Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2087–2092

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE DESIGN, SYNTHESIS, AND STRUCTURE–ACTIVITY RELATIONSHIPS OF A SERIES OF MACROCYCLIC MMP INHIBITORS

Douglas H. Steinman,* Michael L. Curtin, Robert B. Garland, Steven K. Davidsen, H. Robin Heyman, James H. Holms, Daniel H. Albert, Terry J. Magoc, Ildiko B. Nagy, Patrick A. Marcotte, Junling Li, Douglas W. Morgan, Charles Hutchins, and James B. Summers

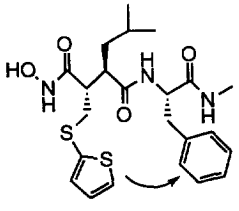
Cancer Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064, U.S.A.

Received 3 March 1998; accepted 29 June 1998

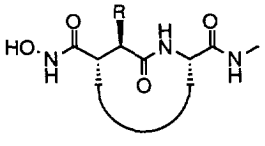
Abstract: A series of succinate-derived hydroxamic acids incorporating a macrocyclic ring were designed, synthesized, and evaluated as inhibitors of matrix metalloproteinases. The inhibitors were designed based on the published X-ray crystal structure of batimastat (**1**) complexed with human neutrophil collagenase (MMP-8). The synthesized compounds were shown to inhibit selected MMPs in vitro with low nanomolar potency.

The matrix metalloproteinases (MMPs) are a family of zinc dependent proteolytic enzymes which are responsible for the degradation and remodeling of the extracellular matrix.[1] Various MMPs have been implicated in joint destruction seen in osteoarthritis and rheumatoid arthritis, development of periodontal disease and growth and metastatic spread of various cancers.[2] A variety of succinate-derived hydroxamic acids have been reported as both broad spectrum and sub-type selective MMP inhibitors.[3] Some of these inhibitors are currently being evaluated in clinical studies.[4] As part of our research efforts in the cancer area we investigated novel succinate-derived hydroxamic acids as broad spectrum inhibitors of MMPs.

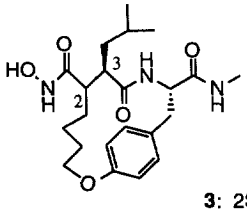
Structural studies, both by NMR and X-ray crystallography, of various MMPs have been published.[5] One such study describes the X-ray crystal structure of batimistat (**1**) when complexed with the active site of human neutrophil collagenase.[6] As expected, the hydroxamate group was coordinated with the zinc atom of the active site. It was also apparent that the phenyl ring of the phenylalanine moiety and the thiophene ring of the side chain at the position adjacent to the hydroxamic acid extend away from the active site and were in proximity to one another. Accordingly, it seemed possible that a cyclic structure connecting these two side-chain positions as shown in structure (**2**) would not perturb the backbone conformation. Such a cyclic compound could benefit from the conformational restriction placed on the molecule and potentially provide a compound with better potency. In addition, the incorporation of a macrocyclic ring into a peptide-mimetic structure can lead to improvements in pharmacokinetics via a reduction in metabolic processing.[7] Researchers at DuPont Merck have recently described the results of their work based on the same concept.[8]



batimastat, **1**



proposed cyclic structure, **2**



3: 2S
4: 2R

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PII: S0960-894X(98)00396-5

Molecular modeling studies which supported this design concept were carried out using InsightII.[9] Batimastat (**1**) was modeled in the active site of neutrophil collagenase (MMP-8) and minimized for 100 cycles of conjugate gradient minimization using the CFF95 forcefield and Discover. The minimized inhibitor was used to build a model of compound **3** and its 2*R* epimer, **4**, in the active site of the enzyme. These were then minimized for 100 steps of conjugate gradient energy minimization, followed by 1 psec of molecular dynamics. The average structures from the dynamics were then subjected to 20 steps of minimization. Comparison of the minimized structures (Figure 1) shows that compound **3** adopts a conformation which allows it to remain tightly bound in the active site and predicts compound **3** should have potency comparable to batimastat (**1**). The epimeric compound **4** however, resides further away from the protein. This substantially weakens the zinc binding of the hydroxamate portion of the inhibitor and moves the P_1' isobutyl group somewhat out of the S_1' pocket. The combination of altered hydrogen bonds of the hydroxamate and the shallower position of the P_1' substituent predicts that compound **4** should be a significantly weaker MMP inhibitor than either compound **1** or **3**.

