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N-((8-Hydroxy-5-substituted-quinolin-7-yl)(phenyl)methyl)-2-phenyloxy/amino-acetamide inhibitors of ADAMTS-5 (Aggrecanase-2)

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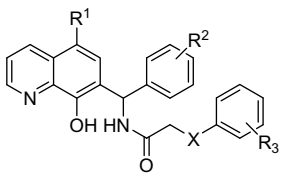
ABSTRACT

N-((8-Hydroxy-5-substituted-quinolin-7-yl)(phenyl)methyl)-2-phenyloxy/amino-acetamide inhibitors of ADAMTS-5 (Aggrecanase-2) have been prepared. Selected compounds **10, 14, 25**, and **53** show sub-μM ADAMTS-5 potency and good selectivity over the related metalloproteases ADAMTS-4 (Aggrecanase-1), MMP-13, and MMP-12. Compound **53** shows a good balance of potent ADAMTS-5 inhibition, moderate CYP3A4 inhibition and good rat liver microsome stability. This series of compounds represents progress towards selective ADAMTS-5 inhibitors as disease modifying osteoarthritis agents.

Osteoarthritis (OA), the most common of musculoskeletal diseases, is rapidly becoming a significant medical and financial burden.[1] This debilitating disease is 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 that is lost in the initial phases of OA. This loss of aggrecan fragments via proteolysis is attributable to Aggrecanase activity.[2] 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.[3]

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) cleave aggrecan at the Glu373–Ala374 peptide bond.[2] Recent studies have demonstrated reduced OA severity for ADAMTS-5 knockout mice 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] 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 low-μM rhodanines and selective, sub-μM thiadiazole inhibitors of ADAMTS-5.[6–8] In this letter, we report our initial studies on a series of ADAMTS-5 inhibitors **I** found via high throughput screening[9] and show their potential utility as selective ADAMTS-5 therapeutics to treat OA. Whereas several metalloprotease scaffolds have been disclosed, to our knowledge the compounds presented herein represent a new class of metalloprotease inhibitors based on the hydroxyquinoline scaffold.[10]



I

Initial efforts to explore the *N*-((8-hydroxy-5-substituted-quinolin-7-yl)(phenyl)methyl)-2-phenyloxy/amino-acetamide scaffold began with the development of a parallel synthesis via a Mannich reaction as outlined in Scheme 1.[11,12] Reaction of 2-phenoxyacetamide

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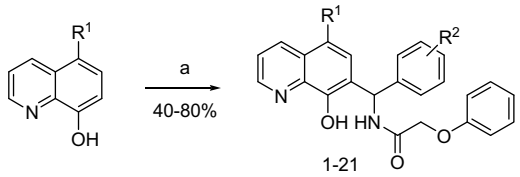
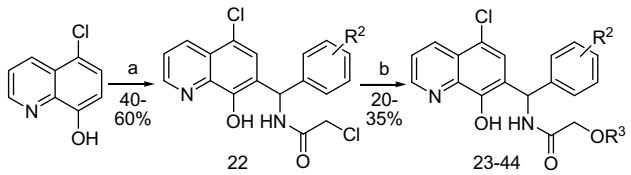


Scheme 1. Reagents and conditions: (a) 2-phenoxyacetamide, aldehyde, 180 °C.



Scheme 2. Reagents and conditions: (a) 2-chloroacetamide, substituted benzaldehyde, 150 °C; (b) substituted phenol, NaH, DMF, 0 °C to room temperature.

with either commercially- or readily-available[13] 5-substituted 8-hydroxy quinolines and various substituted benzaldehydes afforded the desired target hydroxyquinolines **1–21** (Table 1).

That the 5-quinoline position (R^1 substituent) plays a significant role in ADAMTS-5 inhibitory activity is shown in compounds **1–4** (Table 1). Whereas compound **1** (R^1 = H) shows low-μM activity, this potency was decreased twofold with the incorporation of a 5-fluoro substituent in **2**. The potency could be improved with the incorporation of a 5-nitro substituent in **3** and further increased with a 5-chloro substituent to afford sub-μM ADAMTS-5 inhibitor **4** (IC_{50}: 0.78 μM).

