of La Réunion, that the severity of the nature of CHIKV transmission was realized.[25] The spread of CHIKV was accelerated by increasing globalization, whereby CHIKV was introduced into non-endemic regions by travellers returning from CHIKV-epidemic regions.[26] In <10 years, CHIKV had re-emerged as a global pathogen, spreading from Africa throughout the Indian Ocean Islands,[27] the Pacific Islands[28] and the Americas,[29,30] causing millions of cases in almost 100 countries.[31–34] Although death due to CHIKV infection is rare, clinical cases in Asia, especially in countries where health services and procedures are poorly developed, have resulted in high morbidity.[1]

2. Progress towards the development of small-molecule inhibitors of alphaviruses

There is no recognized antiviral therapy to treat alphavirus infections. The current treatment, i.e. administration of non-steroidal anti-inflammatory drugs (NSAIDS) and paracetamol, only alleviates the symptoms of the disease. Over the past 50 years, there have been reports on small-molecule alphavirus inhibitors for development as potential antivirals.[1,35–37] Nearly half of them were discovered and obtained from natural sources.[38] There are many virus-specific and host targets involved in virus replication that can be targeted by antiviral therapy. Direct-acting inhibitors are designed to act on virus-specific targets, i.e. the nsP(s) and structural protein(s), while host-targeting inhibitors inhibit the functions of host-derived proteins that are actively involved in alphavirus replication. Figure 2 illustrates the list of direct-acting and host-targeting inhibitors and their mechanisms of action in disrupting virus attachment and entry, intracellular replication and virus maturation and budding. Table 2 summarizes the *in vitro* antiviral properties of these inhibitors described in terms of CC_{50} (i.e. concentration of inhibitor required for the reduction of cell viability by 50%) and EC_{50} or IC_{50} (i.e. concentration of inhibitor required to produce 50% of the total anti-alphaviral effect) unless stated otherwise.

3. Direct-acting inhibitors

3.1 Inhibitors of virus attachment and entry

Doxycycline, a semi-synthetic tetracycline antibiotic, is commonly used to treat bacterial infections. This drug was discovered to have synergistic *in vitro* anti-CHIKV effects when administered with ribavirin (i.e. around 3-fold improvement in EC_{50} values compared with doxycycline or ribavirin alone).[39] Doxycycline inhibited virus attachment and computational studies revealed that doxycycline binds to E2 glycoprotein, hence impairing the important conformational changes of E2 protein for binding to the cell surface receptors.[39] The observed synergistic effects of doxycycline and ribavirin could be due to doxycycline targeting the entry stage and ribavirin targeting the intracellular replication stage.

Arbidol (Figure 3) was originally licensed in Russia for treatment of influenza and other respiratory viral infections.[40] Time-of-addition studies showed that arbidol demonstrated greater antiviral activity against CHIKV when treatment was before infection, suggesting that arbidol blocks the earliest stages of the CHIKV replication cycle (i.e. virus attachment and/or virus entry).[41] Structure–activity relationship (SAR) studies of similar analogues of arbidol were also investigated.[42] Of these analogues, two arbidol *tert*-butyl ester derivatives, **1** and **2**, possessing a sulphoxide group, demonstrated similar activity to arbidol but better cytotoxic profiles (Figure 3).[42] Signs of resistance were observed when cells infected with a mutant CHIKV were treated with arbidol.[41] Since the position of this mutation was localized in the E2 domain, where interactions between E2 and cell receptors occurred, it was believed that arbidol and its derivatives demonstrate anti-CHIKV activity through blocking the interactions between E2 and surface receptors during CHIKV attachment.

Phenothiazine compounds, i.e. chlorpromazine, ethopropazine, methdilazine, perphenazine, thiethylphenazine and thioridazine, are drugs that have been used in the treatment of psychotic and allergy diseases (Figure 3). When these compounds were tested in an entry inhibition assay that employed a heat-sensitive SFV strain (SFVts9-*Rluc*), an effective inhibition of SFV entry into baby hamster kidney (BHK) cells was observed.[43] One of these phenothiazines, chlorpromazine, has been reported to inhibit HCV entry by blocking the formation of clathrin-coated pits at the plasma membrane for clathrin-mediated endocytosis of viral particles.[44,45] Hence, it was believed that the inhibition of SFV entry is likely to be the consequence of misassembly of clathrin lattices in the presence of these phenothiazine compounds.[43]

