Host cell kinases are important for the replication of a number of hemorrhagic fever viruses. We tested a panel of kinase inhibitors for their ability to block the replication of multiple hemorrhagic fever viruses. OSU-03012 inhibited the replication of Lassa, Ebola, Marburg and Nipah viruses, whereas BIBX 1382 dihydrochloride inhibited Lassa, Ebola and Marburg viruses. BIBX 1382 blocked both Lassa and Ebola virus glycoprotein-dependent cell entry. These compounds may be used as tools to understand conserved virus–host interactions, and implicate host cell kinases that may be targets for broad spectrum therapeutic intervention.

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1. Introduction

Viral hemorrhagic fevers (VHFs) cause significant morbidity and mortality globally. The infectious etiologies of VHFs include members of several RNA virus families, including the Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. The leading cause of VHF worldwide is Lassa virus (LASV), an arenavirus endemic to West Africa that causes 300,000–500,000 infections annually (Ogbu et al., 2007). Ebola virus (EBOV), Marburg virus (MARV), Junin virus (JUNV), Alkhurma hemorrhagic fever virus (AHFV, called Alkhumra virus in some reports), and Crimean Congo hemorrhagic fever virus (CCHFV), as well as the encephalitic Nipah virus (NiV), cause sporadic outbreaks, often with high case-fatality rates (Aljofan, 2013; Kortekaas et al., 2010; MacNeil and Rollin, 2012; Madani, 2005). These highly pathogenic agents are all classified as biosafety level 4 (BSL-4) pathogens; there are no approved therapeutics or vaccines, and medical care for patients is generally only supportive. Despite the challenges inherent in studying BSL-4 agents, research into therapies for these viruses is critical because of the potential for large outbreaks with high case-fatality rates, as demonstrated by the 2013–2015 EBOV outbreak in West Africa.

Host cell kinases have been implicated in the replication of several BSL-4 viruses. One signaling pathway, the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, was reported to be essential for the propagation of LASV in cell culture. Inhibition of the PI3K/Akt pathway by the small molecule BEZ-235 impeded the budding of LASV virus-like particles (VLPs) (Urata et al., 2012). Another inhibitor, LY294002, blocked EBOV entry (Saeed et al., 2008) and an early event in JUNV infection (Linero and Scolaro, 2009). The replication of Andes virus, a bunyavirus, was blocked by temsirolimus, an inhibitor of mTOR, another kinase in the PI3K/Akt pathway (McNulty et al., 2013). We therefore hypothesized that a cellular kinase could be essential for the replication of multiple highly pathogenic viruses. Identifying such a kinase...
might shed light on conserved virus–host interactions. In addition, therapies targeting such kinases could have broad-spectrum antiviral activity, a desirable property given the difficulty of developing therapies for individual hemorrhagic fever viruses. Here, we report the identification of 2 inhibitors of cellular kinases which impeded the replication of multiple highly pathogenic viruses.

2. Materials and methods

2.1. Biosafety

All work with infectious virus was conducted in a BSL-4 laboratory at the Centers for Disease Control and Prevention (CDC, Atlanta, GA). All laboratory adhered to international practices appropriate for this biosafety level. Experiments involving cDNA encoding viral sequences were approved by the CDC Institutional Biosafety Committee.

2.2. Cell lines, viruses, and compounds

A549, Vero-E6, HeLa, and HT-1080 cells were from the CDC Biologics Branch and HEK-293 cells were from ATCC. These cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies, Grand Island, NY, USA) supplemented with 10% (v/v) fetal calf serum (FCS; Hyclone, Thermo Scientific, Waltham, MA, USA) and penicillin–streptomycin (Life Technologies). Huh7 cells were from Apath, LLC (Brooklyn, NY, USA) and were propagated in DMEM, 10% (v/v) FCS, and 1 x non-essential amino acids (Life Technologies). Viruses were from the CDC Viral Special Pathogens Branch reference collection: LASV (strain Josiah); EBOV (strain Mayinga); AHFV (strain 200300001); CCHFV (strain IbAr10200). The Kinase Inhibitor Toolbox library and BIBX 1382 (strain Mayinga); AHFV (strain 200300001); CCHFV (strain IbAr10200). The Kinase Inhibitor Toolbox library and BIBX 1382 dihydrochloride were from Tocris Bioscience (Bristol, UK). The P13K Signaling Inhibitor Library and OSU-03012 were from Selleck Chemicals (Houston, TX, USA). Compounds were diluted in dimethylsulfoxide (DMSO; Sigma–Aldrich, St. Louis, MO, USA) as indicated.

