Journal of Medicinal Chemistry



Drug Annotation

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Discovery of an Orally Available Diazabicyclooctane Inhibitor (ETX0282) of Class A, C and D Serine #-lactamases

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Discovery of an Orally Available Diazabicyclooctane Inhibitor (ETX0282) of Class A, C

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2	and D Serine β-lactamases
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13	Abstract: Multi-drug resistant Gram-negative bacterial infections are an increasing public
14	health threat due to rapidly rising resistance towards β -lactam antibiotics. The hydrolytic
15	enzymes called β -lactamases are responsible for a large proportion of the resistance
16	phenotype. β -lactamase inhibitors (BLIs) can be administered in combination with β -
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17	lactam antibiotics to negate the action of the β -lactamases, thereby restoring activity of
18	the β -lactam. Newly developed BLIs offer some advantage over older BLIs in terms of
19	enzymatic spectrum but are limited to the intravenous route of administration. Reported
20	here is a novel, orally bioavailable, diazabicyclooctane (DBO) β -lactamase inhibitor. This
21	new DBO, ETX1317, contains an endocyclic carbon-carbon double bond and a
22	fluoroacetate activating group, and exhibits broad spectrum activity against Class A, C
23	and D serine β -lactamases. The ester prodrug of ETX1317, ETX0282, is orally
24	bioavailable and, in combination with cefpodoxime proxetil, is currently in development
25	as an oral therapy for multi-drug resistant and carbapenem-resistant Enterobacterales
26	infections.
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	29	The global threat of antibacterial resistance is growing rapidly, and immediate worldwide
0 1 2	30	attention is warranted to ensure new antibiotics become available to treat resistant
3 4 5	31	infections. ¹ Without effective antibiotics, surgeries, organ transplants and cancer
6 7 8 9	32	chemotherapy are also at risk. Of particular concern are infections caused by multi-drug
0 1 2	33	resistant (MDR) Gram-negative bacteria. Recently the WHO ² and the CDC ³ highlighted
3 4 5 6	34	"priority pathogens" that pose the greatest threat to humanity from an infection point of
7 8 9	35	view. Included as critical are carbapenem-resistant Acinetobacter baumannii,
0 1 2 3	36	carbapenem-resistant Pseudomonas aeruginosa; and carbapenem-resistant, extended-
4 5 6	37	spectrum β -lactamase- (ESBL-) producing <i>Enterobacterales</i> , for which new treatment
7 8 9 0	38	options are urgently needed.
1 2 3 4	39	Since the introduction of penicillin in the 1940s, β -lactams have become the safest and
5 6 7 8	40	most effective class of antibiotics to cure infections, especially those caused by Gram-
9 0 1	41	negative bacteria. ⁴ Unfortunately, many β -lactams have lost their activity over the years
2 3 4 5 6	42	as bacteria relentlessly evolve to become resistant to these agents. One of the main
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43	resistance mechanisms in contemporary Gram-negative clinical isolates is mediated by
44	the expression of multiple β -lactamases, enzymes made by the bacteria that hydrolyze
45	and destroy the β -lactams. 4 One approach to counter the action of β -lactamases is to
46	combine a β -lactamase inhibitor (BLI) with the β -lactam to protect it from degradation and
47	restore its antibacterial activity. The only approved oral BLI is clavulanic acid, which
48	became available to the healthcare community in 1984. ⁵ Its spectrum of activity is
49	unfortunately limited to the Ambler Class A β -lactamases, 6 minimizing its clinical utility
50	against contemporary isolates. Recently, three new BLIs with greater spectra of inhibition
51	were approved: avibactam, 7 vaborbactam and relebactam. 8 Unfortunately, none of
52	these are suitable for oral dosing and they all need to be administered in a hospital setting,
53	resulting in a high cost burden on the healthcare system. A potent, broad-spectrum, safe
54	and orally available BLI would therefore represent a welcome addition to the
55	armamentarium against MDR Gram-negative infections. Specifically, an oral β -lactam-
56	BLI combination therapy for urinary tract infections (UTI) remains an important unmet
57	medical need ^{9,10} because the prevalence of ESBL-producing and carbapenem-resistant
58	Enterobacterales (CRE) is increasing at an alarming rate. ¹¹

3 4 5	59	This work describes the discovery and characterization of ETX0282, an orally available
6 7 8 9	60	BLI with a broad spectrum of activity against Class A, C and D serine β -lactamases. The
10 11 12 12	61	combination of this novel BLI with a β -lactam has the potential to become an effective
13 14 15 16 17	62	oral treatment option against MDR Gram-negative infections.
18 19 20 21	63	
22 23 24 25	64	RESULTS AND DISCUSSION
26 27 28 29	65	Design and testing
30 31 32 33	66	The diazabicyclooctane (DBO) scaffold is the basis for several previously developed
34 35 36 37	67	serine β -lactamase inhibitors such as avibactam and durlobactam (Figure 1). 12,13 The
38 39 40	68	high polarity and low pKa of these compounds are well-suited to intravenous
41 42 43 44	69	administration, but lead to low oral bioavailability. We therefore investigated a prodrug
45 46 47	70	approach to improve oral absorption. Our effort was focused on replacing the sulfate
48 49 50 51	71	activating group of the endocyclic double bond series of DBOs. This starting point had
52 53 54	72	several advantages. (1) We possessed a deep understanding of the SAR of β -lactamase
55 56 57 58	73	inhibitors. (2) These compounds have a covalent mechanism of inhibition. (3) We had
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74	available advanced intermediates from well-established syntheses. The very high polarity
75	(logD <0) and low pKa of avibactam and durlobactam are beneficial for their antibacterial
76	activity, urinary excretion and intravenous route of administration, but result in minimal
77	oral bioavailability (%F <10% 14). We therefore turned our attention to the corresponding
78	prodrugs to increase oral bioavailability, an approach that should deliver high oral
79	absorption and release the active BLI upon liver-mediated metabolism. While it seemed
80	possible to prepare a sulfate prodrug of avibactam (as exemplified by ARX-1796 ¹⁵), we
81	decided to focus our efforts on the endocyclic double bond (EDB) series and replace the
82	sulfate activating group by an acetate for several reasons. First, the tetrahydropyridine
83	core in the EDB series is more reactive than the piperidine core, ¹³ which is critical to
84	achieve potent inhibition of a broad spectrum of serine β -lactamases. Second, the
85	carboxylic acid group and its ester prodrugs are among the best-studied moieties in drug
86	discovery to enhance oral absorption of poorly permeable compounds. ¹⁶ The acetate
87	also presents more opportunities for SAR exploration compared to sulfate prodrugs,
88	which are considered to be unstable and potentially toxic alkylating agents. ¹⁷ Several
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	100	1). It was important to keep the BLI to a relatively low molecular weight (MW <450) to
	101	ensure favorable bacterial permeation and provide inhibition of the largest number of β -
0 1 2	102	lactamase enzymes possible. Based on the SAR generated during the durlobactam
3 4 5	103	project, 13 a combination of a hydrogen bond donor and acceptor was required at $R_1,$
6 7 8 9	104	while small alkyl groups at R_2 and R_3 were favored. Attention was also paid to the pKa
0 1 2	105	of the carboxylic acid to optimize the interaction with the β -lactamase conserved KTG
3 4 5 6	106	region, the part of the β -lactam substrate binding pocket that recognizes their carboxylic
7 8 9	107	acid or sulfate group. ²⁰ We also sought to maintain the required low logD. This was
0 1 2 3	108	achieved by varying the substitution at the R_4 and R_5 positions (Figure 1).
4 5 6 7	109	The new analogs were tested for inhibition of representative enzymes from each class of
8 9 0	110	serine β -lactamases. Due to the time-dependent covalent inhibition by these compounds,
1 2 3 4	111	we used the $IC_{\rm 50}$ measured at 10 and 60 minutes of incubation to drive the SAR
5 6 7	112	exploration. 21 $$ IC_{50} values were used as it allowed comparison of the potencies of
o 9 0 1	113	compounds when some showed time-dependent inhibition and others did not. This was
2 3 4	114	followed by evaluation of their ability (at a fixed concentration of 4 $\mu\text{g}/\text{mL})$ to restore the
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115	antibacterial potency of the β -lactam cefpodoxime against two resistant clinical strains of
116	Escherichia coli and Klebsiella pneumoniae, as measured by the minimal inhibitory
117	concentration (MIC) required to prevent bacterial growth. A combination MIC of less than
118	1 μ g/mL was chosen as favorable criteria for progression as it would be similar to the
119	potency observed for avibactam and durlobactam. Their intrinsic antibacterial activity
120	was also evaluated as other DBOs in this series have showed activity on their own. ¹³
121	After optimizing the active form of the BLI (Figure 1), we turned our attention to the ester
122	prodrugs and their metabolic and pharmacokinetic profiles. Four parameters were taken
123	into consideration to achieve the desired profile: (1) high stability to spontaneous
124	hydrolysis in aqueous medium (pH 7.4 buffer, unproductive hydrolysis, Figure 1), (2)
125	stability to intestinal esterases in vitro for stability during absorption, (3) rapid conversion
126	to the active form by liver esterases in vitro (productive metabolism of the ester to the
127	acid, Figure 1), and (4) high oral bioavailability in rats. Different linear and branched alkyl
128	esters were prepared and subjected to this screening cascade to identify an orally

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129 bioavailable BLI with microbiological and DMPK profiles that could be matched with an 130 oral β-lactam. 131 Structure-activity relationship of analogs 132 The initial SAR exploration started from the R₂-methyl hydroxyurea core. Sulfate 133 replacement by acetic acid gave compound 1, which was less potent overall than avibactam or durlobactam (Table 1). Since the pKa of the acidic moiety and chemical 134 135 reactivity of the urea carbonyl are critical for potency, the difluoroacetate analog 2 was 136 prepared. The addition of the electronegative fluorine atoms was predicted to reduce the 137 pKa of the acid to favor binding into the electropositive KTG binding subpocket and 138 increase the electrophilicity of the urea carbonyl to generate a more reactive covalent 139 inhibitor. As expected, analog 2 showed significantly improved potency against β -140 lactamases, with all IC₅₀ values less than 15 nM at 60 minutes. Unfortunately, its 141 antimicrobial activity was not measurable due to rapid decomposition under the conditions 142 of the MIC assay. In order to mitigate this stability issue, we replaced one of the fluorine 143 atoms by a methyl group (compound 3). This compound showed weak inhibition of the

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	144	Class D OXA-48 β -lactamase (IC_{50} = 22 μM at 60 minutes), likely due to a conformational
	145	constraint around the acetate side chain. The monofluoroacetic acid analogs 4 and 5
) <u>)</u>	146	were potent β -lactamase inhibitors, with the (<i>R</i>)-diastereomer being the more potent.
3 1 5	147	Compound 4 was equipotent with difluoroacetate 2, but without the instability under MIC
> 7 3 €	148	measurement conditions. The compound had favorable MICs in combination with
) <u>2</u>	149	cefpodoxime (0.06 µg/mL vs. <i>E. coli</i> ARC3627 and 0.25 µg/mL vs. <i>K. pneumoniae</i>
5 5 5	150	ARC561) with a greater than 128-fold reduction compared to the MIC with no BLI.
7 3 9	151	Compound 4 also showed measurable intrinsic antibacterial activity against both strains.
) <u>2</u> 	152	The (\mathcal{S})-diastereomer 5 showed slightly lower potency against the Class A TEM-1 and
1 5 5	153	Class C AmpC β -lactamases, and significantly lower antibacterial activity. The
' 3 9)	154	unexpected higher MIC values for 5 compared to 4 is not fully understood at this time but
 <u>2</u> 8	155	we hypothesize that the unique stereochemistry of the fluoroacetate sidechain could
1 5 5 7	156	influence bacterial permeation and efflux. A cyclopropyl group at R_2 (6) gave similar
3)	157	potency as a methyl group (5).
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158	In an attempt to improve the activity of compound ${f 5}$, we explored substitutions at the ${\sf R}_1$
159	position (Table 2). The sulfonamide- (7) and sulfonyl urea- (11) containing analogs
160	displayed the best antibacterial activity (MIC \leq 1 µg/mL), but lost potency against OXA-
161	48. The MIC improvement may, in part, be explained by the increased polar surface area
162	compared to the R ₁ -primary amide (from TPSA 113.2 Å ² for 5 to TPSA 167.7 Å ² for 7 and
163	TPSA 179.8 Å ² for 11) and additional hydrogen bond donors and acceptors, leading to
164	better bacterial uptake through porins and lower efflux. ²² These two analogs also showed
165	the lowest intrinsic MICs of the set against the <i>E. coli</i> strain while inactive against the <i>K.</i>
166	pneumonia strain (MIC > 32 μ g/mL). Introduction of ionizable sidechains was not
167	explored due to their likely reduction of oral absorption.
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		IC ₅₀ (μM) 10 min/60 min ^a			MIC (μg/mL) of BLI alone		MIC (μg/mL) of cefpodoxime in the presence of 4 μg/mL of BLI ^b	
ID BLI structure		<i>E. coli</i> TEM-1 (Class A)	E. coli TEM-1P. aeruginosa AmpC(Class A)(Class C)		<i>E. coli</i> ARC3627 ^c	K. pneumoniae ARC561 ^d	E. coli ARC3627 °	K. pneumoniae ARC561 ^d
	Avibactam	0.050/0.013	1.7/0.52	4.2/0.88	32	>32	0.5	1
	Durlobactam	0.0014±0.0005/ 0.0008±0.0003	0.008±0.003/ 0.0058±0.0004	0.024±0.006/ 0.005±0.001	0.25	8	<0.06	<0.06
1	нали и стран	1.5/0.43	4.1/1.2	0.15/0.051	32	>32	4	2
2	H2N ^N N CFOH	0.0012/0.0015	0.05/0.012	0.075/0.014	NM	NM	NM	NM
3	H ₂ N ^J , N N N N N N N N N N N N N N N N N N N	0.68/0.23	1.4/0.4	89/22	>32	>32	2	2
4	H ₂ N ¹ , _N _F	0.00073/0.0014	0.041/0.011	0.071/0.015	2	32	0.06	0.25
5	H ₂ N ^M , F H ₂ N ^M , F NO ^K OH	0.013/0.019	0.18/0.071	0.063/0.015	8	>32	1	2
6	H ₂ N , F H ₂ N , F O , O , O , O , O , O , O , O , O , O ,	0.018/0.024	0.29/0.095	0.017/0.0052	32	>32	2	2

