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PHOSPHINIC ACID INHIBITORS OF MATRIX METALLOPROTEINASES

Charles G. Caldwell, Soumya P. Sahoo, Scott A. Polo, Randall R. Eversole, Thomas J. Lanza, Sander G. Mills, Lisa M. Niedzwiecki,† Maria Izquierdo-Martin,† Benedict C. Chang,† Richard K. Harrison,†◊ David W. Kuo,† T.-Y. Lin,† Ross L. Stein,† Philippe L. Durette, and William K. Hagmann*

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

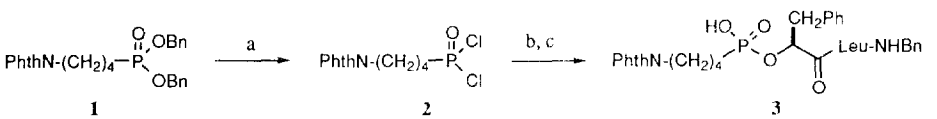
Abstract: The matrix metalloproteinase stromelysin-1 (MMP-3) is inhibited more strongly by peptidyl phosphinic acid **7** than by its corresponding phosphonamidate and phosphonate analogs. Extending a benzyl group at P_1' to a phenylethyl group in **8** further increases the potency (K_i = 1.4 nM). Enhanced potency with an extended substituent into the P_3 region was observed.

Matrix metalloproteinases (MMPs) have been implicated as causative agents in the degradation of articular cartilage in diseases such as rheumatoid and osteoarthritis and thus have been targets of intensive efforts to discover potent, selective inhibitors.[1-3] Given the discoordinate expression[4,5] and unknown activation process(es) of these enzymes, highly selective inhibitors of each MMP may help to define the role of these proteinases in both normal and pathological cartilage remodeling. To that end, we sought to develop selective inhibitors of stromelysin-1 (MMP-3), a proteoglycanase present in large amounts in arthritic joints.[6] Herein is described the preparation of phosphorus-containing dipeptides and their evaluation as inhibitors of several members of the MMP family.

Chemistry

The sodium salt of the phosphonamidate-containing inhibitor **39** was prepared using the method of Portoghese.[7] Conversion of dibenzyl 4-phthalimidobutylphosphonate into the corresponding phosphonochloridate with phosphorus pentachloride, condensation with the appropriate dipeptide and hydrogenolysis in the presence of 1 equivalent of sodium bicarbonate gave **39**. The synthesis of the phosphonate analog **3** is shown in Scheme 1.[8] Dibenzyl 4-phthalimidobutylphosphonate **1** was treated with two equivalents of phosphorus pentachloride in carbon tetrachloride to yield the phosphonyl chloride **2**. Reaction of **2** with L-3-phenyllactic acid followed by treatment with the benzylamide of L-leucine gave the phosphonate **3**.

Scheme 1



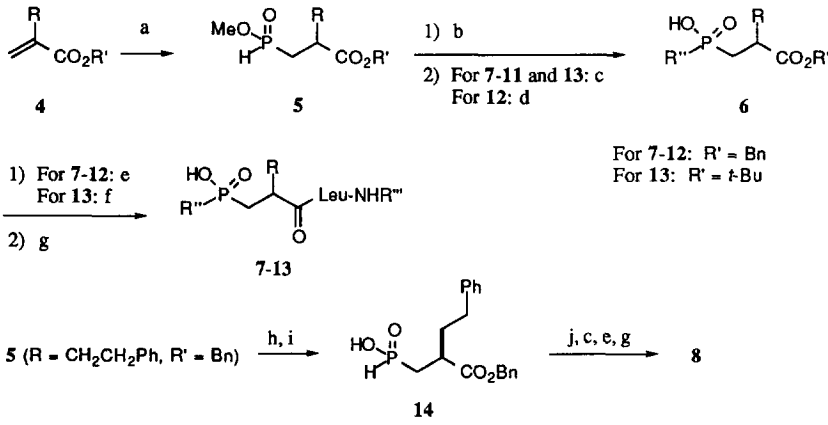
1 **2** **3**

Reagents and conditions: (a) 2 eq. PCl_5, CCl_4, 80 °C; (b) L-3-phenyllactic acid, Et_3N, THF, -78 to 25 °C; (c) L-Leu-NHPh.

