Bioorganic & Medicinal Chemistry Letters, Vol.3, No.12, pp. 2547-2552, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

ENANTIOSELECTIVE SYNTHESIS AND ANTIELASTASE ACTIVITY OF 1,3,4-TRISUBSTITUTED AND 3,4-DISUBSTITUTED β-LACTAM ANTIBIOTICS

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(*Received in Belgium* 5 *May* 1993; *accepted* 5 *August* 1993)

Abstract: The synthesis and elastase inhibitory properties of 1,3,4-trisubstituted and 3,4-disubstituted 2-azetidinones are described. Nine of the synthesized derivatives showed elastase inhibitory activities and four of them were specific against human leukocyte elastase (HLE). 2-Deoxy-2-dithianyl-3,4;5,6-di-*O*-isopropylidene-*D*-2-glucosyl group attached to the β-lactam nitrogen atom is related to the specificity against HLE.

Human leukocyte elastase (HLE), a serine proteinase isolated from the azurophilic granules of polymorphonuclear leucocytes, hydrolyzes several connective tissue components such as elastin, proteoglycan, and certain types of collagen. This enzyme is thought to play an important role in the destructive processes associated with chronic inflammatory diseases such as emphysema[1], glomerulonephritis[2] and rheumatoid arthritis.[3] In these diseases, the leukocyte elastase is inadequately controlled by plasmatic macromolecular inhibitors such as α_1-proteinase inhibitor and α_2-macroglobulin. There is considerable interest in the development of low molecular weight synthetic inhibitors of elastase which may be used in prophylactic replacement therapy.

In a number of laboratories, there is an extended effort to design non-toxic, specific and potent drugs able to control elastase-related disorders. Scores of valuable structures have been made, which at present may be categorized chemically as:[4] 1.- Peptidic reversible/irreversible inhibitors, such as boronic acids, peptide chloromethylketones, 2.-Heterocyclic reversible/irreversible inhibitors, such as chloropyrans, chloroisocoumarins and, more recently, β-lactam compounds.[5,6]

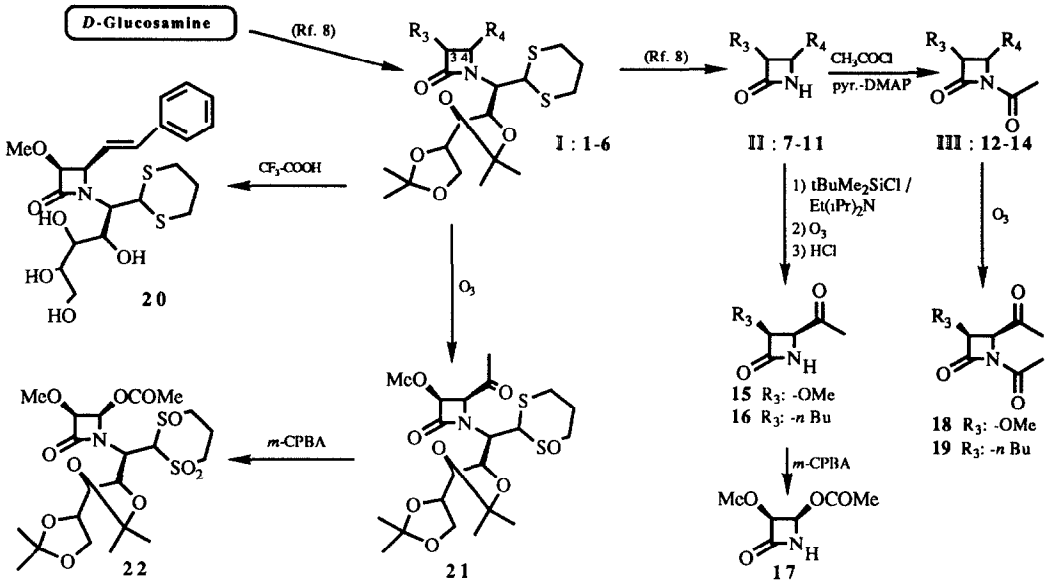
The discovery that monocyclic β-lactam nuclei with appropriate substituents at 1, 3 and 4 positions are inhibitors of HLE,[6a, 6b] prompted us to study the inhibitory activity of the 1,3,4-trisubstituted and 3,4-disubstituted 2-azetidinones against three different enzymes: pancreatitic porcine elastase (PPE), rat leukocyte elastase (RLE) and human leukocyte elastase (HLE).

