



IID572: A New Potentially Best-In-Class β -Lactamase Inhibitor

Folkert Reck,^{*,†} Alun Bermingham,[†] Johanne Blais,[†] Anthony Casarez,[†] Richard Colvin,[§] Charles R. Dean,[†] Markus Furegati,[¶] Luis Gamboa,[†] Ellena Growcott,[†] Cindy Li,[†] Sara Lopez,[†] Louis Metzger,[†] Sandro Nocito,[¶] Flavio Ossola,[¶] Kaci Phizackerley,[†] Dita Rasper,[†] Jacob Shaul,[†] Xiaoyu Shen,[†] Robert L. Simmons,[†] Dazhi Tang,[†] Kyuto Tashiro,[†] and Qin Yue[†]

[†]Novartis Institutes for BioMedical Research, 5300 Chiron Way, Emeryville, California 94608, United States

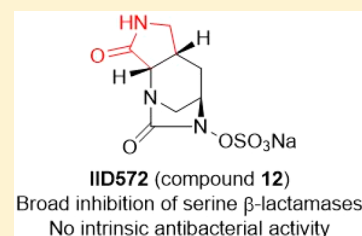
[§]Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States

[¶]Synthesis and Technologies Group, Novartis Institutes for BioMedical Research, Klybeckstrasse 141, Basel 4057, Switzerland

Supporting Information

ABSTRACT: Resistance in Gram-negative bacteria to β -lactam drugs is mediated primarily by the expression of β -lactamases, and co-dosing of β -lactams with a β -lactamase inhibitor (BLI) is a clinically proven strategy to address resistance. New β -lactamases that are not impacted by existing BLIs are spreading and creating the need for development of novel broader spectrum BLIs. IID572 is a novel broad spectrum BLI of the diazabicyclooctane (DBO) class that is able to restore the antibacterial activity of piperacillin against piperacillin/tazobactam-resistant clinical isolates. IID572 is differentiated from other DBOs by its broad inhibition of β -lactamases and the lack of intrinsic antibacterial activity.

KEYWORDS: β -lactamase inhibitors, diazabicyclooctane, DBOs, piperacillin, antibiotic



Multidrug-resistant (MDR) Gram-negative bacteria are increasing in prevalence in many regions of the world and present a threat to the health care system, especially in the hospital. Treatment of infections caused by these pathogens still relies in large part on the use of β -lactam antibiotics.¹ Resistance in Gram-negative bacteria to β -lactam antibiotics is mediated primarily through the expression of β -lactamases, which degrade the β -lactam.² Extended spectrum serine β -lactamases (ESBLs) are spreading and cause resistance to penicillins, cephalosporins, and monobactams. Physicians are therefore relying increasingly on carbapenems, which are stable to ESBLs, but this now appears to be driving the global spread of organisms producing carbapenemases.³

β -Lactamase-mediated resistance can in principle be addressed through the development of novel β -lactams with increased stability against β -lactamases⁴ or by co-dosing with a β -lactamase inhibitor (BLI). The approved BLIs clavulanic acid, sulbactam, and tazobactam have become limited in their clinical usefulness, because they are not effective inhibitors of ESBLs or class C serine β -lactamases (cephalosporinases) and are not active against serine carbapenemases such as *Klebsiella pneumoniae* carbapenemases (KPCs). Although piperacillin/tazobactam (TZP) is still a very important broad-spectrum hospital drug, the BLI tazobactam does not effectively protect piperacillin against ESBLs and serine carbapenemases. As a consequence, the proportion of clinical strains expressing ESBLs or carbapenemases that are susceptible to TZP is moderate (60%) and low (0.3%), respectively, according to a recent study.⁵

Recently, avibactam, a novel BLI of the diazabicyclooctane (DBO) class (Figure 1) was launched in combination with

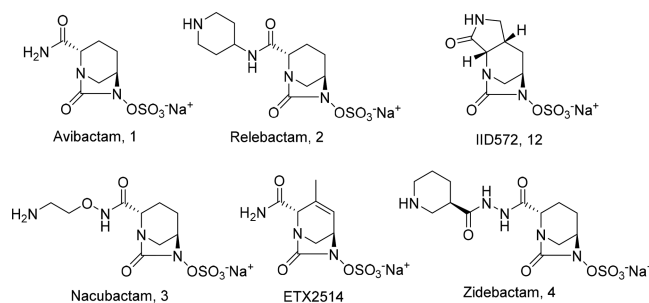
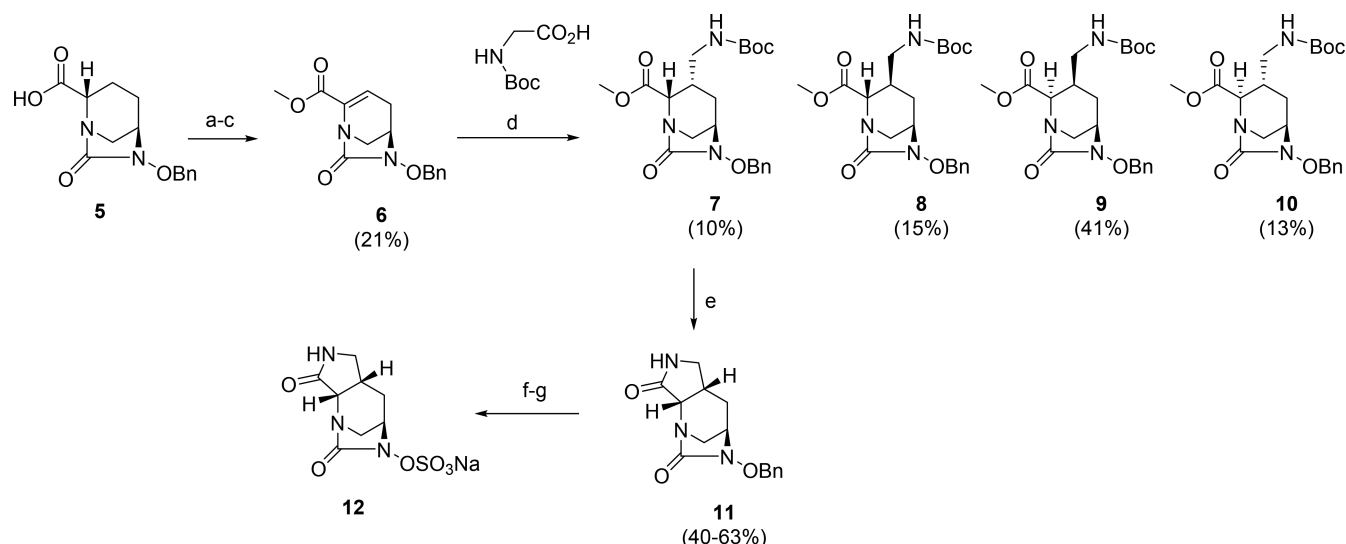


