

ANA598, a Novel Non-Nucleoside Inhibitor of HCV NS5B Polymerase, Exhibits Favorable Pharmacokinetic Properties in Multiple Preclinical Species

Leo Kirkovsky, Yuefen Zhou, Daniel Norris, Ellen Okamoto, Thomas G. Nolan, Darian Bartkowski, Julia Khandurina, Maria Sergeeva, Douglas Murphy, Benjamin Ayida, Alan Xiang, David Ellis, Julie Blazel, Zhongxiang Sun

Anadys Pharmaceuticals, Inc.
3115 Merryfield Row, San Diego, CA 92121 USA

ABSTRACT

INTRODUCTION: Inadequate responses to current HCV therapies create an urgent unmet need for new antiviral agents to complement today's standard of care, particularly for treatment of genotype 1 infections. Probable future HCV therapies will add multiple direct-acting antiviral agents selected from differing functional classes to enhance response and suppress the emergence of resistant HCV variants. The essentiality of the NS5B polymerase predicts that inhibitors of this enzyme should be attractive additions to current standard of care. However, first generation development candidates targeting this essential enzyme suffer notable defects in potency, metabolism, or pharmacokinetic properties that limit their potential. We describe here the superior pharmacokinetic properties of ANA598, a novel, orally available and potent "palm site" inhibitor of HCV genotype 1 NS5B polymerase.

RESULTS AND DISCUSSION: ANA598 demonstrated high oral bioavailability in all four species evaluated. ANA598 plasma exposures were lower in the rat than the monkey; exposure did not differ significantly for solution and suspension formulations in these species. An oral dose of 5 mg/kg ANA598 to monkeys provided C_{12} and C_{24} levels (21,600 nM and 7,600 nM, respectively) that were in excess of the replicon EC_{50} (52 nM for genotype 1a and 3 nM for genotype 1b) even after adjustment for binding to plasma proteins. ANA598 accumulated in rat liver and reached a liver-to-plasma ratio of ~20 at 12 hours after an oral 5 mg/kg dose, suggesting significant additional antiviral coverage in the primary target organ for HCV replication.

CONCLUSIONS: Preclinical studies of ANA598 in four species have demonstrated high oral bioavailability and plasma and liver trough levels that are substantial multiples of replicon EC_{50} . The favorable combined characteristics of ANA598 support additional investigation of the compound and clearly differentiate it from other molecules of this functional class.

ANA598, a Novel Non-Nucleoside Inhibitor of HCV NS5B Polymerase, Exhibits Favorable Pharmacokinetic Properties in Multiple Preclinical Species

Leo Kirkovsky, Yuefen Zhou, Daniel Norris, Ellen Okamoto, Thomas G. Nolan, Darian Bartkowski, Julia Khandurina, Maria Sergeeva, Douglas Murphy, Benjamin Ayida, Alan Xiang, David Ellis, Julie Blazel, Zhongxiang Sun

Anadys Pharmaceuticals, Inc. 3115 Merryfield Row, San Diego, CA 92121 USA

Abstract

INTRODUCTION: Inadequate responses to current HCV therapies create an urgent unmet need for new antiviral agents to complement today's standard of care, particularly for treatment of genotype 1 infections. Probable future HCV therapies will add multiple direct-acting antiviral agents selected from differing functional classes to enhance response and suppress the emergence of resistant HCV variants. The essentiality of the NS5B polymerase predicts that inhibitors of this enzyme should be attractive additions to current standard of care. However, first generation development candidates targeting this essential enzyme suffer notable defects in potency, metabolism, or pharmacokinetic properties that limit their potential. We describe here the superior pharmacokinetic properties of ANA598, a novel, orally available and potent "palm site" inhibitor of HCV genotype 1 NS5B polymerase.

RESULTS AND DISCUSSION: ANA598 demonstrated high oral bioavailability in all four species evaluated. ANA598 plasma exposures were lower in the rat than the monkey; exposure did not differ significantly for solution and suspension formulations in these species. An oral dose of 5 mg/kg ANA598 to monkeys provided C_{12} and C_{24} levels (21,600 nM and 7,600 nM, respectively) that were in excess of the replicon EC_{50} (52 nM for genotype 1a and 3 nM for genotype 1b) even after adjustment for binding to plasma proteins. ANA598 accumulated in rat liver and reached a liver-to-plasma ratio of ~20 at 12 hours after an oral 5 mg/kg dose, suggesting significant additional antiviral coverage in the primary target organ for HCV replication.

