Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3531–3536

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Evaluation of Tripeptidyl α-Ketoamides as Human Rhinovirus 3C Protease Inhibitors

Shu-Hui Chen,[a,*] Jason Lamar,[a] Frantz Victor,[a] Nancy Snyder,[a] Robert Johnson,[b] Beverly A. Heinz,[b] Mark Wakulchik[b] and Q. May Wang[b,*]

[a]*Discovery Chemistry Research and Technology, Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA*

[b]*Department of Infectious Diseases, Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA*

Received 16 April 2003; accepted 8 July 2003

Abstract—We describe herein the synthesis and biological evaluation of a series of tripeptidyl α-ketoamides as human rhinovirus (HRV) 3C protease inhibitors. The most potent inhibitor discussed in this manuscript, **4I**, exhibited impressive enzyme inhibitory activity as well as antiviral activity against HRV-14.

Introduction

Human rhinoviruses (HRV) are a group of plus-strand RNA viruses belonging to the picornavirus family. As the leading cause of common cold in humans, over 100HRV serotypes have been identified.[1] Approximate 100 million annual incidence of common cold and up to 25 million office visits in USA are reported. Furthermore, several HRV infections related complications such as acute exacerbation, sinusitis, acute bronchitis, and otitis media in children may occur. In contrast to the unmet medical needs, there are no antiviral drugs available for the treatment of HRV infections today.

HRV 3C protease is a cysteine protease that is responsible for the generation of mature viral proteins and the enzyme required for viral replication and infections.[2,3] HRV 3C protease recognizes specific amino acid sequence and makes a cleavage primarily at the Gln/Gly bond.[4] Due to its essential roles in viral infection and unique protein structure,[3] HRV 3C protease has been viewed as an attractive target for the development of antiviral agents (Fig. 1).[5]

In view of the urgent medical needs for anti-HRV drugs, several reports of substrate based reversible and irreversible enzyme inhibitors have recently appeared in the literature. These include Michael acceptors (e.g., G7088 **1**),[6,7] substrate derived peptidyl aldehydes (e.g., **2**),[8] C-termini heterocyclic ketones (e.g., **3**),[9] azapeptides,[10] and C-termini bromomethyl ketones,[11] S-nitrosothiols,[12] as well as a novel series of tripeptidyl α-ketoamides (e.g., **4A**). The initial hit emerging from α-ketoamide series, **4A** showed good inhibitory activity against HRV-14 in both the enzyme and cellular assays with IC_{50} values around 2–3 μM. In this manuscript, we report our recent progress achieved through **4A**-based SAR modifications at P3, P2, P1, and P1′ positions.

Chemical Synthesis

All tripeptidyl α-ketoamides discussed in this manuscript (**4A**–**4N**) were synthesized via two sequential peptide coupling reactions between their respective P3/P2 units and P3-P2/P1-P1′ units as shown in Schemes 1 and 2. The structures of the α-ketoamides discussed herein are secured on the basis of their proton NMR and mass spectra analyses.

The synthetic route employed for the preparations of two bicyclo-Pro P2 bearing inhibitors **4A** and **4B** is outlined in Scheme 1. In this case, the known bicycloproline

*Corresponding author. Tel.: +1-317-276-2076; fax: +1-317-276-1177; e-mail: chen_shu-hui@lilly.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00753-4

