


## REVIEW ARTICLE

Non-ribosomally synthesized lipopeptides:  
Promising novel therapeutics for cancer  
treatment

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## Abstract

Bacteria-derived non-ribosomally synthesized lipopeptides (NRLPs) present promising potential for cancer treatment, alongside their known antimicrobial, anti-inflammatory, and other pharmacological effects, due to their unique properties and modular assembly. However, addressing challenges such as toxicity, pharmacokinetics, and regulatory considerations necessitates an in-depth understanding of lipopeptides. This review provides extensive insights into the modular synthesis pathways, molecular mechanisms, structural diversity, and bioactivities of NRLPs. It highlights the remarkable potential of these lipopeptides as innovative therapeutic agents for cancer treatment. A significant portion of the review is dedicated to unraveling the sources, types, and bioactivities of NRLPs, with particular emphasis on their anti-cancer properties. The mechanisms underlying their efficacy against cancer cells, including apoptosis induction, cell cycle modulation, and interference with signaling pathways, are discussed. Envisioning the future of cancer therapeutics, the review concludes by outlining strategies for improved peptide design, integration with existing therapies, innovative and targeted cancer treatments, and the incorporation of emerging technologies. This comprehensive overview underscores the transformative potential of NRLPs in reshaping the landscape of cancer treatment.

**Keywords:** Non-ribosomally synthesized lipopeptides; Non-ribosomally synthesized lipopeptide; Synthesis; Cytotoxicity; Anticancer drugs

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## 1. Introduction

The quest for innovative and effective therapeutics against cancer has propelled exploration into diverse molecular entities, with non-ribosomally synthesized lipopeptides (NRLPs)

emerging as captivating candidates in this pursuit.<sup>1-4</sup> The modular assembly of these lipopeptides, orchestrated by non-ribosomal peptide synthetases (NRPSs), gives rise to a myriad of structurally diverse molecules, each exhibiting distinct bioactivities.<sup>5</sup> This review endeavors to provide comprehensive insights into the potential of NRLPs as novel therapeutics for cancer treatment.

The modular nature of NRPSs, akin to nature's molecular assembly lines, governs the biosynthesis of lipopeptides, enabling the incorporation of various amino acids and imparting exquisite structural complexity to these peptides.<sup>6</sup> As a result, these NRLPs exhibit unique properties that render them promising candidates for therapeutic applications. The molecular mechanisms involved in NRLP synthesis are multifaceted, involving a series of enzymatic steps and post-translational modifications.<sup>7,8</sup> Understanding these mechanisms is crucial for unraveling the diverse array of lipopeptides and deciphering their biological activities.

The structural diversity of NRLPs forms a cornerstone of their therapeutic potential.<sup>9</sup> Classification and characterization of these molecules provide a framework for exploring their distinct functions and tailoring them for specific applications, particularly in the realm of cancer treatment. While the focus of this review is primarily on the potential of NRLPs as cancer therapeutics, it is essential to contextualize their broader biological activities. These peptides have demonstrated antimicrobial, anti-inflammatory, and other pharmacological effects, contributing to their versatility as therapeutic agents.<sup>10,11</sup>

As research progresses, preclinical studies have sought to validate the therapeutic efficacy of NRLPs. Investigations into *in vitro* cell culture models and *in vivo* xenograft or orthotopic models have provided valuable insights into their anti-cancer properties and safety profiles.<sup>12</sup> Despite the promising potential, challenges in developing NRLPs as cancer therapeutics persist. Toxicity concerns, pharmacokinetics, and regulatory considerations represent significant hurdles that necessitate careful consideration and strategic mitigation strategies.<sup>13</sup>

In addressing these challenges and optimizing the translational potential of NRLPs, this review also offers guidance on several future directions. Potential improvements in peptide design, integration with existing therapies, and the incorporation of emerging technologies are envisioned to shape the landscape of cancer treatment. Through this comprehensive exploration, we aim to provide a thorough understanding of NRLPs, with a primary focus on those of bacterial origin, thereby offering a glimpse into their promising potential as innovative therapeutics in the ongoing battle against cancer.

## 2. Non-ribosomally synthesized lipopeptides

Non-ribosomally synthesized lipopeptides constitute a diverse group of peptides characterized by the presence of a fatty acid (lipid) moiety attached to a peptide chain. They are produced by enzymes independent of the ribosomal machinery, often referred to as NRPSs, and may self-assemble into a variety of forms. These compounds are produced by certain soil bacteria and fungi as metabolic byproducts (Table 1). Due to their chemical nature and structure, they can disrupt the cell membrane integrity and cellular function, ultimately promoting cell death.<sup>14</sup> Lipopeptides often exhibit surfactant, antibacterial, antifungal, insecticidal, or hemolytic properties, attracting considerable interest from the agricultural, chemical, food, and pharmaceutical industries. Moreover, they show promise as antitumor agents.<sup>11,15</sup> The key sources of lipopeptides are briefly discussed below:

