Antibiotic Discovery: from mechanistic studies to target ID

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Chapter 6

Summary and conclusions

1. Summary

In light of the imminent threat of a post-antibiotic era there is currently an urgent need for fundamental research in antibiotics. Antibacterial drug discovery, however, is a particularly challenging field of medicinal research. The main goal of the work described in this thesis is to introduce strategies to discover and characterize unique drug-target combinations and facilitate a molecular level understanding of the mechanism of action of antimicrobials.

Chapter 1 highlighted the importance of antibiotic research and the urgent need for new antibiotics. When comparing the current pipeline of antibiotics in development with the increasing incidence of antimicrobial resistance (AMR), it is evident that we are in a global healthcare crisis. Beyond the scientific difficulties associated with antibiotic discovery and development, there are numerous regulatory and market-related issues that must also be addressed in order to protect and maximize the potential of the existing and future clinical agents.

Already at the discovery phase, there are various challenges that can slow down novel antibacterial candidates from entering medicinal chemistry programs and hit-to-lead optimization. For example, uncertainties relating to mechanism of action (MoA) and target identification can impede antibiotic development. In this regard, MoA studies that inform the optimization of the molecular structure, as well as, the physicochemical properties that can help mapping the drug-target interplay are of great importance. The elucidation of the mechanism of action of an antibacterial candidate leads to better assessing the relevance of the target, helps predict the propensity for resistance, and facilitates knowledge-able decisions in the optimization process.

Chapter 1 also discussed the existing wealth of MoA information that is ready to be mined among the already validated targets. One such example is the bacterial cell wall biosynthesis network and specifically the unique bacterial cell wall building block lipid II. A number of recent discoveries are discussed that exploit this validated target in the development of cell wall biosynthesis inhibitors. Lipid II is targeted by a variety of different antibiotics and represents the "Achilles' heel" of peptidoglycan biosynthesis. Mechanistic studies are pivotal in explaining the efficacy of lipid II targeting molecules and are important in further optimizing the chemical scaffolds of the antibiotic candidates while also supporting the discovery and development of new antibacterial strategies.

In **Chapter 2** a quantitative approach for the characterization of cell-wall active antibiotics binding to their bacterial targets is described. The data presented verify the interactions of antimicrobial peptides with natural undecaprenyl cell wall precursors and help explain their subsequent bactericidal effect. This chapter specifically illustrates the use of Isothermal Titration Calorimetry (ITC) to reveal a nanomolar binding affinity for teixobactin. Teixobactin is a natural peptide that belongs to an entirely novel antibiotic class and holds promising properties in terms of activity, *in vivo* efficacy and low propensity for resistance development. Also reported in **Chapter 2** is the characterization of the lipid II binding interactions with a new generation of semisynthetic lipoglycopeptide antibiotics. It is shown that compound **C7**, a semisynthetic vancomycin derivative under development in our lab, has an increased affinity for lipid II as compared to vancomycin and is also able to bind well to lipid II variants associated with vancomycin resistance. Notably, these studies include the first calorimetric analysis of vancomycin binding to lipid II in a membrane environment. Finally, in a similar manner, empedopeptin, a putative lipid II binding lipopeptide, was found to tightly engage with lipid II in the membrane phase. The structurally related tripropeptin was confirmed to share its binding partners with empedopeptin. A deep dive into the thermodynamic aspects underpinning the target binding of these two lipopeptide antibiotics was also possible given the experimental approach. Notably, the ITC analyses provided evidence for the role of calcium ions in promoting the interactions of the antibiotics with their individual targets. These applications have provided a comprehensive thermodynamic profile for their target engagement mechanism (**Figure 1),** and will pave the way for the characterization of compounds that have unique targets in the bacterial cell wall.

