Design and Synthesis of a Series of (2*R*)-*N*^{4}-Hydroxy-2-(3-hydroxybenzyl)-*N*^{1}-[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]butanediamide Derivatives as Potent, Selective, and Orally Bioavailable Aggrecanase Inhibitors†

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Abstract: A pharmacophore model of the P1′ site, specific for aggrecanase, was defined using the specificity studies of the matrix metalloproteinases and the similar biological activity of aggrecanase and MMP-8. Incorporation of the side chain of a tyrosine residue into compound **1** as the P1′ group provided modest selectivity for aggrecanase over MMP-1, -2, and -9. A *cis*-(1*S*)(2*R*)-amino-2-indanol scaffold was incorporated as a tyrosine mimic (P2′) to conformationally constrain **2**. Further optimization resulted in compound **11**, a potent, selective, and orally bioavailable inhibitor of aggrecanase.

Degenerative joint diseases are commonly characterized by destruction of the cartilage extracelluar matrix where loss of aggrecan from cartilage is believed to be the early event that leads to breakdown of extracellular matrix macromolecules.[1] The loss of aggrecan can be attributed to proteolytic cleavage of the core protein within the interglobular domain (IGD). Two significant cleavage sites have been identified within the IGD: one between residue Asn^{341} and Phe^{342} and the other between Glu^{373} and Ala^{374}. Many matrix metalloproteinases (MMP-1, 2, 3, 7, 8, 9, and 13) have been shown to cleave aggrecan in vitro at the Asn^{341}-Phe^{342} site.[2] G-1 fragments resulting from this cleavage site have been identified within articular cartilage bound to hyaluronic acid.[2b] Recently, aggrecanase, an ADAM-TS of the reprolysin family, was isolated, cloned, and expressed by Arner and co-workers.[3] This enzyme effectively cleaves at the Glu^{373}-Ala^{374} bond of the IGD. Aggrecan fragments resulting from this cleavage have been identified in the synovial fluid of patients with osteoarthritis, inflammatory joint disease, and joint injury, suggesting that aggrecanase plays a pivotal role in the catabolism of aggrecan in human arthritic disease.[4] There has been substantial interest in developing a selective aggrecanase inhibitor to prevent the progression of joint destruction,[5] and to delineate its role in normal and disease states.

There are several known classes of MMP inhibitors, distinguished by their zinc-chelating groups such as hydroxamates, thiols, and *N*-carboxyalkyls, which have been extensively reviewed.[6,7] Targeted screening of DuPont's compound collection for inhibitors of aggrecanase provided a potent lead **1**.[8] Although this compound has a K_i of 368 nM against aggrecanase, it is also potent against other MMPs such as MMP-1, -2, and -9. (Table 1). In this communication, we describe the conversion of a broad spectrum MMP inhibitor **1** into a potent, selective, and orally bioavailable inhibitor of aggrecanase by a structure-based approach using an enzyme homology model and by the similarity in biological activity of aggrecanase and MMP-8.

Results and Discussion. Our initial screen was conducted using a partially purified preparation from IL-1 stimulated bovine nasal cartilage. Thus, we began the medicinal chemistry effort without having amino acid sequence information for the enzyme. To assist us in the rational design of specific aggrecanase inhibitors, we examined the general patterns for substrate preference among the MMPs. We and others[9] observed that human neutrophil collagenase (MMP-8) is able to clip aggrecan at the specific aggrecanase cleavage site, albeit with relatively poor efficiency. This result suggested that MMP-8 and aggrecanase might share some common residues involved in substrate binding.

Nagase and Fields[10] have extensively reviewed specificity studies of the matrix metalloproteinases using a collagen sequence-based synthetic six-mer peptide spanning the scissle bond. Incorporation of a tyrosine residue at the P1′ position of the peptide substrate dramatically increased cleavage efficiency for MMP-8, while either decreasing or maintaining affinity for MMP-1, -2, and -9.[11] This suggested to us that a P1′ tyrosine residue of the peptide substrate provided good recognition for the S1′ site of MMP-8 but is less favorable in the S1′ sites of MMP-1, -2, and -9.

On the basis of the above specificity studies and on our hypothesis that aggrecanase and MMP-8 share a topologically similar active site, we envisioned that incorporation of a tyrosine side chain into the broad-spectrum MMP inhibitor **1** at the P1′ (i.e. **2**) position might provide some selectivity for aggrecanase over MMP-8.

