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P4 cap modified tetrapeptidyl α-ketoamides as potent HCV NS3 protease inhibitors

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Abstract—We describe herein the design, syntheses, and biological evaluation of new series of P4 tetrazole and adipic acid, ester, amide capped tetrapeptidyl α-ketoamide based HCV protease inhibitors.

1. Introduction

Hepatitis C virus (HCV) infection has become a serious health threat to our society, which poses risk of developing significant morbidity and mortality.[1] Chronic hepatitis C is the leading cause for liver transplantation in the USA. The Centers for Disease Control and Prevention estimates that chronic HCV infection is responsible for more than 10 thousands deaths annually. The combination of pegylated-IFN-α and ribavirin is now the standard therapy and produces a sustained virologic response in about 80% of those infected with genotypes 2 and 3 and 40% of patients infected with genotype 1. Clearly, in order to provide better treatment options for patients infected with genotype 1 virus, it is imperative to develop antiviral therapeutic agents with excellent efficacy and acceptable side effects profiles.[2] Of the various viral targets found with HCV, the NS3-4A serine protease has been studied extensively.[3] Thus, discovery of inhibitors for this enzyme has become the focus of intense research for academic institutions and pharmaceutical industry.[4–6] As documented in several recent publications from Lilly[7] and Vertex,[8] we embarked on the design and systematic optimization (P1 through P4) of tetrapeptidyl α-ketoamides as HCV protease inhibitors. Parallel with these efforts, we were also interested in P4 cap modification in hopes of improving enzyme inhibitory potency as well as enzyme selectivity (e.g. human neutrophil elastase). Toward this end, we designed a new set of P2 hydroxyproline bearing inhibitors **3**–**5** with adipic acid, ester, or amide incorporated as their respective P4 caps. It should be mentioned that these initial designs were built upon the finding discovered by our Vertex colleagues.[9a] Consistent with the NS3 protease substrate preference at P5 and P6 sites (Asp and Glu),[9b] Perni et al. reported that the P4 adipic acid capped aldehyde **2** exhibited 2–4-fold improved enzyme inhibitory potency relative to the P4 neutral (pyrazine) capped inhibitor **1**.[9a] In addition, we decided to prepare three P4 tetrazole[10] capped derivatives **6**–**8** as potential HCV protease inhibitors because compound **6** was bioisostere of the P4 adipic acid capped inhibitor **3**.

In view of the excellent enzyme inhibitory potency exhibited by **6** ($K_i = 6$ nM as listed in Table 1), we decided later to expand the use of P4 tetrazole capping strategy to P2 bicycloproline α-ketoamide inhibitor series. Toward that end, in conjunction with P1′ variations, we designed inhibitors **10**, **12**, and **15** for direct scaffold comparison with their P2 TIQ-hydroxyproline bearing counterparts **6**–**8** as shown in Figure 1. Furthermore, with the intention to modulate cellular activity or/and enzyme selectivity of the P2 bicycloproline inhibitor series, we decided to incorporate additional neutral or polar P1′ functionalities as seen in compounds **11**, **13**, **14**, and **16**. On the other hand, to take advantage of our recent P3 and P4 SAR findings,[7c,d] we planed to prepare inhibitors **17**–**19** for comparison with **9**. In this manuscript, we report the syntheses and antiviral activity evaluation of these P4

