Small molecule inhibitors of ebola virus infection

Edwige Picazo and Fabrizio Giordanetto

Medicinal Chemistry, Taros Chemicals GmbH & Co. KG, Emil-Figge-Str. 76a, 44227 Dortmund, Germany

Ebola viruses are extremely virulent and highly transmissible. They are responsible for sporadic outbreaks of severe hemorrhagic fevers with human mortality rates of up to 90%. No prophylactic or therapeutic treatments in the form of vaccine, biologicals or small molecule, currently exist. Yet, a wealth of antiviral research on ebola virus is being generated and potential inhibitors have been identified in biological screening and medicinal chemistry programs. Here, we detail the state-of-the-art in small molecule inhibitors of ebola virus infection, with >60 examples, including approved drugs, compounds currently in clinical trials, and more exploratory leads, and summarize the associated in vitro and in vivo evidence for their effectiveness.

Introduction

The Ebolavirus genus is a member of the Filoviridae family of viruses of the Mononegavirales order [1] and includes five species. These have significant differences in terms of virulence and geographical distribution. For instance, Reston Ebolavirus (RESTV) is not pathogenic for humans, whereas Zaire Ebolavirus (EBOV) represents the most pathogenic form for humans, with lethality rates of up to 90% [2]. They are enveloped nonsegmented negative single-stranded RNA viruses, of a filamentous morphology [3].

The approximately 19 kb RNA genome of ebola viruses encodes seven genes that produce a nucleoprotein, three glycoproteins (GP1,2), the membrane-bound surface protein responsible for entry, and soluble and small soluble glycoprotein: sGP and sSGP, respectively), four viral proteins (VP24, VP30, VP35 and VP40) and the viral RNA-dependent RNA polymerase. The matrix protein VP40 drives the formation of virus-like particles (VLP) [4,5] that, owing to GP1,2 exposed on their surfaces, are presented to the host cell. The subsequent virus fusion and entry occur through a complex cascade of micropinocytosis–endocytosis [6–8], endosome trafficking [9,10] and proteolytic activation [11,12] steps, among others. This results in virions being internalized and the viral genome replicated. The virus infection is characterized by massive production of proinflammatory cytokines, severe host immunosuppression and rapid viremia, and often manifests in the form of a fulminant hemorrhagic fever [2,13,14].

The ease of ebola virus transmission from bodily fluids [15], the high virulence and rapid progression of infection, coupled with the high fatality rate, have prompted its classification as a hazard group 4 pathogen by the Advisory Committee on Dangerous Pathogens (ACDP). Despite several therapeutic options, including vaccines [2], monoclonal antibodies [16,17], recombinant proteins [18,19], antibody–interferon (IFN) combinations [20] and small interfering (si)RNA [21] having been developed and tested with success in nonhuman primate models of ebola virus infection, none is currently approved for use in humans. Additionally, because most of these approaches build on virus-specific designs, they are likely to have a limited spectrum of activity. The lack of therapy and the recent cases of ebola virus infection outside the African region have created a high level of public concern, and highlight the need to identify effective therapeutic agents targeting ebola viruses.

A large volume of biomedical research is devoted to the investigation of the molecular basis of ebola virus infection as a way to develop strategies to combat it. Here, we review the body of literature detailing the identification and characterization of small molecules acting as ebola virus infection inhibitors. The compounds identified from a systematic literature survey have been categorized based on their reported mechanism of anti-ebola virus...
action (e.g. inhibition of viral replication) and documented molecular mechanisms (e.g. kinase inhibitor). When mechanistic information on their ebola virus inhibitory activity was not available, small molecules have been organized based on the type of their documented anti-ebola virus activity (e.g. in vitro). For each literature record, the most relevant molecular entities and associated data are described, as summarized in the supplementary material online. A total of 65 compounds belonging to more than 50 chemical classes, including approved drugs, antiviral agents in clinical trials, lead compounds, exploratory chemical probes and screening hits, are discussed.

