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ORALLY ACTIVE INHIBITORS OF STROMELYSIN-1 (MMP-3)

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Abstract: Further development of *N*-carboxyalkyl dipeptide inhibitors of stromelysin-1 (MMP-3) led to the discovery of *C*-carboxyalkyl dipeptide analogs with improved oral bioavailability. An in vivo assay of human MMP-3 mediated degradation of a macromolecular substrate in an extravascular space is described and inhibition studies are reported.

Stromelysin-1 (MMP-3) is a member of a family of extracellular matrix-degrading zinc metalloenzymes (matrix metalloproteinases, MMPs) that have been implicated in the tissue destruction associated with a number of diseases.[1] In particular, MMP-3 mRNA and protein are found in very high levels in the synovial fluid and cartilage from rheumatoid and osteoarthritic patients.[2] This enzyme has a very broad activity profile for macromolecular substrates including proteoglycan and may be involved in a cascade of proteolytic activation of other members of the family. However, the concurrent presence of other MMPs in many of the same tissues with MMP-3 makes it difficult to delineate the specific role of each enzyme in a disease process or model thereof. Only by selective inhibition of each enzyme (or utilizing transgenic animals lacking the gene for each enzyme) can the individual contributions of each MMP family member be determined. Our goal was to obtain a selective, orally active inhibitor of stromelysin-1 with suitable pharmacokinetics that might prove useful in examing the role of this enzyme in models of cartilage degradation.

An in vivo model was designed to conveniently and rapidly measure the ability of an MMP-3 inhibitor to block the degradation of a macromolecular substrate in an extravascular space.[3] Radiolabeled transferrin was used as a convenient substrate and was injected into a mouse pleural cavity followed by the addition of activated human MMP-3. After an incubation period, the cavity was lavaged and the amount of MMP-3-mediated degradation of the transferrin was quantitated (*vide infra*). The inhibition of this degradation by MMP-3 inhibitors administered by intravenous (iv) or oral (po) routes could then be measured.

Our initial efforts towards the discovery of a selective, orally active inhibitor of MMP-3 led to the *N*-carboxyalkyl dipeptide **2**. This compound was sufficiently potent as to be effective by iv administration in a model MMP-3-mediated joint destruction in the rabbit.[4] However, the relatively poor oral activity of **2** in the mouse pleural cavity assay (ED_{50} ~ 100 mg/kg po) necessitated the continued pursuit of more potent MMP-3 inhibitors with better oral activity. The results of these efforts led to *C*-carboxyalkyl dipeptide inhibitors that were more potent in the mouse pleural cavity assay by oral administration than the corresponding *N*-carboxyalkyl dipeptide series and are described herein.

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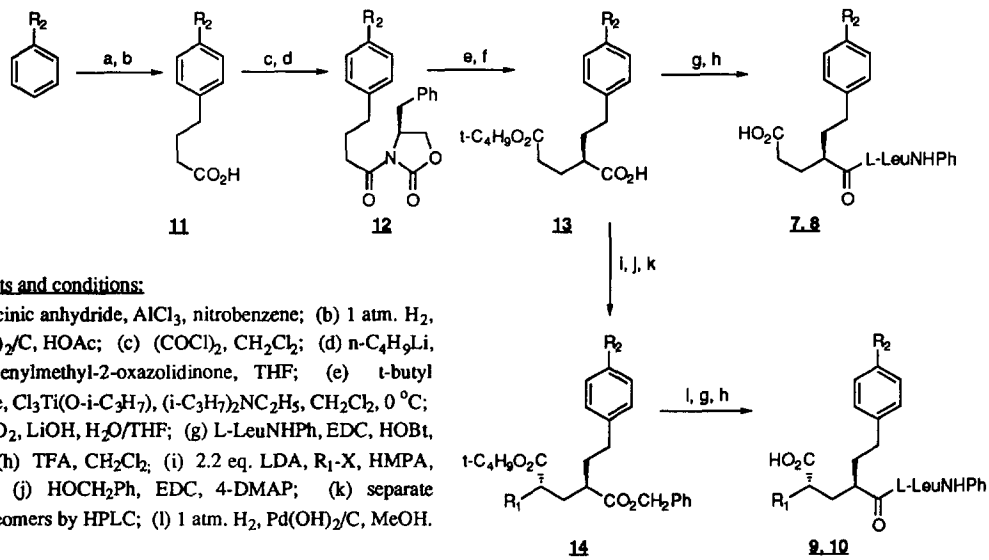
Chemistry

The synthesis of *N*-carboxyalkyl dipeptides **1-4** has been described.[5-7] Compounds **5** and **6** were prepared by analogous procedures. The *C*-carboxyalkyl analogs **7-10** (X = CH_2) were prepared as outlined in Scheme 1. Lewis acid catalyzed acylation of benzene derivatives followed by reduction of the aryl ketone gave 4-aryl-butyric acids **11**. The Evans chiral auxillary was added to yield **12**. Titanium isopropoxide trichloride mediated Michael addition of **12** to *tert*-butyl acrylate followed by lithium hydroperoxide removal of the oxazolidinone gave **13** as a single enantiomer. Standard peptide coupling conditions with L-leucine phenylamide and subsequent removal of the *tert*-butyl ester group gave **7** (R_2 = H) and **8** (R_2 = n-C_3H_7).

