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Design and Synthesis of Irreversible Depsipeptidyl Human Rhinovirus 3C Protease Inhibitors

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Abstract—Novel tripeptidyl C-terminal Michael acceptors with an ester replacement of the P₂–P₃ amide bond were investigated as irreversible inhibitors of the human rhinovirus (HRV) 3C protease (3CP). When screened against HRV serotype-14 the best compound was shown to have very good 3CP inhibition ($k_{\text{obs}}/[I]=270,000 \text{ M}^{-1} \text{ s}^{-1}$) and potent in vitro antiviral activity ($\text{EC}_{50}=7.0 \text{ nM}$). © 2001 Elsevier Science Ltd. All rights reserved.

Human rhinoviruses (HRVs),¹ the primary cause of the common cold, are small single stranded RNA viruses belonging to the picornaviridae family.² Proteins essential for HRV replication are generated by the proteolysis of a large polypeptide. The virally encoded HRV 3C protease (3CP)³ is predominantly responsible for this operation. 3CP is a cysteine protease that lacks sequence homology to common host enzymes and structurally resembles trypsin-like serine proteases.^{4,5} Inhibition of this enzyme has recently become the topic of interesting research directed toward the development of an antirhinoviral therapy.⁶ In particular, significant attention has been devoted to a class of irreversible inhibitors based on the peptide substrate binding determinants incorporating C-terminal Michael acceptors that are reactive with the 3CP catalytic cysteine.⁷ Recent work describes structural modifications to this class of molecules to remove their inherent peptidic characteristics while improving physical properties without adversely affecting activity.^{8–10} Inhibitory activity and/or physical properties of several of these molecules were improved by *N*-methylation of the P₂–P₃ amide linkage or isosteric replacement of this amide with a ketomethylene group.^{8,9} Close examination of the X-ray crystal structures of irreversibly bound peptidic inhibi-

tors to HRV-2 3CP, specifically at the P₂ site, reveals a hydrogen bond between the P₂-phenylalanine N–H and the hydroxyl of residue Ser-128.^{7a,9} The hydroxymethyl side chain of Ser-128 partially defines the S₂ subsite and is also exposed to solvent. The mobility of this side chain allows for the design of P₂–P₃ amide modifications. Replacement of this linkage with an ester was logical since modeling suggested the formation of a hydrogen bond to Ser-128 with reversed ordering compared to an amide. As illustrated in Figure 1, the serine hydroxyl would now serve as the proton donor while the alkoxy oxygen the acceptor.

In order to test this hypothesis, and determine whether depsipeptides possessing a C-terminal Michael acceptor are useful as irreversible inhibitors of HRV 3CP, we have prepared and evaluated some representative examples. Three depsipeptidyl irreversible inhibitors of HRV 3CP were prepared. This work was guided by our

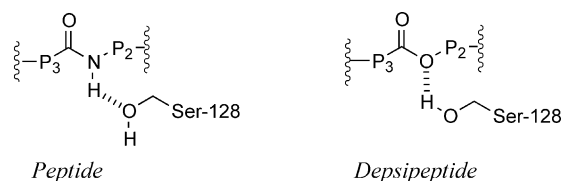


Figure 1. Observed H-bond between P₂–P₃ amide and Ser-128 versus postulated P₂–P₃ ester.

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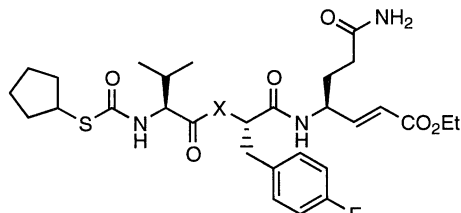
initial protein-structure-based design and structure–activity relationship (SAR) optimization of substrate-derived tripeptides with incorporated C-terminal α,β -unsaturated ethyl esters.^{7a,b} These new P₂–P₃ ester derivatives, compounds **1**, **4**, and **7**, are directly compared to their corresponding amide and ketomethylene analogues as shown in Tables 1–3. The Michael acceptor, P₂-4-fluorophenylalanine and P₃-valine groups are common structural features in the molecules.^{7a,b} Derivatives **7**–**9** include the optimal P₁-(S)- γ -lactam and N-terminal methylisoxazole carboxamide identified previously.¹⁰

Compared to their amide and ketomethylene counterparts, depsipeptides **1**, **4**, and **7** displayed reduced HRV-

14 3CP activity¹¹ except in the case of **1** versus **2**. Excluding the data from compound **1**¹² a possible explanation for these results was made after inspection of the HRV-2 3CP cocrystal structure of **4**. As illustrated in Figure 2, the distance between Ser-128 and the P₂–P₃ ester is 3.6 Å indicating that our originally hypothesized H-bond is not optimal in this case.