2.3. Assays for antiviral activity and cell viability

To test for the inhibition of LASV replication, A549 cells were seeded at a density of 1 x 10^4 cells/well of a 96-well plate the day prior to infection. Compounds were added to the cells, and 1 h later, the cells were infected with LASV at an MOI of 0.2. After 48 h, the monolayers were fixed with 10% (v/v) formalin (Sigma–Aldrich) and γ-irradiated with 2 x 10^6 rads. The cells were permeabilized with 0.1% (v/v) Triton X-100 in phosphate buffered saline (PBS) for 10 min at room temperature, and LASV proteins were detected with monoclonal antibodies directed against the LASV glycoprotein and nucleoprotein (1:10,000 dilution in PBS supplemented with 2% w/v bovine serum albumin) and goat anti-mouse Alexa 488 (1:1000; Life Technologies). Cells were stained with CellMask Red and NucBlue (Life Technologies) and immunofluorescence microscopy was performed using the Operetta Imaging System (PerkinElmer, Waltham, MA).

The assay for the inhibition of AHFV-induced cytopathic effect in A549 cells was as described previously (Flint et al., 2014). The recombiant reporter viruses NiV-luc and EBOV and MARV expressing green fluorescent protein (GFP) reporter (EBOV-GFP and MARV-GFP) have also been described (Albariño et al., 2013; Lo et al., 2014; Towner et al., 2005). The assay for the inhibition of CCHFV-induced cytopathic effects was based on one described previously (Paragas et al., 2004). Briefly, compounds were added to SW13 cells in an 80% confluent monolayer in a 96-well plate, followed by infection with CCHFV at an MOI of 0.1, 1 h later. Cell viability, as measured by CellTiter-Glo (Promega, Madison, WI, USA), was measured 72 h post infection.

Cell viability was determined concurrently with the virus inhibition assays, but on compound-treated and mock-infected cells, using CellTiter-Glo (Promega) or PrestoBlue (Life Technologies) according to the manufacturer’s instructions. Viability was also assessed by nuclei number, as determined by counting the NucBlue-stained organelles with Harmony image analysis software (PerkinElmer). For each assay, values were normalized to vehicle-only DMSO controls.

For compound titrations, GraphPad Prism (GraphPad Software, La Jolla, CA, USA) was used to fit a 4-parameter equation to semilog plots of the concentration–response data and to interpolate the concentration of compound that inhibited 50% of the virus replication (EC50). The 50% cytopathic effect (CC50) was similarly derived using viability data from mock-infected cells. The selectivity index (SI) was calculated by dividing the CC50 by the EC50.

2.4. Viral titer reduction assay

Titer reduction assays for LASV and EBOV were performed in A549 and Huh7 cells, respectively. Cells were treated with compounds for 1 h prior to infection. Two days later, culture supernatants were harvested and virus titrations were performed in Vero-E6 cells. Three days post infection, the cells were fixed, permeabilized, and stained to visualize viral proteins. End-point viral titers were determined, and the 50% tissue culture infectious dose (TCID50) was calculated using the method of Reed and Muench (Reed and Muench, 1938).

2.5. Quantitative reverse transcription polymerase chain reaction assay

Cells were seeded and treated with compounds for 1 h before infection with LASV at an MOI of 0.1. The medium was removed 24 h post-infection, lysis buffer (MagMax Total RNA isolation kit; Life Technologies) was added, and RNA was extracted using a MagMax-96 deep-well magnetic particle processor (Life Technologies). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed with the Express One-Step Superscript qRT-PCR kit (Life Technologies) and analyzed on an Applied Biosciences 7500 real-time PCR machine (Life Technologies). LASV nucleoprotein RNA was quantitated using forward (5′-AATCAGTTCGGGACCATGC-3′) and reverse (5′-GTGTTGGGATACATTGCTGTG-3′) primers and a probe oligonucleotide (5′/5′-6-FAM/AGTCAACCT/ZEN/GCCCCTGTTTTGTCA/Iowa Black FQ/3′) from Integrated DNA Technologies (Coralville, IA). Levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) RNA, or 18S ribosomal RNA in Vero-E6 cells, were determined using control primer-probe sets (Life Technologies). Viral RNA levels were normalized to GAPDH or 18S RNA and expressed relative to infected, vehicle-treated controls.