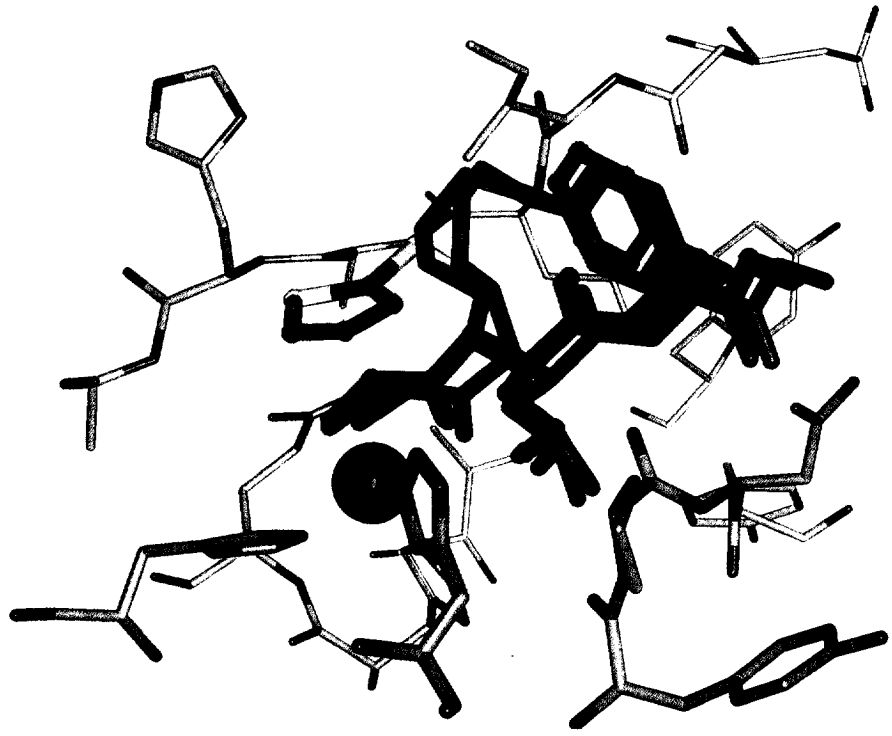
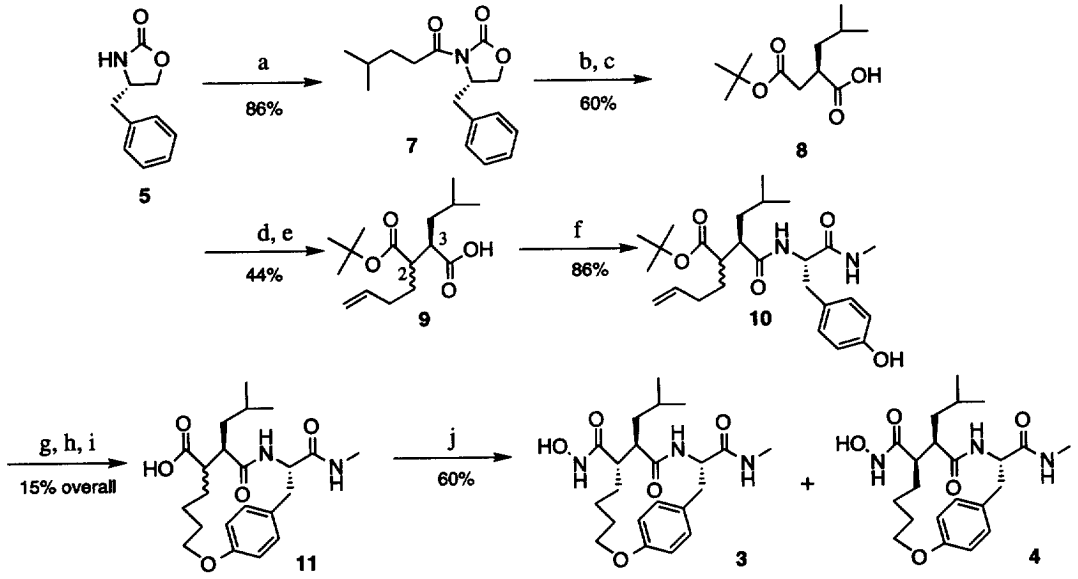


