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Potent aza-peptide derived inhibitors of HCV NS3 protease

Srikanth Venkatraman *, Wanli Wu, Neng-Yang Shih, F. George Njoroge

Schering Plough Research Institute, K15-MS 3545, 2015, Galloping Hill Road, Kenilworth, NJ 07033, United States

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ABSTRACT

Chronic hepatitis C infection is the primary cause for cirrhosis of the liver and hepatocellular carcinoma leading to liver failure and transplantation. The etiological agent hepatitis C virus produces a single positive strand RNA that is processed further with the help of NS3 serine protease to produce mature virus. Inhibition of this protease can potentially be used to develop drugs for HCV infections. Boceprevir is a ketoamide derived novel inhibitor of HCV NS3 protease that has been progressed to clinical trials and proven to be efficacious in humans. Herein, we report our efforts in identifying an aza-peptide derivative as a potential second generation compound, that lacks electrophilic ketoamide group and are potent in enzyme and replicon assay.

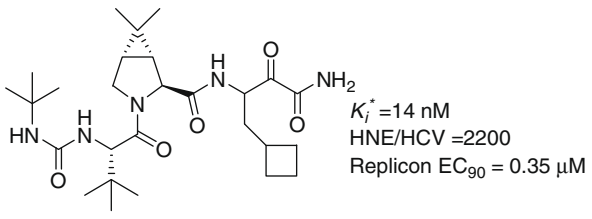
Hepatitis C virus is the primary etiological agent responsible for non-A, non B infections of the liver.[1] The prognosis for patients infected with hepatitis C is poor with a majority of these infections turning chronic progressing to cirrhosis of the liver and hepatocellular carcinoma. Pegylated alpha-interferon in combination with ribivirin is the primary standard of care which is effective in ~50% of genotype-1 infected patients.[2] Patients infected with genotype-2 respond better to Pegylated alpha-interferon with >80% of patients demonstrating sustained reduction in viral RNA.[2]

Lack of effective methods to treat genotype-1 infections and patients relapsing from interferon therapy necessitate discovery of new drugs. Significant efforts are now directed towards development of therapies that target key enzymes vital for HCV replication and maturation.[3]

Hepatitis C virus is a positive strand virus that encodes a single polyprotein of ~3000 amino acids. This polyprotein which contains all the structural and functional proteins is post translationally modified by HCV NS3 protease.[4] This enzyme with the assistance of cofactor NS4B catalyzes the cleavage of NS2–NS3, NS3–NS4A, NS4A–NS5B junctions to form functional proteins. HCV NS3, a serine protease has a shallow active site on the surface of the enzyme catalyzes the cleavage of cysteine–serine or a cysteine–threonine bond. Inhibition of this vital enzyme has been extensively investigated in an effort to develop potential drugs for the treatment of HCV infections. Boceprevir **1**[5] and VX-950 are two ketoamide derived inhibitors that have been advanced to Phase-III clinical trails and demonstrated excellent efficacy in humans. In addition, macrocyclic inhibitors ITMN-191, MK7006 and TMC435350 have also been progressed to clinical studies and have demonstrated excellent viral load reduction in patients. These compounds are currently being evaluated at various phases of clinical development.

Boceprevir **1** (Fig. 1), is a ketoamide containing inhibitor that readily epimerizes in human serum under physiological conditions at the P_1 center. The (*S*)-diastereomer at this center is the more potent isomer whereas the (*R*)-isomer is less active. Several attempts to avoid this epimerization by introduction of quaternary amino acids have resulted in significant loss in potency. A recent publication from a group at Boehringer Ingelhiem demonstrated that novel hydrazide derived inhibitor **2** spanning from P_6 to P'_1 demonstrated excellent enzyme binding[6] (Fig. 2). However these compounds were peptidic and no cellular activity in the replicon based assay was reported.

From our experience in developing ketoamide derived clinical candidate **1**, we discovered appropriate modifications of P_2, P_3 and P_4 moieties allowed truncation of inhibitors spanning from P_6 to P'_1 achieving compounds with lower molecular weights, desirable binding and pharmacokinetic profile. In this present work we envisioned that introduction of hydrazide analogs in our class of inhibitors may potentially provide compounds with better binding



1, Boceprivir

Figure 1.

