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Article

Zidovudine-β-Lactam Pronucleoside Strategy for Selective Delivery into Gram-Negative Bacteria Triggered by β-Lactamases

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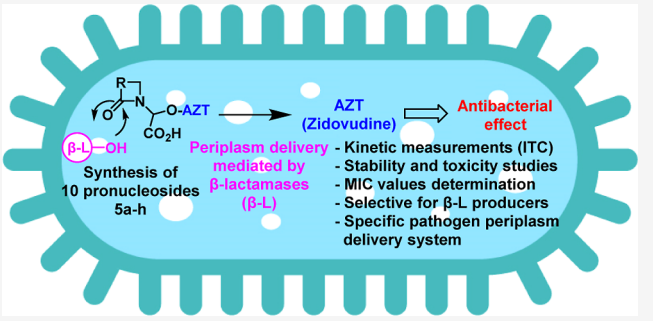
ABSTRACT: Addressing antibacterial resistance is a major concern of the modern world. The development of new approaches to meet this deadly threat is a critical priority. In this article, we investigate a new approach to negate bacterial resistance: exploit the β-lactam bond cleavage by β-lactamases to selectively trigger antibacterial prodrugs into the bacterial periplasm. Indeed, multidrug-resistant Gram-negative pathogens commonly produce several β-lactamases that are able to inactivate β-lactam antibiotics, our most reliable and widely used therapeutic option. The chemical structure of these prodrugs is based on a monobactam promoiety, covalently attached to the active antibacterial substance, zidovudine (AZT). We describe the synthesis of 10 prodrug analogues (**5a−h**) in four to nine steps and their biological activity. Selective enzymatic activation by a panel of β-lactamases is demonstrated, and subsequent structure−activity relationships are discussed. The best compounds are further evaluated for their activity on both laboratory strains and clinical isolates, preliminary stability, and toxicity.



KEYWORDS: *prodrug, β-lactamase, bacterial resistance, Gram-negative pathogen, zidovudine*

In the early 1900s, bacterial infections represented one of the most significant causes of death worldwide. This plague has been circumvented by the development of several classes of antibiotics, including β-lactams, which show optimal efficacy and selectivity.[1] Almost immediately after the introduction of penicillin, penicillin-resistant strains of *Staphylococcus aureus* emerged in the clinical setting. More generally, due to the natural selective pressure when exposed to exogenous agents, bacteria developed an arsenal of mechanisms to escape the therapeutic effect of antibiotics.[2] Although the fast discovery and development of new antibacterial drugs allowed us to adequately address the problem of resistance (a period known as the golden age of antibiotic discovery), the situation drastically changed in the late 1990s with the emergence of multidrug-resistant bacteria and the progressive disinterest of pharmaceutical companies in antibiotic R&D, leading to a substantial erosion of available therapeutic options.[3] Nowadays, besides multidrug-resistant bacteria, the prevalence of isolates showing extensively drug or pan drug resistance phenotypes is increasing in the clinical setting.[4,5] Currently, global deaths attributable to antimicrobial resistance are estimated to exceed 1.2 million, raising worldwide awareness.[6] Among other responses (antibiotic stewardship and better and faster diagnosis), the development of new antibiotics remains urgent, especially against the so-called "critical priority" pathogens, as established by the WHO, which include carbapenem-resistant Gram-negative bacteria (*Enterobacterales, Acinetobacter*, and *Pseudomonas*).[7] β-lactam antibiotics still represent the mainstay of antibacterial therapy, accounting for more than 50% of antibiotics prescribed worldwide.[8] Numerous families were developed, such as penicillins, cephalosporins, monobactams, and carbapenems, and are all characterized by a four-membered β-lactam ring, crucial for their antibacterial activity. A major mechanism of β-lactam resistance in Gram-negative pathogens is the production of enzymes that are able to hydrolyze the amide bond of the β-lactam ring, called β-lactamases.[9] They are classified into four molecular classes (A, B, C, and D) but can be divided into two main families based on their mechanism of action. Serine-β-lactamases (SBLs, classes A, C, and D) are related to serine hydrolase enzymes,[10] and metallo-β-lactamases (MBLs, class B) are characterized by the presence of one or two zinc atoms into the active site.[11] Their hydrolytic action results from the nucleophilic attack of the serine residue or water molecule, according to the β-lactamase type, on the β-lactam carbonyl. β-

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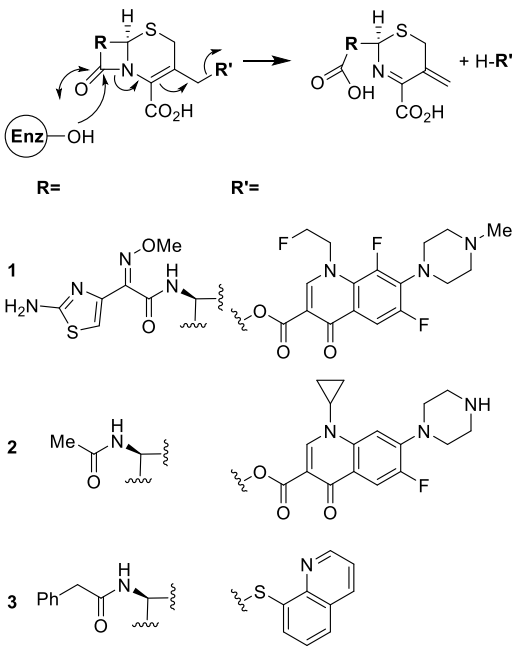
Lactamases show an extraordinary diversity (with >7800 unique enzymes) and evolutionary potential, leading to the emergence of clinical variants (by either a mutation or the acquisition of novel from the environmental resistome) with a peculiar substrate profile, such as extended-spectrum β-lactamases or carbapenem-hydrolyzing enzymes (e.g., KPC or OXA-type carbapenemases).[12] The latter represent a major threat as they confer resistance to the last resort and life-saving carbapenems, a major therapeutic resource for the treatment of infections caused by MDR isolates. Furthermore, resistant bacterial strains commonly produce several β-lactamases, sometimes both serine- and metallo-carbapenemases. In this context, the development of new effective β-lactam-based therapies is still necessary but extremely challenging.[13] One strategy is represented by the development of combinations with β-lactamase inhibitors, such as clavulanate or, more recently, avibactam or vaborbactam. Nevertheless, resistance to such new combinations is already observed in the clinical setting, highlighting the need for further innovation in the field.[14] The development of new drugs based on original strategies is fundamental to tackle this growing major threat.[15]

In this context, the design of compounds that are able to reverse bacterial resistance to β-lactam agents by exploiting the specific β-lactam fission mechanism leads to a new type of hybrid antibacterial drugs.[16−18] A first generation of dual agents was developed based on a fissile covalent combination of two antibiotics (Scheme 1). Several studies have explored

Scheme 1. Some Dual β-Lactam Agents Described in the Literature That Are Enzymatically Activated (Enz: PBPs or β-Lactamases)



the association of cephalosporin with a fluoroquinolone agent (such as Ro 23-9424, **1**).[19] With such compounds, the penicillin-binding proteins (PBPs) are initially irreversibly inactivated through the formation of a covalent adduct with the β-lactam moiety, which, after a subsequent spontaneous rearrangement, release the fluoroquinolone moiety, inhibiting bacterial DNA gyrases (Scheme 1). The chemical codrug activation has been widely studied, and different leaving groups as well as self-immolative linkers have been introduced to improve the stability and other biological properties.[20−22] Due to the ever-expanding heterogeneity and simultaneous and increased production of β-lactamases in MDR clinical isolates, inactivation of PBPs mediated by such codrugs is seriously compromised. Prodrug **2**, based on a similar approach by exploiting the β-lactamase-mediated release of a fluoroquinolone from a β-lactam scaffold, was also developed.[23] In this case, inactivation of PBPs was not desirable, and only β-lactamases producers were targeted. Recently, specific prodrugs to fight MBLs were designed with a cephalosporin-MBL inhibitor conjugate **3**.[24−26] The hydrolysis of the β-lactam ring is mediated by the MBL enzymes that will then be inhibited by the released entity. This adjuvant strategy needs a combination with a β-lactam agent (e.g., meropenem) to provide bacterial inhibition.

