ORGANIC LETTERS

1999
Vol. 1, No. 2
249−252

Heterocyclic HIV-1 Protease Inhibitors

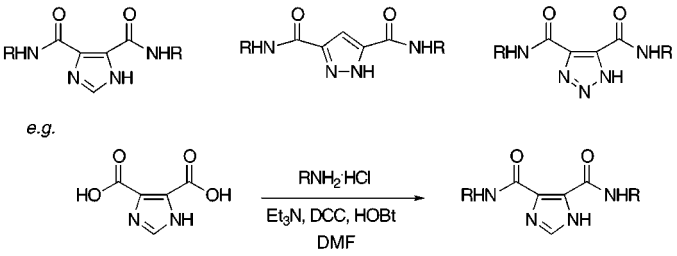
Paul W. Baures

111 Willard Hall, Department of Chemistry, Kansas State University, Manhattan, Kansas 66506

baures@ksu.edu

Received April 13, 1999

ABSTRACT



A series of simple heterocyclic HIV-1 protease inhibitors were developed on the basis of size, shape, and electronic complementarity to the active site of the enzyme. The C_2-symmetric heterocycles do not contain a transition-state isostere nor are they active site directed irreversible inhibitors; thus, they represent the success of a new design strategy. The first generation heterocycles inhibit the protease in the micromolar range, whereas control compounds show no bioactivity at the same concentrations.

The development of the HIV-1 protease inhibitors now clinically available has brought hope to thousands of people infected with the HIV virus.[1] These inhibitors were developed around transition-state design elements previously used against other aspartic proteases such as renin.[2] Clinical candidates having suitable potency and specificity against HIV-1 protease were identified by researchers rapidly. However, modifications of the drug candidates were required to improve the pharmacokinetics of these early compounds.

This paper details the development of a simple class of HIV-1 protease inhibitors designed on the basis of the size, shape, and electronic complementarity of the core structures to the active site of the enzyme. The heterocyclic core structures exclude the use of transition-state mimics, eliminating the complications of stereochemistry which often accompany these mimics. Moreover, the heterocycles offer potential benefits in the final pharmacokinetic properties of the compounds due to their amphoteric nature.

Inhibitor Design. Our goal at the outset of this project was to consider an alternative design strategy for HIV-1 protease inhibitors which would reasonably be assumed to offer potential benefits in the ease of synthesis, aqueous solubility, and oral bioavailability of the compounds. The choice of our core heterocycles was made on the basis of three important pieces of information described in the following paragraphs.

Heterocycles were first considered as possible core structures after examination of imidazolium hydrogen maleate (Figure 1), which forms an extended hydrogen bonded salt in the solid state.[3] The molecules are organized intermolecularly by the formation of NH···O hydrogen bonds as well as short CH···O contacts. While the carboxylate distances in this structure (carbon-to-carbon distance of 6.5 Å) are significantly greater than a typical separation observed for HIV-1 protease active site residues (carbon-to-carbon distance of 5.1 Å),[4] some degree of active site flexibility is considered possible given the substrate diversity recognized by the protease.[2c]

Development of novel C_2-symmetric inhibitors has led to potent compounds for which X-ray cocrystal structures are available.[5] Most of the symmetric compounds do not bind

(1) Havlir, D. V.; Lange, J. M. A. *AIDS* **1998**, *12*, S165.

(2) Selected reviews of HIV-1 protease inhibitor development: (a) West, M. L.; Fairlie, D. P. *Trends Pharmacol. Sci.* **1995**, *16*, 67. (b) Kempf, D. J.; Sham, H. L. *Curr. Pharm. Design* **1996**, *2*, 225. (c) Wlodawer, A.; Vondrasek, J. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249.

(3) Hsu, B.; Schlemper, E. O. *Acta Crystallogr.* **1980**, *B36*, 3017.

(4) This is the average distance in the HIV-1 protease structures with the PDB codes 1HVI, 1HVJ, 1HVK, and 1HVL.

