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# The Identification of α-Ketoamides as Potent Inhibitors of Hepatitis C Virus NS3-4A Proteinase

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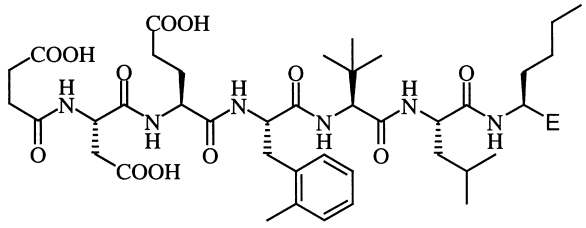
**Abstract**—Peptides based upon the non-prime side residues of the NS4A-4B cleavage site of hepatitis C virus (HCV) NS3-4A proteinase containing an α-ketoamide moiety in place of the scissile amide bond are potent inhibitors of this enzyme. 

Hepatitis C virus (HCV) is the cause of the majority of cases of transfusion-associated hepatitis and a significant proportion of community-acquired hepatitis worldwide. Infection by HCV can lead to a range of clinical conditions including an asymptomatic carrier state, severe chronic active hepatitis, cirrhosis and, in some cases, hepatocellular carcinoma.[1] Current therapies for HCV infection include treatment with interferon-α in combination with ribavirin, but this therapy is of only limited efficacy. Hence, a new treatment for the disease would be of great interest.[2]

The HCV NS3 protein encodes a serine proteinase which is responsible for the cleavage at the NS3-4A, NS4A-4B, NS4B-5A, and NS5A-5B junctions in the viral polyprotein. The 54 amino acid NS4A protein is a co-factor which binds to the NS3 protein and enhances its proteolytic activity.[3] This NS3-4A proteinase is one of the most intensively studied targets for HCV antiviral therapy.[2]

We recently described the design and synthesis of molecules such as **1** and **2**, derived from the non-prime side residues of the NS4A-4B cleavage site of the proteinase.



1. E = CHO
2. E = $B(OH)_2$
3. E = COCONHR

These compounds incorporate an electrophile at the C-terminus of the P1-residue and are potent inhibitors of this enzyme.[4–6] The use of aldehydes and boronic acids to terminate the inhibitors at that point prevented exploration of the structure–activity relationships of the prime-side. Potent α-keto acids have been reported[7] but again these terminate the inhibitor. The introduction of an α-keto amide functionality, to give inhibitors such as **3**, should afford an electrophilic carbonyl, whilst retaining the opportunity of prime-side exploration.[8,9] We therefore undertook the synthesis of this class of inhibitors to determine their efficacy against HCV NS3-4A proteinase.[10] The synthesis of one such inhibitor[11] is outlined in Scheme 1.[12]

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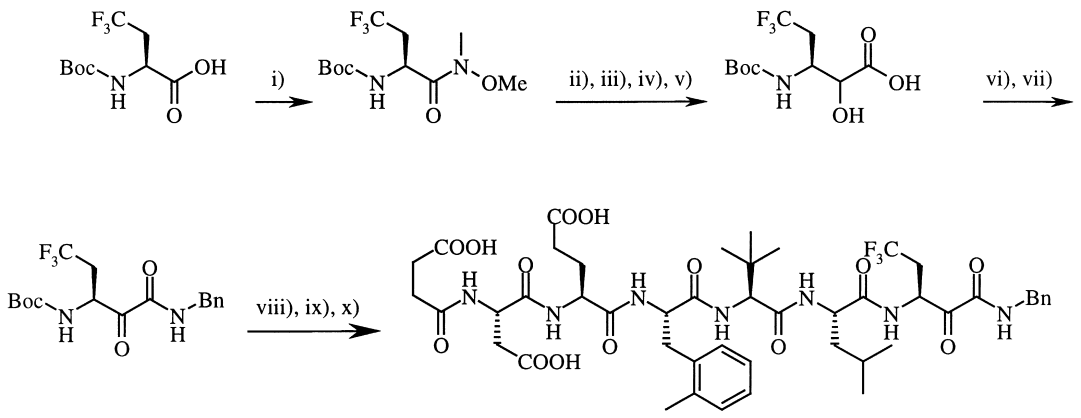
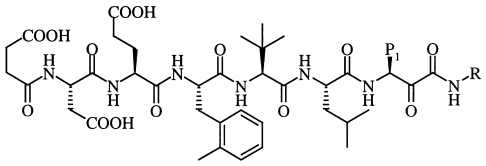


**Scheme 1.** (i) *N*,*O*-Dimethylhydroxylaminehydrochloride, diisopropylethylamine, hydroxybenzotriazole (HOBT), 1-(3-diethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), tetrahydrofuran, room temperature (rt), 16 h, 91%; (ii) $LiAlH_4$, tetrahydrofuran, 0 °C, 30 min, 87%; (iii) acetone cyanohydrin, $Et_3N$, $CH_2Cl_2$, rt, 1.5 h, 93%; (iv) HCl, reflux, 17 h; (v) $Boc_2O$, $NaHCO_3$, $H_2O$, 3 days, 84% over two steps; (vi) $BnNH_2$, HOBT, EDCI, $CH_2Cl_2$, rt, 2 h, 76%; (vii) Dess–Martin periodinane, $CH_2Cl_2$, rt, 1 h, 65%; (viii) TsOH, MeCN, rt, 1 h, 91%; (ix) protected pentapeptide fragment EDCI, HOBT, *N*-ethyl morpholine, $CH_2Cl_2$, rt, 6 h, 32%; (x) TFA, 30 min, 76%.

