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Discovery of 3-OH-3-methylpipecolic hydroxamates: Potent orally active inhibitors of aggrecanase and MMP-13

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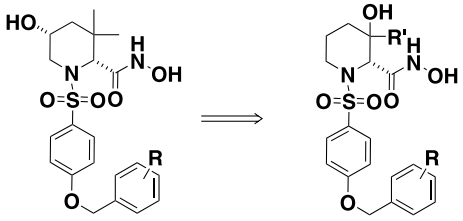
Abstract—A series of 3-hydroxy-3-methylpipecolic hydroxamate inhibitors of MMP-13 and aggrecanase was designed based on the observation of increased aggrecanase activity with substitution at the 3-position of the piperidine ring. Potency versus aggrecanase was optimized by modification of the benzyloxyarylsulfonamide group that binds in the S1′ pocket. These compounds also possess markedly improved bioavailability and lower metabolic clearance compared to analogous 3,3-dimethyl-5-hydroxypipecolic hydroxamates. These improvements are attributed to lowered lipophilicity proximal to the metabolically labile hydroxamic acid. Synthesis, structure activity relationships, and in vivo efficacy data are described.

Osteoarthritis is a highly debilitating disease characterized by progressive cartilage degradation that leads to pain and reduced motility in affected joints. The degeneration of proteoglycan and disruption of the type II collagen network in cartilage are pathological processes that reduce joint function.[1] Inhibition of the enzymes that mediate cartilage catabolism (aggrecanases and collagenases) is a strategy for developing disease modifying therapies for osteoarthritis. Previously, we described the discovery of potent inhibitors of MMP-13 (collagenase **3**) and aggrecanase activity with selectivity versus MMP-1 and TACE based on the series exemplified by **1**.[2] Herein we describe another series of pipecolic hydroxamates with similar functional activity but greatly improved metabolic stability and bioavailability.

Appropriately substituted hydroxamic acids are effective competitive inhibitors of matrix metalloproteases, ADAMS and ADAM-TS family members. Structural studies point to a critical bidentate interaction between the active site zinc atom and the hydroxamic acid ligand with additional hydrogen bond interactions with the active site glutamic acid residue and other H-bond donor and acceptor groups present in the protein.[3] However, the presence of a hydroxamic acid presents significant challenges for the optimization of pharmacokinetic properties necessary for an effective oral therapy. The hydroxamic acid group is particularly prone to hydrolysis, reduction, and glucuronidation.[4] As part of the strategy to reduce the metabolic lability of our lead aggrecanase series **1**, we explored the effects of introducing polar functionality at the 3-position of the piperidine ring as exemplified by **2**.



1 2

The synthesis of these compounds was carried out as described in Scheme 1. Starting with glycine *tert*-butyl ester, sulfonylation with benzyloxyphenylsulfonyl chloride afforded the sulfonamide **4** in 81% yield. Alkylation of

