



INTRODUCTION

Lefamulin is the first semi-synthetic pleuromutilin antibiotic for IV and oral use in humans. It is currently in Phase 3 trials for the treatment of community-acquired bacterial pneumonia in adults.

Pleuromutilins inhibit translation by binding to the peptidyl transferase center (PTC). The pleuromutilin core binds to the A-site, while the C-14 side chain binds in the P-site. Lefamulin interacts with the PTC via four H-bonds and other interactions resulting in an “induced fit” whereby nucleotides in the PTC shift and tighten the binding pocket around lefamulin.

This study investigated the inhibition of bacterial and eukaryotic translation by lefamulin to determine its relative specificity for bacteria.

METHODS

Inhibition of protein synthesis was measured by coupled *in vitro* transcription-translation (TT) using *E. coli* or *S. aureus* ribosomal extracts which measure the expression of functional luciferase (Promega).

Eukaryotic TT inhibition was evaluated with the rabbit TNT SP6 coupled reticulocyte lysate systems assay (Promega). Functional luciferase was detected by the Steady-Glo Luciferase Assay System.

RESULTS

- Lefamulin displayed potent inhibition of bacterial translation with an IC_{50} of 0.58 μ M and 0.29 μ M for *E. coli* and *S. aureus*, respectively (Table 1).
- In contrast, lefamulin was ineffective at inhibiting mammalian protein synthesis (IC_{50} of 952 μ M).
- All controls results were consistent with data in the literature.¹
 - Controls included retapamulin (a topical pleuromutilin), puromycin (a non-selective prokaryotic and eukaryotic protein synthesis inhibitor), and cycloheximide (a selective inhibitor of eukaryotic TT).
- TT inhibition results correlate well with the potent activity of lefamulin against Gram-positive and fastidious Gram-negative bacterial pathogens.

REFERENCES

- (1) Yan K., et al. (2006) Antimicrobial Agents and Chemotherapy 50(11):3875
- (2) Eyal Z., et al. (2016) Scientific Reports 6:39004

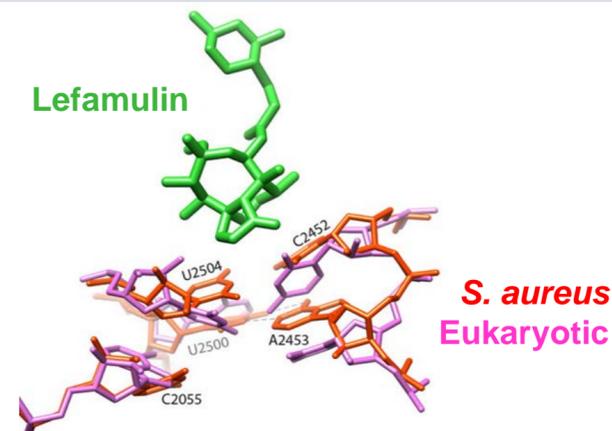


Figure 1. Binding pocket of lefamulin in the PTC

In the eukaryotic PTC, nucleotides 2453 and 2500 are both uridines and no interaction between them occurs owing to their orientation, whereas in *S. aureus* nucleotides C2452 and U2504 interact. In eukaryotes, U2504 has different orientation than in bacteria which allows pi stacking to A2055 (C in bacteria, A in eukaryotes). This interaction stabilizes the “open” conformation of the PTC which is the key for the pleuromutilins’ selectivity.²

Table 1. Selective inhibition of protein synthesis. IC_{50} [μ M] of lefamulin and comparators in *in vitro* bacterial and eukaryotic coupled transcription-translation assay (CI_{95})

	<i>E. coli</i>	<i>S. aureus</i>	Eukaryotic
Lefamulin	0.58 (0.52-0.64)	0.29 (0.26-0.32)	952 (732-1238)
Retapamulin	0.69 (0.64-0.76)	0.35 (0.32-0.39)	850 (562-1287)
Puromycin	0.39 (0.34-0.46)	0.19 (0.16-0.23)	0.31 (0.27-0.36)
Cycloheximide	>100	>100	0.44 (0.29-0.68)

CONCLUSIONS

- This study demonstrates that lefamulin is a selective inhibitor of bacterial TT.
- These results are consistent with:
 - the known structural differences between the PTC of bacterial and eukaryotic ribosomes (Fig. 1);
 - a lack of target organ toxicity reported in preclinical studies with lefamulin; and
 - a good tolerability profile observed in clinical trials of lefamulin conducted to date.

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