Ebola Virus Bayesian Machine Learning Models Enable New in Vitro Leads

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INTRODUCTION

In 2014, the outbreak of Ebola virus (EBOV) in West Africa highlighted the direct need for broad-spectrum antiviral drugs for this and other emerging viruses and remains currently relevant. EBOV was the causative agent responsible for over 11,310 deaths in 10 countries, making it one of the deadliest viral pathogens in modern human history based on the percentage fatality. Although no drug has been approved for the treatment of EBOV, multiple medium and high-throughput screens (HTS) of large molecular libraries have identified small molecules effective against EBOV in vitro (Table S1), but so far few have advanced to clinical testing. This is still important as we are currently in the midst of an EBOV outbreak in the Congo.

To date numerous compounds have been validated in vivo (mouse and or nonhuman primate), and two small molecules are in early clinical trials (BCX4430 and favipiravir). BCX4430 is an adenosine analog that inhibits RNA transcription, whereas favipiravir is a nucleotide analog that inhibits viral RNA-dependent RNA polymerase. In contrast, several antimalarials, and compounds of this class like pyronaridine and quinacrine have previously been shown to inhibit EBOV. We identified the antimalarial drug arterolane (IC_{50} = 4.53 μM) and the anticancer clinical candidate lucanthone (IC_{50} = 3.27 μM) as novel compounds that have EBOV inhibitory activity in HeLa cells and generally lack cytotoxicity. This work provides further validation for using machine learning and medicinal chemistry expertise to prioritize compounds for testing in vitro prior to more costly in vivo tests. These studies provide further corroboration of this strategy and suggest that it can likely be applied to other pathogens in the future.

ABSTRACT: We have previously described the first Bayesian machine learning models from FDA-approved drug screens, for identifying compounds active against the Ebola virus (EBOV). These models led to the identification of three active molecules in vitro: tilorone, pyronaridine, and quinacrine. A follow-up study demonstrated that one of these compounds, tilorone, has 100% in vivo efficacy in mice infected with mouse-adapted EBOV at 30 mg/kg/day intraperitoneal. This suggested that we can learn from the published data on EBOV inhibition and use it to select new compounds for testing that are active in vivo. We used these previously built Bayesian machine learning EBOV models alongside our chemical insights for the selection of 12 molecules, absent from the training set, to test for in vitro EBOV inhibition. Nine molecules were directly selected using the model, and eight of these molecules possessed a promising in vitro activity (EC_{50} < 15 μM). Three further compounds were selected for an in vitro evaluation because they were antimalarials, and compounds of this class like pyronaridine and quinacrine have previously been shown to inhibit EBOV. We identified the antimalarial drug arterolane (IC_{50} = 4.53 μM) and the anticancer clinical candidate lucanthone (IC_{50} = 3.27 μM) as novel compounds that have EBOV inhibitory activity in HeLa cells and generally lack cytotoxicity. This work provides further validation for using machine learning and medicinal chemistry expertise to prioritize compounds for testing in vitro prior to more costly in vivo tests. These studies provide further corroboration of this strategy and suggest that it can likely be applied to other pathogens in the future.

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Supporting Information

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properties. These repurposed drugs may represent a more advanced starting point for therapeutic development as compared with new chemical entities since their safety has already been clinically validated. It appears that most of the FDA-approved drugs described as having EBOV activity in vitro were not dosed in patients during the 2014 epidemic in Africa, likely due to their lack of availability or efficacy data in higher order species at the time of the outbreak and their low potency, requiring higher doses than for the original indication. A recent review summarized many of the known pharmacological interventions for EBOV and describes the mechanism of action of the treatment when known.

Computational approaches have also been used to suggest compounds to experimentally test or propose potential key features for activity. Previously, Bayesian machine learning models were developed using datasets from prior drug screens against EBOV. These Bayesian models were then used to score the MicroSource Spectrum library to predict compounds that would display anti-Ebola activity. Quinacrine, pyronaridine, and tilorone were identified using these models, and their activities were successfully confirmed in vitro as having good potency. In subsequent studies, tilorone was also shown to have 100% efficacy at 30 mg/kg/day when dosed intraperitoneal in a mouse model of Ebola infection. In vivo efficacy testing of the other two compounds is currently ongoing. The current study was initiated to find additional, novel compounds active against EBOV using a similar machine learning methodology, to provide additional evidence of how this approach can be integrated into the drug discovery paradigm for antivirals.

