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Synthesis, Reactivity and Biochemical Evaluation of 1,3-Substituted Azetidin-2-ones as Enzyme Inhibitors

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Abstract : A series of monocyclic azetidinones were prepared, bearing, at position C-3, an acetylamino or a bromo substituent, at position N-1, a carboxymethyl group protected as p-nitrobenzyl ester (PNB) and α-functionalized with a potential leaving group (LG). These structures were designed as potential suicide-inhibitors of enzymes containing a serine nucleophile in their active site. The β-lactam ring of these molecules was found to be stable in phosphate buffer (pH 7.5), but the PNB ester was rapidly cleaved. This constitutes a practical method of *in situ* deprotection. Depending on the nature of the LG group on the carboxymethyl chain, substitution of this group (LG = F) or decarboxylation (LG = SO_2Ph) was observed under hydrolytic conditions. The 1,3-disubstituted azetidinones were inactive against β-lactamases of classes A, B, C, and D. Three compounds behaved as weak reversible inhibitors of porcine pancreatic elastase (PPE).

Keywords : monocyclic β-lactam, PNB ester hydrolysis, suicide-inhibition, β-lactamase, elastase.

INTRODUCTION

Since the discovery of penicillin and cephalosporin antibiotics [1], the β-lactam ring (azetidin-2-one) is considered as a general lead-structure for the design of new inhibitors of enzymes containing an essential serine nucleophile in their active site [2-5]. Presently, the most important medicinal targets are elastases [6] and β-lactamases [7,8], whose inactivation is observed in the presence of adequately functionalized monocyclic azetidinones. In the inhibitors, the azetidinone ring is equipped with substituents required for specific enzyme recognition (Z,Z'), on the one hand, and for chemical activation of the lactam bond towards nucleophilic attack (EWG), on the other hand (Scheme 1). Moreover, the presence of a potential leaving group (LG) is generally planned for promoting suicide-type irreversible inhibition [9]. Accordingly, monocyclic β-lactams considered as potential β-lactamase inhibitors have been heterofunctionalized at positions C-4 [10,11], or N-1 [12]; bridged sulfactams (N-1 substituent = OSO_3H) were recently found to be effective inhibitors of class A and class C β-lactamases [13].

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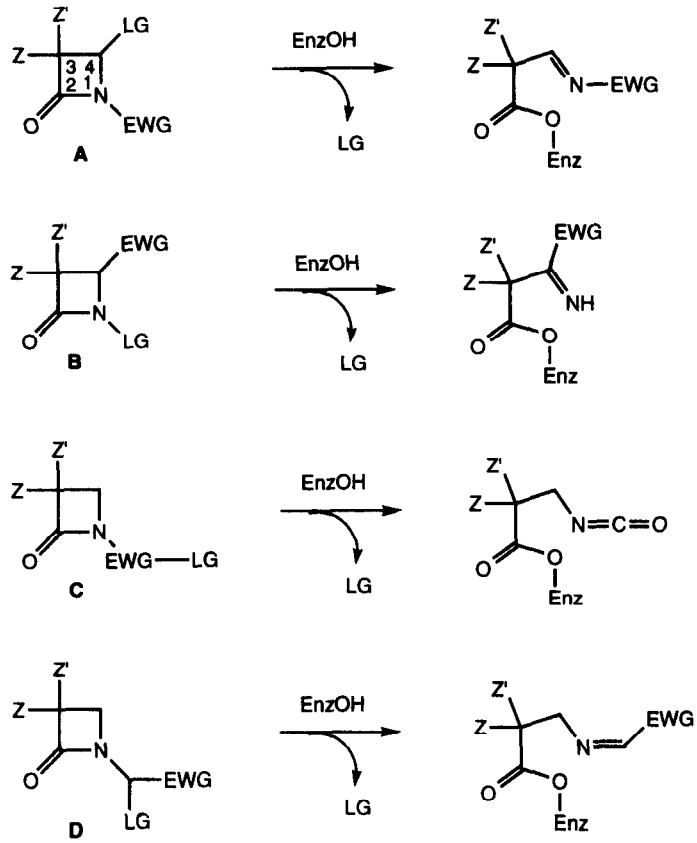


Z,Z' : substituents for specific recognition by elastases or β-lactamases
EWG : electron - withdrawing group (Ar, CO, SO_2) for chemical activation of the lactam bond
LG : leaving group (OR, $S(O)_nR$, F) designed to promote irreversible inhibition

Scheme 1

N-aryl azetidinones susceptible to release of a latent quinonimmonium methide function into the enzymatic cavity on β-lactam cleavage were proposed by Wakselman *et al.* as human leukocyte elastase (HLE) inhibitors [14,15]. Other suicide-inhibitors were constructed following the design outlined in the general structures **A** and **B** (Scheme 1) [16,17]. Processing of such β-lactams by the target enzyme (*i.e.* nucleophilic attack of the active serine onto the azetidinone carbonyl) creates a Schiff base (after elimination of the leaving group) that could be quenched by a nucleophilic residue of the active site. This strategy led to the discovery of potent, orally active, HLE inhibitors such as L-680,833 [18] and L-694,458 [19], corresponding to structure **A** in which LG = O-Aryl, EWG = CONH-CHR'-Aryl, Z and Z' = Et; the loss of the aryloxy moiety in the enzymatic cavity has been experimentally proved [20]. HLE Inhibitors related to structure **B** (Scheme 1), in which LG = SO_2Aryl, EWG = CO_2R, Z = Et and Z' = H, were also disclosed [21]; but in this case, the potential leaving group was not expelled from the acyl-enzyme intermediate [22].

Recently, we considered the possibility of putting the EWG and LG substituents together in position N-1. The first molecules prepared were 1-alkoxycarbonyl-3-bromoazetidin-2-ones, corresponding to structure **C** (Scheme 1) in which EWG-LG = CO-OR, Z = Br and Z' = H [23]. We hypothesised that, on ring opening by a

serine-enzyme, the loss of the leaving group could release a highly electrophilic cumulene (*i.e.* an isocyanate function), leading to a covalent inhibitor-enzyme complex by reaction with a nucleophilic residue in the active site. In fact, β-lactams **C** behaved as transient inhibitors of the porcine pancreatic elastase (PPE). In control experiments on the chemical reactivity, we found that basic hydrolysis led to the cleavage of the lactam ring, but not of the urethane bond [23]. Thus, the design of β-lactams which will form stable complexes (*via* a suicide mechanism or not) with serine-enzymes appears to remain a difficult task [22].

In this paper, we describe another approach for combining the EWG and LG substituents on the azetidinone nitrogen atom. According to the general structure **D** (Scheme 1), we have considered substituting the N-1 position with various α-heterofunctionalized carboxymethyl chains (LG = F, OR, SR, $S(O)_nR$ and EWG = CO_2R); in such compounds, β-lactam ring opening could unmask a reactive Schiff base into the enzymatic cavity of serine-proteases. The recognition selectivity would mainly depend on the nature and orientation of the C-3 substituent, and the nature of the EWG group. Potential β-lactamase inhibitors feature a short (3*S*)-acylamino chain, and a free carboxylic acid as EWG group (for interaction with carboxypeptidases [24]), while potential elastase inhibitors contain a (3*R*)-bromo substituent (or eventually a short (3*R*)-acylamino chain), and a lipophilic ester as EWG group (for interaction with endopeptidases [25]). This paper deals with the synthesis, the chemical reactivity, and the inhibition potential of a series of monocyclic β-lactams of general structure **D** (Scheme 1).

RESULTS

Synthesis

3-(*t*-Butyloxycarbonyl)aminoazetidin-2-one **1** was used as starting material [26]; the (*S*) and (*R*)-enantiomers could be prepared in five steps from (*L*) and (*D*)-serine, respectively. Most of our syntheses were conducted in both enantiomeric series, but for conciseness, we will describe here the reactions concerning the 3-(*S*) enantiomers (Scheme 2).

Condensation of **1** with *p*-nitro-[27,28] or *p*-methoxybenzylglyoxylate [29] gave the N-hydroxyacetyl derivatives **2a** or **2b** [28,30] as approximately 60 : 40 mixtures of diastereoisomers, according to the ^1H NMR analysis. The substitution of the C-5 hydroxyl group with fluorine was performed with diethylaminosulfur trifluoride (DAST) in dichloromethane at low temperature; the resulting fluoride derivatives **3a** or **3b** were isolated as 60 : 40 mixtures of diastereoisomers (Scheme 2).

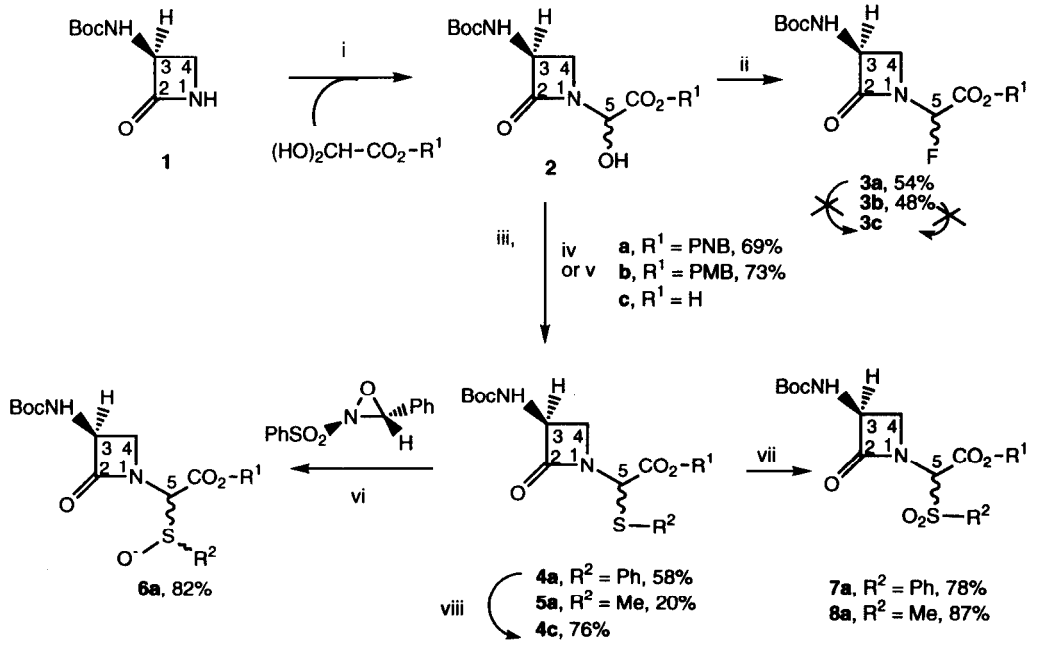
Replacement of the C-5 hydroxyl group with a thioaryl or thioalkyl substituent required an activation step. Thus, the alcohol **2a** was first treated with mesyl chloride and triethylamine, then added to a solution of thiophenol and triethylamine in dichloromethane, or of sodium thiomethoxide in dimethylformamide, to furnish respectively **4a** or **5a** (≈ 60 : 40 mixtures of diastereoisomers; Scheme 2). Controlled oxidation of **4a** into sulfoxide could be performed using an oxaziridine reagent; reaction with 2-(phenylsulfonyl)-3-phenyloxaziridine [31] gave **6a** as a 51.5 : 34 : 8.5 : 6 mixture of four diastereoisomers. Complete oxidation of **4a** and **5a** with potassium permanganate in aqueous acetic acid led to the corresponding sulfones **7a** and **8a** (≈ 60 : 40 mixtures of two diastereoisomers; Scheme 2).

