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Novel 2-oxoimidazolidine-4-carboxylic acid derivatives as Hepatitis C virus NS3-4A serine protease inhibitors: synthesis, activity, and X-ray crystal structure of an enzyme inhibitor complex

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Abstract—Synthesis and HCV NS3 serine protease inhibitory activity of some novel 2-oxoimidazolidine-4-carboxylic acid derivatives are reported. Inhibitors derived from this new P2 core exhibited activity in the low μM range. X-ray structure of an inhibitor, **15c** bound to the protease is presented.

Hepatitis C virus (HCV) is the etiologic agent of non-A, non-B hepatitis leading to liver cirrhosis, heptacellular carcinoma, and liver failure in humans.[1] It has been estimated that 3% of the human population worldwide is infected by HCV.[2] Currently, α-interferon monotherapy and α-interferon–ribavirin combination therapy are the only approved treatment regimen.[3] Recently pegylated version of α-interferon, PEG-INTRON and PEGASYS has been approved by the FDA as improved therapy. Due to the seriousness of the disease there is an urgent need for new and more effective protocol for the treatment of HCV infections.

Since identification of this virus, the NS3 serine protease contained within the N-terminal region of the NS3 protein has been studied extensively.[4] This chymotrypsin-like serine protease is implicated in the viral replication and hence is an attractive target for HCV antiviral therapeutics.[5] While a number of HCV NS3 serine protease inhibitors have been reported by different groups, most of these are peptide-like molecules derived from the cleavage site sequences. Based on the P2 moiety, these reported inhibitors could be classified as leucine[6] or proline based[7] analogs. Replacement of naturally occurring proline residue with a non-natural proline surrogate may impart beneficial biological and pharmacological properties to the peptide inhibitors. Herein, we report the incorporation of one such proline surrogate, 2-oxoimidazolidine-4-carboxylic acid derivative,[8] as a promising P2 scaffold in the design and synthesis of potent HCV NS3 serine protease inhibitors.[9] Noteworthy feature of this scaffold is the ability to introduce additional functionality off the N-1 position; a mission that was undertaken in the SAR development of potential inhibitors. In the present study, an α-ketoamide functional group was incorporated as electrophilic serine trap (see Fig. 1), since this entity facilitated probing the P prime region of the protease.[10]

Synthetic approach for the preparation of the designed inhibitors was initiated from 2-oxoimidazolidine derivative **1** prepared by the method of Hayashi et al.[12] (Scheme 1). Migration of the benzyloxycarbonyl (Z) protecting functionality was carried out in DMF using sodium hydride as the base at room temperature to afford **2**. *N*-Acylation of the potassium salt of **2** with the activated ester derived from *N*-Boc-Val-OH at −40 °C provided the benzyl ester **3**. Removal of the benzyloxycarbonyl and benzyl protecting groups were carried out in a single step under hydrogenation conditions to give

