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3-Hydroxy-4-arylsulfonyltetrahydropyranyl-3-hydroxamic acids are novel inhibitors of MMP-13 and aggrecanase

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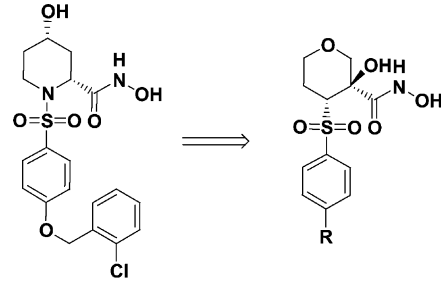
Abstract—*N*-Hydroxy-3-hydroxy-4-arylsulfonyltetrahydropyranyl-3-carboxamides were designed as novel inhibitors of MMP-13 and aggrecanase based on known endocyclic hydroxamate inhibitors of matrix metalloproteinases. These compounds offer favorable physicochemical properties and low metabolic clearance. Synthesis and structure–activity relationships are reported.

Osteoarthritis is a disease of progressive cartilage degradation that leads to pain and reduced mobility in affected joints. The pathogenesis of osteoarthritis can be traced to multiple factors that lead to degeneration of proteoglycan, and disruption of the type II collagen network in cartilage. These two cartilage components maintain the tissue's structural integrity and impart critical biomechanical properties necessary for the normal function of articulating joints. The aggregating proteoglycan, aggrecan is composed of two N-terminal globular domains that are separated by an interglobular domain, followed by a glycosaminoglycan attachment region and a C-terminal globular domain. Aggrecan monomers interact with hyaluronic acid and link proteins to form a heavily hydrated high molecular weight aggregate that imparts resistance to compression. Type II collagen is a triple-helical protein, which forms a highly organized three-dimensional structure that is responsible for tissue stiffness and resistance to shear forces. Because incorporation of functional type II collagen is not an active process in adult cartilage, the loss of type II collagen is considered to be an irreversible process in osteoarthritis.[1] Both aggrecan and type II collagen are degraded by proteolytic enzymes that have elevated activity in osteoarthritic joints. 'Aggrecanases' are defined by their functional activity in cleaving aggrecan at specific sites in the aggrecan protein backbone. Two members of the ADAMTS (a disintegrin and metalloprotease possessing thrombospondin domains) family, ADAMTS-4[2] and ADAMTS-5,[3] are believed to be primarily responsible for cartilage aggrecan degradation. Collagenases are the principle enzymes that cleave type II collagen and are members of the MMP (matrix metalloprotease) family. MMP-13 is particularly efficient at cleaving type II collagen, and its expression has been shown to be elevated in osteoarthritic joints.[4] In order to protect both the aggrecan and type II collagen components of cartilage, we sought to discover compounds that possessed inhibitory activity versus both MMP-13 and aggrecanase. Such compounds could serve as effective therapeutic agents for the treatment of osteoarthritis.



