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Design and Synthesis of 4-Alkylidene-β-lactams: Benzyl- and Phenethyl-carbamates as Key Fragments to Switch on Antibacterial Activity

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The emergence of multidrug-resistant bacterial strains is particularly important in chronic pathologies such as cystic fibrosis (CF), in which persistent colonization and selection of resistant strains is favored by the frequent and repeated use of antibacterial agents. *Staphylococcus aureus* is a common pathogen in CF patients that has an associated increased multidrug resistance. In previous studies we demonstrated that the presence of a 4-alkylidene side chain directly linked to a β-lactam appeared to strengthen the potency against *S. aureus*, especially against methicillin-resistant *S. aureus* (MRSA) strains. In the present study, 21 new 4-alkylidene-β-lactams were synthesized and evaluated for antibacterial activity. We designed the new compounds to have aryl, benzyl, or phenethyl-carbamate groups on the C3 hydroxyethyl side chain. We found a correlation between biological activity and the nitrogen substituent of the carbamate group, and two phenethyl-carbamate β-lactams were shown to be valuable antibacterial agents against selected linezolid-resistant strains, with a minimum inhibitory concentrations of 2–4 mg L^{-1}.

Introduction

The battle against bacterial infections remains a significant challenge, as drug-resistant infections in hospitals and in the communities, caused by both Gram-positive and Gram-negative bacterial pathogens, are growing. The World Health Organization has identified antimicrobial resistance as one of the greatest threats to human health and has adopted a global action plan that outlines five objectives:[1] to increase awareness of the problem with effective communication and education; to strengthen knowledge on the mechanisms and on the spread of resistance; to decrease the incidence of infections; and to optimize the use of antimicrobial agents, with particular attention being given to increase investments in the development of new antimicrobial agents. In 2010, the Infectious Diseases Society of America (IDSA) launched the "10×'20" initiative, aimed at developing ten new antibacterial drugs by the year 2020.[2] More recently, CARB-X, a new global public–private partnership program, has launched a program to invest US$350 million in the next five years to address the growing problem of antibiotic resistance, with the aim of accelerating the progression of innovative antibacterial products into clinical trials.[3] The emergence of multidrug-resistant (MDR) bacterial strains is particularly important in some chronic pathologies, such as cystic fibrosis (CF), where persistent colonization by pathogenic bacteria occurs, and selection of resistant strains is favored by the frequent and repeated use of antibacterial agents. Among Gram-positive pathogens such as staphylococci and enterococci, *Staphylococcus aureus* is a common pathogen in CF patients with an associated increased multidrug-resistance phenotype.

A recent study reported that, in CF patients, methicillin-resistant *Staphylococcus aureus* (MRSA) strains accounted for 62.6% of all isolated *S. aureus*, while overall, MDR *S. aureus* incidence was 73.1% in all cultures.[4] Moreover, community-acquired MRSA strains (CA-MRSA) have been described in Europe, as highlighted by a recent pilot multicenter study that showed an increasing prevalence from north to south of CA-MRSA, characterized by multidrug resistance patterns unrelated to the USA300 CA-MRSA clone.[5]

In the last ten years, we have been involved in an interdisciplinary project to study the design and synthesis of new monocyclic β-lactam compounds with antibacterial activity against resistant bacterial strains.[6] From these studies, a new β-lactam scaffold with improved features (from a reactivity standpoint) was developed: the 4-alkylidene-β-lactam (**A** Figure 1).[7] The double bond directly linked to the C4 position of the azetidinone ring favors nucleophilic ring opening reactions by suitable enzymes, thus developing new β-lactam-based enzyme inhibitors.[8] Notably, some 4-alkylidene-β-lactams armed with a polyphenolic ester residue on the C3 hydroxyethyl side chain showed significant antibacterial activity when tested in vitro against both MRSA and methicillin-susceptible *S. aureus* (MSSA) strains isolated from patients with CF.[9] In these stud-

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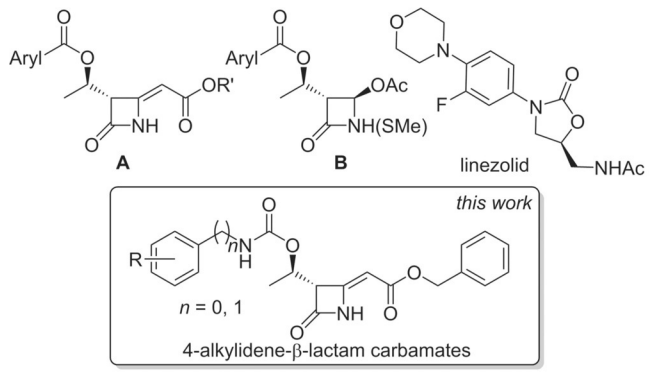


Figure 1. General structures of previously developed monocyclic β-lactam compounds with antibacterial activity, linezolid as an antibiotic model, and the new carbamate derivatives that are the focus of this work.

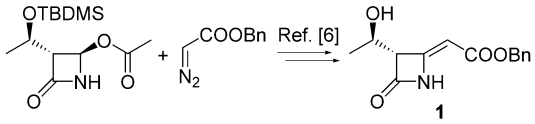
ies, it was observed that the presence of a 4-alkylidene side chain or a 4-acetoxy group, rather than an N-methylthio group (**B**, Figure 1), appeared to increase the potency against Gram-positive bacteria.[9]

In the present work, we synthesized 21 new 4-alkylidene-β-lactams and evaluated them for antibacterial activity (Figure 2). We designed the new molecules to have an aryl-, benzyl-, or phenethyl-carbamate group on the C3 hydroxyethyl side chain (Figure 2). This choice reflects the properties of organic carbamates, which have frequently been employed as pharmaceuticals, with linezolid (cyclic carbamate, Figure 1) being one such example in the class of antibiotic oxazolidinones.[10] In recent years, several reports indicated that a carbamate linkage present between the active pharmacophores of various synthetic or semisynthetic molecules increases their biological activities.[11,12] Very recently, Stephen et al. reported analogues of ethyl *N*-(2-phenethyl) carbamate as biofilm inhibitors of MRSA strains.[13] Moreover, considering bicyclic β-lactams, Yoshizawa et al. reported enhanced antibacterial activity for some cephalosporins with a carbamate derivatization,[14a] whereas Yan et al. incorporated a linezolid-like moiety to obtain cephalosporin–oxazolidinone conjugates.[14b] In this respect, we also designed, synthesized and evaluated two β-lactam carbamates armed with a linezolid-like molecular fragment.

Results and Discussion

Chemistry

Previous studies of structure–activity relationships (SAR) of new β-lactams demonstrated an enhancement in antibacterial potency with the presence of an alkylidene moiety conjugated to a benzyl ester at the C4 position of the azetidinone ring.[6,9] Thus, starting from this scaffold, we decided to explore the effect of a carbamate group on the C3 hydroxyethyl side chain and synthesized 21 novel β-lactam derivatives (**3a**–**u**; (Figure 2). The carbamate library was developed starting from a common precursor—the 3-hydroxyethyl-4-alkylidene β-lactam **1**—obtained using a two-step protocol beginning from the commercially available (3*R*,4*R*)-4-acetoxy-3-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one and benzyl diazoacetate (Scheme 1).[6,9]



Scheme 1. Synthesis of the β-lactam precursor.

