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Synthesis and evaluation of aryl thioxothiazolidinone inhibitors of ADAMTS-5 (Aggrecanase-2)

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Abstract—5-Benzylidene-2-thioxo-thiazolidin-4-one inhibitors of ADAMTS-5 (Aggrecanase-2) have been prepared via commercially available starting materials. The identified compounds show micromolar ADAMTS-5 potency and demonstrate SAR trends for both the aryl group and thioxothiazolidinone zinc chelator. This series of compounds represents steps toward a metalloprotease inhibitor as a disease-modifying osteoarthritis drug.

Osteoarthritis (OA), the most common of musculoskeletal diseases, is rapidly becoming a significant medical and financial burden upon the world.[1] This debilitating disease is characterized by progressive articular cartilage breakdown leading to chronic pain, inflammation, and reduced mobility in the affected joint. More than 20 million Americans suffer the significant reduction in quality of life associated with OA with this number expected to grow with the aging population. Current treatments provide only symptomatic relief (NSAIDs, intra-articular injections of hyaluronic acid conjugates, and surgical joint replacement) with no therapy available to halt and/or reverse the progression of this disease.[2]

Aggrecan, a multidomain proteoglycan, is a major component of cartilage that interacts with hyaluronic acid and link proteins to provide compressive resistance to articular cartilage. An early and important characteristic of the osteoarthritis process is loss of aggrecan from the extracellular matrix with increased loss of aggrecan fragments via aggrecanase proteolysis activity.[3] If this degradation is not halted or reversed the cartilage will be subject to degradation by additional metalloproteinases resulting in irreversible damage to the joint.

Aggrecanases are members of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family of zinc metalloproteases. Aggrecanase-2 has been designated ADAMTS-5 and shown to be responsible for cleavage of aggrecan at the physiologically relevant Glu373-Ala374 peptide bond.[3] ADAMTS-5 knock-out mice have been shown to have significantly reduced OA severity in a surgically induced instability model.[4] ADAMTS-5 has also been shown to be the major ADAMTS in a mouse model of inflammatory arthritis.[5] The inhibition of ADAMTS-5 may therefore protect cartilage from damage and provide the first potential therapy to halt and/or reverse the progression of OA.

In our ADAMTS-5 HTS screening efforts several inhibitor scaffolds possessing zinc-chelating groups were identified.[6] These included, among others, hydroxamic acids (I), carboxylic acids (II), hydantoins (III), and thioxothiazolidinones (IV) (Fig. 1). The significant prior

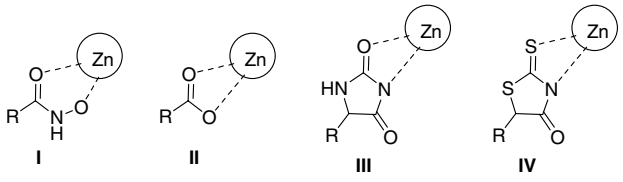

Figure 1. Zinc binding modes associated with various known bidentate zinc chelators: (I) hydroxamic acid, (II) carboxylic acid, (III) hydantoin, and (IV) thioxothiazolidinone.

Keywords: Aggrecanase; ADAMTS-5; Thioxothiazolidinone; Metalloprotease inhibitor; Zinc chelator.
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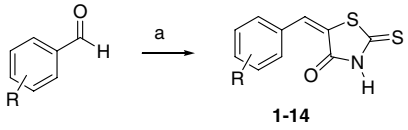
art describing hydroxamic acid, carboxylic acid, and hydantoin-based metalloprotease inhibitors[7] led us to pursue a series of less-explored thioxothiazolidinones as potential inhibitors of the zinc metalloprotease ADAMTS-5. Whereas several general aggrecanase inhibitors have been disclosed, to our knowledge, this class of compounds[8] represents the first disclosure of ADAMTS-5 inhibitors.[9–12]

Initial efforts to explore the thioxothiazolidinone scaffold began with the synthesis outlined in Scheme 1.[13] Knoevenagel condensation[14] of various substituted benzaldehydes and 2-thioxo-thiazolidin-4-one in the presence of β-alanine in refluxing acetic acid[15] afforded the desired target thioxothiazolidinone compounds **1**–**14** (Table 1).

From the synthesis of the initial array it was apparent that a benzyloxy substituted aryl thioxothiazolidinone **1**–**5** greatly enhanced activity as analogs bearing smaller substituents showed no activity against ADAMTS-5 (data not shown). Whereas compound **1** possessing only one meta-benzyloxy group showed modest activity, this potency was increased 10-fold with the incorporation of a second benzyloxy group in either the *p*-position **2** or other meta-position **3**. To reduce the molecular weight, the replacement of one of the bis(benzyloxy) groups of **2** with a methoxy group was investigated with synthesis of **4**. This replacement leads to a significant drop in activity ($IC_{50} > 22$ μM), but upon further substitution of the remaining *p*-benzyloxy analogs with micromolar activity **5** and **6** were identified.



