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Potent ketoamide inhibitors of HCV NS3 protease derived from quaternized P_1 groups

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ABSTRACT

Blood borne hepatitis C infections are the primary cause for liver cirrhosis and hepatocellular carcinoma. HCV NS3 protease, a pivotal enzyme in the replication cycle of HCV virus has been the primary target for development of new drug candidates. Boceprevir and telaprevir are two novel ketoamide derived inhibitors that are currently undergoing phase-III clinical trials. These inhibitors include ketoamide functionality as serine trap and have an acidic alpha-ketoamide center that undergoes epimerization under physiological conditions. Our initial attempts to arrest this epimerization by introducing quaternary amino acids at P_1 had resulted in significantly diminished activity. In this manuscript we describe alpha quaternized P_1 group that result in potent inhibitors in the enzyme assay and demonstrate cellular activity comparable to boceprevir.

Hepatitis C virus is the primary etiological agent responsible for non A, non B infections of the liver.[1,2] The prognosis for patients infected with hepatitis C is poor with majority of these infections turning chronic. In many cases these infections progress to liver cirrhosis and liver carcinoma.[3,4] Peginterferon in combination with antiviral ribavirin is the primary standard of care, which is effective in ~40% of genotype-1 infected patients.[5–9] Patients infected with genotype-2 or 3 respond better to peginterferon with >80% of patients demonstrating a sustained virologic response after treatment.

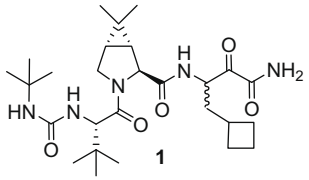
Lack of effective methods to treat genotype-1 HCV infections and patients relapsing from failed peginterferon/ribavirin therapy necessitates development of new drugs. Significant efforts are now directed towards development of therapies that target key viral enzymes vital to HCV replication and maturation.

Hepatitis C virus is a positive strand virus which encodes for a single polyprotein of ~3000 amino acids. This polyprotein that contain all the structural and functional proteins is post-translationally modified by a single HCV NS3 serine protease. This protease with the assistance of the cofactor NS4A catalyzes the cleavage of the NS3-NS4A, NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B junction to form functional proteins.[10–12] HCV NS3 protease has a shallow active site on the surface of the enzyme that catalyzes cleavage of a cysteine–serine or a cysteine–threonine bond.[13–15] Inhibition of this key enzyme has been extensively investigated in an effort to develop potential drugs for the treatment of HCV infections. Boceprevir (Fig. 1) (**1**)[16] and telaprevir[17] are two novel ketoamide derived inhibitors that has been progressed to phase-III clinical trials.

Boceprevir and telaprevir are ketoamide containing inhibitors that have a P_1 stereocenter that readily epimerizes in human serum under physiological conditions. The (*S*)-diastereomer at P_1 site is the active antipode whereas the (*R*)-isomer is less potent.

Several unsuccessful attempts have been made to avoid this epimerization by introducing a quaternary amino acid at P_1 resulting in significant loss in potency.[18,16] The significant loss in activity could potentially be attributed to the presence of quaternary center at the alpha position of the ketoamide carbonyl group, that could hinder serine-139 from attacking the carbonyl moiety. In this manuscript, we disclose a series of ketoamide derived inhibitors containing small cyclic quaternary amino acid derived P_1 residues that demonstrate similar potency to our first generation compound **1**.

An investigation of X-ray structure of **1** bound to the NS3 protease revealed that the catalytic serine-139 attacked the ketoamide group of the inhibitor from the *Re* face. This resulted in orientation of amide group towards His-57 into the oxy-anion hole. This is in



Boceprevir
K_i^* = 14 nM
EC_{90} = 0.35 μM

Figure 1.

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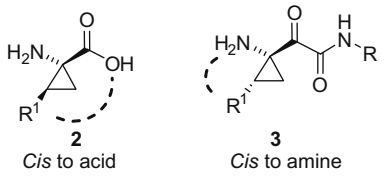


Figure 2.

