

Protease Inhibitors: Synthesis of a Series of Bacterial Collagenase Inhibitors of the Sulfonyl Amino Acyl Hydroxamate Type

Brian W. Clare,*[†] Andrea Scozzafava,[‡] and Claudiu T. Supuran*[‡]

Department of Chemistry, The University of Western Australia, W.A. Australia 6009, and Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121, Florence, Italy

Received February 27, 2001

A series of sulfonyl amino acyl hydroxamates incorporating alkyl/arylsulfonyl-*N*-2-nitrobenzyl-L-alanine was prepared. Related compounds were obtained by reaction of *N*-2-nitrobenzyl-L-Ala with aryl isocyanates, arylsulfonyl isocyanates, or benzoyl isothiocyanate, followed by the conversion of the COOH into the CONHOH moiety. The new compounds were assayed as inhibitors of the *Clostridium histolyticum* collagenase (ChC), a bacterial protease involved in the degradation of extracellular matrix. Many of the obtained hydroxamates proved to be effective bacterial collagenase inhibitors, the main contributor to activity being the substitution pattern at the sulfonamido moiety. The best ChC inhibitors were those containing pentafluorophenylsulfonyl and 3- and 4-protected-aminophenylsulfonyl P₁' groups among others, with affinities in the low nanomolar range. This study also proves that the 2-nitrobenzyl- moiety, similarly to the 4-nitrobenzyl one previously investigated (Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* 2000, 43, 1858–1865) is an efficient P₂' anchoring moiety for obtaining potent bacterial collagenase inhibitors.

Introduction

Protease inhibitors proved recently to be of high clinical value in the management of many diseases, especially considering the extensively investigated viral proteases of the human immunodeficiency virus (HIV) or human hepatitis B virus (HBV) among others.¹ In contrast to such viral proteases, bacterial proteases have only marginally been studied for the development of specific inhibitors, although such agents would constitute a new generation of antibiotics,² free (at least in the beginning) of the pathogen resistance to antibiotics problem, observed with almost all the bacterial cell wall biosynthesis inhibitors used nowadays as antibacterial agents.^{1–3}

Another class of proteolytic enzymes much investigated ultimately for the development of specific inhibitors with pharmaceutical applications is constituted by the matrix metalloproteinases (MMPs). Inhibitors of these zinc enzymes were extensively studied in recent years, as these proteases are involved in many crucial physiologic and pathologic processes connected with extracellular matrix (ECM) degradation.^{4–10} The same situation is not true for the inhibitors of related enzymes that degrade ECM, such as the bacterial collagenases isolated from *Clostridium histolyticum*, which were much less investigated. This collagenase (EC 3.4.24.3) is a 116 kDa protein belonging to the M31 metalloproteinase family,^{11,12} being able to efficiently hydrolyze triple helical regions of collagens under physiological conditions as well as an entire range of synthetic peptide

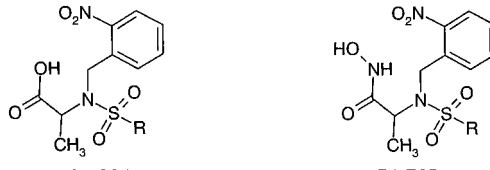
substrates. In fact the crude homogenate of *Clostridium histolyticum*, which contains several distinct collagenase isozymes,^{11,12} is the most efficient system known for the degradation of connective tissue, being also involved in the pathogenicity of this and related clostridia, such as, among others, *C. perfringens*, which causes human gas gangrene and food poisoning.¹³ Typically, these bacteria (and their collagenases) cause so much damage and so quickly, that common antibiotics now available are ineffective. Thus, development of inhibitors against these collagenases represents an interesting target for drug design. Similarly to the vertebrate MMPs, *Clostridium histolyticum* collagenase (abbreviated as ChC) contains the conserved HEXxH zinc-binding motif, which in this specific case is constituted by His⁴¹⁵ExxH, 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.^{14,15} Like the MMPs, ChC is a multiunit protein, consisting of four segments, S1, S2a, S2b, and S3, with S1 incorporating the catalytic domain.¹⁴ 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.^{11–15} One must also mention that this bacterial collagenase has not been crystallized to the present, and no X-ray crystallographic/NMR structures are available in order to assist the design of active site directed inhibitors, although much effort has been spent in our and other laboratories for this task.

In previous contributions^{16–19} we have shown that potent inhibitors of ChC of the sulfonylated amino acid hydroxamate type can be developed, in analogy with the same type of compounds reported to act as MMP inhibitors.^{19–22} In this paper we extend our previous

* Corresponding authors. B.W.C.: (address) Department of Chemistry, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 Australia; (phone) +61-8-9337 7824; (fax) +61-8-9380-1005; (e-mail) bwc@crystal.uwa.edu.au. C.T.S.: (fax) +39-055-2757555; (e-mail) claudiu.supuran@unifi.it.

[†] The University of Western Australia.

[‡] Università degli Studi.

Table 1. Inhibition of Clostridium Histolyticum Collagenase (ChC) with the Carboxylic Acids **A1–A35** and the Corresponding Hydroxamates **B1–B35**