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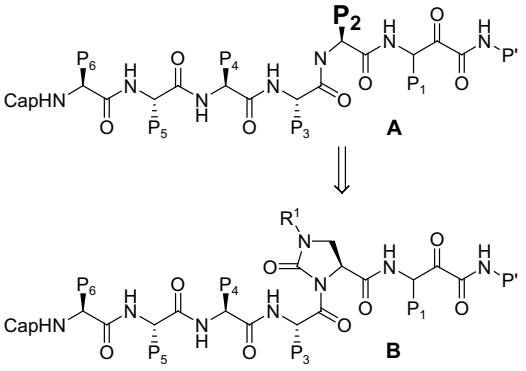
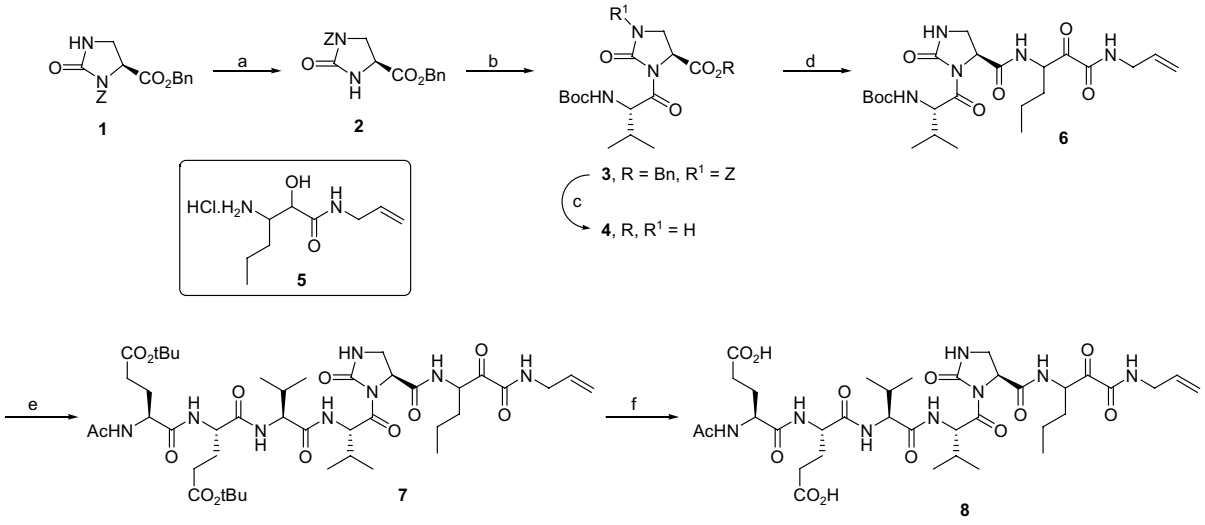


Figure 1.

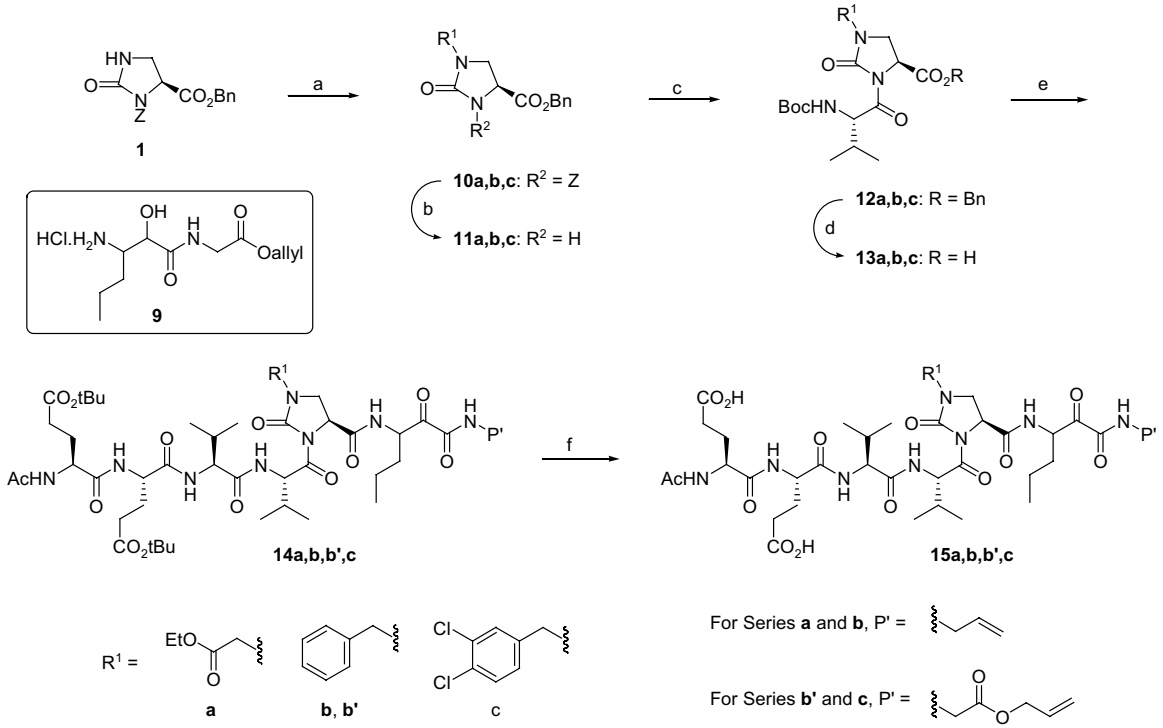
acid **4**. Installation of the P1-P′ residue was done by coupling the acid **4** with amine **5** under EDCI, HOBt conditions. The resulting mixture of hydroxyl amide diastereomers was oxidized using Dess–Martin's periodinane to provide the ketoamide **6**. Deprotection of the Boc group of **6** and subsequent elaboration of the P region was then accomplished by treatment with the P6-P4 tripeptide, Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-OH [prepared from commercially available protected amino acid derivatives employing standard protocol] using peptide coupling methodology, which afforded the protected ketoamide **7**. The target compound **8** was obtained by deprotection of the ester functionalities using 4 M HCl in dioxane.

