Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1009–1013

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Heterocycle-Based MMP Inhibitors with P2′ Substituents

Stanislaw Pikul,* Kelly McDow Dunham, Neil G. Almstead, Biswanath De, Michael G. Natchus, Yetunde O. Taiwo, Lisa E. Williams, Barbara A. Hynd, Lily C. Hsieh, Michael J. Janusz, Fei Gu and Glen E. Mieling

Procter and Gamble Pharmaceuticals, Health Care Research Center, 8700 Mason-Montgomery Road, Mason, OH 45040, USA

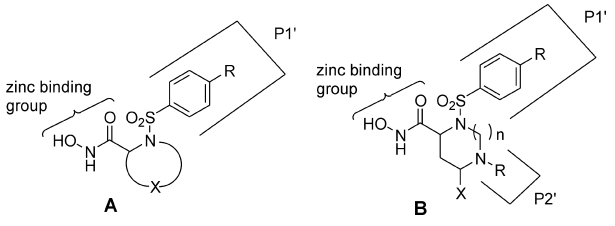
Received 27 July 2000; accepted 16 February 2001

Abstract—Potent and selective inhibition of matrix metalloproteinases was demonstrated for a series of sulfonamide-based hydroxamic acids. The design of the heterocyclic sulfonamides incorporates a six- or seven-member central ring with a P2′ substituent that can be modified. Binding interactions of this substituent at the S2′ site are believed to contribute to high inhibitory potency against stromelysin, collagenase-3 and gelatinases A and B, and to provide selectivity against collagenase-1 and matrilysin. An X-ray structure of a stromelysin–inhibitor complex was obtained to provide insights into the SAR and selectivity trends observed for the series.

Matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that are capable of degrading many proteinaceous components of the extracellular matrix.[1] Under normal physiological conditions, the proteolytic activities of the enzymes are controlled by tissue inhibitors of matrix metalloproteinases (TIMPs).[2] In pathological conditions this balance is shifted towards overactivation of MMPs leading to excessive degradation of the matrix components. Excessive MMP activity has been implicated in numerous disease states involving matrix degradation, which include arthritis,[3] cancer,[4] and periodontal diseases.[5] Consequently, there has been a significant interest in developing MMP inhibitors that may control the aberrant activity of MMP production.[6]

The Design Concept of Constrained Inhibitors

Earlier work at P&GP[7,8] and other laboratories had demonstrated that a central, heterocyclic ring could be effectively used in the design of potent MMP inhibitors (see structure **A** below). For many of these inhibitors the zinc binding group (ZBG) and the P1′ substituent appear to be primarily responsible for the binding interactions with the enzymatic target. We believed that an additional ring substituent capable of interacting with the S2′ binding site could provide an opportunity to modify not only the potency but also the selectivity of such inhibitors against various MMPs. Since inhibition of collagenase-1 (MMP-1) may be responsible for the musculoskeletal side effects observed clinically with broad-spectrum inhibitors, we were especially interested in molecules that would spare this enzyme. Modification of the P2′ substituent could provide such selectivity due to the hydrophilic nature of the S2′ site of MMP-1 containing Ser-222 as compared to the hydrophobic nature of our target enzymes: stromelysin (MMP-3) and collagenaase-3 (MMP-13), which contain Leu-222 and Ile-222, respectively. Similar effects were expected for matrilysin (MMP-7), which has a hydrophilic threonine in the 222 position. In an effort to provide a heterocyclic template capable of additional P2′ binding interactions, we designed a new series of MMP inhibitors based on a 1,5-piperazine/diazepine framework as represented by structure **B**. In this paper, we wish to report our early SAR work that produced a series of potent inhibitors with a unique range of selectivities for various MMPs.



