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Protease Inhibitors: Synthesis of *Clostridium histolyticum* Collagenase Inhibitors Incorporating Sulfonyl-L-alanine Hydroxamate Moieties

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Abstract—A series of hydroxamates was obtained by the reaction of *N*-(4-nitrobenzyl)-L-alanine with alkyl/arylsulfonyl halides, followed by conversion of the COOH group into CONHOH. Structurally-related compounds were prepared similarly by using arylsulfonyl isocyanates, aryl isocyanates or arylsulfenyl halides instead of the sulfonyl halides. Many of the new compounds showed nanomolar affinity for the bacterial collagenase isolated from the pathogen *Clostridium histolyticum*.

Introduction

Clostridium histolyticum collagenase (EC 3.4.24.3) is a 116 kDa zinc-protein belonging to the M9 metalloproteinase family,[1] being able to hydrolyze triple helical regions of collagen under physiological conditions, as well as an entire range of synthetic peptide substrates,[2–8] being also involved in the pathogenicity of this and related clostridia, such as *Clostridium perfringens*, which causes human gas gangrene and food poisoning among others.[2–8]

Similar to the vertebrate MMP's,[9,10] *C. histolyticum* collagenase (abbreviated as ChC) has the conserved HExxH zinc-binding motif, which in this specific case is His^{415}ExxH, with two histidine residues (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 cleavage.[2–10] Similarly to the MMP's, ChC is also a multiunit protein, consisting of four segments, S1, S2a, S2b and S3,[5,6] with S1 incorporating the catalytic domain. Amino acid hydroxamates act as efficient inhibitors of MMPs,[11] and presumably also of ChC. Thus, we hypothesized that this class of compounds which strongly inhibit MMPs (collagenases, gelatinases, etc.) would also act as potent ChC inhibitors. Our interest in this type of enzyme inhibitors is related to the development of pharmacological agents for the treatment of bacterial corneal keratitis, a condition leading to serious complications for which efficient cures are difficultly envisageable.[12–14] It was in fact recently reported[12–14] that collagen shields applied to the cornea of patients with bacterial keratitis degrade rapidly, due to the collagenases secreted by the pathogen bacterial species, but these shields also protect, to some extent, the corneal collagen degradation and thus the ocular surface.[12–14] The use of such a shield impregnated with an antibiotic agent specific for the collagen-degrading bacteria would thus have a double benefit for the patient: (i) the collagenase inhibitor would kill (or impair the growth of) bacteria present on the cornea, improving and accelerating healing of the keratitis; (ii) the protective collagen shield would possess an augmented stability, as its degradation by the secreted collagenases would be delayed, promoting/accelerating in this way the healing of the wound.[12–14]

Chemistry

In this paper we report the preparation of a series of ChC inhibitors incorporating alkyl/arylsulfonamido-L-alanine hydroxamate as well as arylsulfonylureido-/arylureido-L-alanine hydroxamate moieties in their molecule. As the ChC enzyme catalyzes the cleavage of the Xaa-Gly peptide bond of the repeating sequence of collagen: -Gly-Pro-Xaa-Gly-Pro-Xaa- (Xaa = amino acid residue), it appears that the S_3, S_2 and S_1 subsites of the enzyme are occupied by Gly, Pro and Xaa, respectively.[15] Analogously, the $S_{1'}$, $S_{2'}$ and $S_{3'}$ subsites are

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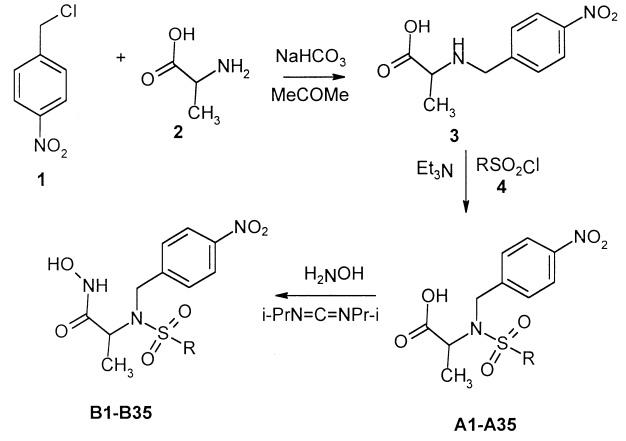
also occupied by Gly, Pro and Xaa, respectively. Thus, we opted for the following structural elements in the design of the ChC inhibitors reported in the present study: (i) a strong zinc-binding function (of the carboxylic acid, or hydroxamic acid type); (ii) a small (compact) group in α to it; (iii) the already optimized benzyl[16–18] group at the $S_{2'}$ site, converted to a 4-nitrobenzyl one; and (iv) variable alkyl/arylsulfonyl-; arylsulfonyl-ureido/arylureido-; or arylsulfenyl, moieties at $S_{1'}$.[16–18] The new inhibitors were obtained as shown in Scheme 1.

