Development of small-molecule viral inhibitors targeting various stages of the life cycle of emerging and re-emerging viruses

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Abstract   **In recent years, unexpected outbreaks of infectious diseases caused by emerging and re-emerging viruses have become more frequent, which is possibly due to environmental changes. These outbreaks result in the loss of life and economic hardship. Vaccines and therapeutics should be developed for the prevention and treatment of infectious diseases. In this review, we summarize and discuss the latest progress in the development of small-molecule viral inhibitors against highly pathogenic coronaviruses, including severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus, Ebola virus, and Zika virus. These viruses can interfere with the specific steps of viral life cycle by blocking the binding between virus and host cells, disrupting viral endocytosis, disturbing membrane fusion, and interrupting viral RNA replication and translation, thereby demonstrating potent therapeutic effect against various emerging and re-emerging viruses. We also discuss some general strategies for developing small-molecule viral inhibitors.**

Keywords   emerging and re-emerging viruses; small-molecule inhibitor; coronavirus; Ebola virus; Zika virus; life cycle

Introduction

In recent years, increasing outbreaks of emerging and re-emerging virus diseases have threatened human public health and economic stability worldwide. These epidemics are possibly caused by anthropogenic, social, and behavioral changes [1].

In the late 2002 and early 2003, many patients with acute respiratory disease symptoms in Guangdong Province of China were infected with a new coronavirus called severe acute respiratory syndrome coronavirus (SARS-CoV) [2]. This virus spread rapidly in over 27 countries and causes over 8000 cases of SARS, which results in nearly 800 deaths (~10% case fatality rate). In 2012, another new coronavirus, named Middle East respiratory syndrome coronavirus (MERS-CoV), also spread throughout 27 countries, which results in 2029 confirmed cases and 704 deaths (~35% case fatality rate) [3–5]. In 2014–2016, the latest outbreak of Ebola virus (EBOV) disease (EVD)

occurred in West Africa, with 28 646 EBOV-infected cases and 11 323 EVD-related deaths (case fatality rate of approximately 50%) [6]. Finally, starting from 2015, Zika virus (ZIKV) initially causes a local outbreak in Brazil and quickly spread to 84 countries and areas in Africa, the United States, Asia, and the Pacific Rim [7]. ZIKV infection can harm the human nervous system and male reproductive system [8–10], and it may cause the development of microcephaly in fetuses of ZIKV-infected pregnant women [11–13].

With increasing globalization, many emerging and re-emerging viral infectious diseases have been reported worldwide, thereby highlighting the importance of developing effective vaccines and therapeutics for the prevention and treatment of these infectious diseases.

Most antiviral drugs are small-molecule viral inhibitors targeting various stages of the viral life cycle [14]. For example, anti-HIV drugs inhibit viral infection by targeting viral proteins functioning at different stages of HIV replication, such as surface glycoprotein (GP), reverse transcriptase, integrase, and protease. Small-molecule viral inhibitors can be produced on a large scale and applied to considerable populations with lower cost than that of antibody-based drugs. Their high thermostability makes

them easy to store and transport when used in tropical and subtropical areas. These inhibitors can also be taken orally, which is the most acceptable administration route for small-molecule viral inhibitors.

This review summarizes the advances in research and development of small-molecule viral inhibitors against emerging and re-emerging viruses, including SARS-CoV, MERS-CoV, EBOV, and ZIKV.

Research and development of small-molecule viral inhibitors against emerging and re-emerging viruses

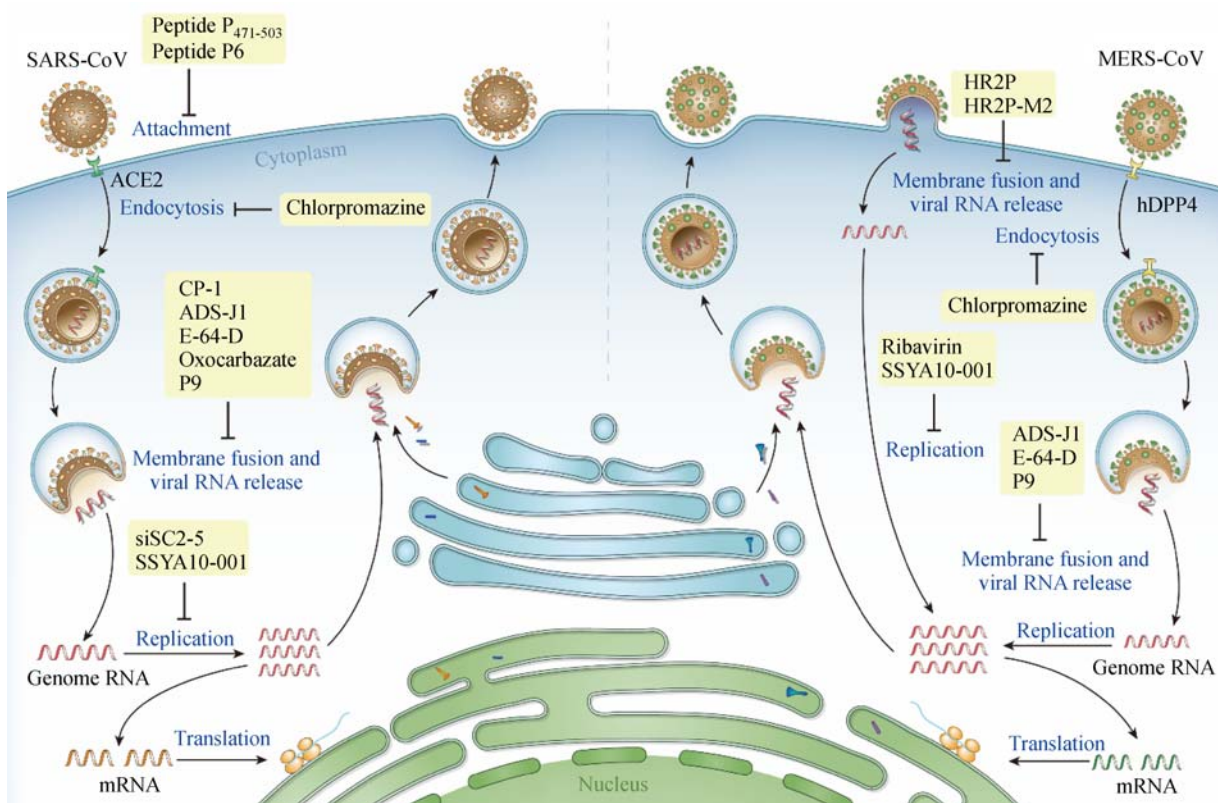## Small-molecule viral inhibitors against SARS-CoV and MERS-CoV

Both SARS-CoV and MERS-CoV belong to coronaviruses, which are enveloped viruses consisting of single-stranded positive RNA that encodes nonstructural and structural proteins, including spike (S), envelope (E), membrane, and nucleocapsid proteins [15].

SARS-CoV attaches to the target cell through binding between the receptor-binding domain (RBD) in the S1 subunit of S protein and the cellular receptor angiotensin-converting enzyme 2 (ACE2) on the target cell [16]. This

coronavirus enters the target cell mainly via the endosomal pathway [17]. After endocytosis, S protein changes conformation under acidic environment, which results in the formation of a six-helix bundle (6-HB) fusion core [18,19]. Afterward, the viral genome RNA is released through the fusion pore into the cytoplasm for replication [20]. Finally, the progeny virions are released by exocytosis. MERS-CoV binds to the target cell via interaction of its RBD with its cellular receptor, namely, dipeptidyl peptidase 4 (hDPP4) [21], and subsequently enters the cell mainly through plasma membrane fusion [22]. The life cycles of SARS-CoV and MERS-CoV are shown in Fig. 1.

