Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 389–394

BIOORGANIC & MEDICINAL CHEMISTRY

# Inhibition of Adamalysin II and MMPs by Phosphonate Analogues of Snake Venom Peptides

Silvana D'Alessio,[a] Carlo Gallina,[b,*] Enrico Gavuzzo,[c] Cesare Giordano,[d] Barbara Gorini,[d] Fernando Mazza,[e] Mario Paglialunga Paradisi,[d] Gabriella Panini,[d] Giorgio Pochetti[c] and Antonio Sella[a]

[a]*Polifarma Research Center, Via Tor Sapienza 138, 00185 Roma, Italy*
[b]*Istituto di Scienze del Farmaco, Università G. d'Annunzio, Via dei Vestini, 66100 Chieti, Italy*
[c]*Istituto di Strutturistica Chimica del CNR, C. P. n. 10, 00016 Monterotondo Stazione (Roma), Italy*
[d]*Centro di Studio per la Chimica del Farmaco del CNR, Università La Sapienza, P. le A. Moro 5, 00185 Roma, Italy*
[e]*Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università degli Studi, 67010 L'Aquila, Italy*

Received 22 June 1998; accepted 15 October 1998

**Abstract**—Phosphonate analogues of the peptidomimetic *N*-(Furan-2-yl)carbonyl-Leu-Trp-OH were prepared with the goal of evaluating the effect of phosphonate for carboxylate replacement on binding with snake venom metalloproteinases and MMPs. *N*-(Furan-2-yl)carbonyl-Leu-L-Trp(P)-$(OH)_2$ showed a 75-fold increase of the inhibiting activity against adamalysin II, a snake venom metalloproteinase structurally related to MMPs and TACE. Both the phosphonate and carboxylate peptidomimetics fit into the active site adopting a retrobinding mode and provide the structural base for a new class of metalloproteinases inhibitors. 

## Introduction

Matrix metaloproteinases (MMPs) are a family of zinc endopeptidases that can degrade virtually all of the constituents of the extracellular matrix. They are involved in different physiological processes such as connective tissue turnover and tissue remodeling. Misregulation of MMPs is believed to be implicated in a number of disease states characterized by unwanted degradation of the extracellular matrix, including arthritis, bone destruction, inflammation, tumor invasion and metastasis.[1]

A number of low-molecular weight, synthetic, pseudopeptide inhibitors of MMPs, which could be of clinical interest in the control of these pathologies, have been reported.[1,2] Their structures (Fig. 1) include a peptide chain that is generally accommodated at the 'primed'[3] binding region of the active site, and a zinc binding function (ZBF) capable of ligating the catalytic zinc ion. Hydroxamate, carboxylate, phosphonate, and thiolate groups are the most effective ZBFs. A large part of these pseudopeptide inhibitors have been grouped[4] into two classes related to the primary and secondary tetrahedral intermediates of the enzyme catalysis, respectively. Class I inhibitors include succinamide hydroxamates and carboxylates, for instance, presenting two $sp^3$ carbons between the ZBF and the first peptide bond; Class II inhibitors comprise carboxyalkyl α-aminoamide ($Z = NH$) and glutaramide ($Z = CH_2$) carboxylates possessing three $sp^3$ hybridized atoms between the ZBF and the first peptide bond. In both cases the peptide backbone of the inhibitors adopts a substrate like binding mode. In the present paper we report on a new class of zinc proteinases inhibitors including carboxylates and phosphonates of general structure **1**: they fit at the active site of the snake venom metalloproteinase adamalysin II in a retrobinding mode and contain only one $sp^3$ carbon between the ZBF and the first peptide bond.

## Results and Discussion

Pyroglutamate containing tri- and hexapeptides isolated from snake venoms, as well as the structurally related peptidomimetic Fur-Leu-Trp-OH (**5**),[5] were known as inhibitors of zinc metalloproteinases ($K_i$ in the lower micromolar range). The two diastereomeric peptidomimetics **6a** and **6b**, phosphonate analogues of **5**, have

Key words: Retrobinding pseudopeptide inhibitors; adamalysin II; matrix metalloproteinases.
*Corresponding author. Carlo Gallina, Centro di Studio per la Chimica del Farmaco del CNR, Dipartimento di Studi Farmaceutici, Università La Sapienza, P. le A. Moro 5, 00185 Roma, Italy; Tel: +39 6 49913892; fax: +39 6 491491.