Compound **2** was prepared and found to have IC$_{50}$'s of 332 nM and 0.45 nM against aggrecanase[12] and MMP-8, respectively, very similar to those of **1**. This result supports our hypothesis that the S1′ pockets are similar, and that the residues comprising the S1′ pocket of aggrecanase and MMP-8 could accept this inhibitor without requiring energetically demanding movement of the enzyme. This result also suggests that the 4-hydroxylbenzyl group (P1′) of **2** might be involved in a specific hydrogen-bond interaction within the S1′ pocket of aggrecanase and MMP-8. X-ray structural studies of MMP-8 revealed that its S1′ pocket projects several carbonyl groups such as that of Tyr 237, which could serve as a hydrogen bond acceptor.[13] As expected, **2** was significantly less potent than **1** against MMP-1, -2, and

† Dedicated to Professor Ralph F. Hirschmann on the occasion of his 79th birthday.

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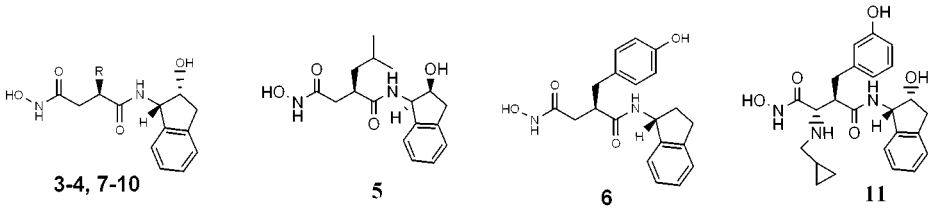
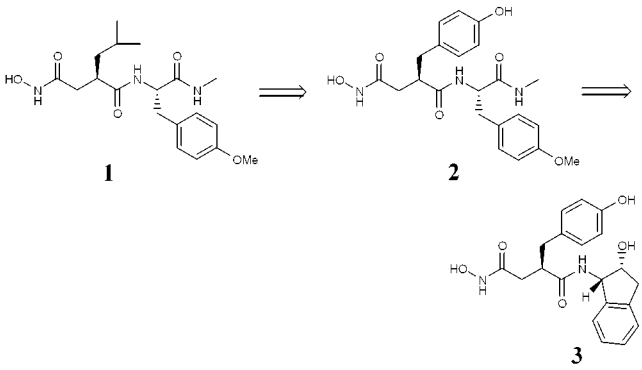
Table 1. In Vitro Binding Data of Inhibitors **1**−**11** against Aggrecanase, MMP-8, MMP-1, MMP-2, and MMP-9



compd	R	IC_{50} (nM)[a] aggrecanase	K_i (nM)[a,b] MMP-8	MMP-1	MMP-2	MMP-9
1		368	0.70	<1	<1	<1
2		332	0.45	10.7	4.3	3.2
3	4-OH-Bn	182	70	946	408	166
4	i-butyl	1700	37	82	73	162
5		13333	303	1087	1533	4200
6		1309	15300	75000	31600	21200
7	4-OMe-Bn	16.4	9.2	1163	52	203
8	3-OH-Bn	64	8600	30953	1507	3324
9	3-OMe-Bn	3139	1192	95500	6600	15500
10	1,3-benzodioxol-5-ylmethyl	790	40000	50000	35000	22000
11		12	171	33160	6300	4468

[a] Values are ±SD of three determinations unless otherwise noted. [b] The IC_{50} values on aggrecanase and K_i values on MMPs were determined as previously described.[18] All of the hydroxamic acids studies here were assumed to act as competitive inhibitors of the enzyme, binding to the active site Zn atom as previously demonstrated by crystallographic and NMR studies of MMP complexed with related hydroxamic acids.[19] On the basis of the assumption of competitive inhibition, the IC_{50} values were converted to K_i values from the equation, $K_i = IC_{50}/(1 + [S]/K_m)$, where IC_{50} is the concentration of competing compound producing 50% inhibition of the enzymatic activity at the substrate concentration [S]. K_m is the substrate concentration that provides a reaction velocity that is half the maximal velocity obtainable under saturating substrate conditions.

Chart 1



-9, (Table 1). The tight SAR is consistent with Fields and co-worker's results and suggests that the S1′ sites of MMP-1, -2, and -9 are less tolerant of polar functional groups such as the 4-hydroxylbenzyl of **2**.

