Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 13 (2005) 6120–6132

4-Alkyliden-β-lactams conjugated to polyphenols: Synthesis and inhibitory activity

Gianfranco Cainelli,[a] Paola Galletti,[a] Spiridione Garbisa,[b,*] Daria Giacomini,[a,*] Luigi Sartor[b] and Arianna Quintavalla[a]

[a]*Department of Chemistry ''G. Ciamician'', University of Bologna, Via Selmi 2, 40126 Bologna, Italy*
[b]*Department of Experimental Biomedical Sciences, Medical School of Padova, Viale G. Colombo 3, 35121 Padova, Italy*

Received 7 April 2005; revised 9 June 2005; accepted 13 June 2005
Available online 3 August 2005

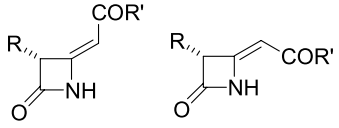
Abstract—A series of compounds combining the β-lactam and polyphenol scaffold have been prepared and evaluated for inhibition of human leukocyte elastase and matrix metallo-proteases MMP-2 and MMP-9. The design of these compounds has been based on the 'overlapping-type' strategy where two pharmacophores are linked in a single molecule. The most powerful compound against elastase was an *N*-galloyl-4-alkyliden β-lactam, [3-[1-(*tert*-butyl-dimethyl-silanyloxy)-ethyl]-4-oxo-1-(3,4,5-tris-benzyloxy-benzoyl)-azetidin-2-ylidene]-acetic acid ethylester, with an IC_{50} of 0.5 μM; while the most powerful against MMP-2 was a 4-alkyliden β-lactam arylated on the C-3 hydroxy side chain (3,5-bis-benzyloxy-4-hydroxy-benzoic acid 1-(2-benzyloxycarbonylmethylene-4-oxo-azetidin-3-yl)-ethyl ester) with an IC_{50} of 4 μM. Of the total 35 compounds tested, high levels of inhibition of elastase and of MMPs were separately exerted by distinct molecules.

1. Introduction

β-Lactam compounds really are 'evergreen' bioactive molecules. Starting from the antimicrobial potency exerted by naturally occurring bicyclic compounds (penicillins and cephalosporins), today new variants with monocyclic structure (azetidinones) are showing new and specific biological activities. In fact, β-lactam related compounds are irreversible inhibitors of a wide range of serine proteases including elastase,[1] β-lactamase,[2] phospholipase A2,[3] and bacterial signal peptidase,[4] the key step in this process being the acylation of the serine at the enzyme active site by the β-lactam ring.

We recently developed the synthesis of new monocyclic β-lactam derivatives, 4-alkyliden-azetidinones, characterized by a C=C bond on the C-4 position of the ring (Fig. 1).[5]

As compared to other β-lactam inhibitors, this novel structure was responsible for an increased inhibition of human leukocyte elastase (LE), a very potent degradative weapon released by inflammatory cells, and mediator of some severe pathologies such as pulmonary emphysema. Furthermore, some of these new derivatives were the first β-lactams shown to be active at micromolar concentrations against two matrix metalloproteases instrumental in cancer invasion and metastasis, MMP-2 and MMP-9.[6]



R = 1'-OTBS-ethyl, H, Cl, Br

R' = OEt, SPh, Ph, CH=CHPh

Figure 1.

In animal models, generic matrix protease inhibitors are able to reduce inflammatory tissue damage, and prevent tumor dissemination.[7] The possibility of acquiring an extraprotection against excessive proteolytic activities through designer drugs has led to the development of a number of synthetic inhibitors; however, when assayed in clinical trials, these compounds failed to live up to expectations, mostly because of their systemic toxicity.[8]

Keywords: β-Lactams; Polyphenols; Inhibitors; Gelatinase; Elastase.
* Corresponding authors. Tel.: +39 051 209 9528; fax: +39 051 209 9456 (D.G.); fax: +39 049 8276089 (S.G.); e-mail addresses: garbisa@unipd.it; daria.giacomini@unibo.it

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.06.041

Certain plant compounds can exert anti-inflammatory, anti-tumor growth, anti-angiogenic, anti-invasive, and anti-metastatic effects without apparent toxicity,[9–12] and (−) epigallocatechin-3-gallate (EGCG), the major flavonoid contained in green tea, has proved to be a good inhibitor of MMP-2 and MMP-9,[13] macrophage metallo-elastase MMP-12,[14] and leukocyte serine-elastase LE.[15]

To test whether the introduction of phenolic moieties to the β-lactam scaffold can increase binding affinity with specific enzymes, we have designed and synthesized new molecules with a central 4-alkyliden β-lactam scaffold to which aromatic—in particular, polyphenolic—branches are anchored, and for some, we have indeed found improved efficacy in inhibition of LE, MMP-2, and MMP-9.

2. Results and discussion

2.1. Chemistry

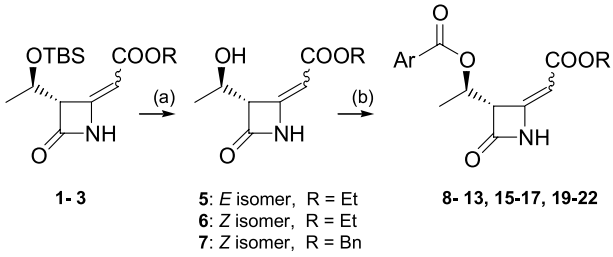
Our earlier work demonstrated that 4-alkyliden functionality is essential for switching on inhibitory properties.[6] In the design of these new compounds, we make use of a central 4-alkyliden β-lactam scaffold which presents three sites for introduction of molecular diversity: (1) the C-3 hydroxyethyl side chain, (2) the C-4 carboxy function, and (3) the β-lactam nitrogen atom.

The Lewis acid-mediated reaction of 4-acetoxy-azetidinones with α-diazo carbonyls represents the key step in the synthesis of 4-alkylidene-azetidinone precursors. Thus, compounds **1**–**4** were prepared starting from commercially available (3*R*,4*R*)-4-acetoxy-3-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one and the corresponding diazoacyl compound (Scheme 1) following a procedure already developed in our laboratory.[5]

The reaction proceeds smoothly, yielding *E* and/or *Z* isomers of the corresponding 4-alkylidene-β-lactams with ethyl- or benzyldiazoacetate. Critical to the success of the reaction was a stoichiometric amount of $TiCl_4$ or $AlCl_3$, and an excess of the diazo-compound associated with a requirement for trimethylsilyl protection of the β-lactam nitrogen atom.

The diastereomeric products *E* and *Z* were easily separated by column chromatography, allowing access to stereochemically pure compounds.

Treatment of **1**, **2**, and **3** with HCl in acetonitrile produced deprotected derivatives **5**–**7** (Scheme 2). The conditions of the O-acylation of these compounds required an acidic catalyst, because, as we demonstrated, 4-alkyliden β-lactams are quite sensitive to basic conditions.[16] Thus, the aromatic or phenolic function was introduced via a tin tetrachloride catalyzed reaction (Scheme 2).



Scheme 2. Reagents and conditions: (a) HCl 1 M, CH_3CN. (b) ArCOCl, $SnCl_4$ 5%, benzene, reflux.

Hydrogenolysis of benzyl esters and of *O*-benzyl phenols yielded the corresponding carboxylic acids and phenols **14**, **18**, **23**, **24**, and **25** (Scheme 3).

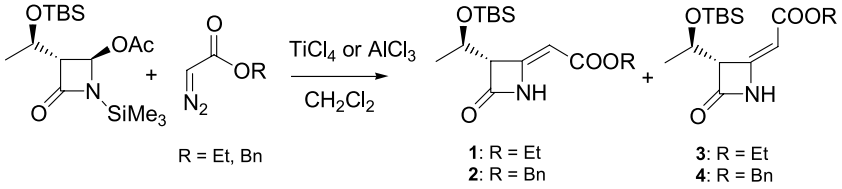
Starting from carboxylic acid **26**, obtained from benzyl ester **2** via hydrogenolysis, we introduced different aromatic moieties by treatment with amines, phenols, or benzyl alcohols (compounds **27**–**34**, Scheme 4).

Starting from the 4-alkyliden β-lactams **3** and **4**, we obtained N-benzoylated derivatives **35**–**39** by coupling with the corresponding benzoyl chlorides (Scheme 5). Compounds **37** and **38** were subsequently treated with H_2 and Pd on C, giving the fully debenzylated derivatives **40** and **41**, respectively (Scheme 5).

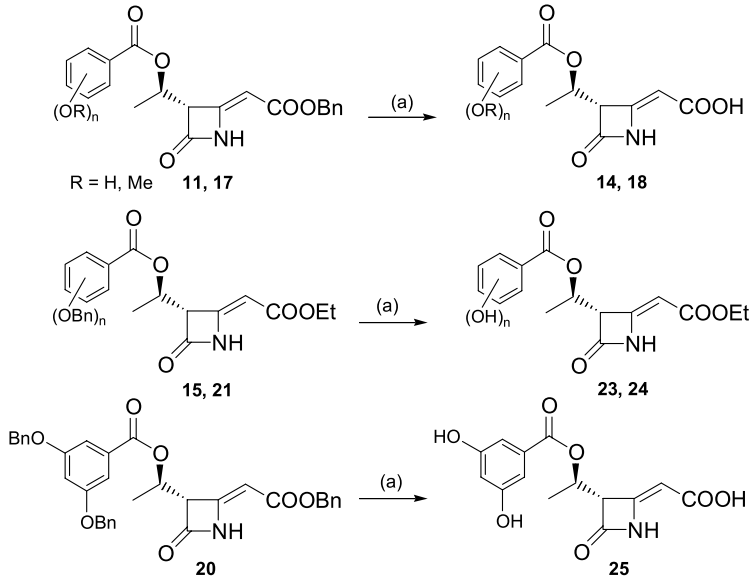
All compounds were purified by silica gel chromatography and new derivatives fully characterized (see Section 5).