2.6. VLP assembly assay

HEK-293 cells were transfected with plasmids encoding FLAG-tagged LASV Z protein, or the non-budding G2A mutant (Perez et al., 2004), using Lipofectamine 2000 (Life Technologies). Following overnight incubation, the transfection media was removed and compounds were added to a final DMSO concentration of 0.5%. After 48 h, cell lysates were prepared with radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitors (Complete Protease Inhibitor, Roche, Indianapolis, IN, USA). The culture medium was clarified by centrifugation at 4000×g for 20 min, and VLPs were concentrated from the supernatants by centrifugation at 300,000×g through a 20%
sucrose cushion. Cell lysates and pelleted VLPs were analyzed by Western blotting with anti-FLAG M2-peroxidase antibody (Sigma–Aldrich) or anti-β actin antibody (Genscript, Piscataway, NJ, USA).

2.7. Assay for LASV and EBOV glycoprotein-dependent entry

HIV pseudotyped particles bearing the glycoproteins of LASV, EBOV from the 1976 Zaire (Genbank accession No. U23187.1) and 2014 West African (KP178538.1) outbreaks, vesicular stomatitis virus (VSV) G protein, or no glycoprotein were prepared and used as previously (Flint et al., 2004). Briefly, LentiX-293T cells (Clontech, Mountain View, CA, USA) were co-transfected with plasmid DNA encoding the HIV genome containing the firefly luciferase gene (pNL4–3.Luc.R–.E–) and expression vectors encoding the viral glycoprotein or empty vector in a 32:1 ratio. Pseudotyped viruses were quantitated by determining HIV matrix protein (p24) content. Viral glycoprotein-dependent entry assays were performed using HT-1080 cells and 6 ng of p24 pseudotyped particles, with firefly luciferase activity determined using BrightGlo (Promega) 72 h post-transduction. Cell viability was concurrently determined by CellTiter-Glo (Promega) in compound-treated and mock-transduced cells.

3. Results

3.1. Identification of kinase inhibitors with activity against hemorrhagic fever viruses

We hypothesized that a cellular kinase would be essential for the replication of multiple BSL-4 viruses. Therefore, we initially tested a total of 163 kinase inhibitors, focusing on the PI3K/Akt pathway. PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PAK, p21-activated kinase; mTOR, mammalian target of rapamycin; PDK-1, 3-phosphoinositide-dependent kinase 1; GSK-3, glycogen synthase kinase 3; VEGFR, vascular endothelial growth factor receptor; EGFR, epidermal growth factor receptor; PKB, protein kinase B; n/a, not applicable.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>% infected cells</th>
<th>% cell viability</th>
<th>Reported targets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 µM</td>
<td>500 nM</td>
<td>50 nM</td>
</tr>
<tr>
<td>BEZ-235</td>
<td>14 ± 3</td>
<td>19 ± 5</td>
<td>43 ± 7</td>
</tr>
<tr>
<td>OSU-03012</td>
<td>0 ± 0</td>
<td>32 ± 9</td>
<td>94 ± 18</td>
</tr>
<tr>
<td>AZD8055</td>
<td>4 ± 3</td>
<td>17 ± 15</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>TWS119</td>
<td>24 ± 9</td>
<td>41 ± 16</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>SU 4312</td>
<td>32 ± 6</td>
<td>80 ± 19</td>
<td>83 ± 29</td>
</tr>
<tr>
<td>IBX 1382 dihydrochloride</td>
<td>8 ± 1</td>
<td>69 ± 5</td>
<td>75 ± 7</td>
</tr>
<tr>
<td>KU 8751</td>
<td>23 ± 1</td>
<td>70 ± 14</td>
<td>90 ± 18</td>
</tr>
<tr>
<td>10-DEAC hydrochloride</td>
<td>11 ± 5</td>
<td>85 ± 25</td>
<td>81 ± 7</td>
</tr>
</tbody>
</table>

a Mean % infected cells or % viability ± standard deviation from 3 replicate wells, normalized to the vehicle-only control.

b PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PAK, p21-activated kinase; mTOR, mammalian target of rapamycin; PDK-1, 3-phosphoinositide-dependent kinase 1; GSK-3, glycogen synthase kinase 3; VEGFR, vascular endothelial growth factor receptor; EGFR, epidermal growth factor receptor; PKB, protein kinase B; n/a, not applicable.