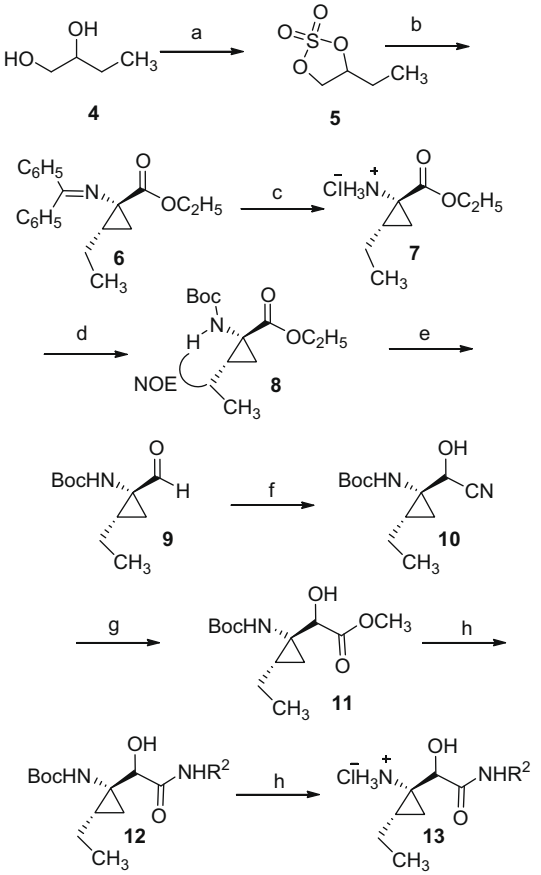
contrast to acid derived inhibitors such as BILN-2061 which binds from the *Si* face of the carbonyl group with one of the oxygens of the carboxylic acid being directed into the oxy-anion hole while other forming a hydrogen bond with His-57.[19] It is also well known that BILN-2061 has a vinyl cyclopropyl derived amino acid at P_1. We reasoned that the difference in binding modes of the ketoamide and acid derived inhibitors required different spatial arrangement of the alkyl groups attached to the cyclopropyl moiety (R^1, Fig. 2) in the ketoamide and acid series of inhibitors to occupy the S_1 pocket effectively.

For an optimum occupation of the S_1 pocket in the acid series of inhibitors the R_1 group should be *cis* to the carboxylic acid, whereas in the ketoamide series of inhibitors the R_1 residue should be oriented away from the acid such that it is *cis* to the amino group. With this paradigm we decided to explore quaternary amino acids at P_1 in the ketoamide series derived from cyclopropyl and cyclobutyl groups.

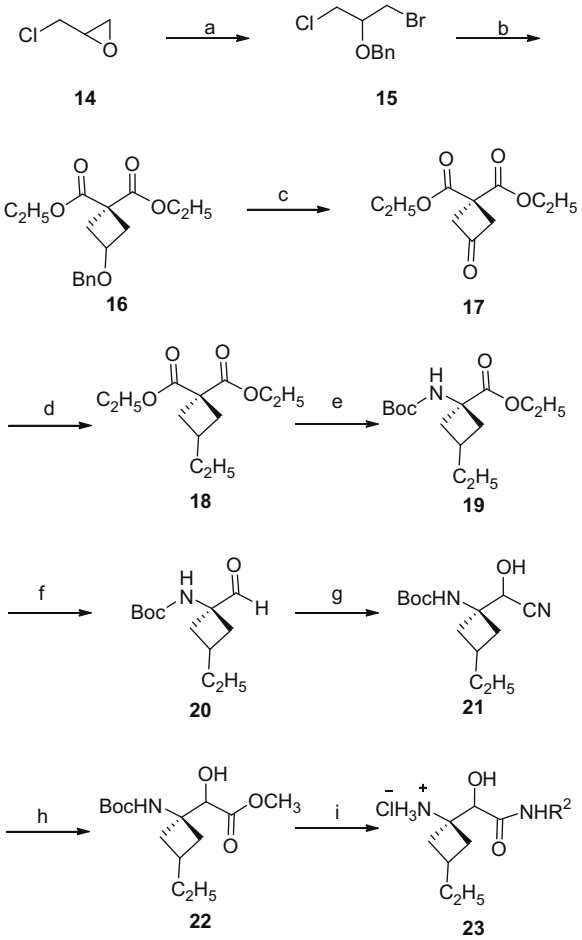
The synthesis of P_1 cyclopropyl derived hydroxy amide fragment is shown in Scheme 1. Reaction of diol **4** with $SOCl_2$ resulted in cyclic sulfinate that was oxidized with ruthenium trichloride resulting in cyclic sulfate **5**.[20] Reaction of **5** with NaH and ethyl 2-(diphenylmethyleneamino)acetate yielded cyclopropyl derived amino acid[21] **6** which was hydrolyzed to form amine salt **7** using aq 1 M HCl. The amine salt **7** was treated with di-*tert*-butyl dicarbonate to form Boc-protected compound **8**. Analysis of **8** by NMR demonstrated that the ethyl group was *cis* to the amino group. This was confirmed by a positive NOE enhancement between the carbamate NH proton and the ethyl group attached to the cyclopropyl ring. Reduction of ester **8** with $LiBH_4$ followed by oxidation of the resultant alcohol with Dess–Martin reagent resulted in aldehyde **9**. Aldehyde **9** was treated with acetone cyanohydrin resulting in **10**, which was converted to hydroxy ester **11** by refluxing with methanolic HCl followed by Boc protection, Hydrolysis of methyl ester of compounds **11** followed by coupling with appropriate amines using HATU yielded amides of type **12**, which were deprotected with 4 M HCl in dioxane to form amine salts of type **13**.

