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Hepatitis C virus NS3-4A serine protease inhibitors: SAR of P_2' moiety with improved potency

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Abstract—We have discovered that introduction of appropriate amino acid derivatives at P_2' position improved the binding potency of P_3-capped α-ketoamide inhibitors of HCV NS3 serine protease. X-ray crystal structure of one of the inhibitors (**43**) bound to the protease revealed the importance of the P_2' moiety.

Hepatitis C virus (HCV) is the etiologic agent of non-A and 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 with HCV.[2] Currently, the FDA-approved treatment regimens for this infection are α-interferon monotherapy and α-interferon–ribavirin combination therapy.[3] While recent approval of pegylated version of α-interferon, PEG-INTRON, and PEGASYS, have improved the therapeutic effectiveness, it is still far from ideal. 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 the identification of hepatitis C 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 has been implicated in the viral replication and hence, is an attractive target for HCV antiviral therapeutics.[5]

Hexapeptide derivatives containing α-ketoamide electrophilic trap have been reported by our group[6] and others[7] to be potent inhibitors of HCV NS3 serine protease. A noteworthy feature of the α-ketoamide moiety is the ability to introduce functionality at both ends, that is, amino and carboxyl terminus, of the molecule. A generic structure of an α-ketoamide inhibitor describing the non-prime (P) region and prime (P′) region is shown in Figure 1.

Most of the work reported so far on HCV NS3 serine protease inhibitors describes truncation/variation of the P region in an attempt to improve the binding potency.[8] Limited work has been undertaken in the P′ region to enhance the binding affinity.[9] Herein, we disclose our efforts in this area, specifically discussing variation of the P_2' side chain, which resulted in highly potent inhibitors of HCV NS3 serine protease.

Both solution phase and solid phase methods were applied for synthesis of compounds reported in this communication. For solution phase approach, synthetic

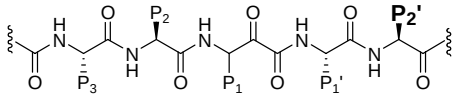

Figure 1.

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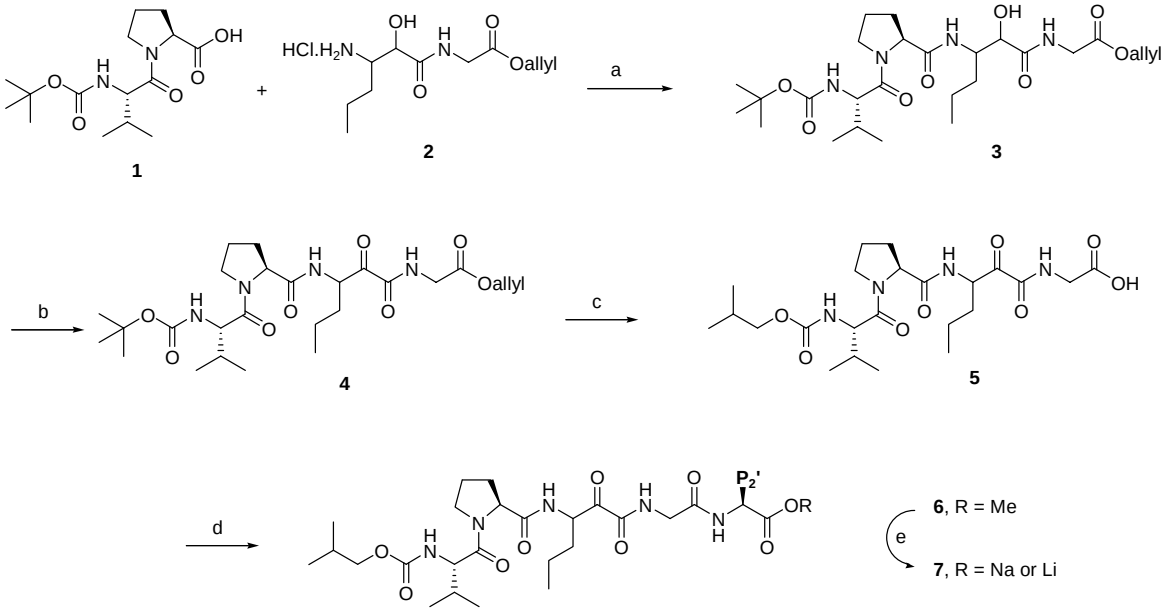
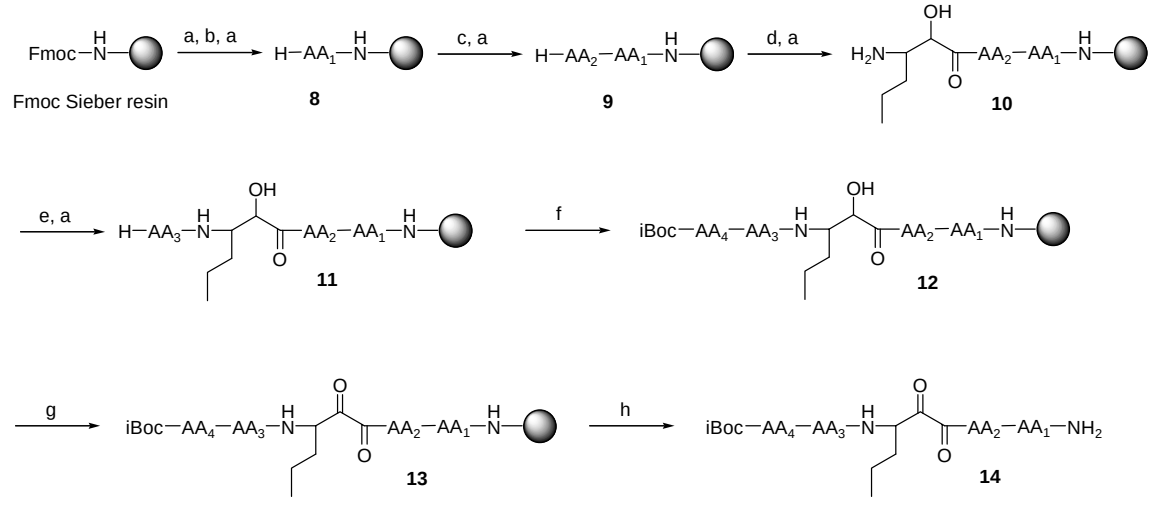
efforts for the designed targets were initiated by treating the commercially available dipeptide **1** with the P_1–P' intermediate **2**[6] under coupling conditions (EDCI, HOBt, and NMM) to provide the hydroxy amide **3**. Oxidation of **3** to the corresponding ketoamide was carried out using Dess–Martin's periodinane.[10] Protecting group manipulation on the amino end, followed by unraveling the allyl moiety gave the acid **5**. Introduction of the P'_2 moiety was carried out by coupling with appropriate amino acid derivative to give **6**. Hydrolysis using aqueous sodium (or lithium) hydroxide provided **7** (Scheme 1).[11]

