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Potent P1′ Biphenylmethyl Substituted Aggrecanase Inhibitors

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Abstract—A series of *cis*-1(*S*)2(*R*)-amino-2-indanol based compounds with a biphenylmethyl group at the P1′ position was found to be potent aggrecanase inhibitors. Both compounds **2j** and **2n** possessed very high aggrecanase affinity ($IC_{50} = 1.5$ nM), and showed excellent selectivity over MMP-1 and MMP-9, with moderate selectivity against MMP-2.

Aggrecanase, an ADAM-TS of the reprolysin family, was recently isolated, cloned and expressed.[1] Recent studies suggest that aggrecanase plays a pivotal role in the catabolism of aggrecan in human arthritic disease.[2] Hence, there has been substantial interest in developing a selective aggrecanase inhibitor to prevent the progression of joint destruction, and to delineate its role in normal and disease states.[3]

We recently reported the discovery of a series of *cis*-1(*S*)2(*R*)-amino-2-indanol based aggrecanase inhibitors represented by structure **1** ($IC_{50} = 64$ nM), having a 3-hydroxy benzyl and *cis*-1-amino-2-indanol at the P1′ and P2′ positions, respectively.[4] Although compound **1** shows excellent selectivity for aggrecanase relative to neutrophil collagenase 1 (MMP-1), human gelatinase A (MMP-2), and human gelatinase B (MMP-9), it is only moderately potent for aggrecanase. This selectivity is postulated to be due to a specific hydrogen-bond interaction between the hydroxyl group of the 3-hydroxylbenzyl (P1′) and a threonine residue in the aggrecanase S1′ pocket at a position occupied by valine in MMP-1, -2, and -9. In order to advance the lead from this series into a viable drug candidate targeting degenerative joint diseases, it was necessary to increase the potency of **1** by modifying its general structure without loss of the selectivity over MMP-1, -2 and -9. In this communication, we describe our efforts to optimize compound **1** into a series of highly potent and selective aggrecanase inhibitors by employing a biphenylmethyl group at the P1′ position (Fig. 1).

Comparison of the X-ray structure of MMP-1 to the aggrecanase homology model indicates that the S1′ pockets differ considerably. Aggrecanase has a deep hydrophobic S1′ pocket, whereas MMP-1 is much shallower.[5] We envisioned that large substitutents such as the biphenyl methyl moiety could fit tightly into the S1′ pocket of aggrecanase without perturbing the conformation of the native enzyme. In addition, a larger biphenylmethyl group at the P1′ position might provide additional binding energy through strong hydrophobic interaction to compensate the loss of a specific hydro-

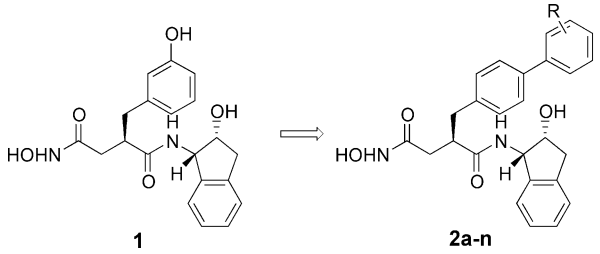

Figure 1.

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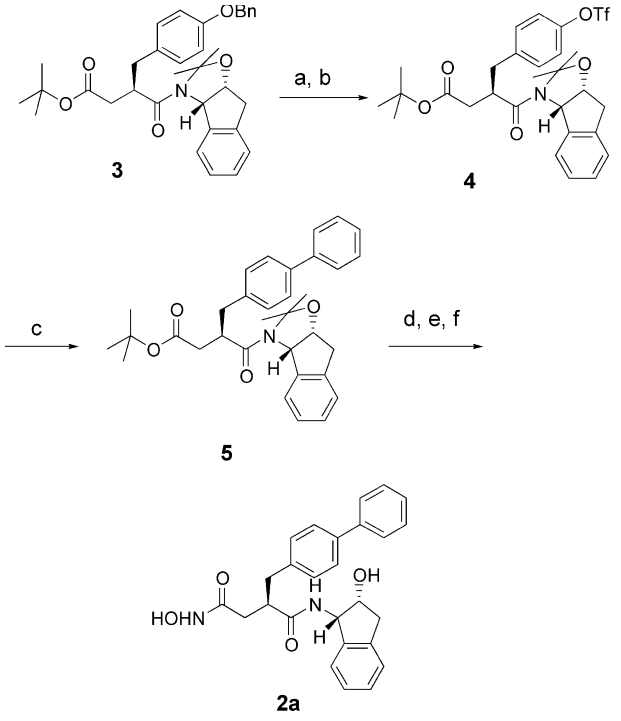
gen-bonding interaction between the 3-hydroxybenzyl and the threonine residue of aggrecanase. It was anticipated that selectivity against MMP-1 could be achieved by this large P1′ substitutent due to its shallower S1′ pocket.