3. Biochemistry

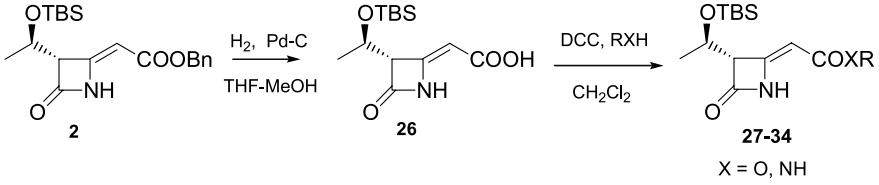
The inhibition exerted on human LE and gelatinases MMP-2 and MMP-9 by the various molecules was assayed using a chromogenic substrate for elastase, and gelatin zymography for the gelatinases, as described in Section 5. The results are shown in the tables as percentage of inhibition at 100 μM and IC_{50} values: Table 1 reports data for 4-alkyliden β-lactams arylated on the hydroxy side chain, Table 2 for those modified on the C-4 side chain, and Table 3 for *N*-benzoyl-4-alkyliden β-lactams.



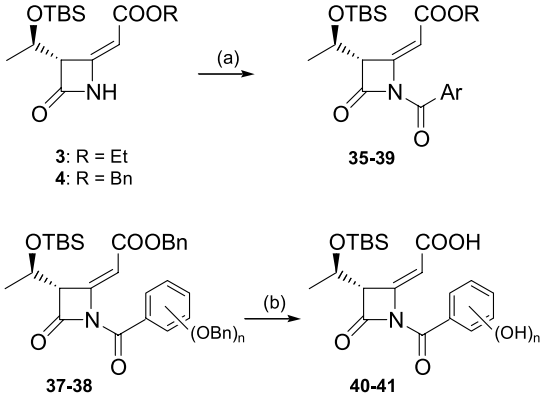
Scheme 1.



Scheme 3. Reagents: (a) H_2, Pd on C, THF–MeOH.



Scheme 4.



Scheme 5. Reagents: (a) ArCOCl, K_2CO_3, acetone. (b) H_2, Pd on C, THF–MeOH.

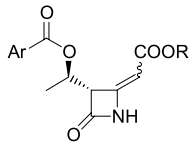
When used at 100 μM, the majority of the 18 compounds of the first group (Table 1) showed a certain degree of LE inhibition, but for six of them the inhibition was greater than 75%, with compounds **15** and **22** close to 100%, and **15** showing the lowest IC_{50} (1 μM). The substitution pattern on the C-3 aromatic fragment was important; in particular a higher number of substituents afforded higher activity. It should be underlined that the inhibitory potency against LE increases with hydrophobic substituents on the aromatic fragment (compounds **13**, **15**, and **22**), with a significant effect of the *para*-benzyloxy one (cf. compounds **19**, **21**, and **15**). Among the OH derivatives, only compound **23**, characterized by a galloyl fragment, presented a significant potency. Regarding the substituent R on the 4-alkyliden-carboxy side chain, in general benzyl esters exerted a better inhibitory activity, while carboxylic acid had a lower potency.

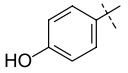
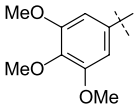
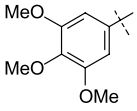
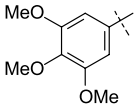
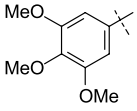
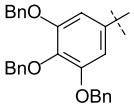
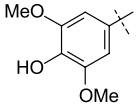
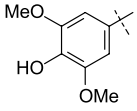
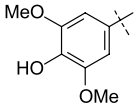
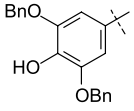
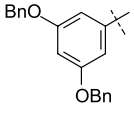
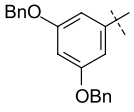
Four compounds in Table 1 showed some activity against MMPs. Only two members of this group—namely **19** and **23**—were effective in blocking MMP-2 activity, with **19** showing the lowest IC_{50} (4 μM), but **23** significant against MMP-9 with an IC_{50} of 50 μM. If we compare **19**, **21**, and **23**, it is interesting to note that the structural motif that switched on the inhibition against MMPs was the 3,4,5-trihydroxy framework (galloyl moiety) with improved and specific activity of the 3,5-dibenzyloxy-4-hydroxy derivative **19** against MMP-2. Moreover, when compared to EGCG—which has a 15 μM IC_{50} against MMP-2—compound **19** represents an excellent inhibitor with a potency equivalent to EGCG and the best of the β-lactams.

Data in Table 2 show that modifications of the C-4 carboxy residue had little influence on the anti-LE activity, even if of the 10 members of this group **27** and **34** are worthy of mention, with LE inhibition percentages at 100 μM of 74 and 81, respectively.

Structural modifications on C-4 conferred anti-gelatinasic potency to some extent, even if the majority of these derivatives showed weak inhibition of MMP-2 and

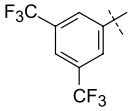
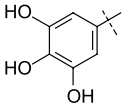
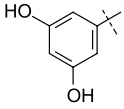
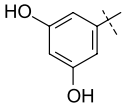
Table 1. 4-Alkyliden β-lactams arylated on the C-3 hydroxy side chain: human LE, MMP-2, and MMP-9 inhibition results



Compound	Ar	R	Isomer	Human LE		MMP-2		MMP-9	
				Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)
8		Et	*Z*	11	—	—	—	—	—
9		Et	*E*	39	—	—	—	—	—
10		Et	*Z*	29	—	—	—	—	—
11		Bn	*Z*	50	50	—	—	—	—
12		Et	*Z*	82	25	33	—	24	—
13		Et	*E*	50	100	—	—	—	—
14		H	*Z*	11	—	—	—	—	—
15		Et	*Z*	98	1	43	—	10	—
16		Et	*Z*	15	—	—	—	—	—
17		Bn	*Z*	77	10	—	—	—	—
18		H	*Z*	59	60	—	—	—	—
19		Et	*Z*	59	25	81	4	36	—
20		Bn	*Z*	79	15	—	—	—	—
21		Et	*Z*	58	n.d.[a]	—	—	—	—

(*continued on next page*)

Table 1 (*continued*)

Compound	Ar	R	Isomer	Human LE		MMP-2		MMP-9	
				Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)
22		Et	*Z*	97	6	—	—	—	—
23		Et	*Z*	82	20	61	70	70	50
24		Et	*Z*	5	—	—	—	—	—
25		H	*Z*	37	—	—	—	—	—

[a] n.d., not determined, due to non-constant slope of the inhibition exerted.

MMP-9 activity. Only compounds **29** and **33** had significant potency against MMP-2, with IC_{50} of 40 and 70 μM, respectively.

Acylation of the β-lactam nitrogen atom is crucial for LE inhibition; in fact, five out of seven members of the third group (Table 3) exerted an LE inhibition >75% at 100 μM, with **36** close to 100% and showing the best IC_{50} (0.5 μM). This is an important result given that one of the best inhibitors of LE was EGCG, with an IC_{50} of 0.3 μM. Ethyl ester **36** worked better than benzyl ester **37** with a 100-fold improvement in IC_{50}. Results in Table 3 confirm the preference for LE inhibitor of a higher lipophilic character, with the exception of **41**.

We have already demonstrated the efficiency of N-acylation for LE inhibition.[6] The electron-withdrawing character of this N1 side chain should have an influence on the β-lactam reactivity, that is, the sensitiveness of the C-2 carbonyl toward nucleophilic attack.[17] This fact has a counterpart in a lower stability of these compounds at pH values higher than 7.8, and in a higher sensitivity to alcohols.[18]

Gelatinases MMP-2 and MMP-9 are poorly inhibited by compounds in Table 3, with the exception of **40** which, bearing a galloyl moiety, showed >90% inhibition at 100 μM, and an IC_{50} of 15 μM against MMP-2, and 70 against MMP-9. The inhibition of MMPs by 4-alkyliden β-lactams could depend on a specific interaction with the NH of the azetidinone;[6] however, the special characteristics of compound **40** could result from the presence of the 3,4,5-phenolic triad that may replace the loss of NH interaction in this N-acylated compound.

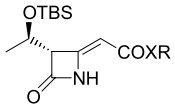
The results in Tables 1–3 revealed that the potency of these new 4-alkyliden β-lactams was specifically located at particular substitutions: N-acylation with lipophilic benzyloxy-galloyl moiety against LE, whereas for MMPs at *O*-galloyl or *N*-galloyl derivatives with free phenolic OH.

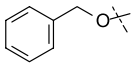
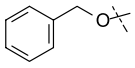
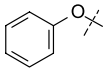
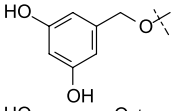
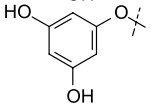
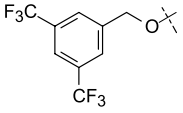
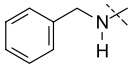
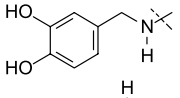
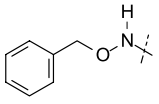
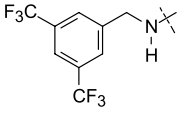
The K_i was studied for one member of each group against both LE and MMP-2 (the most sensitive of the two gelatinases). With the exception of compounds **29** and **40** (see below), the K_i was in good agreement with the calculated IC_{50}, and indicates that the type of inhibition was non-competitive, as shown by the example, double-reciprocal plots in Figure 2 and Table 4. The non-competitive inhibition inferred for these compounds indicates that the inhibitory effects observed are due to allosteric binding rather than binding to the active site.[19]

In the case of **29** and **40**, the chromogenic assay for MMP-2 gave absorbance values higher in the presence of compound (potential inhibitor) than in its absence, preventing full deduction of the K_i (n.d. in Table 4); comprehension of this unexpected effect requires further investigation.

