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Potent inhibitors of HCV-NS3 protease derived from boronic acids

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ABSTRACT

Chronic hepatitis C infection is the leading causes for cirrhosis of the liver and hepatocellular carcinoma, leading to liver failure and liver transplantation. The etiological agent, HCV virus produces a single positive strand of RNA that is processed with the help of serine protease NS3 to produce mature virus. Inhibition of NS3 protease can be potentially used to develop effective drugs for HCV infections. Numerous efforts are now underway to develop potent inhibitors of HCV protease that contain ketoamides as serine traps. Herein we report the synthesis of a series of potent inhibitors that contain a boronic acid as a serine trap. The activity of these compounds were optimized to 200 pM. X-ray structure of compound **17** bound to NS3 protease is also discussed.

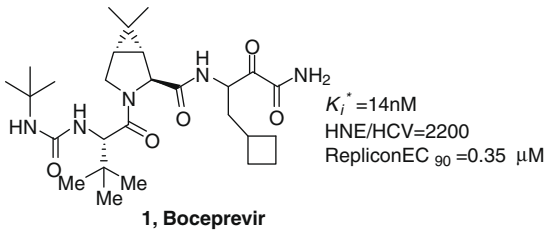
Hepatitis C virus is the primary etiological agent responsible for chronic HCV infections of liver leading to liver cirrhosis and hepatocellular carcinoma.[1] Nearly 200 million people are infected worldwide and up to 80% of them turn chronic infections. Current therapeutic regimens include α-interferon and its combinations with Ribavirin®, which is effective only in 25–40% of patients for sustained response. The newly introduced pegylated interferon has reduced the frequency of injection and has a sustained response in lowering viral titers and improved histological growth.[2] The lack of efficient methods for the treatment of chronic HCV infections requires the identification of new chemical entities.

Hepatitis C virus is a *Flavivirdae* family virus that encodes a polyprotein of ~3000 amino acids from a positive strand RNA genome. This polyprotein is post translationally spliced to produce various proteins essential for viral replication. Autocatalytic cleavage at the NS2–NS3 junction followed by cleavage of NS3–NS4A, NS4A–NS4B, NS4B–NS5A and NS5A–NS5B by the NS3 protease produces functionally mature virions. The inhibition of NS3 protease would possibly impair the ability of the enzyme to process the polyprotein, thus arresting maturation of virus and inhibiting viral production.[3]

HCV NS3 protease is a serine protease which enables the lesion of a Cys-Ser bond with the assistance of the cofactor NS4A. The hydrolysis of the amide bond proceeds with the attack of Ser-139 followed by the stabilization of the tetrahedral transition state by His-57 and Asp-81. We and others have developed various novel inhibitors containing a α-ketoamide moiety as an electrophilic trap that trap Ser-139 reversibly. ***Sch 503034*, Boceprevir** (**1**),[4] is one of the compounds that is currently undergoing Phase III clinical trials and shown to be efficacious in humans (Fig. 1).

As a possible back-up to Sch 503034 (**1**), we explored inhibitors that contained alternative electrophiles to ketoamides. Boronic acids have been extensively investigated as possible electrophiles that could react with nucleophilic serine in the context of developing inhibitors of thrombin receptor and other serine proteases including HCV NS3 protease.[5] Valcade®, a boronic acid derived dipeptide is an inhibitor of 26S proteosome that has been successfully developed into a drug for treatment of multiple myeloma.[6] We therefore decided to investigate the replacement of ketoamide group in **1** with a boronic acid derivative. From our previous SAR exploration of inhibitors spanning from P_6–P_1, it was determined that an ethyl group at P_1 was well tolerated. We therefore decided to explore compound **2** as our first boronic acid target (Fig. 2).

Intermediates for the synthesis of the inhibitors of type **2** were accomplished using methods outlined in Scheme 1. Deprotonation of dichloromethane with BuLi at −100 °C, followed by treatment with trimethylborate resulted in compound **4**. Methyl ester **4** was converted to the pinene diol ester **5** by transesterification with (+)-pinenediol. Treatment of **5** with ethyl or cyclobutyl Grignard



1, Boceprevir

Figure 1.