CONCLUSIONS: Preclinical studies of ANA598 in four species have demonstrated high oral bioavailability and plasma and liver trough levels that are substantial multiples of replicon EC_{50} . The favorable combined characteristics of ANA598 support additional investigation of the compound and clearly differentiate it from other molecules of this functional class.

Updated abstract

Acknowledgements

The authors thank Mei Tran and Amit Shah for generating the data in Table 1, Laurie LeBrun for generating the data in Table 2, Richard Showalter for providing recombinant NS5B enzyme for *in vitro* studies, and Oleg Khatsenko for TK analysis.

This work would not be possible without Medicinal Chemistry contribution of Chinh Tran, Frank Ruebsam, Douglas Murphy, Peter Dragovich, and Yuefen Zhou to the discovery of ANA598.

The authors are grateful to Stephen Worland, Devron Averett, James Appleman and Stephen Webber for their support and helpful discussions.

Summary of *in vitro* and *in vivo* preclinical data for ANA598 was presented at the 14th International Symposium on Hepatitis C Virus and Related Viruses, September, 2007, Glasgow, UK (Kirkovsky L, Zhou Y, Shah A, Tsan M, LeBrun L, Sergeeva M, Norris D, Bartkowski D, Nolan T, Khandurina J. **Preclinical Characterization of a Novel, Potent, and Pharmacokinetically Appealing Non-Nucleoside Inhibitor of HCV NS5B Polymerase**).

The first part of this work was presented at the 41st Western Regional Meeting of the American Chemical Society, October, 2007, San Diego, CA, (Maria Sergeeva, Amit Shah, Michael Noble, Mei Tsan, Rupal Patel, Richard Showalter, Ruhi Kamran, Laurie LeBrun, Chinh Tran, Frank Ruebsam, Douglas Murphy, Peter Dragovich, Yuefen Zhou, and Leo Kirkovsky. **ANA598, a Novel Non-Nucleoside Inhibitor of HCV NS5B Polymerase, Exhibits Potent Anti-HCV Activity and Favorable ADME Characteristics *in Vitro***).



