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Novel N-substituted 2-phenyl-1-sulfonylamino-cyclopropane carboxylates as selective ADAMTS-5 (Aggrecanase-2) inhibitors

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ABSTRACT

A series of N-substituted sulfonylamino-alkanecarboxylate ADAMTS-5 (Aggrecanase-2) inhibitors has been synthesized and the in vitro enzyme SAR is discussed. This report is the first example of carboxylate-based ADAMTS-5 inhibitors which show strong potency of $IC_{50} < 0.1$ μM with excellent selectivity over MMP-1 and TACE.

Human osteoarthritis (OA) is a progressive disease of the joints characterized by degradation of articular cartilage, which appears to be a result of uncontrolled proteolytic extracellular matrix destruction. A major component of the cartilage extracellular matrix is aggrecan, and its loss from cartilage is believed to be the early event that leads to further breakdown of extracellular matrix macromolecules.[1]

Aggrecanases are members of the ADAMTS (A Disintegrin and Metalloprotease with Thrombospondin Motifs) family of zinc metalloproteases, and comprised of two isozymes including ADAMTS-4 (Aggrecanase-1) and ADAMTS-5 (Aggrecanase-2). They are known to cleave the aggrecan interglobular domain at the Glu373-Ala374 peptide bond,[2] and are 1000-fold more efficient than any other MMP enzymes at cleaving aggrecan.

Until recently, it has been unclear which ADAMTS isozyme is the primary source of devastating aggrecanase activity in cartilage. In 2005, two independent groups reported that ADAMTS-5 knockout mice showed protective effects against the erosion of cartilage and the occurrence of arthritis.[3,4] With these reports, ADAMTS-5 has begun to receive greater attention as an important target for the prevention of OA. Since the first discovery of ADAMTS-4 inhibitors in 2001,[5] intensive efforts have been made by several groups to target inhibition of this isozyme.[6–8] By contrast, there have been only a few examples of ADAMTS-5 inhibitors reported to date.[9–11]

Historically, the vast majority of Zn metalloprotease inhibitors including ADAMTS inhibitors contain a hydroxamic acid zinc-binding group (ZBG). While this ZBG group imparts high levels of potency to these molecules due to the strong binding affinity to the Zn ion, the enzyme selectivities of these types of inhibitors tend to be problematic. For this reason, the use of milder ZBGs,

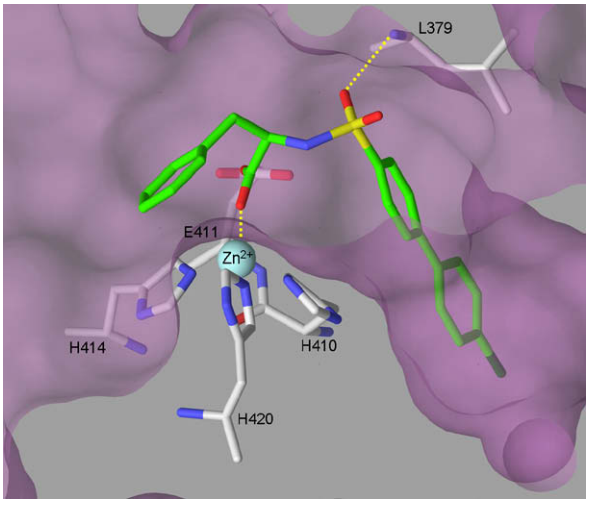

Figure 1. Proposed binding mode for compound **1**. The inhibitor was docked manually into the X-ray structure of ADAMTS-5 (PDB accession code 3B8Z[12]). Compound **1** is depicted in green, the solvent-accessible surface of the protein is violet, and selected side chains are shown in white. The zinc ion is portrayed as a blue sphere. Electronic contacts are shown as yellow dashed lines.