### 2.1. Lipopeptides from *Bacillus* and *Paenibacillus* species

Bacteria of the Gram-positive genus *Bacillus* produce numerous cyclic lipopeptides, several of which possess appreciable antibacterial or antifungal properties.<sup>16</sup> Lipopeptides in *Bacillus* and *Paenibacillus* species are synthesized in a ribosome-independent manner. Structural diversity in these lipopeptides arises from factors such as the type of fatty acids, chain length, amino acid composition and configuration, and the presence of hydroxyl groups and/or iso- or anteiso-methyl branches.<sup>7</sup>

### 2.2. Lipopeptides from actinomycetes (*Streptomyces*)

Actinomycetes, particularly the genus *Streptomyces*, serve as sources of a large number of antifungal and antibiotic compounds. For example, *Streptomyces roseosporus* (actinobacteria) produces daptomycin, an acidic, cyclic lipopeptide comprised of 13 amino acids, including three D-amino acid residues (D-asparagine, D-alanine, and D-serine), linked through the N-terminal trypsin to decanoic acid.<sup>17,18</sup>

### 2.3. Lipopeptides from *Pseudomonas* species

The genus *Pseudomonas* produces many cyclic lipopeptides with surfactant, antibacterial, and antifungal properties, and few are reported to exhibit anti-cancer activity. Despite evolutionary differences, the non-ribosomal mechanism for lipopeptide assembly in *Pseudomonas* species shares commonalities with that of *Bacillus* species.<sup>19,20</sup>

### 2.4. Lipopeptides from cyanobacteria

An increasing number of cyanobacteria species, particularly those of marine origin, have been found to produce lipopeptides and glycolipopeptides with novel structures.<sup>21,22</sup>

Table 1. A summary of key characteristics of non-ribosomally synthesized lipopeptides

Family	Properties					
	Lipopeptide	Producing microbe	Use	Peptide length (with isomeric variants)	Fatty acid type and length	Structure
Surfactins	Surfactin	<i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i>	Antifungals, antibiotics, surfactants	Cyclic heptapeptide	$\beta$ -hydroxy FA, 13 – 15 carbons (carbon number may differ for each variant.)	Cyclic lactone ring
	Lichenysin	<i>Bacillus licheniformis</i>	Antibiotics, surfactants			
	Pumilacidin	<i>Bacillus pumilus</i>	Antivirals			
Iturins	Iturin	<i>Bacillus subtilis</i>	Biopesticides	Cyclic heptapeptide	$\beta$ -amino FA, 14 – 17 carbons (carbon number may differ for each variant.)	Cyclic peptide ring
	Bacillomycin	<i>Bacillus megaterium</i>	Antifungals			
Fengycins	Fengycin	<i>Bacillus subtilis</i>	Biopesticides	Cyclic decapeptide	$\beta$ -hydroxy FA, 16 – 19 carbons (carbon number may differ for each variant.)	Cyclic lactone ring
	Plipastatin	<i>Bacillus thuringiensis</i>	Antibiotics			
Kurstakins	Kurstakin	<i>Bacillus thuringiensis kurstakin HD-1</i>	Antifungals	Cyclic heptapeptide	$\beta$ -hydroxy FA, 11 – 14 carbons	Cyclic lactone ring
Polymyxins	Polymyxin B	<i>Bacillus polymyxa</i>	Antibiotics	Cyclic heptapeptide with a tripeptide side chain		
	Polymyxin E (Colistin)	<i>Bacillus polymyxa</i>	Antibiotics			
<i>Streptomyces</i> sp. lipopeptides	Daptomycin	<i>Streptomyces roseoporous</i>	Antibiotics	Cyclic decapeptide with a tripeptide side chain		
<i>Paenibacillus</i> sp. lipopeptides	Fusaricidins	<i>Paenibacillus</i> sp.	Antibiotics	Cyclic hexadepsipeptides		
	Paenibacterin	<i>Paenibacillus thiaminolyticus</i>	Antibiotics			
	Octapeptin	<i>Paenibacillus tianmuensis</i>	Antibiotics			
<i>Pseudomonas</i> sp. lipopeptides	Viscosin	<i>Pseudomonas libanensis</i> , <i>Paenibacillus Fluorescens</i>	Antibiotics, antifungals			
	Syringopeptin	<i>Paenibacillus syringae</i>	Antibiotics			
	Xantholysin	<i>Paenibacillus putida</i>	Antibiotics			

Abbreviation: FA: Fatty acid.