* To whom correspondence and reprint requests should be sent.
◊ Present address: 3D Pharmaceuticals, 665 Stockton Drive, Exton, PA 19341

The synthesis of the phosphinic acid inhibitors **7-11** began with the 1,4-addition of methyl hypophosphite[9] to the appropriately substituted benzyl acrylate **4** in the presence of 1,1,3,3-tetramethylguanidine (Scheme 2).[10] Alkylation on phosphorus was accomplished by treatment with *N,O*-bis(trimethylsilyl)acetamide followed *N*-(4-bromo-2-butenyl)phthalimide.[11,12] Hydrogenolysis of the benzyl ester **6** and reduction of the olefin produced a diacid which was activated with carbonyldiimidazole prior to coupling with an amide derivative of L-leucine to give phosphinic acids **7-11**.[13] Compound **12** was prepared by an analogous route. The route to compound **13** required the use of an acid labile *tert*-butyl ester which could be deprotected without reduction of the olefinic side chain. Compounds **7-13** were initially synthesized as mixtures of diastereomers which were subsequently separated by reverse-phase HPLC. The need for this separation in the case of **8** and its derivatives was obviated by using the resolved phosphonous acid intermediate **14**, obtained by recrystallization of its salt with (*S*)-α-methylbenzylamine from ethyl acetate.

Scheme 2

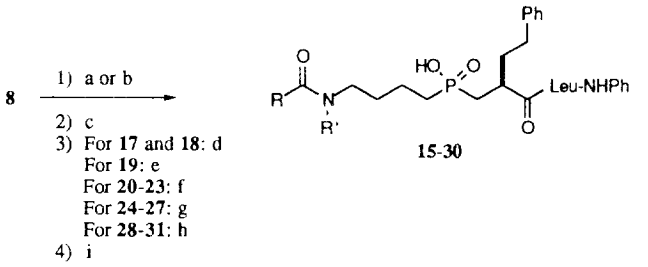


Reagents and conditions: (a) methyl hypophosphite, 1,1,3,3-tetramethylguanidine; (b) 1.8 eq. *N,O*-bis(trimethylsilyl)acetamide, $(i\text{-Pr})_2NEt$, CH_2Cl_2; (c) *N*-(4-bromo-2-butenyl)phthalimide; (d) CH_3I; (e) H_2, 10% Pd/C, EtOH; (f) TFA, CH_2Cl_2; (g) carbonyldiimidazole, L-Leu-NHR''', THF; (h) 1 eq. NaOH, MeOH, H_2O; (i) resolution with (*S*)-α-methylbenzylamine; (j) 2.9 eq. *N,O*-bis(trimethylsilyl)-acetamide, $(i\text{-Pr})_2NEt$, CH_2Cl_2.

In the route to the aminobutyl derivatives **15-30** (Scheme 3), phosphinic acid **8** was esterified by reaction with diazomethane or by refluxing a methanol solution containing chlorotrimethylsilane. After hydrazinolysis of the phthaloyl protecting group, the resulting free amine was derivatized. Deprotection of the phosphinic acid methyl esters then produced the free acids.

