Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1111–1114

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Glycine α-Ketoamides as HCV NS3 Protease Inhibitors

Wei Han,* Zilun Hu, Xiangjun Jiang, Zelda R. Wasserman and Carl P. Decicco

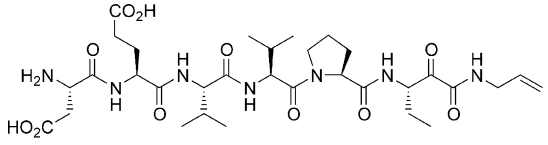
Department of Discovery Chemistry, Pharmaceutical Research Institute, Bristol-Myers Squibb Company, PO Box 5400, Princeton, NJ 08543, USA

Received 29 October 2002; accepted 3 January 2003

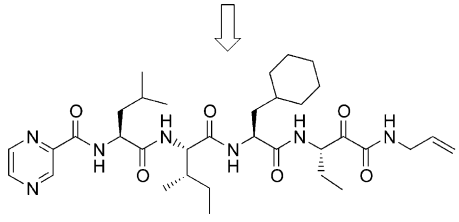
Abstract—Using a tetrapeptide-based α-ketoamide template, various amines and amino acids were incorporated to explore the prime side of the HCV NS3 protease catalytic site. Glycine carboxylic acid was found to be the most effective prime group. Further optimization yielded an inhibitor with IC_{50} of 0.060 μM.

The hepatitis C virus (HCV) is the principal etiologic agent of both parenterally transmitted and sporadic non-A non-B hepatitis.[1] HCV infection most commonly results in chronic hepatitis that eventually develops into cirrhosis, hepatocellular carcinoma or liver failure. Current therapies for HCV infection include treatment with interferon-α alone and in combination with ribavirin.[2] These therapies have limited efficacy and frequently are accompanied by side effects. Given these drawbacks, new treatments for the disease are currently being explored through multiple approaches. One promising approach is the inhibition of the serine protease associated with the NS3 protein.[3] In a chimpanzee study, the NS3 protease has been shown to be required for HCV infectivity, supporting NS3 as a viable drug discovery target.[4] Moreover, the NS3 protease is well characterized[5] and its crystal structure has been obtained.[6]

We and others reported the use of α-ketoamides as templates for HCV NS3 protease inhibitors.[7,8] Our original hexapeptide lead **1** has an IC_{50} of 0.42 μM. Truncation and modification led to tetrapeptide **2**, which has an IC_{50} of 2.7 μM. In the proposed binding mode of **2** with HCV NS3 protease, the ketoamide forms a covalent bond with the catalytic serine and projects the side chains of Cha, Ile, and Leu to the S2, S3, and S4 sites, respectively. The ethyl substituent on the ketoamide is believed to occupy the S1 site. The ketoamide group offers an advantage of extending into the prime side through the amide linker. Even though prime site binding inhibitors have been reported using peptide carboxamides,[9] information on prime site interactions of the α-ketoamide series is limited. This communication discloses our SAR efforts exploring the prime site interactions using α-ketoamide **2** as a template, and brief optimization of P1 group.



1 IC_{50} = 0.42 μM



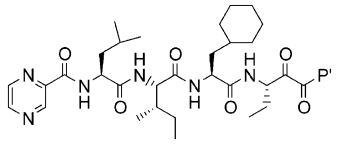
2 IC_{50} = 2.7 μM

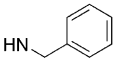
Several simple amide derivatives were first synthesized and tested to explore the prime site interaction. As shown in Table 1,[10] amides derived from small primary amines, such as allyl-, ethyl-, propargyl- and cyclopropylamine, gave comparable potency in the NS3 protease assay (2.7, 1.8, 1.6 and 2.2 μM, respectively, entries 1–4). The more bulky *N*-benzyl amide resulted in significant loss of potency (11 μM, entry 5). α-Ketoamides derived from pyrrolidine and morpholine were even less active (42 and >60 μM, entries 6 and 7).

