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Synthesis and biological evaluation of ((4-keto)-phenoxy)methyl biphenyl-4-sulfonamides: A class of potent aggrecanase-1 inhibitors

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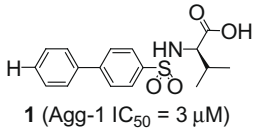
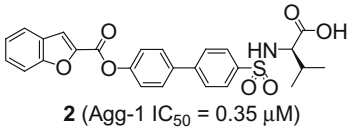
ABSTRACT

The prevention of aggrecan (a key component of cartilage) cleavage via the inhibition of aggrecanase-1 may provide a unique opportunity to stop the progression of cartilage degradation in osteoarthritis. The evaluation of a series of biphenylsulfonamides resulted in the identification of the ((4-keto)-phenoxy)methyl biphenyl-4-sulfonamides analogs (**19**–**21** and **24**) with improved Agg-1 inhibition and MMP-2, MMP-13 activity.

Osteoarthritis (OA) is an incapacitating joint disease resulting from the progressive loss and breakdown of articular cartilage. OA is characterized by pain, joint dysfunction and inflammation, which can be debilitating and reduce quality of life. Loss of aggrecan, a multidomain proteoglycan, via proteolysis attributable to 'aggrecanase' activity throughout the disease eventually results in cartilage erosion and ultimately replacement joint surgery.[1,2]



1 (Agg-1 IC_{50} = 3 μM)



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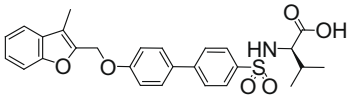
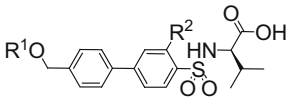


3 (Agg-1 IC_{50} =0.7 μM)



4

Aggrecanase-1 (Agg-1, ADAMTS-4)[3] and Aggrecanase-2 (Agg-2, ADAMTS-5) are members of the ADAMTS (a disintegrin and metalloprotease possessing thrombospondin domain) family of zinc-containing metalloproteases and are the only known enzymes that cleave aggrecan IGD (Interglobular domain) at Glu^{373}–Ala^{374} in osteoarthritis. In mice, ADAMTS-5 (but not ADAMTS-4) is responsible for disease progression in a surgically-induced model of OA.[4,5] However, questions remain regarding the relative contribution of ADAMTS-4 and ADAMTS-5 in human disease. In addition, ADAMTS-4/ADAMTS-5 double knockout mice are physiologically normal[6] and also protected from developing osteoarthritis. Therefore, the inhibition of Aggrecanases represent very attractive targets for the development of therapeutics that could alter the progression of OA.[1,2]

In our previously published research it was shown that substitution beyond the biphenyl P1′ group could improve activity (**1**–**3**).[7] Using this as a starting point, we decided to investigate the SAR of a variety of substituted aromatic systems with the goal of improving the Agg-1 inhibition potency. As part of our ongoing research efforts toward Agg-1 inhibitors, we describe herein the design, synthesis and activity of a variety of analogs (**4**) to explore the

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SAR of substitution, particularly at the para position, of the biphenyl group. Analogs **1**–**3** show the importance of substitution of the biphenyl system for Agg-1 inhibition potency. In order to improve the inhibition potency further, we investigated a variety of substitutions to the biphenyl ring system as shown in Tables 1 and 2.

The substituted quinoline analogs **17** and **18** (Table 1) showed improved Agg-1 inhibition potency[8] compared to **2** and **3**. In addition, the 4-phenoxybenzene analog **5** and two 4-phenoxypyridine analogs **6** and **7** showed equivalent potency. Analogs **8**–**11** containing smaller non-aromatic meta substituents on the R^1 phenyl group showed diminished activity. Analogs **12** and **13**, which contain smaller non-aromatic substituents at the para position of the R^1 phenyl showed similarly reduced potency as Agg-1 inhibitors. However, analog **15**, containing a 2,5-substituted pyridine, showed potency similar to that of **3**. Interestingly, analogs with a larger extended aromatic R^1 group (**5**,**6**,**7**,**16**–**18**) showed either comparable or improved potency, However, the morpholine substituted analog **14** containing an extended bicyclic R^1 group exhibited a >6-fold loss of inhibition potency.

