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From Peptides to Non-Peptide Peptidomimetics: Design and Synthesis of New Piperidine Inhibitors of Aspartic Peptidases

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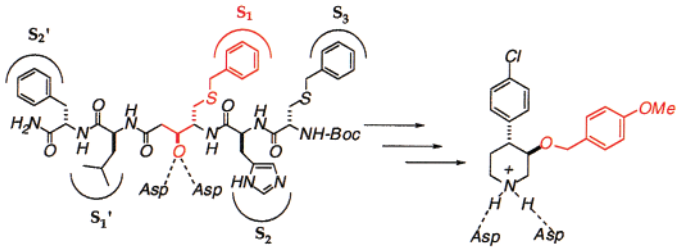
ABSTRACT



The 3-alkoxy-4-arylpiperidine inhibitors of aspartic peptidases are shown to be a new type of non-peptide peptidomimetic inhibitor. These piperidines can be designed from peptide-derived inhibitors by use of a structure-generating program but only after the enzyme active site conformation has been modified in a mechanistically related fashion. New enantioselective syntheses of 3-alkoxy-4-arylpiperidine analogues are described.

Structure-based design of peptidomimetic peptidase inhibitors is important for developing new drugs.[1] This approach has proven highly successful with the aspartic peptidase inhibitors,[2,3] as illustrated by the development of the HIV protease inhibitors for treating AIDS.[4] Most of these inhibitors have been designed to emulate the ligand-bound extended β-strand conformation[5] observed with most substrate-based peptidase inhibitors.[6] In this Letter we define these inhibitors as peptide-derived peptidomimetics,[7−9] due to their close relationship with the enzyme-bound peptide−substrate conformation. A few structurally distinct aspartic protease inhibitors[10,11] have been discovered by high-throughput screening methods and developed into useful HIV protease inhibitors, e.g., **1** and **2** (Figure 1). We define these inhibitors as non-peptide peptidomimetics[12] because of their remote structural relationship to peptide substrates. However, X-ray crystal structures of inhibitors **1** and **2**, as well as the rationally

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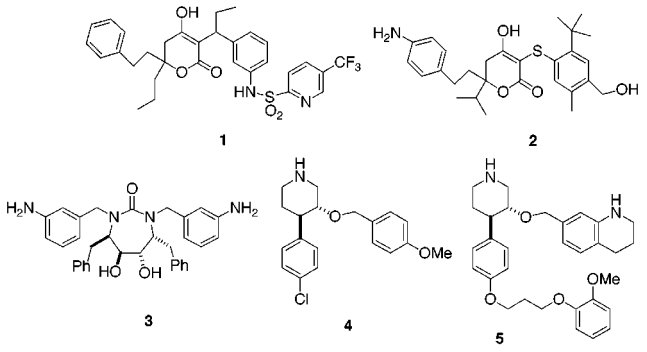

Figure 1. Non-peptide peptidomimetic peptidase inhibitors.

designed non-peptide peptidomimetic **3**,[13] complexed with HIV protease revealed that the inhibited active site topography is very similar to the inhibited active site topography for the peptide-derived peptidomimetics.

In this Letter we show that GrowMol, a computer program for generating libraries of structures complementary to an enzyme active site,[14] can be used to identify known "non-peptide peptidomimetics" that bind to a structurally distinct (nonextended β-strand conformation) aspartic peptidase active site conformation. Piperidine **4** inhibits renin at about 26 μM while the optimized piperidine inhibitor **5** inhibits renin at low nM concentrations.[15] The discovery of these piperidines as a new class of aspartic peptidase inhibitor represents a major advance in the design of inhibitors. These compounds are simple and contain no amide bonds, and some are orally active. Moreover, portions of both lead compound **4** and optimized inhibitor **5** bind in the active site of human renin as mechanism-based inhibitors. The binding of the piperidine nitrogen to the enzyme catalytic carboxyl groups is similar to the binding of the statine hydroxyl[16] and aminostatine nitrogen[17] in peptide-derived inhibitors. In addition, the binding of the 3-alkoxy group in the S_1-S_3 enzyme subsite is close to where the P_1 side chain of peptide-derived inhibitors bind. However, the topography of the inhibited active site is fundamentally different from the extended β-strand conformational topography. Piperidine **5** constitutes a new type of non-peptide peptidomimetic inhibitor.

