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SOLUTION-PHASE SYNTHESIS OF A COMBINATORIAL MONOCYCLIC β-LACTAM LIBRARY: POTENTIAL PROTEASE INHIBITORS

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Abstract: A 126-member library of monocyclic β-lactams was generated in parallel fashion by solution-phase Ugi four-component condensation reaction between β-amino acids, aldehydes, and isocyanides. The library was designed to identify potential human leukocyte elastase inhibitors. The approach is also capable of optimizing the lead compounds generated in the original library. © 1997 Elsevier Science Ltd.

Combinatorial chemistry and solid-phase synthesis are rapidly developing methods in medicinal chemistry and have emerged among the most promising tools in new lead generation.[1] Many think that these approaches are at least equally powerful in speeding up lead optimization processes. In the last few years significant advances have been made in adapting organic reactions to solid-phase synthesis.[2] However, solution-phase chemistry still prevails in achieving diversity. Therefore, several groups have recently attempted to use solution-phase chemistry in their combinatorial library generation.[3] The use of multiple component condensation reactions (MCCs) in conjunction with combinatorial chemistry can be used very efficiently to generate chemical diversity in short reaction sequences.[4] Although only a handful of MCCs are suitable for combinatorial chemical purposes,[5,3f] post-condensation modification of the molecule clusters generated by some of these reactions can lead to a wide variety of important pharmacophores.[6] Such approaches provided, among others, libraries of small heterocycles, e.g., pyrroles,[6,7] imidazoles,[8] benzodiazepines,[6] and γ-lactams.[9] Herein we report an approach to the combination of library generation, lead optimization, and MCC in search of potent inhibitors of pathologically important human serine proteases.

In our studies we targeted human leukocyte elastase (HLE),[10] whose degradative effect on lung elastin has been implicated as a major causative factor of serious degenerative diseases (e.g., emphysema, respiratory distress syndrome, chronic bronchitis, cystic fibrosis, and rheumatoid arthritis). These effects make the enzyme a promising medicinal target. We also utilized the successful efforts by Merck scientists, who were able to obtain several highly potent and selective monocyclic β-lactam based inhibitors of the enzyme, exemplified by **1** and **2** in Scheme 1. They used classical medicinal chemistry approaches to obtain these compounds and generated extensive structure–activity relationship data. Profiting from their observations, we planned to adapt the synthesis to combinatorial methods to provide a somewhat different carbon skeleton for the inhibitor candidates.

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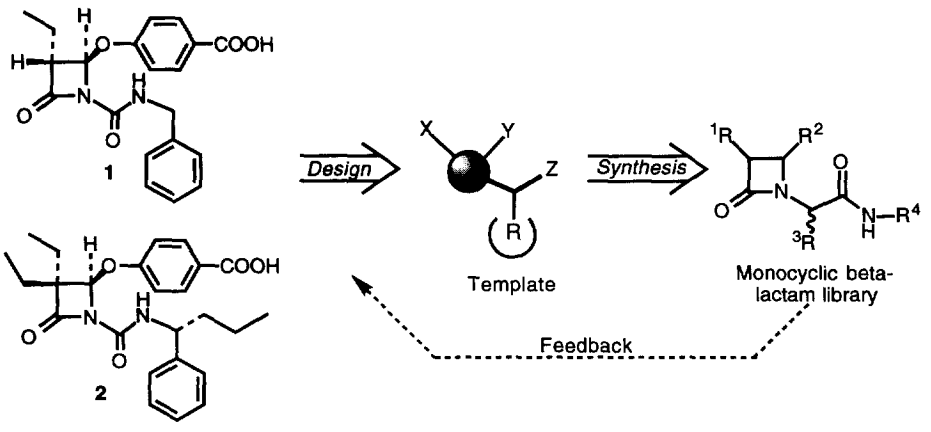
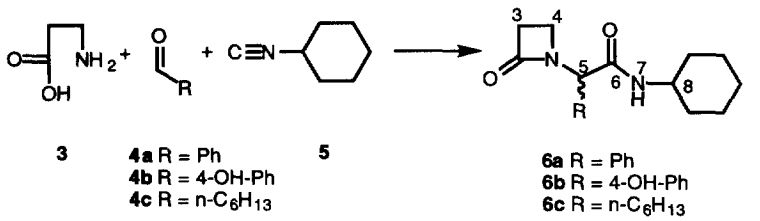


Scheme 1. Experimental design.

Our approach (Scheme 1) consisted of the following steps: (i) Retain the highly active β-lactam ring, successful structural element of several serine protease inhibitors.[11] (ii) Retain crucial H-bonding and hydrophobic interactions observed in the case of compounds **1** and **2**. (iii) Design a structural motif that allowed combinatorial synthesis. (iv) Combine these elements to generate a combinatorial library of potential inhibitors yielding, ideally, a small set of molecules sufficiently active to serve as leads in further inhibitor design. (v) Then, by gaining some SAR data by testing members of the library (and from the Merck approach), we could redesign our library optimize to active compounds. (vi) Repetition of these cycles could lead to highly potent compounds readily accessible by total synthesis.

