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Synthesis and evaluation of N1/C4-substituted β-lactams as PPE and HLE inhibitors

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Abstract—4-(Alkylamino)carbonyl-1-(alkoxy)carbonyl-2-azetidinones (**9**–**11**) have been prepared in five steps from 4-(benzyloxy)-carbonyl-1-(*t*-butyldimethyl)silyl-2-azetidinone (**1**). The β-lactam reactivity of **9** has been established by ^{1}H NMR experiment. Compound **11** was a good reversible inhibitor of PPE and HLE. Based on theoretical design, series of 2-azetidinones (**12**–**17**) and 4-(alkoxy)carbonyl-2-azetidinones (**18**–**21**) bearing various carbonyl (ester, thiolester, amide) and thiocarbonyl (thioamide) functionalities at position N1 were similarly prepared. In the absence of C4-substituent, the compounds were inactive against elastases. On the other hand, 4-(benzyloxy)carbonyl-1-(ethylthioxy)carbonyl-2-azetidinone (**19**) and 4-(benzyloxy)carbonyl-1-(benzylamino)-thiocarbonyl-2-azetidinone (**21**) were both good reversible inhibitors, but acting most probably via different mechanisms (enzymic processing of the exocyclic ester function or β-lactam ring opening).

1. Introduction

One of the current research lines in medicinal chemistry for the discovery of novel anti-inflammatory drugs is based on the design of human leukocyte elastase (HLE) inhibitors.[1] This enzyme, released by neutrophils, exerts numerous and important biological functions, including the degradation of connective tissue proteins. The HLE proteolitic activity is regulated by endogenous inhibitors; but, under pathological conditions, increased production or inadequate neutralisation of HLE can lead to uncontrolled connective tissue degradation, generating inflammatory diseases such as pulmonary emphysema, cystic fibrosis and chronic bronchitis.[2] Thus, synthetic HLE inhibitors able to restore the protease/antiprotease balance are of potential therapeutic interest.[3]

HLE belongs to the superfamily of serine proteases like chymotrypsin.[4] The hydrolysis catalytic mechanism involves the formation of an acyl-enzyme connecting the active serine residue (Ser-195) and the reactive carbonyl function of the substrate/inhibitor; this intermediate is stabilized in the so-called oxy-anion hole. Porcine pancreatic elastase (PPE)[5] is usually considered as a good model of HLE (the respective aminoacid sequences share 40% residue identities); this enzyme is more readily available and crystallographic studies have demonstrated the structural similarity between the active sites of both enzymes (conserved catalytic triad, namely His-57, Asp-102 and Ser-195).

Nowadays, numerous peptidic and non peptidic HLE inhibitors of low molecular weight have been reported, including for example, trifluoromethylketones (TFMK),[6] thiazolidinones[7] and coumarinic derivatives.[8] Lactams have also been considered as lead-structures for serine proteases inhibition, such as δ-lactams,[9] γ-lactams,[10] and β-lactams.[11] Our interest in this last family led us to propose *N*-(alkoxy)carbonyl derivatives as potential suicide-substrates (mechanism-based inhibitors) designed to operate according to Figure 1 (Y = X = O), but this mechanism has not actually been demonstrated.

We have previously studied two series of compounds, namely, 3-halogeno-[12] and 4-(alkoxy)carbonyl-1-

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(alkoxy)carbonyl-2-azetidinones [13] shown in Figure 2. These β-lactams behaved as reversible inhibitors of PPE [activities (K_i) in the micromolar range], but their mode of action was different, depending on the C3/C4 substitution. Indeed, after a transient inhibition of PPE, enzymic hydrolysis of 3-halogeno-2-azetidinones **A** (active configuration: 3*R*) led to the β-lactam bond cleavage without expulsion of the OR leaving group, while PPE processing of 4-(alkoxy)carbonyl-2-azetidinones **B** (active configuration: 4*S*) led to the OR′ ester cleavage, but not to the β-lactam ring opening. Thus, in molecules **B**, the ester carbonyl appeared to be more sensitive towards serine nucleophilic attack than the β-lactam carbonyl; the same reactivity was also observed under smooth basic chemical hydrolysis (phosphate buffer, pH 7.5). On the other hand, in molecules **A**, the *N*-carbamate function did not behave as an isocyanate precursor under enzymic processing of the β-lactam (see Fig. 1; Y = X = O). Accordingly, we have designed structural modifications of β-lactams **B** in two directions: (i) the decrease of the reactivity of the C4 carbonyl substituent; (ii) the increase of the β-lactam carbonyl reactivity and the subsequent ability of the N1 substituent to expulse a leaving group, after C2–N1 bond cleavage.

In this paper, we describe the synthesis and evaluation against elastases (PPE and HLE) of 4-(alkylamino)-carbonyl-1-(alkoxy)carbonyl-2-azetidinones on the one hand, and series of 2-azetidinones and 4-(alkoxy)-carbonyl-2-azetidinones bearing various carbonyl and thiocarbonyl functionalities at position N1 (Fig. 1; Y = O, S and X = O, S, NH), on the other hand. The replacement of the oxygen atoms (Y = X = O) in the N1 side chain with other heteroatoms (S, NH) should impact on several factors (binding interactions with the enzyme, hydrogen bond formation, lipophilicity, polarizability, leaving group ability of XR^2,...) in a way which is hardly predictable.

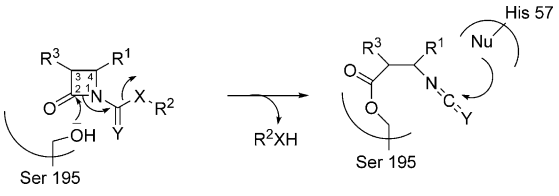


Figure 1. Design of β-lactamic inhibitors — possible suicide mechanism.

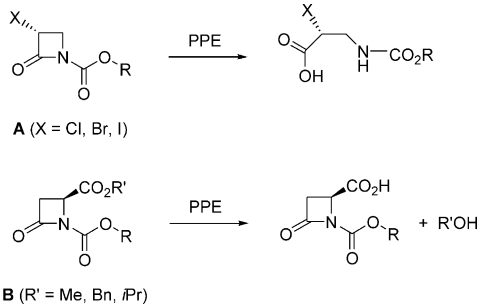


Figure 2. Reversible inhibition of PPE by β-lactams — products of the enzymic reaction.

2. Results and discussion

2.1. Modification of the C4 substituent

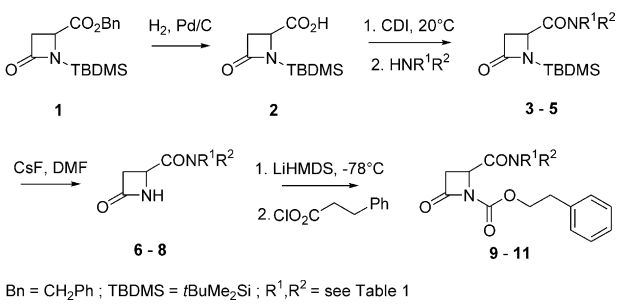
(*S*)-4-(Benzyloxy)carbonyl-1-(phenethyloxy)carbonyl-2-azetidinone[13] (molecule **B** with R = CH_2CH_2Ph and R′ = CH_2Ph) is a good reversible inhibitor of PPE (K_i = 10 μM), fitting into the enzymic cavity in such a manner that Ser-195 selectively attacks the ester carbonyl and not the β-lactam carbonyl. However, docking experiments[14] showed that other positioning should be possible, similar to that occurring in the case of (*R*)-3-bromo-1-(benzyloxy)carbonyl-2-azetidinone[12a] (molecule **A** with R = CH_2Ph and X = Br). In order to avoid competitive enzymic hydrolysis of the C4 substituent with respect to the N1–C2 bond cleavage, we decided to replace the ester group of compounds **B** (Fig. 2) with amide functions, leading to the target-molecules **9–11** (Scheme 1).

