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REVIEW ARTICLE

The War on Superbugs: Teixobactin, and a New Frontier in Antibiotic Resistance

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ABSTRACT: An increasing rise in antimicrobial resistance is an alarming threat to health and well-being globally. Antibiotic resistance specifically is cause for alarm, as it renders many commonly used classes of antibiotics ineffective. The World Health Organization has developed different strategies to combat antibiotic resistance, one of those being research and development of new classes of drugs that are immune to antibiotic resistance. The objective of this paper is to evaluate traditional teixobactin and its different analogues, looking at their accessibility and potency. Data and research were collected from a variety of different studies and papers, which are listed in the references. Through this literature review, a few important conclusions about teixobactin and its analogues can be made. First, teixobactin has a unique structure and rare amino acid enduracididine, enabling it to use a "dual pronged" mechanism in killing off bacteria. Different teixobactin analogues attempt to solve the difficulty in synthesizing traditional teixobactin at a low cost, while also maintaining effectiveness. One study was able to achieve gram-scale total synthesis, while increasing the teixobactin analogue's potency, bringing teixobactin closer to becoming a clinically accessible antibiotic. More research needs to be done in the future to find teixobactin analogues effective against gram-negative bacteria.

KEYWORDS: Biochemistry, Medical Biochemistry, Antibiotic Resistance, Teixobactin.

Introduction

Antimicrobial resistance, specifically antibiotic resistance, is an increasingly dangerous threat to global health. In 2019, antimicrobial resistance was directly responsible for 1.27 million deaths and contributed to 4.95 million deaths globally. Antibiotic resistance occurs because bacteria evolve due to selective pressures and begin to resist the effects of antibiotic medicines, making standard treatments harder and leading to persistent infections.[1] Antibiotic resistance could result in 40 million deaths by 2050, and 2 million could die yearly by the middle of this century. Medical procedures can also become harder, as most require the use of antibiotics to prevent infections. Antibiotic resistance is also a significant economic burden, as it can lead to up to 3.4 trillion dollars in global costs by 2030.[1] Antibiotic resistance poses a significant health challenge and must be dealt with appropriately.[2]

There have been efforts to help prevent the severe harms of antibiotic resistance from spreading globally. The World Health Organization (WHO) has its own Global Action Plan. Adopted in 2015, it encourages countries to develop methods to combat antimicrobial resistance. Antimicrobial stewardship is another method they suggest, where governments seek to educate the public on how to use antimicrobials responsibly. Research and development is another strategy, where new antibiotics, vaccines, and diagnostics are created to fight infections.[1] Currently, there is not enough attention on research and development of new antibiotics, which is a major issue globally.[1]

Several factors contribute the most to antibiotic resistance. The first is the overuse and misuse of antibiotics. Excessive use in humans, animals, and agriculture directly contributes to the resistance, as overuse puts greater pressure on bacteria to evolve. Poor control of infections also contributes to the issue, as a lack of sanitation facilitates the spread of pathogens.[1]

One of the most dangerous bacteria that develops antibiotic resistance is the gram-negative ones. They often cause the deadliest infections and have a particularly strong drug resistance. Gram-negative bacteria are also resistant to carbapenem drugs (Figure 1), which are used to treat severe microbial infections. Deaths from carbapenem-resistant gram-negative bacteria have risen by almost 150% from 1990 to 2021.[2]

Teixobactin (Figure 2) is a novel antibiotic that differs in structure and class from other antibiotics, such as carbapenems. This review paper will focus on recent research and development of teixobactin and its general immunity to antibiotic resistance. This paper aims to analyse whether teixobactin and its different derivatives and analogues could contribute to the fight against antibiotic resistance.

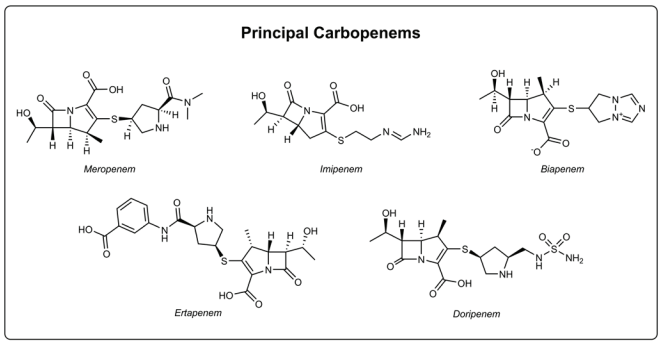

Figure 1: Chemical structures of representative carbapenems (meropenem, imipenem, biapenem, ertapenem, doripenem) are last-resort antibiotics for multidrug-resistant gram-negative bacteria.

