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Inhibition of Metalloproteinase by Futoenone Derivatives

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Abstract: Futoenone and a series of its derivatives have shown inhibitory activities against matrix metalloproteinases. The molecular modelings of these compounds indicate the preferred binding of the P2' site of the enzymes.

Introduction

Futoenone is a neolignan, with an unique spirocyclohexadienone skeleton, originally isolated in 1968 by Ogiso et al[1]. Recently it was identified as one of the active ingredients in the Chinese herbal plant, *Piper futokadsura* Sieb et Zucc. (Haifengteng), which has been used in the treatment of inflammatory and pulmonary allergic conditions[2]. Futoenone inhibits platelet activating factor (PAF) at submicro molar concentrations. In this study, we have found that futoenone and several derivatives possess some inhibitory activities against matrix metalloproteinases (MMP), especially stromelysin with IC50 < 1μM. The structure-activity relationships of metalloproteinases inhibition of futoenone and its derivatives are discussed.

Results

Futoenone and several derivatives have been found to possess moderate inhibitory activities against several matrix metalloproteinases, including stromelysin, interstitial collagenase and 92 kD gelatinase, as shown in Table 1. Notably, while the rigid tricyclic futoenone skeleton is preferred for binding to the PAF receptor [3], the ring-opened, more flexible dihydrobenzofuran derivatives are more active as MMP inhibitors. The presence of a metal-chelating sulfhydryl group in the most potent compound #1148 is important for MMP inhibition; its S-acetyl derivative #1146, which is stable and not hydrolyzed to the sulfhydryl precursor in the assay medium, is less active. The two phenolic analogs, #1149 and #1145, are moderately active. The hydrophobic allyl side-chain in #1145 apparently does not contribute to enzyme inhibition. Addition of one or two more hydrophobic bromo substituents to futoenone, as in #1147 and #1144, does not improve the activity. The scheme for MMP binding to the inhibitor is illustrated in Fig. 1.

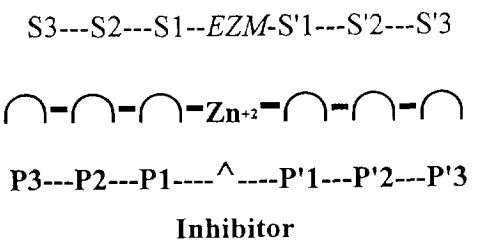


Figure 1. Active Site of MMPs

The P' sites of MMPs are well defined and form a basis for designing tight binding inhibitors. The P1' site forms an important hydrophobic pocket, inhibitors which are peptide mimetics usually have leucine or isoleucine here. The P2' pocket is also hydrophobic, but is more tolerant of different groups such as those belonging to leucine, O-methyl-tyrosine and tryptophane. Compared to the P1' and P2' pockets, the P3' pocket is less defined and appears to fit small amino acids such as alanine. Sites beyond P3' are probably extended into solvent, as implicated in the published truncated x-ray crystal structure for collagenase[4,5]. The P sites are solvent exposed. Inhibitors designed solely for the "P-site surface" are not potent (usually having IC_{50}'s in the μM to mM range). To design a potent inhibitor usually follows groups for selective binding to the P'-sites.

The starting structures for this set of futoenone derivatives is #1136 which is a highly constrained molecule. Compound #1144 was initially modeled and built graphically according to x-ray crystallography and energy minimization data of GM6001. Models of other molecules on the list (Table 1) were constructed by modifying the structure of #1144. The comparisons were made through overlapping and superposition of all the compounds. Compound #1148 was found to contain specific MMP binding activity possibly through P-site binding based on the following observations. All compounds seem to favor binding to stromelysin than other MMPs. This suggests P-site bindings due to the greater hydrophobicity at this site for stromelysin based on sequence comparisons. The sulfhydryl group on #1148 enhances the potency probably through binding to the catalytic zinc. #1136, #1144 and #1150 are highly constrained and unable to obtain the active conformation compared to the structure of #1148 (Fig. 2).

Table 1

% Inhibition of MMP Activities

OAS#	% Inhi. at 10 μM Stromelysin	% Inhi. at 10 μM Collagenase	% Inhi. at 10 μM gelatinase
1136	35	16	0
1144	38	0	0
1145	36	0	0
1146	58	23	8
1147	38	0	22
1148	98 (IC_{50}:0.6 μM)	75	44
1149	34	0	0
1150	42	0	0
GM6001	100 (IC_{50}: 28 nM)	100 (IC_{50}: 2 nM)	100 (IC_{50}: 0.2 nM)

