

Development of Small-Molecule Antivirals for Ebola

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Abstract: Ebola hemorrhagic fever is a deadly disease caused by infection with one of the Ebola virus species. Although a significant progress has recently been made in understanding of Ebola virus biology and pathogenesis, development of effective anti-Ebola treatments has not been very productive, compared to other areas of antiviral research (e.g., HIV and HCV infections). No approved vaccine or medicine is available for Ebola but several are currently under development. This review summarises attempts in identification, evaluation, and development of small-molecule candidates for treatment of Ebola viral disease, including the most promising experimental drugs brincidofovir (CMX001), BCX4430, and favipiravir (T-705). © 2015 Wiley Periodicals, Inc. Med. Res. Rev., 35, No. 6, 1175–1194, 2015

Key words: antiviral; filovirus; Ebola virus; Marburg virus; hemorrhagic fever

1. INTRODUCTION

Ebolavirus, *Marburgvirus* (MARV), and *Cuevavirus* are the only genera of the *Filoviridae* family of enveloped viruses with nonsegmented negative-sense RNA genomes.¹ They are causative agents of severe viral hemorrhagic fevers (VHFs) and are classified as biosafety level-4 (BSL-4) pathogens and Category A agents (in terms of bioterrorism).² The taxonomy of the *Filoviridae* family has kept changing over time and several virus names and abbreviations have been created. Currently, five ebola species (earlier they were considered strains or subtypes of one species) are recognized, namely *Zaire ebolavirus* (EBOV), *Sudan ebolavirus* (SUDV), *Tai Forest ebolavirus* (TAFV), *Reston ebolavirus* (RESTV), and *Bundibugyo ebolavirus* (BDBV).^{1,3} Especially, EBOV and SUDV are responsible for serious outbreaks of Ebola hemorrhagic fever (EHF), or Ebola virus disease (EVD), among humans and nonhuman primates in the regions of sub-Saharan Africa. EBOV is the most virulent ebola virus with fatality rate ranging from 50 to 90%.

EVD was first identified in 1976 in Sudan (now South Sudan)⁴ and Zaire (now the Democratic Republic of the Congo)⁵ and 24 outbreaks were reported by the World Health Organization (WHO) since then through 2013.⁶ The transmissions from animals to humans are believed

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to involve direct contact with an infected wild animal or fruit bat, which are considered to be the most likely natural reservoir for Ebola virus.

The recent outbreak in West Africa,⁷ which started in March 2014, represents the biggest Ebola outbreak so far and it is considered to be the first Ebola epidemic the world has ever seen.⁶ It has brought a substantial attention of both scientific community and the public. Over 20,000 confirmed, probable, and suspected cases of EVD have been reported by WHO from six African countries (with main incidence in Guinea, Liberia, and Sierra Leone) and several isolated cases also from other countries (Spain and United States).⁸ The reported case fatality rate across the most-affected countries is estimated to be at least 70%.⁹

VHFs caused by filoviruses are usually characterized by nonspecific flu-like symptoms including high fever, severe headache, myalgia, and prostration, followed by gastrointestinal symptoms such as diarrhea, nausea, and vomiting, and further signs as bleeding, petechiae, rash, dry cough, chest pain, behavioral disorders, and seizures, resulting in multiorgan failure and, ultimately, death.^{10–12} Death, due to multiorgan failure and a syndrome resembling septic shock, typically occurs within 6–16 days after development of the clinical signs.^{13,14}

Commonly applied standard supportive care is based on replacement of the body fluids patients lose during the infection, and on treatment of other opportunistic infections. Currently, there are no approved drugs to treat EHF. Although several small-molecule candidates were developed and approved for the treatment of various RNA virus infections,¹⁵ most of them did not show to be really potent in case of filovirus diseases. Only a handful of potential antiviral agents are in the pipeline for filovirus infections,^{16–23} but these experimental drugs actually represent promising options for the prevention and treatment of EVD.

While development of EBOV vaccines is highly desirable, especially for protection of high-risk groups (e.g., medical personal or family members of patients), vaccines cannot completely prevent single cases or even new EBOV outbreaks in remote areas of West Africa. The use of vaccines is, furthermore, accompanied with more or less serious adverse effects and it is not even clear how efficient it would be in areas endemic with various other serious human diseases (e.g., malaria), where, moreover, the local community may strongly disagree with vaccination. Development and use of humanized monoclonal antibodies (e.g., ZMapp)²⁴ also has significant limitations, including stability, difficulty with transport, and problematic scale-up production. For these reasons, usage of small-molecule antivirals is invaluable approach in treatment and prevention of viral infections in general and development of potent small-molecule anti-EBOV agents is clearly of high priority.

The EBOV genome contains seven genes (NP, VP35 (where VP is viral protein), VP40, GP, VP30, VP24, and L) that encode the corresponding VPs. The filovirus replication complex consists of the genomic RNA molecule and four proteins: NP (nucleoprotein), VP30 (transcription activator), VP35 (polymerase cofactor), and L (RNA-dependent RNA polymerase).¹⁶ The matrix proteins VP24 and VP40 connect glycoprotein (GP) (actually, its GP₂ segment) to the central ribonucleoprotein.¹⁶ Number of steps in the filovirus replication cycle can, theoretically, be targeted with small-molecule inhibitors, namely attachment of the virion to a cell-surface receptor, fusion of the viral envelope with cellular membranes, replication/transcription process, assembly/maturation of new viral particles, and budding.¹⁶

This work summarizes discovery and identification of number of small molecules with important anti-Ebola virus properties. Promising treatments based on antisense technology and RNA interference (RNAi) are also briefly mentioned while monoclonal antibodies (e.g., ZMapp)²⁴ and Ebola virus vaccine^{25–27} development is not addressed in this review and can be found elsewhere.