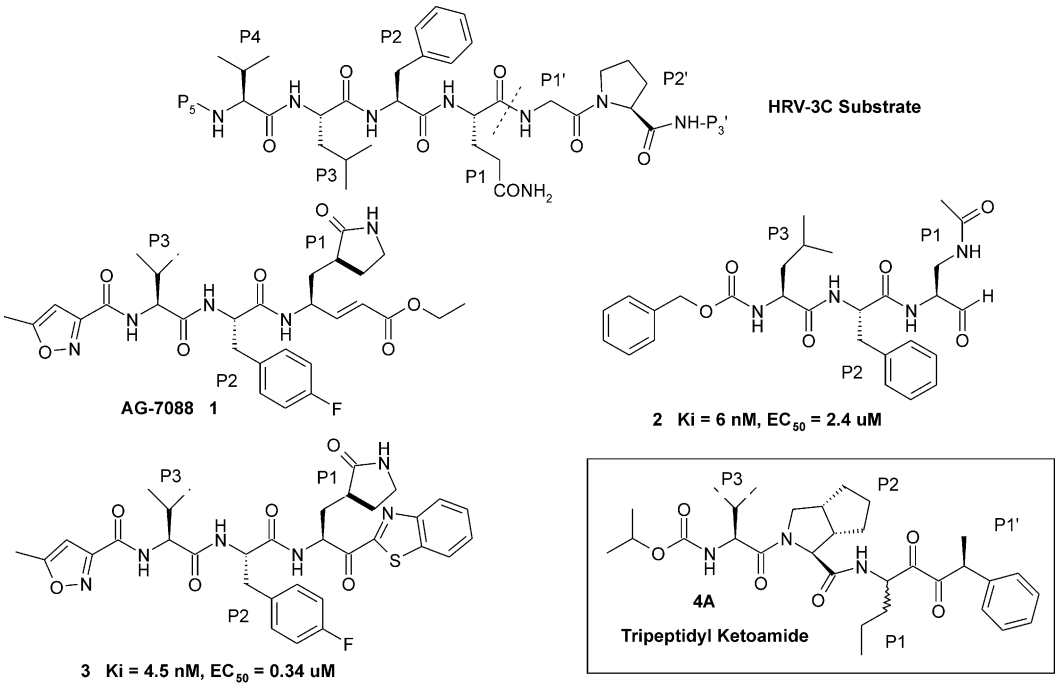


Figure 1. Representative HRV 3C protease inhibitors.

ketone **AA**[13] was reduced with sodium borohydride to gave a mixture of the respctive carbinol **BB**, which was then converted to the deoxy bicyclo-Pro acid **EE** via Barton deoxygenation reaction[14] and hydrogenation. Coupling of the P3 acid **FF** with **EE** was promoted by HOAt/EDCI,[15] and afforded the expected adduct **HH**, which was converted next to the acid **II** via saponification. Final PyBOP mediated coupling[16] of **II** with the P1-P1′ unit **LL** (itself prepared in two steps from the α-hydroxyacid RR as shown in Scheme 2) provided the desired α-hydroxyamide adduct **NN**, which was then oxidized with Dess–Martin periodinane[17] to furnish the desired α-ketoamide **4A**. On the other hand, treatment of the α-isomer of carbinol **BB** (the major isomer) with DAST furnished the desired β-fluoro bc-Pro derivative (via SN_2 inversion),[18] which was further converted to its free amine **GG** via hydrogenalysis. Following the identical peptide coupling sequence described for **4A**, the P2 fluorinated intermediate **GG** was incorporated into the final product **4B**.

The general synthetic route for the preparation of P2 Phe bearing α-ketoamides is shown in Scheme 2. Coupling of the P3-Val acid **FF** (itself prepared from Val-OBn via a two-step sequence) with phenylalanine methyl ester was mediated by HOAt/DCC,[15] and provided the dipeptide methyl ester **OO**, which was then saponified to give its corresponding acid **PP** in 93% yield. Subsequent coupling of **PP** with the P1-P1′ unit **QQ** (prepared from the known β-amino-α-hydroxyacid **RR**[19] via a two-step sequence) was promoted by PyBOP[16] and *i*-Pr_2EtN and provided the expected adduct **SS**, which was further oxidized to the final α-ketoamide **4I** using Dess–Martin periodinane.[17] The low yield (~20%) obtained in the final coupling and oxidation steps was due largely to the gel-like nature of **4I**.

