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Short communication

Arylsulfonamide inhibitors of aggrecanases as potential therapeutic agents for osteoarthritis: Synthesis and biological evaluation

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ABSTRACT

Aggrecanases, in particular aggrecanase-2 (ADAMTS-5), are considered the principal proteases responsible for aggrecan degradation in osteoarthritis. For this reason, considerable effort has been put on the discovery and development of aggrecanase inhibitors able to slow down or halt the progression of osteoarthritis. We report herein the synthesis and biological evaluation of a series of arylsulfonamido-based hydroxamates as aggrecanase inhibitors. Compound **18** was found to have a nanomolar activity for ADAMTS-5, ADAMTS-4 and MMP-13 and high selectivity over MMP-1 and MMP-14. Furthermore, this compound proved to be effective in blocking *ex vivo* cartilage degradation without having effect on cell cytotoxicity.

1. Introduction

Osteoarthritis (OA) is a joint disorder characterized by destruction of articular cartilage. Pain related to this pathology is the leading cause of disability in the elderly population in the USA [1] and UK [2]. OA treatment is currently limited to steroidal and non-steroidal anti-inflammatory drugs (NSAIDS), which provide symptomatic relief for pain and inflammation but do not arrest or slow down the progression of the disease [3]. Recently, considerable effort has been put on the discovery and development of drugs able to reduce or halt the progression of OA, i.e. disease-modifying osteoarthritis drugs (DMOADs).

Articular cartilage is composed by chondrocytes embedded in an extracellular matrix (ECM) which bestows the biomechanical properties that are essential for movement. The two major ECM components are type II collagen fibrils and aggrecan proteoglycans; while collagen is the structural component of cartilage and provides tensile strength to the tissue, aggrecan provides hydration, flexibility, elasticity and compressibility by interacting with hyaluronan, link protein and collagen fibrils [4]. Under physiological conditions, cartilage homeostasis is maintained by a balance between the synthesis and degradation of aggrecan and collagen, but in OA and other joint disorders this equilibrium shifts toward catabolism. Loss of aggrecan is an early event in the onset of cartilage destruction, occurring initially at the joint surface and progressing to deeper zones [5]. Two members of ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin motifs) family of zinc metallopeptidases, ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) have been identified as the major enzymes responsible for aggrecan cleavage [6]. These enzymes cleave aggrecan proteoglycan at $Glu^{373}\downarrow^{374}Ala$ site within the interglobular domain, thus releasing aggrecan core protein from the matrix meshwork [7]. Synovial fluid of patients with various forms of arthritis, such as OA and rheumatoid arthritis, and joint injury, all show aggrecan products of cleavage at this site, which is distinctive of aggrecanase activity [8]. It has been shown that cleavage of aggrecan in cartilage is blocked by a general metalloproteinase inhibitor but not by a MMP-specific inhibitor, thus

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ruling out a role of these enzymes in primary aggrecan degradation [9]. Under physiological conditions, ADAMTS-5 is the most active aggrecanase [10]. Studies with *Adamts-5* null mice showed that the lack of ADAMTS-5 protected against cartilage destruction when OA [11] or inflammatory arthritis [12] was induced. Protection from cartilage erosion in *Adamts-5* knockout mice was observed in other murine models of OA [13]. On the other hand, *Adamts-4* null mice are not protected by cartilage aggrecan loss [14]. These data indicate *Adamts-5* as the major aggrecanase in mouse cartilage and may suggest a similar role in humans. Since the first studies reporting cloning and characterization of aggrecanases, a lot of effort has been spent to develop small molecule inhibitors targeting these enzymes which will pave the way for their use as DMOADs in clinics [15]. Just to cite a few examples, in 2001 a peptidomimetic hydroxamate (**1**, Fig. 1) was reported by Yao et al. [16] as a general inhibitor of aggrecanases. Analogously, Pfizer's MMP-13 sulfonamido-based inhibitor **2** (Fig. 1) [17] showed strong aggrecanase inhibitory activity and, most recently, Cappelli et al. [18] reported a new series of hydroxamate-based aggrecanase inhibitors, among which compound **3** (Fig. 1) was the most active on isolated enzymes. Many efforts [19] were directed to find out scaffolds possessing a zinc-binding group (ZBG) other than the hydroxamate moiety, which is known to be a strong zinc-chelating group that generally confers poor pharmacokinetic properties, but the vast majority of these studies produced only aggrecanase inhibitors active in the low-micromolar range. An exception, among these non-hydroxamate reported compounds, is represented by a potent and selective aggrecanase inhibitor (**4**, Fig. 1) bearing a carboxylate ZBG described by Shiozaki et al. in 2011 [20].

Recently, we have reported [21] a new series of *N*-isopropoxy-arylsulfonamide hydroxamate inhibitors for MMP-13, selective over MMP-1 and MMP-14. Among the novel analogs, a very promising compound was discovered (**5**, Fig. 2), which showed nanomolar activity for MMP-13 and high selectivity over MMP-1, -14, and TACE.

Furthermore, this compound was demonstrated to be effective in an *in vitro* collagen assay and in a model of cartilage degradation since MMP-13 is the enzyme that catalyzes the hydrolysis of type II collagen. On this basis, we undertook the modification of compound **5** in order to identify new potent aggrecanase inhibitors possibly able to maintain activity for MMP-13 and selectivity over MMP-1 and MMP-14. In fact the inhibition of both proteolytic enzymes responsible for destruction of articular cartilage, ADAMTS-5 and MMP-13, could improve therapeutic efficacy in OA.

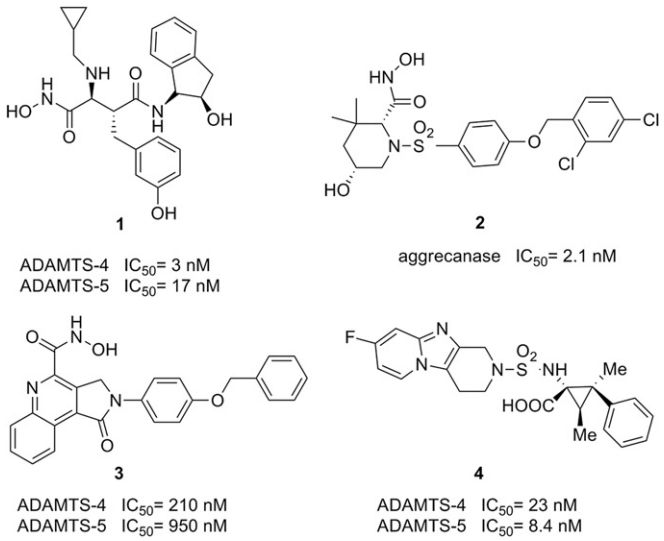


Fig. 1. Examples of aggrecanase inhibitors.

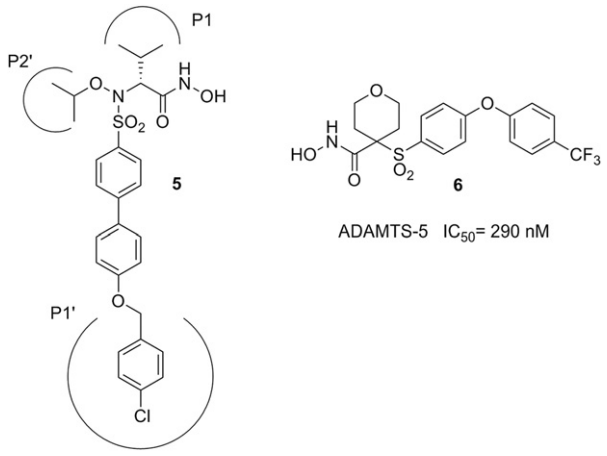


Fig. 2. Structures of the previously reported MMP-13 inhibitor **5** and of the hydroxamate inhibitor **6** used for determining the X-ray structure of the ADAMTS-5 catalytic domain.

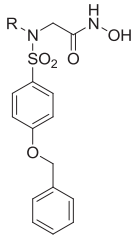
Moreover, it's important to preserve selectivity over MMP-1 and MMP-14 because the inhibition of these enzymes has been hypothesized to cause the musculoskeletal syndrome (MSS) clinically observed with broad-spectrum MMP inhibitors [22].

In this study we report the synthesis and biological evaluation of a new series of arylsulfonamido-based hydroxamates, compounds **7**–**18**, and the discovery of an aggrecanase inhibitor, **18**, which is effective in blocking *ex vivo* cartilage degradation without having effect on cell cytotoxicity.

1.1. Chemistry

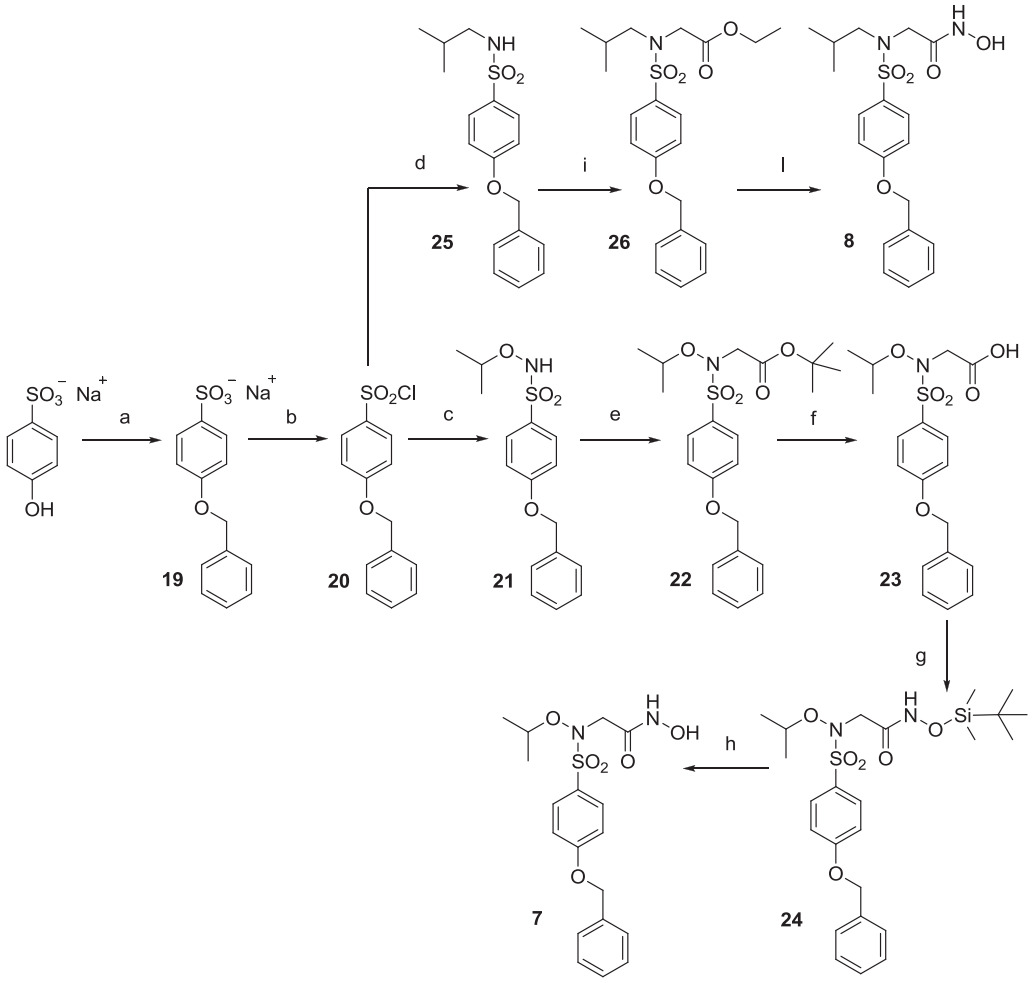
The synthesis of tertiary 4-(benzyloxy)phenyl sulfonamides **7** and **8** (Table 1) is described in Scheme 1. Sulfonyl chloride **20** was synthesized starting from sodium 4-hydroxybenzenesulfonate dihydrate which was alkylated with benzyl bromide using sodium hydroxide as base to give **19**. Reaction of sodium salt **19** with oxalyl chloride in the presence of DMF in dichloromethane afforded sulfonyl chloride **20** which was coupled with *O*-isopropylhydroxylamine hydrochloride [23] upon treatment with *N*-methylmorpholine (NMM) in THF to give sulfonamide **21**. The latter was then reacted with *tert*-butyl bromoacetate in the presence of cesium carbonate to generate ester **22**. Acid hydrolysis of ester **22** yielded carboxylate **23**, which was finally converted to hydroxamate **7** by condensation with *O*-(*tert*-butyldimethylsilyl) hydroxylamine and acid cleavage with TFA [24]. Alternatively,

Table 1
ADAMTS-5 inhibitory activity[a] of compounds **5** [21] and **7**–**9**.



