A Novel Ketolide, RBx 14255, with Activity against Multidrug-Resistant *Streptococcus pneumoniae*

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We present here the novel ketolide RBx 14255, a semisynthetic macrolide derivative obtained by the derivatization of clarithromycin, for its *in vitro* and *in vivo* activities against sensitive and macrolide-resistant *Streptococcus pneumoniae*. RBx 14255 showed excellent *in vitro* activity against macrolide-resistant *S. pneumoniae*, including an in-house-generated telithromycin-resistant strain (*S. pneumoniae* 3390 NDDR). RBx 14255 also showed potent protein synthesis inhibition against telithromycin-resistant *S. pneumoniae* 3390 NDDR. The binding affinity of RBx 14255 toward ribosomes was found to be more than that for other tested drugs. The *in vivo* efficacy of RBx 14255 was determined in murine pulmonary infection induced by intranasal inoculation of *S. pneumoniae* ATCC 6303 and systemic infection with *S. pneumoniae* 3390 NDDR strains. The 50% effective dose (ED₅₀) of RBx 14255 against *S. pneumoniae* ATCC 6303 in a murine pulmonary infection model was 3.12 mg/kg of body weight. In addition, RBx 14255 resulted in 100% survival of mice with systemic infection caused by macrolide-resistant *S. pneumoniae* 3390 NDDR at 100 mg/kg four times daily (QID) and at 50 mg/kg QID. RBx 14255 showed favorable pharmacokinetic properties that were comparable to those of telithromycin.

*S. pneumoniae* is an important human pathogen, and it has been identified as the primary cause of community-acquired bacterial pneumonia (CAPB) (1). *S. pneumoniae* is a major cause of morbidity and mortality associated with bacterial pneumonia, bacteremia, sinusitis, and bacterial meningitis (2). In recent years, antimicrobial drug resistance in *S. pneumoniae* has increased worldwide and has become a major health concern. The treatment of these infections remains challenging because of the antibacterial resistance and the emergence of multidrug-resistant (MDR) phenotypes (1, 3).

Macrolides are antimicrobial agents for the treatment of mild to moderate CAPB, and they are clinically useful for pediatric and penicillin-allergic patients. In fact, an increasing prevalence of β-lactam- and macrolide-resistant *S. pneumoniae* strains has been observed in many countries (4). In the Asian Network for Surveillance of Resistant Pathogens (ANSORG) study, Kim et al. (5) demonstrated that the world’s highest level of antimicrobial resistance in *S. pneumoniae* is in Asian countries.

Ketolides represent the latest generation of macrolide antibiotics, displaying improved activities against both erythromycin-resistant and -sensitive *S. pneumoniae* (6, 7). Telithromycin, the first member of the ketolide family, was developed to overcome macrolide resistance, and *in vitro* data showed activity against erythromycin-resistant strains (8–10). Telithromycin demonstrated high efficacy against *S. pneumoniae* isolates that cause community-acquired respiratory tract infection. Telithromycin was approved in 2004 for treating respiratory tract infections, but concerns over liver toxicity and other serious adverse events were noted soon after its launch (11). After several telithromycin-related deaths, its approval was withdrawn for all indications except for CABP (11, 12). The emergence of clinical isolates of *S. pneumoniae* with telithromycin resistance was also reported (13, 14). In this situation, exploration and further development of new ketolides require resolving these issues with telithromycin (11).

Still, there is an urgent need for alternative drugs to treat both sensitive and multidrug-resistant *S. pneumoniae* (4, 11, 14). In this regard, many pharmaceutical companies have synthesized and evaluated new macrolides and ketolides against multidrug-resistant respiratory pathogens (15–20). Recently, a randomized double-blind multicenter phase 2 study that compared the efficacy and safety of oral solithromycin (CEM-101) in the treatment of patients with community-acquired bacterial pneumonia was reported by Cempra, Inc. (21). In this study, we present the novel ketolide RBx 14255, a semisynthetic macrolide derivative obtained by the derivatization of clarithromycin, and its excellent *in vitro* and *in vivo* activities against macrolide-susceptible and -resistant *S. pneumoniae* strains. The mode of action of RBx 14255 showed potent protein synthesis inhibition and a strong binding affinity toward ribosomes.

