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DISCOVERY OF NOVEL HDV ENTRY INHIBITORS WITH SELECTIVITY OVER BILE ACID INHIBITION

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ALIGOS THERAPEUTICS

BACKGROUND AND AIMS

Chronic hepatitis delta virus (HDV) infection affects about 4.5% of patients chronically infected with hepatitis B virus (HBV), corresponding to an estimated 12 million people.[1] HDV is a satellite virus of HBV and is only found as a co-infection with HBV due to its dependence on the HBV surface antigen (HBsAg) for viral particle assembly. HBV and HDV enter hepatocytes via the sodium taurocholate cotransporting polypeptide (NTCP) receptor.[2] A 47-amino acid lipopeptide inhibitor blocking NTCP, bulevirtide (BLV), was recently approved by the European Medicines Agency for chronic HDV infection. Two potential drawbacks of BLV are the required daily subcutaneous (SC) administration and the inhibition of bile acid (BA) transport, the natural function of NTCP. An entry inhibitor that is selective for antiviral inhibition over BA transport blockade could provide patients with a potentially superior antiviral treatment.

METHODS

A screening library was assembled based on natural product-based substrates of NTCP or viral entry. Inhibition of HBV and HDV entry was evaluated in HepG2-NTCP cells by adding compound with the inoculum and measuring secreted HBeAg or intracellular HDAg and HDV RNA as read-outs. Inhibition of BA transport was assessed using a fluorescently labeled BA derivative.[3] Entry specificity was confirmed using an HBV time-of-addition setup where compound was added before, during or after infection. Competitive binding with BLV was investigated using flow cytometry with fluorescently labeled BLV as a probe. Structure-activity relationship (SAR) exploration generated potent and selective inhibitors of viral entry. Metabolic stability was evaluated in rodent and human liver microsomes. In vivo pharmacokinetics were studied in mice and rats after SC or intravenous (IV) bolus dosing.

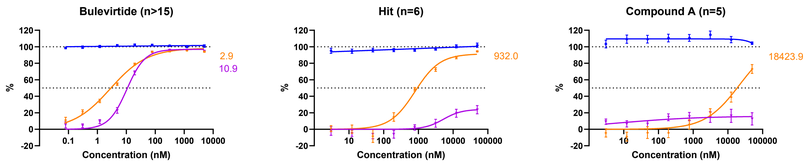
CONCLUSIONS

A lead series of entry inhibitors with potent antiviral activity against both HBV and HDV was identified. Lead compounds had pronounced selectivity (>100-fold) over bile acid transport and were metabolically stable in liver microsomes. An in vivo pharmacokinetic study was conducted in mice where compound D demonstrated a potential long-acting profile after SC injection. Following IV administration in rats, compound D showed a high liver/plasma ratio and long plasma half-life. Further optimization of activity, selectivity, and oral bioavailability is ongoing.

RESULTS

POTENT HBV ENTRY INHIBITION WITH SELECTIVITY OVER BILE ACID TRANSPORT

Screening of focused compound libraries for HBV entry and NTCP substrates led to the identification of a hit compound with antiviral potency and selectivity over BA transport. Compound A, a novel derivative of the hit demonstrated lower potency but clear selectivity over BA transport (Figure 1), acceptable metabolic stability in human liver microsomes (Table 1), and was amenable to modular synthesis.



Exploration of the structure-activity relationship and further chemical optimization yielded a lead series with sub-micromolar EC_{50} antiviral potency and pronounced selectivity (> 100-fold). Selected molecules demonstrated HBV entry inhibition with EC_{50} values under 100 nM for HBV entry and CC_{50} values > 50 µM (Compounds D to G).

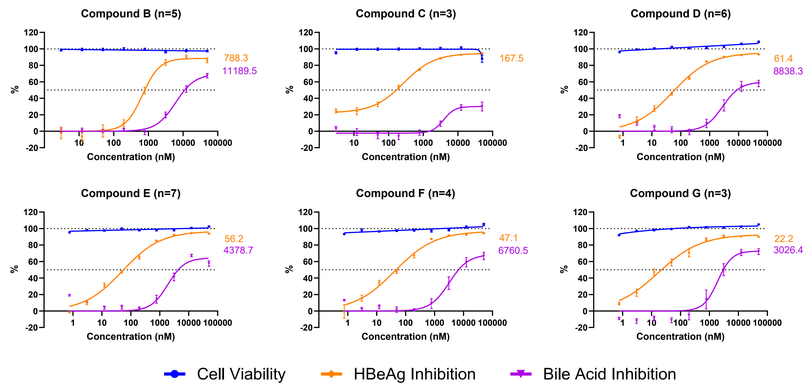


Figure 1. Potent antiviral activity with high selectivity over BA transport. The graphs show percentage inhibition of HBeAg secretion and BA transport, and cell viability. Data are expressed as mean ± SEM. The number of replicates (n) is shown in each panel. EC_{50} values are displayed on the graph in their corresponding curve colors.

CONFIRMATION OF ENTRY SPECIFICITY

Entry specificity of the hit series was confirmed using an HDV entry assay and HBV time-of-addition assay. The selected molecules demonstrated HDV entry inhibition in the low nanomolar range (Fig 2A). In a time-of-addition assay, the selected molecules showed in the post-treatment condition a loss of antiviral activity, confirming its activity is through inhibition of HBV entry (Fig 2B).