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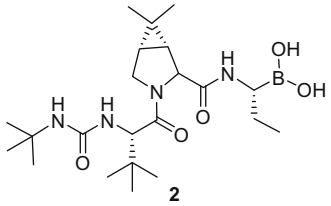
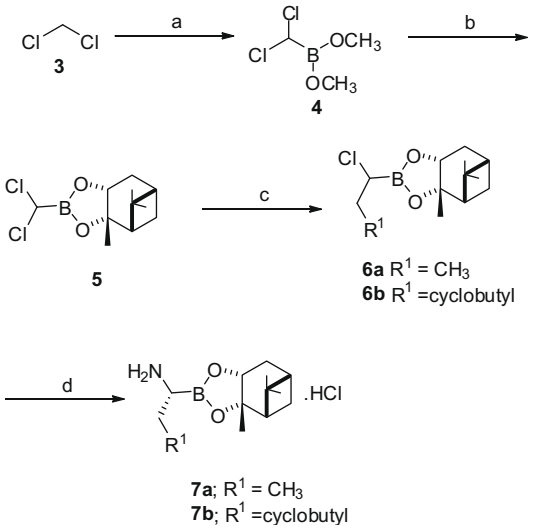


Figure 2.

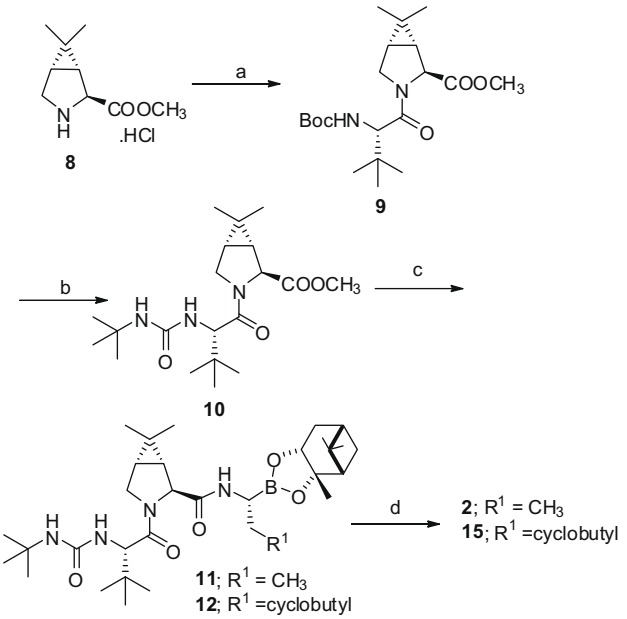


Scheme 1. Reagents and conditions: (a) BuLi, −100 °C → rt. $(CH_3O)_3B$. (b) (+)-Pinenediol, rt., THF (c) R^1MgCl, THF, −78 °C→ rt., 12 h (d) i—LiHMDS, THF, rt. ii—HCl in ether.

reagent resulted in compounds of type **6** in excellent yields. Further displacement of the chloride of **6** with LiHMDS followed by treatment with HCl yielded amine salts of type **7**.