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3	170	$^{8N-1}$ determination except N=3 for durlobactam for which the average + standard deviation is shown 6 MIC of defined aviation in the absence of a RLI: A RC3627 > 64 ug/mL and
4	171	$-N-1$ determination, except $N-5$ for durinovaciani, for which the average T standard deviation is shown, "which is exposition of a BLI. ARC5027 > 04 µg/mL and ARC561 = 22 µg/mL \cdot SE coli (OXA 1: AmpC: CTX M 15: TEM 1): dK programping (OKP 6: AmpC: OXA 2: SHV 18) ATCC700602: NM: Non-monotorial
5	171	ARC301 – 32 μ g/mL, *2. con (OAA-1, AmpC, CTX-M-13, TEM-1), *A. preunomae (ORF-0, AmpC, OAA-2, SHV-16) ATCC700003, NM. Non-measurable.
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172 173 Table 2. β-Lactamase inhibition and antibacterial activity in combination with

174 cefpodoxime of R₁-substituted inhibitors

	R _{1/} , N O O O O O O O O O H	IC ₅₀ (μM) 10 min/60 min ^a			MIC (µg/mL) of BLI alone		MIC (µg/mL) of cefpodoxime in the presence of 4 µg/mL of BLI ^b	
ID	R ₁	E. coli TEM-1 (Class A)	P. aeruginosa AmpC (Class C)	K. pneumoniae OXA-48 (Class D)	E. coli ARC3627°	K. pneumoniae ARC561 ^d	E. coli ARC3627°	K. pneumoniae ARC561 ^d
5	H_2N	0.013/ 0.019	0.18/ 0.071	0.063/ 0.015	8	>32	1	2
7	0,0 H ₂ N ^{-S} N ^{-U} , s ⁵	0.048/ 0.057	0.022/ 0.0041	2.2/ 0.41	4	>32	<0.06	1
8	N N N N N N N N N N N N N N N N N N N	0.44/ 0.66	0.26/ 0.054	0.055/ 0.012	16	>32	>32	4
9	O N N N N N N N N N N N N N N N N N N N	0.018/ 0.026	0.7/ 0.13	0.4/ 0.087	>32	>32	16	4
10		0.017 /0.024	0.36/ 0.18	0.026/ 0.0073	16	>32	2	2
11	$H_2N_{S} N_{N} H_{N} N_{S} N_{N} H_{N} H$	0.055/ 0.083	0.053/ 0.014	2.0/ 0.42	2	>32	<0.06	0.125

<sup>aN=1 determination; bMIC of cefpodoxime in the absence of a BLI: ARC3627 > 64 μg/mL and ARC561 = 32 μg/mL; cE. coli
(OXA-1; AmpC; CTX-M-15; TEM-1); dK. pneumoniae (OKP-6; AmpC; OXA-2; SHV-18) ATCC700603; NM: Non-measurable.</sup>

The SAR around the acetate side chain was explored on the R₃-methyl scaffold (Table The des-fluoro and both monofluoro acetic acid analogs all showed similar 3). antibacterial activity in combination with cefpodoxime (MIC \leq 0.25 µg/mL, Table 3).

$^{3}_{4}$ 182 Compound 12 had low potency against AmpC (IC ₅₀ = 4.6 μ M at 60 mi	nutes).
⁶ 7 183 Unexpectedly, the position of the methyl group on the core (R_2 vs. R_3) had	a very
 10 11 184 significant effect on the activity of the monofluoro diastereomers, particularly with r 12 	espect
13 14 185 to the MICs. Compounds 4 and 5 (R_2 -methyl series, Table 1) showed a large diff	erence
16 17 18 186 in antibacterial potency, whereas 13 and 14 (R ₃ -methyl series, Table 3) were much 19	closer
 187 in biological activity (alone or in combination with cefpodoxime). This result clearly 22 	shows
 that the antibacterial activity from such structurally similar compounds will remain that the antibacterial activity from such structurally similar compounds will remain 	hard to
 27 28 189 predict, even with the most advanced computational chemistry and biology 29 	v tools
³⁰ ³¹ ₃₂ 190 available.	
34 35 36 191 37 38	
³⁹ ⁴⁰ 192 Table 3. β-Lactamase inhibition and antibacterial activity in combination with ⁴¹	
 193 cefpodoxime of R₃-methyl-substituted inhibitors 193 <l< td=""><td></td></l<>	
59 16 60 ACS Paragon Plus Environment	

			IC ₅₀ (μM) 10 min/60	min ^a	MIC (µg a	/mL) of BLI lone	MIC cefpode presence	μg/mL) of oxime in the of 4 μg/mL of BLI ^b
	ID	BLI structure	E. coli TEM-1 (Class A)	P. aeruginosa AmpC (Class C)	K. pneumoniae OXA-48 (Class D)	E. coli ARC3627°	K. pneumoniae ARC561 ^d	<i>E. coli</i> ARC3627°	K. pneumoniae ARC561 ^d
	12	H ₂ N N O OH	0.34/ 0.15	14/4.6	0.066/0.053	2	32	<0.06	0.125
	13	H ₂ N , F O O O O O O O	0.0023±0.0002/ 0.0032±0.0002	0.45±0.03/ 0.14±0.02	0.37±0.02/ 0.070±0.006	0.25	16	<0.06	0.25
	14	H ₂ N ^N , F OF OF	0.020/ 0.026	1.3/0.3	0.067/0.019	0.5	>32	<0.06	0.06
194 195 196 197	^a N=1 d absenc <i>pneur</i>	letermination exce ee of a BLI: ARC3(<i>moniae</i> (OKP-6; <i>F</i>	pt N=3 for 13 , for wh 627 > 64 μg/mL and AmpC; OXA-2; SH\	hich the average ± ARC561 = 32 μg/ /-18), ATCC7006	standard deviatic /mL; ^c <i>E. coli</i> (OXA 603; NM: Non-mea	on is shown; A-1; AmpC; C asurable.	^b MIC of cefpodo CTX-M-15; TEM	oxime in the I-1); ^d <i>K</i> .	
198	The	best analogs	from each se	ries were se	lected (4, 11,	13 and 1	4) for testin	g prodru	gs.
199	Seve	eral esters w	ere prepared a	and profiled	<i>in vitro</i> to as	sess thei	r aqueous s	stability a	nd
200	their	stability to	intestinal an	d liver este	erases, and	<i>in vivo</i> t	o measure	e their o	ral
201	bioa	vailability (Ta	able 4). The g	goal was to	identify an or	ally avail	able DBO a	acetate tl	nat
202	woul	ld be stable e	enough to read	ch the liver a	and subseque	ently to b	e efficiently	hydrolyz	ed
203	by c	arboxylester	ases ²³ to un	mask the a	ictive DBO a	acid prior	to tissue	distributio	on.
204	Carb	ooxylesterase	e CES-1 and C	ES-2 are th	e predomina	nt isoform	is found in	human liv	/er
				ACS Paragon P	17 Ilus Environment				

2 3 4 5	205	and intestinal tissues, respectively. In addition to tissue expression level differences,
6 7 8	206	these isoforms also demonstrate different substrate affinity SAR. ²⁴ Targeted conversion
9 10 11 12	207	in the liver during first-pass absorption and metabolism was a strategy we explored based
13 14 15 16	208	upon the success of other approved and marketed prodrugs which have utilized
17 18 19	209	conversion by liver (CES-1) carboxylesterase to maximize the production of active drug.
20 21 22 23	210	Interestingly prodrug conversion in the intestinal enterocyte by CES-2 has shown mixed
23 24 25 26	211	results with multiple mechanisms often required for optimum conversion and transfer of
27 28 29 30	212	active drug to systemic circulation. ^{24, 25}
31 32 33 34	213	Linear and branched alkyl esters of the R_2 -methyl series had relatively low bioavailability,
35 36 37	214	with the best bioavailability of 45% in rats obtained with ethyl ester 15 . Isopropyl ester 16
38 39 40 41	215	had similar bioavailability despite having greater aqueous stability as well as greater
42 43 44	216	stability to intestinal esterases ($t_{1/2} > 2$ h). Notably, the proxetil sidechain rendered the
45 46 47 48	217	ester 17 very unstable despite being the prodrug of choice for the marketed oral β -lactam
49 50 51	218	cefpodoxime. R_2 -methyl analogs with a modified R_1 had poor oral bioavailability, as
52 53 54 55	219	exemplified by compound 19 (F = 4%). In contrast, esters of the R_3 -methyl series (20-23)
56 57 58		18
59 60		ACS Paragon Plus Environment

2 3 4	220	had high oral bioavailabilities of 71 to 98%), which was independent of the chirality at
6 7 8	221	R_4/R_5 . Within the R_3 -methyl series, compound 23 showed markedly better stability than
9 10 11 12	222	closely related analogs 20, 21 and 22. While we do not fully understand the reasons for
12 13 14 15	223	this remarkable feature, we hypothesize that a unique conformation and orbital overlap is
16 17 18	224	adopted by compound 23. That conformation could translate into a differentiated steric
19 20 21 22	225	environment around the ester carbonyl and could influence the electrophilicity of the sp ²
23 24 25	226	carbon.
26 27 28 29 30	227	
32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57	228	Table 4. Stability data and rat oral bioavailability of ester prodrugs
50 59 60		19 ACS Paragon Plus Environment

2										
3 4				Half-life (min) Bioavailabi						Bioavailability
5 6 7		ID	Prodrug Structure	R_6	pH 7.4 buffer	Rat intestinal S9	Rat liver S9	Human intestinal S9	Human liver S9	%F (against IV AUC of corres- ponding acid)
8		15		Et	41	39	4.4	38	6.5	45
9 10		16	N F	<i>i</i> -Pr	236	233	14	132	16	38
10 11 12		17	$0 \qquad 0^{-1} \sum_{0}^{-1} R_{6}$	₽ ²⁵ 0 0	4.8	4.2	2	<1	1.7	11
13		18	H ₂ N ₂ N ₁ H ₁ N ₁	<i>n</i> -Hex	76	39	0.45	5.7	0.84	NT
14 15		19		Et	26	20	10	22	9.9	4
16 17		20		Et	22	18	2.4	6.9	2.4	71
18 19		21	H ₂ N ⁻ N - F - O - C - C - C - C - C - C - C - C - C	<i>i</i> -Pr	91	68	3	36	8.5	84
20		22	0 II	Et	44	42	20	35	27	75
21 22 23		23	H ₂ N ^N , F of C _{R6}	<i>i</i> -Pr	186	240	32	163	39	98
24 25	229									
29 30 31 32 33 34 35 36 37	230 231 232	At the profilin	e end of this SAR ng based on its h 317 in human live	exploratio high oral t er S9, and	n, comp pioavaila I the ab	oound 23 ability in a ility of the	(ETX rats, o e corr	0282) wa efficient o respondir	as selec conversi ng active	ted for further on <i>in vitro</i> to e BLI acid 13
38 39 40 41 42	233	(ETX	1317) to restore the	e antibacte	erial activ	vity of β-la	actam	S.		
43 44 45 46	234	<u>In vitr</u>	oprofiling of ETX1	<u>317</u>						
47 48 49 50	235	The s	pectrum of Class A	A, C and D	β-lactar	nase inhi	bition	by ETX1	317 was	evaluated by
51 52 53 54	236	meas	uring its ability to p	rotect the	β-lactan	nase subs	strate	nitrocefin	from de	gradation
54 55 56 57	237	using	previously describ	ed methoo	is . ¹³ ET	FX1317 sl	hows	potent inl	nibition o	of all serine β -
58 59						20				
60				AC	S Paragon	Plus Enviror	nment			

1 ว		
2 3 4 5	238	lactamases tested with k_{inact}/K_i acylation rate constants greater than 1.2 x 10 ⁴ M ⁻¹ s ⁻¹ for
6 7 8	239	Class A and C enzymes and greater than 6.8 x 10^2 M ⁻¹ s ⁻¹ for Class D enzymes (Table
9 10 11 12	240	5). ETX1317 is not only more potent than avibactam, but also broadens the activity to
13 14 15 16	241	include the large family of Class D OXA enzymes.
17 18 19 20	242	Table 5. Kinetic values of β -lactamase and penicillin-binding protein inhibition by
21 22 23	243	ETX1317
24 25 26		
26 27		
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β-lactamase	ETX1317 k _{inact} /K _i (M ⁻¹ s ⁻¹)	durlobactam k _{inact} /K _i (M ⁻¹ s ⁻¹)	avibactam k _{inact} /K _i (M ⁻¹ s ⁻¹)
	Class	А	
CTX-M-14	2.0 (± 0.2) x 10 ⁶	$2.2 (\pm 0.3) \ge 10^6$	1.81 (± 0.09) x 10
CTX-M-15	$4.8 (\pm 0.4) \ge 10^6$	$7 (\pm 2) \ge 10^6$	8 x 10 ⁵
KPC-2	$4.9 (\pm 0.2) \ge 10^4$	9.3 (± 0.6) x 10^5	6 x 10 ³
SHV-5	$3.2 \pm (0.1) \ge 10^6$	$6.4 (\pm 0.5) \ge 10^6$	1 x 10 ⁵
TEM-1	NV	$1.4 (\pm 0.6) \ge 10^7$	4 x 10 ⁵
	Class	C	
P. aeruginosa AmpC	1.20 (± 0.04) x 10 ⁴	9 (± 5) x 10 ⁵	3 x 10 ³
E. cloacae P99	$3.9 (\pm 0.2) \ge 10^4$	$2.3 (\pm 0.4) \ge 10^6$	8 x 10 ³
	Class	D	
OXA-10	$6.8 \pm (0.3) \ge 10^2$	9 (± 2) x 10 ³	7 x 10 ¹
OXA-23	$1.54 \pm (0.06) \ge 10^3$	$5.1 (\pm 0.2) \ge 10^3$	1 x 10 ²
OXA-24	$4.6 (\pm 0.2) \ge 10^3$	9 (± 2) x 10 ³	8 x 10 ¹
OXA-48	5.3 (± 0.2) x 10^4	8 (± 2) x 10 ⁵	5 x 10 ³
	Penicillin-bind	ing protein	
E. coli PBP2	9.3 (± 0.7) x 10 ³	1.7 (± 0.3) x 10 ⁴	240 ± 40
V: no value because the inhib	um of activity is neces	ssary for potent activ	vity against curre
Gram-negative clinic	al isolates, which ver	y often bear a wide used on its structure	variety of β-lacta e and mode of in
covalent binding to	active site serine),	ETX1317 is not ac	tive against Cla
actamases, as these	e enzymes use a meta	l ion in the active site	e to hydrolyze β-l
		22	
	ACC Daragon [