Alternatively aldehyde **9** was subjected to Passerini reaction[22,23] with alkylisonitriles and acetic acid to directly install secondary hydroxyamide groups resulting in intermediate of type **12** that was converted to amine salt **13**. Other cyclopropyl P_1 analogs were also synthesized using similar methods.

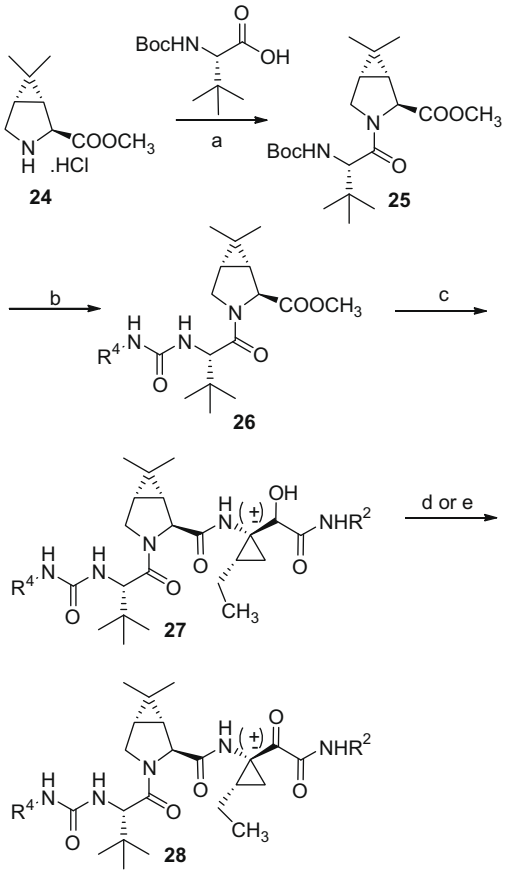
Synthesis of cyclobutyl derived P_1 fragment was initiated from epichlorohydrin **14** as outlined in Scheme 2. Treatment of **14** with



Scheme 1. Reagents and conditions: (a) (i) $SOCl_2$, CCl_4, 80 °C; (ii) $RuCl_3{\cdot}3H_2O$, HIO_5, CCl_4/CH_3CN; (b) NaH, DME, $(C_6H_5)_2C{=}NCH_2COOC_2H_5$ reflux, 2 h; (c) (i) aq HCl, THF; (d) Boc_2O, $^iPr_2NEt$, CH_2Cl_2; (e) (i) $LiBH_4$, THF; (ii) Dess–Martin reagent; (f) acetone cyanohydrin, Et_3N; (g) (a) 6 M methanolic HCl reflux; (b) Boc_2O, CH_2Cl_2 (h) (i) aq LiOH, THF/MeOH; (ii) R^2NH_2, HATU, NMM; (i) 4 M HCl in dioxane, rt.



