# Development of a Novel Hepatitis C Protease Inhibitor

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## Executive Summary

Hepatitis C virus (HCV) infects 150-200 million people worldwide. Americans account for 4.1 million of the people affected with hepatitis C. There are six hepatitis C genotypes; the most common genotype in the United States, Japan, and Europe is genotype 1. At present, the most commonly prescribed drug used to treat HCV is pegylated interferon, which affects patients' immune responses, but HCV research is now focused on attacking the virus directly through the inhibition of viral proteins' essential activities. There are several proteins, which may be inhibited, including those involved in cell entry and viral replication, but one particularly promising target is the NS3/NS4A complex. This complex forms a potent serine protease, which cleaves several of the viral polyprotein's peptide bonds. This activity releases three of the most important functional proteins of the virus. If an inhibitor molecule can bind to the NS3/NS4A complex active site, a catalytic triad, the formation of infectious virions can be prevented.

Sixteen potential drug candidates have been identified. These were identified through modifying the functional groups of a known HCV protease inhibitor, boceprevir. The functional groups were modified to choose the combinations that would likely give the highest binding affinity, which corresponds to a low value of $K_i$, and highest HNE/HCV. These drug candidates were tested for their binding strength using DockingServer. One candidate, (3S)-3-{[(1R,2S,5S)-3-[(2R)-2-[(tert-butylcarbamoyl)amino]-2-cyclohexylacetyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2-yl]formamido}-4-cyclobutyl-2-oxobutanamide, was determined to be have a better inhibition coefficient and also a better selectivity against human neutrophil elastase than boceprevir.

# Table of Contents

## Introduction

Approximately 150-200 million people worldwide are affected with hepatitis C; this is equivalent to approximately 2-3% of the world's population. In America, 4.1 million people are affected with hepatitis C which causes the death of 10,000 to 20,000 Americans each year. Hepatitis C, although typically asymptomatic, can lead to liver damage. In fact, 50% of all cases of cirrhosis of the liver are caused by hepatitis C.

Hepatitis C Virus (HCV) is a complicated disease with six known genotypes and more than fifty subtypes. Genotype 1 is the most common genotype in America, Japan, and Europe. There are currently no vaccines for hepatitis C. Current medications work reasonably well for genotypes 2 and 3, but current treatments, as discussed below, only provide a 40% to 50% response rate for genotype 1 patients.

Hepatitis C is spread through contaminated blood. There are a variety of ways that an individual can be exposed to hepatitis C infected blood. The most common way that hepatitis C is spread is through needles shared during drug use, tattooing, or body piercing. Other individuals may have contracted HCV due to blood transfusions or organ transplants before 1992. This is because prior to 1992 blood was not tested for the presence of HCV or other blood-borne pathogens. Rarely, babies born to HCV infected mothers may become infected, this occurs in less than 5% of births to HCV infected mothers.

Hepatitis C is typically asymptomatic. When symptoms occur however they often can be misdiagnosed as the flu, these symptoms include fatigue, nausea, muscle or joint pains, and tenderness near the liver. Later stage symptoms are also rare, if they appear they may include fatigue, lack of appetite, nausea, and jaundice. Cirrhosis of the liver is typically the first symptom that causes the diagnosis of hepatitis C; this usually occurs 20 to 30 years after the infection is contracted.

Current medications used to treat hepatitis C are pegylated interferon alfa and ribavirin. There are two types of pegylated interferon alfa, peginterferon alfa-2a (trade name: pegasys) and peginterferon alfa-2b (trade name: peg-intron), these are given in weekly injections in conjunction with twice daily oral doses of ribavirin (trade name: rebetol). These treatments are given for 24 weeks for patients infected with genotype 2 or 3 HCV with a 70%-80% rate of sustained virological response. For patients infected with genotype 1 HCV a higher dose of these medications is given and patients must remain on the medicine for 48 weeks. Even with the higher dose for genotype 1 patients, the current medication only provides a sustained virological response of 40%-50%. The current medications also

have adverse side effects including, flu-like symptoms, irritability, depression, memory problems, fatigue, insomnia, anemia, and severe birth defects.

The low response rate to current medications for genotype 1 HCV, as well as the high incidence of genotype 1 in America, Europe, and Japan has led drug developers to focus on developing a new drug that can more effectively treat HCV genotype 1.

## Problem Statement

Propose new molecules that can act as protease inhibitors for hepatitis C. These molecules should have a binding affinity equal to or greater than that of SCH 503034.

## Background

### *HCV Structure and Function*

HCV is a small enveloped virus with ten functional proteins and a single strand of RNA. HCV virions are spherical and only 50 to 55 nm in diameter. Their viral envelope in composed of 90 copies of glycoproteins E1 and E2. Within this envelope is a lipid bilayer covering the viral nucleocapsid, which consists of the HCV core (C) protein. E1, E2, and C are the structural proteins of the virus. The nucleocapsid contains the 9.6kb single-stranded positive-sense RNA molecule, which functions as mRNA for the translation of viral proteins. This molecule contains two non-translated regions (NTRs) which are located on either side of an open reading frame, which codes for a precursor polyprotein of about 3000 amino acid residues. This polyprotein is eventually cleaved into the structural proteins earlier described as well as seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B).

Viral entry into the cell begins with adsorption of HCV to a target cell, and multiple receptors are required in the process. HCV interacts with an LDL/HDL receptor, scavenger receptor B type 1 (SR-BI), and then with CD81, a tetraspan expressed on the surface of hepatocytes and peripheral blood mononuclear cells. An integral membrane protein, which is highly expressed in the liver, called claudin-1 (CLDN1) is then able to interact with the virus. After binding, the virus is internalized by endocytosis through a clathrin-coated pit. Endosomes are highly acidic and able to fuse with viral envelope glycoproteins, which allows for the release of the HCV RNA into the cytoplasm of the liver cell.

