Direct acting HCV antiviral agent, Sofosbuvir, makes one pill that fits all

Zahra Rashidi

01/25/2017
CONTENT

- Introduction
  I. hepatitis C
  II. treatment 1990-2013
  III. current treatment
  IV. HCV life cycle
    - Developing of sofosbuvir
    - Mechanism of inhibitory effect of sofosbuvir
    - synthesis of sofosbuvir
A major public health problem worldwide
180 million individuals infected worldwide 4 times more than HIV
The most common indication for liver transplantation, accounting for 40–50% of liver transplants

http://www.educatingwomen.org/hepatitis-c/
Genotype diversity:

hepatitis C virus (HCV) exhibits enormous genetic variability due to high mutation rates, There are six major HCV genotypes that differ from each other by 30%-35%, and also over 100 subtypes. There is no approved vaccine.

Nat. Rev. Drug Discov. 2013, 12, 595 -610
General standard of care:
Current problems:

Potential side effects:
- influenza-like symptoms
- fatigue
- hemolytic anemia
- depression
Is it possible to design a new efficient drug against hepatitis C?

efficient drug with following features:
• High potency
• Pan genotypic coverage
• High barrier to resistance
• Oral – single dose therapy
• INF-free
• Minimal toxicity and side effect

development of direct acting antivirals (DAAs)

targeting essential viral proteins
Sofosbuvir treatment:

- discovered in 2007 and approved for medical use in the United States in 2013
- $10.3 billion sold in 2014, the best-selling drug in the world in only its first year on the market
- the cost of a 12-week regimen of Sovaldi is around $84,000
- up to 97% cure rate

J. Hepatol. 2015, 62, s87-s99
Hepatitis C virus:

http://www.medicalnewstoday.com/articles/294705.php
HCV life cycle:
targeting RNA polymerase:

Nature Rev. Microbiol. 2013,11,482-496
Introduction

I. hepatitis C

II. treatment 1990-2013

III. current treatment

IV. HCV life cycle

- Developing of sofosbuvir
- Mechanism of inhibitory effect of sofosbuvir
- synthesis of sofosbuvir
Adenine
Guanine
Uracil
Cytosine

nucleoside triphosphate
nucleoside diphosphate
nucleoside monophosphate
nucleoside

pentose

2’ OH = Ribose → RNA
2’ H = Deoxyribose → DNA

Adenine
Guanine
Uracil
Cytosine
Design a RNA polymerase inhibitor:
Design a RNA polymerase inhibitor:
Design experiment to measure IC$_{50}$ and EC$_{50}$:

The IC50 is the concentration of an inhibitor where the response (or binding) is reduced by half. The EC50 is the concentration of a drug that gives half-maximal response.

- binding affinity of NTP to RNA polymerase
- inhibitory effect of NTP

- cellular uptake of nucleosides
- conversion to NTP
- metabolism and deactivation
- and other factors
Modification of nucleosides to get active inhibitors:

- modification of ribose ring
- substitution on C1’ and C4’

- substitution on C2’ and C3’
Role of 3’-hydroxyl group:

Isolated enzyme assay

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Km(µM)</th>
<th>Ki(µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP</td>
<td>0.06± 0.001</td>
<td></td>
</tr>
<tr>
<td>GTP</td>
<td>0.23 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>UTP</td>
<td>0.24 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>10 ± 2</td>
<td></td>
</tr>
<tr>
<td>3’-dCTP</td>
<td></td>
<td>0.72 ± 0.2</td>
</tr>
<tr>
<td>3’-dGTP</td>
<td></td>
<td>0.93 ± 0.08</td>
</tr>
<tr>
<td>3’-dUTP</td>
<td></td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>3’-dATP</td>
<td></td>
<td>23 ± 5</td>
</tr>
<tr>
<td>2’,3’-Dideoxycytidine</td>
<td></td>
<td>9100</td>
</tr>
<tr>
<td>3’-fluoro -3’-dGTP</td>
<td></td>
<td>1.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>EC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytidine</td>
<td>200</td>
</tr>
<tr>
<td>3’-Deoxycytidine</td>
<td>45</td>
</tr>
<tr>
<td>3’-Deoxyuridine</td>
<td>200</td>
</tr>
<tr>
<td>3’-Deoxyguanosine</td>
<td>500</td>
</tr>
<tr>
<td>3’-Deoxyadenosine</td>
<td>150</td>
</tr>
<tr>
<td>2’,3’-Dideoxycytidine</td>
<td>200</td>
</tr>
<tr>
<td>Zalcitabine</td>
<td></td>
</tr>
<tr>
<td>3’-fluoro -3’-deoxyguanosin</td>
<td>1.2</td>
</tr>
</tbody>
</table>

removal of 3’-hydroxyl group reduces a nucleotide’s affinity to the enzyme complex

