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Selectivity of Inhibition of Matrix Metalloproteases MMP-3 and MMP-2 by Succinyl Hydroxamates and their Carboxylic Acid Analogues is Dependent on P3′ Group Chirality

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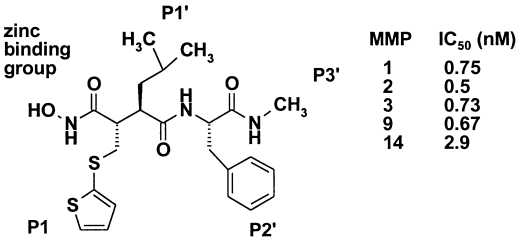
Abstract—Structure–activity relationships are described for a series of succinyl hydroxamic acids **1a**–**o** and their carboxylic acid analogues **2a**–**o** as inhibitors of matrix metalloproteases MMP-3 and MMP-2. For this series (P1′ = $(CH_2)_3$Ph, P2′ = *t*-Bu) selectivity for the inhibition of MMP-2 was found to be strongly dependent on P3′.

Matrix metalloproteases (MMPs) constitute a family of structurally similar zinc-dependent proteases, which are involved in the remodelling, repair and degradation of extracellular matrix proteins, both as part of normal physiological processes and in pathological conditions.[1] For example, MMPs are thought to play a key role in the post-partum remodelling of reproductive tissue,[2] angiogenesis, and tissue repair and re-epithelialisation of wounds by invading fibroblasts, keratinocytes and endothelial cells.[3] In these conditions, MMP function is closely regulated due to their high destructive potential. In contrast, abnormal levels of MMPs have been implicated in conditions such as arthritis (MMP-3 and 13),[4] tumour metastasis (MMP-2 and 9)[5] and periodontal disease (MMP-8).[6] In order to help understand the role of a particular MMP in pathological processes it would be useful to have selective inhibitors available.

This paper describes our initial work to optimise potency and selectivity for MMP-3 inhibition over MMP-2 and the discovery that certain P3′ binding groups can dramatically influence activity against MMP-2.

At the time we started this project, several structural classes of MMP inhibitor with nanomolar potency were known, for example, BB94 (batimastat; Fig 1).



MMP	IC_{50} (nM)
1	0.75
2	0.5
3	0.73
9	0.67
14	2.9

Figure 1. Structure and enzyme inhibition profile of BB94 (batimastat) (data generated in our laboratories).

Although it was known that selectivity over MMP-1 could be readily achieved by increasing the size of the P1′ substituent,[7] there were few examples of MMP-3 inhibitors with appreciable selectivity over MMP-2,[8] and several authors have reasoned that the difficulty in obtaining MMP-3 selective compounds is due to the relatively open nature of the S3′ binding site.[9,10] However, this site should be smaller in MMP-2 because a Tyr occupies the corresponding position of a Thr in MMP-3.[11] Thus, we prepared a series of hydroxamic and carboxylic acids (**1a**–**o** and **2a**–**o**) with fairly large and rigid P3′ substituents which we hoped would be accommodated into the S3′ site of MMP-3 but not MMP-2.

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Chemistry

The syntheses of **1a**–**o** and **2a**–**o** are shown in Scheme 1.