We used racemic 1-(*t*-butyldimethylsilyl)-4-(benzyloxy)-carbonyl-2-azetidinone (**1**)[15] as the starting material. Catalytic hydrogenation furnished quantitatively the corresponding acid **2**.[15] Activation of **2** with carbonyldiimidazole (CDI)[16] followed by addition of benzylamine, *N*-(methyl)benzylamine and dibenzylamine gave the amides **3**, **4** and **5**, respectively, in modest to good yields after chromatographic purifications. Deprotection of the silyl group was performed, in moderate yield, with cesium fluoride in a polar non protic solvent in order to avoid β-lactam ring opening. The resulting NH-derivatives **6–8** were directly engaged in the two step reaction of *N*-functionalisation, that is deprotonation with lithium hexamethyldisilazanate (LiHMDS) at low temperature and acylation with phenethyl chloroformate, followed a previously established protocol.[13] Good yields of 1-(phenethyloxy)carbonyl-4-(alkylamino)-carbonyl-2-azetidinones (**9–11**) were recovered after chromatography on silica gel (Table 1). The compounds were characterized by the usual spectroscopies (see

Table 1. Yields of compounds **3–11** and activity of compounds **9–11** against PPE

R^1	R^2	Compd (yield)	K_i (μM)[a]	(Compd)
H	CH_2Ph	**3** (68%); **6** (50%); **9** (71%)	115	(**9**)
Me	CH_2Ph	**4** (92%); **7** (54%); **10** (81%)	290	(**10**)
CH_2Ph	CH_2Ph	**5** (27%); **8** (55%); **11** (74%)	36	(**11**)

[a] Racemic mixture; experiments were made three times.



Bn = CH_2Ph ; TBDMS = $tBuMe_2Si$; R^1,R^2 = see Table 1

Scheme 1. Synthesis of 4-(alkylamino)carbonyl-1-phenethyloxy-carbonyl-2-azetidinones.

Experimental). In particular, the ^{1}H NMR spectra showed a typical ABX pattern around 4.50, 3.20 and 3.00 ppm corresponding to protons H4, H3 and H3′. For compound **10**, two rotamers (ratio 60/40) were visible in NMR. The ^{13}C NMR spectra displayed three carbonyl functions around 168, 162 and 149 ppm attributed, respectively, to the amide, carbamate and β-lactam functions. Lastly, the three carbonyl motifs gave IR signals around 1670, 1730 and 1820 cm^{-1}.

The chemical reactivity of β-lactam **9** has been evaluated in a ^{1}H NMR experiment. The evolution of a 10^{-3} M solution of **9** in deuteriated phosphate buffer (pD 7.5; 50 mM) containing 5% of DMSO-d_6 was followed at 500 MHz in function of time. Within 15 h, compound **9** was slowly hydrolyzed to give the corresponding β-aminoacid derivative resulting from nucleophilic attack onto the β-lactam carbonyl. The small ring opening was ascertained by a significant deshielding of the former's H3 and H3′ protons (Fig. 3). Cleavage of the carbamate function could not be observed (absence of signals at 2.68 and 3.65 ppm typical of phenethyl alcohol). Thus, the replacement of benzyloxy residue with benzylamino residue at C4 has well restored a higher chemical reactivity to the β-lactam ring. Similar NMR experiments could not be performed with amides **10** and **11** because these derivatives were poorly soluble in aqueous media at the concentrations required for NMR spectroscopy. More disappointingly, the PPE catalyzed hydrolysis of **9** was really too slow to be followed by NMR, without significant competition with chemical hydrolysis.

β-Lactams **9–11** were evaluated for their potential inhibitory effect on PPE in competitive experiments with a good chromophoric substrate. The rates of PPE-catalyzed hydrolysis of *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide were measured in the presence of various concentrations of tested compounds. The variation of absorbance at 410 nm (*p*-nitroaniline) was recorded in function of time. Transient inhibitions were observed. Plots of V/Vi, corresponding to the ratios of initial rates of substrate hydrolysis in the absence and in the presence of inhibitors, versus the concentration of inhibitor gave a straight line where the slope corresponds to $1/K_i$. The measured inhibition constants (K_i) are collected in Table 1. Comparatively to the reference **B** (C4 ester substituent), the amide derivatives **9–11** were less active. Surprisingly, the most sterically constrained amide **11** revealed to be the most potent inhibitor. From our ^{1}H NMR experiments, we could reasonably suppose that the mode of action of compounds **9–11** is different from that of the reference **B**. The inhibition should result from the slow deacylation of the acyl-enzyme intermediate formed with the C2 carbonyl. The experimental order of activity of tested azetidinones (**11** > **9** > **10**) was hardly predictable and most probably reflects the best fit of the C4 side chains into the enzymic pocket, rather than a different electronic activating effect.

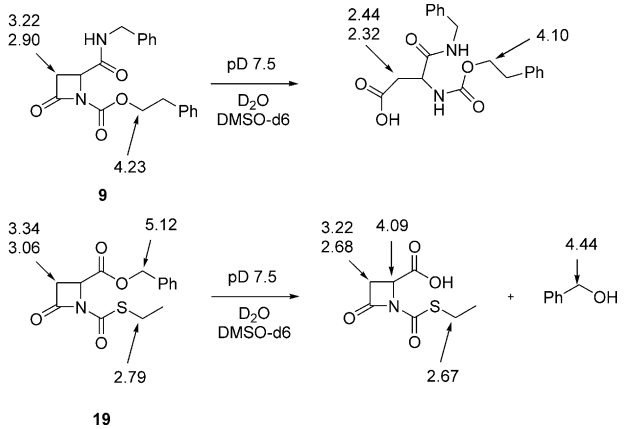


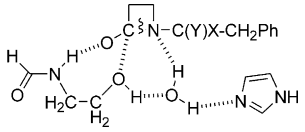
Figure 3. ^{1}H NMR data (δ) of chemical hydrolysis.

2.2. Modification of the N1 side chain

The electron withdrawing character of the N1 side chain should have an influence on the β-lactam reactivity, that is the sensitiveness of the C2 carbonyl towards nucleophilic attack (see Figure 1). This has been theoretically evaluated at the ab initio level with a minimal basis set, using a previously established model of the elastase active site.[13,17] The catalytic environment was mimicked by an imidazole (model of His-57), a water molecule (transient vehicle of the proton) and *N*-(β-hydroxyethyl)formamide (model of Ser-195 side chain including the amide bond of the backbone which is involved in the oxyanion hole stabilization). The ΔG values (calculated energies at the transition states) have been computed by reference to the isolated partners. The high values thus obtained give an indication of the complexation effort produced by the enzyme to generate the Michaelis complex as the starting point of the reaction path towards the transition state. On the other hand, the optimization of the complexes connected by the transition state could generate non representative structures as the active site geometric constraints are not taken into account in the ab initio calculations.

