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Discovery and in vivo evaluation of alcohol-containing benzothiazoles as potent dual-targeting bacterial DNA supercoiling inhibitors

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ABSTRACT

A series of dual-targeting, alcohol-containing benzothiazoles has been identified with superior antibacterial activity and drug-like properties. Early lead benzothiazoles containing carboxylic acid moieties showed efficacy in a well-established in vivo model, but inferior drug-like properties demanded modifications of functionality capable of demonstrating superior efficacy. Eliminating the acid group in favor of hydrophilic alcohol moieties at C^5, as well as incorporating solubilizing groups at the C^7 position of the core ring provided potent, broad-spectrum Gram-positive antibacterial activity, lower protein binding, and markedly improved efficacy in vivo.

Increasing emergence of drug resistance in pathogenic bacteria has resulted in higher human mortality and increased healthcare costs.[1,2] We therefore targeted a new class of agents with no cross-resistance to existing therapies. Recently, we reported the discovery of a new class of dual-targeting, benzothiazole-containing small molecule inhibitors of bacterial DNA gyrase and topoisomerase IV.[3] Bacterial type II topoisomerases form heterotetramers consisting of two subunits: DNA gyrase forms an A_2B_2 complex comprised of GyrA and GyrB, and topoisomerase IV forms a C_2E_2 complex made up of ParC and ParE monomers.[3] In addition, the GyrB and ParE subunits are functionally similar with an ATPase active site within their N-terminal domains. The C-terminal domains of GyrB and ParE, by contrast, interact with the GyrA and ParC subunits of the heterotetramers, and also form the DNA binding grooves necessary for supercoiling and decatenation.[4] Highly conserved among bacterial species, these enzymes are ideal for broad spectrum inhibitor approaches. Fluoroquinolones interact with the DNA binding grooves of the GyrA and ParC subunits, but the similarity between the ATP binding regions of GyrB and ParE suggests a motif for simultaneous targeting.[5] GyrB/ParE inhibitors offer the advantage of no pre-existing resistance and a low propensity for the development of resistance.

A number of groups have recently described ATP site-binding GyrB/ParE inhibitor series.[6–10] We recently reported the discovery of isonipecotic acid-containing benzothiazole ureas with enhanced solubility and pharmacokinetic properties,[11,12] along with microbiological assessment of well-characterized examples.[13] To establish the potential for further development of this chemotype, we evaluated compounds in a neutropenic mouse model of MSSA *Staphylococcus aureus* infection.[14] In this acute model, which was not designed to establish PK/PD parameters, compounds showed a dose-dependent drop in colony-forming units (CFUs) versus controls (Fig. 1).

Compounds **1** and **2** (MIC versus MSSA type strain *S. aureus* ATC 29213 of 0.06 and 0.12 μg/mL, respectively) were dosed intravenously at 2, 4, and 6 h after inoculation. Assessment of bacterial load 24 h post infection showed the compounds to have been efficacious. Although the lower doses of 3 × 30 mg/kg afforded

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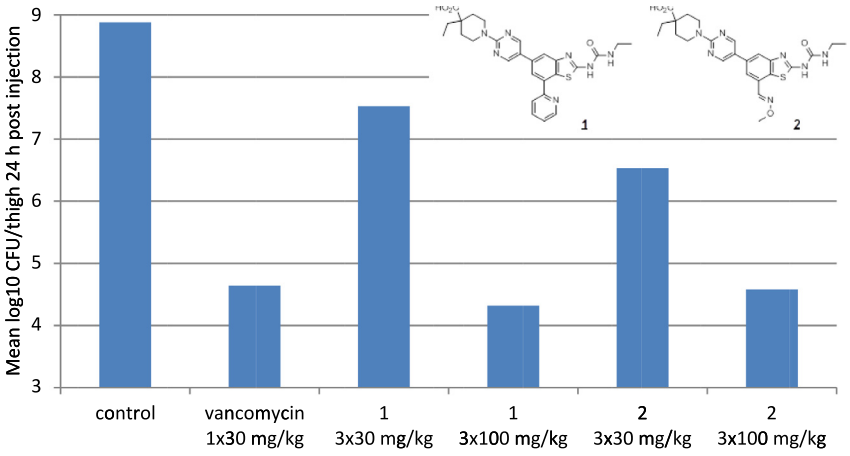


Figure 1. Efficacy of isonipecotic acids in neutropenic mouse thigh model.

