Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 571–574

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Discovery of Potent and Selective Succinyl Hydroxamate Inhibitors of Matrix Metalloprotease-3 (Stromelysin-1)

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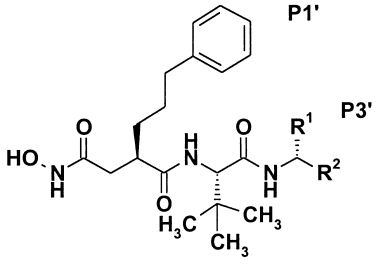
Received 9 June 2000; revised 27 November 2000; accepted 19 December 2000

Abstract—Structure–activity relationships are described for a series of succinyl hydroxamic acids **4a**–**o** as potent and selective inhibitors of matrix metalloprotease-3 (stromelysin-1). Optimisation of P1′ and P3′ groups gave compound **4j** (MMP-3 $IC_{50} = 5.9$ nM) which was >140-fold less potent against MMP-1 ($IC_{50} = 51{,}000$ nM), MMP-2 ($IC_{50} = 1790$ nM), MMP-9 ($IC_{50} = 840$ nM) and MMP-14 ($IC_{50} = 1900$ nM).

The previous paper[1] described SAR for a series of succinyl hydroxamic acids designed to optimise inhibition of MMP-3 and selectivity over MMP-2 inhibition. Starting from **1**, appropriate choice of P3′ group, for example, **2** and **3**, diminished inhibition of MMP-2 by up to 1000-fold whilst retaining inhibition of MMP-3.



	MMP-3 IC_{50} (nM)	MMP-2 IC_{50} (nM)
1 $R^1 = R^2 = H$	48	0.38
2 $R^1 = CH_3$, $R^2 = Ph$	40	61
3 $R^1 = R^2 = Ph$	48	414

However, the selectivity of **2** and **3** was modest and we felt the limit of what could be achieved from the P3′ substituent alone had been reached. We therefore decided to synthesise P1′ analogues **4a**–**o** in the hope of gaining further increases in potency and selectivity for MMP-3.

Chemistry

Two routes were used for the preparation of **4a**–**o**. In the first (Scheme 1), amine **5**[2] was coupled to succinic acid derivatives **6**–**8**[3] to give compounds **9**–**11**. The *t*-butyl esters were cleaved and the resulting acids **12**–**14** converted to hydroxamic acids **4a**–**c**.

A flexible route[4] (Scheme 2) was employed for compounds **4d**–**o** in which a biarylpropyl group was introduced into P1′. The amines **5**, **15** and **16**[1] were coupled to acid **17**[4] as above, affording **18**–**20**, and then a biaryl group was introduced via a Heck reaction. The reaction partners for the Heck reaction are listed below.[5] The major product (~70%) in each case was the (*E*)-3-(biaryl)-3-propenyl derivative (**21**), but other alkene products were evident by NMR. These were tentatively identified as the (*Z*)-3-(biaryl)-3-propenyl, (*E*)- and (*Z*)-3-(biaryl)-2-propenyl and 2-(biaryl)-3-propenyl isomers (~7% each). The last product (arising from attack of the biaryl group on the more substituted end of the alkene) could be removed by (a) careful chromatography at this stage, (b) following the next step, or (c) by recrystallisation of carboxylic acid **22**. The mixture of alkenes was then reduced and the *t*-butyl ester cleaved to give carboxylic acids **22**, which were usually converted to the hydroxamic acids as described previously.[1] Compounds **4l**, **4n** and **4o** were prepared from the carboxylic acid using hydroxylamine and the peptide coupling agent HATU.

