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DESIGN, COMBINATORIAL CHEMICAL SYNTHESIS AND IN VITRO CHARACTERIZATION OF NOVEL UREA BASED GELATINASE INHIBITORS

Yingsheng Zhang, Dongmei Li,[‡] Jon C. Houtman,[‡] Donald T. Witiak, Jo Seltzer,[†] Paul J. Bertics[‡] and Charles T. Lauhon*

Pharmaceutical Science Division, School of Pharmacy, [‡]Department of Biomolecular Chemistry, Medical School, University of Wisconsin-Madison, Madison, WI 53706, U.S.A.
[†]Division of Dermatology, School of Medicine, Washington University, St. Louis, MO 63110, U.S.A.

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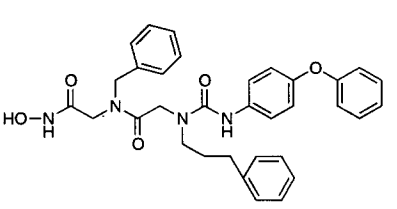
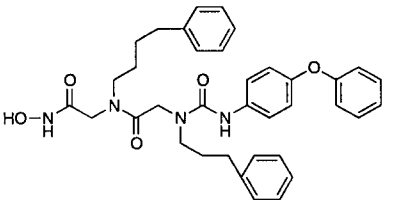
Abstract: A novel series of hydroxamate/urea-based inhibitors of gelatinases has been discovered via solid-phase combinatorial chemistry. SAR of P_1', P_2', and P_3' has been exploited and structures different from traditional succinate-based MMP inhibitors have been found.

The matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases involved in extracellular matrix remodeling and degradation.[1] Gelatinases (MMP-2 and MMP-9) are a subgroup of the MMP family and are shown to be major enzymes involved in the degradation of basement membrane and metastasis.[2] MMP-2 is widely expressed, including fibroblast cells around malignant cancer tissue, and localized in a proteolytically active form on the surface of invasive cells, based on its ability to bind directly integrin $\alpha_v\beta_3$.[3] Increased MMP-2 expression has been detected in many different human tumors, and various synthetic MMP inhibitors have been shown to restrict tumor growth, and thus inhibit the progress of metastasis, in animal models.[4] Poor pharmacokinetics due to the hydrolyzable amide bonds in MMP inhibitors is a potential problem. The P_2'/P_3' amide bond in N-cayboxyalkyl peptide inhibitors, for example, has been reported to be sensitive to enzymatic hydrolysis.[5] Here we report one series of novel hydroxamate/urea-based gelatinase inhibitors as shown in Scheme 1, which was identified on the basis of rational design and solid-phase combinatorial synthesis.



IC_{50} 0.3 μM



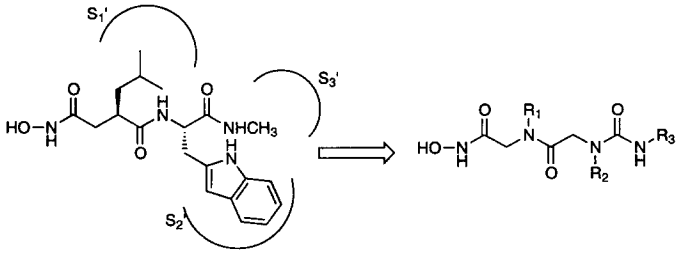
IC_{50} 0.3 μM

Scheme 1

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00498-9

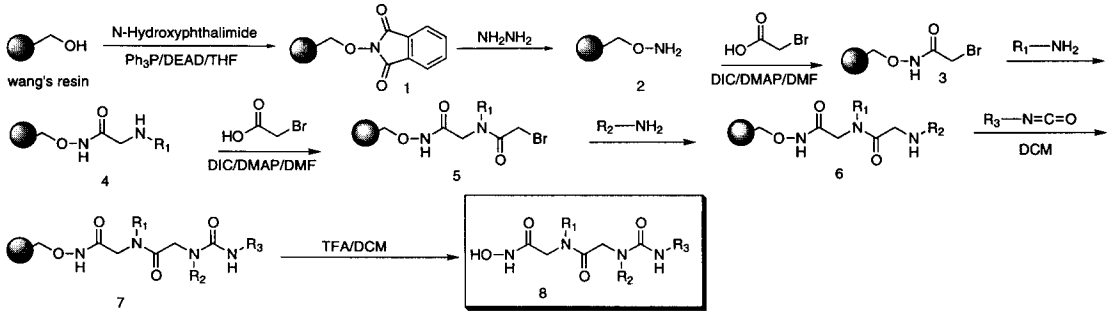
Previous studies with MMPs have explored the S_1' pocket in detail by using an asymmetric synthetic method to install diversified P_1' substituents.[6] The S_2' binding site currently has not contributed to selectivity. However, a bulky P_2' group improves water solubility, bioavailability, as well as activity against TNF-α converting enzyme (TACE).[7] Owing to the limit of the availability of unnatural amino acids, the SAR of P_2' group has not been as well established as for the P_1' group.

