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Orally active achiral *N*-hydroxyformamide inhibitors of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) for the treatment of osteoarthritis

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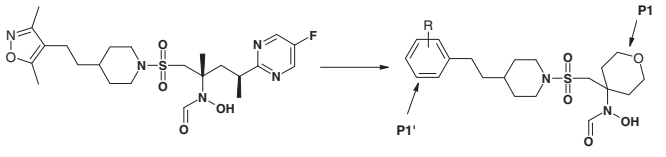
ABSTRACT

A new achiral class of *N*-hydroxyformamide inhibitor of both ADAM-TS4 and ADAM-TS5, **2** has been discovered through modification of the complex P1 group present in historical inhibitors **1**. This structural change improved the DMPK properties and greatly simplified the synthesis whilst maintaining excellent cross-MMP selectivity profiles. Investigation of structure–activity and structure–property relationships in the P1 group resulted in both ADAM-TS4 selective and mixed ADAM-TS4/5 inhibitors. This led to the identification of a pre-clinical candidate with excellent bioavailability across three species and predicting once daily dosing kinetics.

Osteoarthritis (OA) remains the most common form of arthritis and is the leading cause of disability in elderly adults both in the United States[1] and the United Kingdom.[2] Treatment options are limited to steroidal and non-steroidal anti-inflammatory drugs (NSAIDS)[3] and although providing symptomatic relief for pain and inflammation in arthritis they have failed to modify the progression of the disease.[4,5] The goal of OA treatment is to control symptoms, prevent disease progression, minimize disability and improve the quality of life. It is believed that inhibition of aggrecanase has the potential to prevent further damage to the joint and/or slow disease progression by stopping cartilage catabolism. 'Aggrecanase-1' (ADAM-TS4)[6] and 'Aggrecanase-2' (ADAM-TS5)[7] have been identified as the most likely members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin) family to be responsible for cartilage aggrecan degradation and remain attractive drug targets for intervention in OA.[8,9] ADAM-TS5 is responsible for disease progression in a surgically-induced model of OA in mice[10] nonetheless the relative individual contributions of ADAM-TS4 and ADAM-TS5 proteases to cartilage pathology in human disease is not fully understood.[11] We have previously reported the discovery of a potent series of chiral *N*-hydroxyformamide ADAM-TS4 inhibitors with excellent selectivity versus all tested matrix metalloproteinases which led to the identification of an advanced pre-clinical candidate **1**.[12] The strategy of the subsequent medicinal chemistry programme was to explore SAR for both ADAM-TS4 and ADAM-TS5.

We now wish to report further advances in improving DMPK properties whilst maintaining cross-MMP selectivity and significantly simplifying the core structure associated with **1** via modifications to the P1 group, ultimately leading to simple achiral homologues **2**.



1 2

Compound **1** was shown to be a potent inhibitor of both ADAM-TS4 and ADAM-TS5, >1000× selective versus all tested metalloproteinases (with respect to ADAM-TS4). It possessed only moderate oral bioavailability in mouse and dog and poor bioavailability in rat. Although a stereoselective synthesis of **1** was achieved, the synthesis consisted of 17 discrete synthetic transformations from

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commercially available starting materials. The optimised medicinal chemistry route was used to deliver >320 g of drug substance for early rat and dog toxicology profiling but developing a cost-efficient route suitable for multi-kilogram production was a significant challenge.

The primary goal therefore was to design a molecule that maintained all the desirable properties associated with **1** (viz. ADAM-TS4 potency and excellent cross-MMP selectivity) but with significant reductions to synthetic complexity and associated cost-of-goods. In addition, a molecule with higher bioavailability across three pre-clinical species was highly desirable in order to increase confidence in achieving good oral exposure in humans.