In the preceding two Letters we described our use of a structure-generating computer program to generate novel aspartic peptidase inhibitors and to "rediscover" known peptide-derived inhibitors related to pepstatin.[18] Therefore, we attempted to generate the Roche-type structures in the active sites of pepsin and *Rhizopus chinensis* pepsin, two enzymes not inhibited by the Roche compounds. We began with the X-ray structure of bis-*S*-benzyl peptide **6**[19] bound to porcine pepsin (Figure 2, Supporting Information) and attempted to grow the piperidine unit from the P_1 *S*-benzyl side chain.[20] Growth from that point on CySta **7** toward the catalytic carboxyls (Figure 3) generated only acyclic amines,

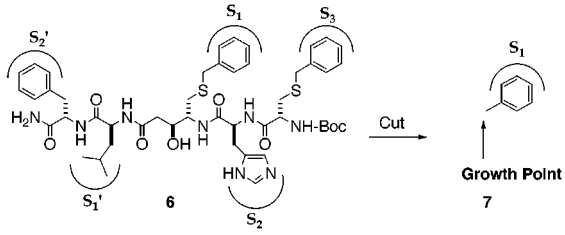

Figure 3. GrowMol growth point defined.

e.g., **8** (Figure 4), and it became evident that we could not generate piperidines such as **4** without altering the active

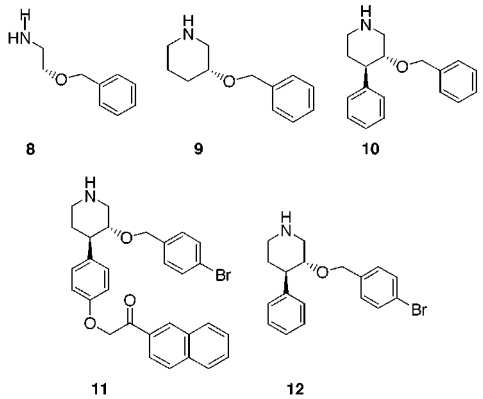

Figure 4. GrowMol-generated structures.

site conformation. Aspartic peptidases contain a hairpin turn structure, or "flap" region, in the enzyme active site that

(12) For the original definition of non-peptide peptidomimetic, see: Farmer, P. S. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1980; Vol. 10, p 119. However, those HIV protease inhibitors in refs 3−5 that do not contain a dipeptide unit are a different type of non-peptide peptidomimetic.

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moves in a "hinge motion" during peptide inhibitor binding, with the end of the flap moving up to 4 Å.[21] Since flap opening is a low-energy process that can occur up to 100 times per second for good substrates,[22] we decided to open this enzyme flap about 1 Å (Figure 5A); GrowMol now

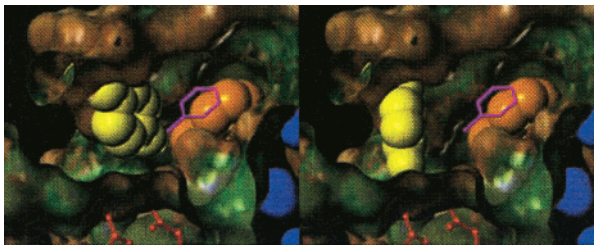


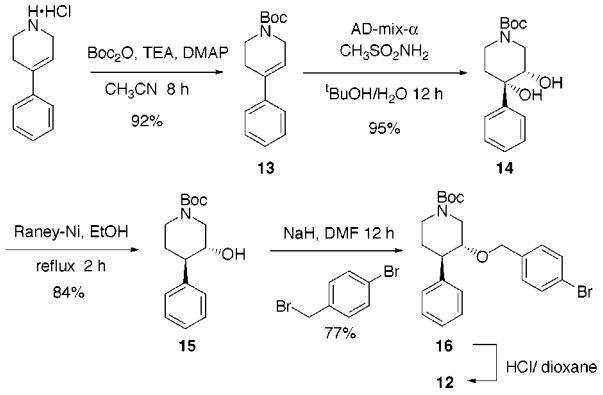
Figure 5. (**A**, left) Growth point in porcine pepsin active site after opening of flap region: flap, light brown; *S*-benzyl, pink; Asp32 and Asp215, red; Try75, yellow; Trp39, orange. (**B**, right) Active site of porcine pepsin after flap opening and Tyr75 side-chain rotation. Note the opening between Tyr75 and Trp39 where C4 phenyl growth occurs.

generated a series of piperidines, e.g., **9**, but not the 4-arylpiperidines. Further examination of the active site by molecular modeling revealed that a simple −120° rotation of χ^1 in Tyr75 would provide the space needed for growth at C4 of the piperidine system (Figure 5B). Running GrowMol now created the 3,4-disubstituted piperidine **10**, a direct analogue of the Roche HTS lead **4**. In addition, Roche scientists had discovered that the C4′-position of the 4-phenylpiperidines could be substituted to produce tight-binding renin inhibitors. This was possible because a tryptophan indole in human renin rotated out of the way to provide an additional binding site for the C4′ substituents. Therefore, we moved Trp39 in pepsin the same way (Figure 6, Supporting Information) and GrowMol now created acetonaphthone analogue **11**, which is closely related to the optimized Roche renin inhibitors.

