AT-337, AT-511 and its Salt Form, AT-527: Novel Potent and Selective Pan-genotypic Purine Nucleotide Prodrug Inhibitors of HCV Polymerase

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Introduction

Hepatitis C virus (HCV) infection remains a global concern with approximately 170 million patients chronically infected worldwide. Although HCV treatment outcomes have been greatly improved recently with direct-acting antivirals (DAAs), current marketed therapies still require 12 weeks of treatment. More potent DAAs could significantly shorten treatment duration and improve compliance while achieving high SVR rates across genotype and disease stage. Nucleotide analogs with potent pan-genotypic activity and high barrier to drug resistance are considered backbone therapeutics in the treatment of HCV infection. Previously reported results (1) with AT-337 and AT-511, novel purine-based nucleotide prodrugs with both base and sugar modifications, demonstrated activity against HCV genotype 1b and favorable *in vitro* selectivity profiles. Here we further report potent pan-genotypic activities and additional *in vitro* selectivity of these compounds, as well as the *in vivo* pharmacokinetics and formation of the active analog triphosphate after oral administration of AT-527, a salt form of AT-511.

Methods

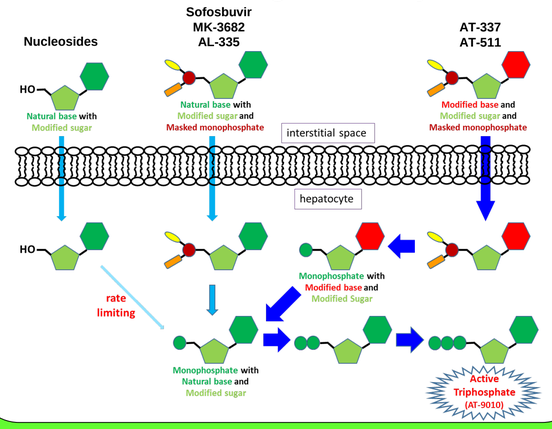
A panel of replicons containing the NS5B sequences from various HCV genotypes derived from 6 laboratory reference strains (GT1a, 1b, 2a, 3a, 4a and 5a) and from 8 HCV patient plasma samples (GT1a, 1b, 2a, 2b, 3a-1, 3a-2, 4a and 4d), as well as replicons containing wild-type or sofosbuvir (SOF) resistance-associated variants (RAVs) from laboratory constructs (1a(C316N), 1a(S282T), 1b(L159F), 1b(S282T) and 2b(L159N)) and from clinical isolates (1a(L159F), 1a(L159F/S282T) and 1b(C316N)) were used to determine the IC_{50} and/or IC_{95} values for AT-337, AT-511 and sofosbuvir.

$K_{d,app}$ and k_{pol} for the incorporation of the analog nucleoside triphosphates of sofosbuvir, INX-189, and AT-337 and AT-511 by human mitochondrial RNA-dependent RNA polymerase (POLRMT) were determined according to published methods (2).

Nucleobase formation from the purine-based nucleotide prodrugs PSI-938, PSI-661, INX-189, AT-337 and AT-511 (5 µM starting concentrations) was measured by LC-MS/MS after 2-hr incubations with recombinant human CYP3A4 as described (3).

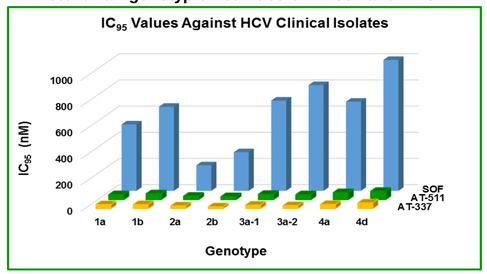
Male Sprague-Dawley rats and cynomolgus monkeys (3 animals per dose group) were given single oral doses of AT-527 (a salt form of AT-511). Aliquots of plasma prepared from blood samples treated with Dichlorvos, were analyzed by LC-MS/MS for concentrations of AT-511 and AT-273 (the nucleoside metabolite of the active triphosphate of AT-511), and pharmacokinetic parameters were determined using WinNonlin. Samples of liver and heart were obtained from separate animals (2 per dose group) at 4 or 8 hrs after a single oral dose of AT-527, flash-frozen, homogenized and analyzed by LC-MS/MS for intracellular levels of the active triphosphate, AT-9010.