All these approaches are mainly based on the cephalosporin core as the promoiety. The use of clinically valuable β-lactam antibiotic scaffolds, such as especially third-generation cephalosporin (e.g., ceftazidime, ceftriaxone, and cefotaxime), which still represents the most widely used β-lactam subclass, is expected to maintain and even increase global bacterial resistance and compromise the antibacterial activity of potentially life-saving new β-lactam agents (e.g., cefiderocol).[27] We here propose an alternative approach to disrupt this domino effect, leading to worsening resistance, by protecting crucial β-lactam therapeutic subclasses through the investigation of a novel design of β-lactamase-activated prodrugs.

RESULTS AND DISCUSSION

Prodrug Design. Antibiotic therapies are never specific to bacterial pathogens, and gut microbiota disturbance[28] or superinfection phenomenon[29] is a very commonly observed side effect. To provide selective treatments, it is necessary to specifically target (opportunistic) pathogens, especially multidrug-resistant strains, which represent a common cause of difficult-to-treat hospital-acquired infections. Our approach relies on the consideration that, in Gram-negative bacteria, the production of one- or more β-lactamases, more importantly, carbapenem-hydrolyzing enzymes, represents a common resistance mechanism found in resistant strains, where the level of β-lactamase production often correlates with the level of resistance.[9] Therefore, the use of the β-lactam motif as a prodrug carrier will provide a new way to hijack one of the most common bacterial resistance mechanisms and offer the opportunity to remodulate the spectrum of activity of antibacterial compounds, limited to β-lactamase producers, with potentially beneficial effects such as (a) reducing the selective pressure contributing to the dissemination of resistant strains and (b) limiting the undesirable effect of broad-spectrum antibiotics on the gut microbiota and the damaging consequences of antibiotic-associated dysbiosis. We decided to focus on the structurally simplest β-lactam core, thus leading to a novel monobactam-based prodrug approach. Interestingly, this β-lactam subclass is underexploited, and aztreonam is the only monobactam agent, thus sharing this structural minimalism (although with some structural specificities), to be approved for the treatment of bacterial infections in humans.[30] To prevent any anti-PBP activity and avoid β-lactamase stability (as the efficient cleavage of the β-lactam is actually desired here), all usual β-lactam designs, such as the aminothiazole ring, oxime moiety, or β-lactam substitutions, will be avoided.[31−33] At position 3 (R part, Scheme 2) of the

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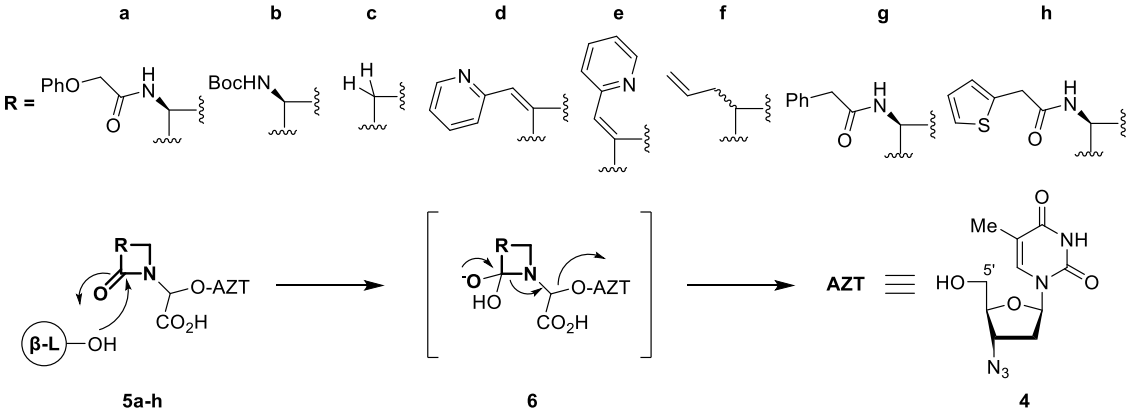
Scheme 2. Main Envisioned Substitutions and Targeted Pronucleosides 5a−h and Their Activation Mechanisms by β-Lactamase Enzymes (β-L)

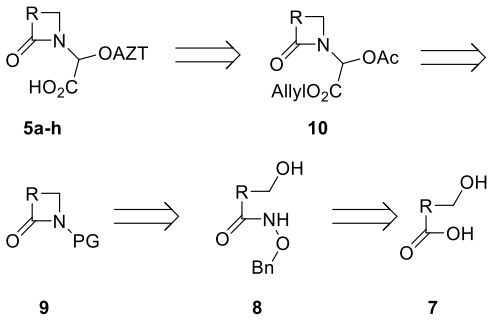


azetidinone ring, we focused on the first-generation side chains of β-lactam agents to combine optimal β-lactamase recognition while protecting the most effective substitutions present in marketed drugs. Thus, a list of side chains was drawn up from known derivatives (Scheme 2): penicillin V (a), clavulanic acid (c), penicillin G (g), cephalothin (h), reported SBL inhibitors (d and e),[34] inspired from serine protease inhibitors (f),[35] or chemical intermediate (b). The next step consisted in the selection of the antibacterial agent to be released. Drug repurposing, i.e., the use of existing drugs for a different therapeutic application, is a common strategy presenting potential advantages, such as decreased costs and minimized risks during drug development.[36] In the complicated field of antibiotics, numerous active substances have been considered, and one of the most promising classes is represented by biomolecules belonging to the nucleoside analogue (NA) class.[37] NAs are indeed a major therapeutic class, mainly known as antiviral and anticancer agents. They display pleiotropic effects in all fundamental genetic information processes in both eukaryotic and prokaryotic cells. For now, the most relevant NA studied for its antibacterial activity is zidovudine (AZT, **4**, Scheme 2). Initially developed as an anticancer agent, it is currently intensively used in AIDS treatment.[38] Several studies show that it is a potent antibacterial agent against relevant opportunistic pathogens, such as *Escherichia coli*, one of the first causes of hospital-acquired infections, including on β-lactamase-producing isolates.[39,40] To obtain the desired pronucleoside **5**, AZT has to be covalently attached to the monobactam promoiety. This linker will be achieved by an ether function between the 5′ hydroxyl group of AZT and the β-lactam moiety. By masking the phosphorylation site at position 5′ of AZT, all potential intrinsic biological activities of the corresponding pronucleoside **5a−h** are prevented (Scheme 2). β-Lactamase-mediated hydrolysis of the β-lactam ring will lead to the unstable intermediate **6**, which will spontaneously rearrange to release AZT through a rearrangement and elimination process similar to that described previously.

