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Novel Ribosome Inhibitors are Efficacious in a Murine Respiratory Tract Infection Model Caused by *Streptococcus pneumoniae*

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Modified Abstract

Background: There is a dearth of novel antibacterial agents with which to treat serious Gram-positive and Gram-negative infections. We have used structure-based drug design to generate novel compounds in the RX-04 program targeting the large subunit of the bacterial ribosome. RX-04 compounds have demonstrated robust broad-spectrum in vitro activity as well as efficacy in several *in vivo* infection models against pathogens of clinical interest. The goal of the present study was to evaluate the abilities of exemplar compounds to reduce lung bacterial burdens and protect mice in a pulmonary infection model caused by *Streptococcus pneumoniae*.

Methods: For PD_{50} studies, murine pulmonary infection was initiated by intranasal instillation of bacteria into the lungs; BID therapy was initiated 18 hours later and survival was monitored for 7 days. For lung bacterial burden studies, therapy was administered as a single dose at 4 hours post-challenge. Efficacy was measured by determining bacterial burden in lungs at 24 hours. Tissue samples were homogenized, diluted and plated for colony-forming unit (cfu) enumeration.

Results: Compounds were used to treat pulmonary infections caused by *S. pneumoniae* and efficacy was evaluated by cfu reduction over 24 hours and survival over 5 days. Lung bacterial burden studies corroborated these findings, with many RX-04 compounds demonstrating cfu reductions in this model. Dose titration studies indicated that several compounds from this program had single-digit PD_{50}s against this pathogen.

Conclusion: Exemplar compounds from the RX-04 program were used to demonstrate efficacy in a pulmonary infection model against *S. pneumoniae*. Compounds were efficacious as demonstrated by cfu reductions in lung tissues and protection with a single dose. These results add further value to compounds from the RX-04 program, which have already demonstrated efficacy in murine models of peritonitis, kidney and thigh abscess infection against key bacterial pathogens.

Introduction

There is a dearth of novel antibacterial agents with which to treat serious Gram-positive and Gram-negative infections (1,2). Of particularly challenge to treat are nosocomial pulmonary infections due to a range of etiologic agents, so-called HAP (hospital-acquired pneumonia), HCAP (health care-associated pneumonia) and VAP (ventilator-acquired pneumonia). Antibacterial agents to treat serious Gram-positive and Gram-negative infections often fall short due to bacterial resistance, poor tissue penetration or toxicity, leaving clinicians with few therapeutic options [3, 4]. Using structure-based drug design we have generated novel compounds in the RX-04 program that target the large subunit of the bacterial ribosome and have activity against a broad range of clinically-relevant pathogens. Many compounds in this series have excellent properties in terms of in vitro potency, whole-cell activity, pharmacokinetics, tolerability and efficacy in several murine models, such as peritonitis, kidney, and thigh abscess infections. In models of peritonitis and thigh abscess several compounds from this program are capable of delivering single-digit, single-dose efficacy.

For the present study, we focused on a subset of compounds that have robust activity against *S. pneumoniae*, excellent mouse exposure, and demonstrated single-dose efficacy in the murine lung infection model. In addition, we show here that several compounds can protect mice from lethal *S. pneumoniae* challenge and reduce bacterial burdens in lungs to below detectable levels. Such robust demonstrations of efficacy at low doses against *S. pneumoniae* as well as against a broad range of infection models and organisms bode well for these compounds, which would present a great clinical benefit.

Methods

Bacteria were prepared for infection studies by inoculating from frozen stocks onto Tryptic Soy Agar plates containing 5% sheep blood and incubating overnight at 37˚ C in 5% CO_2. The next day, colonies were inoculated into pre-warmed TSB, incubated without shaking at 37˚ C in 5% CO_2 for 4 hours, and adjusted to ~2.5 x 10^6 cfu/ml.

For lung bacterial burden studies, female Swiss-Webster mice (average weight 21 – 25g) were anesthetized with isoflurane (3% in oxygen) and infected via intranasal instillation with 40µl of bacterial inoculum containing ~1.5 x 10^5 cfu of *S. pneumoniae* 10813 (5) per mouse; therapy was administered as a single dose at 1 - 4 hours post-challenge. RX-04 compounds were administered via subcutaneous injection; the positive control compound telithromycin (Ketek™) was dosed at 50 mg/kg via oral gavage. Efficacy was measured by determining bacterial burden in lungs at 24 hours. Tissue samples were homogenized, diluted and plated for colony-forming unit (cfu) enumeration. Data are presented as Δcfu compared to untreated controls at the start of therapy.

For PD_{50} studies, murine pulmonary infection was initiated by intranasal instillation of 40µl of bacterial inoculum containing ~ 1 x 10^5 cfu of *S. pneumoniae* 02J1016 (6) into the lungs of isoflurane-anesthetized female Swiss-Webster mice (average weight 21 – 25 g); BID therapy was initiated 18 hours later and survival was monitored for 7 days. The PD_{50} value of each compound was calculated with Prizm Compiled Pharmacodynamic Model (Sigmoidal Dose-Response Variable Slope Model).

Results

Table 1. Structures, MICs and AUCs of compounds used in the present studies.