Figure 1. Selected BLIs of the DBO type.

ceftazidime (CZA), addressing serine β -lactamase mediated resistance in Enterobacteriaceae and *Pseudomonas aeruginosa*.⁶ However, CZA has a narrower spectrum than TZP, lacking coverage of methicillin-susceptible *Staphylococcus aureus* and of anaerobes.^{7,8} Avibactam is a broad spectrum BLI, providing coverage of ESBLs, serine carbapenemases, class C enzymes, and some class D β -lactamases. However, emergence of resistance in the clinic upon treatment of carbapenem-resistant Enterobacteriaceae (CREs) with CZA has been reported,^{9,10} and it is currently not clear what the extent of this vulnerability is and if other DBO BLIs in the pipeline will show a similar development of resistance upon treatment.¹¹ In addition to their BLI activity, the DBOs zidebactam, nacubactam, and ETX-2514 and to some extent avibactam are inhibitors of penicillin binding protein 2 (PBP-2) and exert antibacterial activity against some Gram-

Received: January 23, 2019

Published: March 12, 2019

Scheme 1. Synthesis of Compound 12 by Photoredox Coupling^a

^aReagents and conditions: (a) MeOH, DMAP, DCC, 0 °C → rt; (b) NH(ipr)₂, *n*-BuLi, PhSeCl, −78 °C → −10 °C; (c) H₂O₂/AcOH, K₂SO₃, 0 °C; (d) Boc-Gly-OH, Ir[*df*(CF₃)ppy₂(dtbpy)]PF₆, K₂HPO₃, 8W UVA fluorescence tube; (e) TFA, rt, then N(Et)₃, 0 °C → rt; (f) Pd-C, MeOH; (g) SO₃/py.

negative organisms.^{12–15} PBP-2 inhibition by these DBOs acts synergistically with PBP-3-inhibitory β -lactams.¹⁶ However, *in vitro*, reduced susceptibility to the antibacterial activity of PBP-2 inhibitors, including DBOs, is selected at high frequency via a multiplicity of mutations related to the stringent response, and it is not clear at this point if this will translate into rapid resistance development in the clinic.¹² Another concern with BLIs that show intrinsic antibacterial activity is that the use of minimal inhibitory concentrations (MICs) for the combinations with β -lactams as a biomarker to predict efficacy is not straightforward, owing to multiple modes of actions for efficacy and potential differences in PK/PD for both partners.

We initiated a program aiming to discover novel DBOs with improved properties. Our initial goal was to identify novel DBOs with clinically relevant intrinsic antibacterial activity due to inhibition of multiple PBPs as we hypothesized that inhibition of multiple PBPs would reduce the frequency of selecting single-step mutants of the stringent response associated with earlier DBOs which inhibit PBP-2 only, following the original vision of chemists at Hoechst Marion Roussel.¹⁷ Fortuitously, we identified a DBO that was a very potent BLI, providing broad inhibition of serine β -lactamases but that did not engage PBP-2 and correspondingly lacked intrinsic antimicrobial activity altogether. Our vision for this BLI was for it to replace tazobactam in combination with piperacillin to restore broad activity of piperacillin against drug-resistant Enterobacteriaceae.

RESULTS AND DISCUSSION

Chemistry. The preparation of DBOs is challenging, due to long and linear synthetic routes and the high ring strain of the diazabicyclooctane scaffold,^{18,19} leading to instability toward nucleophiles. Therefore, we chose to access novel analogs of DBOs through late stage modification, which has the advantage of divergent synthetic routes for more efficient analoging. Our initial strategy was to utilize the α/β unsaturated ester 6 (Scheme 1) for accessing 3-substituted analogs, which had not been explored much in the literature, in part probably due to the synthetic difficulties. Conventional Michael additions to 6 failed

to give the desired products, resulting instead in opening of the cyclic urea moiety. However, radical Michael additions via visible light-mediated photoredox catalysis²⁰ proved successful, leading to the diastereomeric methylene amino-substituted analogs 7–10 in 79% overall yield (Scheme 1). Boc-deprotection of the amino group with TFA in 7 resulted in cyclization, providing after neutralization the lactam 11. No lactam formation was observed for isomers 8–10 following similar treatment. Formation of the lactam was initially not intended; however, we were interested in profiling a final compound with this novel structural feature. The benzyl group in 11 was removed by hydrogenation, and a sulfonyl group was introduced using SO₃/pyridine to give 12. Because of the low overall yield of 12 from 5 of only 0.1%, we subsequently designed a more efficient and scalable route for 12, which we intend to publish elsewhere.

Since 12 showed an interesting profile as a β -lactamase inhibitor (discussed in the next section), we prepared a number of analogs, including those with substitutions on the amide, as well as the corresponding six-membered lactam, the synthesis of which is described in the patent.²¹ These analogs were synthetically more complex than 12, with similar properties to 12 in early profiling and are therefore not discussed in this report.

Hydrolytic Stability. The chemical hydrolytic stability of 12 was determined in phosphate buffer pH 7.4 at 37 °C and was found to be similar to other DBOs ($t_{1/2}$ = 55 h, Table 1). As a point of reference, the carbapenem imipenem and the monobactam aztreonam show a $t_{1/2}$ of 46 and >2000 h, respectively, in this assay.⁴ Since DBOs are hydrolytically labile, similar to carbapenems, care must be taken to ensure sufficient chemical solution stability of analogs for development and for biological assays.

