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Structural variations in keto-glutamines for improved inhibition against hepatitis A virus 3C proteinase

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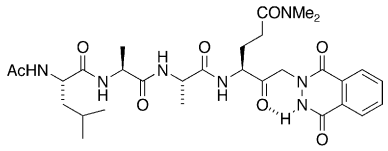
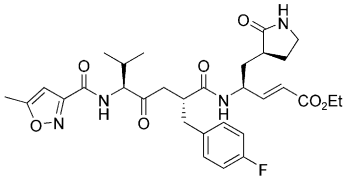
Abstract—A series of keto-glutamine tetrapeptide analogs containing a 2-oxo-pyrrolidine ring as a glutamine side chain mimic were synthesized with both *R* and *S* configuration at the β-carbon. Compounds bearing a phthalhydrazide moiety show improved reversible inhibition of HAV 3C proteinase in the low micromolar range.

Hepatitis A virus (HAV) is a causative agent of an acute form of infectious hepatitis,[1] and belongs to the picornavirus family that contains more than 200 known members, including other pathogens such as human rhinovirus (HRV), foot and mouth disease virus, poliovirus (PV), and encephalomyocarditis virus (EMCV). Picornaviruses possess a small positive single-stranded RNA genome whose translation in the host cells produces a single ~250 kDa polyprotein, proteolytic processing of which is a core feature of the viral replication strategy. In hepatitis A virus, the 3C cysteine proteinase is the key enzyme necessary for cleavage of the primary polyprotein.[2] Hence, potent and selective inhibitors of 3C proteinase could serve as attractive targets for the development of new antiviral therapeutic agents.

Our previous studies on picornaviral 3C proteinases have focused on synthesis and evaluation of several glutamine analogs as effective inhibitors of these enzymes from human rhinovirus-14 (HRV-14) and hepatitis A virus (HAV).[3] In particular, we have recently reported[3b] that the keto-glutamine analog **1** (Fig. 1) is a good reversible inhibitor of HAV 3C proteinase, with an IC_{50} value in the low micromolar range (13 μM). This is probably due to the combined effect of the β and β′ amino functionalities adjacent to the keto group as well as intramolecular hydrogen bonding to the carbonyl, which makes it more electrophilic and potentially susceptible to hemithioacetal formation with the active site cysteine. We now report synthesis and evaluation of structurally modified keto-glutamines that exhibit improved inhibition of the HAV 3C proteinase.



1
Reversible inhibitor of HAV 3C
(IC_{50} = 13 μM)



2, AG7088
Irreversible inhibitor of HRV 3C
(IC_{50} = 0.023 μM)

Figure 1. HAV 3C and HRV 3C proteinase inhibitors.

Our attention focused on the 2-oxo-pyrrolidine ring in the glutamine side chain of AG7088 (**2**, Fig. 1), a potent, nontoxic antirhinoviral agent that irreversibly inhibits HRV 3C proteinase from different virus serotypes.[4] Based on the close structural and mechanistic similarities of various 3C viral proteinases, it appeared that

Keywords: Cysteine protease; Hepatitis A virus; Inhibitors; Glutamine analogs; Phthalhydrazides.
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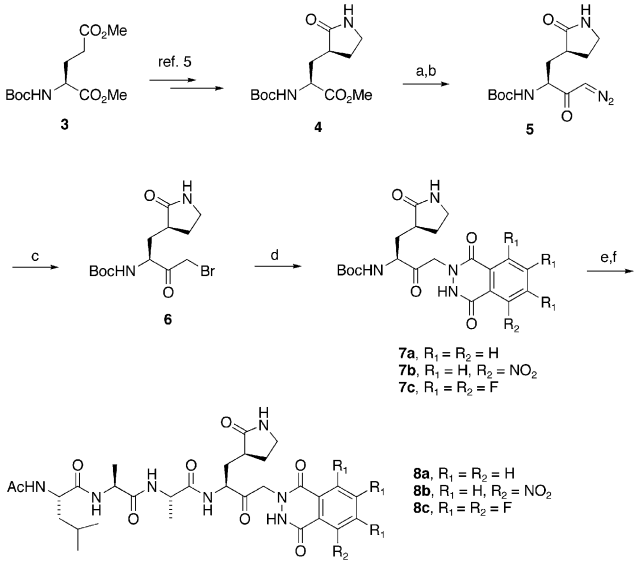
incorporation of the 2-oxo-pyrrolidine ring moiety of **2** into the P_1 position of **1** could generate potent inhibitors of the HAV 3C enzyme.

