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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) enzymes1,2 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 32-35556 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-sulphone9,10 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 (XR1 = OMe) or 4-phenoxythiophenol, which was prepared via Newmann rearrangement\textsuperscript{11} from 4-phenoxyphenol as we reported previously for our γ-sulphone thiol series of MMP inhibitors.\textsuperscript{12} Oxone\textsuperscript{®} 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\textsuperscript{2} reagent and direct conversion of the methyl ester to the hydroxamate with aqueous hydroxylamine in methanol/tetrahydrofuran afforded the hydroxamic acid 3.

\begin{align*}
\text{MeO}_2\text{C} & \equiv \text{AcNH} \\
\text{HCl, HOAc} & \rightarrow \text{MeO}_2\text{C} \equiv \text{NH} \\
\text{SOCl}_2 & \rightarrow \text{MeO}_2\text{C} \equiv \text{NH} \\
\text{R}^2 & \text{derivitization} \\
\text{HONH}_2 &
\end{align*}

\textbf{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\textsuperscript{®} 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\textsuperscript{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.

\begin{align*}
\text{MeO}_2\text{C} & \equiv \text{AcNH} \\
\text{HCl, HOAc} & \rightarrow \text{MeO}_2\text{C} \equiv \text{NH} \\
\text{HONH}_2 & \rightarrow \text{MeO}_2\text{C} \equiv \text{NH} \\
\text{R}^2 & \text{derivitization} \\
\text{HONH}_2 &
\end{align*}

\textbf{Scheme 2}
Chiral (R)-\(\alpha\)-amino-\(\beta\)-phenylthiophenyl sulphones were synthesized from N-BOC-L-serine-\(\beta\)-lactone which was prepared from BOC-L-serine by the procedure of Vederas\(^{13}\) (Scheme 3). Nucleophilic ring-opening of 8 with sodium 4-fluorothiophenoxide gave the sulfide, which was oxidized directly with Oxone\(^\circ\) 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 THPONH\(_2\) and deprotection.

Scheme 3

Chiral \(\alpha\)-amino \(\beta\)-phenoxyphenyl sulphones were prepared by sodium thiolate displacement\(^{14}\) of the tosylate\(^{15}\) of Cbz-L-serine methyl ester (11) to afford 12 after oxidation with Oxone\(^\circ\). 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)-\(\alpha\)-amino \(\beta\)-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\(^\circ\) gave sulphone 15. Removal of the tert-butylcarbamate protecting group under acidic conditions was followed by functionalization with the appropriate R\(^2\) 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 \(\alpha\)-position of the ester.
Scheme 5

We elected to initially 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 P1’ (XR2 = OMe) afforded lower potency and selectivity\textsuperscript{16–18} with the notable exception of compound 20 bearing a Cbz-glycine moiety in the P1 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 (XR2 = SPh) afforded a 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 (>12,000X) versus MMP-1. Utilization of the diaryl ether in P1’ (XR1 = 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\textsubscript{50} (nM)\textsuperscript{19} values for racemic α-amino-β-sulphone hydroxamates

<table>
<thead>
<tr>
<th>Compound</th>
<th>XR\textsuperscript{1}</th>
<th>R\textsuperscript{2}</th>
<th>MMP-13</th>
<th>MMP-1</th>
<th>MMP-1/13</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>OMe</td>
<td>Ac</td>
<td>15</td>
<td>300</td>
<td>20</td>
</tr>
<tr>
<td>18</td>
<td>OMe</td>
<td>BOC</td>
<td>15</td>
<td>1500</td>
<td>100</td>
</tr>
<tr>
<td>19</td>
<td>OMe</td>
<td>H</td>
<td>15</td>
<td>250</td>
<td>17</td>
</tr>
<tr>
<td>20</td>
<td>OMe</td>
<td>CbzGly</td>
<td>&lt;1.0</td>
<td>40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>21</td>
<td>SPh</td>
<td>Ac</td>
<td>5.9</td>
<td>&gt;10,000</td>
<td>&gt;1700</td>
</tr>
<tr>
<td>22</td>
<td>SPh</td>
<td>Cbz</td>
<td>3.7</td>
<td>&gt;10,000</td>
<td>&gt;2700</td>
</tr>
<tr>
<td>23</td>
<td>SPh</td>
<td>Tos</td>
<td>0.4</td>
<td>600</td>
<td>1500</td>
</tr>
<tr>
<td>24</td>
<td>SPh</td>
<td>H</td>
<td>24</td>
<td>&gt;10,000</td>
<td>420</td>
</tr>
<tr>
<td>25</td>
<td>SPh</td>
<td>CbzGly</td>
<td>0.8</td>
<td>&gt;10,000</td>
<td>&gt;12,000</td>
</tr>
<tr>
<td>26</td>
<td>OPh</td>
<td>Ac</td>
<td>1.1</td>
<td>770</td>
<td>700</td>
</tr>
<tr>
<td>27</td>
<td>OPh</td>
<td>Cbz</td>
<td>1.1</td>
<td>1400</td>
<td>1300</td>
</tr>
<tr>
<td>28</td>
<td>OPh</td>
<td>Tos</td>
<td>0.6</td>
<td>400</td>
<td>670</td>
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Table 2 shows the enzyme results of chiral \((R)\-\alpha\)-amino-\(\beta\)-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 \((X = O \text{ vs } X = S)\), although the thioethers are more selective.