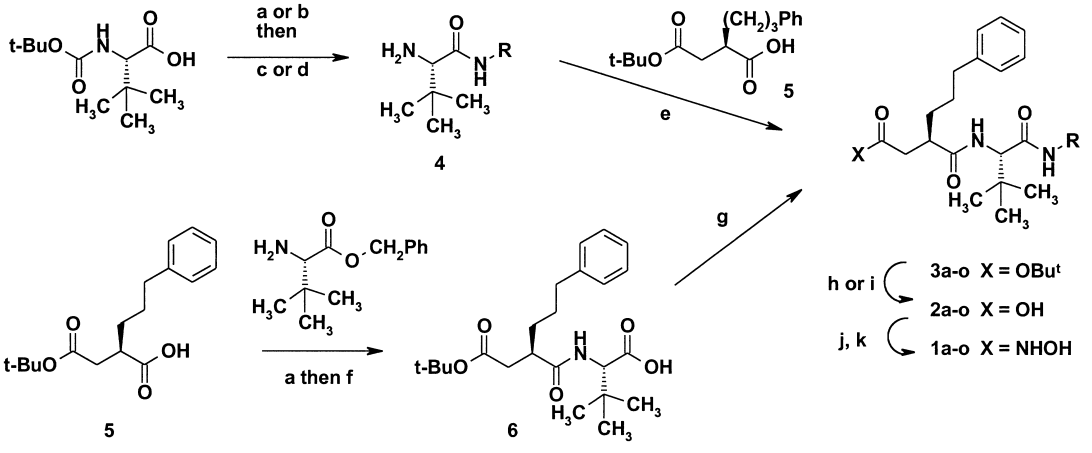
Two routes were used to access intermediates **3a**–**o**. In the first, coupling of *N*-Boc-*tert*-leucine with various P3′ amines (EDC, HOBt, Et_3N or *i*-Pr_2NEt, CH_2Cl_2) followed by removal of the Boc protection afforded amines **4**. However, coupling of tertiary amines by this method led to significant racemisation, but this was readily overcome by replacing HOBt with HOAt.[12] The amines **4** were then coupled to acid **5**.[13] Alternatively, coupling *tert*-leucine benzyl ester with **5** followed by hydrogenolysis gave acid **6** which was coupled with amines without epimerisation using Carpino's conditions.[14] Deprotection of the *t*-butyl esters **3a**–**o** afforded the carboxylic acids **2a**–**o** which were converted in two steps to the hydroxamic acids **1a**–**o** by reaction with *O*-allylhydroxylamine followed by palladium catalysed reductive removal of the allyl group.[15]

Results and Discussion

Inhibition of MMP-3 and MMP-2 was measured by the method of Knight et al.[16] (with minor modifications) including using the fluorogenic peptide substrate Mca-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2[17] which has approximately equal k_{cat}/K_m values for each enzyme and results are shown in Table 1. The selectivities shown are expressed as the ratios of the two IC_{50}s (MMP-2/MMP-3), and ratios for MMP-3 selective compounds are indicated in bold type. Our starting point for exploration of SAR was the *N*-methyl analogue **1a**[13] which is highly selective for MMP-2. The phenpropyl P1′ group was chosen because it enhanced MMP-3 inhibition,[6] and we kept P2′ = *t*-butyl because succinyl hydroxamates without bulky P2′ groups are less stable at high and low pH.[18]

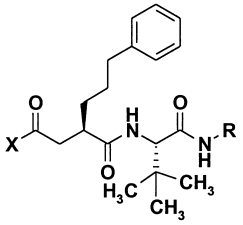
Aryl P3′ groups were initially investigated because they had been reported to increase potency and selectivity for MMP-3.[19] As can be seen, **1b** and **1c** were indeed more potent but retained selectivity for MMP-2. The cycloalkyl analogues **1d** and **1e** increased MMP-3 inhibition slightly and the selectivity for MMP-2 was diminished, and even a *t*-butyl group (**1f**) was tolerated by both enzymes. Examination of the X-ray structure of MMP-3[20] suggested introducing more bulky P3′ groups with a bend, such as benzyl, but since the S3′ binding site is solvent-exposed, we decided to use branched groups to ensure that at least one of the groups would protude into S3′. For the α-methylbenzylamides **1g** and **1h**, potency and selectivity was found to be strongly dependent on the chirality of the P3′ group, with the *R*-enantiomer retaining similar potency to **1a** against MMP-3, but causing a loss in potency of about 200-fold against MMP-2. Levy et al. have reported P3′ SARs in a related series (**7**) and observed a loss of potency against both MMP-2 and 3.[10] The same stereochemical preference was observed for **1i**–**l**. It was interesting that the tertiary P3′ groups in **1m** and **1o** did not increase the MMP-3 selectivity, whereas the benzyhydryl analogue **1n** was the most selective of all. Workers at British Biotech have independently reported that the benzhydryl group can increase selectivity for MMP-3: for example, compound **8**.[8b]

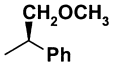
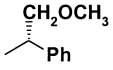
Broadly similar potency and selectivity trends were observed for the corresponding carboxylic acids **2a**–**o**, except that the potency against both enzymes was markedly lower than the hydroxamates. Selectivity for MMP-2 was also higher for the carboxylates, with only the benzhydryl analogue **2n** showing any selectivity for MMP-3.