**RESULTS**

**Machine Learning.** The Assay Central Bayesian model for EBOV cell entry had a 5-fold cross-validation receiver operating characteristic (ROC) of 0.82, precision 0.16, recall 0.79, Specificity 0.78, F1-score 0.27, Cohen’s Kappa (CK) 0.20, and Matthews correlation coefficient (MCC) 0.29 (Figure 1A). The replication model had a 5-fold cross-validation ROC of 0.83, precision 0.36, recall 0.61, Specificity 0.97, F1-score 0.45, CK 0.43, and MCC 0.44 (Figure 1B).

To further evaluate these models, we used recent publications as a source of independent test sets. One described a set of compounds identified by another computational model and then tested in vitro which led to 14 hits with <10 μM potencies. Of these 14 compounds, 11 were also scored as active by our own computational approach using the EBOV entry model (Table S2). Out of 9 compounds, 4 compounds were found as active from another recent high-throughput screen against EBOV and Marburg (MARV), which were also predicted to be active by the same EBOV entry model (Table S3).

In summary, both 5-fold cross-validation statistics and external testing results on literature test sets not included in the EBOV entry model indicated that it was able to reliably identify actives against EBOV in vitro.

**In Vitro Testing.** Of the 7 known antimalarials tested in this study, 5 were identified as having activity against EBOV, e.g., the ozonides such as arterolane, artemether, and artesunate. The clinical antimalarial candidate cipargamin was cytotoxic at close to the concentration at which it had EBOV activity, and the antimalarial lumefantrine was inactive (Table S4 and Figure 2). Although others have previously determined that several CNS active compounds are active against EBOV, we now show that 5 antipsychotics have similar activity (Table S4 and Figure 3). In addition, we tested 5 known antivirals (including favipiravir; an EBOV treatment candidate), and these were determined to be inactive under the conditions used, apart from NCS 62914 which had activity (1.91 μM) (Table S5 and Figure 4). Various analogs of the
previously identified EBOV-active tilorone (Table S6) were all inactive at the concentrations tested, and interestingly the anticancer drug lucanthone was found to have EBOV activity (Figure 5 and Table S5). Cytotoxicity was also assessed for the compounds tested for EBOV activity, and several of the molecules (arterolane, lucanthone, fluphenazine, and cipargamin) had suboptimal selectivity indexes <5 (Table 1 and Figures S1, S2).

One of the challenges of high-throughput screening is the identification of compounds with promiscuous activities (frequent hitters). Several filters or substructural alerts for Pan assay interference compounds (PAINS) are now widely accessible. PAINS and other filter scores for the compounds selected for testing were performed (Tables S7 and S8). Out of the 12 compounds tested for PAINS, only phenothiazine and promazine were identified by the PAINS analysis, both containing the substructure het_thio_666_A flag.

## DISCUSSION

Even though the EBOV was discovered in 1976, the intensive search for potential therapeutic drugs against it is a relatively recent occurrence and has been focused on molecules that are already approved for other uses. In 2013, an assay developed previously was used to screen multiple molecular probes, FDA-approved and other drugs approved outside of the U.S. and identified several compounds with activity against Ebola Zaire virus. These molecules included selective estrogen receptor antagonists: clomiphene, toremifene, tamoxifen, and

| Molecule | Function | Compound name | EC_{50} Activity (µM) | HeLa CC_{50} (µM) |
|----------|----------|---------------|-----------------------|------------------|
| Tilorone | Antimalarial | NSC 62914 | 1.91 | > 50 |
| Lucanthone HCl | Chemotherapy Drug | | 3.27 | 11.5 |
| Artemether | Antimalarial | | 6.83 | > 50 |
| Arterolane Maleate | Antimalarial | | 4.53 | 23.2 |
| Artesunate | Antimalarial | | 8.21 | > 50 |
| Cipargamin | Antimalarial | | ~6 | 8.7 |
| Fluphenazine 2HCl | Antipsychotic | | 2.54 | 15.0 |
| Periclazine | Antipsychotic | | 4.76 | > 50 |
| Phenothiazine | Insecticide (antimalarial) | | 7.58 | > 50 |
| Pippermerone 2HCl | Antipsychotic | | 8.28 | > 50 |
| Promazine HCl | Antipsychotic | | 4.4 | > 50 |
| Ziprasidone | Antipsychotic | | 7.84 | > 50 |

Figure 3. EBOV activity of various antipsychotic compounds using EBOV-infected HeLa cells 24 h post-infection.