Scheme 2. Reagents and conditions: (a) BnBr, HgCl, 12 h, 150 °C, 70%; (b) $H_2C(COOC_2H_5)_2$, NaH, 110 °C, 72 h, 35%; (c) (i) $Pd(OH)_2$, H_2, EtOAc, 2 h, 70%; (ii) Dess Martin reagent, CH_2Cl_2, 70%; (d) (i) $C_2H_5PPH_3Br$, ^{t}BuOK, THF; (ii) H_2/Pd/C, MeOH; (e) (i) LiOH, THF/MeOH; (ii) DPPA, ^{t}BuOH, reflux; (f) (i) $LiBH_4$, THF; (ii) Dess–Martin reagent; (g) acetone cyanohydrin, Et_3N; (h) (a) 6 M methanolic HCl; (b) Boc_2O, CH_2Cl_2 (i) (i) aq LiOH, THF/H_2O; (ii) R^2NH_2, HATU, NMM; (iii) 4 M HCl in dioxane.



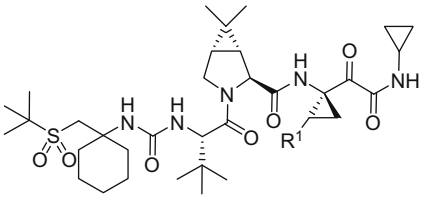
Scheme 3. Reagents and conditions: (a) HATU, NMM, CH_2Cl_2/DMF; (b) (i) 4 M HCl in dioxane; (ii) R^4NCO, NMM; (c) (i) aq LiOH, THF/MeOH; (ii) **13**, HATU, NMM; (d) when (R^2 = cyclopropyl) Dess–Martin reagent CH_2Cl_2; (e) when (R^2 = H), EDCI, $Cl_2CHCOOH$, toluene.

benzyl bromide in the presence of HgCl yielded benzylated alcohol **15** in 70% yield.[24] Treatment of **15** with diethylmalonate and NaH formed cyclobutyl derivative **16**. The benzyl ether of **16** was catalytically hydrogenated with H_2/Pd(OH)$_2$ and the resulting alcohol was further oxidized to ketone **17** using Dess–Martin reagent. Ketone **17** was elaborated using Wittig reaction by treatment of ethyl phosphonium bromide and K^{t}BuO. The resulting olefin was catalytically hydrogenated to generate ethyl substituted cyclobutyl derivative **18**. Basic hydrolysis of ethyl ester with one equivalent of lithium hydroxide resulted in the selective hydrolysis of the ethyl ester *syn* to the alkyl group. The obtained acid was treated with DPPA and *tert*-butanol to induce a Curtius rearrangement to form Boc-protected amino ester **19**, which was converted to the P_1 hydroxy amides of type **23** using similar steps outlined for the synthesis of the cyclopropyl derivative in Scheme 1.

The methodology used for the synthesis of desired inhibitors is outlined in Scheme 3. Coupling of dimethyl cyclopropylproline derivative **24**[25,26] with *tert*-butylglycine using HATU resulted in compound **25**.[16] Deprotection of the Boc group **25** followed by treatment of the resultant amine salt with appropriate isocyanate yielded compounds of type **26**, which was further coupled with P_1 segment of type **13** to yield hydroxy amides of type **27** that were oxidized to form the corresponding ketoamide inhibitors of type **28**. P_1 diastereomers of synthesized inhibitors were separated using YMC diol column under normal phase HPLC conditions.