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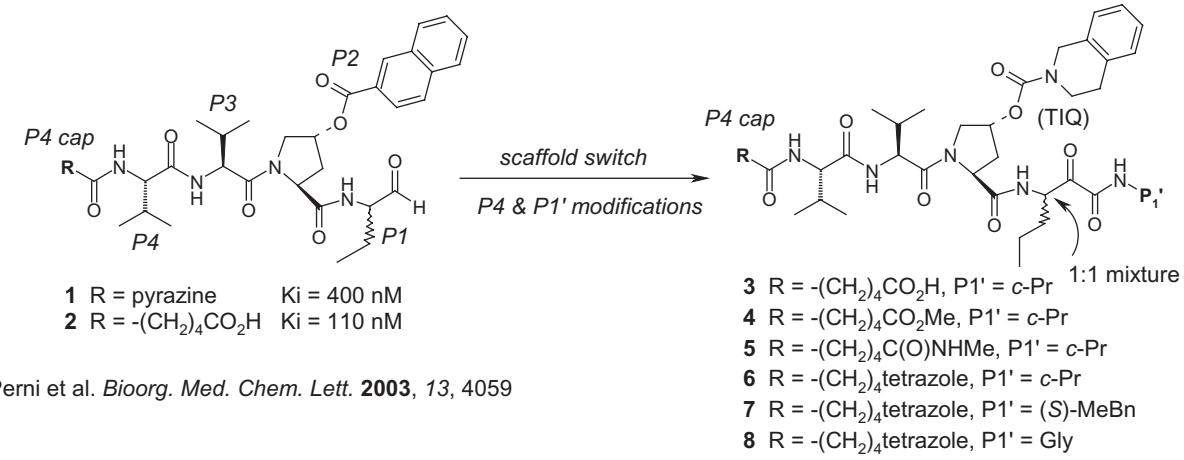


Figure 1. TIQ-proline P2 bearing α-ketoamides.

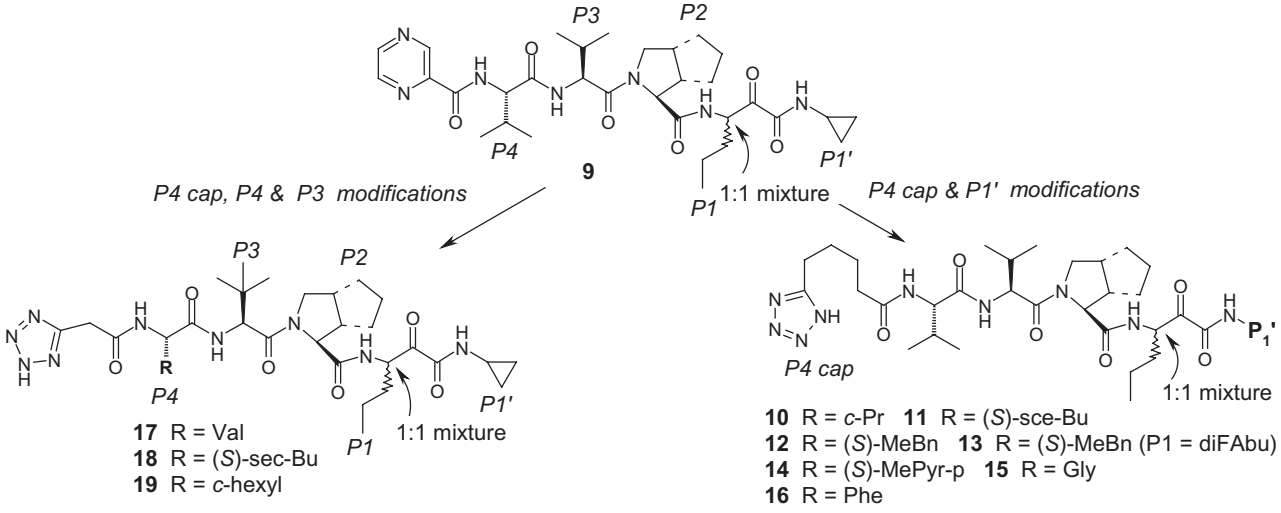


Figure 2. Bicycloproline P2 bearing α-ketoamides.

cap modified HCV protease inhibitors as shown in Figures 1 and 2.

