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Synthesis and biological evaluation of biphenylsulfonamide carboxylate aggrecanase-1 inhibitors

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Abstract—Aggrecanases are recently discovered enzymes that cleave aggrecan, a key component of cartilage. Aggrecanase inhibitors may provide a unique means to halt the progression of cartilage destruction in osteoarthritis. The synthesis and evaluation of biphenylsulfonamidocarboxylic acid inhibitors of aggrecanase-1 are reported. Compound **24** demonstrated 89% inhibition of proteoglycan degradation at 10 μg/mL and has an oral bioavailability in rat of 35%.

Osteoarthritis (OA) is a debilitating disease resulting from the breakdown of articular cartilage and characterized by chronic joint pain and inflammation, which results in significant reduction in the quality of life. Currently there is no therapy available to halt the progression of this disease.

Aggrecan, a multidomain proteoglycan, is a major component of cartilage and provides compressive resistance to articular cartilage. During the early stages of osteoarthritis, and then throughout the disease, there is increased loss of GAG (glycosaminoglycan)-rich aggrecan fragments via proteolysis attributable to 'aggrecanase' activity. Eventually, the cartilage is eroded and replacement joint surgery is usually required.

Aggrecanase-1 (agg-1)[1] is a member of ADAMTS (A Disintegrin and Metalloprotease possessing Thrombospondin domain) family of zinc containing metalloproteases, responsible for the cleavage of aggrecan IGD (Interglobular domain) at the Glu^{373}-Ala^{374} peptide bond, a unique site untouched by any previously identified enzyme. Inhibition of agg-1 may impart overall cartilage protection and offer a potential therapy that could alter the progression of OA.[2]

Several reports in the literature described aggrecanase inhibitors that contain hydroxamic acid zinc-chelating groups.[3,4] Modification around hydroxamic acid functional group by introducing polar functionality was reported to have a favorable effect on absorption and clearance through steric hindrance and intramolecular hydrogen bonding.[3a] The use of other zinc-chelating groups, such as the carboxylic acid, has become a popular approach for hydroxamic acid replacement in other matrix metalloproteinase programs.[5]

In our research efforts toward agg-1 inhibitors, we conducted high throughput screening of our corporate library and discovered that the hydroxamate compound **1a**, reported by Novartis as a non-selective stromelysin inhibitor (CGS 27023A),[6] showed 92% inhibition of agg-1 at a concentration of 25 μM.[7] To our surprise, **1b** (the *S*-isomer of **1a**) was inactive toward agg-1 (Table 1).

This enantiomeric preference for an *R*-configuration was examined using an agg-1 homology model derived from the venom metalloproteinase, Atrolysin C (PDB code: 1DTH).[8] The catalytic domains of the two proteins

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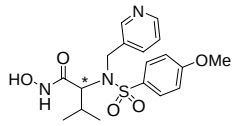
Table 1. High throughput screening hit



Compound	Enantiomer	Agg-1 inhibition at 25 μM
1a	*R*	92%
1b	*S*	Not active

share ~43% homology.[9] The homology model was built in Sybyl 6.7 (Tripos, Inc.) using the Composer module for Homology Modeling. Docking of compound **1a** to the homology model placed the hydroxamic acid moiety around the Zn, the sulfonyl group in H-bonding distance to backbone NHs of Leu330 and Gly331, the methoxy phenyl within the S1′ pocket, and the pyridine substituent facing the solvent near the S2′ area of the active site. Only the *R*-enantiomer **1a** can dock in this manner, as the comparable conformation of the *S*-enantiomer **1b** would clash with Met332 and other residues within the S2′ area of the active site (the βC–βD loop) (Fig. 1).

The binding model of compound **1a** led us to explore carboxylic acid derivatives with the same enantiomeric preference. Structure-based explorations using the homology model suggested that a biphenyl P1′ ligand (**2**) might be best because of the potential for: (1) enthalpic π-stacking interactions with the active site His (His361), (2) enthalpic van der Waals interactions due to the shape of the S1′ pocket (flattened cylinder), and (3) entropic gains from the hydrophobic effect, as this area is encapsulated by hydrophobic residues (e.g., Leu330, Met395, and Phe357).

