Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1157–1162

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

MALONYL α-MERCAPTOKETONES AND α-MERCAPTOALCOHOLS, A NEW CLASS OF MATRIX METALLOPROTEINASE INHIBITORS

David A. Campbell,*[a] Xiao-Yi Xiao,[a] David Harris,[a] Satoru Ida,[a] Reza Mortezaei,[a] Khehyong Ngu,[a] Lihong Shi,[a] David Tien,[a] Yongwen Wang,[a] Marc Navre,[a] Dinesh V. Patel,[a] Michele A. Sharr,[b] John F. DiJoseph,[b] Loran M. Killar,[b] Christina L. Leone,[b] Jeremy I. Levin,[c] and Jerauld S. Skotnicki*[c]

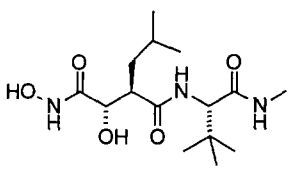
[a]*Affymax Research Institute, 3410 Central Expressway, Santa Clara, CA 95051, U.S.A.*
[b]*Wyeth-Ayerst Research, Princeton, NJ 08543, U.S.A.*
[c]*Wyeth-Ayerst Research, Pearl River, New York 10965, U.S.A.*

Received 28 January 1998; accepted 2 April 1998

Abstract: A novel series of matrix metalloproteinase (MMP) inhibitors is described. Incorporation of a terminal α-mercaptoketone or α-mercaptoalcohol in the zinc binding domain of a series of inhibitors led to compounds exhibiting low nanomolar activity against collagenase-1 (MMP-1), stromelysin (MMP-3), and gelatinase-B (MMP-9).

The matrix metalloproteinases (MMP's) are a family of zinc dependent enzymes involved in the degradation and remodeling of the extracellular matrix.[1] They are important therapeutic targets with indications in cancer,[2] arthritis,[3] auto-immunity,[4] and cardiovascular disease.[5] Recent observations that certain MMP inhibitors can inhibit the metalloproteinase mediated cleavage and release of soluble cytokine receptors,[6] as well as several growth factors,[7] and the shedding of L-selectin during leukocyte migration,[8] demonstrate additional applications for metalloproteinase inhibitors. As a consequence of these diverse biological roles, MMP inhibitors are expected to provide therapeutic agents for a number of disease states, as well as assist in delineating some of the biological activities of metalloproteinases in general.

Figure 1



1 (Marimastat; **BB-2516**)

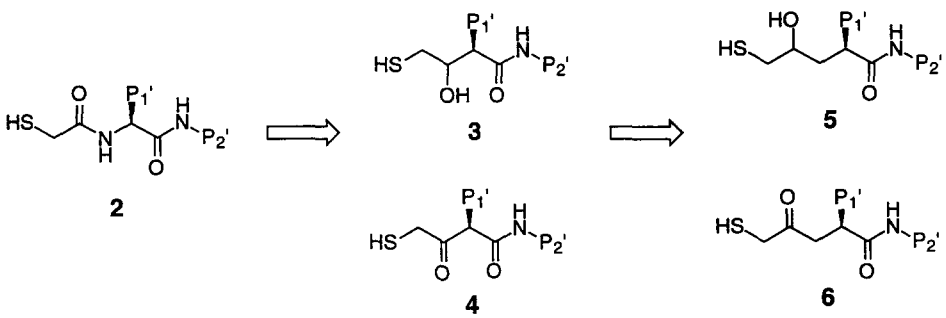
Structural features common to most MMP inhibitors include a ligand that interacts with the catalytic site zinc metal and a peptidic fragment that binds to a subset of the enzyme's specificity pockets.[9] The most commonly used zinc ligands are hydroxamates, carboxylates, and thiols. Marimastat (**BB-2516**) which has recently entered Phase III studies for evaluation as a cancer treatment exemplifies the peptidyl-hydroxamate class of inhibitors and is the furthest advanced catalytic site MMP inhibitor to date.

0960-894X/98/$19.00
PII: S0960-894X(98)00185-1

Our strategy was to design inhibitors that utilize thiols as replacements for the more commonly investigated hydroxamic acid zinc ligands (Figure 2). Since initiating this work, mercaptan based MMP inhibitors have gained increased recognition following Chiroscience's reports of oral efficacy in animal models of arthritis with mercaptoacyl based compounds.[10]

Initially, we investigated α-mercaptoacetamides **2** but some members of this series displayed a time dependent loss of inhibition that was not due to oxidation of the mercaptan, but was most likely a result of proteolysis of the acetamide bond. Removal of the scissile amide bond by deletion of the amino group resulted in the malonyl alcohols **3** and ketones **4**, while replacement of the amide nitrogen with a methylene group yielded the succinyl alcohols **5** and ketones **6**. Ketone **4** is reminiscent of a series of β-mercapto carboxylate inhibitors that have been previously described that have the carbonyl and mercaptan groups transposed.[11] In this paper we describe the synthesis and SAR for the malonyl series of MMP inhibitors; the succinyl mercaptoalcohols and mercaptoketones are described in the following paper.