Intrinsic Antibacterial Activity for 12 and Reference DBOs. Antibacterial activity was determined by the broth microdilution assay following the recommended CLSI methodology²² against quality control strains of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Table 1). Avibactam showed weak

Table 1. Antibacterial Activity and Hydrolytic Stability of Compound 12 and Reference DBOs

DBO	<i>E. coli</i> ATCC 25922	MIC [$\mu\text{g/mL}$] <i>K. pneumoniae</i> ATCC 43816	<i>P. aeruginosa</i> ATCC 27853	hydrolytic stability $t_{1/2}$ [h] ^a
1 (avibactam)	16	32	>64	61
2 (relebactam)	>64	>64	>64	60
3 (nacubactam)	2	>64	>64	32
4 (zidebactam)	0.125	>64	2	53
12	>64	>64	>64	55

^aHydrolytic chemical stability in phosphate buffer pH 7.4 at 37 °C.

antibacterial activity against *E. coli* and *K. pneumoniae*, whereas nacubactam and zidebactam showed stronger antibacterial activity against *E. coli* and for the latter also against *P. aeruginosa*, in agreement with reports in the literature.^{23–25} As discussed above, the antibacterial activity observed for some DBOs is a result of selective PBP-2 inhibition, a feature that selects for high frequencies of resistance *in vitro*. Compound 12 did not show measurable antibacterial activity against any of the isolates tested (MICs > 64 $\mu\text{g/mL}$), similar to relebactam.²⁶

Potential of Piperacillin by Compound 12 against Strains Expressing β -Lactamases. Potentiation of piperacillin by 12 in the presence of a wide range of serine β -lactamases was determined using isogenic strains of *E. coli* expressing individual β -lactamases from a multi-copy plasmid.⁴ Comparative data were generated for the BLIs tazobactam, avibactam, relebactam, and vaborbactam.²⁷ MICs for piperacillin/BLI combinations were determined with a fixed concentration of BLI at 4 $\mu\text{g/mL}$. Vaborbactam was tested at 8 $\mu\text{g/mL}$ and relebactam, at 2 $\mu\text{g/mL}$, tentatively reflecting the relative high and low human doses and exposures for vaborbactam²⁸ and relebactam,²⁹ respectively (Tables 2 and S1).

Table 2. *In Vitro* Activity of Piperacillin in Combination with β -Lactamase Inhibitors against Isogenic Strains of *E. coli* Expressing Individual Serine β -Lactamases

strain (β -lactamase)	ambler class ^b	piperacillin MIC [$\mu\text{g/mL}$] when tested alone or in combination with BLI ^a					
		no BLI	12	TAZ	AVI	REL	VAB
parent	N/A	4	2	4	1	4	2
CTX-M-15	A	>64	4	4	2	16	4
KPC-2	A	>64	4	>64	2	4	4
TEM-1	A	>64	4	32	2	8	>32
TEM-10	A	>64	4	4	2	>32	>32
SHV-12	A	>64	4	4	2	>32	>32
PER-1	A	32	2	4	2	4	4
AmpC	C	>64	4	8	2	4	8
CMY-2	C	32	2	2	2	4	4
DHA-1	C	64	4	4	2	2	4
OXA-10	D	>64	4	32	16	>32	>32
OXA-23	D	>64	4	64	8	>32	>32
OXA-40	D	>64	8	>64	16	>32	>32
OXA-48	D	>64	4	>64	2	>32	>32
OXA-146	D	>64	16	>64	32	>32	>32

^a12, TAZ, and AVI tested at 4 $\mu\text{g/mL}$, vaborbactam at 8 $\mu\text{g/mL}$, relebactam at 2 $\mu\text{g/mL}$; TAZ: tazobactam; AVI: avibactam; REL: relebactam; VAB: vaborbactam. ^bAmbler β -lactamase class.^{44–46}

Piperacillin is a substrate for all four classes of β -lactamases, the serine β -lactamases classes A, C, and D, as well as the metallo β -lactamases (class B, Table S1), as is apparent from the large shift of the MIC against strains expressing β -lactamases relative to the parental strain.

Tazobactam did not effectively potentiate piperacillin in the presence of KPCs, TEM-1, BEL-1, and OXA-type enzymes, which is apparent from the elevated MICs for strains expressing these enzymes. A smaller MIC shift is observed for tazobactam and the class C enzymes AmpC and P99, indicating suboptimal inhibition by tazobactam. This is also reflected in the clinic, where TEM-1 hyperproduction, as well as AmpC upregulation and OXA enzymes have been associated with elevated MICs for TZP against clinical strains,³⁰ which often express multiple of these β -lactamases. Tazobactam's inability to inhibit KPC-2 is probably at least in part due to tazobactam being a substrate for KPC-2.³¹

Piperacillin/relebactam showed elevated MICs for strains expressing CTX-M-15, TEM-10, SHV-12, and all OXAs, indicating the limitations for relebactam to inhibit class A enzymes other than KPCs and its lack of activity against class D enzymes. A similar profile of narrow spectrum inhibition of class A and lack of activity against class D enzymes was also observed for vaborbactam when combined with piperacillin. Relebactam and vaborbactam are intended for use in combination with carbapenems, which are relatively stable to most serine β -lactamases. However, the lack of activity against OXA carbapenemases like OXA-48 limits the utility of these combinations to carbapenemases of the class A type (mainly KPCs). Our data also show that these BLIs are not able to effectively potentiate a β -lactam that is more labile to serine β -lactamases than a carbapenem, like piperacillin.

Avibactam has been reported to have a broader spectrum of β -lactamase inhibitory activity, resulting in better protection against class A, with some coverage of class D enzymes, especially OXA-48,³² and our data are in agreement with these findings. Compound 12 provided the broadest protection of piperacillin from this set of BLIs, including better activity against the OXA-type enzymes than avibactam and good coverage of class A and C enzymes, including KPC-2. None of the serine β -lactamase inhibitors studied potentiated piperacillin in the presence of metallo β -lactamases, as expected (Table S1).