In **Chapter 3** ITC is used to further characterize the structural features of the bacterial phospholipid target, phosphatidylglycerol, that drive the binding of the lipopeptide antibiotic daptomycin to bacterial cells. Daptomycin is the only clinically used calcium-dependent lipopeptide antibiotic, and unlike other members of its class, selectively disrupts Gram-positive bacterial membranes by hijacking phospholipids. By studying the daptomycin-phosphoglycerol-Ca^{2+} complex we were able to elucidate the affinities of daptomycin to negatively charged phospholipid headgroups and in doing so demonstrate that its activity is specifically dependent on the hydroxyl functionalities present in the phosphoglycerol (**Figure 2**) and cardiolipin moieties. Also of note is our finding that calcium binding by daptomycin is strongly dependent on the presence of phosphatidylglycerol (**Figure 3**). These investigations provided new insights into the interactions that drive the antibacterial efficacy of this clinically used antibiotic.

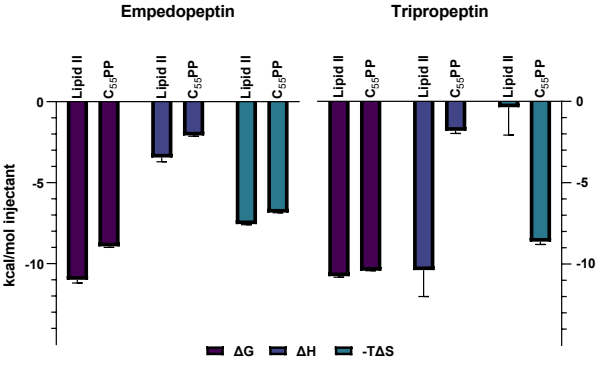
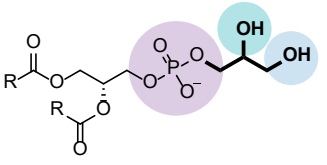


Figure 1 | Empedopeptin and tripropeptin share binding partners but not binding mechanisms Thermodynamic signature for empedopeptin (left) and tripropeptin C (right) binding to pyropshosphate containing precursors anchored in neutral LUVs, in $CaCl_2$ containing buffer. Athough empedopeptin binds both precursors with an entropic contribution, tripropeptin does not, as Lipid II binding is dominated by enthalpy.



R: lipid chain

Figure 2 | Phosphatidylglycerol Daptomycin recognizes the hydroxyl moieties of the phosphoglycerol headgroup. The 2-hydroxyl (green) of the glycerol unit is enough for binding, while the distal 3-hydroxyl (blue) increases affinity. The phosphate plays a role in the calcium dependency of daptomycin's affinity to

Chapter 4 reported the discovery of a new class of small molecules with potent antibiotic activity against clinically relevant Gram-negative bacteria. Bacterial susceptibility testing was used to screen a small molecule library and establish structure activity relationships (SAR), for the isoquinoline sulfonamide hit **1 (Figure 4a).** These investigations led to the rational design of compound LEI-800, containing a cis-2R,5S pyrrolidine core (**Figure 4a**), a novel compound with enhanced antimicrobial activity against *E. coli* and *K. pneumoniae*. **Chapter 4** also described studies aimed at the elucidation of the MoA and subsequent target identification for LEI-800 in *E. coli*. Bacterial cytological profiling (BCP) in combination with whole-genome sequencing of resistant *in vitro* isolates revealed that the isoquinoline sulfonamides disrupt DNA topoisomerase activity. Selected mutations and biochemical studies led to the verification of DNA gyrase as the target responsible for their antimicrobial activity. DNA gyrase is a well-validated, essential bacterial topoisomerase that is targeted by ciprofloxacin (CIP), a fluoroquinolone for which widespread

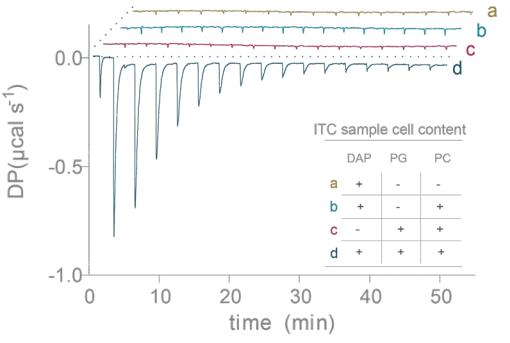


Figure 3 | Ca^{2+} binds a daptomycin in presence of PG/PC membrane models The calcium dependency of daptomycin is revealed by calcium chloride titrations in a set of different preparations. No interaction is observed when the $CaCl_2$ is titrated in: **a)** daptomycin (DAP) solution; **b)** daptomycin premixed with homogeneous membrane preparations consisting of the neutral diacyl phospholipid phosphatidylcholine (PC); **c)** mixed membrane preparations consisting of the negatively charged diacyl phospholipid phosphatidylglycerol (PG) and PC. **d)** An exothermic signal is observed when Ca^{2+} is mixed with daptomycin premixed with mixed membrane preparations consisting of PG and PC.