The synthesis of new carbamates was carried out to exploit the reaction between alcohols and isocyanates.[15] For the development of a suitable method tailored for 4-alkylidene-β-lactams, we chose the condensation between commercially avail-

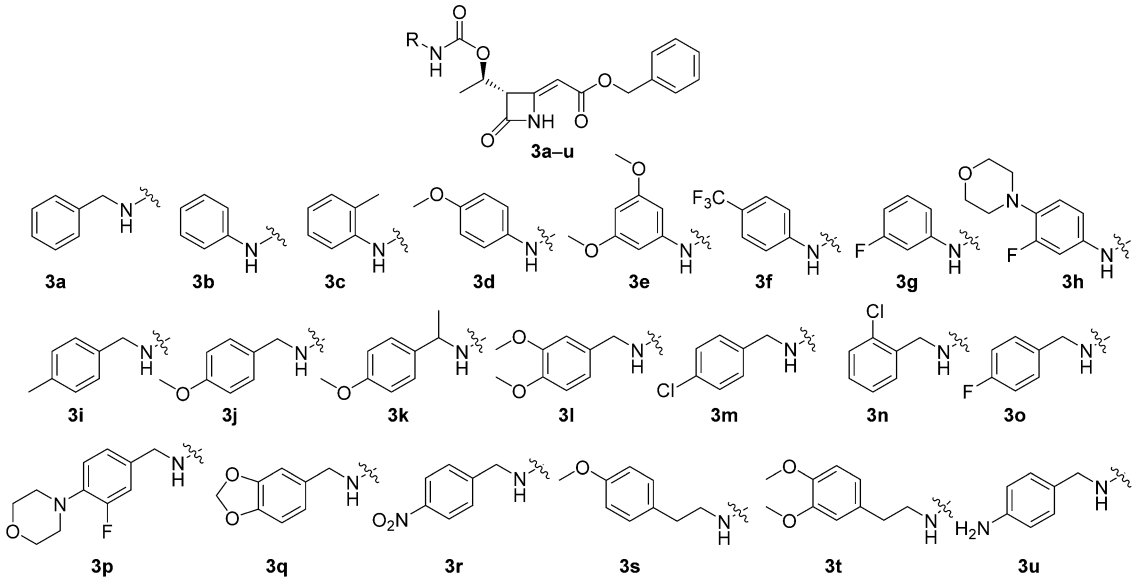
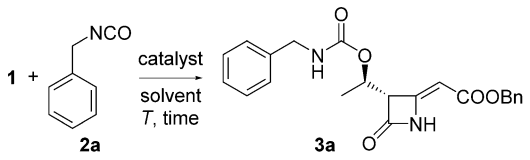


Figure 2. New β-lactam carbamate derivatives evaluated in this study.

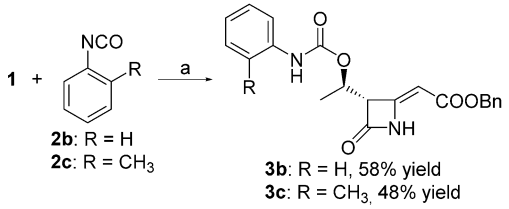
able *N*-benzylisocyanate **2a** and alkylidene azetidinone **1** as a model reaction to optimize the reaction conditions (Table 1). Initially, a base-mediated reaction in CH_2Cl_2 or acetonitrile (entries 1–4) was investigated,[14a] but potassium carbonate, triethylamine, or 4-dimethylaminopyridine (DMAP) gave a crude mixture of several products.[16] After liquid chromatography, carbamate **3a** was obtained with only DMAP as catalyst in very low yields (8%; Table 1, entry 4). On the other hand, the uncatalyzed reaction did not proceed at all (Table 1, entry 5). Next, we attempted neat reaction conditions by mixing azetidinone **1** with liquid isocyanate **2a** in the absence of any catalyst or solvent. In this case, the reaction was very slow but selective, and no by-products were detected. However, conversions were not complete, and consequently, yields of **3a** were poor, even after increasing the isocyanate equivalents (Table 1, entries 6–8). However, encouraging results were obtained by enhancing the reaction temperature or using microwave irradiation (MW;[17] Table 1, entries 8–10). With MW, a better result was obtained under solvent-free conditions than by using a suitable solvent for MW irradiation, such as chlorobenzene (Table 1, entries 9–10). The MW methodology was then exploited with other two commercially available isocyanates: phenyl isocyanate **2b** and *o*-tolyl isocyanate **2c**. The corresponding carbamates (**3b** and **3c**, respectively) were successfully isolated in acceptable yields after flash chromatography (Scheme 2).

Table 1. Study of reaction conditions for the synthesis of compound **3a**.[a]



Entry	Solvent	[**2a**] (equiv)	Cat. (equiv)	*T* [°C][b]	Time [h]	Yield [%][c]
1	CH_3CN	1.2	K_2CO_3 (1.5)	RT	3	–
2	CH_2Cl_2	1.2	TEA (1.5)	RT	3	trace
3	CH_2Cl_2	1.2	TEA (1.5)	RT	16	trace
4	CH_2Cl_2	1.5	DMAP (0.1)	RT	3	8
5	CH_2Cl_2	1.5	–	RT	6	trace
6	–	1.5	–	RT	19	23
7	–	2.5	–	RT	20	25
8	–	1.5	–	40	7	50
9	–	1.5	–	MW 400W	40 min	56
10	PhCl	1.5	–	MW 400W	40 min	38
11	CH_2Cl_2	1.1	$Ti(OBu)_4$ (0.1)	RT	20	20[d]
12	CH_2Cl_2	1.5	$Ti(OBu)_4$ (0.1)	RT	20	28[d]
13	CH_2Cl_2	1.1	$TiCl_4$ (0.1)	RT	19	16
14	CH_2Cl_2	1.1	$TiCl_4$ (0.1)	RT	72	50
15	CH_2Cl_2	1.5	$TiCl_4$ (0.1)	RT	20	35
16	CH_2Cl_2	2	$TiCl_4$ (0.1)	RT	20	56
17	CH_2Cl_2	1.5	$TiCl_4$ (0.1)	40	6	91
18	CH_2Cl_2	1.5	HCl (0.1)	RT	20	27

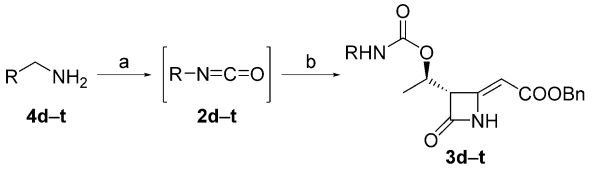
[a] Reaction conditions: compound **1** (1 equiv) in 7 mL mmol^{-1} solvent (except for neat reactions). [b] RT: room temperature. [c] Isolated yields of **3a** after column chromatography. [d] $PhCH_2NH(C{=}O)OBu$ was also detected as a by-product.



Scheme 2. Synthesis of β-lactam compounds **3b** and **3c**: a) MW 400 W, neat, 40 min.

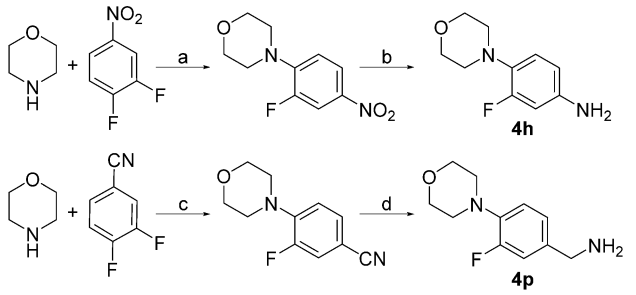
Spino et al. reported the reaction between alcohols and highly hindered and sensitive isocyanates with titanium tetrabutoxide as a Lewis acid catalyst under mild conditions.[18] Moreover, Feledziak et al. used $Ti(OBu)_4$ in carbamate synthesis on the C3-hydroxyethyl chain of N-acylated β-lactams with isocyanates.[19] Thus, the synthesis of **3a** was evaluated under acid catalysis conditions (Table 1, entries 11–18). Nevertheless, $Ti(OBu)_4$ was not satisfying to use for 4-alkylidene β-lactams (entries 11–12) because of the formation of considerable amounts of the corresponding benzyl butoxycarbamate as by-product. The use of $TiCl_4$ gave better results (Table 1, entries 13–17), and using isocyanate (1.5 equiv) in CH_2Cl_2 at reflux (Table 1, entry 17), carbamate **3a** was obtained in excellent yields (91%), together with a relative decrease in the reaction time for a complete consumption of the starting material (6 h instead of 20 h when the reaction was carried out at reflux instead of room temperature). A reaction mediated by HCl (12 M) as catalyst was attempted (Table 1, entry 18), but it was found to be much less efficient than $TiCl_4$.