0968-0896/99/$ - see front matter 
*PII:* S0968-0896(98)00243-0

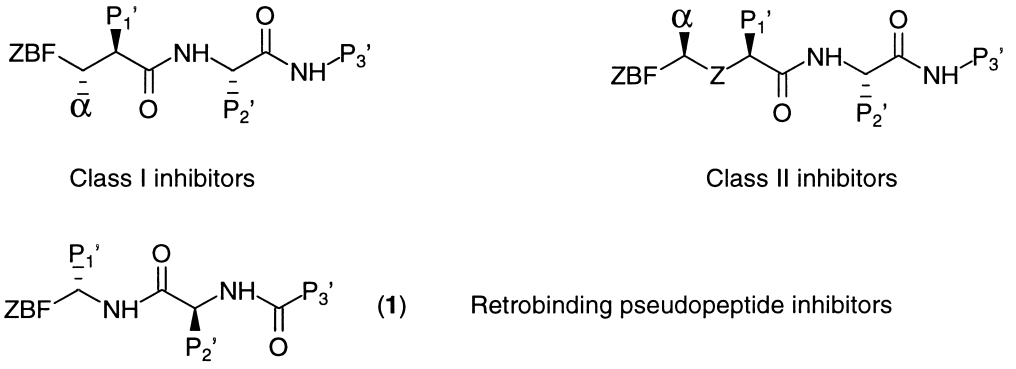



**Figure 1.** Classes of peptidomimetic zinc protenases inhibitors. Alkyl, aralkyl, or hydoxy groups in α may be present.

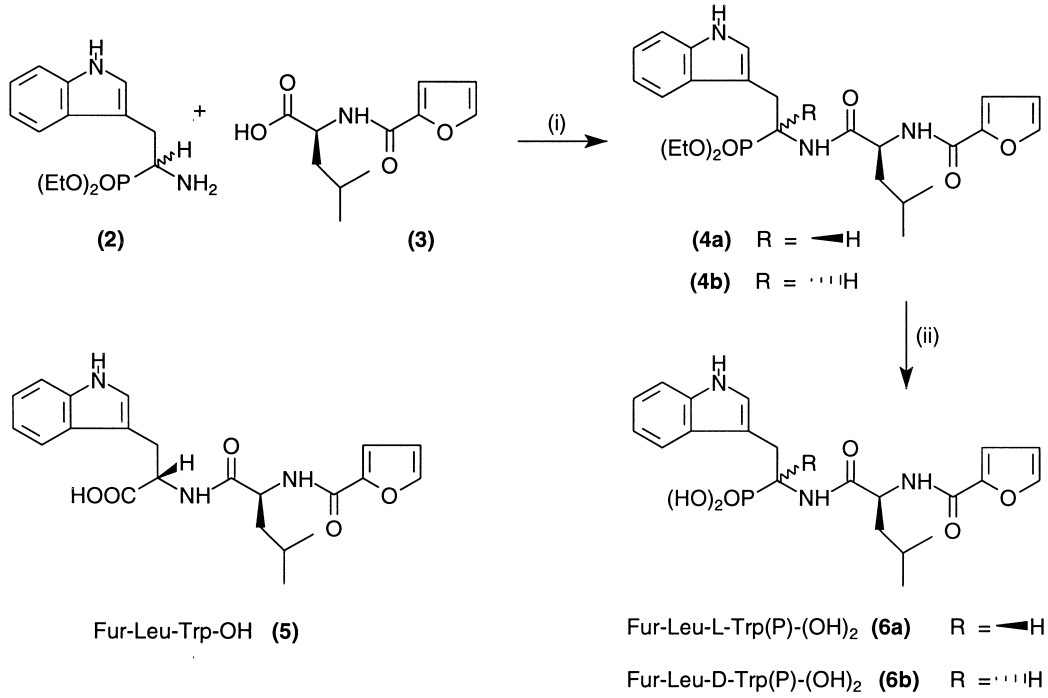
been synthesized by us. They have been evaluated as inhibitors of adamalysin II and representative MMPs. The proposal of **6a** and **5** as the prototypes of a new class (**1**) of zinc proteinases inhibitors is supported by the X-ray structure of the complexes with adamalysin II.[6,7]

Previous examples of MMPs inhibitors with phosphorous-containing ZBFs include phosphoramidates[8] which have very poor in vivo stability, with $K_i$ values in the micromolar range, and phosphinic acids,[9] which are even weaker collagenase inhibitors. Class I phosphonate dipeptides[10] proved to be comparable in potency to the carboxylate analogues. Class II phosphonoalkyl dipeptides which are at least tenfold more potent than their corresponding *N*-carboxyalkyl analogues have been described.[11]

The tripeptide phosphonates **6a** and **6b** were prepared (Scheme 1) by coupling furan-2-carbonyl-L-leucine with D,L-phosphotryptophane diethylester (**2**)[12] by the mixed anhydride method,[13] followed by resolution of the two diastereomeric phosphonate esters **4a** and **4b** by silica–gel chromatography. Final hydrolysis by treatment with trimethylsilyl iodide gave satisfactory yields only in the presence of bis-trimethylsilyl-acetamide.[14] Phosphonates **6a** and **6b** were isolated, purified, and fully characterized as crystalline cyclohexylamine salts.

