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Research Highlight

Clovibactin: A revolutionary antibiotic unleashing lethal efficacy against pathogens with little drug resistance

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The decades spanning from the 1940s to the 1960s are renowned as the heyday of antibiotic discovery. It was during this era that game-changing antibiotics such as streptomycin, vancomycin, and tetracycline were unearthed, fundamentally reshaping the landscape of modern medicine. However, antibiotic discovery faces alarming challenges and traditional screening has not yielded new drugs in many decades. The crisis of antimicrobial resistance (AMR) emerges as a consequence of the unintended disruption of the antibiotic discovery pipeline and the subsequent uncontrolled proliferation of resistant pathogens [1]. According to the World Health Organization (WHO), in 2019, approximately 1.2 million people worldwide died of bacterial infections exacerbated by AMR, surpassing the number of deaths caused by HIV/AIDS. If this trend continues, by 2050, AMR could potentially result in over 10 million deaths, surpassing the mortality rate attributed to cancer. Thus, we are in dire need of new antibiotics to counter the rising resistance of bacteria to most currently employed clinical antibiotics.

In a recent study published in *Cell*, Shukla et al. [2] discovered a novel antibiotic, clovibactin, isolated from an uncultured solid bacterium, which demonstrates efficient pathogen-killing capabilities and leaves no resistance by targeting the pyrophosphate of multiple essential peptidoglycan precursors. The discovery of clovibactin represents a groundbreaking advancement with profound implications for the future of antibiotics, as the stable clovibactin supramolecular structures persist on bacterial cell walls to evade AMR, raising the possibility of designing the first-generation antibiotics with potentially life-long effects.

Utilizing iChip technique and commencing with the extended incubation of environmental bacteria, Shukla et al. [2] successfully isolated the novel antibiotic, clovibactin. The bacterial strain from which clovibactin originates was identified as *E. terrae* ssp. *carolina*, belongs to the Gram-negative β-proteobacteria and was initially isolated from soil samples in North Carolina. It is worth noting that this particular strain falls within the category of uncultured bacteria, a group of microbes that cannot be cultivated using conventional laboratory techniques. The discovery of clovibactin from this strain highlights the potential of exploring previously uncultured bacteria as a rich source of novel bioactive compounds, particularly in the context of antibiotic discovery [3]. Clovibactin may be a significant new weapon against superbugs. Clovibactin exhibited superior antibacterial activity against a broad spectrum of Gram-positive bacterial pathogens without cytotoxicity to mammalian cells. Also, clovibactin demonstrated efficacy similar to vancomycin, an effective antibiotic against Gram-positive pathogens, and effectively treated mice infected with *Staphylococcus aureus*. Clovibactin is greatly departing from the conventional antibiotics as it was derived from previously unculturable bacteria. Pathogenic bacteria have not encountered such an antibiotic before, leaving them insufficient time to develop detectable resistance.

Why did clovibactin stand out among all described antibiotics and what are the killing mechanisms of clovibactin? Shukla et al. [2] conducted a comprehensive series of experiments, including biochemical assays, solid-state nuclear magnetic resonance (ssNMR), and atomic force microscopy (HS-AFM) analysis. The results obtained from these experiments effectively addressed these questions. First, clovibactin defies novel structural features. Unlike traditional antibiotics that often target mutable portions of precursors, clovibactin targets the immutable pyrophosphate moiety found in various bacterial cell wall precursors, such as C_{55}PP, lipid II, and lipid III WTA. Clovibactin's specificity to this invariant region is a remarkable departure from established antibiotic paradigms. Second, clovibactin has a broad spectrum of activities against Gram-positive pathogens. Among clovibactin's most compelling attributes is its broad-spectrum antibacterial activity. Extending its efficacy across a wide range of bacterial strains, including the notorious *Staphylococcus aureus*, positions clovibactin as a promising candidate in the battle against antibiotic-resistant pathogens. Its ability to target multiple essential cell wall precursors enhances its potential clinical utility. Third, the selective binding and resistance avoidance abilities enhance clovibactin antibacterial potency. Clovibactin employs an unconventional hydrophobic interface to securely envelop the pyrophosphate while disregarding the variable structural elements of precursors. Clovibactin's remarkable selectivity in binding to the pyrophosphate group while disregarding the variable sugar-peptide

