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*Review*

# A review on novel therapies to combat hepatitis C

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**The major cause of chronic liver disease, cirrhosis, liver carcinoma and liver failure is Hepatitis C virus (HCV). NS3 is one of the attractive targets for therapy development for HCV as the N-terminal domain of the NS3 protein is a serine protein. NS3 protease acts by interfering with cellular mechanisms which are involved in the host immune response to an HCV infection and the inhibition of the protease is an efficient antiviral approach. NS3 protease inhibitors initially developed were: BILN 2061 (Ciluprevir), VX-950 (Telaprevir) and SCH503034. A combination therapy of injected pegylated interferon-α and oral ribavirin is regarded as the current standard therapy for HCV infection. Leucopenia, flu-like symptoms, thrombocytopenia, depression and anemia are some of the adverse effects of ribavirin. The compounds which constitute a benzoxaborole moiety interact with many biological targets and presents appreciable qualities. The acyl sulfinamide and the acyl cyanamide were shown to be potent P1 C-terminal groups in the enzyme assay. In a study, it is indicated that the aminobenzoyl sulfonamide fragment was identified as a novel P1 structural motif. New P2–P4 macrocycle inhibitors of NS3/4A were made up of a P1 C-terminal carboxylic acid have recently been developed. Potent tetrapeptidic inhibitors of the HCV NS3 protease have been developed by incorporating 4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid as a new N-acyl-L-hydroxyproline were developed for the treatment of Hepatitis C. α-Amino cyclic boronates was designed and synthesized and incorporated successfully in several acyclic templates at the P1 position as HCV inhibitors. The boronic acid compounds acts as the HCV NS3 serine protease inhibitors. Some of the HCV-protease inhibitors are developed which are based on a 2(1H)-pyrazinone P3 scaffold in combination with either a P2 phenylglycine or a glycine. NS3/4A protease inhibitors constituting of quinazoline derivatives as P2 substituent were synthesized. The tripeptide-based inhibitors of the HCV NS3 protease containing a novel P2-triazole had been synthesized. A protease domain (NS3pro) and RNA helicase domain (NS3hel) makes a multifunctional enzyme: Flaviviridae non-structural 3 proteins (NS3). Crude ethanol extract from rhizomes of the Chinese medicinal herb *Rhodiola kirilowii* (Regel) Maxim was also used for the cure of Hepatitis C. A new series of HCV NS3/4A protease inhibitors bearing a P2-P4 macrocycle and a P1- P10 a-ketoamide serine trap has been studied.**

**Key words**: Hepatitis C virus, non-structural protein inhibitors.

## INTRODUCTION

“Hepatitis” means inflammation of the liver. Hepatitis is most often caused by a virus. The most common types of viral hepatitis are hepatitis A, hepatitis B and hepatitis C. Heavy alcohol use, toxins, some medications, and certain medical conditions can also cause hepatitis. Hepatitis may be of two types:

1. Acute hepatitis C is a short-term illness that occurs within the first 6 months after someone is exposed to the HCV.

2. Chronic hepatitis C is a long-term illness that occurs when the HCV remains in a person's body.

It can lead to serious liver problems, including liver damage, cirrhosis, liver failure and liver cancer. Since the characterization of the HCV, from last 15 years, the understanding of the natural history of chronic hepatitis C has been greatly expanded and more effective and useful therapeutic strategies have been developed. Sustained virological response (SVR) rates have been improved from > 20% of patients treated for 48 weeks with conventional interferon (IFN) alfa, to ≈ 40% of patients treated with the combination of IFN alfa-2b plus ribavirin, and 54 to 61% of patients treated with a pegylated IFN and ribavirin, the current treatment of choice. Regulatory T cell markers are increased in chronically infected individuals with the HCV. The naturally occurring viral variants inhibit the T cell responses to cognate NS3358−375 in an antigen-specific manner (Duan et al., 2004). Hepatitis C is usually spread when blood from a person infected with the hepatitis C virus enters the body of someone who is not infected. Most people become infected with hepatitis C by sharing needles or other equipment to inject drugs. Hepatitis C was also commonly spread through blood transfusions and organ transplants. Symptoms for both acute and chronic hepatitis C can include fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, dark urine, grey-colored stools, joint pain and jaundice.

**Diagnosis of hepatitis C**

The diagnosis of hepatitis C can be done by specific blood tests. A person first gets a screening test that looks for "antibodies" to the HCV. The antibodies remain in the bloodstream, even if the person clears the virus.