Results and Discussion

Inhibition of catalytic domain MMP-3 and MMP-2 was measured as described previously using the Nagase fluorogenic substrate,[6] and results are shown in Table 1. Initially, we surveyed the long chain aliphatic, biphenethyl, cyclohexylpropyl and biphenpropyl derivatives

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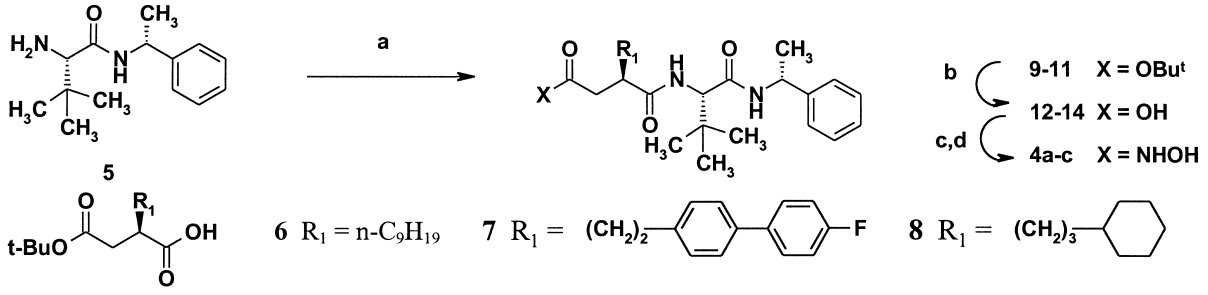
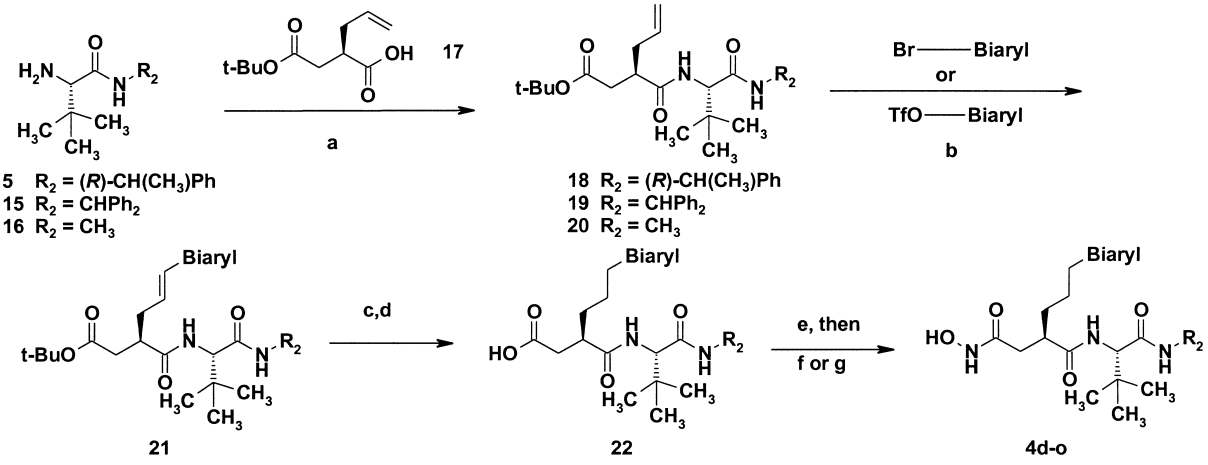
PII: S0960-894X(00)00720-4



Scheme 1. Reagents and conditions: (a) **6**–**8**, EDC, HOAt, NMM, CH_2Cl_2, 0–20 °C, 18 h, 80–97%; (b) $HCl_{(g)}$, CH_2Cl_2 or dioxane, 0 °C, 54–89% or TFA/H_2Cl_2 (1:1), 0–20 °C, 4 h, 80–100%; (c) *O*-allylhydroxylamine, EDC, HOAt, Et_3N or *i*-Pr_2NEt, CH_2Cl_2 or DMF, 0–20 °C, 18 h; or *O*-allylhydroxylamine, PyAOP, *i*-Pr_2NEt, DMF, 0–20 °C, 18 h, 57–95%; (d) NH_4^+ HCO_2^-, 5 mol% $Pd(OAc)_2{\cdot}2PPh_3$, EtOH/H_2O (4:1), reflux, 2 h, 17–88%. EDC = *N*-ethyl-*N*′-dimethylaminopropylcarbodiimide, NMM = *N*-methylmorpholine, HOAt = 7-aza-1-hydroxy-1,2,3-benzotriazole, PyAOP = 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate.



