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Review Article

NS3/4A Serine Protease Inhibitors for Hepatitis C Virus Therapy (Telaprevir and Boceprevir)

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Received: March 11, 2019; **Published:** July 04, 2019

Abstract

Hepatitis C Virus (HCV) is a chronic and challenging disease to eradicate. However, there are a lot of antiviral drugs have been introduced to target the HCV proteins which are involved in the replication process. Telaprevir and Boceprevir are one of the effective antiviral drugs to cleave the NS3/4A serine protease. The general structures and mechanism of action of both the drugs has been discussed along with their clinical trials.

Keywords: *Hepatitis C Virus (HCV); NS3/4A Serine Protease; Telaprevir; Boceprevir*

Introduction

Hepatitis C Virus (HCV) processes 10 proteins after the proteolysis process by host cellular and viral proteases [1], which are divided into structural proteins, indicating C, E1, E2 and p7 and non-structural proteins, representing NS2, NS3, NS4A, NS4B, NS5A, and NS5B [2,3]. Among the non-structural proteins, NS3 protease is crucial for viral replication [4]. Telaprevir (TRV) and Boceprevir (BOC) are first NS3/4A serine protease inhibitors. Both the protease inhibitors belong to the ketoamide group of peptidomimetic inhibitors that are reversibly incorporated with the active site of the serine protease enzyme [5,6]. They prevent the cleavage of the polypeptides to produce different non-structural proteins, which are indispensable for the HCV viral replication. These antivirals are intended for the genotype 1 of Hepatitis C Virus therapy [7]. The combination of Boceprevir [8] or Telaprevir [9] with the standard therapy of pegylated interferon and ribavirin (PR) considerably increases the sustained virologic response rate (SVR) to 77% for 48 weeks and 69% for 24 weeks of treatment respectively in genotype 1 infected patients.

Functions of NS3/4A serine protease of hepatitis C virus

NS3/4A serine protease of HCV is an enzyme resulting from the combination of two different macromolecules, NS3 protein and NS4A protein. NS3 protein is a bi-functional protein with serine protease at the N- terminal and helicase domain at the C- terminal. The N-terminal serine protease catalyses the HCV polyproteins at the NS3/4A, NS4A/4B, NS4B/5A and NS5A/5B junctions [10]. However, NS3 alone is not adequate for cleavage at these junctions. NS4A protein is necessary for the effective cleavages [11]. Primarily, the middle portion of NS4A (amino acid 21-30) is required as a cofactor to provide a tight complex with NS3 and to activate the catalytic process. Mutations in NS4A destabilize the NS3 protease to cleave at these junctions [12]. The specific role of NS4A is to stabilize the NS3 from cellular protease by preventing it from degradation [13] and to promote the proteolytic activity. Autocatalytic, cis cleavage is formed when the NS3/NS4A serine protease catalyses at NS3/4A junction [14], whereas cleavage at NS4A/4B, NS4B/5A and NS5A/5B junctions is known as trans cleavage [15].

Love., *et al.* [16] and Kim., *et al.* [17] solved the 3-dimensional structure of NS3-4A protease. The X-ray crystallography of NS3 protease determined that the protease has a chymotrypsin like fold with two beta barrel subdomains and a zinc-binding site where the zinc metal ion is responsible for structural functions of the serine protease but not for catalytic purposes. Histidine 57, Aspartate 81 and Serine139 form the catalytic triad of the NS3-4A protease enzyme. The catalytic triad is the active site of NS3 protease [16]. The crystal structure of NS3 protease along with NS4A revealed that the 21 - 32 positioned amino acids of NS4A are necessary for an appropriate folding of NS3 through the generation of a β-strand that joined with the N-terminal β-barrel of NS3 [17] (Figure 1). Rost., *et al.* [18], estimated that the N-terminal hydrophobic portion of NS4A (amino acid 1 - 21) forms a transmembrane helix and the cofactor, NS4A pulls the NS3 protease adjacent to the membrane and anchors the HCV replication.