## 2.5. Fungal lipopeptides

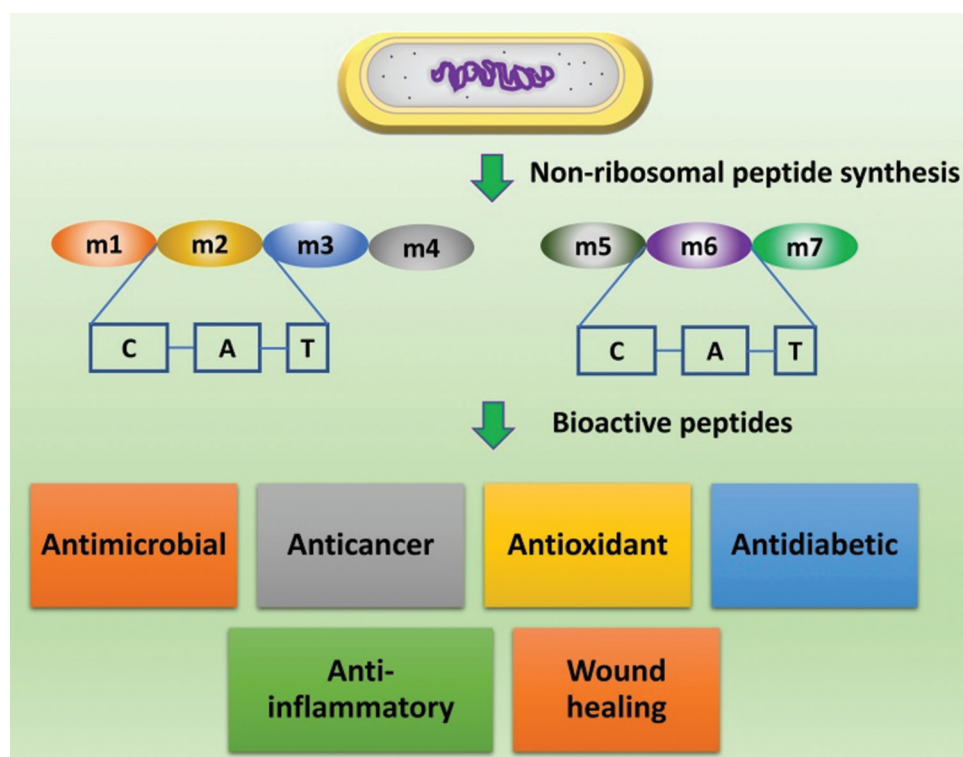
Approximately 30 genera of fungi produce cyclic and linear lipopeptides with antibiotic and antifungal properties, some of which are mycotoxins. For instance, echinocandins, non-ribosomal cyclic hexapeptides produced by fungi such as *Glarea lozoyensis* exhibit potent antifungal properties.<sup>23</sup>

Genetic modifications to both the peptide and fatty acid moieties offer avenues for producing novel compounds with fewer side effects and greater efficacy. Therefore, microbial lipopeptides offer a wide range of development prospects and are in high demand in the market due to their low toxicity, high efficiency, and adaptability compared to synthetic surfactants.<sup>24</sup> Dedicated research into lipopeptide synthesis, such as that conducted by Yaraguppi *et al.*,<sup>25</sup> is essential to overcome the low efficiency and high production

costs limiting the mass production of lipopeptides and their widespread use in industry. The challenge now lies in translating research findings into clinical practice, as only a few of the thousands of identified lipopeptides have been approved by the United States Food and Drug Administration (FDA) for therapeutic applications. One well-known example of lipopeptides is surfactin, produced by bacteria such as *Bacillus subtilis*. Surfactin possesses surfactant properties and can form micelles, making it useful in various industrial and pharmaceutical applications.<sup>4</sup>

## 3. Principle of non-ribosomal peptide synthesis

The intricate metabolic network and precursor requirements for synthesis, the specific and rigorous synthesis pathway, and the coexistence of multiple



**Figure 1.** Biosynthesis and bioactivity of non-ribosomal peptides (NRPs). The modular structure of NRP synthetase reveals that modules comprise several domains. Synthesized NRPs exhibit several bioactivities, including anticancer, antimicrobial, antioxidant, antidiabetic, and anti-inflammatory.<sup>31,32</sup> Notes: m1 – m7 denotes module1–module7; C, A, and T denote condensation domain, adenylation domain, and thiolation domain, respectively.

homologous substances pose challenges to the large-scale production of lipopeptides.<sup>24</sup> Genes encoding for NRPSs, genes necessary for the monomer biosynthesis, and tailoring enzymes responsible for introducing additional alterations in the peptide are organized in biosynthetic gene clusters known as assembly lines, which serve as the home for NRPSs. These gene clusters frequently encode several peptide synthetases, often interconnected by communication domains. The clustered nature of these genes facilitates the cloning of entire NRPS pathways. Each NRPS is arranged into modules, with each module functioning as a catalytic unit that adds one amino acid to the elongating peptide chain. The resulting non-ribosomal peptides (NRPs) follow the sequence and chemical properties dictated by the order of the catalytic units.<sup>26,27</sup>

The process of NRP synthesis requires the involvement of three domains: adenylation, thiolation, and condensation domains. Thiolation domains (T domains) are activated by phosphopantetheinyl transferases (PPTases), which modify the amino acid residue to carry the sulfhydryl group essential for thioester bond formation (Figure 1). Adenylation domains (A domains) subsequently attach the amino acid to the activated T domain, displaying high substrate specificity for a single monomer. Condensation

domains catalyze the formation of peptide bonds when two neighboring monomers are activated.<sup>28–30</sup>

