

Hepatitis C Helicase Inhibitors

(OTT ID 1287)

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Shortfalls of current therapies for Hepatitis C Virus (HCV)

- Currently used protease inhibitors for HCV (telaprevir and boceprevir) must be used in combination with interferon and ribavirin
- Many patients poorly tolerate these new therapies
- HCV evolves to become resistant to therapies
- Therapies are expensive and are not equally effective against all HCV genotypes

Technological Solution:

- The invention consists of new direct acting antivirals (DAAs) that act against the Hepatitis C virus (HCV) replicon and inhibit the NS3 (non-structural protein 3) helicase activity
- Some helicase inhibitors are highly fluorescent and can be used to stain HCV-infected cells.
- These DAAs are most effective against hard to treat genotypes, like 1b.
- Cell culture experiments show no detectable toxicity
- Helicase inhibitors work together with protease inhibitors to yield synergistic effects
- Helicase inhibitors are active against NS3 encoded by similar viruses, like Dengue virus and West Nile virus.

Market, Intellectual Property, and Partnering

Market

- Hepatitis C in combination with hepatitis B, accounts for about 75% of all liver disease around the world
- 170-200 million people are infected with HCV worldwide with 3-5 million in the USA
- The unmet need in the HCV market is approximately 70%, which equals about \$3 billion
- The global Hepatitis C market was worth approximately \$4.4 billion in 2009 and is expected to reach \$9.8 billion by 2016

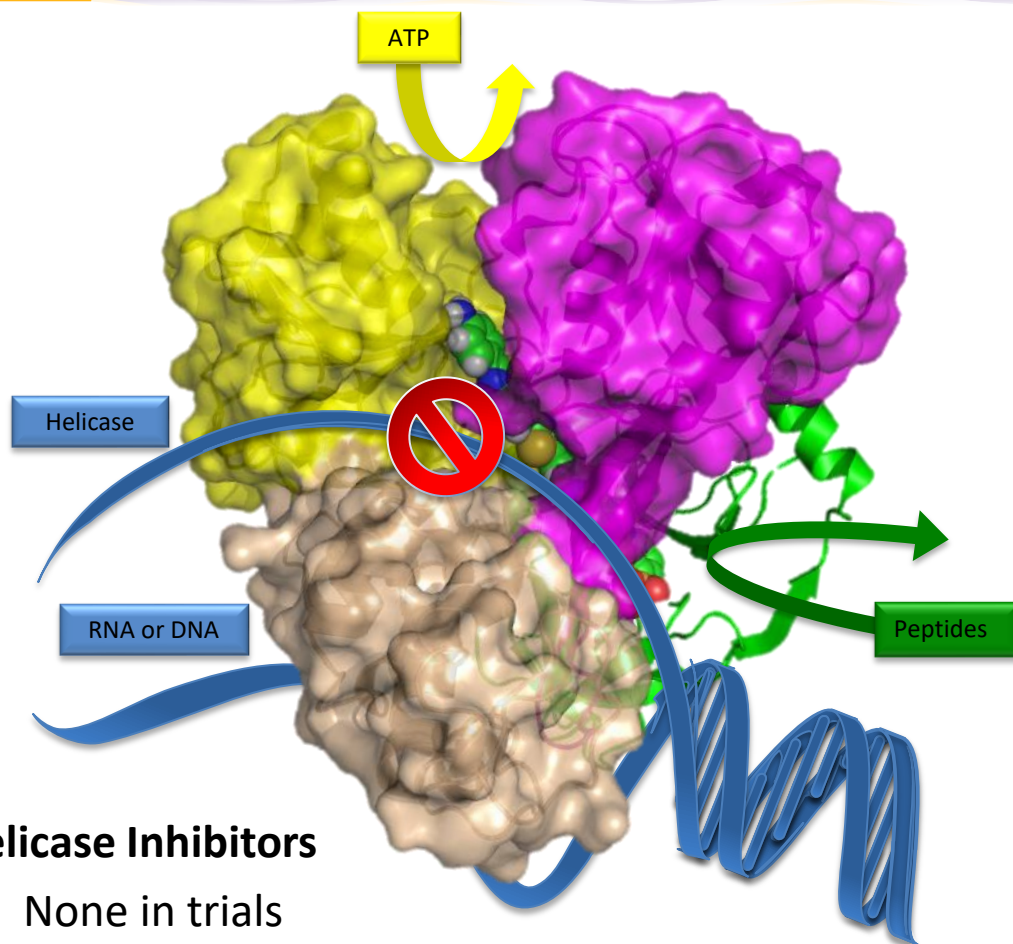
Intellectual Property

- WO Patent 2,013,036,749

Partnering

- Looking for a development partner to:
 - License novel compounds as molecular probes for research and drug discovery

- HCV replicates mainly in the liver, has a wide variety of genotypes, and mutates rapidly
- Once inside the liver cell, HCV takes over some of the cell's machinery to replicate
- HCV needs a functional helicase to replicate in cells
- The HCV helicase,
 - C-terminal domain of non-structural protein 3 (NS3)
 - unwinds double-stranded DNA and RNA
 - N-terminal domain is a protease
- Helicase inhibitors stop the replication of HCV
- Some types of inhibitors that have already been studied are aptamers, antibodies, and small molecules
- Direct acting antiviral drugs (DAAs) are in development which target specific HCV proteins/enzymes
- The Frick lab has identified a compounds that inhibits the NS3 helicase activity