1 2		
2 3 4 5	250	Like durlobactam, ¹³ ETX1317 additionally inhibits <i>E. coli</i> penicillin-binding protein 2
6 7 8	251	(PBP2), with a k_{inact}/K_i acylation rate constant close to 10 ⁴ M ⁻¹ s ⁻¹ , a value very similar to
9 10 11 12	252	that of durlobactam and ~40 times more potent than avibactam.
13 14 15 16	253	The crystal structure of the ETX1317 and CTX-M-14 (Class A β -lactamase) complex was
17 18 19 20	254	solved at 1.28 Å resolution with a refined R_{work}/R_{free} of 0.1427/0.1625, offering a clear
21 22 23	255	depiction of the inhibitor's post-reaction binding mode (Figure 2A). ETX1317 retains
24 25 26 27	256	many of the chemical characteristics of other BLIs in the DBO class, including avibactam
28 29 30	257	and durlobactam, and therefore their binding poses are very similar (Figure 2B). 26
32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50	258	AFigure 2. ETX1317 acvl-enzyme complex with CTX-M-14 β-lactamase. (A) Complex
50 51 52	259	Figure 2. ETATST7 acyl-enzyme complex with CTA-IM-14 p-lactamase. (A) Complex
53 54 55	260	crystal structure of ETX1317 with CTX-M-14 β -lactamase determined at 1.28-Å resolution
56 57 58 59 60	261	(PDB ID: 6VHS). An unbiased F_o - F_c map, shown in green, is contoured at 3 σ . The ligand 23 ACS Paragon Plus Environment

2 3	262	and protein are shown in purple and blue, respectively. Hydrogen bonds between the
4 5 6	202	and protein are shown in purple and blue, respectively. Hydrogen bonds between the
7 8 9	263	ligand and protein are depicted as black dashed lines. The catalytic water is shown as a
10 11 12 13	264	red sphere. (B) Superimposition of ETX1317 with CTX-M-14 (purple/blue) and avibactam
14 15 16	265	with CTX-M-14 (yellow/orange, PDB ID: 6MZ2, showing only the major conformation of
17 18 19 20	266	avibactam).
21 22 23 24 25	267	
26 27 28	268	The cyclic urea of the diazabicyclooctane core reacts with the catalytic serine, Ser70,
29 30 31 32	269	forming a carbamoyl acyl-enzyme covalent linkage. Here, the carbonyl oxygen occupies
33 34 35	270	the oxyanion hole formed by the backbone amide groups of Ser237 and Ser70. Following
36 37 38 39	271	ring opening, ETX1317 adopts a half chair conformation due to the planarity of the C-C
40 41 42	272	double bond, allowing the methyl substituent to project upwards and form hydrophobic
43 44 45 46	273	interactions with Tyr105. In contrast, avibactam lacks a C-C double bond and methyl
47 48 49	274	group. Consequently, avibactam adopts a full chair conformation, and Tyr105 adopts an
50 51 52 53	275	alternative conformation where it moves closer to the piperidine ring. Like avibactam, the
53 54 55 56 57	276	carboxamide moiety of ETX1317 forms extensive hydrogen bonding interactions with
58 59		24
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1 2 3		
4 5 6	277	Asn132 of the SXN motif, Asn104, and the backbone carbonyl of Ser237. The
7 8 9	278	carboxamide and hydroxylamine substituents adopt a pseudo-axial orientation similar to
10 11 12	279	the one observed for other DBO / β -lactamase co-crystal structures. This unique
13 14 15 16	280	conformation in the active site has been hypothesized to facilitate the recyclization of the
17 18 19	281	urea and release of the active inhibitor from the enzyme. ²⁷ Indeed, ETX1317 was shown
20 21 22 23	282	to recyclize and be released intact from β -lactamases as detected by acylation exchange
24 25 26 27	283	from KPC-3 to OXA-48. ²⁸
28 29 30 31	284	Consistent with all known β -lactamase substrates and inhibitors, ETX1317 has an anionic
32 33 34	285	activating group, in this case a fluoroacetate group, that interacts with the highly
35 36 37 38	286	conserved KTG motif. The carboxylate of the fluoroacetate group forms hydrogen bonds
39 40 41	287	with Thr235 and Ser237, while the fluoride atom projects towards Lys234, Thr216, and
42 43 44	288	Thr235, and is within hydrogen bonding distance with Thr235 (2.7 Å), in a similar manner
45 46 47 48	289	to the third oxygen of the avibactam sulfate. This important interaction is further facilitated
49 50 51	290	by a hydrogen bond between Ser130 and the NH group of the hydroxylamine side chain,
52 53 54 55 56	291	which helps orient the flexible N-O side chain towards the electropositive KTG subpocket.
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1 2		
3 4 5	292	The unique conformation of the monofluoroacetate activating group could explain the
6 7 8 9	293	differences in potency between ETX1317 and its diastereomer (14), especially against
10 11 12 13	294	Class A β-lactamases.
14 15 16	295	ETX1317 displaces six water molecules from the active site upon binding, compared with
17 18 19 20	296	the apo CTX-M-14 structure (PDB ID: 4UA6). ²⁹ Interestingly, the catalytic water molecule
21 22 23	297	remains undisturbed and lies just 3 Å from the CTX-M-14-ETX1317 carbamate carbon.
24 25 26 27	298	Since ETX1317 remains covalently bound, this suggests that ETX1317 possesses
28 29 30	299	intrinsic hydrolytic stability, and/or perturbs the protonation state of the catalytic Glu166,
31 32 33 34	300	as has been suggested for avibactam. ^{26,27}
35 36 37 38 39	301	
40 41 42	302	The potency and spectrum of β -lactamase inhibition by ETX1317 supported further
43 44 45 46	303	antibacterial profiling in combination with β -lactams against relevant Gram-negative
47 48 49	304	clinical isolates. Approved penicillins and cephalosporins administered orally were
50 51 52 53	305	evaluated, not only using MIC as a criterion but also oral bioavailability, approved dose
54 55 56	306	levels and unbound plasma exposures. Cefpodoxime (CPD) emerged as the lead β -
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54 55 56	U
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 - 	307	lactam candidate. ³⁰ Its approved use as an orally bioavailable proxetil ester prodrug to
, ,	308	treat UTI infections was also aligned with our potential target product profile. A fixed 1:2
0 1 2	309	ratio of cefpodoxime:ETX1317 was utilized as the testing paradigm for antimicrobial
3 4 5	310	susceptibility. This was found to give MIC values consistent with the outcome of in vivo
6 7 8 9	311	antibacterial efficacy experiments. ³¹ The clinical isolates used in this study (Table 6)
0 1 2	312	were all highly resistant to CPD (MIC>64 $\mu g/mL),$ due to the expression of multiple $\beta\text{-}$
23 24 25 26	313	lactamases, including ESBLs and carbapenemases. This panel did not contain any
.7 .8 .9	314	strains with Class B metallo- β -lactamases, as DBOs do not inhibit those. ETX1317 itself
0 1 2 3	315	showed various levels of antibacterial activity (MIC from 0.25 to 64 $\mu\text{g/mL})$ due to the
4 5 6	316	inhibition of PBP2. The addition of ETX1317 to CPD restored its activity to \leq 0.5 µg/mL
7 8 9	317	across all Enterobacterales isolates tested, including the CRE strains (identified by * in
1 2 3	318	Table 6). These results demonstrate that inhibition by ETX1317 of a broad spectrum of
4 5 6 7	319	β -lactamases translates into potent antibacterial activity in combination with CPD against
8 9 0	320	MDR Enterobacterales clinical isolates.
1 2 3	321	
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8		27

322 Table 6. MIC values of ETX1317, cefpodoxime and the cefpodoxime:ETX1317 (1:2)

323 combination against a set of MDR *Enterobacterales* clinical isolates

S-rasion		β-lactamase genes encoded			MIC (µg/mL)			
Species	Strain ID	Class A	Class C	Class D	ETX1317	CPD	CPD:ETX1317#	
	ARC2687	CTX-M-14	AmpC		0.5	>64	0.25	
	ARC6059	LAP-2	AmpC, CMY-2		16	>64	0.125	
E. coli	ARC6064	CTX-M-15, TEM-40	AmpC		8	>64	0.5	
	ARC6077	CTX-M-15	AmpC	OXA-1	4	>64	0.125	
	ARC6078		AmpC, CMY-2		0.25	>64	0.25	
	ARC6082	CTX-M-2, SHV-11, TEM-1		OXA-9	64	>64	0.125	
K. pneumoniae	ARC6088*	CTX-M-15, SHV-11, TEM-1		OXA-1, OXA-48	16	>64	0.125	
	ARC6098	CTX-M-15, SHV-1	DHA-1	OXA-1	16	>64	0.125	
	ARC6107*	CTX-M-15, SHV-1, TEM-1		OXA-1, OXA-244	4	>64	0.25	
K. oxytoca	ARC5389*	OXY-1, KPC-2 , PER-2			16	>64	0.5	
C. freundii	ARC3518*	TEM-1, KPC-2	AmpC	OXA-1	0.25	>64	0.13	
E. cloacae	ARC6049	CTX-M-15, TEM-1	AmpC	OXA-1	1	>64	0.125	
	ARC6055	SHV-5, TEM-1	ACT variant		1	>64	0.125	

324
325*CRE (carbapenem-resistant *Enterobacterales*) strains express β-lactamases capable of hydrolyzing carbapenems, (highlighted
with bold font); #MIC value shown is that of the CPD component of the combination; CPD = cefpodoxime; CPD:ETX1317 =
cefpodoxime titrated with ETX1317 in a 1:2 ratio; *C. freundii = Citrobacter freundii*; *E. cloacae = Enterobacter cloacae*. All
MIC values are mode of at least three replicates.

In vivo profiling of oral prodrug ETX0282

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2 3 4 5	329
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30 31 32	337
33 34 35 36	338
37 38 39	339
40 41 42 43	340
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54 55 56	344
57 58 59 60	

329	The intravenous (IV) and oral (PO) pharmacokinetics of ETX1317 and its isopropyl ester
330	prodrug ETX0282 were determined in rats (N= 3 /arm). Pharmacokinetic parameters are
331	summarized in Table 7. Moderate clearance (CL) and a low volume of distribution (Vdss)
332	resulted in a short half-life of 0.4 h which is consistent with other members of the DBO
333	class. ¹³ Nearly 60% of the administered IV dose of ETX1317 was eliminated in the urine
334	as unchanged drug suggesting renal excretion was the predominant clearance
335	mechanism. Oral administration of ETX0282 at a 10 mg/kg equivalent dose of ETX1317
336	resulted in high exposure of ETX1317 (C_{max} = 5.8 µg/mL and AUC = 7.0 µg.h/mL) with no
337	circulating concentrations of ETX0282 observed suggesting rapid conversion of ETX0282
338	to ETX1317 and high bioavailability (F%) in this species. The metabolism of ETX0282
339	was studied in vitro and in vivo. In addition to ester prodrug cleavage of ETX0282 to yield
340	ETX1317, hydrolytic cleavage of the DBO core was observed to yield the diamine
341	metabolite of both ETX1317 and ETX0282 (Figure S1, Supplementary Information). The
342	diamine metabolite of ETX1317 was synthesized and used as a standard to quantify the
343	exposure of the metabolite in definitive toxicology studies. We also looked for evidence
344	of N-O bond cleavage to yield the mono-fluoro acetate and have not observed these
	29