Fig. 1. OSU-03012 inhibits LASV and EBOV in cell culture. (A) OSU-03012 induced a concentration-dependent reduction in LASV replication. A549 cells were treated for 1 h with varying concentrations of OSU-03012 before infection with LASV at an MOI of 0.2. Two days post infection, the cells were fixed, permeabilized, and stained. Green, LASV proteins; blue, cell nuclei; red, cell cytoplasm. (B) A representative concentration–response curve showing the quantitation of LASV-infected cells (%) normalized to the vehicle-only control; each point is the mean of 9 fields from each of quadruplicate wells, with error bars indicating the standard deviation. Cell viability (%) of compound-treated, mock-infected cells is also shown; each point is the mean of quadruplicate wells, with error bars indicating standard deviation. (C) A representative concentration–response curve showing viability of mock-infected cells, or inhibition of GFP expression in Huh 7 cells infected with the EBOV-GFP reporter virus. Points represent mean values, and error bars indicate standard deviations calculated from 4 replicate wells.
pathway, for their ability to inhibit the replication of LASV. A549 cells were treated with compounds 1 h prior to mock-infection or infection with LASV at an MOI of 0.2. Two days post infection, the cells were fixed, permeabilized, and stained to visualize LASV proteins and the mock-infected cells were tested for viability (Supplemental Table 1). BEZ-235, a known inhibitor of LASV VLP formation (Urata et al., 2012), was used as a positive control. In addition to BEZ-235, 7 compounds had anti-LASV activity with minimal cytotoxicity (Table 1). These compounds were tested for their ability to inhibit the replication of EBOV-GFP, MARV-GFP, and NIV-luc recombinant reporter viruses, as well as AHFV and CCHFV. Two compounds, OSU-03012 (also known as AR-12) and BIBX 1382 dihydrochloride, demonstrated inhibition of both LASV and other viruses and were further characterized.

Table 2
OSU-03012 activity against selected viruses.

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Cell line</th>
<th>Multiplicity of infection</th>
<th>Duration (h)</th>
<th>EC50 (µM)</th>
<th>CC50 CellTiter-Glo (µM)</th>
<th>SI CellTiter-Glo</th>
</tr>
</thead>
<tbody>
<tr>
<td>LASV</td>
<td>A549</td>
<td>0.2</td>
<td>48</td>
<td>0.5 ± 0.1</td>
<td>5.7 ± 2.5</td>
<td>11</td>
</tr>
<tr>
<td>AHFV</td>
<td>A549</td>
<td>0.1</td>
<td>72</td>
<td>2.9 ± 0.8</td>
<td>6.5 ± 1.1</td>
<td>2</td>
</tr>
<tr>
<td>NIV-Luc</td>
<td>293T</td>
<td>0.2</td>
<td>48</td>
<td>0.4 ± 0.2</td>
<td>8.2 ± 0.9</td>
<td>21</td>
</tr>
<tr>
<td>EBOV-GFP</td>
<td>HuH7</td>
<td>0.2</td>
<td>48</td>
<td>0.3 ± 0.07</td>
<td>6.4 ± 1.1</td>
<td>23</td>
</tr>
<tr>
<td>MARV-GFP</td>
<td>HuH7</td>
<td>0.2</td>
<td>48</td>
<td>0.3 ± 0.1</td>
<td>7.1 ± 0.6</td>
<td>20</td>
</tr>
<tr>
<td>CCHFV</td>
<td>SW13</td>
<td>0.1</td>
<td>72</td>
<td>No inhibition</td>
<td>6.7 ± 0.8</td>
<td>n/a</td>
</tr>
</tbody>
</table>

a CPE, cytopathic effect; Luc, luciferase; GFP, green fluorescent protein.
b Mean EC50 and CC50 values ± the standard deviation from at least 3 independent determinations are shown.

Table 3
Activity of BIBX 1382 dihydrochloride against selected viruses.

<table>
<thead>
<tr>
<th>Assay type</th>
<th>EC50 (µM)</th>
<th>CC50 CellTiter-Glo (µM)</th>
<th>SI CellTiter-Glo</th>
<th>CC50 PrestoBlue (µM)</th>
<th>SI PrestoBlue</th>
<th>CC50 nuclei number (µM)</th>
<th>SI nuclei number</th>
</tr>
</thead>
<tbody>
<tr>
<td>LASV</td>
<td>3.2 ± 2.4</td>
<td>15.3 ± 6.7</td>
<td>6</td>
<td>21.0 ± 1.4</td>
<td>7</td>
<td>63.4 ± 16.7</td>
<td>24</td>
</tr>
<tr>
<td>EBOV-GFP</td>
<td>1.1 ± 0.6</td>
<td>19.8 ± 0.3</td>
<td>18</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MARV-GFP</td>
<td>1.8 ± 0.2</td>
<td>29.1 ± 16.4</td>
<td>19</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not done.

a Mean EC50 and CC50 values ± the standard deviation from at least 3 independent determinations are shown.

