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Computer-Assisted Drug Design

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Transformation of Peptides into Non-Peptides. Synthesis of Computer-Generated Enzyme Inhibitors

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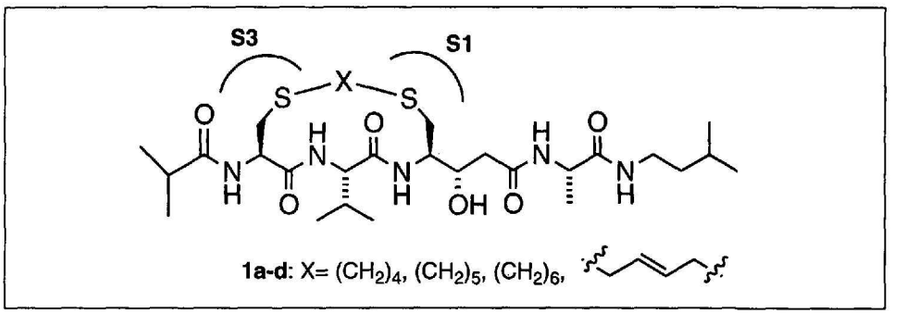
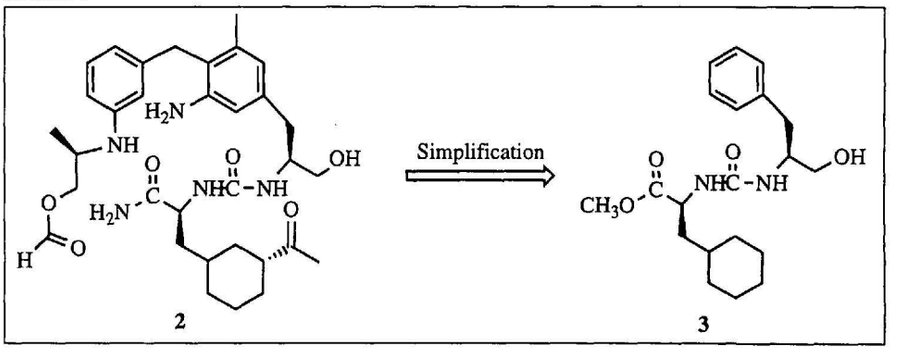
Abstract. With the intent of discovering novel molecular scaffolds for designing protease inhibitors, the computer program GrowMol was used to generate peptidemimetic inhibitors of aspartic proteases. Beginning with the X-ray crystal structure of A66702 complexed to pepsin, GrowMol successfully generated a series of known cyclic inhibitors of pepsin in which the cysteine side chains in the P1 and P3 inhibitor subsites are connected. GrowMol also created a series of novel urea-derived inhibitors of pepsin, a series of α,α-disubstituted amino-alcohol-derived structures, and a series of cyclohexanol-derived inhibitors in which the core portion of the inhibitor lacks nitrogen. The paper describes the iterative process of selection and synthesis of computer-generated structure, determination of activity, and reassessment of potency with respect to the beginning crystal structure. These efforts led to the synthesis of analogs **9–12** which are novel pepsin inhibitors with moderate inhibitory activity. Attempts to crystallize these inhibitors in the active site of pepsin to determine if compounds **9–12** bind as predicted by GrowMol are in progress.

Mechanism-based design of enzyme inhibitors, and its companion approach of structure-based drug design [1], have become so powerful and successful for developing prototype enzyme inhibitors that the major roadblock for drug discovery today is not the discovery of the inhibitor but the discovery of **bioavailable inhibitors**. We are interested in devising systematic ways for transforming the biologically active conformation of a peptide bound to an enzyme into a non-peptide-'mimetic' that will have improved pharmacokinetic properties. In the same way that principles have been discovered for designing enzyme inhibitors by mimicking the topography of reaction pathway intermediates [1], medicinal chemists today seek to discover an equivalent strategy for developing molecular replacements for the *binding* components of both enzyme inhibitors and receptor antagonists and agonists. The hope is that these novel molecular scaffolds will possess the pharmacodynamic properties needed for efficient drug use. The importance to medicine is enormous since this step is one of the few remaining barriers to systematic drug development from peptides.