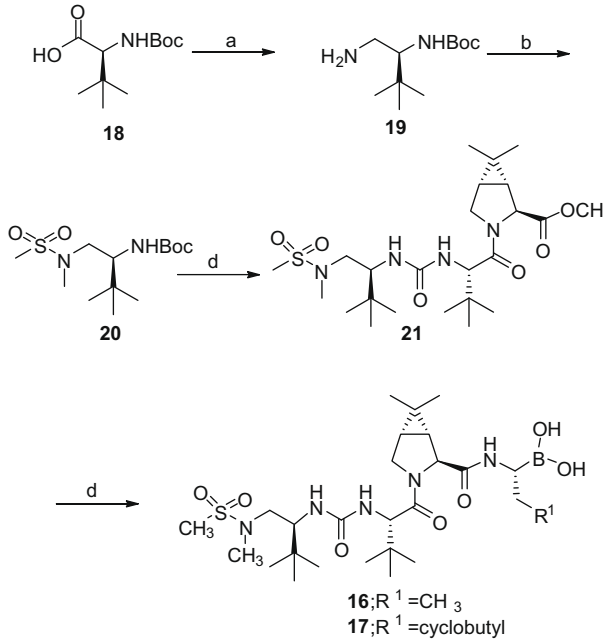
Completion of synthesis of inhibitor **2** was accomplished using reactions outlined in Scheme 2. Thus, coupling of Boc protected *tert*-leucine with 3,4-dimethylcyclopropyl fused proline **8**[7] using HATU and NMM resulted in dipeptide **9** in 60% yield. The Boc group of the dipeptide **9** was deprotected using 4 M HCl in dioxane and further treatment of the resultant amine salt with *tert*-butyl isocyanate yielded *tert*-butyl urea **10**. Hydrolysis of methyl ester of compound **10** with aq LiOH, followed by coupling of the resulting acid with the amine salt **7a** using isobutyl chloroformate and Et_3N yielded inhibitor precursor **11**, which was converted to **2** by reaction with $NaIO_4$.

Compound **2** was tested for its inhibitory activity in a HCV protease continuous assay. Its ability to prevent the NS3 protease catalyzed hydrolysis of chromogenic 4-phenylazophenyl [PAP] ester from peptide fragment Ac-DTEDVVP(Nva)-O-4-PAP was measured using a spectrophotometric assay.[8] The binding constant for the inhibitor **2** was determined as K_i = 380 nM. We also evaluated the pinene ester **11** for its ability to inhibit HCV NS3 protease and determined its binding constant K_i = 500 nM. Since these compounds were less potent than the corresponding first generation compound **1**, we decided to synthesize the corresponding cyclobutylmethyl containing amino boronic acid **7b** and incorporate it into the inhibitor. The synthesis of cyclobutyl derived amino acid **7b** was accomplished in a similar manner to that of **7a** except that the cyclobutylmethyl was introduced using the appropriate Grignard reagent. The synthesis of subsequent inhibitors was also similar to that of **2** and is as shown in Scheme 2. The introduction of a lipophilic cyclobutyl group at P_1 enhanced binding of both the



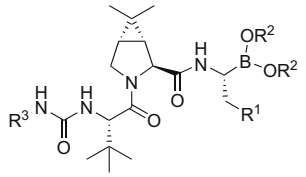
Scheme 2. Reagents and conditions: (a) Boc-*tert*-Leu, HATU, NMM, 0 °C → rt. (b) i—4 M HCl in dioxane, rt. 1 h. ii—*tert*-C_4H_9NCO, NMM, CH_2Cl_2, 0 °C → rt. 12 h. (c) i—aq LiOH, THF, rt, 2 h. ii—IBCF, $(C_2H_5)_3N$, −15 °C, **7a**, −15 °C → rt., 3 h. (d) $NaIO_4$, acetone/0.1 M soln. NH_4OAc, rt, 48 h.

