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Synthesis and antiviral activity of HCV NS3/4A peptidomimetic boronic acid inhibitors

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ABSTRACT

A new series of NS3/4A protease boronic acid inhibitors is described. The compounds show good biochemical potency and cellular activity. The peptidomimetic inhibitors were evaluated against proteases from different HCV genotypes and clinically relevant NS3/4A mutants. Compound **28** displayed subnanomolar to single digit nanomolar potencies in the enzymatic assays and an EC_{50} of 25 nM in the replicon cell-based assay.

HCV virus infection is a leading cause of chronic hepatitis, cirrhosis, liver transplant and hepatocellular carcinoma. Presently, it is estimated that HCV infects more than 170 million people worldwide and thus represents a viral pandemic.[1] The current therapy for chronic HCV consists of pegylated IFN-α, a cytokine with immunomodulatory and antiviral activity, together with the synthetic guanosine nucleoside analogue ribavirin.[2] This combination therapy has significant side effects, involves a costly course of treatment and shows incomplete efficacy in many patients. Given the high prevalence of the disease, the development of more effective, convenient and tolerable therapeutic strategies is needed in the battle against HCV.[3]

The HCV genome encodes a single large polyprotein of approximately 3000 amino acids.[4] Proteolytic processing by both host peptidases and viral proteases results in at least 10 viral proteins. These include a core protein C, two envelope proteins El and E2, and nonstructural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B that are essential for viral replication, translation, and polyprotein processing. The N-terminal domain of NS3 contains a chymotrypsin-like serine protease which is required for proteolytic processing of four cleavage sites in the nonstructural region of the HCV polyprotein.[5] The NS3 protease becomes fully activated after the formation of a complex with its cofactor protein NS4A. A large number of pharmaceutical and biotechnology companies have focused on the HCV NS3/4A protease as a target for small molecule antiviral drug development.[6] The initial observation that the enzyme was susceptible to inhibition by its N-terminal cleavage products led to the synthesis of the first hexapeptide inhibitors.[7] Further truncation and optimization of these product-based peptides has been conducted within the last decade and resulted in the development of a number of potent and efficacious inhibitors that have entered clinical trials (Fig. 1).[8] Due to the error prone nature of HCV replication and the resulting prevalence of mutant enzymes, there is a constant need for new agents with complementary profiles amenable for use as combination therapies.

Most of the NS3/4A inhibitors described in the literature fall under two main categories according to their warhead: carboxylic acids and acyl sulfonamide bioisosteres like BILN2061, ITMN191 (**1**), TMC435350 (**2**) and MK7009; or electrophilic trap-containing inhibitors like VX950 (**3**) and SCH503034 where reversible covalent binding to the catalytic serine is an alternative to the electrostatic binding of the afore mentioned. We were particularly interested in the use of the boronic acid electrophile within the latter class. The usual mechanism of protease inhibition by boronic acids is the formation of a tetracoordinate boronate complex between the boronic acid and the active site serine hydroxyl group. This pharmacophore has been previously described in the literature. Kettner and Decicco synthesized potent NS3/4A peptide and peptidomimetic boronic acid inhibitors such as compounds **4** and

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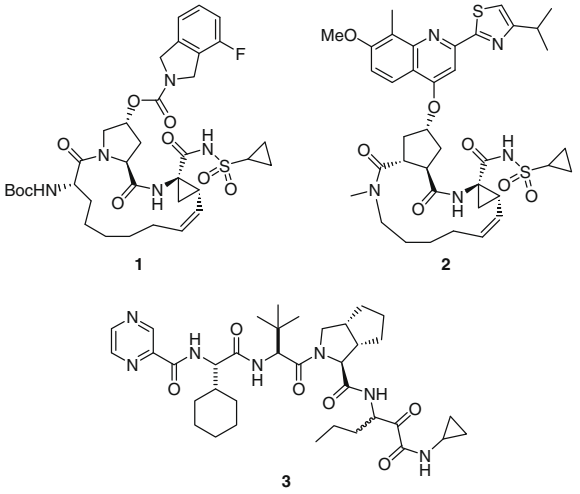
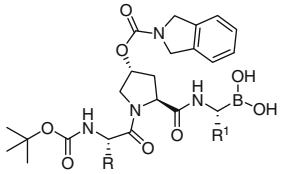


Figure 1. Selected examples of clinical stage NS3/4A inhibitors.