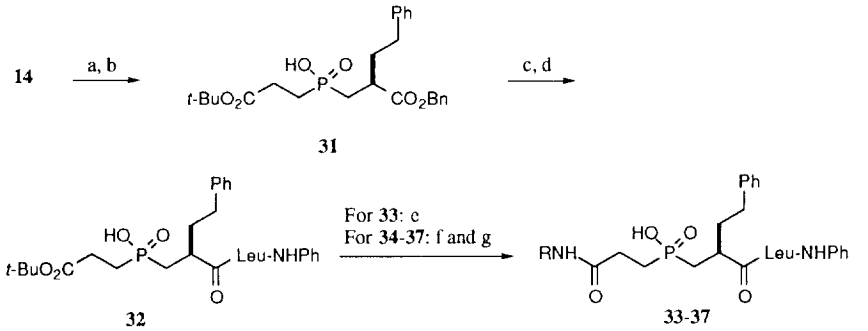
The route to inhibitors **33-37** utilized a 1,4-addition of the resolved phosphonous acid **14** to *tert*-butyl acrylate, yielding intermediate **31** with differentially protected carboxylic acid groups (Scheme 4).[14] Hydrogenolysis and coupling with the phenylamide of L-leucine was followed by deprotection of the *tert*-butyl ester. The resulting diacid intermediate underwent facile esterification in methanol solution to give compound **33**. Alternatively, activation of the diacid with carbonyldiimidazole and coupling with aniline or phenylalkylamines produced inhibitors **34-37**. Compounds were evaluated for inhibition as described.[15]

Scheme 3



Reagents and conditions: (a) CH_2N_2; (b) TMSCl, MeOH, Δ; (c) H_2NNH_2, MeOH; (d) RC(O)Cl, 4-methylmorpholine, CH_2Cl_2; (e) methyl 2-(bromomethyl)benzoate, $(i\text{-Pr})_2NEt$, THF; (f) alkyl chloroformate; (g) alkyl isocyanate; (h) RCO_2H, EDC, HOBt; (i) NaOH, MeOH, H_2O.

Scheme 4



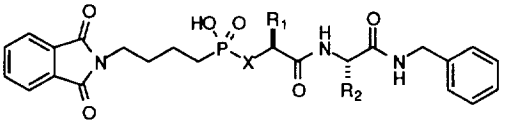
Reagents and conditions: (a) *N,O*-bis(trimethylsilyl)acetamide, $(i\text{-Pr})_2NEt$, CH_2Cl_2; (b) *tert*-butyl acrylate; (c) H_2, 10% Pd/C, EtOH; (d) carbonyldiimidazole, L-Leu-NHPh, THF; (e) MeOH; (f) TFA, CH_2Cl_2; (g) carbonyldiimidazole, RNH_2, THF.

Results and Discussion

Phosphorus-containing inhibitors of MMPs have been described.[2,16-18] In our previous studies of peptidyl phosphinic acids,[19] we noted a lack of sensitivity to amino-acid substituents at the P_1 and P_2 positions which was consistent with substrate specificity studies.[20] Thus the inclusion of an amide bond between the phosphorus ligand and the potency enhancing P_3 substituent was deemed unnecessary. The original lead for our efforts in this area was the sodium salt of compound **38**, a phosphonamidate designed around the cleavage site of collagen,[21] which had been found to be more potent against collagenase (MMP-1) than for MMP-3 (see Table 1). Since phenylalanine often occurs at the P_1' position in stromelysin substrates and arylalkyl substituents in *N*-carboxyalkyl inhibitors were found to offer greater selectivity for MMP-3,[15] the alkyl and aryl side chains at the P_1' and P_2' positions were reversed in compound **39**, resulting in a ~10-fold loss of potency versus MMP-3 and a ~660-fold loss versus MMP-1.

The type of phosphorus ligand was also varied. The phosphonate **3** was inactive but the phosphinic acid **7** was more potent than phosphonamidate **39**. The order of potency in this series of MMP inhibitors (X = CH_2 > NH > O) is very different than that reported for series of phosphorus-containing thermolysin

Table 1. Inhibition of Stromelysin-1 (MMP-3) and Collagenase-1 (MMP-1) by Various Phosphorus-containing Inhibitors.

