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Synthesis and SAR of 2-phenyl-1-sulfonylaminocyclopropane carboxylates as ADAMTS-5 (Aggrecanase-2) inhibitors

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ABSTRACT

A series of 1-sulfonylaminocyclopropanecarboxylates was synthesized as ADAMTS-5 (Aggrecanase-2) inhibitors. After an intensive investigation of the central cyclopropane core including its absolute stereochemistry and substituents, we found compound **22** with an Agg-2 IC_{50} = 7.4 nM, the most potent ADAMTS-5 inhibitor reported so far.

Osteoarthritis (OA) is a degenerative disease that occurs in a large proportion of the elderly. It is characterized by degradation of articular cartilage that leads to significant joint pain and inflammation and eventually results in impaired mobility. No effective treatment to halt the progression is currently available, and the discovery of disease modifying osteoarthritis drugs (DMORDs) has been eagerly awaited for a long time.

Aggrecan, the major proteoglycan in cartilage, endows articular cartilage with its unique compressive resistance. In osteoarthritic cartilage, aggrecan is cleaved at the Glu373-Ala374 site[1] by aggrecanases which are members of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family of zinc metalloproteases. Among aggrecanases, much attention has been recently paid to ADAMTS-5 (Aggrecanase-2), since ADAMTS-5 knock-out mice have been shown to be more resistant to progression of cartilage degradation in a surgically induced osteoarthritis model as well as in a short term inflammatory arthritis model.[2,3] Thus, this enzyme is currently believed to be a promising pharmacologic target to prevent OA.

In a preceding paper[4] we have disclosed a series of N-substituted 2-phenyl-1-sulfonylaminocyclopropane carboxylates as ADAMTS-5 inhibitors. A representative analog (compound **1**, Agg-2 IC_{50} = 0.073 μM) is shown in Table 1; for this series, appropriate heterocycles tethered to the sulfonamide nitrogen (e.g., the imidazole-4-carboxylate ester in **1**) are a significant contributor to potency. The preferred configuration is (1*R*,2*S*), as the enantiomer **2** displayed a significant loss of potency. In the course of pursuing this N-substituted series, we found that non N-substituted 1-sulfonylamino-2-phenylcyclopropane carboxylates are also potent, but their enantiomeric preference is (1*S*,2*R*), in sharp contrast to the N-substituted series. Compound **4,** having the (1*S*,2*R*) configuration, inhibited ADAMTS-5 with an IC_{50} of 0.084 μM, whereas its enantiomer **3** was slightly less active. Since the ligand efficiency[5,6] of **4** is much greater than that of **1**, which is one of the most potent compounds in the previous N-substituted series, we decided to investigate the non-N-substituted series in our ADAMTS-5 inhibitor program. In this paper, we report on our efforts to optimize sulfonamide **4**, which led to discovery of compound **22**, the most potent ADAMTS-5 inhibitor reported to date.

Figure 1 illustrates our proposed binding modes for compounds **1** and **4**. The inverted stereochemical preferences are consistent with orientation of the sulfonamide nitrogen toward solvent when substituted (**1**) or hydrogen-bonded to the backbone carbonyl of Gly380 when unsubstituted (**4**). The lower activity of **3** results from loss of this hydrogen bond. Such alternate sulfonamide orientations have been observed crystallographically for metalloproteinase inhibitors of inverted stereochemistry.[7]

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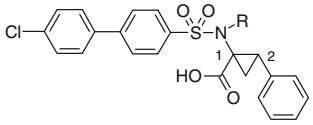
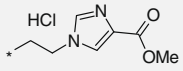
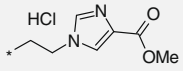
Table 1
In vitro ADAMTS-5 inhibitory activity of 1-[4-(4-Cl-phenyl)phenyl]sulfonylamino-2-phenylcyclopropanecarboxylates



Compd	Configuration	R	%Inhibition at 0.3 μM	Agg-2 IC_{50} (μM)
1	(1*R*,2*S*)			0.073
2	(1*S*,2*R*)		18%	
3	(1*R*,2*S*)			0.210
4	(1*S*,2*R*)			0.084