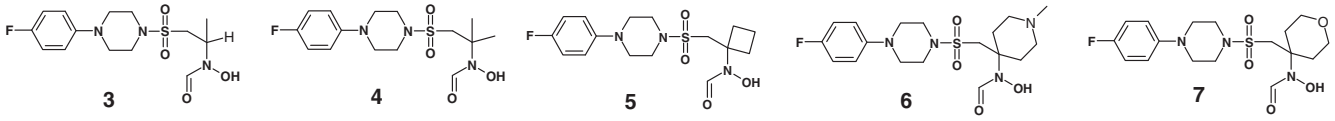
Previously, we showed that the addition of a methyl group adjacent to the *N*-hydroxyformamide to produce a quaternary reverse hydroxamate significantly reduced Cyp 3A4 activity consistently across multiple series (ethyl piperidine and piperazines).[12] This interesting finding prompted us to explore other sterically encumbered reversed hydroxamates to further understand Cyp 3A4 SAR (Fig. 1). By focusing on molecules with symmetry at the reverse hydroxamate the problem of controlling chirality is removed. The fully substituted *gem*-dimethyl compound **4**, is not a potent Cyp 3A4 inhibitor unlike its di-substituted analogue, racemate **3**. On the other hand, the cyclobutyl analog **5** is presumably due to the angle strain of 60° between carbon atoms associated with the cyclobutane providing less steric hindrance. The cyclic analogues, piperazine **6** and tetrahydropyran **7** are also free of Cyp 3A4 inhibition.

Due to both the clean Cyp3A4 profile and the good ADAM-TS4 potency associated with tetrahydropyran **7**, we chose to fix the tetrahydropyran P1 group and explore P1′ SAR. A generalized convergent scheme for the synthesis of analogues **13** is shown in Scheme 1.

The triphenylphosphonium halides **8** were sourced commercially or could be accessed from the reaction of a commercial aryl or heteroaryl halide with PPh_3 using standard conditions.[13] The ylides were formed in the presence of KO^{t}Bu and reacted with 1-(methylsulfonyl)piperidine-4-carbaldehyde[14] to generate the corresponding alkene **9** as a mixture of *E*/*Z* isomers in high yield (70–80%). Typically, the crude alkenes were subjected to hydrogenation to form the Peterson precursor sulfonamide **10** in 95–100% yields. The Peterson reaction of **10** with tetrahydropyran-4-one proceeded smoothly to deliver alkene **11** which was subjected to reaction with hydroxylamine to deliver compound **12**. Subjection of **12** to acylation conditions described by us previously[12] generated the final reverse hydroxamates **13** in moderate to good yields. This synthesis of achiral reverse hydroxamates consists of only nine discrete steps (vs 17 steps for synthesis of chiral compound **1**) and results in high overall yields of up to 44% of final compounds.

Analogues **13a–n** were tested for inhibition of both ADAM-TS4 and ADAM-TS5 and a selection of MMPs to elucidate aggrecanase potency and selectivity. The majority of P1′ groups in this SAR exploration had previously been used in the fluoro-pyrimidine piperidine series **1**,[12] enabling a Matched Molecular Pair Analysis to be performed.[15] Generally, the ADAM-TS4 potency of the pyran P1 compounds was reduced by 1 to 1.5 log units compared to their fluoropyrimidine P1 matched pair analogues (Fig. 2, mean difference in ADAM-TS4 $pIC_{50} = -1.6$, standard error of mean = 0.24, $N = 14$ pairs), however the compounds maintained good to excellent selectivity profiles versus the MMPs tested (Table 1). The most selective analogues were compounds **13b**, **13l** and **13n** achieving up to >7000-fold selectivities across all MMPs tested.