The virus travels to the endoplasmic reticulum where the 5'NTR binds to the 40S and 60S ribosomal subunits and the initiator tRNA, which forms the translational active complex for HCV polyprotein synthesis. Host peptidases cleave the core protein, E1, E2, and p7. The junction between NS2 and NS3 is autocatalytically cleaved by a zinc-dependent NS2-NS3 cysteine protease. This frees

NS3 serine protease to cleave the rest of the nonstructural proteins. NS3 binds to the closest downstream protein, NS4A, which enhances peptidase activity.

One cleaved protein, NS4B, induces a membranous platform for viral RNA replication. NS5B, an RNA-dependent RNA polymerase, uses the HCV positive-sense RNA strand as a template for the synthesis of an antisense RNA molecule. NS3A is necessary for this process as well, because it unwinds the secondary structures of the template RNA.

The synthesized viral components are then assembled into potentially infectious virions in the endoplasmic reticulum. Most viral components, some cellular factors, and lipid droplets are involved in the formation and release of virions, but the exact mechanism by which this is done is still unknown (Mauss, 2009).

The mechanism by which HCV causes tissue damage is also uncertain, but several controversial explanations exist. Unlike many viruses that cause apoptosis or necrosis, it does appear that high levels of HCV only rarely result in direct cytotoxicity. Most damage may actually be done by the body's own immune responses, both humoral and cell-mediated. B-cells reactive to HCV proteins, especially glycoproteins E1 and E2, undergo clonal expansion during infection. E1 and E2 exhibit very high variability and thus epitopic alterations can help the virus escape the immune response while instigating the production of even more different antibodies. Therefore, chronic exposure to HCV is likely to eventually produce self-reactive antibodies. Aggressive T-cell reactions can also harm cells. Slow clearance of the virus can activate cytotoxic T lymphocytes, which produce toxic granules to induce necrosis. As infected cells die and are released into the surrounding tissue, inflammation triggers stellates, the liver's fat cells which normally regulate blood flow and store fat and vitamin A, to alter their function. With the help of leukocytes and Kupffer cells, the stellates produces collagen fibers in the extracellular matrix in order to help inhibit the spread of the infection. When the infection continues, the collagen matrix is not dissolved as it would be in a healthy organ but is instead built up even more. Liver cells cut off from their blood supply by the scar tissue then atrophy and even die due to the resulting lack of oxygen and nutrients. When blood flow is diverted from parts of the liver, drugs and metabolic waste products are not removed properly, and thus can cause symptoms arising from toxicity in the rest of the body (Freeman, 2001). Another mode of tissue damage might be the overproduction of free radicals during infection, which leads to lipid peroxidation and cell membrane deterioration. HCV also damages mitochondrial membranes, which are essential for cellular energy production. This is a likely reason for the common symptom of fatigue in patients.

*Standard of Care*

Despite the likelihood that HCV-associated liver damage is actually caused by a prolonged immune response, the current most effective drug used in the treatment of HCV is interferon alfa, an immunoregulatory cytokine. Interferon alfa-2b transcribes interleukin 4, which induces T helper cells to become Type 2 T cells, which stimulate B cell proliferation. Therefore, the mechanism by which interferon produces a sustained virological response (SVR) is the creation of a strong and rapid immune response. Treatment with interferon is associated with a myriad of side effects including flu-like symptoms, mitochondrial membrane damage, autoimmune phenomena, bone marrow deterioration, and neuropsychiatric problems. Treatment duration should be kept short; 24 to 48 weeks are recommended for genotypes 2 or 3 and genotype 1 patients, respectively. The treatment should not be used at all for HCV-infected patients who have failed to respond to previous interferon therapy. The ineffectiveness of the drug in these non-responders is illustrated by Figure 1 and is a major reason for the continuing search for new HCV drugs.

