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Communications to the Editor

Design and Synthesis of Peptidomimetic Inhibitors of HIV-1 Protease and Renin. Evidence for Improved Transport

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Introduction

Although the development of captopril[1] and the prodrug enalapril[2] has profoundly influenced the treatment of hypertension and angina, the great therapeutic potential of proteolytic enzyme inhibition remains largely unfulfilled, primarily because peptidal inhibitors and related pseudopeptides suffer from unsatisfactory pharmacokinetic behavior. This deficiency is not due solely to the susceptibility of peptides to degradation by proteolytic enzymes.[3] Thus, a 20-year search has furnished many potent peptidal inhibitors of renin,[4] yet none have become marketed drugs.[5] HIV-1 protease, required for infectivity by the virus responsible for AIDS, has generated an even more intensive quest for an orally effective inhibitor. However, to our knowledge, the oral bioavailabilities of compounds fully evaluated to date in patients have not exceeded 2%. Herein we report the design and synthesis of novel pyrrolinone-based inhibitors of the HIV-1 protease and renin. The HIV-1 protease inhibitor **5** proved to be less active than the corresponding peptide-based **4** in direct enzyme inhibition (IC) but more potent than **4** in the cellular inhibition assay (CIC), suggesting that the pyrrolinone structural motif affords improved transport into the cells. Pyrrolinone inhibitor **6** likewise displayed a ratio of CIC/IC more favorable than that reported for more conventional HIV-1 protease inhibitors.

Premise: Amide Bonds Limit Oral Bioavailability. In 1988 we began[6,7] to design novel peptideomimetic scaffolds devoid of the amide backbone on the basis of the postulate[3] that the secondary amide functionality is itself a principal impediment to the oral bioavailability of peptides. This premise is consistent with a 1967 report by Stein[8] that the ease of transport of a compound into cells correlates inversely with its ability to form hydrogen bonds with water. In 1969 Diamond and Wright[9] demonstrated that cell permeation of diverse molecules is adversely affected by intermolecular hydrogen bonding with water but less so by intramolecular hydrogen bonds; the adverse effect of the secondary amide bonds was attributed to solvation with approximately three molecules of water. The favorable oral bioavailability of cyclosporin,[10] which contains several N-methylated and intramolecularly hydrogen bonded amide moieties, further supports these concepts and our underlying surmise. Research by the Upjohn company[11,12] has recently explicitly linked poor oral bioavailability with the energy required for desolvation. They stressed the adverse effect of charge and chain length over the benefits of lipophilicity. In addition, they provided experimental evidence that N-methylation of secondary amide bonds facilitates transport across Caco-2 cell membranes. Interestingly, secondary amides do not interfere with clearance by the liver and kidneys.[12] This fact is consistent with the observation that the cyclic hexapeptide MK-678[13] has both poor oral bioavailability and rapid excretion.[13,3]

Linked Pyrrolinones: Mimics of β-Strands. Recently we demonstrated that crystalline 3,5-linked pyrrolin-4-ones (e.g., **1**, Figure 1) adopt a backbone conformation mimicking a β-strand; moreover, the pyrrolinone N-H protons, although displaced from the backbone, do serve as interstrand hydrogen-bond donors, permitting these peptidomimetics to form parallel and antiparallel β-pleated sheets in the solid state.[7] Earlier crystallographic studies had shown that diverse proteases such as kallikrein A,[14] penicillopepsin,[15] endothiapepsin,[16] rhizopuspepsin,[17] human renin,[18] and the HIV-1 protease[19] bind inhibitors in the extended β-strand orientation, likewise generating β-pleated sheets. Accordingly, we predicted[7] that the pyrrolinone structural motif should provide an alternative to amide-derived peptidomimetic inhibitors of proteolytic enzymes. In addition, we reasoned that intrastrand hydrogen bonding of the N-H proton with the carbonyl of the neighboring pyrrolinone ring would both stabilize

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the β-strand[7] and reduce solvation,[9] thereby improving the pharmacokinetic properties of our novel inhibitors.

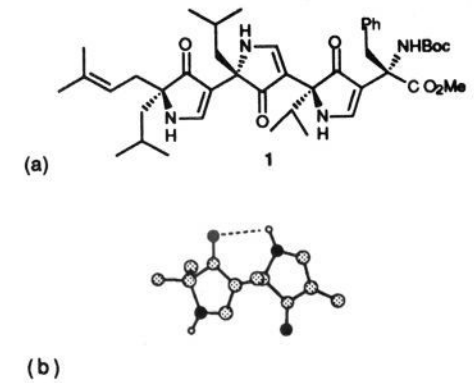

Figure 1. (a) Prototype 3,5-linked pyrrolin-4-one peptidomimetic (1) which adopts a β-sheet conformation in the solid state. (b) Six-membered-ring hydrogen bond joining adjacent pyrrolinone rings of (1); O-to-N distance 2.86 Å.

