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Design and Synthesis of Potent, Non-peptide Inhibitors of HCV NS3 Protease

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Abstract—Starting from a hexapeptide boronic acid lead, 3-amino bicyclic pyrazinones as novel β-sheet dipeptide mimetics have been designed and synthesized. Side-chain manipulation of this scaffold generated a series of potent, nonpeptidic inhibitors of HCV NS3 protease.

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease leading to cirrhosis, hepatocellular carcinoma or liver failure.[1] Current therapies involve treatment with interferon-α, either alone or in combination with ribavirin; however, poor efficacy and severe side effects are frequent.[2] There is thus an urgent need to discover inhibitors of HCV-specific proteins for the development of anti-HCV drugs. One of the most intensively studied and best understood targets is the NS3 serine protease.[2] Recent work has demonstrated that NS3 protease activity is required for HCV replication in chimpanzee.[3] A low-molecular-weight inhibitor of NS3 protease thus would be therapeutically useful against HCV replication and infection.

Many efforts have been made to identify potent inhibitors of HCV NS3.[4] X-ray crystal structures of the protease domain reveal a substrate-binding pocket that is shallow and relatively solvent exposed.[5,6] These features underscore the difficulties of designing small molecule inhibitors.[7] Many of the reported inhibitors are peptides derived from the C-terminal NS3 cleavage product.[8–12] Potent peptide-based inhibitors were obtained when the C-terminal carboxylates are activated as 'serine traps' such as α-ketoacid, ester and amide.[13–15] Replacement of the penultimate amino acid with an α-amino boronic acid also gave potent inhibitors via a reversible, covalent interaction of the boron with the serine hydroxyl group.[16,17] Starting from a hexapeptide boronic acid lead, we report herein the design and synthesis of a series of potent non-peptidic inhibitors of NS3 protease.

Hexapeptide boronic acid **1** (Fig. 1) was shown by Kettner to be very potent against NS3 with $K_i = 4$ nM in

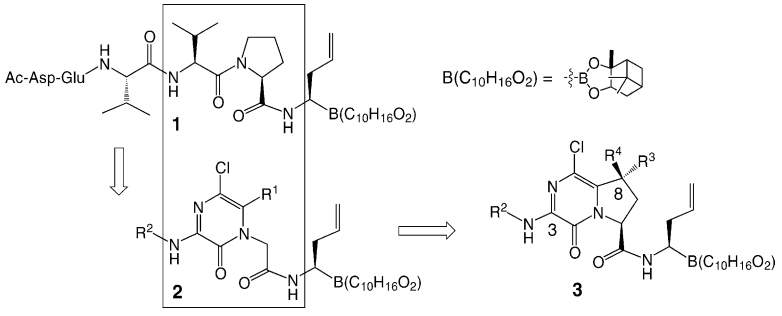

Figure 1. From hexapeptide inhibitor **1** to a non-peptide inhibitor **3**.

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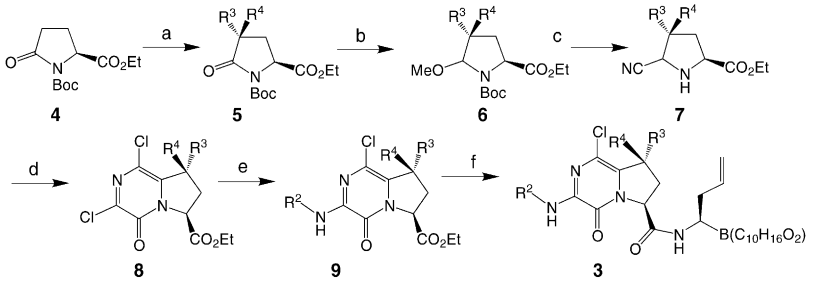


Scheme 1. (a) LHMDS, THF, −78 °C, R^3X, R^4 = H, 50–75%; (b) $LiBEt_3H$, THF, −78 °C, then MeOH, H^+(cat), >80%; (c) TMSCN, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C, then TFA; (d) $(COCl_2)_2$, toluene, 90 °C, 35–60% for three steps; (e) R^2NH_2, EtOAc, 80 °C, >90%; (f) LiOH, THF/H_2O, then $NH_2CH(allyl)B(O_2C_{10}H_{16}){\cdot}HCl$, PyAOP, DIEA, DMF, 50–65%.