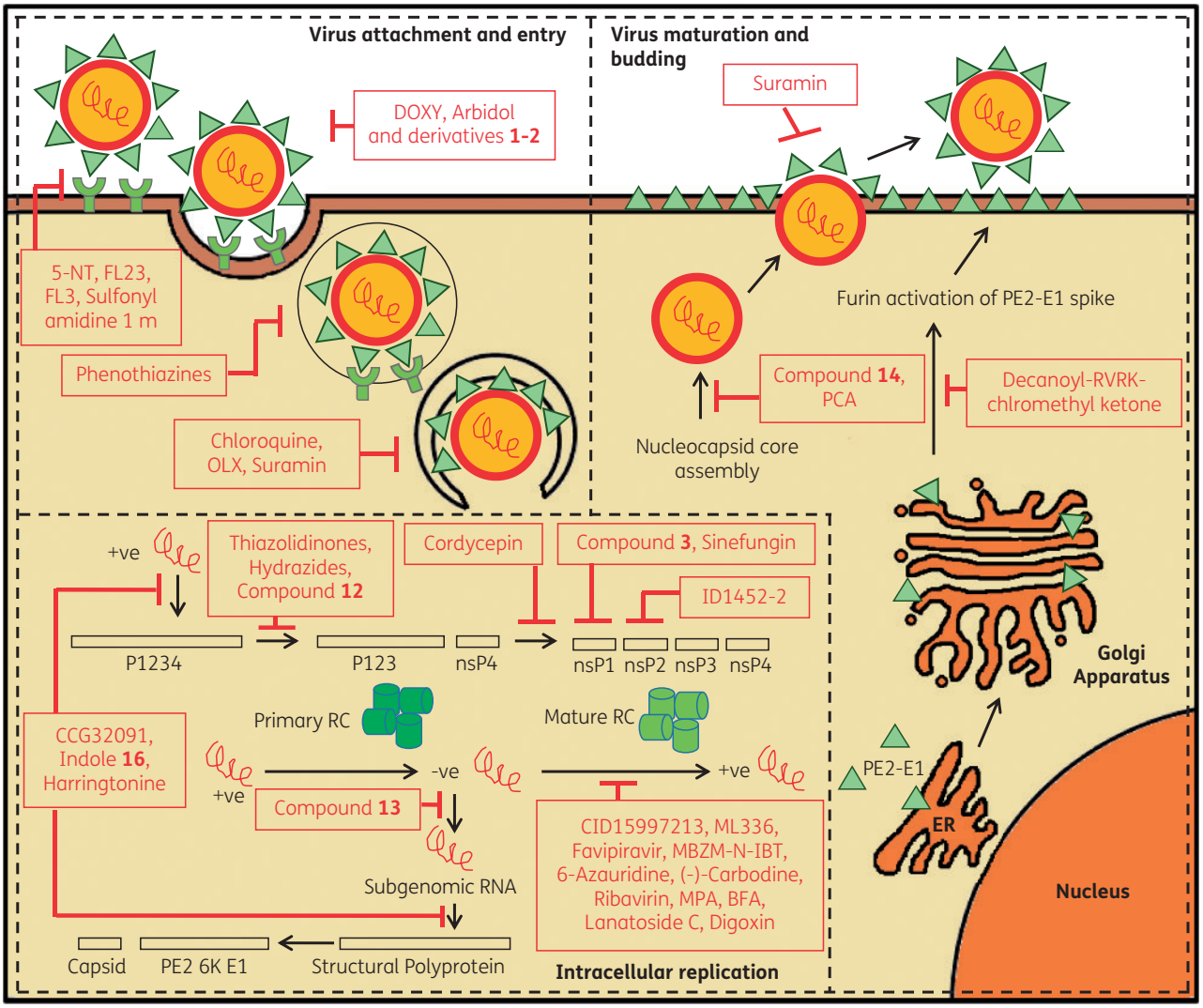

Figure 2. Different stages of the alphavirus replication cycle (virus entry and attachment, intracellular replication and virus maturation) targeted for the development of direct-acting and host-targeting inhibitors of alphaviruses. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Chloroquine, a commonly used antimalarial drug, has been extensively investigated against viral infections such as HIV.[46] This drug demonstrated significant *in vitro* inhibition of SFV, SINV and CHIKV infections.[47–49] The antiviral action of chloroquine involves inhibition of virus entry by increasing the endosomal pH above the critical value needed for the low-pH-dependent fusion reaction to occur, hence preventing the fusion of E1 protein and transfer of virus nucleocapsid into the cytoplasm.[47] However, chloroquine was not effective when administered in mice infected with SFV. Instead the drug enhanced SFV replication *in vivo* and aggravated the disease.[50] In addition, results of a double-blind placebo-controlled randomized trial in CHIKV-infected patients did not yield convincing data on its efficacy.[51]

Obatoclax, an anticancer drug, is an antagonist of the prosurvival Mcl-1 protein, which triggers apoptosis in cancer cells. This drug was screened against alphavirus infections, i.e. SINV, SFV and CHIKV, and demonstrated anti-alphaviral activity at submicromolar concentrations.[52] Both time-of-addition and entry inhibition assays showed that this drug had inhibitory activity against SFV entry.[52] Mechanistically, this drug neutralizes the acidic environment of the late endosomes and hence inhibits virus fusion.[52] In addition, resistance studies after 30 rounds of passaging SFV in the presence of obatoclax obtained a partially resistant mutant that has mutations at the amino residues, i.e. L369 and S395, found in the E1 membrane fusion protein.[52]

3.2 Inhibitors of virus replication and protein synthesis

Among the [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones that were evaluated in a screening programme against CHIKV infection *in vitro*, a lead compound, [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one **3** (Figure 3), was identified and found to possess antiviral activity against various CHIKV strains *in vitro*.[53] Interestingly, these small-molecule inhibitors are selective towards inhibition of CHIKV

Table 2. Biological data of selected small-molecule direct-acting and host-targeting inhibitors

Compound name	Active against	*In vitro* efficacy	*In vivo* efficacy	Reference(s)
Inhibitors of virus entry				
doxycycline	CHIKV	EC_{50} 10.9 μM against CHIKV replication in Vero cells; EC_{50} 4.52 μM (with ribavirin)	considerable reduction in pathological signs and virus titre in blood of infected mice	39
arbidol and its derivatives **1** and **2**	CHIKV	EC_{50} 30–35 μM against CHIKV-induced CPE in Vero cells	ND	41,42
phenothiazines	SFV	EC_{50} 11.3–25.1 μM against SFV replication in BHK cells	ND	43
chloroquine	CHIKV	IC_{50} 7.0 μM against CHIKV-induced CPE upon pre-treatment in Vero cells	did not demonstrate clinical efficacy in infected patients	47,51,148,149
	SINV	SINV replication was reduced by 20% at 0.1 mM in BHK cells	ND	48
	SFV	EC_{50} 0.05 mM against SFV replication in BHK cells	enhanced SFV replication in infected mice	49,50
obatoclax	CHIKV	EC_{50} 0.03 μM against CHIKV replication in BHK cells	ND	52
	SFV	EC_{50} 0.11 μM against CHIKV replication in BHK cells	ND	52
	SINV	virus titre was reduced 5-fold with 0.5 μM in BHK cells	ND	52
Inhibitors of virus replication and protein synthesis				
[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one **3**	CHIKV	CC_{50} >668 μM; EC_{50} 0.75–2.9 μM against CHIKV replication in Vero cells	ND	53
	VEEV	EC_{50} 6.8 μM against VEEV replication in Vero cells	ND	54
thiazolidinone derivatives **4–8**	CHIKV	IC_{50} 0.1–10.0 μg/mL against CHIKV-induced CPE in Vero cells	ND	57
hydrazides **9** and **10**	CHIKV	EC_{50} 4.3-4.9 μM against CHIKV-induced CPE in Vero cells	ND	58
hydrazide **11**	CHIKV	CC_{50} >200 μM; EC_{50} 1.5 μM against CHIKV replication in BHK cells	ND	59
peptidomimetic **12**	CHIKV	EC_{50} 16.4 μg/mL against CHIKV-induced CPE in Vero cells	ND	61
CID15997213	VEEV	CC_{50} >25 μM; EC_{50} 1–2 μM against VEEV-induced CPE in BHK cells	survival rate in infected mice improved from 0% to 60%	62
	WEEV	EC_{50} 10 μM against WEEV-induced CPE in BHK cells	ND	62
ML336	VEEV	EC_{50} 0.03 μM against VEEV-induced CPE in BHK cells	survival rate in infected mice improved from 20% to 80%	63
ID1452-2	CHIKV	EC_{50} 31 μM against CHIKV replication in human embryonic kidney (HEK) 293T cells	ND	65
favipiravir	WEEV	EC_{50} 7.5 μM against WEEV replication in Vero cells	survival rate in infected mice improved from 20% to 40%	67,68
	VEEV	EC_{50} 11 μM against VEEV replication in Vero cells	ND	68
	EEEV	EC_{50} 18 μM against EEEV replication in Vero cells	ND	68
	CHIKV	EC_{50} 2–12 μM against CHIKV replication in Vero cells	survival rate in infected mice improved from 0% to 60%–80%	68
MBZM-N-IBT	CHIKV	CC_{50} >800 μM; EC_{50} 38.68 μM against CHIKV-induced CPE in Vero cells	ND	69
secopregnane steroid glycoside **13**	SINV	EC_{50} 1.5 nM against SINV replication in BHK cells	ND	70
	EEEV	EC_{50} 2 nM against EEEV-induced CPE in BHK cells	ND	70
ribavirin	SFV	EC_{50} 47.0 μg/mL against SFV-induced CPE in Vero cells	ND	93
	CHIKV	EC_{50} 83.3 μg/mL against CHIKV-induced CPE in Vero cells	ND	93