Substituting the pendant phenyl ring in both the *ortho*- and *para*-positions is important to obtain optimum selectivity for MMP13 and MMP14—the simple phenyl homologue **13e** has moderate ADAM-TS4, MMP13 and MMP14 potency. The addition of an *ortho*-methyl to the aryl group (**13f**) significantly improves ADAM-TS4 potency and introduces moderate selectivity over MMP13. The addition of a further substituent to the *para*-position (**13a** and **13c**, chloro and sulfone groups respectively) significantly improves selectivity over both MMP13 and MMP14, whilst maintaining very good ADAM-TS4 activity. Generally, the pyridyl analogues (**13d**, **13g**, **13h**, **13i**) were less potent than analogues containing substituted aryl P1′ groups. Moving the pyridyl nitrogen from the 3 to



3
Cyp 3A4 IC_{50}: 1 μM
ADAM-TS4 IC_{50}: nt
MMP-13 IC_{50}: 154 nM
MMP-14 IC_{50}: 226 nM
MMP-1 IC_{50}: >10 μM

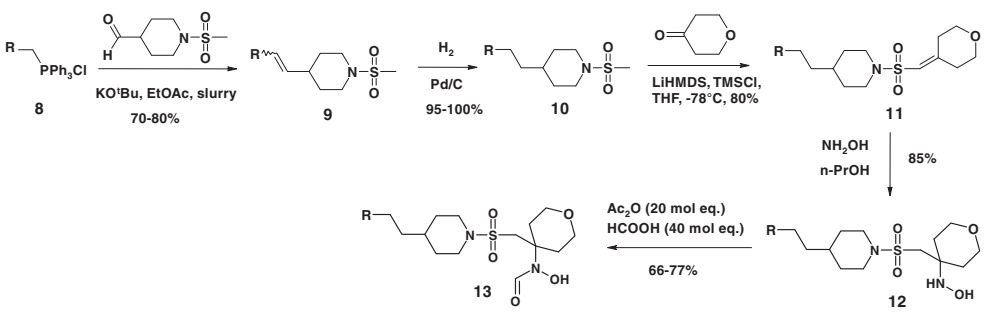
4
Cyp 3A4 IC_{50}: >10 μM
ADAM-TS4 IC_{50}: 92 nM
MMP-13 IC_{50}: 1400 nM
MMP-14 IC_{50}: 1400 nM
MMP-1 IC_{50}: >10 μM

5
Cyp 3A4 IC_{50}: 1.9 μM
ADAM-TS4 IC_{50}: 91 nM
MMP-13 IC_{50}: 81%[a]
MMP-14 IC_{50}: nt
MMP-1 IC_{50}: nt

6
Cyp 3A4 IC_{50}: >10 μM
ADAM-TS4 IC_{50}: 530 nM
MMP-13 IC_{50}: 570 nM
MMP-14 IC_{50}: 2900 nM
MMP-1 IC_{50}: >6700 nM

7
Cyp 3A4 IC_{50}: >10 μM
ADAM-TS4 IC_{50}: 21 nM
MMP-13 IC_{50}: 97%[a]
MMP-14 IC_{50}: nt
MMP-1 IC_{50}: nt

Figure 1. Cyp 3A4, ADAM-TS4 and MMP inhibitory activity for di-substituted reverse hydroxamates; nt = not tested; [a]percent inhibition of enzyme activity at 15 μM.



Scheme 1. General route to tetrahydropyrans **13**.

