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INHIBITORS OF MMP-1: AN EXAMINATION OF P_1' C_α GEM-DISUBSTITUTION IN THE SUCCINAMIDE HYDROXAMATE SERIES

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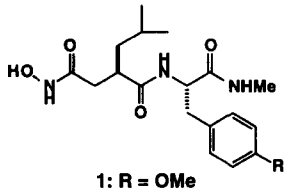
Abstract. The effect of P_1' C_α gem-disubstitution in a series of succinamide hydroxamate inhibitors of MMP-1 has been investigated. While in all cases P_1' gem-disubstitution led to loss of potency relative to the corresponding P_1' isobutyl and phenyl compounds **1** and **3**, respectively, the loss of activity was less pronounced in certain instances, e.g., the P_1' gem-cyclohexyl analogue **12** (IC_{50} = 0.15 μM).

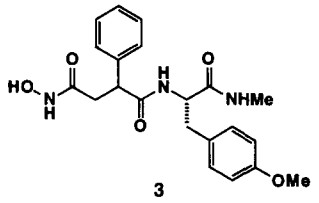
Introduction

Members of the matrix metalloproteinase (MMP) family of zinc-containing enzymes are thought to play major roles in the destruction of articular cartilage during the advancement of osteoarthritis (OA). Interstitial collagenase (MMP-1), for example, is believed to cleave triple-helical type II collagen in cartilage, an irreversible step leading to collagen denaturation, its further degradation by other proteinases and ultimately to the loss of the structural integrity of the tissue. Orally active inhibitors of MMP-1 (and/or other MMPs) are sought as potential drugs for the treatment of OA.[1,2]

Among the several classes of MMP inhibitors, the succinamide hydroxamates (e.g., **1**[3]) have been perhaps the most extensively investigated for the purposes of drug discovery. This is undoubtedly a result of their high degree of intrinsic potency which can be achieved with compounds having relatively low molecular weights. Recently, X-ray crystallographic studies using a truncated form of MMP-1 have revealed how various inhibitors, including the succinamide hydroxamate **2**,[4] bind in the active site. As expected, the hydroxamate function is bound in a bidentate fashion to the catalytic Zn atom and makes important hydrogen bonds to nearby amino acid residues. Another very important interaction involves the P_1' isobutyl side chain which projects into the pronounced hydrophobic S_1' pocket in the enzyme.



1: R = OMe
2: R = H



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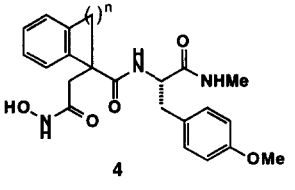

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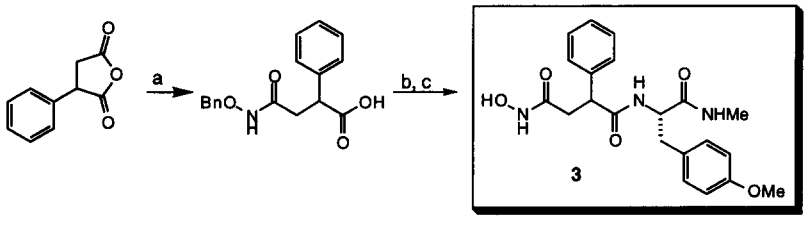
In this paper we report on the synthesis and MMP-1 inhibition activity of a series of P_1' C_α gem-disubstituted succinamide hydroxamates. Our interest in exploring this substitution was based primarily on the potential for increasing oral activity; the newly introduced quaternary center would act as an amide-shielding device similar to a *tert*-butyl group at P_2' which has been reported to increase the oral activity of MMP inhibitors.[2] Of course, the introduction of additional C_α substitution would impart a degree of conformational rigidity which could positively or negatively affect potency. Furthermore, even if proper presentation of the P_1' substituent were to be achieved, there remained the possibility that unfavorable interactions between the additional C_α substituent and the enzyme could result.

Our reference points for the investigation of P_1' C_α disubstitution were **1**[4] and its P_1' phenyl analog **3**. We found the latter compound to retain significant inhibitory activity against MMP-1 (IC_{50} = 40 nM as a 1:1 mixture of diastereomers). The attraction of **3** as a basis for our study was that cyclic analogs of general structure **4** would be readily available. By varying n (general structure **4**) the phenyl group could be displayed in a variety of orientations to maximize the potential for achieving acceptable potency.

Chemistry

The P_1' phenyl analogue **3** was prepared as 1:1 mixture of diastereomers as shown in Scheme 1. The first step gave a separable 2:1 mixture of isomeric O-benzylhydroxamates, the desired isomer being predominant.