N-Boc deprotection was conducted as usual by dissolution in trifluoroacetic acid at room temperature; treatment of compounds **2a** (X = OH), **4a** (X = SPh), **6a** (X = SOPh), **7a** (X = SO_2Ph) and **8a** (X = SO_2Me)

quantitatively furnished the corresponding ammonium trifluoroacetates **9** (Scheme 3), while under similar conditions, **3a** (X = F) led to unidentified products. The crude salts **9** were directly reacted with acetic anhydride under Schotten-Baumann conditions : **10a** resulted from the NH_2 (C-3) and OH (C-5) acylation; **11a** to **14a** corresponded to the expected NH_2 (C-3) acylation (Scheme 3). All the compounds were isolated as mixtures of diastereoisomers (ratios of 54 : 46 to 70 : 30, after column chromatography).

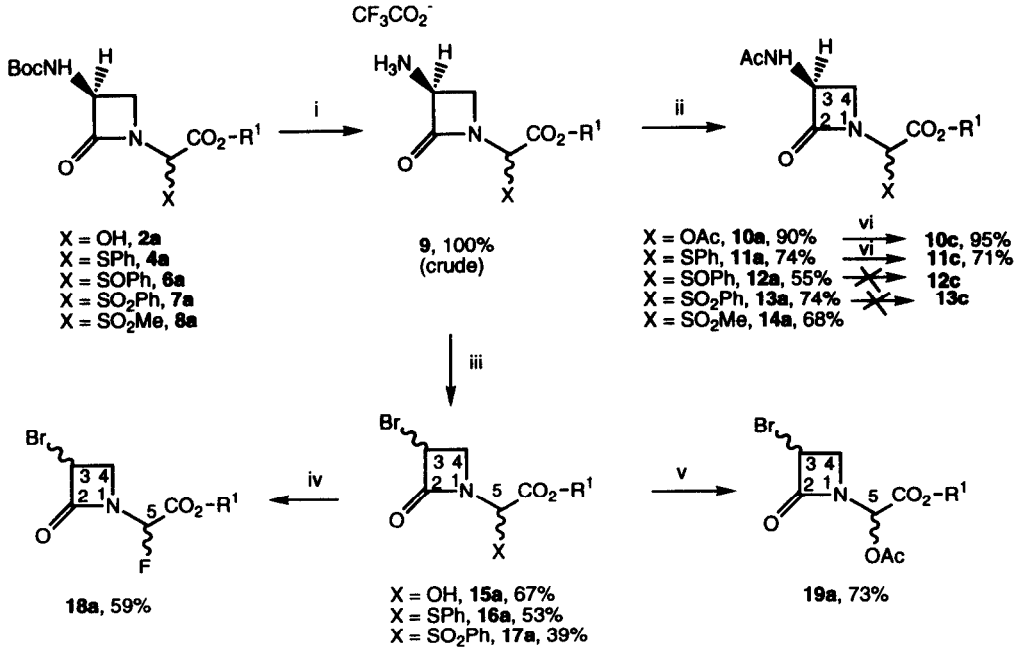
The 3-bromo-azetidinones **15a** (X = OH), **16a** (X = SPh) and **17a** (X = SO_2Ph) were prepared from the corresponding 3-amino-precursors **9** by diazotization in the presence of sodium bromide (Scheme 3) [32,33]. Due to the absence of a C-4 substituent in compounds **9**, chiral control at C-3 by steric effect was not achieved during the substitution. Thus, contrary to what is observed in the penam series, the former reaction led to C-3 racemization. This had been observed previously in the β-lactam **C** series (Scheme 1) [23]; moreover, epimerization of α-bromo-azetidinones in aqueous acidic solution, *via* an enolization process, has been reported [34]. Reaction of **15a** (X = OH) with DAST or acetic anhydride gave respectively the fluoro- derivative **18a** (X = F) and the acetoxy- derivative **19a** (X = OAc). All the bromo-azetidinone compounds were isolated as mixtures of 3,5- stereoisomers (Scheme 3).

Using the classical hydrogenolysis conditions for PNB deprotection, we were able to obtain the free acids **4c** (Z = BocNH, X = SPh; Scheme 2), **10c** (Z = AcNH, X = OAc; Scheme 3) and **11c** (Z = AcNH, X = SPh; Scheme 3). But similar treatment led to intractable mixtures in the case of the PNB precursors **3a** (Z = BocNH, X = F; Scheme 2), **12a** (Z = AcNH, X = SOPh; Scheme 3) and **13a** (Z = AcNH, X = SO_2Ph;



Reagents and conditions : (i) glyoxylate (1.3 equiv.), benzene, reflux, 10 - 19h; (ii) DAST (1.3 equiv.), CH_2Cl_2, -78°C to 20°C, 5h; (iii) $MeSO_2Cl$ (1.3 equiv.), Et_3N (1.3 equiv.), CH_2Cl_2, 0°C to 20°C, 30 min; (iv) PhSH (1.3 equiv.), Et_3N (1.3 equiv.), CH_2Cl_2, 20°C, 3h; (v) MeSNa (1.1 equiv.), DMF, 20°C,2h; (vi) oxaziridine (1 equiv.), CH_2Cl_2, 0°C to 20°C, 3h; (vii) $KMnO_4$ (2 equiv.), HOAc - H_2O (4:1), -10°C, 3h; (viii) H_2, Pd - C, MeOH, 20°C, 2h.

Scheme 2



Reagents and conditions : (i) CF_3CO_2H, 15 min, 20°C; (ii) Ac_2O, CH_3CN - H_2O + K_2CO_3, pH 7, 20°C, 30 min; (iii) KBr, $NaNO_2$, 2N H_2SO_4, 5°C - 8°C, 4h30 min; (iv) DAST, CH_2Cl_2, -78°C to 20°C, 1h; (v) Ac_2O, pyridine, CH_2Cl_2, 20°C, 3h; (vi) H_2, Pd - C, MeOH, 20°C, 2h (addition of N-ethyl morpholine)

Scheme 3

Scheme 3). However, the PNB esters could be smoothly hydrolysed in a mixture of phosphate buffer (pH 7.5) and DMSO (see the following section: chemical reactivity). Finally, various conditions were tested for PMB deprotection of compound **3b** (Z = BocNH, X = F; Scheme 2) , *i.e.* (i) oxidative treatments with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) or cerium ammonium nitrate (CAN); (ii) reaction with trimethylsilyl iodide; (iii) hydrolysis with trifluoroacetic acid-anisole or aluminium trichoride-anisole; (iv) catalytic hydrogenolysis. All attempts were unsuccessful, leading to intractable mixtures, the ^1H and ^{19}F NMR analyses of which revealed the disappearance of the fluorine atom.

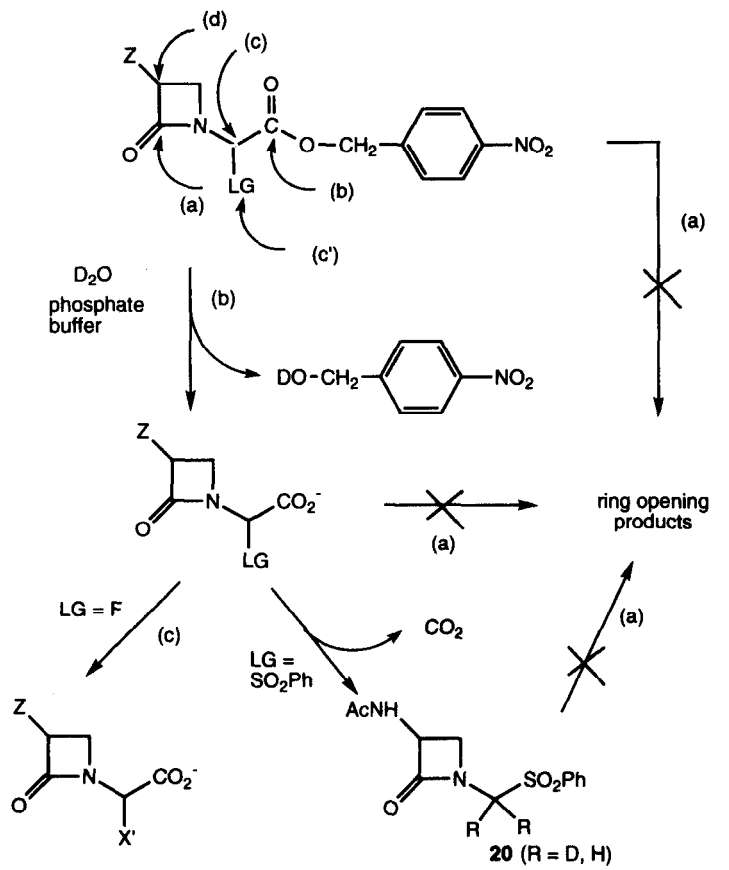
The various derivatives were characterised in ^1H NMR by a typical ABX pattern due to the β-lactam protons H-4, H-4' and H-3. The coupling constants $J_{44'}$, J_{34cis}, and $J_{34'trans}$ varied, respectively, between 3.8-6.8 Hz, 4.9-7.0 Hz, and 2.1-5.5 Hz. The chemical shifts of H-3, H-4 (cis) and H-4'(trans) appeared, respectively, at 4.6-4.9 δ, 3.6-4.0 δ, and 3.4-3.8 δ. The H-5 proton, characteristic of the functionalized acetyl chain, was visible at 5.5-6.3 δ. In the ^{13}C NMR spectra, four typical lines were found at 164-168 ppm (C-2), 46-50 ppm (C-4), 60-85 ppm (C-5), and 56-59 ppm (C-3 linked to NH) or 41-42 ppm (C-3 linked to Br).

Chemical Reactivity

As the potential inhibitory effect of the 1,3-substituted azetidinones will be tested on several target enzymes, measurements of the hydrolytic stability in aqueous buffer were performed as a prerequisite.

Moreover, the rate constants of hydroxide-ion catalysed hydrolysis of β-lactams are usually considered as a good measure of their intrinsic chemical reactivity. Since nucleophilic attack on the β-lactam ring is a key step in the inhibition of enzymes containing an essential serine in the active site, susceptibility towards nucleophilic attack has been used in structure-activity relationships of serine enzyme inhibition [35-37]. The presence of an EWG-substituent at position N-1 of the β-lactam can dramatically increase the rate of hydrolysis [23,38] or aminolysis [39-41]. In structures **11a**, **12a**, **13a**, **16a**, **18a**, and **19a**, this effect should be reduced since the EWG-group (PNB ester) is not directly linked to the β-lactam nitrogen atom. Being multifunctional, the 1,3-substituted azetidinones are susceptible to nucleophilic attack on several electrophilic centres. Reactions at the sp2 carbon atoms of the carbonyl functions (scheme 4 : routes (a), (b) and (c') when LG = AcO), and at the sp3 carbon atoms bearing a potential leaving group (scheme 4 : routes (c) and (d)) can be envisaged.

To determine the products formed under hydrolytic conditions, the PNB ester of β-lactams **11a**, **13a**, **16a**, **18a** and **19a** were dissolved in DMSO-d6 and added to deuterated phosphate buffer (pH 7.5, final concentration : between 5 x 10^{-4} and 10^{-3} M, DMSO : 7% for **11a**, 40% for the others). The solutions were analysed by ^{1}H NMR (500 MHz) as a function of time. The following reactions should be easily detected: (i) β-



route (a) : azetidinone hydrolysis (ring opening) ; route (b) : ester hydrolysis
route (c) : nucleophilic substitution of LG ; route (c') : LG = OAc; acetyl hydrolysis
route (d) : Z = Br; nucleophilic substitution of Z

Scheme 4

lactam ring opening should lead to a modification of the coupling constants of the cyclic protons H-3, H-4 and H-4'; (ii) PNB ester hydrolysis would release p-nitrobenzyl alcohol, while the acetyl hydrolysis (compounds **11a**, **13a**, and **19a**) would produce acetate, two known references; (iii) nucleophilic substitution at C-3 or C-5 should lead to the modification of the chemical shifts of the H-3 and H-5 protons. After 24 hours in phosphate buffer, quantitative deprotection of the PNB ester was the only reaction observed for compounds **11a** (Z = AcNH, X = SPh), **16a** (Z = Br, X = SPh) and **19a** (Z = Br, X = OAc). For them, incubation in a phosphate buffer-DMSO mixture constitutes a practical and smooth method of *in situ* deprotection of the ester function. With compound **13a** (Z = AcNH, X = SO_2Ph), ester hydrolysis was accompanied by H–5 hydrogen exchange and decarboxylation leading to the β-lactam **20** (R = D; scheme 4); the non deuterated product (R = H) has been isolated and fully characterized from a reaction run in H_2O. Compound **18a** (Z = Br, X = F) evolved into a complex mixture of products : the first NMR spectrum recorded during the course of hydrolysis pointed to the cleavage of the ester function as the first event; however, on standing, the initial mixture of diastereoisomers decomposed into a mixture of six secondary products in which the fluorine was lost, as evidenced by the disappearance of the typical H/F couplings for the protons H-5, H-4 and H-4'. These products still contain a β-lactam nucleus, they could not be isolated, nor further characterized.