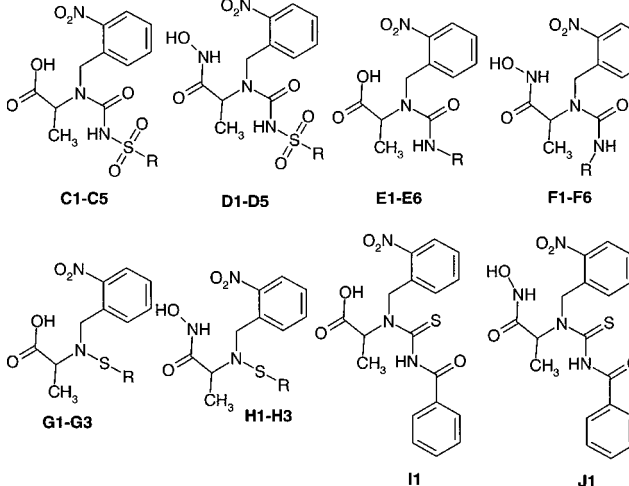
R	compd	K_I^a (μ M)	compd	K_I^a (nM)
CH ₃	A1	22	B1	86
CF ₃	A2	5.1	B2	70
CCl ₃	A3	5.0	B3	64
<i>n</i> -C ₄ F ₉ -	A4	2.1	B4	10
<i>n</i> -C ₈ F ₁₇	A5	1.7	B5	8
Me ₂ N-	A6	43	B6	79
C ₆ H ₅ -	A7	27	B7	59
PhCH ₂ -	A8	18	B8	55
4-F-C ₆ H ₄ -	A9	12	B9	30
4-Cl-C ₆ H ₄ -	A10	11	B10	32
4-Br-C ₆ H ₄ -	A11	10	B11	33
4-I-C ₆ H ₄ -	A12	11	B12	34
4-CH ₃ -C ₆ H ₄ -	A13	17	B13	41
4-O ₂ N-C ₆ H ₄ -	A14	5.0	B14	13
3-O ₂ N-C ₆ H ₄ -	A15	5.2	B15	10
2-O ₂ N-C ₆ H ₄ -	A16	4.4	B16	11
3-Cl-4-O ₂ N-C ₆ H ₃ -	A17	3.1	B17	9
4-AcNH-C ₆ H ₄ -	A18	3.3	B18	12
4-BocNH-C ₆ H ₄ -	A19	2.6	B19	9
3-BocNH-C ₆ H ₄ -	A20	2.5	B20	8
4-Ac-C ₆ H ₄ -	A21	2.0	B21	10
C ₆ F ₅ -	A22	0.3	B22	5
3-CF ₃ -C ₆ H ₄	A23	0.4	B23	5
2,5-Cl ₂ C ₆ H ₃	A24	3.4	B24	14
4-CH ₃ O-C ₆ H ₄ -	A25	5.7	B25	19
2,4,6-(CH ₃) ₃ -C ₆ H ₂ -	A26	6.0	B26	21
4-CH ₃ O-3-BocNH-C ₆ H ₃ -	A27	2.5	B27	9
2-HO-3,5-Cl ₂ -C ₆ H ₂ -	A28	2.7	B28	10
3-HOOC-C ₆ H ₄ -	A29	2.1	B29 ^b	8
4-HOOC-C ₆ H ₄ -	A30	1.9	B30 ^b	7
1-naphthyl	A31	1.6	B31	6
2-naphthyl	A32	1.8	B32	8
5-Me ₂ N-1-naphthyl-	A33	2.1	B33	9
2-thienyl	A34	2.0	B34	10
quinoline-8-yl	A35	2.0	B35	9

^a K_I -s values were obtained from Dixon plots using a linear regression program, from at least three different assays. Errors were around $\pm 10\%$ (from at least three determinations). ^b The C₆H₄-COOH moiety transformed into C₆H₄-CONHOH.

work in the design of bacterial protease inhibitors^{16–19} and report the preparation of a series of ChC inhibitors incorporating alkyl/arylsulfonamido-L-alanine hydroxamate as well as arylsulfonylureido/arylsulfonamide-L-alanine hydroxamate moieties in their molecule. Some of the new compounds, assayed for the inhibition of ChC, showed high affinity for this enzyme (in the nanomolar range), behaving as some of the best ChC sulfonated inhibitors reported up to now. The compounds prepared and tested in this study are presented in Tables 1 and 2.

Results and Discussion

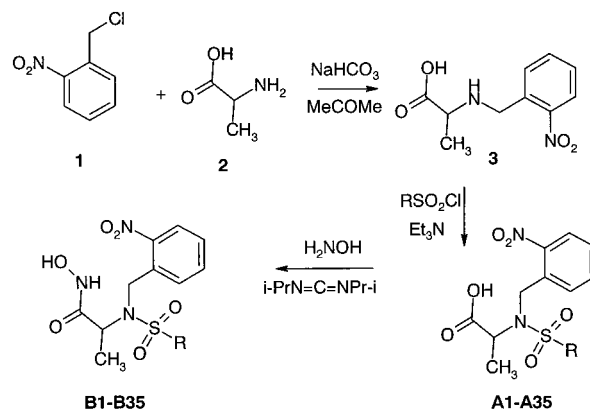
Synthesis. The compounds reported here were obtained as shown in Schemes 1–3, as for similar derivatives previously reported by our or other groups.^{16–22} Reaction of 2-nitrobenzyl chloride **1** with L-alanine **2** afforded the key intermediate, *N*-2-nitrobenzyl-L-Ala **3**. Carboxylic acids **A1–A35** were then prepared by reac-

Table 2. Inhibition of ChC with the Carboxylic Acids of Types **C, E, G, I** and the Corresponding Hydroxamates of Types **D, F, H, J**


R	compd	K_I^a (μ M)	compd	K_I^a (nM)
Ph	C1	3.6	D1	12
4-F-C ₆ H ₄ -	C2	3.0	D2	8
4-Cl-C ₆ H ₄ -	C3	2.7	D3	7
4-CH ₃ -C ₆ H ₄ -	C4	3.1	D4	10
2-CH ₃ -C ₆ H ₄ -	C5	3.0	D5	9
4-F-C ₆ H ₄ -	E1	6.2	F1	15
3-Cl-C ₆ H ₄ -	E2	6.6	F2	17
4-Cl-C ₆ H ₄ -	E3	6.8	F3	19
2,4-F ₂ -C ₆ H ₃ -	E4	6.5	F4	12
3,4-Cl ₂ C ₆ H ₃	E5	5.3	F5	14
1-naphthyl	E6	5.0	F6	10
4-O ₂ N-C ₆ H ₄ -	G1	6.0	H1	13
2-O ₂ N-C ₆ H ₄ -	G2	6.2	H2	12
2,4-(O ₂ N) ₂ -C ₆ H ₃ -	G3	5.2	H3	9
	I1	0.7	J1	8

^a K_I -s values were obtained from Dixon plots using a linear regression program, from at least three different assays. Errors were around $\pm 10\%$ (from at least three determinations).