We have considered carbonyl (Y = O) and thiocarbonyl (Y = S) functions bearing different potential leaving groups (X-benzyl, where X = O, S, NH). Results of Table 2 showed a significant difference between the *N*-carbonyl (entries 1–3) and the *N*-thiocarbonyl derivatives (entries 4–6), the later being more reactive ($\Delta\Delta G$ = 4.4–6.6 kcal/mol). In each series, the ΔG values

Table 2. Theoretical evaluation of reactivity



Entry	Y	X	ΔG (kcal/mol)[a,b]
1	O	O	40.1
2	O	S	40.2
3	O	NH	41.8
4	S	O	34.9
5	S	S	33.6
6	S	NH	37.4

[a] Calculated energy at the transition state.
[b] Azetidinone C–N bond cleavage.

calculated to reach the transition state of the N1–C2 bond cleavage appeared weakly dependent on the nature of the potential leaving group (X = O, S); however, (thio)amide substituents (X = NH) were the less favourable. The important activating effect of sulfur-containing side chains (particularly, entry 5) could be attributed mainly to a polarizability effect.

We first prepared C3/C4-unsubstituted β-lactams representative of the N1 substituted families (Y = O) with ester (X = O), thiolester (X = S) and amide (X = NH) groups, respectively. Commercially available 2-azetidinone (Scheme 2; $R^1 = H$) was first deprotonated with LiHMDS at low temperature, then acylated with ethyl chloroformate and ethyl thiochloroformate to furnish β-lactams **12** and **13** (Table 3). According to a procedure developed by Aoyama et al. for the synthesis of human chymase inhibitors,[18] we prepared β-lactams **14** and **15** by reacting 2-azetidinone with phenyl- and benzylisocyanate, respectively. Compounds **12–15** were characterized in ^{1}H NMR by two triplets around 3.0 and 3.6 δ for H4 and H3 protons. In ^{13}C NMR, the β-lactam carbonyl gave a signal around 164–167 ppm; carbonyls of carbamate, thiocarbamate and urea functions were visible at 149, 165 and 148 ppm, respectively.

Preparation of the corresponding thiocarbonyl families (Scheme 2; $R^1 = H$ and Y = S) was a very difficult task: the required reagents (chlorothioformates, chlorodithioformates; for X = O, S) are not commercially available, nor easily prepared. Moreover, all our attempts to quench the anion derived from 2-azetidinone with carbondisulfide and an alkylating agent failed (Y = X = S), though this sequence of reaction has been successfully applied in the case of aliphatic amides, γ-, δ- and ε-lactams.[19] Reactions of 2-azetidinone with thiophosgene or thiocarbonyldiimidazole, followed by treatment with alcohols or thiols (Y = S; X = O, S) also gave untractable mixtures. Finally, in our hands, the only readily synthesized compounds were the thiocarbamate derivatives **16** and **17** (Scheme 2, Table 3) resulting from the reaction of 2-azetidinone with LiHMDS and phenyl- or benzylisothiocyanate. Similar conditions were used by Anderson during the synthesis of PSA inhibitors.[20] The thiocarbonyl function of **16–17** gave a typical line at 176–178 ppm in the ^{13}C NMR spectra.

Starting from racemic 4-(benzyloxy)carbonyl-2-azetidinone (Scheme 2, $R^1 = CO_2CH_2Ph$),[21] we prepared the N1-substituted β-lactams **18–21** (Table 3) by using the protocols validated above. A typical ABX pattern was visible in the ^{1}H NMR spectra around 3.0 δ, 3.3 δ and 4.5 δ corresponding to protons H3′, H3 and H4.

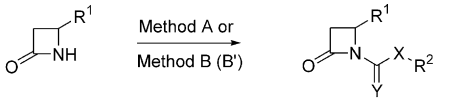
The chemical reactivity of β-lactam **19** has been assayed by ^{1}H NMR as described for β-lactam **9** (Fig. 3): the benzyl ester was hydrolyzed without opening of the β-lactam ring; the ABX pattern of protons H3, H3′ and H4 was still visible. The formed benzyl alcohol gave a typical singlet at 4.44 δ. Thus, the N1 thiolcarbamate function does not improve the β-lactam carbonyl reactivity versus the exocyclic ester function. The same result was previously obtained for β-lactam **18**.[13] Compounds **20** and **21** could not be similarly analyzed because their water solubility was to low. Enzymic processing of β-lactam **19** with PPE, followed by ^{1}H NMR, showed also the exocyclic ester hydrolysis.

β-Lactams **12–21** were evaluated against PPE as described for compounds **9–11**. Results, collected in Table 3 (K_i values), showed that the C4 unsubstituted derivatives are inactive, except the 1-(ethylthio)carbonyl β-lactam **13** which is very poorly active. On the other hand, all the compounds bearing a benzyl ester group at position C4 were active against PPE, the more active β-lactams being the sulfur-containing derivatives **19** and **21**. The inhibition was reversible (native enzyme activity was recovered after 1–2 h); the possible suicide mechanism outlined in Scheme 1 did not occur, most probably because the enzyme hydrolyzes the C4 ester group faster than the β-lactam function. This has been experimentally proved (^{1}H NMR) in the case of β-lactams **18**[13] and **19**, but not for compounds **20** and **21**. However, we could suppose that the same mechanism operates for compound **20** (Y = O) and that the differences of K_i values only reflect the goodness of fit of the N1 side chains into the enzymic pocket. On the other hand, the mode of action of azetidinone **21** (Y = S) could not be determined presently.

Table 3. Yields of compounds **12–21** and activity against PPE

R^1	Y	X	R^2	Compd (yield)	K_i (μM)[a]
H	O	O	C_2H_5	**12** (76%)	Inactive
H	O	S	C_2H_5	**13** (21%)	> 500
H	O	NH	Ph	**14** (74%)	Inactive
H	O	NH	CH_2Ph	**15** (96%)	Inactive
H	S	NH	Ph	**16** (52%)	Inactive
H	S	NH	CH_2Ph	**17** (50%)	Inactive
CO_2Bn	O	O	C_2H_5	**18** (78%)	160
CO_2Bn	O	S	C_2H_5	**19** (20%)	7
CO_2Bn	O	NH	CH_2Ph	**20** (74%)	> 500
CO_2Bn	S	NH	CH_2Ph	**21** (20%)	37

[a] See Table 1.



R^1 = H, CO_2Bn

Y = O, S ; X = O, S, NH

Method A : LiHMDS, -78°C then $ClCO_2R^2$ or $ClCOSR^2$
Method B : R^2NCO, Et_3N, DMAP
Method B' : LiHMDS, -78°C then R^2NCS

Scheme 2. Synthesis of N1-substituted β-lactams with ester, thiolester, amide and thioamide groups.

2.3. Inhibition of HLE

In view to confirm the inhibitory activities recorded with PPE, we evaluated representative compounds of both families (C4 modified substitution and N1 modified substitution) against HLE. The results collected in Table 4 are expressed as percentages of enzyme residual

activity. Thus, HLE was incubated with a good substrate (namely *N*-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide) and the tested inhibitor. The residual activity was obtained by comparison of the variation of the absorbance (410 nm, *p*-nitroaniline) in function of time, between this sample and the reference (sample without inhibitor). For water-solubility reasons, β-lactams **9**–**11** (first family) were assayed at a concentration of 6 μM, while the other β-lactams **12**–**19** (second family) were tested at 60 μM.

