A NOVEL HBV CAPSID INHIBITOR COMPOUND SERIES DEMONSTRATES IMPROVED INHIBITION OF HBV WT, T33N AND I105T CORE PROTEIN VARIANTS AND SHOWS A UNIQUE BINDING MODE TO CORE PROTEIN


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BACKGROUND

- The process of core protein assembly into pregenic RNA (pgRNA) containing nucleocapsids is an essential step during replication of the hepatitis B virus.
- The successful encapsidation of pgRNA by 240 core protein molecules signals the synthesis of the viral genome (relaxed circular DNA, rDNA) and downstream events of infectious virion production and replenishment of the nuclear pgDNA pool.
- A number of small molecule inhibitors of HBV capsid assembly have moved into clinical development and have demonstrated potent antiviral activity.
- Capsid inhibitors thus represent a novel mechanistic class of anti-HBV agents with potential to be part of future curative regimens (Cole, 2016).
- Capsid inhibitors have been classified into two mechanistically differentiating categories: Class I capsid inhibitors form non-capsid polymers while Class II capsid inhibitors form capsids that look normal but are devoid of viral nucleic acids (pgRNA/rDNA).

OBJECTIVES

- Optimize the antiviral profile of novel HBV capsid inhibitor compound series for improved potency against WT and core variants T33N and I105T that confer resistance to AB-506 and other capsid inhibitors as well as for improved inhibition of nucleocapsid uncoating.

MATERIALS AND METHODS

- Antiviral activity was determined in different cell culture models of HBV using branched DNA and ELISA to measure on rcDNA or secreted e-antigens as described previously (Mani et al, 2016).
- HepG2/NTCP/HBV infection systems were used to study effects on capsid uncoating (Ag) and rcDNA establishment.
- Activity against HBV core protein variants was determined using a HepG2 transient transfection assay.
- Capsid activity of compounds was evaluated in various cell lines using Cell-Tracker DiO (Cm) assay (Promo).
- A cryo-crystallography studies were conducted to determine the binding mode of representative compounds to core protein and were consistent with the assembly defect motif as described previously (Klamp et al, 2015).
- Mode of action studies were conducted using HepG2 or 293A/AD2/HBV infected cells to evaluate compound effects on HBV pgRNA encapsidation, core particle density, size and core protein phosphorylation, abundance, sucrose CsCl density gradient centrifugation, electron microscopy and western blot analysis as described previously (Campagna et al, 2011, Mar et al, 2014).
- Immunofluorescence microscopy studies were conducted to visualize the effects of compounds on core protein localization in HBV infected PHLW ABNL2/HBV/T33A was used for studying effect of capsid inhibitor on core protein phosphorylation.
- Sucrose and CsCl gradient analysis of HBV capsids: HepG2/AD2 cells were mock treated (DMSO) or treated with the compound indicated for 6 days in the presence of tetracycline. The cell lysates were centrifuged and loaded onto a 250,000 x g centrifugation and centrifuged at 46,000 rpm for 3 h (Beckman, Rotor SW28). Pellets were dissolved in 1 mL of TNE buffer containing phosphatase and proteinase inhibitors and loaded onto a linear sucrose gradient in TNE buffer and spun at 27,000 rpm for 4 h (Beckman, Rotor SW28). Fractions were collected from the bottom of the centrifugation tubes using a left collector kit and analyzed by Western Blot to detect in uncoated and anti-Hilg-labeled capsids. By HBV capsids from sucrose gradient centrifugation were fractionated and dissolved as described above (Mani et al, 2016).
- Molecular analysis of HBV pgRNA encapsidation: HepG2/AD2/HBV infected cells were treated with different compound and pgRNA was extracted and visualized as described previously (Mani et al, 2016).
- Sucrose and CsCl density gradient centrifugation analyses and electron microscopy studies suggested that compound treatment did not alter the particle density or modified the shape/conformation of core protein during pgRNA encapsidation attributing the differences to potential alterations in capsid shape/conformation induced upon capsid inhibitor binding.
- Multiple examples of compounds from series 3A were shown in improved potency against a T33N and a 1007F variant of core protein as well as improved inhibition of capsid uncoating and ncDNA establishment in comparison to AB-506.
- Compounds from series 3A are being evaluated for advancement into IND-enabling studies.

RESULTS

- Figure 1: Immunofluorescence microscopy and core protein phosphorylation studies A) Core-protein localization in HBV infected human hepatocytes treated with inhibition. B) Western blot analysis of core protein phosphorylation status in AB-506 infected cells upon compound treatment.
- Figure 2: Molecular analysis of HBV pgRNA encapsidation: HepG2/AD2/HBV infected cells were treated with different compound and pgRNA was extracted and visualized as described previously (Mani et al, 2016).
- Figure 3: Space-filling model of capsid core protein dimer density. Cap1 and Cap2 are the structural core protein dimer density. INHIBITORS are a structurally diverse set of small molecule compounds that target the core protein binding site. An overlay of X-ray structures from 5 different capsid inhibitors shows the differences in conformational space in terms of core protein configurations. Binding of capsid inhibitors to the dimer interface residues is a common feature of all inhibitors. Cap1 and Cap2 are the structural core protein dimer density.
- Figure 4: Sucrose gradient centrifugation analyses and electron microscopy studies suggested that compound treatment did not alter the particle density or modified the shape/conformation of core protein during pgRNA encapsidation attributing the differences to potential alterations in capsid shape/conformation induced upon capsid inhibitor binding.
- Figure 5: Sucrose gradient centrifugation analyses and electron microscopy studies suggested that compound treatment did not alter the particle density or modified the shape/conformation of core protein during pgRNA encapsidation attributing the differences to potential alterations in capsid shape/conformation induced upon capsid inhibitor binding.
- Figure 6: Sucrose gradient centrifugation analyses and electron microscopy studies suggested that compound treatment did not alter the particle density or modified the shape/conformation of core protein during pgRNA encapsidation attributing the differences to potential alterations in capsid shape/conformation induced upon capsid inhibitor binding.
- Figure 7: Electron microscopy and particle size measurements. HBV capsids isolated from HepG2/AD2/HBV infected cells treated with series compounds representing series 2A, 3A and 3B show capped particles with similar size suggesting a potential altered capsid conformation as the most likely basis of different gel migration pattern observed induced by differential interactions with capsid inhibitors.

REFERENCES


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