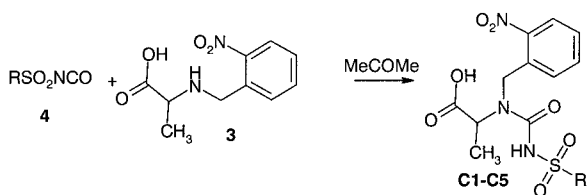
Scheme 1



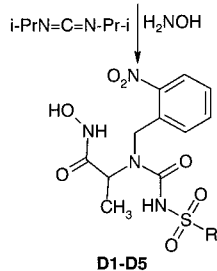
tion of alkyl/arylsulfonyl chlorides with the L-alanine derivative **3** (Scheme 1). Conversion of the carboxylic acids **A1–A35** into the corresponding hydroxamates **B1–B35** was done with hydroxylamine and diisopropyl carbodiimide (Scheme 1).^{9,23–26}

Other derivatives (**C1–C5**; **D1–D5** and **E1–E6**; **F1–F6**) were obtained by reaction of arylsulfonylisocyanates **4** or aryl isocyanates **5** with *N*-2-nitrobenzyl-L-Ala **3**, followed by the conversion of the COOH moiety into the CONHOH one, as described above (Schemes 2 and 3).^{25,27}

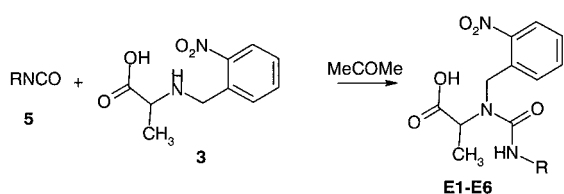
Scheme 2



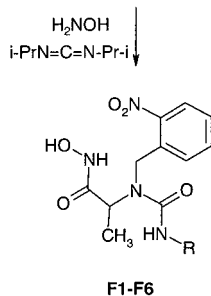
No	R
1	Ph
2	4-F-C ₆ H ₄
3	4-Cl-C ₆ H ₄
4	4-Me-C ₆ H ₄
5	2-Me-C ₆ H ₄



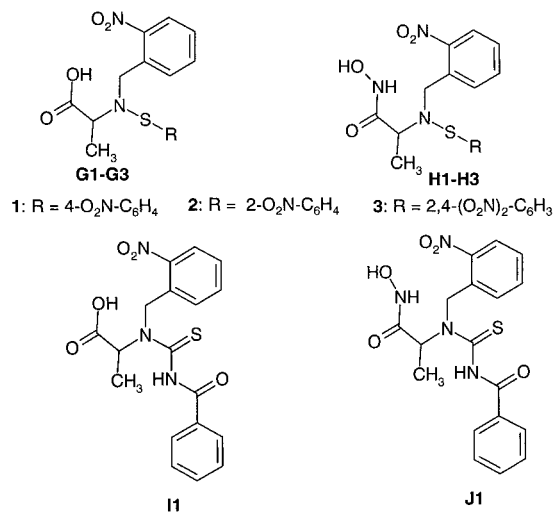
Scheme 3



No	R
1	4-F-C ₆ H ₄
2	3-Cl-C ₆ H ₄
3	4-Cl-C ₆ H ₄
4	2,4-F ₂ -C ₆ H ₃
5	3,4-Cl ₂ -C ₆ H ₃
6	1-naphthyl



By applying synthetic strategies related to the previously described ones, the sulfenamido derivatives **G1–G3** and **H1–H3** as well as the thioureas **I1** and **J1** were also prepared.



MacPherson et al.^{20a} originally reported that sulfonylated amino acid hydroxamates act as powerful MMP inhibitors, and this type of compounds was then further explored by other researchers.^{9,16–19,20b} It was thus

observed that, in addition to acting as strong MMP inhibitors,^{4,20} compounds of this type are also effective bacterial collagenase (ChC) inhibitors, with potencies in the nanomolar range in some cases.^{16–19} It appeared thus of interest to extend the study of this type of protease inhibitors as well as the relationship governing structure–activity correlations in this class of biologically active compounds.

The new inhibitors reported here were obtained by routine procedures involving the reaction of amino acids with benzyl/alkyl/arylsulfonyl halides, followed by conversion of the carboxy moiety to the hydroxamate one (Scheme 1).^{16–20} Derivatives **A1–A35** and **B1–B35** were obtained in this way. Alternatively, derivatization of the amino acid derivative **3** with arylsulfonyl isocyanates **4** or aryl isocyanates **5** afforded urea derivatives which were then similarly transformed into the corresponding hydroxamic acids **D1–D5** and **F1–F6** (Schemes 2 and 3).

ChC Inhibitory Activity. Inhibition data against type II ChC with the compounds reported in the present paper are shown in Tables 1 and 2. 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.²⁸

The following should be noted regarding ChC inhibition data of Tables 1 and 2: (i) all hydroxamates were 50–500 more active as ChC inhibitors as compared to the corresponding carboxylic acids, probably due to the enhanced Zn(II) coordinating properties of the CONHOH moiety (bidentate binding) as compared to the COOH group (generally monodentate binding to the zinc ion);^{6–9} (ii) potent inhibitors were obtained from all classes of hydroxamates investigated here, such as the alkyl/arylsulfonyl-*N*-2-nitrobenzyl-Ala derivatives (**B5**; **B15–B24**; **B27**, **B29–B35**), the arylsulfonylureas- and arylureas (such as **D2–D5**, **F5**, **F6**), the sulfenamido-benzyl-Ala derivatives (such as **H3**), or the thiourea **J1**. Thus, it seems that the S₁-binding moiety of the arylsulfonamide type, previously investigated for the obtaining of MMP inhibitors,^{9,10,21} can be efficiently substituted by related moieties such as alkylsulfonyl-; arylsulfonyl-; arylsulfonylureido-; arylureido-; or benzoyl-thioureido, without loss of the ChC/MMP inhibitory properties; (iii) in the subseries of alkyl/arylsulfonamido derivatives (of types **A**, **B**(1–35)) the best ChC inhibitory properties were correlated with the presence of perfluoroalkylsulfonyl- (**B4**, **B5**), perfluorophenylsulfonyl- (**B22**); 3-trifluoromethylphenylsulfonyl- (**B23**); 3-chloro-4-nitrophenylsulfonyl- (**B17**); 3- or 4-protected-aminophenylsulfonyl- (**B18–B20**; **B27**); 3- or 4-carboxyphenylsulfonyl- (**B29**, **B30**); and 1- or 2-naphthylsulfonyl as well as 8-quinolinesulfonyl moieties (**B30–B35**). All these derivatives possessed inhibition constants in the range of 5–12 nM against ChC, being among the most potent such inhibitors reported up to now. A second group of sulfonamide inhibitors, containing moieties such as 4-fluorophenyl (**B9**), 4-nitrophenyl (**B14**), 2,5-dichlorophenyl- (**B24**), 4-methoxyphenyl- (**B25**), or 2,4,6-trimethylphenyl- (**B26**) substituting the *N*-2-nitrobenzyl-L-Ala hydroxamate, behaved as medium potency inhibitors, with affinities in the 13–30 nM range (Table 1). The least active sulfonamides were those containing

methyl-; trihalomethyl-; dimethylamino-; phenyl-; and benzyl moieties (Table 1); (iv) the arylsulfonylureido compounds **D1–D5** were more active than the corresponding arylsulfonyl derivatives (compare for instance **D2** with **B9**; **D3** and **B10**, etc.), acting as strong ChC inhibitors. The ureas of type **F** and the sulfenamides of type **H** behaved similarly, except for **F2** and **F3**, which are somehow weaker inhibitors. A potent inhibitor is the thiourea derivative **J1** (Table 2).

