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GroEL/ES inhibitors as potential antibiotics

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ABSTRACT

We recently reported results from a high-throughput screening effort that identified 235 inhibitors of the *Escherichia coli* GroEL/ES chaperonin system [*Bioorg. Med. Chem. Lett.* **2014**, *24*, 786]. As the GroEL/ES chaperonin system is essential for growth under all conditions, we reasoned that targeting GroEL/ES with small molecule inhibitors could be a viable antibacterial strategy. Extending from our initial screen, we report here the antibacterial activities of 22 GroEL/ES inhibitors against a panel of Gram-positive and Gram-negative bacteria, including *E. coli*, *Bacillus subtilis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*. GroEL/ES inhibitors were more effective at blocking the proliferation of Gram-positive bacteria, in particular *S. aureus*, where lead compounds exhibited antibiotic effects from the low-μM to mid-nM range. While several compounds inhibited the human HSP60/10 refolding cycle, some were able to selectively target the bacterial GroEL/ES system. Despite inhibiting HSP60/10, many compounds exhibited low to no cytotoxicity against human liver and kidney cell lines. Two lead candidates emerged from the panel, compounds **8** and **18**, that exhibit >50-fold selectivity for inhibiting *S. aureus* growth compared to liver or kidney cell cytotoxicity. Compounds **8** and **18** inhibited drug-sensitive and methicillin-resistant *S. aureus* strains with potencies comparable to vancomycin, daptomycin, and streptomycin, and are promising candidates to explore for validating the GroEL/ES chaperonin system as a viable antibiotic target.

The number of lives saved by antibiotics is a hallmark of the success of this class of drugs. However, resistant bacterial strains have been identified for every class of antibiotic, usually within a few years of general therapeutic use.[1–3] The threat of antibiotic resistance is epitomized by the emergence of six multi-drug resistant bacteria referred to as the *ESKAPE* pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species.[4–8] The Centers for Disease Control (CDC) and Prevention Antibiotic Resistance Threat Report lists these bacteria as serious threats (level 4 out of 5) requiring prompt and sustained action.[9] Most alarming is that antibiotic resistance has mounted to the point where therapeutics are severely limited or ineffective for once easily treated infections. For example, ~10,000 people per year are estimated to die from methicillin-resistant *S. aureus* (MRSA) infections in the United States.[10] Moreover, the CDC estimates the direct medical cost of treating antibiotic resistant bacterial infections in the US is more than $20 billion per year.[9] Clearly, the rise of resistant bacterial strains requires enhanced research efforts to ensure an ongoing antibiotic pipeline.

Current antibiotics primarily function by blocking cell wall construction, structure and function of the cell membrane, protein synthesis, DNA structure and function, or folic acid synthesis.[11] Recently developed therapeutics for infections caused by drug-resistant bacteria include the injectable carbapenem beta-lactam, doripenem, which targets penicillin-binding proteins and inhibits

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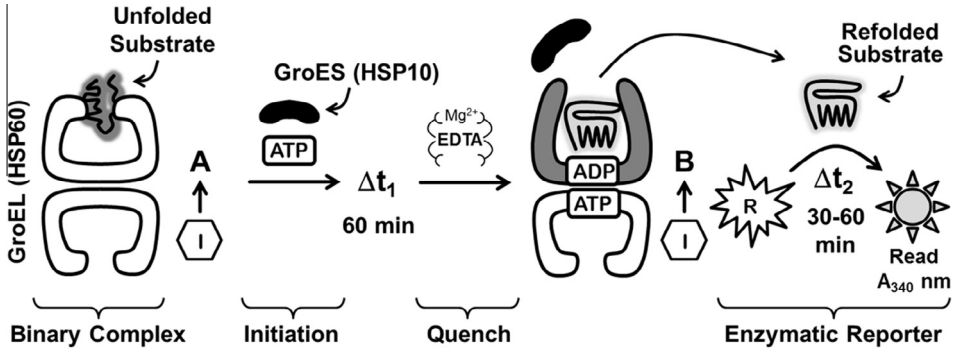

Figure 1. General protocol for chaperonin-mediated biochemical assays. Compounds (**I**) are added at point A to a solution containing GroEL (or HSP60) with bound substrate protein (e.g., malate dehydrogenase, MDH). Addition of GroES (or HSP10) and ATP initiates the refolding cycle, which is quenched with EDTA after a 60 min incubation. Substrates (R) for the refolded reporter enzyme are added and after another 30–60 min incubation (until the DMSO control wells have reached ~90% consumption of NADH), absorbance is measured to evaluate the amount of refolded enzyme present, and by association the extent of chaperonin inhibition. Alternatively, addition of compounds at point B enables determination of off-target inhibition of the reporter enzyme (i.e., native MDH enzyme activity). Chaperonin-mediated ATP hydrolysis is also evaluated using a malachite green assay. Biochemical assays employing Rhodanese (Rho) are performed similarly (refer to Supporting information for detailed protocols).

