

In Vitro Activity of RX-P873 against *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*

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RX-P873 is a novel antibiotic from the pyrrolocytosine series which exhibits high binding affinity for the bacterial ribosome and broad-spectrum antibiotic properties. The pyrrolocytosines have shown *in vitro* activity against multidrug-resistant Gram-negative and Gram-positive strains of bacteria known to cause complicated urinary tract, skin, and lung infections, as well as sepsis. *Enterobacteriaceae* (657), *Pseudomonas aeruginosa* (200), and *Acinetobacter baumannii* (202) isolates from North America and Europe collected in 2012 as part of a worldwide surveillance program were tested *in vitro* by broth microdilution using Clinical and Laboratory Standards Institute (CLSI) methodology. RX-P873 (MIC₉₀, 0.5 µg/ml) was >32-fold more active than ceftazidime and inhibited 97.1% and 99.5% of *Enterobacteriaceae* isolates at MIC values of ≤1 and ≤4 µg/ml, respectively. There were only three isolates with an MIC value of >4 µg/ml (all were indole-positive *Protea*). RX-P873 (MIC_{50/90}, 2/4 µg/ml) was highly active against *Pseudomonas aeruginosa* isolates, including isolates which were nonsusceptible to ceftazidime or meropenem. RX-P873 was 2-fold less active against *P. aeruginosa* than tobramycin (MIC₉₀, 2 µg/ml; 91.0% susceptible) and colistin (MIC₉₀, 2 µg/ml; 99.5% susceptible) and 2-fold more potent than amikacin (MIC₉₀, 8 µg/ml; 93.5% susceptible) and meropenem (MIC₉₀, 8 µg/ml; 76.0% susceptible). RX-P873, the most active agent against *Acinetobacter baumannii* (MIC₉₀, 1 µg/ml), was 2-fold more active than colistin (MIC₉₀, 2 µg/ml; 97.0% susceptible) and 4-fold more active than tigecycline (MIC₉₀, 4 µg/ml). This novel agent merits further exploration of its potential against multidrug-resistant Gram-negative bacteria.

Multidrug-resistant (MDR) Gram-negative bacilli represent a serious public health problem (1–7). It is estimated that there are more than two million infections with antibiotic-resistant bacteria and at least 23,000 deaths annually (7). The CDC report *Antibiotic Resistance Threats in the United States* categorizes pathogens based on threat level categories (7). Among the Gram-negative bacilli, carbapenem-resistant *Enterobacteriaceae* (CRE) are listed as an urgent threat. Multidrug-resistant *Acinetobacter* spp., *Pseudomonas aeruginosa*, and extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* are included in the serious threat category.

RX-P873 (Fig. 1) is a novel antibiotic from a series of compounds that target a biologically conserved region in the bacterial ribosome. RX-P873 is from the pyrrolocytosine series, one of three *de novo*-designed molecular scaffolds with high binding affinity and properties that have been rationally optimized for broad-spectrum activity (8–10). The pyrrolocytosines have shown *in vitro* activities and preclinical efficacies against multidrug-resistant (MDR) Gram-negative and Gram-positive strains of bacteria known to cause complicated urinary tract, skin, and lung infections, as well as sepsis (11–15).

In this study, we evaluated the spectrum and activity of RX-P873 (a late preclinical stage compound) when tested against contemporary *Enterobacteriaceae* and nonfermentative Gram-negative bacilli collected in 2012 as part of a worldwide surveillance program.

(This work was presented in part at the 54th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 5 to 9 September 2014 [11].)

MATERIALS AND METHODS

Bacterial collection. A total of 657 *Enterobacteriaceae* and 402 nonfermentative Gram-negative bacilli collected during 2012 as part of a global

surveillance program were selected. Isolates were chosen from various medical institutions located in North America and Europe to represent the contemporary frequency distributions of antimicrobial susceptibility profiles within each organism species or genus group. We selected 202 *Escherichia coli* strains, 202 *Klebsiella pneumoniae* strains, 50 *Enterobacter cloacae* strains, 50 *Enterobacter aerogenes* strains, 51 *Citrobacter freundii* strains, 51 *Proteus* species strains, 51 *Serratia marcescens* strains, 200 *Pseudomonas aeruginosa* strains, and 202 *Acinetobacter baumannii* strains.