The majority of NRPSs feature a thioesterase (TE) domain at their terminus, which aids in terminating NRP assembly. For TE domains to operate, they must initially attach to the peptide's conserved serine residue. Subsequently, they use a nucleophile to sever the connection. TE domains are categorized into two groups: macrocyclization and product cleavage. The former cleaves the thioester bond between the product and the enzyme complex, facilitating a nucleophilic attack with water. The latter introduces a macrocycle into the product, connecting the two peptide termini or introducing any other peptide bond-based cycle. In addition, independent but cluster-encoded thioesterases can function as rescue proteins for stalled NRPSs.<sup>33–36</sup>

#### 4. Critical bacterial non-ribosomally synthesized lipopeptides

Bacterial lipopeptides are amphiphilic molecules consisting of short linear chains or cyclic structures of amino acids. These structures include peptides where one or more amide bonds are substituted by an ester bond (or lactone in cyclic structures). These peptides are linked by either an ester or



an amide bond to a fatty acid of variable chain length, often accompanied by the presence or absence of substituents, primarily hydroxyl groups or methyl branches. Notably, the amino acid mixture predominantly consists of those of the D-configuration rather than the typical L-configuration, likely serving to resist protease activity.<sup>37</sup> A single bacterial species can generate numerous structural variants, or isoforms, differing by the composition of one or two amino acids and fatty acids. These lipopeptides can be divided into three types, namely, surfactin, iturin, and fengycin.<sup>20</sup> Within each family, variants share the same peptide length but exhibit different residues at specific positions, with each variant possessing several isomers.

Classification of lipopeptide surfactants mainly relies on amino acid sequences and the diverse strains of *Bacillus* spp. responsible for their production, such as *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Bacillus pumilus*, and *Bacillus licheniformis*.<sup>32</sup> The subsequent discussion highlights some representative lipopeptides:

#### 4.1. Surfactin

Surfactin (1036 Da) is an amphipathic cyclic lipopeptide biosurfactant produced by numerous species within the bacterial genus *Bacillus*, initially isolated by Arima *et al.* in 1968, reported by Roongsawang *et al.*<sup>7</sup> Originally screened from the culture media of *B. subtilis* strains, surfactin found application as a clotting inhibitor.<sup>38</sup> Comprising a heptapeptide (ELLVDLL) coupled with a chiral sequence (LLDLLDL), surfactin forms a close cyclic lactone ring structure linked with the  $\beta$ -hydroxy (fatty acid chain) of the carbon chain (C12 – C16). Its structure includes both hydrophobic (located at 2 – 4, 6, and 7) and hydrophilic (located at 1 and 5) components.<sup>39</sup> In aqueous solutions, surfactin displays stable and conserved folding, with negatively charged amino acids (Glu and Asp) exhibiting polar domains. Moreover, it exhibits solubility in organic solvents, such as dichloromethane, ethanol, chloroform, butanol, and methanol.<sup>32</sup>

The peptide portion adopts a topology akin to a “horse saddle” and is referred to as the  $\beta$ -sheet structure in the backbone folding. These structural characteristics are believed to contribute to the broad spectrum of biological properties associated with surfactin.<sup>40</sup> Naturally occurring isoforms of surfactin exhibit variations solely in their physicochemical properties, such as (i) the type of amino acid within the peptide ring at the second, fourth, and seventh positions and (ii) the branching of the hydroxyl fatty acid moiety and chain length. Isoforms are influenced by *Bacillus* spp. and other factors such as media composition, environmental conditions, and nutritional substrate content.<sup>41</sup> Previous studies have reported

surfactin's potent antitumoral, antiviral, anticoagulant, enzyme inhibition, and antimycoplasmal activities.<sup>15</sup> In addition, *in silico* analyses have determined surfactin's affinity to cancer cell ligands.<sup>4</sup>

