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Novel Five-membered Ring Imino Sugar Derivatives Inhibit Flaviviruses via Distinct Mechanisms

Jinhong Chang[1,*], Lijuan Wang[1], Dongling Ma[1], Robert Moriarty[3], Benjamin Davis[4], Peter Mason[5], Baohua Gu[1], Xiaodong Xu[6], Ju-Tao Guo[1], Timothy Block[1,2]

[1] Drexel University College of Medicine, Doylestown, USA; [2] Hepatitis B Foundation, Doylestown, USA; [3] University of Illinois, Chicago, USA; [4] University of Oxford, Oxford, United Kingdom; [5] University of Texas Medical Branch, Galveston, USA; [6] Pharmabridge Inc., Doylestown, USA

Classical imino sugars, such as deoxynojirimycin (DNJ), are six-membered ring glucose analogues. Previous studies demonstrated that DNJ and its derivatives selectively inhibited cellular α-glucosidase that processes N-glycan in glycoproteins and as a consequence, exhibited a broad spectrum of antiviral activities against enveloped viruses. To obtain imino sugars with potent antiviral activity against flaviviruses as well as improved toxicity profile, we set out to (i) further modify the six-membered ring imino sugar with a DNJ head by diversifying the nitrogen-linked alkylated side chain and (ii) synthesize two classes of novel imino sugars with a five-membered ring head and side chains attached to nitrogen and its adjacent carbon atom. The antiviral activities of those compounds were tested in bovine viral diarrhea virus (BVDV)-infected MDBK cells. Our results demonstrated that among the compounds that showed antiviral activity, all six-membered ring and one class of five-membered ring imino sugar derivatives, represented by OSL-95II and CM-7-28, respectively, exhibited inhibitory activities on glucosidase. Consistently, both groups of compounds did not inhibit viral RNA replication and protein synthesis, but reduced viral particle secretion and compromised infectivity of progeny virions. On the contrary, another class of five-membered ring imino sugars, represented by A005, potently inhibited BVDV infection, but had no effect on α-glucosidase. Unlike the glucosidase inhibitors, this class of compounds prevented cytopathic effects (CPE) induced by BVDV at high m.o.i. and inhibited the synthesis of viral proteins and RNA in infected cells, suggesting a distinct antiviral mechanism. Evaluation of the antiviral activity of all the three classes of compounds against other flaviviruses such as dengue virus and West Nile virus are currently under way and will be presented.

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A Novel and Selective Inhibitor of Hepatitis B Virus Surface Antigen Secretion

Anne Marie Dougherty[1,*], Haitao Guo[2], Gael Westby[1], Yuanjie Liu[2], Ju-Tao Guo[2], Anand S. Mehta[2], Pamela Norton[2], Baohua Gu[2], Timothy M. Block[1,2], Andrea Cuconati[1]

[1] Institute for Hepatitis and Virus Research, Doylestown, USA; [2] Drexel Institute for Biotechnology and Virology Research, Doylestown, USA

The high levels of hepatitis B surface antigen (HBsAg)-bearing subviral particles in the serum of chronically infected individuals are thought to play a role in suppressing hepatitis B virus (HBV)-specific immune response. Current hepatitis B therapeutics does not significantly reduce this viral antigenemia. Our group has focused on enhancing immune response through the inhibition of viral antigen secretion in infected hepatocytes, with the goal of using vaccination for the treatment of chronic infection. High-throughput screening of 80,288 drug-like compounds was undertaken to discover novel inhibitors of HBsAg secretion. Using the human hepatoma cell line HepG2.2.15.we developed an HTS-amenable ELISA protocol for the detection of secreted HBsAg. The screen identified a tetrahydro-tetrazolo-pyrimidine with halogenated aromatic ring substituents, which was resynthesized and termed HBF-0259. EC_{50} in the HBV-expressing cell line HepG2-DE19 was in the range of 0.4–1.4 mM in the primary screening assay, while CC_{50} was >50.0 mM. Testing of analogues suggested nascent structure–activity relationship. HBF-0259 strongly inhibited secretion of large (L) and medium (M) antigens in the form of both subviral and infectious particles, while secretion of cellular glycoproteins was unaffected. HBV e antigen, which is not a constituent of HBV particles, was also unaffected, suggesting that only secretion of particles bearing HBV structural glycoproteins is targeted. Inhibitory activity was also confirmed by transient transfection of surface antigen into HepG2 cells. HBF-0259 was found to have no effect on HBV DNA synthesis, demonstrating that inhibition is independent of viral genomic replication. Finally, HBF-0259 had little or no effect on cell-to-cell spread of two unrelated viruses, suggesting that it is a specific inhibitor of secretion of HBV surface antigen. The compound is undergoing characterization in tissue culture and animal models, and pharmacokinetics, possible mechanism of action, and directions for chemical optimization will be discussed.

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