Continued

Table 2. *Continued*

Compound name	Active against	*In vitro* efficacy	*In vivo* efficacy	Reference(s)
ribavirin-5′-sulfamate	SFV	IC_{50} 10 μM against SFV-induced CPE in Vero cells	survival rate in infected mice improved from 0% to 92%	95
6-azauridine	CHIKV	EC_{50} 0.8 μM against CHIKV replication in chick embryo cells	ND	93
	SFV	EC_{50} 1.6 μM against SFV replication in chick embryo cells	ND	93
(−)-carbodine	VEEV	EC_{50} 0.3 μg/mL against VEEV-induced CPE in Vero cells	slight, but significant extension in mean time to death was observed after prophylactic treatment	98
mycophenolic acid	CHIKV	IC_{50} 0.2 μM against CHIKV-induced CPE in Vero cells	ND	103
harringtonine	CHIKV	EC_{50} 0.24 μM against CHIKV replication in BHK cells	ND	106
digoxin	CHIKV	EC_{50} 48.8 nM against CHIKV replication in U-2-OS cells; only showed cytotoxic effect at 1 μM	ND	108
	RRV	EC_{50} 126.5 nM against RRV replication in U-2-OS cells	ND	108
	SINV	EC_{50} 198.9 nM against SINV replication in U-2-OS cells	ND	108
CCG32091	WEEV	CC_{50} >200 μM; IC_{50} 9.3 μM against WEEV replicon in BSR-T7 cells	ND	110
indole-2-carboxamide **15**	WEEV	CC_{50} 89.9 μM; IC_{50} 6.5 μM against WEEV replicon in BSR-T7 cells	ND	111
indole-2-carboxamide **16**	WEEV	CC_{50} 69.9 μM; IC_{50} 0.58 μM against WEEV replicon in BSR-T7 cells	survival rate in infected mice improved from 10% to 30%	112,113
	VEEV	virus titre was reduced 10-fold with 2.5 μM in BE(2)-C cells	ND	113
anthranilamides **17** and **18**	WEEV	CC_{50} >75 μM; IC_{50} 0.56–1.6 μM against WEEV replicon in BSR-T7 cells	ND	114
	VEEV	virus titre was reduced 10-fold with 25 μM in HEK293 cells	ND	114
Inhibitors of virus maturation				
dioxane-based compound **14**	SINV	CC_{50} >1 mM; EC_{50} 1–3.4 μM against CHIKV replication in BHK cells	ND	72,73
picolinic acid	CHIKV	viral load was reduced by up to 2 logs with 2 mM in Vero cells	ND	75
Inhibitor of multiple stages of virus replication cycle				
suramin	CHIKV	CC_{50} >700 μM in BHK cells and U2OS cells; EC_{50} 79–80 μM against CHIKV CPE in Vero cells	showed reduced viral burden and decreased foot swelling in infected mice	78,79,81
	SFV	EC_{50} 40 μM against SFV-induced CPE in Vero cells	ND	79
	SINV	EC_{50} 141 μM against SINV-induced CPE in Vero cells	ND	79
Modulators of cellular functions that assist virus replication				
WP1130	SINV	virus titre was reduced by nearly 2 logs after pretreatment with 5 μM in Vero cells	ND	120
WP1130 derivatives **19** and **20**	SINV	virus titre was reduced by nearly 2 logs after pretreatment with 5 μM in Vero cells	ND	121
Ag-126	VEEV	virus titre was reduced by 4 logs after pretreatment with 10 μM in U87MG cells	ND	123
	EEEV	virus titre was reduced by 1 log after pretreatment with 10 μM in U87MG cells	ND	123
	WEEV		ND	123

Continued

Table 2. *Continued*

Compound name	Active against	*In vitro* efficacy	*In vivo* efficacy	Reference(s)
		virus titre was reduced by 2 logs after pretreatment with 10 μM in U87MG cells		
berberine	CHIKV	CC_{50} 202.6 μM; EC_{50} 4.5 μM against CHIKV replication in HEK 293T cells	showed reduced joint inflammation in infected mice	126
	ONNV	CC_{50} >800 μM; EC_{50} 29.2 μM against ONNV replication in CRL-2522 cells	ND	126
	SFV	virus titre was reduced by nearly 4 logs with 3 μM in BHK cells	ND	127
	SINV	virus titre was reduced by 3–4 logs with 3 μM in BHK cells	ND	127
CND0335 and CND3514	CHIKV	CC_{50} >50 μM; EC_{50} 2.2–3.3 μM against CHIKV-induced CPE in HuH-7 cells	ND	128
SKI-417616	SINV	virus titre was reduced by over 3–5 logs with 10 μM in HEK293 cells	ND	122
leptomycin B	VEEV	virus titre was reduced by 5 logs after pretreatment with 45 nM in U87MG cells	ND	140
KPT-185, KPT-335 and KPT-350	VEEV	CC_{50} >10 μM; EC_{50} 0.09–0.62 μM against VEEV replication in Vero cells	ND	141
bortezomib	VEEV	virus titre was reduced by 4 logs after pretreatment with 0.1 μM in U87MG cells	ND	143
	WEEV	virus titre was reduced by 2 logs after pretreatment with 0.1 μM in U87MG cells	ND	143
	EEEV	virus titre was reduced by 3 logs after pretreatment with 0.1 μM in U87MG cells	ND	143

ND, not determined.

replication and not of related viruses, such as SINV and SFV.[53] Delang *et al.*[54] discovered that compound **3** demonstrates inhibition of CHIKV replication at a post-entry step, other than viral protein translation or viral RNA synthesis. Since they could not obtain enzymatically active CHIKV nsP1, they investigated the mechanism of **3** against VEEV nsP1 and results showed that **3** inhibited the activity of VEEV nsP1, specifically the *in vitro* guanylyltransferase (GT) activity, hence causing significant inhibition of VEEV replication.[54] In addition, a CHIKV mutant possessing a P34S substitution in nsP1 was also found to be highly resistant to the antiviral effect of compound **3**.[54]

S-Adenosyl methionine (AdoMet) and *S*-adenosyl homocysteine (AdoHcy) are natural substrates of alphavirus nsP1 mRNA capping machineries. Being an analogue of AdoMet and AdoHcy, sinefungin has been shown to be a potent inhibitor of RNA capping activities.[55] Sinefungin was evaluated against methyltransferase (MT) and GT activities in VEEV nsP1 in an enzymatic assay and demonstrated reasonably good activities against MT and GT.[56] As demonstrated in the studies, sinefungin is likely to function at two levels, i.e. it inhibits methyltransfer by competing with the methyl donor, AdoMet, and blocks the activation of AdoHcy in the GT reaction.[56]

In the past decade, small-molecule inhibitors of CHIKV replication with inhibitory effects against nsP2 have been reported. Thiazolidinones, i.e. compounds **4–8** (Figure 3), demonstrate antiviral activities against CHIKV and limited cytotoxic liabilities at their active concentrations.[57] A molecular docking study revealed that these inhibitors established crucial hydrophobic interactions with S2 and S3 pockets of CHIKV nsP2 and hydrogen bonding interactions with a key residue (Tyr1047), suggesting that these thiazolidinone derivatives could be inhibitors of CHIKV nsP2 protease.[57]

In another strategy for small-molecule drug discovery, a virtual screening method was utilized. A homology model of CHIKV nsP2 based on a VEEV nsP2 protease template was created and screened with a commercially available library of ∼5 million compounds for binding activities with the protease active site. Of these, compound **9** (Figure 3), which possesses a hydrazide structure, demonstrated significant inhibition in both virus yield and CHIKV-induced cytopathic effect (CPE) reduction assays.[58] Using hydrazide **9** as a lead, an SAR approach was adopted by designing and evaluating a series of hydrazide compounds. Replacement of the cyclopropane ring in hydrazide **9** with an alkene moiety resulted in hydrazide **10** (Figure 3), which demonstrated a slightly improved antiviral profile.[58] A new class of hydrazide-based nsP2 inhibitors was also designed and generated by employing computational pharmacophoric replacement and using hydrazide **9** as a lead.[59] Of these, hydrazide **11** (Figure 3) not only showed inhibition of the cleavage of peptide substrate by CHIKV nsP2 in a fluorescence resonance energy transfer (FRET)-based cell-free protease assay, but also demonstrated anti-CHIKV activities at low micromolar concentrations.[59] Interestingly, reduction of both viral RNA synthesis