Similar experiments were performed with PPE (substrate = *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide) (Table 4). The results recorded with HLE and PPE were very similar, confirming that PPE is a valuable model of elastase. Except in the case of the sulfur-containing derivatives **13** and **19** (but not **17**), all β-lactams were slightly more active against HLE than PPE. This could be explained by structural differences in the close environment of the respective active sites; for instance, residue 192 corresponds to a phenyl-alanine in HLE and a glutamine in PPE.[4,5] This residue appeared to be in interaction with the N1 side chains when the inhibitors are placed in the enzymic cavity in such a way that serine 195 attacks the C4 ester substituent.[14]

2.4. Conclusion

Our results showed the potential interest of N1-(thio)-carbonyl-2-azetidinones as reactive structures for the design of novel elastase inhibitors. However, an electron-withdrawing substituent (activating group) placed at position C4 (or C3) was systematically required to reach a good level of enzyme inhibition. The nature of this group could dramatically change the mode of action of the azetidinone derivatives: a C4-ester substituent is more reactive towards nucleophilic attack than the β-lactam function itself, while a C4-amide substituent is less reactive. Thus compound **11** (Scheme 1, Table 1) is a good reversible inhibitor of PPE and HLE, acting most probably like 3-halogeno-1-(alkoxy) carbonyl-2-azetidinones (structure **A** in Fig. 2). Compound **19** (Scheme 2, Table 3) is more active against PPE and HLE, but behaves in the same way as 1,4-bis[(alkoxy)carbonyl]-2-azetidinones (structure **B** in Fig. 2). In this case, the nature of the potential leaving group (*S*-alkyl instead of *O*-alkyl) of the N1 side chain did not influence the mode of action, nor promote the suicide mechanism proposed in Figure 1.

Table 4. Biochemical evaluation

Compd (concn)	Residual activity (%)	
	PPE	HLE
9 (6 μM)	87	83
10 (6 μM)	92	78
11 (6 μM)	85	63
12 (60 μM)	100	89
13 (60 μM)	81	88
15 (60 μM)	100	73
17 (60 μM)	98	81
18 (60 μM)	51	37
19 (60 μM)	14	29

According to our theoretical evaluation, N1 substituents belonging to the thiocarbonyl family (Y = S; Table 2) should enhance the β-lactam reactivity versus the C4 ester group (ΔG of ester cleavage = 40.0–42.8 kcal/mol).[13] Docking experiments[14] showed that the good positioning of potential inhibitors required for the N1–C2 bond cleavage, instead of the C4-ester hydrolysis, is also well accessible. Actually, one representative of this family, the thioamide **21** (Y = S, X = NH; Scheme 2, Table 3), has been prepared and found to be active against PPE (K_i = 37 μM). We are now studying novel synthetic routes allowing the preparation of N1-thionoester (Y = S; X = O) and N1-dithioester derivatives (Y = X = S), since we could not carry out the direct *N*-functionalisation of a preformed azetidinone with CS_2-type reagents or synthetic equivalents.

3. Experimental

3.1. General

Reagents and solvents were purchased from Acros chimica, Aldrich or Fluka. Porcine pancreatic elastase (type 1), human leukocyte elastase and *N*-succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide were obtained from Sigma Chemical Co. *N*-Methoxysuccinyl-L-alanyl-L-alanyl-L-prolin-L-valine-*p*-nitroanilide was obtained from Calbiochem. Tetrahydrofuran was dried over sodium/benzophenone, then distilled. Column chromatographies were carried out with silica gel 60 (70–230 mesh ASTM) supplied by Merck. The IR spectra were recorded with a Perkin–Elmer 1710 instrument, only the most significant absorption bands being reported. The mass spectra were obtained with a Finnigan MAT TSQ-70 instrument. The microanalyses were performed at the Christopher Ingold Laboratories of the University College, London (Dr A. Stones). The melting points were determined with an Electrothermal microscope and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 300 (at 300 MHz for proton and 75 MHz for carbon) or Brucker AM-500 spectrometers (at 500 MHz for proton and 125 MHz for carbon); the chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (internal standard). The high resolution mass spectra were obtained at the University of Mons, Belgium (Prof. R. Flammang).

3.2. Synthesis

3.2.1. General procedure for 4-(alkylamino)carbonyl-1-(*tert*-butyldimethylsilyl)-2-azetidinones. A solution of **2**[15] (equiv) and CDI (1.2 equiv) in dry CH_2Cl_2 (10 mL/mmol) was stirred at room temperature for 30 min. Amine (1.2 equiv) was slowly added and stirring was continued for 24 h. The solution was washed with 1 N HCl. The aqueous layer was extracted with EtOAc. The combined extracts were dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting β-lactam was

purified by column chromatography on silica gel (CH_2Cl_2/EtOAc, 90/10 to 80/20).

3.2.1.1. 4 - (Benzylamino)carbonyl - 1 - (*tert* - butyldimethylsilyl)-2-azetidinone (3). Reaction of **2** (115 mg; 0.5 mmol) with CDI (97 mg; 0.6 mmol) and benzylamine (66 μL; 0.6 mmol) yielded **3** (108 mg, 68%) as a white solid; mp 130–131 °C; IR (KBr) 3429, 1744, 1674 cm^{-1}; ^1H NMR (500 MHz; $CDCl_3$) δ 0.09 and 0.22 (2×s, 2×3H, 2×$SiCH_3$), 0.90 (s, 9H, $C(CH_3)_3$), 2.98 (dd, J = 15.5 Hz, 3.0 Hz, 1H, H-3), 3.32 (dd, J = 15.5 Hz, 6.1 Hz, 1H, H-3′), 3.96 (dd, J = 6.1 Hz, 3.0 Hz, 1H, H-4), 4.44 (m, 2H, CH_2Ph), 6.55 (br s, 1H, NH), 7.34-7.42 (m, 5H, Ph); ^{13}C NMR (125 MHz; $CDCl_3$) δ −6.3 and −5.7 ($SiCH_3$), 18.5 ($C(CH_3)_3$), 26.1 ($C(CH_3)_3$), 43.6 and 44.5 (CH_2Ph and C-3), 50.3 (C-4), 127.9, 128.5 and 128.7 (CH_{Ar}), 137.5 (C_{Ar}), 171.1 and 171.7 (2×CO); MS (EI) m/z 318, 261, 185, 91. Anal. calcd for $C_{17}H_{26}N_2O_2Si$: C, 64.11; H, 8.23; N, 8.79. Found: C, 64.25; H, 8.25; N, 8.72%.

3.2.1.2. 4 - [(*N* - Methyl)benzylamino]carbonyl - 1 - (*tert* - butyldimethylsilyl)-2-azetidinone (4). Reaction of **2** (115 mg; 0.5 mmol) with CDI (97 mg; 0.6 mmol) and methylbenzylamine (78 μL; 0.6 mmol) yielded **4** (152 mg, 92%) as a white solid; mp 80–81 °C; IR (KBr) 1738, 1655, 1263 cm^{-1}; ^1H NMR (500 MHz; $CDCl_3$) δ 0.12 and 0.35 (2×s, 2×3H, 2×$SiCH_3$), 0.98 (s, 9H, $C(CH_3)_3$), 2.86 (s, 3H, CH_3), 2.89 (dd, J = 14.7 Hz, 3.2 Hz, 1H, H-3), 3.34 (dd, J = 14.7 Hz, 6.2 Hz, 1H, H-3′), 4.32 (dd, J = 6.2 Hz, 3.2 Hz, 1H, H-4), 4.53 and 4.63 (2×d, J = 14.8 Hz, 2×1H, CH_2Ph), 7.20–7.31 (m, 5H, Ph); ^{13}C NMR (125 MHz; $CDCl_3$) δ −6.5 and −5.8 ($SiCH_3$), 18.4 ($C(CH_3)_3$), 26.3 ($C(CH_3)_3$), 33.6 (CH_3), 43.3 (C-3), 47.8 (C-4), 51.1 (CH_2Ph), 127.5, 127.9 and 128.5 (CH_{Ar}), 136.5 (C_{Ar}), 170.0 and 170.5 (2×CO); MS (EI) m/z 332, 275, 149, 91. Anal. calcd for $C_{18}H_{28}O_2N_2Si$: C, 65.02; H, 8.49; N, 8.42. Found: C, 65.33; H, 8.48; N, 8.22%.

