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Novel P4 truncated tripeptidyl α-ketoamides as HCV protease inhibitors

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Abstract—We describe herein the synthesis and evaluation of two series of P-4 truncated tripeptidyl α-ketoamides as HCV serine protease inhibitors. The most promising compound disclosed in this communication **7b** demonstrated enzyme binding affinity (K_i) at 0.27 uM.

1. Introduction

Hepatitis C virus (HCV) infection is widely recognized today as a huge public health concern, with more than 170 million people infected worldwide, most of them unknowingly.[1] Majority of the HCV infected patients developed slowly progressing liver diseases which can lead to liver cirrhosis and hepatocellular carcinoma. Unfortunately, neither a generally effective treatment nor a preventive vaccine is available so far.[2] To address this unmet medical need, we embarked on the discovery of novel ketoamide based inhibitors for HCV serine protease, a key enzyme involved in viral polyprotein cleavage and maturation.[3] As depicted in Figure 1, in contrast to rather large ketoamide inhibitor **1** reported by DuPont,[4] we focused our SAR effort on the design and evaluation of novel bicycloproline P2 bearing tetrapeptidyl ketoamides such as **2**.[5] Replacement of the P3 valine in **2** with *tert*-Leu led to another series of more impressive inhibitors including **3a**.[6] As documented in our recent publications, inhibitors **2** and **3a** were identified as promising HCV serine protease inhibitors endowed with excellent overall antiviral activity, cytotoxicity, and pharmacokinetics profiles. To make further improvement on these inhibitors, we decided to carry out (1) P4 and P4 cap modifications,[7] and (2) P4 truncation to reduce synthetic complexity.[8] In this communication, we will discuss two types of P4 truncated tripeptidyl ketoamide inhibitors as shown in Figure 1. Inhibitors **4**–**7** contain isopropyl carbamate moiety as their P3 *N*-capping group, whereas inhibitors **8** and **9** carry 2-hydroxyl-3-methylbutyric acid as the P3 capping group. It is hoped that the isopropyl moieties embedded in these P3 capping groups could mimic the P4 valine seen in the tetrapeptidyl ketoamides such as **2** and **3**. As can be seen in Figure 1, bc-proline moieties (deoxy and gem-difluoro) and nor-valine were incorporated at the respective P2 and P1 sites in all of the target molecules. In addition, Val and *t*-Leu were chosen for P3 pocket. Both neutral and acidic P1′ moieties were incorporated into each set of tripeptidyl ketoamides (**a** or **b**).

2. Series 1

Tripeptidyl ketoamides **4**–**7** were prepared according to the synthetic route outlined in Scheme 1 for compound **5a**. HOAt/DIC mediated coupling[9] between isopropyl carbamate protected *t*-leucine **10a** with the previously reported deoxy-bicycloproline derivative **11a**[5,10] provided the dipeptide ester **14** (64%), which was then converted to its corresponding carboxylic acid **15** (81%) via NaOH mediated hydrolysis. Further reaction of **15** with the known P1–P1′ amino-alcohol **12b**[11] was promoted by HOAt/DIC and yielded the expected adduct **16** (82%), which was then oxidized with Dess–Martin periodinane to provide the desired tripeptidyl α-ketoamide inhibitor **5a** in 49% yield. The reaction yields for other inhibitors within this series (**4a**, **4b**, **5b**, **6a**, **6b**, **7a**, and **7b**) are included in ref. 18.