The most active compounds of each group were tested for short-term (16 h) cytotoxicity at 1, 10, and 100 μM on non-transformed breast epithelial (HBL-100) and fibrosarcoma (HT-1080) cell lines. As shown in Table 4, the most potent compound against LE (**36**, IC_{50} 0.5 μM) was totally non-cytotoxic on non-transformed cells when used at concentration 20× its IC_{50}, whereas the cell viability of transformed cells was affected 14% at the same concentration. The second most effective compound against LE, **15** (IC_{50} 1 μM), was completely non-cytotoxic on non-transformed cells even when used at concentration 100× its IC_{50}, whereas it appreciably affected the viability of transformed cells even at 10×. Compound **27**, the most effective of the second group against LE, was considerably cytotoxic on both cell lines at 4× its IC_{50} (25 μM). The most effective compound against MMP-2 was **19**, showing a K_i of

Table 2. 4-Alkyliden β-lactams modified on the C-4 side chain: human LE, MMP-2, and MMP- 9 inhibition results



Compound	XR	Human LE		MMP-2		MMP-9	
		Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)
2		48	—	—	—	—	—
4		32[a]	—	—	—	—	—
27		74	25	4	—	—	—
28		—	—	46	—	30	—
29		—	—	65	40	—	—
30		40	—	32	—	18	—
31		26	—	13	—	5	—
32		19	—	47	—	4	—
33		43	—	55	70	20	—
34		81	35	—	—	—	—

[a] Compound **4** has an *E* geometry of the C=C bond.

1.6 μM, with only 26% in viability at 100 μM on HBL-100 cells (Table 4).

4. Conclusions

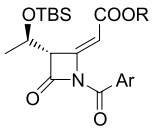
In summary, we designed and synthesized a series of substituted 4-alkyliden β-lactams combining the β-lactam with a polyphenol scaffold, and evaluated these new compounds for inhibition of human LE and matrix metallo-proteases, MMP-2 and MMP-9. The presence of polyphenolic substituents in these β-lactams was determinant for the corresponding biochemical activity: the most potent non-competitive inhibitor against LE (IC_{50} 0.5 μM) was **36**, an *N*-galloyl-4-alkyliden-β-lactam. While the latter results to be thus the best β-lactam-based inhibitor of human LE (the reference leader-compound azetidin-2-one L-680,833 shows, in fact, an IC_{50} of 9 μM[20]), compound **19** represents the best inhibitor of MMP-2, with an IC_{50} of 4 μM. Of the total 35 compounds tested, high levels of inhibition of elastase and MMPs were separately exerted by distinct molecules, and in absence of cytotoxic effects.

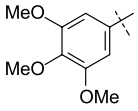
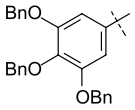
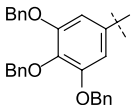
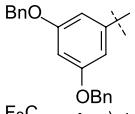
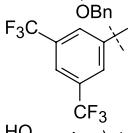
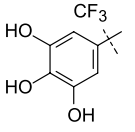
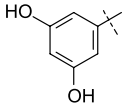
5. Experimental

5.1. General

Commercial reagents were used as received without additional purification. Anhydrous solvents were obtained commercially and used without further drying. ^{1}H and ^{13}C NMR values were recorded on a VARIAN Mercury 400, INOVA 300 or GEMINI 200 instrument using a

Table 3. *N*-benzoyl-4-alkyliden β-lactams: human LE, MMP-2, and MMP-9 inhibition results



Compound	Ar	R	Human LE		MMP-2		MMP-9	
			Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)	Inhibition % at 100 μM	IC_{50} (μM)
35		Et	94	4.5	15	—	40	—
36		Et	97	0.5	25	—	—	—
37		Bn	85	35	21	—	21	—
38		Bn	2	—	—	—	—	—
39		Et	78	8	15	—	—	—
40		H	24	—	92	15	52	70
41		H	87	9	—	—	—	—

5 mm probe. All chemical shifts have been quoted relative to deuterated solvent signals, delta in ppm, *J* in Hz. FT-IR: Nicolet 205 measured as films or nujol mull between NaCl plates and reported in cm^{-1}. TLC: Merck 60 F_{254}; Column chromatography: Merck silica gel 200–300 mesh, HPLC–MS: HPLC: Agilent Technologies HP1100, column ZOBRAX-Eclipse XDB-C8 Agilent Technologies. The products were eluted with CH_3CN/H_2O, gradient from 30 to 80% of CH_3CN, 0.5 mL min^{-1}. Some acid products were eluted with CH_3CN/H_2O mixture containing 0.1% of formic acid. MS: Agilent Technologies MSD1100 single-quadrupole mass spectrometer, full-scan mode from *m*/*z* 50 to *m*/*z* 2600, scan time 0.1 s in positive ion mode, ESI spray voltage 4500 V, nitrogen gas 35 psgi, drying gas flow 11.5 mL min^{-1}, fragmentor voltage 20 V. The $[\alpha]_D^{25}$ was determined with a Perkin Elmer 343 polarimeter. Melting points were determined using a Büchi apparatus and are uncorrected.

5.2. Materials for biological assays

Elastase from human leukocytes (SE563) was from Elastin Products Company, Owensville MO, USA; elastase substrate *N*-methoxysuccinyl-ala-ala-pro-val *p*-nitroanilide was from Sigma.

5.3. Cytotoxicity test

Human breast epithelial (HBL-100) and fibrosarcoma (HT-1080) cells were used for cytotoxicity tests. Cells (10^4) were seeded onto 96-well plastic plates (Falcon, BD Bioscience) and incubated at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, with or without 1, 10, and 100 μM β-lactams. After 16 h, the number of viable cells was determined by CellTiter 96® assay (Promega, Madison WI, USA) and expressed as ratio of absorbance (A_{490}) of treated versus control cells, as already reported.[1]

5.4. Substrate degradation by LE

Human LE was solubilized (250 mU mL^{-1}) in Hepes buffer (0.1 M Hepes, 0.5 M NaCl, and 10% DMSO, pH 7.8). All the compounds and elastase substrate were freshly prepared 20× in DMSO. Dilutions of the compounds were pre-mixed with the enzyme in micrometers wells, and maintained for 15 min at 4 °C. Then, 5 μL of the substrate (8 mM concentrated) were added to 100 μL final volume, and the mixture was incubated at 37 °C. At 20-min intervals, the intensity of the color developed by the digested substrate was measured at

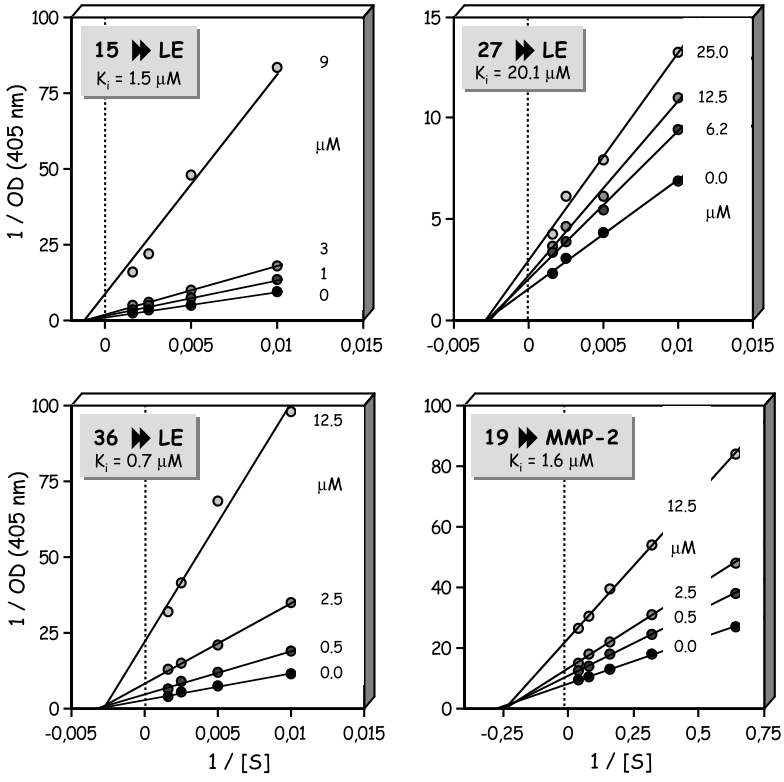


Figure 2. Double-reciprocal plots demonstrating non-competitive inhibition of human LE (**15**, **27**, **36**) and MMP-2 (**19**) by increasing concentration of compound (S substrate), and the calculated K_i. Examples of triplicate experiments, analyzed at 60 min; the points represent the mean values of three wells, with SD <10%.

Table 4. Inhibition constant (K_i) and short-term (16 h) cytotoxicity of some 4-alkyliden β-lactams on non-transformed epithelial (HBL-100) and transformed fibrosarcoma (HT-1080) cells, expressed as percent viability

Compound	K_i (μM)	HBL-100			HT-1080		
		Compound conc. (μM)			Compound conc. (μM)		
		1	10	100	1	10	100
15	1.5[a]	100	100	100	99	62	55
27	20.1[a]	100	92	27	97	91	41
36	0.7[a]	100	100	41	95	86	1
19	1.6[b]	100	94	74	100	90	61
29	n.d.	99	98	2	100	88	14
40	n.d.	91	73	23	97	95	72

n.d., not determined.
[a] Inhibition constant for LE.
[b] Inhibition constant for MMP-2.

405 nm using a Titertek Mutiskan (Flow Laboratories), and the control background subtracted (in triplicate experiments). The reactions developed linearly for as long as 120 min; data from 60 min were used for determining all IC_{50}s. Double-reciprocal plot of the results allowed deduction of the type and K_i of inhibition exerted over LE by compounds **15**, **27**, and **36**.

5.5. Zymographic analysis

Aliquots of gelatinase-containing medium conditioned by human neuroblastoma cells (for MMP-2) or HT-1080 human fibrosarcoma cells (for MMP-9) were assayed as described.[5] Without heating the samples, zymography was performed by electrophoresing 15 μL of medium in 0.1% gelatin-containing 8% polyacrylamide, in the presence of SDS. After electrophoresis, the gels were washed twice for 15 min with 2.5% Triton X-100, incubated overnight at 37 °C in Tris buffer (50 mM Tris–HCl, 200 mM NaCl, and 10 mM $CaCl_2$, pH 7.4). For gelatinase inhibition assays, compounds were freshly solubilized in DMSO and diluted in Tris buffer used for developing the zymogram. The gel slab was then cut into slices corresponding to the lanes and then put in different tanks containing the stated concentrations of inhibitors.