Thus, N-Boc-L-glutamic acid dimethyl ester (**3**) was converted into cyclic glutamine derivative **4** by following the literature route.[5] This protocol involved stereoselective β-alkylation[6] of **3** with bromoacetonitrile followed by reduction of nitrile and base promoted ring closure to form 2-oxo-pyrrolidine ring with '*S*' stereochemistry (final cyclization step simplified: satd aq $NaHCO_3/CH_2Cl_2$, rt, 70% yield). Hydrolysis of methyl ester in **4** (LiOH/THF, 0 °C) produced the corresponding acid in quantitative yield. This was then converted into diazo ketone **5** in 85% yield. Treatment of **5** with aq HBr generated the bromomethyl ketone **6** (89% yield) that was reacted with sodium phthalhydrazide in DMF[3b] to produce **7a**–**c** in 15–33% isolated yield. Removal of Boc group in **7a**–**c** (TFA/CH_2Cl_2) and coupling with the tripeptide Ac-Leu-Ala-Ala-OH (HBTU or HATU, DIPEA, DMF, rt) afforded the tetrapeptides **8a**–**c** in 39–55% yield (Scheme 1).

Assay of **8a**–**c** for inhibition of HAV 3C proteinase employed an overexpressed C24S mutant in which the nonessential surface cysteine is replaced with serine and which displays catalytic parameters indistinguishable from the wild-type proteinase.[7] The enzyme activity is monitored using a continuous fluorometric assay. Cleavage reactions (700 μL) were performed at 22 °C in a solution containing 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 2 mM EDTA, 0.1 mg/mL bovine serum albumin (BSA), 10 μM fluorogenic substrate Dabcyl-GLRTQSFS-Edans (Bachem), 0.1 μM of proteinase and 1% DMF. Increase in fluorescence (λ_{ex} 336 nm, λ_{em} 472 nm) was monitored using a Shimadzu RF5301 spectrofluorometer. Samples were pre-incubated with inhibitor for 15 min. Initial rates were calculated using the first 3 min of the progress curves. The results (Table 1) indicate that compounds **8a**–**c** show considerable improvement of the reversible inhibition ($IC_{50} = 1.6$–$2.5\,\mu M$) compared to the parent compound **1** ($IC_{50} = 13\,\mu M$).



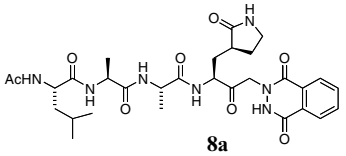
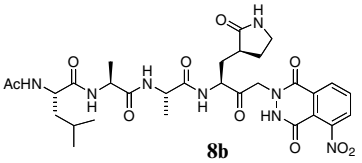
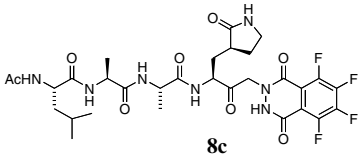
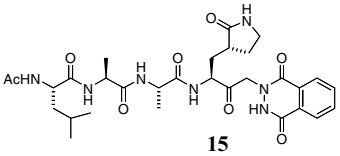
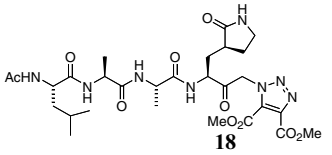
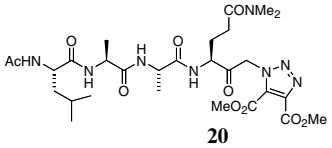
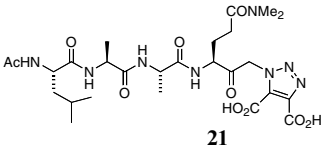
Scheme 1. Reagents and conditions: (a) LiOH, THF/H_2O, 0 °C, quant.; (b) EtOCOCl, Et_3N, THF, −30 °C, then CH_2N_2/Et_2O, −30 °C to rt, 85%; (c) 48% aq HBr, THF, 0 °C, 89%; (d) phthalhydrazide, NaH, DMF, rt, 15–33%; (e) $TFA–CH_2Cl_2$ 1:1, 0 °C, 1.5 h, quant.; (f) Ac-Leu-Ala-Ala-OH, HBTU, or HATU, DIPEA, DMF, rt, 39–55%.