1
rTACE IC_{50}: 36 nM
MMP-13 IC_{50}: 12 nM
MMP-1 IC_{50}: 29000 nM

2a - 2u

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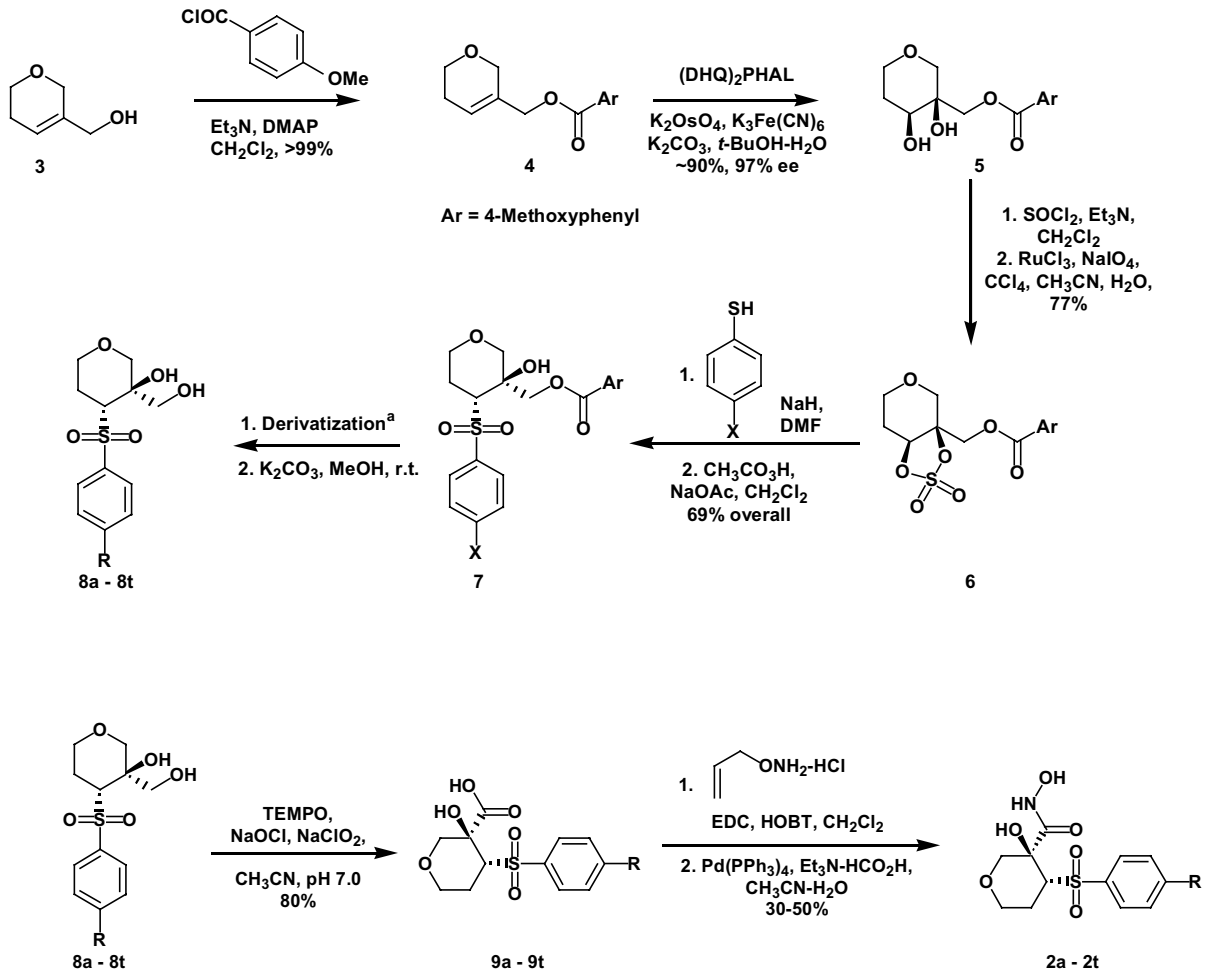


Scheme 1. Synthesis of 3-hydroxy-4-arylsulfonyltetrahydropyranyl-3-hydroxamic acids from dihydropyranylmethanol. (a) Conditions for R = OCH_2Ar: X = OH, $ArCH_2Br$, Cs_2CO_3, DMF, 23 °C, 4–12 h; for R = OAr: X = OH, $ArB(OH)_2$, $Cu(OAc)_2$, Et_3N, MS4A, CH_2Cl_2, O_2, rt, 24 h; for R = Ar: X = Br, $ArB(OH)_2$, $Pd(OAc)_2$, Bu_4NBr, K_2CO_3, toluene, reflux, 30 min.

Previous MMP and TNF-α converting enzyme (TACE) programs at Pfizer led to the discovery of pipecolic acid based inhibitors such as **1**.[5] These compounds generally lacked potent aggrecanase inhibition and suffered from metabolic lability of the hydroxamic acid moiety. We envisioned the series represented by **2** to be superior due to increased polarity, steric encumbrance of the hydroxamic acid moiety and proximity of the polar hydroxy group to the hydroxamate functionality.[6]