resistance has been observed in the clinic.[1,2] Importantly, LEI-800 also showed activity against CIP-resistant and multidrug resistant clinical isolates. Also of note, LEI-800 was found to inhibit the supercoiling activity of DNA gyrase more effectively than CIP (LEI-800 IC_{50} = 35 ± 13 nM; CIP IC_{50} = 925 ± 370 nM) despite having a lower antibacterial activity (LEI-800 MIC = 3.1 µM, 1.4 µg/mL; CIP MIC = 24 nM, 8 ng/mL). We subsequently found that this inconsistency is due to the reduced ability of LEI-800 in crossing the Gram-negative outer membrane (OM): both genetic and pharmacological perturbation of the complex lipopolysaccharide (LPS) layer of the OM increased the potency of LEI-800. Structural studies using cryo-EM revealed an unprecedented allosteric binding mode for LEI-800 at a site on DNA gyrase that is distinct from the binding site of CIP. Our discovery of the isoquinoline sulfonamides as allosteric DNA gyrase inhibitors presents a novel approach against Gram-negative pathogens, which holds promise to counteract fluoroquinolone resistance.

Chapter 5 described the identification of an oxadiazolone scaffold with activity against methicillin-resistant *S. aureus* (MRSA). Susceptibility testing against strains of *S. aureus*, including an MRSA clinical isolate, was used to establish a SAR map, leading to the design of compound **2** (**Figure 4b**) which exhibits submicromolar minimum inhibitory concentrations (MIC = 0.8 µM, 0.3 µg/mL).

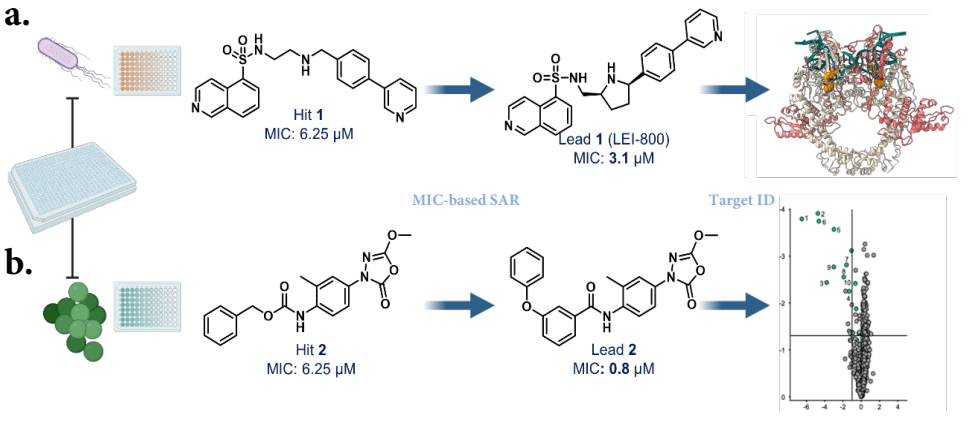


Figure 4 | Susceptibility screens led to the discovery of lead antibiotic compounds and their bacterial target pathways Hit-to-lead and target identification workflow reveal **a)** the isoquinoline sulfonamides as potent inhibitors of the DNA gyrase of *E. Coli*, and **b)** oxadiazolone derivatives interfering with an array of essential and non-essential enzymes, to inhibit MRSA growth.