The rates of hydrolysis of compounds **11a**, **12a**, **16a**, **18a**, and **19a** have been followed by UV spectrophotometry (10^{-4} - 10^{-5} M solution in phosphate or borate buffer containing 10% of CH_3CN), at 240 or 270 nm. First order rate constants (k_{obs}) were measured at different pHs (see experimental section). Plots of k_{obs} *versus* hydroxide ion concentration are linear. The pH dependence of the rate of hydrolysis of **17a**, on the other hand, is consistent with a kinetic scheme including a deprotonation equilibrium (pKa = 7.9 ± 0.1) and a hydroxide ion catalyzed hydrolysis of the neutral ester (scheme 5) :

$$S^{\ominus} + H^{\oplus} \underset{Ka}{\rightleftharpoons} SH \xrightarrow[k_{OH}]{} P$$

Scheme 5

This scheme is supported by the observation of H-5 exchange in D_2O by NMR. Table 1 summarizes the second order rate constants k_{OH} obtained; they are observed to increase with the electron withdrawing character of the C-5 substituent.

Table 1: Calculated second order rate constants for the hydroxide-catalyzed hydrolysis of PNB esters

Cmpd[a]	Z[b]	LG[b]	k_{OH} ($M^{-1}.min^{-1}$)
11a	NHAc	SPh	$(2.4 \pm 0.3)\ 10^4$
12a	NHAc	SOPh	$(3.4 \pm 0.3)\ 10^4$
16a	Br	SPh	$(4.7 \pm 2.3)\ 10^4$
17a	Br	SO_2Ph	$(8.4 \pm 1.0)\ 10^4$ [c]
18a	Br	F	$(5.7 \pm 1.3)\ 10^5$
19a	Br	OAc	$(1.4 \pm 0.3)\ 10^5$

(a) see Scheme 3 ; (b) see Scheme 1; (c) see Scheme 5 , pKa = 7,9 ± 0.1

Biological Evaluation

The free acids **4c** (Z = BocNH, X = SPh), **10c** (Z = AcNH, X = OAc), **11c** (Z = AcNH, X = SPh), and the PNB esters **12a** (Z = AcNH, X = SOPh), **13a** (Z = Br, X = SO_2Ph), **14a** (Z = AcNH, X = SO_2Me) were evaluated, as such or after *in situ* hydrolysis of the PNB ester, for their potential inhibitory effect on various representatives β-lactamases. All the tested compounds (with (S)-configuration at C-3) were inactive against the RTEM (*E.coli*) [42] and NMCA [43] β-lactamases of class A, the 5/B/6 β-lactamase [44] of class B, the Q908R β-lactamase [45] of class C, and the OXA2 β-lactamase [46] of class D.

The bromo-azetidinones (racemic mixtures of PNB esters) **16a** (Z = Br, X = SPh), **17a** (Z = Br, X = SO_2Ph), **18a** (Z = Br, X = F), and **19a** (Z = Br, X = OAc) were tested for their inhibitory effect on porcine pancreatic elastase (PPE) [47]. Three compounds (**16a, 18a, 19a**) behaved as weak reversible inhibitors at concentrations between 10^{-4} and 2 x 10^{-4} M with percentages of inhibition between 12% and 35% (see experimental). The hydrolysis of these compounds was not catalyzed by the enzyme. Replacement of the C-3 bromo substituent with the bulky BocNH group (in the (R)-configuration) totally suppressed the PPE inhibitory activity : the tested PNB esters were **3'a** (Z = (R)BocNH, X = F) and **4'a** (Z = (R)BocNH, X = SPh).

CONCLUSION

A series of monocyclic β-lactams sharing the general structure **D** (Scheme 1) have been prepared. They display various substituents or side-chains at positions C-3 and N-1 and were designed to promote the suicide-inhibition of serine-enzymes. Before testing these compounds as potential inhibitors of carboxypeptidases or β-lactamases, it is essential to deprotect the carboxyl function. For several compounds, this could not be achieved by hydrogenolysis of the p-nitrobenzyl function. The problem could, however, be solved by the discovery of an *in situ* deprotection of the PNB ester by smooth basic hydrolysis in the phosphate buffer. Under these conditions, the β-lactam ring of compounds **D** and their potential leaving group at C-5 were found to be stable, as controlled by ^1H NMR at 500 MHz. However, after 24 h, we observed that the fluorine leaving group was removed and that the presence of a strong electron withdrawing substituent at C-5 induced the loss of CO_2 from the carboxylate obtained by PNB hydrolysis. Indeed, the SO_2Ph substituent is able to stabilize a negative charge; accordingly, the H-5 proton of **13a** was also rapidly exchanged with deuterium in deuterated phosphate buffer.

All the tested compounds were found to be inactive against β-lactamases of classes A, B, C, and D. Yet, a modeling study has shown a good docking of one representative structure (**11c**: Z = (S)AcNH, X = (R)SPh) into the enzymatic cavity of RTEM β-lactamase [48]. On the other hand, the β-lactams **D** bearing a bromo-substituent at position C-3 were recognised by the porcine pancreatic elastase, but without leading to the expected irreversible inhibition. This could be due to their low level of intrinsic chemical reactivity. From the ratios of the apparent hydrolysis rates of the natural substrate by PPE in the absence or in the presence of the synthetic inhibitors, we could estimate that the inhibition constants (Ki) of **16a, 18a,** and **19a** are superior to 2.10^{-4}M.

EXPERIMENTAL SECTION

General

The IR spectra were recorded on Perkin Elmer Serie 1700 or Bio-Rad FTS 135 spectrometers. The NMR spectra were obtained on a Brucker AM-500 apparatus. The Mass spectra were recorded on Finnigan MAT-TSQ70 (70 eV, EI) and ION TECH (8 KeV, FAB) equipments. Melting points were determined with an Electrothermal microscope and are uncorrected. Elemental analyses were performed at the Imperial College (London, UK). HRMS analyses (VG-Autospec-Q, 20 KeV) were obtained from the University of Liège (Belgium). The UV measurements were made on a Gilford Response spectrophotometer and on a Varian Cary 3 BIO apparatus. Reagents and solvents were purchased from Acros, Janssen, Aldrich, Fluka or Sigma. RTEM β-lactamase and porcine pancreatic elastase (PPE) were obtained from Sigma. The β-lactamases Q908R, OXA2, 5/B/6 and NMCA were supplied by Prof.J.M.Frère (U.Lg, Belgium). The column-chromatographies were performed with Merck 60 silica gel (70-230 Mesh ASTM); the analytical plates were Merck 60 F254.

Synthesis

(3*S*)-3-(t-Butyloxycarbonyl)aminoazetidin-2-one (1).This compound was prepared from (*L*)-serine (5.25 g, 50 mmol), in five steps, according to reference [26], with an overall yield of 23 % (2.15 g of **1**, purified by crystallization in EtOAc). $[\alpha]_D^{20}$ - 19.8 (c = 0.1, MeOH; Lit [26]: - 19.8); mp 171.5 – 172.5 °C; ν_{max} (Nujol) 1760, 1730, 1690 cm^{-1}; δ_H (acetone – d6) 4.80 (1H, dd, *J* 5.2, 8.0 Hz, H-3), 3.52 (1H, dd, *J* 5.0, 5.2 Hz, H-4), 3.24 (1H, dd, *J* 3.0, 5.0 Hz, H-4'), 1.42 (9H, s); δ_C (acetone - d6) 169.02 (CO azetidinone), 155.79 (CO carbamate), 79.51, 59.69 (C-3), 44.21 (C-4), 28.52.

(3*R*)-3-(t-Butyloxycarbonyl)aminoazetidin-2-one (1). This compound was prepared as above, from (*D*)-serine. $[\alpha]_D^{20}$ + 19.8 (c = 0.1, MeOH; Lit [26]: + 19.8).

(3*S*)-1-[(p-Nitrobenzyloxy)2-hydroxyacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (2a). A solution of p-nitrobenzyl glyoxylate monohydrate (1.8 g, 8.6 mmol, 1.6 equiv.) in benzene (50 mL) was refluxed during 3 h in a flask (100 mL) equipped with a Dean-Stark condenser. After cooling, (3*S*)-3-(t-butyloxycarbonyl)amino - azetidin-2-one **1** (1 g, 5.3 mmol, 1 equiv.) was added, and the mixture was refluxed again for 7 h. After distillation of the solvent, the residue was dissolved in ethyl acetate (30 mL), washed with brine, and dried over $MgSO_4$. The crude product was purified by column chromatography on silica gel (elution with dichloromethane – ethyl acetate, 90 : 10 then 70 : 30) to furnish **2a** (1.46 g, 69 %) as a white solid, m.p. 144-145 °C; [Found: C,51.82; H, 5.34; N, 10.46. $C_{17}H_{21}0_8N_3$ requires C, 51.64; H, 5.35; N, 10.62%]; the two diastereoisomers (60 : 40 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 50 : 50) 0.43; ν_{max} (KBr) 3476, 3343, 2984, 1784, 1732, 1682, 1529, 1349 cm^{-1}; δ_H (acetone – d6) major isomer, 8.27 (2H, d, *J* 7.5 Hz), 7.75 (2H, d, *J* 7.5 Hz), 6.83 (1H, d, *J* 8.3 Hz, NH), 5.63 (1H, d, *J* 7 Hz, H-5), 5.39 (2H, s, CH_2Ar), 4.77 (1H, ddd, *J* 8.3, 5.5, 3.0 Hz, H-3), 3.76 (1H, dd, *J* 5.5, 5.3 Hz, H-4), 3.49 (1H, dd, *J* 3.0, 5.3 Hz, H-4'), 1.41 (9H, s) – minor isomer, 6.80 (1H, d, *J* 8.3 Hz, NH), 5.40 (1H, d, *J* 7 Hz, H-5), 3.58 (1H, dd, *J* 5.5, 5.3 Hz, H-4), 3.37 (1H, dd, *J* 3.0, 5.3 Hz, H-4'), 1.40 (9H, s); δ_C ($CDCl_3$) major isomer, 168.6 (CO ester), 168.03 (CO azetidinone), 155.81 (CO carbamate), 148.74, 144.21, 129.62, 124.44, 79.87, 72.82 (C-5), 66.45 (CH_2-Ar), 58.29 and 58.19 (C-3, two conformers), 45.95 (C-4), 28.5 – minor isomer, 168.51, 167.72, 72.71 (C-5), 66.40, 58.62 and 58.52 (C-3, two conformers), 46.15 (C-4); MS (FAB^+) *m/z* 396 (M + 1), 136, 56; MS (FAB^-) *m/z* 394 (M-1), 277, 122.

