Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1359–1364

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The synthesis of novel matrix metalloproteinase inhibitors employing the Ireland-Claisen Rearrangement

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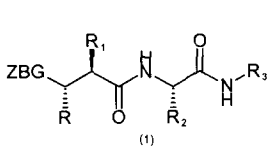
Received 17 February 1998; accepted 21 April 1998

Abstract: Matrix metalloproteinase inhibitors of general formula (1) were synthesised by a route involving an Ireland-Claisen rearrangement which enables systematic modification of the substituent alpha to the hydroxamic acid. An analogue (12c) possessing an α-cyclopentyl group is a potent broad spectrum inhibitor that displays high and sustained blood levels following oral dosing in both the rat and marmoset *ex-vivo* bioassays. This compound and analogues are also potent inhibitors of TNFα release.

Introduction

The matrix metalloproteinases (MMPs)[1] are a family of zinc containing proteinases that collectively can degrade all of the major components of the extracellular matrix. Over production of MMPs is thought to be responsible for a range of biological processes observed in diseases[2] such as the arthritides, tumour metastasis, periodontal diseases and multiple sclerosis.

In the course of our MMP inhibitor (MMPI) programme, derivatives of the pseudopeptide (1) that feature a zinc binding group (ZBG) and mimic the sequence of the natural substrate to the right of the cleavage site (P1'-P3') have been widely studied.[3] For this class of inhibitor the hydroxamic acid ZBG is preferred, leading to broad spectrum inhibitors of which marimastat[3a] (2) is one example. Marimastat (2) is an orally active compound that is currently in human clinical trials for the treatment of cancer.



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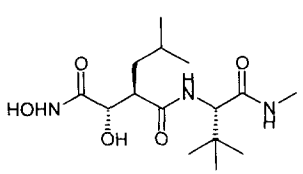

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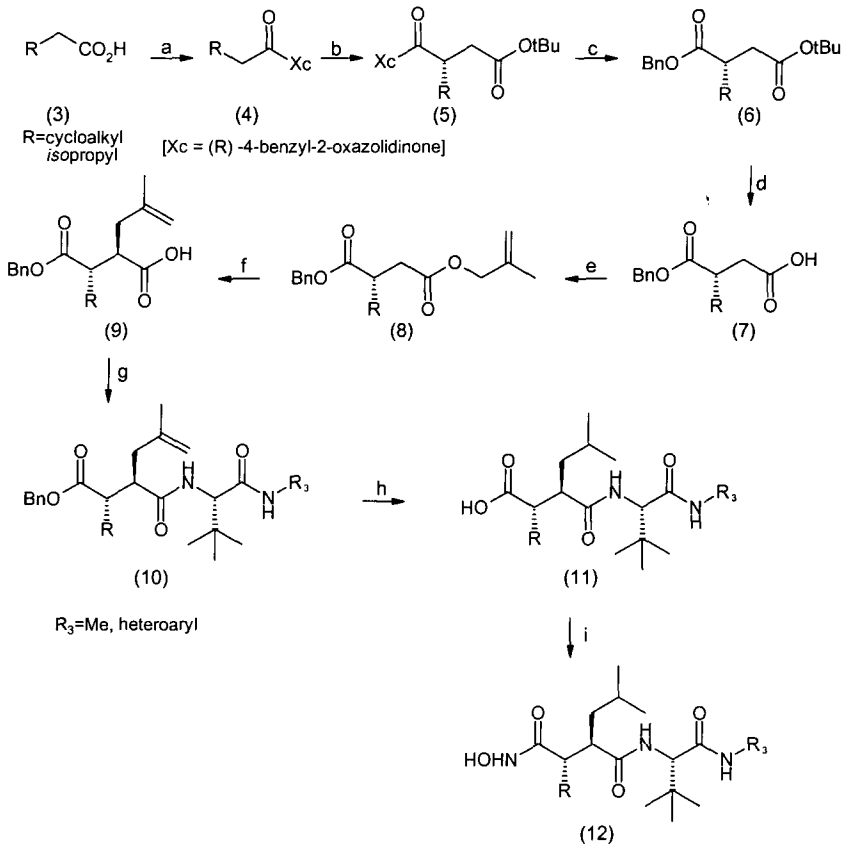
0960-894X/98/$19.00
PII: S0960-894X(98)00218-2

We were interested in the effect that the introduction of a bulky (secondary alkyl) α-substituent (R=cycloalkyl) might have on the *in vitro* and *in vivo* properties of analogues of marimastat (2). In this preliminary account we report on the use of the Ireland-Claisen rearrangement for the preparation of these analogues and their biological evaluation.

