Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 733–738

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. Part 7: Structure–Activity Studies of Bicyclic 2-Pyridone-Containing Peptidomimetics

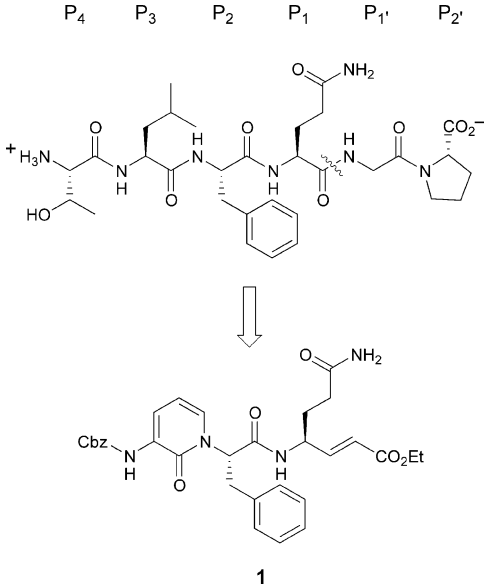
Peter S. Dragovich,* Thomas J. Prins, Ru Zhou, Theodore O. Johnson, Edward L. Brown, Fausto C. Maldonado, Shella A. Fuhrman, Leora S. Zalman, Amy K. Patick, David A. Matthews, Xinjun Hou, James W. Meador, III, Rose Ann Ferre and Stephen T. Worland†

Pfizer Global Research and Development-La Jolla/Agouron Pharmaceuticals, Inc., 10777 Science Center Drive, San Diego, CA 92121-1111, USA

Received 9 October 2001; accepted 12 December 2001

Abstract—The structure-based design, chemical synthesis, and biological evaluation of bicyclic 2-pyridone-containing human rhinovirus (HRV) 3C protease (3CP) inhibitors are described. An optimized compound is shown to exhibit antiviral activity when tested against a variety of HRV serotypes (EC_{50}'s ranging from 0.037 to 0.162 μM).

The human rhinoviruses (HRVs) are members of the picornavirus family and are the single most significant cause of the common cold.[1,2] The replication of these viruses is entirely dependent on the proteolytic processing of a large polyprotein produced by cellular translation of the viral RNA genome. This processing is primarily accomplished by the human rhinovirus 3C protease (3CP),[3] a cysteine protease with structural similarity to the trypsin protein family but possessing minimal homology to prevalent mammalian enzymes.[4] Due to its prominence in the viral replication cycle, 3CP is an ideal target for the development of novel antirhinoviral agents and multiple examples of 3CP inhibitors have recently appeared in the literature.[5] In a previous report, we described the discovery and development of a new class of orally bioavailable 3CP inhibitors that display in vitro antiviral activity against multiple rhinovirus serotypes.[6] These inhibitors are comprised of a peptidomimetic 2-pyridone-containing binding determinant that provides affinity for the target protease and a Michael acceptor moiety, which irreversibly forms a covalent adduct with the active site cysteine residue of the 3C enzyme (e.g., compound **1**, Fig. 1 and Table 1).[7] In this report, we describe the further elaboration of



1

Figure 1. Design of 2-pyridone-containing HRV 3CP inhibitors.

*Corresponding author. Fax: +1-858-622-7998; e-mail: peter.dragovich@pfizer.com

†Present address: Anadys Pharmaceuticals, 9050 Camino Santa Fe, San Diego, CA 92121, USA.

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00008-2

Table 1. Monocyclic and bicyclic 2-pyridone-containing 3C protease inhibitors

Compd	k_{obs}/[I] ($M^{-1}s^{-1}$)[a]	$\Delta\Delta E_{calc}$ (kcal/mol)[b]	EC_{50} (μM)[c]	CC_{50} (μM)[d]
1	114,000	0.00	0.033	50
2	26	16.1	>10	>10
3	4540	8.7	9.0	>100
4[6]	348	9.9	>10	>10
5	177,000	ND	0.162	>10

[a]Inhibition activity against HRV-14 3C protease; see ref 22 for assay method and error.
[b]Calculated binding energetics of ligand and HRV-2 3C protease interactions (relative to compound **1**); see text for details.
[c]Antirhinoviral activity versus HRV-14; see ref 22 for assay method and error.
[d]Cytotoxicity; see ref 22 for assay method and error.
ND = not determined.

such irreversible 3CP inhibitors by the incorporation of a *bicyclic* 2-pyridone moiety into the inhibitor design.