Susceptibility testing. MIC values were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology as described in CLSI document M07-A9 (16). The compound RX-P873 and comparator agents were tested in 96-well frozen-form panels produced by JMI Laboratories (North Liberty, IA) and consisted of one reference medium type, cation-adjusted Mueller-Hinton broth. RX-P873 powder was provided by Melinta Therapeutics, Inc. (Lincolnshire, IL). Quality control (QC) strains were tested daily, and inoculum densities were monitored by colony counts. QC ranges and interpretive criteria for the comparator compounds were as published in CLSI M100-S24 (17); we used *Escherichia coli* ATCC 25922 and ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 as the QC strains.

RX-P873. The chemical structure of RX-P873 is shown in Fig. 1.

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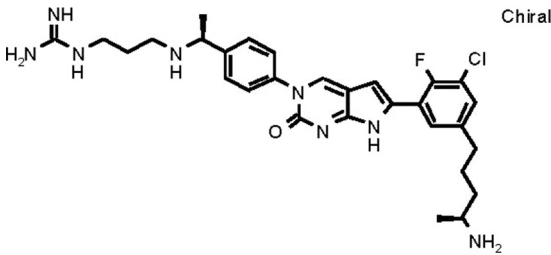


FIG 1 Chemical structure of RX-P873.

RESULTS

Activity against Enterobacteriaceae. The range of RX-P873 MIC values was 0.06 to ≥ 32 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.25 and 0.5 $\mu\text{g/ml}$, respectively (Table 1). Only 4 of the 657 isolates (0.6%) had MIC values of > 2 $\mu\text{g/ml}$ for RX-P873; these isolates include one strain (*Proteus mirabilis*) with an MIC of 4 $\mu\text{g/ml}$, one strain (*Providencia stuartii*) with an MIC of 8 $\mu\text{g/ml}$, one strain (*Providencia rettgeri*) with an MIC of 16 $\mu\text{g/ml}$, and one strain (*P. stuartii*) with an MIC of > 32 $\mu\text{g/ml}$. Against the majority of isolates, RX-P873 was the second most potent agent tested (MIC₉₀, 0.5 $\mu\text{g/ml}$) after meropenem (MIC₉₀, ≤ 0.06 $\mu\text{g/ml}$; Table 2).

Against *E. coli*, the range of RX-P873 MIC values was 0.06 to 2 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.12 and 0.25 $\mu\text{g/ml}$, respectively (Tables 1 and 2). RX-P873 had a potency identical to that of tigecycline (MIC₅₀ and MIC₉₀, 0.12 and 0.25 $\mu\text{g/ml}$, respectively, for each agent), with meropenem being the most potent (MIC₉₀, ≤ 0.06 $\mu\text{g/ml}$; Table 2). Against *K. pneumoniae*, the range of RX-P873 MIC values was 0.12 to 2 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.25 and 0.5 $\mu\text{g/ml}$, respectively (Table 1). Meropenem resistances were 5.0% and 4.5% according to CLSI and EUCAST criteria, respectively, and resistances to all other comparator agents (except tigecycline) ranged from 2.5% for colistin (EUCAST criteria) to 23.3% for ceftazidime (EUCAST criteria; Table 2). RX-P873 inhibited all isolates at an MIC value of ≤ 2 $\mu\text{g/ml}$, which is similar to the inhibition by tigecycline (all isolates inhibited at an MIC of ≤ 4 $\mu\text{g/ml}$).