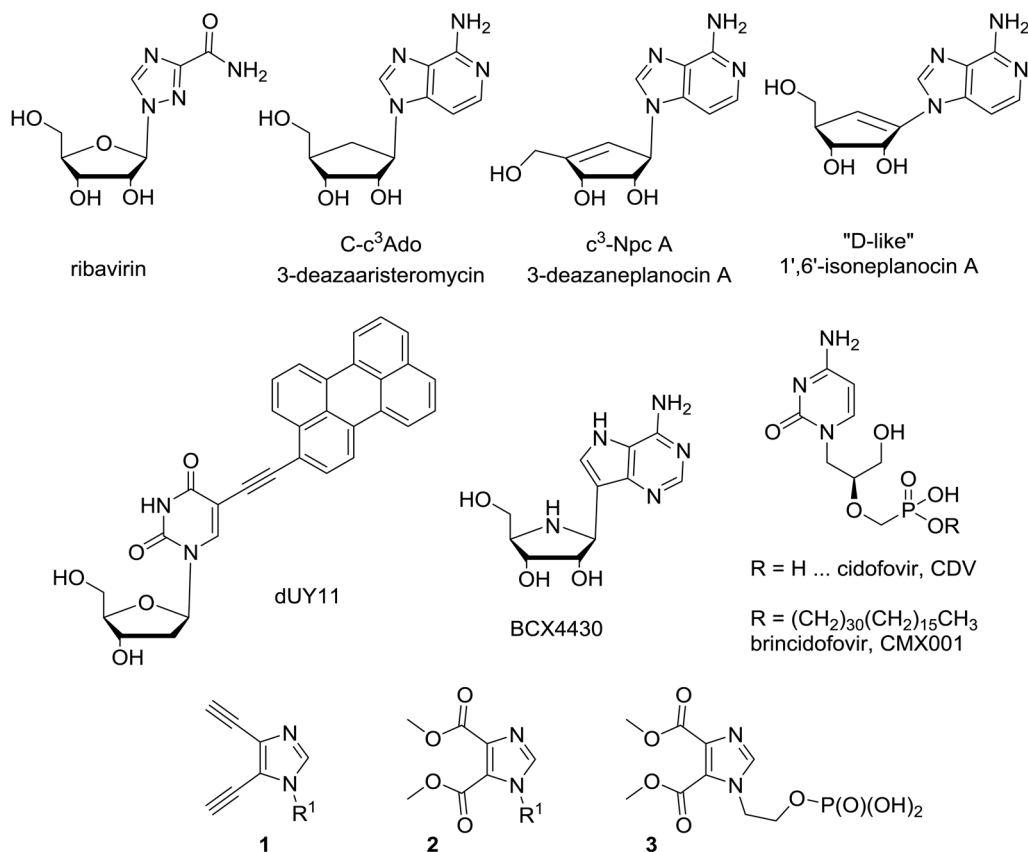


Figure 1. Chemical structures of nucleoside analogues with various modes of action: ribavirin (not active against EBOV); 3-deazaaristeromycin, 3-deazaneplanocin A and "D-like" 1',6'-isoneplanocin A (SAH hydrolase inhibitors); dUY11 (fusion inhibitor); BCX4430, CDV, and CMX001 (viral transcription inhibitors); compounds **1–3** (unknown mechanism of action).

2. NUCLEOSIDE AND NUCLEOTIDE ANALOGUES

This group of compounds is represented by structurally modified nucleosides and nucleotides with various modes of antiviral action. Acyclic nucleoside phosphonates, and compounds that can be metabolized to nucleotide analogues in cells are also included here.

Among nucleoside analogues, ribavirin (Virazole, Fig. 1), a broad-spectrum antiviral drug, has received a lot of attention.²⁸ Ribavirin has been reported to be active against some hemorrhagic fever viruses (e.g., Rift Valley fever virus and Crimean-Congo hemorrhagic fever virus), but it had no in vitro or in vivo effects on Ebola and Marburg viruses.²⁹ Later, number of structural adenosine analogues, for example, 3-deazaaristeromycin (C-c³Ado, Fig. 1) and 3-deazaneplanocin A (c³-Npc A, Fig. 1), were discovered to inhibit replication of EBOV in vitro by blocking *S*-adenosyl-L-homocystein (SAH) hydrolase.^{30–32}

SAH hydrolase is a key enzyme in methylation reactions depending on *S*-adenosylmethionine (SAM) as the methyl donor and it has a key role in the methylation of 5'-end guanine of viral messenger RNA (regulation of capping process). Since the discovery of SAH hydrolase as a valuable pharmacological target for antiviral chemotherapy,³³ a large variety of adenosine (Ado) analogues as potential SAH hydrolase inhibitors have been reported.^{34–36} Such inhibitors block the cleavage of *S*-adenosyl-L-homocystein (SAH) into

homocysteine (Hcy) and adenosine, which itself can be further metabolized into AMP, adenine, and inosine. As a consequence of the SAH hydrolase inhibition, SAH accumulates in the cell and leads to an inhibition of the SAM-dependent methylation processes, including those that are required for the maturation (i.e., 5'-capping) of viral mRNAs. As a consequence, maturation of viral mRNAs is suppressed, and so is the production of progeny virus particles.