Atea's Antiviral Drug Discovery Platform



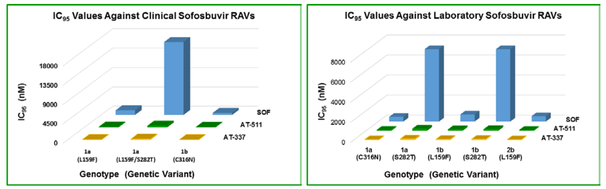
Results

Potent Pan-genotypic Activities of AT-337 and AT-511



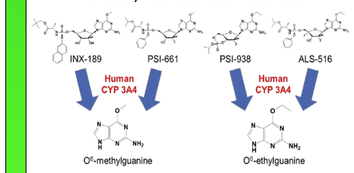
- IC_{95} values for AT-337 and AT-511 are 7-33 times lower than sofosbuvir against clinical isolates of all HCV genotypes tested.
- IC_{50} values for AT-337 and AT-511 are 6-27 times lower than sofosbuvir against laboratory strains of HCV genotypes 1-5 (data not shown).

Potent Activities of AT-337 and AT-511 Against Sofosbuvir RAVs



- AT-337 and AT-511 maintain their activities against clinical isolates of sofosbuvir RAVs, with IC_{95} values that are >50-fold lower than those of sofosbuvir against the S282T variant.
- AT-337 and AT-511 maintain their activities against single variant S282T constructs, with IC_{95} values that are >40-fold lower than those of sofosbuvir.

Potential for Formation of Genotoxic Nucleobases from the Purine Nucleotide Prodrugs INX-189, PSI-661, PSI-938 and ALS-516



- O^6-methylguanine and O^6-ethylguanine are known mutagens that cause aneuploidy and apoptosis via inhibition of DNA synthesis (4-6).
- The formation of these mutagens may be the underlying mechanism for the liver toxicity observed in extended clinical trials with PSI-938.

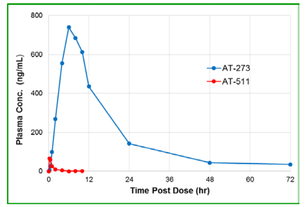
Nucleobase Formation After Incubation of Nucleotide Prodrugs with Human CYP3A4

Test Article	Nucleobase Formation (% of starting concentration converted)		
	O^6-Methyl-guanine	O^6-Ethyl-guanine	Other Modified Nucleobases
AT-337	Not Detected	Not Detected	2.1
AT-511	Not Detected	Not Detected	1.8
PSI-661	52	Not Detected	Not Detected
PSI-938	Not Detected	32	Not Detected
INX-189	8.7	Not Detected	Not Detected

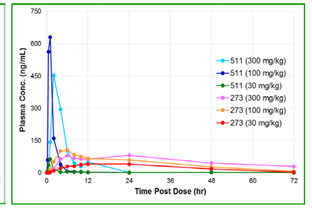
5 µM nucleotide prodrug was incubated for 2 hr with recombinant human cytochrome P450 3A4

- AT-337 and AT-511 are not metabolized to the mutagenic nucleobases O^6-methyl-guanine or O^6-ethylguanine, in contrast to PSI-938, PSI-661 and INX-189.
- AT-337 and AT-511 do not significantly inhibit human cytochrome P450 isoforms 1A2, 2B6, 2C8, 2C9, 2C19, 3A4 and 2D6 (data not shown).

Plasma Profiles of AT-511 and AT-273 (metabolite of the active TP) in Rats Given Single 500 mg/kg Oral Doses of AT-527 (salt form of AT-511)



Plasma Profiles of AT-511 and AT-273 (metabolite of the active TP) in Monkeys Given Single 30, 100 or 300 mg/kg Oral Doses of AT-527 (salt form of AT-511)



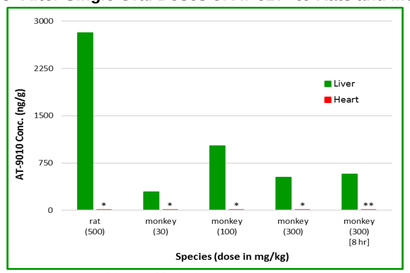
Plasma Pharmacokinetic Parameters for AT-511 and AT-273 in Rats and Monkeys Given Single Oral Doses of AT-527 (salt form of AT-511)

Species	Dose (mg/kg)	AT-511				AT-273			
		C_{max} (ng/mL)	T_{max} (hr)	$T_{½}$ (hr)	$AUC_{0\text{-}last}$ (hr*ng/mL)	C_{max} (ng/mL)	T_{max} (hr)	$T_{½}$ (hr)	$AUC_{0\text{-}last}$ (hr*ng/mL)
Rat[a]	500	60.8	0.25	N.D.[c]	78.2	541	6-8	15.3	9640
Monkey[b]	30	63.5	0.5-2	N.D.[d]	176	42.5	12-24	11.5	1620
	100	783	1-2	N.D.[d]	1100	131	4	15.0	3030
	300	501	2-4	N.D.[d]	1600	93.6	4-24	18.8	3660

