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New non-hydroxamic ADAMTS-5 inhibitors based on the 1,2,4-triazole-3-thiol scaffold

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ABSTRACT

In this Letter we describe the design, synthesis, screening, and optimization of a new family of ADAMTS-5 inhibitors. These inhibitors display an original 1,2,4-triazole-3-thiol scaffold as a putative zinc binding-group. In vitro results are rationalized by in silico docking of the compounds in ADAMTS-5's crystal structure.

Osteoarthritis (OA) is a progressive disease of the joints characterized by the degradation of the articular cartilage. The glycoprotein aggrecan is the major component of the cartilage extracellular matrix and its extensive degradation leads to further breakdown of other extracellular matrix macromolecules.[1] Aggrecanase-2, also called ADAMTS-5 (A Disintegrin and Metalloproteinase with Thrombospondin motifs 5) is a metalloprotease that degrades components of the extracellular matrix, and in particular aggrecan.[2,3] In that context, inhibitors of this enzyme could result in treatments for osteoarthritis.

When targeting zinc metalloproteases, a zinc binding-group (ZBG) is helpful with respect to facilitating inhibitor orientation, and hence binding, in the enzyme's active site. First described inhibitors were hydroxamates that resulted from research on the related MMPs (Matrix MetalloProteinases).[4]

Recent research on ADAMTS-5 describes other ZBGs such as carboxylic acids,[5] hydroxyquinolines,[6] spirothiazolones,[7] or thioxothiazolidinones.[8] Our team has recently published a few examples of squaric acid *N*-hydroxylamide amides inhibitors.[9]

To continue our work on original ZBGs, we described earlier the synthesis of 1,2,4-triazole-3-thiols (Fig. 1).[10,11] We hypothesized that this heterocycle can bind zinc thanks to its exocyclic sulfur atom as already shown for a series of TACE (ADAM17) inhibitors.[12]

We have designed and synthesized a focused library of 500 1,2,4-triazole-3-thiols. The compounds were prepared from the amine and di-2-pyridylthionocarbonate followed by reaction with the required hydrazide and subsequent cyclization into 1,2,4-triazole-3-thiols under basic conditions (Scheme 1).

Amines and most hydrazides were commercially available.[13] Some derivatives of 1,2,4-oxadiazol-5-yl-acetic acid hydrazide were synthesized to complete the hydrazide set (Scheme 2). Amidoximes **1a–c** were prepared as previously described by reaction of hydroxylamine with the corresponding nitrile.[14] Then the 1,2,4-oxadiazole ring was obtained by reaction with acetyl chloride. The resulting ethyl esters were converted to hydrazides by direct reaction with hydrazine.

The screening of the library was performed using previously published conditions at 30 μM.[15] The hit rate was 2.5% for a 80% inhibition threshold. The hits and close inactive analogs (compounds **4–19**) were resynthesized at a larger scale, fully

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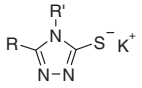
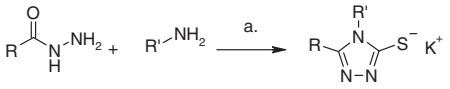
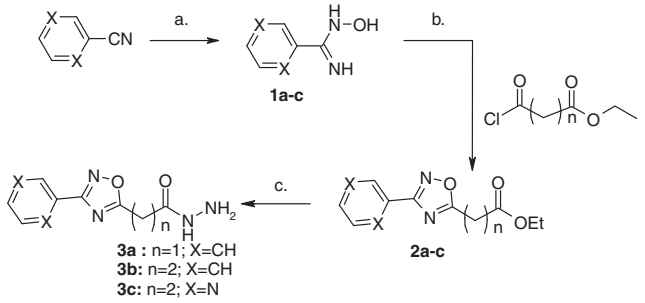


Figure 1. 1,2,4-Triazole-3-thiol scaffold.



Scheme 1. Reagents and conditions: (a) (i) dipyridylthionocarbonate 0.25 M in DMF (1.05 equiv), amine (free base) 0.1 M in DMF (1 equiv), 55 °C, 1.5 h; (ii) hydrazide (free base), 0.1 M in DMF (1 equiv) 55 °C, 1.5 h, solvent evaporation, then (iii) 1 equiv KOH 0.1 M in H2O/EtOH (40/60) 85 °C, 5 h.



Scheme 2. Reagents and conditions: (a) 1 equiv NH_2OH/HCl, DIEA, EtOH, 3 h, reflux. (b) DIEA, dioxane, reflux, 4 h then TBAF, 75%. (c) N_2H_4, ethanol, 18 h, 95%.

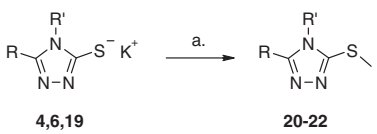
characterized and their IC_{50} on the target measured. All the results are shown in Tables 1 and 2.