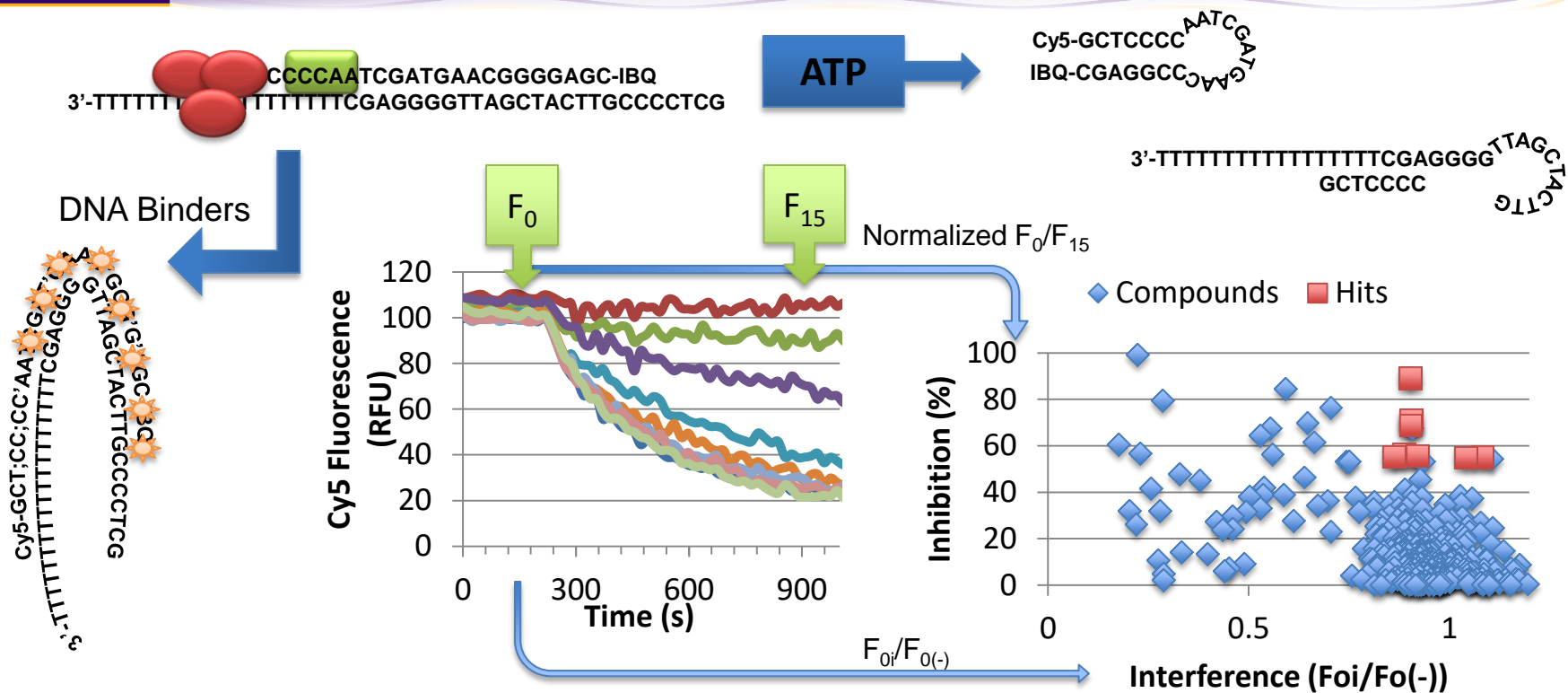


Helicase Inhibitors

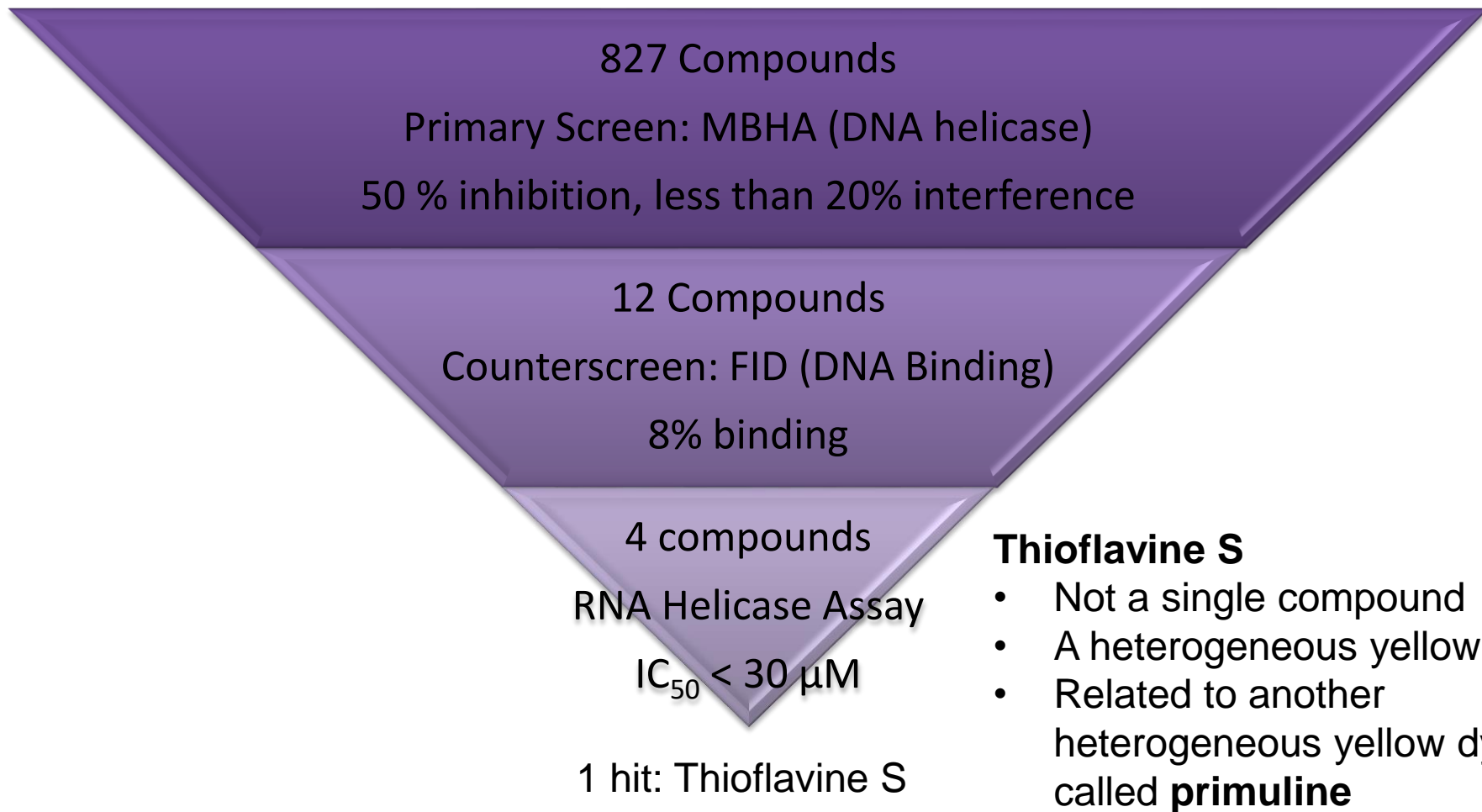
- None in trials
- Most potent helicase inhibitors reported to date act through the nucleic acid
- Non-specific inhibitors are toxic

Protease Inhibitors

- Approved
 - Incivek (Vertex)
 - Victrelis (Merck)
- Phase 3
 - TMC435 (Tibotec)
 - BI201335 (Boehringer Ingelheim)
- Phase 2
 - ABT-450 (Abbott)
 - ACH-1625 (Achillion)
 - BIT225 (Biotron)
 - GS-9256 (Gilead)
 - MK-5172 (Merck)
 - Danoprevir (Intermune)
 - Vaniprevir (Merck)

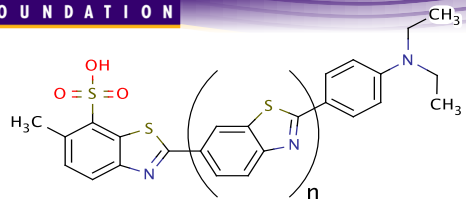


- The PI invented a new assay that uses molecular beacons to detect helicase activity
- This molecular beacon helicase assay (MBHA) can simultaneously detect compound DNA interactions and effects on helicase activity
- The inventors use this assay analyze existing inhibitors and discover new ones.

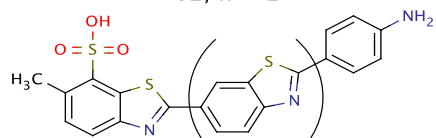


Thioflavine S

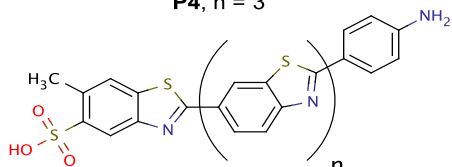
- Not a single compound
- A heterogeneous yellow dye
- Related to another heterogeneous yellow dye called **primuline**