**Prevention of hepatitis**

1. Do not share needles or other equipment to inject cosmetic substances, drugs, or steroids.
2. Do not use personal items that may have come into contact with an infected person's blood, such as razors, nail clippers, toothbrushes, or glucose monitors.
3. Do not get tattoos or body piercings from an unlicensed facility or in an informal setting (hepc general fact sheet).

S370P induced regulatory T cell markers in comparison to NS3358−375-stimulated CD4 T cells. The HCV is able to induce antigen-specific regulatory T cells to suppress the antiviral T cell response in an antigen-specific manner. The variants of an HCV immunodominant epitope, which develop during the chronic infection in a human, induced Foxp3 expression in an antigen-specific manner. It had a dose-dependent suppressive effect *in vitro*. The major cause of chronic liver disease is HCV. HCV causes cirrhosis, liver carcinoma and liver failure, and ultimately liver transplants. About 200 million people have been infected chronically with HCV (Xianfeng et al., 2010). HCV genome is a single- stranded, positive sense RNA molecule of 9600 nucleotides (Ronn et al., 2007). HCV genome is 9.6 kb and the structural proteins C, E1, E2 and non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B are encoded by HCV genome. NS3 is one of the attractive targets for therapy development for HCV as the N-terminal domain of the NS3 protein is a serine protein (Duan et al., 2004). The NS3 protein is basically a bifunctional enzyme with a helicase/NTPase domain and a protease domain. NS3 protease acts by interfering with cellular mechanisms which are involved in the host immune response to an HCV infection. The inhibition of the protease is an efficient antiviral approach that will block viral replication and will also restore the host immune response. NS3 protease inhibitors to enter research were: BILN 2061 (Ciluprevir), VX-950 (Telaprevir) and SCH503034. Telaprevir and SCH 503034 are being used in US as useful remedies to treat HCV-infected patients but the clinical evaluation of Ciluprevir was stopped because of cardiac toxicity in animal model (Ronn et al., 2007).

A combination therapy of injected pegylated interferon-α and oral ribavirin is regarded as the current standard of care for HCV infection, leukopenia, flu-like symptoms, thrombocytopenia, depression, anemia are some of the adverse effects of ribavirin (Xianfeng et al., 2010). BILN 2061 is mainly characterized by three unnatural amino acid residues (P1, P2, P3) in a macrocycle. The oral administration of BILN 2061 to the patients of hepatitis C caused an impressive reduction of viral RNA levels showing the proof for HCV NS3/4A protease inhibitors (Raboisson et al., 2008a). NS3/4A is the virally encoded serine protease which is one of the important constituent of life cycle of the HCV and is responsible for chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The development for potent and selective HCV NS3/4A protease inhibitors is the target for pharmaceutical industry for administering efficacious drugs to cure HCV – infected patients (Raboisson et al., 2008b).

**NOVEL THERAPIES FOR HEPATITIS C**

HCV is responsible for infecting approximately 3% of

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world's population. It has been observed that the compounds which constitute a benzoxaborole moiety interacts with many biological targets and shows appreciable drug qualities. The benzoxaborole moiety can potentially form polar interactions with Thr 42 and positively charged Lys 136. Studies have been performed on the inhibitory potency of different regioisomers of acylsulfamoyl benzoxaboroles-based HCV NS3 protease inhibitors. Appropriate optimization of the benzoxaborole moiety may rebalance the physicochemical properties of the resulting compounds and enhance their membrane absorption, potency and bioavailability (Xianfeng et al., 2010). The major problem in the use of antiviral drugs is the development of drug resistance. Mutations causing resistance to BILN-2061, VX-950, and SCH 503034 have been observed in the subgenomic HCV replicon assay. The acyl sulfinamide and the acyl cyanamide were shown to be potent P1 C-terminal groups, in the enzyme assay (Ro¨nn et al., 2007).

In a study, it is indicated that the aminobenzoyl sulfonamide fragment was identified as a novel therapy for hepatitis C. A microwave irradiated, palladium catalyzed, amidocarbonylation protocol caused the facile preparation of a series of compounds depicting sub-micromolar potencies in the full-length NS3 assay. According to the study, the inhibitors have two sites that can be used in optimization process (Ro¨nn et al., 2008). HCV–NS3 protease is necessary for viral replication hence; NS3 protease inhibitors have proved to be efficient targets in clinical trials. A series of analogs were developed in which the carboxylic residue is replaced by phosphorous acid functionalities and worked as the inhibitors of the NS3 protease. The methylphosphinate analogue showed nanomolar level of enzyme inhibition and sub-micromolar potency in the replication assay. A novel class of P2–P4 macrocycle analogues acted as the inhibitors of HCV–NS3 protease, MK-7009; a potent inhibitor bearing a cyclopropylacylsulfonamide in P1 showed strong antiviral activity in HCV infected chimpanzees and is currently being evaluated in clinical trials (Pompei et al., 2009).