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3	345	metabolite	es or poter	ntially as	sociated s	structures	s in both	<i>in vitro</i> and	in vivo pla	asma ai	nd
3	346	liver tissue	e samples	s. Rat p	rotein bin	ding of I	ETX1317	was low w	vith a mear	n fractio	on
3	347	unbound c	of 0.91 acr	oss a co	ncentratio	n range o	of 5 to 10	0 µM (Table	e 7). Protei	n bindiı	ng
3	348	of ETX028	32 was not	t determi	ned due to	o its limite	ed relevai	nce.			
3	349										
3	350										
3	351	Table 7. P	harmacok	inetic val	lues of ET	X0282/E	TX1317 i	n rats			
			Dose/Route	Comer	AUC	Tup	Vd	CI	Ronal CI	F(%)	PPB (%
		Compound	(mg/kg)	(μg/mL)	(μg*h/mL)	(h)	(L/kg)	(mL/min/kg)	(mL/min/kg)		unbound)
		ETX1317	(mg/kg) 10/IV	(μg/mL) 17.5±2.0	(μg*h/mL) 7.19±0.34	(h) 0.4±0.02	(L/kg)	(mL/min/kg) 23.1±1.1	(mL/min/kg) 13.4±0.3	N/A	unbound) 91±7
0		ETX1317 ETX0282	(mg/kg) 10/IV 11.6/PO ^a	(μg/mL) 17.5±2.0 5.8±0.16	(μg*h/mL) 7.19±0.34 7.04±0.62	(h) 0.4±0.02 1.1±0.3	(L/kg) 0.70±0.01 N/A	(mL/min/kg) 23.1±1.1 N/A	(mL/min/kg) 13.4±0.3 N/A	N/A 98	unbound) 91±7 ND
3	352	ETX1317 ETX0282 ^a Equivalent to	(mg/kg) 10/IV 11.6/PO ^a 0 10 mg/kg ET	(μg/mL) 17.5±2.0 5.8±0.16	(μg*h/mL) 7.19±0.34 7.04±0.62 e; C _{max} : peak	(h) 0.4±0.02 1.1±0.3	(L/kg) 0.70±0.01 N/A on; AUC: are	(mL/min/kg) 23.1±1.1 N/A ea under the cu	(mL/min/kg) 13.4±0.3 N/A rve; T _{1/2} : Appa	N/A 98 rent half-li	unbound) 91±7 ND
3	352 353	ETX1317 ETX0282 ^a Equivalent to based upon li	(mg/kg) 10/IV 11.6/PO ^a 10 mg/kg ET near regressio	(μg/mL) 17.5±2.0 5.8±0.16	$\frac{(\mu g^*h/mL)}{7.19\pm0.34}$ 7.04 ±0.62 e; C _{max} : peak og plot of cor	(h) 0.4±0.02 1.1±0.3 concentration-f	(L/kg) 0.70±0.01 N/A on; AUC: are	(mL/min/kg) 23.1±1.1 N/A ea under the cu	(mL/min/kg) 13.4±0.3 N/A rve; T _{1/2} : Appar dss: volume of	N/A 98 rent half-li distributic	unbound) 91±7 ND fe
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360	of ETX0282 and cefpodoxime proxetil (CPDP) is for the treatment of complicated urinary
361	tract infections (cUTI) the murine neutropenic thigh model is the standard model utilized
362	in exposure-efficacy PK/PD analysis of tissue and non-tissue based infections. ³³
363	Achievement of PK/PD exposure endpoints determined in this model have been shown
364	to correlate to clinical success. ³⁴ The isolate ARC2687 is resistant to fluoroquinolones
365	(MIC > 4 μ g/mL for levofloxacin) and cephalosporins (MIC > 64 μ g/mL for cefpodoxime),
366	but susceptible to meropenem (MIC = 0.03 μ g/mL) and the CPD:ETX1317 combination
367	(MIC = 0.25 μ g/mL). For all arms, three CD-1 female mice were inoculated in each thigh
368	resulting in 2 datapoints per animal. At 24 h, the vehicle control showed a large increase
369	in colony-forming unit (CFU) count compared to the initial inoculum (+4.26 log(CFU/g))
370	confirming the virulence of this isolate (Figure 3). As expected based on its MIC, CPDP
371	alone (PO, q6h) was not efficacious, whereas meropenem was (-1.46 log(CFU/g)
372	compared to pretreatment). Upon oral dosing of the combination of CPDP and ETX0282
373	every 6 hours, a significant decrease in CFU count was observed for all three doses of
374	the BLI. A reduction of 1.11 log(CFU/g) was obtained with a dose of 50 mg/kg and 100
375	mg/kg (PO, q6h) of CPDP and ETX0282, respectively.
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3 4 5	384	The oral efficacy of this combination has also been demonstrated in a murine ascending
6 7 8	385	pyelonephritis (cUTI) infection model where cidal activity was observed at much lower
9 10 11 12	386	doses, consistent with high urinary excretion of both ETX1317 and CPD. 35
13 14 15 16	387	The safety of ETX0282 was investigated with a 14-day toxicology study under Good
17 18 19	388	Laboratory Practice (GLP) in rats and dogs following daily oral administration. The doses
20 21 22	389	of 500 mg/kg/day and 400 mg/kg/day were established as the NOAELs (No Observed
23 24 25 26	390	Adverse Effect Level) for rats and dogs, respectively. These results support continuing
27 28 29	391	development of ETX0282. Phase 1 clinical trials in healthy volunteers showed that
30 31 32 33	392	ETX0282 was generally well-tolerated, either alone or in combination with cefpodoxime
34 35 36	393	proxetil, with no serious adverse events reported (ClinicalTrials.gov Identifier:
37 38 39 40	394	NCT03491748).
41 42 43 44	395	
45 46 47	396	CHEMICAL SYNTHESIS
48 49 50	397	The ester and carboxylic acid analogs were all synthesized in a similar manner from the
52 53 54	398	corresponding substituted, enantiopure hydroxyureas (Scheme 1). ³⁶ The esters were
55 56 57	399	obtained by alkylation of the hydroxy group with the appropriate bromo acetates. When
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413	ethyl bromofluoroacetate by saponification, followed by chiral salt resolution using (S)-1-
414	phenylethanamine. The chiral salt was then re-esterified using isopropanol for 26 and
415	ethanol for 27 to give the (R)-bromofluoroacetates in 79% and 50% yield, respectively.
416	The key intermediate to the synthesis of ETX0282 and ETX1317 was the hydroxyurea
417	37 , which was prepared in ten steps from commercially available material. ³⁶ Ethyl 2-
418	oxoacetate and (S)-2-methylpropane-2-sulfinamide were condensed to afford the chiral
419	imine 28. An Aza-Diels Alder reaction with isoprene, followed by deprotection of the <i>t</i> -
420	butylsulfinyl and subsequent Boc protection, gave compound 30 in 43% yield for three
421	steps and in 99.6% ee. Saponification of the ester followed by amide coupling afforded
422	compound 32 in 62% yield, 99.4% ee. At this stage, many strategies were developed to
423	install the hydroxylamine functionality in a regio and stereoselective manner. While the
424	$S_N 2$ reaction on a chiral alcohol intermediate gave the right product, we identified the
425	nitroso ene reaction ³⁷ as the most efficient transformation. As hypothesized from the
426	concerted nature of its mechanism and the orientation of the primary amide and Boc
427	group, the fully regio- and stereoselective nitroso ene reaction of alkene 32, with N-Boc-
428	hydroxylamine gave compound 33 in a single step in 40% yield. This chiral intermediate
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2 3 4 5	429	was then protected with a TBS group, and Boc removal using zinc bromide gave
6 7 8	430	compound 35 . Cyclization of the diamine with triphosgene provided the corresponding
9 10 11 12	431	cyclic urea, and the TBS was deprotected with HF-pyridine to give key intermediate 37 .
13 14 15	432	ETX0282 and 22 were prepared from the hydroxyurea 37 by alkylation with intermediates
17 18 19	433	26 and 27, respectively. ETX1317 was prepared in 63% yield from 22 by saponification
20 21 22	434	with lithium hydroxide.
23 24 25 26 27	435	
28 29 30 31	436	Scheme 2. Synthesis of ETX0282 and ETX1317. (A) Preparation of the chiral
32 33 34	437	bromofluoroacetates. (B) Stereoselective synthesis of ETX1317 and its ester prodrug
36 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59	438	ETX0282.
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	444	of durlobactam, and the ester prodrug ETX0282 provides high oral bioavailability.
	445	Cefpodoxime proxetil is an orally bioavailable cephalosporin approved for treatment of a
0 1 2	446	variety of bacterial infections. Its clinical utility is currently limited by β -lactamase-
3 4 5	447	mediated resistance. In vitro, ETX1317 restores cefpodoxime's antimicrobial activity
6 7 8 9	448	against a variety of pathogens, including Enterobacterales resistant to fluoroquinolones,
0 1 2	449	cephalosporins and carbapenems. The combination of their orally bioavailable prodrugs,
3 4 5 6	450	ETX0282 and cefpodoxime proxetil, respectively, was efficacious in murine infection
7 8 9	451	models and had favorable tolerability in preclinical safety studies. ETX0282CPDP, the
0 1 2 3	452	combination of ETX0282 and cefpodoxime proxetil, is currently being developed for the
4 5 6	453	treatment of infections caused by Enterobacterales, including multidrug-resistant (MDR)
7 8 9 0	454	and carbapenem-resistant Enterobacterales (CRE). By providing the option of an
1 2 3	455	effective course of oral antibiotic treatment, ETX0282CPDP has the potential to benefit
4 5 6 7	456	patients as well as the healthcare systems by reducing the risk of nosocomial infections
8 9 0	457	and avoiding the healthcare costs associated with hospitalizations. Moreover, if potent,
1 2 3 4	458	orally available β -lactams with activity against non-fermenter Gram-negative species
5 6 7	459	(Pseudomonas aeruginosa and Acinetobacter baumannii, for example) become
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available, ETX0282 could be an oral BLI partner to treat infections due to these highly

461 resistant and problematic pathogens.

463 EXPERIMENTAL SECTION

464 Chemical Synthesis. General Methods, Reagents and Materials

of the solvents and reagents used were obtained commercially and used as received unless 1 otherwise. ¹H and ¹³C NMR spectra were recorded in CDCl₃, D₂O or DMSO- d_6 solutions 0 K using a Brucker Ultrashield 300 MHz instrument. Chemical shifts are reported as parts million relative to tetramethylsilane (TMS) (0.00) for ¹H and ¹³C NMR. Silica gel natographies were performed on ISCO Combiflash Companion Instruments using ISCO Sep Flash Cartridges (particle size: 35-70 microns) or Silicycle SilicaSep Flash Cartridges cle size: 40-63 microns). Reverse phase chromatography was performed on ISCO biflash Rf 200 Instruments using RediSep High Performance Gold C18 and C18Aq columns. arative reverse phase HPLC was carried out using a Gilson instrument. When not indicated, bound intermediates and reagents were purchased from chemical supply houses. All rospray ionization mass spectra (ESI-MS) were recorded via reverse phase UPLC-MS with a ers Acquity UPLC instrument with diode array and electrospray ionization detectors, a UPLC T3, 2.1 x 30 mm, 1.8 µm column and a gradient of 2 to 98% acetonitrile in water with 0.1% ic acid over 2.0 minutes at 1 mL/min. Injection volume was 1 µL and the column temperature 30 °C. Detection was based on electrospray ionization (ESI) in positive and negative polarity Waters ZQ mass spectrometer (Milford, MA, USA), diode-array UV detector from 210 to nm, and evaporative light scattering detector (Cedex 75, Sedere, Alfortville, France). Purities nal compounds were determined using a Waters Acquity UPLC instrument with diode array etor, a UPLC ACE Excel 2 C18-AR 50 x 4.6 mm, 2 µM column and a gradient of 5% to 95% onitrile in water with 0.1% formic acid over 6.0 minutes at 1 mL/min. Injection volume was and the column temperature was 30 °C. All compounds were isolated with \geq 95% purity ss otherwise noted.

488 <u>ethyl (*S,E*)-2-((tert-butylsulfinyl)imino)acetate (28)</u>

1 2		
- 3 4 5	489	To a solution of ethyl 2-oxoacetate (66.0 mL, 321 mmol, 50% in toluene) in DCM (1 L) at
6 7 8 9	490	0 °C was added (S)-2-methylpropane-2-sulfinamide (30.0 g, 248 mmol) and molecular
10 11 12	491	sieves (4Å, 500 g). The resulting solution was stirred at room temperature for 18 hours.
13 14 15 16	492	Molecular sieves were removed by filtration. Filtrate was concentrated by distillation under
17 18 19	493	vacuum to give a crude product, which was purified by flash silica chromatography (0%
20 21 22 23 24	494	to 5% EtOAc in petroleum ether) to give a colorless oil, 45.0 g, 88%.
25 26 27 28	495	¹ H NMR (400 MHz, CDCl ₃ - <i>α</i>) δ: 1.28 (s, 9H), 1.39 (t, 3H, <i>J</i> = 12 Hz), 4.38 (q, 2H, <i>J</i> = 12
29 30 31 32 33	496	Hz), 8.01 (s, 1H).
34 35 36 37 38	497	ethyl (S)-1-((S)-tert-butylsulfinyl)-4-methyl-1,2,3,6-tetrahydropyridine-2-carboxylate (29)
39 40 41 42 42	498	To a solution of (<i>S,E</i>)-ethyl 2-(tert-butylsulfinylimino)acetate (28, 50.0 g, 244 mmol) in
43 44 45 46	499	DCM (600 mL), at -78°C was added isoprene (97.2 mL, 972 mmol), followed by addition
47 48 49	500	of TMSOTf (97.4 mL, 417 mmol). The resulting solution was stirred at -78°C for 3 hours
50 51 52 53	501	and quenched slowly at -78°C with phosphate buffer solution (pH = 7.4, 1 L). After
54 55 56	502	warming to room temperature, the mixture was extracted with DCM (3 x 500 mL). The
57 58 59 60		40 ACS Paragon Plus Environment