Hydrophilic groups including isonicotinyl (compound 33) were incorporated into \(R^2\) to increase water solubility, and the unsubstituted analog 34 \((R^2 \text{ and } R^3 = H)\) 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 \((R^3 = \text{CH}\_3)\). 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 IC\(_{50}\) (nM)\(^{19}\) values for \((R)\-\alpha\)-amino-\(\beta\)-sulphone hydroxamates

<table>
<thead>
<tr>
<th>Compound</th>
<th>(X)</th>
<th>(R^2)</th>
<th>(R^3)</th>
<th>MMP-13</th>
<th>MMP-1</th>
<th>MMP-1/13</th>
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<tbody>
<tr>
<td>29</td>
<td>S</td>
<td>BOC</td>
<td>H</td>
<td>4.0</td>
<td>&gt;10,000</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>30</td>
<td>S</td>
<td>Ac</td>
<td>H</td>
<td>2.9</td>
<td>10,000</td>
<td>3400</td>
</tr>
<tr>
<td>31</td>
<td>S</td>
<td>Cbz</td>
<td>H</td>
<td>0.4</td>
<td>8,000</td>
<td>20,000</td>
</tr>
<tr>
<td>32</td>
<td>O</td>
<td>BOC</td>
<td>H</td>
<td>0.3</td>
<td>500</td>
<td>1600</td>
</tr>
<tr>
<td>33</td>
<td>O</td>
<td>isonicotinyl</td>
<td>H</td>
<td>0.8</td>
<td>900</td>
<td>1100</td>
</tr>
<tr>
<td>34</td>
<td>O</td>
<td>2,6-dimethoxybenzoyl</td>
<td>H</td>
<td>0.4</td>
<td>350</td>
<td>870</td>
</tr>
<tr>
<td>35</td>
<td>O</td>
<td>H</td>
<td>CH_3</td>
<td>0.4</td>
<td>440</td>
<td>1100</td>
</tr>
<tr>
<td>36</td>
<td>O</td>
<td>Ac</td>
<td>CH_3</td>
<td>0.2</td>
<td>90</td>
<td>450</td>
</tr>
<tr>
<td>37</td>
<td>O</td>
<td>BOC</td>
<td>CH_3</td>
<td>0.3</td>
<td>1600</td>
<td>5300</td>
</tr>
<tr>
<td>38</td>
<td>O</td>
<td>4-pyridineacetyl</td>
<td>CH_3</td>
<td>2.0</td>
<td>258</td>
<td>130</td>
</tr>
<tr>
<td>39</td>
<td>O</td>
<td>benzyl</td>
<td>CH_3</td>
<td>0.2</td>
<td>475</td>
<td>2400</td>
</tr>
</tbody>
</table>

Selected analogs were dosed orally in rats at 20 mpk to assess absorption by measuring \(C_{\text{max}}\), 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 \(C_{\text{max}}\) of 6.66 ug/mL, with 0.049 ug/mL remaining at 6 h, and acetyl analogue 36 exhibited a \(C_{\text{max}}\) of 2.45 ug/mL (0.060 ug/mL at 6 h). Pyridineacetyl analog 38 had a somewhat lower \(C_{\text{max}}\) of 0.71 ug/mL,
but was not detected at 6 h. These analogs exhibited higher $C_{\text{max}}$ values than compounds 32, 33, and 34, which exhibited $C_{\text{max}}$ values of <0.2 ug/mL.

In summary, we have described a promising series of $\alpha$-amino-$\beta$-sulphone hydroxamates that are potent inhibitors of MMP-13 that spare MMP-1. Potency and selectivity were modulated by varying the P1’ moiety (XR1), and a wide variety of substituents are tolerated in the P1 (solvent exposed) $\alpha$-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 $\beta$-sulphone hydroxamates in animal models of arthritis and cancer will be disclosed in due course.

References and Notes


19. Inhibitors were assayed against purified hMMP-13 and hMMP-1 using an enzyme assay based on cleavage of the fluorogenic peptide MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂. This is similar to conditions described by C. G. Knight et al. in *FEBS Lett.* **1992**, *296*, 263, except that the assay buffer contained 0.02% 2-mercaptoethanol (Sigma). All basic compounds were tested as their hydrochloride salts.