Antiviral Res. 2003, 58, 243–251
Phosphorylation is necessary:

Nucleoside triphosphate is the substrate for RNA polymerases.
Phosphorylation is necessary:

### Role of 2’-hydroxyl group:

Comparison between substrate efficiency of UTP analogs

<table>
<thead>
<tr>
<th>UTP analogue</th>
<th>relative substrate affinity</th>
<th>comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTP</td>
<td>1</td>
<td>natural substrate</td>
</tr>
<tr>
<td>2’-F-uridine</td>
<td>0.16</td>
<td>substrate but not inhibitor</td>
</tr>
<tr>
<td>2’-NH2 -uridine</td>
<td>0.03</td>
<td>substrate but not inhibitor</td>
</tr>
<tr>
<td>2’-arabinouridine</td>
<td>0.0015</td>
<td>poor substrate</td>
</tr>
<tr>
<td>2’-N3 -uridine</td>
<td>0.0005</td>
<td>poor substrate</td>
</tr>
</tbody>
</table>

2’-arabinouridine

2’-hydroxyl group is crucial for substrate efficiency and inhibitory activity.


Nucleoside analogue should have hydrogen bond donor/acceptor in 2’ position.

Unable to inhibit HCV NS5B.
Modification of \( a \) and \( b \) on C2\(^{\prime} \):

### Inhibitory Potency of 2\(^{\prime} \)-Modified Nucleosides/Nucleoside Triphosphates

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>B</th>
<th>Inhibitory potency (IC(_{50}), ( \mu )M)</th>
<th>Replicon EC(_{50}) ( \mu )M</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>OCH(_3)</td>
<td>adenine</td>
<td>47</td>
<td>&gt;50</td>
</tr>
<tr>
<td>H</td>
<td>OCH(_3)</td>
<td>guanine</td>
<td>1.6</td>
<td>&gt;50</td>
</tr>
<tr>
<td>H</td>
<td>OCH(_3)</td>
<td>cytosine</td>
<td>3.8</td>
<td>21.2</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>OH</td>
<td>adenine</td>
<td>1.9</td>
<td>0.3</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>OH</td>
<td>guanine</td>
<td>0.13</td>
<td>3.5</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>OH</td>
<td>cytosine</td>
<td>0.09-0.18</td>
<td>1.23</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>OCH(_3)</td>
<td>adenine</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Et</td>
<td>OH</td>
<td>adenine</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

J. Med. Chem. 2004, 47, 2283-2295
**Effect of substitution on C3:**

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
<th>Inhibitory potency (IC$_{50}$), µM</th>
<th>Replicon EC$_{50}$ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>OH</td>
<td>CH$_3$</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

**no substitution at c**

Pharmacokinetics study:

2′-O-methylcytidine

2′-C-methylcytidine

HPLC of extract from cells incubated with 2′-O-methylcytidine

J. Biol. Chem. 2003, 278, 11979-11984
Human clinical trial of 2’-C-methylcytidine:

2’-C-methylcytidine

NM283

discontinuation of human clinical trial due to significant gastrointestinal toxicity
Efficacy of 2’-F-2’-C-methyl class of nucleosides:

<table>
<thead>
<tr>
<th>Structure</th>
<th>Potential Toxicity</th>
<th>Inhibitory Effect</th>
<th>Selectivity &amp; Inhibitory Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>-</td>
<td>&gt;1000 µM</td>
<td>Good selectivity &amp; inhibitory effect</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>Lack of inhibitory effect</td>
<td>IC₅₀ = 0.21 ± 0.05</td>
<td></td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 3" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pharmacokinetics study 2’-F-2’-C-methyl cytidine:

HPLC profile of an extract of primary human hepatocytes incubated with 2’-F-2’-C-methyl cytidine (PSI-6130)

Reference compounds

Extract from hepatocytes incubated with PSI-6130

PSI-6130

J. Biol. Chem. 2007, 282, 29812-29820
Pharmacokinetics study 2’-F-2’-C-methyl cytidine:

HPLC profile of an extract of primary human hepatocytes incubated with 2’-F-2’-C-methyl cytidine (PSI-6130)

Reference compounds

Extract from hepatocytes incubated with PSI-6130

J. Biol. Chem. 2007, 282, 29812-29820
Second metabolic pathway for 2’-F-2’-C-methyl cytidine:

<table>
<thead>
<tr>
<th>process</th>
<th>Enzyme Efficiency (K_{cat}/K_m) ( \mu \text{M}^{-1} \text{S}^{-1} ) for each substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1^{st} phosphorylation</td>
<td>2’-F-2’-C-methyl cytidine 2’-F,2’-methyl uridine</td>
</tr>
<tr>
<td>2^{nd} phosphorylation</td>
<td>0.012</td>
</tr>
<tr>
<td>3^{rd} phosphorylation</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Inhibitory effect of 2’-F-2’-C-methyl uridine:

<table>
<thead>
<tr>
<th>compound</th>
<th>Inhibitory potency (IC$_{50}$), µM</th>
<th>Replicon EC$_{50}$ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2’-F-2’-C-methyl cytidine</td>
<td>ND</td>
<td>0.6 ± 0.04</td>
</tr>
<tr>
<td>2’-F,2’-methyl uridine</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2’-F-2’-methyl cytidine-TP</td>
<td>0.023 ± 0.002</td>
<td>ND</td>
</tr>
<tr>
<td>2’-F,2’-methyl uridine-TP</td>
<td>0.141 ± 0.03</td>
<td>ND</td>
</tr>
</tbody>
</table>

Mean half-lives: 4.7 h (PSI-6130) and 38 h (2’-F,2’-methyl uridine)
Skip the first phosphorylation:
Design monophosphate prodrug1:

Antivir. Chem. Chemother. 2011, 22, 23–49
Design monophosphate prodrug2:

\[
\begin{align*}
\text{Nucleoside} & \quad \xrightarrow{\text{Esterase}} \quad \text{Nucleoside} \\
\text{Nucleoside} & \quad \xrightarrow{\text{Esterase}} \quad \text{Nucleoside}
\end{align*}
\]
Design monophosphate prodrug3:
Different activity of two diastereoisomer of PSI-7851:

EC$_{50}$ µM = 0.149 ± 0.001

EC$_{50}$ µM = 1.07 ± 0.04

EC$_{50}$ µM = 0.092 ± 0.005

J. Biol. Chem. 2010, 285, 34337–34347
Comparison of different agents:

<table>
<thead>
<tr>
<th></th>
<th>first generation</th>
<th>second generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>800 mg twice daily</td>
<td>1000 mg twice daily</td>
<td>400 mg once daily</td>
</tr>
<tr>
<td>status</td>
<td>discontinued</td>
<td>Completed Phase II</td>
</tr>
<tr>
<td></td>
<td>diverse metabolism</td>
<td>longer half life</td>
</tr>
<tr>
<td></td>
<td>no toxicity</td>
<td>no toxicity</td>
</tr>
</tbody>
</table>

Antivir. Chem. Chemother. 2011, 22, 23–49
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  ▪ synthesis of sofosbuvir
structural study of RNA replication by RNA polymerase:
Complementary networks for recognizing ribonucleotide substrates:

Science, 2015, 347, 771-775
Hydrogen bond donor/ acceptor in 2’ position:
Chain termination of sofosbuvir:

Science, 2015, 347, 771-775
Infect Disord Drug Targets 2006, 6, 17-29
J. Biol. Chem. 2003, 278, 49164-49170
CONTENT

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Synthesis of sofosbuvir:
1. Late-Stage Fluorination Approach to provide Nucleoside Core

1. Bz$_2$O, DMF, rt
2. TIPDSCI$_2$, DMF

4. (61%, 2 steps)