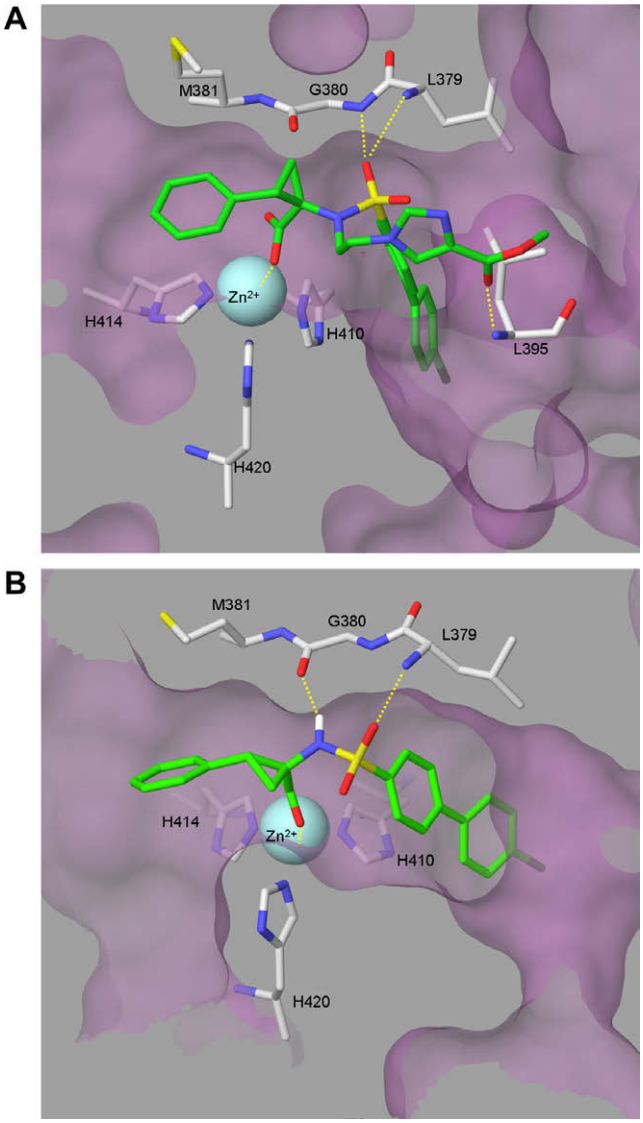
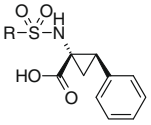


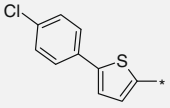
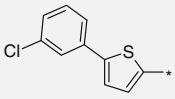
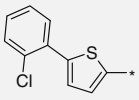
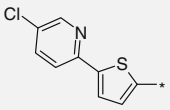
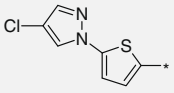
Figure 1. Proposed binding modes for compounds **1** and **4**. The inhibitors were docked manually into the X-ray structure of ADAMTS-5 (PDB accession code 3B8Z[8]). The inhibitors are depicted in green, the protein surface is violet, and selected sidechains are shown in white. The zinc ion is portrayed as a blue sphere. Electronic contacts are shown as dashed yellow lines.

In terms of its structural features, compound **4** is divided into two components, an arylsulfonyl headpiece and a cyclopropane core. We initially varied the arylsulfonyl headpiece to see its effect on inhibitory activity.

The results are summarized in Table 2. Compound **5**, possessing a thiophene ring instead of the proximal benzene ring of **4**, was of

Table 2
In vitro ADAMTS-5 inhibitory activity of 2-phenyl-1-sulfonylaminocyclopopanecarboxylates with various head groups



Compd	R	ADAMTS-5	
		%Inhibition at 1.0 μM	IC_{50} (μM)
5			0.060
6			0.094
7		48	
8			0.100
9			0.080