With the optimized conditions in hand, the scope of the reaction was then investigated. As many aryl- or benzyl-isocyanates are not commercially available, we developed a method that allowed us to synthesize isocyanates in situ starting from the corresponding amines, triphosgene, and triethylamine (TEA) in CH_2Cl_2 (Scheme 3).[20] This method was applied to a series of commercially available anilines, benzylamines, and phenethylamines, with the exception of linezolid-like amines **4h** and **4p**, necessary for the synthesis of β-lactam carbamates **3h** and **3p**, which were prepared according to reported procedures[21] (Scheme 4), and for amine **4u**, which was prepared following a procedure previously established in our research group (Scheme 5).[22]

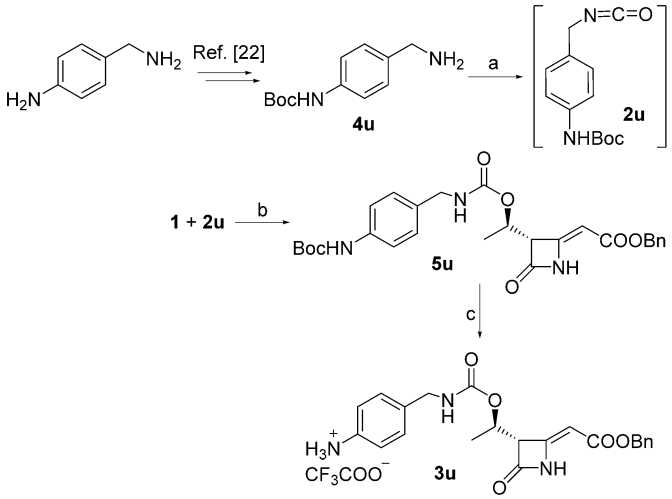


Scheme 3. Synthesis of β-lactam compounds **3d–t**: a) triphosgene, TEA, CH_2Cl_2, 0 °C to reflux, 3 h; b) **1**, $TiCl_4$ (10 mol%), CH_2Cl_2, RT, 18 h, (52–84%).

In all cases, the formation of isocyanates was confirmed through FT-IR analysis by monitoring the intensity of the characteristic N=C=O stretching absorption at around 2270 cm^{-1}.



Scheme 4. Synthesis of amines **4h** and **4p**: a) DMSO, 75 °C, 2 h (35%); b) H_2, Pd/C, MeOH, RT, 2 h (97%); c) K_2CO_3, DMSO, 90 °C, 3 h (83%); d) Red-Al, THF, 0 °C to RT, 3 h (71%).



Scheme 5. Synthesis of the azetidinone **3u**: a) triphosgene, TEA, CH_2Cl_2, 0 °C to reflux, 3 h; b) **1**, $TiCl_4$ (10 mol%), CH_2Cl_2, RT, 18 h, (55%); c) TFA, CH_2Cl_2, 0 °C to RT (96%). Boc = *tert*-butyloxycarbonyl.

The freshly prepared isocyanate was immediately reacted with alcohol **1** and $TiCl_4$ in CH_2Cl_2 to yield the desired β-lactam carbamate. In the case of carbamate **5u**, the NBoc protection was easily removed in the last step to obtain **3u** (Scheme 5) in very good isolated yields (96%, see Experimental Section).

Antibacterial activity

Screening for antibacterial activity for compounds **3a**–**u** was carried out against recent, well-characterized clinical isolates. Gram-positive and Gram-negative bacterial pathogens used for the in vitro antimicrobial susceptibility testing included: *S. aureus*, *Staphylococcus hominis*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Enterococcus faecium* as Gram-positive species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as Gram-negative species, respectively. Some bacterial strains were expressly selected to exhibit a multidrug-resistant phenotype against amoxicillin, linezolid, or vancomycin (see Experimental Section for details). Antimicrobial activities of the compounds are listed in Table 2, with potency being expressed as minimum inhibitory concentration (MIC) values in $\mu g\,mL^{-1}$ and micromolar in brackets (μM). Compounds demonstrating at least one MIC value equal to or less than 128 $\mu g\,mL^{-1}$ against corresponding bacterial species are reported in Table 2. Data are not shown for compounds without demonstrated antibacterial activity or for bacterial species not found to be susceptible to any of the tested molecules.

Notably, antibacterial potency was observed only against Gram-positive bacteria, while none of the tested compounds exhibited significant activities against Gram-negative strains (data not shown). This could be due to decreased uptake through the outer membrane of Gram-negative bacteria or to

Table 2. Antimicrobial activity (MIC values) for the new carbamate azetidinones.[a]

Compd	*S. aureus*				*S. epidermidis*	*S. hominis*	*E. faecalis*	*E. faecium*
	ATCC 29213	69856	44674	SAU-1	G1027	α26	ATCC 29212	VRE2
3a	16 (41)	16 (41)	8 (20)	8 (20)	32 (81)	8 (20)	>128 (325)	>128 (345)
3e	>128 (290)	>128 (290)	>128 (290)	>128 (290)	>128 (290)	>128 (290)	>128 (290)	32 (73)
3i	>128 (313)	>128 (313)	>128 (313)	>128 (313)	128 (313)	8 (19)	>128 (313)	>128 (313)
3j	128 (302)	64 (151)	32 (75)	128 (302)	64 (151)	8 (19)	>128 (302)	>128 (302)
3k	>128 (292)	128 (292)	128 (292)	>128 (292)	128 (292)	16 (36)	>128 (292)	>128 (292)
3l	32 (70)	32 (70)	32 (70)	64 (141)	16 (35)	4 (9)	>128 (282)	>128 (282)
3m	>128 (298)	>128 (298)	>128 (298)	>128 (298)	128 (298)	16 (37)	>128 (298)	>128 (298)
3n	>128 (298)	>128 (298)	>128 (298)	>128 (298)	>128 (298)	16 (37)	>128 (298)	>128 (298)
3o	>128 (311)	>128 (311)	>128 (311)	>128 (311)	128 (311)	8/16 (19/39)[c]	>128 (311)	>128 (311)
3p	32 (64)	>128 (257)	>128 (257)	32 (64)	>128 (257)	16 (32)	>128 (257)	>128 (257)
3q	128 (292)	64 (146)	64 (146)	128 (292)	64 (146)	16 (36)	>128 (>292)	>128 (>292)
3r	32 (73)	>128 (291)	>128 (291)	16 (36)	>128 (291)	32 (73)	>128 (291)	>128 (291)
3s	2 (4)	>128 (292)	>128 (292)	8 (18)	>128 (292)	>128 (292)	>128 (292)	>128 (292)
3t	32 (68)	>128 (273)	>128 (273)	4 (8)	>128 (273)	8 (17)	>128 (273)	>128 (273)
3u	16 (30)	16 (30)	8 (15)	32 (61)	16 (30)	2 (4)	>128 (245)	>128 (245)
linezolid[b]	2 (6)	1 (3)	2(6)	16(47)	32(95)	16(47)	2 (6)	32 (95)
cefuroxime[b]	2 (4)	1 (2)	8 (16)	>128 (251)	32 (63)	8 (16)	>128 (251)	>128 (251)
vancomycin[b]	0.5 (0.3)	0.5 (0.3)	4 (3)	0.5 (0.3)	2 (1)	1 (0.7)	2 (1)	>128 (0.7)

[a] MIC values are reported in $\mu g\,mL^{-1}$, with μM values given in parentheses. [b] Reference compounds. [c] Slightly different values from different experiments.

deactivation mediated by β-lactamases in the periplasmic space between outer and cytoplasmic membranes.

Promising potency against Gram-positive pathogens was observed for benzyl- (**3a**, **3i–r**, and **3u**) and phenethyl-carbamates (**3s**, **3t**), whereas no activity was detected for aniline-carbamates **3b–d** and **3f–h** (data not shown). Compounds **3a** and **3u**, with a benzyl-carbamate residue, showed interesting and broad activities across the staphylococcal strains tested, especially against resistant strains *S. aureus* 44674 and SAU-1, with MIC values of 8 μg mL^{-1} for **3a** against both strains and for **3u** against the *S. aureus* 44674 strain. Moreover, **3u** demonstrated the best antimicrobial activity against the MDR *S. hominis* α26 strain, with an MIC value of 2 μg mL^{-1}. Among the alkoxy-benzyl carbamates, only compound **3l** retained some antibacterial activity against *S. aureus* (MICs = 32–64 μg mL^{-1}); it also showed potency against *S. hominis* (MIC = 4 μg mL^{-1}). Chloro- and fluoro-aryl substituents lost activity, indicating a requirement for an electron-rich aryl fragment on the benzylamine. Among those compounds with a linezolid-like chain anchored on the azetidinones, **3h** (belonging to the group of aniline-carbamates) was inactive, while **3p** showed some potency against *S. aureus* and *S. hominis*, with MIC values of 32 and 16 μg mL^{-1}, respectively.