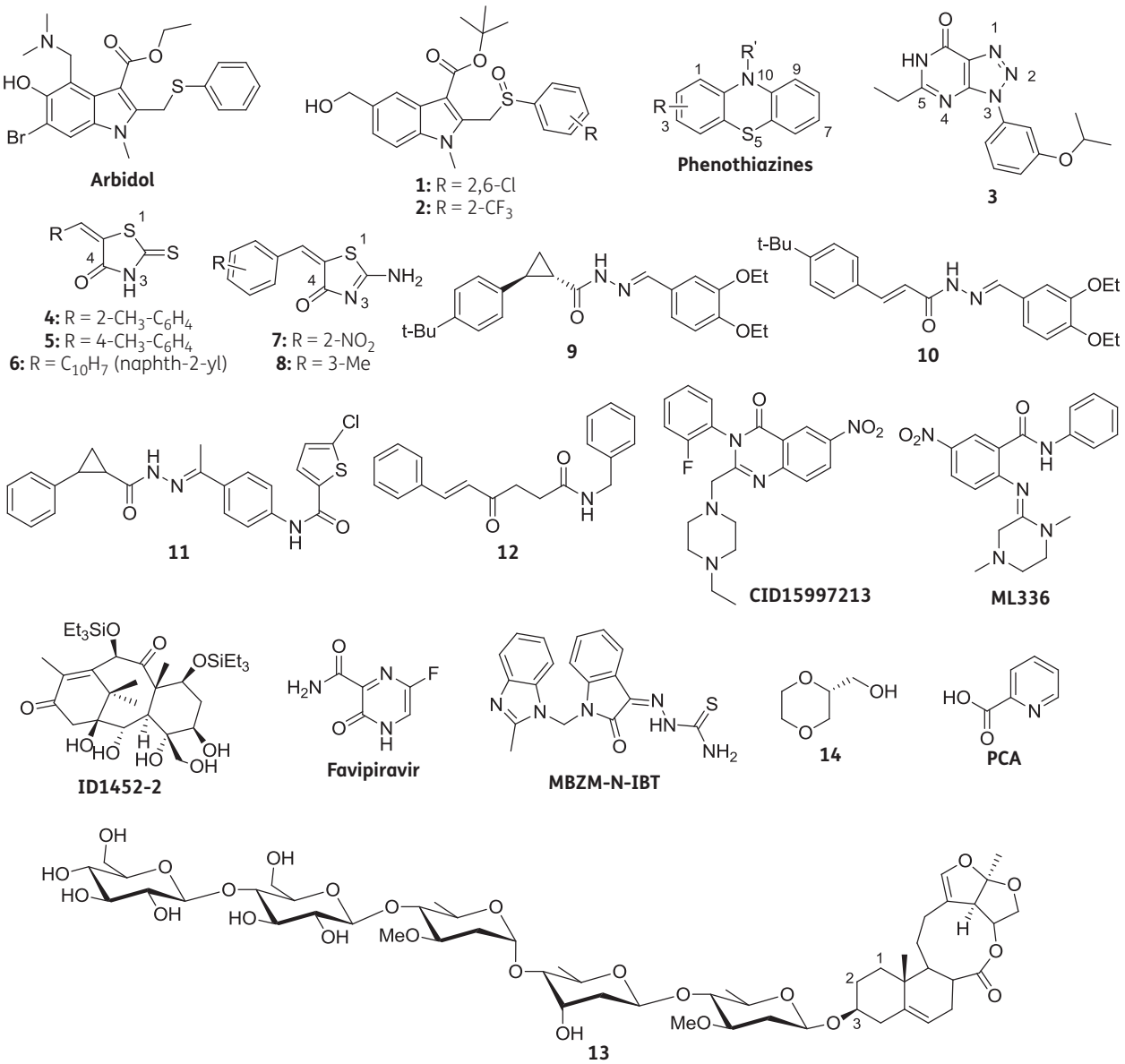

Figure 3. Chemical structures of selected direct-acting inhibitors. Inhibitors of virus attachment and entry: arbidol and its derivatives **1** and **2** and phenothiazines. Inhibitors of virus replication: compounds **3–12**, CID15997213, ML336, ID1452-2, favipiravir, MBZM-N-IBT and seconpregnane steroid glycoside **13** are inhibitors of virus replication. Inhibitors of virus maturation: compound **14** and picolinic acid.

and infectious virus production was observed when cells were pre-treated with hydrazide **11**, suggesting there could be other modes of action associated with this compound.[59] Moving forward, it would be interesting to analyse the inhibitory properties of the individual *cis*/*trans* isomers of hydrazide **11** as stereochemistry could be a major determinant of compound activity.

A peptidomimetic strategy was also employed in the design of small-molecule inhibitors of CHIKV nsP2 in which these peptidomimetic inhibitors were modified from specific amino acid sequences, such as Ala1861-Gly1862-Gly1863-Tyr1864 (AGGY), which is the natural substrate of CHIKV nsP2 protease.[60,61] The studies led to the identification of peptidomimetic **12** (Figure 3), which demonstrated a maximum of 100% inhibition of CHIKV replication at 68.2 μg/mL concentration.[61] Molecular modelling revealed that peptidomimetic **12** binds to CHIKV nsP2 via covalent interaction between its α–β unsaturated ketone functionality and the catalytic residue S1013 in nsP2.[61]

Quinazolinone compound CID15997213 (Figure 3) demonstrates potent *in vitro* activity against various VEEV strains (TC-83 and V3526) and WEEV as well as good *in vivo* antiviral efficacy.[62] A mutation at two key residues (Y102C and D116N) in the N-terminal region of nsP2 in a drug resistance study indicated that the

site of action of CID15997213 could be the nsP2 viral domain.[62] CID15997213 was utilized as a starting point for a classical structure–activity optimization study due to its preliminary promising anti-VEEV activity and good physiochemical profile. In the study, amidine ML336 (Figure 3) was identified as possessing nearly a 7-fold improvement in antiviral potency over the best quinazolinone-based analogues and good *in vivo* efficacy.[63] Mechanistic studies using mutant VEEV, which carried a mutation in nsP2, showed that ML336 possibly targets a critical function of nsP2/nsP4 in the VEEV RC, and hence inhibits viral replication.[63]

nsP2 is not only involved in viral RNA synthesis by being a cofactor of the RC, but it is also a virulence factor that blocks cellular gene transcription, i.e. transcriptional shutoff by inducing the degradation of the Rpb1, a catalytic subunit of RNA polymerase II.[64] Employing a high-throughput phenotypic functional assay to identify small molecules targeting nsP2-mediated transcriptional shutoff, a natural product derivative, ID1452-2 (Figure 3), which partially blocks nsP2 activity and inhibits CHIKV replication *in vitro*, was identified when screened with a chemical library of 3040 molecules.[65]

Favipiravir, an analogue of pyrazine (Figure 3), has been reported to have broad-spectrum antiviral activity against various RNA viruses, including WEEV and CHIKV.[66] Favipiravir not only demonstrated potent *in vitro* and *in vivo* activities against CHIKV and WEEV infections,[67,68] but also demonstrated inhibitory activities against CHIKV RNA synthesis in [^{3}H]uridine labelling experiments.[68] A phenotypic resistance to favipiravir was also observed when cells were infected with a mutant CHIKV genotype (with a K291R mutation in CHIKV nsP4). This suggests that favipiravir possibly inhibits CHIKV replication via interference with RNA-dependent RNA polymerase (RdRp) activity.[68]