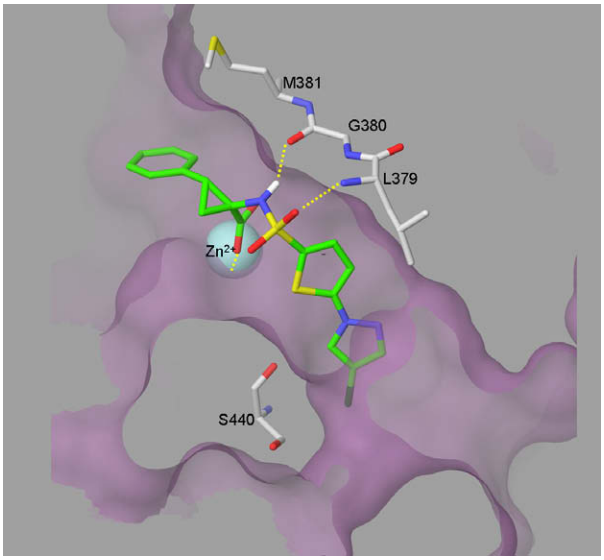
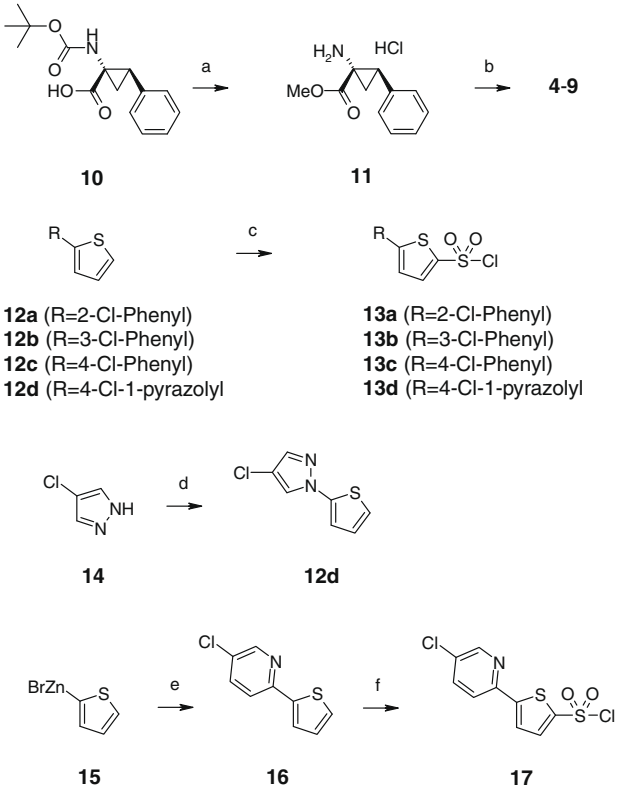


Figure 2. Proposed binding mode for **9**. The color scheme is as described for Figure 1. Note that an alternate rotamer of the distal pyrazole is sterically tolerated, but would suffer from an electronic repulsion from the carbonyl of Ser440. The resulting orientation places the distal chlorine atom into a small depression within the S1′ pocket.

comparable potency to **4** (IC_{50} = 0.060 μM). Models comparing **4** and **5** suggest that the terminal chlorine atom can reside in a similar position in the enzyme pocket, consistent with their similar activities. From a synthetic point of view, we decided to keep the thiophene ring constant while varying the distal aromatic ring because the corresponding sulfonyl chlorides could be prepared under much milder conditions. There was a clear positional preference of the chlorine atom on the distal benzene ring and ortho-substituted analog **7** had greatly reduced potency; this is expected to arise from a significantly larger torsion between the two aryl rings and resulting steric incompatibility with the S1′ pocket. The meta-substituted analog **6** had comparable potency to **5**. Replacement of the distal benzene ring with heteroaromatics was well tolerated. The pyridine and pyrazole analogs (**8** and **9**) had IC_{50}s of 0.10 and 0.080 μM, respectively.

Figure 2 illustrates the proposed binding mode for compound **9**. For the illustrated orientation, the pyrazole nitrogen is not involved in a direct hydrogen bonding interaction; we hypothesize that an electronic repulsion to the backbone carbonyl of Ser440 (ca. 2.8 Å distance from the pyrazole ring) drives the orientation as depicted, which in turn allows the chlorine atom to occupy a small surface depression near the sidechain of Ser440.

