ACS Medicinal Chemistry Letters

PATENT HIGHLIGHT

pubs.acs.org/acsmedchemlett

Halting HCV Replication with NS5A Inhibitors and NS5B Polymerase Inhibitors: Effective New Treatments of HCV Infection

Ahmed F. Abdel-Magid*

Therachem Research Medilab (India) Pvt. Ltd., Jaipur, India

General Background: Chronic hepatitis C infections affect nearly 3−4 million people in the United States and 170 million globally. The virus may cause damage to the liver leading potentially to serious complications such as liver failure and liver cancer over time. Until very recently, the combination of pegylated interferon alpha and ribavirin was the standard treatment for hepatitis C infections. This combination treatment is limited in scope as it does not cure all patients with genotype 1 virus, and it causes severe adverse effects intolerable to most patients.

The modern research on the life cycle and replication of HCV over the past decade revealed new potential viral and host targets for the development of effective anti-HCV therapy. Advances such as the establishment of HCV replicon system in 1999 and the development of robust HCV cell culture models in 2005 are credited with improving and accelerating the testing of potential antiviral compounds and consequently the advancement of HCV replication knowledge leading to new anti-HCV therapies.

Hepatitis C virus genome contains a positive-strand RNA that encodes a large polypeptide of nearly 3000 amino acids. This polypeptide is cleaved in the infected cells into at least 10 structural and nonstructural (NS) proteins. The NS-proteins are named NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The formation of NS-proteins is caused by the action of two viral proteases. The first is a metalloprotease that cleaves at the N52−NS3 junction. The second is a serine protease contained within the *N*-terminal region of NS3 (named NS3 protease) that mediates all the subsequent cleavages downstream of NS3. The NS4A protein is believed to serve multiple functions including the formation of a NS4A/NS3 complex, which enhances the proteolytic efficiency of the NS3 protein. The nonstructural protein 5A (NS5A) plays an important role in viral replication, modulation of cell signaling pathways, and the interferon (IFN) response. While no known enzymatic function has been attributed to NS5A, it is an essential component of the HCV replicase and exerts a wide range of effects on cellular pathways and processes, including innate immunity and host cell growth and proliferation. NS5A is highly phosphorylated by host cell kinases and interacts with host cell membranes. The nonstructural 5B protein (NS5B; referred to as HCV polymerase) is an RNA-dependent RNA polymerase that is involved in HCV replication via the synthesis of double-stranded RNA from the single-stranded viral RNA genome, which serves as a template.

In pursuit of better treatment, researchers have targeted the inhibition of enzymatic targets such as the NS3 protease and NS5B (HCV polymerase), nonenzymatic targets such as NS5A, and some host targets such as microRNAs and cyclophilins to develop therapeutic tools for the treatment of HCV infection. The goal is to produce effective, direct-acting, interferon-free treatments through slowing or stopping the virus replication. Their efforts produced two approved HCV drugs in 2011. Both drugs are NS3 protease inhibitors: boceprevir (trade name victrelis) developed by Merck and Telaprevir (trade names incivek and incivo) developed jointly by Vertex and Johnson & Johnson. However, these drugs are used in combination with interferon and ribavirin; thus, patients still have to deal with the serious intolerable adverse effects of interferon. In addition, they are not effective with all types of HCV such as genotype 1 virus.

The next generation experimental HCV drugs are very promising. There are several effective NS5A inhibitors in late phase development including daclatasvir (BMS) and leidipasvir (Gilead). There are also several NS5B polymerase inhibitors in late development including the sofosbuvir (Gilead) and mericitabine (Genentech). However, the most promising experimental therapies are combination drugs with different mechanisms of action. The Gilead three-drug combination including the NS5A inhibitor leidipasvir, the NS5B polymerase inhibitor sofosbuvir, and ribavirin. The AbbVie five-drug combination treatment includes the two protease inhibitors ABT-450 and ritonavir, the NS5A inhibitor ABT-267, the non-nucleoside polymerase inhibitor ABT-333 and ribavirin. These combination drugs are showing good clinical trials data and high cure percentage even against genotype 1 virus.

The following are highlights of two recent patent applications dealing with inventions of new inhibitors of NS5A and NS5B.

Special Issue: HCV Therapies

Received: November 12, 2013
Published: November 20, 2013

1. NS5A INHIBITORS FOR THE TREATMENT OF HEPATITIS C INFECTIONS

Title: Potent and Selective Inhibitors of Hepatitis C Virus

Patent Application Number: US 2013/0210774 A1 **Publication date:** August 15, 2013

Priority Application: PCT/US11/49426 **Priority date:** August 26, 2011

Inventors: Coats, S. J.; Amblard, F.; Zhang, H.; Zhou, L.; Whitaker, R. A.; McBrayer, T. R.; Schinazi, R. F.; Shi, J.

