Bioorganic & Medicinal Chemistry Letters 23 (2013) 2111–2116

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and SAR investigation of thienosultam derivatives as ADAMTS-5 (aggrecanase-2) inhibitors

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ARTICLE INFO

Article history:
Received 27 November 2012
Revised 24 January 2013
Accepted 28 January 2013
Available online 9 February 2013

Keywords:
Osteoarthritis
Aggrecanase-2
ADAMTS-5
Thienosultam
S1′ pocket

ABSTRACT

A series of 1,1-dioxothieno[2,3-*d*]isothiazole (thienosultam) derivatives were designed and synthesized as novel ADAMTS-5 inhibitors for an investigation into a side chain of thienosultam for the S1′ pocket. The resulting compounds (**19** and **24**) show high ADAMTS-5 inhibition and other MMP selectivity, and these compounds show good oral bioavailability.

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Osteoarthritis (OA) is characterized by loss of joint cartilage and thickening of bone, and may lead to bony outgrowths. Joint cartilage functions to absorb impacts when pressure is applied to the joint or the joint is put into motion.[1] The pathogenesis of OA is poorly understood, but a major feature is the loss of the two most important components of cartilage extracellular matrix (ECM); Type II collagen and aggrecan.[2]

Aggrecan, a major glycoprotein that is expressed in cartilage tissues, is highly hydrated due to the presence of long, negatively charged polysaccharide chains, thus providing cartilage with its ability to resist compressive loads.[3] In addition, aggrecan has a protective effect against collagen degradation. Mechanistically, Type II collagen is exposed due to degradation of aggrecan, the exposed collagen becomes an easy target for enzymatic degradation by collagenase, a proteolytic enzyme specific for collagen chains, and absorptivity of the cartilage matrix is reduced by loss of aggrecan, resulting in reduced elasticity of the matrix and increased mechanical stress on the collagen chain, thus causing collagen chain breakdown.[4]

Aggrecanase is a protease that cleaves the aggrecan molecule. Specifically, it cleaves the peptide bonds between Glu373 and Ala374 that are present in the aggrecan interglobular domain.[5] Two types of aggrecanase, aggrecanase-1 and aggrecanase-2, are found in joint tissue. These enzymes belong to the family of A Disintegrin and Metalloprotease with ThromboSpondin motifs (ADAMTS), and are also referred to as ADAMTS-4 and ADAMTS-5, respectively.[6]

In 2005, Glasson et al.[7] and Stanton et al.[8] reported that in a mouse genetically modified to show resistance to cleavage in the interglobular domain of aggrecan, surgically induced OA showed reduced loss of aggrecan; thus, destruction of cartilage is inhibited and regeneration of cartilage is promoted. ADAMTS-5 may therefore be a suitable target for the development of novel drugs designed to inhibit cartilage destruction in OA.

To date, small-molecule ADAMTS-5 inhibitors have been described from hydroxamic acid analogues[9] or non-hydroxamic acid analogues,[10] no doubt due to the presence of a zinc atom at the active site of these enzymes. More recently, Shiozaki et al. reported a series of sulfonamide compounds bearing cyclopropane carboxylate to show high ADAMTS-5 inhibition and high selectivity over other Matrix Metalloproteases (MMPs).[11] To design a novel class of ADAMTS-5 inhibitors, we explored 1,1-dioxothieno[2,3-*d*]isothiazole (thienosultam) derivatives, and aimed to create an original structure and to give different profile compound by fixing the conformation of sulfonamide **1a** (Fig. 1).

Unrestricted compound **1a** was previously reported by O'Brien et al.[12] and Hu et al.[13] This compound has ADAMTS-5 inhibitory activity, but was low selectivity against ADAMTS-4, MMPs (Table 1).[14] MMP-2,[15] -3[16] and -14[17] knockout mice studies have been conducted reported, and inhibition of MMP-2, -3 and -14 may facilitate proteoglycan degradation. In addition, an investigation in ADAMTS-4 knockout mice confirmed that ADAMTS-4 is not responsible for aggrecan degradation in murine osteoarthritis.[18] Therefore, we reasoned that an ADAMTS-5 selective inhibitor would constitute an ideal OA therapeutic agent.

