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THE SYNTHESIS AND BIOLOGICAL EVALUATION OF NON-PEPTIDIC MATRIX METALLOPROTEINASE INHIBITORS

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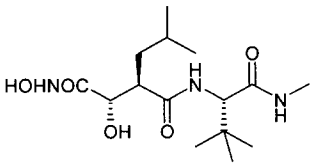
Abstract: Novel sulfonamide matrix metalloproteinase inhibitors of general formula (**9**) were synthesised by a route involving a stereoselective conjugate addition reaction. Enzyme selectivity was found to be dependant on the nature of the sulfonamide substituents. Compounds (**9f, 9q**) are potent selective collagenase inhibitors with good oral bioavailability.

Introduction

The matrix metalloproteinases (MMPs) are a family of zinc containing proteinases that collectively can degrade all of the major components of the extracellular matrix.[1] While MMP's play a role in many physiological processes, over production of MMPs is thought to be responsible for a range of biological processes observed in disease states such as arthritis[2] and cancer.[3] In the course of our MMP inhibitor (MMPI) programme, the succinyl hydroxamate based inhibitor marimastat (**1**) was identified.[4] This orally available broad spectrum MMPI is currently in human clinical trials for the treatment of cancer. In cancer individual MMPs are rarely, if ever, expressed singly but are produced co-ordinately in a group; thus a broad spectrum inhibitor is likely to be advantageous for the treatment of such a disease state. However, specific inhibition of the collagenases (MMP-1, MMP-8, MMP-13) represents an attractive target for the treatment of arthritis.[5]



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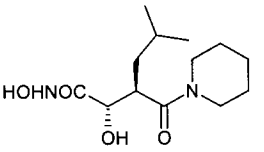

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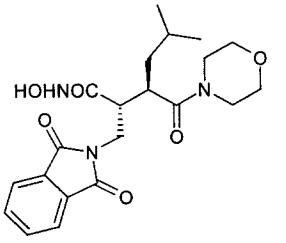

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During the course of our work on analogues of marimastat directed to the identification of selective inhibitors we chose to examine alternative groups in the position alpha to the hydroxamic acid zinc binding group (the P_1 group) capable of hydrogen bonding to the enzyme, in conjunction with truncation of the P_2' amino acid group. This

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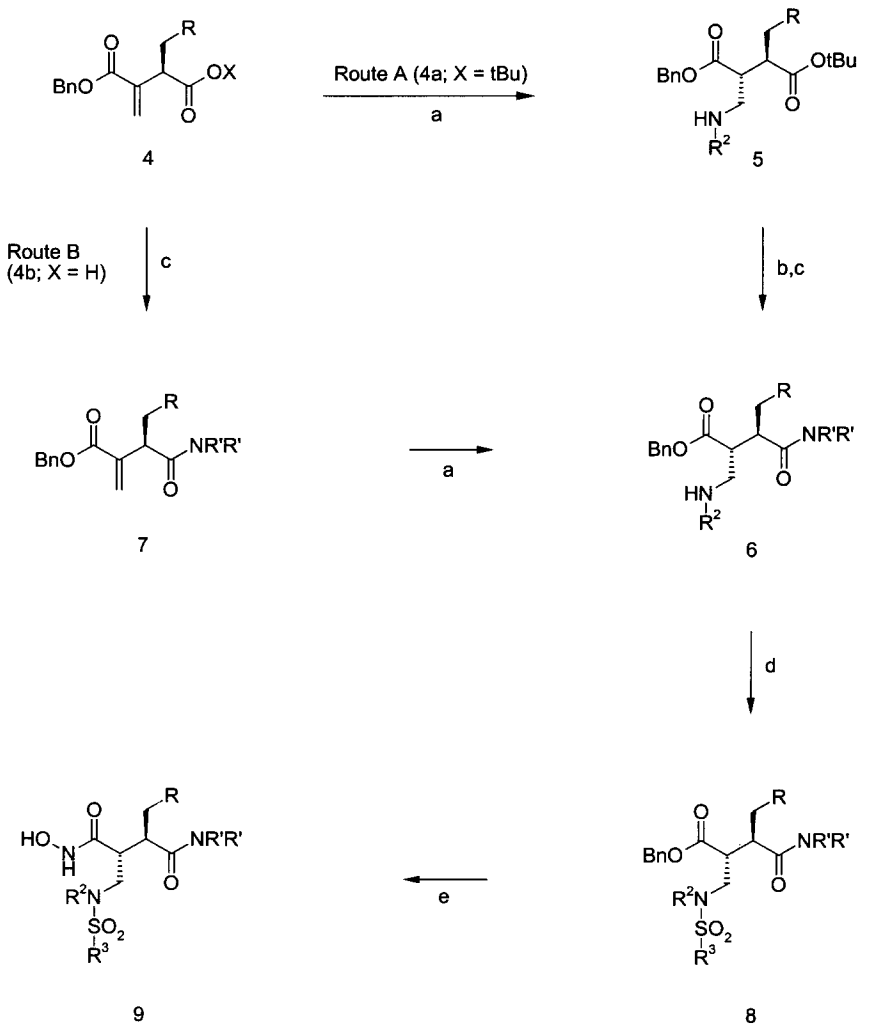
was because early MMP structural studies revealed hydrogen bonding interactions in the P_1 region to either the backbone of a conserved alanine residue (Ala-163 in MMP-8)[6a] or to the side chain of a variable residue (Asn-180 in MMP-1).[6] Replacement of the *tert*-butyl glycine P_2' fragment of marimastat with piperidine gave the truncated derivative (**2**). However, this compound possessed poor inhibitory potency in comparison to marimastat and was a weaker inhibitor of fibroblast collagenase (MMP-1) than the Roche compound (**3**)[8] (Table 1). We felt that the incorporation of a tertiary sulfonamido group at P_1 could fulfill a similar hydrogen bond acceptor role to that provided by a cyclic imido in this position[6c] as in the phthalimido derivative (**3**).[7] This letter describes the synthesis of novel MMPIs exploiting a stereoselective conjugate addition reaction[8a] for the generation of key intermediate (**6**) and the biological evaluation of the series of compounds generated from it by traditional and array methods.