Cursory analysis of the HRV-2 3CP-**1** X-ray crystal structure[6] suggested that, since no obvious steric clashes were apparent, the P_2-P_3 pyridone-Phe portion of **1** could be replaced with some kind of bicyclic entity.[8] Such replacement was anticipated to further diversify the physical and biological properties (e.g., mw, mp, water solubility, metabolic stability, etc.) of our existing anti-3CP compounds and presumably thereby improve our ability to identify such agents suitable for oral administration. However, many literature examples exist that demonstrate successful incorporation of bicyclic peptidomimetic moieties into inhibitors of trypsin-like proteases,[9,10] and it was not obvious which of these (often synthetically complex) possibilities was most suitable for use in targeting 3CP.

An initial attempt at identifying bicyclic 3CP inhibitors was therefore made with the examination of the easily-prepared compound **2**, which incorporated a commercially available bicyclic peptide mimetic.[11,12] Unfortunately, this molecule displayed drastically reduced 3CP inhibition properties relative to the monocyclic 2-pyridone-containing compound **1** and was not an active antirhinoviral agent in cell culture (Table 1). The precise reasons for the difference in activities between **1** and **2** were not apparent from simple, non-quantitative modeling experiments conducted with the 3CP X-ray crystal structure. Although such modeling indicated that **2** would not occupy the S_2 3CP peptide binding subsite filled by the benzyl substituent of **1**, this alteration did not appear to be responsible for the near-total loss in 3CP inhibition activity exhibited by the bicyclic compound. Accordingly, more detailed quantitative molecular modeling studies were performed to better define and predict the interactions between bicyclic molecules and the 3CP enzyme.

A summary of the quantitative computational method employed is outlined below and a more extensive discussion of the procedure will be published elsewhere.[13] The covalent irreversible inactivation of 3CP by Michael acceptors was modeled based on a kinetic mechanism that was divided into two parts as shown below.

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EI \overset{k_3}{\rightarrow} E - I^*$$

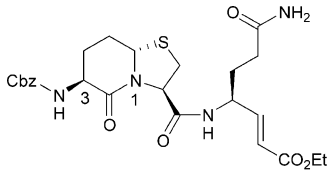
The inhibitor (**I**) initially forms a reversible encounter complex with 3CP (**E**), which can then undergo a chemical reaction (nucleophilic attack by Cys-147) leading to an irreversible covalent complex (**E-I***). The observed inactivation (k_{obs}/[I]) depends upon both the equilibrium binding constant (k_2/k_1) and the chemical rate (k_3) of covalent bond formation.[14] For the purposes of this study, we postulated that (1) the energetics of compound equilibrium binding are determined by noncovalent molecular recognition and (2) that the subsequent chemical reaction is dominated by the intrinsic electrophilicity of the inhibitor and its ability to adopt a favorable binding orientation for the reaction to occur. These assumptions are consistent with previous structure–activity studies of 3CP inhibitors.[15,16] We further assumed that the reactive contribution to k_{obs}/[I] is approximately the same for compounds **1**–**4** (which contain the same reactive center and adjacent functional groups) and thus anticipated relative k_{obs}/[I] values to approximately correspond to relative noncovalent binding energetics. The computational study therefore sought to compute relative noncovalent binding energetics of inhibitors in an effort to rank compounds and guide their optimization.