It is well-known that restricting the flexibility of a molecule can enhance the potency and selectivity of an inhibitor. To enhance potency against our target enzyme, we next studied conformational restraint on compound **2**. *cis*-1-Amino-2-indanol has been reported as a phenylglycine mimetic and has been successfully employed in the development of Crixivan, an HIV protease inhibitor.[14] We replaced the 4-methoxy-tyrosine moiety of compound **2** with *cis*-(1*S*)(2*R*)-1-amino-2-indanol, which led to compound **3** (Chart 1). Compound **3** was found to be about 2-fold more potent than **2** in the aggrecanase inhibitory assay with an IC_{50} of 182 nM. More importantly, the structural change significantly reduced the potency against MMP-1, -2, -9 and MMP-8 (Table 1).

The stereochemistry of *cis*-(1*S*)(2*R*)-1-amino-2-indanol moiety is important for aggrecanase activity. Compound **5**, a distereomer of *cis*-(1*S*)(2*R*)-1-amino-2-indanol analogue **4**, was found to be inactive in the aggrecanase assay. The importance of the indanol hydroxyl group was also demonstrated by testing compound **6** against aggrecanase, which was found to show only modest activity (IC_{50} = 1309 nM, Table 1). Molecular modeling studies show that this hydroxyl group mimics the carbonyl oxygen of the tyrosine residue of **2**.

The 4-methoxybenzyl derivative **7** maintains similar potency for aggrecanase and MMP-8, and has IC_{50}s of 1163 nM, 52 nM, and 203 nM against MMP-1, -2, and -9, respectively. The 10-fold potency increases for **7** over **3** against aggrecanase can be rationalized by two explanations. The methoxy group of **7** might act as a hydrogen bond acceptor. However, the lower potency of **3** could also suggest that there may be a binding energy liability, perhaps due to the desolvation of the hydrophilic hydroxyl group in a highly hydrophobic S1′ pocket.

The SAR investigation around positional isomers of the phenol hydroxyl group reveals that the 3-hydroxylbenzyl analogue **8** is significantly more potent than **3** against aggrecanase (Table 1). A different and possibly unique hydrogen-bonding interaction between the hydroxyl phenol of **8** and residues in the S1′ pocket of aggrecanase was proposed, different from that between the 4-hydroxylbenzyl group of **2** and residues of the S1′ pocket of MMP-8 and aggrecanase. Additionally, compound **8** is more than 2 orders of magnitude selective for aggrecanase over other MMPs (Table 1) and shows no inhibitory activity against MMP-8. This observation suggests that although the active sites of MMP-8 and aggrecanase are quite similar as described earlier, there exist exploitable differences.

After much of the work described here was completed, the aggrecanase protein sequence became available to us. Noting the active site sequence similarity with those of atrolysin C[15] and adamalysin II,[16] we constructed a homology model of aggrecanase. Docking compound **8** into the active site of the model[17] revealed a unique threonine residue in the aggrecanase S1′ pocket at a

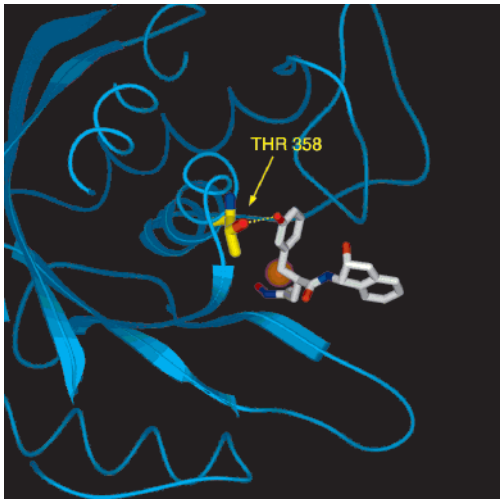
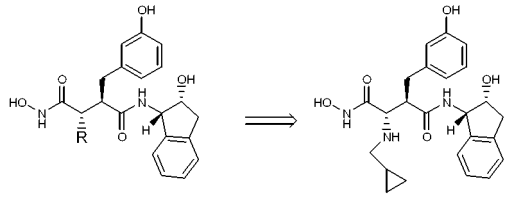


Figure 1. Model of possible binding mode for compound **8** in the active site of aggrecanase homology model.