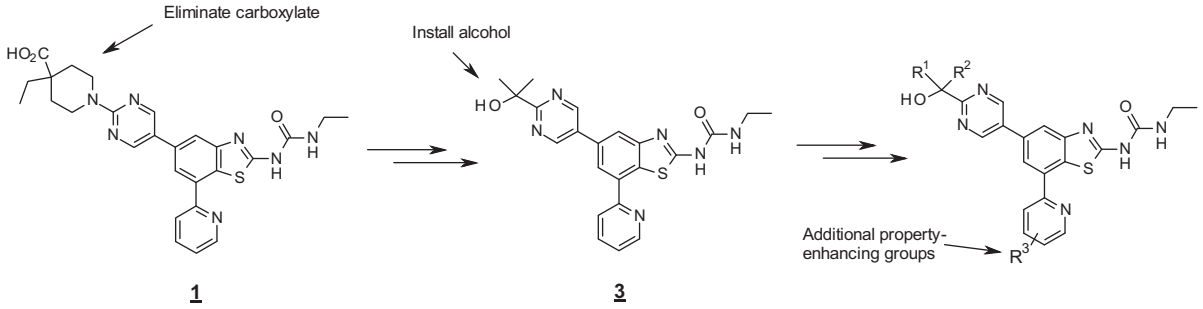
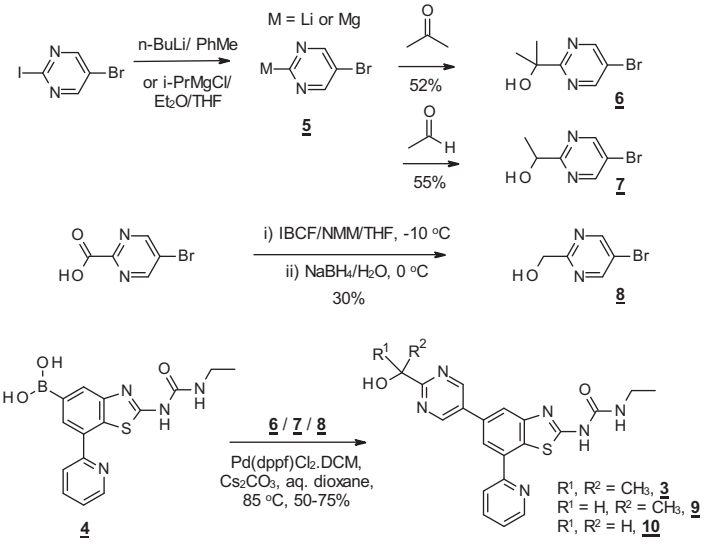


Figure 2. Evolution of alcohol-containing benzothiazole ureas.



Scheme 1. Synthesis of alcohol-containing C^5-substituted benzothiazoles.

modest reductions in CFUs (<3 logs) the higher doses of 3×100 mg/kg demonstrated greater than 4 log drops in CFUs, comparable to a single dose of vancomycin at 30 mg/kg. While calculating CFU reductions from an early time point is commonly used, it is not improper to calculate reductions from the matched control time point; when a compound has a static or slow cidal mode of action, calculating reductions in CFUs to the matched controls is more informative.[6,14]

Armed with these encouraging proof-of-concept data, we sought to improve beneficial physical properties further. In particular, we focused on reducing plasma protein binding, while retaining solubility sufficient to permit formulation within acceptable pH ranges for hospital-based intravenous administration. Lastly, we required demonstration of efficacy by both intravenous and oral routes, laying the groundwork for both inpatient and outpatient treatments.

To this end, we eliminated the carboxylic acid functionality in favor of an alcohol-substituted pyrimidine (R^1R^2COH) attached to the C^5 position of the benzothiazole (Fig. 2). This moiety provided an alternative hydrophilic, yet neutral group suitable for prodrugging as needed. At the same time, we recognized a further