3 4 5	503	combined organic extracts were washed with water (2 x 500 mL) and brine. The organic
6 7 8 9	504	layer was dried over Na_2SO_4 , filtered and evaporated to afford 60.0 g of crude product as
10 11 12 13 14	505	a brown oil. The product was used in the next step without further purification.
15 16 17	506	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 1.08(s, 9H), 1.18 (t, 3H, <i>J</i> = 12 Hz), 1.55 (m, 2H), 1.64
19 20 21	507	(s, 3H), 2.15 (m, 2H), 3.55 (m, 1H), 4.11 (dq, 2H, <i>J</i> = 2, 4 Hz), 5.37 (m, 1H).
22 23 24 25 26 27	508	MS (ESI ⁺) [M+H] ⁺ = 274 (C ₁₃ H ₂₃ NO ₃ S)
28 29 30 31 32 33	509	<u>1-(tert-butyl) 2-ethyl (S)-4-methyl-3,6-dihydropyridine-1,2(2H)-dicarboxylate (30)</u>
34 35 36 37	510	To a solution of the crude (S)-ethyl 1-((S)-tert-butylsulfinyl)-4-methyl-1,2,3,6-
38 39 40 41	511	tetrahydropyridine-2-carboxylate (29, 100 g, 366 mmol) in MeOH (1.00 L) at 0 °C was
42 43 44	512	added hydrogen chloride (100 mL, 4M in dioxane, 400 mmol). The resulting solution was
45 46 47 48	513	stirred at room temperature for 18 hours. MeOH and HCI/dioxane were removed by
49 50 51	514	distillation under vacuum to give a crude product, which was dissolved in water (1.00 L)
52 53 54 55	515	and extracted with EtOAc (3 x 500 mL). The pH of the aqueous solution was adjusted to
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516	7 with solid NaHCO $_3$. The aqueous was extracted with EtOAc until LCMS showed no
517	product detected. The organic phases were combined and dried over Na_2SO_4 , filtered
518	and concentrated to afford crude product (30.0 g, 177 mmol) as a light-yellow oil. The oil
519	was dissolved in THF (500 mL) and cooled by ice-water bath. To the cooled solution was
520	added a solution of sodium bicarbonate (22.3 g, 266 mmol) in water (500 mL), followed
521	by di-tert-butyl dicarbonate (57.8 g, 266 mmol). The resulting solution was stirred at room
522	temperature for 18 hours. The two layers were separated. The aqueous layer was
523	extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 ,
524	filtered and evaporated. Crude product was purified by flash silica chromatography (0%-
525	30% EtOAc in PE) to afford the title compound 47.5 g, 43% yield, 99.6% ee from 28 .
526	¹ H NMR (400 MHz, DMSO- <i>d_δ</i>) δ: 1.12 (t, 3H), 1.40 (m, 9H), 1.63 (s, 3H), 2.34 (m, 2H),
527	3.58 (m, 1H), 3.87 (m, 1H), 4.08 (m, 2H), 4.80 (m, 1H), 5.34 (m, 1H).
528	MS (ESI+) [M+Na]+ = 292 (C ₁₄ H ₂₃ NO ₄ Na)
529	tert-butyl (S)-2-carbamoyl-4-methyl-3,6-dihydropyridine-1(2H)-carboxylate (31)
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2 3 4	530	To a solution of 1-(tert-butyl) 2-ethyl (<i>S</i>)-4-methyl-3,6-dihydropyridine-1,2(2H)-
5 6 7 8	531	dicarboxylate (30 , 47.5 g, 176 mmol) in THF (1000 mL) and water (500 mL) at 0 °C was
9 10 11 12	532	added dropwise lithium hydroxide (1 M, 440 mL, 440 mmol). The reaction mixture was
13 14 15 16	533	warmed to room temperature and stirred for 16 hours. Solvent was removed; residue was
17 18 19	534	diluted with water. The pH of the solution was adjusted to \sim 3 with HCl (1N) solution. The
20 21 22 23	535	mixture was extracted with EtOAc (3 x 300 mL). Organic layers were combined, washed
24 25 26	536	with water and brine, dried over MgSO ₄ , filtered and concentrated to give a colorless oil
27 28 29 30 31	537	(40.3 g).
32 33 34 35	538	¹ H NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 1.38 (m, 9H), 1.64 (s, 3H), 2.36 (m, 2H), 3.60 (m, 1H),
36 37 38 39	539	3.89 (m, 1H), 4.75 (m, 1H), 5.35 (dd, 1H, J=3, 15 Hz), 12.73 (s, 1H).
40 41 42 43 44 45 46	540	MS (ESI+) [M+Na]+ = 264 (C ₁₂ H ₁₉ NO ₄ Na)
47 48 49 50 51 52 53 54 55 56 57	541	tert-butyl (S)-2-carbamoyl-4-methyl-3,6-dihydropyridine-1(2H)-carboxylate (32)
58 59 60		43 ACS Paragon Plus Environment

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1 2		
3 4 5	542	To a solution of (S) -1-(tert-butoxycarbonyl)-4-methyl-1,2,3,6-tetrahydropyridine-2-
6 7 8 9	543	carboxylic acid (31, 40.3 g, 167 mmol) in THF (500 mL) at 0 °C was added N,N'-
10 11 12	544	carbonyldiimidazole (32.5 g, 201 mmol) in portions. The crude was stirred at 0 $^\circ C$ for 5
13 14 15	545	hours. Then ammonium acetate (38.2 g, 503 mmol) was added. The reaction was stirred
17 18 19	546	at room temperature for an additional 18 hours, quenched with water and extracted with
20 21 22	547	EtOAc. The combined organic layers were washed with water and brine, dried over
23 24 25 26	548	Na ₂ SO ₄ , filtered and concentrated. Crude product was purified by flash silica
27 28 29	549	chromatography (0%-30% EtOAc in PE) to give a white solid, 25.0 g, 62% yield, 99.4%
30 31 32 33 34 35	550	ee.
36 37 38 30	551	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 1.41 (s, 9H), 1.66 (s, 3H), 2.35 (s, 2H), 3.84 (m, 2H),
40 41 42 43	552	4.66 (m, 1H), 5.35 (m, 1H), 6.96 (s, 1H), 7.19 (s, 1H).
44 45 46 47 48 49	553	MS (ESI ⁺) [M+Na] ⁺ = 263 (C ₁₂ H ₂₀ N ₂ O ₃ Na)
50 51 52	554	tert-butyl (3R,6S)-3-((tert-butoxycarbonyl)(hydroxy)amino)-6-carbamoyl-4-methyl-3,6-
53 54 55 56	555	dihydropyridine-1(2H)-carboxylate (33)
57 58		4.4
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3 4 5	556	To a solution of tert-butyl (S)-2-carbamoyl-4-methyl-3,6-dihydropyridine-1(2H)-
6 7 8 0	557	carboxylate (32 , 25.0 g, 104 mmol) in DCM (250 mL) was added BocNHOH (70.6 g, 531
9 10 11 12	558	mmol), CuCl (6.10 g, 62.5 mmol) and pyridine (107 mL, 1.30 mmol). The resulting solution
13 14 15	559	was stirred at room temperature for 44 hours under oxygen. The solids were removed by
16 17 18 19	560	filtration. The filtrate was washed with water (6 x 500 mL) and brine, dried over Na_2SO_4 ,
20 21 22	561	filtered and concentrated. The crude product was purified by flash silica chromatography
23 24 25 26	562	(0%-50% EtOAc in PE) to give the title compound as a white solid, 40% yield. Starting
27 28 29	563	material was recovered (10 g). The same procedure was repeated three times to afford
30 31 32 33	564	15.0 g of product in total.
34 35 36 37 38 39	565	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 1.34-1.42 (m, 18H), 1.68 (s, 3H), 3.31 (dd, <i>J</i> = 4.4, 13.6
40 41 42	566	Hz, 1H), 3.92 (dd, J = 13.6, 48.8 Hz, 1H), 4.21 (d, J = 78 Hz, 1H), 4.61 (d, J = 72.4 Hz,
43 44 45 46 47	567	1H), 5.69 (d, <i>J</i> = 42 Hz, 1H), 6.98 (s, 1H), 7.40 (s, 1H), 8.77 (d, <i>J</i> = 62.4 Hz, 1H).
48 49 50 51 52 53 54 55	568	MS (ESI+) [M+Na]+ = 394 (C ₁₇ H ₂₉ N ₃ O ₆ Na)
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1 2		
- 3 4 5	569	tert-butyl (3R,6S)-3-((tert-butoxycarbonyl)((tert-butyldimethylsilyl)oxy)amino)-6-
6 7 8 9 10	570	carbamoyl-4-methyl-3,6-dihydropyridine-1(2H)-carboxylate (34)
11 12 13 14	571	To a solution of tert-butyl (<i>3R,6S</i>)-3-((tert-butoxycarbonyl)(hydroxy)amino)-6-carbamoyl-
15 16 17 18	572	4-methyl-3,6-dihydropyridine-1(2H)-carboxylate (33, 12.0 g, 32.3 mmol) in DCM (96 mL)
19 20 21	573	at 0 $\pm 5^{\circ}$ C was added imidazole (4.40 g, 64.6 mmol). The resulting solution was stirred at
22 23 24	574	room temperature for 10 mins, then TBS-CI (4.80 g, 32.3 mmol) in DCM (10 mL) was
25 26 27 28	575	added dropwise. The reaction mixture was stirred at 0°C for an additional 18 hours,
29 30 31	576	washed with water and brine, dried over Na_2SO_4 , filtered and concentrated. The crude
32 33 34 35	577	product was purified by flash silica chromatography (0%-20% EtOAc in PE) to afford the
36 37 38 39 40	578	title compound as a white solid, 10.0 g, 63%.
41 42 43 44	579	¹ H NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 0.08-0.12 (m, 6H), 0.84 (s, 9H), 1.31-1.40 (m, 18H),
45 46 47	580	1.75 (s, 3H), 3.20-3.29 (m, 1H), 4.00-4.15 (m, 2H), 4.59 (d, J = 25.8 Hz, 1H), 5.69 (d, J =
48 49 50 51 52 53	581	13.8 Hz, 1H), 7.01 (d, <i>J</i> = 15.3 Hz, 1H), 7.49 (s, 1H).
54 55 56	582	MS (ESI ⁺) [M+H] ⁺ : 486 (C ₂₃ H ₄₃ N ₃ O ₆ Si)
57 58 59 60		46 ACS Paragon Plus Environment

1 2		
3 4 5	583	(2S,5R)-5-(((tert-butyldimethylsilyl)oxy)amino)-4-methyl-1,2,5,6-tetrahydropyridine-2-
6 7 8	584	carboxamide (35)
9 10 11 12	505	
13 14 15	585	Io a solution of tert-butyl (<i>3R,6S</i>)-3-[tert-butoxycarbonyl-[tert-butyl(dimethyl)silyl]oxy-
16 17 18	586	amino]-6-carbamoyl-4-methyl-3,6-dihydro-2H-pyridine-1-carboxylate (34, 21.7 g, 44.7
19 20 21	587	mmol) in DCM (250 mL) at 0 $^\circ\text{C}$ was added zinc bromide (40.2 g, 179 mmol). The resulting
22 23 24	588	suspension was allowed to warm to room temperature and stir ~66 hours. The reaction
25 26 27 28	589	mixture was cooled by ice-water bath, to which a slurry of NaHCO $_3$ (38.23 g, 10
29 30 31	590	equivalents) in water (300 mL) was added. The resulting mixture was stirred for 1 hr. Solid
32 33 34 35	591	was removed by filtration and washed 3-4 times with DCM until no product was detected
36 37 38	592	from the rinsing solution. The two layers from the filtrate were separated. The aqueous
39 40 41 42	593	layer was extracted with DCM three times (until no product was detected from aqueous
43 44 45	594	layer). The combined DCM solution was concentrated to remove most of the solvent. The
46 47 48 49	595	residue was partially dissolved in 10% MeOH in DCM and was loaded onto a short silica
50 51 52	596	gel pad and eluted with 10% MeOH in DCM. The filtrate was evaporated and dried under
53 54 55	597	vacuum to give a yellow foam solid (crude 9.9 g, 77%).
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3 4 5	598	¹ H NMR (300 MHz, CDCl ₃ - <i>d</i> ₁) δ: 0.00 (s, 6H), 0.80 (s, 9H), 1.71-1.72 (m, 3H), 2.55 (dd, <i>J</i>
6 7 8	599	= 13.8 Hz, 3.0 Hz, 1H), 2.54 (dd, J = 3.0, 10.8, 1H), 3.12 (dd, J = 1.8, 13.8 1H), 2.91 (s,
9 10 11 12	600	1H), 3.69 (d, J= 1.2 Hz, 1H), 4.95 (bs, 1H), 5.50 (s, 1H), 5.92 (d, J= 1.5 Hz, 1H), 7.15 (s,
13 14 15 16	601	1H).
17 18 19 20 21 22 23	602	MS (ESI+) [M+H]+: 286 (C ₁₃ H ₂₇ N ₃ O ₂ Si)
24 25 26	603	(2S,5R)-6-((tert-butyldimethylsilyl)oxy)-4-methyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-3-ene-
27 28 29 30 21	604	2-carboxamide (36)
32 33 34 35 36	605	To a clear solution of (<i>3R,6S</i>)-3-[[tert-butyl(dimethyl)silyl]oxyamino]-4-methyl-1,2,3,6-
37 38 39	606	tetrahydropyridine-6-carboxamide (35, 7.66 g, 26.8 mmol) in MeCN (150 mL) and DCM
40 41 42 43	607	(200 mL) at 0 $^\circ\text{C}$ was added N,N'-diisopropylethylamine (19.1 mL, 107 mmol) followed by
44 45 46	608	a solution of triphosgene (2.71 g, 9.12 mmol) in MeCN (50 mL) dropwise (2 mL/hour by
47 48 49 50	609	a syringe pump). After addition, the solution was allowed to warm to room temperature
51 52 53	610	and stirred overnight. The reaction mixture was concentrated to dryness. The resulting
54 55 56 57	611	residue was diluted with EtOAc and washed with brine. The aqueous layer was extracted
58 59 60		48 ACS Paragon Plus Environment