Viral transcription modulators

T-705 (favipiravir, Fig. 1) was first described by Toyama Chemicals as a selective inhibitor of influenza virus replication with minimal cytotoxicity [22] and is currently in Phase III clinical trials for the treatment of influenza. T-705 closely resembles naturally occurring primary nucleobases (Fig. 1). It was shown to inhibit the viral RNA-dependent RNA polymerase via an active metabolite and to induce a high rate of lethal RNA mutation [23–26]. Oesterlich et al. showed that T-705 was also effective at inhibiting ebola virus replication in vitro without any observed cytotoxicity under the experimental conditions used [27]. When dosed orally twice daily to type I IFN-α/β receptor knockout (IFNAR−/−) mice, T-705 was able to prevent mortality in 100% of the animals. Importantly, T-705 treatment was started 6 days post infection (pi) and resulted in a significant production of ebola virus antibodies, indicating the occurrence of a virus-specific adaptive immune response [27]. Similar results (100% protection) were obtained by Smith et al. when administering oral T-705 to (IFNAR−/−) 129/Sv mice, 1 hour after aerosol ebola virus E718 infection [28].

BCX4430 (Fig. 1) is an adenosine analog rapidly metabolized to its 5′-monophosphate derivative, which in turn acts as a nonobligate RNA chain terminator upon incorporation into viral RNA but not human RNA or DNA [29]. BCX4430 is active in vitro against ebola virus and multiple negative-sense RNA viruses and did not display any significant mutagenicity, as determined by the Ames assay. Its pharmacokinetic profile is reminiscent of that of a nucleotide, with the parent compound being rapidly cleared (Rat t1/2 = 5 min) and the phosphorylated metabolites residing longer (Rat t1/2 = 6.2 hours for the 5′-triphosphate-BCX4430) [29]. Intramuscular or oral, twice-daily administration of BCX4430 to ebola virus-infected C57Bl/6 mice, 4 hours before infection, resulted in 100% and 90% survival, respectively. Further studies evaluating the ebola virus protection by BCX4430 in nonhuman primates are reportedly ongoing [29].

In 1991 and 1995, Huggins et al. first reported on the ability of two 5′-adenosylyhomocysteine (SAH) hydrolase inhibitors, carbocyclic 3′-deazaadenosine (c3Ado) and 3′-deazaneplanocin A (c3Nep) (Fig. 1), to inhibit ebola virus replication in vitro [30,31], confirming their original broad antiviral profile [32]. Twice-daily dosing of c3Ado or c3Nep prolonged survival of SCID mice infected with the Mayinga strain of ebola virus [30,31]. Further studies with c3Ado confirmed these initial results: when c3Ado was administered at day 0 or 1 pi, ebola virus-infected BALB/c mice were protected in a dose-dependent manner, with c3Ado doses ≥0.7 mg/kg/8 hours preventing mortality completely [33]. Survival and virus protection decreased with increased time between infection and start of treatment (90% versus 40% protection when C-c3Ado dosing started at days 2 and 3, respectively) [33]. Similar deterioration of efficacy because of delay in therapy start was obtained when administering c3Nep.
Ye and Schneller recently reported that the two enantiomers of C-1’/C-6’ isonaplanocin (‘D-like’ and ‘L-like’, Fig. 1) effectively inhibited ebola virus replication in vitro [35]. Despite their structural similarity to c3Nep, a difference in their ability to inhibit SAH hydrolase was observed (IC50 = 0.9 and 27 nM, for the D-like and L-like enantiomers, respectively). This difference notwithstanding, the two enantiomers had comparable activity against ebola virus [35]. The postulated link between SAH hydrolase inhibition and its indirect reduction of methylation of the S’ cap of viral mRNA resulting in impaired ebola virus replication inspires further investigation. For instance, c3Nep administration massively increased interferon-α production in ebola virus-infected but not uninfected BALB/c mice [36]. This can reverse the suppression of innate antiviral responses, thus offering an additional mechanism of action for the class of SAH hydrolase inhibitors. Additionally, the role of any phosphorylated metabolites deriving from such nucleoside-like compounds, analogously to metabolic activation pathways for well-established antiviral therapies such as ribavirin [37], together with their pharmacokinetic and pharmacodynamics profiles, needs to be considered [38].

An indoline-based alkaloid-like derivative (CMLDBU3402, Fig. 1) originated from diversity-oriented synthesis was also found to significantly inhibit viral transduction and, thus, ebola virus infection in A549 cells [39].