Accordingly, compounds (**1**–**4**) were covalently modeled into a protein binding site constructed from the cocrystal structure of HRV-2 3CP-**1**.[6] Ab initio partial atomic charges were computed and assigned to all ligands and AMBER* charge parameters were assigned to protein residues. The complexes were energetically minimized using the Batchmin program of MacroModel V5.5[17] with the AMBER* force field and a GB/SA solvation model. Inhibitors and protein binding site residues were fully flexible and protein residues further away from the ligand (>5 Å) were harmonically constrained to their crystallographic positions during the minimization. Protein–ligand interactions and desolvation energetics were computed from the minimized complexes ($E_{PL}{}^{Bound}$). To compute appropriate conformational strain energies, inhibitor and protein structures were independently extracted from the minimized complexes and further minimized in solvent to obtain the unbound free states ($E_L{}^{Free}$ and $E_P{}^{Free}$). The energetics of bond making, along with conformational and configurational entropic terms were assumed to be constant and to cancel within the same series of inhibitors. The compound binding energetics (relative to inhibitor **1**) were then approximated with the following thermodynamic path using the principle of cancellation of errors.

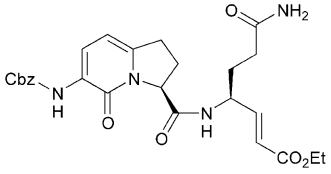
$$\Delta E^{L}{}_{calc} = E_{PL}{}^{Bound} - E_P{}^{Free} - E_L{}^{Free}$$

$$\Delta\Delta E_{calc}\ (L_1 \rightarrow L_2) = \Delta E^{L2}{}_{calc} - \Delta E^{L1}{}_{calc}$$

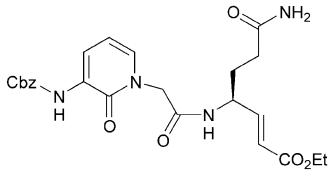
The above computational efforts correctly assessed the significant difference in anti-3CP activity between inhibitors **1** and **2** (compare $\Delta\Delta E_{calc}$ values, Table 1). Importantly, the calculations also indicated that the interactions between **2** and 3CP could be greatly improved by changing the sp^3 C-3 carbon atom present in the bicyclic molecule to the sp^2 hybridization state. This alteration was anticipated to better position the appended Cbz-amino functionality in the S_4 3CP binding subsite. The bicyclic 2-pyridone moiety contained in structure **3** was eventually identified as a synthetically-accessible fragment which met the above C-3 sp^2 hybridization requirement and compound **3** performed well relative to **2** in the computational binding energy calculations (Table 1). The structural similarity between **3** and the known inhibitor **1** was also expected to increase the probability of favorable 3CP recognition.



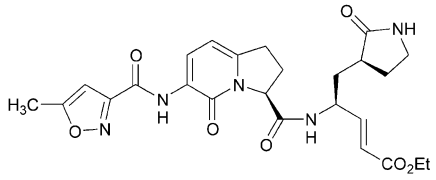
2



3



4



5

In the event, the bicyclic 2-pyridone-containing compound **3** was prepared and displayed dramatically improved (>100-fold) 3CP inhibition properties relative to the previously examined bicyclic molecule **2** (Table 1). Inhibitor **3** also exhibited measurable antirhinoviral activity in cell culture experiments (Table 1). The importance of proper conformational constraint imparted by the bicyclic 2-pyridone moiety contained in **3** was demonstrated with the examination of the monocyclic inhibitor **4**.[6] The latter molecule exhibited significantly poorer anti-3CP properties relative to **3** (Table 1) even though both compounds were anticipated to contact the 3CP enzyme in roughly the same manner (see X-ray analysis below). Although compound **3** was not as potent an anti-3CP agent as the monocyclic 2-pyridone-containing inhibitor **1**, the activity displayed by the bicyclic molecule was sufficient to justify additional optimization experiments.

Accordingly, modifications known from our previous work[6] to improve the anti-3CP properties of monocyclic 2-pyridone-containing molecules were incorporated into the bicyclic inhibitor design. Specifically, simultaneous introduction of a P_1 (*S*)-γ-lactam fragment[16] and a P_4 amide derived from 5-methylisoxazole-3-carboxylic acid[18] afforded a molecule (**5**), which displayed greatly improved 3CP inhibition properties relative to **3** (Table

Table 2. Antirhinoviral activity of **5** versus multiple HRV serotypes

HRV Serotype	EC_{50} (μM)[a]	EC_{90} (μM)[a]
2	0.037	0.119
3	0.043	0.133
9	0.121	0.257
10	0.041	0.175
14	0.162	0.298
16	0.072	0.303
25	0.162	0.616
39	0.016	0.152
87	0.058	0.110

[a]Cytotoxicity >10 μM in all cases; see ref 22 for assay method and error.