The dianion of **13** was generated with lithium diisopropylamide and quenched with excess alkyl halide (CH_3I when R_1 = CH_3 and 4-phthalimido-2-butenyl bromide when R_1 = 4-phthalimidobutyl) to give a ~3:1 mixture of diastereomers which were separated by HPLC as their benzyl esters. Hydrogenolysis of the benzyl esters (and catalytic hydrogenation of the olefin leading to **10**) followed by peptide coupling and cleavage of the *tert*-butyl ester with trifluoroacetic acid gave *C*-carboxylates **9** and **10**, respectively.

Scheme 1.



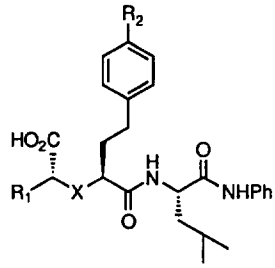
Reagents and conditions:
(a) succinic anhydride, $AlCl_3$, nitrobenzene; (b) 1 atm. H_2, $Pd(OH)_2$/C, HOAc; (c) $(COCl)_2$, CH_2Cl_2; (d) n-C_4H_9Li, 4(S)-phenylmethyl-2-oxazolidinone, THF; (e) t-butyl acrylate, $Cl_3Ti(O\text{-}i\text{-}C_3H_7)$, $(i\text{-}C_3H_7)_2NC_2H_5$, CH_2Cl_2, 0 °C; (f) H_2O_2, LiOH, H_2O/THF; (g) L-LeuNHPh, EDC, HOBt, THF; (h) TFA, CH_2Cl_2; (i) 2.2 eq. LDA, R_1-X, HMPA, THF; (j) $HOCH_2Ph$, EDC, 4-DMAP; (k) separate diastereomers by HPLC; (l) 1 atm. H_2, $Pd(OH)_2$/C, MeOH.

Biological Evaluation:

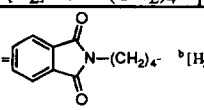
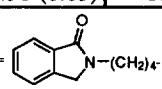
Compounds **1-10** were evaluated as inhibitors of human MMP-3, MMP-1 and MMP-2 by the methods previously described,[5] and the results are reported in Table 1. The methodology to evaluate the inhibition of MMP-3-mediated degradation of radiolabeled transferrin in the mouse pleural cavity by these compounds is similar to that described for the rat pleural cavity, except that transferrin was used as substrate instead of aggrecan.[3] Briefly, mice were predosed (iv or po) with compound or vehicle[8] at the designated time. After the dosing time, ^{3}H-transferrin[9] (500 μg; 0.2 mL of a 2.5 mg/mL solution) was injected into the pleural cavity, followed immediately by the injection of 0.3 mL of a solution of trypsin-activated MMP-3 (at a concentration of 100 μg/mL in buffered solution[10]). After 30 minutes, the animals were euthanized by carbon dioxide asphyxiation and the pleural cavity lavaged with buffer[10] containing 20 mM phenanthroline to prevent continued

transferrin degradation in vitro. The fluid was centrifuged (10 min @ 3000 RPM). Either a 3% solution of trichloroacetic acid (TCA, 100 μL) or buffer[10] (100 μL) was added to 400 μL of the supernatant. TCA preparations were precipitated on ice for 30 min and then centrifuged (5 min @ 10,000 RPM). The supernatant (250 μL) was then counted and a percent inhibition relative to controls was calculated.[11] The results for both iv and po dosing are reported in Table 1.

Table 1.
Inhibition of Matrix Metalloproteinases and MMP-3-Mediated Degradation of Radiolabeled Transferrin in the Mouse Pleural Cavity (PLCAV) by *N*-Carboxyalkyl- and *C*-Carboxyalkyl Dipeptides.



