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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4515–4519
Bioorganic & Medicinal Chemistry Letters

Hepatitis C virus NS3-4A serine protease inhibitors: Use of a P_2–P_1 cyclopropyl alanine combination for improved potency

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Received 18 May 2005; revised 6 July 2005; accepted 6 July 2005
Available online 19 August 2005

Abstract—Modification of the P_2 and P_1 side chains of earlier P_3-capped α-ketoamide inhibitor of HCV NS3 serine protease **1** resulted in the discovery of compound **24** with about 10-fold improvement in potency.

1. Introduction

Hepatitis C virus (HCV), a blood-borne virus previously referred to as non-A, non-B hepatitis, infects 200 million people worldwide.[1] Untreated HCV infections can progress to liver cirrhosis and hepatocellular carcinoma.[2] Currently, α-interferon and PEG-interferon monotherapies or in combination with the antiviral drug ribavirin are the only approved treatment options.[3] Although combination therapy is reasonably successful with genotypes 2 and 3, its efficacy against the predominant genotype 1 is moderate at best. Therefore, several research groups have been working toward the development of a more effective, convenient, and tolerable treatment. Because of its vital role in viral replication,[4] HCV NS3 serine protease has been actively pursued as a viral protein target.[5] Oligopeptide 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.

More recently, we demonstrated that introduction of a phenylglycine residue at P'_2 position improved the binding potency of P_3-capped ketoamide inhibitor of type **1** (Fig. 1).[8] From the X-ray crystal structure of compound

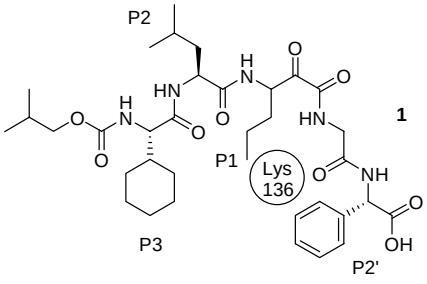

Figure 1.

1 bound to the protease, it was evident that the P_1–P'_2 side chains formed a C-clamp around Lys136 of the protease and thus provided extensive hydrophobic interaction that resulted in improved potency. X-ray crystal structure analysis also revealed a close proximity of P_2 side chain to arginine 155. Unsuccessful attempts from our group[6] and others[9] to make a salt bridge between Arg155 and a variety of charged species at P_2 prompted us to evaluate the possibility of hydrophobic interactions with the side chain of that residue and hydrophobic moieties of the P_2 position. To assess the effect of replacement of leucine at P_2 with more lypophilic residues, we decided to take advantage of hydrophobic sulfide moiety by introducing thioketals in the P_2 position. Better hydrophobic contact could also be accomplished via incorporation of larger alkyl and cycloalkyl chains at that position (Fig. 2).

Keywords: Hepatatis C; P3 capped ketoamide; Inhibitor.
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doi:10.1016/j.bmcl.2005.07.009

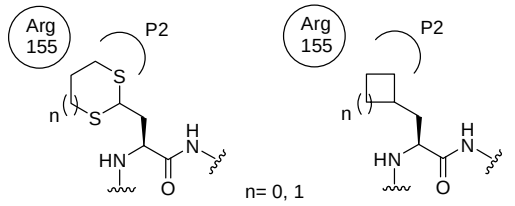
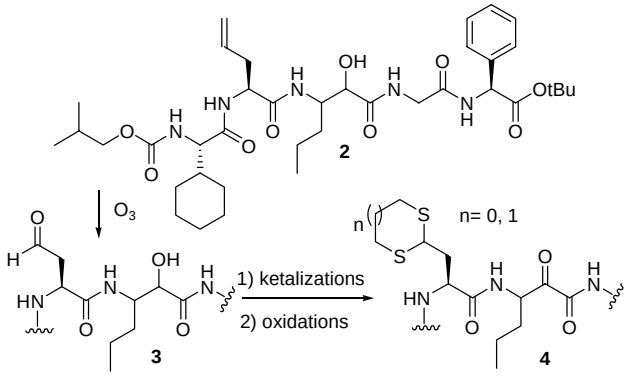


Figure 2.