Synthesized inhibitors were evaluated for inhibition of NS3 protease in a continuous enzyme binding assay to obtain binding constant K_i^*.[27] Compounds that demonstrated good binding were further evaluated in a replicon based cellular assay to establish

Table 1



Entry	R^1	K_i^* (μM)
29	H	18.0
30	CH_3	3.0
31	CH_3CH_2	0.11
32	$CH_3CH_2CH_2$	0.70

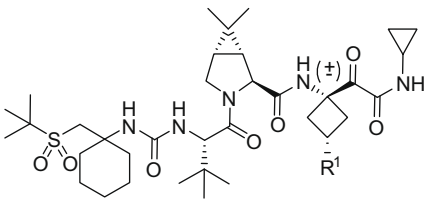
the EC_{90}.[28] The effect of substitution on the cyclopropyl group with various alkyl groups is shown in Table 1.

Incorporation of unsubstituted cyclopropyl ketoamide at P_1 resulted in compound **29** with a K_i^* = 18 μM. The corresponding norvaline P_1 analog demonstrated a K_i^* = 5 nM indicating a huge loss in potency by quaternizing P_1. The effect of substitution at P_1 cyclopropyl ring was evaluated by incorporating alkyl group syn to amine. Introduction of a methyl group on the cyclopropyl ring resulted in compound **30** with a K_i^* = 3.0 μM, a six fold improvement in activity compared to the unsubstituted cylopropyl derivative **29**. Replacement of the methyl group with ethyl substituent resulted in compound **31** which had a binding of K_i^* = 0.11 μM. Thus, the ethyl group had a profound effect on binding with a 160-fold improvement in binding compared to the cyclopropyl compound **29**. However, extension of **29** with propyl group resulted in compound **32** (K_i^* = 0.7 μM) a sevenfold loss in activity compared to ethyl compound **31**.

We also evaluated the effect of cyclobutyl group at P_1 (Table 2). P_1 diastereomers in the cyclobutyl series were not readily separable in HPLC; therefore they were assayed as mixtures. Introduction of unsubstituted cyclobutyl at P_1 resulted in compound **33** with a binding (K_i^* = 15 μM) similar in activity to the cyclopropyl derivative **29**. Addition of a methyl group on the cyclobutyl ring resulted in compound **35** with a K_i^* = 0.42 μM whereas addition of an ethyl group resulted in compound **36** with a K_i^* = 0.19 μM. The introduction of an ethyl substituent resulted in a dramatic improvement in binding, similar to the effect of this substitution observed in the cyclopropyl series. However, incorporation of a polar substituent such as a hydroxyl group was not well tolerated resulting in compound **34** (K_i^* = 89 μM).

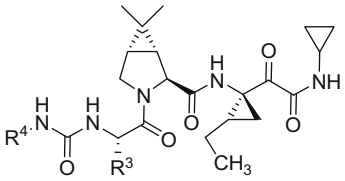
Having identified that a cylopropyl or a cyclobutyl group with an ethyl substitution provided potent inhibitors, we next explored the effect of variation of the P_3 and P_3 capping groups to optimize the binding activity and cellular potencies. Since P_1 diasteromer in the cyclopropyl series were readily separable it was used for fur-

Table 2



Entry	R^1	K_i^* (μM)
33	H	15
34	OH	89
35	CH_3	0.42
36	CH_3CH_2	0.19

Table 3



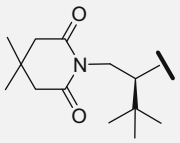
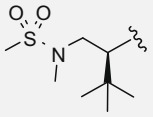
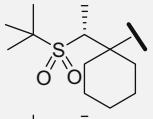
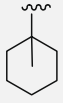
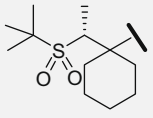
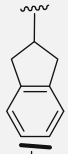
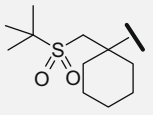
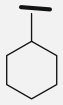
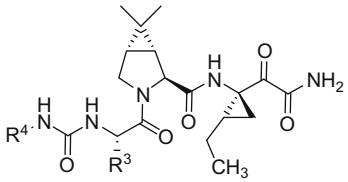
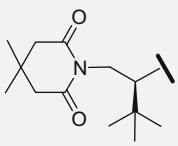
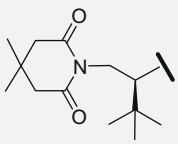
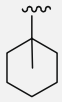
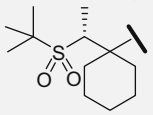
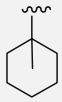
Entry	R^4	R^3	K_i^* (μM)	EC_{90} (μM)
37			0.082	0.5
38			0.20	2.7
39			0.95	–
40			0.50	–
41			0.6	–

Table 4



Entry	R^4	R^3	K_i^* (μM)	EC_{90} (μM)
42			0.058	1.6
43			0.023	0.40
45			0.015	0.40

ther SAR studies. The effects of these modifications are summarized in Table 3.