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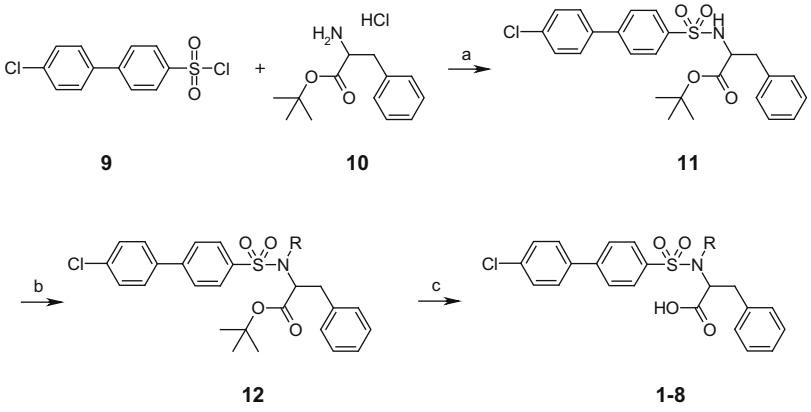
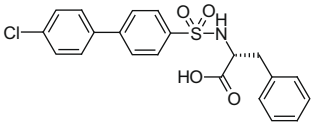


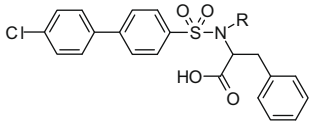
Scheme 1. Reagents and conditions: (a) Et_3N, DCM, rt, 1 h (98%); (b) RX, K_2CO_3, DMF, 60 °C, 5 h or ROH, DIAD, PPh_3, THF, rt, 2 h (75–98%); (c) TFA, DCM, rt, 2 h (56–99%).

such as the carboxylic acid, the hydantoin, and the 2-thioxothiazolidin-4-one, has become a popular approach in recent ADAMTS inhibitor programs.[9–11]



As a starting point for our research efforts toward ADAMTS-5 inhibitors, we focused on weaker carboxylic acid ZBG based inhibitors, reasoning that these would allow us to obtain good selectivity over other MMPs. From an initial carboxylic acid focused combinatorial library, we found (*R*)-phenylalanine derivative **1** to be a moderate ADAMTS-5 inhibitor. We were attracted to this

Table 1
In vitro ADAMTS-5 inhibitory activity of N-substituted 2-[4-(4-Cl-phenyl)phenyl]sulfonylamino-3-phenyl-propionates



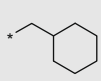
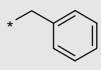
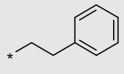
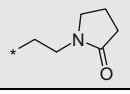
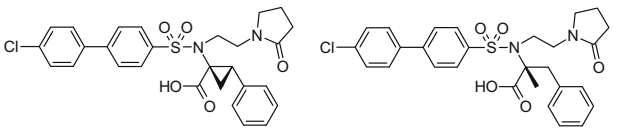
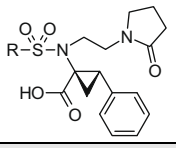
Compounds	Enantiomer	R	% Inhibition at 10 μM	Agg-2 IC_{50} (μM)
1	*R*			3.2
2	*R*			8.0
3	*S*		−0.12	
4	*R*		46	
5	*R*		22	
6	*R*		30	
7	*R*			7.3
8	*R*			1.6

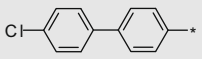
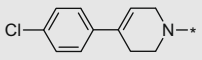
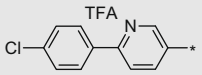
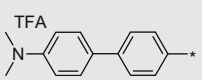
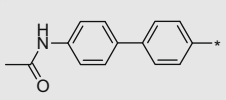
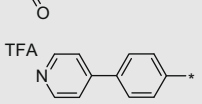
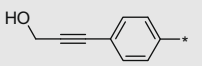
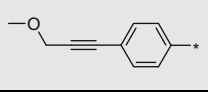
Table 2
In vitro ADAMTS-5 inhibitory activity of constrained analogues of compound **8**



Compounds	Enantiomer	% Inhibition at 10 μM	Agg-2 IC_{50} (μM)
13	(1*R*, 2*S*)		0.20
14	*R*	0.10	

Table 3
Modification of the substituent on the N-atom of N-substituted 2-phenyl-1-sulfonylamino-cyclopopanecarboxylate