3-Deazaaristeromycin (C-c³Ado, Fig. 1) was the first compound that demonstrated to cure mice from otherwise lethal EBOV infection.³¹ Bray et al.³² showed that 3-deazaneplanocin A (Fig. 1), as a single inoculation of 1 mg/kg, given on the first or second day after virus infection, also afforded a significant protection of mice against a lethal infection with EBOV without causing acute toxicity. In later study,³⁷ it was discovered that the protective effect of 3-deazaneplanocin A might result from massively increased production of interferon- α in Ebola-infected, but not uninfected mice. SAH hydrolase inhibitors have received only limited clinical evaluation and should be further tested for their potential antiviral properties as they exert a broad-spectrum antiviral activity and represent an attractive antiviral strategy.³⁸

Ye and Schneller³⁹ have reported 1',6'-isoneplanocin A enantiomers (e.g., "D-like" 1',6'-isoneplanocin A, Fig. 1) as compounds potent against a variety of important viruses, including EBOV with submicromolar EC₅₀ values (e.g., EC₅₀ = 0.38 for the "D-like" enantiomer, Fig. 1). The author also speculated that SAH hydrolase inhibition is not the only site of action of the "L-like" enantiomer and more studies are needed to fully understand the antiviral potential of L-like carbocyclic nucleosides.³⁹

Rigid amphipathic fusion inhibitors (RAFIs),⁴⁰ for example, compound dUY11 (Fig. 1), are uridine nucleoside analogues bearing a bulky hydrophobic group in the C-5 position. RAFIs represent another group of synthetic compounds that inhibit infectivity of several unrelated enveloped viruses, including HCV and HSV-1 and HSV-2 at submicromolar range and with no cytotoxic or cytostatic effects (selectivity index > 3000).⁴⁰ It was shown that RAFIs inhibit virion fusion as a result of their shape and amphipathicity. RAFIs should be further evaluated against other emerging viruses, such as EBOV and MARV.

Another promising EBOV therapy represents compound BCX4430 (Fig. 1),⁴¹ which has been reported in 2014 as a novel broad-spectrum antiviral agent. It is an adenine analogue of the so-called Immucillin H,⁴² a powerful transition-state analogue⁴³ inhibitor of purine nucleoside phosphorylase, which has a potential for treatment of human T-cell leukemia and lymphoma. BCX4430 exhibits broad-spectrum activity against numerous viruses, including filo-, bunya-, arena-, paramyxo-, corona-, and flaviviruses. BCX4430 was shown to inhibit infection of distinct filoviruses in human cells and postexposure intramuscular administration of BCX4430 protected rodents both against EBOV and MARV viral disease.⁴¹ It, moreover, completely protected cynomolgus macaques from MARV infection when administered as late as 48 hr following infection. BCX4430 appeared to inhibit viral RNA polymerase function, acting probably as a non-obligate RNA chain terminator.⁴¹ BCX4430 also effectively treated yellow fever virus (YFV) infection in a hamster model, even when treatment was initiated at the peak of viral replication.⁴⁴ The first-in-man Phase I study to evaluate the safety, tolerability, and pharmacokinetic properties of BCX4430 administered via intramuscular injection in healthy volunteers was announced by BioCryst Pharmaceuticals (Durham, NC, USA) in the middle of December 2014.⁴⁵

Brincidofovir (CMX001, BCV, HDP-CDV, Fig. 1) is an oral nucleotide analogue with broad-spectrum in vitro and in vivo antiviral activity against dsDNA viruses that infect humans,⁴⁶ including adenoviruses,^{47,48} poxviruses,⁴⁹ and herpesviruses.⁵⁰ CMX001 is a hexadecyloxypropyl prodrug of cidofovir (Fig. 1),⁵¹ acyclic nucleoside phosphonate⁵² approved by FDA for treatment of cytomegalovirus (CMV) infections. CMX001, being developed by Chimerix (Durham, NC, USA), has several key advantages compared to the parent compound:

3. POTENTIAL VIRAL ENTRY INHIBITORS

This section includes structurally diverse compounds that are reported to inhibit the cell entry of filoviruses. Several of the agents discussed are repurposed FDA-approved drugs.

Number of compounds of distinct structural features has been reported as potential entry inhibitors. EBOV entry requires functioning cholesterol transporter protein Niemann–Pick C1 (NPC1).⁶⁵ It was shown that cells defective NPC1 function, which binds to the viral GP, are resistant to infection by EBOV and MARV. Small-molecule inhibitors, derived from benzylpiperazine adamantane diamides (e.g., compounds 3.0 and 3.47, Fig. 3),⁶⁶ have been described that interfere with GP binding to NPC1. Since this process is essential for EBOV infection, it seems to represent a good target for potential antiviral therapy.

Wolf et al.⁶⁷ have reported the discovery of promising broad-spectrum antiviral agent, LJ001 (Fig. 3), active against an impressive number of enveloped viruses. It was effective against influenza A, filo-, pox- arena-, bunya-, paramyxo-, flaviviruses, and HIV, but had no effect on the infection of nonenveloped viruses. LJ001 intercalates into viral membranes preventing virus–cell fusion, but the host cells can overcome the toxic effects of LJ001 due to their repair by cellular lipid biosynthesis. The rhodanine derivative LJ001 was suggested to inhibit viral entry at a step after virus binding and before virus–cell fusion, but the molecular target and molecular mechanism remained elusive. Later, Vigant et al.⁶⁸ identified the unsaturated fatty acid chains of viral membrane phospholipids as the major target of LJ001 antiviral activity. In the membrane bilayer, LJ001 generates singlet oxygen ($^1\text{O}_2$) and subsequent lipid oxidation results in changes to the biophysical properties of the viral membrane that disrupts the virus ability to undergo virus–cell fusion. Furthermore, elucidation of the mode of action and subsequent structure–activity relationship (SAR) optimization of LJ001 led to a new class of oxazolidine-2,4-dithiones, for example, compound JL103 (Fig. 3),⁶⁸ as membrane-targeted photosensitizers with increased potencies, $^1\text{O}_2$ quantum yields, and red-shifted absorption spectra.