3.2.1.3. 4 - (Dibenzylamino)carbonyl - 1 - (*tert* - butyldimethylsilyl)-2-azetidinone (5). Reaction of **2** (115 mg; 0.5 mmol) with CDI (97 mg; 0.6 mmol) and dibenzylamine (115 μL; 0.6 mmol) yielded **5** (54 mg, 27%) as a colourless oil; IR (film) 1749, 1653, 1264 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 0.12 and 0.35 (2×s, 2×3H, 2×$SiCH_3$), 0.98 (s, 9H, $C(CH_3)_3$), 3.14 (dd, J = 15.2 Hz, 3.0 Hz, 1H, H-3), 3.34 (dd, J = 15.2 Hz, 6.1 Hz, 1H, H-3′), 4.32 (dd, J = 6.1 Hz, 3.0 Hz, 1H, H-4), 4.63 (m, 4H, 2×CH_2Ph), 7.20–7.31 (m, 5H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$;Me_4Si) δ −6.6 and −5.5 ($SiCH_3$), 18.6 ($C(CH_3)_3$), 26.1 ($C(CH_3)_3$), 43.2 (C-3), 47.9 (C-4), 51.1 and 52.9 (2×CH_2Ph), 127.5, 127.8, 127.9 and 128.3 (CH_{Ar}), 136.5 and 138.2 (C_{Ar}), 170.1 and 170.3 (2×CO); MS (CI) m/z 409 ($C_{24}H_{32}N_2O_2Si$), 302, 91.

3.2.2. General procedure for 4-(alkylamino)carbonyl-2-azetidinones (*N*-deprotection). A solution of *N*-silylated azetidin-2-one (1 equiv) and cesium fluoride (1.5 equiv) in dry DMF (5 mL/mmol) was stirred for 2 h at room temperature. The crude mixture was concentred and after addition of EtOAc, the organic layer was washed twice with water, dried over $MgSO_4$, filtered and concentrated under vacuum. The deprotected β-lactam was directly used in the acylation reaction without purification.

3.2.2.1. 4 - (Benzylamino)carbonyl - 2 - azetidinone (6). Reaction of **3** (190 mg; 0.6 mmol) and cesium fluoride (137 mg; 0.9 mmol) yielded the *N*-deprotected azetidin-2-one (64 mg, 50%) as a colourless oil; IR (film) 3225, 1734, 1670 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 3.01 (dd, J = 15.3 Hz, 2.8 Hz, 1H, H-3), 3.34 (dd, J = 15.3 Hz, 6.1 Hz, 1H, H-3′), 4.15 (dd, J = 6.1 Hz, 2.8 Hz, 1H, H-4), 4.42 (m, 2H, CH_2Ph), 6.30 and 6,65 (2×br s, 2×1H, 2×NH), 7.25–7.40 (m, 5H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ 42.8 (C-3), 45.6 (CH_2Ph), 47.9 (C-4), 126.2, 127.5 and 128.6 (CH_{Ar}), 136.3 (C_{Ar}), 166.1 and 169.9 (2×CO).

3.2.2.2. 4-[(*N*-Methyl)benzylamino]carbonyl-2-azetidinone (7). Reaction of **4** (332 mg; 1 mmol) and cesium fluoride (228 mg; 1.5 mmol) yielded *N*-deprotected azetidin-2-one (117 mg, 54%) as a colourless oil; IR (film) 3245, 1772, 1654 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 2.90 (s, 1H, CH_3), 3.06 (m, 2H, H-3 and H-3′), 4.52 (m, 3H, H-4 and CH_2Ph), 7.10 (br s, 1H, NH), 7.15–7.48 (m, 5H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ 33.6 (CH_3), 42.9 (C-3), 47.1 (C-4), 51.1 (CH_2Ph), 126.2, 127.5 and 128.6 (CH_{Ar}), 136.3 (C_{Ar}), 166.1 and 169.9 (2×CO).

3.2.2.3. 4-(Dibenzylamino)carbonyl-2-azetidinone (8). Reaction of **5** (111 mg; 0.3 mmol) and cesium fluoride (60 mg; 0.4 mmol) yielded *N*-deprotected azetidin-2-one (45 mg, 55%) as a colourless oil; IR (film) 1748, 1685 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 3.01 (dd, J = 15.2 Hz, 2.7 Hz, 1H, H-3), 3.19 (dd, J = 15.2 Hz, 6.1 Hz, 1H, H-3′), 4.48 (m, 5H, 2×CH_2Ph and H-4), 6.25 (br s, 1H, NH), 7.20–7.31 (m, 10H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ 43.5 (C-3), 47.1 (C-4), 48.8 and 49.3 (2×CH_2Ph), 126.4, 127.5, 127.8, 127.9, 128.1 and 128.3 (CH_{Ar}), 135.4 and 136.5 (2×C_{Ar}), 165.8 and 170.4 (2×CO).

3.2.3. General procedure for 1-(phenethyloxy)carbonyl-4-(alkylamino)carbonyl-2-azetidinones. A solution of LiHMDS (1 equiv) in dry THF (5 mL/mmol) was added dropwise to a solution of *N*-deprotected azetidinone (1 equiv) in dry THF (5 mL/mmol), cooled at −78 °C under argon atmosphere. The mixture was stirred for 30 min at −78 °C, then phenethyl chloroformate (1 equiv) was added with a syringe through a rubber stopper. Stirring was continued for 1 h at this temperature and the mixture was allowed to reach room temperature and further stirred for 45 min at 20 °C. After addition of water and extraction with CH_2Cl_2, the organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting β-lactam was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc, 90/10).

3.2.3.1. 1 - (Phenethyloxy)carbonyl - 4 - (benzylamino)-carbonyl-2-azetidinone (9). Yield from 4-(benzylamino)-carbonyl-2-azetidinone (64 mg; 0.3 mmol), LiHMDS (72 mg; 0.3 mmol) and phenethyl chloroformate (55 mg; 0.3 mmol): 75 mg (71%) as a colourless oil; IR (film) 3367, 1819, 1731, 1684 cm^{-1}; ^1H NMR (500 MHz; $CDCl_3$) δ 2.96 (t, $J = 7.3$ Hz, 2H, CH_2CH_2Ph), 3.19 (dd, $J = 16.2$ Hz, 6.7 Hz, 1H, H-3), 3.29 (dd, $J = 16.2$ Hz, 3.7 Hz, 1H, H-3′), 4.35 (dd, $J = 6.7$ Hz, 3.7 Hz, 1H, H-4), 4.39 (t, $J = 7.3$ Hz, 2H, CH_2CH_2Ph), 4.42 (dd, $J = 14.6$ Hz, 5.9 Hz, 1H, CH_2Ph), 4.47 (dd, $J = 14.6$ Hz, 5.9 Hz, 1H, CH_2Ph), 7.03 (br s, 1H, NH), 7.18–7.38 (m, 10H, Ph); ^{13}C NMR (125 MHz; $CDCl_3$) δ 34.8 (CH_2CH_2Ph), 41.1 (C-3), 43.6 (CH_2Ph), 51.3 (C-4), 67.5 (CH_2CH_2Ph), 126.7, 127.5, 128.5, 128.6 and 128.9 (CH_{Ar}), 136.7 and 137.4 (C_{Ar}), 149.5 (CO carbamate), 162.8 (CO azetidinone), 167.4 (CO amide); MS (CI) m/z 353 (M + 1), 243, 105; HRMS (CI) calcd for $C_{20}H_{21}N_2O_4$: 353.1501. Found: 353.1489.