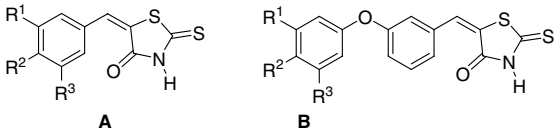
Scheme 1. Reagents and condition: (a) 2-Thioxo-thiazolidin-4-one, β-alanine, AcOH, reflux.

To follow up the initial SAR trends observed with the benzyloxy analogs, a series of *m*-biphenyl ether thioxothiazolidinones **7**–**14** were synthesized. Again various substitutions on the biphenyl ether were investigated and shown to play a significant role in developing active analogs. Compounds with no substitution on the biphenyl ether were found to be inactive in **7**, whereas incorporation of *p*-chloro group in **8** provided a low micromolar analog. Subsequent substitution of the *p*-chloro for either a *p*-methoxy in **9** or *p*-methyl in **10** afforded a 3-fold reduction in potency. However increasing the steric bulk of the *p*-substituent to a *t*-butyl in **11** led to an increase of potency. Keeping the *p*-chloro and adding another *m*-chloro resulted in a small increase in potency for **12**. Switching from a 3,4-di-chloro substitution to a 3,5-di-chloro substitution in **13** also provided a modest increase in potency. Moving the electron withdrawing substitution around the ring proved unfruitful except for the *m*-trifluoromethyl analog **14** that maintained low micromolar activity.

To explore the importance of the thioxothiazolidinone-binding motif, various other chelators were investigated. This substitution was explored for the *m*-biphenyl ether series of thioxothiazolidinones. Knoevenagel condensation of the desired *m*-biphenyl ether benzaldehydes with either thiazolidine-2,4-dione, imidazolidine-2,4-dione, or 1-methyl-imidazolidine-2,4-dione in the presence of β-alanine in refluxing acetic acid afforded the desired targets **15**–**22** as shown in Scheme 2.

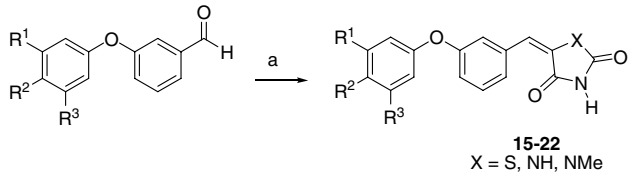
From the focused array synthesis (Table 2) the importance of the thioxothiazolidinone chelator is evident. Simply replacing the thioxothiazolidinone sulfur to an oxygen **15**–**18** led to at least a 3-fold decrease in activity (compared to **8** and **11**–**13**). Similarly the

Table 1. ADAMTS-5 inhibition for thioxothiazolidinones **1**–**14**



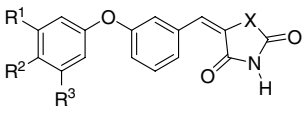
Compound	R^1	R^2	R^3	Scaffold	ADAMTS-5 IC_{50}[a] (μM)
1	O–Bn			A	20.1
2	O–Bn	O–Bn		A	1.8
3	O–Bn		O–Bn	A	1.4
4	OMe	O–Bn		A	>22
5	OMe	*p*-Cl O–Bn		A	3.5
6	OMe	*p*-OMe O–Bn		A	11.6
7	H	H	H	B	>22
8		Cl		B	4.9
9		OMe		B	16.2
10		Me		B	14.8
11		*t*-Bu		B	1.7
12	Cl	Cl		B	3.2
13	Cl		Cl	B	2.5
14	CF3			B	5.3

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



Scheme 2. Reagents and condition: (a) thiazolidine-2,4-dione, imidazolidine-2,4-dione, or 1-methyl-imidazolidine-2,4-dione, β-alanine, AcOH, reflux.

Table 2. ADAMTS-5 inhibition for substituted *m*-biphenyl ethers thiazolidinedione, imidazolidinedione, or 1-methyl-imidazolidinedione analogs **15**–**22**

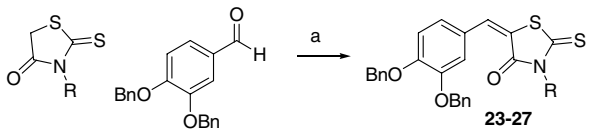


Compound	R^1	R^2	R^3	X	ADAMTS-5 IC_{50}[a] (μM)
15		Cl		S	>22
16	Cl	Cl		S	10.0
17	Cl		Cl	S	9.2
18		*t*-Bu		S	6.8
19		Cl		NH	>67
20	Cl		Cl	NH	>67
21		*t*-Bu		NH	>67
22		Cl		NMe	>22

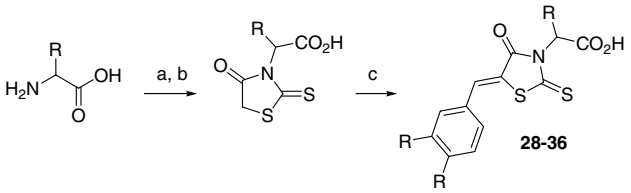
[a] Values are means of two experiments, standard deviations are ±10%.