The new compounds were evaluated for inhibitory activities against adamalysin II, MMP-2, MMP-9, MMP-8, and MMP-3 by evaluation of the residual enzyme activity by continuous fluorimetric assays (Table 1).

Replacement of the carboxylate with a phosphonate group in the peptidomimetic Fur-Leu-Trp-OH (5) caused a 75-fold increase of potency of the inhibitor against adamalysin II. To explain this result and to




**Scheme 1.** Reagents and conditions: (i) *i*-butyl chloroformate, *N*-methylmorpholine, 4 °C, 12 h, 65%; (ii) *N,O*-bis-trimethylsilyl-triflurooacetamide, $Me_3SiI$, $CH_2Cl_2$, 25 °C, 2 h,79–82%.

**Table 1.** In vitro inhibition of adamalysin II, gelatinase A (MMP-2), gelatinase B (MMP-9), human neutrophyl collagenase (MMP-8), and stromelysin I (MMP-3) by inhibitors using phosphonic or carboxylic zinc binding groups

| No. | Inhibitor | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | Adamalysin II | MMP-2 | MMP-9 | MMP-8 | MMP-3 |
| **6a** | Fur-Leu-L-Trp(P)-$(OH)_2$ | 0.4 | 60 | n.i.[a] | > 100 | n.i. |
| **6b** | Fur-Leu-D-Trp(P)-$(OH)_2$ | 70 | n.i. | n.i. | n.i. | n.i. |
| **5** | Fur-Leu-Trp-OH | 30 | 40 | > 100 | 50 | > 100 |

[a]Not inhibitory at 100 μM.

assist in the structure based design of more potent inhibitors of this enzyme and structurally related zinc proteinases, the crystal structure of the complex between adamalysin II and Fur-Leu-L-Trp(P)-$(OH)_2$ was determined.[6] Like Class I and Class II pseudopeptide inhibitors,[4] phosphonate **6a** occupies the 'primed' region of the enzyme active site. While the peptide chain of Class I and Class II inhibitors adopts a substrate like binding mode, lying antiparallel to the 'upper rim' and completing a β-strand like structure, the peptide chain of **6a** is oriented in the opposite direction (Figs 1 and 2). In spite of that, the key backbone hydrogen bonds of substrate like inhibitors are maintained.

Dominant hydrophobic interactions are made through the $P_1'$ indolyl side chain that is buried in the unusually large $S_1'$ hydrophobic pocket. In addition, the indole NH is hydrogen bonded to the Arg167 CO (not represented in Figure 1). It is interesting to note that the $S_1'$ pocket is not completely filled by the Trp(P) side chain and its lower part still contains two ordered water molecules. These solvent positions are important for the design of bulkier hydrophobic groups at the $P_1'$ position of more potent and selective analogous inhibitors.

The phosphonate group of **6a** (Fig. 2) binds the catalytic zinc ion in an asymmetric bidentate mode (2.0 and 2.8 Å Zn–O distances). In the crystal structure[7] of the complex between adamalysin II and 5, the carboxylate oxygen atoms form longer contacts (2.7 Å Zn–O distances). Since superimposition of the two complexes reveals that the peptide chains occupy very nearly the same position, the shorter Zn–O distances in the phosphonate complex should be caused by the different hybridization and geometry of this group. Shortening of Zn-O contacts and involvement of the O atoms into a close network of hydrogen bonds with complementary polar groups of the protein and ordered water molecules, probably account for the increase of affinity of the phosphonate (**6a**) with respect to the carboxylate ligand (**5**).

Surprisingly, the phosphonate diastereomer **6b**, containing D-Trp(P)-$(OH)_2$, also inhibits adamalysin II, even though at a much lower extent. Inspection of the active site of the enzyme by molecular graphics, clearly excludes that the phosphonate group can ligate the zinc ion, if occupation of the primary specificity site $S_1'$ by the Trp(P) side chain of **6b** is maintained. Its modest inhibiting activity seems therefore to be attributed to a nonspecific binding mode.

