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

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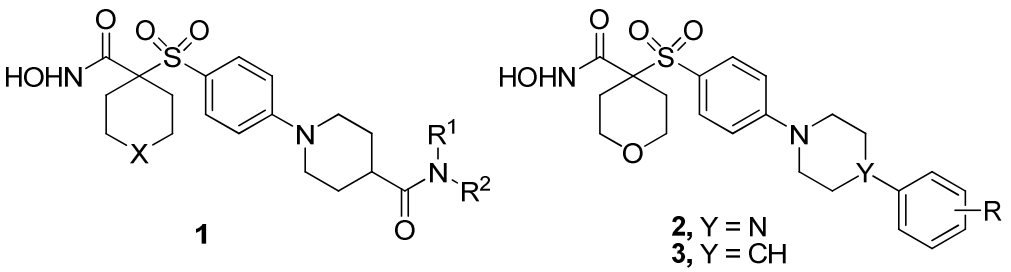
Abstract—A series of phenyl piperidine α-sulfone hydroxamate derivatives has been prepared utilizing a combination of solution-phase and resin-bound library technologies to afford compounds that are potent and highly selective for MMP-13, are dual sparing of MMP-1 and MMP-14 (MT1-MMP) and exhibit oral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

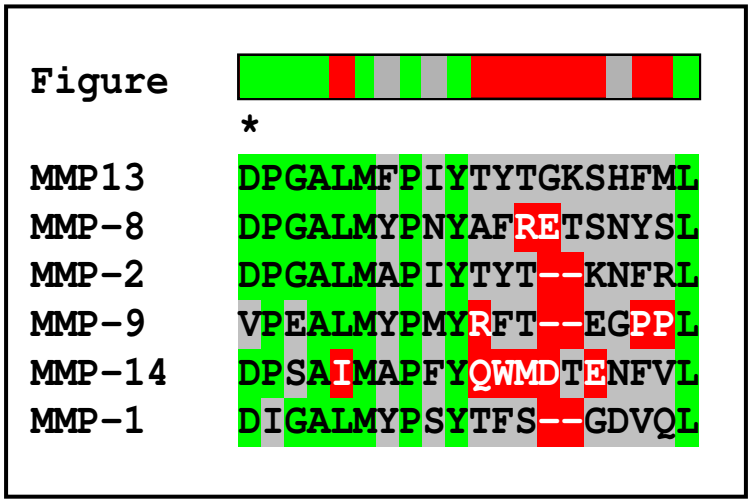
The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that degrade all components of the extracellular matrix.[1-2] There are at least 24 isozymes in the MMP family, and they are roughly classified on the basis of their substrate specificity: collagenases (MMP-1, -8, -13 and -18), 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



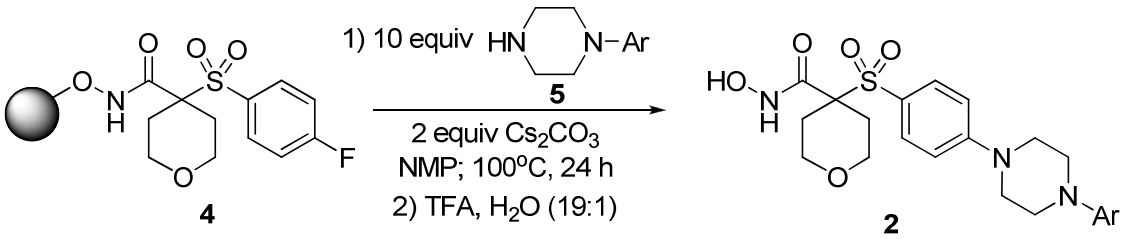
In the preceding paper,[4] we demonstrated that the N-substituted phenyl isonipecotamide hydroxamic acid template **1** (Figure 1) yielded potent and selective MMP-13 inhibitors. Figure 2 shows a sequence comparison for a set of MMP family members focused on the S_1' loop. There are differences in both 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 S_1' 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