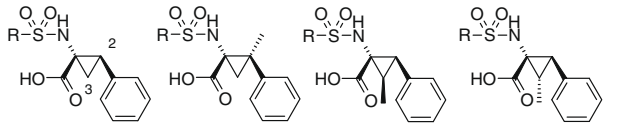
Compounds **4**–**9** were prepared as shown in Scheme 1. Commercially available carboxylic acid **10** was treated with $SOCl_2$ in MeOH to give ester **11**, which was further transformed to **4**–**9** via coupling reactions with sulfonyl chlorides followed by ester hydrolysis. 5-(Chlorophenyl)thiophenesulfonyl chlorides (**13a**–**c**) were prepared in two steps: sulfonylation with sulfuric acid in the presence of Ac_2O, and successive chlorination with $SOCl_2$. **13d** was similarly prepared from **12d**, which was obtained from **14** and 2-bromothiophene in the presence of a copper catalyst.[9] A Negishi coupling was applied to make **16**, which was subjected to $ClSO_3H$ to give **17** in a single step.

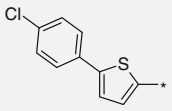
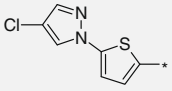


Scheme 1. Reagents and conditions: (a) $SOCl_2$, MeOH, rt, 12 h (99%); (b) (i) 4-(4-Cl-phenyl)phenyl]sulfonyl chloride or **13a**–**d** or **17**, Et_3N, $CHCl_3$, rt, 6 h, (ii) 4 N NaOH, MeOH, THF, 100 °C, 4 h (37–86%, two steps); (c) (i) Ac_2O, c-H_2SO_4, AcOEt, 0 °C to rt, 24 h, (ii) DMF, $SOCl_2$, 90 °C, 8 h (47–80%, 2 steps); (d) 2-bromothiophene, Cu_2O, salicyl aldoxime, Cs_2CO_3, DMF, 120 °C, 15 h (68%); (e) 2,5-dichloropyridine, Pd(dppf)Cl_2·CH_2Cl_2, THF, 50 °C, 8 h (30%); (f) $ClSO_3H$, rt, 12 h (27%).

After the exploration of the arylsulfonyl headpiece discussed above, we next focused on the cyclopropane core portion. To investigate the effects of substituents on the inhibitory activity, a methyl group was initially introduced at the 2-, cis-3-, or trans-3-position of the cyclopropane ring in a stereoselective fashion. Results using the newly-discovered two thienylsulfonyl groups as headpieces are shown in Table 3. A marked improvement in potency was observed for the 2- and cis-3-methylated compounds, while introduction of a methyl group in the trans-3-position virtually showed no effect. It is interesting to note that methyl group introduction in the 2-position is slightly more effective than in the cis-3 position for the 4-chlorophenyl series (compare **18** and **19**), while methylation of the cis-3-position increased the potency more markedly than 2-

Table 3
Effects of substituents on the cyclopropane ring for 2-phenyl-1-sulfonylamin ocyclopopanecarboxylates



Compd	R	Cyclopropane Methylation	ADAMTS-5 IC_{50} (μM)
5		Unmethylated	0.060
18		2-Methyl	0.010
19		*cis*-3-Methyl	0.021
20[a]		*trans*-3-Methyl	0.120
9		Unmethylated	0.080
21		2-Methyl	0.032
22		*cis*-3-Methyl	0.0074

[a] Racemic compound.