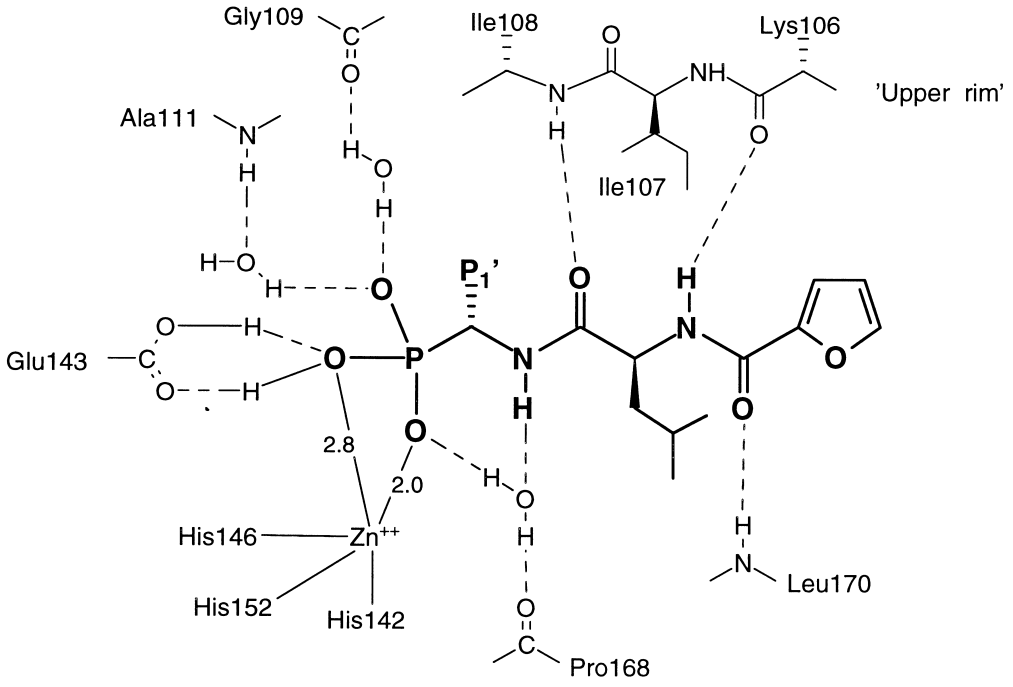



**Figure 2.** Schematic representation of the zinc-ligand and hydrogen bond interactions in the complex between adamlysin II and **6a**. Inhibitor chain is in bold and the zinc-phosphonate distances are in Å.

## Conclusion

Owing to the highly conserved overall topology and virtual identical zinc environment of adamalysins and MMPs, included with other zinc endopeptidases in the superfamily of 'metzincins',[15] determination of inhibiting activity of **6a** and **6b** and **5** was extended to representative MMPs. Interestingly, while the potency against adamalysin II increases on going from the carboxylate (**5**) to the phosphonate ligand (**6a**), the opposite occurs for MMPs inhibition (Table 1). This circumstance suggests that more potent inhibitors against MMPs should preferably be designed by modeling the carboxylate **5** in the tridimensional structure of the required enzyme. Modeling of the phosphonate **6a**, on the contrary, seems more suitable for the design of selective inhibitors of adamalysin II and strictly related enzymes[15] like TACE (TNF-α converting enzyme),[16] a zinc endopeptidase involved in the release of the inflammatory cytokine TNF-α. In the absence of the crystal structure of TACE, two adamalysin-based active-site models have been reported,[6,7] and we found that the phosphonate **6a** ideally fits in the proposed active site of this enzyme.

## Experimental

Melting points (Büchi oil bath apparatus) are uncorrected. $[\alpha]_D$ were determined with a Schmidt–Haensch 1604 digital polarimeter. Silica gel for column chromatography and TLC silica gel plates were from Merck AG (Darmstadt, Germany). IR spectra were obtained with a Perkin–Elmer 16 FPC FT-IR spectrophotometer. $^1$H NMR spectra were recorded on a Varian XL-300 spectrometer using TMS as internal standard. Continuous fluorimetric assays were performed by using a Perkin–Elmer spectrofluorimeter LS 50B.