Against *Enterobacter cloacae*, the range of RX-P873 MIC values was 0.12 to 1 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.5 and 0.5 $\mu\text{g/ml}$, respectively (Table 2). All the isolates were inhibited by meropenem at an MIC value of ≤ 2 $\mu\text{g/ml}$ (98.0% and 100.0% susceptible according to CLSI and EUCAST interpretive criteria, respectively). Resistances (CLSI criteria) for the other agents ranged from 2.0% for tigecycline to 46.0% for ceftriaxone. For colistin, 12.0% of the isolates were found to be resistant by EUCAST interpretive criteria (Table 2). RX-P873 retained activity against all *E. cloacae* resistance phenotypes, inhibiting all 50 strains at an MIC value of ≤ 1 $\mu\text{g/ml}$. The range of RX-P873 MIC values against the 50 *E. aerogenes* strains was 0.12 to 1 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.25 and 0.5 $\mu\text{g/ml}$, respectively (Table 1). All the isolates were inhibited by meropenem at an MIC value of ≤ 0.25 $\mu\text{g/ml}$ (100.0% susceptible according to CLSI and EUCAST interpretive criteria). Resistances (CLSI criteria) for other agents ranged from 0% for tigecycline to 46.0% for ceftriaxone. For colistin, 4.0% of the isolates were resistant according to EUCAST interpretive criteria (Table 2). RX-P873 retained activity against all resistance phenotypes, inhibiting all 50 strains at an MIC value of ≤ 1 $\mu\text{g/ml}$.

TABLE 1 Cumulative MIC distribution for RX-P873 when tested against 657 Enterobacteriaceae and 402 nonfermentative Gram-negative bacilli

Organism group (no. of strains tested)	No. of strains (cumulative %) at an MIC ($\mu\text{g/ml}$) of:											MIC ($\mu\text{g/ml}$)		
	≤ 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥ 32	MIC ₅₀	MIC ₉₀
Enterobacteriaceae (657)	0 (0.0)	0 (0.0)	2 (0.3)	188 (28.9)	287 (72.6)	122 (91.2)	39 (97.1)	15 (99.4)	1 (99.5)	1 (99.7)	1 (99.8)	1 (100.0)	0.25	0.5
<i>E. coli</i> (202)	0 (0.0)	0 (0.0)	2 (1.0)	111 (55.9)	78 (94.6)	8 (98.5)	2 (99.5)	1 (100.0)					0.12	0.25
<i>K. pneumoniae</i> (202)	0 (0.0)	0 (0.0)	0 (0.0)	52 (25.7)	117 (83.7)	28 (97.5)	2 (98.5)	3 (100.0)					0.25	0.5
<i>E. cloacae</i> (50)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.0)	21 (48.0)	23 (94.0)	3 (100.0)						0.5	0.5
<i>E. aerogenes</i> (50)	0 (0.0)	0 (0.0)	0 (0.0)	14 (28.0)	30 (88.0)	5 (98.0)	1 (100.0)						0.25	0.5
<i>C. freundii</i> (51)	0 (0.0)	0 (0.0)	0 (0.0)	5 (9.8)	22 (52.9)	19 (90.2)	4 (98.0)	1 (100.0)					0.25	0.5
<i>Proteus</i> spp. (51) ^a	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)	4 (9.8)	9 (27.5)	24 (74.5)	9 (92.2)	1 (94.1)	1 (96.1)	1 (98.0)	1 (100.0)	1	2
<i>S. marcescens</i> (51)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.9)	15 (33.3)	30 (92.2)	3 (98.0)	1 (100.0)					0.5	0.5
Nonfermentative bacilli (402)	0 (0.0)	0 (0.0)	0 (0.0)	9 (2.2)	41 (12.4)	85 (33.6)	136 (67.4)	77 (86.6)	45 (97.8)	9 (100.0)			1	4
<i>P. aeruginosa</i> (200)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	5 (3.0)	76 (41.0)	70 (76.0)	39 (95.5)	9 (100.0)			2	4
<i>A. baumannii</i> (202)	0 (0.0)	0 (0.0)	0 (0.0)	9 (4.5)	40 (24.3)	80 (63.9)	60 (93.6)	7 (97.0)	6 (100.0)				0.5	1

^a Includes the following species (no.): *Proteus mirabilis* (30), *Morganella morganii* (11), *Proteus vulgaris* (4), *Providencia rettgeri* (4), and *Providencia stuartii* (2).