3.2.3.2. 1 - (Phenethyloxy)carbonyl - 4 - [(*N* - methyl)benzylamino]carbonyl-2-azetidinone (10). Yield from 4-[(*N*-methyl)benzylamino]carbonyl-2-azetidinone (153 mg; 0.6 mmol), LiHMDS (145 mg; 0.6 mmol) and phenethyl chloroformate (110 mg; 0.6 mmol): 176 mg (81%) as a colourless oil; IR (film) 1818, 1731, 1662 cm^{-1}; ^1H NMR (500 MHz; $CDCl_3$) major rotamer (values in parentheses correspond to the minor rotamer) δ 2.95 (s, 3H, CH_3) (3.05), 3.03 (t, $J = 7.2$ Hz, 2H, CH_2CH_2Ph), 3.06 (dd, $J = 15.4$ Hz, 3.2 Hz, 1H, H-3), 3.24 (dd, $J = 15.4$ Hz, 6.4 Hz, 1H, H-3′), 4.35 (dd, $J = 6.4$ Hz, 3.2 Hz, 1H, H-4), 4.43 (t, $J = 7.2$ Hz, 2H, CH_2CH_2Ph), 4.60 (d, $J = 14.7$ Hz, 1H, CH_2Ph) (4.50), 4.65 (d, $J = 14.7$, 1H, CH_2Ph), 7.11–7.45 (m, 10H, Ph); ^{13}C NMR (125 MHz; $CDCl_3$) major rotamer (values in parentheses correspond to the minor rotamer) δ 34.0 (CH_3) (34.8), 34.9 (CH_2CH_2Ph), 41.2 (C-3) (41.3), 47.5 (C-4) (47.4), 51.4 (CH_2Ph), 67.2 (CH_2CH_2Ph), 125.9, 126.6, 127.6, 127.9, 128.4, 128.7, 128.9 and 129.0 (CH_{Ar}), 136.2 and 136.9 (C_{Ar}), 148.5 (CO carbamate), 161.8 (CO azetidinone) (161.9), 167.6 (CO amide) (168.1); MS (CI) m/z 367 (M + 1), 105, 91; HRMS (CI) calcd for $C_{21}H_{23}N_2O_4$: 367.1657. Found: 367.1660.

3.2.3.3. 1-(Phenethyloxy)carbonyl-4-(dibenzylamino)-carbonyl-2-azetidinone (11). Yield from 4-(dibenzylamino)carbonyl-2-azetidinone (45 mg; 0.15 mmol), LiHMDS (37 mg; 0.15 mmol) and phenethyl chloroformate (29 mg; 0.15 mmol): 49 mg (74%) as a colourless oil; IR (film) 1819, 1732, 1663 cm^{-1}; ^1H NMR (500 MHz; $CDCl_3$) δ 2.93 (dd, $J = 16.4$ Hz, 5.7 Hz, 1H, H-3), 2.99 (dd, $J = 16.4$ Hz, 3.7 Hz, 1H, H-3′), 3.01 (t, $J = 7.4$ Hz, 2H, CH_2CH_2Ph), 4.40 (d, $J = 17.0$ Hz, 1H, CH_2Ph), 4.43 (t, $J = 7.4$ Hz, 2H, CH_2CH_2Ph), 4.54 (d, $J = 17.0$ Hz, 1H, CH_2Ph), 4.64 (dd, $J = 5.7$ Hz, 3.7 Hz, 1H, H-4), 4.65 (d, $J = 14.6$ Hz, 1H, CH_2,Ph), 4.72 (d, $J = 14.6$ Hz, 1H, CH_2,Ph), 7.11–7.45 (m, 15H, Ph); ^{13}C NMR (125 MHz; $CDCl_3$) δ 34.9 (CH_2CH_2Ph), 41.6 (C-3), 47.5 (C-4), 49.6 (2×CH_2Ph), 67.2 (CH_2CH_2Ph), 126.6, 127.6, 127.9, 128.1, 128.2, 128.4, 128.7, 128.9 and 129.1 (CH_{Ar}), 135.6, 136.3 and 136.9 (C_{Ar}), 148.4 (CO carbamate), 161.8 (CO azetidinone), 168.5 (CO amide); MS (CI) m/z 443 (M + 1), 105, 91; HRMS (CI) calcd for $C_{27}H_{27}N_2O_4$: 443.1970. Found: 443.1987.

3.2.4. General procedure for 1-ethyloxy(thio)carbonyl-2-azetidinones. A solution of LiHMDS (1 equiv) in dry THF (5 mL/mmol) was dropwise added to a solution of azetidinone (1 equiv) in dry THF (5 mL/mmol), cooled at −78 °C under argon atmosphere. The mixture was stirred for 30 min at −78 °C, then ethylchloroformate or thiochloroformate (1.2 equiv) was added with a syringe through a rubber stopper. Stirring was continued for 1 h at this temperature and the mixture was allowed to reach room temperature and stirred for 45 min at 20 °C. After addition of water and extraction with CH_2Cl_2, the organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting β-lactam was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc, 90/10 to 70/30).

3.2.4.1. 1 - (Ethyloxy)carbonyl - 2 - azetidinone (12). Reaction of 2-azetidinone (71 mg; 1 mmol), LiHMDS (241 mg; 1 mmol) and ethyl chloroformate (96 μL; 1.2 mmol) yielded **12** (109 mg, 76%) as a yellow oil; IR (film) 2927, 1802, 1717 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, $J = 7.1$ Hz, 3H, CH_3), 3.06 (t, $J = 5.2$ Hz, 2H, H-4), 3.64 (t, $J = 5.2$ Hz, 2H, H-3), 4.30 (q, $J = 7.1$ Hz, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3 (CH_3), 36.5 and 37.7 (C-3 and C-4), 62.7 (OCH_2), 149.3 (CO carbamate), 164.1 (CO azetidinone); MS (CI) m/z 144 (M + 1, $C_6H_{10}NO_3$), 116, 98.

3.2.4.2. 1-(Ethyloxy)thiocarbonyl-2-azetidinone (13). Reaction of 2-azetidinone (71 mg; 1 mmol), LiHMDS (241 mg; 1 mmol) and ethyl thiochloroformate (104 μL; 1.2 mmol) yielded **13** (33 mg, 21%) as a white solid; mp 71–72 °C; IR (KBr) 2959, 1791, 1653 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.33 (t, $J = 7.4$ Hz, 3H, CH_3), 3.01 (q, $J = 7.4$ Hz, 2H, CH_2), 3.09 (t, $J = 5.3$ Hz, 2H, H-4), 3.69 (t, $J = 5.3$ Hz, 2H, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7 (CH_3), 23.6 (SCH_2), 36.1 and 38.1 (C-3 and C-4), 163.8 (CO azetidinone), 165.5 (CO thiocarbamate); MS (EI) m/z 159 ($C_6H_9NO_2S$), 98, 89, 70.

