



# **Synergy of Small Molecular Inhibitors of Hepatitis C Virus (HCV) Replication Directed at Different Viral Targets**

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# Synergy of Small Molecular Inhibitors of HCV Replication Directed at Different Viral Targets

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# Introduction

Interferon based therapy for HCV has significant limitations

- ◆ Marginal Efficacy ~50-55%
  - ◆ Genotype 1 ~45%
  - ◆ HIV co-infection ~20-30%
- ◆ Poor Tolerability
  - ◆ Interferon: flu-like symptoms, depression, neutropenia
  - ◆ Ribavirin: hemolytic anemia

# Hypothesis

As virus specific anti-HCV therapies are developed- viral resistance, as in HIV-1, will become a major problem.

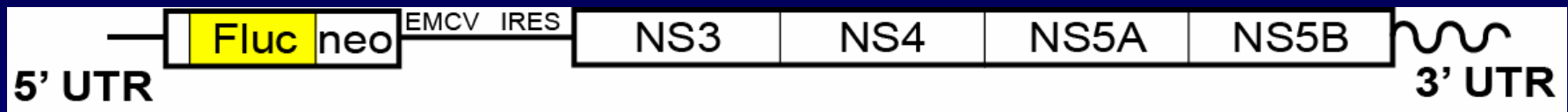
(Trozzi C. J Virol, 2003. Sarrasin C. AASLD, 2005)

Multi-drug (multi-target) therapy will be necessary to prevent the development of resistance. In vitro synergy studies will aid in the design of effective multi-drug therapeutic strategies.

# Experimental System

## Luciferase Replicon

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



## Compound Activity Assay

- ◆ 96-well plates (10,000 cells/well)
- ◆ Conditions run in triplicate
- ◆ 48 hour incubation
- ◆ Luciferase activity determined (BrightGlo-Promega)

# Synergy Testing

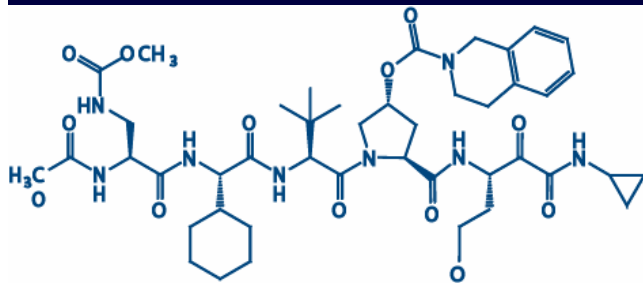
Based on the median effect principle and expressed as a combination index. (Chou T. Adv Enzyme Reg, 1984.)

$$CI = \frac{(D)_1}{(D_X)_1} + \frac{(D)_2}{(D_X)_2} + \alpha \frac{(D)_1 (D)_2}{(D_X)_1 (D_X)_2}$$

|        |   | Drug 1                                    |   |  |  |  |  |
|--------|---|---|---|--|--|--|--|
|        |   | 0   | 0.25x<br>(IC <sub>50</sub> ) <sub>1</sub> | 0.5x<br>(IC <sub>50</sub> ) <sub>1</sub> | 1x<br>(IC <sub>50</sub> ) <sub>1</sub> | 2x<br>(IC <sub>50</sub> ) <sub>1</sub> | 4x<br>(IC <sub>50</sub> ) <sub>1</sub> |
| Drug 2 | 0   | Control<br>(f <sub>a</sub> ) <sub>0</sub> | (f <sub>a</sub> ) <sub>1</sub>            | (f <sub>a</sub> ) <sub>1</sub>           | (f <sub>a</sub> ) <sub>1</sub>         | (f <sub>a</sub> ) <sub>1</sub>         | (f <sub>a</sub> ) <sub>1</sub>         |
|        | 0.25x<br>(IC <sub>50</sub> ) <sub>1</sub> | (f <sub>a</sub> ) <sub>2</sub>            | (f <sub>a</sub> ) <sub>1,2</sub>          |  |  |  |  |
|        | 0.5x<br>(IC <sub>50</sub> ) <sub>1</sub>  | (f <sub>a</sub> ) <sub>2</sub>            |   | (f <sub>a</sub> ) <sub>1,2</sub>         |  |  |  |
|        | 1x<br>(IC <sub>50</sub> ) <sub>1</sub>    | (f <sub>a</sub> ) <sub>2</sub>            |   |  | (f <sub>a</sub> ) <sub>1,2</sub>       |  |  |
|        | 2x<br>(IC <sub>50</sub> ) <sub>1</sub>    | (f <sub>a</sub> ) <sub>2</sub>            |   |  |  | (f <sub>a</sub> ) <sub>1,2</sub>       |  |
|        | 4x<br>(IC <sub>50</sub> ) <sub>1</sub>    | (f <sub>a</sub> ) <sub>2</sub>            |   |  |  |  | (f <sub>a</sub> ) <sub>1,2</sub>       |