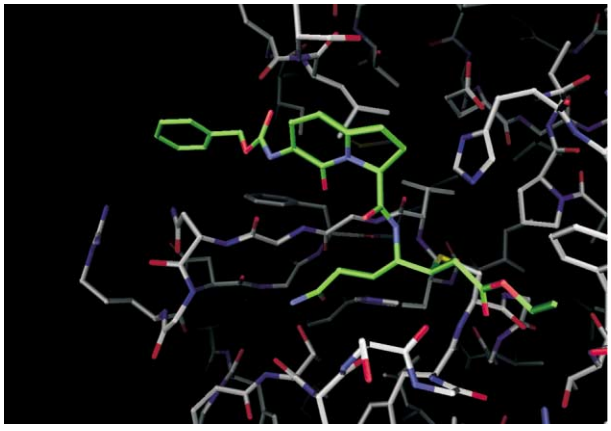


Figure 2. Crystal structure of **3** complexed with HRV-2 3CP (1.55 Å resolution).

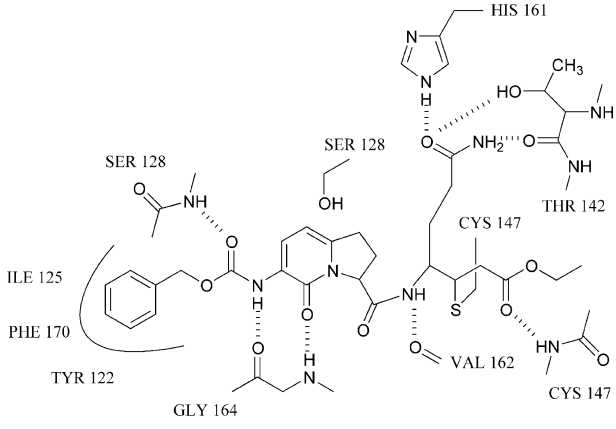
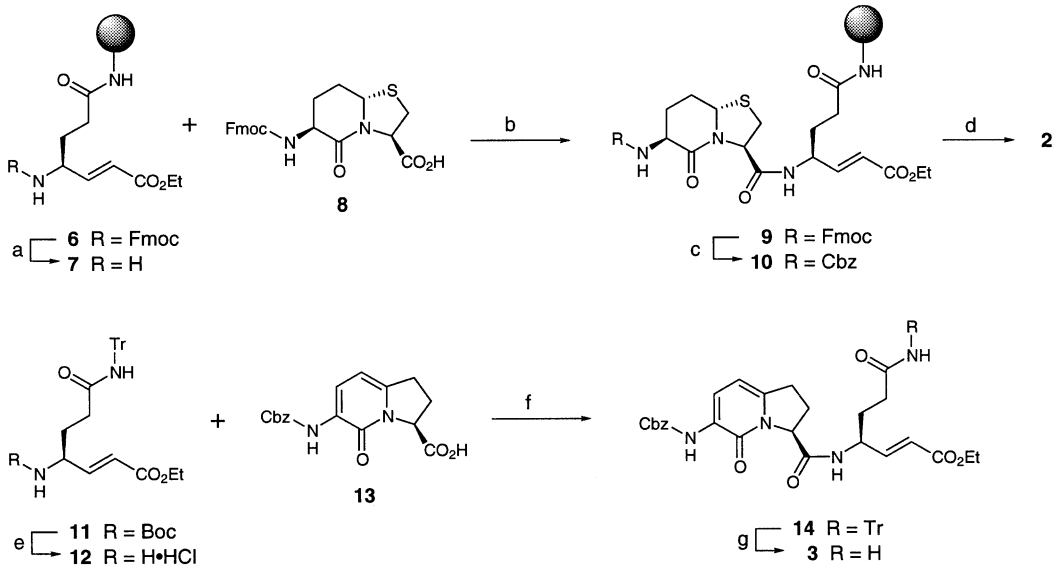


Figure 3. Schematic diagram of **3** bound in the HRV-2 3CP active site. Hydrogen bonds are represented as dashed lines and the residues which make up the enzyme binding subsites are depicted.