These results show that piperidines **9** and **10** bind to an active site conformation that is fundamentally different from the extended β-strand topography, yet the new active site conformation can be reached via mechanistically related local conformational changes.

We developed a new and expedient enantioselective synthesis to prepare piperidine **12** (Scheme 1). N-Protection of 4-phenyl-1,2,3,6-tetrahydropyridine with Boc_2O gave **13**. Sharpless asymmetric dihydroxylation (AD)[23] was employed to generate diol **14**. Stereoselective reduction[24] of benzylic alcohol **14** with Raney nickel in refluxing EtOH gave **15**

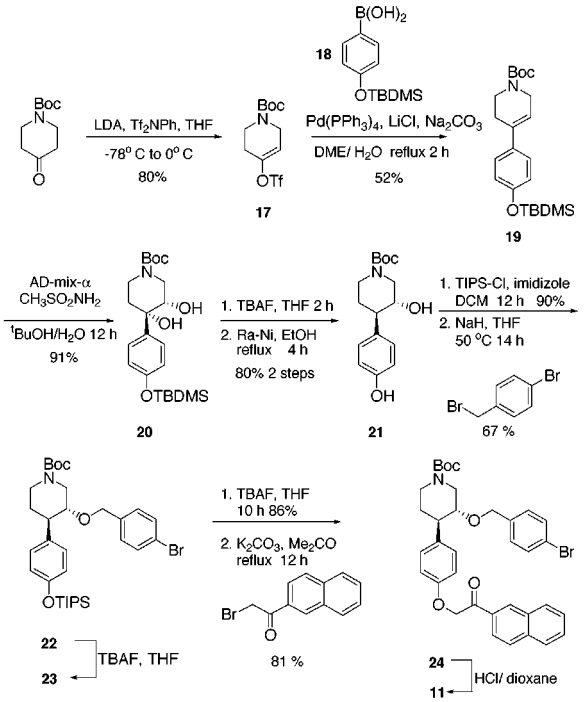
Scheme 1



(>95% ee). The remaining secondary hydroxyl of **15** was alkyated with NaH and *p*-bromobenzyl bromide in DMF to form the Boc-protected piperidine **16**. Subsequent removal of the Boc protecting group with HCl−dioxane provided piperidine **12**, which was used directly in the enzyme assays.

The enantioselective synthesis of piperidine **11** (Scheme 2) required slight modifications in this route. *tert*-Butyl

Scheme 2



4-oxypiperidine-1-carboxylate was converted to the corresponding tetrahydropyridinyl triflate **17** by use of LDA and *N*-phenyltrifluoromethanesulfonamide.[25] Palladium-mediated coupling of triflate **17** with the readily prepared arylboronic

(19) Szewczuk, Z.; Satyshur, K,; Rich, D. H. Unpublished results. K_i = 320 nM against porcine pepsin.

(20) The same process was carried out with *R. chinensis* pepsin.

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(22) K_{cat} for aspartic peptidases typically varies from 1 to 100 s^{-1}.

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(25) Wustriw, D. J.; Wise, L.D. *Synthesis* **1991**, 993.

acid **18** afforded the desired aryltetrahydropyridine **19** in moderate yield. Utilizing Sharpless AD methods, the enantiomerically pure diol **20** was synthesized. However, Raney nickel reduction of the benzylic alcohol in phenol-protected analogues of **20** was not successful.[26] After much experimentation, we determined that the desired Raney nickel reduction of **20** to **21** could be achieved after first removing the silyl-protecting group. Selective TIPS-protection of the phenol followed by alkylation of the secondary alcohol with NaH and *p*-bromobenzyl bromide in THF provided **22**. Deprotection of the TIPS groups with TBAF in THF gave phenol **23** whose stereochemical assignments were confirmed by X-ray structure.[27] Alkylation of **23** with potassium carbonate and 2-bromo-2′-acetonaphthone in acetone afforded the desired Boc-protected piperidine **24**. Removal of the Boc protecting group with HCl−dioxane provided piperidine **11**, which was used directly in the enzyme assays.

