Product name:	1,8-Cineol
In vitro (Ebola):	Yes
Comments:	Screening result for entry of beta-lactamase containing Ebola VLPs in to HeLA cells loaded with beta-lactamase substrate CCF2-AM.

Data From: NCATS

Data:

Compound ID	Compound Name	Maximal inhibition (%)	Concentration-1 (M)	Inhibition (%) Concentration-1	Concentration-2 (M)	Inhibition (%) Concentration-2	Concentration-3 (M)	Inhibition (%) Concentration-3	Concentration-4 (M)	Inhibition (%) Concentration-4
NCGC00091666-04	1,8-Cineol	4.11	5.75e-005	4.11	1.15e-005	1.3	2.3e-006	1.76	4.6e-007	-2.84

Method:

qHTS Assay for Identifying Compounds that block Entry of Ebola Virus

Description:

The current outbreak of Ebola virus disease in West Africa has escalated to a never before seen scale. As the outbreak continues, exportations of virus infections to Europe and to the United States have taken place, accompanied in some cases by secondary infections. Currently, there is no proven effective treatment for Ebola virus infection. Although antibody-based therapy has been shown to be effective in a macaque model and has been used for the treatment of a few patients, the current supply of such drugs is very limited. The estimated mortality rate of the current Ebola outbreak is around 70%, which is dramatically high. A specific vaccine might eventually control the Ebola virus infection, but its development and deployment may take some time. Drug repurposing screens that identify approved drugs with the potential for new indications might be a good approach to rapidly discover and develop anti-ebola virus drugs for the treatment of patients with Ebola virus infection.

We have developed a BSL-2 1536-well plate assay to screen for entry inhibitors of Ebola virus-like particles (VLP) containing the GP protein and the matrix VP40 protein fused to a beta-lactamase reporter

protein. HeLa cells are loaded with the beta-lactamase substrate CCF2-AM that turns HeLa cells to green fluorescence. After the VLP is loaded into cells, Bla hydrolyzes the substrate CCF2-AM disrupting the Fluorescence Resonance Energy Transfer (FRET) in the substrate and thus turning cells to blue fluorescence. The ratio of blue/red fluorescence intensities represents the activity of Bla inside cells. We have miniaturized this assay into 1536-well plate format for high throughput screening (HTS). We were also able to eliminate the washing steps by using a dye quenching solution in the beta-lactamase loading buffer. This assay was applied for a quick drug repurposing screen of 600 FDA-approved drugs and additional 2216 drugs from our NPC collection that also contains experimental and animal drugs.

Protocol:

This 1536-well plate assay was adapted from the original 6-wells assay with a modification that eliminated cell wash steps (Kouznetsova et al., 2014). HeLa cells were plated at 750 cells/well in 3 µl of assay medium (DMEM + 10% FBS) in 1536-well assay plates and incubated for 16 hours at 37°C and 5% CO_2. Compounds in the 1536-well drug source plates were added to the 1536-well assay plates in a volume of 23 nl/well via a NX-TR pintool station (WAKO Scientific Solutions, San Diego, CA). Following 1-hour incubation at 37°C with 5% CO_2, 1 µl/well of VLP solution was added to the assay plates using a BioRapTR FRD dispenser (the VLP solution was diluted in Opti-MEM to a final concentration of 1:16). Plates were then spinoculated by centrifugation at 1500 rpm at 4°C for 45 minutes followed by incubation at 37°C with 5% CO_2 for 4.5 hours. The CCF2-AM beta-lactamase substrate was prepared at 6x concentration following the manufacturer's instruction which was added to assay plates at 1 µl/well. Following a 2-hour incubation at room temperature, dual fluorescence intensities (Ex_1 = 405±20, Em_1 = 460±20, and Ex_2 = 405±20, Em_2 = 530±20 nm) were measured in an EnVision plate reader (PerkinElmer, Boston, MA). The ratio of fluorescence intensities (Em_1/Em_2) was calculated to represent the beta-lactamase activity that is proportional to the amount of VLP entry into the host cells.

Compounds with maximal inhibition < 50% were considered negative.

Reference

Kouznetsova J, Sun W, Martínez-Romero C, Tawa G, Shinn P, Chen CZ, Schimmer A, Sanderson P, McKew JC, Zheng W, and García-Sastre A. (2014) Identification of 23 compounds that block Ebola virus like particle entry from a repurposing screen of approved drugs. Emerging Microbes and Infections 3, e84; doi:10.1038/emi.2014.88