Biological Evaluation

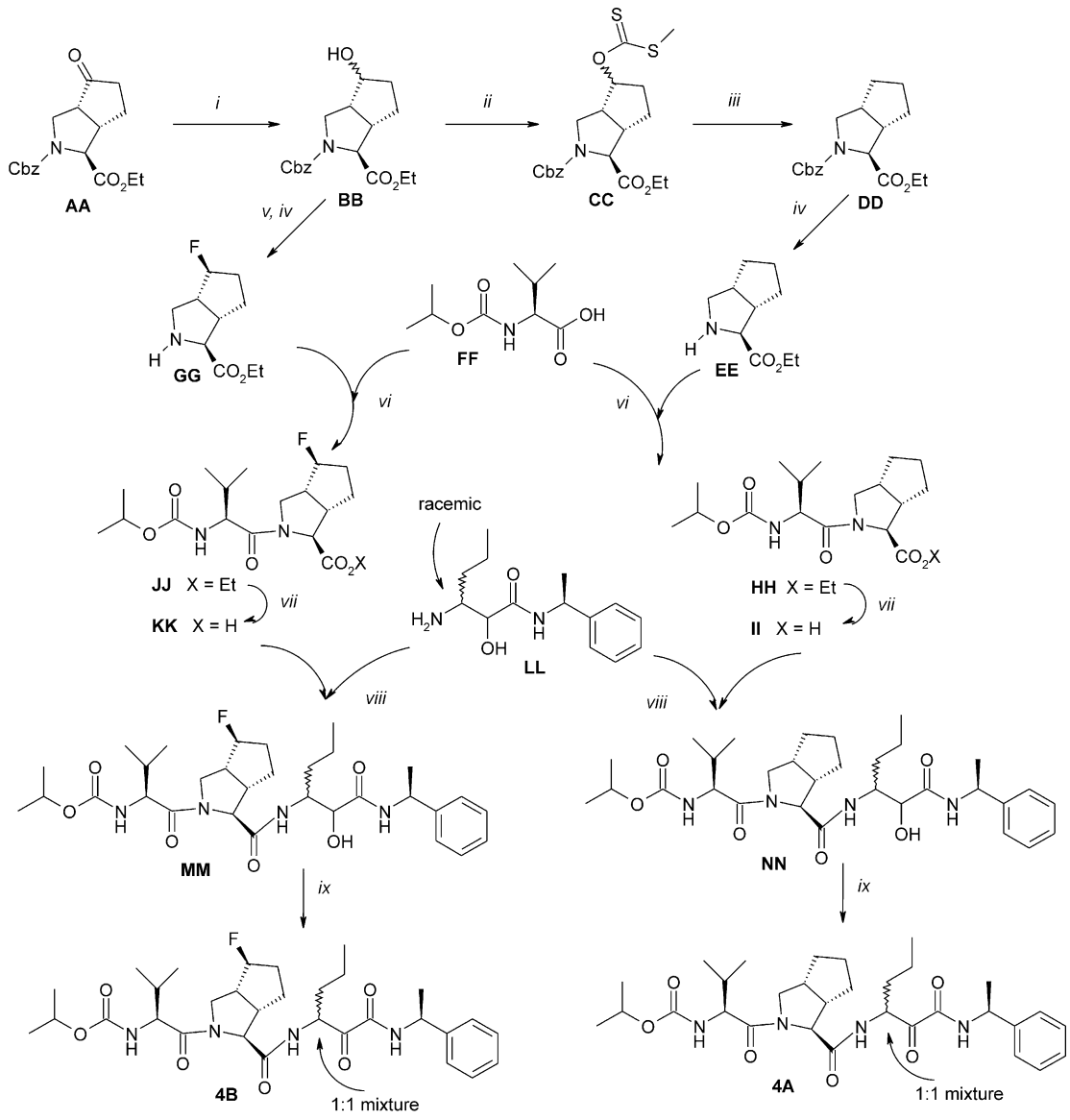
All tripeptidyl α-ketoamides (**4A**–**4N**) synthesized were tested sequentially in the following assays: (1) HRV-14 3C enzyme assay;[20] (2) antiviral cell culture assays against both HRV-14 and HRV-16;[8a,21] and (3) XTT cytotoxicity assay.[22]