Inhibition of substrate hydrolysis by porcine pepsin and *R. chinensis* pepsin were determined using reported assay conditions.[28] Piperidine **11** inhibited porcine pepsin with an $IC_{50} = 0.2\ \mu M$ and piperidine **12** inhibited *R. chinensis* pepsin with an $IC_{50} = 2\ \mu M$. Several of these piperidines are poorly water-soluble, a property first noted by Oefner et al.[11] Some compounds saturated the buffer before full inhibition was obtained, which was detected by titrating enzyme activity from low to high inhibitor concentration.

Our results show that judicious application of a structure-generating program by medicinal chemists can be used to convert peptide-derived inhibitors into non-peptide peptidomimetics related to those found by HTS methods. To achieve these results, it was necessary to alter the conformation of portions of the enzyme active site. These conformational changes were implemented after careful consideration of plausible enzyme intermediates that could be formed during catalysis. All changes were predictable, low-barrier conformational changes. However, it should be emphasized that our success was made possible only because we knew this type of piperidine inhibited human renin. In our earlier efforts to identify novel structures,[29] various potential inhibitors were generated that contained amines but these were not studied further as we focused on hydroxyl inhibitors. Even when we decided to evaluate amines as a result of the Roche publications, the generation of piperidine structures by GrowMol was rare; only about 1% of the grown structures contained the piperidine nucleus. Furthermore, these piperidines were obtained only when the growth point (Figure 3) was moved along the hydrophobic surface comprising the S_1-S_3 subsites. Finally, successful growth was achieved only when we began with X-ray structures of moderate inhibitors (e.g., **6**) bound to the enzyme.

Despite these caveats, our work demonstrates that it is possible to identify non-peptide peptidomimetics utilizing GrowMol and the crystal structure of a peptide-derived inhibitor bound to the enzyme. The process we have described represents a simple protocol for altering enzyme active sites to permit design of non-peptide peptidomimetic inhibitors that bind to novel enzyme active site conformers. Our results are consistent with recent calculations that show ligands bind to the dynamic ensemble of *preexisting* enzyme conformations[30] such that "binding of an inhibitor selectively stabilizes those conformational states in which the binding site is formed." [31] Thus, we propose that the surprising renin conformations discovered by Oefner et al.[11] are not "induced" but simply *preexisting enzyme conformations* selectively stabilized by the inhibitor to afford the crystallographically observed complex.

Our work demonstrates that a third approach to peptidomimetic design can include inhibitors designed to stabilize *potential preexisting* enzyme active site conformations in addition to those observable in both native and inhibitor−enzyme crystal structures. Rather than design peptidomimetics to emulate only the enzyme-bound conformation of the peptide-derived inhibitor (the extended β-strand topography), as is currently done,[5] design should be targeted to the complete ensemble of potential preexisting active site conformations. Structure-generating programs that allow systematic variation of the position of the starting growth point, that permit systematic evaluation of the dynamic ensembles of *preexisting* enzyme active site conformations, and that provide systematic evaluation (scoring) of the grown structures will greatly accelerate the discovery of non-peptide peptidomimetic inhibitors.[32] Finally, inhibition of two additional aspartic peptidases by these piperidines supports the proposal of Oefner et al.[11] that the piperidines may become general scaffolds for inhibiting this enzyme class. Since piperidines **11** and **12** are structurally related to paroxetine,[33] a known CNS active drug, this scaffold may become especially effective at inhibiting aspartic peptidases located in the CNS.

Acknowledgment. We thank the NIH (GM50113) for financial support, Dr. George Flentke for providing *R. chinensis* pepsin and initial assistance with the biological assays, Drs. Ken Satyshur and Regine Bohacek for assistance with molecular modeling, Dr. Colin McMartin for the use of his software Flo-01, Dr. Ilia Guzei for the X-ray crystal structure of **23**, and Dr. Martha Vestling for MS data.

Supporting Information Available: Figures 2 and 6−9 as well as experimental procedures for the synthesis and characterization of compounds **11**−**24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) The silyl-ether analogue did not react presumably for steric reasons; other ether analogues underwent reductive cleavage of the C4′ C−O bond.

(27) See Figure 7 in Supporting Information.

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(32) Programs to perform these calculations have not been reported to date, but seem possible.

(33) For structural comparison, see Figure 8 in Supporting Information.