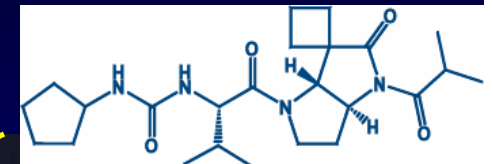
# NS3/4A Protease Inhibitors

## Peptidomimetics



**Vertex PI**  
**IC<sub>50</sub> 70nM**

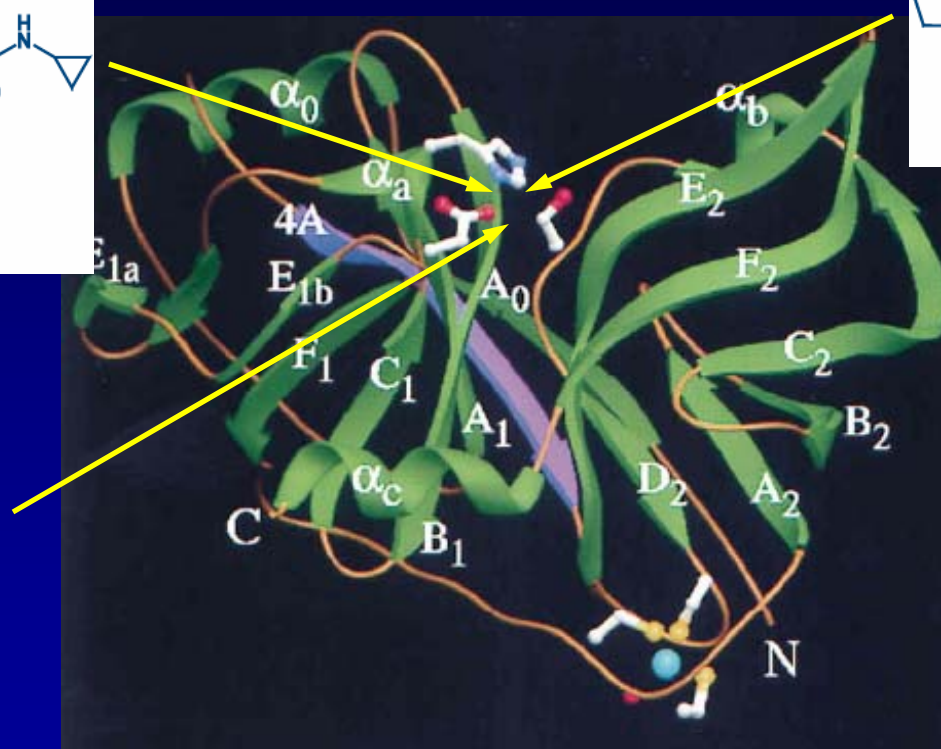
## Trans-lactam



**GSK PI**  
**IC<sub>50</sub> 200nM**

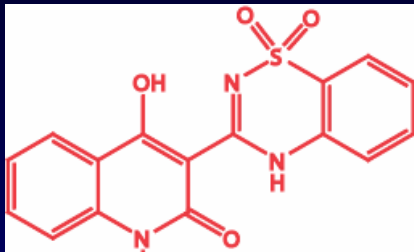


**BILN-2601**  
**IC<sub>50</sub> 5nM**

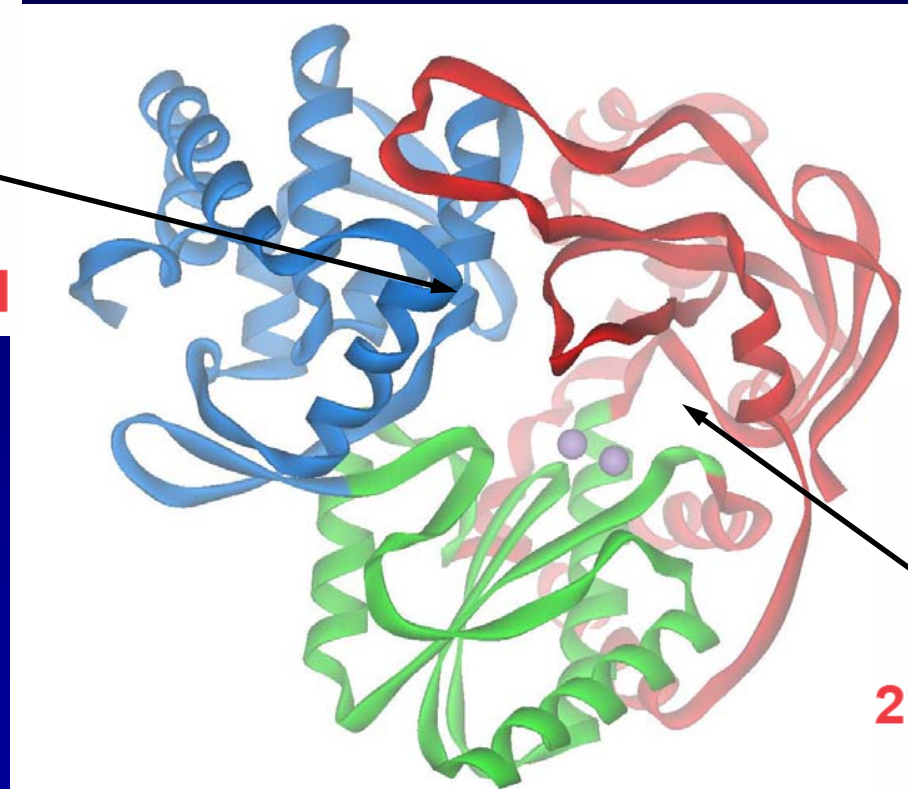


# NS5B polymerase inhibitors

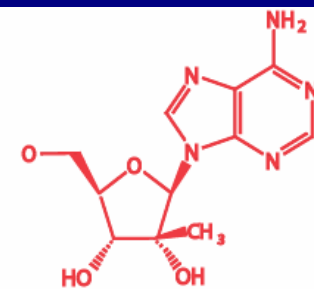
## Thiadiazine



**GSK NNI**  
**IC50 1600nM**

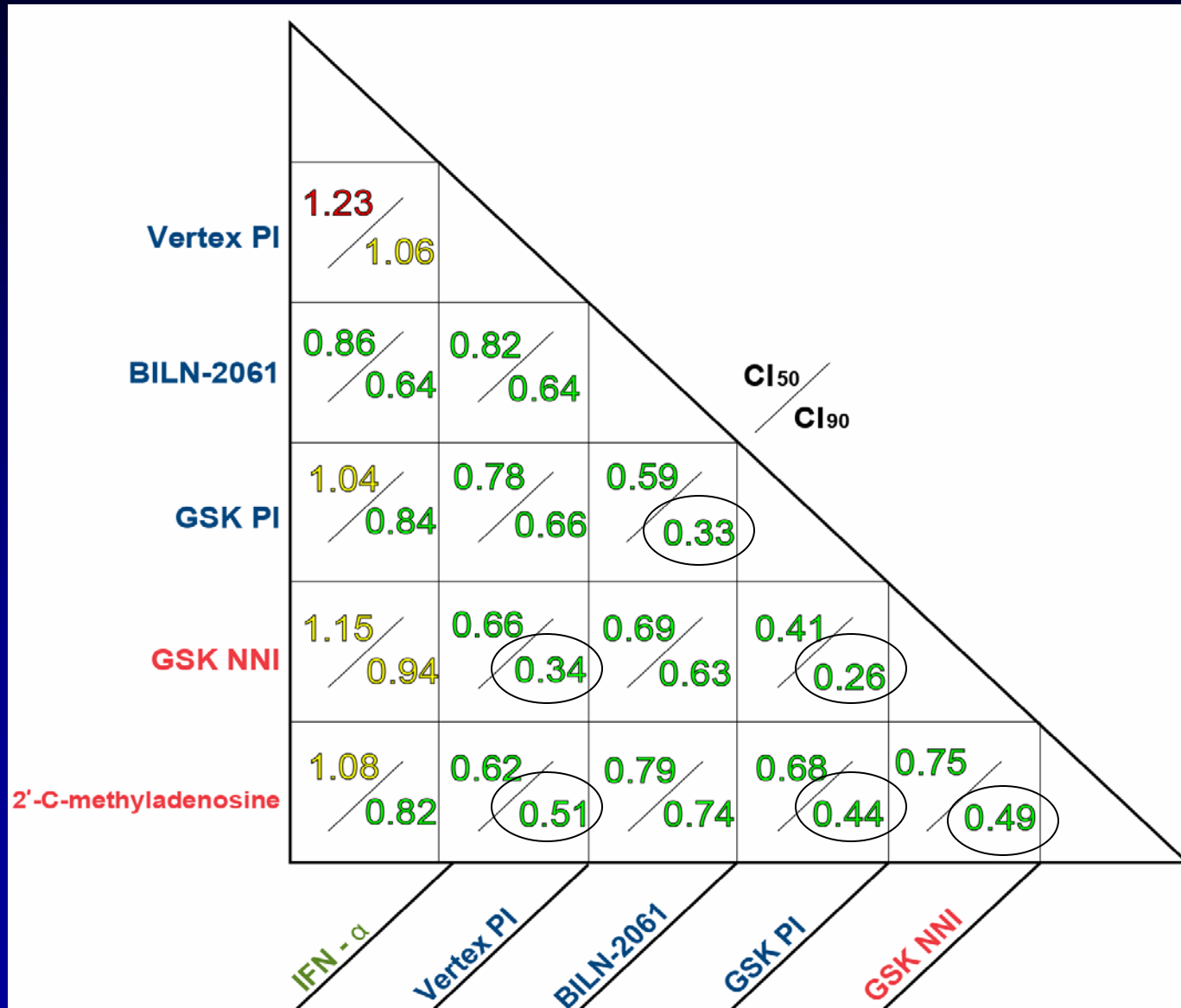


## Nucleoside



**2'-C-methyladenosine**  
**IC50 400nM**

# Synergy Summary



# Conclusions

- Compounds tested displayed additivity when combined with IFN
- Combinations targeting different viral proteins (or different sites within the protein) tended to have the lowest CIs (most synergy)
- Results of in vitro synergy studies should be considered when prioritizing drug candidates for clinical trials of combination therapy

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