

Discovery of 3,3-dimethyl-5-hydroxypipicolic hydroxamate-based inhibitors of aggrecanase and MMP-13

Mark C. Noe,* Vijayalakshmi Natarajan, Sheri L. Snow, Peter G. Mitchell, Lori Lopresti-Morrow, Lisa M. Reeves, Sue A. Yocum, Thomas J. Carty, John A. Barberia, Francis J. Sweeney, Jennifer L. Liras, Marcie Vaughn, Joel R. Hardink, Joel M. Hawkins and Christopher Tokar

Pfizer Global Research and Development Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA

Received 31 January 2005; revised 7 March 2005; accepted 25 March 2005

Available online 28 April 2005

Abstract—A series of pipicolic hydroxamate inhibitors of MMP-13 and aggrecanase was discovered based on screening known inhibitors of TNF- α converting enzyme (TACE). Potency versus aggrecanase was optimized by modification of the benzyloxyaryl-sulfonamide group. Incorporation of geminal alkyl substitution at the 3-position of the piperidine ring improved metabolic stability, presumably by increasing steric hindrance around the metabolically labile hydroxamic acid. This modification also resulted in dramatic improvement of aggrecanase activity with a slight reduction in selectivity versus MMP-1. Synthesis, structure activity relationships, and strategies to reduce metabolic clearance are described.

© 2005 Elsevier Ltd. All rights reserved.

Osteoarthritis is a disease of progressive cartilage degradation that leads to pain and reduced mobility in affected joints. Approximately 21 million patients are afflicted with some form of the disease in the United States, and current treatments are limited to symptomatic relief with NSAIDs or COXIBs (selective COX-2 inhibitors), intra-articular injections of hyaluronic acid conjugates (Synvisc) or surgical joint replacement.^{1,2} The efficacy of nutritional supplements (glucosamine and chondroitin sulfate) is somewhat controversial, and there are currently no treatments available that retard or reverse the gradual destruction of cartilage associated with the disease.^{3–5} The pathogenesis of osteoarthritis can be attributed to multiple factors that affect the degeneration of proteoglycan and disruption of the type II collagen network in cartilage.⁶ These critical components of cartilage maintain its structural integrity and impart mechanical properties associated with normal joints. Aggrecan monomers interact with hyaluronic acid and link proteins to form high molecular weight networks with ordered hydration that provides cartilage with resilience and compressibility. Type II collagen is a triple-helical protein that forms a

highly organized three-dimensional structure responsible for tissue stiffness and integrity. Both aggrecan and type II collagen can be degraded by proteolytic enzymes that have elevated expression in osteoarthritic joints. Aggrecanase refers to a collection of proteases that cleave aggrecan and belong to the ADAM-TS (A Disintegrin and Metalloprotease possessing Thrombospondin domains) family of zinc containing metalloproteases.⁷ On the other hand, collagenases are the principle enzymes that cleave type II collagen and are members of the MMP (matrix metalloprotease) family.⁸ MMP-13 is particularly efficient at cleaving type II collagen; its expression is regulated by pro-inflammatory mediators and elevated in osteoarthritic joints.⁹ We sought to discover compounds that possessed inhibitory activity versus both MMP-13 and aggrecanase and could therefore serve as therapeutically useful agents for the treatment of this disease.

Unfortunately, the vast majority of broad-spectrum MMP inhibitors that have advanced to clinical trials exhibit musculoskeletal side effects characterized by joint stiffness and pain, particularly in the hands and shoulders.^{8,10} In rodents, joint fibroplasia and accumulation of type I collagen in the affected joints accompanies the observed side effect.¹¹ Others have speculated that sheddase inhibition (specifically inhibition of TACE)

* Corresponding author. Tel.: +1 860 441 8825; fax: +1 860 715 2467; e-mail: mark_c_noe@pfizer.com

could be responsible for the observed side effects, possibly through the failure to downregulate receptor signaling by proteolytic release of the extracellular domain.¹² We therefore desired to spare both MMP-1 inhibition and TACE inhibition (as measured by suppression of TNF- α release in human blood stimulated with LPS).

Previous MMP and TNF- α converting enzyme (TACE) programs at Pfizer led to the discovery of pipecolic acid based inhibitors such as **1**.¹³ Screening these compounds in a cell based aggrecanase assay revealed several analogs with modest activity. Only compounds possessing an *ortho*-methyl or halo group exhibited potent inhibitory activity (<500 nM), with **2** providing the most potent inhibition of both MMP-13 and aggrecanase activities. Compounds from this series generally exhibit poor pharmacokinetic properties due to high metabolic clearance (Rat PK data for **2**: Clp: 86 mL min⁻¹ kg⁻¹; $T_{1/2}$: 1.7 h; bioavailability: 12%). Therefore, we incorporated additional substituents at the piperidine 3-position with the intent of improving the metabolic stability of the hydroxamate group by increasing steric hindrance, thereby limiting access to metabolic enzymes (Fig. 1).