***N*-(Furan-2-yl)carbonyl-L-leucine methyl ester.** *i*-Butyl chloroformate (2.33 mL, 17.8 mmol) was added dropwise, under stirring, to a solution of 2-furancarboxylic acid (2 g, 17.8 mmol) and *N*-methylmorpholine (1.95 mL, 17.8 mmol) in THF (25 mL) at −10 °C. After 30 min, a mixture of L-Leu-OMe·HCl (3.23 g, 17.8 mmol) and *N*-methylmorpholine (1.95 mL, 17.8 mmol) in THF (15 mL) was slowly added, the reaction mixture further stirred for 3 h at −10 °C and stored for 12 h at 4 °C. After filtration, the solvent was removed under reduced pressure, the residue dissolved in AcOEt and the solution washed with 2 N HCl and saturated aqueous $NaHCO_3$ solution. The organic phase was dried over $Na_2SO_4$ and evaporated under reduced pressure. The residue was crystallized from petroleum ether (bp 40–70 °C) to give *N*-(furan-2-yl)carbonyl-L-leucine methylester as colorless crystals (3.32 g, 80%): mp 88–90 °C; $[\alpha]_D^{22} = -23°$(*c* 1; MeOH); IR ($CHCl_3$) main peaks at 3424, 2956, 1741, 1663, 1595, 1517, 1351, 1177 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.96 (6H, m, $CH_2CH(C\underline{H}_3)_2$), 1.45–1.95 (3H, m, $C\underline{H}_2C\underline{H}(CH_3)_2$), 3.85 (3H, s, $O\underline{CH}_3$), 4.93 (1H, m, αCH), 6.39–7.45 (3H, m, furan CH) and 6.89 (1H, d, NH, $J$ = 8 Hz); Anal. calcd for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.15; H, 7.22; N, 5.88.

***N*-(Furan-2-yl)carbonyl-L-leucine (3).** A solution of *N*-(furan-2-yl)carbonyl-L-leucine methylester (2.73 g, 11.4 mmol) in dioxane (70 mL), MeOH (10 mL) and aqueous 1 N NaOH (22.8 mL) was stored at room temperature overnight. After concentration under reduced pressure, the alkaline aqueous phase was diluted with $H_2O$ (20 mL), washed with $Et_2O$, acidified with excess 2 N HCl and re-extracted with $CHCl_3$. After washing with brine, the organic phase was dried over $Na_2SO_4$ and evaporated under reduced pressure. Crystallization of the residue from $CH_2Cl_2$/petroleum ether, gave **3** as colorless crystals (2.24 g, 90%): mp 80–83 °C; $[\alpha]_D^{22} = -10°$ (*c* 1; MeOH); IR ($CHCl_3$) 3426, 2957, 1721, 1659, 1593, 1419, 1179, 1011 cm$^{-1}$; $^1$H NMR (MeOD) δ 0.75–1.08 (6H, m, $CH_2CH(C\underline{H}_3)_2$), 1.50–1.83 (3H, m, $C\underline{H}_2C\underline{H}(CH_3)_2$), 4.56 (1H, m, αCH), 6.30–7.46 (3H, m, furan); Anal. calcd for $C_{11}H_{15}NO_4$: C, 58.66; H, 6.71; N, 6.22. Found: C, 58.34; H, 6.33; N, 6.01.