A molecular hybrid of isatin-β-thiosemicarbazone and benzimidazole, MBZM-N-IBT (Figure 3), was developed for investigation against *in vitro* CHIKV infection.[69] This hybrid compound not only reduced viral protein and RNA production at 200 μM concentration, but also inhibited CHIKV infection in the early and late phases of replication, which indicates multiple mechanisms for its anti-CHIKV activity.[69] In addition, molecular docking studies revealed favourable binding affinities of MBZM-N-IBT with the homology models of CHIKV nsP1, nsP3 and nsP4.[69]

A class of natural products (i.e. secopregnane steroid glaucogenin C and its monosugar-glycoside cynatratoside A of *Strobilanthes cusia* and three new pentasugar glycosides of glaucogenin C of *Cyananchum paniculatum*) were found to possess effective inhibition against alphaviruses such as SINV and EEEV at nanomolar concentrations.[70] These steroid-containing compounds, including secopregnane steroid glycoside **13** (Figure 3), suppress the expression of SINV subgenomic RNA (sgRNA), predominantly without affecting the accumulation of viral genomic RNA.[70] From the study, the mode of action of these compounds may involve alteration of the structure of the sgRNA promoter, thereby affecting the binding of the transcription complex to the sgRNA promoter, resulting in a decreased expression of sgRNA.[70]

3.3 Inhibitors of virus maturation

Dioxane was discovered as a suitable ligand that bound nicely to the hydrophobic pocket of the SINV CP in protein crystallization studies.[71] Employing the crystal structure of this hydrophobic pocket in a molecular docking study, a series of dioxane-based antivirals that were predicted to bind to the hydrophobic pocket were synthesized and evaluated against SINV replication.[72,73] Although the most potent dioxane-based compound, **14** (Figure 3), demonstrated inhibition against SINV replication, it did not demonstrate any inhibition of the nucleocapsid assembly in the CP assembly assay.[72,73]

Picolinic acid (PCA; Figure 3) was previously reported to have antiviral properties against HIV and human herpes simplex virus.[74] In a molecular docking study, PCA showed stronger binding affinity with the conserved hydrophobic pocket of homology-modelled CHIKV CP as compared with dioxane.[75] PCA also showed strong binding affinity with purified CHIKV CP in isothermal titration calorimetry, surface plasmon resonance and fluorescence spectroscopy studies.[75] PCA was non-toxic up to 2 mM and demonstrated anti-CHIKV activity by causing significant inhibition of viral RNA production and plaque formation at 2 mM concentration.[75] Taking these findings together, PCA may inhibit CHIKV maturation via interfering with CP formation.

3.4 Inhibitor of multiple stages of virus replication cycle

Suramin, a symmetrical sulphonated naphthylurea compound, was first used as an anti-parasitic agent for the treatment of African trypanosomiasis in the 1920s. Since then, research on suramin has gained momentum after its anticancer and antiviral potential was discovered between the 1970s and 1990s.[76,77] Its antiviral activity against CHIKV, SFV and SINV was recently demonstrated.[78,79] Suramin restricted CHIKV multiplication via inhibition of CHIKV RNA synthesis.[79] Furthermore, various studies also demonstrated that suramin interferes with post-attachment stages of the CHIKV replication cycle (i.e. virus entry or the fusion step).[78–80] An SAR study on suramin showed that removal of any moieties from suramin resulted in a loss of activity or 3- to 10-fold drops in activities.[79] A molecular docking study showed that suramin docks in the cavity between CHIKV E1 domain II and E2 domain C. This interaction may inhibit the process of virus release, resulting in reduced cell–cell transmission.[78] Treatment with suramin was shown to reduce viral loads as well as reducing foot swelling, inflammation and cartilage damage in CHIKV-infected C57BL/6 mice.[81] The clinical efficacy of suramin in ameliorating CHIKV-induced arthritis in patients would be worth exploring in the near future.

4. Host-targeting inhibitors

4.1 Inhibitors of virus entry

5-Nonyloxytryptamine (5-NT; Figure 4), a C5 unbranched nonyl-substituted serotonin, has been shown to possess affinity for most serotonin receptors.[82] In antiviral evaluation studies against *in vitro* reovirus infection, 5-NT impeded virus entry and delayed intracellular transport of incoming virions by affecting the distribution of early endosomes, thereby leading to an inhibition of virus infection.[83] 5-NT exhibited potent anti-CHIKV activity,[83] which strongly suggests that serotonin receptor signalling could be one of the crucial regulatory factors involved in the entry of viruses of diverse families, including CHIKV.

Synthetic flavaglines (i.e. FL23 and FL3) and sulfonyl amidine 1 m are known to bind to host cellular receptors such as prohibitin (PHB),[84,85] which a number of different pathogens, including CHIKV, dengue virus (DENV) and HIV, use for entry into the host

