Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 20, pp. 2441-2446, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00425-4

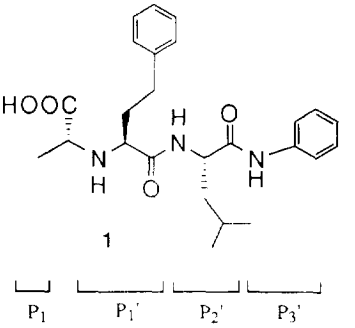
INHIBITION OF MATRIX METALLOPROTEINASES BY N-CARBOXYALKYL DIPEPTIDES: ENHANCED POTENCY AND SELECTIVITY WITH SUBSTITUTED P_1' HOMOPHENYLALANINES.

Soumya P. Sahoo*, Charles G. Caldwell, Kevin T. Chapman, Philippe L. Durette, Craig K. Esser, Ihor E. Kopka, Scott A. Polo, Kelly M. Sperow, Lisa M. Niedzwiecki,[†] Maria Izquierdo-Martin,[†] Benedict C. Chang,[‡] Richard K. Harrison,[†◊] Ross L. Stein,[†‡] Malcolm MacCoss, William K. Hagmann

Departments of Medicinal Chemistry and Enzymology[†]
Merck Research Laboratories, Rahway, NJ 07065-0900

Abstract - A series of N-carboxyalkyl dipeptides were synthesized to evaluate their inhibitory activities against human stromelysin-1(MMP-3), collagenase(MMP-1), and gelatinase-A(MMP-2). Structures with a homophenylalanine residue at P_1' substituted at the *para* position with small alkyl groups are potent inhibitors of (MMP-3) and (MMP-2) (K_i' s 2-40 nM), but weak inhibitors of (MMP-1).

The matrix metalloproteinases (MMP's) constitute a family of related zinc metalloenzymes proposed as primary agents of extracellular matrix degradation and remodeling.[1] It is widely recognized that the MMP's are responsible for the excessive cartilage and bone destruction that leads to joint dysfunction in osteo- and rheumatoid arthritis.[2] Hence, inhibition of these proteases may provide a novel disease modifying approach to the treatment of arthritic conditions. As part of an ongoing effort in our laboratories to develop potent and selective inhibitors of several key members of the MMP's, we described the results of an extensive study of the binding requirements for inhibition by N-carboxyalkyl dipeptides.[3] This resulted in the discovery of N-[1(R)-carboxy-ethyl]-α-(S)-(2-phenylethyl)glycine-(L)-leucine N-phenylamide **1** as a moderately potent, nonselective inhibitor of several MMP's.



* To whom correspondence and reprint requests should be sent.

◊ Present address: 3D Pharmaceuticals, 3700 Market Street, Philadelphia, PA 19104

‡ Present address: Mycogenics, Inc., 61 Moulton Road, Cambridge, MA 02139

The SAR for MMP-3 inhibition resulting from that study revealed little potency enhancement from varying the substitution pattern at the P_1, P_2', and P_3' subsites. However, introduction of a P_1' β-phenethyl side-chain afforded inhibitors with potencies in the submicromolar range. These findings, along with those obtained with N-carboxyalkyl peptides containing extended alkyl groups at P_1',[4] suggested that the S_1' subsite comprised a deep hydrophobic pocket and that additional binding interactions could be derived from further chemical modification at the P_1' inhibitor subsite. The present communication describes the results obtained from substitution of the P_1' phenyl ring in **1** on MMP inhibitory potency and selectivity.

Chemistry:

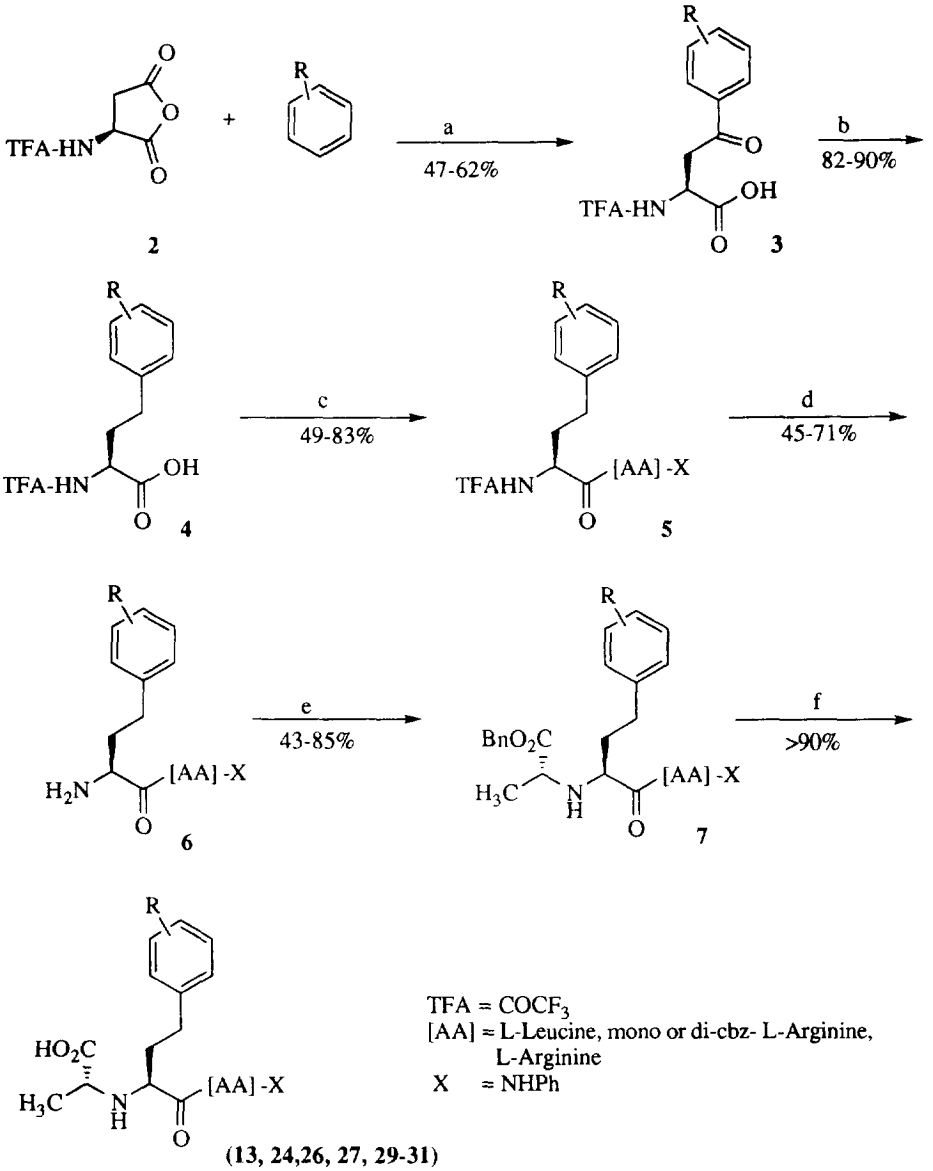
The synthesis of the inhibitors (**8-12, 14-23, 25 and 28**) was accomplished by previously described procedures.[3] The synthesis of the inhibitors (**13, 24, 26-27**) and (**29 -31**) required the enantioselective synthesis of various *para* substituted linear and branched alkyl homophenylalanine derivatives. Scheme 1 illustrates our general approach for the synthesis of such inhibitors. The synthesis of *para*- substituted homophenylalanine was based on the use of N-(trifluoroacetyl)-L-aspartic acid anhydride as a chiral synthon.[5,6] The Friedel-Crafts acylations of substituted benzenes with N-(trifluoroacetyl)-L-aspartic acid anhydride[7] **2** were carried out in presence of anhydrous aluminum chloride in methylene chloride at room temperature. Homochiral aryl ketones **3** were obtained, which were further reduced under catalytic hydrogenation conditions to give pure para substituted N-(trifluoroacetyl)-L-homophenylalanines **4**. Coupling of the substituted homophenylalanines to amino acid anilides (L-leucine anilide or di-cbz-L-arginine anilide) was accomplished under standard EDC/HOBt conditions.[8] Deprotection of the dipeptide **5** with ammonia in methanol yielded the free amine **6**. Displacement of triflate derived from benzyl-(S)-lactate with the amino dipeptides,[9] followed by the catalytic hydrogenolysis of the benzyl esters **7**, afforded the desired N-carboxyalkyl dipeptide inhibitors (**13, 24, 26, 27**) and (**29-31**) as listed in Table 1.

