Orally Bioavailable Dual MMP-1/MMP-14 Sparing, MMP-13 Selective Alpha-sulfone Hydroxamates

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Orally Bioavailable Dual MMP-1/MMP-14 Sparing, MMP-13 Selective α-Sulfone Hydroxamates

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Abstract—A series of phenyl piperidine α-sulfone hydroxamate derivatives has been prepared utilizing a combination of solution-phase and resin-bound library technologies to afford compounds that are potent and highly selective for MMP-13, are dual sparing of MMP-1 and MMP-14 (MT1-MMP) and exhibit oral bioavailability in rats.

The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that degrade all components of the extracellular matrix.1,2 There are at least 24 isozymes in the MMP family, and they are roughly classified on the basis of their substrate specificity: collagenases (MMP-1, -8, -13 and -18), gelatinases (MMP-2, and-9), stromelysins (MMP-3, -10 and -11) and membrane-type MMPs (MMP-14, -15, -16, -17, and -24) and others (MMP-7, -11, -12, -19, -20, -21, -22, and -23). Clinical experience with the pan MMP inhibitor Marimastat has revealed a constellation of adverse effects collectively referred to as musculoskeletal syndrome (MSS).3 We have hypothesized that this is predominantly a result of inhibiting both MMP-1 and MMP-14, and that MMP inhibitors sparing these two isozymes should be devoid of MSS.4 Toward obtaining efficacy in mitigating damage suffered in osteoarthritic patients, it has been reported that MMP-13 mRNA levels are increased in osteoarthritic cartilage.5 Thus, we envisioned the development of a selective inhibitor of MMP-13 sparing both MMP-1 and MMP-14 as a safe means of treating osteoarthritis (OA) which we refer to as the dual-sparing hypothesis.

Figure 1. 4-Substituted piperidine/piperazine sulfone hydroxamic acids
In the preceding paper, we demonstrated that the N-substituted phenyl isonipecotamide hydroxamic acid template 1 (Figure 1) yielded potent and selective MMP-13 inhibitors. Figure 2 shows a sequence comparison for a set of MMP family members focused on the S₁’ loop. There are differences in both amino acid identity and in length of the loop, as MMP-1, -2 and -9 are two residues shorter than MMP-13, -8 and -14. Interaction of the amide N-substituents of 1 deep in the S₁’ pocket was expected to affect isozyme selectivity across the MMP family. In an effort to further investigate SAR in this region, we designed templates 2 and 3, where the distal phenyl group resides in approximately the same region as the amide N-substituents of 1. To explore the effect of substituents on the distal aryl rings of 2 and 3, we undertook a parallel synthetic approach with the goal of optimizing MMP-13 potency and selectivity.

Figure 2: S₁’ loop sequence variation across selected MMP family members

<table>
<thead>
<tr>
<th>Figure</th>
<th>MMP13</th>
<th>MMP-8</th>
<th>MMP-2</th>
<th>MMP-9</th>
<th>MMP-14</th>
<th>MMP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DPGALMFP IYTYTGKSHFML</td>
<td>DPGALMPNYAF RE TSNYSL</td>
<td>DPGALMPIYTYT KNFRL</td>
<td>VPEALMPYMYRFT EG PF L</td>
<td>DPSALMPY QWMDT ENFVL</td>
<td>DIGALMYPSYTFS GDVQL</td>
</tr>
</tbody>
</table>

A solid-phase parallel synthesis approach was used to create a small library of N-arylpiperazine α-sulfone hydroxamic acid derivatives (2) from commercially available N-arylpiperazines 5. Nucleophilic aromatic substitution of the previously reported polymer-bound aryl fluoride 4 with N-arylpiperazines was found to require a 10-fold excess of 5 and presence of 2 equivalents of cesium carbonate in N-methylpyrrolidinone (NMP) at 100 degrees Celsius overnight to achieve good conversions. Acidic deprotection with trifluoroacetic acid afforded α-sulfone hydroxamic acids 2 in good yields (Scheme 1).

Scheme 1. Solid phase synthesis of N-arylpiperazine sulfone hydroxamates 2
Alternatively, a solution phase approach was developed to synthesize analogs with a basic amine in the α-heterocycle (8). THP-protected hydroxymate 7 was obtained by reaction of carboxylic acid 6 with THP-protected hydroxylamine using the water-soluble carbodiimide EDC.\textsuperscript{6} Subsequent nucleophilic aromatic substitution with the requisite N-arylpiperazines followed by acidic deprotection afforded α-sulfone hydroxamic acids 6 in high yields.