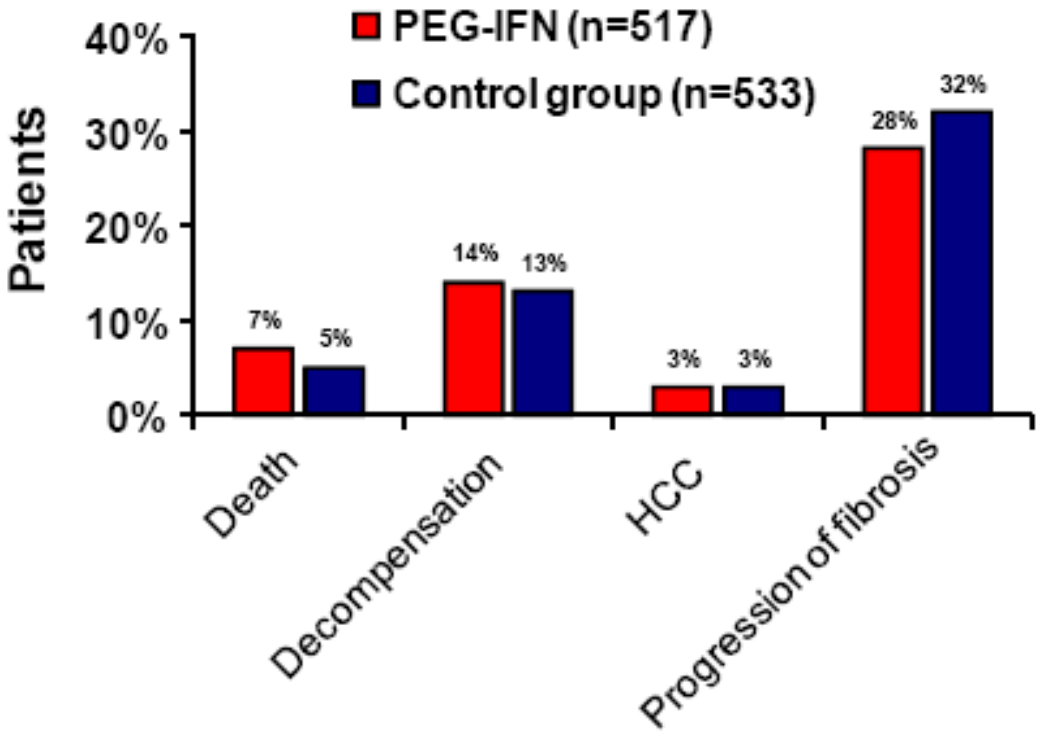



**Figure 1: Effect of interferon treatment in HCV-infected subjects who have been previously non-responsive to interferon treatment**

Interferon alfa may either be IFNα-2a (brand name: Pegasys) or IFNα-2b (brand name: Peg Intron). Both are prescribed in pegylated forms. Pegylation increases the biological half-life of

interferon by protecting it from proteolytic breakdown. The two PEG-IFNs generate similar SVRs, but have slightly different pharmacokinetic profiles. Pegasys is prescribed in fixed doses of 180 µg once weekly. Peg Intron should be taken once weekly according to body weight, 1.5 µg/kg. The efficacy of PEG-IFN as determined by multiple separate studies can be seen in Table 1.

| Study | n | Treatment | Start of therapy | Duration | Efficacy |
|---|---|---|---|---|---|
| (Jaeckel 2001) | 44 re-cruited in 24 centers | IFN α-2b (4 weeks 5 MU daily, 20 weeks 5 MU tiw | 89 days after infec-tion (range 30-112 days) | 24 weeks | 43/44 (98%) |
| (Santanto nio 2005) | 28 | PEG-IFN α-2b (1.5 µg/kg/week) | 12 weeks after onset of disease (17/28 chronic, 16 treated) | 24 weeks | 15/16 (94%) |
| (Broers 2005) | 27 *(22 IVDU)* | PEG-IFN α-2b (1.5 µg/kg/week) | 100±82 days after onset of symptoms, 63±82 days after diagnosis (asymp-tomatic) (22/27 chronic, 14 were treated) | 24 weeks | 8/14 (57%) 7/8 (88%) of adherent pts. |
| (Wiegand 2006) | 89 re-cruited in 53 centers | PEG-IFN α-2b (1.5 µg/kg/week) | 76 days after infec-tion (range 14-150 days), 27 days after onset of symptoms (range 5-131) | 24 weeks | 63/89 (71% 58/65 (89%) of adherent pts. |

**Table 1: Efficacy (based on SVR) of PEG-IFN treatment**

The second most prescribed drug for HCV treatment is ribavirin (brand names: Rebetol or Copegus). Ribavirin's morphology is similar to that of an RNA nucleotide. Ribavirin interferes with RNA metabolism thus preventing viral replication. The exact mechanism by which it does this is unknown. Rebetol is prescribed based on body weight, 11 mg/kg. Higher doses are known to produce better sustained virological responses, but the drug commonly causes severe hemolytic anemia at high doses. Ribavirin is often given in combination with interferon.

The effectiveness of the two main treatments for HCV, interferon and ribavirin, for different genotypes and different durations is shown by Figure 3.

| Study | Treatment | HCV genotype | Duration | SVR |
|---|---|---|---|---|
| (Manns 2001) | 1.5µg/kg PEG-IFN α-2b 800 mg ribavirin | HCV-1 | 48 weeks | 42% |
| | | HCV-2/3 | 48 weeks | 82% |
| | 1.5µg/kg PEG-IFN α-2b >10.6 mg/kg ribavirin | HCV-1 | 48 weeks | 48% (retrospective) |
| | | HCV-2/3 | 48 weeks | 88% (retrospective) |
| (Fried 2002) | 180µg PEG-IFN α-2a 1000/1200 mg ribavirin | HCV-1 | 48 weeks | 46% |
| | | HCV-2/3 | 48 weeks | 76% |
| (Hadziyannis 2004) | 180µg PEG-IFN α-2a 800 mg ribavirin | HCV-1 | 24 weeks | 29% |
| | | | 48 weeks | 40% |
| | | HCV-2/3 | 24 weeks | 78% |
| | | | 48 weeks | 73% |
| | 180µg PEG-IFN α-2a 1000/1200 mg ribavirin | HCV-1 | 24 weeks | 41% |
| | | | 48 weeks | 51% |
| | | HCV-2/3 | 24 weeks | 78% |
| | | | 48 weeks | 77% |
| (Zeuzem 2004) | 1.5µg/kg PEG-IFN α-2b 800-1400 mg ribavirin | HCV-2 | 24 weeks | 93% |
| | | HCV-3 | | 79% |
| (Kamal 2005) | 1.5µg/kg PEG-IFN α-2b 1000/1200 mg ribavirin | HCV-4 | 24 weeks | 29% |
| | | | 36 weeks | 66% |
| | | | 48 weeks | 69% |

**Table 2: Sustained virological responses (SVR) based on drug dosage, treatment duration, and genotype**

*New Agents*

Interferon and ribavirin are very useful in diminishing HCV activity, but their functions are broad and not limited to simply attacking virions. It is partly this lack of specificity that results in the current treatments' extensive and severe side effects. These side effects can be so severe that treatment must be terminated before the intended duration is complete. Therefore, researchers are attempting to discover molecules that inhibit the essential activities of specific viral proteins.