Results and Discussion:

A previous report from these laboratories described the elaboration of moderately potent non-selective inhibitors of MMP-1, MMP-2, and MMP-3 with K_i's in the range 0.2-0.8 μM.[3] The effect of substitution on the phenyl ring of the P_1' homoPhe residue in **1** was subsequently investigated in an effort to derive more potent and selective inhibitors of MMP-3. A similar approach to MMP-2 inhibitors has been reported.[10]

Substitution at the *para* position of **1** with short-chain aliphatic groups yielded significant enhancement of inhibitory potency against MMP-3. Thus, analogs containing a 4-chloro (**15**), 4-fluoro (**16**), or 4-methyl (**20**) group were several-fold more potent than the parent compound **1**. In general, *para* substitution proved more efficacious than *meta* or *ortho* substitution (compare **20** vs **18** and **19**; **15** vs **14**). *Ortho* substitution significantly weakened activity (see compounds **8**, **11**, and **18**).

Scheme-1

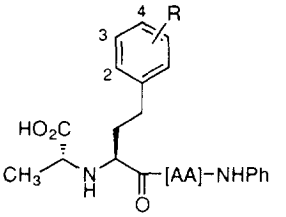


(13, 24,26, 27, 29-31)

Reaction Conditions:

(a) anh. $AlCl_3$, CH_2Cl_2, room temp.; (b) H_2, Pd/C, EtOAc, HOAc; (c) (L)-LeuNHPh or di-cbz-(L)-ArgNHPh, HOBt, EDC, THF, 25 °C; (d) NH_3, MeOH; (e) benzyl-(S)-lactate, Tf_2O, 2,6-lutidine, $Et(i\text{-}Pr)_2N$, CH_2Cl_2, 0-25 °C ; (f) H_2, $Pd(OH)_2/C$, MeOH.

Table 1. Inhibition of Matrix Metalloproteinases by N-Carboxyalkyl Dipeptides Containing Substituted P_1' Homophenylalanines



COMPD. #	R	[AA]	MMP-3 Ki [a] (μM) ± SE	MMP-1 Ki [a] (μM) ± SE	MMP-2 Ki [a] (μM) ± SE
1	H	L-Leu	0.47 (.08)	0.76 (.22)	0.20 (0.04)
8	2-OH	L-Leu	1.6 (.4)	2.7 (.35)	71% [b]
9	3-OH	L-Leu	0.23 (.05)	0.51 (.05)	70%
10	4-OH	L-Leu	0.33 (.05)	1.0 (.1)	57%
11	2-OCH_3	L-Leu	>10	>10	24%
12	3-OCH_3	L-Leu	4.7 (.7)	>10	42%
13	3,4-$(CH_3O)_2$	L-Leu	>10	>10	>10
14	3-Cl	L-Leu	1.8 (.2)	3.3 (.4)	NT [c]
15	4-Cl	L-Leu	0.19 (.03)	2.1 (.3)	0.077 (.03)
16	4-F	L-Leu	0.19 (.02)	0.87 (.07)	0.22 (.04)
17	4-CF_3	L-Leu	0.83 (.11)	5.1 (.4)	0.11 (.009)
18	2-CH_3	L-Leu	>10	>10	14%
19	3-CH_3	L-Leu	0.22 (.01)	1.5 (.2)	71%
20	4-CH_3	L-Leu	0.11 (.01)	1.9 (.2)	91%
21	3,4-$(CH_3)_2$	L-Leu	0.22 (.02)	7.7 (1.1)	0.025 (.004)
22	3,5-$(CH_3)_2$	L-Leu	>10	>10	4.84 (.50)
23	4-C_2H_5	L-Leu	0.072 (.007)	2.3 (.1)	0.012 (.001)
24	4-n-C_3H_7	L-Leu	0.018 (.002)	5.9 (.8)	0.0035 (.0004)
25	4-i-C_3H_7	L-Leu	0.18 (.02)	>10	0.13 (.02)
26	3-i-C_4H_9	L-Leu	1.8 (.1)	>10	6.1 (.5)
27	4-i-C_4H_9	L-Leu	0.043 (.006)	>10	0.011 (.001)
28	H	L-Arg	0.23 (.03)	0.47 (.07)	0.21 (.03)
29	4-n-C_3H_7	L-Arg	0.033 (.004)	2.9 (.2)	0.0030 (.0004)
30	4-n-C_4H_9	L-Arg	0.036 (.004)	>10	0.0021 (.0002)
31	4-OC_2H_5	L-Arg	0.22 (.02)	2.3 (.2)	0.0061 (.0003)

