**Synthesis and Optimization of Novel Bacterial Topoisomerase Inhibitors: Structure-Activity Relationships from Modifications to the DNA Gyrase-Binding Domain**

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**Background:** Multidrug-resistant (MDR) bacterial infections continue to pose an enormous threat to human health. Worldwide mortality rates are estimated to be approximately 700,000 individuals per year, and this number will rapidly increase in the years to come.1 Of particular concern is the bacterial pathogen *Staphylococcus aureus* and its methicillin-resistant variant (MRSA) due to its leading contribution to mortality rates in the United States.2 Therefore, there is an urgent need to develop novel therapeutics to combat this issue of resistance. Novel Bacterial Topoisomerase Inhibitors (NBTIs) are a promising class of compounds that have been reported to have potent antibacterial activity. Previously we have generated a series of dioxane linked NBTIs that inhibit both DNA gyrase and topoisomerase IV enzymes of *S. aureus* strains. Our current focus is to synthesize NBTIs that have improved pharmacokinetic (PK) properties while maintaining potency through structural modifications to the gyrase-binding domain. Ideal inhibitors will also demonstrate minimal hERG inhibition, a general safety concern for the NBTIs.

**Materials and Methods:** A series of aldehydes with desired physicochemical properties was prepared by multistep synthesis. Final compounds were then prepared by reductive amination of these aldehydes using a 5-amino-1,3-dioxane derivative. Aldehyde intermediates as well as final compounds were purified by flash column chromatography and characterized by NMR spectroscopy. Minimum inhibitory concentrations (MIC) were measured in both drug-sensitive and methicillin-resistant *S. aureus* as well as an isolate from an individual with cystic fibrosis. Additional MIC determinations were conducted using an NBTI-resistant *S. aureus* strain with a D83N amino acid substitution in DNA gyrase. Fifty percent inhibitory concentrations (IC50 values) were determined using purified DNA gyrase and TopoIV enzymes, as well as with DNA gyrase enzyme with the D83N amino acid substitution. Compounds were assessed for metabolic stability using mouse microsomes and for cardiovascular safety in the stability assessment hERG assay.

**Results:** The synthesized NBTIs possessed potent anti-staphylococcal activity. NBTIs were also tested for activity in biochemical assays, with low ratios of TopoIV decatenation / DNA gyrase supercoiling IC50 values indicating improved dual-targeting capability. Improvements were also seen in antibacterial activity against the D83N mutant *S. aureus* strain. Select compounds showed improvement in metabolism and hERG safety profiles.

**Conclusion:** Multi-step synthetic approaches for the generation of aldehyde intermediates, followed by reductive amination afforded a new set of dioxane-linked NBTIs. This series of compounds enabled further insight into the physicochemical properties driving metabolic stability and cardiovascular safety and also delivered superior antibacterial activity in several cases.

**References:**

1. Mitton-Fry, M. J. *Med. Chem. Rev.* **2017,** *52,* 281.
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