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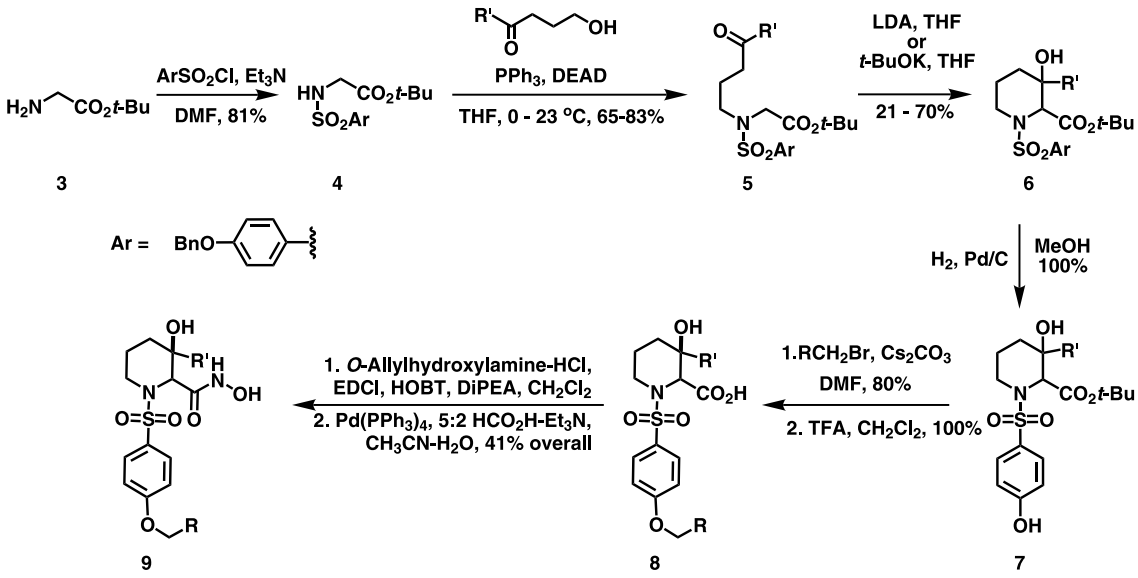
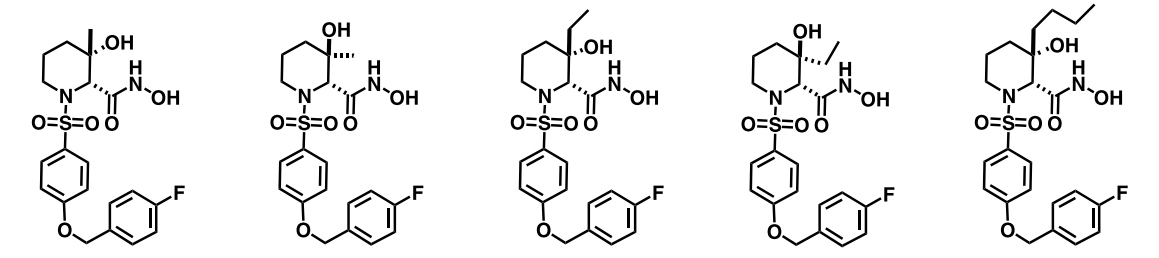


Scheme 1. Synthesis of 3-hydroxy-3-alkylpipecolic-3-hydroxamic acids from glycine *tert*-butyl ester.

the sulfonamide nitrogen using the appropriate γ-hydroxyketone under Mitsunobu conditions afforded the cyclization precursor **5** in moderate yield. Intramolecular aldol condensation proceeded in somewhat variable yield that was dependent on the size of the ketone substituent R′ and the type of base. For substrate **5** where R′ = methyl, potassium *tert*-butoxide was the optimum base, affording the cyclization product in ca. 70% yield. However, compounds with larger R′ groups failed to cyclize under these conditions, and a much cleaner reaction was observed using LDA in THF initially at −78 °C. In either case, a 1:1 to 8:1 mixture of diastereomers was obtained, depending on the size of the R′ group. The resulting pipecolate ester diastereomers could be separated by silica gel chromatography. Preparative chiral separation using a Chiralpak AD column gave each of the four stereoisomers of **6**. Deprotection of the phenol, followed by alkylation with the appropriate alkyl halide and acidic removal of the *tert*-butyl ester afforded the pipecolic acid **8**. Formation of the hydroxamate was best accomplished by coupling with *O*-allylhydroxylamine–HCl in the presence of EDCI and HOBT with sufficient Hunig's base to neutralize the hydrochloride salt. Palladium catalyzed deallylation afforded the test compounds in 41% overall yield from the pipecolic acid.[5]

The effect of stereochemistry and substitution at the 3-position of the piperidine ring was examined first. Biological data for several of these analogues appear in Scheme 2.[6] Both aggrecanase and MMP-1 potency are slightly influenced by the pendant alkyl group and stereochemistry of the 3-position, and compound **11** exhibited the most potent inhibition of aggrecanase. Using this compound as the lead, we next explored SAR at the aryl sulfonamide. Biological data for these analogues are shown in Table 1.