* Corresponding author. Tel.: +1 908 740 3758; fax: +1 908 740 7152.
E-mail address: Srikanth.Venkatraman@spcorp.com (S. Venkatraman).

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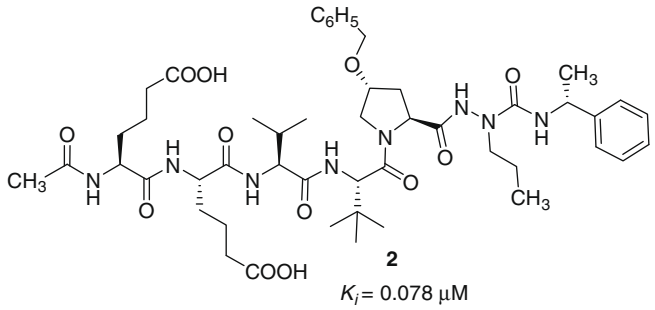


Figure 2.

than **2** and compounds with desirable cellular activity. In addition these modifications would depeptidize our compounds and provide inhibitors that are free of labile stereocenters which epimerized under physiological conditions. Pursuant to our identification of **1** we had also discovered that introduction of novel depeptidized P_3 caps allowed improvement in binding and cellular activity of **1.**[7] In this Letter we disclose our efforts to identify novel hydrazide derived inhibitors with excellent binding and cellular activity.

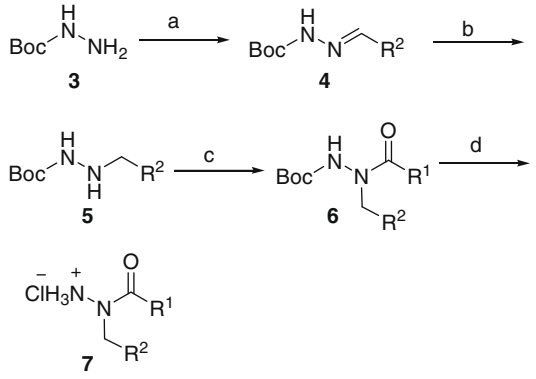
Synthesis of these hydrazide inhibitors is outlined in Schemes 1 and 2.

Condensation of Boc-carbazate **3** with desired aldehyde resulted in hydrazone of type **4** which were reduced to alkylated hydrazides using $NaBH_4$ and toluenesulfonic acid. The resulting alkylated hydrazides were acylated with acid chlorides or isocyanates to yield desired amides or ureas of type **6**. These acylated derivatives were deprotected to form hydrazide salts of type **7** that were further used for syntheses of inhibitors.

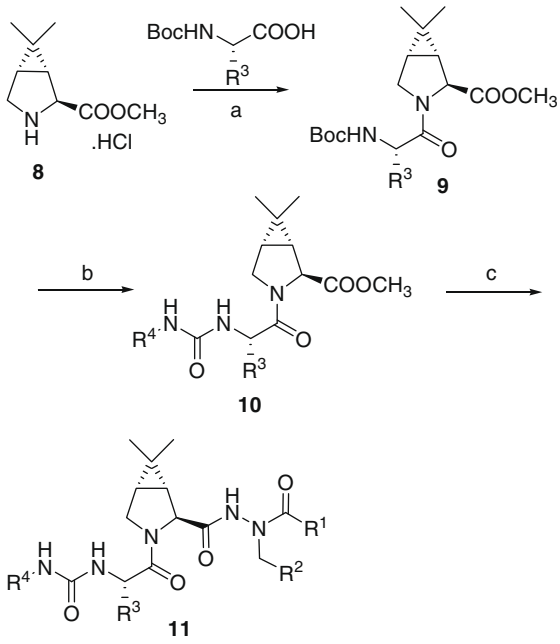
Coupling of dimethylcyclopropanated proline derivative **8**[8] with P_3 amino acid using HATU coupling agent[9] resulted in dipeptides of type **9.** The Boc group of **9** was deprotected using 4 M HCl in dioxane and capped with appropriate P_3-capping group using corresponding isocyanate to yield compounds of type **10.** The methyl ester **10** was hydrolyzed with aq lithium hydroxide and the resulting acid was coupled with hydrazide salt of type **7** to obtain azapeptide inhibitors of type **11**.