The solid phase synthesis of α-ketoamides is depicted in Scheme 2. Fmoc protected α-amino acids (P'_2 moiety) were coupled to Sieber resin under HATU/DIEA conditions.[12] Deprotection of the Fmoc group followed by coupling with Fmoc-α-amino acid or Fmoc-α-amino hydroxyacid in an iterative mode resulted in **11**. The resin-bound α-hydroxyamide **12** could be obtained by capping the P_2 N-terminus in **11** with *i*-Boc-Val-OH or *i*-Boc-Chg-OH. It is worth noting that the hydroxyl group was not acylated under the repeated HATU/DIEA amide bond formation conditions. The solid phase oxidation of the resin-bound α-hydroxyamide **12**



Scheme 1. Reagents: (a) EDCI, HOBt, NMM, CH_2Cl_2; (b) Dess–Martin's periodinane, CH_2Cl_2; (c) (i) 4 M HCl, dioxane; (ii) *i*-BuOCOCl, DIPEA, CH_2Cl_2; (iii) aq 1 N NaOH, THF; (d) $HCl{\cdot}H_2NCH(P'_2)CO_2Me$, EDCI, HOBt, NMM, CH_2Cl_2; (e) aq 1 N NaOH or aq 1 M LiOH, THF.



AA_1 = P_2' moiety (varied); AA_2 = P_1' moiety, Gly; AA_3 = P_2 moiety, Pro or Leu; AA_4 = P_3 moiety, Val or Chg.

Scheme 2. Solid phase synthesis of ketoamides. Reagents: (a) 20% piperidine–DMF; (b) Fmoc-AA_1-OH, HATU, DIEA, DMF; (c) Fmoc-AA_2-OH, HATU, DIEA, DMF; (d) Fmoc-NH-CH(*n*-Pr)-CH(OH)-CO_2H, HATU, DIEA, DMF; (e) Fmoc-AA_3-OH, HATU, DIEA, DMF; (f) *i*-Boc-AA_4-OH, HATU, DIEA, DMF; (g) (i) Dess–Martin periodinane, *t*-BuOH, CH_2Cl_2; (ii) *i*-PrOH, CH_2Cl_2; (iii) 50% aq THF; (h) 2% TFA–CH_2Cl_2.

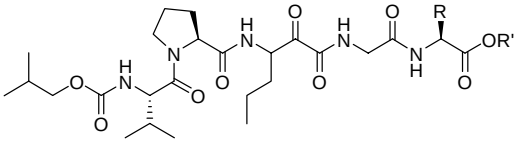
to the α-ketoamide **13** was achieved using Dess–Martin's periodinane. Excess periodinane was quenched with isopropanol before work-up. It was found that the solid by-product from the Dess–Martin oxidation could be removed by washing with aqueous THF. The desired α-ketoamide **14** was cleaved from the Sieber resin under mild 2% TFA/CH_2Cl_2 conditions.