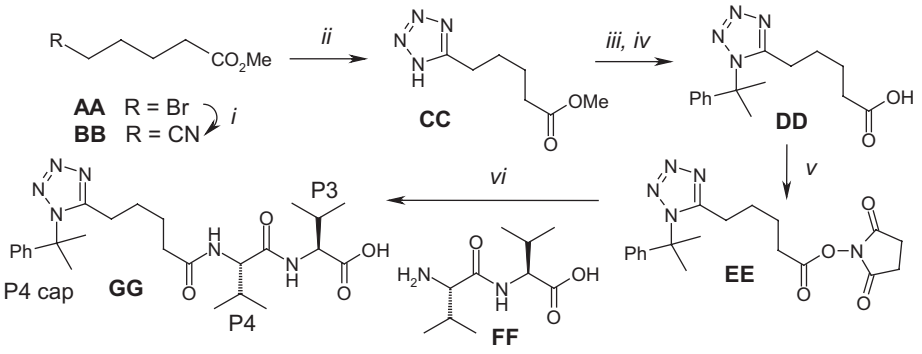
2. Chemical synthesis

2.1. Synthesis of N-termini tetrazole bearing inhibitors

The representative synthetic route employed for the preparation of novel P4 tetrazole capped inhibitors (e.g. **6** and **10**) is outlined in Schemes 1 and 2. As shown in Scheme 1, the P4 tetrazole capped dipeptide acid **GG** was assembled via reaction of the commercial available H_2N-Val-Val-OH **FF** with the requisite P4 cap **EE** in 91% yield. Intermediate **EE** was obtained in three steps from its corresponding tetrazole ester **CC** via *N*-protection followed by ester hydrolysis and subsequent acid activation with HOSu. The tetrazole ester **CC** was in turn prepared via a two-step sequence from **AA** in overall 50% yield.

With the key building block **GG** in hand, we carried out the remaining steps toward the syntheses of inhibitors **6** and **10** as illustrated in Scheme 2. Coupling of **GG** with the bicycloproline P2 ester **II**[7a] was mediated by HOAt/DCC[11] and provided the tripeptidyl (P4-P2) adduct **LL** (58%), which was then saponified to its corresponding acid **MM** in almost quantitative yield. Coupling of **MM** with the P1-P1′ unit **NN**[7a] was promoted by PyBOP[12] and yielded the desired α-hydroxyamide **PP** (60%). Final conversion of **PP** to the desired inhibitor **10** was accomplished via Dess–Martin oxidation[13] and TFA mediated *N*-deprotection in an overall yield of 31%. Following the identical sequence as outlined in Scheme 2, coupling of P4-P3 dipeptide acid **GG** with the 4-hydroxyproline P2 unit **HH**[14] provided the adduct **JJ** (95%), thereafter the respective acid **KK** (100%). Coupling of **KK** with the α-hydroxyamine **NN**[7a] afforded the adduct **OO** (60%), which was next converted to the final product **6** in 27% overall yield.

The syntheses of other P4 tetrazole capped inhibitors **11**–**16** were accomplished via coupling of the tripeptidyl



Scheme 1. Synthesis of P4 tetrazole capped dipeptidyl acid GG. Reagents and conditions: (i) KCN/aq EtOH, 74%; (ii) NaN_3/Et_3NHCl, 68%; (iii) $Ph(Me)=CH_2/Cl_3CCO_2H$, 95%; (iv) aq NaOH, 75%; (v) HOSu/EDCI/DIEA, 75%; (vi) **FF**/DIEA, 91%.

acid **MM** with the appropriate P1-P1′ units required. Likewise, two P1′ modified inhibitors **7** and **8** were prepared via condensation of the tripeptidyl acid **KK** with the requisite P1-P1′ units bearing either (*S*)-MeBn or Gly as their respective P1′ moieties.

On the other hand, each of the P4 capped dipeptidyl acids needed for the syntheses of **17**–**19** was prepared via a four-step sequence consisting of (a) P4/P3 coupling, (b) P4 *N*-deprotection, (c) P4 *N*-acylation with 1H-tetrazole-5-acetic acid, and (d) P3 ester hydrolysis.[7c] Sequential coupling of these requisite P4-P3 acids with the bicycloproline P2 unit **II**,[7a] and later with the P1-P1′ unit **NN**[7a] provided, upon final oxidation, the desired tetrapeptidyl α-ketoamide inhibitors.