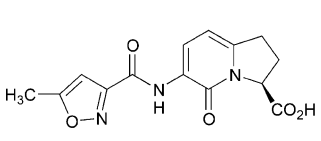
Scheme 1. Reagents and conditions (solid support = Rink amide resin, Tr = CPh_3): (a) 3% DBU in CH_2Cl_2, (b) 1.5 equiv **8**, HATU, *i*Pr_2NEt, DMF; (c) 10% collidine in CH_2Cl_2, Cbz-Cl; (d) 9:1 TFA:CH_2Cl_2, 45% from **6**; (e) 2.0 M HCl in 1,4-dioxane; (f) 1.0 equiv **13**, EDC, HOBt, 4-methylmorpholine, 60% from 11; (g) 1:1 TFA:CH_2Cl_2, 5 drops *i*Pr_3SiH, 74%.

1). Compound **5** also exhibited potent antirhinoviral activity in cell culture when tested against a number of different HRV serotypes (Table 2). Thus, the present work demonstrates that properly optimized bicyclic 2-pyridone-containing 3CP inhibitors can function as potent, broad-spectrum antirhinoviral agents.

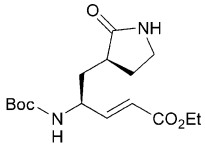
The 1.55 Å X-ray crystal structure of the covalent adduct formed between compound **3** and HRV-2 3CP[19,20] is shown in Figure 2, and key protein–inhibitor interactions are illustrated in Figure 3. As expected, the inhibitor filled a series of shallow grooves on the protein surface N-terminal to the scissile amide of the enzyme's substrate (S_1–S_4)[8] and a covalent bond was observed between the 3CP active site cysteine residue (Cys-147) and the β-carbon of the Michael acceptor of **3**. A hydrogen bond between the Michael acceptor ester moiety and the 3CP Cys-147 amide NH was also present in the above complex, but, as with all such 3CP inhibitors studied to date, it is uncertain whether this interaction facilitates addition of the cysteine to **3** or merely arises after the covalent adduct has been formed.

The most significant departure from previously examined 3CP–ligand structures observed in the above complex was the absence of any appreciable contact between **3** and the S_2 binding subsite of the enzyme. Not unexpectedly, the 3CP residues that comprise the S_2 binding pocket (Leu-127, Ser-128, and Asn-130 on one side and His-40 on the other) deviated somewhat from their observed locations in other 3CP–ligand complexes in which the S_2 subsite was filled. The remaining interactions between the bicyclic inhibitor **3** and 3CP were essentially identical to those observed in previous crystal structures of peptide,[21] peptidomimetic,[21] and monocyclic 2-pyridone-containing 3CP inhibitors.[6] These included the important anti-parallel β-sheet interaction formed between the pyridone moiety of **3** and Gly-164 along with five additional protein-ligand hydrogen bonds (Fig. 3). As expected, the conformationally mobile side chain of 3CP Ser-128 which was previously observed to form hydrogen bonds between the P_2-P_3 amide NH of non-methylated tripeptide-derived 3CP inhibitors[22] was rotated out of the active site by the bicyclic P_2-P_3 moiety contained in **3**.

The bicyclic 2-pyridone-containing 3CP inhibitors described in this study were prepared by two related synthetic methods and both are illustrated in Scheme 1. The synthesis of compound **2** was accomplished on solid support and began with the Fmoc deprotection of resin-bound entity **6**[18] to afford the corresponding free amine (**7**). Subsequent HATU-mediated coupling with the commercially available bicyclic carboxylic acid **8**[11,12] provided resin-bound intermediate **9**. The Fmoc moiety present in **9** was then replaced with a benzyl carbamate to give intermediate **10**. Cleavage of **10** from the solid support was effected by exposure to strong acid and the resulting primary amide (**2**) was purified by reverse-phase preparative HPLC. The described solid-phase synthesis represents a convenient alternative to the solution-phase methods typically employed in the preparation of 3CP inhibitors and afforded **2** in 45% yield for the five-step process.