A number of small-molecule viral inhibitors targeting different stages of the coronavirus life cycle, including both SARS-CoV and MERS-CoV, were reported [23]. The first group of inhibitors consists of those with the ability to block the attachment of the virus to host cells. The RBD in the S1 subunit of S protein plays critical roles in the viral entry stage based on its specific binding with host receptors. Peptides, which overlap with either the SARS-CoV RBD region (termed as $S_{471-503}$ peptide) [24] or its binding motifs of ACE2 (termed peptide P6), inhibit the entry of SARS-CoV into Vero cells and that of pseudo-typed virus into ACE2-expressing HeLa cells [25]. The second group of inhibitors consists of those that can disrupt

Fig. 1 Schematic diagram of the life cycle of coronaviruses (SARS-CoV and MERS-CoV). Small-molecule viral inhibitors are classified into specific groups according to their different mechanisms of action. ACE2, angiotensin-converting enzyme 2; hDPP4, human dipeptidyl peptidase 4.

viral endocytosis. Chlorpromazine, an inhibitor of endocytosis, was identified as a suppressor of MERS-CoV infection at micromolar concentration through the screening of 348 approved compounds [26]. The third group of inhibitors consists of those with the capacity to interrupt membrane fusion. Tetra-*O*-galloyl-β-D-glucose blocks SARS-CoV infection by binding with the S2 domain of S protein, which suggests that it possibly hinders virus–cell fusion [27]. The peptides overlapping the heptad repeat 2 (HR2) domain in S2 domain of S protein inhibit coronavirus infection at a micromolar level through interrupting the formation of a 6-HB. For instance, CP-1 blocks SARS-CoV entry into Vero E6 cells [28], and HR2P inhibits MERS-CoV infection efficiently [18]. Intranasal application of HR2P-M2, the analog of HR2P, but with improved solubility and stability, could significantly reduce the titers of MERS-CoV in the lung of Ad5-hDPP4-transduced mice [29,30]. Peptide P9, which is derived from mouse β-defensin-4, could enter into cells, along with virions, to prevent endosomal acidification, thereby obstructing the membrane fusion of SARS-CoV and MERS-CoV [31]. ADS-J1 penetrates into the deep pocket of HR1 to interfere with interactions between HR1 and HR2 of coronavirus by hydrophobic force and consequently inhibit the entry of pseudotyped SARS-CoV and MERS-CoV [32,33]. Given that cathepsin L (CatL) facilitates the conformational changes of S protein in endosomes with low pH, CatL inhibitors, such as oxocarbazate and E-64-D, are effective in inhibiting coronavirus infection [34–36]. The fourth group of inhibitors includes those that can interrupt viral RNA replication and translation. Small interfering RNA (siRNA), which aims to silence the leader sequence of SARS-CoV, reduces the mRNA abundance and consequently suppresses viral replication in Vero E6 cells [37]. Furthermore, siSC2–5, which is siRNA duplexes directed against both S protein-coding and the ORF1b region of SARS-CoV, could reduce viral copies in the respiratory tract and relieve the symptoms of SARS-CoV-infected rhesus macaques [38]. Ribavirin, a nucleoside analog, can suppress MERS-CoV infection *in vitro* [39]. SSYA10-001, the helicase nsp13 inhibitor, blocks the replication of SARS-CoV and MERS-CoV [40]. Some coronavirus protease inhibitors, such as the compound 5c, can also suppress viral replication [41,42]. The fifth group of inhibitors includes those with undefined mechanism of action. According to high-throughput screening of FDA drug libraries, some clinically used drugs, including estrogen receptor inhibitors (tamoxifen citrate and toremifene citrate) and DNA metabolism inhibitor (gemcitabine hydrochloride), display significant antiviral effects with undefined mechanism [36]. Further studies on their mechanisms of action against SARS-CoV and MERS-CoV infection and potential repurposing using the described approaches are warranted. Inhibitors of SARS-CoV and MERS-CoV are shown in Table 1 and Fig. 1.

Small-molecule viral inhibitors against EBOV

EBOV is a negative-sense single-stranded enveloped RNA virus with approximately 19 kb genome, which encodes seven structural proteins, including two GPs, four virion proteins (VPs), one nucleoprotein (NP), and one non-structural protein, namely, RNA-dependent RNA polymerase (RdRp) (L protein) [43]. Similar to other filoviruses, EBOV is a viral pathogen causing hemorrhagic fever and other EVDs with high mortality [44]. The recent epidemics of EVD in West Africa have claimed many lives, thereby highlighting the importance of developing anti-EBOV therapeutics.

The entry of EBOV into the host cell, which is the first critical step in its life cycle, is initiated by the interaction between viral surface protein (GP1) and receptors on the host cell, such as T cell immunoglobulin and mucin domain 1 (TIM-1). When this virus attaches to the cell surface, it will be internalized into endosome with enclosed acidic environment [45]. Within this acidic compartment, GP1 binds to Niemann–Pick C1 (NPC1), which is cleaved by the proteases CatL and CatB, and triggers membrane fusion. Afterward, the viral RNA genome is released into the cytoplasm of host cell for replication [46]. The host's metabolic pathway is utilized for viral replication and transcription, where viral genome and NP, VP35, VP30, and L protein participate [47–49]. Final processing of assembly and budding allow newly infectious virions to invade the neighboring cell. These life cycle steps are attractive targets for the development of therapeutic agents against EBOV infection (Fig. 2).

A number of small-molecule viral inhibitor-based anti-EBOV drug candidates targeting different stages of the viral life cycle are under preclinical and clinical development [50,51]. First, inhibitors can disrupt viral endocytosis. Macropinocytosis is the primary endocytic pathway for internalizing EBOV, demanding equilibria of diverse ions both inside and outside the cell. Amiloride and its derivatives, such as 5-(N-ethyl-N-isopropyl) amiloride, can disturb such balance to inhibit the entry of EBOV into host cells [52,53]. Second, inhibitors can also disturb membrane fusion. LJ001, a broad-spectrum antiviral compound against enveloped viruses, restricts the entry of EBOV by intercalating into viral membranes to disrupt the critical step of membrane fusion [54]. Moreover, modified anti-EBOV peptides (e.g., Tat-Ebo), which consists of residues 610-633 of EBOV GP2 and the arginine-rich sequence from HIV-1 Tat spaced by a Gly-Ser-Gly linker, can accumulate in endosome and block 6-HB formation [55]. Oxocarbazate, the inhibitor of CatL, which is responsible for processing GP, blocks pseudo-

Table 1 Small-molecule viral inhibitors against SARS-CoV and/or MERS-CoV

| Virus	Inhibitor	Testing model	Efficacy (IC50)	Ref.
Inhibitors blocking the binding between virus and host cells				
SARS-CoV	Peptide $S_{471-503}$	*In vitro*	41.6 μmol/L	[24]
	Peptide P6	*In vitro*	100 nmol/L	[25]
Inhibitors disrupting endocytosis				
SARS-CoV MERS-CoV	Chlorpromazine	*In vitro*	8.8 μmol/L; 4.9 μmol/L	[26]
Inhibitors disturbing membrane fusion				
SARS-CoV	CP-1	*In vitro*	19 μmol/L	[28]
	Oxocarbazate	*In vitro*	273 nmol/L	[35]
	Tetra-O-galloyl-β-D-glucose	*In vitro*	4.5 μmol/L	[27]
MERS-CoV	HR2P	*In vitro*:		[18]
		293T cells	0.8 μmol/L	
		Vero cells	0.6 μmol/L	
		Calu-3 cells	0.6 μmol/L	
		HFL cells	13.9 μmol/L	
	HR2P-M2	*In vitro*	0.55 μmol/L	[29,30]
		In vivo Ad5-hDPP4-transduced mouse	Decreasing viral titer in lung tissue	
SARS-CoV	P9	*In vitro*	5 μg/mL	[31]
MERS-CoV	ADS-J1	*In vitro*	0.6 and 3.89 μmol/L	[32,33]
	E-64-D	*In vitro*	0.76 and 1.28 μmol/L	[36]
Inhibitors interrupting viral RNA replication and translation				
SARS-CoV	siSC2-5	*In vivo* NHP[a]	Reducing viral copies in respiratory tract	[38]
	Compound 5c	*In vitro*	0.35 μmol/L	[41,42]
MERS-CoV	Ribavirin	*In vitro*	9.99 μg/mL	[39]
SARS-CoV MERS-CoV	SSYA10-001	*In vitro*	7 μmol/L; 25 μmol/L	[40]
Inhibitors with undefined mechanism				
SRAS-CoV MERS-CoV	Tamoxifen citrate	*In vitro*	10.12 and 92.89 μmol/L	[36]
	Toremifene citrate	*In vitro*	11.97 and 12.92 μmol/L	
	Gemcitabine hydrochloride	*In vitro*	1.2 and 4.9 μmol/L	

