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Potent and Selective Aggrecanase Inhibitors Containing Cyclic P1 Substituents

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Abstract—Anti-succinate hydroxamates with cyclic P1 motifs were synthesized as aggrecanase inhibitors. The *N*-methanesulfonyl piperidine **23** and the *N*-trifluoroacetyl azetidine **26** were the most potent aggrecanase inhibitors both having an $IC_{50} = 3$ nM while maintaining >100-fold selectivity over MMP-1, -2, and -9. The cyclic moieties were also capable of altering in vivo metabolism, hence delivering low clearance compounds in both rat and dog studies as shown for compound **14**.

An early step in degenerative joint disease is cartilage degradation resulting from the proteolysis of aggrecan within the interglobular domain (IGD).[1] This proteoglycan cleavage occurs between the G1 and G2 domains of the IGD at the Glu373-Ala374 bond.[2] The aggrecanases are the proteolytic enzymes responsible for this cleavage, which has been observed in the synovial fluid of patients with osteoarthritis, inflammatory joint disease, and joint injury.[3,4] This aggrecanase cleavage site is distinct from the matrix metalloproteinase (MMP) site which occurs between residues Asn341 and Phe342 within the IGD.[5] Two human aggrecanases (aggrecanase-1 and aggrecanase-2) have been isolated, cloned and expressed.[6,7] They are members of the ADAM-TS (a disintegrin and metalloproteinase with thrombospondin motifs) sub-family which is part of the adamalysin/reprolysin family contained within the metzincins (which includes the MMPs).[8] Studies indicate that aggrecanase-1 (ADAM-TS-4) and aggrecanase-2 (ADAM-TS-5) are responsible for the aggrecan degradation within osteoarthritic cartilage.[9,10] As a result of this unique proteoglycan cleavage, there has been much interest in the design of aggrecanase inhibitors. Recently, we described the discovery of α-amino hydroxamate **1** as a potent and selective aggrecanase inhibitor.[11] In order to optimize the potency and pharmacokinetic profile of this inhibitor, we investigated hydroxamates **2** with cyclic P1 substituents and report the results herein (Fig. 1).

A general synthesis of the cyclic P1 hydroxamates is illustrated in Scheme 1 using compounds **14** and **18** as examples. The essential anti-succinate core was synthesized using Evans chemistry starting from the substituted acetic acid **3** (or R = a ring).[12,13] The requisite imide **4** was formed via the coupling of **3** and *R*-4-ben-

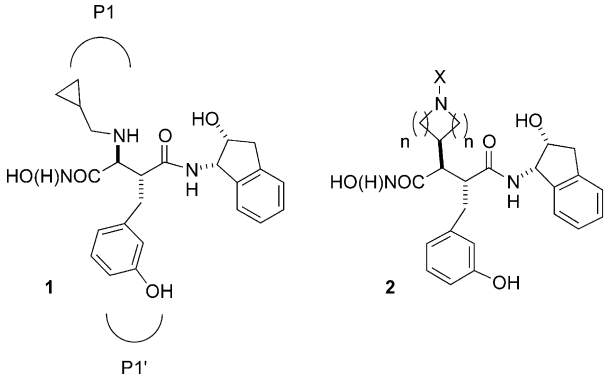

Figure 1. α-Amino (**1**) and cyclic P1 (**2**) inhibitors of aggrecanase.