In order to probe the effect of *S* stereocenter in the pyrrolidone ring of **8a**–**c** on the inhibition of the HAV 3C proteinase, compound **15** was synthesized with '*R*' configuration at the ring stereocenter, as outlined in Scheme 2. Thus, L-pyroglutamic acid (**9**) was converted into pyrrolidone **10** as reported.[8] This involved reduction of carboxylic acid moiety of **9** followed by silyl protection of the resulting alcohol and Boc protection of amide nitrogen. Alkylation of **10** with LiHMDS/bromoacetonitrile gave compound **11** as 12:1 diastereomeric mixture (by ^1H NMR analysis) from which the desired major *anti*-isomer was readily separated by silica gel chromatography (85% yield). Hydrogenation of the nitrile functionality in **11** (PtO_2, 50 psi H_2, EtOH/$CHCl_3$) generated the amine **12** that upon treatment with LiOH (THF/H_2O) undergoes intramolecular acyl transfer to afford **13**. Removal of TBDPS group (TBAF, THF/AcOH) and oxidation of resulting alcohol ($RuCl_3/NaIO_4$) produced the carboxylic acid **14** that was converted into **15** by following steps similar to those used for synthesis of **8a**–**c**.

Other heterocyclic keto-glutamines, such as triazole derivatives **18**, **20**, and **21**, which lack the activation of ketone carbonyl group by hydrogen bonding, were also synthesized as outlined in Scheme 3. Thus, bromide **6** was converted into the corresponding azide **16** with NaN_3/KF (58% yield). Treatment of **16** with dimethyl acetylenedicarboxylate generated the triazole **17** in quantitative yield, which upon removal of Boc-protection (TFA/CH_2Cl_2) and coupling with Ac-Leu-Ala-Ala-OH (HATU, DIPEA, DMF, rt) gave **18** in 65% yield. Similarly, bromide **19** was converted into **20** in 58% overall yield. Hydrolysis of methyl esters in **20** (LiOH, $MeOH/H_2O$) produced the diacid **21** in 80% yield. Assay of **15**, **18**, **20**, and **21** for inhibition of HAV 3C proteinase were done as described for **8a**–**c** (Table 1).

As outlined in Table 1, the keto-glutamine **15** ($IC_{50} = 62.6$) with *R* configuration at pyrrolidone ring shows 25-fold weaker reversible inhibition of the HAV 3C enzyme as compared to the corresponding *S* diastereomer **8a** ($IC_{50} = 2.5\,\mu M$). The triazoles **18**, **20**, and **21** that lack ketone activation by intramolecular hydrogen bonding exhibit very poor inhibition (1–14% inhibition at 100 μM inhibitor concentration), despite being diamino ketones. These results indicate that the cyclic glutamines with *S* configuration at pyrrolidone stereocenter are good reversible inhibitors of HAV 3C proteinase and support the proposal[3b] that the activation of 'keto-glutamine' war-head by intramolecular H-bonding may be essential for strong inhibition. Although structural studies on these enzyme–inhibitor complexes are not yet available, it seems likely that the enzyme's active site thiol (Cys172) adds reversibly to the keto function-

Table 1. Evaluation of compounds **8a–c**, **15**, **18**, and **20–21** as reversible inhibitors of HAV 3C proteinase