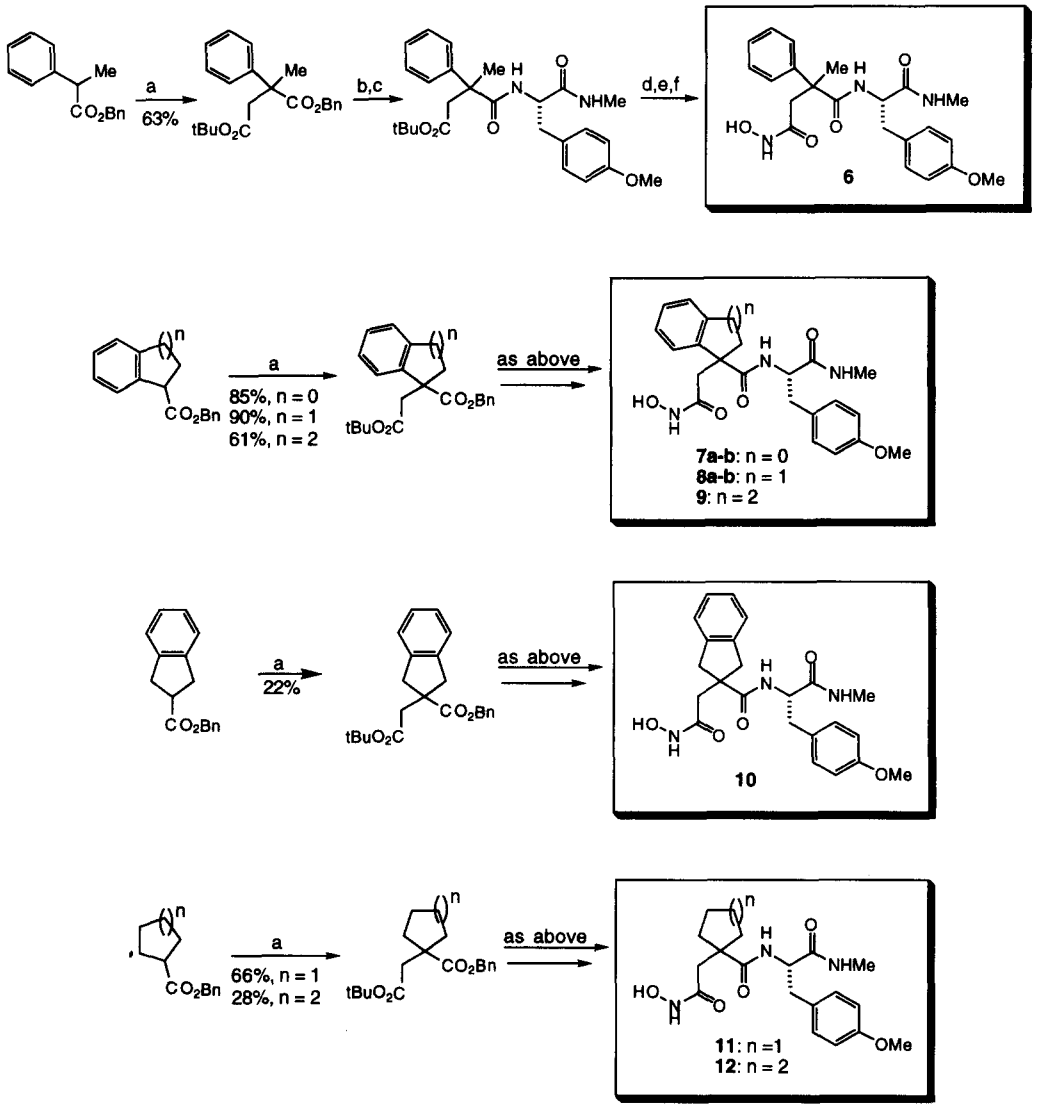
Scheme 1



Reagents and conditions: a) $BnONH_2$·HCl/Et_3N/CH_2Cl_2; b) EtOCOCl/NMM/THF/-20° then H-Tyr(Me)NHMe·HCl/NMM; c) H_2/5%Pd(C)/MeOH

The C_α methyl analog of **3** (compound **6**) was prepared by the route shown in Scheme 2 involving ester enolate alkylation with *tert*-butyl bromoacetate. All cyclic analogs (i.e. compounds **7a-b**, **8a-b**, **9-12**) were prepared by the same sequence starting with the appropriate cycloalkane or benzo-fused cycloalkane carboxylic acid benzyl ester (Scheme 2). In the cases where the ester α proton was also benzylic (i.e. in the syntheses of **6**, **7a-b**, **8a-b** and **9**), the yields of the benzyl *tert*-butyl succinate diesters from the enolate alkylation step were good to excellent (61-90%). The range of yields was markedly lower (22-66%) for the enolate alkylations in which the ester α proton was not also benzylic (i.e., in the syntheses of **10-12**).