In this paper we describe the synthetic methods involved in the preparation of the monobactams **1-22**[7] (Scheme 1), their inhibitory activities and a study of their structure-activity relationships carried out by molecular modeling.

The stereospecific synthesis of compounds **1-6** (class I monobactams) and **7-11** (class II monobactams), has been carried out with good yields (70 to 90%) by applying our method[8] which is based on the use of *D*-glucosamine as a chiral auxiliary in the Staudinger reaction.[9] The *cis*-relationship of the C-3 and C-4 substituents in compounds **1-11** was deduced from the coupling constants of the β-lactam H-3 and H-4 ($4.3Hz < J_{3,4} < 6.0Hz$)

and the absolute configuration of these substances was unequivocally assigned from the $[\alpha]_D$ data, circular dichroism (CD) of the monobactams **II**[10] and the X-ray crystal analysis of compounds **1b**[8] and **6**.[10]

Compounds **12-14** (class **III** monobactams) were obtained, in a low to medium yield (20 to 57 %), by *N*-acylation of the monobactams **II** with acetyl chloride and 4-dimethylaminopyridine in pyridine.



I	II	III	R3	R4
1a (3α, 4α) **1b** (3β, 4β)	**7a** (3α, 4α) **7b** (3β, 4β)		- OMe	- CH=CH-Ph
2a (3α, 4α) **2b** (3β, 4β)	**8b** (3β, 4β)	**12b** (3β, 4β)	- OMe	- C(CH3)=CHPh
3a (3α, 4α) **3b** (3β, 4β)	**9a** (3α, 4α) **9b** (3β, 4β)	**13b** (3β, 4β)	- OMe	- Ph
4a (3α, 4α) **4b** (3β, 4β)			- OMe	- Ph(p-OMe)
5 (3β, 4β) **6** (3β, 4β)[1]	**10** (3β, 4β) **11** (3β, 4β)	**14** (3β, 4β)	- *n*-Bu	- C(CH3)=CHPh

[1] *cis*-1-methyl-styryl

Scheme 1

Silylation of **8b** and **10** with *tert*-butyldimethylchlorosilane and diisopropylethylamine in CH_2Cl_2 followed by ozonolysis and removal of the silyl group with HCl (0.1N) in MeOH, gave the 3-acetyl derivatives **15** and **16** in 33% and 82% yields, respectively. Oxidation of compound **16** with *m*-chloroperbenzoic acid in CH_2Cl_2 gave the 3β-butyl-4β-acetoxyazetidinone **17**[6a] in 49% yield.

Compounds **18** and **19** were obtained from the monobactams **12b** and **14** by ozonolysis in 62% and 87% yields, respectively.

Deprotection of the isopropylidene groups of monobactam **1b** with trifluoracetic acid in MeOH gave the tetraol derivative **20** in 90% yield.[11] Ozonolysis of compound **2b** (O_3/Me_2S) gave the 4β-acetyl derivative **21** (70%) which was transformed into the 4β-acetoxyazetidinone **22** (76%) by Baeyer-Villiger reaction.

We have investigated the antielastase activity of 1,3,4-trisubstituted and 3,4-disubstituted 2-azetidinones against three different enzymes (PPE, RLE and HLE) in order to design potent and specific HLE inhibitors.

The inhibition of elastases by monocyclic β-lactams is summarized in Table 1. Inhibitory potency was determined as the concentration of the tested compound (IC_{50}, μM) to give 50% inhibition of enzymatic activity at an arbitrarily chosen time of 5 min after mixing.[12]

Table 1 - *In vitro*, inhibition of pancreatitic porcine elastase (PPE), rat leukocyte elastase (RLE) and human leukocyte elastastase (HLE) by 1,3,4-trisubstituted 2-azetidinones.