Figure 2



Chemistry

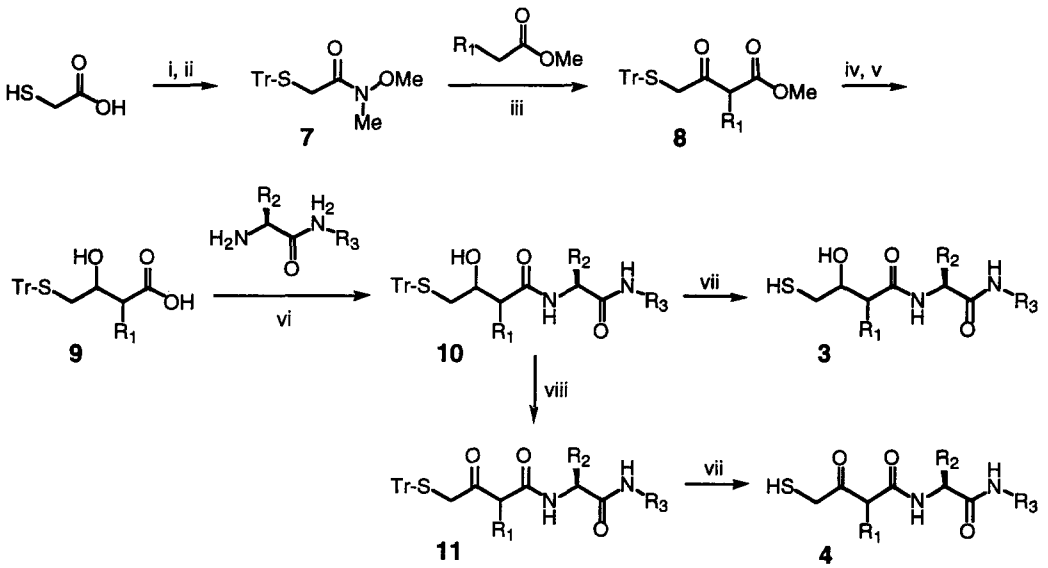
The inhibitors were synthesized as mixtures of diastereomers as shown in Scheme 1. Commercially available α-mercaptoacetic acid was first converted to the S-trityl-Weinreb amide **7**, then treated with a methyl ester enolate to produce the β-keto ester **8**. In order to avoid the facile decarboxylation of β-keto acids, ketone **8** was first reduced to the alcohol followed by saponification of the methyl ester to yield **9**. Standard peptide couplings of **9** with a variety of amino acid derivatives produced the intermediate **10**. Detritylation of **10** yielded the desired mercaptoalcohol **3** directly. The mercaptoketone **4** was obtained from **10** via oxidation to the ketone **11** with Dess-Martin periodinane followed by deprotection.

Results and Discussion

The effects of modifying the P1'-P3' substituents on inhibitory potency against collagenase-1 (MMP-1), stromelysin (MMP-3), and gelatinase-B (MMP-9) in both the mercaptoalcohol **3** and mercaptoketone **4** inhibitor series were investigated and the results are summarized in Tables 1 and 2, respectively.[12] In general, the mercaptoketone inhibitors are more active against all of the MMP's than their mercaptoalcohol analogues. The

mercaptoketones are more active against gelatinase-B than collagenase-1 and stromelysin, with the most active inhibitor **4e** exhibiting a picomolar IC_{50} against MMP-9.

Scheme 1

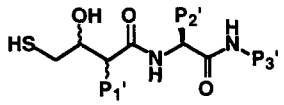


Reagents and conditions: (i) trityl alcohol, TFA; (ii) HNMe(OMe), EDC, HOBt, DMF; (iii) LDA, THF, -78° C; (iv) $NaBH_4$, MeOH, 0° C; (v) KOH, H_2O, MeOH; (vi) EDC, HOBt, DIEA, DMF; (vii) TFA, triethylsilane, DCM; (viii) Dess Martin periodinane, pyridine, DCM.

The SAR that is observed for these inhibitors versus gelatinase-B and stromelysin is in agreement with the SAR that has been described for hydroxamic acid based inhibitors.[13] In general, the most potent inhibitors of gelatinase-B and stromelysin, both of which have deep S1' pockets, incorporate a heptyl group at P1'. Further support for this observation is obtained by the direct comparison of inhibitors that differ only in their P1' substituent. Compounds that contain a heptyl group are more active than those that contain an isobutyl group against both stromelysin (**3a**, **4a**, **4c**, and **4d** versus **3d**, **4e**, **4k**, and **4i**, respectively) and gelatinase-B (**4a** versus **4e**). Mercaptoketone inhibitors that replaced the P1' heptyl group with a phenylpropyl group also exhibited a loss in stromelysin binding affinity (**4i** and **4k** versus **4p** and **4o**, respectively). Overall, these results are consistent with structural analyses indicating that the S1' pocket is the primary determinant of binding affinity for MMP's and that modification of P1' has a substantial effect on inhibitor potency and selectivity profiles.