Assignee Company: Emory University, Atlanta, GA (US) and RFS Pharma, LLC, Tucker, GA(US)

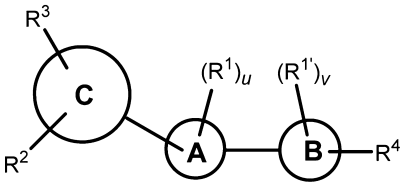
Disease Area: Hepatitis C virus (HCV) infections **Biological Target:** Nonstructural protein 5A (NS5A)

Summary: The invention in this patent application relates to substituted aromatic compounds represented generally by formulas (I, II, and III). Most of these compounds are inhibitors of NS5A phosphoprotein and may be useful in the treatment and/or prevention of hepatitis C virus infections.

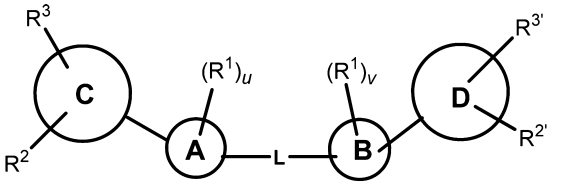
The nonstructural protein 5A (NS5A) is a hydrophilic phosphoprotein that plays an important role in viral replication, modulation of cell signaling pathways, and the interferon (IFN) response. NS5A has no known enzymatic function; however, it is an essential component of the HCV replicase. It is also implicated in a wide range of cellular pathways and processes, including innate immunity and host cell growth and proliferation. Thus, inhibition of NS5A is an attractive therapeutic target for the treatment of chronic HCV infections.

The inventors stated that the compounds of this invention that inhibit the HCV-NS5A may potentially provide new antiviral agents that may be advantageous in the treatment of drug-resistant HCV.

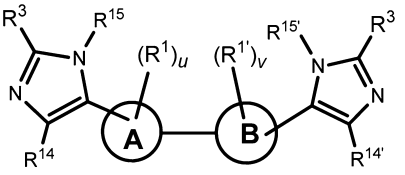
Important Compound Classes:



Formula (I)



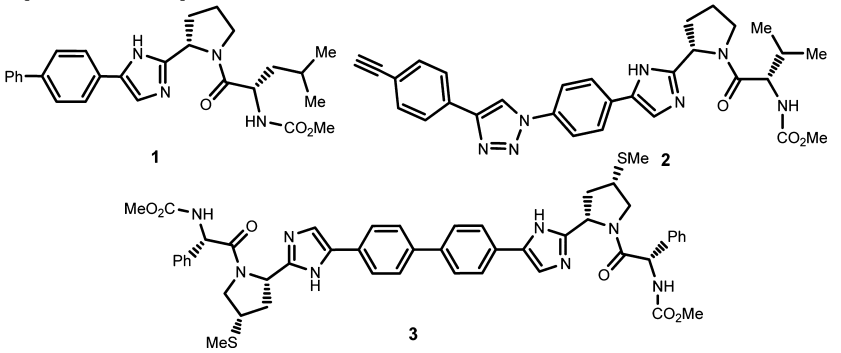
Formula (II)



Formula (III)

Key Structures:

Representative examples:



Biological Assay:

- Cellular Toxicity Assays
- Mitochondrial Toxicity Assays in HepG2 Cells
- Mitochondrial Toxicity Assays in Neuro2A Cells
- Assay for Bone Marrow Cytotoxicity
- HCV Replicon Assay

dx.doi.org/10.1021/ml400456r |ACS Med. Chem. Lett. 2014, 5, 234–237

Biological Data: Representative data for compounds 1−3 from the HCV Replicon Assay:

Compound	Replicon activity (EC_{50})
1	B
2	C
3	D

Note: Median effective concentration (EC_{50}) ranges against HCV 1b are as follow: **A** = 1−10 μM, **B** = 100−999 nM, **C** = 1−99 nM, **D** = <1 nM

Recent Review Articles:

(1.) Shah, N.; Pierce, T.; Kowdley, K. V. *Expert Opin. Investig. Drugs* **2013**, *22* (9), 1107−1121.
(2.) Belda, O.; Targett-Adams, P. *Virus Res.* **2012**, *170*, 1−14.
(3.) Bartenschlager, R.; Lohmann, V.; Penin, F. *Nat. Rev. Microbiol.* **2013**, *11*, 482−496.
(4.) Pawlotsky, J.-M. *J. Hepatol.* **2013**, *59* (2), 375−382.
(5.) Hamatake, R.; Maynard, A.; Kazmierski, W. M. *Annu. Rep. Med. Chem.* **2012**, *47*, 331−345.