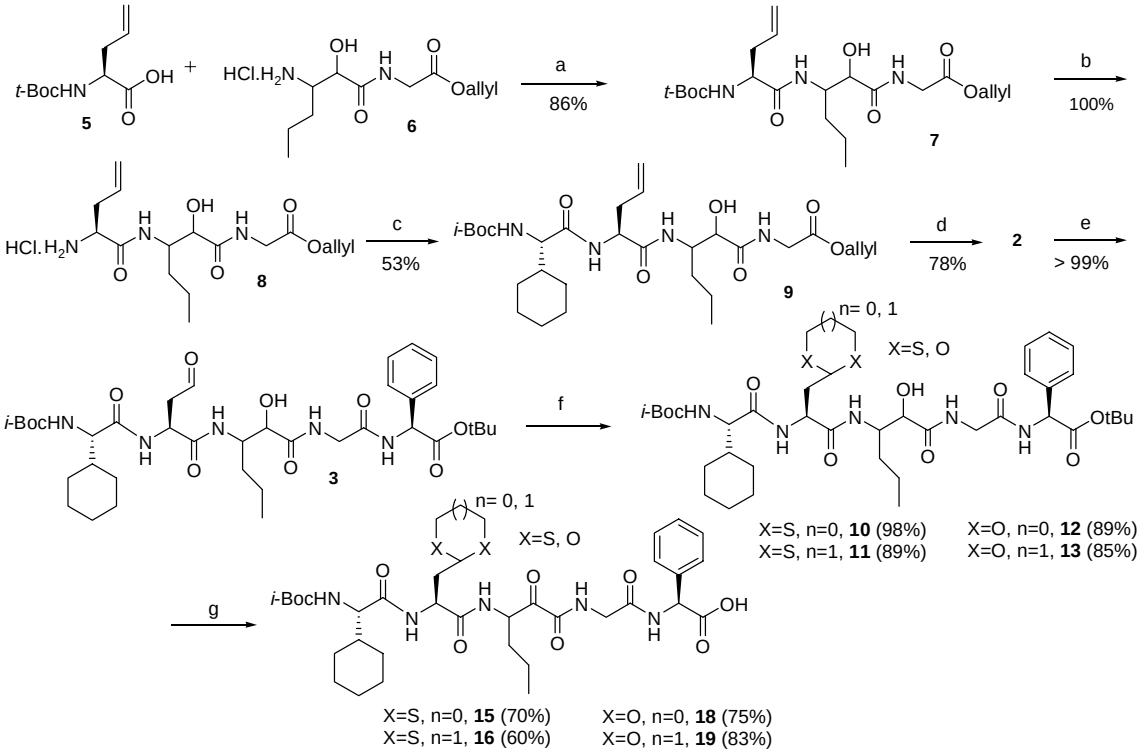


Scheme 1.

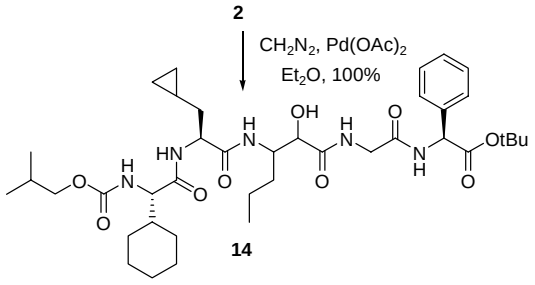
Hydroxyl amide **2**, containing P3–P2′ residues, was identified as a key intermediate for the synthesis of ketals at P_2 (Scheme 1).

Aldehyde **3** generated through ozonolysis of the allyl moiety could provide, after ketalization and subsequent oxidation, inhibitors of type **4** bearing desirable lypophilic P_2 surrogates.