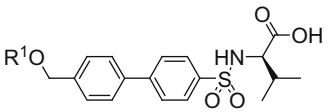
The largest improvement in Agg-1 inhibition potency was observed with the incorporation of a carbonyl group into the extended aromatic P1' substituent (Table 2). The 4-fluorobenzoyl (**19**, >4-fold), 4-cyclohexanecarbonyl (**20**, >3-fold) and 4-isobutyryl (**21**, >3-fold) substituents all demonstrated increased Agg-1 inhibitor potency relative to compound **2**. In addition a more modest potency enhancement was observed when the carbonyl was constrained within a fused system (**24**). When the carbonyl was oriented meta to the ether linkage, inhibition potency was diminished (e.g., **22** *para* vs **23** *meta*). However, the benzophenone analog **28** with the carbonyl in the meta position showed a slight increase in activity relative to **3**. If the carbonyl was incorporated as a carbamate (**29**) or an amide (**30**, **31**), potency was diminished. Additionally, substitution of the biphenyl system ortho to the sulfonamide (R^2, Table 2) resulted in decreased potency as the substituent increased in size (**26**,**27**). However, when R^2 = F (**25**) potency was retained compared to the unsubstituted analog (**24**).

Recently, the crystal structure for Aggrecanase-1 (ADAMTS-4) was described with **21** bound (Fig. 1).[9] As expected, the carboxylic functionality binds the Zn ion while the opposite end of the molecule binds deeply into the S1′ pocket, making extensive hydrophobic and some polar contacts with Agg-1 residues. Specifically, the interaction mediated by a molecule of water between the carbonyl group of **21** and A248 could account for the increased inhibitor potency of **21** and the analogs with similar structures (**19**, **20** and **24**).

Compounds **19**–**21** and **24** were also tested to determine their ability to inhibit MMP-1, MMP-2, MMP-13, MMP-14 and Agg-2 (Table 3). These four compounds were significantly less potent for the inhibition of MMP-1 as compared to Agg-1, and only weakly active as inhibitors of MMP-14. Additionally, compounds **19**–**21** and **24** were shown to be selective for inhibition of Agg-1 over Agg-2. All four compounds were shown to be single-digit nanomolar inhibitors of MMP-2 and MMP-13. It has been shown that MMP-13 plays an important role in cartilage degradation. Consequently, inhibition of MMP-13 may likewise be beneficial in treating OA.[10]

Analogs **19**–**21** and **24** were also assessed for inhibition of proteoglycan degradation in an interleukin-1 (IL-1) stimulated bovine cartilage explant assay.[11] In this very common in vitro model of cartilage degradation exposure of bovin articular cartilage explants to inflammatory IL-1 induces aggrecanase activity and release of degraded extracellular matrix aggrecan. Analogs **19** (71%), **20** (69%) and **24** (53%) showed significant percent inhibition of proteoglycan degradation at 10 μg/mL after a 3-day incubation (Fig. 2). According to previous reports[10,12] the aggrecan degrades early in the first week of culture (days 3–7), whereas the collagen starts to degrade rapidly only later in the culture period (days 8–

Table 1



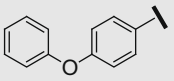
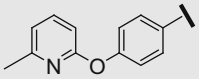
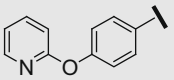
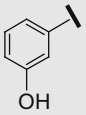
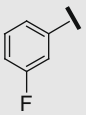
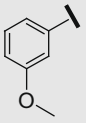
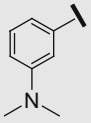
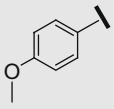
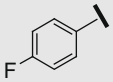
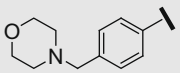
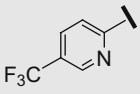
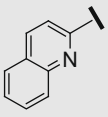
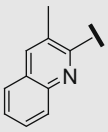
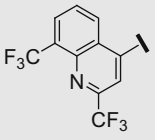
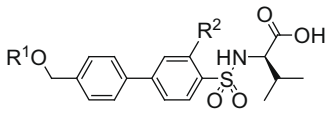
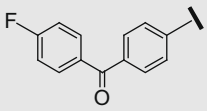
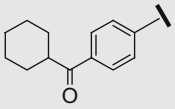
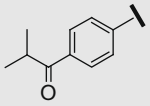
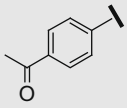
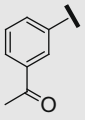
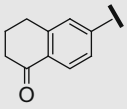
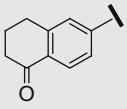
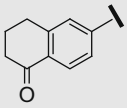
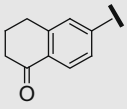
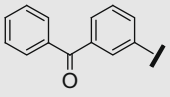
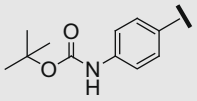
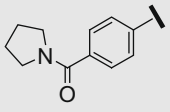
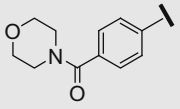
Compound	R^1	Agg-1 IC_{50} (μM)
5		0.4
6		0.73
7		0.9
8		1.1
9		1.2
10		1.8
11		4.9
12		1.6
13		1.7
14		6.0
15		0.8
16		0.4
17		0.2
18		0.2