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Discussion

Introduction to Teixobactin:

One important discovery in the recent fight against antibiotic resistance is teixobactin. Teixobactin is isolated from *Eleftheria terrae*, which is an uncultured gram-negative bacterium. *Eleftheria terrae* is cultivated using iChip technology. Teixobactin is a cyclic undecapeptide with four D-amino acids. It has one rare amino acid, which is L-allo-enduracididine (Figure 2). Enduracididine occurs rarely in nature, and teixobactin is one of the places the amino acid can be found in.[3] The mechanism through which teixobactin functions enables it to reduce antibiotic resistance. Teixobactin uses dual inhibition, where it targets both the peptidoglycan and wall teichoic acid precursors through binding to lipid II and lipid III, respectively. The way that teixobactin avoids antibiotic resistance is by binding to cell wall precursors rather than proteins.[3] Teixobactin binds in a 2:1 molar ratio with lipids, those being lipid I, II, III, and undecaprenyl pyrophosphate. The minimal binding motif of teixobactin is the pyrophosphate moiety and polyprenyl chain. Attacking the precursors curtails the genetic alteration of teixobactin's drug target in pathogenic bacteria, which prevents a common method of antibiotic resistance.[3] Additionally, because teixobactin targets lipid precursors, bacteria cannot mutate easily without disabling the cell wall itself and causing cell death, thus further lowering the chance of developing resistance.[4]

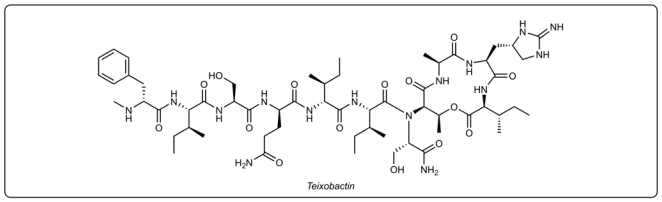

Figure 2: Chemical structure of teixobactin, a novel antibiotic with a unique scaffold that shows activity against multidrug-resistant gram-positive bacteria.

Studies claim that there are no resistant *S. aureus* mutants, a gram-positive bacterium, that have emerged in sub-MIC serial passaging. It is relevant to note that modification of the residues of the core ring structure of teixobactin can inhibit its biological activity. However, in Arg10-teixobactin, L-Ser3, D-Gln4, and Ala9 residues can be substituted with the corresponding enantiomer of lysine, and it will have similar functionality to that of Arg10-teixobactin.[3]

Comparison of Teixobactin to Other Antibiotics:

One of the studies investigated whether a bacterium like Staphylococcus aureus could evolve resistance to Arg10-teixobactin, and then compared it to moenomycin A and rifampicin, two other antibiotics. The experiment lasted 75 days, which is 500 generations, with antibiotic pressure. MICs were measured every 5 days, and whole genome sequencing was used to identify mutations. What the research found was that Arg10-teixobactin resistance evolved around 2,500 times slower than rifampicin, and around 320 times slower than moenomycin A. After withdrawal of the antibiotic after day 45 of the experiment, Arg10-teixobactin resistance dropped back to baseline within 30 days. This indicates a high fitness cost to teixobactin resistance, while antibiotics like moenomycin A and rifampicin experienced more persistent resistance. What this study shows is that Arg10-teixobactin has slow resistance and a high cost of resistance, which the other antibiotics do not possess to a high degree.[5]

Effectiveness of Teixobactin on Drug-resistant Bacteria:

One study investigated the antimicrobial activity of three teixobactin derivatives (Figure 3) against drug-resistant clinical isolates, specifically Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus* (VRE). The aim was to identify the most effective derivative by comparing its minimum inhibitory concentration (MIC). The MIC values for each compound against MRSA and VRE are summarized in Tables 1 and 2.

The results showed that compound **2**, specifically Lys10-teixobactin, exhibited the best performance in terms of MIC, although compound **3** was also effective. They are either comparable or superior to traditional drugs, showing promising signs that some teixobactin derivatives are effective against antibiotic-resistant bacteria.