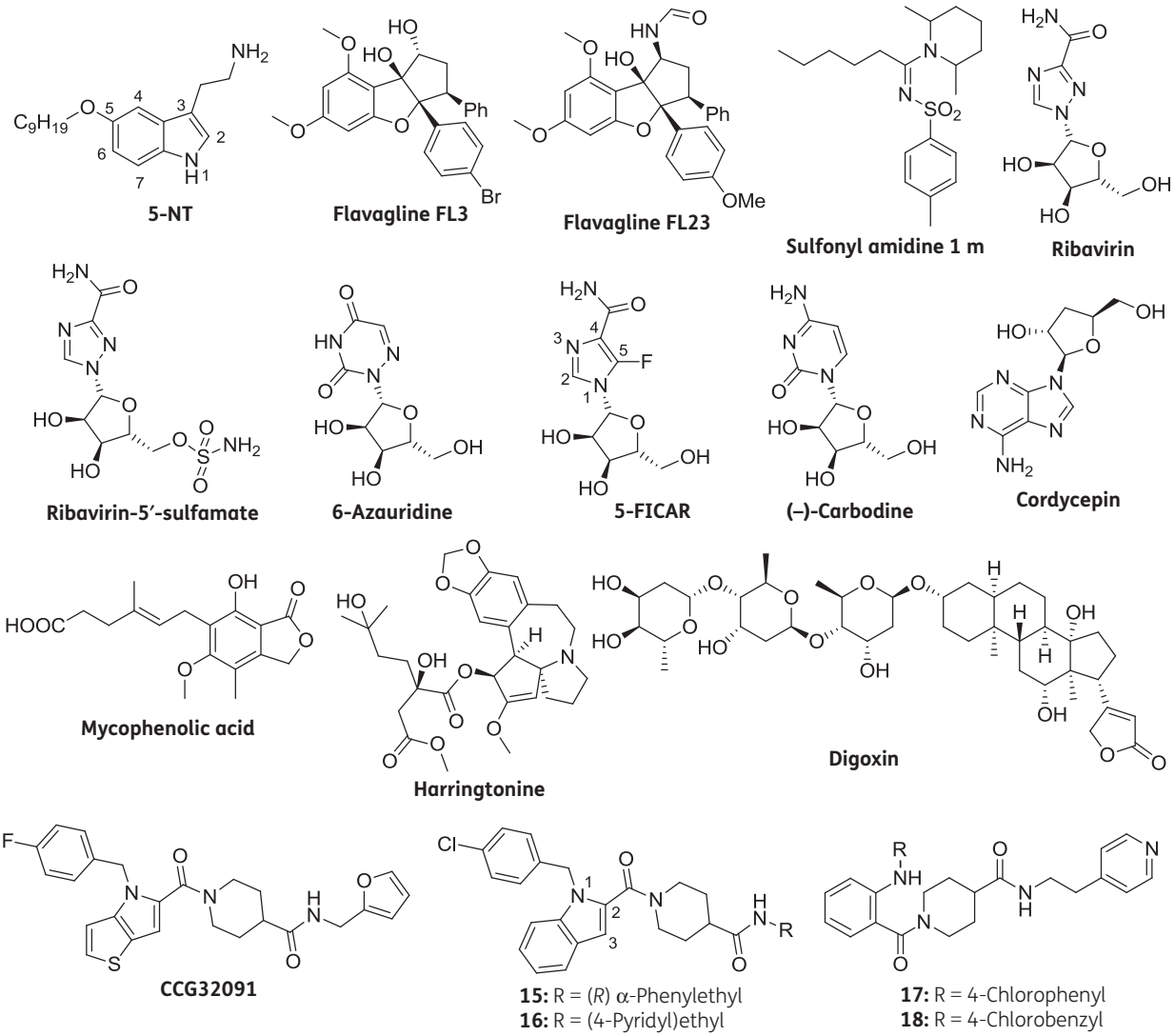

Figure 4. Chemical structures of selected host-targeting inhibitors. Inhibitors of virus entry: 5-NT, flavaglines FL3 and FL23 and sulfonyl amidine 1 m. Inhibitors of virus replication: ribavirin, ribavirin-5′-sulfamate, 6-azauridine, 5-FICAR, (−)-carbodine, cordycepin, mycophenolic acid, harringtonine, digoxin, CCG32091 and compounds **15–18.**

cell.[86–88] These compounds (Figure 4) were assessed for antiviral evaluation on CHIKV production in PHB-expressing cells, and significantly reduced CHIKV production.[89] In addition, co-localization studies between PHB and CHIKV in the presence of these compounds showed interference in CHIKV E2-PHB binding.[89]

4.2 Inhibitors of virus replication and protein synthesis

Nucleoside analogues (containing sugars such as ribose or deoxyribose) are by far the most important class of antiviral drugs.[90,91] Ribavirin (Figure 4) was one of the first anti-alphaviral inhibitors reported when it was show to reduce SFV-induced CPE in chick embryo fibroblasts.[92] This compound was once considered as a treatment for alphaviral infections when studies showed that the combination of IFN-α and ribavirin had a subsynergistic antiviral effect on CHIKV and SFV replication.[93] A number of mechanisms associated with ribavirin have been proposed. Of these, the predominant mechanism of action is inhibition of inosine-5′-monophosphate dehydrogenase (IMPDH), which leads to a depletion of cellular GTP pools and is needed for virus replication.[94] A derivative of ribavirin, ribavirin-5′-sulfamate (Figure 4), was also described to inhibit SFV replication.[95] Another nucleoside analogue, 6-azauridine (Figure 4), is a broad-spectrum antimetabolite that inhibits both DNA and RNA virus replication of CHIKV and SFV,[93] while replacement of the adenine or guanine core in a nucleoside with 4-fluoroimidazole resulted in 5-fluoro-1-β-D-ribofuranosylimidazole-4-carboxamide (5-FICAR; Figure 4), which showed antiviral activity against SINV.[96]

The carbocylic analogue of cytidine, carbodine (Figure 4), has been shown to deplete CTP pools by inhibiting CTP synthetase (which converts UTP into CTP) and thereby inhibits RNA synthesis in the replication cycle of a range of DNA and RNA viruses.[97] Two enantiomeric

pure carbodine compounds [(+)-carbodine and (−)-carbodine] were screened against VEEV-induced CPE.[98] Interestingly, (+)-carbodine did not exhibit any activity whereas (−)-carbodine demonstrated potent *in vitro* and *in vivo* antiviral efficacy.[98] Cordycepin (3′-deoxyadenosine; Figure 4) is known to influence replication of several viruses via reducing the poly(A) content of viral mRNA.[99,100] However, antiviral studies against SFV infection demonstrated that cordycepin did not specifically inhibit the synthesis of poly(A) sequences; instead it inhibited the synthesis of the virus RC.[101]

Mycophenolic acid (Figure 4), a weak organic acid and well-known immunosuppressive agent, was first isolated from the fungus *Penicillium stoloniferum* and demonstrated broad-spectrum antiviral activity against replication of several viruses, including CHIKV.[102,103] Similar to ribavirin, the mechanism of action of mycophenolic acid *in vitro* is based on inhibition of cellular IMPDH activity.[94,103] Brefeldin A, a macrolide lactone antibiotic produced by the fungus *Eupenicillium brefeldianum*, inhibits SINV protein synthesis and RNA replication, possibly due to its interference with the formation of vesicles that were required for viral RNA synthesis.[104]

Harringtonine (Figure 4), a cephalotaxine ester derived from the Japanese plum yew, *Cephalotaxus harringtonia*, is known to be an inhibitor of eukaryotic protein synthesis.[105] It not only demonstrated dose-dependent inhibition of CHIKV during the early events of CHIKV replication after virus entry, but significantly reduced CHIKV RNA and the synthesis of nsP3 and E2 proteins at its non-cytotoxic concentrations (1 or 10 μM).[106] The mechanism of action of harringtonine has been suggested not to be specific to CHIKV, instead being exercised through inhibition of the eukaryotic large ribosomal unit, thereby suppressing viral protein translation, leading to a decrease in the levels of RCs and viral RNA.[106]

Lanatoside C, an approved cardiac glycoside that acts by inhibiting the Na^+-K^+-ATPase ion pump, was demonstrated to have potent inhibitory activity against various RNA viruses, such as DENV, CHIKV and SINV.[107] As optimum levels of intracellular Na^+ and K^+ in the cytosol environment are important for proper replication of various DNA and RNA viruses, increased levels of intracellular Na^+ and reduced intracellular K^+ caused by lanatoside C affected the replication of CHIKV and SINV.[107] Similarly, another inhibitor of the Na^+-K^+-ATPase ion pump, digoxin (Figure 4), showed enhanced inhibition of CHIKV when extracellular Na^+ was introduced, but exhibited no or marginal inhibition of CHIKV when extracellular K^+ was introduced.[108] Digoxin displayed a broad-spectrum inhibitory effect against other alphaviruses, such as RRV and SINV.[108] In addition, mutation of the valine at residue 209 in nsP4 to isoleucine was observed in digoxin-resistant CHIKV populations, suggesting that digoxin could be inhibiting CHIKV replication by disrupting RNA synthesis.[108] Although digoxin is known to be cytotoxic owing to its narrow therapeutic index in treating heart diseases,[109] the observed *in vitro* CHIKV inhibition by digoxin was not due to its cytotoxicity (i.e. toxicity only occurred at a dose 20 times its EC_{50} for antiviral activity).[108] Preclinical studies using an *in vivo* mouse model might be employed to verify the margin between its toxicity and antiviral efficacy.