A series of benzodiazepine compounds, represented by derivative 4 (Fig. 3), has been reported as potential entry inhibitors for filoviruses.⁶⁹ Compound 4 was validated as an inhibitor of EBOV and MARV in cell-based assays, with 50% inhibitory concentrations (IC_{50} s) of 10 and 12 μM , respectively. It was hypothesized that it binds to the hydrophobic pocket of the EBOV GP1–GP2 interface and as a consequence inhibits EBOV infection of cells.

Pyridinyl imidazole inhibitors of p38 MAP kinase, for example, compound SB202190 (Fig. 3), were found to impair viral entry and reduce cytokine induction by EBOV.⁷⁰ SB202190 reduced viral replication in macrophage-like human THP-1 cells with an $\text{IC}_{50} = 4.73 \mu\text{M}$ and primary human monocyte derived dendritic cells (MDDCs) with an $\text{IC}_{50} = 2.67 \mu\text{M}$. Kinase, as well as phosphatase inhibitors may represent new leads and a unique strategy for antifilovirus therapeutic development and such compounds with reported anti-EBOV activity have recently been reviewed in depth.²¹

Yermolina et al.⁷¹ have reported a novel group of selective inhibitors of filoviral entry that selectively inhibit the EBOV and MARV GP mediated infection of human cells. Extensive SAR study led to an identification of lead compound 5 (Fig. 3) as a selective inhibitor of filoviral entry with an IC_{50} of 30 μM .⁷¹ Also several natural products that are able to impair microfilament function, including latrunculin A (Fig. 3) and cytochalasins, were shown to be potent inhibitors of EBOV virus GP mediated entry and fusion.⁷²

A novel high-throughput screening (HTS) assay of some 5000 small molecules led to an identification of novel broad-spectrum compounds able to block cathepsin L (CatL) cleavage of viral GPs derived from SARS-CoV and EBOV, Hendra, and Nipah viruses that are required for their entry into the host cell.⁷³ Parent compound 5705213 (Fig. 3) and its derivative 7402683 (Fig. 3) showed IC_{50} s of 15 and 10 μM against EBOV-GP, respectively, and are not cytotoxic.