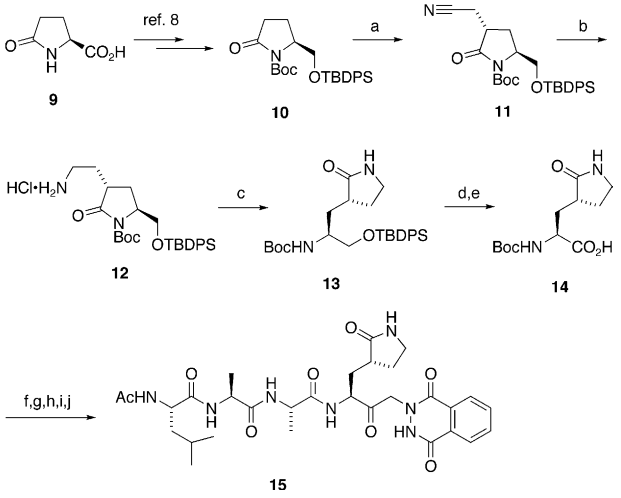
Compound	IC_{50} values[a] (μM)	% Inhibition[a,b]
 **8a**	2.5	
 **8b**	1.6	
 **8c**	2.0	
 **15**	62.6	
 **18**		14
 **20**		9
 **21**		1

[a] HAV 3C (0.1 μM), Dabcyl-GLRTQSFS-Edans (10 μM), EDTA (2 mM), BSA (0.1 mg/mL), KH_2PO_4/K_2HPO_4 (100 mM) at pH 7.5, 22 °C, 15 min preincubation of the enzyme with inhibitor. The IC_{50} values reported are within ±10% error.

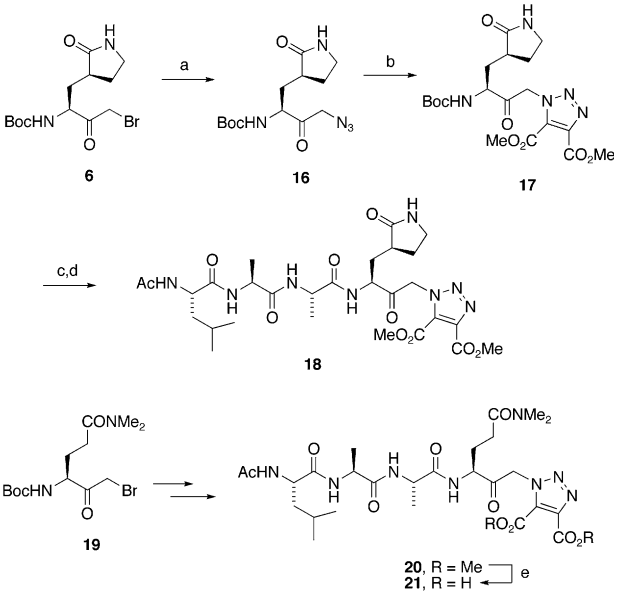
[b] Inhibition at [I] 100 μM.

ality of the keto-glutamine P1 mimic in these substrate analogs to transiently form a hemithioacetal.

In conclusion, inhibition studies of HAV 3C proteinase were performed with various keto-glutamine inhibitors and the structural factors affecting the inhibition were examined. These studies indicate that there is further scope of improvement in inhibition of HAV 3C by



Scheme 2. Reagents and conditions: (a) $BrCH_2CN$, LiHMDS, THF, −78 °C, 85%; (b) PtO_2, 50 psi H_2, EtOH/$CHCl_3$, rt, 48 h; (c) LiOH, THF/H_2O, rt, 12 h, 63% over two steps; (d) TBAF, THF/AcOH, 0 °C–rt, 18 h, 85%; (e) $RuCl_3/NaIO_4$, MeCN/CCl_4/H_2O, 0 °C–rt, 64%; (f) EtOCOCl, Et_3N, THF, −30 °C, then CH_2N_2/Et_2O, −30 °C to rt, 82%; (g) 48% aq HBr, THF, 0 °C, 80%; (h) phthalhydrazide, NaH, DMF, rt, 32%; (i) TFA–CH_2Cl_2 1:1, 0 °C, 1.5 h, quant.; (j) Ac-Leu-Ala-Ala-OH, HATU, DIPEA, DMF, rt, 45%.