Carbamylation and Decarbamylation Kinetics against CTX-M-15 and KPC-2 β -Lactamases. DBOs cause carbamylation of the active site serine of β -lactamases, with reversible but slow decarbamylation.^{33–35} The kinetic constants for carbamylation of the enzymes CTX-M-15 and KPC-2 by compound 12 and avibactam were determined using a stopped-flow spectrophotometric assay following nitrocefin (NCF) substrate hydrolysis (see Supplementary Methods). The values for the carbamylation efficiencies k_2/K_i of avibactam for CTX-M-15 and KPC-2 were $5.9 \pm 2.6 \times 10^5$ and $7.2 \pm 3.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, respectively (Table 3), similar to the values reported by Ehmann et al.³⁶ (1.3×10^5 and $1.3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, respectively). Compound 12 showed ~32- and 7-fold higher carbamylation efficiencies than avibactam with CTX-M-15 and KPC-2, respectively. It is possible that the fused five-membered lactam ring in compound 12 exerts additional ring strain on the cyclic urea warhead in 12, leading to a higher reactivity of the cyclic urea moiety toward the active site serine of these β -lactamases. Both avibactam and especially 12 appear to carbamylate CTX-M-15 faster than KPC-2. The residence times of binding for compound 12 to CTX-M-15 and KPC-2 were 12 and 36 min,

Table 3. Kinetic Values for Carbamylation and Decarbamylation of Compound 12 against CTX-M-15 and KPC-2 in Comparison to Avibactam^a

parameter		CTX-M-15	KPC-2
carbamylation k_2/K_i ($M^{-1} s^{-1}$)	12	$1.9 \pm 0.8 \times 10^7$	$5.6 \pm 2.8 \times 10^5$
	avibactam	$5.9 \pm 2.6 \times 10^5$	$7.2 \pm 3.6 \times 10^4$
decarbamylation K_{off} (s^{-1})	12	0.0014	0.0005
	avibactam	0.0003	0.0001
residence time (min)	12	12	36
	avibactam	48	219
K_m nitrocefin (μM)		15 ± 7	29 ± 4

^aThe values represent an average of 4 or 5 replicates for the carbamylation and 3 replicates for decarbamylation experiments.

respectively, shorter than those observed for avibactam (33 and 152 min). It is possible that the strain through the lactam moiety in the adduct of 12 to the β -lactamase is leading to higher off-rates. However, we have not yet confirmed the structure of the adduct between 12 and CTX-M-15 and KPC-2, so it is also conceivable that 12 may react with the active site serine of β -lactamases through ring opening of the five membered lactam. We have not confirmed reversibility for the inhibition of β -lactamases by 12, so it is also possible that 12 is not regenerated upon cleavage from the β -lactamases but is hydrolyzed. Further studies are needed to elucidate the mechanism of inhibition of β -lactamases by 12 and to understand the impact of mechanism and kinetics on human dose selection.

Potential of Piperacillin by Compound 12 against Clinical Strains. *In vitro* activity of piperacillin and compound 12 against 190 TZP-nonsusceptible clinical isolates of Enterobacteriaceae was determined by broth microdilution, with 12 tested at a fixed concentration of 4 $\mu g/mL$ (Tables 4 and S2).

Table 4. *In Vitro* Activity of Piperacillin/Compound 12 against 190 Piperacillin/Tazobactam-Nonsusceptible Enterobacteriaceae Isolates^a

antibiotic	MIC ($\mu g/mL$)			susceptible ^a (%)
	range	MIC ₅₀	MIC ₉₀	
piperacillin/12 ^b	0.5 to >64	8	16	NA ^c
TZP ^b	32 to >64	>64	>64	0
piperacillin	64 to >64	>64	>64	0
CZA ^b	≤ 0.06 to 16	1	4	99.5
meropenem	≤ 0.06 to >64	8	>64	37.4

^aSusceptibility as defined by CLSI (CLSI M100-S28). ^bBLIs were tested at a fixed concentration of 4 $\mu g/mL$. ^cNot applicable, breakpoint not defined. TZP: piperacillin/tazobactam; CZA: ceftazidime/avibactam. Strains tested include: *Citrobacter freundii* (18), *Enterobacter aerogenes* (3), *E. cloacae* (22), *E. hormaechei* (1), *E. coli* (28), *Klebsiella oxytoca* (2), *K. pneumoniae* (99), *Salmonella* spp. (5), and *Serratia marcescens* (12). For the MIC distribution, MIC₅₀ and MIC₉₀ values of PIP/12 against the various species tested; see Table S2.

Annotated mechanisms of resistance for the strains included de-repressed class C enzymes, KPCs, upregulated TEM-1, and ESBLs like SHV-5 and -7 as well as OXA-48-like enzymes. This panel did not include metallo- β -lactamase producing strains. Potentiation of piperacillin by 12 was observed for the majority of the strains, with MIC_{50/90} values of 8/16 $\mu g/mL$, respectively, for the combination, representing broad susceptibility of most of the TZP-resistant strains, based on the approved breakpoint for TZP of 16 $\mu g/mL$. Exceptions, aside from metallo- β -lactamase

expressing strains, which were not investigated here, were several OXA-181 or CTX-M-15/CMY-42 expressing strains of *E. coli* from Turkey, Thailand, and the Philippines (Table S3). According to our isogenic panel studies, compound 12 inhibits these β -lactamases, suggesting that there may be additional β -lactamases that are not inhibited by compound 12 or that non- β -lactamase mediated mechanisms that decrease susceptibility to piperacillin (PIP or compound 12) are present in these isolates. Consistent with the latter, we found that these isolates harbored an alteration of the *ftsI* gene encoding a YRIN insertion in the target of PIP (PBP-3³⁷) that was previously shown to impact susceptibility to aztreonam³⁸ and LYS228.³⁹ We showed that an *E. coli* ATCC25922 derivative harboring the YRIN insertion (or a previously described YRIK insertion³⁸) was also 4-fold less susceptible to PIP than ATCC 25922 (MIC shifting from 4 to 16 $\mu g/mL$, Table S4). Therefore, these insertions directly reduce susceptibility to PIP via a target-based mechanism that would not be overcome by the BLI activity of compound 12. It is possible that other non- β -lactamase mechanisms of resistance are also present in these isolates. The impact of PBP-3 alteration on susceptibility to PIP/12 found here is reminiscent of a recent report implicating a TIPY insertion in PBP-3 in reducing susceptibility of a carbapenem resistant *E. coli* clinical isolate to ceftazidime, ceftaroline, or aztreonam in combination with avibactam.⁴⁰

The activity of PIP/12 against clinical strains of the anaerobic species *Bacteroides fragilis* group, methicillin-susceptible *Staphylococcus aureus* (MSSA), *Pseudomonas aeruginosa*, and *Haemophilus influenzae* isolates was determined as well (Table 5). PIP/12 was equipotent compared to TZP against *B. fragilis* group, *P. aeruginosa*, and *H. influenzae* isolates and 2-fold more potent than TZP against MSSA (piperacillin is not active against methicillin-resistant *S. aureus* (MRSA), due to changes in the target PBP2a,⁴¹ which cannot be rescued by addition of a BLI). Thus, the combination of PIP/12 has the potential for empiric broad coverage, including the treatment of drug-resistant Enterobacteriaceae.