Although a direct comparison between these inhibitors, in particular ketones **4**, and the β-mercapto carboxylate inhibitors previously described in the literature[11] is not possible, both series give rise to low nanomolar MMP-1 inhibitors. In order to account for the potent inhibition that was observed for the β-mercapto carboxylates the authors proposed either a bidentate interaction between the active site zinc and the mercaptan and carboxylate carbonyl, or a model in which the carboxylate contributes to binding affinity by forming a hydrogen bond in the active site. At this time we are unable to differentiate between the potential binding modes.

Table 1. *In vitro* activity of mercaptoalcohols.

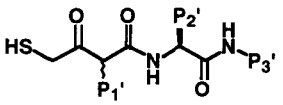


Cpd	P_1'	P_2'	P_3'	IC_{50}'s (nM)*		
				MMP-1	MMP-3	MMP-9
3a	i-butyl	t-butyl	methyl	380	>20K	NT
3b	i-butyl	t-butyl	2-pyridyl	890	4600	4500
3c	i-butyl	CHM	phenethyl	NT	1300	NT
3d	n-heptyl	t-butyl	methyl	NT	120	NT
3e	n-heptyl	t-butyl	$(CH_2)_2Ph(p\text{-}SO_2NH_2)$	NT	120	NT
3f	n-heptyl	i-butyl	phenethyl	>20K	1500	NT
3g	n-heptyl	i-butyl	$(CH_2)_2$morpholino	>10K	5100	640
3h	n-heptyl	i-butyl	Leu(N-ethyl)	NT	210	NT
3i	n-heptyl	CHM	$(CH_2)_2$morpholino	>20K	4100	NT
3j	n-heptyl	CHM	$(CH_2)_2Ph(p\text{-}SO_2NH_2)$	1700	290	NT
3k	n-heptyl	$(CH_2)_4NHTs$	Leu(N-ethyl)	>10K	110	780
3l	phenpropyl	i-butyl	Leu(N-ethyl)	NT	140	NT
3m	phenpropyl	CHM	phenethyl	NT	>2K	NT

***The synthesis produces diastereomeric mixture combinations. The most active constitutional isomer(s) is listed in the table (stereochemistry not determined).**
NT = not tested
CHM = cyclohexylmethyl

Collagenase-1, with a shallower S1' subsite resulting from extension of the Arg_{214} residue into the binding pocket,[14] would be expected to prefer the shorter isobutyl group at P1'. Indeed, compounds incorporating an isobutyl group were amongst the most potent mercaptoalcohol (**3a** and **3b**) and mercaptoketone (**4a** and **4b**) collagenase-1 inhibitors. However, this is complicated by the fact that compound **4e**, possessing a P1' heptyl group, exhibits collagenase-1 inhibition that is comparable to **4a**. This observation is analogous to reports describing unexpectedly potent hydroxamic acid collagenase-1 inhibitors that incorporate extended phenolic ethers[15] and alkyl groups[13] at P1'. Whether these exceptions are the result of conformational changes in the S1' pocket or a different mode of binding for these inhibitors is yet to be determined.

Table 2. *In vitro* activity of mercaptoketones.



Cpd	P_1'	P_2'	P_3'	IC_{50}'s (nM)		
				MMP-1	MMP-3	MMP-9
4a	i-butyl	t-butyl	methyl	14	500	6
4b	i-butyl	t-butyl	2-pyridyl	69	160	19
4c	i-butyl	CHM	phenethyl	NT	98	NT
4d	i-butyl	i-butyl	Leu(N-ethyl)	NT	860	NT
4e	n-heptyl	t-butyl	methyl	15	16	0.3
4f	n-heptyl	t-butyl	$(CH_2)_2Ph(p\text{-}SO_2NH_2)$	200	22	NT
4g	n-heptyl	i-butyl	phenethyl	>20K	39	5
4h	n-heptyl	i-butyl	$(CH_2)_2$morpholino	1300	130	6
4i	n-heptyl	i-butyl	Leu(N-ethyl)	>10K	26	30
4j	n-heptyl	i-butyl	Ph	NT	11	NT
4k	n-heptyl	CHM	phenethyl	1500	88	1.4
4l	n-heptyl	CHM	$(CH_2)_2Ph(p\text{-}SO_2NH_2)$	430	43	4
4m	n-heptyl	$(CH_2)_4NHTs$	Leu(N-ethyl)	NT	13	NT
4n	n-heptyl	n-Bu	Leu(N-ethyl)	NT	28	NT
4o	phenpropyl	CHM	phenethyl	NT	210	NT
4p	phenpropyl	i-butyl	Leu(N-ethyl)	NT	140	NT

