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α -Amino- β -Sulphone Hydroxamates as Potent MMP-13 Inhibitors That Spare MMP-1

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Abstract—A series of α -amino- β -sulphone hydroxamates was prepared and evaluated for potency versus MMP-13 and selectivity versus MMP-1. Various substituents were employed on the α -amino group (P₁ position), as well as different groups attached to the sulphone group extending into P₁'. Low nanomolar potency was obtained for MMP-13 with selectivity versus MMP-1 of >1000X for a number of analogues.

Matrix metalloproteinase (MMP) enzymes^{1,2} are endopeptidases that mediate the breakdown of structural proteins of the extracellular matrix and are involved in many normal physiological processes such as the remodeling of connective tissues and basement membranes.³ The activity of these enzymes is closely controlled through expression as a latent proform that requires prior activation for activity, and also through direct inhibition by tissue inhibitors of metalloproteinases (TIMPs). Elevated levels of MMPs are associated with certain pathologies including osteoarthritis (OA) and rheumatoid arthritis (RA). Recently, Freemont et. al.⁴ reported the use of in situ zymography to localize type II collagen degrading activity in osteoarthritic human articular cartilage, and in situ hybridization to localize MMP-13 mRNA. Significantly, they found that MMP-13 mRNA expression co-distributed with type II collagenase activity in articular chondrocytes, and that MMP-13 was upregulated in articular osteoarthritic cartilage but not in cartilage from normals. Thus inhibition of the relevant collagenase enzymes, particularly MMP-13, may prove to be clinically effective in halting the advance of OA and RA.⁵ Roche collagenase inhibitor **Ro 32-3555**⁶ inhibits collagenases 1, 2 and 3 (MMP-1, MMP-8, and MMP-13, respectively) and prevents cartilage breakdown both in vitro and in vivo. For the treatment of arthritis we targeted inhibitors of MMP-13 that spare interstitial collagenase (MMP-1) in order to avoid the musculoskeletal side effect observed clinically with the broad-spectrum inhibitor marimastat.⁷ The hypothesis⁸ that sparing MMP-1 will eliminate the fibroplasia seen with broad-spectrum inhibitors is still speculative. We wish to report preliminary SAR of a series of potent α -amino- β -sulphone^{9,10} hydroxamate MMP-13 inhibitors that are selective in sparing MMP-1.

Racemic α -amino compounds bearing methoxyphenyl sulphones and phenoxyphenyl sulphone moieties were prepared as shown in Scheme 1. The Michael acceptor methyl-2-acetamidoacrylate (**1**) was reacted with 4-methoxythiophenol ($\text{XR}^1 = \text{OMe}$) or 4-phenoxythiophenol, which was prepared via Newmann rearrangement¹¹ from 4-phenoxyphenol as we reported previously for our γ -sulphone thiol series of MMP inhibitors.¹² Oxone[®] treatment of the crude thioether afforded the sulphone **2** in excellent yield from the acrylate. Vigorous hydrolysis of **2** with concentrated hydrochloric acid in glacial acetic acid afforded the free amino acid which was re-esterified with thionyl chloride in methanol. Derivitization with the appropriate R^2 reagent and direct conversion of the methyl ester to the hydroxamate with aqueous hydroxylamine in methanol/tetrahydrofuran afforded the hydroxamic acid **3**.

Scheme 1

Racemic compounds bearing the phenylthiophenyl sulphone moiety were prepared as illustrated in Scheme 2. 4-Fluorothiophenol was added in a conjugate fashion to methyl-2-acetamidoacrylate (**1**), and treatment of the crude thioether with Oxone[®] gave the sulphone **4**. Controlled hydrolysis of the methyl ester gave the carboxylic acid with the acetamide intact. Nucleophilic aromatic displacement of the fluoride proceeded cleanly on the carboxylate salt to afford **5**, whereas attempts to perform the fluoride displacement directly on ester **4** did not afford the desired product due to competing beta-elimination. Hydrolysis of the acetamide **5** followed by derivitization with the appropriate R^2 reagent gave carboxylic acid **6**. The hydroxamic acid **7** was then prepared by employing the standard EDC/HOBT coupling with tetrahydropyranyl hydroxylamine followed by deprotection under acidic conditions.