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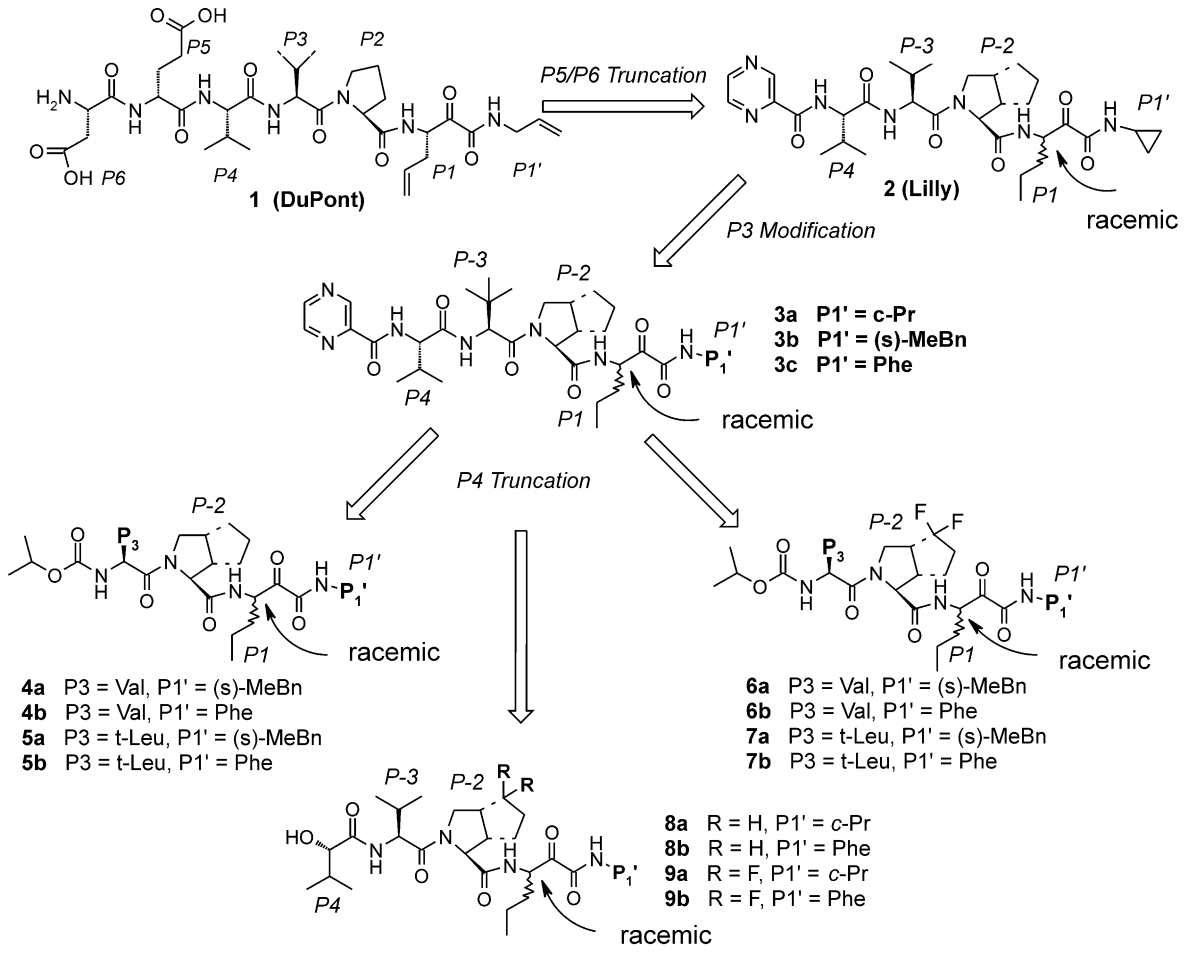
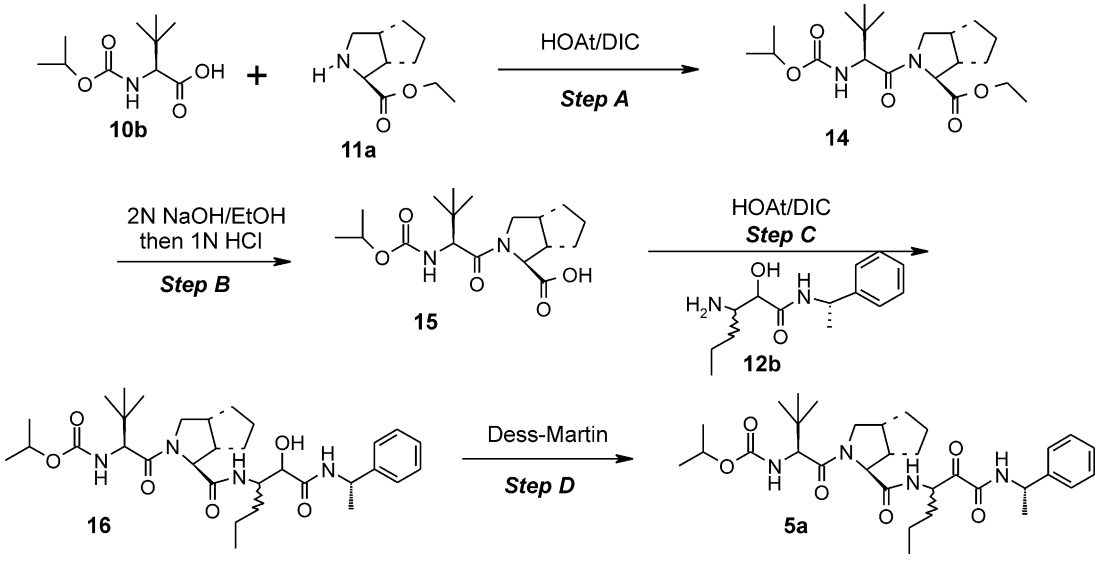


Figure 1. Representative structures of HCV protease inhibitors.