Experimental Section

General Methods. Melting points: heating plate microscope (not corrected); IR spectra: KBr pellets, 400–4000 cm^{-1} Perkin-Elmer 16PC FTIR spectrometer; ^1H NMR spectra: Varian Gemini 200 apparatus (chemical shifts are expressed as δ values relative to Me_4Si as standard); elemental analysis ($\pm 0.4\%$ 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 \times 250 mm), with a Beckman EM-1760 instrument. The detection wavelength was 254 nm.

Amino acids (L-Ala), 4-nitrobenzyl chloride, sulfonyl chlorides, arylsulfonyl isocyanates, aryl isocyanates, benzoyl isothiocyanate, triethylamine, carbodiimides, hydroxylamine, FAL-GPA, buffers, and other reagents used in the syntheses were commercially available compounds, from Sigma, Acros, or Aldrich.

Preparation of *N*-2-Nitrobenzyl-L-alanine **3.** An amount of 8.6 g (0.10 M) of L-Ala **2** and the stoichiometric amount of 2-nitrobenzyl chloride (16.1 g) were suspended/dissolved in 150 mL of anhydrous acetonitrile, and the equivalent amount of triethylamine (0.10 mM, 14.7 mL) was added. The reaction mixture was stirred at room temperature for 10 h, and then the solvent was evaporated in vacuo. The obtained reaction mixture was taken in 200 mL of water, the pH was brought to 7 with citric acid, and the crude product **3** precipitated by leaving the mixture overnight at 4 $^\circ\text{C}$. Recrystallization from ethanol afforded the pure title compound in almost quantitative yield.

General Procedure for the Preparation of *N*-2-Nitrobenzyl-alkyl/arylsulfonyl L-Alanines **A1–A35.** An amount of 2.10 g (10 mMol) of *N*-2-nitrobenzyl-L-Ala **3** and 10 mmol of sulfonyl chloride were suspended/dissolved in 100 mL of acetone + 25 mL of water. The 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 was 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 was extracted in ethyl acetate. After evaporation of the solvent, the compounds **A1–A35** were recrystallized from EtOH or MeOH. Yields were around 75–90%.

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 diisopropyl-carbodiimide. The reaction mixture was magnetically stirred at room temperature for 15 min, then 180 μL (12 mM) of triethylamine was added, and stirring was continued for 12 h at 4 $^\circ\text{C}$. The solvent was evaporated in vacuo, and the residue was 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 was removed in vacuo. Preparative HPLC (Dynamax-60A column (25 \times 250 mm); 90% acetonitrile/10% methanol; flow rate of 30 mL/min) afforded the pure hydroxamic acids.

General Procedure for the Preparation of Compounds **C1–C5; **E1–E6**; and **I1**.** An amount of 2.10 g (10 mMol) of *N*-2-nitrobenzyl-L-Ala **3** and the stoichiometric amount of arylsulfonyl isocyanate **4**, aryl isocyanate **5**, or benzoyl isothiocyanate was suspended in 50 mL of anhydrous acetone, and 150 μL (10 mM) of triethylamine was added. The reaction mixture was either stirred at room temperature (in the case of derivatives prepared from **4**) 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.

General Procedure for the Preparation of Compounds **G1–G3.** The general procedure described above for the preparation of compounds **A1–A35** has been followed, except that arylsulfonyl halides were used instead of alkyl/arylsulfonyl halides. The yields in the title sulfenamides were around 70%.

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

***N*-4-Toluenesulfonyl-*N*-2-nitrobenzyl-L-alanine **A13**.** Pale yellow crystals, mp 171–2 $^\circ\text{C}$; ^1H NMR (DMSO- d_6), δ , ppm: 1.53 (d, $^3J_{\text{HH}} = 6.5$, 3H, CHCH_3 of Ala), 2.55 (s, 3H, $\text{CH}_3\text{C}_6\text{H}_4$), 3.73 (s, 2H, CH_2 of benzyl); 3.90 (q, 1H, CH of Ala); 7.20–7.59 (m, 6H, H_{ortho} of $\text{CH}_3\text{C}_6\text{H}_4$ and H_{arom} of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$), 7.92 (d, $^3J_{\text{HH}} = 8.1$, 2H, H_{meta} of $\text{CH}_3\text{C}_6\text{H}_4$); 11.54 (br s, 1H, COOH); ^{13}C NMR (DMSO- d_6), δ , ppm: 20.1 (s, CHCH_3 of Ala); 26.4 (s, $\text{CH}_3\text{C}_6\text{H}_4$), 34.5 (s, CHCH_3 of Ala); 43.0 (s, CH_2 of benzyl), 127.2 (s, C-5 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 129.2 (C-4 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 129.8 (C-3 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 130.1 (s, C_{meta} of $\text{CH}_3\text{C}_6\text{H}_4$), 130.9 (C-6 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 134.0 (C-2 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 135.4 (C-1 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 135.7 (s, C_{ortho} of $\text{CH}_3\text{C}_6\text{H}_4$), 145.5 (s, C_{ipso} of $\text{CH}_3\text{C}_6\text{H}_4$), 148.8 (s, C_{para} of $\text{CH}_3\text{C}_6\text{H}_4$), 177.3 (s, CO_2H). Anal. Found: C, 52.48; H, 4.61; N, 7.67%. $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_6\text{S}$ requires: C, 52.74; H, 4.43; N, 7.69%.