We decided to replace the urea backbone of the Merck inhibitors by a peptide-like skeleton that possessed similar hydrogen-bonding properties (represented by thick bonds in the template in Scheme 1), but could more easily be generated by combinatorial approaches. The highly effective Ugi four-component condensation reaction (U4CC)[12] of β-amino acids, aldehydes, and isocyanides was the reaction of choice to create the oligopeptide-like backbone and the β-lactam "warhead" in a single step. By using substituted β-amino acids one can successfully place different substituents at positions **X** and **Y** of the template. The **R** substituent, which lies in a relatively small hydrophobic pocket of the enzyme, derives from the aldehyde, whereas the **Z** side-chain is from the isocyanide. We planned to obtain small sub-libraries of inhibitor candidates by keeping **R** (i.e., the aldehyde) constant and altering the β-amino acids and isocyanides (variables **X**, **Y**, and **Z**). The U4CC yields mixtures of diastereomers at C-5, which is particularly useful because we can test both possible enantiomers simultaneously.

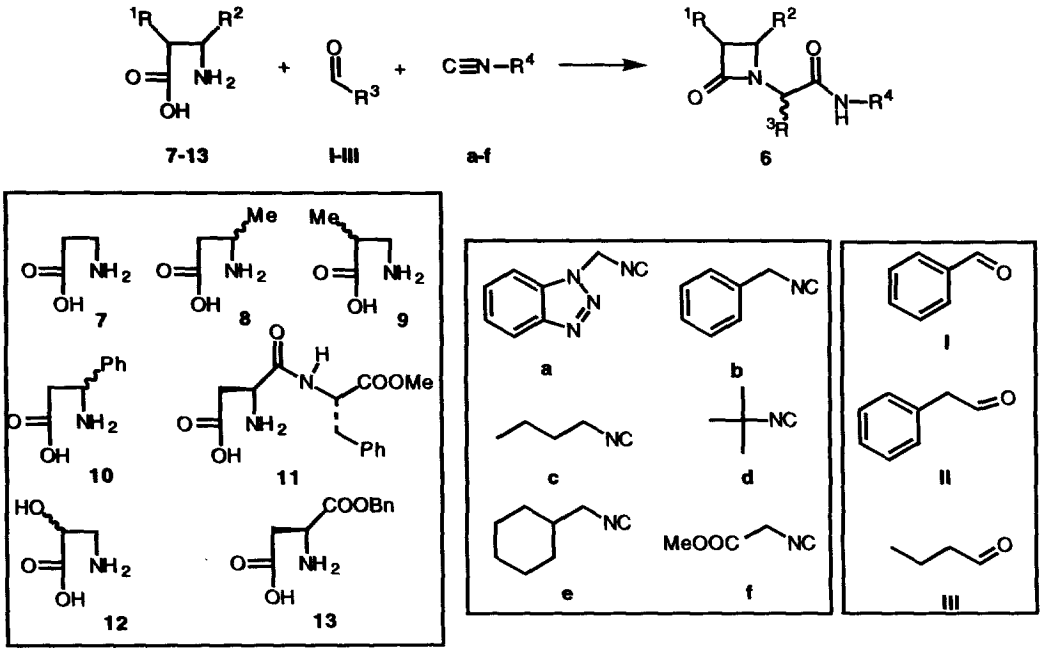


3 **4a** R = Ph **5** **6a** R = Ph
4b R = 4-OH-Ph **6b** R = 4-OH-Ph
4c R = n-C_6H_{13} **6c** R = n-C_6H_{13}

Scheme 2. Model Ugi 4CC reactions to optimize library generation conditions.

In model experiments (Scheme 2), β-alanine (**3**), the simplest β-amino acid, three different aldehydes (**4a–c**), and a moderately reactive isocyanide (**5**) were condensed to form β-lactam dipeptides **6a–c**.[13] The reactions were monitored by TLC. It was easily determined that methanol is the best solvent for the reaction, which can proceed at a reasonable rate at room temperature in the case of all three aldehydes examined. The reaction was fastest in the case of the aliphatic aldehyde **4c** (3 h, 91%), whereas benzaldehyde (**4a**) reacted rather slowly, providing **6a** in 56% yield after 48 h. *p*-Hydroxybenzaldehyde (**4b**) reacted quicker than the parent aldehyde **4a** (24 h, 72%). These experiments also led to very significant conclusions concerning the workup of the reactions. Although analytical samples were purified, there was really no need for column chromatography to obtain the products **6a–c** in reasonable yield and purity ready for initial biological evaluation. The volatile aldehydes **4a** and **4c** and the isocyanide (**5**) could easily be removed under high vacuum. The unreacted β-alanine was removed by filtration through a silica gel pad using hexane:ethyl acetate (1:4) as eluent. In light of these findings and building on the information that HLE and chymotrypsin (an enzyme with similar specificities to be used to study the selectivity of the potential inhibitors) have preference for small hydrophobic substrates and inhibitors, we decided to use a solution-phase approach to generate a combinatorial library of compound **6**. Later, if needed, virtually any component of the four-component condensation reaction could be linked to a solid support,[14] thus enabling an automated synthesis of related compounds. We planned to generate the library in a parallel fashion,[15] obtaining each compound as a single entity. Thus, difficulties with compound identification and activity determination, often a problem if a mix-and-split protocol[16] is used, can be avoided.