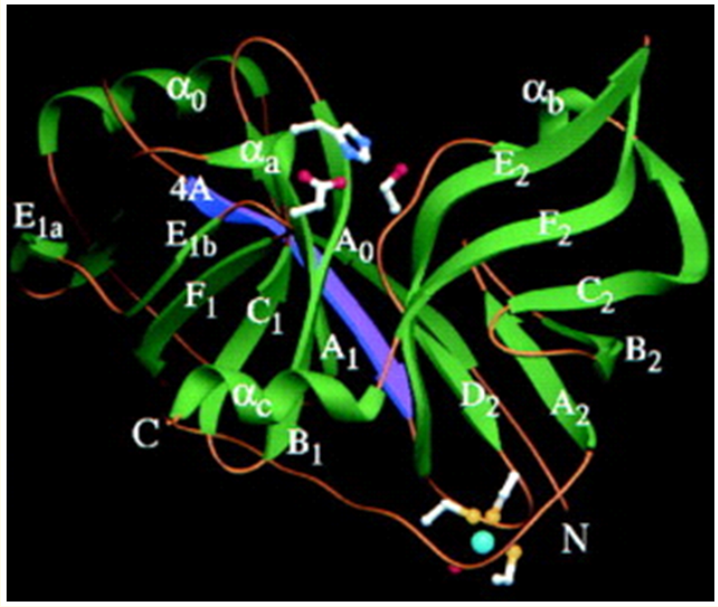

Figure 1: *'N' on the left side of the figure represents the N-terminal and 'C' on the right side stands for C-terminal of the NS3. The catalytic triad and the zinc ions in blue are illustrated in ball and stick configurations. The beta- strand resulted from the NS4A in a purple colour joined the N-terminal β-barrel of NS3 [17].*

According to Schechter and Berger nomenclature system, the substrate of HCV NS3/4A protease encompasses with 10 amino-acid comprising polypeptides, which are represented as P6, P5, P4, P3, P2 and P1 at the N-terminal and P1', P2', P3' and P4' at the C-terminal. There is a scissile bond positioned between P1 and P1' amino acids. S1 pocket is formed when the P1 residues combines with the serine protease [19].

A flexible polypeptide linker that separates the two-beta domain of NS3 protein provides substrate-binding pocket of HCV protease [20]. Three conserved places of NS3-4A substrates are obtained as an aspartate or glutamate at P6, a cysteine or threonine at P1, a serine or alanine at P1' after performing the sequence alignment in all cleavage sites. The trans cleavage sites have a cysteine at the P1 location, while threonine is present in the cis cleavage site. Noticeably, the aromatic ring of Phenyline-1180 is located at the bottom of the S1 pocket which is small and hydrophobic [16]. Structural information of NS3 protease with its substrate binding sites is useful in producing structure-based inhibitors [21]. However, HCV NS3 protease does not have 60A-60G loops, which are considered to interact with P2, P3 and P4 moieties of inhibitors in other members of serine proteases such as trypsin, thrombin and factor Xa. Because of the absence of

these loops, it is quite difficult to produce a low molecular weight inhibitor for HCV serine protease [16]. Moreover, Shrivave., *et al.* [22] observed that, despite having continuous mutations in HCV during replication process, these mutations could not deactivate the NS3/4A cleavage junctions over 5000 known HCV isolates.

Besides the role of cleavage in polyprotein process, the HCV protease activity restores the innate antiviral defences by controlling the interferon 3 (IRF-3) activation [23]. The serine protease cleaves Toll-interleukin1 receptor domain, which contains an adaptor protein for Toll-like receptor 3 signaling (TRIF). TRIF allows the cellular stimulation of kinases, which in turns triggers the antiviral innate immune responses in the cells. The cleavage might effectively weaken the host response and support the viral replication in hepatitis C virus infected patients [24]. Besides the cleavage of polyprotein and TRIF, NS3/4A protease also cleave a subunit of Cul4-based ubiquitin ligase complex, which is known as DDB1. DDB1 is the actual cellular substance of NS3/4A protease and take parts in viral replication process [25].

Mechanism of action of telaprevir (VX-950)

Perni., *et al.* [5] explained the mechanism of action of Telaprevir on the NS3/4A protease. Telaprevir (VX-950) is a tetrapeptide mimetic, highly selective, effective and reversible HCV protease inhibitor. Sequences of NS5A/5B cleavage site was used to produce the inhibitor. Combination of active site serine139 with the conventional electrophiles such as the non-cleavable alpha ketoamide group, results in keto-carbonyl inhibitors (Figure 2A), which is a constant, covalent and reversible complex with the serine protease. The substrate- binding pockets of S1, S2, S3 and S4 are filled with the hydrophobic side chains of the inhibitors. Additionally, hydrogen bond is formed between the amino groups of the antiviral and main chain of the protease.

