# 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**

1. World Health Organization. Hepatitis E. Updated July 2023. Accessed February 6, 2026. https://www.who.int/news-room/fact-sheets/detail/hepatitis-e
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**Acknowledgements**
These studies were funded by Atea Pharmaceuticals, and several authors are employees of and may own stock in Atea Pharmaceuticals, Boston, MA, USA.