We prepared thienosultam **2** bearing a phenyl group, and found that **2** has moderate ADAMTS-5 inhibitory activity (Table 1).[19]

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http://dx.doi.org/10.1016/j.bmcl.2013.01.120

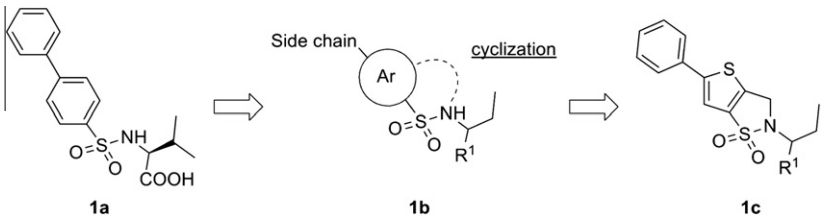
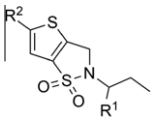
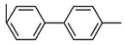
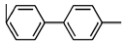


Figure 1. Strategy for the design 1,1-dioxothieno[2,3-*d*]isothiazole.

Table 1
Activity of selected sultams (**1**–**5**)



Compounds	R^1	R^2	ADAMTS-5	ADAMTS-4	MMP-2 (μM)	MMP-3 (μM)	MMP-14
1a			8.2 μM[a]	3.0 μM[b]	0.040[c]	0.038[c]	ND
2	–CONHOH		2.1 μM	1.1 μM	0.03	5.1	1.2 μM
3	–COOH		~3% (10 μM inh)	~1.7% (10 μM inh)	16	12	>30 μM
4	–CONHOH		~3% (10 μM inh)	~1.7% (10 μM inh)	0.3	20	<30 μM
5	–COOH		2.3 μM	~1.6% (10 μM inh)	1.8	12	<30 μM

ND = not determined.
[a] In house data.
[b] Assay details for the inhibition of ADAMTS-4 is described in Ref. 13.
[c] Assay details for the inhibition of MMP-2 is described in Ref. 12.

Unfortunately, **2** showed lower selectivity against both MMP-2 (30 nM) and MMP-14 (1.2 μM), while carboxylic acid **3** lost ADAMTS-5 inhibitory activity. However, thienosultam **4** bearing a biphenyl group showed reduced MMP-2 inhibitory activity, and carboxylic acid **5** had enhanced anti-ADAMTS-5 activity.

These results suggested that pocket size of the side chain occupying the S1′ pocket provided good selectivity over both MMP-2 and MMP-3.[20] The crystal structures of both ADAMTS-5 and MMP-2 have been reported.[21,22] X-ray structures co-crystallized with different ligands show that the S1′ pocket of ADAMTS-5 is deep, as a result of a compact S1′-loop. In contrast, the MMP-2 S1′ pocket is entirely enclosed in the S1′-loop, as if in a box, and longer groups led to decreased activity.

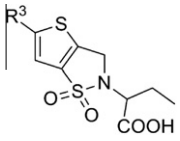
Based on this analysis, our initial investigation focused on the side chain of thienosultam for the S1′ pocket. We report on the enzyme activity in a series of analogs of **5** prepared in an effort to increase selectivity by taking advantage of differences between ADAMTS-5 and MMPs.

We evaluated various thienosultam linkers between the thienosultam and the terminal phenyl group. As shown in Table 2, the linkers for phenyl **5** and olefin-linkered **6** enhanced potency, but these linkers were nearly equipotent against ADAMTS-5 and MMP-2. The corresponding pyrazolyl **7** or piperazinyl **8** were not beneficial for ADAMTS-5 potency, while phenyl-olefin-linkered **9** enhanced ADAMTS-5 potency and showed 2.79-fold greater selectivity.

However, alkyne-linkered **10** showed significantly better selectivity over MMP-2 (7.5-fold). In addition, the terminal phenyl substituent (**5**) was indispensable for potency, as loss of MMP-2 potency was observed with the replacement of the 3-thiophene **11**. Thus, we next investigated the effects of terminal ring replacement of other heterocyclic systems.