In chlorophenoxylmethyl series (Table 1), *para*- and *ortho*-biphenyl ring are slightly better than *meta*-derivative (**4**–**6**). The biphenyl system is preferred to phenylalkyl group (**4** vs **7**–**9**). Interestingly, 3-(*N*-imidazolyl)propyl displays an IC_{50} of 11 μM whereas its (3-phenyl)propyl analog is inactive (**9** vs **10**). Interestingly, isosteric replacement of the chlorine atom by a fluorine (compound **11**) decreases activity, consistently with the less hydrophobic properties of fluorine. Cyclization in dihydrobenzoxazine (compound **12**) resulted in a loss of activity. This could be attributed to both the steric constraint and the introduction of a NH group.

In the biaryl series (Table 2), the 3-phenylpropyl derivatives are equipotent (**13**–**15**). Introduction of an *o*-biphenyl system, like in **6**, gives similar results (compound **15**). *o*-Biphenyl derivative **16** displays an activity of 22 μM similar to **13** but isosteric replacement of 1,2,4-oxadiazole by a phenyl in **17** leads to complete loss of activity. This could be attributed to the high lipophilicity of the compound precluding its solubility in the assay. Interestingly, like in the chlorophenoxymethyl series, 3-(*N*-imidazolyl)propyl **18** is the most active compound (6 μM). Introduction of a methylene moiety completely abolishes activity (**19**) evidencing an optimal spacer between the 1,2,4-oxadiazole ring and the ZBG.

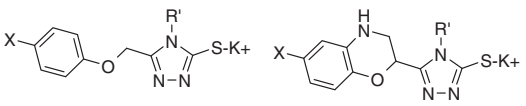
Also, to evaluate our primary hypothesis on binding to the target by the exocyclic sulfur atom, we synthesized a few *S*-methylated analogs (Scheme 3). Table 3 gathers inhibition results for the *S*-methylated compounds **20**–**22**. As expected, these compounds do not inhibit ADAMTS-5 due to the absence of ZBG.

The catalytic domain of ADAMTS-5 is known to be flexible in several regions of the binding site.[16] X-ray structures co-crystallized with different ligands (e.g., pdb codes: 3B8Z, 2RJQ, and 3HYG) show that the His^{373}-loop is capable to adapt to the ligand by closing onto the binding site as for the highly hydrophobic S1′ pocket through the Ser^{441}-Ile^{442}-Leu^{443}-loop and the His^{403}-loop. Hydrophobic moieties within the ADAMTS-5 ligands are known to plunge into the S1′ pocket while nucleophilic groups such as the often observed hydroxamate function complement the Zn^{2+}-chelating residues His^{410}, His^{414}, and His^{420}.



Scheme 3. Reactions and conditions: (a) MeI (5 equiv), MeOH 18 h (quant.).

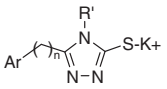
Table 1
Inhibition on ADAMTS-5 for compounds **4**–**12**

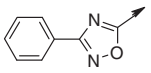
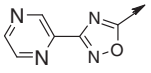
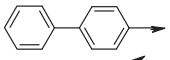
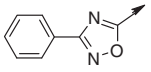
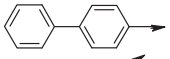
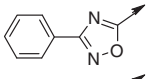
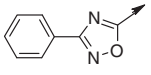


Compd	X	R′	ADAMTS-5 IC_{50}[a] (μM)
4	Cl–	–CH_2-*p*-biphenyl	13
5	Cl–	–CH_2-*m*-biphenyl	27
6	Cl–	–CH_2-*o*-biphenyl	13
7	Cl–	–Benzyl	>100
8	Cl–	–Phenethyl	>100
9	Cl–	–(3-Phenyl)propyl	>100
10	Cl–	–3-(*N*-Imidazolyl)propyl	11
11	F–	–CH_2-*p*-biphenyl	>100
12	Cl–	–CH_2-*p*-biphenyl	>100

[a] Values are means of two experiments minimum, standard deviations are ±10%.

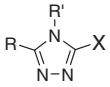
Table 2
Inhibition on ADAMTS-5 for compounds **13**–**29**

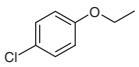
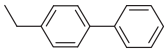
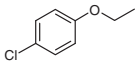
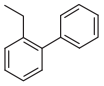
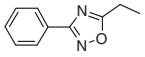
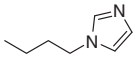


Compd	*n*	Ar–	R′	ADAMTS-5 IC_{50}[a] (μM)
13	1		–(3-Phenyl)propyl	38
14	1		–(3-Phenyl)propyl	47
15	1		–(3-Phenyl)propyl	22
16	1		–CH_2-*o*-biphenyl	22
17	1		–CH_2-*o*-biphenyl	>100
18	1		–3-(*N*-Imidazolyl)propyl	6
19	2		–3-(*N*-Imidazolyl)propyl	>100

[a] Values are means of two experiments minimum, standard deviations are ±10%.