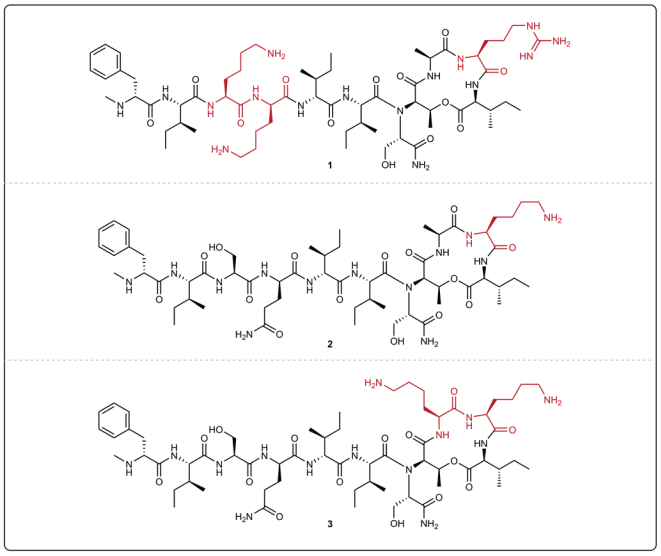

Figure 3: Chemical structures of teixobactin derivatives **1**, **2**, and **3** were investigated for activity against MRSA and VRE. Structural modifications compared to native teixobactin are highlighted in red. Derivative 2 showed the greatest resistance to MRSA and VRE.

Table 1: Minimum inhibitory concentration (μg/ml) of teixobactin derivatives against methicillin-resistant *Staphylococcus aureus* (MRSA).[6]

Isolates	Origin[a]	Species	1	2	3	Vancomycin	Ampicillin
			MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)
B11970	Blood	*S. aureus*	32	2	2	1	>512
P10781	Nasal	*S. aureus*	32	2	2	1	>512
P10747	CVP	*S. aureus*	32	2	2	1	>512
S37938	-	*S. aureus*	32	2	2	1	>512
S18155	ETT	*S. aureus*	32	2	2	0.5	>512
B13178	Blood	*S. aureus*	32	2	22	1	>512
440260	-	*S. aureus*	32	4	4	1	>512
S18970	-	*S. aureus*	32	2	2	1	>512
P11520	Pus	*S. aureus*	32	4	4	1	512
T5683	Nasal	*S. aureus*	32	2	2	1	>512
	MIC50		32	2	2	1	>512

[a]ETT, Endotracheal tube; CVP, Central venous catheter, -, Missing data.

Table 2: Minimum inhibitory concentration (μg/ml) of teixobactin derivatives against vancomycin-resistant *Enterococcus faecium* (VRE).[6]

Isolates	Species	1	2	3	Vancomycin
		MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)
951245262 (A)	*Enterococcus faecium*	8	4	4	>128
951234856 (B)	*Enterococcus faecium*	16	4	4	>128
951208931 (C)	*Enterococcus faecium*	16	4	4	>128
938636470 (D)	*Enterococcus faecium*	16	8	4	>128
938666613 (E)	*Enterococcus faecium*	16	16	4	>128
938600912 (F)	*Enterococcus faecium*	16	2	8	>128
938072607 (G)	*Enterococcus faecium*	16	8	4	>128
944414000 (H)	*Enterococcus faecium*	16	8	4	>128
945530665 (I)	*Enterococcus faecium*	16	4	4	>128
U43821 (J)	*Enterococcus faecium*	16	8	4	>128
	MIC50		4	4	>128

Limitations of Teixobactin and the Promise of Its Derivatives:

While teixobactin certainly opens a path in the fight against antibiotic resistance, some challenges and setbacks currently need to be resolved. The first problem is that while teixobactin is effective, it's difficult and expensive to synthesize. This is because of two major reasons. First, L-allo-enduracididine takes seven steps to synthesize, making it very complicated. L-allo-enduracididine is generally a non-natural amino acid, making it even harder to access. Teixobactin also requires four D-amino acids. Together, these two problems limit teixobactin's production, as it's difficult to mass-produce and also make it cost-effective.[7] Another problem with teixobactin is that it is not effective against gram-negative bacteria. That is because gram-negative bacteria have an outer membrane barrier that renders teixobactin's binding mechanism unviable.[3] This is problematic because, as stated above, gram-negative bacteria can often cause the deadliest infections as they tend to have the highest antibiotic resistance, and there needs to be a solution.

