Antiviral drug discovery against arthritogenic alphaviruses: tools and molecular targets

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Abstract

Alphaviruses are (mainly) arthropod-borne viruses that belong to the family of the *Togaviridae*. Based on the disease they cause, alphaviruses are divided into an arthritogenic and an encephalitic group. Arthritogenic alphaviruses such as the chikungunya virus (CHIKV), the Ross River virus (RRV) and the Mayaro virus (MAYV) have become a serious public health concern in recent years. Epidemics are associated with high morbidity and the infections cause in many patients debilitating joint pain that can persist for months to years. The recent (2013-2014) introduction of CHIKV in the Americas resulted in millions of infected persons. Massive outbreaks of CHIKV and other arthritogenic alphaviruses are likely to occur in the future. Despite the worldwide (re-)emergence of these viruses, there are no antivirals or vaccines available for the treatment or prevention of infections with alphaviruses. It is therefore of utmost importance to develop antiviral strategies against these viruses. We here review the possible molecular targets in the replication cycle of these viruses for the development of antivirals. In addition, we provide an overview of the currently available *in vitro* systems and mouse infection models that can be used to assess the potential antiviral effect against these viruses.

1. Burden of arthritogenic alphaviruses

Alphaviruses (family *Togaviridae*) comprise a group of human and animal viruses, which are mainly transmitted by the bite of hematophagous arthropods [1]. Based on their historical geographical location, alphaviruses are classified into the New World and Old World alphaviruses. The New World alphaviruses such as the Venezuelan and Western equine encephalitis viruses (VEEV and WEEV) are usually associated with encephalitic disease symptoms [1]. On the other hand, infections by the Old World alphaviruses e.g. chikungunya virus (CHIKV) and Ross river virus (RRV) result mainly in arthralgia and debilitating pain similar to pains caused by rheumatoid arthritis [1]. Acute infections by arthritogenic alphaviruses are self-limiting and mainly associated with fever, rash and arthritis [2]. However, many patients develop a chronic polyarthritis that can severely incapacitate the patient for weeks and even up to several years after the acute stage [2]. Recent CHIKV outbreaks have also been associated with severe complications such as Guillain-Barré syndrome [3] and meningoencephalitis [4].

Some arthritogenic alphaviruses are currently endemic in specific regions of the world, for example, O'nyong'nyong virus (ONNV) in Sub-Saharan Africa [5], RRV [6] and Barmah forest virus (BFV) [7] in Australia, and MAYV in the South and the Central Americas [8]. CHIKV on the other hand has re-emerged in several parts of the world in the last decade resulting in massive outbreaks that were associated with high morbidity rates [2]. At the end of 2013, the first local transmission of CHIKV in the Americas has been reported on the Island of Saint Martin. Since then, millions of CHIKV infections have been reported in the Caribbean region and countries of Central and South America [2]. Potential expansion of infections with other arthritogenic alphaviruses are expected in the future.

24   In general, arthritogenic alphaviruses are transmitted in enzootic/epizootic transmission

25   cycles between an arthropod vector and animal reservoirs [1,9]. Transmission to humans

26   typically occurs by direct spillover from these cycles via the bite of mosquitoes [1,9]. Until

27   now, only CHIKV showed the ability for inter-human transmission, similar to dengue and Zika

28   virus [9].

29   A wide variety of mosquito species has been reported to transmit arthritogenic alphaviruses.

30   *Aedes* mosquitoes are the main vectors for CHIKV transmission [1]. ONNV is transmitted by

31   Anopheles species [5]. For MAYV, Haemagogus species, mainly *H. janthinomys*, are the

32   primary vectors for virus transmission [8]. However, other mosquitoes such as *Ae. aegypti*, *Ae.*

33   *albopictus* [10] and four Anopheles species [11] have been reported to be competent vectors

34   for MAYV in laboratory settings. On the other hand, RRV is transmitted by several mosquito

35   species such as those belonging to the genera of *Culex, Aedes, Anopheles and Mansonia* [12].

36   Despite the worldwide (re-) emergence of CHIKV and other arthritogenic alphaviruses and

37   their major clinical impact, there is no antiviral drug for the treatment or vaccine for the

38   prevention of these viral infections. Therefore, the current treatment relies on symptomatic

39   relief via the use of analgesics, antipyretics, non-steroidal anti-inflammatory drugs and, in

40   severe cases, methotrexate [1]. Here, we review the potential molecular targets for inhibitors

41   of arthritogenic alphaviruses replication **(Fig 1)**. Moreover, we discuss the available *in vitro*

42   and mouse models to assess the potential efficacy of such antiviral drugs.