Results and discussion

The general strategy for the synthesis of the α-modified MMPIs is as shown in Scheme 1.



Scheme 1

Reagents and conditions: a) pivaloyl chloride, Et_3N, nBuLi, (*R*)-(+)-4-benzyl-2-oxazolidinone, THF, -78°C to rt overnight; b) *t*-butyl bromoacetate, NaHMDS, THF, -78°C to rt overnight; c) nBuLi, BnOH, THF, -5°C overnight; d) TFA/DCM, 4°C overnight; e) EDC, DMAP, DCM, 2-methyl-2-propen-1-ol, rt overnight; f) LDA,THF, -78°C 10 min, TESCl, -78 °C 10 min, warm and heat at 55°C overnight (yields 32-58% product); g) HOBt, EDC, *t*-butylglycine N-methyl amide, EtOAc, reflux 2h; h) Pd/C, H_2, EtOH, rt overnight; i) HOBt, EDC, DMF, hydroxylamine.HCl, NMM, rt overnight.

Synthesis

The required homochiral succinates (6) were prepared using methods based on the asymmetric alkylation

methodology of Evans.[4] Deprotection of the t-butyl ester (6) followed by esterification with 2-methyl-2-propen-1-ol generated the required unsaturated ester (8) as a single enantiomer. A stereoselective Ireland-Claisen rearrangement based on the methods described by Barlett *et al*[5] using the homochiral succinates (8) resulted in the formation of the desired 2,3-disubstituted succinates (9). The diastereomeric ratio of the products (9) from the Ireland-Claisen rearrangement varied depending on the nature of the cycloalkyl group present in the starting homochiral succinate (8). A single diastereomer was obtained when R= cyclopentyl while a 4:1 diastereomeric mixture resulted when R= cyclopropyl. These diastereomeric ratios were estimated from analysis of the ^{1}H nmr spectra of the coupled materials (10) as they could not be determined readily from spectroscopic analysis of the acids (9). The amides (10) were easily obtained by coupling of the acids (9) with either the N-methyl amide or N-heteroaryl amides of L-t-butyl glycine. Subsequent hydrogenation of the benzyl ester and concomitant reduction of the double bond generated the required acids (11). Conversion of the acids to the hydroxamic acids (12) was achieved by direct coupling using hydroxylamine. For comparison studies the isopropyl group was selected as a bulky alkyl substituent and compound (12i, R=iPr) was prepared in a similar manner.

Biological results

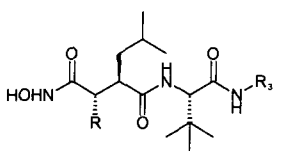
We were interested in exploring whether the introduction of bulky substituents alpha to the hydroxamic acid zinc binding group in a series of MMPIs would provide potent inhibition and increased oral bioavailability. Thus a series of inhibitors were prepared that incorporated a cycloalkyl substituent in the α-position (12a-h) as well as one example with a bulky alkyl substituent (12i). The *in vitro* MMP enzyme activities for these compounds are detailed in Table 1.[6] All compounds show broad spectrum MMPI activity similar to that observed for marimastat (2). The observation that certain MMPIs also inhibit the production of TNFα from activated cells, a process that is believed to involve a TNF convertase enzyme (TCE)[7a,b] prompted us to test this series of compounds for this activity. Promising inhibition of TNFα release from activated cells[7c] along with an improvement in activity against stromelysin-1 was observed for selected compounds (12b, c, d, e , g, i). The IC_{50} values for the corresponding carboxylic acids (11) were much higher, as expected, and are not included in this communication.

We were also interested in ascertaining whether the introduction of a P3' heteroaryl substituent would improve the level of TCE inhibition observed *in vitro* for the α-cycloalkyl series of compounds. We have previously observed[8] in the α-OH series that incorporation of a heteroaryl P3' substituent can give enhanced TCE activity. The *in vitro* MMPI enzyme assay results for this series are detailed in Table 1. The expected improvement in TCE activity was observed in compounds (12j, l).