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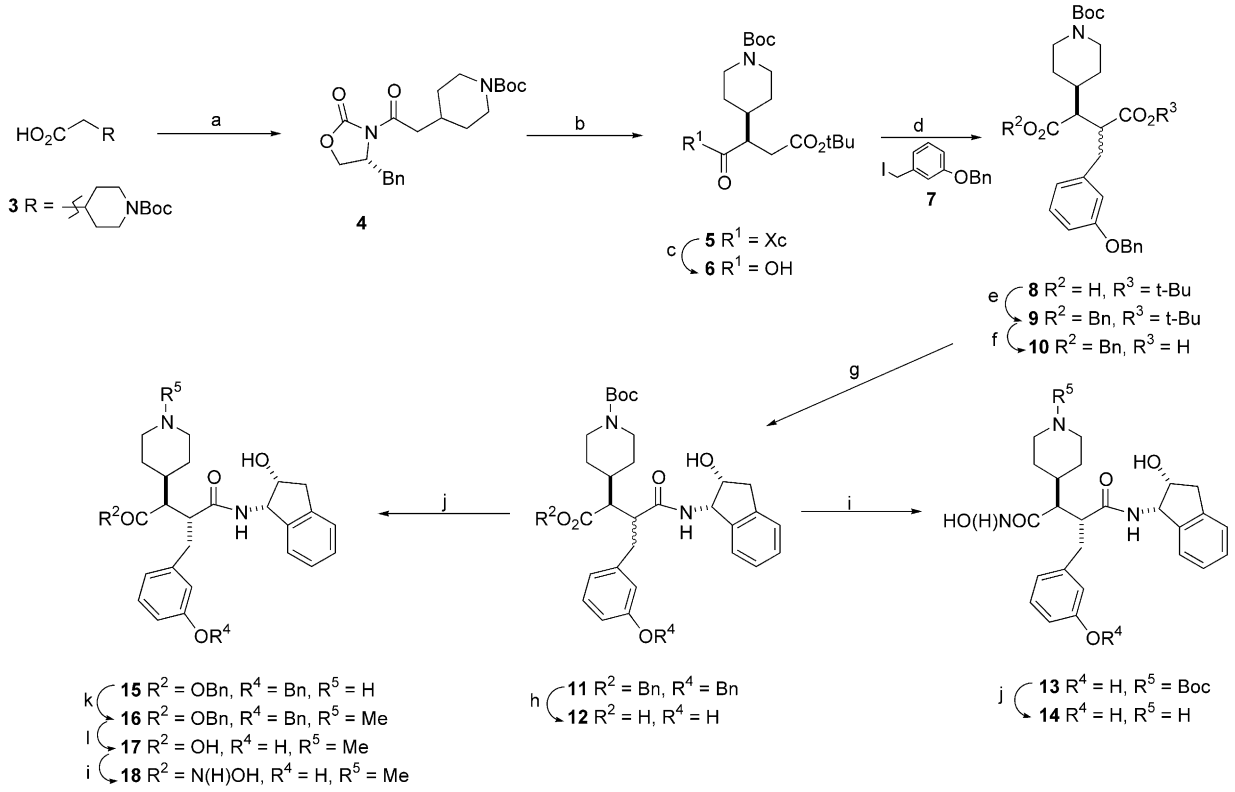
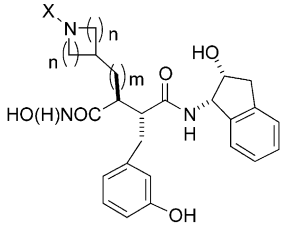


Scheme 1. Reagents and conditions: (a) LiCl, Piv-Cl, *R*-4-benzyl-2-oxazolidinone THF; (b) $NaN(TMS)_2$, $BrCH_2CO_2tBu$, THF, −78 to −22 °C; (c) H_2O_2, LiOH; (d) LDA, HMPA, **7**, THF, −78 °C to rt; (e) DBU, BnBr, Ph-H; (f) (i) TFA, CH_2Cl_2; (ii) Boc_2O, Et_3N, dioxane, H_2O; (g) BOP reagent (1*S*,2*R*)-*cis*-1-amino-2-indanol, DMF; (h) H_2, $Pd(OH)_2$, MeOH; (i) BOP reagent, H_2NOH–HCl, $(iPr)_2NEt$, DMF; (j) TFA, CH_2Cl_2; (k) 37% HCHO, $NaBH(OAc)_3$, DCE; (l) H_2, $Pd(OH)_2$, MeOH.

zyl-2-oxazolidinone.[14] Asymmetric alkylation of **4** with *tert*-butylbromoacetate gave the single diastereomer **5**. Hydrolysis gave the carboxylate **6** which was used in the next alkylation with benzyl iodide **7**. The resulting succinate **8** was isolated as a mixture of diastereomers (1:1) and was taken into the next step. A benzyl ester was installed to afford **9** before the *tert*-butyl ester was converted to the carboxylate **10**. Treatment of **10** with BOP reagent and (1*S*,2*R*)-*cis*-1-amino-2-indanol gave the amide **11**. The benzyl groups were removed to **12**, and direct hydroxamate formation was accomplished with BOP reagent and hydroxylamine. The resulting anti-succinate **13** was isolated via reverse-phase HPLC. Treatment of **13** with TFA gave **14**. Other *N*-substituted analogues were synthesized from compound **11** by Boc removal with TFA to afford **15**. Using **18** as an example, reductive amination of **15** gave the N-Me **16**. As above, the benzyl ester was converted to the target hydroxamate **18** through the carboxylate **17**. Additional analogues differed only in the functionalization (step k) of **15** as acylation gave **20**, sulfonylation gave **23**, and carbamoylation gave **24**. The homologated analogues (**21** and **22**) and the azetidines (**25** and **26**) were made in a similar manner starting, in each case, from the requisite substituted acetic acid **3**.