Commercial availability and appropriate side-chain functionalities were deemed selection factors to generate a 126-member library of monocyclic β-lactam dipeptides (**6**). Seven β-amino acids (**7–13**), six isocyanides (**a–f**), and three aldehydes (**I–III**) (Scheme 3) were combined in one-well/one-compound fashion using methanol as solvent (0.1 mmol reactants in 5 mL MeOH). The reaction vessels were vigorously shaken in a vertical shaker at ambient temperature. The reaction times were determined by the aldehyde component. In the case of aldehyde **I**, 48 h reaction time was used, for **II** 24 h, and for **III** overnight. The reaction vessels were then placed under high vacuum for 12 h in a SpeedVac apparatus (Savant). The crude products were redissolved in methanol, insoluble materials were removed with Pasteur pipettes containing cotton plugs and the filtrates were evaporated under high vacuum again. Filtration through silica gel, the last planned purification step, was not carried out at this time. The crude library was analyzed directly.



Scheme 3. Construction of a combinatorial library of 126 monocyclic β-lactam dipeptides (**6**).

Twenty diversely substituted samples **6** were submitted for electron-ionization mass spectral (EI-MS) analysis to determine the success of library generation. Both the stability and purity of the samples could be assessed in this manner. The EI-MS analysis provided unambiguous evidence that the synthesis was very effective. Only one sample failed to provide the correct M^+. The yield and product purity were clearly dependent upon the substitution patterns of the reactants. Yields were highest in sub-library **III** (butanal) and lowest in the case of sub-library **I** (benzaldehyde). Isocyanides **a** and **f** (purchased dark colored and used without purification) appeared somewhat unstable, and provided impure products in low yields. Probably distillation/use of an automated synthesizer would have been helpful in these cases. Use of isocyanide **b** resulted in moderate yields, whereas **c–e** gave excellent results in all cases. As far as the β-amino acids are concerned, solubility seemed to be the most important factor in determining the yield of the reactions. Compounds **11–13** provided the dipeptides in moderate yields and significant amounts of unreacted β-amino acids were removed by filtration, especially in the case of isoserine (**12**). Owing to these factors, one dipeptide, **I/12a** did not give the correct M^+ by EI-MS.

In conclusion, elements of structure-based rational drug design and solution-phase combinatorial library generation techniques were successfully applied for the synthesis of a monocyclic β-lactam dipeptide library, potential elastase inhibitors. This strategy could provide a very powerful alternative to "conventional" medicinal chemistry in lead generation and optimization. Biological evaluation of the monocyclic β-lactam dipeptide library against elastase and chymotrypsin is under design and results will be reported in due course.

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13. Selected pysical data for **6a–c**: Compound **6a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02–1.2, 1.2–1.4, 1.52–1.76, and 1.82–1.96 (m, 10H, cyclohexyl), 2.88 (dd, 1H, J = 3.05, 5.5 Hz, H-4), 2.98 (dd, 1H, J = 3.05, 5.5 Hz, H-4), 3.16 (m, 1H, H-3), 3.63 (m, 1H, H-3), 3.76 (m, 1H, H-8), 5.28 (s, 1H, H-5), 6.11 (d, 1H, J = 7.3 Hz, NH), 7. 36 (m, 5H, aryl) ppm. Ms (EI/CI, NH_3, 4.9 V): *m/z* 287 (MH^+, 100%). Compound **6b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08–1.2, 1.24–1.38, 1.54–1.72, and 1.82–1.92 (m, 10H, cyclohexyl), 2.87 (dd, 1H, J = 3.05, 5.5 Hz, H-4), 2.96 (dd, 1H, J = 3.05 , 5.5 Hz, H-4), 3. 16 (m, 1H, H-3), 3.57 (m, 1H, H-3), 3.76 (m, 1H, H-8), 5.25 (s, 1H, H-5), 6.24 (d, 1H, J = 7.9 Hz, NH), 6.75 (d, 2H, J = 8.5 Hz, aryl), 7.13 (d, 2H, J = 8.5 Hz, aryl) ppm. Ms (EI/CI, NH_3, 3.4 V): *m/z* 303 (M^+, 100%). Compound **6c**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.9, 1.02–1.2, and 1.24–1.95 (m, 23H, hexyl and cyclohexyl), 2.9 (m, 2H, H-4), 3.35 (m, 1H, H-3), 3.44 (m, 1H, H-3), 3.71 (m, 1H, H-8), 4.07 (dd, 1H, J = 6.1, 8.54 Hz, H-5), 6.42 (d, 1H, J = 7.5 Hz, NH) ppm. Ms (EI/CI, NH_3, 9.0 V): *m/z* 295 (M^+, 100%).

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