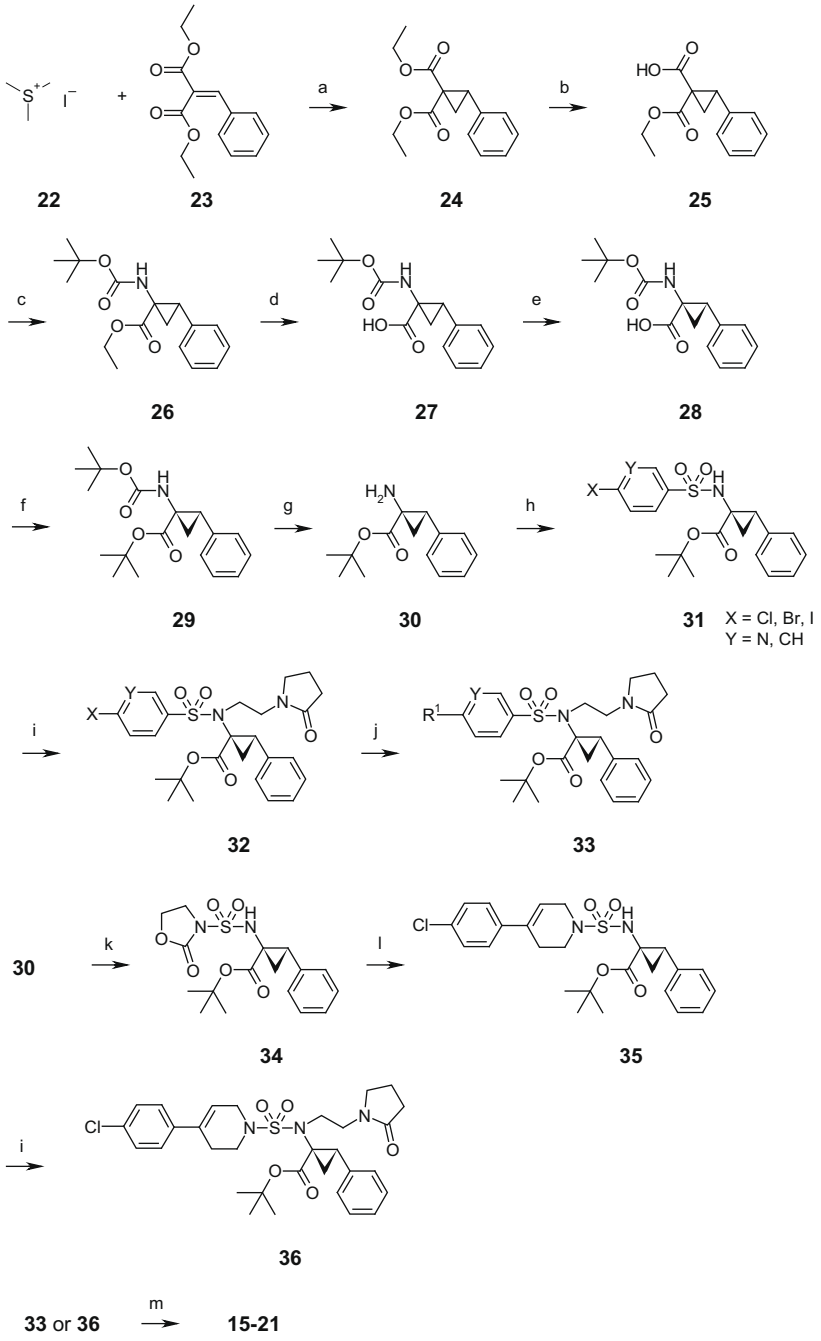


Compounds	R	% Inhibition at 10 μM	Agg-2 IC_{50} (μM)
13			0.20
15			0.88
16			1.1
17			6.6
18			4.5
19		18	
20		−1.5	
21		2.9	

structure because we believed the synthetic flexibility of the α-amino acid derived sulfonamide would allow us to rapidly evaluate the ADAMTS-5 SAR.

During the course of this work, we utilized homology models and X-ray crystal structures of other ADAM family members to guide our compound design and SAR evaluation. Recently, X-ray structures of ADAMTS-4 and ADAMTS-5 have become available[12,13] and we have incorporated them here (PDB code 3B8Z[12]) to illustrate refined models of selected compounds. Figure 1 depicts the expected binding mode of compound **1** in ADAMTS-5; all of the conventional interactions are present, including chelation of the zinc by the carboxylic acid and deep association of the biaryl moiety in the hydrophobic S1′ pocket. Additionally, the benzyl group rests above the surface formed by the imidazole ring of His414.