The oral bioavailability of these inhibitors in a rat *ex-vivo* bioassay[9] was measured and the results are given in Figure 1 and Table 2. The cyclopentyl analogue compound (12c) displayed high blood levels in this model, in excess of those observed for marimastat (2). However, the other cycloalkyl analogues and the α-isopropyl

analogue (12i) had lower blood level profiles which had returned to baseline levels at the 24h time point. We thought that there may have been a correlation between the AUC and ClogP[10] but none was apparent. Further studies on compound (12c) in the marmoset (Cmax 20325.5 ng/ml at 1h and C@ 24h 4547.1 ng/ml; AUC 207256 ng/ml.h) confirmed the high sustained blood levels observed in the rat model. Interestingly, the heteroaryl P3' series proved not to be as orally bioavailable in the rat *ex-vivo* bioassay (eg 12j; Table 2) as compound (12c).

Table 1 *In vitro* results for a series of α-cycloalkyl analogues



Compound*	R	R_3	HFC MMP-1	Gel. A MMP-2	Strom-1 MMP-3	Matrilysin MMP-7	TCE†
2	OH	Me	5	6	200	20	3000
12a	cyclopropyl	Me	20	100	300	200	4600
12b	cyclobutyl	Me	3	2	50	20	3000
12c	cyclopentyl	Me	4	3	30	20	900
12d	cyclohexyl	Me	4	10	30	20	1350
12	4-methyl cyclohexyl	Me	4	9	40	7	1500
12f	4-*tert*-butyl cyclohexyl	Me	40	30	100	50	8000
12g	3-(tetrahydro thiophenyl)	Me	5	5	40	4	2000
12h	4-(N-methyl piperidinyl)	Me	30	3	500	200	-
12i	isopropyl	Me	1	1	40	5	800
12j	cyclopentyl	2-pyridyl	3	30	20	3	300
12k	cyclohexyl	2-pyridyl	4	10	20	-	300
12l	4-methyl cyclohexyl	2-pyridyl	5	40	20	3	800
12m	cyclopentyl	2-pyridyl	20	100	50	-	1160

(*All compounds gave satisfactory NMR spectra and analytical data. † Enyzme assays for MMP-1, MMP-2, MMP-3 and MMP-7 used human recombinant MMP enzymes according to standard protocols[6] whereas the TCE assay (TNFα convertase) involved phorbol 12-myristate 13-acetate (PMA) stimulation of U937 cells[7] and is therefore a cellular assay)

Figure 1 Rat *ex-vivo* bioassay graphs for compound (12c) and marimastat

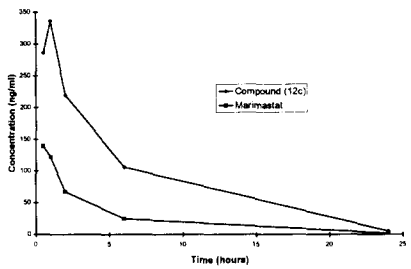


Table 2 Oral bioavailabilty data in the rat for selected inhibitors

Compound	R	R3	C_{max} (ng/ml)[time]	AUC (ng/ml.h)
2	OH	Me	139 [30min]	582.4
12a	cyclopropyl	Me	105.9 [30min]	1024
12c	cyclopentyl	Me	335.9 [1h]	2087
12d	cyclohexyl	Me	9.75 [2h]	155
12i	isopropyl	Me	376 [30min]	895
12j	cyclopentyl	2-pyridyl	70.7 [2h]	899

The oral activity of (12c) was confirmed in an *in vivo* LPS (bacterial lipopolysaccharide) challenge model in the rat.[11] Compared to marimastat (2), compound (12c) gave a consistently higher percentage inhibition of TNFα production when administered orally at various dose levels (Table 3).

Table 3 LPS challenge results

		TNFα %I @ (mg/kg po)		
Compound	R	2.5	5	10
2	OH	11	50	69
12c	cyclopentyl	94	92	98

Summary

In summary, we have developed a versatile new synthetic route[12] for the introduction of either novel α-cycloalkyl or α-alkyl substituents into succinic acid based MMPIs. We have demonstrated that α-cycloalkyl substituents in these succinic acid based MMPIs give broad spectrum enzyme activity. The α-cyclopentyl is a preferred substituent imparting both good bioavailability and a prolonged duration

of action. Furthermore, compound (12c) possesses a promising oral *ex-vivo* bioassay profile in the marmoset and potent activity in a rat LPS challenge model and is the subject of on going pharmocological evaluation.

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