*Corresponding author. Fax: +1-609-818-6570; e-mail: wei.han1@bms.com

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00031-3

Table 1. SAR of analogues with simple amide groups



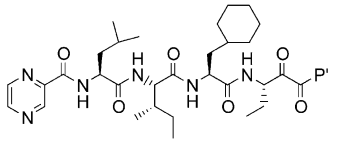
Entry	P′	IC_{50} (μM)
1		2.7
2		1.8
3		1.6
4		2.2
5		11
6		42
7		>60

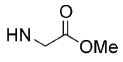
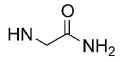
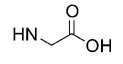
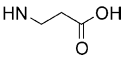
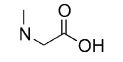
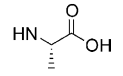
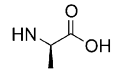
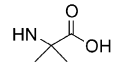
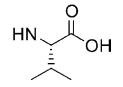
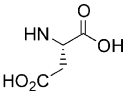
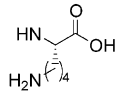
A series of amino acids was coupled with the ketoamide to further explore the prime site interaction. The methyl ester and the primary amide of the glycine derivatives gave inhibitors at IC_{50} around 5 μM (entries 1 and 2, Table 2). In contrast, the free acid afforded a 0.23 μM inhibitor, a 20-fold enhancement (entry 3). The ketoamide of β-alanine was less potent, and that of *N*-methylglycine was totally inactive (entries 4 and 5). The L-alanine analogue was 5-fold less potent than the glycine compound (entries 6 vs 3), indicating that the methyl side chain was tolerated but not preferred. D-Alanine and aminoisobutyric acid derivatives were not tolerated at the prime site and gave inactive compounds (entries 7 and 8). L-Amino acids with side chains larger than methyl groups further attenuated the activity, regardless of the electronic properties of the side chain (entries 9–11).

To help understanding the structure–activity relationships, the α-ketoamide analogue with glycine free acid (Table 2, entry 3) was modeled in the active site of NS3 protease (Fig. 1). According to the model, the carboxylic acid of the glycine appears to interact with Lys136 and Arg 109 of the protein.[11] Loss of binding affinity of the glycinamide compared to the glycine free acid suggested that the carboxylic acid group of glycine serves either as hydrogen bond acceptor or forms charge comlex with Lys136 and Arg109. The narrow channel of the prime site adjacent to the catalytic serine could explain the preference for more linear groups, such as allylamine and glycine, over branched groups such as pyrrolidine and α-substituted amino acids.

Keeping the glycine carboxylic acid constant, we briefly investigated the effect of P1 substitution. NS3 protease is known to have a shallow and hydrophobic S1 pocket

Table 2. SAR of α-ketoamides with amino acids as prime groups



Entry	P′	IC_{50} (μM)
1		5.8
2		4.3
3		0.23
4		12
5		>60
6		1.3
7		>60
8		>60
9		3.6
10		5.3
11		7.7

and cysteine residue is one of the most preferred P1 groups.[12] Attempts at replacing the ethyl P1 group with disubstituted analogues (α,α′-dimethyl or α,α′-cyclized) proved detrimental to activity (entries 2 and 3, Table 3). In contrast, the α,α′-cyclized (ethylenyl) P1 was reported to be tolerated in carboxylate series.[13] Significantly, replacement of ethyl P1 with 2,2-difluoroethyl, a known CH_2SH mimetic,[14] increased the potency to 0.060 μM (entry 4). This potency enhancement was also seen in other peptide-based HCV NS3 protease inhibitors.[14]

In summary, prime site interactions were investigated using a tetrapeptide-based α-ketoamide template. Glycine carboxylic acid is the preferred prime group. Further modification of P1 group yielded a potent HCV NS3 protease inhibitor with IC_{50} of 0.060 μM.