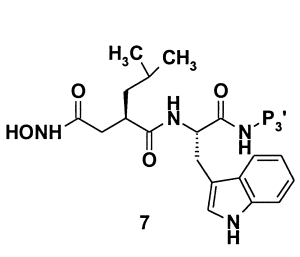
Scheme 1. Reagents and conditions: (a) RNH_2, EDC, HOBt, Et_3N or *i*-Pr_2NEt, CH_2Cl_2, 20 °C, 18 h, 73–96%; (b) RNH_2, EDC, HOAt, Et_3N or *i*-Pr_2NEt, CH_2Cl_2, 38–96%; (c) $HCl_{(g)}$, CH_2Cl_2 or EtOAc, 0 °C, 82–100%; (d) TFA, CH_2Cl_2, 0 °C, 78% for **4o** (only example); (e) EDC, HOBt, *i*-Pr_2NEt, CH_2Cl_2, 20 °C, 18 h, 61–100%; (f) H_2 (3 bar), Pd/C, EtOH/H_2O, 20 °C, 99%; (g) RNH_2, PyAOP, collidine, CH_2Cl_2, 0–20 °C, 3–18 h, 86–97%; (h) $HCl_{(g)}$, CH_2Cl_2 or EtOAc, 0–20 °C, 81–100%; (i) TFA/CH_2Cl_2 (1:1), 20 °C, 4 h, 67–100%; (j) *O*-allylhydroxylamine, EDC, HOBt or HOAt, DMF, 20 °C, 18 h or PyAOP, *i*-Pr_2NEt, 20 °C, 3–8 h, 22–95%; (k) NH_4^+ HCO_2^-, cat. $Pd(OAc)_2{\cdot}2PPh_3$, EtOH/H_2O (4:1), reflux, 30 min–2 h, 35–90%. EDC = *N*-ethyl-*N*′-dimethylaminopropylcarbodiimide, HOBt = 1-hydroxy-1*H*-1,2,3-benzotriazole, HOAt = 7-aza-1-hydroxy-1,2,3-benzotriazole, PyAOP = 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate.

Table 1. Inhibition of MMP-3 and MMP-2 by succinyl hydroxamates **1a–o** and related acids **2a–o**



Cmpd	R	**1** X = NHOH			**2** X = OH		
		MMP-3 IC_{50} (nM)	MMP-2 IC_{50}[a] (nM)	Selectivity ratio MMP-2/MMP-3	MMP-3 IC_{50} (nM)	MMP-2 IC_{50} (nM)	Selectivity ratio MMP-2/MMP-3
a	CH_3	48	0.34	1/141	410	11	1/37
b	Ph	13	1	1/13	475	30	1/17
c	4-Pyridyl	4.4	0.2	1/22	275	6.4	1/46
d	c-C_5H_9	6.8	1.6	1/4.2	3400	54	1/63
e	c-C_6H_{11}	19	1.3	1/15	560	38	1/15
f	*t*-Bu	21	5.8	1/3.6	10,000	84	1/15
g		9	0.38	1/24	2800	50	1/56
h		40	61	**1.5**/1	5350	1580	1/3.4
i		18	3.3	1/5.5	5000	330	1/15
j		40	62	**1.7**/1	6100	5100	1/1.2
k		22	15	1/1.5	8500	6200	1/1.4
l		25	110	**4.4**/1	3200	3500	1.1/1
m	$C(CH_3)_2Ph$	73	3.4	1/21	>10,000	280	1/>35
n	$CHPh_2$	48	414	**8.6**/1	625	2430	**3.9**/1
o	$C(CH_3)Ph_2$	570	26	1/22	>10,000	780	1/>13

[a]IC_{50}s are an average of two determinations.