3 4 5	612	with EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered and
6 7 8 9	613	concentrated. Crude product was purified by silica gel chromatography (0%-100% EtOAc/
10 11 12 13 14	614	hexane) to give the title compound as a white solid 4.36 g, 52%.
15 16 17 18	615	¹ H NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 0.12 (s, 3H), 0.14 (s, 3H), 0.90 (s, 9H), 1.78 (s, 3H),
19 20 21	616	3.19 (m, 2H), 3.57 (s, 1H), 4.12 (q, J = 2.1 Hz, 1H), 5.47 (s, 1H), 7.32 (s, 1H), 7.51 (s,
22 23 24	617	1H).
23 26 27 28 29 30 31	618	MS (ESI+) [M+H]+: 312 (C ₁₄ H ₂₅ N ₃ O ₃ Si)
32 33 34 35 36 37	619	(2S,5R)-6-hydroxy-4-methyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-3-ene-2-carboxamide (37)
38 39 40 41	620	To a solution of (<i>2S,5R</i>)-5-(((tert-butyldimethylsilyl)oxy)amino)-4-methyl-1,2,5,6-
42 43 44 45 46 47 48 49 50 51	621	tetrahydropyridine-2-carboxamide (36 , 214 mg, 0.690 mmol) in ethyl acetate (5 mL) at 0
	622	°C was added HF-pyridine (0.070 mL, 2.75 mmol). The reaction mixture was warmed to
	623	room temperature and stirred for 2 hrs. The reaction mixture was concentrated to afford
52 53 54	624	title compound as a tan solid, 135 mg (100%).
55 56 57 58		
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2 3 4 5	625	¹ H NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 1.79 (m, 3H), 3.16 (m, 2H), 3.54 (m, 1H), 4.05-4.09 (m,
6 7 8	626	1H), 5.41-5.43 (m, 1H), 7.27 (s, 1H), 7.49 (s, 1H), 9.60 (s, 1H).
9 10 11 12 13 14 15	627	MS (ESI ⁺) [M+H] ⁺ : 198(C ₈ H ₁₁ N ₃ O ₃)
16 17 18 19 20 21	628	<u>2-bromo-2-fluoroacetic acid (24)</u>
22 23 24 25	629	To a 50 L reactor at 0-5 °C was charged a solution of ethyl 2-bromo-2-fluoroacetate (3.5
26 27 28	630	kg) in tetrahydrofuran (7L, 2V) and a solution of sodium hydroxide (830 g) in water (7L,
29 30 31 32	631	2V) dropwise over 1 hour. The resulting solution was stirred at 0-5 $^\circ$ C for 1 hour. HCl (160
33 34 35	632	mL) was added dropwise at 0-5 °C. Water and tetrahydrofuran were removed by
36 37 38 39	633	concentration under vacuum. The residue was suspended in tetrahydrofuran (35 L, 10V)
40 41 42	634	and conc. HCl (1.57 L, 1.0 equiv.) was added dropwise. Anhydrous sodium sulfate was
43 44 45 46	635	added, and the resulting mixture was stirred for 2 hours. The solid was filtered off and
47 48 49	636	washed with THF (1L x 2). The filtrate was concentrated under vacuum to give 2-bromo-
50 51 52 53	637	2-fluoroacetic acid (2.2 kg) as a yellow oil, which was combined with a previous batch
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3 4 5	638	made by the same method (940 g, Purity: 72%) and distilled in vacuum (65-70 $^\circ$ C 100 Pa)
6 7 8	639	to give 2-bromo-2-fluoroacetic acid (2.55 kg, total yield 67%) as a colorless oil.
9 10 11 12 13 14 15 16	640	¹ Η NMR (400 MHz, CDCl ₃ - <i>d</i>) δ: 6.66 (d, 1H, <i>J</i> = 68 Hz), 11.15 (s, 1H).
17 18 19 20 21	641	(S)-1-phenylethan-1-amine (R)-2-bromo-2-fluoroacetate (25)
22 23 24 25	642	To a 10 L reactor at 0-5 °C was charged a solution of 2-bromo-2-fluoroacetic acid (24,
26 27 28 20	643	2.0 kg) in 1 L of chloroform (1V), to which, a solution of (S)-1-phenylethanamine (1.4 kg)
30 31 32	644	in 1 L of chloroform (1V) was added dropwise. The mixture was stirred at room
33 34 35	645	temperature overnight and the resulting white solid was collected by filtration to give a
37 38 39	646	salt of (S)-1-phenylethanamine 2-bromo-2-fluoroacetate (2.5 kg; ee: 6%), which was
40 41 42	647	charged into a 10 L reactor, followed by addition of chloroform (5L, 2V). The resulting
43 44 45 46	648	mixture was stirred for 2 hours at 50 °C (solid was partially dissolved in chloroform),
47 48 49	649	cooled to 0 $^\circ\text{C},$ and was allowed to stand for 2 hours. Solid was collected by filtration, and
50 51 52 53	650	washed with cooled chloroform (500 mL, 0.2V). The recrystallization procedure was
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2 3 4	651	repeated 4 times to afford 1.09 kg (97% ee) of the title compound as a white solid with an
5 6 7 8	652	overall yield of 31% (2 steps).
10 11 12 13	653	¹ H NMR (400 MHz, CD ₃ OD <i>-d</i> ₄) δ: 1.65 (d, <i>J</i> = 6.8 Hz, 3H), 4.47 (q, <i>J</i> = 6.8 Hz, 1H), 6.51
15 16 17 18	654	(d, <i>J</i> _{H-F} = 53.2 Hz, 1H), 7.40-7.50 (m, 5H).
20 21 22 23 24	655	pentan-3-yl (<i>R</i>)-2-bromo-2-fluoroacetate (26)
25 26 27 28	656	To a 2 L reactor at room temperature was charged (S)-1-phenylethanamine (R)-2-bromo-
29 30 31 32	657	2-fluoroacetate (25, 450 g), dichloromethane (900 mL, 2V) and iPrOH (2.0 equiv.).
33 34 35 36	658	Chlorotrimethylsilane (1.12 L) was added slowly, and a white precipitate was formed. The
37 38 39	659	resulting mixture was stirred at room temperature overnight. White precipitate was filtered
40 41 42	660	off, and the filter cake was washed with hexane (450 mL, 1V). The combined filtrate was
43 44 45 46	661	washed with water (3x100 mL). Organic solution was dried with anhydrous sodium
47 48 49	662	sulfate, filtered, concentrated under vacuum. Residue was distilled (54-60°C, 100 Pa) to
50 51 52 53	663	give the title compound as a colorless oil (290 g, 79% yield, 95% purity, 97.2% ee).
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1 2 3 4 5 6	664	¹ H NMR (400 MHz, CDCl ₃ - <i>d</i>) δ: 1.32 (m, 6H), 5.17 (m, 1H), 6.53 (d, 1H, <i>J</i> = 51.2 Hz).
7 8 9 10 11 12	665	ethyl (<i>R</i>)-2-bromo-2-fluoroacetate (27)
13 14 15 16	666	Into a 50 mL 3-necked round-bottom flask, purged and maintained with an inert
17 18 19	667	atmosphere of nitrogen, was placed (1R)-1-phenylethan-1-amine; (2S)-2-bromo-2-
20 21 22 23	668	fluoroacetic acid (25, 30.0 g, 108 mmol) and ethanol (34.7 g, 755 mmol). This was
24 25 26	669	followed by the addition of chlorotrimethylsilane (82.0 g, 755 mmol) dropwise with stirring
27 28 29 30	670	at room temperature. The resulting solution was stirred for 4 h at room temperature, then
31 32 33	671	quenched by the addition of 10 mL of water/ice. The resulting solution was extracted with
34 35 36 37	672	3 times 20 mL of petroleum ether (30-60 degree) and the organic layers combined. The
38 39 40	673	resulting mixture was washed with 2 times 20 mL of brine. The mixture was dried over
41 42 43	674	anhydrous sodium sulfate, filtered and concentrated. The residue was applied onto a
44 45 46 47	675	silica gel column with petroleum ether (30-60 degree). This resulted in 10.0 g (50%) of
48 49 50 51 52	676	the title compound as a colorless oil.
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677	¹ H NMR (300 MHz, CDCl ₃ - <i>d</i>) δ:1.38 (t, 3H, <i>J</i> = 6 Hz), 4.38 (q, 2H, <i>J</i> = 6 Hz), 6.58 (d, 1H,
678	J= 51 Hz).
679	(2R)-isopropyl 2-(((2S,5R)-2-carbamoyl-4-methyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-3-en-
680	<u>6-yl)oxy)-2-fluoroacetate (23, ETX0282)</u>
681	To a solution of (2S,5R)-6-hydroxy-4-methyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-3-ene-2-
682	carboxamide (37 , 582 mg, 2.95 mmol) in 1,4-dioxane (16 mL) and DMF (2 mL) was added
683	isopropyl (2R)-2-bromo-2-fluoro-acetate (26, 881 mg, 4.43 mmol). The reaction mixture
684	was cooled to 0 $^\circ\text{C}$ and DBU (0.440 mL, 2.95 mmol) was added dropwise. The reaction
685	mixture was stirred for 10 minutes, then diluted with ethyl acetate and washed with 1:1
686	brine water twice. The organics were dried over magnesium sulfate, filtered and
687	concentrated. Silica gel chromatography (0%-90% ethyl acetate/hexanes) afforded a
688	white foam. The foam was dissolved in 1:1 acetonitrile:water, frozen and lyophilized to
689	afford a white solid, 614 mg, 66% yield, 99.4% de. HPLC purity: 98% (220nm).
690	Optical rotation: -3.71 (acetone, c = 1.25 g/dL)
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	677 678 679 680 681 682 683 684 685 685 686 685 685 685 685 685 685

1 2 3 4 5 6	691	MS (ESI ⁺) [M+H] ⁺ : 316 (C ₁₃ H ₁₈ FN ₃ O ₅)
 7 8 9 10 11 12 13 14 15 16 17 	692	HRMS (ESI+): (m/z) calculated for $C_{13}H_{19}FN_3O_5$ [M+H] ⁺ 316.1303, found 316.1313
	693	¹ H NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 1.24 (m, 6H), 1.82 (t, 3H, <i>J</i> = 1.79 Hz), 3.21 (m, 1H),
17 18 19	694	3.33 (m, 1H), 3.95 (d, 1H, J = 2.1 Hz), 4.22 (m, 1H), 5.01 (m, 1H), 5.52 (m, 1H), 6.15-6.33
20 21 22 23 24	695	(d, 1H, <i>J =</i> 55.8 Hz), 7.32 (s, 1H), 7.55 (s, 1H).
25 26 27 28 29	696	¹³ C NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 21.62, 21.76, 23.00, 46.49, 62.56, 64.16, 70.66,
30 31 32 33	697	104.37, 107.49, 120.98, 138.69, 162.63, 163.07, 169.45, 169.90.
34 35 36 37 38	698	ethyl (2R)-2-[[(2S,5R)-2-carbamoyl-4-methyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-3-en-6-
 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 	699	<u>yl]oxy]-2-fluoro-acetate (22)</u>
	700	The title compound was prepared from (37 , 215 mg, 1.09 mmol) and ethyl (<i>2R</i>)-2-bromo-
	701	2-fluoro-acetate (27, 342.9 mg, 1.85 mmol) according to the procedure for 23, ETX0282.
	702	HPLC purity: 96% (diode array detection).
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1 2 3 4 5	703	MS (ESI ⁺) [M+H] ⁺ : 302 (C ₁₂ H ₁₆ FN ₃ O ₅)
6 7 8 9 10 11	704	HRMS (ESI+): (m/z) calculated for $C_{12}H_{17}FN_3O_5$ [M+H] ⁺ 302.1147, found 302.1151
12 13 14 15 16	705	¹ H NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 1.21 (t, 3H, <i>J</i> = 7.2 Hz), 1.82 (m, 3H), 3.19 (m, 1H),
17 18 19	706	3.29 (m, 1H), 3.95 (d, 1H, J= 1.8 Hz), 4.23 (m, 1H), 4.24 (m, 2H), 5.52 (m, 1H), 6.19-6.37
20 21 22 23 24	707	(d, 1H, J= 56.1 Hz), 7.35 (br, 1H), 7.58 (br, 1H).
25 26 27 28 29	708	(2R)-2-(((2S,5R)-2-carbamoyl-4-methyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-3-en-6-yl)oxy)-
30 31 32 33 34	709	2-fluoroacetic acid lithium salt (13, ETX1317)
35 36 37 38	710	To a solution of (2R)-ethyl 2-(((2S,5R)-2-carbamoyl-4-methyl-7-oxo-1,6-diazabicyclo-
39 40 41	711	[3.2.1]oct-3-en-6-yl)oxy)-2-fluoroacetate (22, 107 mg, 0.36 mmol) in THF (3 mL) and
42 43 44 45	712	water (1 mL) at 0 $^\circ\text{C}$ was added 1M lithium hydroxide (0.360 mL, 0.360 mmol). The
46 47 48	713	reaction mixture was stirred at 0 $^\circ\text{C}$ for 10 minutes. Another 0.2 equiv. of lithium hydroxide
49 50 51	714	were added. After 10 minutes, the reaction mixture was adjusted to $pH = 7$ with 0.5N
52 53 54 55 56	715	HCI. The THF was evaporated and the remaining aqueous was frozen and lyophilized to
57 58 59 60		56 ACS Paragon Plus Environment