As observed for the 3,3-dimethyl-5-hydroxypipecolic hydroxamates, incorporation of a lipophilic *ortho*-substituent in the benzyl ether group results in potent aggrecanase activity.[2] This observation is highlighted by the dramatic effect of placement of a fluoro substituent on activity in substituted benzyl ether analogues **15**–**17**. The reduced potency observed for the *ortho* cyano substituted analogue **22** and the *ortho* methoxy



Scheme 2. Aggerecanase and MMP inhibitory activity of 3-hydroxy-3-alkyl pipecolic hydroxamates.

Table 1. Aggrecanase and MMP-13 inhibitory activity of benzyl ether analogues of **9** (R′ = (3*R*) Me)

Compound	R	Aggrecanase IC_{50} (nM)	MMP-13 IC_{50} (nM)	MMP-1 IC_{50} (nM)	rTACE IC_{50} (nM)	TNF HWB IC_{50} (uM)
15	2-Fluorophenyl	14	0.49	180	—	33
16	3-Fluorophenyl	85	1.5	340	—	15
17	4-Fluorophenyl	120	3.7	420	14	7.2
18	2-Chlorophenyl	3.7	1.0	270	—	8.3
19	3-Chlorophenyl	78	2.5	860	—	17
20	2-Methylphenyl	6.9	0.65	310	—	2.2
21	2-Methoxyphenyl	73%	18	2600	—	14
22	2-Cyanophenyl	55%	9.7	2700	—	100
23	2-Methyl-3-fluorophenyl	11	3.2	1300	10	1.6
24	2-Methyl-4-fluorophenyl	4.7	2.9	220	15	2.5
25	2-Methyl-5-fluorophenyl	15	2.5	600	—	1.9
26	2-Chloro-4-fluorophenyl	1.5	0.9	310	—	10.4
27	2-Fluoro-4-chlorophenyl	7.9	0.87	120	—	73

Values expressed as a percentage represent percent inhibition of enzyme activity at 500 nM.

substituted analogue **21** relative to the *ortho* methyl (**20**), chloro (**18**) or fluoro (**15**) analogues is consistent with the notion that a lipophilic residue at this position imparts potent aggrecanase activity. MMP-1 inhibition is also sensitive to the presence of a lipophilic *ortho* substituent, and a ~8-fold reduction in MMP-1 activity is observed for **21** relative to **20**. The addition of a second halogen substituent in the *para* position results in comparable potency for *ortho* alkyl or halo substituted analogues (compare **24** with **20** and **26** with **18**). Selectivity for aggrecanase over MMP-1 inhibition improves for 2,3-disubstituted benzyl ethers relative to the monosubstituted analogues (compare **23** with **20**). The 2,4-disubstituted compounds provide the best aggrecanase potency, with the 2-chloro-4-fluoro analogue **26** providing low nanomolar inhibitory activity for aggrecanase and MMP-13, and > 100× selectivity over MMP-1.

Several substituted heterocyclic analogues were prepared, and some of these compounds provide good MMP-13 and aggrecanase activity with reduced lipophilicity compared to the substituted phenyl analogues. Biological data for these analogues are shown in Table 2. Consistent with SAR observed with simple substituted benzyl ethers, aggrecanase potency is strongly influenced by the presence of a lipophilic substituent *ortho* to the benzylic ether linkage (compare **32** with **28**). Only the 4-isoquinolinyl derivative **30** shows > 100× selectivity for aggrecanase inhibition over MMP-1.