Figure 1. Model of batimastat **1**, compound **3** and compound **4** complexed with neutrophil collagenase (MMP-8) green: batimastat **1**, blue: compound **3**, purple: compound **4**

The initial strategy chosen for the synthesis is shown in Scheme 1. Known succinate **8**[10] was alkylated with 1-bromo-4-butene and equilibrated to a 1:1 mixture of the 2*R* and 2*S* diastereomers (**9**), which was coupled with L-tyrosine-*N*-methyl amide to give **10**. Hydroboration with oxidative workup was followed by treatment of

the phenolic alcohol under Mitsunobu conditions and the cyclized product was hydrolyzed to carboxylic acid **11**. To our knowledge, there is only one report in the literature of the formation of cyclic ethers larger than seven membered via the Mitsunobu reaction.[11] By performing the reaction at a concentration not greater than 0.05 M and using at least a 6:1 mixture of benzene to THF with 150 mol percent tri-*n*-butylphosphine and 150 mol percent 1,1′-(azodicarbonyl)dipiperidine, yields of the cyclic ether exceeding 70% were obtained. Cyclized products were characterized by a diagnostic shift in the ^{1}H NMR observed for one proton of the aliphatic methylenes in the macrocyclic compound **4**. A significant upfield shift of about 0.5 ppm to δ −0.05 was observed. A similar shift has been reported previously for a related macrocyclic hydroxamic acid MMP inhibitor.[12] Conversion to the hydroxamic acid and separation of the diastereomers by reverse-phase HPLC gave **3** and **4**.

Scheme 1



Reagents and conditions: (a) *n*-BuLi, THF, -78 °C; $(CH_3)_2CH(CH_2)_2COCl$, **6**; (b) $NaN[Si(CH_3)_3]_2$, THF; $BrCH_2CO_2C(CH_3)_3$; (c) LiOH, H_2O_2, THF; (d) LDA, DMPU, THF, -78 °C; $Br(CH_2)_2CH{=}CH_2$; LiI; (e) LDA, THF, -78 °C; CH_3OH; (f) 1-hydroxybenzotriazole (HOBt), DMF, 0 °C; 4-methylmorpholine (NMM); 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC); L-Tyr-NHMe; (g) BH_3•THF, THF; H_2O_2, pH 7; (h) Ph_3P, diethylazodicarboxylate; (i) trifluoroacetic acid, CH_2Cl_2, 0 °C; (j) NMM, DMF, 0 °C; HOBt; EDC; O-(*t*-butyldimethylsilyl)hydroxylamine.