***N*-(Furan-2-yl)carbonyl-L-leucyl-L-phosphotryptophan diethylester (4a) and *N*-(furan-2-yl)carbonyl-L-leucyl-D-phosphotryptophan diethylester (4b).** A mixture of *i*-butyl chloroformate (0.31 mL, 2.38 mmol) and *N*-methylmorpholine (0.26 mL, 2.38 mmol) in THF (4 mL) was added dropwise to a solution of *N*-(furan-2-yl)carbonyl-L-leucine (536 mg, 2.38 mmol) in THF (4 mL) at −10 °C, under stirring. After 30 min, a solution of D,L-phosphotryptophane diethylester (**2**)[8] (705 mg, 2.38 mmol) in THF (2 mL) was slowly added, the reaction mixture further stirred for 3 h at −10 °C and stored for 12 h at 4 °C. After filtration, the solvent was removed under reduced pressure, the residue was dissolved in AcOEt and washed with 2 N HCl and saturated aqueous $NaHCO_3$ solution. The AcOEt solution was dried over $Na_2SO_4$ and concentrated under reduced pressure to give the crude product as a yellow syrup (1.14 g) which was resolved into the two diastereomers by silica gel (114 g) column chromatography ($CHCl_3$/*i*-propanol, 98/2): **4a** (439 mg, 37%); TLC ($CHCl_3$/*i*-propanol, 95/5) $R_f$ 0.56; $[\alpha]_D^{22} = -54°$(*c* 1; $CHCl_3$); IR ($CHCl_3$) 3478, 3418, 1660, 1474, 1244, 1026 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.87 (6H, d, $J$ = 6.1 Hz, two $CH_2CH(C\underline{H}_3)_2$), 1.20–1.60 (9H, m, $C\underline{H}_3$-$CH_2$- and $C\underline{H}_2C\underline{H}(CH_3)_2$), 3.11 and 3.35 (2H, two m, Trp(P) β-$CH_2$), 4.12 (4H, m, two $CH_3$-$C\underline{H}_2$-) 4.64 (1H, m, Leu αCH), 4.77 (1H, m, Trp(P) αCH), 6.56 (1H, d, $J$ = 8.8 Hz, Leu NH), 6.90 (1H, d, $J$ = 9.8 Hz, Trp(P) NH), 6.48–7.61 (8H, m, furan and indole CH), 8.10 (1H, s, indole NH); **4b** (294 mg, 25%); TLC ($CHCl_3$/*i*-propanol, 95/5) $R_f$ 0.41; $[\alpha]_D^{22} = -25°$(*c* 1; $CHCl_3$); IR ($CHCl_3$) 3477, 3417, 1659, 1512, 1233, 1025 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 0.67 and 0.73 (6H, two d, $J$ = 6.0 Hz, $CH_2CH(C\underline{H}_3)_2$), 1.15–1.35 (9H, m, $C\underline{H}_3$-$CH_2$- and two $C\underline{H}_2C\underline{H}(CH_3)_2$), 3.19 and 3.35 (2H, two m, Trp(P) β-$CH_2$), 4.11 (4H, m, two $CH_3$-$C\underline{H}_2$-), 4.55 (1H, m, Leu αCH), 4.83 [1H, m, Trp(P) αCH], 6.44–7.65 (9H, m, furan and indole CH and Trp(P) NH), 6.90 (1H, d, $J$ = 8.8 Hz, Leu NH), 8.52, (1H, s, indole NH).

***N*-(Furan-2-yl)carbonyl-L-leucyl-L-phosphotryptophan (6a) cyclohexylamine salt.** A solution of *N*-(furan-2-yl)carbonyl-L-leucyl-L-phosphotryptophan diethylester (150 mg, 0.298 mmol) in anhydrous $CH_2Cl_2$ (3 mL) and *N,O*-

bis(trimethylsilyl)acetamide (0.80 mL, 3.27 mmol) was stored under nitrogen, for 1 h, at room temperature. After cooling at −20 °C, iodotrimethylsilane (0.32 mL, 2.38 mmol) was added dropwise, via syringe, under stirring. The reaction mixture was further stirred for 1 h at 0 °C and 2 h at room temperature. Removal of the solvent under reduced pressure gave a brown oily residue which was hydrolyzed by treatment with $CH_3CN/H_2O$ (7/3, 1.5 mL) for 1 h. After evaporation of the solvents under reduced pressure, the residue was dissolved in AcOEt and the solution washed with 1 N HCl and brine. Drying over $Na_2SO_4$ and removal of the solvent under reduced pressure gave the crude product which was dissolved in AcOEt (2 mL) and treated dropwise, under stirring, with a solution of cyclohexylamine (29 mg, 0.3 mmol) in AcOEt (2 mL). The **6a** cyclohexylamine salt was collected by filtration as hygroscopic, colorless crystals: 128 mg (79%); $[\alpha]_D^{22} = -67°$ (*c* 1, MeOH); IR (KBr) 3291, 2937, 1631, 1528 $cm^{-1}$; $^1H$ NMR of the free acid (DMSO-$d_6$) δ 0.82 (6H, apparent t, $CH(C\underline{H}_3)_2$), 1.31–1.58 (3H, m, $C\underline{H}_2$-$C\underline{H}(CH_3)_2$), 2.87 and 3.21 (2H, two m, Trp(P) β-$C\underline{H}_2$), 4.28 (1H, m, Trp(P) αCH), 4.51 (1H, m, Leu αCH), 6.59–7.84 (8H, m, furan and indole CH), 8.01 (1H, d, $J = 9.3$ Hz, Trp(P) NH), 8.07 (1H, d, $J = 9.3$ Hz, Leu NH), 10.72 (1H, s, indole NH). Assignment of the configuration of the carbon α to the phosphorous has been made by X-ray crystallographic analysis of the **6a**-adamalysin II complex.[6] Anal. calcd for $C_{25}H_{34}N_3O_6P{\cdot}2/3\ H_2O$: C, 58.85; H, 6.98; N, 8.24. Found: C, 58.40; H, 6.66; N, 7.85.