P3 SAR

Antiviral activities of two pairs of HRV 3C inhibitors containing P3 modifications (*t*-Leu vs Val) were compared and the data is summarized in Table 1. When compared with their respective P3 *t*-Leu counterparts **4C** and **4D**, two P3 Val bearing analogues **4A** and **4B** exhibited 2–3-fold improved activity against HRV-14 in both the enzyme and cellular assays. Consistent with this finding, similar trend was again observed in the antiviral assay against HRV-16. Careful data comparison between **4A** versus **4B** and **4C** versus **4D** clearly indicates that fluorination at the P2 residue is detrimental to antiviral activity. The antiviral activity could be clearly distinguished from the cytotoxicity, with therapeutic indexes (TC_{50}/EC_{50}) of at least 6. In view of the SAR trends observed thus far, we decided to use Val as the standard P3 residue for the tripeptidyl α-ketoamide series.

P2 SAR

With the aim of simplifying P2 residue, we prepared four additional analogues bearing Pro (**4E**), α,α-cylopentyl (**4F**), Phe (**4G**), and *p*-F-Phe (**4H**) as their respective P2 units. The testing results clearly indicate that **4E** showed about 5-fold weaker antiviral activity relative to **4A**; and **4F** was devoid of any antiviral

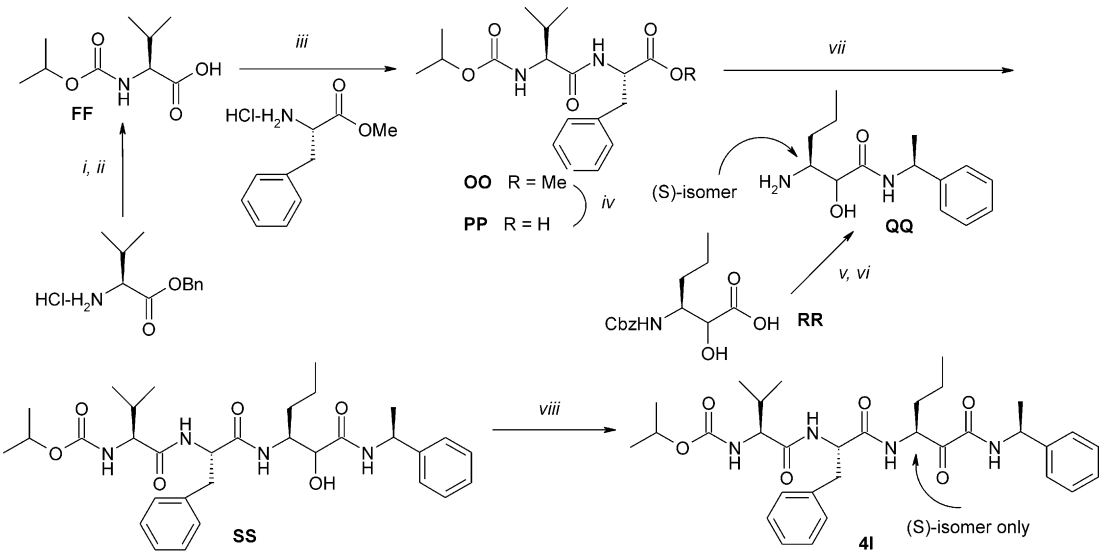


Scheme 1. Syntheses of P2-bc-proline bearing inhibitors **4A** and **4B**. Reagents and conditions: (i) $NaBH_4$/EtOH, 97%; (ii) NaH/CS_2 + THF/MeI; (iii) *n*-Bu_3SnH/AIBN/Tol. 66% for two steps; (iv) $H_2/Pd(OH)_2$/EtOH, 95%; (v) DAST/CH_2Cl_2, 90%; (vi) **FF**/HOAt/EDCI/THF, 73% for **HH**; 79% for **JJ**; (vii) 1 N NaOH/EtOH, 85% for **II**; 69% for **KK**; (viii) PyBOP/THF, then **LL**, 63% for **NN**; 73% for **MM**; (ix) Dess–Martin periodinane, 71% for **4A**; 90% for **4B**.