The gels then were stained for 30 min with 30% methanol/10% acetic acid containing 0.5% Coomassie brilliant blue R-250, and destained in the same solution without

dye. Clear bands on the blue background represent areas of gelatinolysis. Digestion bands were quantitated using an image analyzer system with GelDoc 2000 and Quantity One software (Bio-Rad); the densitometric values were expressed as percentage of control bands on the same gel, and the values used for IC_{50} determination.

5.6. Classification of MMP-2 inhibition

To further characterize the type of inhibition exerted by β-lactams **19**, **29**, and **40** (those with the lowest IC_{50} of the respective group) on the gelatinase, a BIOTRAK™ MMP-2-activity assay system (Amersham Pharmacia Biotech) was used, following the manufacturer's instructions, with slight modifications.[21] The microtitre wells—coated with anti-MMP-2 antibodies—were saturated with the enzyme contained in 50 μL of medium conditioned by SK-N-BE human neuroblastoma cells; after overnight incubation at 4 °C and exhaustive washing, the gelatinase was activated by treatment with 0.25 mM APMA. The wells were then incubated at 37 °C in the presence of increasing amount of a peptide substrate, with increasing concentrations of compound, and the intensity of the color developed by the digested substrate was measured at 405 nm after 4 h. A double-reciprocal plot of the results allowed the type and constant (K_i) of inhibition to be deduced.

Compounds **2** and **4** have been prepared according to the analogous previously reported procedure[5] using benzyldiazoacetate[22] as diazocarbonyl compound.

5.6.1. Compound 2. The obtained yield 54%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.06 (s, 3H), 0.07 (s, 3H), 0.87 (s, 9H), 1.31 (d, J = 6.3 Hz, 3H), 3.67 (d, J = 5.1 Hz, 1H), 4.24 (dr q, J = 5.1 Hz, J = 6.3 Hz, 1H), 5.16 (d, J = 12.3 Hz, 1H), 5.21 (d, J = 12.3 Hz, 1H), 5.28 (s, 1H), 7.37 (m, 5H), 8.45 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −5.1, −4.3, 17.9, 22.2, 25.6, 64.8, 65.0, 65.8, 90.2, 127.9, 128.1, 128.5, 136.1, 153.8, 166.5, 166.9. $[\alpha]_D^{25}$ −14.6 (c 2.42, $CHCl_3$). IR (film): 3295, 1818, 1700, 1653, 1264 cm^{-1}. HPLC–MS: t_R = 27.93 min, m/z: 377.3 $[M+2H]^+$, 399.4 $[M+H+Na]^+$, 414.5 $[M+K]^+$.

5.6.2. Compound 4. The obtained yield 6%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.09 (s, 3H), 0.10 (s, 3H), 0.91 (s, 9H), 1.21 (d, J = 6.3 Hz, 3H), 4.12 (m, 1H), 4.65 (dr q, J = 4.2 Hz, J = 6.3 Hz, 1H), 5.17 (s, 2H), 5.36 (d, J = 1.2 Hz, 1H), 7.37 (m, 5H), 7.53 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ −5.0, −4.8, 17.9, 19.4, 25.7, 64.6, 64.7, 65.7, 92.0, 127.9, 128.1, 128.5, 136.1, 153.7, 166.1, 167.9. $[\alpha]_D^{25}$ +92.6 (c 1.63, $CHCl_3$). IR (film): 3263, 1822, 1715, 1652, 1258 cm^{-1}. HPLC–MS: t_R = 25.99 min, m/z: 377.3 $[M+2H]^+$, 399.4 $[M+H+Na]^+$, 789.3 $[2M+K]^+$.

Compounds **8**–**13**, **15**–**17**, and **19**–**22** were obtained by $SnCl_4$ catalyzed O-acylation of 3-hydroxyethyl chain following the representative procedure: compound **1** (0.313 g, 1 mmol) was dissolved in CH_3CN (6 mL) and HCl 1 N (1 mL) was added. The reaction was monitored by TLC and further 1 mL portions of HCl 1 N as added until total conversion. After, saturated NaCl solution was added and the mixture extracted with CH_2Cl_2 (5× 10 mL), dried on Na_2SO_4, concentrated and the residue purified, when necessary, by flash chromatography (cyclohexane/ethylacetate 4:6) or by trituration in pentane/Et_2O. Yield 87%.

The desilylated compound **6** (0.045 g, 0.226 mmol) was dissolved in anhydrous benzene (4 mL), then 3,4,5-trimethoxybenzoyl chloride (0.104 g, 0.452 mmol) and finally $SnCl_4$ (0.011 mmol, 12 μL of a solution 1 M in CH_2Cl_2) were added. The solution was heated to reflux and stirred for 4 h. The reaction mixture was then quenched in a solution of $NaHCO_3$ (5%), extracted with ethylacetate (4× 10 mL), dried on Na_2SO_4 and concentrated. The residue was purified by flash chromatography (cyclohexane/ethylacetate 8:2) to give compound **12**.

5.6.3. Compound 8. The obtained yield 55%, white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.29 (t, J = 7.0 Hz, 3H), 1.54 (d, J = 6.6 Hz, 3H), 4.04 (d, J = 6.6 Hz, 1H), 4.19 (q, J = 7.0 Hz, 2H), 5.22 (s, 1H), 5.51 (quintet, J = 6.6 Hz, 1H), 7.40–7.63 (m, 3H), 8.03 (m, 2H), 8.67 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 14.3, 18.1, 60.3, 61.5, 67.5, 90.9, 128.4, 129.6, 129.7, 133.2, 151.4, 164.5, 165.2, 166.8. $[\alpha]_D^{25}$ −46.2 (c 0.70, $CHCl_3$). IR (film): 3397, 1819, 1706, 1653 cm^{-1}. HPLC–MS: t_R = 11.13 min, m/z: 260.1 $[M-43]^+$, 304.1 $[M+H]^+$, 321.2 $[M+H_2O]^+$, 326.1 $[M+Na]^+$, 342.0 $[M+K]^+$, 629.2 $[2M+Na]^+$. mp 125–127 °C.

5.6.4. Compound 9. The obtained yield 20%, white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.33 (t, J = 6.8 Hz, 3H), 1.45 (d, J = 6.6 Hz, 3H), 4.24 (q, J = 6.8 Hz, 2H), 4.48 (d, J = 4.6 Hz, 1H), 5.41 (d, J = 1.0 Hz, 1H), 5.96 (dr q, J = 4.6 Hz, J = 6.6 Hz, 1H), 7.42–8.14 (m, 6H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 14.4, 15.4, 60.4, 61.6, 66.8, 93.6, 128.3, 129.7, 133.0, 133.6, 150.6, 164.0, 165.5, 167.0. $[\alpha]_D^{25}$ +24.0 (c 0.70, $CHCl_3$). IR (film): 1818, 1712, 1652 cm^{-1}. HPLC–MS: t_R = 11.78 min, m/z: 260.3 $[M-43]^+$, 304.1 $[M+H]^+$, 321.0 $[M+H_2O]^+$, 326.1 $[M+Na]^+$, 607.5 $[2M+H]^+$, 629.2 $[2M+Na]^+$. mp 119–121 °C.

5.6.5. Compound 10. Yield 36%, white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.29 (t, J = 7.2 Hz, 3H), 1.52 (d, J = 6.2 Hz, 3H), 4.02 (d, J = 6.6 Hz, 1H), 4.20 (q, J = 7.2 Hz, 2H), 5.22 (d, J = 0.8 Hz, 1H), 5.49 (quintet, J = 6.2 Hz, 1H), 5.98 (br s, 1H), 6.86 (m, 2H), 7.92 (m, 2H), 8.67 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 14.3, 18.1, 60.4, 61.6, 67.1, 91.0, 115.3, 122.0, 132.0, 151.5, 160.2, 164.8, 165.0, 166.9. $[\alpha]_D^{25}$ −22.1 (c 0.50, $CHCl_3$). IR (film): 3337, 1825, 1706, 1660 cm^{-1}. HPLC–MS: t_R = 9.43 min, m/z: 276.0 $[M-43]^+$, 320.0 $[M+H]^+$, 337.1 $[M+H_2O]^+$, 342.0 $[M+Na]^+$, 661.1 $[2M+Na]^+$. mp 161–163 °C.

5.6.6. Compound 11. The obtained yield 78%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.54 (d, J = 6.3 Hz, 3H), 3.87 (s, 6H), 3.91 (s, 3H), 4.04 (d, J = 6.3 Hz, 1H), 5.18 (s, 2H), 5.31 (s, 1H), 5.49 (quintet, J = 6.3 Hz, 1H), 7.27 (m, 2H), 7.36 (m, 5H), 8.91 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 18.1, 56.1, 60.8,

61.7, 66.2, 67.3, 90.5, 106.8, 124.5, 128.2, 128.3, 128.6, 135.7, 142.5, 152.3, 152.9, 164.7, 164.8, 166.7. $[\alpha]_D^{25}$ +31.1 (*c* 4.25, CH_3OH). IR (film): 3294, 1824, 1720, 1653 cm^{-1}. HPLC–MS: t_R = 12.00 min, *m/z*: 412.1 $[M-43]^+$, 456.0 $[M+H]^+$, 473.1 $[M+H_2O]^+$, 478.1 $[M+Na]^+$, 494.0 $[M+K]^+$, 933.3 $[2M+Na]^+$.

5.6.7. Compound 12. The obtained yield 50%, white solid. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (t, *J* = 7.2 Hz, 3H), 1.54 (d, *J* = 6.3 Hz, 3H), 3.90 (s, 6H), 3.92 (s, 3H), 4.03 (d, *J* = 6.3 Hz, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 5.24 (d, *J* = 0.9 Hz, 1H), 5.49 (quintet, *J* = 6.3 Hz, 1H), 7.20 (m, 2H), 8.61 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.3, 18.1, 56.2, 60.3, 60.9, 61.7, 67.4, 91.0, 106.9, 124.6, 142.6, 151.5, 153.0, 164.6, 164.9, 166.9. $[\alpha]_D^{25}$ −16.4 (*c* 0.60, $CHCl_3$). IR (film): 3297, 1825, 1700, 1660, 1222 cm^{-1}. HPLC–MS: t_R = 12.78 min, *m/z*: 196.2 $[M-197]^+$, 417.2 $[M+H+Na]^+$. mp 142–144 °C.