#### 4.2. Lichenysin

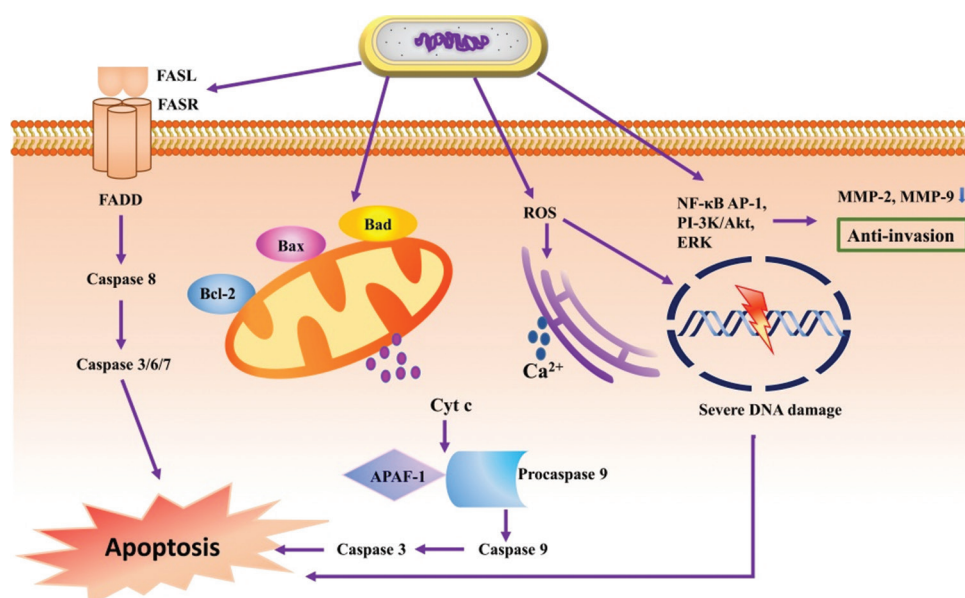
Lichenysin, a lipopeptide produced by *B. licheniformis*, shares structural similarities with surfactin produced by *B. subtilis*. Its structure, cyclo-[L-Gln1 $\rightarrow$ D-Leu2 $\rightarrow$ L-Leu3 $\rightarrow$ L-Val4 $\rightarrow$ L-Asp5 $\rightarrow$ D-Leu6 $\rightarrow$ L-Ile7- $\beta$ -OH fatty acid], differs from surfactin by the substitution of glutamine with glutamic acid in the first amino acid position.<sup>42</sup> However, this minor variation markedly enhances lichenysin's surfactant properties, rendering it an excellent chelating agent for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .<sup>43</sup> Lichenysin also exhibits antimicrobial, anti-inflammatory, antitumor, and immunosuppressive properties alongside hemolytic activity.<sup>32</sup> These traits stem from the amphiphilic nature of the lipopeptide. Structurally, lichenysin consists of amino acids and a  $\beta$ -hydroxy fatty acid along with C12 – C17 carbon atoms. Various isoforms of lichenysin exist in nature, including lichenysin A.<sup>44</sup>

#### 4.3. Kurstakin

Kurstakin, a low-molecular-weight lipopeptide primarily produced and isolated from *B. thuringiensis kurstakin* HD-1, features the following amino acid sequence: Thr-Gly-Ala-Ser-His-Gln-Gln. The fatty acyl chain of kurstakin is linked with the N-terminal amino acid residue via an amide bond, while each lipopeptide consists of a lactone linkage between the carboxyl-terminal amino acid and the hydroxyl group in the side chain of the serine residue.<sup>45</sup>

#### 4.4. Iturin

Iturin is an important class of lipopeptides with a molecular mass of approximately 1.1 kDa. Iturin A consists of two components: (i) C14 – C17 (amino fatty acids) and (ii) seven amino acid residues (heptapeptides; Asn-Tyr-Asn-Gln-Pro-Asn-Ser). Iturins D and E vary from iturin A due to the inclusion of mojavensin, mycosubtilin, bacillomycin D, bacillomycin F, and bacillomycin L. This variation arises from the presence of a free carboxyl group in iturin D and a carboxymethyl group in iturin E. The amphiphilic character of iturin's structure is noteworthy.<sup>46</sup> Iturin molecules are of great interest due to their biological activities and physicochemical properties, finding application in the oil, pharmaceutical, and food industries. Almost all strains of *B. subtilis* produce iturin lipopeptide, with its operon ranging from 38 to 40 kb in size and containing four open reading frames such as *ituA*, *ituB*, *ituC*, and *ituD*, differing in the amino acid sequences of the heptapeptides.<sup>47</sup> Iturin has been reported to exhibit potent



**Figure 2.** Anti-cancer mechanism of lipopeptides from *Bacillus subtilis*. These lipopeptides induce apoptosis in cancer cells through both intrinsic and extrinsic pathways. They upregulate the expression of apoptosis-related genes such as *BAX* and *BAD* while concurrently downregulating the *BCL2* gene. This modulation leads to caspase activation and ultimately culminates in apoptosis. Furthermore, they inhibit cell proliferation through ROS-dependent mitochondrial and caspase pathways. Additionally, lipopeptides hinder the invasion of cancer cells by inhibiting MMP-2 and MMP-9.<sup>64</sup>

antifungal activity against *Botrytis cinerea*, *Alternaria alternata*, and *Penicillium expansum*. Moreover, it displays robust surface activity and destabilizing effects.<sup>48</sup>

#### 4.5. Fengycins

Fengycins are lipopeptides primarily produced by the genera of *Bacillus* and *Paenibacillus*, renowned for their potent antifungal activity, particularly against filamentous fungi.<sup>49,50</sup> Comprising decapeptides and C14 – C19 to  $\beta$ -hydroxy fatty acid chains, fengycins demonstrate potent antifungal activity.<sup>51</sup> Two subclasses of fengycins, namely, fengycin A and fengycin B, differ only by the amino acid attached at position 6. Fengycin B features Val at position 6, whereas fengycin A contains Ala. Fengycins A and B were initially identified in the *B. subtilis* strain by Vanittanakom *et al.*<sup>51</sup> A closely related fengycin type, named plipastatin, was also reported distinguished by the position of amino acids L-Tyr and D-Tyr.<sup>52</sup>