Recently, we described how the computer program GrowMol generates peptidemimetic inhibitors of aspartic proteases [2], which was based on the work of *Bohacek* and *McMartin* [3]. Beginning with the X-ray crystal structure of A66702 complexed to pepsin [4], GrowMol generated 50000–100000 potential structures quickly, classified them according to structural type, and produced a manageable file of 200–400 distinct structures that were examined for synthetic feasibility by the chemists. To illustrate our progress to date, GrowMol successfully generated the known cyclic inhibitors of pepsin **1a–d** by bridging cysteine side chains in the P1 and P3 inhibitor subsites.



Scheme 1



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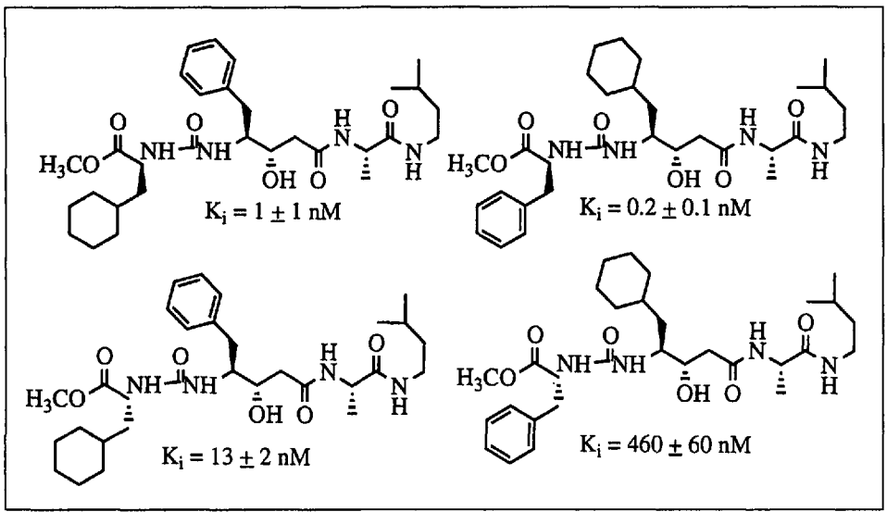
GrowMol also created a series of novel urea-derived inhibitors of pepsin, which we simplified in the fashion shown for **2–3**. A variety of low molecular weight, micromolar inhibitors were generated. When functionalized by addition of P' substituents, the potent inhibitors shown in the *Figure* were produced.

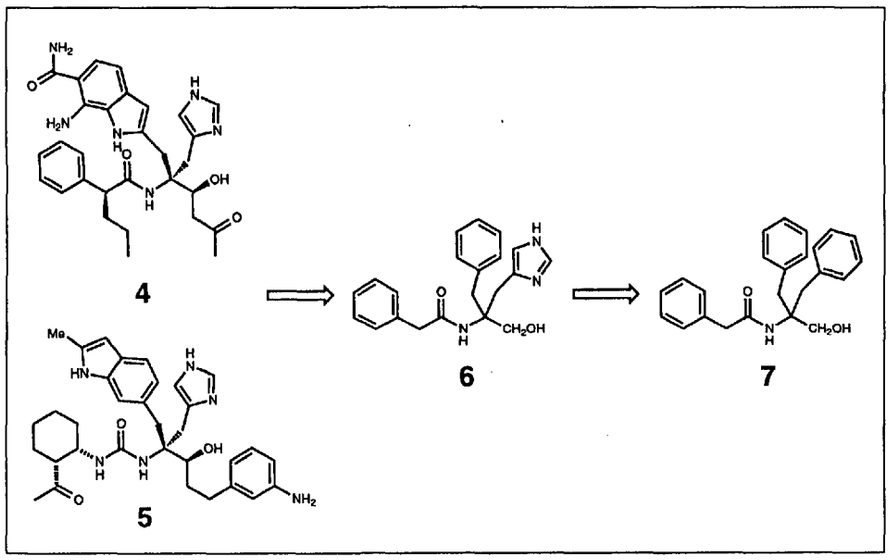
The α,α-disubstituted amino-alcohol-derived structures (**4** and **5**, *Scheme 2*) illustrate another series of successful structures generated by GrowMol in these early studies. These structures were simplified to remove non-essential substructures in order to obtain the synthetically accessible derivatives of α,α-dibenzylglycine **7** and α-benzylhistidine **6**. The α,α-dibenzylglycinol analogue **7** inhibited pepsin moderately, $K_i = 25 \pm 5$ μM.