2.2. Syntheses of N-termini adipic acid, ester, amide bearing inhibitors

It should also be pointed out that inhibitors **4** and **5** were assembled via sequential coupling of the P4 adipic ester or amide capped P4 (Val)-P3 (Val) acid with **HH** (P2 unit) and next with **NN** (P1-P1′ unit). The P4-P3 dipeptidyl acids needed for the syntheses of **4** and **5** were in turn prepared via capping the P4 amino functionality of di-valine **FF** with the requisite adipic ester or amide. Inhibitor **3** was obtained from its corresponding methyl ester **4** via saponification.

All tetrapeptidyl α-ketoamides discussed in this manuscript were obtained via semi-preparative reverse phase HPLC purification and subsequent lyophilization. The structures of these α-ketoamides were established on the basis of their NMR and mass spectroscopic analyses.

3. Biological evaluation

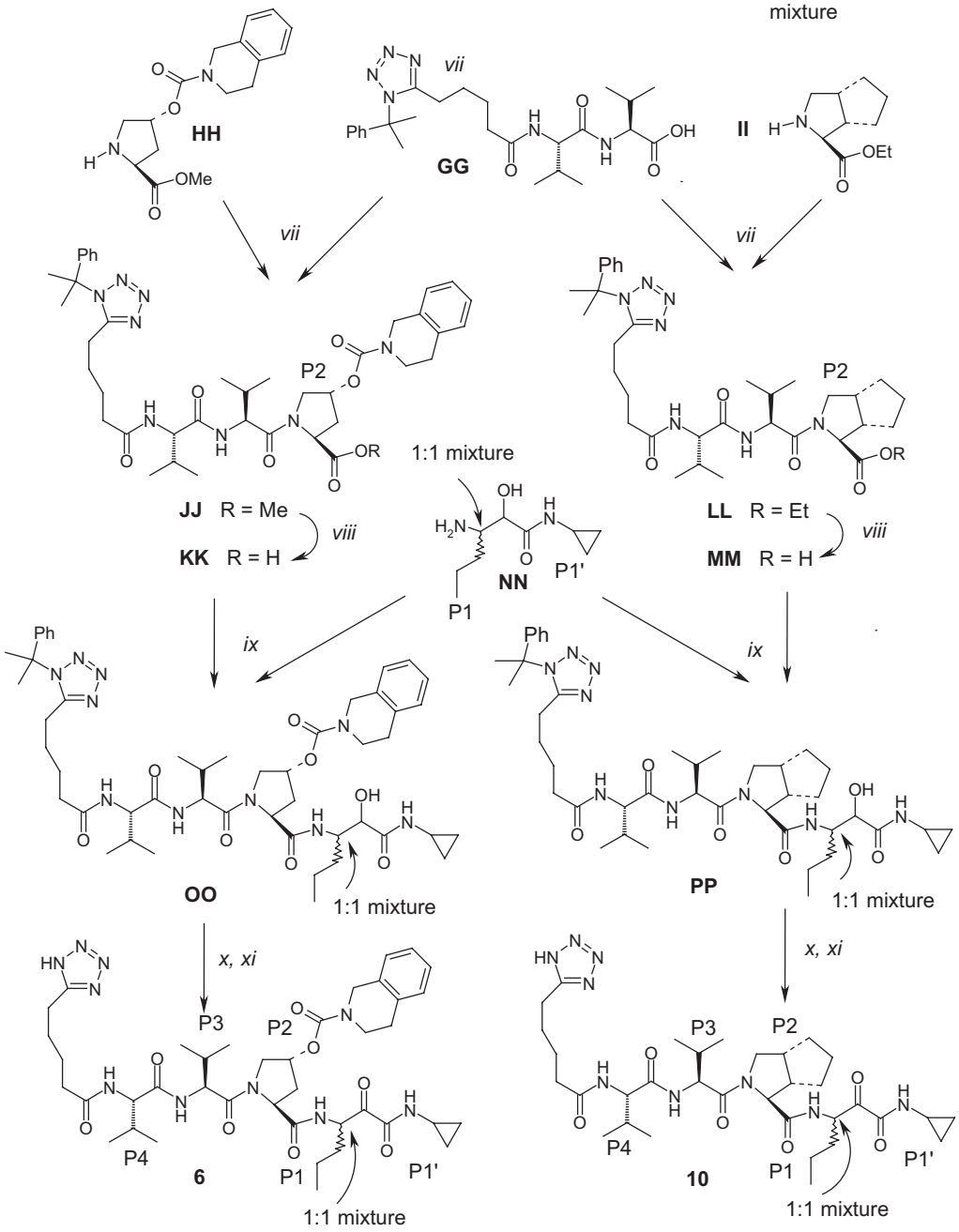
All P4 cap modified tetrapeptidyl α-ketoamides synthesized (**3**–**19**) were evaluated in the pNA based enzyme inhibition assay against truncated NS3 enzyme.[15] Selected inhibitors were further tested in the luciferase based replicon assay with built-in cytotoxicity controls.[16c] Since many closely related peptidyl α-ketoamide based inhibitors evaluated previously showed excellent enzyme selectivity against thrombin, chymotrypsin, trypsin, plasmin, kallikrein, and cathepsins G and L,[7c,d] a few selected inhibitors discussed in this manuscript were evaluated only in human neutrophil elastase (HNE) assay.[17] The results of these testing are listed in Table 1.

