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In Vitro Evaluation of Novel Compounds against Selected Resistant *Pseudomonas aeruginosa* Isolates

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We describe the activities of RX-P763, RX-P766, RX-P770, RX-P792, RX-P793, and RX-P808 against strains of resistant *Pseudomonas aeruginosa*. These compounds target the large subunit of the bacterial ribosome and have broad-spectrum activities against multidrug-resistant pathogens. All compounds demonstrated *in vitro* activity against *P. aeruginosa*, with MIC_{90} values of 4 to 8 μg/ml (range, 0.5 to 64). These novel compounds had narrow MIC distributions and maintained activity despite resistance phenotypes to other commonly utilized agents.

Multidrug-resistant (MDR) *P. aeruginosa* is on the rise, limiting the choice of available anti-infective agents. The National Nosocomial Infections Surveillance System reports that *P. aeruginosa* resistance rates have increased to available agents (9), increasing morbidity and mortality (1, 6). Further, *P. aeruginosa* was the second most common type of pathogen isolated from nosocomial pneumonia patients between 1986 and 2003 (7), and MDR *P. aeruginosa* increased by 10%, from 4% in 1993 to 14% in 2002 (10). Given the high morbidity and mortality and the increased economic burden, new antimicrobial agents are desperately needed to combat highly resistant *P. aeruginosa* infections.

The RX-04 program at Rib-X Pharmaceuticals has developed a new series of anti-infectives designed to target novel sites of action. These compounds, known as the pyrrolocytosines, bind to the P-loop of the large ribosomal subunit and inhibit translation by stabilizing a distorted mode of P-tRNA binding, a new and unique mechanism of inhibition among antibiotics in clinical use (11). RX-P763, RX-P766, RX-P770, RX-P792, RX-P793, and RX-P808 are fruits of the RX-04 program (Fig. 1) (2). Previous *in vitro* work demonstrated activities against many Gram-positive pathogens and Gram-negative pathogens, including MDR *P. aeruginosa*, *Enterobacteriaceae* including carbapenemase-producing *Klebsiella pneumoniae*, and *Acinetobacter baumanii* (2). In this study, we evaluated the potency of six novel compounds, RX-P763, RX-P766, RX-P770, RX-P792, RX-P793, and RX-P808, against a collection of highly resistant *P. aeruginosa* isolates.

A total of 200 *P. aeruginosa* isolates were selected from a population of 1,788 nonduplicate, nonurine isolates collected from 41 hospitals distributed throughout the United States over the period of October 2005 to June 2010 (4, 5). The isolates were selected to represent the following drug-resistant phenotypes: imipenem resistant (R-IMI; MIC, ≥16 μg/ml); ceftazidime resistant (R-TAZ; MIC, ≥32 μg/ml); piperacillin-tazobactam-nonsusceptible (R-TZP; MIC, ≥32 μg/ml), ciprofloxacin resistant (R-CIPRO; MIC, ≥4 μg/ml), tobramycin resistant (R-TOB; MIC, ≥16 μg/ml), and MDR (resistant to ≥3 classes of antimicrobials). Of the 200 isolates selected, 181 had accompanying patient information. The mean ± standard deviation age was 54 ± 23 years, and 54% of patients were males. Further, 50% of isolates were collected in the intensive care unit (ICU), and the respiratory tract was the most common collection site (61%), followed by blood (11%) and bodily fluids (11%), skin wounds (9%), and other sites (8%).

MICs were determined in duplicate with the reference Clinical and Laboratory Standards Institute broth microdilution method (3). MICs for the antimicrobials imipenem, meropenem, piperacillin-tazobactam, cefepime, ceftazidime, ciprofloxacin, levofloxacin, and tobramycin were also determined by broth microdilution. *P. aeruginosa* ATCC 27853 was used as the quality control organism for all six novel compounds. Acceptable ranges for all six compounds were 2 to 8 μg/ml. Of the 200 isolates, 67% were R-TZP, 42% were R-TAZ and R-IMI, 32% were R-CIPRO, and 21% were R-TOB. Further, 21% were considered MDR, with resistance to 3 or more drug classes.