Compd	R	Inhibition % (5 μM)
5		0
7	*O*-*i*-Pr	0
8	*i*-Bu	80
9	H	91

[a] Percent inhibition of ADAMTS-5 observed at 5 μM concentration of the tested compounds. Values are the average from at least three independent experiments. Variations were generally within ±10%.



Scheme 1. Reagents and conditions: (a) benzyl bromide, NaOH, *i*-PrOH, 70 °C; (b) $(COCl)_2$, DMF, CH_2Cl_2; (c) *O*-isopropylhydroxylamine hydrochloride, NMM, THF; (d) isobutylamine, $CHCl_3$; (e) $BrCH_2COOt$-Bu, Cs_2CO_3, Bu_4NHSO_4, DMF; (f) TFA, CH_2Cl_2, 0 °C; (g) $TBDMSiONH_2$, EDC, CH_2Cl_2; (h) TFA, CH_2Cl_2, 0 °C; (i) NaH, $BrCH_2CO_2Et$, THF; (l) NaOMe, $HONH_2$ HCl, MeOH.

sulfonyl chloride **20** was reacted with isobutylamine to give sufonamide **25**. Alkylation with ethyl bromoacetate, followed by treatment with sodium methoxide and hydroxylamine, efficiently provided hydroxamic acid **8**.

Secondary sulfonamido-based hydroxamates **9** and **18** (Table 3) were synthesized as shown in Scheme 2. Sulfonyl chlorides **20** and **28** were prepared as described above and then were coupled with glycine to give carboxylic acids **29** and **30**. Carboxylates were condensed with *O*-(*tert*-butyldimethylsilyl)hydroxylamine in presence of EDC to give silyl intermediates **31** and **32** which afforded hydroxamates **9** and **18** after acid cleavage with TFA.

The compounds listed in Table 2 **10**, **11**, **13**, **14**, and compounds **15**, **17** reported in Table 3, were easily synthesized starting from commercially available sulfonyl chlorides as described in Scheme 3. Sulfonyl chlorides **33**–**37** were made reacting in water and dioxane with glycine or β-alanine using Et_3N as base. Carboxylates **38**–**43** were then condensed with *O*-(*tert*-butyldimethylsilyl)hydroxylamine in the presence of EDC and subsequently deprotected with TFA to yield hydroxamic acids **10**, **11**, **13**–**15** and **17**.

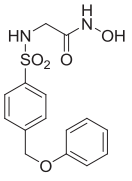
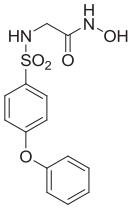
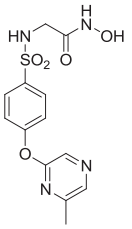
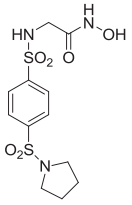
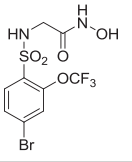
In order to overcome some problems occurred during acid/base work up necessary for isolating pure carboxylates, commercially available sulfonyl chlorides gifted with basic P_1' group underwent the alternative route shown in Scheme 4. Sulfonyl chlorides **50** and **51** were first converted in sulfonamides **52** and **53** by reaction with ammonia aq. solution and then protected by treatment with di-(*tert*-butyl)dicarbonate in CH_2Cl_2 using 4-(dimethylamino)pyridine as acylation catalyst. Boc-sulfonamides **54** and **55** were thus alkylated [25] with *tert*-butyl-bromoacetate using sodium hydride as base to yield *tert*-butyl esters **56** and **57**. Acid cleavage with TFA gave the deprotected carboxylates **58** and **59** that were converted into hydroxamates **12** (Table 2) and **16** (Table 3) as described above.

2. Results and discussion

2.1. Design of ADAMTS inhibitors and in vitro ADAMTS inhibitory activity

Recently, a high resolution crystal structure of the ADAMTS-5 catalytic domain in the presence of a hydroxamate inhibitor **6** (Fig. 2) has been published [26]. The catalytic site has a funnel shape that opens up at the zinc site at its widest point and then forms an L-shaped, hydrophobic channel through the S1' site at its distal end. The enzyme–inhibitor complex exhibits a three-point interaction in which the hydroxamate group plays a key role by chelating the zinc atom and interacting with E411. Moreover, the phenyl-*O*-phenyl group establishes strong hydrophobic interactions with the highly lipophilic and specific S1' pocket. Based on these findings, we aimed to modify the arylsulfonyl moiety of **5** in order to better fit the ADAMTS-5 L-shaped S1' subsite. We synthesized a first series of arylsulfonamides (compounds

Table 2
Structure and ADAMTS-5 inhibitory activity[a] of secondary arylsulfonamides **10**–**14**.

Compd	Inhibition % (5 μM)
 **10**	17
 **11**	98
 **12**	3.7
 **13**	7
 **14**	0.4

[a] Percent inhibition of ADAMTS-5 observed at 5 μM concentration of the tested compounds. Values are the average from at least three independent experiments. Variations were generally within ±10%.

7–**9**) with a short and bended P1′ group, such as the 4-(benzyloxy)-phenyl group (Table 1).

These compounds were tested *in vitro* on human recombinant ADAMTS-5 in comparison with the previously described *N*–O-isopropyl arylsulfonamide **5** to verify the importance of the P2′ substituent on sulfonamido nitrogen. As we have already seen with a series of arylsulfonamide inhibitors of ADAM-17 [27], the *N*–O-alkyl substituent on sulfonamido nitrogen is detrimental also for activity toward ADAMTS-5. In fact, both compounds **5** and **7**, bearing a *N*–O-isopropyl group, resulted devoid of any inhibitory activity on ADAMTS-5. Otherwise, derivatives **8** and **9**, bearing respectively an isobutyl group and a hydrogen atom in P2′, showed a comparable good inhibition of ADAMTS-5 (80 and 91% respectively at 5 μM). On the basis of these preliminary results, we developed a second small set of compounds, secondary sulfonamides **10**–**14** (Table 2), with the aim to find a proper arylsulfonyl substituent to interact with ADAMTS-5 S1′ subsite.

Among these, only the phenoxy-phenyl derivative **11** proved to inhibit ADAMTS-5 (98% inhibition at 5 μM). After this preliminary evaluation, we further investigated possible modifications of compounds **9** and **11** in order to improve activity for ADAMTS-5. Analogs **15**–**18** were easily synthesized and tested *in vitro* on human recombinant aggrecanases and MMPs using a fluorogenic peptide as the substrate (Table 3). The broad spectrum MMP inhibitor GM6001 [28] was used as reference compound.

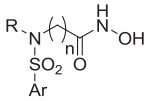
The starting point was the phenoxy-phenyl derivative **11**, a nanomolar inhibitor of MMPs, with a IC_{50} of 330 nM for ADAMTS-5. The elongation of the distance between the sulfonamido nitrogen and the ZBG by insertion of a methylene group adjacent to the hydroxamic functionality, as in derivative **15**, caused a decrease of activity on both MMPs and ADAMTSs. The replacement of the phenoxy-phenyl group with a 6-phenoxypyridine-3-sufonamido group as in **16** provided a 10-fold decrease in ADAMTS-5 inhibitory activity, still retaining nanomolar activity for MMPs. Also the substitution of the phenoxy group with a butoxy group as in **17** gave analogous disappointing results. Thus, we pointed our attention on the 4-(benzyloxy)-phenyl derivative **9** which showed a promising activity on ADAMTS-5 (IC_{50} = 620 nM) and a higher selectivity over ADAMTS-4 and MMP-1. On the basis of literature data regarding similar compounds [31], we planned the introduction of halogen atoms on this scaffold in order to improve ADAMTS-5 inhibitory activity. In particular we synthesized the 2-chloro-4-fluorobenzyloxy derivative **18** which showed a 18-fold increase of potency against ADAMTS-5. Compound **18** proved to be the best of this series exhibiting a nanomolar activity for ADAMTS-5 (IC_{50} = 35 nM) and a high selectivity over MMP-1 and MMP-14. This compound was also active on ADAMTS-4 (IC_{50} = 80 nM) and MMP-13 (IC_{50} = 62 nM), two enzymes implicated cartilage degradation, and MMP-2.

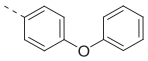
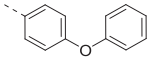
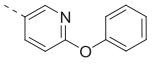
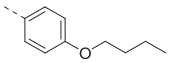
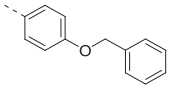
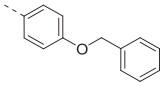
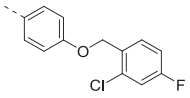
2.2. Docking studies

Molecular docking of **18** and of some close analogs was performed to rationalize the SARs reported in Table 3.

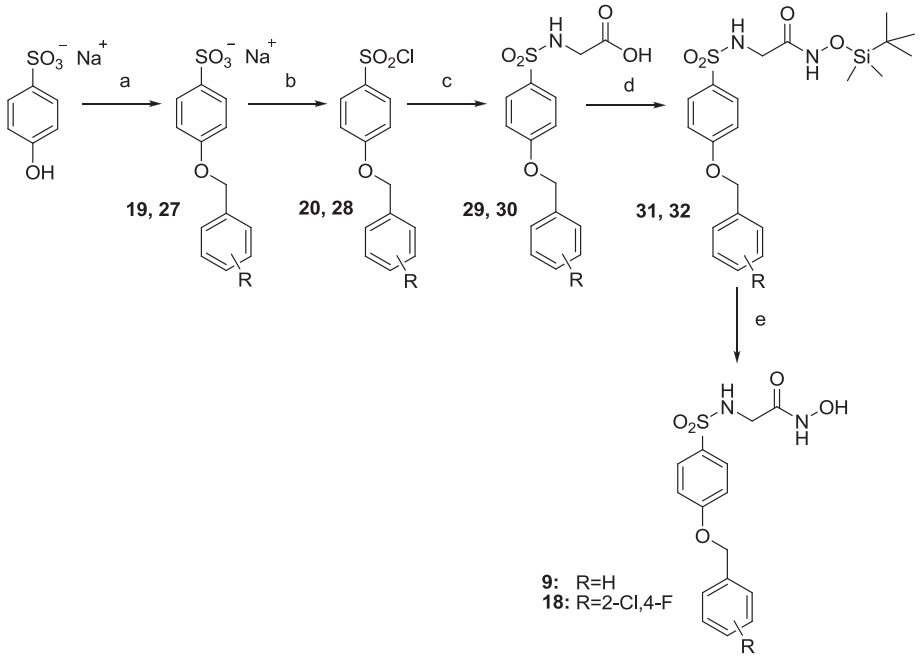
Docking of **18** in ADAMTS-5 resulted in a binding mode (lowest energy docked conformation) where the hydroxamic acid moiety coordinates the catalytic zinc atom in a bidentate fashion and establishes two hydrogen bonds with the backbone CO of G117 and with the E148 side chain (Fig. 3). From the docking results, it is clear that the sulfonamide moiety is particularly important as it forms a hydrogen bond with the backbone amine of L116 and with the carbonyl oxygen of S178 and it enables the *p*-F-*o*-Cl-benzyloxy-phenyl P1′ group to plunge deep into the S1′-specificity pocket (Fig. 3). In the S1′ pocket, the *p*-F-*o*-Cl-benzyl ring establishes an alogen-reinforced T-shaped interaction with the H140 aromatic ring and forms multiple hydrophobic interactions with F143, L175, I183 and L180 side chains. The phenyl ring adjacent to the sulfonamide group is in a suitable position to establish a π–π interaction with H147 side chain and hydrophobic contacts with T144 and I179 side chain. The binding mode here described for **18** in ADAMTS-5 clearly explains its nanomolar activity and it is in perfect accordance with the activities of its analogs toward ADAMTS-5. For example, compounds **9**, lacking both alogens on the benzyl ring, and **17**, missing the entire benzyl moiety, have IC_{50s} toward ADAMTS-5 higher than **18** due to the presence of an alogen-reinforced T-shape interaction between the P1′ group of **18** and the H140. As regards compound **8**, it is clear that the substitution of the sulfonamide hydrogen atom with a bulky group (isobutyl) generates a massive clash with the S1′ pocket entrance, thus causing an evident drop of the inhibitory activity on ADAMTS-5. As for the phenoxyphenyl series, when **11** is docked into the