Introduction of substitution on the N-1 position of the P2 imidazolidinone core and subsequent conversion to target compounds are described in Scheme 2. Thus, *N*-alkylation of **1** to give **10** was carried out in DME (or acetone) using potassium carbonate as the base rather than NaH, to avoid migration of the Z protecting group. Selective removal of the Z functionality was achieved by treatment with 30% HBr in glacial acetic acid to provide ester **11**. Installation of the P3 valine residue and further processing to afford the targets **15** essentially followed procedures described in Scheme 1.

HCV NS3 serine protease inhibitory data[13] for the imidazolidineone targets were obtained using the continuous spectrophotometric assay described earlier[14] and the results are summarized in Table 1. The parent molecule **8** containing the unsubstituted imidazolidineone moiety at P2 and the corresponding diester **7** exhibited moderate inhibition with K_i* of 46 and 69 μM, respectively. It has been previously reported that ethers derived from *trans*-4-hydroxyproline as P2 core improved the potency considerably.[15] Hence, we decided to investigate the effect of substitution on the ring nitrogen of our imidazolidineone P2 core. Introduction of the methylene carbethoxy moiety at the N-1 position improved the activity significantly; while the protected compound **14a** displayed an activity of 30 μM against the NS3 serine protease, the corresponding fully deprotected target **15a** exhibited an improved activity of 4 μM. Importance of the P6-P5 residue is clearly evident from the data for the truncated derivatives **16** and **17** (synthesis not shown), which were less potent. Installation of the benzyl group at the N-1 position on the ring was well tolerated as exemplified by compound **15b**, which displayed K_i* of 1 μM. In an attempt to probe the P prime region, compound **15b**′ (glycine allyl ester as P′ residue) was synthesized. This P prime extended inhibitor **15b**′ was found to be equipotent (K_i* = 1 μM) as the allyl amide **15b**. Further exploration of the structure–activity relationship (SAR) established that substitution of the aromatic ring as 3,4-dichloro derivative in the P prime extended series enhanced the potency further, resulting in **15c** with HCV NS3 serine protease inhibitory activity of 0.31 μM.

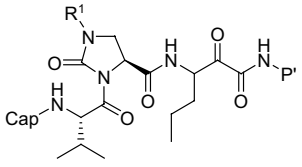


Scheme 1. Reagents and conditions: (a) NaH, DMF (66%); (b) **2**, KO*t*-Bu, THF; add Boc-Val-OSu, THF (80%); (c) H_2, 10% Pd/C, EtOH (quant); (d) (i) **5**, EDCI, HOBt, NMM, CH_2Cl_2; (ii) Dess–Martin's periodinane, CH_2Cl_2 (60%); (e) (i) 4 M HCl, dioxane; (ii) Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-OH, EDCI, HOOBt, NMM, CH_2Cl_2 (31%); (f) 4 M HCl, dioxane (quant).



Scheme 2. Reagents and conditions: (a) R_1Br, K_2CO_3, TBAI (cat), DME (62–83%); (b) 30% HBr/AcOH (50–70%); (c) **11**, KO*t*-Bu, THF; add Boc-Val-OSu, THF (50–55%); (d) for series **a** and **b**—H_2, 10% Pd/C, EtOH; for series **c**—aq 1 M LiOH, THF, MeOH (quant); (e) for **14a** see method A, for **14b** see method B, for **14b′** see method C, for **14c** see method D;[11] (f) 4 M HCl, dioxane (quant).

