Hepatitis C Helicase Inhibitors
(OTT ID 1287)

Inventor: David Frick, Department of Chemistry and Biochemistry
UW-Milwaukee; Jeffrey Aube, Frank Schoenen, Brian Blagg, Kevin Frankowski, Kelin Li, University of Kansas

For further information please contact:
Jessica Silvaggi
Senior Licensing Manager
1440 East North Ave.
Milwaukee, WI 53202
Tel: 414-906-4654
jsilvaggi@uwmfdn.org
Problems for Hepatitis C Virus (HCV)

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 again 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
Hepatitis C Virus Replication

- 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
The target: The ‘Other’ function of NS3

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)
A Better High-throughput Helicase Assay

- 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 to analyze existing inhibitors and discover new ones.

©UWMRF 2016
NCI screen revealed 1 hit

827 Compounds
Primary Screen: MBHA (DNA helicase)
50% inhibition, less than 20% interference

12 Compounds
Counterscreen: FID (DNA Binding)
8% binding

4 compounds
RNA Helicase Assay
IC<sub>50</sub> < 30 µM

1 hit: Thioflavine S

Thioflavine S
- Not a single compound
- A heterogeneous yellow dye
- Related to another heterogeneous yellow dye called primuline
Purification of New Helicase Inhibitors

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 µM, 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

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

- 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

<table>
<thead>
<tr>
<th>Aqueous solubility (µg/mL) a (@ pH)</th>
<th>PAMPA Pe (x 10^4 cm/s) b (@ pH)</th>
<th>Plasma protein binding (% Bound)</th>
<th>Plasma stability c</th>
<th>Aqueous stability d</th>
<th>hepatic microsome stability e</th>
<th>hepatic toxicity f LC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prisma HT buffer a</td>
<td>PBS b</td>
<td>assay matrix c</td>
<td>human 1 µM/10 µM</td>
<td>mouse 1 µM/10 µM</td>
<td>human/mouse</td>
<td>human/mouse</td>
</tr>
<tr>
<td>36.7 (5.0)</td>
<td>0.12 (7.4)</td>
<td>29.2 (6.5)</td>
<td>0 (5.0)</td>
<td>0.22 (6.2)</td>
<td>98/99</td>
<td>83.57</td>
</tr>
<tr>
<td>&gt;60 (6.2)</td>
<td></td>
<td></td>
<td></td>
<td>0 (7.4)</td>
<td>98/99</td>
<td>83.11</td>
</tr>
</tbody>
</table>

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 µg/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 μM > 50 %, Viability @10 μM > 50%
- IC$_{50}$ < 50 μM

2 compounds

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

3 compounds

HCV inhibition or Toxicity (%)

Replicon Staining (Net RFU)

ML283 Stained Huh7.5/Replicon

©UWMRF 2016
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 µM primuline (no effect)
D: 10 µM of CID50930744 (disrupts complexes)
• **CID50930749** decreases HCV RNA levels 15-fold in 10 days (IC$_{50}$ = 15 µM)
• **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.
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.
Peer-Reviewed Research


HCV HELICASE INHIBITORS AND METHODS OF USE THEREOF

J AUBE, B BLAGG, S Jonathan, K FRANKOWSKI, D FRICK, K LI, F SCHOENEN

WO Patent 2,013,036,749

Pub. No.: WO/2013/036749

International Application No.: PCT/US2012/054130

Publication Date: 14.03.2013

International Filing Date: 07.09.2012
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

(OTT ID 1287)

For further information please contact:

Jessica Silvaggi
Senior Licensing Manager
1440 East North Ave.
Milwaukee, WI 53202
Tel: 414-906-4654
jsilvaggi@uwmfdn.org