Table 1
Exploration of the P1 position



Compounds	R	R^1	NS3/4A[a] IC_{50} (nM)	HUH7-Rep EC_{50} (nM)
7	^{t}Bu	Et	2	98
8	^{t}Bu	^{n}Pr	1	69
9	^{t}Bu	CyclobutylCH$_2$	2	171
10	Cy	Et	1	71
11	Cy	^{n}Pr	2	110
12[b]	Cy	(CH_2CH_2)	3353	ND[c]

[a] Genotype **1b**.
[b] Boronic acid pinanediol ester.
[c] ND: not determined.

5 (Fig. 2).[9] Concurrent with our studies, a series of NS3/4A inhibitors possessing a cyclopropyl fused proline core was very recently disclosed by a group at Schering (compound **6**).[10] However, minimal data on cellular activity, mutant protease activity, or genotype coverage has been disclosed for those inhibitors. Herein, we report our optimization of a series of peptidomimetic boronic acids bearing a carbamate substituted proline core as potent inhibitors of the NS3/4A protease as evaluated biochemically and cellularly.

We focused our medicinal chemistry program on tripeptide inhibitors, based on reports that peptides of this length were able to achieve potent NS3/4A inhibition.[11] Screening of a limited set of substituents on the proline core including benzylic and heteroaryl ethers resulted in the identification of an isoindoline carbamate as the preferred group for NS3/4A biochemical and HCV replicon cellular activity in these tripeptide boronic acids.[12] For the P3 position of the inhibitors, the previously optimized P3 surrogates *tert*-leucine and cyclohexyl glycine amino acids were selected.[13] Initial SAR efforts involved exploration of the P1 position (Table 1). Except for the cyclopropyl analogue **12** that exhibited reduced activity, all other analogues displayed single digit nanomolar biochemical potencies. Despite having promising biochemical results with IC50s in the low nM range, these inhibitors exhibited only moderate activities in the cell-based HCV replicon assay.

The possibility of truncating these capped tripeptides was investigated. Removal of the P3 amino acid led to a significant decrease in biochemical potency. Truncation of the isoindoline ring to pyrrolidine or introduction of the *seco* analogue *N*-methyl *N*-benzyl carbamate, while tolerated by the purified enzyme, had a negative impact on cellular activity of the compounds (EC_{50} >1000 nM). Additional modifications on the isoindoline ring such as the introduction of fluoro, chloro and trifluoromethyl groups or replacement with 4-azaisoindoline were also explored. Except for the fluoro substitution, these modifications had a detrimental effect on the cellular potency of these compounds.

The modular nature of the structure of these molecules allowed for a number of different synthetic approaches. Synthesis of the inhibitors was generally carried out following the procedures depicted in Scheme 1 for compound **11**. Commercially available *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester **13** was first coupled with isoindoline by using triphosgene to afford the corresponding carbamate. After the resulting ester was hydrolyzed, IBCF coupling of the pinanediol protected (*R*)-aminobutyl boronic acid **15** proceeded smoothly to provide dipeptide **16**. Sequential deprotection of the Boc group under acidic conditions and of the pinanediol bor-

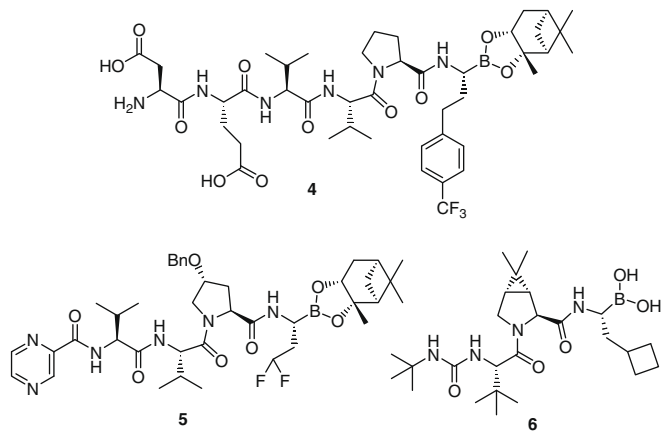
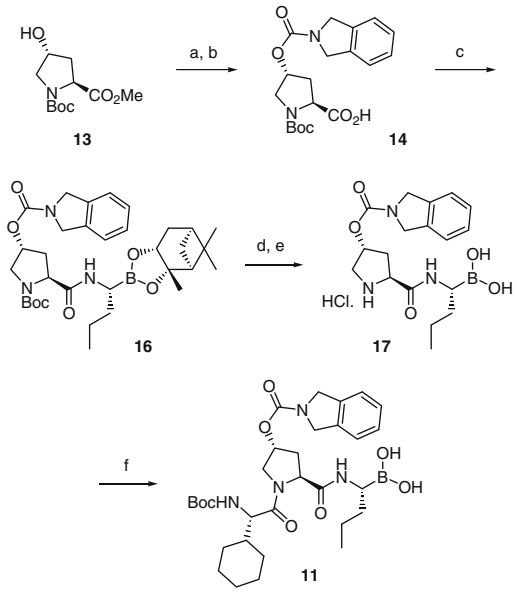


Figure 2. Previously reported NS3/4A boronic acid inhibitors.