As a beginning to our SAR exploration, we investigated substitution effects on the sulfonamide N-atom (Table 1). Initially, a simple methyl group was introduced on the sulfonamide nitrogen atom for both *R*- and *S*-phenylalanine derivatives. The *R*-configuration was found to be favorable and complete loss of potency was observed for the corresponding *S*-isomer **3**. This remarkable enantiometric preference led us to use the (*R*)-phenylalanine as a core



Scheme 2. Reagents and conditions: (a) NaH, DMSO, 12 h (76%); (b) 4 N NaOH, EtOH/H_2O, 12 h (92%); (c) Et_3N, DPPA, *t*-BuOH, 120 °C, 12 h (86%); (d) 4 N NaOH, EtOH/H_2O, 110 °C, 6 h (92%); (e) (−)-cinchonidine, AcOEt/2–propanol, rt, 12 h (31%, 97.7% ee,); (f) $Me_2NCH(Ot\text{-}Bu)_2$, toluene, 100 °C, 5 h (92%); (g) *p*-TsOH·H_2O, MeOH, 12 h (85%); (h) $ArSO_2Cl$, pyridine, $CHCl_3$, 12 h (59–81%); (i) 1-(2-hydroxyethyl)-pyrrolidine-2-one, DIAD, PPh_3, THF, 2 h or 1-(2-bromoethyl)-pyrrolidine-2-one, K_2CO_3, DMF, 90 °C, 12 h (68–96%); (j) $R^1B(OH)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3/DME, 90 °C, 1 h or R^2OCH_2CCH, $PdCl_2(PPh_3)_2$, CuI, Et_3N, $CHCl_3$, rt, 0.5 h (21–99%); (k) $ClSO_2NCO$, 2-Cl-ethanol, NMM, CH_3CN, 0–50 °C, 2 h (56%); (l) 4-(4-Cl-phenyl)-1,2,3,6-tetrahydropyridine·HCl, Et_3N, 1,4-dioxane, 100 °C, 12 h (40%); (m) TFA, $CHCl_3$, 12 h (43–77%).

skeleton for further study. A small set of hydrophobic R-substituents were introduced with intent to pick up another interaction in the binding pocket of ADAMTS-5. Although most of the compounds with R-substituents larger than a methyl group (**4**–**6**) were less potent than compound **2**, compound **8** showed subtle but significant improvement in potency. This discovery was quite encouraging since phenyl analogue **7** showed reduced activity. We hypothesized that the increased potency of **8** may be the result of a new hydrogen-bonding interaction between the terminal lactam and the enzyme.

Compounds **1**–**8** were prepared according to Scheme 1. Phenylalanine *t*-Bu ester was first sulfonylated with [4-(4-Cl-phenyl)phenyl]sulfonyl chloride under basic conditions to give compound **11**, which was then alkylated with the corresponding halides in the presence of base or with the alcohols under Mitsunobu conditions to give **12**. Finally, ester cleavage by TFA led **12** to compounds **1–8**.

Starting from compound **8**, modification of the amino acid core skeleton was investigated next. The transformation of compound **8** into the D-cyclo-phenylalanine analogue **13**, by means of a methylene group addition between the alpha position of the carboxylate and the beta position of **8**, caused an increase of the binding affinity for ADAMTS-5 of one order of magnitude (Table 2).

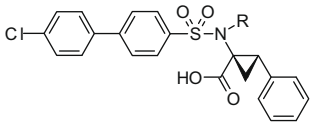
The increased potency of compound **13** can be attributed to rigidifying the compound into a favorable conformation for binding in the active site of the enzyme. This hypothesis is supported by the significant loss of potency observed for the more flexible analogue **14**.