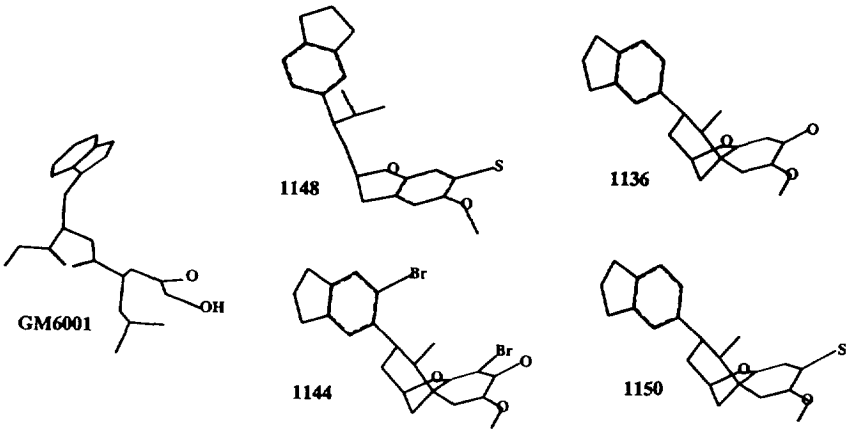


Figure 2. Structure Comparisons

An alternative hypothesis is for this series of compounds to bind within the P' sites of MMP's. Judging from the inhibition data (Table 1), compound #1148 is the only submicromolar inhibitor which also shows reasonable selectivity towards stromelysin. Inhibitors designed towards the P' sites are known to be highly potent, such as GM6001, which is a low nanomolar inhibitor for both collagenase and stromelysin. Fig. 3 shows a comparison of #1148 with GM6001 after energy minimization. The sulfhydryl group is positioned to bind the zinc. The conformation of the molecule is adjusted to simulate the configuration of GM6001. Using a flexible "fit" procedure, by keeping the GM6001 as a rigid structure, #1148 is allowed to change its torsional angle in order to match the structure of GM6001.

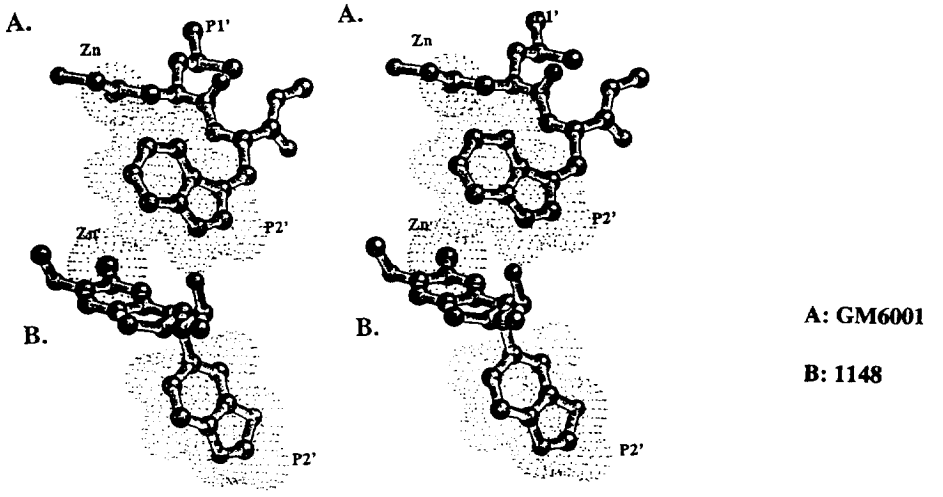


Figure 3. Stereoplot of P-prime Site Binding Comparing 1148 to GM6001

When the two inhibitors are superimposed on top of each other as shown in Fig. 4, it is clear that although the zinc binding groups and P2' groups line up well, #1148 lacks some essential features in GM6001, such as the P1' site binding group and a flexible of hydroxamate group in contrast with a more rigid sulfhydryl group. Although #1148 is only 0.6 μM, it may represent an interesting lead compound for further analogues. It is apparent that #1148 is completely non-peptido and does not resemble other MMP inhibitors already published. Since the x-ray structures of MMPs are known and published, molecular modeling tools can be effectively utilized for optimizing the potency of #1148 analogues. The validity of these analysis remain to be tested by further structure based modifications of #1148 as a lead compound.

