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Protease inhibitors
Part 12. Synthesis of potent matrix metalloproteinase and bacterial collagenase inhibitors incorporating sulfonylated *N*-4-nitrobenzyl-β-alanine hydroxamate moieties

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Abstract

N-4-Nitrobenzyl-β-alanine was reacted with alkyl/arylsulfonyl halides, followed by conversion of the COOH to the CONHOH group. Structurally related compounds were obtained by reaction of *N*-4-nitrobenzyl-β-alanine with aryl isocyanates, arylsulfonyl isocyanates or benzoyl isothiocyanate, followed by similar conversion of the COOH into the CONHOH moiety. Another subseries of derivatives was prepared from sulfanilyl- or metanilyl-4-nitrobenzyl-β-alanine by reaction with arylsulfonyl isocyanates, followed by the introduction of the hydroxamate moiety. The new compounds were assayed as inhibitors of four matrix metalloproteinases (MMPs), MMP-1, MMP-2, MMP-8 and MMP-9, and of the *Clostridium histolyticum* collagenase (ChC). Some of the prepared hydroxamate derivatives proved to be very effective collagenase/gelatinase inhibitors, depending on the substitution pattern at the sulfonamido moiety. Substitutions leading to the best inhibitors of MMP-1, a short-pocket enzyme, were those involving pentafluorophenylsulfonyl or 3-trifluoromethyl-phenylsulfonyl at $P_{1'}$ (K_I of 3–5 nM). For MMP-2, MMP-8 and MMP-9 (deep-pocket enzymes), the best inhibitors were those containing perfluoroalkylsulfonyl- and substituted-arylsulfonyl moieties, such as pentafluorophenylsulfonyl, 3- and 4-protected-aminophenylsulfonyl-, 3- and 4-carboxy-phenylsulfonyl-, arylsulfonylureido- or arylsulfonylureido-sulfanilyl-/metanilyl moieties at $P_{1'}$. Bulkier groups in this position, such as 1- and 2-naphthyl-, substituted-naphthyl or quinoline-8-yl- moieties, among others, led to less effective MMP/ChC inhibitors. The best ChC inhibitors were again those containing pentafluorophenylsulfonyl, 3- and 4-protected-aminophenylsulfonyl $P_{1'}$ groups. This study demonstrates that the 4-nitrobenzyl moiety, investigated here for the first time, is an efficient $P_{2'}$ anchoring moiety, whereas the β-alanyl scaffold can successfully replace the α-amino acyl one, for obtaining potent MMP/ChC inhibitors.

Keywords: *N*-4-Nitrobenzyl-β-alanine; Hydroxamate; Sulfonyl halide; Matrix metalloproteinase; Bacterial collagenase; Enzyme inhibitor

1. Introduction

The extracellular matrix (ECM) plays a crucial role for the structure and integrity of various tissue types in higher vertebrates (Johnson et al., 1998; Whittaker et al., 1999). ECM turnover is involved in important physiological and physiopathological events, such as embryonic development, blastocyst implantation, nerve growth, ovulation, morphogenesis, angiogenesis, tissue resorption and remodeling (such as in the case of wound healing), bone remodeling, apoptosis, cancer invasion and metastasis, arthritis, atherosclerosis, aneurysm, breakdown of blood–brain barrier, periodontal disease, skin ulceration, corneal ulceration, gastric ulcers, and liver fibrosis, among others (Bottomley et al., 1998; Johnson et al., 1998; Kahari and Saarialho-Kere, 1999; Scozzafava and Supuran, 1999a, 2000; Williams et al., 1999). The matrix metalloproteinases (MMPs), a family of zinc-containing endopeptidases (also called matrixins), are thought to play a central role in the above processes (Dioszegi et al., 1995; Tschesche, 1995; Johnson et al., 1998; Nagase and Woessner, 1999; Whittaker et al., 1999).

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At least 20 members of this enzyme family, sharing significant sequence homology, have been reported up to now (Table 1) (Johnson et al., 1998; Nagase and Woessner, 1999; Whittaker et al., 1999). They can be subdivided (considering the macromolecular substrate requirements) into: (i) collagenases (MMP-1, -8, -13 and -18); (ii) gelatinases (MMP-2 and -9); (iii) stromelysins (MMP-3, -10 and -11); and (iv) membrane-type MMPs (MT-MMPs) (MMP-14, -15, -16 and -17). Recently, some new members of the family have been evidenced, but little is known for the moment regarding their properties, substrate specificity, or inhibition (Table 1).

Matrix metalloproteinases (MMPs) have recently become interesting targets for drug design, in the search of novel types of anticancer, antiarthritis or other pharmacological agents useful in the management of inflammatory processes (Babine and Bender, 1997; Supuran and Scozzafava, 2000a,b; Whittaker et al., 1999). It is thus possible to envisage many medicinal chemistry applications by inhibiting the activity of these enzymes, and several pharmacological agents of the hydroxamate type, such as batimastat (**1**), marimastat (**2**), trocade (**3**), or the sulfonyl amino acid derivatives such as CGS 27023A (**4a**), or the structurally related **4b** are currently in advanced clinical trials as antimetastasis, anticancer or antiarthritis drugs (Babine and Bender, 1997; Supuran and Scozzafava, 2000a; Whittaker et al., 1999) (Fig. 1).

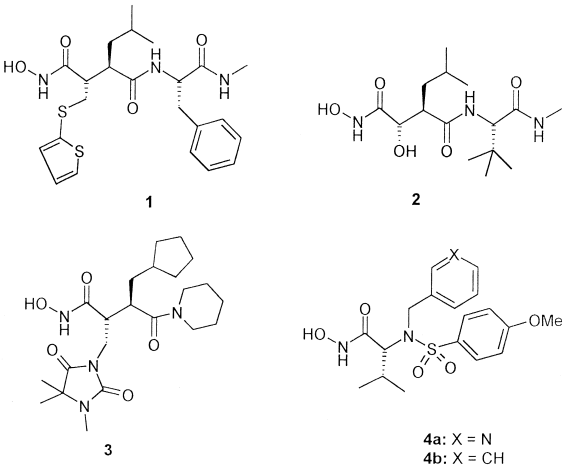


Fig. 1. Structures **1**–**4**.