**MATERIALS AND METHODS**

**Bacterial strains and drugs.** The bacterial strains used for the *in vitro* studies are listed in Table 1. *S. pneumoniae* ATCC 6303 and *S. pneumoniae* 3390 NDDR were used for the *in vivo* studies. *Escherichia coli* MRE 600 was used as a control strain. *E. coli* MRE 600 does not harbor the β-lactamase gene, so it is sensitive to β-lactam antibiotics. *E. coli* MRE 600 was used as a control strain. *E. coli* MRE 600 does not harbor the β-lactamase gene, so it is sensitive to β-lactam antibiotics.
TABLE 1 MICs for RBx 14255 and other drugs against S. pneumoniae

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<th>Clindamycin</th>
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In vitro susceptibility testing. The MICs of RBx 14255, telithromycin, clarithromycin, erythromycin, clindamycin, azithromycin, and other drugs (listed in Table 1) against S. pneumoniae strains were determined using the broth microdilution methods recommended by the Clinical and Laboratory Standards Institute (CLSI) (22).

Generation of telithromycin-resistant S. pneumoniae strains. Macrolide-resistant S. pneumoniae 3390, with an MIC of >32 μg/ml against erythromycin, clarithromycin, azithromycin, and clindamycin, was used to develop resistance against telithromycin by serial passaging, and the resistant strain was named S. pneumoniae 3390 NDDR. The selected resistant strain was checked for stability by subculturing on drug-free medium plates. The MICs of telithromycin were determined by the broth microdilution method against the resistant strain to confirm resistance (22).

Macromolecular synthesis inhibition studies. Macromolecular biosynthesis inhibition in S. pneumoniae was studied as described by Kalia et al. (23), with slight modification. We used the radiolabeled precursors [14C]isoleucine (protein), [3H]thymidine (DNA), [3H]uridine (RNA), N-acetylglucosamine (cell wall), and [14C]acetate (fatty acid).

Briefly, radioactive precursors (1 μCi/ml for 3H-labeled and 0.1 μCi/ml for 14C-labeled compounds) were added during the early logarithmic phase of S. pneumoniae 3390 NDDR cultures. After 5 min, 0.5 μg/ml RBx 14255 (1× MIC) was added, and the wells were harvested at 15, 30, 60, and 120 min. In another experiment with the radiolabeled precursor [14C]isoleucine for protein synthesis inhibition studies, various concentrations of both telithromycin and RBx 14255 were added during the early logarithmic phase of S. pneumoniae 3390 NDDR cultures, and the cells were harvested at 60 min. The macromolecules were precipitated with ice-cold trichloroacetic acid (final concentration, 5% [wt/vol]) and filtered on glass fiber filters (1.0 μm A/B glass multiwell filter plates; Pall Corporation). The plates were dried overnight at 37°C, and radioactivity counting was done in a Wallac scintillation counter.

Competition with [14C]erythromycin for binding to ribosomes by RBx 14255. The ribosomes from E. coli were prepared as described by Kiel et al. (24). E. coli MRE 600 cells were suspended in buffer A (20 mM Tris-HCl (pH 7.5), 10 mM Mg(OAc)2, 50 mM NH4Cl, 1 mM dithiothreitol (DTT)), and the cells were lysed. The cell suspension was centrifuged at 30,000 × g for 15 min, and the pellets were discarded. The supernatant was layered onto sucrose solutions [20 mM Tris-HCl (pH 7.5), 10 mM Mg(OAc)2, 500 mM NH4Cl, 2 mM DTT, 1 mM sucrose] and centrifuged at 30,000 rpm in a Ti70 rotor for 4 h. The crude ribosome pellets were resuspended in buffer B (20 mM Tris-HCl (pH 7.5), 10 mM Mg(OAc)2, 500 mM NH4Cl, 2 mM DTT) (no sucrose) cleared by a 15-min centrifugation step at 30,000 × g, and pelleted at 35,000 rpm in a Ti70 rotor for 2 h. The washed ribosome pellet was resuspended in storage buffer (buffer A without the 50 mM NH4Cl) and stored at −80°C. The binding of telithromycin, RBx 14255, and other drugs at a 2 μM concentration was observed in competition experiments with [14C]erythromycin. The competition experiments for the drugs with [14C]erythromycin were performed as described by Xiong et al. (25).