We chose compound **26** for further profiling based on its excellent aggrecanase and MMP-13 potency, selectivity versus MMP-1 and poor potency against TNF-α release in LPS treated whole blood. The complete MMP-inhibition profile for this compound appears below:

MVP	1	2	3	8	9	13	14
IC_{50} (nM)	310	4	5	1	11	0.9	83

Compound **26** showed potent inhibition of IL-1 induced aggrecan (IC_{50} = 10.3 nM) and collagen (IC_{50} = 100 nM) degradation in bovine nasal cartilage explants.[7] In human osteoarthritic cartilage, **26** inhibited IL-1 induced aggrecan degradation with an IC_{50} of 6.0 nM (n = 2).[8]

Compound **26** showed moderate clearance in rat (Clp = 13 mL min^{-1} kg^{-1}, $t_{1/2}$ = 2 h) and dog (Clp = 11 mL $min^{-1}kg^{-1}$, $t_{1/2}$ = 4.6 h) that was consistent with predicted clearance based on incubations with rat and dog hepatocytes (ER = 0.20 and 0.28, respectively).[9,10] The markedly lower metabolic clearance with this compound compared to the original lead **1** (R = 2,4-dichloro; rat Clp = 44 mL min^{-1} kg^{-1}, $t_{1/2}$ = 2 h; dog Clp = 25 mL min^{-1} kg^{-1}, $t_{1/2}$ = 2 h) is attributed to the presence of the polar hydroxyl group as well as equivalent steric hindrance proximal to the metabolically labile hydroxamic acid. It is also possible that intramolecular hydrogen bonding between the hydroxyl group and the hydroxamic acid influences absorption and clearance. When dosed orally in hamsters, **26** exhibited potent inhibition of IL-1 induced aggrecan degradation (ED_{50} = 4.6 mg kg^{-1}) and MMP-13 induced collagen degradation (ED_{50} = 13 mg/kg) in hamster knee joints.[11]

Table 2. Aggrecanase and MMP-13 inhibitory activity of heteroarylmethyl ether analogues of **9** (R′ = (3*R*) Me)

Compound	R	Aggrecanase IC_{50} (nM)	MMP-13 IC_{50} nM	MMP-1 IC_{50} (nM)	rTACE IC_{50} (nM)	TNF HWB IC_{50} (uM)
28	Pyridin-4-yl	8.1%	2.0	190	—	7.1
29	Pyrazinyl	8.0%	7.4	1600	—	60
30	4-Isoquinolinyl	17	18	7800	—	2.5
31	4-Quinolinyl	19	4.1	120	—	0.48
32	2-Chloro-4-pyridinyl	15	0.43	520	—	6.5
33	2-Methyl-3-pyridinyl	51	1.5	1600	—	3.3

Values expressed as a percentage represent percent inhibition of enzyme activity at 500 nM.

We have discovered a novel series of MMP inhibitors structured around a 3-alkyl-3-hydroxy pipecolic hydroxamate template. Many of these compounds possess potent inhibitory activity versus aggrecanase and MMP-13 with selectivity versus MMP-1 and TACE (as measured by TNF-α release in LPS-stimulated human blood). The pharmacokinetic properties and in vivo activity observed with compound **26** suggest that it could provide a useful starting point for the discovery of agents for the treatment of osteoarthritis. Further studies are in progress and will be reported in due course.