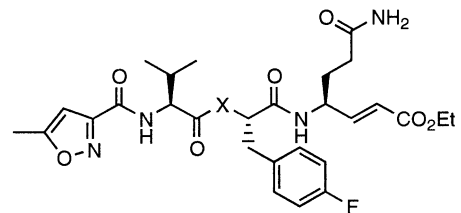
Again, with the exception of compound **1**,¹² depsipeptide inhibitors **4** and **7** displayed improved or comparable antiviral activity¹¹ relative to the corresponding peptides **5** and **8** and ketomethylene analogues **6** and **9**. These results imply that inhibitors **4** and **7** have membrane

Table 1.



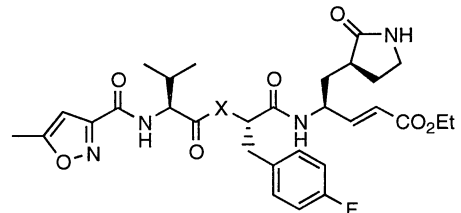
Compd	X	$k_{\text{obs}}/[I]$ (M ⁻¹ s ⁻¹) ^a	EC ₅₀ (μ M) ^b	CC ₅₀ (μ M) ^b
1	O	99,800	1.0	> 10
2	NH	59,900	0.19	> 100
3	CH ₂	293,000	0.02	> 100

Table 2.



Compd	X	$k_{\text{obs}}/[I]$ (M ⁻¹ s ⁻¹) ^a	EC ₅₀ (μ M) ^b	CC ₅₀ (μ M) ^b
4	O	68,400	0.1	> 10
5	NH	248,000	0.42	> 100
6	CH ₂	240,000	0.10	> 100

Table 3.



Compd	X	$k_{\text{obs}}/[I]$ (M ⁻¹ s ⁻¹) ^a	EC ₅₀ (μ M) ^b	CC ₅₀ (μ M) ^b
7	O	270,000	0.007	> 100
8	NH	1,500,000	0.01	> 100
9	CH ₂	1,090,000	0.005	> 100

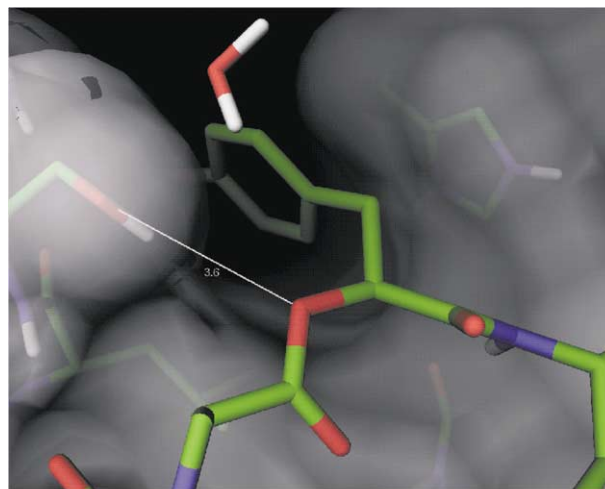
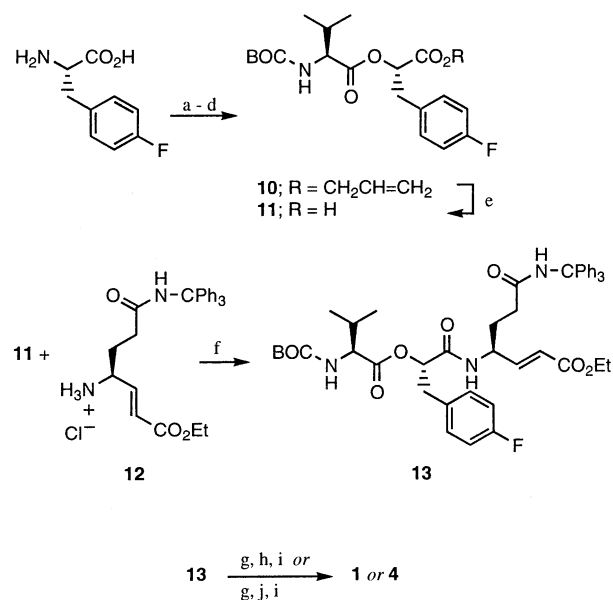
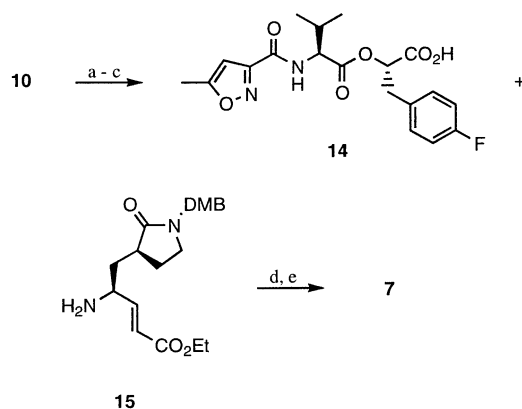


Figure 2. View of the P₂ and S₂ regions of the 1.9 Å cocrystal structure of compound **4** covalently bound to HRV-2, 3CP. The distance between Ser-128 and the P₂ ester oxygen is 3.6 Å. The isopropyl side chain of the P₃ Val is clipped out of view.