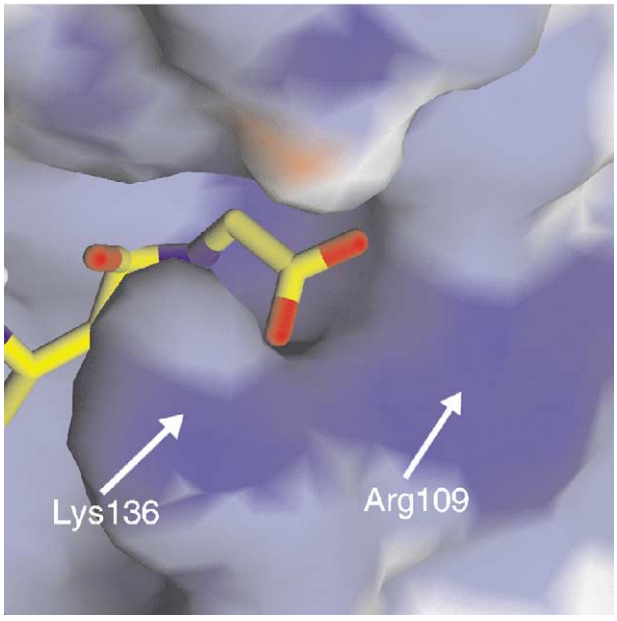
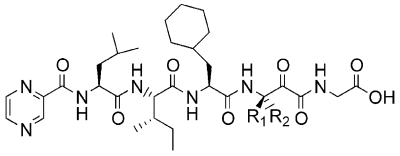


Figure 1. Sectional view of a computer generated model of the glycine carboxylic acid analogue (in stick presentation) in the active site of HCV NS3 protease (in surface presentation).

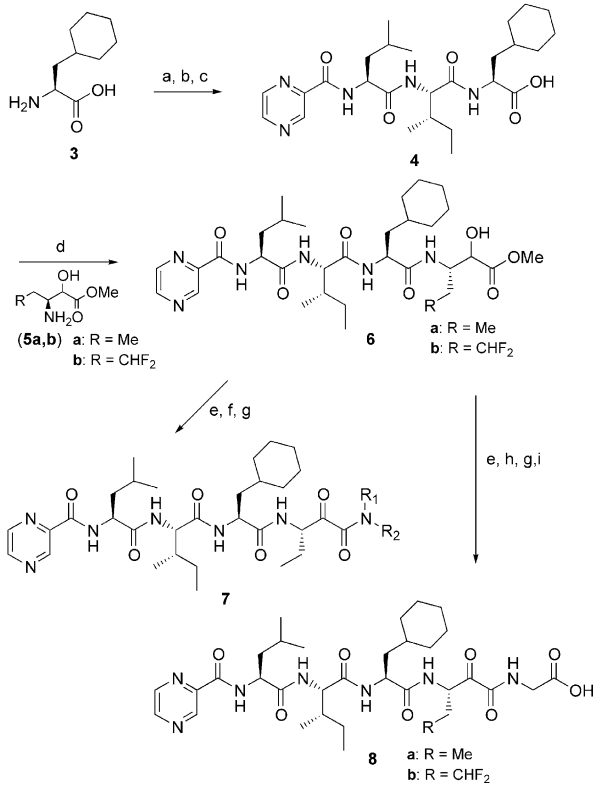
Table 3. SAR of glycine α-ketoamide with different P1 group



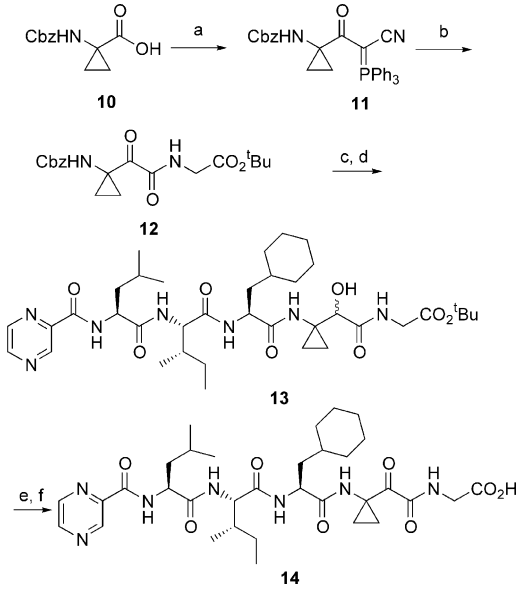
Entry	R_1	R_2	IC_{50} (μM)
1	H	Et	0.23
2	Me	Me	>60
3		$-CH_2CH_2-$	>60
4	H	CHF_2CH_2	0.060