Thus, pronucleosides **5a−h** will be evaluated as substrates on a range of different β-lactamases, and their antibacterial activity investigated on β-lactamase-producing strains. Some ADME aspects will also be explored, such as stability, solubility, and toxicology. This study represents a new attractive approach to fight bacterial resistance by exploiting the major mechanism of β-lactam resistance in relevant pathogens, leading to an antibacterial drug delivery system with a selective activity on β-lactamase-producing isolates.

Chemical Synthesis. Due to the structural relevance of the 2-azetidinone core, retrosynthetic analyses have been extensively studied in the literature. Numerous synthetic pathways are available according to the desired substitutions on the β-lactam ring.[41] We explored several of them, such as the Reformatsky reaction, β-amino acid intramolecular cyclization, and the Mitsunobu reaction. Unfortunately, most of them failed at the β-lactam cyclization step. We deeply explored retrosynthesis based on the hydroxamate approach described by Miller (Scheme 3).[42,43] The carboxylic function of a

Scheme 3. Retrosynthetic Analysis



properly acylated serine residue **7** was activated to react with hydroxylamine and form the corresponding hydroxamate compound **8**. Several experimental conditions were described to carry on the subsequent β-lactam cyclization through a Mitsunobu reaction such as CCl_4/PPh_3 or $DEAD/PPh_3$.[44,45] All these conditions led to a weak conversion rate and a complex mixture to separate. Finally, the best conditions have been identified and consist of activation of the hydroxyl group by a mesyl group, followed by cyclization in basic media to get the key cyclized intermediate **9**.[46] After removal of the protecting group (PG), the NH of the β-lactam function was substituted by a glyoxalate reagent, which was acetylated to give the corresponding promoiety **10**. The introduction of the AZT drug will be performed, thanks to the *N*-acylium chemistry providing final pronucleoside compounds **5a−h**.

Two sequential approaches were implemented according to the nature of R moiety. First, in order to explore, optimize, and develop the synthetic pathway, the side chain of penicillin V was introduced by acylation reaction of L-serine compound **11**

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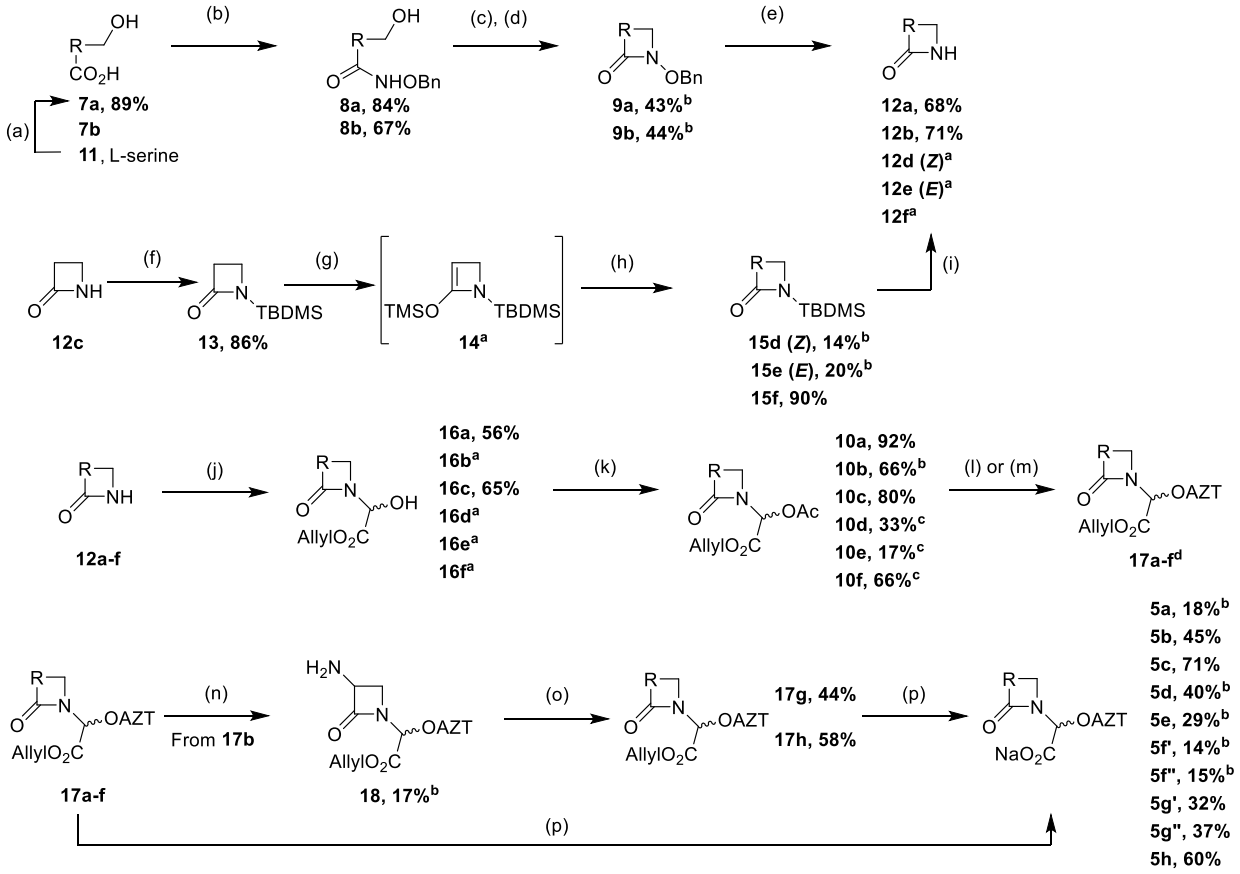
Scheme 4. Synthetic Pathway of Pronucleosides 5a−g



[a]Not isolated, [b] yield over two steps, [c] yield over three steps, and [d] not isolated except for **17c** with 20% yield. Reagents and conditions: (a) phenoxyacetyl chloride (1.3 equiv), Na_2CO_3, CH_3CN-H_20, and RT; (b) benzylhydroxylamine (1.3 equiv), EDCI.HCl (1.1 equiv), HOBt (1.1 equiv), DIPEA (1.5−3 equiv), THF, and 0 °C; (c) MsCl (3 equiv), pyridine, 0 °C, and Ar; (d) K_2CO_3 (1.5−3 equiv), acetone, and 60−80 °C; (e) SmI_2 [0.1 M] (5 equiv), THF-H_2O, 0 °C, and Ar; (f) TBDMSCl (1.15 equiv), DIEA, DCM anhy., RT, and Ar; (g) TMSCl (1.2 equiv), LDA [1 M], THF anhy., −78 °C, and Ar; (g) electrophile (1.2 equiv), LDA [1 M], THF, −78 °C, and Ar; (i) TBAF [1 M], THF anhy., RT, and Ar; (j) allyl glyoxalate (3 equiv), DMF anhy., and 90 °C; (k) Ac_2O (10 equiv), pyridine, and RT; (l) BF_3-Et_2O (anhy.), DCM anhy.,0 °C, and Ar; (m) $AlCl_3$ (2 eq.), THF anhy., RT, and Ar; (n) TFA 50%, DCM, and RT; (o) carboxylic acid compound (1.3 eq.), EDCI.HCl (1.1 equiv), HOBt (1.1 equiv), DIPEA (3.5 equiv), THF, and 0 °C; and (p) NaOH [0.1 M], THF, H_2O, and RT.