Scheme 2. Reagents and conditions: (a) EDC, HOAt or HOBt, NMM, DMF or CH_2Cl_2, 0–20 °C, 18 h, 87–100%; (b) 5 mol% $Pd(OAc)_2{\cdot}2P(o\text{-tol})_3$, Et_3N, DMF or MeCN, 100 °C, 18–24 h, 58–98%; or 5 mol% $Pd(OAc)_2{\cdot}2P(o\text{-tol})_3$, Na_2CO_3, *n*-Bu_4NCl, DMF, 80 °C, 68–24 h, 68%; (c) H_2 (3 bar), 10% Pd/C, EtOH, 20 °C, 6–18 h, 62–98%; or, for Ar = chlorobiphenyl, $TsNHNH_2$, toluene, reflux, 4 h, 74%. (d)–(f) see steps b–d, Scheme 1; (g) HATU, *i*-Pr_2NEt, DMF, 0 °C then $NH_2OH{\cdot}HCl$, 0–20 °C, 44–74%. HOBt = 1-hydroxy-1*H*-1,2,3-benzotriazole, HATU = *O*-(7-aza-1,2,3-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

4a–**d**, since these groups had featured in literature MMP inhibitors. From this set of compounds it was clear that biphenpropyl afforded increased selectivity and potency compared to **1**. The increase in potency can be ascribed to a favourable interaction between the terminal phenyl and His-224[3b,7] when the biaryl is linked via a propylene spacer group.

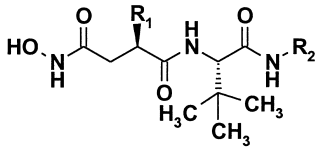
Suitably encouraged, we prepared analogue **4e** in the expectation that selectivity for MMP-3 would increase. We were gratified to find it did, although potency against MMP-3 was reduced by approximately 3-fold. Examination of molecular models of MMP-3 and MMP-2 incorporating **4d** suggested there might be less room in MMP-2 to accommodate an *ortho* substituent on the biaryl, since the enzyme loop which defines S1′ is three amino acid residues shorter in MMP-2.[8] We therefore prepared compounds **4f**–**h**. Surprisingly, introduction of an *ortho* fluorine into **4d** to give **4f** increased selectivity to 47-fold, but **4g** was only 4-fold selective (cf. 35-fold for **4e**).

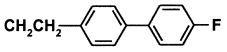
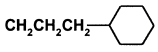
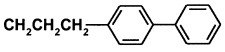
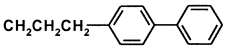
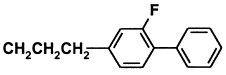
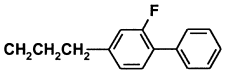
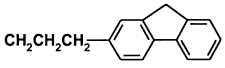
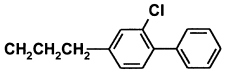
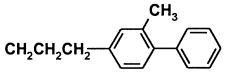
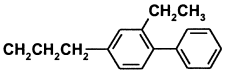
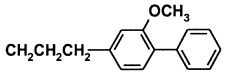
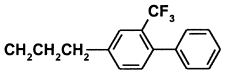
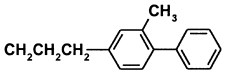
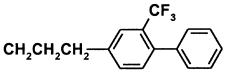
Even more remarkable was the very low selectivity of the fluorenyl derivative **4h**.[9] Presumably, a planar biphenyl moiety is tolerated by MMP-2, whereas an *ortho* substituent disrupts this planarity and causes a steric clash in the MMP-2 binding pocket. Potency for MMP-3 inhibition was relatively unaffected. We then sought to optimise selectivity by systematically increasing the size of the *ortho* substituent (compounds **4i**–**m**).

Inhibition of MMP-3 and selectivity over MMP-2 was remarkably sensitive to the size of the substituent and is clearly optimal for a methyl group (compound **4j**). Larger groups (compounds **4k**–**m**) led to a significant loss of inhibition of both MMP-3 and MMP-2.

