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Structure–activity study on a series of α-glutamic acid scaffold based compounds as new ADAMTS inhibitors

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ABSTRACT

A series of α-glutamic acid scaffold based 4-(benzamido)-4-(1,3,4-oxadiazol-2-yl) butanoic acids were designed and synthesized as new ADAMTS inhibitors. The compounds dose-dependently inhibited the enzymatic activities of ADAMTS-4 and ADAMTS-5. One of the most active compound **2h** potently inhibited ADAMTS-4 and ADAMTS-5 with IC_{50} values of 1.2 and 0.8 μM, respectively. These inhibitors may serve as new lead compounds for further development of therapeutics to treat osteoarthritis.

Osteoarthritis (OA) is a degenerative disease of the joints, resulting from degradation of articular cartilage. The aggrecan is a major component of the cartilage extracellular matrix. The extensive loss of aggrecan via proteolysis is believed to be the early event that leads to further deterioration of weigh-bearing properties and eventually irreversible joint dysfunction.[1]

Aggrecanase-1 and aggrecanase-2, also known as ADAMTS-4[2] and ADAMTS-5,[3] respectively, are members of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family of zinc-containing metalloproteases.[4] Although the relative contributions of ADAMTS-4 and ADAMTS-5 in different stages of human disease remain illusive, they are the only identified enzymes that cleave the interglobular domain (IGD) of aggrecan at Glu373-Ala374 in osteoarthritis. In addition, a recent study revealed that ADAMTS-5 knockout mice were physiologically normal and protected from developing osteoarthritis.[5] A short-interfering RNA study showed that both ADAMTS-4 and ADAMTS-5 were involved in human cartilage degeneration in OA.[6] Therefore, ADAMTS-4 and ADAMTS-5 have been considered as new potential drug targets for the development of new therapeutic agents to treat osteoarthritis.[7,8]

Since the first discovery of the ADAMTS-4 inhibitors in 2001,[9] several classes of small-molecular inhibitors of ADAMTS have been identified.[10–12] Structural feature analysis of these aggrecanase inhibitors suggested that almost all the compounds possessed a zinc-binding group (ZBG, i.e., a hydroxamic acid, carboxylic acid, or semisquaric acid group) and several hydrophobic moieties (P1′ and P2′ groups) to interact with the S1, S1′, and S2′ binding pockets, respectively.[11,13] Compound **1** has been reported as a highly potent aggrecanase inhibitor with an in vitro IC_{50} value of 1.5 nM.[12h] The detailed contributions to the binding with aggrecanase from decomposed moieties of **1** are illustrated in Figure 1. In light of compound **1** and its corresponding interactions with aggrecanase, we designed and synthesized a series of α-glutamic acid scaffold based 4-(benzamido)-4-(1,3,4-oxadiazol-2-yl) butanoic acids (**2**) as new potential ADAMTS inhibitors. In the structure of compound **2**, the carboxylic acid group may serve as a zinc-binding group; while the biphenyl system and the substituted phenyl group may mimic the corresponding moieties in compound **1** to interact with the S1′ and S2′ pockets, respectively. A highly rigid and metabolic stable fragment, 1,3,4-oxadiazol-2-yl, was also introduced to bridge the scaffold (ZBG and P1′ group) and the P2′ group (**2**).

The synthesis of designed compounds **2a**–**2n** was outlined in Scheme 1.[14,15] Briefly, hydrazide **3** coupled with Boc-Glu(Ochx)-OH to afford the diacylhydrazide **4**. Cyclodehydration of **4** with Ph_3P, CBr_4 and triethylamine provided oxadiazole **5**. With intermediate **5** in hands, compound **6** was readily prepared by selectively removing the *N*-Boc group via TFA and following acylation with different carboxylic acids. Finally, compounds **2a**–**2n** were easily obtained by hydrolysis of **6**.

The designed compounds were preliminarily screened for their inhibitory activities against ADAMTS-4 at 1.0 and 10.0 μM by using