to get the starting material **7a** (Scheme 4). Second, the same synthesis was performed with a Boc residue as the PG (from **7b**), allowing access to a broad range of acyl substituents on this position at the end of the synthetic pathway. Carboxylic acid functions of **7a−b** were activated by the EDCI/HOBt coupling condition, and then benzylhydroxylamine was introduced to give the corresponding hydroxamates **8a−b**. The hydroxyl functions were replaced by a mesyl leaving group, and then the cyclization step was performed in the presence of K_2CO_3 to provide β-lactam compounds **9a−b**. The deprotection of the hydroxamate function needed the N−O bond cleavage. The use of Raney-Ni amalgam[47] or a two-step process based on hydrogenation on Pd/C and then hydroxylamine deprotection by $TiCl_3$[48] were shown to be ineffective conditions in our case. Finally, deprotected β-lactam rings **12a−b** were obtained by a solution of SmI_2 in THF in good yields.[49] However, this step was highly sensitive to dissolved oxygen level and quality of commercial solution of samarium.

Non-acyl substituents were also introduced at position 3 (substituent R). The commercially available azetidinone **12c** was protected by a TBDMS group to provide the corresponding β-lactam **13**. The enolate **14** was obtained after the action of LDA and anion trapping by the addition of the chlorotrimethylsilane reagent. This intermediate was not isolated and directly engaged in the next step in the presence of LDA to trap an electrophile reagent.[50] First, 2-pyridinecarboxaldehyde was used to condense on the enolate form to give a separable mixture of isomers **15d** (*Z*) and **15e** (*E*). In the same way, allyl bromide electrophile was also selected to decorate the β-lactam ring by a substitution reaction to provide the allyl derivative **15f** in good yield. Finally, the silyl ether PG was removed by a solution of TBAF in THF to give the corresponding β-lactam compounds **12d−f**.[51] The correct identification between the two *Z* and *E* isomers was based on the ^{1}H NMR chemical shift of the vinylic proton and confirmed by NOE experiments (see the Supporting Information for a detailed spectrum of **12d**).[52] The next stage of the synthesis was done on the different synthesized or commercially available β-lactam precursors **12a−f**. To prepare intermediates **16a−f**, the nitrogen atom has to be condensed on the glyoxalate moiety. The allyl group on the ester function as the PG has been chosen, and the corresponding glyoxalate reagent was prepared in two steps according to the literature method.[53] Hydroxyl intermediates **16a−f** can be purified or directly engaged in the next step. The acylation reaction was performed by acetic anhydride to give the corresponding

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Table 1. β-Lactamase-Mediated Release of AZT Measured by Disk Diffusion after Incubation in the Presence of β-Lactamases Representative of the Four Structural Classes

	E. coli growth inhibition zone diameter (mm) after preincubation of **5a** with a β-lactamase[a]											
	class A			class B			class C			class D		
[enzyme] mg mL^{-1}	KPC-2	SHV-12	TEM-1	IMP-1	NDM-4	VIM-2	AmpC	CMY-4	FOX-7	FUS-1	OXA-46	OXA-48
0.5	38	36	35	28	36	32	24	25	ND	19	31	33
0.01	38	28	19	[b]	ND	13	ND	ND	ND	ND	ND	ND

[a]Performed in duplicate with 30 min of preincubation at RT between **5a** and the corresponding β-lactamase and transfer onto an *E. coli* XL1-inoculated agar plate (see Methods for details). [b]No growth inhibition detected.

protected compounds **10a−f** in good yields. The last reaction consisted of Lewis acid activation of acetyl function to generate an *N*-acyl-iminium intermediate that is able to react with AZT.[54] Several experimental conditions were investigated: Lewis acid ($AlCl_3$, BF_3-Et_2O, $ZnCl_2$, LiCl, and $Mg(OCl_4)_2$), solvents (THF, DCM, and DMF), or temperatures (−20 °C to reflux). Initially, acetylated compound **10a** was used as a reference. Two procedures quickly emerged as being the more efficient based on BF_3-Et_2O in CH_2Cl_2 at 0 °C or $AlCl_3$ in THF at RT. In the first case, the use of BF_3−Et_2O leads to the *N*-acyl iminium intermediate that was trapped by the hydroxyl group of AZT to provide the corresponding conjugate **17a** in the form of an inseparable diastereomeric mixture. Nevertheless, in the presence of $AlCl_3$, only one diastereomer was obtained with a good yield, and the next synthesis steps will be done on it. This specificity was valid only for **10a**, and with other acetylated compounds **10b−f**, only BF_3−Et_2O Lewis acid proved to be efficient, leading each time to a mixture of diastereomers when possible. The last stage of the synthetic pathway consisted of a sequence of successive deprotection reactions with a diversity step on the amino function at position 3 of the β-lactam heterocycle. The Boc group of **17b** was removed by a solution of TFA in CH_2Cl_2 at RT to give the deprotected compound **18** with a low yield over two steps. To introduce the *N*-acyl side chain, the carboxylic acid function of the corresponding substituent was activated by the EDCI/HOBt mixture and then condensed on amino compound **18** to provide conjugates **17g−h**. It was initially considered the use of a palladium catalyst to remove the allyl PG. Finally, the more economical and convenient based-catalyzed ester hydrolysis was successfully applied to give final pronucleosides **5a−h** without any significant side product formation.[31] To prevent any solubility issue, all final compounds **5a−h** were purified in the reverse phase in the presence of ammonium acetate to provide the corresponding sodium salt. During this purification step, some diastereoisomeric mixtures have proven to be separable. In this context, compounds **5f** and **5g** led to pure diastereoisomers **5f′**, **5f″**, **5g′**, and **5g″** for further biological evaluations. Inspired by described methods coupled with the *N*-acyl iminium chemistry strategy, a straightforward and original synthetic pathway was developed. This allowed the obtention of 10 final pronucleosides **5a−h** in four to nine steps with an overall yield in the 5−37% range. All compounds were fully characterized by nuclear magnetic resonance (NMR) and mass spectroscopy with a UV purity ≥95% (see the Supporting Information for all spectra). All the pronucleoside compounds obtained were subjected to biological evaluation.

Enzyme Assays and Reactivity toward Various β-Lactamases. Initially, kinetic experiments to monitor compound hydrolysis by UV spectroscopy were carried out. β-Lactams are well known to absorb light in the UV range (220−300), allowing the development of direct spectrophotometric assays.[55] Sadly, AZT also displayed an even stronger absorbance at these wavelengths, thus preventing any discernible measurement (see Figures S1−S4). Furthermore, such a method would not unambiguously identify the nature of the released product as AZT. To overcome this issue, an indirect approach was implemented by preincubating the compounds with various β-lactamases, with the release of AZT being measured as a result of bacterial growth inhibition in a disk diffusion assay of *E. coli* XL-1 susceptible laboratory strain [minimum inhibitory concentration (MIC) value of 0.015 mg mL^{-1} for AZT], with a suitable negative control (compound incubated without β-lactamase). As a positive control of growth inhibition of *E. coli* XL-1 strain, AZT was spotted on blank disks (quantities, 0.005−10 μg), showing a dose-dependent effect. The final control, performed in parallel in all subsequent experiments, consisted of 0.625 μg of AZT, resulting in a growth inhibition zone diameter of 40 ± 2 mm (see Table S1). A representative panel of 12 β-lactamases was assayed with all possible molecular classes including various substrate specificities and different modes of action (Table 1). As a model, the pronucleoside **5a** was chosen and preincubated for 30 min at 2 μM concentration with each β-lactamase (tested at concentrations 0.5 and 0.01 mg mL^{-1}). Results are reported in Table 1 (all pictures of diffusion disks are provided in the Supporting Information, Figure S5).

