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A novel series of highly selective inhibitors of MMP-3

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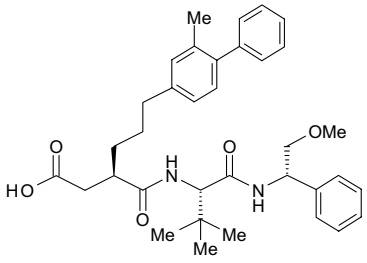
Abstract—The design and synthesis of a series of highly selective hydroxamate inhibitors of stromelysin-1 (MMP-3) is described. Substitution of a 4-biaryl piperidine sulfonamide core, which binds at the S1′ subsite of MMP-3, was optimised to give potent inhibitors of MMP-3, with greater than 300-fold selectivity over MMP-1, MMP-2, MMP-9 and MMP-14. Compounds **26** and **27** were identified as having the best balance of pharmacology and properties required for topical drug delivery.

Matrix metalloproteinases (MMPs) are a family of zinc-dependant endopeptidases which are involved in normal tissue remodelling and extracellular matrix degradation.[1] The overexpression of MMPs has been implicated in a number of pathological conditions including cancer,[2,3] arthritis[4] and chronic non-healing wounds.[5] In particular, MMP-3 (stromelysin-1) over-activity has been implicated in the pathology of chronic non-healing wounds,[6] and therefore selective MMP-3 inhibition represents an attractive target for the treatment of this condition.

Co-workers at Pfizer recently described the discovery of UK-370106 **1** (Fig. 1),[7] a highly selective peptidic MMP-3 inhibitor, which was identified as a clinical candidate for the topical treatment of chronic dermal ulcers. As part of a multi-template approach to selective MMP-3 inhibitors, we now wish to describe our efforts to discover non-peptidic MMP-3 inhibitors with good selectivity over other MMPs, which may have utility in the topical treatment of chronic non-healing wounds.[8] In this paper we describe the SAR for MMP-3 potency, selectivity over other MMPs along with strategies to deliver good physicochemical properties for topical drug candidates.

With the advent of non-selective MMP inhibitors based on sulfonamides derived for α-amino acids,[9] we proposed a new sulfonamide template where the nitrogen and sulfur were reversed (Fig. 2).



1 UK-370106
MMP-3 IC_{50} 23nM
MMP-2 IC_{50} 34.2μM
MMP-1 IC_{50} > 100μM
MMP-9 IC_{50} 30.4μM
MMP-13 IC_{50} 2.3μM
MMP-14 IC_{50} 66.9μM

Figure 1. MMP pharmacology of UK-370106.

This strategy could deliver a template which was novel,[10] synthetically simple to make and lacked chiral centres. By using the S1′ SAR for selectivity from the UK-370106 series combined with in silico modelling, we hoped to rapidly identify a series of reversed sulfonamides with good selectivity for MMP-3 inhibition.

The synthesis of analogues **4**–**30** was accomplished by coupling of the appropriate amine with the sulfonyl chloride **2**[11] to give sulfonamides **3**. When the P_1 group remained as hydrogen, direct condensation of the ester with hydroxylamine then afforded the desired hydroxamic acids **4**–**23** (Scheme 1). In the case where P_1 was gem-dimethyl, dialkylation of **3** was then followed by hydrolysis and coupling with hydroxylamine to give analogues **24**–**30**.

Our initial medicinal chemistry strategy was to find the optimal sulfonamide linker to extend a lipophilic biphe-

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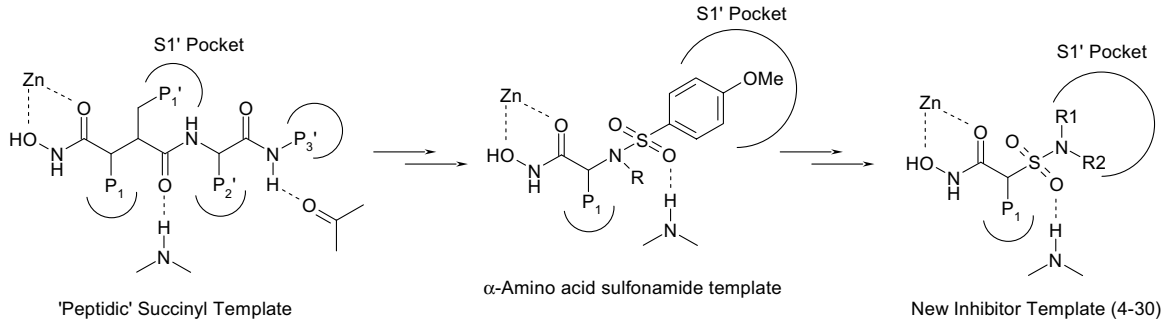
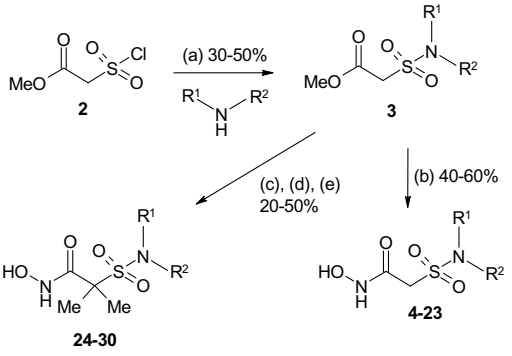