The synthesis of the 3,3-dimethyl-5-hydroxy pipecolic hydroxamates is shown in Scheme 1. The first chiral center is established through a highly diastereoselective Strecker reaction of 2,2-dimethyl-4-pentenal with potassium cyanide and (*R*)- α -methylbenzylamine-hydrochloride.¹⁴ The resulting α -aminonitrile can be purified to homogeneity by recrystallization from aqueous methanol. Attempted hydrolysis of the nitrile by treatment with concentrated acid or base in refluxing aqueous methanol was unsuccessful, presumably due to extreme steric hindrance imparted by the geminal dimethyl and bulky amine substituents. Reasoning that derivatization of the nitrile might be more easily effected by intramolecular lactonization with a hydroxy substituent, we decided to functionalize the olefin first. Reaction of **4** with catalytic OsO₄ and NMO in aqueous acetone afforded the diol product as a 1:1 mixture of diastereomers. Asymmetric dihydroxylation using the DHQD-PHN ligand under standard conditions afforded **5** with

improved diastereoselectivity.^{15,16} We were gratified to find that treatment of **5** with concentrated aqueous HCl at reflux afforded the desired six-membered lactone **6** in 97% yield. Sulfonylation with *p*-toluenesulfonyl chloride in pyridine was followed by spontaneous cyclization to form the bicyclic intermediate **7**. Removal of the α -methylbenzyl group by hydrogenolysis, followed by sulfonylation with the appropriate arylsulfonyl chloride and triethylamine afforded the bicyclic sulfonamide **9** in good yield. For the preparation of analogs, the unsubstituted benzyloxysulfonamide **9** (R = Ph) was treated with hydrogen in the presence of Pd-C and methanol, affording the intermediate phenol, which was then alkylated with the appropriate benzyl halide in the presence of cesium carbonate and acetonitrile to afford the desired derivative **9** in good yield. Opening of the lactone under Weinreb's conditions using *O*-allylhydroxylamine hydrochloride, followed by removal of the allyl group afforded the desired compounds in moderate yield.¹⁷ Substituted 4-benzyloxy aryl sulfonamides were prepared to probe structure activity relationships about this group, which is thought to bind in the S1' pocket by analogy with the previously discovered pipecolic hydroxamate TACE inhibitors. The biological data obtained for each of these compounds are shown in Table 1.^{18,19}

The 3,3-dimethyl-5-hydroxypipelic hydroxamates generally had improved aggrecanase activity and reduced selectivity for MMP-13 over MMP-1 compared to the corresponding 5-hydroxy pipecolic hydroxamates (compare **21** with **2**, other data not shown). As observed for the 5-hydroxypipelic hydroxamates, incorporation of an *ortho*-substituted benzyl ether on the sulfonamide moiety results in potent aggrecanase inhibition. The reduced potency observed for the isopropyl substituted analog **18** relative to the *ortho*-methyl (**11**), bromo (**14**), or ethyl (**17**) analogs suggests a size limitation for this substituent. MMP-13 inhibition is also sensitive to the presence of a large *ortho*-substituent, and a 10-fold reduction in MMP-13 activity is observed for **18** relative to **11**. Interestingly, the opposite effect is observed for the inhibition of TNF- α release in LPS-stimulated human whole blood. This observation is consistent with the previously noted influence of *ortho*-alkyl substitution on TACE potency, the pipecolic hydroxamate series **1**.¹³ The presence of a second halogen substituent in the *meta*- or *para*-positions results in enhanced potency for *ortho*-alkyl or halo substituted analogs. The addition of a *para*-substituent erodes selectivity for MMP-1 (e.g., compounds **13**, **16**, and **20**). The 2,4-disubstituted compounds provide the best potency, with the 2-chloro-4-fluoro analog **22** displaying nanomolar inhibitory activity and >100 $\times$ selectivity for MMP-13 and aggrecanase over MMP-1.

Several substituted heterocyclic analogs were prepared with the intent of reducing lipophilicity while retaining good MMP-13 and aggrecanase activity. Biological data for these analogs are shown in Table 2. Aggrecanase potency is strongly influenced by the position of the heteroatoms on the terminal heterocycle, despite the presence of *ortho*-substitution in each case tested. The same is