Scheme 1. Reagents and conditions: (a) NaNO₂, 1 N H₂SO₄, 79%; (b) K₂CO₃, CH₃I, acetone, 66%; (c) Ti(OⁱPr)₄, allyl alcohol, 90 °C, 68%; (d) BOC-Val, DCC, DMAP, CH₂Cl₂, 90%; (e) Pd(PPh₃)₄, morpholine/THF, 86%; (f) HATU, (*i*Pr)₂NEt, DMF, 39%; (g) 1.3 M HCl/1,4-dioxane, 83%; (h) cyclopentyl chlorothioformate, NEt₃, CH₂Cl₂, 59%; (i) TFA, CH₂Cl₂, 60–63%; (j) 5-methylisoxazole-3-carbonyl chloride, NEt₃, CH₂Cl₂, 45%.



Scheme 2. Reagents and conditions: (DMB = 2,4-dimethoxybenzyl) (a) 1.3 M HCl/1,4-dioxane, satd K_2CO_3 , 99%; (b) 5-methylisoxazole-3-carboxyl chloride, pyr, CH_2Cl_2 , 82%; (c) $Pd(PPh_3)_4$, morpholine/THF, 95%; (d) HATU, $(iPr)_2NEt$, DMF, 72%; (e) DDQ, $CHCl_3$, H_2O , 50 °C, 90%.

Table 4.

Compd	% Metabolism	
	–NADPH	+ NADPH
7	44	67
8	7	22
9	27	50

Incubation time = 30 min; 25 μM compound; 1.0 mg/mL human liver microsomes; 2.0 mM NADPH.

permeabilities similar to **6** and **9**, respectively, and are desolvated more readily than **5** and **8**.⁹

The HRV 3CP depsipeptide inhibitors **1**, **4**, and **7** were synthesized from common intermediate **10**, as outlined in Schemes 1 and 2.¹³ Starting with L-4-F-Phe, (S)-methyl-2-OH-3-(4-F-phenyl)propionate was prepared according to the procedure of Hoffmann and Kim.¹⁴ The methyl ester was transformed to the more versatile allyl ester without any loss of enantiomeric purity. Intermediate **13** was generated via amide formation with carboxylic acid **11** and the P_1 amino-ester **12**^{7a} using HATU.¹⁵ Removal of the BOC group, followed by N-terminus modification and deprotection of the S_1 amide readily afforded products **1** and **4**. In a similar fashion inhibitor **7** was synthesized from carboxylic acid **14** and the P_1 amino-ester **15**.¹⁰ The synthesis of the peptidyl and ketomethylene 3CP inhibitors used for comparative purposes were described previously.^{7a,7b,9,10,13}

One possible drawback of depsipeptide inhibitors may be their potential to behave as better substrates for proteolytic hydrolysis relative to their corresponding peptides.¹⁶ Other than esterases, another concern is their possible susceptibility toward hepatic metabolism. As shown in Table 4, depsipeptide **7** was the least stable of the three analogues when exposed to human liver microsomes either in the presence or absence of cofactor.¹⁷

In conclusion, we have prepared potent irreversible HRV 3CP inhibitors where an ester is substituted for

the P_2 – P_3 amide or ketomethylene unit of optimally modified tripeptides with C-terminal Michael acceptors. Depsipeptide inhibitors may not be ideal therapeutic candidates due to their lack of in vitro stability.

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- Enzymatic and antiviral assays were performed as described in ref 7a.
- In comparing the inhibition data for depsipeptides **1**, **4**, and **7** directly to that of peptides **2**, **5**, and **8**, it is unclear why compound **1** is more potent than **2** as an HRV-14 3CP

inhibitor but a poorer antiviral agent against HRV-14 in cell culture.

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16. For example, see: Holmquist, B.; Vallee, B. L. *Biochem.* **1976**, *15*, 101.

17. Metabolism was determined as follows: 25 μM of compound was incubated with 1.0 mg/mL HLMS with or without 20 mM NADPH for 30 min. A 200 μL aliquot of the incubation mixture was then precipitated with 2.0 mL of CH_3CN . Separation was performed using an Agilent 110 HPLC with a MetaChem Metasil Basic reverse phase column (5 μ , 4.6 \times 150 mm) at rt. Sample volume = 100 μL . Gradient solvent system: $\text{CH}_3\text{CN}/\text{CH}_3\text{OH}/0.01\%$ TFA, pH 3.5 adjusted with NEt_3 from 5:20:75 to 60:20:20 over 20 min and held for 10 min at a flow rate = 1.0 mL/min. The compounds were monitored by UV; $\lambda = 230$ nm. % Metabolism = $100 - (\text{peak area at 30 min}/\text{peak area at time 0}) \times 100$.