NT = not tested
CHM = cyclohexylmethyl

Variations at P2' or P3' have a significantly reduced effect on IC_{50} values compared with changes at P1' for all the MMP's, typically ≤20-fold, with most variations between 2- to 5-fold. The one exception to this trend is compound **4e** which has a 10^2 to 10^3 higher binding affinity for collagenase-1 than compounds **4g**, **4h**, **4i**, and **4k**, while exhibiting binding affinity differences of <10-fold against stromelysin. However, the collagenase-1 disparity is most likely due to differences in binding of the heptyl group in the S1' subsite, as described above, rather than significant changes in P2'/P3' binding interactions.

Conclusion

A series of inhibitors have been prepared that incorporate novel malonyl mercaptoalcohols or mercaptoketones as zinc ligands in place of hydroxamic acid zinc-chelators, while retaining inhibitor potency against a panel of MMP's. This now provides an alternative zinc ligand for the design of matrix metalloproteinase inhibitors in the search for more bioavailable therapeutic agents. The *in vivo* activity of these compounds will be discussed in due course.

References

1. Birkedal-Hansen, H. *Curr. Opin. Cell Biology* **1995**, *7*, 728. (b) Matrisian, L. M. *Trends Genet.* **1990**, *6*, 121. (c) Giannelli, G.; Falk-Marzillier, J.; Schiraldi, O.; Stetler-Stevenson, W. G.; Quaranta, V. *Science*, **1997**, *277*, 225.
2. Coussens, L. M.; Werb, Z. *Chemistry & Biology* **1996**, *3*, 895. (b) MacDougall, J. R.; Matrisian, L. M. *Cancer and Metastasis Reviews* **1995**, *14*, 351.
3. Murphy, G.; Hembry, R. M. *J. Rheum.* **1992**, *19*, 61.
4. Goetzl, E. J.; Banda, M. J.; Leppert, D. *J. Immunol.* **1996**, *156*, 1.
5. Dollery, C. M.; McEwan, J. R.; Henney, A. M.. *Circ. Res.* **1995**, *77*, 863.
6. Arribas, J.; Coodly, L.; Vollmer, P.; Kishimoto, T. K.; Rose-John, S.; Massague, J. *J. Biol. Chem.* **1996**, *271*, 11376.
7. Kayagaki, N.; Kawasaki, A.; Ebata, T.; Ohmoto, H.; Ikeda, S.; Inoue, S.; Yoshino, K.; Okumura, K.; Yagita, H. *J. Exp. Med.* **1995**, *182*, 1777.
8. Preece, G.; Murphy, G.; Ager, A. *J. Biol. Chem.* **1996**, *271*, 11634.
9. Beckett, R. P.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Whittaker, M. *Drug Discovery Today* **1996**, *1*, 16. (b) Zask, A.; Levin, J. I.; Killar, L. M.; Skotnicki, J. S. *Curr. Pharm. Design* **1996**, *2*, 624.
10. Baxter, A. D.; Bird, J.; Bhogal, R.; Massil, T.; Minton, K. J.; Montana, J.; Owen, D. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 897.
11. Beszant, B.; Bird, J.; Gaster, L. M.; Harper, G. P.; Hughes, I.; Karran, E. H.; Markwell, R. E.; Miles-Williams, A. J.; Smith, S. A. *J. Med. Chem.* **1993**, *36*, 4030.
12. *In vitro* assay was performed as described in: Knight, C. G.; Willenbrock, F.; Murphy, G. *FEBS Letters* **1992**, *296*, 263.
13. Miller, A.; Askew, M.; Beckett, R. P.; Bellamy, C. L.; Bone, E. A.; Coates, R. E.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Martin, F. M.; Saroglou, L.; Thompson, A. J.; van Dijk, S. E.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 193.
14. Browner, M. F.; Smith, W. W.; Castelhano, A. L. *Biochemistry* **1995**, *34*, 6602.
15. Tomczuk, B. E.; Gowravaram, M. R.; Johnson, J. S.; Delecki, D.; Cook, E. R.; Ghose, A. K.; Mathiowetz, A. M.; Spurlino, J. C.; Rubin, B.; Smith, D. L.; Pulvino, T.; Wahl, R. C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 343. (b) Wahl, R. C.; Pulvino, T. A.; Mathiowetz, A. M.; Ghose, A. K.; Johnson, J. S.; Delecki, D.; Cook, E. R.; Gainor, J. A.; Gowravaram, M. R.; Tomczuk, B. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 349.