43   **2. Genome organization of alphaviruses**

44   Alphaviruses are small (about 60-70 nm-diameter) positive-strand RNA viruses with an

45   icosahedral-like nucleocapsid surrounded by a lipid envelope with embedded viral

46   glycoproteins. The viral genome is approximately 12 kb and encodes two open reading frames

47   (ORF), flanked by 5' and 3' untranslated regions with 5'cap structures and a 3' poly(A) tail [13].

48   The 5' ORF is translated from genomic RNA by a cap-dependent mechanism, which results in

49   the formation of four non-structural proteins (nsP1-4) responsible for cytoplasmic RNA

50   replication and modulation of cellular antiviral responses. The 3' ORF is translated from a

51   subgenomic RNA, which is also capped, to yield three major structural virus proteins (capsid,

52   E2 and E1 envelope glycoproteins) and two small peptides (6k and E3) [13].

53   **3. Viral targets for anti-alphavirus compounds**

54   **3.1.   Viral entry**

55   Alphaviruses enter the host cell by receptor-mediated endocytosis following the interaction

56   of the viral E2 glycoprotein with specific cellular receptors **(Fig 2)** [14]. Within the endosome,

57   conformational changes of the viral envelope glycoprotein E1 are triggered by the low pH

58   leading to its fusion with the endosomal membrane **(Fig 2)** [14]. Consequently, the viral

59   nucleocapsid is released into the cytoplasm where it disassembles to release the viral RNA

60   genome [14]. Molecules that raise the endosomal pH, such as the antimalarial drug

61   chloroquine [15,16] and the anticancer drug obatoclax [17], have therefore been shown to

62   inhibit the *in vitro* replication of alphaviruses (including CHIKV and SFV) through preventing

63   the viral fusion step. Disruption of the alphavirus envelope structure by amphipathic

64   molecules such as porphyrins was also shown to inhibit the binding and entry of CHIKV, MAYV

65   and Sindbis virus (SINV) [18]. Another strategy to inhibit viral entry is the use of molecules that

66   target alphavirus-specific receptors. For example, flavaglines are natural products that were

67   reported to target prohibitin-1, a signaling protein that was identified as a receptor for CHIKV

68   in mammalian cells [19]. Two synthetic flavaglines derivatives were shown to inhibit CHIKV

69   replication in cell culture and to reduce the co-localization of prohibitin-1 and the CHIKV E2

70   glycoprotein which may suggest an effect on CHIKV binding to this receptor [20]. Arbidol is a

71   broad-spectrum antiviral that has been reported to be an early stage inhibitor of CHIKV

72   replication in cell cultures [21]. An arbidol-resistant variant was identified that carries a glycine

73   to an arginine (G407R) mutation in the CHIKV E2 glycoprotein, which is the protein involved

74   in the viral binding to host receptors [21]. Recent studies report that the anti-trypanosomiasis

75   drug suramin has antiviral activity against different CHIKV isolates and related alphaviruses *in*

76   *vitro* [22,23]. In addition, suramin reduced CHIKV-induced arthritis in CHIKV-infected C57BL/6

77   mice [24]. Suramin inhibits CHIKV entry and cell-to-cell transmission, most probably through

78   binding to the cavity between CHIKV glycoprotein E1 domain II and E2 domain C [22,23].

79   Several human and murine monoclonal antibodies (mAbs, reviewed in [25]) inhibit the entry

80   of CHIKV and other arthritogenic alphaviruses. The mechanism of viral entry inhibition by such

81   mAbs is mainly via interference with receptor attachment or via blocking of post-attachment

82   steps required for the viral membrane fusion [25]. Recently, the adhesion molecule Mxra8 has

83   been identified as an entry receptor for several arthritogenic alphaviruses including CHIKV,

84   ONNV, RRV and MAYV [26]. An Mxra8–Fc fusion protein and anti-Mxra8 mAbs inhibit CHIKV

85   infection in various cell types and were also shown to be able to reduce CHIKV and ONNV

86   infection in C57BL/6 mice [26].