In Vitro Assays of NS5B Inhibition

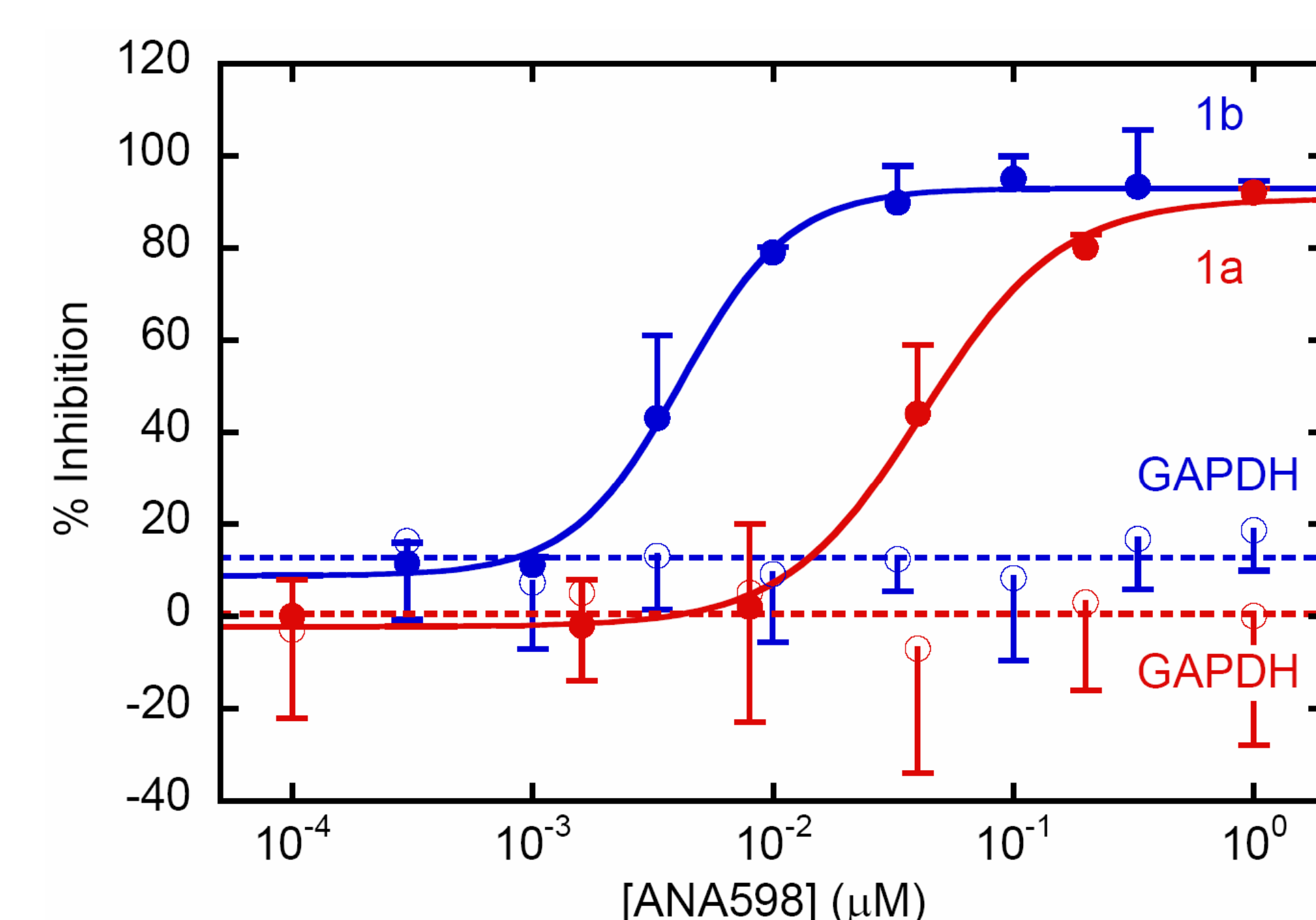


Figure 1. ANA598 is a Potent Inhibitor of 1b and 1a Replicons

• Representative data from a single experiment.

Table 1. Summary of *In Vitro* Efficacy Data

Genotype	NS5B IC_{50} (nM) (steady-state)	NS5B IC_{50}^* (nM) (transient-state)	Replicon EC_{50} (nM)
1b (Consensus)	< 10	<< 1	3 ± 2 (n=10)
1b (BK)	< 10	<< 1	Not Determined
1b (BK M414T)	11 ± 5	8	Not Determined
1a (H77)	14 ± 1	< 1	52 ± 13 (n=5)

Assay Conditions:

- Inhibition of soluble recombinant HCV NS5B was assayed using radiolabeled nucleotide incorporation.
- IC_{50} values were calculated from the dose-dependence of inhibition of product formation during steady state by fitting to a standard 4-parameter logistic equation. Enzyme concentrations: 1b consensus and 1b BK wt – 20 nM, 1a H77 NS5B and 1b BK M414T – 50 nM.
- IC_{50}^* values were calculated from the dose-dependence of inhibition of product formation in the transient state by fitting to a standard 4-parameter equation for tight-binding inhibitors where the enzyme concentration is one of the parameters. Enzyme concentrations: 1b consensus and 1a H77 NS5B – 5 nM, 1b BK wt and 1b BK M414T – 1 nM.
- Permissive Huh7 lineage cells bearing either the dicistronic 1b or the dicistronic 1a replicon were incubated in presence of increasing concentrations of ANA598 for 72 hours. HCV and cellular GAPDH RNA levels were determined by bDNA assay (1b) and RT-PCR assay (1a).

• ANA598 is a sub-nanomolar inhibitor of soluble recombinant HCV NS5B: for 1b (consensus), 1b BK wt, and 1a H77 NS5B enzymes, $IC_{50}^* < 1$ nM.

• For the 1b BK M414T mutant, $IC_{50}^* = 8$ nM; thus, ANA598 remains a potent inhibitor of this palm-site mutation.

• ANA598 exhibits potent and highly selective activity against both 1b and 1a HCV replicons in cellular assays: EC_{50} (1b) ~ 3 nM and EC_{50} (1a) ~ 52 nM (Figure 1).

• ANA598 shows no appreciable cellular cytotoxicity as indicated by XTT $CC_{50} > 100$ µM (data not shown) and cellular GAPDH $EC_{50} > 100$ µM (Figure 1).

In Vitro Predictors of Safety

• **Target specificity:** >1000-fold greater potency for NS5B 1a and 1b than for any enzymes or receptors in MDS Pharma Adverse Reaction Enzymes and Spectrum Screen panels.