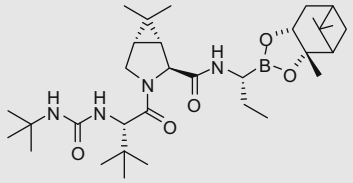
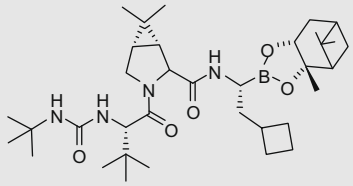
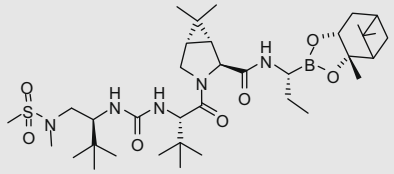
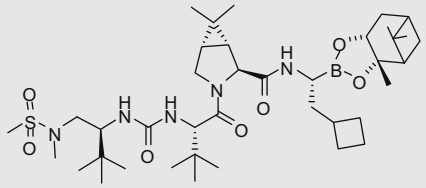
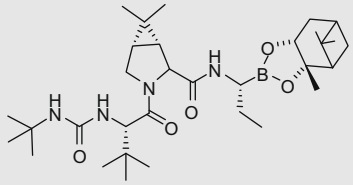
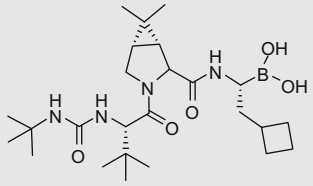
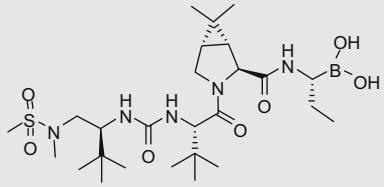
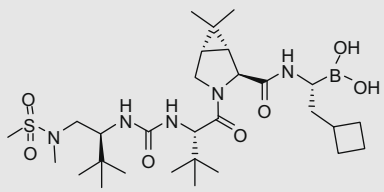
boronate ester compound **12** (K_i = 10 nM) as well as the boronic acid derivative **15** (K_i = 10 nM), a 50-fold improvement in binding over **2**. Our studies in the ketoamide series of inhibitors had previously demonstrated that the replacement of the P_3 *tert*-butyl urea cap with various modified urea moieties enhanced binding of ketoamide inhibitors. To evaluate the effect of this novel capping group we decided to synthesize the sulfonamide derived compound **16**. The synthesis of inhibitor **16** is outlined in Scheme 3. Table 1 summarizes the activity of these compounds.



Scheme 3. Reagents and conditions: (a) i—NH_4Cl, EDCI, DMF, NMM, rt, ii—BH_3·DMS, THF, reflux, 2 h. (b) i—CH_3SO_2Cl, pyridine, −78 °C → rt, 12 h. ii—Cs_2CO_3, DMF, CH_3I (c) i—4 M HCl, dioxane, ii—amine salt[9], CH_3CN, $(C_2H_5)_3N$, rt. (d) i—aq LiOH, THF, rt, 2 h. ii—IBCF, $(C_2H_5)_3N$, −15 °C, **7**, −15 °C→rt, 3 h. iii—$NaIO_4$, acetone/0.1 M soln. NH_4OAc, rt, 48 h.

Table 1



Entry	Structure	K_i (nM)
11		500
12		10
13		34
14		0.5
2		380
15		10
16		52
17		0.2

The coupling of Boc protected *tert*-leucine with ammonium chloride using EDCI resulted in Boc protected *tert*-leucine amide, which was further reduced to amine **19** using $BH_3{\cdot}SMe_2$. The amine **19** was reacted with methanesulfonyl chloride and the resulting sulfonamide was methylated with methyl iodide and Cs_2CO_3 to yield N-methylated sulfonamide **20**. Deprotection of Boc group of **20** followed by treatment of formed amine salt with nitrophenyl carbamate of intermediate **9** yielded compounds **21**. The methyl ester of intermediate **21** was hydrolyzed with aq lithium hydroxide and the intermediate acid was coupled with amine salt of type **7** using mixed anhydride method with IBCF and Et_3N. The formed pinene diol ester was once again hydrolyzed to the corresponding boronic acid using $NaIO_4$ to yield compounds of type **16**.

The replacement of the P_3 *tert*-butyl urea cap with sulfonamide derived cap **20** had a profound effect on binding. Compound **13** obtained by the replacement of *tert*-butyl urea P_3 cap of compound **11** with sulfonamide demonstrated a $K_i = 34$ nM, a fifteen folds improvement in potency. Similarly replacement of P_3 *tert*-butyl cap in the P_1 cyclobutylmethyl derived compound **12** resulted in inhibitor **14** ($K_i = 500$ pM); a twenty fold improvement in activity over **12**. This was the first time we had identified a compound that bound to NS3 protease in the picomolar range. We next evaluated the boronic acid analogs of compounds **13** and **14**. The corresponding boronic acid analogs of compound **13**, compound **16** demonstrated a $K_i = 52$ nM; whereas the boronic acid derivative of compound **14**, compound **17** had a $K_i = 200$ pM.