Table 3
Selectivity profiles[a] of compounds **8**, **9**, **11**, **15**–**18** and the reference compound GM6001.[b]

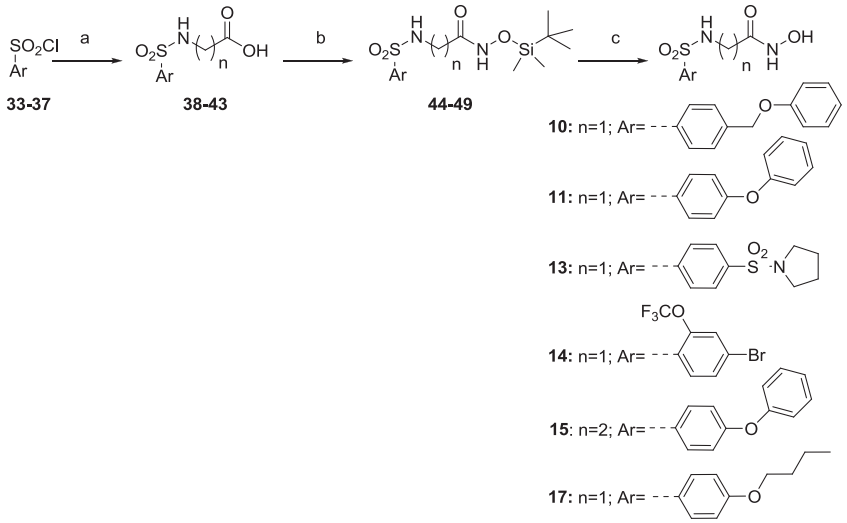


Compd	IC_{50} (nM)								
	Ar	R	n	ADAMTS5	ADAMTS4	MMP1	MMP2	MMP13	MMP14
11		H	1	330	580	720	0.04	0.1	4.0
15[c]		H	2	1410	2100	>5000	10	18	135
16		H	1	3500	5800	2900	0.43	0.65	24
17		H	1	3400	3300	11,000	3.4	7.0	71
8		*i*-Bu	1	1770	630	>5000	1.4	1.5	82
9		H	1	620	3500	27,000	3.8	19	310
18		H	1	35	80	>5000	46	62	3700
GM6001	–	–	–	500	480	3.3	10	5.2	4.7

[a] The IC_{50} values are the average of at least two determinations with a standard deviation of <10%.
[b] Activity data taken from Ref. [29] except those for ADAMTSs (data from our lab).
[c] Compound already reported [30].



Scheme 2. Reagents and conditions: (a) appropriate aryl bromide, NaOH, *i*-PrOH, 70 °C; (b) $(COCl)_2$, DMF, CH_2Cl_2; (c) glycine, TEA, H_2O, dioxane; (d) $TBDMSiONH_2$, EDC, CH_2Cl_2; (e) TFA, CH_2Cl_2, 0 °C.



Scheme 3. Reagents and conditions: (a) glycine or β-alanine (compd **42**), TEA, H_2O, dioxane; (b) $TBDMSiONH_2$, EDC, CH_2Cl_2; (c) TFA, CH_2Cl_2, 0 °C.

ADAMTS-5 X-ray structure, the lowest energy pose is generally superimposable with that of **18**, but the bent geometry of the P1′ group causes a less suitable accommodation in the site. Particularly, in order to accommodate the phenoxyphenyl moiety in the S1′ pocket, the sulfonamide group has to assume distorted geometries and the hydroxamate is not able to chelate the active site zinc in a bidentate fashion. The same happens when **11** is docked into ADAMTS-4 which has a linear S1′ pocket.

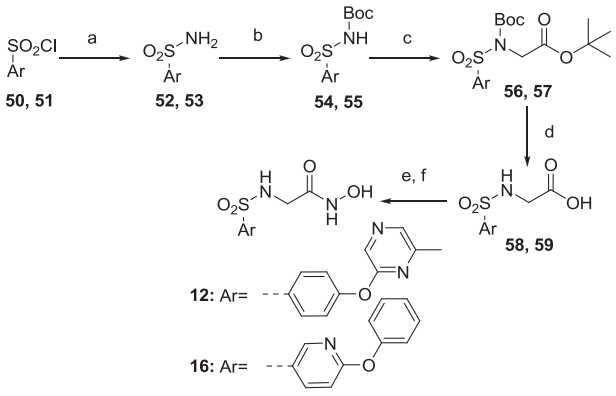
As regards docking of **18** in ADAMTS-4, the Glide program suggests a slight different binding mode with respect to that found in ADAMTS-5. This might be dependent on the differences between the two enzymes. Indeed, in ADAMTS-4 S1′-specificity pocket, ADAMTS-5 H140 is replaced by Q136, so that the above mentioned T-shaped interaction established by **18** with ADAMTS-5 is lost but a parallel π–π interaction with ADAMTS-4 F139 is present and hydrophobic contacts with V167, M172 and V175 side chains are visible (Fig. 2 in SI). In ADAMTS-5, L38, L175 and I183 are replaced by A39, V167 and V175 in ADAMTS-4 thus resulting in different shapes of the S1′ specificity pockets. As a consequence, in ADAMTS-4 the sulfonamide moiety of **18** has to shift its position more into the S1′ pocket where it is not able to establish the usual hydrogen bond with the enzyme. This uncommon position of the sulfonamide group affects also the interaction of the hydroxamic acid with the zinc ion which is chelated in a monodentate fashion by the acid oxygen while the carbonyl oxygen engages a hydrogen bond with the Q144 side chain (see Fig. 2 in SI). However, it should be stated that the monochelation by the hydroxamic acid could also be an artifact aroused by the well-known limits of semiflexible docking procedures.

As regards the low activity of **18** toward MMP-14, in the lowest energy-binding pose, the inhibitor chelates the zinc atom and lays along the binding site reaching the S3′ and the S2′ pockets (see Fig. 1 in SI). In fact, the bulky halogenated-biphenyl moiety is not able to fit into the narrow S1′ pocket. Thus, the low activity of **18** toward MMP-14 can be mainly attributed to the different S1′ pocket shape with respect to that of ADAMTS-4 and -5.

Although the molecular modeling studies herein presented provide valuable explanations for the ligands activity and selectivity, experimental techniques, such as X-ray crystallography, would be finally needed to prove these predictions.

2.3. Inhibition of aggrecan degradation in vitro

To check whether the inhibition of the FRET peptide substrate by ADAMTS-5 reflected inhibition on the native aggrecan substrate, different concentrations of compounds **9**, **11** and **18** were incubated with recombinant human ADAMTS-5 before adding purified aggrecan from bovine nasal cartilage. ADAMTS-5 activity was



Scheme 4. Reagents and conditions: (a) NH_3 aq., CH_3CN, 0 °C to rt; (b) $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2; (c) *tert*-butyl bromoacetate, NaH, DMF; (d) TFA, CH_2Cl_2 0 °C; (e) $TBDMSiONH_2$, EDC, CH_2Cl_2; (f) TFA, CH_2Cl_2, 0 °C.

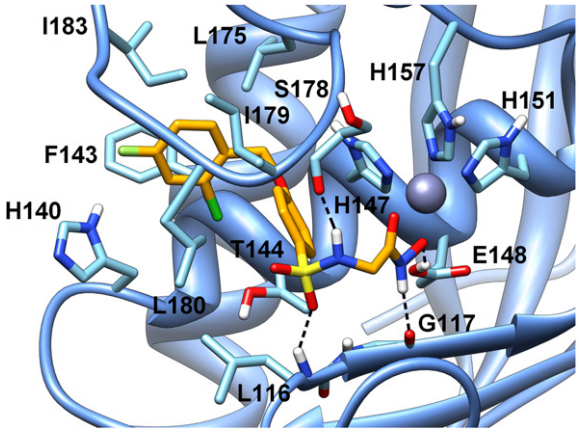
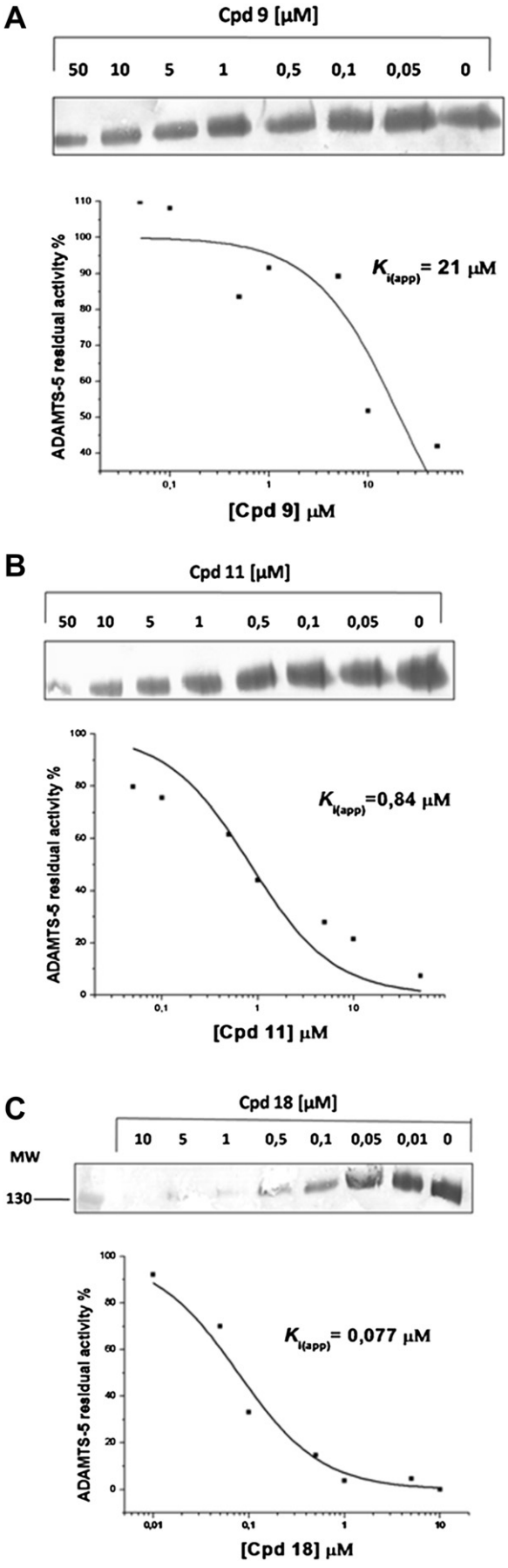


Fig. 3. Docked conformations of **18** in the ADAMTS-5 catalytic site. Hydrogen atoms are omitted for the sake of clarity. Ligands carbon atoms are displayed in golden, and key binding site residues as light blue sticks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



evaluated by Western Blot using anti-AGEG antibody to detect the ^{1772}AGEG neoepitope generated after cleavage in the chondroitin sulfhate-2 (CS-2) domain of aggrecan core protein. Since the sensitivity of the neoepitope antibody used and the fact that cleavage in the CS-2 domain of bovine aggrecan is a particularly favored event, as little as 2 pM of recombinant ADAMTS-5 were required to generate a strong band on Western Blot. All three compounds were able to inhibit ADAMTS-5 activity in a dose-dependent manner (Fig. 4). From this screening, compound **18** emerged as the most potent one, being able to block aggrecan degradation at 1 μM concentration ($K_{i(app)}$ = 0.077 μM) (Fig. 4C). As a consequence, **18** was chosen to carry on further studies in *ex vivo* systems.

2.4. Inhibition of IL-1α-stimulated aggrecan breakdown in cartilage explants

Porcine articular cartilage explants were stimulated with the pro-inflammatory cytokine interleukin-1α (IL-1α) in the presence or absence of **18** or GM6001 for 24 h. Explants treated with IL-1α alone showed approximately three-fold increase in glycosaminoglycan (GAG) release into the medium over unstimulated controls (Fig. 5). The IL-1α-stimulated GAG release was significantly inhibited by the addition of compound **18** in a dose-dependent manner, with a concentration of 10 μM being able to reduce aggrecan degradation to the basal level of the unstimulated controls. However, a concentration of reference compound GM6001 as high as 100 μM was not sufficient to block IL-1α-stimulated GAG release (approximately 30% inhibition).