GrowMol predicted an even more remarkable series of potential inhibitors of pepsin. The cyclohexanol-derived inhibitor **8** (*Scheme 3*) represents a completely novel inhibitor structure in that the core portion of the inhibitor lacks nitrogen. At the time we began this work, no non-nitrogen-containing tight-binding inhibitors of pepsin were known, and we simplified **8** to produce a series of compounds designed to determine whether GrowMol had produced a realistic prediction. The cyclohexanol derivative **9** was synthesized by using a modification of the route to a phylanthocin intermediate [5]. Assay for inhibition of pepsin revealed that **9** did not inhibit the enzyme at the highest concentrations tested (400 μM).

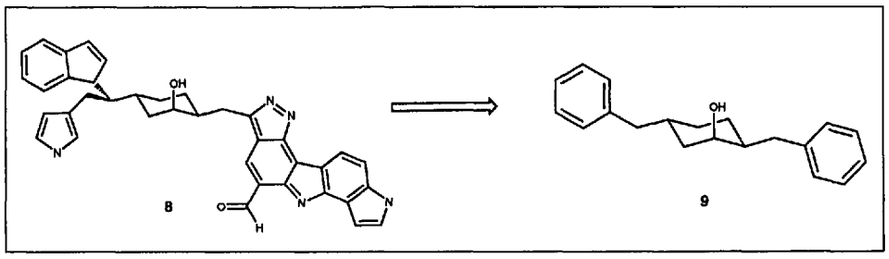


Figur. *Statine-derived urea inhibitors of pepsin*

Scheme 2



Scheme 3



Careful reexamination of the predicted GrowMol structure against the simplified structure showed that at least two critical enzyme-inhibitor interactions were missing in **9** that are present in A66702 and in **8**. Consequently, we synthesized analog **10**, which was designed to restore the α-disubstituted carbon branching and the extra hydrogen-bond donor. Control compounds **11** (contains the α-branching) and **12** (contains the extra hydroxy group) are also much more potent than **9**. Interestingly, both structural changes increase potency but do not act in an additive fashion, which may suggest different binding modes for each compound. We are currently trying to crystallize these inhibitors in the active site of pepsin to determine if compounds **9–12** bind as predicted by GrowMol.

Discussion

Enzyme inhibitors are typically developed by mimicking the reaction pathway intermediates formed in the active site of an enzyme as it transforms the substrate into product [1]. Substrate-derived compounds are synthesized in which the labile amide bonds are replaced by non-hydrolyzable isosteres. Ancillary portions of the resulting inhibitor are modified until inhibitors with the proper pharmacodynamic properties needed for drug use are obtained. The difficulty in this approach is that there is no rational method for transforming the peptide-like structure into a non-peptide-like structure with good pharmacodynamic properties, *e.g.* oral activity. GrowMol offers a new approach to this problem by generating a diverse array of molecules from which medicinal chemists can extract suitable potential lead structures for further evaluation and synthesis. The work reported here demonstrates that GrowMol can generate known and novel inhibitors in the active site of pepsin and *R. chinensis* pepsin, and complements the