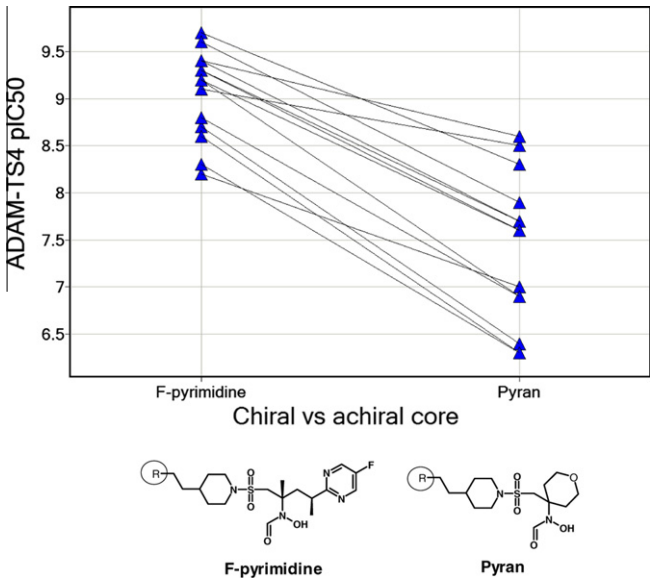


Figure 2. Matched pair analysis of ADAM-TS4 potency (pIC_{50}) versus sub-core structure (F-pyrimidine core vs achiral tetrahydropyran core); lines linking matched R group pairs.

4 position (**13k** vs **13l**) resulted in a significant increase in ADAM-TS4 potency and an excellent selectivity profile, suggesting this was an optimal position for the pyridyl variants. Five-membered heterocycles **13j** and **13n** also showed good to moderate potency versus ADAM-TS4 with good to excellent selectivity profiles compared to multiple MMPs (this was also seen for matched pair compounds in the historical fluoropyrimidine series). The majority of compounds tested were only moderate inhibitors of ADAM-TS5, nonetheless 2-methylphenyl derivative **13f** was potent versus ADAM-TS5 (IC_{50} = 31 nM) but suffered from a poorer cross-MMP selectivity profile. Furthermore, compounds **13b** and **13l** were potent ADAM-TS4 inhibitors showing enhanced selectivity over ADAM-TS5 (700- and 200-fold selectivity ratios, respectively). A comprehensive exploration of novel cyclic P1 groups was next undertaken to probe the S1 pocket and its impact on ADAM-TS4 and -TS5 selectivity (Table 2). From previous ADAM-TS1 protein X-ray crystal structures and modelling to ADAM-TS4 it was known there are polar residues around this area for potential interactions, and also extending this group would eventually access a solvent channel so this could also be an ideal physical property modifying handle. The P1′ group was fixed as the 3,5-dimethylisoxazolyl moiety (a potent and selective group) and P1 changes primarily focused on symmetrical cyclic groups that were predicted not to introduce major clashes and be complementary with the S1 pocket of ADAM-TS1 from modelling, and could be synthesised from readily available ketone precursors.[17] As predicted all compounds (**14a–14l**) tested were quite selective compared to other MMPs tested, presumably due to the selective isoxazole P1′ group common to all compounds. Modest increases in ADAM-TS4 potency were achieved in analogues **14a**, **14e** and **14i** (cf. lead compound **13n**). However, the more lipophilic cyclohexyl derivative (**14l**) offered no particular advantages in potency or selectivity. Four-, five- and seven-membered variants (**14c**, **14k** and **14d**) were all less potent in ADAM-TS4.

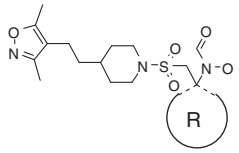
Compound **14e** showed potent ADAM-TS4 inhibition, good ADAM-TS5 potency and excellent selectivity albeit it suffered from high in vitro metabolic turnover. We tried to optimize this desirable TS4/TS5 mixed profile by synthesising a range of compounds with P1′ groups, which had previously shown good

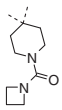
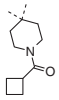
Table 1
ADAM-TS4, ADAM-TS5, cross-MMP inhibitory activity and in vitro/in vivo rat PK of piperidine ether analogues of **13**