87   **3.2.   Viral RNA capping**

88   The mechanism by which the alphavirus non-structural protein 1 (nsP1) is responsible for the

89   capping of the viral RNA is distinct from the conventional capping mechanism of the host cell.

90   A methylgroup transfer and subsequent formation of an m^7GMP-nsP1 covalent complex is

91   mediated by the methyl- and guanylyltransferase activities of nsP1. In a final nsP1 mediated

92   reaction, m^7GMP is attached to the 5' end of the viral RNA, resulting in the formation of a cap0

93   structure [27]. In addition, it has been suggested that interaction of nsP1 with all other nsPs is

94   essential to keep the replication complexes intact and functional [28]. These observations

95   make nsP1 an attractive antiviral drug target [27]. The first class of small molecules reported

to target nsP1 (the MADTP-series) were shown to be active also against clinical CHIKV isolates in cell culture. This class of molecules inhibits the guanylyltransferase activity of enzymatically active VEEV nsP1 *in vitro* [29–31]. The guanosine analog, ribavirin, depletes intracellular GTP pools, which prevents proper capping of newly synthesized viral RNA by nsP1 [32]. Consequently, this may impede viral RNA translation and allow host 5' exonucleases to degrade uncapped viral RNA [28]. More recently, the naturally derived compound lobaric acid was identified in a high-throughput screen as an inhibitor of CHIKV nsP1 GTP binding and guanylation [33]. Antiviral efficacy of lobaric acid was demonstrated in cell-based antiviral assays against both SINV and CHIKV [33].

3.3. Viral protease activity

The non-structural protein 2 (nsP2) of alphaviruses is a multifunctional protein that encodes RNA helicase, nucleoside triphosphatase and RNA triphosphatase activities within its N-terminal. It possesses also a cysteine protease activity at its C-terminal that is involved in cleavage of the non-structural viral polyprotein **(Fig 2)** [28]. nsP2 has also been reported to be involved in shutting off host cell mRNA transcription and translation and to inhibit cellular antiviral response (shut off of the JAK-STAT signaling pathway) [28]. Targeting the viral protease has been shown to be a promising and a powerful strategy to inhibit viral replication in patients infected with either HIV or HCV [34]. It is thus conceivable that the nsP2 protease of alphaviruses could be also a promising target for drug discovery and development.

The crystal structures or homology models of the nsP2 protease of different arthritogenic alphaviruses were explored in an attempt to identify potential inhibitors through a combination of molecular docking and molecular dynamics studies [35–37]. Based on this approach, some hit compounds and five potential binding pockets of the CHIKV nsP2 protease were identified [35]. Some arylalkylidene derivatives of 1,3-thiazolidin-4-one that inhibit the

7

120 *in vitro* CHIKV replication (EC$_{50}$ values in the low μM range) have been proposed, based on

121 molecular docking studies, to target the nsP2 protease domain [38]. A panel of *in silico*

122 predicted inhibitors of the CHIKV protease [37] as well as some peptidomimetics [39] were

123 confirmed to inhibit the CHIKV nsP2 protease activity and were shown to also inhibit the viral

124 replication in cell culture.

125 **3.4.   Viral genome replication**

126 For viruses such as HIV, HBV, HCV, influenza and herpes, the viral polymerase has shown to

127 be an excellent target for antivirals. The alphavirus nsP4, functions as the viral RNA-dependent

128 RNA polymerase (RdRp) [14], may thus be considered one of the most attractive targets for

129 the development of (broad-spectrum) anti-alphavirus compounds. The anti-influenza drug

130 favipiravir and its defluorinated analog, T-1105, have been proven to inhibit the replication of

131 CHIKV and related (arthritogenic) alphaviruses in cell culture [40]. Favipiravir treatment (300

132 mg/kg/day for 7 days) of CHIKV-infected AG129 mice protected from severe neurological

133 disease and markedly increased the survival rate [40]. Furthermore, treating CHIKV-infected

134 C57BL/6J mice with favipiravir (300 mg/kg/day for 4 days) reduced viral replication in the

135 joints of the extremities during the acute phase of infection [41].

136 **4. Targeting host factors**

137 **4.1.   Intracellular nucleotide depletion**

138 Ribavirin (a guanosine analogue) and mycophenolic acid have been reported to inhibit CHIKV

139 replication. Both molecules inhibit the inosine monophosphate dehydrogenase enzyme

140 (IMPDH) resulting in the depletion of GTP pools [42,43]. 6-Azauridine, which inhibits orotidine

141 monophosphate decarboxylase enzyme (OMP) resulting in depletion of the intracellular UTP

142 pools, inhibits the *in vitro* replication of CHIKV and SFV [44]. However, the cytotoxicity of this

143   compound increased in exponentially growing cells and should thus be considered when

144   interpreting the antiviral efficacy.