The synthesis of compound **2a** is outlined in Scheme 1. Compound **3** was prepared as described previously.[4] The benzyl group of **3** was removed by hydrogenation to furnish the phenol, which was then converted to the corresponding triflate **4**.[6] Palladium catalyzed Suzuki cross coupling of triflate **4** with phenyl boronic acid in toluene afforded the cross coupling product **5** in high yield.[7] The *tert*-butyl group of **5** was removed by TFA in methylene chloride, followed by coupling with *O*-benzyl hydroxylamine and hydrogenation to afford the desired product **2a**.

Compound **2a**, the first compound prepared in this series, incorporates an unsubstituted biphenyl group at the P1′ position. Figure 2 shows the Vander Waals surface of compound **2a** docked in the active site of the aggrecanase homology model.

Compound **2a** was found to have an IC_{50} of 9.8 nM in the aggrecanase enzymatic assay, more than a 6-fold increase in potency compared to the 3-hydroxybenzyl analogue **1.** We ascribe the higher potency of **2a** to its enhanced hydrophobic interaction. As expected, compound **2a** was extremely weak against MMP-1. However, **2a** also binds tightly to MMP-2 and MMP-9 with an IC_{50} of <1.0 and 5.4 nM, respectively. These results suggested that additional potency and enhanced selectivity might be achievable by further modification of the biphenyl group.



Scheme 1. (a) H_2, Pd/C, MeOH; (b) $PhN(Tf)_2$, Et_3N, CH_2Cl_2 (55%); (c) $PhB(OH)_2$, $Pd(OAc)_2$, PPh_3, toluene, H_2O, Na_2CO_3 (96%); (d) TFA, CH_2Cl_2 (100%); (e) $BnONH_2$–HCl, TBTU, DMF; (f) H_2, Pd/$BaSO_4$, MeOH (43%).

Methyl substitution on the distal (or proximal) phenyl ring (**2b**) at the 2′ position is well tolerated by aggrecanase, MMP-2 and MMP-9. However, the chlorine substituted analogue **2c** decreased the affinity for aggrecanase, but slightly increased the selectivity against MMP-2 and -9. Compounds **2e–g**, which have a large and polar substituent at the 2′ position, bind weakly to aggrecanase and other MMPs. The poor activity of these compounds is probably due to the desolvation energy required for these polar groups to bind in the relatively hydrophobic S1′ pocket. The fact that the methoxy analogue **2d**, which has a substitutent similar in size to the hydroxylmethyl analogue **2g**, binds tightly to aggrecanase suggested that the poor activity of compounds **2e–g** was probably not the result from steric hindrance of these substitutents. Compound **2d** was the first compound prepared to have an IC_{50} in the low nanomolar range. However, **2d** was found to be only moderately selective against MMP-9, and was not selective over MMP-2.

A variety of small substitutions at the 3′ position are well tolerated for aggrecanase as well as MMP-2 and -9 (Table 1). The methyl analogue **2h**, which has an IC_{50} of 11.5 nM, shows similar potency to the parent compound **2a**. However, the larger isopropyl substitution was not tolerated by aggrecanase. The poor activity of this compound suggested that the steric nature of this substitutent imparts unfavorable interactions in this region, since the relative small methoxy **2k** and halogen **2l** analogues have IC_{50} values of 3.7 and 2.0 nM, respectively.

In contrast to the isopropyl analogue, compound **2j** binds tightly to aggrecanase, more importantly, this compound displayed more than 200-fold selectivity against MMP-1 and MMP-9, and moderate selectivity against MMP-2. Similarly, the methylsulfonamide analogue **2n** was also found to be highly potent in the aggrecanase enzyme assay with an IC_{50} of 1.5 nM, and selectivity profile is similar to that of **2j**.