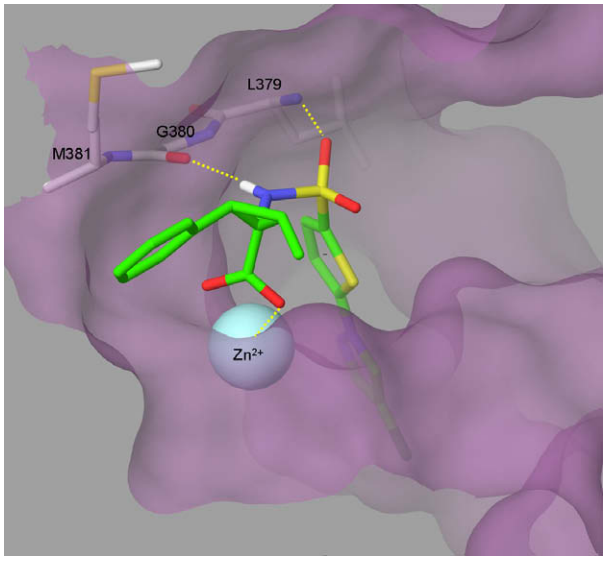


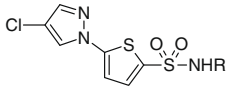
Figure 3. Hypothesized binding mode for compound **22**. The color scheme is as described for Figure 1. The rotamer of the phenyl group at C2 is expected to be twisted slightly by the presence of the cis-3-methyl group, which may enhance hydrophobic contact to the cleft surface near Met381.

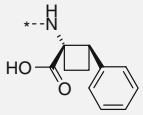
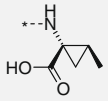
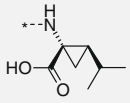
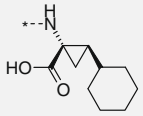
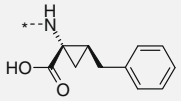
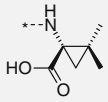
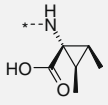
methyl-substitution for the 4-pyrazole series (compare **21** and **22**). Models for these compounds suggest that for both series the 2-methyl substituent introduces an additional hydrophobic contact to Thr378, which resides above the active site cleft, while cis-3-methylation leads to a slight rotation of the phenyl substituent at the 2-position. This rotation may enhance contact to Met381, which forms a hydrophobic patch on the cleft surface (Fig. 3). Compound **22**, with an IC_{50} of 7.4 nM, is the most potent ADAMTS-5 inhibitor reported to date. Although a definitive explanation for the larger enhancement of cis-3-methylation on the pyrazole series remains elusive, we propose that the activity arises from the compact nature of the S1′ biaryl substituent, and a resultant ability to adjust position for maximal contact of the phenyl ring.

Compounds **18–22** were prepared as shown in Scheme 2. Selective hydrolysis of the less hindered ester of racemic ***rac*-23a–c**,[10–12] followed by a Curtius rearrangement, gave compounds ***rac*-24a–c**, which were subjected to hydrolysis conditions to give ***rac*-25a–c**. Optically pure **25b–c**, obtained by chiral resolution as (+)-quinidine salts, and ***rac*-25a** were then esterified before Boc deprotection in the presence of $TsOH{\cdot}H_2O$. Coupling of thus obtained **26b**, **26c** and ***rac*-26a** with **13c**, and successive cleavage of the *t*-Bu ester under acidic conditions provided **18**, **19** and **20**, respectively. Compounds **21** and **22** were similarly prepared via coupling of **26b** and **26c** with **13d**, respectively.

