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5′-Phenyl-3′H-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-one inhibitors of ADAMTS-5 (Aggrecanase-2)

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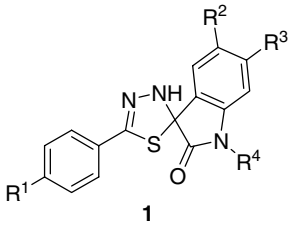
Abstract—5′-Phenyl-3′*H*-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-one inhibitors of ADAMTS-5 (Aggrecanase-2) have been prepared via commercially available starting materials. Selected compounds **23**, **33**–**35** show sub-micromolar ADAMTS-5 potency and strong SAR trends with selectivity over the related metalloproteases ADAMTS-4 (Aggrecanase-1), MMP12, and MMP13. This series of compounds represents progress toward a selective ADAMTS-5 inhibitor as a disease modifying osteoarthritis drug.

Osteoarthritis (OA) is a debilitating disease caused by degradation of aggrecan and collagen in the articular cartilage matrix leading to progressive and chronic joint pain and inflammation. Aggrecan is a multidomain proteoglycan that provides the elasticity and compressive resistance to the articular cartilage which is lost in the initial phases of OA. This loss of aggrecan fragments via proteolysis is attributable to aggrecanase activity.[1] If this degradation is not halted or reversed, the cartilage will be subject to further breakdown by additional metalloproteases resulting in irreversible damage to the joint. While current OA treatments provide only symptomatic relief (NSAIDs, intra-articular injections of hyaluronic acid conjugates, and surgical joint replacement), there is no therapy available to halt and/or reverse the progression of this debilitating disease.[2]

Aggrecanases are members of the ADAMTS (A Disintegrin And Metalloprotease with Thrombospondin Motifs) family of zinc metalloproteases. Both ADAMTS-4 (Aggrecanase-1) and ADAMTS-5 (Aggrecanase-2) have been shown to cleave aggrecan at the physiologically relevant Glu373-Ala374 peptide bond.[1] Recent work has demonstrated significantly reduced OA severity for ADAMTS-5 knockout mice in a surgically induced instability model.[3] ADAMTS-5 has also been shown to be the major ADAMTS in a mouse model of inflammatory arthritis.[4] Thus, 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.

Recently we reported a series of rhodanine-based inhibitors of ADAMTS-5.[5,6] In this letter, we report our initial studies on a series of 5′-phenyl-3′H-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-one ADAMTS-5 inhibitors **1** found via high throughput screening[7] and show their potential utility as selective ADAMTS-5 therapeutics to treat OA. While other general aggrecanase inhibitors have been disclosed,[8–11] to our knowledge the compounds presented herein represent the first disclosure of submicromolar, selective ADAMTS-5 inhibitors.



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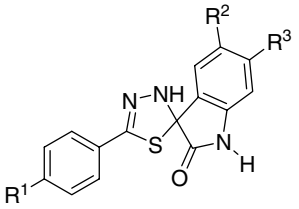
Spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-one (thiadiazole) ADAMTS-5 inhibitors **1** are prepared according to Scheme 1.[12] Thus 2-(arylcarbonothioylthio)acetic acids **3** are synthesized by quenching the corresponding Grignard reagent of **2** with carbon disulfide and subsequent alkylation with chloroacetic acid.[13] Arylthiohydrazides **4** are prepared by treatment of **3** with hydrazine under basic conditions.[14] The non-commercially available isatins **6** and **7** are prepared using the Sandmeyer isatin synthesis (treating anilines **5** with 2,2,2-trichloroacetaldehyde monohydrate, hydroxylamine hydrochloride, and concentrated sulfuric acid to form **6**).[15] Alkylation of **6** is accomplished using NaH and alkyl/benzyl bromides in THF.[16] Conversely, arylation of **6** can be carried out using Cu and aryl boronic acids.[17] The synthesis of thiadiazoles **1** is completed by condensing arylthiohydrazides **4** with isatins **6** or **7** in EtOH at 40 °C.