TABLE 2 Activities of RX-P873 and comparator antimicrobial agents when tested against 657 *Enterobacteriaceae* and 402 nonfermentative bacilli

Organism group (no. tested) and antimicrobial agent	MIC (μg/ml)			Susceptible/intermediate/resistant (%) according to:	
	MIC ₅₀	MIC ₉₀	Range	CLSI ^a	EUCAST ^a
<i>Enterobacteriaceae</i> (657) ^b					
RX-P873	0.25	0.5	0.06->32		
Piperacillin-tazobactam	2	32	≤0.5->64	87.5/6.3/6.2	83.9/3.6/12.5
Ceftriaxone	0.06	>2	≤0.015->2	79.6/0.3/20.1	79.6/0.3/20.1
Ceftazidime	0.25	>16	≤0.12->16	82.3/1.9/15.8	79.3/3.0/17.7
Cefepime	≤0.12	8	≤0.12->16	87.1/3.9/9.0	85.5/4.1/10.4
Ciprofloxacin	0.03	>8	≤0.004->8	83.1/2.0/14.9	81.6/1.5/16.9
Colistin	0.5	>8	≤0.12->8		83.3/0.0/16.7
Gentamicin	0.5	2	≤0.06->8	92.4/0.3/7.3	91.0/1.4/7.6
Meropenem	≤0.06	≤0.06	≤0.06->8	98.0/0.2/1.8	98.2/0.1/1.7
Tigecycline ^c	0.5	2	0.06->4	97.1/2.6/0.3	88.9/8.2/2.9
<i>E. coli</i> (202)					
RX-P873	0.12	0.25	0.06-2		
Piperacillin-tazobactam	2	8	≤0.5->64	93.1/3.4/3.5	90.6/2.5/6.9
Ceftriaxone	0.06	>2	≤0.015->2	88.6/0.0/11.4	88.6/0.0/11.4
Ceftazidime	0.25	2	≤0.12->16	93.1/1.0/5.9	89.1/4.0/6.9
Cefepime	≤0.12	2	≤0.12->16	91.1/1.5/7.4	89.1/3.0/7.9
Ciprofloxacin	0.015	>8	0.008->8	78.2/1.0/20.8	77.7/0.5/21.8
Colistin	0.25	0.5	0.25-0.5		100.0/0.0/0.0
Gentamicin	0.5	1	≤0.06->8	93.1/0.5/6.4	92.1/1.0/6.9
Meropenem	≤0.06	≤0.06	≤0.06	100.0/0.0/0.0	100.0/0.0/0.0
Tigecycline ^c	0.12	0.25	0.06-1	100.0/0.0/0.0	100.0/0.0/0.0
<i>K. pneumoniae</i> (202)					
RX-P873	0.25	0.5	0.12-2		
Piperacillin-tazobactam	2	32	≤0.5->64	88.1/3.0/8.9	83.7/4.4/11.9
Ceftriaxone	0.06	>2	≤0.015->2	77.7/0.0/22.3	77.7/0.0/22.3
Ceftazidime	0.25	>16	≤0.12->16	76.7/3.5/19.8	75.2/1.5/23.3
Cefepime	≤0.12	>16	≤0.12->16	79.2/4.0/16.8	78.7/2.5/18.8
Ciprofloxacin	0.03	>8	0.008->8	80.2/2.5/17.3	79.7/0.5/19.8
Colistin	0.5	1	0.25->8		97.5/0.0/2.5
Gentamicin	0.25	>8	≤0.06->8	89.1/0.0/10.9	87.6/1.5/10.9
