

cent of the infected cells in each well. The average percent infected in each well was calculated from 9 individual spots read in each well. The percent infected data from the drug-treated wells were then normalized to the control by setting the percent infected cells in control wells (average of three wells) as 100 and calculating the efficiency of infection in drug-treated wells as percent of control infection according to the following equation:

$$\% \text{ of control infection} = \left(\frac{\% \text{ infected in experimental well}}{\text{mean } \% \text{ infected in control wells}} \right) \times 100$$

[0184] The data were then graphed by plotting % of control infection on Y-axis against compound concentration on the X-axis and is shown in FIG. 10A.

[0185] B. To determine if pre-treatment of cells with the NSC 369723 increases their antiviral activity, i.e. induces an antiviral state in the cells, the following experiment was conducted. Vero E6 cells were plated on 96 well assay plates (5×10^4 cells/well) in 100 μ l cEMEM medium. The cells were cultured for 3 days at 37° C. in a humidified incubator (5% CO₂). Three sets of 24 wells were labeled on the plated as:

[0186] 1st set: wells 1-24

[0187] 2nd set: wells 25-48

[0188] 3rd set: wells 49-72

[0189] Media were removed from the wells and replaced by 100 μ l of media containing NSC 369723 at various concentrations: 0, 0.31, 0.63, 1.25, 2.50, 5.00, 10.00, and 20.00 μ M in the following manner: The first three wells of the first set received medium only (0 concentration), followed by the next 3 wells of the first set receiving 0.31 μ M, and this was continued in the same manner until the 8th three wells of the first set received the 20 μ M compound dilution. All the wells in the second and third set only received medium. The cells were then incubated again in the incubator at 37° C. for an additional 24 hours.

[0190] Then the media on the second and third sets were removed and replaced with compound dilutions in the same manner as described above for the 1st set. Cells were then incubated again in the incubator at 37° C. for additional 24 hours. Then the media on 1st and 2nd sets were removed. The media on the first two sets was replaced with compound free medium. After adding 50 μ l of GFP-EBOV (10^6 infectious virus particles per ml) to each well, the cells were incubated at 37° C. for 1 hour. Then the media in wells of all three sets was removed and cells were washed with 200 μ l PBS three times. Medium without compound as added to the first and second set wells. For the third set of wells, the compound concentrations as described above were added in the same manner. In this way the following treatment profile was achieved:

[0191] 1st Set: Received compound dilutions 48 hour before infection but not during or after infection.

[0192] 2nd Set: Received compound dilutions 24 hour before infection but not during or after infection.

[0193] 3rd Set: Received compound dilutions 24 hour before infection as well as during and after infection.

[0194] The cells were incubated for an additional 40 to 48 hours. Then the media was aspirated and the assay plates with the cells were submerged in 10% buffered formalin (VT450D, ValTech Diagnostics, Pittsburg, Pa.) in plastic bags (one assay plate per bag) to inactivate the virus. The bags were sealed and incubated at room temperature for three days.

Then, the formaldehyde solution was removed from the assay plates and replaced with 100 μ l of PBS/well. The assay plates were then subjected to high throughput screening as described above to measure the percent of the infected cells in each well. The average percent infected in each well was calculated from 9 individual spots read in each well. The percent infected data from the drug-treated wells were then normalized to the control by setting the percent infected cells in control wells (average of three wells) as 100 and calculating the efficiency of infection in drug-treated wells as percent of control infection according to the following equation:

$$\% \text{ of control infection} = \left(\frac{\% \text{ infected in experimental well}}{\text{mean } \% \text{ infected in control wells}} \right) \times 100$$

[0195] The data were then graphed by plotting % of inhibition on Y-axis against compound concentration on the X-axis and is shown in FIG. 10B.

[0196] C. To determine if pre-treatment of the virus (not cells) with NSC 369723 and/or NSC 294202 results in antiviral activity, the following experiment was conducted. Because of the carry-over of the compounds to the cells there is some compound also in the culture and those concentrations are indicated on the X axis of FIG. 10C in red. The serial dilution results in decreasing multiplicity of infection (MOI). The resulting MOIs are indicated on the X axis of FIG. 10C in black. Vero E6 cells were plated on 96 well assay plates (5×10^4 cells/well) in 100 μ l cEMEM medium. The cells were cultured for 3 days at 37° C. in a humidified incubator (5% CO₂).

[0197] 100 μ l of a stock of GFP-EBOV containing 10^8 pfu/ml was incubated for 30 minutes at room temperature with 100 μ M concentration of either NSC 369723 (Tube 1) or NSC 294202 (Tube 2) or dimethyl sulfoxide (DMSO; the solvent of the drugs) as control (Tube 3). After the incubation the treated virus in each tube was diluted in medium to a total volume of 200 μ l. This resulted in a compound concentration of 50 μ M in Tube 1 and Tube 2. Seven serial dilutions were made. Then 33 μ l of each dilution was added to triplicate wells of cells. Since the wells already contained 100 μ l medium, a 1:4 dilution of the compound was achieved, thereby resulting in the following compound concentrations and MOIs:

	Drug μ M	MOI
Dilution 1:	12.5	1
Dilution 2:	6.25	0.5
Dilution 3:	3.13	0.25
Dilution 4:	1.56	0.13
Dilution 5:	0.79	0.065
Dilution 6:	0.39	0.033
Dilution 7:	0.2	0.0165
Dilution 8:	0.1	0.00825

[0198] The cells were then incubated at 37° C. for 48 hours. Then the media was aspirated and the assay plates with the cells were submerged in 10% buffered formalin (VT450D, ValTech Diagnostics, Pittsburg, Pa.) in plastic bags (one assay plate per bag) to inactivate the virus. The bags were sealed and incubated at room temperature for three days. Then, the formaldehyde solution was removed from the assay plates and replaced with 100 μ l of PBS/well. The assay plates were then