

analysis of cell viability and compound cytotoxicity. This reagent is a stable, single solution that does not require preparation before use. At termination of the assay, 20-25  $\mu\text{L}$  of MTS reagent is added per well and the microtiter plates are then incubated for 4-6 hrs at 37° C., 5% CO<sub>2</sub> to assess cell viability. Adhesive plate sealers were used in place of the lids, the sealed plate was inverted several times to mix the soluble formazan product and the plate was read spectrophotometrically at 490/650 nm with a Molecular Devices SPECTRA-max plate reader.

#### Assay Results

**[0043]** The PBMC data were normalized by dividing by either the average control, infected, untreated value for the infection measurements (% Viral Control) or by the control, uninfected, untreated value for the cytotoxicity measurements (% Cell Control). The normalized values were then analyzed for IC<sub>50</sub> (50% inhibition of virus replication), CC<sub>50</sub> (50% cytotoxicity), and therapeutic index values (TI=CC/IC; also referred to as Antiviral Index or AI).

**[0044]** Cohex was tested for antiviral efficacy against one CXCR4-tropic HIV-1 isolate and one CCR5-tropic HIV-1 isolate in PBMCs. For this study PBMCs were pre-treated with the compound for two hours prior to infection. FIG. 2 illustrates the decrease in RT activity (left), as a measure of viral activity, or uninfected cell viability (right) for HIV-1 NL4-3 isolate. FIG. 3 illustrates of the decrease in RT activity (left), as a measure of viral activity, or uninfected cell viability (right) for HIV-1 Ba-L isolate. In these Figures, “% VC” means “% Virus Control” and “% CC” means “% Cell Control.” The results of the testing are summarized in Table 1.

**[0045]** Cohex displayed definite antiviral activity against the virus isolates evaluated in this study, with an average IC<sub>50</sub> value of 31.2  $\mu\text{M}$ . There did not appear to be any difference in the activity of the compound based on co-receptor tropism, as the compound had approximately equal activity against both virus isolates tested. Cytotoxicity was observed with the compound at concentrations above 100  $\mu\text{M}$  (TC<sub>50</sub>=833  $\mu\text{M}$ ), resulting in an average Therapeutic Index value of 26.7. These results can be summarized with IC<sub>50</sub>, CC<sub>50</sub>, and TI values given in Table 1.

TABLE 1

Summary of Cohex Activity Against HIV-1 in PBMCs				
Compound	HIV-1 Isolate	IC <sub>50</sub>	CC <sub>50</sub>	Therapeutic Index
Cohex	Ba-L	33.8 $\mu\text{M}$	833 $\mu\text{M}$	24.7
	NL4-3	28.6 $\mu\text{M}$		29.1

**[0046]** The results show that Cohex displays very similar activity against HIV as against other types of viruses, attesting to the very broad-spectrum nature of the compound. The antiviral activity is not as high as specific antiviral drugs, like AZT, but there are situations where the use of Cohex can be an advantage.

#### Cohex Activity Against Ebola Virus

**[0047]** Ebola was first discovered simultaneously in 1976 in Sudan and in the Democratic Republic of the Congo (formerly Zaire). While its origins are still not firmly established, Ebola likely came from the rain forests of Africa. The primary reservoir is likely not nonhuman primates, but rather that the

virus is zoonotic, transmitted to humans from ongoing life cycles in animals or arthropods.

**[0048]** Ebola viruses belong to the filoviridae family and has five known strains (subtypes): Bundibugyo, Côte d'Ivoire, Sudan, Zaire, and Reston. The Bundibugyo, Sudan, and Zaire strains have caused outbreaks of Ebola hemorrhagic fever among humans in Africa, killing up to 90% of those infected. Of the Ebola viruses, the Zaire strain is the most virulent and the Reston strain is the least virulent.

**[0049]** The Ebola virus is transmitted via contact with bodily fluids of infected persons and can take from two days to three weeks for symptoms to appear. Disease symptoms start with fever, muscle aches and a cough before progressing to severe vomiting, diarrhea and rashes, along with kidney and liver problems. Death generally occurs as the result of either one or a combination of dehydration and/or massive bleeding from leaky blood vessels, kidney, and liver failure. The World Health Organization has documented 1,850 cases of Ebola (mostly in sub-Saharan Africa) since its discovery; only 600 (32 percent) of the victims survived. (32 percent) of the victims survived.

**[0050]** As with all viruses of the order Mononegavirales, filoviruses, such as Ebola, contain a single-stranded, negative-sense RNA molecule as their genome. The genomes of filoviruses are quite large at approximately 19,000 bases in length and contain seven sequentially arranged genes. Filovirus proteins can be subdivided into two categories, those that form the ribonucleoprotein (RNP) complex and those that are associated with the envelope. The proteins associated with the nucleocapsid are involved in the transcription and replication of the genome, whereas the envelope-associated proteins primarily have a role either in assembly of the virion or in receptor binding and virus entry.

**[0051]** There is no known cure for Ebola disease. Existing antiviral drugs do not work well against this virus and the best doctors can do is attempt to maintain the patient's body fluids and electrolytes levels under intensive care; while bleeding problems may require transfusions of platelets and/or fresh blood.

#### Activity of Cohex Against Ebola Virus in Cell Culture

**[0052]** For EC<sub>50</sub> assays, cells were plated onto 96-well plates and incubated at 37° C. for 24 hours before adding compound followed by cell infection with Zaire Ebola GFP virus, a virus strain that contains a GFP gene. The infected cells were allowed to grow for an additional 48 hours before reading on a Molecular Devices spectrofluorometer (X=485 nm, M=515 nm). Controls were done for +virus/-compound and -virus/-compound. The -virus/+compound controls were part of the CC<sub>50</sub> tests. Dosage of Cohex ranged from 2.5  $\mu\text{M}$  to 5 mM and were done in triplicates. Error bars for the figures are for standard error (SE) of the mean.

**[0053]** The results for A549 cells and HepG2 cells are shown in the left and right panels of FIG. 4, respectively. It is seen that there appears to be a general flat response from 2.5  $\mu\text{M}$  until around 0.1 mM Cohex, at which point, GFP expression drops until there is nearly 100% suppression (-100%) of viral expression at concentrations above 1 mM Cohex.

**[0054]** The results for 293T and VeroE6 cells are shown in the left and right panels of FIG. 5, respectively. For 293T cells, there is a monotonic decrease in GFP expression with increasing Cohex, even starting as low as 2.5  $\mu\text{M}$  Cohex. For VeroE6 cells, there is also a decrease in GFP expression with increasing Cohex, but the slope of the decrease is much less