Chart 2

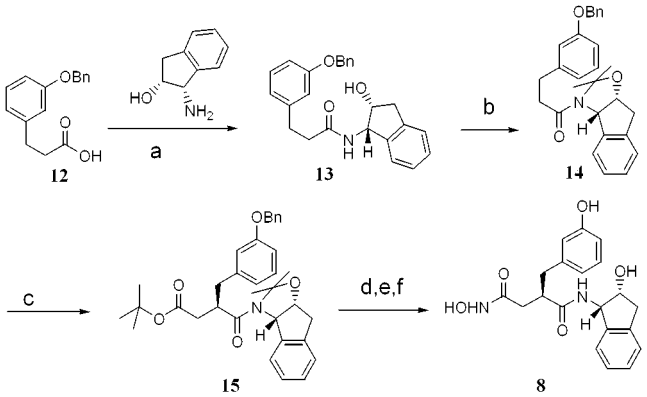


position occupied by valine in MMPs 1, 2, 3, 8, and 9 (Figure 1). This finding supported our hypothesis that the 3-hydroxyl group of inhibitor **8** provides selectivity through a specific hydrogen-bonding interaction with this residue in the S1′ pocket. The proof that **8** forms a specific hydrogen bond to the hydroxyl group of the threonine in the S1′ pocket of aggrecanase awaits further enzyme−inhibitor crystallographic evidence.

Compounds **9** and **10** bind only weakly to aggrecanase with IC_{50} of 3139 nM and 790 nM, respectively. The weak activity of **9** and **10** might suggest that the steric nature of these two P1′ substituents of **9** and **10** possibly imparts unfavorable interactions in this region, which prevent these inhibitors from binding to the enzyme.

In an attempt to further improve the binding potency of **8** for aggrecanase and modify the physicochemical properties to improve the pharmacokinetic profile, we investigated a wide selection of substituents at the position α to the hydroxamic acid. Hirayama and co-workers have shown that P1 substitution can significantly increase the potency of these succinate-based hydroxamic acids.[20] Moreover, the introduction of a bulky substituent α to the hydroxamic acid has been investigated as a means of preventing the hydrolysis of hydroxamic acids in vivo, which could result in an improved pharmacokinetic profile.[21] On the basis of this analysis and the need to have a water-soluble molecule to facilitate in vivo animal efficacy model studies, an amino functional group was selected and introduced into the P1 position of **8**. This analysis and extensive SAR studies eventually led to the synthesis of molecule **11** (Chart 2). This compound was found to have an IC_{50} of 12 nM in the aggrecanase assay and maintained the desired selectivity for aggrecanase over MMP-1, -2, and -9.

Scheme 1. Synthesis of Compound **8**[a]



[a] Reagents: (a) Bop, DMF, Hunig base (85%); (b) 2-methoxypropene, PPTS (cat), CH_2Cl_2 (91%); (c) *n*Bu-Li, THF, −78 °C, $BrCH_2CO_2tBu$ (61%); (d) TFA, CH_2Cl_2, H_2O (96%); (e) $BnONH_2$, TBTU, DMF; (f) H_2, $Pd/BaSO_4$, MeOH.

Following oral administration of **11** to dogs, substantial absorption was found with an average oral bioavailability of 43%. The terminal half-life was 6 h with a low systemic clearance of 0.5 L/h/kg.

A representative synthesis of **8** is shown in Scheme 1. Starting from acid **12**,[22] amide **13** was obtained in 85% by coupling the acid with *cis*-(1*S*)(2*R*)-amino-2-indanol. The hydroxyl group of compound **13** was protected as the acetonide. Subsequent alkylation afforded the desired product **15** in 61% yield.[23] Removal of the *tert*-butyl group with TFA, followed by coupling with *O*-benzyl hydroxyamine and subsequent hydrogenation afforded the desired final product **8**.