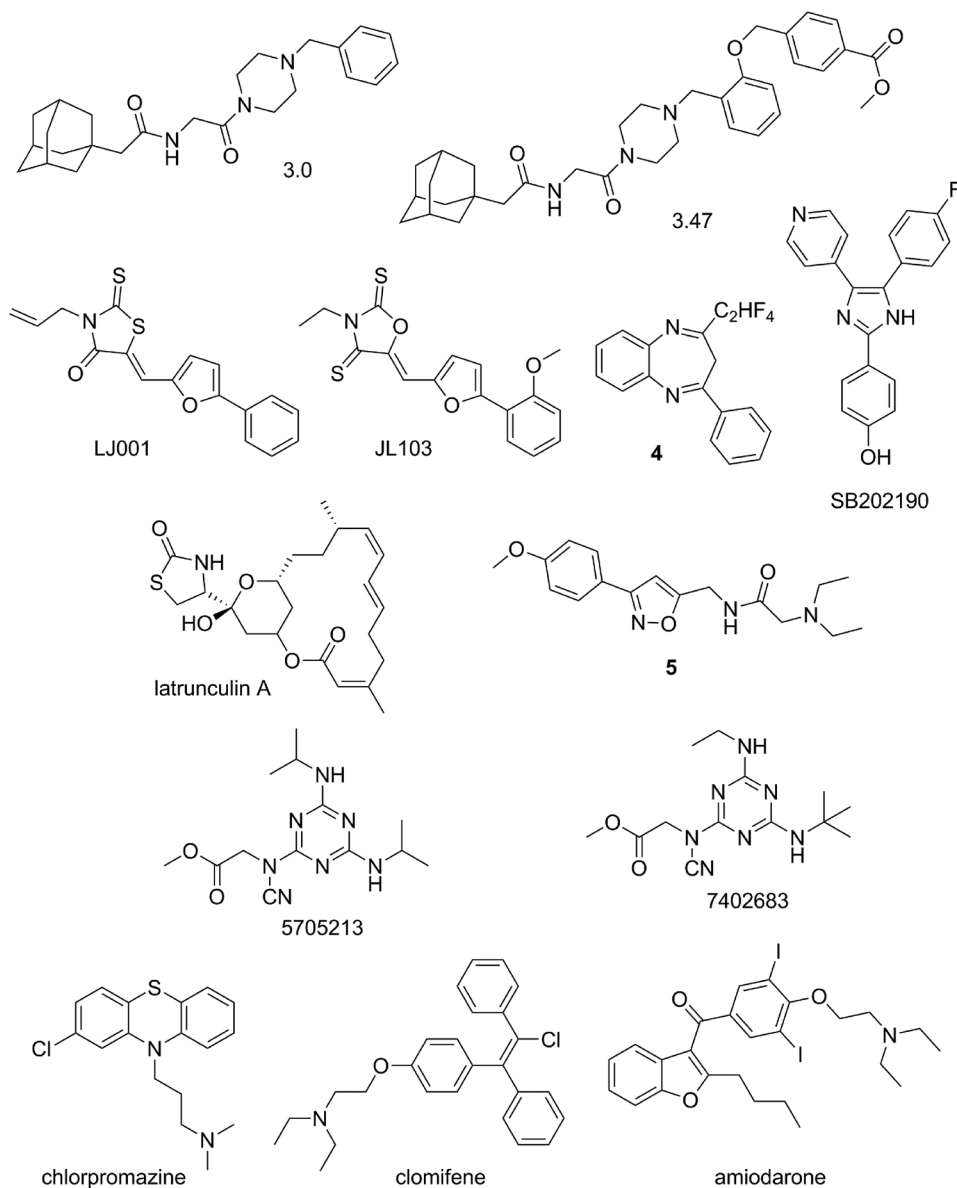


Figure 3. Chemical structures of potential viral entry inhibitors of EBOV: 3.0, 3.47, LJ001, JL103, compound **4**, SB202190, latrunculin A, compound **5**, 5705213, 7402683; FDA-approved drugs chlorpromazine, clomifene, and amiodarone, representing examples of potential drug-repurposing.

Fullerene sugar balls represent a new class of biologically active compounds.⁷⁴ Water-soluble glycofullerenes were found to efficiently inhibit a DC-SIGN-dependent (DC-SIGN is a C-type lectin) cell infection by virus-like particles.⁷⁵ Several mannosylated fullerene sugar balls showed remarkable IC_{50} s of $2 \mu M$ against EBOV and thus can be considered as a very promising tool to interfere with the EBOV entry.

Drug repurposing (or drug repositioning) is potential application of known compounds to new indications. Therefore, a systematic screening of FDA-approved drugs could rapidly become available for a new indication in an emergency, including EBOV infections.^{76–78}

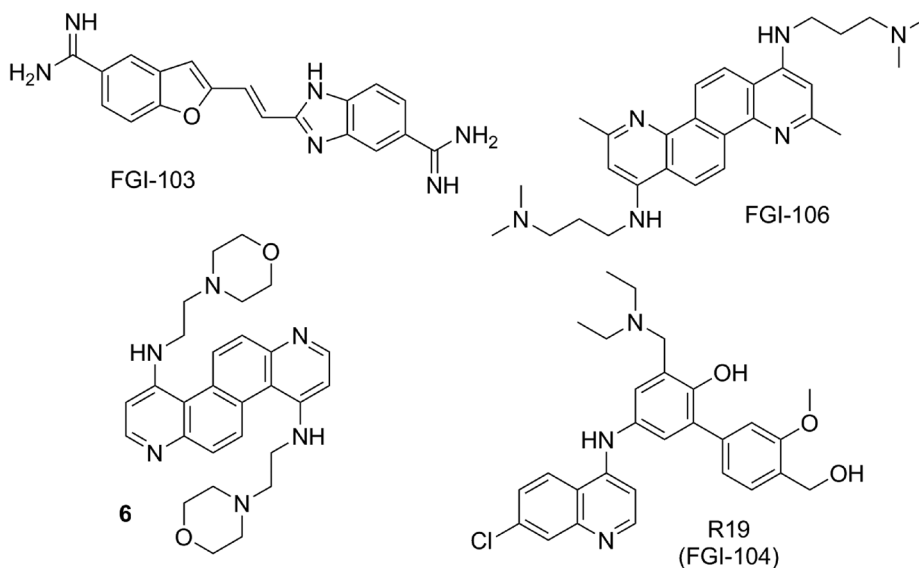


Figure 4. Chemical structures of antiviral inhibitors developed by Functional Genetics, Inc. (Gaithersburg, USA) and related compounds.

Chlorpromazine (trademarked as Thorazine, Largactil, and Megaphen, Fig. 3),⁷⁹ a known psychotropic drug approved by the FDA, was reported as potential inhibitor of EBOV entry, possibly through inhibition of clathrin-mediated endocytosis.⁸⁰

FDA-approved selective estrogen receptor modulators (SERMs), including clomifene (trademarked as Androxal, Clomid, and Omifin, Fig. 3) and toremifene (brand name Fareston) were identified as potent inhibitors of EBOV infection from an in vitro screening of readily available approved drugs.^{77,81,82} The authors suggested that mode of the action of SERMs did not involve classical pathways associated with the estrogen receptor, but instead, interfere with a late step in viral entry.⁸¹

Antiarrhythmic agents amiodarone (a multi-ion channel inhibitor, Fig. 3) and dronedarone are other examples of FDA-approved drugs that could be repurposed.⁸³ Amiodarone⁸⁴ was found to inhibit filovirus entry at concentrations (1.5–2.5 $\mu\text{g}/\text{mL}$) that are routinely reached in human serum during antiarrhythmic therapy.⁸³ The above examples show that drug repurposing may be a viable approach for identification of potent anti-EBOV therapeutics.

4. MISCELLANEOUS SMALL MOLECULES

Functional Genetics, Inc. (Gaithersburg, MD, USA) has reported a series of polyaromatic compounds active against distinct viruses. A small-molecule inhibitor of filovirus infection, designated as FGI-103 (Fig. 4),⁸⁵ was identified via HTS of compound library from National Cancer Institute (NCI). FGI-103 exhibited antiviral activity against wild-type EBOV and SUDV, as well as multiple strains of MARV.⁸⁵ Although the mechanism of its action is unknown, it was shown in the murine model of EBOV infection that FGI-103 reduces viremia and viral burden in organ tissues and that it could be applicable for both prophylactic and therapeutic treatments.