(3*S*)-1-[(p-Methoxybenzyloxy)2-hydroxyacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (2b). **2b** Was prepared as described for **2a**, from p-methoxybenzyl glyoxylate monohydrate (2.7 g, 9.6 mmol, 1.3 equiv.) and **1** (1.36 g, 7.3 mmol, 1 equiv.) in refluxing benzene (50 mL) during 16 h. Column chromatography on silica gel of the crude mixture gave pure **2b** (2 g, 73 %) as a white gummy material; [Found: C, 56.09; H, 6.59; N, 6.78. $C_{18}H_{24}N_2O_7.0.3\ H_2O$ requires C, 56.03; H, 6.42; N, 7.16%]; the two diastereoisomers (67 : 33 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 - EtOAc, 50 : 50) 0.35; ν_{max} (Film) 3450, 3338, 2954, 1747, 1614, 1517, 1178 cm^{-1}; δ_H ($CDCl_3$) major isomer, 7.29 (2H, d, *J* 7.6 Hz), 6.82 (2H, d, *J* 7.6 Hz), 5.80 (1H, d, *J* 7.5 Hz, NH) 5.56 (1H, d, *J* 7 Hz, H-5), 5.12 (2H, s, CH_2Ar), 5.0 (1H, d, *J* 7 Hz, OH), 4.78 (1H, m, H-3), 3.75 (3H, s, OMe), 3.63 (1H, t, *J* 5 Hz, H-4), 3.11 (1H, dd, *J* 5, 3 Hz, H-4'), 1.46 (9H, s) – minor isomer, 7.25 (2H, d, *J* 8 Hz), 5.60 (1H, d, *J* 7.5 Hz, NH), 5.42 (1H, d, *J* 7 Hz, H-5), 5.10 (2H, s); δ_C ($CDCl_3$) 168.51 (CO ester), 167.64 (CO azetidinone), 160.45, 155.29 (CO carbamate), 130.87, 127.34, 114.49, 80.9, 72.67 (C-5), 68.43 (CH_2Ar), 57.99 (C-3), 55.60, 47.13 (C-4), 28.62.

(3*S*)-1-[(p-Nitrobenzyloxy)2-fluoroacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (3a). To a cold (- 78° C) solution of diethylaminosulfur trifluoride (DAST, 0.7 mL, 7 mmol, 1.375 equiv.) in CH_2Cl_2 (5 mL) was added dropwise, under argon atmosphere, a solution of **2a** (2 g, 5.11 mmol, 1 equiv.) in CH_2Cl_2 (5 mL). At the end of the addition, the mixture was allowed to reach room temperature and stirred for 5 h at 20° C. After washing with water, drying over $MgSO_4$ and concentration, the crude product was purified by column chromatography on silica gel (elution with dichloromethane – ethyl acetate, 98 : 2 then 90 : 10) to furnish **3a** (1.1 g, 54 %) as a pale yellow gum; [Found: C, 50.98; H, 4.89; N, 10.15. $C_{17}H_{20}FN_3O_7$ requires C, 51.38; H, 5.07; N, 10.57%]; the two diastereoisomers (59 : 41 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 90 : 10) 0.60; ν_{max} (KBr) 3009, 2984, 1803, 1764, 1739, 1714, 734 cm^{-1}; δ_H (acetone – d6) major isomer, 8.27 (2H, d, *J* 7.2 Hz), 7.76 (2H, d, *J* 7.2 Hz), 6.92 (1H, d, *J* 8.1 Hz, NH), 6.27 (1H, d, $J_{H\text{-}F}$ 51.5 Hz, H-5), 5.47 (2H, s, CH_2Ar), 4.92 (1H, ddd, *J* 8.1, 4.9, 3.8 Hz, H-3), 3.75 (1H, dd, *J* 4.9, 4.8 Hz, H-4), 3.64 (1H, dd, *J* 3.8, 4.8 Hz, H-4'), 1.42 (9H, s); δ_C (acetone – d6) major isomer, 168.86 (CO ester), 164.74 (CO azetidinone), 155.7 (CO carbamate), 148.75, 143.5, 129.77, 124.36, 85.07 (C-5), 80.05, 67.09, 59.26 (C-3), 48.81 (C-4), 28.44; MS (FAB^-) *m/z* 396.1 (M - 1), 261, 122.

(3*S*)-1-[(p-Methoxybenzyloxy)2-fluoroacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (3b). **3b** Was prepared as described for **3a**, from DAST (0.36 mL, 3.61 mmol, 1.375 equiv.) in CH_2Cl_2 (5 mL) at – 78° C and **2b** (1g, 2.63 mmol, 1 equiv.) in CH_2Cl_2 (5 mL). Purification by chromatography on silica gel gave **3b** (0.48 g, 48 %) as a oil; [Found: C, 56.01; H, 5.97; N, 6.99. $C_{18}H_{23}FN_2O_6$ requires C, 56.53; H, 6.06; N, 7.32%]; the two diastereoisomers (60 : 40 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 - EtOAc, 90 : 10) 0.64; ν_{max} (Film) 2984, 1803, 1764, 1680, 1529, 1349, 850, 832 cm^{-1}; δ_H ($CDCl_3$) major isomer, 7.31 (2H, d, *J* 7.5 Hz), 6.90 (2H, d, *J* 7.5 Hz), 6.05 (1H, d, $J_{H\text{-}F}$ 51 Hz, H-5), 5.19 (2H, s, CH_2Ar), 5.12 (1H, br d, NH), 4.90 (1H, ddd, *J* 8, 5, 3.8 Hz, H-3), 3.82 (3H, s, OMe), 3.79 (1H, t, *J* 5 Hz, H-4), 3.48 (1H, dd, *J* 5, 3.8 Hz, H-4'), 1.44 (9H, s) – minor isomer, 6.03 (1H, d, $J_{H\text{-}F}$ 51 Hz, H-5), 5.07 (1H, br d, NH), 4.88 (1H, ddd, *J* 8, 5, 3.8 Hz, H-3), 3.59 (1H, t, *J* 5 Hz, H-4), 3.35 (1H, dd, *J* 5, 3.8 Hz, H-4'); δ_C ($CDCl_3$) major isomer, 167.1 (CO ester), 163.43 (CO azetidinone), 160.1, 154.38 (CO carbamate), 130.55, 126.21, 114.02, 83.56 (d, $J_{C\text{-}F}$ 210 Hz, C-5), 80.73, 68.1, 58.2 (C-3), 55.18, 47.29 (C-4), 28.09 – minor isomer, 167.42 (CO ester), 163.71 (CO azetidinone), 58.34 (C-3), 46.92 (C-4); MS (FAB^+) *m/z* 383 (M + 1), 121.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thiophenylacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (4a). To a solution of **2a** (0.7 g, 1.8 mmol, 1 equiv.) and mesyl chloride (0.18 mL, 2.33 mmol, 1.3 equiv.) in CH_2Cl_2 (5 mL) was added dropwise (with a syringe through a rubber stopper) triethylamine (0.335 mL, 2.33 mmol, 1.3 equiv.). The mixture was stirred for 30 min. at room temperature, then thiophenol (0.255 mL, 2.33 mmol, 1.3 equiv.) and triethylamine (0.335 mL, 2.33 mmol, 1.3 equiv.) were added successively. After 3 h at 20° C, the organic solution was washed with water, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (elution with CH_2Cl_2, then CH_2Cl_2 - EtOAc, 90 : 10) furnished **4a** (0.50 g, 58 %) as a pale yellow gum; [Found: C, 55.89; H, 5.37; N, 8.19. $C_{23}H_{25}N_3O_7S.0.3\ H_2O$ requires C, 56.04; H, 5.23; N, 8.52%]; the two diastereoisomers (64 : 36 mixture) were co-eluted; R_F

(SiO_2; CH_2Cl_2 - EtOAc, 90 : 10) 0.52; ν_{max} (Film) 2977, 1749, 1766, 1716, 1522, 1347 cm^{-1}; δ_H (acetone – d6) major isomer, 8.26 (2H, d, *J* 7.2 Hz), 7.71 (2H, d, *J* 7.2 Hz), 7.58 (2H, d, *J* 7.3 Hz), 7.38 (3H, m), 6.80 (1H, d, *J* 8 Hz, NH), 6.02 (1H, s, H-5), 5.40 (2H, s, CH_2Ar), 4.59 (1H, m, H-3), 3.84 (1H, t, *J* 4.9 Hz, H-4), 3.58 (1H, dd, *J* 4.9, 3.8 Hz, H-4'), 1.39 (9H, s) – minor isomer, 7.67 (2H, d, *J* 7.2 Hz), 7.65 (2H, d, *J* 7.2 Hz), 6.68 (1H, d, *J* 8 Hz, NH), 5.91 (1H, s, H-5), 5.35 (2H, s, CH_2Ar), 4.84 (1H, m, H-3), 3.65 (1H, t, *J* 4.9 Hz, H-4'), 1.42 (9H, s); δ_C (acetone – d6) major isomer, 167.8 (CO ester), 166.54 (CO azetidinone), 155.54 (CO carbamate), 148.51, 143.6, 134.6, 131.51, 130.21, 129.86, 129.47, 124.3, 79.79, 66.82, 59.5 (C-5), 58.01 (C-3), 47.26 (C-4), 28.50 – minor isomer, 166.75 (CO azetidinone), 143.5, 134.2, 131.34, 130.08, 129.57, 129.4, 59.92 (C-5), 58.11 and 57.88 (C-3, two rotamers), 47.66 (C-4); MS (FAB^+) *m/z* 487.7 (M + 1), 307, 136, 89, 77, 57; MS (FAB^-) *m/z* 486 (M - 1), 135.5, 121.6, 109, 46.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thiomethylacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (5a). To a solution of **2a** (0.1 g, 0.25 mmol, 1 equiv.) and mesyl chloride (25 µL, 0.28 mmol, 1.1 equiv.) in CH_2Cl_2 (3 mL) was added triethylamine (40 µL, 0.28 mmol, 1.1 equiv.). After 30 min. at 20° C, the mixture was concentrated under vacuum and the residue dissolved in DMF (3 mL). Sodium thiomethoxide (21 mg, 0.28 mmol, 1.1 equiv.) was added. After 2 h at 20° C, the mixture was poured into cold water (20 mL) and extracted with EtOAc (3 x 5 mL). Drying of the organic layers over $MgSO_4$, concentration and column chromatography on silica gel (elution with CH_2Cl_2 – EtOAc, 95 : 5) gave **5a** as a yellow oil (21 mg, 20 % yield, R_F 0.75). This product was directly oxidized into **8**.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thioxyphenylacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (6a). To a cold (0° C) solution of **4a** (330 mg, 0.68 mmol, 1 equiv.) in CH_2Cl_2 (5 mL) was added dropwise a solution of 2-phenylsulfonyl-3-phenyloxaziridine (218 mg, 68 mmol, 1 equiv.) in CH_2Cl_2 (3 mL). After 3 h at 20° C, the organic solution was washed with water, concentrated and purified by column chromatography on silica gel (elution with CH_2Cl_2 - EtOAc, 90 : 10 then 50 : 50) to furnish **6a** (280 mg, 82 %) as a pale yellow gum; [Found: C, 54.64; H, 4.94; N, 8.04. $C_{23}H_{25}N_3O_8S$ requires C, 54.86; H, 5.0; N, 8.34%]; the four diastereoisomers (51.5 : 34 : 8.5 : 6 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 - EtOAc, 90 : 10) 0.24; ν_{max} (KBr) 2978, 2933, 1772, 1713, 1523, 1348, 1163, 852 cm^{-1}; δ_H (acetone – d6) major isomer, 8.3 (2H, d, *J* 7.3 Hz), 7.81 (3H, m), 7.63 (4H, m), 6.77 (1H, d, *J* 8.3 Hz, NH), 5.66 (1H, s, H-5), 5.55 (2H, s, CH_2Ar), 4.91 (1H, m, H-3), 4.04 (1H, t, *J* 5 Hz, H-4), 3.75 (1H, dd, *J* 5, 3 Hz, H-4'), 1.39 (9H, s) – minor isomer (34 %), 6.44 (1H, d, *J* 8.3 Hz, NH), 5.63 (1H, s, H-5), 5.5 (2H, s, CH_2Ar), 4.69 (1H, m, H-3), 4.1 (1H, t, *J* 5 Hz, H-4), 3.88 (1H, dd, *J* 5, 3 Hz, H-4') – minor isomer (8.5 %), 6.96 (1H, d, *J* 8.3 Hz, NH), 5.65 (1H, s, H-5), 5.41 (2H, sharp m, CH_2Ar), 4.45 (1H, m, H-3), 4.0 (1H, t, *J* 5 Hz, H-4), 1.45 (9H, s) – minor isomer (6 %), 6.67 (1H, d, *J* 8.3 Hz, NH), 5.36 (2H, sharp m, CH_2Ar), 1.43 (9H, s); δ_C (acetone – d6) major isomer, 168.15 (CO azetidinone), 165.06 (CO ester), 155.65 (CO carbamate), 148.85, 143.61, 142.56, 132.81, 130.26, 129.78, 126.07, 124.44, 79.96, 74.99 (C-5), 67.34, 59.3 (C-3), 50.78 (C-4), 28.48 – minor isomer (34 %), 133.05, 75.47 (C-5), 66.95, 59.7 (C-3) – minor isomer (8.5 %), 50.27 (C-3); MS (FAB^+) *m/z* 504 (M + 1), 323, 136, 77, 57.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thiodioxophenylacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (7a). To a cold (- 10° C) solution of **4a** (200 mg, 0.412 mmol, 1 equiv.) in 4 : 1 acetic acid – water (12.5 mL) was added very slowly (during about 2 h) a solution of potassium permanganate (137 mg, 0.866 mmol, 2.1 equiv.) in water (4 mL). After the addition, the mixture was stirred further for 1 h at – 10° C, then 10 % H_2O_2 was added dropwise, until decolourization occurred. Extraction with CH_2Cl_2 (2 x 50 mL), washing of the organic phase with water (2 x 20 mL), 5 % $NaHCO_3$ (20 mL) and water (20 mL), followed by drying ($MgSO_4$) and concentration gave the crude sulfoxide which was purified by column chromatography on silica gel (elution with CH_2Cl_2 – EtOAc, 90 : 10); **7a** was recovered (166 mg, 78 %) as a pale yellow solid, m.p. 173.4-174.1 °C; [Found: C, 53.03; H, 4.84; N, 8.0. $C_{23}H_{25}N_3O_9S$ requires C, 53.17; H, 4.85; N, 8.09%]; the two diastereoisomers (60 : 40 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 90 : 10) 0.65; ν_{max} (KBr) 2976, 1762, 1749, 1716, 1522, 1347, 1162 cm^{-1}; δ_H (DMSO – d6) major isomer, 8.23 (2H, d, *J* 7.5 Hz), 7.93 (2H, d, *J* 7.5 Hz), 7.78 (1H, t, *J* 7.2 Hz), 7.64 (4H, m), 6.38 (1H, s, H–5), 5.42 (2H, s, CH_2Ar), 4.43 (1H, ddd, *J* 8.5, 6.7, 5.5 Hz, H-3), 3.83 (1H, dd, *J* 6.7, 5.5 Hz, H-4), 3.67 (1H, t, *J* 5.5 Hz,