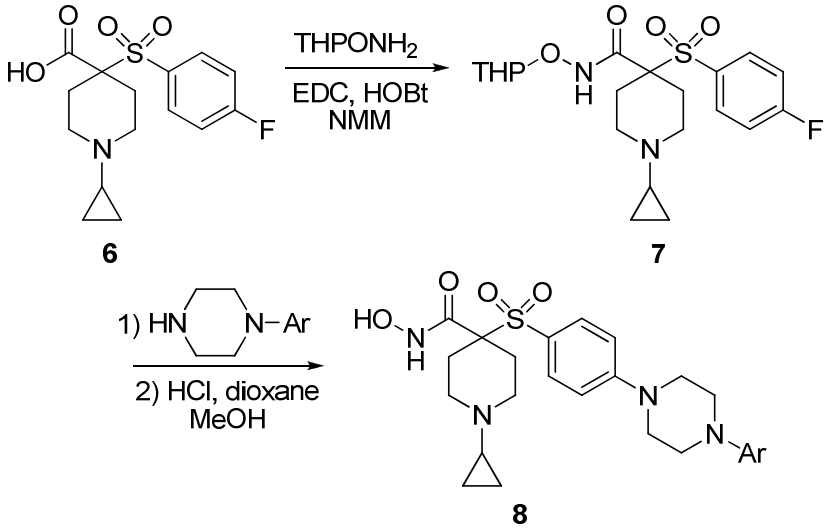
A solid-phase parallel synthesis approach was used to create a small library of N-arylpiperazine α-sulfone hydroxamic acid derivatives (**2**) from commercially available N-aryl piperazines **5**. Nucleophilic aromatic substitution of the previously reported polymer-bound aryl fluoride **4**[4] with 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 over night 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.[6] Subsequent nucleophilic aromatic substitution with the requisite N-arylpiperazines followed by acidic deprotection afforded α-sulfone hydroxamic acids **6** in high yields.

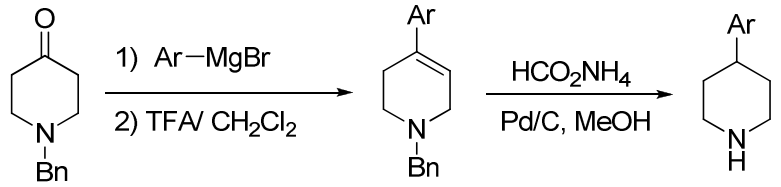
Scheme 2. Solution phase synthesis of N-arylpiperazine sulfone hydroxamic acids **8**



6 **7** **8**

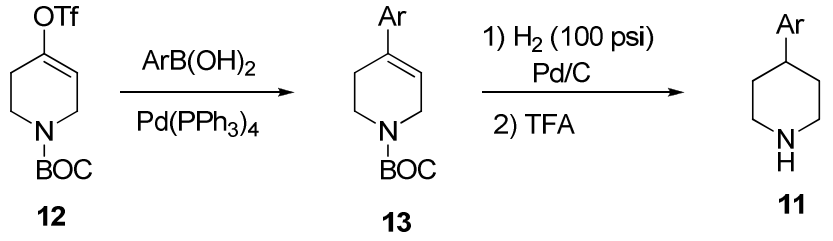
For the synthesis of 4-arylpiperidine phenyl sulfones **3,** the required 4-aryl piperidines **11** were purchased commercially or prepared either via the Grignard route (Scheme 3) or via Suzuki coupling (Scheme 4). For the Grignard route, similar to the method of Burns,[7] the appropriate aryl Grignard 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[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



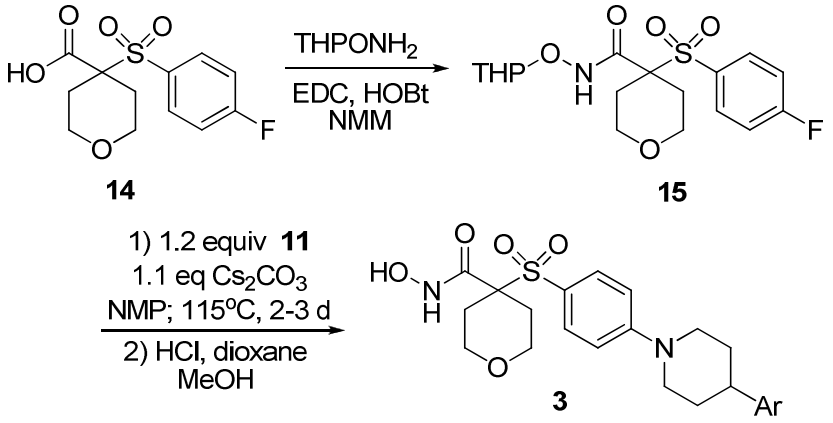
9 **10** **11**

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**[6] 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-aryl piperazines (**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,[6] 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 (IC_{50} = 28.6 nM), although selectivity against MMP-2 was the highest of all N-arylpiperazines 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 IC_{50}'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 < CH_3, CF_3 < OMe, OEt, 4-F-C_6H_4. 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 IC_{50}'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 $t_{1/2}$ 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 P_1', 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.[4] 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[9] favors the limited bioavailability that was observed. We therefore turned our attention to lower molecular weight species, while applying our learnings about P_1' manipulations toward optimizing MMP-13 selectivity.

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