Figure 4. EBOV activity of various antiviral compounds using EBOV-infected HeLa cells 24 h post-infection.

Figure 5. EBOV activities of tilorone and similar compounds using EBOV-infected HeLa cells 24 h post-infection.
raloxifene as well as diethylstilbestrol. Several estrogen receptor agonists also had EBOV antiviral activity. The same group screened a library of 2600 drugs in vitro and found 30 compounds selected for further testing, and 4 compounds were followed up in vivo. Kouznetsova et al. screened 600 FDA-approved drugs in an assay that identifies compounds that block Ebola virus-like particles (VLP) entry into host cells and identified 23 compounds including microtubule inhibitors. A screen of 1280 FDA-approved drugs using a pseudovirus, based on the Zaire strain of Ebola virus with a GP/HIV core containing firefly luciferase reporter gene, identified teicoplanin and toremiphene as EBOV inhibitors. In short, many groups have used high- and medium-throughput screens to identify approved drugs and additional compounds. These may now number several hundred potential compounds which provides the opportunity to build and test computational models for predicting new anti-EBOV small molecules.

For some of these molecules, the mechanism has been studied and partially identified, with some having various direct viral effects and or some indirect host effects. Several molecule types with dual action against EBOV have been shown to modulate the innate immune system, such as topoisomerase II inhibitors, which both induce interferon responses and suppress replication of the virus. Other compounds could affect the viral life-cycle, including the assembly and or budding of the virus. The various high-throughput screens have identified FDA-approved drugs belonging to numerous classes (G-protein coupled receptor (GPCR) antagonists, the selective estrogen receptor modulators, antidepressants, L-type calcium channel inhibitors, antimalarials, with in vitro and/or in vivo growth inhibitory activities against EBOV. For example, three FDA-approved ion channel blockers (amiodarone, dronedarone, and verapamil) were found to have μM EBOV cellular entry inhibition in vitro. Several of these compounds had been shown to be effective against multiple filoviruses, and the antiviral concentrations needed in vitro correlate well with in vivo tolerated doses. Interestingly, all of these compounds have a common tertiary amine feature. In addition, some GPCR antagonists, including histamine neurotransmitter receptors, are potent, selective EBOV entry inhibitors. The principal conclusion of this is there are many different classes of EBOV inhibitors that potentially have very different mechanisms of action. The best pathway(s) to target is currently underetermined, which validates the continual search for new inhibitory compounds.

We had previously generated Bayesian models for Ebola datasets which we used to select pyranoazine, quinacrine, and tilorone from the MicroSource compound library for in vitro testing. These compounds scored highly and were not in the machine learning model training sets. In vitro testing identified that these three compounds possessed EC_{50} values of between 230 and 420 nM, much lower than the positive control chloroquine (EC_{50} 4.0 μM). Repeated testing of these compounds for this study suggested slightly higher EC_{50} values (Table S6) than originally reported, but all possessed low μM potency and likely reflects the different cell types used. A recent EBOV computational modeling publication used a combinatorial methodology, and an initial computational filtering of ~17 million compounds using EBOV activity and cytotoxicity (HeLa/HEK) models led to 14 hits with <10 μM potencies using a pseudovirus assay. Five of these hits were further evaluated in a live green fluorescent protein (GFP)-virus model and were shown to have dose−response inhibition against EBOV infection. Vindesine, an anticancer microtubule inhibitor, was found to be the most potent, confirming previous work showing that microtubule inhibitor compounds are active against EBOV. Most of the compounds identified in this study were similar, based on Tanimoto similarity scores (T_s), with previous hits founds in the literature. However, six of the compounds had novel structures as compared to other known active compounds (T_s < 0.75). Our Laplacian-corrected Naive Bayesian classifier models were used to score these hits. Importantly, 11 out of the 14 compounds scored as active in our EBOV entry blocking model (Table S2), built with ECFP6 descriptors (842 compounds, 23 active) based on our recently published data of inhibition of pseudotype virus (GP-pseudotyped vesicular stomatitis virus-encoding (VSV) firefly luciferase). Of these molecules, vindesine scored the highest. Our group recently screened 319 855 small molecules against MARV pseudovirus and identified 9 compounds active against Ebola and MARV. When we used our previously developed EBOV machine learning models to predict these same molecules, we identified 4 of the 9 compounds (that were absent from the training set) as active (Table S3). These results suggest that our Bayesian machine learning models can identify known active compounds that are absent from the training set used and in addition would have found many of the compounds identified by others.