10.1021/ol990586y CCC: $18.00
Published on Web 05/29/1999

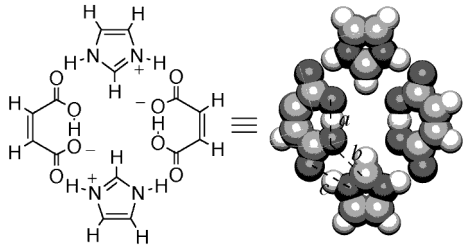


Figure 1. Small molecule X-ray structure of imidazolium hydrogen maleate[6] organized intermolecularly by short NH···O and CH···O contacts. The hydrogen bond distances are (a) 2.39 Å, (b) 3.03 Å, and (c) 2.78 Å. The CH···O angle in b is 117°.

symmetrically to the active site of HIV-1 protease; however, A-76928 does bind symmetrically with the two hydroxyl groups of the dihydroxyethylene core of this compound *gauche* to one another (Figure 2).[5] A conformational

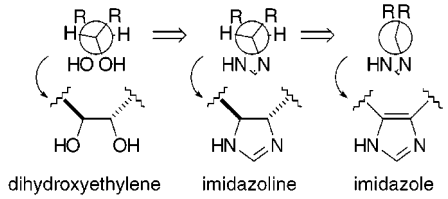


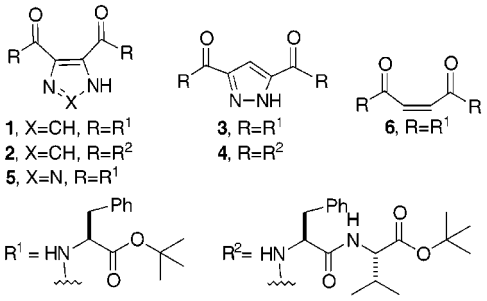
Figure 2. Design rationale for the heterocyclic core structure on the basis of the *gauche* hydroxyl groups in A-76928 and the small molecule crystal structure of imidazolium hydrogen maleate.

constraint involving these *gauche* hydroxyl groups (Figure 2) was considered on the basis of this information. A *trans* imidazoline would place the appended R groups in the same general location as found in A-76928. Yet, the imidazole was chosen for its simplicity and its ready availability for the testing of this hypothesis.

There was one final consideration in choosing the imidazole and the other aromatic heterocycles as a component of our design: namely, that these heterocycles have already been utilized in HIV-1 protease inhibitors to improve the water solubility and oral bioavailability of the final compound.[6] The major difference between the prior use of the heterocycles and the use outlined in this paper is that the heterocycles are proposed to interact directly within the active site in this work rather than their previous peripheral inclusion.

Inhibitor Synthesis. The compounds **1**−**6** synthesized for this study were prepared from the commercially available dicarboxylic acids (Chart 1). The conformationally con-

Chart 1. Compounds Synthesized for This Study



strained control compound, **6**, was synthesized to support the hypothesis that the heterocyclic rings in **1**−**5** would be necessary for enzyme inhibition. The compounds were synthesized by standard coupling reactions[7] in CH_2Cl_2 employing dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, and triethylamine, giving poor yields (2−25%). Compounds **1**, **3**, **5**, and **6** were prepared by coupling the appropriate dicarboxylic acid with L-phenylalanine *tert*-butyl ester hydrochloride. The syntheses of **2** and **4**, which also contain two valine residues, were done from **1** and **3**, respectively, by deprotecting the *tert*-butyl ester with 4 N HCl in dioxane before coupling the product of the reaction with L-valine *tert*-butyl ester hydrochloride. The synthesized compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy as well as mass spectrometry.[8]

Enzyme Inhibition Studies. The ability of these compounds to inhibit HIV-1 protease was tested by using the previously reported colorimetric peptide substrate (H-Lys-Ala-Arg-Val-Nle-*p*-nitro-Phe-Glu-Ala-Nle-NH_2) with some modifications.[9]

In addition to testing the synthesized compounds, a known HIV-1 protease inhibitor, acetylpepstatin (**7**), was included in the study.[10] Three heterocycles, imidazole (**8**), imidazole-4,5-dicarboxylic acid (**9**), and 4,5-diphenylimidazole (**10**), were included to verify the necessity of both the heterocyclic ring and the appended amino acids for bioactivity against the enzyme.