Fig. 2. OSU-03012 (A and C) and BIBX 1382 (B and D) inhibit LASV and EBOV titers in a concentration-dependent manner. Cells were in treated with varying concentrations of compound for 1 h. A549 cells were then infected with LASV at an MOI of 0.2 (A and B), and HuH7 cells were infected with EBOV at an MOI of 0.02 (C and D). Two days post infection, culture supernatants were harvested and viral titers determined in Vero-E6 cells. Mean titers are shown with error bars indicating standard errors calculated from 3 replicate wells.
Fig. 3. OSU-03012 and BIBX 1382 induce a concentration-dependent decrease in LASV RNA in multiple cell lines. (A) A549, (B) Huh7, (C) Vero-E6, and (D) HT-1080 cells were treated with the indicated compound concentrations for 1 h, and then infected with LASV at an MOI of 0.1. RNA was extracted 24 h post infection, and qRT-PCR for LASV RNA or endogenous cell RNA was performed (white bars). Mock-infected cell monolayers were processed for viability assays using CellTiter-Glo (black bars). Columns represent mean values, and error bars indicate standard deviations calculated from 8 replicate wells.
OSU-03012 was reported to be an inhibitor of the ErbB kinases, including ErbB1, the epidermal growth factor receptor (EGFR) kinase (Egeblad et al., 2001). In our study, it inhibited LASV, EBOV-GFP, and MARV-GFP, with EC_{50} values ranging 1.1–3.2 μM and SI values 6–24 (Table 3). OSU-03012 had no specific antiviral effects against NiV-Luc, CCHFV, or AHFV (data not shown).

Next, we tested the effect of OSU-03012 and BIBX 1382 on viral titers. A549 or HuH7 cells were treated with the compounds for 1 h prior to infection with LASV or wild-type EBOV, respectively. Two days post infection, the cell supernatants were harvested and viral titers were determined. Both OSU-03012 and BIBX 1382 reduced LASV and EBOV titers by 2–3 logs in a concentration-dependent manner (Fig. 2A–D) at concentrations below their CC_{50} values (compare concentrations used to CC_{50} values in Tables 2 and 3).

To further confirm the antiviral effects of OSU-03012 and BIBX 1382, we measured LASV RNA in various infected cell lines. A549, Vero-E6, HT-1080, and HuH7 cell lines were treated with the compounds to inhibit LASV and EBOV glycoprotein-dependent entry by use of HIV particles pseudotyped with the viral glycoproteins. Amiodarone, a recently reported inhibitor of EBOV-glycoprotein dependent entry, was used as a control (Gehring et al., 2014). OSU-03012 and BIBX 1382 reduced LASV RNA in each of the cell lines, with minimal effects on cell viability (Fig. 3).

### 3.3. Mechanism of action studies

Fig. 4. Effect of (A) BIBX 1382 and (B) amiodarone on EBOV and LASV glycoprotein-dependent entry. HT-1080 cells were transduced with HIV particles pseudotyped with EBOV, LASV, or vesicular stomatitis virus (VSV) glycoproteins in cell culture medium containing inhibitor compounds. Cell culture media containing the pseudotype particles and compounds were removed 6 h post transduction and were replaced with cell culture media without compound. Transduction was measured by firefly luciferase activity 3 days post transduction. Mock-transduced, compound-treated cell monolayers were processed for viability assays using CellTiter-Glo. A representative of 3 independent experiments is shown. Points represent mean values, and error bars indicate standard deviations calculated from 4 replicate wells.