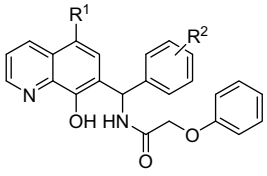
To follow up these initial results compounds **5**–**16**, incorporating various R^2 substituents, were prepared. ADAMTS-5 inhibition data for these compounds demonstrates that R^2 substitution plays a role for activity as ADAMTS-5 potency varies ~1 log in these compounds (Table 1). Optimal R^2 substituents are seen in **8** (R^2 = 4-NO_2 IC_{50}: 0.46 μM), **9** (R^2 = 3-F; IC_{50}: 0.62 μM), **10** (R^2 = 3-NO_2; IC_{50}: 0.49 μM) and **14** (R^2 = 2-Cl; IC_{50}: 0.35 μM). R^2 substituents that give less potent compounds are seen in **6** (R^2 = 4-OMe; IC_{50}: 1.0 μM), **12** (R^2 = 2-Me; IC_{50}: 1.85 μM) and **16** (R^2 = 2-CN: IC_{50}: 2.32 μM). Variation of R^1 to Br (**17** and **18**), NO_2 (**19**), and Me (**20** and **21**) shows that these substituents are also tolerated. Bromine appears to give the best ADAMTS-5 potency (**17**, IC_{50}: 0.62 μM; **18**, IC_{50}: 0.80 μM). Finally compounds where the 8-hydroxy quinoline was changed to an 8-methoxy quinoline show reduced ADAMTS-5 inhibition (data not shown).

Overall, the compounds in Table 1 show low to moderate CYP3A4 inhibition[14] (these analogs display low CYP2D6 and CYP2C9 inhibition) and poor rat liver microsome (RLM) stability.[15] The CYP inhibition does not fluctuate greatly over these compounds; however the microsome stability does. Increasing rat liver microsome stability is seen with compounds **13** ($t_{1/2}$ = 16 min), **17** ($t_{1/2}$ = 30 min) and **18** ($t_{1/2}$ = 10 min).

To explore additional diversity points on the most potent ADAMTS-5 analogs, a parallel synthesis was developed to allow variation of R^3 substituents where R^1 = Cl as outlined in Scheme 2.[12] Mannich reaction of 2-chloroacetamide with 5-chloro 8-hydroxy quinoline and various substituted benzaldehydes affords the desired alkylchloro intermediate hydroxyquinolines **22**. Subsequent displacement of the chloride with various substituted phenols is accomplished using NaH DMF to afford the desired target hydroxyquinoline compounds **23–42** (Table 2).

The compounds in Table 2 show that the substituent on the phenol moiety (R^3) plays a role in ADAMTS-5 inhibition as well as modulating CYP3A4 inhibition and RLM stability. When R^2 = 4-

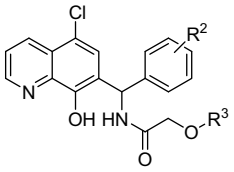
Table 1
In vitro ADAMTS-5 inhibition Data for various R^1 and R^2 substituted *N*-((8-hydroxy-5-substituted-quinolin-7-yl)(phenyl)methyl)-2-phenoxyacetamides.



Compound	R^1	R^2	ADAMTS-5 IC_{50}[a] (μM)	CYP3A4 inhib.[b] (% at 30 μM)	RLM $t_{1/2}$[b] (min)
1	H	H	2.28	56	
2	F	H	4.84	22	4
3	NO_2	H	1.35	20	8
4	Cl	H	0.78	5	
5	Cl	4-Me	0.76	45	
6	Cl	4-OMe	1.0	54	
7	Cl	4-Cl	0.83	38	
8	Cl	4-NO_2	0.46	13	10
9	Cl	3-F	0.62	57	
10	Cl	3-NO_2	0.49	26	6
11	Cl	2-F	1.22	24	5
12	Cl	2-Me	1.85	10	
13	Cl	2-CF_3	0.83	37	16
14	Cl	2-Cl	0.35	43	
15	Cl	2-NO_2	1.21	38	5
16	Cl	2-CN	2.32	26	11
17	Br	3-NO_2	0.62	38	30
18	Br	2-Cl	0.80	33	10
19	NO_2	2-Cl	0.94	35	7
20	Me	3-NO_2	1.32	33	6
21	Me	2-Cl	2.42	55	5

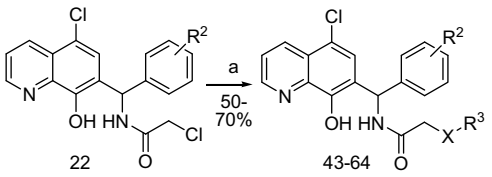
[a] Values are means of 2 experiments, standard deviations are ±10%.
[b] Standard deviations are ±10%.

Table 2
In vitro ADAMTS-5 inhibition data for various R^2 and R^3 substituted *N*-((8-hydroxy-5-substituted-quinolin-7-yl)(phenyl)methyl)-2-phenoxyacetamides.