15



16

The preparation of inhibitor **3** began with acidic Boc deprotection of the known trityl-containing L-glutamine derivative **11**.[22] Subsequent carbodiimide-mediated coupling of the resulting amine salt (**12**) with the known bicyclic 2-pyridone moiety **13**[23] (approximately 60% ee) afforded intermediate **14** as a single diastereomer in 60% yield after purification by flash column chromato-

graphy.[24] Removal of the trityl protecting group present in **14** was then accomplished by treatment with trifluoroacetic acid and compound **3** was isolated in 74% yield after concentration of the reaction mixture, trituration with diethyl ether, and filtration. Inhibitor **5** was synthesized from carboxylic acid **15**[25] (approximately 60% ee) and lactam **16**[26] in a manner completely analogous to that described above for the preparation of **3**.

The studies presented above demonstrate that bicyclic 2-pyridone-containing irreversible inhibitors of the human rhinovirus 3C protease can function as potent, broad-spectrum antirhinoviral agents. The studies also illustrate how such bicyclic molecules can be optimized via a combination of computational methods and crystallographic information.

Acknowledgements

We are grateful for many helpful discussions throughout the course of this work with Professor Larry Overman and Drs. Benjamin Burke, Robert Kumpf, Steven Bender, and Siegfried Reich.