a All assays were performed at pH = 7.5 and 25 °C according to the procedures in Reference-3.
b A value followed by a % sign indicates percent inhibition at 1 μM. c NT = Not tested.

The 4-fold increase in MMP-3 inhibitory activity obtained with the 4-methyl analog **20** prompted further exploration of the dimensions of the S_1' pocket with the synthesis of structures substituted at the *para* position with longer linear and branched alkyl groups. Keeping leucine at P_2' constant, MMP-3 inhibitory potency was found to correlate with the length of the linear alkyl group at the *para* position (activity of **24>23>20>1**). α-Branching (**25**) considerably diminished activity, whereas β-branching (**27**) had minimal effect. A related series with arginine at P_2' (**28-30**) gave similar results, maximal activity being obtained with the n-propyl (**29**) and n-butyl (**30**) analogs. *Bis*-substitution did not yield any further increase in potency (**21** vs **20**); in fact, 3,5-disubstitution, as in **22**, was deleterious to binding, certainly suggestive of an asymmetric S_1' pocket.

Analogs **24** and **29** having 4-n-propyl-homoPhe at P_1' residue represent potent inhibitors of MMP-3 (K_i's 18 and 33 nM, respectively) and MMP-2 (K_i's 3-3.5 nM), with ~100-fold selectivity against MMP-1. The significant increase in inhibitory activity against MMP-3 obtained with a 4-n-propyl-β-phenethyl group at P_1' provided additional evidence that the S_1' subsite is a deep hydrophobic pocket, corroborated by other chemical modification at P_1' [4,10,11] as well as subsequent NMR and X-ray crystal structural studies of the inhibited catalytic domain of the enzyme.[12] A similar study on gelatinase B(MMP-9), a 92kDa MMp belonging to the same subgroup as gelatinase A(MMP-2), which contains a similar deep hydrophobic S_1' subsite based on inhibitor SAR and sequence homology of the MMPs.[13] The weak MMP-1 activity observed with these compounds fits the model of a shallower S_1' pocket as confirmed by X-ray crystal structures of human fibroblast collagenase.[14] In summary, P_1' homophenylalanine analogs of **1** containing a C3-C4 linear alkyl group at the *para* position of the phenyl ring constitute potent dual MMP-3 and MMP-2 inhibitors with good selectivity *vis-a-vis* MMP-1.

References:

1. (a) Murphy, G. J. P.; Murphy, G.; Reynolds, J. J. *FEBS Lett.* **1991**, *289*, 4; (b) Edonard, H.; Grimaud, J.-A. *Cell. Molec. Biol.* **1990**, *36*, 131; (c) Matrisian, L. M. *Trends Genet.* **1990**, *6*, 121; (d) Matrisian, L. M. *Bioessays* **1992**, *14*, 455.
2. (a) Dean, D. D.; Martel-Pelletier, J.; Pelletier, J.-P.; Howell, D. S.; Woessner, J. F. *J. Clin. Invest.* **1989**, *84*, 678; (b) Hasty, K. A.; Reife, R. A.; Kang, A. H.; Stuart, J. M; *Arthr. Rheum.* **1990**, *33*, 388; (c) Okada, Y.; Shinmei, M.; Tanaka, O.; Naka, K.; Kimura, A.; Nakanishi, I.; Bayliss, M. T.; Iwata, K.; Nagase, H. *Lab. Invest.* **1992**, *66*, 680; (d) Walakovits, L. A.; Bhardwaj, N.; Gallick, G. S.; Lark, M. W. *Arthr. Rheum.* **1992**, *35*, 35.
3. Chapman, K. T.; Kopka, I. E.; Durette, P. L.; Esser, C. K.; Lanza, T. J.; Izquierdo-Martin, M.; Niedzwiecki, L.; Chang, B.; Harrison, R. K.; Kuo, D. W.; Lin, T.-Y.; Stein, R. L.; Hagmann, W. K. *J. Med. Chem.* **1993**, *36*, 4293.
4. Esser, C. K.; Kopka, I. E.; Durette, P. L.; Harrison, R. K; Niedzwiecki, L. M.; Izquierdo-Martin, M.; Stein, R. L.; Hagmann, W. K. *Bioorg . Med. Chem. Lett.* **1995**, *6*, 539.