Synthesized inhibitors of type **11** were evaluated for their ability to inhibit HCV NS3 protease in a continuous assay as previously described[10] to obtain a K_i. Compounds with good binding were further evaluated in a replicon based cellular assay to determine EC_{90}.

Based on our experience from ketoamide derived inhibitors we decided to evaluate inhibitors derived from dimethylcylclopropanated proline as P_2, and glutarimide derived P_3 capping. We first



Scheme 1. Reagents and conditions: (a) R^2CHO, toluene, $TsOH{\cdot}H_2O$, reflux; (b) $TsOH{\cdot}H_2O$, $NaBH_4$, CH_2Cl_2; (c) R^1COCl, Et_3N, CH_2Cl_2 −78 °C; (d) 4 M HCl/dioxane.



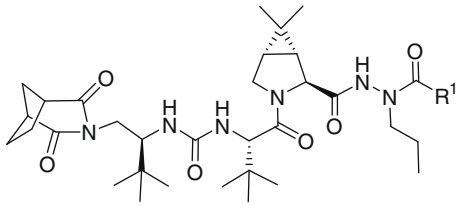
Scheme 2. Reagents and conditions: (a) HATU, NMM, CH_2Cl_2/DMF; (b) (i) 4 M HCl/dioxane; (ii) R^4NCO, NMM; (c) (i) aq LiOH, THF/MeOH; (ii) **7,** HATU, NMM.

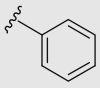
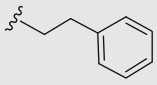
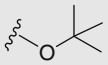
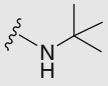
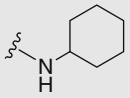
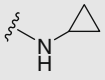
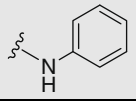
evaluated various novel P_1' groups derived from amides, carbamates and ureas (Table 1).

Introduction of benzamide at P_1' resulted in compound **12** that demonstrated a K_i = 52.0 μM (Table 1). Replacement of benzamide moiety with acetamide group resulted in compound **13** with K_i = 22 μM, a marginal improvement in potency compared to **12**. However, introduction of cyclopropyl amide resulted in compound **14** with improved binding K_i = 3.0 μM and EC_{90} = 15.0 μM. Similarly, incorporation of a phenethyl amide at P_1' resulted in compound **15** with much improved binding K_i = 0.72 μM and EC_{90} = 3.5 μM. This was our first compound in this series to demonstrate a binding less than 1 μM. We next explored the incorporation of carbamates and ureas at this position. Synthesis of the *tert*-butyl carbamate analog resulted in compound **16** with K_i = 3.1 μM and EC_{90} = 24.0 μM whereas introduction of *tert*-butyl urea resulted in compound **17** with K_i = 1.6 μM. Since the urea analogs demonstrated partially better binding than carbamates, we further explored SAR of the ureas analogs by synthesizing cyclohexyl, cyclopropyl and phenyl urea derivatives. The cyclohexyl urea analog **18** demonstrated a K_i = 1.3 μM with an EC_{90} = 16 μM whereas the cyclopropyl analog demonstrated a binding activity K_i = 0.75 μM and EC_{90} = 6.0 μM. The phenyl urea analog **20** had a K_i = 0.95 μM a similar activity as cyclopropyl derivative **19** but demonstrated a better cellular activity (EC_{90} = 2.4 μM).

A close analysis of Table 1 showed that the phenethyl amide derivative **15** and phenyl urea analog **20** were the more active compounds with enzyme binding less than 1 μM. To further improve binding in this series we decided to evaluate the SAR by incorporating α-methylbenzyl urea present in **2** (Table 2). This was also observed as the desirable group in identification of **2**. Concomitant to this modification we evaluated the effect of modification of the P_1, P_3 and P_3-capping groups. Introduction of α-methylbenzyl urea had a profound effect on the binding activity of these inhibitors. α-methylbenzyl urea analog with bicyclic imide cap and P_1 cyclopropylmethyl group resulted in compound **21** with K_i = 0.25 μM and EC_{90} = 1.1 μM. Replacement of bicyclic P_3 cap with dimethylcyclohexylimide cap resulted in compound **22** with K_i = 0.20 μM similar in activity to compound **21** and five fold improvement in activity compared to phenylurea **20.**