H-4'), 1.34 (9H, s) – minor isomer, 6.20 (1H, s, H-5), 5.36 (2H, s, CH_2Ar), 4.70 (1H, ddd, *J* 8.5, 6.7, 5.5 Hz, H-3), 3.57 (1H, t, *J* 5.5 Hz, H-4'), 1.39 (9H, s); δ_C (DMSO – d6) major isomer, 167.22 (CO azetidinone), 161.38 (CO ester), 154.46 (CO carbamate), 147.25, 142.31, 136.3, 135.09, 129.64, 128.84, 128.7, 123.46, 78.78, 71.94 (C-5), 66.47, 57.30 (C-3), 47.96 (C-4), 27.96 – minor isomer, 167.31 (CO azetidinone), 161.26 (CO ester), 154.59 (CO carbamate), 136.62, 134.91, 72.33 (C-5), 66.42, 57.38 (C-3), 47.77 (C-4), 28.03; MS (FAB^-) *m/z* 518.2 (M - 1), 462, 418, 141.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thiodioxomethylacetyl]-3-(t-butyloxycarbonyl)aminoazetidin-2-one (8a). 8a Was prepared as described for **7a** from **5a** (45 mg, 0.1 mmol, 1 equiv.) dissolved in 4 : 1 AcOH-H_2O (5 mL) and $KMnO_4$ (35 mg, 0.21 mmol, 2.1 equiv.) dissolved in H_2O (3 mL). The crude sulfone **8a** (40 mg, 87 %) was directly used for the preparation of **14a**.

(3*S*)-3-Aminoazetidin-2-ones (9) : general procedure for t-butyloxycarbonyl deprotection. A solution of azetidinone (**2a**, **4a**, **6a**, **7a** or **8a**) in trifluoroacetic acid (± 0.4 mmol/5 mL) was stirred for 15 min. at 20° C, then concentrated under vacuum. Addition of toluene and evaporation (2 x) allowed removal of all the acid. The residue was washed with diethylether and dried under vacuum to furnish crude **9** as a trifluoroacetate salt (100 %).

(3*S*)-1-[(p-Nitrobenzyloxy)2-acetyloxyacetyl]-3-(acetylamino)azetidin-2-one (10a). Deprotection of **2a** (150 mg, 0.38 mmol, 1 equiv.) gave **9** (155 mg, 100 %) which was dissolved in H_2O (20 mL). The pH was adjusted to 7 with 0.5 M K_2CO_3. A 1 M solution of acetic anhydride in CH_3CN (0.840 mL, 0.42 mmol, 2 equiv.) was added dropwise. During this addition, the pH was maintained at 7 by addition of 0.5 M K_2CO_3. Extraction with EtOAc (2 x 15 mL), drying over $MgSO_4$, concentration and column chromatography on silica gel (elution with CH_2Cl_2 - MeOH, 99 : 1 then 95 : 5) gave **10a** (130 mg, 90 %) as a colourless oil; [Found: C,50.33; H, 4.67; N, 10.98. $C_{16}H_{17}O_8N_3$ requires C, 50.66; H, 4.52; N, 11.07%]; the two diastereoisomers (63 : 37 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 50 : 50) 0.17; ν_{max} (Film) 3067, 2959, 1761, 1683, 1522, 1348 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.31 (2H, d, *J* 7.5 Hz), 7.50 (2H, d, *J* 7.5 Hz), 6.68 (1H, d, *J* 7.3 Hz, NH), 6.39 (1H, s, H-5), 5.30 (2H, s, CH_2Ar), 4.83 (1H, ddd, *J* 7.3, 5.8, 3.1 Hz, H-3), 3.69 (1H, t, *J* 5.8 Hz, H-4), 3.55 (1H, dd, *J* 3.1, 5.8 Hz, H-4'), 2.14 (3H, s), 1.98 (3H, s) – minor isomer, 8.19 (2H, d, *J* 7.5 Hz), 7.53 (2H, d, *J* 7.5 Hz), 6.74 (1H, d, *J* 7.3 Hz, NH), 6.34 (1H, s, H-5), 4.87 (1H, ddd, *J* 7.3, 5.8, 3.1 Hz, H-3), 3.73 (1H, t , *J* 5.8 Hz, H-4), 3.51 (1H, dd, *J* 3.1, 5.8 Hz, H-4'), 2.13 (3H, s), 1.99 (3H, s); δ_C ($CDCl_3$) major isomer, 170.58 (CO amide), 169.42 (CO ester), 166.84 (CO ester PNB), 164.21 (CO azetidinone), 147.77, 141.59, 128.48, 123.7, 71.84 (C-5), 66.4, 57.08 (C-3), 47.26 (C-4), 22.47, 20.23 – minor isomer, 170.65 (CO amide), 167.07 (CO ester PNB), 164.3 (CO azetidinone), 147.84, 141.47, 123.76, 71.58 (C-5), 46.82 (C-4); MS (FAB^+) *m/z* 379.9 (M + 1), 320, 136.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thiophenylacetyl]-3-(acetylamino)azetidin-2-one (11a). Deprotection of **4a** (200 mg, 0.41 mmol, 1 equiv.) gave **9** (205 mg, 100 %) which was dissolved in water (20 mL). The pH was adjusted to 7 with 0.5 M K_2CO_3, and maintained at 7 during the dropwise addition of a 1 M solution of acetic anhydride in CH_3CN (0.45 mL, 0.45 mmol, 1.1 equiv.). Work-up as for **10a**, and column chromatography on silica gel (elution with CH_2Cl_2 – EtOAc 50 : 50) furnished **11a** (130 mg, 74 %) as a white gum; [Found: C, 55.49; H, 4.41; N, 9.36. $C_{20}H_{19}N_3O_6S.0.2H_2O$ requires C, 55.48; H, 4.48; N, 9.69%]; the two diastereoisomers (54 : 46 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc 50 : 50) 0.34; ν_{max} (Film) 3076, 2967, 1761, 1748, 1521, 1347, 1223 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.24 (2H, d, *J* 7.4 Hz), 7.50 (4H, m), 7.37 (3H, m), 5.90 (1H, s, H-5), 6.06 (1H, d, *J* 7 Hz, NH), 5.26 (2H,sharp m, CH_2Ar), 4.75 (1H, ddd, *J* 7.0, 5.8, 2.8 Hz, H-3), 3.82 (1H, t, *J* 5.8 Hz, H-4), 3.36 (1H, dd, *J* 2.8, 5.8 Hz, H-4'), 1.98 (3H, s) – minor isomer, 5.95 (1H, s, H-5), 5.46 (1H, d, *J* 7 Hz, NH), 5.29 (2H, sharp m, CH_2Ar), 4.95 (1H, ddd, *J* 7.0, 5.8, 2.8 Hz, H-3), 3.87 (1H, t, *J* 5.8 Hz, H-4), 3.50 (1H, dd, *J* 2.8, 5.8 Hz, H-4'), 1.94 (3H, s); δ_C ($CDCl_3$) major isomer, 169.94 (CO amide), 166.3 (CO ester), 165.87 (CO azetidinone), 147.94, 141.47, 133.58, 130.04, 129.51, 129.34, 128.46, 123.84, 66.23, 58.18 (C-5), 56.08