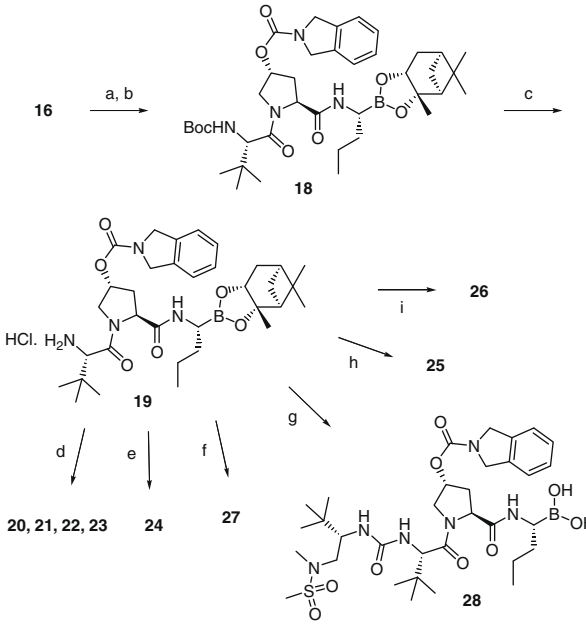
Scheme 1. Reactions and conditions: (a) triphosgene, TEA, isoindoline, DMAP, THF, 88%; (b) LiOH, THF/H_2O, 84%; (c) IBCF, NMM, **15**, DIEA, THF, −8 °C, 92%; (d) 4 N HCl, dioxane; (e) $PhB(OH)_2$, aq 1 N HCl/Hexane, 90% (two steps); (f) PyBroP, HOAt, DIEA, DCM, 70%.

onate ester using phenyl boronic acid in a biphasic solvent system gave the free amino boronic acid **17**.[14] Finally, the target compound was obtained by coupling the Boc protected cyclohexyl glycine amino acid in the presence of PyBroP/HOAt.[15] The required amino boronic acids were prepared according to the methodology of Matteson,[16] except for the cyclopropyl substituted analogue used in the preparation of compound **12**.[17]

Introduction of the different capping groups to expand our SAR was carried out by a four step sequence from intermediate **16** (Scheme 2). Boc removal and coupling of the protected *tert*-Leucine amino acid provided compound **18**. Subsequent unmasking of the Boc protecting group followed by derivatization of the amino group led to compounds **20**–**28**.

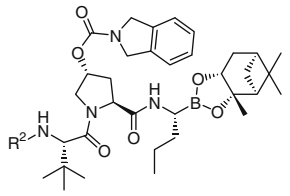
The inhibitors bearing different capping groups were evaluated in the NS3/4A biochemical and HCV replicon assays (Table 2).[18] The presence of the *tert*-butyl carbamate group in the initial leads was a concern because of its documented low stability under acidic conditions. Several alkyl carbamates were prepared and the biochemical and cellular inhibition improved somewhat for those containing the bulkier isobutyl and neopentyl groups (compounds **21** and **22**). A series of analogues incorporating polar groups was also synthesized. While the NS3/4A enzyme showed good tolerability for this type of functionality, considerable loss of cellular activity was observed presumably due to poor cellular penetration. Interestingly, compound **28** bearing an *N*-methyl sulfonamide substituted urea group displayed the best replicon activity of this series (EC_{50} = 25 nM, EC_{90} = 117 nM).[19] This result is in contrast to the reported cell-based activity of the P1 ethyl analogue bearing a 3,4-dimethylcyclopropyl proline in the P2 position that exhibited an EC_{90} >5 μM.[10] Although both polar and non-polar groups were well tolerated by the NS3/4A enzyme as capping groups, a significant drop in activity was observed with compound **19** containing the free amino group.

Compounds **8**, **10** and **28** possessing good in vitro potency profiles were selected for further evaluation. Since the HCV virus exhibits multiple genotypes with different response rates to the standard of care treatment,[2] it is desirable to achieve good genotype coverage. The compounds retained biochemical potency against proteases from multiple HCV genotypes, displaying the best activity against genotypes **1a** and **1b** that are the most difficult to treat in patients (Table 3). Compound **28** was remarkable in that it displayed subnanomolar potencies for each of the genotypes tested.