As a general trend, the amine fragment of the carbamate considerably impacted the antibacterial activity: only benzylamine and phenethylamine residues showed potency. The loss of flexibility of an aniline-carbamate compared with a benzyl- or phenethyl-carbamate could be considered an explanation for this general observation. This argument was supported by the low potency of **3k**, which has a more sterically demanding α-methyl-benzyl-carbamate and, consequently, minor flexibility. Upon extending the chain length between the aryl fragment and the carbamate functional group, as in compounds **3s** and **3t**, the activity became more selective against *S. aureus* ATCC 29213 and *S. aureus* SAU-1 (a methicillin- and linezolid-resistant *S. aureus strain*, LIN-R MRSA) and with a more valuable potency (compound **3s** exhibited an MIC value of 2 μg mL^{-1} against ATCC 29213, while **3t** exhibited an MIC value of 4 μg mL^{-1} against *S. aureus* SAU-1, see Table 2). Notably, **3s** and **3t** are more active than cefuroxime and linezolid against *S. aureus* SAU-1, a strain characterized by a MRSA phenotype responsible for resistance to cefuroxime and an evolved mutational profile related to linezolid resistance. Compounds **3s** and **3t** showed a noticeable difference in activities between MSSA (69856 or ATCC 29213) and MRSA (SAU-1 or 44674) strains. The same behavior was also observed for **3p** and **3r,** but to a lesser extent. The resistance factor in MRSA strains is mainly the production of the modified penicillin-binding protein 2a (PBP2a) in place of the original PBP2, but the selective potency of **3s** and **3t** against both MSSA and MRSA strains suggests that PBP2a is not the only possible target for these compounds but also other PBPs, such as PBP4.[23] When examining their antimicrobial activities, the susceptibility of these compounds against β-lactamases, such as the one coded by the *blaZ* gene in *S. aureus*, is also worthy of consideration.

Compounds **3a**, **3i**, **3j**, **3l**, **3t**, and **3u** were shown to be more active than linezolid against an *S. hominis* α26 isolate, another strain which possesses an important multidrug-resistant (MDR) phenotype and is characterized as linezolid-resistant. Compound **3t** was shown to be as active as cefuroxime against *S. hominis* α26.

The working hypothesis for the different activities of benzyl-carbamate versus aniline-carbamate being due to different conformations was tentatively investigated by NOESY experiments on compounds **3d** and **3j**, chosen for their highly differentiated resonance patterns in the aromatic region (Supporting Information). The observed NOE contacts for the two molecules are depicted in Figure 3. Azetidinone **3d** showed few

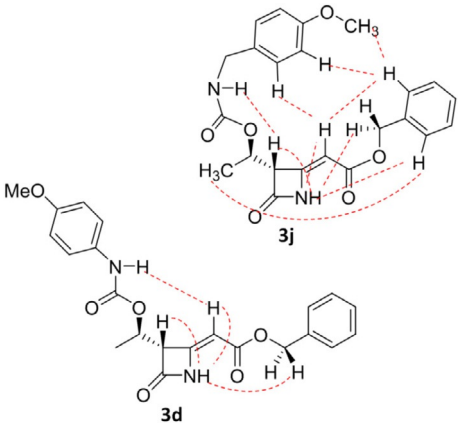


Figure 3. Structures of azetidinones **3d** and **3j**. Dashed red lines indicate the available NOEs. Contacts at a distance of less than four bonds were omitted.

contacts involving exclusively the two NH groups. On the contrary, **3j** significantly showed more contacts, in particular between the benzyl ester fragment and the pOMe-benzyl carbamate. This data could suggest a different spatial arrangement between the two compounds: **3j** seems to prefer a folded conformation in which the two side chains get closer, thanks to the more flexible benzyl amine residue, whereas the more rigid aniline carbamate in **3d** prevents this possibility.

Conclusions

In conclusion, we developed and optimized a two-step protocol for obtaining a small library of highly functionalized azetidinones. The novel derivatives can be considered a new class of monocyclic β-lactams armed with an aryl carbamate moiety on the C3 hydroxyethyl side chain and an 4-alkylidene-carboxybenzyl chain on the C4 of the azetidinone core. All new compounds were tested for their antibacterial efficacy against Gram-positive and Gram-negative recent clinical isolates, including MDR strains.

Most of the new compounds showed a selective and appreciable activity against MDR Gram-positive species, with MIC values of 2–8 μg mL^{-1} for seven azetidinones, whereas none were active against Gram-negative species. Notably, seven β-lactams emerged from the new library that were more active than linezolid against the MDR strain *S. hominis* α26, as well as

three compounds active against another MDR strain, *S. aureus* SAU-1. In addition, a correlation between antibacterial activity and the N-substituent of the carbamate group was found: aniline derivatives **3b–h** were inactive, **3i–r** and **3u** with a benzyl-carbamate residue were more or less active, depending on the aromatic ring substituents, and overall, phenethyl-carbamates **3s** and **3t** were selective antibacterials against *S. aureus* ATCC 29213 and linezolid- and methicillin-resistant SAU-1, with MIC values of 2–4 $\mu g\,mL^{-1}$. Further studies will further examine the correlation of the hit compounds, selected for their antimicrobial activity, with the differences in their resistance mechanisms relative to known classes of antibiotics with different well-characterized bacterial strains.

Experimental Section

General: Commercial reagents (reagent grade, >99%) were used as received without additional purification. Anhydrous solvents (CH_2Cl_2, DMSO) were obtained commercially. All reactions were performed under an inert atmosphere (N_2). ^1H and ^{13}C NMR spectra were recorded with an INOVA 400 instrument with a 5 mm probe. All chemical shifts are quoted relative to deuterated solvent signals (δ in ppm and J in Hz). 2D NOESY experiments were run using a 200 ms mixing time. Polarimetric analyses were conducted on a Unipol L1000 "Schmidt–Haensch" polarimeter at 598 nm. FTIR spectra were measured on a Bruker instrument (ALPHA) as films between NaCl plates; wave numbers are reported in cm^{-1}. Elemental analysis were performed on a Thermo Flash 2000 CHNS/O Analyzer. The purities of the target compounds were assessed as >95% using HPLC-MS. HPLC-MS was carried out on an Agilent Technologies HP1100 instrument, equipped with a ZOBRAX-Eclipse XDB-C8 Agilent Technologies column; mobile phase: H_2O/CH_3CN, 0.4 mL min^{-1}, gradient from 30 to 80% of CH_3CN over 8 min, 80% of CH_3CN until 25 min, coupled with an Agilent Technologies MSD1100 single-quadrupole mass spectrometer, full scan mode from $m/z = 50$ to 2600, in positive ion mode. Isocyanates **2a–c** and amines **4a** and **4d–t** are commercial; compounds **1**, **4h**, and **4p** were synthesized following reported procedures.[6,20]

General procedure for synthesis of isocyanates 2d–u: In a 25 mL two-necked flask with a reflux condenser under nitrogen atmosphere, a solution of the corresponding amine (**4d–t**; 0.375 mmol, 1 equiv) and triethylamine (105 µL, 0.75 mmol, 2 equiv) in anhydrous CH_2Cl_2 (3.75 mL) was cooled to 0 °C, and bis(trichloromethyl) carbonate (223 mg, 0.75 mmol, 2 equiv) was added in one portion. The mixture was warmed to room temperature and then stirred at reflux. The reaction was monitored by IR spectroscopy (N=C=O stretching at 2274 cm^{-1}). After 3 h, the solvent was evaporated under vacuum, avoiding any air exposure. Crude isocyanates **2d–t** were washed with anhydrous Et_2O and filtered under nitrogen (5× 4 mL) to remove the white precipitate, stored under nitrogen atmosphere, and used immediately.