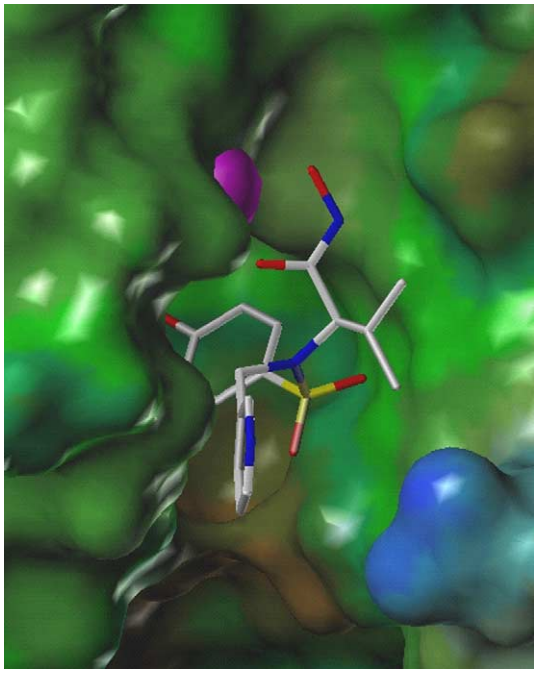
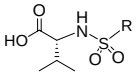


Figure 1. A close-up and schematic view of the predicted binding mode of compound **1a** to agg-1 homology model derived from Atrolysin C.

Compound **2** was synthesized and showed an agg-1 IC_{50} of 3.0 μM (Table 2).[10] Biophysical studies confirmed that **2** binds to agg-1 in 1:1 stoichiometry. A compound with a bent biphenyl configuration (**3**) was inactive presumably because it does not fit into the S1′ narrow hydrophobic channel. Attempts to generate a hydrogen bond between the enzyme and the inhibitor from the biphenyl backbone (**4**–**7**) were not successful. It was thought that the substituents may either disrupt the flattened cylindrical shape of the molecule (**4**–**6**) or were too congested to fit into the narrow S1′ pocket (**4**–**7**), leading to reduced activity against agg-1. A para hydroxy group (**8**), which retained its flattened cylindrical shape, retained activity. Therefore, this para-position provided a good attachment point for further modifications.

Another point of modification was the amino acid headpiece. A variety of amino acids have been explored (Table 3). Compound **9** with an L-valine headpiece had an $IC_{50} > 100$ μM toward agg-1, further confirming the enantiomeric preference of this enzyme. Agg-1 IC_{50} of the homologated acid **10** was ~100 μM. The extra carbon atom increases the spacing between the carboxylic acid and the P1′ group such that they cannot simultaneously achieve the required interactions. Most other modifications (**11**–**14**) were tolerated, suggesting that these side chains might reside in a spacious domain. As suggested by the homology model, there is limited space for N-substitution, and indeed, a small *N*-alkyl substituent (**15**) yielded good

Table 2. Modifications on biphenyl backbone



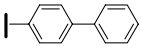
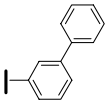
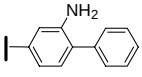
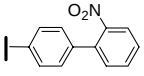
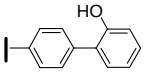
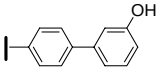
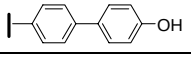
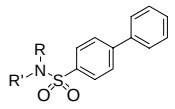
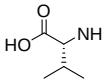
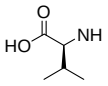
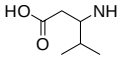
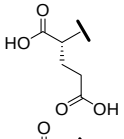
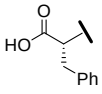
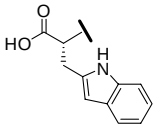
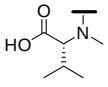
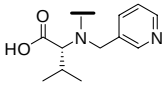
Compound	R	Agg-1 IC_{50} (μM)
2		3.0
3		>100
4		~30
5		~50
6		~20
7		9.0
8		4.6