Literature data show (Grams et al., 1995a,b; Graff von Roedern et al., 1998; Jeng et al., 1998; Krumme et al., 1998; Hanessian et al., 1999; Kiyama et al., 1999; Supuran et al., 2000) that MMP inhibitors have been studied extensively in the last 15 years in order to find medicinal chemistry applications. The same situation is not true for inhibitors of the collagenases isolated from *Clostridium*

Table 1
Vertebrate MMPs, their molecular weights, substrates and preferred scissile amide bonds

Protein	MMP	MW (kDa)	Principal substrate(s)	Preferred scissile amide bond(s)
Collagenase 1	MMP-1	52	Fibrillar and nonfibrillar collagens (types I, II, III, VI and X); gelatins	Gly–Ile
Gelatinase A	MMP-2	72	Basement membrane and nonfibrillar collagens (types IV, V, VII, X); fibronectin, elastin	Ala–Met
Stromelysin 1	MMP-3	57	Proteoglycan, laminin, fibronectin, collagen (types III, IV, V, IX); gelatins; pro-MMP-1	Gly–Leu
Matrilysin	MMP-7	28	Fibronectins; gelatins; proteoglycan	Ala–Ile
Collagenase 2	MMP-8	64	Fibrillar collagens (types I, II, III)	Gly–Leu; Gly–Ile
Gelatinase B	MMP-9	92	Basement membrane collagens (types IV, V); gelatins	Gly–Ile; Gly–Leu
Stromelysin 2	MMP-10	54	Fibronectins; collagen (types III, IV); Gelatins, pro-MMP-1	Gly–Leu
Stromelysin 3	MMP-11	45	Serpin	Ala–Met
Macrophage elastase	MMP-12	53	Elastin	Ala–Leu; Tyr–Leu
Collagenase 3	MMP-13	51.5	Fibrillar collagens (types I, II, III); gelatins	Gly–Ile
MT1-MMP	MMP-14	66	Pro-72 kDa gelatinase	Not determined
MT2-MMP	MMP-15	61	Not determined	Not determined
MT3-MMP	MMP-16	55	Pro-72 kDa gelatinase	Not determined
MT4-MMP	MMP-17	58	Not determined	Ala–Gly
Collagenase 4 (*Xenopus*)	MMP-18	53	Not determined	Gly–Ile
RASI 1	MMP-19	?	Gelatin	Not determined
Enamelysin	MMP-20	?	Amelogenin (dentine); gelatin	Not determined
XMMP (*Xenopus*)	MMP-21	?	Not determined	Not determined
CMMP (chicken)	MMP-22	?	Not determined	Not determined
(No trivial name)	MMP-23	?	Not determined	Not determined

histolyticum, which were much less investigated. This collagenase (EC 3.4.24.3) is a 116 kDa protein belonging to the M31 metalloproteinase family (Bond and Van Wart, 1984a,b; Rawlings and Barrett, 1995; Van Wart, 1998), which is able to hydrolyze triple helical regions of collagen under physiological conditions, as well as an entire range of synthetic peptide substrates. In fact, the crude homogenate of *Clostridium histolyticum*, which contains several distinct collagenase isozymes (Bond and Van Wart, 1984a,b; Rawlings and Barrett, 1995; Van Wart, 1998), is the most efficient system known for the degradation of connective tissue, also being involved in the pathogenicity of this and related clostridia, such as *C. perfringens*, which causes human gas gangrene and food poisoning, among others (Rood, 1998). Typically, these bacteria (and their collagenases) cause so much damage and so quickly, that antibiotics are ineffective. Thus, development of inhibitors against these collagenases is also an interesting target for drug design.

Similarly to the vertebrate MMPs, *Clostridium histolyticum* collagenase (abbreviated as ChC) has the conserved HExxH zinc-binding motif, which in this specific case is His^{415}ExxH, with the two histidines (His 415 and His 419) acting as Zn(II) ligands, whereas the third ligand seems to be Glu 447, and a water molecule/hydroxide ion acting as nucleophile in the hydrolytic scission (Matsushita et al., 1998, 1999; Jung et al., 1999). Similarly to the MMPs, ChC is also a multiunit protein, consisting of four segments, S1, S2a, S2b and S3, with S1 incorporating the catalytic domain. Although the two types of enzymes mentioned above (the MMPs and the bacterial ChC) are relatively different, it is generally considered that their mechanism of action for the hydrolysis of proteins and synthetic substrates is rather similar. One must also mention that these enzymes could not be crystallized, for X-ray crystallographic experiments, and in order to assist the design of their inhibitors.

In this paper we report the preparation of a series of MMP and ChC inhibitors incorporating alkyl/arylsulfonamido-β-alanine hydroxamate as well as arylsulfonylureido-/arylureido-β-alanine hydroxamate moieties in the molecule. Some of the new compounds, assayed for the inhibition of purified collagenases (MMP-1 and MMP-8), gelatinases (MMP-2 and MMP-9) and of ChC, showed high affinity for these enzymes (in the nanomolar range), behaving as some of the best MMP/ChC sulfonylated inhibitors reported up to now. SAR is also discussed both for MMP inhibition as well as bacterial collagenase inhibition with these new types of derivatives.

2. Materials and methods

Melting points, heating plate microscope (not corrected); IR spectra, KBr pellets, 400–4000 cm^{-1} Perkin-Elmer 16PC FTIR spectrometer; ^{1}H-NMR spectra, Varian Gemmini 200 apparatus (chemical shifts are expressed as δ values relative to Me_4Si as standard); elemental analysis (±0.4% of the theoretical values, calculated for the proposed formulas for all the compounds reported here), Carlo Erba Instrument CHNS Elemental Analyzer, Model 1106. All reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm precoated silica gel plates (E. Merck). Preparative HPLC was performed on a Dynamax-60A column (25×250 mm) with a Beckman EM-1760 instrument. The detection wavelength was 254 nm.

Amino acids (β-alanine), 4-nitrobenzyl chloride, sulfonyl chlorides, arylsulfonyl isocyanates, aryl isocyanates, benzoyl isothiocyanate, triethylamine, carbodiimides, hydroxylamine, 5,5′-dithiobis-(2-nitrobenzoic acid), FALGPA, buffers and other reagents used in the syntheses were commercially available compounds from Sigma, Acros or Aldrich. The thioester MMP substrate, AcProLeuGly-S-LeuLeuGlyOEt, was from Bachem.

2.1. Chemistry

2.1.1. Preparation of N-4-nitrobenzyl-β-alanine (7)

An amount of 7.8 g (0.10 M) of β-alanine (**6**) and a stoichiometric amount of 4-nitrobenzyl chloride (16.1 g) were suspended/dissolved in 150 ml of anhydrous acetonitrile and an equivalent amount of triethyl amine (0.10 mM, 14.7 ml) was added. The reaction mixture was stirred at room temperature for 20 h, then the solvent was evaporated in vacuo. The obtained reaction mixture was taken in 250 ml of water, the pH was brought to 7 with citric acid, and crude product **7** precipitated by leaving the mixture overnight at 4°C. Recrystallization from ethanol afforded the pure title compound in almost quantitative yield.

2.1.2. General procedure for the preparation of N-4-nitrobenzyl-alkyl/arylsulfonyl β-alanines (A1–A35)

An amount of 2.10 g (10 mmol) of *N*-2-nitrobenzyl-β-alanine (**7**) and 10 mmol of sulfonyl chloride were suspended/dissolved in 100 ml of acetone+25 ml of water. A stoichiometric amount (10 mmol) of base ($NaHCO_3$, $KHCO_3$, NaOH or Et_3N) dissolved in a small amount (20 ml) of water was added and the mixture stirred at room temperature for 4–10 h (TLC control). The solvent was evaporated, the reaction mixture was retaken in 100 ml of water and the crude product extracted in ethyl acetate. After evaporation of the solvent, compounds **A1**–**A35** were recrystallized from EtOH or MeOH. Yields were around 75–90%.