The current study expands utilization of the machine learning approach. We report the identification of novel EBOV inhibitors with desirable activities (EC_{50} < 10 μM and cytotoxicity >50 μM) as validation of this approach. Several of the compounds we identified, demonstrated promising activity against EBOV, although some had suboptimal cytotoxicity. The compounds identified have been approved for other indications or are in clinical trials. For example, lucanthone is an inhibitor of the DNA repair enzyme apurinic/apyrimidinic endodeoxyribonuclease (APEX1) and acts as an antineoplastic agent. Arterolane is an antimalarial agent and is used as a part of a combination treatment with piperazine and has not previously been shown to possess antiviral activity alone.

We have previously analyzed HTS datasets for malaria and tuberculosis using chemical rules as filters for flagging of undesirable molecules, false positives, and frequent hitters from HTS screening libraries. In the current study, we used several of these methods for determining PAINS and frequent hitters (Tables S7 and S8) and have identified phenothiazine and promazine as the only compounds suggested to be potentially problematic out of the compounds selected by the machine learning model. It should be noted that all of the molecules proposed here are approved drugs or clinical candidates. Although PAINS filters may be useful for HTS using enzymes, receptors, etc., it remains to be seen whether they are as relevant for the repurposing of these compounds against a different target. These are already approved drugs or clinical candidates which have attained some degrees of validation in vitro and in vivo, which may negate any concerns about their PAINS status.

Our results suggest that machine learning can be used to identify active compounds and evaluate novel chemistries before expanding time and resources on synthesis or purchase. An example is artemolane, which was tested along with another clinical antimalarial candidate cipargamin. An initial search scored two other clinical stage compounds (KAF156 and OZ439) as likely actives against EBOV. We were unable to
purchase these compounds, so arterolane, a molecule sharing a similar scaffold, was evaluated. Although our model initially predicted arterolane as inactive, the prediction of the analogous clinical stage compounds justified the in vitro testing (Table S4). Arterolane also led to the selection and EBOV testing of other commercially available antimalarials, such as artemisinin and analogs. As arterolane is dosed as a combination therapy with piperazine, we also tested this for EBOV activity, and found that this compound was inactive (Table S4). Pyronaridine, an antimalarial active against EBOV (Table S6), is used in combination with artesunate, and we have now shown that this latter molecule has an IC_{50} of 8.2 μM. Although not as active as pyronaridine, it might suggest some utility in using this, as it is already available and approved in the EU as a combination therapy. These examples show that several of these compounds have poor scores with the machine learning model, but they are still active in vitro, which demonstrates both limitations of the model and also opportunities for further refinement and learning.

There is scarce clinical data on the use of antimalarial combination therapy during an EBOV outbreak apart from a study, which showed that artesunate–amodiaquine patients had reduced mortality vs artemether–lumefantrine. The basis for testing the amodiaquine regimen was the prior discovery of its activity in vitro with an IC_{50} of 2.6 μM for the inhibition of virus entry in cells. Interestingly, our in vitro data suggests that both artesunate and artemether are similarly active (8.21 and 6.83 μM, respectively), and this may account for the decreased mortality and better clinical outcome for the amodiaquine–artesunate combination. Accessibility for physical testing is clearly important. The availability of other antimalarials, such as artemisinin and analogs, is dosed as a combination therapy, and thus the advantage of knowing that the compound has in vivo potential, whereas pseudovirus or other assays with modified virus or VLP will still require whole cell assays before proceeding to in vivo. Our approach therefore offers perhaps a more direct approach for getting to in vivo testing by utilizing a whole cell infection model (e.g., ebola virus replication model). This also ensures that we may capture compounds that have host effects as well as direct effects on the virus which in vitro pseudovirus and VLP entry would fail to capture. This may also be important as it becomes increasingly recognized how EBOV manipulates the immune response in macrophages, and we may need to identify compounds that have an effect on the host.