[a]Nonhuman primate.

typed EBOV from infecting 293T cells [35]. Some viral inhibitors discovered through high-throughput screening, such as compound 7, can bind to a hydrophobic pocket in GP of EBOV [56]. Seventeen cationic amphiphilic drugs identified from FDA-approved drug libraries show considerably potent anti-EBOV activity by targeting NPC1 [57]. For example, bepridil and sertraline could block the membrane fusion step and protect C57BL/6 mice against EBOV infection [58]. Third, inhibitors can interrupt viral RNA replication and translation. Favipiravir (T-705), a broad-spectrum inhibitor of RNA polymerase, suppresses EBOV replication in Vero E6 cells and protects type I interferon receptor-deficient mice from EBOV infection [59,60]. The adenosine analog BCX4430 could suppress infections and confer protection in a rodent model against EBOV and other filoviruses [61]. Atovaquone and azacitidine, which disrupt the biosynthesis of pyrimidine, and mycophenolate mofetil, which deletes the guanosine triphosphate pool, could all inhibit EBOV infection [58,62–64]. In addition, siRNA acts as a kind of inhibitor that can hinder mRNA translation. AVI-6002, a mixture of phosphorodiamidate morpholino oligomers (PMOs), and TKM-Ebola interfere with VP24 and VP35 mRNA to suppress infection [65,66]. Fourth, some inhibitors exhibit undefined antiviral mechanisms. Strophanthin, which is typically used for heart diseases, displays anti-EBOV effect in drug screens [58]. These anti-EBOV drugs acting on different stages of viral life cycle are shown in Table 2 and Fig. 2.

Small-molecule viral inhibitors against ZIKV

ZIKV, a mosquito-borne flavivirus, is a single-stranded positive RNA virus with approximately 10 kb genome, which contains an open reading frame that encodes three structural proteins and seven nonstructural proteins [67].
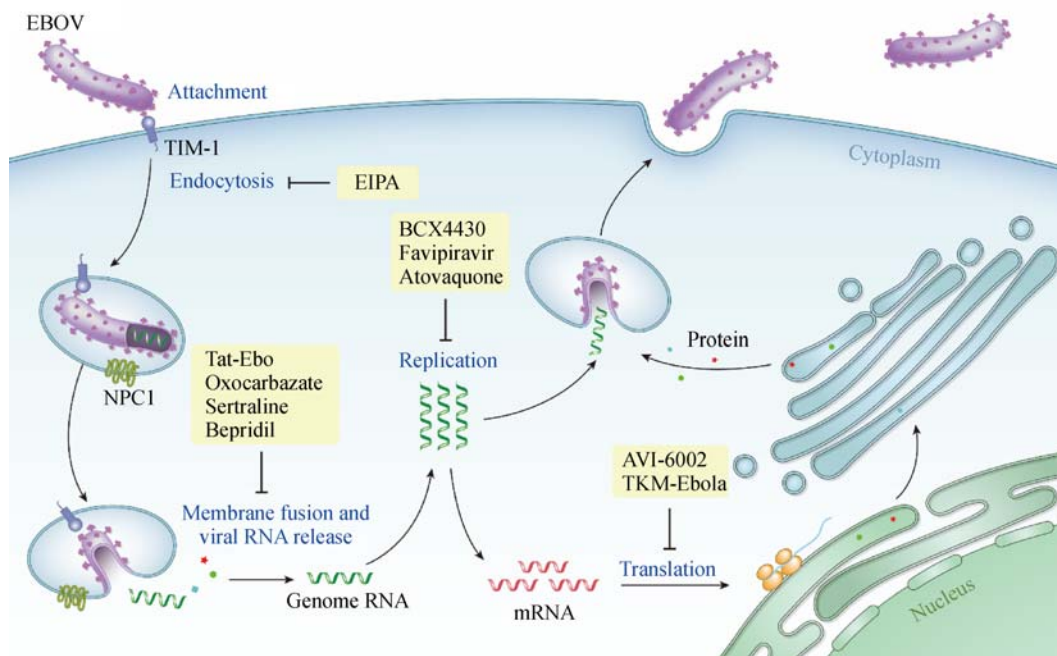
Fig. 2 Schematic diagram of the life cycle of Ebola virus. Small-molecule viral inhibitors are classified into specific groups according to their different mechanisms of action. TIM-1, T cell immunoglobulin and mucin domain 1; NPC1, Niemann–Pick C1.

After its isolation from a rhesus macaque, ZIKV is ignored for a long time, until intermittent outbreaks occur in the Pacific islands and the United States. The recent global pandemic that began in Brazil has attracted extensive attention from WHO due to its possible association with neurological complications [8–13]. Despite the progress in targeting the underlying molecular mechanisms of this pathogen, no anti-ZIKV drug has been approved for clinical use to date.

The first step of ZIKV's life cycle is its attachment to the host cell mediated by interaction between the virus and specific receptor on the host cell, such as AXL and its ligand Gas6 [68]. After internalization through clathrin-dependent endocytosis, the virus undergoes uncoating, which is induced by the special acidic environment of the endosome, where the fusion between viral envelope and the endosomal membrane is facilitated by the transformation of viral envelope proteins into a fusion-active state [69]. Subsequently, the viral RNA genome is released into the cytoplasm for replication. The replication complex is formed by viral nonstructural proteins (NS3 and NS5) and probably some host proteins; this complex also assists with the synthesis of the viral genomic RNA [70,71]. The capsid protein, a viral structural protein, combines with the RNA genome to form the nucleocapsid core. Viral assembly occurs in the endoplasmic reticulum where the budding obtains a lipid envelope; the progeny virus is finally released through the exocytotic pathway [69,72]. The life cycle of ZIKV is shown in Fig. 3.

Following the outbreak of ZIKV epidemic, a wide variety of small-molecule viral inhibitors were reported. The first category of inhibitors blocks the binding between virus and host cells. We found that a peptide-based anti-ZIKV inhibitor (Z2), derived from the stem region of E protein, is highly effective in inhibiting ZIKV infection in type I or type I/II interferon receptor-deficient mice; this inhibitor also prevents the vertical transmission of ZIKV from pregnant C57BL/6 mice to their fetuses through its interaction with viral surface envelope (E) proteins to form a membrane pore and disrupt the integrity of the viral membrane [73]. ZINC33683341, a small-molecule inhibitor with preferential binding affinity to ZIKV E protein, can reduce virus titer at the noncytotoxic concentration of 100 μmol/L using an *in vitro* assay [74]. Curcumin inhibits the infection of ZIKV and other enveloped viruses by blocking interactions between virus and host cells [75]. The second category of inhibitors can disrupt the viral endocytosis process. Nanchangmycin, a natural bacterial product, inhibits ZIKV infection *in vitro* through blocking clathrin-mediated endocytosis [76]. The third category of inhibitors can disturb membrane fusion. Chloroquine and niclosamide, anthelmintic medications that are effective against cestodes, inhibit the acidification of endosome and the low pH-dependent conformational changes of E protein that is necessary for membrane fusion. Both chloroquine and niclosamide can block ZIKV infection through *in vitro* experiments [77,78]. Moreover, 25-hydroxycholesterol can inhibit ZIKV infection in both *in vitro* and *in vivo*

