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## Antibiotics Made to Order

New lipopeptide antibiotics provide hope in the fight against multidrug resistant bacteria

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Antimicrobial drug resistance is a global threat to human health. There is an urgent need to discover new antibiotics whose modes of action circumvent prevalent clinical resistance mechanisms. Most antibiotics in clinical use are microbial natural products or their derivatives, whose production is encoded by a biosynthetic gene cluster (BGC) (1). Traditional antibiotic discovery strategies involve screening large microbial strain collections for antibiotic activity, followed by a resource-intensive pursuit of pure material for further characterisation. This pipeline is hampered by challenges isolating unexplored microbial taxa and because most BGCs are not expressed during laboratory studies (2, 3). On page XXX of this issue, Wang *et al.* (4) use *in silico* discovery of BGCs and chemical synthesis of their predicted products to identify a new lipopeptide that is active against multidrug resistant (MDR) clinical isolates. A previous report by this group also used this approach to identify a promising new antibiotic (5), highlighting its utility in antibiotic discovery.

Wang *et al.* (4) analysed ~10,000 bacterial genomes hunting for BGCs encoding lipopeptides, a clinically deployed antibiotic class with diverse modes of action (6). The authors prioritized BGCs phylogenetically unrelated to those previously characterized in hopes that they would produce new antibiotics. The authors identified a distinct lipopeptide BGC harboured by *Paenibacillus mucilaginosus*. Rather than pursue a time-consuming, culture-dependent approach to produce and purify the compound, the authors capitalized on the power of bioinformatic algorithms to predict possible compounds produced by the enzymatic machinery encoded by the BGC and then chemically synthesised them. They used this so-called “synthetic-bioinformatic natural product (synBNP)” approach to synthesise eight possible compounds predicted from the *P. mucilaginosus* BGC. One compound, which the authors named cilagicin, possessed potent bactericidal activity against several MDR Gram-positive bacteria. Cilagicin was active against difficult to treat *Clostridioides difficile* and vancomycin-resistant enterococci *in vitro*, which are considered urgent and serious threats by the U.S. Centers for Disease Control and Prevention (CDC) (7).

During their experiments, Wang *et al.* (4) discovered that a cell wall precursor accumulated in cilagicin-treated cultures. This observation suggested cilagicin inhibits cell wall biosynthesis, the same target of important classes of antibiotics, such as  $\beta$ -lactams (*e.g.*, penicillins, and

carbapenems) and glycopeptides (e.g., vancomycin) (8, 9). The authors established that cilagicin inhibits cell wall biosynthesis through sequestration of the lipid carrier molecule, undecaprenyl phosphate (C55-P) and its inactive form, undecaprenyl pyrophosphate (C55-PP). C55-PP is produced *de novo* and is dephosphorylated to C55-P during transport of cell wall precursors across the cytoplasmic membrane. Upon delivery of its cargo, C55-P is re-phosphorylated and returns to the inner leaflet of the membrane to replenish the dwindling supply of C55-PP. Thus, by sequestering both C55-PP and C55-P, cilagicin blocks bacterial transport of essential cell wall building blocks, which arrests production of the cell wall and ultimately causes cell death.

A handful of other antibiotics can bind to either C55-PP or C55-P, but overall, this mode of action is underexploited, and resistance to antibiotics that target only one occurs readily. Notably, Wang *et al.* did not observe evolution of resistance to cilagicin over the course of a 25-day experiment in which *Staphylococcus aureus* was serially passaged in culture medium containing a sub-inhibitory concentration of cilagicin, whereas resistance readily developed to bacitracin or amphotericin, agents that bind only to C55-PP or C55-P, respectively (10, 11). The lack of detectable resistance to cilagicin is likely linked to its ability to bind both C55-PP and C55-P, because changes to two distinct molecular targets must evolve for development of resistance. The binding of multiple targets simultaneously may be an important consideration when developing future antibiotics.