*Corresponding author at current address: Avalon Pharmaceuticals, 19 Firstfield Road, Gaithersburg, MD 20878, USA. Fax: +301-556-1100; e-mail: spikul@avalonrx.com

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00137-8

Chemistry

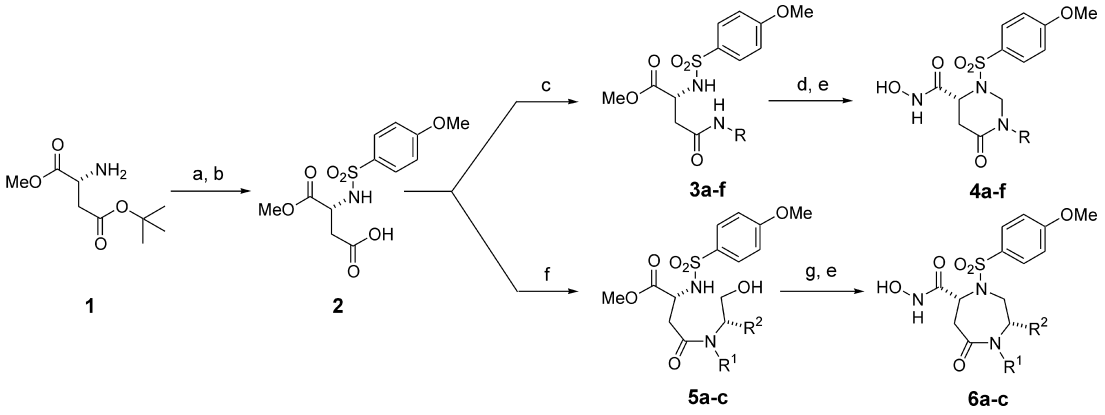
Compounds discussed below were prepared using synthetic routes shown in Schemes 1 and 2. An orthogonally protected D-aspartic acid **1** served as a common starting material for the synthesis of the 6-oxohexahydropyrimidines **4** and the 7-oxo-[1,4]diazepine **6**. First, the free amino group of **1** was derivatized using 4-methoxybenzene sulfonyl chloride, and then the *tert*-butyl ester was cleaved with trifluoroacetic acid. The resulting carboxylic acid **2** was converted to amide **3** using the requisite amine component in the EDAC mediated coupling reaction. Compounds **3a–f**, upon treatment with trioxane and catalytic amounts of sulfuric acid in methylene chloride, cyclized cleanly to form the 6-oxohexahydropyrimidine ring. Exposure of the resulting intermediates to hydroxylamine in basic methanol provided the target hydroxamic acids **4**[10] in good overall yield.[11,12]

Alternatively, carboxylic acid **2** was coupled with an appropriate aminoalcohol derived from the corresponding L-amino acid to form hydroxysulfonamide **5**. Formation of the desired seven-member ring was cleanly accomplished via a Mitsunobu reaction. The intermediate methyl esters were converted into their corresponding hydroxamic acids[10] using standard conditions as described above.

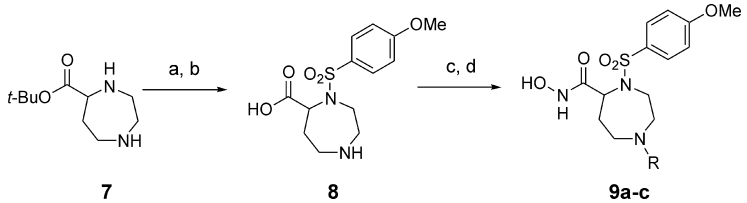
A known racemic [1,4]diazepane **7**[13] was used as the starting material in the synthesis of compounds **9a–c** (Scheme 2). The two amino groups of **7** were differentially functionalized in a two-step, one-pot sequence. First, the distal amino group was selectively protected using Boc_2O and then the proximal amino group was derivatized using methoxybenzene sulfonyl chloride. The resulting intermediate was treated with trifluoroacetic acid to produce the key intermediate amino acid **8**. The P2′ substituent was introduced by acylation or sulfonylation of the amino group under standard Schotten–Baumann conditions. Finally, the carboxylic group was converted into the hydroxamic functionality through intermediate formation of the corresponding acid chloride, which, upon treatment with hydroxylamine, provided the target inhibitor **9**.[10]