2.5. Aggrecanase activity is specifically inhibited by compound *18*

Conditioned media from the experiments above were analyzed using monoclonal antibodies that recognize the aggrecanase-generated neoepitope ARGSV in the aggrecan interglobular domain. In concordance with the GAG release data determined by the 1,9-dimethylmethylene blue (DMMB) assay, an increase in the amount of aggrecanase-generated fragments released upon IL-1α treatment was observed (Fig. 6). The release of aggrecanase-generated fragments was partially inhibited by 1 μM of compound **18** and completely blocked by 10 μM of compound **18**. 100 μM of reference compound GM6001 was not as effective as 1 μM compound **18**. No MMP-generated fragments were detected (data not shown).

2.6. Effect of compound *18* on cell viability

To exclude that the inhibition of aggrecan degradation in stimulated cartilage explants by compound **18** was due to cell death, primary porcine chondrocytes were incubated with 1 and 10 μM inhibitor for a period of 24 h and then assessed for cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Fig. 7). This assay indicated that overall cell viability did not change in the presence of compound **18** at concentrations up to 10 μM. These results confirmed our previous

Fig. 4. Dose-dependent inhibition of ADAMTS-5 by compounds **9**, **11** and **18**. ADAMTS-5 at a concentration of 2 pM was incubated with various concentrations of compound **9** (A), **11** (B) or **18** (C) in a total volume of 45 μL for 2 h at 37 °C. Bovine aggrecan (5 μL, 50 μg) was added and the mixture was incubated for 2 h at 37 °C. The reactions were stopped with 50 mM EDTA and the product was deglycosylated overnight with chondroitinase ABC and keratanase and analyzed by Western Blotting for AGEG-containing fragments using the anti-AGEG polyclonal antibody. Band intensity was determined using the 1D Phoretix software package and plotted as a percentage of ADAMTS-5 residual activity *vs* inhibitor concentration. $K_{i(app)}$ were determined with Origin software.

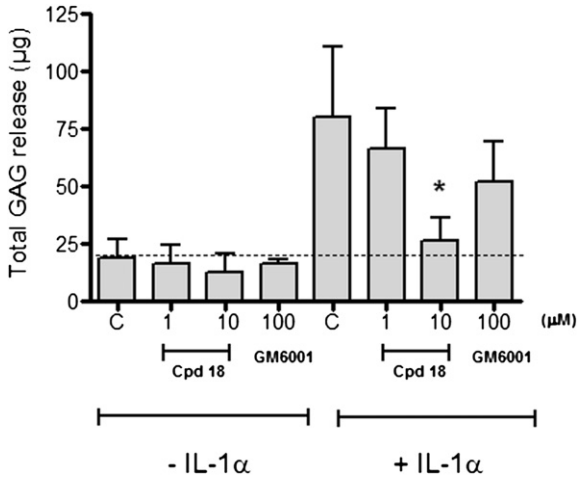


Fig. 5. Inhibition of GAG release by compound **18**. Porcine articular cartilage explants were incubated with DMEM with or without 10 ng/mL IL-1α plus various concentrations of compound **18** (1 and 10 μM) or GM6001 (100 μM) for 24 h. C, DMSO control. The GAG content released into the media was measured using the DMMB assay. The results are from a representative experiment ($n = 3$). *$P < 0.05$ as compared with the IL-1α treatment without the inhibitor.

observations in the field of hydroxamic-based MMP-inhibitors according to which proper substituents around this type of ZBG could be able to avoid cell toxicity [32].

3. Conclusions

A new series of arylsulfonamido-based hydroxamates as aggrecanase inhibitors was designed and synthesized by optimizing the structural characteristics of the previously described MMP-13 inhibitor **5**. Among the novel analogs, a promising compound was discovered (**18**), endowed with nanomolar activity for ADAMTS-5, ADAMTS-4 and MMP-13 and high selectivity over MMP-1 and MMP-14. Because of its promising properties, **18** was chosen for further studies in a model of cartilage degradation stimulated by IL-1α. Aggrecan breakdown in cartilage explants was significantly inhibited by compound **18** in a dose-dependent manner, with a concentration of 10 μM being able to reduce aggrecan degradation to the basal level. No toxicity was detected on parallel cultures of chondrocytes treated with compound **18** by MTT assay.

These preliminary results encourage us to carry on further studies on this class of potential therapeutic agents for osteoarthritis in order to improve their aggrecanase inhibitory activity and selectivity over MMPs.

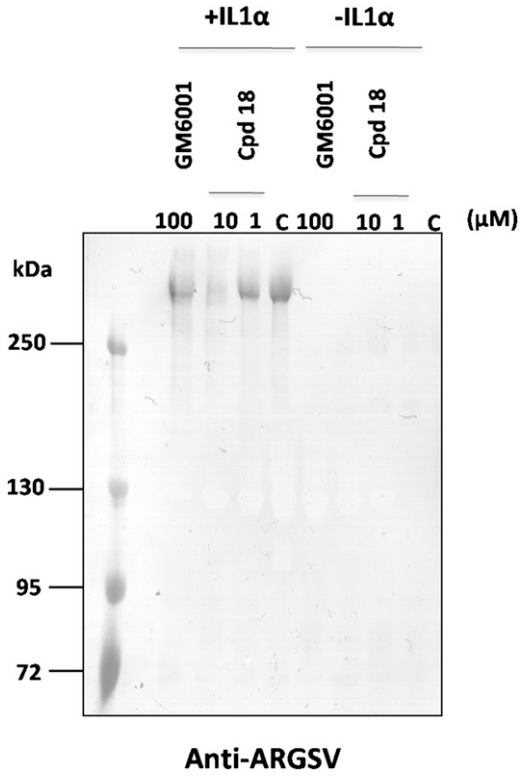


Fig. 6. Detection of aggrecanase-generated aggrecan fragments by anti-ARGSV neoepitope antibody. The conditioned media from porcine articular cartilage explants treated with IL-1α were deglycosylated and analyzed by Western Blotting. C, DMSO controls.

4. Experimental section

4.1. Chemistry

Melting points were determined on a Kofler hotstage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Coupling constants J are reported in hertz; ^{13}C NMR spectra were fully decoupled. The following abbreviations are used: singlet (s), doublet (d), triplet (t), double-doublet (dd), broad (br) and multiplet (m). An Applied Biosystems-MDS Sciex API 4000 triple quadrupole mass spectrometer equipped with Turbo-V IonSpray (TIS) source and interfaced to Perkin–Elmer Series 200 Micro High pressure mixing pump and a Series 200 Autosampler was used for mass analysis (flow injection of sample using MeOH 0.1% NH_4OAc). Where indicated, the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns by flash column chromatography (Kieselgel 40, 0.040–0.063 mm; Merck) or using Isolute Flash Si II cartridges (Biotage). Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F254) sheets that were visualized under a UV lamp and hydroxamic acids were visualized with FeCl3 aqueous solution. Evaporation was performed *in vacuo* (rotating evaporator). Sodium sulfate was always used as the drying agent. 2-Chloro-4-fluorobenzyl bromide was purchased from ABCR (Germany).

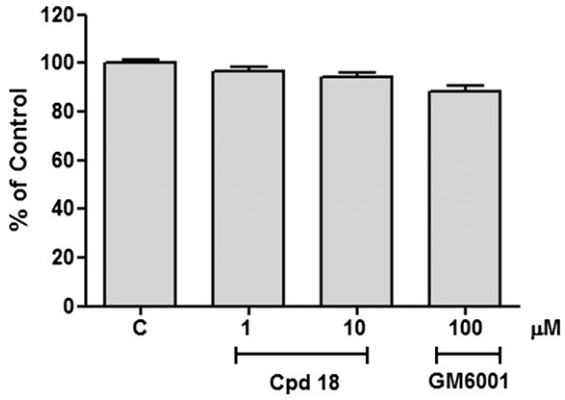


Fig. 7. Analysis of chondrocyte viability. Porcine articular chondrocytes were cultured in the presence of two different concentrations of compound **18** (1 and 10 μM) and reference compound GM6001 (100 μM) for 24 h. The MTT assay way then carried out to test cell viability as described in Section 4.8. The change in the absorbance for each treatment ($n = 3$) is reported. C, DMSO control.

Sulfonyl chlorides **33**–**37** and **50**, **51** were purchased from Maybridge (Novachimica). All other commercially available chemicals were purchased from Sigma–Aldrich.

*4.1.1. Sodium 4-(benzyloxy)benzenesulfonate (**19**)*

To a suspension of sodium 4-hydroxybenzenesulfonate dihydrate (2.0 g, 8.6 mmol) in isopropanol (38.7 mL), containing 8.6 mL of NaOH 1 N freshly prepared, benzyl bromide (2.05 mL, 17.2 mmol) was added. The reaction was refluxed (70 °C) overnight. Isopropanol was evaporated and the precipitate was collected by filtration and washed with isopropanol. The solid was dried *in vacuo*. White solid (2.45 g, 88% yield). ^{1}H NMR (DMSO-d_6) δ: 5.11 (s, 2H); 6.91–6.96 (m, 2H); 7.36–7.55 (m, 7H).

*4.1.2. 4-(Benzyloxy)benzene-1-sulfonyl chloride (**20**)*

To a 0 °C solution of oxalyl chloride (1.93 mL, 22.8 mmol) in dry CH_2Cl_2 (7.6 mL) under Ar atmosphere, *N*,*N*-dimetilformammide was added dropwise (1.76 mL, 22.8 mmol) followed by the sodium salt **19** (2.45 g, 7.6 mmol). The reaction was stirred at 0 °C for 10 min and then at room temperature (RT) for 2 days. The crude was poured into ice and the product was extracted with EtOAc. Organic layers were washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to give sulfonyl chloride **20** as a yellow oil (2.02 g, 83% yield). ^{1}H NMR ($CDCl_3$) δ: 5.18 (s, 2H); 7.09–7.13 (m, 2H); 7.42 (s, 5H); 7.96–8.00 (m, 2H).

*4.1.3. 4-(Benzyloxy)-N-isopropoxybenzenesulfonamide (**21**)*

A solution of sulfonyl chloride **20** (900 mg, 2.82 mmol) in anhydrous THF (8.7 mL) was added dropwise to a stirred and cooled (0 °C) solution of *O*-isopropylhydroxylamine hydrochloride (315 mg, 2.82 mmol) and *N*-methylmorpholine (0.62 mL, 5.65 mmol) in anhydrous THF (5.0 mL). After stirring at RT for 4 days, the reaction mixture was diluted with EtOAc and washed with H_2O. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by trituration with *n*-hexane to afford **21** as a white solid (266 mg, 26% yield). ^{1}H NMR ($CDCl_3$) δ: 1.17 (d, J = 6.2 Hz, 6H), 4.23 (septet, J = 6.2 Hz, 1H), 5.13 (s, 2H); 6.70 (s, 1H); 7.05–7.10 (m, 2H); 7.37–7.43 (m, 5H); 7.83–7.87 (m, 2H). MS (ESI+) m/z: 322 $[M + H]^+$.

*4.1.4. tert-Butyl 2-(4-(benzyloxy)-N-isopropoxyphenylsulfonamido) acetate (**22**)*

A solution of sulfonamide **21** (266 mg, 0.74 mmol) in anhydrous DMF (1.6 mL) was treated with *tert*-butyl bromoacetate (0.13 mL, 0.89 mmol), cesium carbonate (242 mg, 0.74 mmol) and tetrabutylammonium hydrogen sulfate (253 mg, 0.74 mmol). The reaction mixture was stirred for 3 days at RT, diluted with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to afford **22** as a white solid (140 mg, 43% yield). ^{1}H NMR ($CDCl_3$) δ: 1.24 (d, J = 6.2 Hz, 6H); 1.46 (s, 9H); 3.57 (s, 2H); 4.53 (septet, J = 6.4 Hz, 1H); 5.13 (s, 2H); 7.07–7.11 (m, 2H); 7.38–7.44 (m, 5H); 7.78–7.82 (m, 2H). MS (ESI+) m/z: 436 $[M + H]^+$.

*4.1.5. 2-(4-(Benzyloxy)-N-isopropoxyphenylsulfonamido)acetic acid (**23**)*

TFA (1.4 mL, 17.67 mmol) was added dropwise to a stirred, ice-chilled solution of *tert*-butyl ester **22** (135 mg, 0.31 mmol) in freshly distilled dichloromethane (2.4 mL). The mixture was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo*. The crude product was purified by trituration with *n*-hexane to give **23** as a white solid (104 mg, 88% yield). ^{1}H NMR ($CDCl_3$) δ: 1.08 (d, J = 6.2 Hz, 6H), 3.60 (s, 2H); 4.36 (septet, J = 6.2 Hz, 1H); 4.98 (s, 2H); 6.92–6.97 (m, 2H); 7.22–7.28 (m, 5H); 7.63–7.67 (m, 2H). MS (ESI–) m/z: 378 $[M - H]^-$.