Table 3. Modification of amino acid headpieces

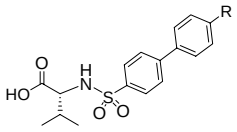


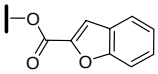
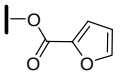
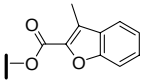
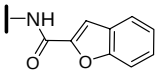
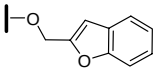
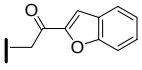
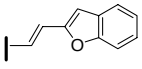
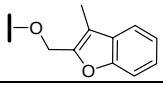
Compound	R-N-R′	Agg-1 IC_{50} (μM)
2		3.0
9		>100
10		~100
11		5.5
12		1.1
13		4.3
14		2.8
15		1.6
16		12

activity, while a bigger group (**16**) led to diminished activity. For simplicity, we kept the D-valine headpiece for further modifications.

Since the homology between agg-1 and Atrolysin C is much lower in the depths of the S1′ pocket, our model was less useful in predicting substitutions beyond the biphenyl P1′ group. Table 4 shows the SAR on further modification of the para-position of the biphenyl backbone. A benzofuran ester P1′ ligand (**17**) improved agg-1 activity by 10-fold. The smaller furan analog (**18**) resulted in decreased activity. A C3-methyl group (**19**) on the benzofuran further improved agg-1 activity by 4-fold (IC_{50} = 86 nM). Other linkages intended to improve metabolic stability while mimicking ester binding, including amide (**20**), ether (**21**), and ketone (**22**), gave reduced activity. A trans double bond configuration (**23**) retained activity; however, this group is prone to metabolism. The C3-methyl ether **24** is 2-fold more active than ether **21**.[11]

Table 4. Linker modification



Compound	R	Agg-1 IC_{50} (μM)
17		0.35
18		11.6
19		0.086
20		7.5
21		1.4
22		3.8
23		0.4
24		0.7

The pharmacokinetics of compound **24** were examined via two different routes of administration, intravenous (iv) and oral (po), to male Sprague–Dawley rats. Animals received a single iv bolus of 2 mpk and a po dose of 10 mpk. The compound exhibited low clearance (16.1 mL/min/kg). The oral bioavailability was 35% ($T_{1/2}$ = 277 min, C_{max} = 1360 ng/mL).

Compound **24** was tested for its ability to inhibit MMP-1, MMP-2, MMP-13, and MMP-14. This compound is selective against MMP-1 and -14, but is a more potent inhibitor of MMP-2 and MMP-13 (Table 5). Since MMP-13 plays an important role in cartilage degradation, inhibition of MMP-13 is also beneficial to preventing osteoarthritis.[2,14]

Compound **24** demonstrated excellent cartilage penetration properties.[12] In an interleukin-1 stimulated bovine cartilage explant assay,[13] it gave 89% inhibition of proteoglycan inhibition at 10 μg/mL after a 3-day incubation (Fig. 2). According to early work published by Pratta[2] and Little,[14] the aggrecan is degraded early, during the first week of culture (day 3–7), whereas the col-

Table 5. Inhibitory activities (IC_{50}, nM) of compound **24**

MMP-1	MMP-2	MMP-13	MMP-14
>100,000	28	4.4	3000

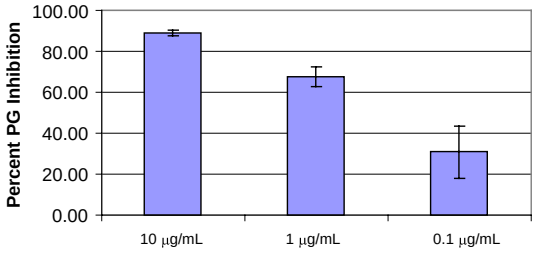
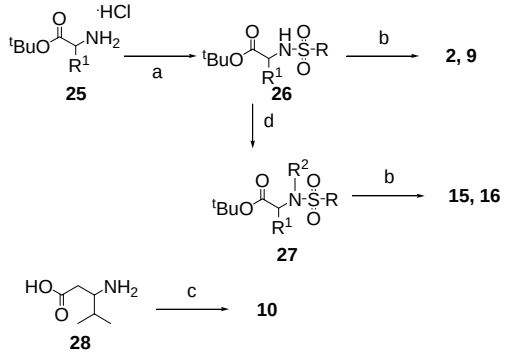


Figure 2. Proteoglycan inhibition of compound **24**.