The same research team also recently used their synBNP approach to overcome colistin resistance (5). Resistance to colistin, another lipopeptide antibiotic, raised substantial concern when a resistance determinant encoded by a gene called *mcr-1* (mobilized colistin resistance 1) spread rapidly in pathogenic enteric bacteria around the globe. Widespread dissemination of the *mcr-1* gene jeopardised the utility of colistin as the last line of defense against infections caused by MDR Gram-negative bacteria (12). Gram-negative bacteria have a cell wall that is encased by an additional lipid outer membrane, which is a permeability barrier to many small molecules. This limits the number of antibiotics in our anti-Gram-negative arsenal that target the cell wall or other targets within. Colistin has potent antibiotic activity against Gram-negative bacteria because it binds to lipopolysaccharides and phospholipids in the outer membrane, displacing divalent cations, which disrupts membrane integrity and ultimately leads to cell death (13, 14).

Wang *et al.* (5) set out to identify BGCs that encoded the production of colistin analogues, with the clever rationale that nature may have figured out how to diversify the antibiotic to overcome resistance. Like their work with cilagicin, the authors focused their attention on a single BGC and synthesized its predicted product, which they named macolacin, which

possessed antibacterial activity against colistin-resistant bacteria. The authors were able to further improve macolacin activity by optimizing its lipid moiety (see the figure), which facilitates interaction with the membrane. One improved derivative, biphenyl-macolacin, outperformed the parent molecule and possessed potent *in vitro* activity against intrinsically colistin-resistant *Neisseria gonorrhoeae*, and carbapenem-resistant and extensively drug-resistant *Acinetobacter baumannii*, which are recognised as urgent threats by the CDC (7).

Although cilagicin and macolacin showed promising *in vitro* activity against problematic MDR bacterial pathogens, the real question was how well do these agents perform in an infection model? The answer will dictate the future of these agents as therapeutics. Wang *et al.* (4, 5) assessed each of these compounds in a mouse infection model. For cilagicin there was an initial setback. High levels of serum binding to cilagicin blocked its antibacterial activity. The authors overcame this hurdle by altering the lipid component of cilagicin, ultimately utilizing the same biphenyl moiety used to improve macolacin, as a strategy to reduce serum binding. Such hit-to-lead optimization is a key feature in antibiotic development (15). The new structure, cilagicin-BP (see the figure), was as efficacious as vancomycin when used to treat mice infected with MDR *S. aureus* and even more so when used to treat *Streptococcus pyogenes* infection. The efficacy of biphenyl-macolacin was also evaluated using mice infected with either carbapenem-resistant *A. baumannii* engineered to express the *mcr-1* colistin resistance gene, or an *mcr-1*-expressing clinical isolate of *A. baumannii* that is resistant to all antibiotics tested. Treatment with colistin did not reduce the bacterial load beyond that used to establish the infection, whereas treatment with biphenyl-macolacin reduced the bacterial load by five orders of magnitude.

Many promising antibiotic compounds fall by the wayside because of low production titre during microbial fermentation. Aside from a rare handful of compounds, chemical synthesis is ultimately used to produce the quantity, and importantly, the chemical diversity of analogues necessary to define the clinical potential of a lead pharmacophore. In two separate studies, Wang *et al.* not only produced two new biologically inspired antibiotics but established a route for their synthesis and generation of analogues. The next major step for development of cilagicin and macolacin are absorption, distribution, metabolism, excretion, and toxicity studies, which may reveal the need for further structural optimization prior to entry into clinical trials. Although clinical deployment of cilagicin and macolacin may take time, Wang *et al.* have established an inspirational interdisciplinary roadmap for future antibiotic discovery that may tip the scales in our fight against antimicrobial resistance.

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## Figure legend:

Discovery of cilagicin and macolacin.

Bioinformatics was used to identify biosynthetic gene clusters (BGCs) within bacterial genomes that produce lipopeptide antibiotics. Chemical synthesis of the predicted compounds from the cilagicin and macolacin BGCs and further chemical optimization resulted in two new promising antibiotics active against multidrug resistance bacteria.