the enzyme assay.[18] Considering the likely unfavorable metabolic stability of structure 1, our goal was to design a scaffold to significantly reduce the peptidic character of inhibitor **1**. Based on the assumption that proteases typically bind their substrate in an extended conformation, we looked for β-sheet mimetics that have been successfully used in other serine protease inhibitors. One such mimetic, 3-amino pyrazinone, has been reported effective against thrombin.[19] It was postulated that the CO and NH of the pyrazinone ring could form a pair of β-sheet H-bonds with the enzyme backbone. We thus replaced Val-Pro in **1** with a chloropyrazinone and the Ac-Asp-Glu-Val of **1** with a non-peptide side chain R^2 to give compound **2**. A library of about 100 compounds was prepared, but the binding affinities were moderate (1–50 μM). However, studies of SAR suggested that a bulky R^1 (e.g., *tert*-butyl, *neo*-pentyl) increased the binding affinity to low or sub μM level. This is likely a conformational effect due to the 1,2-relationship of R^1 with the side chain. A crowded R^1 would retard the rotation of the N–CH_2 bond, producing a relatively rigid conformation. We reasoned that by a further constraint using a bicyclic pyrazinone as in compound **3**, more rigid conformation could be generated, creating more potent compounds. Our results demonstrate that this is indeed the case.

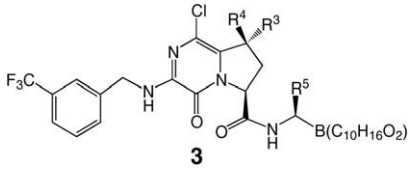
Synthesis of **3** (Scheme 1) started with a stereoselective alkylation of pyroglutamate **4** to **5**.[20,21] Chemo-selective reduction of the ring carbonyl with $LiBEt_3H$ in **5** to a hemiaminal, followed by solvent exchange with MeOH gave **6**. TMSCN under Lewis acid conditions converted **6** to aminonitrile **7** through an *N*-acyl iminium intermediate. Cyclization of **7** with oxaly chloride smoothly afforded dichloropyrazinone **8**, which reacted with a wide variety of primary amines to afford 3-amino bicyclic pyrazinone **9** in high yields. Hydrolysis of **9** and coupling with $NH_2CH(allyl)B(C_{10}H_{16}O_2){\cdot}HCl$ gave target **3** in good yield. It should be noted that the boronic acid was protected as a pinanediol ester to simplify the chemistry, the active species is the acid which should be in fast equilibrium with the ester under the assay condition. We made the discrete free boronic acids of **3h** and **3i** (Table 1), which displayed identical IC_{50} with the corresponding pinanediol esters as expected. Compound **3h** was shown to be active-site directed, reversible inhibitor.

The parent pyrazinone **3a** ($R^3 = R^4 =$ H, Table 1) is a low μM inhibitor with IC_{50} = 4.6 μM. A benzyl substitution (R^3) at the 8-position increased the potency 18-fold, giving **3b** with IC_{50} = 0.26 μM. *para*-Substitution on the benzyl with methyl, methoxy, or phenyl (**3c**, **3e**, and **3g**) further increased the potency to about 50 nM, but *meta*-substitution (**3d** and **3f**) decreased the binding affinity. Phenylpropyl (**3h**) and naphthylpropyl (**3i**) substitution increased potency, with naphthylpropyl proving the best (IC_{50} = 20 nM). Replacing the P1 allyl in **3i** with an ethyl gave an equally potent compound **3j**. Compounds **3i** and **3j** represent a significant step towards completely non-peptide NS3 inhibitors.

The enzyme is sensitive to the stereochemistry at the 8-position of **3**. Though **3i** is potent with IC_{50} = 20 nM, its diastereomer **3l** is 25-fold less potent. Similarly, **3k** is 35-fold less potent than its diastereomer **3h**.

We have also investigated the SAR of the amino capping group R^2 (Table 2). Aliphatic chains of different sizes (**3m–s**) had little effect on the potency, suggesting that the R^2 group did not strongly interact with the enzyme. However, electron-withdrawing aromatics (**3i** and **3w–y**) slightly (ca. 5-fold) increased the binding affinity with the enzyme. To gain more insight of the SAR, we have generated a computer model illustrating