activity. In contrast to these findings, the P2 Phe bearing inhibitor **4G** demonstrated 6-fold greater enzyme inhibitory activity as compared with **4A**. When tested in the antiviral assay, only minimal improvement (2×) in cellular activity was observed with **4G** ($EC_{50} = 1$ μM) relative to **4A** ($EC_{50} = 2.2$ μM). Replacement of the P2 Phe in **4G** with *p*-F-Phe in **4H** ($EC_{50} = 2.1$ μM) resulted in 2–4-fold reduction in enzyme inhibitory potency and antiviral activity. It is worthwhile to note that the P2 preference observed with the tripeptidyl α-ketoamide series is different from that reported for the Michael acceptors.[6b] As far as cytotoxicity is concerned, it is evident that none of the inhibitors discussed in Tables 2 and 3 was considered to be cytotoxic ($TC_{50} > 50$ μM). The ratio of TC_{50}/EC_{50} ranged from 6 (for **4D**) to 30 (for **4A**).

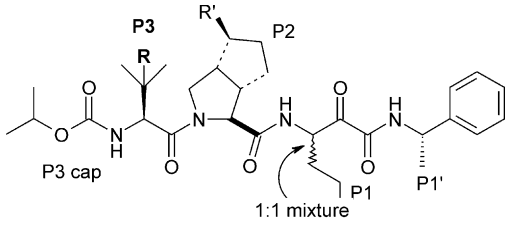
P1 SAR

Having found the preferred P2 residue (Phe) for α-ketoamide series, we shifted our attention to P1 modifications. Replacement of the achiral Nva P1 residue in **4G** with the chiral (*s*)-Nva led to **4I**. As expected, inhibitor **4I** was found to be 2-fold more potent than **4G** in the enzyme assay. Interestingly, when evaluated in the antiviral assay, **4I** exhibited similar antiviral activity to that of **4G**. Based on our previous experience with similar α-ketoamide bearing inhibitors designed for HCV protease,[23] we believed that the current finding suggests that the chiral center at P1 (alpha to the ketoamide moiety) epimerized rapidly in the cell-culture media. Like compound **4G**, the newly prepared single isomer (at P1) 4I was found to be non-cytotoxic



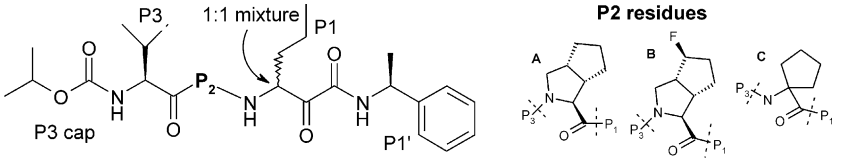
Scheme 2. Synthesis of Phe-P2 bearing inhibitor **4I**. Reagents and conditions: (i) *i*-PrOC(O)Cl/*i*-Pr_2EtN/HCl-ValOBn, %; (ii) H_2, Pd/C, ~100%; (iii) HOAt/DCC/*i*-Pr_2EtN/HCl-PheOMe, 78%; (iv) 1 N NaOH/MeOH, 93%; (v) **RR**/PyBOP/(s)-MeBnNH$_2$, %; (vi) H_2, $Pd(OH)_2$/C, ~100%; (vii) PyBOP/*i*-Pr_2EtN /**PP**, then **QQ**; (viii) Dess–Martin periodinane 20% (last two steps).