Scheme 1. Synthesis of inhibitor **5a**.

3. Series 2

The representative synthetic route employed for the 2-hydroxy-3-methylbutyric acid capped inhibitors **8** and **9** is shown in Scheme 2. The requisite *O*-silyl-acid **13** was prepared from HCl-Val-OMe via the following three-step sequence: (1) *N*-acylation, (2) *O*-silyl protection, and (3) NaOH mediated hydrolysis. HOAt/DCC mediated coupling[9] of **13** with the known difluoro-bc-Pro-OEt **11b** afforded the adduct **19** (66%), which was saponified to provide the corresponding carboxylic acid **20** (90%). PyBOP mediated coupling[12] of **20** with the

requisite P1–P1′ unit **12a**[11] provided the carbinol adduct **21** (94%), which was further converted to the *O*-silyl protected ketoamide **22** (92%). Further treatment of a dichloromethane solution of **22** with TFA yielded the target HCV protease inhibitor **9a** in 92% yield.

All other α-ketoamides discussed herein were prepared according to the identical sequences described in Scheme 1 or 2. The overall yields for each inhibitor are listed in ref 18. The structures of these tripeptidyl α-ketoamides (**4**–**9**) were secured on the basis of their proton NMR and mass spectra analyses.

All tripeptidyl α-ketoamides synthesized (**4**–**9**) were evaluated in the following bioassays: (1) enzyme binding assay against truncated NS3 enzyme;[13] (2) HCV replication surrogate assay (replicon) for cellular activity;[14] (3) cytotoxicity assay in a liver cell line (Huh-7 cells).[15] The testing results obtained with the newly synthesized inhibitors along with four previously reported tetrapeptidyl α-ketoamides (**2** and **3**) are listed in Table 1.

Table 1. Biological activity of tripeptidyl α-ketoamides

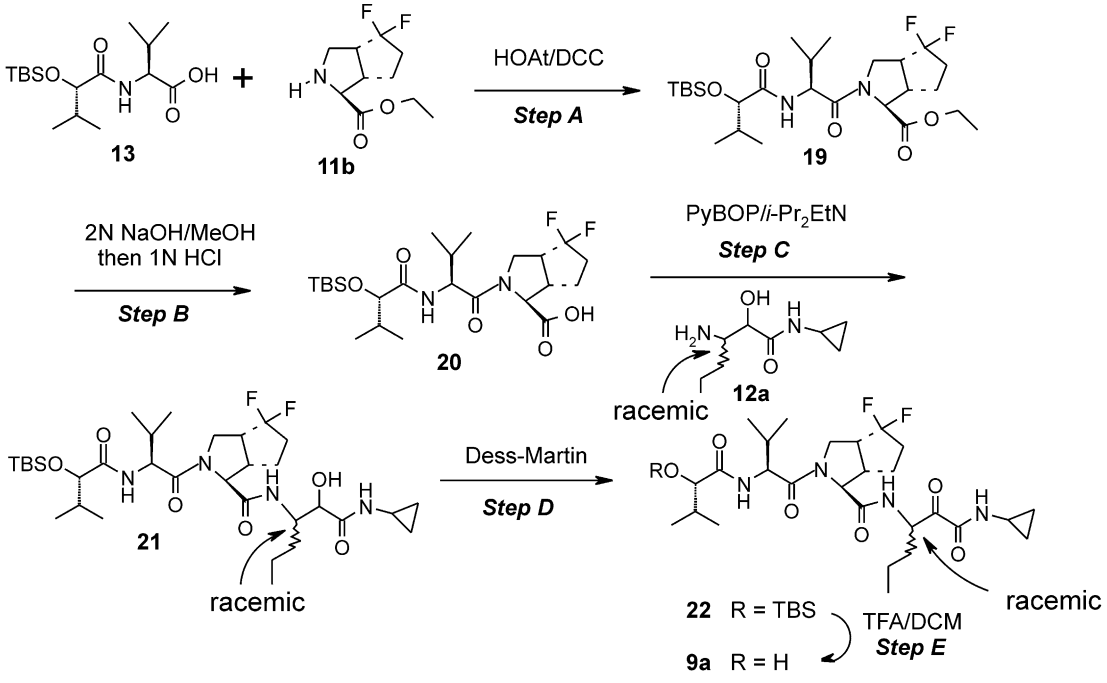
Compd	P-3	P-1′	K_i (pNA) (μM)	Replicon inhibition @50 μM	Cytotoxicity (μM)
4a	Val	(s)MeBn	1.91	7%	>100
4b	Val	Phe	0.42	Not active	>100
5a	*t*-Leu	(s)MeBn	1.75	53%	>100
5b	*t*-Leu	Phe	0.82	Not active	Not tested
6a	Val	(s)MeBn	6.7	22%	>100
6b	Val	Phe	2.7	Not active	>100
7a	*t*-Leu	(s)MeBn	2.1	51%	>100
7b	*t*-Leu	Phe	0.27	Not active	>100
8a	Val	c-Pr	14.5	Not active	>100
8b	Val	Phe	1.8	Not active	>100
9a	Val	c-Pr	16.5	Not active	not tested
9b	Val	Phe	2.0	Not active	>100
2	Val	c-Pr	0.123	IC_{50} = 7.0 μM	>100
3a	*t*-Leu	c-Pr	0.084	IC_{50} = 2.2 μM	>100
3b	*t*-Leu	(s)MeBn	0.19	IC_{50} = 0.78 μM	>100
3c	*t*-Leu	Phe	0.023	16%	>100