Pharmacokinetics of Compound 12 in Preclinical Species. Pharmacokinetic parameters for 12 were determined in mouse, rat, and dog. Data in rat were also generated for avibactam for comparison (Table 6). The profile of 12 was similar to other DBOs and β -lactams in all species, showing low clearance with a significant portion of the dose being recovered unchanged in urine in rat and dog. Plasma protein binding of 12 was low to moderate for all preclinical species and 23% for human. The clearance of 12 in rat was somewhat lower than clearance determined for avibactam (9.7 vs 18 mL/min/kg, respectively); however, the doses for 12 and avibactam were different and the values were close. Thus, the difference may not be significant. Clearance for avibactam in rat has been reported in the literature as 24 mL/min/kg.⁴² Clearance for 12 in dog was lower than the reported value for avibactam⁴³ (2.3 vs 6.1 mL/min/kg, respectively). The half-life for 12 in rat was longer than for typical β -lactams (6.2 h), due to a long $t_{1/2}$ β . If the relatively low clearance observed for 12 in rat and dog is also observed in man, then this may lower the dose requirement for 12, without risk of accumulation in q4 dosing typically used for piperacillin.

Efficacy of PIP/12 in the Murine Thigh Infection Model. The combination of compound 12 with piperacillin was profiled for efficacy in the neutropenic mouse thigh infection model (Table 7). We chose the same ratio for PIP/BLI as for TZP (8:1) for this study. However, the human dose prediction has not been performed yet and may indicate a different ratio for

Table 5. *In Vitro* Activity of Piperacillin/Compound 12 and Reference Compounds against *Bacteroides fragilis* Group, Methicillin-Susceptible *Staphylococcus aureus* (MSSA), and *Pseudomonas aeruginosa*^a

microorganism (no. of isolates)	antibiotic	MIC ($\mu\text{g/mL}$)			susceptible ^a (%)
		range	MIC ₅₀	MIC ₉₀	
<i>B. fragilis</i> group ^b (27)	piperacillin/12 ^c	0.25–64	4	32	NA ^d
	TZP ^c	≤ 0.06 to >64	4	32	93
	piperacillin	2 to >64	32	>64	59
	meropenem	0.125 to >16	0.5	1	96
MSSA (40)	piperacillin/12 ^c	0.5–1	1	1	NA ^d
	TZP ^c	0.5–8	1	2	100
	piperacillin	1 to >64	4	>64	NA ^d
	vancomycin	0.5–1	0.5	1	100
<i>P. aeruginosa</i> (124)	piperacillin/12 ^c	≤ 0.06 –64	8	16	NA ^d
	TZP ^c	0.25–64	8	16	90
	piperacillin	0.25 to >64	8	32	77
	meropenem	≤ 0.06 –32	1	8	82
<i>H. influenzae</i> (25)	piperacillin/12 ^c	≤ 0.06 –0.5	≤ 0.06	≤ 0.06	NA ^d
	TZP ^c	0.25–0.5	≤ 0.06	≤ 0.06	100
	piperacillin	0.25 to >64	≤ 0.06	64	NA ^d
	azithromycin	0.25 to >32	4	>32	52

^aSusceptibility as defined by CLSI (CLSI M100-S28). For susceptibility of MSSA to piperacillin/TAZ, the USA FDA breakpoint was applied (ZOSYN package insert). ^b*B. fragilis* (9), *B. ovatus* (6), *B. thetaiotaomicon* (7), and *B. vulgatus* (5). ^cBLIs were tested at a fixed concentration of 4 $\mu\text{g/mL}$. ^dNot applicable.

Table 6. Pharmacokinetics in Mouse, Rat, and Dog and Plasma Protein Binding^a

	compound	clearance [mL/min/kg]	volume [L/kg]	half-life [h]	AUC [$\mu\text{M h}$]	% dose in urine	PPB ^b [% bound]
mouse	12	9.2	0.2	1.4	60	ND ^d	45
rat	12	9.7	0.5	6.2	187	61	20
	avibactam	18	0.4	1.5	18 ^c	30	ND ^d
dog	12	2.3	0.2	2.5	80	88	24

^aPharmacokinetic parameters following administration of 10 mg/kg (mouse), 30 and 5 mg/kg for 12 and avibactam (rat), respectively, or 3 mg/kg (dog) by iv infusion. ^bPlasma protein binding. ^cDifferent dose for 12 and avibactam; see ^a. ^dNot determined.

Table 7. *In Vivo* Efficacy of Piperacillin/Compound 12 and Piperacillin/TAZ against an SHV-12 and KPC-11 Expressing Strain of *K. pneumoniae* in the Neutropenic Mouse Thigh Infection Model^a

	MIC [$\mu\text{g/mL}$]	static dose [mg/kg/day]	maximum efficacy [$\Delta\log_{10}$ CFU/thigh]	highest dose tested PIP/BLI [mg/kg/day]
piperacillin/12	16	1695	-0.84 ± 0.09	5120:640
TZP	>64	stasis not achieved	not efficacious	5120:640

^aAnimals were inoculated with approximately 1×10^6 CFU/thigh. After treatment with vehicle for 24 h, bacterial burden increased by 2.34 ± 0.61 $\log_{10}$ CFU compared to static levels in the thigh (0 h, 5.96 ± 0.24 $\log_{10}$ CFU/thigh). Maximum efficacy was benchmarked against the bacterial load in the thigh at the time treatment began (stasis). Compounds were dosed subcutaneously every 3 h. PIP and BLIs were dosed in a ratio of 8:1. TZP: piperacillin/tazobactam.

optimal efficacy. We chose a relatively resistant strain of *K. pneumoniae* expressing KPC-11 and SHV-12 for this experiment. The MIC for TZP against this strain was >64 $\mu\text{g/mL}$, higher than the clinical breakpoint for TZP of 16 $\mu\text{g/mL}$. At the doses administered, TZP did not achieve efficacy against this strain in this model, consistent with the elevated MIC and the presence of a KPC β -lactamase, which is not inhibited by tazobactam. PIP/12 was efficacious against this strain, consistent with the lower MIC of 16 $\mu\text{g/mL}$ reflecting inhibition of class A β -lactamases including KPCs by 12.