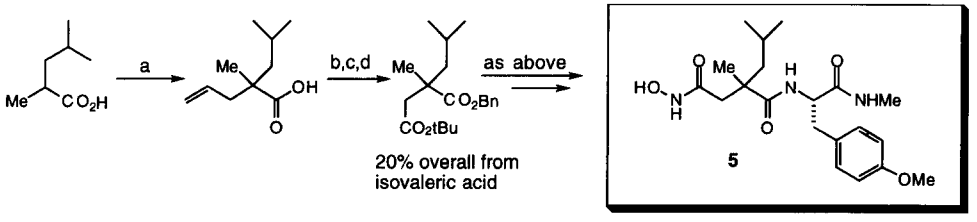
Scheme 2



Reagents and conditions: a) LDA/THF/-78° then $BrCH_2CO_2tBu$/-78°; b) H_2/10% Pd(C)/EtOH; c) H-Tyr(Me)NHMe·HCl/DEC/HOBT/Et_3N/CH_2Cl_2; d) TFA/CH_2Cl_2; e) $BnONH_2$·HCl/BOP/Et_3N/CH_2Cl_2; f) H_2/5% Pd(C)/MeOH

In an attempted synthesis of the C_α methyl analog of **1** (compound **5**), ester enolate alkylation failed altogether. In this case, C-C bond formation was successfully carried out by carboxylate dianion alkylation with allyl bromide (Scheme 3).

Scheme 3



Reagents and conditions: a) LDA (2.1 equiv.)/THF/-78° then allyl bromide/-78° to -20°;
b) BnBr/Cs_2CO_3/MeCN; c)$NaIO_4$/$RuCl_3$/CCl_4/MeCN/H_2O; d) $(tBuO)_2(Me_2N)CH$/benzene/reflux

In two of the instances where a chiral center was created during the alkylation step (i.e. in the syntheses of **7a-b** and **8a-b**), chromatographic separation of the diastereomers arising after the coupling to the Tyr(Me)NHMe residue was possible allowing separate advancement to the diastereomerically pure hydroxamates.

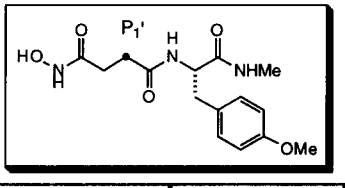
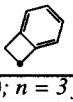


Table 1

Compound[5]	P_1'	IC_{50} (μM)[6] MMP-1[a]	Compound	P_1'	IC_{50} (μM) MMP-1[a]
1		0.0019 (0.00053)	**8a**[c]		17.5[d]
3[b]		0.040 (0.017)	**8b**[c]		>30
5[b]		0.46 (0.21)[7]	**9**[b]		0.77 (0.25)
6[b]		3.8 (0.87)	**10**		0.57 (0.35)
7a[c]		0.80 (0.36)	**11**		2.8 (1.9)
7b[c]		2.2 (1.2)	**12**		0.15 (0.087)

a) Avg. IC_{50}s: μM (s.d.); n = 3 for all compounds unless otherwise indicated; b) 1:1 mixture of diastereomers; c) Single diastereomer, unassigned stereochemistry; d) Avg. of 2 determinations; the third gave IC_{50} > 30 μM.