4.2.   Protein synthesis machinery

146   Some molecules have been shown to inhibit alphavirus replication by interfering with the

147   translation of viral proteins via aspecific mechanisms. Harringtonine, an inhibitor of eukaryotic

148   protein synthesis, was shown to inhibit the *in vitro* replication of CHIKV (including in primary

149   human skeletal myoblasts) and SINV [45].

4.3.   Cellular chloride channels

151   Cellular ion channels has been proven to play an important role during the entry and

152   replication of several viruses [46]. Recently, two chloride channels (CLIC1 and CLIC4) have

153   been identified as pro-viral factors for CHIKV replication using siRNA-mediated knock down

154   [47]. The chloride channel inhibitors diisothiocyanostilbene-2,20-disulfonic acid (DIDS), 9-

155   anthracene carboxylic acid (9-ACA) and 5-nitro-2-3-phenylpropylamino benzoic acid (NPPB)

156   result in *in vitro* antiviral activity against CHIKV in Huh7 cells [47]. NPPB also inhibited CHIKV

157   replication in mosquito (C6/36) cells [47].

4.4.   Cellular furins

159   During alphavirus replication, the viral glycoprotein precursor is processed by cellular furins

160   to produce mature virions **(Fig 2)**. Treating CHIKV-infected human muscle satellite cells with

161   the furin inhibitor decanoyl-RVKR-chloromethyl ketone (dec-RVKR-cmk) has been reported to

162   inhibit viral infection [48]. As a result of furin inhibition by dec-RVKR-cmk, immature viral

163   particles were produced and in turn the viral spreading was markedly reduced [48].

4.5.   Cellular kinases

165   Some cellular kinases have been identified to play a key role during arthritogenic alphavirus

166   replication. The Src family kinases (SFKs) are a family of membrane-associated kinases that

167 mediate signal transduction of several receptors and that have been reported to promote the

168 replication of various viruses such as hepatitis C virus (HCV) and dengue virus (DENV) [49]. A

169 Kinome study was performed, in CHIKV-infected human dermal fibroblasts, to identify which

170 kinase pathways are significantly altered as a result of CHIKV infection [49]. It was shown that

171 the SFK-phosphatidylinositol 3-kinase (PI3K)-AKT-mTORC-signaling pathway is activated in

172 CHIKV infected cells. Treating human fibroblasts with the SFK inhibitor (dasatinib) or the

173 mTORC1/2 inhibitor (Torin 1) reduced the virus yield of CHIKV, ONNV, RRV and MAYV [49].

174 This antiviral effect was mediated by inhibition of the alphavirus subgenomic RNA translation

175 and hence inhibition of the structural protein synthesis. Also the major mitogen-activated

176 protein kinase (MAPK) signaling pathways are activated during CHIKV infection and play a role

177 in infectious alphavirus particles formation [50]. Reducing the MAPK pathway activation by

178 berberine, a plant-derived alkaloid, was reported to inhibit the *in vitro* replication of ONNV

179 and different CHIKV isolates. Furthermore, berberine treatment significantly reduced the joint

180 swelling and inflammation at its peak (i.e. day 6 post-infection) in CHIKV-infected mice [50].

181 Activation of the protein kinases C (PKCs) by phorbol esters e.g. prostratin [51] or salicylate-

182 based bryostatin analogs [52] has been reported to inhibit the *in vitro* replication of CHIKV.

183 Unlike phorbol esters, bryostatin has no tumor-promoting effect. Interestingly, byrostatin-

184 based analogs with a cap in their scaffold that inhibit or reduce binding to PKCs were still able

185 to inhibit CHIKV replication [52]. Combination with different PKC inhibitors counteracted the

186 antiviral activity of a non-capped analog but did not affect that of capped analogs, which

187 further confirmed that the capped analogs inhibit CHIKV replication via a PKC-independent

188 mechanism [52].