In summary, we have discovered potent and selective inhibitors of aggrecanase by a structure-based approach using an enzyme homology model and the similar biological activity profile of aggrecanase and MMP-8. Extensive specificity studies of the matrix metalloproteinases using collagen sequence-based synthetic peptide reported by Nagase and Fields provided crucial information about the active site of MMP-8. This allowed us to incorporate the side chain of a tyrosine residue into the broad spectrum MMP inhibitor **1** to achieve modest selectivity for aggrecanase over MMP-1, -2, and -9. The *cis*-(1*S*)(2*R*)-amino-2-indanol scaffold was selected and incorporated as a P2′ peptide mimic, further enhancing the potency and selectivity for aggrecanase. Finally, compound **11**, a potent and selective inhibitor of aggrecanase, was synthesized and found to be orally bioavailable in the dog, with an excellent pharmacokinetic profile ($T_{1/2}$ = 6 h). The discovery of a selective and orally bioavailable aggrecanase inhibitor provides an important tool to further study the biological function of this enzyme and in the development of new medications for degenerative joint diseases.

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Supporting Information Available: Experimental details and analytical data for the preparation of compounds **8**, **13**−**15** as well as spectral characteristics of the target com-

pounds **2**−**3**, **6**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Mankin, H. J.; Lippiello, L. J. Biochemical and metabolic abnormalities in-articular cartilage from osteo-arthritic human hips. *J. Bone Joint Surg* **1970**, *52A*, 424−434.

(2) (a) Fosang, A. J.; Neame, P. J.; Last, K.; Hardhingham, T. E.; Murphy, G.; Hamilton, J. A. The interglobular domain of cartilage aggrecan is cleaved by PUMP, gelatinases, and cathepsin B. *J. Biol. Chem*. **1992**, *267*, 19470−19474. (b) Flannery, C. R.; Lark, M. W.; Sandy, J. D. Identification of a stromelysin cleavage site within the interglobular domain of human aggrecan. Evidence for proteolysis at this site in vivo in human articular cartilage. *J. Biol. Chem*. **1992**, *267*, 1008−1014. (c) Fosang, A. J.; Last, K.; Knauper, V.; Neame, P. J.; Murphy, G.; Hardingham, T. E.; Tschesche, H.; Hamilton, J. A. Fibroblast and neutrophil collagenases cleave at two sites in the cartilage aggrecan interglobular domain. *Biochem. J*. **1993**, *295*, 273−276. (d) Fosang, A. J.; Last, K.; Knauper, V.; Murphy, G.; Neame, P. J. Degradation of cartilage aggrecan by collagenase-3 (MMP-13). *FEBS Lett*. **1996**, *380*, 17−20.

(3) Tortorella, M. D.; Burn, T. C.; Pratta, M. A.; Abbaszade, I.; Hollis, J. M.; Liu, R.; Rosenfeld, S. A.; Copeland, R. A.; Decicco, C. P.; Wynn, R.; Rockwell, A.; Yang, F.; Duke, J. L.; Solomon, K.; George, H.; Bruckner, R.; Nagase, H.; Itoh, Y.; Ellis, D. M.; Ross, H.; Wiswall, B. H.; Murphy, K.; Hillman, M. C., Jr.; Hollis, G. F.; Newton, R. C.; Magolda, R. L.; Trzaskos, J. M.; Arner, E. C. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. *Science* **1999**, *284*, 1664−1666.

(4) (a) Arner, E. C.; Hughes, C. E.; Decicco, C. P.; Caterson, B.; Tortorella, M. D. Cytokine-induced cartilage proteoglycan degradation is meadiated by aggrecanase *Osteoarthritis Cartilage* **1998**, *6*, 214−228. (b) Lohamnder, L. S.; Neame, P. J.; Sandy, J. D.; The structure of aggrecan fragments in human synovial fluid. Evidence that aggrecanase mediates cartilage degradation in inflammatory joint disease, joint injury, and osteoarthritis. *Arthritis Rheum*. **1993**, *36*, 1214−1222. (c) Sandy, J. D.; Boynton, R. E.; Flannery, C. R. Analysis of the catabolism of aggrecan in cartilage explants by quantitation of peptides from the three globular domains. *J. Biol. Chem*. **1991**, *266*, 8198−205. (c) Arner, E. C.; Pratta, M. A.; Trzaskos, J. M.; Decicco, C. P.; Tortorella, M. D. Generation and characterization of aggrecanase. A soluble, cartilage-derived aggrecan-degrading activity. *J. Biol. Chem*. **1999**, *274*, 6594−6601. (d) Van Meurs, Joyce B. J.; Van Lent, Peter L. E. M.; Holthuysen, Astrid E. M.; Singer, Irwin I.; Bayne, Ellen K.; Van den Berg, Wim B. Kinetics of aggrecanase and metalloproteinase-induced neoepitopes in various stages of cartilage destruction in murine arthritis. *Arthritis Rheum*. **1999**, *42*, 1128−1139. (e) Lark, Michael W.; Gordy, John T.; Weidner, Jeffrey R.; Ayala, Julia; Kimura, James H.; Williams, Hollis R.; Mumford, Richard A.; Flannery, Carl R.; Carlson, Steven S.; Cell-mediated catabolism of aggrecan. Evidence that cleavage at the "aggrecanase" site (Glu373-Ala374) is a primary event in proteolysis of the interglobular domain. *J. Biol. Chem*. **1995**, *270*, 2550−6.