**Scheme 2.** Solution phase synthesis of N-arylpiperazine sulfone hydroxamic acids 8

\[
\begin{align*}
\text{HO}_2\text{C-SO}_2\text{N} & \quad \text{THPONH}_2 \\
\text{6} & \quad \text{EDC, HOBt, NMM} \\
& \quad \text{THPO} N \text{O} \quad \text{SO}_2 \text{N} \\
\text{7} & \quad \text{6} & \quad \text{Ar} \\
1) \text{HN} & \quad \text{N-Ar} \\
2) \text{HCl, dioxane} & \quad \text{MeOH} \\
\text{8} & \quad \text{N-BOC} & \quad \text{4-aryl tetrahydroopyridine}
\end{align*}
\]

For the synthesis of 4-arylpiperidine phenyl sulfones 3, the required 4-aryl piperidines 11 were purchased commercially or prepared either via the Grignard route (Scheme 3) or via Suzuki coupling (Scheme 4). For the Grignard route, similar to the method of Burns,\textsuperscript{7} the appropriate aryl Grignard was added to N-benzyl piperidine-4-one followed by dehydration with TFA in methylene chloride. Reduction then gave 4-arylpiperidines 11. Alternatively, enol triflate 12 was reacted with the requisite arylboronic acid with a catalytic amount of tetrakistriphenylphosphine palladium to afford N-BOC-4-aryl tetrahydropyridine 13, following the procedure of Wustrow and Wise\textsuperscript{8} (Scheme 2). Catalytic hydrogenation of 13 followed by removal of the N-Boc protecting group afforded 4-arylpiperidines 11.

**Scheme 3.** Syntheses of 4-arylpiperidines via the Grignard route

\[
\begin{align*}
\text{N} & \quad \text{Bn} \\
9 & \quad \text{1) Ar-MgBr} \\
& \quad \text{2) TFA/CH}_2\text{Cl}_2 \\
\text{N} & \quad \text{Bn} \\
10 & \quad \text{Ar} \\
& \quad \text{HCO}_2\text{NH}_4 \\
\text{Ar} & \quad \text{Pd/C, MeOH} \\
11 & \quad \text{N}
\end{align*}
\]

**Scheme 4:** Syntheses of 4-arylpiperidines via Suzuki coupling
Assembly of the N-arylpiperidine sulfone hydroxamic acids 3 was accomplished by a solution phase approach (Scheme 5) similar to that described above. Carboxylic acid 14 was coupled with THP-protected hydroxylamine, then aromatic nucleophilic substitution with 11 followed by acidic deprotection afforded α-sulfone hydroxamic acids 3 in good yields.

Scheme 5. Solution phase synthesis of 4-arylpiperidine sulfone hydroxamates

The MMP inhibitory potency values for N-aryl piperazine α-sulfone hydroxamic acids (2 and 8) are summarized in Table 1. All compounds were determined to have no measurable potency at MMP-1 (IC$_{50} >$10,000 nM), hence selectivity for MMP-13 over MMP-1 is >1000X in most cases. The N-arylpiperazines (2a-2j and 8a-8b) exhibited mostly single-digit nanomolar potency for MMP-13, but most were also potent for MMP-2, resulting in only very modest selectivity for MMP-13 over MMP-2 with a number of compounds which were nearly equipotent. N-Phenyl piperazine 2a was very potent for MMP-13 (IC$_{50} = 1.7$ nM) with a 14-fold selectivity versus MMP-2, and nearly 6000-fold selectivity versus MMP-14, whereas the corresponding α-piperidine 8a was nearly equipotent at MMP-13 and MMP-2 (IC$_{50} = 3.3$ and 5.4 nM, respectively.) The reduction in selectivity due to the change from $X = O$ to $X = N$-cyclopropyl was unexpected given the continuity between α-tetrahydropyran and α-piperidine analogs in our earlier MMP-1 sparing series, although this single pair does not necessarily constitute a trend. The ortho-fluorinated derivative 2b exhibited similar potency to the N-phenyl parent 2a, whereas the bulkier ortho-methyl and chloro derivatives 2c-2d dropped five-fold in potency for MMP-13, and the ortho-methoxy derivative 2e dropped 76-fold in potency. The meta-derivatives 2f and 2g suffered a similar loss in potency for MMP-13. On the other hand, para-substituted derivatives maintained high potency and selectivity for MMP-13, in particular para-methoxy derivative 2h with an IC$_{50} = 0.5$ nM for MMP-13, a 40-fold selectivity versus MMP-2, and the highest selectivity observed versus MMP-14 (>20,000). para-Methyl and para-
trifluoromethyl derivatives 2i and 8b exhibited good potency for MMP-13 (1.9 and 2.4 nM, resp.) and selectivity versus MMP-14 (both >4000X), noting that 8b is an α-piperidine. The more sterically demanding 2,4-dimethylphenylpiperazine 2j suffered a drop in potency at MMP-13 (IC50 = 28.6 nM), although selectivity against MMP-2 was the highest of all N-arylpipersazines at approximately 50X.