### Viral entry and fusion modulators

The first phase of ebola virus infection involves fusion of the viral and host cell membranes. Here, proteolysis of the ebola virus membrane glycoprotein (GP1,2) has been shown to represent a necessary step [11]. Proteolytic degradation of ebola virus GP1,2 was blocked in vitro by the unspecific cysteine protease inhibitor E-64d [11] and E-64 [40], the selective cathepsin B (CatB) inhibitors CA-074 [11] and CA-074Me [12], the mixed CatB/L inhibitor FY-DMK [11] and the cathepsin L (CatL) inhibitor Z-FY (t-Bu)-DMK [12] (Fig. 2), resulting in reduced EBOV multiplication [11,12]. Confirming these initial findings, the cysteine and serine protease inhibitor Leupeptin and the CatL inhibitor CID23631927 (Fig. 2) were able to reduce EBOV infection in macrophages [41] and human embryonic kidney 293T cells [42], respectively. Recently, an assay monitoring CatL-based degradation of ebola virus GP1,2-derived peptides identified triazine derivatives 5705213 and 7402683 (Fig. 2) as CatL inhibitors that reduced host cell entry for pseudotyped viruses bearing ebola virus-GP1,2 [43]. It remains to be seen whether protease inhibitors could have utility beyond an in vitro setting, because CatB and CatL activity has been shown not to be required for ebola virus replication in vivo [44].

By using a haploid genetic screen, Carette et al. identified the endo/lysosomal cholesterol transporter protein Niemann–Pick C1 (NPC1) as a key host element required for ebola virus cellular entry [10]. Here, impairment of NPC1 function (NPC1 phenotype) by genetic manipulation resulted in complete resistance to ebola virus infection in vitro and in vivo. Treatment with U18666A [45] and imipramine [46] (Fig. 3), two agents known to induce a NPC1 phenotype, probably via targeting of the NPC1 pathway directly [46] or acid sphingomyelinase (ASMase) inhibition [47], respectively, reduced ebola virus infectivity in vitro. Interestingly, this reduced infectivity was ebola virus specific, because the entry of other viruses was not affected [10]. In line with these findings, a concomitant study by Côté et al. discovered piperazine derivative 3.47 (Fig. 3) as an effective inhibitor of cellular entry by viruses pseudotyped with EBOV-GP1,2 [48] and biochemical experiments revealed that 3.47 inhibited binding of EBOV GP1,2 to NPC1. Furthermore, an affinity-labeling agent based on 3.47 cross-linked directly with NPC1, indicating NPC1 as the target protein [48,49].

Selective estrogen receptor (ER) modulators clomiphene and toremiphene (Fig. 3) showed potent in vitro inhibition of ebola virus infection in a screen of US Food and Drug Administration (FDA)-approved drugs [50]. Dosing of clomiphene and toremiphene to ebola virus-challenged C57Bl/6 mice 1 hour pi (60 mg/kg) yielded survival rates of 90% and 50%, respectively, at day 28 pi. The observed in vitro antiviral effects of clomiphene and toremiphene were independent of ER expression [50] but were affected by NPC1 overexpression [51]. Similarly to U18666A, clomiphene and toremiphene, several cationic amphiphilic small molecules (e.g. Ro 48 8071 and terconazole, Fig. 3) strongly inhibited ebola virus infection in vitro [51]. These compounds induced cholesterol accumulation in endosomes, a typical trait of the NPC1 phenotype, and ebola virus entry inhibition was reduced by NPC1 overexpression. Remarkably and by contrast with what has been observed with 3.47, none of the tested cationic

**FIGURE 2**

Protease inhibitors with reported anti-ebola virus activity.
amphiphiles disrupted binding of ebola virus-GP1,2 to NPC1, indicating yet an additional way of NPC1 pathway interference [51].

