Antimicrobial Activity of a Novel Program of Protein Synthesis Inhibitors Against Clinical Isolates Containing KPC and NDM-1 Carbapenemases

J. DeVito, J. Remy, A. Bhattacharjee, Z. Kanyo and E. Duffy

Rib-X Pharmaceuticals, Inc., New Haven, CT, USA

ABSTRACT

Objective: Multidrug-resistant Gram-negative bacteria are an increasing risk to public health. In these organisms, multiple antibiotic elements confer resistance to most antimicrobial agents currently used in the clinic. Of particular concern are those species of enteric pathogens containing extended spectrum β-lactamases and carbapenemases such as KPC and NDM-1. Clinical isolates with such resistant elements have emerged as a worldwide threat with few therapeutic options remaining. For this reason, we undertook a detailed study of resistance mechanisms in these isolates. We tested representatives from these novel classes of antibiotics for activity against a number of these highly resistant Gram-negative organisms. These compounds inhibit protein synthesis and bind to the 30S ribosomal subunit, a molecular target that has not been accessed by current marketed β-lactam antibiotics.

Methods: Clinical isolates were obtained from various laboratories and commercial repositories to include the Louis Stokes Cleveland Department of Veterans Affairs, Massachusetts General Hospital, Eurofins Medinet Inc., and the American Type Culture Collection. Susceptibility testing was performed using Kirby-Bauer and broth microdilution methods.

Results: Lead molecules from three unique scaffolds in this program were tested against contemporary clinical isolates of E. coli isolated from patients with urinary tract and wound infections, many having ESBL, AmpC, and quinolone-resistance mechanisms. The compounds were highly potent, inhibiting growth of these organisms as concentrations in the range of 0.25-8 mg/L. Similar results were obtained against urine and respiratory isolates of KPC, pneumovirus with ESBL and KPC resistance mechanisms. MICs ranging from 0.125-4 mg/L. These same compounds, when tested against NDM-1 strains of K. pneumoniae and E. cloacae susceptible to currently marketed antibiotics were also highly potent.

Conclusion: These protein synthesis inhibitors represent novel families of antimicrobial agents with promise in vitro activity against multidrug-resistant Gram-negative pathogens, including isolates expressing the KPC and metallo-b-lactamase activities.

INTRODUCTION

A growing number of community and hospital-acquired infections are caused by drug-resistant bacteria belonging to the family Enterobacteriaceae. The 2008 SMART study showed that the frequency of complicated intra-abdominal infections caused by E. coli is still on the rise, while the susceptibility to known antibiotic classes continues to decrease (1). A similar pattern is emerging for infections caused by K. pneumoniae (2). Traditionally, β-lactam antibiotics have been an effective treatment option for infections caused by these organisms. Since the development of penicillins and cephalosporins, resistance mechanisms have evolved in the form of hydrolytic enzymes that inactivate these drugs. Such β-lactamases belong to a diverse family of enzymes, often carried on mobile genetic elements, that have evolved to and extend an expanded spectrum of activity. ESBL render inactivates a broad range of β-lactam antibiotics. While β-lactamase inhibitors, such as clavulanate, have decreased the usefulness of the cephalosporins, newly evolved enzymes of the non-TEM, non-SHV type resistances β-lactamase inhibitors have emerged. The introduction of carbapenems presented another effective treatment option for certain of their resistance profiles. Resistance to these antibiotics can be mediated through the acquisition of plasmid-containing hydrolytic enzymes (the carbapenemases) with broad specificity, to be inactivated most if not all β-lactam antibiotics (3). Carbapenemase and other metallo-β-lactamases in E. coli, K. pneumoniae, and Enterobacter spp. represent a significant challenge in not only their intrinsic resistance, but also their plasmid- and transmissible nature. They provide a very real threat to the overall viability of this class of antibiotics.

