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DESIGN AND SYNTHESIS OF THE CARTILAGE PROTECTIVE AGENT (CPA, Ro32-3555)

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Abstract: A novel series of MMP inhibitors has been identified. The compounds are potent selective inhibitors of collagenase with good solubility and oral bioavailability. One compound, designated Ro32-3555, has been selected for development as a cartilage protective agent for use in the treatment of rheumatoid- and osteo-arthritis. © 1997 Elsevier Science Ltd.

The matrix metalloproteinases (MMP's) are a family of zinc containing proteinases that have the combined ability to degrade all of the components of cartilage. The key irreversible step in this process is the cleavage of triple helical collagen by collagenase. This rationale has provided the impetus for the design and syntheses of a large number of inhibitors of the MMP's. [1,2] Many of these compounds have excellent potencies against collagenase induced cleavage of triple helical collagen fibrils but poor oral bioavailability [3] has resulted in few of these compounds being tested in animal models of arthritis.[4] Ro31-9790 (**9a**) [5], a potent inhibitor of human collagenase, protected bovine nasal cartilage explants against degradation induced by interleukin 1α [6,7]. It was also active in the *in vivo* sponge/cartilage degradation model[8] and in P. Acnes induced arthritis in rats [9] when given orally. Although Ro31-9790 was a drug candidate, there were still a number of properties which required optimisation. The compound had low solubility, oral bioavailability was not ideal and a drug related histopathological event in the joints was observed in rats. This report describes the strategy employed to modify Ro31-9790 in order to obtain a follow-up compound with improved physicochemical, pharmacokinetic and toxicological properties.

Synthesis[5,10,11]

The inhibitors were prepared using the route outlined in the scheme. The starting material was a 2(R)-hydroxyacid (**1**). Protection of the carboxylate as a benzyl ester, conversion to the triflate and reaction with the sodium salt of benzyl tertiary-butylmalonate gave the selectively protected triester (**2**). Alkylation with a bromide gave the triester derivative (**3**) and hydrogenation gave the diacid (**4**). Decarboxylation in the presence of one equivalent of N-methylmorpholine gave **5** as an unequal mixture of diastereoisomers, favouring the required isomer. The ratio varied from 3:1 to greater than 10:1 depending on the nature of the substituents on the succinate. The succinate was coupled to an amine, to give **6** and the minor isomer was removed by

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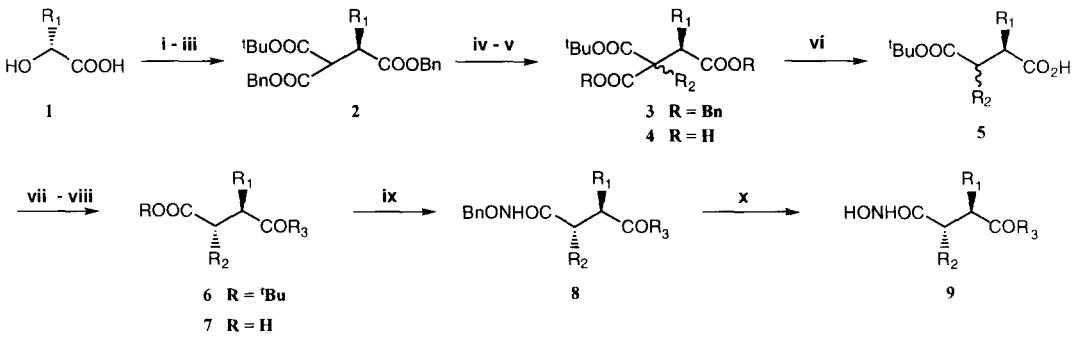
chromatography. Treatment with trimethylsilyltrifluoromethanesulphonate / triethylamine[12] gave (**7**) which was reacted with O- benzylhydroxylamine to give (**8**). Hydrogenolysis gave the inhibitor (**9**).

Scheme



Reagents : i:BnBr / NEt_3 / EtOAc / reflux. ii: $(CF_3SO_2)_2O$ / pyridine. iii: benzyl t-butyl malonate / NaH. iv: alkyl bromide / NaH. v: H_2 / 5%Pd-C. vi: N-methylmorpholine / toluene / reflux. vii:amine / ethyl-3-(3-dimethylamino)-propylcarbodiimide (EDAC) / 1- hydroxybenzotriazole (HOBT). viii. $CF_3SO_2OSiMe_3$ / NEt_3 / dioxan / reflux. ix: $BnONH_2$ / EDAC / HOBT. x: H_2 / 5% Pd-C / EtOH.

Structure-activity relationships

For inhibitors based on the collagenase substrate sequence C-terminal to the cleaved amide, a one carbon spacer group between the hydroxamic acid function and the carbon bearing the P_1' [13] side-chain was optimal for potency.[2] The spacer could be substituted giving improved activity for the 2R,3R diastereoisomer. The compound containing the phthaloylaminomethyl group (**9c**) had significantly more activity than the alkyl containing compound (**9b**) and suggested an additional binding interaction between the phthalimide group and the enzyme. The phthaloyl group could be replaced by a range of imide containing heterocycles, for example, the trimethylhydantoin derivative (**9d**). The increased binding from this $P_1'(\alpha)$ group, allowed the replacement of the aminoacid amide group in the $P_2'P_3'$ subsites by simple amides, with retention of good *in vitro* potency against collagenase. In particular, cyclic tertiary amides (**9e**) retained good activity and possessed significantly different physicochemical properties to the earlier inhibitors. Further optimisation of the molecule, suggested by studies on the crystal structure of collagenase[14], resulted in the replacement of the isobutyl group in the P_1' site by the cyclopentylmethyl group (**9f**), which gave an improvement in binding to collagenase.