(C-3), 47.36 (C-4), 22.71 – minor isomer, 169.74 (CO amide), 166.44 (CO ester), 165.75 (CO azetidinone), 133.75, 130.7, 129.31, 129.01, 128.49, 56.25 (C-3), 47.59 (C-4), 22.65; MS (FAB^+) *m/z* 430 (M + 1), 320, 167, 136; MS (FAB^-) *m/z* 428 (M – 1), 293, 249, 109.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thioxyphenylacetyl]-3-(acetylamino)azetidin-2-one (12a). Deprotection of **6a** (110 mg, 0.22 mmol, 1 equiv.) gave **9** (113 mg, 100 %) which was treated with acetic anhydride (1.1 equiv.) as described for **11a**. Column chromatography on silica gel (elution with CH_2Cl_2 – EtOAc, 70 : 30 then 50 : 50) gave **12a** (56 mg, 55 %) as a white solid, m.p. 71-72 °C; [Found: C, 52.89; H, 4.29; N, 8.98. $C_{20}H_{19}N_3O_7S.0.5H_2O$ requires C, 52.86; H, 4.40; N, 9.25%]; the four stereoisomers (43 : 27 : 17 : 13 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 70 : 30) 0.16; ν_{max} (KBr) 3067, 2917, 1744, 1684, 1518, 1349, 1224 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.23 (2H, d, *J* 7.5 Hz), 7.63 (2H, d, *J* 7.5 Hz), 7.56 (3H, m), 7.54 (2H, d, *J* 7.3 Hz), 6.41 (1H, d, *J* 7.6 Hz, NH), 5.36 (3H, s, H-5 and CH_2Ar), 4.84 (1H, ddd, *J* 7.6, 5.8, 2.7, H-3), 4.09 (1H, dd, *J* 6.1, 5.8 Hz, H-4), 3.87 (1H, dd, *J* 2.7, 6.1 Hz, H-4') 1.94 (3H, s) – minor isomer (27 %), 8.23 (2H, d, *J* 7.5 Hz), 7.56 (5H, m), 7.48 (2H, d, *J* 7.3 Hz), 6.33 (1H, d, *J* 7.6 Hz, NH), 5.47 (1H, s, H-5), 5.30 (2H, s, CH_2Ar), 5.3 (1H, ddd, *J* 7.6, 5.8, 2.7 Hz, H-3), 4.07 (1H, dd, *J* 6.1 and 5.8 Hz, H-4), 3.55 (1H, dd, *J* 2.7, 6.1 Hz, H-4'), 1.99 (3H, s) – minor isomer (17 %), 8.17 (2H, d, *J* 7.5 Hz), 7.68 (2H, d, *J* 7.5 Hz), 7.48 (3H, m), 7.33 (2H, d, *J* 7.3 Hz), 6.76 (1H, d, *J* 7.6 Hz, NH), 5.59 (1H, s, H-5), 5.08 (2H, sharp m, CH_2Ar), 4.98 (1H, ddd, *J* 7.6, 5.8, 2.7 Hz, H-3), 4.0 (1H, dd, *J* 5.8, 6.1 Hz, H-4), 3.74 (1H, dd, *J* 2.7, 6.1 Hz, H-4'), 2.02 (3H, s) – minor isomer (13 %), 8.17 (2H, d, *J* 7.5 Hz), 7.68 (2H, d, *J* 7.5 Hz), 7.58 (3H, m), 7.38 (2H, d, *J* 7.3 Hz), 6.50 (1H, d, *J* 7.6 Hz, NH), 5.42 (1H, s, H-5), 5.13 (2H, sharp m, CH_2Ar), 4.94 (1H, ddd, *J* 7.6, 5.8, 2.7 Hz, H-3), 3.81 (1H, dd, *J* 5.8, 6.1 Hz, H-4), 3.77 (1H, dd, *J* 2.7, 6.1 Hz, H-4'), 1.96 (3H, s); δ_C ($CDCl_3$) major isomer, 170.14 (CO amide), 167.05 (CO azetidinone), 163.51 (CO ester), 147.49, 141.23, 140.6, 132.32, 129.5, 128.61, 124.47, 123.83, 73.85 (C-5), 66.7, 57.22 (C-3), 50.54 (C-4), 22.61 – minor isomer (27 %), 169.79 (CO amide), 168.35 (CO azetidinone), 163.51 (CO ester), 148.05, 129.47, 128.7, 123.7, 75.13 (C-5), 57.22 (C-3), 50.1 (C-4) – minor isomer (17 %), 170.41 (CO amide), 167.55 (CO azetidinone), 161.96 (CO ester), 147.84, 129.24, 128.78, 125.22, 123.9, 73.75 (C-5), 66.15, 57.47 (C-3), 49.76 (C-4), 22.75 – minor isomer (13 %), 170.34 (CO amide), 167,2 (CO azetidinone), 162.2 (CO ester), 129.31, 124.84, 123.87, 73.55 (C-5), 66.2, 57.47 (C-3), 49.96 (C-4); MS (FAB^+) *m/z* 446.3 (M + 1), 402, 320, 136.

(3*S*)-1-[(p-Nitrobenzyloxy)2-thiodioxophenylacetyl]-3-(acetylamino)azetidin-2-one (13a). Deprotection of **7a** (155 mg, 0.299 mmol, 1 equiv.) gave **9** (159 mg, 100 %) which was treated with Ac_2O (1.1 equiv.) as described for **11a**. Column chromatography on silica gel (elution with CH_2Cl_2 - MeOH, 99 : 1 then 95 : 5) furnished **13a** (102 mg, 74 %) as a white solid, m.p. 169-170 °C; [Found: C, 52.09; H, 3.99; N, 8.77. $C_{20}H_{19}N_3O_8S$ requires C, 52.04; H, 4.15; N, 9.14%]; the two diastereoisomers (66 : 34 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 50 : 50) 0.16; ν_{max} (KBr) 3079, 2934, 1754, 1677, 1520, 1323, 1233 cm^{-1}; δ_H (DMSO – d6) major isomer, 8.55 (1H, d, *J* 7.9 Hz, NH), 8.24 (2H, d, *J* 7.5 Hz), 7.94 (2H, d, *J* 7.3 Hz), 7.79 (1H, t, *J* 7.3 Hz), 7.66 (2H, d, *J* J.5 Hz), 7.62 (2H, t, *J* 7.3 Hz), 6.4 (1H, s, H-5), 5.42 (2H, s, CH_2Ar), 4.66 (1H, ddd, *J* 7.9, 5.8, 3.1 Hz, H-3), 3.83 (1H, t, *J* 5.8 Hz, H-4), 3.52 (1H, dd, *J* 3.1, 5.8 Hz, H-4'), 1.8 (3H, s) – minor isomer, 8.63 (1H, d, *J* 7.9 Hz, NH), 8.23 (2H, d, *J* 7.5 Hz), 7.96 (2H, d, *J* 7.3 Hz), 7.77 (1H, t, *J* 7.3), 6.27 (1H, s, H-5), 5.34 (2H, s, CH_2Ar), 4.96 (1H, ddd, *J* 7.9, 5.8, 3.1 Hz, H-3), 3.88 (1H, t, *J* 5.8 Hz, H-4), 3.66 (1H, dd, *J* 3.1, 5.8 Hz, H-4'), 1.87 (3H, s); δ_C (DMSO – d6) major isomer, 169.51 (CO amide), 166.91 (CO azetidinone), 161.43 (CO ester), 147.27, 142.29, 136.33, 135.08, 129.65, 128.68, 128.81, 123.46, 72.01 (C-5), 66.49, 56.21 (C-3), 47.83 (C-4), 22.17 – minor isomer, 161.33, 129.41, 136.68, 134.96, 129.48, 129.11, 128.77, 72.44 (C-5), 66.42, 56.25 (C-3), 47.96 (C-4); MS (FAB^+) *m/z* 462 (M + 1), 136; MS (FAB^-) *m/z* 460 (M – 1), 306.

(3*S*)-1-[(p-Nitrobenzyloxy)-2-thiodioxomethylacetyl]-3-(acetylamino)azetidin-2-one (14a). Deprotection of **8a** (40 mg, 0.09 mmol, 1 equiv.) gave **9** (41 mg, 100 %) which was treated with Ac_2O (1.1 equiv.) as described for **11a**. Column chromatography on silica gel (elution with CH_2Cl_2 – EtOAc, 50 : 50) furnished **14a** (24 mg, 68 %) as a pale yellow gum; [Found: C, 43.38; H, 4.38; N, 10.17. $C_{15}H_{17}N_3O_8S.H_2O$ requires C, 43.16; H, 4.55; N, 10.07%]; the two diastereoisomers (70 : 30 mixture) were

co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 50 : 50) 0.12; ν_{max} (Film) 2927, 1775, 1740, 1671, 1654, 1381 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.24 (2H, d, *J* 7.5 Hz), 7.58 (2H, d, *J* 7.5 Hz), 6.26 (1H, d, *J* 6.7 Hz, NH), 5.73 (1H, s, H-5), 5.44 (2H, s, CH_2Ar), 4.68 (1H, ddd, *J* 6.7, 5.8, 3.3 Hz, H-3), 4.12 (1H, t, *J* 5.8 Hz, H-4), 4.05 (1H, dd, *J* 5.8, 3.3 Hz, H-4'), 3.3 (3H, s), 2.03 (3H, s) – minor isomer, 6.23 (1H, d, *J* 6.7 Hz, NH), 5.59 (1H, s, H-5), 5.35 (2H, s, CH_2Ar), 4.86 (1H, ddd, *J* 6.7, 5.8, 3.3 Hz, H-3), 4.03 (1H, t, *J* 5.8 Hz, H-4), 3.99 (1H, dd, *J* 5.8, 3.3 Hz, H-4'), 3.13 (3H, s), 2.02 (3H, s); δ_C ($CDCl_3$) major isomer, 170.59 (CO amide), 166.97 (CO azetidinone), 161.26 (CO ester), 147.96, 140.96, 128.51, 123.87, 70.20 (C-5), 67.12, 57.94 (C-3), 47.71 (C-4), 40.85, 22.56 – minor isomer, 170.42 (CO amide), 167.21 (CO azetidinone), 161.53 (CO ester), 128.71, 71.57 (C-5), 67.22, 57.77 (C-3), 49.08 (C-4), 40.95, 22.62; MS (FAB^+) *m/z* 399.9 (M + 1), 341.

1-[(p-Nitrobenzyloxy)2-hydroxyacetyl]-3-bromoazetidin-2-one (15a). Deprotection of **2a** (500 mg, 1.26 mmol) gave **9** (400 mg, 0.978 mmol, 78 %) which was dissolved in 2.5 N H_2SO_4 (10 mL) at 5° C. After addition of KBr (580 mg, 4.89 mmol, 5 equiv.) and ethanol (2 mL), a solution of $NaNO_2$ (100 mg, 1.47 mmol, 1.5 equiv.) in water (1 mL) was added dropwise during 1 h. The mixture was stirred further for 3 h 30 min. at 8° C, then extracted with $CHCl_3$ (2 x 15 mL; 4 x 10 mL; 2 x 5 mL). The organic layer was washed with brine, dried over $MgSO_4$, concentrated and chromatographied on silica gel (elution with CH_2Cl_2 – EtOAc, 95 : 5) to furnish **15a** (235 mg, 67 %) as a yellow oil; [HRMS (FAB^+): Found : 358.9872. $C_{12}H_{12}BrN_2O_6$ requires 358.9879]; the two diastereoisomers (29 : 71 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 50 : 50) 0.61; ν_{max} (Film) 3286, 2932, 1759, 1726, 1526, 1347, 1093 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.24 (2H, d, *J* 7.6 Hz), 7.54 (2H, d, *J* 7.6 Hz), 5.49 (1H, s, H-5), 5.35 (2H, sharp m, CH_2Ar), 4.80 (1H, dd, *J* 5.2, 2.4 Hz, H-3), 4.40 (1H, br s, OH), 3.90 (1H, dd, *J* 6.4, 5.2 Hz, H-4), 3.62 (1H, dd, *J* 6.4, 2.4 Hz, H-4') – minor isomer, 5.59 (1H, s, H-5), 5.42 and 5.32 (2H, two d, *J* 12.8 Hz, CH_2Ar), 4.26 (1H, br s, OH), 4.01 (1H, dd, *J* 6.4, 5.2 Hz, H-4), 3.32 (1H, dd, *J* 6.4, 2.4 Hz, H-4'); δ_C ($CDCl_3$) major isomer, 167.45 (CO ester), 163.46 (CO azetidinone), 147.98, 141.19, 128.77, 123.84, 72.57 (C-5), 66.80, 48.39 (C-4), 41.34 (C-3) – minor isomer, 167.65 (CO ester), 163.38 (CO azetidinone), 128.71, 123.86, 72.14 (C-5), 48.0 (C-4), 41.49 (C-3); MS (FAB^+) *m/z* 360.9 (M + 1), 136, 107.