There are several viral proteins and a few host proteins that are promising targets for HCV treatment. Several drugs have been developed for their capability of inhibiting viral activity. These drugs and their individual stages of development are given in Table 3.

| Drug name | Company | Study Phase |
|---|---|---|
| **NS3/4A protease inhibitors** | | |
| Ciluprevir (BILN 2061) | Boehringer Ingelheim | Stopped |
| Telaprevir (VX-950) | Vertex | Phase III |
| Boceprevir (SCH503034) | Schering-Plough | Phase III |
| TMC435350 | Tibotec & Medivir | Phase II |
| ITMN-191 (R7227) | InterMune, Roche | Phase II |
| VX-500 | Vertex | Phase II |
| MK-7009 | Merck | Phase II |
| BILN 12202 | Boehringer Ingelheim | Phase II |
| **Nucleoside analoge NS5B polymerase inhibitors** | | |
| Valopicitabine (NM283) | Idenix/Novartis | Stopped |
| R7128 (prodrug) / PSI-6130 | Roche /Pharmaset | Phase I |
| MK-0608 | Merck | Phase I |
| R1626 (prodrug) / R1479 | Roche | Stopped |
| **Non-nucleoside NS5B polymerase inhibitors** | | |
| HCV-796 | ViroPharma/Wyeth | Phase II |
| BILB 1941 | Boehringer Ingelheim | Stopped |
| PF-868554 | Pfizer | Phase I |
| GS 9190 | Gilead | Phase I |
| GSK625433 | GlaxoSmithKline | Phase I |
| VCH-759 | ViroChem Pharma | Phase I |
| **NS5A inhibitors** | | |
| BMI-790052 | BMI | Phase I |
| **Host protein inhibitors** | | |
| Celgosivir (α-glucosidase inhibitor) | Migenix | Phase II |
| Debio-025 (cyclophilin B inhibitor) | Debiopharm | Phase II |
| Nitazoxanide (unknown mechanism) | Romark Laboratories | Phase II |

**Table 3: Drugs currently being developed to inhibit HCV infectivity**

As described earlier, HCV RNA is hypervariable due to the mechanism and frequency of viral replication so mutations in viral proteins develop easily. When administered in monotherapy, HCV inhibitors tend to cause mutations in the active sites that they are supposed to be binding to. Therefore, if these drugs gain FDA approval, they will most likely be prescribed in combination with interferon. Nucleoside analog inhibitors are an exception. Because NS5B is responsible for polymerase activity, it has a particularly highly conserved active site. Treatments with nucleoside inhibitors, which bind to this highly conserved site, are not likely to result in a viable mutant.

Despite multiple options available, one of the most promising targets for drug treatment is nonstructural protein 3, so its function and its inhibitors will be discussed in detail.

### *NS3 Protease*

NS3's protease and helicase activity make it essential for viral infectivity. NS3 catalyzes binding and unwinding of the viral RNA during replication with its C-terminal portion, but because the mechanism by which it does this it still poorly understood, the C-terminal portion is not currently a good target for drug treatment.

NS3 protease (NS3P) activity is better understood than C-terminal activity. NS3P has two β-barrels forming a long, shallow groove, which is also the active site of NS3/4A protease. Histidine-57 and aspartate-81 located in the N-terminal β-barrel and serine-139 in the C-terminal β-barrel form a catalytic triad (Penin, 2004). The method by which the catalytic triad breaks peptide bonds in the viral polyprotein is as follows:

1. After binding to the polyprotein, aspartate's carboxylic group forms a hydrogen bond with histidine, which increases the pKa of its imidazole nitrogen. At low pH values, the imidazole ring has two NH bonds and a positive charge that is shared between the two nitrogen atoms, but when pH increases, protonation is lost. This leaves one basic nitrogen, which is then able to deprotonate serine.
2. The now nucleophilic serine attacks the carbonyl carbon at its own C-terminal side, which forces the carbonyl oxygen to accept an electron. This creates a tetrahedral intermediate.
3. In order for the intermediate to return to carbonyl, the α-carbon must accept a proton from histidine. Nitrogen and its attached peptide fragment are then able to diffuse away.
4. Another tetrahedral intermediate is formed when water donates a proton to histidine causing the hydroxyl group to attack the carbonyl carbon. When the intermediate returns to its original form, histidine returns a proton to serine.

5. The peptide thus gets a carboxyl group during cleavage. After process is done, the protein diffuses away.

This mechanism is shown in Figure 2 (Dodson, 1998).