FGI-106 (Fig. 4)⁸⁶ is a diazachrysene (DAAC) based analogue that was discovered in a cell-based HTS as a potent and broad-spectrum inhibitor of lethal VHF pathogens, including

EBOV, Rift Valley, and Dengue Fever viruses. FGI-106 protected mice from otherwise lethal EBOV infection both in prophylactic and therapeutic settings. FGI-106 also revealed potential inhibitory activity against other viral pathogens including HIV and HCV,⁸⁶ but the precise mode of action remains unclear.²² The broad-spectrum nature of the antiviral activity of DAAC-based analogues may suggest targeting of a conserved host pathway.⁸⁶

Conventional antivirals are designed to target virally encoded proteins/enzymes and mechanisms. The disadvantage of the conventional antivirals is often their toxicity to the host and development of the resistant viral strains rendering them relatively quickly ineffective. To prevent the resistance problem, combination therapies using a cocktail of drugs with various modes of actions were successfully introduced and approved. Host-directed therapeutics represents another important approach to combat established, as well as emerging viral diseases.⁸⁷ Such approach is based on targeting host to deny the viral pathogen the ability to cause disease.

Structural modification studies of the promising DAAC-based inhibitors were performed,^{88,89} and compound **6** (Fig. 4) was identified as highly efficacious EBOV and MARV inhibitor with IC₅₀ values of 0.70 and 2.76 μM, respectively, with little or no associated cellular toxicity.⁸⁹

Recently, a broad-spectrum small-molecule inhibitor of EBOV, FGI-104 (structure originally not given),⁹⁰ has been reported that might target host protein TSG101 that plays an essential role in the viral life cycle. The interaction of filovirus matrix protein VP40, the key VP that drives the budding process,⁹¹ with TSG101 facilitates the viral budding.⁹² In addition, FGI-104 demonstrated inhibition of multiple emerging viruses (e.g., EBOV, Cowpox) and blood-borne pathogens (e.g., HBV, HCV, HIV). In the patent,⁹³ chaotically, FGI-104 is a name used for the whole family of compounds and R19 (Fig. 4) is mentioned as the preferred compound listed there.

Retinoid thiosemicarbazone derivative, retinazone (RTZ, Fig. 5),⁹⁴ was described as a broad-spectrum antiviral agent active against HIV, HCV, VZV, and CMV. RTZ was found to be a potent suppressor of HCV RNA replicon replication.⁹⁴ Later, RTZ has also been reported to be potent and efficacious inhibitor of EBOV with an IC₅₀ value of 1.1 μM,⁹⁵ but since the SI₅₀ was only 3.4, the activity may be linked to cytotoxicity.

Iminosugar 1-deoxynojirimycin (DNJ) and its derivatives (as glucose mimics) can serve as glucosidase inhibitors and were shown to exhibit antiviral effects against a number of enveloped viruses. DNJ derivative CM-10-18 (Fig. 5) was shown to exhibit *in vitro* and *in vivo* inhibitory activity against endoplasmic reticulum (ER) α-glucosidases I and II,^{96,97} and demonstrated *in vivo* efficacy against lethal Dengue virus infection in mice.⁹⁸ Further extensive SAR studies of CM-10-18 derivatives lead to an identification of novel iminosugars, for example, compound IHVR17028 (Fig. 5), that significantly reduced the mortality of MARV and EBOV infections in mice.⁹⁹ A significant survival rate was, for example, observed for 25 mg/kg of IHVR17028 in a murine protection-of-death model of EBOV infection, when the treatment was initiated 4 hr post virus challenge.

The multifunctional VP35 is another attractive therapeutic target as it plays a critical role in Ebola viral replication, and knowledge of high-resolution structures of the VP35 C-terminal domain (termed VP35 IID)¹⁰⁰ provides an opportunity for further structure-based antiviral research.¹⁰¹ Using *in silico* and NMR-based screening methods, Brown et al.¹⁰² identified several compounds, for example, representative compound GA017 (Fig. 5), capable of binding of VP35 IID with high affinity and specificity. Some of the compounds were also shown to inhibit a replication-competent EBOV in a cell-based assay.¹⁰²

Recent HTS of a subset of FDA-approved drugs has reported that also antimalarials amodiaquine and chloroquine were active *in vitro* and *in vivo* against EBOV in single digit micromolar range,^{76,77} but the mechanism of action was unclear. Later it was shown¹⁰³ that these compounds docked favorably in VP35 suggesting they may be targeting this VP. Furthermore,