Table 2 Small-molecule viral inhibitors against Ebola virus

Inhibitor name	Testing model	Efficacy (IC50)	Ref.
Inhibitors disrupting endocytosis			
5-(N-ethyl-N-isopropyl) amiloride	*In vitro*	<50 μmol/L	[53]
Inhibitors disturbing membrane fusion			
LJ001	*In vivo* BALB/c mouse	Protection rate: 80%	[54]
Tat-Ebo	*In vitro*	<50 μmol/L	[55]
Oxocarbazate	*In vitro*	193 nmol/L	[35]
Compound 7	*In vitro*	10 μmol/L	[56]
Bepridil	*In vitro*:		[58]
	Vero E6 cells	5.08 μmol/L	
	HepG2 cells	3.21 μmol/L	
	In vivo C57BL/6 mouse	Protection rate: 100%	
Sertraline	*In vitro*		
	Vero E6 cells	3.13 μmol/L	
	HepG2 cells	1.44 μmol/L	
	In vivo C57BL/6 mouse	Protection rate: 70%	
Inhibitors interrupting viral RNA replication and translation			
BCX4430	*In vitro*	11.8 μmol/L	[61]
Favipiravir	*In vitro*	67 μmol/L	[60]
	In vivo IFNAR$^{-/-}$ C57BL/6 mouse	Protection rate: 100%	
Atovaquone	*In vitro* Vero E6 cells	0.44 μmol/L	[58,62–64]
Azacitidine	*In vitro*:		
	Vero E6 cells	8.97 μmol/L	
	HepG2 cells	10.3 μmol/L	
Mycophenolate mofetil	*In vitro* HepG2 cells	0.29 μmol/L	
TKM-Ebola	*In vivo* NHP	Protection rate: 66%	[66]
AVI-6002	*In vivo* NHP	Protection rate: 60%	[65]
Inhibitors with undefined mechanism			
Strophanthin	*In vitro*:		[58]
	Vero E6 cells	0.035 μmol/L	
	HepG2 cells	0.021 μmol/L	

assays, especially protecting rhesus monkeys against infection by reducing viremia duration and shortening viral shedding [79]. The fourth category of inhibitors can interrupt viral RNA replication and translation. NS2B and NS3 form a viral protease complex that is essential for ZIKV replication. Ten inhibitors of HCV NS3/NS4A can inhibit ZIKV replication based on the structural similarity between ZIKV NS2B/NS3 and HCV NS3/NS4A [80]. Furthermore, NS3 shows an NTP-dependent RNA helicase domain at the C terminus for unwinding RNA, and NS5 contains domains of methyltransferase and RdRp to assist the replication process, thereby providing attractive targets for designing ZIKV therapies [81,82]. Sofosbuvir and DMB213, inhibitors of ZIKV RdRp, suppress viral replication in Huh7 cells [83]. Recently, temoporfin was demonstrated to inhibit ZIKV infection both *in vitro* and *in*

vivo by disturbing polyprotein processing through blocking the interactions between NS2B and NS3 [84]. Additionally, the polymerase inhibitor 7-deaza-2′-C-methyladenosine inhibits *in vitro* ZIKV replication efficiently and relieves the viremia of infected AG129 mice [85]. Another class of antiviral agents consists of nucleoside analogs that can terminate viral RNA synthesis. The 2′-C-methylated nucleosides and derivatives can inhibit ZIKV replication in cellular assays in a dose-dependent manner [86]. NITD008, an adenosine analog, also protects mice from ZIKV infection [87]. To differentiate between viral translation and RNA synthesis, ZIKV replicon systems were established for the screening and characterization of viral replication inhibitors [88]. The fifth category of inhibitors consists of those without defined mechanism of action. To control ZIKV epidemics,

researchers carried out high-throughput screening of many compounds for ZIKV therapies in multidimension, including inhibitors of ZIKV infection in placental trophoblast cells and neuroprotective agents [76]. Emricasan, an inhibitor of caspase-3 that is essential in the pathogenicity of ZIKV, relieves the neural damage caused by ZIKV [78]. A new mouse model recapitulates the adulthood sequelae of congenital ZIKV infection, which enables the screening and evaluation of small-molecule drugs that repair the impaired nervous system of fetuses or directly suppress viral replication to improve prognosis [89,90]. Potential therapies according to life cycle are shown in Table 3 and Fig. 3.

General strategies for developing small-molecule viral inhibitors

We have reviewed relevant inhibitors against representative viruses in the context of viral life cycle. On the basis of this summary, we can extrapolate some general strategies that may guide further research and development of small-molecule viral inhibitors.

Viral entry into host cell is the first stage for viral infections. Hence, this stage is the most attractive target for designing and developing inhibitors against various viruses [90]. For all of the enveloped viruses with class I fusion protein, such as HIV, SARS-CoV, MERS-CoV, and EBOV, 6-HB formation is required to facilitate the fusion between virus and cell membrane. Small molecules that can block the formation of 6-HB are generally effective in inhibiting the infection of such viruses [91]. Since the first discovery of the potent HIV fusion inhibitory peptide SJ-2176 and clinical application of T20 for treatment of HIV infection [92,93], many viral fusion inhibitory peptides that can block the formation of 6-HB, such as HR2P and HR2P-M2 peptides against MERS-CoV and Tat-Ebo peptide against EBOV [18,30,55], have been reported. Recently, we developed a new tripartite model for designing viral fusion inhibitory peptides with improved efficacy to disturb the formation of 6-HB [94]. This model can be adapted for designing viral inhibitors against other enveloped viruses, including those that may emerge in the future. Another general strategy is to suppress the viral endocytosis pathway utilized by many enveloped viruses. For example, we have reviewed small molecules that can interfere with clathrin-mediated endocytosis and/or caveolin-mediated endocytosis (e.g., chlorpromazine). We have also reviewed the compounds that can prevent the acidification of endosome and consequently inhibit the activities of host proteases in endosome critical for proteolysis and conformational change of viral envelope proteins during fusion between virus and endosome membrane. For example, chloroquine and CatL/CatB inhibitors fall into this category; these inhibitors generally suppress the infection of viruses internalized through endocytosis. The inhibitors against common cellular pathways utilized by different viruses can suppress virus
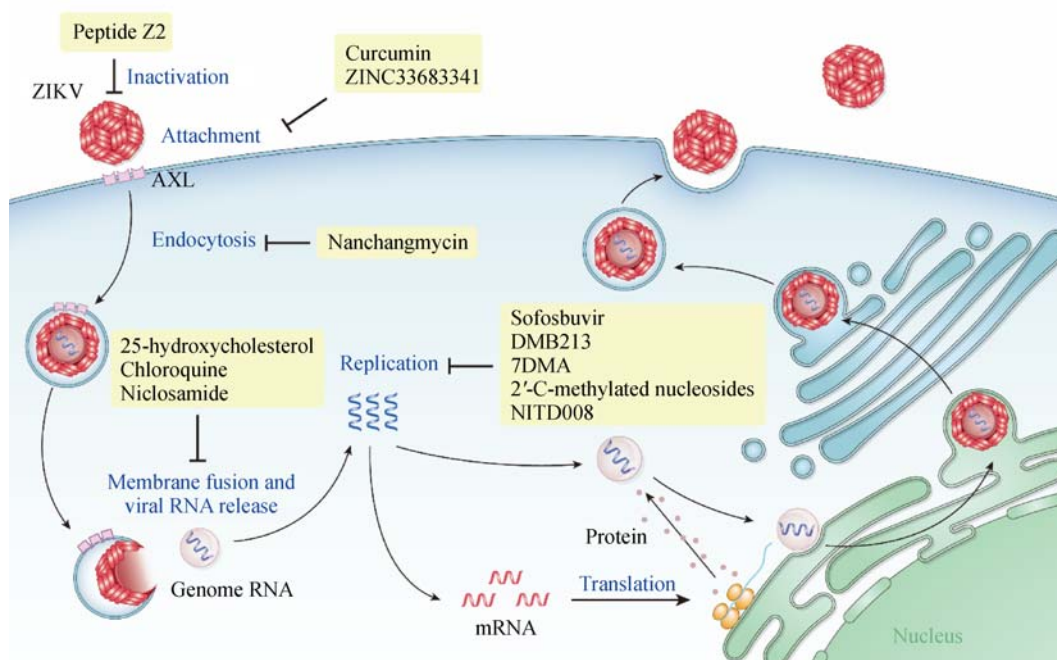
Fig. 3 Schematic diagram of the life cycle of Zika virus. Small-molecule viral inhibitors are classified into specific groups according to their different mechanisms of action.
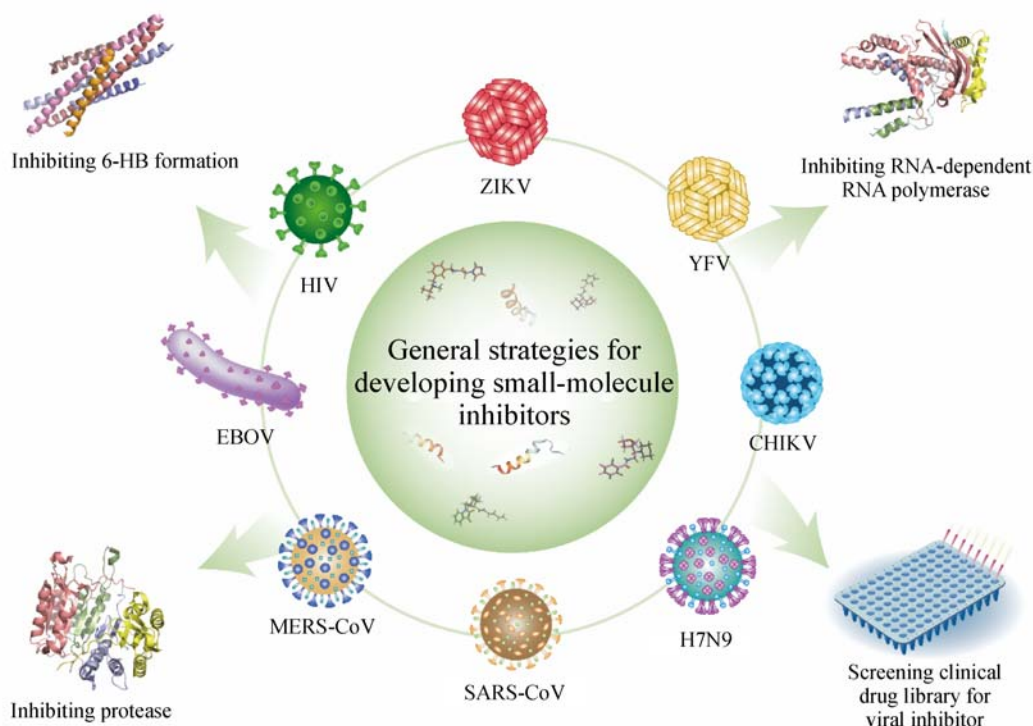
Table 3   Small-molecule viral inhibitors against Zika virus