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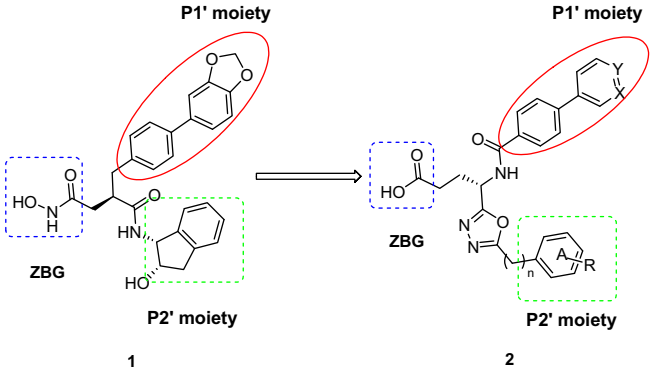
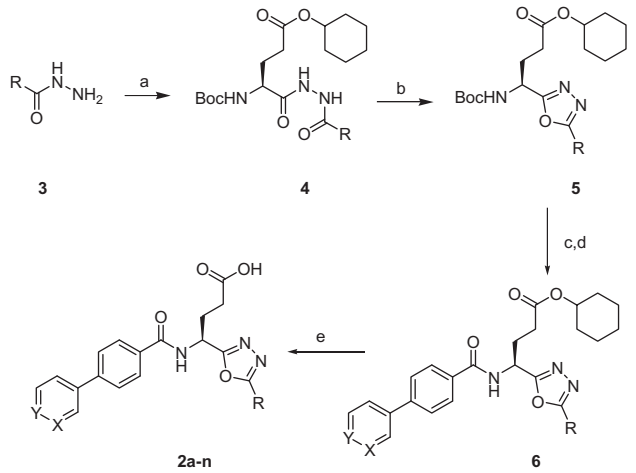


Figure 1. The designed 4-(benzamido)-4-(1,3,4-oxadiazol-2-yl) butanoic acids **2.**

a well established fluorimetric assay (SensoLyte 520 aggrecanase-1 assay kit).[16] Although (*S*)-4-(5-benyl-1,3,4-oxadiazol-2-yl)-4-biphenyl-4-ylcarbosamido butanoic acid (**2a**) did not show obvious inhibition against ADAMTS-4 at 10.0 μM, compound **2b** dose-dependently inhibited the enzymatic activity of ADAMTS-4 with inhibitory rates of 30% or 59% at 1.0 or 10.0 μM, respectively. Further evaluation revealed that compound **2b** possessed an IC_{50} value of 9.7 μM against ADAMTS-4 (Table 1). However, when the terminal phenyl group in compound **2b** was replaced with 3-pyridinyl or 4-pyridinyl group (compounds **2c** and **2d**, respectively), obviously lower bioactivity than that of original compound **2b** was observed.

The structure–activity study revealed that although the introduction of stereo hindrant dimethyl group at α or β position of the 2-phenyl-ethyl group in compound **2b** did not obviously affect its ADAMTS-4 inhibition activity (Table 1, compounds **2i** and **2j**), when the benzyl group in compound **2a** was substituted with α,α-dimethyl or cyclopropyl group, significantly lower ADAMTS-4 inhibitory potency was observed (Table 1, compounds **2e** and **2f**). Further investigations demonstrated that the ADAMTS-4 inhibitory activity was barely affected by a mono-substitution at R position (Table 1 and Figure 1, compounds **2g**, and **2k**–**2m**).

Interestingly, when the 3,4,5-trimethoxyl groups were introduced to the bottom phenyl ring (ring A in compound **2a** or **2b**, Figure 1and Table 1), the potency against ADAMTS-4 was obviously improved. For instance, the 3,4,5-trimethoxyl substituted compound **2n** had an IC_{50} value of 3.3 μM against ADAMTS-4, which



Scheme 1. Reagents and conditions: (a) Boc-Glu(Ochx)-OH, HATU, DIPEA, CH_2Cl_2, rt, 65–75%; (b) PhP_3, CBr_4, Et_3N, CH_2Cl_2, rt, 60–70%; (c) TFA, CH_2Cl_2, rt, 98%; (d) diarylcarboxylic acid, EDCI, HOBT, Et_3N, CH_2Cl_2, rt, 85%; (e) LiOH, THF/MeOH/H_2O, rt, 99%.

was threefold more potent than the parental compound **2b**. (Table 1) The corresponding tri-substituted compound **2h** based on **2a** displayed the best inhibitory activity against ADAMTS-4 with IC_{50} value of 1.2 μM (Table 1).

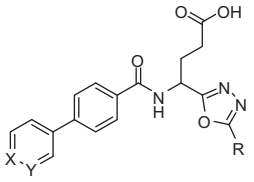
To investigate the selectivity, the inhibitory potencies of four most potent compounds **2b, 2h, 2k**, and **2n** against ADAMTS-5 were also evaluated by using a similar biochemical assay.[16] Although compounds **2b** and **2k** displayed modest selectivity over ADAMTS-4 (five to sixfold lower potency against ADAMTS-5), compounds **2h** or **2n** showed almost equal potency against ADAMTS-4 and ADAMTS-5 (1.2 μM vs 0.8 μM for compound **2h**; 3.3 μM vs 4.3 μM for compound **2n**).