3.2.4.3. 1-(Ethyloxy)carbonyl-4-(benzyloxy)carbonyl-2-azetidinone (18). This compound was obtained from 4-(benzyloxy)carbonyl-2-azetidinone (205 mg; 1 mmol) by treatment with LiHMDS (241 mg; 1 mmol) and ethyl chloroformate (96 μL; 1.2 mmol) to yield **18** (215 mg, 78%) as a yellow oil; IR (film) 2959, 1819, 1730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, $J = 7.2$ Hz, 3H, CH_3), 3.03 (dd, 1H, $J = 15.9$ Hz, 3.3 Hz, H-3′), 3.31 (dd, 1H, $J = 15.9$ Hz, 6.6 Hz, H-3), 4.26 (q, $J = 7.2$ Hz, 2H, CH_2), 4.48 (dd, 1H, $J = 6.6$ Hz, 3.3 Hz, H-4), 5.27 (AB pattern, 2H, $J_{AB} = 13.6$ Hz, CH_2Ph), 7.36–7.41 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (CH_3), 41.6 (C-3), 49.4 (C-4), 63.1 (OCH_2), 67.6 (CH_2Ph), 128.3, 128.6 and 128.7 (CH_{Ar}), 134.9 (C_{Ar}), 148.3 (CO carbamate), 161.6 (CO azetidinone), 168.8 (CO ester); MS (CI) m/z 278 (M + 1), 250, 144, 91. Anal. calcd for $C_{14}H_{15}NO_5$:

C, 60.64; H, 5.45; N, 5.05. Found: C, 60.31; H, 5.51; N, 5.06%.

3.2.4.4. 1 - (Ethyloxy)thiocarbonyl - 4 - (benzyloxy)-carbonyl-2-azetidinone (19). This compound was obtained from 4-(benzyloxy)carbonyl-2-azetidinone (205 mg; 1 mmol) by treatment with LiHMDS (241 mg; 1 mmol) and ethyl thiochloroformate (104 μL; 1.2 mmol) to yield **19** (61 mg, 20%) as a yellow oil; IR (film) 2960, 1796, 1751, 1667 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, $J = 7.4$ Hz, 3H, CH_3), 2.98 (q, $J = 7.4$ Hz, 2H, CH_2), 3.01 (dd, 1H, $J = 16.1$ Hz, 3.5 Hz, H-3′), 3.31 (dd, 1H, $J = 16.1$ Hz, 6.6 Hz, H-3), 4.54 (dd, 1H, $J = 6.6$ Hz, 3.5 Hz, H-4), 5.23 (AB pattern, 2H, $J_{AB} = 12.3$ Hz, CH_2Ph), 7.29–7.38 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (CH_3), 23.8 (SCH_2), 41.0 (C-3), 49.6 (C-4), 67.7 (CH_2Ph), 128.2, 128.6 and 128.7 (CH_{Ar}), 134.9 (C_{Ar}), 161.4 (CO azetidinone), 165.0 (CO thiocarbamate), 168.4 (CO ester); MS (CI) m/z 294 (M + 1, $C_{14}H_{16}NO_4S$), 232, 91.

3.2.5. General procedure for 1-(alkylamino)carbonyl-2-azetidinones. To a solution of azetidinone (1 equiv) and DMAP (0.2 equiv) in dry CH_2Cl_2 (5 mL/mmol), under argon atmosphere, were successively added dropwise isocyanate (1.1 equiv) and TEA (0.4 equiv). The mixture was stirred and heated to reflux for 24 h. The crude mixture was then washed successively with a saturated solution of $NaHCO_3$, 1.2 M HCl and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting β-lactam was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc, 95/5).

3.2.5.1. 1-(Phenylamino)carbonyl-2-azetidinone (14). Reaction of 2-azetidinone (108 mg; 1.5 mmol), DMAP (24 mg; 0.3 mmol), phenyl isocyanate (179 μL; 1.65 mmol) and TEA (84 μL; 0.60 mmol) yielded **14** (210 mg, 74%) as a white solid; mp 112–113 °C; IR (KBr) 3381, 2964, 1767, 1714 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.16 (t, $J = 4.8$ Hz, 2H, H-4), 3.76 (t, $J = 4.8$ Hz, 2H, H-3), 7.05–7.52 (m, 5H, Ph), 8.43 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.9 and 37.2 (C-3 and C-4), 119.4, 124.0 and 128.9 (CH_{Ar}), 136.9 (C_{Ar}), 147.6 (CO urea), 167.1 (CO azetidinone); MS (CI) m/z 191 (M + 1), 149, 98, 94, 72. Anal. calcd for $C_{10}H_{10}N_2O_2$: C, 63.14; H, 5.30; N, 14.72. Found: C, 62.73; H, 5.17; N, 14.42%.

3.2.5.2. 1-(Benzylamino)carbonyl-2-azetidinone (15). Reaction of 2-azetidinone (53 mg; 0.75 mmol), DMAP (12 mg; 0.15 mmol), benzyl isocyanate (102 μL; 0.85 mmol) and TEA (42 μL; 0.40 mmol) yielded **15** (148 mg, 96%) as a colourless oil; IR (film) 3370, 2985, 1762, 1721 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.07 (t, $J = 5.3$ Hz, 2H, H-4), 3.67 (t, $J = 5.3$ Hz, 2H, H-3), 4.49 (d, $J = 6.7$ Hz, 2H, CH_2Ph), 6.82 (br s, 1H, NH), 7.28–7.42 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36.0 and 37.2 (C-3 and C-4), 43.6 (CH_2Ph), 127.5, 127.6 and 128.7 (CH_{Ar}), 137.8 (C_{Ar}), 149.8 (CO urea), 167.0 (CO azetidinone); MS (CI) m/z 205 (M + 1, $C_{11}H_{13}N_2O_2$), 91, 70.

3.2.5.3. 1-(Benzylamino)carbonyl-4-(benzyloxy)carbonyl-2-azetidinone (20). This compound was obtained from 4-(benzyloxy)carbonyl-2-azetidinone (205 mg; 1 mmol) by treatment with DMAP (16 mg; 0.2 mmol), benzyl isocyanate (68 μL; 1.1 mmol) and TEA (56 μL; 0.4 mmol) to yield **20** (322 mg, 95%) as a colourless oil; IR (film): 3383, 3052, 1782, 1715 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.98 (dd, 1H, $J = 10.6$ Hz, 2.1 Hz, H-3′), 3.25 (dd, 1H, $J = 10.6$ Hz, 4.2 Hz, H-3), 4.48 (m, 3H, H-4 and $NHCH_2$), 5.21 (AB pattern, 2H, $J_{AB} = 8.8$ Hz, CH_2Ph), 6.72 (br s, 1H, NH), 7.22–7.41 (m, 10H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 40.6 (C-3), 43.3 ($NHCH_2$), 48.3 (C-4), 67.2 (CH_2Ph), 127.1, 127.2, 127.8, 128.1, 128.2 and 128.5 (CH_{Ar}), 134.6 and 137.5 (C_{Ar}), 149.1 (CO urea), 164.3 (CO azetidinone), 168.8 (CO ester); MS (FAB) m/z 339 ($C_{19}H_{19}N_2O_4$), 247, 135, 107, 91.