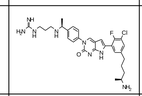
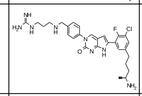
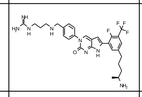
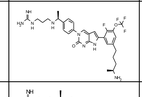
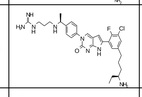
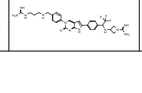
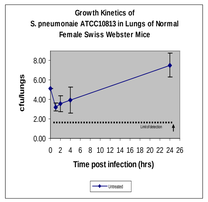
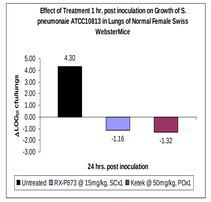
Compound	Structure	MIC vs. *S. pneumoniae* 10813 (µg/mL)	AUC_{0-24} in mouse at 10 mg/kg sc dose (µg*hr/mL)
RX-P873		≤ 0.06	27.9
RX-P907		≤ 0.06	18.5
RX-P914		≤ 0.06	14.5
RX-P954		≤ 0.06	24.7
RX-P1011		≤ 0.06	40.5
RX-P1032		0.125	19.4

Figure 1. Growth kinetics of *S. pneumoniae* 10813 in lungs of female Swiss-Webster mice (A). Efficacy of RX-P873 in a lung infection model caused by *S. pneumoniae* 10813 following single-dose treatment at 1 hour (B), 2 hours (C) or 4 hours (D) post-infection.

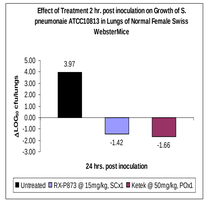
A.



B.



C.



D.

Figure 2. Dose-titration efficacy of RX-P873 in lung infection model caused by *S. pneumoniae* 10813 following single-dose treatment at 4 hours post-infection.

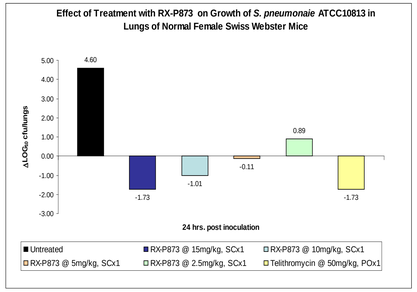
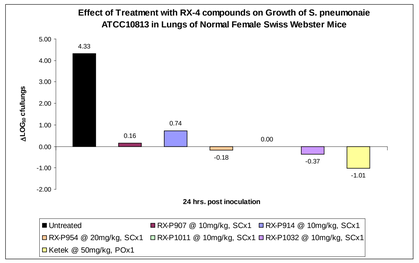


Figure 3. Efficacy of RX-04 compounds in lung infection model caused by *S. pneumoniae* 10813 following single-dose treatment at 4 hours post-infection.



Results

- Structures and properties of compounds used in these studies are shown in **Table 1**. These novel compounds have all demonstrated efficacy in other tissue-based infection models and so were evaluated here for efficacy in a model involving infection in a pulmonary organ system.
- The lung bacterial burden model used is a lethal model, with animals in the untreated control groups succumbing to the infection within 48 hours.
- The model involves a single dose given 4 hours post-intranasal infection; lungs were harvested, homogenized, diluted and plated for cfu burdens at 24 hours post-infection. **Figure 1A** shows the kinetics of *S. pneumoniae* 10813 growth in mouse lungs
  - **Figures 1B, 1C** and **1D** show the 24-hour lung cfu burdens when RX-P873 therapy is administered at 1, 2 or 4 hours post-infection. As the greatest Δ was observed with treatment at 4 hours but with similar efficacy and protection to the other regimens, this dosing regimen was chosen for the remainder of the studies.
- **Figure 2** shows the results of a dose-titration study with compound RX-P873; whereas bacterial growth in the untreated control group increased by 4.6 log_{10} cfu, RX-P873 demonstrated efficacy at 24 hours when dosed at 15 and 10 mg/kg, with 1.73 and 1.01 log_{10} cfu decreases at 24 hours, respectively, and had a static effect at the 5 mg/kg dose. Telithromycin demonstrated a 1.73 log_{10} cfu decrease at 24 hours.
- Five other RX-04 compounds were evaluated in this model, and the results are shown in **Figure 3**. In this study, the bacterial burden in the untreated control group increased by 4.33 log_{10} cfu; all the RX-04 compounds had a static effect except for RX-P914, which was not efficacious, though all compounds protected 100% of the mice at 24 hours. Telithromycin had a 1.01 log_{10} cfu decrease at 24 hours.
- **Table 2** shows survival data for the different doses of RX-P873 and RX-P954 over 5 days, compared to untreated controls (typically 0% survival at 48 hours) and telithromycin.
- PD_{50}s were generated for two of the most active compounds in this model and are shown in **Table 3**. Both are highly active in this model.

Table 2. 5-day survival of mice treated with RX-04 compounds following *S. pneumoniae* 10813 pulmonary infection

Compound	Dose (mg/kg/day)	% Survival on Day 5
RX-P873	30	100
	20	100
	10	100
	2	40
RX-P954	40	100
	20	100
	10	100
	2	60
Telithromycin	50	80
Untreated controls	None	0

Table 3. PD_{50}s of RX-04 compounds in *S. pneumoniae* 02J1016 pulmonary infection

Compound	MIC vs. *S. pneumoniae* 02J1016, µg/mL	PD_{50} (mg/kg/day)
RX-P873	≤ 0.06	3.4
RX-P954	≤ 0.06	2.8

Conclusions

- Several compounds from the novel RX-04 program were able to protect mice from lethal *S. pneumoniae* lung infection with a single dose given 4 hours post-infection.
- One compound profiled in detail, RX-P873, demonstrated good dose-response efficacy in terms of lung cfu burden reduction at 24 hours, compared to untreated controls at the start of therapy, as well as protection from lethality over 5 - 7 days.
- The organism used in the cfu burden model, *S. pneumoniae* 10813 demonstrated > 4 log_{10} cfu of growth in the lungs over the 24 hours of the study; most of the compounds studied demonstrated protection at 24 hours along with bacterial stasis.
- Efficacy in this pulmonary infection model augments the favorable properties of the RX-04 compounds, along with efficacy in peritonitis, thigh abscess and kidney infection models.

References

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