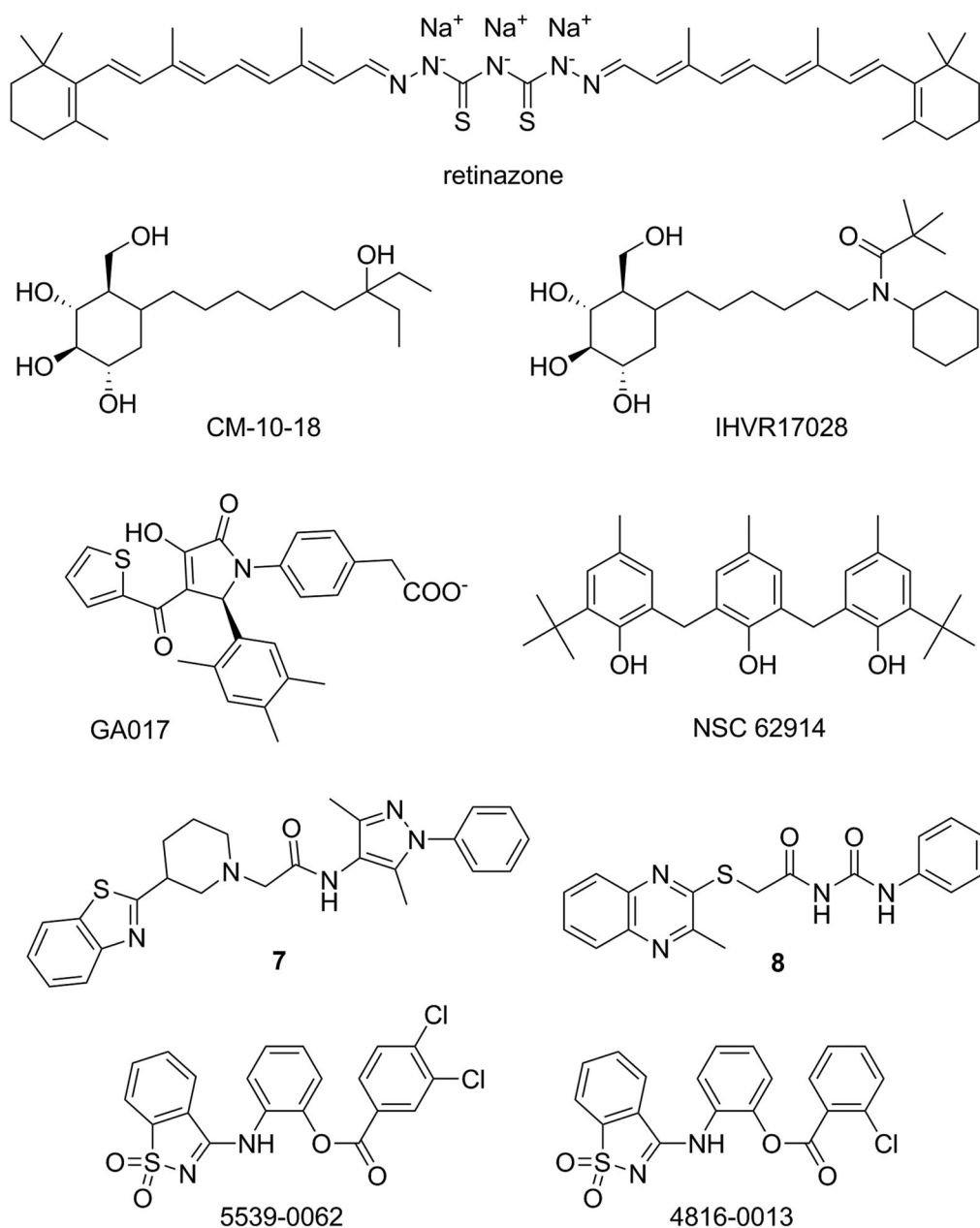


Figure 5. Miscellaneous small-molecule agent with potential anti-EBOV properties: retinazone; endoplasmic reticulum (ER) glucosidase inhibitors CM-10-18 and IHVR17028; GA017; antioxidant compound NSC 62914; compounds **7**, **8**, 5539-0062, and 4816-0013 as potential budding inhibitors.

chloroquine was shown to block EBOV virus like particle entry at an $IC_{50} \sim 15 \mu M$ with selectivity index $SI > 32$.⁷⁷

Small-molecule screening for EBOV inhibitors leads to identification of NSC 62914 (Fig. 5).¹⁰⁴ The compound acts as a scavenger of reactive oxygen species (ROS) and it up-regulates oxidative stress induced genes. ROS contribute to the pathogenesis of a wide array of

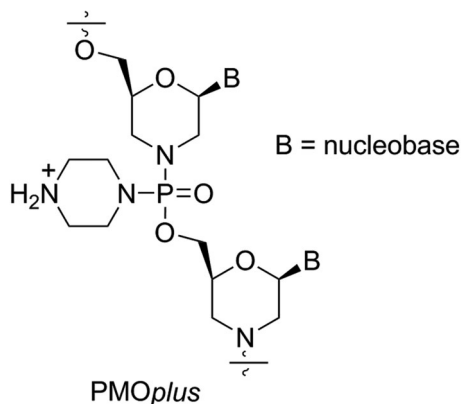


Figure 6. General structure of PMOplus.

diseases including viral infections. NSC 62914 was shown to inhibit EBOV, MARV, Rift Valley fever virus, Lassa virus, and Venezuelan equine encephalitis virus in cell-based assays, and in vivo protected mice following challenge with EBOV or MARV.

Budding of a broad range of RNA viruses is facilitated by subversion of host proteins (e.g., Nedd4) by viral PPxY late budding domains expressed within the matrix proteins of these viruses. In silico design and subsequent SAR study resulted in an identification of lead compounds **7** and **8** (Fig. 5) with ability to inhibit these critical viral–host (PPxY–Nedd4) interactions.¹⁰⁵ In addition, compounds **7** and **8** exhibited antibudding activity against EBOV and other RNA viruses and can thus serve as the lead structures for the development of novel broad-spectrum antivirals.

PTAP type L domain is another domain utilized by number of RNA viruses (e.g., Junin virus, EBOV, HIV-1) during the budding process and, thus, recently identified PTAP inhibitors, such as compound 5593–0062 and its structural analogue 4816–0013 (Fig. 5), have the potential to act as potent broad-spectrum, host-oriented antiviral drugs.

5. SEQUENCE-SPECIFIC ANTIVIRAL AGENTS

RNA viruses present a good target for the rapidly advancing field of sequence-specific therapeutics.^{107–110} Antisense strategy usually utilize single-stranded DNA oligonucleotides to inhibit protein production by binding to specific sites on mRNA essential for translation, or by mediating the catalytic degradation of target mRNA,¹¹¹ while double-stranded RNA oligonucleotides, known as short-interfering RNAs (siRNAs), also mediate the catalytic degradation of complementary mRNAs. Thus, both antisense and RNAi strategies can find therapeutic applications for treatment of highly pathogenic RNA viral infections.