1-[(p-Nitrobenzyloxy)2-thiophenylacetyl]-3-bromoazetidin-2-one (16a). Deprotection of **4a** (240 mg, 0.5 mmol) gave **9** (195 mg, 0.5 mmol, 100 %) which was dissolved in 2.5 N H_2SO_4 (10 mL) and ethanol (1 mL) at 5° C. Treatment with KBr (300 mg, 2.5 mmol, 5 equiv.) and $NaNO_2$ (51 mg, 0.75 mmol, 1.5 equiv.) in H_2O (1 mL) was performed as described for **15a**. Purification by column chromatography on silica gel (elution with CH_2Cl_2 – EtOAc, 90 : 10) furnished **16a** (120 mg, 53 %) as a yellow oil; [Found : C, 48.05; H, 3.55; N, 5.96. $C_{18}H_{15}BrN_2O_5S$ requires C, 47.90; H, 3.35; N, 6.21%]; the two diastereoisomers (52 : 48 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 90 : 10) 0.85, ν_{max} (Film) 3078, 2965, 1775, 1747, 1521, 1347, 1224 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.24 (2H, d, *J* 7.6 Hz), 7.50 (4H, m), 7.37 (3H, m), 5.89 (1H, s, H-5), 5.28 (2H, sharp m, CH_2Ar), 4.55 (1H, dd, *J* 4.9, 2.2 Hz, H-3), 4.02 (1H, dd, *J* 4.9, 6.8 Hz, H-4), 3.67 (1H, dd, *J* 6.8, 2.2 Hz, H-4') – minor isomer, 5.87 (1H, s, H-5), 4.74 (1H, dd, *J* 4.9, 2.2 Hz, H-3), 4.12 (1H, dd, *J* 4.9, 6.8 Hz, H-4), 3.64 (1H, dd, *J* 6.8, 2.2 Hz, H-4'); δ_C ($CDCl_3$) major isomer, 165.7 (CO ester), 163.24 (CO azetidinone), 147.93, 141.43, 134.52, 133.82, 129.50, 129.40, 128.46, 123.82, 66.26, 58.6 (C-5), 49.0 (C-4), 40.99 (C-3) – minor isomer, 165.48 (CO ester), 163.33 (CO azetidinone), 133.55, 129.57, 129.44, 58.67 (C-5), 49.12 (C-4), 41.15 (C-3); MS (FAB^+) *m/z* 453 (M + 1), 272, 136, 77.

1-[(p-Nitrobenzyloxy)2-thiodioxophenylacetyl]-3-bromoazetidin-2-one (17a). Deprotection of **7a** (200 mg, 0.385 mmol) gave **9** (160 mg, 0.385 mmol, 100 %) which was dissolved in 2.5 N H_2SO_4 (10 mL) and ethanol (1 mL). Treatment with KBr (230 mg, 1.92 mmol, 5 equiv.) and $NaNO_2$ (40 mg, 0.58 mmol, 1.5 equiv.) in H_2O (1 mL) was performed as described for **15a**. Purification by column chromatography on silica gel (elution with CH_2Cl_2 – EtOAc, 90 : 10) furnished **17a** (73 mg, 39 %) as a yellow gum; [Found: C, 43.38; H, 3.13; N, 5.46. $C_{18}H_{15}BrN_2O_7S.0.7H_2O$ requires C, 43.58; H, 3.30; N,

5.65%]; the two diastereoisomers (50 : 50 mixture) were co-eluted; R_F (SiO_2 CH_2Cl_2 - EtOAc, 90 : 10) 0.80, ν_{max} (Film) 2941, 1781, 1752, 1522, 1348, 1227, 482 cm^{-1}; δ_H ($CDCl_3$) one stereoisomer, 8.25 (2H, d, *J* 7.5 Hz), 7.94 (2H, d, *J* 7.3 Hz), 7.58 (2H, d, *J* 7.5 Hz), 7.75 (1H, t, *J* 7.3 Hz), 7.61 (2H, t, *J* 7.3 Hz), 5.73 (1H, s, H-5), 5.44 and 5.35 (2H, two d, *J* 10 Hz, CH_2Ar), 4.77 (1H, dd, *J* 5.2, 2.4 Hz, H-3), 4.38 (1H, dd, *J* 7, 5.2 Hz, H-4), 3.92 (1H, dd, *J* 7, 2.4 Hz, H-4') – other isomer, 8.23 (2H, d, *J* 7.5 Hz), 7.86 (2H, d, *J* 7.3 Hz), 7.72 (1H, t, *J* 7.3 Hz), 7.57 (2H, t, *J* 7.3 Hz), 7.51 (2H, d, *J* 7.3 Hz), 5.72 (1H, s, H-5), 5.38 and 5.32 (2H, two d, *J* 10 Hz, CH_2Ar), 4.79 (1H, dd, *J* 5.2, 2.4 Hz, H-3), 4.46 (1H, dd, *J* 7, 5.2 Hz, H-4), 4.03 (1H, dd, *J* 7, 2.4 Hz, H-4'); δ_C ($CDCl_3$) one stereoisomer, 163.58 (CO azetidinone), 161.09 (CO ester), 147.98, 140.67, 136.59, 135.25, 129.8, 128.78, 128.67, 123.83, 71.4 (C-5), 67.17, 51.13 (C-4), 41.79 (C-3) – other isomer, 163.07 (CO azetidinone), 140.82, 136.08, 135.18, 129.61, 128.7, 128.58, 72.58 (C-5), 67.06, 51.51 (C-4), 41.58 (C-3); MS (FAB^+) *m/z* 484.9 (M + 1), 136, 107.

1-[(p-Nitrobenzyloxy)2-fluoroacetyl]-3-bromoazetidin-2-one (18a). To a cold solution (- 78° C) of DAST (40 µL, 0.38 mmol, 1.375 equiv.) in CH_2Cl_2 (3 mL) under argon atmosphere, was added a solution of **15a** (0.1 g, 0.28 mmol, 1 equiv.) in CH_2Cl_2 (3 mL). The mixture was stirred for 1 h at 20° C, then concentrated and chromatographied on silica gel (elution with CH_2Cl_2 – EtOAc, 90 : 10) to give **18a** (60 mg, 59 %) as a yellow oil; [HRMS (FAB^+): Found: 360.9839. $C_{12}H_{11}BrN_2O_5F$ requires 360.9835]; the two diastereoisomers (79 : 21 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2 – EtOAc, 90 : 10) 0.88; ν_{max} (Film) 2972, 1791, 1767, 1522, 1348, 1034 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.25 (2H, d, *J* 7.5 Hz), 7.56 (2H, d, *J* 7.5 Hz), 6.09 (1H, d, $J_{H\text{-}F}$ 50.8 Hz, H-5), 5.38 (2H, s, CH_2Ar), 4.89 (1H, dd, *J* 5.3, 2.4 Hz, H-3), 4.13 (1H, ddd, *J* 5.3, 6.8 Hz, $J_{H\text{-}F}$ 4.1 Hz, H-4), 3.57 (1H, ddd, *J* 2.4, 6.8 Hz, $J_{H\text{-}F}$ 4.1 Hz, H-4') - minor isomer, 6.03 (1H, d, $J_{H\text{-}F}$ 50.8 Hz, H-5), 4.05 (1H, ddd, *J* 5.3, 6.8 Hz, $J_{H\text{-}F}$ 4.1 Hz, H-4), 3.69 (1H, ddd, *J* 2.4, 6.8 Hz, $J_{H\text{-}F}$ 4.1 Hz, H-4'); δ_C ($CDCl_3$) major isomer, 163.53 (CO azetidinone), 162.87 (CO ester, d, $J_{C\text{-}F}$ 34.1 Hz), 148.1, 140.9, 128.77, 123.93, 83.74 (C-5, d, $J_{C\text{-}F}$ 213.6 Hz), 66.63, 49.0 (C-4), 41.65 (C-3) - minor isomer, δ 163.0 (CO ester, d, $J_{C\text{-}F}$ = 34.1 Hz), 83.94 (C-5, d, $J_{C\text{-}F}$ = 213.6 Hz), 66.69; Ms (FAB^+) *m/z* 361 (M + 1), 341, 178, 136.

1-[(p-Nitrobenzyloxy)2-acyloxyacyl]-3-bromoazetidin-2-one (19a). To a solution of **15a** (235 mg, 0.65 mmol, 1 equiv.) in CH_2Cl_2 (2 mL) were added successively Ac_2O (0.123 mL, 1.3 mmol, 2 equiv.) and pyridine (0.105 mL, 1.3 mmol, 2 equiv.) with a syringe through a rubber-stopper. After 3 h stirring at 20° C, the solution was concentrated and chromatographied on silica gel (elution with CH_2Cl_2, then CH_2Cl_2 - EtOAc, 95 : 5) to give **19a** (180 mg, 73 %) as a yellow oil; [HRMS (FAB^+): Found : 400.9975. $C_{14}H_{14}BrN_2O_7$ requires 400.9984]; the two diastereoisomers (54 : 46 mixture) were co-eluted; R_F (SiO_2; CH_2Cl_2) 0.46; ν_{max} (Film) 2966, 1789, 1757, 1653, 1636, 1522, 1348, 1211 cm^{-1}; δ_H ($CDCl_3$) major isomer, 8.22 (2H, d, *J* 7.5 Hz), 7.52 (2H, d, *J* 7.5 Hz), 6.27 (1H, s, H-5), 5.31 (2H, s, CH_2-Ar), 4.84 (1H, dd, *J* 5.2, 2.4 Hz, H-3), 4.06 (1H, dd, *J* 5.2, 6.7 Hz, H-4), 3.60 (1H, dd, *J* 2.4, 6.7 Hz, H-4'), 2.16 (3H, s) - minor isomer, 6.33 (1H, s, H-5), 5.36 (2H, sharp m, CH_2Ar), 4.83 (1H, dd, *J* 5.2, 2.4 Hz, H-3), 4.02 (1H, dd, *J* 5.2, 6.7 Hz, H-4), 3.56 (1H, dd, *J* 2.4, 6.7 Hz, H-4'), 2.14 (3H, s); δ_C ($CDCl_3$) major isomer, 169.38 (CO acetate), 163.84 (CO ester), 163.33 (CO azetidinone), 147.87, 141.3, 128.53, 123.77, 72.01 (C-5), 66.55, 50.12 (C-4), 41.59 (C-3), 20.20 - minor isomer, 169.27 (CO acetate), 163.76 (CO ester), 163.24 (CO azetidinone), 71.91 (C-5), 66.49, 49.9 (C-4); MS (FAB^+) *m/z* 401 (M + 1), 343, 149, 136.

Deprotection of p-nitrobenzyl ester (PNB) : general procedure. The PNB ester dissolved in dry (m)ethanol was hydrogenated in a Parr apparatus (p = 40 psi), in the presence of 10 % Pd on charcoal as catalyst, during 30 min. to 2 h at 20° C under vigorous stirring. Filtration and concentration under high vacuum gave the crude acid.

Acid 4c. Deprotection of **4a** (130 mg, 0.27 mmol) in MeOH (10 mL) containing Pd-C (130 mg) gave **4c** (70 mg, 76 %) as a gum (57 : 43 mixture of stereoisomers); [HRMS (FAB^+): Found : 353.1176. $C_{16}H_{21}N_2O_5S$

requires 353.1171]; ν_{max} (Film) 2977, 2932, 1751, 1716, 1368, 1163, 485 cm^{-1}; δ_H (acetone - d6) major isomer, 7.68 - 7.55 (3H, m), 7.38 (2H, m), 5.82 (1H, s, H-5), 4.82 (1H, m, H-3), 3.81 (1H, t, *J* 5.5 Hz, H-4), 3.59 (1H, dd, *J* 5.5, 3 Hz, H-4'), 1.4 (9H, s) - minor isomer, 5.71 (1H, s, H-5), 4.59 (1H, m, H-3), 3.70 (1H, t, *J* 5.5 Hz, H-4), 3.48 (1H, dd, *J* 5.5, 3 Hz,H-4'), 1.42 (9H, s); MS (FAB^+) *m/z* 353 (M + 1), 297, 251, 57; MS (FAB^-) *m/z* 351 (M - 1), 307, 234, 109.