A siRNA screening for kinome gene products revealed mitogen-activated protein kinase (MAPK), phosphoinositide 3 kinases (PI3K) and calcium/calmodulin kinases (CAMK2) as cell proteins that are significantly related to EBOV infectivity [52]. Accordingly, the PI3K inhibitor LY294002 and CAMKII inhibitor KN-93 (Fig. 4) effectively reduced ebola virus infection in Vero E6 cells, when tested at 50 mM concentration [52]. Furthermore, a series of pyridinyl imidazole inhibitors of p38 MAPK (e.g. SB203280, p386K III, Fig. 4) inhibited viral entry in dendritic cells [53]. In line with the proinflammatory function of MAPK, dosing of the MAPK inhibitors resulted in much-reduced cytokine and chemokine release upon ebola virus infection, an important feature to minimize ebola virus virulence.

Given that protein phosphorylation regulates several protein–protein interactions that are relevant to endosome formation and endocytosis of viruses, Kolokoltsov et al. tested the broad tyrosine kinase inhibitor genistein and the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor tyrphostin AG1478 (Fig. 4) for their anti-ebola virus activity. Both compounds concentration-dependently inhibited ebola virus infection to host cells and displayed a high degree of antiviral synergy when dosed in combination [54]. Furthermore, the c-Ab1 tyrosine kinase was shown to phosphorylate and activate VP40, a key matrix protein involved in the transport of the viral genome–protein complex to the cell surface and subsequent budding [55]. Consequently, c-Ab1 kinase inhibitors nilotinib and imatinib (Fig. 4) reduced the release of virus-like particles and significantly inhibited EBOV replication in vitro [55]. By contrast, dephosphorylation of VP30, a component of the ebola virus nucleocapsid complex, resulted in sustained ebola virus transcription. Okadaic acid (Fig. 4) inhibits protein phosphatases 1 (PP1) and 2A (PP2A), which together with PP2C, are responsible for VP30 dephosphorylation, and significantly blocked ebola virus growth in vitro [56].

Perturbation of cell signaling processes, as shown in the above protein kinase examples, can affect the complex process of viral entry. Results with the multiple ion channel blockers amiodarone, dronedarone and the L-type calcium channel blocker verapamil (Fig. 5) support this notion. All three agents were shown to inhibit ebola virus GP1,2-mediated cell entry [57]. Interestingly, in the same experiments, T-type calcium and potassium blockers had no effect. Consistently with the viral entry results, amiodarone concentration-dependently reduced EBOV infection in EAh9 cells [57]. In this context, rottlerin (Fig. 5), a potent large conductance potassium channel (BKCa2+) opener, a reported mitochondrial uncoupler [58] and an ambiguous protein kinase C (PKC) inhibitor [59], has also been demonstrated to inhibit ebola virus-like particle entry to MDCK cells [60].

The process of ebola virus entry and fusion to host cells requires dynamic trafficking of viral payloads via endocytosis and, as such, is dependent on a functional cytoskeleton. Here, the microtubule-stabilizer taxol enhanced ebola virus entry, whereas the microtubule-disrupting agents nocodazole and colchicine (Fig. 6) significantly impaired it [61]. Additionally, intact and functional actin filaments are required for ebola virus infectivity, as indicated by the EBPV inhibitory effects of actin-specific reagents cytochalasin B and D, latrunculin A and jasplakinolide [61] (Fig. 6).

The ebola virus GPs represent a key recognition element for viral maturation and are crucial mediators of viral budding. Their structure and function are dependent on their glycosylation profile. For instance, treatment with tunicamycin (Fig. 7) an N-linked glycosylation suppressor, decreased EBOV infection of HeLa cells by >90% [62]. Moreover, a series of imino sugars (IHVR11029, IHVR17028 and IHVR19029; Fig. 7) was recently reported to inhibit endoplasmic reticulum (ER) a-glucosidase I, a deglycosylating enzyme required for proper folding and maturation of nascent proteins. Importantly, the three imino sugars yielded 50–80% survival when administered to EBOV-challenged C57Bl/6 mice 4 hours pi [63].