(5) Hosur, M. V.; Bhat, T. N.; Kempf, D. J.; Baldwin, E. T.; Liu, B.; Gulnik, S.; Wideburg, N. E.; Norbeck, D. W.; Appelt, K.; Erickson, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 847.

(6) (a) Thompson, S. K.; Murthy, K. H.; Zhao, B.; Winborne, E.; Green, D. W.; Fisher, S. M.; DesJarlais, R. L.; Tomaszek, T. A., Jr.; Meek, T. D.; Gleason, J. G.; Abdel-Meguid, S. S. *J. Med. Chem.* **1994**, *37*, 3100. (b) Abdel-Meguid, S. S.; Metcalf, B. W.; Carr, T. J.; Demarsh, P.; DesJarlais, R. L.; Fisher, S.; Green, D. W.; Ivanoff, L.; Lambert, D. M.; Murthy, K. H. M.; Petteway, S. R., Jr.; Pitts, W. J.; Tomaszek, T. A., Jr.; Winborne, E.; Zhao, B.; Dreyer, G. B.; Meek, T. D. *Biochemistry* **1994**, *33*, 11671. (c) Turner, S. R.; Strohbach, J. W.; Tommasi, R. A.; Aristoff, P. A.; Johnson, P. D.; Skulnick, H. I.; Dolak, L. A.; Seest, E. P.; Tomich, P. K.; Bohanon, M. J.; Horng, M.-M.; Lynn, J. C.; Chong, K.-T.; Hinshaw, R. R.; Watenpaugh, K. D.; Janakiraman, M. N.; Thaisrivongs, S. *J. Med. Chem.* **1998**, *41*, 3467. (d) Han, Q.; Chang, C.-H.; Li, R.; Ru, Y.; Jadhav, P. K.; Lam, P. Y. S. *J. Med. Chem.* **1998**, *41*, 2019. (e) De Lucca, G. V.; Kim, U. T.; Liang, J.; Cordova, B.; Klabe, R. M.; Garber, S.; Bacheler, L. T.; Lam, G. N.; Wright, M. R.; Logue, K. A.; Erickson-Viitanen, S.; Ko, S. S.; Trainer, G. L. *J. Med. Chem.* **1998**, *41*, 2411.

(7) Klausner, Y. S.; Bodansky, M. *Synthesis* **1972**, 453.

(8) Spectra are included with the Supporting Information.

(9) Richards, A. D.; Phylip, L. H.; Farmerie, W. G.; Scarborough, P. E.; Alvarez, A.; Dunn, B. M.; Hirel, P.-H.; Konvalinka, J.; Strop, P.; Pavlickova, L.; Kostka, V.; Kay, J. *J. Biol. Chem.* **1990**, *265*, 7733. Our inhibitor assays were done at 320 nm instead of 300 nm because this wavelength minimized background variations.

(10) Richards, A. D.; Broadhurst, A.; Ritchie, A. J.; Dunn, B. M.; Kav, J. *FEBS Lett.* **1989**, *253*, 214.

The results of the bioassay are shown in Figure 3 following the normalization of the absorbances at 5 min and against the observed substrate hydrolysis over the course of the experiment. Compounds **1**−**6** and **8**−**10** were tested at 1 μM,

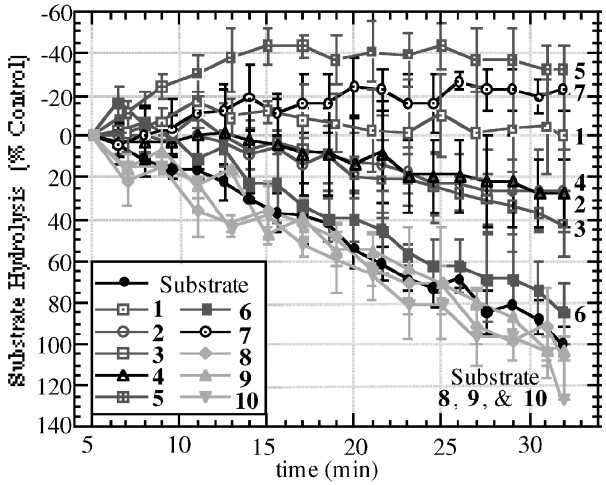


Figure 3. Bioassay of compounds **1**−**6** as well as **8** (imidazole), **9** (imidazole-4,5-dicarboxylic acid), and **10** (4,5-diphenylimidazole) all tested at 1 μM in DMSO alongside the known HIV-1 protease inhibitor acetylpepstatin (**7**) at 3.5 μM.[11]