2.1.3. General procedure for the preparation of compounds B1–B35, D1–D5, F1–F6, H1–H3 and J1

An amount of 5 mM of carboxylic acid derivative **A1**–**A35**, **C1**–**C5**, **E1**–**E6**, **G1**–**G3** or **I1** was dissolved/suspended in 50 ml of anhydrous acetonitrile or acetone,

and treated with 420 mg (6 mM) of hydroxylamine·HCl and 1.10 g (6 mM) of EDCI·HCl or di-isopropyl-carbodiimide. The reaction mixture was magnetically stirred at room temperature for 15 min, then 180 μl (12 mM) of triethylamine were added and stirring was continued for 12 h at 4°C. The solvent was evaporated in vacuo and the residue taken up in ethyl acetate (5 ml), poured into a 5% solution of sodium bicarbonate (5 ml) and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and filtered, and the solvent removed in vacuo. Preparative HPLC (Dynamax-60A column (25× 250 mm); 90% acetonitrile/10% methanol; flow rate 30 ml/min) afforded the pure hydroxamic acids.

*2.1.4. General procedure for the preparation of compounds **C1–C5, E1–E6** and **I1***

An amount of 2.20 g (10 mmol) of *N*-4-nitrobenzyl-β-alanine (**7**) and a stoichiometric amount of arylsulfonyl isocyanate (**8**), aryl isocyanate (**9**) or benzoylisothiocyanate were suspended in 50 ml of anhydrous acetonitrile and 150 μl (10 mM) of triethylamine were added. The reaction mixture was either stirred at room temperature (in the case of derivatives prepared from **8**) or refluxed (for the other two types of derivatives) for 2–6 h. The solvent was evaporated and the reaction mixture worked up as described above. The new compounds were recrystallized from ethanol. Yields were almost quantitative.

*2.1.5. General procedure for the preparation of compounds **G1–G3***

The general procedure described above for the preparation of compounds **A1–A35** was followed, except that arylsulfenyl halides were used instead of alkyl/arylsulfonyl halides. The yields of the title sulfenamides were around 60%.

*2.1.6. General procedure for the preparation of derivatives **M,N(1–5)***

An amount of 10 mM of boc-protected derivative, **A19** or **A20**, was treated with a small excess of TFA at room temperature, under energetic magnetic stirring, for 30 min. The excess TFA was evaporated in vacuo, the residue taken in water and neutralized with sodium bicarbonate until pH 5.5. The precipitated amino derivatives **10** and **11** were filtered and air dried. Reaction of these derivatives with arylsulfonyl isocyanates **8** in acetone, as described above, afforded the carboxylates **K**,**L**(**1–5**), which were converted into the corresponding hydroxamates as described above. Overall yields were around 60–63%.

All new compounds were characterized by ^{1}H- and ^{13}C-NMR spectroscopy and elemental analysis (±0.4% of the theoretical data). Data for a representative compound of each series is provided below.

*2.1.7. N-4-Toluenesulfonyl-N-4-nitrobenzyl-β-alanine (**A13**)*

Pale yellow crystals, m.p. 211–212°C; ^{1}H-NMR (DMSO-d_6), *δ*, ppm: 2.52 (s, 3H, C$\underline{H}_3C_6H_4$), 3.62 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.36 (s, 2H, C$\underline{H}_2$ of benzyl); 7.25–7.64 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$+$\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 7.97 (d, $^{3}J_{HH}$ = 8.1, 2H, $\underline{H}_{meta}$ of $CH_3C_6\underline{H}_4$); 8.21 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 11.69 (br s, 1H, COOH); ^{13}C-NMR (DMSO-d_6), *δ*, ppm: 26.0 (s, $\underline{C}H_3C_6H_4$), 36.5 (s, $\underline{C}H_2$ of β-Ala), 43.5 (s, $\underline{C}H_2$ of β-Ala), 44.7 (s, $\underline{C}H_2$ of benzyl), 123.8 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4$), 129.4 ($\underline{C}_{ortho}$ of $O_2NC_6H_4$), 130.5 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 144.5 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4$), 145.0 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 147.8 (s, $\underline{C}_{para}$ of $O_2NC_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 177.3 (s, $\underline{C}O_2H$). Anal. found: C, 52.57; H, 4.76; N, 7.54%. $C_{17}H_{18}N_2O_6S$ requires: C, 52.74; H, 4.43; N, 7.69%.

*2.1.8. N-4-Toluenesulfonyl-N-4-nitrobenzyl-β-alanine hydroxamate (**B13**)*

Pale yellow crystals, m.p. 233–234°C; ^{1}H-NMR (DMSO-d_6), *δ*, ppm: 2.62 (s, 3H, C$\underline{H}_3C_6H_4$), 3.79 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.39 (s, 2H, C$\underline{H}_2$ of benzyl); 7.21–7.67 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$+$\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 8.00 (d, $^{3}J_{HH}$ = 8.1, 2H, $\underline{H}_{meta}$ of $CH_3C_6\underline{H}_4$); 8.21 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 8.70 (br s, 1H, N$\underline{H}$OH); 10.51 (br s, 1H, NHO$\underline{H}$); ^{13}C-NMR (DMSO-d_6), *δ*, ppm: 26.5 (s, $\underline{C}H_3C_6H_4$), 36.5 (s, $\underline{C}H_2$ of β-Ala), 44.6 (s, $\underline{C}H_2$ of β-Ala), 45.1 (s, $\underline{C}H_2$ of benzyl), 123.8 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4$), 129.4 ($\underline{C}_{ortho}$ of $O_2NC_6H_4$), 130.7 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 135.6 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 144.3 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4$), 145.3 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 147.9 (s, $\underline{C}_{para}$ of $O_2NC_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 174.0 (s, $\underline{C}$ONHOH). Anal. found: C, 50.77; H, 4.39; N, 11.02%. $C_{17}H_{19}N_3O_6S$ requires: C, 50.65; H, 4.52; N, 11.08%.