*4.1.6. 2-(4-(Benzyloxy)-N-isopropoxyphenylsulfonamido)-N-(tert-butyldimethylsilyloxy)acetamide (**24**)*

1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDC) was added portionwise (75.9 mg, 0.40 mmol) to a stirred and cooled solution (0 °C) of the carboxylic acid **23** (100 mg, 0.26 mmol), and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (38.9 mg, 0.26 mmol) in freshly distilled CH_2Cl_2 (6.9 mL). After stirring at RT overnight, the mixture was washed with water and the organic phase was dried and evaporated *in vacuo*. The crude was purified by flash chromatography (*n*-hexane/EtOAc 4:1) using a ISOLUTE Flash Si II cartridge to afford the *O*-silylate derivative **24** as a white solid (73 mg, 54% yield). ^{1}H NMR ($CDCl_3$) δ: 0.09 (s, 3H); 0.21 (s, 3H); 0.97 (s, 9H); 1.22 (d, J = 6.2 Hz, 6H); 3.66 (s, 2H); 4.42 (septet, J = 6.2 Hz, 1H); 5.14 (s, 2H); 7.08–7.13 (m, 2H); 7.38–7.44 (m, 5H); 7.78–7.82 (m, 2H). MS (ESI+) m/z: 509 $[M + H]^+$.

*4.1.7. 2-(4-(Benzyloxy)-N-isopropoxyphenylsulfonamido)-N-hydroxyacetamide (**7**)*

TFA (0.61 mL, 7.87 mmol) was added dropwise to a stirred and ice-chilled solution of *O*-silylate derivative **24** (70 mg, 0.14 mmol) in CH_2Cl_2 (0.5 mL). The solution was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo*. The crude product was purified by trituration with Et_2O to give the hydroxamate **7** as a white solid (20 mg, 38.6% yield). Mp 129–131 °C; MS (ESI+) m/z: 395 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ: 1.22 (d, J = 6.0 Hz, 6H); 3.73 (s, 2H); 4.42 (septet, J = 6.2 Hz, 1H); 5.14 (s, 2H); 7.08–7.13 (m, 2H); 7.38–7.41 (m, 5H); 7.78–7.83 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 21.31, 56.43, 70.73, 80.00, 115.39, 124.06, 127.61, 128.58, 128.90, 132.07, 135.71, 163.74. Elemental analysis for $C_{18}H_{22}N_2O_6S$. Calculated %C, 54.81; %H, 5.62; %N, 7.10; found %C, 54.95; %H, 5.80; %N, 7.00.

*4.1.8. 4-(Benzyloxy)-N-isobutylbenzenesulfonamide (**25**)*

Isobutylamine (0.94 mL, 9.41 mmol) was dissolved in chloroform (7.1 mL), and the solution was cooled to 0 °C. To this solution was added sulfonyl chloride **20** (1.0 g, 3.14 mmol). The reaction was stirred at RT for 1 h and then refluxed for 1 h. After being cooled back to RT, the reaction mixture was washed three times with 4 N hydrochloric acid, twice with water, and once with brine and then dried (Na_2SO_4), and the solvent was evaporated to give **25** (550 mg, 49% yield). ^{1}H NMR ($CDCl_3$) δ: 0.71 (d, J = 6.6 Hz, 6H); 1.55 (septet, J = 6.7 Hz, 1H); 2.58 (t, J = 6.4 Hz, 2H); 4.34 (t, 1H); 4.97 (s, 2H); 6.88–6.92 (m, 2H); 7.26–7.28 (m, 5H); 7.62–7.66 (m, 2H). MS (ESI+) m/z: 320 $[M + H]^+$.

*4.1.9. Ethyl 2-(4-(benzyloxy)-N-isobutylphenylsulfonamido) acetate (**26**)*

Sodium hydride (37 mg, 1.55 mmol) was suspended in THF (4 mL). To this was added a solution of sulfonamide **25** (550 mg, 1.55 mmol) also in THF (23 mL), and the reaction mixture was stirred for 30 min at RT. Then ethyl bromoacetate (0.3 mL, 2.32 mmol) was added, and the reaction mixture was stirred overnight at RT. The reaction was quenched with a small amount of water, and all the solvent was removed. The crude mixture was partitioned between ethyl acetate and water, the aqueous phase was extracted several times with ethyl acetate, the combined organic layers were dried (Na_2SO_4), and the solvent was evaporated. The product was purified by flash chromatography (*n*-hexane/EtOAc 10:1) using a Isolute Flash Si II cartridge to give **26** (145 mg, 21% yield). ^{1}H NMR ($CDCl_3$) δ: 0.88 (d, J = 6.6 Hz, 6H); 1.17 (t, J = 7.1 Hz, 3H); 1.70–1.89 (m, 1H); 3.01 (d, J = 7.7 Hz, 2H); 3.98–4.09 (m, 4H); 5.11 (s, 2H); 7.00–7.04 (m, 2H); 7.36–7.42 (m, 5H); 7.73–7.78 (m, 2H). MS (ESI+) m/z: 406 $[M + H]^+$.

*4.1.10. 2-(4-(Benzyloxy)-N-isobutylphenylsulfonamido)-N-hydroxyacetamide (**8**)*

Ester **26** (145 mg, 0.33 mmol) was dissolved in methanol (1.5 mL). To this solution was added hydroxylamine hydrochloride (45.6 mg, 0.66 mmol), followed by the addition of sodium methoxide, freshly prepared from sodium (23 mg) dissolved in methanol (0.6 mL). The reaction mixture was stirred overnight at RT. The reaction was worked up by partitioning between dilute HCl and ethyl acetate. The aqueous phase was extracted with ethyl acetate, the combined organic layers were dried (Na_2SO_4), and the solvent was evaporated. The crude product was purified by silica gel chromatography ($CHCl_3$/MeOH 60:1) to give **8** as a white solid (43 mg, 33.5% yield). Mp 140–142 °C; MS (ESI+) *m/z*: 393 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 0.80 (d, J = 6.4 Hz, 6H); 1.75–1.88 (m, 1H); 2.83 (d, J = 7.3 Hz, 2H); 3.64 (s, 2H); 5.19 (s, 2H); 7.14–7.19 (m, 2H); 7.34–7.45 (m, 5H); 7.73–7.78 (m, 2H); 8.91 (s, 1H); 10.56 (s, 1H). ^{13}C NMR (acetone-d_6) δ: 20.35, 27.48, 49.51, 57.64, 70.84, 115.65, 128.48, 128.79, 129.27, 130.43, 137.41, 162.82. Elemental analysis for $C_{19}H_{24}N_2O_5S$. Calculated %C, 58.15; %H, 6.16; %N, 7.14; Found %C, 58.32; %H, 6.29; %N, 7.03.

*4.1.11. Sodium 4-(2-chloro-4-fluorobenzyloxy)benzenesulfonate (**27**)*

To a suspension of sodium 4-hydroxybenzenesulfonate dihydrate (1.3 g, 5.6 mmol) in isopropanol (25 mL), containing 5.6 mL of NaOH 1 N freshly prepared, 2-chloro-4-fluorobenzyl bromide (2.5 g, 11.2 mmol) was added. The reaction was refluxed (70 °C) overnight. Isopropanol was evaporated and the precipitate was collected by filtration and washed with isopropanol. The solid was dried *in vacuo*. White solid (1.93 g, 91.7% yield). ^{1}H NMR (DMSO-d_6) δ: 5.13 (s, 2H); 6.92–6.97 (m, 2H); 7.26 (dt, J_1 = 10 Hz, J_2 = 2.6 Hz, 1H); 7.49–7.54 (m, 3H); 7.64 (dd, J = 6.4 Hz, 1H).

*4.1.12. 4-(2-Chloro-4-fluorobenzyloxy)benzene-1-sulfonyl chloride (**28**)*

To a 0 °C solution of oxalyl chloride (1.3 mL, 15.3 mmol) in dry CH_2Cl_2 (5.1 mL) under Ar atmosphere, *N*,*N*-dimetilformammide was added dropwise (1.2 mL, 15.3 mmol) followed by the sodium salt **27** (1.92 g, 5.11 mmol). The reaction was stirred at 0 °C for 10 min and then at rt for 2 days. The crude was poured into ice and the product was extracted with EtOAc. Organic layers were washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to give sulfonyl chloride **28** as a white solid (1.68 g, 88.6% yield). ^{1}H NMR ($CDCl_3$) δ: 5.22 (s, 2H), 7.00–7.23 (m, 4H); 7.49 (dd, J = 6.0 Hz, 1H); 7.98–8.02 (m, 2H).

*4.1.13. General procedure for the synthesis of carboxylates **29** and **30***

The appropriate sulfonyl chloride **20** or **28** (2.16 mmol) was added to a solution of glycine (162 mg, 2.16 mmol) in H_2O (3 mL) and dioxane (3 mL) containing Et_3N (0.6 mL, 4.31 mmol). The mixture was stirred at RT overnight, dioxane was evaporated and the residue was treated with EtOAc and washed with HCl 1 N and brine. Organic layers were then collected, dried over Na_2SO_4, and evaporated *in vacuo*.

*4.1.14. 2-(4-(Benzyloxy)phenylsulfonamido)acetic acid (**29**)*

The title compound was prepared from sulfonyl chloride **20** following the general procedure. White solid (87% yield). ^{1}H NMR (DMSO-d_6) δ: 3.53 (d, J = 6.04 Hz, 2H); 5.18 (s, 2H); 7.15–7.19 (m, 2H); 7.34–7.49 (m, 2H); 7.70–7.74 (m, 2H); 7.87 (t, J = 6.04 Hz, 1H). MS (ESI−) *m/z*: 320 $[M - H]^-$.

*4.1.15. 2-(4-(2-Chloro-4-fluorobenzyloxy)phenylsulfonamido)acetic acid (**30**)*

The title compound was prepared from sulfonyl chloride **28** following the general procedure. White solid (85.7% yield). ^{1}H NMR (DMSO-d_6) δ: 3.65 (d, J = 5.6 Hz, 2H); 5.32 (s, 2H); 7.29–7.33 (m, 2H); 7.42 (dt, J_1 = 8.6 Hz, J_2 = 2.4 Hz, 1H); 7.67 (dd, J_1 = 8.6 Hz, J_2 = 2.8 Hz, 1H); 7.77–7.81 (m, 1H); 7.84–7.88 (m, 2H); 8.02 (t, J = 6.4 Hz, 1H). MS (ESI−) *m/z*: 372 $[M - H]^-$, 374 $[M + 2 - H]^-$.

*4.1.16. General procedure for the synthesis of N-(tert-butyldimethylsilyloxy)acetamides **31** and **32***

1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDC) (292 mg, 1.56 mmol) was added portionwise to a stirred and cooled solution (0 °C) of the carboxylic acid **29** or **30** (0.61 mmol), and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (359 mg, 2.44 mmol) in freshly distilled CH_2Cl_2 (16 mL). After stirring at RT overnight, the mixture was washed with water and the organic phase was dried and evaporated *in vacuo*.

*4.1.17. 2-(4-(Benzyloxy)phenylsulfonamido)-N-(tert-butyldimethylsilyloxy)acetamide (**31**)*

The title compound was prepared from carboxylic acid **29** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to give **31** as a white solid (45% yield). ^{1}H NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 3.57 (br s, 1H); 3.77 (br s, 1H); 5.12 (s, 2H); 5.19 (br s, 1H); 7.03–7.07 (m, 2H); 7.76–7.80 (m, 2H); 8.50 (br s, 1H). MS (ESI+) *m/z*: 451 $[M + H]^+$.

*4.1.18. N-(tert-Butyldimethylsilyloxy)-2-(4-(2-chloro-4-fluorobenzyloxy)phenylsulfonamido)acetamide (**32**)*

The title compound was prepared from carboxylic acid **30** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2.5:1) using a Isolute Flash Si II cartridge to give **32** as a white solid (44% yield). ^{1}H NMR (DMSO-d_6) δ: 0.04 (s, 6H); 0.87 (s, 9H); 5.20 (s, 2H); 7.18–7.22 (m, 2H); 7.29 (m, 1H); 7.55 (m, 1H); 7.67 (m, 1H); 7.72–7.76 (m, 2H). MS (ESI+) *m/z*: 503 $[M + H]^+$, 505 $[M + 2 + H]^+$.