References and Notes

1. (a) Couch, R. B. *Fields Virology*, 3rd Ed., Fields, B. N., Knipe, D. M., Howley, P. M., Chanock, R. M., Monath, T. P., Melnick, J. L., Roizman, B., Eds.; Lippincott-Raven: Philadelphia, 1996, Vol. 1, Chapter 23; p 713. (b) McKinlay, M. A.; Pevear, D. C.; Rossmann, M. G. *Annu. Rev. Microbiol.* **1992**, *46*, 635. (c) Phillpotts, R. J.; Tyrrell, D. A. J. *Br. Med. Bull.* **1985**, 41, 386. (d) Gwaltney, J. M.; *Principles and Practices of Infectious Diseases*, Mandell, G. L., Douglas, R. G., Bennett, J. E., Eds.; John Wiley & Sons: New York, 1985; Chapter 38, p 351 (e) Gwaltney, J. M.; *Viral Infections of Humans*, Evans, A. S., Ed.; Plenum: New York, 1982; Chapter 20, pp 491.
2. (a) Rueckert, R. R.; *Fields Virology*, 3rd Ed., Fields, B. N., Knipe, D. M., Howley, P. M., Chanock, R. M., Monath, T. P., Melnick, J. L., Roizman, B., Eds.; Lippincott-Raven: Philadelphia, 1996; Vol. 1., Chapter 21, p 609 (b) Kräusslich, H.-G.; Wimmer, E. *Annu. Rev. Biochem.* **1988**, *57*, 701.
3. (a) Cordingley, M. G.; Callahan, P. L.; Sardana, V. V.; Garsky, V. M.; Colonno, R. J. *J. Biol. Chem.* **1990**, *265*, 9062. (b) Orr, D. C.; Long, A. C.; Kay, J.; Dunn, B. M.; Cameron, J. M. *J. Gen. Virol.* **1989**, *70*, 2931. (c) Cordingley, M. G.; Register, R. B.; Callahan, P. L.; Garsky, V. M.; Colonno, R. J. *J. Virol.* **1989**, *63*, 5037.
4. (a) Matthews, D. A.; Smith, W. W.; Ferre, R. A.; Condon, B.; Budahazi, G.; Sisson, W.; Villafranca, J. E.; Janson, C. A.; McElroy, H. E.; Gribskov, C. L.; Worland, S. *Cell* **1994**, *77*, 761. (b) Allaire, M.; Chernaia, M. M.; Malcolm, B. A.; James, M. N. G. *Nature* **1994**, *369*, 72. (c) Bazan, J. F.; Fletterick, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7872. (d) Gorbalenya, A. E.; Blinov, V. M.; Donchenko, A. P. *FEBS Lett.* **1986**, *194*, 253.
5. Dragovich, P. S. *Exp. Opin. Ther. Pat.* **2001**, *11*, 177.
6. 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.* accepted for publication.
7. (a) For other examples of Michael acceptor-containing cysteine protease inhibitors, see: Roush, W. R.; Gwaltney, S. L., II; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, 10994. (b) McGrath, M. E.; Klaus, J. L.; Barnes, M. G.; Brömme, D. *Nat. Struct. Biol.* **1997**, *4*, 105. (c) Brömme, D.; Klaus, J. L.; Okamoto, K.; Rasnick, D.; Palmer, J. T. *Biochem. J.* **1996**, *315*, 85. (d) Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Brömme, D. *J. Med. Chem.* **1995**, *38*, 3193. (e) Liu, S.; Hanzlik, R. P. *J. Med. Chem.* **1992**, *35*, 1067. (f) Hanzlik, R. P.; Thompson, S. A. *J. Med. Chem.* **1984**, *27*, 711.
8. The nomenclature used for describing the individual amino acid residues of a peptide substrate (P_2, P_1, $P_{1'}$, $P_{2'}$, etc.) and the corresponding enzyme subsites (S_2, S_1, $S_{1'}$, $S_{2'}$, etc.) is described in: Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
9. (a) For some recent examples, see: Hanessian, S.; Balaux, E.; Musil, D.; Olsson, L.-L.; Nilsson, I. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 243. (b) Boatman, P. D.; Ogbu, C. O.; Eguchi, M.; Kim, H.-O.; Nakanishi, H.; Cao, B.; Shea, J. P.; Kahn, M. *J. Med. Chem.* **1999**, *42*, 1367. (c) Bachand, B.; DiMaio, J.; Siddiqui, M. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 913. (d) St.-Denis, Y.; Augelli-Szafran, C. E.; Bachand, B.; Berryman, K. A.; DiMaio, J.; Doherty, A. M.; Edmunds, J. J.; Leblond, L.; Lévesque, S.; Narasimhan, L. S.; Penvose-Yi, J. R.; Rubin, J. R.; Tarazi, M.; Winocour, P. D.; Siddiqui, M. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3193.
10. (a) For recent reviews of peptide mimics, see: Hruby, V. J.; Balse, P. M. *Curr. Med. Chem.* **2000**, *7*, 945. (b) Ripka, A. S.; Rich, D. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 441. (c) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699. (d) Liskamp, R. M. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 1. (e) Adang, A. E. P.; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; van Staveren, C. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 63. (f) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039. (g) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244. (h) Kahn, M. *Synlett* **1993**, 821.
11. The bicyclic intermediate **8** required for the synthesis of **2** was purchased from Neosystem (SNPE North America LLC): FMOC-BTD, catalog number FB02601.
12. The synthesis of **8** is described in: Nagi, U.; Sato, K.; Nakamura, R.; Kato, R. *Tetrahedron* **1993**, *49*, 3577.
13. Hou, X.; Burke, B. J. manuscript in preparation.
14. Meara, J. P.; Rich, D. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2277.
15. Webber, S. E.; Tikhe, J.; Worland, S. T.; Fuhrman, S. A.; Hendrickson, T. F.; Matthews, D. A.; Love, R. A.; Patick, A. K.; Meador, J. W., III; Ferre, R. A.; Brown, E. L.; DeLisle, D. M.; Ford, C. E.; Binford, S. L. *J. Med. Chem.* **1996**, *39*, 5072.
16. 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.
17. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comp. Chem.* **1990**, *11*, 440.
18. Dragovich, P. S.; Zhou, R.; Skalitzky, D. J.; Fuhrman, S. A.; Patick, A. K.; Ford, C. E.; Meador, J. W., III; Worland, S. T. *Bioorg. Med. Chem.* **1999**, *7*, 589.
19. HRV-2 3CP provided diffraction quality crystals more routinely than the serotype 14-derived protease. The inhibitor-binding regions of the two enzymes do not differ significantly.