Perni found that Telaprevir reversibly binds to the HCV protease in two phases. Firstly, it combines inadequately to the protease to provide a transient-collision complex. Later, the complex is gently readjusted to form a tighter covalent bond between the protease and the ketoamide group of Telaprevir (Figure 2B). Then the covalent complex dissociates slowly, with a half-life of 58 minutes unlike the noncovalent inhibitor of ciluprevir, which dissociates rapidly with a half-life of seconds [26].

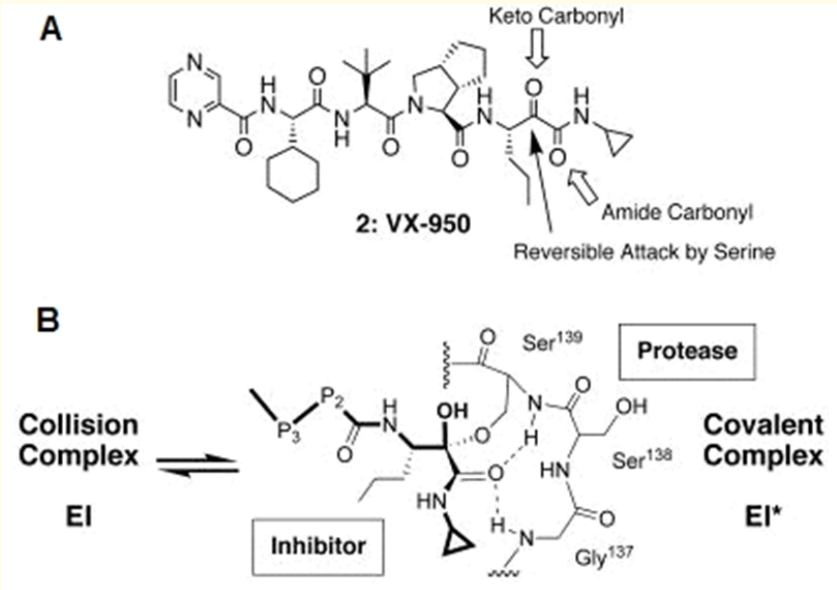

Figure 2: *(A): Chemical structure of Telaprevir which is reversibally attacked by Serine and forming two carobonyl groups with (1) Keto carbony and (2) Amide carbonyl. (B): Combination of enzyme and inhibitor: reversibly covalent bond is formed between the active Serine139 of serine protease and inhibitor (VX-950). Two hydrogen bonds are shown in dash lines between the protease and VX-950 [5].*

The inhibitory activity of telaprevir was performed and the results are as follows. 50% inhibitory concentration (IC_{50}) was examined by incubating telaprevir on HCV replicon cells at 24, 48, 72 and 120 hours and the results exhibited that there was a rise in blocking effect Telaprevir with time, showing 0.574, 0.288, 0.210 and 0.139 µM respectively [27]. Telaprevir disturbs the NS3 activity completely by blocking the RNA synthesis (Emax, 92%) and viral production in a 12-hour period [28].

Mechanism of action of boceprevir (SCH 503034) on NS3/NS4A protease

Boceprevir is another HCV protease inhibitor. Venkatraman., *et al.* [29], developed highly potent and selective inhibitor, Boceprevir (SCH 503034) by the process of modification of compound 4 in which 3,4-dimethylcyclopropylproline is combined at the P2 position. Modification process was performed by altering the side chain residues at P1', P1, P3, and P3 capping sites. Boceprevir was obtained when the compound 4 was reformed with the integration of tertiary-butylurea at the P3 capping site (Figure 3). Since it is a ketoamide group, it binds to the serine 139 of NS3/4A protease to offer a reversible covalent bond, whereas the hydrogen bond is formed from the amide group and help to anchor the inhibitor to the surface [6].