Results and Discussion

All compounds were tested for the inhibition of various MMP enzymes including collagenases-1, -2, and -3 (MMP-1, -8, and -13, respectively), gelatinases A and B (MMP-2 and -9, respectively), stromelysin (MMP-3) and matrilysin (MMP-7) and the data are summarized in Tables 1 and 2. First, we explored a series of 6-oxohexahydropyrimidines containing a six-member central ring. We chose this ring size after observing potent inhibition of MMPs in a related series of achiral compounds.[7] Compound **4a** was prepared to test the intrinsic inhibitory activity associated with its novel ring structure. Its inhibitory activity against MMP-13 and MMP-2 was found to be comparable to that observed for the reference compound **CGS27023A**. However, it was interesting to see a significant improvement in selectivity against MMP-1 and MMP-7. Next, the *N*-methyl group of **4a** was replaced by increasingly bulky alkyl substituents (compounds **4b–d**) in order to improve contact with the S2′ pocket. As expected, we observed progressively more potent inhibition of all tested enzymes with the most significant response from



Scheme 1. Reagents and conditions: (a) $MeOC_6H_4SO_2Cl$, Et_3N, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) RNH_2, EDAC, HOBT, DMF; (d) trioxane, H_2SO_4, CH_2Cl_2; (e) NH_2OH, KOH, MeOH; (f) $R^1NHCHR^2CH_2OH$, EDAC, HOBT, DMF; (g) DEAD, Ph_3P, CH_2Cl_2.



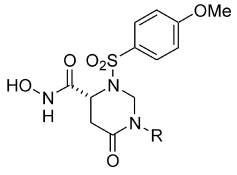
Scheme 2. Reagents and conditions: (a) Boc_2O, NaOH, dioxane–water, then Et_3N followed by $MeOC_6H_4SO_2Cl$; (b) TFA, CH_2Cl_2; (c) CbzCl, Et_3N, dioxane–water or $ArSO_2Cl$, Et_3N, dioxane–water; (d) $(COCl)_2$, DMF, CH_2Cl_2, then Et_3N followed by NH_2OH.

stromelysin. Compound **4d** containing a *tert*-butyl P2′ group was found to have 1 nM potency for MMP-2, -3, -8, -9, and subnanomolar potency for MMP-13. At the same time the inhibition of MMPs-1 and -7 was not highly affected, producing a considerable improvement in selectivity (e.g., MMP-1/MMP-3 selectivity is 5 for **4a** vs 246 for **4d**; MMP-1/MMP-13 selectivity is 278 for **4a** vs 941 for **4d**). Introduction of the benzyl group in **4e** brought a noticeable improvement in inhibition of MMP-1, while inhibition of MMP-7 decreased markedly. This result and significantly lower overall potency of **4f**, containing P2′ substituent with a heteroatom, pointed to relatively low tolerance for structural variation of the P2′ substituent.

Next, we turned our attention to inhibitors containing a [1,4]diazepine central ring and prepared compound **6a**. It was interesting to notice that while inhibition of MMP-13 was not affected, the inhibition of MMP-1 and MMP-7 increased significantly compared to the six-member homologue **4b**. A marked improvement in potency against MMP-7 was also observed for compound **9b** with an *N*-benzyl substituent, while the inhibition of other MMPs was only slightly improved. The broad-spectrum character of these seven-member ring-based inhibitors was confirmed by compound **6c** derived from L-prolinol. Potent inhibition of all MMP enzymes observed for these seven-member ring-based molecules was in stark contrast to the high selectivity displayed by the six-member ring-based analogues.

To investigate the effect of increased flexibility of the central ring on inhibitory potency we decided to remove the ring oxo substituent. The distal amino group was then functionalized to form carbamate **9a** or sulfonamides **9b** and **9c**. Interestingly, all three analogues showed increased inhibition of MMP-1 and decreased inhibition of MMP-7. The inhibition of other enzymes

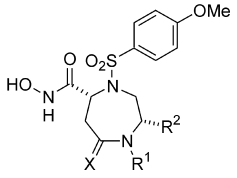
Table 1. SAR of 6-oxohexahydropyrimidines



Compound	R	IC_{50} (nM)[14]						
		MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13[a]
4a	Me	2200	8.8	404	>10,000	47.3	24.1	7.9
4b	*iso*-Pr	643	5.3	5.3	3460	6.3	4.2	2.9
4c	*cyclo*-Hex	465	0.7	3.5	776	2.8	3.3	0.75
4d	*tert*-Bu	320	1.0	1.3	909	1.5	1.1	0.34
4e	CH_2Ph	22.2	1.0	3.1	>10,000	6.4	6.8	2.56
4f	CH_2CH_2OMe	696	3.4	180	>10,000	102	52.5	nd
CGS27023A		49.5	9.1	16.9	106	4.4	4.3	4.3

[a]Data obtained using rat MMP-13; nd, not determined.