Figure 2. Evolution of 'Reversed' sulfonamide template.



Scheme 1. Reagents and conditions: (a) DBU, CH_2Cl_2, 0 °C to rt; (b) $HONH_2{\cdot}HCl$, K_2CO_3, MeOH, reflux; (c) MeI, K_2CO_3, DMSO, rt; (d) NaOH, MeOH, H_2O, reflux; (e) HATU, *N*-ethyl-diisopropylamine, $HONH_2{\cdot}HCl$, NMP, rt.

nyl unit into the S1′ pocket (Table 1), as this region of UK-370106 delivered both MMP-3 potency and MMP-2 selectivity. Flexible alkyl linkers in compounds **4**, **5** and **6** afforded modest MMP-3 potency, with the chain length having no significant effect on MMP-3 potency. Cyclisation and conformational restriction was then investigated. Piperidine **7** was significantly more potent than the acyclic analogue **6**, and a further increase in potency was achieved with dihydropiperidine **8**. The azetidines **9** and **10** lost MMP-3 potency, indicating that this linker was either too short, or conformationally suboptimal, to extend the biphenyl into the S1′ pocket. Interestingly a significant degree of potency was also lost when a phenoxyphenyl substituent was incorporated (compound **11**), indicating the enzyme is also sensitive to the linearity of the P1′ group.

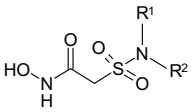
Compounds **4**–**11** were also assessed for their MMP-2 activity,[13] however we found no separation of activity.

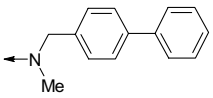
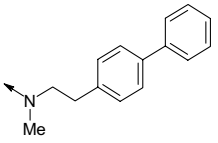
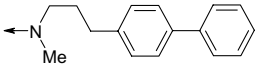
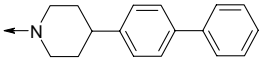
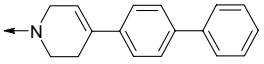
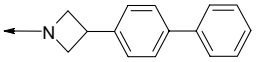
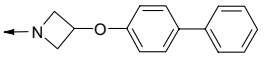
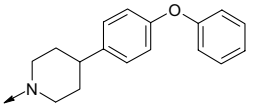
We then investigated substitution on the biphenyl as a strategy for delivering selectivity for MMP-3 over MMP-2 (Table 2). This approach has been successfully employed during the discovery of UK370106 **1**. Incorporation of an R^3 substituent in the 3-position of the A-ring was initially investigated whilst keeping the distal B-ring unsubstituted.

The MMP-3 inhibitory potency of compounds **12**–**17** tended to decrease as the size of the R^3-group was increased, but activity against MMP-2 was even more sensitive. Based on its good balance of MMP-3 potency and MMP-2 selectivity, the methyl derivative **15** was selected for further investigation of substitution of the distal phenyl, ring B.

Modelling of **15** into the published MMP-2 catalytic domain crystal structure[14] suggested substitution in the 3-position of ring B could induce unfavourable interactions with the loop forming the S1′ pocket and therefore

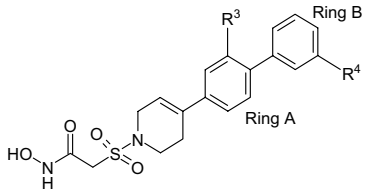
Table 1. In vitro inhibition of MMP-3 activity[a,b] for compounds **5**–**12**



Compound	NR^1R^2	MMP-3 IC_{50} (nM)
4		378
5		164
6		174
7		26
8		6
9		161
10		84
11		205

[a] See Ref. 12 for description of assay conditions.
[b] MMP IC_{50} values are geometric means of at least three experiments.