Scheme 1 outlines the synthesis of ketoamides **7** and **8**. Cyclohexylalanine (**3**) was coupled sequentially with appropriately functionalized isoleucine, leucine and pyrazinecarboxylic acid to give tripeptide **4**. Upon coupling with **5a** or **5b**,[4] the corresponding α-hydroxyl ester **6** was obtained. Saponification of ester **6** provided the corresponding carboxylic acid, which was coupled with various amines to give the desired amides. Dess–Martin oxidation then completed the synthesis of α-ketoamides **7**. Alternatively, the acid derived from ester **6** was coupled with glycine *t*-butyl ester. Oxidation and acid deprotection provided glycine α-ketoamides **8**.

Preparation of glycine α-ketoamides with α,α′-cyclized P1 group (**14**) is depicted in Scheme 2. The aminocyclopropane carboxylic acid **10** was activated with EDCI and displaced with (cyanomethylene)-triphenylphosphorane to give cyano keto ylide **11**.[15] Ozonolysis followed by in situ trapping with glycine *t*-butyl ester provided the glycine ketoamide **12**. The α-carbonyl was reduced to alcohol to prevent interference with subsequent coupling reaction. After removal of Cbz, the resultant amine was coupled with tripeptide **15** to give



Scheme 1. (a) Boc-Ile-OSu, DIEA, DMF, 0 °C to rt, 8 h (92%); (b) (i) TFA, CH_2Cl_2 (>95%); (ii) Boc-Leu-OSu, DIEA, DMF, 0 °C to rt, 8 h (90%); (c) (i) TFA, CH_2Cl_2 (>95%); (ii) pyrazinecarbonyl-OSu, DIEA, DMF, 0 °C to rt, 4 h (79%); (d) PyBOP, DIEA, DMF, 0 °C to rt, 4 h (71–84%); (e) LiOH, THF, H_2O, 0 °C, 1.5 h (90%); (f) NHR_1R_2, BOP, DIEA, 0 °C to rt, 4 h (78–88%); (g) Dess–Martin reagent, CH_2Cl_2, 2–8 h (50–83%); (h) H-Gly-O*t*Bu, BOP, DIEA, DMF, 0 °C to rt, 4 h (65–87%); (i) TFA, CH_2Cl_2, rt, 1 h (74–91%).



Scheme 2. (a) $Ph_3P{=}CHCN$, EDCI, DMAP, CH_2Cl_2, 0 °C to rt, 8 h (63%); (b) O_3, CH_2Cl_2, −78 °C, then H-Gly-O*t*Bu HCl, 1 h (84%); (c) (i) $NaBH_4$, THF, 0 °C, 0.5 h; (ii) H_2, Pd/C, MeOH, 0.5 h (56% for two steps); (d) pyrazinyl-CO-Leu-Ile-Cha-OH (15), PyBOP, DIEA, DMF, 0 °C to rt, 4 h (90%); (e) Dess–Martin, CH_2Cl_2, rt, 8 h (45%); (f) TFA, CH_2Cl_2, rt, 1 h (90%).

α-hydroxy amide **13**, which was oxidized and deprotected to give glycine α-ketoamide **14**. The same route was used to prepare the dimethyl P1 analogue.

Acknowledgements

We would like to thank Drs. Bruce D. Korant and Marina G. Bukhtiyarova for providing HCV protease, Dr. James L. Meek and Ms. Lorraine Gorey-Feret for determination of IC_{50} values.