Table 3
Inhibition on ADAMTS-5 for compounds **20**–**22**



Compd	R	R′	X	ADAMTS-5 IC_{50}[a] (μM)
20			–SMe	>100
4			$-S^-K^+$	13
21			–SMe	>100
6			$-S^-K^+$	13
22			–SMe	>100
18			$-S^-K^+$	6

[a] Values are means of two experiments minimum, standard deviations are ±10%.

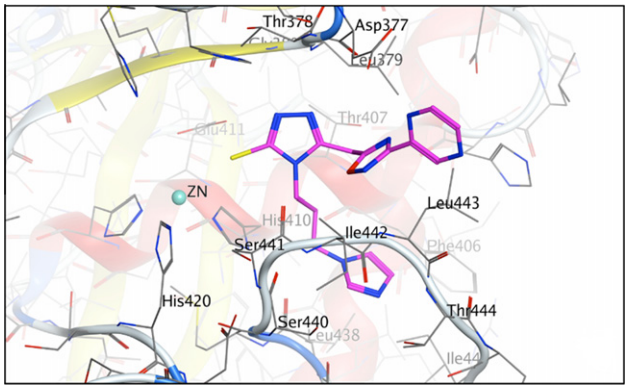


Figure 2. Binding mode of 1,2,4-triazole-3-thiol. ADAMTS-5 structure as in pdb code 3HYG with docked compound 18.

Based on these observations, compounds **6**, **10**, and **18** were docked into ADAMTS-5 (pdb code: 3HYG) in an attempt to rationalize their binding mode. The combination of several docking programs,[17] has allowed the identification of a consensual binding mode for the three compounds (Fig. 2 and Supplementary data). Thus, the binding modes show that the thiol function complements the chelating of the zinc ion by His^{410}, His^{414}, and His^{420}. This confirms the poor activity of the *S*-methylated analogs. The 1,2,4-triazole ring forms a Hydrogen bond with the backbone nitrogen of Leu^{379}.[18] The S1′ pocket is occupied by either the –3-(*N*-imidazolyl)propyl moiety (compounds **10** and **18**) or the $-CH_2$-*o*-biphenyl moiety (compound **6**). For the former, a Hydrogen bond is formed with the backbone Nitrogen of Thr^{444}. This may explain the better inhibition of **10** versus **9**, that is, devoided of any H-bond acceptor in that position. Finally, the *R* moiety either a 1,2,4-oxadiazole-based biaryl (compound **18**) or a chlorophenoxymethyl group (compounds **6** and **10**) always occupied the tip of the S1′ pocket where it binds ADAMTS-5 through hydrophobic contact to Leu^{379}, Ile^{442}, and Leu^{443}. This may explain why compound **11** bearing a fluorine is less active than the more hydrophobic Chlorine analogs.

Table 4

Compd	IC_{50} (μM) TS5	IC_{50} (μM) TS4	Log *D*[a] (pH 7.4)	Solubility[a] (μg/mL)
6	13	12	3.6	0.4
10	11	>30	1.6	14
14	>30	8	1.7	49
18	6	>30	1.1	64

[a] Solubility and Log *D* are measured from a DMSO stock solution.

Table 4 shows selectivity results on ADAMTS-4, measured log *D* between 1-octanol and PBS buffer (pH 7.4) and solubility in PBS for some selected compounds.

Both structurally close ADAMTS-4 and five cleave aggrecan. ADAMTS-5 has been shown to be the major aggrecanase in a model of arthritis.[2] Interestingly, differences in four residues in the catalytic site result in a roomier S1′ pocket of ADAMTS-4. It may thus be difficult to reach selectivity on ADAMTS-5 explaining why only a few examples of selective compounds have been described in the literature.[7] In our series, some selectivity was obtained. Compounds **10** and **18** have the best selectivity ratio for ADAMTS-5 and **14** displays on the contrary a lower IC_{50} on ADAMTS-4.

Compounds **10**, **14**, and **18** display reasonable Log *D* in comparison with **6**, that is, highly hydrophobic and poorly soluble. Compound **18** is the most promising compound both in terms of activity and physico-chemical properties.

In conclusion, we have developed a series of ADAMTS-5 inhibitors containing a 1,2,4-triazole-3-thiol metal binding-group. These molecules display IC_{50}s of 6–47 μM in a similar range to other non-hydroxamic acid inhibitors reported in the literature. Based on the potency, selectivity, and physico-chemical properties, we consider the 3-(*N*-imidazolyl)propyl derivative **18** as a suitable starting point for further optimization of this chemical series.

Acknowledgments

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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.2010.08.108.

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18. Similarly the Oxygen of the amide function of the side chain of the hydroxamate-based inhibitor co-crystallized in structure pdb code 3HYG, makes a Hydrogen bond with the backbone of Leu^{379}. See Supplementary data.