Visualizing the novel veterinary macrolide tildipirosin bound to its ribosomal target

Stephen Douthwaite,1 Jacob Poehlsgaard,1 Benoit Desmolaize,1 Simon Rose,1 Niels M. Andersen,1 and Ralf Warrass2

1 Dept. Biochemistry & Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark.

Respiratory tract infections in cattle are commonly associated with the bacterial pathogens *Mannheimia haemolytica* and *Pasteurella multocida*. These infections can generally be successfully treated with several types of veterinary antibiotics including macrolides. Tildipirosin (20,23-dipiperidinyl-mycaminosyl-tylonolide) is a semi-synthetic 16-membered ring macrolide derived from the naturally occurring compound tylosin. Tildipirosin (Zuprevo®) was recently approved for veterinary use in Europe, the US and Canada. Compared to tylosin and tilmicosin (an earlier tylosin-derivative), tildipirosin is more effective against macrolide-susceptible isolates, and retains activity against some of the resistant strains that have recently emerged (1-3). Here, the molecular interactions of the macrolides are mapped and visualized at their inhibitory target on the bacterial ribosome.

Chemical footprinting and computer modelling show that tildipirosin, tilmicosin and tylosin all bind and inhibit the well-documented drug site that is centred at 23S rRNA nucleotide A2058 within the large subunit of the bacterial ribosome. There are, however, subtle differences in how the compounds occupy the site. Interactions of the two piperidine components, which are particular to tildipirosin (green), indicate how its mode of action is distinct from tylosin, tilmicosin (magenta) and the 15-membered macrolide tulathromycin (red). The 23-piperidine of tildipirosin contacts specific ribosomal residues on the tunnel wall while its 20-piperidine is oriented into the tunnel lumen and is positioned to interfere with the growing nascent peptide (4).

We measured the IC₅₀ for tildipirosin at 0.23 ± 0.01 μM in an *in vitro* assay. The IC₅₀ for tilmicosin was 0.36 ± 0.02 μM, while tylosin and tulathromycin fall between these values. Drug binding is lowered by mutations and methylations of rRNA nucleotides within the target site, and consequently raise MICs (5). Collectively, the data show how the mode of action of a naturally-occurring antimicrobial compound has been improved by derivatization, and provide a basis for further development of macrolide drugs by ration design.


**Keywords:** Ribosome inhibitors; IC₅₀; rRNA methyltransferases; efflux; *Pasteurellaceae*