Inhibitor name	Testing model	Efficacy (IC50)	Ref.
Inhibitors blocking the binding between virus and host cells			
Peptide Z2	*In vitro*:		[73]
	BHK21 cells	1.75 μmol/L	
	Vero cells	3.69 μmol/L	
	In vivo:	Protection rate: 75% and 67%	
	A129 mouse		
	AG6 mouse		
Curcumin	*In vitro*	1.90 μmol/L	[75]
Inhibitors disrupting endocytosis			
Nanchangmycin	*In vitro*:		[76]
	Human HBMECs	0.4 μmol/L	
	Human U2OS Cells	0.1 μmol/L	
Inhibitors disturbing membrane fusion			
Chloroquine	*In vitro*	50 μmol/L	[77]
	In vivo	Inhibiting ZIKV infection in mouse	
	Swiss mouse	neurospheres	
Niclosamide	*In vitro*	0.2 μmol/L	[78]
25-Hydroxycholesterol	*In vitro*	188 nmol/L	[79]
	In vivo:	Reducing viremia and improving	
	A129 mouse	survival	
	NHP	Reducing viremia	
Inhibitors interrupting viral RNA replication and translation			
Compounds 1-10	*In vitro*	<50 μmol/L	[80]
Sofosbuvir	*In vitro*	8.3 μmol/L	[83]
DMB213	*In vitro*	4.6 μmol/L	
Temoporfin	*In vitro*	0.024 μmol/L	[84]
	In vivo		
	BALB/C mouse	Reducing viremia	
	A129 mouse	Protection rate: 83%	
7-Deaza-2′-C-methyladenosine	*In vivo*	Delaying Zika diseases	[85]
	AG129 mouse		
2′-C-methylated nucleosides	*In vitro*	2.7–47.3 μmol/L	[86]
NITD008	*In vitro*	137–241 nmol/L	[87]
	In vivo	Protection rate: 50%	
	A129 mouse		
Inhibitors with undefined mechanism			
Emricasan	*In vitro*:		[78]
	SNB-19 cells	0.87 μmol/L	
	Astrocyte cells	4.11 μmol/L	
	hNPC cells	3.88 μmol/L	
	Ex vivo	Showing neuroprotective activity for	
	3D brain organoids	hNPC cells	

infections with broad spectrum. However, they target host proteins, instead of specific viral proteins, which may raise the concern about side effects because of their nonspecificity. Therefore, further extensive *in vitro* experiments and *in vivo* animal studies should be conducted to evaluate the potential toxicity of drug candidates targeting host proteins. In addition, small molecules, such as nucleoside analogs (e.g., BCX4430, favipiravir, 2′-C-methylated nucleoside, and NITD008), siRNAs (e.g., TKM-Ebola), and PMOs (e.g., AVI-6002), can inhibit the activity of viral RdRp or target viral RNA and interfere with viral RNA replication, transcription, and translation. Consequently, the RNA virus infection is suppressed. Finally, timely, effective therapies are available for emerging and re-emerging viruses through repurposing clinical small-molecule drugs. Taking the recent ZIKV epidemic as an example, both emricasan, a pan-caspase inhibitor, and niclosamide, an anthelmintic drug, can protect against ZIKV infection. These general strategies (Fig. 4) can be adapted for the development of small-molecule viral

Fig. 4 Schematic diagram of general strategies for developing small-molecule viral inhibitors. The strategies include the (1) inhibition of viral entry by blocking the formation of six-helix bundle, (2) inhibition of viral replication by targeting the viral RNA-dependent RNA polymerase, (3) inhibition of viral replication by targeting cellular protease, and (4) screening of clinical drug library for viral entry and replication inhibitors. ZIKV, Zika virus; YFV, yellow fever virus; CHIKV, Chikungunya virus; H7N9, H7N9 influenza A virus; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; EBOV, Ebola virus; HIV, human immunodeficiency virus.

inhibitors against emerging and re-emerging viruses that may cause future pandemics.

Conclusions

Most viruses utilize host cellular components to satisfy various physiological processes, including viral entry, genomic replication, and the assembly and budding of virions, thereby resulting in pathological damage to the host. Therefore, any key stage through the life cycle could be a potential target for developing small-molecule viral inhibitors. Upon the emergence or re-emergence of viral outbreak, researchers use high-throughput screening approaches to determine rapidly effective small-molecule viral inhibitors and pharmacological compounds for clinical treatment. The research and development stages of small molecules are relatively inexpensive. Small-molecule viral inhibitors are also convenient for oral administration. Generally, therapeutic small molecules are superior to other antiviral therapies, such as antibodies. Hence, their use is widespread in both developing and developed countries. The antiviral activities of small-molecule viral inhibitors are typically not as potent as those

of antibodies, and small molecules exhibit shorter half-life *in vivo* than those of antibodies. Their relatively high toxicity also restricts their use, especially in pregnant women and neonates infected with ZIKV. To overcome this problem, repurposing of approved clinically safe drugs in pregnant women is an advisable alternative solution.

We have observed a consistent lag time between the emergence or re-emergence of outbreaks and the development of effective antiviral drugs. In addition, many large pharmaceutical companies are reluctant to develop antiviral drugs against viruses with potential to cause short-term epidemic because of their unpredictable market values. This challenge may be addressed with the development of broad-spectrum, cross-reactive drugs, which may represent an important future trend.

Acknowledgements

Compliance with ethics guidelines

Xiaohuan Wang, Peng Zou, Fan Wu, Lu Lu, and Shibo Jiang declare no conflict of interest. This manuscript is a review article. It does not involve a research protocol requiring approval by relevant institutional review board or ethics committee.

References

1. Pybus OG, Tatem AJ, Lemey P. Virus evolution and transmission in an ever more connected world. Proc Biol Sci 2015; 282(1821): 20142878.

2. Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D, Laman JD, de Jong T, van Doornum G, Lim W, Ling AE, Chan PK, Tam JS, Zambon MC, Gopal R, Drosten C, van der Werf S, Escriou N, Manuguerra JC, Stöhr K, Peiris JS, Osterhaus AD. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 2003; 362(9380): 263–270

3. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012; 367(19): 1814–1820

4. Lu L, Liu Q, Du L, Jiang S. Middle East respiratory syndrome coronavirus (MERS-CoV): challenges in identifying its source and controlling its spread. Microbes Infect 2013; 15(8-9): 625–629

5. World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV). Available from: http://www.who.int/emergencies/mers-cov/en/ (Accessed on June 19, 2017)

6. World Health Organization. Ebola Situation Reports. Available from: http://apps.who.int/ebola/ebola-situation-reports (Accessed on March 27, 2016)

7. World Health Organization. Media centre: Zika virus. Available from: http://www.who.int/mediacentre/factsheets/zika/en/ (Accessed on September 6, 2016)

8. Ma W, Li S, Ma S, Jia L, Zhang F, Zhang Y, Zhang J, Wong G, Zhang S, Lu X, Liu M, Yan J, Li W, Qin C, Han D, Qin C, Wang N, Li X, Gao GF. Zika virus causes testis damage and leads to male infertility in mice. Cell 2016; 167(6): 1511–1524. e1510

9. Li C, Xu D, Ye Q, Hong S, Jiang Y, Liu X, Zhang N, Shi L, Qin CF, Xu Z. Zika virus disrupts neural progenitor development and leads to microcephaly in mice. Cell Stem Cell 2016; 19(1): 120–126

10. Wang A, Thurmond S, Islas L, Hui K, Hai R. Zika virus genome biology and molecular pathogenesis. Emerg Microbes Infect 2017; 6 (3): e13

11. Calvet G, Aguiar RS, Melo ASO, Sampaio SA, de Filippis I, Fabri A, Araujo ESM, de Sequeira PC, de Mendonça MCL, de Oliveira L, Tschoeke DA, Schrago CG, Thompson FL, Brasil P, Dos Santos FB, Nogueira RMR, Tanuri A, de Filippis AMB. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis 2016; 16(6): 653–660

12. Goodfellow FT, Tesla B, Simchick G, Zhao Q, Hodge T, Brindley MA, Stice SL. Zika virus induced mortality and microcephaly in chicken embryos. Stem Cells Dev 2016; 25(22): 1691–1697

13. Schuler-Faccini L, Ribeiro EM, Feitosa IM, Horovitz DD, Cavalcanti DP, Pessoa A, Doriqui MJ, Neri JI, Neto JM, Wanderley HY, Cernach M, El-Husny AS, Pone MV, Serao CL, Sanseverino MT; Brazilian Medical Genetics Society–Zika Embryopathy Task Force. Possible association between Zika virus infection and microcephaly — Brazil, 2015. MMWR Morb Mortal Wkly Rep 2016; 65(3): 59–62

14. De Clercq E, Li G. Approved antiviral drugs over the past 50 years. Clin Microbiol Rev 2016; 29(3): 695–747

15. de Haan CA, Rottier PJ. Molecular interactions in the assembly of coronaviruses. Adv Virus Res 2005; 64: 165–230

16. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003; 426(6965): 450–454

17. Matsuyama S, Ujike M, Morikawa S, Tashiro M, Taguchi F. Protease-mediated enhancement of severe acute respiratory syndrome coronavirus infection. Proc Natl Acad Sci USA 2005; 102 (35): 12543–12547

18. Lu L, Liu Q, Zhu Y, Chan KH, Qin L, Li Y, Wang Q, Chan JF, Du L, Yu F, Ma C, Ye S, Yuen KY, Zhang R, Jiang S. Structure-based discovery of Middle East respiratory syndrome coronavirus fusion inhibitor. Nat Commun 2014; 5: 3067

19. Xia S, Liu Q, Wang Q, Sun Z, Su S, Du L, Ying T, Lu L, Jiang S. Middle East respiratory syndrome coronavirus (MERS-CoV) entry inhibitors targeting spike protein. Virus Res 2014; 194: 200–210

20. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV—a target for vaccine and therapeutic development. Nat Rev Microbiol 2009; 7(3): 226–236

21. Raj VS, Mou H, Smits SL, Dekkers DH, Müller MA, Dijkman R, Muth D, Demmers JA, Zaki A, Fouchier RA, Thiel V, Drosten C, Rottier PJ, Osterhaus AD, Bosch BJ, Haagmans BL. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 2013; 495(7440): 251–254

22. Shirato K, Kawase M, Matsuyama S. Middle East respiratory syndrome coronavirus infection mediated by the transmembrane serine protease TMPRSS2. J Virol 2013; 87(23): 12552–12561

23. Liu Q, Xia S, Sun Z, Wang Q, Du L, Lu L, Jiang S. Testing of Middle East respiratory syndrome coronavirus replication inhibitors for the ability to block viral entry. Antimicrob Agents Chemother 2015; 59(1): 742–744

24. Hu H, Li L, Kao RY, Kou B, Wang Z, Zhang L, Zhang H, Hao Z, Tsui WH, Ni A, Cui L, Fan B, Guo F, Rao S, Jiang C, Li Q, Sun M, He W, Liu G. Screening and identification of linear B-cell epitopes and entry-blocking peptide of severe acute respiratory syndrome (SARS)-associated coronavirus using synthetic overlapping peptide library. J Comb Chem 2005; 7(5): 648–656

25. Han DP, Penn-Nicholson A, Cho MW. Identification of critical determinants on ACE2 for SARS-CoV entry and development of a potent entry inhibitor. Virology 2006; 350(1): 15–25

26. de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC,

van Nieuwkoop S, Bestebroer TM, van den Hoogen BG, Neyts J, Snijder EJ. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. Antimicrob Agents Chemother 2014; 58(8): 4875–4884

27. Yi L, Li Z, Yuan K, Qu X, Chen J, Wang G, Zhang H, Luo H, Zhu L, Jiang P, Chen L, Shen Y, Luo M, Zuo G, Hu J, Duan D, Nie Y, Shi X, Wang W, Han Y, Li T, Liu Y, Ding M, Deng H, Xu X. Small molecules blocking the entry of severe acute respiratory syndrome coronavirus into host cells. J Virol 2004; 78(20): 11334–11339

28. Liu S, Xiao G, Chen Y, He Y, Niu J, Escalante CR, Xiong H, Farmar J, Debnath AK, Tien P, Jiang S. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet 2004; 363(9413): 938–947

29. Channappanavar R, Lu L, Xia S, Du L, Meyerholz DK, Perlman S, Jiang S. Protective effect of intranasal regimens containing peptidic Middle East respiratory syndrome coronavirus fusion inhibitor against MERS-CoV infection. J Infect Dis 2015; 212(12): 1894–1903

30. Lu L, Xia S, Ying T, Jiang S. Urgent development of effective therapeutic and prophylactic agents to control the emerging threat of Middle East respiratory syndrome (MERS). Emerg Microbes Infect 2015; 4(6): e37

31. Zhao H, Zhou J, Zhang K, Chu H, Liu D, Poon VK, Chan CC, Leung HC, Fai N, Lin YP, Zhang AJ, Jin DY, Yuen KY, Zheng BJ. A novel peptide with potent and broad-spectrum antiviral activities against multiple respiratory viruses. Sci Rep 2016; 6(1): 22008

32. Chu LH, Chan SH, Tsai SN, Wang Y, Cheng CH, Wong KB, Waye MM, Ngai SM. Fusion core structure of the severe acute respiratory syndrome coronavirus (SARS-CoV): in search of potent SARS-CoV entry inhibitors. J Cell Biochem 2008; 104(6): 2335–2347

33. Zhao G, Du L, Ma C, Li Y, Li L, Poon VK, Wang L, Yu F, Zheng BJ, Jiang S, Zhou Y. A safe and convenient pseudovirus-based inhibition assay to detect neutralizing antibodies and screen for viral entry inhibitors against the novel human coronavirus MERS-CoV. Virol J 2013; 10(1): 266

34. Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. Proc Natl Acad Sci USA 2005; 102 (33): 11876–11881

35. Shah PP, Wang T, Kaletsky RL, Myers MC, Purvis JE, Jing H, Huryn DM, Greenbaum DC, Smith AB 3rd, Bates P, Diamond SL. A small-molecule oxocarbazate inhibitor of human cathepsin L blocks severe acute respiratory syndrome and ebola pseudotype virus infection into human embryonic kidney 293T cells. Mol Pharmacol 2010; 78(2): 319–324