The highest antibacterial activity, i.e., reflecting the optimal release of AZT from the prodrug, was obtained with class A β-lactamases (19−38 mm). Interestingly, a significant compound conversion was also observed at low enzyme concentrations (0.01 mg mL^{-1}), particularly with the KPC-2 carbapenemase (Table 1). Almost similar inhibition levels were obtained at 0.5 mg mL^{-1} for class B metallo-β-lactamases, although AZT conversion was drastically reduced at lower concentrations, except, to some extent, in the presence of the VIM-2 enzyme. This result is consistent with the substrate profile of metallo-β-lactamases for which monobactams are poor substrates.[56] Preferred substrates of class C enzymes (AmpC-type) are generally cephalosporins over monobactams or carbapenems (cephalosporinase activity). Nonetheless, weak **5a** conversion was detected with *Enterobacter cloacae* AmpC and the plasmid-encoded CMY-4 β-lactamase. Among class D enzymes, the narrow-spectrum FUS-1 (OXA-85) yielded a limited release of AZT, but the activity of OXA-46 and, more interestingly, the carbapenem-hydrolyzing OXA-48 β-lactamase allowed for a conversion rate similar to that observed with other enzymes, although no conversion could be observed in the presence of lower enzyme concentrations. Since class A β-lactamases, and notably the epidemiologically relevant KPC-2 carbapenemase, appeared as the most active enzymes on compound **5a**, further biochemical experiments were carried out to further character-

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ize the interaction and KPC-2-mediated hydrolysis of this compound.

Characterization of β-Lactamase-Mediated Hydrolysis by ITC. Based on previous panel results, the KPC-2 enzyme was chosen as a model for the determination of the kinetic parameters for the hydrolysis of pronucleosides **5a−h** using isothermal titration calorimetry (ITC).[57] Based on the enthalpy value of β-lactam amide bond hydrolysis, k_{cat} and K_M values were determined.[58] The method was validated by determining the kinetic parameters of hydrolysis of meropenem, yielding values of k_{cat} and K_M comparable to that reported in the literature (Table 2).[59] All considered prodrugs are structurally equivalent except for the nature of the side chain at the 3-position of the β-lactam ring. These experiments allowed us to obtain relevant SAR data. First, the effect of this substitution was investigated on the affinity and hydrolytic activity of the KPC-2 enzyme. No enzymatic activity was highlighting the requirement of a more complex side chain for enzyme recognition and hydrolysis. Substitutions by allyl groups, such as in the diastereoisomers **5f′** or **5f″**, did not improve compound reactivity toward KPC-2. The introduction of a pyridine moiety seemed more favorable. Indeed, with the *E*-configuration of the carbon−carbon double bond, compound **5e** displayed low but measurable turnover rates, although with a relatively high K_M value, leading to an overall poor catalytic efficiency (k_{cat}/K_M). However, the *Z*-configurated pyridine **5d** showed a 10-fold higher k_{cat} and a 10-fold lower K_M value compared to *E*-configuration, allowing a significant improvement of the catalytic efficiency. Based on literature data, only *E*-configuration was explored to design β-lactamase inhibitors (due to the necessary intramolecular cyclization step for the inhibition mechanism),[34] but *Z*-configurated substrates seemed better suited to accommodate themselves into the active site of KPC-type enzymes. The main type of explored substitution at the 3-position was acyl side chain like most marketed β-lactam agents. The Boc-protected compound **5b** displayed unexpected pretty good recognition even, to the best of our knowledge, it was never described in the literature as an appropriate side chain. The two isolated epimers **5g′** and **5g″** inspired by the benzylic penicillin G side chain were evaluated separately to understand the significance of the stereochemistry of the hemiaminal carbon atom. As expected, only one epimer, the compound **5g″**, was strongly active, whereas the other one **5g′** displayed almost no activity. It is a consistent result because, in the β-lactam agent series, it is well known that only one stereochemistry is allowed, the *R* configuration. The pure epimer **5a** with the side chain of penicillin V showed a similar activity profile to that of **5g″**, probably with the same carbon stereochemistry. Unfortunately, the other epimer of **5a** was never obtained in pure form to confirm this hypothesis. Finally, the best result was achieved by the analogue of cephalothin **5h** characterized by a thiophene residue. The K_M value was in the same range as the other best compounds (around 1−4 mM), but the k_{cat} value was significantly better (350 s^{-1}). The resulting catalytic efficiency for **5h** hydrolysis was interestingly similar to that of the substrate meropenem (≈250 mM^{-1} s^{-1}), indicating that a significant rate of conversion of our compounds should be expected with at least some clinically relevant β-lactamases. However, this series of pronucleosides **5a−g** is characterized by high values of K_M when compared to that of β-lactam substrates, which is probably due to the presence of the monobactam core, generally known to display lower affinity against KPC-type β-lactamase. Hopefully, this is compensated by significantly high turnover rates, exceeding that of meropenem by up to 60-fold.

Table 2. Kinetic Parameters of KPC-2 and NDM-1 for the Hydrolysis of Pronucleosides, Determined by ITC

Cpd	k_{cat} (s^{-1})[a]	K_M (mM)[a]	k_{cat}/K_M (mM^{-1} s^{-1})
		KPC-2	
meropenem[b]	5.8 ± 0.1 (4.1)	0.023 ± 0.002 (0.016)	252.2 (256)
5a[e]	105 ± 3	4.1 ± 0.2	25.6
5b	96.1 ± 2.2	5.3 ± 0.2	18.1
5c	<0.003[c]		
5d	3.2 ± 0.1	0.45 ± 0.04	7.1
5e	0.21 ± 0.01	3.1 ± 0.3	0.07
5f′[e]	0.02[d]		
5f″[e]	0.09[d]		
5g′[e]	≥0.06[d]	≥3.8[d]	
5g″[e]	127.6 ± 2.1	3.3 ± 0.1	38.7
5h	351.4 ± 8.8	1.5 ± 0.08	234.3
		NDM-1	
meropenem[b]	109 ± 1 (144.9)	0.06 ± 0.003 (0.06)	1816.7 (2415)
5a[e]	15.5 ± 0.4	3.6 ± 0.1	4.3

[a]Values are mean ± SD. [b]KPC-2 data are from Wang et al.,[59] and NDM-1 data are from Ali et al.[60] [c]Not detected, below the detection limit. [d]Approximate values due to the very low activity. [e]Pure diastereomer compound.