cell wall synthesis;[12] the cyclic lipopeptide, daptomycin, which inserts into the bacterial membrane and leads to pore formation;[13] quinupristin/dalfopristin, which bind to two different sites on the 50S ribosomal subunit and interfere with protein synthesis;[14] the oxazolidinone, linezolid, which also binds the 50S ribosomal subunit;[15] the tetracycline derivative, tigecycline, which targets protein synthesis via the 30S ribosomal subunit;[16] and the lipoglycopeptide, dalbavancin, which has the same mode of action as vancomycin, binding to the D-Ala-D-Ala motif in the cell wall.[17]

As these examples illustrate, most new antibiotics are derivatives of existing drugs that also target the aforementioned pathways. Unfortunately, bacterial resistance to these drugs is quick to develop. These data argue for the continued pursuit of antibiotics with entirely new modes of action, which may better avoid mechanisms of resistance and have longer effective life times.

An attractive strategy for the development of novel antibiotics is to target bacterial protein homeostasis (proteostasis) mechanisms, in particular molecular chaperones. Molecular chaperones

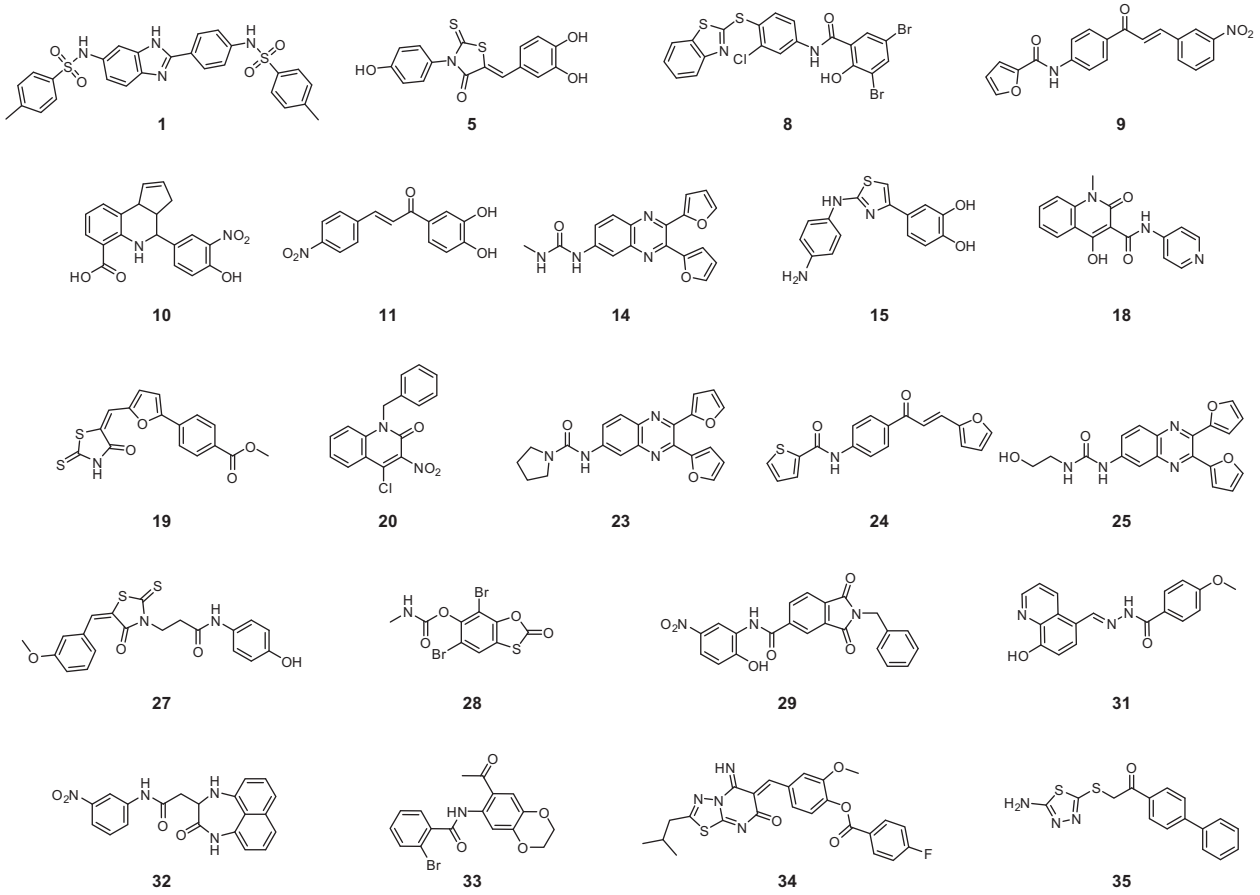

Figure 2. Structures of the 22 compounds under evaluation. For ease of comparison, compound numbering from **1** to **36** was maintained as presented in our previous high-throughput screening study.[37] Compounds **2**–**4**, **6**, **7**, **12**, **13**, **16**, **17**, **21**, **22**, **26**, **30**, and **36** were omitted from evaluation as they were either not commercially available, or purchased compounds were not readily identified by LC–MS and/or did not have acceptable purities confirmed by HPLC.