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Metalloproteases Involved in Breast Cancer Metastasis

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13. ABSTRACT (Maximum 200 words) Matrix metalloproteases (MMPs) are endopeptidases with a diverse set of physiological and pathological functions. Two of these proteases, MMP-2 and MMP-9, also known as gelatinases A and B, respectively, have been implicated in breast tumor metastasis. This research endeavors to prepare the first mechanism-based inactivators for these enzymes, molecules which are expected to show high selectivity in inactivation of the targeted enzymes. Toward this goal, six such putative inactivators were designed and synthesized in the Mobashery laboratory. Initial characterization with the enzyme indicated that three of these molecules showed the desired inactivating trait, but they exhibited relatively low affinities for the gelatinases. Work in on-going in the design of derivatives of higher affinity.				
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FOREWORD

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PI - Signature Date

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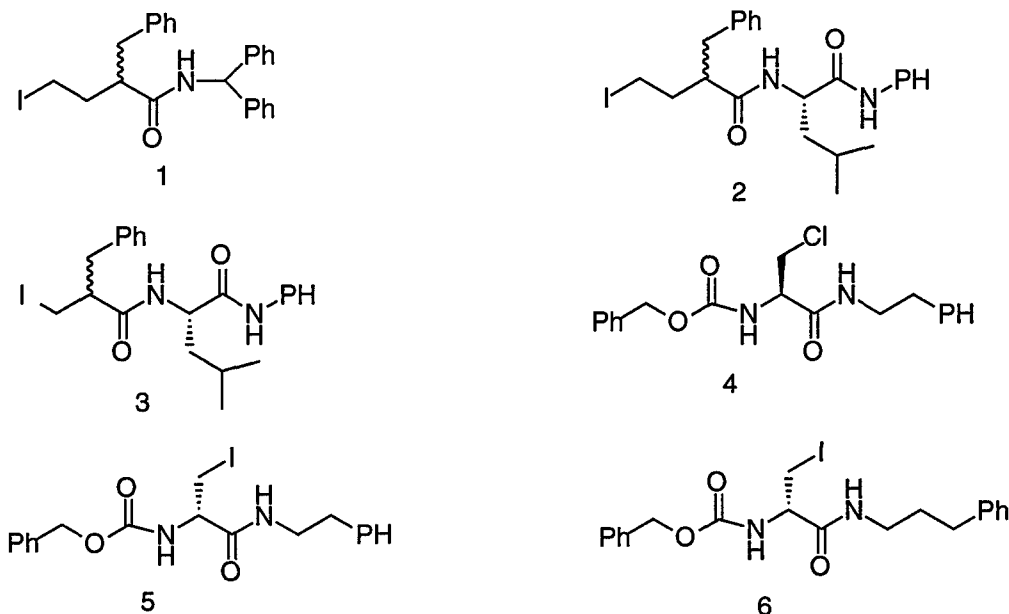
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Introduction

Matrix metalloproteases (MMPs), zinc-dependent endopeptidases that degrade extracellular matrix components. Two of these proteinases, MMP-2 and MMP-9, also known as gelatinases A and B, respectively, are involved in breast tumor metastasis and the process of neovascularization. Evidence indicates that the activity of gelatinases is critical for breast cancer tumor invasion and angiogenesis. We have undertaken a multidisciplinary research approach for inhibition of the gelatinase-mediated tumor cell invasion and angiogenesis using the first novel synthetic mechanism-based inactivators targeted for gelatinases. These inactivators are expected to show inherent high selectivity for gelatinases, as previously demonstrated by us with similar types of inactivators for other families of metalloproteases. The efficacy and selectivity of the inactivators will be evaluated with purified preparations of recombinant gelatinases A and B (MMP-2, MMP-9), stromelysin-1 (MMP-3) and collagenase-3 (MMP-13). Provisions have been made so that the best inactivator of our design would be evaluated in *in vitro* invasion and angiogenesis assays, as well as in animal models for breast tumor metastasis in collaborations with other groups in the foreseeable future.