Compds	*R*	ADAM-TS4 IC_{50},[a] nM	ADAM-TS5 IC_{50},[a] nM	MMP-2 IC_{50},[b] nM	MMP-13 IC_{50}, nM	MMP-14 IC_{50}, nM	MMP-9 IC_{50}, nM	Hu Cl_{int},[c] (μL/min/mg)	Rat Cl_{int},[d] (μL/min/10^6 cells)	Rat Cl[e] (mL/min/kg)	$t_{1/2}$ h
13a	4-Chloro-2-methyl-phenyl	2.7	65	1400	780	>10,000	1200	–	–	–	
13b	2-Cyclopropyl-4-methylsulfonyl-phenyl	1.4	950	>10,000	>10,000	>10,000	>10,000	3.7	–	–	
13c	2-Methyl-4-methylsulfonyl-phenyl	5.8	530	3300	1700	>10,000	>10,000	<2	<2	–	
13d	4-Methyl-3-pyridyl	110	630	3500	5900	>7200	>10,000	3.4	–	–	
13e	Phenyl	470	1700	150	260	1200	670	8.8	–	–	
13f	2-Methylphenyl	5.6	31	620	>600	>770	>1800	<2	–	–	
13g	3-Methyl-2-pyridyl	100	580	1100	1100	2900	8500	3.8	–	–	
13h	2-Methyl-3-pyridyl	400	5400	3800	2000	8000	>10,000	<2	–	–	
13i	4,6-Dimethyl-3-pyridyl	130	1400	7600	>10,000	>10,000	8900	–	–	–	
13j	3,5-Dimethylisothiazol-4-yl	13	450	>10,000	>10,000	>10,000	>10,000	8.5	16.7	18	6.3
13k	2,5-Dimethyl-3-pyridyl	380	5200	>10,000	8400	>10,000	>10,000	–	–	–	
13l	2,5-Dimethyl-4-pyridyl	18	3400	61,000	46,000	>100,000	>100,000	4.3	<2	20	4
13m	4-Fluoro-2-methyl-phenyl	4.5	69	1300	940	3100	3100	<2	11	15	nv
13n	3,5-Dimethylisoxazol-4-yl	26	860	>23,000	>31,000	>23,000	>25,000	3.1	3.6	24	8.9

[a] IC_{50}'s were derived from triplicate measurements whose standard errors were normally <5% in a given assay. Assay to assay variability was within twofold based on the results of a standard compound.
[b] All compounds tested for MMP1 were >10 μM.
[c] Cl_{int} reported is mean of two separate experiments; – = not tested; nv = no value.
[d] For procedure see Ref. 16.
[e] Compounds dosed at 2 mg/kg iv, *n* = 2 animals.

Table 2
ADAM-TS4/5 and cross-MMP inhibitory activity of P1 changes to compound **13n**



Compds	*R*	ADAM-TS4 IC_{50},[a] nM	ADAM-TS5 IC_{50},[a] nM	MMP-2 IC_{50},[b] nM	MMP-13 IC_{50}, nM	MMP-14 IC_{50}, nM	MMP-9 IC_{50}, nM	Hu Cl_{int}[c] (μL/min/mg)	Rat Cl_{int}[d] (μL/min/10^6 cells)	Cyp 3A4 IC_{50} μM
14a		11	480	>10,000	>10,000	>10,000	>10,000	31		
14b		29	310	>10,000	4600	>10,000	>10,000	6.5		
14c		51	1500	>10,000	>10,000	>10,000	>10,000	3.3		
14d		48	1500	>10,000	>10,000	>10,000	>10,000	59		
14e		10	270	>10,000	>10,000	>10,000	>10,000	65	92	>10
14f		20	410	>10,000	>10,000	>10,000	>10,000	13	126	>10
14g		16	270	>10,000	>10,000	>10,000	>10,000	4.9	30	>10
14h		24	820	>10,000	>10,000	>10,000	>10,000	<2		
14i		9.7	250	>10,000	>10,000	>10,000	>10,000	12	47	>10
14j		19	1200	>10,000	>10,000	>10,000	>10,000	<2	<2	>10
14k		52	1900	>10,000	>10,000	>10,000	>10,000			
14l		20	850	>10,000	>4100	>7500	>10,000			

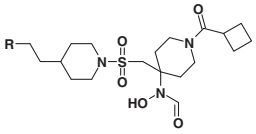
[a–d] See footnotes from Table 1.