CONCLUSIONS

Compound 12 is a potent and broad spectrum β -lactamase inhibitor that does not inhibit PBP-2 and lacks measurable intrinsic antibacterial activity. This may remove potential development and therapeutic liabilities attributed to selective PBP-2 inhibition. Compound 12 effectively protects piperacillin

against degradation by serine β -lactamase-expressing drug-resistant Enterobacteriaceae and other organisms, restoring activity against the majority of TZP-resistant strains tested. The pharmacokinetic parameters for 12 were evaluated in rodents and dog and found to be similar to avibactam, except for 12 having a longer half-life, especially in dog. PIP/12 but not TZP showed efficacy against a KPC-expressing strain of *K. pneumoniae* in the murine thigh infection model.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.9b00031.

Supplementary methods; Table S1: MICs for piperacillin $\pm$ BLI against isogenic strains of *E. coli* expressing individual β -lactamases; Table S2: MIC distribution of

PIP/12 against 190 Enterobacteriaceae isolates; Table S3: presence of *ftsI* mutations in *E. coli* clinical isolates that are not highly susceptible to PIP/12; Table S4: impact of YRIN and YRIK insertions in PBP-3 on susceptibility of *E. coli* to β -lactam antibiotics; supplementary references (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: Folkert.Reck@Novartis.com.

ORCID

Folkert Reck: 0000-0001-7250-2526

Charles R. Dean: 0000-0001-9858-9818

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank JMI Laboratories, Inc. and International Health Management Associates (IHMA) for clinical isolates and Jennifer Leeds for critical review of the manuscript.

ABBREVIATIONS

BLI, β -lactamase inhibitor; DBO, diazabicyclooctane; MDR, multidrug-resistant; ESBL, extended spectrum β -lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; TZP, piperacillin/tazobactam; CZA, ceftazidime/avibactam; CRE, carbapenem-resistant Enterobacteriaceae; PBP, penicillin binding protein; MIC, minimal inhibitory concentration; PIP, piperacillin; REL, relebactam; VAB, vaborbactam; AVI, avibactam; TAZ, tazobactam

REFERENCES

- (1) Van Boeckel, T. P., Caudron, Q., Gandra, S., Ashok, A., Grenfell, B. T., Levin, S. A., and Laxminarayan, R. (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* 14, 742–750.
- (2) Bush, K., and Fisher, J. F. (2011) Epidemiological expansion, structural studies, and clinical challenges of new β -lactamases from Gram-negative bacteria. *Annu. Rev. Microbiol.* 65, 455–478.
- (3) Bonomo, R. A., Burd, E. M., Conly, J., Limbago, B. M., Poirel, L., Segre, J. A., and Westblade, L. F. (2018) Carbapenemase-Producing Organisms: A Global Scourge. *Clin. Infect. Dis.* 66, 1290–1297.
- (4) Reck, F., Birmingham, A., Blais, J., Capka, V., Cariaga, T., Casarez, A., Colvin, R., Dean, C. R., Fekete, A., Gong, W., Growcott, E., Guo, H., Jones, A. K., Li, C., Li, F., Lin, X., Lindvall, M., Lopez, S., McKenney, D., Metzger, L., Moser, H. E., Prathapam, R., Rasper, D., Rudewicz, P., Sethuraman, V., Shen, X., Shaul, J., Simmons, R. L., Tashiro, K., Tang, D., Tjandra, M., Turner, N., Uehara, T., Vitt, C., Whitebread, S., Yifru, A., Zang, X., and Zhu, Q. (2018) Optimization of novel monobactams with activity against carbapenem-resistant Enterobacteriaceae - Identification of LYS228. *Bioorg. Med. Chem. Lett.* 28, 748–755.
- (5) Castanheira, M., Mendes, R. E., Jones, R. N., and Sader, H. S. (2016) Changes in the frequencies of β -lactamase genes among Enterobacteriaceae isolates in U.S. Hospitals, 2012 to 2014: activity of ceftazidime-avibactam tested against β -lactamase-producing isolates. *Antimicrob. Agents Chemother.* 60, 4770–4777.
- (6) Stone, G. G., Bradford, P. A., Yates, K., and Newell, P. (2017) In vitro activity of ceftazidime/avibactam against urinary isolates from patients in a Phase 3 clinical trial programme for the treatment of complicated urinary tract infections. *J. Antimicrob. Chemother.* 72, 1396–1399.
- (7) Zhanel, G. G., Lawson, C. D., Adam, H., Schweizer, F., Zelenitsky, S., Lagace-Wiens, P. R. S., Denisuik, A., Rubinstein, E., Gin, A. S., Hoban, D. J., Lynch, J. P., 3rd, and Karlowsky, J. A. (2013) Ceftazidime-Avibactam: a Novel Cephalosporin/ β -lactamase Inhibitor Combination. *Drugs* 73, 159–177.
