Activity of novel pyrrolocytosine agents against multidrug and carbapenem resistant *Klebsiella pneumoniae*

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Revised Abstract

Background: The prevalence of multidrug-resistant (MDR) *Klebsiella pneumoniae*, including carbapenem resistant strains associated with KPC production, is increasing, and new antimicrobial agents are needed to combat infections due to these highly resistant pathogens. Pyrrolocytosines are a new class of agents that bind to the P-loop of the large ribosomal subunit and inhibit translation by stabilizing a distorted mode of P-tRNA binding. In this study, we evaluated the potency of four of these novel compounds against a collection of MDR *K. pneumoniae* isolates, including strains deficient in porins and containing ESBLs and KPCs.

Methods: Susceptibilities of 55 carbapenem susceptible and resistant *K. pneumoniae* isolates, including 6 wild-type control strains, 36 KPC-producers, 8 ESBL-producers, 5 characterized porin-deficient strains, were determined by CLSI broth microdilution against RX-P792, RX-P873, RX-P933 and RX-P934 (Rib-X Pharmaceuticals). Isolates included 5 colistin and two tigecycline resistant strains.

Results: Unimodal MIC distributions were found for all 4 agents, with MIC values (ug/ml) as follows:

Agent	RX-P792	RX-P873	RX-P933	RX-P934
MIC range	0.25-1	0.25-1	0.5->16	0.5-8
MIC50	0.25	0.5	4	2
MIC90	0.5	1	>16	8

MICs were not affected by resistance to other agents, including carbapenems, quinolones, aminoglycosides and colistin. RX-P792 was the most potent agent tested, with MIC_{90} of 0.5 ug/ml, followed by RX-P873 and RX-P934, while RX-P933 was the least potent, with MIC_{90} of >16 ug/ml.

Conclusions: Two of the 4 pyrrolocytosine agents tested showed potent in vitro activity against *K. pneumoniae*, including MDR, carbapenem, tigecycline and colistin resistant isolates. Further development of these agents is warranted to determine their potential for clinical use.

Introduction

Rib-X's RX-04 drug discovery process from initial crystal structure analysis to lead optimization and efficacy validation has led to the description of a clinically unexploited site on the large bacterial ribosome using natural products to generate crystallographic evidence of desired target binding and protein synthesis inhibition. After detailed structural analysis, three completely novel chemical scaffolds were developed, each representing a novel antibiotic class: the pyrrolocytosines, the phenoxazinocytosines and the isocytosines. Rib-X conducted in-depth analyses of the molecular properties of each scaffold with the objectives of demonstrating on-target activity, confirming that selected resistance mechanisms do not affect activity and optimizing each scaffold to provide microbiological activity against contemporary MDR Gram-negative strains of bacteria.

Of the three RX-04 classes, the pyrrolocytosines emerged as the lead series. Pyrrolocytosines bind to the P-loop of the large ribosomal subunit and inhibit translation by stabilizing a distorted mode of P-tRNA binding. These agents were developed along several chemical lines to yield multiple series of compounds with broad spectrum activity. Several distinct series showed consistent MICs of ≤ 4 µg/mL against MDR Gram-positives (enterococci and staphylococci) and MDR Gram-negatives, including *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* (MIC ranges 0.5 - 16 µg/mL). Three preclinical efficacy studies with RX-04 compounds in relevant infection models showed that compounds from all three scaffolds with diverse architectures demonstrated *in vivo* efficacy in models when used to treat infections caused by Gram-positive and Gram-negative organisms. Representative RX-04 compounds demonstrated efficacy in a murine skin and soft tissue infection model caused by *K. pneumoniae*, with bacterial load reductions of 1 - 2 log10 cfu over 22 hours, compared to bacterial levels in untreated control tissues increasing by approximately 2 log10 cfu.

Methods and Materials

Susceptibilities of 55 carbapenem susceptible and resistant *K. pneumoniae* isolates, including 6 wild-type control strains, 36 KPC-producers, 8 ESBL-producers and 5 characterized porin-deficient strains, were determined by CLSI broth microdilution[1] against RX-P792, RX-P873, RX-P933 and RX-P934 (Rib-X Pharmaceuticals). Isolates included 5 colistin and two tigecycline resistant strains. Agents were tested at concentrations of 0.03 to 16 µg/mL. The structures of the agents are shown in Figure 1.

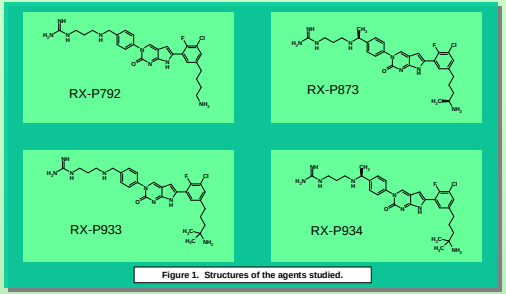

Figure 1. Structures of the agents studied.

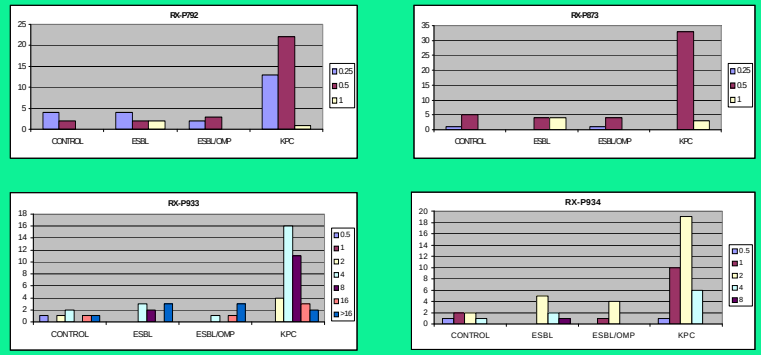

Figure 2. Histograms of MICs of the 4 RIB-X agents

Results

Unimodal MIC distributions were found for all 4 agents, with MIC values (ug/ml) shown in Figure 2. MICs were not affected by resistance to other agents, including carbapenems, quinolones, aminoglycosides, tigecycline and colistin. RX-P792 was the most potent agent tested, with MIC90 of 0.5 ug/ml, followed by RX-P873 and RX-P934, while RX-P933 was the least potent, with MIC90 of >16 ug/ml.

Discussion

Until recently, carbapenems were active against the vast majority of Gram negative pathogens encountered clinically and could be used empirically without much concern for resistance. This situation has changed considerably in recent years, and a wide variety of these pathogens have become resistant to carbapenems, associated with a variety of enzymatic and other mechanisms, as well as to most other drug classes. The Gram negative pathogens most associated with resistance include the "ESKAPE Group," consisting of *Escherichia coli*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae* and *K. oxytoca*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*. To overcome the growing problem of microbial resistance, researchers and pharmaceutical companies have adopted a number of strategies, including development both of new classes of agents and new beta-lactamase inhibitors to overcome the production of beta-lactamases.[2-4]

Two of the 4 pyrrolocytosine agents tested showed potent in vitro activity against *K. pneumoniae*, including MDR, carbapenem and colistin resistant isolates. Further development of these agents is warranted to determine their potential for clinical use.

References

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