There is now further research and studies that explore possibilities in resolving the problems that teixobactin faces. One of those solutions is to develop simpler and more cost-effective teixobactin analogues. One study approached this issue by replacing the complex peptide tail, residues 1-6, of teixobactin with lipid chains to create lipopeptidomimetics.[7] The study also tested substitutions of enduracididine with other amino acids. This teixobactin analogue they looked to research would also be focused on its antibacterial effects on *S. aureus* in particular. The following table contains the results of the different teixobactin analogues against *S. aureus* and *E. coli* bacterial strains in minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (Table 3). The lower both values are, the more effective an antibiotic is. What the study discovered is that farnesylation, which elongates the lipid chain, improves activity significantly in comparison to geranyl, a shorter chain, or no lipid tail at all. The study also found that basic amino acids at position 10, being the enduracididine, are essential because they have a positive charge, which facilitates interactions with bacterial membranes. Neutral, acidic, or non-polar substitutions at position 10 led to a loss in activity, while Lys10 was better than Arg10, despite the latter being more structurally similar to native allo-enduracididine.

Table 3: MIC and MBC values of teixobactin analogues (in μg/mL) against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.[7]

	S. aureus ATCC 25923		*E. coli* ATCC 25922
Compound	MIC	MBC	MIC
Arg_{10}-farnesylbactin (1)	16	>32	>32
Lys_{10}-farnesylbactin (2)	8	16	16
Orn_{10}-farnesylbactin (3)	8	16	32
Cit_{10}-farnesylbactin (4)	>32	>32	>32
His_{10}-farnesylbactin (5)	>32	>32	>32
Ala_{10}-farnesylbactin (6)	>32	>32	>32
Glu_{10}-farnesylbactin (7)	>32	>32	>32
Arg_{10}-geranylbactin (8)	>32	>32	>32
Lys_{10}-geranylbactin (9)	>32	>32	>32
Orn_{10}-geranylbactin (10)	>32	>32	>32
Cit_{10}-geranylbactin (11)	>32	>32	>32
His_{10}-gernaylbactin (12)	>32	>32	>32
Ala_{10}-geranylbactin (13)	>32	>32	>32
Glu_{10}gernaylbactin (14)	>32	>32	>32

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This study provides examples of farnesylated teixobactin analogues that show antimicrobial activity. These simplified teixobactin analogues can lead to new antibiotics that are cheaper and more accessible compared to conventional teixobactin, while still maintaining partial effectiveness and resistance to antibiotic resistance.[7]

Another study also looked to make teixobactin analogues so the antibiotic could become more accessible. The approach they took was to research whether teixobactin analogues with a modified tail stereochemistry, notably replacing the D-amino acids with L-amino acids, could still retain antibacterial activity. Previous studies found that any stereochemical change in the tail would eliminate all activity, so the researchers tried to introduce "swapmers". Swapmers are teixobactin analogues where pairs of D-amino acids are replaced with their L counterparts.[8] The study incorporated an O-acyl linkage to serine to address some synthesis challenges. The antibacterial efficacy of different teixobactin analogues against multiple bacterial species is presented in Table 4.

Table 4: MIC values of teixobactin, teixobactin prodrugs, and "swapmer" analogues in μg/mL.[a]

Compound	*Bacillus subtilis* ATCC 6051 MIC	*Staphylococcus epidermidis* ATCC 14990 MIC	*Staphylococcus aureus* (MSSA) ATCC 29213 MIC	*Staphylococcus aureus* (MRSA) ATCC 700698 MIC	*Escherichia coli* ATCC 10798 MIC
Lys_{10}-teixobactin (1)	≤0.0313	1	2	2	>32
[Ile_8-O-Ser_7],Lys_{10}-teixobactin (Lys_{10}-teixobactin prodrug 2)	≤0.0313	1	2	1-2	>32
Ile_4,Gln_5,[Ile_8-O-Ser_7],Lys_{10}-teixobactin (Lys_{10}-swapmer prodrug 3)	4	4	8	8	>32
N-Me-L-Phe_1,Ile_4,Gln_5,[Ile_8-O-Ser_7],Lys_{10}-teixobactin (L-tail swapmer 4)	≥32	>32	>32	>32	>32
N-Bn-Gly_1,Ile_4,Gln_5,[Ile_8-O-Ser_7],Lys_{10}-teixobactin (peptoid swapmer 5)	32	>32	>32	>32	>32
Arg_{10}-teixobactin (6)	≤0.0313	0.5	2	2	>32
[Ile_8-O-Ser_7],Arg_{10}-teixobactin (Arg_{10}-teixobactin prodrug 7)	0.0625	0.5	2	1	>32
Ile_4,Gln_5,[Ile_8-O-Ser_7],Arg_{10}-teixobactin (Arg_{10}-swapmer prodrug 8)	2	2	4	4	>32
N-Me-Phe_8,Gln_1,Ile_4,Gln_5,[Ile_8-O-Ser_7],Arg_{10}-teixobactin (extended-tail swapmer 9)	>32	>32	>32	>32	>32
vancomycin	0.125-0.25	1-2	1-2	4	>32