It has been established[9a] that glycine moiety at P_1' position enhanced the potency of P_4 capped inhibitors. Interaction with Lys136 and Arg 109 of the protein was reasoned for the improved potency of these inhibitors. Based on extensive analyses of X-ray crystal structures of our hexapeptide inhibitors bound to the protease,[13] we rationalized that strategically positioned groups off the P_1' position could interact favorably with Lys136 and hence lead to further enhancement in potency. To test this hypothesis, a small set of compounds (Table 1) was synthesized using solution phase chemistry (Scheme 1). We chose to truncate these molecules at P_3 (*i*-Boc cap at P_3) in order to maintain the molecular weight of the target compounds in the 'drug-like' region.

HCV NS3 serine protease inhibitory activity[14] for the targets synthesized was obtained using the continuous spectrophotometric assay described earlier.[15] Interestingly, the targets containing hydrophobic side chain at P_2' (compounds **19** and **20**, Table 1) turned out to be more potent (K_i* = 15 μM) in comparison with targets containing acidic side chain (compounds **15**–**18**, Table 1).

Encouraged by the results obtained for compounds **19** and **20**, we then embarked on varying the P_2' moiety. These compounds were synthesized using solid phase chemistry described in Scheme 2. Target compounds were assayed as described previously[15] and the results are listed in Table 2. From the HCV protease inhibitory data obtained it was clear that polar functionality at P_2' position was less potent (compounds **22**–**24**, Table 2). While hydrophobic moiety was accommodated at this position, disubstitution at the β carbon was more preferred (compound **27**, Table 2), compared to trisubstitution (compound **26**, Table 2) or monosubstitution (compounds **28**–**32**, Table 2). Most interestingly, an aromatic group off the α-carbon (compound **33**, Table 2, phenylglycine at P_2') exhibited the best potency in this series, with K_i* = 2.1 μM. Replacement of P_3 valine with cyclohexylglycine resulted in compound **34** with further improvement in potency (K_i* = 0.36 μM).

Table 1.



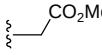
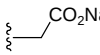
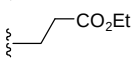
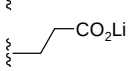
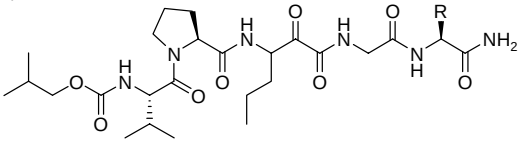
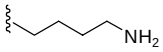
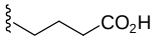
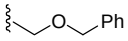
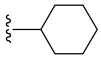
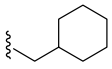
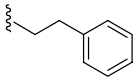
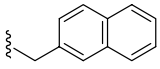
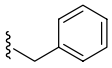
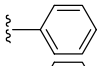
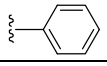
Compound No.	R	R′	K_i* (μM)
15		Me	55
16		Na	>100
17		Me	20
18		Li	>100
19		Me	15
20		Li	15

Table 2.

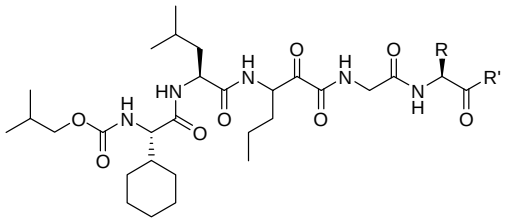


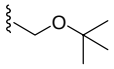
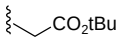
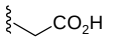
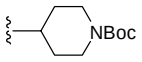
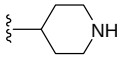
Compound No.	R	K_i*(μM)
21		—
22		82
23		18
24		111
25		3.5
26		53
27		2.6
28		16
29		8
30		6.7
31		12
32		15
33		2.1
34[a]		***0.36***

[a] P_3 = Chg instead of Val.

The targets included in Tables 1 and 2 were prepared using proline as the P_2 residue. However, after extensive exploration, we found that leucine at P_2 was comparable, if not better than proline. Thus, we carried out a similar study using leucine as P_2 with cyclohexylglycine moiety at P_3 because P_3 residue showed a synergistic effect in improving the potency. Most of these targets were prepared using the solid phase chemistry described in Scheme 2. Selected compounds that were prepared via solution phase chemistry followed procedures described in Scheme 1. The SAR of the P_2 leucine series (Table 3) paralleled the proline series. Thus, polar functionalities at P_2' were less potent (compounds **37**–**39**, Table 3). Small hydrophobic

Table 3.