Experimental animals. All animal studies were approved by the institutional animal ethics committee (IAEC) at Ranbaxy Research Laboratory (Gurgaon, Haryana, India) per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (New Delhi, India). Swiss albino mice (n = 6) weighing 20 ± 2 g of either sex were used for the study. The animals were purchased from an animal breeding and housing facility (Ranbaxy Research Laboratories, Gurgaon, India) 2 to 3 days prior to the start of experiments to acclimatize them to the experimental environment (temperature, 25°C ± 2°C; rela-
tive humidity, 30% to 70%). Food and water were provided ad libitum during the study.

**Mouse pulmonary infection with sensitive *S. pneumoniae* ATCC 6303.** Pulmonary infection was established with *S. pneumoniae* ATCC 6303 as described by Barman et al. (26). Briefly, a culture grown overnight was suspended in sterile saline to obtain $1 \times 10^8$ CFU/ml. The mice were anesthetized with isoflurane by using an inhalant anesthesia machine and then infected intranasally with 50 μl of the infecting dose. RBx 14255 was administered twice daily (BID) orally for 3 days at a dose of 3.12 mg/kg of body weight and 12.5 mg/kg at 30 min and 4 h postinfection on day 1 and 6 h apart on days 2 and 3. Telithromycin was used as a reference standard during the study.

**Mouse systemic infection with macrolide-resistant *S. pneumoniae* 3390 NDDR.** The minimum lethal dose (MLD) of *S. pneumoniae* 3390 NDDR for systemic infection was determined as the minimum number of cells required to cause 90% to 100% mortality. The *in vivo* efficacy of RBx 14255 was determined for murine systemic infection caused by the telithromycin-resistant *S. pneumoniae* 3390 NDDR strain. RBx 14255 and telithromycin were administered 12 h apart. The animals were observed for 7 days, and the survival of the mice was recorded and analyzed as described above.

**In vivo pharmacokinetic studies.** Male Swiss mice (20 ± 2 g, n = 3) were used for the pharmacokinetic study. Animals were fasted for 4 h before and 2 h after dosing. The animals were dosed with either 25 mg/kg orally or 5 mg/kg intravenously. RBx 14255 and telithromycin were prepared in pH 4.5 acetate buffer. The test compounds were administered orally using an 18-gauge stainless steel feeding needle (5 ml/kg) and intravenously as a bolus (2 ml/kg) through the tail vein. Blood samples were collected by sampling at 5, 15, and 30 min and 1, 2, 4, 8, 12, and 24 h postdose (for the mice given the dose intravenously) or at 15 and 30 min and 1, 2, 4, 8, 12, and 24 h postdose (for the mice given the dose orally). For pharmacokinetic profiling of lung concentrations, 21 mice were used per test compound. At each sampling point, three mice were sacrificed, and the lungs of three animals were pooled. The sampling time points were predose and 0.25, 0.5, 1, 2, 4, 8, and 24 h postdose. The pooled lungs were homogenized in double the weight volume of ice-cold phosphate buffer (pH 7.4) and stored at −80°C until analysis. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays of plasma and lung samples were performed on a SCIEX API-4000 mass spectrophotometer equipped with a PerkinElmer 200 series high-performance liquid chromatograph (HPLC). Noncompartmental analysis was performed using WinNonlin 4.0 Pro software (Pharsight, Mountain View, CA). Pharmacokinetic parameters were calculated using the mean concentration values of three animals.

**RESULTS**

The macrolide-resistant *S. pneumoniae* 3390 NDDR strain showed resistance to macrolides as well as the ketolide telithromycin. As shown in Table 1, RBx 14255 was active against the telithromycin-resistant *S. pneumoniae* 3390 NDDR strain and had an MIC of 0.5 μg/ml. Telithromycin, erythromycin, clarithromycin, azithromycin, and clindamycin had MICs of >32 μg/ml against *S. pneumoniae* 3390 NDDR. All these drugs, including RBx 14255, showed excellent *in vitro* activity against the *S. pneumoniae* ATCC 6303 macrolide-susceptible strain (Table 1). Overall, RBx 14255 showed good *in vitro* activity against telithromycin-susceptible and -resistant *S. pneumoniae* strains; the highest MIC was 0.5 μg/ml against a telithromycin-resistant strain.

