

# A Next Generation HBV Capsid Inhibitor, AB-506: *In Vitro* and *In Vivo* Antiviral Characterization

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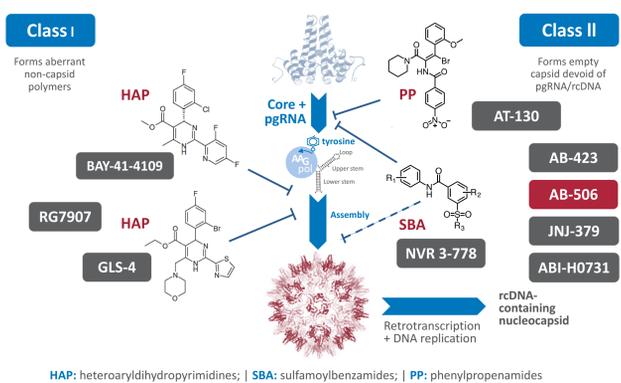
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Abstract  
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## BACKGROUND

- Hepatitis B virus (HBV) replication is strictly dependent upon capsid assembly around pregenomic RNA (pgRNA)
- Proper assembly of HBV nucleocapsid is essential for viral genome relaxed circular DNA (rcDNA) synthesis, infectious virion production and maintenance of a nuclear covalently closed circular DNA (cccDNA) pool
- The capsid assembly process thus represents a *bona fide* antiviral target, and constitutes a novel mechanism that is distinct from the nucleos(t)ide analogues currently available for clinical use
- Interfering with HBV capsid assembly with small molecule inhibitors has been shown to translate into antiviral activity *in vitro* and *in vivo* (Cole, 2016)



**Figure 1:** HBV capsid assembly pathway and examples of capsid inhibitors.

## OBJECTIVES

Characterize the *in vitro* and *in vivo* antiviral activities of AB-506, a potent, next generation small-molecule inhibitor of HBV capsid assembly.

## MATERIALS AND METHODS

- AB-506 was tested in a biochemical assay of capsid assembly as described previously (Zlotnick, 2007)
- The ability of AB-506 to bind and thermally stabilize core protein was determined in thermal shift assays using differential scanning fluorimetry
- X-ray crystallography studies were conducted to determine the binding mode of AB-506 to core protein Cp-Y132A mutant
- Antiviral activity was determined in different cell culture models of HBV using branched DNA, quantitative PCR, and AlphaLISA<sup>®</sup> assays to measure effects on rcDNA or secreted e-antigen
- Activity against HBV genotypes and nucleoside analog inhibitor-resistant (Nuc<sup>R</sup>) variants was determined using a transient transfection assay system
- Cytotoxicity of compounds was evaluated in various cell lines using CellTiter-Glo<sup>®</sup> or MTT assay
- Antiviral activity against viruses of various families was determined using cell culture assays
- Pharmacokinetic profiles of AB-506 was determined in CD-1 mice, SD rats, and Beagle dogs
- The *in vivo* antiviral activity was assessed in a hydrodynamic injection (HDI) HBV mouse model utilizing pHBV1.3 (Guidotti 1995). Test article was administered orally for 7 days starting on Day 0, AB-506 and vehicle twice daily and ETV once daily. HBV DNA was measured using qPCR. Reported liver HBV DNA values were vector-subtracted

## CONCLUSIONS

- AB-506 is a 2nd generation highly selective HBV capsid inhibitor
- In vitro* AB-506:
  - showed potent inhibition of HBV replication in cell culture models
  - bound at the dimer:dimer interface of core protein in X-ray crystallography studies
  - inhibited pgRNA encapsidation in HepAD38 cells
  - accelerated rate of capsid assembly in a biochemical assay
  - conferred increased thermal stability to core protein indicating improved target engagement compared to first generation capsid inhibitors
- Dosing performed in multiple species suggest QD potential and significant liver concentrations achieved
- AB-506 showed potent *in vivo* activity in a HDI mouse model of HBV
- Even low-dose AB-506 substantially reduced liver HBV DNA
- AB-506 is being evaluated for advancement into clinical development