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3 4 5	716	afford a pale yellow solid. Reverse phase HPLC (YMC Carotenoid C30, 19 mm x 150
7 8 9	717	mm, 5 μm coupled with Synergi Polar RP 21.2 mm x 100 mm, 4 μm , 0%-40% acetonitrile
10 11 12	718	in water, 20 mL/min, 15 min) afforded the title compound as a white solid after
13 14 15 16 17 18	719	lyophilization, 64.6 mg, 63%. HPLC purity: 96% (220nm).
19 20 21 22 23 24	720	Optical rotation: -3.64 (water, c = 1.23 g/dL)
25 26 27 28 29	721	MS (ESI ⁺) [M+H]+: 274 (C ₁₀ H ₁₂ FN ₃ O ₅)
 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 52 	722	HRMS (ESI+): (m/z) calculated for $C_{10}H_{13}FN_3O_5$ [M+H] ⁺ 274.0834, found 274.0823
	723	¹ H NMR (300 MHz, DMSO- d_6) δ : 1.83 (m, 3H), 3.21 (m, 2H), 3.91 (d, 1H, J = 2.7 Hz),
	724	4.16 (m, 1H), 5.19-5.41 (d, 1H, J = 65.4 Hz), 5.44 (m, 1H), 7.26 (br, 1H), 7.52 (br, 1H).
	725	¹³ C NMR (300 MHz, DMSO- <i>d</i> ₆) δ: 22.34, 46.94, 62.64, 63.59, 106.43, 109.62, 117.77,
	726	140.66, 169.01, 169.37, 171.20, 173.39.
54 55 56 57	727	Enzyme Inhibition Assays
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	728	The methods used to measure inhibition of β -lactamases were the same as those
	729	described in the supplement to Durand-Reville, <i>et al.</i> ¹³ Reaction progress curves at 490
) 2	730	nm for hydrolysis of the chromogenic β -lactamase substrate nitrocefin (SynGene,
3 4 5	731	Bangalore, India) at 100 μM were measured for a range of inhibitor concentrations in 0.1
5 7 3 9	732	M sodium phosphate (pH 7.0), 10 mM NaHCO ₃ , and 0.005% Triton X-100 at ambient
) 2	733	temperature using a Spectramax absorbance plate reader (Molecular Devices,
3 4 5 5	734	Sunnyvale, CA). The set of 12 progress curves were fit globally to a 2nd-order kinetic
7 3 9	735	model of enzyme inactivation. The 10-min and 60-min time points on the set of best-fit
) 2 3	736	curves were used to calculate the $\rm IC_{50}$ by nonlinear regression to the Hill equation: %
4 5 5	737	inhibition = $100[I]^n/(IC50^n + [I]^n)$, where [I] is the inhibitor concentration and n is the Hill
7 3 9)	738	coefficient. The second-order inactivation rate constant k_{inact}/K_i was calculated from the
1 2 3	739	set of progress curves using Global Kinetic Explorer (Kintek) as previously described. ¹³
4 5		
5 7 3	740	Inhibition of <i>E. coli</i> PBP2 was measured according to the method published in Shapiro et
) 2	741	al. 38 A set of 2-fold serial dilutions of each inhibitor from 61.44 to 0.06 and 0 μM were
3 1 5 5	742	prepared from a fresh solution of the compound dissolved in assay buffer consisting of
7 3		59
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1 2		
3 4 5	743	0.1 M sodium phosphate (pH 6.0) and 0.01% Triton X-100. Inhibitor solution (2 $\mu L)$ and 2
6 7 8	744	μL of 90 nM 5-TAMRA-ampicillin were added to the wells of a low-volume, shallow-well,
9 10 11 12	745	black polystyrene, 384-well microplate (Corning). Reactions were initiated by addition of
13 14 15	746	2 µL of 300 nM <i>E. coli</i> PBP2. Reactions were followed by the change in fluorescence
16 17 18 19	747	anisotropy. The second-order inactivation rate constant k_{inact}/K_i was calculated from the
20 21 22 23	748	set of 12 progress curves using Global Kinetic Explorer.
23 24 25 26 27 28 29	749	Antimicrobial Susceptibility Testing
30 31 32 33	750	The broth microdilution susceptibility testing was conducted according to CLSI ³⁹ using
34 35 36 37	751	CAMHB. Durlobactam and avibactam were synthesized at Entasis Therapeutics.
38 39 40	752	Cefpodoxime (catalog # J66225) was purchased from Alfa Aesar. The minimal inhibitory
41 42 43 44	753	concentration (MIC) of cefpodoxime combined with ETX1317 against MDR
45 46 47	754	Enterobacterales clinical isolates was tested by titrating two-fold dilutions of the
48 49 50 51 52	755	combination in a fixed 1:2 weight ratio.
53 54 55 56 57 58 59	756	Crystallization Experiments and Structure Determination
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3 4 5	757	CTX-M-14 was purified as previously described. ⁴⁰ Apo crystals of CTX-M-14 were grown
6 7 8 9	758	in a hanging drop apparatus consisting of a 1:1 mixture of 20 mg•mL ⁻¹ CTX-M-14 and 1.2
10 11 12	759	M potassium phosphate pH 7.9 at 20°C. Apo crystals were transferred into a
13 14 15 16	760	crystallization solution containing 3 mM ETX1317, for 15 minutes, then cryoprotected with
17 18 19	761	30% sucrose and 1.8M potassium phosphate (pH 7.9) and flash frozen in liquid nitrogen.
20 21 22 23	762	Data was collected on the SERCAT-22ID beamline at the Advanced Photon Source
24 25 26	763	(APS) in Argonne, IL, and processed with <i>iMOSFLM</i> . ⁴¹ The CCP4 versions of Scala and
27 28 29 30	764	REFMAC were used for scaling and refinement. ^{42,43} All model building was performed
31 32 33 34 35	765	with COOT ⁴⁴ and figures 2A and 2B were generated with PyMol (Schrödinger, LLC).
36 37 38 39	766	In vitro DMPK Experiments
40 41 42 43	767	Liver and intestinal S9 sub-cellular fractions (Xenotech) from rat and human tissue were
44 45 46 47	768	diluted to a protein concentration of 0.8 mg/mL in 100 mM potassium phosphate buffer,
48 49 50	769	pH 7.4 and pre-incubated in a 37°C water bath for 5 minutes prior to addition of 10 μM
51 52 53 54	770	(final) of compound of interest. Serial aliquots were removed at 0, 2, 5, 10, 20, 40, and
55 56 57	771	60 minutes and quenched in acetonitrile with internal standard prior to LC/MS/MS 30 to
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5 4 5	772	determine concentrations of prodrug. First order degradation half-lives were determined
0 7 8 9	773	from the slope of the log-linear plots of the depletion data.
10 11 12 13	774	Buffer stability (potassium phosphate buffer, pH 7.4) was evaluated using the same
14 15 16 17	775	protocol but without adding the S9 sub-cellular fractions.
18 19 20 21 22	776	In vivo Pharmacokinetics and Pharmacology Experiments
23 24 25 26	777	All in vivo procedures were completed in compliance with the Animal Welfare Act
27 28 29	778	Regulations (9 CFR 3) under Entasis-reviewed IACUC protocols and under the
30 31 32 33	779	supervision of a site attending veterinarian.
34 35 36 37	780	Intravenous pharmacokinetics of ETX1317 were investigated in jugular vein-cannulated
38 39 40	781	male Sprague Dawley rats (Charles River Laboratories, 180-200 g, n = 3/route) at a dose
41 42 43 44	782	of 10 mg/kg formulated in 0.9% saline. Oral pharmacokinetics of ETX0282 were
45 46 47	783	evaluated at a 10 mg/kg equivalent dose of ETX1317 formulated in 25:75 PEG 400:WFI.
48 49 50 51	784	The pH of each formulation was verified to fall within a range of 4-7, and compounds were
52 53 54	785	confirmed to be in solution prior to dosing. Potency was verified by LC/MS/MS. Dose
55 56 57 58	786	volumes were 5 and 10 mL/kg for ETX1317(IV) and ETX0282(PO), respectively. Blood
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787 samples to be processed for plasma using K_2EDTA as an anticoagulant were taken at 0.08, 0.17, 788 0.25, 0.5, 1, 2, 4, 8 hours (IV) and 0.25, 0.5, 1, 2, 4, 8, and 24 hours (PO) post-dose in rats. Blood 789 samples collected into 0.5-mL BD microtainers (Becton, Dickinson and Company) containing 1.0 790 mg K₂EDTA were centrifuged in a microfuge for 10 minutes. Plasma was transferred to 96-well 791 cryo tubes, and stored at -80°C prior to analysis. 792 In vivo neutropenic infection models in the mouse were conducted as previously 793 described. ^{32,45} All procedures were performed to Entasis-approved IACUC policies and 794 guidelines as well as OLAW standards. Briefly, female CD-1 mice (N=3 per arm, 20-22 795 g, Charles River Laboratories) were acclimated for 5 days prior to start of study. Animals 796 were housed 3 per cage with free access to food and water. Mice were rendered 797 neutropenic via two doses of cyclophosphamide on days -4 and -1 with 150 mg/kg and 798 100 mg/kg delivered intraperitoneally in a dose volume of 10 mL/kg, respectively. E. coli 799 isolate ARC2687 was prepared for infection from an overnight plate culture. A portion of 800 the plate was resuspended in sterile saline and adjusted to an OD of 0.1 at 625 nm. The 801 adjusted bacterial suspension was further diluted to target an infecting inoculum of 802 approximately 5.0 x 10⁶ - 1.0 x 10⁷ CFU/mouse. The actual inoculum size varied between 803 $5.5 \times 10^6 - 1.6 \times 10^7$ CFU/thigh and was administered via intramuscular injection of 100

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- 3 4 5	804	µL. Plate counts of the inoculum were performed to confirm inoculum concentration.
6 7 8	805	Treatment was initiated 2 hours after bacterial challenge. ETX0282 and CPDP were
9 10 11 12	806	formulated in 0.5% HPMC/0.1% Tween 80 suspension. Meropenem was dissolved in
13 14 15 16	807	water for injection. The <i>in vivo</i> efficacy study used meropenem dosed at 600 mg/kg q6h
17 18 19	808	subcutaneously as positive control. For dose arms utilizing a combination of ETX0282
20 21 22 23	809	and CPDP, both agents were reconstituted in the same vehicle. All dose concentrations
24 25 26	810	were adjusted to deliver targeted mg/kg doses within a dose volume of 10 mL/kg.
27 28 29 30	811	Formulation potency was verified by LC/MS/MS. ³⁰ ETX0282 and CPDP were orally
31 32 33	812	administered via q6h dose intervals in order to achieve targeted unbound exposures. At
34 35 36 37	813	24 hours post initiation of therapy, animals were euthanized, and thighs (2 separate
38 39 40	814	samples/animal) were aseptically collected and homogenized in 1 mL of sterile saline.
41 42 43	815	Bacterial colony enumeration of tissue homogenate was performed by serial dilution on
44 45 46 47	816	tryptic soy agar (TSA) plates, which were incubated overnight at 35°C prior to colony-
48 49 50 51	817	forming unit (CFU) counting.
52 53 54 55 56 57	818	
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2 3 4 5 6 7	819	ASSOCIATED CONTENT
7 8 9 10 11	820	Supporting Information
12 13 14 15	821	The Supporting Information is available free of charge on the ACS Publications website
16 17 18	822	at DOI: TBD.
19 20 21 22 23	823	Synthesis and characterization (NMR, LC/MS, HPLC, HRMS) of intermediates and final
24 25 26	824	compounds, synthetic schemes (Schemes S1 and S2), crystallographic data collection
27 28 29	825	and refinement statistics (Table S1), LC/MS/MS conditions (Table S2) and metabolism
31 32 33	826	pathways (Figure S1) (PDF)
34 35 36 37 38	827	Molecular Formula Strings (Supporting Information for Publication)
39 40 41 42	828	
43 44 45 46 47 48 49 50 51 52 53 54 55 56	829	Accession Codes
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3 4 5	830	The coordinates and structure factors of ETX1317 acyl-enzyme complex with CTX-M-14
7 8 9	831	β -lactamase (PDB code: 6VHS) has been deposited with the Protein Data Bank. Authors
10 11 12 13 14	832	will release the atomic coordinates and experimental data upon article publication.
15 16 17 18 19 20	833	
21 22 23 24 25	834	AUTHOR INFORMATION
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20 21 22 23 24 25	871	assistance with CTX-M-14 crystallization.
26 27 28 29 30	872	
31 32 33 34 35 36	873	ABBREVIATIONS USED
37 38 39 40	874	BOC, tert-butyloxycarbonyl; CDC, centers for disease control and prevention; DMPK,
40 41 42 43	875	drug metabolism and pharmacokinetics; IACUC, institutional animal care and use
44 45 46 47	876	committee; MS, mass spectrometry; NMR, nuclear magnetic resonance; OLAW, office of
48 49 50	877	laboratory animal welfare; PE, petroleum ether; SAR, structure-activity relationship; TBS,
51 52 53	878	tert-butyldimethylsilyl; TPSA, topological polar surface area; UPLC, ultra-performance
55 56 57	879	liquid chromatography; WHO, world health organization.
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1 2 3 4 5 6	880	
7 8 9 10 11	881	REFERENCES
12 13	882	1. Antibacterial Agents in Clinical Development: an Analysis of the Antibacterial
14	883	<i>Clinical Development Pipeline;</i> World Health Organization: Geneva, Switzerland, 2019.
16	884	2. Global Priority List of Antibiotic-resistant Bacteria to guide Research, Discovery
17 18	885	and Development of New Antibiotics; World Health Organization: Geneva, Switzerland,
19 20	886	2017.
21	887	3. Antibiotic Resistance Threats in the United States; Centers for Disease Control
22	888	and Prevention, US Department of Health and Human Services: Atlanta, Georgia, 2019.
24 25	889	4. Bush, K.; Bradford, P. A. β-lactams and β-lactamase inhibitors: an overview. <i>Cold</i>
26 27	890	<i>Spring Harb. Perspect. Med.</i> 2016, 6, a025247. DOI: 10.1101/cshperspect.a025247.
28	891	5. Reading, C.; Cole, M. Clavulanic acid: a β -lactamase-inhibiting β -lactam from
29 30 31 32 33 34 35 26	892	Streptomyces clavuligerus. Antimicrob. Ag. Chemother. 1977, 11, 852-857.
	893	6. Drawz, S. M.; Papp-Wallace, K. M.; Bonomo, R. A. New β-lactamase inhibitors: a
	894	therapeutic renaissance in an MDR world. Antimicrob. Ag. Chemother. 2014, 58, 1835-
	895	1846.
36 37	896	7. Shirley, M. Ceftazidime-avibactam: A review in the treatment of serious Gram-
38 39	897	negative bacterial Infections. Drugs 2018, 78, 675-692.
40 41	898	8. Zhanel, G. G.; Lawrence, C. K.; Adam, H.; Schweizer, F.; Zelenitsky, S.; Zhanel,
42	899	M.; Lagace-Wiens, P. R. S.; Walkty, A.; Denisuik, A.; Golden, A.; Gin, A. S.; Hoban, D.
43 44	900	J.; Lynch, J. P., 3rd; Karlowsky, J. A. Imipenem-relebactam and meropenem-
45 46	901	vaborbactam: two novel carbapenem-β-lactamase inhibitor combinations. Drugs 2018,
47 48	902	78, 65-98.
49	903	9. Zowawi, H. M.; Harris, P. N.; Roberts, M. J.; Tambyah, P. A.; Schembri, M. A.;
50 51	904	Pezzani, M. D.; Williamson, D. A.; Paterson, D. L. The emerging threat of multidrug-
52 53	905	resistant Gram-negative bacteria in urology. Nature reviews. Urology 2015, 12, 570-
54 55	906	584.
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907 Bush, K. A resurgence of β-lactamase inhibitor combinations effective against 10. 908 multidrug-resistant Gram-negative pathogens. Internat. J. Antimicrob. Ag. 2015, 46, 909 483-493. 910 11. Bush, K.; Bradford, P. A. Epidemiology of β -lactamase-producing pathogens. 911 *Clin. Microbiol. Rev.* 2020, 33, e00047-19. DOI: 10.1128/CMR.00047-19. 912 12. Coleman, K. Diazabicyclooctanes (DBOs): a potent new class of non- β -lactam β -913 lactamase inhibitors. Curr. Opin. Microbiol. 2011, 14, 550-555. 914 13. Durand-Reville, T. F.; Guler, S.; Comita-Prevoir, J.; Chen, B.; Bifulco, N.; Huynh, 915 H.; Lahiri, S.; Shapiro, A. B.; McLeod, S. M.; Carter, N. M.; Moussa, S. H.; Velez-Vega, 916 C.: Olivier, N. B.: McLaughlin, R.: Gao, N.: Thresher, J.: Palmer, T.: Andrews, B.: 917 Giacobbe, R. A.; Newman, J. V.; Ehmann, D. E.; de Jonge, B.; O'Donnell, J.; Mueller, J. 918 P.; Tommasi, R. A.; Miller, A. A. ETX2514 is a broad-spectrum β-lactamase inhibitor for 919 the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter* 920 *baumannii. Nature Microbiol.* **2017**, 2, e17104. DOI: 10.1038/nmicrobiol.2017.104. 921 Merdian, H.; Rangaraju, M.; Tarral, A. Safety and pharmacokinetics of single and 14. 922 multiple ascending doses of avibactam alone and in combination with ceftazidime in 923 healthy male volunteers: results of two randomized, placebo-controlled studies. Clin. 924 Drug Invest. 2015, 35, 307-317. 925 15. Gordon, E. M.; Duncton, M. A. J.; Gallop, M. A. Orally Absorbed Derivatives of 926 the β-lactamase inhibitor avibactam. Design of novel prodrugs of sulfate-containing 927 drugs. J. Med. Chem. 2018, 61, 10340-10344.