Notably, an appropriate toxicity against antibacterial activity ratio was also ascertained for **2** ($CC_{50} \geq 10 \times MIC_{MRSA}$, *i.e.,* 10-fold less toxic to human cell lines than bacterial cells). Compound **2** showed antibacterial activity against a variety of clinical isolates of MRSA, including vancomycin-resistant *S. aureus* (VRSA), while stable spontaneous resistant mutants were not isolated in laboratory conditions. Building from these findings, an activity-based probe related to compound **2** was also synthesized and used to reveal the polypharmacological MoA of the oxadiazolones. Target identification was carried out with MS-based and gel-based competitive chemical proteomics protocols which led to the identification of 10 protein candidates, including a number of cysteine and serine proteases. The protein hits were validated by pharmacological inhibition for FabH (the only essential target for MRSA among the 10 proteins identified) and competitive labelling of transposon mutants. The appearance of resistant isolates was also used to probe the role of the protein hits, revealing significant increases in the expression of FphE, AdhE, and FabH, and a significant reduction in AdhE activity. Principal component analysis suggested AdhE and FphC as targets of the active inhibitors. Interestingly, the essential protein FabH was still inhibited strongly by oxadiazolone derivatives that had no antibacterial action, which suggested that FabH inhibition alone is not responsible for the observed antibacterial effect. Also, AdhE and FphC transposon mutants were susceptible to the same inactive derivatives. These data suggest a

polypharmacological MoA for oxadiazolones and highlight FabH, FphC and AdhE as new, auxiliary targets for MRSA. This MoA study further showcases chemical proteomics as a valuable chemical biology technique for antibiotic drug discovery.

2. Conclusions in perspective

The AMR threat, as eloquently described by Cook and Wright in a recent review,[3] is in its foundation a kinetics problem, as the rate of resistance development is currently outpacing the rate at which novel therapies are brought to the clinic. The closer we come to shift this balance, the more chances there are to reverse the imminent post-antibiotic era.

An antibiotic's cellular mechanism of action or specific molecular target(s) are sometimes not known at the hit-to-lead stage. In fact, in some cases, the elucidation of the precise MoA of an antibiotic has been achieved years after the approval of the drug. For example, the cellular effects of polymyxin[4] or the specific molecular target of daptomycin[5] and its corresponding bactericidal MoA,[6] have, and continue to be, the subject of significant research. Modern methods such as those described in this thesis have the potential to enable early characterization of an antibiotic's MoA and provide the key insights needed for the design and development of agents with promising antibacterial and resistance profiles.

The lipid II binders In addition to new insights into the cellular roles of lipid II and the undecaprenyl intermediates (*outlined in* **Chapter 1**, *reviewed in refs:* 7 *and* 8), mechanistic studies have also greatly enhanced our understanding of lipid II targeting antibiotics. Emerging discoveries on mechanisms of action provide fresh insights into the parameters that affect antibacterial efficacy and in some cases reveal multimodal capacities for lipid II-targeting compounds. Nisin, a very well characterized lipid II-binder,[9–12] shows activity in nanomolar concentrations, which results from its ability to use the membrane-bound lipid II as a docking molecule with subsequent formation of pores in the cytoplasmic membrane.[10] In another example of a dual antibacterial attack, teixobactin[13] highjacks lipid II to disrupt the cytoplasmic membrane via the formation of a complicated fibrous complex.[14] More research into the molecular drivers of antibiotic efficacy will hopefully lead to the rational design of molecules with superior properties for their targeting pathway(s). The applications of microcalorimetry as described in **Chapter 2** for studying drug-target interactions in membrane bilayers, enhances our

ability to understand the full spectrum of antibiotic-undecaprenyl precursor recognition. This allows one to differentiate cell-wall precursor binding compounds from inhibitors of biosynthetic enzymes or other disruptors of the biosynthetic machinery. The thermodynamic properties of the binding interaction as provided by ITC can also provide valuable insights, useful for the optimization of cell-wall active molecules.