Compd. No.	IC_{50} (μM) PPE	IC_{50} (μM) RLE	IC_{50} (μM) HLE	Compd. No.	IC_{50} (μM) PPE	IC_{50} (μM) RLE	IC_{50} (μM) HLE
1a	IN*	IN	76	**13b**	0.7	0.4	1.6
2b	IN	IN	18	**14**	61	>160	43
5	IN	IN	11	**18**	26	11	94
6	IN	IN	13	**19**	>160	37	45
12b	1.1	0.5	1.4	**Ref.#**	0.8	0.7	0.1

* IN= Not active (IC_{50} >200 μM).

\# Reference in test evaluation: 3,4-dichloroisocoumarin.

We found that the 3,4-disubstituted 2-azetidinones, compounds **7-11**, **15**, **16** and **17**, do not show elastase inhibition (not included in Table 1); however, the introduction of an acyl group on the nitrogen atom, compounds **12-14**, **18** and **19**, activates the β-lactam ring and showed non selective antielastase activity.

Compounds **1a**, **2b**, **5** and **6** (monobactams II), containing a sugar residue attached to the β-lactam nitrogen atom, gave selective inhibition to HLE. The inhibition IC_{50} values of these compounds (Table 1) are rather modest as compared with those of the most active cephalosporins,[5] but compare favorably with the most potent monocyclic β-lactam HLE inhibitors.[6]

Apart from **1b** and **2a** (synthetic products not tested) the remaining class II monobactams do not show elastase inhibition.

It is clear, from the above results, that the 2-deoxy-2-dithianyl-3,4;5,6-di-*O*-isopropylidene-*D*-2-glucosyl group is related to the selective HLE inhibition, but it is intriguing to observe that compounds **3a**, **3b**, **4a**, **4b** and **20** are not active.

In order to clarify the role played by the sugar moiety and the C-4 substituents of monobactams in the HLE inhibition, we have carried out a molecular modeling study with INSIGHT II and MACROMODEL[13] programs

using the atomic coordinates obtained for the X-ray structure (Figure 1) of the enzyme complexed with a peptide chloromethyl ketone inhibitor.[14]

Compound **12b**, the HLE inhibitor with a lesser degree of freedom, was placed in the active site of the enzyme and the best fit showed interatomic distances of 1.86 Å and 1.72 Å between the carbonyl group of the β-lactam and the catalytic residues of HLE Ser-195 and His-57, respectively. The remaining compounds were superimposed over **12b,** as shown in Figure 2. The side chains were allowed to relax so as to avoid unfavorable interactions between the inhibitor and the enzyme.

The behaviour of the monobactams studied can be explained according to the molecular modelling as follows:

1.- For the best fitting of the β-lactam ring and HLE structure (Figure 3), the 2-deoxy-2-dithianyl-3,4;5,6-di-*O* -isopropylidene-*D* -2-glucosyl substituent at N-1 (yellow and red) occupies the same place as the peptide inhibitor (violet), S1 to S3 pockets.[14] This observation is consistent with the results we have obtained for the inhibition of HLE by compounds **1a**, **2b**, **5** and **6**.

The inactivity of the tetrahydroxy derivative **20** could be explained by the higher water solvation that could hamper the approach of this substance to the enzyme active site. A similar reason (higher polarity and water solubility)[16] could perhaps explain the inactivity of the sulfoxide and sulfone derivatives **21** and **22**.

2.- The steric crowding originated by the C-4 substituent can also explain the inactivity of the compounds **3a-4b**. For these compounds, strong steric interactions can be observed (Figure 4) between the phenyl groups (pink) and the convoluted surface of HLE (orange).

In the active compounds **1a**, **2b**, **5** and **6**, the phenyl groups, which are placed two atoms apart from C-4, can be oriented in such a way (blue) that there are no unfavourable interactions with the HLE structure.