*4.1.19. General procedure for the synthesis of hydroxamates **9** and **18***

TFA (1.2 mL, 15.3 mmol) was added dropwise to a stirred and ice-chilled solution of *O*-silylate derivative **31** or **32** (0.27 mmol) in CH_2Cl_2 (2.1 mL). The solution was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo*. The crude products were purified by trituration with *n*-hexane/Et_2O to give the desired hydroxamates.

*4.1.20. 2-(4-(Benzyloxy)phenylsulfonamido)-N-hydroxyacetamide (**9**)*

The title compound was prepared from *O*-silylate derivative **31** following the general procedure. White solid (95% yield). MS (ESI+) *m/z*: 337 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 3.27 (d, J = 5.31 Hz, 2H); 5.19 (s, 2H); 7.16–7.20 (m, 2H); 7.35–7.45 (m, 5H); 7.72–7.76 (m, 2H); 7.80 (t, J = 5.31 Hz, 1H); 10.10 (br s, 1H); 10.53 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 43.13, 69.62, 114.96, 127.81, 128.03, 128.45, 128.70, 132.04, 135.46, 136.28, 161.15. Elemental analysis for $C_{15}H_{16}N_2O_5S$. Calculated: %C, 53.56; %H, 4.79; %N, 8.33; found %C, 53.71; %H, 4.89; %N, 8.30.

*4.1.21. 2-(4-(2-Chloro-4-fluorobenzyloxy)phenylsulfonamido)-N-hydroxyacetamide (**18**)*

The title compound was prepared from *O*-silylate derivative **32** following the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 40:1) using a Isolute Flash Si II cartridge to give **18** as a white solid (36% yield). Mp 128–130 °C; MS (ESI+) *m/z*: 389 $[M + H]^+$, 391 $[M + 2 + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 3.32 (s, 2H); 5.25 (s, 2H); 7.22–7.27 (m, 2H); 7.34 (dt, J_1 = 8.6 Hz, J_2 = 2.4 Hz, 1H); 7.58 (dd, J_1 = 8.6 Hz, J_2 = 2.8 Hz, 1H); 7.69–7.73

(m, 1H); 7.76–7.81 (m, 2H); 8.86 (br s, 1H); 10.60 (br s, 1H). ^{13}C NMR (acetone-d_6) δ: 44.35, 67.96, 115.20 (d, J_{C-F} = 21.0 Hz), 115.94, 117.62 (d, J_{C-F} = 24.7 Hz), 130.16, 131.40, 132.37, 133.60, 162.63, 165.76. Elemental analysis for $C_{15}H_{14}ClFN_2O_5S$. Calculated: %C, 46.34; %H, 3.63; %N, 7.21; found %C, 46.58; %H, 3.79; %N, 7.02.

*4.1.22. General procedure for the synthesis of carboxylates **38**–**43***

The appropriate arylsulfonyl chloride **33**–**37** (1.83 mmol) was added to a solution of glycine or β-alanine (0.91 mmol) in H_2O (0.9 mL) and dioxane (0.9 mL) containing Et_3N (0.25 mL, 1.83 mmol). The mixture was stirred at RT overnight, dioxane was evaporated and the residue was treated with EtOAc and washed with HCl 1 N, brine and with a satured solution of $NaHCO_3$. Aqueous basic phases were then acidified with HCl 1 N until pH = 4 and the product was extracted with EtOAc. Organic layers were collected, dried over Na_2SO_4 and concentrated *in vacuo*.

*4.1.23. 2-(4-(Phenoxymethyl)phenylsulfonamido)acetic acid (**38**)*

The title compound was prepared from sulfonyl chloride **33** and glycine following the general procedure. White solid (73% yield). ^{1}H NMR (DMSO-d_6) δ: 3.59 (s, 2H); 5.19 (s, 2H); 6.93–7.05 (m, 3H); 7.28–7.34 (m, 2H); 7.62–7.66 (m, 2H); 7.79–7.84 (m, 2H); 8.06 (m, 1H). MS (ESI−) *m*/*z*: 320 $[M - H]^-$.

*4.1.24. 2-(4-Phenoxyphenylsulfonamido)acetic acid (**39**)*

The title compound was prepared from sulfonyl chloride **34** and glycine following the general procedure. White solid (28% yield). ^{1}H NMR (DMSO-d_6) δ: 3.56 (s, 2H); 7.07–7.15 (m, 4H); 7.21–7.29 (m, 2H); 7.43–7.50 (m, 2H); 7.76–7.80 (m, 2H); 7.91 (br s, 1H). MS (ESI−) *m*/*z*: 306 $[M - H]^-$.

*4.1.25. 2-(4-(Pyrrolidin-1-ylsulfonyl)phenylsulfonamido)acetic acid (**40**)*

The title compound was prepared from sulfonyl chloride **35** and glycine following the general procedure. White solid (62% yield). ^{1}H NMR (DMSO-d_6) δ: 1.66 (s, 4H); 3.17 (s, 4H); 3.68 (d, J = 5.31 Hz, 2H); 7.99 (s, 4H); 8.35 (t, J = 5.31 Hz, 1H). MS (ESI−) *m*/*z*: 347 $[M - H]^-$.

*4.1.26. 2-(4-Bromo-2-(trifluoromethoxy)phenylsulfonamido)acetic acid (**41**)*

The title compound was prepared from sulfonyl chloride **36** and glycine following the general procedure. White solid (73% yield). ^{1}H NMR (MeOD-d_6) δ: 3.85 (s, 2H); 7.64–7.69 (m, 2H); 7.88–7.92 (m, 1H). MS (ESI−) *m*/*z*: 376 $[M - H]^-$, 378 $[M + 2 - H]^-$.

*4.1.27. 3-(4-Phenoxyphenylsulfonamido)propanoic acid (**42**)*

The title compound was prepared from 4-phenoxybenzene-1-sulfonyl chloride **34** and β-alanine following the general procedure. White solid (80.6% yield). ^{1}H NMR (DMSO-d_6) δ: 2.36 (t, J = 6.9 Hz, 2H); 2.86–3.00 (m, 2H); 7.09–7.30 (m, 5H); 7.43–7.51 (m, 2H); 7.63 (t, 1H); 7.75–7.80 (m, 2H); 12.25 (s, 1H). MS (ESI−) *m*/*z*: 320 $[M - H]^-$.

*4.1.28. 2-(4-Butoxyphenylsulfonamido)acetic acid (**43**)*

The title compound was prepared from sulfonyl chloride **37** and glycine following the general procedure. White solid (83% yield). ^{1}H NMR (DMSO-d_6) δ: 0.93 (t, J = 6.96 Hz, 3H); 1.38–1.49 (m, 2H); 1.68–1.75 (m, 2H); 3.52 (d, J = 5.30 Hz, 2H); 4.04 (t, J = 6.23 Hz, 2H); 7.05–7.09 (m, 2H); 7.68–7.72 (m, 2H); 7.83 (t, J = 5.30 Hz, 1H). MS (ESI−) *m*/*z*: 286 $[M - H]^-$.

*4.1.29. General procedure for the synthesis of N-(tert-butyldimethylsilyloxy)acetamides **44**–**49***

1-[3-(Dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDC) (93.5 mg, 0.48 mmol) was added portionwise to a stirred and cooled solution (0 °C) of the carboxylic acids **38**–**43** (0.32 mmol), and *O*-(*tert*-butyldimethylsilyl)hydroxylamine (47.9 mg 0.32 mmol) in freshly distilled CH_2Cl_2 (8.5 mL). After stirring at RT overnight, the mixture was washed with water and the organic phase was dried and evaporated *in vacuo*.

*4.1.30. N-(tert-Butyldimethylsilyloxy)-2-(4-(phenoxymethyl) phenylsulfonamido)acetamide (**44**)*

The title compound was prepared from carboxylic acid **38** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to give **44** as a white solid (34% yield). ^{1}H NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 3.60 (br s, 1H); 3.82 (br s, 1H); 5.14 (s, 2H); 5.35 (br s, 1H); 6.94–7.03 (m, 3H); 7.31–7.35 (m, 2H); 7.58–7.62 (m, 2H); 7.85–7.90 (m, 2H); 8.45 (br s, 1H). MS (ESI+) *m*/*z*: 451 $[M + H]^+$.

*4.1.31. N-(tert-Butyldimethylsilyloxy)-2-(4-phenoxyphenylsulfonamido)acetamide (**45**)*

The title compound was prepared from carboxylic acid **39** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to give **45** as a white solid (28% yield). ^{1}H NMR ($CDCl_3$) δ: 0.16 (s, 6H); 0.95 (s, 9H); 3.60 (br s, 1H); 3.77 (br s, 1H); 5.23 (br s, 1H); 7.02–7.09 (m, 4H); 7.18–7.31 (m, 1H); 7.38–7.43 (m, 2H); 7.77–7.81 (m, 2H); 8.43 (br s, 1H). MS (ESI+) *m*/*z*: 437 $[M + H]^+$.

*4.1.32. N-(tert-Butyldimethylsilyloxy)-2-(4-(pyrrolidin-1-ylsulfonyl)phenylsulfonamido)acetamide (**46**)*

The title compound was prepared from carboxylic acid **40** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **46** as a white solid (25% yield). ^{1}H NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 1.77–1.84 (m, 4H); 3.24–3.31 (m, 2H); 3.70 (br s, 1H); 3.89 (br s, 1H); 5.45 (br s, 1H); 7.94–7.98 (m, 2H); 8.00–8.04 (m, 2H). MS (ESI+) *m*/*z*: 478 $[M + H]^+$.

*4.1.33. 2-(4-Bromo-2-(trifluoromethoxy)phenylsulfonamido)-N-(tert-butyldimethylsilyloxy)acetamide (**47**)*

The title compound was prepared from carboxylic acid **41** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **47** as a yellow oil (42% yield). ^{1}H NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.93 (s, 9H); 3.65 (br s, 1H); 3.96 (br s, 1H); 5.53 (br s, 1H); 7.52–7.60 (m, 2H); 7.85 (d, J = 8.0 Hz, 1H). MS (ESI+) *m*/*z*: 507 $[M + H]^+$, 509 $[M + 2 + H]^+$.

*4.1.34. N-(tert-Butyldimethylsilyloxy)-3-(4-phenoxyphenylsulfonamido)propanamide (**48**)*

The title compound was prepared from carboxylic acid **42** following the general procedure. The crude product was purified by flash chromatography on silica gel (*n*-hexane/EtOAc 2:1) to give **48** as a colorless oil (46% yield). ^{1}H NMR ($CDCl_3$) δ: 0.17 (s, 6H); 0.95 (s, 9H); 2.31–2.45 (m, 2H); 3.22 (q, J = 5.5 Hz, 2H); 7.00–7.08 (m, 4H); 7.18–7.27 (m, 1H); 7.37–7.45 (m, 2H); 7.77–7.82 (m, 2H); 7.87 (br s, 1H). MS (ESI+) *m*/*z*: 451 $[M + H]^+$.

*4.1.35. 2-(4-Butoxyphenylsulfonamido)-N-(tert-butyldimethylsilyloxy)acetamide (**49**)*

The title compound was prepared from carboxylic acid **43** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 2:1) using a Isolute Flash Si II cartridge to give **49** as a white solid (57% yield). ^{1}H NMR ($CDCl_3$) δ: 0.15 (s, 6H); 0.94 (s, 9H); 0.98 (t, J = 7.32 Hz, 3H); 1.41–1.59 (m, 2H); 1.68–1.86 (m, 2H); 3.58 (br s, 1H); 3.78 (br s, 1H); 4.01 (t, J = 6.4 Hz,

2H); 5.15 (br s, 1H); 6.94–6.99 (m, 2H); 7.74–7.79 (m, 2H); 8.45 (br s, 1H). MS (ESI+) *m/z*: 417 $[M + H]^+$.

4.1.36. General procedure for the synthesis of hydroxamates **10**, **11**, **13**–**15** and **17**

TFA (0.22 mL, 2.8 mmol) was added dropwise to a stirred and ice-chilled solution of *O*-silylate derivatives **44**–**49** (0.06 mmol) in CH_2Cl_2 (1.0 mL). The solution was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo*. The crude products were purified by trituration with Et_2O to give the desired hydroxamates.