*2.1.9. N-4-Toluenesulfonylureido-N-4-nitrobenzyl-β-alanine (**C3**)*

Pale yellow crystals, m.p. 277–278°C; ^{1}H-NMR (DMSO-d_6), *δ*, ppm: 2.60 (s, 3H, C$\underline{H}_3C_6H_4$), 3.82 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.38 (s, 2H, C$\underline{H}_2$ of benzyl); 7.29–7.73 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$+$\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 7.99 (d, $^{3}J_{HH}$ = 8.1, 2H, $\underline{H}_{meta}$ of $CH_3C_6\underline{H}_4$); 8.16 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 8.29 (br s, 2H, NHCONH); 11.73 (br s, 1H, COOH); ^{13}C-NMR (DMSO-d_6), *δ*, ppm: 26.5 (s, $\underline{C}H_3C_6H_4$), 36.3 (s, $\underline{C}H_2$ of β-Ala), 43.9 (s, $\underline{C}H_2$ of β-Ala), 45.3 (s, $\underline{C}H_2$ of benzyl), 123.9 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4$), 129.4 ($\underline{C}_{ortho}$ of $O_2NC_6H_4$), 131.9 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (s, NH$\underline{C}$ONH), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 144.5 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4$), 145.7 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 147.8 (s, $\underline{C}_{para}$ of $O_2NC_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 177.3 (s, $\underline{C}O_2H$). Anal. found: C, 50.34; H, 4.08; N, 10.15%. $C_{17}H_{17}N_3O_7S$ requires: C, 50.12; H, 4.21; N, 10.31%.

*2.1.10. N-4-Toluenesulfonylureido-N-4-nitrobenzyl-β-alaninehydroxamate (**D3**)*

Pale yellow crystals, m.p. 273–274°C; ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.63 (s, 3H, C$\underline{H}_3C_6H_4$), 3.80 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.36 (s, 2H, C$\underline{H}_2$ of benzyl); 7.20–7.74 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$ + $\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 7.99 (d, $^3J_{HH}$ = 8.1, 2H, $\underline{H}_{meta}$ of $CH_3C_6\underline{H}_4$); 8.21 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 8.32 (br s, 2H, NHCONH); 8.76 (br s, 1H, N$\underline{H}$OH); 10.59 (br s, 1H, NHO$\underline{H}$); ^{13}C-NMR (DMSO-d_6), δ, ppm: 26.3 (s, $\underline{C}H_3C_6H_4$), 36.6 (s, $\underline{C}H_2$ of β-Ala), 44.9 (s, $\underline{C}H_2$ of β-Ala), 45.2 (s, $\underline{C}H_2$ of benzyl), 123.7 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4$), 129.5 ($\underline{C}_{ortho}$ of $O_2NC_6H_4$), 131.3 (s, $\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (s, NH$\underline{C}$ONH), 135.0 (s, $\underline{C}_{ortho}$ of $CH_3C_6H_4$), 144.5 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4$), 145.6 (s, $\underline{C}_{ipso}$ of $CH_3C_6H_4$), 147.7 (s, $\underline{C}_{para}$ of $O_2NC_6H_4$), 148.6 (s, $\underline{C}_{para}$ of $CH_3C_6H_4$), 174.6 (s, $\underline{C}$ONHOH). Anal. found: C, 48.09; H, 4.43; N, 13.12%. $C_{18}H_{20}N_4O_7S$ requires: C, 48.34; H, 4.30; N, 13.26%.

*2.1.11. N-4-Fluorophenylureido-N-4-nitrobenzyl-β-alanine (**E1**)*

Yellow crystals, m.p. 238–239°C; ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.80 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.35 (s, 2H, C$\underline{H}_2$ of benzyl); 7.18–7.66 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$ + $\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 7.94 (d, $^3J_{HH}$ = 8.0, 2H, $\underline{H}_{meta}$ of $FC_6\underline{H}_4$); 8.05 (br s, 2H, NHCONH); 8.22 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 11.44 (br s, 1H, COOH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 36.8 (s, $\underline{C}H_2$ of β-Ala), 44.3 (s, $\underline{C}H_2$ of β-Ala), 44.6 (s, $\underline{C}H_2$ of benzyl), 123.8 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4$), 129.4 ($\underline{C}_{ortho}$ of $O_2NC_6H_4$), 131.4 (s, $\underline{C}_{meta}$ of FC_6H_4), 132.0 (s, NH$\underline{C}$ONH), 135.2 (s, $\underline{C}_{ortho}$ of FC_6H_4), 144.5 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4$), 147.4 (s, $\underline{C}_{para}$ of $O_2NC_6H_4$), 148.9 (s, $\underline{C}_{ipso}$ of FC_6H_4), 149.7 (s, $\underline{C}_{para}$ of FC_6H_4), 177.8 (s, $\underline{C}O_2H$). Anal. found: C, 55.60; H, 4.13; N, 12.00%. $C_{17}H_{16}FN_3O_5$ requires: C, 55.33; H, 4.06; N, 12.10%.

*2.1.12. N-4-Fluorophenylureido-N-4-nitrobenzyl-β-alaninehydroxamate (**F1**)*

Yellow crystals, m.p. 200–201°C; ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.78 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.42 (s, 2H, C$\underline{H}_2$ of benzyl); 7.22–7.69 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$ + $\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 7.90 (d, $^3J_{HH}$ = 8.2, 2H, $\underline{H}_{meta}$ of $FC_6\underline{H}_4$); 8.04 (br s, 2H, NHCONH); 8.24 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 8.70 (br s, 1H, N$\underline{H}$OH); 10.62 (br s, 1H, NHO$\underline{H}$); ^{13}C-NMR (DMSO-d_6), δ, ppm: 36.9 (s, $\underline{C}H_2$ of β-Ala), 44.1 (s, $\underline{C}H_2$ of β-Ala), 45.4 (s, $\underline{C}H_2$ of benzyl), 123.8 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4$), 129.4 ($\underline{C}_{ortho}$ of $O_2NC_6H_4$), 130.7 (s, $\underline{C}_{meta}$ of FC_6H_4), 131.7 (s, NH$\underline{C}$ONH), 135.5 (s, $\underline{C}_{ortho}$ of FC_6H_4), 144.7 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4$), 145.6 (s, $\underline{C}_{ipso}$ of FC_6H_4), 147.7 (s, $\underline{C}_{para}$ of $O_2NC_6H_4$), 148.9 (s, $\underline{C}_{para}$ of FC_6H_4), 174.8 (s, $\underline{C}$ONHOH). Anal. found: C, 52.84; H, 4.32; N, 15.40%. $C_{17}H_{16}FN_4O_5$ requires: C, 53.04; H, 4.17; N, 15.46%.

*2.1.13. N-4-Nitrophenylsulfenyl-N-4-nitrobenzyl-β-alanine (**G1**)*

Yellow crystals, m.p. 223–224°C; ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.87 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.37 (s, 2H, C$\underline{H}_2$ of benzyl); 6.75 (s, 1H, SNH), 7.21–7.62 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$ + $\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 8.05 (d, $^3J_{HH}$ = 8.3, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$); 8.21 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 11.68 (br s, 1H, COOH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 36.5 (s, $\underline{C}H_2$ of β-Ala), 44.0 (s, $\underline{C}H_2$ of β-Ala), 44.8 (s, $\underline{C}H_2$ of benzyl), 123.8 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4CH_2$), 129.4 ($\underline{C}_{ortho}$ of $O_2NC_6H_4CH_2$), 130.3 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4S$), 135.9 (s, $\underline{C}_{ortho}$ of $O_2NC_6H_4S$), 144.5 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4CH_2$), 145.9 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4S$), 147.8 (s, $\underline{C}_{para}$ of $O_2NC_6H_4CH_2$), 150.4 (s, $\underline{C}_{para}$ of $O_2NC_6H_4S$), 177.5 (s, $\underline{C}O_2H$). Anal. found: 49.45; H, 3.77; N, 11.24%.$C_{16}H_{15}N_3O_6S$ requires: C, 49.58; H, 3.61; N, 11.56%.