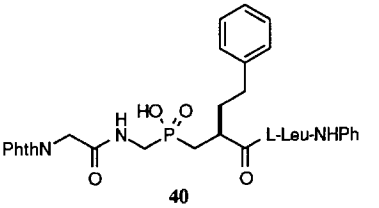


Cmpd. #	X	R_1	R_2	MMP-3 (IC_{50}, μM)	MMP-1 (IC_{50}, μM)
38	NH	i-C_4H_9	CH_2-3-indolyl	0.26	0.05
39	NH	CH_2Ph	i-C_4H_9	2.8 (0.6)	30 (0.5)
3	O	CH_2Ph	i-C_4H_9	> 200	>32
7	CH_2	CH_2Ph	i-C_4H_9	0.43 (0.02)	4.1 (0.1)

inhibitors[22] (X = NH > CH_2 > O), carboxypeptidase-A inhibitors[23] (X = O > NH), and ACE inhibitors[24] (X = NH > O > CH_2). Clearly the active sites of each of these zinc metalloproteinases offer different binding elements to their respective inhibitors.

As with the *N*-carboxyalkyl inhibitors,[15] extension of the benzyl group at P_1' to a phenylethyl group as in **8** results in greatly enhanced potency and selectivity for MMP-3. None of the compounds in Table 2 inhibited MMP-1 (K_i's > 10 μM) but the potencies with respect to gelatinase-A (MMP-2) generally tracked those of MMP-3. 3- and 4-Methoxy substituents around the P_1' phenylethyl group (**10-11**) had no effect on inhibition whereas the 2-methoxy analog **9** resulted in a ≥300-fold loss of potency. The importance of the large P_{1-3} phthalimidobutyl substitutent in **8** is noted in the >1000-fold loss of MMP-3 activity in the methyl analog **12.**

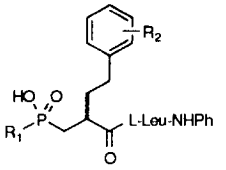
The comparable potencies of the phthalimido-*trans*-butenyl analog **13** with the saturated phthalimidobutyl compound **8** suggests that this P_{1-3} alkyl group is bound to MMP-3 (and also MMP-2) in an extended linear conformation. It is reasonable to speculate that the *trans* double bond in **13** is a conformational mimic of the amide bond that would exist between the P_2 and P_1 amino acids in a protein or peptide substrate. However, the peptidyl phosphinic acid inhibitor that does contain an amide bond at that position (**40**) was a much poorer inhibitor of MMP-3 (K_i = 410 nM) than either **8** or **13.**[19] Thus the amide bond at P_2-P_1 is unnecessary in these peptide-based inhibitors.

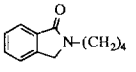


40

As with most of the described metalloproteinases, the major recognition elements for binding and cleavage lie on the "prime side" or C-terminus of the scissile bond. This preference for C-terminal binding is particularly evident in peptidyl hydroxamic acid inhibitors that are much more potent when they contain P_1'-$P_{3(4)}$' amino acids than when only P_4-P_1 substituents are present.[2,16] Thus, in order to make the same binding interactions with the potency-enhancing phthalimido group in **8** and **13**, MMP-3 cannot accommodate the amide bond in **40.** With respect to transistion-state characterization of these phosphinic acid inhibitors, it is interesting to speculate whether a peptide substrate lacking the P_2-P_1 amide bond (-P(O2)CH would be cleaved.

Table 2. Inhibition of Stromelysin-1 (MMP-3) and Gelatinase-A (MMP-2) by Phosphinic Acid Inhibitors. [a]