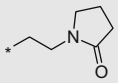
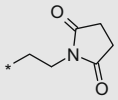
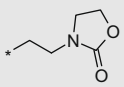
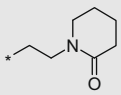
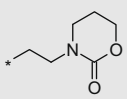
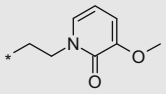
Compounds **13** and **14** were prepared essentially the same way as described in Scheme 1. Each amino acid portion was commercially available or prepared according to known literature procedures.[14,15] Subjecting these amino acids to the earlier described sequence of sulfonylation, alkylation, and subsequent ester cleavage gave the desired compounds.

Having identified potent amino acid and sulfonamide nitrogen substitutions, we next turned our attention to the sulfonamide S1′ moiety. Our SAR investigation of the biphenyl portion is shown in Table 3. The importance of the internal benzene ring of **13** was made apparent when a 4-fold reduction of inhibitory activity was observed by replacement of this ring with the 1,2,3,6-tetrahydropyridine ring or pyridine ring (**15** and **16**). Additionally, the terminal 4-Cl-phenyl substituent was indispensable for potency as loss of potency was observed by the replacement of either the chlorine atom (**17** and **18**) or the phenyl ring itself (**19**–**21**).

The synthesis of compounds **15**–**21** is shown in Scheme 2. Cyclopropanation was first performed with benzylidene malonate to give **24**. The sterically less hindered ester was selectively hydrolyzed and the resultant mono-ester was transformed to compound **26** by Curtius rearrangement. After another hydrolysis, an optical resolution was performed with (−)-cinchonidine to give desired enantiomer in over 97% ee. After protection of the carboxylate as the *t*-Bu ester, the Boc group was selectively deprotected with stoichiometric amounts of *p*-TsOH·H_2O, then sulfonylation and

Table 4
Modification of the substituent on the N-atom of 2-phenyl-1-sulfonylamino-cyclopopanecarboxylate: cyclic amide and urethane series



Compounds	R	Agg-2 IC_{50} (μM)
13		0.20
37		0.31
38		0.22
39		0.21
40		0.18
41		0.078

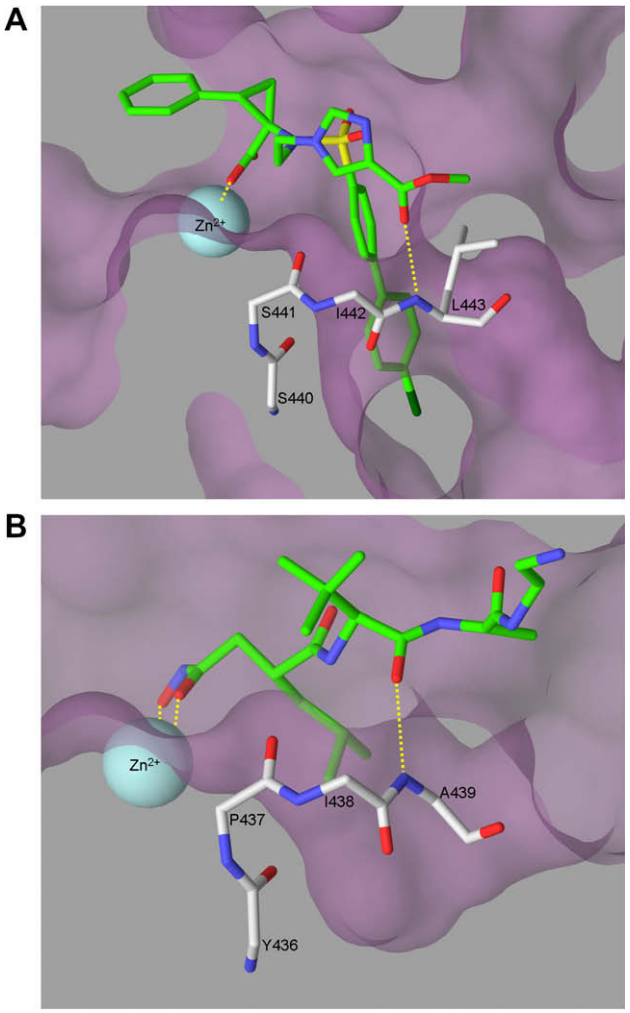
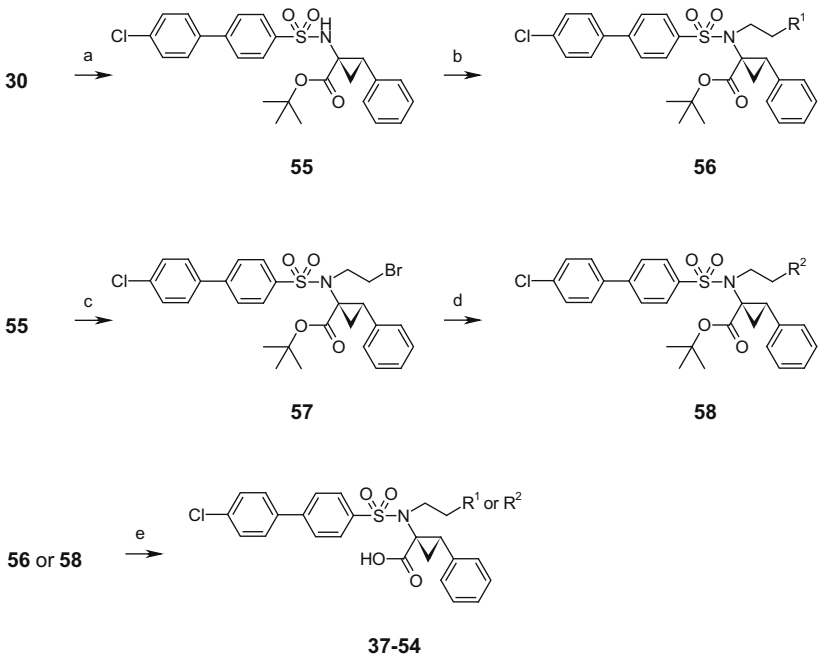