36. Dyall J, Coleman CM, Hart BJ, Venkataraman T, Holbrook MR, Kindrachuk J, Johnson RF, Olinger GG Jr, Jahrling PB, Laidlaw M, Johansen LM, Lear-Rooney CM, Glass PJ, Hensley LE, Frieman MB. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob Agents Chemother 2014; 58(8): 4885–4893

37. Li T, Zhang Y, Fu L, Yu C, Li X, Li Y, Zhang X, Rong Z, Wang Y, Ning H, Liang R, Chen W, Babiuk LA, Chang Z. siRNA targeting the leader sequence of SARS-CoV inhibits virus replication. Gene Ther 2005; 12(9): 751–761

38. Li BJ, Tang Q, Cheng D, Qin C, Xie FY, Wei Q, Xu J, Liu Y, Zheng BJ, Woodle MC, Zhong N, Lu PY. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat Med 2005; 11(9): 944–951

39. Chan JF, Chan KH, Kao RY, To KK, Zheng BJ, Li CP, Li PT, Dai J, Mok FK, Chen H, Hayden FG, Yuen KY. Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. J Infect 2013; 67(6): 606–616

40. Adedeji AO, Singh K, Kassim A, Coleman CM, Elliott R, Weiss SR, Frieman MB, Sarafianos SG. Evaluation of SSYA10-001 as a replication inhibitor of severe acute respiratory syndrome, mouse hepatitis, and Middle East respiratory syndrome coronaviruses. Antimicrob Agents Chemother 2014; 58(8): 4894–4898

41. Wang H, Xue S, Yang H, Chen C. Recent progress in the discovery of inhibitors targeting coronavirus proteases. Virol Sin 2016; 31(1): 24–30

42. Báez-Santos YM, Barraza SJ, Wilson MW, Agius MP, Mielech AM, Davis NM, Baker SC, Larsen SD, Mesecar AD. X-ray structural and biological evaluation of a series of potent and highly selective inhibitors of human coronavirus papain-like proteases. J Med Chem 2014; 57(6): 2393–2412

43. Hoenen T, Groseth A, Falzarano D, Feldmann H. Ebola virus: unravelling pathogenesis to combat a deadly disease. Trends Mol Med 2006; 12(5): 206–215

44. Mahanty S, Bray M. Pathogenesis of filoviral haemorrhagic fevers. Lancet Infect Dis 2004; 4(8): 487–498

45. Nanbo A, Imai M, Watanabe S, Noda T, Takahashi K, Neumann G, Halfmann P, Kawaoka Y. Ebolavirus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner. PLoS Pathog 2010; 6(9): e1001121

46. Hunt CL, Lennemann NJ, Maury W. Filovirus entry: a novelty in the viral fusion world. Viruses 2012; 4(2): 258–275

47. Noda T, Kolesnikova L, Becker S, Kawaoka Y. The importance of the NP: VP35 ratio in Ebola virus nucleocapsid formation. J Infect Dis 2011; 204(Suppl 3): S878–S883

48. Noda T, Ebihara H, Muramoto Y, Fujii K, Takada A, Sagara H, Kim JH, Kida H, Feldmann H, Kawaoka Y. Assembly and budding of Ebolavirus. PLoS Pathog 2006; 2(9): e99

49. Xu W, Luthra P, Wu C, Batra J, Leung DW, Basler CF, Amarasinghe GK. Ebola virus VP30 and nucleoprotein interactions modulate viral RNA synthesis. Nat Commun 2017; 8: 15576

50. Li H, Yu F, Xia S, Yu Y, Wang Q, Lv M, Wang Y, Jiang S, Lu L. Chemically modified human serum albumin potently blocks entry of Ebola pseudoviruses and viruslike particles. Antimicrob Agents Chemother 2017; 61(4): e02168-16

51. Li H, Ying T, Yu F, Lu L, Jiang S. Development of therapeutics for treatment of Ebola virus infection. Microbes Infect 2015; 17(2): 109–117

52. Kleyman TR, Cragoe EJ Jr. Amiloride and its analogs as tools in the study of ion transport. J Membr Biol 1988; 105(1): 1–21

53. Saeed MF, Kolokoltsov AA, Albrecht T, Davey RA. Cellular entry of Ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes. PLoS Pathog 2010; 6(9): e1001110

54. Wolf MC, Freiberg AN, Zhang T, Akyol-Ataman Z, Grock A, Hong PW, Li J, Watson NF, Fang AQ, Aguilar HC, Porotto M, Honko AN, Damoiseaux R, Miller JP, Woodson SE, Chantasirivisal S,

Fontanes V, Negrete OA, Krogstad P, Dasgupta A, Moscona A, Hensley LE, Whelan SP, Faull KF, Holbrook MR, Jung ME, Lee B. A broad-spectrum antiviral targeting entry of enveloped viruses. Proc Natl Acad Sci USA 2010; 107(7): 3157–3162

55. Miller EH, Harrison JS, Radoshitzky SR, Higgins CD, Chi X, Dong L, Kuhn JH, Bavari S, Lai JR, Chandran K. Inhibition of Ebola virus entry by a C-peptide targeted to endosomes. J Biol Chem 2011; 286 (18): 15854–15861

56. Basu A, Li B, Mills DM, Panchal RG, Cardinale SC, Butler MM, Peet NP, Majgier-Baranowska H, Williams JD, Patel I, Moir DT, Bavari S, Ray R, Farzan MR, Rong L, Bowlin TL. Identification of a small-molecule entry inhibitor for filoviruses. J Virol 2011; 85(7): 3106–3119

57. Shoemaker CJ, Schornberg KL, Delos SE, Scully C, Pajouhesh H, Olinger GG, Johansen LM, White JM. Multiple cationic amphiphiles induce a Niemann-Pick C phenotype and inhibit Ebola virus entry and infection. PLoS One 2013; 8(2): e56265

58. Johansen LM, DeWald LE, Shoemaker CJ, Hoffstrom BG, Lear-Rooney CM, Stossel A, Nelson E, Delos SE, Simmons JA, Grenier JM, Pierce LT, Pajouhesh H, Lehár J, Hensley LE, Glass PJ, White JM, Olinger GG. A screen of approved drugs and molecular probes identifies therapeutics with anti-Ebola virus activity. Sci Transl Med 2015; 7(290): 290ra89

59. Madelain V, Oestereich L, Graw F, Nguyen TH, de Lamballerie X, Mentré F, Günnther S, Guedj J. Ebola virus dynamics in mice treated with favipiravir. Antiviral Res 2015; 123: 70–77

60. Oestereich L, Lüdtke A, Wurr S, Rieger T, Muñoz-Fontela C, Günther S. Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. Antiviral Res 2014; 105: 17–21

61. Warren TK, Wells J, Panchal RG, Stuthman KS, Garza NL, Van Tongeren SA, Dong L, Retterer CJ, Eaton BP, Pegoraro G, Honnold S, Bantia S, Kotian P, Chen X, Taubenheim BR, Welch LS, Minning DM, Babu YS, Sheridan WP, Bavari S. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature 2014; 508(7496): 402–405

62. Knecht W, Henseling J, Löffler M. Kinetics of inhibition of human and rat dihydroorotate dehydrogenase by atovaquone, lawsone derivatives, brequinar sodium and polyporic acid. Chem Biol Interact 2000; 124(1): 61–76

63. Leone G, Voso MT, Teofili L, Lübbert M. Inhibitors of DNA methylation in the treatment of hematological malignancies and MDS. Clin Immunol 2003; 109(1): 89–102

64. Khan M, Dhanwani R, Patro IK, Rao PV, Parida MM. Cellular IMPDH enzyme activity is a potential target for the inhibition of Chikungunya virus replication and virus induced apoptosis in cultured mammalian cells. Antiviral Res 2011; 89(1): 1–8

65. Warren TK, Warfield KL, Wells J, Swenson DL, Donner KS, Van Tongeren SA, Garza NL, Dong L, Mourich DV, Crumley S, Nichols DK, Iversen PL, Bavari S. Advanced antisense therapies for postexposure protection against lethal filovirus infections. Nat Med 2010; 16(9): 991–994