The MIC distributions of all six compounds are shown in Fig. 2. The MIC profiles of each of the six novel compounds were narrow, and modal MICs of 2 μg/ml were observed for all compounds except RX-P770, which had a modal MIC of 4 μg/ml. RX-P763, RX-P766, RX-P792, RX-P793, and RX-P808 had similar MIC distributions, while RX-P770 seemed to be 1 dilution less potent than its fellow novel compounds, as illustrated in Fig. 2. Marketed compounds tended to have higher modal MICs (in μg/ml, with ranges shown in parentheses): 16 (0.25 to ≥128) for imipenem, 32 (≤0.5 to ≥128) for piperacillin-tazobactam, 32 (1 to ≥128) for ceftazidime, 0.125 (≤0.06 to ≥128) for ciprofloxacin, and 1 (0.25 to ≥128) for tobramycin. Imipenem and piperacillin-tazobactam had elevated MIC_{50} and MIC_{90} values of 4 and 32 and of 32 and 256 μg/ml, respectively. Ceftazidime MIC_{50} and MIC_{90} values were 16 and 128 μg/ml, respectively. The final two marketed compounds, ciprofloxacin and tobramycin, had MIC_{50} and MIC_{90} values of 0.5 and 1 and of 16 and 128 μg/ml, respectively. The MIC distributions of each resistant phenotype are shown in Table 1. The MIC profile of the six novel compounds did not change when R-IMI was broken out, with the exception of the RX-P763 MIC_{50}, which increased by one dilution to 4 μg/ml. None of the novel compounds' MIC profiles changed based on

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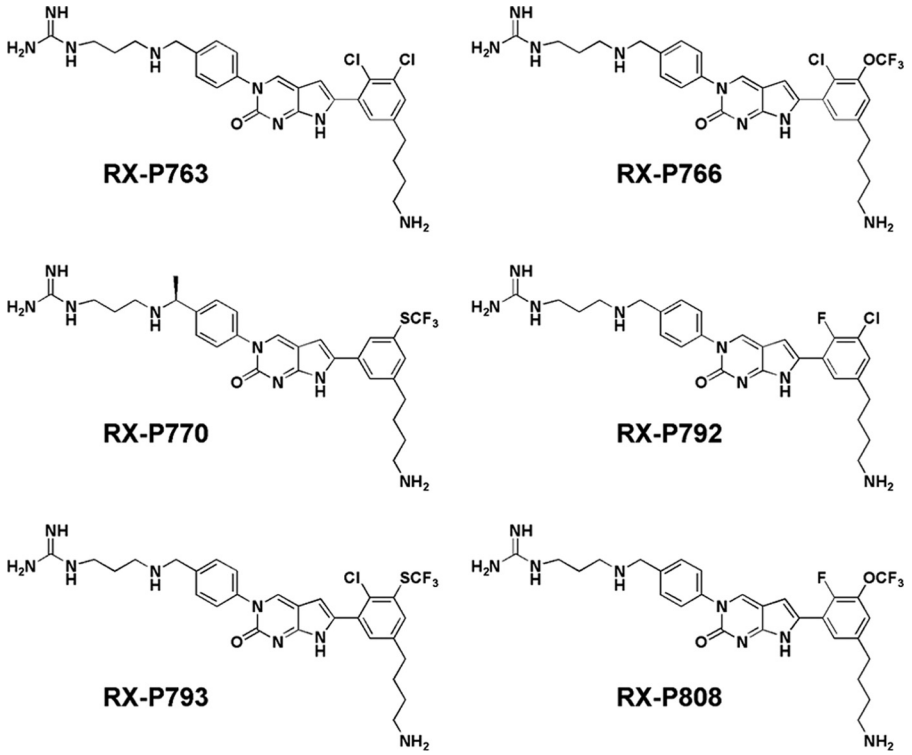

FIG 1 Chemical structures of the novel compounds.

R-TAZ isolates, while the potency of ceftazidime decreased by approximately 2 dilutions. Similar results were seen for all agents against R-CIPRO isolates. MIC_{50} and MIC_{90} values for the novel compounds changed by ≤1 dilution, while the MIC_{50} for ciprofloxacin increased by 5 dilutions and the MIC_{90} increased by 1 dilution, to 16 and 32 μg/ml, respectively. After separating those isolates that were multidrug resistant, the MIC_{50} values for RX-P763 and RX-P792 changed by 1-fold dilutions from 2 to 4 μg/ml, while the other four compounds did not show any changes in MIC profiles. Of note, six isolates contributed to all of the MICs that were ≥16 μg/ml for the novel compounds. The isolates were collected from geographically diverse locations (two isolates from Ohio and one each from Alabama, the District of Columbia, Georgia, and Florida), were generally not MDR, and were obtained from different sites of infection. Of these six isolates, three were R-IMI, three were R-TAZ, four were R-TZP, one was R-TOBRA, and two were R-CIPRO. The modal MICs for each of the compounds against these six isolates were 4 μg/ml for RX-P793 (range, 4 to 16 μg/ml), 8 μg/ml for RX-P766 (range, 4 to 16 μg/ml) and RX-P808 (range, 4 to 16 μg/ml), and 16 μg/ml for RX-P763 (range, 4 to 32 μg/ml), RX-P770 (range, 8 to 16 μg/ml), and RX-P792 (range, 4 to 64 μg/ml).