Mode of action of daptomycin Daptomycin's rapid bactericidal activity against a broad spectrum of resistant Gram-positive microorganisms (MRSA, VRSA, VRE), led to its approval for clinical use in 2003 as a new 'last resort' antibiotic. Early mechanistic studies indicated that daptomycin's MoA was driven by disruption of bacterial membranes.[15,16] Daptomycin's low efficacy in treating pulmonary infection (implicating lung surfactant as an antagonist of daptomycin activity) and evolution of resistance mechanisms (indicating a role for phosphoglycerol-containing membrane lipids) subsequently directed research efforts towards the negatively charged membrane constituents. This ultimately led to the identification of phosphatidylglycerol as a primary target for daptomycin. The studies in **Chapter 3** made clear that the hydroxyl functionalities of the phosphoglycerol headgroup are an essential structural feature for daptomycin recognition. The compelling evidence for the specificity of daptomycin to phosphoglycerol, together with novel methods in synthesis,[15] have enabled a more thorough and systematic exploration.[16] Specifically, during the course of the work reported in this thesis, Taylor and coworkers published a series of new findings regarding daptomycin.[5,18–20] Among them, the specific stereoisomer 2R,2′S of phosphoglycerol was shown to be its target. Although the absolute configuration of PG in most bacteria has not been determined,[21] the finding that the enantiomer of daptomycin (also prepared by the Taylor group) is inactive strongly suggests that the PG in bacterial membranes bears the 2R,2′S stereochemistry.

Discovery of an inhibitor of E. coli DNA gyrase All pathogens classified as critical on the WHO priority list are Gram-negative bacterial strains.[22] Despite substantial efforts, only a few novel compounds currently in development are intended for the treatment of Gram-negative infections.[23,24] In this regard, the discovery of the isoquinoline sulfonamides described in **Chapter 4** as a new anti-Gram-negative scaffold is a notable finding. While LEI-800 shows potent DNA gyrase inhibition *in vitro*, its antibacterial activity is affected by its limited capacity to cross the Gram-negative outer membrane. OM permeability is a function of numerous factors including the presence of varying lipopolysaccharide structures, efflux systems, and multiple porins. Our studies with

mutant strains such as ΔrfaD (*rfaD*, disrupted LPS biosynthesis) as well as ΔTolC (*tolC*, disrupted efflux systems) indicate that poor OM penetration is a key factor in limiting the activity of LEI-800. It should, however, also be noted that porin-mediated transport still needs to be investigated. This knowledge will help the optimization of the isoquinoline sulfonamides using structural elements known to facilitate bacterial membrane permeability and will hopefully increase their intracellular accumulation. Thanks to cryogenic electron microscopy (cryoEM) experiments, such optimization ventures can also be based on structural information. **Chapter 4** describes the identification of a unique binding pocket (**Figure 5**) in each GyrA subunit wherein two serine residues (Ser97 and Ser172, **Figure 5**) can coordinate LEI-800 via hydrogen bonds. In this way, LEI-800, as well as several of its analogues, bind the DNA gyrase complex and inhibit its DNA supercoiling activity.

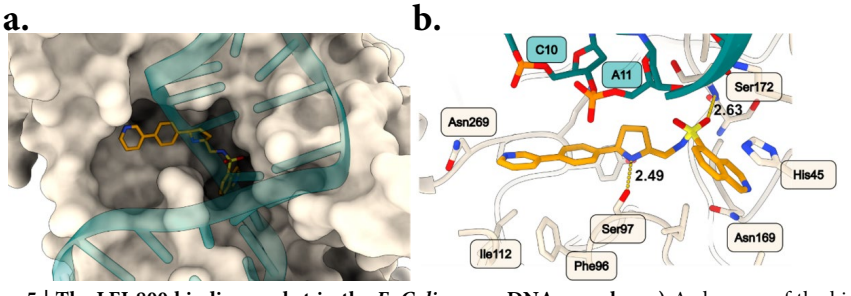


Figure 5 | The LEI-800 binding pocket in the *E. Coli* gyrase-DNA complex **a)** A close-up of the binding pocket on the DNA-binding surface of GyrA subunit. GyrA is shown as molecular surface (beige), DNA as semi-transparent cartoon (teal green), and LEI-800 as stick representation. **b)** Molecular interactions between GyrA and LEI-800. LEI-800 is shown as stick representation. Main GyrA residues important for LEI-800 binding are labelled. Two key hydrogen bonds between the Ser97 side-chain and the central pyrrolidine nitrogen of LEI-800, and the Ser172 backbone nitrogen and sulfonic acid of LEI-800 are shown in gold; distances in Angstrom are indicated. *Experiments, analysis and figure design were conducted by Dmitry Ghilarov.*