In conclusion, our data have shown how Bayesian machine learning models identified hits in several recently published HTS and may assist us in identification of further active compounds against EBOV, building on our earlier work in this area.

## EXPERIMENTAL SECTION

### Chemicals and Materials.

Ziprasidone (TCL, Portland OR), pipamperone 2HCl, NSC 62914, oseltamivir phosphate, 2,7-dinintro-9-fluorenone, fluorenone (Sigma, St. Louis, MO), luctanone HCl, pericazaine, promazine HCl, phenothiazine, fluphenazine, artesether, artesunate, favipiravir (7-TOS) (TRC Canada, North York, ON, Canada), lumefantrine, cinpargmin (MCE, Monmouth Junction, NJ), arterolane maleate (Clear synth, Mississauga, Ontario, Canada), piperazine phosphate tetrahydrate (Avachem, San Antonio, TX), 7-deaza-2′‘-C-methyladenosine (MK-608), ayclovir, pyronaridine tetraphosphate, tilorone, quinacrine (Cayman Chemical, Ann Arbor, Michigan), 2,7-dihydroxy-9H-fluorene-9-one (OxChem, Wood Dale, IL) and were dissolved in either dimethyl sulfoxide or water as 10 mM stock solutions and were stored at −20 °C. The nucleus staining dye, Hoechst 33342, CellMask Deep Red cytoplasmic/nuclear stain, NHS-Alexa-488 dye, the Dual-Glo Luciferase Assay System, and CytoTox 96 assay kit were purchased from Promega (Promega, Madison, WI). The modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay Cell Counting Kit 8 was procured from Dojindo Molecular Technologies (Gaithersburg, MD). MTT assays were performed in 96-well high-content imaging plates from BD (BD Biosciences, Franklin Lakes, NJ), and 96-well white-walled tissue culture plates were from Corning (Corning Life Sciences, MA). Plates were read using the Opera QHES confocal imaging reader, Acapella and Definiens image analysis packages were purchased from PerkinElmer (PerkinElmer).

### Training Datasets.

A total of 842 molecules from previously reported screens for inhibitors of the viral pseudotype entry and EBOV replication were used to construct the computational models. Compounds with reported IC_{50} values less than 50 μM were selected as actives. This high threshold for activity was set, as there are relatively few active compounds in these two datasets. Entry and replication models possess 43 and 23 active compounds, respectively.

### Assay Central.

We utilized Assay Central to prepare and merge datasets collated in Molecular Notebook as well as generate Bayesian models of either training data alone or combined with testing data, using the ECFP6 descriptor. The Assay Central project uses the source code management system Git to gather and store molecular datasets from diverse sources, in addition to scripts for curating well-defined structure–activity databases. These scripts employ a series of rules for the detection of problem data that is
corrected by a combination of automated structure standardization including removing salts, neutralizing unbalanced charges, merging duplicate structures with finite activities, and identifying advanced problems resolved by human recuration. Each model in Assay Central includes the following metrics for evaluative predictive performance: recall, precision, Specificity, F1-Score, receiver operating characteristic (ROC) curve, Cohen’s Kappa (CK), and the Matthews correlation coefficient (MCC). For the metric definitions during 5-fold cross-validation, we will use the following abbreviations: the number of true positives (TP), the number of false positives, the number of true negatives (TN), and the number of false negatives. Specificity or the TN rate is defined by the percentage of false class labels correctly identified by 5-fold cross-validation (specificity = \( \frac{TN}{TN + FP} \)). Model recall also known as sensitivity, or the true positive rate (TPR), is the percentage of positive class labels (i.e., compound is active at a target) correctly identified by the model out of the total number of actual positives and is defined: recall = \( \frac{TP}{TP + FN} \). Precision, also known as the positive predictive value, is the percentage of positive class labels correctly identified out of total predicted positives and is defined: precision = \( \frac{TP}{TP + FP} \).