Table 2. In vitro inhibition of MMP-3 amd MMP-2 activity[a,b] for compounds **12–23**



Compound	R^3	R^4	MMP-3 IC_{50} (nM)	MMP-2 IC_{50} (nM)
12	H	H	6	9
13	F	H	3	17
14	Cl	H	55	620
15	Me	H	16	320
16	Et	H	420	4380
17	CF_3	H	960	3080
18	Me	Me	4	776
19	Me	OMe	5	222
20	Me	Et	31	1208
21	Me	OEt	4	998
22	Me	CH_2OMe	3	196
23	Me	OCF_3	51	173

[a] See Ref. 12 for description of assay conditions.
[b] MMP IC_{50} values are geometric means of at least three experiments.

reduce MMP-2 enzyme inhibition (Fig. 3). This loop region is three residues shorter in MMP-2 compared to MMP-3.

This strategy gave a number of compounds with improved potency and selectivity, that is, substitution not only reduced inhibition of MMP-2 but also increased MMP-3 enzyme inhibition. In particular 3-OEt analogue **21** was selected for further evaluation.

Having identified **21** as a highly potent and selective MMP-3 inhibitor, we used this compound to assess the series against key topical drug-like properties. We envisaged that MMP-3 inhibitor drug candidates would be delivered topically as sterile solution or suspension formulations and therefore aqueous solubility and autoclave stability were important parameters to measure.[16] Compound **21** was found to have very low aqueous solubility (≪1 μg/ml) and poor autoclave solution stability across a range of pH. Hydrolysis of the hydroxamic acid was found to be a major degradation pathway, as was oxidation of the tetrahydropiperidine ring, followed by fragmentation of the N–S bond of the sulfonamide. We reasoned that by introducing steric bulk next to the hydroxamic acid, and by reducing the dihydropiperidine ring to a piperidine we may improve stability. To increase solubility, we focused on adding polar solubilising groups onto the 3-OEt group of compound **21**. These design principles led to a series of further analogues (Table 3).

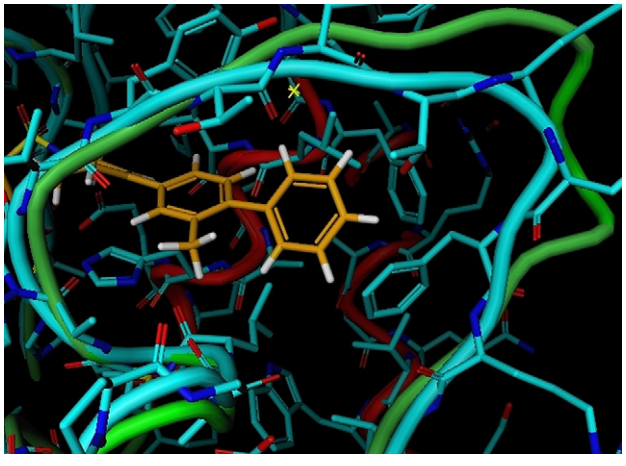
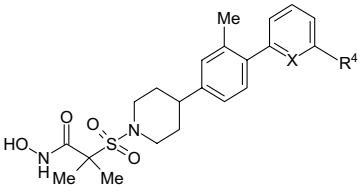


Figure 3. Docking of compound **15** (gold) into X-ray structures of catalytic domain MMP-3[15] (green) and MMP-2 (blue).

Incorporation of steric bulk adjacent to the hydroxamic acid increased MMP-3 potency (examples **21** vs **24**), and retained MMP-2 selectivity. Encouragingly this additional steric bulk also improved solution autoclave stability. We then investigated polar additions onto the R^4 substituent. Methoxyethoxy, hydroxyethoxy and aminoethoxy substitutions also increased potency without compromising MMP-2 selectivity. Examples **26** and **27** had improved aqueous solubility (1–2 μg/ml) and excellent solution stability. Incorporation of a pyridyl ring in example **27** also retained excellent MMP-3 activity and slightly improved MMP-2 selectivity. Solubility was increased significantly for the basic analogues **28–30**, however these compounds suffered from poor

Table 3. In vitro inhibition of MMP-3 amd MMP-2 activity[a,b] for compounds **24–30**



Compound	R^4	X	MMP-3IC_{50} (nM)	MMP-2IC_{50} (nM)
24	OEt	CH	2	457
25	OCH_2CH_2OMe	CH	3	853
26	OCH_2CH_2OH	CH	1	262
27	OCH_2CH_2OH	N	1	529
28	$OCH_2CH_2NH_2$	CH	0.4	188
29	OCH_2CH_2NHMe	CH	0.3	196
30	$OCH_2CH_2NMe_2$	CH	1	534

[a] See Ref. 12 for description of assay conditions.
[b] MMP IC_{50} values are geometric means of at least three experiments.