Compound	R^2	R^3	ADAMTS-5 IC_{50}[a] (μM)	CYP3A4 inhib.[b] (% at 30 μM)	RLM $t_{1/2}$[b] (min)
23	4-OMe	3-Pyridyl	2.98	33	16
24	4-OMe	(4-Me)Ph	0.77	78	
25	4-OMe	(4-OMe)Ph	0.94	74	
26	4-OMe	(3-NMe_2)Ph	1.67	56	5
27	4-OMe	(4-NHCOMe)Ph	3.39	37	15
28	4-OMe	(3-Me, 4-Cl)Ph	>10	25	9
29	4-OMe	(4-OPh)Ph	>10	21	
30	3-NO_2	3-Pyridyl	1.66	67	30
31	3-NO_2	(4-COMe)Ph	2.33	18	
32	3-NO_2	(3-Me, 4-Cl)Ph	>10	19	30
33	3-NO_2	(3,5-Cl)Ph	>10	20	
34	H	(4-Me)Ph	1.90	69	
35	H	(4-Cl)Ph	1.02	2	
36	3-F	(3-NMe_2)Ph	1.33	53	4
37	3-F	(4-NHCOMe)Ph	3.37	31	8
38	3-F	3-Pyridyl	>10	35	30
39	2-Cl	(3-NMe_2)Ph	1.72	38	
40	2-Cl	(4-NHCOMe)Ph	2.34	4	
41	2-Cl	(3-Me, 4-Cl)Ph	>10	30	23
42	2-Cl	(4-tBu) Ph	>10	10	24

[a] Values are means of 2 experiments, standard deviations are ±10%.
[B] Standard deviations are ±10%.



Scheme 3. Reagents and conditions: (a) substituted aniline, DIEA, DMF, 90 °C or benzylamine/benzylalcohol, NaH, DMF, 0 °C to room temperature different R^3 substituents demonstrate low-μM ADAMTS-5 activity.

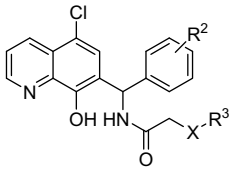
OMe (**23**–**29**), several R^3 substituents show potent ADAMTS-5 activity—especially **24** (R^3 = (4-Me)Ph; IC_{50}: 0.77 μM) and **25** (R^3 = (4-OMe)Ph; IC_{50}: 0.94 μM). Unfortunately both of these compounds show a significant level of CYP3A4 inhibition. When R^2 = 3-NO_2, various R^3 substituents show reduced ADAMTS-5 inhibition. When R^2 = H (**34**, **35**), low-μM ADAMTS-5 activity is seen (IC_{50}: 1.90, and 1.02 μM, respectively). When R^2 = 3-F, analogs **36** and **37** with R^3 = (3-NMe_2)Ph or (4-NHCOMe)Ph show low-μM activity (IC_{50}: 1.33, and 3.37 μM, respectively). Conversely analog **38** with R^3 = 3-pyridyl shows minimal ADAMTS-5 activity IC_{50} > 10 μM) despite its moderate CYP3A4 inhibition and excellent RLM stability. When R^2 = 2-Cl, analogs **39** and **40** with R^3 = (3-NMe2)Ph or (4-NHCOMe)Ph show low-μM activity (IC_{50}: 1.72, and 2.34 μM, respectively). Conversely analogs **41** and **42** with R^3 = (3-Me, 4-Cl)Ph or (4-*t*Bu)Ph demonstrate minimal ADAMTS-5 activity (IC_{50}s > 10 μM) despite showing moderate CYP3A4 inhibition and good RLM stability.

To further investigate the substituted X-aryl moiety (**I**), the alkylchloro intermediate **22** was reacted with several different nucleophiles as outlined in Scheme 3.[12] Displacement of the chloride in **22** with various commercial- or readily-available anilines, and benzyl amines was accomplished using DIEA in DMF to afford the desired target compounds **43**–**62**. Displacement of the chloride in **22** with benzyl alcohol was accomplished using NaH in DMF to afford the target compounds **63** and **64**.

From the synthesis of this focused array (**43**–**64**) it was apparent that the substituted heteroatom linker (X = NH, NMe, or O) combined with the R^3 substituent plays a role in ADAMTS-5 inhibition (Table 3). When X = NH and R^2 = 4-Me several analogs (**43–47**) with different R^3 substituents demonstrate low-μM ADAMTS-5 activity. Compounds **48** and **49** with X = NMe demonstrate slightly improved ADAMTS-5 activity (IC_{50}: 0.78, and 1.28 μM, respectively). Similarly analogs **50**–**52** with X = NH and R^2 = 3-NO_2 having different R^3 substituents show low μM ADAMTS-5 activity, but analogs **53** and **54** with X = NMe show improved ADAMTS-5 inhibition (IC_{50}: 0.56, and 0.82 μM, respectively). Analogs with X = NH or NMe and R^2 = 3-F (**55**–**58**) and analogs with X = NH or NMe and R^2 = 2-Cl (**59**–**62**) combined different R^3 substituents demonstrate low-μM ADAMTS-5 activity. Compounds **63** and **64** possessing X = O, an R^3 benzyl substituent, and an R^2 substituent either 4-Me or 3-Cl show sub-μM ADAMTS-5 activity (IC_{50}: 0.70, and 0.83 μM, respectively). Overall the analogs in Table 3 show reduced CYP3A4 inhibition especially compared to analogs in Table 2. It is also gratifying to see that several compounds show good ADAMTS-5 inhibition and moderate to good RLM stabilities ($t_{1/2}$ > 15 min). The reduced lipophilicity of the amide- analogs in Table 3 may be responsible for this trend.