Scheme 3. Reagents and conditions: (a) NaN_3, KF, DMF, rt, 1 h, 58%; (b) dimethyl acetylenedicarboxylate, toluene, rt, 3 d, quant.; (c) TFA–CH_2Cl_2 1:1, 0 °C, 1.5 h, quant.; (d) Ac-Leu-Ala-Ala-OH, HATU, DIPEA, DMF, rt, 65%; (e) LiOH, MeOH/H_2O, 80%.

modifications in the glutamine side chain as well as by substitutions on the phthalhydrazide ring. Investigations on the detailed structure of the inhibitor enzyme complexes and on improved inhibitors are in progress.

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References and notes

1. Hollinger, F. B.; Ticehurst, J. R. In *Fields Virology*; Fields, B. N., Knipe, D. M., Howley, P. M., Channock, R. M., Melnick, J. L., Monath, T. P., Roizmann, B. E., Strauss, S. E., Eds.; Lippincott–Raven: Philadelphia, 1996.
2. (a) Bergmann, E. M.; James, M. N. G. In *Proteases as Targets for Therapy*; Von der Helm, K., Korant, B., Eds.; Springer: Heiderlberg, 1999; (b) Gorbalenya, A. E.; Snijder, E. J. *Perspect. Drug Discov. Des.* **1996**, *6*, 64–86; (c) Rueckert, R. R. In *Fields Virology*; Fields, B. N., Knipe, D. M., Howley, P. M., Channock, R. M., Melnick, J. L., Monath, T. P., Roizmann, B. E., Strauss, S. E., Eds.; Lippincott–Raven: Philadelphia, 1996.
3. (a) Ramtohul, Y. K.; Martin, N. I.; Silkin, L.; James, M. N. G.; Vederas, J. C. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1351–1359; (b) Ramtohul, Y. K.; James, M. N. G.; Vederas, J. C. *J. Org. Chem.* **2002**, *67*, 3169–3178; (c) Lall, M. S.; Ramtohul, Y. K.; James, M. N. G.; Vederas, J. C. *J. Org. Chem.* **2002**, *67*, 1536–1547; (d) Ramtohul, Y. K.; Martin, N. I.; Silkin, L.; James, M. N. G.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **2001**, 2740–2741.
4. Dragovich, P. S.; Prins, T. J.; Zhou, R.; Brown, E. L.; Maldonado, F. C.; Fuhrman, S. A.; Zalman, L. S.; Tuntland, T.; Lee, C. A.; Patick, A. K.; Matthews, D. A.; Hendrickson, T. F.; Kosa, M. B.; Liu, B.; Batugo, M. R.; Gleeson, J.-P. R.; Sakata, S. K.; Chen, L.; Guzman, M. C.; Meador, J. W., III; Ferre, R. A.; Worland, S. T. *J. Med. Chem.* **2002**, *45*, 1607–1623.
5. Tian, Q.; Nayyar, N. K.; Babu, S.; Chen, L.; Tao, J.; Lee, S.; Tibbetts, A.; Moran, T.; Liou, J.; Guo, M.; Kennedy, T. P. *Tetrahedron Lett.* **2001**, *42*, 6807–6809.
6. Hanessian, S.; Margarita, R. *Tetrahedron Lett.* **1998**, *39*, 5887–5890.
7. Malcolm, B. A.; Chin, S. M.; Jewell, D. A.; Stratton-Thomas, J. R.; Thudium, K. B.; Rosenberg, S. *Biochemistry* **1992**, *31*, 3358–3363.
8. Woo, K.-C.; Jones, K. *Tetrahedron Lett.* **1991**, *32*, 6949–6952.