Mudhasani et al. performed a high content image-based screening for inhibitors of Rift Valley fever virus (RVFV) to HeLa cells [64]. Among the hits that were subsequently screened for additional antiviral activity, G202-0362 (Fig. 8) proved to be the most
potent against ebola virus infection (EC₅₀: 16.1 mM). Interestingly, additional mechanistic studies using RVFV indicated that G202-0362 affected viral budding from the Golgi to the cell surface [64]. Another two compounds found to impair ebola virus budding directly, 4 and 5 (Fig. 8), were determined to inhibit the interaction between the VP40 PPxY late budding domains and the host Nedd4 E3 ubiquitin ligase [65]. Benzodiazepine derivative 7 (Fig. 8) was also shown to inhibit EBOV infection in Vero E6 cells and mechanistic experiments indicated that it directly binds ebola virus GP₁₂ [66], normally required for initiating virus budding and entry.

**Small molecule ebola virus modulators in vitro**

Several different chemotypes have shown to affect ebola virus-related biology and impair ebola virus infection in vitro, as summarized in Fig. 9. These include the heat shock protein 90 (HSP90)

---

**FIGURE 4**
Kinase and phosphatase inhibitors with reported anti-ebola virus activity.

**FIGURE 5**
Ion channel modulators with reported anti-ebola virus activity.
inhibitor **17-AAG** [67], the sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1) inhibitor **ouabain** (probably affecting the function of ebola virus VP24 [68]) the 11beta-hydroxysteroid dehydrogenase inhibitor **glycyrrhizic acid** [69], the specific vacuolar ATPase (V-ATPase) inhibitors **bafilomycin A1** and **concanamycin A**, because of their alkalinizing effect on the endosome [12,61,70] and the nonspecific V-ATPase inhibitor and RAB5A GTPase activator **vacuolin-1** [47,71]. Furthermore, **retinazone** (Fig. 9) was shown to covalently bind glucocorticoid response elements and disrupt EBOV infection [72], whereas isoxazole derivative **8j** (Fig. 9) inhibited EBOV GP1,2-pseudotyped HIV particles entry in 293T cells (IC50 = 2.5 mM) [73]. Lastly, in a drug-repurposing effort, Madrid *et al.* screened 1012 FDA-approved drugs for their antiviral effect against several viral pathogens, including ebola viruses [74]. Among the compounds able to inhibit pseudotype viral entry, **amiodaquin, diphenoxylate, diphenylpyraline and ketotifen** (Fig. 9) also displayed the ability to inhibit ebola virus replication [74].
FIGURE 8
Budding modulators with reported anti-ebola virus activity.

FIGURE 9
Diverse small molecules with reported anti-ebola virus activity in vitro.
Small molecule ebola virus modulators in vivo

Screening of the National Cancer Institute compound library (NCI, Frederick, MD) using an EBOV variant that expresses GFP (GFP-EBOV) in a fluorescence-based high-throughput assay identified FGI-103 (Fig. 10) as an effective EBOV replication inhibitor (EC50: 330 nM) with no overt in vitro cytoxicity at the compound concentration range tested and an in vivo 50% lethal intraperitoneal (ip) dose greater than than 200 mg/kg [75]. Prophylactic administration (1 hour before ebola virus infection) of FGI-103 to C57Bl/6 mice resulted in 100% protection. Therapeutic administration (1–5 days pi) yielded dose- and administration time-dependent protection, with a therapeutically window of less than 2 days. Ongoing studies are aiming at delineating the antiviral mechanism of action of FGI-103 [75]. In 2009, the same research group at Functional Genetics Inc. was issued a patent covering 4-amino-quinoline derivatives (Fig. 10) as methods of inhibiting viral infection [76]. One such derivative, FGI-104 (Fig. 10), an analog of the antimalarial drug amodiaquine (cf. Figs 9 and 10), exhibited broad-spectrum antiviral activity in vitro, including ebola virus, Hepatitis B (HBV) and C (HCV), and Cowpox viruses, among others [77]. In a prophylactic mouse model of ebola virus infection, a 10 mg/kg dose of FGI-104 (2 hours before infection) yielded a 100% survival rate. Mechanistic studies with HCV and HBV material indicated that FGI-104 does not interfere with viral replication [77].