ADAMTS-5 inhibition data for N–H thiadiazoles (**8**–**25**) is shown in Table 1. Compounds **8**–**12** demonstrate that isatin substitution at the R^2 position plays a key role in ADAMTS-5 activity. When $R^1 = R^2 = R^3 = H$ (**8**), weak ADAMTS-5 activity is seen. The corresponding $R^2 = Br$ analog **9** shows a nearly twofold increase in ADAMTS-5 potency ($IC_{50} = 11.1$ μM). Other R^2 substitutions also demonstrate enhanced ADAMTS-5 activity ($R^2 = Me$ **10**: $IC_{50} = 11.1$ μM; $R^2 = Et$ **11**: $IC_{50} = 9.6$ μM). Further improvement is demonstrated with a OCF_3 group in **12** ($IC_{50} = 7.2$ μM).

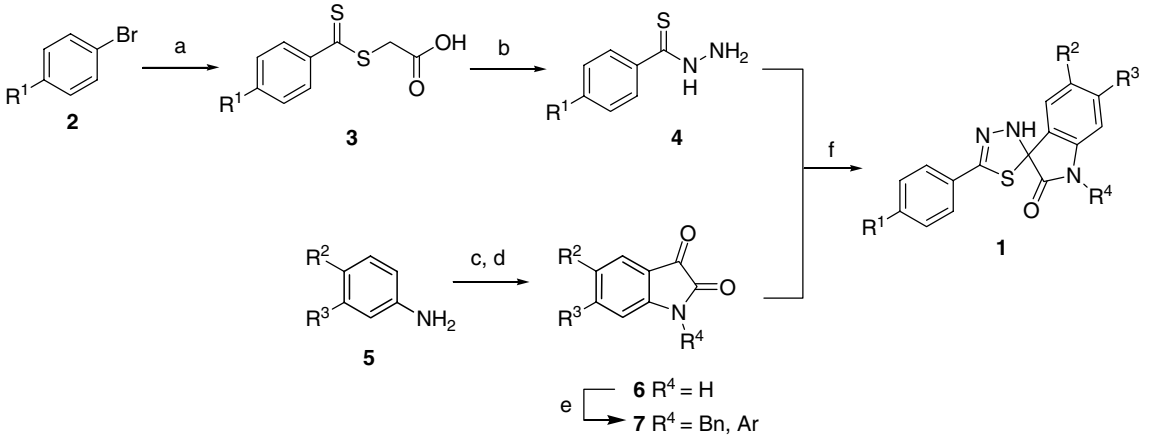
A series of R^1 substituted thiadiazoles **13**–**25** continue to show improved ADAMTS-5 potency. The $R^1 = Me$ $R^2 = R^3 = H$ analog **13** leads to a sixfold potency increase over **8** ($IC_{50} = 3.7$ μM vs 24.2 μM). Analogs where $R^2 = Me$ (**14**: $IC_{50} = 3.0$ μM) or $R^2 = Et$ (**15**: $IC_{50} = 1.9$ μM) show similar or slightly greater potency. Incorporation of a $R^3 = n$-Pr substituent shows similar low micromolar ADAMTS-5 potency (**16**: $IC_{50} = 1.4$ μM). Thiadiazole analogs where $R^1 = OMe$ **17**–**26** were also pursued to further expand the scope of the R^1 substituent. The $R^1 = OMe$, $R^2 = R^3 = H$ analog **17** shows a twofold potency increase over **8** ($IC_{50} = 13.4$ μM vs 24.2 μM). As shown above, R^2 substitution ($R^2 = Me$ **18**: $IC_{50} = 2.5$ μM; $R^2 = Et$ **19**: $IC_{50} = 1.4$ μM) produces analogs with increased ADAMTS-5 potency. Analogs where $R^2 = Cl$ (**20**: $IC_{50} = 2.0$ μM), $R^2 = Br$ (**21**: $IC_{50} = 2.4$ μM), and $R^2 = NO_2$ (**22**: $IC_{50} = 4.4$ μM) also show low micromolar activity. Substitution of the R^3 position affords analog **23** ($R^1 = OMe$, $R^2 = H$, $R^3 = n$-Pr) that demonstrates sub-micromolar ADAMTS-5 inhibition ($IC_{50} = 0.78$ μM). Further investigation of the R^3 position affords analogs **24** ($R^3 = Me$: $IC_{50} = 1.4$ μM) and **25** ($R^3 = Cl$: $IC_{50} = 1.2$ μM) with minimal loss in ADAMTS-5 activity.