[a]MIC assays were performed in the presence of 0.002% polysorbate 80.

The results showed that swapmers 3 and 8 maintained partial activity, although effectiveness was around 2-4 times worse compared to native teixobactin. D-stereochemistry at position 1 was also essential for the teixobactin to function properly. The conclusion of the study is thus that pairwise stereochemical swapping can be tolerated, maintaining its structure and preserving partial activity. The D-amino acid in position 1 is indispensable, however. This study is another effort at finding more synthetically accessible analogues that still retain activity. The next step is ensuring similar effectiveness to native teixobactin.[8]

Finally, one report surrounding teixobactin looked to, once again, find a way to make the antibiotic more accessible. While previous researchers had been able to achieve milligram-scale total synthesis, this study's goal was to achieve gram-scale total synthesis of teixobactin. It also wanted to further discover more potent analogues for clinical development. The study was able to innovate by developing a one-pot, one-hour synthesis of the L-allo-End building block with 66% yield and excellent stereoselectivity. This is crucial, as L-allo-End is one of the complicated portions of teixobactin to synthesize. The study then used a convergent 3+2+6 strategy to construct a cyclic pentapeptide and a linear hexapeptide separately, then joined them together to form the full-length teixobactin. To go even further, the researchers reasoned that the methyl group on N-Me-D-Phe1 was not essential, so they created a series of different teixobactin analogs and tested their efficacy. The most effective ones were found to be compounds **4** and **5** (Figure 4). Compound 4 demonstrated higher potency than traditional teixobactin, while compound **5** was 8 times better.[9] This is a major advancement in teixobactin development, as this study finds an efficient synthetic route for larger-scale teixobactin production, while also producing analogues with improved potency.

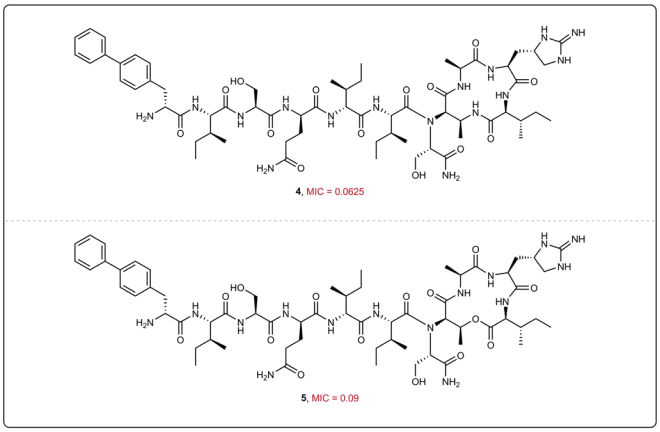

Figure 4: Chemical structure of compound **4** and compound **5**.

Conclusion

Teixobactin offers a new path forward in fighting antibiotic resistance. The different teixobactin derivatives and analogues also help improve upon the accessibility and cost-effectiveness issues that traditional teixobactin faces. While the scalability and economic issues are being addressed, one big possibility moving forward is to make teixobactin analogues that are effective against gram-negative bacteria. Many gram-negative bacteria pose the largest threat to human life, as they are highly resistant to many classes of antibiotics. There is research currently that shows highly hydrophobic derivatives of teixobactin can have some activity against gram-negative bacteria at high concentrations, but more work needs to be done to increase its potency.[9] Antibiotic resistance will become an ever-growing threat to human health and wellbeing, which is why developments in drugs like teixobactin are crucial for the future.

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