Considering that metallo-β-lactamases showed a surprising ability to convert our monobactam-based pronucleoside, the hydrolysis of compound **5a** was also further investigated in the presence of NDM-1 (Table 2). As previously shown, the kinetic parameters of meropenem hydrolysis were measured and compared to that reported in the literature, showing a satisfactory agreement.[60] Although high K_M values were obtained, an expected data based on our current knowledge of the MBL spectrum of activity, a significantly high turnover rate (15 s^{-1}) could be measured, in agreement with what was observed in earlier disk diffusion-based experiments. This is relevant because it might translate into a moderate, though potentially sufficient, conversion rate to observe AZT release, which exerts its antibacterial activity at very low concentrations.

Chemical and Enzymatic Stability of Pronucleosides. Before any microbiological evaluations, the chemical stability of conjugates in an aqueous solution has to be carefully determined. Indeed, zidovudine (AZT) displays a very strong antibacterial activity with MIC values well under 0.5 μg mL^{-1} on susceptible stains.[39] Thus, even a minor chemical instability with subsequent AZT release may represent a significant interference biasing the interpretation of in vitro antibacterial susceptibility testing. To monitor the chemical stability, a two-step process was implemented. In the first step, a representative panel of prodrugs (**5a**, **5d−e**, and **5h**) were evaluated by tandem mass spectroscopy coupled to HPLC. A 0.9% NaCl physiological solution, previously used in enzyme assays (see Table 1), was used as the aqueous media, and the compounds were further incubated at 37 °C. Calibration curves were obtained for each compound and AZT, and then, stability was monitored over a period of 16 h. Almost all considered compounds proved to be completely stable, with no detectable AZT formation, except for compound **5e**. In the

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case of *E*-pyridine **5e**, a quick degradation was observed with the detection of AZT from the first sample associated with decreasing intensity of the starting compound (all chromatograms and result tables are provided in the Supporting Information, Table S4). The underlying degradation mechanism is unknown, but it seems specific for the *E*-configuration (the intensity of the *Z* isomer **5d** decreases slightly but with no detection of AZT release). To fully demonstrate the specific enzymatic activation of the prodrugs, the same experiments were conducted in the presence of CTX-M-15 class A β-lactamase. The same preincubation conditions were used as those described in Table 1, and a full conversion of **5a** into AZT was observed after 30 min. To confirm these stability data, the two best compounds **5a** and **5h** and the presumably unstable **5e** were selected for a further NMR-based study. Compounds were solubilized in deuterium water at 37 °C, and several NMR spectra were generated for 14 h. The NMR characteristic peaks of AZT were carefully monitored, and no significant degradation was observed with **5a** and **5h** (all spectra are provided in the Supporting Information, Figures S15 and S16). In the case of **5e**, AZT peaks emerged after 20 min only, confirming its propensity to self-degradation. Based on NMR integration of proton 5′ of AZT, 50% of the starting compound **5e** was hydrolyzed with subsequent release of the corresponding AZT after a period of 6.7 h (see Figures S17 and S18).

In Vitro Antibacterial Activity of Pronucleosides. Only the best and most stable compounds **5a** and **5h** were selected for further microbiological evaluations. AZT was used as the reference compound in such assays. MIC values were first determined using a set of isogenic *E. coli* BL21 (DE3)-derived laboratory strains producing a single β-lactamase (Table 3, entries 1−5). Based on the ITC results and epidemiology relevance, strains producing the KPC-3, NDM-1, IMP-1, and *E. cloacae* AmpC β-lactamases were used. It was also verified that all the corresponding *E. coli* strains were highly susceptible to AZT (MIC ranging from 0.03 to 0.5 μg mL^{-1}). No significant antibacterial activity (16−32 μg mL^{-1}) could be measured for compounds **5a** and **5h** with the parent strain in the absence of the vector. In agreement with our previous results (including ITC), the strain producing the KPC-3 carbapenemase was highly susceptible to both prodrugs **5a** and **5h**, with MIC values very similar to that of AZT, indicating a significant rate of conversion of the prodrug. This further confirms the ability of KPC-type β-lactamases to accommodate and efficiently hydrolyze our β-lactam-based carrier moiety also in the context of the bacterial periplasm. Metallo-β-lactamase-producing laboratory strains (IMP-1 and NDM-1) were less susceptible to the pronucleosides, although a moderate level of compound conversion could be observed, more particularly **5a** with NDM-1 and **5h** with IMP-1. Although difficult to interpret, these data may also reflect the different functional properties of these two enzyme subtypes. Similarly, the *E. cloacae* AmpC-producing strain showed a strong dependence on the nature of the compound, being able to efficiently convert prodrug **5h** only whereas acyl-side-chain equivalent β-lactam agents, amoxicillin and cephalothin, are both known to be good substrates.[61] To rule out any spontaneous activation of the prodrug in the culture medium (matrix effect), the MIC values of **5a** were also determined on all *E. coli* BL21 (DE3) strains after 6 h of incubation at 37 °C in the culture medium. No significant modification of the MIC value was observed compared to the provided values in Table 3 without the incubation step, confirming that lower MICs were relying specifically on the β-lactamase-mediated release of AZT. These results, as previously mentioned, showed that the prodrug compounds were also able to enter into the bacterial periplasm and that these β-lactamases could exert their hydrolytic activity on such substrates in a cellular context. To further confirm this aspect, two types of strains were selected with no or very weak β-lactamase production (Table 3, entries 6 and 7). The prodrug **5a** showed no significant growth inhibition on both strains, demonstrating the necessity of β-lactamase enzymes to trigger it. The narrower antibacterial spectrum of conjugates allows for specific targeting of β-lactamase-producing opportunistic pathogens and not antibiotic-susceptible bacteria found in the healthy gut microbiota. Finally, the activity of such prodrugs was also determined with a small panel of carbapenemase-producing MDR clinical isolates (Table 2, entries 8−11). Although these isolates were susceptible to AZT, the MIC values of the prodrugs were significantly higher. However, we found it extremely encouraging to observe MIC values as low as 4−8 μg mL^{-1} on a NDM-1-producing clinical isolate, further supporting the rationale and validity of our approach. In the case of KPC-producing bacteria, only the *Klebsiella pneumoniae* SI-353 strain displayed a moderate inhibition with MIC values of 32 and 16 μg mL^{-1} with **5a** and **5h**, respectively, reflecting the importance of other factors in the susceptibility to our prodrug, such as modifications of the outer membrane permeability. Indeed, these clinical isolates are genetically heterogeneous and thus characterized by a different combination of the mechanism of resistance and potentially unknown other factors compromising the activity of our prodrugs. To rule out any potential permeability-related issue, the MICs of the prodrugs were determined in the presence of a sub-inhibitory concentration of colistin, a polymyxin antibiotic known for its membrane-permeabilizing activity, but no modification was observed. Thus, outer membrane diffusion does not seem to be the main factor involved. It is likely to result from a combination of factors, which deserves further detailed investigation. Nevertheless, in almost every case, the thiophene substitution of **5h** seems slightly favorable compared to the penicillin V side chain of **5a**.