Meropenem	≤0.06	≤0.06	≤0.06->8	95.1/0.1/5.0	95.1/0.4/4.5
Tigecycline ^c	0.5	2	0.12-4	96.5/3.5/0.0	88.6/7.9/3.5
<i>E. cloacae</i> (50)					
RX-P873	0.5	0.5	0.12-1		
Piperacillin-tazobactam	4	>64	1->64	70.0/12.0/18.0	64.0/6.0/30.0
Ceftriaxone	1	>2	0.03->2	54.0/0.0/46.0	54.0/0.0/46.0
Ceftazidime	0.5	>16	≤0.12->16	60.0/2.0/38.0	54.0/6.0/40.0
Cefepime	≤0.12	8	≤0.12->16	76.0/16.0/8.0	72.0/14.0/14.0
Ciprofloxacin	0.015	8	0.008->8	80.0/6.0/14.0	80.0/0.0/20.0
Colistin	0.5	8	0.25->8		88.0/0.0/12.0
Gentamicin	0.25	>8	0.12->8	88.0/0.0/12.0	84.0/4.0/12.0
Meropenem	≤0.06	0.12	≤0.06-2	98.0/2.0/0.0	100.0/0.0/0.0
Tigecycline ^c	0.5	2	0.25->4	90.0/8.0/2.0	84.0/6.0/10.0
<i>E. aerogenes</i> (50)					
RX-P873	0.25	0.5	0.12-1		
Piperacillin-tazobactam	4	64	≤0.5->64	64.0/32.0/4.0	56.0/8.0/36.0
Ceftriaxone	0.25	>2	≤0.015->2	54.0/0.0/46.0	54.0/0.0/46.0
Ceftazidime	1	>16	≤0.12->16	54.0/2.0/44.0	52.0/2.0/46.0
Cefepime	≤0.12	2	≤0.12-16	92.0/6.0/2.0	88.0/10.0/2.0
Ciprofloxacin	0.015	0.5	0.008->8	94.0/0.0/6.0	90.0/4.0/6.0
Colistin	0.25	0.5	0.25->8		96.0/0.0/4.0
Gentamicin	0.25	1	0.12->8	96.0/0.0/4.0	96.0/0.0/4.0
Meropenem	≤0.06	0.12	≤0.06-0.25	100.0/0.0/0.0	100.0/0.0/0.0
Tigecycline ^c	0.5	1	0.12-4	98.0/2.0/0.0	92.0/6.0/2.0
<i>C. freundii</i> (51)					
RX-P873	0.25	0.5	0.12-2		
Piperacillin-tazobactam	2	64	≤0.5->64	82.4/7.8/9.8	78.4/4.0/17.6
Ceftriaxone	0.25	>2	0.06->2	76.5/0.0/23.5	76.5/0.0/23.5
Ceftazidime	0.5	>16	0.25->16	78.4/0.0/21.6	72.5/5.9/21.6
Cefepime	≤0.12	4	≤0.12->16	88.2/4.0/7.8	86.3/5.9/7.8
Ciprofloxacin	0.06	2	0.008->8	86.3/3.9/9.8	80.4/5.9/13.7
Colistin	0.5	1	≤0.12-1		100.0/0.0/0.0
Gentamicin	0.5	1	0.12->8	92.2/1.9/5.9	90.2/2.0/7.8
Meropenem	≤0.06	≤0.06	≤0.06->8	96.1/0.0/3.9	96.1/0.0/3.9
Tigecycline ^c	0.25	1	0.12-2	100.0/0.0/0.0	92.2/7.8/0.0

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TABLE 2 (Continued)