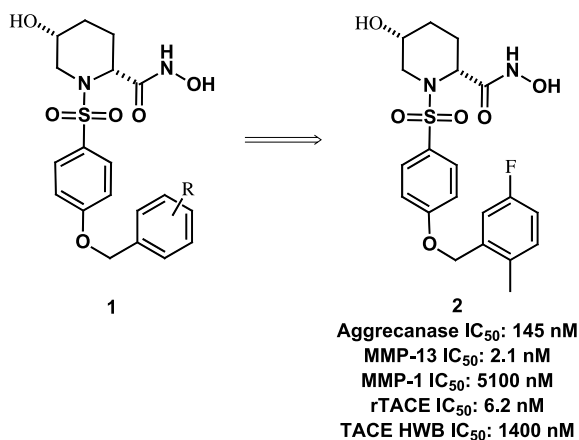
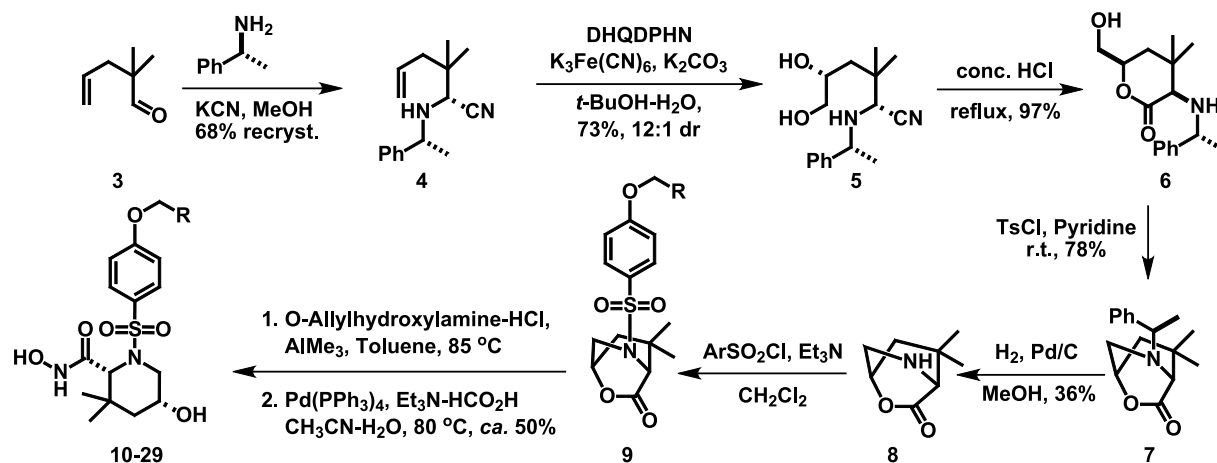


Figure 1.



Scheme 1. Synthesis of 3,3-dimethyl-5-hydroxypipercolic-3-hydroxamic acids from 2,2-dimethyl-4-pental.

Table 1. Aggrecanase and MMP-13 inhibitory activity of benzyl ether analogs of **10**

Compds	R	Aggrecanase IC ₅₀ (nM) ^a	MMP-13 IC ₅₀ (nM)	MMP-1 IC ₅₀ (nM)	rTACE IC ₅₀ (nM)	TNF HWB IC ₅₀ (nM) ^b
11	2-Methylphenyl	38	14	690	7.3	4900
12	3-Methylphenyl	>100	2.8	773	12	25,850
13	4-Methylphenyl	19%	1.2	198	11	19,150
14	2-Bromophenyl	16	8	715	11	8900
15	3-Bromophenyl	57%	ND	ND	15	18,950
16	4-Bromophenyl	>100	1.4	100	10	7700
17	2-Ethylphenyl	67	36	1500	6.7	1100
18	2-Isopropylphenyl	78%	188	2300	12	1800
19	2-Methyl-3-fluorophenyl	3.9	4.3	679	17	2875
20	2-Methyl-4-fluorophenyl	2.7	3.5	255	5.2	5483
21	2-Methyl-5-fluorophenyl	40	6.5	790	7.3	3450
22	2-Chloro-4-fluorophenyl	0.5	4.1	592	ND	10,200
23	2,4-Dichlorophenyl	2.1	3.0	430	19	31,000

^aValues expressed as a percentage represent percent inhibition of enzyme activity at 500 nM.^bHWB = human whole blood.Table 2. Aggrecanase and MMP-13 inhibitory activity of heteroaryl methyl ether analogs of **10**

Compds	R	Aggrecanase IC ₅₀ (nM) ^a	MMP-13 IC ₅₀ (nM)	MMP-1 IC ₅₀ (nM)	rTACE IC ₅₀ (nM)	TNF HWB IC ₅₀ (nM) ^b
24	3-Methylisothiazol-4-yl	76%	ND	ND	12	5200
25	4-Methylthiazol-5-yl	55%	ND	ND	16	4950
26	4-Isoquinoliny	>100	53	290	10	2425
27	4-Quinoliny	15	7.8	170	ND	950
28	2-Chloro-4-pyridiny	18	19	1100	ND	10,150
29	2-Methyl-3-pyridiny	91	ND	ND	6.7	2000

^aValues expressed as a percentage represent percent inhibition of enzyme activity at 500 nM.^bHWB = Human whole blood.

observed for MMP-1 and TNF- α inhibition, with the 4-quinoliny derivative **27** showing the greatest potency against all of the endpoints.