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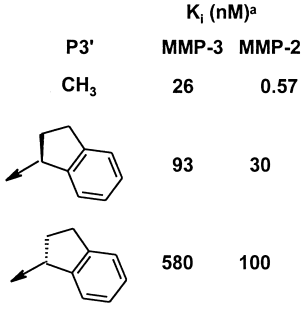
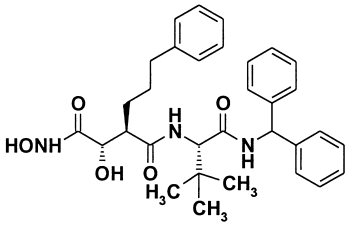
[a] data from ref. 10



K_i (nM)[a]		
P3'	MMP-3	MMP-2
CH_3	26	0.57
	93	30
	580	100



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MMP-3 IC_{50} = 10 nM
MMP-2 IC_{50} = 270 nM
data from our laboratories

Conclusions

We have shown that varying the size of the P3′ group has relatively little impact on the potency of MMP-3 inhibition in accord with X-ray crystallographic structures of MMP-3 which show the S3′ binding site to be open and solvent exposed. However, potency of inhibition of MMP-2 can be reduced by up to 1000-fold through a suitable choice of an α-substituted benzylic group. Where the substituent is not phenyl, the chirality of the

P3′ group is critical. Whilst we do not have X-ray structural evidence, we propose that one of the phenyl groups in **1n** points in the direction of Tyr395 in MMP-2[11] and the resulting steric clash reduces binding affinity. It is striking that the SARs at P3′ are different to **7**,[10] suggesting that the choice of the other substituents (P1′, P2′, etc.) can crucially influence SARs at another binding site. Although the selectivity in favour of MMP-3 in this series is clearly not useful therapeutically, we were encouraged to build on these observations and combine some of the best P3′ groups shown here with novel P1′ substituents. The results of that study are the subject of the following paper.[21]

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References

1. Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev*. **1999**, *99*, 2735. Johnson, L. L.; Dyer, , R.; Hupe, D. J. *Curr. Opin. Chem. Biol*. **1998**, *2*, 466. Beckett, R. P.; Whittaker, M. *Expert Opin. Ther. Pat*. **1998**, *8*, 259. Denis, L. J.; Verweij, J. *Invest. New Drugs* **1997**, *15*, 175. Wojtowicz-Praga, S. M.; Dickson, R. B.; Hawkins, M. J. *Invest. New Drugs* **1997**, *15*, 61. Davidson, A.; Drummond, A. H.; Galloway, W. A.; Whittaker, M. *Chem. Ind. (London)* **1997**, 258. Beckett, R. P. *Expert Opin. Ther. Pat*. **1996**, *6*, 1305. Hagmann, W. K.; Larkk, M. W.; Becker, J. W. *Annu. Rep. Med. Chem*. **1996**, *31*, 231. Beckett, R. P.; Davidson, A.; Drummond, A. H.; Huxley, P.; Whittaker, M. *Drug Discovery Today* **1996**, *1*, 16. Zask, A.; Levin, J. I.; Killar, L. M.; Skotnicki, J. S. *Curr. Pharm. Design* **1996**, *2*, 624.

2. Balbin, M.; Fuego, A.; Knauper, V.; Pendas, A. M.; Lopez, J. M.; Jimenez, M. G.; Murphy, G.; Lopez-Otin, C. *J. Biol. Chem*. **1998**, *273*, 23959.

3. Agren, M. S. *Brit. J. Dermatology* **1996**, *131*, 634. Salo, T.; Mäkänen, M.; Kylmäniemi, M. *Lab. Invest*. **1994**, *70*, 176. Saarialho-Kere, U. K.; Kovacs, S. O.; Pentland, A. P. *J. Clin. Invest*. **1993**, *92*, 2858. Di Colandrea, T.; Wang, L.; Wille, J.; D'Armiento, J.; Chada, K. K. *J. Invest. Dermatol*. **1998**, *111*, 1029.

4. Cawston, T. *Pharmacol. Ther*. **1996**, *70*, 163.

5. Lochter, A.; Sternlicht, M. D.; Werb, Z.; Bissell, M. J. *Ann. N.Y. Acad. Sci*. **1998**, *857*, 180. Summers, J. B.; Davidsen, S. K. *Annu. Rep. Med. Chem*. **1998**, *33*, 131.