Scheme 2

Chiral (*R*)- α -amino- β -phenylthiophenyl sulphones were synthesized from N-BOC-L-serine- β -lactone which was prepared from BOC-L-serine by the procedure of Vederas¹³ (Scheme 3). Nucleophilic ring-opening of **8** with sodium 4-fluorothiophenoxide gave the sulfide, which was oxidized directly with Oxone® to afford **9** with (*R*)-stereochemistry. Nucleophilic aromatic displacement of the aryl fluoride with thiophenol followed by acidic deprotection of the BOC group and acylation afforded the functionalized carboxylic acid, which was converted to hydroxamate **10** via coupling with THPONH₂ and deprotection.

Scheme 3

Chiral α -amino β -phenoxyphenyl sulphones were prepared by sodium thiolate displacement¹⁴ of the tosylate¹⁵ of Cbz-L-serine methyl ester (**11**) to afford **12** after oxidation with Oxone®. Hydrogenolysis, acylation and conversion of the methyl ester of **12** to the hydroxamate with hydroxylamine afforded compounds of type **13** with (*R*)-stereochemistry (Scheme 4). It may be noted that Schemes 3 and 4 are complementary with respect to amine protecting groups and also with respect to the state of the carboxylate. Specifically, the nucleophilic aromatic fluoride displacement accomplished on free carboxylic acid **9** was not viable on the corresponding Cbz-protected methyl ester prepared by the general method of Scheme 4 due to beta-elimination.

Scheme 4

Chiral (*R*)- α -amino β -phenoxyphenyl sulphones were prepared with minor modifications of the above procedures according to Scheme 5. Commercially available (*S*)-BOC-*N*-methyl-serine (**14**) was esterified with methyl iodide, then treated with 4-phenoxythiophenol under Mitsunobu conditions. Oxidation of the resulting sulfide with tetra-*N*-butylammonium Oxone® gave sulphone **15**. Removal of the *tert*-butylcarbamate protecting group under acidic conditions was followed by functionalization with the appropriate R² reagent. The methyl ester was converted directly to the hydroxamate **16** by treating with aqueous hydroxylamine, albeit in lower yields as expected, due to the increased steric hindrance in the α -position of the ester.

Scheme 5

We elected to initially to examine racemic α -amino- β -sulphones in order to explore potency for MMP-13 and selectivity versus MMP-1 (Table 1). A 4-methoxyphenyl sulphone moiety in P_1' ($XR^2 = \text{OMe}$) afforded lower potency and selectivity^{16–18} with the notable exception of compound **20** bearing a Cbz-glycine moiety in the P_1 position appended to the α -amino group. The Cbz-glycine group was selected to probe for an additional binding region to enhance potency. Incorporation of the phenylthiophenyl moiety ($XR^2 = \text{SPh}$) afforded an modest increase in potency (**17** vs **21**) and very high levels of selectivity (compounds **21–25**). The Cbz-glycine moiety again afforded a large jump in potency for MMP-13, which translated to exceptional selectivity ($>12,000\times$) versus MMP-1. Utilization of the diaryl ether in P_1' ($XR^1 = \text{OPh}$, **26–28**) led to compounds of excellent potency for MMP-13 but which had somewhat lower selectivity than the phenylthiophenyl compounds versus MMP-1.