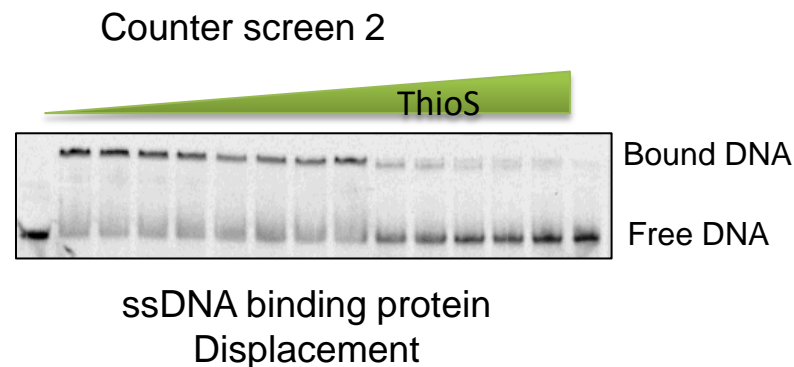
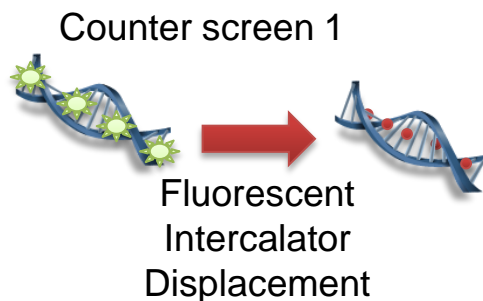
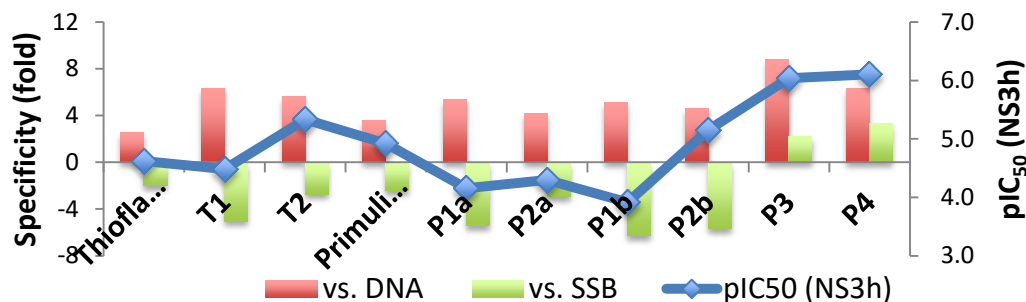
T1, $n = 0$
T2, $n = 1$



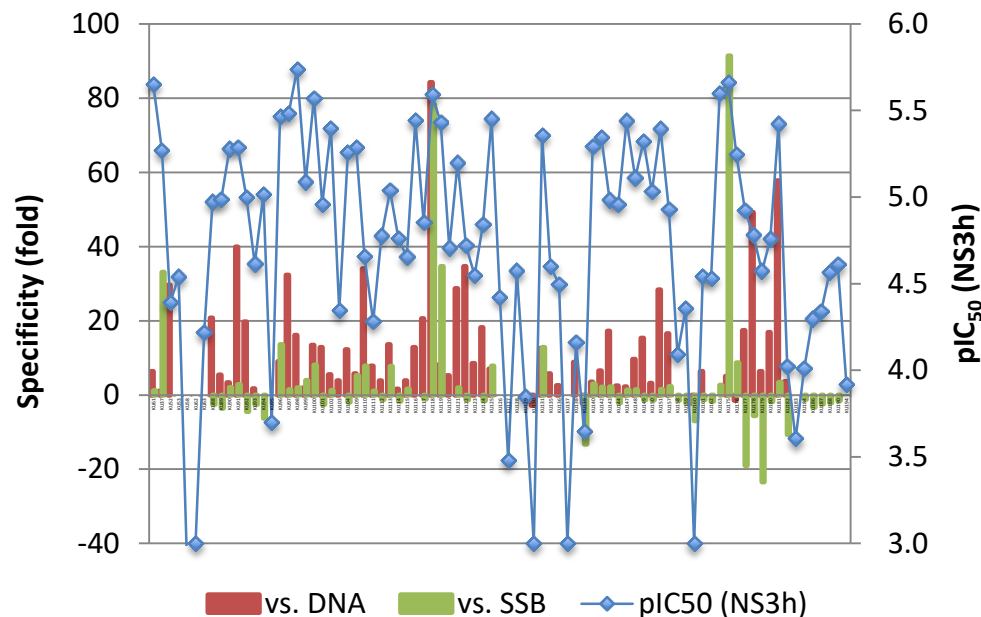
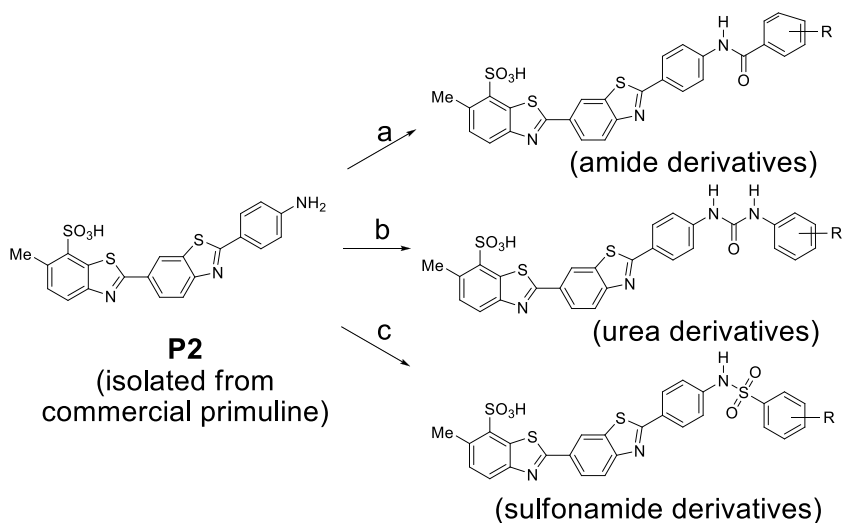
P1a, $n = 0$
P2a, $n = 1$
P3, $n = 2$
P4, $n = 3$