4.1.37. N-Hydroxy-2-(4-(phenoxymethyl)phenylsulfonamido) acetamide (**10**)

The title compound was prepared from *O*-silylate derivative **44** following the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 18:1) using a Isolute Flash Si II cartridge to give **10** as a white solid (46% yield). MS (ESI+) *m/z*: 337 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 3.32 (s, 2H); 5.20 (s, 2H); 6.92–7.04 (m, 3H); 7.27–7.35 (m, 2H); 7.62–7.66 (m, 2H); 7.80–7.84 (m, 2H); 8.83 (br s, 1H); 10.15 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ: 43.07, 68.21, 114.70, 120.89, 126.66, 127.74, 129.47, 139.54, 141.75, 157.95, 164.19. Elemental analysis for $C_{15}H_{16}N_2O_5S$. Calculated: %C, 53.56; %H, 4.79; %N, 8.33; found %C, 53.62; %H, 4.81.12; %N, 8.23.

4.1.38. N-Hydroxy-2-(4-phenoxyphenylsulfonamido)acetamide (**11**)

The title compound was prepared from *O*-silylate derivative **45** following the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 18:1) using a Isolute Flash Si II cartridge to give **11** as a white solid (32% yield). MS (ESI+) *m/z*: 323 $[M + H]^+$; ^{1}H NMR (acetone-d_6) δ: 3.58 (s, 2H); 3.93 (br s, 1H); 7.08–7.16 (m, 4H); 7.22–7.29 (m, 1H); 7.43–7.51 (m, 2H); 7.85–7.89 (m, 2H). ^{13}C NMR (acetone-d_6) δ: 44.40, 118.44, 120.88, 125.58, 130.28, 131.04, 156.33, 162.03. Elemental analysis for $C_{14}H_{14}N_2O_5S$. Calculated: %C, 52.17; %H, 4.38; %N, 8.69; found %C, 52.22; %H, 4.41.12; %N, 8.73.

4.1.39. N-Hydroxy-2-(4-(pyrrolidin-1-ylsulfonyl)phenylsulfonamido)acetamide (**13**)

The title compound was prepared from *O*-silylate derivative **46** following the general procedure. White solid (94% yield). MS (ESI+) *m/z*: 364 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 1.60–1.77 (m, 4H); 3.14–3.20 (m, 4H); 3.40 (s, 2H); 8.00 (s, 4H); 8.30 (br s, 1H); 8.85 (br s, 1H); 10.54 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ: 24.77, 42.94, 47.89, 127.54, 127.98, 131.34, 139.43, 144.22, 163.99. Elemental analysis for $C_{12}H_{17}N_3O_6S_2$. Calculated: %C, 39.66; %H, 4.72; %N, 11.56; found %C, 39.86; %H, 4.77.12; %N, 11.61.

4.1.40. 2-(4-Bromo-2-(trifluoromethoxy)phenylsulfonamido)-N-hydroxyacetamide (**14**)

The title compound was prepared from *O*-silylate derivative **47** following the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 9:1) using a Isolute Flash Si II cartridge to give **14** as a white solid (53% yield). MS (ESI+) *m/z*: 392 $[M + H]^+$, 394 $[M + 2 + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 3.50 (s, 2H); 7.76–7.87 (m, 3H); 8.28 (br s, 1H); 8.85 (br s, 1H); 10.52 (br s, 1H). ^{13}C NMR (MeOD-d_6) δ: 44.23, 118.96, 125.09, 128.66, 131.27, 132.70, 133.52, 147.47, 167.41. Elemental analysis for $C_9H_8BrF_3N_2O_5S$: Calculated %C, 27.50; %H, 2.05; %N, 7.13; found %C, 27.61; %H, 2.12; %N, 7.00.

4.1.41. N-Hydroxy-3-(4-phenoxyphenylsulfonamido)propanamide (**15**)

The title compound was prepared from *O*-silylate derivative **48** following the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 18:1) using a Isolute Flash Si II cartridge to give **15** as a light pink solid (30% yield). MS (ESI+) *m/z*: 337 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 2.08–2.16 (m, 2H); 2.83–2.98 (m, 2H); 7.09–7.28 (m, 5H); 7.43–7.50 (m, 2H); 7.60 (m, 1H); 7.75–7.79 (m, 2H); 8.76 (s, 1H); 10.44 (s, 1H). ^{13}C NMR (acetone-d_6) δ: 33.31; 40.23, 118.45, 120.88, 125.56, 130.07, 131.04, 156.33, 161.86. Elemental analysis for $C_{15}H_{16}N_2O_5S$. Calculated: %C, 53.56; %H, 4.79; %N, 8.33; found %C, 53.72; %H, 4.93; %N, 8.09.

4.1.42. 2-(4-Butoxyphenylsulfonamido)-N-hydroxyacetamide (**17**)

The title compound was prepared from *O*-silylate derivative **49** following the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 9:1) using a Isolute Flash Si II cartridge to give **17** as a white solid (45% yield). MS (ESI+) *m/z*: 303 $[M + H]^+$; ^{1}H NMR (MeOD-d_6) δ: 0.99 (t, $J = 7.32$ Hz, 3H); 1.42–1.60 (m, 2H); 1.72–1.85 (m, 2H); 3.46 (s, 2H); 4.06 (t, $J = 6.23$ Hz, 2H); 7.03–7.07 (m, 2H); 7.75–7.79 (m, 2H). ^{13}C NMR (MeOD-d_6) δ: 14.15, 20.25, 32.29, 44.63, 69.23, 115.73, 130.25, 164.02. Elemental analysis for $C_{12}H_{18}N_2O_5S$. Calculated: %C, 47.67; %H, 6.00; %N, 9.27; found % C, 47.70; %H, 6.12; %N, 9.34.

4.1.43. General procedure for the synthesis of sulfonamides **52** and **53**

To a cooled (0 °C) solution of sulfonyl chloride **50** or **51** (4.12 mmol) in CH_3CN (0.4 mL), a solution of aqueous ammonia (1.32 mL) was added dropwise. The reaction was stirred at RT for 30 min, and then it was diluted with water and extracted with EtOAc. Organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give the desired sulfonamides.

4.1.44. 4-(6-Methylpyrazin-2-yloxy)benzenesulfonamide (**52**)

The title compound was prepared from sulfonyl chloride **50** following the general procedure. Yellow oil (63% yield). ^{1}H NMR (DMSO-d_6): 2.34 (s, 3H); 7.34–7.39 (m, 2H); 7.41 (br s, 2H); 7.84–7.88 (m, 2H); 8.33–8.39 (m, 2H). MS (ESI+) *m/z*: 266 $[M + H]^+$.

4.1.45. 6-Phenoxypyridine-3-sulfonamide (**53**)

The title compound was prepared from sulfonyl chloride **51** following the general procedure. White solid (94% yield). ^{1}H NMR (DMSO-d_6): 7.18–7.31 (m, 4H); 7.43–7.46 (m, 2H); 7.52 (br s, 2H); 8.17–8.26 (m, 1H), 8.52 (s, 1H). MS (ESI+) *m/z*: 251 $[M + H]^+$.

4.1.46. General procedure for the synthesis of Boc-sulfonamides **54** and **55**

Sulfonamides **52** or **53** (10.3 mmol) were suspended in dry CH_2Cl_2 (12.9 mL) containing DMAP (125.84 mg, 1.03 mmol) and Et_3N (1.59 mL, 11.33 mmol). A solution of di-(*tert*-butyl)dicarbonate (2.58 g, 11.85 mmol) in dry CH_2Cl_2 (20.6 mL) was added dropwise, and the reaction was stirred at RT overnight. The solution was concentrated *in vacuo* and the residue was treated with EtOAc and HCl 1 N. The organic phase was then washed with water and brine, dried over Na_2SO_4 and evaporated.

4.1.47. tert-Butyl 4-(6-methylpyrazin-2-yloxy)phenylsulfonylcarbamate (**54**)

The title compound was prepared from sulfonamide **52** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 3:1) using a Isolute Flash Si II cartridge to give **54** as a white solid (71% yield). ^{1}H NMR ($CDCl_3$) δ: 1.41 (s, 9H); 2.44 (s, 3H); 7.29–7.33 (m, 2H); 7.41 (br s, 1H); 8.04–8.08 (m, 2H); 8.26–8.28 (m, 2H). MS (ESI+) *m/z*: 366 $[M + H]^+$.

4.1.48. tert-Butyl 6-phenoxypyridin-3-ylsulfonylcarbamate (**55**)

The title compound was prepared from sulfonamide **53** following the general procedure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 4:1) using a Isolute Flash Si

II cartridge to give **55** as a white solid (71% yield). ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H); 6.99–7.04 (m, 2H); 7.14–7.19 (m, 2H); 7.28–7.32 (m, 1H); 7.42–7.49 (m, 2H); 8.25–8.30 (m, 1H); 8.75–8.76 (m, 1H). MS (ESI+) *m*/*z*: 351 $[M + H]^+$.

4.1.49. General procedure for the synthesis of tert-butyl esters *56* and *57*

Carbamates **54** or **55** (1.37 mmol) were added to a cooled suspension of NaH 60% in mineral oil (49.2 mg, 2.05 mmol) in dry DMF (37 mL). After 10 min stirring, *tert*-butyl-bromoacetate (0.20 mL, 1.37 mmol) was added and the mixture was stirred under Argon atmosphere for 16 h. The reaction was then diluted with EtOAc and washed with water and HCl 1 N, dried over Na_2SO_4, and evaporated.

4.1.50. tert-Butyl 2-(N-(tert-butoxycarbonyl)-4-(6-methylpyrazin-2-yloxy)phenylsulfonamido)acetate (*56*)

The title compound was prepared from carbamate **54** following the general procedure. Yellow oil (57% yield). ^{1}H NMR ($CDCl_3$) δ: 1.47 (s, 9H); 1.48 (s, 9H); 2.42 (s, 3H); 4.47 (s, 2H); 7.29–7.34 (m, 2H); 8.11–8.15 (m, 2H); 8.23–8.26 (m, 2H). MS (ESI+) *m*/*z*: 480 $[M + H]^+$.

4.1.51. tert-Butyl 2-(N-(tert-butoxycarbonyl)-6-phenoxypyridine-3-sulfonamido)acetate (*57*)

The title compound was prepared from carbamate **55** following the general procedure. Yellow oil (73% yield). ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 9H); 1.46 (s, 9H); 4.45 (s, 2H); 6.97–7.02 (m, 1H); 7.14–7.19 (m, 2H); 7.28–7.31 (m, 1H); 7.41–7.49 (m, 2H); 8.33–8.39 (m, 1H); 8.78–8.79 (m, 1H). MS (ESI+) *m*/*z*: 465 $[M + H]^+$.

4.1.52. General procedure for the synthesis of carboxylic acids *58* and *59*

TFA (1.9 mL, 25 mmol) was added dropwise to a stirred, ice-chilled solution of *tert*-butyl ester **56** or **57** (0.44 mmol) in freshly distilled dichloromethane (2.5 mL). The mixture was stirred under these reaction conditions for 5 h and the solvent was removed *in vacuo*. The crude product was purified by trituration with Et_2O.

4.1.53. 2-(4-(6-Methylpyrazin-2-yloxy)phenylsulfonamido)acetic acid (*58*)

The title compound was prepared from *tert*-butyl ester **56** following the general procedure. Brown solid (52% yield). ^{1}H NMR (DMSO-d_6) δ: 2.36 (s, 3H); 3.62 (d, J = 5.5 Hz, 2H); 7.35–7.39 (m, 2H); 7.82–7.86 (m, 2H); 8.08 (t, J = 5.5 Hz, 1H); 8.36–8.38 (m, 2H). MS (ESI−) *m*/*z*: 322 $[M - H]^-$.

4.1.54. 2-(6-Phenoxypyridine-3-sulfonamido)acetic acid (*59*)

The title compound was prepared from *tert*-butyl ester **57** following the general procedure. White solid (51% yield). ^{1}H NMR (DMSO-d_6) δ: 3.66 (d, J = 6.05 Hz, 2H); 7.17–7.31 (m, 4H); 7.43–7.50 (m, 2H); 8.15–8.26 (m, 2H); 8.49 (br s, 1H). MS (ESI−) *m*/*z*: 307 $[M - H]^-$.

4.1.55. General procedure for the synthesis of hydroxamates *12* and *16*

Hydroxamates **12** and **16** were obtained respectively from carboxylic acids **58** and **59** following the procedure in two steps previously described for **10**, **11**, **13**–**15** and **17**.