⁴⁰ 928 16. Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Järvinen, T.;
 ⁴² 929 Savolainen, J. Prodrugs: design and clinical applications. *Nature Rev. Drug Disc.* 2008,
 ⁴³ 930 7, 255-270.

931 17. Wolfenden, R.; Yuan, Y. Monoalkyl sulfates as alkylating agents in water,
 932 alkylsulfatase rate enhancements, and the "energy-rich" nature of sulfate half-esters.
 933 *Proc. Nat. Acad. Sci.USA* 2007, 104, 83-86.

934 18. Burns, C. J.; Trout, R.; Zulli, A.; Mesaros, E.; Jackson, R.; Boyd, S.; Liu, B.;
 935 McLaughlin, L.; Chatwin, C.; Hamrick, J.; Daigle, D.; Pevear, D. Discovery of VNRX ⁵⁴ 936 7145: A Broad-Spectrum Orally Bioavailable β-lactamase inhibitor (BLI) for Highly

1 2		
3 4 5 6	937	Resistant Bacterial Infections ("Superbugs"). Poster MEDI259 at ACS Spring 2019
	938	National Meeting & Exposition, Orlando, FL, 2019.
0 7	939	19. Hecker, S. J.; Reddy, K. R.; Lomovskaya, O.; Griffith, D. C.; Rubio-Aparicio, D.;
8 9	940	Nelson, K.; Tsivkovski, R.; Sun, D.; Sabet, M.; Tarazi, Z.; Parkinson, J.; Totrov, M.;
10 11	941	Boyer, S. H.; Glinka, T. W.; Pemberton, O. A.; Chen, Y.; Dudley, M. N. Discovery of
12	942	cyclic boronic acid QPX7728, an ultra broad-spectrum inhibitor of serine and metallo- β -
14	943	lactamases. <i>J. Med. Chem.</i> 2020. DOI: 10.1021/acs.jmedchem.9b01976.
15 16	944	20. Tondi, D.; Venturelli, A.; Bonnet, R.; Pozzi, C.; Shoichet, B. K.; Costi, M. P.
17 18	945	Targeting class A and C serine β -lactamases with a broad-spectrum boronic acid
19	946	derivative. <i>J. Med. Chem.</i> 2014, 57, 5449-5458.
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35	947	21. Stachyra, T.; Pechereau, M. C.; Bruneau, J. M.; Claudon, M.; Frere, J. M.;
	948	Miossec, C.; Coleman, K.; Black, M. T. Mechanistic studies of the inactivation of TEM-1
	949	and P99 by NXL104, a novel non- β-lactam β-lactamase inhibitor. <i>Antimicrob. Ag.</i>
	950	<i>Chemother.</i> 2010 , 54, 5132-5138.
	951	22. Brown, D. G.; May-Dracka, T. L.; Gagnon, M. M.; Tommasi, R. Trends and
	952	exceptions of physical properties on antibacterial activity for Gram-positive and Gram-
	953	negative pathogens. <i>J. Med. Chem.</i> 2014, 57, 10144-10161
	954	23. Laizure, S. C.; Herring, V.; Hu, Z.; Witbrodt, K.; Parker, R. B. The role of human
	955	carboxylesterases in drug metabolism: have we overlooked their importance?
36 37	956	<i>Pharmacother.</i> 2013 , 33, 210-222.
38 39	957	24. Imai, T. Human carboxylesterase isozymes: catalytic properties and rational drug
40 41	958	design. Drug Metabol. Pharmacokinet. 2006, 21, 173-185.
42	959	25. Chanteux, H.; Van Bambeke, F.; Mingeot-Leclercq, MP.; Tulkens, P. M.
43 44	960	Accumulation and oriented transport of ampicillin in Caco-2 cells from its
45 46	961	pivaloyloxymethylester prodrug, Pivampicillin. Antimicrob. Ag. Chemother. 2005, 49,
47 48	962	1279-1288.
49	963	26. Pemberton, O. A.; Noor, R. E.; Kumar, M. V. V.; Sanishvili, R.; Kemp, M. T.;
50 51	964	Kearns, F. L.; Woodcock, H. L.; Gelis, I.; Chen, Y. Mechanism of proton transfer in class
52 53	965	A β-lactamase catalysis and inhibition by avibactam. Proc. Nat. Acad. Sci.USA 2020,
54 55	966	117, 5818-5825.
56		
57 58		- 4
2		
---	-----	--
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23	967	27. Lahiri, S. D.; Mangani, S.; Durand-Reville, T.; Benvenuti, M.; De Luca, F.;
	968	Sanyal, G.; Docquier, J. D. Structural insight into potent broad-spectrum inhibition with
	969	reversible recyclization mechanism: avibactam in complex with CTX-M-15 and
	970	Pseudomonas aeruginosa AmpC β-lactamases. Antimicrob. Ag. Chemother. 2013, 57,
	971	2496-2505.
	972	28. Shapiro, A. B.; Moussa, S. H.; Carter, N. M.; Gao, N.; Miller, A. A. Cefpodoxime-
	973	ETX1317 Susceptibility is Unaffected by Ceftazidime-Avibactam Resistance Mutations
	974	V240G, D179Y and D179Y/T243M in KPC-3 β -lactamase, Poster AAR-715 at ASM
	975	Microbe; San Francisco, CA, 2019.
	976	29. Nichols, D. A.; Hargis, J. C.; Sanishvili, R.; Jaishankar, P.; Defrees, K.; Smith, E.
	977	W.; Wang, K. K.; Prati, F.; Renslo, A. R.; Woodcock, H. L.; Chen, Y. Ligand-induced
	978	proton transfer and low-barrier hydrogen bond revealed by X-ray crystallography. J.
24 25	979	Amer. Chem. Soc. 2015, 137, 8086-8095.
26 27 28	980	30. O'Donnell, J.; Tanudra, A.; Chen, A.; Hines, D.; Tommasi, R.; Mueller, J.
	981	Pharmacokinetic/pharmacodynamic determination and preclinical pharmacokinetics of
29 30	982	the β -lactamase inhibitor ETX1317 and its orally available prodrug ETX0282. ACS
31 32 33 34	983	<i>Infect. Dis.</i> 2020 , 6, 1378-1388.
	984	31. Miller, A. A.; Shapiro, A. B.; McLeod, S. M.; Carter, N. M.; Moussa, S. H.;
35	985	Tommasi, R.; Mueller, J. P. In vitro characterization of ETX1317, a broad-spectrum β -
36 37	986	Lactamase inhibitor that restores and enhances β -lactam activity against multidrug-
38 39	987	resistant Enterobacterales, including carbapenem-resistant strains. ACS Infect. Dis.
40	988	2020 , 6, 1389-1397.
41	989	32. Gerber, A. U.; Craig, W. A.; Brugger, HP.; Feller, C.; Vastola, A. P.; Brandel, J.
43 44	990	Impact of dosing intervals on activity of gentamicin and ticarcillin against Pseudomonas
45 46	991	aeruginosa in granulocytopenic mice. J. Infect. Dis. 1983, 147, 910-917.
47	992	33. Bulitta, J. B.; Hope, W. W.; Eakin, A. E.; Guina, T.; Tam, V. H.; Louie, A.;
48 49	993	Drusano, G. L.; Hoover, J. L. Generating robust and informative nonclinical in vitro and
50 51	994	in vivo bacterial infection model efficacy data to support translation to humans.
52 53	995	Antimicrob. Ag. Chemother. 2019, 63, e02307-18. DOI: 10.1128/AAC.02307-18.
54		
55 56		
57 58		
59		12

60

1

Page 73 of 75

60

1 2		
3 4 5 6 7	996	34. Ambrose, P. G.; Bhavnani, S. M.; Rubino, C. M.; Louie, A.; Gumbo, T.; Forrest,
	997	A.; Drusano, G. L. Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's
	998	not just for mice anymore. Clin. Infect. Dis. 2007, 44, 79-86.
8 9	999	35. Weiss, W. J.; Carter, K.; Pulse, M. E.; Nguyen, P.; Valtierra, D.; Peterson, K. M.;
10 11	1000	Tommasi, R.; Mueller, J.; O'Donnell, J. Efficacy of Cefpodoxime Proxetil and ETX0282
12 13	1001	in a Murine UTI model with <i>E. coli</i> and <i>K. pneumoniae.</i> Poster P1991 at <i>ECCMID</i> ;
14	1002	Amsterdam, Netherlands, 2019.
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34	1003	36. McGuire, H.; Bist, S.; Bifulco, N.; Zhao, L.; Wu, Y.; Huynh, H.; Xiong, H.; Comita-
	1004	Prevoir, J.; Dussault, D.; Geng, B.; Chen, B.; Durand-Reville, T.; Guler, S. β -lactamase
	1005	Inhibitor Compounds. US9309245B2, 2016.
	1006	37. Adam, W.; Krebs, O. The Nitroso Ene Reaction: A Regioselective and
	1007	Stereoselective Allylic nitrogen functionalization of mechanistic delight and synthetic
	1008	potential. <i>Chem. Rev.</i> 2003, 103, 4131-4146.
	1009	38. Shapiro, A. B.; Comita-Prevoir, J.; Sylvester, M. 5-Carboxytetramethylrhodamine-
	1010	ampicillin fluorescence anisotropy-based assay of Escherichia coli penicillin-binding
	1011	protein 2 transpeptidase inhibition. ACS Infect. Dis. 2019, 5, 863-872.
	1012	39. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow
	1013	Aerobically; Clinical and Laboratory Standards Institute M07, 11th Edition.: Wayne, PA,
35 36	1014	2018.
37	1015	40. Chen, Y.; Delmas, J.; Sirot, J.; Shoichet, B.; Bonnet, R. Atomic resolution
38 39	1016	structures of CTX-M β -lactamases: extended spectrum activities from increased mobility
40 41	1017	and decreased stability. <i>J. Mol. Biol.</i> 2005 , 348, 349-362.
42 43	1018	41. Battye, T. G.; Kontogiannis, L.; Johnson, O.; Powell, H. R.; Leslie, A. G.
44	1019	iMOSFLM: a new graphical interface for diffraction-image processing with MOSFLM.
45 46	1020	<i>Acta cryst.</i> 2011 , D67, 271-281.
47 48	1021	42. Murshudov, G. N.; Skubak, P.; Lebedev, A. A.; Pannu, N. S.; Steiner, R. A.;
49 50	1022	Nicholls, R. A.; Winn, M. D.; Long, F.; Vagin, A. A. REFMAC5 for the refinement of
51	1023	macromolecular crystal structures. Acta cryst. 2011, D67, 355-367.
52 53	1024	43. Evans, P. Scaling and assessment of data quality. <i>Acta cryst.</i> 2006 , D62, 72-82.
54 55	1025	44. Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. <i>Acta</i>
56 57	1026	<i>cryst.</i> 2004, D60, 2126-2132.
58 59		73

1

2		
3 4	1027	45. Gudmundsson, S.; Vogelman, B.; Craig, W. A. The <i>in vivo</i> postantibiotic effect of
5 6	1028	imipenem and other new antimicrobials. J. Antimicrob. Chemother. 1986, 18 Suppl E,
7	1029	67-73.
8 9	1030	
10 11		
12		
13 14		
15		
16 17		
18 19		
20		
21 22		
23		
24 25		
26 27		
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