ADAM-TS5 potency (see **13a**, **13f** and **13m**) and the P1 group from **14e** (Table 3). The majority of these P1′ groups have an *ortho*-methyl group with/without a halogen in the *para*-position. These compounds represent potential probe compounds to test the hypothesis that a dual aggrecanase inhibitor void of collagenase activity is beneficial for treating OA.

The achiral reverse hydroxamate compound class was relatively stable in both human microsomes and rat hepatocytes and a selected number of examples (**13j**, **13l**, **13m** and **13n**) were dosed in vivo to rat (see Table 1). These selected compounds all showed moderate clearance with V_{dss} generally within the range of 2–3 L/kg leading to good iv half-lives. Isoxazole **13n** showed particularly good $t_{1/2}$ (8.9 h) in rat which warranted both oral profiling and further investigation in other species.

Compound **13n** showed further good pharmacokinetic properties in both mouse and dog (Table 4) showing low to moderate clearance with high volumes and relatively prolonged elimination half-lives ($t_{1/2}$ = 2.6, 8.9 and 19.1 h in mouse, rat and dog, respectively) coupled with excellent oral absorption in all pre-clinical species dosed (*F*% 45–54; F_{abs} 0.62–1.0). A small amount of excretion of parent compound was observed in rat and dog urine and no parent compound was detected in rat bile. Cl_{int} data generated in hepatic microsomes and hepatocytes from both species predicted in vivo clearance well (within twofold of observed data). Taken together, these observations suggested that elimination of this compound was predominantly via hepatic metabolism. Furthermore, compound **13n** showed no evidence of competitive or time dependent CYP inhibition or induction of major inducible CYPs and no evidence for the presence of reactive products. Assuming that free exposure must exceed fivefold ADAM-TS4 IC_{50} over the entire dosing period, human doses of **13n** required for efficacy were predicted to be low (7–126 mg/day b.i.d.), a result of good predicted bioavailability (68%), low human clearance (3.5 mL/min/kg) and moderate volume (5.2–10.6 L/kg), resulting in an elimination half-life entirely consistent with either once or twice daily dosing (range 17–60 h).[19]

Table 3
ADAM-TS4 and ADAM-TS5 inhibitory activities of selected achiral analogues of **14e**



Compds	*R*	ADAM-TS4 IC_{50}, nM	ADAM-TS5 IC_{50}, nM
15a	4-Fluoro-2-methylphenyl	1.9	11
15b	2-Methylphenyl	4.1	13
15c	4-Chloro-2-methylphenyl	1.2	20
15d	4-Fluoro-2-(trifluoromethyl)phenyl	8.7	61
15e	2-Methyl-4-(trifluoromethyl)	1.2	79

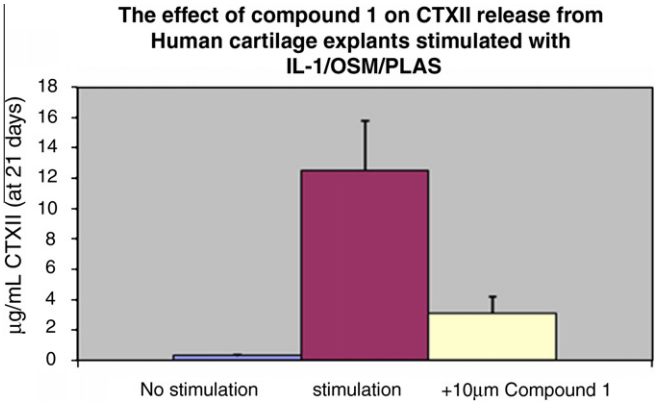


Figure 3. Human cartilage was stimulated with interleukin-1, oncostatin-M and plasminogen for 21 days in the absence or presence of 10 µM compound **1**.