General procedure for synthesis of carbamates 3d–t and 5u: In a 10 mL two-necked flask under nitrogen, freshly prepared isocyanate (**2d–t**; 0.375 mmol, 2.5 equiv) diluted in anhydrous CH_2Cl_2 (1 mL) was added to a solution of alcohol **1** (40 mg, 0.15 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1.5 mL). After stirring for 5 min, $TiCl_4$ (1 M in CH_2Cl_2, 15 µL, 0.015 mmol, 0.1 equiv) was added dropwise. After completion (TLC monitoring, 16 h), the mixture was quenched with water and extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, concentrated under vacuum, and purified by flash chromatography (CH_2Cl_2/acetone, 97:3 then 95:5), affording carbamates **3d–t** as waxy white solids.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-((benzylcarbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3a): In a 10 mL two-necked flask with a reflux condenser under nitrogen atmosphere, commercial benzylisocyanate **2a** (20 µL, 0.17 mmol, 1.5 equiv) and $TiCl_4$ (1 M in CH_2Cl_2, 11 µL, 0.011 mmol, 0.1 equiv) were added to a solution of alcohol **1** (29 mg, 0.11 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1.1 mL) The mixture was then stirred at reflux for 6 h. After completion (TLC monitoring), the mixture was quenched with water and extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, concentrated under vacuum, and purified by flash chromatography (cyclohexane/EtOAc, 1:1) affording carbamate **3a** as a waxy white solid (40 mg, 0.10 mmol, 91% yield): $[\alpha]^{20}_D$ +0.01 ($c = 1.00$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) $\delta = 1.41$ (d, $J = 6.4$ Hz, 3H), 3.89 (d, $J = 6.4$ Hz, 1H), 4.36 (d, $J = 5.9$ Hz, 2H), 5.07 (bt, $J = 5.9$ Hz, 1H), 5.16 (s, 2H), 5.21 (quintet, $J = 6.4$ Hz, 1H), 5.25 (s, 1H), 7.22–7.40 (m, 10H), and 8.59 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 18.1$, 45.1, 61.7, 66.2, 67.3, 90.6, 127.5, 127.6, 128.3, 128.4, 128.6, 128.7, 135.8, 138.0, 152.0, 155.1, 164.7, 166.7 ppm; IR (film) $\tilde{\nu} = 3330$, 1818, 1699, and 1657 cm^{-1}; ESI-MS m/z 412 $[M + H_2O]^+$; Anal. calcd for $C_{22}H_{22}N_2O_5$: C 66.99, H 5.62, N 7.10, found: C 67.02, H 5.63, N 7.08.

Benzyl-(*Z*)-2-((*S*)-4-oxo-3-((*R*)-1-((phenylcarbamoyl)oxy)ethyl)azetidin-2-ylidene)acetate (3b): A mixture of alcohol **1** (27 mg, 0.10 mmol, 1 equiv) and commercial phenylisocyanate **2b** (16 µL, 0.15 mmol, 1.5 equiv) in a 10 mL single-necked flask was subjected to microwave irradiation (400 W, 40 min) under stirring. The crude mixture was directly purified by flash chromatography (cyclohexane/EtOAc, 3:2) affording carbamate **3b** as a waxy white solid (22 mg, 0.06 mmol, 58% yield): $[\alpha]^{20}_D$ +0.13 ($c = 1.00$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) $\delta = 1.46$ (d, $J = 6.4$ Hz, 3H), 3.96 (d, $J = 6.0$ Hz, 1H), 5.18 (s, 2H), 5.27 (quintet, $J = 6.4$ Hz, 1H), 5.29 (s, 1H), 6.72 (bs, 1H), 7.07 (t, $J = 7.3$ Hz, 1H), 7.28–7.40 (m, 9H), and 8.61 ppm (bs, 1H); ^{13}C NMR (100 MHz, CD_3CN) $\delta = 18.7$, 62.5, 66.3, 68.2, 90.6, 119.6, 124.1, 128.9, 129.0, 129.5, 129.8, 137.7, 139.5, 153.6, 154.1, 166.6, 167.2 ppm; IR (film) $\tilde{\nu} = 3314$, 1815, 1698, and 1658 cm^{-1}; ESI-MS m/z 337 $[M-43]^+$; Anal. calcd for $C_{21}H_{20}N_2O_5$: C 66.31, H 5.30, N 7.36, found: C 66.39, H 5.28, N 7.34.

Benzyl-(*Z*)-2-((*S*)-4-oxo-3-((*R*)-1-((*o*-tolylcarbamoyl)oxy)ethyl)azetidin-2-ylidene)acetate (3c): A mixture of alcohol **1** (21 mg, 0.08 mmol, 1 equiv) and commercial *o*-tolylisocyanate **2c** (15 µL, 0.12 mmol, 1.5 equiv) in a 10 mL single-necked flask was subjected to microwave irradiation (400 W, 40 min) under stirring. The crude was directly purified by flash chromatography (cyclohexane/EtOAc, 1:1), affording carbamate **3c** as a waxy white solid (15 mg, 0.04 mmol, 48%): $[\alpha]^{20}_D$ +0.28 ($c = 1.00$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) $\delta = 1.47$ (d, $J = 6.4$ Hz, 3H), 2.25 (s, 3H), 3.96 (d, $J = 6.2$ Hz, 1H), 5.18 (s, 2H), 5.24–5.31 (m, 2H), 6.43 (bs, 1H), 7.03–7.21 (m, 3H), 7.32–7.40 (m, 5H), 7.70–7.72 (m, 1H), and 8.58 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 17.7$, 18.0, 61.6, 66.2, 67.7, 90.6, 124.6, 126.8, 128.2, 128.4, 128.6, 130.5, 135.3, 135.8, 151.9, 159.3, 164.6, 166.6 ppm; IR (film) $\tilde{\nu} = 3293$, 1807, 1694, and 1640 cm^{-1}; ESI-MS m/z 351 $[M\text{-}43]^+$; Anal. calcd for $C_{22}H_{22}N_2O_5$: C 66.99, H 5.62, N 7.10, found: C 66.90, H 5.63, N 7.09.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-methoxyphenyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3d): Following the general procedure, carbamate **3d** was obtained as a waxy white solid in 52% yield (32 mg, 0.08 mmol): $[\alpha]^{20}_D$ +0.23 ($c = 1.00$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) $\delta = 1.44$ (d, $J = 6.4$ Hz, 3H), 3.77 (s, 3H), 3.94 (d, $J = 5.9$ Hz, 1H), 5.18 (s, 2H), 5.25 (quintet, $J = 6.4$ Hz, 1H),

5.29 (s, 1H), 6.68 (bs, 1H), 6.83 (d, J=8.9 Hz, 2H), 7.28–7.37 (m, 7H), and 8.76 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=17.9, 55.5, 61.7, 66.2, 67.6, 90.6, 114.3, 120.7, 128.2, 128.3, 128.6, 130.4, 135.8, 152.0, 152.1, 156.3, 164.8, 166.7 ppm; IR (film) $\tilde{\nu}$=3054, 1824, 1708, and 1656 cm^{-1}; ESI-MS m/z 367 $[M-43]^+$; Anal. calcd for $C_{22}H_{22}N_2O_6$: C, 64.38, H 5.40, N, 6.83, found: C 64.21, H 5.46, N 6.81.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((3,5-dimethoxyphenyl)carbamoyl)oxy)-ethyl)-4-oxoazetidin-2-ylidene)acetate (3e): Following the general procedure, carbamate **3e** was obtained as a waxy white solid in 53% yield (35 mg, 0.08 mmol): $[\alpha]^{20}_D$ +0.20 (c=1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3CN) δ=1.38 (d, J=6.4 Hz, 3H), 3.72 (s, 6H), 3.99 (d, J=6.1 Hz, 1H), 5.15 (s, 2H), 5.17 (quintet, J=6.4 Hz, 1H), 5.27 (s, 1H), 6.19 (t, J=2.2 Hz, 1H), 6.63 (d, J=1.9 Hz, 2H), 7.30–7.37 (m, 5H), 8.03 (bs, 1H), and 9.27 ppm (bs, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ=18.5, 55.8, 62.3, 66.3, 68.1, 90.5, 95.9, 97.6, 128.8, 128.9, 129.3, 137.5, 141.2, 153.4, 153.9, 161.9, 166.7, 167.1 ppm; IR (film) $\tilde{\nu}$=3317, 1819, 1699, and 1659 cm^{-1}; ESI-MS m/z 397 $[M-43]^+$; Anal. calcd for $C_{23}H_{24}N_2O_7$: C 62.72, H 5.49, N 6.36, found: C 62.81, H 5.50, N 6.34.