***N*-4-Toluenesulfonyl-*N*-2-nitrobenzyl-L-alanine hydroxamate **B13**.** Pale yellow crystals, mp 213–5 $^\circ\text{C}$; ^1H NMR (DMSO- d_6), δ , ppm: 1.53 (d, $^3J_{\text{HH}} = 6.5$, 3H, CHCH_3 of Ala), 2.65 (s, 3H, $\text{CH}_3\text{C}_6\text{H}_4$), 3.79 (s, 2H, CH_2 of benzyl); 3.98 (q, 1H, CH of Ala); 7.14–7.68 (m, 6H, H_{ortho} of $\text{CH}_3\text{C}_6\text{H}_4$ and H_{arom} of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$), 8.10 (d, $^3J_{\text{HH}} = 8.1$, 2H, H_{meta} of $\text{CH}_3\text{C}_6\text{H}_4$); 8.78 (br s, 1H, NHOH); 10.56 (br s, 1H, NHOH); ^{13}C NMR (DMSO- d_6), δ , ppm: 20.4 (s, CHCH_3 of Ala); 26.1 (s, $\text{CH}_3\text{C}_6\text{H}_4$), 34.3 (s, CHCH_3 of Ala); 44.7 (s, CH_2 of benzyl), 127.0 (s, C-5 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 129.5 (C-4 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 129.9 (C-3 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 130.6 (s, C_{meta} of $\text{CH}_3\text{C}_6\text{H}_4$), 130.8 (C-6 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 134.7 (C-2 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 135.3 (C-1 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 135.8 (s, C_{ortho} of $\text{CH}_3\text{C}_6\text{H}_4$), 145.2 (s, C_{ipso} of $\text{CH}_3\text{C}_6\text{H}_4$), 148.0 (s, C_{para} of $\text{CH}_3\text{C}_6\text{H}_4$), 175.6 (s, CONHOH). Anal. Found: C, 50.69; H, 4.87; N, 10.98%. $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_6\text{S}$ requires: C, 50.65; H, 4.52; N, 11.08%.

***N*-4-Toluenesulfonylureido-*N*-2-nitrobenzyl-L-alanine **C3**.** Pale yellow crystals, mp 274–5 $^\circ\text{C}$; ^1H NMR (DMSO- d_6), δ , ppm: 1.52 (d, $^3J_{\text{HH}} = 6.5$, 3H, CHCH_3 of Ala), 2.57 (s, 3H, $\text{CH}_3\text{C}_6\text{H}_4$), 3.79 (s, 2H, CH_2 of benzyl); 3.95 (q, 1H, CH of Ala); 7.17–7.68 (m, 6H, H_{ortho} of $\text{CH}_3\text{C}_6\text{H}_4$ and H_{arom} of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$), 7.99 (d, $^3J_{\text{HH}} = 8.2$, 2H, H_{meta} of $\text{CH}_3\text{C}_6\text{H}_4$); 8.25 (br s, 2H, NHCONH); 11.73 (br s, 1H, COOH); ^{13}C NMR (DMSO- d_6), δ , ppm: 20.1 (s, CHCH_3 of Ala); 26.4 (s, $\text{CH}_3\text{C}_6\text{H}_4$), 34.7 (s, CHCH_3 of Ala); 43.8 (s, CH_2 of benzyl), 127.0 (s, C-5 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 129.3 (C-4 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 129.9 (C-3 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 130.5 (s, C_{meta} of $\text{CH}_3\text{C}_6\text{H}_4$), 130.9 (C-6 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 132.7 (s, NHCONH), 134.1 (C-2 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 135.0 (C-1 of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$); 135.6 (s, C_{ortho} of $\text{CH}_3\text{C}_6\text{H}_4$), 145.8 (s, C_{ipso} of $\text{CH}_3\text{C}_6\text{H}_4$), 148.6 (s, C_{para} of $\text{CH}_3\text{C}_6\text{H}_4$), 177.9 (s, CO_2H). Anal. Found: C, 50.09; H, 4.35; N, 10.21%. $\text{C}_{17}\text{H}_{17}\text{N}_3\text{O}_7\text{S}$ requires: C, 50.12; H, 4.21; N, 10.31%.

***N*-4-Toluenesulfonylureido-*N*-2-nitrobenzyl-L-alanine Hydroxamate **D3**.** Pale yellow crystals, mp 229–30 $^\circ\text{C}$; ^1H NMR (DMSO- d_6), δ , ppm: 1.54 (d, $^3J_{\text{HH}} = 6.5$, 3H, CHCH_3 of Ala), 2.61 (s, 3H, $\text{CH}_3\text{C}_6\text{H}_4$), 3.82 (s, 2H, CH_2 of benzyl); 3.95 (q, 1H, CH of Ala); 7.12–7.60 (m, 6H, H_{ortho} of $\text{CH}_3\text{C}_6\text{H}_4$ and H_{arom} of 2- $\text{O}_2\text{N}-\text{C}_6\text{H}_4$), 7.99 (d, $^3J_{\text{HH}} = 8.3$ Hz, 2H, H_{meta} of

CH₃C₆H₄); 8.29 (br s, 2H, NHCONH); 8.75 (br s, 1H, NHOH); 10.66 (br s, 1H, NHOH); ¹³C NMR (DMSO-*d*₆), δ , ppm: 20.6 (s, CHCH₃ of Ala); 26.7 (s, CH₃C₆H₄), 34.4 (s, CHCH₃ of Ala); 44.5 (s, CH₂ of benzyl), 127.0 (s, C-5 of 2-O₂N-C₆H₄); 129.3 (C-4 of 2-O₂N-C₆H₄); 129.9 (C-3 of 2-O₂N-C₆H₄); 130.5 (s, C_{meta} of CH₃C₆H₄), 130.9 (C-6 of 2-O₂N-C₆H₄); 132.8 (s, NHCONH), 134.0 (C-2 of 2-O₂N-C₆H₄); 135.1 (C-1 of 2-O₂N-C₆H₄); 135.6 (s, C_{ortho} of CH₃C₆H₄), 145.6 (s, C_{ipso} of CH₃C₆H₄), 148.9 (s, C_{para} of CH₃C₆H₄), 174.3 (s, CONHOH). Anal. Found: C, 48.60; H, 4.13; N, 13.21%. C₁₇H₁₈N₄O₇S requires C, 48.34; H, 4.30; N, 13.26%.