Starting from compound **11**, modification of the terminal ring replacement was subsequently investigated (Table 3). We had prepared compounds bearing 2-thiophene **12**, 1-*N*-Methyl 6-indole **13** and 1-*N*-Methyl 6-indazole **14**, and found that these compounds had increased ADAMTS-5 activity and retained good selectivity for MMP-2. These compounds lead to higher lipophilicity; thus, we synthesized nitrogen-containing heterocyclic compounds. But pyridine **15** and pyrazole **16** lost ADAMTS-5 activity. To exploit further activity, we prepared an additional set of substituted pyridines, including dimethylamino group **17** and *N*-Benzyl-*N*-

Table 2
Activity of linker (**5**–**11**)



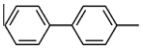
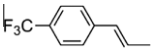
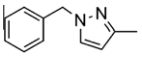
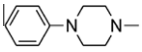
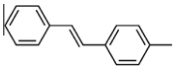
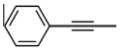
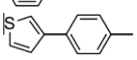
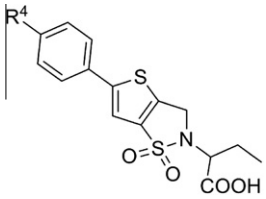
Compounds	R^1	ADAMTS-5	MMP-2 (μM)	MMP-2/ ADAMTS5
5		2.3 μM	1.8	0.78
6		3.2 μM	2.0	0.63
7		43.2% (10 μM inh)	>30	
8		~9% (10 μM inh)	>30	
9		1.4 μM	3.9	2.79
10		4.0 μM	>30	>7.5
11		2.3 μM	>30	>13

Table 3
Activity of selected sultams (**11**–**19**)



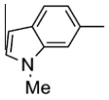
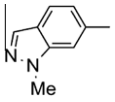
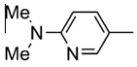
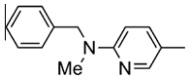
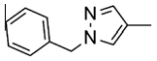
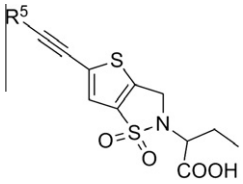
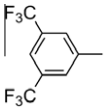
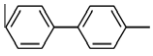
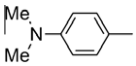
Compounds	R^4	ADAMTS-5 (μM)	MMP-2 (μM)	MMP-2/ ADAMTS5
11		2.3	>30	>13
12		1.3	>30	>23
13		1	16.5	16.5
14		0.3	4	13.3
15		>10	>30	
16		>10	>30	
17		7	>30	4.5
18		1	>30	>30
19		0.4	>30	>75

Table 4
Activity of selected sultams (**8**, **20–24**)



Compounds	R^5	ADAMTS-5 (μM)	MMP-2 (μM)	MMP-2/ ADAMTS5
8		4.0	>30	>7.5
20		9.3	>30	>3.23
21		8.9	>30	>3.37
22		4.9	>30	>6.12
23		1.5	>30	>20
24		1.5	>30	>20

methyl amino group **18**. These compounds had enhanced activity and **18** showed improved selectivity for MMP-2 (>30-fold). When the pyrazole in compound **16** was induced by an *N*-1-benzyl group (**19**), potency increased significantly and retained selectivity over MMP-2 (>75-fold). These results suggested that long groups, such as the benzyl group, may form additional hydrophobic interactions with the ADAMTS-5 S1′ pocket.

Investigation of the alkyne linker (Table 4) revealed that analogs bearing pyridine **20**, cyclohexanemethyl **21** and 3,5-di-(trifluoromethyl)phenyl group **22** displayed lower anti-ADAMTS-5 potency. However, analogues bearing biphenyl group **23** and 4-*N*,*N*-dimethylamino group **24** showed increased anti-ADAMTS-5 activity and displayed no MMP-2 inhibitory effect.

Compounds **19** and **24** were tested for ability to inhibit ADAMTS-4, MMP-2, MMP-3 and MMP-14. As listed in Table 5, each compound showed excellent ADAMTS-5 selectivity over the four other Zn metalloproteases.

