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Determination of absolute configuration and binding efficacy of benzimidazole-based FabI inhibitors through the support of electronic circular dichroism and MM-GBSA techniques

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ABSTRACT

We have previously reported benzimidazole-based compounds to be potent inhibitors of FabI for *Francisella tularensis* (*Ft*FabI), making them promising antimicrobial hits. Optically active enantiomers exhibit markedly differing affinities toward *Ft*FabI. The IC_{50} of benzimidazole (−)-**1** is ∼100× lower than the (+)-enantiomer, with similar results for the **2** enantiomers. Determining the absolute configuration for these optical compounds and elucidating their binding modes is important for further design. Electronic circular dichroism (ECD) quantum calculations have become important in determining absolute configurations of optical compounds. We determined the absolute configuration of (−)/(+)-**1** and (−)/(+)-**2** by comparing experimental spectra and theoretical density functional theory (DFT) simulations of ECD spectra at the B3LYP/6-311+G(2d, p) level using Gaussian09. Comparison of experimental and calculated ECD spectra indicates that the *S* configuration corresponds to the (−)-rotation for both compounds **1** and **2**, while the *R* configuration corresponds to the (+)-rotation. Further, molecular dynamics simulations and MM-GBSA binding energy calculations for these two pairs of enantiomers with *Ft*FabI show much tighter binding MM-GBSA free energies for *S*-**1** and *S*-**2** than for their enantiomers, *R*-**1** and *R*-**2**, consistent with the *S* configuration being the more active one, and with the ECD determination of the *S* configuration corresponding to (−) and the *R* configuration corresponding to (+). Thus, our computational studies allow us to assign (−) to (*S*)- and (+) to (*R*)- for compounds **1** and **2**, and to further evaluate structural changes to improve efficacy.

The need for new antimicrobial agents is increasing consequent to the spread of antibiotic-resistant bacteria, the emergence of new infections, and the potential of using serious pathogenic bacteria in bioweapons.[1–3] Hence, it is important to develop new, highly potent antibiotics with novel mechanisms for treatment of bacterial infections. In this regard, the enoyl-acyl carrier protein reductase, FabI, is a promising target for antibacterial drug development.[4] FabI is an NAD(P)H-dependent oxidoreductase that acts to reduce enoyl-ACP substrates in the final and rate-controlling step of the bacterial fatty acid biosynthesis pathway (FAS II).[5] The FAS II pathway is the result of a series of discrete enzymes at separate steps and is essential in the biosynthesis of the bacterial fatty acid components of bacterial lipid membranes and energy stores.[6–8] The mammalian counterpart (FAS I) uses a single, large, multifunctional enzyme. Because of the differing structure and mechanism, FAS-II is an attractive and potentially safe target for antibacterial agent development. Although there are multiple isozymes (including FabK, FabL, and FabV)[9–11] that can be present in addition to, or in place of FabI in various bacterial species, FabI is recognized as a target for specific, narrow-spectrum antibacterial agents, for treating species that express FabI as the sole or dominant enoyl reductase enzyme in the FAS II pathway. *Francisella tularensis* (*F. tularensis*) is a bacterial species for which FabI is an essential enzyme in fatty acid synthesis.[12,13] *F. tularensis* is a Gram-negative bacterium responsible for tularemia and is a potential bioweapon due to its ease of cultivation and spread by aerosol as well as its high virulence.[14] Although streptomycin, ciprofloxacin and tetracycline have been used for treatment of tularemia,[15] each

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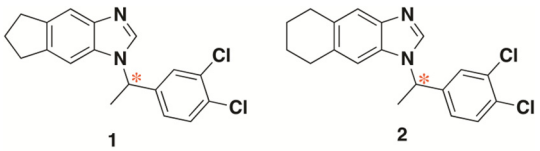

Fig. 1. Structures and *Ft*FabI IC_{50} inhibitory activities of enantiomers of compounds **1** and **2**.

has limitations, such as ototoxicity of streptomycin, teeth staining by tetracyclines or even the emergence of ciprofloxacin-resistant strains of *F. tularensis*.[16] Therefore, development of novel agents with improved efficacy and different mechanisms of action against *F. tularensis* is an important priority.

In prior work, we identified benzimidazole compounds as novel and potent FabI inhibitors, with antibacterial activities against both *F. tularensis* and *Staphylococcus aureus* (*S. aureus*).[17–21] Our current work demonstrates that enantiomers of optical benzimidazole compounds possess markedly differing inhibitory efficacies toward *Ft*FabI. The IC_{50} of benzimidazole (−)-**1** is about one hundred times lower than its (+)-enantiomer, with similar results for enantiomers of **2** (Fig. 1). This motivated us to determine the absolute configuration (AC) for the optical benzimidazole compounds to correlate the differing efficacies with their distinctive binding modes to *Ft*FabI.