***N*-(Furan-2-yl)carbonyl-L-leucyl-D-phosphotryptophan (6b) cyclohexylamine salt.** According to the previous procedure, *N*-(furan-2-yl)carbonyl-L-leucyl-D-phosphotryptophan diethylester (230 mg, 0.46 mmol) gave **6b** cyclohexylamine salt as hygroscopic, colorless crystals: 206 mg (82%); $[\alpha]_D^{22} = 42°$ (*c* 1, MeOH); IR (KBr) 3409, 2933, 1639, 1525 $cm^{-1}$; $^1H$ NMR of the free acid (DMSO-$d_6$) δ 0.72 and 0.75 (6H, two d, $J = 6.5$ Hz, $CH(C\underline{H}_3)_2$), 1.00–1.37 (3H, m, $C\underline{H}_2$-$C\underline{H}(CH_3)_2$), 2.87 and 3.21 (2H, two m, Trp(P) β-$C\underline{H}_2$), 4.22 (1H, m, Trp(P) αCH), 4.52 (1H, m, Leu αCH), 6.58–7.82 (8H, m, furan and indole CH), 7.95 (1H, d, $J = 8$ Hz, Leu NH), 8.21 (1H, d, $J = 9.5$ Hz, Trp(P) NH), 10.77 (1H, s, indole NH). Anal. calcd for $C_{25}H_{34}N_3O_6P{\cdot}2/3\ H_2O$: C, 58.85; H, 6.98; N, 8.24. Found: C, 58.55; H, 6.78; N, 7.98.

**Matrix metalloproteinases activation and inhibition assays.** The proenzymes[17] were activated immediately prior to use with *p*-aminophenylmercuric acetate (APMA) 1 mM for 1 h at 25 °C for pro-MMP-2 and pro-MMP-9;[18] with APMA 2 mM for 2 h at 37 °C for pro-MMP-8[19] and with α-chymotrypsin 6 μg/mL for 2 h at 37 °C followed by phenylmethylsulphonyl fluoride 0.2 mM for pro-MMP-3.[20]

For assays measurements, 0.05 mM solutions of the inhibitors in MeOH were further diluted as required in the assay buffer, Tris·HCl 100 mM, NaCl 100 mM, $CaCl_2$ 10 mM, pH 7.5, Brij 35 0.05%. The activated enzyme plus inhibitor solution was incubated in the assay buffer for 3 h. The assay temperature was 25 °C for MMP-2 and MMP-9, and 37 °C for MMP-8 and MMP-3. After addition of 0.1 or 0.05 mM solution of the appropriate fluorogenic substrate[21] in DMSO, the hydrolysis was monitored[22] by continuously recording the increase in fluorescence ($\lambda_{ex}$ 328 nm, $\lambda_{em}$ 393 nm) using a Perkin–Elmer spectrofluorimeter LS 50B. The $IC_{50}$ were calculated from control reactions without the inhibitor. The mean $IC_{50}$ were obtained from at least three independent measurements, and the standard deviation of the mean was less than 10%.

**Adamalysin II inhibition assay.** Similarly to the previous procedure, adamalysin II[17] (6 μg) in the assay buffer (Tris·HCl 50 mM, $CaCl_2$ 10 mM, pH 7.5) plus inhibitor solution in MeOH, was immediately added with a solution of 2-aminobenzoyl-Ala-Gly-Leu-Ala-*p*-nitrobenzylamide[23] in MeOH (1.5 μM, final concentration). Hydrolysis of the fluorogenic substrate was monitored by continuously recording the increase in fluorescence ($\lambda_{ex}$ 320 nm, $\lambda_{em}$ 420 nm) for 30 min at 30 °C.

## Acknowledgements

The authors wish to thank Mr. M. Petrilli and F. Dianetti, Servizio Microanalisi del CNR, Area della Ricerca di Roma, Montelibretti who performed elemental microanalyses. This work was supported by Italian CNR, MURST, Cofin MURST 97 CFSIB and Izogetto Finalizzato Biotecnologie.