Table 1: Inhibition of MMPs by compounds **1-3** (IC_{50} nM)

Compound	HFC MMP-1	Gel-A MMP-2	Strom-1 MMP-3	HNC MMP-8	Coll-3 MMP-13
1	5	6	200	2	2
2	800	500	60% @ 100,000	1,000	ND
3	20	2,000	5,000	600	100

Synthesis

The required homochiral benzyl α,β-unsaturated succinates (**4**) were prepared by literature methods.[8] In Route A (Scheme 1) stereoselective conjugate addition of various amines to **4a** gave β-amino benzyl esters (**5**) with high diastereomeric purity as determined from ^{1}H NMR analysis. The absolute stereochemistry (2*R*, 3*R*) is inferred from crystal studies performed on a related system by Todd.[8a] The amides (**6**) were obtained by sequential acidolysis and amide bond formation using standard peptide coupling conditions. Subsequent reaction with a variety of alkyl and arylsulfonyl chlorides gave the required sulfonamides (**8**). Hydrogenolysis of the benzyl ester group followed by coupling with hydroxylamine gave the hydroxamic acids (**9**). Rapid analogue generation was achieved in an array fashion by conversion of **4b** directly into the amide derivatives (**7**) prior to secondary amine formation (Route B; Scheme 1) and subsequent conversion to hydroxamic acid derivatives (**9**) as before. For the arrays, the overall yields were in the range (10-42%) following purification by automated reverse phase preparative HPLC.

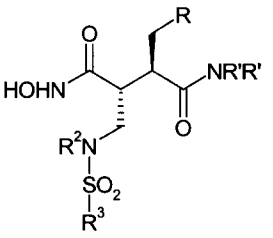


Scheme 1
Reagents and conditions a) R_2NH_2, MeOH, rt, overnight (34-88%); b) TFA, DCM (quant); c) EDC, HOBt, DCM, R'R'NH (60-88%); d) R_3SO_2Cl, DCM, Et_3N (62-90%); e) (i) HCO_2H, MeOH, 10% Pd-C (quant); (ii) HOBt, EDC, DMF, hydroxylamine.HCl, NMM, rt, overnight (22-26%).

Biological results

The *in vitro* MMP enzyme activities[9] for selected analogues are given in Table 2. As suggested in the literature[7] the amino acid residue in the P_2'-P_3' position of MMPIs, which is normally required for activity, may be truncated if a cyclic imide, for example a phthaloyl group as in **3,** occupies the α-position. This interaction was clearly not satisfied by an α-hydroxy group as in the truncated marimastat derivative **2**. The phthaloyl group is reported as

Table 2: Inhibition of MMPs by compounds **9a** - **9t** (IC_{50} nM)



