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| |  | | --- | | **Quinoxaline-6-carboxamides inhibit HBV infection *in vitro*** | | Vadim Bichko, Alexei Rjakhovskiy, Eugenia Remeeva, Boris Rogovoy. *Viriom Inc., San Diego, CA, United States*  Currently, more than 350 million people suffer from chronic HBV infection. Chronic hepatitis B frequently progresses to liver cirrhosis and hepatocellular carcinoma, a leading cause of cancer-related morbidity and mortality worldwide. This study is focused on the discovery and characterization of small molecules that reduce or eliminate HBV cccDNA from the nuclei of infected cells. Drug candidates with such mechanism of action, in contrast to the currently available HBV drugs, would have a potential to eradicate HBV and cure chronically-infected HBV patients. A robust HBV *in vitro*-infection model has been developed (Seeger and Sohn, 2014). A human hepatoma cell line HepG2, stably expressing the sodium taurocholate cotransporting polypeptide (NTCP), has been constructed and characterized. An efficient HBV replication in the infected cells was confirmed with cell ELISA for several viral intracellular antigens (including HBsAg- Large, HBsAg-Middle and HBcAg), as well as with immunofluorescence and immunohistochemistry. The HTS was automated and run on the Biomek robotic workstation. The HepG2/NTCP cells in the 96-well plates were infected with HBV, treated with test compounds at 10 µM for 7 days, and HBV replication inhibition was measured using ELISA for the secreted HBeAg as readout. Compound cytotoxicity was determined in parallel, using cells in same 96-well plates. The antiviral activity and cytotoxicity of the identified hits was further evaluated at multiple concentrations, and the EC50 and CC50 values were determined. As a result, at least five distinct chemistry series of HBV inhibitors were identified, with the EC50 values ranging from 0.08+/- 0.03 µM to < 10 µM, and CC50 values >30 µM (the highest concentration tested). The HBV inhibition was also confirmed by immunofluorescence staining for the intracellular HBcAg. The mechanism of molecular action studies for the most promising inhibitor series are in progress. The results of these studies, including time of drug addition experiments, compound effects on HBV core DNA and HBV cccDNA in cell cultures using the real-time quantitative PCR technique, will be presented. In conclusion, further pre-clinical studies of these newly discovered HBV inhibitors are warranted. | |
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