Table 1 IC_{50} (nM)¹⁹ values for racemic α -amino- β -sulphone hydroxamates

Compound	XR^1	R^2	MMP-13	MMP-1	MMP-1/13
17	OMe	Ac	15	300	20
18	OMe	BOC	15	1500	100
19	OMe	H	15	250	17
20	OMe	CbzGly	<1.0	40	>40
21	SPh	Ac	5.9	$>10,000$	>1700
22	SPh	Cbz	3.7	$>10,000$	>2700
23	SPh	Tos	0.4	600	1500
24	SPh	H	24	$>10,000$	420
25	SPh	CbzGly	0.8	$>10,000$	$>12,000$
26	OPh	Ac	1.1	770	700
27	OPh	Cbz	1.1	1400	1300
28	OPh	Tos	0.6	400	670

Table 2 shows the enzyme results of chiral (*R*)- α -amino- β -sulphones derived from L-serine. Direct comparison of the single enantiomers and racemates of *N*-acetyl (**30** vs **21**) and *N*-Cbz (**31** vs **22**) showed an increase in potency expected for the eutomers, and an accompanying (apparent) increase in selectivity. Comparison of BOC derivatives **29** and **32** reveal again the order of magnitude increase in potency of the diphenyl ether versus the diaryl thioether (X = O vs X = S), although the thioethers are more selective. Hydrophilic groups including isonicotinyl (compound **33**) were incorporated into R² to increase water solubility, and the unsubstituted analog **34** (R² and R³ = H) was also prepared. Bulky groups (such as dimethoxybenzoyl in compound **34**) were introduced to increase the half-life of these compounds by adding steric hindrance to block the metabolism of the hydroxamate moiety. This was the reason for preparation of the disubstituted compounds **36–39** (R³ = CH₃). These compounds exhibited sub-nanomolar potency for MMP-13 and good selectivity versus MMP-1. The acetyl analog **36** showed unexpected potency versus MMP-1.

Table 2 IC₅₀ (nM)¹⁹ values for (*R*)- α -amino- β -sulphone hydroxamates

<u>Compound</u>	<u>X</u>	<u>R²</u>	<u>R³</u>	<u>MMP-13</u>	<u>MMP-1</u>	<u>MMP-1/13</u>
29	S	BOC	H	4.0	>10,000	>2500
30	S	Ac	H	2.9	10,000	3400
31	S	Cbz	H	0.4	8,000	20,000
32	O	BOC	H	0.3	500	1600
33	O	isonicotinyl	H	0.8	900	1100
34	O	2,6-dimethoxybenzoyl	H	0.4	350	870
35	O	H	CH ₃	0.4	440	1100
36	O	Ac	CH ₃	0.2	90	450
37	O	BOC	CH ₃	0.3	1600	5300
38	O	4-pyridineacetyl	CH ₃	2.0	258	130
39	O	benzyl	CH ₃	0.2	475	2400

Selected analogs were dosed orally in rats at 20 mpk to assess absorption by measuring C_{max}, and the concentration remaining at 6 h was used as an initial rough indicator of the half-life. *N*-Methyl derivative **35** showed a high C_{max} of 6.66 ug/mL, with 0.049 ug/mL remaining at 6 h, and acetyl analogue **36** exhibited a C_{max} of 2.45 ug/mL (0.060 ug/mL at 6 h). Pyridineacetyl analog **38** had a somewhat lower C_{max} of 0.71 ug/mL,

but was not detected at 6 h. These analogs exhibited higher $C_{\max}$ values than compounds **32**, **33**, and **34**, which exhibited $C_{\max}$ values of <0.2 ug/mL.

In summary, we have described a promising series of α -amino- β -sulphone hydroxamates that are potent inhibitors of MMP-13 that spare MMP-1. Potency and selectivity were modulated by varying the P_1' moiety (XR^1), and a wide variety of substituents are tolerated in the P_1 (solvent exposed) α -amino position of MMP-13. This position was utilized to modulate solubility and pharmacokinetic parameters. Compounds **35** and **36** showed good absorption when administered orally in the rat. The activity of these MMP-1 sparing β -sulphone hydroxamates in animal models of arthritis and cancer will be disclosed in due course.

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