2. NS5B POLYMERASE INHIBITORS FOR THE TREATMENT OF HEPATITIS C INFECTIONS

Title: Viral Polymerase Inhibitors

Patent Application Number: EP2626354 A1 **Publication date:** August 14, 2013

Priority Application: US 546213 P **Priority date:** February 20, 2004

Inventors: Tsantrizos, Y. S.; Chabot, C.; Beaulieu, P.; Brochu, C.; Poirier, M.; Rancourt, J.; Stammers, T.; Thavonekham, B.

Assignee Company: Boehringer Ingelheim International GmbH, 55216 Ingelheim am Rhein (DE)

Disease Area: Hepatitis C virus (HCV) infections **Biological Target:** Nonstructural protein 5B (NS5B polymerase)

Summary:

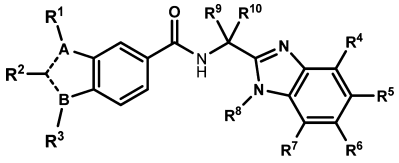
The invention in this patent application describes substituted indole derivatives represented generally by formula (IV). These compounds possess good to very good inhibitory activity against HCV NS5B polymerase and may be useful in the treatment and/or prevention of hepatitis C virus infections.

The recent research on anti-HCV drug development has targeted the specific functions essential for the replication of hepatitis C virus. Inhibition of NS5B polymerase seems an ideal target for the development of effective anti-HCV therapeutics for the following reasons:

- The enzyme appears to be essential to viral replication.
- The absence of RNA-dependent RNA polymerases in humans and mammals, the likely hosts of the virus.

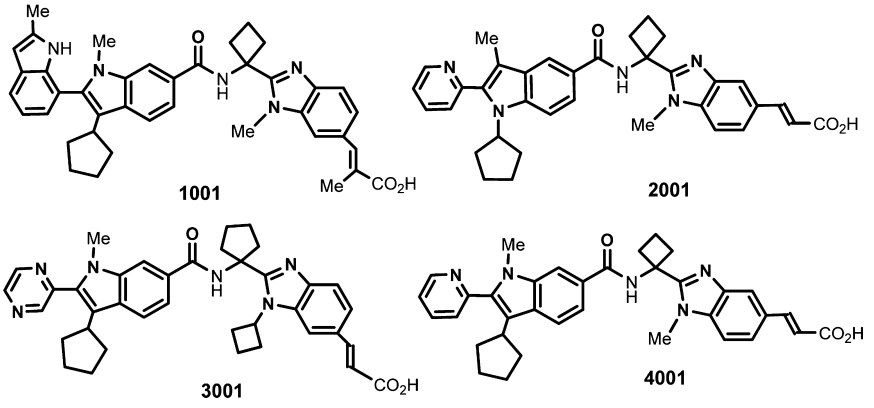
The inventors commented that while other indole inhibitors of the NS5B HCV-polymerase were previously disclosed, the inhibitors described in this current invention exhibit unexpectedly good activity in a cell-based HCV RNA replication assay.

Important Compound Classes:



Formula (IV)

Key Structures: The inventors listed 403 compounds in four groups numbered 1001−1153, 2001−2074, 3001−3132, and 4001−4044. One representative example of each group is shown here:



1001 2001 3001 4001

Biological Assay:
- Inhibition of NS5B RNA-dependent RNA polymerase activity
- Specificity of NS5B RNA-dependent RNA polymerase inhibition
- Cell-based luciferase reporter HCV RNA Replication Assay

Biological Data: The inventors reported that all tested compounds were found to have unexpectedly good activity in the cell-based HCV RNA replication assay.

Recent Review Articles:
(1.) Shah, N.; Pierce, T.; Kowdley, K. V. *Expert Opin. Investig. Drugs* **2013**, *22* (9), 1107−1121.
(2.) Soriano, V.; Vispo, E.; de Mendoza, C.; Labarga, P.; Fernandez-Montero, J. V.; Poveda, E.; Trevino, A.; Barreiro, P. *Expert Opin. Pharmacother.* **2013**, *14* (9), 1161−1170.
(3.) Varshney, J.; Sharma, P.; Sharma, A. *Eur. Rev. Med. Pharmacol. Sci.* **2012**, *16*, 667−671.

AUTHOR INFORMATION

Corresponding Author

*Address: 1383 Jasper Drive, Ambler, Pennsylvania 19002, United States. Tel: 215-913-7202. E-mail: afmagid@comcast.net.

Notes

The authors declare no competing financial interest.