RBx 14255 was found to be a potent protein synthesis inhibitor, and it inhibited the incorporation of the specific radiolabeled precursor [14C]isoleucine into the nascent polypeptide chain during protein synthesis in macrolide-resistant *S. pneumoniae* 3390 NDDR (Fig. 2a). RBX 14255 did not inhibit DNA, RNA, lipid, or cell wall synthesis in macrolide-resistant *S. pneumoniae* 3390 NDDR (Fig. 2a). Insignificant inhibition of RNA and cell wall synthesis was observed at 60 and 120 min. This indicates that protein synthesis inhibition is the primary mode of action of RBx 14255. In another experiment, the known protein synthesis inhibitor and ketolide telithromycin inhibited the protein synthesis of *S. pneumoniae* 3390 NDDR with less potency, but potent protein synthesis inhibition was observed with RBx 14255, even at sub-MICs (Fig. 2b). RBx 14255 inhibited protein synthesis (for ≥50%
inhibition) at a 0.125-µg/ml concentration, while ≥16 µg/ml telithromycin was needed to inhibit protein synthesis (Fig. 2b). In addition, a membrane disruption assay was performed for RBx 14255, and it was observed that RBx 14255 did not show any membrane disruption activity (data not shown).

Ribosomes from *E. coli* MRE 600 were prepared, and the binding of [14C]erythromycin to the 70S ribosome and competition with RBx 14255 were assessed (Fig. 3). The quality of the ribosomes was checked by the sucrose density gradient method with ultracentrifugation, and RBx 14255 was found to contain only 70S ribosomes. The binding of RBx 14255 to ribosomes was found to be more than with other drugs, and RBx 14255 displaced [14C]erythromycin, as depicted in Fig. 3. The 50% inhibitory concentrations (IC50) for binding to 70S ribosomes were 148 nM for RBx 14255 and 408 nM for telithromycin.

**FIG 2** (a) Macromolecular synthesis inhibition by RBx 14255 in macrolide-resistant *S. pneumoniae* 3390 NDDR. The concentration of RBx 14255 was 0.5 µg/ml (1× MIC), and macromolecular synthesis inhibition was observed at 15, 30, 60, and 120 min. (b) Protein synthesis inhibition in macrolide-resistant *S. pneumoniae* 3390 NDDR by RBx 14255 and telithromycin. NAG, *N*-acetylglucosamine.

The *in vivo* efficacy of RBx 14255 was determined for murine pulmonary infection caused intranasally by *S. pneumoniae* ATCC 6303. As shown in Fig. 4a, all the mice treated with 12.5 mg/kg RBx 14255 BID for 3 days survived, whereas treatment with telithromycin resulted in a 76.7% survival rate. Similarly, at 3.12 mg/kg, RBx 14255 and telithromycin resulted in 53.3% and 28.9% survival rates, respectively.

**FIG 3** Inhibition of [14C]erythromycin binding to ribosome by RBx 14255: competition studies for the binding of RBx 14255 and comparison with other drugs.

The *in vivo* efficacy of RBx 14255 against systemic infection with *S. pneumoniae* 3390 NDDR was analyzed, and RBx 14255 showed 87.8% and 100% survival rates, respectively, at 50-mg/kg QID and 100-mg/kg QID doses, compared to the 0% survival rate with telithromycin at 50 mg/kg QID and the 32.8% survival rate with telithromycin at 100 mg/kg QID (Fig. 4b). In addition, RBx 14255 resulted in a 100% survival rate at 100 mg/kg BID and a 47.8% survival rate at 50 mg/kg BID (Fig. 4c). Telithromycin resulted in a 0% survival rate at the 100-mg/kg BID and 50-mg/kg BID doses. RBx 14255 showed *in vivo* efficacy against both macrolide-resistant and -susceptible *S. pneumoniae*.