Results and Discussion

As can be seen by comparing the relative potencies of **1** and **3** with **5**[7] and **6** respectively (Table 1), introduction of a P_1' C_α methyl substituent in the succinamide hydroxamate series was associated with about a 100-fold loss of activity (one diastereomer of **5** presumed essentially inactive). Conformational restriction of the phenyl ring in **3** by formation of a benzo-fused gem-cycloalkane ring (compounds **7a,b**, **8a,b** and **9**) gave, in some cases, increased activity over the acyclic P_1' C_α methyl compound **6** (e.g., **9**: MMP-1 IC_{50} = 0.77 μM) although activities approaching those of **1** or **3** were not attained. Interestingly, **8a** and **8b**, the benzo-fused gem-cyclopentane (1-indanyl) analogues of **3**, were appreciably less active against MMP-1 than the corresponding benzo-fused gem-cyclobutyl and gem-cyclohexyl compounds. Apparently conformation rather than purely the size of the cycloalkyl ring determines activity in this series. Reasoning that analogs with simple gem-cycloalkyl or benzo-fused gem-cycloalkyl having a homobenzylic quaternary center were likely to exhibit further changes in conformation, compounds **10-12** were prepared. Relative to **8a,b** and the gem-cyclopentyl analogue **11** (IC_{50} = 2.8 μM), the benzo-fused cyclopentyl (2-indanyl) analogue **10** showed markedly improved activity (IC_{50} = 0.57 μM) indicating both the presence and position of the aromatic ring to be important for binding. Of the two simple gem-cycloalkyl analogues, the gem-cyclohexyl compound **12** gave the highest activity (IC_{50} = 0.15 μM). Indeed, **12** was the most potent of all the analogues prepared in the P_1' C_α gem-disubstituted succinamide hydroxamate series. While being about 80-fold less active than **1**, **12** was about 20 times more potent than the corresponding gem-cyclopentyl compound **11**. This result suggests that **12** can adopt a conformation more suitable for binding possibly allowing the larger cycloalkyl ring of **12** to project more deeply into the S_1' pocket of the enzyme to gain additional binding energy by making more extensive hydrophobic interactions.

Conclusion

While P_1' C_α gem-disubstitution is in all cases detrimental to the inhibition of MMP-1 in the succinamide hydroxamate series, the degree to which potency is lost is dependent on the nature of the P_1' group. By comparing the activities of **6** and **5** with those of **9** (or **7a**) and **12** respectively, conformational restriction by formation of a geminal ring at P_1' appears to improve activity relative to the corresponding acyclic geminally disubstituted analogues. Our finding that P_1' C_α gem-cyclohexyl substitution gives compounds having greater activity relative to other P_1' gem-disubstituted succinamide hydroxamates, together with a similar result in a series of glutaramide carboxylate MMP-1 inhibitors,[8] suggests that this substitution may warrant further investigation in the design of novel inhibitors of MMP-1. With the current availability of X-ray structural information on members of the matrix metalloproteinase family (including MMP-1), the guided design of more potent P_1' C_α gem-disubstituted succinamide hydroxamates may be possible, e.g., by determining the optimal substitution of the gem-cyclohexyl ring for maximal interaction with the S_1' pocket of the enzyme.

Acknowledgment

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Footnotes and References

1) Morphy, J. R.; Millican, T. A.; Porter, J. R *Current Med. Chem.* **1995**, *2*, 743.
2) Beckett, R. P.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Whittaker, M. *Drug Discovery Today* **1996**, *1*, 16.
3) Dickens, J. P.; Donald, D. K.; Kneen, G.; McCay, W. R. U.S. Patent 4,599,361.
4) Spurlino, J. C.; Smallwood, A. M.; Carlton, D. D.; Banks, T. M.; Vavra, K. J.; Johnson, J. S.; Cook, E. R.; Falvo, J.; Wahl, R. C.; Pulvino, T. A.; Wendoloski, J. J.; Smith, D. L. *Proteins: Struct. Funct. Genet.* **1994**, *19*, 98.Dickens, J. P.; Donald, D. K.; Kneen, G.; McCay, W. R. U.S. Patent 4,599,361.
5) All compounds reported in Table 1 were determined to be pure by ^{1}H NMR and elemental analysis.
6) Enzyme assay: MMP-1 was activated with trypsin and assayed using a quenched fluorescent peptide substrate: Bickett, D. M.; Green, M. D.; Berman, J.; Dezube, M.; Howe, A. S.; Brown, P. J.; Roth, J. T.; McGeehan, G. M. *Anal. Biochem.* **1993**, *212*, 58.
7) Compound **5** (one diastereomer with stereochemistry corresponding to **1**) was recently reported to inhibit MMP-1 with K_i = 0.63 μM: Ghose, A. K.; Logan, M. E.; Treasurywala, A. M.; Wang, H.; Wahl, R. C.; Tomczuk, B. E.; Gowravaram, M. R.; Jaegar, E. P.; Wendoloski, J. J. *J. Am. Chem. Soc.* **1995**, *117*, 4671.
8) Robinson, R. P.; Cronin, B. J.; Donahue, K. M.; Jones, B. P.; Lopresti-Morrow, L. L.; Mitchell, P. G.; Rizzi, J. P.; Reeves, L. M.; Yocum, S. A. following paper in this issue.

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