DNA gyrase is a well-characterized and validated target, as exemplified by the clinical success of the fluoroquinolones, aminocoumarins,[25,26] as well as agents currently in clinical development[23] (a first in class spiropyrimidinetrione,[27] zoliflodacin, in Phase III, the "NBTI"[28] triazaacenaphthylene gepotidacin,[29,30] Phase III and indoline-containing BWCO977,[31] Phase I). Our studies highlight LEI-800 and its analogues as unique binders with superior DNA gyrase inhibitory activity when compared to the fluoroquinolones. The discovery of LEI-800 and the elucidation of the structure of the complex it forms with DNA gyrase provide opportunities to design new inhibitors of this highly mutable

target.[1] In addition, as a potent small molecule inhibitor, LEI-800 has the potential for further optimization as a single antibacterial agent as well as the possibility to be used in combination with existing antibacterial agents.

In search of druggable pathways An important premise in recent approaches to antibiotic discovery is that the bacterial target to be exploited maintains its validity in the drug resistant pathogens encountered in the clinical setting. To this end, target validation measures should also consider the potential for resistance development in addition to pre-existing resistance elements. While the oxadiazolones described in **Chapter 5** were initially identified as anti-MRSA hits, other multidrug resistant clinical isolates were also found to be susceptible and spontaneous selection for stable resistance was not observed. Investigating the possible oxadiazolone targets by chemical proteomics, brought us a step further in characterizing new druggable target(s) of clinically relevant *S. aureus* strains. These investigations revealed FabH as a clear candidate. FabH is the only protein among the proteomic hits that is essential for MRSA growth *in vitro*.[32,33] Although it has previously been implicated as an antibiotic target,[34,35] it is not yet known if FabH inhibition presents a clinically relevant strategy.[36] Among the proteomic hits identified, AdhE is a well-characterized bi-functional enzyme, with dual aldehyde- and alcohol-dehydrogenase activities.[37–39] In addition, among the fluorophosphonate-binding hydrolase (Fph) enzymes identified, FphB is reported to be essential for *S. aureus* virulence,[40] while FphC and FphE are largely uncharacterized. Given the growing interest in exploitable bacterial pathways,[41] the function of these targets and the apparent polypharmacology of the oxadiazolone scaffold hold particular interest.

To conclude, the investigations described in this thesis lay out strategies aimed at advancing antibiotic research and development. The examples presented revolve around two main approaches: understanding drug-target interactions and target identification. The applications of microcalorimetry for characterization of drug-target complexes provide a thermodynamic profile within a complex membrane system. These studies assist in the target identification of cell wall active compounds and the thermodynamic parameters generated can support antibiotic design and structural optimization. Furthermore, a suite of target identification methods, including whole genome sequencing and MS-based chemical proteomics, led to the discovery of a new class of DNA gyrase inhibitors as well as the identification of previously unmapped pathways in *S. aureus*, orchestrated by series of known and unknown enzymes. While significant development will be required to advance the isoquinoline sulfonamides and

oxadiazolones, towards the clinic, the approaches employed in their identification as antibiotic leads rely on advanced techniques with the potential to detect unprecedented mechanisms of action. Increasing our knowledge of bacterial cell systems by using such approaches is imperative to expanding our understanding of druggable targets.