Table 2. SAR of [1,4]diazepanes



Compound	R^1	R^2	X	IC_{50} (nM)[14]						
				MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13[a]
6a	*iso*-Pr	H	O	22	2.4	6.5	369	3.7	4.1	1.9
6b	CH_2Ph	H	O	13.6	0.68	3.3	88	1.5	1.0	nd
6c	$CH_2CH_2CH_2$		O	1.2	5.2	3.4	nd	nd	nd	2.0
9a[b]	Cbz	H	H,H	97	1.6	14	737	3.3	1.8	2.1
9b[b]	$SO_2C_6H_4OMe$	H	H,H	28.9	0.5	6.3	905	1.8	0.5	0.84
9c[b]		H	H,H	346	2.7	39.2	2780	6.6	5.5	2.4

[a]Data obtained using rat MMP-13.
[b]Racemic material; nd, not determined.

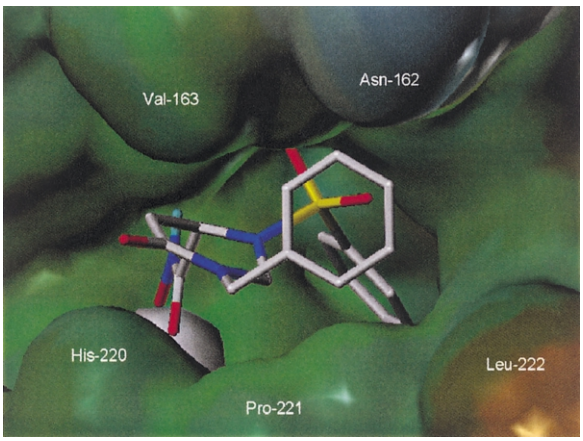


Figure 1. X-ray structure of a complex of **4e** with truncated stromelysin.

was not significantly altered except for somewhat weaker inhibition of MMP-2 and MMP-3 by compound **9c**.

X-ray Crystal Structure

In order to better understand the interactions of this new series of MMP inhibitors with stromelysin we have obtained X-ray crystal data of compound **4e** with truncated stromelysin (Fig. 1).[15] All interactions involving the hydroxamic acid group, the P1′ methoxyphenyl group and the sulfonamide system linking them were found to be similar to those previously described.[16] The 6-oxohexahydropyrimidine ring in its half-chair conformation provided the desired conformation placing the hydroxamic acid in the pseudo-axial position and the sulfone group in pseudo-equatorial position. The ring oxo group was found to point towards the solvent and did not develop any characteristic interactions with the enzyme. As intended in the original design of this series of MMP inhibitors, the alkyl group attached to the distal nitrogen atom of the hexahydropyrimidine ring was found to extend towards the S2′ pocket. This explains the increased potency and selectivity observed for six-member ring-based inhibitors with substituents rigidly attached to the ring (compounds **4a**–**d**). The benzylic phenyl group of **4e** developed good van der Waals contacts (within 3 Å) with Leu-222 of stromelysin. Flexibility of the benzyl group may allow alternative conformations supported by favorable interactions with neighboring amino acid residues (e.g., Pro-221 or Val-163). This arrangement could also save **4e** from unfavorable interactions with the S2′ pocket of MMP-1, which may help explain the relatively low selectivity of this inhibitor. Similar reasoning can be used to explain the very good inhibitory potency, but fairly low selectivity observed for the [1,4]diazepine-based series. In this case, different ring size and conformational flexibility allow the P2′ substituents to orient themselves away from the S2′ pocket if necessary. The unusual inhibitory profile of **4f** containing a flexible methoxyethyl substituent is difficult to explain and may be attributed to the presence of a heteroatom in the P2′ position. For some reasons, such a presence does not seem to be well tolerated by selected MMPs. This provides additional evidence that specific geometry requirements must be met by MMP inhibitors if high selectivity is to be achieved through modulation of interactions at the S2′ site.

