

Synergy of a Hepatitis C virus  
NS4A antagonist, ACH-806, in  
combination with HCV  
protease or polymerase  
inhibitors

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# HCV NS4A

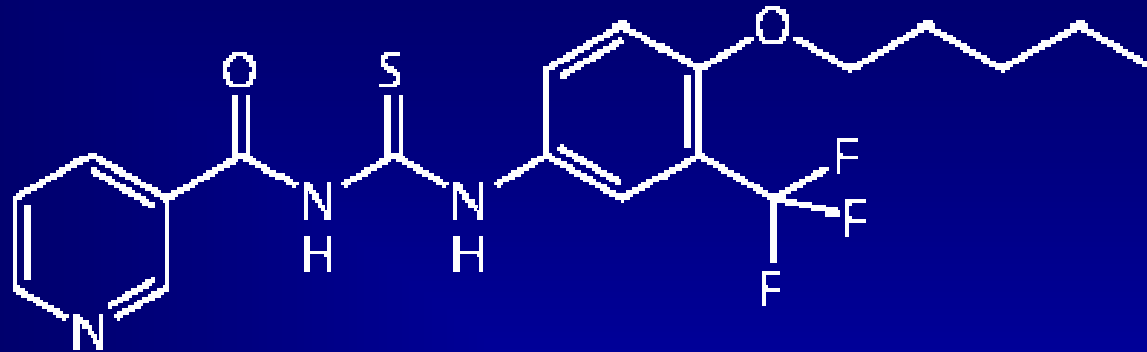
## 54 amino acid NS3 protease co-factor

- Hydrophobic N-terminal: transmembrane  $\alpha$ -helix
- Central amino acids(21-34): essential for co-factor function

## Co-factor functions

- Stabilizes NS3 structure and anchors to replication complex
- Improves cleavage efficiency
- Required for cleavage at NS4B/5A junction

# NS4A Antagonist: ACH-806



## Acythiourea NS4A antagonist

- Blocks formation of functional replication complexes
- IC<sub>50</sub> 20nM (genotype 1 replicon)
- Unique resistance profile
  - No cross resistance in vitro

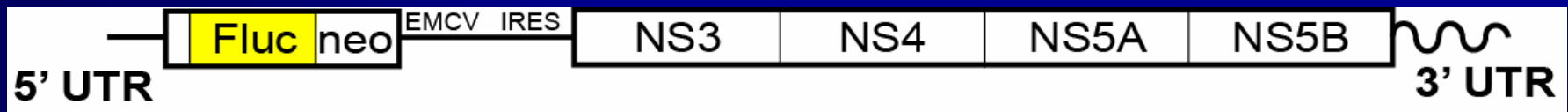
# Aim

Given its unique mechanism of action, we sought to evaluate ACH-806 in vitro in combination with interferon  $\alpha$  and small molecular inhibitors of HCV replication.

# Experimental System

## Luciferase Replicon

- Modification of the BM4-5 replicon (Guo JT. J Virol, 2001.)



## Assay Conditions

- 96-well plates (10,000 cells/well)
- 48 hour compound incubations
- Synergy determinations based on the multiple drug effect equation of Chou and Talalay (Calculusyn).
- Cytotoxicity evaluated using a MTS viability assay (Promega)