5.6.8. Compound 13. The obtained yield 25%, white solid. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.33 (t, *J* = 7.0 Hz, 3H), 1.44 (d, *J* = 6.2 Hz, 3H), 3.91 (s, 3H), 3.93 (s, 6H), 4.23 (q, *J* = 7.0 Hz, 2H), 4.45 (m, 1H), 5.40 (d, *J* = 1.2 Hz, 1H), 5.93 (m, 1H), 7.40 (m, 2H), 7.70 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.3, 15.4, 56.2, 60.4, 60.9, 61.5, 67.0, 93.5, 107.1, 107.4, 124.1, 125.1, 150.9, 153.0, 165.2, 165.7. $[\alpha]_D^{25}$ +22.0 (*c* 0.70, $CHCl_3$). IR (film): 3271, 1820, 1713, 1660, 1222 cm^{-1}. HPLC–MS: t_R = 11.30 min, *m/z*: 350.3 $[M-43]^+$, 394.2 $[M+H]^+$, 411.2 $[M+H_2O]^+$, 416.3 $[M+Na]^+$, 809.3 $[2M+Na]^+$. mp 144–146 °C.

5.6.9. Compound 15. The obtained yield 51%, white solid. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.27 (t, *J* = 7.0 Hz, 3H), 1.52 (d, *J* = 6.6 Hz, 3H), 4.00 (d, *J* = 6.2 Hz, 1H), 4.15 (m, 2H), 5.14 (s, 6H), 5.20 (s, 1H), 5.45 (quintet, *J* = 6.6 Hz, 1H), 7.20–7.50 (m, 17H), 8.55 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.5, 18.3, 60.6, 61.8, 67.7, 71.5, 75.4, 91.3, 109.5, 124.9, 127.8, 128.2, 128.3, 128.5, 128.8, 136.8, 137.6, 143.1, 151.7, 152.8, 164.8, 165.0, 167.2. $[\alpha]_D^{25}$ −24.0 (*c* 1.00, $CHCl_3$). IR (film): 3271, 1825, 1693, 1646, 1587 cm^{-1}. HPLC–MS: t_R = 28.62 min, *m/z*: 460.4 $[M-161]^+$, 645.3 $[M+H+Na]^+$, 661.2 $[M+H+K]^+$, 1266.3 $[2M+H+Na]^+$. mp 144–146 °C.

5.6.10. Compound 16. The obtained yield 51%, pale yellow oil. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.27 (t, *J* = 7.2 Hz, 3H), 1.52 (d, *J* = 6.2 Hz, 3H), 3.92 (s, 6H), 4.02 (d, *J* = 6.4 Hz, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 5.22 (s, 1H), 5.47 (quintet, *J* = 6.4 Hz, 1H), 6.00 (s, 1H), 7.28 (m, 2H), 8.75 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.2, 18.1, 56.3, 60.3, 61.6, 67.2, 90.8, 106.6, 120.5, 139.6, 146.6, 151.7, 164.9, 165.0, 167.0. $[\alpha]_D^{25}$ −29.2 (*c* 1.33, $CHCl_3$). IR (film): 3295, 1820, 1699, 1653, 1229 cm^{-1}. HPLC–MS: t_R = 9.44 min, *m/z*: 336.2 $[M-43]^+$, 380.1 $[M+H]^+$, 397.2 $[M+H_2O]^+$, 402.2 $[M+Na]^+$, 418.1 $[M+K]^+$, 781.2 $[2M+Na]^+$.

5.6.11. Compound 17. The obtained yield 60%, pale yellow oil. ^1H NMR (acetone-d_6, 300 MHz): δ 1.52 (d, *J* = 6.3 Hz, 3H), 3.82 (s, 6H), 4.23 (d, *J* = 5.1 Hz, 1H), 5.18 (s, 2H), 5.34 (s, 1H), 5.45 (quintet, *J* = 6.3 Hz, 1H), 7.20–7.40 (m, 7H), 8.10 (br s, 1H), 9.94 (br s, 1H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 18.0, 56.0, 62.0, 65.4, 67.2, 89.9, 107.2, 120.3, 128.2, 128.4, 128.7, 137.2, 141.3, 147.8, 153.7, 164.8, 165.9, 166.2. $[\alpha]_D^{25}$ −10.1 (*c* 1.58, $CHCl_3$). IR (film): 3295, 1820, 1700, 1658, 1611, 1227 cm^{-1}. HPLC–MS: t_R = 10.47 min, *m/z*: 398.1 $[M-43]^+$, 442.1 $[M+H]^+$, 459.2 $[M+H_2O]^+$, 480.2 $[M+K]^+$, 905.2 $[2M+Na]^+$.

5.6.12. Compound 19. The obtained yield 14%, pale yellow oil. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.17 (t, *J* = 7.2 Hz, 3H), 1.42 (d, *J* = 6.2 Hz, 3H), 3.89 (d, *J* = 6.6 Hz, 1H), 4.07 (m, 2H), 5.08 (s, 4H), 5.11 (s, 1H), 5.34 (quintet, *J* = 6.6 Hz, 1 H), 5.95 (br s, 1 H), 7.20–7.40 (m, 12H), 8.52 (br s 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 14.3, 18.1, 60.3, 61.6, 67.3, 71.5, 90.9, 108.9, 120.3, 127.8, 128.3, 128.6, 136.1, 140.6, 145.9, 151.4, 164.5, 164.7, 166.8. $[\alpha]_D^{25}$ −28.3 (*c* 0.60, $CHCl_3$). IR (film): 3510, 3277, 1819, 1706, 1660 cm^{-1}. HPLC–MS: t_R = 16.34 min, *m/z*: 334.2 $[M-197]^+$, 460.2 $[M-43-29]^+$, 489.4 $[M+H-43]^+$, 555.2 $[M+H+Na]^+$, 571.3 $[M+H+K]^+$, 1086.3 $[2M+H+Na]^+$.

5.6.13. Compound 20. The obtained yield 23%, pale yellow oil. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.54 (d, *J* = 6.4 Hz, 3H), 4.02 (d, *J* = 6.2 Hz, 1H), 5.08 (s, 4H), 5.12 (d, *J* = 12.4 Hz, 1H), 5.19 (d, *J* = 12.4 Hz, 1H), 5.27 (s, 1H), 5.49 (quintet, *J* = 6.2 Hz, 1H), 6.84 (m, 1H), 7.28–7.47 (m, 17H), 8.60 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 18.1, 61.5, 66.2, 67.6, 70.3, 90.6, 107.5, 108.5, 127.6, 128.1, 128.2, 128.3, 128.6, 131.5, 135.7, 136.3, 152.0, 159.8, 164.4, 164.9, 166.6. $[\alpha]_D^{25}$ −29.8 (*c* 0.64, $CHCl_3$). IR (film): 3294, 1821, 1699, 1655, 1445 cm^{-1}. HPLC–MS: t_R = 22.05 min, *m/z*: 578.2 $[M+H]^+$, 595.2 $[M+H_2O]^+$, 600.0 $[M+Na]^+$, 616.1 $[M+K]^+$, 1178.4 $[2M+H+Na]^+$.

5.6.14. Compound 21. The obtained yield 20%, pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.25 (t, *J* = 7.2 Hz, 3H), 1.52 (d, *J* = 6.3 Hz, 3H), 3.99 (d, *J* = 6.6 Hz, 1H), 4.16 (m, 2H), 5.07 (s, 4H), 5.19 (s, 1H), 5.47 (quintet, *J* = 6.6 Hz, 1H), 6.83 (m, 1H), 7.26–7.45 (m, 12H), 8.76 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.2, 18.1, 60.3, 61.4, 67.7, 70.3, 90.9, 107.4, 108.5, 127.6, 128.1, 128.6, 131.5, 136.3, 151.5, 159.8, 164.6, 164.9, 166.9. $[\alpha]_D^{25}$ −32.6 (*c* 1.67, $CHCl_3$). IR (film): 3278, 1822, 1700, 1652, 1595, 1445 cm^{-1}. HPLC–MS: t_R = 18.88 min, *m/z*: 472.2 $[M-43]^+$, 516.2 $[M+H]^+$, 533.2 $[M+H_2O]^+$, 538.1 $[M+Na]^+$, 1053.3 $[2M+Na]^+$.

5.6.15. Compound 22. The obtained yield 14%, pale yellow oil. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.30 (t, *J* = 7.0 Hz, 3H), 1.59 (d, *J* = 6.6 Hz, 3H), 4.10 (d, *J* = 6.2 Hz, 1H), 4.21 (q, *J* = 7.0 Hz, 2H), 5.22 (s, 1H), 5.58 (quintet, *J* = 6.2 Hz, 1H), 8.35–8.45 (m, 3H), 8.66 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.2, 17.9, 60.5, 61.3, 68.8, 91.3, 122.8 (q, *J* = 273.0 Hz), 127.1 (m), 130.3 (m), 131.4, 132.5 (q, *J* = 34.2 Hz), 150.8, 164.3, 166.8, 167.7. $[\alpha]_D^{25}$ +7.9 (*c* 0.67, $CHCl_3$). IR (film): 3272, 1825, 1733, 1655, 1281 cm^{-1}. HPLC–MS:

t_R = 14.79 min, *m/z*: 395.9 $[M-43]^+$, 440.1 $[M+H]^+$, 457.1 $[M+H_2O]^+$, 461.9 $[M+Na]^+$, 478.0 $[M+K]^+$.