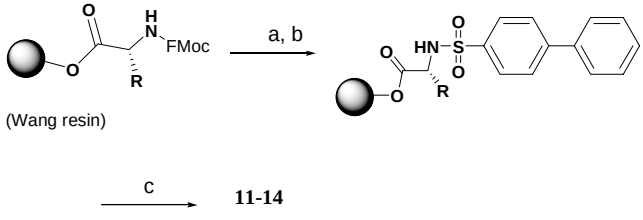
lagen is not rapidly degraded until later in the culture period (day 8–14). Therefore, the activity in this 3-day assay is indicative of aggrecanase inhibition. There is no significant involvement of MMPs during this early stage of proteoglycan degradation.[2,14]

The synthesis of compounds **2**–**24** is shown in Schemes 1–3. Due to the presence of a chiral center sensitive to racemization, the synthesis of these compounds was carried out in the absence of strong basic media.[15]

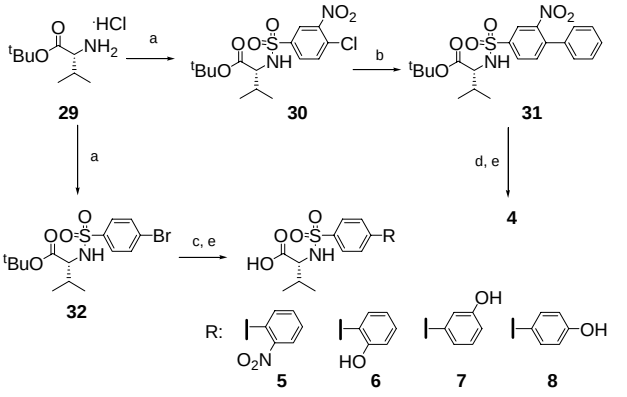
As shown in Scheme 1, sulfonylation of the amino ester **25** gave **26**, which upon hydrolysis formed compounds **2** and **9** in excellent yield. Alkylation of the sulfonamide nitrogen atom and hydrolysis led to compounds **15** and **16**. Sulfonamide formation was performed on β-amino acid **28** using transient silylation condition[16] to give **10**. Compounds **11**–**14** were synthesized from Wang resin through Fmoc deprotection, sulfonamide formation, and TFA cleavage, in excellent yields (Scheme 2).



Scheme 1. Reagents and conditions: (a) RSO_2Cl, DIEA, DCM, 3 h (93–100%); (b) TFA, DCM, 3 h (95–100%); (c) (i)—Me_3SiCl, DCM, reflux, 5 h; (ii)— Et_3N, Ph-$PhSO_2Cl$ (33%); (d) R^2X, K_2CO_3, CH_3CN, reflux, 5 h (45–77%).



Scheme 2. Reagents and conditions: (a) piperidine, DMF, 1 h; (b) biphenyl-4-sulfonyl chloride, iPr_2NEt, CH_2Cl_2; (c) TFA.

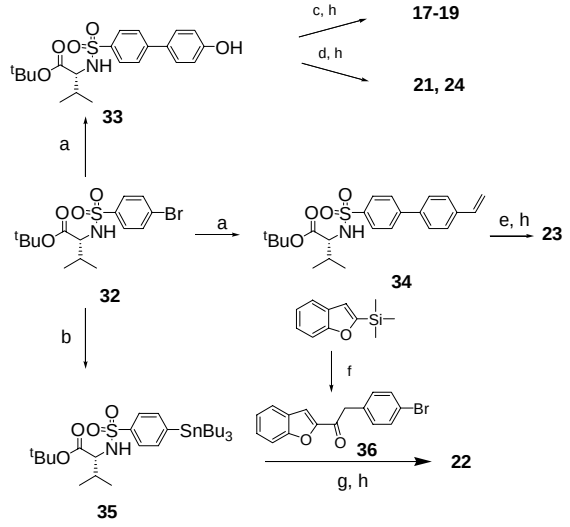


Scheme 3. Reagents and conditions: (a) DIEA, DCM, 3 h (91–95%); (b) $PhB(OH)_2$, $Pd(PPh_3)_2Cl_2$, CsF, NMP, 100 °C, 18 h (55%); (c) for **5**, boronic acid, $Pd(OAc)_2$, PPh_3, Et_3N, DMF, 100 °C, 2 h (43%). For **6**–**7**, boronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O, 80 °C, 12 h (71–83%); (d) Pd/C, H_2, THF, 12 h (100%); (e) TFA, DCM, 3 h (100%).