Phosphorodiamidate morpholino oligomers (PMOs) were designed to inhibit translation of EBOV VP35, VP24, and L transcripts.^{112,113} All anti-EBOV PMOs reported showed reduced viral titer in cell cultures and provided complete protection to rodents when administered in both pre- and postexposure therapeutic regimens. PMOs also protected 75% of rhesus macaques in a prophylactic regimen.¹¹³

Sarepta Therapeutics (Cambridge, MA, USA, formerly AVI BioPharma) has developed PMO containing up to five positively charged linkages (PMOplus, Fig. 6)^{114,115} that have significantly improved the stability, efficacy, specificity, delivery, and safety of antisense complexes. Chemical evolution of the antisense molecules led to the discovery of two new therapeutic

agents, AVI-7537 targeting the VP24 transcript of EBOV and AVI-7288 targeting the NP transcript of MARV.¹¹⁶ The VP24 protein is an inhibitor of type I interferon responses. It also forms homodimers and binds to VP35 or NP and, thus, may play an important role in the switch from viral replication to transcription, a function that is critical to the viral life cycle. Inhibition of VP24 may lead to an efficient host response to viral infection.

Recently, Heald et al.¹¹⁷ evaluated the safety and pharmacokinetic properties of two combination drugs AVI-6002 (a combination of AVI-7537 and AVI-7539) and AVI-6003 (a combination of AVI-7287 and AVI-7288) that are under evaluation for postexposure prophylaxis of EBOV and MARV, respectively. Additional studies in nonhuman primates and humans are in progress to estimate the protective human doses.¹¹⁷

RNAi may also prove to be an effective and druggable therapy against filovirus infections.^{118–120} siRNAs targeting EBOV RNA polymerase, formulated in stable nucleic acid lipid particles (SNALPs),¹²¹ completely protected guinea pigs when administered shortly after an EBOV challenge.¹¹⁸ The siRNA proof-of-concept experiment in non-human primates against a lethal Ebola virus infection showed 66% and full postexposure protection of rhesus monkeys and macaques, respectively.¹²² Although the observation of adverse events (as fever) in some subjects in a Phase I study caused TKM-Ebola, siRNA developed by Tekmira Pharmaceuticals (Burnaby, Canada), to be placed on partial clinical hold, the FDA has still authorized its use in treating patients with confirmed or suspected EBOV infection under expanded access protocols.

6. CONCLUSIONS

The development of successful antiviral therapies to treat filovirus diseases is under way. The research programs should be facilitated by use of specific technologies and strategies,^{123,124} as well as high-throughput systems^{125,126} and suitable animal models,^{127–130} that have been recently reported. Especially, the use of laboratory animals is fundamental for the development of potent antifiloviral agents. Since guinea pigs are, for their size, less useful, newborn mice and immunodeficient adult mice represent a suitable model for preliminary testing of potential vaccines and antiviral agents.¹⁶ But namely use of non-human primates (NHPs), in which filoviruses cause severe VHF, is crucial for the successful development of efficient anti-EBOV treatments.¹⁶

All of the filoviral proteins (GP, L, NP, VP24, VP30, VP35, and VP40)^{16,131} can potentially be chosen as a suitable target for development of druggable anti-EBOV agents. For example, VP24 and VP35, and RNA-dependent RNA polymerase L have been shown so far to be exploitable targets for potential antiviral therapy. It has also been demonstrated that host-directed therapeutics, those targeting host proteins (e.g., TSG101, SAH hydrolase), represent another viable approach to combat various viral diseases. The number of small-molecule inhibitors was shown to interfere with the filoviral entry/fusion step, but mode of action of many other inhibitors of viral replications is not yet known or fully understood.

Two potential small-molecule antivirals were intended to be tested in human trials during the 2014 epidemic in Africa: brincidofovir (as orally bioavailable prodrug of cidofovir), an experimental drug originally developed by Chimerix to treat DNA viruses,⁵⁵ and favipiravir from Toyama Chemicals, approved in Japan to treat influenza.¹³² These agents were considered to receive the Fast Track designation from FDA to speed up the development of potent anti-EBOV drugs as much as possible.

Although this most frightening disease is endemic mainly to developing and third-world African countries, the recent Ebola outbreak has triggered new drug- and vaccine-development programs by a number of pharmaceutical companies, as well as by many academic research

teams. It has become evident that remaining challenge for development of any VHF treatment currently is to move the most promising vaccine and drug candidates forward into human trials, so we are ready when the next Ebola outbreak strikes.

7. ABBREVIATIONS

BDBV	=	<i>Bundibugyo ebolavirus</i>
CMV	=	cytomegalovirus
EBOV	=	<i>Zaire ebolavirus</i> or ebola
EHF	=	Ebola hemorrhagic fever
EVD	=	Ebola virus disease
HTS	=	high-throughput screening
MARV	=	<i>Marburgvirus</i>
PMO	=	phosphorodiamidate morpholino oligomer
RAFI	=	rigid amphipathic fusion inhibitor
RESTV	=	<i>Reston ebolavirus</i>
SAH	=	<i>S</i> -adenosyl-L-homocystein
SAR	=	structure–activity relationship
siRNA	=	short-interfering RNA
SNALP	=	stable nucleic acid lipid particle
SUDV	=	<i>Sudan ebolavirus</i>
TAFV	=	<i>Tai Forest ebolavirus</i>
VHF	=	viral hemorrhagic fever
VP	=	viral protein

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