

IN VITRO ACTIVITY OF RX100472 AGAINST CLINICALLY IMPORTANT BACTERIA

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ABSTRACT

Background:

With bacterial drug resistance increasing, new drugs with novel mechanisms of action are urgently needed. RX100472 (Fig. 1) is a potent antibacterial compound from a novel pyrimidine series targeting methionyl-tRNA synthetase (MetRS). We evaluated RX100472 against various Gram-positive and Gram-negative bacteria, including a large panel of *Streptococcus pneumoniae* strains with varying levels of penicillin resistance.

Methods:

RX100472 was evaluated using the standard CLSI broth microdilution method to determine MIC values for forty-eight different Gram-positive and Gram-negative bacterial strains from ATCC and other sources. MIC values were determined for 109 *S. pneumoniae* clinical isolates (mostly of US origin) from the following susceptibility groups: 39 penicillin-susceptible (PSSP), 35 penicillin-intermediate (PISP), and 35 penicillin-resistant (PRSP). Comparator agents were vancomycin and trimethoprim.

Results:

The initial MIC screen showed RX100472 to be active only against Gram-positive organisms, with MIC values of 0.06 µg/mL to 2 µg/mL. The exception was poor activity of RX100472 against two *Bacillus cereus* strains and some of the *S. pneumoniae* strains. RX100472 and trimethoprim exhibited similar potency against the 35 PSSP isolates tested. There were 15 PISP isolates with trimethoprim MIC values significantly higher than those of RX100472. Out of 35 PRSP isolates, 28 showed resistance to trimethoprim and only 7 showed resistance to RX100472.

Conclusions:RX100472 was a very potent antibacterial agent against most of the Gram-positive organisms tested but lacked potent activity against Gram-negative bacterial species.

MATERIALS AND METHODS

Organisms

The test organisms for the assay were 109 recent *S. pneumoniae* clinical isolates from the following susceptibility groups: 39 penicillin-susceptible, 35 penicillin intermediate, and 35 penicillin-resistant. The isolates were mostly from the United States, with the exception that a few of the penicillin-resistant isolates were from Europe. The other 48 strains were from ATCC or private collection. Also included was *S. pneumoniae* QA1442 and derivatives for direct study of resistance to MetRS inhibitors.¹

Test Media

The medium employed for the MIC assay was Mueller Hinton II Broth. MHB II was supplemented with 2% lysed horse blood to accommodate the growth of *S. pneumoniae*.

Test Procedure

The MIC assay method followed the procedure described by the Clinical and Laboratory Standards Institute, CLSI² and employed automated liquid handlers to conduct serial dilutions and liquid transfers for the *S. pneumoniae* experiments. MICs for the Gram-positive/Gram-negative testing were determined by broth microdilution according to Clinical and Laboratory Standards Institute method M7-A7,² and interpreted using Alamar Blue to visualize cell viability/cell killing.³ Assays were conducted in Mueller Hinton-cationic adjusted medium over a two-fold serial dilution range from 64 to 0.5µg/mL of test compounds.

RESULTS

Table 1. MIC Range for RX100472, Vancomycin and Trimethoprim vs. various Gram-positive and Gram-negative Isolates

Organism (No. tested)	MIC Range µg/mL		
	RX100472	VAN	TMP
<i>A. baumannii</i> (1)	>4	>8	16
<i>B. anthracis</i> (3)	0.125 - 0.25	2	>64
<i>B. cereus</i> (2)	>4	2	>64
<i>B. megaterium</i> (1)	0.5	0.25	≤0.5
<i>B. subtilis</i> (1)	0.25	0.25	≤0.5
<i>E. cloacae</i> (1)	>4	>8	>64
<i>E. coli</i> (3)	>4	0.5 - >8	≤0.5 - 1
<i>E. faecalis</i> (5)	0.06 - 0.25	0.5 - >8	0.5 - >64
<i>E. faecium</i> (2)	0.06	0.5 - >8	>64
<i>H. influenzae</i> (2)	>4	>8	≤0.5 - 2
<i>M. catarrhalis</i> (1)	>4	>8	16
<i>P. aeruginosa</i> (3)	>4	>8	1->64
<i>S. aureus</i> (11)	1 - 2	0.5 - 2	1 - 2
<i>S. epidermidis</i> (1)	1	2	≤0.5
<i>S. pneumoniae</i> (10)	2 - >64	0.25 - 1	1 - >64
<i>S. pyogenes</i> (1)	2	0.5	2

Table 2. Further study of the *S. pneumoniae* shows resistance to RX100472 by some of the ATCC strains tested. PCR results confirmed which of these *S. pneumoniae* strains possess *metRS1* or *metRS1&2*. Previous data has shown that resistance to MetRS-specific antibiotics was traced to the presence of a second functionally expressed *metRS* allele¹. QA1442 strains¹ containing *metRS1* plus *metRS2* or deletions for either *metRS1* or *metRS2* confirmed that RX100472 strains possessing *metRS2* were resistant to RX100472.