- (8) Zasowski, E. J., Rybak, J. M., and Rybak, M. J. (2015) The β -Lactams Strike Back: Ceftazidime-Avibactam. *Pharmacotherapy* 35, 755–770.
- (9) Both, A., Büttner, H., Huang, J., Perbandt, M., Belmar Campos, C., Christner, M., Maurer, F. P., Kluge, S., König, C., Aepfelbacher, M., et al. (2017) Emergence of ceftazidime/avibactam non-susceptibility in an MDR *Klebsiella pneumoniae* isolate. *J. Antimicrob. Chemother.* 72, 2483–2488.
- (10) Shields, R. K., Potoski, B. A., Haidar, G., Hao, B., Doi, Y., Chen, L., Press, E. G., Kreiswirth, B. N., Clancy, C. J., and Nguyen, M. H. (2016) Clinical outcomes, drug toxicity, and emergence of ceftazidime-avibactam resistance among patients treated for carbapenem-resistant enterobacteriaceae infections. *Clin. Infect. Dis.* 63, 1615–1618.
- (11) Giddins, M. J., Macesic, N., Annavaiah, M. K., Stump, S., Khan, S., McConville, T. H., Gomez-Simmonds, A., Uhlemann, A., and Mehta, M. (2018) Successive Emergence of Ceftazidime-Avibactam Resistance through Distinct Genomic Adaptations in blaKPC-2-Harboring *Klebsiella pneumoniae* Sequence Type 307 Isolates. *Antimicrob. Agents Chemother.* 62, e02101-17.
- (12) Livermore, D. M., Warner, M., Mushtaq, S., and Woodford, N. (2016) Interactions of OP0595, a novel triple-action diazabicyclooctane, with β -lactams against OP0595-resistant Enterobacteriaceae mutants. *Antimicrob. Agents Chemother.* 60, 554–560.
- (13) Livermore, D. M., Mushtaq, S., Warner, M., Vickers, A., and Woodford, N. (2017) In vitro activity of cefepime/zidebactam (WCK 5222) against Gram-negative bacteria. *J. Antimicrob. Chemother.* 72, 1373–1385.
- (14) Asli, A., Brouillette, E., Krause, K. M., Nichols, W. W., and Malouin, F. (2016) Distinctive binding of avibactam to penicillin-binding proteins of gram-negative and gram-positive bacteria. *Antimicrob. Agents Chemother.* 60, 752–756.
- (15) Durand-Reville, T. F., Guler, S., Comita-Prevoir, J., Chen, B., Bifulco, N., Huynh, H., Lahiri, S., Shapiro, A. B., McLeod, S. M., Carter, N. M., Moussa, S. H., Velez-Vega, C., Olivier, N. B., McLaughlin, R., Gao, N., Thresher, J., Palmer, T., Andrews, B., Giacobbe, R. A., Newman, J. V., Ehmann, D. E., de Jonge, B., O'Donnell, J., Mueller, J. P., Tommasi, R. A., and Miller, A. A. (2017) ETX2514 is a broad-spectrum β -lactamase inhibitor for the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter baumannii*. *Nat. Microbiol.* 2, 17104.
- (16) Livermore, D. M., Mushtaq, S., Warner, M., and Woodford, N. (2015) Activity of OP0595/ β -lactam combinations against Gram-negative bacteria with extended-spectrum, AmpC and carbapenem-hydrolysing β -lactamases. *J. Antimicrob. Chemother.* 70, 3032–3041.
- (17) Coleman, K. (2011) Diazabicyclooctanes (DBOs): a potent new class of non- β -lactam β -lactamase inhibitors. *Curr. Opin. Microbiol.* 14, 550–555.
- (18) Yin, J., Weisel, M., Ji, Y., Liu, Z., Liu, J., Wallace, D. J., Xu, F., Sherry, B. D., and Yasuda, N. (2018) Improved Preparation of a Key Hydroxylamine Intermediate for Relebactam: Rate Enhancement of Benzyl Ether Hydrogenolysis with DABCO. *Org. Process Res. Dev.* 22, 273–277.
- (19) Ball, M., Boyd, A., Ensor, G. J., Evans, M., Golden, M., Linke, S. R., Milne, D., Murphy, R., Telford, A., Kalyan, Y., Lawton, G. R., Racha, S., Ronsheim, M., and Zhou, S. H. (2016) Development of a Manufacturing Route to Avibactam, a β -Lactamase Inhibitor. *Org. Process Res. Dev.* 20, 1799–1805.
- (20) Chu, L., Ohta, C., Zuo, Z., and MacMillan, D. W. C. (2014) Carboxylic Acids as A Traceless Activation Group for Conjugate Additions: A Three-Step Synthesis of ($\pm$)-Pregabalin. *J. Am. Chem. Soc.* 136, 10886–10889.
- (21) Casarez, A., Furegati, M., Koch, M., Lin, X., Ossola, F., Reck, F., Simmons, R. L., and Zhu, Q. (2018) U.S. Pat. Appl. Publ. US20180086762.
- (22) Clinical and Laboratory Standards Institute (2015) *CLSI Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard*, 10th ed., CLSI document M07-A10, Clinical and Laboratory Standards Institute, Wayne, PA.