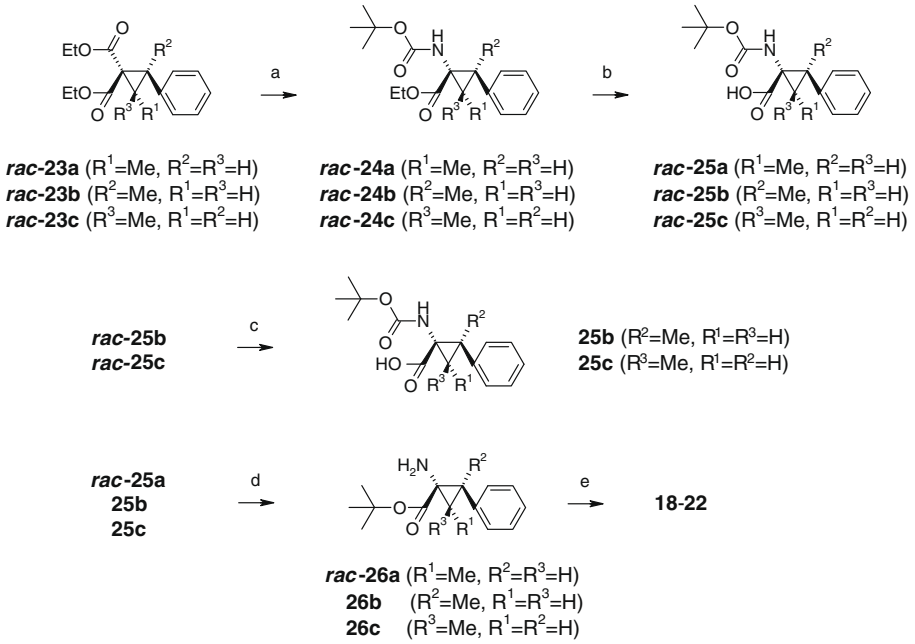
The significant improvement in potency achieved by introduction of a methyl group on the cyclopropane core prompted us to pursue further investigation of this part of the molecule. A variety of alkyl-substituted cyclopropane analogs, as well as a cyclobutane analog, was synthesized in racemic form (Table 4). Diminishing the contact of the C-2 phenyl group by removal (**28**) or homologation (**31**) resulted in significant loss of activity. Re-introduction of hydrophobicity with alkylation at C2 led to restoration of some activity (**29** and **30**), while enhanced activity by addition of a second methyl group (**32** and **33**) is consistent with the positional preference of methylation found in the phenyl substituted series possessing a chloropyrazole headpiece (see **21** and **22**). Although a gain in potency was observed by increasing the size and hydrophobicity of the 2-alkyl substituent in **28** from methyl to *i*-propyl and cyclohexyl (**28**–**30**), the marked inhibitory activity found in

Table 4
Investigation of the amino acid portion of [5-(4-Cl-pyrazole-1-yl)thiophene-2-yl]sulfonamides



Compd[a]	NHR	Agg-2	
		%Inhibition at 1.0 μM	IC_{50} (μM)
27		−0.84	
28		3.5	
29			0.77
30			0.36
31		25	
32		19.6	
33			0.80

[a] All compounds are racemic.



***rac*-23a** (R^1=Me, R^2=R^3=H)
***rac*-23b** (R^2=Me, R^1=R^3=H)
***rac*-23c** (R^3=Me, R^1=R^2=H)

***rac*-24a** (R^1=Me, R^2=R^3=H)
***rac*-24b** (R^2=Me, R^1=R^3=H)
***rac*-24c** (R^3=Me, R^1=R^2=H)

***rac*-25a** (R^1=Me, R^2=R^3=H)
***rac*-25b** (R^2=Me, R^1=R^3=H)
***rac*-25c** (R^3=Me, R^1=R^2=H)

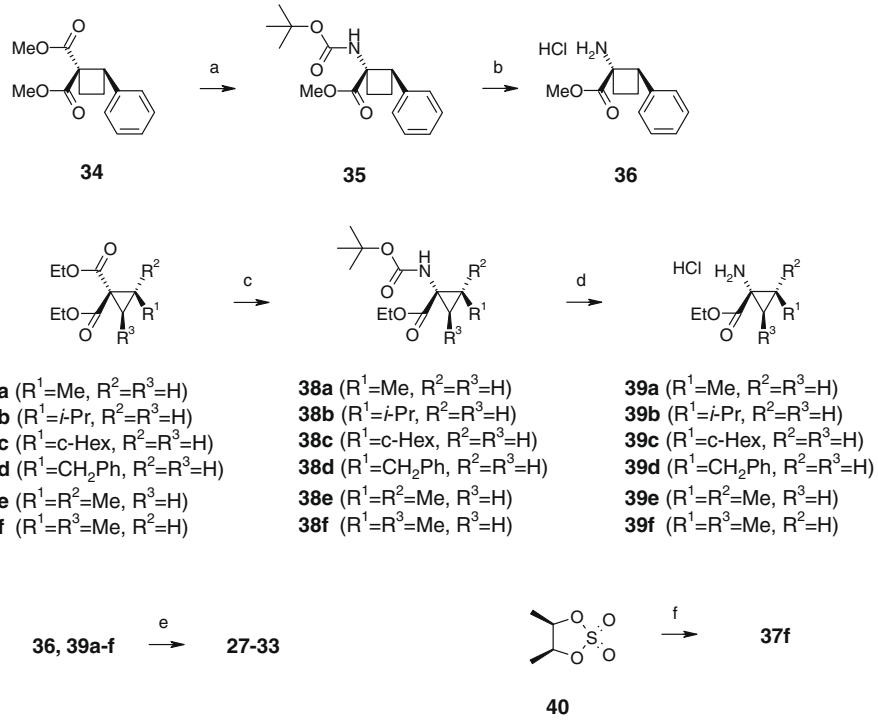
***rac*-25b**
***rac*-25c**

25b (R^2=Me, R^1=R^3=H)
25c (R^3=Me, R^1=R^2=H)

