42

Establishment of an ELISA-Based Primary Antiviral Screen and Plaque Reduction Based Confirmatory Assay for Evaluation of Compounds against the Filovirus Ebola Under Maximum Biological Containment Conditions (BL-4).

J. W. Huggins, Z. X. Zhang and T.P. Monath. Department of Antiviral Studies, Virology Division, U.S. Army Medical Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, 21701-5011, USA

Primary Ebola outbreaks have been associated with high mortality (40-90%) in man. The requirement for maximum biological containment facilities (BL-4) and lack of a high-volume drug screen have inhibited drug development. Recently, infections associated with imported macaques in a U.S. primate colony, coupled with Ebola's potential for serious disease outbreaks, emphasized the need for a priority effort to identify potential antiviral compounds. Confluent monolayers of Vero E6 cells in microculture plates were infected with Ebola virus at an MOI of 10-2. Dilutions (1:2) of test compound were added (in triplicate), incubated at 37°C (Mayinga strain 5 days, Boniface strain 4 days), cell monolayers fixed with formalin-10% calcium chloride, and decontaminated with 106 rads of y irradiation prior to removal from BL-4 containment. Viral antigen was detected on fixed cell monolayers with a mixture of Ebola monoclones by ELISA. The assay measures viral replication and subsequent infection of additional cells. Toxicity was determined on microtiter plates of confluent monolayers of Vero E6 cells in parallel with an MTT assay based on mitochondrial enzyme reduction of colorless MTT tetrazolium salt to the colored MTT formazan by viable cells. Active compounds were confirmed in a plaque reduction assay in Vero E6 cells under Bl-4 conditions. Two active compounds have been identified.

43

Summary of Natural Products Isolated from Marine Organisms with Antiviral Activity against Mouse Coronavirus. S. Cross and L. Moritz. Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Ft. Pierce, Florida, 34946, U. S. A.

Marine organisms including algae, sponges, soft corals and species from other phyla have been screened for antiviral activity against mouse coronavirus strain A59. This strain was used for the antiviral screen. because samples could be processed and results obtained in 24 hours. A number of new and novel compounds have been isolated from sponges by following the bioactivity of A59 for the isolation schemes. Other pure compounds isolated from marine organisms have also been identified as Some few examples of active compounds to be discussed include active. onnamide A from the marine sponge Theonella, isospongiadiol from Spongia, reiswigins A and B from Epipolasis reiswigi, brianthein V from Briareum asbestinum, topsentin, bromotopsentin, and dihydrodeoxybromotopsentin from Caribbean deep-sea sponges of the family Halichondriidae. None of the compounds have been tested against the Some of these compounds may show interesting human coronaviruses. activity against human coronaviruses, and because of their novel structures could provide the backbone for the synthesis of new, active compounds.