The key intermediate **3** was obtained by alkylation of L-alanine with 4-nitrobenzyl chloride. Its reaction with sulfonyl halides **4**,[19] arylsulfonyl isocyanates,[20] aryl isocyanates,[20] sulfenyl halides,[21] gave the ChC inhibitors shown in Tables 1 and 2 after the conversion of the COOH into the CONHOH, moiety.[23,24]

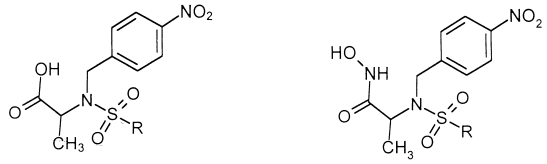
Enzyme Inhibition

The following should be noted regarding ChC inhibition data of Tables 1 and 2 with the new compounds reported here: (i) all hydroxamates were 100–500 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);[9–16] (ii) potent inhibitors were obtained from all classes of hydroxamates investigated here, such as the alkyl/arylsulfonyl-*N*-4-nitrobenzyl-Ala derivatives (**B5**; **B20**–**B22**; **B26**, **B27**, **B29**, etc.), the arylsulfonylureas- and arylureas (such as **D2**, **F5**, **F6**), the sulfenamido-4-nitrobenzyl-Ala derivatives (such as **H2**, **H3**) or the thiourea **J1**. Thus, it seems that the $S_{1'}$-binding moiety of the arylsulfonamide type, previously investigated for the obtaining of MMP inhibitors of type **7**,[16–19] can be efficiently substituted by related moieties such as alkylsulfonyl-; arylsulfenyl-; arylsulfonylureido-; arylureido- or benzoyl-thioureido, without loss of the ChC inhibitory properties; (iii) in the subseries of alkyl/arylsulfonamido derivatives (of types **A**,**B(1-35)**) 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- (**B18**–**B20**; **B26**); 3- or 4-carboxy-phenylsulfonyl- (**B28**, **B29**), 1- or 2-naphthylsulfonyl as well as 8-quinolinesulfonyl moieties (**B30**–**B32**; **B34**). All these derivatives possessed inhibition constants in the range of 5–12 nM against ChC, being among the most potent such inhibitors ever reported. A second group of sulfonamide inhibitors, containing moieties such as 4-bromophenyl; 4-iodophenyl; 2-, 3- or 4-nitrophenyl; 2,5-dichlorophenyl-; 2,4,6-trimethylphenyl-; 4-methoxyphenyl- or 2-thienyl, substituting the *N*-benzyl-glycine hydroxamate, behaved as medium potency inhibitors, with affinities in the 15–30 nM range (Table 1). The least active sulfonamides were those



Scheme 1.

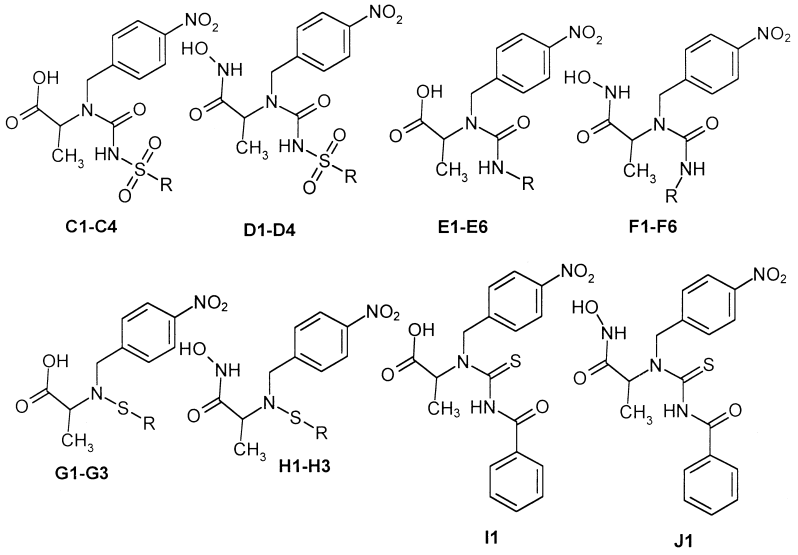
Table 1. Inhibition[22] of *Clostridium histolyticum* collagenase (ChC) with the carboxylic acids **A1**–**A35** and the corresponding hydroxamates **B1**–**B35**