### 5. The general mode of action

The antimicrobial and antitumor activities of lipopeptides involve multiple mechanisms. Lipopeptides disrupt the cell membranes of target microorganisms, ultimately leading to cell death. This disruption primarily stems from the lipophilic (fat-attracting) and amphiphilic (both hydrophobic and hydrophilic) properties inherent to lipopeptides. They electrostatically interact with the charged head groups of membrane lipids and the

hydrophobic region of lipid bilayers.<sup>53</sup> Consequently, electrostatic and mechanical changes, along with reduced surface tension and enhanced metal ion sequestration, destabilize microbial cell membrane lipid bilayers. This disruption promotes pore formation, resulting in the leakage of cellular contents and eventual cell lysis, rendering lipopeptides effective against a range of pathogens.<sup>20</sup>

### 6. Cancer cell cytotoxic properties of non-ribosomally synthesized lipopeptides

The bioactivities of NRLPs position them as promising candidates for cancer treatment, showcasing their potential as innovative therapeutics. They exhibit diverse mechanisms of action against cancer cells. Non-ribosomally synthesized disrupt key signaling pathways involved in cancer cell survival, growth, and metastasis (Figure 2). This interference contributes to their anti-cancer effects and offers opportunities for targeted therapies.<sup>54</sup> By regulating cell cycle progression, these peptides impede the uncontrolled proliferation characteristic of cancer cells<sup>55</sup> and induce programmed cell death (apoptosis) in cancer cells.<sup>56</sup> Understanding these bioactivities is crucial for evaluating the therapeutic potential of NRLPs and their specific applications in cancer treatment.

Microbial lipopeptides, a class of amphiphilic molecules, feature fatty acid moieties covalently connected with peptide moieties.<sup>57</sup> Many of these molecules, possessing membrane-active properties, play an important

role in inducing apoptosis in tumor cells. For example, surfactin, a cyclic lipopeptide produced by *B. subtilis*, is well-known for its antitumor activity against Ehrlich carcinoma cells and LoVo cells.<sup>58</sup> In addition, surfactin induces membrane leakage through pore formation, ion channel formation, cation carrier activity, and detergent-like effects,<sup>59</sup> all of which eventually lead to cytotoxic effects. Surfactin inhibits 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced invasion, migration, and colony formation of human breast carcinoma cells by inhibiting matrix metalloproteinase-9 (MMP-9).<sup>60</sup> In addition, it has demonstrated utility in preventing platelet aggregation and enhancing fibrinolysis.<sup>61</sup>

A study has suggested that cyclic lipopeptides possess an anti-proliferative effect on K562 cells by inducing apoptosis.<sup>62</sup> Characterization of lipopeptide structures has revealed surprising diversity and, along with their unique functional groups, points to an encouraging area of anticancer research. However, the precise mechanism by which the membrane-active properties of lipopeptides contribute to the induced apoptosis of tumor cells remains unknown. Lipopeptides produced by *B. subtilis* HSO121 have demonstrated selective cytotoxicity against Bcap-37 cell lines, inducing apoptosis with a significant decrease in the unsaturated degree of cellular fatty acids.<sup>1</sup> Lipopeptides from *B. subtilis* exhibit a significant impact on various cancers, due to the production of stable bioactive molecules and enzymes by *B. subtilis*.<sup>63</sup>

Cancerous cells from breast, colon, lung, liver, pancreas, cervix, and stomach can interact with lipopeptides if introduced, inducing aberrant regulation of the cell cycle by influencing cancer cell regulatory checkpoints and cell cycle checkpoints.<sup>64</sup> They also impact signaling pathways, interfering with downstream signaling. Potential bioactive compounds produced by *B. subtilis* efficiently treat various cancer cell lines through apoptosis, paraptosis, and autophagy.<sup>64</sup> On the other hand, the molecular mechanism of lipopeptides such as iturin, surfactin, and fengycin, derived from *B. subtilis*, targets several cancer types by primarily disrupting the cell cycle, as increased proliferation of cancerous cells exacerbates the disease. Surfactin molecules effectively inhibit cancer cell invasion and migration, acting as antagonists for colony development of tumor cell lines by modulating various regulatory proteins, thus exhibiting anticancer activity.<sup>65</sup> Iturin is reported to inhibit the proliferation of breast cancer cells MDA-MB-231<sup>58,66</sup> and MCF-7, alveolar adenocarcinoma A549, renal carcinoma A498, chronic myelogenous leukemia cells K562, and colon adenocarcinoma HCT-15.<sup>67</sup> Fengycin can block non-small cell lung cancer cell 95D and inhibit the growth of xenografted 95D cells in denuded

mice.<sup>3</sup> Overall, *B. subtilis* lipopeptides (consisting of a majority of iturin) exhibit promising potential in inhibiting chronic myelogenous leukemia *in vitro* by simultaneously inducing paraptosis, apoptosis, and inhibiting autophagy.<sup>56</sup>

In addition, lipopeptides exhibit immunomodulatory effects, enhancing the body's immune response against cancer. However, it is important to note that research in this area is still underway at the time of writing, and the specific mechanisms may vary among different lipopeptides.