The synthesis of the tetrahydropyranyl template that was used for analog preparation is shown in Scheme 1. The chiral centers are introduced through a highly enantioselective asymmetric dihydroxylation of the 4-methoxybenzoate **4** using catalytic $(DHQ)_2PHAL$ and potassium osmate under standard conditions.[7] Reaction of the diol **5** with thionyl chloride followed by oxidation with $RuCl_3$ and NMO affords the cyclic sulfate **6** in high yield.[8] Upon treatment with 4-hydroxythiophenol sodium salt in DMF at elevated temperature, the cyclic sulfate opens at the less hindered position to afford the arylsulfide in 93% yield. Oxidation of the sulfide with peracetic acid afforded the sulfone **7** in 74% yield. At this point, the phenol could be alkylated with benzylic halides to form benzyl ether analogs, converted to the corresponding aryl triflate and coupled with aryl boronic acids under palladium catalysis to form biarylsulfones, or reacted with arylboronic acids under copper(II) catalysis in the presence of molecular oxygen to form biaryl ether sulfones.[9,10] Alkylation of the 3-hydroxy group could be effected by treatment of the appropriate intermediate **7** with methyl iodide and silver nitrate. Removal of the 4-methoxybenzoyl group, followed by oxidation of the hydroxymethyl group using sodium chlorite with catalytic TEMPO and sodium hypochlorite at neutral pH affords the carboxylic acid **9** in 80% yield.[11] This intermediate was converted to the *O*-allyl hydroxamate using standard peptide coupling conditions, followed by removal of the allyl group with triethylammonium formate under palladium catalysis to afford the final compounds.[12]

Initial analog generation was focused around 4-benzyloxy aryl sulfones that were prepared to probe structure–activity relationships about this group, which is thought to bind in the S1′ pocket by analogy with the previously discovered pipecolic hydroxamate TACE inhibitor **1**. The biological data obtained for each of

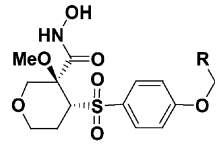
Table 1. Aggrecanase, MMP-13, and MMP-1 inhibitory activity of benzyl ether analogs **2a**–**n**

Compounds	R	Aggrecanase IC_{50} (nM)	MMP-13 IC_{50} (nM)	MMP-1 IC_{50} (nM)
2a	2-Methylbenzyloxy	55	21	12,000
2b	3-Methylbenzyloxy	>1000	5.0	22,000
2c	4-Methylbenzyloxy	>1000	1.3	5400
2d	2-Chlorobenzyloxy	40	2.7	2800
2e	3-Chlorobenzyloxy	>1000	2.4	7900
2f	4-Chlorobenzyloxy	330	0.88	1400
2g	2-Trifluoromethylbenzyloxy	220	28	>30,000
2h	2,3-Dichlorobenzyloxy	140	15	13,000
2i	2,4-Dichlorobenzyloxy	8.1	0.95	920
2j	2,5-Dichlorobenzyloxy	87	13	27,000
2k	2,6-Dichlorobenzyloxy	>1000	>300	>30,000
2l	2,4-Dimethylbenzyloxy	370	17	19,000
2m	2,5-Dimethylbenzyloxy	26	300	>30,000
2n	2,6-Dimethylbenzyloxy	>1000	>300	>30,000

these compounds is shown in Table 1.[13] Electron rich groups were avoided in an effort to optimize metabolic stability of the benzyl ether. Aggrecanase potency is strongly dependent on the presence of an *ortho* substituent on the terminal aromatic ring. Generally chloro substituted analogs possess better potency versus both MMP-13 and aggrecanase compared with their methyl substituted counterparts (compare **2d** with **2a**, **2i** with **2l**, and **2j** with **2m**). This observation is fortuitous in that the halo substituted compounds are expected to have better metabolic stability due to the lack of an additional benzylic position that could be oxidatively labile. Replacement of the *ortho* chloro or methyl substituent with the bulkier trifluoromethyl group (**2g**) led to a reduction in potency against both of these enzymes. Optimum potency for aggrecanase inhibition is obtained with one *ortho* substituent, but the presence of two *ortho* substituents (**2k**,**n**) results in a dramatic reduction in both aggrecanase and MMP-13 potency. The presence of *meta* or *para* substituents generally provides an improvement in MMP-13 potency. Each of these structure–activity relationships is additive across the two enzymes, and combination of an *ortho* and *para* chloro substituent results in optimum dual inhibition of MMP-13 and aggrecanase. All of the benzylic ether analogs show poor MMP-1 inhibitory potency.