4.1.56. N-Hydroxy-2-(4-(6-methylpyrazin-2-yloxy)phenylsulfonamido)acetamide (*12*)

O-Silyl intermediate was purified by flash chromatography Isolute Si (II) (*n*-hexane/EtOAc 2:1) and a white solid was obtained (37% yield). ^{1}H NMR ($CDCl_3$) δ: 0.17 (s, 6H); 0.95 (s, 9H); 2.44 (s, 3H); 3.63 (br s, 1H); 3.85 (br s, 1H); 5.24 (br s, 1H); 7.27–7.32 (m, 3H); 7.87–7.92 (m, 2H); 8.25 (s, 1H). The hydroxamate was obtained after trituration with *n*-hexane. White solid (65% yield). MS (ESI+) *m*/*z*: 339 $[M + H]^+$; ^{1}H NMR (acetone-d_6) δ: 2.39 (s, 3H); 3.60 (s, 2H); 6.78 (br s, 1H); 7.38–7.42 (m, 2H); 7.93–7.97 (m, 2H); 8.31 (m, 1H); 10.17 (br s, 1H). ^{13}C NMR (acetone-d_6) δ: 24.81, 40.73, 121.65, 128.32, 135, 63, 137.92, 142.61, 148.43, 155.81, 158.42, 166.01. Elemental analysis for $C_{13}H_{14}N_4O_5S$. Calculated: %C, 46.15; %H, 4.17; %N, 16.56; found %C, 46.23; %H, 4.19; %N, 16.33.

4.1.57. N-Hydroxy-2-(6-phenoxypyridine-3-sulfonamido) acetamide (*16*)

O-silyl intermediate was purified by flash chromatography Isolute Si (II) (*n*-hexane/EtOAc 2:1) and a white solid was obtained (51% yield). ^{1}H NMR ($CDCl_3$) δ: 0.16 (s, 6H); 0.94 (s, 9H); 3.61 (br s, 1H); 3.85 (br s, 1H); 5.52 (br s, 1H); 6.99–7.03 (m, 2H); 7.12–7.17 (m, 2H); 7.24–7.31 (m, 1H); 7.41–7.48 (m, 1H); 7.86 (br s, 1H); 8.07–8.12 (m, 1H); 8.39 (br s, 1H); 8.61–8.62 (m, 1H). The hydroxamate was obtained after trituration with Et_2O ed *n*-hexane followed by purification by flash chromatography Isolute Si (II) ($CHCl_3$/MeOH 18:1). White solid (32% yield). MS (ESI+) *m*/*z*: 324 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ: 3.66 (s, 2H); 6.93 (br s, 1H); 7.11–7.31 (m, 4H); 7.42–7.50 (m, 2H); 8.21–8.26 (m, 1H); 8.54 (m, 1H); 9.45 (br s, 1H); 10.13 (br s, 1H). ^{13}C NMR (acetone-d_6) δ: 45.04, 113.23, 123.26, 126.92, 131.38, 140.53, 148.75, 155.17, 167.42. Elemental analysis for $C_{13}H_{13}N_3O_5S$. Calculated: %C, 48.29; %H, 4.05; %N, 13.00; found %C, 48.40; %H, 4.11; %N, 12.89.

4.2. Aggrecanase fluorometric assay

Recombinant human ADAMTS-5 and ADAMTS-4 were expressed and purified from HEK293/EBNA cells following the procedure established in our laboratory [10].

All enzyme assays were conducted in TNC buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij-35 and 0.02% NaN_3) at 37 °C, using a Gemini microplate spectrofluorimeter (Molecular Devices, Sunnyvale, CA, USA). Each reaction was performed in a total volume of 200 μL per well in 96-well microtiter plates (Corning).

The activity of ADAMTS-4 was monitored for 15 min using the fluorescent peptide substrate carboxyfluorescein-Ala-Glu∼Leu-Asn-Gly-Arg-Pro-Ile-Ser-Ile-Ala-Lys-*N*,*N*,*N*′,*N*′-tetramethyl-6-carboxyrhodamine (FAM-AE∼LQGRPISIAK-TAMRA) (custom-made by Bachem) at a final concentration of 0.5 μM with an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

The activity of ADAMTS-5 was monitored for 2 h using the fluorescent peptide substrate *ortho*-aminobenzoyl-Thr-Glu-Ser-Glu∼Ser-Arg-Gly-Ala-Ile-Tyr-(*N*-3-[2,4-dinitrophenyl]-L-2,3-diamino-propionyl)-Lys-Lys-NH_2 (Abz-TESE∼SRGAIY-Dpa-KK) (custom-made by Bachem) at a final concentration of 20 μM with an excitation wavelength of 300 nm and an emission wavelength of 430 nm.

Fluorescence was expressed in relative fluorescence units (RFU) and normalized against a blank containing only buffer and substrate. The inhibitor stock solutions (DMSO, 10 mM) were diluted at seven different concentrations and incubated with ADAMTS-4 or ADAMTS-5 (final concentration: 4 nM) in the assay buffer for 2 h at 37 °C. Percent of inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula: $v_i/v_0 = 1/(1 + [I]/IC_{50})$, where v_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration $[I]$ and v_0 is the initial velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro software and GraphPad Prism software (San Diego, CA, USA).

4.3. MMP inhibition assays

Recombinant human MMP-14 catalytic domain (MMP-14cd) was a kind gift of Prof. Gillian Murphy (Department of Oncology,

University of Cambridge, UK). Pro-MMP-1, pro-MMP-2, and pro-MMP-13, were purchased from Calbiochem.

Proenzymes were activated immediately prior to use with *p*-aminophenylmercuric acetate (APMA 2 mM for 1 h at 37 °C for MMP-2, APMA 2 mM for 2 h at 37 °C for MMP-1 and 1 mM for 30 min at 37 °C for MMP-13). For assay measurements, the inhibitor stock solutions (10 mM in DMSO) were further diluted, at 7 different concentrations for each MMP in the fluorometric assay buffer (FAB: Tris 50 mM, pH = 7.5, NaCl 150 mM, $CaCl_2$ 10 mM, Brij 35 0.05% and DMSO 1%). Activated enzyme (final concentration 0.5 nM for MMP-2, 1.0 nM for MMP-14cd, 2.0 nM for MMP-1 and 0.3 nM for MMP-13) and inhibitor solutions were incubated in the assay buffer for 2 h at 25 °C. After the addition of the fluorogenic substrate Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 (Bachem) for all enzymes in DMSO (final concentration 2 μM), the hydrolysis was monitored every 15 s for 15 min, recording the increase in fluorescence ($\lambda_{ex} = 325$ nm, $\lambda_{em} = 395$ nm) with a Molecular Devices SpectraMax Gemini XS plate reader. The assays were performed in duplicate in a total volume of 200 μL per well in 96-well microtiter plates (Corning black, NBS). The MMP inhibition activity was expressed in relative fluorescent units (RFU). Percent of inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula: $v_i/v_o = 1/(1 + [I]/IC_{50})$, where v_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration $[I]$ and v_o is the initial velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro software [33] and GraFit software [34].

4.4. Aggrecan digestion assay

Bovine nasal aggrecan was prepared in this lab according to the method of Hascall and Sajdera [35]. Purified recombinant human ADAMTS-5 (2 pM) was incubated with bovine aggrecan (50 μg) in 50 μL TNC buffer at 37 °C for 2 h. The reaction was stopped by adding 1 volumes of double strength glycosaminoglycan (GAG) buffer (200 mM sodium acetate, 250 mM Tris HCl pH 8.0 and 100 mM EDTA). Aggrecan was then deglycosylated by incubating with 0.1 mU/μL of chondroitinase ABC and 0.1 mU/μL of keratanase (Seikagaku, Tokyo, Japan) overnight at 37 °C. The samples were precipitated by adding 5 volumes of cold acetone, incubated at −20 °C for 4 h, and then centrifuged at 13,000×*g* for 30 min. The pellet was dissolved in 50 μL of reducing sample buffer. The products were subjected to SDS PAGE on 6% total acrylamide gels and analyzed by Western Blot analysis as described by Little et al. [7b]. Pre-stained Precision Protein Standards™ from BioRad (Hemel Hempstead, UK) were used. The primary antibodies used to detect aggrecanase-generated aggrecan-fragments were anti-AGEG antibody (which recognizes the AGEG neoepitope generated by aggrecanase cleavage at the $TAQE^{1771}\downarrow^{1772}AGEG$ site in the CS-2 region of bovine aggrecan) (Qiagen, Tokyo, Japan) [10] and anti-ARGSV antibody (which recognizes the *N*-terminal neoepitope sequence $^{374}ARGXX$ generated by aggrecanase-mediated cleavage of the Glu^{373}–Ala^{374} peptide bond with the aggrecan interglobular domain) (Abcam, Cambridge, UK) [36]. Antigen-antibody complexes were detected by anti-mouse alkaline phosphatase-linked donkey antibody (Promega, Southampton, UK) and the AP substrate (Promega, Southampton, UK). Gels and blots were scanned using a Bio-Rad GS-710 scanning densitometer, and the band intensity was quantified using the 1D Phoretix quantification software (Nonlinear Dynamics, Newcastle upon Tyne, UK).

4.5. Cartilage explants cultures and inhibition studies

Porcine articular cartilage from the metacarpophalangeal joints of 3–9-month-old pigs was dissected into small pieces approximately 3 mm long and 3–4 mm wide. Each cartilage piece placed in one well of a round bottom 96-well plate was incubated in 100 μL of serum-free DMEM (Dulbecco's modified Eagle's medium) with or without IL-1α (10 ng/mL) and two concentrations (1 and 10 μM) of compound **18** or 100 μM GM6001. Three pieces of cartilage were subjected to each treatment. After incubation for the various periods of time, the conditioned media were harvested. Content of glycosaminoglycan (GAG) was analyzed by 1,9-dimethylmethylene blue (DMMB) assay (see Section 4.6). Aggrecan fragments released into the conditioned medium were deglycosylated using chondroitinase ABC and keratanase, acetone-precipitated and subjected to SDS-PAGE as described in Section 4.4.

4.6. Analysis of glycosaminoglycan (GAG) release

GAG released into the conditioned medium was measured in duplicate using a modification of the DMMB assay as described by Farndale et al. [37]. Shark chondroitin sulfate was used as a standard. All data were analyzed by unpaired one-tail *t* tests with Welch's correction using the software package GraphPad Prism (San Diego, CA, USA).

4.7. Primary chondrocytes isolation and culture

Diced porcine and human cartilage were incubated with 1.5 mg/mL collagenase 2 (Worthington) with DMEM containing 10% FCS at 37 °C for 24 h. Isolated cells were then passed through a cell strainer, pelleted, washed twice with DMEM, and plated in DMEM containing 10% FCS. Primary porcine cells were maintained in DMEM with 10% FCS.

4.8. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

Chondrocyte viability and apoptosis were examined by MTT assay. Isolated chondrocytes were seeded at 2.5×10^4 cells per well in a 96-well plate. Sterile-filtered MTT in phosphate-buffered saline (PBS) was added (0.5 mg/mL final concentration) and incubated for 1 h at 37 °C. A final concentration of 5% SDS/5 mM HCl was then added to solubilize the formazan product and incubated overnight at 37 °C in a shaking incubator. The absorbance was read at 590 nm.

4.9. Molecular modeling. Docking simulations

Molecular docking of **18** and its analogs **8**, **9**, **11** and **17** into the X-ray structure of ADAMTS-4, -5, MMP-2, -13 and -14 (PDB ID: 2RJP, 3B8Z, 3AYU, 3ZXH, 3MA2, respectively) was carried out using the Glide 5.5 program [38]. Maestro 9.0.211 [39] was employed as the graphical user interface and Fig. 3 was rendered by the Chimera software package [40].

4.9.1. Ligand and protein setup

The inhibitor structures were first generated through the Dundee PRODRG2 Server [41]. Then, CM1 atomic charges [42,43] were computed via single-point AM1 calculations using OPLS-AA [44] optimized geometries and the BOSS program [45,46]. The target proteins were prepared through the Protein Preparation Wizard of the graphical user interface Maestro and the OPLS-2001 force field. Water molecules were removed, hydrogen atoms were added, a +2 charge was assigned to the zinc ion in the active site and minimization was performed until the RMSD of all heavy atoms was within 0.3 Å of the crystallographically determined positions. The binding pockets were identified by placing a 20 Å cube around the catalytic ion.

4.9.2. Docking setting

Molecular docking calculations were performed with the aid of Glide 5.5 in extra-precision (XP) [47,48] mode, using Glidescore for ligand ranking. A constraint that forced the interaction with the metal ion was included. For multiple ligand docking experiments, an output maximum of 5000 ligand poses per docking run with a limit of 100 poses for each ligand was adopted.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.058.

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