N-4-Fluorophenylureido-N-2-nitrobenzyl-L-alanine E1. Pale yellow crystals, mp 169–70 °C; ¹H NMR (DMSO-*d*₆), δ , ppm: 1.54 (d, ³J_{HH} = 6.5, 3H, CHCH₃ of Ala), 3.78 (s, 2H, CH₂ of benzyl); 3.96 (q, 1H, CH of Ala); 7.11–7.69 (m, 6H, H_{ortho} of 4-FC₆H₄ and H_{arom} of 2-O₂N-C₆H₄), 7.95 (d, ³J_{HH} = 8.1, 2H, H_{meta} of 4-FC₆H₄); 8.15 (br s, 2H, NHCONH); 11.47 (br s, 1H, COOH); ¹³C NMR (DMSO-*d*₆), δ , ppm: 20.2 (s, CHCH₃ of Ala); 34.5 (s, CHCH₃ of Ala); 43.8 (s, CH₂ of benzyl), 127.8 (s, C-5 of 2-O₂N-C₆H₄); 129.7 (C-4 of 2-O₂N-C₆H₄); 129.9 (C-3 of 2-O₂N-C₆H₄); 130.1 (s, C_{meta} of FC₆H₄), 130.8 (C-6 of 2-O₂N-C₆H₄); 132.6 (s, NHCONH), 134.1 (C-2 of 2-O₂N-C₆H₄); 135.0 (s, C_{ortho} of FC₆H₄), 135.7 (C-1 of 2-O₂N-C₆H₄); 148.9 (s, C_{ipso} of FC₆H₄), 149.8 (s, C_{para} of FC₆H₄), 177.9 (s, CO₂H). Anal. Found: C, 55.29; H, 4.24; N, 12.06%. C₁₆H₁₄FN₃O₅ requires: C, 55.33; H, 4.06; N, 12.10%.

N-4-Fluorophenylureido-N-2-nitrobenzyl-L-alanine Hydroxamate F1. Pale yellow crystals, mp 219–20 °C; ¹H NMR (DMSO-*d*₆), δ , ppm: 1.56 (d, ³J_{HH} = 6.5, 3H, CHCH₃ of Ala), 3.80 (s, 2H, CH₂ of benzyl); 3.97 (q, 1H, CH of Ala); 7.10–7.62 (m, 6H, H_{ortho} of 4-FC₆H₄ and H_{arom} of 2-O₂N-C₆H₄), 7.93 (d, ³J_{HH} = 8.1, 2H, H_{meta} of 4-FC₆H₄); 8.16 (br s, 2H, NHCONH); 8.79 (br s, 1H, NHOH); 10.64 (br s, 1H, NHOH); ¹³C NMR (DMSO-*d*₆), δ , ppm: 20.2 (s, CHCH₃ of Ala); 34.7 (s, CHCH₃ of Ala); 43.7 (s, CH₂ of benzyl), 127.0 (s, C-5 of 2-O₂N-C₆H₄); 129.5 (C-4 of 2-O₂N-C₆H₄); 129.9 (C-3 of 2-O₂N-C₆H₄); 130.3 (s, C_{meta} of FC₆H₄), 130.8 (C-6 of 2-O₂N-C₆H₄); 132.6 (s, NHCONH), 134.4 (C-2 of 2-O₂N-C₆H₄); 135.1 (s, C_{ortho} of FC₆H₄), 135.7 (C-1 of 2-O₂N-C₆H₄); 135.6 (s, C_{ortho} of FC₆H₄), 145.7 (s, C_{ipso} of FC₆H₄), 148.5 (s, C_{para} of FC₆H₄), 174.5 (s, CONHOH). Anal. Found: C, 52.90; H, 4.13; N, 15.33%. C₁₆H₁₅FN₄O₅ requires: C, 53.04; H, 4.17; N, 15.46%.

N-4-Nitrophenylsulfenyl-N-2-nitrobenzyl-L-alanine G1. Yellow crystals, mp 228–9 °C; ¹H NMR (DMSO-*d*₆), δ , ppm: 1.55 (d, ³J_{HH} = 6.5, 3H, CH₃ of Ala), 3.79 (s, 2H, CH₂ of benzyl); 3.90 (q, 1H, CH of Ala); 6.75 (s, 1H, SNH), 7.10–7.66 (m, 6H, H_{ortho} of 4-O₂N-C₆H₄ and H_{arom} of 2-O₂N-C₆H₄), 8.09 (d, ³J_{HH} = 8.3, 2H, H_{meta} of 4-O₂N-C₆H₄); 11.78 (br s, 1H, COOH); ¹³C NMR (DMSO-*d*₆), δ , ppm: 20.4 (s, CHCH₃ of Ala); 34.3 (s, CHCH₃ of Ala); 43.8 (s, CH₂ of benzyl), 127.1 (s, C-5 of 2-O₂N-C₆H₄); 129.8 (C-4 of 2-O₂N-C₆H₄); 129.9 (C-3 of 2-O₂N-C₆H₄); 130.0 (s, C_{meta} of 4-O₂N-C₆H₄), 130.3 (C-6 of 2-O₂N-C₆H₄); 134.1 (C-2 of 2-O₂N-C₆H₄); 135.2 (C-1 of 2-O₂N-C₆H₄); 135.5 (s, C_{ortho} of 4-O₂N-C₆H₄), 145.0 (s, C_{ipso} of 4-O₂N-C₆H₄), 150.8 (s, C_{para} of 4-O₂N-C₆H₄), 177.6 (s, CO₂H). Anal. Found: 49.62; H, 3.70; N, 11.49%. C₁₅H₁₃N₃O₆S requires: C, 49.58; H, 3.61; N, 11.56%.

N-4-Nitrophenylsulfenyl-N-2-nitrobenzyl-L-alanine Hydroxamate H1. Yellow crystals, mp 248–9 °C; ¹H NMR (DMSO-*d*₆), δ , ppm: 1.55 (d, ³J_{HH} = 6.5, 3H, CH₃ of Ala), 3.78 (s, 2H, CH₂ of benzyl); 3.96 (q, 1H, CH of Ala); 6.83 (s, 1H, SNH), 7.15–7.68 (m, 6H, H_{ortho} of 4-O₂N-C₆H₄ and H_{arom} of 2-O₂N-C₆H₄), 8.17 (d, ³J_{HH} = 8.2, 2H, H_{meta} of 4-O₂N-C₆H₄); 8.75 (br s, 1H, NHOH); 10.70 (br s, 1H, NHOH); ¹³C NMR (DMSO-*d*₆), δ , ppm: 20.1 (s, CHCH₃ of Ala); 34.5 (s, CHCH₃ of Ala); 43.8 (s, CH₂ of benzyl), 127.1 (s, C-5 of 2-O₂N-C₆H₄); 129.6 (C-4 of 2-O₂N-C₆H₄); 129.9 (C-3 of 2-O₂N-C₆H₄); 130.2 (s, C_{meta} of 4-O₂N-C₆H₄), 130.5 (C-6 of 2-O₂N-C₆H₄); 134.5 (C-2 of 2-O₂N-C₆H₄); 135.0 (C-1 of 2-O₂N-C₆H₄); 135.9 (s, C_{ortho} of 4-O₂N-C₆H₄), 145.1 (s, C_{ipso} of 4-O₂N-C₆H₄), 150.5 (s, C_{para} of 4-O₂N-C₆H₄), 174.2 (s, CONHOH). Anal. Found: C, 47.54; H, 3.85; N, 14.73%. C₁₅H₁₄N₄O₆S requires: C, 47.62; H, 3.73; N, 14.81%.