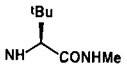
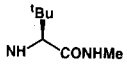
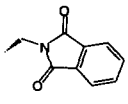
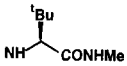
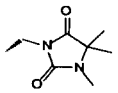
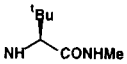
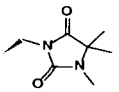
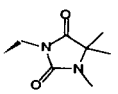
Selectivity of collagenase inhibitors

The compounds, which were designed as inhibitors of collagenase-1 (MMP1), have inhibitory activity against other members of the MMP family of enzymes. A selection of the compounds was tested against human stromelysin-1 (MMP3) and human gelatinase B (MMP9)[6,7,15]. The parent compound (**9a**) had good activity against collagenase and gelatinase but was less active against stromelysin. The incorporation of a simple alkyl group in the $P_1'(\alpha)$ position (**9b**) gave an improvement in potency against all three enzymes. The use of the phthaloylaminomethyl group (**9c**) had a marked effect on both the collagenase and stromelysin activities but gave no improvement against gelatinase. The tertiary amide containing compounds (**9e, 9f**), containing no $P_2'P_3'$ amide bond were more selective than earlier compounds. Compound **9e** had a selectivity of 215 for collagenase over stromelysin and 23 over gelatinase.

Oral bioavailability of selected inhibitors

The octanol/water partition coefficients (logD) of a range of hydroxamate containing inhibitors, selected on the basis of their structural diversity, were measured and their oral bioavailability (%F) was measured in the rat. The compounds which contain a secondary amide group (CONHMe) in $P_2'P_3'$ subsites, for example **9a-9d**, have poor oral bioavailability and there is no relationship between bioavailability and partition coefficient. Compounds from the new series, exemplified by **9e** and **9f**, have a range of oral bioavailabilities from 3% (logD -0.6) to 41% (logD 1.54) and the values correlate with the measured logD (r=0.92: n= 5).

Table: *In vitro* data and properties of inhibitors

	R_1	R_2	R_3	MMP 1	MMP 3 IC_{50} (nM) (n)	MMP 9	Sol. (mg/ml)	%F
9a		—H		**4.9** (99)	**160** (25)	**10.4** (29)	**0.8**	**3.0**
9b		—Me		**2.6** (5)	**47** (2)	**2.4** (2)	-	**2.7**
9c				**0.5** (8)	**9.1** (3)	**4.3** (3)	-	**1.0**
9d				**1.0** (2)	-	**2.8** (2)	-	**1.5**
9e				**10.1** (5)	**2175** (2)	**237** (6)	**>100**	**30**
9f				**7.0** (4)	**527** (3)	**59** (4)	**73**	**25**

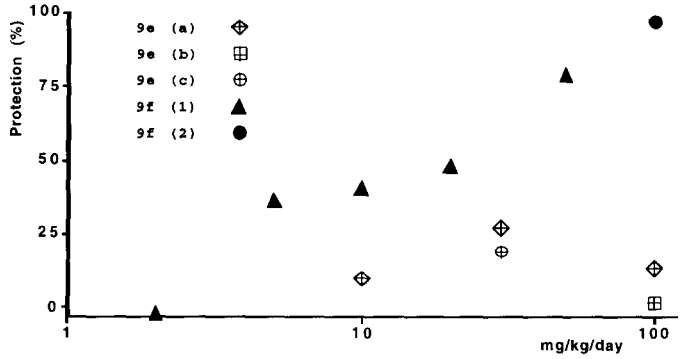
MMP1 = collagenase 1: MMP 3 = stromelysin 1: MMP 9 = gelatinase B: Sol. = solubility
%F- compounds detected by a specific HPLC-MS method

A number of the compounds studied have solubilities one to two orders of magnitude greater than that of the parent molecule (**9a**). For example, compounds **9e** and **9f**, have aqueous solubilities of greater than 70mg/mL.

Biology

In efficacy experiments *in vitro* [7], compounds **9e** and **9f** were equally effective in protecting cartilage explants against degradation induced by interleukin 1α, with IC_{50} values below 100nM. However, in the sponge-cartilage model in rats[8], there was a significant difference in activity between **9e** and **9f**. In three experiments (Figure: experiments a, b and c), compound **9e** showed only weak and non-dose related cartilage protection, while **9f** gave a dose-response (Figure: experiments 1 and 2) approaching 100% protection of the implanted cartilage, when administered orally at 100mg/kg/day .

Figure: Efficacy of 9e and 9f in the sponge-cartilage model[8] when given orally



Summary

The change in structure from a mono or di-substituted succinyl-aminoacid amide type inhibitor to a di-substituted succinic acid tertiary amide has resulted in compounds with selectivity for collagenase, oral bioavailability and increased solubility. The compound **9f**, designated Ro32-3555, has the best combination of potency, selectivity, oral bioavailability and low toxicity and has been selected for development as a cartilage protective agent (CPA). This compound could form the basis of a novel disease modifying drug for the treatment of rheumatoid and osteoarthritis.

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