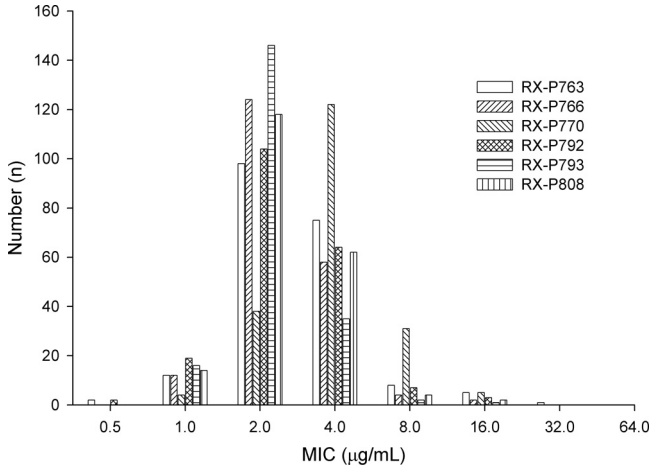

FIG 2 MIC distributions for the six novel compounds, RX-7957, RX-7960, RX-7973, RX-7999, RX-8000, and RX-8015, against 200 clinical *P. aeruginosa* isolates.

Respiratory isolates ($n = 111$) were separated, and three novel compounds, RX-P766, RX-P770, and RX-P793, were compared against this cache of isolates (8). The MIC distributions of the novel compounds were very similar to the whole collection of 200 isolates. When broken out according to those isolates collected in the ward and those collected in the ICU, there were no changes to the MIC distributions of the three compounds (data not shown). Further, the 3 compounds demonstrated greater potencies *in vitro* than currently utilized antimicrobials for *P. aeruginosa* respiratory infections.

Overall, these data showed that all six novel compounds demonstrated *in vitro* activity against a collection of highly resistant *P. aeruginosa* isolates. All of the novel compounds demonstrated a unimodal MIC distribution with this collection of isolates. However, elevated MICs were observed. These six isolates (3%) contributed to all of the observed MICs that were ≥16 μg/ml, suggesting some type of cross-resistance between the novel compounds. While the genetic profiles of these isolates have not

TABLE 1 MIC distributions of novel compounds and comparator agents against 200 *P. aeruginosa* isolates, by resistance phenotype

