



PRECLINICAL CHARACTERIZATION OF POTENT CORE PROTEIN ASSEMBLY MODIFIERS FOR THE TREATMENT OF CHRONIC HEPATITIS B

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Background

Approximately 240 million people worldwide are chronically infected with Hepatitis B virus (HBV). A significant proportion will develop chronic liver diseases, such as hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Two classes of treatment options are currently approved for chronic HBV: nucleos(t)ide analogs (Nuc) (e.g. adefovir, entecavir (ETV), lamivudine, telbivudine, tenofovir) and interferons (IFN and pegylated-interferon (PEG-IFN)). These therapies are effective in inhibiting HBV replication and reducing the risk of chronic liver diseases, however they rarely succeed in fully eliminating HBV and providing a cure to chronic HBV. Thus, there is a need for a chronic HBV curative treatment, which will most likely be facilitated with the addition of a novel class of anti-HBV molecules. The HBV core protein is a highly conserved viral protein that has no human homolog and is involved in multiple steps of the HBV life cycle. Core Protein Allosteric Modifiers (CpAMs) represent a novel class of direct acting antivirals for the treatment of chronic HBV, and have been shown to interfere with the HBV capsid assembly process and reduce HBV replication^{1,2}. Here we report the preclinical characterization of a promising novel series of CpAMs.

Materials and Methods

Compounds: Entecavir³ (ETV) (Nuc inhibitor) was purchased from ACME Bioscience (USA). CpAM inhibitor GLS4 was synthesized by published procedures⁴.

Cell lines and compound treatments: AD38 cells⁵ were seeded in 96-well plates, induced and treated with compounds. Supernatants and cells were harvested at 5 days post-induction. HepG2-NTCP cells were infected with AD38 virus at MOI 25 and treated with CpAMs at indicated concentrations. Supernatants and cells were harvested at Day 8.

HBV DNA/pgRNA quantification: HBV DNA/pgRNA levels were quantified by Taqman quantitative PCR (qPCR or RT-qPCR) using primers and probe specific to the HBV core gene.

HBV encapsidated DNA/RNA extraction: HBV capsids were precipitated using 10% PEG and treated with MNase to remove residual RNA/DNA. Encapsidated HBV DNA/RNA was extracted using commercial kits.

HBeAg/HBsAg levels quantification: HBe and HBs antigens were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (Autobio Diagnostics and Beijing Wantai Biological Pharmacy Enterprise Co, respectively).

EC₅₀ calculations: EC₅₀ values were derived from non-linear regression analyses calculated using GraphPad Prism software.

Virus specificity assays: Counter assays included MDCK cells (ATCC CCL-34) infected with Influenza A/Puerto Rico/8/1934 (H1N1) (ATCC VR-1469), Vero cells (ATCC CCL-81) infected with HSV-1 (ATCC VR-260) and HeLa H1-A cells infected with HRV-16 and treated with compounds. After 72 hr, cell survival was determined by CellTiter-Glo Luminescent Cell Viability (CTG) Assay (Promega).