3.1. TIQ-proline P2 bearing inhibitor series

As shown in Table 1, as expected, incorporation of adipic acid as the P4 cap indeed led to inhibitor **3** endowed with high enzyme inhibitory potency ($K_i = 65$ nM). Further derivatization of this acid functionality resulted in its corresponding methyl ester **4** and methylamide **5** possessing 3–4-fold reduced enzyme potency. Replacement of the P4 acid moiety in **3** with its bioisostere tetrazole produced, to our satisfaction, an excellent HCV NS3 protease inhibitor **6** ($K_i = 6.1$ nM), which is 10-fold more potent than its corresponding carboxylic acid P4 capped counterpart **3**. Additional P1′ modification was performed on **6** and provided less potent NS3 protease inhibitors **7** (>60×) and **8** (12×). When tested in the enzyme selectivity

Table 1. Enzyme inhibitory potency, HNE selectivity, replicon IC_{50}, and cytotoxicity

Comp#	HCV NS3 K_i (nM)	HNE selectivity	Replicon IC_{50} (μM)	Cytotoxicity CC_{50} (μM)
3	65	—	>50	>50
4	271	—	—	—
5	193	4	—	>10
6	**6.1**	13	>50	>50
7	404	—	—	—
8	74	*131*	—	>10
9	123	33	11.6	>50
10	170	7	>50	>50
11	248	—	>50	>50
12	**31**	—	>50	>50
13	**27**	—	>50	>50
14	112	—	—	—
15	82	*172*	—	>10
16	**22**	*1185*	—	>10
17	808	—	—	—
18	3112	—	>50	>50
19	**30**	—	>50	>50



Scheme 2. Syntheses of tetrapeptidyl α-ketoamides **6** and **10**. Reagents and conditions: (vii) **GG**/HOAt/DCC/**HH** or **II**, 95% for **JJ**, 58% for **LL**; (viii) aq NaOH, ~100% for **KK** or **MM**; (ix) PyBOP/DIEA/**NN**, 60% for **OO** or **PP**; (x) Dess–Martin periodinane and followed by (xi) TFA, 27% for **6**, 31% for **10** (two-step).

assay, compounds **5** and **6** exhibited modest (4–13-fold) selectivity against human neutrophil elastase (HNE). In contrast, the P1′ glycine bearing inhibitor **8** exhibited 131-fold selectivity toward HNE. When tested in the newly invented luciferase based replicon assay,[16c] none of the P4 polar moiety capped inhibitors **3** (carboxylic acid) and **6** (tetrazole) showed any activity up to 50 μM. This is likely due to poor membrane permeability associated with these inhibitors, which is in turn resulted from having acid or its equivalent as P4 capping groups. When evaluated in the cytotoxicity assay in Huh-7 liver cells, compounds **3** and **6** were found to be noncytotoxic up to 50 μM (the highest concentration tested).