The pharmacokinetics of compounds **19** and **24** were examined via two different routes of administration, intravenous (iv) and oral (po), to male Sprague–Dawley rats. Animals received a single iv bolus of 1 mpk and a p.o. dose of 5 mpk. Compound **19** exhibited low clearance (16.7 mL/min/kg), and oral bioavailability was 47%. Most strikingly, compound **24** had a clearance of 3.5 mL/min/kg and the best oral bioavailability (179%) (Table 6).

Synthesis of thienosultam derivatives is shown in Schemes 1–3. All compounds are racemate. As shown in Scheme 1, sulfonylation of methyl 3-amino-5-phenyl-2-thiophencarboxylate **25**, as a starting material, under the Sandmeyer condition gave sulfonyl chloride **26**, which yielded sulfonamide **27** on aminolysis. After reduction of **27** as primary alcohol **28**, bromination of primary alcohol **28** afforded the corresponding bromide **29**, which underwent NaH-mediated cyclization at 60 °C to give sultam derivative **30**. Alkylation of **30** with NaH and bromide was converted **31**, which upon hydrolysis formed carboxylic acid **3**. Hydroxamic acid formation was formed by two-step sequence via amidation, followed by hydrolysis with HCl to give **2** (R^6 = H). Suzuki–Miyaura coupling of compound **31** (R^6 = Br) was used to construct the second aromatic ring or vinyl moiety, followed by hydrolysis to give **5**, **9** or **11**–**19**. Hydroxamic acid formation was formed by above condition to give **4** (R^6 = Ph).

In the case of methyl 2-{5-bromo-1,1-dioxothieno[2,3-*d*]isothiazole-2(3*H*)-yl}butyrate, the synthetic route started from the commercially available methyl 3-chlorosulfonyl-2-thiophene carboxylate **32** (Scheme 2), which was first amidated to the corresponding sulfamoyl **33** with NH_4OH aq and then reduced with DIBAL-H to give primary alcohol **34**. After protection of **34** as TBDPS ether **35**, lithiation of the 2-position of thiophene and subsequent quenching with CBr_4 resulted in the formation of 2-bromo

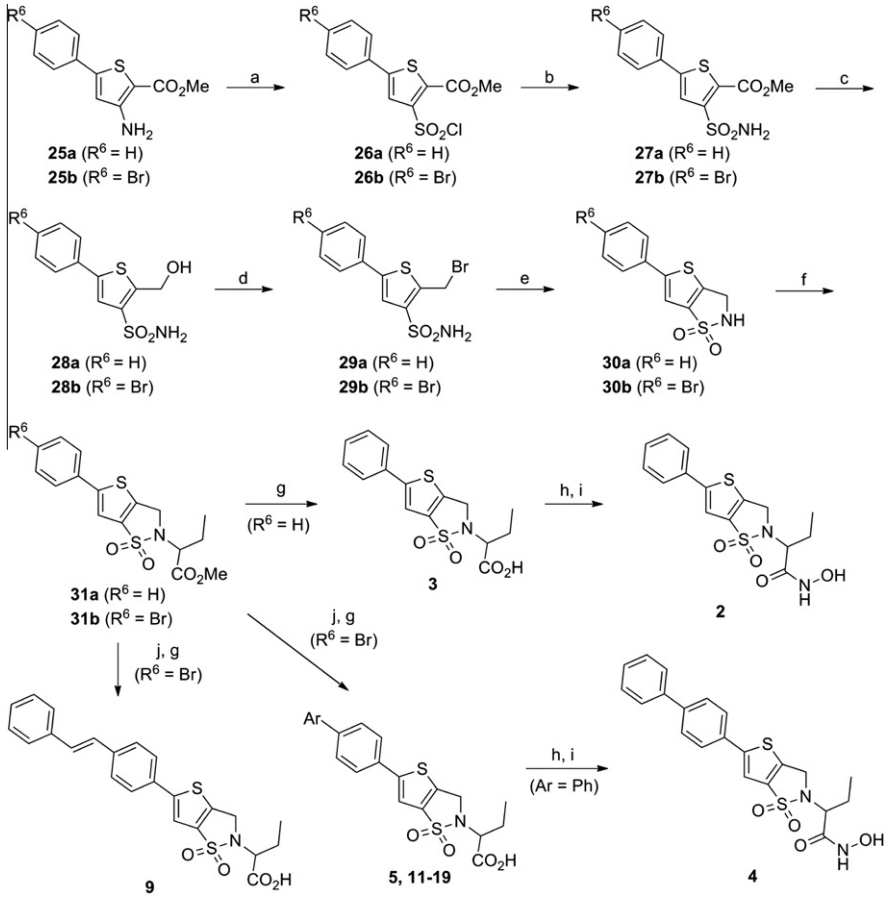
Table 5
Activity of selected sultams (**5**, **19**, **24**)