<table>
<thead>
<tr>
<th>Virus glycoprotein</th>
<th>BIBX 1382</th>
<th>Amiodarone HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC_{50} (μM)^{a}</td>
<td>CC_{50} (μM)^{b}</td>
</tr>
<tr>
<td>EBOV (1976)^{b}</td>
<td>1.2 ± 0.2</td>
<td>24.3 ± 3.9</td>
</tr>
<tr>
<td>EBOV (2014)</td>
<td>1.6 ± 0.3</td>
<td>15</td>
</tr>
<tr>
<td>LASV</td>
<td>7.5 ± 3.0</td>
<td>3</td>
</tr>
<tr>
<td>VSV</td>
<td>30.3 ± 8.5</td>
<td>1</td>
</tr>
</tbody>
</table>

a Mean EC_{50} and CC_{50} values ± the standard deviation from at least 3 independent determinations are shown.

formation (Perez et al., 2004) served as a negative control. Each transfeceted cell monolayer expressed similar amounts of wild-type Z or G2A mutant regardless of treatment (Fig. 5). As expected, the G2A mutant was not secreted, while the wild-type Z protein assembled into VLPs. Neither OSU-03012 nor BIBX 1382 inhibited the release of LASV Z VLPs (Fig. 5).

4. Discussion

Host cell kinases have been implicated in the replication of several BSL-4 viruses (Linero and Scolaro, 2009; Saeed et al., 2008; Urata et al., 2012). Here, we identified 2 inhibitors of cellular kinases that inhibit multiple such viruses in cell culture.

OSU-03012 (AR-12) is a celecoxib derivative that does not inhibit cyclooxygenase-2, but has been reported to inhibit PDK-1 and p21-activated kinase (Porchia et al., 2007; Zhu et al., 2004). We tested several other reported inhibitors of PDK-1 for their ability to inhibit LASV replication, including PHT-427, BX-912, BX-795, PS 48, and GSK 2334470. None of these had a specific anti-LASV activity (data not shown), suggesting that the antiviral effect of OSU-03012 is probably not mediated through PDK-1 inhibition. It is important to note that kinase inhibitors, especially those that bind within the highly-conserved ATP binding site, frequently inhibit multiple cellular kinases. OSU-03012 was reported to induce apoptosis or autophagy (Gao et al., 2008; Johnson et al., 2005; Lee et al., 2009; Liu et al., 2013; Zhu et al., 2004) and was the subject of a phase I clinical trial in patients with advanced or recurrent solid tumors or lymphoma. Recently, OSU-03012 was reported to reduce expression of the endoplasmic reticulum chaperone HSP90 also called GRP78 or BiP) (Booth et al., 2015). Such a reduction could inhibit the assembly of enveloped viruses that rely on HSP90 activity for glycoprotein folding. HSP90 is incorporated into the EBOV virion (Spurgers et al., 2010), and knocking down HSP90 in mice conferred protection from lethal challenge with EBOV (Reid et al., 2014). Although OSU-03012 was reported to decrease the expression of NPC1 and LAMP1 (Booth et al., 2015), molecules involved in the entry of EBOV and LASV (Carette et al., 2011; Cote et al., 2011; Jae et al., 2014), it did not inhibit EBOV or LASV glycoprotein-dependent entry in our HIV pseudotype system (Fig. 4). We are currently investigating the effect of OSU-03012 on the maturation of the EBOV and LASV glycoproteins.

BIBX 1382 was reported to be an inhibitor of the ErbB kinases, including EGFRI (Egeblad et al., 2001). Further work is required to understand the mechanism by which BIBX 1382 inhibits LASV and EBOV glycoprotein-dependent entry. These two viruses appear to use different routes of entry, with LASV apparently using clathrin-mediated endocytosis and EBOV using macropinocytosis (Nanbo et al., 2010; Saeed et al., 2010; Vela et al., 2007). Since BIBX 1382 appears to inhibit the entry of both viruses, this suggests that some aspect of the entry mechanism is shared between the two. The development of BIBX 1382 was halted after a phase I study revealed low bioavailability and a dose-limiting increase of liver enzymes (Dittrich et al., 2002). It may therefore be difficult to justify repurposing this compound as a candidate VHF therapeutic.

In conclusion, we have identified 2 compounds with antiviral activity against LASV, EBOV and MARV in cell culture. Our data suggest that BIBX 1382 is an inhibitor of LASV and EBOV entry. OSU-03012 reduces LAV and EBOV virus titers and RNA levels, but does not inhibit entry or LASV Z-protein mediated assembly. We believe that testing OSU-03012 for antiviral efficacy in appropriate animal models is warranted. In addition, OSU-03012 and BIBX 1382 may be useful tools for understanding interactions between these viruses and their host cells.

Acknowledgements

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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.antiviral.2015.05.003.

References