while acetylpepstatin (**7**) was used at 3.5 μM.[11] The error bars are calculated standard deviations obtained by averaging three separate experiments except for compounds **6** and **8**−**10** which were done in duplicate. The increase in absorbance for **5** and **7** is likely due to some precipitation (turbidity) which occurs with these inhibitors over the course of the experiment.

Discussion. Although an oversimplification of ligand/inhibitor design, the important features include appropriately matching the size and shape of the small molecule to the macromolecular binding site as well as presenting an electronic and hydrophobic surface which complements the macromolecular surface.[12]

Traditional aspartic protease inhibition has generally utilized two design approaches, either the use of transition-state isosteres[13] or active-site directed irreversible inhibitors.[14] Transition-state isosteres have been used with the greatest success in the development of clinical drugs against HIV-1 protease.[2]

The outcome of this project was the development of noncovalent inhibitors of HIV-1 protease with achiral heterocyclic core structures that might logically improve the pharmacokinetics of the compounds. While this work does not attempt to mimic the transition-state geometry, recent reports on the development of achiral, potent, and specific serine protease inhibitors support the hypothesis that this could be attained.[15] For example, the active site chemical functionality in HIV-1 protease has been reported to form a copper(II) chelate—designed by comparison with small molecule organometallics.[16] In addition, researchers at Pharmacia & Upjohn, Inc. are developing a class of HIV-1 protease inhibitors which do contain a hydroxyl group to hydrogen bond to the active site, but which is not part of a stereogenic center.[6d]

Incorporation of heterocyclic rings into existing HIV-1 protease inhibitors had previously been done on the premise of improving water solubility or oral bioavailability of the compound.[6] The results of these modifications have thus far been mixed, with both successes and failures being reported. Dupont-Merck's cyclic ureas were reported to have poor aqueous solubilities despite the presence of two substituted heterocyclic rings (pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, or tetrazole) or benzo-fused analogues.[6a] These researchers hypothesized that the symmetric structures had greater crystal packing energies, thereby making them less soluble.[6d,e] The preparation of asymmetric ureas containing one benzo-fused pyrazole ring did result in a series of inhibitors with improved oral bioavailability.[17]

It appears the compounds reported herein bind to HIV-1 protease with occupation of the active site by the heterocyclic ring, since the observed enzyme inhibition data indicates a dependence on both the presence of the ring as well as the presence of the linked amino acids. On this basis, the proposed binding with the HIV-1 protease active site is shown schematically in Figure 4 for the imidazole and pyrazole inhibitors. The utilization of the C−H bond of the 2-position of the imidazole-containing inhibitors may provide some additional stabilization. This position is known to be acidic enough to exchange with D_2O at room temperature[18] and to form short CH···O contacts in small molecule X-ray structures.[3,19]

The triazole inhibitors could potentially interact in either version of the proposed interactions. The similarity of the observed *in vitro* enzyme inhibition of **1**−**5** precludes a current judgment regarding the possible contributions of pK_a versus hydrogen bonding for the heterocycle core structures.

Conclusions. HIV-1 protease inhibitors have been developed which contain heterocyclic rings complementary to the

(11) Inhibition by **1** and **5** drops off quickly below 1 μM. Determination of the compounds K_i values will be published at a later date.

(12) (a) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359. (b) Böhm, H. J.; Klebe, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2588.

(13) Dreyer. G. B.; Metcalf, B. W.; Tomaszek, T. A., Jr.; Carr, T. J.; Chandler, A. C., III; Hyland, L.; Fakhoury, S. A.; Magaard, V. W.; Moore, M. L.; Strickler, J. E.; Debouck, C.; Meek, T. D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9752.