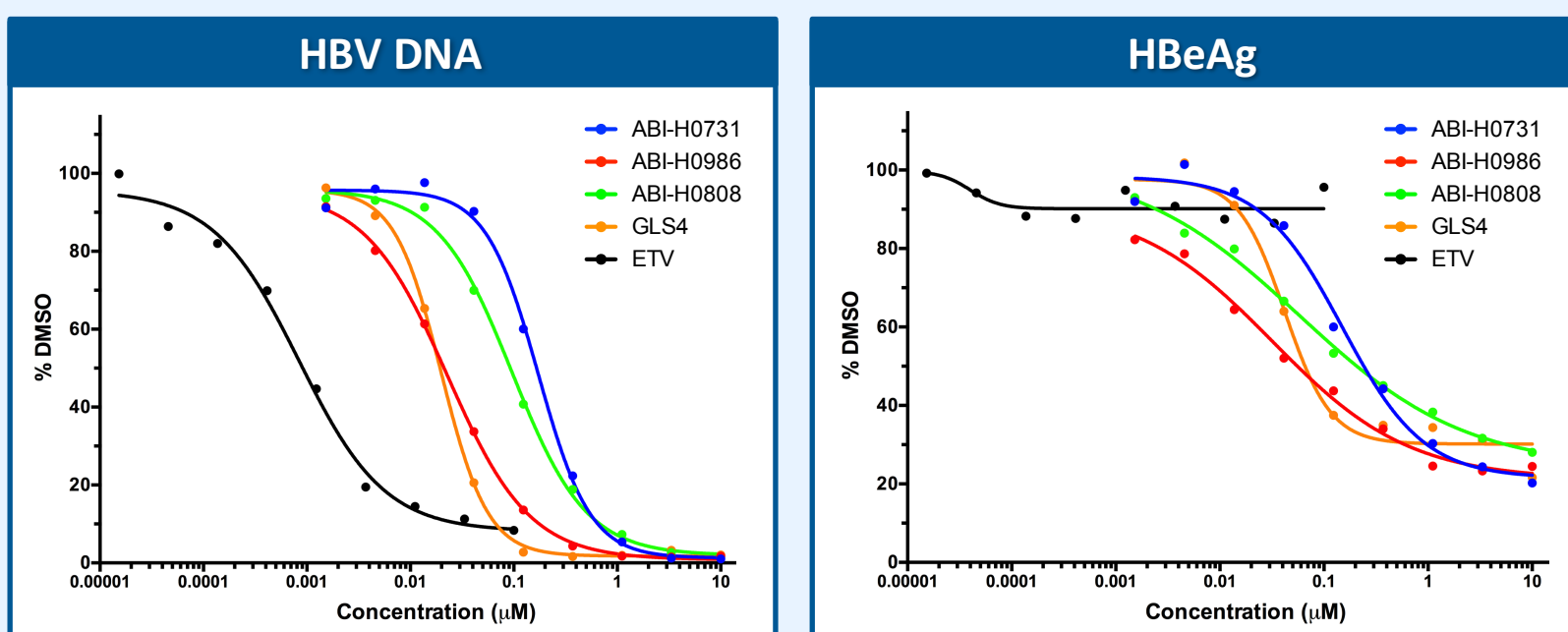
Cytotoxicity assays: CC₅₀ values were determined in six cell lines (HepG2, Huh7, HeLa H1-A, PBMC, HEK293, MOLT-4) representative of different tissues. Log phase cells were treated with compounds for 4 days and cell viability was determined using CTG assay. Uninfected cells treated with DMSO were used as controls (100% cell viability).

Drug combination studies: AD38 cells were induced and treated with various ratios of ABI-H0731 and ETV for 5 days. Synergy analyses were performed using both MacSynergy II⁶ and CompuSyn⁷ software.

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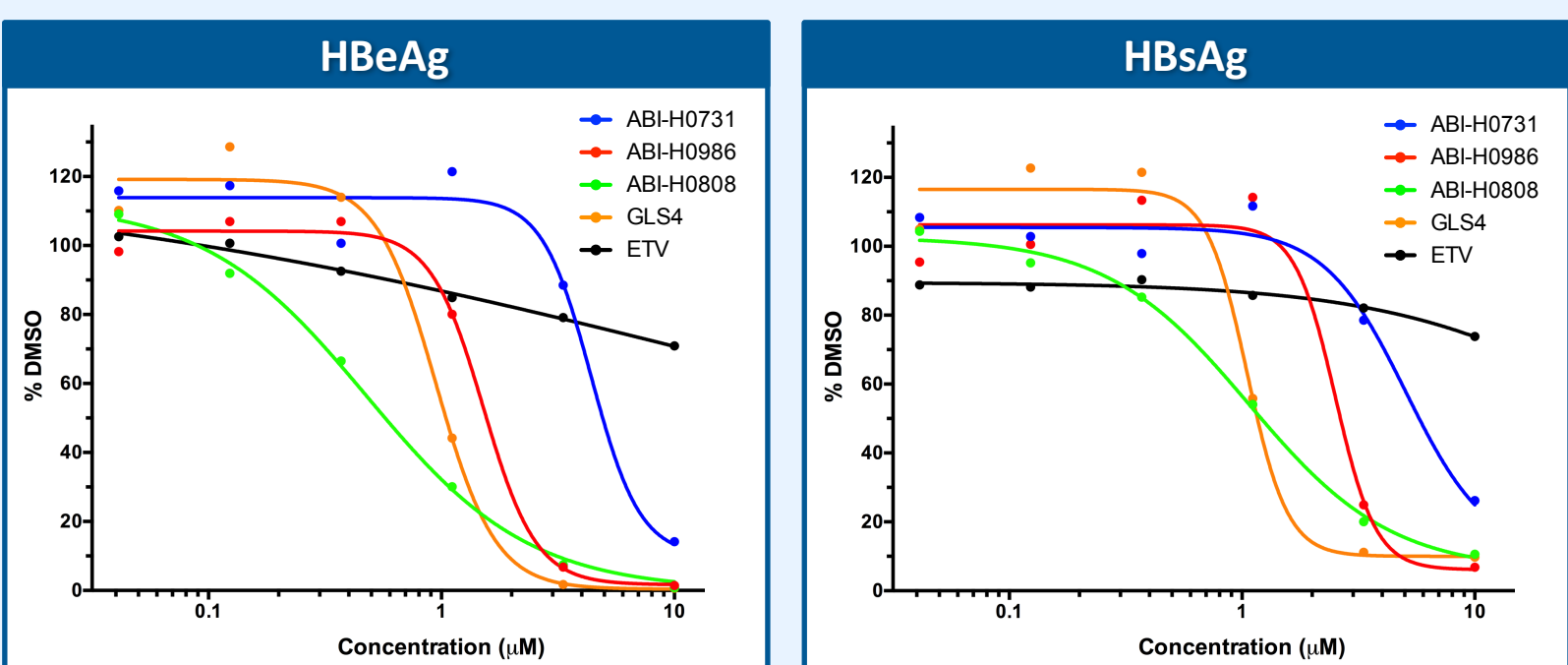
HBV Replication and HBeAg Production are Reduced in CpAM Treated AD38 Cells