To improve the poor *in vitro* metabolic properties of the reported inhibitor of WEEV, thieno[3,2-*b*]pyrrole CCG32091 (Figure 4),[110] a class of indole compounds, i.e. bioisosteres of thieno[3,2-*b*]pyrrole, was evaluated for activity against the WEEV replicon.[111] The investigation led to the discovery of an indole analogue, (*R*)-enantiomer **15** (Figure 4), which not only possesses better metabolic stability in mouse liver microsomes (MLMs) as compared with CCG32091 (half-life 31 versus 1.7 min), but also potent antiviral activities, a good cytotoxic profile and *in vivo* efficacy.[111] In subsequent SAR studies, various structural modifications, such as varying substituents at the N1 and C2 position of the indole core as well as scaffold hopping (i.e. replacement of the indole with pyrrole, benzimidazole and imidazole), were made.[112] The investigation led to the discovery of indole-2-carboxamide **16** (with a C2 terminal pyridinyl group; Figure 4), which exhibited 10-fold improvement in potency as compared with **15** in a WEEV replicon assay.[112] Mechanistically, both classes of thieno[3,2-*b*]pyrrole and indole compounds, i.e. CCG32091 and **16**, did not directly inhibit WEEV RdRp or other viral enzymatic activities; instead they possibly targeted a host factor that modulates a cellular cap-dependent translation pathway such as the eukaryotic initiation factor 2 signalling pathway.[113]

Continuing the efforts to improve the physiochemical properties that contribute to the *in vivo* blood–brain barrier (BBB) permeability of indole **16**, the indole core was replaced with a lower molecular weight core structure such as pyrrole or a simple phenyl ring.[114] Through these efforts, two anthranilamide analogues, **17** and **18** (Figure 4), were discovered as possessing better metabolic stability in MLMs (half-life 15–19 versus 9 min), improved aqueous solubility and nearly equivalent passive permeability as measured in a BBB–parallel artificial membrane permeability assay without losing anti-WEEV potency.[114]

4.3 Inhibitors of virus maturation

Earlier reports showed that during alphavirus maturation the envelope glycoprotein precursor, pE2, is usually cleaved at short multibasic motifs by furin or furin-like convertases.[115–117] To inhibit the maturation of CHIKV virions, a synthetic peptide mimic of the conserved sequence (K/R)X(K/R)R↓ of the cleavage site of CHIKV pE2, namely decanoyl-RVRK-chloromethyl ketone (FI), was screened for anti-CHIKV activity. This peptidomimetic behaved like furin inhibitors, which induce inhibition of CHIKV infection by preventing the processing of pE2.[118] Interestingly, FI also showed inhibition of CHIKV entry when used as a pretreatment, suggesting there could be other modes of action associated with FI.[118]

4.4 Modulators of cellular functions that assist virus replication

Deubiquintinases (DUBs) are a class of cysteine proteases involved in proteasomal degradation and regulation of cellular processes such as the unfolded protein response.[119] Studies showed that many viruses depend on the ubiquitin (Ub) cycle by hijacking cellular Ub-modifying enzymes, including DUBs, to assist their post-entry events.[119] To investigate the relationship between DUBs and virus infection, an inhibitor of DUB, WP1130 (Figure 5), was evaluated to determine whether DUBs promoted norovirus infection.[120] Results showed that WP1130 inhibited a proteasome-associated DUB known as USP14 and restricted replication of several RNA viruses, including SINV, through the IRE1-dependent decay of viral proteins, which was activated upon inhibition of DUBs.[120] Derivatives of WP1130 possessing fluoro-substitution or no substitution on the pyridinyl group and a solubilizing group on the phenyl group were explored to improve the aqueous solubility

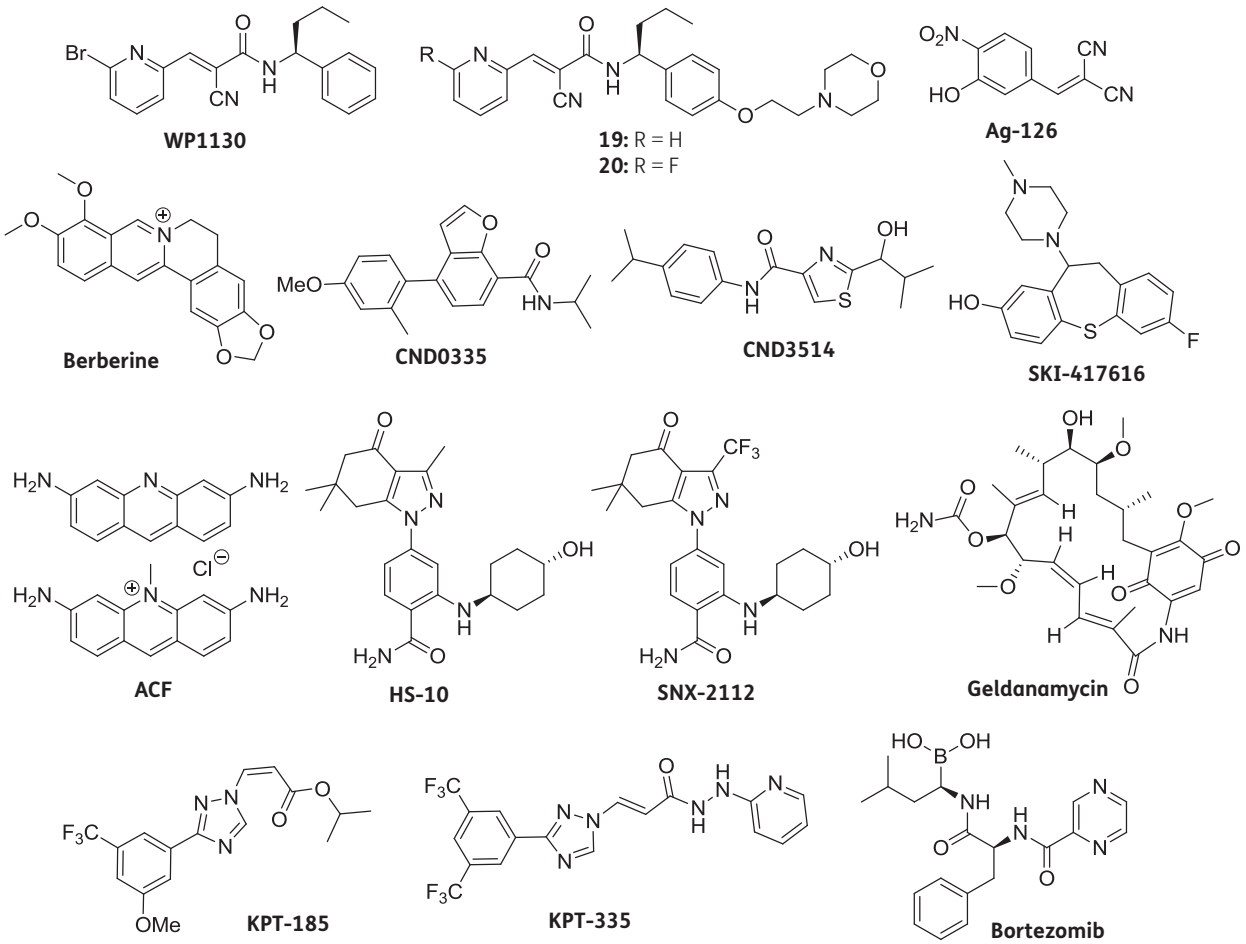

Figure 5. Chemical structures of selected host-targeting inhibitors that are modulators of cellular functions that assist alphavirus replication. Inhibitors of virus fusion and/or replication: WP1130 and its derivatives **19** and **20**, Ag-126, berberine, CND0335, CND3514, SKI-417616, ACF, HS-10, SNX-2112, geldanamycin and bortezomib. Inhibitors of virus assembly: KPT-185 and KPT-335.

of WP1130.[121] Of these, derivatives **19** and **20** (Figure 5) significantly reduced virus titres in SINV-infected Vero cells.[121]