Table 2



Compound	R^1	R^2	Agg-1 IC_{50} (μM)
19		H	0.08
20		H	0.09
21		H	0.1
22		H	0.43
23		H	1.0
24		H	0.23
25		F	0.18
26		CF_3	3.2
27		OCF_3	20
28		H	0.23
29		H	0.59
30		H	1.9
31		H	5.2

14). Consequently, the activity in this 3-day assay is most likely attributable to aggrecanase inhibition.

Table 3

Compound	IC_{50} (nM)				
	MMP-1	MMP-2	MMP-13	MMP-14	Agg-2
19	>100,000	3.6	6.3	220	55% inhib. @ 2.5 μM
20	>100,000	5.3	15	1641	2600
21	>100,000	1.3	1.1	915	8400
24	>100,000	6.3	2.2	1305	6600

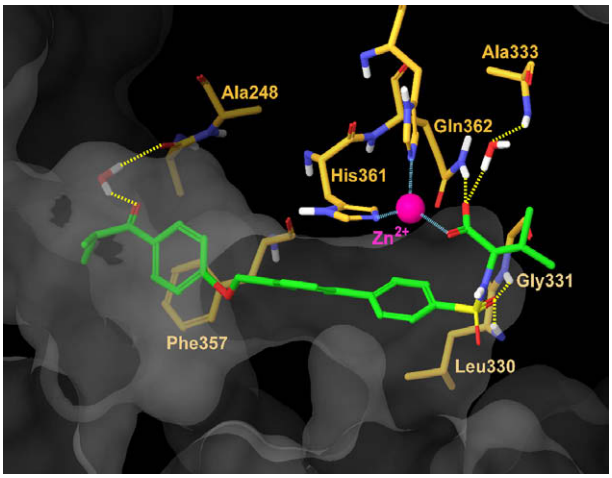


Figure 1. Crystal structure of Agg-1 with **21** (green) bound.[9] H-bonds are represented as yellow dash lines and coordination bonds are represented as blue dash lines.

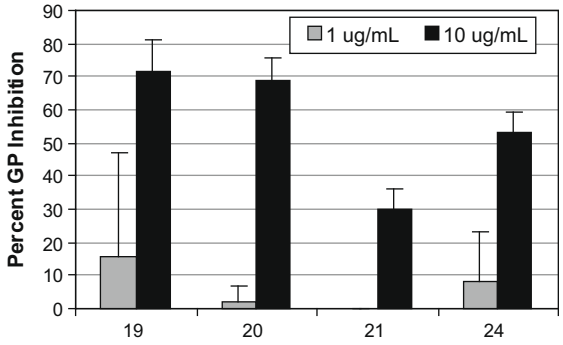
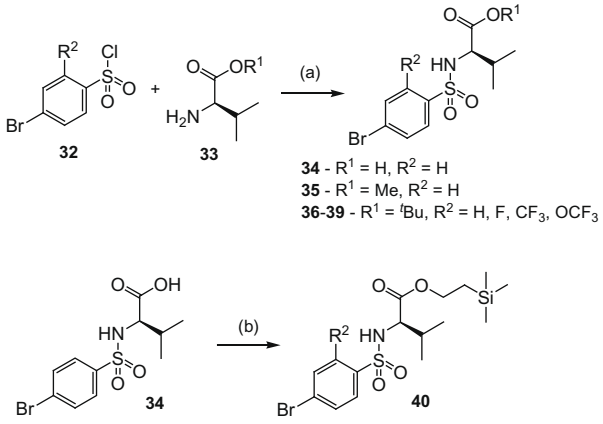


Figure 2. Proteoglycan (PG) inhibition of compounds **19**–**21**, **24**.