Organism group (no. tested) and antimicrobial agent	MIC ($\mu\text{g/ml}$)			Susceptible/intermediate/resistant (%) according to:	
	MIC ₅₀	MIC ₉₀	Range	CLSI ^a	EUCAST ^a
<i>Proteus</i> spp. (51) ^d					
RX-P873	1	2	0.12->32		
Piperacillin-tazobactam	≤ 0.5	≤ 0.5	$\leq 0.5-4$	100.0/0.0/0.0	100.0/0.0/0.0
Ceftriaxone	≤ 0.015	0.06	$\leq 0.015->2$	96.1/0.0/3.9	96.1/0.0/3.9
Ceftazidime	≤ 0.12	≤ 0.12	$\leq 0.12-8$	98.0/2.0/0.0	96.1/1.9/2.0
Cefepime	≤ 0.12	≤ 0.12	$\leq 0.12-8$	96.1/3.9/0.0	96.1/1.9/2.0
Ciprofloxacin	0.03	2	$\leq 0.004->8$	88.2/2.0/9.8	86.3/1.9/11.8
Colistin	>8	>8	>8		0.0/0.0/100.0
Gentamicin	0.5	2	0.12->8	98.0/0.0/2.0	96.1/1.9/2.0
Meropenem	≤ 0.06	0.12	$\leq 0.06-0.12$	100.0/0.0/0.0	100.0/0.0/0.0
Tigecycline ^e	1	2	0.12->4	92.2/5.8/2.0	52.9/39.3/7.8
<i>Serratia marcescens</i> (51)					
RX-P873	0.5	0.5	0.12-2		
Piperacillin-tazobactam	2	8	$\leq 0.5-64$	96.1/3.9/0.0	94.1/2.0/3.9
Ceftriaxone	0.25	2	0.03->2	88.2/4.0/7.8	88.2/4.0/7.8
Ceftazidime	0.25	0.5	$\leq 0.12-4$	100.0/0.0/0.0	98.0/2.0/0.0
Cefepime	≤ 0.12	0.25	$\leq 0.12->16$	98.0/0.0/2.0	98.0/0.0/2.0
Ciprofloxacin	0.06	0.12	0.015->8	98.0/0.0/2.0	94.1/3.9/2.0
Colistin	>8	>8	1->8		9.8/0.0/90.2
Gentamicin	0.5	1	0.12->8	98.0/0.0/2.0	98.0/0.0/2.0
Meropenem	≤ 0.06	0.12	$\leq 0.06-0.25$	100.0/0.0/0.0	100.0/0.0/0.0
Tigecycline ^e	1	2	0.5-4	96.1/3.9/0.0	80.4/15.7/3.9
<i>P. aeruginosa</i> (200)					
RX-P873	2	4	0.25-8		
Piperacillin-tazobactam	4	64	$\leq 0.5->64$	80.5/11.0/8.5	80.5/0.0/19.5
Ceftazidime	2	>16	0.5->16	83.0/4.0/13.0	83.0/0.0/17.0
Cefepime	2	16	0.25->16	84.5/10.5/5.0	84.5/0.0/15.5
Ciprofloxacin	0.12	>8	0.008->8	78.0/2.0/20.0	76.0/2.0/22.0
Tobramycin	0.5	2	$\leq 0.25->16$	91.0/1.5/7.5	91.0/0.0/9.0
Amikacin	4	8	$\leq 0.25->32$	93.5/2.0/4.5	91.0/2.5/6.5
Meropenem	0.5	8	$\leq 0.06->8$	76.0/6.0/18.0	76.0/14.5/9.5
Colistin	1	2	0.25-4	99.5/0.5/0.0	100.0/0.0/0.0
<i>Acinetobacter baumannii</i> (202)					
RX-P873	0.5	1	0.12-4		
Ampicillin-sulbactam	16	>32	0.5->32	48.0/11.4/40.6	
Ceftazidime	>16	>16	0.5->16	38.1/2.5/59.4	
Cefepime	16	>16	0.25->16	36.6/13.4/50.0	
Ciprofloxacin	>8	>8	0.06->8	36.1/0.0/63.9	36.1/0.0/63.9
Tobramycin	4	>16	$\leq 0.25->16$	53.5/3.4/43.1	53.5/0.0/46.5
Amikacin	8	>32	$\leq 0.25->32$	58.4/6.0/35.6	53.5/4.9/41.6
Meropenem	>8	>8	$\leq 0.06->8$	46.0/1.5/52.5	45.5/2.0/52.5
Colistin	1	2	0.25->8	97.0/0.0/3.0	97.0/0.0/3.0
Tigecycline	1	4	0.12->4		

^a 2013 CLSI and EUCAST criteria.