demonstrated success of GrowMol for generating thermolysin inhibitors [3]. Although not yet done, GrowMol should be able to re-create known inhibitor databases, *e.g.* HIV protease inhibitors, from the component substructures, in a fashion analogous to that described for inhibitors **1a–d** [2]. It is important to note for all examples reported here that we do not know if the inhibitors are binding to the enzyme in the computed fashion; additional X-ray studies of the enzyme-inhibitor complexes are needed to determine the bound conformations.

The use of GrowMol to generate libraries of potential inhibitors for a target enzyme represents a combinatorial process of enormous power. Clearly, potent inhibitors have been obtained and when structure-generating programs are combined with powerful synthetic efforts, it is reasonable to expect that optimization will lead to more potent inhibitors. Combinatorial synthesis of molecules is likely to be a particularly effective way to optimize lead structures to obtain tight-binding inhibitors [6].

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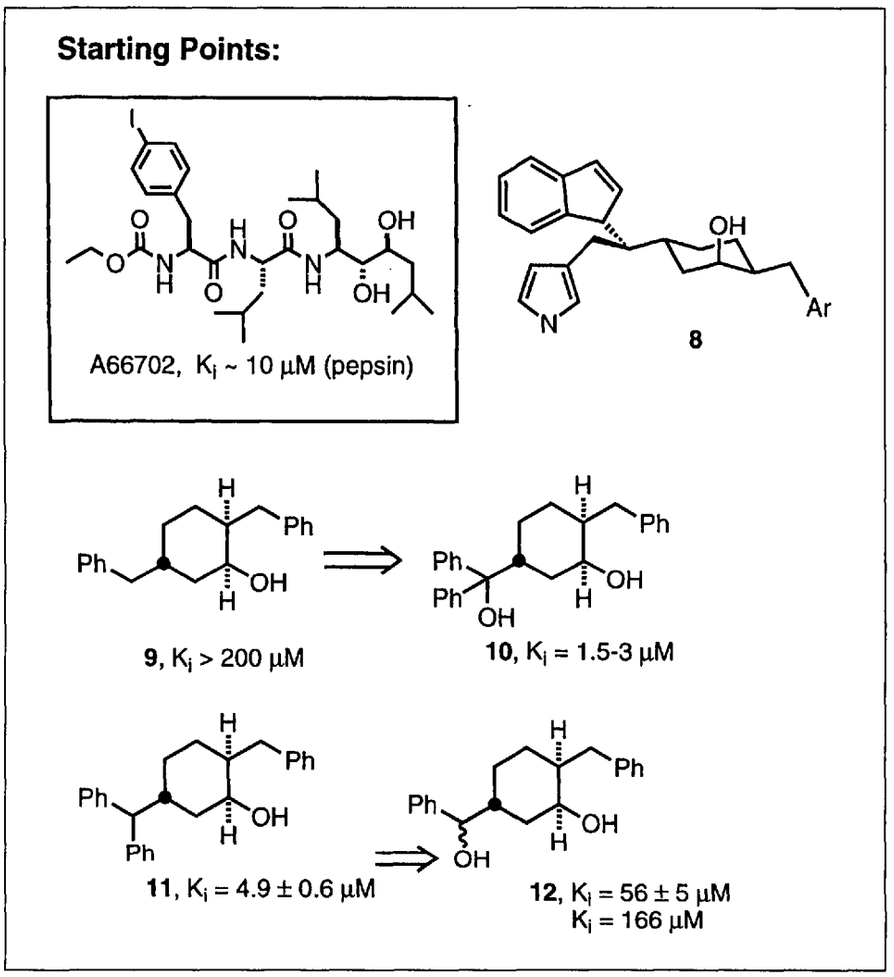
Scheme 4



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