P1b, $n = 0$
P2b, $n = 1$

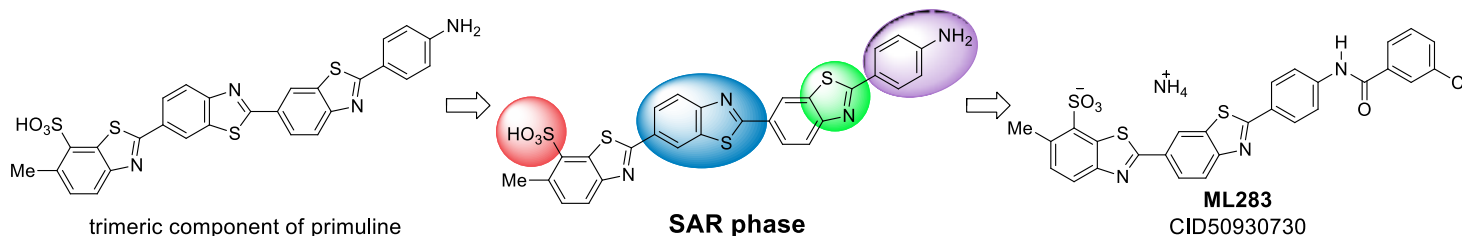


- Two compounds were purified from Thioflavine S (T1, T2)
- Six compounds were purified from primuline
- **Mechanism:** Dyes prevent NS3 from binding DNA
- The best compound inhibited helicase with an IC_{50} of 2 μ M, but it also bound DNA and prevented other proteins from binding DNA



- Over 88 primuline derivatives were synthesized
- DNA binding capacity varies widely but many retain an ability to inhibit helicase
- Most specific compounds are 10-times more potent than previously disclosed helicase inhibitors.

NIH Molecular Probe: ML283



- sulfonic acid necessary for potency
- replacement of the second benzothiazole with amide/phenyl ring linker tolerated
- replacement of the third benzothiazole with amide, urea, thiourea or amine tolerated
- *p*-amino group not necessary for potency
diverse substituted phenyl or benzene fused polycyclic moieties afforded active analogs

A Specific
Fluorescent
Molecular Probe for
HCV helicase with
promising PK
properties

Aqueous solubility ($\mu\text{g/mL}$) ^a (@ pH)			PAMPA Pe ($\times 10^{-6}$ cm/s) ^d (@ pH)	Plasma protein binding (% Bound)		Plasma stability ^d human/ mouse	Aqueous stability ^f	hepatic microsome stability ^g		hepatic toxicity ^h LC ₅₀ (μM)
Prisma HT buffer ^a	PBS ^b	assay matrix ^c		human 1 μM /10 μM	mouse 1 μM /10 μM			human mouse	human	
36.7 (5.0) >60 (6.2) >60 (7.4)	0.12 (7.4)	29.2 (6.5)	0 (5.0) 0.22 (6.2) 0 (7.4)	98/99	98/99	96.6/ 95.0	100	83.57	83.11	>50

^a in aqueous pION's Prisma HT buffer, pH's 5.0/6.2/7.4, ^b in aqueous PBS, pH 7.4, ^c 24 mM MOPS, 1.25 mM MgCl₂, 0.05 mM DTT, 5 $\mu\text{g/mL}$ BSA, 0.01% v/v final Tween 20] and 5% v/v final [DMSO], pH 6.5, ^d in aqueous buffer; donor compartment pH's 5.0/6.2/7.4; acceptor compartment pH 7.4. ^e remaining at 3 hr, ^f in aqueous PBS buffer with 50% acetonitrile, pH 7.4; % remaining after 48 hr at room temperature. ^g % remaining at 1 hr. ^h towards Fa2N-4 immortalized human hepatocytes