(5) Arner, E. C.; Pratta, M. A.; Decicco, C. P.; Xue, Chu-Biao; Newton, R. C.; Trzaskos, J. M.; Magolda, R. L.; Tortorella, M. D. Aggrecanase: A target for the design of inhibitors of cartilage degradation *Ann. N. Y. Acad. Sci*. **1999**, *878*, 92−107.

(6) Michaelides, M. R.; Curtin, M. L.; Recent Advances in Matrix Metalloproteinase Inhibitor Research. *Curr. Pharm. Des*. **1999**, *5*, 787−819.

(7) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem. Rev*. **1999**, *99*, 2735−2776.

(8) Compound **1** was originally designed and synthesized as collagenase inhibitors by Dickens and co-workers; see: Dickens, J. P.; Donald, D. K.; Kneen, G.; Mckay, W. R. [(Hydroxycarbamoyl)-propionyl]tyrosinamides as collagenase inhibitors. U.S. Patent 4,599,361, 1986.

(9) (a) Fosang, A. J.; Last, K.; Neame, P. J.; Murphy, G.; Knaeuper, V.; Tschesche, H.; Hughes, C. E.; Caterson, B.; Hardingham, T. E. Neutrophil collagenase (MMP-8) cleaves at the aggrecanase site E373-A374 in the interglobular domain of cartilage aggrecan. *Biochem. J*. **1994**, *304*, 347−351. (b) Arner, Elizabeth C.; Decicco, Carl P.; Cherney, R.; Tortorella, M. D. Cleavage of native cartilage aggrecan by neutrophil collagenase (MMP-8) is distinct from endogenous cleavage by aggrecanase. *J. Biol. Chem*. **1997**, *272*, 9294−9299.

(10) Nagase, H.; Fields, G. B. Human Matrix Metalloproteanase Specificity Studies Using Collagen Sequence Based Synthetic Peptides. *Biopolymers* **1996**, *40*, 399−416.

(11) (a) Fields, G. B.; Van Wart, H. E.; Birkedal-Hansen, H. Sequence specificity of human skin fibroblast collagenase. Evidence for the role of collagen structure in determining the collagenase cleavage site. *J. Biol. Chem*. **1987**, *262*, 6221−6226, (b) Netzel-Arnett, S.; Fields, G. B.; Birkedal-Hansen, H.; Van Wart, H. E. Sequence specificities of human fibroblast and neutrophil collagenases. *J. Biol. Chem*. **1991**, *266*, 6747−6755.

(12) Tortorella, M.; Pratta, M.; Liu, R−Q.; Abbaszade, I.; Ross, H.; Burn, T.; Arner, E. The Thrombospondin Motif of Aggrecanase-1 (ADAMTS-4) is Critical for Aggrecan Substrate Recognition and Cleavage. *J. Biol. Chem* **2000**, *275*, 25791−25797.

(13) Gowravaram, M. R.; Tomczuk, B. E.; Johnson, J. S.; Delecki, D.; Cook, E. R.; Ghose, A. K.; Mathiowetz, A. M.; Spurlino, J. C.; Rubin, B.; Smith, D. L.; Pulvino, T. A.; Wahl, R. C. Inhibition of matrix metalloproteinases by hydroxamates containing heteroatom-based modifications of the P1′ group. *J. Med. Chem*. **1995**, *38*, 2570−2581.

(14) Dorsey, B. D.; Levin, R. B.; McDaniel, S. L.; Vacca, J. P.; Guare, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; et al. L-735,524: The Design of a Potent and Orally Bioavailable HIV Protease Inhibitor. *J. Med. Chem*. **1994**, *37*, 3443−51.