R	Compound	K_I[a] (μM)	Compound	K_I[a] (nM)
CH_3	**A1**	18	**B1**	76
CF_3	**A2**	4.7	**B2**	75
CCl_3	**A3**	5.1	**B3**	52
n-C_4F_9-	**A4**	2.3	**B4**	11
n-C_8F_{17}	**A5**	1.8	**B5**	7
Me_2N-	**A6**	36	**B6**	69
C_6H_5-	**A7**	21	**B7**	54
$PhCH_2$-	**A8**	15	**B8**	50
4-F-C_6H_4-	**A9**	10	**B9**	35
4-Cl-C_6H_4-	**A10**	9	**B10**	32
4-Br-C_6H_4-	**A11**	10	**B11**	30
4-I-C_6H_4-	**A12**	12	**B12**	37
4-CH_3-C_6H_4-	**A13**	13	**B13**	36
4-O_2N-C_6H_4-	**A14**	5.3	**B14**	10
3-O_2N-C_6H_4-	**A15**	5.5	**B15**	12
2-O_2N-C_6H_4-	**A16**	4.8	**B16**	13
3-Cl-4-O_2N-C_6H_3-	**A17**	3.3	**B17**	9
4-AcNH-C_6H_4-	**A18**	3.1	**B18**	11
4-BocNH-C_6H_4-	**A19**	2.4	**B19**	10
3-BocNH-C_6H_4-	**A20**	2.6	**B20**	8
4-Ac-C_6H_4-	**A21**	2.1	**B21**	9
C_6F_5-	**A22**	0.4	**B22**	5
3-CF_3-C_6H_4	**A23**	0.3	**B23**	6
2,5-$Cl_2C_6H_3$	**A24**	3.6	**B24**	13
4-CH_3O-C_6H_4-	**A25**	5.2	**B25**	21
2,4,6-$(CH_3)_3$-C_6H_2-	**A26**	6.0	**B26**	17
4-CH_3O-3-BocNH-C_6H_3-	**A27**	2.1	**B27**	8
2-HO-3,5-Cl_2-C_6H_2-	**A28**	2.5	**B28**	12
3-HOOC-C_6H_4-	**A29**	2.0	**B29**[b]	9
4-HOOC-C_6H_4-	**A30**	1.4	**B30**[b]	6
1-Naphthyl	**A31**	1.2	**B31**	7
2-Naphthyl	**A32**	1.3	**B32**	8
5-Me_2N-1-naphthyl-	**A33**	1.1	**B33**	7
2-Thienyl	**A34**	2.1	**B34**	11
Quinoline-8-yl	**A35**	1.2	**B35**	8

[a] K_I-s values were obtained from Dixon plots using a linear regression program,[4] from at least three different assays. Errors were around ± 10% (from at least three determinations).
[b] COOH transformed into -CONHOH.

Table 2. Inhibition[22] of ChC with the carboxylic acids of types **C**, **E**, **G**, **I** and the corresponding hydroxamates of types **D**, **F**, **H**, **J**



R	Compound	K_I[a] (μM)	Compound	K_i[a] (nM)
4-F-C_6H_4-	**C1**	3.3	**D1**	9
4-Cl-C_6H_4-	**C2**	2.4	**D2**	7
4-CH_3-C_6H_4-	**C3**	2.5	**D3**	12
2-CH_3-C_6H_4-	**C4**	3.1	**D4**	8
4-F-C_6H_4-	**E1**	5.4	**F1**	12
3-Cl-C_6H_4-	**E2**	6.1	**F2**	14
4-Cl-C_6H_4-	**E3**	6.5	**F3**	18
2,4-F_2-C_6H_3-	**E4**	6.2	**F4**	15
3,4-$Cl_2C_6H_3$	**E5**	5.0	**F5**	13
1-Naphthyl	**E6**	4.6	**F6**	10
4-O_2N-C_6H_4-	**G1**	5.5	**H1**	11
2-O_2N-C_6H_4-	**G2**	6.3	**H2**	7
2,4-$(O_2N)_2$-C_6H_3-	**G3**	5.1	**H3**	9
—	**I1**	0.9	**J1**	6

[a]K_I-s values were obtained from Dixon plots using a linear regression program, from at least three different assays.[4] Errors were around ±10% (from at least three determinations).

containing methyl-; trihalomethyl-; dimethylamino-; phenyl- and benzyl moieties (Table 1); (iv) 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 ChC inhibitors. Similarly behaved are the ureas of type **F**, and the sulfenamides of type **H**, except for **F5** and **F6**, as well as **H2** and **H3**, which are strong inhibitors. A very potent inhibitor is the thiourea derivative **J1** (Table 2).