Table 1. In vitro ADAMTS-5 inhibition data for N–H 5′-phenyl-3′H-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-ones



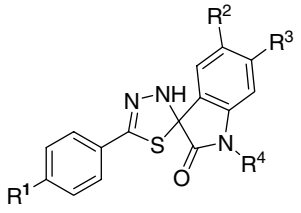
Compound	R^1	R^2	R^3	ADAMTS-5 IC_{50}[a] (μM)
8	H	H	H	24.2
9	H	Br	H	13.6
10	H	Me	H	11.1
11	H	Et	H	9.6
12	H	OCF_3	H	7.2
13	Me	H	H	3.7
14	Me	Me	H	3.0
15	Me	Et	H	1.9
16	Me	H	*n*-Pr	1.4
17	OMe	H	H	13.4
18	OMe	Me	H	2.5
19	OMe	Et	H	1.4
20	OMe	Cl	H	2.0
21	OMe	Br	H	2.4
22	OMe	NO_2	H	4.4
23	OMe	H	*n*-Pr	0.78
24	OMe	H	Me	1.4
25	OMe	H	Cl	1.2

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



Scheme 1. Reagents and conditions: (a) i.Mg, Et_2O; ii.CS_2, Et_2O then chloroacetic acid; (b) H_2NNH_2, 1 N NaOH; (c) chloral hydrate, Na_2SO_4, H_2NOH–HCl, H_2O, HCl; (d) H_2SO_4; (e) BnBr, NaH, THF or $ArB(OH)_2$, $Cu(OAc)_2$, pyridine, CH_2Cl_2; (f) EtOH, 40 °C.

Table 2. In vitro ADAMTS-5 inhibition data for R^4 N-substituted 5′-phenyl-3′H-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-ones



Compound	R^1	R^2	R^3	R^4	ADAM TS-5 IC_{50}[a] (μM)
26	H	H	H	Me	24.1
27	H	H	H	*i*-Pr	3.1
28	H	H	H	Ph	2.6
29	Me	H	H	Me	4.6
30	Me	Me	H	(*o*-Me)Ph	1.2
31	Me	Me	H	Bn	0.61
32	OMe	H	H	Me	7.5
33	OMe	H	H	Bn	0.87
34	OMe	Me	H	Bn	0.64
35	OMe	Me	H	(*o*-Me)Ph	0.90
36	OMe	Me	H	(*p*-Cl)Ph	1.5
37	OMe	Me	*n*-Pr	Bn	0.95