METHODS

Each E. coli and K. pneumoniae strains were purchased from Eurofins Medinet Inc. (Chantilly, VA) and represent urinary, wound, blood and hospital-acquired infections patients from across the United States. These strains encode resistance mechanisms, as confirmed through susceptibility testing. The E. coli isolate with plasmid pMIV-5 and the K. pneumoniae isolates 958, 238, 267, 273, 361, 427 (5) were generously gifted of Robert Bonomo (Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH). The E. coli strain containing NDM-1 (8) is a strain obtained from Massachusetts General Hospital (a gift of Mary Jane Ferrero). The K. pneumoniae strains CM1024 containing KPC-2, CM1025 containing NDM-1, and CM1024 containing VIM-6-like Carbapenemases were obtained from Kenneth Thompson (Crongilton University School of Medicine, Idaho, US). E. coli strain 5952 and K. pneumoniae strain 4814 containing NDM-1 were purchased from ARI (Alliance, WA). Susceptibility testing was performed in cation-adjusted Mueller-Hinton broth (CAMHB) according to CLSI methods (7). LB broth plates were dissolved in 100% DMSO at the appropriate concentration and serial diluted 1:2.4/mL in 96-well plates. Stock solutions of standard antibiotics and controls were prepared according to the manufacturer’s instructions. Organisms were plated from frozen stock plates and incubated overnight prior to testing to ensure viability and sterility.

RESULTS AND DISCUSSION

Figure 1. Compounds from the RX-04 class of Protein Synthesis Inhibitors

Table 1: MIC values for E. coli ATCC 25922. Quality assurance of protein synthesis inhibitor concentrations (MIC) values for subsequent tests was performed to ensure that this strain. Categorization of interpretative criteria were determined according to CLSI criteria.

Table 2: RX-04 compounds are highly potent against E. coli clinical isolates.

Thirty (30) clinical isolates were used as a measure of the potential utility of RX-04 compounds to combat infections caused by E. coli. This panel of organisms was weighted toward drug resistant isolates, with 18/30 strains classified as ESBL and 6/30 isolates classified as AmpC. RX-04 compounds were significantly more potent, with exemplar compounds from series I showing MICs of ≤0.06 mg/L, or lower.

Table 3: RX-04 compounds are highly potent against K. pneumoniae isolates.

Thirty (30) clinical isolates were used as a measure of the potential utility of RX-04 compounds to combat infections caused by K. pneumoniae. This panel of organisms was weighted toward drug resistant isolates with 10/30 strains classified as CARB-RES. All strains were classified as AmpC, 10/30 isolates were susceptible to ceftriaxone, and 2/30 isolates were impervious to cefepime. MICs 50/50 range for the KPC-2 strain contained against these organisms was ≤3.2 mg/L. MICs 50/50 range for the RX-04 compounds against these organisms was 0.25-32 mg/L. RX-04 compounds were significantly more potent, with exemplar compounds from series I showing MICs of ≤0.06 mg/L, or lower.

Table 4: Susceptibility of drug-resistant Enterobacteriaceae to RX-04 compounds

Perhaps the most serious threat to current antibiotic treatment regimens is the emergence of resistant isolates that encode metallo-β-lactamases. Metallo-β-lactamases of the β-Lactam and VMH4-like classes are represented in Table 5 for two key E. coli and the carbenemase-positive β-Lactamase. RX-04 compounds from the RX-04 program have MICs for the NDM-1, RP, and VMH strains as low as 0.5 mg/L. E. coli resistance mechanisms of β-Lactamase colistin, or IMP-1. The resistance results in this work demonstrate that:

1. The RX-04 class of novel protein synthesis inhibitors have robust activity against a number of highly drug resistant isolates of the Enterobacteriaceae family.

2. Compounds from the RX-04 program can overcome the resistance mechanisms encoded by the KPC and NDM-1 carbapenemases as well as the multidrug resistance phenotypes associated with these problematic β-lactamase enzymes.

3. The potent activities of these novel chemical scaffolds against Gram-negative organisms are encouraging and support further preclinical development.

CONCLUSIONS