- (23) Lagace-Wiens, P. R. S., Taylor, F., Simner, P., DeCorby, M., Karlowsky, J. A., Walkty, A., Hoban, D. J., and Zhanel, G. G. (2011) Activity of NXL104 in combination with β -lactams against genetically characterized *Escherichia coli* and *Klebsiella pneumoniae* isolates producing class A extended-spectrum β -lactamases and class C β -lactamases. *Antimicrob. Agents Chemother.* 55, 2434–2437.
- (24) Morinaka, A., Tsutsumi, Y., Yamada, M., Suzuki, K., Watanabe, T., Abe, T., Furuuchi, T., Inamura, S., Sakamaki, Y., Mitsuhashi, N., Ida, T., and Livermore, D. M. (2015) OP0595, a new diazabicyclooctane: mode of action as a serine β -lactamase inhibitor, antibiotic and β -lactam 'enhancer'. *J. Antimicrob. Chemother.* 70, 2779–2786.
- (25) Papp-Wallace, K. M., Nguyen, N. Q., Jacobs, M. R., Bethel, C. R., Barnes, M. D., Kumar, V., Bajaksouzian, S., Rudin, S. D., Rather, P. N., Bhavsar, S., Ravikumar, T., Deshpande, P. K., Patil, V., Yeole, R., Bhagwat, S. S., Patel, M. V., van den Akker, F., and Bonomo, R. A. (2018) Strategic Approaches to Overcome Resistance against Gram-Negative Pathogens Using β -Lactamase Inhibitors and β -Lactam Enhancers: Activity of Three Novel Diazabicyclooctanes WCK 5153, Zidebactam (WCK 5107), and WCK 4234. *J. Med. Chem.* 61, 4067–4086.
- (26) Livermore, D. M., Warner, M., and Mushtaq, S. (2013) Activity of MK-7655 combined with imipenem against Enterobacteriaceae and *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 68, 2286–2290.
- (27) Hecker, S. J., Reddy, K. R., Hirst, G. C., Lomovskaya, O., Griffith, D. C., King, P., Tsivkovski, R., Sun, D., Sabet, M., Tarazi, Z., Boyer, S. H., Loutit, J. S., Morgan, E. E., Durso, S., Dudley, M. N., Totrov, M., Clifton, M. C., Atkins, K., Raymond, A., Potts, K. T., and Abendroth, J. (2015) Discovery of a Cyclic Boronic Acid β -Lactamase Inhibitor (RPX7009) with Utility vs Class A Serine Carbapenemases. *J. Med. Chem.* 58, 3682–3692.
- (28) Rubino, C. M., Bhavnani, S. M., Loutit, J. S., Morgan, E. E., White, D., Dudley, M. N., and Griffith, D. C. (2018) Phase 1 Study of the Safety, Tolerability, and Pharmacokinetics of Vaborbactam and Meropenem Alone and in Combination following Single and Multiple Doses in Healthy Adult Subjects. *Antimicrob. Agents Chemother.* 62, e02228-17.
- (29) Rhee, E. G., Rizk, M. L., Calder, N., Nefliu, M., Schwartz, M. S., Mangin, E., Boundy, K., Bhagunde, P., Colon-Gonzalez, F., Jumes, P., Liu, Y., Butterton, J. R., and Warrington, S. J. (2018) Pharmacokinetics, Safety, and Tolerability of Single and Multiple Doses of Relebactam, a β -Lactamase Inhibitor, in Combination with Imipenem and Cilastatin in Healthy Participants. *Antimicrob. Agents Chemother.* 62, e00280-18.
- (30) Lee, J., Oh, C. E., Choi, E. H., and Lee, H. J. (2013) The impact of the increased use of piperacillin/tazobactam on the selection of antibiotic resistance among invasive *Escherichia coli* and *Klebsiella pneumoniae* isolates. *Int. J. Infect. Dis.* 17, e638–e643.
- (31) Papp-Wallace, K. M., Bethel, C. R., Distler, A. M., Kasuboski, C., Taracila, M., and Bonomo, R. A. (2010) Inhibitor resistance in the KPC-2 β -lactamase, a preeminent property of this class A β -lactamase. *Antimicrob. Agents Chemother.* 54, 890–897.
- (32) Livermore, D. M., Mushtaq, S., Warner, M., Zhang, J., Maharjan, S., Doumith, M., and Woodford, N. (2011) Activities of NXL104 combinations with ceftazidime and aztreonam against carbapenemase-producing Enterobacteriaceae. *Antimicrob. Agents Chemother.* 55, 390–394.
- (33) King, D. T., King, A. M., Lal, S. M., Wright, G. D., and Strynadka, N. C. J. (2015) Molecular Mechanism of Avibactam-Mediated β -Lactamase Inhibition. *ACS Infect. Dis.* 1, 175–184.
- (34) Ehmann, D. E., Jahic, H., Ross, P. L., Gu, R., Hu, J., Kern, G., Walkup, G. K., and Fisher, S. L. (2012) Avibactam is a covalent, reversible, non- β -lactam β -lactamase inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* 109, 11663–11668.
- (35) Shapiro, A. B., Gao, N., Jahić, H., Carter, N. M., Chen, A., and Miller, A. A. (2017) Reversibility of covalent, broad-spectrum serine β -lactamase inhibition by the diazabicyclooctenone ETX2514. *ACS Infect. Dis.* 3, 833–844.
- (36) Ehmann, D. E., Jahic, H., Ross, P. L., Gu, R., Hu, J., Durand-Reville, T. F., Lahiri, S., Thresher, J., Livchak, S., Gao, N., Palmer, T., Walkup, G. K., and Fisher, S. L. (2013) Kinetics of Avibactam Inhibition against Class A, C, and D β -Lactamases. *J. Biol. Chem.* 288, 27960–27971.
- (37) Kocaoglu, O., and Carlson, E. E. (2015) Profiling of beta-lactam selectivity for penicillin-binding proteins in *Escherichia coli* strain DC2. *Antimicrob. Agents Chemother.* 59, 2785–2790.
- (38) Alm, R. A., Johnstone, M. R., and Lahiri, S. D. (2015) Characterization of *Escherichia coli* NDM isolates with decreased susceptibility to aztreonam/avibactam: role of a novel insertion in PBP3. *J. Antimicrob. Chemother.* 70, 1420–1428.
- (39) Dean, C. R., Barkan, D. T., Bermingham, A., Blais, J., Casey, F., Casarez, A., Colvin, R., Fuller, J., Jones, A. K., Li, C., Lopez, S., Metzger, L. E., Mostafavi, M., Prathapam, R., Rasper, D., Reck, F., Ruzin, A., Shaul, J., Shen, X., Simmons, R. L., Skewes-Cox, P., Takeoka, K. T., Tamrakar, P., Uehara, T., and Wei, J. (2018) The monobactam LYS228: mode of action and mechanisms decreasing in vitro susceptibility of *Escherichia coli* and *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.*, e01200-18.
- (40) Zhang, Y., Kashikar, A., Brown, C. A., Denys, G., and Bush, K. (2017) Unusual *Escherichia coli* PBP 3 Insertion Sequence Identified from a Collection of Carbapenem-Resistant Enterobacteriaceae Tested In Vitro with a Combination of Ceftazidime-, Ceftaroline-, or Aztreonam-Avibactam. *Antimicrob. Agents Chemother.* 61, e00389–17.
- (41) de Lencastre, H., de Jonge, B. L., Matthews, P. R., and Tomasz, A. (1994) Molecular aspects of methicillin resistance in *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 33, 7–24.
- (42) Ouchi, S., Matsumoto, K., Uchida, M., Shibasaki, S., Tsuchiya, T., and Ida, T. (September 5–9, 2014) 54th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC), Washington, DC.
- (43) Chavan, R. P., Patel, A. M., Zope, V. S., Shaikh, J. U., Patil, A. D., Yeole, R. D., and Patel, M. V. (June 16–20, 2016) WCK5107 (zidebactam) and WCK5222 (cefepime-zidebactam) intravenous pharmacokinetics in beagle dogs. In 56th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC), Poster Sunday 450.
- (44) Ambler, R. P. (1980) The structure of beta-lactamases. *Philos. Trans. R. Soc., B* 289, 321–331.
- (45) Jaurin, B., and Grundstrom, T. (1981) ampC cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of beta-lactamases of the penicillinase type. *Proc. Natl. Acad. Sci. U. S. A.* 78, 4897–4901.
- (46) Ouellette, M., Bissonnette, L., and Roy, P. H. (1987) Precise insertion of antibiotic resistance determinants into Tn21-like transposons: nucleotide sequence of the OXA-1 beta-lactamase gene. *Proc. Natl. Acad. Sci. U. S. A.* 84, 7378–7382.