Table 1. P3 SAR trends



Compd	P3-R	P2-R′	HRV-14 enzyme K_i (μM)	HRV-14 EC_{50} (μM)	HRV-16 EC_{50} (μM)	Cytotoxicity TC_{50} (μM)
4A	**H**	**H**	**3.0**	**2.2**	**~10**	**65.4**
4C	Me	H	5.4	7.7±0.3	25.7	57.8
4B	H	F	5.5	5.8	—	82
4D	Me	F	9.8	10	—	60.4

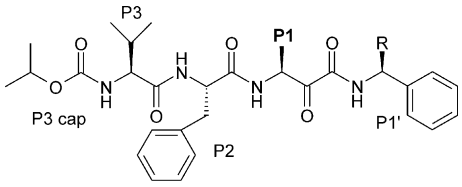
Table 2. P2 SAR trends



Compd	P2	HRV-14 enzyme K_i (μM)	HRV-14 EC_{50} (μM)	HRV-16 EC_{50} (μM)	Cytotoxicity TC_{50} (μM)
4A	A	3.0	2.2	~10	65
4B	B	5.5	5.8	—	82
4E	Proline	17.4	10.4±2.9	43.3	>100
4F	C	Inactive	>20	Inactive	>100
4G	**Phe**	**0.50**	**1.0±0.2**	**10.1**	**62**
4H	*para*-F-Phe	1.36	2.1	37.3	>100

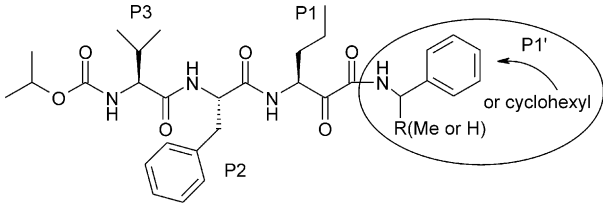
(TC_{50} > 100 μM). On the other hand, comparative evaluation of **4J** (IC_{50} = 0.9 μM) and **4K** (IC_{50} = 4.6 μM) clearly shows that the Gln functionality at P1 is preferred (5×) over P1-Nva bearing counterpart for the inhibition of HRV 3C enzyme activity. This trend is in good agreement with that reported for the Michael acceptor series[6] as well as the peptidyl aldehyde series inhibitors.[8b] The poor antiviral activity (EC_{50} > 20 μM) displayed by **4J** was due to the high polarity of its P1 residue (Gln).

Table 3. P1 SAR Trends



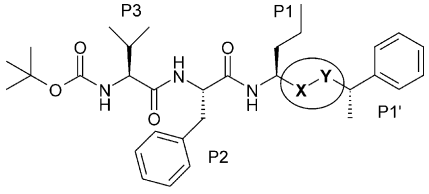
Compd	P1	R	HRV-14 enzyme K_i (μM)	HRV-14 EC_{50} (μM)	Cytotoxicity TC_{50} (μM)
4G	(*rac*)-Nva	Me	0.50	1.0±0.2	62
4I	**(*S*)-Nva**	**Me**	**0.17±0.06**	**0.85±0.15**	**>100**
4J	(*S*)-Gln	H	0.94±0.59	>20	>100
4K	(*S*)-Nva	H	4.60	5.3±2.6	>100

Table 4. P1′ SAR trends



Compd	P1′	HRV-14 Enz. K_i (μM)	HRV-14 EC_{50} (μM)	HRV-16 EC_{50} (μM)	Cytotoxicity TC_{50} (μM)
4I	***(S)-MeBn***	***0.17±0.06***	***0.85±0.15***	***~10***	***>100***
4L	(*R*)-MeBn	6.5±1.9	11.1±1.5	—	Not tested
4K	Bn	4.6	2.7	—	>100
4M	(*S*)-Me-Hexyl-*c*	6.5	>20	—	54.6

Table 5. Ketoamide versus amide



LY	X	Y	HRV-14 enzyme K_i (μM)	HRV-14 antiviral EC_{50} (μM)	Cytotoxicity TC_{50} (μM)
4N	–C(O)C(O)–	NH	0.71±0.25	1.3±0.3	30.3
5A	–C(O)–	NH	7.4±1.0	>20	>100
5B	–C(O)–	$NHCH_2$	29.1±4.7	>15	>100