References and notes

1. Hedbom, E.; Hauselmann, H. J. *Cell. Mol. Life Sci.* **2002**, *59*, 45.
2. Noe, M. C.; Natarajan, V.; Snow, S. L.; Mitchell, P. G.; Lopresti-Morrow, L. L.; Reeves, L. M.; Yocum, S. A.; Carty, T. J.; Barberia, J. T.; Sweeney, F. J.; Liras, J. L.; Vaughn, M.; Hardink, J.; Hawkins, J. M.; Tokar, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2808.
3. Letavic, M. A.; Axt, M. Z.; Barberia, J. T.; Carty, T. J.; Danley, D. E.; Geoghegan, K. F.; Halim, N. S.; Hoth, L. R.; Kamath, A. V.; Laird, E. R.; Lopresti-Morrow, L. L.; McClure, K. F.; Mitchell, P. G.; Natarajan, V.; Noe, M. C.; Pandit, J.; Reeves, L. M.; Schulte, G. K.; Snow, S. L.; Sweeney, F. J.; Tan, D. H.; Yu, C. H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1387.
4. Aranapakam, V.; Davis, J. M.; Grosu, G. T.; Baker, J.; Ellingboe, J.; Zask, A.; Levin, J. I.; Sananayaka, V. P.; Du, M.; Skotnicki, J. S.; DiJoseph, J. F.; Sung, A.; Sharr, M. A.; Killar, L. M.; Walter, T.; Jin, G.; Cowling, R.; Tillett, J.; Zhao, W.; McDevitt, J.; Xu, Z. B. *J. Med. Chem.* **2003**, *46*, 2376.
5. All new compounds exhibited satisfactory ^{1}H NMR and MS analysis
6. TACE, MMP, and whole blood TNF-α release assays were conducted as described in Ref. 3. Aggrecanase activity was assessed using a cell based assay: porcine chondrocytes were plated in 48-well tissue culture plates. Glycosaminoglycan (GAG) chains were labeled by including [^{35}S]sulfate (5 mCi/ml) in the culture medium. Unincorporated label was washed out and aggrecan degradation was stimulated by the addition of IL-1α (5 ng/ml). Ten hours later, aggrecan degradation was quantified by scintillation counting of ^{35}S in both the conditioned medium and cell layers
7. Plugs (4 mm diameter and length) of bovine nasal cartilage (BNC) were cultured in serum-free DMEM + IL-1α and + **26**] Samples were taken at 48 h for analysis of GAG content using the DMMB assay (Farndale, R., et al., *Biochim. Biophys. Acta* **1986**, *883*, 173). Medium was replaced every 3–4 days with fresh IL-1 and inhibitor. Assays were terminated after 2–3 weeks of culture and collagen degradation was determined via hydroxyproline analysis of hydrolyzed conditioned medium samples (Otterness, I. et al., *Arthritis Rheum.* **1998**, *41*, 2068)
8. Human articular cartilage was obtained at total knee replacement. Pieces (1 × 2 mm) were cultured in DMEM containing 0.5% fetal bovine serum. Aggrecan degradation was stimulated by the addition of IL-1α (50 ng/ml) + oncostatin M (50 ng/ml). After 48 h, media samples were assayed for GAG content using the DMMB assay
9. Male Sprague–Dawley rats were obtained with jugular vein catheters from Charles River Labs, Wilmington, MA. iv bolus doses were administered in glycerol formal (5 mg/kg). Blood samples (600 mL) were collected from the jugular vein (t = 5, 15, 30 min, 1, 2, 4, 6, 8, and 24 h). Plasma was diluted with an equal volume of water and extracted with MTBE. Drug levels were determined by LC–MS/MS analysis. Pharmacokinetic parameters were calculated using the non-compartmental method in WinNonlin v2.1 (Pharsight, Mountain View, CA)
10. Predicted clearances were obtained from the disappearance of drug in cryopreserved rat and dog hepatocytes according to the half-life method of Shibata, Y.; Takahashi, H.; Ishii, Y. *Drug Metab. Dispos.* **2000**, *28*, 1518.
11. Aggrecan degradation was initiated in hamster knees by the intraarticular injection of 40 ng of murine IL-1α. Animals were orally dosed with **26** 4 h after IL-1. At 7 h post IL-1, the animals were sacrificed and the GAG content in lavaged synovial fluid was determined via the DMMB assay. Collagen degradation was initiated by the intraarticular injection of 2 μg of recombinant human MMP-13. **26** was administered orally 1 h prior to MMP-13 injection. Two hours after MMP-13 injection, the animals were sacrificed and type II collagen fragments were measured in the joint lavage using an ELISA specific for collagenase cleaved type II collagen (Downs, J., et al., *J. Immunol. Methods* **2001**, *247*, 25).