Figure 2. (A) Proposed binding mode for compound **51**. The color scheme is as described for Figure 1. Residues that contribute to the outer rim of the S1′ pocket are shown in white; for clarity, only the side chain of Leu443 is shown explicitly. (B) The X-ray crystal structure of TACE in complex with a succinate-based inhibitor (PDB accession code 1BKC).[16] A hydrogen-bond to the main chain NH of Ala439 is comparable to the contact to Leu443 illustrated in Figure 2A.



Scheme 3. Reagents and conditions: (a) [4-(4-Cl-Phenyl)phenyl]sulfonyl chloride, pyridine, $CHCl_3$, 12 h (90%); (b) $R^1CH_2CH_2Br$, K_2CO_3, DMF, 60 °C, 2.5 h or $R^1CH_2CH_2OH$, DIAD, PPh_3, THF, rt, 2 h (37–99%); (c) 1,2-dibromoethane, K_2CO_3, DMF, 60 °C, 4 h (89%); (d) R^2H, K_2CO_3, DMF, 80 °C, 12 h (13–62%); (e) 4 N HCl in AcOEt, rt, 12 h or TFA, $CHCl_3$, rt, 12 h (21–99%).

successive N-alkylation gave compound **32** which was ready for the following Suzuki- or Sonogashira-coupling. Both couplings proceeded in moderate to good yield under typical conditions, and a final acidic ester cleavage gave the target compounds **16–21**. Amino intermediate **30** could also be converted to oxazolidine-2-one-3-sulfonamide **34**, which in turn could be reacted with 4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine to give sulfamide **35**. N-Alkylation of **35** followed by the final ester cleavage gave compound **15**.

The last point of modification was the substituent on the N-atom of the sulfonamide. According to the SAR of D-phenylalanine derivatives shown in Table 1, the 2-(2-oxopyrrolidinyl)ethyl was identified as a favorable substituent at that position, and we envisioned the existence of its putative hydrogen-bonding partner in the binding pocket of ADAMTS-5.

At this point, we decided to explore this 2-(2-oxopyrrolidinyl)ethyl analogues further with the goal of identifying better binders and to bolster the inhibitory activity. The initial attempts of the introduction of an extra heteroatom or the ring expansion were discouraging since virtually no improvement was observed in potencies (compounds **37**–**40**, Table 4) of the carbonyl containing ring systems. Replacement of the pendent ring with an aromatic ring and subsequent introduction of a methoxy group was effective to increase the potency as seen in compound **41**, which showed an IC_{50} of 78 nM.