Using a GFP-EBOV-based high-throughput-screening assay, Aman et al. identified a diazachrysene derivative (FGI-106, Fig. 10) with significant in vitro antiviral activity (EBOV EC50: 0.6 mM) and limited cytoxicity in VERO E6 cells (CC50: 10 mM) [78]. When administered ip 1 hour before infection to C57Bl/6 mice, FGI-106 showed a dose-dependent decrease in mortality rate with a 5 mg/kg dose offering 100% survival. FGI-106 also conferred protection when used in a therapeutic setting, although mortality increased with time of first dose [78]. The mechanism of antiviral action of FGI-106 has not been elucidated. However, based on the wide antiviral in vitro profile reported for FGI-106 (inhibiting replication of both negative and positive-strand RNA viruses), the authors speculate that it might target a host factor or pathway that is conservatively used by different viruses for replication [78]. Interestingly, FGI-106 analogs with 3-(morpholin-4-yl)propan-1-amine side chains and 2,8-dimethyl-diazaheyrnene scaffold (FGI-106-a and FGI-106-b, Table S1 in the supplementary material online) also exhibited ebola virus inhibition in vitro [79,80].

Chloroquine (Fig. 10) emerged as one of the best in vitro ebola virus inhibitors following a drug-repurposing screening [74]. Follow-up in vivo studies revealed that chloroquine was the only tested drug able to reduce mortality significantly (90% survival rate at day 13 pi) when dosed at 90 mg/kg 4 hours before infection [74]. Chloroquine has been shown to exert multiple biological actions in cells, notably endosomal trafficking interference, all likely to contribute to its observed antiviral effect [74].

Chemical screening for EBOV inhibitors identified triphenolic derivative NSC62914 (Fig. 10) as an effective inhibitor of EBOV infection in Vero E6 cells [81]. NSC62914 displayed marked antioxidant properties, similar to known reactive oxygen species scavengers. In a prophylaxis study, 2 mg/kg NSC62914 1 hour before infection protected C57Bl/6 from EBOV infection (80% survival rate). The same dose was less effective in a therapeutic model, yielding 50% survival when administered to EBOV-challenged C57Bl/6 mice 1 day pi, with the 5 mg/kg dose decreasing survival even further, possibly because of compound toxicity [81].

Concluding remarks

Despite the wide public concern associated with ebola virus infection, it is comforting to witness the continuous advances in dissecting crucial mechanisms of ebola virus infection and the identification of potential therapeutic targets. Likewise, the promising preclinical results obtained from existing experimental antiviral agents [28] or approved medicaments [50,74] in the small-molecule space seem to indicate opportunities to tackle ebola viruses beyond vaccines [2], biologicals [16–20] and RNA interference [21]. Importantly, the manifest ability of the immune system to counteract ebola virus infection, as from asymptomatic individuals [82] or antiviral treatment [27], represents a crucial resource to harness further. Still, several important challenges need to be resolved. First, the translational potential of the preclinical findings highlighted here needs to be verified. Even ebola virus hemorrhagic fever (EHF) models as advanced as those in nonhuman primates do not completely recapitulate the immunological aspects of ebola virus infection, and there is considerable difference in treatment efficacy across different animal models of ebola virus infection [83]. Here, rodent species are easier to protect from ebola virus infection compared with higher species, particularly humans, and their true predictive power to a clinical situation needs to be assessed. Likewise, the relevance of in vivo treatment results using rodent-adapted ebola virus forms, and that of in vitro data using vesicular stomatis virus particles, needs to be evaluated in greater details. Second, the optimal window of efficacy for any ebola virus therapeutic needs to be defined. All the various vaccines and biologicals, as well as the small molecules described
here, have been administered to animals very close in time to the
given Ebola virus challenge. Although this might still provide
protection benefits for personnel at risk of infection, their prophylactic
and therapeutic potential for wider use remain unclear.
Small molecule-based, oral treatment of Ebola virus infections is
of particular appeal in remote outbreak settings, because the
logistical challenges associated with it are reduced compared
with biologicals or intravenous treatment in general. Effective,
small-molecule antiviral agents have been successfully discovered
and developed for a large range of viruses. Here, nucleoside
derivatives and their ability to impair virus transcription might
offer an important therapeutic option. Alternatively, small
molecules targeting the process of virus entry could yield treat-
ment options that are less susceptible to virus mutations. The
current study represents the first systematic analysis of small
molecules reported to inhibit Ebola virus infection. As the
gathered data were sparse and unstructured, we believe that this
article could serve as a useful survey for researchers, especially
medicinal chemists, embarking on Ebola virus infection projects
to evaluate available chemical matters and anti-Ebola virus
mechanistic hypotheses.