Renin Inhibitor Design. To expeditiously test the potential of the 3,5,5-linked pyrrolin-4-one unit for proteolytic enzyme inhibition, we patterned the selection of side chains, N and C termini, and transition-state isosteres largely on the prior art. Thus, to mimic the renin inhibitors R-Phe-His-Ts, we selected the Abbott erythro glycol transition-state analog[20] (Ts) and adopted the Kissei morpholine amide as the N-terminal R group.[21] To simplify the synthesis we replaced the His side chain by the isobutyl group of Leu,[20] generating the bispyrrolinone **2** (Figure 2) as our initial target. Molecular mechanics calculations[22] suggested that **2** could mimic the published backbone conformation of bound inhibitors.[23] However, attempts to dock **2** into a renin active-site model[24] indicated that ring B of **2** would experience unfavorable steric interactions with the flap of the enzyme. To test these ideas, we chose to evaluate both **2** and **3**, predicting that the latter would circumvent the potential steric liabilities of **2**. Both compounds were synthesized via the iterative protocol described earlier for **1**.[7,25] Indeed, *in vitro* assays[26] of **2** and **3** revealed IC_{50}s of 18 μM and 600 nM, respectively; the greater potency of **3** is in accord with the above analysis. Neither compound inhibited the HIV-1 protease, indicating specificity.

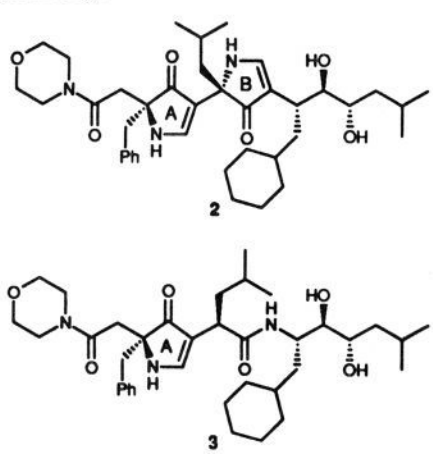

Figure 2. Designed renin inhibitors embodying the pyrrolinone scaffolding.

HIV-1 Protease Inhibitors. Having demonstrated the effectiveness of the pyrrolinone scaffold as a replacement for the amide backbone for protease inhibitor design, we concentrated our efforts on the medically more important HIV-1 protease since safe and effective ACE inhibitors are readily available. Using the known peptidal inhibitor **4** as a model[27] (Figure 3), we designed and synthesized **5** to permit direct experimental comparison with its exact peptidal counterpart. Molecular modeling predicted that **5** can adopt the β-strand/sheet conformation required for inhibition. Moreover, docking into the HIV-1 protease active site[19,28] revealed excellent potential for inhibitor–enzyme binding with no unfavorable steric interactions. We also undertook the synthesis of **6**. While there exists no exact peptidal counterpart, the N-terminal 3-hydroxytetrahydrofuran functionality had been shown to enhance potency.[29]

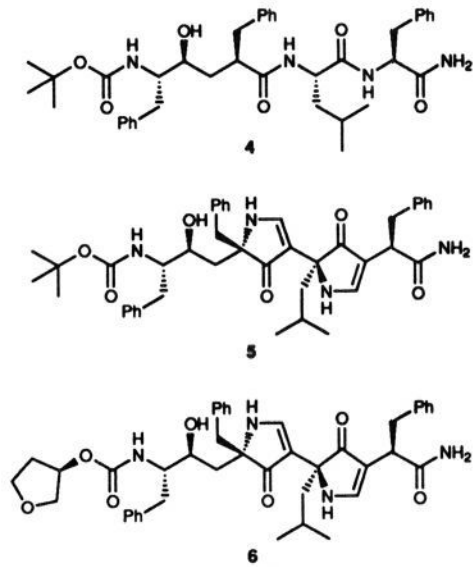

Figure 3. Peptidal HIV-1 protease inhibitor **4** and designed pyrrolinone-based inhibitors **5** and **6**.

In the direct enzyme inhibition assay,[30] **5** afforded an IC_{50} of 10 nM, compared with 0.6 nM for **4**. Importantly, the relative potencies were reversed in the cellular antiviral assay[31] (CIC_{95} of 1.5 and 6.0 μM, respectively), suggesting that the bispyrrolinone **5** is more readily transported into the cell than the corresponding dipeptide **4**.[32] The 3-hydroxy-THF derivative **6** proved to be our most active compound to date, with an IC_{50} (1.3 nM) and CIC_{95} (800 nM) again indicative of improved transport properties.

In summary, we have demonstrated the efficacy of the 3,5-linked pyrrolinone unit as a novel scaffolding for proteolytic enzyme inhibitors. Pyrrolinones contain no peptidal amide bonds susceptible to cleavage by peptidases or proteases and also appear to provide improved transport properties. Oral bioavailability studies will be reported in due course.

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assays, respectively. We are also indebted to Dr. Paul S. Anderson (Merck Research Laboratories) for his critical review of the manuscript and Dr. Perry V. Halushka (Medical University of South Carolina) for a stimulating discussion.

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(32) It is not known whether the inhibitor must penetrate beyond the plasma membrane in order to be effective as an antiviral agent. If it must, it is possible that peptidal inhibitors are less effective because the potential for intracellular degradation. However, we are not aware of any evidence to support this hypothesis.