(14) Rose, R. B.; Rose, J. R.; Salto, R.; Craik, C. S.; Stroud, R. M. *Biochemistry* **1993**, *32*, 12498.

(15) Katz, B. A.; Clark, J. M.; Finer-Moore, J. S.; Jenkins, T. E.; Johnson, C. R.; Ross, M. J.; Luong, C.; Moore, W. M.; Stroud, R. M. *Nature* **1998**, *391*, 608.

(16) Florence, L.; Ledecq, M.; Ben atallah, Z.; Sicsic, S.; Rosny, E. de; Arnaud, G.; Michèle, R.-R.; René, L.; Olivier, K.; François, D. "Copper(II) Chelates as a New Generation of Non-Peptide Inhibitors of the Human Immunodeficiency Virus", presented at the 26th National Medicinal Chemistry Symposium, Richmond, VA, June 14−18, 1998; Abstract A−21.

(17) Rodgers, J. D.; Lam, P. Y. S.; Johnson, B. L.; Wang, H.; Ko, S. S.; Seitz, S. P.; Trainor, G. L.; Anderson, P. S.; Klabe, R. M.; Bacheler, L. T.; Cordova, B.; Garber, S.; Reid, C.; Wright, M. R.; Chang, C.-H.; Erickson-Viitanen, S. *Chem. Biol.* **1998**, *5*, 597.

(18) Harris, T. M.; Randall, J. C. *Chem. Ind.* **1965**, 1728.

(19) Gorbitz, C. H.; Husdal, J. *Acta Chem. Scand.* **1998**, *52*, 218.

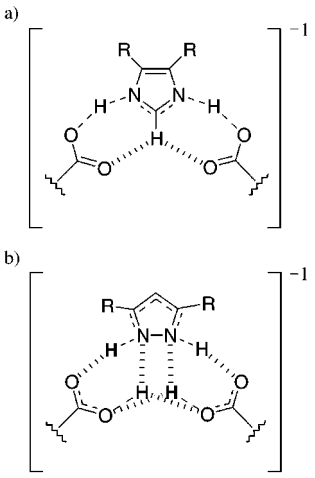


Figure 4. (a) The proposed interaction of an imidazole-based inhibitor with the two catalytic aspartic acid side chains. The diagram indicates contributors resulting from symmetry-equivalent hydrogen-bonded structures plus interactions resulting from proton transfer by an aspartic acid side chain (partial bonds). In addition, the potential interaction of the C−H group of the 2-position in the imidazole ring is indicated by dashes. (b) The proposed interaction of a pyrazole-based inhibitor with the two catalytic aspartic acid side chains. The partial bonds indicate symmetry-related structures in the absence of proton transfer from the aspartic acid side chain, whereas the dashes represent hydrogen-bonding interactions only. The emboldened hydrogens serve to indicate the pairwise relationship of the hydrogens in this diagram.

size and shape of the active site. Furthermore, despite differences in the structure of the imidazole, pyrazole, and triazole rings, all of the heterocycles could plausibly form favorable electrostatic interactions with the catalytic aspartic acid side chains. This may imply that other new classes of inhibitors remain to be discovered and that some of these classes may offer advantages in the synthesis and development of the drug candidate(s) or both.

In addition, this work addresses electronic complementarity with functionality not traditionally considered in drug design, such as CH groups as hydrogen bond donors. In an era of data mining, the importance of subtleties can often go unnoticed in the wealth of data. The energy of association of the imidazole CH group is yet undetermined, but it is apparent that its inclusion did not preclude bioactivity. This suggests that medicinal chemists may be able to design alternative structures for a variety of known drugs, with each alternative structure having potential differences in potency, specificity, toxicity, pharmacokinetics, or other important properties.

Future efforts are directed at discovering more potent compounds through analogue synthesis. Specificity is also being determined by measuring the bioactivity of these inhibitors against other aspartic proteases.

Acknowledgment. The author thanks Kansas State University and the Kansas State University Department of Chemistry for the funds, space, and opportunity to pursue this research.

Supporting Information Available: Experimental details, ^{1}H and ^{13}C NMR spectra of **1**−**6**, and a larger Figure 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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