Compounds **35**–**39** were obtained by β-lactam N-acylation using the corresponding acyl-chlorides. A representative procedure is the following: compound **4** (0.381 g, 1.017 mmol) and 3,4,5-tribenzyloxybenzoyl chloride (0.513 g, 1.119 mmol) were dissolved in anhydrous acetone (10 mL); K_2CO_3 (0.141 g, 1.017 mmol) was added and the reaction mixture stirred at room temperature for 4 h. Then the K_2CO_3 was filtered, the solvent removed, and the crude oily residue immediately purified by flash chromatography (cyclohexane/ethylacetate 95:5) to give compound **37**. Compounds **35**–**39** are eluted in HPLC analysis as single peaks; however in the ESI ionization they are N-deacylated.

5.6.16. Compound 35. The obtained yield 41%, white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.12 (s, 3H), 0.14 (s, 3H), 0.90 (s, 9H), 1.28 (d, J = 6.3 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H), 3.92 (s, 6H), 3.95 (s, 3H), 4.23 (q, J = 7.2 Hz, 2H), 4.33 (dd, J = 1.8 Hz, J = 4.5 Hz, 1H), 4.79 (dr q, J = 4.5 Hz,J = 6.3 Hz, 1H), 6.65 (d, J = 1.5 Hz, 1H), 7.28 (m, 2H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −4.9,−4.6, 14.3, 17.9, 19.8, 25.7, 56.3, 60.3, 61.0, 64.2, 65.4, 100.6, 107.6, 116.2, 125.8, 143.0, 149.3, 152.8, 164.3, 164.8, 166.2. $[\alpha]_D^{25}$ +57.3 (*c* 0.89, $CHCl_3$). IR (film): 1841, 1719, 1683, 1330 cm^{-1}. HPLC: t_R = 27.50 min. mp 120–122 °C.

5.6.17. Compound 36. The obtained yield 44%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.15 (s, 3H), 0.16 (s, 3H), 0.93 (s, 9H), 1.24 (d, J = 6.3 Hz, 3H), 1.33 (t, J = 7.2 Hz, 3H), 4.23 (q, J = 7.2 Hz, 2H), 4.31 (dd, J = 1.8 Hz, J = 4.5 Hz, 1H), 4.79 (dr q, J = 4.5 Hz, J = 6.3 Hz, 1H), 5.17 (s, 6H), 6.63 (d, J = 1.8 Hz, 1H), 7.26–7.46 (m, 17H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ −4.9, −4.6, 14.3, 17.9, 19.8, 25.7, 60.3, 64.1, 65.4, 71.4, 75.2, 100.5, 109.9, 125.8, 127.4, 127.5, 128.0, 128.2, 128.4, 128.5, 136.6, 137.3, 143.3, 149.3, 152.4, 164.1, 164.6, 166.2. $[\alpha]_D^{25}$ +47.3 (*c* 4.16, $CHCl_3$). IR (film): 1841, 1718, 1683, 1328, 1197 cm^{-1}. HPLC: t_R = 9.64 min.

5.6.18. Compound 37. The obtained yield 30%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.16 (s, 3H), 0.17 (s, 3H), 0.96 (s, 9H), 1.26 (d, J = 6.2 Hz, 3H), 4.35 (d, J = 4.8 Hz, 1H), 4.81 (dr q, J = 6.2 Hz, J = 4.8 Hz, 1H), 5.20 (s, 6H), 5.26 (s, 2H), 6.74 (s, 1H), 7.30–7.49 (m, 22H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −4.9, −4.7, 17.9, 19.8, 25.7, 64.2, 65.3, 66.1, 71.4, 75.2, 100.2, 109.9, 125.8, 127.4, 127.5, 128.0, 128.1, 128.2, 128.5, 128.6, 135.9, 136.6, 137.3, 149.9, 152.4, 164.1, 164.6, 166.1. $[\alpha]_D^{25}$ +78.2 (*c* 1.60, $CHCl_3$). IR (film): 1845, 1718, 1685, 1335, 1127 cm^{-1}. HPLC–MS: t_R = 20.27 min, *m/z*: 376.3 $[M+H-423]^+$, 398.1 $[M+Na-423]^+$.

5.6.19. Compound 38. The obtained yield 39%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.15 (s, 3H), 0.16 (s, 3H), 0.94 (s, 9H), 1.29 (d, J = 6.3 Hz, 3H), 4.36 (d, J = 4.2 Hz, 1H), 4.79 (m, 1H), 5.10 (s, 4H), 5.25 (s, 2H), 6.75 (m, 1H), 6.87 (m, 1H), 7.18 (m, 2H), 7.34–7.47 (m, 14H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −5.0, −4.7, 17.9, 19.9, 25.6, 64.4, 65.3, 66.1, 70.3, 100.3, 108.0, 108.6, 127.5, 128.0, 128.1, 128.2, 128.5, 128.6, 133.0, 135.8, 136.3, 149.7, 159.6, 163.8, 165.0, 166.0. $[\alpha]_D^{25}$ +54.8 (*c* 2.77, $CHCl_3$). IR (film): 1843, 1716, 1659, 1595, 1306 cm^{-1}. HPLC: t_R = 9.38 min.

5.6.20. Compound 39. The obtaiend yield 37%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.12 (s, 6H), 0.88 (s, 9H), 1.30 (d, J = 6.2 Hz, 3H), 1.34 (t, J = 7.0 Hz, 3H), 4.25 (q, J = 7.0 Hz, 2H), 4.40 (dd, J = 2.0 Hz, J = 4.4 Hz, 1H), 4.76 (m, 1H), 6.73 (d, J = 2.0 Hz, 1H), 8.11 (m, 1H), 8.34 (m, 2H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −5.1, −4.6, 14.3, 17.9, 20.3, 25.6, 60.6, 65.0, 65.6, 102.1, 122.7 (q, J = 273.2 Hz), 126.8m, 129.9m, 132.0 (q, J = 34.3 Hz), 133.5, 148.0, 162.5, 164.3, 165.9. $[\alpha]_D^{25}$ +132.6 (*c* 0.76, $CHCl_3$). IR (film): 1845, 1717, 1665, 1280 cm^{-1}. HPLC: t_R = 26.33 min.

All the following compounds were obtained by hydrogenolysis of the corresponding benzylesters and benzylethers. A representative procedure is the following: compound **11** (0.444 g, 0.976 mmol) was dissolved in an anhydrous mixture THF/MeOH (4 mL/4 mL) and Pd, 10 wt% on activated carbon (0.045 g) was added. Finally, the reaction mixture was treated with H_2 (1 atm) and stirred at room temperature for 4 h. It was then filtered and concentrated to give compound **14**.

5.6.21. Compound 14. Quantitative yield, white syrup. ^{1}H NMR (acetone-d_6, 200 MHz): δ 1.53 (d, J = 6.2 Hz, 3H), 3.79 (s, 3H), 3.86 (s, 6H), 4.22 (d, J = 5.2 Hz, 1H), 5.26 (s, 1H), 5.46 (dr q, J = 6.2 Hz, J = 5.2 Hz, 1H), 7.29 (m, 2H), 9.81 (br s, 1H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 17.9, 55.8, 60.0, 61.7, 67.6, 90.3, 107.0, 125.2, 142.9, 153.2, 153.6, 164.6, 165.8, 167.0. $[\alpha]_D^{25}$ −20.8 (*c* 2.31, $CHCl_3$). IR (film): 3281, 1824, 1705, 1654 cm^{-1}. HPLC–MS: t_R = 8.07 min, *m/z*: 322.1 $[M-43]^+$, 366.2 $[M+H]^+$, 383.1 $[M+H_2O]^+$, 388.1 $[M+Na]^+$, 404.0 $[M+K]^+$, 753.2 $[2M+Na]^+$, 769.2 $[2M+K]^+$.

5.6.22. Compound 18. Quantitative yield, white syrup. ^{1}H NMR (acetone-d_6, 300 MHz): δ 1.52 (d, J = 6.3 Hz, 3H), 3.86 (s, 6H), 4.21 (d, J = 5.1 Hz, 1H), 5.26 (s, 1H), 5.46 (dr q, J = 6.3 Hz,J = 5.1 Hz, 1H), 7.30 (m, 2H), 8.10 (br s, 1H), 9.81 (br s, 1H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 18.6, 56.6, 62.4, 67.9, 90.8, 107.9, 121.0, 141.9, 148.4, 154.0, 165.4, 166.4, 167.7. $[\alpha]_D^{25}$ −17.5 (*c* 1.43, $CHCl_3$). IR (film): 3308, 1824, 1710, 1654 cm^{-1}. HPLC–MS: t_R = 6.57 min, *m/z*: 308.1 $[M-43]^+$, 352.1 $[M+H]^+$, 369.2 $[M+H_2O]^+$, 374.1 $[M+Na]^+$, 390.0 $[M+K]^+$, 725.2 $[2M+Na]^+$.

5.6.23. Compound 23. Quantitative yield, white syrup. ^{1}H NMR (CD_3CN, 200 MHz): δ 1.23 (t, J = 7.0 Hz, 3H), 1.42 (d, J = 6.4 Hz, 3H), 4.07 (dd, J = 6.3 Hz, J = 0.8 Hz 1H), 4.14 (q, J = 7.0 Hz, 2H), 5.14 (s, 1H), 5.37 (quintet, J = 6.4 Hz, 1H), 7.02 (m, 2H). ^{13}C NMR (CD_3CN, 75.5 MHz): δ 14.6, 18.4, 60.7, 62.3, 67.8, 91.2, 109.9, 121.8, 138.5, 145.7, 153.2, 165.7, 166.5, 167.2. $[\alpha]_D^{25}$ −21.9 (*c* 0.32, CH_3OH). IR (film): 3353,

1813, 1695, 1378, 1236 cm^{-1}. HPLC–MS: t_R = 6.88 min, *m/z*: 308.1 $[M-43]^+$, 352.1 $[M+H]^+$, 369.2 $[M+H_2O]^+$, 374.0 $[M+Na]^+$, 390.0 $[M+K]^+$, 725.2 $[2M+Na]^+$.