• **Cellular cytotoxicity:** 300-fold difference between 1a replicon EC_{50} and CC_{50} of HK-2 kidney proximal tubule cell line, the most sensitive cell line in a broad panel of cell lines representing many tissue types.

• ***In vitro* genotoxicity:** non-mutagenic *in vitro* at 0.15 mM (Ames) and 9 mM (chromosomal aberration, ± S9).

• ***In vitro* cardiotoxicity:** hERG $IC_{50} = 84$ µM (plasma protein binding adjusted value equal to 168 mM).

• **Drug-drug interactions:** no significant inhibition of CYPs at >1000 x IC_{50}^* for inhibition of HCV NS5B.

DMPK

Table 2. ANA598 Microsomal Stability

Species	$t_{1/2}$ (min)	% remaining at 60 minutes
Human		
Male	>60 min	79 %
Female	>60 min	113%
Cynomolgus Monkey		
Male	>60 min	86%
Female	>60 min	84%
Beagle Dog		
Male	>60 min	100%
Female	>60 min	92%
CD-1 Mouse		
Male	60 min	
Female	>60 min	56%
Sprague-Dawley Rat		
Male	43 min	
Female	>60 min	103%

Assay Conditions:

- Stability was assessed with 1 µM test compound in the presence of 0.5 mg/mL microsomes with a re-generation system at 37 °C over 60 minutes.

• ANA598 demonstrates excellent *in vitro* metabolic stability in human, monkey and dog liver microsomes.

• Significant sex-dependent microsomal stability of ANA598 is observed in Sprague-Dawley rat liver microsomes.

Table 3. Plasma PK Parameters of ANA598 after a Single Oral Dose of 5 mg/kg in Different Species

Species	F_{oral} %	AUC_{inf} h*µM	** C_{12} µM	** C_{24} µM	C_{max} µM	T_{max} h	$T_{1/2}$ h
Cynomolgus Monkey	65	602	21.6	7.6	42	5.5	8
Beagle Dog	85	441	13.8	3.7	40	2	2
CD-1 Mouse *	45	42 (M) 114 (F)	0.10 (M) 0.74 (F)		11 (M) 22 (F)	0.5 (M) 4 (F)	3
Sprague-Dawley Rat *	~115	94	0.3 (M) 2.0 (F)		24	0.4	5

* mean values in rat and mouse are given separately for males (M) and females (F)
** C_{12} and C_{24} - plasma levels at 12 and 24 hours post dose, respectively

• ANA598 demonstrated high oral bioavailability in all four species evaluated.

• ANA598 plasma exposures were lower in rodents than in monkey and dog.

• Exposure did not differ significantly for solution and suspension formulations in monkey (data not shown).

• An oral dose of 5 mg/kg ANA598 to monkeys provided C_{12} and C_{24} levels (21.6 µM and 7.6 µM, respectively) that were in excess of the replicon EC_{50} adjusted for binding to plasma proteins.

Table 4. Oral Bioavailability of ANA598 in Rat and Monkey at Different Dose Levels

Dose (mg/kg)	Oral Bioavailability (%F)	
	Sprague-Dawley Rat	Cynomolgus Monkey
1	55	52
5	115	65
30	96	73

• Oral bioavailability of ANA598 in rat and monkey is high for doses that are anticipated to be clinically relevant.