Comparison of binding activities of the pinene diol esters **12–14** to their corresponding boronic acid inhibitors **15–17** suggested that the boronate esters were readily hydrolyzed in the assay conditions and the activity of the boronate esters probably arose from the corresponding boronic acid derivatives.

The X-ray structure of inhibitor **17** bound to HCV NS3 protease was solved and is shown in Figure 3.[10] From the structure of **17** bound to protease, it was clear that the cyclobutyl group at P_1 occupy the S_1 pocket. The P_2 (1R,5S)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane ring adopted a bent conformation that allowed maximum overlap of the methylenes of proline and cyclopropyl ring to Ala-156. The conformation adopted by cyclopropyl group allowed the methyl group proximal to the carbonyl to interact with His-57 and the methyl group distal to carbonyl to interact with Ala-156 and Arg-155. The *tert*-butyl group of P_3 sulfonamide capping occupied the S_4 region of space making excellent van der Waals contact with the protein and the oxygens of the sulfonamide group formed a hydrogen bond with Cys-159. The oxygen of Ser-139 is bound to the lewis acidic boron to form a tetrahedral boronate derivative.

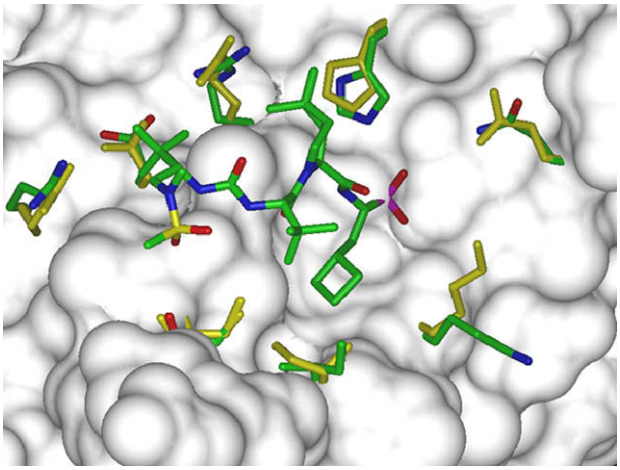


Figure 3. X-ray structure of inhibitor **17** bound to NS3 protease.

In addition to van der Waals contacts, inhibitor **17** formed a series of specific hydrogen bonds with the protein surface. Mapping out the various hydrogen bonding interactions that existed between inhibitor **17** and NS3 protease it is evident that the urea nitrogens donated two hydrogen bonds to alanine-157 thus improving potency and HCV specificity. Additionally, the nitrogen of the P_1 residue donated a hydrogen bond to Arg-155 and oxygen of P_3 carbonyl group accepted a hydrogen bond from the Ala-157.

We next evaluated the activity of these potent inhibitors in the replicon based cellular assay. Evaluation of these compounds demonstrated an $EC_{90} > 5.0$ μM suggesting that cell permeability may be limiting and be responsible for poor cellular activity.

In search of a possible second generation compound to Sch 503034 (**1**) we evaluated the possibility of replacements of the ketoamide with a boronic acid electrophile. The replacement of this group with boronic acid resulted in inhibitor **12** which was equipotent to our first generation compound **1**. We next evaluated the replacement of P_3 capping with modified P_3 cap derived such as *N*-(2-amino-3,3-dimethylbutyl)-*N*-methylmethanesulfonamide which resulted in compound **17** that had markedly improved activity (K_i = 200 pM). This was the first time we identified compounds in the picomolar range. Analysis of X-ray structure of **17** bound to the protease revealed binding of the boronic acid to Ser-139, where the boronic acid forms a tetrahedral borate. Evaluation of these inhibitors in the replicon based cellular assay showed $EC_{90} > 5$ μM suggesting that the cell penetration was probably an issue. Further studies are on way to improve cellular activity of these inhibitors.

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9. Compound **9** was treated with 4 M HCl to deprotect the Boc group and the resultant amine salt was treated with 4-nitrophenyl carbonate to form the 4-nitrophenyl carbamate derivative which was used for coupling.
10. X-ray coordinates of compound **17** bound to HCV protease has been deposited in the PDB database with the RCSB ID code rcsb049921 and PDB ID code 3EYD.