Inhibitors	EC ₅₀ (nM)		CC ₅₀ (μM)
	HBV DNA	HBeAg	
ABI-H0731	172	150	> 10
ABI-H0986	23	32	> 10
ABI-H0808	96	58	> 10
GLS4	20	42	> 10
ETV	1	> 1000	> 0.1

- CpAMs displayed EC₅₀s ranging from 23 to 172 nM in reducing viral replication
- CpAMs reduced HBeAg (surrogate for establishment of cccDNA) with a similar range of EC₅₀s (32 to 150 nM), while ETV failed to inhibit HBeAg at any concentration tested
- No loss in cell viability was observed for any CpAMs within the range of concentrations tested

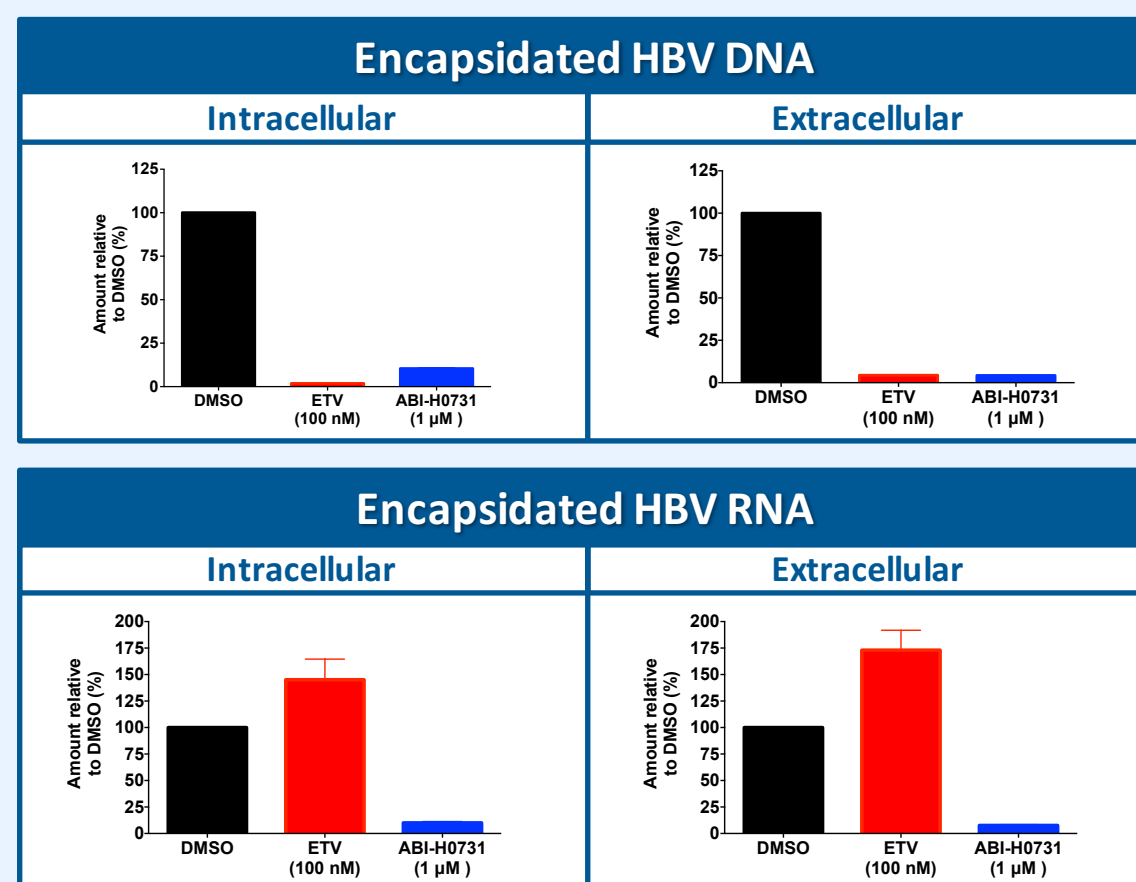
CpAMs Inhibit Establishment of cccDNA in a HepG2-NTCP HBV Infection Model