Given the suggestive mouse ADAMTS-5 knockout data discussed above,[4,5] we assessed ADAMTS-5/ADAMTS-4 selectivity for several of the more potent hydroxyquinolines presented in this manuscript (Table 4). Analogs **10**, **14**, **25**, and **53** each show significant selectivity over ADAMTS-4 with **25** and **53** demonstrating excellent selectivity for ADAMTS-5. We also assessed selectivity against MMP-13, another metalloprotease implicated in osteoarthritis. Once again **10**, **14**, **25**, and **53** demonstrate excellent selectivity for ADAMTS-5 as none of the analogs show appreciable activity against MMP-13. These compounds were also tested for selectivity against MMP-12 and showed moderate to good selectivity.

Table 3
In vitro ADAMTS-5 inhibition data for various R^2, R^3 and X-heteroatom linker *N*-((8-hydroxy-5-substituted-quinolin-7-yl)(phenyl)methyl)-2-2-benzyloxy/amino-acetamides.



Compound	X	R^2	R^3	ADAMTS-5 IC_{50}[a] (μM)	CYP3A4 inhib.[b] (% at 30 μM)	RLM $t_{1/2}$[b] (min)
43	NH	4-Me	(4-COMe) Ph	1.12	45	4
44	NH	4-Me	(4-CN) Ph	1.01	29	5
45	NH	4-Me	(3-NMe_2)Ph	1.93	37	5
46	NH	4-Me	(3-Me, 4-Cl)Ph	1.58	12	
47	NH	4-Me	Bn	1.83	29	10
48	NMe	4-Me	Ph	0.78	20	6
49	NMe	4-Me	Bn	1.28	30	16
50	NH	3-NO_2	(4-COMe)Ph	1.07	27	7
51	NH	3-NO_2	(3-Me, 4-Cl)Ph	1.19	17	12
52	NH	3-NO_2	(4-NHCOMe)Ph	2.77	8	30
53	NMe	3-NO_2	Ph	0.56	42	19
54	NMe	3-NO_2	Bn	0.82	20	11
55	NH	3-F	(3-Me, 4-Cl)Ph	1.43	14	6
56	NH	3-F	Bn	1.70	32	13
57	NMe	3-F	Ph	1.25	41	11
58	NMe	3-F	Bn	1.44	32	16
59	NH	2-Cl	(3-Me, 4-Cl)Ph	1.48	11	8
60	NH	2-Cl	Bn	1.89	36	7
61	NMe	2-Cl	Ph	0.99	34	6
62	NMe	2-Cl	Bn	1.31	16	5
63	O	4-Me	Bn	0.70	45	21
64	O	3-F	Bn	0.83	46	24

[a] Values are means of 2 experiments, standard deviations are ±10%.
[b] Standard deviations are ±10%.

Table 4
In vitro selectivity data for *N*-((8-hydroxy-5-substituted-quinolin-7-yl)(phenyl)-methyl)-2-phenoxyacetamides.

Compound	ADAMTS-5 IC_{50}[a] (μM)	ADAMTS-4 IC_{50}[a] (μM)	MMP-13 IC_{50}[a] (μM)	MMP-12 IC_{50}[a] (μM)
10	0.49	1.9	>200	45% at 2.5 μM
14	0.35	2.4	>67	50% at 2.5 μM
25	0.94	>50	>200	37% at 2.5 μM
53	0.56	>22	>100	25

[a] Values are means of 2 experiments, standard deviations are ±10%.

In conclusion, we have presented a series of hydroxyquinoline inhibitors of ADAMTS-5. This series of compounds has tractable SAR with several analogs of sub-μM potency demonstrating functional selectivity for ADAMTS-5 over ADAMTS-4, MMP-13 and MMP-12. In addition compounds were identified that possess good ADAMTS-5 inhibition, low CYP3A4 inhibition and moderate to good RLM stability. The continued development of selective ADAMTS-5 inhibitors is currently ongoing and will be reported in due course.

Acknowledgments

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