Table 1. SAR of the substituents at the 8-position of compound **3**

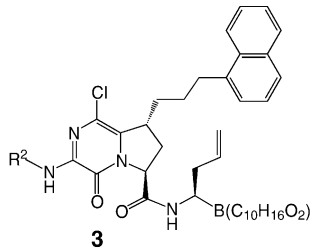


3

Compd	R^3	R^4	R^5	IC_{50}, μM[a]
3a	H	H	Allyl	4.6
3b	$C_6H_5CH_2$	H	Allyl	0.26
3c	*p*-$CH_3C_6H_4CH_2$	H	Allyl	0.06
3d	*m*-$CH_3C_6H_4CH_2$	H	Allyl	0.40
3e	*p*-$CH_3OC_6H_4CH_2$	H	Allyl	0.05
3f	*m*-$CH_3OC_6H_4CH_2$	H	Allyl	0.26
3g	*p*-$PhC_6H_4CH_2$	H	Allyl	0.06
3h	$C_6H_5(CH_2)_3$	H	Allyl	0.06
3i	2-naph$(CH_2)_3$	H	Allyl	0.02
3j	2-naph$(CH_2)_3$	H	Et	0.02
3k	H	$C_6H_5(CH_2)_3$	Allyl	2.0
3l	H	2-naph$(CH_2)_3$	Allyl	0.51

[a]All in vitro assays were performed as described previously;[13] standard deviation for the enzyme assays were typically ±50% of the means or less.

Table 2. SAR of the side chain R_2 in compound 3



3

Compd	R^2	IC_{50}, μM
3m	H	0.19
3n	Me	0.32
3o	Et	0.23
3p	Pr	0.18
3q	*n*-Bu	0.16
3r	*i*-Bu	0.17
3s	$C_6H_{11}CH_2$	0.20
3t	$C_6H_5CH_2$	0.24
3u	3,4-$HOC_6H_3CH_2$	0.11
3v	*m*-$MeOC_6H_4CH_2$	0.06
3w	2-Picolyl	0.09
3x	*m*-$FC_6H_4CH_2$	0.06
3y	*m*-$NO_2C_6H_4CH_2$	0.04
3i	*m*-$CF_3C_6H_4CH_2$	0.02

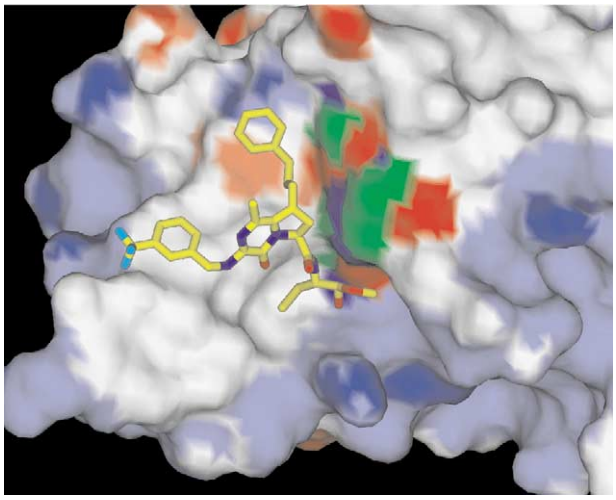


Figure 2. Inhibitor **3h** modeled into the active site of HCV-NS3.

the important features that maybe involved in binding of **3h** in the active site. The computer model was based on the structure of HCV protease, PDB file 1jxp.[22] Because of the flexibility of R^2 and R^3 of **3h**, multiple calculations having different starting conformations were carried out. Other than covalent attachment of the ligand to the catalytic serine, the initial conformations were generated at random and had little overlap. After minimization and short molecular dynamics simulation runs, using the method of Luty et al.,[23] a predominant binding mode emerged and is illustrated in Figure 2. In this model, the R^2 group points away from the enzyme surface into the solvent, explaining the flat SAR of R^2. Inhibitor **3h** makes three H-bond interactions with the enzyme backbone, a pair from the pyrazinone CO and NH, the third from the P1 amide NH. We believe the electron-withdrawing R^2 groups increase the acidity of the NH off the pyrazinone ring, making it a better H-bond donor, thus increasing binding affinity. The phenylpropyl seems to cover a salt bridge formed by Asp-Arg of the enzyme. However, the low solubility of **3h** prevented it from being further evaluated in a functional assay. Modification of the physical properties is desired to further progress this series of inhibitors.

In summary, we have designed a 3-amino bicyclic pyrazinone scaffold that effectively replaces a dipeptide segment in a hexapeptide lead. By further manipulation of the side chains of the scaffold, a series of potent non-peptidic inhibitors of HCV NS3 was obtained. Because of the correct alignment of the CO and NH, coupled with rigidity of the bicyclic pyrazinone ring, we believe this scaffold could be effective as a general β-sheet mimetic that will find use in peptide chemistry and protease inhibitor design.

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