**FIG 4** (a) In vivo efficacy of RBx 14255 against macrolide-resistant *S. pneumoniae* ATCC 6303. All mice treated with 12.5 mg/kg RBx 14255 BID for 3 days survived, whereas 76.7% of mice treated with telithromycin survived. (b) In vivo efficacy of RBx 14255 against systemic infection with *S. pneumoniae* 3390 NDDR. RBx 14255 showed 87.8% and 100% survival rates, respectively, at 50-mg/kg QID and 100-mg/kg QID doses, whereas telithromycin resulted in only a 32.8% survival rate at 100 mg/kg QID. (c) In vivo efficacy of RBx 14255 against systemic infection with *S. pneumoniae* 3390 NDDR. RBx 14255 resulted in an 87.8% survival rate at 50 mg/kg BID and a 100% survival rate at 100 mg/kg BID. Telithromycin resulted in a 0% survival rate at 100 mg/kg BID and 50 mg/kg BID.

**DISCUSSION**

Antibacterial resistance, and macrolide resistance in *S. pneumoniae* in particular, is increasing worldwide, and this presents treatment challenges to physicians (8, 27). Ketolides are active against pneumococci that have a macrolide efflux pump (9). Telithromycin is active against macrolide-resistant pneumococci that contain either the methylase or the efflux mechanism of resistance (8, 9). However, the greater frequency of liver damage with telithromycin than with other macrolides resulted in its withdrawal of approval for two of three indications after postmarketing reports (11, 28). RBx 14255, a fluoroketolide was evaluated for its *in vitro* and *in vivo* potential against macrolide-resistant *S. pneumoniae*.

The *in vitro* activity of RBx 14255 against drug-resistant *S. pneumoniae* is comparable with that of other ketolides, such as telithromycin and cethromycin (29). *In vitro* activities similar to those of RBx 14255 were reported for cethromycin (ABT-773), another ketolide, and telithromycin against many resistant phenotypes, including activity against penicillin and macrolide-resistant Gram-positive bacteria (29). In a study by Sato et al. (30), modithromycin (EDP-420, EP-013420, S-013420), a novel 6,11-
bridged bicyclolide, showed excellent in vitro activity against clinical isolates of Gram-positive and macrolide-resistant Gram-negative pathogens. In 2010, McGhee et al. (18) reported the in vitro activity of a fluoroketolide, solithromycin (CEM-101), against S. pneumoniae, and the in vitro activities were comparable to those of our fluoroketolide, RBx 14255. Solithromycin is currently being developed in phase 3 clinical trials at Cempra, Inc., for the once-daily oral treatment of mild to moderate community-acquired bacterial pneumonia (CAPB), and phase 2 clinical trial results were recently reported (21). In 2010, Bertrand et al. (12) reported that a pyridine-imidazole group of the ketolide telithromycin could be the possible cause of its adverse reactions, and the fluoroketolide solithromycin lacks this pyridine-imidazole side chain.

RBx 14255, a fluoroketolide, also lacks the combination pyridine-imidazole side chain, and the adverse reactions may be less than those with telithromycin and comparable to those with solithromycin. In addition, RBx 14255 showed good in vitro activity against the telithromycin-resistant S. pneumoniae 3390 NDDR strain.

RBx 14255 showed potent protein synthesis inhibitory activity using ribosomes from telithromycin-resistant S. pneumoniae 3390 NDDR. It also showed specific inhibitory activity toward protein synthesis and not toward any other macromolecules. Macrolide and ketolide antibiotics interfere with the elongation of the growing polypeptide chain on the 50S ribosome, and protein synthesis is terminated by the activity of these drugs (29, 31). During extensive study on the mechanism of action of ketolides, Mankin’s group and others (32–34) described mistranslation as part of the mechanism of action of the ketolides. It has been noted that translational frameshifting contributes to mistranslated proteins that are potentially toxic, converting macrolides to being bactericidal (32–34). In addition, the researchers noted that instead of blocking protein synthesis in general, ketolides may inhibit protein synthesis selectively (34).