66. Geisbert TW, Lee AC, Robbins M, Geisbert JB, Honko AN, Sood V, Johnson JC, de Jong S, Tavakoli I, Judge A, Hensley LE, Maclachlan I. Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study. Lancet 2010; 375(9729): 1896–1905

67. Wang Z, Wang P, An J. Zika virus and Zika fever. Virol Sin 2016; 31 (2): 103–109

68. Meertens L, Labeau A, Dejarnac O, Cipriani S, Sinigaglia L, Bonnet-Madin L, Le Charpentier T, Hafirassou ML, Zamborlini A, Cao-Lormeau VM, Coulpier M, Missé D, Jouvenet N, Tabibiazar R, Gressens P, Schwartz O, Amara A. Axl mediates ZIKA virus entry in human glial cells and modulates innate immune responses. Cell Reports 2017; 18(2): 324–333

69. Stiasny K, Heinz FX. Flavivirus membrane fusion. J Gen Virol 2006; 87(Pt 10): 2755–2766

70. Yi Z, Yuan Z, Rice CM, MacDonald MR. Flavivirus replication complex assembly revealed by DNAJC14 functional mapping. J Virol 2012; 86(21): 11815–11832

71. Zhao B, Yi G, Du F, Chuang YC, Vaughan RC, Sankaran B, Kao CC, Li P. Structure and function of the Zika virus full-length NS5 protein. Nat Commun 2017; 8: 14762

72. Mukhopadhyay S, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. Nat Rev Microbiol 2005; 3 (1): 13–22

73. Yu Y, Deng YQ, Zou P, Wang Q, Dai Y, Yu F, Du L, Zhang NN, Tian M, Hao JN, Meng Y, Li Y, Zhou X, Fuk-Woo Chan J, Yuen KY, Qin CF, Jiang S, Lu L. A peptide-based viral inactivator inhibits Zika virus infection in pregnant mice and fetuses. Nat Commun 2017; 8: 15672

74. Fernando S, Fernando T, Stefanik M, Eyer L, Ruzek D. An approach for Zika virus inhibition using homology structure of the envelope protein. Mol Biotechnol 2016; 58(12): 801–806

75. Mounce BC, Cesaro T, Carrau L, Vallet T, Vignuzzi M. Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. Antiviral Res 2017; 142: 148–157

76. Rausch K, Hackett BA, Weinbren NL, Reeder SM, Sadovsky Y, Hunter CA, Schultz DC, Coyne CB, Cherry S. Screening bioactives reveals Nanchangmycin as a broad spectrum antiviral active against Zika virus. Cell Reports 2017; 18(3): 804–815

77. Delvecchio R, Higa LM, Pezzuto P, Valadão AL, Garcez PP, Monteiro FL, Loiola EC, Dias AA, Silva FJ, Aliota MT, Caine EA, Osorio JE, Bellio M, O'Connor DH, Rehen S, de Aguiar RS, Savarino A, Campanati L, Tanuri A. Chloroquine, an endocytosis blocking agent, inhibits Zika virus infection in different cell models. Viruses 2016; 8(12): E322

78. Xu M, Lee EM, Wen Z, Cheng Y, Huang WK, Qian X, Tcw J, Kouznetsova J, Ogden SC, Hammack C, Jacob F, Nguyen HN, Itkin M, Hanna C, Shinn P, Allen C, Michael SG, Simeonov A, Huang W, Christian KM, Goate A, Brennand KJ, Huang R, Xia M, Ming GL, Zheng W, Song H, Tang H. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat Med 2016; 22(10): 1101–1107

79. Li C, Deng YQ, Wang S, Ma F, Aliyari R, Huang XY, Zhang NN, Watanabe M, Dong HL, Liu P, Li XF, Ye Q, Tian M, Hong S, Fan J, Zhao H, Li L, Vishlaghi N, Buth JE, Au C, Liu Y, Lu N, Du P, Qin FX, Zhang B, Gong D, Dai X, Sun R, Novitch BG, Xu Z, Qin CF, Cheng G. 25-Hydroxycholesterol protects host against Zika virus infection and its associated microcephaly in a mouse model. Immunity 2017; 46(3): 446–456

80. Lee H, Ren J, Nocadello S, Rice AJ, Ojeda I, Light S, Minasov G, Vargas J, Nagarathnam D, Anderson WF, Johnson ME. Identification of novel small molecule inhibitors against NS2B/NS3 serine

protease from Zika virus. Antiviral Res 2017; 139: 49–58

81. Cao X, Li Y, Jin X, Li Y, Guo F, Jin T. Molecular mechanism of divalent-metal-induced activation of NS3 helicase and insights into Zika virus inhibitor design. Nucleic Acids Res 2016; 44(21): 10505–10514

82. Ramharack P, Soliman MES. Zika virus NS5 protein potential inhibitors: an enhanced in silico approach in drug discovery. J Biomol Struct Dyn 2017 Apr 17 [Epub ahead of print] https://doi.org/10.1080/07391102.2017.1313175

83. Xu HT, Hassounah SA, Colby-Germinario SP, Oliveira M, Fogarty C, Quan Y, Han Y, Golubkov O, Ibanescu I, Brenner B, Stranix BR, Wainberg MA. Purification of Zika virus RNA-dependent RNA polymerase and its use to identify small-molecule Zika inhibitors. J Antimicrob Chemother 2017; 72(3): 727–734

84. Li Z, Brecher M, Deng YQ, Zhang J, Sakamuru S, Liu B, Huang R, Koetzner CA, Allen CA, Jones SA, Chen H, Zhang NN, Tian M, Gao F, Lin Q, Banavali N, Zhou J, Boles N, Xia M, Kramer LD, Qin CF, Li H. Existing drugs as broad-spectrum and potent inhibitors for Zika virus by targeting NS2B-NS3 interaction. Cell Res 2017; 27 (8): 1046–1064

85. Zmurko J, Marques RE, Schols D, Verbeken E, Kaptein SJ, Neyts J. The viral polymerase inhibitor 7-deaza-2′-C-methyladenosine is a potent inhibitor of in vitro Zika virus replication and delays disease progression in a robust mouse infection model. PLoS Negl Trop Dis 2016; 10(5): e0004695

86. Hercík K, Kozak J, Šála M, Dejmek M, Hřebabecký H, Zborníková E, Smola M, Ruzek D, Nencka R, Boura E. Adenosine triphosphate analogs can efficiently inhibit the Zika virus RNA-dependent RNA polymerase. Antiviral Res 2017; 137: 131–133

87. Deng YQ, Zhang NN, Li CF, Tian M, Hao JN, Xie XP, Shi PY, Qin CF. Adenosine analog NITD008 is a potent inhibitor of Zika virus. Open Forum Infect Dis 2016; 3(4): ofw175

88. Xie X, Zou J, Shan C, Yang Y, Kum DB, Dallmeier K, Neyts J, Shi PY. Zika virus replicons for drug discovery. EBioMedicine 2016; 12: 156–160

89. Cui L, Zou P, Chen E, Yao H, Zheng H, Wang Q, Zhu JN, Jiang S, Lu L, Zhang J. Visual and motor deficits in grown-up mice with congenital Zika virus infection. EBioMedicine 2017; 20: 193–201

90. Zou J, Shi PY. Adulthood sequelae of congenital Zika virus infection in mice. EBioMedicine 2017; 20: 11–12

91. Lu L, Yu F, Cai L, Debnath AK, Jiang S. Development of small-molecule HIV entry inhibitors specifically targeting gp120 or gp41. Curr Top Med Chem 2016; 16(10): 1074–1090

92. Jiang S, Lin K, Strick N, Neurath AR. HIV-1 inhibition by a peptide. Nature 1993; 365(6442): 113

93. Lalezari JP, Henry K, O'Hearn M, Montaner JS, Piliero PJ, Trottier B, Walmsley S, Cohen C, Kuritzkes DR, Eron JJ Jr, Chung J, DeMasi R, Donatacci L, Drobnes C, Delehanty J, Salgo M; TORO 1 Study Group. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N Engl J Med 2003; 348(22): 2175–2185

94. Su S, Wang Q, Xu W, Yu F, Hua C, Zhu Y, Jiang S, Lu L. A novel HIV-1 gp41 tripartite model for rational design of HIV-1 fusion inhibitors with improved antiviral activity. AIDS 2017; 31(7): 885–894