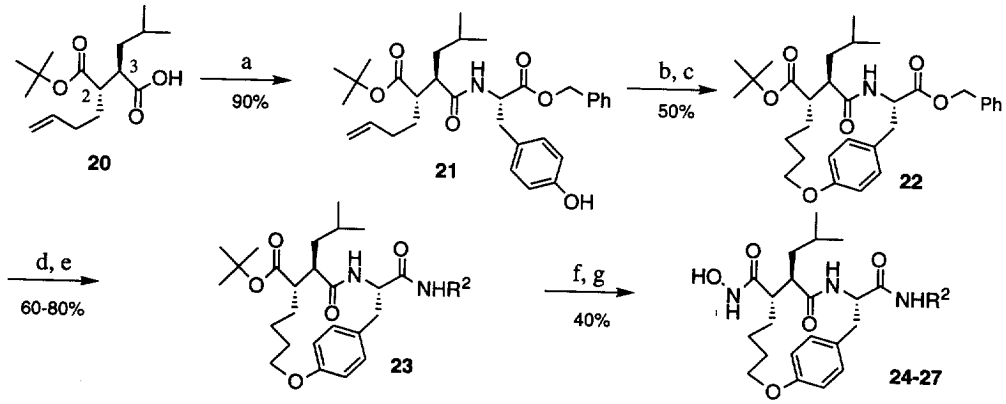
We began an exploration of the SAR of the series by synthesizing compounds with modifications in the P1′ substituent (compounds **12–14**) and evaluating their in vitro inhibition of fibroblast collagenase (MMP-1), gelatinase A (MMP-2), matrilysin (MMP-7), and stromelysin (MMP-3) as seen in Table 1.[13] These compounds were available by replacing **6** with the appropriate acid chloride and completing the synthesis as described for compounds **3** and **4**. Yields and reaction conditions were similar to those reported in Scheme 1.[14]

The effect of variation in the macrocyclic ring size on MMP inhibition was also evaluated. Substitution of 1-bromo-4-butene with the appropriate alkenyl halide for the alkylation of carboxylate **8**, and completion of the

synthesis as shown in Scheme 1 led to compounds **16**, **18**, and **19**. It was noted that the yield of the Mitsunobu cyclization reaction was lower in the case of the synthesis of both **16** and **19**.

Variations in the substituent at the C-terminus were accessible using the synthesis shown in Scheme 2. Separation of the diastereomers of **9** by flash chromatography followed by coupling of the 2*S* diastereomer **20** with L-tyrosine benzyl ester gave **21**. Hydroboration with oxidative workup followed by application of the optimized Mitsunobu conditions gave **22**; conversion to the carboxylic acid and coupling with the appropriate amine gave compound **23**. Hydrolysis of the *t*-butyl ester and conversion of the carboxylic acid to the hydroxamic acid gave compounds **24–27**.

Scheme 2



Reagents and conditions: (a) 1-hydroxybenzotriazole (HOBt), DMF, 0 °C; 4-methylmorpholine (NMM); 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC); L-Tyr-OCH_2Ph; (b) BH_3•THF, THF, 0 °C; H_2O_2, pH 7; (c) Bu_3P, 1,1'-(azodicarbonyl)dipiperidine, THF, benzene; (d) H_2, Pd/C, CH_3OH; (e) HOBt, DMF, 0 °C; NMM; EDC; R^2NH_2; (f) trifluoroacetic acid, CH_2Cl_2, 0 °C; (g) HOBt, DMF, 0 °C; NMM; EDC; O-(*t* butyldimethylsilyl)hydroxylamine.

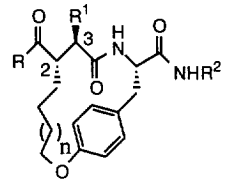
As predicted by molecular modeling, compound **4** (2*R* stereochemistry) is significantly less active than compound **3** (2*S* stereochemistry), which is in agreement with what has been reported previously for related acyclic MMP inhibitors.[6] Also consistent with the SAR reported for acyclic MMP inhibitors of comparable structure was the inferior potency of the macrocyclic carboxylic acids relative to the corresponding hydroxamic acids (**15** vs. **16** and **17** vs. **18**). Although there are reports in the literature of potent MMP inhibitors which are carboxylic acids, the most potent succinate-derived inhibitors are hydroxamic acids.[15]

