Antibacterial Discovery via Phenotypic DNA-Encoded Library Screening

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ABSTRACT: The global rise of multidrug resistant infections poses an imminent, existential threat. Numerous pipelines have failed to convert biochemically active molecules into bona fide antibacterials, owing to a lack of chemical material with antibacterial-like physical properties in high-throughput screening compound libraries. Here, we demonstrate scalable design and synthesis of an antibacterial-like solid-phase DNA-encoded library (DEL, 7488 members) and facile hit deconvolution from whole-cell Escherichia coli and Bacillus subtilis cytotoxicity screens. The screen output identified two low-micromolar inhibitors of B. subtilis growth and recapitulated known structure–activity relationships of the fluoroquinolone antibacterial class. This phenotypic DEL screening strategy is also potentially applicable to adherent cells and will broadly enable the discovery and optimization of cell-active molecules.

Multidrug-resistant (MDR) pathogens are on the rise, while antibacterial discovery has stalled. The pharmaceutical industry has largely abandoned antibacterial discovery and development in the wake of substantial failures to produce clinical candidates in the postgenome era. This stands in sharp contrast to the record-setting numbers of non-antibacterial FDA drug approvals. Expanded efforts both to understand the nature of antibacterial drug efficacy and to develop technology for identifying novel antibacterial scaffolds are sorely needed in the face of this impending global health crisis.

Most approved antibacterials are natural products or their derivatives; therefore recent efforts to identify novel scaffolds have focused on screening libraries of synthetics. However, these efforts were manifestly unproductive. High-throughput screening (HTS) campaigns have yielded many biochemically active compounds, but their subsequent optimization for antibacterial activity has proven to be a Sisyphean task: hydrophobic interactions improve target binding, but increased hydrophilicity confers bacterial accumulation. Moreover, HTS collections are devoid of novel bacterial cell-active compounds, suggesting that alternative sources of chemical diversity with more advantageous property distributions would be key to rekindling antibacterial discovery.

DNA-encoded libraries (DELs) can provide ready access to chemical space that is underrepresented in HTS compound collections, and emergent screening modalities suggest potential opportunities for cellular screening. DEL affinity selection is a widely deployed hit finding technology that has produced several clinical candidates, including lead molecules targeting HTS-refractory protein–protein interactions and antibacterial targets. Additionally, recent technology development efforts have afforded activity-based screening of individual solid-phase DEL beads in microfluidic water-in-oil droplets. Integrating solid-phase DEL technology with bead diffusion assays would address two fundamental challenges of antibacterial discovery: libraries could feature designable chemical diversity and that diversity could be screened directly for cellular activity.

We designed a 3-cycle solid-phase DEL (7488 members) to sample the physicochemical property space of known antibacterials. Hydrophobicity and molecular weight were key design considerations, as FDA-approved antibacterial drugs are less hydrophobic and have higher molecular masses than CNS-active FDA-approved drugs (Figure 1A), among other divergent properties (Figures S1 and S2). The DEL was prepared by split-and-pool synthesis with each building block covalently attached to resin (Figure 1B) via photocleavable linker, allowing mild (λ = 365

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nm) library member release from the bead for bioactivity assays.

We screened the DEL for antibacterial compounds using a whole cell bead diffusion assay and identified multiple cell-active compounds. In the assay modified from previous approaches, UV irradiation of DEL beads distributed on inoculated agar plates liberated compounds from beads, allowing their diffusion through the surrounding agar to yield a sufficient screening hit rate. This approach for phenotypic DEL screening was validated using photocleavable ciprofloxacin (PC-cipro) beads. PC-cipro beads only formed growth inhibition zones on bacterial plates upon UV irradiation, and the same UV irradiation did not affect bacterial growth (Figures S3 and S4). Similarly, a growth inhibition zone (GIZ) formed around DEL hit beads after overnight incubation (Figure S5). In total, 97 GIZ-forming hit beads (24 Escherichia coli, 73 Bacillus subtilis) were isolated from screening approximately 85,000 beads (45,000 E. coli, 40,000 B. subtilis; 0.05% and 0.2% hit rates, respectively; Table S2). Hit bead DNA tags were PCR amplified, sequenced, and decoded. Position 2 of the hit pool was overrepresented by amine-containing building blocks (65% of library; 98% of hits) but was devoid of related hydroxyl analogues (19% of library; 0% of hits). A fluoronaphthyridone building block was highly enriched at position 3 (53/97 hits, Figure 2A). Structurally related pyrrolopiiperidine and piperazine monomers in position 2 appeared frequently with the fluoronaphthyridone in screening hits (Figure 2B,C), suggesting that the position 1 building block identity may be inconsequential to these
Derivatives in Broth Microdilution Assays