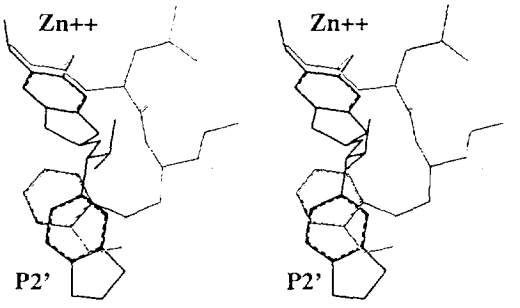


Figure 4. Stereoplot Superposition Comparing GM6001 and 1148

Material and Methods

Metalloproteinase Assays

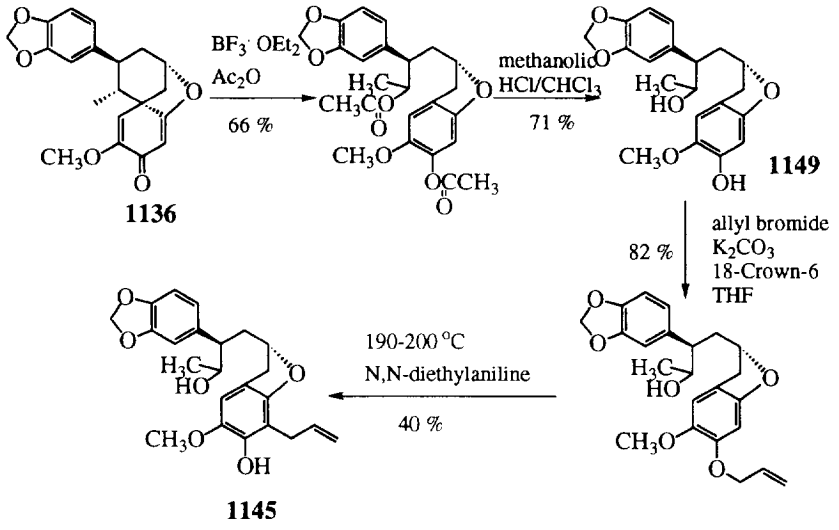
Human recombinant metalloproteinases (stromelysin, interstitial collagenase and 92 kD gelatinase) were purified to nealy homogenous as pro-enzyme form. Metalloproteinase activities were assayed according to Knight[6] with some modifications. Briefly, 50 nM purified recombinant proenzymes (stromelysin, collagenase and gelatinases) were incubated with trypsin (4 ug/ml) in a buffer containing 20 mM Tris-HCl, 5 mM $CaCl_2$ and 0.15 N NaCl (pH 7.5) for 30 minutes at 37 degree. Soybean trypsin inhibitor was added to quench the reaction. Substrate (7-methoxy coumarin 4-yl) acetyl-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2 at 10 uM (SynPep. Corp., Dublin, CA) was then added to the reaction mixture. Incubation was carried out for another 60 minutes. The reaction was terminated by addition of 0.1 N sodium acetate buffer (pH4.0). The hydrolysis of substrate was assessed by fluorescence with a luminescence spectrometer (Perkin Elmer LS-50B, Norwalk, CT) with excitation at 328 nm and emmission at 393 nm.

Chemical Synthesis

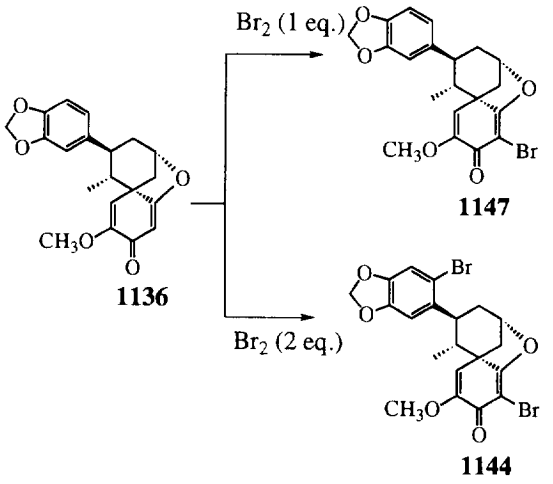
The chemical synthesis of futoenone derivatives[3] are illustrated in the Scheme 1,2 and 3. GM6001 was synthesized according to Grobelny, et al[7].

Molecular Modeling

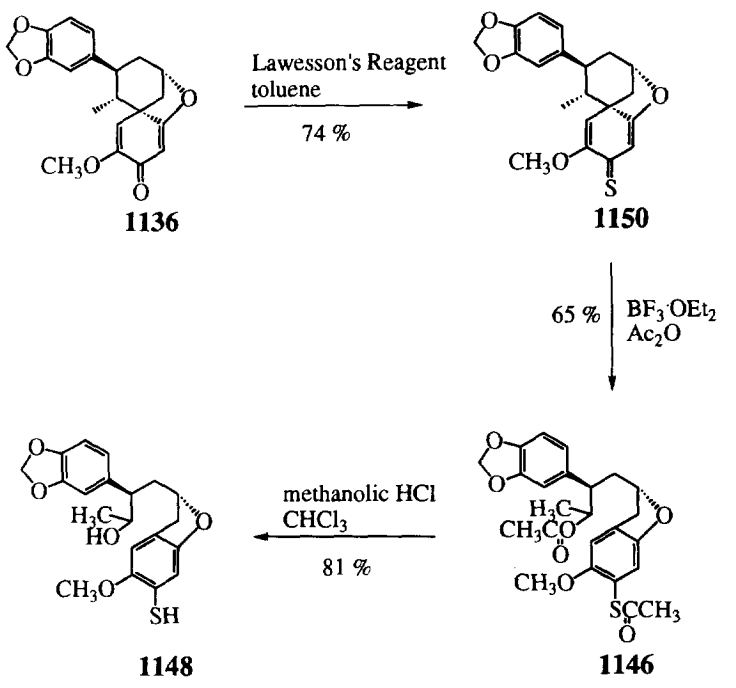
Tripos Software (version 6.0) was used for molecular modeling.



Scheme 1. Modifications of Futoenone



Scheme 2. Bromination of Futoenone



Scheme 3. Preparation of Thiofutoenone Derivatives

Acknowledgement

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