Compounds	ADAMTS-5 (μM)	ADAMTS-4 (μM)	MMP-2 (μM)	MMP-3 (μM)	MMP-14 (μM)
5	2.1	1.1	0.03	1.2	5.1
19	0.4	8	>30	>30	>30
24	1.5	>10	>30	>30	>30

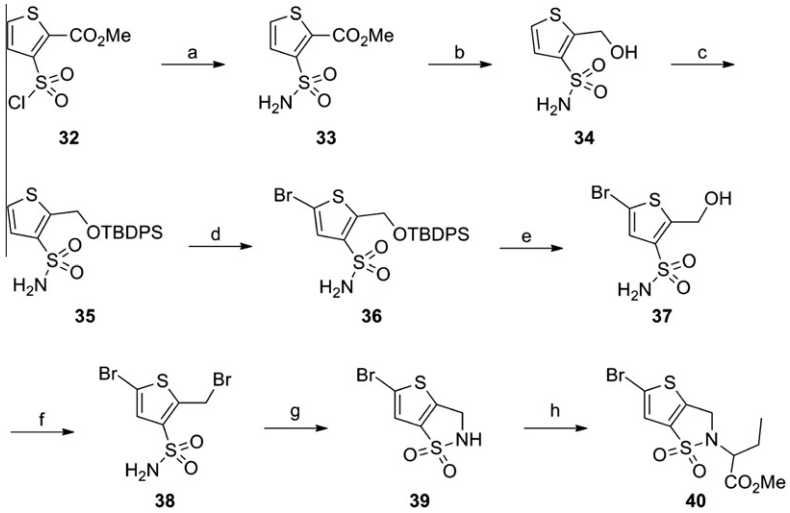
Table 6
Pharmacokinetics data[a] for compounds **19** and **24**

Compounds	AUC[b] (μg h/mL)	C_{max} (mg/mL)	$T_{1/2}$ (h)	V_{ss} (L/kg)	Cl (L/h/kg)	*F* (%)
19	4.73	3.09	1.8	0.33	16.7	47
24	106	29.5	2.8	0.34	3.5	179

[a] Dose at 1 mg/kg (iv) and 5 mg/kg (p.o.).
[b] AUC of 0 → ∞.