5. Lapidus, M., Sweeny, M. J. *J. Med. Chem.*, **1973**, *16*, 163.
6. Hanessian, S. In Total synthesis of Natural Products:the Chiron Approach; Baldwin, J. E.; Ed.; Pergamon : Oxford, 1983.
7. Norlander, J. E.; Payne, M. J.; Njoroge, F. G.; Balk, M. A.; Laikos, G. D.; Viswanath, V. M. *J. Org. Chem.* **1984,** *49*, 4107.
8. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984.
9. Attwood, M. R.; Hassall, A. K.; Krohn, A.; Lawton, G.; Redshaw, S. *J. Chem. Soc., Perkin Trans.* 1,**1986**, 1011.
10. Porter, J. R., Beeley, N. R. A., Boyce, B. A., Mason, B., Millican, A., Millar, K., Leonard, J., Morphy, J. R., O'Connell, J. P. *Bioorg. Med. Chem. Lett.* **1994**, *4* , 2741.
11. Gowravaram, M. R.; Tomczuk, B. E.; Johnson, J. S.; Delecki, K.; Cook, E. R.; Ghose, A. K.; Mathiowetz, A. M.; Spurlino, J. C.; Rubin, B.; Smith, D. L.; Pulvino, T.; Wahl, R. C. *J. Med. Chem.*,**1995**, *38*, 2570.
12. (a) Gooley, P. R.; O'Connell, J. F.; Marcy, A. I.; Cuca, G. C.; Salowe, S. P.; Bush, B. L.; Hermes, J. D.; Esser, C. K.; Hagmann, W. K.; Springer, J. P.; Johnson, B. A. *Structural Biology* **1994**, *1*, 111; (b) Becker, J. W.; Marcy, A. I.; Rotisz, L. L.; Axel, M. G.; Burbaum, J. J.; Fitzgerald, P. M. D.; Cameron, P. M.; Esser, C. K.; Hagmann, W. K.; Hermes, J. D.; Springer, J. P.; *Protein Science*, **1995**, in press.
13. Wahl, R. C.; Pulvino, T. A.; Mathiowetz, A. M.; Ghose, A. K.; Johnson, J. S.; Delecki, D.; Cook, E. R.; Gainor, J. A.; Gowaravaram, M. R.; Tomczuk, B. E.; *Bioorg. Med. Chem. Lett.* **1995,** *5*, 349.
14. (a) Lovejoy, B.; Cleasby, A.; Hassell, A. M.; Longley, K.; Luther, M. A.; Weigl, D.; McGeehan, G.; McElroy, A. B.; Drewry, D.; Lambert, M. H.; Jordan, S. R. *Science* **1994**, *263*, 375; (b) Borkakoti, N.; Winkler, F. K.; Williams, D. H.; Arcy, A. D.; Broadhurst, M. J.; Brown, P. A.; Johnson, W. H.; Murray, E. J. *Structural Biology* **1994**, *1*, 106; (c) Spurlino, J. C.; Smallwood, A. M.; Carlton, D. D.; Banks, T. M.; Vavra, K. J.; Johnson, J. S.; Cook, E. R.; Falvo, J.; Wahl, R. C.; Pulvino, T. A.; Wendoloski, J. J.; Smith, D. L. *Proteins: Structure, Function, and Genetics* **1994**, *19*, 98.

(*Received in USA* 14 *August* 1995; *accepted* 18 *September* 1995)