4. Enzyme inhibition assay

All tripeptidyl α-ketoamides were found to be tight-binding inhibitors for HCV NS3 protease. Therefore, the inhibition constant, K_i, was determined using Morrison equation.[13] Careful inspection of the data listed in Table 1 revealed the following trends: (1) All P1′ Phe bearing inhibitors were found to be more potent than their corresponding neutral P1′ containing counterparts. A number of P1′ Phe containing analogues (e.g., **4b**, **5b**, and **7b**) displayed K_i values less than 1 uM, with compound **7b** being the most potent one (K_i = 0.27 μM). It is worthwhile to mention that the enzyme inhibitory activity demonstrated by **7b** is comparable to a recently reported difluoroAbu P1 bearing tripeptidyl α-ketoacid (K_i = 0.38 μM).[16] (2) P3-isopropyl carbamate capped analogues such as **4b** (K_i = 0.42 μM) and **7b** (K_i = 0.27 μM) showed superior activity to that found with 2-hydroxy-3-methylbutyric acid capped analogues **8b** (K_i = 1.8 μM) and **9b** (K_i = 2.0 μM), respectively. (3) Careful comparison of the K_i data generated for three pairs of inhibitors (**8a** vs **2**, **5a** vs **3b**, and **5b** vs **3c**) clearly showed that the tetrapeptidyl α-ketoamides were 10- to 100-fold more potent than their corresponding P4 truncated tripeptidyl ketoamide based inhibitors.

5. Replicon assay and cytotoxicity assay[14,15]

In view of the data listed in Table 1, we discovered the following SAR trends: (1) All of the P1′ Phe bearing inhibitors (**4b**–**9b**) were devoid of cellular activity. This



Scheme 2. Synthesis of inhibitor **9a**.

is likely due to their poor membrane permeability. (2) P3 *t*-Leu bearing analogues **5a** and **7a** displayed better activity than their respective P3 Val containing counterparts **4a** and **6a**. (3) When compared with their corresponding tetrapeptidyl α-ketoamides **3b** ($IC_{50} = 0.78$ μM) and **2** ($IC_{50} = 7.0$ μM), both tripeptidyl α-ketoamides **5a** ($IC_{50} \sim 50$ μM) and **8a** (inactive at 50 μM) showed significantly reduced activity. (4) Judged from the IC_{50} values listed in Table 1, all newly prepared inhibitors (**4**–**9**) were found to be non-cytotoxic with IC_{50} values greater than 100 μM.

6. Conclusion

We prepared and evaluated two series of P4 truncated tripeptidyl α-ketoamide inhibitors **4**–**9**. The most potent inhibitor discussed in this manuscript was **7b** with K_i value of 0.27 M. Despite its enzyme inhibitory activity, compound **7b** was inactive in the replicon assay. Compared with their respective tetrapeptidyl ketoamide counterparts, the newly prepared tripeptidyl α-ketoamides possessed significantly reduced enzyme and cellular activity.[17]

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