Molecular docking study was performed to investigate the binding modes of compound **2h** with ADAMTS-4 and ADAMTS-5 using Glide.[17] The results are illustrated in Figure 2A and B. Since the active sites of ADAMTS-4 and ADAMTS-5 shared highly conserved residues with similar configurations,[13] **2h** bound to the proteins with similar modes. The carboxylic acid of **2h** chelated with the zinc ions as expected, and the rigid biphenyl group protruded into the S1′ pocket and made extensive hydrophobic contacts with the residues like His361, Ala392, Ala396, and Val398 in ADAMTS-4 (His410, Ile442, Leu443, and ILE446 in the case of ADAMTS-5). Moreover, the amide group formed hydrogen bonds with the main chain residues on the S2′-loops (Leu330 in ADAMTS-4 and Leu379 in ADAMTS-5). The significant improvement of the inhibitory potency of **2h** can be attributed to the hydrogen bonds formed between the methoxyl groups on the phenyl ring and the Met395 in ADAMTS-4 (the Ser375 in ADAMTS-5). Overall, the low selectivity of the most potent compounds **2h** may also stem from the putative similar binding modes in ADAMTS-4 and ADAMTS-5. Not surprisingly, the inactive compound **2e** could not bind to the pockets of ADAMTS-4 or ADAMTS-5 properly. Due to the steric hindrance caused by the dimethyl group between the oxadiazol and phenyl rings, the P2′ moiety of **2e** was not able to fully occupy the S2′ binding sites in the proteins. Moreover, lacking of polar substituents on the P2′ phenyl also prohibits forming hydrogen bonds with the S2′ site and further decreases the potency of **2e** (as shown in the Supplementary data).

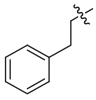
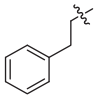
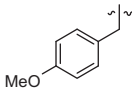
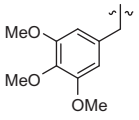
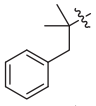
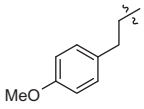
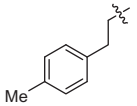
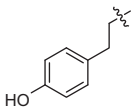
Very recently, the S1′ loop of ADAMTS-5 was proved flexible upon binding with compound **1** by creating a novel pocket to accommodate the rigid and hydrophobic P1′ moiety, which might be crucial for designing new potent and selective aggrecanase inhibitors.[18] In light of this new site flexibility, we also modeled the binding poses of **2a** and **2h** using this new structure (altered S1′ pocket, PDB ID: 3LJT). The biphenyl moiety of **2h** could occupy the same S1′ pocket induced by the phenylbenzodioxole of the compound **1** and make variant hydrophobic contacts with the residues in S1′ like Phe406, Leu438, and Ile446 (as shown in Figure 2**C**). The carboxylic acid still chelated with the zinc ion and the ring B protruded deeper into the S2′ site with slightly different orientations from the binding pose in the conventional S1′ pocket. Similarly, the improvement of the inhibitory potency of **2h** over **2a** could be attributed to the hydrogen bond formed between the *m*-methoxyl group on the phenyl ring and Thr444 (as shown in the Supplementary data). However, it is difficult to decide the actual binding modes of the inhibitors just from the docking results, because the docking scores for **2h** in the conventional and altered S1′ pockets are very close (GScores are −11.34 and −10.85, respectively). Further designs of the new inhibitors based on two different molecular docking studies are undergoing and will be reported in due course.

In summary, a series of 4-(benzamido)-4-(1,3,4-oxadiazol-2-yl) butanoic acids were designed and synthesized as new dual inhibitors of ADAMTS-4 and ADAMTS-5. These compounds dose-dependently inhibited the enzymatic activities of both ADAMTS-4 and ADAMTS-5. One of the most potent compound **2h** potently inhib-

Table 1
4-(Benzamido)-4-(1,3,4-oxadiazol-2-yl) butanoic acids as new AMDAMTS inhibitors[a]

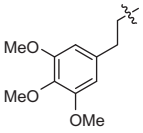


2

Compd	X	Y	R	ADAMTS-4 inhibition			ADAMTS-5 inhibition (IC_{50}, μM)
				@ 1.0 μM	@ 10.0 μM	IC_{50}, μM	
2a	CH	CH		17	26	—	—
2b	CH	CH		30	59	9.7	57.3
2c	CH	N		12	39	—	—
2d	N	CH		8	18	—	—
2e	CH	CH		-5	5	—	—
2f	CH	CH		5	20	—	—
2g	CH	CH		5	21	—	—
2h	CH	CH		47	86	1.2	0.8
2i	CH	CH		23	47	—	—
2j	CH	CH		19	45	—	—
2k	CH	CH		35	66	4.9	24.3
2l	CH	CH		27	56	—	—
2m	CH	CH		22	51	—	—