It was also concluded that the insertion of only one heteroatom (O or S) between the aryl group and the β-lactam ring could be enough to avoid the steric crowding of the C-4 substituent and the HLE structure.

3.- One of the active compounds, **1a**, has 3α configuration (see Figure 2) while the remaining active substances have 3β configuration. It seems that the configuration at C-3 is not essential for the antielastase activity of monobactams.

The preceding experimental data, the molecular study and the proposed reaction mecanism to explain the inhibition of HLE by β-lactams,[6a,16] suggest the following structural requirements of a monobactam for a better and specific inhibition of HLE:

a) The N-1 substituent should be a low polar group like 2-deoxy-2-dithianyl-3,4;5,6-di-*O* -isopropylidene-*D* -2-glucosyl that can occupy the S1-S3 pockets of HLE.

b) A better leaving group on C-4 such as an alcoxy, aryloxy, acyloxy or the respective thioderivatives, will increase the reactivity and hence the antielastase activity.

c) The C-3 substituents can be small alkyl or alcoxyl substituents (MeO-, Et-, Bu-). The $3\beta,4\beta$ derivatives seem to be more active than the $3\alpha,4\alpha$ isomers.

Further studies dealing with the synthesis of molecules that fulfil the former structural requirements are now underway in our laboratory.

Figure 1.- X-ray structure of the complex formed upon reaction of HLE with methoxysuccinyl-Ala-Ala-Ala-Pro-Ala-chloromethylketone.[14]

The catalytic residues Ser-195 and His-57 (in green) and the inhibitor (in yellow) are highlighted.

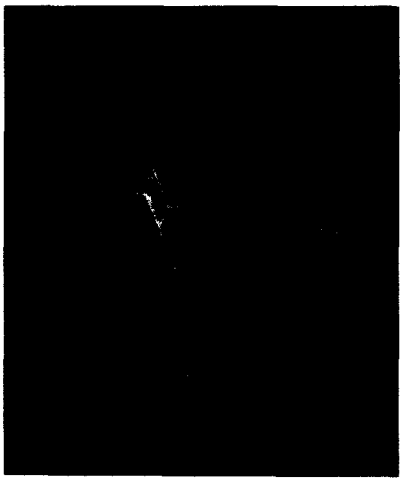


Figure 2.- Interatomic distances between carbonyl group of the b-lactam ring and the catalytic residues of enzyme.

Figure 3.- Monobactams in structure of the enzyme. 2-Deoxy-2-dithianyl-3,4;5,6-di-O-isopropylidene-D-2-glucosyl group (yellow and red) and the peptide HLE inhibitor (violet).

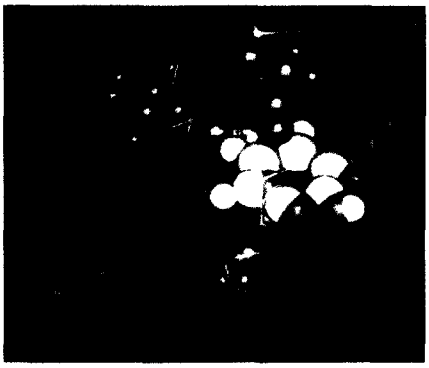


Figure 4.- Interations between the phenyl groups of monobactams I (pink and blue) and HLE structure (orange).

Acknowledgments. The authors are grateful to Institut Servier for financial support. We also thank MEC (Spain) and MAE (France) for generous financial assistance (Acción Integrada HF 298 A, 1991).

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7.-All the new compounds gave IR, ^{1}H, ^{13}C, mass spectra and elemental analyses consistent with the assigned structures.

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9.- A manuscript describing the synthesis of these and other monobactams of type I and II is in preparation. The same synthesis, using *D* -glucosamine as chiral auxiliary in the Staudinger reaction, has been carried out to prepare substituted methyl carbapenem antibiotic presursors[11] as well as 2-isocephems and 2-oxa-isocephems.[17]

10.- Optical rotations, CD and X-Ray crystal data will be reported elsewhere.

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