Table 1

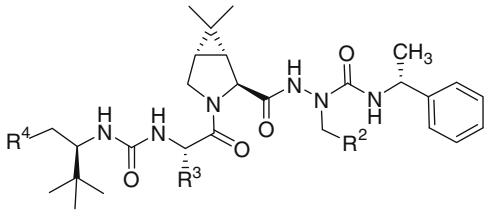


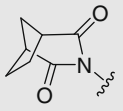
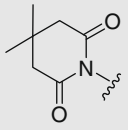
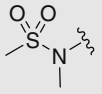
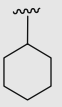
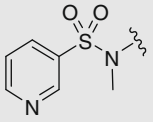
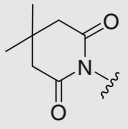
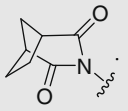
Entry	R^1	K_i (μM)	EC_{90} (μM)
12		52.0	NA
13		22.0	NA
14		3.0	15.0
15		0.72	3.5
16		3.1	24.0
17		1.6	8.0
18		1.3	16.0
19		0.75	6.0
20		0.95	2.4

We next evaluated the effect of modification of the P_3 group and its capping. Incorporation of methylsulfonamide derived cap with *n*-propyl P_1 residue resulted in compound **23** with $K_i = 0.11$ μM and $EC_{90} = 0.7$ μM; a 10-fold improvement in binding compared to **20.** This was the first compound with sub micro-molar cellular activity in this series of inhibitors. Introduction of pyridylsulfonamide derived P_3 cap with P_3 cyclohexyl glycine and P_1 propyl residue resulted in compound **24** with $K_i = 0.23$ μM a two fold loss in binding compared to methylsulfonamide derivative **23**.

Compound containing, dimethylglutarimide P_3-cap, P_1 propyl group, P_1' α-methylbenzyl urea resulted in inhibitor **25** with much improved binding ($K_i = 0.064$ μM) and $EC_{90} = 0.3$ μM. This was an aza-peptide analog with an EC_{90} comparable to our first generation clinical candidate **1**. Analog **25** was evaluated for its selectivity against human neutrophil elastase a structural homologous enzyme to HCV NS3 protease. The selectivity was determined to be K_i(HNE)/K_i(HCV) = 960. Incorporation of bicyclic imide derived P_3 cap with P_1 propyl group and P_1' α-methylbenzyl urea resulted in compound **26** with a binding of $K_i = 0.032$ μM and $EC_{90} = 0.25$ μM. This compound was more potent in binding and replicon cellular activity than the dimethylcyclohexylimide analog **25**. It was also slightly more active than our first generation compound **1.**

Table 2



Entry	R^2	R^3	R^4	K_i (μM)	EC_{90} (μM)
21				0.25	1.1
22				0.2	NA
23				0.11	0.7
24				0.23	NA
25				0.064	0.3
26				0.032	0.25

In conclusion we have identified a series of novel aza-peptide derived series of HCV NS3 protease inhibitors that demonstrated excellent binding and cellular activity. This provides a possible avenue for depetidization of peptidic analogs of our first generation compound **1**. By truncation of P_6—P_1' derived inhibitors we have identified a series of novel imide P_3-capped aza-peptide analogs that demonstrated excellent inhibition of HCV NS3 protease. SAR investigation of P_1' identified a phenethyl amide analog **15** and phenylurea derivative **20** which demonstrated submicro-molar enzyme binding activity and moderate cellular activities. Further modification of P_1' P_3 and P_3-capping residue resulted in identification of potent analogs such as **25** ($K_i = 0.064$ μM; $EC_{90} = 0.30$ μM) and **26** ($K_i = 0.032$ μM; $EC_{90} = 0.25$ μM) that contained imide P_3 capping and α-methylbenzyl urea P_1' and a propyl P_1 groups. These inhibitors are similar in potency to our first generation clinical candidate **Sch 503034**, but desirably lack the electrophilic trap and epimerizable ketoamide center. In addition the urea carbonyl moiety is non-electrophilic and serine does not make a covalent bond with the inhibitor.

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