Synthesis of α-hydroxyl amide **2** and inhibitors of type **4** are depicted in Scheme 2. Thus, dipeptide **6**,[6] bearing the hydroxyl amide moiety, was reacted with P_2 allylglycine residue **5** under standard coupling procedure (EDCI, HOOBt, NMM) to provide allyl ester **7**. After removal of the *t*-Boc protecting group of **7**, the *i*-Boc protected cyclohexyl glycine moiety at P_3 was incorporated, via **8**, using the coupling conditions described earlier. Saponification of the allyl ester **9** followed by incorporation of the P_2' moiety by coupling to phenylglycine amino acid derivative gave key intermediate **2** (Scheme 2). Ozonolysis of **2** generated the desired aldehyde intermediate **3** as the minor product with methoxy ketals as the major products of the reaction as established by NMR and mass spectrometry analysis. The mixture was then subjected to transketalization procedure; thus, use of $Et_2O{\cdot}BF_3$ efficiently promoted thioketal formation but unfortunately cleaved the *tert*-butyl ester moiety at P_2'. Control of temperature, reaction time or amount of $Et_2O{\cdot}BF_3$ did not afford the desired chemoselectivity. However, the side reaction was totally eliminated by employing a milder Lewis acid for activation. Use of 1.1 equiv of zinc triflate and 2.5 equiv of 1,2-ethanedithiol afforded in <24 h the desired thioketal **10** in high yield.



Scheme 2. Reagents and conditions: (a) EDCI, HOOBt, NMM, DMF/CH_2Cl_2; (b) 4 M HCl, dioxane; (c) *i*-Boc-cyclohexyl glycine, EDCI, HOOBt, NMM, DMF; (d) (i) aq 1 N LiOH, THF/H_2O; (ii) H-PhG-O-*t*-Bu, EDCI, HOOBt, DIPEA, DMF; (e) (i) O_3, CH_2Cl_2/MeOH, −78 °C; (ii) DMS, rt, 12 h; (f) X = S, di-thiols (2.5 equiv), $Zn(OTf)_2$, (1.1 equiv), CH_2Cl_2, X = O, diols (10 equiv), molecular sieves, TsOH cat, CH_2Cl_2, (g) (i) Swern (X = S), Dess–Martin (X = O), (ii) 50% TFA/CH_2Cl_2.



Scheme 3.

Oxidation of **10** to generate the corresponding α-ketoamide **15** was rather challenging. Thus, with a stoichiometric amount of Dess–Martin's periodinane,[10] the reaction was totally chemoselective toward the sulfide oxidation. Use of tetrapropyl ammonium perruthenate[11] (TPAP) or SO_3–pyridine[12] complex as oxidizing reagent led mainly to degradation products. Moffatt[13] oxidation furnished the desired ketoamide albeit with a M+16 adduct that was detected by mass spectrometry analysis. Interestingly, Swern[14] oxidation of thioketal **10** provided, chemoselectively, the corresponding α-ketoamide in good yield (70%). Finally, hydrolysis of the terminal *tert*-butyl ester group with 50% TFA in CH_2Cl_2 gave the desired target **15**. Compounds **16** and **17** were prepared in a similar fashion using 1,2-propanethiol and 1,2-benzenedime-thanethiol, respectively. The oxygen analogs **18** and **19** were also prepared from common intermediate **3**. In the case of the oxygen analogs, transketalization was performed in the presence of molecular sieves and a catalytic amount of TsOH. Dess–Martin's periodinane oxidation followed by TFA deprotection of the terminal *tert*-butyl ester group furnished the desired α-ketoamides **18** and **19** without affecting the ketal group. P_2 alkyl and cycloalkyl chain analogs **20**, **21**, and **22** were prepared via solid-phase synthesis[8] using Sasrin resin. Inhibitor **23**, bearing a cyclopropyl alanine at P_2 was prepared from key intermediate **2**. As described in Scheme 3, cyclopropanation of the allyl group was efficiently performed with CH_2N_2 and a catalytic amount of $Pd(OAc)_2$.[15] Dess–Martin oxidation of **14** followed by deprotection of the *tert*-butyl ester moiety with TFA provided **23** in high yield.