References

1. Bhatnagar, K. & Wong, A. The Mutational Landscape Of Quinolone Resistance In *Escherichia Coli*. *PLoS One* **14**, (2019).
2. Nitiss, J. L. DNA Topoisomerase II And Its Growing Repertoire Of Biological Functions. *Nature Reviews Cancer* **9,** 327–337 (2009).
3. Cook, M. A. & Wright, G. D. The Past, Present, And Future Of Antibiotics. *Sci. Transl. Med.* **14**, eabo7793 (2022).
4. Manioglu, S. *et al.* Antibiotic Polymyxin Arranges Lipopolysaccharide Into Crystalline Structures To Solidify The Bacterial Membrane. *Nat. Commun.* **13**, 6195 (2022).
5. Moreira, R. & Taylor, S. D. The Chiral Target Of Daptomycin Is The 2R,2′S Stereoisomer Of Phosphatidylglycerol. *Angew. Chemie - Int. Ed.* **61**, (2022).
6. Zuttion, F. *et al.* High-Speed Atomic Force Microscopy Highlights New Molecular Mechanism Of Daptomycin Action. *Nat. Commun.* **11**, 6312 (2020).
7. Müller, A., Klöckner, A. & Schneider, T. Targeting A Cell Wall Biosynthesis Hot Spot. *Nat. Prod. Rep.* **34**, 909–932 (2017).
8. Grein, F., Schneider, T. & Sahl, H.-G. Docking On Lipid II—A Widespread Mechanism For Potent Bactericidal Activities Of Antibiotic Peptides. *J. Mol. Biol.* **431**, 3520–3530 (2019).
9. Breukink, E. *et al.* Use Of The Cell Wall Precursor Lipid II By A Pore-Forming Peptide Antibiotic. *Science (80-.).* **286**, 2361–2364 (1999).
10. Wiedemann, I. *et al.* Specific Binding Of Nisin To The Peptidoglycan Precursor Lipid II Combines Pore Formation And Inhibition Of Cell Wall Biosynthesis For Potent Antibiotic Activity. *J. Biol. Chem.* **276**, 1772–1779 (2001).
11. Breukink, E. *et al.* Lipid II Is An Intrinsic Component Of The Pore Induced By Nisin In Bacterial Membranes. *J. Biol. Chem.* **278**, 19898–19903 (2003).
12. Kleijn, L. H. J. *et al.* Total Synthesis Of Laspartomycin C And Characterization Of Its Antibacterial Mechanism Of Action. *J. Med. Chem.* **59**, 3569–3574 (2016).
13. Ling, L. L. *et al.* A New Antibiotic Kills Pathogens Without Detectable Resistance. *Nature* **517**, 455–459 (2015).
14. Shukla, R. *et al.* Teixobactin Kills Bacteria By A Two-Pronged Attack On The Cell Envelope. *Nature* **608**, 390–396 (2022).
15. Alborn, W. E., Allen, N. E. & Preston, D. A. Daptomycin Disrupts Membrane Potential In Growing *Staphylococcus Aureus*. *Antimicrob. Agents Chemother.* **35**, 2282–2287 (1991).
16. Allen, N. E., Alborn, W. E. & Hobbs, J. N. Inhibition Of Membrane Potential-Dependent Amino Acid Transport By Daptomycin. *Antimicrob. Agents Chemother.* **35**, 2639–2642 (1991).
17. Lohani, C. R., Taylor, R., Palmer, M. & Taylor, S. D. Solid-Phase Total Synthesis Of Daptomycin And Analogs. *Org. Lett.* **17**, 748–751 (2015).
18. Moreira, R. & Taylor, S. D. Establishing The Structure–Activity Relationship Between Phosphatidylglycerol And Daptomycin. **8**, 1674–1686 (2022).

19. Moreira, R. & Taylor, S. D. A54145 Factor D Is Not Less Susceptible To Inhibition By Lung Surfactant Than Daptomycin. *ACS Infect. Dis.* **8**, 1935–1947 (2022).

20. Barnawi, G. *et al.* Discovery Of Highly Active Derivatives Of Daptomycin By Assessing The Effect Of Amino Acid Substitutions At Positions 8 And 11 On A Daptomycin Analogue. *ACS Infect. Dis.* **8**, 778–789 (2022).

21. Sohlenkamp, C. & Geiger, O. Bacterial Membrane Lipids: Diversity In Structures And Pathways. *FEMS Microbiol. Rev.* **40**, 133–159 (2016).

22. World Health Organization. *Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis (September 2017)*. (2017).

23. World Health Organization. *Antibacterial products in clinical development for priority pathogens (June 2022)*. (2022).

24. Butler, M. S. *et al.* Analysis Of The Clinical Pipeline Of Treatments For Drug-Resistant Bacterial Infections: Despite Progress, More Action Is Needed. *Antimicrob. Agents Chemother.* **66**, (2022).

25. Collin, F., Karkare, S. & Maxwell, A. Exploiting Bacterial DNA Gyrase As A Drug Target: Current State And Perspectives. *Appl. Microbiol. Biotechnol.* **92**, 479–497 (2011).