Replacement of *tert*-butyl sulfone derived P_3 capping of **31** with dimethylglutarimide derived cap[29] resulted in compound **37** with a $K_i^* = 0.082$ μM and $EC_{90} = 0.5$ μM. Introduction of methylsulfonamide[30] derived P_3 cap resulted in compound **38** with a $K_i^* = 0.2$ μM and $EC_{90} = 2.7$ μM. We next evaluated the effect of modification of P_3 substitution. Incorporation of β-methylcyclohexylglycine at P_3 position resulted in compound **39** with diminished activity ($K_i^* = 0.95$ μM) compared to *tert*-butyl derivative **31**. Similarly incorporation of indanylglycine at P_3 resulted in compound **40** with binding $K_i^* = 0.5$ μM and introduction of cyclohexylglycine yielded compound **41** with $K_i^* = 0.6$ μM. Both these modifications yielded compounds with lower activity than the *tert*-butyl glycine P_3 substitution.

We next evaluated the replacement of P_1' cyclopropyl amide with a primary amide (Table 4). Replacement of cyclopropylamide of compound **37** with primary amide resulted in compound **42** with improved binding ($K_i^* = 0.058$ μM); however it was less potent in the cellular assay ($EC_{90} = 1.6$ μM) than **37**. The replacement of P_3 *tert*-butyl group of compound **42** with β-methylcyclohexylglycine resulted in compound **43** with $K_i^* = 0.023$ μM and $EC_{90} = 0.4$ μM; comparable cellular activity to the compound **1**. Similarly replacement of P_1' cyclopropyl amide of P_3 β-methycylohexylglycine containing compound **39** with primary amide resulted in compound **45** which had a $K_i^* = 0.015$ μM and $EC_{90} = 0.40$ μM.

In an effort to address the epimerization of ketoamide inhibitor in plasma we investigated the possibility of quaternizing the P_1 center. Our initial efforts in this direction by introducing a α-methyl substituent had resulted in complete loss in binding. We therefore investigated introduction of small cyclic groups such as cyclopropyl and cyclobutyl derived amino acids. Aided by modeling we also reasoned that this amino acid required an alkyl substituent projecting from the ring such that it was oriented *syn* to the amino group to effectively occupy S_1 pocket. Efficient syntheses of these P_1 amino acids were developed and incorporating them into the inhibitors demonstrated early that an ethyl substituent was preferred yielding compounds **31** and **36** with modest binding activity. In an effort to further improve binding and cellular activity, a systematic variation of P_3 and P_3 capping groups were investigated. Introduction of dimethylglutarimide derived P_3 capping resulted in compound **37** with improved binding activity ($K_i^* = 0.082$ μM) and $EC_{90} = 0.5$ μM. Further SAR investigation at P_1' identified the beneficial effect of introducing the primary amide functionality. Incorporation of P_1' primary ketoamide resulted in inhibitors **43** and **45** that demonstrated $K_i^* = 0.023$ μM and 0.015 μM. This was similar in potency to our first generation clinical candidate **1**. These compounds also demonstrated good cellular activity in the replicon based cellular assay achieving an $EC_{90} = 0.40$ μM which is similar to that of **1**. Thus, we have demonstrated that incorporation of a P_1 quaternary amino acid is well tolerated in the ketoamide series of inhibitors achieving similar binding and cellular potency to boceprevir. However, it is unclear that if these compounds inhibit HCV protease by reversibly trapping serine-139. Further studies are in progress to establish if the gain in potency was mainly due to hydrogen bonds and lipophilic interactions only or do these compounds still interact with the enzyme covalently.

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