2. Results and discussion

Because of the close proximity of the P_2 side chain to Arg155, we anticipated that introduction of larger hydrophobic moieties off the P_2 position of our inhibitors would present favorable interactions with this residue. Toward this end, we investigated replacement of leucine at P_2 with more lipophilic moieties. Thus, with a K_i^* of 0.12 μM, inhibitor **1** was a good candidate for P_2 optimization. HCV NS3 serine protease inhibitory activity[16] for the targets synthesized was obtained using the continuous spectrophotometric assay previously reported.[17] For the thioketals series, the steric factor seemed to be critical for the activity, thus, the five-membered thioketals **15** with a K_i^* of 0.09 μM was more potent than the six-membered analog **16** ($K_i^* = 0.25$ μM). The aromatic ring of the benzenedimethane thioketal **17** did not provide any additional hydrophobic interaction with the enzyme backbone as demonstrated by its poorer potency ($K_i^* = 2.3$ μM). The smaller the ring, the better was the activity; thus, compound **15**, with a five-membered thioketals group, turned out to be the most potent ($K_i^* = 0.09$ μM) of all the inhibitors evaluated in this series. For the oxygenated compounds, the same trend was also observed with the five-membered ketal **18** ($K_i^* = 0.30$ μM) being more potent than the six-membered analog **19** ($K_i^* = 0.43$ μM). In the two series evaluated, the thioketal showed better activity than the oxygen counterpart with more than 3-fold loss in activity for the five-membered ketal **18** compared to the thioketals analog **15**. This was in good agreement with the hydrophobic character of sulfide versus ether and confirmed the need for a hydrophobic side chain at P_2.

To achieve better hydrophobic contact with Arg155, we also tried to incorporate bulkier leucine analogs at P_2. From the HCV protease inhibitory data obtained for compounds **20** and **21** ($K_i^* = 0.31$ and 0.30 μM, respectively) compared to compound **1** ($K_i^* = 0.12$ μM), it was clear that steric factors were critical for activity. Incorporation of long side chains homo-leucine in **20** and homo-norleucine in **21** resulted in about 3-fold loss in activity. Although we anticipated that hydrophobic moiety should be well accommodated at this position, we noticed that larger substitutions at P_2 did not provide enhancement in activity. SAR of the ketals series indicated that the smaller the size of P_2 modification, the better was the activity. Using those observations, we prepared compound **22** bearing a cyclobutyl alanine moiety at P_2. Inhibitor **22**, with a K_i^* of 0.14 μM, was equipotent to the leucine analog **1** ($K_i^* = 0.12$ μM). Incorporation of the smaller cyclopropyl ring in **23** resulted in more than 2-fold improvement in activity ($K_i^* = 0.05$ μM). From the X-ray analysis (Fig. 4), we could see that the cyclopropyl ring exhibited good van der Waals contacts with the two terminal NH groups of Arg155. Distances were 3.52 and 3.26 Å from the nearest methylene group of the ring. The Cγ of Arg 155, the nearest methylene group, was 4.41 Å from the same methylene group of the cyclopropyl ring. These observations were in good agreements with the cyclopropyl ring filling the S_2 pocket in an optimum manner.

Targets included in Table 1 were prepared using *n*-valine as the P_1 residue. We carried out modifications aimed at optimizing the P_1 residue by retaining cyclopropyl alanine at P_2 since it had previously demonstrated

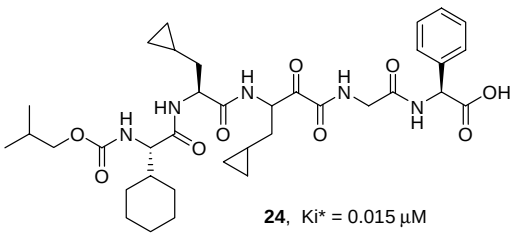


Figure 3.