6. Overall, C. M.; Wiebkin, O. W.; Thonad, J. C. *J. Periodontal Res*. **1987**, *22*, 81.

7. Porter, J. R.; Beeley, N. R. A.; Boyce, B.; Mason, B.; Millican, A.; Millar, K.; Leonard, J.; Morphy, J. R.; O'Connell, J. P. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 2741.

8. (a) Tanzawa, K.; Ishii, M.; Ogita, T.; Shimad, K. *J. Antibiotics* **1992**, *45*, 1733. Bottomley, K. M.; Borkakoti, N.; Bradshaw, D.; Brown, P. A.; Broadhurst, M. J.; Budd, J. M.; Elliott, L.; Eyers, P.; Hallam, T. J.; Handa, B. K.; Hill, C. H.; James, M.; Lahm, H.-W.; Lawton, G.; Merritt, J. E.; Nixon, J. S.; Rothlisberger, U.; Whittle, A.; Johnson, W. *Biochem. J*. **1997**, *323*483. Esser, C. K.; Bugianesi, R. L.; Caldwell, C. G.; Chapman, K. T.; Durette, P. L.; Girotra, N. N.; Kopka, I. E.; Lanza, T. J.; Levorse, D. A.; MacCoss, M.; Owens, K. A.; Ponpipom, M. M.; Simeone, J. P.; Harrison, R. K.; Niedzwiecki, L.; Becker, J. W.; Marcy, A. I.; Axel, M. G.; Christen, A. J.; McDonnell, J.; Moore, V. L.; Olszewski, J. M.; Saphos, C.; Visco, D. M.; Shen, F.; Colletti, A.; Krieter, P. A.; Hagmann, W. K. *J. Med. Chem*. **1997**, *40*, 1026. (b) World Pat. Appl. WO 96/33165, 1996; *Chem. Abstr*. **1997**, *126*, 18654. (c) Bender, S. J. 2nd Winter Conference on Medicinal and Bioorganic Chemistry, Steamboat Springs, Colorado, USA, 26–31 January 1997.

9. See for example Jacobsen, I. C.; Reddy, P. G.; Wasserman, Z. R.; Hardman, K. D.; Covington, M. B.; Arner, E. C.; Copeland, R. A.; Decicco, C. P.; Magolda, R. L. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 837.

10. Levy, D. E.; Lapierre, F.; Liang, W.; Ye, W.; Lange, C. W.; Li, X.; Grobelny, D.; Casabonne, M.; Tyrrell, D.; Holme, K.; Nadzan, A.; Galardy, R. E. *J. Med. Chem*. **1998**, *41*, 199.

11. Tyr-395 (MMP-2) becomes Thr-194 (MMP-3), see Massova, I.; Fridman, R.; Mobashery, S. *J. Molec. Model*. **1997**, *3*, 17.

12. Carpino, L. A. *J. Am. Chem. Soc*. **1993**, *115*, 4397.

13. World Pat. Appl. WO 95/04033, 1995; *Chem. Abstr*. **1995**, *122*, 264925.

14. Fray, M. J.; Ellis, D. *Tetrahedron* **1998**, *54*, 13825 and references therein.

15. cf. Yamada, T.; Goto, K.; Mitsuda, Y.; Tsuji, J. *Tetrahedron Lett*. **1987**, *28*, 4557.

16. Knight, C. G.; Wilenbrock, F.; Murphy, G. *Fed. Euro. Biochem. Soc*. **1992**, *296*, 263.

17. Nagase, H.; Fields, C. G.; Fields, G. B. *J. Biol. Chem*. **1994**, *269*, 20952.

18. Unpublished observations by K. N. Dack and T. J. Evans in our laboratories.

19. World Pat. Appl. WO 95/19956, 1995; *Chem. Abstr*. **1996** *124*, 56708.

20. Becker, J. W.; Marcy, A. I.; Rokosz, L. L.; Axel, M. G.; Burbaum, J. J.; Fitzgerald, P. M. D.; Cameron, P. M.; Esser, C. K.; Hagmann, W. K., et al. *Protein Sci*. **1995**, *4*, 1966.

21. Fray, M. J.; Dickinson, R. P. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 571.