Suzuki coupling was used to construct the second benzene ring of the biphenyl backbone, leading to compounds **31**, **5**–**8** (Scheme 3), **33** and **34** (Scheme 4). Hydrogenation of **31**, followed by hydrolysis, gave **4**.

Esterification (**17**–**19**) or alkylation (**21**, **24**) of the OH group on compound **33** led to compounds with P1′ ester or ether linkages. Heck coupling on **34** gave compound **23**. 2-Acylbenzofuran **36** was synthesized according to the literature procedure.[17] Stille coupling of tributyltin compound **35** (synthesized from **29**) with **32** gave **22**.

In summary, we described herein the design, synthesis, and biological evaluation of biphenylsulfonamide car-



Scheme 4. Reagents and conditions: (a) boronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O, 80 °C, 12 h (80–82%); (b) $(Bu_3Sn)_2$, $Pd(PPh_3)_4$, toluene, reflux, 12 h (56%); (c) carboxylic acid, DCC, DMAP, DCM, 3.5 h (31–71%); (d) benzofuran-2-CH_2Br, K_2CO_3, DMF, 90 °C, 18 h (35–48%); (e) 2-bromobenzofuran, $Pd(dba)_3$, $[tBu_3PH]^+BF_4^-$, Cy_2NMe, dioxane, microwave, 180 °C, 1 h (25%); (f) 2-(4-bromophenyl)acetyl chloride, $TiCl_4$, DCM, −78 °C, 20 min (17%); (g) $Pd(PPh_3)_4$, toluene, reflux, 12 h (20%); (h) TFA, DCM, 3 h (95–100%).

boxylic acids as aggrecanase-1 inhibitors. The design was based on HTS results and a homology model derived from Atrolysin C. This limited SAR study suggested that biphenylsulfonamide carboxylic acids provided a good starting point and the benzofuran moiety can improve agg-1 inhibition. Compounds such as **24** show good oral bioavailability and inhibition of proteoglycan degradation in a cell-based assay. Compound **24** also shows inhibitory activity against MMP-13. Detailed study of this dual Agg-1/MMP-13 inhibition will be disclosed in due course.