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portions of its target molecules. This exquisite specificity ensures efficient and targeted disruption of bacterial cell wall synthesis, enhancing its robustness to resistance development. Finally, clovibactin can do even more: the formation of stable supramolecular complexes represents a groundbreaking aspect of clovibactin's mechanism. These large fibrils serve as focal points for its antibacterial activity, allowing for the concentration of the antibiotic on bacterial membranes. This sustained presence and action, even after the soluble compound has been cleared from the body, likely elevate clovibactin's potential as a clinical agent to kill bacteria.

Notably, Shukla and colleagues [4] carefully elucidated the bactericidal action of a powerful new antibiotic, teixobactin in *Nature* in 2022. Just a year later, they made a game-changing discovery with the novel antibiotic clovibactin. Such rapid progress in antibiotic discovery is truly exhilarating. Similar to clovibactin, teixobactin delivers potent antibacterial effects through precise binding to conserved lipid precursors, disrupting cell wall biosynthesis and forming supramolecular complex that destabilizes membranes (Fig. 1). However, clovibactin and teixobactin exhibit notable differences. Clovibactin inhibits cell wall synthesis by targeting C_{55}PP, lipid II, and lipid III WTA, whereas teixobactin primarily targets lipid II (Fig. 1b). Both of them bind lipid II on the membrane surface (Fig. 1c). Clovibactin is only loosely anchored into the membrane by a single long hydrophobic residue (Leu2), explaining why clovibactin supra-structures are quite mobile. The side chains of the depsi-cycle that face the lipid II sugars are exclusively hydrophobic (Ala6, Leu7, and Leu8). GlcNAc is situated away from the complex interface. Clovibactin primarily targets the conserved pyrophosphate group in a specific manner, which also explains why it exhibits a strong affinity for other essential precursor molecules (lipid I and C_{55}PP). This multi-target mechanism is a significant factor contributing to clovibactin's resistance to the development of antimicrobial resistance. Teixobactin is firmly secured within the membrane by three extended hydrophobic residues (Ile2, Ile5, and Ile6), thereby enhancing the rigidity of teixobactin's supramolecular complexes. The pentapeptide is highly mobile and does not participate in complex formation, which is one of the reasons why clovibactin and teixobactin are less prone to the development of antimicrobial resistance. At the membrane surface, both clovibactin and teixobactin bind to lipid II, forming small oligomers that act as nuclei for fibril formation (Fig. 1d). The formation of these fibrils facilitates the stable binding of lipid II and other cell wall precursors, effectively blocking cell wall biosynthesis. It is noteworthy that the fibrils formed by the clovibactin-lipid II complex are less deeply inserted and rest on top of the membrane surface. This is in contrast to teixobactin, which penetrates the membrane quite deeply.

However, some questions remain unanswered. The exact arrangement of the clovibactin-lipid II supramolecular structure is not conclusively established, and alternative arrangements involving clovibactin interactions cannot be ruled out. Interestingly, Chen et al. [5] recently unveiled the supramolecular assembly of mosaic RIP1-RIP3 complexes reported in *Nature Cell Biology*, and these complexes promote the oligomerization of MLKL, leading to membrane disruption and kill target cells in various mammalian cells. Supramolecular structures can serve as biochemical reaction centers, substantially elevating the local effector protein concentrations, thus enabling proximity-driven protein activation and spatial regulation of cellular signaling [6]. Therefore, an important question arises: Is the formation of supramolecular complex assemblies a fundamental principle in cell fate determination across different species? Thus far, scientists have only scratched the surface of supramolecular assembly signaling, with many more discoveries awaiting us in the future.