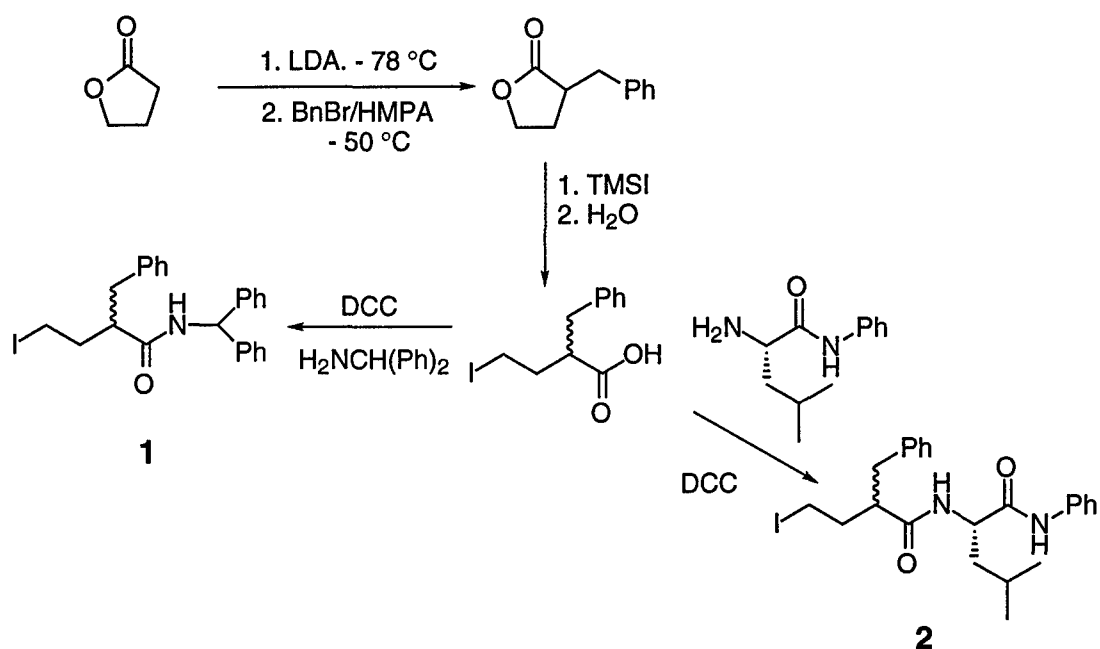
Body

As described above, we intend to prepare inactivators which will be selective against gelatinases. The design concepts have evolved over the past year, and we have developed new designs for the first such molecules; so the design has been updated. Toward our goal, we have synthesized in the first year of funding putative inactivators **1-6**; these molecules are based on our recent conceptualization of targeting for gelatinases. The design of these molecules was based on our computational models for the catalytic domains of the two gelatinases and reports of other competitive inhibitors for these enzymes.

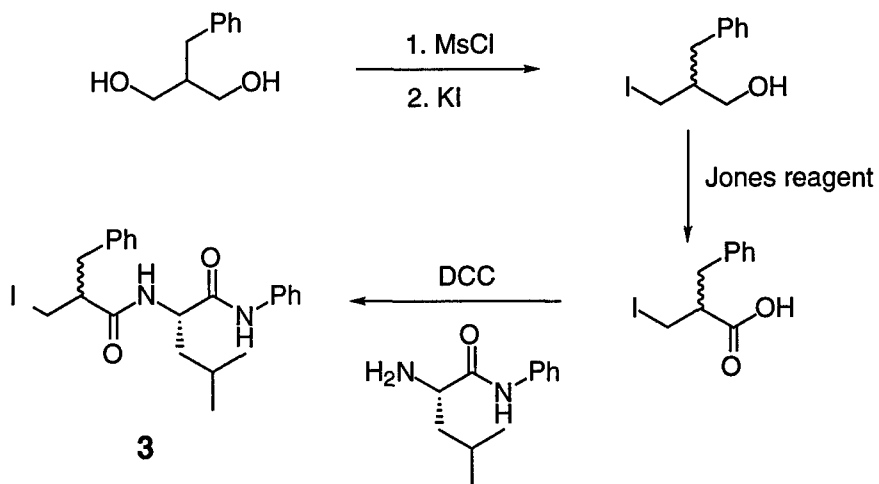


These compounds were synthesized according to the following methodologies, as outlined in Schemes 1, 2, and 3.