Compound No.	R	R′	K_i* (μM)
35		NH_2	0.46
36		NH_2	—
37		NH_2	8.4
38		OH[b]	3.3
39		OH[b]	6.4
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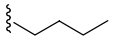
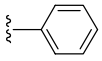
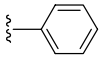
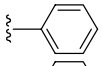
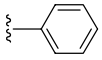
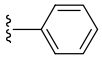
41		NH_2	0.28
42		OH[b]	0.33
43		NH_2	*0.066*
44		O′Bu[b]	0.65
45		OH[b]	0.12
46[a]		OBn[b]	0.60
47[a]		OH[b]	0.098

[a] P_3 cap = *t*-Boc.
[b] These compounds were prepared via solution phase synthesis.

groups were well tolerated, with phenyl glycine at P_2' exhibiting the best potency (compound **43**, Table 3, K_i* = 0.066 μM). Changing the P_2' terminal carboxamido group to carboxylic acid functionality retained most of the potency (compound **45**, Table 3). Similarly, both the *i*-Boc and *t*-Boc P_3 caps were essentially equipotent (compounds **45** and **47**, Table 3).

To demonstrate the importance of the P_2' carboxyl terminus (as carboxamido or carboxylic acid), target compounds lacking the P_2' carboxyl residue were synthesized in P_2 proline and P_2 leucine series. In both cases, these derivatives (**48** and **49**) had lost most of the binding potency, thus, clearly indicating the importance of the carboxyl functionality (Fig. 2).

X-ray crystal structure of the inhibitor **43** bound to the protease is shown in Figure 3.[16] The peptidic core is bound to the protease through a series of hydrogen bonding interactions. The catalytic Ser139 attacked the electrophilic keto functionality in a covalent and reversible manner. Interestingly, the amide carbonyl group adjacent to

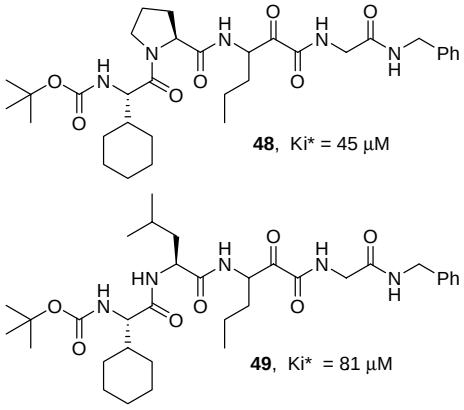


Figure 2.

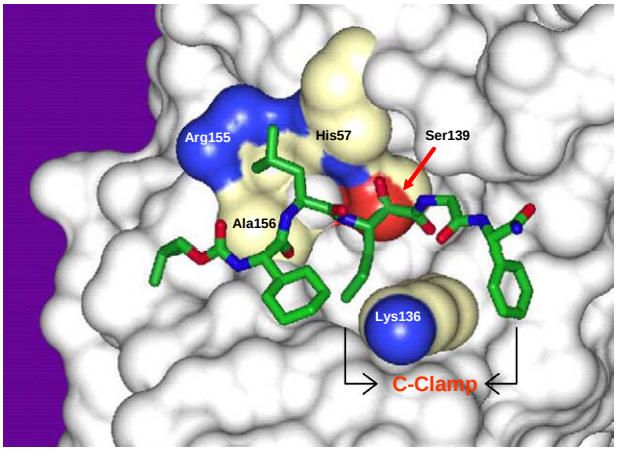


Figure 3. X-ray structure of **43** bound to the protease.

the keto moiety occupied the oxyanion hole, instead of the tetrahedral oxygen anion resulting from the attack of Ser139, as expected. The norvaline residue at P_1 is buried into the shallow hydrophobic S_1 pocket. The cyclohexyl moiety at P_3 occupied the S_3 pocket, providing additional hydrophobic contact with the enzyme, in comparison with isopropyl group (i.e., Val) at P_3. The P′ residue wrapped over the side-chain of Lys136. Most notably, the P_1–P_2' moiety formed a C-clamp locking Lys136 in place. The resultant increase in extensive hydrophobic interaction was translated into enhanced binding potency.

In summary, we have discovered that introduction of appropriate amino acid derivatives, specifically phenylglycine, at P_2' position improved the binding potency of P_3-capped ketoamide inhibitors of HCV NS3 serine protease. X-ray crystal structure of one of the inhibitors (**43**) bound to the protease revealed the importance of the P_2' moiety in enhancing the potency. It can be seen that the P_1–P_2' moiety formed a C-clamp locking Lys136, thereby making extensive hydrophobic interaction with the Lys side chain. This hydrophobic interaction, along with the hydrogen bonding interaction resulting from the P_2' carboxyl terminus, played a critical role in improving the binding potency. We are currently using this information advantageously in the design of other analogs in this series.

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