*2.1.14. N-4-Nitrophenylsulfenyl-N-4-nitrobenzyl-β-alaninehydroxamate (**H1**)*

Yellow crystals, m.p. 197–198°C; ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.84 (m, 4H, C$\underline{H}_2$ of β-Ala), 4.38 (s, 2H, C$\underline{H}_2$ of benzyl); 6.75 (s, 1H, SNH), 7.20–7.67 (m, 4H, $\underline{H}_{ortho}$ of $CH_3C_6\underline{H}_4$ + $\underline{H}_{ortho}$ of $O_2NC_6\underline{H}_4$), 8.09 (d, $^3J_{HH}$ = 8.2, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$); 8.23 (d, 2H, $\underline{H}_{meta}$ of $O_2NC_6\underline{H}_4$), 8.74 (br s, 1H, N$\underline{H}$OH); 10.68 (br s, 1H, NHO$\underline{H}$); ^{13}C-NMR (DMSO-d_6), δ, ppm:, 36.5 (s, $\underline{C}H_2$ of β-Ala), 44.1 (s, $\underline{C}H_2$ of β-Ala), 44.6 (s, $\underline{C}H_2$ of benzyl), 123.8 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4CH_2$), 129.6 ($\underline{C}_{ortho}$ of $O_2NC_6H_4CH_2$), 130.6 (s, $\underline{C}_{meta}$ of $O_2NC_6H_4S$), 135.9 (s, $\underline{C}_{ortho}$ of $O_2NC_6H_4S$), 144.5 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4CH_2$), 147.9 (s, $\underline{C}_{para}$ of $O_2NC_6H_4CH_2$), 148.5 (s, $\underline{C}_{para}$ of $O_2NC_6H_4S$), 150.3 (s, $\underline{C}_{ipso}$ of $O_2NC_6H_4S$), 174.6 (s, $\underline{C}$ONHOH). Anal. found: C, 47.91; H, 3.65; N, 14.69%. $C_{15}H_{14}N_4O_6S$ requires: C, 47.62; H, 3.73; N, 14.81%.

2.2. Enzymology

Human purified MMPs (MMP-1, MMP-2, MMP-8 and MMP-9) were purchased from Calbiochem (Inalco, Milano, Italy). They were activated (Nagase, 1997) in the assay buffer by adding bovine trypsin (50 μl, 0.6 mg/ml) to the proenzyme, followed by incubation at 37°C for 10 min. The trypsin was then inactivated with aprotinin (50 μl, 1.2 mg/ml). Initial rates for the hydrolysis of the thioester substrate AcProLeuGly-S-LeuLeuGlyOEt, coupled to the reaction with 5,5′-dithiobis-(2-nitrobenzoic acid), were used for assessing the catalytic activity and inhibition of the four MMPs mentioned above, by the spectrophotometric method of Powers and Kam (1995) and Johnson et al. (1999). The change of absorbance (ε = 19,800 M^{-1} cm^{-1}) at 405 nm was monitored continuously at room temperature using a Cary 3 spectrophotometer interfaced to a PC. A typical 100 μl reaction contained 50 mM MES, pH 6.0, 10 mM $CaCl_2$, 100 μM substrate, 1

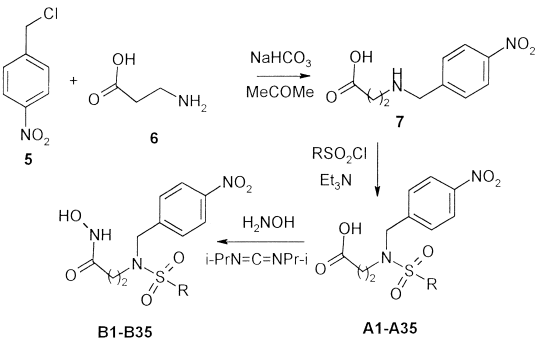
mM 5,5′-dithiobis-(2-nitrobenzoic acid) and 5 nM MMP. For the K_I determinations, DMSO solutions of the inhibitor were included in the assay, resulting in a final concentration of 2% DMSO in the reaction mixture. Under these conditions, K_I values varied from 5 to 10% in replicate experiments. K_I were then determined using Easson–Stedman (Bieth, 1995) plots and a linear regression program.

Clostridium histolyticum highly purified collagenase and its substrate, FALGPA (furanacryloyl-leucyl-glycyl-prolyl-alanine), were purchased from Sigma (Milano, Italy), and their concentrations were determined from the absorbance at 280 nm and the extinction coefficients furnished by the supplier. The activity of such preparations was in the range of 10 NIH units/mg solid. The potency of standard and newly obtained inhibitors was determined from the inhibition of the enzymatic (amidolytic) activity of the collagenase at 25°C using FALGPA as substrate by the method of Van Wart and Steinbrink (1981). The substrate was reconstituted as 5 mM stock in 50 mM Tricine buffer, 0.4 M NaCl, 10 mM $CaCl_2$, pH 7.50. The rate of hydrolysis was determined from the change in absorbance at 324 nm using an extinction coefficient for FALGPA $\varepsilon_{305} = 24{,}700\ M^{-1}\ cm^{-1}$ in the above-mentioned reaction buffer. Measurements were made using a Cary 3 spectrophotometer interfaced to a PC. Initial velocities were thus estimated using the direct linear plot-based procedure reported by Van Wart and Steinbrink (1981). K_I were then determined according to Easson–-Stedman (Bieth, 1995) plots and a linear regression program.

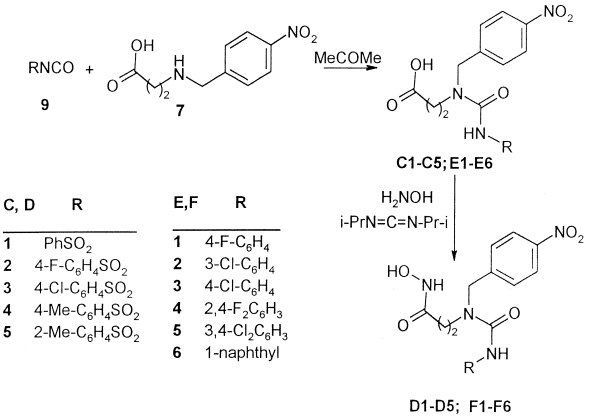
3. Results

Non-exceptional routine synthetic procedures were used for the preparation of the MMP inhibitors reported here, related to those applied by MacPherson et al. (1997), Jeng et al. (1998), Hanessian et al. (1999) and our group (Scozzafava and Supuran, 1999b, 2000) for sulfonylated amino acid hydroxamates. Reaction of 4-nitrobenzyl chloride **5** with β-alanine **6** afforded the key intermediate, *N*-4-nitrobenzyl-β-alanine (**7**). Carboxylic acids **A1**–**A35** were then prepared by reaction of alkyl/arylsulfonyl chlorides with compound **7** (Scheme 1). Conversion of the carboxylic acids **A1**–**A35** into the corresponding hydroxamates **B1**–**B35** was done with hydroxylamine and diisopropyl carbodiimide (Scheme 1) (Tamura et al., 1998; Borras et al., 1999; Supuran et al., 1999; Scozzafava et al., 1999a,b).