***rac*-25a**
25b
25c

***rac*-26a** (R^1=Me, R^2=R^3=H)
26b (R^2=Me, R^1=R^3=H)
26c (R^3=Me, R^1=R^2=H)

Scheme 2. Reagents and conditions: (a) (i) 4 N NaOH, EtOH, rt, 12 h, (ii) DPPA, *t*-BuOH, Et_3N 110 °C, 12 h (32–88%, 2 steps); (b) 4 N NaOH, EtOH, 100 °C, 12 h (96–99%); (c) (+)-quinidine, *i*-PrOH, AcOEt, rt, 12 h (30%, 99% ee for **25b**, 36%, 100% ee for 25c); (d) (i) $Me_2NCH(Ot\text{-}Bu)_2$, toluene, 100 °C, 5 h, (ii) *p*-$TsOH{\cdot}H_2O$, MeOH, 12 h (74–99%, 2 steps); (e) (i) **13c** or **13d**, pyridine, $CHCl_3$, 12 h; (ii) TFA, $CHCl_3$, 12 h (24–84%, 2 steps).



Scheme 3. All compounds are racemic. Reagents and conditions: (a) (i) 4 N NaOH, MeOH, rt, 12 h; (ii) DPPA, Et_3N, *t*-BuOH, 130 °C, 12 h (95%, two steps); (b) 4 N HCl in 1,4-dioxane, 12 h (30%); (c) (i) 4 N NaOH, MeOH, rt, 12 h; (ii) DPPA, Et_3N, *t*-BuOH, 130 °C, 12 h (40–87%, 2 steps); (d) 4 N HCl in 1,4-dioxane, 12 h (63–99%); (e) (i) **13d**, pyridine, $CHCl_3$, rt, 12 h; (ii) 8 N NaOH, EtOH, 100 °C, 12 h (23–81%, 2 steps); (f) diethyl malonate, NaH, DMF, 75 °C, 12 h (57%).

the phenyl derivative **9** could not be reproduced in the alkyl series. Expansion of the cyclopropane core of **9** to cyclobutane (**27**) resulted in a complete loss of activity. The expanded ring would be expected to create a steric clash between the phenyl ring and Met281. Given these observations, the presence and the position of the phenyl group on the cyclopropane core appear to be crucial for potent inhibitory activity.

Synthesis of compounds **27–33** is shown in Scheme 3. Following the same procedure described in Scheme 2, **36** and **39** were synthesized from **34** and **37** through **35** and **38**, respectively, via three steps: selective hydrolysis of the less hindered ester, Curtius rearrangement and deprotection of the amino group. **37a–e** were prepared according to known procedures,[13–20] and **37f** was synthesized by alkylation of diethyl malonate with **40**.[21] Coupling reactions of thus obtained **36** and **39** with **13d** followed by saponification of the ester moiety gave **27**-**33** as racemates.

In summary, we have found a series of non-N-substituted 2-phenyl-1-sulfonylamino-cyclopropane carboxylates possessing (1*S*,2*R*) configuration as novel ADAMTS-5 inhibitors. A dramatic potency gain was observed by introduction of a methyl group on the 2- or cis-3 position of the cyclopropane core, and compound **22** (IC_{50} = 7.4 nM) was found to be the most potent ADAMTS-5 inhibitor reported to date. **22** also displayed good to excellent selectivity over MMP-1 and TACE (IC_{50}s were 180 nM and 4000 nM, respectively). In addition, this compound is orally bioavailable and shows a good pharmacokinetic profile in rats (BA = 81%, $T_{1/2}$ = 4.5 h).

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