189 **4.6.   Host immune response**

190   A recent study reported on elucidating the subtype-specific mechanisms by which IFN-α and

191   IFN-β exert a protective role during acute alphavirus infection, highlighting their importance

192   in controlling the infection [53]. We here discuss how the possibility of treating arthritogenic

193   alphavirus disease by targeting the host immune response has been explored using different

194   strategies. Recombinant IFN-α inhibits the replication of CHIKV and SFV *in vitro* [44]. However,

195   suppressing CHIKV production with IFN-α monotherapy requires concentrations exceeding

196   feasible clinical treatment regimens [54]. Mathematical modelling was used to investigate the

197   potential of ribavirin and IFN-α combination therapy against CHIKV. The simulation outputs

198   were validated experimentally showing a 99% reduction in CHIKV levels by combining ribavirin

199   and IFN-α at standard clinical regimens [54]. Type I IFN associated factors have also been

200   considered as potential therapeutics. For example, viperin, an IFN-inducible protein has been

201   reported to inhibit a broad spectrum of DNA and RNA viruses [55]. Evidence of its antiviral

202   function was further supported by *in vivo* data from a study involving SINV [56]. Another

203   example is polyinosinic acid:polycytidylic acid (poly(I:C)). This synthetic analogue of dsRNA has

204   been shown to upregulate the toll-like receptor-3 (TLR3) which results in the induction of IFN-

205   α/β and other antiviral genes (e.g. OAS and MxA) in mice [57,58]. Activation of retinoic acid

206   inducible gene-I (RIG-I) by viral nucleic acids results in downstream signaling leading to

207   increased levels of type I IFNs [59]. Previous studies have described the therapeutic potential

208   of RIG-I agonists as antiviral agents against different viruses including alphaviruses, both *in*

209   *vitro* and *in vivo* [60,61]. Recently, a small-molecule agonist of the adaptor protein STING was

210   suggested to be a novel drug candidate due to its ability to produce an antiviral type I IFN

211   response [62]. In this study, the molecule C11 was shown to elicit conditions in human

212   telomerase-transduced foreskin fibroblast cells that are refractory to the growth of multiple

213   alphaviruses [62].

214   Heparan sulfate mimetics which can modulate the levels of inflammatory infiltrates and

215   cytokines, were found to reduce the severity of alphavirus-induced pathologies in mice

216   [63,64]. Particularly, pentosan polysulfate is currently undergoing phase II clinical trials

217   (PARA_004, Paradigm BioPharmaceuticals) for patients diagnosed with RRV-induced arthritic

218   disease [64].

219   **5. *In vitro* and *in vivo* infection models**

220   **5.1.   Viruses**

221   ***Surrogate and bio-safe alphaviruses***

222   Since some arthritogenic alphaviruses, such as CHIKV, require high biosafety level facilities to

223   carry out antiviral studies, several surrogate systems have been developed to allow

224   conducting such studies at a lower biosafety level. One example is the use of an attenuated

225   strain of the virus e.g. CHIKV vaccine strain 181/25 [49]. Another example is to use stable

226   mammalian cell lines that contain a persistently replicating CHIKV replicon which allow to

227   screen for replication inhibitors [65,66]. To assess whether certain molecules act as entry

228   inhibitors, pseudo-viral particles that carry the alphavirus envelope proteins and express a

229   luciferase reporter can be used [67,68]. In addition, arthritogenic alphaviruses with a lower

230   biosafety level such as the Semliki Forest virus (SFV) are of value for initial screening for

231   alphavirus inhibitors, hits can be then profiled against alphaviruses of a higher biosafety level

232   [65].

233   ***Pathogenic alphaviruses***

234   Various laboratory strains and clinical isolates of arthritogenic alphaviruses have been used to

235   evaluate the efficacy of antiviral compounds. For CHIKV, the laboratory adapted strains

236   include ROSS [69,70], LR2006_OPY1 (GenBank: DQ443544.2) [38], CHIKV-S27 (ATCC: VR-64,

237   African prototype)[23] and CHIKV 899 (GenBank: FJ959103.1, an Indian Ocean strain) [40].

238 Clinical CHIKV isolates have also been used for antiviral screening purpose such as CHIKV-0708

239 (GenBank: FJ513654) [69,71], DRDE-06 (GenBank: EF210157) [42], Venturini and Bianchi and

240 Congo 95 [31]. In addition, CHIKV isolates that carry the mosquito adapting A226V mutation

241 in the E1 protein [e.g. CHIKV-122508 (GenBank: FJ445502.2) and the 0810bTw (GenBank:

242 FJ807899)] are available [23,71,72].

243 Unlike for CHIKV, antiviral studies for the other arthritogenic alphaviruses are sparse.

244 Examples for available strains of the other arthritogenic alphaviruses include: MAYV (ATCC VR

245 66, strain TR4675) [73], MAYV BeAr20290 strain (GenBank: KY618127) [74], ONNV strain IPD

246 A234 (GenBank: AF192890.1) [31], BFV strain BH2193 (GenBank: U73745.1) [31] and RRV T48

247 (GenBank: GQ433359, a laboratory strain) [64]. Convenient tools for high-throughput

248 screening for alphavirus inhibitors are recombinant infectious alphaviruses that express a

249 fluorescence protein or a luciferase gene such as the green-fluorescent protein (GFP) [75],

250 Renilla luciferase (Rluc) [65] or Gaussia luciferase (Gluc) [50].

251 **5.2.   Cell culture models**

252 Cell-based antiviral assays that rely on the infection of susceptible cells with a virus are

253 commonly used in the initial stage of alphavirus drug discovery [14,76,77]. The antiviral

254 efficacy can be expressed as a reduction in virus-induced cytopathic effect (CPE), which can

255 be scored microscopically and/or quantified using colorimetric assays [78,79]. Alternatively,

256 when the cell type is not susceptible to the virus-induced CPE, the antiviral efficacy can be

257 expressed as a reduction in fluorescence or luminescence using specific reporter viruses [76].

258 Arthritogenic alphaviruses infect a wide range of cell types and tissues, including monocytes

259 and/or macrophages, dendritic cells, synovial and dermal fibroblasts, endothelial cells and

260 muscle cells [80]. However, other cell types are most commonly used for antiviral screening,

261 including African green monkey kidney (Vero) cells, baby hamster kidney (BHK) cells, HeLa

262 cells, human fetal lung fibroblast (MRC-5) cells and human embryonic kidney 293 (HEK-293T)

263 cells [76,77,81]. Although these cells have little to no clinical relevance to alphavirus

264 infections, they are very suitable for high-throughput screening campaigns. Recently, four

265 mammalian cells lines; Huh7 (hepatocyte, human), C2C12 (myoblast, mouse), SVG-A

266 (astroglia, human) and dermal fibroblasts (transformed cell line, human); were proposed as

267 good cell culture models for *in vitro* CHIKV research [82]. These cell lines are biologically and

268 clinically relevant in the context of arthritogenic alphavirus infections. Two of these proposed

269 cell lines were previously used in high-throughput screening applications [83,84]. These

270 reports implicate the potential of using clinically relevant cell lines in future drug discovery

271 campaigns for alphaviruses.

272 **5.3.   Mouse models**

273 Mouse models are commonly used to preclinically assess the efficacy of antiviral drug

274 candidates *in vivo*. Both immunocompetent and immunocompromised mouse models are

275 available to evaluate the efficacy of antivirals against arthritogenic alphaviruses. Using these

276 models, the antiviral efficacy can be assessed based on quantification of infectious virus titers

277 in serum and target organs, histopathology and clinical disease scores. Adult C57BL/6 mice are

278 the main immunocompetent model for arthritogenic alphavirus infections [85–90]. For some

279 alphaviruses, newborn and adult Swiss, adult CD-1 and BALB/c mice have been used as

280 alternative immunocompetent models [87,89,91,92]. To recapitulate the pathologies

281 associated with acute human infections, the mice are inoculated subcutaneously at the ventral

282 side of the rear foot pad [93,94]. On day 6-8 post infection, animals display peak swelling of

283 the inoculated foot and occur with signs of moderate arthritic disease defined by hunching,

284 lethargy and gait alteration. Infectious virus can be detected in serum, skeletal muscle and

285 distant joint tissues such as ankles and wrists. Signs of joint and skeletal muscle inflammation

286 can be observed after histopathological examination. Infection of C57BL/6 mice with SFV or

287 SINV also leads to viral replication in the central nervous system, resulting in paralysis and

288 death [95–97]. Thus, this particular model is mainly used to study the effect on alphavirus

289 neurological pathogenesis. A limited number of immunocompromised mouse models are

290 available to study CHIKV and the closely related viruses, ONNV and MAYV [40,90,94]. The mice

291 used in these models are deficient in type I or type I and type II interferon pathways. Infection

292 of these mice results in viral replication in skeletal muscle, myositis and high mortality, which

293 can be used as end-point markers for testing the antiviral efficacy.