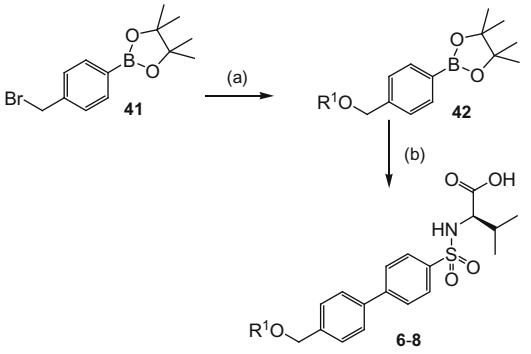
In addition, the pharmacokinetics of compound **20** were examined using intravenous (iv) and oral (po) modes of administration to male Sprague–Dawley rats. Animals received a single iv bolus of 2 mpk and a po dose of 25 mpk. Compound **20** exhibited low clearance (6.9 mL/min/kg) and the oral bioavailability was 13% ($T_{1/2}$ = 184 min, C_{max} = 694 ng/mL). The low bioavailability is most likely attributable to the high molecular weight and high clog*P* (e.g., **20**, MW = 549, clog*P* = 7.27) for this series of compounds.

The synthesis of compounds **5**–**31** and their intermediates is shown in Schemes 1–5. As shown in Scheme 1 the aryl bromide intermediates **34**–**39** are formed through the sulfonylation of the appropriate amino ester or acid. Aryl bromide **40** was formed through the protection of **34** as the 2-(trimethylsilyl)ethyl ester.

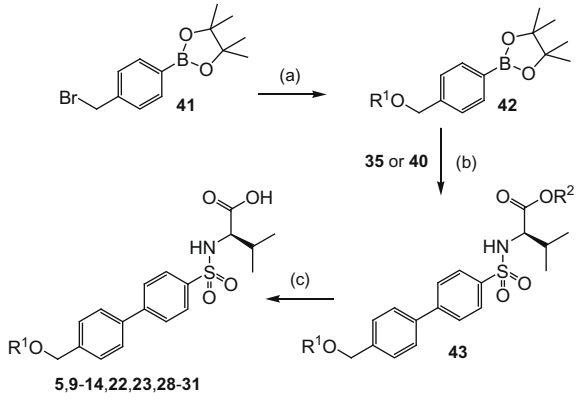
Compounds **6**–**8** were synthesized by Suzuki coupling of **34** as shown in Scheme 2. In addition, compounds **5**, **9**–**14**, **22**, **23**, **28**–**31** were also synthesized through a Suzuki coupling of **35** or **40**



Scheme 1. Reagents and conditions: (a) R^1 = Me or *t*Bu, DIEA, CH_2Cl_2 (87–98%); R^1 = H, H_2O, THF, TEA (80%); (b) $HOCH_2CH_2Si(CH_3)_3$, EDCI, TEA, DMAP, CH_2Cl_2 (100%).



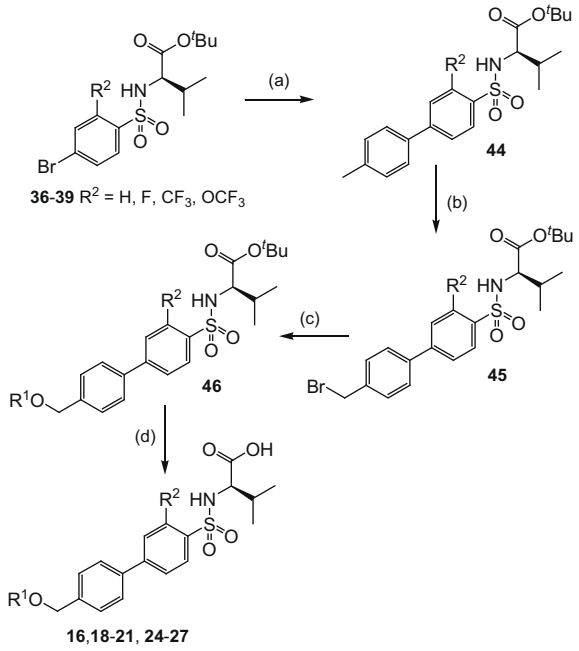
Scheme 2. Reagents and conditions: (a) R^1OH, THF, *n*BuNOH (37–73%) or acetone, K_2CO_3, 50 °C (25–64%); (b) **34**, $Pd(PPh_3)_4$, K_2CO_3, DME, H_2O, 80 °C (9–19%).