3.2. Bicycloproline P2 bearing inhibitor series

Careful analysis of the data shown in Table 1 reveals the following SAR trends at enzyme inhibition level: (1) replacement of the P2 TIQ-proline as seen in **6** ($K_i = 6.1$ nM) with [3,4]-fused bicycloproline led to inhibitor **10** with reduced potency ($K_i = 170$ nM); (2) whilst incorporation of the (*S*)-*sec*-butyl amine as the

P1′ moiety produced a slightly less potent enzyme inhibitor **11**, introduction of (*S*)-MeBn moiety as the P1′ binder indeed gave rise to 6-fold more potent inhibitors **12** ($K_i = 31$ nM) and **13** ($K_i = 27$ nM) relative to **10**; (3) replacing the C-termini phenyl with basic pyridine moiety led to **14** endowed with 4-fold weaker potency; and (4) incorporation of carboxylic acid C-termini resulted in more potent inhibitors **15** (2×) and **16** (8×) in comparison to the neutral C-termini bearing inhibitor **10**. As far as enzyme selectivity is concerned, it is evident that the C-termini acid bearing ones, **15** and **16**, exhibited much greater HNE selectivity (as high as >1000-fold detected for **16**) relative to **10** (7-fold only). When evaluated in the cell based luciferase replicon assay (at 50 μM), compounds **10** through **13** were found to be inactive, presumably due to having polar tetrazole as their P4 caps. In contrast, the P4 pyrazine capped inhibitor **9** (included as the positive control) demonstrated whole cell activity with IC_{50} value of 11.6 μM. When tested for their cytotoxicity in the Huh-7 liver cells, four compounds tested (**10**–**13**) were considered to be noncytotoxic at the highest concentration tested (50 μM).

Three P3 and P4 modified HCV protease inhibitors **17**–**19** were synthesized and evaluated in the bioassays. Consistent with our previous finding regarding P4 SAR, the P4 Chg bearing inhibitor **19** was found to be the most potent one within this set ($K_i = 30$ nM). Inhibitors **18** and **19** failed to show activity in the luciferase replicon assay and were also found to be noncytotoxic at the highest dose tested.

4. Conclusions

Prompted by the preliminary finding by our collaborators from Vertex, we designed and synthesized a number of novel P4 cap modified tetrapeptidyl α-ketoamides as HCV NS3 protease inhibitors. We found that compound **6** bearing tetrazole moiety as P4 cap demonstrated excellent enzyme inhibitory activity ($K_i = 6.1$ nM), which was 10-fold more potent than its corresponding P4 adipic acid capped inhibitor **3**. Subsequently, we synthesized and evaluated a series of P2 bicycloproline containing inhibitors **10**–**19**. Of those inhibitors bearing P4 tetrazole caps, compounds **12**, **13**, **16**, and **19** were found to be highly potent enzyme inhibitors with K_i values ranging from 22 to 31 nM. Furthermore, inhibitors **8**, **15**, and **16**, all bearing carboxylic acid as their C-termini, showed excellent selectivity (131–1185×) against human neutrophil elastase (HNE). Perhaps as a result of having polar tetrazole or adipic acid as P4 cap, none of the newly synthesized inhibitors (e.g. **3**, **6**, **10**–**13**) showed cell based activity. On the other hand, all inhibitors tested were found to be noncytotoxic (up to 50 μM).[18]

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18. We have not investigated the inhibition kinetics on those HCV-NS3 inhibitors disclosed in this manuscript. However, we indeed performed inhibition kinetic study on our clinical candidate VX-950. The results of this work will be published in due time.