Table 4. In vitro inhibition of MMP-1, MMP-9 and MMP-14 activity[a,b] for compounds **26** and **27**

Compound	MMP-1 IC_{50} (nM)	MMP-9 IC_{50} (nM)	MMP-14 IC_{50} (nM)
26	3230	406	1710
27	14,000	2420	20,100

[a] See Ref. 7b for description of assay conditions.
[b] MMP IC_{50} values are geometric means of at least two experiments.

aqueous stability. Further MMP selectivity screening for examples **26** and **27** demonstrated their excellent selectivity for MMP-3 over MMP-1, MMP-9 and MMP-14 (Table 4).

In summary, we have described the discovery of a novel series of highly selective inhibitors of MMP-3. By reversing the nitrogen and sulfur of previous non-selective MMP inhibitor series, we were able to work in a novel area of chemical space and in a simplified template without chiral centres. Identification of the optimal linker for the key pharmacophoric biaryl S1′ substituent was rapidly achieved, and using learning and selectivity SAR from an unrelated peptidic series, we were able to deliver compounds which were highly selective for MMP-3. Drug-like properties for topical delivery, namely solution autoclave stability and solubility, were also measured during the evolution of the series. It was found that steric bulk adjacent to the hydroxamic acid, and reduction of a tetrahydropiperidine to a piperidine significantly improved aqueous autoclave stability. Polar groups could be tolerated on the distal aryl ring of the biaryl which improved solubility. The combination of these features led to compounds **26** and **27** which had the best balance of pharmacological and physicochemical properties as potential candidates for the topical treatment of chronic dermal ulcers.

Acknowledgments

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12. The MMP-2 and MMP-3 enzyme inhibition assays are based on the original protocol described by Knight et al.[17] with the modifications described below. Catalytic domain MMP-2 and MMP-3 were prepared at Pfizer Global R&D. A stock solution of MMP-2 or MMP-3 (1 μM) was activated by the addition of aminophenylmercuric acetate (APMA, 1 mM for MMP-2, 2 mM for MMP-3) followed by incubation at 37 °C (1 h for MMP-2, 3 h for MMP-3). The enzymes were then diluted in Tris–HCl assay budder to a concentration of 10 nM. The final assay concentration of enzyme used in the assays was 0.1 nM. The fluorogenic substrate used in these assays was Mca-Arg-Pro-Lys_Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH2[16] (Bachem Ltd). This substrate was selected because it had balanced hydrolysis rates against MMP-2 and MMP-3 (k_{cat}/k_m 54,000 and 59,400 $s^{-1}M^{-1}$, respectively). The final substrate concentration used in the assay was 5 μM. Test compounds were dissolved in DMSO then diluted with test buffer solution (as above) so that not more than 1% DMSO was present. Test compound and enzyme were added to each well of a 96-well plate and allowed to equilibrate for 15 min at 37 °C prior to addition of substrate. Plates were then incubated for 1 h at 37 °C prior to determination of fluorescence using a fluorimeter (Fluostar, BMG Lab-Technologies) at an excitation wavelength of 328 nm and emission wavelength of 393 nm. The potency of inhibitors was measured from the amount of substrate cleavage obtained from a range of test compound concentrations, and IC_{50} values were calculated from the resulting concentration–response curves.
13. MMP-2 inhibition data for compounds **4**–**11** demonstrated that selectivity for MMP-3 over MMP-2 was <2-fold.
14. (a) Dhanaraj, V.; Williams, M. G.; Ye, Q.-Z.; Molina, F.; Johnson, L. L.; Ortwine, D. F.; Pavlovsky, A.; Rubin, J. R.; Skeean, R. W.; White, A. D.; Humblet, C.; Hupe, D. J.; Blundell, T. L. *Croat. Chem. Acta* **1999**, *72*, 575; The crystal structure of full length MMP-2 has also been described (b) Morgunova, E.; Tuuttila, A.; Bergmann, U.; Isupov, M.; Lindqvist, Y.; Schneider, G.; Tryggvason, K. *Science* **1999**, *284*, 1667.
15. Docking of compound **15** into the MMP-3 active site was based on the X-ray structure of a close analogue bound to catalytic domain MMP-3 (unpublished data).
16. Aqueous solubility was measured at pH 1, 5, 7, 9 and 11. Solution stability was measured after 15 min autoclave at 120 °C across the same range of pH. For a compound to be suitable as a candidate for topical delivery, it was felt that aqueous solubility needed to be >1 μg/ml to enable sufficient exposure within the site of action. In addition <5% degradation of parent after autoclave stability assessment was required for this sterilisation technique to be viable.
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