Another series of derivatives (**C1**–**C5**, **D1**–**D5** and **E1**–**E6**, **F1**–**F6**) was obtained by reaction of arylsulfonylisocyanates **8** or aryl isocyanates **9** with the *N*-benzyl-β-alanine derivative **7**, followed by conversion of the COOH moiety into the CONHOH one, as described above (Scheme 2) (Tamura et al., 1998; Borras et al., 1999;



Scheme 1.



Scheme 2.

Supuran et al., 1999; Scozzafava et al., 1999a,b; Scozzafava and Supuran, 2000).

By applying synthetic strategies related to those described previously, the sulfenamido derivatives **G1**–**G3** and **H1**–**H3**, as well as the thioureas **I1** and **J1** were also obtained (Fig. 2).

Another approach for obtaining MMP and ChC inhibitors consisted of deprotection of the Boc-derivatives **A19** and **A20** (with TFA), followed by reaction of the

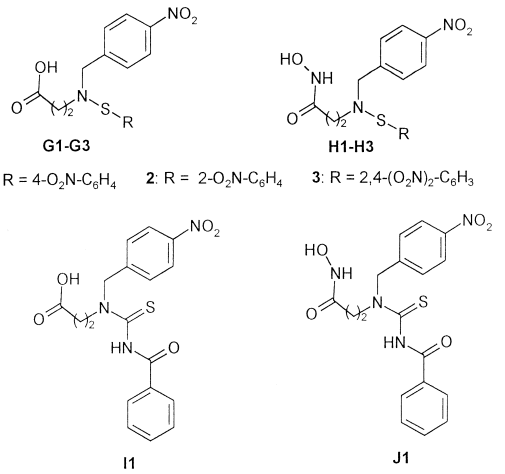
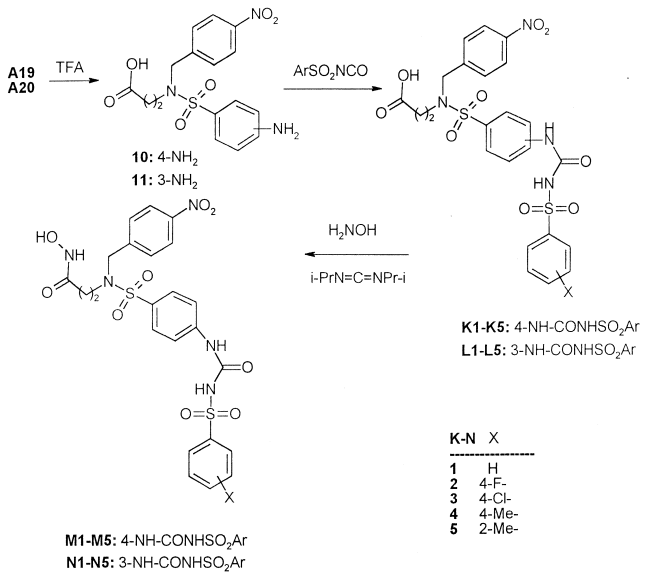


Fig. 2. Structures **G1**–**G3**, **H1**–**H3**, **I1** and **J1**.



Scheme 3.

amino derivatives **10** and **11** with arylsulfonyl isocyanates, and conversion of the COOH into the hydroxamate moiety, thus leading to the carboxylates **K**,**L**(**1**–**5**) and the corresponding hydroxamates **M**,**N**(**1**–**5**) (Scheme 3).

Inhibition data against four MMPs (MMP-1, MMP-2, MMP-8 and MMP-9) and type II ChC with the compounds reported in the present paper are shown in Tables 2 – 4.

4. Discussion

Sulfonylated amino acid hydroxamates were recently discovered to act as efficient MMP inhibitors (MacPherson et al., 1997; Bottomley et al., 1998; Almstead et al., 1999; Cheng et al., 1999; Pavlovsky et al., 1999; Whittaker et al., 1999; O'Brien et al., 2000). The first compound from this class to be developed for clinical trials, type **4** (CGS 27023A), possesses the following structural features. (i) An isopropyl substituent α to the hydroxamic acid moiety, considered to slow down metabolism of the zinc-binding function. It was shown to bind (by X-ray crystallography, Babine and Bender, 1997; Pavlovsky et al., 1999, and NMR spectroscopy, Moy et al., 1999) within the S_1 subsite. (ii) A bulkier pyridyl-methyl (or benzyl, in structurally related compounds) moiety substituting the amino nitrogen atom, and binding within the $S_{2'}$ pocket of the enzyme. (iii) A arylsulfonyl group, which occupies (but generally does not fill) the specificity $S_{1'}$ pocket. For reasons little explained for the moment, this group was a 4-methoxybenzenesulfonyl one in most of the investigated derivatives (MacPherson et al., 1997; Jeng et al., 1998).

The promising MMP inhibition and clinical data of some of these sulfonamide inhibitors prompted us to investigate them in some detail. Our lead compound was **4**, to which we performed the following structural modifications. (i) The P_1 substituent is absent in the compounds designed by us, but the carbon atom chain was extended, since in contrast to the previously reported compounds, which are all α-amino acid derivatives, the inhibitors reported here are β-alanine derivatives. (ii) The already optimized (Jeng et al., 1998) benzyl group at the $S_{2'}$ site was modified to the 4-nitrobenzyl moiety, as we have shown previously that substituents at the benzyl moiety with acidifying properties, such as 2-nitro, 2-chloro, etc., lead to better ChC inhibitors as compared to the corresponding unsubstituted derivatives (Scozzafava and Supuran, 1999b, 2000). (iii) A large number of diverse alkyl/arylsulfonyl-, arylsulfonyl-ureido/arylureido-, or arylsulfenyl moieties at $S_{1'}$, as these moieties are the major determinants of activity/selectivity towards different MMPs (Dioszegi et al., 1995; Tschesche, 1995; Johnson et al., 1998; Nagase and Woessner, 1999; Whittaker et al., 1999).