## 7. Microbial lipopeptide-based drugs

Lipopeptide-based therapeutics are currently undergoing investigation for various applications, including antimicrobial and anticancer treatments, with ongoing research and development in this field. Several NRPL-based drugs have demonstrated promising results in both preclinical and clinical studies, showcasing high success rates in treating specific diseases. Notable NRPL-based drugs are discussed as follows:

- (i) Daptomycin (Cubicin) stands as a lipopeptide antibiotic approved by the FDA for treating skin and bloodstream infections caused by Gram-positive bacteria. Clinical trials have revealed daptomycin's high efficacy and low resistance rates, with success rates ranging from 80% to 95% in treating infections caused by methicillin-resistant *Staphylococcus aureus* and other Gram-positive bacteria.<sup>68</sup> It is administered intravenously.

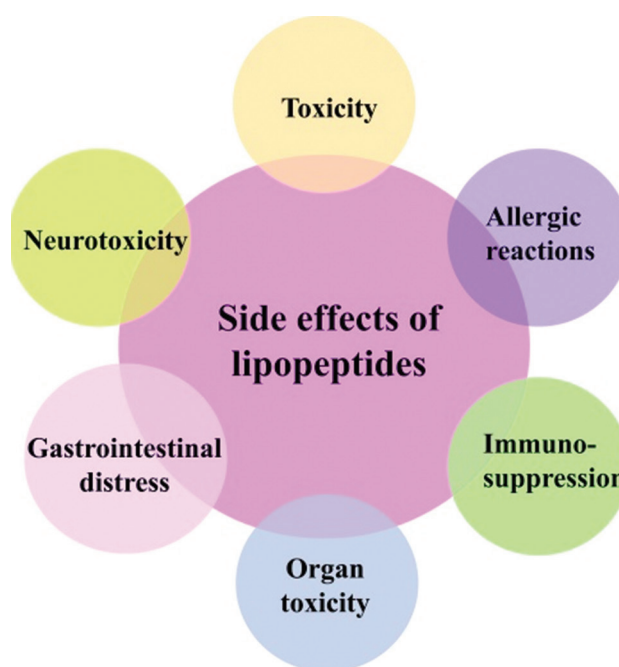


Figure 3. Side effects of lipopeptides.

- (ii) Telaprevir is a lipopeptide-based drug that has been approved for the treatment of hepatitis C virus (HCV) infection. Clinical trials involving telaprevir have demonstrated high rates of sustained virologic response, ranging from 70% to 80%, in treating HCV genotype 1 infection.<sup>69</sup>
- (iii) Polymyxin B, a cationic lipopeptide antibiotic, is used to treat multidrug-resistant Gram-negative bacterial infections. It has demonstrated high efficacy in treating infections caused by *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and other Gram-negative bacteria, with success rates ranging from 60% to 70%.<sup>70</sup> It is often reserved as a last-resort treatment option due to potential toxicity.
- (iv) Surfactin is a lipopeptide-based drug studied for its potential therapeutic applications, demonstrating effectiveness against a range of bacteria, fungi, and viruses. Research has shown that surfactin effectively inhibited the growth of several pathogenic bacteria, including *S. aureus* and *Escherichia coli*.<sup>71</sup> In addition, surfactin has exhibited an 80% success rate in inhibiting the growth of *S. aureus* at a concentration of 0.2%.<sup>72</sup> Furthermore, surfactin has shown promising antiviral activity against the influenza virus.
- (v) Iturin is among the most studied lipopeptide-based drugs, exhibiting similar potential to surfactin. Several studies have reported iturin's success rate in various applications, including antimicrobial activity against a range of bacteria and fungi. Iturin demonstrated a success rate of 90 – 97% in inhibiting the growth of *Fusarium oxysporum*<sup>11</sup> and has also proven its antiviral activity against viruses such as the herpes simplex virus and human immunodeficiency virus (HIV).
- (vi) In terms of anticancer effects, both surfactin and iturin have demonstrated potent cytotoxic effects against several cancer cell lines, including breast cancer and lung cancer.

Overall, the success rate of lipopeptide-based drugs such as surfactin and iturin can be high, but it can also depend on the specific application and target organism. Further research is warranted to fully understand the potential of these drugs.