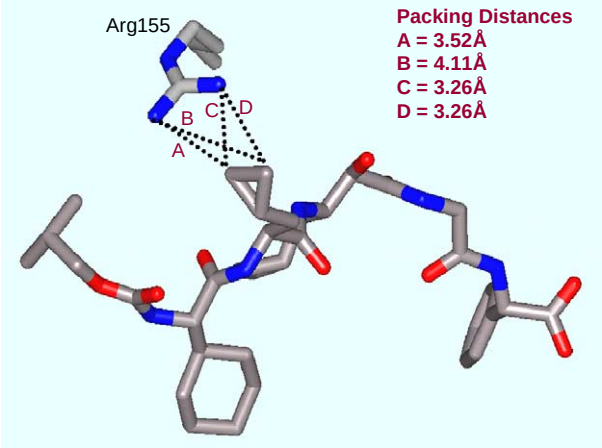


Figure 4. Packing distances of cyclopropyl alanine at P2 with Arg 155.

improved potency. We discovered that incorporation of a cyclopropyl alanine at P_1 also provided a real boost in activity. Thus, inhibitor **24** (Fig. 3) exhibited the best potency in that series with $K_i^* = 0.015$ μM.

X-ray crystal structure of the inhibitor **24** bound to the protease is shown in Figure 5.[18] It can be seen that the peptidic core binds to the protease through a series of hydrogen bonding interactions. The cyclopropyl alanine residues at P_2 and P_1 fit well in the S_2 and S_1 pockets, respectively, making tight hydrophobic contacts. The cyclohexyl moiety at P_3 provided additional hydrophobic contacts in comparison with smaller groups at P_3.[8] The P_1–P_2' residues form a 'C-clamp' that wraps over the side chain of lys136.

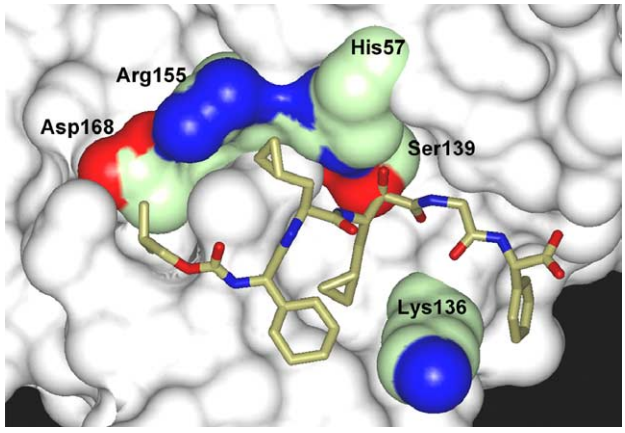
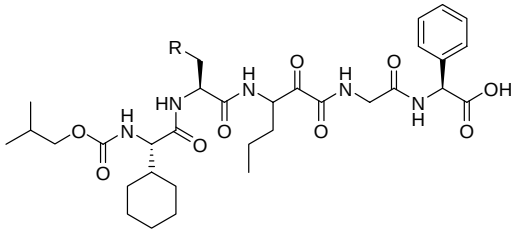
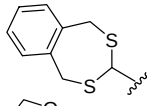
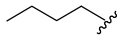


Figure 5. X-ray structure of **24** bound to the protease.

In summary, we have identified potent inhibitors of the HCV NS3 serine protease. Incorporation of a cyclopropyl alanine side chain at both P_2 and P_1 improved the binding potency by about 10-fold compared to our earlier P_3-capped inhibitors **1**. Further work aimed at the depeptidization of **24** is under progress and will be reported shortly.

Table 1. Synthesis of inhibitors **15**–**23**



Entry	Compound no.	R	K_i^* (μM)
1	**15**		0.09
2	**16**		0.25
3	**17**		2.3
4	**18**		0.30
5	**19**		0.43
6	**20**		0.31
7	**21**		0.30
8	**22**		0.14
9	**23**		0.05

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18. Crystallographic data for the structures in this article have been deposited with the RCSB Protein Data Bank as PDB ID 2A4R. The structural details can be viewed at www.rcsb.org using the ID number above.