References and Notes

1. Houghton, M. In *Fields Virology*, 3rd ed.; Raven: New York, 1996; p 1035.
2. Bartenschlager, R. *Antiviral Chem. Chemother*. **1997**, *8*, 281.
3. (a) LaPlante, S. R.; Cameron, D. R.; Aubry, N.; Lefebvre, S.; Kukolj, G.; Maurice, R.; Thibeault, D.; Lamarre, D.; Llinàs-Brunet, M. *J. Biol. Chem*. **1999**, *274*, 18618. (b) DiMarco, 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.
4. (a) Kolykhalov, A. A.; Agapov, E. V.; Blight, K. J.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *Science* **1997**, *277*, 570. (b) Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *J. Virol*. **2000**, *74*, 2046.
5. Bartenschlager, R. *J. Viral Hepatitis* **1999**, *6*, 165.
6. (a) Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Hostomska, Z. *Cell* **1996**, *87*, 331. (b) Kim, J. L.; Morgenstern, K. A.; Lin, C.; Fox, T.; Dwyer, M. D.; Landro, J. A.; Chambers, S. P.; Markland, W.; Lepre, C. A.; O'Malley, E. T.; Harbeson, J. A. *Cell* **1996**, *87*, 343. (c) Yao, N.; Reichert, P.; Taremi, S. S.; Prosise, W. W.; Weber, P. C. *Structure* **1999**, *7*, 1353.
7. (a) Han, W.; Hu, Z.-L.; Jiang, X.-J.; Decicco, C. P. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 711 and references therein. (b) Han, W.; Jiang, X.-J.; Hu, Z.-L.; Wasserman, Z. R.; Decicco, C. P. *Abstract of Presentation*, National Meeting of the American Chemical Society, San Diego, Apr. 2001; MEDI-119 and MEDI-121.
8. (a) Bennett, J. M.; Campbell, A. D.; Campbell, A. J.; Carr, M. G.; Dunsdon, R. M.; Greening, J. R.; Hurst, D. N.; Jennings, N. S.; Jones, P. S.; Jordan, S.; Kay, P. B.; O'Brien, M. A.; King-Underwood, J.; Raynham, T. M.; Wilkinson, C. S.; Wilkinson, T. C. I.; Wilson, F. X. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 355. (b) Llinàs-Brunet, M.; Bailey, M.; Déziel, R.; Fazal, G.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2719.
9. Ingallinella, P.; Fattori, D.; Altamura, S.; Steinkühler, C.; Koch, U.; Cicero, D.; Bazzo, R.; Cortese, R.; Bianchi, E.; Pessi, A. *Biochemistry* **2002**, *41*, 5483.
10. The same assay conditions were used as described in ref 7a.
11. Binding mode for α-ketoacid has been reported: Colarusso, S.; Gerlach, B.; Koch, U.; Muraglia, E.; Conte, I.; Stansfield, I.; Matassa, V.G.; Narjes, F. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 705.
12. Ingallinella, P.; Altamura, S.; Bianchi, E.; Taliani, M.; Ingenito, R.; Cortese, R.; De Francesco, R.; Steinkühler, C.; Pessi, A. *Biochemistry* **1998**, *37*, 8906.
13. (a) Poupart, M.; Cameron, D. R.; Chabot, C.; Ghiro, E.; Goudreau, N.; Goulet, S.; Poirier, M.; Tsantrizos, Y. S. *J. Org. Chem*. **2001**, *66*, 4743. (b) Llinàs-Brunet, M.; Bailey, M.; Fazal, G.; Ghiro, E.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2267.
14. Narjes, F.; Koehler, K. F.; Koch, U.; Gerlach, B.; Colarusso, S.; Steinkühler, C.; Brunetti, M.; Altamura, S.; De Francesco, R.; Matassa, V. G. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 701.
15. Wasserman, H. H.; Ho, W. B. *J. Org. Chem*. **1994**, *59*, 4364.