Inhibitors	EC ₅₀ (μM)		CC ₅₀ (μM)
	HBeAg	HBsAg	
ABI-H0731	4.40	5.05	> 10
ABI-H0986	1.52	2.52	> 10
ABI-H0808	0.48	1.07	> 10
GLS4	0.95	1.05	> 10
ETV	> 10	> 10	> 10

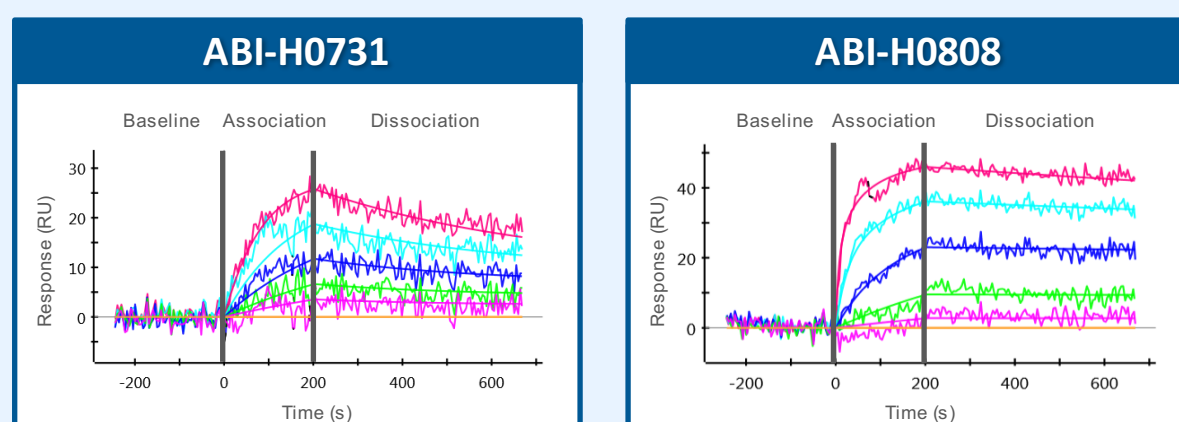
- CpAMs reduced both HBeAg and HBsAg production in HBV-infected HepG2-NTCP cells (surrogate for establishment of cccDNA)
- CpAMs reduced HBeAg and HBsAg levels over a ~10-fold range (EC₅₀s 0.48 to 5.05 μM), whereas ETV showed minimal inhibition of antigen production when tested up to 10 μM
- No effect on viability was seen with any ABI compound in the range of doses tested

CpAMs Interfere with HBV RNA Packaging in Induced AD38 cells



- ABI-H0731 inhibited packaging of both viral RNA and viral DNA in HBV capsids
- While ETV reduced the quantity of encapsulated HBV DNA, an increase (~50%) in packaged HBV RNA and the release of virions containing HBV RNA was observed

Surface Plasmon Resonance Evaluation of CpAM/Core Protein Interaction



- Surface plasmon resonance (SPR) sensorgrams of CpAM binding to the HBV core protein demonstrate differences in behavior bonding between different CpAMs
- ABI-H0731 exhibited slower on- and off-rates, with a half-life on the order of minutes
- ABI-H0808 exhibited a stronger binding affinity and an even slower off-rate, with a half-life on the order of hours

CpAMs Show Activity Specific to HBV and are Generally Non-Cytotoxic

Inhibitors	EC ₅₀ (μM)		
	Flu	HSV	HRV
ABI-H0731	> 10	> 10	> 10
ABI-H0986	> 10	> 10	> 10
ABI-H0808	> 10	> 10	> 10

- CpAMs exhibited no measureable activity (EC₅₀ >10 μM) in viral selectivity assays
- No cytotoxicity was observed for any of the CpAMs (CC₅₀ >10 μM) in the six cell lines tested