P1′ SAR

The antiviral activity and cytotoxicity of three P1′ modified analogues of **4I** is listed in Table 4. Careful data analysis reveals the following SAR trends: (1) the C-termini benzylic (*S*)-chirality as seen in **4I** is preferred at least 10× over its opposite (*R*)-stereochemistry as seen in **4L**; (2) aromaticity at the P1′ position (e.g., **4I**) is preferred (>3×) over its corresponding saturated cyclohexyl functionality (e.g., **4M**); and (3) introduction of a methyl group with (s)-stereochemistry at the C-termini benzylic position led to significant improvement in enzyme inhibitory activity in **4I** relative to the corresponding desmethyl derivative **4K**.

α-Ketoamides versus simple amides

Having discovered a new series of α-ketoamide bearing HRV 3C inhibitors such as **4I**, we decided to prepare a few structurally related tripeptides without the electrophilic α-keto moiety for comparison. Judging from of the enzyme inhibitory potencies as well as the antiviral activities displayed by three compounds included in

Table 5, it is clear that the eletrophilic keto moiety in **4N** is needed for optimal antiviral activity. In contrast, analogues lacking such 'keto' functionality (e.g., **5A** and **5B**) are devoid of significant antiviral activities.

Conclusion

Starting from our initial screen hit **4A**, we synthesized and evaluated a number of **4A** based α-ketoamides containing modification(s) at P3, P2, P1 and P1′ position. The most potent HRV 3C inhibitor identified through this effort, **4I** exhibited impressive activity in both the enzyme assay ($IC_{50}=0.17$ μM) and cell based assay (EC_{50} ~0.85 μM against HRV-14). Furthermore, we also found that deletion of the α-keto moiety from **4** resulted in significant loss of antiviral activity (**4N** vs **5A**).

Acknowledgements

We would like to thank Drs. J. Munroe, J. Colacino, and G. H. Cassell for helpful discussion and encouragement.