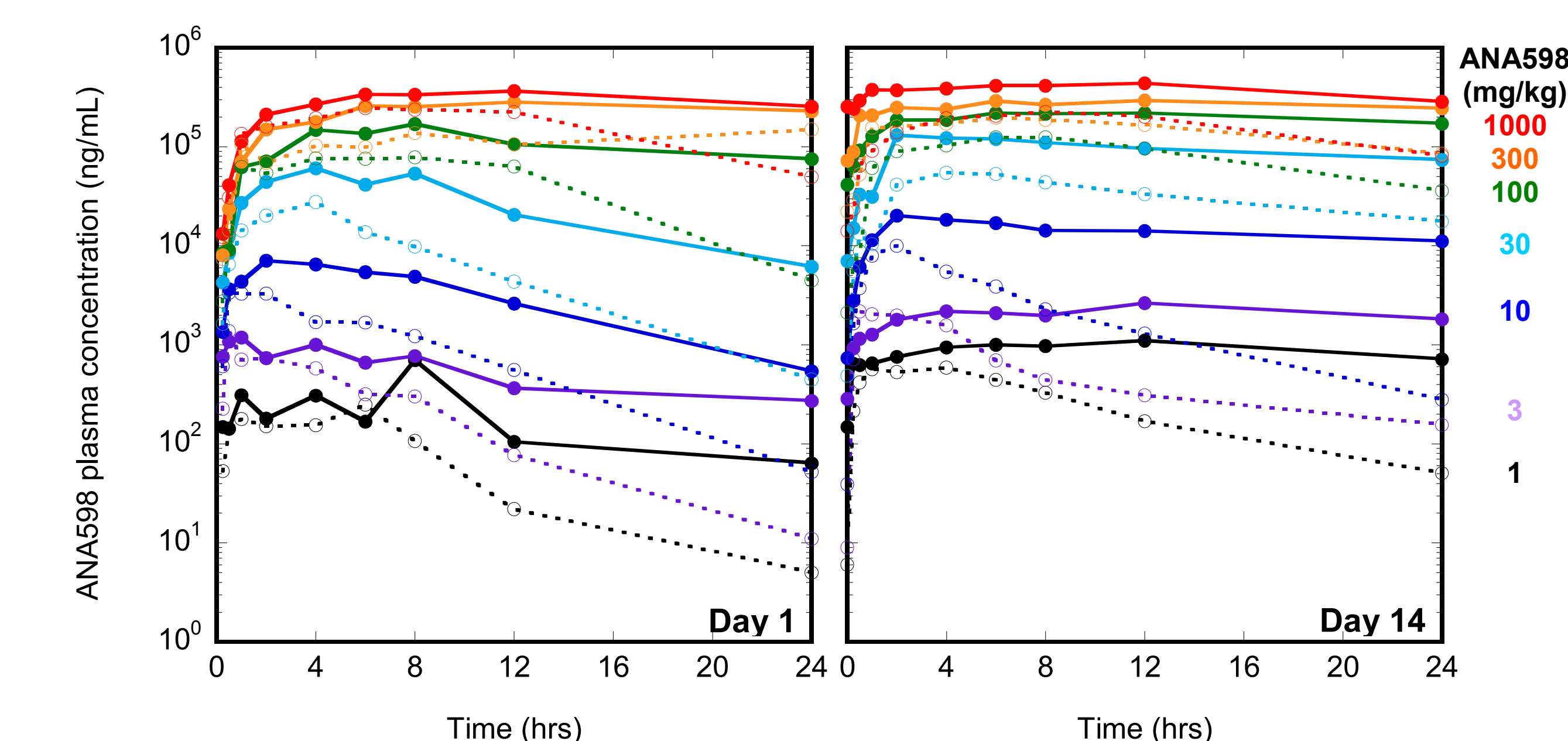


Figure 2. TK of ANA598 in Sprague-Dawley Rats Dosed QD for 14 Days

ANA598 was administered as an aqueous suspension containing 3% CMC in this study. Solid lines/closed symbols and dashed lines/open symbols represent ANA598 concentrations in plasma of female and male rats, respectively.