Resistance phenotype (MIC; *n*)	Agent	No. of isolates (cumulative %) inhibited at MIC (μg/ml) of:								
		≤0.5	1	2	4	8	16	32	64	≥128
R-IMI (≥16 μg/ml; 84)	RX-P763	2 (2)	5 (8)	33 (48)	38 (93)	3 (96)	3 (100)	0 (100)	0 (100)	0 (100)
	RX-P766	0 (0)	7 (8)	48 (65)	26 (96)	2 (99)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P770	0 (0)	3 (4)	12 (18)	54 (82)	13 (98)	2 (100)	0 (100)	0 (100)	0 (100)
	RX-P792	2 (2)	8 (12)	35 (54)	34 (94)	3 (98)	2 (100)	0 (100)	0 (100)	0 (100)
	RX-P793	0 (0)	7 (8)	62 (82)	13 (98)	2 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P808	0 (0)	8 (10)	42 (60)	30 (95)	3 (99)	1 (100)	0 (100)	0 (100)	0 (100)
	Imipenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	53 (63)	22 (89)	5 (95)	4 (100)
R-TAZ (≥32 μg/ml; 83)	RX-P763	2 (2)	6 (10)	39 (57)	30 (93)	3 (96)	2 (99)	1 (100)	0 (100)	0 (100)
	RX-P766	0 (0)	8 (10)	51 (71)	20 (95)	2 (98)	2 (100)	0 (100)	0 (100)	0 (100)
	RX-P770	0 (0)	3 (4)	18 (25)	48 (83)	12 (98)	2 (100)	0 (100)	0 (100)	0 (100)
	RX-P792	2 (2)	9 (13)	41 (63)	24 (92)	5 (98)	1 (99)	1 (100)	0 (100)	0 (100)
	RX-P793	0 (0)	11 (13)	57 (82)	13 (98)	1 (99)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P808	0 (0)	11 (13)	45 (67)	23 (95)	2 (98)	2 (100)	0 (100)	0 (100)	0 (100)
	Ceftazidime	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	37 (45)	24 (73)	22 (100)
R-TZP (≥32 μg/ml; 133)	RX-P763	2 (2)	9 (8)	59 (53)	52 (92)	7 (97)	3 (99)	1 (100)	0 (100)	0 (100)
	RX-P766	0 (0)	11 (8)	77 (66)	40 (96)	3 (98)	2 (100)	0 (100)	0 (100)	0 (100)
	RX-P770	0 (0)	4 (3)	22 (20)	80 (80)	24 (98)	3 (100)	0 (100)	0 (100)	0 (100)
	RX-P792	2 (2)	11 (10)	65 (59)	45 (92)	7 (98)	2 (99)	1 (100)	0 (100)	0 (100)
	RX-P793	0 (0)	15 (11)	88 (77)	28 (98)	1 (99)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P808	0 (0)	13 (10)	72 (64)	43 (96)	3 (98)	2 (100)	0 (100)	0 (100)	0 (100)
	Piperacillin-tazobactam	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	50 (38)	21 (53)	62 (100)
R-CIPRO (≥4 μg/ml; 64)	RX-P763	1 (2)	4 (8)	22 (42)	31 (91)	4 (97)	2 (100)	0 (100)	0 (100)	0 (100)
	RX-P766	0 (0)	5 (8)	33 (59)	24 (97)	1 (98)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P770	0 (0)	2 (3)	9 (17)	37 (75)	15 (98)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P792	1 (2)	6 (11)	24 (48)	28 (92)	4 (98)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P793	0 (0)	6 (9)	43 (77)	14 (98)	1 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P808	0 (0)	6 (9)	30 (56)	25 (95)	2 (98)	1 (100)	0 (100)	0 (100)	0 (100)
	Ciprofloxacin	0 (0)	0 (0)	0 (0)	13 (20)	16 (45)	14 (67)	15 (91)	0 (91)	6 (100)
R-TOB (≥16 μg/ml; 42)	RX-P763	0 (0)	2 (5)	17 (46)	19 (93)	3 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P766	0 (0)	3 (7)	26 (71)	12 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P770	0 (0)	0 (0)	10 (24)	23 (80)	7 (98)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P792	0 (0)	5 (12)	18 (56)	18 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P793	0 (0)	3 (7)	31 (83)	7 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P808	0 (0)	4 (10)	23 (66)	14 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	Tobramycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (12)	8 (32)	6 (46)	22 (100)
MDR (resistance to ≥3 drug classes; 42)	RX-P763	0 (0)	3 (7)	15 (43)	19 (88)	4 (98)	1 (100)	0 (100)	0 (100)	0 (100)
	RX-P766	0 (0)	4 (10)	23 (64)	14 (98)	1 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P770	0 (0)	1 (2)	6 (17)	26 (79)	9 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P792	0 (0)	4 (10)	15 (45)	20 (93)	3 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P793	0 (0)	5 (12)	29 (81)	8 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	RX-P808	0 (0)	5 (12)	20 (60)	15 (95)	2 (100)	0 (100)	0 (100)	0 (100)	0 (100)
	Imipenem	1 (2)	2 (7)	6 (21)	2 (26)	3 (33)	12 (62)	9 (83)	4 (93)	3 (100)
	Piperacillin-Tazobactam	1 (2)	0 (2)	0 (2)	1 (5)	1 (7)	2 (12)	2 (17)	2 (21)	33 (100)
	Ceftazidime	0 (0)	0 (0)	1 (2)	4 (12)	2 (17)	5 (29)	10 (52)	6 (67)	14 (100)
	Ciprofloxacin	0 (0)	0 (0)	6 (14)	7 (31)	9 (52)	8 (71)	9 (93)	0 (93)	3 (100)
	Tobramycin	2 (5)	4 (14)	2 (19)	1 (21)	4 (31)	5 (43)	4 (52)	3 (60)	17 (100)

been elucidated and the resistance mechanism(s) has not been identified, it is suspected that multiple resistance mechanisms, possibly including efflux pumps, are involved, given the differing resistance phenotypes (2). With a new mechanism of action and these *in vitro* data, these novel compounds could provide new options for clinicians against multidrug-resistant *P. aeruginosa*.

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