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References and notes

1. 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. *Science* **1999**, *284*, 1664.
2. Pratta, M. A.; Yao, W.; Decicco, C.; Tortorella, M. D.; Liu, R.-Q.; Copeland, R. A.; Magolda, R.; Newton, R. C.; Trzaskos, J. M.; Arner, E. C. *J. Biol. Chem.* **2003**, *278*, 45539.
3. Dual MMP-13 and aggrecanase-1 inhibitors: (a) Noe, M. C.; Natarajan, V.; Snow, S. L.; Wolf-Gouveia, L. A.; Mitchell, P. G.; Lopresti-Morrow, L.; Reeves, L. M.; Yocum, S. A.; Otterness, I.; Bliven, M. A.; Carty, T. J.; Barberia, J. T.; Sweeney, F. J.; Liras, J. L.; Vaughn, M. *Bioorg. Med. Chem.* **2005**, *15*, 3385; (b) Noe, M. C.; Natarajan, V.; Snow, S. L.; Mitchell, P. G.; Lopresti-Morrow, L.; Reeves, L. M.; Yocum, S. A.; Carty, T. J.; Barberia, J. A.; Sweeney, F. J.; Liras, J. L.; Vaughn, M.; Hardink, J. R.; Hawkins, J. M.; Tokar, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2808; (c) Noe, M. C.; Snow, S. L.; Wolf-Gouveia, L. A.; Mitchell, P. G.; Lopresti-Morrow, L.; Reeves, L. M.; Yocum, S. A.; Liras, J. L.; Vaughn, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4727.
4. (a) Yao, W.; Wasserman, Z. R.; Chao, M.; Reddy, G.; Shi, E.; Liu, R.-Q.; Covington, M. B.; Arner, E. C.; Pratta, M. A.; Tortorella, M.; Magolda, R. L.; Newton, R.; Qian, M.; Ribadeneira, M. D.; Christ, D.; Wexler, R. R.; Decicco, C. P. *J. Med. Chem.* **2001**, *44*, 3347; (b) Yao, W.; Chao, M.; Wasserman, Z. R.; Liu, R.-Q.; Covington, M. B.; Newton, R.; Christ, D.; Wexler, R. R.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 101; (c) Cherney, R. J.; Mo, R.; Meyer, D. T.; Wang, L.; Yao, W.; Wasserman, Z. R.; Liu, R.-Q.; Covington, M. B.; Tortorella, M. D.; Arner, E. C.; Qian, A.; Christ, D. D.; Trzaskos, J. M.; Newton, R. C.; Magolda, R. L.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1297.
5. For a review of MMP inhibitors, see: (a) Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. *Curr. Med. Chem.* **2004**, *11*, 2911; (b) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735; (c) Zask, A.; Levin, J. I.; Killar, L. M.; Skotnicki, J. S. *Curr. Pharm. Design* **1996**, *2*, 624.
6. MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.-I.; 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. *J. Med. Chem.* **1997**, *40*, 2525.
7. Compounds are assessed by their ability to inhibit cleavage of a fluorescent peptide substrate (Abz-TEGARGSVI-Dap(Dnp)) (Abz, *o*-aminobenzoyl; Dnp, 2,4 dinitrophenyl) (Anaspec Inc.). The peptide sequence TEGARGSVI is based on the amino acid sequence of the Glu373-Ala374 cleavage site of aggrecan in osteoarthritis. Inhibitors are pre-incubated with purified full-length human recombinant agg-1 for 10 min followed by the addition of substrate, at temperatures ranging from 25 to 37 °C, typically at 30 °C. Cleavage of the Glu-Ala bond releases the fluorophore from internal quenching. This results in an increase in fluorescence monitored at λ_{ex} 340 nm and λ_{ex} 420 nm over a period of 40 min. The initial rate (v) at each concentration of the substrate is fit to the following equation $V = V_{max} \cdot S^h/(S^h_{0.5} + S^h)$, where h is the Hill constant and $S_{0.5}$ is the substrate concentration at half the V_{max}. The percentage activity remaining in the presence of inhibitor is plotted as a function of inhibitor concentration and the IC_{50} value is determined by fitting the data to the following equation: % activity = 100 IC_{50}/(I_o + IC_{50}).
8. Botos, I.; Scapozza, L.; Zhang, D.; Liotta, L. A.; Meyer, E. F. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2749.
9. The most significant primary sequence differences around the active site were in the loop that defines the S2′ area (βC–βD loop; 4 residue insertion for agg-1), and in the loop that defines the shape of the deep end of the S1′ pocket (the metalloprotease 'specificity' loop; 5 residue insertion for agg-1). Nevertheless, most residues close to the active site Zn were conserved and thus we felt confident in using this model for atom-based simulations.
10. All final compounds were characterized by ^{1}H NMR and either HRMS, LC/MS or CHN.
11. Compound **19** has an IC_{50} of 86 nM against aggrecanase-1, as compared to single digit nM activity of hydroxamate aggrecanase-1 inhibitors reported.[2–4]
12. Bovine articular cartilage was cultured in the absence and presence of rabbit serum albumin for 3 days. Conditioned media were collected and cartilage was stored at −20 °C. Cartilage was milled in the presence of liquid nitrogen. Twenty-five milligram of wet control cartilage was extracted with 1.5-mL acetonitrile twice, dried under nitrogen, and reconstituted in 100 μL acetonitrile. Cartilage standards (0.1, 0.5, 5, 10, 50, 100, 500, and 1000 ng/mL) and quality control samples (0.5, 50, and 500 ng/mL) were prepared by adding appropriate amounts of M-369 stock solution to the control samples. M-048 was used as the internal standard (IS) and was added to each cartilage sample or standard prior to the extraction. HPLC was performed on a Perkin Elmer Series 200 HPLC system (Perkin Elmer, Norwalk, CT) using XTerra MS C18, 2.1 × 20 mm, 2.5 mm (Waters, Milford, MA). The mobile phase was: solvent A = 0.1% HCOOH in H_2O and solvent B = 0.1% HCOOH in acetonitrile. The elution gradient was isocratic at 0% B for 1 min, followed by a linear gradient to 100% B over 4 min for the quantitative analysis and over 14 min for the identification of the metabolites. The column was allowed to equilibrate at 0%