However, neither the crystal structures for the optical compounds nor the crystal complexes with *Ft*FabI have been determined, which could directly determine the AC for these chiroptical compounds. We thus used two strategies to determine the AC of chiral benzimidazole compounds by comparing experimental and theoretical results. The first strategy focused on the optically active benzimidazoles themselves by comparing the experimental and calculated spectra for the chiroptical compounds. That benefits from the dramatic growth in the applications of chiroptical spectroscopy for molecular structure determination, and the development of computational resources with faster processor speeds for quantum chemical spectral predictions in recent years.[22] The use of electronic circular dichroism (ECD) can provide reliable compound structure determination.[23,24] We thus used ECD to determine the AC for both optical benzimidazole compounds **1** and **2**. We have also previously used molecular mechanics-poisson boltzmann/generalized born surface area (MM-PB/GBSA) methods to evaluate the detailed correlation between experimental and predicted results for benzimidazoles binding to *Ft*FabI.[25] Herein, we report similar computations to predict the binding affinity of the enantiomers of compounds **1** and **2** to *Ft*FabI. Entropy calculations were combined in this study with MM-GBSA to improve predicted binding affinity. The resulting MM-GBSA calculations, in conjunction with experimental IC_{50} measurements, are highly consistent with the ECD absolute configuration calculations, allowing us to unambiguously correlate optical enantiomers with inhibitory activity.

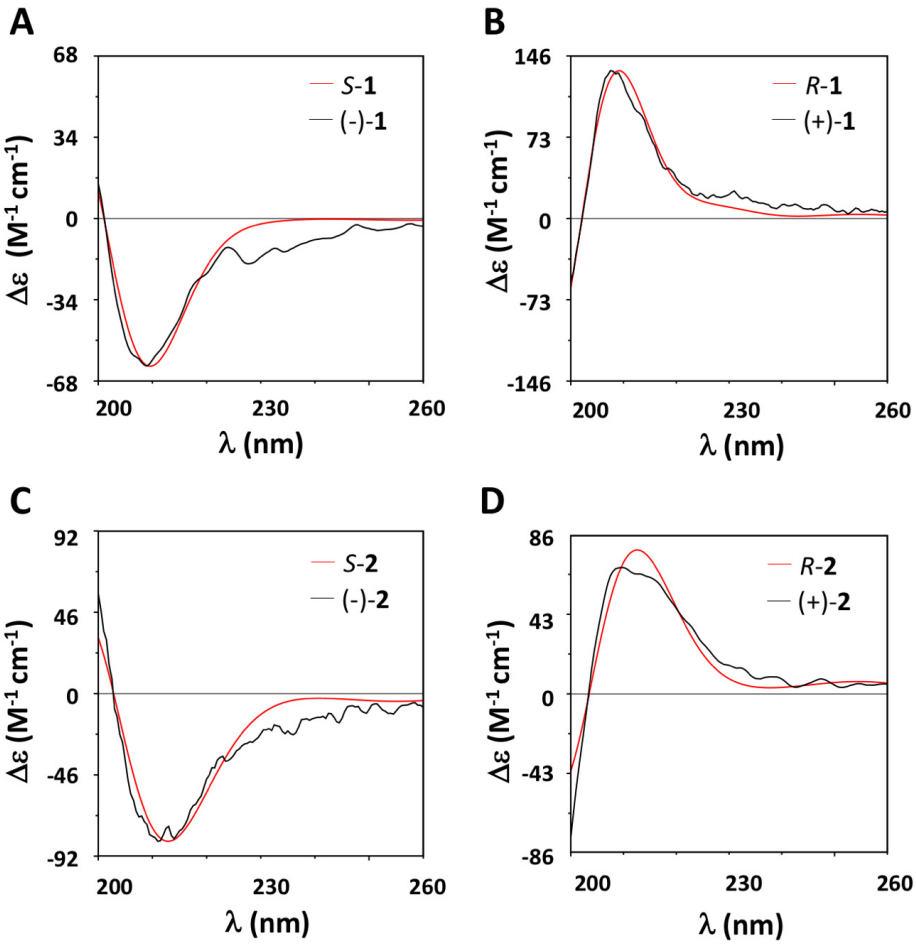

Fig. 2. Comparison of experimental (black) and calculated (red) ECD spectra for (A) *S*-**1** and (−)-**1**; (B) *R*-**1** and (+)-**1**; (C) *S*-**2** and (−)-**2**; and (D) *R*-**2** and (+)-**2**. To facilitate the comparison between experimental and computational spectra, which is sometimes hampered by misalignment of Cotton Effects (CE), we have blue shifted the computational spectrum by 1 nm.