The new compounds reported here were obtained by non-exceptional synthetic procedures, as outlined in Schemes 1–3. These involved reaction of *N*-4-nitrobenzyl-β-alanine (**7**) with alkyl/arylsulfonyl chlorides, arylisocyanates, arylsulfonyl-isocyanates or benzoyl-isothiocyanate, followed by conversion of the COOH moiety to the hydroxamate one. Related synthetic strategies led to some sulfenamides of type **G** and **H**, as well as to the thioureas **I** and **J**. As it has recently been reported (Hanessian et al., 1999; Whittaker et al., 1999) that elongated moieties in $P_{1'}/P_{2'}$ may lead to selectivity of the obtained inhibitors for the deep-pocket enzymes, relative to the short-pocket ones, we also studied an approach for preparing inhibitors containing such moieties (Scheme 3). Thus, the Boc-protected derivatives **A19** and **A20** were treated with TFA in order to eliminate the protecting group, and the amino derivatives thus obtained were reacted with arylsulfonyl isocyanates **8**. The carboxy groups of derivatives **K**,**L**(**1**–**5**) were then converted to the hydroxamate moieties by the standard procedure, as mentioned above, thus leading to derivatives **M**,**N**(**1**–**5**).

Inhibition data of Tables 2–4 show that the entire class of sulfonylated β-alanine hydroxamate derivatives reported here act as efficient metallo-peptidase inhibitors, but important differences in activity were detected against the diverse enzymes for the different substitution patterns of the alkyl/arylsulfonamide moiety contained in the molecules of the new inhibitors.

Thus, for MMP-1, an enzyme possessing a short specificity $S_{1'}$ pocket, aliphatic (**B1**–**B6**, **B35**), bulky aromatic (**B25**, **B30**–**B32**, **D1**–**D5**, **F5**, **F6**, **J1**, **M1**–**M5**, **N1**–**N5**) or heterocyclic (**B34**) moieties as $P_{1'}$ groups led to the least effective hydroxamate inhibitors, with K_I in the 30–80 nM range. The carboxylates **K**,**L**(**1**–**5**) were also ineffective in inhibiting this collagenase. Obviously, these data may be explained by the restricted space available around the $S_{1'}$ subsite of MMP-1, which cannot accommodate bulky groups in it, discriminating in this way between the bulky

Table 2
Inhibition of MMPs and ChC with the hydroxamates **B1**–**B35**

R	Compound	K_I[a] (nM)				
		MMP-1[b]	MMP-2[b]	MMP-8[b]	MMP-9[b]	ChC[c]
CH_3	**B1**	87	24	36	31	>100
CF_3	**B2**	22	14	22	28	95
CCl_3	**B3**	23	19	20	27	78
n-C_4F_9-	**B4**	69	1.8	2.0	2.5	14
n-C_8F_{17}	**B5**	77	0.6	1.1	1.1	9
Me_2N-	**B6**	38	33	37	44	88
C_6H_5-	**B7**	25	15	26	19	53
$PhCH_2$-	**B8**	27	18	28	21	56
4-F-C_6H_4-	**B9**	21	17	15	13	40
4-Cl-C_6H_4-	**B10**	23	12	19	16	48
4-Br-C_6H_4-	**B11**	24	13	20	21	42
4-I-C_6H_4-	**B12**	32	15	16	11	36
4-CH_3-C_6H_4-	**B13**	30	21	28	32	44
4-O_2N-C_6H_4-	**B14**	16	10	7	9	15
3-O_2N-C_6H_4-	**B15**	15	9	10	11	14
2-O_2N-C_6H_4-	**B16**	43	24	18	23	34
3-Cl-4-O_2N-C_6H_3	**B17**	30	7	8	10	15
4-AcNH-C_6H_4-	**B18**	13	9	8	11	13
4-BocNH-C_6H_4-	**B19**	12	9	10	15	18
3-BocNH-C_6H_4-	**B20**	20	12	15	13	8
C_6F_5-	**B21**	2	0.9	0.1	0.2	3
3-CF_3-C_6H_4	**B22**	6	1.3	0.6	0.7	6
2,5-$Cl_2C_6H_3$	**B23**	11	12	14	15	21
4-MeO-C_6H_4-	**B24**	23	24	23	30	25
2,4,6-$Me_3C_6H_2$-	**B25**	35	29	34	36	32
4-MeO-3-BocNH-C_6H_3-	**B26**	24	4	7	6	11
2-HO-3,5-Cl_2-C_6H_2-	**B27**	13	11	15	18	10
3-HOOC-C_6H_4-	**B28**[d]	31	2.1	3.0	2.7	12
4-HOOC-C_6H_4-	**B29**[d]	30	2.0	2.2	2.1	9
1-Naphthyl	**B30**	82	38	55	62	18
2-Naphthyl	**B31**	65	36	49	54	16
5-Me_2N-1-naphthyl-	**B32**	89	54	60	67	18
2-Thienyl	**B33**	12	7	10	13	15
Quinoline-8-yl	**B34**	55	49	37	41	10
Camphor-10-yl	**B35**	57	45	46	65	17

[a] K_I values were obtained from Easson–Stedman (Bieth, 1995) plots using a linear regression program, from at least three different assays. Standard errors were 5–10%.
[b] With the thioester substrate Ac-ProLeuGly-S-LeuLeuGlyOEt, spectrophotometrically.
[c] With FALGPA as substrate, spectrophotometrically.
[d] The C_6H_4-COOH moiety transformed into C_6H_4-CONHOH.

and less voluminous inhibitors. Inhibitors with K_I in the 15–30 nM range against MMP-1 were also obtained. These contained aromatic rings, substituted *meta* or *para* with electron-attracting groups such as nitro, halogeno, methoxy, etc. Efficient MMP-1 inhibitors (K_I in the range 3–15 nM) were those containing the following moieties: 4-acetamido-phenyl- (**B18**), perfluorophenyl- (**B21**), 3-trifluoromethyl-phenyl- (**B22**), 2,5-dichlorophenyl- (**B23**) or 2-hydroxy-3,5-dichlorophenyl- (**B27**), among others.

The deep-pocket enzymes MMP-2, MMP-8 and MMP-9 showed a more similar inhibition behavior, relatively different from that of the previously discussed MMP-1. Thus, again short aliphatic moieties at $P_{1'}$ led to the least effective inhibitors, such as **B1**, **B6** and **B35**. The same was true for the bulky naphthyl-derived moieties, such as in **B30**–**B32**, **B34** and **F6**. All these derivatives possessed $K_I > 20$ nM against these three MMPs. More effective inhibitors (K_I in the range 5–20 nM) were the aromatic derivatives, containing moieties such as 4-halogenophenyl, nitrophenyl, boc-aminophenyl, etc. (**B9**–**B12**, **B14**–**B20**, **B23**, **B24**, **B27**, **D1**–**D5**, **F1**–**F5**, **H1**–**H3** and **J1**). Several of the new inhibitors showed affinities in the 0.5–3.0 nM range. These were: (i) the perfluoroalkyl/aryl-sulfonyl compounds **B4**, **B5** and **B21**/**B22**; (ii) the carboxyphenyl-substituted derivatives **B28** and **B29**; and (iii) the elongated derivatives **M1**–**M5** and **N1**–**N5**.