## RESULTS

**Table 1:** *In vitro* antiviral activities of next generation capsid inhibitors

Compound	HepDE19 (rcDNA_bDNA) (μM)			HepBHAe82 (HBeAg AlphaLISA) (μM)			HepG 2.2.15 (HBV DNA qPCR) (μM)		
	EC <sub>50</sub>	EC <sub>90</sub>	CC <sub>50</sub>	EC <sub>50</sub>	EC <sub>90</sub>	CC <sub>50</sub>	EC <sub>50</sub>	EC <sub>90</sub>	CC <sub>50</sub>
AB-506	0.07 ± 0.02	0.28 ± 0.10	>25	0.04 ± 0.02	0.20 ± 0.06	>25	0.04 ± 0.01	>10	>10

- In a primary human hepatocyte assay, AB-506 inhibited HBV replication with an EC<sub>50</sub> of 0.03 ± 0.02 μM
- Maintains activity in the presence human serum with a modest ~6 fold increase in EC<sub>50</sub> in 40% human serum

**Table 2:** Antiviral activity against HBV genotypes A through D and potency of AB-506 against Nuc<sup>R</sup> variants

HBV Nuc <sup>R</sup> Variant	HBV DNA qPCR		HBV Genotype	HBV DNA qPCR	
	AB-506 (EC <sub>50</sub> μM)			AB-506 (EC <sub>50</sub> μM)	
rtM204I	0.059 ± 0.013		A1	0.008 ± 0.002	
rtM204I + V173L	0.038 ± 0.008		A2	0.023 ± 0.005	
rtM204I + S202G	0.052 ± 0.009		B1	0.017 ± 0.005	
rtM204V + L180M	0.055 ± 0.010		B2	0.020 ± 0.004	
rtM204I + S202G + M250V	0.061 ± 0.004		C1	0.015 ± 0.006	
WT, GtD	0.040 ± 0.010		C2	0.009 ± 0.002	
			D	0.040 ± 0.010	

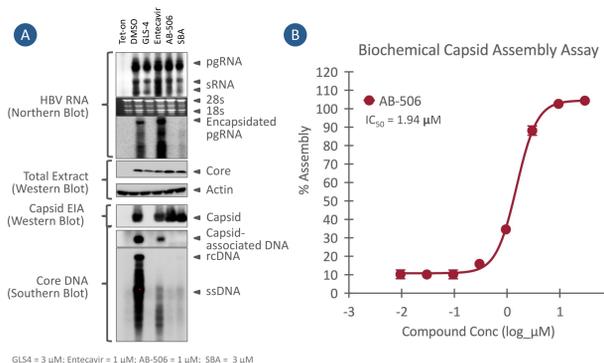
- No cross-resistance with Nuc<sup>R</sup> variants. Consistent with its distinct mechanism of action
- AB-506 shows activity against the most prevalent HBV genotypes globally

**Table 3:** Antiviral selectivity of AB-506

Virus	Family	Genome	AB-506		Host Cell Line
			EC <sub>50</sub> (μM)	CC <sub>50</sub> (μM)	
HCV	Flaviviridae	(+) ssRNA	>30	>30	Huh7
WNV	Flaviviridae	(+) ssRNA	>30	>30	VERO
RSV	Paramyxoviridae	non-segmented (-) ssRNA	>30	>30	HEp2
IFA	Orthomyxoviridae	segmented (-) ssRNA	>30	>30	MDCK
HIV	Retroviridae	ssRNA to DNA	>30	>30	CEMSS
HSV1&2	Herpesviridae	dsDNA	>30	>30	VERO
HCMV	Herpesviridae	dsDNA	>30	>30	MRC5
DENV	Flaviviridae	(+) ssRNA	>22	22	BHK21
HRV	Picornaviridae	(+) ssRNA	>30	>30	H1/HeLa