(*continued on next page*)

Table 1 (*continued*)

Compd	X	Y	R	ADAMTS-4 inhibition			ADAMTS-5 inhibition (IC_{50}, μM)
				@ 1.0 μM	@ 10.0 μM	IC_{50}, μM	
2n	CH	CH		35	74	3.3	4.3

[a] The data were reported as the means of at least three independent experiments, standard deviation are ±15% of the data presented.

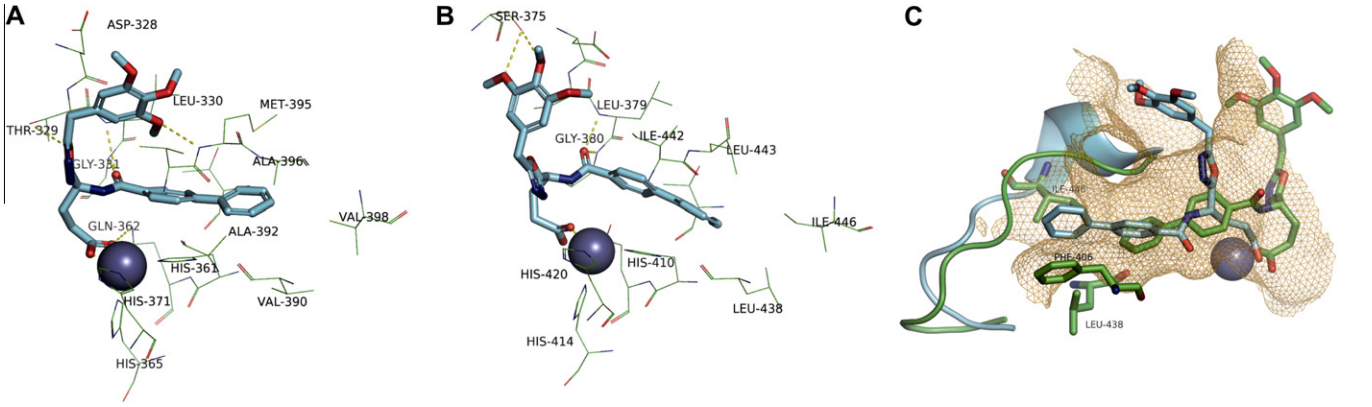


Figure 2. Docking poses of compound **2h** into the active sites of (A) ADAMTS-4, (B) conventional S1′ site of ADAMTS-5, and (C) altered S1′ site of ADAMTS-5. The zinc ions are depicted as violet spheres and the predicted inter-molecule hydrogen bonds were shown as yellow dash lines. The binding sites of the ADAMTS-5 structures with different S1′ pockets conformations were superposed, featuring the pocket of the altered S1′ site structure shown in orange mesh and S1′ loop colored in cyan. The binding pose predicted in the altered S1′ pocket is colored in cyan and the binding pose in the conventional S1′ pocket as well as the conventional S1′ loop are shown in reference colored in green. The three hydrophobic residues at the bottom of the altered S1′ in contact with the biphenyl moiety of **2h** are shown in green sticks.

ited ADAMTS-4 and ADAMTS-5 with IC_{50} values of 1.2 and 0.8 μM, respectively. These inhibitors might serve as new lead compounds for further development of therapeutics to treat osteoarthritis.

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.2011.06.009.

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16. The biological evaluation was performed following the manufactory instruction from the ANASPEC (www.anaspect.com).
17. Docking protocol: Prior to docking, compound **1h** was prepared to create meaningful protonation states and cover potential tautomers (LigPrep, Schrödinger Inc., www.schrodinger.com). Crystal structures of ADAMTS-4 (2RJP) and ADAMTS-5(2RJQ) were selected for docking (Glide, Schrödinger Inc., www.schrodinger.com). Amino acid residues located within 14 Å from the complexed inhibitors were defined as part of the binding site and prepared with the Protein Preparation Wizard of Schrödinger to add hydrogen atoms and appropriate protonation states without further energy minimization. The Glide XP mode was applied for docking with default settings and the top ranked configurations were observed as the predicted binding poses. Results are presented in Supplementary data.
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