Pharmacokinetic/Pharmacodynamic Determination and Preclinical Pharmacokinetics of the Beta-lactamase Inhibitor ETX1317 and Its Orally Available Prodrug ETX0282

Supporting Information File

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Table S1. Pharmacokinetics of ETX1317 following oral administration of ETX0282 to mice, rats, dog, and monkeys.

Species	Dose (mg/kg)	C_{max} (µg/mL)	AUC (µg*h/mL)	$T_{1/2}$ (h)	F (%)
Mouse	10	4.20	5.03	1.2	--
Mouse	50	22.4	24.5	1.6	--
Mouse	200	61.4	65.1	0.8	--
Mouse	400	97.2	78.4	1.4	--
Rat	10 equiv.	5.8±0.16	7.04±0.62	1.1±0.3	98
Dog	1 equiv.	1.27±0.02	2.68±0.24	1.3±0.6	97
Monkey	0.94 equiv.	1.32±0.02	2.23±0.43	0.8±0.4	78

AUC = area under the curve. C_{max} = peak concentration. Equiv. = mg/kg ETX1317 equivalent dose of ETX0282. F = bioavailability. $T_{1/2}$ = half-life.

Table S2. Plasma protein binding of ETX1317 in humans and non-clinical species.

Species	Proportion unbound at different concentrations		
	1 µM	5 µM	100 µM
Rat	94%	97%	83%
Mouse	88%	100%	90%
Dog	92%	100%	81%
Human	82%	99%	80%
Monkey	77%	97%	--

Figure S1. ETX1317 Plasma concentration vs. time profiles following IV (ETX1317) and oral (ETX0282) administration to rats (A), dogs (B), and monkeys (C).

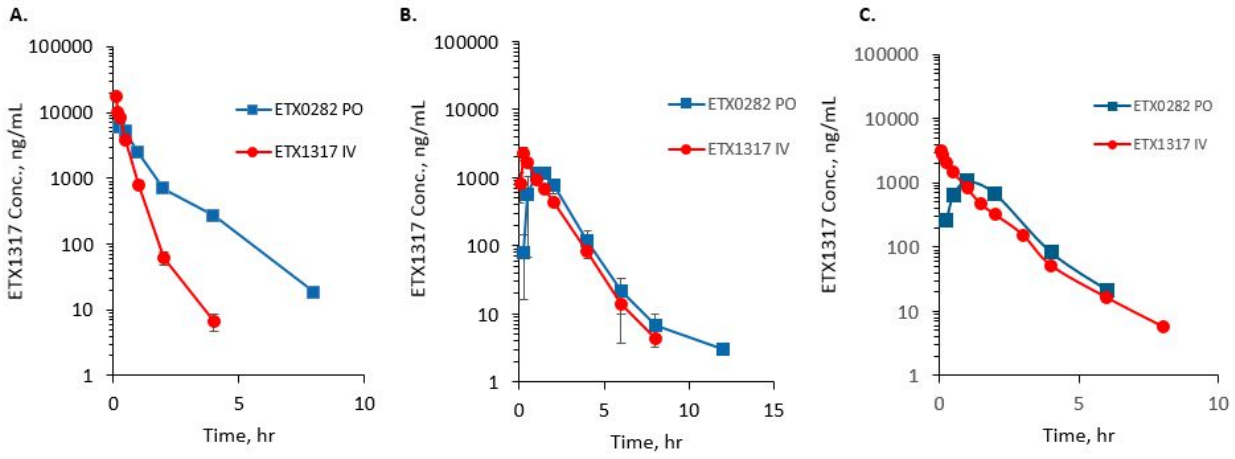


Table S3. Percent Time > C_T of 1 µg/mL of a 500 mg equivalent dose of ETX1317 based upon clearance, bioavailability, and absorption rate.

F%	V/F (L)	CL/F (L/h)	Absorption rate		
			0.5 h^{-1}	1.0 h^{-1}	1.5 h^{-1}
60	41	17.6 - 23.7	69 - 58%	58 - 48%	54 - 42%
70	35	15.1 - 20.3	75 - 61%	61 - 51%	57 – 47%
80	31	13.2 – 17.8	78 - 63%	65 - 52%	60 – 49%
CL/F = oral clearance. F = bioavailability. h = hour. V/F = oral volume of distribution.					