Benzyl-(*Z*)-2-((*S*)-4-oxo-3-((*R*)-1-(((4-(trifluoromethyl)phenyl)carbamoyl)oxy)ethyl)azetidin-2-ylidene)acetate (3f): Following the general procedures, carbamate **3f** was obtained as a waxy white solid in 76% yield (51 mg, 0.11 mmol): $[\alpha]^{20}_D$ +0.17 (c=1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3CN) δ=1.41 (d, J=6.4 Hz, 3H), 4.02 (d, J=6.0 Hz, 1H), 5.22 (quintet, J=6.4 Hz, 1H), 5.16 (s, 2H), 5.30 (s, 1H), 7.33–7.38 (m, 5H), 7.57–7.62 (m, 4H), 8.15 (bs, 1H), and 9.05 ppm (bs, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ=17.8, 61.7, 65.5, 67.8, 89.8, 114.7 (q, J=103 Hz), 118.3, 126.2 (q, J=3.9 Hz), 126.3, 128.1, 128.2, 128.7, 136.9, 152.6, 153.2, 165.6, 166.4 ppm; IR (film) $\tilde{\nu}$=3308, 1809, 1694, and 1654 cm^{-1}; ESI-MS m/z 405 $[M-43]^+$; Anal. calcd for $C_{22}H_{19}F_3N_2O_5$: C 58.93, H 4.27, N 6.25, found: C 59.02, H 4.28, N 6.24.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((3-fluorophenyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3g): Following the general procedure, carbamate **3g** was obtained as a waxy white solid in 53% yield (32 mg, 0.08 mmol): $[\alpha]^{20}_D$ +0.21 (c=1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3CN) δ=1.39 (d, J=6.4 Hz, 3H), 4.01 (d, J=6.1 Hz, 1H), 5.16 (s, 2H), 5.19 (quintet, J=6.4 Hz, 1H), 5.28 (s, 1H), 6.75–6.80 (m, 1H), 7.13 (dd, J=8.2, 1.0 Hz, 1H), 7.25–7.38 (m, 7H), 8.14 (bs, 1H), and 9.17 ppm (bs, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ= 18.6, 62.5, 66.4, 68.4, 90.6, 106.3 (d, J=25.9 Hz), 110.3 (d, J= 21.4 Hz), 115.0, 128.9, 129.0, 129.5, 131.3 (d, J=9.7 Hz), 137.7, 141.4 (d, J=11.1 Hz), 153.4, 154.0, 163.9 (d, J=220 Hz), 166.7, 167.2 ppm; IR (film) $\tilde{\nu}$=3407, 1810, 1693, and 1656 cm^{-1}; ESI-MS m/z 355 $[M-43]^+$; Anal. calcd for $C_{21}H_{19}FN_2O_5$: C 63.31, H 4.81, N 7.03, found: C 63.28, H 4.80, N 7.01.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((3-fluoro-4-morpholinophenyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3h): Following the general procedure, carbamate **3h** was obtained as a waxy white solid in 72% yield (52 mg, 0.11 mmol): $[\alpha]^{20}_D$ +0.38 (c=1.00, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.46 (d, J=6.4 Hz, 3H), 3.09 (s, 4H), 3.87–3.93 (m, 4H), 3.97 (d, J=5.9 Hz, 1H), 5.18 (s, 2H), 5.25–5.31 (m, 2H), 6.69 (bs, 1H), 6.96–7.01 (m, 2H), 7.34–7.41 (m, 6H), and 8.51 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=17.9, 51.2, 61.6, 66.2, 66.9, 67.6, 90.8, 107.8, 108.1, 114.6, 119.1, 128.3, 128.4, 128.7, 132.7, 135.7, 151.7, 152.0, 155.6 (d, J=246 Hz), 164.5, 166.6 ppm; IR (film) $\tilde{\nu}$=3419, 1813, 1719, 1694, and 1657 cm^{-1}; ESI-MS m/z 440 $[M-43]^+$; Anal. calcd for $C_{25}H_{26}FN_3O_6$: C 62.10, H 5.42, N 8.69, found: C 62.11, H 5.43, N 8.67.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-methylbenzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3i): Following the general procedure, carbamate **3i** was obtained as a waxy white solid in 69% yield (42 mg, 0.10 mmol): $[\alpha]^{20}_D$ −0.01 (c=1.10, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.40 (d, J=6.4 Hz, 3H), 2.31 (s, 3H), 3.88 (d, J=6.4 Hz, 1H), 4.31 (d, J=5.7 Hz, 2H), 5.09 (bt, J=5.2 Hz, 1H), 5.17 (s, 2H), 5.22 (quintet, J=6.4 Hz, 1H), 5.26 (s, 1H), 7.10–7.17 (m, 4H), 7.34–7.38 (m, 5H), and 8.79 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=18.1, 21.0, 44.8, 61.7, 66.1, 67.2, 90.4, 127.5, 128.2, 128.3, 128.6, 129.3, 135.0, 135.8, 137.2, 152.3, 155.1, 165.0, 166.7 ppm; IR (film) $\tilde{\nu}$=3315, 1810, 1693, and 1654 cm^{-1}; ESI-MS m/z 426 $[M+H_2O]^+$; Anal. calcd for $C_{23}H_{24}N_2O_5$: C 67.63, H 5.92, N 6.86, found: C 67.72, H 5.93, N 6.85.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-methoxybenzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3j): Following the general procedure, carbamate **3j** was obtained as a waxy white solid in 63% yield (40 mg, 0.09 mmol): $[\alpha]^{20}_D$ +0.01 (c=1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.39 (d, J=6.4 Hz, 3H), 3.77 (s, 3H), 3.88 (d, J=6.3 Hz, 1H), 4.28 (d, J=5.3 Hz, 2H), 5.07 (bt, J=5.4 Hz, 1H), 5.16 (s, 2H), 5.19 (quintet, J=6.5 Hz, 1H), 5.25 (s, 1H), 6.84 (d, J=8.4 Hz, 2H), 7.19 (d, J=8.4 Hz, 2H), 7.32–7.37 (m, 5H), and 8.73 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=18.1, 44.5, 55.2, 61.7, 66.1, 67.2, 90.4, 114.0, 128.1, 128.3, 128.6, 128.9, 130.2, 135.8, 152.2, 155.1, 159.0, 164.8, 166.6 ppm; IR (film) $\tilde{\nu}$=3329, 1811, 1692, and 1654 cm^{-1}; ESI-MS m/z 442 $[M+H_2O]^+$; Anal. calcd for $C_{23}H_{24}N_2O_6$: C 65.08, H 5.70, N 6.60, found: C 65.12, H 5.71, N 6.59.

Benzyl-(*Z*)-2-((3*S*)-3-((1*R*)-1-(((1-(4-methoxyphenyl)ethyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3k): Following the general procedure, carbamate **3k** was obtained as a waxy white solid in 69% yield (45 mg, 0.10 mmol): $[\alpha]^{20}_D$ −0.24 (c=1.20, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.41 (d, J=5.6 Hz, 3H), 1.44 (d, J=7.6 Hz, 3H), 3.75 (s, 3H), 3.85 (d, J=6.2 Hz, 1H), 4.77 (bt, J= 6.4 Hz, 1H), 5.07 (d, J=7.2 Hz, 1H), 5.12–5.17 (m, 3H), 5.21 (s, 1H), 6.83 (d, J=8.2 Hz, 2H), 7.21 (d, J=8.2 Hz, 2H), 7.29–7.38 (m, 5H), and 8.82 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=17.9, 22.1, 50.1, 55.2, 61.7, 66.1, 67.0, 90.3, 113.7, 113.9, 126.6, 127.0, 128.1, 128.2, 128.6, 135.2, 135.8, 152.3, 154.3, 158.7, 165.0, 166.7 ppm; IR (film) $\tilde{\nu}$=3317, 1819, 1698, and 1658 cm^{-1}; ESI-MS m/z 456 $[M+H_2O]^+$; Anal. calcd for $C_{24}H_{26}N_2O_6$: C 65.74, H 5.98, N 6.39, found: C 65.65, H 5.96, N 6.37.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((3,4-dimethoxybenzyl)carbamoyl)oxy)-ethyl)-4-oxoazetidin-2-ylidene)acetate (3l): Following the general procedure, carbamate **3l** was obtained as a waxy white solid in 75% yield (51 mg, 0.11 mmol): $[\alpha]^{20}_D$ +0.01 (c=1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.39 (d, J=6.4 Hz, 3H), 3.84 (s, 6H), 3.88 (d, J=6.3 Hz, 1H), 4.28 (d, J=5.7 Hz, 2H), 5.09 (bt, J=5.5 Hz, 1H), 5.12–5.18 (m, 2H), 5.20 (quintet, J=6.1 Hz, 1H), 5.24 (s, 1H), 6.67–6.81 (m, 3H), 7.32–7.36 (m, 5H), and 8.72 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=18.1, 44.9, 55.7, 55.8, 61.7, 66.1, 67.2, 90.4, 110.8, 111.1, 119.8, 128.2, 128.3, 128.6, 130.6, 135.7, 148.4, 149.1, 155.1, 164.9, 152.2, 166.6 ppm; IR (film) $\tilde{\nu}$=3356, 1819, 1700, and 1657 cm^{-1}; ESI-MS m/z 472 $[M+H_2O]^+$; Anal. calcd for $C_{24}H_{26}N_2O_7$: C 63.43, H 5.77, N 6.16, found: C 63.55, H 5.78, N 6.16.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-chlorobenzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3m): Following the general procedure, carbamate **3m** was obtained as a waxy white solid in 69% yield (45 mg, 0.10 mmol): $[\alpha]^{20}_D$ +0.02 (c=1.10, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.39 (d, J=6.3 Hz, 3H), 3.88 (d, J=6.3 Hz, 1H), 4.29 (d, J=5.9 Hz, 2H), 5.16–5.20 (m, 4H), 5.24 (s, 1H), 7.17 (d, J= 8.0 Hz, 2H), 7.25 (d, J=8.0 Hz, 2H), 7.31–7.39 (m, 5H), and 8.83 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=18.1, 44.3, 61.6,