Acknowledgements
The research leading to these results has received support from the
Innovative Medicines Initiative Joint Undertaking under grant
agreement no. 115489, resources of which comprise financial
assistance from the European Union’s Seventh Framework
Programme (FP7/2007–2013) and a EFPIA companies’ in-kind
contribution.

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in
the online version, at http://dx.doi.org/10.1016/j.drudis.2014.12.
010.

References
1 Sanchez, A. et al. (2006) Filoviridae: Marburg and Ebola viruses. In Fields Virol-
yogy (Knipe, D.M. and Howley, P.M., eds), pp. 1409–1448, Lippincott Williams &
Wilkins
849–862
microscopy. Virus Res. 39, 129–150
4 Hardy, R.N. et al. (2000) A PXX motif within the VP40 protein of Ebola virus
interacts physically and functionally with a ubiquitin ligase: implications for
5 Noda, T. et al. (2002) Ebola virus VP40 drives the formation of virus-like filamentous
particles along with GP. J. Virol. 76, 4855–4865
6 Nanbo, A. et al. (2010) Ebola virus is internalized into host cells via macroinocytosis
in a viral glycoprotein-dependent manner. PLoS Pathog. 6, e1000121
7 Saed, M.F. et al. (2010) Cellular entry of ebola virus involves uptake by a
macroinocytosis-like mechanism and subsequent trafficking through early and
late endosomes. PLoS Pathog. 6, e1000110
8 Aleksandrowicz, P. et al. (2011) Ebola virus enters host cells by macroinocytosis
and clathrin-mediated endocytosis. J. Infect. Dis. 204 (Suppl 3), 5957–5967
of entry. Future Virol. 4, 621–635
10 Carette, J.E. et al. (2011) Ebola virus entry requires the cholesterol transporter
Niemann-Pick C1. Nature 477, 340–343
11 Chandran, K. et al. (2005) Endosomal proteolysis of the Ebola virus glycoprotein
is necessary for infection. Science 308, 1643–1645
12 Schomberg, K. et al. (2006) Role of endosomal cathepsins in entry mediated by the
Ebola virus glycoprotein. J. Virol. 80, 4174–4178
30, 161–177
14 Kortepeter, M.G. et al. (2011) Basic clinical and laboratory features of filoviral
hemorrhagic fever. J. Infect. Dis. 204, S810–S816
15 Bausch, D.G. et al. (2007) Assessment of the risk of ebola virus transmission from
bodily fluids and fomites. J. Infect. Dis. 196, S142-S147
16 Qui, X. et al. (2012) Successful treatment of ebola virus-infected Cynomolgus
macaques with monoclonal antibodies. Sci. Transl. Med. 4, 138ra81
17 Olinger, G.G. et al. (2012) Delayed treatment of Ebola virus infection with plant-
derived monoclonal antibodies provides protection in rhesus macaques. Proc. Natl.
Acad. Sci. U. S. A. 109, 18030–18035
18 Geisbert, T.W. et al. (2003) Treatment of Ebola virus infection with a recombinant
inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. Lancet 362, 1953–
1958
19 Smith, L.M. et al. (2013) Interferon-β therapy prolongs survival in rhesus macaque
models of Ebola and Marburg hemorrhagic fever. J. Infect. Dis. 208, 310–318
20 Qui, X. et al. (2013) Monoclonal antibodies combined with adenovirus-vectored
interferon significantly extend the treatment window in Ebola virus-infected guinea
pigs. J. Virol. 87, 7754–7757
a lethal Ebola virus challenge with RNA interference: a proof-of-concept study.
Lancet 375, 1896–1905
22 Furuta, Y. et al. (2002) In vitro and in vivo activities of anti-influenza virus compound
Antimicrob. Agents Chemother. 49, 981–986
24 Sme, D.F. et al. (2009) Intracellular metabolism of favipiravir (T-705) in uninfected
and influenza A (H5N1) virus-infected cells. J. Antimicrob. Chemother. 64, 741–746