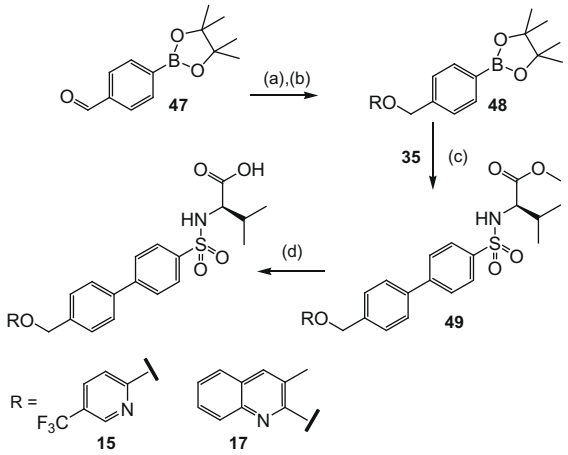
Scheme 3. Reagents and conditions: (a) ROH, THF, *n*BuNQH (37–73%) or acetone, K_2CO_3, 50 °C (25–64%); (b) $Pd(PPh_3)_4$, K_2CO_3, DME, H_2O, 80 °C (51–66%); (c) R^2 = Me **5**, **11**, **29**, LiOH, MeOH, THF (70–93%). $R^2 = CH_2CH_2Si(CH_3)_3$ **9**, **10**, **12**–**14**, **22**, **23**, **28**, **30**, **31**, CH_2Cl_2, $MgBr_2$–Et_2O (73–100%). For compounds **5**, **22**, **23**, **28**, $PdCl_2$(dppf) (22–67%) was used in the Suzuki coupling rather than $Pd(PPh_3)_4$.

and the desired aryl boronic ester followed by the deprotection of the ester to the carboxylic acid (Scheme 3). The aryl boronic esters used in Scheme 2 and 3 were synthesized using the commercially available 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and the desired phenol derivative.

Compounds **16**, **18**–**21**, **24**–**27** were synthesized (Scheme 4) starting with the Suzuki coupling of the appropriate aryl bromide



Scheme 4. Reagents and conditions: (a) $Pd(PPh_3)_4$, *p*-tolylboronic acid, K_2CO_3, DME, H_2O, 80 °C (58–81%); (b) CCl_4 NBS, AIBN, 70 °C (69–98%); (c) R^1OH, acetone, K_2CO_3, reflux (11–88%); (d) TFA, CH_2Cl_2 (47–100%).



Scheme 5. Reagents and conditions: (a) $NaBH_4$, MeOH (74%); (b) RCl, NaH, DMF (80–85%); (c) $Pd(PPh_3)_4$, K_2CO_3, DME, H_2O, 80 °C (50–66%); (d) NaOH, MeOH (70–93%).

(**36**–**39**) and *p*-tolylboronic acid to form **44,** which was converted to the benzyl bromide **45** using NBS. Displacement of the benzylic bromide **45** with the desired phenol derivative furnished **46**. Cleavage of the *t*butyl ester gave the desired analogs **16**, **18**–**21**, **24**–**27**.

Compounds **15** and **17** were synthesized (Scheme 5) by the reduction of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde and alkylation of the resulting alcohol followed by a Suzuki coupling with the appropriate aryl bromide. Deprotection of the ester formed the desired carboxylic acids **15** and **17**.

In conclusion, we have described the synthesis and biological evaluation of a series of biphenylsulfonamide Agg-1 inhibitors. In so doing, we identified a series of ((4-keto)-phenoxy)methyl biphenyl-4-sulfonamide analogs with improved Agg-1 inhibition potency, however, this series retained significant activity for MMP-2 and MMP-13. The enhancement of inhibition potency for Agg-1

is likely attributable to the interaction of the ligand's ketone carbonyl with residue A248, which was observed in the recently reported crystal structure of Agg-1 with compound **21** bound.[9] In addition, this series of analogs showed significant inhibition of proteoglycan degradation at 10 μg/mL in a cell based assay. Additional analogs will be designed using the crystals structure of compound **21** bound in aggrecanase-1 and these results will be reported in due course.

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