Antiviral Efficacy Assay

Sponsor: Dr. Raghavan
Start date: 12 April 2015
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Compound: Metadichol (USU-2239)

Method:

Sample were tested using standard neutral red assays with 8 concentrations. Compound was eluted to 1000 ug/mL in MEM then prepared in eight half-log dilutions in MEM or DMEM solution with 2% FBS and 50 µg/mL gentamicin. Each dilution was added to 5 wells of a 96-well plate with 80-100% confluent cells. After 24 hrs, three wells of each dilution were infected with an equal volume of virus, and two wells had MEM solution added as toxicity controls. A known active drug was tested in parallel as a control. After untreated virus control wells reached maximum cytopathic effect (CPE), plates were stained with neutral red dye for approximately 2 hours, then supernatant dye was removed and the incorporated dye was extracted in 50:50 Sorensen citrate buffer/ethanol, then read on a spectrophotometer. OD values were normalized based on cell and virus controls, then the EC50 (50% effective concentration) and CC50 (50% toxic concentration) was calculated by regression analysis.

Table 1. Antiviral results of NanoRx compound vs virus.

Virus	Cell Line	EC50	IC50	SI
Adenovirus-5	A549	> 9.9	9.9	0
Tacaribe	vero	2.8	7.3	2.6
Rift valley fever	vero	> 8.4	8.4	0.0
SARS	vero	1.6	6.7	4.2
Powassan[a]				
Japanese encephalitis	vero	> 7.2	7.2	0
West Nile	vero	> 8.5	8.5	0
Yellow Fever	vero	> 5.0	5.0	0

was reported.

CC_{50} = 50% toxic concentration of compound without virus added

EC_{50} = 50% effective antiviral concentration

SI = CC_{50}/EC_{50}

Units are ug/mL unless noted

[a]Powassan showed some slight activity, but controls were not within specification so a retest is in process.

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