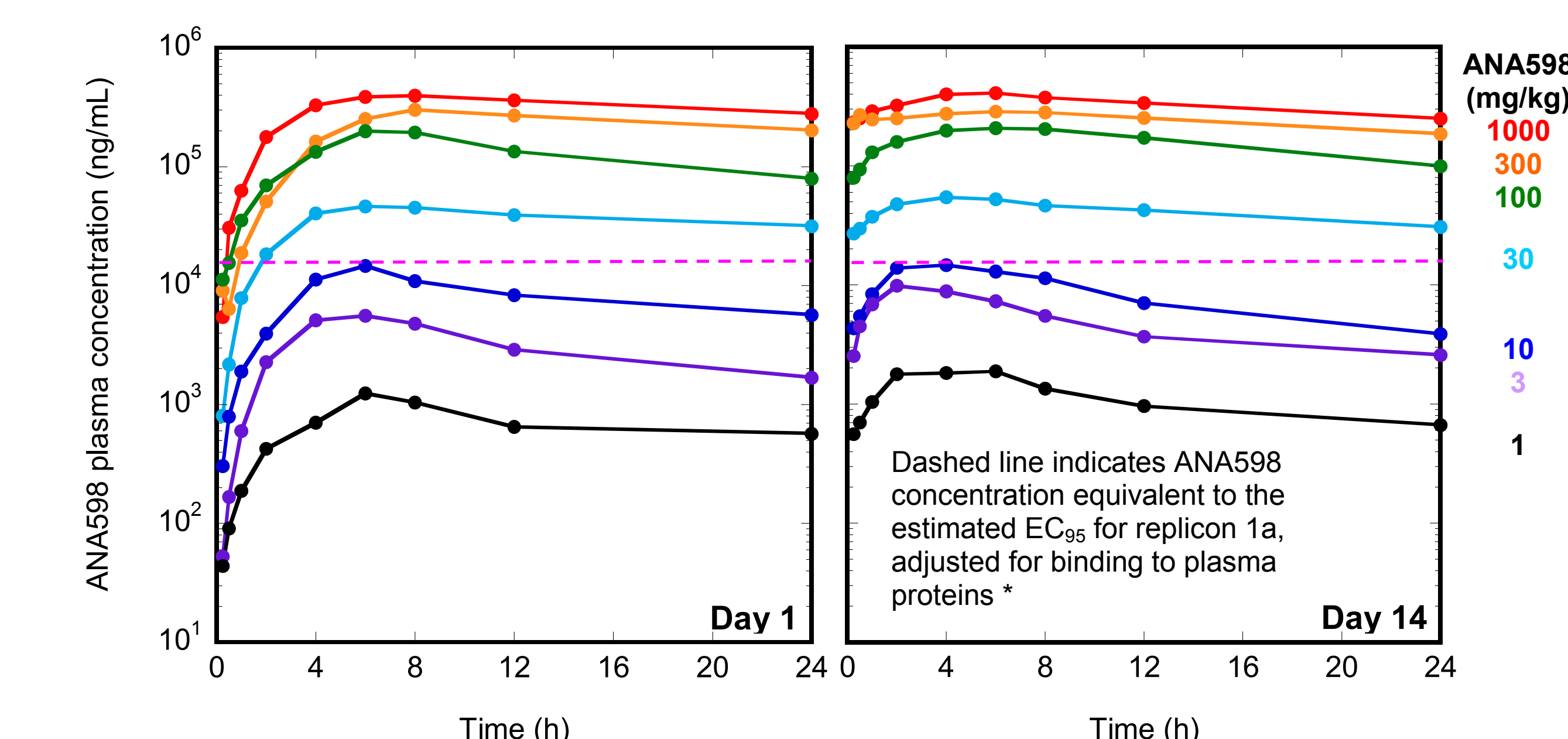


Figure 3. TK of ANA598 in Cynomolgus Monkeys Dosed QD for 14 Days

ANA598 was administered as an aqueous suspension containing 3% CMC in this study.

* The adjustment for binding to plasma proteins is the ratio of the free fraction of ANA598 in replicon media to the free fraction in human plasma. This is a very conservative approach to estimating the effect of binding to plasma proteins on the potency of an antiviral *in vivo*. For the 1a replicon, $EC_{50} \sim 10 * EC_{50}^*$.

• ANA598 is well-tolerated at all dose levels (n=2, one M and one F monkey at each dose level and n=12, six M and six F rats at each dose level in this DRF study).

• Intra- and inter-day plasma concentrations of ANA598 are relatively constant throughout the 14-day dosing period at all doses.

• AUC and C_{max} are essentially dose-proportional from 1 – 100 mg/kg. These values continue to increase at 300 and 1000 mg/kg, but in a less than dose-proportional fashion.

• Trough plasma concentrations of ANA598 in monkeys well in excess of those required to inhibit HCV genotype 1 replicons, including the less sensitive genotype 1a, were easily achieved with once daily oral dosing.

• ANA598 liver concentrations in monkeys were approximately 2-fold higher than ANA598 plasma concentrations on Day 14, 24 hours post-administration at the expected therapeutic dose. In contrast, ANA598 liver concentrations in rodents exceed the plasma concentration by ~ 20-fold at the corresponding time point (data not shown).

Conclusions

• ANA598 is a highly potent and selective inhibitor of HCV genotypes 1a and 1b NS5B RNA polymerases ($IC_{50}^* < 1$ nM) and of HCV replication in cell culture (EC_{50} values for genotypes 1a and 1b HCV replicons are 52 and 3 nM, respectively).

• Preclinical studies of ANA598 in four species have demonstrated high oral bioavailability and plasma and liver trough levels that are substantial multiples of replicon EC_{50} .

• These favorable antiviral, metabolic, pharmacokinetic and preliminary toxicologic properties clearly differentiate it from other molecules of this functional class and strongly support further evaluation of ANA598 for the treatment of HCV infection.