^b Includes *Citrobacter freundii* (51 strains), *Enterobacter aerogenes* (50 strains), *Enterobacter cloacae* (50 strains), *Escherichia coli* (202 strains), *Klebsiella pneumoniae* (202 strains), *Morganella morganii* (11 strains), *Proteus mirabilis* (30 strains), *Proteus vulgaris* (4 strains), *Providencia rettgeri* (4 strains), *Providencia stuartii* (2 strains), and *Serratia marcescens* (51 strains).

^c In the absence of a CLSI breakpoint, U.S. FDA breakpoints were applied when available (22).

^d Includes *Morganella morganii* (11 strains), *Proteus mirabilis* (30 strains), *Proteus vulgaris* (4 strains), *Providencia rettgeri* (4 strains), and *Providencia stuartii* (4 strains).

The range of RX-P873 MIC values against 51 *C. freundii* strains was 0.12 to 2 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.25 and 0.5 $\mu\text{g/ml}$, respectively (Table 1). Meropenem resistance was 3.9% according to CLSI and EUCAST criteria, and resistances (CLSI criteria) to all other comparator agents (except tigecycline) ranged from 5.9% for gentamicin to 23.5% for ceftriaxone (Table 2). RX-P873 and tigecycline inhibited all the isolates at an MIC value of ≤ 2 $\mu\text{g/ml}$ (U.S. FDA susceptibility breakpoint). All the isolates were susceptible to colistin (EUCAST criteria). RX-P873 inhibited 47/51 (92.2%) isolates of *Proteus* spp. at an MIC value of ≤ 2 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 1 and 2 $\mu\text{g/ml}$, respectively, which represent the highest MIC parameters observed for all seven species/groups tested (Table 1). The range of RX-P873

MIC values against 51 *S. marcescens* strains was 0.12 to 2 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.5 and 0.5 $\mu\text{g/ml}$, respectively (Table 1).

Activities against *P. aeruginosa* and *Acinetobacter baumannii*. Against all 402 strains of *P. aeruginosa* and *Acinetobacter baumannii* tested, the range of RX-P873 MIC values was 0.12 to 8 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 1 and 4 $\mu\text{g/ml}$, respectively (Table 1). The range of RX-P873 MIC values against the 200 *P. aeruginosa* strains was 0.25 to 8 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 2 and 4 $\mu\text{g/ml}$, respectively (Table 1). For β -lactam agents, nonsusceptibilities for the agents against *P. aeruginosa* ranged from 15.5% for cefepime to 24.0% for meropenem. For non- β -lactam agents, nonsusceptibilities ranged from 0.5% for

colistin to 22.0% (CLSI criteria) for ciprofloxacin (Table 2). RX-P873 and colistin were the most potent agents tested. Against *A. baumannii*, the range of RX-P873 MIC values was 0.12 to 4 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.5 and 1 $\mu\text{g/ml}$, respectively (Table 1). For β -lactam agents, nonsusceptibilities ranged from 52.0% for ampicillin-sulbactam to 63.4% for cefepime. Meropenem resistance was 52.5%. For non- β -lactam agents, nonsusceptibilities ranged from 3.0% for colistin to 63.9% for ciprofloxacin (Table 2). RX-P873 was the most potent agent tested.