For the treatment of hepatitis C, potent tetrapeptidic inhibitors of the HCV NS3 protease have been developed by incorporating 4-hydroxy-cyclopent-2-ene-1,2-di-carboxylic acid as a new N-acyl-L-hydroxyproline. The hydroxylcyclopentene template was synthesized from commercially available (syn)-tetrahydrophthalic anhydride. Three different amino acids were discovered in the P1-position, and in the P2-position the hydroxyl group of the cyclopentene template was substituted with 7-methoxy-2-phenyl-quinolin-4-ol. The P3/P4-positions were optimized from a set of six amino acid derivatives. All inhibitors were evaluated in an *in vitro* assay using the full-length NS3 protease. Several potent inhibitors were identified, with the most promising exhibiting a Ki value of 1.1 nM (Thorstensson et al., 2007).

There are many potent novel HCV NS3 protease inhibitors which have been developed from two inhibitor series constituting either a P2 trisubstituted macrocyclic cyclopentane- or a P2 cyclopentene dicarboxylic acid moiety as surrogates for the widely used Nacyl-( 4R)-hydroxyproline in the P2 position. According to the studies, it was found that the 14-membered ring system had a better potency in these two series and that the corresponding 13-, 15-, and 16-membered macrocyclic rings exhibited lesser potency. It has been observed that P1 acylsulfonamides had better potencies than the corresponding P1 carboxylic acids. Trisubstituted cyclopentane- and cyclopentene dicarboxylic acid moieties have been used to replace the commonly used N-acyl-(4R)-hydroxyproline, and have been incorporated in the P2 position of macrocyclic functionalized HCV NS3 inhibitors (Back et al., 2007).

The inhibitors of the HCV NS3 serine protease, α-amino cyclic boronates, was designed and synthesized and incorporated them successfully in several acyclic templates at the P1 position. The structural studies depict that they inhibit the NS3 protease by trapping the Ser-139 hydroxyl group at the active site. The first peptidomimetic boronic acid is velcade that has been developed into a therapeutic agent for the cure of relapsed multiple myeloma. The boronic acid compounds acts as the HCV NS3 serine protease inhibitors by many research groups in pharmaceutical companies such as Schering-Plough, Phenomix, Dupont, and BMS (Xianfeng et al., 2010).

A new class of phosphonate derivatives was developed to copy the interaction of product-like carboxylate based inhibitors of HCV NS3 protease. A phosphonic acid was reported to be a potent HCV NS3 protease inhibitor and utilized for the treatment of HCV infection (Sheng et al., 2009).

HCV- protease inhibitors are developed which are based on a 2(1H)-pyrazinone P3 scaffold in combination with either a P2 phenylglycine or a glycine. These HCV-protease inhibitors were further evaluated on the wild type as well as on two resistant variants of the enzyme, A156T and D168V. According to the molecular modeling the aromatic side-chain of the P2 phenylglycine occupies the same space as the substituent in position 6 on the pyrazinone core. The aromatic P1–P10 scaffold was found to be better in combination with the new P3–P2 building block and an entirely new type of achiral and rigidified inhibitors was discovered with enhanced potency. These developed inhibitors may be utilized for further optimization in a combinatorial fashion because of the rapid synthetic scheme for pyrazinone synthesis, the easy access to structurally diverse starting materials for the pyrazinone synthesis, like aldehydes, primary amines and a cyanide source, and the short (five steps) overall synthetic route of the inhibitor synthesis (Nilsson et al., 2010).

NS3/4A protease inhibitors constituting of quinazoline derivatives as P2 substituent were synthesized. By the optimization of quinazoline P2 substituents in three series

having macrocyclic P2 cyclopentane dicarboxylic acid and P2 proline urea motifs, better potency inhibitors depicting valuable pharmacokinetic properties can be obtained. An enhanced metabolic stability in human liver microsomes was obtained by 8-methyl substitution in the P2 cyclopentane dicarboxylic acid series in the quinazoline moiety. The proline urea series exhibited useful $CaCO^{-2}$ permeability than the cyclopentane series. A better pharmacokinetic property was observed *in vivo* in rats (Naud et al., 2008).