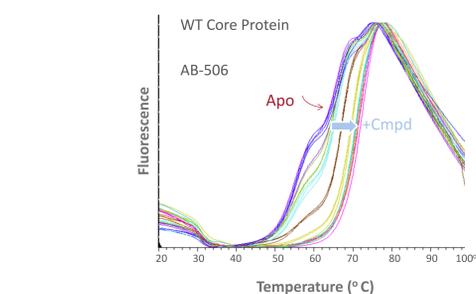
HCV = Hepatitis C Virus; WNV = West Nile Virus; RSV = Respiratory Syncytial Virus; IFA = Influenza A Virus; HIV = Human Immunodeficiency Virus; HSV = Herpes Simplex Virus; hCMV = Human Cytomegalovirus; DENV = Dengue Virus; HRV = Human Rhinovirus

- No significant inhibition of a panel of RNA & DNA viruses demonstrating high selectivity for HBV

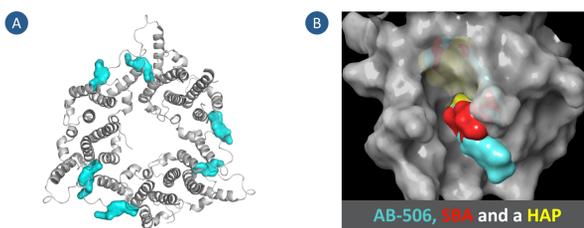


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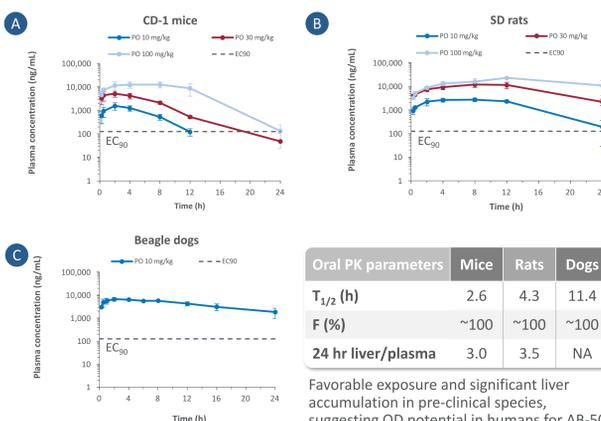
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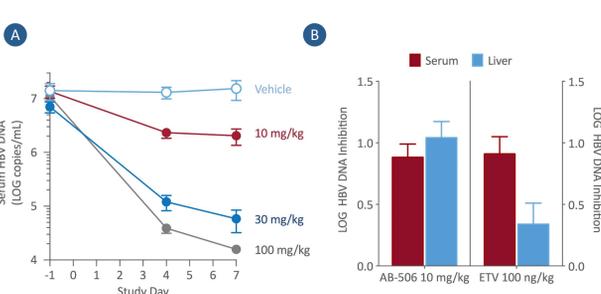
**Figure 3:** Increased thermal stabilization of HBV core protein by AB-506. AB-506 binding increases thermal stability of WT core protein by up to 6°C.



**Figure 4:** X-ray crystallography studies. A) AB-506 binds to core protein at the dimer:dimer interface similar to other known Class I and Class II capsid inhibitors. B) X-ray structure overlay of AB-506, a SBA and a HAP.



**Figure 5:** Pharmacokinetics of AB-506 in A) mice, B) rat, and C) dog.



**Figure 6:** *In vivo* antiviral activity of AB-506. A) Reduction in serum HBV DNA is dose responsive following AB-506 administration. B) AB-506 surpassed ETV at inhibiting liver HBV DNA, at dosages where the serum HBV DNA inhibition was equivalent (data relative to vehicle at Day 7).

## CONTACT INFORMATION

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