66.2, 67.4, 90.4, 128.2, 128.3, 128.4, 128.6, 128.8, 133.3, 135.7, 136.7, 152.2, 155.2, 165.0, 166.7 ppm; IR (film) $\tilde{\nu}$ = 3329, 1810, 1691, and 1649 cm^{-1}; ESI-MS *m/z* 446 $[M+H_2O]^+$; Anal. calcd for $C_{22}H_{21}ClN_2O_5$: C 61.61, H 4.94, N 6.53, found: C 61.60, H 4.95, N 6.52.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((2-chlorobenzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3n): Following the general procedure, carbamate **3n** was obtained as a waxy white solid in 59% yield (38 mg, 0.09 mmol): $[\alpha]^{20}_D$ +0.01 (*c* = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.39 (d, *J* = 6.4 Hz, 3H), 3.89 (d, *J* = 6.4 Hz, 1H), 4.44 (d, *J* = 6.2 Hz, 2H), 5.16 (s, 2H), 5.19–5.26 (m, 3H), 7.19–7.21 (m, 2H), 7.33–7.37 (m, 7H), and 8.67 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.1, 43.0, 61.7, 66.2, 67.4, 90.5, 127.1, 128.2, 128.3, 128.6, 129.0, 129.5, 129.7, 133.4, 135.5, 135.8, 152.1, 155.1, 164.8, 166.7 ppm; IR (film) $\tilde{\nu}$ = 3318, 1819, 1699, and 1658 cm^{-1}; ESI-MS *m/z* 446 $[M+H_2O]^+$; Anal. calcd for $C_{22}H_{21}ClN_2O_5$: C 61.61, H 4.94, N 6.53, found: C 61.74, H 4.96, N 6.52.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-fluorobenzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3o): Following the general procedure, carbamate **3o** was obtained as a waxy white solid in 69% yield (43 mg, 0.10 mmol): $[\alpha]^{20}_D$ +0.01 (*c* = 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.40 (d, *J* = 6.4 Hz, 3H), 3.89 (d, *J* = 6.3 Hz, 1H), 4.31 (d, *J* = 5.8 Hz, 2H), 5.08 (bt, *J* = 5.9 Hz, 1H), 5.16 (s, 2H), 5.20 (quintet, *J* = 6.4 Hz, 1H), 5.24 (s, 1H), 6.96–7.00 (m, 2H), 7.21–7.24 (m, 2H), 7.33–7.40 (m, 5H), and 8.61 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.1, 44.4, 61.7, 66.2, 67.4, 90.5, 115.5 (d, *J* = 21.5 Hz), 128.2, 128.3, 128.6, 129.1 (d, *J* = 9.1 Hz), 133.9, 135.8, 152.1, 155.1, 162.2 (d, *J* = 240 Hz), 164.6, 166.6 ppm; IR (film) $\tilde{\nu}$ = 3330, 1817, 1697, and 1656 cm^{-1}; ESI-MS *m/z* 430 $[M+H_2O]^+$; Anal. calcd for $C_{22}H_{21}FN_2O_5$: C 64.07, H 5.13, N 6.79, found: C 63.96, H 5.11, N 6.77.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((3-fluoro-4-morpholinobenzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3p): Following the general procedure, carbamate **3p** was obtained as a waxy white solid in 74% yield (55 mg, 0.11 mmol): $[\alpha]^{20}_D$ +0.04 (*c* = 1.20, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.39 (d, *J* = 6.1 Hz, 3H), 3.03 (s, 4H), 3.84 (s, 4H), 3.88 (d, *J* = 6.0 Hz, 1H), 4.26 (s, 2H), 5.13–5.28 (m, 5H), 6.86–6.94 (m, 3H), 7.16–7.35 (m, 5H), and 8.80 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.0, 44.1, 50.8, 61.6, 66.1, 66.8, 67.3, 90.4, 115.4 (d, *J* = 21.3 Hz), 118.7 (d, *J* = 3.2 Hz), 123.4 (d, *J* = 3.0 Hz), 128.1, 128.3, 128.6, 133.0 (d, *J* = 7.4 Hz), 135.8, 139.1 (d, *J* = 9.0 Hz), 152.2, 155.1, 155.5 (d, *J* = 240 Hz), 164.9, 166.6 ppm; IR (film) $\tilde{\nu}$ = 3329, 1814, 1696, and 1658 cm^{-1}; ESI-MS *m/z* 498 $[M+H]^+$; Anal. calcd for $C_{26}H_{28}FN_3O_6$: C 62.77, H 5.67, N 8.45, found: C 62.75, H 5.68, N 8.44.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((benzo[*d*][1,3]dioxol-5-ylmethyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3q): Following the general procedure, carbamate **3q** was obtained as a waxy white solid in 76% yield (50 mg, 0.11 mmol): $[\alpha]^{20}_D$ −0.03 (*c* = 1.10, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.39 (d, *J* = 6.4 Hz, 3H), 3.88 (d, *J* = 6.3 Hz, 1H), 4.19–4.29 (m, 2H), 5.13–5.22 (m, 4H), 5.24 (s, 1H), 5.91 (s, 2H), 6.71–6.76 (m, 3H), 7.31–7.37 (m, 5H), and 8.82 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.0, 44.8, 61.6, 66.1, 67.2, 90.4, 101.0, 108.1, 108.2, 120.8, 128.2, 128.3, 128.6, 132.0, 135.8, 146.9, 147.8, 152.3, 155.1, 165.0, 166.7 ppm; IR (film) $\tilde{\nu}$ = 3301, 1819, 1701, and 1656 cm^{-1}; ESI-MS *m/z* 456 $[M+H_2O]^+$; Anal. calcd for $C_{23}H_{22}N_2O_7$: C 63.01, H 5.06, N 6.39, found: C 63.33, H 5.08, N 6.38.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-nitrobenzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3r): Following the general procedure, carbamate **3r** was obtained as a waxy white solid in 66% yield (44 mg, 0.10 mmol): $[\alpha]^{20}_D$ +0.05 (*c* = 1.10, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.41 (d, *J* = 6.2 Hz, 3H), 3.91 (d, *J* = 6.0 Hz, 1H), 4.45 (d, *J* = 5.9 Hz, 2H), 5.17 (s, 2H), 5.19–5.23 (m, 1H), 5.25 (s, 1H), 5.33 (bt, *J* = 5.6 Hz, 1H), 7.33–7.38 (m, 5H), 7.42 (d, *J* = 8.3 Hz, 2H), 8.15 (d, *J* = 8.3 Hz, 2H), and 8.71 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.1, 44.3, 61.6, 66.2, 67.6, 90.5, 123.9, 127.9, 128.2, 128.4, 128.6, 135.7, 145.7, 147.3, 152.0, 155.3, 164.8, 166.6 ppm; IR (film) $\tilde{\nu}$ = 3322, 1817, 1699, and 1657 cm^{-1}; ESI-MS *m/z* 457 $[M+H_2O]^+$; Anal. calcd for $C_{22}H_{21}N_3O_7$: C 60.13, H 4.82, N 9.56, found: C 60.04, H 4.82, N 9.54.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-methoxyphenethyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3s): Following the general procedure, carbamate **3s** was obtained as a waxy white solid in 84% yield (55 mg, 0.13 mmol): $[\alpha]^{20}_D$ −0.08 (*c* = 1.20, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.37 (d, *J* = 6.3 Hz, 3H), 2.74 (t, *J* = 6.8 Hz, 2H), 3.34–3.43 (m, 2H), 3.77 (s, 3H), 3.86 (d, *J* = 6.6 Hz, 1H), 4.77 (bt, *J* = 5.4 Hz, 1H), 5.12–5.21 (m, 3H), 5.24 (s, 1H), 6.84 (d, *J* = 8.2 Hz, 2H), 7.09 (d, *J* = 8.2 Hz, 2H), 7.32–7.37 (m, 5H), and 8.72 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.1, 35.0, 42.3, 55.2, 61.7, 66.2, 67.1, 90.4, 114.0, 128.2, 128.3, 128.6, 129.7, 130.5, 135.8, 152.3, 155.0, 158.2, 164.9, 166.7 ppm; IR (film) $\tilde{\nu}$ = 3302, 1819, 1700, and 1658 cm^{-1}; ESI-MS *m/z* 456 $[M+H_2O]^+$; Anal. calcd for $C_{24}H_{26}N_2O_6$: C 65.74, H 5.98, N 6.39, found: C 65.82, H 6.00, N 6.38.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((3,4-dimethoxyphenethyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (3t): Following the general procedure, carbamate **3t** was obtained as a waxy white solid in 57% yield (40 mg, 0.09 mmol): $[\alpha]^{20}_D$ −0.05 (*c* = 1.20, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.37 (d, *J* = 6.3 Hz, 3H), 2.74 (t, *J* = 7.1 Hz, 2H), 3.38–3.43 (m, 2H), 3.83–3.87 (m, 7H), 4.79 (bt, *J* = 5.6 Hz, 1H), 5.13–5.19 (m, 3H), 5.23 (s, 1H), 6.69–6.80 (m, 3H), 7.32–7.36 (m, 5H), and 8.67 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.1, 35.6, 42.3, 55.7, 55.8, 61.7, 66.2, 67.1, 90.4, 111.4, 111.8, 120.7, 128.2, 128.3, 128.6, 131.0, 135.7, 147.7, 149.0, 152.3, 155.0, 164.9, 166.6 ppm; IR (film) 3355, 1819, 1701, and 1657 cm^{-1}; ESI-MS *m/z* 486 $[M+H_2O]^+$; Anal. calcd for $C_{25}H_{28}N_2O_7$: C 64.09, H 6.02, N 5.98, found: C 64.13, H 6.04, N 5.87.