Table 3. MIC Determination by the Broth Microdilution Method[a]

entry	bacterial strain	MIC[b] (μg mL^{-1})		
		AZT[f]	5a	5h
1[c]	*E. coli* BL21(DE3)	0.06	32	16
2[d]	*E. coli* BL21(DE3)/ pET9a-KPC-3	0.5	1	0.25
3[d]	*E. coli* BL21(DE3) / pET9a-IMP-1	0.03	8	2
4[d]	*E. coli* BL21(DE3) / pET9a-NDM-1	0.06	2	4
5[d]	*E. coli* BL21(DE3) / pET9a-AmpC	0.125	32	2
6[c]	*E. coli* ATCC 25922	4	>256	ND
7[c]	*K. pneumoniae* ATCC 13833	0.25	128	ND
8[e]	*E. coli* SI-44 KPC-3	2	64	64
9[e]	*K. pneumoniae* SI-353 (KPC-3)	4	32	16
10[e]	*K. pneumoniae* SI-109 (KPC-3)	2	>128	>128
11[e]	*E. coli* B28 (NDM-1)	0.25	8	4

[a]Triplicate experiment. [b]Minimal inhibitory concentration. [c]Reference isolate. [d]Isogenic β-lactamase producer. [e]Clinical isolate. [f]Positive control experiment with AZT alone; ND, not determined.

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Solubility and Preliminary Toxicity Evaluations. To consider further potential clinical developments, the solubility and toxicity profiles of prodrugs **5a**−**h** were investigated. Final compounds were initially isolated in neutral form (e.g., with carboxylic acid function), displaying a moderate aqueous solubility capacity. Sodium salts were later obtained, which showed a significantly high solubility (≥100 μM). The potential cytotoxicity of compounds AZT, **5a**, and **5h** was evaluated on human HeLa cells. No membrane-damaging activity or cytotoxic effects could be detected at concentrations up to 128 μg mL^{-1}, indicating that our prodrugs are comparable to AZT. These preliminary results are favorable for further optimizations and developments.

CONCLUSIONS

The development of new approaches leading to antibacterial agents is a crucial step toward addressing and minimizing the potentially dramatic consequences of antimicrobial resistance, already accounting for approximately 1.3 M deaths worldwide. This study addresses this expectation to use bacterial resistance to trigger the activation of β-lactam conjugates: the higher will be the level of β-lactamase expression, the more vulnerable will be the bacteria. First, based on the literature, a synthetic pathway was developed to obtain prodrugs in good yields. AZT was chosen as the antibacterial agent while several substitutions on the β-lactam ring were considered. Finally, 10 final compounds **5a**−**h** were obtained and characterized. It was possible in some cases to separate the diastereomers, allowing us to measure the impact of the configuration on the biological activity. Nevertheless, it is still necessary to optimize this separation step or to develop a stereoselective synthesis. The unambiguous determination of the absolute configuration of this asymmetric center also remains to be established.

Second, the reactivity of the various synthesized prodrugs toward a representative panel of β-lactamases was investigated. To this aim, indirect detection of released AZT after preincubation with different β-lactamases provided interesting preliminary information. KPC-2 was identified as the β-lactamase able to catalyze compound conversion more efficiently, although some metallo-β-lactamases also surprisingly, considering our use of a monobactam core, showed this ability. A calorimetric titration study was used to determine the enzymatic parameters of KPC-2 for the hydrolysis of our prodrugs and allowed us to establish some interesting SAR considerations. Different pharmacomodulations were evaluated, showing the necessity of specific substitution motifs particularly based on acyl side chains of regular β-lactam agents (penicillins V and G and cephalothin). Into the class A β-lactamase-β-lactam binary complex, these substituents are generally oriented toward a Ω-loop of the enzyme involved in the stabilization of the acyl-enzyme intermediate.[62] This crucial loop is located next to the catalytic pocket, and it is described to well accommodate small aromatic side chains, especially in the case of KPC variants.[63] In this context, compound **5c**, with no substituents, is not able to favorably interact with this Ω-loop and probably explains the lack of activity. Several hydrogen bond networks define the interaction between the Ω-loop and a β-lactam substrate, although these may vary in different β-lactamases. However, a conserved Asn residue is involved in a crucial hydrogen bond with the carbonyl of the β-lactam acyl amide function.[64] The necessity of the carbonyl motif is well illustrated with the better affinities of acylated compounds **5a**, **5g**, and **5h** compared to that exhibited by carbon−carbon derivatives **5d**−**f**. Finally, the thiophene moiety of **5h** seems to be slightly more favorable than other acyl derivatives, which is consistent with the literature data.[65] Moreover, the specific chirality of the stereogenic center proved to be crucial for the activity. Even if the affinities were globally lower compared to that of meropenem, two compounds, **5a** and **5h**, emerged with interesting values, especially because of their high turnover rates. Even in the case of the metallo-enzyme NDM-1, the monobactam prodrug remained an appropriate substrate contrary to aztreonam. That was demonstrated with a good MIC value on the clinical isolate expressing the NDM-1 enzyme. The chemical stability was also evaluated, showing that most compounds were not prone to self-conversion or degradation in aqueous medium. Microbiological assays, first performed using isogenic laboratory strains producing different β-lactamases, resulted in low MIC values, confirming the specific and β-lactamase-mediated release of AZT in a cellular context. Nevertheless, the results were more contrasted on clinical isolates, although undoubtfully interesting. Preliminary solubility and toxicity studies showed that the compounds **5a** and **5h** also presented a favorable profile for future clinical development.

This pioneering study demonstrated that it was possible to target pathogenic bacteria by using the production of β-lactamases to selectively release antibacterial agents, thus obtaining compounds specifically targeting antibiotic-resistant bacteria. This could lead to the possibility of developing targeted antibiotic therapies, and a potentially drastic reduction of off-target effects, particularly on intestinal microbiota. A larger study is underway to incorporate other active substances than AZT as well as to modify the nature of the vector to increase affinity toward β-lactamases.

METHODS

General Methods. NMR spectra were recorded with a Bruker AVANCE 400 or 500 or 600 spectrometer (for ^{1}H and ^{13}C NMR) and a Bruker AVANCE 400 spectrometer (376.5 for ^{19}F). Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively, using residual solvent signals as a reference for ^{1}H and ^{13}C. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, br s = broad signal, dd = double doublet, dt = double of triplet, and m = multiplet. High-resolution mass spectra were registered on a JEOL JMS-SX-102A mass spectrometer. Reactions were monitored with a Merck Kieselgel 60F254 precoated aluminum silica gel plates (0.25 mm thickness), which were visualized under 254 nm light or by charring with a ninhydrin solution in butanol or with a vanillin solution in ethanol, followed by heating or iodine stains. Purification experiments were carried out on a silica gel premium Rf grade (40−63 μm) or were performed on a Biotage Isolera One using a 20−50 μm packed silica cartridge or using reversed-phase preparative HPLC on an Akta pure C18 column (Luna 21.2 × 100 mm, 5 μm, 100 Å). Eluent A: Water (10 mM $AcONH_4$ or 0.1% TFA or neutral); B: acetonitrile (neutral or 0.1% TFA, respectively) with flow rate: 20 mL/min. LC−MS analyses were obtained on the Waters Alliance 2690 HPLC coupled to a Waters Micromass Quattro micro API (electrospray ionization mode, ESI+). Conditions: RP C18 Chomolith high-resolution column (25 × 4.6 mm) using a linear gradient (0−100%) of water and acetonitrile (each containing 0.1% of formic acid) over 3 min and at a flow rate of 3 mL/min, mono λ detection (214 nm). Positive-ion electrospray mass spectra

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were acquired at a solvent flow rate of 0.15 μL min^{-1}. Nitrogen was used as both the nebulizing and drying gas. The data were obtained in the scan mode in 0.7 s intervals. UPLC analyses were obtained on the Waters Acquity H-Class UPLC coupled to a Waters Zspray (electrospray ionization mode, ESI+). Conditions: Acquity UPLC peptide HSS T3 column (2.1 × 50 mm, 1.8 μm, 100 Å) using a linear gradient (0−100%) of water and acetonitrile (each containing 0.1% of formic acid) over 5 min and at a flow rate of 0.8 mL min^{-1} flow rate, photodiode array detection (TUV—214 nm). Positive-ion electrospray mass spectra were acquired at a solvent flow rate of 0.8 μL min^{-1}. Nitrogen was used as both the nebulizing and drying gas. The data were obtained in the scan mode in 0.2 s intervals. The purity of all synthetic compounds was determined by UPLC analysis and was greater than 95%.