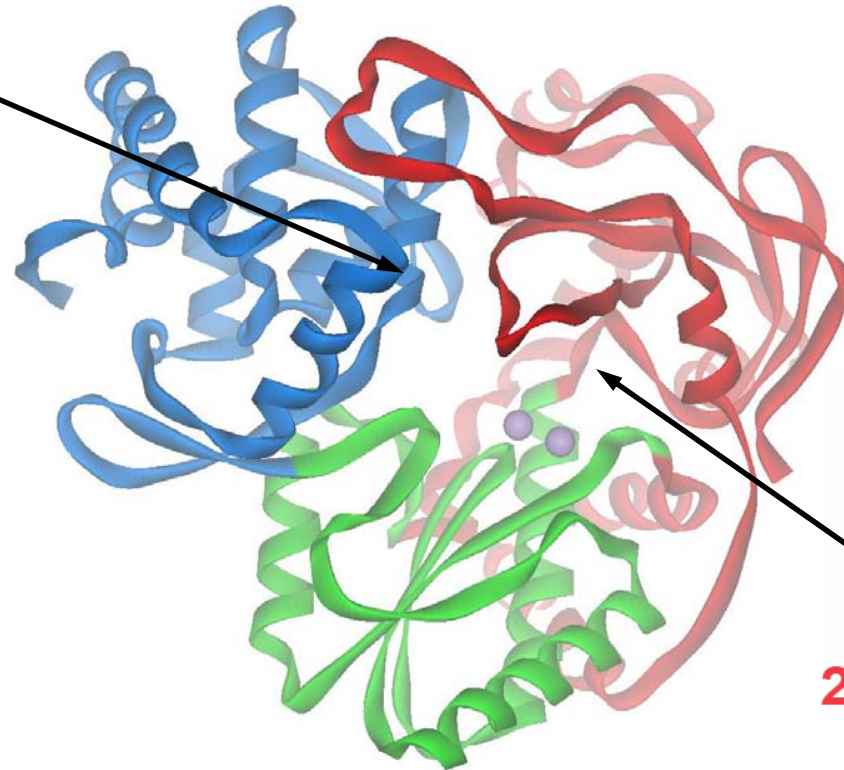


# NS5B polymerase inhibitors

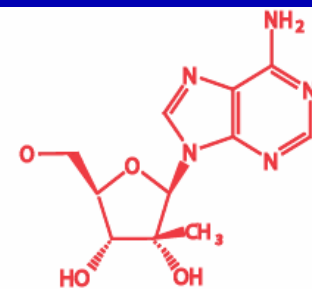
## Thiadiazine



$IC_{50}$  3.5 $\mu$ M



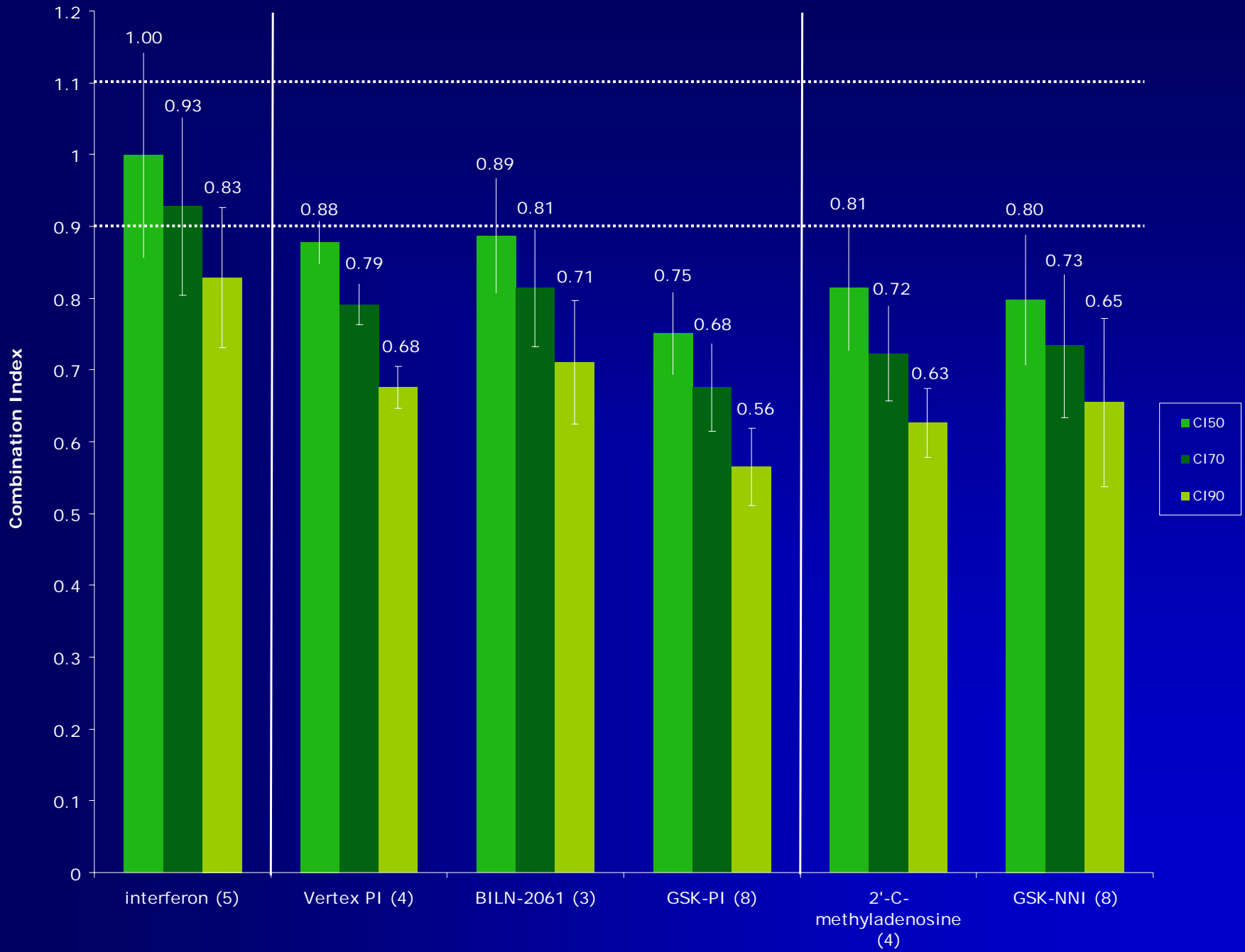
## Nucleoside



**2'-C-methyladenosine**

$IC_{50}$  450nM

# ACH-806 combination studies



# Conclusions

The NS4A antagonist, ACH-806, displayed mild to moderate synergy with other small molecular inhibitors of HCV replication. Importantly, no antagonism was noted between ACH-806 and NS3 protease inhibitors.

An additive interaction was seen with interferon

Chip Schooley  
Kelly Kaihara  
Aleem Siddiqui

UCSD CFAR

- Doug Richman

Achillion Pharmaceuticals

- John Pottage

Christoph Seeger

Vicki Sato

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William Lee

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**TEAMWORK**

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