3,4-di-chloro and 3,5-di-chloro substituted analogs **16** and **17** were more potent than the single *p*-chloro analog **15** (IC_{50} > 22 μM). The potency could also be increased as the *p*-*t*-Bu analog **18** was more potent than the *p*-chloro analog (IC_{50} > 22 μM). The substituted *m*-biphenyl ether imidazolidinedione analogs, replacing the sulfur with nitrogen, were even worse, as none of the substituted imidazolidinediones **19**–**21** or substituted N–Me imidazolidinedione **22** showed any ADAMTS-5 inhibition.

To further explore the importance of the thioxothiazolidinone chelator, an array of N-substituted thioxothiazolidinone analogs were synthesized. Using previously identified conditions, a variety of commercially available N-substituted 2-thioxo-thiazolidin-4-ones were condensed with 3,4 dibenzyloxy benzaldehyde to afford the desired target thioxothiazolidinone compounds **23**–**27** (Scheme 3). The desired *N*-acid substituted thioxothiazolidinones were synthesized (Scheme 4) via reaction of the desired amino acid and carbon disulfide under basic conditions followed by ring closure with chloroacetic acid.[16] Knoevenagel condensation of the thioxothiazolidinone with substituted benzaldehydes in



Scheme 3. Reagents and condition: (a) β-alanine, AcOH, reflux.

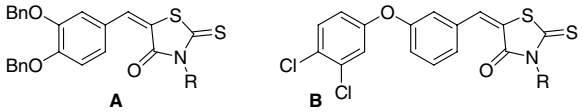


Scheme 4. Reagents and condition: (a) CS2, KOH, H_2O; (b) Na_2CO_3, $ClEtCO_2H$; (c) benzaldehyde derivative, β-alanine, AcOH, reflux.

the presence of β-alanine in refluxing acetic acid afforded the desired target *N*-acid substituted thioxothiazolidinones **28**–**36**.

From this focused array of N-substituted thioxothiazolidinone analogs the importance of the chelator was once again evident (Table 3). Substitution of the dibenzyloxy *N*-thioxothiazolidinone analogs with alkyl, phenyl, benzyl or phenethyl groups **23**–**27** resulted in a complete loss of activity. Interestingly substitution of the NH with an additional acid via glycine **28** afforded a compound of modest activity that could be improved slightly via chain extension to the β-glycine amino acid analog **29**. Substituted amino acid derivatives **30**–**32** provided a further increase of potency with the Ph-Gly analog **30** identified with sub-micromolar potency against ADAMTS-5. The activity trend was also evident in the biphenyl-ether *N*-thioxothiazolidinone analogs **33**–**36.** In this scaffold substitution of the NH with an additional acid via glycine afforded compound **33** of modest activity that could be significantly improved via substituted amino acid derivatives **34**–**36**.

Table 3. ADAMTS-5 inhibition for N-substituted thioxothiazolidinone analogs **2** and **23**–**36**



Compound	R	Scaffold	ADAMTS-5 IC_{50}[a] (μM)
2	H	A	1.8
23	Me	A	>67
24	Et	A	>67
25	Ph	A	>67
26	Bn	A	>67
27	Phenethyl	A	>67
28	CH_2CO_2H	A	6.7
29	$CH_2CH_2CO_2H$	A	5.3
30	$CH(Ph)CO_2H$	A	0.9
31	CH(*R*-*i*-Pr)CO2H	A	1.1
32	CH(*R*-*i*-Bn)CO2H	A	1.3
12	H	B	3.2
33	CH_2CO_2H	B	11.1
34	$CH(Ph)CO_2H$	B	1.3
35	$CH(R\text{-}i\text{-}Pr)CO_2H$	B	1.8
36	$CH(R\text{-}i\text{-}Bn)CO_2H$	B	1.6

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

In summary, we have presented a series of aryl thioxothiazolidinones with varying activities as inhibitors of ADAMTS-5. This series of compounds has tractable SAR for both the thioxothiazolidinone aryl substituents and zinc chelator. The continued development of ADAMTS-5 inhibitors using both zinc chelating and non-chelating scaffolds is currently ongoing and will be reported in due course.

Acknowledgments

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