5.6.24. Compound 24. Quantitative yield, pale yellow oil. ^1H NMR (acetone-d_6, 200 MHz): δ 1.22 (t, J = 6.8 Hz, 3H), 1.48 (d, J = 6.6 Hz, 3H), 4.13 (q, J = 6.8 Hz, 2H), 4.21 (d, J = 7.0 Hz, 1H), 5.18 (s, 1H), 5.43 (m, 1H), 6.60 (m, 1H), 7.00 (m, 2H), 8.66 (br s, 2H), 9.80 (br s, 1H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 14.6, 18.1, 60.2, 62.1, 68.3, 90.7, 108.2, 108.6, 132.8, 153.3, 159.4, 165.6, 166.0, 166.7. $[\alpha]_D^{25}$ −13.0 (*c* 1.60, acetone). IR (film): 3351, 1815, 1695, 1603, 1234, 1160 cm^{-1}. HPLC–MS: t_R = 8.66 min, *m/z*: 292.3 $[M-43]^+$, 336.2 $[M+H]^+$, 353.1 $[M+H_2O]^+$, 358.3 $[M+Na]^+$, 375.2 $[M+H+K]^+$, 693.2 $[2M+Na]^+$.

5.6.25. Compound 25. Quantitative yield, white syrup. ^1H NMR (acetone-d_6, 200 MHz): δ 1.49 (d, J = 6.2 Hz, 3H), 4.21 (d, J = 6.2 Hz, 1H), 5.20 (s, 1H), 5.44 (quintet, J = 6.2 Hz, 1H), 6.60 (m, 1H), 6.95–7.04 (m, 2H), 8.70 (br s, 2H), 9.76 (br s, 1H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 18.1, 62.0, 68.3, 90.7, 108.1, 108.6, 132.8, 153.4, 159.4, 165.6, 165.9, 167.5. $[\alpha]_D^{25}$ −2.6 (*c* 0.98, acetone). HPLC–MS: t_R = 5.18 min, *m/z*: 264.4 $[M-43]^+$, 308.1 $[M+H]^+$, 325.4 $[M+H_2O]^+$, 330.0 $[M+Na]^+$, 346.3 $[M+K]^+$, 637.3 $[2M+Na]^+$.

5.6.26. Compound 40. Quantitative yield, white syrup. ^1H NMR (CD_3CN, 200 MHz) δ −0.01 (s, 6H), 0.77 (s, 9H), 1.13 (d, J = 6.2 Hz, 3H), 4.18 (dd, J = 1.8 Hz, J = 6.0 Hz, 1H), 4.66 (m, 1H), 6.23 (d, J = 1.8 Hz, 1H), 6.85 (m, 2H), 7.05 (br s, 2H), 7.17 (br s, 1H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ −4.8, −4.7, 18.2, 19.6, 26.1, 65.1, 66.0, 99.5, 110.7, 122.9, 141.1, 145.9, 151.7, 164.8, 165.5, 167.3. $[\alpha]_D^{25}$ +9.1 (*c* 0.34, acetone). HPLC–MS: t_R = 12.11 min, *m/z*: 394.1 $[M-43]^+$, 438.2 $[M+H]^+$, 460.1 $[M+Na]^+$, 476.1 $[M+K]^+$, 897.3 $[2M+Na]^+$.

5.6.27. Compound 41. Quantitative yield, white syrup. ^1H NMR (acetone-d_6, 200 MHz) δ 0.14 (s, 3H), 0.15 (s, 3H), 0.91 (s, 9H), 1.30 (d, J = 6.2 Hz, 3H), 4.43 (d, J = 3.6 Hz, 1H), 4.88 (m, 1H), 6.50 (s, 1H), 6.63 (m, 1H), 6.88 (m, 2H), 8.78 (br s, 3H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ −6.0, −5.8, 17.2, 18.6, 24.9, 64.2, 64.9, 99.2, 107.4, 107.5, 133.3, 149.9, 158.1, 163.5, 165.0, 166.5. $[\alpha]_D^{25}$ +14.5 (*c* 2.82, acetone). HPLC–MS: t_R = 12.99 min, *m/z*: 378.2 $[M-43]^+$, 422.3 $[M+H]^+$, 444.2 $[M+Na]^+$, 460.2 $[M+K]^+$, 865.4 $[2M+Na]^+$.

Compounds **27**–**34** were obtained starting from **26** by coupling with the corresponding amine, phenol or alcohol. A representative procedure is the following: compound **26** (0.065 g, 0.228 mmol) was dissolved in anhydrous CH_2Cl_2 (4 mL) and the solution brought to 0 °C. Then DCC (0.047 g, 0.228 mmol) and benzylamine (37 μL, 0.342 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 2 h, then filtered and concentrated. The residue was purified by flash chromatography (cyclohexane/ethylacetate 8:2) to give compound **31**.

5.6.28. Compound 27. The obtained yield 44%, pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 0.13 (s, 3H), 0.14 (s, 3H), 0.93 (s, 9H), 1.40 (d, J = 6.3 Hz, 3H), 3.78 (d, J = 4.8 Hz, 1H), 4.32 (m, 1H), 5.48 (s, 1H), 7.14–7.46 (m, 5H), 8.53 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ−5.1, −4.2, 17.9, 22.3, 25.6, 65.0, 65.1, 89.6, 121.7, 125.8, 129.4, 150.5, 155.4, 165.6, 166.1. $[\alpha]_D^{25}$ −36.3 (*c* 1.63, $CHCl_3$). IR (film): 3356, 1821, 1799, 1714, 1653 cm^{-1}. HPLC–MS: t_R = 20.41 min, *m/z*: 318.2 $[M-43]^+$, 362.1 $[M+H]^+$, 384.2 $[M+Na]^+$.

5.6.29. Compound 28. The obtained yield 68%, white solid. ^1H NMR (acetone-d_6, 200 MHz): δ 0.08 (s, 3H), 0.09 (s, 3H), 0.87 (s, 9H), 1.32 (d, J = 6.2 Hz, 3H), 3.84 (d, J = 4.4 Hz, 1H), 4.30 (dr q, J = 4.4 Hz, J = 6.4 Hz, 1H), 4.97 (d, J = 12.4 Hz, 1H), 5.05 (d, J = 12.4 Hz, 1H), 5.21 (s, 1H), 6.30 (m, 1H), 6.36 (m, 2H), 8.28 (br s, 2H), 9.68 (br s, 1H). ^{13}C NMR (THF-d_8, 50.3 MHz): δ −5.0, −4.3, 18.5, 22.4, 26.0, 65.4, 65.6, 66.3, 89.8, 102.7, 106.6, 139.6, 155.3, 159.7, 166.7, 167.3. $[\alpha]_D^{25}$ −10.1 (*c* 2.90, CH_3OH). IR (film): 3311, 1810, 1696, 1654 cm^{-1}. HPLC–MS: t_R = 11.93 min, *m/z*: 408.2 $[M+H]^+$, 430.2 $[M+Na]^+$, 446.0 $[M+K]^+$. mp 79–81 °C.

5.6.30. Compound 29. The obtained yield 20%, white syrup. ^1H NMR (acetone-d_6, 300 MHz): δ 0.10 (s, 3H), 0.12 (s, 3H), 0.90 (s, 9H), 1.37 (d, J = 6.3 Hz, 3H), 3.90 (d, J = 4.8 Hz, 1H), 4.35 (m, 1H), 5.32 (s, 1H), 6.12 (m, 2H), 6.24 (m, 1H), 8.46 (br s, 2H), 9.81 (br s, 1H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ −4.9, −4.1, 18.5, 22.5, 26.1, 65.7, 65.9, 89.6, 100.7, 101.7, 153.4, 156.7, 159.7, 165.0, 167.4. $[\alpha]_D^{25}$ −3.9 (*c* 0.77, $CHCl_3$). IR (film): 3325, 1806, 1700, 1653, 1625 cm^{-1}. HPLC–MS: t_R = 12.05 min, *m/z*: 394.2 $[M+H]^+$, 416.1 $[M+Na]^+$, 432.0 $[M+K]^+$, 809.3 $[2M+Na]^+$.

5.6.31. Compound 30. The obtained yield 50%, pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.07 (s, 3H), 0.08 (s, 3H), 0.86 (s, 9H), 1.33 (d, J = 6.3 Hz, 3H), 3.70 (d, J = 5.1 Hz, 1H), 4.35 (dr q, J = 5.1 Hz, J = 6.3 Hz, 1H), 5.28 (s, 2H), 5.33 (s, 1H), 7.82 (m, 2H), 7.85 (m, 1H), 8.49 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −5.1, −4.3, 17.9, 22.3, 25.6, 64.0, 65.0, 65.1, 89.3, 122.0m, 123.2 (q, J = 272.5 Hz), 127.8 (m), 132.0 (q, J = 33.6 Hz), 138.9, 155.1, 166.3, 166.6. $[\alpha]_D^{25}$ −21.2 (*c* 1.18, $CHCl_3$). IR (film): 3297, 1820, 1699, 1653 cm^{-1}. HPLC–MS: t_R = 25.41 min, *m/z*: 512.2 $[M+H]^+$, 529.3 $[M+H_2O]^+$, 534.0 $[M+Na]^+$.

5.6.32. Compound 31. The obtained yield 62%, pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.10 (s, 3H), 0.11 (s, 3H), 0.91 (s, 9H), 1.34 (d, J = 6.3 Hz, 3H), 3.66 (d, J = 5.4 Hz, 1H), 4.24 (quintet, J = 6.0 Hz, 1H), 4.51 (dd, J = 6.0 Hz, J = 15.0 Hz, 1H), 4.57 (dd, J = 6.0 Hz, J = 15.0 Hz, 1H), 5.13 (s, 1H), 5.64 (t, J = 6.0 Hz, 1H), 7.30–7.41 (m, 5H), 8.96 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −4.9, −4.3, 17.9, 22.5, 25.7, 43.3, 64.8, 65.4, 92.2, 127.5, 127.6, 128.7, 138.3, 150.3, 166.6, 167.0. $[\alpha]_D^{25}$ +6.0 (*c* 1.66, $CHCl_3$). IR (film): 3346, 1804, 1682, 1627 m^{-1}. HPLC–MS: t_R = 14.69 min, *m/z*: 375.2 $[M+H]^+$, 397.2 $[M+Na]^+$, 771.5 $[2M+Na]^+$.