				Enzyme Inhibition			Mouse Pleural Cavity Assay	
#	X	R_1	R_2	MMP-3 K_i, nM (±S.E.)	MMP-1 K_i, nM (±S.E.)	MMP-2 K_i, nM (±S.E.)	% Inh @ 30 mg/kg iv (±S.E.)	% Inh @ 100 mg/kg po (±S.E.)
1	NH	H	H	3600 (400)	4620 (360)	470 (60)	ND	ND
2	NH	CH_3	H	310 (20)	770 (70)	200 (40)	50 (4)	55% (8)
3	NH	CH_3	n-C_3H_7	18 (2)	5900 (800)	3.5 (0.4)	63 (4)	ED_{50} = 65 (12)
4	NH	Phth-N$(CH_2)_4$ [a]	H	8 (1)	720 (120)	86 (9)	91 (1)	1% (2)
5	NH	Phth-N$(CH_2)_4$ [a]	n-C_3H_7	1.9 (0.2)	>10,000	2.6 (0.2)	89 (4)	ND
6	NH	[H_2]Phth-N$(CH_2)_4$ [b]	n-C_3H_7	9.5 (1)	1600 (300)	110 (20)	81 (6)	4% (5)
7	CH_2	H	H	5800 (900)	>10,000	> 1000	ND	ND
8	CH_2	H	n-C_3H_7	140 (10)	>10,000	220 (10)	ND	ED_{50} = 47 (10)
9	CH_2	CH_3	n-C_3H_7	68 (4)	>10,000	310 (20)	ND	ED_{50} = 32 (11)
10	CH_2	[H_2]Phth-N$(CH_2)_4$ [b]	n-C_3H_7	0.36 (0.03)	>10,000	5.7 (0.5)	82 (5)	20% (9)

[a] Phth-N$(CH_2)_4$ =  [b] [H_2]Phth-N$(CH_2)_4$ = 

Results and Discussion:

As had been reported, potency versus MMP-3 was generally enhanced with the addition of substituents in the P_1 (R_1) and P_1' (R_2) positions.[5-7] This was also seen in the *N*-carboxyalkyl (**1** → **2** → **3** → **5**) and *C*-carboxyalkyl series (**7** → **8** → **9** → **10**). Selectivity for MMP-3 over MMP-1 was determined by the larger substituents in the P_1' site (eg., **2** → **3**; **4** → **5**). The enchanced MMP-3 potency and selectivity over MMP-1 obtained with larger P_1' substituents was consistent with the X-ray structures of these two enzymes.[12,13] MMP-3 has a large deep S_1' pocket that extends completely through the enzyme whereas the S_1' pocket of MMP-1 has the guanidine of Arg^{214} that traverses the pocket, thus making it shallower. There is generally less selectivity for MMP-3 over MMP-2 in these compounds. Substituents at P_1' that enhanced MMP-3 activity also enhanced MMP-2 inhibition (eg., **2** → **3**; **4** → **5**; **7** → **8**). Where modest selectivity for MMP-3 was obtained,

it was usually associated with the substituted butyl group at P_1 (R_1) (eg., **4**, **6**, and **10**). These and other data[6] suggest that the structural features of the S_1' pocket of MMP-2 may be very similar to that of MMP-3.

The *N*-carboxyalkyl compounds were generally more potent MMP-3 inhibitors than the analogous *C*-carboxyalkyl derivatives (compare **1** → **7** and **3** → **9**) but not always (**6** → **10**). As the compounds became better enzyme inhibitors versus MMP-3, they were also more potent inhibitors of MMP-3-mediated transferrin degradation in the mouse pleural cavity when dosed intravenously (**2** → **3** → **4-6**, **10**). When dosed orally, only the compounds without the P_1 phthalimidobutyl group (**2**, **3**, **8**, **9**) had significant activity in the pleural cavity assay. Among these compounds, **3** and **9** offer a direct comparison of analogues in the two series. The *C*-carboxyalkyl **9** is 2-fold more potent than **3** when dosed orally despite being a 4-fold less potent MMP-3 inhibitor in vitro.

The mouse pleural cavity assay was designed to conveniently measure functional pharmacokinetics of MMP-3 inhibitors. Many inhibitors, often available in only small amounts, were rapidly screened and some relative measure of compound efficacy versus MMP-3-mediated degradation of a macromolecular substrate was obtained. While the absolute potencies of these MMP-3 inhibitors evaluated in this assay are modest and probably irrevelant to the objective of protecting against MMP-3 mediated damage in a joint space, relative bioavailability and duration of action parameters provided a good measure of progress towards the goal of obtaining a potent orally active MMP-3 inhibitor with good duration of action in an extravascular space.

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8. Dosing vehicles: (iv) 2% DMSO, 2% Cremaphore, 96% PBS; (po) 2% DMSO, 2% Cremaphore, 96% aq. solution of 0.25% carboxymethylcellulose.
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10. MMP-3 buffer: 20 mM Tris, pH = 7.5; 0.15 M NaCl, 10 mM $CaCl_2$, 0.02% NaN_3, 0.05% Brij.
11. $$\% \text{ degradation} = \frac{\text{TCA soluble counts}}{\text{total counts}}$$

$$\% \text{ inhibition} = 1 - \frac{(\% \text{ degradation w/ inhibitor solution}) - (\% \text{ degradation w/ MMP-3 buffer})}{(\% \text{ degradation w/o MMP-3 added}) - (\% \text{ degradation w/ MMP-3 buffer})}$$

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