ATCC	MIC µg/mL			PCR results
	RX100472	VAN	TMP	
49619	>64	0.5	4	<i>metRS</i> 1&2
700677	>64	0.5	8	<i>metRS</i> 1&2
700676	>64	0.5	2	<i>metRS</i> 1&2
700671	>64	0.5	>64	<i>metRS</i> 1&2
700674	4	0.5	>64	<i>metRS</i> 1
700669	2	0.5	>64	<i>metRS</i> 1
51916	4	0.25	>64	<i>metRS</i> 1
700675	4	0.5	8	<i>metRS</i> 1
CP1250 (Lab)	2	0.25	1	<i>metRS</i> 1
QA1442	>64	≤0.5	4	<i>metRS</i> 1&2
QA1442 Δ <i>metS</i> 1	>64	1	2	<i>metRS</i> 2
QA1442 Δ <i>metS</i> 2	4	≤0.5	2	<i>metRS</i> 1

Figure 1. Structure of RX100472

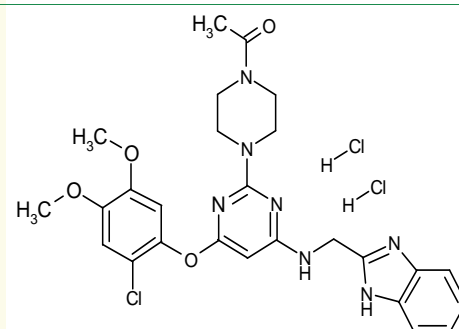


Table 3. MIC Range, MIC₅₀ and MIC₉₀ Values for RX100472 and Trimethoprim vs. a Battery of *Streptococcus pneumoniae* Clinical Isolates

Group	Drug	Number of Strains Inhibited at Concentration (µg/mL)											MIC Range (µg/mL)	MIC ₅₀ ¹ (µg/mL)	MIC ₉₀ ² (µg/mL)			
		0.06	0.12	0.25	0.5	1	2	4	8	16	32	64				>64		
PSSP ³ (39)	RX100472				2	14	18	3							2	0.5->64	2	4
	Trimethoprim		1	1	3	6	22	4							2	0.12->64	2	4
PISP ⁴ (35)	RX100472					18	16								1	1->64	1	2
	Trimethoprim				1	4	9	6			3	6	6		6	0.5->64	4	>64
PRSP ⁵ (35)	RX100472						12	15		1					7	1->64	2	>64
	Trimethoprim				1	3	3					3	25		0.5->64	>64	>64	

1 MIC at which 50% of strains were inhibited
2 MIC at which 90% of strains were inhibited
3 Penicillin-susceptible *Streptococcus pneumoniae*
4 Penicillin-intermediate *Streptococcus pneumoniae*
5 Penicillin-resistant *Streptococcus pneumoniae*

RESULTS AND DISCUSSION

Gram-positive/ Gram-negative Study (Table 1)

- RX100472 has equivalent or higher potency against some of the Gram-positive organisms tested compared to vancomycin or trimethoprim.
- The potency against *B. anthracis* is explained by the non-essentiality of the *metRS2* gene.⁴
- RX100472 did not show potency against the Gram-negative organisms tested.
- Table 2 shows that resistance to RX100472 in PRSP strains is correlated with the presence of a second *metRS* gene.

S. pneumoniae Study (Table 3)

- RX100472 and trimethoprim exhibited similar potency against the 35 PSSP isolates tested, producing identical MIC₅₀ and MIC₉₀ values of 2 µg/mL and 4 µg/mL, respectively.
- Testing against 35 PISP isolates showed 15 isolates with trimethoprim MIC values significantly higher than those of RX100472.
- For PRSP, there were 7 isolates found with MIC values of >64 µg/mL of RX100472. The clinical isolates have not been analyzed for MetRS genes or other genes that could explain their resistance to RX100472.
- Six isolates were highly resistant to both compounds.

CONCLUSIONS

- RX100472 represents a new class of compounds targeting MetRS1
- RX100472 is a potent compound against Gram-positive organisms
- RX100472 is potent against PSSP and PISP strains; however strains carrying *metRS2* gene are nonsusceptible

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