## References and Notes

1. (a) Morphy, J. R.; Millican, T. A.; Porter, J. R. *Curr. Med. Chem.* **1995**, *2*, 743. (b) Hagmann, W. K.; Lark, M. V.; Becker, J. W. *Ann. Rep. Med. Chem.* **1996**, *31*, 231.
2. For recent reviews, see (a) Schwartz, M. A.; Van Wart, H. E. In *Progress in Medicinal Chemistry*; Ellis, G. P.; Luscombe, D. K., Eds.; Elsevier Science Publishers: The Netherlands 1991; Vol 29, pp 239–270. (b) Porter, J. R.; Millican, T. A.; Morphy, J. R. *Exp. Opin. Ther. Patents* **1995**, *5*, 1287. (c) Becket, R. P.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Whittaker, M. *Drug Design Today* **1996**, *1*, 16. (d) Beckett, R. P. *Exp. Opin. Ther. Patents* **1996**, *5*, 1305. (d) Beckett, R. P.; Whittaker, M. *Exp. Opin. Ther. Patents* **1998**, *8*, 259.
3. Berger, A.; Schechter, J. *Phil. Trans. Roy. Soc. Lond.* **1970**, *B257*, 249.
4. Babine, E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
5. Robeva, A.; Politi, V.; Shannon, J. D.; Bjarnason, J. B.; Fox, J. W. *Biomed. Biochim. Acta* **1991**, *50*, 769.
6. Cirilli, M.; Gallina, C.; Gavuzzo, E.; Giordano, C.; Gomis-Rüth, F. X.; Gorini, B.; Kress, L. F.; Mazza, F.; Paglialunga-Paradisi, M.; Pochetti, G.; Politi V. *FEBS Letters*, **1997**, *418*, 319.
7. Gomis-Rüth, F. X.; Politi, V.; Meyer, E. F.; Kress, L. F.; Bode, W. *Protein Sci.* **1998**, *7*, 283.
8. Kortylewicz, Z. P.; Galardy, R. E. *J. Med. Chem.* **1990**, *33*, 263.
9. Galardy, R. E.; Grobelny, D.; Kortylewicz, Z. P.; Poncz, L. *Metalloproteinases Inhib., Proc. Matrix Metalloproteinase Conf., 1989, Matrix Suppl. n 1* **1992**, 259.
10. Morphy, J. R.; Beeley, N. R. A.; Boyce, B. A.; Leonard, J.; Mason, B.; Millican, A.; Millar, K.; O'Connell, J. P.; Porter, J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2747.
11. Bird J.; De Mello, R. C.; Harper, G. P.; Hunter, D. J.; Karran, E. H.; Markwell, R. E.; Miles-Williams, A. J.; Rahman, S. S.; Ward, R. W. *J. Med. Chem.* **1994**, *37*, 158.

12. Subotkwski, W.; Kowalik, J.; Tyka, R.; Mastalerz, P. *Pol. J. Chem*. **1981**, *55*, 853.
13. Chen, F. M.; Steinhauer, R.; Benoiton, N. L. *J. Org. Chem*. **1983**, *26*, 2939.
14. Solas, D.; Hale, R. L.; Patel, D. V. *J. Org. Chem*. **1996**, *61*, 1537.
15. Stöcker, W.; Grams, F.; Baumann, U.; Reinemer, P.; Gomis-Rüth, F. X.; McKay, D. B.; Bode, W. *Protein Sci*. **1995**, *4*, 823.
16. See 7 and literature cited therein.
17. Pro-MMP-2, pro-MMP-3, pro-MMP-8 and pro-MMP-9 were supplied by G. Murphy (Strangeways Research Laboratories, Cambridge, UK). Adamalysin II was a kind gift from L. F. Kress (Roswell Park Cancer Institute, Buffalo, New York).
18. Murphy, G.; Crabbe, T. *Methods in Enzymol*. **1995**, *248*, 470.
19. Tschesche, H. *Methods in Enzymol*. **1995**, *248*, 431.
20. Nagase, H. *Methods in Enzymol*. **1995**, *248*, 449.
21. Fluorogenic substrates (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitrophenyl]-L-2,3-diaminopropionyl)-Ala-Arg-$NH_2$ (MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$)[22] for MMP-2 and MMP-9, MCA-Pro-Cha-Gly-Nva-His-Ala-Dpa-$NH_2$ for MMP-8 (G. Knight, private communication) and MCA-Pro-Leu-Ala-Nva-Dpa-Ala-Arg-$NH_2$ for MMP-3 (G. Knight, private communication) were supplied by G. Knight (Strangeways Research Laboratories, Cambridge, UK). 2-Aminobenzoyl-Ala-Gly-Leu-Ala-*p*-nitrobenzylamide was purchased from Bachem (Budendorf, Switzerland).
22. Knight, C. G.; Willenbrock, F.; Murphy, G. *FEBS Letters* **1992**, *296*, 263.
23. Fox, J. W.; Campbell, R.; Beggerly, L.; Bjarnason, J. B. *Eur. J. Biochem*. **1986**, *156*, 65.