Ketolides are known to interact with multiple sites in addition to the macrolide binding site on domain V. Douthwaite et al. showed that telithromycin was able to bind more tightly than erythromycin (35). This was attributed to the binding of telithromycin not only on domain V of 23S rRNA (the binding site of macrolide is adenosine 2058) but also on domain II (adenosine 752) (35). As a result, telithromycin showed activity against erythromycin-resistant and multidrug-resistant isolates of S. pneumoniae (36). RBx 14255 interacts with ribosomes, and its binding affinity was greater than that of telithromycin and other macrolides. The higher affinity of RBx 14255 to ribosomes may be partially responsible for its increased in vitro potency. The differences in IC50s between the compounds correlate well with the overall in vitro efficacy of RBx 14255 compared to that of telithromycin in a murine pulmonary infection model against an S. pneumoniae ATCC 6303 drug-sensitive strain. Shown is a Kaplan-Meier survival curve of mice treated with 12.5 mg/kg RBx 14255 (○), 3.12 mg/kg RBx 14255 (▲), 12.5 mg/kg telithromycin (●), or 3.12 mg/kg telithromycin (●) in a murine pulmonary infection experiment with an S. pneumoniae ATCC 6303 drug-sensitive strain and in untreated infection control (●) and negative control (○) mice.

(b) In vivo efficacy of RBx 14255 in a QID dosing regimen compared to that of telithromycin in a murine systemic infection model against the macrolide-resistant S. pneumoniae 3390 NDDR strain. Shown is a Kaplan-Meier survival curve of mice treated with 100 mg/kg RBx 14255 (△), 50 mg/kg RBx 14255 (▲), 100 mg/kg telithromycin (●), or 50 mg/kg telithromycin (●) in a murine systemic infection experiment with an S. pneumoniae 3390 NDDR strain and in untreated infection control (●) and negative control (○) mice. (c) In vivo efficacy of RBx 14255 in a twice-daily dosing regimen compared to that of telithromycin in a murine systemic infection model against the macrolide-resistant S. pneumoniae 3390 NDDR strain. Shown is a Kaplan-Meier survival curve of mice treated with 100 mg/kg RBx 14255 (△), 50 mg/kg RBx 14255 (▲), 100 mg/kg telithromycin (●), or 50 mg/kg telithromycin (●) in a murine systemic infection experiment with an S. pneumoniae 3390 NDDR strain and in untreated infection control (●) and negative control (○) mice.

**TABLE 2** Pharmacokinetic parameters for RBx 14255 and telithromycin in male Swiss albino mice

<table>
<thead>
<tr>
<th>Dose and parameters</th>
<th>Plasma</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBx 14255</td>
<td>Telithromycin</td>
</tr>
<tr>
<td>5 mg/kg intravenously</td>
<td>AL/Cmax (μg · h/ml)</td>
<td>5.25</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>CLp (ml/min/kg)</td>
<td>15.9</td>
<td>16.2</td>
</tr>
<tr>
<td>Vp (liter/kg)</td>
<td>1.10</td>
<td>1.56</td>
</tr>
<tr>
<td>25 mg/kg orally</td>
<td>Cmax (μg/ml)</td>
<td>6.13</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>3.4</td>
<td>1.7</td>
</tr>
<tr>
<td>ALUCmax (μg · h/ml)</td>
<td>22.4</td>
<td>39.98</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>85</td>
<td>~100</td>
</tr>
</tbody>
</table>

*Vp* volume of distribution at steady state; *Cmax* maximum concentration of drug in plasma; *T1/2* time to maximum concentration of drug in plasma; %F, percent bioavailability.

*NA, not applicable.