Inhibition of the bacterial collagenase ChC with the new compounds reported here generally paralleled the MMP-1 inhibition data. Thus, potent inhibitors were obtained from all classes of hydroxamates investigated here, such as the alkyl/arylsulfonyl-4-nitrobenzyl-Gly derivatives (**B5**, **B20**–**B22**, **B26**, **B27**, **B29**, **B33**, **B34**, etc.), the arylsul-

Table 3
Inhibition of MMPs and ChC with hydroxamates of types **D**, **F**, **H** and **J**

R	Compound	K_I[a] (nM)				
		MMP-1[b]	MMP-2[b]	MMP-8[b]	MMP-9[b]	ChC[c]
Ph	**D1**	52	5	4	3	15
4-F-C_6H_4-	**D2**	47	4	5	7	18
4-Cl-C_6H_4-	**D3**	40	3.5	3.9	6	23
4-CH_3-C_6H_4-	**D4**	42	5	7	10	25
2-CH_3-C_6H_4-	**D5**	69	8	5	7	10
4-F-C_6H_4-	**F1**	21	10	13	14	27
3-Cl-C_6H_4-	**F2**	26	13	9	8	19
4-Cl-C_6H_4-	**F3**	24	10	11	9	18
2,4-F_2-C_6H_3-	**F4**	18	6	8	7	17
3,4-$Cl_2C_6H_3$	**F5**	36	5	5	8	15
1-Naphthyl	**F6**	>100	39	44	37	62
4-O_2N-C_6H_4-	**H1**	24	13	11	16	25
2-O_2N-C_6H_4-	**H2**	28	15	14	19	30
2,4-$(O_2N)_2$-C_6H_3-	**H3**	27	15	8	9	32
–	**J1**	32	2	1.8	1.6	15

[a] K_I values were obtained from Easson–Stedman (Bieth, 1995) plots using a linear regression program, from at least three different assays. Standard errors were 5–10%.
[b] With the thioester substrate Ac-ProLeuGly-S-LeuLeuGlyOEt, spectrophotometrically.
[c] With FALGPA as substrate, spectrophotometrically.

fonylureas- and arylureas (such as **D3**, **D5** and **F5**), the sulfenamido-4-nitrobenzyl-Gly derivatives (such as **H2** and **H3**) or the thiourea **J1**. Thus, it seems that the $S_{1'}$-binding moiety of the arylsulfonamide type, investigated for obtaining MMP inhibitors, can be efficiently substituted by related moieties such as alkylsulfonyl-, arylsulfenyl-, arylsulfonylureido-, arylureido- or benzoyl-thioureido, without loss of the MMP/ChC inhibitory properties. In the subseries of alkyl/arylsulfonamido derivatives (of types **B**(**1**–**35**)) the best ChC inhibitory properties were correlated with the presence of perfluoroalkylsulfonyl- (**B4**, **B5**), perfluorophenylsulfonyl- (**B21**), 3-trifluoromethylphenylsulfonyl- (**B22**), 3-chloro-4-nitro-phenylsulfonyl- (**B17**), 3- or 4-protected-amino-phenylsulfonyl-

Table 4
Inhibition of MMPs and ChC with carboxylates and hydroxamates of types **K**, **L**, **M** and **N**

Compound	K_I[a] (nM)				
	MMP-1[b]	MMP-2[b]	MMP-8[b]	MMP-9[b]	ChC[c]
K1	>100	19	23	30	36
K2	>100	15	24	29	35
K3	>100	23	25	38	39
K4	>100	27	27	27	38
K5	>100	29	32	29	31
L1	>100	21	31	31	32
L2	>100	18	34	28	34
L3	>100	19	35	29	37
L4	>100	25	38	29	39
L5	>100	27	36	28	46
M1	69	0.7	0.7	0.8	15
M2	55	0.6	0.8	1.5	11
M3	56	0.7	1.4	1.3	10
M4	57	0.9	1.7	0.8	17
M5	70	1.0	1.5	0.9	21
N1	62	1.1	1.3	1.1	9
N2	55	1.4	1.9	2.7	13
N3	57	1.6	1.6	1.6	20
N4	50	2.3	2.3	3.0	24
N5	55	2.5	2.5	3.0	25

[a] K_I values were obtained from Easson–Stedman (Bieth, 1995) plots using a linear regression program, from at least three different assays. Standard errors were 5–10%.
[b] With the thioester substrate Ac-ProLeuGly-S-LeuLeuGlyOEt, spectrophotometrically.
[c] With FALGPA as substrate, spectrophotometrically.

(**B18**–**B20**, **B26**), and 3- or 4-carboxy-phenylsulfonyl- (**B28**, **B29**). All these derivatives possessed inhibition constants in the range 5–10 nM against ChC. A second group of sulfonamide inhibitors, containing moieties such as 4-bromophenyl, 4-iodophenyl, 2-, 3- or 4-nitrophenyl, 2,5-dichlorophenyl-, 4-methoxyphenyl- or 2-thienyl substituting the *N*-4-nitrobenzyl-β-alanine hydroxamate, behaved as medium potency inhibitors, with affinities in the 12–30 nM range. The least active sulfonamides were those containing methyl-, trihalomethyl-, dimethylamino-, phenyl- and benzyl moieties. The arylsulfonylureido compounds **D1**–**D4** were more active than the corresponding arylsulfonyl derivatives (compare, for instance, **D1** with **B9**, **D2** and **B10**, etc.), acting as strong–medium potency ChC inhibitors. Behaving similarly were the ureas of type **F**, and the sulfenamides of type **H**. A very potent inhibitor was the thiourea derivative **J1**. The elongated molecule compounds of types **K**–**N** were slightly less effective as compared to the previously mentioned derivatives.

In conclusion, we describe here a novel class of strong inhibitors of the zinc proteases MMP-1, MMP-2, MMP-8, MMP-9 and ChC (EC 3.4.24.3), a collagenase from *Clostridium histolyticum*. The drug design has been realized by utilizing X-ray data for the adduct of some MMPs, with inhibitors of the sulfonyl amino acid hydroxamate type. Reaction of *N*-4-nitrobenzyl-β-alanine with sulfonyl chlorides, arylsulfonyl isocyanate, aryl isocyanates or benzoyl isothiocyanate afforded the β-alanine derivatives which were subsequently converted into the corresponding hydroxamates. The best substitutions for obtaining high affinity inhibitors (0.5–2.5 nM) involved hydrophobic moieties at $S_{1'}$, such as perfluoroalkylsulfonyl-, substituted-arylsulfonyl, pentafluorophenylsulfonyl, 3- and 4-carboxy-phenylsulfonyl-, 3-trifluoromethyl-phenylsulfonyl, among others. Some of the new inhibitors possessing elongated moieties at $P_{1'}$ also showed selectivity for the deep-pocket enzymes (MMP-2, MMP-8 and MMP-9) over MMP-1 and ChC.

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