DISCUSSION

RX-P873 was identified in a preclinical program focused on the *de novo* design of new classes of antibiotics that bind to an unexploited site in the large subunit (50S) of the bacterial ribosome. The program was designed to optimize the classes to treat multidrug- and extremely drug-resistant Gram-positive and Gram-negative pathogens. The research approach uses crystal structures of the bacterial ribosome to identify antibiotics that bind to the ribosome and inhibit its function. Three novel families of antibiotics with broad-spectrum activities were designed, optimized, and evaluated (fewer than 900 total compounds) to deliver broad-spectrum compounds (18).

The lead chemical series of the program, the pyrrolocytosine class, consists of broad-spectrum antibacterial agents that are bactericidal and have activities against multidrug-resistant *Enterococcus* spp., *Staphylococcus aureus* (including methicillin-resistant *S. aureus* [MRSA]), *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. coli* (ESKAPE pathogens) and *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei*, and *Burkholderia pseudomallei* (11, 19, 20). There is a lack of cross-resistance to classes used for the treatment of Gram-negative infections and to other 50S ribosome-binding antibiotics. Efficacies for the pyrrolocytosine class have been shown in multiple murine models of infection, including skin, kidney, and lung infections, and in sepsis. RX-P873 is a lead compound in the pyrrolocytosine class (12–15, 21).

In this study, RX-P873 demonstrated a high level of activity and consistent potency against the contemporary collection of *Enterobacteriaceae* bacilli selected from various medical institutions located in North America and Europe. The isolates were selected to represent the frequency distributions of antimicrobial susceptibility profiles for uncomplicated and complicated urinary tract infections and complicated intra-abdominal infections. Only three of the 657 *Enterobacteriaceae* isolates tested exhibited an RX-P873 MIC value of $>4 \mu\text{g/ml}$ (all three were indole-positive *Protea*). The overall MIC₉₀ for RX-P873 (0.5 $\mu\text{g/ml}$) was >32 -fold higher than that of ceftazidime (MIC₉₀, $>16 \mu\text{g/ml}$; 82.3% susceptible). A total of 97.1% of all *Enterobacteriaceae* isolates exhibited an RX-P873 MIC value of $\leq 1 \mu\text{g/ml}$. RX-P873 was shown to be very active against *Enterobacteriaceae* strains with acquired and intrinsic resistance to piperacillin-tazobactam, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, colistin, gentamicin, meropenem, and tigecycline.

RX-P873 was also shown to be highly active (MIC_{50/90}, 2/4 $\mu\text{g/ml}$) against *P. aeruginosa*, including strains which were not susceptible to ceftazidime or meropenem. RX-P873 was 2-fold less active than tobramycin (MIC₉₀, 2 $\mu\text{g/ml}$; 91.0% susceptible) and colistin (MIC₉₀, 2 $\mu\text{g/ml}$; 99.5% susceptible) and 2-fold more potent than amikacin (MIC₉₀, 8 $\mu\text{g/ml}$; 93.5% susceptible) and meropenem (MIC₉₀, 8 $\mu\text{g/ml}$; 76.0% susceptible). RX-P873 was

the most active agent tested against *Acinetobacter* spp. (MIC_{50/90}, 0.5/1 $\mu\text{g/ml}$). Its activity was 2-fold greater than that of colistin (MIC₉₀, 2 $\mu\text{g/ml}$; 97.0% susceptible). Susceptibilities to other agents were compromised. Meropenem, tobramycin, and amikacin susceptibilities ranged from 45.5% to 53.5%. RX-P873 demonstrated activities against isolates of *P. aeruginosa* and *A. baumannii* with acquired and intrinsic resistances to piperacillin-tazobactam, ampicillin-sulbactam, ceftazidime, cefepime, meropenem, ciprofloxacin, colistin, tobramycin, amikacin, and tigecycline.

Overall, RX-P873, a novel agent targeting protein synthesis, exhibited potent activities against contemporary Gram-negative bacteria collected from patients in North American and European medical centers. These activities against *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter baumannii*, including isolates resistant to many commonly used antibacterial agents, indicate that RX-P873 merits further exploration of its potential use against multidrug-resistant Gram-negative bacteria.

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