BACTERIAL TOPOISOMERASE INHIBITORS BEARING DIXANE-LINKED AMIDE DERIVATIVES FIGHTING MULTIDRUG BACTERIAL INFECTIONS

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Background: There is an urgent need for new antibiotics due to the rapid emergence of multidrug-resistant bacteria. Novel bacterial topoisomerase inhibitors (NBTIs) provide a promising option against superbugs by inhibiting the type II bacterial topoisomerases, DNA gyrase and topoisomerase IV (Topo IV). Here, a series of amide NBTIs analogues was designed, synthesized, and evaluated.

Materials and Methods: 36 amide analogues were synthesized by a 5-step route and purified by flash chromatography. Minimum inhibitory concentrations (MICs) were measured with both methicillin-sdrug-sensitive and methicillin-resistant *Staphylococcus aureus*, and selected compounds were also assayed for MICs against different Gram-positive and Gram-negative pathogens. Fifty percent inhibitory concentrations (IC_{50} values) against both *S. aureus* DNA gyrase and Topo IV were determined by supercoiling and decatenation assays, respectively. Additionally, DNA cleavage assays were performed in the presence of DNA gyrase to elucidate the mode of action of these amide inhibitors. A high concentration (200 micromolar) of various amide analogues was used to evaluate the potential for inhibition of human topoisomerase IIα. IC_{50} values against hERG (the human ether-a-go-go related gene) were determined to assess potential cardiotoxicity. Metabolic stability of a representative analogue was assessed using mouse microsomes.

Results: NBTIs with excellent antibacterial activity were discovered; a number of amide analogues possessed *S. aureus* MICs $\leq$ 4 microgram/mL. Most amide analogues showed potent inhibition of bacterial DNA gyrase, and several inhibited both gyrase and Topo IV. Surprisingly, amide analogues with an oxazinone or thiazinone moiety induced both single and double strand breaks to bacterial DNA through their action on DNA gyrase. Compounds displayed minimal inhibition of the human topoisomerase and some of the compounds showed minimal hERG inhibition (IC_{50} > 100 uM).

Conclusion: Novel NBTIs with an amide motif were developed as antibiotics for multidrug-resistant bacteria. These compounds demonstrated excellent potency, selectivity, and safety alongside a relatively unique pharmacological mechanism.

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