Table 2 shows MMP inhibitory potencies of 4-aryl piperidine α-sulfone hydroxamates (3). 4-Phenylpiperidine 3a was 3X less potent at MMP-13 than N-phenylpiperazine 2a but its potency for MMP-2 increased to 4.4 nM, making 3a equipotent for MMP-13 and MMP-2. A substantial boost in MMP-13 selectivity was achieved by the presence of an ortho-methoxy substituent (3b). Potency of 3b for MMP-13 dropped 3-fold from the parent compound (3a), while MMP-2 potency dropped 840-fold, generating a selectivity ratio of 211X. On the other hand, para-chloro analog 3c was found to have an increased potency relative to parent compound 3a at both MMP-13 and -2. The substantial effect of ortho substitution on selectivity prompted further evaluation of additional ortho-substituted analogs (3d-i). Generally, MMP-13 potencies were similar and reduced compared to 3a, but IC50’s for MMP-2 (and thus the MMP-2/13 selectivity ratio) corresponded approximately to the size of the substituent, with methoxy being optimal: H < Cl, OH < CH3, CF3 < OMe, OEt, 4-F-C6H4. The effect of an additional substituent was explored in an attempt to increase potency for MMP-13 while maintaining micromolar affinity for MMP-2. The 1-naphthyl derivative 3j was slightly more potent than the ortho-methoxy analog 3b at MMP-13, but potency at MMP-2 increased 7-fold. Other disubstituted analogs (3k-n) showed a similar trend, except for 3n with a 2-methoxy and a 5-isopropyl substitution where MMP-13 potency dropped 4-fold. Presumably the decreased affinity for MMP-13 was due to steric reasons. Comparison of the MMP-2/13 selectivity for ortho-methoxy substituted N-aryl piperazine 2e (2.8-fold) with that of the 4-arylpiperidine analog 3b (211-fold) is noteworthy. Presumably, 3b adopts a conformation where the aryl group is orthogonal to the piperidine ring, evidenced by the substantial effect of ortho-substitution on selectivity. The energetic penalty for an N-aryl piperazine to adopt such a conformation would be high, which is likely responsible for the reduced potency of 2e at MMP-13 and the increased potency at MMP-2 relative to 3b.

Based on the superior MMP-13 potency and dual MMP-1 and -14 sparing profiles of the p-substituted N-aryl piperazines, additional analogs were prepared for more thorough enzyme and PK evaluation (Table 3). Potent MMP-13 inhibition was observed for compounds 2l, 2m and 8c with IC50’s of 0.6, 1.0, 0.5 nM, respectively. Selectivity versus other MMP family members was generally >>100-fold except for MMP-2 (4-20 fold) and MMP-3 (58-500 fold). Rat PK for these three compounds showed low to moderate values for half-life and bioavailability. Aryl piperazine 8c had an acceptable BA of 20.7%, but a very short t1/2 of only 0.24h. Aryl piperidine 2l exhibited a modest bioavailability of 16%, but a much improved half-life of 2.59 h, which we attribute to the trifluoromethylphenyl moiety in P1’, which has enhanced the PK of other series as well. 4-Chlorophenyl piperidine 2m possessed a longer half-life but a disappointing BA of only 7.4%. Included for comparison is broader-spectrum, MMP-1 sparing α-sulfone SC-276.6 Compound SC-276 lacks selectivity among MMPs, only significantly sparing MMP-1, yet this compound has the very high exposure in the rat that is compelling for development, consistent with its potent and efficacious antitumor activity.6
In summary, the related series of compounds described herein have demonstrated single-digit to sub-nanomolar potency for MMP-13 combined with exceptional selectivity versus MMP-1 and MMP-14 of typically >100X up to 20,000X. Selectivities versus other MMPs when tested varied for MMP-3 (40-500X), MMP-8 (140-2500X) and for MMP-9 (20 to >4000X). Selectivity for MMP-13 over MMP-2 ranged from equipotency to 200X, thus selectivities were somewhat lower relative to the related isonipecotate α-sulfone hydroxamate series.

Rat PK for selected members of these series demonstrated bioavailabilities of up to 20.7% (8c) and a half lives of up to 2.6 h (2l) yet individual compounds lacked a compelling complete package to initiate development. Undoubtedly the high molecular weights of these analogs (551 a.u. for 2m) plays a role given the recommendations of Lipinski although the limited number of rotatable bonds favors the limited bioavailability that was observed. We therefore turned our attention to lower molecular weight species, while applying our learnings about P₁' manipulations toward optimizing MMP-13 selectivity.

References and Notes