(15) Zhang, D.; Botos, I.; Gomis-Ruth, F. X.; Doll, R.; Blood, C.; Njoroge, F. G.; Fox, J. W.; Bode, W.; Meyer, E. F. Structural interaction of natural and synthetic inhibitors with the venom metalloproteinase atrolysin C. *Proc. Natl. Acad. Sci. U.S.A*. **1994**, *91*, 8447−8451.

(16) Gomis-Ruth, F. X.; Kress, L. F.; Kellermann, J.; Mayr, I.; Huber, R.; Bode, W. Refined 2.0A X-ray crystal structure of the snake venom zinc endopeptidase adamalysin II. Primary and tertiary structure determination, refinement, molecular structure and comparison with astacin, collagenase and thermolysin. *J. Mol. Biol*. **1994**, *239*, 513−544.

(17) Luty, B. A.; Wasserman, Z. R.; Stouten, P. F. W.; Hodge, C. N.; Zacharias, M.; McCammon, J. A. A molecular mechanics/grid method for evaluation of ligand−receptor interactions. *J. Comput. Chem*. **1995**, *16*, 454−464.

(18) (a) Tortorella, M.; Pratta, M.; Liu, R-Q.; Abbaszade I.; Ross H.; Burn T.; Arner E. The Thrombosponding Motif of Aggrecanase-1 (ADAMTS-4) is Critical for Aggrecan Substrate Recognition and Cleavage. *J. Biol. Chem* **2000**, *275*, 25791-25797. (b) Xue, C.-B.; He, X.; Roderick, J.; DeGrado, W. F.; Cherney, R. J.; Hardman, K. D.; Nelson, D. J.; Copeland, R. A.; Jaffee, B. D.; Decicco, C. P. Design and synthesis of cyclic inhibitors of matrix metalloproteinases and TNF-alpha production. *J. Med. Chem*. **1998**, *41*, 1745−1748. (c) Miller, J. A.; Arner, E. C.; Copeland, R. A.; Davis, G. L.; Pratta, M., Tortorella, M. D. Assays and peptide substrate for determining aggrecan-degrading metalloprotease activity. WO 0005256, 1998.

(19) (a) Dunten, P.; Kammlott, U.; Crowther, R.; Levin, W.; Foley, L. H.; Wang, P.; Palermo, R. X-ray structure of a novel matrix metalloproteinase inhibitor complexed to stromelysin. *Protein Sci*. **2001,** *10*, 923−926. (b) Pikul, S.; Dunham, K.; M, Almstead, N. G.; De B. Natchus, M. G.; Taiwo, Y. O.; Williams, L. E.; Hynd, B. A.; Hsieh, L. C.; Janusz, M. J.; Gu, F.; Mieling, G. E. Heterocycle-based MMP inhibitors with P2′ substituents. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1009−13. (c) Moy, F. J.; Chanda, P. K.; Chen, J. M.; Cosmi, S.; Edris, W.; Levin, J. I.; Powers, R. High-resolution solution structure of the catalytic fragment of human collagenase-3 (MMP-13) complexed with a hydroxamic acid inhibitor. *J. Mol. Biol*. **2000**, *302*, 671−89.

(20) Hirayama, R.; Yamamoto, M.; Tsukida, T.; Matsuo, K.; Obata, Y.; Sakamoto, F.; Ikeda, S. Synthesis and Biological Evaluation of Orally Active Matrix Metalloproteinase Inhibitors. *Bioorg. Med. Chem*. **1997**, *5*, 765−778.

(21) MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, Shou-Ih; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. Discovery of CGS 27023A, a Non-Peptidic, Potent, and Orally Active Stromelysin Inhibitor that Blocks Cartilage Degradation in Rabbits. *J. Med. Chem*. **1997**, *40*, 2525−2532.

(22) Baenteli, R.; Brun, I.; Hall, P.; Metternich, R. A Synthesis of the C1−N12 Tripeptide Fragment of Sanglifehrin A. *Tetrahedron Lett*. **1999**; *40*, 2109−2112.

(23) Askin, D.; Wallace, M. A.; Vacca, J. P.; Reamer, R. A.; Volante, R. P.; Shinkai, I. Highly diastereoselective alkylations of chiral amide enolates: New routes to hydroxyethylene dipepide isosteres inhibitors of HIV-1 protease. *J. Org. Chem*. **1992**, *57*, 2771−2773.

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