A detailed comparison of the profile of compound **13n** with the more complex chiral compound **1** was performed Although compound **13n** is approximately 40-fold less potent in ADAM-TS4 it maintains an excellent selectivity profile vs all MMPs tested, has low in vitro clearance across three species, has far superior free levels in all three pre-clinical species (f_u = >0.5), and displays superior bioavailability across three clinical species (mouse, rat and dog) ultimately leading to predicted once daily human dosing profiles. The significantly improved absorption profiles of achiral compound **13n** is intriguing and we hypothesise that this could be due to a combination of overall molecule properties of compound **13n** compared to compound **1**: (1) molecular weight (MW_t) is significantly lower[20] (MW_t 429 vs 511), (2) one less aromatic ring,[21] (3) one less rotatable bond, (4) higher fraction of sp^3 C atoms[22] (0.70 vs 0.84, compounds **13n** and **1**, respectively). Furthermore, due to the removal of the two chiral centres in compound **1** and the improved yields in key synthetic steps, compound **13n** is also significantly cheaper to synthesise on multi-kg scale. Although both compounds **1** and **13n** represented excellent pre-clinical candidates in terms of their pharmacokinetic, enzyme selectivity and early in vitro safety profiles, it was important to demonstrate their ability to inhibit breakdown of aggrecan and collagen in disease-relevant tissues.

Ex vivo cartilage explant studies using selective aggrecanase and MMP inhibitors have demonstrated that selective inhibition of aggrecanases results in longer-term protection of the collagen network.[23]

The production and release of the aggrecanase specific neo-epitopes, NITEGE and AGRSVIL, was studied in ex vivo cartilage explant cultures. A good correlation between NITEGE and ARGSVIL readouts has been demonstrated in human articular cartilage with compound **1** (data not shown). Compound **13n** was shown to inhibit both the spontaneous aggrecan degradation in late stage diseased OA cartilage (IC_{50} for release of ARGSVIL neo-epitope: 150–350 nM) and IL-1 stimulated human OA cartilage (IC_{50} for release of ARGSVIL neo-epitope: 120–218 nM).

Compound **1** inhibited NITEGE neo-epitope release in IL-1 stimulated human articular cartilage (IC_{50} = 243nM)[12] and was also used to demonstrated protection of the collagen network. This was determined by reduction of type II collagen C terminal telopeptide (CTXII)[24] release in the presence of compound **1** at day 14 and 21 post stimulation with IL-1, oncostatin-M and plasminogen (Fig. 3). These data support the hypothesis that protection of cartilage aggrecan may ultimately lead to preservation of the intertwined collagen network by sterically hindering the action of collagenases.[23]

Compound **1** was also studied in the Dunkin Hartley guinea pig model of spontaneous osteoarthritis.[25] In these studies compound

Table 4
Profiles of advanced compounds **1** and **13n**

Parameter	**1**	**13n**
ADAM-TS4/TS5 IC_{50}[a]	0.7/23 nM	26/860 nM
Fold selectivity MMP1,2,9,13,14 IC_{50}[a]	>5000×	>5000×
Log *D*	1.6	0.9
Solubility (µM)[b]	150	>1000
Hu/rat/dog %free (%free)	11/22/25	>50 (3/3)
Hu Mics[c]/Heps Cl_{int}[d]	29.4/<2	3.1/<2
Cyp Inhib pIC_{50}[e]	>10 µM (5/5)	>10 µM (5/5)
hERG IC_{50}[18]	65 µM	>100 µM
Caco-2 (@10 µM)[f]	5.3×10^{-6} AB/10.9×10^{-6} BA	8.3×10^{-6} AB/–
Mouse PK $Cl/t_{1/2}/V_{dss}$	16.8 mL/min/kg/5.4 h/7.2 L/kg	30 mL/min/kg/2.6 h/2.5 L/kg
Rat PK[g] $Cl/t_{1/2}/V_{dss}$	11 mL/min/kg/11 h/7.4 L/kg	24 mL/min/kg/8.9 h/3.0 L/kg
Dog PK[h] $Cl/t_{1/2}/V_{dss}$	20.7 mL/min/kg/11.3 h/9.2 L/kg	13 mL/min/kg/19.1 h/10.9 L/kg
Mouse/rat/dog *F*%	34/1/20	54/45/52
Human pDTM dose (mg/day)	14 b.i.d	7–126 b.i.d; 63 qd

