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**Paper Poster Session**  
**Discovery of more new antibacterial drugs**

**Biological profiling of novel bacterial topoisomerase inhibitors with broad-spectrum antibacterial activity**

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**Background:** Bacterial infections are becoming increasingly untreatable due to the ever declining number of effective antibiotics. Novel antibiotics are urgently required to address emerging bacterial pathogens and increased drug resistance. Bacterial DNA gyrase and topoisomerase IV are validated drug targets by the use of the coumarins and fluoroquinolones. Redx Pharma has discovered a series of novel bacterial topoisomerase inhibitors (NBTI) active against a wide range of pathogens including clinically important Gram-negative bacteria, mycobacteria and the anaerobe *Clostridium difficile*.

**Material/methods:** Minimum inhibitory concentrations (MIC) were determined using the broth microdilution and agar dilution methods according to CLSI guidelines M07-A9, M24-A2 and M11-A8. MIC₉₀ values were determined using a range of recent clinical isolates. Intracellular inhibition of mycobacteria was determined by measuring the concentration required to inhibit 90% growth of a luminescent strain of *Mycobacterium tuberculosis* H37Rv infecting THP-1 monocytes. DNA gyrase, topoisomerase IV and Human topoisomerase II activity was evaluated using DNA supercoiling/decatenation assays and a DNA cleavage complex formation assay. An IC₅₀ was calculated based on the concentration required to inhibit 50% of enzyme activity. Mammalian cytotoxicity was determined using HepG2 cell line and THP-1 human monocytic cell line in a CellTiter Glo® (Promega) luminescent cell viability assay.

**Results:** Compounds from this series showed broad-spectrum bactericidal activity against representative ESKAPE pathogens, with MIC values ranging from 0.12 – 8 µg/mL. Activity was also demonstrated against fastidious Gram-negative pathogens such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*, the anaerobic Gram-positive pathogen *Clostridium difficile* and mycobacteria species in various conditions including *M. tuberculosis* H37Rv-infected THP-1 monocytes (IC₉₀ = 1.5 µM). In further MIC₉₀ studies NBTI compounds were active against recent clinical isolates of representative ESKAPE pathogens. Similarly, REDX06224 was shown to be potent against a panel of 21 *C. difficile* clinical isolates with an MIC₉₀ of 4 µg/mL. In the DNA cleavage complex assay with *Escherichia coli* DNA gyrase, NBTI compounds did not stabilize cleaved complexes. NBTIs however showed balanced inhibition of supercoiling and decatenation activity of *E.coli* gyrase and
topoisomerase IV (IC$_{50}$ = 1.47 and 1.17 µM respectively) and *M. tuberculosis* gyrase (IC$_{50}$ = 0.25 µM). \textit{In vitro} data showed a promising safety profile with an IC$_{50}$ ≥ 32 µg/mL against the HepG2 cell line and only moderate inhibition of human topoisomerase II with IC$_{50}$ around 100 µM.

**Conclusions:** This NBTI series has shown broad-spectrum activity against the ESKAPE pathogens, the anaerobic pathogen *C. difficile* and mycobacteria species. Furthermore the distinct mechanism-of-action from the fluoroquinolones, combined with a low mutation frequency and promising \textit{in-vitro} safety profile supports the continued investigation of this NBTI series as potential broad-spectrum antibacterial agents.