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5-((1*H*-Pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-one inhibitors of ADAMTS-5

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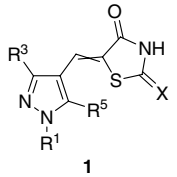
Abstract—A series of 5-((1*H*-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-one inhibitors of ADAMTS-5 (aggrecanase-2) is described. These compounds show μM functional inhibition of ADAMTS-5, and represent a new class of agents with the potential of inhibiting degradation of aggrecan, a major component of cartilage which is lost in osteoporosis. Compound **12** is noteworthy in that it has an ADAMTS-5 IC_{50}: 1.1 μM and shows >40-fold functional selectivity over ADAMTS-4 (aggrecanase-1).

Osteoarthritis (OA), a disease where aggrecan and collagen in the articular cartilage matrix are slowly degraded, often leads to debilitating chronic joint pain and inflammation. While collagen is the major structural component of aggrecan and provides strength to the tissue, aggrecan is responsible for the tissue's flexible and elastic nature. Aggrecan is lost in the initial phases of the disease, while collagen is lost in the disease's later stages. Thus an agent that would inhibit aggrecan breakdown could be a disease-modifying osteoarthritis treatment as opposed to treatments that only provide symptomatic relief (e.g., acetaminophen, COX-2 inhibitors, NSAIDs or intraarticular steroids).[1]

Aggrecan is catabolized by the proteolytic enzymes ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2), two members of the "A Disintegrin And Metalloproteinase with ThromboSpondinmotifs" (ADAMTS) family of proteins. These enzymes cleave the aggrecan interglobular domain at the Glu373–Ala374 peptide bond.[2] Both enzymes are 1000-fold more efficient at cleaving aggrecan than any other enzymes although it is not known which enzyme is the primary source of aggrecanase activity in cartilage. Studies suggest that ADAMTS-5 could be the major player as ADAMTS-5 knockout mice show reduced OA severity after surgical induction of joint instability, whereas the corresponding ADAMTS-4 knockouts show a normal OA progression.[3] ADAMTS-5 is also known to be the major ADAMTS in mouse cartilage and in a mouse model of inflammatory arthritis.[4] In this letter, we report our initial studies on a series of 5-((1*H*-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-one ADAMTS-5 inhibitors **1** found from high throughput screening and show that they are useful as potentially selective ADAMTS-5 therapeutics to treat OA.[5] To our knowledge, these compounds represent the first disclosure of inhibitors of ADAMTS-5. Examples of general aggrecanase inhibitors have been previously disclosed.[6–9]



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Thioxothiazolidin-4-one ADAMTS-5 inhibitors **1** are prepared according to Scheme 1.[10] 5-Hydroxypyrazoles **3** are synthesized by combining acetylacetic esters **2** with hydrazines.[11]

Keywords: ADAMTS-5; Aggrecanase-2; Thioxothiazolidin-4-ones; Osteoporosis.
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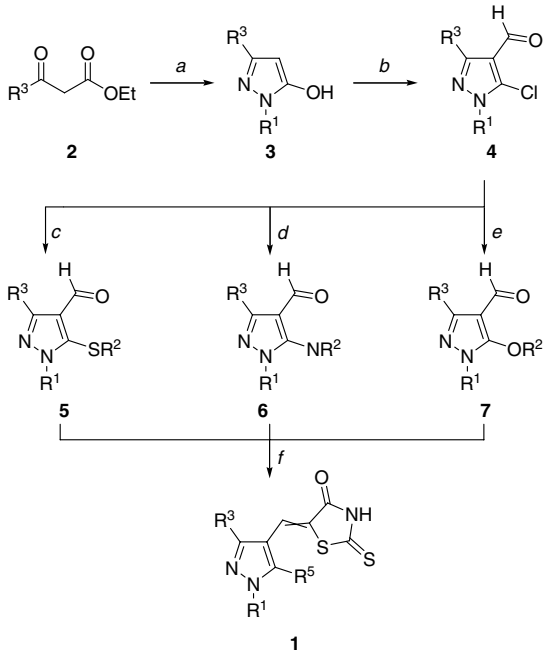


Scheme 1. Reagents and conditions: (a) R^1NHNH_2, H_2SO_4, EtOH, reflux, 17 h; (b) $POCl_3$, DMF, 80 °C, 12 h; (c) R^2SH, Et_3N, DMSO, 40 °C, 12 h; (d) R^2NH, Et_3N, DMF, 100 °C, 12 h; (e) *t*-BuOK, DMF, 23 °C, 12 h; (f) rhodanine, β-alanine, AcOH, 110 °C, 12 h.