A shallow hydrophobic S3′ binding site[8] requires a hydrophobic functionality at P_3' to enhance the binding affinity. However, S3′ is also exposed to solvent[9] and this has made the structural requirements of the P_3' substituent ambiguous. To better explore the SAR of P_2' and P_3' and to eliminate the potentially labile amide bonds, we incorporated urea functionality as a replacement of the P_2'/P_3' amide bond and adopted a peptoid scaffold for our inhibitors (Scheme 2).



Scheme 2

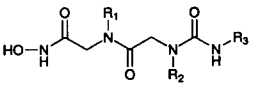
The split-pool method was used to produce and deconvolute our chemical libraries.[10] The preparation of the proposed libraries was carried out as shown in Scheme 3. Departing from general solution-phase MMP inhibitor synthesis, the hydroxamate group was synthesized from a pre-formed hydroxylamine linker. The hydroxyl group in Wang's resin was replaced by an N-hydroxynaphthalimide group, and the naphthyl group is cleaved to produce the hydroxylamine resin **2**. Coupling of α-bromo acetic acid to this resin and replacement of bromide with a set of primary amines afford **4**. Repeating this procedure gives structure **6**. Capture of the secondary amines with a set of isocyanates produces the library **7**, which then can be cleaved from resin to give the target **8**.



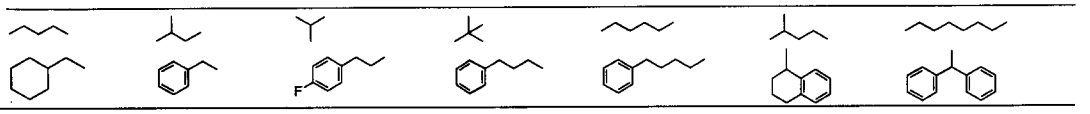
Scheme 3

Fourteen different amines were incorporated as R_1 and R_2 groups and were selected based on previous SAR studies and the characteristics of S_1' and S_2' pockets. Hydrophobic aromatic moieties were chosen for R_3 in order to elucidate structural requirements of the hydrophobic S_3' site. The libraries were assayed against the MMP-2, utilizing a colorimetric substrate in a 96-well plate format.[11] The IC_{50} data is summarized in Tables 1–3. These data clearly show R_3 has a large effect on inhibitory activity against MMP2. The presence of a 4-phenoxyphenyl or naphthyl group is essential in our result for the high potency against gelatinases. The most promising group, 4-;phenoxyphenyl, was selected as R_3 and deconvolution was conducted to evaluate the effect of R_1 and R_2 on inhibitory activity . The results (Table 2 and Table 3) indicate that R_2 group plays a minor role in binding affinity and that aromatic moieties were preferred in this position. The 3-phenyl-1-propyl group gave the most potent activity against MMP2. The effect of R_2 on inhibition is dependent on the presence of aromatic groups at preferred distance while unbranched alkyl chains show greater inhibition over branched ones.

Table 1



R1/R2



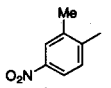
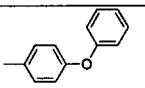
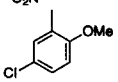
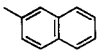
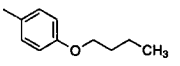
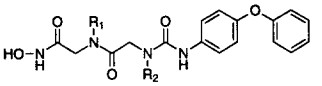
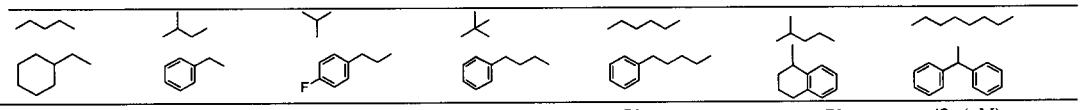
R_3	IC_{50}	R_3	IC_{50}	R_3	IC_{50}	R_3	IC_{50}
	>500		110		90		150
	>500		120		>500		

Table 2



R1



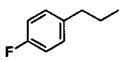
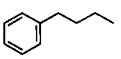
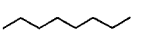
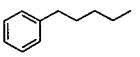
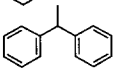
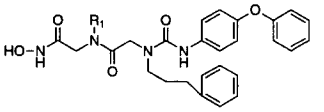
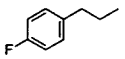
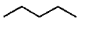
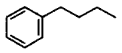
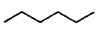
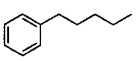
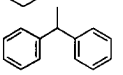
R2	IC_{50} (μM)	R2	IC_{50} (μM)	R2	IC_{50} (μM)	R2	IC_{50}(μM)	R2	IC_{50}(μM)
	51		54		72		155		160
	23		60		150		125		114
	43		52		60		50		

Table 3



R_1	IC_{50} (μM)	R_1	IC_{50} (μM)	R_1	IC_{50} (μM)	R_1	IC_{50}(μM)	R_1	IC_{50}(μM)
	40		230		370		>500		100
	2		100		>500		>500		150
	0.3		0.9		>500		200		

In conclusion, we have discovered a new series of potent (effective at submicromolar concentration) urea-based gelatinase inhibitors. Using the split-pool method, we have begun to elucidate the different structural requirements for this series of inhibitors. In our results, R_3 plays a significant role in the inhibitory activity and the 4-phenoxyphenyl and naphthy groups are promising structures. These results can be used in future MMP inhibitor design.

References and Notes

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