Dose formulations: [a]0.5% carboxymethylcellulose and 0.5% Tween80 in water; [b]powder in capsules
[c]Elimination half-life cannot be determined due to the instability of AT-511 in rat blood ($T_{½}$<2 min)
[d]Apparent enterohepatic recirculation prevents accurate determination of $T_{½}$

- High plasma levels of AT-273, the nucleoside metabolite of the active triphosphate (TP) of AT-511, are indicative of formation of high levels of the TP, even in rats where very low plasma levels of parent nucleotide prodrug are observed due to the short half-life of AT-511 in rat blood (<2 min).
- Persistent plasma levels of AT-273 reflect the long half-life of the TP.
- In monkeys, plasma exposures (AUC) of AT-511 are roughly dose-proportional and AT-273 exposures are somewhat less than dose-proportional, although AUC values for both parent drug and the nucleoside metabolite of the active TP (AT-9010) continue to increase up to the highest dose tested (300 mg/kg).

Tissue Levels[1] of AT-9010 (active triphosphate of AT-511) 4 Hours[2] After Single Oral Doses of AT-527[2] to Rats and Monkeys



[1] Average of 2 animals per group
[2] Unless otherwise indicated [in brackets]
* Concentrations below the LLOQ of 60 ng/g
** Concentrations below the LLOQ of 30 ng/g

- Significant liver levels of the active TP were obtained in both rats and monkeys at all doses and undetectable heart levels indicate selective liver formation of the TP.

Kinetic Parameters for Nucleotide Analogs Evaluated with Human Mitochondrial RNA Polymerase

Nucleotide Analog	K_{pol} (s^{-1})	$K_{d,app}$ (uM)	$K_{pol}/K_{d,app}$ ($uM^{-1}s^{-1}$)	Relative Efficiency*
2'-deoxy-2'-F-2'-C-methyl UTP (active TP of sofosbuvir)	0.00034 ± 0.00005	590 ± 250	5.8×10^{-7} ± 2.6×10^{-7}	1.0×10^{-6}
2'-C-methyl GTP (active TP of INX-189)	0.051 ± 0.002	240 ± 26	2.1×10^{-4} ± 0.2×10^{-4}	5.5×10^{-5}
AT-9010 (active TP of AT-337 and AT-511)	0.0017 ± 0.0002	204 ± 94	8.3×10^{-6} ± 4.0×10^{-6}	2.2×10^{-6}

*Relative efficiency = $(K_{pol}/K_{d,app})_{analog\ nucleotide}$ / $(K_{pol}/K_{d,app})_{natural\ nucleotide}$

- The poor efficiency of incorporation of AT-9010 is similar to that of the triphosphate of sofosbuvir.

Conclusions

- AT-337 and AT-511 are potent pan-genotypic inhibitors of HCV and are 6- to 33-fold more inhibitory of HCV replication *in vitro* than sofosbuvir.
- AT-337 and AT-511 maintain their activities against the HCV S282T variant, with 40- to 124-fold greater potency than sofosbuvir.
- AT-337 and AT-511 are not metabolized to mutagenic nucleobases by recombinant human CYP3A4, in contrast to the purine-based nucleotide prodrugs PSI-661, PSI-938 and INX-189.
- AT-337 and AT-511 are not likely to affect mitochondrial integrity since their active triphosphate is poorly incorporated by human mitochondrial RNA polymerase with an efficiency similar to that of the triphosphate of sofosbuvir; the relative efficiency of incorporation of the triphosphate of INX-189 is up to 55-fold greater.
- The salt form of AT-511 (AT-527) exhibits substantially improved pharmaceutical properties as compared to the free base (data not shown).
- Oral administration of AT527 to rats and monkeys produced high and dose-dependent plasma exposures to AT-273 (the nucleoside metabolite of the intracellular active triphosphate, AT-9010); AT-273 exposures continued to increase up to the highest dose tested, reflecting substantial formation of AT-9010 in these species.
- The high levels of AT-9010 observed in liver and the long half-lives of AT-273 in plasma of rats and monkeys dosed orally with AT-527 suggest clinical antiviral activity with once daily dosing; the undetectable levels of AT-9010 in heart are indicative of liver-specific formation of the active triphosphate.
- IND/CTA-enabling studies with AT-527 are ongoing.

References

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Acknowledgements

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