The discovery of compound **41** possessing a highly functionalized heteroaromatic ring prompted us to explore these analogues further. The pyridine analogues were first synthesized and a 3-pyridyl isomer was found out to be most favorable among the three regioisomers (**42**–**44**). The five-membered azole derivatives were also synthesized and all the compounds showed good potency without strict restriction of the number and the position of N atoms (**45**–**50**). Among these five-membered azole derivatives, imidazole derivative **45** was chosen in consideration of the synthetic flexibility and the ease of further modification. Since the methoxy group of compound **41** was speculated to be a key functionality for the observed improvements in potency, analogous Lewis basic functionalities were introduced on the imidazole ring of compound **45**, and these changes resulted in compounds **51** and **53**, with IC_{50}s of 73 and 71 nM, respectively.

Figure 2 illustrates a rationale for the improved potency of compound **51** and other potent analogues: A correctly placed hydrogen-bond acceptor is capable of reaching the main chain NH of Leu443, which forms part of the outer rim of the S1′ pocket.

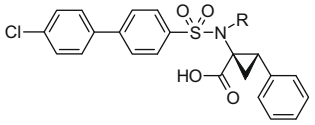
Consideration of X-ray structures for MMPs and ADAM family members that contain peptide-like inhibitors show that this hydrogen-bonding interaction is common (Fig. 2B illustrates this interaction using an X-ray crystal structure of TACE in complex with a succinate-based inhibitor[16]). The methoxy group provides an added hydrophobic contact against the surface created by the branched side chain of Leu443.

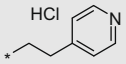
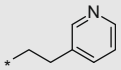
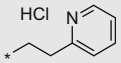
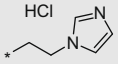
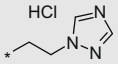
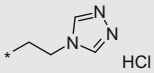
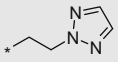
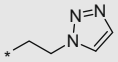
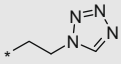
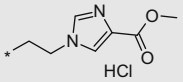
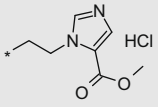
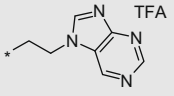
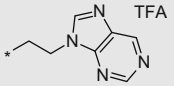
Compounds **37**–**54** were prepared according to Scheme 3. After sulfonylation of compound **30**, the resultant sulfonamide **55** was either alkylated with the appropriate alkyl bromides or reacted with the appropriate alcohols under Mitsunobu conditions to give compound **56**. Since some 2-bromoalky- or 2-hydroxyalkyl-substituted heterocycles were neither commercially available nor easy to synthesize, the corresponding substituents were introduced by way of the ethyl bromide **57** which was easily prepared from compound **55** and 1,2-dibromoethane.

To access our original hypothesis that these carboxylic acid ZBG ADAMTS-5 inhibitors would maintain high selectivity, we examined the potency of the most active compounds in MMP-1 and TACE, representatives of two distinct classes of Zn metalloproteases, the MMP and ADAM family. In fact all the compounds shown in Tables 4 and 5 had no inhibitory activity against these two enzymes below 10 μM (data not shown). This compound series demonstrates a high level of selectivity for inhibition of ADAMTS-5 over these closely related Zn metalloproteases.

In conclusion, we have discovered a series of novel N-substituted 2-phenyl-1-sulfonylamino-cyclopropane carboxylates as ADAMTS-5 inhibitors which are conformationally constrained phenylalanine analogues. To the best of our knowledge, this was the first disclosure of carboxylate-based ADAMTS-5 inhibitors

Table 5
Modification of the substituent on the N-atom of 2-phenyl-1-sulfonylamino-cyclopopanecarboxylate: heteroaromatic series



Compounds	R	Agg-2 IC_{50} (μM)
42		0.75
43		0.29
44		0.82
45		0.32
46		0.18
47		0.19
48		0.26
49		0.27
50		0.27
51		0.073
52		0.23
53		0.071
54		0.17

which show both strong potencies (IC_{50} < 0.1 μM) as well as good selectivities against closely related MMPs like MMP-1 and TACE. The continued efforts to further optimize this series of compounds are currently ongoing and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.024.

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