20. Human rhinovirus 3C protease of serotype 2 was incubated with a 3-fold molar excess of compound **3** in the presence of 2% DMSO for 2 h at room temperature followed by an additional 4 h incubation at 4 °C. The resulting complex was concentrated to 12 mg/mL and then passed through a 0.45 μM cellulose-acetate filter. Crystals were grown at 13 °C using the hanging drop vapor diffusion method in which equal volumes (3 μL) of the protein/ligand complex and reservoir solution were layered on plastic coverslips and sealed over wells filled with 1 mL of reservoir solution containing 0.2 M $CaCl_2$, 0.1M Tris(HCl) pH 8.5, 25% PEG 4K and 5 mM DTT. A rectangular rod-shaped crystal of approximate dimensions 0.4×0.15×0.15 mm (space group P1, two molecules per asymmetric unit, a = 33.02, b = 41.98, c = 67.79 Å, α = 89.99, β = 100.94, γ = 112.20 deg) was prepared for low temperature data collection by transfer to an artificial mother liquor solution consisting of 400 μL of the reservoir solution mixed with 125 μL of glycerol and then flash frozen in a stream of N_2 gas at −170 °C. X-ray diffraction data were collected with a MAR Research 345 mm imaging plate and processed with DENZO.[27] Diffraction data were 88.9% complete to a resolution of 1.55 Å with R_{sym} = 2.2%. Protein atomic coordinates from a previously solved isomorphous cocrystal structure of type 2 3C protease were used to initiate rigid-body refinement in XPLOR[28] followed by simulated annealing and conjugate gradient protocols. Placement of the inhibitor, addition of ordered solvent, and further refinement proceeded as described previously.[29] The final R factor was 20.9% [39009 reflections with $F > 2\sigma(F)$]. The root-mean-square deviations from ideal bond lengths and angles were 0.011 Å and 2.8 deg, respectively. The final model consisted of all atoms for residues 1–180 in both molecules plus 258 water molecules.

21. Matthews, D. A.; Dragovich, P. S.; Webber, S. E.; Fuhrman, S. A.; Patick, A. K.; Zalman, L. S.; Hendrickson, T. F.; Love, R. A.; Prins, T. J.; Marakovits, J. T.; Zhou, R.; Tikhe, J.; Ford, C. E.; Meador, J. W., III; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Brothers, M. A.; DeLisle, D. M.; Worland, S. T. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11000.

22. Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Reich, S. R.; Prins, T. J.; Marakovits, J. T.; Littlefield, E. S.; Zhou, R.; Tikhe, J.; Ford, C. E.; Wallace, M. B.; Meador, J. W., III; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Harr, J. E. V.; DeLisle, D. M.; Worland, S. T. *J. Med. Chem.* **1998**, *41*, 2806.

23. Dragovich, P. S.; Zhou, R.; Prins, T. J. *J. Org. Chem.* in press.

24. No attempt was made to quantitate and/or isolate the other diastereomer of **14** that was presumably also formed.

25. Dragovich, P. S.; Prins, T. J.; Zhou, R.; Johnson, T. O., Jr. WO 01/40189 A1; CAN **135**:33649.

26. (a) Tian, Q.; Nayyar, N. K.; Babu, S.; Chen, L.; Tao, J.; Lee, S.; Tibbetts, A.; Moran, T.; Liou, J.; Guo, M.; Kennedy, T. *Tetrahedron Lett.* **2001**, *42*, 6807. (b) Tian, Q.; Nayyar, N. K.; Babu, S.; Tao, J.; Moran, T. J.; Dagnino, R., Jr.; Remarchuk, T. P.; Melnick, M. J.; Mitchell, L. J., Jr.; Bender, S. L. **2001** WO 01/14329, CAN **134**:193743.

27. Otwinowski, Z.; *Proceedings of the CCP4 Study Weekend*, Sawyer, L.; Isaacs, N.; Baily, S., Eds.; SERC Daresbury Laboratory: Warrington, 1993; p 55.

28. XPLOR, version 3.1. Brünger, A. T. *XPLOR v3.1 Manual*; Yale University Press: New Haven, CT, 1992.

29. 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.