In summary, the synthesis of a new series of *cis*-1-amino-2-indanol based compounds with biphenyl substitutents

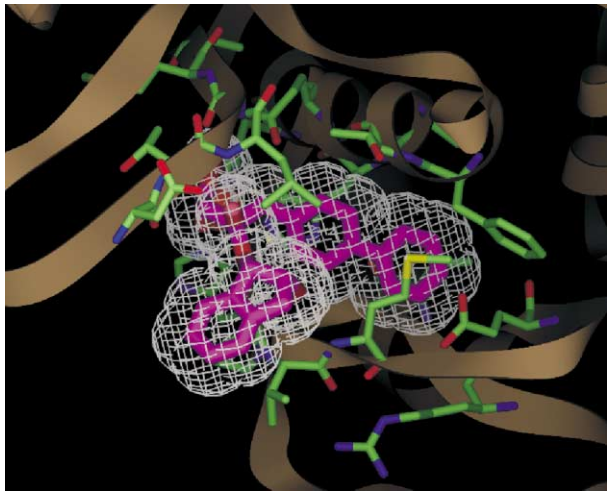
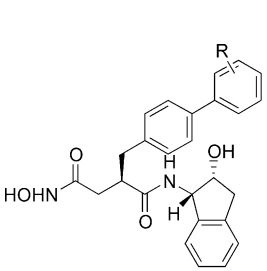
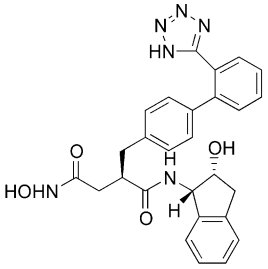


Figure 2. The active site of aggrecanase homology model docked with compound **2a**.

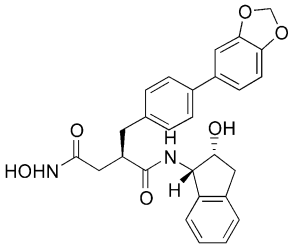
Table 1. Aggrecanase, MMP-1, MMP-2 and MMP-9 binding data



2a-n



2e



2j

Compd	R	IC_{50} (nM)[a,b] Aggrecanase	K_i (nM)[a,b]		
			MMP-1	MMP-2	MMP-9
1	—	64	30,953	1507	3324
2a	H	9.8	>5000	<1	5.4
2b	2-Me	11.6	4107	<2.8	4.2
2c	2-Cl	32	>5000	27	150
2d	2-MeO	3.4	>5000	4.5	347
2e	—	239	>5000	1133	277
2f	2-^{t}BuNHSO$_2$	75	4868	2033	3422
2g	2-HOCH$_2$	144	>5000	500	>2000
2h	3-Me	11.5	>5000	39	1085
2i	3-*i*-Pr	216	>5000	48	87
2j	—	1.5	12,800	43	309
2k	3-MeO	3.7	>5000	<2.8	2.8
2l	3-F	2.0	1820	<2.8	<2.11
2m	3-NO$_2$	5.5	1931	2.8	17.2
2n	3-MeSO$_2$NH	1.5	>5000	31	691

[a]Values are ±SD of three determinations unless otherwise noted.
[b]The IC_{50} values on aggrecanase and K_i values on MMPs were determined as previously described.[8] All of the hydroxamic acids studies here were assumed to act as competitive inhibitors of the enzyme, binding to the active site Zn atom as previously demonstrated by crystallographic and NMR studies of MMPs complexed with related hydroxamic acids.[9] On the basis of the assumption of competitive inhibition, the IC_{50} values were converted to K_i values from the equation, $K_i = C_{50}/(1 + [S]/K_m)$, where IC_{50} is the concentration of competing compound producing 50% inhibition of the enzymatic activity at the substrate concentration [S]. The K_m is the substrate concentration that provides a reaction velocity that is half the maximal velocity obtainable under saturating substrate conditions.

at the P1′ position are reported. Replacing the potential metabolically labile 3-hydroxybenzyl group of **1** with a substituted biphenyl group dramatically increase the potency for aggrecanase. The selectivity was achieved by varying the substitutions on the distal phenyl ring. Among the compounds synthesized, **2j** and **2n** are the most potent aggrecanase inhibitors, and have excellent selectivity over MMP-1, -9 and moderate selectivity over MMP-2. The discovery of a potent and selective aggrecanase inhibitor provides an important tool to further study the biological function of this enzyme and represents a lead in the development of new medications for degenerative joint diseases.

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