References and Notes

1. Couch, R. B. In *Virology*; Fields, B. N., Knipe, D. M., Eds.; Raven: New York, 1990.
2. Porter, A. G. *J. Virol.* **1993**, *67*, 6917.
3. Matthews, D. A.; Smith, W. A.; Ferre, R. A.; Condon, B. *Cell* **1994**, *77*, 761.
4. HRV substrates: (a) Cordingley, M. G.; Callahan, P. L.; Sardana, V. V.; Garsky, V. M.; Colonno, R. J. *J. Biochem.* **1990**, *265*, 9062. (b) Wang, Q. M.; Johnson, R. B.; Cox, G. A.; Villareal, E. C.; Loncharich, R. J. *Anal. Biochem.* **1997**, *252*, 238.
5. Recent Reviews: (a) Dragovich, P. S. *Exp. Opin. Ther. Pat.* **2001**, *11*, 177. (b) Wang, Q. M. *Drugs Future* **2000**, *25*, 279.
6. (a) AG-7088: Dragovich, P. S.; Prins, T. J.; Zhou, R.; Webber, S. E.; Marakovits, J. T.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Ford, C. E.; Burke, B. J.; Rejto, P. A.; Hendrickson, T. F.; Tuntland, T.; Brown, E. L.; Meador, J. W., III.; Ferre, R. A.; Harr, J. E. V.; Kosa, M. B.; Worland, S. T. *J. Med. Chem.* **1999**, *42*, 1213. (b) Dragovich, P. S.; Prins, T. J.; Zhou, R.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Ford, C. E.; Meador, J. W., III; Ferre, R. A.; Worland, S. T. *J. Med. Chem.* **1999**, *42*, 1203.
7. Kong, J. S.; Venkatraman, S.; Furness, K.; Nimkar, S.; Shepherd, T. A.; Wang, Q. M.; Aube, J.; Hanzlik, R. P. *J. Med. Chem.* **1998**, *41*, 2579.
8. (a) Kaldor, S. W.; Hammond, M.; Dressman, B. A.; Labus, J. M.; Chadwell, F. W.; Kline, A. D.; Heinz, B. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2021. (b) Shepherd, T. A.; Cox, G. A.; McKinney, E.; Tang, J.; Wakulchik, M.; Zimmerman, R. E.; Villarreal, E. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2893. (c) Webber, S. E.; Okano, K.; Little, T. L.; Reich, S. H.; Xin, Y.; Fuhrman, S. A.; Matthews, D. A.; Love, R. A.; Hendrickson, T. F.; Patick, A. K.; Meador, J. W., III.; Ferre, R. A.; Brown, E. L.; Ford, C. E.; Binford, S. L.; Worland, S. T. *J. Med. Chem.* **1998**, *41*, 2786.
9. Dragovich, P. S.; Zhou, R.; Webber, S. E.; Prins, T. J.; Kwok, A. K.; Okano, K.; Fuhrman, S. A.; Zalman, L. S.; Maldonado, F. C.; Brown, E. L.; Meador, J. W.; Patick, A. K.; Ford, C. E.; Brothers, M. A.; Binford, S. L.; Matthews, D. A.; Ferre, R. A.; Worland, S. T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 45.
10. Venkatraman, S.; Kong, J.; Nimkar, S.; Wang, Q. M.; Aube, J.; Hanzlik, R. P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 577.
11. Kati, W. M.; Sham, H. L.; McCall, J. O.; Montgomery, D. A.; Wang, G. T.; Rosenbrook, W.; Miesbauer, L.; Buko, A.; Norbeck, D. W. *Arch. Biophys. Biochem.* **1999**, *362*, 363.
12. Xian, M.; Wang, Q. M.; Chen, X.; Wang, K.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2097.
13. c.f. Monn, J. A.; Valli, M. J. *J. Org. Chem.* **1994**, *59*, 2773. The enatiomerically pure material was obtained via chiral HPLC separation.
14. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574.
15. Carpino, L. A.; El-Faham, A. *Tetrahedron* **1999**, *55*, 6813.
16. Costa, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205.
17. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
18. (a) Hudlicky, M. *Org. React* **1987**, *35*, 513. (b) Leory, J.; Hebert, E.; Wakselman, C. *J. Org. Chem.* **1979**, *44*, 3406.
19. P1-Nvaline bearing α-hydroxyacid was prepared from *Z*-Nva(H) via its corresponding cyanohydrin intermediate.
20. HRV enzyme assay: All compounds synthesized were tested for their ability to inhibit HRV 3C protease activity (with 0.1 uM enzyme concentration) using a colorimetric assay with EALFQ-*p*-nitroanilide as substrate. All cleavage reactions were initiated by the addition of 0.4 mM peptide substrate and monitored for 60 min. K_i values were calculated according to the following reference: Morrison, J. F. *Biochim. Biophys. Acta.* **1969**, *185*, 269 for tight binding inhibitors. The Prism program (GraphPad Software) was used for this procedure.
21. Cell-based antiviral assays were performed using confluent monolayers of HeLa cells infected with either HRV14 or HRV16 under the conditions described in the following reference: *J. Med. Chem.* **1999**, *42*, 50.
22. XTT cytotoxicity assay: HeLa cells were cultured under the identical conditions without virus infection but with the test compounds. Also see: Roehm, N. W.; Rodgers, G. H.; Hatfield, S. M.; Glasebrook, A. L. *J. Immunol. Methods* **1991**, *142*, 257.
23. For α-ketoamide based HCV protease inhibitors, see: Babine, R.; Chen, S. H.; Lamar, J.; Snyder, N.; Sun, X.; Tebbe, M.; Victor, F.; Wang, Q. M.; Yip, Y.; Collado, I.; Garcia-Paredes, C.; Parker, R.; Jin, L.; Guo, D.; Glass, J. PCT Patent WO 0218369 A2, March 7, 2002. Detailed reports describing P1 (Nva) racemization under cell culture media and rat plasma will be published in due time.