Having discovered a P1′ group that markedly increased selectivity, we considered whether simplification of the P3′ group would be possible, and therefore prepared compounds **4n** and **4o**. Although **4n** was very potent, selectivity was dramatically reduced, thereby emphasising the vital contributions made by *both* the P3′ and P1′ groups in **4j**. Replacement of the α-methylbenzyl in **4m** by methyl (as in **4o**) led to increased potency for inhibition of MMP-3 and MMP-2 of approximately 19- and 600-fold, respectively, a similar effect on the selectivity at a

Table 1. Inhibition of MMP-3 and MMP-2 by succinyl hydroxamic acids **4a–o**



Compd	R_1	R_2	MMP-3 IC_{50} (nM)[a]	MMP-2 IC_{50} (nM)[a]	Sel.[b]
a	$(CH_2)_8CH_3$	(*R*)-CH(CH_3)Ph	25	100	4
b		(*R*)-CH(CH_3)Ph	26	210	8.1
c		(*R*)-CH(CH_3)Ph	120	920	7.7
d		(*R*)-CH(CH_3)Ph	2.6	34	13
e		$CHPh_2$	8.8	310	35
f		(*R*)-CH(CH_3)Ph	7.2	335	47
g		$CHPh_2$	10	41	4.1
h		(*R*)-CH(CH_3)Ph	3.8	11	2.9
i		(*R*)-CH(CH_3)Ph	13	1300	100
j		(*R*)-CH(CH_3)Ph	5.9	1790	300
k		(*R*)-CH(CH_3)Ph	450	6,800	15
l		(*R*)-CH(CH_3)Ph	110	1700	15
m		(*R*)-CH(CH_3)Ph	770	52,000	68
n		CH_3	0.5	1.0	2
o		CH_3	40	86	2.1

[a]IC_{50}s are an average of two determinations.
[b]Selectivity for MMP-3 = MMP-2 $IC_{50} \div$ MMP-3 IC_{50}.

lower level of potency. The remarkable interdependence of SARs on these two substituents can be rationalised qualitatively from the structures of the S3′ and S1′ binding sites. S3′ can more easily accommodate a large substituent in MMP-3, and thus in MMP-2 a residue may have to move. If this residue is Tyr426 then the next amino acid (Thr427), which starts the loop which defines S1′, may also shift. Thus, whilst MMP-2 can adjust its conformation to alleviate steric interactions with S3′ or S1′ residues, movement to embrace both substituents simultaneously is very difficult, and a large drop in inhibitory potency results. Compound **4j** was more widely profiled against other MMPs (see Table 2).[10]

Table 2. Enzyme inhibition profile of **4j**

MMP	IC_{50} (nM)	±SEM (n)
1	51,000	±27,000 (4)
2	1790	±1000 (5)
3	5.9	±2.2 (5)
9	840	±91 (5)
13	73	±1.4 (4)
14	1900	±450 (4)

As expected from the large P1′ group, inhibition of MMP-1 was very weak, whereas it was a relatively potent inhibitor of MMP-13, consistent with the more open S1′ binding site of the latter. Inhibition potency of MMP-14 was similar to MMP-2. MMP-14 is similar to MMP-3 in the S3′ site, but is more sterically demanding at S2′ (Phe233 replaces Leu222),[11] so it is possible the *t*-butyl P2′ group plays a part in determining the selectivity over MMP-14. Inhibition potency of MMP-9 was about 2-fold greater than MMP-2, and the small difference was anticipated given the high homology between the two gelatinases.

Conclusions

We have demonstrated that inhibitory potency against MMP-2 may be significantly reduced by subtle modifications to P1′ and P3′ groups in a series of succinyl hydroxamic acid inhibitors of MMP-3. Compound **4j** (UK-356,618) is the most potent *and* selective MMP-3 inhibitor reported to date, and may be a useful tool for elucidating the contribution of MMP-3 to pathological conditions in which selectivity may be required over other MMPs.