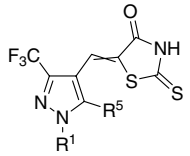
The corresponding 4-formyl-5-chloropyrazoles **3** are prepared using $POCl_3$ in hot DMF.[11] The 5-chloro moiety can be displaced with the following nucleophiles: thiols to produce **5** (Et_3N, DMSO, 40 °C), amines to produce **6** (Et_3N, DMF, 100 °C), and alcohols to produce **7** (*t*-BuOK, DMF, 23 °C). The corresponding thioxothiazolidin-4-ones **1** are produced using rhodanine and β-alanine in hot HOAc.[12] ^1H NMR spectroscopy shows that thioxothiazolidin-4-ones **1** exist in a range from nearly pure Z isomers (as drawn in Scheme 1) to 80:20 Z:E isomeric mixtures.

ADAMTS-5 activity was determined using a fluorescence resonance energy transfer (FRET) assay using a QF-peptide containing the aggrecanase cleavage site. Binding to target studies were conducted using fluorescence polarization experiments using a xanthene-ADAMTS-5 hydroxamic acid inhibitor. Several compounds were analyzed for ADAMTS-4 activity using the same QF-peptide used for the ADAMTS-5 activity studies.

Biological data of 3-CF_3-1*H*-pyrazole thioxothiazolidin-4-ones are shown in Table 1. When R^1 = Me and R^5 = SPh (**9**) or SCH_2Ph (**10**), weak ADAMTS-5 activity is seen. Replacing the Ph in **10** with the isosteric 2-thiophene in **11** produces similarly weak ADAMTS-5 potency. However the addition of a *p*-Cl moiety to **11** produces **12**, a compound which displays micromolar ADAMTS-5 inhibitory activity (IC_{50}: 1.1 μM). Movement of the *p*-Cl moiety in **12** to the *o*-position affords a compound with ~20-fold less activity (**13**: IC_{50}, 23.2 μM). The *p*-Cl moiety in **12** may be replaced by a *p*-*t*-Bu in **14** (IC_{50}, 2.2 μM), but not a *p*-OMe as in **15**. Removing the benzyl CH_2 methylene in **12** gives the *p*-Cl phenyl analog **16** which has reduced ADAMTS-5 inhibition. In addition to R^1 = Me, R^1 can be Ph (**17**: IC_{50}, 2.5 μM) and cyclohexyl (**18**: IC_{50}, 4.2 μM). The SCH_2 moiety in **12** and **17** can be replaced by a piperazine to give compounds with similar ADAMTS-5 activity (**19**: IC_{50}, 9.0 μM and **20**: IC_{50}, 2.1 μM).

The 3-CF_3 moiety in **9**–**20** can be modified to produce active compounds if other pyrazole substituents are modified as well (Table 2). The 1,3-diphenyl-1*H*-pyrazole analog **21** shows ADAMTS-5 inhibition. This contrasts with the R^1 = Me, R^3 = 3-CF_3 analog **11**. The 2-thiophene moiety in **21** may be changed to a furan

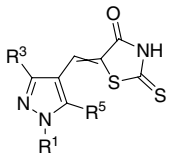
Table 1. In vitro ADAMTS-5 inhibition data for 5-((3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-ones



Compound	R^1	R^5	% Inhib. @ 20[a] (μM)	IC_{50}[a] (μM)
9	Me	SPh	28	
10	Me	SCH_2Ph	44	
11	Me	SCH_2(2-thiophene)	40	
12	Me	SCH_2Ph(4-Cl)		1.1
13	Me	SCH_2Ph(2-Cl)		23.2
14	Me	SCH_2Ph(4-*t*-Bu)		2.2
15	Me	SCH_2Ph(4-OMe)	53	
16	Me	SPh(4-Cl)	47	
17	Ph	SCH_2Ph(4-Cl)		2.5
18	CyH	SCH_2Ph(4-Cl)		4.2
19	Me	PiperazinePh(4-Cl)		9.0
20	Ph	PiperazinePh(4-Cl)		2.1

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

Table 2. In vitro aggrecanase-2 inhibition data for 5-((1*H*-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-ones



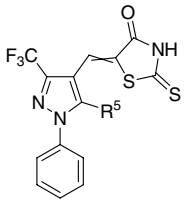
Compound	R^1	R^3	R^5	% Inhib. @ 20[a] (μM)	IC_{50}[a] (μM)
21	Ph	Ph	SCH_2(2-thiophene)		3.7
22	Ph	Ph	SCH_2(2-furan)		6.7
23	CyH	Ph	SCH_2(2-furan)		4.6
24	Ph	CF_3	SCH_2(2-furan)	42	
25	Ph	CF_3	SCH_2Ph		16.9
26	Me	Ph	SCH_2(2-furan)		14.1
27	Me	Ph	SPh(4-Me)		8.3
28	CyH	Ph	Piperazine(2-pyrimidine)		8.0
29	CyH	CF_3	Piperazine(2-pyrimidine)		11.7
30	Ph	Ph	Piperazine(Me)		4.6
31	Ph	CF_3	Piperazine(Me)	45	

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

22 with little change in activity, while the corresponding compound where R^1 = CyH **23** has similar ADAMTS-5 potency. Interestingly, **24** where $R^3 = CF_3$ does not show good ADAMTS-5 activity (compare with **22**), but **25** shows moderate activity where $R^5 = SCH_2Ph$ (compare with **10**). The R^1 = Me and R^3 = Ph substitution pattern gives moderately active compounds (**26** and **27**) and piperazinyl moieties can be incorporated at R^5 (**28**–**31**) as previously shown above (Table 1).