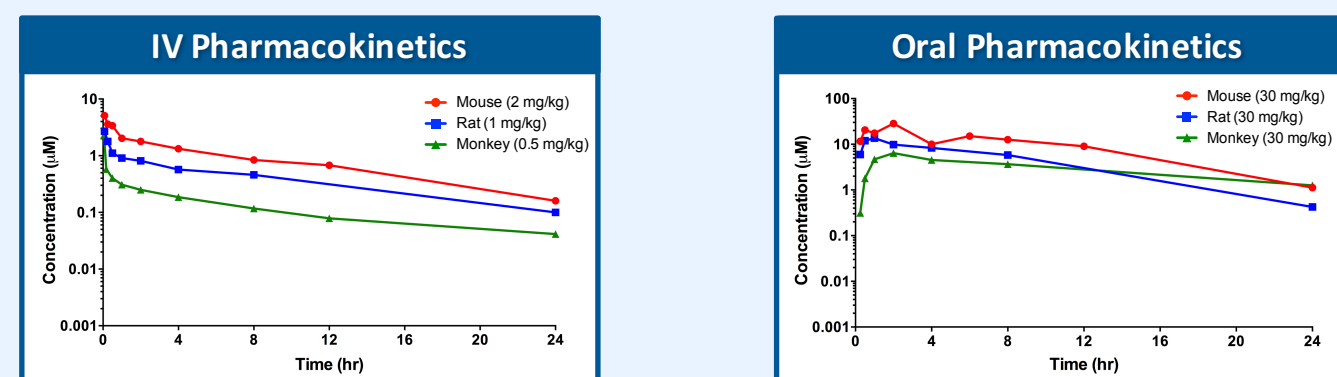
Cell lines	CC ₅₀ (μM)		
	ABI-H0731	ABI-H0986	ABI-H0808
HepG2	> 10	> 10	> 10
HeLa	> 10	> 10	> 10
Huh-7	> 10	> 10	> 10
PBMC	> 10	> 10	> 10
HEK293	> 10	> 10	> 10
MOLT-4	> 10	> 10	> 10

ABI-H0731 + ETV Combination Studies

MacSynergy II Analysis			CompuSyn Analysis		
Synergy (μM ² %)	Antagonism (μM ² %)	Degree of synergy	CI values	Overall CI	Degree of synergy
14.9	-1.2	Insignificant	ED ₅₀ 1.1 ED ₇₅ 0.8 ED ₉₀ 0.6	0.8 +/- 0.3	Slight/moderate

- MacSynergy II analysis of ABI-H0731 + ETV combination in AD38 cells were obtained within the 95% confidence interval, with no significant synergism or antagonism detected
- CompuSyn analysis of HBV inhibition from constant ratios of ABI-H0731 and ETV yielded the indicated Combination Index (CI) values, with increased synergy observed at higher concentrations of compounds

ABI-H0731 PK Profile



Species	Route	Dose (mg/kg)	T _{max} (h)	Co/C _{max} (μM)	AUC _{last} (μM·h)	T _{1/2} (h)	CL (ml/min/kg)	V _s (L/kg)	F (%)
Mouse	IV	2		5.99	20.8	6.5	3.18	1.33	
	PO	30	2.0	28.4	233	NR			75
Rat	IV	1		3.27	10.2	7.6	3.16	1.74	
	PO	5	5.7	0.980	18.7	NR			37
Monkey	IV	0.5		4.20	3.55	13	4.86	4.06	
	PO	2	3.3	0.756	8.74	12			62

NR = not reported

- Prototype compound ABI-H0731 exhibited low clearance (4-11% of liver blood-flow) following IV administration
- Terminal half-lives ranged from 6.5-13 hr, with similar values derived from IV and oral profiles
- Good oral bioavailability was achieved (37-75%) in solution formulation following oral gavage administration in mice, rats and monkeys
- ABI-H0731 exposures increased with dose from 30-100 mg/kg (data not shown)
- Pharmacokinetic behavior of ABI-H0731 is consistent with having potential for once daily dosing in humans

Conclusions

- A new series of potent and selective CpAMs have been identified and their effects upon the viral life cycle characterized
- CpAMs potentially inhibit HBV replication and reduce HBV antigens (surrogate for establishment of cccDNA) in both induced AD38 cells and HBV infected cells
- Different CpAMs within this series have distinct antiviral and core protein binding profiles suggesting differential allosteric effects on core protein
- CpAMs are non-cytotoxic and their activity appears specific to HBV
- When dosed with Nucs such as ETV, ABI-H0731 exhibited additive/synergistic effects on suppressing viral replication
- Prototype compound ABI-H0731 exhibits low clearance and good oral bioavailability in preclinical animal studies
- These data support ongoing advancement of CpAMs into IND enabling toxicology programs in support of human clinical trials