5.6.33. Compound 32. The obtained yield 31%, white syrup. ^{1}H NMR (acetone-d_6, 200 MHz): δ 0.08 (s, 3H), 0.09 (s, 3H), 0.87 (s, 9H), 1.29 (d, J = 6.2 Hz, 3H), 3.71 (d, J = 5.0 Hz, 1H), 4.20 (dd, J = 5.8 Hz, J = 14.2 Hz, 1H), 4.24 (m, 1H), 4.32 (dd, J = 5.8 Hz, J = 14.2 Hz, 1H), 5.31 (s, 1H), 6.59–6.79 (m, 3H), 7.29 (t, J = 5.8 Hz, 1H), 7.81 (br s, 2H), 9.47 (br s, 1H). ^{13}C NMR (acetone-d_6, 50.3 MHz): δ −4.8, −4.2, 18.5, 22.5, 26.1, 42.8, 65.2, 66.2, 93.4, 115.7, 115.8, 119.9, 132.3, 144.9, 145.8, 149.8, 166.8, 167.9. $[\alpha]_D^{25}$ −18.8 (*c* 0.16, acetone). IR (film): 3392, 1804, 1683 cm^{-1}. HPLC–MS: t_R = 11.34 min, *m*/*z*: 407.1 $[M+H]^+$, 429.2 $[M+Na]^+$, 835.4 $[2M+Na]^+$.

5.6.34. Compound 33. The obtained yield 56%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.07 (s, 3H), 0.08 (s, 3H), 0.87 (s, 9H), 1.31 (d, J = 6.2 Hz, 3H), 3.66 (d, J = 5.4 Hz, 1H), 4.22 (quintet, J = 6.2 Hz, 1H), 4.88 (s, 3H), 7.39 (m, 5H), 7.92 (br s, 1H), 8.81 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −4.9, −4.2, 17.9, 22.4, 25.7, 65.2, 65.3, 79.1, 87.8, 128.7, 128.9, 129.2, 134.9, 152.8, 166.7, 166.8. $[\alpha]_D^{25}$ +19.4 (*c* 3.27, $CHCl_3$). IR (film): 3226, 1813, 1684 cm^{-1}. HPLC–MS: t_R = 12.49 min, *m*/*z*: 391.1 $[M+H]^+$, 413.1 $[M+Na]^+$, 430.1 $[M+H+K]^+$, 803.3 $[2M+Na]^+$.

5.6.35. Compound 34. The obtained yield 98%, pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.07 (s, 3H), 0.08 (s, 3H), 0.87 (s, 9H), 1.32 (d, J = 6.3 Hz, 3H), 3.66 (d, J = 5.7 Hz, 1H), 4.23 (quintet, J = 5.7 Hz, 1H), 4.63 (d, J = 6.6 Hz, 2H), 5.16 (s, 1H), 5.79 (t, J = 6.6 Hz, 1H), 7.75 (m, 2H), 7.79 (m, 1H), 8.84 (br s, 1H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ −5.0, −4.3, 17.9, 22.5, 25.6, 42.3, 64.9, 65.4, 91.5, 121.4m, 123.2 (q, J = 271.2 Hz), 127.5m, 131.9 (q, J = 33.4 Hz), 141.3, 151.5, 166.7, 166.8. $[\alpha]_D^{25}$ −2.9 (*c* 3.67, $CHCl_3$). IR (film): 3330, 3226, 1800, 1683, 1635 cm^{-1}. HPLC–MS: t_R = 19.34 min, *m*/*z*: 511.1 $[M+H]^+$, 533.2 $[M+Na]^+$.

Acknowledgments

This research was supported by MURST (60% and PRIN), University of Bologna (funds for selected topics), University of Padova, and the Associazione Italiana per la Ricerca sul Cancro. We are grateful to Dr. S. Biggin for revision of the English manuscript and to Mrs. Maurizia Soverini for experimental assistance.

Supplementary data

^{1}H NMR and ^{13}C NMR for all new compounds (**2**, **4**, **8**–**25**, **27**–**41**), whose synthesis is reported in the experimental part. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.06.041.

References and notes

1. Green, B. G.; Chabin, R.; Mills, S.; Underwood, D. J.; Shah, S. K.; Kuo, D.; Gale, P.; Maycock, A. L.; Liesch, J.; Burgey, C. S.; Doherty, J. B.; Dorn, C. P.; Finke, P. E.; Hagmann, W. K.; Hale, J. J.; Maccoss, M.; Westler, W. M.; Knight, W. B. *Biochemistry* **1995**, *34*, 14331.
2. Strynadka, N. C. J.; Adachi, H.; Jensen, S. E.; Johns, K.; Sielecki, A.; Betzel, C.; Sutoh, K.; James, M. N. G. *Nature* **1992**, *359*, 700.
3. Tew, D. G.; Boyd, H. F.; Ashman, S.; Theobald, C.; Leach, C. A. *Biochemistry* **1998**, *37*, 10087.
4. Paetzel, M.; Dalbey, R. E.; Strynadka, N. C. J. *Nature* **1998**, *396*, 186.
5. Cainelli, G.; Giacomini, D.; Galletti, P.; Quintavalla, A. *Eur. J. Org. Chem.* ***2003***, *9*, 1765.
6. Cainelli, G.; Galletti, P.; Garbisa, S.; Giacomini, D.; Sartor, L.; Quintavalla, A. *Biorg. Med. Chem.* **2003**, *11*, 5391.
7. Eccles, S. A.; Box, G. M.; Court, W. J.; Bone, E. A.; Thomas, W.; Brown, P. D. *Cancer Res.* **1996**, *56*, 2815.
8. Coussens, L. M.; Fingleton, B.; Matrisian, L. M. *Science* ***2002***, *295*, 2387.
9. Wang, Z. Y.; Huang, M. T.; Ho, C. T.; Chang, R.; Ma, W.; Ferraro, T.; Reuhl, K. R.; Yang, C. S.; Conney, A. H. *Cancer Res.* ***1992***, *52*, 6657.
10. Cao, Y.; Cao, R. *Nature* **1999**, *398*, 381.
11. Garbisa, S.; Biggin, S.; Cavallarin, N.; Sartor, L.; Benelli, R.; Albini, A. *Nat. Med.* **1999**, *5*, 1216.
12. Taniguchi, S.; Fujiki, H.; Kobayashi, H.; Go, H.; Miyado, K.; Sadano, H.; Shimokawa, R. *Cancer Lett.* **1992**, *65*, 51.
13. Garbisa, S.; Sartor, L.; Biggin, S.; Salvato, B.; Benelli, R.; Albini, A. *Cancer* **2001**, *91*, 822.
14. Demeule, M.; Brossard, M.; Page, M.; Gingras, D.; Beliveau, R. *Biochim. Biophys. Acta* **2000**, *1478*, 51.
15. Sartor, L.; Pezzato, E.; Garbisa, S. *J. Leuk. Biol.* **2002**, *71*, 73.
16. Cainelli, G.; Giacomini, D.; Gazzano, M.; Galletti, P.; Quintavalla, A. *Tetrahedron Lett.* **2003**, *44*, 6269.
17. Gerard, S.; Galleni, M.; Dive, G.; Marchand-Brynaert, J. *Bioorg. Med. Chem.* **2004**, *12*, 129; Beauve, C.; Bouchet, M.; Touillaux, R.; Fastrez, J.; Marchand-Brynaert, J. *Tetrahedron* **1999**, *55*, 13301.
18. Cainelli, Giacomini, Galletti unpublished results.
19. cf. for instance the X-ray structures of complexes between β-lactams and porcine pancreas elastase: Taylor, P.; Anderson, V.; Dowden, J.; Flitsch, S. L.; Turner, N. J.; Loughrans, K.; Walkinshaw, M. D. *J. Biol. Chem.* **1999**, *35*, 24901; Navia, M. A.; Springer, J. P.; Lin, T.-Y.; Williams, H. R.; Firestone, R. A.; Pisano, J. M.; Doherty, J. B.; Finke, P. E.; Hoogsteen, K. *Nature* **1987**, *327*, 79.
20. (a) Durette, P. L.; Chabin, R. M.; Fletcher, D. S.; Green, B. G.; Hanlon, W. A.; Humes, J. L.; Knight, W. B.; Lanza, T. J., Jr.; Mumford, R. A.; Pacholok, S.; MacCoss, M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 271; (b) Doherty, J. B.; Shah, S. K.; Finke, P. E.; Dorn, C. P., Jr.; Hagmann, W. K.; Hale, J. J.; Kissinger, A. L.; Thompson, K. R.; Brause, K.; Chandler, G. O.; Knight, W. B.; Maycock, A. L.; Ashe, B. M.; Weston, H.; Gale, P.; Mumford, R. A.; Andersen, O. F.; Williams, H. R.; Nolan, T. E.; Frankenfield, D. L.; Underwood, D.; Vyas, K. P.; Kari, P. H.; Dahlgren, M. E.; Mao, J.; Fletcher, D. S.; Dellea, P. S.; Hand, K. M.; Osinga, D. G.; Peterson, L. B.; Williams, D. T.; Metzger, J. M.; Bonney, R. J.; Humes, J. L.; Pacholok, S. P.; Hanlon, W. A.; Opas, E.; Stolk, J.; Davies, P. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8727.
21. Donà, M.; Dell'Aica, I.; Pezzato, E.; Sartor, L.; Calabrese, F.; Della Barbera, M.; Donella-Deana, A.; Appendino, G.; Borsarini, A.; Caniato, R.; Garbisa, S. *Cancer Res.* **2004**, *64*, 6225.
22. Angle, S. R.; Belanger, D. S. *J. Org. Chem.* ***2004***, *69*, 4361, and references cited therein; Danheiser, R. L.; Miller, R. F.; Brisbois, R. G.; Park, S. Z. *J. Org. Chem.* **1990**, *55*, 1959.