Biological data for biaryl ether and biaryl analogs of **2** are shown in Tables 2 and 3, respectively. These compounds are generally potent inhibitors of MMP-13 but have dramatically reduced potency versus aggrecanase compared to their benzyl ether counterparts. Moreover, potency versus MMP-1 generally increases as the terminal aryl group is moved closer to the central aromatic ring. This is consistent with the shorter S1′ pocket in MMP-1 compared with many of the other MMPs.[14] In the biaryl series, incorporation of an *ortho* substituent on the terminal aromatic ring results in a dramatic reduction of MMP-13 and MMP-1 potency (**2r**).

In order to determine the importance of the 3-hydroxyl group, the potency of several methyl ether analogs (**10a**–**b**) was determined. Interestingly, this modification is detrimental to potency for both MMP-13 and aggrecanase. These studies were only conducted on benzyl ether analogs of **10**, since the parent biaryl and biaryl ether sulfone analogs of **2** that were tested demonstrated minimal activity versus aggrecanase (Table 4).



10a-10b

Compound **2i** is a potent inhibitor of both MMP-13 and aggrecanase. Other MMP inhibitory activity is summarized below.

MMP	2	3	9	12	14
IC_{50} (nM)	12	55	5.3	0.95	150

The pharmacokinetic parameters of compound **2i** in rat show low clearance (Clp = 9.0 mL/min/kg), and a long $t_{1/2}$ (5 h).[15] Unfortunately, oral bioavailability was poor (7% when dosed as a suspension in 0.5% aqueous methylcellulose) despite good P_{app} values obtained in CACO-2 experiments (P_{app} A:B = 30×10^{-6}; B:A = 15×10^{-6}).

Table 2. Aggrecanase, MMP-13, and MMP-1 inhibitory activity of biaryl ether analogs **2o**–**q**

Compounds	R	Aggrecanase IC_{50} (nM)	MMP-13 IC_{50} (nM)	MMP-1 IC_{50} (nM)
2o	2-Methylphenoxy	>1000	9.8	4700
2p	3-Methylphenoxy	>1000	1.1	270
2q	4-Methylphenoxy	>1000	1.0	120

Table 3. Aggrecanase, MMP-13, and MMP-1 inhibitory activity of biaryl analogs **2r–t**

Compounds	R	Aggrecanase IC_{50} (nM)	MMP-13 IC_{50} (nM)	MMP-1 IC_{50} (nM)
2r	2-Methylphenyl	>1000	140	10,000
2s	3-Methylphenyl	>1000	0.43	<30
2t	4-Methylphenyl	>1000	0.39	<30

Table 4. Aggrecanase and MMP-13 inhibitory activity of benzyl ether analogs **10a–b**

Compounds	R	Aggrecanase IC_{50} (nM)	MMP-13 IC_{50} (nM)
10a	2-Chlorophenyl	100	38
10b	2,4-Dichlorophenyl	100	46

We have discovered a novel series of MMP-inhibitors structured around a 3-hydroxy-4-arylsulfone tetrahydropyranyl hydroxamate template. Despite the fact that these compounds were designed based on analogy with an earlier endocyclic hydroxamate series of TACE inhibitors, we were unable to find any that potently inhibit this enzyme in human whole blood ($IC_{50} > 100\,\mu M$ in human whole blood assays for **2d–f**). Several of these inhibitors possess potent inhibitory activity versus aggrecanase and MMP-13 and could provide a useful starting point for the discovery of agents for the treatment of osteoarthritis.

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13. TACE, MMP, and whole blood TNF-a release assays were conducted as described in Ref. 5. Aggrecanase activity was assessed using a cell based assay: porcine chondrocytes were plated in 48-well tissue culture plates. Glycosaminoglycan (GAG) chains were labeled by including ^{35}S sulfate (5 mCi/mL) in the culture medium. Unincorporated label was washed out and aggrecan degradation stimulated by the addition of IL-1α (5 ng/mL). 10 h later, aggrecan degradation was quantified by scintillation counting of ^{35}S in both the conditioned medium and cell layers.
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