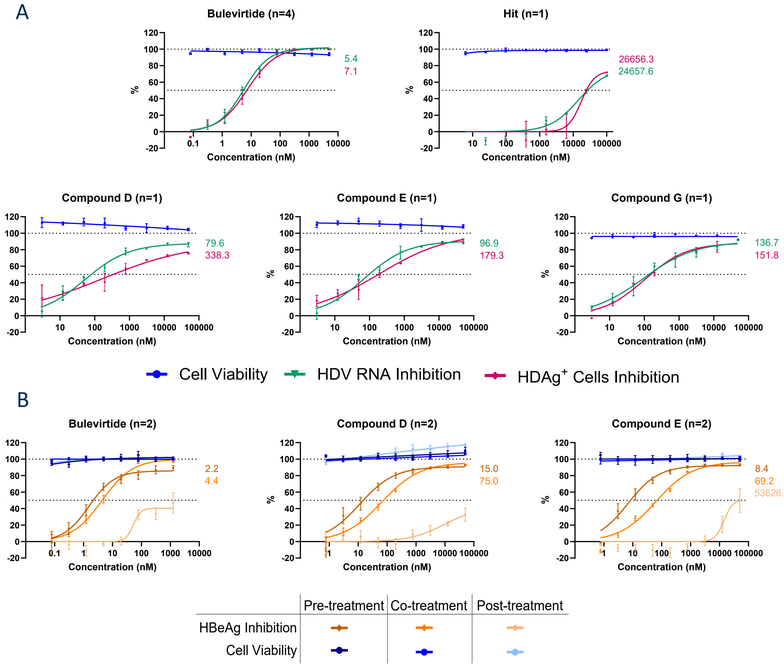


Figure 2. Confirmation of entry specificity through HDV entry and HBV time-of-addition assays. (A) Graphs show HDV entry inhibition through percentage inhibition of HDV RNA replication and number of infected ($HDAg^+$) cells, and cell viability. **(B)** Graphs show HBV entry inhibition in a time-of-addition setting through percentage inhibition of HBeAg secretion and cell viability. Data are expressed as mean ± SEM. The number of replicates (n) is shown in each panel. EC_{50} values are displayed on the graph in their corresponding curve colors.

Table 1. Stability in Human Liver Microsomes (LM).

Compound	Human LM $T_{1/2}$ (min) (% remaining at 60 min)	Compound	Human LM $T_{1/2}$ (min) (% remaining at 60 min)
A	>60 (51)	E	>60 (101)
B	>60 (102)	F	>60 (104)
C	>60 (99)	G	>60 (88)
D	>60 (99)		

COMPETITIVE BINDING WITH BULEVIRTIDE

Using a fluorescently labeled BLV probe, the selected molecules showed competitive binding with BLV.

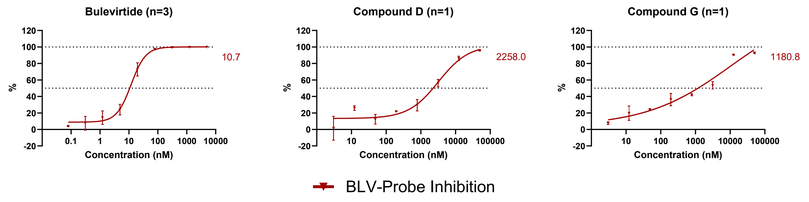


Figure 3. Competitive binding of hit series with fluorescently labelled BLV. Graphs show percentage inhibition of BLV-probe binding when adding BLV, compound D and G. Data are expressed as mean ± SEM. The number of replicates (n) is shown in each panel. EC_{50} values are displayed on the graph in their corresponding curve colors.

PHARMACOKINETICS OF COMPOUND D IN MICE AND RATS

Compound **D** demonstrated good metabolic stability in mouse and rat liver microsomes with a half-life of >60 min. The dose vehicle for SC PK in mice was 0.5% CMC and 0.2% Tween 80 in water. Three non-fasted male C57Bl/6J mice were used. The dose for IV PK in rats was formulated in 80% PEG400 in water.

Sustained high exposures were achieved through 5-days post dose in mice demonstrating the potential of compound **D** as a long-acting injectable. The liver to plasma ratio was 0.33 at 120 h after the single SC dose. Three male Wistar Han rats in fed state were used for the IV PK study. Following a single IV dose, compound **D** had a long plasma half-life (16.2 h). High liver concentrations (16,542 nM) with a liver to plasma ratio of 131 were achieved at 24 h post dose in rats.

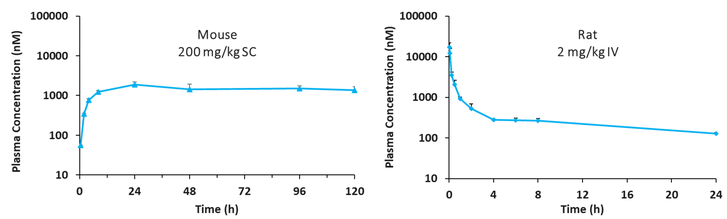


Figure 4. In vivo pharmacokinetics. Mean plasma concentration time profile of Compound D following a single SC dose at 200 mg/kg in mice (n=3) and a single IV dose at 2 mg/kg in rats (n=3). Data are expressed as mean with SD (upper bars only).

Table 2. Mean Plasma PK Parameters of Compound D in Mice and Rats.

Parameter	Mouse	Rat
Dose (mg/kg)	200	2
Administration	SC	IV
C_{max} or C_0 (nM)	1,881	21,632
$AUC_{0\text{-last}}$ (nM·h)	174,641	9,452
$T_{1/2}$ (h)	NC	16.2

NC=not calculable; $AUC_{0\text{-inf}}$ was not reportable; CL and Vss could not be calculated

REFERENCES

1. Stockdale et al. 2020 *J Hepatol*, 73(3):523-532.

2. Yan et al. 2012 *Elife*, 13:1.

3. De Bruyn et al. 2014 *J Pharm Sci*, 103(6):1872-81.

DISCLOSURE AND CONTACT INFORMATION

Authors are current employees of Aligos Therapeutics and may own stock in Aligos Therapeutics.

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