130

A Rapid DNA Hybridization Assay for the Evaluation of Antiviral Compounds Against Epstein-Barr Virus

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There is a need for additional therapies for Epstein-Barr virus (EBV) infections, but the routine screening of large numbers of potential inhibitors has been difficult due to the laborious nature of traditional assays. Our laboratory has used ELISA, in situ hybridization, and flow cytometry assays to assess antiviral activity. We sought to develop a more efficient assay in 96-well plates to facilitate the routine evaluation of large numbers of compounds. A new assay was developed using latently infected Akata cells that were induced to undergo a lytic infection using an antibody to human IgG. Three days post infection, viral DNA synthesis was measured by a non-radioactive DNA hybridization assay. Drug concentrations sufficient to inhibit DNA synthesis by 50% were calculated using uninduced Akata cells as a negative control. This assay was validated in a series of experiments using a set of compounds with known activity against EBV. Results from these and other studies compared favorably with historical results from our laboratory, as well as results obtained with a confirmatory real-time PCR assay. Our subsequent experience with this assay has confirmed that it offers improved efficiency and robustness and is used routinely to evaluate candidate compounds.

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131

Design, Synthesis, Antiviral Activity and Cytotoxicity of Novel Sulphonamide Derivatives

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Sulphonamide is a versatile lead molecule for designing potential bioactive agents, and its heteroaryl derivatives were reported to possess broad-spectrum antiviral activity including HIV (Selvam et al., 2006. 19th ICAR, USA). Recently much attention has been devoted to searching for potent antiviral agents for treatment of acute and chronic viral diseases. We designed and synthesized novel heteroaryl sulphonamides by microwave technique and characterized them by spectral analysis. Synthesized compounds were screened for antiviral activity against influenza A (H1N1, H3N2, H5N1), influenza B, Dengue, yellow fever, Venezuelan Equine encephalitis, West Nile, Rift Valley fever, and Tacaribe viruses in cell culture. Cytotoxicity of the test compounds was also tested in mock-infected Vero, MDCK, and BSC-1 cells by neutral red uptake assay. The lead molecule is suitable for designing newer derivatives against avian influenza (H5N1), West Nile and Tacaribe virus, based upon promising antiviral activity seen. Detailed chemistry, antiviral activity and cytotoxicity will be presented.

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132

Inhibition of the Tax-Dependent Human T-Lymphotropic Virus Type 1 (HTLV-1) Replication in Persistently Infected Cells by the Naphthalene Derivative JTK-101

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In the search for anti-HTLV-1 compounds, we have identified the naphthalene derivative JTK-101 to be a potent and selective inhibitor of HTLV-1 replication. This compound could suppress HTLV-1 production in persistently infected MT-4 cells at nanomolar concentrations. Its EC_{50} and CC_{50} were 0.018 and 1.7 μM, respectively. The compound also displayed selective inhibition of HTLV-1 production in peripheral blood mononuclear cells obtained from patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Quantitative reverse transcription (RT)-PCR analysis revealed that JTK-101 potently and selectively suppressed viral mRNA synthesis in MT-4 cells in a dose-dependent fashion. The compound could inhibit the endogenous Tax-induced HTLV-1 long terminal repeat (LTR)-driven reporter gene expression in MT-4 cells. Western blot analysis confirmed reduced Tax expression in MT-4 cells exposed to JTK-101. Furthermore, when Tax was introduced into uninfected cells with a tax-expression plasmid under the control of human cytomegalovirus promoter, the compound could also inhibit exogenous Tax-induced HTLV-1 LTR-driven reporter gene expression. These results suggest that JTK-101 inhibits HTLV-1 replication in persistently infected cells through the suppression of viral gene expression, presumably a Tax-dependent mechanism.

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