Clovibactin, as a peptide antibiotic, demonstrates a unique mode of action that sets it apart from conventional antibiotics. Structurally, it is classified as a novel depsipeptide, characterized by the presence of two *D*-amino acids in its linear N-terminus and a distinctive *D*-3-hydroxyasparagine residue within its depsipeptide ring (Fig. 1a). This peptide antibiotic displays a potent bactericidal effect primarily against various Gram-positive pathogens, including drug-resistant strains, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Staphylococcus aureus*, and vancomycin-resistant *enterococci*. However, its efficacy against Gram-negative bacteria, such as *E. coli* and *Pseudomonas aeruginosa*, is comparatively limited, suggesting a certain limitation of spectrum in its antimicrobial activity.

Clovibactin's limited efficacy against Gram-negative pathogens is likely due to several factors. First, Gram-positive bacteria feature a simpler cell wall with a thick peptidoglycan layer, while Gram-negative bacteria possess an outer membrane composed of lipopolysaccharides and phospholipids, creating a permeability challenge for clovibactin. Second, clovibactin's mode of action may be more tailored to Gram-positive bacterial cell wall components, leading to reduced effectiveness against Gram-negative pathogens. Third, resistance genes in Gram-negative bacteria can expel or neutralize clovibactin, diminishing its bactericidal action [7]. Finally, enzyme systems, mutation rates, and membrane charge variations could further influence clovibactin's antibacterial performance. Hence, further research is still necessary for a better understanding of clovibactin's mechanisms of action on different types of bacteria.

The biosynthetic pathway of clovibactin involves a series of enzymatic reactions governed by specific biosynthetic genes (*cloA, cloB, cloC,* and *cloD*), leading to the stepwise assembly and cyclization of amino acid precursors into the final active compound. The intricate interplay of these biosynthetic elements contributes to the unique structure and bioactivity of clovibactin. Clovibactin demonstrates highly effective bactericidal activity against various drug-resistant Gram-positive pathogens, with a low risk of resistance development, which gives it the potential for long-term antibiotic use. Its specific mechanism of action targeting the immutable pyrophosphate structure in cell wall precursors appears to confer an advantage in avoiding common antibiotic resistance mechanisms. This suggests that over extended periods of use, it may exhibit a lower tendency for the development of resistance, providing a certain level of reliability for long-term treatment.

Nevertheless, regarding the concern about whether clovibactin leads to antibiotic resistance, it is important to recognize the complexity of the resistance issue. Although Shukla et al.'s study [2] indicates the effectiveness of clovibactin against certain bacterial strains, while being ineffective against others, such as *E. coli* K12, this suggests that different bacterial strains may elicit varying responses to the antibiotic. These differences may involve strain specificity and potential genetic variations, among other factors. Additionally, despite their study mentioning the current low levels of resistance and the relatively slow development of resistance due to clovibactin's targeting of an "immutable" bacterial cell wall structure, this does not imply that resistance will never emerge. Considering the adaptability and evolutionary capacity of bacteria, the development of resistance is a dynamic process that may be influenced by various factors [8]. Future prolonged clinical use and exposure may pose a potential risk for bacteria to develop resistance to clovibactin. While its mode of action seems to avoid common resistance mechanisms, over longer timescales, bacteria may gradually evolve resistance to it. Therefore, while clovibactin as a first-generation antibiotic may have the potential for long-term effectiveness, it is imperative to exercise caution, maintain continual monitoring, and undertake further research endeavors to gain a comprehensive understanding of the potential pathways of resistance development.

