Optimization of Coumarin-Based Inhibitors of Staphylococcus aureus and Bacillus anthracis Replicating DNA Helicases


ABSTRACT (Revised)

Background: New antibiotics are needed to treat drug-resistant infections. Previously, we described coumarin-based inhibitors of an underexplored bacterial target, the replicative DNA helicase (Aiello et al., 2009, Bioorg. Med. Chem. 17:4048). Here we report the synthesis and evaluation of optimized coumarin-based inhibitors with 10-fold increased potency against S. aureus (Bai) and B. anthracis (Bai) helicases. Methods: Enzyme inhibition was measured in a fluorescent assay of helicase-mediated dissociation of two annealed oligonucleotides bearing fluorescent and quencher moieties. MIC values were determined by the CLSI method. Membranial cytotoxicity (CC50) was determined using HeLa cells in serum-free medium. Results: Forty-six analogs of the original reported coumarin series of helicase inhibitors were synthesized and evaluated for structure-activity relationships (SARs). Addition of a methyl group at the 8-position of the coumarin nucleus consistently provided increased potency. A thorough examination of substituents at the 7-position revealed that a halogen-substituted biphenyl group provided the best potency, with IC50 values of 1.5 and 1.0 µM vs. Ba and Sa MIC values of 40-65. Conclusions: We have optimized a validated coumarin series of helicase inhibitors to generate compounds with 1 µM potency and favorable bacterial selectivity.

METHODS AND RESULTS

Background: Essential Role of Helicase in Bacterial DNA Replication

During DNA replication, helicase-DNA complexes unwind the double-stranded DNA to form the Okazaki fragment: a single-stranded template for the lagging strand is uncovered, it discontinuously as an Okazaki fragment. The two core polymerase-leading-strand polymerase, together with its -subunit clamp, remains at the end of the leading strand as its single-1) adds nucleotides to the 3'end of the lagging strand; 2) removes the RNA primer from the 3'end of the lagging strand; and 3) for the synthesis of RNA strands, the two polymerases function as a single enzyme.

Results:

1. Chemical optimization of a previously reported coumarin-based bacterial helicase inhibitor series (ref. 1) resulted in the discovery of several analogs exhibiting more potency and selectivity.

2. SAR is responsible for biological activity and exhibits clear trends such as preferences for a hydrophobic side group at position 7, an acido- or alkyl group at position 3, and for methyl at positions 1 and 4. 3. MBX 2101 and MBX 2353 are up to 25-50-fold more potent in bacterial helicase inhibition assays (IC50) than in cytotoxicity assays (CC50) vs. HeLa cells in the absence of serum.

4. The coumarin-based helicase inhibitors do not appear to act competitively with either the DNA or ATP substrates.

5. These results indicate that further optimization of coumarin-based helicase inhibitors may provide a new class of antibacterial agents.

ACKNOWLEDGEMENTS

We thank Debra Mills and Tommy Tashjian for HeLa cell cytotoxicity determinations in serum-free medium. This research was funded by an STTR grant from NAINGRNH (AI56540).

REFERENCES