Compound†	R	NR'R'*	R²	R³	HFC MMP-1	Gel. A MMP-2	Strom-1 MMP-3	HNC MMP-8	Coll-3 MMP-13
9a	i-propyl	pip	Me	Me	60	10000	4000	100	ND
9b	i-propyl	pip	Me	Et	50	2000	2000	100	ND
9c	i-propyl	pip	Me	Ph(4-OMe)	80	600	400	20	100
9d	i-propyl	pip	Me	Dansyl	6	80	60	10	ND
9e	i-propyl	NMe_2	Me	Me	1000	2000	ND	4000	2000
9f	c-pentyl	pip	Me	Me	6	900	200	200	400
9g	c-pentyl	pip	Me	Et	6	600	400	10	40
9h	c-pentyl	pip	Me	Ph(4-OMe)	10	60	60	3	20
9i	c-pentyl	pip	Me	Dansyl	2	20	7	2	8
9j	c-pentyl	pip	Me	naphthal-yl	6	50	50	4	8
9k	c-pentyl	pip	n-propyl	Me	30	3000	6000	90	200
9l	c-pentyl	pip	c-pentyl	Me	20	3000	10000	200	200
9m	c-pentyl	pip	c-propyl	Me	10	2000	2000	40	100
9n	c-pentyl	pip	i-propyl	Me	30	7000	7000	100	600
9o	c-pentyl	pip	Me	i-propyl	5	400	300	9	40
8p	c-pentyl	pip	Me	Ph(4-Cl)	9	100	30	4	10
9q	c-pentyl	pip	Me	NMe_2	6	300	400	10	40
9r	c-pentyl	pip	Me	CF_3	9	500	800	10	40
9s	c-pentyl	morp	Me	Me	5	400	800	20	80
9t	c-pentyl	morp	Me	Ph(4-OMe)	10	95	100	3	20

(* pip = piperidinyl, morp = morpholinyl; †All compounds gave satisfactory NMR spectra and analytical data. ‡Enzyme assays for MMP-1, MMP-3, MMP-8 and MMP-13 used human recombinant MMP enzymes and MMP-2 used wild type MMP enzyme according to according to standard protocols[9]).

Table 3: Oral Bioavailability data in the rat for selected inhibitors

Compound	C_{max} (ng/mL) [time]	AUC (ng/mL.h)
1	139 [30 min]	582.4
9f	171.8 [30 min]	662
9q	47.55 [6 h]	904

giving a specific H-bonding interaction between the inhibitor and MMP-1.[6c] We postulated that a sulfonamido group might also act as an H-bond acceptor. Thus, we prepared the compounds with a piperidine amide as the P_2'-P_3' substituent to give non-peptidic analogues (**9a-9r**). Interestingly sulfonamides bearing an alkyl substituent (**9a, 9b**) exhibit a degree of collagenase selectivity. However, inclusion of aryl sulfonamides again leads to a broader spectrum of MMP inhibition (**9c, 9d**). Whilst our investigations were in progress the Roche group reported that the replacement of the *iso*-butyl P_1' group with a cyclopentyl methyl group in their inhibitor series resulted in improved inhibition of the collagenases.[5b] Applying this modification to our series generally resulted in a similar improvement in potency (**9f-9j**). Changing the cyclic amido group (NR'R') to an acyclic tertiary amido group resulted in loss of activity; however no significant difference in potency between piperidine and morpholine amide analogues was observed (**9f** and **9s**; **9h** and **9t**). Variation of the substituent (R^2) on the nitrogen of the sulfonamide (**9k-9n**) does not greatly effect the inhibitor potency. The most significant factor effecting enzyme selectivity was found to be the nature of the sulfonyl substituent (R^3). Large aromatic groups (e.g. dansyl, naphth-l-yl) provide broad spectrum activity as in compounds **9i** and **9j**. Selective inhibition of the three collagenases is achieved by certain medium sized alkyl or other substituents (e.g. ethyl, i-propyl, NMe_2) as in compounds **9g, 9o** and **9q**. The methyl sulfonyl derivatives (e.g. **9f, 9s**) are most active against MMP-1.

Having identified compounds with good potency and interesting selectivity we examined their oral availability in a rat *ex vivo* bioassay.[10] Both the 'MMP-1 selective' compound **9f** and the 'MMP-1, MMP-8, MMP-13 selective' compound **9q** exhibit good blood levels following oral administration (Table 3). The blood levels for marimastat **1** are included as our standard.[4a]

Summary

We have shown that the introduction of a sulfonamido methylene group alpha to the hydroxamic acid in a non-peptidic succinate based series of MMPIs can provide different enzyme selectivity dependant on the nature of the sulfonyl substituent. Further increases in potency can be achieved by the introduction of a cyclopentyl methyl group in P_1'. Representatives of this series show good oral bioavailablity in the rat *ex-vivo* bioassay and are the subject of on going pharmocological evaluation.

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10. Groups of male rats (n=12) were dosed orally with the test compounds (10 mg/kg), serial blood samples were removed and MMPI activity extracted and assayed against HFC. Concentrations were calculated against a standard curve; Bone, E. A; Askew, M; Laber, D. unpublished results.