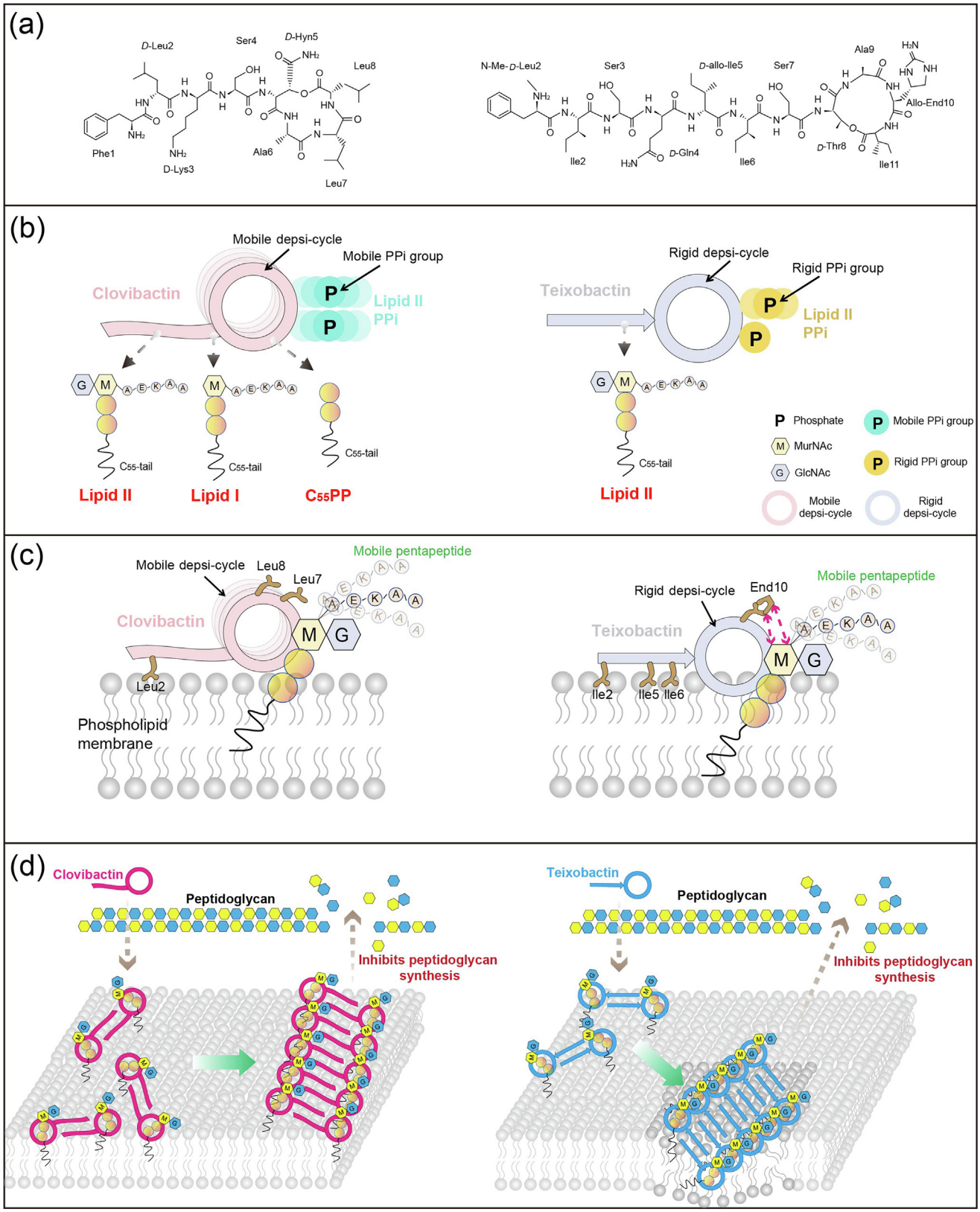

Fig. 1. Comparison of clovibactin and teixobactin and the formation of their complexes. (a) Chemical structures of clovibactin and teixobactin. (b) Molecular targets for clovibactin and teixobactin. (c) Schematic comparison of the binding interfaces between clovibactin and teixobactin. (d) Model of the mode of action of clovibactin and teixobactin. At membrane surface, both clovibactin and teixobactin bind to lipid II, forming small oligomers that act as nuclei for fibril formation.