**Table 1.**



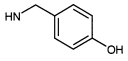
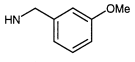
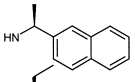
| Compound | $P_1$ | NHR | $IC_{50}$ (nM) |
|---|---|---|---|
| **4** | $CH_2CF_3$ | $NH_2$ | 7 |
| **5** | $CH_2CF_3$ |  | 4 |
| **6** | $CH_2CF_3$ |  | 4 |
| **7** | $CH_2CF_3$ |  | 1100 |
| **8** | $CH_2CF_3$ |  | 7 |
| **9** | $CH_2CF_3$ |  | 7 |
| **10** | $CH_2CF_3$ |  | 4 |
| **11** | $CH_2CF_3$ |  | 6 |
| **12** | $CH_2CF_3$ |  | 4 |
| **13** | $CH_2CF_3$ |  | 8 |
| **14** | Bu | $NH_2$ | 11 |

**Table 2.** Activity of **14** against important human serine proteinases

| | Enzyme | $IC_{50}$ (nM) |
|---|---|---|
| 1. | HCV NS3-4A Proteinase | 11 |
| 2. | Elastase[14] | 12,000 |
| 3. | Chymotrypsin[15] | 300 |
| 4. | Trypsin[16] | >200,000 |

Gratifyingly, as can be seen in Table 1, the activities[13] of the optimal α-ketoamides are all in the nanomolar range and are approximately 10-fold more potent than their corresponding aldehyde or boronic acid analogues. The primary amide in **4** can be substituted with benzyl or (2-naphthyl)-methyl moieties, which in turn can be substituted around the aromatic ring. The stereospecific nature of the interaction is clearly indicated by the effect of substitution at the 1-position where a 250-fold discrimination is observed for introduction of a chiral methyl group (compare compounds **6** and **7**). Replacement of the $CH_2CF_3$ sidechain at P1 by butyl had little effect on potency, but improved the plasma stability of the compound.

These inhibitors also display selectivity for HCV NS3-4A proteinase over other important human serine proteinases. The data for inhibitor 14 is shown in Table 2.

In summary, the introduction of the α-ketoamide electrophile has led to the identification of highly potent inhibitors of the NS3-4A proteinase. Further work on this exciting class of inhibitors will be reported elsewhere.[17]

## References and Notes

1. Cuthbert, J. A. *Clin. Microbiol. Rev.* **1994**, *7*, 505.
2. Dymock, B. W.; Jones, P. S.; Wilson, F. X. *Antiviral Chem. Chemother.* **2000**, *11*, 79.
3. Bartenschlager, R. *Antiviral Chem. Chemother.* **1997**, *8*, 281 and references cited therein.
4. Attwood, M. R.; Bennett, J. M.; Campbell, A. D.; Canning, G. G. M.; Carr, M. G.; Dunsdon, R. M.; Greening, J. R.; Jones, P. S.; Kay, P. B.; Handa, B. K.; Hurst, D. N.; Jennings, N. S.; Jordan, S.; Keech, E.; O'Brien, M. A.; Overton, H. A.; King-Underwood, J.; Raynham, T. M.; Stenson, K. P.; Wilkinson, C. S.; Wilkinson, T. C. I.; Wilson, F. X. *Antiviral Chem. Chemother.* **1999**, *10*, 259.
5. Attwood, M. R.; Hurst, D. N.; Jones, P. S.; Kay, P. B.; Raynham, T. M.; Wilson, F. X. Patent WO9822496A2.

6. Attwood, M. R.; Hurst, D. N.; Jones, P. S.; Kay, P. B.; Raynham, T. M.; Wilson, F. X. Patent GB2337262A1.
7. Di Marco, S.; Rizzi, M.; Volpari, C.; Walsh, M. A.; Narjes, F.; Colarusso, S.; De Francesco, R.; Matassa, V. G.; Sollazzo, M. *J. Biol. Chem.* **2000**, *275*, 7152.
8. Edwards, P. D.; Bernstein, P. R. *Med. Res. Rev.* **1994**, *14*, 127 and references cited therein.
9. In the course of this work others have reported α-keto-amides as HCV NS3-4A proteinase inhibitors: Han, W.; Hu, Z.; Jiang, X.; Decicco, C. P. *Biorg. Med. Chem. Lett.* **2000**, *10*, 711. Also ref 2 and references cited therein.
10. Hurst, D. N.; Jones, P. S.; Kay, P. B.; Raynham, T. M.; Wilson, F. X. Patent GB2338482A1.
11. All novel compounds were characterised by $^1$H NMR and HPLC.
12. The protected peptide fragment is Succ(O*t*Bu)-Asp(O*t*Bu)-Glu(O*t*Bu)-(*o*Me)Phe-*t*BuGly-Leu-OH prepared as described in ref 10.
13. The inhibition of HCV NS3-4A proteinase activity was determined as reported in ref 4.
14. Castillo, M. J.; Nakajima, K.; Zimmerman, M.; Powers, J. C. *Anal. Biochem.* **1979**, *99*, 53.
15. Oleksyszyn, J.; Powers, J. C. *Biochemistry* **1991**, *30*, 485.
16. Kanaoka, Y.; Takahashi, T.; Nakayama, H.; Takada, K.; Kimura, T.; Sakakibara, S. *Chem. Pharm. Bull.* **1977**, *25*, 3126.
17. Manuscript in preparation.