The tripeptide-based inhibitors of the HCV NS3 protease containing a novel P2-triazole had been designed and synthesized. A diverse series of inhibitors can be produced by the replacement of the P2 quinoline with a triazole moiety. Improvement may be done by the incorporation of an aryl-substituted triazole and by the replacement of the P1 acid with an acyl sulfonamide. Thus, these inhibitors produced appreciable cellular activity (Yao et al., 1999).

HCV is responsible for infecting approximately 3% of the world's population. HCV RNA is translated into a polyprotein. It gets cleaved into functional components that is, nonstructural protein 3 (NS3), which is a 631-residue bifunctional enzyme with protease and helicase activities. The scNS3–NS4A structure provides the first atomic view of polyprotein *cis* processing. The NS3 serine protease processes the HCV polyprotein by both *cis* and *trans* mechanisms. The local and global structural rearrangements follow the *cis* cleavage reaction, and large segments of the polyprotein can be folded prior to proteolytic processing. The product complex of the *cis* cleavage reaction exists in a stable molecular conformation and suggests autoinhibition and substrate-induced activation mechanisms for the regulation of NS3 protease activity (Xiaoping et al., 2009).

HCV protease inhibitors targets HCV NS3 which can effectively suppress HCV replication. Luciferase-reporter 1b replicon shuttle vectors allow the cloning of either the HCV full-length NS3/4A gene or the NS3 protease domain gene which only were developed. Initially, chimeric replicons carrying patient derived full-length NS3/4A failed to replicate in cell culture but the poor replication efficiency of the NS3/4A shuttle vector was enhanced by approximately 100-fold when the NS3 helicase domains of clinical isolates were substituted for that of the 1b Con1 lab strain. The HCV replicon system has been utilized to study the potency and mechanism of HCV inhibitors in a cell-based format. The current subgenomic or full-length genomic replicons are established with laboratory-optimized strains. According to the studies, shuttle vector approach may be used for the NS5B gene encoding the RdRp in which the NS5B gene was isolated from the sera of HCV-infected patients and then shuttled to a replicon vector deficient in RdRp activity to restore the RNA replication. A good potency was observed for the inhibitor when the replicons containing patient-derived NS5B from a panel of clinical isolates were tested against a polymerase inhibitor. Hence, this approach is useful for the evaluation of the phenotype of a mixed quasispecies pool. The full-length NS3/4A and NS3 protease domain are useful for the novel therapy of hepatitis C (Sallouma et al., 2010). The mutations causing resistance may reduce the efficiency of NS3/4A serine protease. The level of resistance associated with specific mutations differs from compounds to compounds. The substitutions R155K and A156T reduce the activity of all protease inhibitors. The R155K variant is having a high replication level and there is a substantial loss of cross-recognition by specific CD8 T cells targeting the epitope HAVGIFRAAV1175–1184 (Bogen et al., 2005).

The replication of the HCV takes place by the help of HCV NS3 protease and NS5B polymerase, a number of competitive inhibitors of the NS3 protease as well as nucleoside and non-nucleoside inhibitors of the NS5B polymerase have been identified by combining the power of high-throughput screening with rational, knowledge-based drug discovery. The HCV NS3 protein is a component of a heterodimeric serine protease that requires the noncovalently-associated viral protein NS4A for better catalytic activity. NS3-4A proteinase enzyme is responsible to cause the proteolytic cleavage of the viral polyprotein at the four junctions NS3/NS4A, NS5A/NS5B, NS4A/NS4B and NS4B/NS5A. NS3-4A protease plays an important role in the production of the viral RNA replication machinery by mediating a number of events in the proteolytic processing and maturation of the nonstructural viral proteins. By the help of this enzyme, the virus antagonizes the host cell innate immune response. NS3-4A causes the cleavage of CARDIF and TRIF which are the two essential components by which the cells sense the invasion by viral pathogens thus causing the induction of the antiviral state. NS3-4A counteracts the onset of the cell natural antiviral defenses. NS3-4A inhibitor prevents polyprotein processing and restores the hepatocytes innate antiviral response, thus depicting dual antiviral activity (Francesco et al., 2007).