26. Dighe, S. N. & Collet, T. A. Recent Advances In DNA Gyrase-Targeted Antimicrobial Agents. *Eur. J. Med. Chem.* **199**, 112326 (2020).

27. Bradford, P. A., Miller, A. A., O'Donnell, J. & Mueller, J. P. Zoliflodacin: An Oral Spiropyrimidinetrione Antibiotic For The Treatment Of *Neisseria Gonorrheae*, Including Multi-Drug-Resistant Isolates. *ACS Infect. Dis.* **6**, 1332–1345 (2020).

28. Desai, J., S, S., Kumar, S. & Sharma, R. Novel Bacterial Topoisomerase Inhibitors (NBTIs) – A Comprehensive Review. *Eur. J. Med. Chem. Reports* **3**, 100017 (2021).

29. Vanden Broeck, A., Lotz, C., Ortiz, J. & Lamour, V. Cryo-EM Structure Of The Complete *E. Coli* DNA Gyrase Nucleoprotein Complex. *Nat. Commun.* **10**, 4935 (2019).

30. Scangarella-Oman, N. E. *et al.* Dose Selection For Phase III Clinical Evaluation Of Gepotidacin (GSK2140944) In The Treatment Of Uncomplicated Urinary Tract Infections. *Antimicrob. Agents Chemother.* **66**, (2022).

31. Michael, D. *et al. In Vitro* Activity Of BWC0977 (A Novel Bacterial Topoisomerase Inhibitor) And Comparators Against Recent Clinical And Molecularly Characterized Enterobacteriaceae And Non-Fermenter Isolates From The United States And Europe. *ECCMID, Amsterdam* 13–16 (2019).

32. Perez, M. & Castillo, Y. Bacterial β-Ketoacyl-Acyl Carrier Protein Synthase III (FabH): An Attractive Target For The Design Of New Broad-Spectrum Antimicrobial Agents. *Mini-Reviews Med. Chem.* **8**, 36–45 (2008).

33. Song, H., Ao, G. Z. & Li, H. Q. Novel FabH Inhibitors: An Updated Article Literature Review (July 2012 To June 2013). *Expert Opin. Ther. Pat.* **24**, 19–27 (2014).

34. Martens, E. & Demain, A. L. Platensimycin And Platencin: Promising Antibiotics For Future Application In Human Medicine. *J. Antibiot. (Tokyo).* **64**, 705–710 (2011).

35. Wang, J. *et al.* Discovery Of Novel Antibiotics As Covalent Inhibitors Of Fatty Acid Synthesis. *ACS Chem. Biol.* **15**, 1826–1834 (2020).

36. Parsons, J. B. & Rock, C. O. Is Bacterial Fatty Acid Synthesis A Valid Target For Antibacterial Drug

Discovery? *Curr. Opin. Microbiol.* **14**, 544–549 (2011).

37. Beckham, K. S. H. *et al.* The Metabolic Enzyme AdhE Controls The Virulence Of *Escherichia Coli* O157: H7. *Mol. Microbiol.* **93**, 199–211 (2014).

38. Fuchs, S., Pané-Farré, J., Kohler, C., Hecker, M. & Engelmann, S. Anaerobic Gene Expression In *Staphylococcus Aureus*. *J. Bacteriol.* **189**, 4275–4289 (2007).

39. Lo, J., Zheng, T., Hon, S., Olson, D. G. & Lynd, L. R. The Bifunctional Alcohol And Aldehyde Dehydrogenase Gene, AdhE, Is Necessary For Ethanol Production In *Clostridium Thermocellum* And *Thermoanaerobacterium Saccharolyticum*. *J. Bacteriol.* **197**, 1386–1393 (2015).

40. Lentz, C. S. *et al.* Identification Of A S. Aureus Virulence Factor By Activity-Based Protein Profiling (ABPP) *Nat. Chem. Biol.* **14**, 609–617 (2018).

41. Petchiappan, A. & Chatterji, D. Antibiotic Resistance: Current Perspectives. *ACS Omega* **2**, 7400–7409 (2017).