Supporting Information

Discovery and Structural Optimization of 4-(Aminomethyl)benzamides as Potent Entry Inhibitors of Ebola and Marburg Virus Infections

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Table of content:

CYP450 Inhibition Assay

Inhibitory activity of the compounds for CYP450 enzymes was evaluated using the Vivid® CYP3A4 Green and CYP2C9 Green Screening Kits by ThermoFisher Scientific according to manufacturer's protocol. Briefly, test compounds/DMSO were prepared at 250 µM in 1X Vivid CYP450 Reaction Buffer and were serially diluted two- or three-fold for 9-11 times with the Reaction Buffer to achieve 2.5X of the desired final concentrations. 40 µl of the 2.5X compound solutions was transferred into the wells of the 96-well black plates. 50 µl of the Master Pre-Mix including P450 BACULOSOMES® Plus Reagent and Vivid® Regeneration System in the Reaction Buffer was subsequently added to each well and mixed on a rocking shaker for 10 minutes at room temperature (RT). After 10 minutes of incubation, 10 µl of a mixture of Vivid® Substrate and Vivid® NADP+ was added to each well to start the CYP450 reaction. Plates were placed on a rocking shaker for 25 minutes at RT and fluorescent signals were then immediately measured using a fluorescent micro-plate reader. Final concentrations of 100 µM to 0.39 µM or 100 µM to 0.0017 µM of test compounds with 1% DMSO were achieved for calculation of IC_{50} values. Final concentrations of 10µM ketoconazole and 30µM sulfaphenazole were used as positive control inhibitors for CYP3A4 and CYP2C9, respectively. 1% DMSO was used as a negative control.

***In vitro* Stability in Plasma.**

Stability of the test compounds was studied in human (Human Plasma K2EDTA Pooled Gender HUMANPLK2P2N) and rat (Sprague Dawley Rat Plasma K2EDTA Pooled Gender RAT00PLK2P2N) plasma received from BioIVT. The plasma (80 µl) from each species was placed in 1.5-ml microcentrifuge tubes (for each compound in duplicates) and pre-warmed to 37 °C. The reactions were initiated by the addition of 20 µl of 100 µM solution of the test compounds

in a 0.1 M potassium phosphate buffer (pH 7.4) to each tube to yield a final concentration of 20 µM (final DMSO concentration is 1%). The assays were performed in a shaking water bath at 37 °C and conducted in duplicate. Samples (40 µl) were taken at 0 and 60 min and added to 80 µl of cold acetonitrile containing IS (10 µM). The samples were subjected to vortex mixing for 30 seconds and then centrifugation at 4°C for 15 min at 14,000 rpm. The clear supernatants were analyzed by HPLC.

***In vitro* Stability in Liver Microsomes.**

The metabolism of test compounds was assessed by substrate depletion methodology (percent of test article remaining), using human (X008070 InVitroCYP 150-D HLM, Mixed Gender) and rat (M00011 Male Fischer 344 Rat) liver microsomes received from BioIVT. To 65 µl of a potassium phosphate buffer (0.1 M, pH 7.4) were added 5 µL of microsomes (20 mg/mL) and 10 µl of NADPH (10 mM solution in potassium phosphate buffer) and the mixture was pre-incubated (5 min) at 37 °C. The reactions were initiated by the addition of 20 µl of 100 µM solution of the test compounds in a 0.1 M potassium phosphate buffer (pH 7.4) to each tube to yield a final concentration of 20 µM (final DMSO concentration is 1%). The assays were performed in a shaking water bath at 37 °C and conducted in duplicate. Samples (40 µl) were taken at 0 and 60 min and added to cold acetonitrile (80 µl) containing IS (10 µM). The samples were subjected to vortex mixing for 30 seconds and then centrifugation at 4°C for 15 min at 14,000 rpm. The clear supernatants were analyzed by HPLC.

HPLC Methods.

HPLC was performed using a Shimadzu LC-20AD HPLC system consisting of a UV/VIS detector (SDP-20AV), degasser (DGU-20A), and an autosampler (SIL-HTA) using a Poroshell 120 EC-

C18 column (3 × 50 mm, particle size 2.7 μm) with detection at 254 and 280 nm; flow rate =0.8 mL/min; gradient of 20−95% acetonitrile in water (both containing 0.1 vol % of FA) in 6 min.

Generation of Plasmids with Single-point Mutations in Zaire Ebolavirus Glycoprotein.

Plasmid containing the Zaire Ebolavirus glycoprotein gene was mutated using the Agilent Technologies QuickChange Lightning Site-Directed Mutagenesis Kit (Cat. No. 210518). First, primer pairs containing the mutated DNA sequence of interest were designed using Agilent Technologies web-based QuickChange Primer Design program and purchased through Sigma-Aldrich. Mutant strand synthesis was performed using a thermocycler with 125 ng of each primer and 100 ng of plasmid containing wild-type Zaire Ebolavirus glycoprotein. All other reagents were at the concentrations and amounts described in the QuickChange Lightning Site-Directed Mutagenesis Kit manual. Amplified plasmid was then sequenced to ensure the correct mutation was made and no additional mutations were acquired.

Computer modeling. The X-ray crystal structure of Ebola glycoprotein trimer in complex with toremifene (PDB 5JQ7) was used. The protein was optimized using the Protein Preparation Wizard implementation in the Schrödinger suite. All water molecules were removed from the protein beyond 5 Å from heteroatoms and hydrogen atoms were added by the docking program. To generate binding poses, compounds were docked into the binding site using Glide (Schrödinger, Maestro 11.9.Ink). The distances that determine the displayed interactions are consistent with the default values that are used in Maestro for (a) Hydrogen bonds: The maximum distance is 2.5 Å the minimum donor angle is 120.0°, and the minimum acceptor angle is 90.0°; (b) Pi-pi stacking: A pi-pi interaction is defined as an interaction between two aromatic rings in which either (a) the angle between the ring planes is less than 30° and the distance between the ring centroids is less than 4.4 Å (face-to-face), or (b) the angle between the ring planes is between 60° and 120° and the

distance between the ring centroids is less than 5.5 Å (edge-to-face); (c) Pi-cation interaction: The maximum distance between the cation center and the ring center is 6.6 Å and the angle between the ring plane and the line between the cation center and the ring center does not deviate from the perpendicular by more than 30° (https://www.schrodinger.com/kb/1556)