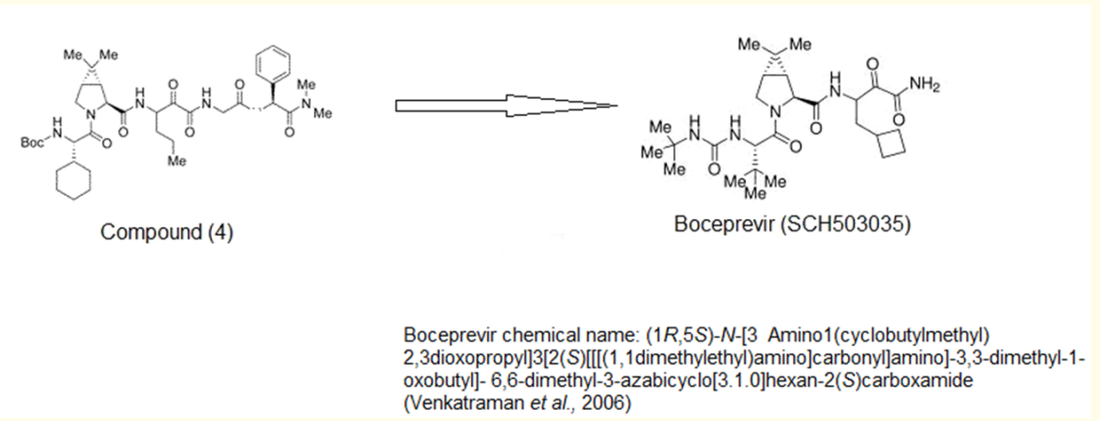

Figure 3: *Structure of Boceprevir (SCH53035) after modifying the compound 4 at the P3 capping site with tertiary-butyl group of urea and its chemical name [29].*

Venkatraman solved the crystal structure of Boceprevir fixed with NS3 protease, which is illustrated in figure 4. The figure showed that most of the S1 pocket area were occupied by cyclobutyl alanine moiety whereas; the bent configuration of dimethylcyclopropane proline residue at P2 enables the proline and cyclopropyl ring to overlap with Alaine -156. The S3 pocket, which is engaged with the side chain of P3 tertiary-butyl glycine, which hydrophobically interact with the protease. P3 urea-capping from the groups of tertiary-butyl had attached to the S4 pocket and offers a three-fold increase of activity than other conventional residues at P4 with low molecular weight. Boceprevir inhibits the active site of protease and prevents its regular function of polyprotein cleavage with the potency of K_i^*= 14 Nm [30].

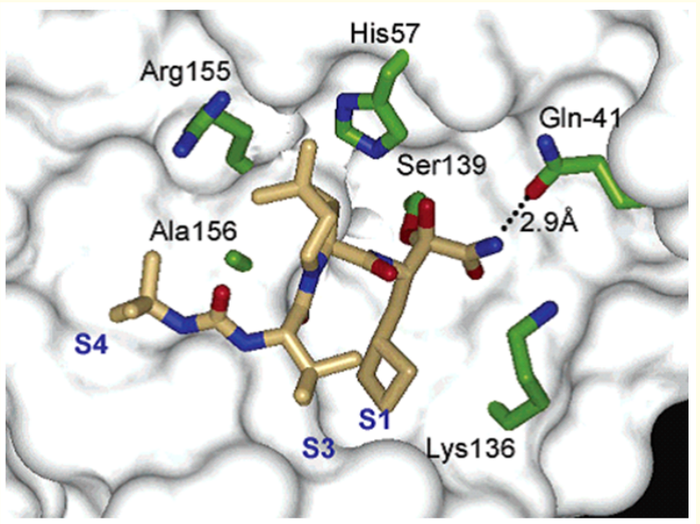

Figure 4: *Three-dimensional structure of Boceprevir bound to the NS3/4A serine protease [29].*

Clinical trial of telaprevir or boceprevir with peginterferon and ribavirin in genotype 1 patients

The main purpose of HCV infection treatment is to obtain high sustained virologic response (SVR), which is described as the HCV RNA level that is undetected at the 24th week after the end of treatment [31]. Telaprevir monotherapy is linked with the increase chance of telaprevir-resistant complication. However, the addition of Interferon and Ribavirin brings about the considerable decline in viral load and reduces the development of telaprevir-resistant problems [32].

McHutchison., *et al.* [33], analysed 250 number of HCV genotype 1 infected patients by using combined therapy of telaprevir with peginterferon and ribavirin. It was observed that there was higher SVR rate in triple therapy received patients than the patients who received only peg-interferon α-2a and ribavirin for 48 weeks (Table 1). This triple therapy proved to be more potent in reducing HCV RNA level.