Antivirals and HCV Stains

HCV Replicon Inhibition

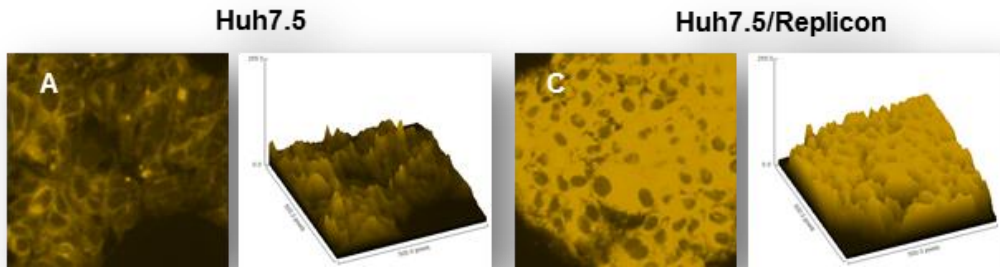
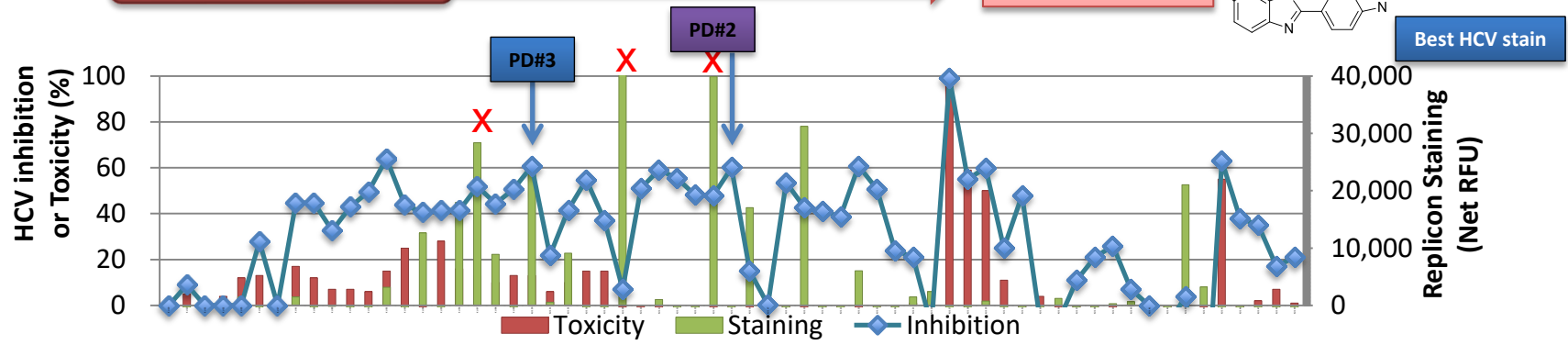
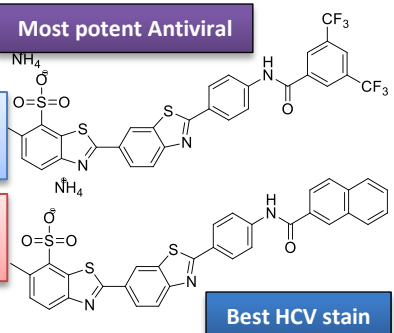
- Inhibition @ 10 μ M > 50 %, Viability @ 10 μ M > 50%
- IC₅₀ < 50 μ M

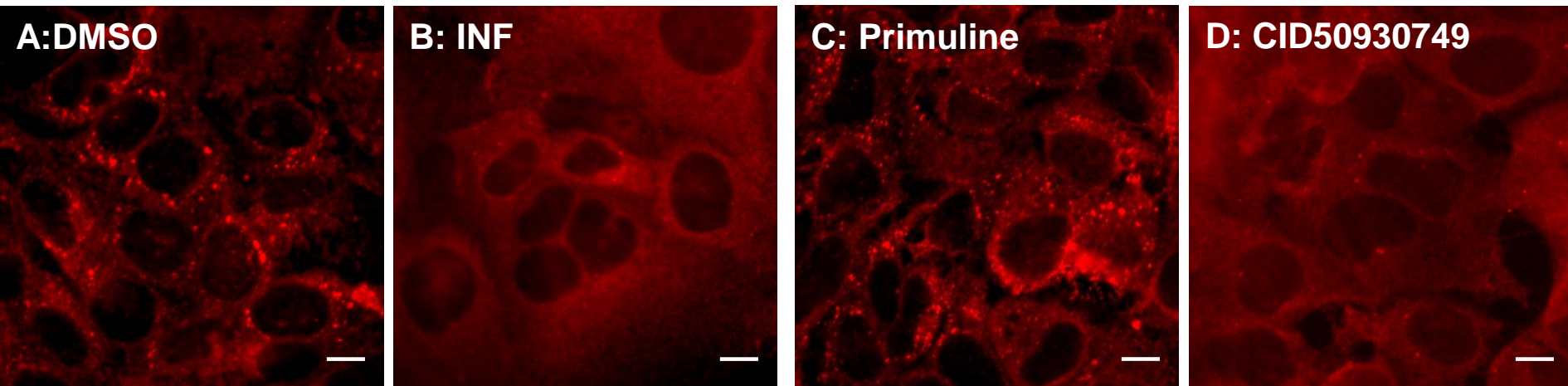
2 compounds

HCV Replicon Staining

- Huh7.5 – Huh7.5/HCV > 25,000
- No visible precipitates

3 compounds





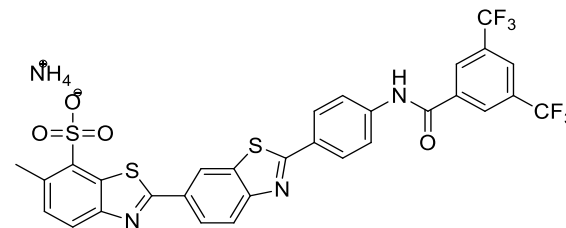
Effect of CID50930749 on the cellular location of HCV Replication complexes seen in the replicon-containing Huh7.5/Con1sg-Rluc cells. Cells were fixed, permeabilized, and stained with 9E10 α -NS5A antibody (obtained from Charles Rice, Rockefeller University) and Alexa 546 secondary antibody after 72 hours of:

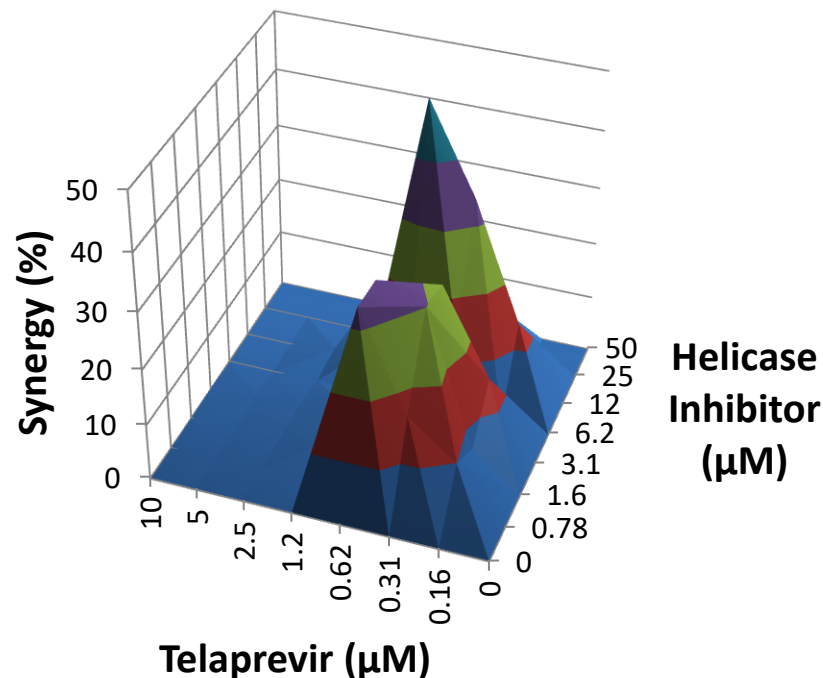
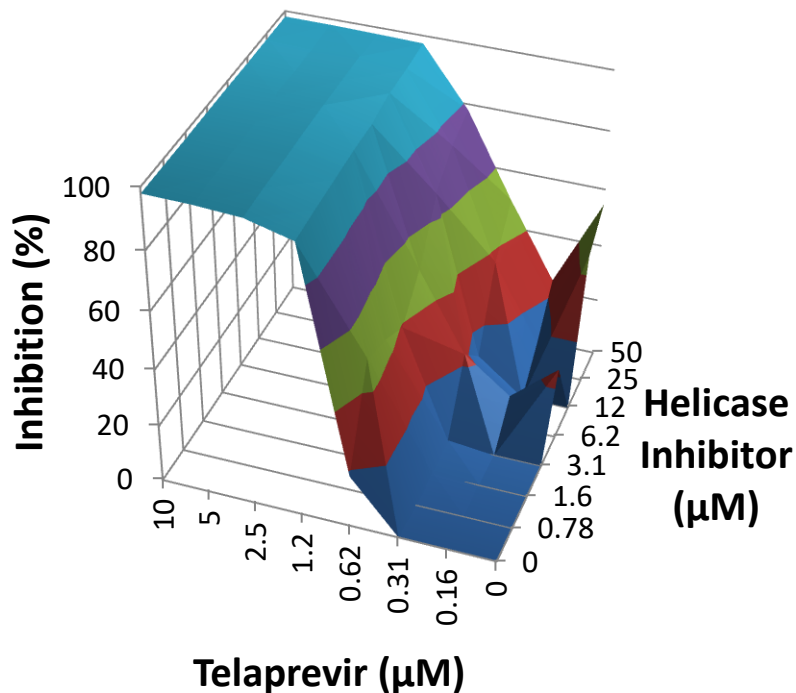
A: mock treatment with 0.5% DMSO (red=HCV replicase)

B: 100 units of interferon (positive control)

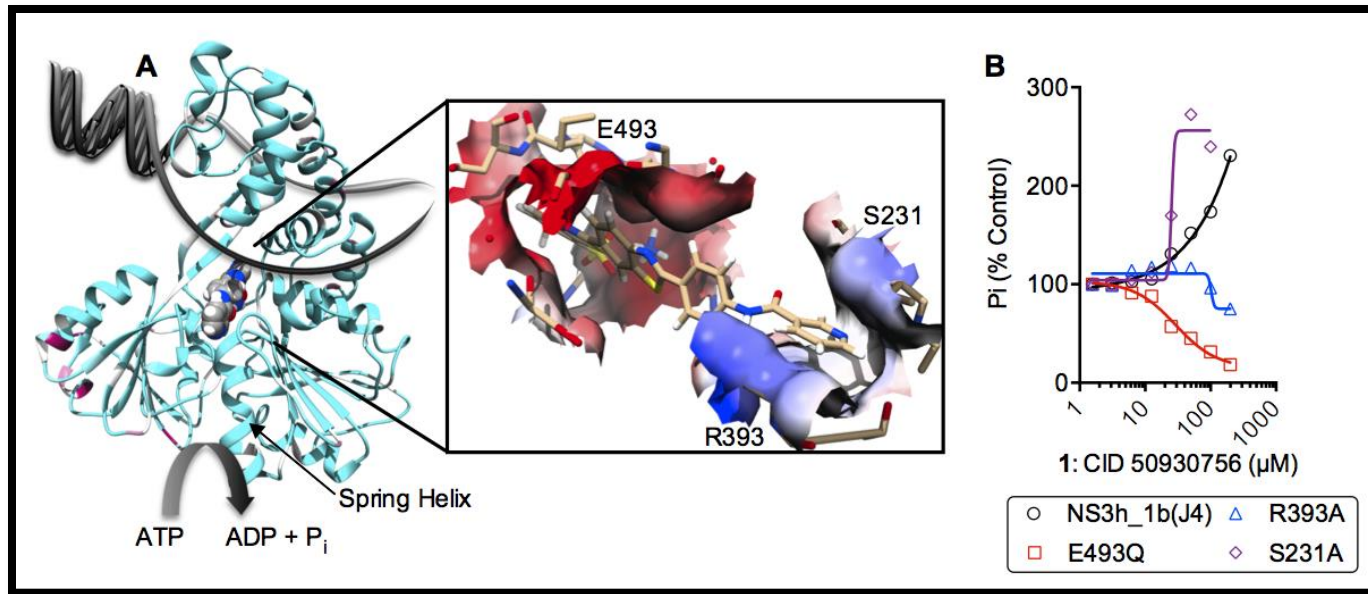
C: 10 μ M primuline (no effect)