294 **6. Future directions**

295 The worldwide re-emergence of arthritogenic alphaviruses and the high morbidity rate

296 associated with their infections make these viruses an emerging health threat. The

297 development of potent and safe antiviral compounds against arthritogenic alphaviruses is

298 therefore urgently needed. Such antivirals will help to reduce the severity of the disease

299 symptoms during the acute phase of infection. In addition, reduction of the viral load in

300 infected patients by antiviral treatment may help to decrease the transmission efficiency of

301 the virus by mosquitoes [98]. Since patients with more severe symptoms during the acute

302 phase of alphavirus infection have a higher chance to develop chronic joint disease [99], the

303 use of a potent antiviral during the acute infection is expected to decrease the likelihood to

304 develop chronic symptoms. Household-prophylaxis may also be a potential application of

305 antivirals as the probability of arthritogenic alphavirus transmission such as CHIKV was shown

306 to be up to 12% between household members [100].

307 To date, highly potent drugs are only available for the treatment of a limited number of viruses

308 such as HIV, HBV, HCV, influenza and herpes viruses. However, with sufficient time and effort,

309 it should also be possible to develop safe and potent antivirals for the treatment and/or

310   prophylaxis of arthritogenic alphavirus infections. Potential targets for development of broad-

311   spectrum antivirals for arthritogenic alphaviruses may for example be the viral nsP1 (capping

312   machinery), nsP2 (protease) and nsP4 (viral polymerase) proteins. Another potential target is

313   Mxra8 which functions as an entry receptor for several arthritogenic alphaviruses [26].

314   Designing molecules that can block this receptor may be therefore a helpful strategy to control

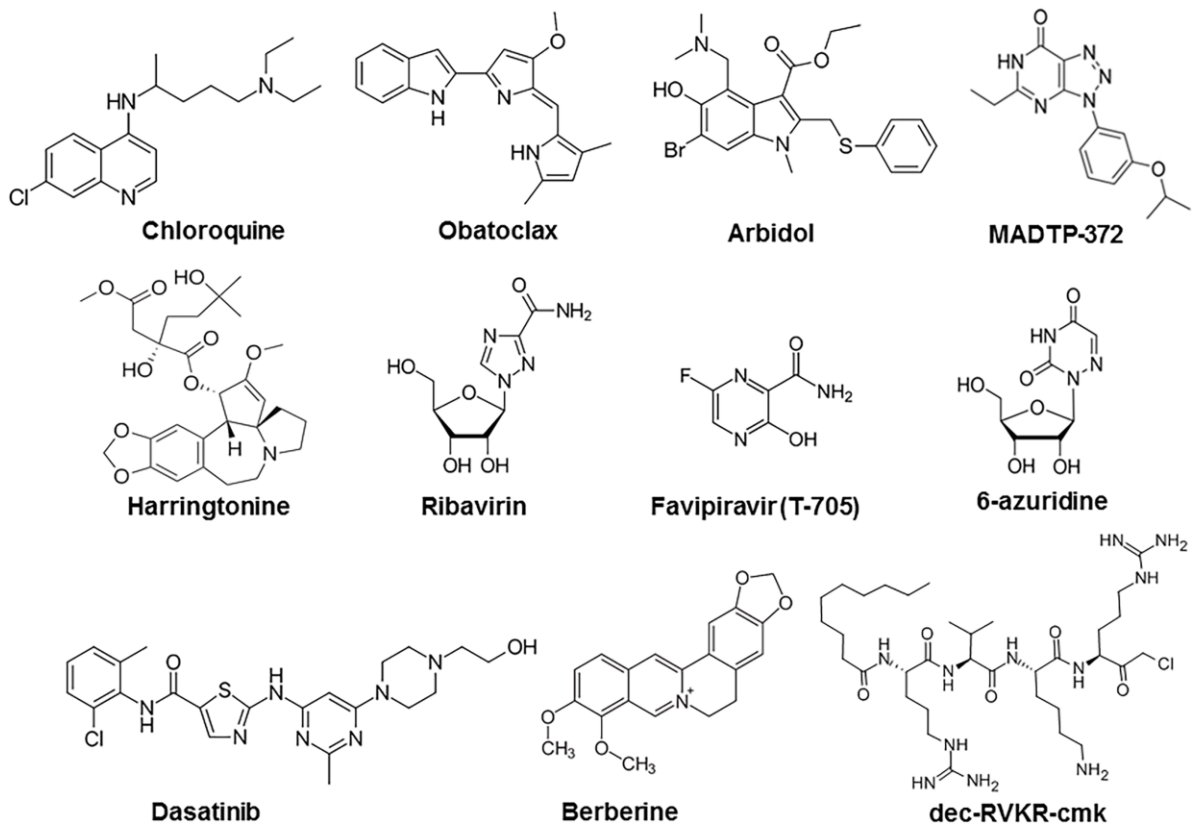315   arthritogenic alphavirus infections.