Cmpd. #	R_1	R_2	MMP-3 K_i, nM (± SD)	MMP-2 K_i, nM (± SD)
8	[b] PhthN$(CH_2)_4$	H	1.4 (0.3)	20 (3)
9	PhthN$(CH_2)_4$	2-OCH_3	440 (60)	4400 (1000)
10	PhthN$(CH_2)_4$	3-OCH_3	2.5 (0.5)	2.1 (0.5)
11	PhthN$(CH_2)_4$	4-OCH_3	2.3 (0.5)	12 (2.0)
12	CH_3	H	1500 (100)	570 (10)
13	PhthNCH_2(*t*-CH=CH)CH_2	H	4.5 (0.2)	4.8 (1)
15	$H_2N(CH_2)_4$	H	3700 (400)[d]	13 % [c]
16	PhCONH$(CH_2)_4$	H	5.7 (1.1)	26 (5)
17	$PhCH_2CONH(CH_2)_4$	H	30 (8)	130 (34)
18		H	5.5 (0.5)	7.3 (2.0)
19	$CH_3OC(=O)NH(CH_2)_4$	H	54 (3)	90% [c]
20	$C_2H_5OC(=O)NH(CH_2)_4$	H	16 (1)	94% [c]
21	$PhOC(=O)NH(CH_2)_4$	H	56 (5)	73% [c]
22	$PhCH_2OC(=O)NH(CH_2)_4$	H	63 (5)	86% [c]
23	$CH_3NHC(=O)NH(CH_2)_4$	H	130 (20)	87% [c]
24	$C_2H_5NHC(=O)NH(CH_2)_4$	H	17 (3)	19 (4)
25	$PhNHC(=O)NH(CH_2)_4$	H	70 (17)	85% [c]
26	$PhCH_2NHC(=O)NH(CH_2)_4$	H	50 (6)	96% [c]
27	Ac-L-Pro-NH$(CH_2)_4$	H	62 (21)	6.1 (1.4)
28	Ac-D-Pro-NH$(CH_2)_4$	H	26 (4)	92% [c]
29	Bz-L-Pro-NH$(CH_2)_4$	H	13 (3)	2.1 (0.4)
30	Bz-D-Pro-NH$(CH_2)_4$	H	24 (2)	89% [c]
33	$CH_3OC(=O)(CH_2)_2$	H	520 (60)	1300 (100)
34	$PhNHC(=O)(CH_2)_2$	H	470 (60)	60% [c]
35	$PhCH_2NHC(=O)(CH_2)_2$	H	540 (70)	ND [e]
36	$Ph(CH_2)_2NHC(=O)(CH_2)_2$	H	170 (20)	58% [c]
37	$Ph(CH_2)_3NHC(=O)(CH_2)_2$	H	670 (60)	46% [c]

[a] K_i's for collagenase-1 (MMP-1) ≥ 10 μM for all compounds. [b]PhthN = phthalimido. [c]% inhibition at 1 μM. [d]This result is an IC_{50}. [e]ND = Not determined.

The phthalimido group in **8** can be replaced by a benzamide **16** or a benzolactam **18** without loss of potency. Carbamates **19-22** and ureas **23-26** generally gave a 10-100-fold loss of activity. L-Proline is found at the P_3 position in many macromolecular and peptidyl substrates of the MMPs.[19,25,26] Proline analogs **27-30** have comparable potencies against MMP-3 and may be more potent versus MMP-2. However, there is no preference for the natural L-configuration in **27** and **29** over the D-isomers **28** and **30**. The arylamidoethyl analogs **34-37** are much less potent than **8.** Even those analogs that would effectively place a phenyl ring at thesame length from phosphorus as the arylamidobutyl analogs are 20 to 30-fold less potent (**36, 37** vs. **16, 17**).

The major focus of this work has been the optimization of binding requirements for phosphorus-containing inhibitors of MMP-3. In particular, peptido-mimetics of the P_{1-3} region of peptide-based phosphinic acids have been explored. Potent and somewhat selective inhibitors have been prepared but, at least in the P_{1-3} region, many of the examples do not appear to 'mimic' their peptide counterparts. Indeed, some of the inhibitors would prefer not to be 'peptidyl' in this region. This appears to be consistent with the very broad substrate selectivity among the MMPs, particularly MMP-3, in the P_{1-3} region.[20]