D: 10 μ M of **CID50930744** (disrupts complexes)



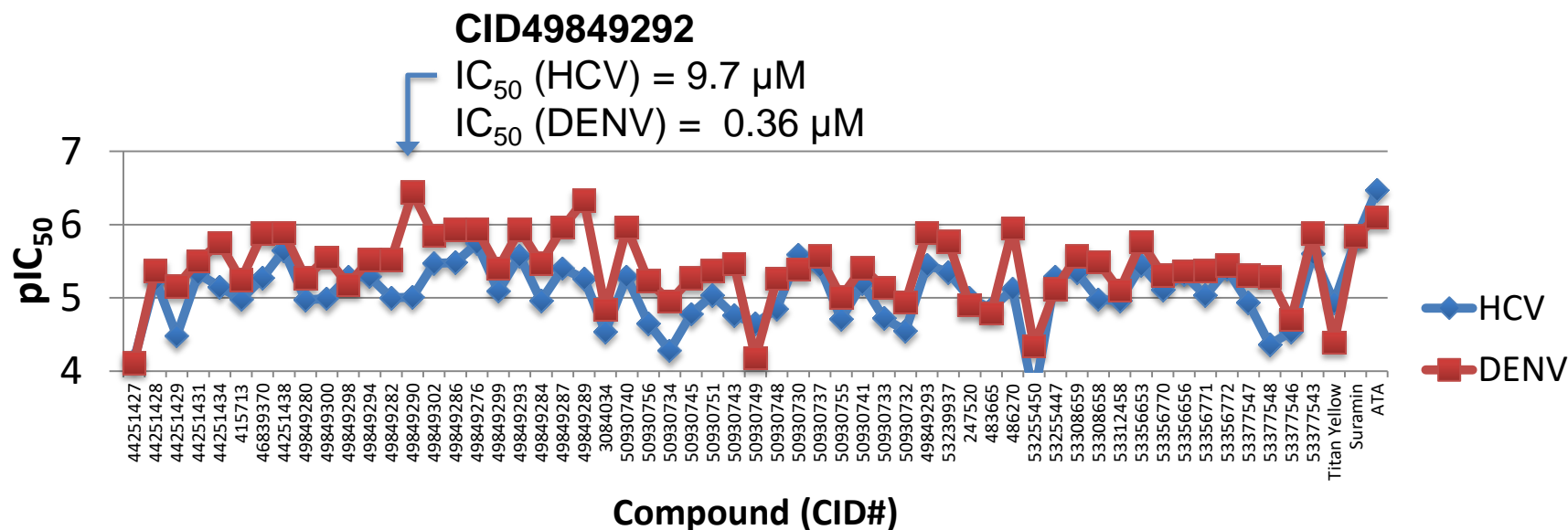


- **CID50930749** decreases HCV RNA levels 15-fold in 10 days ($IC_{50} = 15 \mu M$)
- **CID50930749** treatment enhances the effect of NS3 protease inhibitors
- Low concentrations of **CID50930749** and **telaprevir** are up to 50% more effective than would be expected from the Bliss Independence Model



- Some analogs bind in place of RNA to stimulate helicase-catalyzed ATP hydrolysis
- Molecular modeling predicts compounds interact with key conserved residues
- Site directed mutagenesis alters NS3 response to compounds

Potent inhibition of Dengue Virus NS3



- Most compounds also inhibit NS3 helicase from related viruses, like Dengue virus (DENV), West Nile virus, and yellow fever virus.
- Some compounds inhibit the Dengue virus NS3 helicase much better than they inhibit HCV helicase.
- Some compounds show antiviral activity in assays with DENV replicons.

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HCV HELICASE INHIBITORS AND METHODS OF USE THEREOF

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(54) Title: HCV HELICASE INHIBITORS AND METHODS OF USE THEREOF

(57) Abstract: The present invention discloses thioflavine S and primuline derivatives which inhibit hepatitis C virus helicase and protease activity. Consequently, the compounds of the present invention interfere with the life cycle of the hepatitis C virus and are useful as antiviral agents. The present invention further relates to pharmaceutical compositions containing the aforementioned compounds and methods of treating an HCV infection.

WO 2013/036749 A1

Further investigations

- Resistance selection
- Delivery methods
- Synthesizing and testing of more soluble analogs
- Testing against RNA helicases encoded by other organisms
- Testing against related viruses
- Structural studies using X-ray crystallography
- Structure-based design to enhance specificity
- Combination studies with other direct acting antiviral

Partnering

Looking for a development partner to:

License novel compounds as molecular probes for research and drug discovery

Hepatitis C Helicase Inhibitors

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