We chose compound **23** 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. Compound **23** showed moderate clearance in rat²⁰ (Clp = 44 mL min⁻¹ kg⁻¹, $T_{1/2}$ = 2 h) and dog (Clp = 25 mL min⁻¹ kg⁻¹, $T_{1/2}$ = 2 h) that was consistent with predicted extraction ratios based on incubations with rat and dog hepatocytes

(Clb = 44 mL min⁻¹ kg⁻¹ and 34 mL min⁻¹ kg⁻¹, respectively).²¹ The markedly lower metabolic clearance with this compound compared to the original lead **2**, is attributed to increased steric hindrance proximal to the metabolically labile hydroxamic acid as well as the replacement of the *ortho*-methyl substituent on the terminal aromatic ring with a chlorine. Oral bioavailability in rat was 50% when dosed as a suspension in 0.5% aqueous methylcellulose.

We have discovered a novel series of MMP inhibitors structured around a 3,3-dimethyl-5-hydroxy pipercolic

hydroxamate template. The aggrecanase structure activity relationships that have emerged from this study are reminiscent of those seen for TACE inhibition with the earlier less-substituted pipicolate hydroxamate series **1** (requirement of an *ortho*-substituted benzyl ether for optimum cellular activity). 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) and could provide a useful starting point for the discovery of agents for the treatment of osteoarthritis.

References and notes

- Kuritzky, L.; Weaver, A. J. *Pain Symptom Manag.* **2003**, 25, S6.
- Wollheim, F. A. *Drugs* **1996**, 52, 27.
- McAlindon, T. *Lancet* **2001**, 357, 247.
- Chard, J.; Dieppe, P. *Brit. Med. J.* **2001**, 322, 1439.
- Reginster, J. Y.; Deroisy, R.; Rovati, L. C.; Lee, R. L.; Lejeune, E.; Bruyere, O.; Giacomelli, G.; Henrotin, Y.; Dacre, J. E.; Gossett, C. *Lancet* **2001**, 357, 251.
- Hedbom, E.; Hauselmann, H. J. *Cell. Mol. Life Sci.* **2002**, 59, 45.
- Arner, E. C. *Curr. Opin. Pharmacol.* **2002**, 2, 322.
- Bigg, H. F.; Rowan, A. D. *Curr. Opin. Pharmacol.* **2001**, 1, 314.
- Mitchell, P. G.; Magna, H. A.; Reeves, L. M.; Lopresti-Morrow, L. L.; Yocum, S. A.; Rosner, P. J.; Geoghegan, K. F.; Hambor, J. E. *J. Clin. Inv.* **1996**, 97, 761.
- Brown, P. D. *Expert Opin. Inv. Drug* **2000**, 9, 2167.
- Renkiewicz, R.; Qiu, L.; Lesch, C.; Sun, X.; Devalaraja, R.; Cody, T.; Kaldjian, E.; Welgus, H.; Baragi, V. *Arthritis Rheum.* **2003**, 48, 1742.
- Naglich, J. G.; Jure-Kunkel, M.; Gupta, E.; Fagnoli, J.; Henderson, A. J.; Lewin, A. C.; Talbott, R.; Baxter, A.; Bird, J.; Savopoulos, R.; Wills, R.; Kramer, R. A.; Trail, P. A. *Cancer Res.* **2001**, 61, 8480.
- 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.
- Stout, D. M.; Black, L. A.; Matier, W. L. *J. Org. Chem.* **1983**, 48, 5369.
- Sharpless, K. B.; Amberg, W.; Beller, M.; Chen, H.; Hartung, J.; Kawanami, Y.; Lubben, D.; Manoury, E.; Ogino, Y.; Shibata, T.; Ukita, T. *J. Org. Chem.* **1991**, 56, 4586.
- Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, 94, 2483.
- Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, 18, 4171.
- All new compounds exhibited satisfactory ^1H NMR and MS analysis.
- TACE, MMP, and whole blood TNF- α release assays were conducted as described in Ref. **13**. 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 stimulated by the addition of IL-1 α (5 ng/mL). Ten hours later, aggrecan degradation was quantified by scintillation counting of ^{35}S content of both the conditioned medium and cell layers.
- Male Sprague–Dawley rats were obtained with jugular vein catheters from Charles River Labs, Wilmington, MA. I.V. bolus doses were administered in glycerol formal (5 mg/kg). Blood samples (600 μL) 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).
- In vitro extraction ratios were calculated from predicted blood clearances (CL_b) according to the following equation $\text{ER} = \text{CL}_b/Q$ where 70 and 35 mL/min/kg were assumed as hepatic blood flow (Q) for rat and dog, respectively. 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, 518.