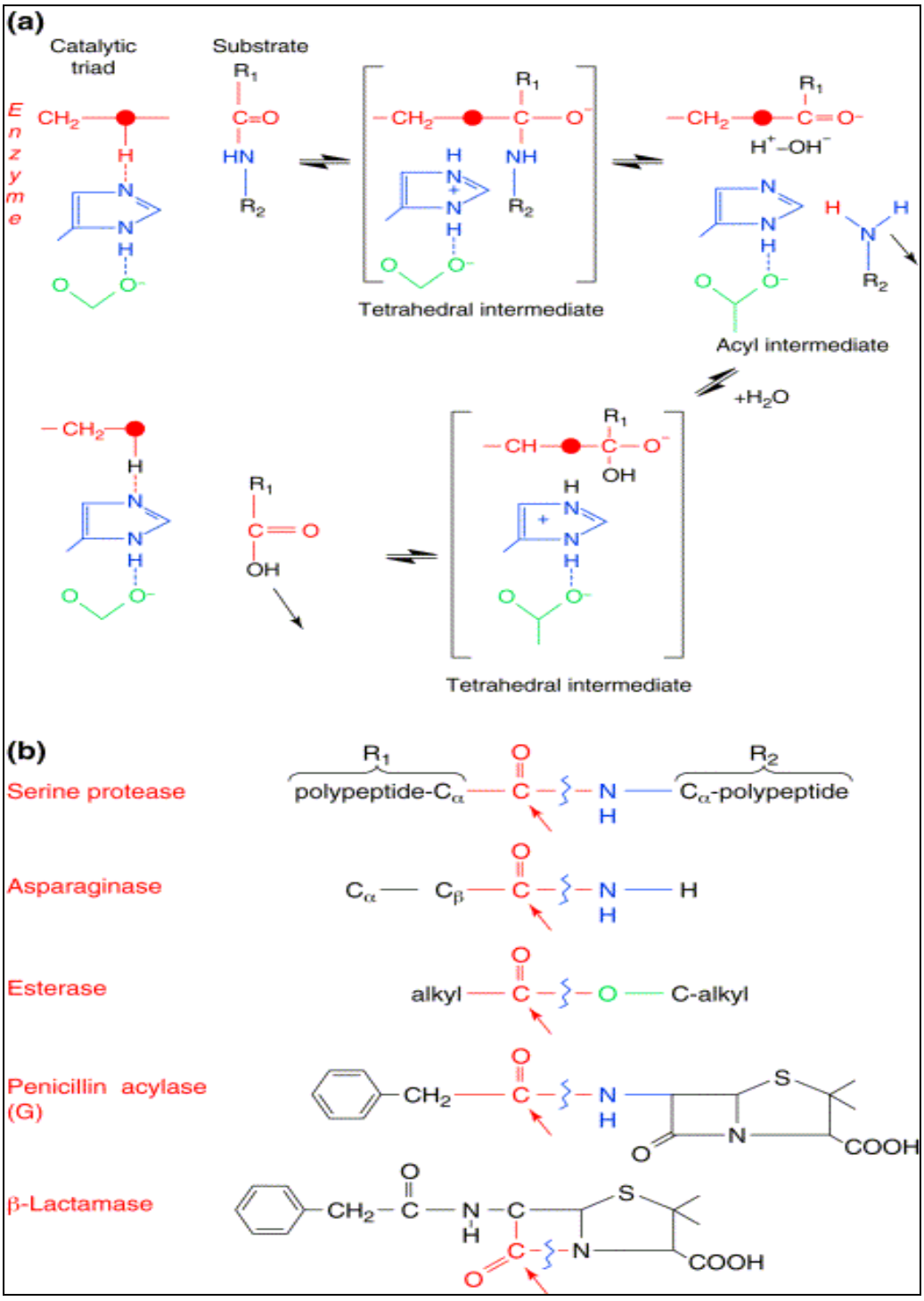



**Figure 2: Catalytic triad mechanism by which the peptide bond is cleaved. The NS3 protease is a serine protease.**

In this fashion, NS3P cleaves the viral polyprotein at Thr/Ser at the NS3/4A junction, Cys/Ala at the NS4A/4B junction, and Cys/Ser at both the NS4B/5A and NS5A/5B junctions. For correct protease activity, NS3 needs to bind with cofactor NS4A. When bound to NS4A, aspartate-81 and histidine-57 form a hydrogen bond, which causes a slight rearrangement of the catalytic triad. The new arrangement allows for better aligning of the active site residues (Lin, 1995).

### *Serine Protease Inhibitors*

Serine protease inhibitors, also called serpins, are groups of proteins, which are able to inhibit proteases. Many serpins occur naturally; over 1000 natural, serpins have been identified. There are 36 known human serpins; research to determine which serine proteases they inhibit is ongoing. It is known that all serpins undergo a unique conformational change when they inhibit target proteases.

When a serpin binds to the catalytic triad of NS3 protease, the triad is blocked and thus unable to cleave the viral polyprotein. Without the viral proteins which only become functional after separation, the viral RNA cannot be replicated and infectious virions cannot be assembled. By modifying the structure of known serpins to specifically bind to the amino acids of and near the catalytic triad, an effective new HCV protease inhibitor could be developed.

### *NS3P Inhibitors (Boceprevir)*

Several NS3P inhibitors are being researched. The structures of some of these molecules are given in Figure 3. Telaprevir and boceprevir have come furthest in development. The effectiveness of telaprevir and boceprevir is illustrated in Figure 4 and Figure 5 respectively. In these studies, the drugs were administered in combination with interferon and/or ribavirin.