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320 **Conflict of interest**

321 None to declare.
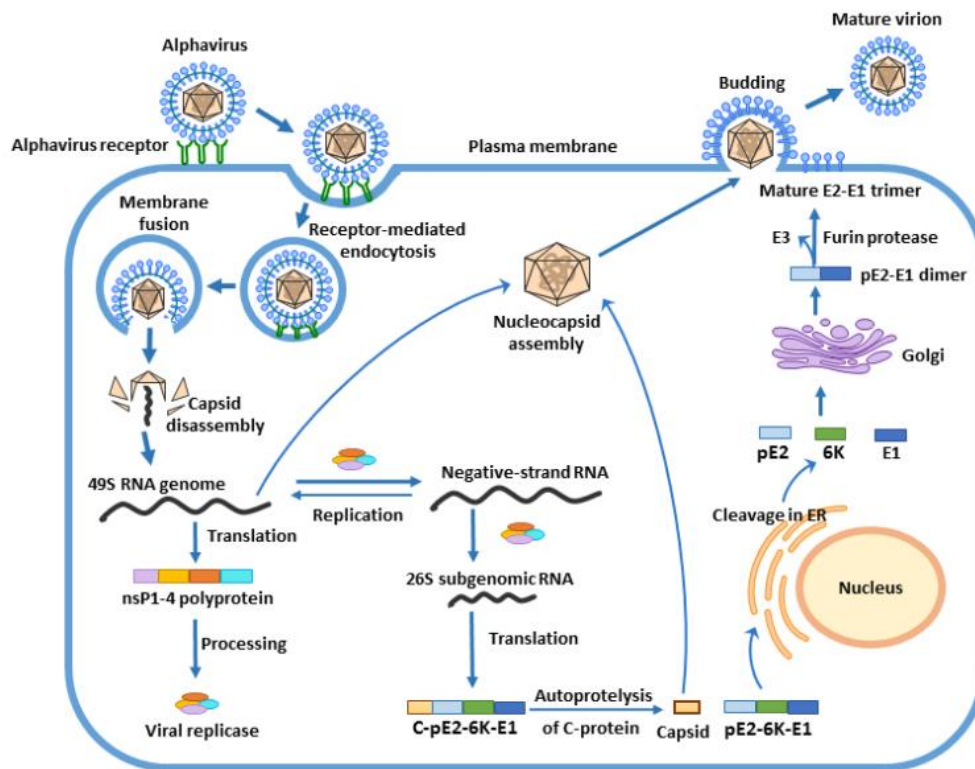
FIGURES

Figure 1. Structural formulae of molecules inhibiting arthritogenic alphaviruses.

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Figure 2. Schematic representation of the replication cycle of alphaviruses. Alphavirus enters the cell by endocytosis following the binding of E2 protein to specific receptor(s) on the cell surface. Within the endosome, the low pH triggers the fusion of the viral envelope with the endosomal membrane leading to the release of the nucleocapsid into the cytoplasm. The nucleocapsid disassembles to liberate the viral genome which is being translated to produce the viral nonstructural proteins (nsP1–4). After processing, the viral nonstructural proteins complex to form the viral replicase which catalyzes the synthesis of a negative-sense RNA strand to serve as a template for synthesis of both the full-length positive-sense genome and the subgenomic (26S) RNA. The subgenomic (26S) RNA is being translated to produce the structural polyprotein (C-E3-E2-6K-E1). The structural polyprotein is then cleaved to produce the individual structural proteins followed by assembly of the viral components. The assembled virus particle is released by budding out through the plasma membrane where it acquires the envelope with embedded viral glycoproteins. The figure was adapted from [14].

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7. References

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