The F1-Score is simply the harmonic mean of the recall and precision: \( F_1 = \frac{2 \cdot \text{precision} \cdot \text{recall}}{\text{precision} + \text{recall}} \). The ROC curve can be computed by first plotting the TPR versus the false positive rate (FPR) at various decision thresholds, \( T \), where FPR = \( \frac{FP}{FP + TN} \). All constructed models are capable of assigning a probability estimate of a sample belonging to the positive class. The TPR and FPR performance are measured when we consider a sample with a probability estimate > \( T \) as being true for various intervals between 0 and 1. The AUC can be computed from this plot and can be interpreted as the ability of the model to separate classes, where 1 denotes perfect separation and 0.5 is random classification. Accuracy is the percentage of correctly identified labels (TP and TN) out of the entire population: accuracy = \( \frac{TP + TN}{TP + FN + TN + FP + FN} \). Cohen’s Kappa (CK), attempts to leverage the accuracy by normalizing it to the probability that the classification would agree by chance (\( p_c \)) and is calculated by CK = \( \frac{\text{accuracy} - p_c}{1 - p_c} \), where

\[
\begin{align*}
    P_c &= P_{\text{true}} + P_{\text{false}}' \\
    P_{\text{true}} &= \frac{TP + FN}{TN + FN} \\
    P_{\text{false}}' &= \frac{TP + FN}{TN + FN} \\
    P_{\text{false}} &= \frac{TP + FN}{TN + FN} \\
    P_{\text{false}}' &= \frac{TP + FN}{TN + FN} \\
    P_{\text{false}} &= \frac{TP + FN}{TN + FN} \\
    \end{align*}
\]

F1-Score, receiver operating characteristic (ROC) curve, Cohen’s Kappa (CK), Kappa (CK), 76,77 and the Matthews correlation coefficient (MCC).78

**In Vitro Testing.** Recombinant, infectious EBOV encoding GFP was used for testing efficacy of compounds and was originally provided by Dr Heinz Feldmann, Rocky Mountain Laboratories. The strain that was used has the GFP gene inserted between the VP30 and VP24 genes. All viral infections were done in the BSL-4 lab at Texas Biomedical Research Institute. Briefly, 4000 HeLa cells per well were grown overnight in 384-well tissue culture plates, the volume of culture medium was 25 \( \mu \)L. On the day of assay, test compounds were diluted to 1 mM concentration in complete medium, mixed in equal volume to medium overlaying the cells, and then serially diluted a total of 12 times in triplicate. Treated cells were then incubated at 37 °C in a humidified CO2 incubator for 1 h. Final concentrations of 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.9, 0.97, 0.48, 0.24, and 0.12 \( \mu \)M were achieved upon addition of 25 \( \mu \)L of infection mix containing Ebola-GFP virus, Bafilomycin at a final concentration of 10 nM was used as a positive control drug. Infections were done to achieve a MOI of 0.05–0.15. The virus-challenged cells were incubated for 24 h. Post-infection for 24 h, cells were fixed and activated by immersing the plates in formalin for 24 h at 4 °C. Plates were washed 3× with PBS. EBOV-infected cells were stained for nuclei using Hoechst at 1:50 000 dilution, and plates were imaged. Nuclei (blue) and infected cells (green) were counted using the CellProfiler software. Total number of nuclei (blue) was used as a proxy for cell numbers, and a loss of cell number was assumed to reflect cytotoxicity. Concentrations in which total cell numbers were 20% less than the control were rejected from analysis.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02948.

In Vito screening of drugs from various types and their hit rates (Table S1); assay central Bayesian model predictions for compounds (Table S2); cytotoxicity activity (24 h) of various antimalarial compounds using HeLa cells (Figure S1); cytotoxicity activity (24 h) of various antipsychotic compounds using HeLa cells (Figure S2) (PDF)

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Notes

The authors declare the following competing financial interest(s): T.L., K.M.Z., and S.E. works for Collaborations Pharmaceuticals, Inc. A.M.C. consults for Collaborations Pharmaceuticals, Inc. M.L. was employed by Collaborations Pharmaceuticals, Inc.
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**ABBREVIATIONS USED**

ADME, absorption, distribution, metabolism, and excretion; CK, Cohen’s Kappa; CNS, central nervous system; EBOV, Ebola virus; FDA, Food and Drug Administration; GPCR, G-protein coupled receptor; HTS, high-throughput screens; ip, intraperitoneal; MARV, Marburg; MCC, Matthews correlation coefficient; PAINS, Pan assay interference compounds; ROC, receiver operating characteristic; VLP, virus-like particles; VSV, vesicular stomatitis virus–encoding

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