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

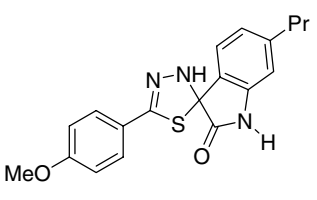
ADAMTS-5 inhibition data for the R^4 N-substituted thiadiazoles **26**–**37** are shown in Table 2. While incorporation of the R^4 = Me group in **26** affords no potency increase over the R^4 = H analog **8** (**24**: IC50 = 24.1 μM vs **8**: IC_{50} = 24.2 μM), the R^4 = *i*-Pr analog **27** shows nearly a 10-fold increase in potency (IC_{50} = 3.1 μM). The R^4 = Ph analog **28** shows similar low micromolar ADAMTS-5 potency (IC_{50} = 2.6 μM). Incorporation of an R^1 = Me into the R^4 substituted thiadiazole analogs **29**–**31** shows continued potency improvement over the R^1 = H analogs. While the R^1 = Me, R^4 = Me analog **29** does not give a potency increase over **13** (**13**: IC_{50} = 3.7 μM vs **29**: IC_{50} = 4.6 μM), combining R^1, R^2 = Me and R^4 = (*o*-Me)Ph substituents affords compound **30** (IC_{50} = 1.2 μM) with low micromolar activity. The R^1 = R^2 = Me, R^4 = benzyl analog **31** gives sub-micromolar ADAMTS-5 inhibition (IC_{50} = 0.61 μM).

The most consistently potent series of thiadiazoles are obtained when R^1 = OMe and R^4 is substituted with either an aryl or benzyl group **33**–**37** (Table 2). While the R^1 = OMe, R^4 = Me analog **32** possesses moderate ADAMTS-5 inhibition (IC_{50} = 7.5 μM), the R^1 = OMe, R^4 = benzyl analog shows sub-micromolar activity (**33**: IC_{50} = 0.87 μM). Adding an R^2 = Me substituent to **33** gives a more potent compound (**34**: IC_{50} = 0.64 μM). Other variations at R^4 when R^1 = OMe and R^2 = Me also give active compounds (R^4 = (*o*-Me)Ph **35**: IC_{50} = 0.90 μM; R^4 = (*p*-Cl)Ph **36**: IC_{50} = 1.5 μM). Attempting to combine optimal substituents in all four positions (R^1 = OMe; R^2 = Me; R^3 = *n*-Pr; R^4 = Bn) affords the sub-micromolar ADAMTS-5 inhibitor **37** (IC_{50} = 0.95 μM).

Given the suggestive mouse ADAMTS-5 knockout data discussed above,[3,4] we assessed ADAMTS-5/ADAMTS-4 selectivity for several of the more potent thiadiazoles presented in this manuscript (Table 3). Analogs **23** and **33**–**35** show high levels of selectivity over ADAMTS-4. Only compound **35** shows any appreciable activity against ADAMTS-4, but still maintains 11-fold selectivity for ADAMTS-5. To follow up the observed ADAMTS selectivity we also assessed metalloprotease selectivity against both MMP-12 and MMP-13. Whereas none of the analogs showed any activity against MMP-13, compounds **23** and **33** show modest activity against MMP-12 (IC_{50} = 1.7 and 5.6 μM, respectively). Compound **34** appears to be very selective over ADAMTS-4, MMP12, and MMP13.

In conclusion, we have presented a series of 5′-phenyl-3′H-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-one ADAMTS-5 inhibitors. This series of compounds has tractable SAR with several analogs of sub-micromolar potency demonstrating functional selectivity for ADAMTS-5 over ADAMTS-4, MMP-12, and MMP-13. The continued development of selective ADAMTS-5 inhibitors is currently ongoing and will be reported in due course.

Table 3. In vitro selectivity data for 5′-phenyl-3′H-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-ones



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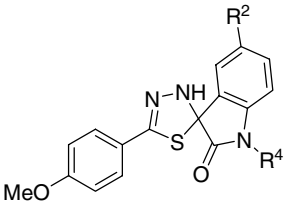

33: R^2 = H, R^4 = Bn
34: R^2 = Me, R^4 = Bn
35: R^2 = Me, R^4 = *o*-Me Ph

Compound	ADAMTS-5 IC_{50}[a] (μM)	ADAMTS-4 IC_{50}[a] (μM)	MMP13 IC_{50}[a] (μM)	MMP12 IC_{50}[a] (μM)
23	0.78	>22	>22	1.7
33	0.87	>22	>100	5.6
34	0.64	>22	>100	>22
35	0.90	10.5	>100	>22

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

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