Differences in the size and shape of the S_1' pocket of the enzymes are known to exist from published reports of the X-ray crystal structures of several MMPs.[16] Consequently, selective inhibition of MMPs can be achieved by varying the size of the P_1' substituent.[17] Fibroblast collagenase and matrilysin have a shallow pocket compared to that of stromelysin and gelatinase A and thus are relatively intolerant of large P_1' substituents. This is due to the presence at the bottom of the S_1' pocket of a large amino acid group (arginine for fibroblast collagenase and tyrosine for matrilysin) which is replaced by leucine in stromelysin and gelatinase A.[16] As shown in Table 1, changes in the P_1' substituent of the macrocycle produced compounds which selectively inhibit stromelysin and gelatinase A in some instances (compounds **13** and **14**) but not in others (compound **12**).

Further investigation of the SAR showed that MMP inhibition was independent of the size of the macrocycle. It seemed clear from modeling studies that the macrocycle projected away from the enzyme binding site. However, it was expected that contracting or expanding the ring would alter the positions of the side chains such that the binding affinity for the enzyme might be affected. That this is not the case is demonstrated by comparing the inhibition potency of compounds **3**, **16**, **18**, and **19**.

The substitution of polar groups for the methyl amide at the C-terminus reportedly affects both the in vitro and in vivo potency of acyclic succinate-derived hydroxamic acid MMP inhibitors.[18] A compound with a terminus that could function as both a hydrogen bond donor and acceptor (compound **24**), one which could act as a hydrogen bond acceptor (compound **25**), and two compounds that could be neither (compounds **26** and **27**) were evaluated. As Table 1 shows, none of these substituents had a significant effect on in vitro MMP inhibition although compound **24** was slightly less potent than most of the hydroxamic acids as an inhibitor of matrilysin.

Table 1. In vitro Inhibition of Selected MMPs by Macrocycles



Compd	n	R	R^1	R^2	IC_{50} (nM) MMP-1	MMP-2	MMP-7	MMP-3
3	1	NHOH	*iso*-butyl	Me	1.8	2.2	1.6	3.9
4[a]	1	NHOH	*iso*-butyl	Me	110	230	440	570
12	1	NHOH	$(CH_2)_3Ph(_4\text{-Me})$	Me	6.1	0.8	2.8	9.1
13	1	NHOH	$(CH_2)_2Ph(CH_2)_2Me$	Me	54	0.1	5.9	1.5
14	1	NHOH	$(CH_2)_4OCH_2Ph$	Me	210	0.3	180	1.8
15	0	OH	*iso*-butyl	Me	>10000	14000	900	2100
16	0	NHOH	*iso*-butyl	Me	3.0	2.6	3.0	7.8
17	2	OH	*iso*-butyl	Me	1400	1400	110	75
18	2	NHOH	*iso*-butyl	Me	2.1	2.3	2.6	6.1
19	3	NHOH	*iso*-butyl	Me	2.2	5.9	5.7	12
24	1	NHOH	*iso*-butyl	$(CH_2)_2Ph(4\text{-}SO_2NH_2)$	3.0	1.9	25	6.9
25	1	NHOH	*iso*-butyl	2-pyridyl	4.6	2.1	1.6	2.6
26	1	NHOH	*iso*-butyl	CH_2CH_2SMe	2.7	3.8	2.5	4.2
27	1	NHOH	*iso*-butyl	$CH_2CH_2NMe_2$	6.6	6.5	4.2	12

[a]Stereochemistry at C-2 is *R*.

In conclusion, we have shown that succinate-derived hydroxamic acids which incorporate a macrocyclic ring between the position adjacent to the hydroxamic acid and P_2' are, as predicted by molecular modeling, potent inhibitors of selected MMPs. The use of a Mitsunobu reaction as the key step in the synthesis allowed us to easily alter the substituents at various positions in the lead structure, **3**. The results reported here are consistent with those reported previously for acyclic MMP inhibitors.

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