Benzyl-(*Z*)-2-((*S*)-3-((*R*)-1-(((4-((*tert*-butoxycarbonyl)amino)benzyl)carbamoyl)oxy)ethyl)-4-oxoazetidin-2-ylidene)acetate (5u): Following the general procedure, carbamate **5u** was obtained as a waxy white solid in 55% yield (42 mg, 0.08 mmol): $[\alpha]^{20}_D$ −0.03 (*c* = 1.08, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.39 (d, *J* = 6.4 Hz, 3H), 1.51 (s, 9H), 3.87 (d, *J* = 6.4 Hz, 1H), 4.27–4.29 (m, 2H), 5.05 (bt, *J* = 4.8 Hz, 1H), 5.16 (s, 2H), 5.20 (quintet, *J* = 6.4 Hz, 1H), 5.24 (s, 1H), 6.47 (bs, 1H), 7.16 (d, *J* = 8.2 Hz, 2H), 7.25 (d, *J* = 8.2 Hz, 2H), 7.32–7.40 (m, 5H), and 8.62 ppm (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.2, 28.3, 44.6, 61.7, 66.2, 67.3, 80.6, 90.5, 114.0, 118.7, 128.2, 128.3, 128.6, 132.6, 135.8, 137.8, 152.2, 152.7, 155.1, 164.8, 166.7 ppm; IR (film) $\tilde{\nu}$ = 3334, 1812, 1698 and 1657 cm^{-1}; ESI-MS *m/z* 527 $[M+H_2O]^+$.

4-(((((*R*)-1-((S,Z)-2-(2-(benzyloxy)-2-oxoethylidene)-4-oxoazetidin-3-yl)ethoxy)carbonyl)amino)methyl)benzenaminium-2,2,2-trifluoroacetate (3u): In a 10 mL 2-neck flask under nitrogen at 0 °C, TFA (48 μL, 0.64 mmol, 8 equiv) was added dropwise to a solution of **5u** (42 mg, 0.08 mmol) in anhydrous CH_2Cl_2 (2.8 mL). The mixture was stirred at room temperature for 1 h, and new TFA aliquots (30 equiv overall) were added at 0 °C until complete conversion as assessed by HPLC monitoring. The solvent was removed under reduced pressure, and the crude mixture was triturated with a few drops of pentane, providing **3u** as a colorless oil (96%, 40 mg, 0.076 mmol): $[\alpha]^{20}_D$ −0.14 (*c* = 1.02, CH_3OH); ^{1}H NMR (400 MHz,

CD_3OD) $\delta = 1.36$ (d, $J = 6.4$ Hz, 3H), 3.95 (d, $J = 6.7$ Hz, 1H), 4.09–4.17 (m, 2H), 5.10 (quintet, $J = 6.4$ Hz, 1H), 5.17 (s, 2H), 5.24 (s, 1H), 6.64 (d, $J = 8.1$ Hz, 2H), 7.00 (d, $J = 8.1$ Hz, 2H), and 7.29–7.38 ppm (m, 5H); ^{13}C NMR (100 MHz, CD_3CN) $\delta = 18.8$, 44.6, 62.7, 66.4, 68.1, 90.6, 122.4, 129.0, 129.1, 129.2, 129.4, 129.5, 131.6, 137.8, 154.2, 156.8, 166.8, 167.3 ppm; IR (film) $\tilde{\nu} = 3356$, 1815, 1701 and 1655 cm^{-1}; ESI-MS *m/z* 410 $[M-TFA+H]^+$; Anal. calcd for $C_{24}H_{24}F_3N_3O_7$: C 55.07, H 4.62, N 8.03, found: C 55.4, H 4.63, N 8.00.

Bacterial strains and antimicrobial susceptibility testing procedures: Representative Gram-positive and Gram-negative bacterial pathogens, hosted in the microbial biobank (MicroMiB Biobank) of the Laboratory of Clinical Microbiology and Virology of the University of Milano-Bicocca, Monza, were used for in vitro antimicrobial susceptibility testing. These included *S. aureus*, *S. hominis*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Enterococcus faecium* as Gram-positive species and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as Gram-negative species. In particular, laboratory stocks of MSSA strain 69856, MRSA strain 44674, LIN-R MRSA strain SAU-1, methicillin-susceptible *S. epidermidis* (MSSE) strain 3226, linezolid-resistant and methicillin-resistant *S. epidermidis* (LIN-R MRSE) strain G1027, linezolid-resistant methicillin-resistant *S. hominis* (LIN-R MRSH) strain α26, and linezolid-resistant vancomycin-resistant *E. faecium* (LIN-R VRE) strain VRE-2 were used as test strains. *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were purchased from the American Type Culture Collection (Manassas, VA, USA). The in vitro antibacterial activity of newly synthetized compounds **3a–t** was studied by determining their MIC values using a broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[24] All chemicals used in this study were of analytical grade. Linezolid, cefuroxime and vancomycin (Sigma–Aldrich, Italy) were used as reference antibiotic compounds for MIC determinations. Briefly, serial twofold dilutions of each compound were made using Mueller–Hinton broth in 96-well microtiter plates. Dimethyl sulfoxide (DMSO) was used as solvent for all of the synthetized compounds. An equal volume of bacterial inoculum (1×10^6 CFU mL^{-1}) was added to each well on the microtiter plate containing serial antibiotic dilutions (50 μL). The microtiter plate was then incubated at 37 °C for 18–24 h, after which time each well was analyzed for the presence of bacterial growth. The MIC value was defined as the lowest concentration of antimicrobial agent able to cause inhibition of bacterial growth, as shown by the lack of visible turbidity of the culture medium. Standard strains *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as controls and for MIC testing validation.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: antibiotics · azetidinones · carbamates · β-lactams · drug resistance

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