A protease domain (NS3pro) and an RNA helicase domain (NS3hel) makes a multifunctional enzyme: Flaviviridae non-structural 3 protein (NS3). The activities of NS3 are critical for the viral replication. The replicative cycle of Flaviviridae needs the coordinated regulation of all the activities of NS3 protein. By the analysis of the root mean square (RMS) variation, NS3pro increases the stability of the subdomain 1 of the RNA helicase. By the utilization of the normal mode analysis, studies characterized slow collective motions of NS3, and observed that the two lowest-frequency normal modes are enough to represent reorientations of NS3pro relative to NS3hel. These movements induced an enhancement in the exposure of the active site of NS3pro that can be important during the proteolytic processing of the viral polyprotein (Rosales-Leon et al., 2007). To develop the

naturally occuring chemical entities as lead compounds from which the novel anti-HCV agents could be developed, the bioassay-guided fractionation and isolation were performed on a crude ethanol extract from rhizomes of the Chinese medicinal herb *Rhodiola kirilowii* (Regel) Maxim by the use of column chromatography (CC) techniques and *in vitro* inhibitory activity against HCV NS3-SP. The partition of the extract between water and different organic solvents caused the isolation and identification of 12 compounds in the ethyl acetate part which was the most active. The compounds were tested for *in vitro* activity against HCV NS3-SP, and the most potent ones are: (−)-Epicatechin, derivatives: 3,3_-digalloylproprodelphinidin B2 (Rhodisin); 3,3_-digalloylprocyanidin B2; (−)-epigallocatechin-3-O-gallate; and (−)-epicatechin-3-O-gallate with $IC_{50}$ of 0.77, 0.91, 8.51 and 18.55 _M, respectively (Zuoa et al., 2007).

A new series of HCV NS3/4A protease inhibitors bearing a P2-P4 macrocycle and a P1- P10 a-ketoamide serine trap has been studied. The NS3 protease which is important for the viral replication has been proved to be one of the attractive targets for developing novel anti-HCV therapies. The optimization of macrocycle led to the discovery of compounds 8b and 8g with a good activity both in the enzyme as well as in the cell based (replicon) assays with favorable PK profile in a preclinical species (Avolio et al., 2009).

### The persons who must be tested for hepatitis c

Patients who should proactively be offered testing for hepatitis C include:

1. Haemodialysis patients.
2. Blood, blood product, tissue and organ donors.
3. All drug users, especially those who have injected drugs or shared ‘works’, including prisoners.
4. Babies born to hepatitis C infected mothers.
5. Immigrants from countries of high endemicity for hepatitis C infection.
6. Persons, including healthcare workers, who have had potential percutaneous or mucous membrane exposure to hepatitis C.
7. HIV or hepatitis B infected patients.
8. Those with unexplained persistently raised serum transaminases.

Hepatitis C testing should be considered on:

1. Sexual partners of people who have hepatitis C (low risk).
2. Men who have sex with men who present for sexually transmitted disease screening (low risk).
3. Those with tattoos or body piercing.

### Laboratory methods for hepatitis c testing

Screening for hepatitis C includes testing of blood for the presence of HCV antibodies or hepatitis C antigen in serum. Initially, enzyme immunoassay (EIA) test was utilized for the screening of hepatitis C virus. Acute, chronic or resolved infection can be identified by antibody and antigen testing. The molecular investigation includes the detection of hepatitis C RNA, hepatitis C genotype testing and estimation of the viral load. Genotyping may be used for the detection of HCV (National Hepatitis C Strategy, 2011).

## CONCLUSION

About 200 million people have been infected chronically with HCV. NS3 is one of the attractive targets for therapy development for HCV as the N-terminal domain of the NS3 protein is a serine protein. Various therapies have been developed for the treatment of hepatitis C and some are currently under clinical trials. BILN 2061 (Ciluprevir), VX-950 (Telaprevir) and SCH503034 are some of the compounds which were developed initially. A combination of pegylated interferon-α and oral ribavirin compounds constituting of benzoxaborole moiety, acyl sulfinamide and acyl cyanamide, α-amino cyclic boronates, boronic acid compounds, NS3/4A protease inhibitors constituting of quinazoline derivatives, crude ethanol extract from rhizomes of the Chinese medicinal herb *Rhodiola kirilowii* (Regel) Maxim are some of the therapies against hepatitis C. By utilizing different approaches and by molecular modification of the existing drug molecules, hepatitis C may be treated efficiently.

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#### Abbreviations:

**NS3,** Non-structural protease; **HCV,** hepatitis C virus.

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