Conclusion

A new series of MMP inhibitors was designed based on a scaffold containing a central ring of six-member hexahydropyrimidines or seven-member [1,4]diazepines. The distal nitrogen present in both ring systems was used to introduce the P2′ substituent which is believed to influence inhibitory selectivity through interactions with the S2′ pocket. Compounds with hexahydropyrimidine ring with short and rigid alkyl P2′ substituents were found to be highly potent against MMP-2, -3, -8, -9 and -13, and very selective against MMP-1 and -7 (e.g., MMP-1/MMP-13 IC_{50} ratio was found to be >200-fold). A considerable loss of selectivity was observed for compounds containing a seven-member [1,4]diazepine ring. An X-ray structure of a stromelysin-inhibitor complex confirmed the predicted arrangement of the inhibitor in the enzyme active site and helped rationalize the observed SAR.

Acknowledgements

The authors wish to thank a number of P&GP scientists associated with the MMP project whose hard work and dedication over many years have contributed towards the completion of this work.

References and Notes

1. Woessner, J. F., Jr. *FASEB* **1991**, *5*, 2145.
2. Matrisian, L. M. *Trends Genet*. **1990**, *6*, 121.
3. (a) Firestein, G. S.; Paine, M. M.; Littman, B. H. *Arthritis Rheum*. **1991**, *34*, 1094. (b) Walakovits, L. A.; Bhardwaj, N.; Gallick, G. S.; Lark, M. W. *Arthritis Rheum*. **1992**, *35*, 35.
4. Pyke, C.; Ralfkiaer, E.; Huhtala, P.; Hurskeinen, T.; Dano, K.; Tryggvason, K. *Cancer Res*. **1992**, *52*, 1336.
5. Overall, C. M.; Wiebkin, O. W.; Thonard, J. C. *J. Periodontal Res*. **1987**, *22*, 81.
6. (a) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev*. **1999**, *99*, 2735. (b) Greenwald, R. A.; Zucker, S.; Golub, L. M., Eds. *Ann. N.Y. Acad. Sci*. **1999**, 87.
7. Pikul, S.; McDow Dunham, K. L.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. B.; McPhail, S. J.; Snider, C. E.; Taiwo, Y. O.; Rydel, T.; Dunaway, C. M.; Gu, F.; Meiling, G. E. *J. Med. Chem*. **1998**, *41*, 3568.
8. Almstead, N. G.; Bradley, R. S.; Pikul, S.; De, B.; Natchus, M. G.; Taiwo, Y. O.; Gu, F.; Williams, L. E.; Hynd, B. A.; Janusz, M. J.; Dunaway, C. M.; Meiling, G. E. *J. Med. Chem*. **1999**, *42*, 4547.
9. Levin, J. I.; DiJoseph, J. F.; Killar, L. M.; Sung, A.; Walter, T.; Sharr, M. A.; Roth, C. E.; Skotnicki, J. S.; Albright, J. D. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2657.

10. Satisfactory analytical data (^{1}H NMR, ^{13}C NMR, MS, EA and/or HRMS) were obtained for all target hydroxamic acids described in this paper.
11. Compounds analogous to **4** with R = H and aryloxyphenylsulfonyl group in the P1′ position were also disclosed by Agouron: Bender, S. L.; Melnick, M. J. US Patent 5753653, 1998; *Chem. Abstr*. **1998**, 324822.
12. Although we have no direct data on the optical purity of compounds **4a**–**f**, we assume little racemization occurred during their synthesis as we observed no sign of formation of the second possible diastereomer during the synthesis of compounds **6a**–**c**.
13. Barraclough, P.; Farrant, R. D.; Kettle, D.; Smith, S. *J. Chem. Res., Synop*. **1991**, 306.
14. All in vitro inhibition assays were performed as described previously: Pikul, S.; McDow Dunham, K. L.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider, C. E.; Taiwo, Y. O.; Chen, L.; Dunaway, C. M.; Gu, F.; Mieling, G. E. *J. Med. Chem*. **1999**, *42*, 87.
15. The atomic coordinates of compound **4e** bound to truncated stromelysin have been deposited to the Protein Data Bank, and the file name is 1G05.
16. Babine, E. R.; Bender, S. L. *Chem. Rev*. **1997**, *97*, 1359.