Acknowledgements

The authors wish to thank Mrs. E. J. Fairman, Mrs. K. E. Holland, Ms. L. M. Reeves and Mr. S. Lewis for measuring enzyme inhibition potencies, Mrs. K. S. Mills, Ms. C. A. Loosley and Messrs. D. Ellis, T. J. Evans and M. Sproates for making the compounds, Drs. A. Alex and M. J. de Groot for molecular modeling studies and staff of the Physical Sciences Dept. for measuring spectroscopic data.

References and Notes

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3. (a) Compound **6**: Broadhurst, M. J.; Brown, P. A.; Johnson, W. H.; Lawton, G. Eur. Pat. Appl. EP 575844, 1993; *Chem. Abstr*. **1994**, *121*, 57993. (b) Compound **7**: prepared from 4′-fluoro-1,1′-biphen-4-ylbutanoic acid (Esser, C. K. et al. *J. Med. Chem*. **1997**, *40*, 1026) by the method of 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. (c) Compound **8**: prepared by catalytic hydrogenation of the phenpropyl analogue (World Pat. Appl. WO 95/04033, 1995; *Chem. Abstr*. **1995**, *122*, 264925) over Rh/Al_2O_3 (4 bar, HOAc, 20 °C, 100%).
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5. Reaction partners for the Heck reaction (Scheme 2): Alkene **18** was reacted with 4-bromo-1,1′-biphenyl, 4-bromo-2-fluoro-1,1′-biphenyl, 2-bromo-fluorene, 2-chloro-1,1′-biphen-4-yl triflate,[12] 4-bromo-2-methyl-1,1′-biphenyl,[13] 4-bromo-2-ethyl-1,1′-biphenyl,[13] 4-bromo-2-methoxy-1,1′-biphenyl[13] and 4-bromo-2-trifluoromethyl-1,1′-biphenyl.[13] Alkene **19** was reacted with 4-bromo-1,1′-biphenyl and 4-bromo-2-fluoro-1,1′-biphenyl. Alkene **20** was reacted with 2-methyl-1,1′-biphen-4-yl triflate[12] and 4-bromo-2-trifluoromethyl-1,1′-biphenyl.
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10. The assays for MMPs 2, 3, 9, and 14 are based upon the original protocol described by Knight et al. (*Fed. Euro. Biochem. Soc*. **1992**, *296*, 263) with slight modifications. Assays for MMPs 2, 3, and 9 used the Nagase substrate,[6] whereas the assay for MMP-14 (purchased from Prof. Tschesche, Department of Biochemistry, Faculty of Chemistry, University of Bielefeld, Germany) used the substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Bachem Ltd, Essex, UK) as described by Will et al. (*J. Biol. Chem*. **1996**, *271*, 17119). The assay for MMP-1 inhibition used the substrate Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys(N-Me-Ala)-NH_2 as originally described by Bickett et al. (*Anal. Biochem*. **1993**, *212*, 58). The assay for MMP-13 inhibition used the substrate Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2. Enzyme and substrate concentrations were typically 1 nM and 5–10 μM, respectively.
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12. Prepared as follows: (a) 4-bromo-3-chloroanisole or 4-bromo-3-methylanisole, $PhB(OH)_2$, 3 mol% $Pd(PPh_3)_4$, CsF, DME, reflux; (b) BBr_3, CH_2Cl_2, −78 to 0 °C; (c) Tf_2O, pyridine, CH_2Cl_2, 0–20 °C.
13. Prepared by a modified Gomberg–Bachmann–Hey reaction of the appropriate 2-substituted-4-bromoaniline and *i*-amyl nitrite in benzene at reflux for 2–3 h (yields 25–40%); cf. Hassanaly, P.; Vernin, G.; Dou, H. J. M.; Metzger, J. *Bull. Soc. Chim. Fr*. **1974**, 560, and Eur. Pat. Appl. EP 455058, 1991; *Chem. Abstr*. **1992**, *116*, 123167.