The newly synthesized hydroxamates were evaluated as aggrecanase inhibitors before ADAM-TS-4 and ADAM-TS-5 were isolated. At the time, a bovine derived aggrecanase assay (measuring total aggrecanase activity: ADAM-TS-4 plus ADAM-TS-5) was used to evaluate these hydroxamates.[11] We also used a counter screen of MMP-1, -2, and -9 to assess initial selectivity.[15] It was our desire to evaluate exclusively the aggrecanase clip site (and its relevance in arthritic disease), hence we required at least 100-fold selectivity for aggrecanase

Table 1. In vitro evaluation of P1 cyclic compounds



Compd	*m*	*n*	X	Agg[a] IC_{50} (nM)	MMP-9 K_i (nM)[a]
1	See Figure 1			12	4468
13	0	2	Boc	19	1302
12	See Scheme 1			>1000	>2128
14	0	2	H	28	4000
18	0	2	Me	210	>2128
19	0	2	Et	45	>2128
20	0	2	C(O)Me	29	1741
21	1	2	C(O)Me	114	>2128
22	1	2	C(O)Et	97	>2128
23	0	2	SO_2Me	3	2099
24	0	2	CO_2Et	7	832
25	0	1	H	7	>2128
26	0	1	$C(O)CF_3$	3	1490

[a]Values are an average from three determinations.

over the MMPs. The α-amino compound **1** displayed excellent selectivity over the MMPs as controlled by the phenol in P1′ and the indanol in P2′. From our modeling studies, the P1 group appeared to project toward the solvent exposed area of the active site, and hence we assumed cyclic substituents could be accommodated. As shown in Table 1, we were gratified to see compound **13** supported this model (aggrecanase $IC_{50} = 19$ nM) without disruption of the excellent MMP selectivity. In fact, **13** displayed greater than 100-fold selectivity over MMP-1 and -2 while maintaining 68-fold selectivity over MMP-9 (all the inhibitors of Table 1 displayed >100-fold selectivity for aggrecanase over MMP-1 and -2, hence only MMP-9 is shown). The hydroxamate was required for these inhibitors, as the carboxylate **12** was inactive. Removal of the Boc group gave the water soluble **14** with good aggrecanase affinity ($IC_{50} = 28$ nM) and greater than 100-fold selectivity versus MMP-1, -2, and -9. The N-Me (**18**) was detrimental toward aggrecanase affinity, as was the ethyl (**19**). Acylation (**20**) was equipotent in aggrecanase inhibition to **14**; however selectivity versus MMP-9 was somewhat compromised (60-fold for MMP-9). Homologation of the P1 ring away from the extended conformation was unfavorable as **21** and **22** lost affinity (3-fold) for aggrecanase versus **14**. Sulfonylation gave **23** which displayed a 9-fold (compared to **14**) increase in aggrecanase affinity while still maintaining excellent MMP selectivity. Carbamoylation was also beneficial as **24** displayed excellent MMP selectivity and was 4-fold more potent versus aggrecanase than **14**. Other rings were explored, and the azetidine (**25**) displayed excellent aggrecanase affinity (4-fold improvement over **14**) and MMP selectivity. Again installation of a carbonyl was beneficial, as the trifluoroacetamide **26** increased the aggrecanase affinity by 2-fold over **25** without affecting the MMP selectivity.

Targeting aggrecanase inhibitors was an unproven mechanism within a clinical setting, and therefore our goal was to advance compounds not only into the clinic, but also into our in vivo rat efficacy models for proof of principal studies. Hence, we needed a subset of compounds with acceptable rat pharmacokinetics (iv or po) for these studies. Although compound **1** displayed excellent dog pharmacokinetic it proved to be a poor rat compound characterized by high clearance ($CL = 9.9$ L/h/kg). We assumed the hydroxamate underwent glucuronidation (or another biotransformation) and/or hydrolysis which contributed to the high clearance. Cyclic P1 substituents were installed to shield the hydroxamate from such transformations and to decrease hydroxamate hydration in order to aid intestinal permeability.[16,17] Compound **14** was selected for pharmacokinetic studies in rat and dog as a result of its water solubility, good aggrecanase affinity, and excellent MMP selectivity. As shown in Table 2, **14** had a dramatic lowering in the rat systemic clearance as compared to **1** while displaying a 4-h half-life. Although this data supported its use as an iv proof of principal compound, the bioavailability in rat appeared to be minimal. Compound **14** was also studied in the dog and proved to be not only low clearance but also bioavailable ($F\% = 20\%$).

Table 2. Rat and dog pharmacokinetic data for **14**[a]

	Parameters	Rat	Dog
iv	Dose (mg/kg)	5.0	0.5
	Cl (L/h/kg)	0.5	0.1
	$t_{1/2}$ (h)	4.0	3.8
	V_{ss} (L/kg)	0.3	0.2
po	Dose (mg/kg)	10.0	1.0
	t_{max} (h)	1.5	4.3
	$F\%$	1%	20%

[a]Data is averaged from two dosings.

In summary, we have identified anti-succinate hydroxamates containing P1 cyclic moieties as potent and selective aggrecanase inhibitors. Cyclic substituents containing a properly displayed carbonyl group were found to be the most potent inhibitors. The cyclic moieties were also effective in creating low clearance compounds that should aid in the development of future aggrecanase inhibitors.

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