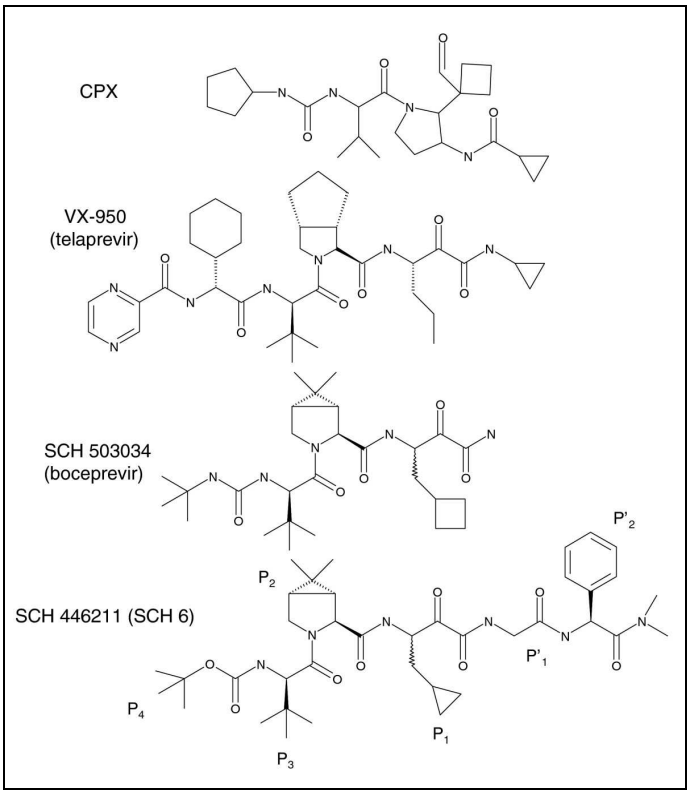



**Figure 3: Molecules intended to inhibit HCV NS3 protease activity.**

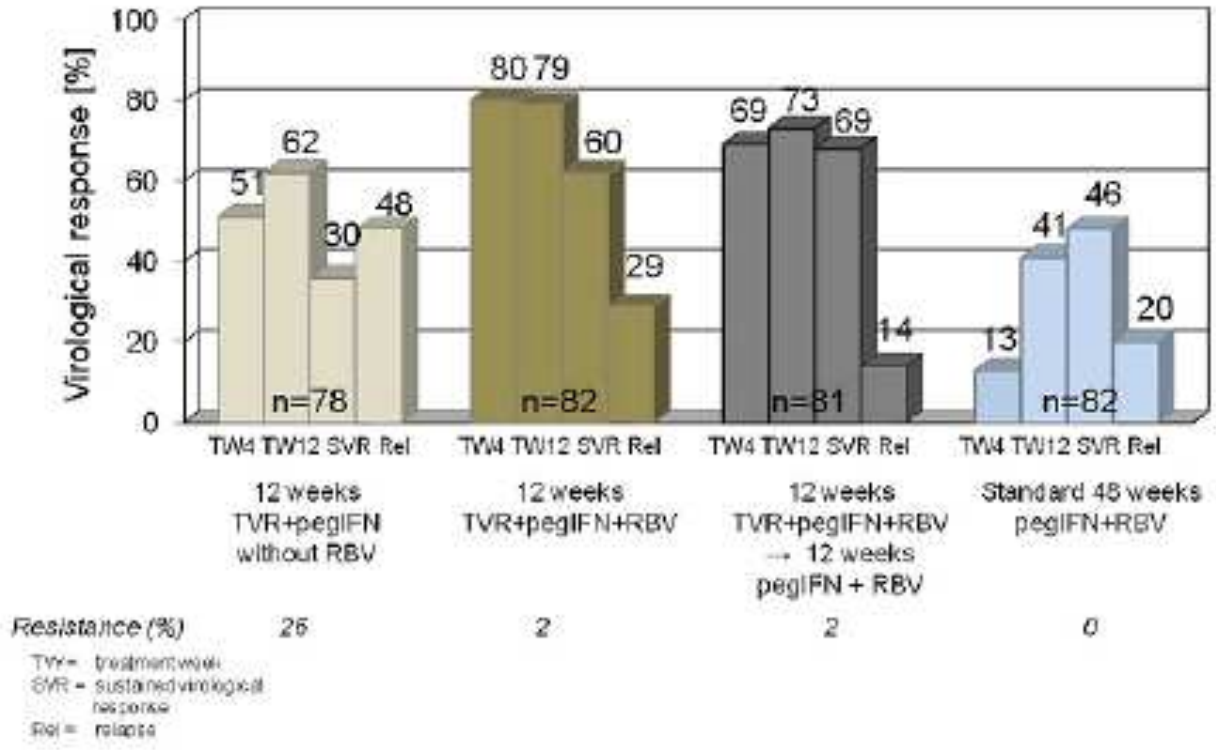



**Figure 4: Virological response after treatment with telaprevir**

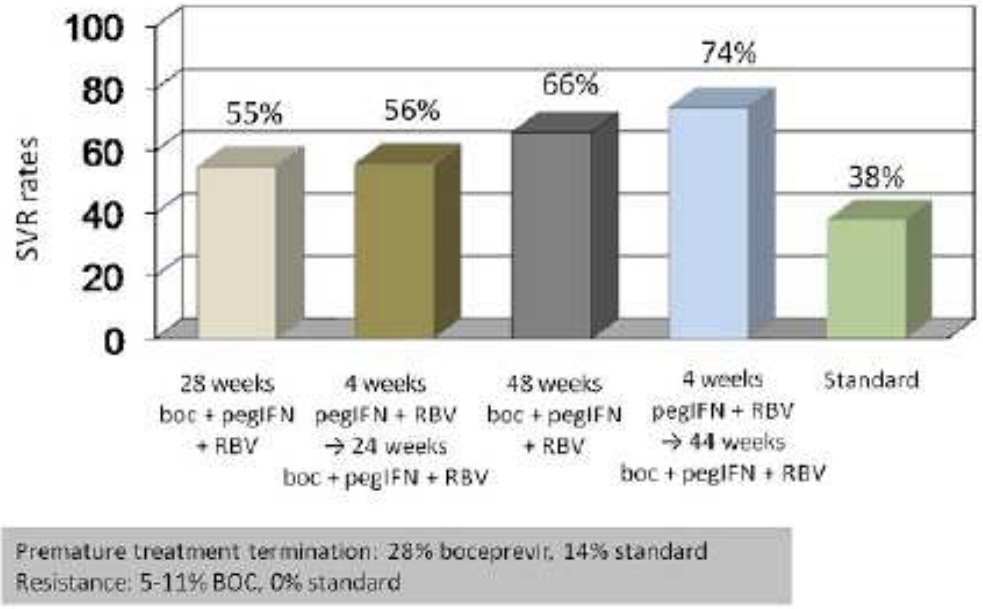


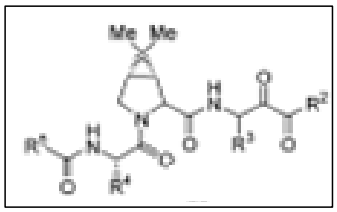

**Figure 5: Virological response after treatment with boceprevir**

Boceprevir is usually given in daily doses of 200 to 400 mg. Side effects associated with boceprevir treatments have been few and mild, but viral breakthrough may occur (Mauss, 2009).

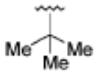
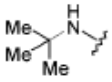
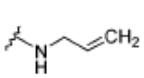
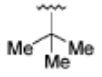
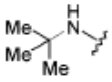
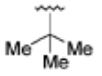
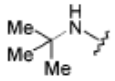
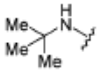
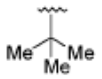
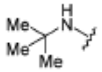
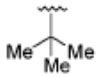
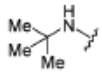
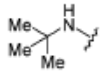
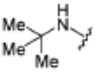
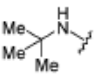
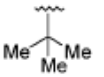
## New Protease Inhibitor Development

### *Proposed Molecules*

A base molecule with variable functional groups has already been proven to be an effective serine protease inhibitor. Functional groups can be substituted into this molecule to give different total binding affinities to NS3P. Drug candidates were made by modifying the functional groups on existing drugs to obtain a drug that has strong binding affinity and a higher HNE/HCV, the selection of functional groups was possible due to data obtained from Schering Plough. These sixteen proposed drug candidates are shown in Figure 6.



Functional Groups

| | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
| 6 |  |  |  |  |
| 7 |  |  |  |  |
| 8 |  |  |  |  |
| 9 |  |  |  |  |
| 10 |  |  |  |  |
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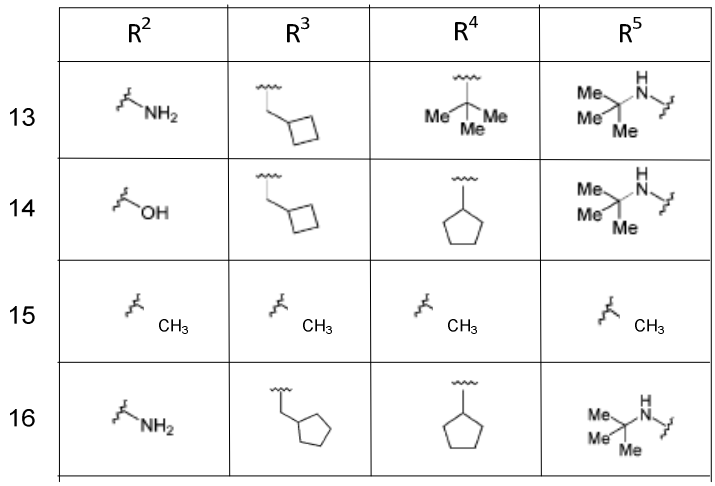


**Figure 6: Proposed Drug Candidates**

*Test Methods*

