Discovery and Preclinical Profiles of Potent Pan-Genotypic Hepatitis E Virus Inhibitors

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BACKGROUND

Hepatitis E virus (HEV), the causative agent of hepatitis E, is endemic worldwide, with an estimated 20 million acute HEV infections each year. HEV infection is typically self-limiting in healthy individuals but can lead to fulminant hepatitis during pregnancy, and chronic disease in those with compromised immunity, such as transplant recipients, that can rapidly progress to cirrhosis[1]. Currently, there are no approved HEV-specific antivirals, and therapeutic options for HEV infection are limited to off-label use of ribavirin (RBV) and pegylated-interferon-α. *In vitro* compound screening identified two prodrugs, AT-2490 and AT-587, that exhibit promising antiviral profiles for the treatment of life-threatening HEV infection. Studies to evaluate *in vivo* therapeutic efficacy are currently ongoing.

METHODS

Pre-selected molecules were screened for anti-HEV activity utilizing a luciferase-based HEV subgenomic replicon and infection system[2]. EC_{50} values for AT-2490, AT-587, and known HEV inhibitors sofosbuvir (SOF) and RBV, were determined against several strains of HEV, genotype 1 & 3.

- AT-2490 and AT-587 were incubated with primary human hepatocytes at 37ºC for 24 h. Cells were extracted and the formation of the active metabolite AT-9068 measured by LC-MS/MS.
- The activity of human DNA polymerases α, β and γ were tested in a fluorescence-based assay after the addition of the active triphosphate AT-9068.
- Cytotoxicity of AT-2490 and AT-587 was evaluated in Huh-7 replicon cells, human iPS cardiomyocytes and human bone marrow CD34+ cells.
- Genotoxicity of AT-2490 and AT-587 was evaluated using Ames and chromosomal aberration assays, and cardiotoxicity was tested with an hERG assay.
- The selectivity of the compounds were established against other RNA and DNA viruses.

Triphosphate AT-9068 $AUC_{0\text{-}24h}$ (h*pmol /10^6 cells)

CELLS	AT-587	AT-2490
Human hepatocytes	**3,920**	**9,297**

The prodrugs formed high levels of the active triphosphate AT-9068 in human hepatocytes.

There is an urgent need for highly effective drugs for the treatment of Hepatitis E virus (HEV) infection which can lead to fulminant hepatitis during pregnancy and hepatic failure in transplant recipients. *In vitro*, the nucleotide prodrugs **AT-2490 and AT-587 are >30-fold more potent than sofosbuvir and ribavirin against HEV** genotypes 1 and 3, including clinically-resistant strains, and they form high levels of active metabolite in liver cells, the tissue of interest. AT-2490 and AT-587 also have **favorable *in vitro* safety profiles,** so they are promising antivirals for development as potential treatments of life-threatening HEV infection.

RESULTS

Inhibition of HEV Replication by Nucleotide Analogs

	HEV-3 p6 WT (n=5)	HEV-3 p6 G1634R (RBV RAS)	HEV-3 83.2.27	HEV-3f Bochum	HEV-3a LB rabbit	HEV-1 SAR55
AT-587	**81 ± 16**	84 ± 2	142 ± 2	106 ± 12	231 ± 53	292 ± 57
AT-2490	**24 ± 8**	20 ± 2	36 ± 19	34 ± 6	101 ± 84	75 ± 33
SOF	**3647 ± 814**	3,925 ± 1,078	8,181 ± 1,671	8,762 ± 3,022	7071 ± 1,465	12,102 ± 1,529
RBV	**> 10000**	12,793 ± 945	19,111 ± 335	15,761 ± 2,264	24,441 ± 2269	12,793 ± 945
Fitness (%)	**100**	144.2	115	12.1	0.6	0.5

EC_{50} values (nM) against HEV GT-3 WT and RBV clinical resistant strains in Huh7 HEV-3 Kernow-C1 p6/Gluc replicon cells show that AT-2490 and AT-587 were significantly more potent than known HEV inhibitors SOF and RBV. Similar efficacy against HEV-1, Sar55 strain, is also presented.

AT-2490 and AT-587 showed no toxicity in *in vitro* studies

- Active metabolite (AT-9068) did not inhibit human DNA polymerases α, β or γ (IC_{50} >100 μM)
- Not cytotoxic in replicon cells and human iPS cardiomyocytes (CC_{50} >100 μM)
- Minimal effect on viability of bone marrow cells (AT-2490 IC_{50} = 24 μM; AT-587 IC_{50} >30 μM)
- Negative for genotoxicity in Ames and chromosomal aberration assays
- Mean hERG inhibition at 10 μM was 9.4% and 5.5% for AT-2490 and AT-587, respectively

Antiviral specificity of AT-2490 & AT-587 (EC_{50} values, nM)

Virus	AT-2490	AT-587	SOF
HCV GT-1	3	7	81
DENV-2	107	194	>10,000
JEV	134	215	5,760
POWV	188	298	>10,000
WNV	60	81	3,210
YFV	26	54	1,908
ZIKV	55	125	>10,000
CHIKV	173	259	>10,000
Norovirus	7,957	4,868	ND
Rubella	57	92	12,425
VEEV	2,930	3,408	>10,000

ND= not determined; DENV, dengue; HCV, hepatitis C; JEV, Japanese encephalitis; POWV, Powassan; WNV, West Nile; YFV, yellow fever; ZIKV, Zika virus; CHIKV, chikungunya; VEEV, Venezuelan equine encephalitis

- AT-2490 and AT-587 were efficacious against all flaviviruses (HCV, DENV, JEV, POWV, WNV, YFV, ZIKV) tested using reporter luciferases in infected Huh7 cells.
- The prodrugs were also active against rubella and CHIKV, but only weakly active against VEEV and human norovirus.
- They were not active against coronaviruses (HCoV-OC43, SARS-Cov-2, MERS), RSV, hMPV, CCHFV, LASV, RVFV, VSV, HIV and Marburg virus, or any of the DNA viruses tested (HBV and HSV-1).

CONCLUSIONS

- AT-2490 and AT-587 were 30-150-fold more potent *in vitro* against HEV than known inhibitors SOF and RBV.
- They formed high levels of AT-9068, the active metabolite, in human hepatocytes, the tissue of interest.
- They showed no toxicity in *in vitro* studies.
- The prodrugs were also active against flaviviruses, rubella and chikungunya (EC_{50} range of 7 – 300 nM).
- AT-2490 and AT-587 are oral nucleotide analogs exhibiting promising antiviral profiles of potential first-in-class inhibitors for the treatment of life-threatening HEV infection.

ADDITIONAL KEY INFORMATION

References

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