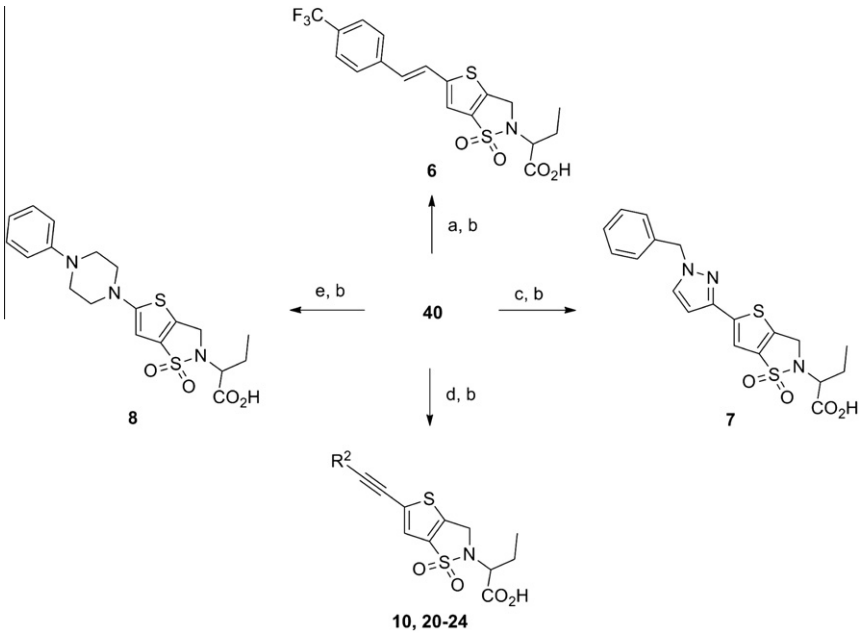
Scheme 1. Reagents and conditions: (a) $NaNO_3$, SO_2, 20% HCl, then CuCl, AcOH, 0 °C (**26a**: 96%, **26b**: 81%); (b) 0.5 M NH_3–dioxane, rt (**27a**: 48%, **27b**: 59%); (c) $LiAlH_4$, THF, 0 °C (**28a**: 77%, **28b**: 77%); (d) PPh_3, CBr_4, CH_2Cl_2–THF (3:1), rt (**29a**: 82%, **29b**: 88%); (e) NaH, DMF, 55 °C (**30a**: 38%, **30b**: 31%); (f) methylbutanoate, NaH, DMF, 60 °C (**31a**: 71%, **31b**: 71%); (g) 1 M NaOH aq, MeOH, rt (12–84%); (h) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole hydrate, Et_3N, DMF, rt; (i) 2 M HCl aq, MeOH, rt, 2 steps (**2**: 57%, **4**: 67%); (j) boronic acid, Pd_2dba_3, (*o*-tol)$_3$P, K_3PO_4, CMF, 140 °C microwave irradiation (16–42%).



Scheme 2. Reagents and conditions: (a) 25%, NH_4OH aq, dioxane, rt (67%); (b) DIBAL-H, CH_2Cl_2, 0 °C (86%); (c) TBDPSCl, imidazole, DMF, rt (32%); (d) LDA (4 equiv), THF, −78 °C to rt (82%); (e) TBAF, THF, rt (99%); (f) PBr_3, pyridine, $CHCl_3$, −20 °C (99%); (g) NaH, DMF, 55 °C (58%); (h) methyl 2-bromobutyrate, NaH, DMF, 50 °C (44%).

thiophene **36**. After deprotection of the TBDPS group with TBAF, bromination of primary alcohol **37** afforded the corresponding bromide **38**, which underwent NaH-mediated cyclization at 60 °C to give the sultam derivative **39**. Alkylation of **39** with NaH and bromide was converted **40**, as a common intermediate.

Suzuki–Miyaura coupling of compound **40** was used to prepare the olefin moiety and the pyrazole-ring moiety (Scheme 3), followed by hydrolysis to give **6** or **7**. Sonogashira coupling of compound **40** was used to construct the alkyne moiety, followed by hydrolysis to give **10** or **20**–**24**. Buchwald N-arylation of compound



Scheme 3. Reagents and conditions: (a) (*E*)-(4-(trifluoromethyl)styryl)boronic acid, Pd_2dba_3, (*o*-tol)$_3$P, K_3PO_4, DMF, 140 °C microwave irradiation (53%); (b) 1 M-NaOH aq, MeOH, rt (41–49%); (c) (1-benzyl-1*H*-pyrazol-3-yl)boronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3 aq, DMF, 100 °C (38%); (d) alkyne, $PdCl_2(PPh_3)_2$, CuI, Et_3N, PPh_3, THF, 80 °C (22%); (e) 1-phenylpiperazine, Cs_2CO_3, xanphos, Pd_2dba_3, dioxane, 100 °C (22%).

40 was used to give the piperazine-ring moiety, followed by hydrolysis to give **8**.

In summary, a series of thienosultam derivatives were designed and synthesized as new ADAMTS-5 inhibitors in an investigation focusing on the side chain of thienosultam for the S1′ pocket. The resulting compounds **19** and **24** showed better ADAMTS-5 inhibition and MMP selectivity; these compounds also showed good oral bioavailability. These inhibitors may serve as lead compounds for further development of disease-modifying OA drugs.

References and notes

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