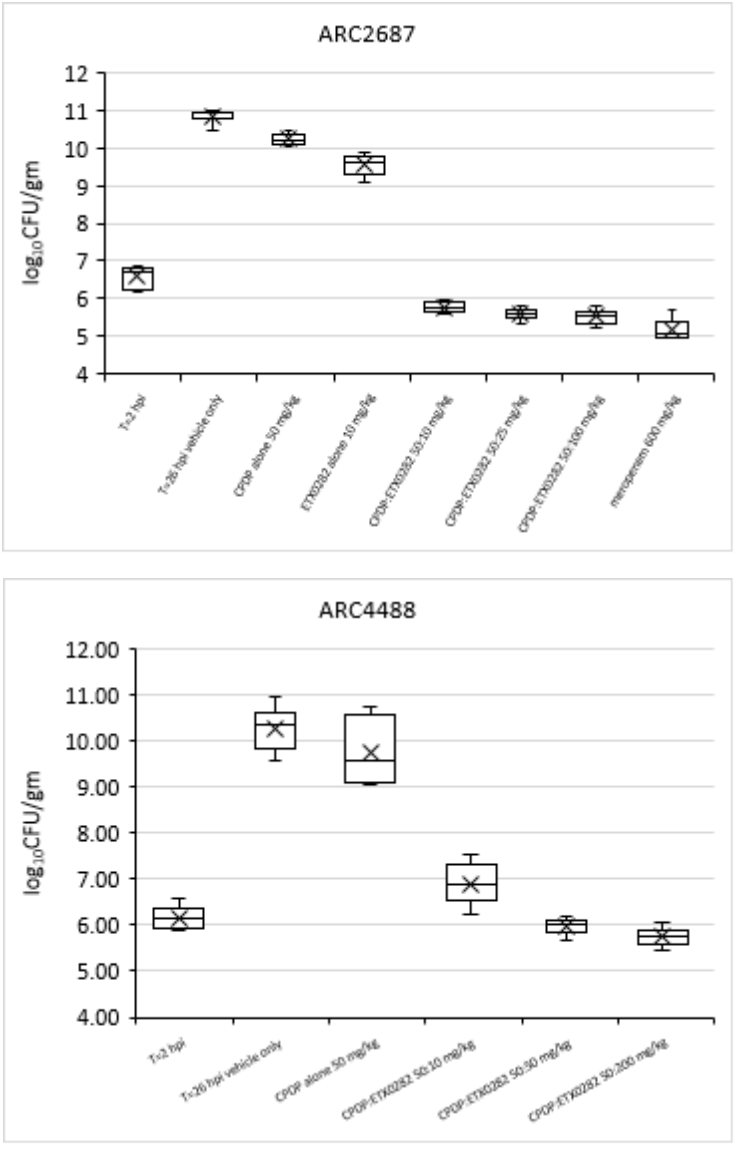
Table S4. LC-MS/MS conditions for ETX1317, ETX0282, CPD CPDP in plasma, urine and MHBII

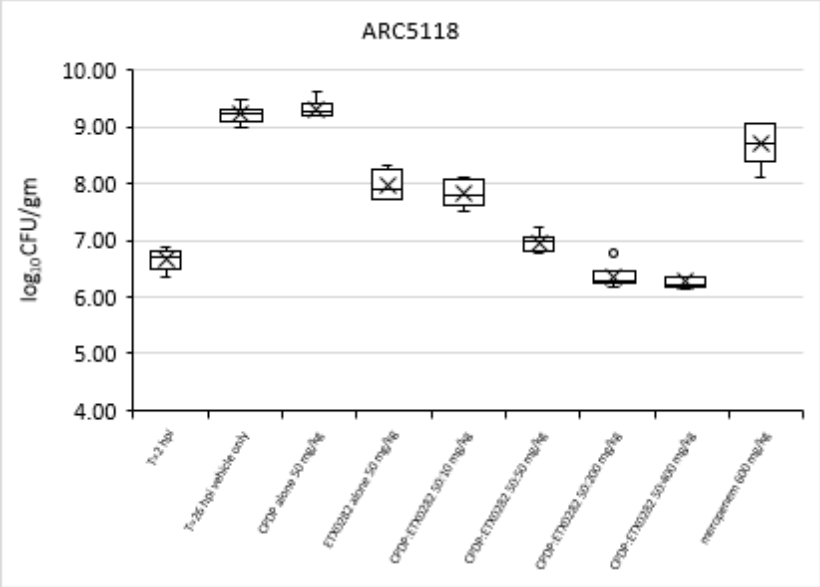
Instrument	Schimadzu UPLC - Sciex QTRAP 6500 Mass Spectrometer		
Column	Atlantis T3, 5µ, 50 x 3.0mm		
Column Temperature	40^0C		
Sample Temperature	15^0C		
Flow rate	1.200 mL/min		
Gradient	Time (min)	%B	Curve
	Initial	2.0	
	0.3	2.0	6
	1.3	98	6

	1.75	98	6
	1.76	2.0	6
	2.00	Stop	
Divert Valve	0.40 min to Mass Spec 1.80 min to waste		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in Acetonitrile		
MRM transitions	<u>ETX1317:</u> Q1: 273.986 → Q3: 94.000 DP 56.00 CXP 12.00 CE 37.00	<u>cefpodoxime:</u> Q1: 428.000 → Q3: 241.000 DP 80.00 CXP 12.00 CE 21.00	
	<u>ETX0282:</u> Q1: 316.082 → Q3: 93.900 DP 41.00 CXP 14.00 CE 47.00	<u>cefpodoxime proxetil:</u> Q1: 558.000 → Q3: 410.000 DP 80.00 CXP 12.00 CE 23.00	
	<u>Carbutamide (IS):</u> Q1: 272.1 → Q3: 156.1 DP 46.00 CXP 10.00 CE 25.00		
MRM conditions	Source Type Turbo Spray Polarity: Positive Resolution Q1: Unit Resolution Q3: Unit IS: 2500.00 TEM: 550.00		

	GS1: GS2: CAD:	60.00 50.00 10.00
Injection volume	0.5 µL	

Figure S2. Box-Whisker plots of CFU burden following treatment with CPDP, ETX0282, and CPDP:ETX0282 in a murine neutropenic thigh model.




ARC5118
10.00
9.00
8.00
7.00
6.00
5.00
4.00
log10CFU/gm
T=2 hpi
T=26 hpi vehicle only
CFDP alone 50 mg/kg
ETX0282 alone 50 mg/kg
CFDP-ETX0282 50:10 mg/kg
CFDP-ETX0282 50:50 mg/kg
CFDP-ETX0282 50:200 mg/kg
CFDP-ETX0282 50:400 mg/kg
meropenem 600 mg/kg