Scheme 2. Reactions and conditions: (a) 4 N HCl, dioxane, rt; (b) EDC, HOBt, DIEA, DCM, 0 °C to rt; (c) 4 N HCl, dioxane, rt; (d) Et–, *i*Bu–, Np–, or FCH_2CH_2–OCOCl, DIEA, DCM, 0 °C; (e) $Py(CH_2)_2OH$, DSC, TEA, CH_3CN/DCM, 0 °C; (f) $(CH_3CH_2)_2N(CH_2)_2NH_2$, CDI, TEA, DCM, rt; (g) (i) (*S*)-$MsN(CH_3)CH_2CH(^tBu)NCO$, DIEA, DCM, 0 °C; (ii) BCl_3, DCM, −78 °C to 0 °C; (h) MsCl, DIEA, DCM, 0 °C; (i) (*S*)-$(CH_3)_3CCH(OH)CO_2H$, EDC, HOBt, DIEA, DCM, rt.

Table 2
Exploration of the capping group



Compounds	R^2	NS3/4A[a] IC_{50} (nM)	HUH7-Rep EC_{50} (nM)
18	^tBuOCO	0.8	93
20	EtOCO	2	230
21	^iBuOCO	0.3	54
22	$(CH_3)_3CCH_2OCO$	0.7	41
23	FCH_2CH_2OCO	2	408
24	$Py(CH_2)_2OCO$	5	>1000
25	$MeSO_2$	11	>1000
26	$(CH_3)_3CCH(OH)CO$	3	>1000
27	$(CH_3CH_2)_2N(CH_2)_2NHCO$	27	>1000
28[b]	$MsN(CH_3)CH_2CH(^tBu)NHCO$	0.5	25
19	H	3197	ND[c]

[a] Genotype **1b**.
[b] Free boronic acid.
[c] ND: not determined.

In addition to the excellent genotype coverage, enhanced biochemical inhibitory activity against a panel of mutant NS3/4A proteases known to arise in patients treated with other NS3/4A inhibitors was also observed (Table 4).[20] Compound **28** displayed very low fold increase in IC_{50} values and retained very good activity across the different mutant proteases.[21] The increased activity of the carbamate substituted proline analogues against the A156 mutants is probably due to the decreased steric congestion around that amino acid as compared to the bicyclic proline derivatives present in the P2 position of VX950 and SCH503034.[20]

In summary, we synthesized and evaluated a new series of HCV NS3/4A boronic acid inhibitors. The compounds showed good biochemical potency and exhibited a variable degree of cellular activity in the replicon assay depending on the substituents present in the molecule. Among the compounds evaluated, compound **28** proved to be the best overall inhibitor with excellent potencies

Table 3
Genotype profile of selected analogues

Compounds	NS3/4A, IC_{50} (nM)			
	wt **1b**	wt **1a**	wt **2a**	wt **3a**
8	1	2	21	61
10	1	0.9	6	23
28	0.5	0.3	0.6	0.9

Table 4
Mutant profile of selected analogues

Compounds	Fold shift in IC_{50} versus wt **1b**					
	A156S	A156T	A156V	R155K	D168A	T54A
8	5	70	158	109	61	121
10	4	40	64	63	47	69
28	<4	<4	12	6	<4	8

in both enzymatic and cell-based assays, superior genotype coverage and good activity against clinically relevant mutants. Further evaluation of these boronic acid inhibitors will be reported in a future Letter.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.017.

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18. Comparison of binding activities of compounds **8** and **18** together with additional data generated in our laboratories imply that the boronate esters are quickly hydrolyzed in the assay conditions to the free boronic acids.
19. This sulfonamide substituted capping group for NS3/4A inhibitors was originally developed by researchers at Schering. See: (a) Chen, K. X.; Njoroge, F. G.; Yang, W.; Vibulbhan, B.; Girijavallabhan, V. M.; Venkatraman, S.; Velazquez, F. PCT Patent Application WO05/021584A2.; (b) Venkatraman, S.; Velazquez, F.; Wu, W.; Blackman, M.; Chen, K. X.; Bogen, S.; Nair, L.; Tong, X.; Chase, R.; Hart, A.; Agrawal, S.; Pichardo, J.; Prongay, A.; Cheng, K.-C.; Girijavallabhan, V.; Piwinski, J.; Shih, N.-Y.; Njoroge, F. G. *J. Med. Chem.* **2009**, *52*, 336.
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21. The negative impact that some mutated residues have on binding of inhibitor **8** to the protein could be attenuated by the additional interactions derived from the presence of the *N*-methyl sulfonamide capping group in compound **28**.