**FIG 4** (a) In vivo efficacy of RBx 14255 compared to that of telithromycin in a murine pulmonary infection model against an S. pneumoniae ATCC 6303 drug-sensitive strain. Shown is a Kaplan-Meier survival curve of mice treated with 12.5 mg/kg RBx 14255 (△), 3.12 mg/kg RBx 14255 (▲), 12.5 mg/kg telithromycin (●), or 3.12 mg/kg telithromycin (●) in a murine pulmonary infection experiment with an S. pneumoniae ATCC 6303 drug-sensitive strain and in untreated infection control (●) and negative control (○) mice. (b) In vivo efficacy of RBx 14255 in a QID dosing regimen compared to that of telithromycin in a murine systemic infection model against a macrolide-resistant S. pneumoniae 3390 NDDR strain. Shown is a Kaplan-Meier survival curve of mice treated with 100 mg/kg RBx 14255 (△), 50 mg/kg RBx 14255 (▲), 100 mg/kg telithromycin (●), or 50 mg/kg telithromycin (●) in a murine systemic infection experiment with an S. pneumoniae 3390 NDDR strain and in untreated infection control (●) and negative control (○) mice. (c) In vivo efficacy of RBx 14255 in a twice-daily dosing regimen compared to that of telithromycin in a murine systemic infection model against the macrolide-resistant S. pneumoniae 3390 NDDR strain. Shown is a Kaplan-Meier survival curve of mice treated with 100 mg/kg RBx 14255 (△), 50 mg/kg RBx 14255 (▲), 100 mg/kg telithromycin (●), or 50 mg/kg telithromycin (●) in a murine systemic infection experiment with an S. pneumoniae 3390 NDDR strain and in untreated infection control (●) and negative control (○) mice.
vitro activities, with RBx 14255 having more affinity for ribosome binding than other drugs. A new fluoroketolide, solithromycin (CEM-101), displaced $^{[14]}$C-erythromycin from E. coli ribosomes, with an IC$_{50}$ of 155 nM (37). The binding of CEM-101 to E. coli ribosomes is comparable to the binding of RBx 14255 to E. coli ribosomes, with an IC$_{50}$ of 148 nM. The potent protein synthesis inhibition against telithromycin-resistant S. pneumoniae 3390 NDDR may be attributed to the additional binding or tight binding of RBx 14255 to ribosomes. Llano-Sotelo et al. characterized the mechanism of action and the binding mode of solithromycin (CEM-101) to ribosomes and identified the additional stronger interaction of the aminopyrimidine moiety with the 23S rRNA of ribosomes at the A752-U2609 base pair, which accounts for the major difference in the binding mode of solithromycin from that of other macrolides (37). It is possible that the aminopyrimidine side chain of fluoroketolide RBx 14255, like solithromycin, has additional binding sites in the 23S rRNA of ribosomes, and that may be one of the reasons for its potent inhibition of protein synthesis and excellent in vitro activity against S. pneumoniae, including telithromycin-resistant S. pneumoniae.

The potent protein synthesis inhibition and the excellent in vitro activity and pharmacokinetics of RBx 14255 were reflected in the in vivo activity against S. pneumoniae in the murine model. In addition, RBx 14255 showed in vivo efficacy against the telithromycin-resistant S. pneumoniae 3390 NDDR strain. The in vitro activity of RBx 14255 against telithromycin-resistant S. pneumoniae 3390 NDDR was also reflected in its in vivo efficacy. RBx 14255 showed high oral bioavailability, good distribution, and low plasma clearance in mice, comparable to those of telithromycin. It was reported that telithromycin has very good oral bioavailability and a favorable pharmacokinetic profile in respiratory infections (7). RBx 14255 was better than telithromycin at half the exposure levels in plasma in Swiss mice. The better efficacy of RBx 14255 over telithromycin could be due to the long half-life ($t_{1/2}$) achieved in both plasma and lung pharmacokinetic studies.

In summary, the ketolide telithromycin was developed to overcome macrolide resistance mechanisms, including target site modification and active efflux. Some telithromycin resistance has been identified, and because of the serious adverse events associated with telithromycin, it is no longer used. In this context, we report a novel fluoroketolide, RBx 14255, which showed good in vitro and in vivo activities against macrolide-resistant and susceptible S. pneumoniae strains. RBx 14255 is a potent protein synthesis inhibitor with a high binding affinity to ribosomes. Since multidrug-resistant pathogens are emerging worldwide, there is an urgent need for the development of new antibiotics. In this situation, RBx 14255 can be further explored as a suitable candidate for development against not only macrolide-resistant S. pneumoniae but also all respiratory pathogens due to its excellent in vitro and in vivo activities and pharmacokinetics. Clinical trials need to be conducted.

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