Overall, in an era plagued by antibiotic resistance, the discovery of clovibactin is an encouraging stepping stone in hunting for antibiotic miracles. Clovibactin's unique structural features, broad-spectrum activity, resistance-avoidant mechanism, and supramolecular stability make it a promising candidate for the battle against antibiotic-resistant bacteria. Even when compared to

the new powerful antibiotic teixobactin, clovibactin boasts several distinct advantages (Fig. 1). As we navigate the evolving landscape of infectious diseases and drug resistance, unconventional approaches to antibiotic discovery, exemplified by the clovibactin story, are indispensable. Clovibactin may well be the harbinger of a paradigm shift in antibiotic discovery, redefining our strategies to combat antibiotic-resistant bacteria.

Latest scientific advancements offer alternative diverse approaches to tackle antimicrobial resistance, including synthetic biology, genome mining, and artificial intelligence in drug discovery [9]. In the field of synthetic biology, novel biosynthetic tool-kits are designed and constructed to produce antibiotics with specific activities. By altering the microbial metabolic pathways, novel antibiotics or antibiotic-like compounds can be generated. This customized biosynthesis approach offers broad prospects for the development of new antibiotics. Synthetic biology enables a significant reduction in the traditional antibiotic development cycle, along with increased antibiotic yield and efficiency, providing robust technical support for addressing antimicrobial resistance. Through the exploration and in-depth study of microbial genomes, gene clusters encoding potential antibiotic molecules can be discovered. These investigations contribute to uncovering new natural product antibiotics and understanding the biosynthetic pathways involved, laying the foundation for their subsequent biosynthesis. Microbial metabolic pathways can be optimized through various new methods such as genome editing and directed evolution to enhance antibiotic production capabilities and improve their pharmacological properties. Additionally, with the aid of machine learning and big data analysis, scientists can predict the biological activity of molecules, expediting the screening of compounds with antibacterial activity. Artificial intelligence and its related products including AlphaFold2 can be utilized to simulate molecular interactions and predict protein structures, assisting in the design of more effective antibiotic molecules. Further research and development in this domain may herald the dawn of a new era in antibiotic therapeutics, offering clinicians new toolkits or drugs to fight against pathogens that currently lack effective cures.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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References

[1] Lewis K. The science of antibiotic discovery. Cell 2020;181:29–45.
[2] Shukla R, Peoples AJ, Ludwig KC, et al. An antibiotic from an uncultured bacterium binds to an immutable target. Cell 2023;186:4059–73.
[3] Wilson MC, Mori T, Rückert C, et al. An environmental bacterial taxon with a large and distinct metabolic repertoire. Nature 2014;506:58–62.
[4] Shukla R, Lavore F, Maity S, et al. Teixobactin kills bacteria by a two-pronged attack on the cell envelope. Nature 2022;608:390–436.
[5] Chen X, Zhu R, Zhong J, et al. Mosaic composition of RIP1–RIP3 signalling hub and its role in regulating cell death. Nat Cell Biol 2022;24:471–82.
[6] Wu H. Higher-order assemblies in a new paradigm of signal transduction. Cell 2013;153:287–92.
[7] Wellington EM, Boxall AB, Cross P, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infect Dis 2013;13:155–65.
[8] Larsson DG, Flach CF. Antibiotic resistance in the environment. Nat Rev Microbiol 2022;20:257–69.
[9] Miethke M, Pieroni M, Weber T, et al. Towards the sustainable discovery and development of new antibiotics. Nat Rev Chem 2021;5:726–49.

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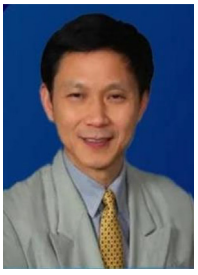

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