The mitogen-activated protein (MAP) signalling pathway has been suggested to be activated by viruses, which potentially confers a prosurvival status on the infected cells in order to have a productive infection cycle for generating sufficient progeny virions.[122–125] For this reason, an inhibitor of the MAP kinase, extracellular signal-regulated kinase (ERK), Ag-126 (Figure 5), was evaluated and found to possess inhibitory activity against VEEV replication in its non-toxic concentration range during early and late events of the virus replication cycle.[123] Another natural product, berberine (Figure 5), was also found to impede alphavirus replication through inhibiting the phosphorylation of ERK, thereby affecting the egress of progeny virions, since it did not affect virus entry and enzymatic activity of the viral RC.[126] *In vivo* antiviral efficacy studies of berberine revealed that it could behave as both an antiviral agent (i.e. reducing viral load in infected mice) and an anti-inflammatory agent (i.e. decreasing joint swelling in infected mice).[126] Interestingly, berberine demonstrated broad-spectrum antiviral activity against other Old World alphaviruses such as SINV and SFV,[127] while Ag-126 demonstrated broad-spectrum antiviral activity against New World alphaviruses such as EEEV and WEEV.[123] On the other hand, a kinase inhibitor library containing 4000 compounds was screened against *in vitro* CHIKV infection and among the 72 primary hits, 6 compounds containing benzofuran, thiazole and pyrrolopyridine core structures were identified. Of these, the benzofuran CND0335 and pyrrolopyridine CND3514 (Figure 5) exhibited significant reduction in virus titres at 20 μM.[128]

Activation of the D4 dopamine receptor generates a series of downstream signals in which phosphorylation of ERK occurs, which in turn regulates viral replication during the replication cycles of a range of DNA and RNA viruses.[129–131] Treatment with an antagonist of D4 dopamine receptor such as SKI-417616 (containing a dihydrodibenzothiepine scaffold; Figure 5) in DENV-infected cells inhibited the phosphorylation of ERK, which results in the inhibition of virus replication (especially at the early stage of the replication cycle).[122] In addition, it inhibited the replication of SINV at 1 or 10 μM concentration,[122] which suggests that D4 dopamine receptor signalling could be one of the regulatory factors involved in the replication of alphavirus.

Before the degradation of a target mRNA, argonaute (Ago) proteins and microRNAs (miRNAs) along with other co-factors become incorporated into the RNA-induced silencing complex (RISC), which in turn associates with the target mRNA.[132,133] The association

between cellular miRNAs, miRNA processing machinery and VEEV replication was shown when a marked decrease in VEEV replication was observed in the absence of Ago2.[134] Acriflavine (ACF; Figure 5)—a mixture of trypaflavine and proflavine—was found to inhibit the association between Ago2 and other co-factors that assist in RNA loading onto the RISC, thereby reducing VEEV replication.[134] Interestingly, ACF demonstrated broad-spectrum antiviral activity against WEEV and EEEV, suggesting that it could act upon a pathway that is conserved among the encephalitic alphaviruses.[134] However, ACF treatment in infected BALB/c mice did not significantly reduce virus replication.[134] This could be due to its short biological half-life, which accounted for the differences between its *in vivo* and *in vitro* effects in VEEV infection.[135]

HSP-90, which assists in proper folding of viral proteins and stabilizes these proteins against heat stress, plays an important role in the replication of many DNA and RNA viruses.[136,137] HSP-90 is involved in the CHIKV RC by interacting with nsP3 and nsP4 to facilitate virus replication.[138] For this reason, HSP-90 inhibitors such as HS-10, SNX-2112 and geldanamycin (Figure 5) were investigated and found to inhibit CHIKV replication.[138]

VEEV CP was known to associate with cellular proteins such as host trafficking proteins [i.e. cellular importin α/β, chromosomal maintenance 1 (CRM1) and nuclear pore complex], which have the unique ability to block the nuclear import of transcription factors required for an antiviral response, and the export of newly synthesized cellular mRNA.[139] Hence, host trafficking proteins are viable targets for antivirals designed specifically to interrupt the interaction between CP and these proteins. Nuclear transport inhibitors such as mifepristone, ivermectin and leptomycin B have been shown to inhibit VEEV replication by altering VEEV CP localization and activity.[140] Of these, leptomycin B, a well-documented CRM1 inhibitor and *Streptomyces* metabolite, was very potent in restricting the CP to the nucleus, making it unavailable at the cytoplasm to form viable virions.[140] Since leptomycin B is rather cytotoxic, there is a need to investigate a new generation of CRM1 inhibitors. Hence, a series of selective inhibitors of nuclear export (SINE) compounds, such as KPT-185, KPT-335 (Figure 5) and KPT-350, which are analogues of selinexor, was explored.[141] These compounds confine VEEV CP to the nucleus, leading to a depletion in the amount of cytoplasmic CP (i.e. intracellular CP) and released CP (i.e. extracellular CP), as demonstrated in western blot analyses.[141] This leads to a decrease in virus assembly and/or release of mature virions. Serial passaging of VEEV-infected cells in the presence of KPT-185 resulted in mutations within the nuclear localization and nuclear export signals in the CP (i.e. T41I, K64E or K64M), confirming that this SINE compound exerts its antiviral activity by targeting CP localization.[141]

Bortezomib (Figure 5) is a dipeptidyl boronic acid that specifically and reversibly inhibits the ubiquitin proteasome.[142] As a result, the early stage of the VEEV infectious cycle was affected by bortezomib during the fusion stage, when the VEEV CP was K48 ubiquitinated for proteasomal degradation for the release of the viral RNA.[143] Bortezomib treatment also decreased the multiplication of other virulent New World alphaviruses.[143]

5. Conclusions and perspectives

A number of reported direct-acting and host-targeting inhibitors of alphaviruses have been highlighted, with the emphasis on their mechanisms of action. These include drugs that are already on the market and currently used for the treatment of other diseases, such as doxycycline, phenothiazines, chloroquine, obatoclax, suramin and digoxin, which have been discovered in drug repurposing screens. The challenge here is to improve the potency of these compounds against CHIKV while retaining good drug-like properties. The inhibitory activities of the tested compounds against alphavirus ranged from strong to weak inhibition depending on the type of assay used, with secopregnane steroid glycoside **13** being the strongest inhibitor, with EC_{50} 1.5 and 2 nM against SINV replication and EEEV-induced CPE, respectively, while suramin displayed the weakest activity against SINV-induced CPE, with EC_{50} 141 μM. Despite the selective antiviral activity of direct-acting inhibitors such as favipiravir, CID15997213 and ML336 against the functions of alphavirus elements compared with host cellular targets, the use of direct-acting inhibitors in treatment regimens can lead to the rapid selection of resistant viruses. On the other hand, host-targeting inhibitors such as digoxin, bortezomib, berberine, Ag-126 and SINE compounds represent an alternative approach, and may increase the barrier to resistance and achieve broad-spectrum antiviral coverage against a range of alphaviruses. However, a possible downside is the potential on-target toxicity, as exemplified by digoxin, an inhibitor of the Na^+–K^+-ATPase ion pump, which shows toxicity at concentrations as low as 1 μM.

Besides developing small-molecule inhibitors as therapy against alphaviruses, other forms of unconventional antiviral therapy, such as RNA interference (RNAi)-based therapy and antiviral immunotherapy, can be considered. Given the ability to specifically silence any gene of interest in the viral RNA, thereby preventing viral proteins from being translated, short interfering RNAs (siRNAs) and miRNAs, which constitute RNAi-based therapy, offer several advantages over conventional drugs as potential therapeutic agents by overcoming patient compliance and drug toxicity issues.[144] As the innate immune system plays a central role in the progression and control of alphavirus infection, small-molecule immunomodulators such as DD264, G10 and 5,6-dimethylxanthenone-4-acetic acid, which stimulate expression of antiviral proteins, have also been explored as potential therapeutic agents against alphavirus infection.[145,146] With the recent advances in RNAi technology and developments in the characterization of the receptors and pathways of the innate immune system associated with alphavirus infection, it should be possible to develop highly targeted RNAi-based and immunomodulatory therapies for the treatment of alphavirus infections.

Transparency declarations

None to declare.

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