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PROBING THE P3' POCKET OF STROMELYSIN WITH PIPERAZIC ACID ANALOGS

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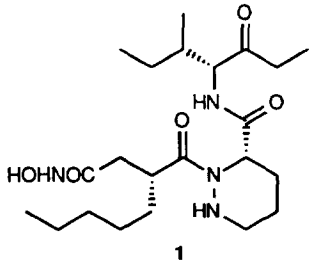
Abstract. The preparation and SAR of several piperazic acid-based stromelysin (MMP-3) inhibitors is presented. The standard P3' methyl amide substituent can be replaced by other carboxy based substituents and maintain good binding affinity. Removal of a hydrogen-bond acceptor results in a 30-fold decrease in activity.

Introduction. Matrix metalloprotienases have received considerable attention recently due to their involvement in several metabolic disorders including rheumatoid and osteoarthritis,[1] and cancer.[2] Several groups have found stromelysin (MMP-3) as an attractive therapeutic target.[3] A recent report revealed the structure of a natural product, matlystatin (**1**), having metalloprotienase inhibition properties.[4] We were especially interested in exploiting the novel piperazic acid moiety found in this molecule. A result of this effort culminated in the preparation of compound **2** which is a potent inhibitor of MMP-3.

Based on molecular modeling studies and X-ray crystallographic information of related analogs, we were able to determine how the different binding motifs of compound **2** interact with the corresponding subsites of the enzyme.[5] The piperazic acid methyl amide establishes two backbone hydrogen-bonds with S3' residues Asp 162 and Tyr 223. The amide carbonyl hydrogen-bonds with a distance of 2.8 Å and the amide NH bonds with a distance of 4.1 Å. We were interested in determining how critical these hydrogen bonds were and if replacing the synthetically unfavorable amide with other functional groups might improve the compound's potency.



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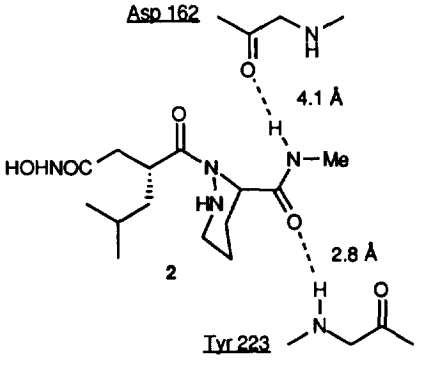


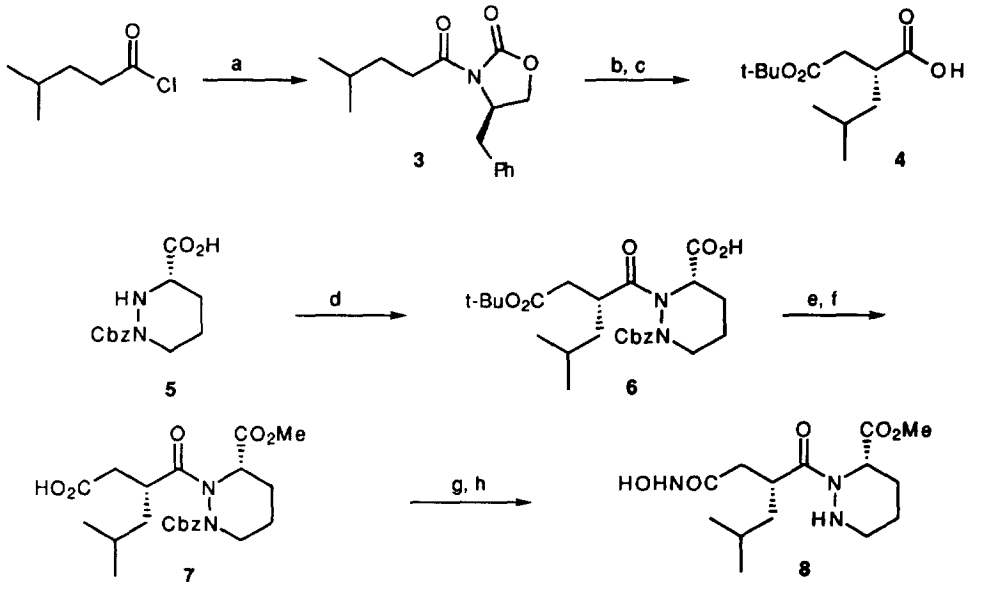
2

Chemistry. Since the piperazic acid parent **5** was known,[6] it provided a convenient starting material for manipulating the functionality at the P3' position. The chemistry used to prepare these analogs is shown in Scheme 1. Using standard Evans based methodology,[7] the desired succinate **4** was obtained in 49% yield as essentially one enantiomer. The acid was converted to the acid chloride using oxallyl chloride and coupled with piperazic acid **5** to give intermediate **6**. Methyl ester formation using diazomethane and subsequent removal of the

tert-butyl ester with 4N HCl in dioxane at rt, gave the desired acid **7** in 54% yield over 2 steps. Dicyclohexylcarbodiimide (DCC) coupling with O-benzylhydroxyl amine followed by protecting group removal gave the desired target **8** in 50% overall yield for the two steps. Compound **8** was a key intermediate for our desired transformations and was used to prepare compounds **14-16** using standard chemical manipulations.