Enzyme Preparations. *Clostridium histolyticum* highly purified collagenase and its substrate FALGPA (furanacryloyl-glycyl-prolyl-alanine) were purchased from Sigma Chemical Co. (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.²⁸ The substrate was reconstituted as 5 mM stock in 50 mM Tricine buffer, 0.4 M NaCl, 10 mM CaCl₂, pH 7.50. The rate of hydrolysis was determined from the change in absorbance at 324 nm using an extinction coefficient for FALGPA $\epsilon_{305} = 24\,700\text{ M}^{-1}\text{ cm}^{-1}$ in the above-mentioned reaction buffer.²⁸ Measurements were made using a Cary 3 spectrophotometer interfaced with a PC. Initial velocities were thus estimated using the direct linear plot-based procedure reported by van Wart and Steinbrink.²⁸ *K_i*-s were then determined according to Dixon plots and a linear regression program.

Acknowledgment. This research was financed in part by the EU grant ERB CIPDCT 940051 and by a grant from the Italian CNR (Target Project Biotechnology).

References

- (1) (a) Leung, D.; Abbenante, G.; Fairlie, D. P. Protease inhibitors: Current status and future prospects. *J. Med. Chem.* **2000**, *43*, 305–341. (b) Wlodawer, A.; Gustchina, A. Structural and biochemical studies of retroviral proteases. *Biochim. Biophys. Acta* **2000**, *1477*, 16–34.
- (2) (a) Travis, J.; Potempa, J. Bacterial proteinases as targets for the development of second generation antibiotics. *Biochim. Biophys. Acta* **2000**, *1477*, 35–50. (b) Supuran, C. T.; Scozzafava, A.; Mastrolorenzo, A. Bacterial proteases: current therapeutic use and future prospects for the development of new antibiotics. *Exp. Opin. Ther. Patents* **2001**, *11*, 221–259.
- (3) Miyoshi, S. I.; Shinoda, S. Bacterial metalloproteases as the toxic factor in infection. *J. Toxicol. Toxin Rev.* **1997**, *16*, 177–194.
- (4) (a) Woessner, J. F., Jr.; Nagase, H. *Matrix metalloproteinases and TIMPs*; Oxford University Press: 2000; pp 1–223. (b) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem. Rev.* **1999**, *99*, 2735–2776. (c) Bottomley, K. M.; Johnson, W. H.; Walter, D. S. Matrix metalloproteinase inhibitors in arthritis. *J. Enzyme Inhib.* **1998**, *13*, 79–102. (d) Babine, R. E.; Bender, S. L. Molecular recognition of protein-ligand complexes: Applications to drug design. *Chem. Rev.* **1997**, *97*, 1359–1472.
- (5) Supuran, C. T.; Scozzafava, A. Matrix metalloproteinase inhibitors. In *Proteinase and Peptidase Inhibition: Recent Potential Targets for Drug Development*; Smith, H. J., Simons, C., Eds.; Harwood Academic Press: London, 2001; in press.
- (6) (a) Nagase, H.; Woessner, J. F., Jr. Matrix metalloproteinases. *J. Biol. Chem.* **1999**, *274*, 21491–21494. (b) Dioszegi, M.; Cannon, P.; Van Wart, H. E. Vertebrate collagenases. *Meth. Enzymol.* **1995**, *248*, 413–431. (c) Tschesche, H. Human neutrophil collagenase. *Meth. Enzymol.* **1995**, *248*, 431–449.
- (7) (a) Johnson, L. L.; Dyer, R.; Hupe, D. J. Matrix metalloproteinases. *Curr. Opin. Chem. Biol.* **1998**, *2*, 466–471. (b) Williams, S.; Barnes, J.; Wakisaka, A.; Ogasa, H.; Liang, C. T. Treatment of osteoporosis with MMP inhibitors. *Ann. N. Y. Acad. Sci.* **1999**, *878*, 191–200. (c) Kahari, V. M.; Saarialho-Kere, U. Matrix metalloproteinases and their inhibitors in tumor growth and invasion. *Ann. Med.* **1999**, *31*, 34–45.
- (8) (a) Grams, F.; Crimmin, M.; Hinnes, L.; Huxley, P.; Pieper, M.; Tschesche, H.; Bode, W. Structure determination and analysis of human neutrophil collagenase complexed with a hydroxamate inhibitor. *Biochemistry* **1995**, *34*, 14012–14020. (b) Grams, F.; Reinemer, P.; Powers, J. C.; Kleine, T.; Pieper, M.; Tschesche, H.; Huber, R.; Bode, W. X-ray structures of human neutrophil collagenase complexed with hydroxamate and peptide thiols inhibitors. Implications for substrate binding and rational drug design. *Eur. J. Biochem.* **1995**, *228*, 830–841. (c) Graff von Roedern, E.; Grams, F.; Brandstetter, H.; Moroder, L. Design and synthesis of malonic acid-based inhibitors of human neutrophil collagenase (MMP-8). *J. Med. Chem.* **1998**, *41*, 339–345.
- (9) Jeng, A. Y.; Chou, M.; Parker, D. T. Sulfonamide-based hydroxamic acids as potent inhibitors of mouse macrophage metalloelastase. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 897–902.