Enzyme Assays and Release of AZT from Prodrugs. The β-lactam derivative **5a** (2.5 μL) at 5 mg mL^{-1} was preincubated with 8 μL of different enzymes at a concentration of 0.5 mg/mL (deposit A), 0.01 mg mL^{-1} (deposit B), and no enzyme (deposit C) in 9.5 μL of 0.9% of NaCl solution. After 30 min at room temperature, 2 μL was plated on Mueller−Hinton (MH) agar containing the *E. coli* XL1 strain and incubated at 37 °C for 24 h. The same method is used for MIC determination described below.

Determination of Kinetic Parameters of Hydrolysis by ITC. The enzyme activity was recorded by ITC on a MicroCal ITC200 (GE-Malvern) equipped with a 200 μL Hastelloy sample cell and an automated 40 μL glass syringe rotating at 1000 rpm. Compounds were solubilized in 10 mM HEPES-NaOH, 0.15 M NaCl, and pH 7.5, and both enzymes were diluted to the desired concentrations with the same buffer. For compounds **5a−h**, the buffer also contained 1% DMSO, and KPC-2 was brought to the same final DMSO concentration. The activity was recorded and calculated as described, using a molar enthalpy value for β-lactam amide bond hydrolysis of 12 kcal/mol at 25 °C. The reaction rate constant k_{cat} (s^{-1}) is defined here as the number of amide bonds hydrolyzed per second and per active site. For K_m determination, activities were recorded at decreasing substrate concentrations, and the data points were fitted to the Michaelis−Menten equation.

MIC Determination. The MICs of considered inhibitors were determined in triplicate using cation-supplemented Mueller−Hinton broth and a bacterial inoculum of 5 × 10^4 cfu/well, as recommended by the CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST document "Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents", 2000). The MIC values of compounds were determined on *E. coli* BL21(DE3) derivative laboratory strains hyperproducing a single β-lactamase and carbapenemase-producing clinical isolate part of our collection.

Stability Assays by Ultraperformance Liquid Chromatography Separation and Tandem Mass Spectrometry. Identification and stability experiments were carried out by reverse-phase liquid chromatography on a Waters Acquity Iclass UPLC system coupled to a high-resolution mass spectrometer (Xevo TQD Waters) equipped with an electrospray ionization source and controlled by MassLynx v.4.2 software for data acquisition. All the analyses were carried out using an Acquity UPLC BEH C18 2.1 × 50 mm column at 40 °C. A flow rate of 0.8 mL min^{-1} and a gradient of (0−100%) B over 3.5 min were used: eluent A, water/0.1% HCO_2H; eluent B, acetonitrile/0.1% HCO_2H. The mass spectrometer operated in the positive or negative ionization mode and parameters were set as follows: source block temperature, 150 °C; nebulizer nitrogen flow rate, 50 L/h; capillary voltage, 2.0 or 2.5 kV; desolvation temperature, 550 or 600 °C; and desolvation nitrogen gas flow, 900 or 1000 L/h. The different investigated parameters are cone voltage, collision energy, ionization mode, and MRM channels. Specific settings for each compound are provided in the Supporting Information (Table S4). All samples were prepared by dilution in a commercial physiological solution (0.9% NaCl). In the case of enzymatic assay, filtration on an Amicon Ultra-0.5 Centrifugal Filter Unit, MWCO 3 kDa, is performed before injection. A calibration curve is determined for each compound as follows: samples used for the calibration were injected (1 μL) in triplicate from the lowest concentration (0.0001 mg mL^{-1}) to the highest one (0.01 mg mL^{-1}), and the calibration curves were plotted with the Targetlynx tool from MassLynx. Then, samples used for stability experiments were injected (1 μL) at 0.005 mg/mL at 37 °C and monitored every hour over 24 h. The stability curves were plotted with the Targetlynx tool from MassLynx. The MRM channel monitoring of AZT apparition and molecule degradation were plotted at the same time to check the release.

Stability Assays by NMR. Pronucleosides were dissolved in 600 μL of D_2O at a concentration of 10 mM. The ^{1}H NMR spectra were measured on a Bruker 600 MHz spectrometer at various time points at 310 K.

Toxicity Assays. The potential cytotoxic activity of compounds was evaluated using the commercially available integrity assay (CytoTox 96 nonradioactive cytotoxicity assay, Promega). The compounds were tested for their ability to induce the lysis of HeLa cells by measuring the release of lactate dehydrogenase (LDH) after incubating the HeLa cell cultures (20,000 cells/well) for 24 h (37 °C, 5% CO_2) in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 4.5 mg mL^{-1} glucose, and 2 mM L-glutamine in the absence and presence of varying concentrations of the compounds (up to 250 μM). Further controls included samples containing the medium only or in which cell lysis was induced by the addition of 9% Triton X-100 (maximum LDH release control). The percentage of cytotoxicity was computed as 100× (sample LDH release)/(maximum LDH release). The variation in the percentage of cytotoxicity allowed us to compute an IC_{50} value (compound concentration inducing 50% cytotoxicity).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.3c00110.

Detailed synthetic procedures with all NMR spectrum associated; data on **5a**, AZT concentrations, kinetic parameters, integration values, SMILES strings, and InChI keys strings; and miscellaneous data from biological and stability evaluations (PDF)

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Author Contributions

M.R.-H. synthesized, purified, and characterized compounds; conducted stability assays; and wrote the Supporting Information; F.S. and F.V. were in charge of enzymatic, microbiological, and toxicity assays; L.L. synthesized, purified, and characterized compounds; G.F. performed and interpreted ITC measurements; E.C. monitored LC−MS/MS stability assays; Y.V.-H. and P.L.-F. performed and interpreted microbiological assays; J.-D. D. designed and interpreted biological evaluations and contributed to project design; and L.G. designed, funded, and supervised the project and wrote the draft. All authors contributed to editing of this manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AIDS, acquired immunodeficiency syndrome; AmpC, ampicillin C; AZT, zidovudine; β-L, beta-lactamase; DEAD, diethyl azodicarboxylate; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; ESBL, extended-spectrum beta-lactamase; HOBt, 1-hydroxybenzotriazole; IMP, imipenemase; ITC, isothermal titration calorimetry; KPC, *K. pneumoniae* carbapenemases; LDA, lithium diisopropylamide; MBL, metallo-beta-lactamase; NA, nucleoside analogue; NDM, New Delhi metallo-beta-lactamase; OXA, oxacillinase; PBPs, penicillin-binding proteins; SARs, structure−activity relationships; SBL, serine-beta-lactamase; TBDMS, *tert*-butyl dimethyl silyl; TFA, trifluoroacetic acid

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