Table 1. Characterization of Antibacterial Hits and Compounds

| ID | Structure | E. coli (μM) | B. subtilis (μM) |
|----|-----------|--------------|-----------------|
| 1  | ![Structure 1](image1) | >32 | 1 |
| 2  | ![Structure 2](image2) | 32 | 2 |
| 3  | ![Structure 3](image3) | 16 | 16 |
| 4  | ![Structure 4](image4) | 4 | 4 |
| 5  | ![Structure 5](image5) | 0.25 | 0.13 |
| 6  | ![Structure 6](image6) | 2 | 8 |
| 7  | ![Structure 7](image7) | 0.016 | 0.063 |
| 8  | ![Structure 8](image8) | 0.004 | 0.063 |

*Reported as MIC (μg/mL).*

The promising results demonstrating activity of 8 against screening strains spurred further mechanistic studies and evaluation of its potential as a broad spectrum antibacterial. Compound 8 inhibited *E. coli* DNA gyrase biochemical activity, albeit with one-fifth the potency of ciprofloxacin (IC₅₀ = 1.43 ± 0.06 and 0.28 ± 0.04 μM, respectively) (Figure S6). Antibacterial disk diffusion assays were conducted to evaluate 5 and 8 against three diverse bacterial panels: standard antibacterial susceptibility ATCC organisms, clinical isolates, and carbapenem-resistant (MDR) enterobacter strains. Disks impregnated with 5 or 8 generated GIZs on lawns of various Gram positive (*B. subtilis, Staphylococcus aureus*) and Gram negative (*E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens*) bacteria. Furthermore, 8 generated GIZs for 2/4 clinical isolates but was inactive against 10/11 MDR strains (Table 2). For all strains tested, GIZ measurements of ciprofloxacin and 8 agreed within 3 mm, while 5 was uniformly less potent.

Table 2. Antibacterial Disk Susceptibility Testing against ATCC Control and Clinical Strains

| organism | 8 (μg) | ciprofloxacin (μg) | cefederocol (30 μg) |
|----------|--------|---------------------|---------------------|
| *E. coli* ATCC 25922 | 31 | 33 | 25 |
| *B. subtilis* ATCC 23857 | 28 | 31 | 15 |
| *P. aeruginosa* ATCC 27853 | 24 | 27 | 25 |
| *K. pneumoniae* ATCC 70063 | 20 | 21 | 23 |
| *K. pneumoniae* BAA 1785 (CRE) | ![Ciprofloxacin](image9) | ![Ciprofloxacin](image10) | 19 |
| *S. aureus* ATCC 25923 | 20 | 23 | 9 |

*Reported as MIC (μg/mL).*
Fluoronaphthyridone 8 is a broad-spectrum synthetic antibacterial compound and, together with the other structures reported here, represents the first hits from cell-based DEL screening. Though its synthesis was previously described, its characterization was limited. Our analysis found that 8 and ciprofloxacin exhibited nearly identical activity against all bacterial strains tested. Failure of 8 in disk diffusion studies against the carbapenem-resistant strain panel underscores the challenge of developing therapeutics to address MDR strains. These strains were resistant to most classes of antibacterials, and some were even resistant to ceftiorocel, an antibacterial of last resort. This encouraging demonstration provides compelling motivation to design DELs around known antibacterial scaffolds and conduct phenotypic screens on clinically relevant MDR strains.

This study introduces cellular DEL screening capabilities to the expanding repertoire of activity-based DEL modalities. Previous combinatorial library screening techniques have shown that cells (prokaryotic or eukaryotic) and beads can be distributed either in wells or on culture dishes to identify active library members based on cellular response in the periphery of the bead. These studies inspired our work, which further integrated the pivotal advantages of DEL. DNA tags were readily amplified, sequenced, and decoded (97% and 35% loss after incubation with B. subtilis and E. coli, respectively). Recapitulating library synthesis conditions when preparing hit compounds revealed that an unplanned side reaction yielded the most potent hits, highlighting the importance of encoding chemical synthesis.

Although synthetic antibacterial discovery via HTS has been fraught with seemingly insurmountable challenges owing to the limited compound library physicochemical property space, phenotypic DEL screening offers a viable path forward. Indeed, synthetic compound collections have been completely and unsuccessfully mined for broad-spectrum antibacterials, and the rare discoveries of novel natural product antibacterial scaffolds are considered to be globally significant scientific advances. DEL technology enables efficient design, synthesis, and scouting of antibacterial-like and potentially many other novel chemical spaces. For example, amines have been observed to confer membrane permeability in Gram negative bacteria, and this hypothesis was supported by the enrichment of amine-containing compounds in the hit collection of this screen. Moreover, additional amine-enriched space is readily accessed using well-established and more recently discovered DNA-compatible reactions. Exploration of these new spaces will demand further technology development to overcome the present bottleneck of manual hit detection and bead picking. We anticipate that these approaches will begin to unravel the complex interplay between chemical properties, bacterial cell pharmacokinetics, and the desired broad-spectrum antibacterial response.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschembio.1c00714.

Experimental methods including DEL synthesis and screening, hit deconvolution, and synthesis, antibacterial activity assays, and supplementary tables and figures describing DEL synthesis, DEL BB and hit structures, DEL screening data, and activity assay data (PDF)

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**Notes**

The authors declare the following competing financial interest(s): B.M.P. declares a significant financial interest in 1859, a company seeking to commercialize some aspects of this work.

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