[a] For general assay procedures see Ref. 12. IC_{50}'s were derived from at least six measurements whose standard errors were normally <5% in a given assay. Assay to assay variability was within twofold based on the results of a standard compound.
[b] Thermodynamic solubility (µM) in 0.1 M phosphate buffer pH 7.4 at constant temperature (25 °C) for 24 h.
[c] Human microsome metabolism intrinsic clearance Cl_{int} (µL/min/mg). Cl_{int} reported is mean of eight separate experiments.
[d] Human hepatocyte metabolism intrinsic clearance Cl_{int} (µL/min/10^6 cells). Cl_{int} reported is mean of six separate experiments.
[e] Inhibition of cytochrome P450 isoforms: 1A2, 2C9, 2C19, 2D6 and 3A4. NA = not active.
[f] Apparent permeability (P_{app} cm/s $\times 10^{-6}$) in human cancer colon cells at 10 µM/pH7.4. AB—apical to basolateral direction; BA—basolateral to apical direction (efflux).
[g] Compounds dosed at 2 mg/kg iv, *n* = 2 animals and 3–10 mg/kg po, *n* = 4 animals.
[h] Compounds dosed at 1 mg/kg iv, *n* = 4 animals and 1 mg/kg po, *n* = 4 animals.

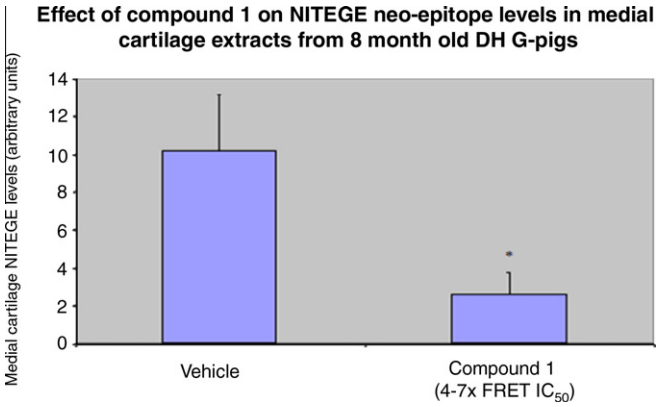


Figure 4. Compound **1** was administered for 7 days by sub-cutaneous mini-pump after which articular cartilage was dissected from the medial tibial plateau of the hind knee joints. Levels of NITEGE neo-epitope were measured in the cartilage extracts (P <0.05*).

1 significantly reduced NITEGE levels in medial cartilage when compared to vehicle treated animals at exposures equivalent to steady state free plasma concentrations 4–7-fold cover over the human ADAM-TS4 FRET IC_{50} (Fig. 4).

In summary, we have discovered a novel series of achiral *N*-hydroxyformamide inhibitors of ADAM-TS4 and ADAM-TS5 with improved pharmacokinetic properties and more cost-efficient large scale synthesis compared to our previous chiral series. Emerging SAR has led to ADAM-TS4 selective and equipotent ADAM-TS4/TS5 potent compounds. Compound **13n** is a potent and highly selective aggrecanase inhibitor with pharmacokinetic properties predicted to be consistent with once daily dosing in humans, and thus has the potential to test the hypothesis that inhibition of aggrecanase activity will have therapeutic benefits in OA. Preliminary testing of compound **1** supports the hypothesis that treatment of diseased cartilage with a selective aggrecanase inhibitor also leads to preservation of the collagen network.

References and notes

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