1. MeLi, -78°C
2. TBAF, conc HOAc

**Synthesis of sofosbuvir:**

Sofosbuvir

\[
\text{NH}_2
\]

\[
\text{NH}_2
\]

\[
\text{NH}_2
\]

\[
\text{NH}_2
\]
2. Early-Stage Fluorination Approach to provide Nucleoside Core

DAST or deoxyfluor
THF, 0°C

DAST or deoxyfluor
THF, 0°C
DIPEA

DAST or deoxyfluor
THF, -78°C, amine

1. Li(O-tBu)₃AlH
THF, -20 0°C

2. Ac₂O, DMAP, -20 0°C

1. SnCl₄, PhCl, 65°C
2. NH₃, MeOH, rt


29% β from 17
2. Early-Stage Fluorination Approach to provide Nucleoside Core

\[ \text{BzCl, pyridine, rt } 71\% \]

\[ \text{DAST or deoxyfluor, THF, 0\degree C } 68\% \]

\[ \text{TFA, MeCN, 80 0\degree C then PhCH}_3, \Delta\]

\[ \text{EtOAc, 60 0\degree C } 91\% \]
3. Industrial early-Stage Fluorination Approach to provide Nucleoside Core

10 | 11 | 12 | 20
---|---|---|---
\[\text{CHO} \rightarrow \begin{aligned} \text{CHO} & \rightarrow \text{CHO} \\ \text{CHO} & \rightarrow \text{CHO} \end{aligned} \]

4. Development of early-Stage Fluorination Approach

Z:E ratio dose not given

20:1 dr isolated

25% overall yield

Phosphoramidation:

Phosphoramidation:

\[
\text{Ph-O-P-Cl} + \text{O-CO-CH}_2\text{NH}_2\cdot\text{HCl} \rightarrow \text{O-CO-NH-Cl} \rightarrow \text{O-CO-NH-Cl} \rightarrow \text{sofosbuvir (crude)}
\]

\[
\text{Ph-O-P-Cl} + \text{O-CO-CH}_2\text{NH}_2\cdot\text{HCl} \rightarrow \text{O-CO-NH-Cl} \rightarrow \text{O-CO-NH-Cl} \rightarrow \text{sofosbuvir (crude)}
\]

\[
\text{sofosbuvir (crude)}
\]

99.74% purity
15.2% yield from 9

Diastereoselective phosphoramidation approach:

\[ \text{Ph-O-P-Cl} + \text{O-CH}_{2}-\text{CONH}_{2} \cdot \text{HCl} \xrightarrow{1. \text{NEt}_3, -70^\circ \text{C}} \text{O-CH}=\text{C(\text{O})}_{2}\text{N-P-OC}_{6}\text{F}_{5} \xrightarrow{2. \text{C}_{6}\text{F}_{5} \text{OH}, 0^\circ \text{C}} \text{recryst.} \]

32
(S\text{p}:S\text{R} 1:1)

\[ \text{O-CH}=\text{C(\text{O})}_{2}\text{N-P-OC}_{6}\text{F}_{5} \xrightarrow{\text{recryst.}} \]

33
34%
(S\text{p}:S\text{R} = 99:1)

\[ \text{O-CH}=\text{C(\text{O})}_{2}\text{N-P-OPh} + \text{HO-CH(OH)OC}_{6}\text{F}_{5} \xrightarrow{\text{t-BuMgCl, THF}} \text{recryst.} \]

then aqueous workup
98.21% purity

99.72% de
99.79% purity

Conclusion:

- HCV infection is a complex disease with large population of patients,
- Targeting the active site of virus RNA polymerase is an interesting idea to develop a drug for multiple genotypes,
- Modification of ribose ring of nucleoside by methyl and fluorine substitution on C2’ provide active inhibitor that can terminate RNA replication,
- Using phosphoramidate prodrug strategies to deliver nucleoside monophosphate drug to cells,
- Phosphorus stereocenter is important in activation of prodrug,
- Improvement in sofosbuvir synthesis
Acknowledgment

Dr. Xuefei Huang
Mehdi, Berm, Zibin, Sherif, Kedar and my group
Saeedeh, Aliakbar