- (10) Krumme, D.; Wenzel, H.; Tschesche, H. Hydroxamate derivatives of substrate analogous peptides containing aminomalonic acid are potent inhibitors of matrix metalloproteinases. *FEBS Lett.* **1998**, *436*, 209–212.
- (11) (a) Bond, M. D.; Van Wart, H. E. Purification and separation of individual collagenases of *Clostridium histolyticum* using red dye ligand chromatography. *Biochemistry* **1984**, *23*, 3077–3085. (b) Bond, M. D.; Van Wart, H. E. Characterization of the individual collagenases from *Clostridium histolyticum*. *Biochemistry* **1984**, *23*, 3085–3091. (c) Van Wart, H. E. Clostridium collagenases. In *Handbook of Proteolytic Enzymes – CD ROM*; Rawlings, N. D., Barrett, A. J., Woessner J. F., Eds.; Academic Press: London, 1998; Chapter 368.
- (12) Rawlings, N. D.; Barrett, A. J. Evolutionary families of metalloproteinases. *Meth. Enzymol.* **1995**, *248*, 183–228.
- (13) Rood, J. I. Virulence genes of *Clostridium perfringens*. *Annu. Rev. Microbiol.* **1998**, *52*, 333–360.
- (14) (a) Matsushita, O.; Jung, C. M.; Minami, J.; Katayama, S.; Nishi, N.; Okabe, A. A study of the collagen-binding domain of a 116-kDa *Clostridium histolyticum* collagenase. *J. Biol. Chem.* **1998**, *273*, 3643–3648. (b) Matsushita, O.; Jung, C. M.; Minami, J.; Katayama, S.; Minami, J.; Takashi, Y.; Okabe, A. Gene duplication and multiplicity of collagenase in *Clostridium histolyticum*. *J. Bacteriol.* **1999**, *181*, 923–933.
- (15) Jung, C. M.; Matsushita, O.; Katayama, S.; Minami, J.; Sakurai, J.; Okabe, A. Identification of metal ligands in the *Clostridium histolyticum* ColH collagenase. *J. Bacteriol.* **1999**, *181*, 2816–2822.
- (16) (a) Scozzafava, A.; Supuran, C. T. Protease inhibitors. Part 5. Alkyl/arylsulfonyl- and arylsulfonylureido-/arylureido- glycine hydroxamate inhibitors of *Clostridium histolyticum* collagenase. *Eur. J. Med. Chem.* **2000**, *35*, 299–307. (b) Supuran, C. T.; Scozzafava, A. Protease inhibitors. Part 7. Inhibition of *Clostridium histolyticum* collagenase with sulfonfylated derivatives of L-valine hydroxamate. *Eur. J. Pharm. Sci.* **2000**, *10*, 67–76.
- (17) Scozzafava, A.; Supuran, C. T. Protease inhibitors. Part 8. Synthesis of potent *Clostridium histolyticum* collagenase inhibitors incorporating sulfonfylated L-alanine hydroxamate moieties. *Bioorg. Med. Chem.* **2000**, *8*, 637–645.
- (18) Scozzafava, A.; Supuran, C. T. Protease inhibitors. Synthesis of *Clostridium histolyticum* collagenase inhibitors incorporating sulfonyl-L-alanine hydroxamate moieties. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 499–502.
- (19) Scozzafava, A.; Supuran, C. T. Protease inhibitors: Synthesis of potent bacterial collagenase and matrix metalloproteinase inhibitors incorporating N-4-nitrobenzylsulfonylglycine hydroxamate moieties. *J. Med. Chem.* **2000**, *43*, 1858–1865.
- (20) (a) MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. Discovery of CGS 27023A, a nonpeptidic, potent, and orally active stromelysin inhibitor that blocks cartilage degradation in rabbits. *J. Med. Chem.* **1997**, *40*, 2525–2532. (b) Hanessian, S.; Bouzbouz, S.; Boudon, A.; Tucker, G. C.; Peyroulan, D. Picking the S₁, S_{1'} and S₂ pockets of matrix metalloproteinases. A niche for potent acyclic sulfonamide inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1691–1696.
- (21) Almstead, N. G.; Bradley, R. S.; Pikul, S.; De, B.; Natchus, M. G.; Taiwo, Y. O.; Gu, F.; Williams, L. E.; Hynd, B. A.; Janusz, M. J.; Dunaway, C. M.; Mieling, G. E. Design, synthesis and biological evaluation of potent thiazine and thiazepine-based matrix metalloproteinase inhibitors. *J. Med. Chem.* **1999**, *42*, 4547–4562.
- (22) Kiyama, R.; Tamura, Y.; Watanabe, F.; Tsuzuki, H.; Ohtani, M.; Yodo, M. Homology modeling of gelatinase catalytic domains and docking simulations of novel sulfonamide inhibitors. *J. Med. Chem.* **1999**, *42*, 1723–1738.
- (23) (a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring? *J. Med. Chem.* **1999**, *42*, 2641–2650. (b) Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, amino acyl/dipeptidyl sulfonamides possessing long-lasting intraocular pressure-lowering properties via the topical route. *J. Med. Chem.* **1999**, *42*, 3690–3700.
- (24) Borrás, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. Carbonic anhydrase inhibitors: Synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/ heterocyclic sulfonamides containing 8-quinoline-sulfonyl moieties: is the tail more important than the ring? *Bioorg. Med. Chem.* **1999**, *7*, 2397–2406.
- (25) (a) Supuran, C. T.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G. Carbonic anhydrase inhibitors. Part 71. Synthesis and ocular pharmacology of a new class of water-soluble, topically effective intraocular pressure lowering sulfonamides incorporating picolinoyl moieties. *Eur. J. Pharm. Sci.* **1999**, *8*, 317–328. (b) Supuran, C. T.; Scozzafava, A.; Menabuoni, L.; Mincione, G.; Briganti, F.; Mincione, F. Carbonic anhydrase inhibitors. Part 70. Synthesis and ocular pharmacology of a new class of water-soluble, topically effective intraocular pressure lowering agents derived from nicotinic acid and aromatic/heterocyclic sulfonamides. *Eur. J. Med. Chem.* **1999**, *34*, 799–808.
- (26) Tamura, Y.; Watanabe, F.; Nakatani, T.; Yasui, K.; Fuji, M.; Komurasaki, T.; Tsuzuki, H.; Maekawa, R.; Yoshioka, T.; Kawada, K.; Sugita, K.; Ohtani, M. Highly selective and orally active inhibitors of type IV collagenase (MMP-9 and MMP-2): N-sulfonylamino acid derivatives. *J. Med. Chem.* **1998**, *41*, 640–649.
- (27) Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors. Arylsulfonylureido and arylureido- substituted aromatic and heterocyclic sulfonamides: toward selective inhibitors of carbonic anhydrase isozyme I. *J. Enz. Inhib.* **1999**, *14*, 343–363.
- (28) Van Wart, H. E.; Steinbrink, D. R. A continuous spectrophotometric assay for *Clostridium histolyticum* collagenase. *Anal. Biochem.* **1981**, *113*, 156–165.

JM010087E