Structures of the NS3/NS4a complex and of human neutrophil elastase were downloaded as pdb files from the RCSB protein data bank. These proteins were uploaded and prepared using DockingServer, a molecular modeling internet service developed by Virtua Drug Ltd. DockingServer is capable of calculating the site, geometry and energy of molecules interacting with proteins. Ligands, the sixteen proposed drug candidates, were drawn and prepared in DockingServer. The boceprevir molecule as well as the sixteen proposed drug candidates were then each docked to the catalytic triad of the NS3 protease. All ligands were also docked to the human neutrophil elastase protein.

Genetic algorithms can be used to predict the bound conformations of flexible ligands to protein targets. The total interaction energy (*fitness*) of the ligand with the protein is calculated for a given translation, orientation, and conformation (*genotype*). Changing these state variables changes the atomic coordinates (phenotype) of the ligand. Individuals that have low fitness die. Individuals that have high fitness survive and mate. New individuals with different phenotypes are created by inheriting genes from either of their two parents. Mutations also occur randomly which can help get fitness away from local maxima. In this way, DockingServer can predict where and how a ligand will bind to be most energetically favorable. DockingServer uses a Lamarckian genetic algorithm which is even more efficient because it can use desirable phenotypes to produce desirable genotypes (Morris, 1998).

*Determination of Drug Quality*

Boceprevir is considered a promising new drug. Therefore, we may compare the properties of our proposed molecules to those of boceprevir to determine their relative expected effectiveness.

Boceprevir binds strongly to NS3/4A and is thus a potent inhibitor. The hydrogen bond interactions between boceprevir and NS3P are shown in Figure 7 (Venkatraman, 2006). Inhibition constants can be used to compare binding affinity. The inhibition constant of boceprevir with the NS3 protease was calculated by DockingServer modeling software. This inhibition constant was higher than the value found in literature, but the DockingServer value was used instead of a literature value in order to fairly compare all of the calculated binding affinities. The inhibition constant, $K_i$, calculated for boceprevir with NS3P was 560 μM. Lower $K_I$ values correspond to better binding, so any proposed drug candidate with a $K_i$<560 μM can be assumed to be an effective inhibitor of the NS3 protease. $K_i$ is the required concentration of a ligand for a specific binding site so that half of those available sites are occupied.