B for 2 min before each injection. The flow rate was 0.2 mL/min and the injection volume was 10 μL. Mass spectrometry was performed on a PE SCIEX API 4000 triple quadrupole mass spectrometer (SCIEX, Concord, Ontario, Canada) using TurboIon Spray source operated under negative electrospray ionization mode. Ion source temperature was 400 °C; spray voltage, − 4500 V; MRM, *m*/*z* 499.1 ⩾ 266.7 (M-369) and *m*/*z* 519.3 ⩾ 403.8 (M-048, IS). Unit mass resolution was utilized for Q1 and low resolution was used for Q3 quadrupoles. The dwell time was 400 ms for each selected reaction monitoring (SRM) channel with a 5-ms pause between the scans. All ion and tandem MS instrument parameters were optimized for high sensitivity by infusing a standard solution of 1000 ng/mL at a flow rate of 10 μL/min using an infusion pump (Harvard Apparatus, South Natick, MA).

13. Bovine carpal joints were obtained from young (1- to 2-week-old) animals. Full-depth articular cartilage plugs were harvested using a cork borer and then sliced on a custom die to generate individual disks ~6 mm wide, 1 mm thick, and 30 mg in weight. Cartilage explants were cultured at 37 °C for 5 days in a humidified atmosphere of 5% CO_2 in air in cartilage explant media (CEM) consisting of Dulbecco's modified Eagle's medium containing 1% antimycotic/antibiotic, 2 mM glutamine, 10 mM HEPES, and 50 μg/ml of ascorbate (all from Sigma, St. Louis, MO). The explants were washed with CEM and 1 weighed disk per well was placed in a 96-well culture dish with 0.2 ml media and 6–8 replicates per treatment and cultured for 3 days in the presence or absence of recombinant human IL-1α (rhIL-1, 5 ng/ml, Sigma) and presence or absence of small molecule compound. Media were replaced every day. The proteoglycan content in the medium was measured as sulfated glucosaminoglycan (GAG) by a colorimetric assay using dimethylmethylene blue (DMMB) and chondroitin sulfate C from shark cartilage (Sigma) as a standard. Measured proteoglycan was expressed per weight of cartilage. Treatment of cartilage with IL-1 results in the induction of catabolic enzymes including aggrecanases that degrade cartilage matrix proteoglycan. The cleaved proteoglycan is released from the matrix into the media. Addition of the compound together with IL-1 to the cartilage results in the decrease of proteoglycan release, indicating the inhibition of proteoglycan degradation. During this early phase of proteoglycan degradation, aggrecanases are the predominant catabolic enzymes that cleave aggrecan with no significant role of other MMPs.[2,14]
14. Little, C.; Hughes, C.; Curtis, C.; Janusz, M.; Bohme, R.; Wang-Weigand, S.; Taiwo, Y.; Mitchell, P.; Otterness, I.; Flannery, C.; Caterson, B. *Matrix Biol.* **2002**, *21*, 271.
15. Chiral purities (HPLC) were determined for selected compounds **24** and **33** to look for possible racemization during Suzuki coupling and base-assisted alkylation conditions. No racemization was observed.
16. Varray, S.; Gauzy, C.; Lamaty, F.; Lazaro, R.; Martinez, J. *J. Org. Chem.* **2000**, *65*, 6787.
17. Gill, M. *Tetrahedron* **1984**, *40*, 621.