The synthesis of compound **13**, lacking functionality in the P3' position, required the preparation of the parent piperazine. The synthetic sequence is shown in Scheme 2. Bubbling 1,3-butadiene into a THF solution of diethylazodicarboxylate gave the desired Diels-Alder product in 75% yield.[8] Removal of the carboethoxy groups with KOH gave the tertahydropyrazine **9**. Selective mono protection with CbzCl gave the parent piperazine **10**. This was coupled with the acid chloride of **4** using the same protocol detailed in Scheme 1, to give compound **11** in 75% yield. We found the lack of a free carboxylate in the piperazine core improves the yield of the coupling reaction substantially. Conversion of **11** to the final target **13** proceeded smoothly. The final step removes the Cbz-protecting group and the ring double bond in one step, using catalytic hydrogenation.

Scheme 1[a]



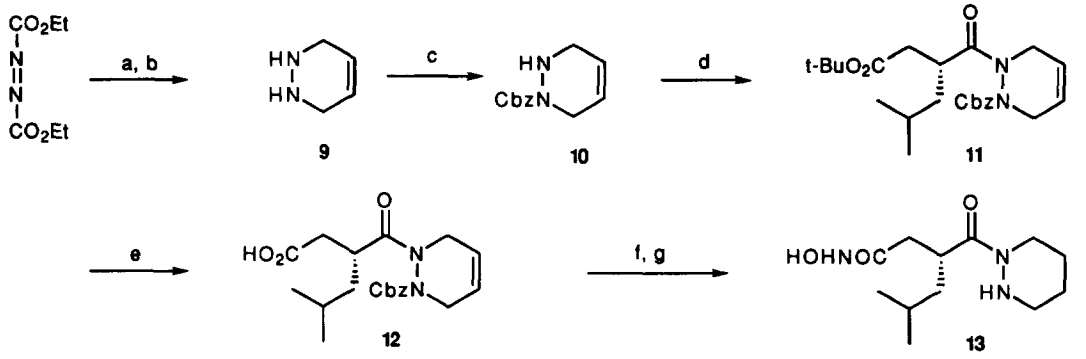
[a]Reagents and conditions. a) ref. 7, 91%; b) LDA, THF, $BrCH_2CO_2t$-Bu, -78°C, 67%; c) H_2O_2, LiOH, THF, 73%; d) the acid chloride of **4**, CH_2Cl_2, rt, 38%; e) CH_2N_2, ether, 63%; f) 4N HCl in dioxane, rt, 85%; g) $BnONH_2$, DCC, DMAP, CH_2Cl_2, rt, 57%; h) H_2, 5% Pd/C, EtOAc, 88%.

Results and Discussion. The compounds of interest were tested in a MMP-3 inhibition assay [9] and the results are shown in Table 1. It was possible to replace the methyl amide with other carboxylic based functionality and retain good binding. Replacing the amide group with a carboxylic acid or methyl ester results in a 2-3 fold loss of activity. Complete removal of the amide functionality shows a thirty-fold decrease in activity (compound **13**).

Retaining a hydrogen-bond donating group in the form of a hydroxyl (compound **15**) cannot compensate for the loss of the carbonyl group and a ten-fold decrease in activity was observed. Capping the hydroxyl group as a methyl ether results in further loss of activity (compound **16**). This loss is greater than compound **13** having no substituent at all. The desolvation energy of the methyl ether upon binding is not compensated for by any hydrogen-bond interactions and may explain the lower binding affinity of compound **16**.

Based on these results, a P3' hydrogen-bond accepting element appears to be critical for high affinity binding. The presence of a hydrogen-bond donating group is less important. Removal of the amide NH does not substantially reduce the compound's activity and the presence of a hydrogen-bond donor is not sufficient for good activity. These results correlate with the modeling studies presented earlier where the amide carbonyl had closer contact with S3' residues. Replacing the amide with a more efficient hydrogen-bond accepting group could improve activity.[10] Since a carboxylic acid is well tolerated at the P3' position, bioisosteres of this functional group could be used to improve both activity and pharmacokinetic parameters.

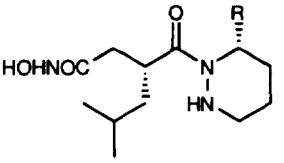
Scheme 2[a]



[a]Reagents and conditions. a) 1,3-Butadiene, THF, rt, 75%; b) KOH, EtOH, reflux, 70%; c) CbzCl, THF, rt, 48%; d) acid chloride of **4**, CH_2Cl_2, NMM, rt, 75%; e) 4N HCl in dioxane, rt, 90%; f) $BnONH_2$, DCC, DMP, CH_2Cl_2, rt, 44%; g) H_2, 5% Pd/C, EtOAc, 58%.

Conclusion. A series of piperazic acid based stromelysin (MMP-3) inhibitors was prepared. It was possible to replace the P3' methyl amide with other hydrogen-bond accepting functionality and maintain good activity. The presence of a hydrogen-bond accepting group is critical for submicromolar potency. More efficient hydrogen-bonding functionality or carboxylic acid bioisosteres could improve activity. A full account detailing the SAR of this class of MMP-3 inhibitors is in progress and will be reported in due course.

Table 1



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
1	CONHMe	0.30	14	CO_2H	0.6
8	CO_2Me	0.81	15	CH_2OH	2.7
13	H	9.67	16	CH_2OMe	20.4

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† Author regarding X-ray crystallography inquiries.

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