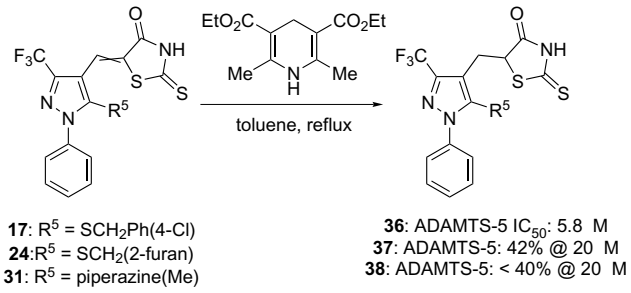
The sulfur at the R^5 position of the pyrazole can be substituted with an oxygen substituent provided that an extra CH_2 group is added to the linker (Table 3). Thus, **32** possesses ADAMTS-5 activity (IC_{50}, 4.2 μM; compare with **17**). The corresponding *p*-F phenyl compound **33** also shows ADAMTS-5 inhibition (IC_{50}, 7.1 μM) as does the 2-thiophene analog **34** (IC_{50}, 9.1 μM). Similarly to **15**, the *p*-OMe-phenyl analog **35** shows reduced ADAMTS-5 activity again showing the importance of the *p*-substituent on phenyl group of the R^5 side chain (Table 4).

Table 3. In vitro ADAMTS-5 inhibition data for 5-((5-alkoxy-1*H*-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-ones



Compound	R^5	% Inhib. @ 20[a] (μM)	IC_{50}[a] (μM)
32	$O(CH_2)_2Ph$(4-Cl)		4.2
33	$O(CH_2)_2Ph$(4-F)		7.1
34	$O(CH_2)_2$(2-thiophene)		9.1
35	$O(CH_2)_2Ph$(4-OMe)	31	

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



17: $R^5 = SCH_2Ph$(4-Cl)
24: $R^5 = SCH_2$(2-furan)
31: R^5 = piperazine(Me)

36: ADAMTS-5 IC_{50}: 5.8 M
37: ADAMTS-5: 42% @ 20 M
38: ADAMTS-5: < 40% @ 20 M

Reduction of the double bond attached to the thioxothiazolidin-4-one headpiece using the Hantzsch ester[13] produces compounds with reduced ADAMTS-5 activity. Thus while **17** only loses 2-fold ADAMTS-5 potency on double bond reduction (**36**: IC_{50}, 5.8 μM), the case is worse for **24** and **31**; both compounds lose substantial potency when converted to their single bond analogs **37** and **38**.

Given the previously cited suggestive mouse ADAMTS-5 knockout data,[3] we have begun to look at ADAMTS-5/ADAMTS-4 selectivity for a few of the thioxothiazolidin-4-ones presented in this manuscript using an ADAMTS-4 FRET assay. Two of the more potent ADAMTS-5 inhibitors show selectivity over ADAMTS-4. The *p*-Cl-Ph analog **12** shows good functional selectivity over ADAMTS-4 (ADAMTS-5 IC_{50}, 1.1 μM; ADAMTS-4 IC_{50}, 44 μM), something that is not surprising since ADAMTS-5 has approximately 40% sequence homology to ADAMTS-4 overall and

Table 4. In vitro ADAMTS-5 and ADAMTS-4 inhibition data for selected 5-((1*H*-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-ones

Compound	ADAMTS-5 IC_{50}[a] (μM)	ADAMTS-4 IC_{50}[a] (μM)
12	1.1	44
14	2.2	7.5

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

50% active site sequence similarity.[14] The closely related *t*-Bu-Ph analog **14** is less functionally selective (ADAMTS-5 IC_{50}, 2.2 μM; ADAMTS-4 IC_{50}, 7.5 μM). In addition, both **12** and **14** successfully displaced a xanthene-ADAMTS-5 hydroxamic acid inhibitor from the ADAMTS-5 protein showing that their inhibitory activity involved binding to ADAMTS-5.[15]

Thus, we have presented a series of 5-((1*H*-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-one ADAMTS-5 inhibitors. This series of compounds has tractable SAR and several analogs show modest functional selectivity for ADAMTS-5 over ADAMTS-4. The continued development of selective ADAMTS-5 inhibitors is currently ongoing and will be reported in due course.

Acknowledgments

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