References

1. Woessner, E. M. *FASEB J.* **1991**, *5*, 2145.
2. Schwartz, M. A.; Van Wart, H. E. in *Progress in Medicinal Chemistry - Vol. 29*; Ellis, G. P. and Luscombe, D. K., Eds.; Elsevier Science Publishers: London, **1992**; Chapter 8, pp. 271.
3. Beeley, N. R. A.; Ansell, P. J. A.; Docherty, A. J. P. *Curr. Opin. Ther. Patents* **1994**, *4*, 7.
4. Tetlow, L. C.; Lees, M.; Ogata, Y.; Nagase, H.; Woolley, D. E. *Rheumatol. Int.* **1993**, *13*, 53
5. Yang, M.; Kurkinen, M. *Eur. J. Biochem.* **1994**, *222*, 651.
6. Okada, Y.; Shinmei, M.; Tanaka, O.; Naka, K.; Kimura, A.; Nakanishi, I.; Bayliss, M. T.; Iwata, K.; Nagase, H. *Lab. Invest.* **1992**, *66*, 680.
7. Elliott, R.L.; Marks, N.; Berg, M.J.; Portoghese, P.S. *J. Med. Chem.* **1985**, *28*, 1208.
8. Thottathil, J.K.; Wong, M.K.Y. *Tetrahedron Lett.* **1986**, *27*, 5441.
9. Fitch, S.J. *J. Am. Chem. Soc.* **1964**, *86*, 61.
10. Broadhurst, M.J.; Handa, B.K.; Johnson, W.H.; Lawton, G.; Machin, P.J. Eur. Pat. 276436-A, 1988.
11. Thottathil, J.K.; Przybyla, C.A.; Moniot, J.L. *Tetrahedron Lett.* **1984**, *25*, 4737.
12. Press, J.B.; Wright, W.B., Jr.; Chan, P.S.; Marsico, J.W.; Haug, M.F.; Tauber, J.; Tomcufik, A.S. *J. Med. Chem.* **1986**, *29*, 816.
13. Weller, H.N.; Rom, M.B. *J. Enzyme Inhibition* **1988**, *2*, 183.
14. Boyd, E.A.; Corless, M.; James, K.; Regan, A.C. *Tetrahedron Lett.* **1990**, *31*, 2933.
15. 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.
16. Johnson, W. H.; Roberts, N. A.; Borkakoti, N. *J. Enzyme Inhibition* **1987**, *2*, 1.
17. Kortylewicz, Z. P.; Galardy, R. E. *J. Med. Chem.* **1990**, *33*, 263.
18. Hunter, D. J.; Bird, J.; Cassidy, F.; De Mello, R. C.; Harper, G. P.; Karran, E. H.; Markwell, R. E.; Miles-Williams, A. J.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2833.
19. Goulet, J. L.; Kinneary, J. F.; Durette, P. L.; Stein, R. L.; Harrison, R. K.; Izquierdo-Martin, M.; Kuo, D. W.; Lin, T.-Y.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1221.
20. Niedzwiecki, L.; Teahan, J.; Harrison, R. K.; Stein, R. L. *Biochemistry* **1992**, *31*, 12618.
21. Purchased from Elastin Products, Pacific, Missouri, USA.
22. Bartlett, P. A.; Marlowe, C. *Science* **1987**, *235*, 569.
23. Kaplan, A.; Bartlett, P. A. *Biochemistry* **1991**, *30*, 8165.
24. Karanewsky, D. S.; Badia, M. C.; Cushman, D. W.; DeForrest, J. M.; Dejneka, T.; Loots, M. J.; Perri, M. G.; Petrillo, E. W.; Powell, J. R. *J. Med. Chem.* **1988**, *31*, 204.
25. Netzel-Arnett, S.; Sang, Q.-X.; Moore, W. G. I.; Navre, M.; Birkedal-Hansen, H.; Van Wart, H. E. *Biochemistry* **1993**, *32*, 6427.
26. Nagase, H.; Fields, C. G.; Fields, G. B. *J. Biol. Chem.* **1994**, *269*, 20952.

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