Boceprevir is also selective against human neutrophil elastase (HNE). HNE is an important serine protease enzyme with broad substrate specificity. Our molecules should exhibit a preference to bind to NS3 over this host enzyme. Selectivity against HNE is calculated as the $K_i$ of HNE over the $K_i$ of HCV; therefore, higher values are preferable.

Pharmacokinetic properties should also be evaluated before deciding that a drug is worthy of production, but these properties must be studied through laboratory research. These properties include bioavailability and AUC. Bioavailability is the fraction of a drug dose that reaches the systemic circulation. This was found to be between 4 and 11% in monkeys for boceprevir. AUC is the area under the concentration time curve which requires information about the changing concentration of the drug in the system for the entire duration that it stays in the system. The AUC found for boceprevir is 0.12 μMh.

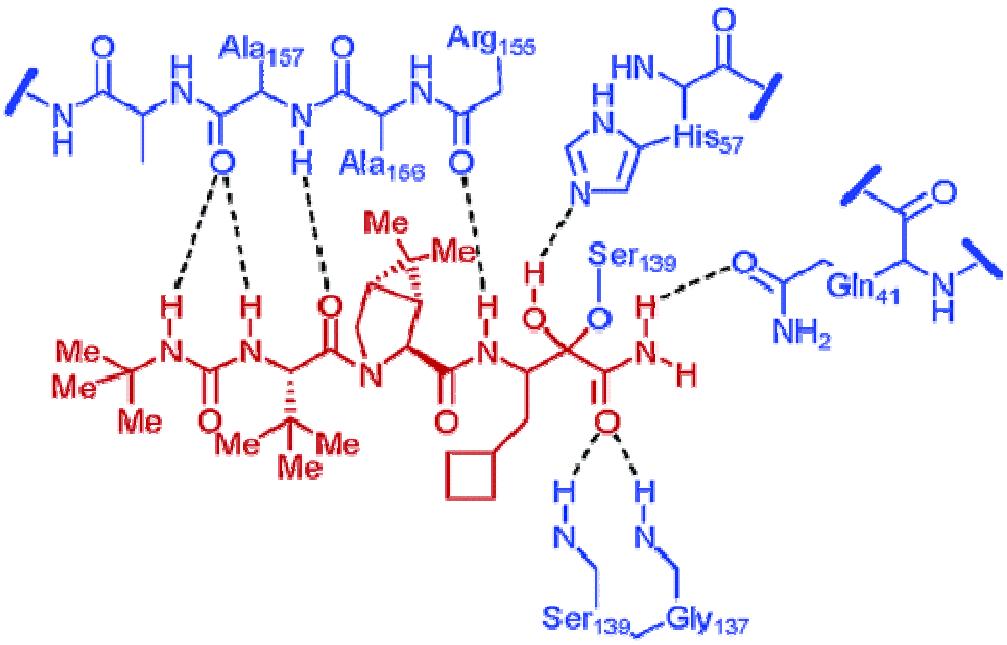


**Figure 7: Hydrogen bond interactions between NS3P and boceprevir**

## Results

All sixteen drug candidates were capable of binding to at least two of the three amino acids constituting the catalytic triad. Therefore, all could to some degree inhibit the binding and cleavage of the viral polyprotein with the NS3 protease. Only one, however, showed both better binding and better HNE selectivity than boceprevir. Other potential candidates did have better binding than boceprevir, but they did not have better binding and better HNE/HCV selectivity than boceprevir. Candidate #9 showed a $K_i$ of 38 µM and 801 µM when it was bound to NS3P and HNE, respectively. The actual nomenclature of this molecule is (3S)-3-{[(1R,2S,5S)-3-[(2R)-2-[(tert-butylcarbamoyl)amino]-2-cyclohexylacetyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2-yl]formamido}-4-cyclobutyl-2-oxobutanamide.

## Recommendations

Due to differences between DockingServer values and literature values for the binding of boceprevir to NS3 protease, more research should be done to verify the accuracy of all DockingServer results. Upon this verification, we recommend that (3S)-3-{[(1R,2S,5S)-3-[(2R)-2-[(tert-butylcarbamoyl)amino]-2-cyclohexylacetyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2-yl]formamido}-4-cyclobutyl-2-oxobutanamide, be synthesized and evaluated in a laboratory for pharmacokinetic properties. If its bioavailability and AUC are comparable or higher than those for boceprevir, then it

should be considered for clinic trials in the treatment of HCV. Proposed molecules 5, 7, 8, 10, and 15 also had a higher binding affinity but because they had a lower selectivity it is recommended that molecule 9 be further evaluated and tested first. After that time proposed molecules 5, 7, 8, 10, and 15 could be evaluated and further studies could be taken on to improve their selectivity without affecting their binding affinity.

## Areas for future work

This study only covered the NS3/NS4A complex; there are a variety of other potential viral proteins that could be studied in the future. These include NS4B, NS5B, NS3 helicase, and NS5A. Adsorption into the hepatocytes could also be targeted. A similar method could be used to determine potential drug candidates which inhibit the function of any of these viral proteins. NS3/NS4A was the focus of our report because its function and active site are known. When studying NS3/NS4A it could also be possible to use a variety of other base molecules and alter the functional groups to generate more potential drug candidates. The area of HCV research is gaining popularity after many years without new drug development. The areas listed above could all be areas of further research.

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