Drug regime	Sustained virologic response rate (SVR) after 24 weeks (%)
T12PR24 (n = 79)	61
T12PR48 (n = 79)	67
T12PR12 (n = 17)	35
PR48 (n = 75)	41

Table 1: *T12PR24 = patients receiving Telaprevir with peginterferon α-2a and ribavirin (PR) for 12 weeks followed by PR for 12 more weeks. T12PR48= patients receiving Telaprevir with PR for 12 weeks followed by PR for 36 more weeks. T12PR12 = patients receiving Telaprevir with PR for 12 weeks. PR48 = patients receiving peginterferon α-2a plus ribavirin (PR) for 12 weeks (control group), n= number of patients.*

Patients treated with Telaprevir with peginterferon and ribavirin (PR) for 12 weeks and 8 weeks achieved 75% and 69% of SVR respectively, whereas PR alone treated, patients yielded only 44% of SVR [34].

Poordad., *et al.* [35], examined the combined therapy of Boceprevir with peginterferon and ribavirin on genotype1-infected patients who were previously untreated. The study depicted that there is a considerable increase SVR in triple therapy received patients as shown in table 2. Group-3 patients obtained highest SVR among the three groups.

Total number of patients	Group-1 SVR%	Group-2 SVR%	Group-3 SVR%
Non-black patients (n = 938)	40 (n = 311)	67 (n = 316)	68 (n = 311)
Black patients (n = 159)	23 (n = 52)	42 (n = 52)	53 (n = 55)

Table 2: *SVR= sustained virologic response, Group-1 = patients who received peginterferon and ribavirin for 44 weeks, Group-2 = patients who received Boceprevir with peginterferon and ribavirin for 22 weeks, Group-3 = patients who received Boceprevir with peginterferon and ribavirin for 44 weeks, n=number of patients.*

In Brazil, Borba., *et al.* [36] determined the effectiveness of Boceprevir and Telaprevir on 117 HCV infected patients. The result was shown in Rapid Virological Response (RVR). It is defined as HCV RNA level that is not detectable after 4 weeks of treatment and it is used for SVR prediction [37]. Patients with higher RVR tends to achieve reasonable SVR rate [38]. Borba mentioned the mean viral load at 4 weeks of treatment with Telaprevir and Boceprevir and RVR as in the table 3. The percentage of RVR in Boceprevir treated patients was

smaller than those of Telaprevir treated patients. Likewise, Bailly., *et al.* [39] reported that Telaprevir treated patients achieved better RVR rate than the Boceprevir received patients (67% and 36%) respectively.

Number of patients receiving inhibitors	Mean viral load at 4 weeks of treatment, (IU/mL)	RVR, n (%)
BOC (n = 15)	34,462.73	4 (26.7)
TVR (n = 102)	5,850.00	89 (87.3)

Table 3: *BOC= Boceprevir, TVR= Telaprevir, n= Number of patients and RVR= Rapid virologic response.*

The inhibitory activity of protease inhibitors with peginterferon and ribavirin considerably increases the cure rate of genotype 1 infection. However, these drugs were discontinued during the experimental process in some patients due to their adverse effect. Anaemia and dysgeusia are the most prominent adverse effect in boceprevir recipients. Poordad reported that there were 29% of PR recipients and 49% of boceprevir received patient got anaemia. In a certain cases, erythropoietin was used to manage the anaemia problem. Lower anaemia rate follows in telaprevir treated patients than in boceprevir recipients. However, rash, pruritus, nausea, and diarrhoea were more common in telaprevir recipients.

Next generation NS3/4A protease inhibitors

The next generation protease drugs are non-covalent reversible macrocyclic inhibitors. Simeprevir (TMC435) [40] and Narlaprevir [41] and MK-5172 [42] belong to the macrocyclic group and they are currently used in some developed countries because they have less adverse effect than Telaprevir and Boceprevir. However, the first generation protease inhibitors are still extensively used in some nations in order to treat the genotype 1 infection because of the financial limitations. Therefore, combination of standard therapy with Boceprevir or Telaprevir might use as an important treatment in the near future [43].

Conclusion

The combination of Boceprevir or Telaprevir with pegylated interferon and ribavirin (PR) significantly raises the SVR to more than 75%. Despite of having new effective HCV antivirals, Boceprevir and Telaprevir are still high demand in many developing countries to cure genotype 1 infection.

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Volume 15 Issue 8 August 2019