The new class of hydroxamate inhibitors reported here presumably binds bidentately to the Zn(II) ion of the enzyme, as the structurally related MMP inhibitors. Probably the methyl group provening from Ala occupies the S_1 site, the nitrobenzyl moiety the $S_{2'}$ site, whereas the arylsulfonyl group the $S_{1'}$ site.[9,10] These interactions would thus explain the very strong ChC inhibitory properties of the new compounds.

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References and Notes

1. Van Wart, H. E. Clostridium collagenases. In *Handbook of Proteolytic Enzymes — CD ROM*; Barrett, A. J.; Rawlings, N. D.; Woessner, J. F., Eds.; Chapter 368, Academic Press, London, 1998.
2. Bond, M. D.; Van Wart, H. E. *Biochemistry* **1984**, *23*, 3077.
3. Bond, M. D.; Van Wart, H. E. *Biochemistry* **1984**, *23*, 3085.
4. Van Wart, H. E.; Steinbrink, D. R. *Anal. Biochem.* **1981**, *113*, 356.
5. Matsushita, O.; Jung, C. M.; Minami, J.; Katayama, S.; Nishi, N.; Okabe, A. *J. Biol. Chem.* **1998**, *273*, 3643.
6. Jung, C. M.; Matsushita, O.; Katayama, S.; Minami, J.; Sakurai, J.; Okabe, A. *J. Bacteriol.* **1999**, *181*, 2816.
7. Schiff, W. M.; Speaker, M. G.; McCormick, S. A. *CLAO J.* **1992**, *18*, 59.
8. Groos, E. B. In *Cornea*: *Fundamentals of Cornea and External Disease*; Krachmer, J. H.; Mannis, M. J.; Holland, E. J., Eds.; Mosby-Year Book, St Louis, MO, 1997; Vol. 6, pp 105–142.
9. Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735.
10. Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
11. Bottomley, K. M.; Johnson, W. H.; Walter, D. S. *J. Enzyme Inhib.* **1998**, *13*, 79–101.

12. Reidy, J. J.; Gebhardt, B. M.; Kaufman, H. E. *Cornea* **1990**, *9*, 196.
13. Sawusch, M. R.; O'Brien, T. P.; Dick, J. D.; Gottsch, J. D. *Am. J. Ophthalmol.* **1988**, *106*, 279.
14. Dorigo, M. T.; De Natale, R.; Miglioli, P. A. *Chemotherapy* **1995**, *41*, 1.
15. Grobelny, D.; Galardy, R. E. *Biochemistry* **1985**, *24*, 6145.
16. MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Caroll, 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. *J. Med. Chem.* **1997**, *40*, 2525.
17. Jeng, A. Y.; Chou, M.; Parker, D. T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 897.
18. Hanessian, S.; Bouzbouz, S.; Boudon, A.; Tucker, G. C.; Peyroulan, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1691.
19. Borras, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *Bioorg. Med. Chem.* **1999**, *7*, 2397.
20. Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib.* **1999**, *14*, 343.
21. Briganti, F.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2043.
22. Collagenase type VII (highly purified) and its substrate, FALGPA (furanacryloyl-leucyl-glycyl-prolyl-alanine) were purchased from Sigma-Aldrich (Milan, Italy); 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.[4] 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 $\epsilon_{305} = 24{,}700$ L mol^{-1} cm^{-1} in the above-mentioned reaction buffer.[4] Measurements were made using a Perkin–Elmer spectrophotometer interfaced with a PC. Initial velocities were estimated using the direct linear plot-based procedure reported by van Wart and Steinbrink.[4] K_I-s were then determined according to Dixon, using a linear regression program. The K_I values determined are the means of at least three determinations.
23. Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.* **1999**, *42*, 2641.
24. Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. *J. Med. Chem.* **1999**, *42*, 3690.