25 Jin, Z. et al. (2013) The ambiguous base-pairing and high substrate efficiency of
T-705 (Favipiravir) ribofuranosyl 5′-triphosphate towards influenza A virus
polymerase. PLOS ONE 8, e68347
26 Baranovich, T. et al. (2013) T-705 (favipiravir) induces lethal mutagenesis in
influenza A H1N1 viruses in vitro. J. Virol. 87, 3741–3751
27 Ostereich, L. et al. (2014) Successful treatment of advanced Ebola virus infection
with T-705 (favipiravir) in a small animal model. Antivir. Res. 105, 17–21
28 Smither, S.J. et al. (2014) Post-exposure efficacy of Oral T-705 (Favipiravir) against
29 Warren, T.K. et al. (2014) Protection against filovirus diseases by a novel broad-
spectrum nucleoside analogue BCX4430. Nature 508, 402–405
30 Huggins, J.W. et al. (1991) Inhibition of ebola virus replication in vitro and in a SCID
mouse model by S-adenosylhomocysteine hydrolase inhibitors. Antivir. Res. 15
(Suppl. 1), 122
31 Huggins, J.W. et al. (1995) Inhibition of Ebola virus by S-adenosylhomocysteine
hydrolase inhibitors. Antivir. Res. 26, A301
32 De Clercq, E. and Montgomery, J.A. (1983) Broad-spectrum antiviral activity of the
carbocyclic analog of 3-deazaadenosine. Antivir. Res. 3, 17–24
33 Huggins, J. et al. (1999) Antiviral drug therapy of filovirus infections: S-
adensylhomocysteine hydrolase inhibitors inhibit ebola virus in vitro and in a
34 Bray, M. et al. (2000) Treatment of Lethal Ebola virus infection in mice with a single
36 Bray, M. et al. (2002) 3-Deazaneplanocin A induces massively increased interferon-
alpha production in Ebola virus-infected mice. Antivir. Res. 55, 151–159
37 Parker, W.B. (2005) Metabolism and antiviral activity of ribavirin. Virus Res. 107,
165–171
38 Sme, D.F. et al. (2001) Intracellular phosphorylation of carbocyclic 3-
RNA synthesis: validation of a prototype virus-based approach. Chem. Biol. 20,
424–433
acidic extracellular milieu exacerbates Ebola virus-induced cell damage. Virology
358, 1–9
41 Gmurh, K. et al. (2012) Cathepsins B and L activate Ebola but not Marburg virus glycoproteins for efficient entry into cell lines and macrophages independent of TMPRSS2 expression. Virolgy 424, 3-10
51 Shoemaker, C.J. et al. (2013) Multiple cationic amphiphiles induce a Niemann-Pick C phenotype and inhibit Ebola virus entry and infection. PLOS ONE 8, e56265
54 Kolokoltsov, A.A. et al. (2012) Inhibition of Lassa virus and Ebola virus infection in host cells treated with the kinase inhibitors genistein and tyrphostin. Arch. Virol. 157, 121-127
58 Soltogl, S.P. (2001) Rottlerin is a mitochondrial uncoupler that decreases cellular ATP levels and indirectly blocks protein kinase Cdelta tyrosine phosphorylation. J. Biol. Chem. 276, 37986-37992
63 Chang, J. et al. (2013) Small molecule inhibitors of ERα glucosidases are active against multiple hemorrhagic fever viruses. Antivir. Res. 98, 432-440
70 Kamiyama, H. et al. (2011) Infection of XCl cells by MLVs and ebola virus is endosom-dependent but acidification-independent. PLoS ONE 6, e26180
71 Lu, Y. et al. (2014) Vacuolin-1 potently and reversibly inhibits autophagosome-lysosome fusion by activating RAB7A. Autophagy 10, 1895-1905
74 Madrid, P.B. et al. (2013) A systematic screen of FDA-approved drugs for inhibitors of biological threat agents. PLOS ONE 8, e60579
76 Kinch, M. and Goldblatt, M. Methods of inhibiting viral infection. WO2009091435 A2
80 Selakovíc, Ž. et al. (2012) A limited structural modification results in a significantly more efficacious diazachrysene-based filovirus inhibitor. Viruses 4, 1279-1288