Table 1.



Compound	Cap	R^1	P′	K_i* (μM)[a]
7	Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-	H	Allyl	69
8	Ac-Glu-Glu-Val-	H	Allyl	46
14a	Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-	CH_2CO_2Et	Allyl	30
15a	Ac-Glu-Glu-Val-	CH_2CO_2Et	Allyl	4
16	*i*-Boc-Val-	CH_2CO_2Et	Allyl	78
17	*t*-Boc-	CH_2CO_2Et	Allyl	150
14b	Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-	CH_2Ph	Allyl	61
15b	Ac-Glu-Glu-Val-	CH_2Ph	Allyl	1
14b′	Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-	CH_2Ph	Gly-Oallyl	>100
15b′	Ac-Glu-Glu-Val-	CH_2Ph	Gly-Oallyl	1
14c	Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-	CH_2Ph(3,4-dichloro)	Gly-Oallyl	>100
15c	Ac-Glu-Glu-Val-	CH_2Ph(3,4-dichloro)	Gly-Oallyl	0.31

[a] K_i* value is reported as a weighted mean of at least three independent determinations.

X-ray crystal structure of the inhibitor **15c** bound to the protease is shown in Figure 2. It can be seen that the peptidic core binds to the protease through a series of hydrogen bonding interactions. The norvaline residue at P1 occupies the shallow hydrophobic S1 pocket. The P′ residue wraps over the side chain of lysine 136, thus resulting in some hydrophobic interaction. Most notably, the dichlorobenzyl group off the N-1 position of the imidazolidineone ring extends towards glutamine 41, and thereby causing a slight increase in the size of the S1′ pocket.

In summary, we have discovered that 2-oxoimidazolidine-4-carboxylic acid derivatives are suitable P2

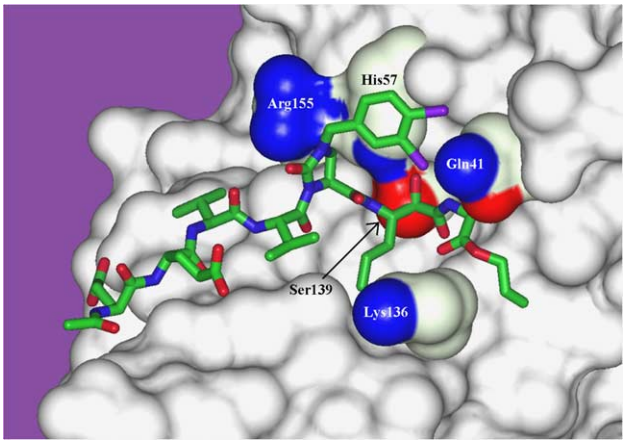


Figure 2. X-ray structure of **15c** bound to the protease.

surrogates with potent HCV NS3 serine protease inhibitory activity. While truncated inhibitors were not as potent, targets containing the P6 residues exhibited potent activity. X-ray crystal structure of one of the inhibitors (**15c**) bound to the protease revealed that the substituent off the N-1 position of the P2 ring extends towards glutamine 41 in an unique fashion. We are currently in the process of using this information in the design of other analogs in this series.

Acknowledgements

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Method B: (i) **5**, EDCI, HOOB*t*, NMM (91%); (ii) 4 M HCl/dioxane; (iii) Boc-Val-OH, EDCI, HOOB*t*, NMM (60%); (iv) Dess–Martin's periodinane (78%); (v) 4 M HCl/dioxane; (vi) Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-OH, EDCI, HOOB*t*, NMM (64%).
Method C: (i) **9**, EDCI, HOOB*t*, NMM (64%); (ii) Dess–Martin's periodinane (83%); (iii) 4 M HCl/dioxane; (iv) Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-OH, EDCI, HOOB*t*, NMM (59%).
Method D: (i) **9**, EDCI, HOOB*t*, NMM (98%); (ii) 4 M HCl/dioxane; (iii) Ac-Glu(*t*-Bu)-Glu(*t*-Bu)-Val-OH, EDCI, HOOB*t*, NMM (72%); (iv) DCC, DMSO, $Cl_2CHCOOH$ (49%).
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