Acid 10c. Deprotection of **10a** (53 mg, 0.14 mmol) in EtOH - EtOAc (80 : 20) containing Pd-C (13 mg) and N-ethyl morpholine (17 µL, 0.14 mmol) gave **10c** (48 mg, 95 %, N-ethyl morpholinium salt) as a 54 : 46 mixture of two stereoisomers; ν_{max} (Film) 3049, 2975, 2870, 1766, 1639, 1371, 1226 cm^{-1}; δ_H (CD_3OD) major isomer, 6.1 (1H, s, H-5), 5.08 (1H, dd, *J* 5.6, 2.5 Hz, H-3), 3.89 (4H, m), 3.74 (1H, t, *J* 5.6 Hz, H-4), 3.33 (1H, dd, *J* 5.6, 2.5 Hz, H-4'), 3.27 (4H, m), 3.18 (2H, q, *J* 7 Hz), 2.11 (3H, s), 1.97 (3H, s), 1.33 (3H, t, *J* 7 Hz) - minor isomer, 6.2 (1H, s, H-5), 4.92 (1H, dd, *J* 5.6, 2.5 Hz, H-3), 3.67 (1H, t, *J* 5.6 Hz, H-4), 3.43 (1H, dd, *J* 5.6, 2.5 Hz, H-4'); δ_C (CD_3OD) major isomer, 173.38 (CO acetate), 171.67 (CO amide), 169.99 (CO carboxylate), 169.23 (CO azetidinone), 75.1 (C-5), 65.14, 57.12 (C-3), 53.52, 52.56, 48.32 (C-4), 22.42, 20.77, 9.25 - minor isomer, 173.14 (CO acetate), 171.61 (CO amide), 170.1 (CO carboxylate), 169.56 (CO azetidinone), 75.35 (C-5), 57.43 (C-3), 47.38 (C-4), 22.3, 20.75; MS (FAB^-) *m/z* 243 (M - 1).

Acid 11c. Deprotection of **11a** (53 mg, 0.12 mmol) in EtOH - EtOAc (80 : 20) containing Pd-C (53 mg) and N-ethyl morpholine (16 µL, 0.12 mmol, 1 equiv.) gave **11c** (45 mg, 78 % purity, 71 % corrected yield) as a 58 : 42 mixture of two stereoisomers (N-ethyl morpholinium salts); [HRMS (FAB^+): Found : 410.1753. $C_{19}H_{28}N_3O_5S$ requires 410.1750]; ν_{max} (Film) 3055, 2973, 1751, 1669, 1654, 1370 cm^{-1}; δ_H (acetone-d6) major isomer, 7.50 (2H, d, *J* 7.3 Hz), 7.32 (3H, m), 5.75 (1H, s, H-5), 4.73 (1H, dd, *J* 5.5, 2.4 Hz, H-3), 3.89 (4H, m), 3.71 (1H, t, *J* 5.5 Hz, H-4), 3.38 (1H, dd, *J* 2.4, 5.5 Hz, H-4'), 3.11 (4H, m), 3.04 (2H, q, *J* 7 Hz), 1.87 (3 H, s), 1.29 (3H, t, *J* 7 Hz) - minor isomer, 7.54 (2H, d, *J* 7.3 Hz), 5.68 (1H, s, H-5), 4.96 (1H, dd, *J* 5.5, 2.4 Hz, H-3), 3.80 (1H, t, *J* = 5.5 Hz, H-4), 3.34 (1H, dd, *J* 5.5, 2.4 Hz, H-4'), 1.86 (3H, s); MS (FAB^+) *m/z* 410 (M + 1), 236, 185, 116.

Chemical reactivity

Products analysis by NMR. Solutions of the azetidinones (10^{-3} to 5 x 10^{-4} M) in deuterated phosphate buffer (25 mM, pH 7.5) containing DMSO-d6 (7 to 40%) were analyzed by ^1H NMR spectrometry at 500 MHz as a function of time.

Acid 19c. This acid (non isolated) was obtained from a 6.6.10^{-4} M solution of **19a** in deuterated phosphate buffer-DMSO-d6 (90:10) left for 2 h at 20°C; δ_H (D_2O, two diastereoisomers) 5.93 and 5.88 (1H, s, H-5), 4.86 and 4.84 (1H, dd, *J* 5.2, 2.1 Hz, H-3), 3.89 and 3.79 (1H, dd, *J* 6.7, 5.2 Hz, H-3), 3.44 and 3.38 (1H, dd, *J* 6.7, 2.1 Hz, H-3'), 1.97 and 1.96 (3H, s).

Acid 13c. This acid was transiently obtained from a 10^{-3} M solution of **13a** in deuterated phosphate buffer-DMSO-d6 (60:40). After 24 h of hydrolysis, the decarboxylation product **20** (crude) was identified by addition of water (D/H exchange) and lyophilization; δ_H ($CDCl_3$) 7.87 (2H, d, *J* 7.4 Hz), 7.65 (1H, t, *J* 7.4 Hz), 7.54 (2H, t, *J* 7.4 Hz), 6.1 (1H, d, *J* 7.3 Hz, NH), 4.83 (1H, ddd, *J* 7.3, 5.6, 2.8 Hz, H-3), 4.58 (1H, d, *J* 10 Hz, H-5), 4.47 (1H, d, *J* 10 Hz, H-5'), 3.82 (1H, t, *J* 5.6 Hz, H-4), 3.58 (1H, dd, *J* 5.6, 2.8 Hz, H-4'), 1.95 (3H, s); δ_C ($CDCl_3$) 170.13 (CO amide), 166.34 (CO azetidinone), 136.92, 134.64, 129.55, 128.47, 62.74 (C-5), 57.58 (C-3), 49.76 (C-4), 22.69.

Kinetics of p-nitrobenzyl ester hydrolysis. 100 µL of solutions of the azetidinones (between 2 x 10^{-3} and 5 x 10^{-4} M) in acetonitrile were added to 2 ml of phosphate buffer at pH 7. The wavelength of maximum absorbance change on hydrolysis was determined by recording the UV spectra every 2 minutes for 15 minutes. Then, the rates of hydrolysis were measured in phosphate (pH = 6.0 to 8.0) or borate (pH = 9.0) buffers. The logarithm of the first order rate constants extracted from the exponential absorbance change (k_{obs}) were plotted as a function of pH: for **11a**: λ = 240 nm, slope = 1.05 (r = 0.989), for **12a**: λ = 237 nm, slope = 0.94 (r = 0.96), for **16a**: λ = 240 nm, slope = 0.93 (r = 0.90) for **18a**: λ = 270 nm, slope = 1.06 (r = 0.936), for **19a**: λ = 279 nm, slope = 0.98 (r = 0.999). Except for **13a**, second order rate constants of hydroxide ion catalyzed hydrolysis were obtained from the ratios between k_{obs}s and hydroxide ion concentrations. For **17a**, λ = 240 nm, the first order rate constants were fitted by an non linear least square program to equation $k_{obs} = k_{OH}[OH] / (1 + K_b [OH])$, where $K_b = K_a/K_w$.

Evaluation of the inhibition of enzymes

Assay of RTEM β-lactamase. The following solutions were prepared: (i) phosphate buffer (50 mM, pH 7.5); (ii) β-lactamase : 5 10^{-8} to 10^{-8} M solution in phosphate buffer; (iii) benzylpenicillin (substrate): 3.5 10^{-4} M solution in phosphate buffer; (iv) 10^{-2} M solution of tested compound in acetonitrile. To the solution of substrate (2 ml) was added the solution of the tested compound (20 µl). When the potential inhibitor was protected as PNB ester, the mixture was left 2 h at 20°C for allowing ester hydrolysis. After addition of the solution of β-lactamase (200 µl), the absorbance decrease at 232 nm was recorded as a function of time.

Assay of NMCA β-lactamase. The following solutions were prepared: (i) phosphate buffer (50 mM, pH 7); (ii) NMCA β-lactamase : 6 10^{-8} M solution in phosphate buffer; (iii) imipenem (substrate) : 6.3 10^{-3} M solution in phosphate buffer; (iv) 10^{-2} M solution of tested compound in acetonitrile. The solution of the tested compound (20 µl) was diluted in phosphate buffer (1.6 ml) and left, if required, for 2 h at 20°C for PNB ester hydrolysis. After addition of the β-lactamase solution (200 µl), and incubation during a well-defined time, the solution of substrate (200 µl) was added. The absorbance change was measured at 300 nm.

Assay of 5/B/6 β-lactamase. The following solutions were prepared: (i) citrate buffer (50 mM and 11 mM in $ZnCl_2$, pH); (ii) benzylpenicillin (substrate) : 10^{-2} M solution in citrate buffer; (iii) β-lactamase : 10^{-7} M solution in citrate buffer; (iv) 10^{-2} M solution of tested compound in acetonitrile. The assay was performed as above with measurement at 232 nm.

Assay of Q980R β-lactamase. The following solutions were prepared: (i) phosphate buffer (50 mM, pH 7); (ii) cephaloridine (substrate) : 3 10^{-3} M solution in phosphate buffer; (iii) β-lactamase : 3 10^{-8} M solution in phosphate buffer; (iv) 10^{-2} M solution of tested compound in acetonitrile. The assay was performed as above with measurement at 260 nm.

Assay of OXA2 β-lactamase. The following solutions were prepared: (i) phosphate buffer (50 mM, pH 7); (ii) β-lactamase : 10^{-7} to 5 10^{-8} M solution in phosphate buffer; (iii) oxacilline (substrate) : 10^{-7} to 5 10^{-8} M solution in phosphate buffer; (iv) 10^{-2} M solution of tested compound in acetonitrile. The assay was performed as above with measurement at 260 nm.

Assay of PPE (porcine pancreatic elastase). The following solutions were prepared: (i) acetate buffer (50 mM, pH 5); (ii) TRIS buffer (100 mM, pH 7.5); (iii) 10^{-2} to 10^{-3} M solution of tested compound (I) in N-methyl pyrrolidone (NMP); (iv) 2 10^{-7} to 6 10^{-8} M solution of elastase (E) in acetate buffer ; (v) solution of N-succinyl-L-alanyl-L-alanyl-L-alanyl-p-nitroanilide (substrate (S); 2.5 mg in 50 µl of NMP) diluted with TRIS buffer (5 ml). To the solution of substrate (S) (2.1 ml) were added the solution of tested compound (I) (21 µl) and the solution of elastase (E) (70 µl). The appearance of the substrate hydrolysis product was measured as a function of time at 410 nm. Inhibition by **19a**: from the apparent rates k_{app} = 4.87 10^{-4} sec^{-1} in the presence of inhibitor (I = 10^{-4} M; E = 6.22 10^{-8} M; S = 5.38 10^{-4} M) and k_{app} = 5.5 10^{-4} sec^{-1} without inhibitor, we calculated 12% of inhibition. Inhibition by **18a**: from the apparent rates k_{app} = 2.95 10^{-4} sec^{-1} in the presence of inhibitor (I = 2 10^{-4} M; E = 3.9 10^{-8} M; S = 8.46 10^{-4} M) and k_{app} = 3.45 10^{-4} sec^{-1} without inhibitor, we calculated 16% of inhibition. Inhibition by **16a**: from the apparent rates k_{app} = 2.5 10^{-4} sec^{-1} in the presence of inhibitor (I = 10^{-4} M; E = 4.41 10^{-8} M; S = 10^{-3} M) and k_{app} = 3.9 10^{-4} sec^{-1} without inhibitor, we calculated 35% of inhibition.

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