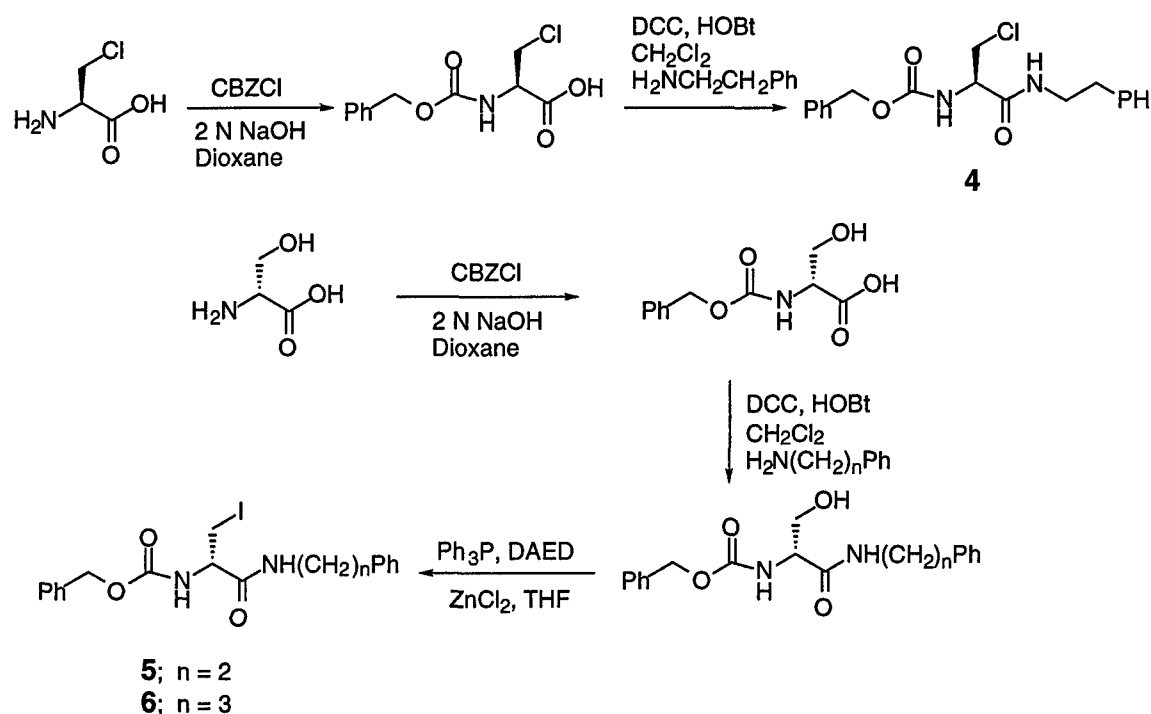
Scheme 1



Scheme 2

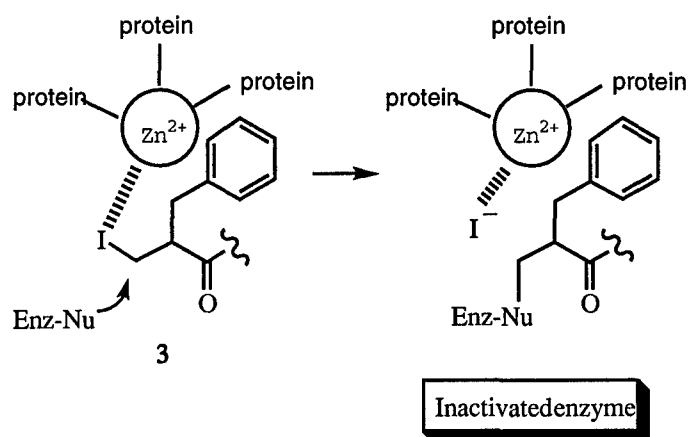


Scheme 3



These molecules were expected to bind the active sites of gelatinases, as depicted in Scheme 4 for compound **3**. After binding, the enzyme active site, the iodo moiety is expected to coordinate with the active site zinc ion and would get activated for the inactivation chemistry by modifying an active-site amino acid which would be nucleophilic in nature.

Scheme 4



Therefore, we should expect to see a time-dependent inactivation of the enzyme, by a type of kinetic analysis which we have published in our earlier work.¹ We do see the time-dependent inactivation with compounds **4**, **5**, and **6**, but not with **1**, **2**, and **3**. Furthermore, the effectiveness of these compounds as inactivators follows the same order (**4**, **5**, and **6**). The problem is that the inactivators are not recognized with high affinity by

gelatinases A and B, and that the inactivation process does not proceed to completion. In the next year of funding, we need to work on generating variants of these compounds which show higher affinity for the enzyme. Molecular modeling to achieve such a design has already begun.

Conclusions

We have synthesized six molecules as potential inactivators of gelatinases. These molecules establish some of the required elements in binding to the active site, and three show time-dependence for loss of activity, which is required for the inactivators of our design. Our recent results point us in the direction of improving our design paradigms, which should lead to molecules of high potency for inhibition of gelatinases.

References

- (1) Tanaka, Y.; Grapsas, I.; Dakoji, S.; Cho, Y.J.; Mobashery, S. Conscripting the Active-Site Zinc Ion in Carboxypeptidase A in Inactivation Chemistry by a New Type of Irreversible Enzyme Inactivator, *J. Am. Chem. Soc.* **1994**, *116*, 7475.



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