3.2.6. General procedure for 1-(alkylamino)thiocarbonyl-2-azetidinones. A solution of LiHMDS (1 equiv) in dry THF (5 mL/mmol) was dropwise added to a solution of azetidinone (1 equiv) in dry THF (5 mL/mmol), cooled at −78 °C under argon atmosphere. The mixture was stirred for 30 min at −78 °C, then isothiocyanate (1.1 equiv) was slowly added. Stirring was continued for 30 min at this temperature and the mixture was allowed to reach room temperature and stirred for 4 h at 20 °C. After addition of a solution of saturated NH_4Cl and extraction with CH_2Cl_2, the organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting β-lactam was purified by column chromatography on silica gel (CH_2Cl_2).

3.2.6.1. 1-(Phenylamino)thiocarbonyl-2-azetidinone (16). Reaction of 2-azetidinone (108 mg; 1.5 mmol), LiHMDS (360 mg; 1.5 mmol) and phenyl isothiocyanate (197 μL; 1.65 mmol) yielded **16** (160 mg, 52%) as a white solid; mp 85–86 °C; IR (KBr) 3281, 1756, 1326 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.09 (t, $J = 5.0$ Hz, 2H, H-4), 3.88 (t, $J = 5.0$ Hz, 2H, H-3), 7.21–7.49 (m, 5H, Ph), 10.2 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.4 and 40.2 (C-3 and C-4), 123.3, 126.3 and 128.7 (CH_{Ar}), 137.1 (C_{Ar}), 165.2 (CO azetidinone), 175.7 (CO thiourea); MS (CI) m/z 207 (M + 1), 136, 70. Anal. calcd for $C_{10}H_{10}N_2OS$: C, 58.23; H, 4.88; N, 13.59; S, 15.54. Found: C, 58.19; H, 4.77; N, 14.14; S, 15.30%.

3.2.6.2. 1-(Benzylamino)thiocarbonyl-2-azetidinone (17). Reaction of 2-azetidinone (108 mg; 1.5 mmol), LiHMDS (360 mg; 1.5 mmol) and benzyl isothiocyanate (219 μL; 1.65 mmol) yielded **17** (156 mg, 50%) as a yellow solid; mp 76–77 °C; IR (KBr) 3326, 1759, 1315 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.01 (t, $J = 4.9$ Hz, 2H, H-4), 3.81 (t, $J = 4.9$ Hz, 2H, H-3), 4.85 (d, $J = 5.7$ Hz, 2H, CH_2Ph), 7.34–7.48 (m, 5H, Ph), 8.72 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.4 and 40.2 (C-3 and C-4), 48.3 (CH_2Ph), 127.5, 127.6 and 128.7 (CH_{Ar}), 137.8 (C_{Ar}), 165.0 (CO azetidinone), 178.1 (CO thiourea); MS (CI) m/z 221 (M + 1), 91, 70. Anal. calcd for $C_{11}H_{12}N_2OS$: C, 59.98; H, 5.49; N, 12.73; S, 14.55. Found: C, 59.77; H, 5.39; N, 12.69; S, 14.93%.

3.2.6.3. 1-(Benzylamino)thiocarbonyl-4-(benzyloxy)-carbonyl-2-azetidinone (21). This compound was obtained from 4-(benzyloxy)carbonyl-2-azetidinone (205 mg; 1 mmol) by treatment with LiHMDS (241 mg; 1 mmol) and benzyl isothiocyanate (146 μL; 1.1 mmol) to yield **21** (64 mg, 20%) as a yellow oil; IR (film) 3336, 1775, 1751, 1269 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.95 (dd, 1H, $J = 15.7$ Hz, 3.2 Hz, H-3′), 3.22 (dd, 1H, $J = 15.7$ Hz, 6.1 Hz, H-3), 4.66 (dd, 1H, $J = 6.1$ Hz, 3.2 Hz, H-3′), 4.81 (d, $J = 5.6$ Hz, 2H, $NHCH_2$), 5.28 (AB pattern, 2H, $J_{AB} = 12.4$ Hz, CH_2Ph), 7.26–7.39 (m, 10H, Ph), 8.52 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.5 (C-3), 48.7 (C-4), 51.4 ($NHCH_2$), 67.7 (CH_2Ph), 127.8, 127.9, 128.1, 128.2, 128.6 and 128.7 (CH_{Ar}), 134.8 and 136.1 (C_{Ar}), 163.0 (CO azetidinone), 168.8 (CO ester), 177.1 (CO thiourea); MS (CI) m/z 355 (M + 1, $C_{19}H_{19}N_2O_3S$), 150, 91.

3.3. Theoretical calculations

All the calculations have been performed at the ab initio RHF level using the minimal basis set MINI-1′. They were performed with Gaussian 98 on two computers, a Dec Alpha 8400 8-processor and a Dec Alpha 4100 4-processor running Digital Unix.[22] The starting geometries were sketched drawn by standard fragments and then completely optimized following all the 3N-6 degrees of freedom either for the minima or the transition state structures. For each equilibrium structure, the thermochemistry data are derived from the analytical frequency calculation at 298.15 K and 1 atm.

3.4. Assay of PPE (porcine pancreatic elastase)

To 2 mL of the solution of substrate (solution of *N*-succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide [2.7 mg in 200 μL of *N*-methyl pyrrolidone (NMP) diluted with TRIS buffer (20 mL at 100 mM, pH 7.5 solution)] were added 20 μL of the solution of the tested compound (10^{-2}–10^{-4} M in NMP) and 67 μL of the solution of elastase [6×10^{-6} M in acetate buffer (50 mM, pH 5)]. The appearance of the substrate hydrolysis product (*p*-nitroaniline) was measured (with a Cary 210 spectrophotometer) at 410 nm as a function of time.

Plots of V/V_I versus [I] (ratios of initial rates of hydrolysis in the absence and in the presence of inhibitors) gave the inhibition constants indicated in Tables 1 and 3. All experiments were performed two or three times.

The reversibility of the inhibition was controlled by the incubation/dilution method. The incubation solution consisted of 5 μL of inhibitor solution (10^{-3} M in DMSO), 34 μL of elastase solution (2.5×10^{-4} M in acetate buffer, 50 mM, pH 5) and 161 μL of Tris buffer (100 mM, pH 7.5). At various times (0, 5, 10, 15, 20, 30 min), 10 μL of the incubation solution were dissolved in 2 mL of the substrate solution (10^{-4} M in TRIS buffer), and the enzyme activity was measured as before. No decrease of activity was recorded as a function of incubation time.

3.5. Assay of HLE (human leukocyte elastase)

To 5 μL of the solution of substrate [solution of methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (50 mM in DMSO)] diluted with 490 μL of buffer (Tris–HCl 0.1 M, pH 7.5 and NaCl 0.5 M solution) were added 5 μL of the solution of elastase (in Tris–HCl and NaCl buffer) and 3 μL of the solution of the tested compound (10^{-2} M in DMSO). The appearance of the substrate hydrolysis product (*p*-nitroaniline) was measured (with a Cary 210 spectrophotometer) at 410 nm as a function of time and the residual activity was obtained by comparison with the variation of the absorbance of a reference (sample without inhibitor).

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