However, while lipopeptides hold therapeutic potential, it is essential to acknowledge potential adverse effects (Figure 3). It is important to note that the specific adverse effects may vary depending on the type of lipopeptide, its formulation, and individual patient factors.<sup>20</sup>

## 8. Challenges and considerations

Research on NRLPs for cancer treatment faces several challenges and considerations that demand careful

attention. Addressing these challenges and considerations is paramount for successfully translating NRLPs into viable cancer therapeutics. The summary of challenges and considerations is outlined as follows:

### 8.1. Toxicity concerns and mitigation strategies

Non-ribosomally synthesized lipopeptides may raise toxicity concerns, necessitating thorough evaluation in preclinical and clinical studies. Understanding the range and nature of potential toxicities is critical for ensuring patient safety.<sup>73</sup> Future research should focus on developing and implementing effective mitigation strategies for identified toxicities. This endeavor may involve refining peptide design to enhance selectivity, minimizing off-target effects, and optimizing dosing regimens.<sup>74</sup>

### 8.2. Pharmacokinetics and bioavailability issues

Factors such as stability, solubility, and delivery mechanisms can limit the efficacy of NRLPs. Addressing these issues is crucial to achieving therapeutic concentrations at the target site.<sup>75</sup> Research efforts should concentrate on optimizing formulations and delivery systems to enhance the pharmacokinetics of these peptides. Exploring nanotechnology applications and innovative delivery strategies may improve overall bioavailability.<sup>76</sup>

### 8.3. Regulatory considerations and approval processes

Translating NRLPs from research to clinical applications requires navigating complex regulatory processes. Researchers must address safety concerns, demonstrate efficacy, and comply with regulatory standards to progress through approval processes.<sup>77</sup>

### 8.4. Collaboration with regulatory bodies

Collaboration between researchers and regulatory bodies is essential to streamlining the approval process. Ongoing communication and transparency can facilitate a smoother transition from preclinical research to clinical trials and eventual market approval.

### 8.5. Adherence to Good Manufacturing Practices

Meeting Good Manufacturing Practices (GMP) standards is critical to ensuring the quality and consistency of peptide production. Researchers must adhere to GMP guidelines to meet regulatory requirements for clinical development and commercialization.<sup>78</sup>

## 9. Future directions

Research on NRLPs for cancer treatment holds promise for advancing therapeutic strategies. The potential improvements and directions are outlined as follows:



## 9.1. Potential improvements in peptide design and synthesis

### 9.1.1. Rational design approaches

Future research may focus on implementing rational design approaches for NRLPs. Leveraging structural and functional insights can aid in optimizing peptide sequences for enhanced specificity, bioavailability, and therapeutic efficacy.<sup>79</sup>

### 9.1.2. Incorporation of novel building blocks

Exploring the incorporation of novel building blocks, including non-canonical amino acids and post-translational modifications, can contribute to diversifying the structural landscape of these peptides. This diversification may lead to improved properties and expanded therapeutic applications.<sup>80</sup>

### 9.1.3. Advancements in synthesis technologies

Continuous advancements in peptide synthesis technologies, such as solid-phase synthesis and automation, may facilitate the production of NRLPs on a larger scale. These advancements could enhance accessibility for clinical applications.<sup>81</sup>

## 9.2. Integration of non-ribosomally synthesized lipopeptides with existing therapies

### 9.2.1. Combination therapies

Future research may explore the integration of NRLPs with existing cancer therapies.<sup>82</sup> Combination therapies could exploit synergistic effects, overcome resistance mechanisms, and improve overall treatment outcomes.

### 9.2.2. Strategic targeting

Identifying specific molecular targets or signaling pathways that can be modulated by NRLPs may enable strategic targeting in combination with conventional treatments.<sup>4</sup> This approach could enhance the precision of cancer therapies.

## 9.3. Emerging technologies and methodologies in the field

### 9.3.1. Genomic and proteomic approaches

Advancements in genomic and proteomic technologies may contribute to a deeper understanding of the molecular signatures associated with the response to NRLPs.<sup>83</sup> This knowledge could inform patient stratification and personalized treatment approaches.

### 9.3.2. Nanotechnology applications

Exploring nanotechnology applications, such as nanoformulations or drug delivery systems, may enhance the pharmacokinetics and bioavailability of NRLPs. These

advancements can improve their targeted delivery to cancer cells.<sup>76</sup>

### 9.3.3. Machine learning and computational modeling

Integration of machine learning and computational modeling approaches may aid in predicting peptide behavior, optimizing design, and accelerating the identification of potential therapeutic candidates.<sup>84</sup>

## 9.4. Immuno-modulatory peptides

Exploration of peptides not only directly targets cancer cells but also modulates the immune system to enhance anti-tumor responses. Immunomodulatory peptides may play a crucial role in the development of cancer immunotherapies.<sup>85</sup>

## 10. Conclusion

Non-ribosomally synthesized lipopeptides are undergoing investigation as potential cancer therapies. These molecules exhibit diverse bioactivities, including anti-cancer, antimicrobial, and anti-inflammatory effects. They have demonstrated the ability to induce apoptosis, modulate cell cycles, and interfere with signaling pathways. However, safety concerns, optimization of pharmacokinetics, and regulatory considerations pose challenges to their clinical application. Strategic mitigation strategies, formulation enhancements, and collaboration with regulatory agencies are crucial for successful clinical translation. The field of NRLP research holds exciting prospects, with potential improvements in peptide design, integration with existing therapies, and the incorporation of emerging technologies. As researchers explore synergies with conventional treatments, the future may witness the emergence of NRLPs as transformative agents in personalized cancer care. Continued exploration and translation of their potential into clinical practice could lead to innovative and targeted cancer treatments.

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The authors declare that they have no competing interests.

## Author contributions

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